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(54) Title: PHYTASE VARIANTS AND POLYNUCLEOTIDES ENCODING SAME

(57) Abstract: The present invention relates to phytase variants. The present invention also relates to polynucleotides encoding the variants; nucleic acid constructs, vectors, and host cells comprising the polynucleotides; and methods of using the variants.

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PHYTASE VARIANTS AND POLYNUCLEOTIDES ENCODING SAME

REFERENCE TO A SEQUENCE LISTING

5 This application contains a Sequence Listing in computer readable form, which is incorporated herein by reference.

REFERENCE TO ATOMIC COORDINATES

This application sets forth in Figure 5, the atomic coordinates of the three-dimensional structure of the phytase variant (var400).

10 FIELD OF THE INVENTION

The present invention relates to phytase variants, polynucleotides encoding the variants, methods of producing the variants, and methods of using the variants.

BACKGROUND OF THE INVENTION

15 Phytases are well-known enzymes, as are the advantages of adding them to foodstuffs for animals, including humans. Phytases have been isolated from various sources, including a number of fungal and bacterial strains.

It is an object of the present invention to provide alternative polypeptides having phytase activity (phytases) and polynucleotides encoding the polypeptides. The phytase variants of the invention exhibit modified or altered preferably improved properties as compared to the parent phytase. Non-limiting examples of such properties are: stability (such as acid-stability, heat-stability, steam stability, pelleting stability, and/or protease stability, in particular pepsin stability), temperature profile, pH profile, specific activity, substrate specificity, performance in animal feed (such as an improved release and/or degradation of phytate), susceptibility to glycation, and/or glycosylation pattern.

As described herein, mutagenesis of a parent polynucleotide encoding a phytase is employed to prepare variant (synthetic) DNAs encoding a phytase having improved properties relative to the phytase encoded by the parent polynucleotide.

Citrobacter

30 The sequence of the phyA gene from a strain of *Citrobacter freundii* has been submitted by Zinin et al to the EMBL/GenBank/DDBJ databases with accession no. AY390262. The corresponding phytase amino acid sequence is found in the UniProt/TrEMBL databases with accession no. Q676V7. The expected mature part of Q676V7 is included in the present sequence listing as SEQ ID NO: 4.

35 WO-2004/085638 (Republic of National Fisheries Research and Development Institute of Korea) discloses, as SEQ ID NO: 7, the amino acid sequence of a phytase from *Citrobacter braakii* YH-15, deposited as KCCM 10427. The mature part of this amino acid sequence is included

herein as SEQ ID NO: 3. This sequence is also found in the database Geneseq with accession no. ADU50737.

WO 2006/037328 (Novozymes A/S) discloses the wildtype phytase of *Citrobacter braakii* ATCC 51113 (i.e., SEQ ID NO: 2 herein), as well as a variant thereof, which is also included in the present sequence listing, viz. as SEQ ID NO: 6.

WO 2006/038062 and WO 2006/038128 (Danisco A/S) both disclose the amino acid sequence of the phytase gene of *Citrobacter freundii* P3-42, deposited under accession number NCIMB 41247 and a number of variants thereof. This amino acid sequence is included herein as SEQ ID NO: 9. These applications disclose only one substitution in position 233 to a cysteine (S233C) according to the numbering used herein this would be S211C. The texts of WO 2006/038062 and WO 2006/038128 seem to be identical.

WO 2007/112739 (Novozymes A/S) discloses a large number of phytase variants with exemplification using *Citrobacter braakii* ATCC 51113 phytase as parent. WO 2007/112739 indicates inter alia the creation of disulfide bridges.

WO2011/117396 (Novozymes A/S) discloses additional phytase variants with exemplification using *Citrobacter braakii* ATCC 51113 phytase as variant by introducing two or more disulfide bridges in the molecule.

SUMMARY OF THE INVENTION

The present invention is directed, in one aspect, to phytase variants which has at least 70% identity to SEQ ID NO: 2 and which comprises the alterations N31C+G52C+A99C+K141C+T177C+V199C as compared to SEQ ID NO: 2, so as to form disulfide bridges between positions 52 and 99, 31 and 177, and 141 and 199 and further comprises a substitution in one or more position(s) selected from the following: 30, 36, 43, 46, 57, 60, 64, 73, 79, 119, 121, 123, 130, 134, 138, 151, 155, 161, 162, 168, 176, 180, 184, 190, 207, 224, 230, 243, 273, 286, 336, 340, 358 and 375 using SEQ ID NO: 2 for numbering.

The present invention relates to phytase variants which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% identity, but less than 100% identity, to SEQ ID NO: 2 and which comprises the alterations N31C+G52C+A99C+K141C+T177C+V199C+N203L as compared to SEQ ID NO: 2 and further comprises a substitution in one or more position(s) selected from the following: 30, 36, 43, 46, 57, 60, 64, 73, 79, 119, 121, 123, 130, 134, 138, 151, 155, 161, 162, 168, 176, 180, 184, 190, 207, 224, 230, 243, 273, 286, 336, 340, 358 and 375 using SEQ ID NO: 2 for numbering, and which have phytase activity.

A further aspect is directed to an isolated polypeptide having phytase activity, selected from the group consisting of

- a) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at

least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 12;

b) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 14;

c) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 16;

d) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 18; and

e) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 20.

Typically said polypeptide is pH stable and thermostable such that it comprises one or more of the following properties

i. an unfolding temperature at pH 4 of at least 75 °C;

ii. an unfolding temperature at pH 3 of at least 70°C; and

iii. an unfolding temperature at pH 2 of at least 55°C.

Alternatively defined, the said polypeptide is acid stable such that it maintains a residual activity level above 90% after 24 hours at each of pH 2, 3, 4, 5, 6, 7 and 8.

The present invention also relates to polynucleotides encoding the variants; nucleic acid constructs, vectors, and host cells comprising the polynucleotides; and methods of producing the variants.

The invention accordingly relates to method of preparing a recombinant polypeptide having phytase activity comprising the steps of:

(a) cultivating a recombinant host cell comprising an exogenous polynucleotide selected from the group consisting of

a. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 13;

b. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 15;

c. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 17;

d. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 19;

e. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO:

21;

wherein the polynucleotide is expressed and the polypeptide is produced;

(b) optionally isolating the polypeptide; and

(c) optionally recovering the polypeptide.

5 The invention further relates to a method of producing a polypeptide of the present invention, said method comprising the steps of:

(a) cultivating a recombinant host cell comprising an exogenous polynucleotide encoding the polypeptide having phytase activity selected from the group consisting of

10 a. a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99% or 100 identity to SEQ ID NO: 12;

b. a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99% or 100 identity to
15 SEQ ID NO: 14;

c. a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99% or 100 identity to SEQ ID NO: 16;

d. a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99% or 100 identity to
20 SEQ ID NO: 18; and

e. a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 20;

25 wherein the polynucleotide is expressed and the polypeptide is produced;

(b) optionally isolating the polypeptide; and

(c) optionally recovering the polypeptide.

The present invention further relates to compositions, in particular animal feed compositions comprising the variants of the invention, and the use of such compositions for
30 improving the nutritional value of an animal feed; reducing the phytate levels in animal manure; treating vegetable proteins; for liberating phosphorous from a phytate substrate; or for increasing weight gain, improving specific growth rate and/or improving Feed Conversion Ratio of an animal; or for improving nutrient retention, and/or nutrient digestibility in an animal.

35 BRIEF DESCRIPTION OF THE SEQUENCE LISTING

In the Sequence listing the sequences apply as follows:

SEQ ID NO: 1 represents the polynucleotide sequence of the phytase from *Citrobacter*

braakii ATCC 51113 (WO 2006/037328).

SEQ ID NO: 2 represents the polypeptide sequence of the phytase from *Citrobacter braakii* ATCC 51113 (WO 2006/037328).

5 SEQ ID NO: 3 represents the polypeptide sequence of the phytase from *Citrobacter braakii* YH-15 (WO-2004/085638).

SEQ ID NO: 4 represents the polypeptide sequence of the phytase from *Citrobacter freundii* (UniProt/TrEMBL accession no. Q676V7).

SEQ ID NO: 5 represents a variant of SEQ ID NO: 2 (18 is Xaa and 323 are Xaa).

SEQ ID NO: 6 represents a variant of SEQ ID NO: 2 (18 is Gly and 323 is Pro).

10 SEQ ID NO: 7 represents *Citrobacter braakii* ATCC 51113 signal peptide.

SEQ ID NO: 8 represents *Citrobacter braakii* ATCC 51113 pro-peptide.

SEQ ID NO: 9 represents the polypeptide sequence of the phytase from *Citrobacter freundii* NCIMB 41247 (WO 2006/038062 and WO 2006/038128).

15 SEQ ID NO: 10 represents the mature polypeptide sequence of a variant of SEQ ID NO: 2, entitled var300.

SEQ ID NO: 11 represents the polynucleotide sequence encoding for SEQ ID NO: 10 with coding sequence (CDS) from nucleotide 11 to nucleotide 1351.

SEQ ID NO: 12 represents the mature polypeptide sequence of a variant of SEQ ID NO: 2, entitled var400.

20 SEQ ID NO: 13 represents the polynucleotide sequence encoding for SEQ ID NO: 12 with CDS from nucleotide 11 to nucleotide 1351.

SEQ ID NO: 14 represents the mature polypeptide sequence of a variant of SEQ ID NO: 2, entitled var404.

25 SEQ ID NO: 15 represents the polynucleotide sequence encoding for SEQ ID NO: 14 with CDS from nucleotide 11 to nucleotide 1351.

SEQ ID NO: 16 represents the mature polypeptide sequence of a variant of SEQ ID NO: 2, entitled var405.

SEQ ID NO: 17 represents the polynucleotide sequence encoding for SEQ ID NO: 16 with CDS from nucleotide 11 to nucleotide 1351.

30 SEQ ID NO: 18 represents the mature polypeptide sequence of a variant of SEQ ID NO: 2, entitled var406.

SEQ ID NO: 19 represents the polynucleotide sequence encoding for SEQ ID NO: 18 with CDS from nucleotide 11 to nucleotide 1351.

35 SEQ ID NO: 20 represents the mature polypeptide sequence of a variant of SEQ ID NO: 2, entitled var411.

SEQ ID NO: 21 represents the polynucleotide sequence encoding for SEQ ID NO: 20 with CDS from nucleotide 1 to nucleotide 1341.

SEQ ID NO: 22 represents a polypeptide sequence of a protease.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an example of an alignment of the phytase of SEQ ID NO: 2 and the phytase of SEQ ID NO: 9.

Figure 2 shows feed consumption ratio (FR) and body weight (BW) evolution during the experimental trial.

Figure 3 shows schema for bone P retention sampling.

Figure 4 shows phosphorus in the plasma of fish from different experimental treatments after 91 days of feeding. Data are presented as mean $\pm$ SD (Standard Deviation). Significant treatments ($p < 0.05$) are shown by different letters.

Figure 5 sets forth the atomic coordinates of the three-dimensional structure of the phytase variant (var400). These atomic coordinates can aid in generating a three-dimensional model depicting the structure of the phytase variant (var400) and a three-dimensional model of homologous structures, such as variants of the aforementioned phytase variant.

DETAILED DESCRIPTION OF THE INVENTION

A phytase with improved properties, including improved intrinsic temperature and pH stability and improved *in vivo* efficacy per enzyme unit (FYT) is herein described. As known to the person skilled in the art, these types of improvements not only allow for flexibility in the formulation of the product and the consequent cost-savings with this flexibility, it provides for improved removal of phytate and anti-nutritional factors, improved release of phosphorous, calcium and myo-inositol and increased digestibility of phosphorous, improved muscle protein accretion by myo-inositol release and minimized P excretion for improved sustainability.

The present invention is directed, in one aspect, to phytase variants which has at least 70% identity to SEQ ID NO: 2 and which comprises the alterations N31C+G52C+A99C+K141C+T177C+V199C as compared to SEQ ID NO: 2, so as to form disulfide bridges between positions 52 and 99, 31 and 177, and 141 and 199.

The present invention relates to phytase variants which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% identity to SEQ ID NO: 2 and which comprises the alterations N31C/G52C/A99C/K141C/T177C/V199C as compared to SEQ ID NO: 2 and further comprises a substitution in one or more position(s) selected from the following: 30, 36, 43, 46, 57, 60, 64, 73, 79, 119, 121, 123, 130, 134, 138, 151, 155, 161, 162, 168, 176, 180, 184, 190, 207, 224, 230, 243, 273, 286, 336, 340, 358 and 375 using SEQ ID NO: 2 for numbering, wherein the variants have phytase activity.

Variants

The present invention is directed, in one aspect, to phytase variants which has at least 70% identity to SEQ ID NO: 2 and which comprises the alterations N31C+G52C+A99C+K141C+T177C+V199C as compared to SEQ ID NO: 2, so as to form disulfide bridges between positions 52 and 99, 31 and 177, and 141 and 199.

5 The present invention provides phytase variants, comprising the substitutions N31C/G52C/A99C/K141C/T177C/V199C/N203L as compared to SEQ ID NO: 2 and further comprises an alteration in one or more position(s) selected from the following: 30, 36, 43, 46, 57, 60, 64, 73, 79, 119, 121, 123, 130, 134, 138, 151, 155, 161, 162, 168, 176, 180, 184, 190, 207, 224, 230, 243, 273, 286, 336, 340, 358 and 375 using SEQ ID NO: 2 for numbering, wherein the
10 variants have phytase activity.

In an embodiment, the alteration is a substitution.

In an embodiment, the variant has sequence identity of at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, but less than 100%, to the amino
15 acid sequence of the parent phytase.

In another embodiment, the variant has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, such as at least 96%, at least 97%, at least 98%, or at least 99%, but less than 100%, sequence identity to the mature polypeptide of SEQ ID NO: 2.

20 In one aspect, the variants of the invention comprises the substitutions N31C/G52C/A99C/K141C/T177C/V199C/N203L as compared to SEQ ID NO: 2 and further alterations, where the number of further alterations in the variants of the present invention is 1-30, e.g., 1-20, 1-10 and 1-5, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 alterations.

In another aspect, the variants of the invention comprises the substitutions
25 N31C/G52C/A99C/K141C/T177C/V199C/N203L as compared to SEQ ID NO: 2 and further one or more substitution in positions corresponding to 57, 73, 121, 134, 155, 207 and 273 using SEQ ID NO: 2 for numbering. In a preferred embodiment, the variants comprises the substitutions N31C/G52C/A99C/K141C/T177C/V199C/N203L as compared to SEQ ID NO: 2 and further two or more substitution, e.g. 2, 3, 4, 5, 6 or 7 substitutions; in positions corresponding to 57, 73, 121,
30 134, 155, 207 and 273 using SEQ ID NO: 2 for numbering.

In another aspect, the variant comprises or consists of a substitution at a position corresponding to position 57. In another aspect, the amino acid at a position corresponding to position 57 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Tyr. In another aspect, the variant comprises or consists
35 of the substitution E57Y of the mature polypeptide of SEQ ID NO: 2.

In another aspect, the variant comprises or consists of a substitution at a position corresponding to position 73. In another aspect, the amino acid at a position corresponding to

position 73 is substituted with Ala, Arg, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Pro. In another aspect, the variant comprises or consists of the substitution N73P of the mature polypeptide of SEQ ID NO: 2.

5 In another aspect, the variant comprises or consists of a substitution at a position corresponding to position 121. In another aspect, the amino acid at a position corresponding to position 121 is substituted with Ala, Arg, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Pro. In another aspect, the variant comprises or consists of the substitution N121P of the mature polypeptide of SEQ ID NO: 2.

10 In another aspect, the variant comprises or consists of a substitution at a position corresponding to position 134. In another aspect, the amino acid at a position corresponding to position 134 is substituted with Ala, Arg, Asp, Asn, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Thr, Trp, Tyr, or Val, preferably with Gln. In another aspect, the variant comprises or consists of the substitution S134Q of the mature polypeptide of SEQ ID NO: 2.

15 In another aspect, the variant comprises or consists of a substitution at a position corresponding to position 155. In another aspect, the amino acid at a position corresponding to position 155 is substituted with Ala, Arg, Asp, Asn, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, or Val, preferably with Phe. In another aspect, the variant comprises or consists of the substitution Y155F of the mature polypeptide of SEQ ID NO: 2.

20 In another aspect, the variant comprises or consists of a substitution at a position corresponding to position 207. In another aspect, the amino acid at a position corresponding to position 207 is substituted with Ala, Arg, Asp, Asn, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, or Val, preferably with Thr. In another aspect, the variant comprises or consists of the substitution P207T of the mature polypeptide of SEQ ID NO: 2.

25 In another aspect, the variant comprises or consists of a substitution at a position corresponding to position 273. In another aspect, the amino acid at a position corresponding to position 273 is substituted with Ala, Arg, Asp, Asn, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Leu. In another aspect, the variant comprises or consists of the substitution M273L of the mature polypeptide of SEQ ID NO: 2.

30 In another aspect, the variant comprises or consists of an alteration at positions corresponding to positions 57 and 73, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57 and 121, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57 and 134, such as those described above.

35 In another aspect, the variant comprises or consists of an alteration at positions corresponding to positions 57 and 155, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57 and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57 and 273, such as those described above.

5 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, and 121, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73 and 134, such as those described above.

10 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, and 155, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73 and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73 and 273, such as those described above.

15 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 121 and 134, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 121 and 155, such as those described above.

20 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 121 and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 121 and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 134 and 155, such as those described above.

25 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 134 and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 134 and 273, such as those described above.

30 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 155 and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 155 and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 207 and 273, such as those described above.

35 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, and 121, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, and 134, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, and 155, such as those described above.

5 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, and 273, such as those described above.

10 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 121, and 134, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 121, and 155, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 121, and 207, such as those described above.

15 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 121, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 155, and 207, such as those described above.

20 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 155, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 207, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 121, and 134, such as those described above.

25 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 121, and 155, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 121, and 207, such as those described above.

30 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 121, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 134, and 155, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 134, and 207, such as those described above.

35 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 134, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 155, and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 134, and 207, such as those described above.

5 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 134, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 121, 134, and 155, such as those described above.

10 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 121, 134, and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 121, 134, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 134, 155, and 207, such as those described above.

15 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 134, 155, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 155, 207, and 273, such as those described above.

20 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 121, and 134, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 121, and 155, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 121, and 207, such as those described above.

25 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 121, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 134, and 155, such as those described above.

30 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 134, and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 134, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 155, and 207, such as those described above.

35 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 155, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 207, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 121, 134, and 155, such as those described above.

5 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 121, 134, and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 121, 134, and 273, such as those described above.

10 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 121, 155, and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 121, 207, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 134, 155, and 207, such as those described above.

15 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 134, 155, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 155, 207, and 273, such as those described above.

20 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 121, 134, and 155, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 121, 134, and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 121, 134, and 273, such as those described above.

25 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 134, 155, and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 134, 155, and 273, such as those described above.

30 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 134, 207, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 155, 207, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 121, 134, 155, and 207, such as those described above.

35 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 121, 134, 155, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 121, 134, 207, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 134, 155, 207, and 273, such as those described above.

5 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 121, 134, and 155, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 121, 134, and 207, such as those described above.

10 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 121, 134, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 121, 155, and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 121, 155, and 273, such as those described above.

15 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 121, 207, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 134, 155, and 207, such as those described above.

20 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 134, 155, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 134, 207, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 155, 207, and 273, such as those described above.

25 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 121, 134, 155, and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 121, 134, 155, and 273, such as those described above.

30 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 121, 134, 207, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 121, 155, 207, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 134, 155, 207, and 273, such as those described above.

35 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 121, 134, 155, and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 121, 134, 155, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 121, 134, 207, and 273, such as those described above.

5 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 121, 155, 207, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 134, 155, 207, and 273, such as those described above.

10 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 121, 134, 155, 207, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 121, 134, 155 and 207, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 121, 134, 155, and 273, such as those described above.

15 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 121, 134, 207, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 121, 155, 207 and 273, such as those described above.

20 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 134, 155, 207, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 121, 134, 155, 207, and 273, such as those described above.

In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 73, 121, 134, 155, 207, and 273, such as those described above.

25 In another aspect, the variant comprises or consists of alterations at positions corresponding to positions 57, 73, 121, 134, 155, 207, and 273, such as those described above.

30 In another aspect, the variant comprises or consists of one or more (e.g., several) substitutions selected from the group consisting of 30Q, 36A, 43C, 46C, 57Y, 60H, 64Q, 73P, 79Q, 119P, 121P, 123C, 130T,C, 134Q, 138A, 151S, 155F, 161T, 162A, 176P, 180N, 184Q, 190T, 207T, 224Q, 230E, 243N, 273L, 286S, 336R, 340L,P, 358Q and 375K.

35 In another aspect, the variant comprises or consists of one or more (e.g., several) substitutions selected from the group consisting of K30Q, Q36A, P43C, W46C, E57Y, Q60H, L64Q, N73P, S79Q, E119P, N121P, P123C, M130T,C, S134Q, L138A, N151S, Y155F, S161T, S162A, N168R, E176P, T180N, S184Q, P190T, P207T, E224Q, Q230E, R243N, M273L, N286S, K336R, T340L,P, D358Q and D375K.

In another aspect, the variant comprises or consists of the substitutions.

In a preferred embodiment, the variants comprises the substitutions N31C/G52C/A99C/K141C/T177C/V199C/N203L as compared to SEQ ID NO: 2, or of a polypeptide having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity to the mature polypeptide of SEQ ID NO: 2 which has phytase activity, and further the variant comprises substitutions in the positions: 57, 73, 121, 134, 155, 207 and 273, preferably 57Y, 73P, 121P, 134Q, 155Y, 207T and 273L; and further comprises one or more substitution in one or more of the positions: 30, 36, 43, 46, 60, 64, 79, 119, 123, 130, 138, 151, 161, 162, 168, 176, 180, 184, 190, 224, 230, 243, 286, 336, 340, 358 and 375.

If a variant comprises the substitution P43C as compared to SEQ ID NO: 2 it is preferred that it also comprises the substitution W46C. If a variant comprises the substitution W46C as compared to SEQ ID NO: 2 it is preferred that it also comprises the substitution P43C. If a variant comprises the substitution P123C as compared to SEQ ID NO: 2 it is preferred that it also comprises the substitution M130C. If a variant comprises the substitution M130C as compared to SEQ ID NO: 2 it is preferred that it also comprises the substitution P123C.

The amino acid changes may be of a minor nature, that is conservative amino acid substitutions or insertions that do not significantly affect the folding and/or activity of the protein; small deletions, typically of 1-30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue; a small linker peptide of up to 20-25 residues; or a small extension that facilitates purification by changing net charge or another function, such as a poly-histidine tract, an antigenic epitope or a binding domain.

Examples of conservative substitutions are within the groups of basic amino acids (arginine, lysine and histidine), acidic amino acids (glutamic acid and aspartic acid), polar amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tryptophan and tyrosine), and small amino acids (glycine, alanine, serine, threonine and methionine). Amino acid substitutions that do not generally alter specific activity are known in the art and are described, for example, by H. Neurath and R.L. Hill, 1979, *In, The Proteins*, Academic Press, New York. Common substitutions are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, and Asp/Gly.

Alternatively, the amino acid changes are of such a nature that the physico-chemical properties of the polypeptides are altered. For example, amino acid changes may improve the thermal stability of the polypeptide, alter the substrate specificity, change the pH optimum, and the like.

Essential amino acids in a polypeptide can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, 1989, *Science* 244: 1081-1085). In the latter technique, single alanine mutations are

introduced at every residue in the molecule, and the resultant mutant molecules are tested for phytase activity to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al.*, 1996, *J. Biol. Chem.* 271: 4699-4708. The active site of the enzyme or other biological interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction, or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos *et al.*, 1992, *Science* 255: 306-312; Smith *et al.*, 1992, *J. Mol. Biol.* 224: 899-904; Wlodaver *et al.*, 1992, *FEBS Lett.* 309: 59-64. The identity of essential amino acids can also be inferred from an alignment with a related polypeptide.

The crystal structure of the phytase variant (var400) was solved at a resolution of 1.33 Å. The atomic coordinates of this structure are shown in Figure 5. These atomic coordinates can be used to generate a three-dimensional model depicting the structure of the phytase variant (var400) or homologous structures (such as more variants of the present invention). The three-dimensional structure shows the molecular consequence of the introduced mutations, and it confirms that disulfide bridges between positions 52 and 99, 31 and 177, and 141 and 199 are formed.

In an embodiment, the variant has improved stability under storage conditions compared to the parent enzyme.

In an embodiment, the variant has improved thermostability compared to the parent enzyme.

Examples of variants according to the invention including variants having following substitution in comparison with SEQ ID NO: 2:

N31C/G52C/E57Y/N73P/A99C/N121P/S134Q/K141C/Y155F/T177C/V199C/N203L/P207T/M273L;

N31C/Q36A/G52C/E57Y/Q60H/L64Q/N73P/A99C/E119S/N121P/M130T/S134Q/L138A/K141C/Y155F/S161T/S162A/E176P/T177C/T180N/S184Q/P190T/V199C/N203L/P207T/E224Q/Q230E/R243N/M273L/K336R/T340L;

N31C/Q36A/P43C/W46C/G52C/E57Y/Q60H/L64Q/N73P/A99C/E119S/N121P/M130T/S134Q/L138A/K141C/Y155F/S161T/S162A/E176P/T177C/T180N/S184Q/P190T/V199C/N203L/P207T/E224Q/Q230E/R243N/M273L/N286S/K336R/T340L;

N31C/Q36A/G52C/E57Y/Q60H/L64Q/N73P/A99C/E119S/N121P/P123C/M130C/S134Q/L138A/K141C/N151S/Y155F/S161T/S162A/E176P/T177C/T180N/S184Q/P190T/V199C/N203L/P207T/E224Q/Q230E/R243N/M273L/K336R/T340L; and

N31C/Q36A/P43C+W46C+G52C+E57Y+Q60H+L64Q+N73P+A99C+E119S+N121P+P123C+M130C+S134Q+L138A/K141C/Y155F/S161T/S162A/E176P/T177C/T180N/S184Q/P190T/V199C/N203L/P207T/E224Q/Q230E/R243N/M273L/N286S/K336R/T340L.

Phytase Polypeptides, Percentage of Identity

In the present context a phytase is a polypeptide having phytase activity, i.e. an enzyme which catalyzes the hydrolysis of phytate (myo-inositol hexakisphosphate) to (1) myo-inositol and/or (2) mono-, di-, tri-, tetra- and/or penta-phosphates thereof and (3) inorganic phosphate.

5 In the present context the term a phytate substrate encompasses, i.e., phytic acid and any phytate (salt of phytic acid), as well as the phosphates listed under (2) above.

The ENZYME site at the internet (<http://www.expasy.ch/enzyme/>) is a repository of information relative to the nomenclature of enzymes. It is primarily based on the recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology
10 (IUB-MB) and it describes each type of characterized enzyme for which an EC (Enzyme Commission) number has been provided (Bairoch A. The ENZYME database, 2000, Nucleic Acids Res 28:304-305). See also the handbook Enzyme Nomenclature from NC-IUBMB, 1992).

According to the ENZYME site, three different types of phytases are known: a so-called 3-phytase (alternative name 1-phytase; a myo-inositol hexaphosphate 3-phosphohydrolase, EC
15 3.1.3.8), a so-called 4-phytase (alternative name 6-phytase, name based on 1L-numbering system and not 1D-numbering, EC 3.1.3.26), and a so-called 5-phytase (EC 3.1.3.72). For the purposes of the present invention, all three types are included in the definition of phytase.

In a particular embodiment, the phytases of the invention belong to the family of acid histidine phosphatases, which includes the *Escherichia coli* pH 2.5 acid phosphatase (gene appA)
20 as well as fungal phytases such as *Aspergillus awamorii* phytases A and B (EC: 3.1.3.8) (gene phyA and phyB). The histidine acid phosphatases share two regions of sequence similarity, each centered around a conserved histidine residue. These two histidines seem to be involved in the enzymes' catalytic mechanism. The first histidine is located in the N-terminal section and forms a phosphor-histidine intermediate while the second is located in the C-terminal section and possibly
25 acts as proton donor.

In a further particular embodiment, the phytases of the invention have a conserved active site motif, viz. R-H-G-X-R-X-P, wherein X designates any amino acid (see amino acids 16 to 22 of SEQ ID NOs: 2, 3, 4, 6 and amino acids 38-44 of SEQ ID NO: 9). In a preferred embodiment, the conserved active site motif is R-H-G-V-R-A-P, i.e. amino acids 16-22 (by reference to SEQ ID
30 NO: 2) are RHGVRAP.

For the purposes of the present invention the phytase activity is determined in the unit of FYT, one FYT being the amount of enzyme that liberates 1 micro-mol inorganic ortho-phosphate per min. under the following conditions: pH 5.5; temperature 37°C; substrate: sodium phytate (C₆H₆O₂₄P₆Na₁₂) in a concentration of 0.0050 mol/l. Suitable phytase assays are the FYT and FTU
35 assays described in Example 1 of WO 00/20569. FTU is for determining phytase activity in feed and premix. Phytase activity may also be determined using the assays of Example 1 ("Determination of phosphatase activity" or "Determination of phytase activity").

In a particular embodiment the phytase of the invention is isolated. The term “isolated” as used herein refers to a polypeptide which is at least 20% pure, preferably at least 40% pure, more preferably at least 60% pure, even more preferably at least 80% pure, most preferably at least 90% pure, and even most preferably at least 95% pure, as determined by SDS-PAGE. In particular, it is preferred that the polypeptides are in “essentially pure form”, i.e., that the polypeptide preparation is essentially free of other polypeptide material with which it is natively associated. This can be accomplished, for example, by preparing the polypeptide by means of well-known recombinant methods or by classical purification methods.

The relatedness between two amino acid sequences is described by the parameter “identity”. For purposes of the present invention, the alignment of two amino acid sequences is determined by using the Needle program from the EMBOSS package (<http://emboss.org>) version 2.8.0. The Needle program implements the global alignment algorithm described in Needleman, S. B. and Wunsch, C. D. (1970) J. Mol. Biol. 48, 443-453. The substitution matrix used is BLOSUM62, gap opening penalty is 10, and gap extension penalty is 0.5.

The degree of identity between an amino acid sequence of the present invention (“invention sequence”) and the amino acid sequence referred to in the claims (SEQ ID NO: 2) is calculated as the number of exact matches in an alignment of the two sequences, divided by the length of the “invention sequence,” or the length of the SEQ ID NO: 2, whichever is the shortest. The result is expressed in percent identity.

An exact match occurs when the “invention sequence” and SEQ ID NO: 2 have identical amino acid residues in the same positions of the overlap (in the alignment example below this is represented by “|”). The length of a sequence is the number of amino acid residues in the sequence (e.g. the length of amino acids 1-411 of SEQ ID NO: 2 is 411).

Example 11 in WO2011/117396 is an example of an alignment of the phytase of SEQ ID NO: 2 and the phytase of SEQ ID NO: 9, and the example illustrates how to calculate the percentage of identity between these two backbones.

In another, purely hypothetical, alignment example below, the overlap is the amino acid sequence “HTWGER-NL” of Sequence 1; or the amino acid sequence “HGWGEDANL” of Sequence 2. In the example a gap is indicated by a “-”.

Hypothetical alignment example:

```
Sequence 1:      ACMSHTWGER-NL
                  |  | |  | |
Sequence 2:      HGWGEDANLAMNPS
```

In a particular embodiment, the percentage of identity of an amino acid sequence of a polypeptide with, or to, SEQ ID NO: 2 is determined by i) aligning the two amino acid sequences using the Needle program, with the BLOSUM62 substitution matrix, a gap opening penalty of 10, and a gap extension penalty of 0.5; ii) counting the number of exact matches in the alignment; iii)

dividing the number of exact matches by the length of the shortest of the two amino acid sequences, and iv) converting the result of the division of iii) into percentage.

In the above hypothetical example, the number of exact matches is 6, the length of the shortest one of the two amino acid sequences is 12; accordingly the percentage of identity is 50%.

5 In particular embodiments of the phytase of the invention, the degree of identity to SEQ ID NO: 2 is at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99%. In still further particular embodiments, the degree of identity is at least 98.0%, 98.2%, 98.4%, 98.6%, 98.8%, 99.0%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or at least 99.9%. In
10 alternative embodiments, the degree of identity is at least 70%, 71%, 72%, or at least 73%.

In still further particular embodiments, the phytase of the invention has no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or no more than 10 modifications as compared to SEQ ID NO: 2 or any other parent phytase; no more than 11, 12, 13, 14, 15, 16, 17, 18, 19, or no more than 20 modifications as compared to SEQ ID NO: 2 or any other parent phytase; no more than 21, 22, 23, 24, 25, 26,
15 27, 28, 29, or no more than 30 modifications as compared to SEQ ID NO: 2 or any other parent phytase; no more than 31, 32, 33, 34, 35, 36, 37, 38, 39, or not more than 40 modifications as compared to SEQ ID NO: 2 or any other parent phytase; no more than 41, 42, 43, 44, 45, 46, 47, 48, 49, or no more than 50 modifications as compared to SEQ ID NO: 2 or any other parent phytase; no more than 51, 52, 53, 54, 55, 56, 57, 58, 59, or no more than 60 modifications as
20 compared to SEQ ID NO: 2 or any other parent phytase; no more than 61, 62, 63, 64, 65, 66, 67, 68, 69, or no more than 70 modifications as compared to SEQ ID NO: 2 or any other parent phytase; no more than 71, 72, 73, 74, 75, 76, 77, 78, 79, or no more than 80 modifications as compared to SEQ ID NO: 2 or any other parent phytase; no more than 81, 82, 83, 84, 85, 86, 87, 88, 89, or no more than 90 modifications as compared to SEQ ID NO: 2 or any other parent
25 phytase; no more than 91, 92, 93, 94, 95, 96, 97, 98, 99, or no more than 100 modifications as compared to SEQ ID NO: 2 or any other parent phytase; no more than 101, 102, 103, 104, 105, 106, 107, 108, 109, or no more than 110 modifications as compared to SEQ ID NO: 2 or any other parent phytase; no more than 111, 112, 113, 114, 115, 116, 117, 118, 119, or no more than 120 modifications as compared to SEQ ID NO: 2 or any other parent phytase; or no more than 121,
30 122, 123, or 124 modifications as compared to SEQ ID NO: 2 or any other parent phytase.

An aspect of the invention is directed to an isolated polypeptide having phytase activity, selected from the group consisting of

a) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 12;

35 b) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 14;

c) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 16; and

d) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, 99% identity or 100% identity to SEQ ID NO: 18; and

5 e) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 20.

Preferably, the polypeptide is obtained or obtainable from *Citrobacter braakii*.

An interesting aspect is directed to an isolated polypeptide having phytase activity, selected from the group consisting of

10 a) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 12;

b) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 14;

15 c) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 16;

d) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 18; and

e) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 20;

20 wherein the polypeptide is pH and thermostable such that it comprises one or more of the following properties

i. an unfolding temperature at pH 4 of at least 75 °C;

ii. an unfolding temperature at pH 3 of at least 70°C; and

iii. an unfolding temperature at pH 2 of at least 55°C.

25 A further interesting aspect is directed to an isolated polypeptide having phytase activity, selected from the group consisting of

a) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 12;

30 b) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 98% identity, at least 99% identity or 100% identity to SEQ ID NO: 14;

c) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 16;

d) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 18; and

35 e) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 20;

wherein said polypeptide is acid stable such that it maintains a residual activity level above 90% after 24 hours at each of pH 2, 3, 4, 5, 6, 7 and 8.

Typically, the polypeptide comprises the alterations N31C/G52C/A99C/K141C/T177C/V199C as compared to SEQ ID NO: 2. Preferably, the polypeptide comprises the alterations N31C/G52C/A99C/K141C/T177C/V199C/N203L as compared to SEQ ID NO: 2. More typically, the polypeptide of the present invention comprises a substitution in one or more position(s) selected from the following: 30, 36, 43, 46, 57, 60, 64, 73, 79, 119, 121, 123, 130, 134, 138, 151, 155, 161, 162, 168, 176, 180, 184, 190, 207, 224, 230, 243, 273, 286, 336, 340, 358 and 375 using SEQ ID NO: 2 for numbering.

Position Numbering

The nomenclature used herein for defining amino acid positions is based on the amino acid sequence of the phytase derived from *Citrobacter braakii* ATCC 51113, the mature sequence of which is given in the sequence listing as SEQ ID NO: 2 (amino acids 1-411 of SEQ ID NO: 2). Accordingly, in the present context, the basis for numbering positions is SEQ ID NO: 2 starting with E1 and ending with E411.

When used herein the term "mature" part (or sequence) refers to that part of the polypeptide which is secreted by a cell which contains, as part of its genetic equipment, a polynucleotide encoding the polypeptide. In other words, the mature polypeptide part refers to that part of the polypeptide which remains after the signal peptide part, as well as a propeptide part, if any, has been cleaved off. The signal peptide part can be predicted by programs known in the art (e.g. SignalP). The expected signal peptide part of SEQ ID NO: 2 is included in the present sequence listing as SEQ ID NO: 8, which is encoded by SEQ ID NO: 7. SEQ ID NO: 2 is the expected mature part. Generally, the first amino acid of the mature part of an enzyme can be determined by N-terminal sequencing of the purified enzyme. Any difference between the signal peptide part and the mature part must then be due to the presence of a propeptide.

Modifications, such as Substitutions, Deletions, Insertions

A phytase variant can comprise various types of modifications relative to a template (i.e. a reference or comparative amino acid sequence such as SEQ ID NO: 2): An amino acid can be substituted with another amino acid; an amino acid can be deleted; an amino acid can be inserted; as well as any combination of any number of such modifications. In the present context the term "insertion" is intended to cover also N- and/or C-terminal extensions.

The general nomenclature used herein for a single modification is the following: XDcY, where "X" and "Y" independently designate a one-letter amino acid code, or a "*" (deletion of an amino acid), "D" designates a number, and "c" designates an alphabetical counter (a, b, c, and so forth), which is only present in insertions. Reference is made to the below Table which

describes purely hypothetical examples of applying this nomenclature to various types of modifications.

Table

5

Type	Description	Example
Substitution	X=Amino acid in template D=Position in template c empty Y=Amino acid in variant	G80A 80 AALNNSIGVLGVAPSAELYAVKVLGASGSG : AALNNSIAVLGVAPSAELYAVKVLGASGSG
Insertion	X="*" D=Position in template before the insertion c="a" for first insertion at this position, "b" for next, etc.	*80aT *80bY *85aS 80 85 AALNNSIG..VLGVA.PSAELYAVKVLGASG AALNNSIGTYVLGVASPSAELYAVKVLGASG
Deletion	X=Amino acid in template D=Position in template c empty Y="*"	V81* 80 AALNNSIGVLGVAPSAELYAVKVLGASGSG AALNNSIG.LGVAPSAELYAVKVLGASGSG
N-terminal extension	Insertions at position "0".	*0aA *0bT *0cG 1 ...AQSVPWGISRVQ ATGAQSVPWGISRVQ
C-terminal extension	Insertions after the N-terminal amino acid.	*275aS *275bT 270 275 ATSLGSTNLYGSLVNAEAATR.. ATSLGSTNLYGSLVNAEAATRST

As explained above, the position number ("D") is counted from the first amino acid residue of SEQ ID NO: 2.

Several modifications in the same sequence are separated by "/" (slash), e.g. the designation "1*/2*/3*" means that the amino acids in position number 1, 2, and 3 are all deleted, and the designation "104A/105F" means that the amino acid in position number 104 is substituted

by A, and the amino acid in position number 105 is substituted by F.

Alternative modifications are separated by “,” (comma), e.g., the designation “119R,K” means that the amino acid in position 119 is substituted with R or K.

The commas used herein in various other enumerations of possibilities mean what they usually do grammatically, viz. often and/or. E.g., the first comma in the listing “53V,Q, 121D, and/or 167Q” denotes an alternative (V or Q), whereas the two next commas should be interpreted as and/or options: 53 V or Q, and/or 121D, and/or 167Q.

In the present context, “at least one” (e.g. modification) means one or more, e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 modifications; or 12, 14, 15, 16, 18, 20, 22, 24, 25, 28, or 30 modifications; and so on, up to a maximum number of modifications of 125, 130, 140, 150, 160, 170, 180, 190, or of 200. The phytase variants of the invention, however, still have to be at least 74% identical to SEQ ID NO: 2, this percentage being determined as described above.

A substitution or extension without any indication of what to substitute or extend with refers to the insertion of any natural, or non-natural, amino acid, except the one that occupies this position in the template.

Identifying Corresponding Position Numbers

As explained above, the mature phytase of *Citrobacter braakii* ATCC 51113 (SEQ ID NO: 2) is used as the standard for position numbering and, thereby, also for the nomenclature.

For another phytase, in particular a phytase variant of the invention, the position corresponding to position D in SEQ ID NO: 2 is found by aligning the two sequences as specified above in the section entitled “Phytase polypeptides, percentage of identity”. From the alignment, the position in the sequence of the invention corresponding to position D of SEQ ID NO: 2 can be clearly and unambiguously identified (the two positions on top of each other in the alignment).

Figure 1 of the present invention is an example of an alignment of the phytase of SEQ ID NO: 2 and the phytase of SEQ ID NO: 9, and the example illustrates how corresponding positions in these two backbones are identified.

Below some additional, purely hypothetical, examples are included which are derived from the above Table which in the third column includes a number of alignments of two sequences.

Consider the third cell in the first row of the above Table: The upper sequence is the template, the lower the variant. Position number 80 refers to amino acid residue G in the template. Amino acid A occupies the corresponding position in the variant. Accordingly, this substitution is designated G80A.

Consider now the third cell in the second row of the above Table: The upper sequence is again the template and the lower the variant. Position number 80 again refers to amino acid residue G in the template. The variant has two insertions, viz. TY, after G80 and before V81 in the template. Whereas the T and Y of course would have their own “real” position number in the

variant amino acid sequence, for the present purposes we always refer to the template position numbers, and accordingly the T and the Y are said to be in position number 80a and 80b, respectively.

Finally, consider the third cell in the last row of the above Table: Position number 275 refers to the last amino acid of the template. A C-terminal extension of ST are said to be in position number 275a and 275b, respectively, although, again, of course they have their own “real” position number in the variant amino acid sequence.

Modified Properties, Reference Phytase

In a particular embodiment, the method of the invention for producing phytase variants provides variants having modified, preferably improved, properties.

The terms “modified” and “improved” imply a comparison with another phytase. Examples of such other, reference, or comparative, phytases are: SEQ ID NO: 2 and/or SEQ ID NO: 6. Still further examples of reference phytases may be SEQ ID NO: 3, and/or SEQ ID NO: 4. A still further example of a reference phytase may be SEQ ID NO: 9, and variants thereof.

Non-limiting examples of properties that are modified, preferably improved, are the following: Thermostability, pH profile, specific activity, performance in animal feed, pelleting stability, protease-sensibility, and/or glycosylation pattern. The phytase variants produced by the method of the invention exhibits improved thermostability and may also have a modified, preferably improved, temperature profile, and/or it may incorporate a change of a potential protease cleavage site.

Thermal Performance

Temperature-stability

Temperature stability may be determined as described in WO2011/117396, Example 3 by determining the activity during 30 minutes incubation at temperatures from 60°C or higher and comparing with a reference experiment performed at 37°C.

Thermostability

Thermostability may be determined as described in WO2011/117396, Example 4, i.e., using DSC measurements to determine the denaturation temperature, T_d, of the purified phytase protein. The T_d is indicative of the thermostability of the protein: The higher the T_d, the higher the thermostability. Accordingly, in a preferred embodiment, the phytase of the invention has a T_d which is higher than the T_d of a reference phytase, wherein T_d is determined on purified phytase samples (preferably with a purity of at least 90% or 95%, determined by SDS-PAGE).

Heat-stability

Heat stability may be determined as described in WO2011/117396, Example 5 by determining the temperature/activity profile of the variant phytases.

Steam stability

- 5 Steam stability may be determined as described in WO 2011/117396, Example 7 by determining the residual activity of phytase molecules after steam treatment at 85°C or 90°C for a short time.

Pelleting stability

- 10 Pelleting stability may be determined as described in WO 2001/117396, Example 8 by using enzyme granulate pre-mixed with feed. This premix is mixed with feed. From the mixer the feed is conditioned with steam to 95°C. After conditioning the feed is pressed to pellets and the residual activity determined.

- In preferred embodiments, the thermal properties such as heat-stability, temperature
15 stability, thermostability, steam stability, and/or pelleting stability as provided by the residual activity, Td or other parameter of the phytase of the invention is higher than the corresponding value, such as the residual activity or Td, of the phytase of SEQ ID NO: 2, more preferably at least 101% thereof, or at least 102%, 103%, 104%, 105%, 106%, 107%, 108%, 109%, or at least 110% thereof. Even more preferably, the value of the parameter, such as residual activity or Td, of the
20 phytase of the invention is at least 120%, 130%, 140%, 150%, 160%, 170%, 180%, or at least 190% of the value for the phytase of SEQ ID NO: 2.

- In still further particular embodiments, the thermostable phytase of the invention has a melting temperature, Tm (or a denaturation temperature, Td), as determined using Differential Scanning Calorimetry (DSC) as described in the Examples (i.e. in 20 mM sodium acetate, pH
25 4.0), of at least 50°C. In still further particular embodiments, the Tm is at least 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 62.5, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or at least 100°C. DSC measurements may also be performed as described in the Examples.

Temperature profile/temperature stability

- 30 Whether or not a phytase of the invention has a modified temperature profile as compared to a reference phytase may be determined as described in WO 2011/117396, Example 5. Accordingly, in a particular embodiment the phytase of the invention has a modified temperature profile as compared to a reference phytase, wherein the temperature profile is determined as
35 phytase activity as a function of temperature on sodium phytate at pH 5.5 in the temperature range of 20-90°C (in 10°C steps). A preferred buffer is in 0.25 M Na-acetate buffer pH 5.5. The activity at each temperature is preferably indicated as relative activity (in %) normalized to the value at optimum temperature. The optimum temperature is that temperature within the tested

temperatures (i.e. those with 5-10°C jumps) where the activity is highest.

Performance in animal feed

In a particular embodiment the phytase of the invention has an improved performance in animal feed as compared to a reference phytase. The performance in animal feed may be determined by the *in vitro* model. Accordingly, in a preferred embodiment the phytase of the invention has an improved performance in animal feed, wherein the performance is determined in an *in vitro* model, by preparing feed samples composed of 30% soybean meal and 70% maize meal with added CaCl₂ to a concentration of 5 g calcium per kg feed; pre-incubating them at 40°C and pH 3.0 for 30 minutes followed by addition of pepsin (3000 U/g feed) and phytase; incubating the samples at 40°C and pH 3.0 for 60 minutes followed by pH 4.0 for 30 minutes; stopping the reactions; extracting phytic acid and inositol-phosphates by addition of HCl to a final concentration of 0.5M and incubation at 40°C for 2 hours, followed by one freeze-thaw cycle and 1 hour incubation at 40°C; separating phytic acid and inositol-phosphates by high performance ion chromatography; determining the amount of residual phytate phosphorus (IP6-P); calculating the difference in residual IP6-P between the phytase-treated and a non-phytase-treated blank sample (this difference is degraded IP6-P); and expressing the degraded IP6-P of the phytase of the invention relative to degraded IP6-P of the reference phytase.

The phytase of the invention and the reference phytase are dosed in the same amount, preferably based on phytase activity units (FYT). A suitable dosage is 100-5000 FYT/kg feed, such as 125 to 4000 FYT/kg feed, such as 125 to 3000 FYT/kg. The phytases may be dosed in the form of purified phytases, or in the form of fermentation supernatants. Purified phytases preferably have a purity of at least 95%, as determined by SDS-PAGE.

In preferred embodiments, the degraded IP6-P value of the purified phytase of the invention, relative to the degraded IP6-P value of the reference phytase, is at least 101%, or at least 102%, 103%, 104%, 105%, 110%, 115%, or at least 120%. In still further preferred embodiments, the degraded IP6-P value of the purified phytase of the invention, relative to the degraded IP6-P value of the reference phytase, is at least 125%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, or at least 200%. Preferably, the degraded IP6-P value of the phytase of the invention, relative to the degraded IP6-P value of the SEQ ID NO: 2 phytase, is at least 105%, 110%, 113%, 115%, 120%, 125%, or at least 130%.

The relative performance of a phytase of the invention may also be calculated as the percentage of the phosphorous released by the reference phytase.

In a still further particular embodiment, the relative performance of the phytase of the invention may be calculated as the percentage of the phosphorous released by the phytase of the invention, relative to the amount of phosphorous released by the reference phytase.

In still further particular embodiments, the relative performance of the phytase of the invention is at least 105%, preferably at least 110, 120, 130, 140, 150, 160, 170, 180, 190, or at least 200%.

Steam stability

Thermostability is an important parameter, but associated with that also steam stability is important. In this respect reference is made to WO 2011/117396, Example 8.

5 Low-allergenic variants

In a specific embodiment, the phytase variants produced by the method of the present invention are (also) low-allergenic variants, designed to invoke a reduced immunological response when exposed to animals, including man. The term immunological response is to be understood as any reaction by the immune system of an animal exposed to the phytase variant.

10 One type of immunological response is an allergic response leading to increased levels of IgE in the exposed animal. Low-allergenic variants may be prepared using techniques known in the art. For example the phytase variant may be conjugated with polymer moieties shielding portions or epitopes of the phytase variant involved in an immunological response. Conjugation with polymers may involve *in vitro* chemical coupling of polymer to the phytase variant, e.g. as described in WO
15 96/17929, WO 98/30682, WO 98/35026, and/or WO 99/00489. Conjugation may in addition or alternatively thereto involve in vivo coupling of polymers to the phytase variant. Such conjugation may be achieved by genetic engineering of the nucleotide sequence encoding the phytase variant, inserting consensus sequences encoding additional glycosylation sites in the phytase variant and expressing the phytase variant in a host capable of glycosylating the phytase variant,
20 see e.g. WO 00/26354. Another way of providing low-allergenic variants is genetic engineering of the nucleotide sequence encoding the phytase variant so as to cause the phytase variants to self-oligomerize, effecting that phytase variant monomers may shield the epitopes of other phytase variant monomers and thereby lowering the antigenicity of the oligomers. Such products and their preparation are described e.g. in WO 96/16177. Epitopes involved in an immunological response
25 may be identified by various methods such as the phage display method described in WO 00/26230 and WO 01/83559, or the random approach described in EP 561907. Once an epitope has been identified, its amino acid sequence may be altered to produce altered immunological properties of the phytase variant by known gene manipulation techniques such as site directed mutagenesis (see e.g. WO 00/26230, WO 00/26354 and/or WO 00/22103) and/or conjugation of
30 a polymer may be done in sufficient proximity to the epitope for the polymer to shield the epitope.

Daily BW gain per bird (BW gain) and feed conversion ratio (FCR)

Daily Body weight gain per bird (BW gain) and feed conversion ratio (FCR) were calculated as follows:

35 Body weight gain per bird: difference between BW per bird at the end and at the beginning of a study

Daily BWgain: difference between BW per bird at the end and at the beginning of the study

divided by the number of days

FCR: total feed consumption of a pen divided by total BW gain of that pen (total BW gain = total BW at the end + weight of removals and losses – total BW at the beginning).

FI = FCR*WG

5

Nucleic Acid Sequences and Constructs

The present invention also relates to nucleic acid sequences comprising a nucleic acid sequence which encodes a phytase variant of the invention.

10 An aspect of the invention is directed to a method of preparing a recombinant polypeptide having phytase activity comprising the steps of:

(a) cultivating a recombinant host cell comprising an exogenous polynucleotide selected from the group consisting of

15 a. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 13;

b. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 15;

20 c. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 17;

d. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 19;

25 e. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 21;

wherein the polynucleotide is expressed and the polypeptide is produced;

(b) optionally isolating the polypeptide; and

30 (c) optionally recovering the polypeptide.

An alternate aspect is directed to method of producing a polypeptide of the present invention, said method comprising the steps of:

(a) cultivating a recombinant host cell comprising an exogenous polynucleotide encoding the polypeptide having phytase activity selected from the group consisting of

35 a. a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 12;

b. a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 14;

c. a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 16;

d. a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 18; and

e. a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 20;

wherein the polynucleotide is expressed and the polypeptide is produced;

(b) optionally isolating the polypeptide; and

(c) optionally recovering the polypeptide.

Typically, in the methods of the invention, the polypeptide having phytase activity comprises the substitutions N31C/G52C/A99C/K141C/T177C/V199C as compared to SEQ ID NO: 2. Preferably, in the methods of the invention, the polypeptide having phytase activity comprises the substitutions N31C/G52C/A99C/K141C/T177C/V199C/N203L as compared to SEQ ID NO: 2. More typically, the polypeptide further comprises a substitution in one or more position(s) selected from the following: 30, 36, 43, 46, 57, 60, 64, 73, 79, 119, 121, 123, 130, 134, 138, 151, 155, 161, 162, 168, 176, 180, 184, 190, 207, 224, 230, 243, 273, 286, 336, 340, 358 and 375 using SEQ ID NO: 2 for numbering.

The term "isolated nucleic acid sequence" refers to a nucleic acid sequence which is essentially free of other nucleic acid sequences, e.g., at least about 20% pure, preferably at least about 40% pure, more preferably at least about 60% pure, even more preferably at least about 80% pure, and most preferably at least about 90% pure as determined by agarose electrophoresis. For example, an isolated nucleic acid sequence can be obtained by standard cloning procedures used in genetic engineering to relocate the nucleic acid sequence from its natural location to a different site where it will be reproduced. The cloning procedures may involve excision and isolation of a desired nucleic acid fragment comprising the nucleic acid sequence encoding the polypeptide, insertion of the fragment into a vector molecule, and incorporation of the recombinant vector into a host cell where multiple copies or clones of the nucleic acid sequence will be replicated. The nucleic acid sequence may be of genomic, cDNA, RNA, semisynthetic, synthetic origin, or any combinations thereof.

The nucleic acid sequences of the invention can be prepared by introducing at least one mutation into a template phytase coding sequence or a subsequence thereof, wherein the mutant

nucleic acid sequence encodes a variant phytase. The introduction of a mutation into the nucleic acid sequence to exchange one nucleotide for another nucleotide may be accomplished by any of the methods known in the art, e.g. by site-directed mutagenesis, by random mutagenesis, or by doped, spiked, or localized random mutagenesis.

5 Random mutagenesis is suitably performed either as localized or region-specific random mutagenesis in at least three parts of the gene translating to the amino acid sequence shown in question, or within the whole gene. When the mutagenesis is performed by the use of an oligonucleotide, the oligonucleotide may be doped or spiked with the three non-parent nucleotides during the synthesis of the oligonucleotide at the positions which are to be changed. The doping
10 or spiking may be performed so that codons for unwanted amino acids are avoided. The doped or spiked oligonucleotide can be incorporated into the DNA encoding the phytase enzyme by any technique, using, e.g., PCR, LCR or any DNA polymerase and ligase as deemed appropriate.

Preferably, the doping is carried out using "constant random doping", in which the percentage of wild-type and mutation in each position is predefined. Furthermore, the doping may
15 be directed toward a preference for the introduction of certain nucleotides, and thereby a preference for the introduction of one or more specific amino acid residues. The doping may be made, e.g., so as to allow for the introduction of 90% wild type and 10% mutations in each position. An additional consideration in the choice of a doping scheme is based on genetic as well as protein-structural constraints.

20 The random mutagenesis may be advantageously localized to a part of the parent phytase in question. This may, e.g., be advantageous when certain regions of the enzyme have been identified to be of particular importance for a given property of the enzyme.

Alternative methods for providing variants of the invention include gene shuffling e.g. as described in WO 95/22625 or in WO 96/00343, and the consensus derivation process as
25 described in EP 897985.

Nucleic Acid Constructs

A nucleic acid construct comprises a nucleic acid sequence of the present invention operably linked to one or more control sequences which direct the expression of the coding
30 sequence in a suitable host cell under conditions compatible with the control sequences. Expression will be understood to include any step involved in the production of the polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

The term "nucleic acid construct" as used herein refers to a nucleic acid molecule, either
35 single- or double-stranded, which is isolated from a naturally occurring gene or which is modified to contain segments of nucleic acids in a manner that would not otherwise exist in nature. The term nucleic acid construct is synonymous with the term "expression cassette" when the nucleic

acid construct contains the control sequences required for expression of a coding sequence of the present invention.

The term “control sequences” is defined herein to include all components, which are necessary or advantageous for the expression of a polynucleotide encoding a polypeptide of the present invention. Each control sequence may be native or foreign to the nucleotide sequence encoding the polypeptide. Such control sequences include, but are not limited to, a leader, polyadenylation sequence, propeptide sequence, promoter, signal peptide sequence, and transcription terminator. At a minimum, the control sequences include a promoter, and transcriptional and translational stop signals. The control sequences may be provided with linkers for the purpose of introducing specific restriction sites facilitating ligation of the control sequences with the coding region of the nucleotide sequence encoding a polypeptide.

The term “operably linked” denotes herein a configuration in which a control sequence is placed at an appropriate position relative to the coding sequence of the polynucleotide sequence such that the control sequence directs the expression of the coding sequence of a polypeptide.

When used herein the term “coding sequence” (CDS) means a nucleotide sequence, which directly specifies the amino acid sequence of its protein product. The boundaries of the coding sequence are generally determined by an open reading frame, which usually begins with the ATG start codon or alternative start codons such as GTG and TTG. The coding sequence may a DNA, cDNA, or recombinant nucleotide sequence.

Expression Vector

The term “expression” includes any step involved in the production of the polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

The term “expression vector” is defined herein as a linear or circular DNA molecule that comprises a polynucleotide encoding a polypeptide of the invention, and which is operably linked to additional nucleotides that provide for its expression.

A nucleic acid sequence encoding a phytase variant of the invention can be expressed using an expression vector which typically includes control sequences encoding a promoter, operator, ribosome binding site, translation initiation signal, and, optionally, a repressor gene or various activator genes.

The recombinant expression vector carrying the DNA sequence encoding a phytase variant of the invention may be any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. The vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

The phytase variant may also be co-expressed together with at least one other enzyme of animal feed interest, such as a phytase, phosphatase, xylanase, galactanase, alpha-galactosidase, protease, phospholipase, amylase, and/or beta-glucanase. The enzymes may be co-expressed from different vectors, from one vector, or using a mixture of both techniques. When using different vectors, the vectors may have different selectable markers, and different origins of replication. When using only one vector, the genes can be expressed from one or more promoters. If cloned under the regulation of one promoter (di- or multi-cistronic), the order in which the genes are cloned may affect the expression levels of the proteins. The phytase variant may also be expressed as a fusion protein, i.e. that the gene encoding the phytase variant has been fused in frame to the gene encoding another protein. This protein may be another enzyme or a functional domain from another enzyme.

Host Cells

The term "host cell", as used herein, includes any cell type which is susceptible to transformation, transfection, transduction, and the like with a nucleic acid construct comprising a polynucleotide of the present invention.

The present invention also relates to recombinant host cells, comprising a polynucleotide of the present invention, which are advantageously used in the recombinant production of the polypeptides. A vector comprising a polynucleotide of the present invention is introduced into a host cell so that the vector is maintained as a chromosomal integrant or as a self-replicating extra-chromosomal vector as described earlier. The term "host cell" encompasses any progeny of a parent cell that is not identical to the parent cell due to mutations that occur during replication. The choice of a host cell will to a large extent depend upon the gene encoding the polypeptide and its source.

The host cell may be a unicellular microorganism, e.g., a prokaryote, or a non-unicellular microorganism, e.g., a eukaryote.

Useful unicellular microorganisms are bacterial cells such as gram positive bacteria including, but not limited to, a *Bacillus* cell, e.g., *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus stearothermophilus*, *Bacillus subtilis*, and *Bacillus thuringiensis*; or a *Streptomyces* cell, e.g., *Streptomyces lividans* and *Streptomyces murinus*, or gram negative bacteria such as *E. coli* and *Pseudomonas* sp. In a preferred aspect, the bacterial host cell is a *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus stearothermophilus*, or *Bacillus subtilis* cell. In another preferred aspect, the *Bacillus* cell is an alkalophilic *Bacillus*.

The introduction of a vector into a bacterial host cell may, for instance, be effected by protoplast transformation (see, e.g., Chang and Cohen, 1979, Molecular General Genetics 168: 111-115), using competent cells (see, e.g., Young and Spizizin, 1961, Journal of Bacteriology 81:

823-829, or Dubnau and Davidoff-Abelson, 1971, Journal of Molecular Biology 56: 209-221), electroporation (see, e.g., Shigekawa and Dower, 1988, Biotechniques 6: 742-751), or conjugation (see, e.g., Koehler and Thorne, 1987, Journal of Bacteriology 169: 5771-5278).

The host cell may also be a eukaryote, such as a mammalian, insect, plant, or fungal cell.

5 In a preferred aspect, the host cell is a fungal cell. "Fungi" as used herein includes the phyla *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, and *Zygomycota* (as defined by Hawksworth et al., In, Ainsworth and Bisby's Dictionary of The Fungi, 8th edition, 1995, CAB International, University Press, Cambridge, UK) as well as the Oomycota (as cited in Hawksworth et al., 1995, supra, page 171) and all mitosporic fungi (Hawksworth et al., 1995, supra).

10 In a more preferred aspect, the fungal host cell is a yeast cell. "Yeast" as used herein includes ascosporogenous yeast (Endomycetales), basidiosporogenous yeast, and yeast belonging to the Fungi Imperfecti (Blastomycetes). Since the classification of yeast may change in the future, for the purposes of this invention, yeast shall be defined as described in Biology and Activities of Yeast (Skinner, F.A., Passmore, S.M., and Davenport, R.R., eds, Soc. App. Bacteriol. Symposium Series No. 9, 1980).

In an even more preferred aspect, the yeast host cell is a *Candida*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Schizosaccharomyces*, or *Yarrowia* cell.

In a most preferred aspect, the yeast host cell is a *Pichia pastoris*, *Pichia methanolica*, *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae*, *Saccharomyces diastaticus*,
20 *Saccharomyces douglasii*, *Saccharomyces kluyveri*, *Saccharomyces norbensis* or *Saccharomyces oviformis* cell. In another most preferred aspect, the yeast host cell is a *Kluyveromyces lactis* cell. In another most preferred aspect, the yeast host cell is a *Yarrowia lipolytica* cell.

In another more preferred aspect, the fungal host cell is a filamentous fungal cell.
25 "Filamentous fungi" include all filamentous forms of the subdivision Eumycota and Oomycota (as defined by Hawksworth et al., 1995, supra). The filamentous fungi are generally characterized by a mycelial wall composed of chitin, cellulose, glucan, chitosan, mannan, and other complex polysaccharides. Vegetative growth is by hyphal elongation and carbon catabolism is obligately aerobic. In contrast, vegetative growth by yeasts such as *Saccharomyces cerevisiae* is by
30 budding of a unicellular thallus and carbon catabolism may be fermentative.

In an even more preferred aspect, the filamentous fungal host cell is an *Acremonium*, *Aspergillus*, *Aureobasidium*, *Bjerkandera*, *Ceriporiopsis*, *Coprinus*, *Coriolus*, *Cryptococcus*, *Filobasidium*, *Fusarium*, *Humicola*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Phanerochaete*, *Phlebia*, *Piromyces*, *Pleurotus*,
35 *Schizophyllum*, *Talaromyces*, *Thermoascus*, *Thielavia*, *Tolypocladium*, *Trametes*, or *Trichoderma* cell.

In a most preferred aspect, the filamentous fungal host cell is an *Aspergillus awamori*,

Aspergillus fumigatus, *Aspergillus foetidus*, *Aspergillus japonicus*, *Aspergillus nidulans*, *Aspergillus niger* or *Aspergillus oryzae* cell. In another most preferred aspect, the filamentous fungal host cell is a *Fusarium bactridioides*, *Fusarium cerealis*, *Fusarium crookwellense*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium graminum*, *Fusarium heterosporum*,
 5 *Fusarium negundi*, *Fusarium oxysporum*, *Fusarium reticulatum*, *Fusarium roseum*, *Fusarium sambucinum*, *Fusarium sarcochroum*, *Fusarium sporotrichioides*, *Fusarium sulphureum*, *Fusarium torulosum*, *Fusarium trichothecioides*, or *Fusarium venenatum* cell. In another most preferred aspect, the filamentous fungal host cell is a *Bjerkandera adusta*, *Ceriporiopsis aneirina*, *Ceriporiopsis aneirina*, *Ceriporiopsis caregiea*, *Ceriporiopsis gilvescens*, *Ceriporiopsis*
 10 *pannocinta*, *Ceriporiopsis rivulosa*, *Ceriporiopsis subrufa*, or *Ceriporiopsis subvermispora*, *Coprinus cinereus*, *Coriolus hirsutus*, *Humicola insolens*, *Humicola lanuginosa*, *Mucor miehei*, *Myceliophthora thermophila*, *Neurospora crassa*, *Penicillium purpurogenum*, *Phanerochaete chrysosporium*, *Phlebia radiata*, *Pleurotus eryngii*, *Thielavia terrestris*, *Trametes villosa*, *Trametes versicolor*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*,
 15 *Trichoderma reesei*, or *Trichoderma viride* strain cell.

Fungal cells may be transformed by a process involving protoplast formation, transformation of the protoplasts, and regeneration of the cell wall in a manner known per se. Suitable procedures for transformation of *Aspergillus* and *Trichoderma* host cells are described in EP 238 023 and Yelton et al., 1984, Proceedings of the National Academy of Sciences USA
 20 81: 1470-1474. Suitable methods for transforming *Fusarium* species are described by Malardier et al., 1989, Gene 78: 147-156, and WO 96/00787. Yeast may be transformed using the procedures described by Becker and Guarente, In Abelson, J.N. and Simon, M.I., editors, Guide to Yeast Genetics and Molecular Biology, Methods in Enzymology, Volume 194, pp 182-187, Academic Press, Inc., New York; Ito et al., 1983, Journal of Bacteriology 153: 163; and Hinnen et al., 1978, Proceedings of the National Academy of Sciences USA 75: 1920.

Methods of Production

The present invention relates to methods for producing a phytase variant comprising (a) cultivating a host cell under conditions conducive for production of the phytase; and (b) recovering
 30 the phytase.

In the production methods of the present invention, the cells are cultivated in a nutrient medium suitable for production of the polypeptide using methods well known in the art. For example, the cell may be cultivated by shake flask cultivation, and small-scale or large-scale fermentation (including continuous, batch, fed-batch, or solid state fermentations) in laboratory or
 35 industrial fermentors performed in a suitable medium and under conditions allowing the polypeptide to be expressed and/or isolated. The cultivation takes place in a suitable nutrient medium comprising carbon and nitrogen sources and inorganic salts, using procedures known in

the art. Suitable media are available from commercial suppliers or may be prepared according to published compositions (e.g., in catalogues of the American Type Culture Collection). If the polypeptide is secreted into the nutrient medium, the polypeptide can be recovered directly from the medium. If the polypeptide is not secreted, it can be recovered from cell lysates.

5 The resulting polypeptide may be recovered using methods known in the art. For example, the polypeptide may be recovered from the nutrient medium by conventional procedures including, but not limited to, centrifugation, filtration, extraction, spray-drying, evaporation, or precipitation.

10 The polypeptides of the present invention may be purified by a variety of procedures known in the art including, but not limited to, chromatography (e.g., ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (e.g., preparative isoelectric focusing), differential solubility (e.g., ammonium sulfate precipitation), SDS-PAGE, or extraction (see, e.g., Protein Purification, J.-C. Janson and Lars Ryden, editors, VCH Publishers, New York, 1989).

15

Compositions and Uses

In still further aspects, the present invention relates to compositions comprising a polypeptide of the present invention, as well as methods of using these.

20 The polypeptide compositions may be prepared in accordance with methods known in the art and may be in the form of a liquid or a dry composition. The polypeptide to be included in the composition may be stabilized in accordance with methods known in the art.

25 For a liquid formulation, the formulating agent may comprise a polyol (such as e.g. glycerol, ethylene glycol or propylene glycol), a salt (such as e.g. sodium chloride, sodium benzoate, potassium sorbate) or a sugar or sugar derivative (such as e.g. dextrin, glucose, sucrose, and sorbitol). Thus in one embodiment, the composition is a liquid composition comprising the polypeptide of the invention and one or more formulating agents selected from the list consisting of glycerol, ethylene glycol, 1,2-propylene glycol, 1,3-propylene glycol, sodium chloride, sodium benzoate, potassium sorbate, dextrin, glucose, sucrose, and sorbitol. The liquid formulation may be sprayed onto the feed after it has been pelleted or may be added to drinking
30 water given to the animals.

35 For a solid formulation, the formulation may be for example as a granule, spray dried powder or agglomerate. The formulating agent may comprise a salt (organic or inorganic zinc, sodium, potassium or calcium salts such as e.g. such as calcium acetate, calcium benzoate, calcium carbonate, calcium chloride, calcium citrate, calcium sorbate, calcium sulfate, potassium acetate, potassium benzoate, potassium carbonate, potassium chloride, potassium citrate, potassium sorbate, potassium sulfate, sodium acetate, sodium benzoate, sodium carbonate, sodium chloride, sodium citrate, sodium sulfate, zinc acetate, zinc benzoate, zinc carbonate, zinc

chloride, zinc citrate, zinc sorbate, zinc sulfate), starch or a sugar or sugar derivative (such as e.g. sucrose, dextrin, glucose, lactose, sorbitol).

In an embodiment, the solid composition is in the form of granulates or microgranulates. The granule may have a matrix structure where the components are mixed homogeneously.

5 However, the granule typically comprises a core particle and one or more coatings, which typically are salt and/or wax coatings. Examples of waxes are polyethylene glycols; polypropylenes; Carnauba wax; Candelilla wax; bees wax; hydrogenated plant oil or animal tallow such as hydrogenated ox tallow, hydrogenated palm oil, hydrogenated cotton seeds and/or hydrogenated soy bean oil; fatty acid alcohols; mono-glycerides and/or di-glycerides, such as glyceryl stearate, 10 wherein stearate is a mixture of stearic and palmitic acid; micro-crystalline wax; paraffin's; and fatty acids, such as hydrogenated linear long chained fatty acids and derivatives thereof. A preferred wax is palm oil or hydrogenated palm oil. The core particle can either be a homogeneous blend of phytase of the invention optionally combined with one or more additional enzymes and optionally together with one or more salts or an inert particle with the phytase of the invention 15 optionally combined with one or more additional enzymes applied onto it.

In an embodiment, the material of the core particles are selected from the group consisting of inorganic salts (such as calcium acetate, calcium benzoate, calcium carbonate, calcium chloride, calcium citrate, calcium sorbate, calcium sulfate, potassium acetate, potassium benzoate, potassium carbonate, potassium chloride, potassium citrate, potassium sorbate, 20 potassium sulfate, sodium acetate, sodium benzoate, sodium carbonate, sodium chloride, sodium citrate, sodium sulfate, zinc acetate, zinc benzoate, zinc carbonate, zinc chloride, zinc citrate, zinc sorbate, zinc sulfate), starch or a sugar or sugar derivative (such as e.g. sucrose, dextrin, glucose, lactose, sorbitol), sugar or sugar derivative (such as e.g. sucrose, dextrin, glucose, lactose, sorbitol), small organic molecules, starch, flour, cellulose and minerals and clay minerals (also 25 known as hydrous aluminium phyllosilicates). In a preferred embodiment, the core comprises a clay mineral such as kaolinite or kaolin.

The salt coating is typically at least 1 μm thick and can either be one particular salt or a mixture of salts, such as Na_2SO_4 , K_2SO_4 , MgSO_4 and/or sodium citrate. Other examples are those described in e.g. WO 2008/017659, WO 2006/034710, WO 1997/05245, WO 1998/54980, 30 WO 1998/55599, WO 2000/70034 or polymer coating such as described in WO 2001/00042.

In another embodiment, the composition is a solid composition comprising the phytase of the invention and one or more formulating agents selected from the list consisting of sodium chloride, sodium benzoate, potassium sorbate, sodium sulfate, potassium sulfate, magnesium sulfate, sodium thiosulfate, calcium carbonate, sodium citrate, dextrin, glucose, sucrose, sorbitol, 35 lactose, starch and cellulose. In a preferred embodiment, the formulating agent is selected from one or more of the following compounds: sodium sulfate, dextrin, cellulose, sodium thiosulfate and calcium carbonate. In a preferred embodiment, the solid composition is in granulated form.

In an embodiment, the solid composition is in granulated form and comprises a core particle, an enzyme layer comprising the phytase of the invention and a salt coating.

In a further embodiment, the formulating agent is selected from one or more of the following compounds: glycerol, ethylene glycol, 1, 2-propylene glycol or 1, 3-propylene glycol, sodium chloride, sodium benzoate, potassium sorbate, sodium sulfate, potassium sulfate, magnesium sulfate, sodium thiosulfate, calcium carbonate, sodium citrate, dextrin, glucose, sucrose, sorbitol, lactose, starch, kaolin and cellulose. In a preferred embodiment, the formulating agent is selected from one or more of the following compounds: 1, 2-propylene glycol, 1, 3-propylene glycol, sodium sulfate, dextrin, cellulose, sodium thiosulfate, kaolin and calcium carbonate.

The phytase of the invention can be used for degradation, in any industrial context, of, for example, phytate, phytic acid, and/or the mono-, di-, tri-, tetra- and/or penta-phosphates of myo-inositol. It is well known that the phosphate moieties of these compounds chelates divalent and trivalent cations such as metal ions, i.e. the nutritionally essential ions of calcium, iron, zinc and magnesium as well as the trace minerals manganese, copper and molybdenum. Besides, the phytic acid also to a certain extent binds proteins by electrostatic interaction.

Accordingly, preferred uses of the polypeptides or polynucleotides of the invention are in animal feed preparations (including human food) or in additives for such preparations.

In a particular embodiment, the polypeptide or polynucleotide of the invention can be used for improving the nutritional value of an animal feed. Non-limiting examples of improving the nutritional value of animal feed (including human food), are: improving feed digestibility; promoting growth of the animal; improving feed utilization; improving bio-availability of proteins; increasing the level of digestible phosphate; improving the release and/or degradation of phytate; improving bio-availability of trace minerals; improving bio-availability of macro minerals; eliminating the need for adding supplemental phosphate, trace minerals, and/or macro minerals; and/or improving egg shell quality. The nutritional value of the feed is therefore increased, and the growth rate and/or weight gain and/or feed conversion (i.e. the weight of ingested feed relative to weight gain) of the animal is/are improved. In another particular embodiment, the polypeptide or polynucleotide of the invention can be used for improving nutrient retention, and/or nutrient digestibility in an animal.

Furthermore, the polypeptide or polynucleotide of the invention can be used for reducing phytate level of manure.

Animals, Animal Feed, and Animal Feed Additives

The term animal includes all animals, including human beings. Examples of animals are non-ruminants, and ruminants. Ruminant animals include, for example, animals such as sheep, goat, and cattle, e.g. cow such as beef cattle and dairy cows. In a particular embodiment, the animal is a non-ruminant animal. Non-ruminant animals include mono-gastric animals, e.g. pig or

swine (including, but not limited to, piglets, growing pigs, and sows); poultry such as turkeys, ducks and chickens (including but not limited to broiler chicks, layers); fish (including but not limited to salmon, trout, tilapia, catfish and carp); and crustaceans (including but not limited to shrimp and prawn).

5 The term feed or feed composition means any compound, preparation, mixture, or composition suitable for, or intended for intake by an animal.

 In the use according to the invention the polypeptide or polynucleotide can be fed to the animal before, after, or simultaneously with the diet. The latter is preferred.

 The invention further relates to a method of enhancing one or more of the group selected
10 from growth rate, phosphorus digestibility, whole-body phosphorus retention and/or reducing the FCR in an animal, said method comprising feeding the animal the phytase as defined herein. The method of the invention typically comprises phytase supplementation doses of 100-5000 FYT/kg feed, such as 125 to 4000 FTY/kg feed, such as 125 to 3000 FTY/kg feed.

 The invention is further directed to a method of enhancing the growth rate or reducing the
15 FCR in a mono-gastric animal, said method comprising feeding the animal the phytase as defined herein. The method of the invention typically comprises phytase supplementation doses of 100-5000 FYT/kg feed, such as 125 to 4000 FTY/kg feed, such as 125 to 3000 FTY/kg feed.

 The invention is further directed to a method of enhancing the growth rate or reducing the FCR in an animal selected from the group consisting of poultry, swine, fish or crustacean, said
20 method comprising feeding the animal the phytase as defined herein. The method of the invention typically comprises phytase supplementation doses of 100-5000 FYT/kg feed, such as 125 to 4000 FTY/kg feed, such as 125 to 3000 FTY/kg feed.

 The invention is further directed to a method of enhancing the growth rate or reducing the FCR in poultry, said method comprising feeding the poultry the phytase as defined herein. The
25 poultry may be typically selected from the group consisting of turkeys, ducks and chickens (including but not limited to broiler chicks, layers), typically chickens, particularly broiler chickens and layer chickens. The method of the invention typically comprises phytase supplementation doses of 100-5000 FYT/kg feed, such as 125 to 4000 FTY/kg feed, such as 125 to 3000 FTY/kg feed.

30 The invention is further directed to a method of enhancing the growth rate or reducing the FCR in swine, said method comprising feeding the swine the phytase as defined herein. The method of the invention typically comprises phytase supplementation doses of 100-5000 FYT/kg feed, such as 125 to 4000 FTY/kg feed, such as 125 to 3000 FTY/kg feed.

 The invention is further directed to a method of enhancing the growth rate or reducing the
35 FCR in fish or crustaceans said method comprising feeding the fish or crustaceans the phytase as defined herein. The fish may typically be selected from the group consisting of salmon, trout, tilapia, catfish, seabream such as gilthead seabream, bass, such as seabass, and carp. The

crustaceans may typically be selected from the group consisting of lobster, crab, crayfish, krill, shrimp and prawn. The method of the invention typically comprises phytase supplementation doses of 100-5000 FYT/kg feed, such as 125 to 4000 FTY/kg feed, such as 125 to 3000 FTY/kg feed.

5 In a particular embodiment, the polypeptide, in the form in which it is added to the feed, or when being included in a feed additive, is substantially pure. In a particular embodiment it is well-defined. The term "well-defined" means that the phytase preparation is at least 50% pure as determined by Size-exclusion chromatography (see Example 12 of WO 01/58275). In other particular embodiments the phytase preparation is at least 60, 70, 80, 85, 88, 90, 92, 94, or at
10 least 95% pure as determined by this method.

A substantially pure, and/or well-defined polypeptide preparation is advantageous. For instance, it is much easier to dose correctly to the feed a polypeptide that is essentially free from interfering or contaminating other polypeptides. The term dose correctly refers in particular to the objective of obtaining consistent and constant results, and the capability of optimising dosage
15 based upon the desired effect.

For the use in animal feed, however, the phytase polypeptide of the invention need not be that pure; it may e.g. include other polypeptides, in which case it could be termed a phytase preparation.

The phytase preparation can be (a) added directly to the feed (or used directly in a
20 treatment process of proteins), or (b) it can be used in the production of one or more intermediate compositions such as feed additives or premixes that is subsequently added to the feed (or used in a treatment process). The degree of purity described above refers to the purity of the original polypeptide preparation, whether used according to (a) or (b) above.

Polypeptide preparations with purities of this order of magnitude are in particular
25 obtainable using recombinant methods of production, whereas they are not so easily obtained and also subject to a much higher batch-to-batch variation when the polypeptide is produced by traditional fermentation methods.

Such polypeptide preparation may of course be mixed with other polypeptides.

The polypeptide can be added to the feed in any form, be it as a relatively pure
30 polypeptide, or in admixture with other components intended for addition to animal feed, i.e. in the form of animal feed additives, such as the so-called pre-mixes for animal feed.

In a further aspect the present invention relates to compositions for use in animal feed, such as animal feed, and animal feed additives, e.g. premixes.

A further aspect of the invention is directed to an animal feed additive comprising the
35 phytase, as defined herein. The animal feed additive may be for use in a feed for a mono-gastric or ruminant, typically a monogastric animal. The animal feed additive is for use in a animal feed for an animal typically selected from the group consisting poultry, swine, fish or crustacean. The

animal feed additive typically comprises the phytase in amount 100-5000 FYT/kg feed, such as 125 to 4000 FTY/kg feed, such as 125 to 3000 FTY/kg feed.

The invention is further directed to an animal feed additive comprising the phytase, as defined herein, for use in a feed for poultry. The feed additive for poultry is typically for feed for poultry selected from the group consisting of turkeys, ducks and chickens (including but not limited to broiler chicks, layers), typically chickens, particularly broiler chickens and layer chickens.

The invention is further directed to to an animal feed additive comprising the phytase, as defined herein, for use in a feed for swine.

The invention is further directed to an animal feed additive comprising the phytase, as defined herein, for use in a feed for fish or crustaceans. The fish is typically be selected from the group consisting of salmon, trout, tilapia, catfish, seabream such as gilthead seabream, bass, such as seabass, and carp. The crustaceans may typically be selected from the group consisting of lobster, crab, crayfish, krill, shrimp and prawn.

Apart from the polypeptide of the invention, the animal feed additives of the invention contain at least one fat-soluble vitamin, and/or at least one water soluble vitamin, and/or at least one trace mineral. The feed additive may also contain at least one macro mineral.

Further, optional, feed-additive ingredients are colouring agents, e.g. carotenoids such as beta-carotene, astaxanthin, and lutein; aroma compounds; stabilisers; antimicrobial peptides; polyunsaturated fatty acids; reactive oxygen generating species; and/or at least one other polypeptide selected from amongst phytase (EC 3.1.3.8 or 3.1.3.26); phosphatase (EC 3.1.3.1; EC 3.1.3.2; EC 3.1.3.39); xylanase (EC 3.2.1.8); galactanase (EC 3.2.1.89); alpha-galactosidase (EC 3.2.1.22); protease (EC 3.4.-.-), phospholipase A1 (EC 3.1.1.32); phospholipase A2 (EC 3.1.1.4); lysophospholipase (EC 3.1.1.5); phospholipase C (3.1.4.3); phospholipase D (EC 3.1.4.4); amylase such as, for example, alpha-amylase (EC 3.2.1.1); and/or beta-glucanase (EC 3.2.1.4 or EC 3.2.1.6).

As demonstrated in Example 14 in feeding broilers, a combination of a protease and the phytase of the invention increased protein digestibility by 0.7 to 1.6%, protein retention by 0.9 to 4.4% and energy retention by 0.6 to 1.3%. This resulted in an improvement in body weight gain (BWG) by 2.3 or 3.7% and feed conversion ratio by 4.6 or 2.8%. Accordingly, an embodiment of the invention is directed to an animal feed or animal feed additive comprising the phytase of the invention and a protease. The protease may be selected from the proteases comprised in Ronozyme ProAct™, Ronozyme ProAct360™, Aextra® PRO, Avizyme 1502, Cibenza, Enolzyme, Poultrygrow-250 or Aquagrow-175, preferably selected from Ronozyme ProAct™, Ronozyme ProAct360™, Aextra® PRO and Cibenza, more preferably selected from Ronozyme ProAct™, Ronozyme ProAct360™, most preferably Ronozyme ProAct360™. The invention is further directed to an animal feed or animal feed additive comprising the phytase of the invention and a protease selected from the group consisting of a protease having at least 70% sequence identity,

to SEQ ID NO:1 or SEQ ID NO: 2 of WO0158276 and of a protease having at least 75% sequence identity to SEQ ID NO:3 of WO2019043191. In one embodiment, the protease is selected from a protease having at least 75% sequence identity, such as at least 80% sequence identity, such as at least 85% sequence identity, such as at least 90% sequence identity, such as at least 95% sequence identity, such as at least 98% sequence identity, such as at least 99% sequence identity, such as 100% sequence identity to a protease comprising the sequence of SEQ ID NO: 22 of the present invention.

Typically, the animal feed comprising the protease and the phytase comprises 100 to 5,000 FYT/kg of the phytase in the feed, such as 500 to 2000 FYT/kg. Typically the animal feed comprising the protease and the phytase comprises 10,000 to 50,000 U/kg of the protease in the feed, such as 15,000 to 35,000 U/kg typically 20,000 to 40,000 U/kg.

In a particular embodiment, these other polypeptides are well-defined (as defined above for phytase preparations).

The phytase of the invention may also be combined with other phytases, for example ascomycete phytases such as *Aspergillus* phytases, for example derived from *Aspergillus ficuum*, *Aspergillus niger*, or *Aspergillus awamori*; or basidiomycete phytases, for example derived from *Peniophora lycii*, *Agrocybe pediades*, *Trametes pubescens*, or *Paxillus involutus*; or derivatives, fragments or variants thereof which have phytase activity.

Thus, in preferred embodiments of the use in animal feed of the invention, and in preferred embodiments of the animal feed additive and the animal feed of the invention, the phytase of the invention is combined with such phytases.

Examples of antimicrobial peptides (AMP's) are CAP18, Leucocin A, Tritrpticin, Protegrin-1, Thanatin, Defensin, Lactoferrin, Lactoferricin, and Ovispirin such as Novispirin (Robert Lehrer, 2000), Plectasins, and Statins, including the compounds and polypeptides disclosed in WO 03/044049 and WO 03/048148, as well as variants or fragments of the above that retain antimicrobial activity.

Examples of antifungal polypeptides (AFP's) are the *Aspergillus giganteus*, and *Aspergillus niger* peptides, as well as variants and fragments thereof which retain antifungal activity, as disclosed in WO 94/01459 and WO 02/090384.

Examples of polyunsaturated fatty acids are C18, C20 and C22 polyunsaturated fatty acids, such as arachidonic acid, docosohexaenoic acid, eicosapentaenoic acid and gamma-linoleic acid.

Examples of reactive oxygen generating species are chemicals such as perborate, persulphate, or percarbonate; and polypeptides such as an oxidase, an oxygenase or a syntetase.

Usually fat- and water-soluble vitamins, as well as trace minerals form part of a so-called premix intended for addition to the feed, whereas macro minerals are usually separately added

to the feed. Either of these composition types, when enriched with a polypeptide of the invention, is an animal feed additive of the invention.

In a particular embodiment, the animal feed additive of the invention is intended for being included (or prescribed as having to be included) in animal diets or feed at levels of 0.01 to 10.0%; more particularly 0.05 to 5.0%; or 0.2 to 1.0% (% meaning g additive per 100 g feed). This is so in particular for premixes.

The following are non-exclusive lists of examples of these components:

Examples of fat-soluble vitamins are vitamin A, vitamin D3, vitamin E, and vitamin K, e.g. vitamin K3.

Examples of water-soluble vitamins are vitamin B12, biotin and choline, vitamin B1, vitamin B2, vitamin B6, niacin, folic acid and panthothenate, e.g. Ca-D-panthothenate.

Examples of trace minerals are manganese, zinc, iron, copper, iodine, selenium, and cobalt.

Examples of macro minerals are calcium, phosphorus and sodium.

The nutritional requirements of these components (exemplified with poultry and piglets/pigs) are listed in Table A of WO 01/58275. Nutritional requirement means that these components should be provided in the diet in the concentrations indicated.

In the alternative, the animal feed additive of the invention comprises at least one of the individual components specified in Table A of WO 01/58275. At least one means either of, one or more of, one, or two, or three, or four and so forth up to all thirteen, or up to all fifteen individual components. More specifically, this at least one individual component is included in the additive of the invention in such an amount as to provide an in-feed-concentration within the range indicated in column four, or column five, or column six of Table A.

The present invention also relates to animal feed compositions. Animal feed compositions or diets have a relatively high content of protein. Poultry and pig diets can be characterised as indicated in Table B of WO 01/58275, columns 2-3. Fish diets can be characterised as indicated in column 4 of this Table B. Furthermore such fish diets usually have a crude fat content of 200-310 g/kg.

WO 01/58275 corresponds to US 09/779334 which is hereby incorporated by reference.

An animal feed composition according to the invention has a crude protein content of 50-800 g/kg, and furthermore comprises at least one polypeptide of the present invention.

Furthermore, or in the alternative (to the crude protein content indicated above), the animal feed composition of the invention has a content of metabolisable energy of 10-30 MJ/kg; and/or a content of calcium of 0.1-200 g/kg; and/or a content of available phosphorus of 0.1-200 g/kg; and/or a content of methionine of 0.1-100 g/kg; and/or a content of methionine plus cysteine of 0.1-150 g/kg; and/or a content of lysine of 0.5-50 g/kg.

In particular embodiments, the content of metabolisable energy, crude protein, calcium, phosphorus, methionine, methionine plus cysteine, and/or lysine is within any one of ranges 2, 3, 4 or 5 in Table B of WO 01/58275 (R. 2-5).

Crude protein is calculated as nitrogen (N) multiplied by a factor 6.25, i.e. Crude protein
5 (g/kg) = N (g/kg) x 6.25. The nitrogen content is determined by the Kjeldahl method (A.O.A.C., 1984, Official Methods of Analysis 14th ed., Association of Official Analytical Chemists, Washington DC).

Metabolisable energy can be calculated on the basis of the NRC publication Nutrient requirements in swine, ninth revised edition 1988, subcommittee on swine nutrition, committee
10 on animal nutrition, board of agriculture, national research council. National Academy Press, Washington, D.C., pp. 2-6, and the European Table of Energy Values for Poultry Feed-stuffs, Spelderholt centre for poultry research and extension, 7361 DA Beekbergen, The Netherlands. Grafisch bedrijf Ponsen & Iooijen bv, Wageningen. ISBN 90-71463-12-5.

The dietary content of calcium, available phosphorus and amino acids in complete animal
15 diets is calculated on the basis of feed tables such as Veevoedertabel 1997, gegevens over chemische samenstelling, verteerbaarheid en voederwaarde van voedermiddelen, Central Veevoederbureau, Runderweg 6, 8219 pk Lelystad. ISBN 90-72839-13-7.

In a particular embodiment, the animal feed composition of the invention contains at least one protein. The protein may be an animal protein, such as meat and bone meal, and/or fish meal;
20 or it may be a vegetable protein. The term vegetable proteins as used herein refers to any compound, composition, preparation or mixture that includes at least one protein derived from or originating from a vegetable, including modified proteins and protein-derivatives. In particular embodiments, the protein content of the vegetable proteins is at least 10, 20, 30, 40, 50, or 60% (w/w).

Vegetable proteins may be derived from vegetable protein sources, such as legumes and
25 cereals, for example materials from plants of the families Fabaceae (Leguminosae), Cruciferae, Chenopodiaceae, and Poaceae, such as soybean meal, lupin meal and rapeseed meal.

In a particular embodiment, the vegetable protein source is material from one or more
30 plants of the family Fabaceae, e.g. soybean, lupine, pea, or bean.

In another particular embodiment, the vegetable protein source is material from one or more plants of the family Chenopodiaceae, e.g. beet, sugar beet, spinach or quinoa.

Other examples of vegetable protein sources are rapeseed, sunflower seed, cotton seed, and cabbage.

35 Soybean is a preferred vegetable protein source.

Other examples of vegetable protein sources are cereals such as barley, wheat, rye, oat, maize (corn), rice, triticale, and sorghum.

In still further particular embodiments, the animal feed composition of the invention contains 0-80% maize; and/or 0-80% sorghum; and/or 0-70% wheat; and/or 0-70% Barley; and/or 0-30% oats; and/or 0-40% soybean meal; and/or 0-25% fish meal; and/or 0-25% meat and bone meal; and/or 0-20% whey.

5 Animal diets can e.g. be manufactured as mash feed (non pelleted) or pelleted feed. Typically, the milled feed-stuffs are mixed and sufficient amounts of essential vitamins and minerals are added according to the specifications for the species in question. Polypeptides can be added as solid or liquid polypeptide formulations. For example, a solid polypeptide formulation is typically added before or during the mixing step; and a liquid polypeptide preparation is typically
10 added after the pelleting step. The polypeptide may also be incorporated in a feed additive or premix.

The final polypeptide concentration in the diet is within the range of 0.01-200 mg polypeptide protein per kg diet, for example in the range of 5-30 mg polypeptide protein per kg animal diet.

15 The phytase of the invention should of course be applied in an effective amount, i.e. in an amount adequate for improving solubilisation and/or improving nutritional value of feed. It is at present contemplated that the polypeptide is administered in one or more of the following amounts (dosage ranges): 0.01-200; 0.01-100; 0.5-100; 1-50; 5-100; 10-100; 0.05-50; or 0.10-10 – all these ranges being in mg phytase polypeptide protein per kg feed (ppm).

20 For determining mg phytase polypeptide protein per kg feed, the phytase is purified from the feed composition, and the specific activity of the purified phytase is determined using a relevant assay. The phytase activity of the feed composition as such is also determined using the same assay, and on the basis of these two determinations, the dosage in mg phytase protein per kg feed is calculated.

25 The same principles apply for determining mg phytase polypeptide protein in feed additives. Of course, if a sample is available of the phytase used for preparing the feed additive or the feed, the specific activity is determined from this sample (no need to purify the phytase from the feed composition or the additive).

30 **Methods for Producing Fermentation Products**

Yet another aspect of the present invention relates to the methods for producing a fermentation product, such as, e.g., ethanol, beer, wine, distillers dried grains (DDG), wherein the fermentation is carried out in the presence of a phytase produced by the present invention. Examples of fermentation processes include, for example, the processes described in WO
35 01/62947. Fermentation is carried out using a fermenting microorganism, such as, yeast.

In a particular embodiment, the present invention provides methods for producing fermentation product, comprising (a) fermenting (using a fermenting microorganism, such as

yeast) a carbohydrate containing material (e.g., starch) in the presence of a phytase of the present invention and (b) producing the fermentation product from the fermented carbohydrate containing material.

5 In a particular embodiment, the present invention provides methods for producing ethanol, comprising fermenting (using a fermenting microorganism, such as yeast) a carbohydrate containing material (e.g., starch) in the presence of a phytase of the present invention and producing or recovering ethanol from the fermented carbohydrate containing material.

10 In another embodiment, the present invention provides methods for producing ethanol comprising a) hydrolyzing starch, e.g., by a liquefaction and/or saccharification process, a raw starch hydrolysis process, b) fermenting the resulting starch in the presence of a phytase of the present invention, and c) producing ethanol.

The phytase may be added to the fermentation process at any suitable stage and in any suitable composition, including alone or in combination with other enzymes, such as, one or more
15 alpha-amylases, glucoamylases, proteases, and/or cellulases.

In another embodiment, the present invention provides methods for producing ethanol comprising hydrolyzing biomass, and fermenting (using a fermenting microorganism, such as yeast) the resulting biomass in the presence of a phytase of the present invention.

20 **Plants**

The present invention also relates to plants, e.g., a transgenic plant, plant part, or plant cell, comprising a polynucleotide of the present invention so as to express and produce the phytase in recoverable quantities. The phytase may be recovered from the plant or plant part. Alternatively, the plant or plant part containing the phytase may be used as such for improving
25 the quality of a food or feed, e.g., improving nutritional value, palatability, and rheological properties, or to destroy an antinutritive factor.

The transgenic plant can be dicotyledonous (a dicot) or monocotyledonous (a monocot). Examples of monocot plants are grasses, such as meadow grass (blue grass, Poa), forage grass such as Festuca, Lolium, temperate grass, such as Agrostis, and cereals, e.g., wheat, oats, rye,
30 barley, rice, sorghum, and maize (corn).

Examples of dicot plants are tobacco, legumes, such as lupins, potato, sugar beet, pea, bean and soybean, and cruciferous plants (family Brassicaceae), such as cauliflower, rape seed, and the closely related model organism *Arabidopsis thaliana*.

35 Examples of plant parts are stem, callus, leaves, root, fruits, seeds, and tubers as well as the individual tissues comprising these parts, e.g., epidermis, mesophyll, parenchyme, vascular tissues, meristems. Specific plant cell compartments, such as chloroplasts, apoplasts, mitochondria, vacuoles, peroxisomes and cytoplasm are also considered to be a plant part. Furthermore, any plant cell, whatever the tissue origin, is considered to be a plant part. Likewise,

plant parts such as specific tissues and cells isolated to facilitate the utilization of the invention are also considered plant parts, e.g., embryos, endosperms, aleurone and seed coats.

Also included within the scope of the present invention are the progeny of such plants, plant parts, and plant cells.

5 The transgenic plant or plant cell expressing a phytase may be constructed in accordance with methods known in the art. In short, the plant or plant cell is constructed by incorporating one or more expression constructs encoding a phytase into the plant host genome or chloroplast genome and propagating the resulting modified plant or plant cell into a transgenic plant or plant cell.

10 The expression construct is conveniently a nucleic acid construct that comprises a polynucleotide encoding a phytase operably linked with appropriate regulatory sequences required for expression of the polynucleotide in the plant or plant part of choice. Furthermore, the expression construct may comprise a selectable marker useful for identifying plant cells into which the expression construct has been integrated and DNA sequences necessary for introduction of
15 the construct into the plant in question (the latter depends on the DNA introduction method to be used).

The choice of regulatory sequences, such as promoter and terminator sequences and optionally signal or transit sequences, is determined, for example, on the basis of when, where, and how the phytase is desired to be expressed. For instance, the expression of the gene
20 encoding a phytase may be constitutive or inducible, or may be developmental, stage or tissue specific, and the gene product may be targeted to a specific tissue or plant part such as seeds or leaves. Regulatory sequences are, for example, described by Tague *et al.*, 1988, *Plant Physiology* 86: 506.

For constitutive expression, the 35S-CaMV, the maize ubiquitin 1, or the rice actin 1
25 promoter may be used (Franck *et al.*, 1980, *Cell* 21: 285-294; Christensen *et al.*, 1992, *Plant Mol. Biol.* 18: 675-689; Zhang *et al.*, 1991, *Plant Cell* 3: 1155-1165). Organ-specific promoters may be, for example, a promoter from storage sink tissues such as seeds, potato tubers, and fruits (Edwards and Coruzzi, 1990, *Ann. Rev. Genet.* 24: 275-303), or from metabolic sink tissues such as meristems (Ito *et al.*, 1994, *Plant Mol. Biol.* 24: 863-878), a seed specific promoter such as the
30 glutelin, prolamin, globulin, or albumin promoter from rice (Wu *et al.*, 1998, *Plant Cell Physiol.* 39: 885-889), a *Vicia faba* promoter from the legumin B4 and the unknown seed protein gene from *Vicia faba* (Conrad *et al.*, 1998, *J. Plant Physiol.* 152: 708-711), a promoter from a seed oil body protein (Chen *et al.*, 1998, *Plant Cell Physiol.* 39: 935-941), the storage protein *napA* promoter from *Brassica napus*, or any other seed specific promoter known in the art, e.g., as described in
35 WO 91/14772. Furthermore, the promoter may be a leaf specific promoter such as the *rbcs* promoter from rice or tomato (Kyoizuka *et al.*, 1993, *Plant Physiol.* 102: 991-1000), the chlorella virus adenine methyltransferase gene promoter (Mittra and Higgins, 1994, *Plant Mol. Biol.* 26: 85-

93), the *aldP* gene promoter from rice (Kagaya *et al.*, 1995, *Mol. Gen. Genet.* 248: 668-674), or a wound inducible promoter such as the potato *pin2* promoter (Xu *et al.*, 1993, *Plant Mol. Biol.* 22: 573-588). Likewise, the promoter may be induced by abiotic treatments such as temperature, drought, or alterations in salinity or induced by exogenously applied substances that activate the promoter, e.g., ethanol, oestrogens, plant hormones such as ethylene, abscisic acid, and gibberellic acid, and heavy metals.

A promoter enhancer element may also be used to achieve higher expression of a phytase in the plant. For instance, the promoter enhancer element may be an intron that is placed between the promoter and the polynucleotide encoding a phytase. For instance, Xu *et al.*, 1993, *supra*, disclose the use of the first intron of the rice actin 1 gene to enhance expression.

The selectable marker gene and any other parts of the expression construct may be chosen from those available in the art.

The nucleic acid construct is incorporated into the plant genome according to conventional techniques known in the art, including *Agrobacterium*-mediated transformation, virus-mediated transformation, microinjection, particle bombardment, biolistic transformation, and electroporation (Gasser *et al.*, 1990, *Science* 244: 1293; Potrykus, 1990, *Bio/Technology* 8: 535; Shimamoto *et al.*, 1989, *Nature* 338: 274).

Agrobacterium tumefaciens-mediated gene transfer is a method for generating transgenic dicots (for a review, see Hooykas and Schilperoort, 1992, *Plant Mol. Biol.* 19: 15-38) and for transforming monocots, although other transformation methods may be used for these plants. A method for generating transgenic monocots is particle bombardment (microscopic gold or tungsten particles coated with the transforming DNA) of embryonic calli or developing embryos (Christou, 1992, *Plant J.* 2: 275-281; Shimamoto, 1994, *Curr. Opin. Biotechnol.* 5: 158-162; Vasil *et al.*, 1992, *Bio/Technology* 10: 667-674). An alternative method for transformation of monocots is based on protoplast transformation as described by Omirulleh *et al.*, 1993, *Plant Mol. Biol.* 21: 415-428. Additional transformation methods include those described in U.S. Patent Nos. 6,395,966 and 7,151,204 (both of which are herein incorporated by reference in their entirety).

Following transformation, the transformants having incorporated the expression construct are selected and regenerated into whole plants according to methods well known in the art. Often the transformation procedure is designed for the selective elimination of selection genes either during regeneration or in the following generations by using, for example, co-transformation with two separate T-DNA constructs or site specific excision of the selection gene by a specific recombinase.

In addition to direct transformation of a particular plant genotype with a construct of the present invention, transgenic plants may be made by crossing a plant having the construct to a second plant lacking the construct. For example, a construct encoding a phytase can be introduced into a particular plant variety by crossing, without the need for ever directly

transforming a plant of that given variety. Therefore, the present invention encompasses not only a plant directly regenerated from cells which have been transformed in accordance with the present invention, but also the progeny of such plants. As used herein, progeny may refer to the offspring of any generation of a parent plant prepared in accordance with the present invention.

5 Such progeny may include a DNA construct prepared in accordance with the present invention. Crossing results in the introduction of a transgene into a plant line by cross pollinating a starting line with a donor plant line. Non-limiting examples of such steps are described in U.S. Patent No. 7,151,204.

10 Plants may be generated through a process of backcross conversion. For example, plants include plants referred to as a backcross converted genotype, line, inbred, or hybrid.

Genetic markers may be used to assist in the introgression of one or more transgenes of the invention from one genetic background into another. Marker assisted selection offers advantages relative to conventional breeding in that it can be used to avoid errors caused by phenotypic variations. Further, genetic markers may provide data regarding the relative degree
15 of elite germplasm in the individual progeny of a particular cross. For example, when a plant with a desired trait which otherwise has a non-agronomically desirable genetic background is crossed to an elite parent, genetic markers may be used to select progeny which not only possess the trait of interest, but also have a relatively large proportion of the desired germplasm. In this way, the number of generations required to introgress one or more traits into a particular genetic
20 background is minimized.

The present invention also relates to methods of producing a phytase of the present invention comprising: (a) cultivating a transgenic plant or a plant cell comprising a polynucleotide encoding the phytase under conditions conducive for production of the phytase; and (b) recovering the phytase.

25 The invention is further defined in the following paragraphs:

1. A phytase variant which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2 and which comprises the alterations N31C/G52C/A99C/K141C/T177C/V199C/N203L as compared to SEQ ID NO: 2 and further comprises a substitution in one or more position(s)
30 selected from the following: 30, 36, 43, 46, 57, 60, 64, 73, 79, 119, 121, 123, 130, 134, 138, 151, 155, 161, 162, 168, 176, 180, 184, 190, 207, 224, 230, 243, 273, 286, 336, 340, 358 and 375 using SEQ ID NO: 2 for numbering.

2. The variant of paragraph 1, comprising substitutions in the positions: 57, 73, 121, 134, 155, 207 and 273.

35 3. The variant of paragraph 2, further comprising substitutions in the positions: 36, 60, 64, 73, 119, 130, 138, 161, 162, 168, 176, 180, 184, 190, 224, 230, 243, 336 and 340.

4. The variant according to any of paragraphs 1-3, where the substitutions are

selected among: 30Q, 36A, 43C, 46C, 57Y, 60H, 64Q, 73P, 79Q, 119P, 121P, 123C, 130T,C, 134Q, 138A, 151S, 155F, 161T, 162A, 168R 176P, 180N, 184Q, 190T, 207T, 224Q, 230E, 243N, 273L, 286S, 336R, 340L,P, 358Q and 375K.

5. The variant according to any of the paragraphs 1-4, comprising the substitutions
5 31C/52C/57Y/ 73P/99C/121P/134Q/141C/155F/177C/199C/203L/207T/273L.

6. The variant according to paragraph 5, selected among variants comprising the substitutions selected from the group consisting of:

31C/ 52C/ 57Y/ 73P/ 99C/ 121P/ 134Q/ 141C/ 155F/ 177C/ 199C/ 203L/ 207T/ 273L;

31C/ 36A/ 52C/ 57Y/ 60H/ 64Q/ 73P/ 99C/ 119S/ 121P/ 130T/ 134Q/ 138A/ 141C/ 155F/
10 161T/ 162A/ 176P/ 177C/ 180N/ 184Q/ 190T/ 199C/ 203L/ 207T/ 224Q/ 230E/ 243N/ 273L/
336R/ 340L;

31C/ 36A/ 43C/ 46C/ 52C/ 57Y/ 60H/ 64Q/ 73P/ 99C/ 119S/ 121P/ 130T/ 134Q/ 138A/
141C/ 155F/ 161T/ 162A/ 176P/ 177C/ 180N/ 184Q/ 190T/ 199C/ 203L/ 207T/ 224Q/ 230E/
243N/ 273L/ 286S/ 336R/ 340L;

31C/ 36A/ 52C/ 57Y/ 60H/ 64Q/ 73P/ 99C/ 119S/ 121P/ 123C/ 130C/ 134Q/ 138A/ 141C/
151S/ 155F/ 161T/ 162A/ 176P/ 177C/ 180N/ 184Q/ 190T/ 199C/ 203L/ 207T/ 224Q/ 230E/
243N/ 273L/ 336R/ 340L; and

31C/ 36A/ 43C/ 46C/ 52C/ 57Y/ 60H/ 64Q/ 73P/ 99C/ 119S/ 121P/ 123C/ 130C/ 134Q/
138A/ 141C/ 155F/ 161T/ 162A/ 176P/ 177C/ 180N/ 184Q/ 190T/ 199C/ 203L/ 207T/ 224Q/
20 230E/ 243N/ 273L/ 286S/ 336R/ 340L.

7. The variant of paragraph 6, having the amino acid sequence of SEQ ID NO: 2 with the substitutions selected from the group consisting of:

N31C/ G52C/ E57Y/ N73P/ A99C/ N121P/ S134Q/ K141C/ Y155F/ T177C/ V199C/
N203L/ P207T/ M273L;

N31C/ Q36A/ G52C/ E57Y/ Q60H/ L64Q/ N73P/ A99C/ E119S/ N121P/ M130T/ S134Q/
L138A/ K141C/ Y155F/ S161T/ S162A/ E176P/ T177C/ T180N/ S184Q/ P190T/ V199C/ N203L/
P207T/ E224Q/ Q230E/ R243N/ M273L/ K336R/ T340L;

N31C/ Q36A/ P43C/ W46C/ G52C/ E57Y/ Q60H/ L64Q/ N73P/ A99C/ E119S/ N121P/
M130T/ S134Q/ L138A/ K141C/ Y155F/ S161T/ S162A/ E176P/ T177C/ T180N/ S184Q/ P190T/
30 V199C/ N203L/ P207T/ E224Q/ Q230E/ R243N/ M273L/ N286S/ K336R/ T340L;

N31C/ Q36A/ G52C/ E57Y/ Q60H/ L64Q/ N73P/ A99C/ E119S/ N121P/ P123C/ M130C/
S134Q/ L138A/ K141C/ N151S/ Y155F/ S161T/ S162A/ E176P/ T177C/ T180N/ S184Q/ P190T/
V199C/ N203L/ P207T/ E224Q/ Q230E/ R243N/ M273L/ K336R/ T340L; or

N31C/ Q36A/ P43C/ W46C/ G52C/ E57Y/ Q60H/ L64Q/ N73P/ A99C/ E119S/ N121P/
35 P123C/ M130C/ S134Q/ L138A/ K141C/ Y155F/ S161T/ S162A/ E176P/ T177C/ T180N/
S184Q/ P190T/ V199C/ N203L/ P207T/ E224Q/ Q230E/ R243N/ M273L/ N286S/ K336R/
T340L.

8. The variant according to any of paragraphs 1-7, wherein the parent phytase has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mature polypeptide of SEQ ID NO: 2.

5 9. The variant of any of paragraphs 1-8, wherein the parent phytase comprises or consists of the mature polypeptide of SEQ ID NO: 2.

10. The variant of any of paragraphs 1-9, which has an improved thermostability in comparison with the phytase having the amino acid sequence of SEQ ID NO: 2 and the substitutions N31C/ G52C/ A99C/ K141C/ T177C/ V199C/ N203L.

10 11. A polynucleotide encoding the variant of any of paragraphs 1-10.

12. A nucleic acid construct or an expression vector comprising the polynucleotide of paragraph 11.

13. A host cell comprising the polynucleotide of paragraph 11.

14. A method of producing a phytase variant according to any of paragraphs 1-10, comprising:

cultivating the host cell of paragraph 13 under conditions suitable for expression of the variant; and

recovering the variant.

15. A plant comprising the phytase variant of any of paragraphs 1-10 and/or the polynucleotide of paragraph 11.

16. A composition comprising at least one phytase variant according to any of paragraphs 1-10.

17. The composition of paragraph 16 further comprising

at least one fat soluble vitamin;

at least one water soluble vitamin; and/or

at least one trace mineral.

18. The composition of paragraph 16 or 17, further comprising at least one enzyme selected from the following group of enzymes: amylase, phytase, phosphatase, xylanase, galactanase, alpha-galactosidase, protease, phospholipase and/or beta-glucanase.

19. The composition of any of paragraphs 16-18, which is an animal feed additive.

20. An animal feed composition having a crude protein content of 50 to 800 g/kg and comprising the phytase variant of any of paragraphs 1-10, the polynucleotide of paragraph 11, or the composition of any of paragraphs 16-19.

21. A method of improving the nutritional value of an animal feed, wherein the phytase variant of any of paragraphs 1-10, the polynucleotide of paragraph 11, or the composition of any of paragraphs 16-19 is added to the feed.

22. A process for reducing phytate levels in animal manure comprising feeding an

animal with an effective amount of the feed composition of paragraph 20.

23. A method for the treatment of vegetable proteins, comprising the step of adding the phytase variant of any of paragraphs 1-10, the polynucleotide of paragraph 11, or the composition of any of paragraphs 16-19 to at least one vegetable protein or protein source.

5 24. A method for increasing weight gain and/or improving Feed Conversion Ratio of an animal, the method comprising the step of applying to the animal a feed with an efficient amount of the phytase variant of any of paragraphs 1-10, the polynucleotide of paragraph 11, or the composition of any of paragraphs 16-19.

10 25. Use of the phytase variant of any of paragraphs 1-10, the polynucleotide of paragraph 11, or the composition of any of paragraphs 16-19 in animal feed; in the preparation of animal feed; for improving the nutritional value of animal feed; for reducing phytate levels in animal manure; for the treatment of vegetable proteins; for liberating phosphorous from a phytate substrate; or for increasing weight gain, improving specific growth rate and/or improving Feed Conversion Ratio of an animal; or for improving nutrient retention, and/or nutrient digestibility in
15 an animal.

20 26. A method for producing a fermentation product, comprising (a) fermenting using a fermenting microorganism, a carbohydrate containing material in the presence of a phytase variant of any of paragraphs 1-10 or the polynucleotide of paragraph 11, and (b) producing the fermentation product of fermentation coproduct from the fermented carbohydrate containing material.

27. The method of paragraph 26, wherein the fermentation product is ethanol, beer, wine or distillers dried grains (DDG).

28. An isolated polypeptide having phytase activity, selected from the group consisting of

- 25 a) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 12;
b) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 14;
c) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least
30 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 16;
d) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 18;
and
e) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least
35 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 20.

29. The polypeptide according to paragraph 28, obtained or obtainable from *Citrobacter braakii*.

30. The isolated polypeptide according to any of paragraphs 28 to 29, wherein said polypeptide is pH stable and thermostable such that it comprises one or more of the following properties

- i. an unfolding temperature at pH 4 of at least 75 °C;
- ii. an unfolding temperature at pH 3 of at least 70°C; and
- iii. an unfolding temperature at pH 2 of at least 55°C.

31. The isolated polypeptide according to any of paragraphs 28 to 30, wherein said polypeptide is acid stable such that it maintains a residual activity level above 90% after 24 hours at each of pH 2, 3, 4, 5, 6, 7 and 8.

32. The polypeptide according to any of paragraphs 28 to 31 comprising the alterations N31C/G52C/A99C/K141C/T177C/V199C as compared to SEQ ID NO: 2.

33. The polypeptide according to any of paragraphs 28 to 32 comprising a substitution in one or more position(s) selected from the following: 30, 36, 43, 46, 57, 60, 64, 73, 79, 119, 121, 123, 130, 134, 138, 151, 155, 161, 162, 168, 176, 180, 184, 190, 207, 224, 230, 243, 273, 286, 336, 340, 358 and 375 using SEQ ID NO: 2 for numbering.

34. A method of preparing a recombinant polypeptide having phytase activity comprising the steps of:

(a) cultivating a recombinant host cell comprising an exogenous polynucleotide selected from the group consisting of

a. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 13;

b. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 15;

c. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 17;

d. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 19;

e. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 21;

wherein the polynucleotide is expressed and the polypeptide is produced;

(b) optionally isolating the polypeptide; and

(c) optionally recovering the polypeptide.

35. A method of producing a polypeptide having phytase activity, said method comprising the steps of:

(a) cultivating a recombinant host cell comprising an exogenous polynucleotide encoding the polypeptide having phytase activity selected from the group consisting of

a. a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 12;

b. a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 14;

c. a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity SEQ ID NO: 16;

d. a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity SEQ ID NO: 18; and

e. a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 20;

wherein the polynucleotide is expressed and the polypeptide is produced;

(b) optionally isolating the polypeptide; and

(c) optionally recovering the polypeptide.

36. The method according to paragraph 35, wherein the polypeptide having phytase activity comprises the substitutions N31C/G52C/A99C/K141C/T177C/V199C as compared to SEQ ID NO: 2.

37. The method according to any of paragraphs 34 to 36 wherein the polypeptide further comprises a substitution in one or more position(s) selected from the following: 30, 36, 43, 46, 57, 60, 64, 73, 79, 119, 121, 123, 130, 134, 138, 151, 155, 161, 162, 168, 176, 180, 184, 190, 207, 224, 230, 243, 273, 286, 336, 340, 358 and 375 using SEQ ID NO: 2 for numbering. 38. An animal feed additive comprising the phytase defined in any of paragraphs 1 to 10, for use in a animal feed for an animal selected from the group consisting poultry, swine, fish or crustacean.

39. The animal feed additive according to paragraph 38, for use in a feed for poultry wherein the poultry is selected from the group consisting of turkeys, ducks and chickens (including but not limited to broiler chicks, layers), typically chickens, particularly broiler chickens and layer chickens.

40. The animal feed additive according to paragraph 38, for use in a feed for swine.

41. The animal feed additive according to paragraph 38, for use in a feed for fish or

crustaceans.

42. The animal feed additive according to paragraph 41, wherein the fish is selected from the group consisting of salmon, trout, tilapia, catfish, seabream such as gilthead seabream, bass, such as seabass, and carp and wherein the crustaceans is selected from the group consisting of lobster, crab, crayfish, krill, shrimp and prawn.

43. The animal feed additive according to any of paragraphs 38 to 42, comprising the phytase of paragraphs 1 to 10 in amount 100-5000 FYT/kg feed, such as 125 to 4000 FTY/kg feed, such as 125 to 3000 FTY/kg feed.

The invention described and claimed herein is not to be limited in scope by the specific embodiments herein disclosed, since these embodiments are intended as illustrations of several aspects of the invention. Any equivalent embodiments are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. In the case of conflict, the present disclosure including definitions will control.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

The present invention is further described by the following examples that should not be construed as limiting the scope of the invention.

EXAMPLES

Chemicals used were commercial products of at least reagent grade.

Example 1: Preparation of variants, and determination of activity

Expression of phytase variants in *Aspergillus oryzae*

The constructs comprising the *C. braakii* phytase variant genes in the examples were used to construct expression vectors for *Aspergillus*. The *Aspergillus* expression vectors consist of an expression cassette based on the *Aspergillus niger* neutral amylase II promoter fused to the *Aspergillus nidulans* triose phosphate isomerase non translated leader sequence (Pna2/tpi) and the *Aspergillus niger* amyloglycosidase terminator (Tamg). Also present on the plasmid was the *Aspergillus* selective marker *pyrG* from *Aspergillus nidulans* enabling growth on minimal media for an aspergillus which is *pyrG* minus. The expression plasmids for phytase variants were transformed into *Aspergillus* as described in Lassen et al. (2001), Applied and Environmental Microbiology, 67, 4701-4707. For each of the constructs 4-6 strains were isolated, purified and cultivated in microtiterplates. Expression was determined using a p-nitrophenyl phosphate substrate. The best producing strain was fermented in Shake flasks.

Purification of *C. braakii* phytase variants

The fermentation supernatant with the phytase variant was filtered through a Fast PES Bottle top filter with a 0.22 μm cut-off. The resulting solution was diluted with water to the double volume and pH was adjusted to 4.5 with acetic acid. Occasionally, the solution became a little cloudy and this removed by filtration through a Fast PES Bottle top filter with a 0.22 μm cut-off.

After pretreatment the phytase variant was purified by chromatography on S Sepharose, approximately 30 ml in a XK26 column, using as buffer A 50 mM sodium acetate pH 4.5, and as buffer B 50 mM sodium acetate + 1 M NaCl pH 4.5. The fractions from the column were analyzed for activity using the phosphatase assay (see below) and fractions with activity were pooled.

In some cases the solution containing the purified phytase variant was concentrated using an Amicon ultra-15 filtering device with a 30 kDa cut-off membrane.

The molecular weight, as estimated from SDS-PAGE, was approximately 45-50 kDa and the purity was > 95%.

Determination of phosphatase activity

75 microliter phytase-containing enzyme solution is dispensed in a microtiter plate well, e. g. NUNC 269620 and 75 microliter substrate is added (for preparing the substrate, two 5 mg p-nitrophenyl phosphate tablets (Sigma, Cat.No. N-9389) are dissolved in 10 ml 0.1 M Na-acetate buffer, pH 5.5). The plate is sealed and incubated 15 min., shaken with 750 rpm at 37°C. After the incubation time 75 microliter stop reagent is added (the stop reagent is 0.1 M disodiumtetraborate in water) and the absorbance at 405 nm is measured in a microtiter plate spectrophotometer. One phosphatase unit is defined as the enzyme activity that releases 1 micromol phosphate/min under the given reaction conditions (buffer blind subtracted). The absorbance of 1 micromol p-nitrophenol is determined to be 56 AU (AU= absorbancy units) under assay conditions.

Determination of phytase activity

75 microliter phytase-containing enzyme solution, appropriately diluted in 0.25M sodium acetate, 0.005% (w/v) Tween-20. pH5.5, is dispensed in a microtiter plate well, e. g. NUNC 269620, and 75 microliter substrate is added (prepared by dissolving 100mg sodium phytate from rice (Aldrich Cat.No. 274321) in 10ml 0.25M sodium acetate buffer, pH5.5). The plate is sealed and incubated 15min. shaken with 750rpm at 37°C. After incubation, 75 microliter stop reagent is added (the stop reagent being prepared by mixing 10 ml molybdate solution (10% (w/v) ammonium hepta-molybdate in 0.25% (w/v) ammonia solution), 10ml ammonium vanadate (0.24% commercial product from Bie&Berntsen, Cat.No. LAB17650), and 20ml 21.7% (w/v) nitric acid), and the absorbance at 405nm is measured in a microtiter plate spectrophotometer. The phytase activity is expressed in the unit of FYT, one FYT being the amount of enzyme that liberates 1 micromole inorganic ortho-phosphate per minute under the conditions above. An absolute value for the measured phytase activity may be obtained by reference to a standard

curve prepared from appropriate dilutions of inorganic phosphate, or by reference to a standard curve made from dilutions of a phytase enzyme preparation with known activity (such standard enzyme preparation with a known activity is available on request from Novozymes A/S, Krogshoejvej 36, DK-2880 Bagsvaerd).

5

Example 2: Single position variants

The parent phytase for this example was a variant having the sequence of SEQ ID NO: 2 with the substitutions: (var300) N31C/G52C/A99C/K141C/T177C/V199C/N203L.

96 single position variants of the parent phytase was prepared as described in Example

10 1. The substitutions were selected based on alignment of known phytases and identification of consensus sequences as known in the art. The thermostability of the variants were tested using Protein Thermal Shift™ (Applied Biosystems, Carlsbad, CA, US) according to the manufacturer's instructions. Variants having improved thermostability compared with the parent are shown in Table 1.

15

Table 1.

Variant	T (°C)
K30Q	70
Q60H	70
L64Q	70
E119S	70
N121P	71
L138A	70
S162A	70
T180N	70
P190T	70
R243N	70
M273L	71
K336R	70
T340L	70
D358Q	72
Q36A	70
E57Y	71
N73P	71
S79Q	70
M130T	70
S134Q	71
Y155F	71
S161T	70
N168R	70
E176P	70
S184Q	70
P207T	72
E224Q	70
Q230E	70
T340P	70
D375K	70

Example 3: Combining beneficial positions

Based on the results disclosed in Example 2, a variant combining several of the beneficial substitutions was generated having the sequence of SEQ ID NO: 2 with the substitutions:

(var400):

5 N31C/G52C/E57Y/N73P/A99C/N121P/S134Q/K141C/Y155F/T177C/V199C/N203L/
P207T/M273L

A gene was designed and the variant produced as described in Example 1. Thermal shift assay was performed in triplicate on the variant showing 81°C.

10 Example 4: Further Combinatorial variants

Based on the results disclosed in Example 2 a variant combining further beneficial substitutions using the variant generated in Example 3 as the parent phytase. Following variants having the sequence of SEQ ID NO: 2 with the substitutions were generated:

(var404)

15 N31C/Q36A/G52C/E57Y/Q60H/N73P/A99C/E119S/N121P/M130T/S134Q/L138A/K141C/
Y155F/S161T/S162A/E176P/T177C/T180N/S184Q/P190T/V199C/N203L/P207T/E224Q/Q230E
/R243N/M273L/K336R/T340L

(var405)

N31C/Q36A/P43C/W46C/G52C/E57Y/Q60H/L64Q/N73P/A99C/E119S/N121P/M130T/S134Q/
20 L138A/K141C/Y155F/S161T/S162A/E176P/T177C/T180N/S184Q/P190T/V199C/N203L/P207T/
E224Q/Q230E/R243N/M273L/N286S/K336R/T340L

(var406)

N31C/Q36A/G52C/E57Y/Q60H/L64Q/N73P/A99C/E119S/N121P/P123C/M130C/S134Q/L138A/
K141C/N151S/Y155F/S161T/S162A/E176P/T177C/T180N/S184Q/P190T/V199C/N203L/
25 P207T/E224Q/Q230E/R243N/M273L/K336R/T340L

(var411)

N31C/Q36A/P43C/W46C/G52C/E57Y/Q60H/L64Q/N73P/A99C/E119S/N121P/P123C/M130C/
S134Q/L138A/K141C/Y155F/S161T/S162A/E176P/T177C/T180N/S184Q/P190T/V199C/N203L
/P207T/E224Q/Q230E/R243N/M273L/N286S/K336R/T340L

30

Example 5: Thermostability of generated variants

The following variants were constructed and tested for thermostability:

Table 2.

Phytase	Substitutions
var400	N31C/G52C/E57Y/N73P/A99C/N121P/S134Q/K141C/Y155F/T177C/V199C/ N203L/P207T/M273L
var404	N31C/Q36A/G52C/E57Y/Q60H/N73P/A99C/E119S/N121P/M130T/S134Q/L138A/ K141C/Y155F/S161T/S162A/E176P/T177C/T180N/S184Q/P190T/V199C/

	N203L/P207T/E224Q/Q230E/R243N/M273L/K336R/T340L
var405	N31C/Q36A/P43C/W46C/G52C/E57Y/Q60H/L64Q/N73P/A99C/E119S/N121P/M130T/S134Q/L138A/K141C/Y155F/S161T/S162A/E176P/T177C/T180N/S184Q/P190T/V199C/N203L/P207T/E224Q/Q230E/R243N/M273L/N286S/K336R/T340L
var406	N31C/Q36A/G52C/E57Y/Q60H/L64Q/N73P/A99C/E119S/N121P/P123C/M130C/S134Q/L138A/K141C/N151S/Y155F/S161T/S162A/E176P/T177C/T180N/S184Q/P190T/V199C/N203L/P207T/E224Q/Q230E/R243N/M273L/K336R/T340L
var411	N31C/Q36A/P43C/W46C/G52C/E57Y/Q60H/L64Q/N73P/A99C/E119S/N121P/P123C/M130C/S134Q/L138A/K141C/Y155F/S161T/S162A/E176P/T177C/T180N/S184Q/P190T/V199C/N203L/P207T/E224Q/Q230E/R243N/M273L/N286S/K336R/T340L

The variants were tested for thermostability by nano Differential Scanning Fluorescence (nanoDSF).

5 Nano-DSF monitors intrinsic tryptophan (Trp) fluorescence of the protein as a function of temperature at 330 and 350 nm. The temperature stability of a protein can be expressed by T_m (the temperature at which there is an equal population of folded and unfolded molecules) found at the inflection point of the fluorescence signal.

10 NanoDSF was performed with a nanoDSF Prometheus NT.48 instrument (NanoTemper Technologies GmbH, München, Germany). Phytase variant samples (purified as described in Example 1, all of them in 50 mM Na-acetate, pH 4.5) were loaded into nanoDSF standard grade capillaries (NanoTemper Technologies GmbH; catalog number PR-C002) through capillary action. Three capillaries were filled for each sample. The capillaries were then placed into the instrument (up to 48 single capillaries can be loaded in a single run) and the laser intensity required for optimum signal generation was determined. The samples were run with the following
15 experimental setting: temperature slope 2 °C/minute, start temperature 20 °C and end temperature 95 °C.

The data was analyzed using the software that is supplied with the instrument (PR. ThermControl v2.0.4, NanoTemper Technologies GmbH) and the T_m (for the ratio 350 nm/330 nm) was determined (results shown in Table 3 below).

20 Further the variants were tested using DSC using the assay disclosed in WO 2011/117396 Example 4. Results are shown in Table 3.

Table 3.

Designation	nDSF (broth) (°C)	nDSF (purified) (°C)	DSC (°C)
var400	83.7	82.5	85.6
var404	86.1	85.4	88.3
var405	86.1	86.2	88.9
var406	88.7	88.1	90.6
var411	88.8	88.1	

The variants were also tested for thermostability in the pH range 1.0 to 8.5 using NanoDSF (described above). The variant samples were in 0.1M glycine, 0.1M acetic acid, 0.1M Bis-Tris, adjusted to the desired pH with either 0.5 M HCl or 0.1 M NaOH. The temperature slope was 3.33 °C/minute in this experiment. Results are shown in Tables 4a and 4b.

5 Table 4a

	pH							
Phytase	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
C.b. wt SEQ ID 2	ns	44.2	46.0	49.3	53.6	58.6	61.5	63.4
var300	44.3	47.9	52.7	58.8	65.2	70.3	73.8	75.2
var400	52.7	56.2	60.2	65.9	71.5	76.8	80.1	81.9
var404	57.1	61.0	64.4	69.7	75.6	80.5	83.3	85.5
var405	58.8	61.8	65.6	70.6	76.3	81.1	84.7	86.4
var406	62.4	64.6	67.9	72.6	77.8	83.5	85.9	88.2
var411	62.9	65.0	68.0	72.8	78.3	83.5	86.0	88.2

Table 4b

	pH							
Phytase	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5
C.b. wt SEQ ID 2	63.9	63.7	63.8	62.7	61.4	60.0	58.7	57.6
var300	76.5	76.2	76.2	75.9	74.8	73.8	73.3	72.3
var400	83.3	83.6	84.1	83.5	83.4	82.0	81.8	81.1
var404	86.9	87.1	87.5	87.6	87.2	86.7	86.1	85.6
var405	87.3	88.0	88.4	89.2	89.2	88.6	88.5	88.0
var406	89.6	90.3	90.5	90.5	91.6	91.4	90.7	89.7
var411	89.2	89.5	89.9	90.0	90.1	89.5	88.9	88.4

Conclusion: The variants have increased unfolding temperatures at all pH-values tested.

10 The variants have improved thermostability compared to the wild type at all pHs.

Example 6: pH-stability

The pH stability of the purified phytases of C.b. Wt (SEQ ID NO: 2) and var400 (SEQ ID NO: 12) at 37°C was determined by measuring residual phytase activity after incubation at 37°C and at various pH values for 1.0 and 24 hours. The phytases were incubated in 0.1M glycine, 0.1M acetic acid, 0.1M Bis-Tris, adjusted to the desired pH. Samples of the respective incubation mixtures were withdrawn after 0, 1.0 and 24 hours, the pH of the samples was adjusted to 5.5 by dilution in 0.25M sodium acetate, 0.005% (w/v) Tween20, pH5.5), and the residual activity at pH5.5 was determined using the method described in Example 1. The results, normalized to the activity found at 0 hours, are shown for 24 hours in Table 5 below.

Table 5. pH stability at 37°C

pH	C.b. Wt (SEQ ID NO: 2)	var400 (SEQ ID NO: 12)
24 hours		
1.0	1	64
2.0	12	96
3.0	61	101
4.0	99	102
5.0	97	105
6.0	101	94
7.0	105	97
8.0	90	103

Var400 has higher residual activity after incubation at very low pH (pH 1.0-3.0) after 24 hours incubation.

Example 7: pH-stability in the presence of pepsin

- 5 The pH stability of the purified phytases of C.b. Wt (SEQ ID NO: 2) and variants (see tables below) at 37°C and in the presence of pepsin was determined by measuring residual phytase activity after incubation at 37°C and at various pH values for 30 and 60 minutes, respectively. The phytases were incubated in 0.1M glycine, 0.1M acetic acid, 0.1M Bis-Tris, adjusted to the desired pH and added 500 U/ml of pepsin (Sigma P7000). Samples of the
- 10 respective incubation mixtures were withdrawn after 0, 30 minutes and 60 minutes, the pH of the samples was adjusted to 5.5 by dilution in 0.25M sodium acetate, 0.005% (w/v) Tween20, pH5.5), and the residual activity at pH5.5 was determined using the method described in Example 1. The results, normalized to the activity found at 0 hours, are shown in Tables 6 and 7 below.

Table 6. Residual activity after 30 minutes incubation.

	pH							
Phytase	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
var300	3	3	24	62	78	86	88	93
var400	28	48	81	93	89	93	96	97
var404	77	87	95	97	98	93	100	95
var405	84	91	94	96	96	100	98	102
var406	89	90	95	100	101	97	96	96
var411	94	93	91	92	91	97	96	93

Table 7. Residual activity after 60 minutes incubation.

	pH							
Phytase	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
var300	5	4	13	59	77	88	92	97
var400	14	36	73	88	81	86	92	99
var404	67	81	90	90	93	87	98	95
var405	78	83	88	90	93	99	95	101
var406	88	86	90	98	92	95	94	94
var411	92	90	86	88	89	92	94	93

The variants have increased pH-stability in the presence of pepsin at pH-values below 3.0 compared to the var300 reference.

Example 8: Efficacy Study of a phytase in Broiler Chickens and Weaned Piglets

5 In the broiler study, birds (Cobb 500, male, commercially available from Yukou Poultry Husbandry Co., Ltd., Beijing, China) were housed in wire-floored battery cages in an environmentally controlled room. At the start of trial (day 8 of age), birds were sorted by weight and divided into replicate groups, each comprising 8 birds. The birds with similar cage weight were randomly allocated to one of the different treatments, and each treatment was replicated
10 with 12 cages. There were 8 dietary treatments consisting of a negative control and the negative control supplemented with 7 levels of the phytase variant (var400): 187.5, 375, 750, 1125, 1500, 1875 or 2250 FYT/kg. A basal diet was prepared with corn and soybean meal as the main ingredients, and was formulated to be deficient only in total P (0.46%). The phytase variant was pre-mixed with a small amount of the basal diet before the complete mixing of the experimental
15 diets to ensure uniformity of mixing. The feed was pelleted at 75°C. The analyzed phytase variant activities of the dietary treatments were 182, 328, 640, 1060, 1347, 1560 and 1931 FYT/kg.

The experimental diets were supplied to birds from day 8 to 18 of age, feed and water were supplied *ad libitum* through the whole trial. At day 8 and 17 of age, feed consumption and body weight (BW) by cage were recorded to calculate the body weight gain (WG), feed intake (FI)
20 and the feed conversion ratio (FCR). Excreta were collected on day 14 through day 17. During this period, the excreta from 12 cages of each treatment were quantitatively collected once per day, and the excreta per cage from the 4 days were pooled together, frozen immediately at -20°C after collection. After thawing, the total excreta of each cage were homogenized, and the representative sub-samples were taken and freeze-dried for the determination of dry matter (DM),
25 P, Ca and phytate-P. The total amount of feed consumption during the excreta collection period was recorded as well. At day 18 of age, the right tibia was taken from 2 birds randomly chosen from each of the 12 replicate cages. Tibias were defleshed, and cartilaginous caps were removed after collection. They were kept frozen in plastic bags at -20°C to maintain wetness until analysis of ash, Ca and total P content.

30 In the piglet study, 140 castrated male piglets (Redon x Large White) were used. The piglets were weaned at 28 days of age and had an average body weight of 7.5 ± 1.1 kg (mean $\pm$ standard deviation) at the start of trial. The piglets were housed in 35 flat-deck cages with 4 animals per cage in an environmentally controlled room. Each cage had a plastic-coated welded wire floor and was equipped with two water nipples and two stainless-steel feeders. The
35 experimental diets were fed for 42 days which were divided into a starter phase of 14 days and a grower phase of 28 days. Water and feed were supplied *ad libitum*. The feed was offered in mash form.

There were 7 dietary treatments consisting of a positive control (PC), a negative control (NC) and the NC supplemented with 187.5, 375, 750, 1500 or 3000 FYT test phytase/kg feed (on analysis: 266, 383, 771, 1445, and 2914 FYT/kg in starter diets; 219, 395, 884, 1408, and 2730 FYT/kg in grower diets). The PC diets for the starter and grower phases met the pig's requirement for energy and nutrients prescribed by NRC (2012) for the body weight range of 7 to 11 kg and 11 to 25 kg, respectively, and were formulated with corn, soybean meal and rapeseed meal as the main ingredients. The NC diets were established by withdrawing the dicalcium phosphate from the PC diets resulting in P deficient diets (0.43% and 0.41% total P for starter and grower, respectively) with adequate Ca. The ingredient and nutrition compositions of diets are shown in

Table 8.

Table 8. ingredient and nutrition composition of experimental diets in broiler and piglet trials, %

Ingredient	Broiler trial	Piglet trial			
	8-18 day of age	Starter		Grower	
	NC	NC	PC	NC	PC
Corn	60.14	54.35	53.60	59.45	58.75
Soybean meal	33.50	24.00	24.00	20.00	20.00
Rapeseed meal	--	12.00	12.00	12.00	12.00
Soybean oil	2.50	4.50	4.70	3.70	3.90
NaCl	0.41	0.20	0.20	0.05	0.05
NaHCO ₃	0.14	0.35	0.35	0.35	0.35
DL-Met	0.58	0.10	0.10	0.05	0.05
L-Lys HCl	0.37	0.25	0.25	0.25	0.25
L-Thr	0.15	0.10	0.10	0.05	0.05
L-Val	--	0.15	0.15	0.10	0.10
Limestone	1.06	1.00	0.00	0.70	0.00
Dicalcium phosphate	0.55	0.00	1.55	0.00	1.20
Vit-Min Premix ⁽¹⁾	0.50	3.00	3.00	3.00	3.00
TiO ₂	0.10	0.00	0.00	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00
CP, %	21.5	20.0	19.9	18.6	18.5
ME, kcal/kg	3066	3404	3396	3374	3367
Dig Lys, %	1.25	1.35	1.35	1.26	1.26
Dig Met, %	0.85	0.43	0.43	0.37	0.37
Dig Thr, %	0.83	0.83	0.83	0.74	0.74
Digestible P, %	--	0.17	0.40	0.16	0.34
Total P, %	0.46	0.43	0.72	0.41	0.64
Phytate P, %	0.24	0.28	0.28	0.27	0.27
Calcium, %	0.70	0.83	0.84	0.71	0.74

⁽¹⁾ Broiler premix supplied per kg of diet: vitamin A, 10,000 IU; vitamin D₃, 2,240 IU; 25-OH-D₃, 69 µg; vitamin E, 50 IU; vitamin K₃, 3 mg; vitamin B₁, 2 mg; vitamin B₂, 7 mg; vitamin B₆, 4 mg; vitamin B₁₂, 20 µg; biotin, 250 µg; folic acid, 2 mg; niacin, 60 mg; D-pantothenic acid, 12 mg; Fe, 40 mg; Cu, 15 mg; Mn, 110 mg; Zn, 90 mg; I, 0.5 mg; Se, 0.25 mg; and choline, 400 mg.

Piglet premix supplied per kg of diet: vitamin A, 15,000 IU; vitamin D₃, 1,998 IU; vitamin E, 100 IU; vitamin K₃, 20 mg; vitamin B₁, 3.0 mg; vitamin B₂, 10 mg; vitamin B₆, 6 mg; vitamin B₁₂, 40 µg; biotin, 200

µg; D-pantothenic acid, 25 mg; folic acid, 1.5 mg; niacin, 35 mg; vitamin C, 100 mg; Cu, 160 mg; I, 2.0 mg; Fe, 200 mg; Mn, 60 mg; Zn, 100 mg; Se, 400 µg; choline, 375 mg; sodium, 1.5 g; chlorine, 3.2 g; Ca, 2.8 g; lysine, 2.9 g; methionine, 0.5 g; threonine, 1.4 g; tryptophan, 0.3 g; and valine, 0.2 g.

The pigs were weighed individually and feed consumption was recorded for each pen to calculate average daily gain (ADG), average daily feed intake (ADFI) and FCR. At the end of the trial, 2 pigs of each pen with body weight closest to the average body weight of their pen were slaughtered for collection of femurs. The right femurs were separated and removed of the soft tissue. A diaphysis section (~3.5 cm in length) of each femur was obtained by sawing and then subjected to compression to determine the force in Newton to break the bone. The broken bones were used for the determination of ash, Ca and P content.

The samples of diets and excreta collected from broiler and pig trials were ground to pass through a 0.5-mm screen before analysis. All samples were analyzed in duplicate. The samples were dried at 105 °C in an oven for 4 hours for dry matter determination (method 934.01; AOAC International, 2006). Ca and P were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; 5100 Dual View, Agilent, Santa Clara, CA, USA; method 985.01; AOAC International, 2006) after sulfuric acid mineralization. Dietary phytate P was calculated as the difference between total P and free P. Total P was determined after treating the dietary samples with megadose of phytase to release the P bound by phytate. The free P, not bound by phytate, was determined after overnight extraction in 0.66 M HCl. Phytase activity was measured by a colorimetric method. One phytase unit was defined as the amount of enzyme that releases 1 µmol of inorganic phosphate from 5.0 mM phytate per minute at 37°C and pH 5.5.

In both broiler and pig trials, the data were analyzed by one-way ANOVA using GLM procedure of SAS (SAS Inc., Gary, NC, USA, version 9.0). Orthogonal contrasts were constructed to test the linear and quadratic effects of phytase variant supplementation to the NC diet. Tukey's multiple comparison test was also applied in broiler trial. The least square means are presented.

Result

In the broiler study, the body weight gain and feed consumption of birds in NC was 10~15% lower than Cobb 500 performance targets during day 8 to 17 of age, which was attributed to the P deficiency in NC diet. The increasing addition of the phytase variant to the NC diet improved the 8-17 d body weight gain and feed intake of birds both linearly and quadratically ($p < 0.01$). The achievement of performance targets was observed in treatments with high doses of phytase variant while no adverse effect was noted. Additionally, the data from excreta demonstrated that the P release from phytate-P degradation, and the corresponding retention of P and Ca were improved both linearly and quadratically ($p < 0.01$) with the increase of phytase variant supplementation. Consistent with the performance and excreta results, Ca and P were increasingly deposited in the bone ash in a dose-dependent manner with the increase of phytase

variant addition.

In the piglet study, the growth performance of piglets was depressed by deficiency of P in feed as demonstrated by the significant reduction in ADG and ADFI of the NC piglets in comparison to PC during day 14 to 42 and the overall trial duration, which was gradually corrected by the increasing addition of the phytase variant. The test phytase improved ADG and ADFI during day 14 to 42 and the overall trial duration both linearly and quadratically ($p < 0.01$) with the increasing dose of phytase variant. This pattern was also observed with the average body weight of the piglets at the end of the trial. In keeping with the growth performance results, the significant improvement ($p < 0.01$) in bone strength and bone content of ash, Ca and P in association with added phytase variant also showed a dose-response relationship. Moreover, the growth performance and bone measurements achieved at 3,000 FYT/kg feed, the highest dose of phytase variant tested in the current study, exceeded the levels of the PC without causing any noticeable adverse effect during the trial. This showed that both the Ca and P supplied in the form of dicalcium phosphate in the PC diets could be completely replaced by phytase variant when included at 3,000 FYT/kg feed.

Table 9. growth performance, retention of Ca (%) and P (%), phytate-P degradation (%) in excreta and bone parameters of broilers.

	Phytase variant treatments, FYT/kg feed								SEM	P-value	Linear	Quadratic
Items	NC	187.5	375	750	1125	1500	1875	2250				
Body weight, g												
D 8	193	192	192	192	192	192	192	193	0.3	0.08	0.68	0.11
D 17	652 ^a	687 ^b	691 ^b	710 ^{bc}	716 ^{bc}	710 ^{bc}	724 ^c	716 ^{bc}	7.3	< 0.01	< 0.01	< 0.01
D8 to 17												
WG, g	460 ^a	495 ^b	499 ^b	518 ^{bc}	524 ^{bc}	518 ^{bc}	532 ^c	523 ^{bc}	7.3	< 0.01	< 0.01	< 0.01
FI, g	572 ^a	600 ^{ab}	609 ^{ab}	618 ^b	621 ^b	608 ^{ab}	640 ^b	622 ^b	10.0	< 0.01	< 0.01	0.05
FCR, g/g	1.244	1.215	1.220	1.195	1.187	1.174	1.205	1.190	0.017	0.10	0.01	0.06
Excreta												
Ca retention	55.08 ^a	63.00 ^b	66.03 ^b	73.14 ^c	74.64 ^c	75.70 ^c	73.43 ^c	73.19 ^c	0.84	< 0.01	< 0.01	< 0.01
P retention	64.41 ^a	71.46 ^b	75.63 ^c	80.52 ^d	82.48 ^d	83.31 ^d	81.95 ^d	81.87 ^d	0.65	< 0.01	< 0.01	< 0.01
Phytate-P degradation	64.01 ^a	77.03 ^b	82.99 ^c	89.17 ^d	92.88 ^{de}	94.38 ^e	91.86 ^{de}	94.56 ^e	0.97	< 0.01	< 0.01	< 0.01
Bone												
Ash, %	46.80 ^a	50.14 ^b	51.22 ^{bc}	52.36 ^{cd}	52.93 ^{cde}	53.56 ^{de}	54.67 ^e	53.74 ^{de}	0.46	< 0.01	< 0.01	< 0.01
Ca, %	17.10 ^a	18.26 ^b	18.57 ^{bc}	19.07 ^{bcd}	19.22 ^{cd}	19.38 ^{cd}	19.96 ^d	19.38 ^{cd}	0.20	< 0.01	< 0.01	< 0.01
P, %	8.05 ^a	8.61 ^b	8.91 ^{bc}	9.22 ^{cd}	9.38 ^{de}	9.44 ^{de}	9.67 ^e	9.39 ^{de}	0.09	< 0.01	< 0.01	< 0.01

Means with different superscript within the same row differ significantly ($P < 0.05$).

Table 10. growth performance and bone parameters of the piglets

			Phytase variant treatments, FYT/kg feed							<i>P</i> - value	
Items	PC	NC	187.5	375	750	1500	3000	SEM	PC vs. NC	Linear	Quadratic
Body weight, kg											
D 0	7.4	7.5	7.5	7.4	7.4	7.4	7.5	0.05	0.23	0.81	0.34
D 14	10.9	10.1	10.6	10.0	10.8	10.5	11.1	0.33	0.13	0.05	0.94
D 42	25.9	19.6	22.5	22.6	25.3	25.7	27.0	0.70	< 0.01	< 0.01	< 0.01
D 0 to 14											
ADG, g/d/pig	247	181	221	184	238	221	258	24	0.06	0.04	0.74
ADFI, g/d/pig	369	324	351	294	384	337	373	26	0.24	0.19	0.86
FCR, g/g	1.52	1.82	1.61	1.64	1.65	1.53	1.44	0.08	0.02	0.01	0.44
D 14 to 42											
ADG, g/d/pig	537	338	425	448	521	541	570	19	< 0.01	< 0.01	< 0.01
ADFI, g/d/pig	721	432	564	578	707	627	717	25	< 0.01	< 0.01	< 0.01
FCR, g/g	1.34	1.27	1.32	1.28	1.36	1.16	1.27	0.06	0.41	0.41	0.43
D 0 to 42											
ADG, g/d/pig	440	286	357	360	426	434	466	17	< 0.01	< 0.01	< 0.01
ADFI, g/d/pig	603	396	493	483	599	530	602	21	< 0.01	< 0.01	< 0.01
FCR, g/g	1.40	1.45	1.42	1.40	1.46	1.28	1.33	0.05	0.48	0.05	0.33
Bone, %											
Strength, N	366	95	171	229	275	344	417	28	< 0.01	< 0.01	< 0.01
Ash	63.1	56.8	59.9	60.9	60.4	61.8	63.6	0.77	< 0.01	< 0.01	0.09
Ca	23.6	21.4	22.7	23.0	22.6	23.3	24.0	0.31	< 0.01	< 0.01	0.12
P	11.0	9.6	10.2	10.4	10.4	10.9	11.3	0.15	< 0.01	< 0.01	0.02

8 replicates for each treatment.

Example 9: Apparent total tract digestibility of calcium and phosphorus in late-gestation and lactating sows supplemented with a phytase variant

- 5 The objective of this study was to evaluate a phytase variant (var400) in late-gestation and lactating sows with a genetic background of Large white, Landrace and Duroc and fed diets formulated using the National Research Council (NRC) (2012) suggested digestible P for feed ingredients. Forty-five late-gestation sows and 45 lactating sows were used in Examples 1 and 2, respectively, in a completely randomized design. The sows were provided with a control diet

(Table 11) and the control diet added with 187.5 or 375 FYT phytase /kg feed. The diets were devoid of any inorganic P supplement and included 3 g/kg TiO₂ as an indigestible marker. Each dietary treatment was replicated with 15 sows individually-housed in farrowing stalls. The sows were allowed to adapt to the experimental diets for 5 days before a 5-d fecal collection by grab sampling. Digestibility of dry matter, Ca and P were calculated using the following equations:

$$D_d = [1 - (T_i/T_o)] \times 100;$$

$$D_m = [1 - (T_i/T_o) \times (M_o/M_i)] \times 100;$$

where D_d and D_m are the digestibility of DM and minerals (%), respectively; T_i and T_o are the titanium concentration in diet and feces, respectively (% of DM); M_i and M_o are the concentrations of minerals in diet and feces (% of DM), respectively. The digested Ca and P were calculated by multiplying the concentration of Ca and P in feed (%) by their corresponding D_m .

The digestibility and performance data were analyzed by a GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with the model including the dietary treatment as the only fixed effect and error term. Orthogonal contrasts were constructed to test the linear and quadratic effects of supplementation of phytase and to compare control with the treatments with added phytase.

The results are shown in Table 12. The phytase released 0.07-0.12% digestible P for sows depending on the phytase dose and physiological stage of sows (more P release for lactating sows).

Table 11. Ingredient and nutrient composition of the basal diets (g/kg of feed, as-is basis)

Items	Gestation	Lactation
Ingredients		
Corn	680.0	620.0
Soybean meal	80.0	140.0
Wheat bran ^a	200.0	120.0
Full-fat soybean	0.0	50.0
Soybean oil	0.0	32.0
NaCl	2.0	3.0
NaHCO ₃	3.5	3.5
L-Lys·HCl	4.0	3.5
DL-Met	1.0	0.5
L-Thr	1.5	1.5
L-Val	0.5	1.5
L-Trp	0.5	0.5
Limestone	19.0	15.5
Vitamin-mineral premix ^b	5.0	5.0
Stay C, 35% vitamin C	0.0	0.5
TiO ₂	3.0	3.0
Total	1000.0	1000.0
Calculated nutrients and energy		
ME, MJ/kg	12.7	13.9

Crude protein	12.8	15.6
Total Ca	8.4	7.3
Total P	4.3	4.0
Phytate P	3.5	3.0
Apparent total tract digestible P	1.6	1.4
Standardized total tract digestible P	2.0	1.8
Standardized ileal digestible		
Lysine	7.1	8.6
Methionine	2.8	2.6
Threonine	4.9	5.9
Tryptophan	1.6	1.9
Valine	4.9	7.0

^a Wheat bran was pelleted at 95°C before the mixing of diet.

^b Premix supplied per kilogram of diet: vitamin A, 12,000 IU; vitamin D₃, 2,000 IU; vitamin E, 115 mg; vitamin K₃, 5.0 mg; vitamin B₁, 2.0 mg; vitamin B₂, 6 mg; vitamin B₆, 3.0 mg; vitamin B₁₂, 28 µg; D-biotin, 700 µg; pantothenic acid, 20 mg; folic acid, 5 mg; niacin, 30 mg; Cu (tribasic copper chloride), 20 mg; I (potassium iodate), 0.45 mg; Fe (ferrous sulfate), 120 mg; Mn (manganese sulfate), 60 mg; Zn (zinc sulfate), 100 mg; Se (sodium selenite), 400 µg; choline (choline chloride), 600 mg; and Ca (calcium carbonate) 0.5 g.

Table 12. Apparent total-tract digestibility of calcium (Ca) and phosphorus (P) and digested Ca and P in feed for gestating and lactating sows, %^a

Items	Treatments, FYT/kg feed			SEM ^b	Significance level		
	Control	187.5	375.0		Phytase	Linear	Quadratic
Gestation							
Dry matter	82.7	81.9	82.1	0.336	0.121	0.254	0.253
Calcium	28.7	32.2	33.9	1.255	0.007	0.006	0.548
Phosphorus	15.3	28.1	33.8	1.071	< 0.001	< 0.001	0.011
Digested Ca	0.23	0.28	0.29	0.011	< 0.001	< 0.001	0.293
Digested P	0.07	0.14	0.17	0.005	< 0.001	< 0.001	0.006
Digested Ca/digested P	3.22	2.02	1.78	0.112	< 0.001	< 0.001	0.001
Lactation							
Dry matter	86.7	86.4	86.3	0.294	0.404	0.384	0.870
Calcium	33.9	36.4	40.7	1.486	0.016	0.003	0.603
Phosphorus	23.6	43.9	50.2	0.903	< 0.001	< 0.001	< 0.001
Digested Ca	0.24	0.25	0.29	0.010	0.046	0.007	0.414
Digested P	0.10	0.19	0.22	0.004	< 0.001	< 0.001	< 0.001
Digested Ca/P	2.43	1.32	1.28	0.045	< 0.001	< 0.001	< 0.001
	Reproductive stage	Treatment	Interaction				

Dry matter	< 0.001	0.222	0.716
Calcium	< 0.001	< 0.001	0.622
Phosphorus	< 0.001	< 0.001	< 0.001
Digested Ca/digested P	0.514	< 0.001	0.313
Digested P	< 0.001	< 0.001	< 0.001
Digested Ca/P	< 0.001	< 0.001	0.222

^a Each least square mean represents 15 observations.

^b SEM: standard error of mean.

Example 10: Phytase efficacy in rainbow trout (*Oncorhynchus mykiss*)

5 *Experimental Diets*

Experimental diets were prepared in the experimental feed mill of the Research Centre for Animal Nutrition & Health, DSM Nutritional Products in Village-Neuf (France) according to the formulation detailed in Table 13. Diets were prepared in mash and produced as extruded pellets using a Bühler twin screw extruder. After extrusion, pellets were coated with a mixture of oils heated at 40°C and phytase (var400). Experimental diets were kept at 4°C during the feeding trial.

Theoretical calculations were 0.821 % for total phosphorus, 0.326 % for phytate phosphorus and 11.5 g/Kg phytic acid (according to Allix 3 software, A-systems, France).

Fish and rearing facilities

Two hundred and forty monosex (all female) Rainbow trout (IBW= 46.3 ± 1.2g) were randomly distributed in twelve tanks (250L; twenty fish per tank).

Table 13. Detailed composition (%) of the basal diet and calculated crude protein/lipid in the diet

Ingredient	Experimental diet
Fish meal	13.20
Soybean meal	8.00
SPC	17.00
Rapeseed meal	7.00
Sunflower meal	8.00
Wheat	11.00
Wheat gluten	12.00
Fish oil	11.00
Rapeseed oil	9.90
Choline chloride 60%	0.4
DSM OVM VMF	0.5
L-Lysine	0.5
L-Methionine	0.2
Yttrium oxide	200 ppm
Guar gum	0.3

Calc. crude protein	40.0
Calc. crude lipid	24.0

Feeding

Animals were fed for 91 days. Trout were fed by hand twice a day (morning and afternoon) during the week and by automatic feeders during weekends. All fish were fed according to feeding ration table for a similar commercial diet fed to fish maintained at the same water temperature. Feed consumption and body weight thorough the experiment are represented in Figure 2. Feed consumption is calculated as percentage, based on the actual intake per week and the growth of the fish (specific growth rate) during the 2-week period. Feed consumption ranged from 2.31 % to 1.65 % throughout the experimental feeding (Figure 2).

Zotechnical parameters

Fish were individually weighed at the beginning of the trial and the mean body weight of the fish per triplicate tanks was determined after bulk weighing of the fish at each time point considered. Bulk weight was recorded every two weeks. Before handling, fish were anesthetized (0.08 g/L tricaine methane sulfonate; MS222; PharmaQ Ltd., Overhalla, Norway).

The following zootechnical parameters were measured/ calculated:

- Survival (%)
- Performance
 - Initial body weight, IBW (g)
 - Final body weight, FBW (g)
 - Weight gain, WG as FBW – IBW (g)
 - Specific growth rate, SGR as $\{100 * (\ln (FBW/IBW))\} * d^{-1}$ (%BW d⁻¹)
- Feed utilization
- Feed conversion ratio, FCR as Feed Intake * biomass gain⁻¹ (as-fed basis)

Phosphorus whole body retention

At the beginning and at the end of the trial, whole fish were sampled and frozen at -80°C to analyze phosphorus whole body retention.

Plasma

At the end of the experimental feeding trial, five fish per tank (15 fish per treatment) were individually anaesthetized using 80 mg MS222·L⁻¹.

Four mL of blood were sampled using a lithium-heparin 4.5 mL syringe and kept in ice until further processing. Blood samples were centrifuged (10 min, 2000g, 4°C) and 1.5 mL plasma aliquots were frozen (-20°C) until analysis. Samples were analyzed individually.

Samples were analyzed individually but each tank was considered as a replicate for statistical purposes.

Apparent digestibility coefficient (ADC)

Two different faeces collection were done 77 and 91 days after the start of the

experimental feeding for the determination of phosphorus ADC. Animals were slightly anaesthetized (80 mg MS222·L⁻¹) and fecal material from all fish in each tank was collected by manually stripping faeces from the distal portion of the intestine by applying pressure to the abdominal cavity using three passes for each fish. Samples from both collection days were pooled and one sample per tank was lyophilized prior to storage and analysis.

The apparent digestibility coefficient (ADC) was calculated as outlined by the NRC (2001) on a dry matter basis:

$$\text{ADC (\%)} = 100 - [(\text{CMf/CMe}) \times (\text{CNe/CNf})] \times 100$$

CMf = concentration of marker in feed; CMe = concentration of marker in faeces;

CNf = concentration of nutrient in feed; CNe = concentration of nutrient in faeces

Phosphorus release represents the % of phosphorus released due to phytase supplementation. It is calculated as follows:

$$(\% \text{ total P of the feed} \times \text{Phosphorus ADCPHY X}) - (\% \text{ total P of the feed} \times \text{Phosphorus ADCtest})$$

Where % total P is the total percentage of phosphorus in the diet; ADCPHY X is the digestibility of the phosphorus at a given phytase dose; and Phosphorus ADCtest is the phosphorus digestibility of the test diet without phytase supplementation.

Bone phosphorus retention

At the beginning and at the end of the trial, a 3-4 cm slide (see figure 3) was sampled in five fish per tank (15 fish per treatment) and frozen at -80°C. Skin and flesh will be removed to further process only the bones for analysis.

Analyses and calculations

The analyses of the nutrient content in the feed, excreta, plasma, whole fish and vertebra samples were performed according to standard methods (VDLUFA 1976; AOAC, 2006).

Phytate-P in feed were measured by enzyme laboratory DNP R&D Solution Center (Kaiseraugst) with an enzymatic method using ammonium molybdate by calculation of the difference between the total P and free P, according to Zhai et al., 2001.

The crude protein was determined by a nitrogen analyzer (FP 528, LECO, St. Joseph, USA) using the Dumas method ($\text{CP} = \text{N} \times 6.25$).

Gross energy measurements were performed using an adiabatic bomb calorimeter (C 2000 basic, IKA, Staufen, Germany).

Calcium, Phosphorus, Zinc and Yttrium oxide concentrations in feed, whole fish, vertebra and excreta were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES, 5100 Dual View, Agilent) according to DIN EN ISO 11885:1997 (DIN EN ISO 1998; AOAC, 2006) after sulfuric acid mineralization. Water analysis of phosphorus were performed on the same instrument on frozen water samples by direct injection.

The plasma concentrations of P concentration were determined by the mean of a

Biomedical automate COBAS 6000 (Roche Diagnostics, CH-4202 Basel) using the respective Roche Diagnostic kits.

The lipid analyses of feed were performed at the external company Larebron by hot acidic etching with hydrochloric acid, followed by continuous extraction with petroleum ether and determination with a gravimetric method.

Phytase analysis in the diets was done at BioPract GmbH, Germany, according to ISO 30024:2009 (Animal feeding stuffs — Determination of phytase activity, <https://www.iso.org/standard/45787.html>).

Result

Table 14 shows the analytical results obtained at BioPract GmbH.

Table 14. Phytase content in the eight experimental treatments. LOD = below level of detection.

Treatment ID	Dose U/Kg (theoretical)	Pellet Ø (mm)	Dose U/Kg (analytical)
P	Control -0	3	LOD
Q	phytase 1000 FYT/kg	3	955
R	phytase 2000 FYT/kg	3	1740
S	phytase 3000 FYT/kg	3	2477
P	Control -0	4	LOD
Q	phytase 1000 FYT/kg	4	977
R	phytase 2000 FYT/kg	4	1719
S	phytase 3000 FYT/kg	4	2895

Fish behavior and water quality

No mortality was recorded through the whole experiment and fish accepted well all the experimental diets.

Performance

Table 15 shows the zootechnical parameters at the end of the 91-day experimental feeding period. Control diet showed the poorest growth rate. Supplementation with phytase had a positive impact in growth. This trend was dose-related and supplementation in 1000 FYT/Kg group growth was significantly higher than the control group; similarly, animals with 2000 and 3000 FYT/Kg enzyme supplementation perform significantly better than the 1000 FYT/Kg.

Specific growth rate and feed conversion ratio were also significantly improved with any inclusion level of phytase but independently from the inclusion level.

Table 15. Survival and growth performance of fish (day 0 to day 91).

ID	Dose	Survival	IBW	FBW	SGR	FCR
P	Control -0	100 ± 0	46.3 ± 0.0	334.7 ± 7.0 c	2.17 ± 0.02 b	0.87 ± 0.0 a
Q	phytase 1000 FYT/kg	100 ± 0	46.3 ± 0.0	357.2 ± 10.1 b	2.25 ± 0.03 a	0.83 ± 0.01 b
R	phytase 2000 FYT/kg	100 ± 0	46.3 ± 0.0	374.4 ± 3.0 a	2.30 ± 0.01 a	0.82 ± 0.01 b
S	phytase 3000 FYT/kg	98.3 ± 2.9	46.3 ± 0.0	376.0 ± 9.7 a	2.30 ± 0.03 a	0.81 ± 0.01 b
	sign	ns	ns	p = 0.0006	p = 0.0006	P=0.0004

Survival percentages referred to the whole duration of the trial; IBW: Initial Body Weight (g); FBW: Final Body Weight (g); SGR: specific growth rate (%BW/day); FCR: feed conversion ratio (as-fed basis). Data are presented as mean ± SD (Standard Deviation). Sign. = significance; Ns: no significant differences were observed between treatments.

5 Whole body phosphorus retention

Table 16 shows the whole body retention (% of intake) for minerals and protein. Significant improvement in whole body zinc and protein retention at any supplementation dose. Phosphorus was significantly improved when phytase was supplemented at 1000 and 3000 FYT/Kg. Surprisingly, phytase inclusion at 2000 FYT/Kg showed an intermediate response.

10 Table 16. Phosphorus, zinc, calcium and protein whole body retention (% of intake; day 0 to day 91).

ID	Dose	Phosphorus	Zinc	Calcium	Protein
P	Control -0	1.64 ± 0.29 b	0.60 ± 0.04 b	0.52 ± 0.12	2.22 ± 0.08 b
Q	phytase 1000 FYT/kg	2.63 ± 0.56 a	1.25 ± 0.25 a	1.68 ± 0.78	2.65 ± 0.32 a
R	phytase 2000 FYT/kg	2.27 ± 0.37 ab	1.15 ± 0.24 a	1.34 ± 0.38	2.48 ± 0.02 ab
S	phytase 3000 FYT/kg	2.60 ± 0.17 a	1.28 ± 0.10 a	1.48 ± 0.30	2.57 ± 0.03 ab
	Sign	p=0.0398	p=0.0055	Ns p = 0.0640	Ns p = 0.0526

Data are presented as mean ± SD (Standard Deviation; n=3). Sign. = significance; Ns: no significant differences were observed between treatments.

Phosphorus content in plasma

15 Plasma phosphorus concentration was significantly increased with the supplementation of phytase. The supplementation of the exogenous enzyme increased the circulating phosphorus 1.99, 2.3 and 2.44 fold when compared to control diet.

Apparent digestibility coefficient (ADC)

20 The faecal digestibility for dry matter, protein, energy and minerals (phosphorus, calcium and zinc) is summarized in the Table 17.

Data show that the supplementation of phytase from 1000 FYT/kg significantly improved the faecal dry matter, energy digestibility as well as the faecal calcium, phosphorus and zinc digestibility. Phosphorus digestibility was increased by 60.1, 82.9 and 89.7% for phytase 1000 FYT/kg, phytase 2000 FYT/kg and phytase 3000 FYT/kg, respectively, when compared to the non-supplemented control group. Accordingly, based on the above results, an aspect of the invention is directed to method of feeding fish comprising adding the phytase, as defined herein,

such as in an amount of 500 to 5000 FYT/kg. In typical embodiments, the amount of phytase is from 500 to 3000 FYT/kg, such as 750 to 3000 FYT/kg, such as 1000 to 3000 FYT/kg.

Table 17. Apparent digestibility coefficient (%) and phosphorus release (Phos. Rel.; % phosphorus / kg feed).

5

ID	Dose	Dry matter	Protein	Energy	Phosphorus	Calcium	Zinc	Phos. Rel.
P	Control -0	69.8 ± 0.18 c	89.6 ± 0.08 b	78.9 ± 0.13c	32.1 ± 1.06d	-0.6 ± 2.03 c	20.0 ± 0.97c	
Q	phytase 1000 FYT/kg	71.3 ±0.55 b	90.0 ± 0.19 b	79.6 ± 0.31 b	51.4 ± 0.62c	5.2 ± 1.46 b	28.6 ± 0.65b	0.14
R	phytase 2000 FYT/kg	71.4 ± 0.16b	90.0 ± 0.21b	79.5 ± 0.07b	58.7 ± 0.31b	7.3 ± 1.26ba	32.0 ± 1.90a	0.20
S	phytase 3000 FYT/kg	72.6 ±0.30a	90.6 ± 0.20 a	80.2 ± 0.30 a	60.9 ± 1.89 a	10.2 ± 1.89a	34.0 ± 2.09 a	0.21
	Sign	p=0.0001	p=0.0013	p = 0.0006	p < 0.0001	p = 0.0003	p < 0.0001	

Newman-Keuls test: Means within a row, not sharing a common superscript, are significantly different (p<0.05). Digestibility were calculated with n=3 tanks per treatment.

10 Bone phosphorus retention

Retention of phosphorus is summarized in Table 18. In the vertebrae, percentage of ash and the concentration of phosphorus increased significantly with phytase incorporation when compared to the negative control.

Table 18. Retention of ash and phosphorus in vertebrae of fish (% of DM) after 91 days of experimental feeding.

15

ID	Dose	Ash	Phosphorus
P	Control -0	21.2 ± 3.4b	3.77 ± 0.66b
Q	phytase 1000 FYT/kg	28.6 ± 1.4a	5.16 ± 0.26a
R	phytase 2000 FYT/kg	28.6 ± 3.0 a	5.17 ± 0.58a
S	phytase 3000 FYT/kg	28.9 ± 1.0 a	5.26 ± 0.19a
	sign	p = 0.0116	p = 0.013

Newman-Keuls test: Means within a row, not sharing a common superscript, are significantly different (p<0.05). Digestibility were calculated with n=3 tanks per treatment.

Conclusion

20

The supplementation of the reference diet (0.74 total phosphorus) with the phytase at 1000, 2000 and 3000 FYT/kg showed a dose-dependent and significant increase in growth, plasma phosphorus and plasma apparent digestibility after 91 days of experimental feeding. The inclusion of phytase at any level increased the retention of phosphorus in whole body and

vertebrae of the animals.

The phytase of the invention is efficient in releasing phosphorus from phytate and the invention is directed in part to the use of the phytase when fish meal in the diets of salmonid fish is replaced by vegetable raw materials rich in this antinutritional factor.

5

Example 11: Effect of graded supplemental levels of phytase on the growth performance, whole-body nutrient retention and nutrient digestibility in European seabass (*Dicentrarchus labrax*) fed a plant-protein rich diet

The trial comprised five dietary treatments: a control diet (CTRL) with a total dietary phosphorus (P) level of 0.7%, in which a significant fraction of P was present in the form of phytate-bound P (0.3%). Three other diets, based on the CTRL formulation, were supplemented with phytase (var400) at graded doses (500, 1000 and 2000 FTY/kg feed) (diets PHY500, PHY1000, PHY2000). Phytase was applied post-extrusion by coating. A fifth diet (MCP), also based on the CTRL formulation, was supplemented with monocalcium phosphate, to a total P level of 0.9% and an available P level higher than the known requirements of the species. All diets were isonitrogenous, isolipidic and isoenergetic. Quadruplicate groups of 38 European seabass, with a mean initial body weight of 57.6 ± 3.8 g were fed one of the five experimental diets during 94 days, with a water temperature profile of $22.1 \pm 0.4^\circ\text{C}$.

Table 19. Formulation of experimental diets and measured phytase activity

Ingredients, %	CTRL	PHY500	PHY1000	PHY2000	MCP
Fishmeal Super Prime	7.50	7.50	7.50	7.50	7.50
Fish protein hydrolysate	2.50	2.50	2.50	2.50	2.50
Wheat gluten	10.10	10.10	10.10	10.10	10.50
Corn gluten meal	20.00	20.00	20.00	20.00	20.00
Soybean meal	24.00	24.00	24.00	24.00	24.00
Rapeseed meal	12.00	12.00	12.00	12.00	12.00
Sunflower meal	5.00	5.00	5.00	5.00	5.00
Wheat bran	3.03	3.03	3.03	3.03	1.43
Fish oil	5.00	5.00	5.00	5.00	5.00
Rapeseed oil	9.60	9.60	9.60	9.60	9.60
Vitamin and mineral premix	1.00	1.00	1.00	1.00	1.00
Antioxidant	0.20	0.20	0.20	0.20	0.20
Monocalcium phosphate					1.20
L-Tryptophan	0.02	0.02	0.02	0.02	0.02
DL-Methionine	0.03	0.03	0.03	0.03	0.03
Yttrium oxide	0.02	0.02	0.02	0.02	0.02
phytase (FTY/kg)		500	1000	2000	

Result

At the end of the trial, the final body weight (FBW) ranged between 142.0 and 156.5 grams. Fish from the best performing treatment (PHY2000) showed a 2.7-fold increase of initial body weight (IBW). At day 94, all supplemented diets resulted on a significantly higher FBW and SGR than those fed the CTRL diet ($P < 0.05$). Fish fed the PHY500, PHY1000, PHY2000 and MCP diets showed a significantly lower FCR than those fed the CTRL diet ($P < 0.05$). The CTRL treatment was consistently associated to significantly lowest values among the various treatments for whole-body phosphorus retention and apparent digestibility of phosphorus. The higher phytase supplementation doses (1000 and 2000 FTY/kg) led to a significant increase of whole-body P content ($P < 0.05$). Fish fed the MCP diet showed a significantly higher whole-body P retention than those fed the CTRL diet, although significantly lower than those fed the PHY1000 and PHY2000 diets. Significant enhancements of total P and phytate-P digestibility were associated to increasing dietary doses of phytase, with dietary treatment PHY2000 having a significantly higher P digestibility than PHY1000, and the later having a significantly higher P digestibility than PHY500. Additionally, the increase of phytase supplementation doses (0, 500, 1000 and 2000 FTY/kg) was also positively associated to a stepwise increase of whole-body phosphorus retention. Accordingly, based on the above results, an aspect of the invention is directed to method of feeding fish comprising adding the phytase, as defined herein, such as in an amount of 250 to 5000 FTY/kg. In typical embodiments, the amount of phytase is from 300 to 4000 FTY/kg, such as 500 to 3000 FTY/kg.

Table 20. Growth performance after 94 days of feeding (end of the trial).

	CTRL	PHY500	PHY1000	PHY2000	MCP	P-value
Survival, %	99.3 ± 1.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	0.438
IBW, g	57.6 ± 0.1	57.5 ± 0.1	57.5 ± 0.1	57.6 ± 0.1	57.5 ± 0.1	0.572
FBW, g	142.0 ± 2.4 ^a	148.6 ± 1.6 ^b	153.0 ± 1.6 ^c	156.5 ± 2.6 ^c	152.5 ± 2.9 ^c	<0.001
SGR, %/d	0.96 ± 0.02 ^a	1.01 ± 0.01 ^b	1.04 ± 0.01 ^c	1.06 ± 0.02 ^c	1.04 ± 0.02 ^c	<0.001
FCR	1.35 ± 0.07 ^b	1.25 ± 0.03 ^a	1.24 ± 0.02 ^a	1.19 ± 0.01 ^a	1.24 ± 0.02 ^a	<0.001
FI, %ABW/d	1.21 ± 0.05	1.18 ± 0.03	1.19 ± 0.02	1.17 ± 0.02	1.19 ± 0.03	0.412
PER	1.62 ± 0.08 ^a	1.75 ± 0.05 ^b	1.77 ± 0.03 ^{bc}	1.83 ± 0.02 ^c	1.74 ± 0.03 ^b	<0.001

Values are means ± standard deviation (n=4).

Values within a row with different superscripts, denote a statistical difference ($P < 0.05$).

Table 21. Whole-body nutrient retention (% of intake).

	CTRL	PHY500	PHY1000	PHY2000	MCP	P-value
Protein, %	21.2 ± 2.1 ^a	23.3 ± 0.5 ^{ab}	23.6 ± 2.0 ^{ab}	24.6 ± 0.7 ^b	22.2 ± 0.4 ^{ab}	0.027
Fat, %	61.5 ± 2.1	67.7 ± 1.3	67.9 ± 3.7	66.4 ± 2.8	65.6 ± 5.7	0.118
Energy, %	31.5 ± 1.9 ^{ab}	34.3 ± 1.6 ^b	32.0 ± 1.8 ^{ab}	31.4 ± 0.7 ^{ab}	29.9 ± 1.0 ^a	0.013
Phosphorus, %	40.3 ± 1.3 ^a	44.9 ± 1.6 ^b	51.8 ± 1.5 ^c	62.0 ± 2.9 ^d	46.3 ± 3.3 ^b	<0.001
Calcium, %	36.9 ± 1.8 ^b	40.7 ± 4.4 ^b	47.0 ± 2.8 ^c	48.5 ± 3.6 ^c	30.1 ± 3.6 ^a	<0.001

Values are average $\pm$ standard deviation (n=4).

Values within a row with different superscripts, denote a statistical difference (P<0.05).

Table 22. Apparent digestibility coefficients (ADC, %) of nutrients.

	CTRL	PHY500	PHY1000	PHY2000	MCP	P-value
Dry matter, %	66.9 $\pm$ 0.3 ^b	70.4 $\pm$ 0.7 ^d	69.0 $\pm$ 1.2 ^{cd}	68.2 $\pm$ 1.1 ^{bc}	64.1 $\pm$ 1.4 ^a	<0.001
Protein, %	89.4 $\pm$ 0.1 ^a	91.2 $\pm$ 0.6 ^c	90.6 $\pm$ 0.3 ^b	90.2 $\pm$ 0.5 ^b	88.9 $\pm$ 0.3 ^a	<0.001
Phosphorus, %	33.5 $\pm$ 1.3 ^a	41.4 $\pm$ 3.0 ^b	50.1 $\pm$ 2.3 ^c	62.8 $\pm$ 0.7 ^d	52.8 $\pm$ 2.3 ^c	<0.001
Phytate-P, %	23.9 $\pm$ 0.8 ^b	33.5 $\pm$ 1.7 ^c	37.2 $\pm$ 3.0 ^c	42.4 $\pm$ 1.8 ^d	18.1 $\pm$ 4.4 ^a	<0.001
Calcium, %	37.8 $\pm$ 1.1 ^b	40.5 $\pm$ 0.7 ^b	38.5 $\pm$ 2.4 ^b	38.2 $\pm$ 3.6 ^b	29.8 $\pm$ 2.7 ^a	<0.001

Values are average $\pm$ standard deviation (n=4).

5 Values within a row with different superscripts, denote a statistical difference (P<0.05).

Conclusion

The phytase at supplementation doses of 500 to 5000 FTY/kg feed, such as 500 to 3000 FTY/kg, such as 500 to 2500 FTY/kg, such as 500 to 2000 FTY/kg such as 1000 to 2000 FTY/kg feed is an effective strategy to enhance the growth rate, phosphorus digestibility, whole-body phosphorus retention and reduce FCR in European seabass fed plant protein-rich diets.

Example 12: Efficacy of a phytase on the growth performance, whole-body nutrient retention and nutrient digestibility in Nile tilapia (*Oreochromis niloticus*) fed plant-protein rich diets

The trial comprised five dietary treatments: a control diet (CTRL) with a total dietary phosphorus (P) level of 0.96%, in which a significant fraction of P was present in the form of phytate-bound P (0.6%). Three other diets, based on the CTRL formulation, were supplemented with phytase (var400) at graded doses (500, 1000 and 2000 FTY/kg feed) (diets PHY500, PHY1000, PHY2000). Phytase was applied post-extrusion by coating. A fifth diet (DCP), also based on the CTRL formulation, was supplemented with dicalcium phosphate, to a total P level of 1.2% and an available P level higher than the known requirements of the species. All diets were isonitrogenous, isolipidic and isoenergetic. Quadruplicate groups of 30 tilapia, with a mean initial body weight of 39.5 $\pm$ 1.4 g were fed one of the five experimental diets during 93 days, with a water temperature profile of 25.5 $\pm$ 0.4°C.

Table 23. Formulation of experimental diets

Ingredients, %	CTRL	PHY500	PHY1000	PHY2000	DCP
Fishmeal 60 ¹	2.50	2.50	2.50	2.50	2.50
Guar korma meal ²	4.50	4.50	4.50	4.50	4.80
Soybean meal	32.50	32.50	32.50	32.50	32.50

Rapeseed meal	20.00	20.00	20.00	20.00	20.00
Wheat bran	14.00	14.00	14.00	14.00	14.00
Rice bran	14.00	14.00	14.00	14.00	14.00
Corn meal	8.20	8.20	8.20	8.20	6.53
Fish oil	0.50	0.50	0.50	0.50	0.50
Rapeseed oil	1.90	1.90	1.90	1.90	2.00
Vit & Min premix	1.00	1.00	1.00	1.00	1.00
Antioxidant	0.20	0.20	0.20	0.20	0.20
Dicalcium phosphate					1.60
Calcium carbonate	0.33	0.33	0.33	0.33	
L-Lysine HCl	0.10	0.10	0.10	0.10	0.10
L-Tryptophan	0.05	0.05	0.05	0.05	0.05
DL-Methionine	0.20	0.20	0.20	0.20	0.20
Yttrium oxide	0.02	0.02	0.02	0.02	0.02
phytase (FTY/kg)		500	1000	2000	

¹ COFACO 60: 62.3% crude protein (CP), 8.4% crude fat (CF), COFACO, Portugal;

² Guar Korma: 55.3% CP, 7.8% CF, KFEED Ltd, Bulgaria.

Result

5 At the end of the trial, fish from the best performing treatment (PHY2000) showed a 4.7-fold increase of initial body weight. Fish fed the CTRL diet showed a significantly lower FBW, SGR, PER, and a higher FCR than those fed all other diets ($P < 0.05$). Moreover, fish fed the PHY1000, PHY2000 and DCP diets showed a significantly higher FBW and SGR than those fed the PHY500 and CTRL diets ($P < 0.05$). Fish fed the PHY2000 diet showed a significantly lower FCR than those

10 fed the PHY500 diet ($P < 0.05$). Feed intake (FI) varied between 1.75 and 1.82 %ABW per day and was not significantly affected by dietary treatments ($P > 0.05$). Fish fed the CTRL diet showed a significantly lower whole-body phosphorus (P) content than those fed all other diets ($P < 0.05$). The graded increase of phytase supplementation doses resulted on significant increases of whole-body P content ($P < 0.05$). The graded increase of dietary phytase resulted on significant

15 increases of whole-body P retention ($P < 0.05$). Fish fed the DCP diet showed a significantly higher whole-body P retention than those fed the CTRL and PHY500 diets ($P < 0.05$), although significantly lower than those fed the PHY1000 and PHY2000 diets ($P < 0.05$). Fish fed the CTRL diet showed a significantly lower P digestibility than those fed all other diets ($P < 0.05$). Significant enhancements ($P < 0.05$) of P digestibility were associated to increasing dietary doses of the

20 phytase, with dietary treatment PHY2000 having a significantly higher P digestibility than PHY1000 ($P < 0.05$), and the later having a significantly higher P digestibility than PHY500 ($P < 0.05$). Phosphorus digestibility of the DCP diet was significantly lower than that of diet

PHY2000 ($P < 0.05$) and significantly higher than that of PHY500 and CTRL diets ($P < 0.05$).

Table 24. Growth performance after 93 days of feeding (end of the trial).

	CTRL	PHY500	PHY1000	PHY2000	DCP	P-value
Survival, %	99.2 $\pm$ 1.7	99.2 $\pm$ 1.7	99.2 $\pm$ 1.7	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	0.736
IBW, g	39.7 $\pm$ 0.1	39.5 $\pm$ 0.3	39.7 $\pm$ 0.2	39.2 $\pm$ 0.3	39.6 $\pm$ 0.4	0.187
FBW, g	144.1 $\pm$ 8.0 ^a	162.6 $\pm$ 3.3 ^b	175.3 $\pm$ 3.1 ^c	183.7 $\pm$ 6.4 ^c	174.6 $\pm$ 4.6 ^c	<0.001
SGR, %/d	1.39 $\pm$ 0.06 ^a	1.52 $\pm$ 0.03 ^b	1.60 $\pm$ 0.02 ^c	1.66 $\pm$ 0.03 ^c	1.60 $\pm$ 0.03 ^c	<0.001
FCR	1.45 $\pm$ 0.06 ^c	1.36 $\pm$ 0.05 ^b	1.31 $\pm$ 0.04 ^{ab}	1.26 $\pm$ 0.05 ^a	1.34 $\pm$ 0.02 ^{ab}	<0.001
FI, %ABW/d	1.79 $\pm$ 0.06	1.78 $\pm$ 0.08	1.78 $\pm$ 0.04	1.75 $\pm$ 0.07	1.82 $\pm$ 0.02	0.672
PER	2.28 $\pm$ 0.10 ^a	2.43 $\pm$ 0.10 ^b	2.54 $\pm$ 0.08 ^{bc}	2.63 $\pm$ 0.10 ^c	2.47 $\pm$ 0.03 ^b	<0.001

Values are means $\pm$ standard deviation (n=4).

Values within a row with different superscripts, denote a statistical difference ($P < 0.05$).

Table 25. Whole-body nutrient retention (% of intake)

	CTRL	PHY500	PHY1000	PHY2000	DCP	P-value
Protein, %	36.7 $\pm$ 3.6 ^a	40.6 $\pm$ 1.8 ^b	42.7 $\pm$ 2.5 ^b	44.0 $\pm$ 1.7 ^b	41.6 $\pm$ 1.7 ^b	0.007
Fat, %	77.2 $\pm$ 10.4	83.5 $\pm$ 6.1	87.1 $\pm$ 7.1	81.5 $\pm$ 2.0	80.1 $\pm$ 5.0	0.324
Energy, %	28.1 $\pm$ 2.2 ^a	30.4 $\pm$ 1.7 ^{ab}	32.2 $\pm$ 2.0 ^b	31.6 $\pm$ 1.0 ^b	30.5 $\pm$ 0.6 ^{ab}	0.025
Phosphorus, %	26.2 $\pm$ 1.5 ^a	35.5 $\pm$ 0.7 ^b	45.2 $\pm$ 0.3 ^d	50.7 $\pm$ 1.3 ^e	38.7 $\pm$ 1.1 ^c	<0.001
Calcium, %	40.9 $\pm$ 2.7 ^b	43.5 $\pm$ 1.9 ^b	48.1 $\pm$ 2.5 ^c	48.8 $\pm$ 3.0 ^c	33.8 $\pm$ 1.3 ^a	<0.000

Values are average $\pm$ standard deviation (n=4).

Values within a row with different superscripts, denote a statistical difference ($P < 0.05$).

Table 26. Apparent digestibility coefficients (ADC, %) of nutrients.

	CTRL	PHY500	PHY1000	PHY2000	DCP	P-value
Dry matter, %	68.3 $\pm$ 1.7 ^a	71.5 $\pm$ 0.5 ^b	71.3 $\pm$ 0.1 ^b	72.9 $\pm$ 0.1 ^c	71.4 $\pm$ 0.5 ^b	<0.001
Protein, %	83.6 $\pm$ 0.9 ^a	84.4 $\pm$ 0.4 ^{ab}	84.2 $\pm$ 0.3 ^{ab}	84.9 $\pm$ 0.3 ^b	84.0 $\pm$ 0.3 ^{ab}	0.020
Phosphorus, %	27.7 $\pm$ 2.2 ^a	37.4 $\pm$ 1.6 ^b	46.8 $\pm$ 1.5 ^c	64.3 $\pm$ 1.2 ^d	47.5 $\pm$ 3.0 ^c	<0.001
Phytate-P, %	21.2 $\pm$ 3.8 ^a	36.5 $\pm$ 3.6 ^b	46.6 $\pm$ 1.8 ^c	51.3 $\pm$ 1.5 ^d	21.5 $\pm$ 1.5 ^a	<0.001
Calcium, %	50.8 $\pm$ 3.7 ^{ab}	52.4 $\pm$ 1.8 ^{ab}	53.1 $\pm$ 2.0 ^{ab}	54.9 $\pm$ 1.7 ^b	48.0 $\pm$ 3.0 ^a	0.019

Values are average $\pm$ standard deviation (n=4).

Values within a row with different superscripts, denote a statistical difference ($P < 0.05$).

Conclusion

The phytase at supplementation doses of 500, 1000 and 2000 FTY/kg feed, such as 500 to 200 FTY/kg is an effective strategy to enhance the growth rate, phosphorus

digestibility, whole-body phosphorus retention and reduce FCR in Nile tilapia fed plant protein-rich diets.

Example 13: Effect of graded supplemental levels of phytase on the growth performance, whole-body nutrient retention and nutrient digestibility in gilthead seabream (*Sparus aurata*) fed a plant-protein rich diet

The trial comprised five dietary treatments: a control diet (CTRL) with a total dietary phosphorus (P) level of 0.78%, in which a significant fraction of P was present in the form of phytate-bound P (0.4%). Three other diets, based on the CTRL formulation, were supplemented with phytase (var400) at graded doses (500, 1000 and 2000 FTY/kg feed) (diets PHY500, PHY1000, PHY2000). Phytase was applied post-extrusion by coating. A fifth diet (MCP), also based on the CTRL formulation, was supplemented with monocalcium phosphate, to a total P level of 1.1% and an available P level higher than the known requirements of the species. All diets were isonitrogenous, isolipidic and isoenergetic. Quadruplicate groups of 37 gilthead seabream, with a mean initial body weight of 55.3 ± 4.1 g were fed one of the five experimental diets during 94 days, with a water temperature profile of $22.4 \pm 0.2^\circ\text{C}$.

Table 27. Formulation of experimental diets.

Ingredients, %	CTRL	PHY500	PHY1000	PHY2000	MCP
Fishmeal Supre Prime	7.50	7.50	7.50	7.50	7.50
Fish protein hydrolysate	2.50	2.50	2.50	2.50	2.50
Wheat gluten	7.40	7.40	7.40	7.40	7.70
Corn gluten meal	19.00	19.00	19.00	19.00	19.00
Soybean meal	24.00	24.00	24.00	24.00	24.00
Rapeseed meal	8.50	8.50	8.50	8.50	8.50
Sunflower meal	14.00	14.00	14.00	14.00	14.00
Wheat bran	3.33	3.33	3.33	3.33	1.83
Fish oil	5.00	5.00	5.00	5.00	5.00
Rapeseed oil	7.50	7.50	7.50	7.50	7.50
Vitamin and mineral premix	1.00	1.00	1.00	1.00	1.00
Antioxidant	0.20	0.20	0.20	0.20	0.20
Monocalcium phosphate					1.20
L-Tryptophan	0.02	0.02	0.02	0.02	0.02
DL-Methionine	0.03	0.03	0.03	0.03	0.03
Yttrium oxide	0.02	0.02	0.02	0.02	0.02
Phytase (FTY/kg)		500	1000	2000	

Result

At the end of the trial, the final body weight (FBW) ranged between 142.8 and 157.5 grams. Fish with the highest weight gain showed a 2.8-fold increase of their initial body weight (IBW). The specific growthrate (SGR) varied between 1.01 and 1.11 %/day. At day 94, all supplemented diets resulted on a significantly higher FBW and SGR than those fed the CTRL diet ($P < 0.05$). Fish fed the PHY500, PHY1000, PHY2000 and MCP diets showed a significantly lower FCR than those fed the CTRL diet ($P < 0.05$). The CTRL treatment was consistently associated to significantly lowest values among the various treatments for whole-body phosphorus retention and apparent digestibility of phosphorus. The higher phytase supplementation doses (1000 and 2000 FTY/kg) led to a significant whole-body P retention in comparison to both CTRL and MCP treatments ($P < 0.05$). Significant enhancements of total P and phytate-P digestibility were associated to increasing dietary doses of phytase, with dietary treatment PHY2000 having a significantly higher P digestibility than PHY500 ($P < 0.05$). Additionally, the increase of phytase supplementation doses (0, 500, 1000 and 2000 FTY/kg) was also positively associated to an increase of whole-body phosphorus retention.

Table 28. Growth performance after 94 days of feeding.

	CTRL	PHY500	PHY1000	PHY2000	MCP	P-value
Survival, %	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	-
IBW, g	55.4 $\pm$ 0.6	55.3 $\pm$ 0.3	55.2 $\pm$ 0.5	55.6 $\pm$ 0.2	55.4 $\pm$ 0.4	0.791
FBW, g	142.8 $\pm$ 2.4 ^a	148.4 $\pm$ 0.7 ^b	156.2 $\pm$ 1.8 ^c	156.7 $\pm$ 1.2 ^c	157.5 $\pm$ 1.5 ^c	<0.001
SGR, %/d	1.01 $\pm$ 0.01 ^a	1.05 $\pm$ 0.01 ^b	1.11 $\pm$ 0.01 ^c	1.10 $\pm$ 0.01 ^c	1.11 $\pm$ 0.01 ^c	<0.001
FCR	1.29 $\pm$ 0.04 ^c	1.21 $\pm$ 0.03 ^b	1.12 $\pm$ 0.03 ^a	1.14 $\pm$ 0.03 ^{ab}	1.16 $\pm$ 0.05 ^{ab}	<0.001
FI, %ABW/d	1.21 $\pm$ 0.03 ^b	1.17 $\pm$ 0.03 ^{ab}	1.14 $\pm$ 0.02 ^a	1.15 $\pm$ 0.02 ^{ab}	1.18 $\pm$ 0.04 ^{ab}	0.048
PER	1.74 $\pm$ 0.05 ^a	1.86 $\pm$ 0.05 ^b	2.01 $\pm$ 0.06 ^c	1.97 $\pm$ 0.05 ^{bc}	1.95 $\pm$ 0.09 ^{bc}	<0.001

Values are means $\pm$ standard deviation (n=4).

Values within a row with different superscripts, denote a statistical difference ($P < 0.05$).

Table 29. Whole-body nutrient and energy retention (% of intake).

	CTRL	PHY500	PHY1000	PHY2000	MCP	P-value
Protein, %	26.5 $\pm$ 1.9 ^a	28.2 $\pm$ 1.5 ^{ab}	30.7 $\pm$ 0.8 ^b	30.6 $\pm$ 2.3 ^b	28.7 $\pm$ 1.9 ^{ab}	0.021
Fat, %	60.4 $\pm$ 4.5	63.1 $\pm$ 7.5	63.7 $\pm$ 3.4	69.5 $\pm$ 6.2	63.6 $\pm$ 3.3	0.210
Energy, %	29.9 $\pm$ 2.5 ^a	33.3 $\pm$ 2.6 ^{abc}	35.2 $\pm$ 1.2 ^{bc}	35.9 $\pm$ 2.4 ^c	31.2 $\pm$ 1.9 ^{ab}	0.006
Phosphorus, %	45.9 $\pm$ 2.6 ^a	53.5 $\pm$ 2.9 ^a	62.6 $\pm$ 5.3 ^b	59.9 $\pm$ 3.6 ^b	48.0 $\pm$ 5.7 ^a	<0.001
Calcium, %	44.7 $\pm$ 2.0 ^b	52.9 $\pm$ 4.4 ^c	59.9 $\pm$ 3.4 ^d	60.7 $\pm$ 5.0 ^d	37.2 $\pm$ 1.1 ^a	<0.001

Values are average $\pm$ standard deviation (n=4).

Values within a row with different superscripts, denote a statistical difference ($P < 0.05$).

Table 30. Apparent digestibility coefficients (ADC, %) of nutrients.

	CTRL	PHY500	PHY1000	PHY2000	MCP	P-value
Dry matter, %	63.4 ± 1.0 ^a	66.3 ± 0.8 ^b	66.5 ± 2.2 ^b	65.1 ± 1.7 ^{ab}	62.5 ± 0.8 ^a	0.004
Protein, %	86.3 ± 0.9	87.5 ± 0.7	88.0 ± 1.3	87.0 ± 1.1	86.3 ± 0.8	0.117
Phosphorus, %	34.5 ± 1.1 ^a	49.4 ± 1.5 ^b	57.2 ± 4.3 ^c	60.4 ± 3.4 ^{cd}	62.6 ± 1.2 ^d	<0.001
Phytate-P, %	32.4 ± 1.0 ^a	39.1 ± 3.2 ^b	39.8 ± 3.8 ^b	45.7 ± 4.4 ^c	30.8 ± 2.3	<0.001
Calcium, %	35.0 ± 1.7 ^b	36.1 ± 4.7 ^b	42.7 ± 5.0 ^c	43.1 ± 2.0 ^c	29.9 ± 2.3	<0.001

Values are average ± standard deviation (n=4).

Values within a row with different superscripts, denote a statistical difference (P<0.05).

5 Conclusion

The phytase at supplementation doses of 500, 1000 and 2000 FTY/kg feed is an effective strategy to enhance the growth rate and phosphorus digestibility. Phytase at supplementation doses of 1000 and 2000 FTY/kg feed significantly increase whole-body phosphorus retention in gilthead seabream fed plant protein-rich diets.

10 Example 14: Efficacy of a phytase with a protease in broilers

The effect on the growth performance of broilers using feed with 1,500 FYT/kg of phytase (var400) and 30,000 U/kg of protease (Ronozyme®ProAct 360) was investigated. Male broilers (Ross 308) were fed one of the four diets as described in Table 31 from hatch to day 28 post-hatch. Each treatment had 8 replicate cages of 6 birds per cage. The basal diets used in the trial were diet with standard Ca, standard crude protein diet or a low Ca, high crude protein diet (Table 32).

Table 31. Treatment designation

Treatment	Basal Diet	Protease, U/kg ¹	Phytase, U/kg ²
1	Standard Ca and CP	0	500
2		30,000	1,500
3	Low Ca and high CP	0	500
4		30,000	1,500

¹ Protease was included in the diets over the top (e.g. no further amino acid or protein matrix was applied).

² 500 U/kg of phytase was included in the diets to provide 0.15% avP, 0.17% Ca and 0.018% Na. No extra nutrients were removed when the additional 1,000 U/kg was included.

Table 32. Starter and grower experimental diets

Ingredient	Treatment 1, 2 (fed from day 0-14)	Treatment 3, 4 (fed from day 0-14)	Treatment 1, 2 (fed from day 15-28)	Treatment 3, 4 (fed from day 15-28)
Corn	59.10%	56.19%	61.52%	58.61%
Soybean meal	34.98%	38.43%	31.79%	35.26%
Soy oil	1.86%	1.93%	3.03%	3.10%
Salt	0.13%	0.12%	0.13%	0.12%
Limestone	0.77%	0.12%	0.66%	
Dicalcium phosphate	1.53%	1.48%	1.33%	1.28%
Lysine HCl	0.23%	0.24%	0.17%	0.18%
DL-Methionine	0.32%	0.36%	0.26%	0.30%
Threonine	0.09%	0.10%	0.05%	0.060%
Valine	0.038%	0.059%		0.013%
Vitamin and mineral premix	1.00%	1.00%	1.00%	1.00%
TiO Marker			0.10%	0.10%
TOTAL	100.0%	100.0%	100.0%	100.0%
Nutrients				
Crude protein %	22.50	24.00	21.00	22.50
Poult ME kcal/kg	3000.00	3000.00	3100.00	3100.00
DM, %	87.07	87.04	87.04	87.00
Calcium %	1.00	0.75	0.90	0.65
Phosphorus %	0.68	0.69	0.63	0.64
Available P %	0.35	0.35	0.31	0.31
Phytate P %	0.26	0.27	0.26	0.26
Crude Fibre %	2.23	2.28	2.18	2.23
D Met+Cys %	0.95	1.02	0.85	0.93
D Lys %	1.28	1.38	1.15	1.25
D Thr %	0.83	0.90	0.75	0.81
Available P %	0.35	0.35	0.31	0.31
Na %	0.18	0.18	0.18	0.18
Cl %	0.36	0.36	0.35	0.35

Results

Table 33. Growth performance of broilers fed standard Ca and crude protein or low Ca and high crude protein diets without or with protease and an extra 1,000 U/kg of phytase from hatch to day 28 post-hatch

Dietary Ca	Dietary crude protein	Protease, U/kg	Phytase, U/kg	Feed intake, g/bird	BW gain, g/bird	FCR, g:g
Standard Ca	Standard CP	0	500	2,376	1,683	1.427
		30,000	1,500	2,341	1,722	1.362
Percent delta				-1.5%	+2.3%	-4.6%
Low Ca	High CP	0	500	2,349	1,795	1.318
		30,000	1,500	2,370	1,861	1.281
Percent delta				+0.9%	+3.7%	-2.8%

Table 34. Apparent nutrient digestibility of broilers fed standard Ca and crude protein or low Ca and high crude protein diets without or with protease and an extra 1,000 U/kg of phytase from hatch to day 28 post-hatch

Dietary Ca	Dietary crude protein	Protease, U/kg	Phytase, U/kg	Ileal protein digestibility, %	Fecal protein retention, %	Fecal energy retention, %
Standard Ca	Standard CP	0	500	82.5	63.4	77.0
		30,000	1,500	83.8	66.2	78.0
Percent delta				+1.6%	+4.4%	+1.3%
Low Ca	High CP	0	500	84.8	66.5	77.8
		30,000	1,500	85.4	67.1	78.3
Percent delta				+0.7%	+0.9%	+0.6%

5

Conclusion

Feeding broilers protease in combination with an extra 1,000 FYT/kg of phytase increased protein digestibility by 0.7 to 1.6%, protein retention by 0.9 to 4.4% and energy retention by 0.6 to 1.3%. This resulted in an improvement in body weight gain (BWG) by 2.3 or 3.7% and feed conversion ratio by 4.6 or 2.8%.

10

CLAIMS

1. A phytase variant which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2 and which comprises the alterations N31C/G52C/A99C/K141C/T177C/V199C/N203L as compared to SEQ ID NO: 2 and further comprises a substitution in one or more position(s) selected from the following: 30, 36, 43, 46, 57, 60, 64, 73, 79, 119, 121, 123, 130, 134, 138, 151, 155, 161, 162, 168, 176, 180, 184, 190, 207, 224, 230, 243, 273, 286, 336, 340, 358 and 375 using SEQ ID NO: 2 for numbering.
2. The variant of claim 1, comprising substitutions in the positions: 57, 73, 121, 134, 155, 207 and 273.
3. The variant of claim 2, further comprising substitutions in the positions: 36, 60, 64, 73, 119, 130, 138, 161, 162, 168, 176, 180, 184, 190, 224, 230, 243, 336 and 340.
4. The variant according to any of claims 1-3, where the substitutions are selected among: 30Q, 36A, 43C, 46C, 57Y, 60H, 64Q, 73P, 79Q, 119P, 121P, 123C, 130T, C, 134Q, 138A, 151S, 155F, 161T, 162A, 168R, 176P, 180N, 184Q, 190T, 207T, 224Q, 230E, 243N, 273L, 286S, 336R, 340L, P, 358Q and 375K.
5. The variant according to any of the claim 1-4, comprising the substitutions 31C/52C/57Y/73P/99C/121P/134Q/141C/155F/177C/199C/203L/207T/273L.
6. The variant according to claim 5, selected among variants comprising the substitutions selected from the group consisting of:
31C/ 52C/ 57Y/ 73P/ 99C/ 121P/ 134Q/ 141C/ 155F/ 177C/ 199C/ 203L/ 207T/ 273L;
31C/ 36A/ 52C/ 57Y/ 60H/ 64Q/ 73P/ 99C/ 119S/ 121P/ 130T/ 134Q/ 138A/ 141C/ 155F/ 161T/ 162A/ 176P/ 177C/ 180N/ 184Q/ 190T/ 199C/ 203L/ 207T/ 224Q/ 230E/ 243N/ 273L/ 336R/ 340L;
31C/ 36A/ 43C/ 46C/ 52C/ 57Y/ 60H/ 64Q/ 73P/ 99C/ 119S/ 121P/ 130T/ 134Q/ 138A/ 141C/ 155F/ 161T/ 162A/ 176P/ 177C/ 180N/ 184Q/ 190T/ 199C/ 203L/ 207T/ 224Q/ 230E/ 243N/ 273L/ 286S/ 336R/ 340L;

31C/ 36A/ 52C/ 57Y/ 60H/ 64Q/ 73P/ 99C/ 119S/ 121P/ 123C/ 130C/ 134Q/ 138A/ 141C/
151S/ 155F/ 161T/ 162A/ 176P/ 177C/ 180N/ 184Q/ 190T/ 199C/ 203L/ 207T/ 224Q/
230E/ 243N/ 273L/ 336R/ 340L; and

5

31C/ 36A/ 43C/ 46C/ 52C/ 57Y/ 60H/ 64Q/ 73P/ 99C/ 119S/ 121P/ 123C/ 130C/ 134Q/
138A/ 141C/ 155F/ 161T/ 162A/ 176P/ 177C/ 180N/ 184Q/ 190T/ 199C/ 203L/ 207T/
224Q/ 230E/ 243N/ 273L/ 286S/ 336R/ 340L.

10 7. The variant of claim 6, having the amino acid sequence of SEQ ID NO: 2 with the
substitutions selected from the group consisting of:

N31C/ G52C/ E57Y/ N73P/ A99C/ N121P/ S134Q/ K141C/ Y155F/ T177C/ V199C/
N203L/ P207T/ M273L;

15 N31C/ Q36A/ G52C/ E57Y/ Q60H/ L64Q/ N73P/ A99C/ E119S/ N121P/ M130T/ S134Q/
L138A/ K141C/ Y155F/ S161T/ S162A/ E176P/ T177C/ T180N/ S184Q/ P190T/ V199C/
N203L/ P207T/ E224Q/ Q230E/ R243N/ M273L/ K336R/ T340L;

20 N31C/ Q36A/ P43C/ W46C/ G52C/ E57Y/ Q60H/ L64Q/ N73P/ A99C/ E119S/ N121P/
M130T/ S134Q/ L138A/ K141C/ Y155F/ S161T/ S162A/ E176P/ T177C/ T180N/ S184Q/
P190T/ V199C/ N203L/ P207T/ E224Q/ Q230E/ R243N/ M273L/ N286S/ K336R/ T340L;

25 N31C/ Q36A/ G52C/ E57Y/ Q60H/ L64Q/ N73P/ A99C/ E119S/ N121P/ P123C/ M130C/
S134Q/ L138A/ K141C/ N151S/ Y155F/ S161T/ S162A/ E176P/ T177C/ T180N/ S184Q/
P190T/ V199C/ N203L/ P207T/ E224Q/ Q230E/ R243N/ M273L/ K336R/ T340L; or

30 N31C/ Q36A/ P43C/ W46C/ G52C/ E57Y/ Q60H/ L64Q/ N73P/ A99C/ E119S/ N121P/
P123C/ M130C/ S134Q/ L138A/ K141C/ Y155F/ S161T/ S162A/ E176P/ T177C/ T180N/
S184Q/ P190T/ V199C/ N203L/ P207T/ E224Q/ Q230E/ R243N/ M273L/ N286S/ K336R/
T340L.

8. The variant according to any of claims 1-7, wherein the parent phytase has at least 75%,
at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least
98%, at least 99% or 100% sequence identity to the mature polypeptide of SEQ ID NO: 2.

35

9. The variant of any of claims 1-8, wherein the parent phytase comprises or consists of the
mature polypeptide of SEQ ID NO: 2.

10. The variant of any of claims 1-9, which has an improved thermostability in comparison with the phytase having the amino acid sequence of SEQ ID NO: 2 and the substitutions N31C/ G52C/ A99C/ K141C/ T177C/ V199C/ N203L.

5

11. A polynucleotide encoding the variant of any of claims 1-10.

12. A nucleic acid construct or an expression vector comprising the polynucleotide of claim 11.

10

13. A host cell comprising the polynucleotide of claim 11.

14. A method of producing a phytase variant according to any of claims 1-10, comprising:
cultivating the host cell of claim 13 under conditions suitable for expression of the variant;
and
recovering the variant.

15

15. A plant comprising the phytase variant of any of claims 1-10 and/or the polynucleotide of claim 11.

20

16. A composition comprising at least one phytase variant according to any of claims 1-10.

17. The composition of claim 16 further comprising
at least one fat soluble vitamin;
at least one water soluble vitamin; and/or
at least one trace mineral.

25

18. The composition of claim 16 or 17, further comprising at least one enzyme selected from the following group of enzymes: amylase, phytase, phosphatase, xylanase, galactanase, alpha-galactosidase, protease, phospholipase and/or beta-glucanase.

30

19. The composition of any of claims 16-18, which is an animal feed additive.

20. An animal feed composition having a crude protein content of 50 to 800 g/kg and comprising the phytase variant of any of claims 1-10, the polynucleotide of claim 11, or the composition of any of claims 16-19.

35

21. A method of improving the nutritional value of an animal feed, wherein the phytase variant of any of claims 1-10, the polynucleotide of claim 11, or the composition of any of claims 16-19 is added to the feed.
- 5 22. A method for the treatment of vegetable proteins, comprising the step of adding the phytase variant of any of claims 1-10, the polynucleotide of claim 11, or the composition of any of claims 16-19 to at least one vegetable protein or protein source.
- 10 23. A method for increasing weight gain and/or improving Feed Conversion Ratio of an animal, the method comprising the step of applying to the animal a feed with an efficient amount of the phytase variant of any of claims 1-10, the polynucleotide of claim 11, or the composition of any of claims 16-19.
- 15 24. Use of the phytase variant of any of claims 1-10, the polynucleotide of claim 11, or the composition of any of claims 16-19 in animal feed; in the preparation of animal feed; for improving the nutritional value of animal feed; for reducing phytate levels in animal manure; for the treatment of vegetable proteins; for liberating phosphorous from a phytate substrate; for increasing weight gain, improving specific growth rate and/or improving Feed Conversion Ratio of an animal; or for improving nutrient retention, and/or nutrient digestibility in an animal.
- 20 25. An isolated polypeptide having phytase activity, selected from the group consisting of
- a) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 12;
 - 25 b) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 14;
 - c) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 16;
 - d) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 18;
 - 30 and
 - e) a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO: 20.
- 35 26. The isolated polypeptide according to claim 25, wherein said polypeptide is pH stable and thermostable such that it comprises one or more of the following properties
- i. an unfolding temperature at pH 4 of at least 75 °C;

- ii. an unfolding temperature at pH 3 of at least 70°C; and
- iii. an unfolding temperature at pH 2 of at least 55°C.

27. A method of preparing a recombinant polypeptide having phytase activity comprising the steps of:

(a) cultivating a recombinant host cell comprising an exogenous polynucleotide selected from the group consisting of

a. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO:

13;

b. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO:

15;

c. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO:

17;

d. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO:

19;

e. a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to SEQ ID NO:

21;

wherein the polynucleotide is expressed and the polypeptide is produced;

(b) optionally isolating the polypeptide; and

(c) optionally recovering the polypeptide.

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SEQ2	1	EEQNGMKLERVVIVSRHGVRAPTKFTPI	28
		.	
SEQ9	1	MSTFIIRLLFFSLLCGSFSIHAEEPNGMKLERVVIVSRHGVRAPTKFTPI	50
SEQ2	29	MKNVTPDQWPQWDVPLGWLTTPRGGELVSELGQYQRLWFTSKGLLNNQTCP	78
		:	
SEQ9	51	MKDVTDPDQWPQWDVPLGWLTTPRGGELVSELGQYQRLWFTSKGLLNNQTCP	100
SEQ2	79	SPGQVAVIADTDQRTKRTGEAFLAGLAPKCQIQVHYQKDEEKNDPLFNPV	128
SEQ9	101	SPGQVAVIADTDQRTKRTGEAFLAGLAPKCQIQVHYQKDEEKTDPLFNPV	150
SEQ2	129	KMGKCSFNTLQVKNAILERAGGNIELYTQRYQSSFRTLENVLNFSQSETC	178
		. :	
SEQ9	151	KMGTCFNTLKVKNAILERAGGNIELYTQRYQSSFRTLENVLNFSQSETC	200
SEQ2	179	KTTEKSTKCTLPEALPSELKVTPDNVSLPGAWSLSSSTLTEIFLLQEAQGM	228
SEQ9	201	KTTEKSTKCTLPEALPSELKVTPDNVSLPGAWSLSSSTLTEIFLLQEAQGM	250
SEQ2	229	PQVAWGRITGEKEWRDLLSLHNAQFDLLQRTPEVARSRATPLLDMDTAL	278
SEQ9	251	PQVAWGRITGEKEWRDLLSLHNAQFDLLQRTPEVARSRATPLLDMDTAL	300
SEQ2	279	LTNGTTENRYGIKLPVSLLEFIAGHDTNLANLSGALDLNWSLPGQPDNTTP	328
SEQ9	301	LTNGTTENRYGIKLPVSLLEFIAGHDTNLANLSGALDLNWSLPGQPDNTTP	350
SEQ2	329	GGELVFEEKWKRTSDNTDWWQVSFVYQTLRDMRDIQPLSLEKPAGKVDLKL	378
SEQ9	351	GGELVFEEKWKRTSDNTDWWQVSFVYQTLRDMRDIQPLSLEKPAGKVDLKL	400
SEQ2	379	IACEEKNSQGMCSLKSFSRLIKEIRVPECAVTE	411
SEQ9	401	IACEEKNSQGMCSLKSFSRLIKEIRVPECAVTE	433

Fig. 1

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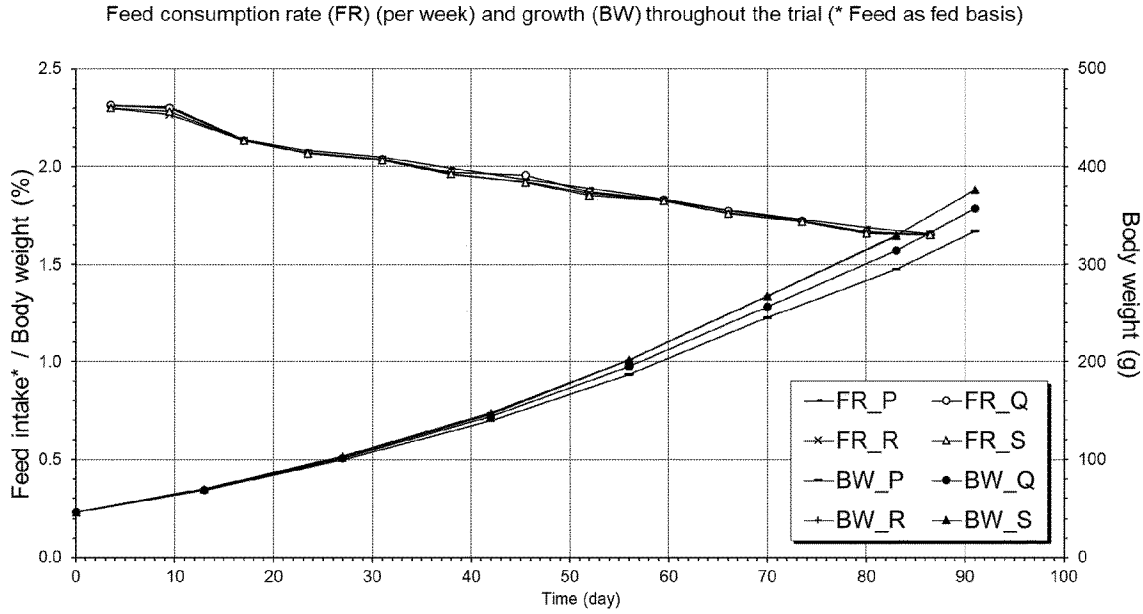


Fig. 2

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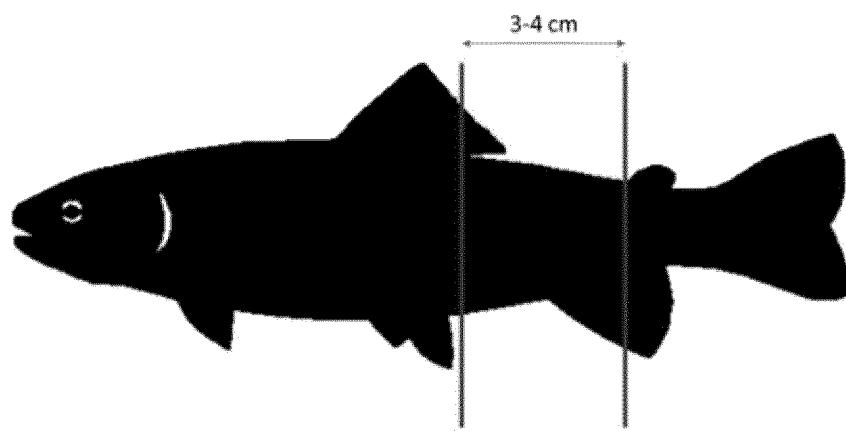


Fig. 3

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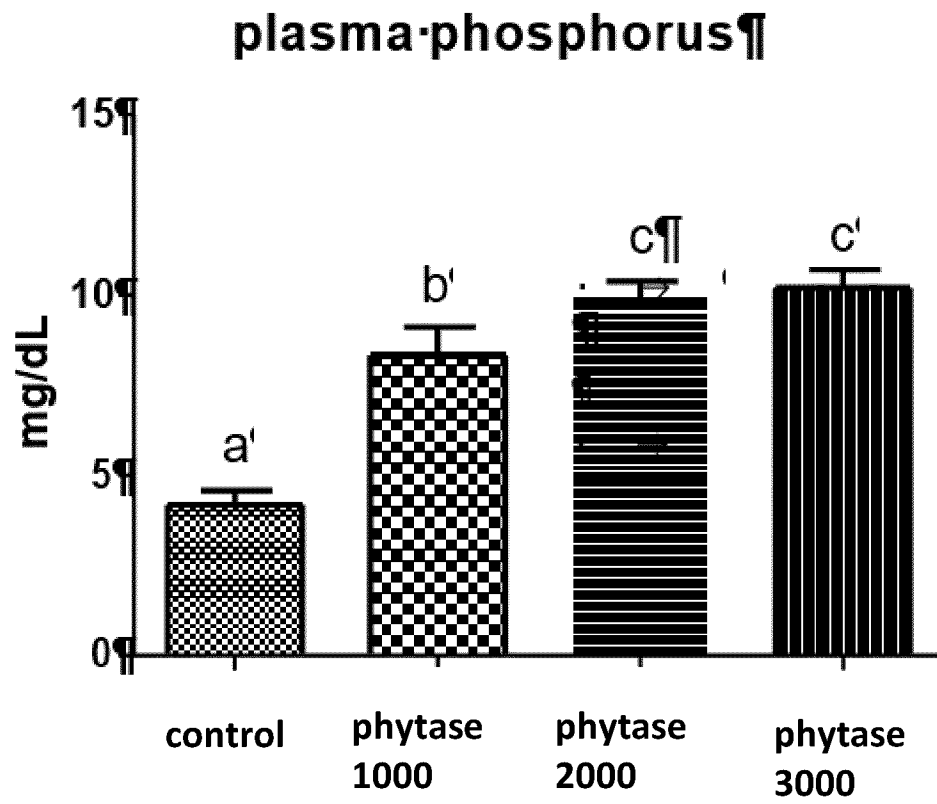


Fig. 4

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ATOM	1	N	GLY	A	5	-14.638	16.094	42.125	1.00	54.10	B	N
ATOM	2	CA	GLY	A	5	-15.897	16.043	41.297	1.00	48.79	B	C
ATOM	3	C	GLY	A	5	-15.811	15.052	40.146	1.00	39.17	B	C
ATOM	4	O	GLY	A	5	-16.655	15.172	39.240	1.00	35.11	B	O
ATOM	5	N	MET	A	6	-14.890	14.075	40.212	1.00	40.74	B	N
ATOM	6	CA	MET	A	6	-14.412	13.242	39.062	1.00	37.72	B	C
ATOM	7	CB	MET	A	6	-12.939	13.539	38.771	1.00	40.34	B	C
ATOM	8	CG	MET	A	6	-12.684	14.989	38.575	1.00	46.28	B	C
ATOM	9	SD	MET	A	6	-13.492	15.429	37.060	1.00	52.01	B	S
ATOM	10	CE	MET	A	6	-12.054	15.422	36.022	1.00	33.09	B	C
ATOM	11	C	MET	A	6	-14.503	11.748	39.358	1.00	31.32	B	C
ATOM	12	O	MET	A	6	-13.995	11.326	40.373	1.00	37.72	B	O
ATOM	13	N	LYS	A	7	-15.046	10.993	38.425	1.00	29.14	B	N
ATOM	14	CA	LYS	A	7	-15.179	9.516	38.476	1.00	31.19	B	C
ATOM	15	CB	LYS	A	7	-16.642	9.128	38.228	1.00	35.92	B	C
ATOM	16	CG	LYS	A	7	-16.987	7.684	38.524	1.00	44.61	B	C
ATOM	17	CD	LYS	A	7	-18.252	7.187	37.855	1.00	50.51	B	C
ATOM	18	CE	LYS	A	7	-18.624	5.809	38.369	1.00	60.08	B	C
ATOM	19	NZ	LYS	A	7	-18.809	4.845	37.258	1.00	65.98	B	N
ATOM	20	C	LYS	A	7	-14.258	8.961	37.385	1.00	27.78	B	C
ATOM	21	O	LYS	A	7	-14.446	9.297	36.192	1.00	24.28	B	O
ATOM	22	N	LEU	A	8	-13.418	7.990	37.744	1.00	27.52	B	N
ATOM	23	CA	LEU	A	8	-12.608	7.241	36.745	1.00	27.34	B	C
ATOM	24	CB	LEU	A	8	-11.411	6.577	37.431	1.00	26.27	B	C
ATOM	25	CG	LEU	A	8	-10.373	5.923	36.501	1.00	25.54	B	C
ATOM	26	CD1	LEU	A	8	-9.827	6.902	35.452	1.00	23.52	B	C
ATOM	27	CD2	LEU	A	8	-9.237	5.355	37.329	1.00	28.69	B	C
ATOM	28	C	LEU	A	8	-13.480	6.207	36.034	1.00	26.90	B	C
ATOM	29	O	LEU	A	8	-14.007	5.267	36.684	1.00	27.75	B	O
ATOM	30	N	GLU	A	9	-13.636	6.329	34.723	1.00	24.05	B	N
ATOM	31	CA	GLU	A	9	-14.540	5.500	33.907	1.00	25.74	B	C
ATOM	32	CB	GLU	A	9	-15.313	6.378	32.931	1.00	30.35	B	C
ATOM	33	CG	GLU	A	9	-16.156	7.426	33.621	1.00	36.12	B	C
ATOM	34	CD	GLU	A	9	-17.584	6.954	33.799	1.00	43.98	B	C
ATOM	35	OE1	GLU	A	9	-18.432	7.351	32.981	1.00	48.09	B	O
ATOM	36	OE2	GLU	A	9	-17.818	6.180	34.736	1.00	50.69	B	O
ATOM	37	C	GLU	A	9	-13.770	4.429	33.126	1.00	25.69	B	C
ATOM	38	O	GLU	A	9	-14.266	3.326	33.045	1.00	25.23	B	O
ATOM	39	N	ARG	A	10	-12.625	4.757	32.542	1.00	23.75	B	N
ATOM	40	CA	ARG	A	10	-11.929	3.817	31.620	1.00	25.30	B	C
ATOM	41	CB	ARG	A	10	-12.341	3.936	30.164	1.00	25.77	B	C
ATOM	42	CG	ARG	A	10	-13.758	3.509	29.881	1.00	26.91	B	C
ATOM	43	CD	ARG	A	10	-14.030	3.555	28.413	1.00	30.15	B	C
ATOM	44	NE	ARG	A	10	-15.471	3.685	28.244	1.00	32.57	B	N
ATOM	45	CZ	ARG	A	10	-16.091	3.865	27.107	1.00	33.88	B	C
ATOM	46	NH1	ARG	A	10	-15.437	3.885	25.969	1.00	28.83	B	N
ATOM	47	NH2	ARG	A	10	-17.407	3.980	27.090	1.00	40.76	B	N
ATOM	48	C	ARG	A	10	-10.469	4.199	31.566	1.00	27.26	B	C
ATOM	49	O	ARG	A	10	-10.167	5.366	31.750	1.00	23.20	B	O
ATOM	50	N	VAL	A	11	-9.605	3.220	31.379	1.00	21.08	B	N
ATOM	51	CA	VAL	A	11	-8.142	3.419	31.258	1.00	21.25	B	C
ATOM	52	CB	VAL	A	11	-7.394	3.054	32.561	1.00	22.53	B	C
ATOM	53	CG1	VAL	A	11	-5.895	3.144	32.405	1.00	24.39	B	C
ATOM	54	CG2	VAL	A	11	-7.804	3.931	33.744	1.00	25.89	B	C
ATOM	55	C	VAL	A	11	-7.637	2.616	30.083	1.00	21.58	B	C
ATOM	56	O	VAL	A	11	-8.061	1.477	29.887	1.00	21.59	B	O

FIG. 5

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ATOM	57	N	VAL	A	12	-6.757	3.215	29.270	1.00	18.76	B	N
ATOM	58	CA	VAL	A	12	-5.894	2.521	28.293	1.00	18.33	B	C
ATOM	59	CB	VAL	A	12	-6.111	2.926	26.824	1.00	20.13	B	C
ATOM	60	CG1	VAL	A	12	-5.227	2.148	25.888	1.00	21.20	B	C
ATOM	61	CG2	VAL	A	12	-7.567	2.794	26.414	1.00	22.29	B	C
ATOM	62	C	VAL	A	12	-4.453	2.727	28.702	1.00	15.60	B	C
ATOM	63	O	VAL	A	12	-4.036	3.890	28.865	1.00	17.78	B	O
ATOM	64	N	ILE	A	13	-3.710	1.631	28.877	1.00	16.65	B	N
ATOM	65	CA	ILE	A	13	-2.253	1.665	29.151	1.00	15.98	B	C
ATOM	66	CB	ILE	A	13	-1.841	0.850	30.369	1.00	17.29	B	C
ATOM	67	CG1	ILE	A	13	-2.512	1.400	31.625	1.00	18.59	B	C
ATOM	68	CG2	ILE	A	13	-0.358	0.716	30.470	1.00	17.26	B	C
ATOM	69	CD1	ILE	A	13	-2.327	0.552	32.892	1.00	20.41	B	C
ATOM	70	C	ILE	A	13	-1.545	1.200	27.901	1.00	16.56	B	C
ATOM	71	O	ILE	A	13	-1.842	0.059	27.452	1.00	17.50	B	O
ATOM	72	N	VAL	A	14	-0.549	1.935	27.460	1.00	16.23	B	N
ATOM	73	CA	VAL	A	14	0.422	1.423	26.463	1.00	15.88	B	C
ATOM	74	CB	VAL	A	14	0.582	2.317	25.197	1.00	17.37	B	C
ATOM	75	CG1	VAL	A	14	1.539	1.672	24.222	1.00	18.75	B	C
ATOM	76	CG2	VAL	A	14	-0.785	2.563	24.559	1.00	19.91	B	C
ATOM	77	C	VAL	A	14	1.737	1.263	27.191	1.00	15.81	B	C
ATOM	78	O	VAL	A	14	2.276	2.251	27.702	1.00	16.08	B	O
ATOM	79	N	SER	A	15	2.230	0.034	27.316	1.00	15.17	B	N
ATOM	80	CA	SER	A	15	3.406	-0.307	28.113	1.00	15.61	B	C
ATOM	81	CB	SER	A	15	3.066	-1.425	29.120	1.00	16.35	B	C
ATOM	82	OG	SER	A	15	4.156	-1.741	29.939	1.00	15.99	B	O
ATOM	83	C	SER	A	15	4.533	-0.736	27.220	1.00	15.08	B	C
ATOM	84	O	SER	A	15	4.323	-1.561	26.311	1.00	17.28	B	O
ATOM	85	N	ARG	A	16	5.731	-0.334	27.529	1.00	14.56	B	N
ATOM	86	CA	ARG	A	16	6.911	-1.048	27.041	1.00	14.68	B	C
ATOM	87	CB	ARG	A	16	8.203	-0.278	27.290	1.00	15.67	B	C
ATOM	88	CG	ARG	A	16	9.442	-0.922	26.702	1.00	16.66	B	C
ATOM	89	CD	ARG	A	16	10.664	-0.091	26.884	1.00	16.63	B	C
ATOM	90	NE	ARG	A	16	11.803	-0.788	26.309	1.00	16.62	B	N
ATOM	91	CZ	ARG	A	16	13.033	-0.629	26.665	1.00	15.92	B	C
ATOM	92	NH1	ARG	A	16	13.388	0.330	27.493	1.00	16.70	B	N
ATOM	93	NH2	ARG	A	16	13.980	-1.407	26.135	1.00	20.39	B	N
ATOM	94	C	ARG	A	16	6.934	-2.426	27.748	1.00	14.45	B	C
ATOM	95	O	ARG	A	16	6.635	-2.543	28.943	1.00	14.95	B	O
ATOM	96	N	HIS	A	17	7.540	-3.393	27.071	1.00	14.74	B	N
ATOM	97	CA	HIS	A	17	7.969	-4.639	27.745	1.00	14.90	B	C
ATOM	98	CB	HIS	A	17	8.576	-5.599	26.718	1.00	15.61	B	C
ATOM	99	CG	HIS	A	17	9.720	-4.978	26.046	1.00	15.93	B	C
ATOM	100	ND1	HIS	A	17	10.930	-4.813	26.637	1.00	17.27	B	N
ATOM	101	CE1	HIS	A	17	11.758	-4.206	25.769	1.00	18.76	B	C
ATOM	102	NE2	HIS	A	17	11.128	-3.959	24.669	1.00	19.69	B	N
ATOM	103	CD2	HIS	A	17	9.832	-4.449	24.810	1.00	16.08	B	C
ATOM	104	C	HIS	A	17	8.906	-4.318	28.894	1.00	15.53	B	C
ATOM	105	O	HIS	A	17	9.562	-3.277	28.974	1.00	14.28	B	O
ATOM	106	N	GLY	A	18	9.019	-5.301	29.783	1.00	14.08	B	N
ATOM	107	CA	GLY	A	18	9.873	-5.191	30.954	1.00	13.96	B	C
ATOM	108	C	GLY	A	18	11.332	-5.453	30.665	1.00	13.89	B	C
ATOM	109	O	GLY	A	18	11.784	-5.562	29.506	1.00	14.62	B	O
ATOM	110	N	VAL	A	19	12.148	-5.511	31.720	1.00	14.53	B	N
ATOM	111	CA	VAL	A	19	13.595	-5.714	31.643	1.00	15.25	B	C
ATOM	112	CB	VAL	A	19	14.250	-5.731	33.023	1.00	15.83	B	C
ATOM	113	CG1	VAL	A	19	15.707	-6.047	32.946	1.00	17.22	B	C
ATOM	114	CG2	VAL	A	19	14.028	-4.392	33.772	1.00	15.90	B	C

Fig. 5 (cont.)

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ATOM	115	C	VAL	A	19	13.916	-7.007	30.899	1.00	15.60	B	C
ATOM	116	O	VAL	A	19	13.268	-8.049	31.160	1.00	17.21	B	O
ATOM	117	N	ARG	A	20	14.741	-6.895	29.908	1.00	16.25	B	N
ATOM	118	CA	ARG	A	20	15.075	-7.992	28.990	1.00	16.35	B	C
ATOM	119	CB	ARG	A	20	14.507	-7.758	27.614	1.00	16.57	B	C
ATOM	120	CG	ARG	A	20	14.819	-6.374	26.998	1.00	16.66	B	C
ATOM	121	CD	ARG	A	20	14.617	-6.414	25.480	1.00	19.04	B	C
ATOM	122	NE	ARG	A	20	14.860	-5.097	24.854	1.00	19.16	B	N
ATOM	123	CZ	ARG	A	20	16.027	-4.615	24.641	1.00	18.99	B	C
ATOM	124	NH1	ARG	A	20	17.148	-5.249	24.926	1.00	19.07	B	N
ATOM	125	NH2	ARG	A	20	16.146	-3.372	24.105	1.00	21.69	B	N
ATOM	126	C	ARG	A	20	16.583	-8.038	28.821	1.00	16.05	B	C
ATOM	127	O	ARG	A	20	17.310	-7.034	29.024	1.00	16.77	B	O
ATOM	128	N	ALA	A	21	17.092	-9.170	28.339	1.00	15.89	B	N
ATOM	129	CA	ALA	A	21	18.458	-9.253	27.859	1.00	15.60	B	C
ATOM	130	CB	ALA	A	21	18.759	-10.726	27.533	1.00	17.00	B	C
ATOM	131	C	ALA	A	21	18.630	-8.375	26.595	1.00	17.00	B	C
ATOM	132	O	ALA	A	21	17.649	-8.062	25.921	1.00	17.14	B	O
ATOM	133	N	PRO	A	22	19.864	-8.026	26.247	1.00	17.23	B	N
ATOM	134	CA	PRO	A	22	20.146	-7.295	25.023	1.00	18.58	B	C
ATOM	135	CB	PRO	A	22	21.680	-7.185	24.971	1.00	18.95	B	C
ATOM	136	CG	PRO	A	22	22.075	-7.281	26.419	1.00	18.80	B	C
ATOM	137	CD	PRO	A	22	21.098	-8.331	26.954	1.00	18.02	B	C
ATOM	138	C	PRO	A	22	19.569	-7.995	23.801	1.00	20.33	B	C
ATOM	139	O	PRO	A	22	19.505	-9.213	23.776	1.00	18.82	B	O
ATOM	140	N	THR	A	23	19.178	-7.250	22.768	1.00	20.99	B	N
ATOM	141	CA	THR	A	23	18.518	-7.856	21.582	1.00	22.36	B	C
ATOM	142	CB	THR	A	23	17.684	-6.859	20.769	1.00	22.97	B	C
ATOM	143	OG1	THR	A	23	18.565	-5.833	20.322	1.00	25.18	B	O
ATOM	144	CG2	THR	A	23	16.515	-6.286	21.551	1.00	24.95	B	C
ATOM	145	C	THR	A	23	19.576	-8.494	20.702	1.00	22.21	B	C
ATOM	146	O	THR	A	23	19.152	-9.283	19.860	1.00	26.58	B	O
ATOM	147	N	LYS	A	24	20.826	-8.115	20.825	1.00	21.87	B	N
ATOM	148	CA	LYS	A	24	21.876	-8.622	19.910	1.00	23.70	B	C
ATOM	149	CB	LYS	A	24	21.882	-7.792	18.622	1.00	27.07	B	C
ATOM	150	CG	LYS	A	24	22.186	-6.325	18.832	1.00	30.11	B	C
ATOM	151	CD	LYS	A	24	21.989	-5.525	17.561	1.00	34.66	B	C
ATOM	152	CE	LYS	A	24	22.162	-4.044	17.776	1.00	34.80	B	C
ATOM	153	NZ	LYS	A	24	21.752	-3.303	16.556	1.00	39.07	B	N
ATOM	154	C	LYS	A	24	23.241	-8.575	20.572	1.00	23.69	B	C
ATOM	155	O	LYS	A	24	23.442	-7.871	21.598	1.00	22.64	B	O
ATOM	156	N	PHE	A	25	24.188	-9.324	20.020	1.00	24.37	B	N
ATOM	157	CA	PHE	A	25	25.603	-9.308	20.429	1.00	22.97	B	C
ATOM	158	CB	PHE	A	25	25.931	-10.434	21.427	1.00	25.44	B	C
ATOM	159	CG	PHE	A	25	27.337	-10.356	21.933	1.00	27.34	B	C
ATOM	160	CD1	PHE	A	25	27.718	-9.316	22.762	1.00	29.83	B	C
ATOM	161	CE1	PHE	A	25	29.013	-9.234	23.249	1.00	32.40	B	C
ATOM	162	CZ	PHE	A	25	29.931	-10.171	22.887	1.00	35.48	B	C
ATOM	163	CD2	PHE	A	25	28.278	-11.305	21.578	1.00	33.41	B	C
ATOM	164	CE2	PHE	A	25	29.577	-11.206	22.049	1.00	35.46	B	C
ATOM	165	C	PHE	A	25	26.434	-9.377	19.159	1.00	25.61	B	C
ATOM	166	O	PHE	A	25	26.583	-10.467	18.566	1.00	26.75	B	O
ATOM	167	N	THR	A	26	26.977	-8.242	18.740	1.00	23.17	B	N
ATOM	168	CA	THR	A	26	27.574	-8.098	17.397	1.00	26.89	B	C
ATOM	169	CB	THR	A	26	26.839	-7.038	16.587	1.00	29.04	B	C
ATOM	170	OG1	THR	A	26	27.096	-5.849	17.332	1.00	28.58	B	O
ATOM	171	CG2	THR	A	26	25.380	-7.325	16.407	1.00	28.03	B	C
ATOM	172	C	THR	A	26	29.064	-7.816	17.504	1.00	25.69	B	C

Fig. 5 (cont.)

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ATOM	173	O	THR	A	26	29.612	-7.437	18.539	1.00	25.41	B	O
ATOM	174	N	PRO	A	27	29.797	-8.109	16.406	1.00	26.53	B	N
ATOM	175	CA	PRO	A	27	31.226	-7.869	16.414	1.00	26.08	B	C
ATOM	176	CB	PRO	A	27	31.661	-8.277	14.991	1.00	30.49	B	C
ATOM	177	CG	PRO	A	27	30.599	-9.276	14.532	1.00	31.98	B	C
ATOM	178	CD	PRO	A	27	29.313	-8.822	15.196	1.00	32.47	B	C
ATOM	179	C	PRO	A	27	31.640	-6.420	16.738	1.00	25.60	B	C
ATOM	180	O	PRO	A	27	32.561	-6.271	17.485	1.00	26.19	B	O
ATOM	181	N	ILE	A	28	30.849	-5.464	16.276	1.00	26.52	B	N
ATOM	182	CA	ILE	A	28	31.164	-4.032	16.546	1.00	26.94	B	C
ATOM	183	CB	ILE	A	28	30.303	-3.111	15.704	1.00	27.80	B	C
ATOM	184	CG1	ILE	A	28	30.893	-1.705	15.669	1.00	32.28	B	C
ATOM	185	CG2	ILE	A	28	28.869	-3.094	16.210	1.00	30.45	B	C
ATOM	186	CD1	ILE	A	28	30.091	-0.793	14.792	1.00	42.98	B	C
ATOM	187	C	ILE	A	28	31.082	-3.761	18.053	1.00	28.88	B	C
ATOM	188	O	ILE	A	28	31.929	-3.045	18.576	1.00	26.61	B	O
ATOM	189	N	MET	A	29	30.208	-4.459	18.794	1.00	26.83	B	N
ATOM	190	CA	MET	A	29	30.136	-4.286	20.259	1.00	25.03	B	C
ATOM	191	CB	MET	A	29	28.940	-5.059	20.802	1.00	23.41	B	C
ATOM	192	CG	MET	A	29	27.661	-4.579	20.269	1.00	23.89	B	C
ATOM	193	SD	MET	A	29	26.244	-5.532	20.857	1.00	27.71	B	S
ATOM	194	CE	MET	A	29	26.623	-5.693	22.569	1.00	30.96	B	C
ATOM	195	C	MET	A	29	31.424	-4.707	20.955	1.00	25.76	B	C
ATOM	196	O	MET	A	29	31.846	-4.123	21.980	1.00	26.76	B	O
ATOM	197	N	LYS	A	30	32.074	-5.768	20.452	1.00	27.82	B	N
ATOM	198	CA	LYS	A	30	33.391	-6.173	20.963	1.00	28.23	B	C
ATOM	199	CB	LYS	A	30	33.675	-7.614	20.491	1.00	31.57	B	C
ATOM	200	CG	LYS	A	30	32.707	-8.624	21.084	1.00	34.50	B	C
ATOM	201	CD	LYS	A	30	33.123	-10.057	20.838	1.00	40.02	B	C
ATOM	202	CE	LYS	A	30	32.654	-10.562	19.501	1.00	45.94	B	C
ATOM	203	NZ	LYS	A	30	33.387	-11.802	19.146	1.00	49.41	B	N
ATOM	204	C	LYS	A	30	34.508	-5.248	20.461	1.00	24.44	B	C
ATOM	205	O	LYS	A	30	35.471	-5.097	21.158	1.00	27.36	B	O
ATOM	206	N	CYS	A	31	34.370	-4.718	19.262	1.00	25.71	B	N
ATOM	207	CA	CYS	A	31	35.395	-3.865	18.601	1.00	27.49	B	C
ATOM	208	CB	CYS	A	31	35.014	-3.626	17.150	1.00	27.39	B	C
ATOM	209	SG	CYS	A	31	36.380	-2.880	16.206	1.00	36.07	B	S
ATOM	210	C	CYS	A	31	35.566	-2.541	19.385	1.00	25.80	B	C
ATOM	211	O	CYS	A	31	36.656	-2.055	19.433	1.00	26.84	B	O
ATOM	212	N	VAL	A	32	34.538	-2.053	20.103	1.00	24.70	B	N
ATOM	213	CA	VAL	A	32	34.583	-0.643	20.638	1.00	23.41	B	C
ATOM	214	CB	VAL	A	32	33.256	0.091	20.365	1.00	21.28	B	C
ATOM	215	CG1	VAL	A	32	32.959	0.271	18.900	1.00	22.24	B	C
ATOM	216	CG2	VAL	A	32	32.108	-0.621	21.091	1.00	22.78	B	C
ATOM	217	C	VAL	A	32	34.962	-0.634	22.101	1.00	21.84	B	C
ATOM	218	O	VAL	A	32	35.055	0.467	22.708	1.00	24.38	B	O
ATOM	219	N	THR	A	33	35.295	-1.778	22.720	1.00	21.94	B	N
ATOM	220	CA	THR	A	33	35.765	-1.885	24.105	1.00	20.84	B	C
ATOM	221	CB	THR	A	33	34.609	-2.094	25.106	1.00	23.14	B	C
ATOM	222	OG1	THR	A	33	35.161	-2.147	26.414	1.00	20.93	B	O
ATOM	223	CG2	THR	A	33	33.735	-3.307	24.788	1.00	23.76	B	C
ATOM	224	C	THR	A	33	36.781	-3.016	24.231	1.00	24.68	B	C
ATOM	225	O	THR	A	33	36.575	-4.086	23.651	1.00	24.71	B	O
ATOM	226	N	PRO	A	34	37.848	-2.811	25.003	1.00	24.49	B	N
ATOM	227	CA	PRO	A	34	38.800	-3.891	25.300	1.00	26.16	B	C
ATOM	228	CB	PRO	A	34	39.988	-3.189	25.925	1.00	28.59	B	C
ATOM	229	CG	PRO	A	34	39.419	-1.904	26.503	1.00	25.40	B	C
ATOM	230	CD	PRO	A	34	38.332	-1.517	25.520	1.00	25.83	B	C

Fig. 5 (cont.)

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ATOM	231	C	PRO	A	34	38.225	-4.871	26.315	1.00	28.57	B	C
ATOM	232	O	PRO	A	34	38.700	-5.994	26.374	1.00	28.67	B	O
ATOM	233	N	ASP	A	35	37.155	-4.494	26.996	1.00	25.86	B	N
ATOM	234	CA	ASP	A	35	36.575	-5.243	28.115	1.00	26.24	B	C
ATOM	235	CB	ASP	A	35	36.077	-4.252	29.181	1.00	28.35	B	C
ATOM	236	CG	ASP	A	35	37.219	-3.446	29.775	1.00	33.11	B	C
ATOM	237	OD1	ASP	A	35	38.228	-4.086	30.163	1.00	37.82	B	O
ATOM	238	OD2	ASP	A	35	37.179	-2.209	29.696	1.00	32.82	B	O
ATOM	239	C	ASP	A	35	35.479	-6.204	27.618	1.00	20.75	B	C
ATOM	240	O	ASP	A	35	35.085	-6.179	26.443	1.00	25.38	B	O
ATOM	241	N	GLN	A	36	35.105	-7.098	28.533	1.00	23.58	B	N
ATOM	242	CA	GLN	A	36	34.182	-8.213	28.236	1.00	22.81	B	C
ATOM	243	CB	GLN	A	36	34.627	-9.493	28.954	1.00	26.53	B	C
ATOM	244	CG	GLN	A	36	36.088	-9.876	28.720	1.00	28.33	B	C
ATOM	245	CD	GLN	A	36	36.406	-10.038	27.255	1.00	26.87	B	C
ATOM	246	OE1	GLN	A	36	35.655	-10.590	26.455	1.00	32.61	B	O
ATOM	247	NE2	GLN	A	36	37.508	-9.396	26.865	1.00	31.19	B	N
ATOM	248	C	GLN	A	36	32.743	-7.870	28.654	1.00	19.61	B	C
ATOM	249	O	GLN	A	36	32.544	-7.464	29.842	1.00	23.42	B	O
ATOM	250	N	TRP	A	37	31.823	-8.070	27.759	1.00	20.37	B	N
ATOM	251	CA	TRP	A	37	30.409	-7.831	28.094	1.00	21.36	B	C
ATOM	252	CB	TRP	A	37	29.601	-7.755	26.835	1.00	20.66	B	C
ATOM	253	CG	TRP	A	37	29.923	-6.532	26.021	1.00	21.31	B	C
ATOM	254	CD1	TRP	A	37	30.803	-6.429	24.992	1.00	23.78	B	C
ATOM	255	NE1	TRP	A	37	30.820	-5.144	24.495	1.00	23.75	B	N
ATOM	256	CE2	TRP	A	37	29.905	-4.400	25.202	1.00	21.99	B	C
ATOM	257	CD2	TRP	A	37	29.316	-5.229	26.175	1.00	22.24	B	C
ATOM	258	CE3	TRP	A	37	28.360	-4.710	27.052	1.00	20.49	B	C
ATOM	259	CZ3	TRP	A	37	27.998	-3.375	26.918	1.00	21.26	B	C
ATOM	260	CH2	TRP	A	37	28.570	-2.579	25.932	1.00	22.38	B	C
ATOM	261	CZ2	TRP	A	37	29.553	-3.057	25.083	1.00	22.90	B	C
ATOM	262	C	TRP	A	37	29.906	-8.930	29.028	1.00	22.60	B	C
ATOM	263	O	TRP	A	37	30.127	-10.124	28.775	1.00	23.43	B	O
ATOM	264	N	PRO	A	38	29.196	-8.627	30.095	1.00	20.66	B	N
ATOM	265	CA	PRO	A	38	28.585	-9.648	30.957	1.00	21.26	B	C
ATOM	266	CB	PRO	A	38	27.999	-8.921	32.142	1.00	25.92	B	C
ATOM	267	CG	PRO	A	38	28.203	-7.457	31.862	1.00	24.54	B	C
ATOM	268	CD	PRO	A	38	28.919	-7.255	30.558	1.00	20.67	B	C
ATOM	269	C	PRO	A	38	27.460	-10.348	30.207	1.00	20.37	B	C
ATOM	270	O	PRO	A	38	26.839	-9.828	29.321	1.00	20.30	B	O
ATOM	271	N	GLN	A	39	27.300	-11.644	30.510	1.00	20.17	B	N
ATOM	272	CA	GLN	A	39	26.372	-12.555	29.824	1.00	19.07	B	C
ATOM	273	CB	GLN	A	39	27.073	-13.885	29.565	1.00	21.09	B	C
ATOM	274	CG	GLN	A	39	28.274	-13.651	28.636	1.00	22.15	B	C
ATOM	275	CD	GLN	A	39	29.181	-14.831	28.419	1.00	25.54	B	C
ATOM	276	OE1	GLN	A	39	29.475	-15.596	29.340	1.00	23.06	B	O
ATOM	277	NE2	GLN	A	39	29.648	-14.928	27.189	1.00	26.55	B	N
ATOM	278	C	GLN	A	39	25.117	-12.754	30.651	1.00	15.99	B	C
ATOM	279	O	GLN	A	39	25.179	-12.680	31.893	1.00	18.63	B	O
ATOM	280	N	TRP	A	40	24.003	-12.978	29.977	1.00	19.06	B	N
ATOM	281	CA	TRP	A	40	22.658	-13.084	30.573	1.00	17.57	B	C
ATOM	282	CB	TRP	A	40	21.644	-12.213	29.812	1.00	18.88	B	C
ATOM	283	CG	TRP	A	40	21.962	-10.734	29.850	1.00	16.98	B	C
ATOM	284	CD1	TRP	A	40	23.067	-10.111	29.377	1.00	18.54	B	C
ATOM	285	NE1	TRP	A	40	22.998	-8.751	29.662	1.00	18.15	B	N
ATOM	286	CE2	TRP	A	40	21.829	-8.518	30.303	1.00	18.74	B	C
ATOM	287	CD2	TRP	A	40	21.138	-9.744	30.487	1.00	17.90	B	C
ATOM	288	CE3	TRP	A	40	19.873	-9.760	31.071	1.00	17.60	B	C

Fig. 5 (cont.)

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ATOM	289	CZ3	TRP	A	40	19.360	-8.568	31.546	1.00	18.65	B	C
ATOM	290	CH2	TRP	A	40	20.060	-7.365	31.366	1.00	18.29	B	C
ATOM	291	CZ2	TRP	A	40	21.284	-7.308	30.758	1.00	18.10	B	C
ATOM	292	C	TRP	A	40	22.259	-14.577	30.625	1.00	19.04	B	C
ATOM	293	O	TRP	A	40	22.963	-15.436	30.006	1.00	21.88	B	O
ATOM	294	N	AASP	A	41	21.205	-14.923	31.320	0.50	17.59	B	N
ATOM	295	N	BASP	A	41	21.155	-14.849	31.296	0.50	18.21	B	N
ATOM	296	CA	AASP	A	41	20.829	-16.367	31.404	0.50	17.73	B	C
ATOM	297	CA	BASP	A	41	20.673	-16.216	31.654	0.50	19.92	B	C
ATOM	298	CB	AASP	A	41	20.989	-16.929	32.821	0.50	19.22	B	C
ATOM	299	CB	BASP	A	41	20.436	-16.277	33.169	0.50	20.61	B	C
ATOM	300	CG	AASP	A	41	20.033	-16.304	33.815	0.50	20.61	B	C
ATOM	301	CG	BASP	A	41	21.708	-16.081	33.984	0.50	23.80	B	C
ATOM	302	OD1AASP	A	41	19.087	-15.599	33.384	0.50	19.46	B	O	
ATOM	303	OD1BASP	A	41	22.652	-16.895	33.835	0.50	27.27	B	O	
ATOM	304	OD2AASP	A	41	20.207	-16.594	35.035	0.50	24.45	B	O	
ATOM	305	OD2BASP	A	41	21.780	-15.065	34.712	0.50	27.66	B	O	
ATOM	306	C	AASP	A	41	19.424	-16.566	30.816	0.50	18.97	B	C
ATOM	307	C	BASP	A	41	19.412	-16.550	30.837	0.50	19.77	B	C
ATOM	308	O	AASP	A	41	18.738	-17.570	31.143	0.50	19.47	B	O
ATOM	309	O	BASP	A	41	18.799	-17.615	31.055	0.50	20.48	B	O
ATOM	310	N	VAL	A	42	18.998	-15.687	29.903	1.00	17.64	B	N
ATOM	311	CA	VAL	A	42	17.844	-15.891	29.031	1.00	16.66	B	C
ATOM	312	CB	VAL	A	42	16.620	-15.038	29.443	1.00	17.15	B	C
ATOM	313	CG1	VAL	A	42	16.120	-15.331	30.816	1.00	18.26	B	C
ATOM	314	CG2	VAL	A	42	16.933	-13.567	29.282	1.00	18.80	B	C
ATOM	315	C	VAL	A	42	18.274	-15.611	27.609	1.00	16.53	B	C
ATOM	316	O	VAL	A	42	19.296	-14.955	27.387	1.00	19.14	B	O
ATOM	317	N	PRO	A	43	17.522	-16.024	26.583	1.00	17.55	B	N
ATOM	318	CA	PRO	A	43	17.873	-15.691	25.226	1.00	17.88	B	C
ATOM	319	CB	PRO	A	43	16.753	-16.336	24.386	1.00	19.72	B	C
ATOM	320	CG	PRO	A	43	16.205	-17.446	25.272	1.00	19.88	B	C
ATOM	321	CD	PRO	A	43	16.348	-16.876	26.676	1.00	18.61	B	C
ATOM	322	C	PRO	A	43	17.903	-14.163	24.943	1.00	18.81	B	C
ATOM	323	O	PRO	A	43	17.237	-13.397	25.622	1.00	18.70	B	O
ATOM	324	N	LEU	A	44	18.718	-13.806	23.979	1.00	18.24	B	N
ATOM	325	CA	LEU	A	44	18.801	-12.389	23.613	1.00	18.85	B	C
ATOM	326	CB	LEU	A	44	19.754	-12.232	22.435	1.00	21.23	B	C
ATOM	327	CG	LEU	A	44	21.230	-12.554	22.646	1.00	22.61	B	C
ATOM	328	CD1	LEU	A	44	21.992	-12.304	21.380	1.00	27.29	B	C
ATOM	329	CD2	LEU	A	44	21.872	-11.777	23.769	1.00	27.76	B	C
ATOM	330	C	LEU	A	44	17.416	-11.873	23.299	1.00	19.06	B	C
ATOM	331	O	LEU	A	44	16.596	-12.535	22.640	1.00	19.24	B	O
ATOM	332	N	GLY	A	45	17.110	-10.688	23.844	1.00	18.13	B	N
ATOM	333	CA	GLY	A	45	15.817	-10.059	23.573	1.00	18.68	B	C
ATOM	334	C	GLY	A	45	14.687	-10.554	24.433	1.00	18.44	B	C
ATOM	335	O	GLY	A	45	13.563	-10.058	24.227	1.00	19.91	B	O
ATOM	336	N	TRP	A	46	14.919	-11.519	25.331	1.00	17.62	B	N
ATOM	337	CA	TRP	A	46	13.831	-12.056	26.159	1.00	16.62	B	C
ATOM	338	CB	TRP	A	46	14.025	-13.573	26.348	1.00	16.16	B	C
ATOM	339	CG	TRP	A	46	13.627	-14.369	25.152	1.00	17.80	B	C
ATOM	340	CD1	TRP	A	46	13.786	-14.064	23.833	1.00	20.29	B	C
ATOM	341	NE1	TRP	A	46	13.251	-15.058	23.047	1.00	20.67	B	N
ATOM	342	CE2	TRP	A	46	12.723	-16.029	23.857	1.00	18.66	B	C
ATOM	343	CD2	TRP	A	46	12.981	-15.672	25.197	1.00	17.30	B	C
ATOM	344	CE3	TRP	A	46	12.527	-16.462	26.250	1.00	17.10	B	C
ATOM	345	CZ3	TRP	A	46	11.905	-17.655	25.941	1.00	20.68	B	C
ATOM	346	CH2	TRP	A	46	11.679	-18.009	24.626	1.00	19.78	B	C

Fig. 5 (cont.)

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ATOM	347	CZ2	TRP	A	46	12.073	-17.238	23.559	1.00	19.41	B	C
ATOM	348	C	TRP	A	46	13.710	-11.337	27.497	1.00	15.65	B	C
ATOM	349	O	TRP	A	46	14.695	-10.956	28.080	1.00	16.90	B	O
ATOM	350	N	LEU	A	47	12.471	-11.222	27.947	1.00	15.26	B	N
ATOM	351	CA	LEU	A	47	12.126	-10.759	29.269	1.00	15.54	B	C
ATOM	352	CB	LEU	A	47	10.617	-10.746	29.493	1.00	17.70	B	C
ATOM	353	CG	LEU	A	47	10.096	-10.088	30.739	1.00	15.87	B	C
ATOM	354	CD1	LEU	A	47	10.141	-8.559	30.616	1.00	18.79	B	C
ATOM	355	CD2	LEU	A	47	8.703	-10.472	31.005	1.00	18.46	B	C
ATOM	356	C	LEU	A	47	12.805	-11.641	30.278	1.00	16.65	B	C
ATOM	357	O	LEU	A	47	12.748	-12.893	30.105	1.00	17.97	B	O
ATOM	358	N	THR	A	48	13.434	-11.118	31.304	1.00	16.61	B	N
ATOM	359	CA	THR	A	48	13.995	-11.924	32.401	1.00	15.66	B	C
ATOM	360	CB	THR	A	48	15.213	-11.277	33.044	1.00	17.09	B	C
ATOM	361	OG1	THR	A	48	14.761	-10.095	33.724	1.00	16.93	B	O
ATOM	362	CG2	THR	A	48	16.296	-10.976	32.064	1.00	17.68	B	C
ATOM	363	C	THR	A	48	12.949	-12.160	33.466	1.00	16.56	B	C
ATOM	364	O	THR	A	48	11.942	-11.478	33.594	1.00	16.09	B	O
ATOM	365	N	PRO	A	49	13.113	-13.208	34.325	1.00	17.34	B	N
ATOM	366	CA	PRO	A	49	12.226	-13.319	35.475	1.00	16.97	B	C
ATOM	367	CB	PRO	A	49	12.788	-14.563	36.203	1.00	18.35	B	C
ATOM	368	CG	PRO	A	49	13.364	-15.359	35.078	1.00	19.48	B	C
ATOM	369	CD	PRO	A	49	14.010	-14.375	34.112	1.00	18.77	B	C
ATOM	370	C	PRO	A	49	12.084	-12.089	36.377	1.00	16.86	B	C
ATOM	371	O	PRO	A	49	11.000	-11.808	36.763	1.00	17.49	B	O
ATOM	372	N	ARG	A	50	13.216	-11.449	36.629	1.00	17.63	B	N
ATOM	373	CA	ARG	A	50	13.156	-10.200	37.402	1.00	17.95	B	C
ATOM	374	CB	ARG	A	50	14.563	-9.697	37.626	1.00	20.13	B	C
ATOM	375	CG	ARG	A	50	14.635	-8.287	38.151	1.00	30.72	B	C
ATOM	376	CD	ARG	A	50	16.034	-8.328	38.694	1.00	41.58	B	C
ATOM	377	NE	ARG	A	50	16.498	-7.060	39.146	1.00	48.22	B	N
ATOM	378	CZ	ARG	A	50	17.231	-6.924	40.221	1.00	43.88	B	C
ATOM	379	NH1	ARG	A	50	17.559	-7.967	40.999	1.00	40.29	B	N
ATOM	380	NH2	ARG	A	50	17.620	-5.717	40.493	1.00	42.38	B	N
ATOM	381	C	ARG	A	50	12.325	-9.161	36.636	1.00	16.26	B	C
ATOM	382	O	ARG	A	50	11.512	-8.494	37.271	1.00	19.14	B	O
ATOM	383	N	GLY	A	51	12.505	-9.104	35.321	1.00	16.05	B	N
ATOM	384	CA	GLY	A	51	11.681	-8.207	34.472	1.00	16.34	B	C
ATOM	385	C	GLY	A	51	10.212	-8.505	34.635	1.00	15.03	B	C
ATOM	386	O	GLY	A	51	9.380	-7.612	34.786	1.00	16.76	B	O
ATOM	387	N	CYS	A	52	9.871	-9.805	34.667	1.00	15.20	B	N
ATOM	388	CA	CYS	A	52	8.498	-10.217	34.877	1.00	16.41	B	C
ATOM	389	CB	CYS	A	52	8.428	-11.752	34.765	1.00	16.79	B	C
ATOM	390	SG	CYS	A	52	6.804	-12.503	35.066	1.00	18.95	B	S
ATOM	391	C	CYS	A	52	7.990	-9.757	36.262	1.00	16.30	B	C
ATOM	392	O	CYS	A	52	6.858	-9.236	36.378	1.00	17.45	B	O
ATOM	393	N	GLU	A	53	8.762	-10.013	37.324	1.00	16.82	B	N
ATOM	394	CA	GLU	A	53	8.338	-9.612	38.666	1.00	17.81	B	C
ATOM	395	CB	GLU	A	53	9.396	-10.088	39.642	1.00	20.10	B	C
ATOM	396	CG	GLU	A	53	9.032	-9.775	41.066	1.00	21.92	B	C
ATOM	397	CD	GLU	A	53	9.972	-10.366	42.099	1.00	24.07	B	C
ATOM	398	OE1	GLU	A	53	10.915	-11.018	41.718	1.00	26.05	B	O
ATOM	399	OE2	GLU	A	53	9.670	-10.172	43.314	1.00	25.80	B	O
ATOM	400	C	GLU	A	53	8.143	-8.072	38.693	1.00	16.18	B	C
ATOM	401	O	GLU	A	53	7.173	-7.659	39.293	1.00	17.52	B	O
ATOM	402	N	LEU	A	54	9.088	-7.308	38.120	1.00	16.78	B	N
ATOM	403	CA	LEU	A	54	8.945	-5.829	38.146	1.00	16.79	B	C
ATOM	404	CB	LEU	A	54	10.184	-5.225	37.510	1.00	16.62	B	C

Fig. 5 (cont.)

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ATOM	405	CG	LEU	A	54	11.445	-5.266	38.358	1.00	17.80	B	C
ATOM	406	CD1	LEU	A	54	12.632	-4.863	37.535	1.00	18.18	B	C
ATOM	407	CD2	LEU	A	54	11.255	-4.338	39.590	1.00	18.09	B	C
ATOM	408	C	LEU	A	54	7.657	-5.389	37.461	1.00	17.06	B	C
ATOM	409	O	LEU	A	54	6.956	-4.500	37.928	1.00	18.24	B	O
ATOM	410	N	VAL	A	55	7.339	-6.039	36.334	1.00	15.71	B	N
ATOM	411	CA	VAL	A	55	6.057	-5.721	35.673	1.00	16.60	B	C
ATOM	412	CB	VAL	A	55	5.942	-6.342	34.261	1.00	17.49	B	C
ATOM	413	CG1	VAL	A	55	4.554	-6.257	33.710	1.00	17.74	B	C
ATOM	414	CG2	VAL	A	55	7.001	-5.736	33.331	1.00	17.97	B	C
ATOM	415	C	VAL	A	55	4.888	-6.100	36.574	1.00	16.61	B	C
ATOM	416	O	VAL	A	55	3.827	-5.435	36.661	1.00	16.79	B	O
ATOM	417	N	SER	A	56	4.961	-7.294	37.190	1.00	17.39	B	N
ATOM	418	CA	SER	A	56	3.884	-7.707	38.111	1.00	18.81	B	C
ATOM	419	CB	SER	A	56	4.158	-9.128	38.606	1.00	19.13	B	C
ATOM	420	OG	SER	A	56	5.058	-9.136	39.699	1.00	23.71	B	O
ATOM	421	C	SER	A	56	3.691	-6.707	39.272	1.00	17.17	B	C
ATOM	422	O	SER	A	56	2.560	-6.507	39.669	1.00	18.06	B	O
ATOM	423	N	TYR	A	57	4.755	-6.072	39.730	1.00	18.44	B	N
ATOM	424	CA	TYR	A	57	4.630	-5.015	40.756	1.00	18.13	B	C
ATOM	425	CB	TYR	A	57	5.967	-4.535	41.255	1.00	20.27	B	C
ATOM	426	CG	TYR	A	57	6.715	-5.500	42.143	1.00	22.90	B	C
ATOM	427	CD1	TYR	A	57	6.235	-6.748	42.450	1.00	24.55	B	C
ATOM	428	CE1	TYR	A	57	6.938	-7.602	43.291	1.00	30.29	B	C
ATOM	429	CZ	TYR	A	57	8.139	-7.201	43.804	1.00	31.54	B	C
ATOM	430	OH	TYR	A	57	8.794	-8.089	44.623	1.00	36.51	B	O
ATOM	431	CE2	TYR	A	57	8.651	-5.953	43.497	1.00	36.25	B	C
ATOM	432	CD2	TYR	A	57	7.951	-5.136	42.624	1.00	31.24	B	C
ATOM	433	C	TYR	A	57	3.845	-3.816	40.223	1.00	19.16	B	C
ATOM	434	O	TYR	A	57	3.036	-3.271	40.960	1.00	20.24	B	O
ATOM	435	N	LEU	A	58	4.027	-3.464	38.945	1.00	18.49	B	N
ATOM	436	CA	LEU	A	58	3.172	-2.392	38.337	1.00	17.29	B	C
ATOM	437	CB	LEU	A	58	3.699	-1.990	36.948	1.00	18.17	B	C
ATOM	438	CG	LEU	A	58	5.021	-1.266	37.046	1.00	19.32	B	C
ATOM	439	CD1	LEU	A	58	5.622	-1.089	35.651	1.00	19.59	B	C
ATOM	440	CD2	LEU	A	58	4.828	0.087	37.692	1.00	21.94	B	C
ATOM	441	C	LEU	A	58	1.754	-2.857	38.242	1.00	16.92	B	C
ATOM	442	O	LEU	A	58	0.817	-2.101	38.462	1.00	18.27	B	O
ATOM	443	N	GLY	A	59	1.523	-4.147	37.937	1.00	17.46	B	N
ATOM	444	CA	GLY	A	59	0.168	-4.664	37.943	1.00	17.46	B	C
ATOM	445	C	GLY	A	59	-0.472	-4.612	39.339	1.00	17.89	B	C
ATOM	446	O	GLY	A	59	-1.670	-4.258	39.424	1.00	18.79	B	O
ATOM	447	N	GLN	A	60	0.284	-4.956	40.365	1.00	19.51	B	N
ATOM	448	CA	GLN	A	60	-0.236	-4.871	41.769	1.00	20.97	B	C
ATOM	449	CB	GLN	A	60	0.800	-5.426	42.750	1.00	23.82	B	C
ATOM	450	CG	GLN	A	60	0.973	-6.932	42.636	1.00	23.63	B	C
ATOM	451	CD	GLN	A	60	1.900	-7.508	43.686	1.00	33.66	B	C
ATOM	452	OE1	GLN	A	60	3.066	-7.164	43.788	1.00	34.90	B	O
ATOM	453	NE2	GLN	A	60	1.379	-8.456	44.431	1.00	40.19	B	N
ATOM	454	C	GLN	A	60	-0.524	-3.406	42.121	1.00	21.60	B	C
ATOM	455	O	GLN	A	60	-1.627	-3.115	42.662	1.00	23.62	B	O
ATOM	456	N	TYR	A	61	0.350	-2.478	41.765	1.00	20.93	B	N
ATOM	457	CA	TYR	A	61	0.162	-1.033	42.014	1.00	22.73	B	C
ATOM	458	CB	TYR	A	61	1.402	-0.251	41.584	1.00	22.35	B	C
ATOM	459	CG	TYR	A	61	1.154	1.234	41.533	1.00	24.65	B	C
ATOM	460	CD1	TYR	A	61	1.121	1.946	42.730	1.00	27.76	B	C
ATOM	461	CE1	TYR	A	61	0.780	3.294	42.749	1.00	31.75	B	C
ATOM	462	CZ	TYR	A	61	0.484	3.930	41.558	1.00	30.83	B	C

Fig. 5 (cont.)

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ATOM	463	OH	TYR	A	61	0.187	5.268	41.588	1.00	41.60	B	O
ATOM	464	CE2	TYR	A	61	0.503	3.246	40.361	1.00	30.22	B	C
ATOM	465	CD2	TYR	A	61	0.793	1.888	40.359	1.00	27.07	B	C
ATOM	466	C	TYR	A	61	-1.067	-0.520	41.280	1.00	22.70	B	C
ATOM	467	O	TYR	A	61	-1.944	0.134	41.875	1.00	21.30	B	O
ATOM	468	N	GLN	A	62	-1.250	-0.881	39.992	1.00	20.71	B	N
ATOM	469	CA	GLN	A	62	-2.413	-0.431	39.207	1.00	21.72	B	C
ATOM	470	CB	GLN	A	62	-2.220	-0.690	37.704	1.00	21.11	B	C
ATOM	471	CG	GLN	A	62	-1.048	0.105	37.112	1.00	20.68	B	C
ATOM	472	CD	GLN	A	62	-1.351	1.574	36.947	1.00	21.08	B	C
ATOM	473	OE1	GLN	A	62	-2.182	2.159	37.660	1.00	23.84	B	O
ATOM	474	NE2	GLN	A	62	-0.643	2.192	36.023	1.00	20.65	B	N
ATOM	475	C	GLN	A	62	-3.714	-1.031	39.754	1.00	20.59	B	C
ATOM	476	O	GLN	A	62	-4.736	-0.360	39.755	1.00	21.95	B	O
ATOM	477	N	AARG	A	63	-3.695	-2.291	40.195	0.50	21.81	B	N
ATOM	478	N	BARG	A	63	-3.677	-2.285	40.199	0.50	22.47	B	N
ATOM	479	CA	AARG	A	63	-4.909	-2.901	40.795	0.50	21.02	B	C
ATOM	480	CA	BARG	A	63	-4.882	-2.896	40.800	0.50	22.28	B	C
ATOM	481	CB	AARG	A	63	-4.712	-4.392	41.113	0.50	21.51	B	C
ATOM	482	CB	BARG	A	63	-4.659	-4.360	41.186	0.50	24.05	B	C
ATOM	483	CG	AARG	A	63	-5.944	-5.083	41.706	0.50	22.20	B	C
ATOM	484	CG	BARG	A	63	-5.882	-4.996	41.843	0.50	26.19	B	C
ATOM	485	CD	AARG	A	63	-5.636	-6.456	42.313	0.50	26.21	B	C
ATOM	486	CD	BARG	A	63	-7.134	-4.936	40.977	0.50	30.47	B	C
ATOM	487	NE	AARG	A	63	-6.785	-7.169	42.855	0.50	32.01	B	N
ATOM	488	NE	BARG	A	63	-8.036	-6.077	41.163	0.50	35.42	B	N
ATOM	489	CZ	AARG	A	63	-7.682	-7.892	42.171	0.50	35.51	B	C
ATOM	490	CZ	BARG	A	63	-9.231	-6.069	41.744	0.50	39.28	B	C
ATOM	491	NH1AARG	A	63	-7.705	-7.957	40.840	0.50	30.88	B	N	
ATOM	492	NH1BARG	A	63	-9.902	-7.207	41.812	0.50	35.41	B	N	
ATOM	493	NH2AARG	A	63	-8.625	-8.508	42.864	0.50	33.49	B	N	
ATOM	494	NH2BARG	A	63	-9.746	-4.962	42.262	0.50	39.30	B	N	
ATOM	495	C	AARG	A	63	-5.278	-2.083	42.036	0.50	20.52	B	C
ATOM	496	C	BARG	A	63	-5.273	-2.072	42.026	0.50	21.05	B	C
ATOM	497	O	AARG	A	63	-6.461	-1.763	42.175	0.50	22.27	B	O
ATOM	498	O	BARG	A	63	-6.463	-1.759	42.148	0.50	22.44	B	O
ATOM	499	N	LEU	A	64	-4.330	-1.747	42.884	1.00	21.84	B	N
ATOM	500	CA	LEU	A	64	-4.658	-0.947	44.098	1.00	23.30	B	C
ATOM	501	CB	LEU	A	64	-3.434	-0.783	44.962	1.00	23.80	B	C
ATOM	502	CG	LEU	A	64	-2.969	-2.075	45.620	1.00	28.58	B	C
ATOM	503	CD1	LEU	A	64	-1.579	-1.882	46.166	1.00	30.76	B	C
ATOM	504	CD2	LEU	A	64	-3.918	-2.487	46.753	1.00	30.73	B	C
ATOM	505	C	LEU	A	64	-5.232	0.402	43.672	1.00	24.94	B	C
ATOM	506	O	LEU	A	64	-6.281	0.833	44.208	1.00	26.72	B	O
ATOM	507	N	TRP	A	65	-4.562	1.086	42.747	1.00	22.92	B	N
ATOM	508	CA	TRP	A	65	-4.985	2.436	42.333	1.00	25.39	B	C
ATOM	509	CB	TRP	A	65	-3.892	3.001	41.410	1.00	29.33	B	C
ATOM	510	CG	TRP	A	65	-3.986	4.478	41.393	1.00	38.65	B	C
ATOM	511	CD1	TRP	A	65	-3.687	5.351	42.402	1.00	43.02	B	C
ATOM	512	NE1	TRP	A	65	-4.033	6.619	42.034	1.00	42.92	B	N
ATOM	513	CE2	TRP	A	65	-4.544	6.583	40.769	1.00	43.76	B	C
ATOM	514	CD2	TRP	A	65	-4.594	5.236	40.359	1.00	38.69	B	C
ATOM	515	CE3	TRP	A	65	-5.052	4.908	39.092	1.00	37.01	B	C
ATOM	516	CZ3	TRP	A	65	-5.530	5.922	38.313	1.00	40.01	B	C
ATOM	517	CH2	TRP	A	65	-5.502	7.254	38.740	1.00	45.81	B	C
ATOM	518	CZ2	TRP	A	65	-4.996	7.620	39.964	1.00	41.83	B	C
ATOM	519	C	TRP	A	65	-6.367	2.363	41.688	1.00	24.46	B	C
ATOM	520	O	TRP	A	65	-7.296	3.143	42.102	1.00	26.04	B	O

Fig. 5 (cont.)

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ATOM	521	N	PHE	A	66	-6.577	1.511	40.685	1.00	22.90	B	N
ATOM	522	CA	PHE	A	66	-7.840	1.405	39.946	1.00	25.27	B	C
ATOM	523	CB	PHE	A	66	-7.736	0.472	38.731	1.00	27.18	B	C
ATOM	524	CG	PHE	A	66	-6.792	0.958	37.658	1.00	29.82	B	C
ATOM	525	CD1	PHE	A	66	-6.595	2.311	37.438	1.00	32.75	B	C
ATOM	526	CE1	PHE	A	66	-5.700	2.755	36.470	1.00	36.97	B	C
ATOM	527	CZ	PHE	A	66	-5.016	1.837	35.711	1.00	34.02	B	C
ATOM	528	CD2	PHE	A	66	-6.091	0.042	36.890	1.00	32.84	B	C
ATOM	529	CE2	PHE	A	66	-5.253	0.486	35.876	1.00	30.85	B	C
ATOM	530	C	PHE	A	66	-8.953	1.005	40.916	1.00	27.84	B	C
ATOM	531	O	PHE	A	66	-10.078	1.490	40.730	1.00	28.32	B	O
ATOM	532	N	THR	A	67	-8.686	0.100	41.854	1.00	26.52	B	N
ATOM	533	CA	THR	A	67	-9.711	-0.339	42.856	1.00	26.80	B	C
ATOM	534	CB	THR	A	67	-9.244	-1.597	43.596	1.00	29.15	B	C
ATOM	535	OG1	THR	A	67	-9.139	-2.638	42.618	1.00	33.08	B	O
ATOM	536	CG2	THR	A	67	-10.242	-1.956	44.676	1.00	31.71	B	C
ATOM	537	C	THR	A	67	-10.047	0.853	43.763	1.00	28.30	B	C
ATOM	538	O	THR	A	67	-11.274	1.092	43.965	1.00	29.58	B	O
ATOM	539	N	SER	A	68	-9.052	1.617	44.205	1.00	26.40	B	N
ATOM	540	CA	SER	A	68	-9.257	2.805	45.089	1.00	28.27	B	C
ATOM	541	CB	SER	A	68	-7.961	3.382	45.551	1.00	28.96	B	C
ATOM	542	OG	SER	A	68	-7.396	4.223	44.547	1.00	34.58	B	O
ATOM	543	C	SER	A	68	-10.133	3.840	44.366	1.00	33.89	B	C
ATOM	544	O	SER	A	68	-10.940	4.528	45.021	1.00	32.70	B	O
ATOM	545	N	LYS	A	69	-10.045	3.955	43.045	1.00	31.80	B	N
ATOM	546	CA	LYS	A	69	-10.888	4.891	42.255	1.00	30.45	B	C
ATOM	547	CB	LYS	A	69	-10.087	5.374	41.037	1.00	31.62	B	C
ATOM	548	CG	LYS	A	69	-8.743	5.966	41.382	1.00	33.45	B	C
ATOM	549	CD	LYS	A	69	-8.842	7.212	42.239	1.00	37.70	B	C
ATOM	550	CE	LYS	A	69	-7.486	7.748	42.631	1.00	44.56	B	C
ATOM	551	NZ	LYS	A	69	-7.591	8.795	43.674	1.00	50.99	B	N
ATOM	552	C	LYS	A	69	-12.198	4.250	41.789	1.00	30.94	B	C
ATOM	553	O	LYS	A	69	-12.916	4.908	41.056	1.00	34.24	B	O
ATOM	554	N	GLY	A	70	-12.519	3.009	42.135	1.00	32.18	B	N
ATOM	555	CA	GLY	A	70	-13.791	2.364	41.770	1.00	35.49	B	C
ATOM	556	C	GLY	A	70	-13.838	1.861	40.340	1.00	39.62	B	C
ATOM	557	O	GLY	A	70	-14.910	1.431	39.919	1.00	37.95	B	O
ATOM	558	N	LEU	A	71	-12.726	1.892	39.596	1.00	36.98	B	N
ATOM	559	CA	LEU	A	71	-12.704	1.363	38.211	1.00	35.32	B	C
ATOM	560	CB	LEU	A	71	-11.411	1.807	37.529	1.00	32.31	B	C
ATOM	561	CG	LEU	A	71	-11.294	1.305	36.094	1.00	33.79	B	C
ATOM	562	CD1	LEU	A	71	-12.302	1.990	35.197	1.00	35.20	B	C
ATOM	563	CD2	LEU	A	71	-9.888	1.491	35.574	1.00	34.48	B	C
ATOM	564	C	LEU	A	71	-12.818	-0.169	38.219	1.00	34.59	B	C
ATOM	565	O	LEU	A	71	-13.417	-0.743	37.281	1.00	38.90	B	O
ATOM	566	N	LEU	A	72	-12.138	-0.836	39.144	1.00	34.02	B	N
ATOM	567	CA	LEU	A	72	-12.130	-2.312	39.208	1.00	34.64	B	C
ATOM	568	CB	LEU	A	72	-10.721	-2.885	39.069	1.00	35.20	B	C
ATOM	569	CG	LEU	A	72	-10.098	-2.747	37.678	1.00	32.11	B	C
ATOM	570	CD1	LEU	A	72	-8.707	-3.362	37.637	1.00	34.13	B	C
ATOM	571	CD2	LEU	A	72	-10.969	-3.333	36.606	1.00	35.51	B	C
ATOM	572	C	LEU	A	72	-12.750	-2.671	40.537	1.00	43.65	B	C
ATOM	573	O	LEU	A	72	-12.522	-1.974	41.521	1.00	36.78	B	O
ATOM	574	N	PRO	A	73	-13.593	-3.719	40.591	1.00	53.78	B	N
ATOM	575	CA	PRO	A	73	-14.381	-3.950	41.793	1.00	54.48	B	C
ATOM	576	CB	PRO	A	73	-15.494	-4.905	41.319	1.00	60.52	B	C
ATOM	577	CG	PRO	A	73	-15.266	-5.071	39.813	1.00	61.04	B	C
ATOM	578	CD	PRO	A	73	-13.797	-4.770	39.584	1.00	59.41	B	C

Fig. 5 (cont.)

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ATOM	579	C	PRO	A	73	-13.354	-4.507	42.789	1.00	49.83	B	C
ATOM	580	O	PRO	A	73	-12.324	-4.985	42.353	1.00	43.78	B	O
ATOM	581	N	ASN	A	74	-13.610	-4.376	44.090	1.00	48.00	B	N
ATOM	582	CA	ASN	A	74	-12.783	-5.043	45.125	1.00	51.16	B	C
ATOM	583	CB	ASN	A	74	-12.711	-4.256	46.444	1.00	56.12	B	C
ATOM	584	CG	ASN	A	74	-11.485	-4.599	47.266	1.00	56.97	B	C
ATOM	585	OD1	ASN	A	74	-10.777	-5.555	46.961	1.00	73.14	B	O
ATOM	586	ND2	ASN	A	74	-11.208	-3.821	48.298	1.00	60.71	B	N
ATOM	587	C	ASN	A	74	-13.372	-6.448	45.274	1.00	60.82	B	C
ATOM	588	O	ASN	A	74	-13.962	-6.750	46.322	1.00	71.20	B	O
ATOM	589	N	GLN	A	75	-13.298	-7.233	44.203	1.00	57.96	B	N
ATOM	590	CA	GLN	A	75	-13.574	-8.691	44.218	1.00	55.06	B	C
ATOM	591	CB	GLN	A	75	-14.798	-9.056	43.376	1.00	58.18	B	C
ATOM	592	CG	GLN	A	75	-14.594	-8.891	41.872	1.00	61.86	B	C
ATOM	593	CD	GLN	A	75	-15.860	-8.631	41.080	1.00	62.12	B	C
ATOM	594	OE1	GLN	A	75	-16.938	-8.376	41.627	1.00	64.51	B	O
ATOM	595	NE2	GLN	A	75	-15.733	-8.668	39.760	1.00	50.92	B	N
ATOM	596	C	GLN	A	75	-12.316	-9.372	43.697	1.00	47.08	B	C
ATOM	597	O	GLN	A	75	-11.410	-8.689	43.146	1.00	45.37	B	O
ATOM	598	N	THR	A	76	-12.286	-10.683	43.772	1.00	40.61	B	N
ATOM	599	CA	THR	A	76	-11.033	-11.435	43.517	1.00	38.98	B	C
ATOM	600	CB	THR	A	76	-11.242	-12.910	43.873	1.00	43.03	B	C
ATOM	601	OG1	THR	A	76	-11.621	-12.896	45.257	1.00	40.48	B	O
ATOM	602	CG2	THR	A	76	-10.001	-13.736	43.611	1.00	40.50	B	C
ATOM	603	C	THR	A	76	-10.614	-11.228	42.057	1.00	38.15	B	C
ATOM	604	O	THR	A	76	-9.384	-11.002	41.767	1.00	39.10	B	O
ATOM	605	N	CYS	A	77	-11.583	-11.317	41.156	1.00	28.81	B	N
ATOM	606	CA	CYS	A	77	-11.300	-11.244	39.714	1.00	25.59	B	C
ATOM	607	CB	CYS	A	77	-11.453	-12.576	39.005	1.00	25.32	B	C
ATOM	608	SG	CYS	A	77	-10.223	-13.807	39.472	1.00	27.52	B	S
ATOM	609	C	CYS	A	77	-12.191	-10.241	39.007	1.00	30.31	B	C
ATOM	610	O	CYS	A	77	-13.391	-10.163	39.259	1.00	29.85	B	O
ATOM	611	N	PRO	A	78	-11.645	-9.534	38.000	1.00	26.33	B	N
ATOM	612	CA	PRO	A	78	-12.464	-8.715	37.112	1.00	28.93	B	C
ATOM	613	CB	PRO	A	78	-11.447	-8.139	36.108	1.00	28.49	B	C
ATOM	614	CG	PRO	A	78	-10.178	-8.095	36.918	1.00	28.02	B	C
ATOM	615	CD	PRO	A	78	-10.203	-9.389	37.726	1.00	28.19	B	C
ATOM	616	C	PRO	A	78	-13.519	-9.571	36.412	1.00	25.95	B	C
ATOM	617	O	PRO	A	78	-13.242	-10.762	36.121	1.00	29.20	B	O
ATOM	618	N	SER	A	79	-14.678	-8.975	36.118	1.00	30.72	B	N
ATOM	619	CA	SER	A	79	-15.745	-9.548	35.267	1.00	31.73	B	C
ATOM	620	CB	SER	A	79	-16.942	-8.648	35.242	1.00	36.68	B	C
ATOM	621	OG	SER	A	79	-17.478	-8.563	36.541	1.00	41.93	B	O
ATOM	622	C	SER	A	79	-15.269	-9.760	33.839	1.00	35.62	B	C
ATOM	623	O	SER	A	79	-14.378	-9.058	33.370	1.00	31.32	B	O
ATOM	624	N	PRO	A	80	-15.906	-10.646	33.053	1.00	34.02	B	N
ATOM	625	CA	PRO	A	80	-15.713	-10.654	31.596	1.00	36.17	B	C
ATOM	626	CB	PRO	A	80	-16.711	-11.702	31.072	1.00	35.82	B	C
ATOM	627	CG	PRO	A	80	-17.009	-12.554	32.295	1.00	35.63	B	C
ATOM	628	CD	PRO	A	80	-16.905	-11.638	33.497	1.00	34.87	B	C
ATOM	629	C	PRO	A	80	-16.003	-9.285	30.966	1.00	34.78	B	C
ATOM	630	O	PRO	A	80	-16.894	-8.564	31.434	1.00	36.03	B	O
ATOM	631	N	GLY	A	81	-15.136	-8.893	30.025	1.00	46.72	B	N
ATOM	632	CA	GLY	A	81	-15.193	-7.595	29.323	1.00	50.35	B	C
ATOM	633	C	GLY	A	81	-14.834	-6.429	30.228	1.00	48.08	B	C
ATOM	634	O	GLY	A	81	-15.187	-5.292	29.858	1.00	53.45	B	O
ATOM	635	N	GLN	A	82	-14.184	-6.661	31.379	1.00	38.39	B	N
ATOM	636	CA	GLN	A	82	-13.700	-5.538	32.201	1.00	31.64	B	C

Fig. 5 (cont.)

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ATOM	637	CB	GLN	A	82	-13.744	-5.745	33.708	1.00	33.94	B	C
ATOM	638	CG	GLN	A	82	-15.046	-5.315	34.333	1.00	40.78	B	C
ATOM	639	CD	GLN	A	82	-14.808	-5.045	35.784	1.00	42.48	B	C
ATOM	640	OE1	GLN	A	82	-14.699	-5.960	36.602	1.00	39.15	B	O
ATOM	641	NE2	GLN	A	82	-14.699	-3.762	36.084	1.00	41.80	B	N
ATOM	642	C	GLN	A	82	-12.228	-5.267	31.857	1.00	28.77	B	C
ATOM	643	O	GLN	A	82	-11.858	-4.072	31.843	1.00	29.47	B	O
ATOM	644	N	VAL	A	83	-11.427	-6.313	31.690	1.00	25.81	B	N
ATOM	645	CA	VAL	A	83	-9.957	-6.161	31.415	1.00	23.86	B	C
ATOM	646	CB	VAL	A	83	-9.095	-6.669	32.564	1.00	22.19	B	C
ATOM	647	CG1	VAL	A	83	-7.597	-6.653	32.194	1.00	23.39	B	C
ATOM	648	CG2	VAL	A	83	-9.370	-5.929	33.833	1.00	23.71	B	C
ATOM	649	C	VAL	A	83	-9.610	-6.872	30.147	1.00	26.32	B	C
ATOM	650	O	VAL	A	83	-10.042	-8.022	29.963	1.00	29.75	B	O
ATOM	651	N	ALA	A	84	-8.805	-6.265	29.312	1.00	22.80	B	N
ATOM	652	CA	ALA	A	84	-8.317	-6.872	28.061	1.00	22.10	B	C
ATOM	653	CB	ALA	A	84	-9.092	-6.482	26.822	1.00	25.27	B	C
ATOM	654	C	ALA	A	84	-6.847	-6.496	27.949	1.00	21.02	B	C
ATOM	655	O	ALA	A	84	-6.436	-5.349	28.264	1.00	19.99	B	O
ATOM	656	N	VAL	A	85	-6.094	-7.448	27.497	1.00	19.70	B	N
ATOM	657	CA	VAL	A	85	-4.643	-7.324	27.221	1.00	17.64	B	C
ATOM	658	CB	VAL	A	85	-3.833	-8.157	28.213	1.00	18.93	B	C
ATOM	659	CG1	VAL	A	85	-2.363	-8.046	27.920	1.00	18.72	B	C
ATOM	660	CG2	VAL	A	85	-4.125	-7.744	29.599	1.00	20.83	B	C
ATOM	661	C	VAL	A	85	-4.344	-7.683	25.792	1.00	19.15	B	C
ATOM	662	O	VAL	A	85	-4.753	-8.779	25.363	1.00	20.80	B	O
ATOM	663	N	ILE	A	86	-3.602	-6.838	25.092	1.00	18.41	B	N
ATOM	664	CA	ILE	A	86	-3.030	-7.098	23.772	1.00	19.16	B	C
ATOM	665	CB	ILE	A	86	-3.511	-6.052	22.755	1.00	19.66	B	C
ATOM	666	CG1	ILE	A	86	-5.030	-6.142	22.572	1.00	21.88	B	C
ATOM	667	CG2	ILE	A	86	-2.782	-6.189	21.458	1.00	22.14	B	C
ATOM	668	CD1	ILE	A	86	-5.600	-4.855	21.973	1.00	23.62	B	C
ATOM	669	C	ILE	A	86	-1.522	-7.063	23.923	1.00	19.53	B	C
ATOM	670	O	ILE	A	86	-0.978	-6.096	24.543	1.00	21.23	B	O
ATOM	671	N	ALA	A	87	-0.809	-7.955	23.296	1.00	18.96	B	N
ATOM	672	CA	ALA	A	87	0.661	-7.937	23.227	1.00	17.23	B	C
ATOM	673	CB	ALA	A	87	1.222	-9.052	24.097	1.00	18.49	B	C
ATOM	674	C	ALA	A	87	1.143	-8.139	21.812	1.00	19.52	B	C
ATOM	675	O	ALA	A	87	0.484	-8.901	21.077	1.00	19.49	B	O
ATOM	676	N	ASP	A	88	2.283	-7.605	21.473	1.00	17.46	B	N
ATOM	677	CA	ASP	A	88	2.984	-7.967	20.236	1.00	18.23	B	C
ATOM	678	CB	ASP	A	88	4.129	-6.989	19.999	1.00	17.83	B	C
ATOM	679	CG	ASP	A	88	4.590	-6.911	18.562	1.00	20.70	B	C
ATOM	680	OD1	ASP	A	88	3.978	-7.647	17.706	1.00	21.28	B	O
ATOM	681	OD2	ASP	A	88	5.548	-6.202	18.304	1.00	21.62	B	O
ATOM	682	C	ASP	A	88	3.430	-9.442	20.352	1.00	17.51	B	C
ATOM	683	O	ASP	A	88	3.412	-9.992	21.468	1.00	19.11	B	O
ATOM	684	N	THR	A	89	3.902	-9.977	19.257	1.00	20.25	B	N
ATOM	685	CA	THR	A	89	4.119	-11.442	19.126	1.00	21.52	B	C
ATOM	686	CB	THR	A	89	4.062	-11.837	17.648	1.00	23.32	B	C
ATOM	687	OG1	THR	A	89	5.138	-11.170	17.021	1.00	25.36	B	O
ATOM	688	CG2	THR	A	89	2.700	-11.584	17.045	1.00	28.07	B	C
ATOM	689	C	THR	A	89	5.430	-11.856	19.769	1.00	21.54	B	C
ATOM	690	O	THR	A	89	5.639	-13.090	19.958	1.00	22.09	B	O
ATOM	691	N	ASP	A	90	6.325	-10.961	20.143	1.00	19.06	B	N
ATOM	692	CA	ASP	A	90	7.603	-11.285	20.808	1.00	17.58	B	C
ATOM	693	CB	ASP	A	90	8.454	-10.028	21.005	1.00	20.81	B	C
ATOM	694	CG	ASP	A	90	8.911	-9.358	19.730	1.00	28.78	B	C

Fig. 5 (cont.)

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ATOM	695	OD1	ASP	A	90	9.041	-10.050	18.713	1.00	29.63	B	O
ATOM	696	OD2	ASP	A	90	9.211	-8.170	19.789	1.00	31.52	B	O
ATOM	697	C	ASP	A	90	7.377	-11.928	22.203	1.00	16.36	B	C
ATOM	698	O	ASP	A	90	6.385	-11.648	22.905	1.00	17.08	B	O
ATOM	699	N	GLN	A	91	8.304	-12.786	22.631	1.00	16.47	B	N
ATOM	700	CA	GLN	A	91	8.255	-13.299	24.014	1.00	17.23	B	C
ATOM	701	CB	GLN	A	91	9.456	-14.208	24.320	1.00	16.45	B	C
ATOM	702	CG	GLN	A	91	9.461	-14.731	25.752	1.00	17.01	B	C
ATOM	703	CD	GLN	A	91	10.196	-13.863	26.729	1.00	19.77	B	C
ATOM	704	OE1	GLN	A	91	10.688	-12.787	26.412	1.00	18.34	B	O
ATOM	705	NE2	GLN	A	91	10.419	-14.325	27.955	1.00	22.05	B	N
ATOM	706	C	GLN	A	91	8.187	-12.093	24.977	1.00	16.76	B	C
ATOM	707	O	GLN	A	91	7.472	-12.133	25.980	1.00	16.61	B	O
ATOM	708	N	ARG	A	92	8.949	-11.037	24.682	1.00	16.89	B	N
ATOM	709	CA	ARG	A	92	9.138	-10.003	25.731	1.00	15.92	B	C
ATOM	710	CB	ARG	A	92	10.288	-9.094	25.394	1.00	17.40	B	C
ATOM	711	CG	ARG	A	92	10.097	-8.278	24.140	1.00	17.91	B	C
ATOM	712	CD	ARG	A	92	11.410	-7.544	24.041	1.00	21.15	B	C
ATOM	713	NE	ARG	A	92	11.565	-6.867	22.790	1.00	24.74	B	N
ATOM	714	CZ	ARG	A	92	12.276	-7.300	21.761	1.00	27.81	B	C
ATOM	715	NH1	ARG	A	92	12.871	-8.483	21.755	1.00	28.64	B	N
ATOM	716	NH2	ARG	A	92	12.328	-6.560	20.678	1.00	28.27	B	N
ATOM	717	C	ARG	A	92	7.828	-9.254	25.964	1.00	15.26	B	C
ATOM	718	O	ARG	A	92	7.498	-8.929	27.116	1.00	15.36	B	O
ATOM	719	N	THR	A	93	7.038	-9.076	24.914	1.00	15.07	B	N
ATOM	720	CA	THR	A	93	5.738	-8.457	25.026	1.00	14.75	B	C
ATOM	721	CB	THR	A	93	5.374	-7.779	23.700	1.00	15.43	B	C
ATOM	722	OG1	THR	A	93	5.586	-8.661	22.605	1.00	17.22	B	O
ATOM	723	CG2	THR	A	93	6.188	-6.535	23.446	1.00	16.55	B	C
ATOM	724	C	THR	A	93	4.647	-9.389	25.561	1.00	16.33	B	C
ATOM	725	O	THR	A	93	3.851	-9.026	26.414	1.00	16.15	B	O
ATOM	726	N	ARG	A	94	4.593	-10.625	25.021	1.00	15.95	B	N
ATOM	727	CA	ARG	A	94	3.598	-11.596	25.522	1.00	17.09	B	C
ATOM	728	CB	ARG	A	94	3.700	-12.944	24.795	1.00	18.99	B	C
ATOM	729	CG	ARG	A	94	3.234	-12.888	23.339	1.00	21.91	B	C
ATOM	730	CD	ARG	A	94	3.399	-14.197	22.592	1.00	23.86	B	C
ATOM	731	NE	ARG	A	94	2.793	-15.301	23.321	1.00	26.12	B	N
ATOM	732	CZ	ARG	A	94	2.867	-16.569	22.982	1.00	30.06	B	C
ATOM	733	NH1	ARG	A	94	3.600	-16.913	21.943	1.00	28.54	B	N
ATOM	734	NH2	ARG	A	94	2.279	-17.467	23.754	1.00	31.51	B	N
ATOM	735	C	ARG	A	94	3.754	-11.833	27.028	1.00	14.41	B	C
ATOM	736	O	ARG	A	94	2.805	-11.747	27.775	1.00	16.28	B	O
ATOM	737	N	LYS	A	95	5.008	-11.999	27.438	1.00	14.99	B	N
ATOM	738	CA	LYS	A	95	5.271	-12.240	28.867	1.00	16.88	B	C
ATOM	739	CB	LYS	A	95	6.634	-12.877	29.115	1.00	18.49	B	C
ATOM	740	CG	LYS	A	95	6.592	-14.393	28.801	1.00	22.72	B	C
ATOM	741	CD	LYS	A	95	5.676	-15.181	29.722	1.00	24.76	B	C
ATOM	742	CE	LYS	A	95	5.491	-16.657	29.401	1.00	26.59	B	C
ATOM	743	NZ	LYS	A	95	4.629	-17.281	30.452	1.00	27.69	B	N
ATOM	744	C	LYS	A	95	5.037	-10.989	29.703	1.00	16.08	B	C
ATOM	745	O	LYS	A	95	4.600	-11.064	30.855	1.00	16.49	B	O
ATOM	746	N	THR	A	96	5.345	-9.807	29.155	1.00	15.68	B	N
ATOM	747	CA	THR	A	96	4.952	-8.576	29.863	1.00	15.64	B	C
ATOM	748	CB	THR	A	96	5.435	-7.303	29.135	1.00	14.15	B	C
ATOM	749	OG1	THR	A	96	6.845	-7.301	29.185	1.00	14.33	B	O
ATOM	750	CG2	THR	A	96	4.887	-6.027	29.745	1.00	15.72	B	C
ATOM	751	C	THR	A	96	3.461	-8.574	30.155	1.00	14.61	B	C
ATOM	752	O	THR	A	96	3.010	-8.226	31.258	1.00	16.89	B	O

Fig. 5 (cont.)

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ATOM	753	N	GLY	A	97	2.650	-8.897	29.153	1.00	15.68	B	N
ATOM	754	CA	GLY	A	97	1.201	-8.963	29.357	1.00	16.27	B	C
ATOM	755	C	GLY	A	97	0.826	-9.967	30.454	1.00	17.07	B	C
ATOM	756	O	GLY	A	97	0.019	-9.675	31.333	1.00	17.08	B	O
ATOM	757	N	GLU	A	98	1.401	-11.167	30.407	1.00	15.92	B	N
ATOM	758	CA	GLU	A	98	1.060	-12.219	31.372	1.00	18.27	B	C
ATOM	759	CB	GLU	A	98	1.731	-13.545	30.991	1.00	18.88	B	C
ATOM	760	CG	GLU	A	98	1.217	-14.116	29.683	1.00	19.11	B	C
ATOM	761	CD	GLU	A	98	1.754	-15.501	29.294	1.00	28.24	B	C
ATOM	762	OE1	GLU	A	98	1.712	-15.889	28.113	1.00	30.80	B	O
ATOM	763	OE2	GLU	A	98	2.079	-16.219	30.192	1.00	31.25	B	O
ATOM	764	C	GLU	A	98	1.541	-11.788	32.743	1.00	16.35	B	C
ATOM	765	O	GLU	A	98	0.835	-12.021	33.735	1.00	18.13	B	O
ATOM	766	N	CYS	A	99	2.726	-11.202	32.829	1.00	16.31	B	N
ATOM	767	CA	CYS	A	99	3.291	-10.784	34.112	1.00	16.35	B	C
ATOM	768	CB	CYS	A	99	4.750	-10.470	33.972	1.00	18.14	B	C
ATOM	769	SG	CYS	A	99	5.726	-11.918	33.425	1.00	19.09	B	S
ATOM	770	C	CYS	A	99	2.477	-9.644	34.703	1.00	16.99	B	C
ATOM	771	O	CYS	A	99	2.352	-9.533	35.947	1.00	19.62	B	O
ATOM	772	N	PHE	A	100	1.994	-8.720	33.872	1.00	16.84	B	N
ATOM	773	CA	PHE	A	100	1.160	-7.619	34.372	1.00	17.94	B	C
ATOM	774	CB	PHE	A	100	0.850	-6.625	33.244	1.00	17.80	B	C
ATOM	775	CG	PHE	A	100	0.008	-5.453	33.691	1.00	17.53	B	C
ATOM	776	CD1	PHE	A	100	0.594	-4.378	34.375	1.00	17.21	B	C
ATOM	777	CE1	PHE	A	100	-0.176	-3.272	34.731	1.00	18.39	B	C
ATOM	778	CZ	PHE	A	100	-1.518	-3.300	34.541	1.00	19.44	B	C
ATOM	779	CD2	PHE	A	100	-1.361	-5.438	33.495	1.00	17.44	B	C
ATOM	780	CE2	PHE	A	100	-2.119	-4.351	33.905	1.00	18.77	B	C
ATOM	781	C	PHE	A	100	-0.110	-8.227	35.003	1.00	17.42	B	C
ATOM	782	O	PHE	A	100	-0.505	-7.785	36.061	1.00	18.31	B	O
ATOM	783	N	LEU	A	101	-0.730	-9.157	34.289	1.00	19.81	B	N
ATOM	784	CA	LEU	A	101	-1.943	-9.829	34.800	1.00	20.53	B	C
ATOM	785	CB	LEU	A	101	-2.522	-10.740	33.735	1.00	20.79	B	C
ATOM	786	CG	LEU	A	101	-3.221	-10.007	32.592	1.00	21.04	B	C
ATOM	787	CD1	LEU	A	101	-3.642	-10.961	31.494	1.00	20.77	B	C
ATOM	788	CD2	LEU	A	101	-4.434	-9.235	33.064	1.00	22.07	B	C
ATOM	789	C	LEU	A	101	-1.636	-10.622	36.067	1.00	20.76	B	C
ATOM	790	O	LEU	A	101	-2.524	-10.646	36.915	1.00	20.62	B	O
ATOM	791	N	ALA	A	102	-0.444	-11.173	36.220	1.00	19.34	B	N
ATOM	792	CA	ALA	A	102	-0.086	-11.913	37.466	1.00	21.77	B	C
ATOM	793	CB	ALA	A	102	1.298	-12.475	37.388	1.00	21.65	B	C
ATOM	794	C	ALA	A	102	-0.168	-10.956	38.642	1.00	21.28	B	C
ATOM	795	O	ALA	A	102	-0.480	-11.403	39.732	1.00	23.63	B	O
ATOM	796	N	GLY	A	103	0.097	-9.650	38.452	1.00	20.65	B	N
ATOM	797	CA	GLY	A	103	0.002	-8.636	39.510	1.00	19.89	B	C
ATOM	798	C	GLY	A	103	-1.386	-8.030	39.611	1.00	20.31	B	C
ATOM	799	O	GLY	A	103	-1.916	-7.798	40.729	1.00	21.90	B	O
ATOM	800	N	LEU	A	104	-2.011	-7.770	38.478	1.00	19.26	B	N
ATOM	801	CA	LEU	A	104	-3.324	-7.113	38.472	1.00	20.43	B	C
ATOM	802	CB	LEU	A	104	-3.652	-6.638	37.062	1.00	19.97	B	C
ATOM	803	CG	LEU	A	104	-4.917	-5.767	36.965	1.00	21.43	B	C
ATOM	804	CD1	LEU	A	104	-4.586	-4.298	37.161	1.00	23.05	B	C
ATOM	805	CD2	LEU	A	104	-5.610	-5.987	35.645	1.00	20.39	B	C
ATOM	806	C	LEU	A	104	-4.426	-8.063	38.962	1.00	22.24	B	C
ATOM	807	O	LEU	A	104	-5.323	-7.572	39.666	1.00	24.02	B	O
ATOM	808	N	ALA	A	105	-4.393	-9.337	38.539	1.00	23.14	B	N
ATOM	809	CA	ALA	A	105	-5.555	-10.259	38.606	1.00	23.22	B	C
ATOM	810	CB	ALA	A	105	-6.436	-10.148	37.388	1.00	23.01	B	C

Fig. 5 (cont.)

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ATOM	811	C	ALA	A	105	-5.025	-11.679	38.745	1.00	21.20	B	C
ATOM	812	O	ALA	A	105	-5.233	-12.493	37.881	1.00	24.24	B	O
ATOM	813	N	PRO	A	106	-4.216	-11.938	39.769	1.00	25.23	B	N
ATOM	814	CA	PRO	A	106	-3.560	-13.245	39.840	1.00	28.49	B	C
ATOM	815	CB	PRO	A	106	-2.768	-13.163	41.139	1.00	26.20	B	C
ATOM	816	CG	PRO	A	106	-3.309	-12.023	41.922	1.00	30.15	B	C
ATOM	817	CD	PRO	A	106	-3.917	-11.081	40.902	1.00	25.10	B	C
ATOM	818	C	PRO	A	106	-4.572	-14.409	39.848	1.00	27.80	B	C
ATOM	819	O	PRO	A	106	-5.587	-14.314	40.546	1.00	28.73	B	O
ATOM	820	N	LYS	A	107	-4.315	-15.412	39.009	1.00	30.64	B	N
ATOM	821	CA	LYS	A	107	-5.076	-16.694	38.942	1.00	31.30	B	C
ATOM	822	CB	LYS	A	107	-5.270	-17.267	40.348	1.00	33.39	B	C
ATOM	823	CG	LYS	A	107	-3.979	-17.523	41.104	1.00	39.37	B	C
ATOM	824	CD	LYS	A	107	-4.188	-18.322	42.367	1.00	46.82	B	C
ATOM	825	CE	LYS	A	107	-3.004	-18.274	43.304	1.00	54.50	B	C
ATOM	826	NZ	LYS	A	107	-3.341	-17.554	44.552	1.00	62.92	B	N
ATOM	827	C	LYS	A	107	-6.427	-16.535	38.243	1.00	32.90	B	C
ATOM	828	O	LYS	A	107	-7.209	-17.543	38.194	1.00	31.26	B	O
ATOM	829	N	CYS	A	108	-6.716	-15.363	37.659	1.00	25.89	B	N
ATOM	830	CA	CYS	A	108	-8.029	-15.063	37.029	1.00	25.19	B	C
ATOM	831	CB	CYS	A	108	-8.332	-13.561	37.115	1.00	25.56	B	C
ATOM	832	SG	CYS	A	108	-8.467	-12.964	38.820	1.00	25.90	B	S
ATOM	833	C	CYS	A	108	-8.081	-15.611	35.599	1.00	25.31	B	C
ATOM	834	O	CYS	A	108	-9.155	-15.538	34.995	1.00	28.93	B	O
ATOM	835	N	GLN	A	109	-6.956	-16.062	35.028	1.00	29.52	B	N
ATOM	836	CA	GLN	A	109	-6.882	-16.625	33.659	1.00	29.24	B	C
ATOM	837	CB	GLN	A	109	-7.663	-17.944	33.528	1.00	38.25	B	C
ATOM	838	CG	GLN	A	109	-7.397	-18.967	34.624	1.00	45.02	B	C
ATOM	839	CD	GLN	A	109	-8.430	-20.071	34.531	1.00	55.94	B	C
ATOM	840	OE1	GLN	A	109	-8.318	-20.982	33.709	1.00	63.17	B	O
ATOM	841	NE2	GLN	A	109	-9.482	-19.967	35.332	1.00	62.89	B	N
ATOM	842	C	GLN	A	109	-7.433	-15.629	32.647	1.00	29.04	B	C
ATOM	843	O	GLN	A	109	-7.993	-16.046	31.675	1.00	30.01	B	O
ATOM	844	N	ILE	A	110	-7.185	-14.323	32.809	1.00	25.11	B	N
ATOM	845	CA	ILE	A	110	-7.478	-13.390	31.712	1.00	24.22	B	C
ATOM	846	CB	ILE	A	110	-7.363	-11.963	32.254	1.00	22.85	B	C
ATOM	847	CG1	ILE	A	110	-8.451	-11.722	33.305	1.00	26.60	B	C
ATOM	848	CG2	ILE	A	110	-7.461	-10.928	31.167	1.00	22.60	B	C
ATOM	849	CD1	ILE	A	110	-8.215	-10.500	34.157	1.00	28.12	B	C
ATOM	850	C	ILE	A	110	-6.527	-13.684	30.554	1.00	23.31	B	C
ATOM	851	O	ILE	A	110	-5.297	-13.922	30.835	1.00	26.34	B	O
ATOM	852	N	GLN	A	111	-7.045	-13.617	29.328	1.00	25.86	B	N
ATOM	853	CA	GLN	A	111	-6.336	-13.915	28.069	1.00	31.45	B	C
ATOM	854	CB	GLN	A	111	-7.381	-13.938	26.939	1.00	34.82	B	C
ATOM	855	CG	GLN	A	111	-6.861	-14.374	25.578	1.00	38.42	B	C
ATOM	856	CD	GLN	A	111	-6.208	-15.738	25.686	1.00	47.49	B	C
ATOM	857	OE1	GLN	A	111	-5.068	-15.870	26.132	1.00	52.02	B	O
ATOM	858	NE2	GLN	A	111	-6.935	-16.777	25.309	1.00	49.44	B	N
ATOM	859	C	GLN	A	111	-5.361	-12.807	27.742	1.00	25.92	B	C
ATOM	860	O	GLN	A	111	-5.631	-11.618	27.971	1.00	31.52	B	O
ATOM	861	N	VAL	A	112	-4.182	-13.145	27.262	1.00	20.94	B	N
ATOM	862	CA	VAL	A	112	-3.383	-12.155	26.519	1.00	20.99	B	C
ATOM	863	CB	VAL	A	112	-1.909	-12.267	26.879	1.00	20.06	B	C
ATOM	864	CG1	VAL	A	112	-0.962	-11.485	25.929	1.00	22.09	B	C
ATOM	865	CG2	VAL	A	112	-1.702	-11.942	28.312	1.00	19.73	B	C
ATOM	866	C	VAL	A	112	-3.611	-12.423	25.050	1.00	25.82	B	C
ATOM	867	O	VAL	A	112	-3.252	-13.558	24.540	1.00	26.67	B	O
ATOM	868	N	HIS	A	113	-4.215	-11.447	24.394	1.00	22.98	B	N

Fig. 5 (cont.)

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ATOM	869	CA	HIS	A	113	-4.444	-11.538	22.943	1.00	23.02	B	C
ATOM	870	CB	HIS	A	113	-5.508	-10.581	22.520	1.00	24.40	B	C
ATOM	871	CG	HIS	A	113	-6.836	-10.872	23.101	1.00	25.05	B	C
ATOM	872	ND1	HIS	A	113	-7.825	-11.540	22.401	1.00	32.90	B	N
ATOM	873	CE1	HIS	A	113	-8.888	-11.623	23.167	1.00	29.18	B	C
ATOM	874	NE2	HIS	A	113	-8.650	-11.077	24.320	1.00	32.43	B	N
ATOM	875	CD2	HIS	A	113	-7.357	-10.591	24.298	1.00	23.86	B	C
ATOM	876	C	HIS	A	113	-3.159	-11.215	22.210	1.00	24.46	B	C
ATOM	877	O	HIS	A	113	-2.513	-10.205	22.565	1.00	24.88	B	O
ATOM	878	N	TYR	A	114	-2.846	-11.917	21.137	1.00	25.01	B	N
ATOM	879	CA	TYR	A	114	-1.685	-11.632	20.273	1.00	24.37	B	C
ATOM	880	CB	TYR	A	114	-0.401	-12.112	20.970	1.00	24.09	B	C
ATOM	881	CG	TYR	A	114	-0.281	-13.612	21.092	1.00	24.54	B	C
ATOM	882	CD1	TYR	A	114	-0.818	-14.286	22.178	1.00	28.95	B	C
ATOM	883	CE1	TYR	A	114	-0.666	-15.666	22.298	1.00	28.78	B	C
ATOM	884	CZ	TYR	A	114	-0.053	-16.385	21.288	1.00	34.79	B	C
ATOM	885	OH	TYR	A	114	0.071	-17.766	21.371	1.00	35.25	B	O
ATOM	886	CE2	TYR	A	114	0.493	-15.721	20.202	1.00	32.41	B	C
ATOM	887	CD2	TYR	A	114	0.385	-14.338	20.109	1.00	31.01	B	C
ATOM	888	C	TYR	A	114	-1.966	-12.251	18.899	1.00	28.39	B	C
ATOM	889	O	TYR	A	114	-2.773	-13.200	18.810	1.00	28.46	B	O
ATOM	890	N	GLN	A	115	-1.309	-11.752	17.872	1.00	27.51	B	N
ATOM	891	CA	GLN	A	115	-1.501	-12.285	16.498	1.00	31.96	B	C
ATOM	892	CB	GLN	A	115	-0.968	-11.245	15.513	1.00	32.82	B	C
ATOM	893	CG	GLN	A	115	-0.714	-11.783	14.114	1.00	39.76	B	C
ATOM	894	CD	GLN	A	115	-0.239	-10.663	13.223	1.00	35.50	B	C
ATOM	895	OE1	GLN	A	115	-0.921	-9.653	13.042	1.00	40.99	B	O
ATOM	896	NE2	GLN	A	115	0.969	-10.805	12.724	1.00	36.59	B	N
ATOM	897	C	GLN	A	115	-0.843	-13.685	16.449	1.00	31.81	B	C
ATOM	898	O	GLN	A	115	0.327	-13.830	16.832	1.00	33.77	B	O
ATOM	899	N	LYS	A	116	-1.580	-14.722	16.047	1.00	40.01	B	N
ATOM	900	CA	LYS	A	116	-1.097	-16.133	16.129	1.00	40.60	B	C
ATOM	901	CB	LYS	A	116	-2.298	-17.080	16.067	1.00	46.42	B	C
ATOM	902	CG	LYS	A	116	-3.168	-17.061	17.321	1.00	50.32	B	C
ATOM	903	CD	LYS	A	116	-2.388	-17.337	18.611	1.00	50.83	B	C
ATOM	904	CE	LYS	A	116	-3.247	-17.200	19.851	1.00	51.85	B	C
ATOM	905	NZ	LYS	A	116	-3.761	-15.816	19.985	1.00	51.73	B	N
ATOM	906	C	LYS	A	116	-0.033	-16.426	15.059	1.00	47.86	B	C
ATOM	907	O	LYS	A	116	0.730	-17.397	15.255	1.00	51.36	B	O
ATOM	908	N	ASP	A	117	0.062	-15.603	14.013	1.00	50.28	B	N
ATOM	909	CA	ASP	A	117	1.083	-15.743	12.941	1.00	53.29	B	C
ATOM	910	CB	ASP	A	117	0.468	-15.331	11.603	1.00	54.74	B	C
ATOM	911	CG	ASP	A	117	1.338	-15.663	10.402	1.00	63.20	B	C
ATOM	912	OD1	ASP	A	117	2.551	-15.961	10.594	1.00	63.75	B	O
ATOM	913	OD2	ASP	A	117	0.799	-15.613	9.292	1.00	59.18	B	O
ATOM	914	C	ASP	A	117	2.342	-14.942	13.324	1.00	55.32	B	C
ATOM	915	O	ASP	A	117	2.488	-13.794	12.854	1.00	55.05	B	O
ATOM	916	N	GLU	A	118	3.233	-15.539	14.129	1.00	53.62	B	N
ATOM	917	CA	GLU	A	118	4.508	-14.931	14.603	1.00	58.20	B	C
ATOM	918	CB	GLU	A	118	5.213	-15.916	15.533	1.00	67.60	B	C
ATOM	919	CG	GLU	A	118	6.333	-15.308	16.346	1.00	71.45	B	C
ATOM	920	CD	GLU	A	118	7.111	-16.354	17.128	1.00	77.55	B	C
ATOM	921	OE1	GLU	A	118	8.307	-16.550	16.809	1.00	77.85	B	O
ATOM	922	OE2	GLU	A	118	6.516	-16.984	18.051	1.00	71.17	B	O
ATOM	923	C	GLU	A	118	5.425	-14.597	13.418	1.00	60.04	B	C
ATOM	924	O	GLU	A	118	6.321	-13.737	13.579	1.00	52.86	B	O
ATOM	925	N	GLU	A	119	5.236	-15.281	12.284	1.00	65.12	B	N
ATOM	926	CA	GLU	A	119	6.045	-15.110	11.044	1.00	68.74	B	C

Fig. 5 (cont.)

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ATOM	927	CB	GLU	A	119	5.828	-16.291	10.089	1.00	76.99	B	C
ATOM	928	CG	GLU	A	119	5.954	-17.652	10.765	1.00	83.81	B	C
ATOM	929	CD	GLU	A	119	6.452	-18.794	9.891	1.00	87.81	B	C
ATOM	930	OE1	GLU	A	119	7.461	-18.602	9.179	1.00	93.36	B	O
ATOM	931	OE2	GLU	A	119	5.840	-19.881	9.939	1.00	91.28	B	O
ATOM	932	C	GLU	A	119	5.682	-13.771	10.380	1.00	61.96	B	C
ATOM	933	O	GLU	A	119	6.576	-13.136	9.796	1.00	57.10	B	O
ATOM	934	N	LYS	A	120	4.418	-13.354	10.468	1.00	58.87	B	N
ATOM	935	CA	LYS	A	120	3.930	-12.067	9.897	1.00	53.86	B	C
ATOM	936	CB	LYS	A	120	2.498	-12.213	9.373	1.00	56.87	B	C
ATOM	937	CG	LYS	A	120	2.220	-11.419	8.105	1.00	69.89	B	C
ATOM	938	CD	LYS	A	120	1.100	-10.404	8.220	1.00	76.26	B	C
ATOM	939	CE	LYS	A	120	1.434	-9.092	7.537	1.00	77.53	B	C
ATOM	940	NZ	LYS	A	120	2.009	-9.295	6.182	1.00	74.84	B	N
ATOM	941	C	LYS	A	120	3.973	-11.006	10.996	1.00	47.19	B	C
ATOM	942	O	LYS	A	120	3.575	-11.300	12.116	1.00	43.74	B	O
ATOM	943	N	PRO	A	121	4.432	-9.759	10.724	1.00	38.48	B	N
ATOM	944	CA	PRO	A	121	4.423	-8.710	11.748	1.00	38.01	B	C
ATOM	945	CB	PRO	A	121	5.120	-7.497	11.123	1.00	39.37	B	C
ATOM	946	CG	PRO	A	121	5.702	-7.980	9.797	1.00	41.84	B	C
ATOM	947	CD	PRO	A	121	5.024	-9.293	9.456	1.00	42.37	B	C
ATOM	948	C	PRO	A	121	2.971	-8.373	12.103	1.00	32.26	B	C
ATOM	949	O	PRO	A	121	2.147	-8.397	11.204	1.00	34.34	B	O
ATOM	950	N	ASP	A	122	2.706	-8.042	13.371	1.00	27.88	B	N
ATOM	951	CA	ASP	A	122	1.461	-7.385	13.883	1.00	25.56	B	C
ATOM	952	CB	ASP	A	122	1.148	-7.617	15.366	1.00	25.61	B	C
ATOM	953	CG	ASP	A	122	-0.208	-7.104	15.819	1.00	28.14	B	C
ATOM	954	OD1	ASP	A	122	-0.778	-6.225	15.126	1.00	25.54	B	O
ATOM	955	OD2	ASP	A	122	-0.671	-7.448	16.887	1.00	27.01	B	O
ATOM	956	C	ASP	A	122	1.553	-5.872	13.674	1.00	24.93	B	C
ATOM	957	O	ASP	A	122	2.272	-5.140	14.356	1.00	24.67	B	O
ATOM	958	N	PRO	A	123	0.782	-5.385	12.708	1.00	26.73	B	N
ATOM	959	CA	PRO	A	123	0.920	-3.990	12.312	1.00	24.42	B	C
ATOM	960	CB	PRO	A	123	0.075	-3.935	11.021	1.00	27.95	B	C
ATOM	961	CG	PRO	A	123	-0.950	-5.007	11.221	1.00	28.33	B	C
ATOM	962	CD	PRO	A	123	-0.187	-6.129	11.877	1.00	26.88	B	C
ATOM	963	C	PRO	A	123	0.428	-2.981	13.362	1.00	23.81	B	C
ATOM	964	O	PRO	A	123	0.755	-1.840	13.185	1.00	22.57	B	O
ATOM	965	N	LEU	A	124	-0.316	-3.381	14.405	1.00	22.20	B	N
ATOM	966	CA	LEU	A	124	-0.630	-2.446	15.518	1.00	21.38	B	C
ATOM	967	CB	LEU	A	124	-1.361	-3.184	16.639	1.00	22.17	B	C
ATOM	968	CG	LEU	A	124	-1.771	-2.333	17.837	1.00	23.42	B	C
ATOM	969	CD1	LEU	A	124	-2.817	-1.313	17.467	1.00	23.55	B	C
ATOM	970	CD2	LEU	A	124	-2.273	-3.173	18.987	1.00	24.57	B	C
ATOM	971	C	LEU	A	124	0.655	-1.796	16.019	1.00	20.50	B	C
ATOM	972	O	LEU	A	124	0.610	-0.630	16.402	1.00	20.56	B	O
ATOM	973	N	PHE	A	125	1.736	-2.547	16.074	1.00	19.96	B	N
ATOM	974	CA	PHE	A	125	3.014	-2.116	16.704	1.00	20.74	B	C
ATOM	975	CB	PHE	A	125	3.649	-3.282	17.475	1.00	21.35	B	C
ATOM	976	CG	PHE	A	125	2.747	-3.794	18.569	1.00	20.89	B	C
ATOM	977	CD1	PHE	A	125	2.754	-3.178	19.812	1.00	20.09	B	C
ATOM	978	CE1	PHE	A	125	1.848	-3.557	20.775	1.00	20.00	B	C
ATOM	979	CZ	PHE	A	125	0.985	-4.597	20.552	1.00	18.98	B	C
ATOM	980	CD2	PHE	A	125	1.847	-4.834	18.352	1.00	20.91	B	C
ATOM	981	CE2	PHE	A	125	0.949	-5.208	19.333	1.00	19.89	B	C
ATOM	982	C	PHE	A	125	3.956	-1.490	15.715	1.00	21.43	B	C
ATOM	983	O	PHE	A	125	4.989	-0.943	16.103	1.00	21.02	B	O
ATOM	984	N	ASN	A	126	3.597	-1.566	14.424	1.00	20.84	B	N

Fig. 5 (cont.)

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ATOM	985	CA	ASN	A	126	4.415	-0.911	13.384	1.00	21.60	B	C
ATOM	986	CB	ASN	A	126	5.817	-1.466	13.231	1.00	22.41	B	C
ATOM	987	CG	ASN	A	126	6.585	-0.749	12.135	1.00	23.29	B	C
ATOM	988	OD1	ASN	A	126	6.073	0.216	11.566	1.00	25.38	B	O
ATOM	989	ND2	ASN	A	126	7.781	-1.229	11.872	1.00	27.93	B	N
ATOM	990	C	ASN	A	126	3.675	-1.073	12.059	1.00	23.60	B	C
ATOM	991	O	ASN	A	126	3.782	-2.099	11.391	1.00	22.76	B	O
ATOM	992	N	PRO	A	127	2.827	-0.099	11.707	1.00	21.96	B	N
ATOM	993	CA	PRO	A	127	1.975	-0.230	10.522	1.00	22.05	B	C
ATOM	994	CB	PRO	A	127	0.985	0.915	10.649	1.00	22.85	B	C
ATOM	995	CG	PRO	A	127	1.776	1.954	11.405	1.00	23.09	B	C
ATOM	996	CD	PRO	A	127	2.549	1.152	12.438	1.00	23.16	B	C
ATOM	997	C	PRO	A	127	2.758	-0.119	9.212	1.00	22.95	B	C
ATOM	998	O	PRO	A	127	2.174	-0.540	8.195	1.00	23.81	B	O
ATOM	999	N	VAL	A	128	4.001	0.315	9.237	1.00	21.89	B	N
ATOM	1000	CA	VAL	A	128	4.813	0.465	8.005	1.00	25.86	B	C
ATOM	1001	CB	VAL	A	128	6.017	1.378	8.265	1.00	29.56	B	C
ATOM	1002	CG1	VAL	A	128	6.742	1.720	6.990	1.00	37.89	B	C
ATOM	1003	CG2	VAL	A	128	5.604	2.668	8.961	1.00	32.13	B	C
ATOM	1004	C	VAL	A	128	5.166	-0.924	7.487	1.00	28.64	B	C
ATOM	1005	O	VAL	A	128	5.478	-1.018	6.283	1.00	26.95	B	O
ATOM	1006	N	LYS	A	129	4.886	-1.988	8.234	1.00	25.78	B	N
ATOM	1007	CA	LYS	A	129	4.990	-3.360	7.674	1.00	30.18	B	C
ATOM	1008	CB	LYS	A	129	4.957	-4.418	8.775	1.00	30.16	B	C
ATOM	1009	CG	LYS	A	129	6.104	-4.323	9.768	1.00	35.31	B	C
ATOM	1010	CD	LYS	A	129	7.452	-4.412	9.121	1.00	39.21	B	C
ATOM	1011	CE	LYS	A	129	8.541	-4.816	10.094	1.00	43.07	B	C
ATOM	1012	NZ	LYS	A	129	9.869	-4.738	9.449	1.00	51.32	B	N
ATOM	1013	C	LYS	A	129	3.852	-3.643	6.686	1.00	30.67	B	C
ATOM	1014	O	LYS	A	129	3.954	-4.660	5.983	1.00	31.85	B	O
ATOM	1015	N	MET	A	130	2.780	-2.865	6.644	1.00	26.60	B	N
ATOM	1016	CA	MET	A	130	1.673	-3.048	5.665	1.00	26.90	B	C
ATOM	1017	CB	MET	A	130	0.362	-2.445	6.158	1.00	26.75	B	C
ATOM	1018	CG	MET	A	130	-0.173	-3.079	7.432	1.00	25.88	B	C
ATOM	1019	SD	MET	A	130	-1.745	-2.401	7.840	1.00	30.21	B	S
ATOM	1020	CE	MET	A	130	-1.283	-0.693	8.045	1.00	28.01	B	C
ATOM	1021	C	MET	A	130	2.078	-2.363	4.364	1.00	28.56	B	C
ATOM	1022	O	MET	A	130	2.709	-1.295	4.417	1.00	27.48	B	O
ATOM	1023	N	GLY	A	131	1.774	-3.001	3.221	1.00	31.19	B	N
ATOM	1024	CA	GLY	A	131	2.136	-2.440	1.907	1.00	34.41	B	C
ATOM	1025	C	GLY	A	131	1.602	-1.029	1.750	1.00	29.65	B	C
ATOM	1026	O	GLY	A	131	2.366	-0.112	1.316	1.00	31.52	B	O
ATOM	1027	N	LYS	A	132	0.368	-0.793	2.179	1.00	27.70	B	N
ATOM	1028	CA	LYS	A	132	-0.282	0.524	1.883	1.00	29.17	B	C
ATOM	1029	CB	LYS	A	132	-1.809	0.498	1.831	1.00	33.37	B	C
ATOM	1030	CG	LYS	A	132	-2.459	-0.639	2.559	1.00	45.28	B	C
ATOM	1031	CD	LYS	A	132	-2.622	-0.272	3.949	1.00	40.74	B	C
ATOM	1032	CE	LYS	A	132	-4.080	-0.093	4.273	1.00	40.50	B	C
ATOM	1033	NZ	LYS	A	132	-4.246	0.481	5.629	1.00	36.00	B	N
ATOM	1034	C	LYS	A	132	0.181	1.625	2.838	1.00	26.79	B	C
ATOM	1035	O	LYS	A	132	-0.157	2.788	2.629	1.00	25.95	B	O
ATOM	1036	N	CYS	A	133	0.966	1.290	3.873	1.00	25.47	B	N
ATOM	1037	CA	CYS	A	133	1.467	2.308	4.805	1.00	25.90	B	C
ATOM	1038	CB	CYS	A	133	1.138	1.885	6.235	1.00	24.20	B	C
ATOM	1039	SG	CYS	A	133	1.844	2.915	7.564	1.00	24.10	B	S
ATOM	1040	C	CYS	A	133	2.958	2.459	4.602	1.00	22.36	B	C
ATOM	1041	O	CYS	A	133	3.732	1.532	4.829	1.00	24.01	B	O
ATOM	1042	N	GLN	A	134	3.372	3.636	4.209	1.00	22.75	B	N

Fig. 5 (cont.)

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ATOM	1043	CA	GLN	A	134	4.775	3.868	3.853	1.00	23.41	B	C
ATOM	1044	CB	GLN	A	134	5.006	3.769	2.320	1.00	24.46	B	C
ATOM	1045	CG	GLN	A	134	4.668	2.412	1.728	1.00	27.23	B	C
ATOM	1046	CD	GLN	A	134	5.066	2.330	0.261	1.00	26.16	B	C
ATOM	1047	OE1	GLN	A	134	6.048	2.941	-0.140	1.00	31.30	B	O
ATOM	1048	NE2	GLN	A	134	4.260	1.595	-0.481	1.00	28.10	B	N
ATOM	1049	C	GLN	A	134	5.146	5.254	4.319	1.00	23.15	B	C
ATOM	1050	O	GLN	A	134	4.332	6.159	4.317	1.00	24.65	B	O
ATOM	1051	N	PHE	A	135	6.385	5.410	4.696	1.00	23.19	B	N
ATOM	1052	CA	PHE	A	135	6.905	6.746	4.988	1.00	23.96	B	C
ATOM	1053	CB	PHE	A	135	8.287	6.712	5.638	1.00	24.24	B	C
ATOM	1054	CG	PHE	A	135	8.309	6.179	7.061	1.00	24.50	B	C
ATOM	1055	CD1	PHE	A	135	7.453	6.719	8.018	1.00	24.68	B	C
ATOM	1056	CE1	PHE	A	135	7.468	6.223	9.326	1.00	25.67	B	C
ATOM	1057	CZ	PHE	A	135	8.291	5.172	9.656	1.00	24.75	B	C
ATOM	1058	CD2	PHE	A	135	9.105	5.091	7.400	1.00	24.40	B	C
ATOM	1059	CE2	PHE	A	135	9.116	4.604	8.711	1.00	26.49	B	C
ATOM	1060	C	PHE	A	135	7.002	7.554	3.712	1.00	25.07	B	C
ATOM	1061	O	PHE	A	135	7.377	6.947	2.684	1.00	26.18	B	O
ATOM	1062	N	ASN	A	136	6.671	8.823	3.826	1.00	24.28	B	N
ATOM	1063	CA	ASN	A	136	6.935	9.840	2.786	1.00	23.54	B	C
ATOM	1064	CB	ASN	A	136	5.954	11.001	2.891	1.00	23.25	B	C
ATOM	1065	CG	ASN	A	136	6.210	12.087	1.867	1.00	23.11	B	C
ATOM	1066	OD1	ASN	A	136	7.362	12.296	1.478	1.00	26.79	B	O
ATOM	1067	ND2	ASN	A	136	5.159	12.818	1.523	1.00	26.56	B	N
ATOM	1068	C	ASN	A	136	8.383	10.226	3.000	1.00	22.45	B	C
ATOM	1069	O	ASN	A	136	8.684	11.020	3.926	1.00	24.63	B	O
ATOM	1070	N	THR	A	137	9.304	9.613	2.271	1.00	23.27	B	N
ATOM	1071	CA	THR	A	137	10.727	9.643	2.628	1.00	21.88	B	C
ATOM	1072	CB	THR	A	137	11.527	8.621	1.830	1.00	23.13	B	C
ATOM	1073	OG1	THR	A	137	11.198	8.866	0.436	1.00	23.16	B	O
ATOM	1074	CG2	THR	A	137	11.138	7.197	2.137	1.00	22.64	B	C
ATOM	1075	C	THR	A	137	11.272	11.058	2.548	1.00	24.34	B	C
ATOM	1076	O	THR	A	137	12.154	11.399	3.372	1.00	24.55	B	O
ATOM	1077	N	LEU	A	138	10.808	11.875	1.595	1.00	23.46	B	N
ATOM	1078	CA	LEU	A	138	11.279	13.274	1.467	1.00	23.81	B	C
ATOM	1079	CB	LEU	A	138	10.676	13.903	0.200	1.00	25.95	B	C
ATOM	1080	CG	LEU	A	138	11.128	15.327	-0.070	1.00	28.35	B	C
ATOM	1081	CD1	LEU	A	138	12.651	15.420	-0.200	1.00	29.29	B	C
ATOM	1082	CD2	LEU	A	138	10.462	15.860	-1.336	1.00	28.01	B	C
ATOM	1083	C	LEU	A	138	10.854	14.014	2.733	1.00	23.22	B	C
ATOM	1084	O	LEU	A	138	11.710	14.713	3.291	1.00	23.73	B	O
ATOM	1085	N	GLN	A	139	9.573	13.917	3.066	1.00	24.50	B	N
ATOM	1086	CA	GLN	A	139	9.012	14.710	4.197	1.00	24.76	B	C
ATOM	1087	CB	GLN	A	139	7.522	14.477	4.321	1.00	29.68	B	C
ATOM	1088	CG	GLN	A	139	6.723	15.613	4.906	1.00	42.90	B	C
ATOM	1089	CD	GLN	A	139	5.270	15.451	4.511	1.00	52.44	B	C
ATOM	1090	OE1	GLN	A	139	4.837	15.926	3.461	1.00	57.93	B	O
ATOM	1091	NE2	GLN	A	139	4.501	14.757	5.341	1.00	50.67	B	N
ATOM	1092	C	GLN	A	139	9.700	14.271	5.497	1.00	23.98	B	C
ATOM	1093	O	GLN	A	139	10.083	15.129	6.276	1.00	24.49	B	O
ATOM	1094	N	VAL	A	140	9.900	12.981	5.670	1.00	23.84	B	N
ATOM	1095	CA	VAL	A	140	10.524	12.469	6.916	1.00	22.44	B	C
ATOM	1096	CB	VAL	A	140	10.405	10.945	7.016	1.00	23.75	B	C
ATOM	1097	CG1	VAL	A	140	11.430	10.344	7.967	1.00	26.19	B	C
ATOM	1098	CG2	VAL	A	140	8.987	10.584	7.329	1.00	24.41	B	C
ATOM	1099	C	VAL	A	140	11.976	12.889	6.959	1.00	23.54	B	C
ATOM	1100	O	VAL	A	140	12.415	13.418	7.996	1.00	22.04	B	O

Fig. 5 (cont.)

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ATOM	1101	N	CYS	A	141	12.756	12.707	5.909	1.00	22.30	B	N
ATOM	1102	CA	CYS	A	141	14.188	13.052	5.944	1.00	21.54	B	C
ATOM	1103	CB	CYS	A	141	14.955	12.545	4.721	1.00	24.14	B	C
ATOM	1104	SG	CYS	A	141	16.140	11.246	5.163	1.00	28.31	B	S
ATOM	1105	C	CYS	A	141	14.341	14.563	6.111	1.00	24.95	B	C
ATOM	1106	O	CYS	A	141	15.193	14.981	6.892	1.00	25.19	B	O
ATOM	1107	N	ASN	A	142	13.576	15.373	5.395	1.00	25.41	B	N
ATOM	1108	CA	ASN	A	142	13.620	16.853	5.563	1.00	26.14	B	C
ATOM	1109	CB	ASN	A	142	12.654	17.574	4.617	1.00	26.59	B	C
ATOM	1110	CG	ASN	A	142	13.147	17.623	3.173	1.00	27.99	B	C
ATOM	1111	OD1	ASN	A	142	14.289	17.334	2.894	1.00	29.48	B	O
ATOM	1112	ND2	ASN	A	142	12.270	17.987	2.263	1.00	28.30	B	N
ATOM	1113	C	ASN	A	142	13.312	17.212	7.034	1.00	25.03	B	C
ATOM	1114	O	ASN	A	142	14.024	18.048	7.567	1.00	25.08	B	O
ATOM	1115	N	ALA	A	143	12.277	16.615	7.626	1.00	23.46	B	N
ATOM	1116	CA	ALA	A	143	11.839	16.950	9.002	1.00	22.52	B	C
ATOM	1117	CB	ALA	A	143	10.519	16.357	9.338	1.00	24.15	B	C
ATOM	1118	C	ALA	A	143	12.931	16.550	9.985	1.00	24.91	B	C
ATOM	1119	O	ALA	A	143	13.231	17.350	10.924	1.00	25.25	B	O
ATOM	1120	N	ILE	A	144	13.500	15.356	9.840	1.00	22.68	B	N
ATOM	1121	CA	ILE	A	144	14.521	14.892	10.802	1.00	22.52	B	C
ATOM	1122	CB	ILE	A	144	14.837	13.409	10.612	1.00	23.52	B	C
ATOM	1123	CG1	ILE	A	144	13.647	12.539	10.990	1.00	23.57	B	C
ATOM	1124	CG2	ILE	A	144	16.109	13.041	11.374	1.00	24.77	B	C
ATOM	1125	CD1	ILE	A	144	13.837	11.078	10.671	1.00	25.72	B	C
ATOM	1126	C	ILE	A	144	15.764	15.763	10.707	1.00	27.84	B	C
ATOM	1127	O	ILE	A	144	16.340	16.124	11.768	1.00	27.22	B	O
ATOM	1128	N	LEU	A	145	16.225	16.080	9.500	1.00	25.12	B	N
ATOM	1129	CA	LEU	A	145	17.438	16.898	9.313	1.00	28.20	B	C
ATOM	1130	CB	LEU	A	145	17.801	16.982	7.827	1.00	28.64	B	C
ATOM	1131	CG	LEU	A	145	18.381	15.675	7.291	1.00	29.26	B	C
ATOM	1132	CD1	LEU	A	145	18.455	15.707	5.771	1.00	30.79	B	C
ATOM	1133	CD2	LEU	A	145	19.743	15.361	7.876	1.00	33.14	B	C
ATOM	1134	C	LEU	A	145	17.218	18.262	9.963	1.00	27.85	B	C
ATOM	1135	O	LEU	A	145	18.183	18.685	10.620	1.00	34.04	B	O
ATOM	1136	N	GLU	A	146	16.034	18.870	9.823	1.00	29.05	B	N
ATOM	1137	CA	GLU	A	146	15.684	20.168	10.490	1.00	30.76	B	C
ATOM	1138	CB	GLU	A	146	14.269	20.623	10.105	1.00	40.12	B	C
ATOM	1139	CG	GLU	A	146	14.220	21.570	8.932	1.00	54.79	B	C
ATOM	1140	CD	GLU	A	146	15.050	22.830	9.149	1.00	64.72	B	C
ATOM	1141	OE1	GLU	A	146	14.628	23.672	9.959	1.00	69.22	B	O
ATOM	1142	OE2	GLU	A	146	16.143	22.940	8.538	1.00	79.12	B	O
ATOM	1143	C	GLU	A	146	15.875	19.989	12.013	1.00	34.12	B	C
ATOM	1144	O	GLU	A	146	16.625	20.787	12.633	1.00	37.92	B	O
ATOM	1145	N	AARG	A	147	15.293	18.943	12.602	0.50	29.75	B	N
ATOM	1146	N	BARG	A	147	15.266	18.952	12.605	0.50	32.38	B	N
ATOM	1147	CA	AARG	A	147	15.280	18.739	14.081	0.50	28.39	B	C
ATOM	1148	CA	BARG	A	147	15.222	18.714	14.083	0.50	32.66	B	C
ATOM	1149	CB	AARG	A	147	14.376	17.570	14.445	0.50	24.37	B	C
ATOM	1150	CB	BARG	A	147	14.225	17.592	14.393	0.50	31.41	B	C
ATOM	1151	CG	AARG	A	147	12.909	17.883	14.226	0.50	23.44	B	C
ATOM	1152	CG	BARG	A	147	13.809	17.429	15.853	0.50	31.70	B	C
ATOM	1153	CD	AARG	A	147	11.994	16.938	14.953	0.50	23.64	B	C
ATOM	1154	CD	BARG	A	147	13.122	16.099	15.980	0.50	35.13	B	C
ATOM	1155	NE	AARG	A	147	12.086	17.180	16.397	0.50	22.81	B	N
ATOM	1156	NE	BARG	A	147	12.561	15.855	17.291	0.50	32.83	B	N
ATOM	1157	CZ	AARG	A	147	12.176	16.235	17.327	0.50	22.96	B	C
ATOM	1158	CZ	BARG	A	147	11.323	16.198	17.625	0.50	34.60	B	C

Fig. 5 (cont.)

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ATOM	1159	NH1AARG	A	147	12.152	14.957	16.984	0.50	19.86	B	N	
ATOM	1160	NH1BARG	A	147	10.545	16.828	16.758	0.50	36.15	B	N	
ATOM	1161	NH2AARG	A	147	12.253	16.595	18.600	0.50	22.66	B	N	
ATOM	1162	NH2BARG	A	147	10.880	15.933	18.836	0.50	36.73	B	N	
ATOM	1163	C	AARG	A	147	16.659	18.399	14.640	0.50	34.01	B	C
ATOM	1164	C	BARG	A	147	16.614	18.370	14.639	0.50	36.59	B	C
ATOM	1165	O	AARG	A	147	16.932	18.666	15.845	0.50	34.67	B	O
ATOM	1166	O	BARG	A	147	16.877	18.664	15.834	0.50	38.80	B	O
ATOM	1167	N	ALA	A	148	17.474	17.745	13.830	1.00	31.68	B	N
ATOM	1168	CA	ALA	A	148	18.826	17.303	14.202	1.00	30.49	B	C
ATOM	1169	CB	ALA	A	148	19.325	16.326	13.152	1.00	31.81	B	C
ATOM	1170	C	ALA	A	148	19.759	18.499	14.249	1.00	32.14	B	C
ATOM	1171	O	ALA	A	148	20.878	18.339	14.736	1.00	35.82	B	O
ATOM	1172	N	GLY	A	149	19.338	19.606	13.660	1.00	34.49	B	N
ATOM	1173	CA	GLY	A	149	20.164	20.821	13.647	1.00	41.38	B	C
ATOM	1174	C	GLY	A	149	20.436	21.278	12.235	1.00	45.06	B	C
ATOM	1175	O	GLY	A	149	21.054	22.330	12.109	1.00	48.33	B	O
ATOM	1176	N	GLY	A	150	20.084	20.477	11.226	1.00	42.51	B	N
ATOM	1177	CA	GLY	A	150	20.249	20.820	9.792	1.00	33.31	B	C
ATOM	1178	C	GLY	A	150	21.013	19.745	9.029	1.00	40.22	B	C
ATOM	1179	O	GLY	A	150	20.781	19.587	7.840	1.00	41.60	B	O
ATOM	1180	N	ASN	A	151	21.921	19.037	9.688	1.00	34.60	B	N
ATOM	1181	CA	ASN	A	151	22.511	17.774	9.194	1.00	36.34	B	C
ATOM	1182	CB	ASN	A	151	23.624	18.000	8.177	1.00	36.22	B	C
ATOM	1183	CG	ASN	A	151	24.777	18.792	8.745	1.00	38.92	B	C
ATOM	1184	OD1	ASN	A	151	25.383	18.413	9.738	1.00	40.68	B	O
ATOM	1185	ND2	ASN	A	151	25.039	19.942	8.164	1.00	42.53	B	N
ATOM	1186	C	ASN	A	151	23.004	16.958	10.385	1.00	34.24	B	C
ATOM	1187	O	ASN	A	151	22.978	17.428	11.544	1.00	30.32	B	O
ATOM	1188	N	ILE	A	152	23.411	15.739	10.128	1.00	28.97	B	N
ATOM	1189	CA	ILE	A	152	23.819	14.797	11.194	1.00	30.90	B	C
ATOM	1190	CB	ILE	A	152	23.826	13.357	10.662	1.00	31.58	B	C
ATOM	1191	CG1	ILE	A	152	22.397	12.910	10.337	1.00	32.56	B	C
ATOM	1192	CG2	ILE	A	152	24.534	12.409	11.615	1.00	33.14	B	C
ATOM	1193	CD1	ILE	A	152	21.390	13.216	11.436	1.00	31.99	B	C
ATOM	1194	C	ILE	A	152	25.148	15.239	11.800	1.00	31.68	B	C
ATOM	1195	O	ILE	A	152	25.342	15.014	12.992	1.00	30.06	B	O
ATOM	1196	N	GLU	A	153	25.993	15.965	11.066	1.00	31.80	B	N
ATOM	1197	CA	GLU	A	153	27.260	16.462	11.658	1.00	33.51	B	C
ATOM	1198	CB	GLU	A	153	28.160	17.134	10.612	1.00	41.08	B	C
ATOM	1199	CG	GLU	A	153	28.987	16.166	9.785	1.00	53.35	B	C
ATOM	1200	CD	GLU	A	153	30.243	16.795	9.180	1.00	61.48	B	C
ATOM	1201	OE1	GLU	A	153	30.168	17.964	8.712	1.00	63.63	B	O
ATOM	1202	OE2	GLU	A	153	31.308	16.135	9.214	1.00	71.91	B	O
ATOM	1203	C	GLU	A	153	26.928	17.437	12.802	1.00	28.89	B	C
ATOM	1204	O	GLU	A	153	27.617	17.351	13.827	1.00	28.06	B	O
ATOM	1205	N	LEU	A	154	25.924	18.297	12.653	1.00	26.06	B	N
ATOM	1206	CA	LEU	A	154	25.581	19.300	13.663	1.00	27.70	B	C
ATOM	1207	CB	LEU	A	154	24.612	20.356	13.110	1.00	30.60	B	C
ATOM	1208	CG	LEU	A	154	25.236	21.351	12.113	1.00	33.82	B	C
ATOM	1209	CD1	LEU	A	154	24.177	22.241	11.491	1.00	35.01	B	C
ATOM	1210	CD2	LEU	A	154	26.285	22.205	12.811	1.00	33.46	B	C
ATOM	1211	C	LEU	A	154	24.972	18.555	14.863	1.00	24.43	B	C
ATOM	1212	O	LEU	A	154	25.167	18.947	16.014	1.00	24.68	B	O
ATOM	1213	N	PHE	A	155	24.171	17.512	14.582	1.00	23.50	B	N
ATOM	1214	CA	PHE	A	155	23.509	16.728	15.652	1.00	23.86	B	C
ATOM	1215	CB	PHE	A	155	22.552	15.722	15.013	1.00	25.25	B	C
ATOM	1216	CG	PHE	A	155	21.755	14.891	15.975	1.00	26.29	B	C

Fig. 5 (cont.)

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ATOM	1217	CD1	PHE	A	155	20.818	15.461	16.799	1.00	28.89	B	C
ATOM	1218	CE1	PHE	A	155	20.057	14.622	17.625	1.00	26.23	B	C
ATOM	1219	CZ	PHE	A	155	20.261	13.279	17.609	1.00	25.42	B	C
ATOM	1220	CD2	PHE	A	155	21.951	13.519	15.983	1.00	28.58	B	C
ATOM	1221	CE2	PHE	A	155	21.195	12.703	16.803	1.00	29.24	B	C
ATOM	1222	C	PHE	A	155	24.589	16.034	16.466	1.00	20.47	B	C
ATOM	1223	O	PHE	A	155	24.557	16.027	17.719	1.00	19.95	B	O
ATOM	1224	N	THR	A	156	25.593	15.468	15.823	1.00	22.67	B	N
ATOM	1225	CA	THR	A	156	26.753	14.886	16.493	1.00	21.16	B	C
ATOM	1226	CB	THR	A	156	27.690	14.229	15.483	1.00	24.43	B	C
ATOM	1227	OG1	THR	A	156	26.937	13.219	14.781	1.00	25.33	B	O
ATOM	1228	CG2	THR	A	156	28.904	13.666	16.154	1.00	23.15	B	C
ATOM	1229	C	THR	A	156	27.464	15.939	17.337	1.00	22.66	B	C
ATOM	1230	O	THR	A	156	27.825	15.719	18.527	1.00	22.20	B	O
ATOM	1231	N	GLN	A	157	27.695	17.130	16.764	1.00	22.55	B	N
ATOM	1232	CA	GLN	A	157	28.379	18.209	17.535	1.00	25.38	B	C
ATOM	1233	CB	GLN	A	157	28.550	19.490	16.700	1.00	28.57	B	C
ATOM	1234	CG	GLN	A	157	29.043	20.698	17.566	1.00	33.59	B	C
ATOM	1235	CD	GLN	A	157	29.150	22.066	16.907	1.00	43.97	B	C
ATOM	1236	OE1	GLN	A	157	29.279	23.132	17.563	1.00	40.30	B	O
ATOM	1237	NE2	GLN	A	157	29.090	22.067	15.583	1.00	37.07	B	N
ATOM	1238	C	GLN	A	157	27.595	18.547	18.809	1.00	21.56	B	C
ATOM	1239	O	GLN	A	157	28.160	18.751	19.900	1.00	23.48	B	O
ATOM	1240	N	ARG	A	158	26.280	18.582	18.717	1.00	20.55	B	N
ATOM	1241	CA	ARG	A	158	25.399	18.881	19.843	1.00	18.95	B	C
ATOM	1242	CB	ARG	A	158	23.956	18.822	19.350	1.00	19.54	B	C
ATOM	1243	CG	ARG	A	158	22.891	18.903	20.413	1.00	20.85	B	C
ATOM	1244	CD	ARG	A	158	21.462	18.756	19.944	1.00	24.19	B	C
ATOM	1245	NE	ARG	A	158	20.983	19.962	19.246	1.00	27.75	B	N
ATOM	1246	CZ	ARG	A	158	19.734	20.429	19.312	1.00	41.39	B	C
ATOM	1247	NH1	ARG	A	158	18.793	19.785	19.994	1.00	43.30	B	N
ATOM	1248	NH2	ARG	A	158	19.429	21.565	18.701	1.00	43.85	B	N
ATOM	1249	C	ARG	A	158	25.599	17.920	21.024	1.00	20.98	B	C
ATOM	1250	O	ARG	A	158	25.404	18.264	22.191	1.00	20.99	B	O
ATOM	1251	N	TYR	A	159	25.957	16.668	20.727	1.00	20.39	B	N
ATOM	1252	CA	TYR	A	159	26.147	15.596	21.730	1.00	20.09	B	C
ATOM	1253	CB	TYR	A	159	25.256	14.401	21.363	1.00	18.47	B	C
ATOM	1254	CG	TYR	A	159	23.796	14.648	21.597	1.00	17.43	B	C
ATOM	1255	CD1	TYR	A	159	23.252	14.468	22.852	1.00	18.82	B	C
ATOM	1256	CE1	TYR	A	159	21.928	14.760	23.102	1.00	17.46	B	C
ATOM	1257	CZ	TYR	A	159	21.053	15.169	22.130	1.00	17.49	B	C
ATOM	1258	OH	TYR	A	159	19.753	15.381	22.393	1.00	18.67	B	O
ATOM	1259	CE2	TYR	A	159	21.585	15.355	20.870	1.00	16.71	B	C
ATOM	1260	CD2	TYR	A	159	22.927	15.104	20.619	1.00	17.66	B	C
ATOM	1261	C	TYR	A	159	27.609	15.189	21.875	1.00	20.15	B	C
ATOM	1262	O	TYR	A	159	27.905	14.130	22.482	1.00	19.59	B	O
ATOM	1263	N	GLN	A	160	28.561	15.953	21.406	1.00	20.72	B	N
ATOM	1264	CA	GLN	A	160	29.987	15.567	21.486	1.00	23.02	B	C
ATOM	1265	CB	GLN	A	160	30.943	16.691	21.014	1.00	30.71	B	C
ATOM	1266	CG	GLN	A	160	30.938	16.886	19.517	1.00	40.95	B	C
ATOM	1267	CD	GLN	A	160	31.869	15.967	18.763	1.00	51.19	B	C
ATOM	1268	OE1	GLN	A	160	31.496	15.345	17.768	1.00	65.06	B	O
ATOM	1269	NE2	GLN	A	160	33.126	15.941	19.165	1.00	60.93	B	N
ATOM	1270	C	GLN	A	160	30.376	15.226	22.906	1.00	20.21	B	C
ATOM	1271	O	GLN	A	160	31.064	14.187	23.120	1.00	20.22	B	O
ATOM	1272	N	SER	A	161	29.972	15.968	23.931	1.00	20.43	B	N
ATOM	1273	CA	SER	A	161	30.367	15.738	25.328	1.00	19.76	B	C
ATOM	1274	CB	SER	A	161	29.981	16.933	26.246	1.00	23.35	B	C

Fig. 5 (cont.)

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ATOM	1275	OG	SER A 161	28.655	16.858	26.711	1.00	27.62	B	O
ATOM	1276	C	SER A 161	29.832	14.379	25.830	1.00	20.55	B	C
ATOM	1277	O	SER A 161	30.492	13.776	26.724	1.00	21.28	B	O
ATOM	1278	N	SER A 162	28.664	14.010	25.364	1.00	18.65	B	N
ATOM	1279	CA	SER A 162	28.084	12.689	25.742	1.00	18.30	B	C
ATOM	1280	CB	SER A 162	26.635	12.662	25.423	1.00	18.48	B	C
ATOM	1281	OG	SER A 162	25.905	13.731	26.067	1.00	19.36	B	O
ATOM	1282	C	SER A 162	28.855	11.564	25.059	1.00	17.92	B	C
ATOM	1283	O	SER A 162	29.048	10.540	25.749	1.00	19.68	B	O
ATOM	1284	N	PHE A 163	29.188	11.669	23.783	1.00	18.48	B	N
ATOM	1285	CA	PHE A 163	30.079	10.653	23.168	1.00	19.06	B	C
ATOM	1286	CB	PHE A 163	30.324	10.961	21.712	1.00	19.54	B	C
ATOM	1287	CG	PHE A 163	29.175	10.650	20.814	1.00	18.67	B	C
ATOM	1288	CD1	PHE A 163	28.863	9.316	20.531	1.00	18.42	B	C
ATOM	1289	CE1	PHE A 163	27.827	9.029	19.689	1.00	21.00	B	C
ATOM	1290	CZ	PHE A 163	27.120	10.014	19.062	1.00	20.91	B	C
ATOM	1291	CD2	PHE A 163	28.436	11.625	20.184	1.00	19.40	B	C
ATOM	1292	CE2	PHE A 163	27.390	11.323	19.339	1.00	19.12	B	C
ATOM	1293	C	PHE A 163	31.342	10.541	23.991	1.00	21.12	B	C
ATOM	1294	O	PHE A 163	31.843	9.451	24.262	1.00	21.42	B	O
ATOM	1295	N	ARG A 164	31.938	11.638	24.453	1.00	20.72	B	N
ATOM	1296	CA	ARG A 164	33.178	11.530	25.251	1.00	20.35	B	C
ATOM	1297	CB	ARG A 164	33.734	12.913	25.687	1.00	21.66	B	C
ATOM	1298	CG	ARG A 164	34.234	13.832	24.565	1.00	23.27	B	C
ATOM	1299	CD	ARG A 164	35.251	13.202	23.628	1.00	25.35	B	C
ATOM	1300	NE	ARG A 164	34.692	12.437	22.534	1.00	26.69	B	N
ATOM	1301	CZ	ARG A 164	34.121	12.920	21.454	1.00	24.54	B	C
ATOM	1302	NH1	ARG A 164	34.093	14.241	21.243	1.00	27.57	B	N
ATOM	1303	NH2	ARG A 164	33.558	12.104	20.582	1.00	25.05	B	N
ATOM	1304	C	ARG A 164	32.896	10.750	26.515	1.00	20.22	B	C
ATOM	1305	O	ARG A 164	33.715	9.854	26.859	1.00	21.13	B	O
ATOM	1306	N	THR A 165	31.791	10.927	27.197	1.00	19.61	B	N
ATOM	1307	CA	THR A 165	31.456	10.155	28.392	1.00	20.40	B	C
ATOM	1308	CB	THR A 165	30.166	10.678	29.023	1.00	19.99	B	C
ATOM	1309	OG1	THR A 165	30.395	12.061	29.405	1.00	22.01	B	O
ATOM	1310	CG2	THR A 165	29.772	9.918	30.241	1.00	21.95	B	C
ATOM	1311	C	THR A 165	31.299	8.657	28.018	1.00	19.90	B	C
ATOM	1312	O	THR A 165	31.812	7.828	28.785	1.00	21.41	B	O
ATOM	1313	N	LEU A 166	30.620	8.346	26.927	1.00	19.12	B	N
ATOM	1314	CA	LEU A 166	30.460	6.908	26.507	1.00	18.11	B	C
ATOM	1315	CB	LEU A 166	29.529	6.828	25.306	1.00	18.06	B	C
ATOM	1316	CG	LEU A 166	29.336	5.422	24.680	1.00	18.84	B	C
ATOM	1317	CD1	LEU A 166	28.876	4.435	25.750	1.00	19.51	B	C
ATOM	1318	CD2	LEU A 166	28.387	5.537	23.537	1.00	19.82	B	C
ATOM	1319	C	LEU A 166	31.828	6.343	26.202	1.00	22.15	B	C
ATOM	1320	O	LEU A 166	32.100	5.168	26.608	1.00	20.34	B	O
ATOM	1321	N	GLU A 167	32.666	7.047	25.473	1.00	19.09	B	N
ATOM	1322	CA	GLU A 167	34.004	6.571	25.143	1.00	21.06	B	C
ATOM	1323	CB	GLU A 167	34.735	7.494	24.164	1.00	19.18	B	C
ATOM	1324	CG	GLU A 167	34.125	7.683	22.827	1.00	22.10	B	C
ATOM	1325	CD	GLU A 167	34.374	8.991	22.102	1.00	20.50	B	C
ATOM	1326	OE1	GLU A 167	35.256	9.762	22.604	1.00	25.17	B	O
ATOM	1327	OE2	GLU A 167	33.675	9.217	21.093	1.00	22.86	B	O
ATOM	1328	C	GLU A 167	34.775	6.303	26.425	1.00	22.01	B	C
ATOM	1329	O	GLU A 167	35.474	5.236	26.500	1.00	21.99	B	O
ATOM	1330	N	ASN A 168	34.659	7.117	27.466	1.00	21.50	B	N
ATOM	1331	CA	ASN A 168	35.327	6.864	28.751	1.00	22.37	B	C
ATOM	1332	CB	ASN A 168	35.197	8.099	29.632	1.00	23.68	B	C

Fig. 5 (cont.)

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ATOM	1333	CG	ASN	A	168	35.969	7.936	30.903	1.00	30.17	B	C
ATOM	1334	OD1	ASN	A	168	37.159	7.609	30.868	1.00	36.02	B	O
ATOM	1335	ND2	ASN	A	168	35.306	8.131	32.021	1.00	39.58	B	N
ATOM	1336	C	ASN	A	168	34.788	5.609	29.422	1.00	22.05	B	C
ATOM	1337	O	ASN	A	168	35.591	4.825	30.002	1.00	25.05	B	O
ATOM	1338	N	VAL	A	169	33.488	5.361	29.368	1.00	20.57	B	N
ATOM	1339	CA	VAL	A	169	32.906	4.120	29.956	1.00	21.32	B	C
ATOM	1340	CB	VAL	A	169	31.378	4.220	30.040	1.00	22.28	B	C
ATOM	1341	CG1	VAL	A	169	30.742	2.920	30.494	1.00	22.96	B	C
ATOM	1342	CG2	VAL	A	169	30.999	5.286	31.027	1.00	22.33	B	C
ATOM	1343	C	VAL	A	169	33.393	2.870	29.201	1.00	20.02	B	C
ATOM	1344	O	VAL	A	169	33.648	1.874	29.887	1.00	21.47	B	O
ATOM	1345	N	LEU	A	170	33.455	2.910	27.886	1.00	19.05	B	N
ATOM	1346	CA	LEU	A	170	33.912	1.779	27.038	1.00	19.52	B	C
ATOM	1347	CB	LEU	A	170	33.569	2.083	25.592	1.00	20.88	B	C
ATOM	1348	CG	LEU	A	170	32.089	2.094	25.242	1.00	19.53	B	C
ATOM	1349	CD1	LEU	A	170	31.871	2.549	23.825	1.00	22.22	B	C
ATOM	1350	CD2	LEU	A	170	31.518	0.693	25.459	1.00	22.08	B	C
ATOM	1351	C	LEU	A	170	35.424	1.577	27.175	1.00	24.43	B	C
ATOM	1352	O	LEU	A	170	35.910	0.494	26.738	1.00	24.64	B	O
ATOM	1353	N	ASN	A	171	36.189	2.559	27.640	1.00	21.64	B	N
ATOM	1354	CA	ASN	A	171	37.655	2.647	27.371	1.00	23.16	B	C
ATOM	1355	CB	ASN	A	171	38.409	1.585	28.179	1.00	29.97	B	C
ATOM	1356	CG	ASN	A	171	39.839	2.014	28.479	1.00	47.49	B	C
ATOM	1357	OD1	ASN	A	171	40.272	3.107	28.076	1.00	54.70	B	O
ATOM	1358	ND2	ASN	A	171	40.586	1.164	29.174	1.00	53.75	B	N
ATOM	1359	C	ASN	A	171	37.907	2.537	25.883	1.00	22.59	B	C
ATOM	1360	O	ASN	A	171	38.810	1.715	25.442	1.00	24.69	B	O
ATOM	1361	N	PHE	A	172	37.131	3.245	25.064	1.00	22.09	B	N
ATOM	1362	CA	PHE	A	172	37.159	3.200	23.599	1.00	23.06	B	C
ATOM	1363	CB	PHE	A	172	36.208	4.240	22.991	1.00	22.64	B	C
ATOM	1364	CG	PHE	A	172	36.234	4.289	21.501	1.00	22.96	B	C
ATOM	1365	CD1	PHE	A	172	35.722	3.252	20.742	1.00	24.38	B	C
ATOM	1366	CE1	PHE	A	172	35.818	3.248	19.365	1.00	26.63	B	C
ATOM	1367	CZ	PHE	A	172	36.453	4.276	18.724	1.00	27.56	B	C
ATOM	1368	CD2	PHE	A	172	36.891	5.327	20.836	1.00	24.95	B	C
ATOM	1369	CE2	PHE	A	172	36.963	5.318	19.455	1.00	24.88	B	C
ATOM	1370	C	PHE	A	172	38.593	3.369	23.054	1.00	25.67	B	C
ATOM	1371	O	PHE	A	172	38.928	2.742	21.999	1.00	26.73	B	O
ATOM	1372	N	SER	A	173	39.399	4.220	23.669	1.00	24.73	B	N
ATOM	1373	CA	SER	A	173	40.734	4.574	23.081	1.00	26.79	B	C
ATOM	1374	CB	SER	A	173	41.348	5.778	23.741	1.00	28.53	B	C
ATOM	1375	OG	SER	A	173	41.732	5.515	25.064	1.00	29.83	B	O
ATOM	1376	C	SER	A	173	41.668	3.366	23.158	1.00	31.39	B	C
ATOM	1377	O	SER	A	173	42.678	3.412	22.444	1.00	33.26	B	O
ATOM	1378	N	GLN	A	174	41.354	2.365	23.995	1.00	29.42	B	N
ATOM	1379	CA	GLN	A	174	42.130	1.089	24.144	1.00	30.26	B	C
ATOM	1380	CB	GLN	A	174	42.181	0.667	25.608	1.00	33.46	B	C
ATOM	1381	CG	GLN	A	174	42.424	1.801	26.584	1.00	42.94	B	C
ATOM	1382	CD	GLN	A	174	43.798	1.885	27.187	1.00	58.52	B	C
ATOM	1383	OE1	GLN	A	174	44.528	2.855	26.979	1.00	77.75	B	O
ATOM	1384	NE2	GLN	A	174	44.146	0.884	27.977	1.00	69.31	B	N
ATOM	1385	C	GLN	A	174	41.493	-0.034	23.329	1.00	31.35	B	C
ATOM	1386	O	GLN	A	174	41.977	-1.190	23.385	1.00	32.74	B	O
ATOM	1387	N	SER	A	175	40.482	0.253	22.536	1.00	28.74	B	N
ATOM	1388	CA	SER	A	175	39.759	-0.763	21.757	1.00	29.51	B	C
ATOM	1389	CB	SER	A	175	38.297	-0.374	21.683	1.00	26.82	B	C
ATOM	1390	OG	SER	A	175	38.040	0.545	20.613	1.00	27.82	B	O

Fig. 5 (cont.)

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ATOM	1391	C	SER A 175	40.386	-0.950	20.376	1.00	30.84	B	C
ATOM	1392	O	SER A 175	41.168	-0.067	19.926	1.00	29.59	B	O
ATOM	1393	N	GLU A 176	40.013	-2.040	19.691	1.00	32.52	B	N
ATOM	1394	CA	GLU A 176	40.443	-2.296	18.292	1.00	33.10	B	C
ATOM	1395	CB	GLU A 176	39.993	-3.675	17.800	1.00	42.72	B	C
ATOM	1396	CG	GLU A 176	40.651	-4.785	18.583	1.00	50.67	B	C
ATOM	1397	CD	GLU A 176	40.527	-6.171	17.970	1.00	57.67	B	C
ATOM	1398	OE1	GLU A 176	39.571	-6.388	17.180	1.00	61.64	B	O
ATOM	1399	OE2	GLU A 176	41.393	-7.026	18.279	1.00	67.87	B	O
ATOM	1400	C	GLU A 176	39.898	-1.258	17.330	1.00	38.52	B	C
ATOM	1401	O	GLU A 176	40.557	-1.030	16.326	1.00	35.18	B	O
ATOM	1402	N	CYS A 177	38.712	-0.704	17.594	1.00	31.76	B	N
ATOM	1403	CA	CYS A 177	37.993	0.201	16.675	1.00	32.12	B	C
ATOM	1404	CB	CYS A 177	36.495	0.136	16.903	1.00	32.25	B	C
ATOM	1405	SG	CYS A 177	35.705	-1.014	15.753	1.00	40.30	B	S
ATOM	1406	C	CYS A 177	38.531	1.632	16.814	1.00	28.03	B	C
ATOM	1407	O	CYS A 177	38.144	2.471	15.988	1.00	36.57	B	O
ATOM	1408	N	CYS A 178	39.316	1.889	17.839	1.00	29.35	B	N
ATOM	1409	CA	CYS A 178	40.094	3.144	17.975	1.00	31.43	B	C
ATOM	1410	CB	CYS A 178	40.773	3.264	19.315	1.00	29.54	B	C
ATOM	1411	SG	CYS A 178	41.811	4.741	19.364	1.00	30.51	B	S
ATOM	1412	C	CYS A 178	41.148	3.216	16.866	1.00	36.00	B	C
ATOM	1413	O	CYS A 178	42.161	2.505	16.956	1.00	39.50	B	O
ATOM	1414	N	LYS A 179	40.964	4.177	15.973	1.00	45.28	B	N
ATOM	1415	CA	LYS A 179	41.715	4.287	14.696	1.00	56.14	B	C
ATOM	1416	CB	LYS A 179	40.692	4.348	13.559	1.00	59.19	B	C
ATOM	1417	CG	LYS A 179	39.850	3.086	13.409	1.00	62.57	B	C
ATOM	1418	CD	LYS A 179	38.668	3.210	12.461	1.00	63.16	B	C
ATOM	1419	CE	LYS A 179	37.986	1.877	12.210	1.00	59.94	B	C
ATOM	1420	NZ	LYS A 179	37.255	1.402	13.407	1.00	51.91	B	N
ATOM	1421	C	LYS A 179	42.638	5.511	14.743	1.00	56.16	B	C
ATOM	1422	O	LYS A 179	43.845	5.317	14.420	1.00	67.81	B	O
ATOM	1423	N	SER A 184	46.494	13.975	15.961	1.00	61.85	B	N
ATOM	1424	CA	SER A 184	46.661	14.769	17.211	1.00	64.98	B	C
ATOM	1425	CB	SER A 184	46.351	16.235	16.979	1.00	69.99	B	C
ATOM	1426	OG	SER A 184	47.144	16.783	15.941	1.00	78.25	B	O
ATOM	1427	C	SER A 184	45.730	14.233	18.305	1.00	58.56	B	C
ATOM	1428	O	SER A 184	46.216	13.780	19.356	1.00	57.57	B	O
ATOM	1429	N	THR A 185	44.429	14.315	18.019	1.00	49.46	B	N
ATOM	1430	CA	THR A 185	43.280	14.180	18.947	1.00	39.98	B	C
ATOM	1431	CB	THR A 185	42.077	14.939	18.378	1.00	35.44	B	C
ATOM	1432	OG1	THR A 185	41.870	14.557	17.019	1.00	33.55	B	O
ATOM	1433	CG2	THR A 185	42.264	16.440	18.411	1.00	38.19	B	C
ATOM	1434	C	THR A 185	42.952	12.694	19.145	1.00	33.05	B	C
ATOM	1435	O	THR A 185	43.168	11.897	18.202	1.00	34.90	B	O
ATOM	1436	N	LYS A 186	42.411	12.362	20.317	1.00	34.92	B	N
ATOM	1437	CA	LYS A 186	42.048	10.970	20.692	1.00	33.50	B	C
ATOM	1438	CB	LYS A 186	41.508	10.895	22.128	1.00	34.17	B	C
ATOM	1439	CG	LYS A 186	41.053	9.519	22.619	1.00	37.89	B	C
ATOM	1440	CD	LYS A 186	40.151	9.555	23.876	1.00	37.28	B	C
ATOM	1441	CE	LYS A 186	38.749	10.116	23.671	1.00	37.82	B	C
ATOM	1442	NZ	LYS A 186	37.825	9.118	23.077	1.00	32.60	B	N
ATOM	1443	C	LYS A 186	40.994	10.514	19.690	1.00	31.56	B	C
ATOM	1444	O	LYS A 186	40.143	11.320	19.284	1.00	28.17	B	O
ATOM	1445	N	CYS A 187	41.083	9.267	19.255	1.00	28.78	B	N
ATOM	1446	CA	CYS A 187	40.047	8.622	18.442	1.00	27.45	B	C
ATOM	1447	CB	CYS A 187	40.349	7.136	18.276	1.00	28.89	B	C
ATOM	1448	SG	CYS A 187	40.587	6.297	19.866	1.00	29.54	B	S

Fig. 5 (cont.)

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ATOM	1449	C	CYS	A	187	38.688	8.766	19.129	1.00	23.42	B	C
ATOM	1450	O	CYS	A	187	38.613	8.681	20.364	1.00	27.92	B	O
ATOM	1451	N	THR	A	188	37.667	8.903	18.319	1.00	26.18	B	N
ATOM	1452	CA	THR	A	188	36.277	9.063	18.819	1.00	27.50	B	C
ATOM	1453	CB	THR	A	188	35.762	10.485	18.573	1.00	25.89	B	C
ATOM	1454	OG1	THR	A	188	35.540	10.643	17.168	1.00	26.36	B	O
ATOM	1455	CG2	THR	A	188	36.676	11.483	19.232	1.00	28.91	B	C
ATOM	1456	C	THR	A	188	35.386	8.031	18.160	1.00	27.26	B	C
ATOM	1457	O	THR	A	188	35.661	7.547	17.050	1.00	27.16	B	O
ATOM	1458	N	LEU	A	189	34.249	7.767	18.794	1.00	25.94	B	N
ATOM	1459	CA	LEU	A	189	33.277	6.848	18.176	1.00	24.57	B	C
ATOM	1460	CB	LEU	A	189	32.178	6.609	19.217	1.00	27.43	B	C
ATOM	1461	CG	LEU	A	189	32.330	5.363	20.057	1.00	29.88	B	C
ATOM	1462	CD1	LEU	A	189	31.258	5.375	21.160	1.00	30.49	B	C
ATOM	1463	CD2	LEU	A	189	32.245	4.091	19.217	1.00	30.56	B	C
ATOM	1464	C	LEU	A	189	32.711	7.405	16.868	1.00	27.50	B	C
ATOM	1465	O	LEU	A	189	32.542	6.669	15.873	1.00	25.57	B	O
ATOM	1466	N	PRO	A	190	32.277	8.683	16.785	1.00	25.66	B	N
ATOM	1467	CA	PRO	A	190	31.732	9.151	15.519	1.00	27.72	B	C
ATOM	1468	CB	PRO	A	190	31.229	10.554	15.851	1.00	26.09	B	C
ATOM	1469	CG	PRO	A	190	30.917	10.445	17.343	1.00	26.03	B	C
ATOM	1470	CD	PRO	A	190	32.076	9.654	17.883	1.00	25.27	B	C
ATOM	1471	C	PRO	A	190	32.731	9.115	14.356	1.00	28.36	B	C
ATOM	1472	O	PRO	A	190	32.267	8.993	13.216	1.00	29.61	B	O
ATOM	1473	N	GLU	A	191	34.011	9.238	14.647	1.00	30.11	B	N
ATOM	1474	CA	GLU	A	191	35.046	9.230	13.572	1.00	35.39	B	C
ATOM	1475	CB	GLU	A	191	36.381	9.856	14.002	1.00	39.83	B	C
ATOM	1476	CG	GLU	A	191	37.331	8.887	14.681	1.00	52.60	B	C
ATOM	1477	CD	GLU	A	191	38.700	9.423	15.090	1.00	56.07	B	C
ATOM	1478	OE1	GLU	A	191	39.697	8.696	14.893	1.00	68.71	B	O
ATOM	1479	OE2	GLU	A	191	38.766	10.538	15.633	1.00	68.86	B	O
ATOM	1480	C	GLU	A	191	35.233	7.787	13.104	1.00	31.49	B	C
ATOM	1481	O	GLU	A	191	35.343	7.603	11.895	1.00	32.55	B	O
ATOM	1482	N	ALA	A	192	35.202	6.808	14.014	1.00	30.18	B	N
ATOM	1483	CA	ALA	A	192	35.395	5.372	13.724	1.00	31.81	B	C
ATOM	1484	CB	ALA	A	192	35.632	4.607	14.989	1.00	32.98	B	C
ATOM	1485	C	ALA	A	192	34.179	4.850	12.975	1.00	31.02	B	C
ATOM	1486	O	ALA	A	192	34.319	3.996	12.090	1.00	31.00	B	O
ATOM	1487	N	LEU	A	193	32.989	5.300	13.371	1.00	27.13	B	N
ATOM	1488	CA	LEU	A	193	31.706	4.829	12.839	1.00	26.67	B	C
ATOM	1489	CB	LEU	A	193	30.981	4.081	13.960	1.00	27.25	B	C
ATOM	1490	CG	LEU	A	193	31.695	2.829	14.421	1.00	29.24	B	C
ATOM	1491	CD1	LEU	A	193	31.013	2.270	15.666	1.00	30.28	B	C
ATOM	1492	CD2	LEU	A	193	31.756	1.784	13.299	1.00	32.25	B	C
ATOM	1493	C	LEU	A	193	30.896	6.016	12.375	1.00	29.53	B	C
ATOM	1494	O	LEU	A	193	29.962	6.472	13.037	1.00	25.45	B	O
ATOM	1495	N	PRO	A	194	31.202	6.542	11.176	1.00	28.79	B	N
ATOM	1496	CA	PRO	A	194	30.424	7.644	10.651	1.00	27.83	B	C
ATOM	1497	CB	PRO	A	194	31.006	7.868	9.241	1.00	30.81	B	C
ATOM	1498	CG	PRO	A	194	32.363	7.238	9.285	1.00	30.16	B	C
ATOM	1499	CD	PRO	A	194	32.290	6.100	10.280	1.00	32.70	B	C
ATOM	1500	C	PRO	A	194	28.946	7.270	10.544	1.00	26.43	B	C
ATOM	1501	O	PRO	A	194	28.628	6.156	10.182	1.00	26.70	B	O
ATOM	1502	N	SER	A	195	28.061	8.198	10.890	1.00	26.31	B	N
ATOM	1503	CA	SER	A	195	26.599	7.990	10.957	1.00	24.88	B	C
ATOM	1504	CB	SER	A	195	26.038	8.504	12.265	1.00	26.46	B	C
ATOM	1505	OG	SER	A	195	26.759	8.032	13.372	1.00	27.68	B	O
ATOM	1506	C	SER	A	195	25.913	8.643	9.777	1.00	27.34	B	C

Fig. 5 (cont.)

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ATOM	1507	O	SER A 195	26.257	9.768	9.430	1.00	33.29	B	O
ATOM	1508	N	GLU A 196	24.954	7.959	9.200	1.00	29.98	B	N
ATOM	1509	CA	GLU A 196	24.177	8.441	8.050	1.00	32.65	B	C
ATOM	1510	CB	GLU A 196	24.622	7.623	6.837	1.00	42.35	B	C
ATOM	1511	CG	GLU A 196	23.699	7.728	5.647	1.00	51.68	B	C
ATOM	1512	CD	GLU A 196	24.198	7.004	4.405	1.00	57.08	B	C
ATOM	1513	OE1	GLU A 196	23.931	7.504	3.297	1.00	64.01	B	O
ATOM	1514	OE2	GLU A 196	24.830	5.930	4.555	1.00	60.00	B	O
ATOM	1515	C	GLU A 196	22.697	8.308	8.408	1.00	28.17	B	C
ATOM	1516	O	GLU A 196	22.297	7.242	8.949	1.00	30.78	B	O
ATOM	1517	N	LEU A 197	21.912	9.308	8.074	1.00	26.08	B	N
ATOM	1518	CA	LEU A 197	20.457	9.221	8.160	1.00	25.97	B	C
ATOM	1519	CB	LEU A 197	19.862	10.620	8.272	1.00	26.19	B	C
ATOM	1520	CG	LEU A 197	18.346	10.650	8.333	1.00	28.20	B	C
ATOM	1521	CD1	LEU A 197	17.809	9.984	9.593	1.00	27.14	B	C
ATOM	1522	CD2	LEU A 197	17.851	12.086	8.231	1.00	32.14	B	C
ATOM	1523	C	LEU A 197	19.955	8.524	6.899	1.00	27.88	B	C
ATOM	1524	O	LEU A 197	20.297	8.981	5.772	1.00	29.25	B	O
ATOM	1525	N	LYS A 198	19.171	7.462	7.064	1.00	26.22	B	N
ATOM	1526	CA	LYS A 198	18.553	6.722	5.939	1.00	28.73	B	C
ATOM	1527	CB	LYS A 198	19.063	5.282	5.863	1.00	29.89	B	C
ATOM	1528	CG	LYS A 198	20.561	5.151	5.671	1.00	35.97	B	C
ATOM	1529	CD	LYS A 198	21.059	3.728	5.475	1.00	44.78	B	C
ATOM	1530	CE	LYS A 198	22.298	3.669	4.604	1.00	53.88	B	C
ATOM	1531	NZ	LYS A 198	23.314	2.746	5.164	1.00	60.59	B	N
ATOM	1532	C	LYS A 198	17.057	6.759	6.155	1.00	27.13	B	C
ATOM	1533	O	LYS A 198	16.574	6.334	7.227	1.00	28.64	B	O
ATOM	1534	N	CYS A 199	16.331	7.273	5.194	1.00	24.86	B	N
ATOM	1535	CA	CYS A 199	14.877	7.332	5.153	1.00	24.44	B	C
ATOM	1536	CB	CYS A 199	14.387	8.762	4.973	1.00	26.78	B	C
ATOM	1537	SG	CYS A 199	15.025	9.867	6.267	1.00	25.75	B	S
ATOM	1538	C	CYS A 199	14.426	6.462	4.004	1.00	25.80	B	C
ATOM	1539	O	CYS A 199	14.506	6.914	2.861	1.00	26.30	B	O
ATOM	1540	N	THR A 200	13.877	5.324	4.297	1.00	24.03	B	N
ATOM	1541	CA	THR A 200	13.292	4.385	3.314	1.00	24.34	B	C
ATOM	1542	CB	THR A 200	13.988	3.033	3.317	1.00	25.22	B	C
ATOM	1543	OG1	THR A 200	13.446	2.242	4.376	1.00	27.42	B	O
ATOM	1544	CG2	THR A 200	15.488	3.176	3.379	1.00	26.41	B	C
ATOM	1545	C	THR A 200	11.809	4.279	3.580	1.00	25.28	B	C
ATOM	1546	O	THR A 200	11.299	4.700	4.628	1.00	24.25	B	O
ATOM	1547	N	PRO A 201	11.031	3.748	2.624	1.00	24.41	B	N
ATOM	1548	CA	PRO A 201	9.592	3.671	2.810	1.00	25.27	B	C
ATOM	1549	CB	PRO A 201	9.115	3.005	1.493	1.00	27.70	B	C
ATOM	1550	CG	PRO A 201	10.136	3.543	0.498	1.00	29.32	B	C
ATOM	1551	CD	PRO A 201	11.454	3.432	1.233	1.00	26.87	B	C
ATOM	1552	C	PRO A 201	9.198	2.835	4.031	1.00	26.80	B	C
ATOM	1553	O	PRO A 201	8.080	3.019	4.510	1.00	25.51	B	O
ATOM	1554	N	ASP A 202	10.038	1.899	4.467	1.00	28.58	B	N
ATOM	1555	CA	ASP A 202	9.590	1.116	5.649	1.00	32.58	B	C
ATOM	1556	CB	ASP A 202	9.328	-0.359	5.350	1.00	42.40	B	C
ATOM	1557	CG	ASP A 202	10.561	-1.071	4.853	1.00	48.70	B	C
ATOM	1558	OD1	ASP A 202	11.660	-0.520	5.027	1.00	51.41	B	O
ATOM	1559	OD2	ASP A 202	10.403	-2.166	4.284	1.00	61.96	B	O
ATOM	1560	C	ASP A 202	10.528	1.301	6.832	1.00	26.88	B	C
ATOM	1561	O	ASP A 202	10.236	0.677	7.860	1.00	29.80	B	O
ATOM	1562	N	LEU A 203	11.517	2.187	6.791	1.00	26.29	B	N
ATOM	1563	CA	LEU A 203	12.436	2.288	7.951	1.00	25.17	B	C
ATOM	1564	CB	LEU A 203	13.422	1.107	7.956	1.00	25.87	B	C

Fig. 5 (cont.)

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ATOM	1565	CG	LEU	A	203	14.372	1.055	9.141	1.00	27.09	B	C
ATOM	1566	CD1	LEU	A	203	13.567	0.715	10.389	1.00	28.35	B	C
ATOM	1567	CD2	LEU	A	203	15.493	0.039	8.924	1.00	29.49	B	C
ATOM	1568	C	LEU	A	203	13.185	3.609	7.929	1.00	24.57	B	C
ATOM	1569	O	LEU	A	203	13.744	3.991	6.886	1.00	25.59	B	O
ATOM	1570	N	AVAL	A	204	13.254	4.302	9.076	0.50	22.84	B	N
ATOM	1571	N	BVAL	A	204	13.272	4.263	9.082	0.50	23.53	B	N
ATOM	1572	CA	AVAL	A	204	14.185	5.444	9.283	0.50	23.23	B	C
ATOM	1573	CA	BVAL	A	204	14.207	5.391	9.260	0.50	24.09	B	C
ATOM	1574	CB	AVAL	A	204	13.496	6.747	9.735	0.50	25.80	B	C
ATOM	1575	CB	BVAL	A	204	13.497	6.678	9.687	0.50	27.26	B	C
ATOM	1576	CG1AVAL	A	204	12.515	7.234	8.693	0.50	28.74	B	C	
ATOM	1577	CG1BVAL	A	204	14.477	7.817	9.730	0.50	25.49	B	C	
ATOM	1578	CG2AVAL	A	204	12.807	6.645	11.090	0.50	22.70	B	C	
ATOM	1579	CG2BVAL	A	204	12.347	7.020	8.762	0.50	30.61	B	C	
ATOM	1580	C	AVAL	A	204	15.273	5.006	10.256	0.50	22.96	B	C
ATOM	1581	C	BVAL	A	204	15.286	4.967	10.244	0.50	23.47	B	C
ATOM	1582	O	AVAL	A	204	14.934	4.408	11.313	0.50	24.16	B	O
ATOM	1583	O	BVAL	A	204	14.963	4.330	11.282	0.50	24.44	B	O
ATOM	1584	N	SER	A	205	16.533	5.274	9.939	1.00	23.46	B	N
ATOM	1585	CA	SER	A	205	17.658	4.779	10.734	1.00	23.46	B	C
ATOM	1586	CB	SER	A	205	18.121	3.406	10.268	1.00	30.10	B	C
ATOM	1587	OG	SER	A	205	18.692	3.534	8.999	1.00	35.64	B	O
ATOM	1588	C	SER	A	205	18.761	5.803	10.713	1.00	23.88	B	C
ATOM	1589	O	SER	A	205	18.793	6.666	9.805	1.00	23.56	B	O
ATOM	1590	N	LEU	A	206	19.528	5.802	11.779	1.00	22.45	B	N
ATOM	1591	CA	LEU	A	206	20.679	6.714	11.916	1.00	24.29	B	C
ATOM	1592	CB	LEU	A	206	20.381	7.896	12.867	1.00	24.63	B	C
ATOM	1593	CG	LEU	A	206	21.507	8.910	13.043	1.00	27.42	B	C
ATOM	1594	CD1	LEU	A	206	21.950	9.543	11.719	1.00	31.65	B	C
ATOM	1595	CD2	LEU	A	206	21.034	10.011	13.987	1.00	27.41	B	C
ATOM	1596	C	LEU	A	206	21.831	5.834	12.326	1.00	26.79	B	C
ATOM	1597	O	LEU	A	206	21.954	5.477	13.480	1.00	26.33	B	O
ATOM	1598	N	THR	A	207	22.659	5.438	11.366	1.00	27.31	B	N
ATOM	1599	CA	THR	A	207	23.624	4.333	11.441	1.00	26.25	B	C
ATOM	1600	CB	THR	A	207	24.032	3.981	9.991	1.00	26.99	B	C
ATOM	1601	OG1	THR	A	207	24.780	5.078	9.464	1.00	31.80	B	O
ATOM	1602	CG2	THR	A	207	22.858	3.696	9.088	1.00	34.38	B	C
ATOM	1603	C	THR	A	207	24.854	4.699	12.272	1.00	27.66	B	C
ATOM	1604	O	THR	A	207	24.942	5.771	12.917	1.00	25.57	B	O
ATOM	1605	N	GLY	A	208	25.823	3.836	12.334	1.00	23.76	B	N
ATOM	1606	CA	GLY	A	208	27.157	4.091	12.874	1.00	22.11	B	C
ATOM	1607	C	GLY	A	208	27.191	4.370	14.370	1.00	21.44	B	C
ATOM	1608	O	GLY	A	208	26.482	3.682	15.151	1.00	21.58	B	O
ATOM	1609	N	ALA	A	209	27.956	5.372	14.796	1.00	21.94	B	N
ATOM	1610	CA	ALA	A	209	28.147	5.705	16.229	1.00	21.64	B	C
ATOM	1611	CB	ALA	A	209	29.082	6.883	16.402	1.00	21.34	B	C
ATOM	1612	C	ALA	A	209	26.768	6.040	16.834	1.00	19.73	B	C
ATOM	1613	O	ALA	A	209	26.565	5.728	18.033	1.00	20.14	B	O
ATOM	1614	N	TRP	A	210	25.849	6.639	16.102	1.00	19.70	B	N
ATOM	1615	CA	TRP	A	210	24.525	6.993	16.691	1.00	19.62	B	C
ATOM	1616	CB	TRP	A	210	23.747	7.919	15.787	1.00	19.98	B	C
ATOM	1617	CG	TRP	A	210	24.086	9.352	16.089	1.00	19.60	B	C
ATOM	1618	CD1	TRP	A	210	24.880	10.222	15.412	1.00	20.83	B	C
ATOM	1619	NE1	TRP	A	210	24.893	11.432	16.060	1.00	21.66	B	N
ATOM	1620	CE2	TRP	A	210	24.203	11.323	17.242	1.00	20.04	B	C
ATOM	1621	CD2	TRP	A	210	23.687	10.019	17.289	1.00	18.48	B	C
ATOM	1622	CE3	TRP	A	210	22.907	9.642	18.392	1.00	20.59	B	C

Fig. 5 (cont.)

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ATOM	1623	CZ3	TRP	A	210	22.739	10.543	19.421	1.00	20.17	B	C
ATOM	1624	CH2	TRP	A	210	23.237	11.856	19.333	1.00	20.28	B	C
ATOM	1625	CZ2	TRP	A	210	23.995	12.252	18.262	1.00	19.31	B	C
ATOM	1626	C	TRP	A	210	23.761	5.727	17.073	1.00	20.58	B	C
ATOM	1627	O	TRP	A	210	23.409	5.556	18.233	1.00	19.33	B	O
ATOM	1628	N	ASER	A	211	23.551	4.837	16.100	0.50	20.28	B	N
ATOM	1629	N	BSER	A	211	23.517	4.824	16.132	0.50	20.57	B	N
ATOM	1630	CA	ASER	A	211	22.803	3.570	16.315	0.50	19.85	B	C
ATOM	1631	CA	BSER	A	211	22.738	3.595	16.437	0.50	19.89	B	C
ATOM	1632	CB	ASER	A	211	22.675	2.743	15.027	0.50	21.63	B	C
ATOM	1633	CB	BSER	A	211	22.499	2.805	15.170	0.50	22.82	B	C
ATOM	1634	OG	ASER	A	211	22.127	1.458	15.317	0.50	23.15	B	O
ATOM	1635	OG	BSER	A	211	23.756	2.529	14.593	0.50	23.92	B	O
ATOM	1636	C	ASER	A	211	23.493	2.799	17.432	0.50	19.18	B	C
ATOM	1637	C	BSER	A	211	23.508	2.796	17.493	0.50	19.40	B	C
ATOM	1638	O	ASER	A	211	22.783	2.301	18.319	0.50	18.91	B	O
ATOM	1639	O	BSER	A	211	22.911	2.264	18.428	0.50	18.54	B	O
ATOM	1640	N	LEU	A	212	24.816	2.704	17.394	1.00	19.13	B	N
ATOM	1641	CA	LEU	A	212	25.581	1.905	18.349	1.00	18.09	B	C
ATOM	1642	CB	LEU	A	212	27.037	1.720	17.982	1.00	20.30	B	C
ATOM	1643	CG	LEU	A	212	27.853	0.822	18.906	1.00	22.01	B	C
ATOM	1644	CD1	LEU	A	212	27.310	-0.590	18.929	1.00	23.03	B	C
ATOM	1645	CD2	LEU	A	212	29.313	0.783	18.449	1.00	25.09	B	C
ATOM	1646	C	LEU	A	212	25.437	2.461	19.753	1.00	19.68	B	C
ATOM	1647	O	LEU	A	212	25.241	1.746	20.704	1.00	19.13	B	O
ATOM	1648	N	ASER	A	213	25.595	3.770	19.910	0.50	19.42	B	N
ATOM	1649	N	BSER	A	213	25.623	3.776	19.876	0.50	19.29	B	N
ATOM	1650	CA	ASER	A	213	25.542	4.395	21.255	0.50	18.95	B	C
ATOM	1651	CA	BSER	A	213	25.568	4.459	21.190	0.50	19.18	B	C
ATOM	1652	CB	ASER	A	213	25.955	5.867	21.170	0.50	19.27	B	C
ATOM	1653	CB	BSER	A	213	25.902	5.974	21.092	0.50	18.17	B	C
ATOM	1654	OG	ASER	A	213	27.290	5.924	20.708	0.50	21.44	B	O
ATOM	1655	OG	BSER	A	213	24.850	6.725	20.516	0.50	21.57	B	O
ATOM	1656	C	ASER	A	213	24.147	4.249	21.870	0.50	15.96	B	C
ATOM	1657	C	BSER	A	213	24.200	4.257	21.842	0.50	16.19	B	C
ATOM	1658	O	ASER	A	213	24.019	4.069	23.092	0.50	17.05	B	O
ATOM	1659	O	BSER	A	213	24.193	4.057	23.085	0.50	17.85	B	O
ATOM	1660	N	SER	A	214	23.127	4.330	21.058	1.00	17.74	B	N
ATOM	1661	CA	SER	A	214	21.756	4.154	21.522	1.00	17.28	B	C
ATOM	1662	CB	SER	A	214	20.793	4.411	20.442	1.00	18.24	B	C
ATOM	1663	OG	SER	A	214	19.468	4.260	20.882	1.00	20.04	B	O
ATOM	1664	C	SER	A	214	21.629	2.736	22.107	1.00	19.43	B	C
ATOM	1665	O	SER	A	214	21.056	2.556	23.222	1.00	18.28	B	O
ATOM	1666	N	THR	A	215	22.176	1.745	21.406	1.00	19.15	B	N
ATOM	1667	CA	THR	A	215	22.111	0.333	21.857	1.00	18.53	B	C
ATOM	1668	CB	THR	A	215	22.628	-0.556	20.729	1.00	18.00	B	C
ATOM	1669	OG1	THR	A	215	21.783	-0.436	19.613	1.00	20.67	B	O
ATOM	1670	CG2	THR	A	215	22.712	-1.999	21.183	1.00	19.86	B	C
ATOM	1671	C	THR	A	215	22.909	0.179	23.135	1.00	15.36	B	C
ATOM	1672	O	THR	A	215	22.429	-0.400	24.137	1.00	17.35	B	O
ATOM	1673	N	LEU	A	216	24.161	0.612	23.166	1.00	16.47	B	N
ATOM	1674	CA	LEU	A	216	25.024	0.305	24.285	1.00	16.18	B	C
ATOM	1675	CB	LEU	A	216	26.487	0.651	23.994	1.00	18.99	B	C
ATOM	1676	CG	LEU	A	216	27.089	-0.036	22.759	1.00	19.54	B	C
ATOM	1677	CD1	LEU	A	216	28.569	0.200	22.720	1.00	21.68	B	C
ATOM	1678	CD2	LEU	A	216	26.837	-1.575	22.796	1.00	20.82	B	C
ATOM	1679	C	LEU	A	216	24.559	0.958	25.572	1.00	17.51	B	C
ATOM	1680	O	LEU	A	216	24.694	0.391	26.675	1.00	16.86	B	O

Fig. 5 (cont.)

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ATOM	1681	N	THR	A	217	24.163	2.255	25.478	1.00	16.76	B	N
ATOM	1682	CA	THR	A	217	23.686	2.933	26.694	1.00	16.61	B	C
ATOM	1683	CB	THR	A	217	23.489	4.435	26.390	1.00	15.80	B	C
ATOM	1684	OG1	THR	A	217	22.556	4.683	25.368	1.00	16.79	B	O
ATOM	1685	CG2	THR	A	217	24.828	5.083	26.114	1.00	15.84	B	C
ATOM	1686	C	THR	A	217	22.446	2.228	27.264	1.00	16.67	B	C
ATOM	1687	O	THR	A	217	22.301	2.175	28.481	1.00	16.66	B	O
ATOM	1688	N	GLU	A	218	21.529	1.779	26.415	1.00	15.78	B	N
ATOM	1689	CA	GLU	A	218	20.371	0.971	26.885	1.00	16.42	B	C
ATOM	1690	CB	GLU	A	218	19.428	0.711	25.739	1.00	17.14	B	C
ATOM	1691	CG	GLU	A	218	18.230	-0.131	26.106	1.00	17.23	B	C
ATOM	1692	CD	GLU	A	218	17.221	0.546	27.014	1.00	19.18	B	C
ATOM	1693	OE1	GLU	A	218	17.353	1.849	27.222	1.00	18.60	B	O
ATOM	1694	OE2	GLU	A	218	16.243	-0.044	27.439	1.00	18.14	B	O
ATOM	1695	C	GLU	A	218	20.835	-0.350	27.513	1.00	16.20	B	C
ATOM	1696	O	GLU	A	218	20.308	-0.725	28.568	1.00	16.69	B	O
ATOM	1697	N	ILE	A	219	21.898	-0.953	26.996	1.00	16.07	B	N
ATOM	1698	CA	ILE	A	219	22.376	-2.229	27.620	1.00	16.43	B	C
ATOM	1699	CB	ILE	A	219	23.464	-2.885	26.773	1.00	18.53	B	C
ATOM	1700	CG1	ILE	A	219	22.832	-3.404	25.472	1.00	18.89	B	C
ATOM	1701	CG2	ILE	A	219	24.173	-3.974	27.590	1.00	19.41	B	C
ATOM	1702	CD1	ILE	A	219	23.802	-4.034	24.470	1.00	19.67	B	C
ATOM	1703	C	ILE	A	219	22.877	-1.920	29.031	1.00	16.57	B	C
ATOM	1704	O	ILE	A	219	22.598	-2.659	29.989	1.00	16.52	B	O
ATOM	1705	N	PHE	A	220	23.626	-0.808	29.218	1.00	15.61	B	N
ATOM	1706	CA	PHE	A	220	24.054	-0.525	30.599	1.00	16.09	B	C
ATOM	1707	CB	PHE	A	220	24.906	0.768	30.626	1.00	16.69	B	C
ATOM	1708	CG	PHE	A	220	26.153	0.756	29.780	1.00	17.95	B	C
ATOM	1709	CD1	PHE	A	220	26.932	-0.389	29.621	1.00	19.91	B	C
ATOM	1710	CE1	PHE	A	220	28.089	-0.381	28.832	1.00	19.87	B	C
ATOM	1711	CZ	PHE	A	220	28.511	0.760	28.212	1.00	21.86	B	C
ATOM	1712	CD2	PHE	A	220	26.589	1.901	29.137	1.00	20.03	B	C
ATOM	1713	CE2	PHE	A	220	27.748	1.902	28.360	1.00	21.19	B	C
ATOM	1714	C	PHE	A	220	22.862	-0.359	31.550	1.00	15.65	B	C
ATOM	1715	O	PHE	A	220	22.848	-0.814	32.702	1.00	16.22	B	O
ATOM	1716	N	LEU	A	221	21.846	0.395	31.069	1.00	16.14	B	N
ATOM	1717	CA	LEU	A	221	20.625	0.571	31.853	1.00	15.89	B	C
ATOM	1718	CB	LEU	A	221	19.638	1.464	31.105	1.00	17.82	B	C
ATOM	1719	CG	LEU	A	221	18.463	1.855	31.960	1.00	18.65	B	C
ATOM	1720	CD1	LEU	A	221	18.420	3.354	32.223	1.00	19.20	B	C
ATOM	1721	CD2	LEU	A	221	17.186	1.380	31.389	1.00	20.95	B	C
ATOM	1722	C	LEU	A	221	19.974	-0.781	32.233	1.00	15.49	B	C
ATOM	1723	O	LEU	A	221	19.525	-0.964	33.365	1.00	16.34	B	O
ATOM	1724	N	LEU	A	222	19.909	-1.680	31.263	1.00	15.63	B	N
ATOM	1725	CA	LEU	A	222	19.301	-3.003	31.513	1.00	16.07	B	C
ATOM	1726	CB	LEU	A	222	19.193	-3.724	30.172	1.00	16.04	B	C
ATOM	1727	CG	LEU	A	222	18.155	-3.175	29.209	1.00	16.90	B	C
ATOM	1728	CD1	LEU	A	222	18.298	-3.745	27.796	1.00	18.66	B	C
ATOM	1729	CD2	LEU	A	222	16.781	-3.407	29.724	1.00	17.32	B	C
ATOM	1730	C	LEU	A	222	20.143	-3.793	32.535	1.00	15.48	B	C
ATOM	1731	O	LEU	A	222	19.563	-4.442	33.382	1.00	16.65	B	O
ATOM	1732	N	GLN	A	223	21.466	-3.742	32.390	1.00	15.08	B	N
ATOM	1733	CA	GLN	A	223	22.365	-4.389	33.388	1.00	16.87	B	C
ATOM	1734	CB	GLN	A	223	23.842	-4.162	33.096	1.00	17.64	B	C
ATOM	1735	CG	GLN	A	223	24.335	-4.729	31.785	1.00	17.53	B	C
ATOM	1736	CD	GLN	A	223	25.739	-4.250	31.490	1.00	18.81	B	C
ATOM	1737	OE1	GLN	A	223	26.236	-3.329	32.170	1.00	19.73	B	O
ATOM	1738	NE2	GLN	A	223	26.373	-4.791	30.476	1.00	20.14	B	N

Fig. 5 (cont.)

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ATOM	1739	C	GLN	A	223	22.051	-3.915	34.802	1.00	16.96	B	C
ATOM	1740	O	GLN	A	223	21.902	-4.679	35.743	1.00	18.21	B	O
ATOM	1741	N	GLU	A	224	21.950	-2.598	34.958	1.00	16.73	B	N
ATOM	1742	CA	GLU	A	224	21.629	-1.998	36.262	1.00	17.71	B	C
ATOM	1743	CB	GLU	A	224	21.701	-0.455	36.147	1.00	17.69	B	C
ATOM	1744	CG	GLU	A	224	21.459	0.274	37.460	1.00	18.40	B	C
ATOM	1745	CD	GLU	A	224	22.658	0.357	38.384	1.00	24.12	B	C
ATOM	1746	OE1	GLU	A	224	23.703	-0.191	38.052	1.00	25.22	B	O
ATOM	1747	OE2	GLU	A	224	22.530	0.976	39.475	1.00	25.92	B	O
ATOM	1748	C	GLU	A	224	20.264	-2.439	36.753	1.00	17.17	B	C
ATOM	1749	O	GLU	A	224	20.093	-2.831	37.899	1.00	19.46	B	O
ATOM	1750	N	ALA	A	225	19.234	-2.323	35.925	1.00	16.43	B	N
ATOM	1751	CA	ALA	A	225	17.852	-2.664	36.253	1.00	17.49	B	C
ATOM	1752	CB	ALA	A	225	16.915	-2.384	35.084	1.00	19.01	B	C
ATOM	1753	C	ALA	A	225	17.758	-4.138	36.678	1.00	17.35	B	C
ATOM	1754	O	ALA	A	225	16.879	-4.461	37.483	1.00	18.67	B	O
ATOM	1755	N	GLN	A	226	18.531	-4.978	35.999	1.00	17.01	B	N
ATOM	1756	CA	GLN	A	226	18.490	-6.441	36.192	1.00	16.61	B	C
ATOM	1757	CB	GLN	A	226	19.082	-7.097	34.956	1.00	16.15	B	C
ATOM	1758	CG	GLN	A	226	19.217	-8.626	35.078	1.00	17.65	B	C
ATOM	1759	CD	GLN	A	226	17.884	-9.264	35.198	1.00	18.20	B	C
ATOM	1760	OE1	GLN	A	226	16.858	-8.755	34.759	1.00	17.50	B	O
ATOM	1761	NE2	GLN	A	226	17.895	-10.508	35.728	1.00	19.66	B	N
ATOM	1762	C	GLN	A	226	19.198	-6.789	37.496	1.00	21.49	B	C
ATOM	1763	O	GLN	A	226	19.081	-7.930	37.916	1.00	21.98	B	O
ATOM	1764	N	GLY	A	227	19.916	-5.885	38.131	1.00	20.15	B	N
ATOM	1765	CA	GLY	A	227	20.643	-6.256	39.360	1.00	20.45	B	C
ATOM	1766	C	GLY	A	227	21.929	-6.985	39.090	1.00	23.14	B	C
ATOM	1767	O	GLY	A	227	22.464	-7.646	40.027	1.00	24.10	B	O
ATOM	1768	N	AMET	A	228	22.494	-6.848	37.906	0.50	20.85	B	N
ATOM	1769	N	BMET	A	228	22.505	-6.835	37.906	0.50	21.37	B	N
ATOM	1770	CA	AMET	A	228	23.773	-7.527	37.609	0.50	21.29	B	C
ATOM	1771	CA	BMET	A	228	23.794	-7.496	37.598	0.50	22.21	B	C
ATOM	1772	CB	AMET	A	228	24.043	-7.514	36.108	0.50	20.90	B	C
ATOM	1773	CB	BMET	A	228	24.160	-7.398	36.121	0.50	23.06	B	C
ATOM	1774	CG	AMET	A	228	22.945	-8.300	35.404	0.50	22.08	B	C
ATOM	1775	CG	BMET	A	228	23.253	-8.219	35.236	0.50	25.52	B	C
ATOM	1776	SD	AMET	A	228	22.950	-8.325	33.604	0.50	21.36	B	S
ATOM	1777	SD	BMET	A	228	23.896	-8.219	33.554	0.50	27.92	B	S
ATOM	1778	CE	AMET	A	228	24.200	-9.555	33.221	0.50	24.06	B	C
ATOM	1779	CE	BMET	A	228	24.959	-9.659	33.613	0.50	32.90	B	C
ATOM	1780	C	AMET	A	228	24.900	-6.916	38.437	0.50	22.85	B	C
ATOM	1781	C	BMET	A	228	24.922	-6.902	38.433	0.50	23.39	B	C
ATOM	1782	O	AMET	A	228	24.877	-5.732	38.795	0.50	24.61	B	O
ATOM	1783	O	BMET	A	228	24.992	-5.699	38.706	0.50	24.02	B	O
ATOM	1784	N	PRO	A	229	25.880	-7.738	38.858	1.00	25.95	B	N
ATOM	1785	CA	PRO	A	229	26.892	-7.283	39.806	1.00	28.89	B	C
ATOM	1786	CB	PRO	A	229	27.524	-8.633	40.211	1.00	31.52	B	C
ATOM	1787	CG	PRO	A	229	27.392	-9.456	38.967	1.00	31.36	B	C
ATOM	1788	CD	PRO	A	229	25.965	-9.166	38.536	1.00	26.57	B	C
ATOM	1789	C	PRO	A	229	27.921	-6.326	39.185	1.00	29.06	B	C
ATOM	1790	O	PRO	A	229	28.439	-5.473	39.894	1.00	35.52	B	O
ATOM	1791	N	GLN	A	230	28.213	-6.458	37.909	1.00	26.61	B	N
ATOM	1792	CA	GLN	A	230	29.136	-5.529	37.215	1.00	29.92	B	C
ATOM	1793	CB	GLN	A	230	30.339	-6.314	36.685	1.00	34.19	B	C
ATOM	1794	CG	GLN	A	230	31.500	-5.434	36.226	1.00	41.40	B	C
ATOM	1795	CD	GLN	A	230	32.438	-6.166	35.291	1.00	49.83	B	C
ATOM	1796	OE1	GLN	A	230	33.620	-6.313	35.578	1.00	55.56	B	O

Fig. 5 (cont.)

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ATOM	1797	NE2	GLN	A	230	31.924	-6.628	34.154	1.00	50.66	B	N
ATOM	1798	C	GLN	A	230	28.374	-4.829	36.092	1.00	25.20	B	C
ATOM	1799	O	GLN	A	230	28.237	-5.425	35.039	1.00	24.41	B	O
ATOM	1800	N	VAL	A	231	28.075	-3.542	36.287	1.00	23.25	B	N
ATOM	1801	CA	VAL	A	231	27.370	-2.726	35.250	1.00	20.72	B	C
ATOM	1802	CB	VAL	A	231	26.291	-1.907	35.975	1.00	20.45	B	C
ATOM	1803	CG1	VAL	A	231	25.627	-0.882	35.084	1.00	19.90	B	C
ATOM	1804	CG2	VAL	A	231	25.244	-2.809	36.614	1.00	21.07	B	C
ATOM	1805	C	VAL	A	231	28.387	-1.830	34.567	1.00	19.95	B	C
ATOM	1806	O	VAL	A	231	29.041	-1.027	35.294	1.00	22.51	B	O
ATOM	1807	N	ALA	A	232	28.542	-1.916	33.272	1.00	20.37	B	N
ATOM	1808	CA	ALA	A	232	29.463	-1.036	32.527	1.00	21.53	B	C
ATOM	1809	CB	ALA	A	232	28.890	0.378	32.469	1.00	23.10	B	C
ATOM	1810	C	ALA	A	232	30.827	-1.030	33.224	1.00	23.21	B	C
ATOM	1811	O	ALA	A	232	31.477	0.047	33.365	1.00	22.01	B	O
ATOM	1812	N	TRP	A	233	31.277	-2.216	33.657	1.00	21.86	B	N
ATOM	1813	CA	TRP	A	233	32.608	-2.406	34.291	1.00	23.02	B	C
ATOM	1814	CB	TRP	A	233	33.663	-2.202	33.213	1.00	24.27	B	C
ATOM	1815	CG	TRP	A	233	33.345	-3.025	32.012	1.00	22.63	B	C
ATOM	1816	CD1	TRP	A	233	33.506	-4.386	31.905	1.00	26.61	B	C
ATOM	1817	NE1	TRP	A	233	33.067	-4.807	30.688	1.00	24.80	B	N
ATOM	1818	CE2	TRP	A	233	32.583	-3.750	29.953	1.00	22.05	B	C
ATOM	1819	CD2	TRP	A	233	32.728	-2.616	30.789	1.00	22.97	B	C
ATOM	1820	CE3	TRP	A	233	32.313	-1.388	30.269	1.00	21.61	B	C
ATOM	1821	CZ3	TRP	A	233	31.727	-1.357	29.030	1.00	21.99	B	C
ATOM	1822	CH2	TRP	A	233	31.568	-2.486	28.237	1.00	23.31	B	C
ATOM	1823	CZ2	TRP	A	233	32.007	-3.702	28.685	1.00	23.11	B	C
ATOM	1824	C	TRP	A	233	32.783	-1.498	35.502	1.00	23.36	B	C
ATOM	1825	O	TRP	A	233	33.958	-1.235	35.855	1.00	25.68	B	O
ATOM	1826	N	GLY	A	234	31.715	-1.094	36.197	1.00	23.96	B	N
ATOM	1827	CA	GLY	A	234	31.817	-0.272	37.420	1.00	23.94	B	C
ATOM	1828	C	GLY	A	234	32.097	1.186	37.099	1.00	23.72	B	C
ATOM	1829	O	GLY	A	234	32.337	1.951	38.056	1.00	28.31	B	O
ATOM	1830	N	ARG	A	235	31.987	1.603	35.835	1.00	24.32	B	N
ATOM	1831	CA	ARG	A	235	32.457	2.957	35.432	1.00	24.67	B	C
ATOM	1832	CB	ARG	A	235	33.191	2.862	34.123	1.00	24.88	B	C
ATOM	1833	CG	ARG	A	235	34.451	2.016	34.244	1.00	26.13	B	C
ATOM	1834	CD	ARG	A	235	34.972	1.816	32.842	1.00	29.02	B	C
ATOM	1835	NE	ARG	A	235	36.012	0.814	32.874	1.00	30.55	B	N
ATOM	1836	CZ	ARG	A	235	36.289	-0.017	31.875	1.00	27.86	B	C
ATOM	1837	NH1	ARG	A	235	35.710	-0.005	30.689	1.00	25.29	B	N
ATOM	1838	NH2	ARG	A	235	37.212	-0.922	32.107	1.00	28.73	B	N
ATOM	1839	C	ARG	A	235	31.346	3.998	35.329	1.00	24.42	B	C
ATOM	1840	O	ARG	A	235	31.679	5.121	34.864	1.00	28.80	B	O
ATOM	1841	N	ILE	A	236	30.127	3.727	35.785	1.00	23.03	B	N
ATOM	1842	CA	ILE	A	236	29.053	4.753	35.845	1.00	22.90	B	C
ATOM	1843	CB	ILE	A	236	27.797	4.302	35.085	1.00	21.99	B	C
ATOM	1844	CG1	ILE	A	236	28.109	3.868	33.664	1.00	22.33	B	C
ATOM	1845	CG2	ILE	A	236	26.808	5.464	35.122	1.00	22.96	B	C
ATOM	1846	CD1	ILE	A	236	26.942	3.193	32.928	1.00	22.57	B	C
ATOM	1847	C	ILE	A	236	28.784	5.053	37.325	1.00	26.43	B	C
ATOM	1848	O	ILE	A	236	28.430	4.149	38.082	1.00	29.91	B	O
ATOM	1849	N	THR	A	237	28.911	6.308	37.729	1.00	29.49	B	N
ATOM	1850	CA	THR	A	237	28.585	6.782	39.099	1.00	32.10	B	C
ATOM	1851	CB	THR	A	237	29.880	7.089	39.863	1.00	35.34	B	C
ATOM	1852	OG1	THR	A	237	30.688	5.913	39.812	1.00	39.75	B	O
ATOM	1853	CG2	THR	A	237	29.654	7.435	41.319	1.00	38.48	B	C
ATOM	1854	C	THR	A	237	27.649	8.011	39.033	1.00	25.09	B	C

Fig. 5 (cont.)

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ATOM	1855	O	THR	A	237	28.048	8.983	38.430	1.00	26.06	B	O
ATOM	1856	N	GLY	A	238	26.447	7.891	39.579	1.00	28.36	B	N
ATOM	1857	CA	GLY	A	238	25.574	9.048	39.841	1.00	28.74	B	C
ATOM	1858	C	GLY	A	238	24.649	9.396	38.698	1.00	24.94	B	C
ATOM	1859	O	GLY	A	238	24.878	8.991	37.540	1.00	24.44	B	O
ATOM	1860	N	GLU	A	239	23.684	10.240	39.012	1.00	25.00	B	N
ATOM	1861	CA	GLU	A	239	22.651	10.684	38.068	1.00	23.47	B	C
ATOM	1862	CB	GLU	A	239	21.658	11.576	38.802	1.00	26.30	B	C
ATOM	1863	CG	GLU	A	239	20.544	12.070	37.927	1.00	24.89	B	C
ATOM	1864	CD	GLU	A	239	19.493	12.970	38.549	1.00	29.69	B	C
ATOM	1865	OE1	GLU	A	239	19.835	13.615	39.555	1.00	33.58	B	O
ATOM	1866	OE2	GLU	A	239	18.353	13.065	38.001	1.00	31.35	B	O
ATOM	1867	C	GLU	A	239	23.251	11.406	36.867	1.00	22.15	B	C
ATOM	1868	O	GLU	A	239	22.808	11.222	35.699	1.00	20.33	B	O
ATOM	1869	N	LYS	A	240	24.209	12.326	37.077	1.00	20.15	B	N
ATOM	1870	CA	LYS	A	240	24.686	13.097	35.937	1.00	19.85	B	C
ATOM	1871	CB	LYS	A	240	25.668	14.198	36.372	1.00	22.64	B	C
ATOM	1872	CG	LYS	A	240	24.964	15.235	37.227	1.00	26.74	B	C
ATOM	1873	CD	LYS	A	240	25.923	16.241	37.810	1.00	27.79	B	C
ATOM	1874	CE	LYS	A	240	25.201	17.285	38.611	1.00	29.12	B	C
ATOM	1875	NZ	LYS	A	240	24.705	16.763	39.898	1.00	32.39	B	N
ATOM	1876	C	LYS	A	240	25.287	12.199	34.861	1.00	19.45	B	C
ATOM	1877	O	LYS	A	240	24.997	12.398	33.686	1.00	20.03	B	O
ATOM	1878	N	GLU	A	241	26.078	11.206	35.265	1.00	20.42	B	N
ATOM	1879	CA	GLU	A	241	26.737	10.342	34.258	1.00	20.66	B	C
ATOM	1880	CB	GLU	A	241	27.856	9.532	34.921	1.00	22.62	B	C
ATOM	1881	CG	GLU	A	241	29.121	10.262	35.313	1.00	31.40	B	C
ATOM	1882	CD	GLU	A	241	30.117	9.401	36.093	1.00	35.76	B	C
ATOM	1883	OE1	GLU	A	241	30.116	8.110	35.881	1.00	29.90	B	O
ATOM	1884	OE2	GLU	A	241	30.910	9.993	36.926	1.00	38.89	B	O
ATOM	1885	C	GLU	A	241	25.706	9.394	33.587	1.00	16.19	B	C
ATOM	1886	O	GLU	A	241	25.675	9.300	32.347	1.00	18.84	B	O
ATOM	1887	N	TRP	A	242	24.792	8.898	34.382	1.00	17.99	B	N
ATOM	1888	CA	TRP	A	242	23.711	8.032	33.856	1.00	18.05	B	C
ATOM	1889	CB	TRP	A	242	22.785	7.560	34.961	1.00	18.89	B	C
ATOM	1890	CG	TRP	A	242	23.143	6.239	35.562	1.00	18.50	B	C
ATOM	1891	CD1	TRP	A	242	23.667	6.007	36.798	1.00	19.37	B	C
ATOM	1892	NE1	TRP	A	242	23.917	4.675	36.985	1.00	21.20	B	N
ATOM	1893	CE2	TRP	A	242	23.623	4.032	35.817	1.00	19.79	B	C
ATOM	1894	CD2	TRP	A	242	23.122	4.963	34.895	1.00	18.97	B	C
ATOM	1895	CE3	TRP	A	242	22.775	4.540	33.607	1.00	19.08	B	C
ATOM	1896	CZ3	TRP	A	242	22.876	3.198	33.296	1.00	19.81	B	C
ATOM	1897	CH2	TRP	A	242	23.371	2.300	34.238	1.00	19.38	B	C
ATOM	1898	CZ2	TRP	A	242	23.729	2.685	35.508	1.00	19.97	B	C
ATOM	1899	C	TRP	A	242	22.933	8.801	32.793	1.00	19.29	B	C
ATOM	1900	O	TRP	A	242	22.632	8.286	31.714	1.00	18.13	B	O
ATOM	1901	N	AARG	A	243	22.555	10.053	33.067	0.50	18.46	B	N
ATOM	1902	N	BARG	A	243	22.506	10.016	33.147	0.50	18.42	B	N
ATOM	1903	CA	AARG	A	243	21.694	10.816	32.117	0.50	17.82	B	C
ATOM	1904	CA	BARG	A	243	21.644	10.798	32.234	0.50	18.39	B	C
ATOM	1905	CB	AARG	A	243	21.026	11.988	32.833	0.50	18.29	B	C
ATOM	1906	CB	BARG	A	243	20.906	11.909	32.987	0.50	18.36	B	C
ATOM	1907	CG	AARG	A	243	19.924	11.552	33.768	0.50	18.73	B	C
ATOM	1908	CG	BARG	A	243	19.896	11.423	34.004	0.50	20.98	B	C
ATOM	1909	CD	AARG	A	243	18.998	12.643	34.329	0.50	19.53	B	C
ATOM	1910	CD	BARG	A	243	18.822	12.453	34.351	0.50	20.40	B	C
ATOM	1911	NE	AARG	A	243	17.787	12.155	34.959	0.50	16.83	B	N
ATOM	1912	NE	BARG	A	243	17.609	12.305	33.579	0.50	20.14	B	N

Fig. 5 (cont.)

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ATOM	1913	CZ	AARG	A	243	16.693	11.757	34.275	0.50	18.03	B	C
ATOM	1914	CZ	BARG	A	243	16.509	11.748	34.069	0.50	21.77	B	C
ATOM	1915	NH1AARG	A	243	16.519	11.973	32.988	0.50	15.14	B	N	
ATOM	1916	NH1BARG	A	243	16.446	11.447	35.355	0.50	27.16	B	N	
ATOM	1917	NH2AARG	A	243	15.701	11.240	34.944	0.50	25.36	B	N	
ATOM	1918	NH2BARG	A	243	15.439	11.616	33.329	0.50	32.24	B	N	
ATOM	1919	C	AARG	A	243	22.498	11.299	30.919	0.50	16.72	B	C
ATOM	1920	C	BARG	A	243	22.441	11.277	31.016	0.50	16.57	B	C
ATOM	1921	O	AARG	A	243	22.031	11.421	29.772	0.50	18.51	B	O
ATOM	1922	O	BARG	A	243	21.836	11.339	29.936	0.50	20.04	B	O
ATOM	1923	N	ASP	A	244	23.738	11.630	31.159	1.00	17.61	B	N
ATOM	1924	CA	ASP	A	244	24.627	11.983	30.058	1.00	18.94	B	C
ATOM	1925	CB	ASP	A	244	25.997	12.340	30.637	1.00	19.73	B	C
ATOM	1926	CG	ASP	A	244	26.864	13.162	29.709	1.00	19.91	B	C
ATOM	1927	OD1	ASP	A	244	26.327	13.743	28.713	1.00	23.91	B	O
ATOM	1928	OD2	ASP	A	244	28.084	13.163	29.943	1.00	21.63	B	O
ATOM	1929	C	ASP	A	244	24.677	10.834	29.025	1.00	17.54	B	C
ATOM	1930	O	ASP	A	244	24.585	11.043	27.834	1.00	19.07	B	O
ATOM	1931	N	LEU	A	245	24.862	9.625	29.520	1.00	19.10	B	N
ATOM	1932	CA	LEU	A	245	24.907	8.422	28.642	1.00	19.39	B	C
ATOM	1933	CB	LEU	A	245	25.276	7.210	29.523	1.00	18.27	B	C
ATOM	1934	CG	LEU	A	245	26.728	7.219	29.971	1.00	19.92	B	C
ATOM	1935	CD1	LEU	A	245	26.955	6.231	31.088	1.00	20.25	B	C
ATOM	1936	CD2	LEU	A	245	27.725	6.974	28.821	1.00	20.71	B	C
ATOM	1937	C	LEU	A	245	23.573	8.191	27.948	1.00	15.50	B	C
ATOM	1938	O	LEU	A	245	23.511	8.076	26.706	1.00	17.22	B	O
ATOM	1939	N	LEU	A	246	22.502	8.155	28.738	1.00	15.50	B	N
ATOM	1940	CA	LEU	A	246	21.159	7.875	28.239	1.00	17.11	B	C
ATOM	1941	CB	LEU	A	246	20.124	7.477	29.275	1.00	15.14	B	C
ATOM	1942	CG	LEU	A	246	20.058	5.950	29.588	1.00	16.98	B	C
ATOM	1943	CD1	LEU	A	246	19.622	5.106	28.364	1.00	20.29	B	C
ATOM	1944	CD2	LEU	A	246	21.329	5.414	30.130	1.00	18.01	B	C
ATOM	1945	C	LEU	A	246	20.654	8.955	27.293	1.00	15.70	B	C
ATOM	1946	O	LEU	A	246	19.757	8.745	26.495	1.00	17.24	B	O
ATOM	1947	N	SER	A	247	21.239	10.169	27.388	1.00	17.62	B	N
ATOM	1948	CA	SER	A	247	20.860	11.242	26.449	1.00	17.42	B	C
ATOM	1949	CB	SER	A	247	21.534	12.573	26.792	1.00	17.91	B	C
ATOM	1950	OG	SER	A	247	22.853	12.641	26.379	1.00	18.70	B	O
ATOM	1951	C	SER	A	247	21.133	10.880	24.985	1.00	15.00	B	C
ATOM	1952	O	SER	A	247	20.383	11.298	24.116	1.00	16.67	B	O
ATOM	1953	N	LEU	A	248	22.095	9.981	24.763	1.00	17.30	B	N
ATOM	1954	CA	LEU	A	248	22.399	9.526	23.380	1.00	17.41	B	C
ATOM	1955	CB	LEU	A	248	23.722	8.788	23.343	1.00	17.92	B	C
ATOM	1956	CG	LEU	A	248	24.943	9.656	23.631	1.00	19.16	B	C
ATOM	1957	CD1	LEU	A	248	26.191	8.826	23.840	1.00	19.70	B	C
ATOM	1958	CD2	LEU	A	248	25.158	10.688	22.500	1.00	20.14	B	C
ATOM	1959	C	LEU	A	248	21.255	8.648	22.884	1.00	16.12	B	C
ATOM	1960	O	LEU	A	248	20.736	8.804	21.773	1.00	16.68	B	O
ATOM	1961	N	HIS	A	249	20.820	7.752	23.766	1.00	16.97	B	N
ATOM	1962	CA	HIS	A	249	19.681	6.861	23.476	1.00	15.90	B	C
ATOM	1963	CB	HIS	A	249	19.526	5.898	24.666	1.00	16.28	B	C
ATOM	1964	CG	HIS	A	249	18.368	4.985	24.539	1.00	16.58	B	C
ATOM	1965	ND1	HIS	A	249	18.447	3.769	23.842	1.00	18.28	B	N
ATOM	1966	CE1	HIS	A	249	17.254	3.206	23.888	1.00	18.17	B	C
ATOM	1967	NE2	HIS	A	249	16.408	3.902	24.631	1.00	18.14	B	N
ATOM	1968	CD2	HIS	A	249	17.109	5.031	25.037	1.00	16.74	B	C
ATOM	1969	C	HIS	A	249	18.424	7.697	23.258	1.00	15.20	B	C
ATOM	1970	O	HIS	A	249	17.652	7.532	22.292	1.00	16.62	B	O

Fig. 5 (cont.)

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ATOM	1971	N	ASN	A	250	18.152	8.631	24.197	1.00	16.76	B	N
ATOM	1972	CA	ASN	A	250	16.949	9.462	24.103	1.00	16.57	B	C
ATOM	1973	CB	ASN	A	250	16.747	10.265	25.408	1.00	15.53	B	C
ATOM	1974	CG	ASN	A	250	16.469	9.392	26.612	1.00	17.86	B	C
ATOM	1975	OD1	ASN	A	250	15.761	8.351	26.493	1.00	16.26	B	O
ATOM	1976	ND2	ASN	A	250	16.917	9.825	27.773	1.00	18.19	B	N
ATOM	1977	C	ASN	A	250	16.974	10.371	22.831	1.00	13.61	B	C
ATOM	1978	O	ASN	A	250	15.924	10.565	22.270	1.00	15.53	B	O
ATOM	1979	N	ALA	A	251	18.155	10.813	22.454	1.00	16.31	B	N
ATOM	1980	CA	ALA	A	251	18.334	11.656	21.242	1.00	17.91	B	C
ATOM	1981	CB	ALA	A	251	19.702	12.203	21.155	1.00	17.27	B	C
ATOM	1982	C	ALA	A	251	17.995	10.869	19.980	1.00	16.88	B	C
ATOM	1983	O	ALA	A	251	17.251	11.356	19.096	1.00	18.16	B	O
ATOM	1984	N	AGLN	A	252	18.467	9.612	19.930	0.50	16.42	B	N
ATOM	1985	N	BGLN	A	252	18.465	9.609	19.926	0.50	17.50	B	N
ATOM	1986	CA	AGLN	A	252	18.229	8.720	18.758	0.50	16.43	B	C
ATOM	1987	CA	BGLN	A	252	18.196	8.757	18.732	0.50	18.84	B	C
ATOM	1988	CB	AGLN	A	252	19.012	7.440	19.002	0.50	15.77	B	C
ATOM	1989	CB	BGLN	A	252	18.895	7.409	18.806	0.50	21.05	B	C
ATOM	1990	CG	AGLN	A	252	18.914	6.428	17.875	0.50	14.39	B	C
ATOM	1991	CG	BGLN	A	252	18.513	6.563	17.606	0.50	22.94	B	C
ATOM	1992	CD	AGLN	A	252	19.838	6.687	16.711	0.50	14.60	B	C
ATOM	1993	CD	BGLN	A	252	19.466	5.436	17.315	0.50	29.30	B	C
ATOM	1994	OE1	AGLN	A	252	20.428	7.764	16.568	0.50	15.34	B	O
ATOM	1995	OE1	BGLN	A	252	19.196	4.295	17.671	0.50	32.77	B	O
ATOM	1996	NE2	AGLN	A	252	19.994	5.649	15.886	0.50	14.78	B	N
ATOM	1997	NE2	BGLN	A	252	20.561	5.742	16.627	0.50	27.53	B	N
ATOM	1998	C	AGLN	A	252	16.719	8.482	18.650	0.50	15.59	B	C
ATOM	1999	C	BGLN	A	252	16.691	8.509	18.650	0.50	16.61	B	C
ATOM	2000	O	AGLN	A	252	16.135	8.560	17.542	0.50	17.29	B	O
ATOM	2001	O	BGLN	A	252	16.089	8.589	17.556	0.50	17.55	B	O
ATOM	2002	N	PHE	A	253	16.061	8.174	19.793	1.00	17.27	B	N
ATOM	2003	CA	PHE	A	253	14.611	7.933	19.817	1.00	17.41	B	C
ATOM	2004	CB	PHE	A	253	14.139	7.354	21.158	1.00	18.17	B	C
ATOM	2005	CG	PHE	A	253	14.236	5.845	21.176	1.00	17.86	B	C
ATOM	2006	CD1	PHE	A	253	15.456	5.212	21.122	1.00	18.64	B	C
ATOM	2007	CE1	PHE	A	253	15.551	3.810	21.111	1.00	19.35	B	C
ATOM	2008	CZ	PHE	A	253	14.392	3.098	21.044	1.00	19.37	B	C
ATOM	2009	CD2	PHE	A	253	13.087	5.087	21.074	1.00	19.56	B	C
ATOM	2010	CE2	PHE	A	253	13.185	3.702	21.065	1.00	19.37	B	C
ATOM	2011	C	PHE	A	253	13.836	9.206	19.431	1.00	17.04	B	C
ATOM	2012	O	PHE	A	253	12.816	9.153	18.805	1.00	17.42	B	O
ATOM	2013	N	ASP	A	254	14.281	10.356	19.942	1.00	19.09	B	N
ATOM	2014	CA	ASP	A	254	13.662	11.650	19.570	1.00	17.70	B	C
ATOM	2015	CB	ASP	A	254	14.380	12.764	20.323	1.00	19.43	B	C
ATOM	2016	CG	ASP	A	254	13.650	14.086	20.310	1.00	22.44	B	C
ATOM	2017	OD1	ASP	A	254	12.482	14.151	20.072	1.00	20.97	B	O
ATOM	2018	OD2	ASP	A	254	14.317	15.046	20.711	1.00	35.48	B	O
ATOM	2019	C	ASP	A	254	13.640	11.840	18.027	1.00	16.15	B	C
ATOM	2020	O	ASP	A	254	12.568	12.048	17.495	1.00	18.97	B	O
ATOM	2021	N	LEU	A	255	14.799	11.705	17.407	1.00	18.84	B	N
ATOM	2022	CA	LEU	A	255	14.845	11.915	15.930	1.00	18.98	B	C
ATOM	2023	CB	LEU	A	255	16.256	11.850	15.392	1.00	22.41	B	C
ATOM	2024	CG	LEU	A	255	17.129	13.045	15.678	1.00	23.76	B	C
ATOM	2025	CD1	LEU	A	255	18.351	13.002	14.754	1.00	22.29	B	C
ATOM	2026	CD2	LEU	A	255	16.411	14.380	15.509	1.00	23.34	B	C
ATOM	2027	C	LEU	A	255	14.062	10.846	15.203	1.00	20.79	B	C
ATOM	2028	O	LEU	A	255	13.237	11.135	14.301	1.00	21.04	B	O

Fig. 5 (cont.)

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ATOM	2029	N	LEU	A	256	14.346	9.566	15.525	1.00	18.91	B	N
ATOM	2030	CA	LEU	A	256	13.808	8.464	14.686	1.00	19.84	B	C
ATOM	2031	CB	LEU	A	256	14.697	7.239	14.805	1.00	19.68	B	C
ATOM	2032	CG	LEU	A	256	16.133	7.416	14.374	1.00	20.16	B	C
ATOM	2033	CD1	LEU	A	256	16.843	6.109	14.440	1.00	21.80	B	C
ATOM	2034	CD2	LEU	A	256	16.201	7.971	12.923	1.00	23.42	B	C
ATOM	2035	C	LEU	A	256	12.365	8.124	14.999	1.00	20.30	B	C
ATOM	2036	O	LEU	A	256	11.696	7.507	14.150	1.00	21.96	B	O
ATOM	2037	N	GLN	A	257	11.875	8.393	16.224	1.00	18.30	B	N
ATOM	2038	CA	GLN	A	257	10.554	7.926	16.636	1.00	18.89	B	C
ATOM	2039	CB	GLN	A	257	10.704	6.894	17.760	1.00	18.24	B	C
ATOM	2040	CG	GLN	A	257	11.551	5.714	17.389	1.00	18.76	B	C
ATOM	2041	CD	GLN	A	257	11.077	4.864	16.242	1.00	17.76	B	C
ATOM	2042	OE1	GLN	A	257	9.913	4.847	15.854	1.00	20.53	B	O
ATOM	2043	NE2	GLN	A	257	12.041	4.095	15.766	1.00	23.48	B	N
ATOM	2044	C	GLN	A	257	9.624	9.037	17.145	1.00	16.01	B	C
ATOM	2045	O	GLN	A	257	8.432	8.865	17.037	1.00	18.29	B	O
ATOM	2046	N	ARG	A	258	10.166	10.189	17.556	1.00	17.43	B	N
ATOM	2047	CA	ARG	A	258	9.269	11.287	18.025	1.00	18.77	B	C
ATOM	2048	CB	ARG	A	258	9.767	11.997	19.280	1.00	17.27	B	C
ATOM	2049	CG	ARG	A	258	9.912	11.089	20.523	1.00	17.82	B	C
ATOM	2050	CD	ARG	A	258	10.373	11.850	21.736	1.00	21.31	B	C
ATOM	2051	NE	ARG	A	258	9.302	12.670	22.256	1.00	22.41	B	N
ATOM	2052	CZ	ARG	A	258	9.211	13.989	22.249	1.00	30.90	B	C
ATOM	2053	NH1	ARG	A	258	10.161	14.729	21.690	1.00	34.50	B	N
ATOM	2054	NH2	ARG	A	258	8.144	14.565	22.808	1.00	29.79	B	N
ATOM	2055	C	ARG	A	258	8.976	12.327	16.928	1.00	20.35	B	C
ATOM	2056	O	ARG	A	258	7.896	12.913	16.960	1.00	23.08	B	O
ATOM	2057	N	THR	A	259	9.870	12.494	15.976	1.00	20.50	B	N
ATOM	2058	CA	THR	A	259	9.578	13.453	14.855	1.00	19.67	B	C
ATOM	2059	CB	THR	A	259	10.648	13.265	13.804	1.00	20.10	B	C
ATOM	2060	OG1	THR	A	259	11.938	13.462	14.354	1.00	18.95	B	O
ATOM	2061	CG2	THR	A	259	10.436	14.236	12.645	1.00	20.33	B	C
ATOM	2062	C	THR	A	259	8.175	13.203	14.333	1.00	18.95	B	C
ATOM	2063	O	THR	A	259	7.877	12.091	13.892	1.00	19.14	B	O
ATOM	2064	N	PRO	A	260	7.265	14.214	14.296	1.00	20.62	B	N
ATOM	2065	CA	PRO	A	260	5.885	13.947	13.950	1.00	21.54	B	C
ATOM	2066	CB	PRO	A	260	5.186	15.285	14.051	1.00	25.33	B	C
ATOM	2067	CG	PRO	A	260	6.021	16.033	15.005	1.00	26.77	B	C
ATOM	2068	CD	PRO	A	260	7.457	15.577	14.811	1.00	24.19	B	C
ATOM	2069	C	PRO	A	260	5.659	13.308	12.581	1.00	22.30	B	C
ATOM	2070	O	PRO	A	260	4.741	12.554	12.403	1.00	22.15	B	O
ATOM	2071	N	GLU	A	261	6.518	13.668	11.621	1.00	24.08	B	N
ATOM	2072	CA	GLU	A	261	6.392	13.152	10.239	1.00	23.95	B	C
ATOM	2073	CB	GLU	A	261	7.377	13.853	9.309	1.00	26.23	B	C
ATOM	2074	CG	GLU	A	261	6.961	15.292	8.973	1.00	26.80	B	C
ATOM	2075	CD	GLU	A	261	7.271	16.365	9.999	1.00	31.63	B	C
ATOM	2076	OE1	GLU	A	261	7.868	16.074	11.103	1.00	27.77	B	O
ATOM	2077	OE2	GLU	A	261	6.900	17.535	9.730	1.00	32.40	B	O
ATOM	2078	C	GLU	A	261	6.605	11.641	10.210	1.00	24.27	B	C
ATOM	2079	O	GLU	A	261	6.091	11.000	9.297	1.00	26.68	B	O
ATOM	2080	N	VAL	A	262	7.316	11.077	11.196	1.00	22.07	B	N
ATOM	2081	CA	VAL	A	262	7.417	9.609	11.402	1.00	22.48	B	C
ATOM	2082	CB	VAL	A	262	8.773	9.250	12.016	1.00	22.35	B	C
ATOM	2083	CG1	VAL	A	262	8.783	7.831	12.506	1.00	23.49	B	C
ATOM	2084	CG2	VAL	A	262	9.949	9.538	11.089	1.00	26.08	B	C
ATOM	2085	C	VAL	A	262	6.294	9.137	12.338	1.00	18.58	B	C
ATOM	2086	O	VAL	A	262	5.619	8.162	12.033	1.00	20.63	B	O

Fig. 5 (cont.)

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ATOM	2087	N	ALA	A	263	6.187	9.845	13.465	1.00	19.67	B	N
ATOM	2088	CA	ALA	A	263	5.378	9.384	14.617	1.00	20.22	B	C
ATOM	2089	CB	ALA	A	263	5.597	10.270	15.807	1.00	20.10	B	C
ATOM	2090	C	ALA	A	263	3.907	9.261	14.305	1.00	20.44	B	C
ATOM	2091	O	ALA	A	263	3.243	8.306	14.732	1.00	20.59	B	O
ATOM	2092	N	AARG	A	264	3.342	10.233	13.560	0.50	20.01	B	N
ATOM	2093	N	BARG	A	264	3.336	10.229	13.561	0.50	20.01	B	N
ATOM	2094	CA	AARG	A	264	1.892	10.222	13.292	0.50	20.34	B	C
ATOM	2095	CA	BARG	A	264	1.889	10.186	13.289	0.50	20.16	B	C
ATOM	2096	CB	AARG	A	264	1.486	11.530	12.594	0.50	21.74	B	C
ATOM	2097	CB	BARG	A	264	1.460	11.424	12.502	0.50	21.45	B	C
ATOM	2098	CG	AARG	A	264	1.430	12.745	13.516	0.50	23.58	B	C
ATOM	2099	CG	BARG	A	264	1.647	12.712	13.281	0.50	22.70	B	C
ATOM	2100	CD	AARG	A	264	1.039	14.043	12.794	0.50	26.17	B	C
ATOM	2101	CD	BARG	A	264	1.113	13.884	12.463	0.50	24.84	B	C
ATOM	2102	NE	AARG	A	264	2.123	14.437	11.898	0.50	30.21	B	N
ATOM	2103	NE	BARG	A	264	0.966	15.064	13.290	0.50	25.42	B	N
ATOM	2104	CZ	AARG	A	264	2.227	15.590	11.254	0.50	32.40	B	C
ATOM	2105	CZ	BARG	A	264	0.289	16.148	12.940	0.50	28.01	B	C
ATOM	2106	NH1AARG	A	264	1.280	16.506	11.365	0.50	34.78	B	N	
ATOM	2107	NH1BARG	A	264	-0.347	16.171	11.774	0.50	30.79	B	N	
ATOM	2108	NH2AARG	A	264	3.277	15.823	10.489	0.50	30.20	B	N	
ATOM	2109	NH2BARG	A	264	0.219	17.172	13.772	0.50	28.23	B	N	
ATOM	2110	C	AARG	A	264	1.541	8.942	12.516	0.50	20.69	B	C
ATOM	2111	C	BARG	A	264	1.550	8.907	12.527	0.50	20.62	B	C
ATOM	2112	O	AARG	A	264	0.554	8.289	12.874	0.50	21.52	B	O
ATOM	2113	O	BARG	A	264	0.570	8.248	12.883	0.50	21.58	B	O
ATOM	2114	N	SER	A	265	2.308	8.589	11.467	1.00	21.33	B	N
ATOM	2115	CA	SER	A	265	2.098	7.338	10.706	1.00	19.93	B	C
ATOM	2116	CB	SER	A	265	3.045	7.233	9.538	1.00	22.49	B	C
ATOM	2117	OG	SER	A	265	2.652	8.159	8.487	1.00	23.93	B	O
ATOM	2118	C	SER	A	265	2.257	6.099	11.614	1.00	18.31	B	C
ATOM	2119	O	SER	A	265	1.345	5.293	11.687	1.00	20.66	B	O
ATOM	2120	N	ARG	A	266	3.391	6.047	12.261	1.00	19.99	B	N
ATOM	2121	CA	ARG	A	266	3.761	4.814	13.015	1.00	19.67	B	C
ATOM	2122	CB	ARG	A	266	5.221	4.856	13.393	1.00	19.96	B	C
ATOM	2123	CG	ARG	A	266	5.874	3.478	13.493	1.00	21.28	B	C
ATOM	2124	CD	ARG	A	266	7.355	3.571	13.707	1.00	21.54	B	C
ATOM	2125	NE	ARG	A	266	8.033	2.263	13.813	1.00	21.66	B	N
ATOM	2126	CZ	ARG	A	266	8.103	1.530	14.924	1.00	22.36	B	C
ATOM	2127	NH1	ARG	A	266	7.484	1.899	16.036	1.00	22.08	B	N
ATOM	2128	NH2	ARG	A	266	8.773	0.384	14.892	1.00	23.39	B	N
ATOM	2129	C	ARG	A	266	2.869	4.606	14.245	1.00	18.76	B	C
ATOM	2130	O	ARG	A	266	2.638	3.422	14.573	1.00	19.76	B	O
ATOM	2131	N	ALA	A	267	2.301	5.656	14.813	1.00	19.61	B	N
ATOM	2132	CA	ALA	A	267	1.380	5.573	15.973	1.00	18.62	B	C
ATOM	2133	CB	ALA	A	267	1.523	6.812	16.817	1.00	19.50	B	C
ATOM	2134	C	ALA	A	267	-0.070	5.414	15.567	1.00	19.64	B	C
ATOM	2135	O	ALA	A	267	-0.911	5.287	16.409	1.00	18.93	B	O
ATOM	2136	N	THR	A	268	-0.435	5.474	14.264	1.00	19.97	B	N
ATOM	2137	CA	THR	A	268	-1.867	5.563	13.879	1.00	20.27	B	C
ATOM	2138	CB	THR	A	268	-2.046	5.817	12.364	1.00	21.25	B	C
ATOM	2139	OG1	THR	A	268	-1.775	7.193	12.079	1.00	21.68	B	O
ATOM	2140	CG2	THR	A	268	-3.451	5.471	11.967	1.00	21.85	B	C
ATOM	2141	C	THR	A	268	-2.644	4.389	14.442	1.00	19.38	B	C
ATOM	2142	O	THR	A	268	-3.724	4.600	14.995	1.00	20.18	B	O
ATOM	2143	N	PRO	A	269	-2.182	3.119	14.338	1.00	19.67	B	N
ATOM	2144	CA	PRO	A	269	-3.022	2.052	14.871	1.00	20.13	B	C

Fig. 5 (cont.)

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ATOM	2145	CB	PRO	A	269	-2.245	0.789	14.523	1.00	20.77	B	C
ATOM	2146	CG	PRO	A	269	-1.462	1.184	13.312	1.00	22.10	B	C
ATOM	2147	CD	PRO	A	269	-1.078	2.633	13.523	1.00	20.65	B	C
ATOM	2148	C	PRO	A	269	-3.285	2.198	16.372	1.00	17.46	B	C
ATOM	2149	O	PRO	A	269	-4.391	1.934	16.778	1.00	19.45	B	O
ATOM	2150	N	LEU	A	270	-2.245	2.562	17.113	1.00	19.90	B	N
ATOM	2151	CA	LEU	A	270	-2.396	2.715	18.578	1.00	18.65	B	C
ATOM	2152	CB	LEU	A	270	-1.023	2.820	19.235	1.00	19.95	B	C
ATOM	2153	CG	LEU	A	270	-0.280	1.483	19.371	1.00	19.37	B	C
ATOM	2154	CD1	LEU	A	270	1.169	1.674	19.701	1.00	18.28	B	C
ATOM	2155	CD2	LEU	A	270	-0.942	0.579	20.387	1.00	19.81	B	C
ATOM	2156	C	LEU	A	270	-3.273	3.913	18.901	1.00	19.62	B	C
ATOM	2157	O	LEU	A	270	-4.117	3.824	19.785	1.00	19.62	B	O
ATOM	2158	N	LEU	A	271	-3.139	5.002	18.138	1.00	19.58	B	N
ATOM	2159	CA	LEU	A	271	-4.056	6.149	18.340	1.00	19.52	B	C
ATOM	2160	CB	LEU	A	271	-3.658	7.318	17.433	1.00	21.24	B	C
ATOM	2161	CG	LEU	A	271	-2.314	7.980	17.714	1.00	21.33	B	C
ATOM	2162	CD1	LEU	A	271	-1.877	8.929	16.600	1.00	23.84	B	C
ATOM	2163	CD2	LEU	A	271	-2.329	8.740	19.021	1.00	22.34	B	C
ATOM	2164	C	LEU	A	271	-5.507	5.756	18.072	1.00	21.01	B	C
ATOM	2165	O	LEU	A	271	-6.400	6.148	18.867	1.00	20.98	B	O
ATOM	2166	N	ASP	A	272	-5.741	5.019	16.991	1.00	20.61	B	N
ATOM	2167	CA	ASP	A	272	-7.111	4.596	16.635	1.00	22.38	B	C
ATOM	2168	CB	ASP	A	272	-7.106	3.831	15.314	1.00	22.54	B	C
ATOM	2169	CG	ASP	A	272	-6.964	4.688	14.080	1.00	25.59	B	C
ATOM	2170	OD1	ASP	A	272	-6.937	5.953	14.201	1.00	25.15	B	O
ATOM	2171	OD2	ASP	A	272	-6.866	4.044	13.021	1.00	25.09	B	O
ATOM	2172	C	ASP	A	272	-7.661	3.720	17.759	1.00	20.61	B	C
ATOM	2173	O	ASP	A	272	-8.818	3.780	18.072	1.00	21.99	B	O
ATOM	2174	N	LEU	A	273	-6.827	2.812	18.272	1.00	21.30	B	N
ATOM	2175	CA	LEU	A	273	-7.273	1.883	19.319	1.00	21.82	B	C
ATOM	2176	CB	LEU	A	273	-6.182	0.823	19.488	1.00	21.19	B	C
ATOM	2177	CG	LEU	A	273	-6.520	-0.231	20.524	1.00	22.10	B	C
ATOM	2178	CD1	LEU	A	273	-7.868	-0.907	20.284	1.00	23.56	B	C
ATOM	2179	CD2	LEU	A	273	-5.388	-1.270	20.584	1.00	22.08	B	C
ATOM	2180	C	LEU	A	273	-7.584	2.626	20.631	1.00	18.98	B	C
ATOM	2181	O	LEU	A	273	-8.626	2.356	21.253	1.00	20.60	B	O
ATOM	2182	N	ILE	A	274	-6.709	3.580	21.003	1.00	20.58	B	N
ATOM	2183	CA	ILE	A	274	-6.942	4.440	22.200	1.00	20.02	B	C
ATOM	2184	CB	ILE	A	274	-5.742	5.348	22.454	1.00	20.53	B	C
ATOM	2185	CG1	ILE	A	274	-4.470	4.591	22.847	1.00	19.66	B	C
ATOM	2186	CG2	ILE	A	274	-6.135	6.357	23.512	1.00	22.86	B	C
ATOM	2187	CD1	ILE	A	274	-3.183	5.345	22.587	1.00	20.42	B	C
ATOM	2188	C	ILE	A	274	-8.255	5.225	22.017	1.00	21.38	B	C
ATOM	2189	O	ILE	A	274	-9.084	5.271	22.921	1.00	22.64	B	O
ATOM	2190	N	ASP	A	275	-8.415	5.836	20.858	1.00	22.89	B	N
ATOM	2191	CA	ASP	A	275	-9.616	6.629	20.494	1.00	24.18	B	C
ATOM	2192	CB	ASP	A	275	-9.468	7.197	19.081	1.00	25.90	B	C
ATOM	2193	CG	ASP	A	275	-10.601	8.091	18.589	1.00	29.70	B	C
ATOM	2194	OD1	ASP	A	275	-11.145	8.813	19.392	1.00	31.97	B	O
ATOM	2195	OD2	ASP	A	275	-10.735	8.171	17.366	1.00	35.28	B	O
ATOM	2196	C	ASP	A	275	-10.853	5.745	20.630	1.00	24.42	B	C
ATOM	2197	O	ASP	A	275	-11.783	6.116	21.354	1.00	26.02	B	O
ATOM	2198	N	THR	A	276	-10.889	4.600	19.968	1.00	22.50	B	N
ATOM	2199	CA	THR	A	276	-12.033	3.674	20.032	1.00	23.00	B	C
ATOM	2200	CB	THR	A	276	-11.845	2.456	19.144	1.00	25.66	B	C
ATOM	2201	OG1	THR	A	276	-11.645	2.886	17.800	1.00	28.19	B	O
ATOM	2202	CG2	THR	A	276	-13.028	1.521	19.190	1.00	28.22	B	C

Fig. 5 (cont.)

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ATOM	2203	C	THR A 276	-12.272	3.272	21.479	1.00	25.27	B	C
ATOM	2204	O	THR A 276	-13.433	3.230	21.900	1.00	26.66	B	O
ATOM	2205	N	ALA A 277	-11.216	2.899	22.210	1.00	22.06	B	N
ATOM	2206	CA	ALA A 277	-11.408	2.343	23.557	1.00	22.76	B	C
ATOM	2207	CB	ALA A 277	-10.093	1.849	24.074	1.00	22.87	B	C
ATOM	2208	C	ALA A 277	-12.009	3.380	24.489	1.00	23.03	B	C
ATOM	2209	O	ALA A 277	-12.857	3.023	25.312	1.00	23.84	B	O
ATOM	2210	N	LEU A 278	-11.608	4.653	24.353	1.00	23.13	B	N
ATOM	2211	CA	LEU A 278	-12.110	5.739	25.237	1.00	23.62	B	C
ATOM	2212	CB	LEU A 278	-11.027	6.811	25.386	1.00	22.61	B	C
ATOM	2213	CG	LEU A 278	-9.752	6.353	26.081	1.00	24.23	B	C
ATOM	2214	CD1	LEU A 278	-8.819	7.520	26.297	1.00	26.00	B	C
ATOM	2215	CD2	LEU A 278	-10.043	5.658	27.413	1.00	26.11	B	C
ATOM	2216	C	LEU A 278	-13.417	6.371	24.733	1.00	24.63	B	C
ATOM	2217	O	LEU A 278	-14.131	6.897	25.566	1.00	26.83	B	O
ATOM	2218	N	LEU A 279	-13.713	6.283	23.440	1.00	26.15	B	N
ATOM	2219	CA	LEU A 279	-14.903	7.006	22.905	1.00	29.30	B	C
ATOM	2220	CB	LEU A 279	-14.536	7.835	21.693	1.00	33.29	B	C
ATOM	2221	CG	LEU A 279	-14.182	9.223	22.178	1.00	38.76	B	C
ATOM	2222	CD1	LEU A 279	-12.663	9.328	22.342	1.00	34.33	B	C
ATOM	2223	CD2	LEU A 279	-14.858	10.285	21.324	1.00	40.98	B	C
ATOM	2224	C	LEU A 279	-16.063	6.091	22.633	1.00	35.40	B	C
ATOM	2225	O	LEU A 279	-17.180	6.495	22.993	1.00	38.96	B	O
ATOM	2226	N	THR A 280	-15.818	4.873	22.200	1.00	35.14	B	N
ATOM	2227	CA	THR A 280	-16.897	3.893	21.923	1.00	35.70	B	C
ATOM	2228	CB	THR A 280	-16.360	2.555	21.414	1.00	40.72	B	C
ATOM	2229	OG1	THR A 280	-17.501	1.928	20.830	1.00	44.97	B	O
ATOM	2230	CG2	THR A 280	-15.789	1.661	22.503	1.00	39.31	B	C
ATOM	2231	C	THR A 280	-17.731	3.594	23.168	1.00	36.91	B	C
ATOM	2232	O	THR A 280	-17.236	3.630	24.309	1.00	37.29	B	O
ATOM	2233	N	ASN A 281	-19.021	3.338	22.950	1.00	38.97	B	N
ATOM	2234	CA	ASN A 281	-19.964	2.959	24.035	1.00	42.25	B	C
ATOM	2235	CB	ASN A 281	-21.130	3.957	24.051	1.00	52.15	B	C
ATOM	2236	CG	ASN A 281	-20.751	5.315	24.612	1.00	57.79	B	C
ATOM	2237	OD1	ASN A 281	-20.886	6.344	23.940	1.00	66.28	B	O
ATOM	2238	ND2	ASN A 281	-20.261	5.324	25.841	1.00	55.13	B	N
ATOM	2239	C	ASN A 281	-20.321	1.466	23.863	1.00	39.96	B	C
ATOM	2240	O	ASN A 281	-20.901	0.859	24.811	1.00	39.94	B	O
ATOM	2241	N	GLY A 282	-19.897	0.870	22.748	1.00	33.44	B	N
ATOM	2242	CA	GLY A 282	-19.999	-0.582	22.495	1.00	41.01	B	C
ATOM	2243	C	GLY A 282	-19.606	-0.909	21.068	1.00	41.89	B	C
ATOM	2244	O	GLY A 282	-20.274	-0.358	20.180	1.00	44.85	B	O
ATOM	2245	N	THR A 283	-18.548	-1.722	20.858	1.00	48.25	B	N
ATOM	2246	CA	THR A 283	-18.070	-2.291	19.554	1.00	51.73	B	C
ATOM	2247	CB	THR A 283	-17.114	-1.410	18.723	1.00	63.90	B	C
ATOM	2248	OG1	THR A 283	-16.371	-0.535	19.580	1.00	67.63	B	O
ATOM	2249	CG2	THR A 283	-17.802	-0.620	17.629	1.00	68.10	B	C
ATOM	2250	C	THR A 283	-17.176	-3.506	19.778	1.00	57.25	B	C
ATOM	2251	O	THR A 283	-16.540	-3.591	20.843	1.00	57.85	B	O
ATOM	2252	N	THR A 284	-17.024	-4.325	18.743	1.00	56.95	B	N
ATOM	2253	CA	THR A 284	-16.084	-5.469	18.717	1.00	64.26	B	C
ATOM	2254	CB	THR A 284	-16.757	-6.778	18.292	1.00	71.46	B	C
ATOM	2255	OG1	THR A 284	-15.676	-7.694	18.130	1.00	65.07	B	O
ATOM	2256	CG2	THR A 284	-17.581	-6.677	17.022	1.00	78.61	B	C
ATOM	2257	C	THR A 284	-14.929	-5.134	17.781	1.00	68.91	B	C
ATOM	2258	O	THR A 284	-15.146	-5.133	16.561	1.00	72.94	B	O
ATOM	2259	N	GLU A 285	-13.751	-4.844	18.339	1.00	67.98	B	N
ATOM	2260	CA	GLU A 285	-12.527	-4.642	17.526	1.00	67.77	B	C

Fig. 5 (cont.)

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ATOM	2261	CB	GLU	A	285	-11.406	-3.997	18.348	1.00	69.16	B	C
ATOM	2262	CG	GLU	A	285	-10.660	-4.948	19.253	1.00	67.10	B	C
ATOM	2263	CD	GLU	A	285	-9.773	-5.920	18.508	1.00	62.69	B	C
ATOM	2264	OE1	GLU	A	285	-9.447	-6.958	19.094	1.00	67.72	B	O
ATOM	2265	OE2	GLU	A	285	-9.447	-5.656	17.327	1.00	60.27	B	O
ATOM	2266	C	GLU	A	285	-12.243	-6.003	16.888	1.00	62.89	B	C
ATOM	2267	O	GLU	A	285	-12.024	-6.986	17.612	1.00	64.01	B	O
ATOM	2268	N	ASN	A	286	-12.367	-6.067	15.567	1.00	72.00	B	N
ATOM	2269	CA	ASN	A	286	-12.453	-7.345	14.813	1.00	69.68	B	C
ATOM	2270	CB	ASN	A	286	-12.958	-7.131	13.379	1.00	74.10	B	C
ATOM	2271	CG	ASN	A	286	-14.452	-6.873	13.289	1.00	79.08	B	C
ATOM	2272	OD1	ASN	A	286	-14.973	-5.927	13.882	1.00	78.54	B	O
ATOM	2273	ND2	ASN	A	286	-15.159	-7.696	12.529	1.00	83.32	B	N
ATOM	2274	C	ASN	A	286	-11.101	-8.069	14.865	1.00	63.79	B	C
ATOM	2275	O	ASN	A	286	-11.108	-9.288	14.589	1.00	69.69	B	O
ATOM	2276	N	ARG	A	287	-9.993	-7.398	15.233	1.00	49.41	B	N
ATOM	2277	CA	ARG	A	287	-8.652	-7.994	14.973	1.00	42.61	B	C
ATOM	2278	CB	ARG	A	287	-7.579	-6.940	14.692	1.00	38.90	B	C
ATOM	2279	CG	ARG	A	287	-7.562	-6.526	13.220	1.00	45.53	B	C
ATOM	2280	CD	ARG	A	287	-6.163	-6.299	12.640	1.00	45.19	B	C
ATOM	2281	NE	ARG	A	287	-5.581	-5.475	13.650	1.00	39.63	B	N
ATOM	2282	CZ	ARG	A	287	-4.455	-5.682	14.323	1.00	37.83	B	C
ATOM	2283	NH1	ARG	A	287	-3.578	-6.625	14.034	1.00	34.62	B	N
ATOM	2284	NH2	ARG	A	287	-4.237	-4.882	15.327	1.00	36.07	B	N
ATOM	2285	C	ARG	A	287	-8.286	-8.991	16.078	1.00	42.30	B	C
ATOM	2286	O	ARG	A	287	-7.762	-10.042	15.717	1.00	50.69	B	O
ATOM	2287	N	TYR	A	288	-8.570	-8.746	17.361	1.00	32.79	B	N
ATOM	2288	CA	TYR	A	288	-8.226	-9.769	18.388	1.00	35.49	B	C
ATOM	2289	CB	TYR	A	288	-7.329	-9.189	19.485	1.00	34.54	B	C
ATOM	2290	CG	TYR	A	288	-6.130	-8.416	18.990	1.00	30.69	B	C
ATOM	2291	CD1	TYR	A	288	-4.949	-9.049	18.654	1.00	31.93	B	C
ATOM	2292	CE1	TYR	A	288	-3.869	-8.324	18.171	1.00	30.34	B	C
ATOM	2293	CZ	TYR	A	288	-3.977	-6.951	18.048	1.00	29.73	B	C
ATOM	2294	OH	TYR	A	288	-2.924	-6.257	17.551	1.00	29.59	B	O
ATOM	2295	CE2	TYR	A	288	-5.158	-6.304	18.349	1.00	30.01	B	C
ATOM	2296	CD2	TYR	A	288	-6.223	-7.042	18.831	1.00	31.96	B	C
ATOM	2297	C	TYR	A	288	-9.509	-10.374	18.965	1.00	36.54	B	C
ATOM	2298	O	TYR	A	288	-9.417	-11.346	19.734	1.00	41.47	B	O
ATOM	2299	N	GLY	A	289	-10.664	-9.828	18.599	1.00	35.64	B	N
ATOM	2300	CA	GLY	A	289	-11.968	-10.330	19.057	1.00	39.33	B	C
ATOM	2301	C	GLY	A	289	-12.324	-9.723	20.386	1.00	36.29	B	C
ATOM	2302	O	GLY	A	289	-13.214	-10.237	21.061	1.00	39.88	B	O
ATOM	2303	N	ILE	A	290	-11.700	-8.603	20.758	1.00	35.99	B	N
ATOM	2304	CA	ILE	A	290	-12.047	-7.971	22.049	1.00	30.73	B	C
ATOM	2305	CB	ILE	A	290	-10.893	-7.094	22.582	1.00	32.22	B	C
ATOM	2306	CG1	ILE	A	290	-9.635	-7.955	22.749	1.00	30.37	B	C
ATOM	2307	CG2	ILE	A	290	-11.321	-6.383	23.856	1.00	32.28	B	C
ATOM	2308	CD1	ILE	A	290	-8.354	-7.177	23.063	1.00	31.00	B	C
ATOM	2309	C	ILE	A	290	-13.347	-7.180	21.886	1.00	34.83	B	C
ATOM	2310	O	ILE	A	290	-13.437	-6.380	20.923	1.00	35.15	B	O
ATOM	2311	N	LYS	A	291	-14.264	-7.348	22.837	1.00	33.83	B	N
ATOM	2312	CA	LYS	A	291	-15.516	-6.558	22.961	1.00	39.88	B	C
ATOM	2313	CB	LYS	A	291	-16.623	-7.315	23.710	1.00	45.71	B	C
ATOM	2314	CG	LYS	A	291	-17.067	-8.630	23.083	1.00	56.45	B	C
ATOM	2315	CD	LYS	A	291	-18.045	-8.446	21.928	1.00	65.71	B	C
ATOM	2316	CE	LYS	A	291	-18.665	-9.743	21.439	1.00	65.18	B	C
ATOM	2317	NZ	LYS	A	291	-18.011	-10.239	20.207	1.00	66.36	B	N
ATOM	2318	C	LYS	A	291	-15.150	-5.288	23.725	1.00	36.02	B	C

Fig. 5 (cont.)

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ATOM	2319	O	LYS	A	291	-14.804	-5.394	24.919	1.00	39.38	B	O
ATOM	2320	N	LEU	A	292	-15.137	-4.157	23.023	1.00	36.06	B	N
ATOM	2321	CA	LEU	A	292	-14.915	-2.826	23.639	1.00	35.67	B	C
ATOM	2322	CB	LEU	A	292	-14.044	-1.960	22.727	1.00	33.11	B	C
ATOM	2323	CG	LEU	A	292	-12.604	-2.429	22.476	1.00	31.74	B	C
ATOM	2324	CD1	LEU	A	292	-11.936	-1.526	21.476	1.00	32.92	B	C
ATOM	2325	CD2	LEU	A	292	-11.800	-2.496	23.763	1.00	29.00	B	C
ATOM	2326	C	LEU	A	292	-16.280	-2.188	23.851	1.00	36.16	B	C
ATOM	2327	O	LEU	A	292	-17.226	-2.472	23.108	1.00	39.69	B	O
ATOM	2328	N	PRO	A	293	-16.415	-1.285	24.823	1.00	31.27	B	N
ATOM	2329	CA	PRO	A	293	-15.309	-0.846	25.664	1.00	32.17	B	C
ATOM	2330	CB	PRO	A	293	-15.806	0.483	26.236	1.00	33.48	B	C
ATOM	2331	CG	PRO	A	293	-17.312	0.234	26.356	1.00	34.09	B	C
ATOM	2332	CD	PRO	A	293	-17.650	-0.536	25.110	1.00	36.56	B	C
ATOM	2333	C	PRO	A	293	-15.039	-1.848	26.791	1.00	33.78	B	C
ATOM	2334	O	PRO	A	293	-15.884	-2.718	27.047	1.00	30.56	B	O
ATOM	2335	N	VAL	A	294	-13.876	-1.700	27.417	1.00	27.28	B	N
ATOM	2336	CA	VAL	A	294	-13.486	-2.366	28.686	1.00	25.20	B	C
ATOM	2337	CB	VAL	A	294	-12.307	-3.342	28.449	1.00	25.17	B	C
ATOM	2338	CG1	VAL	A	294	-12.711	-4.511	27.599	1.00	27.15	B	C
ATOM	2339	CG2	VAL	A	294	-11.109	-2.656	27.806	1.00	25.11	B	C
ATOM	2340	C	VAL	A	294	-13.111	-1.332	29.724	1.00	23.75	B	C
ATOM	2341	O	VAL	A	294	-12.829	-0.178	29.391	1.00	27.58	B	O
ATOM	2342	N	SER	A	295	-13.061	-1.696	30.982	1.00	22.39	B	N
ATOM	2343	CA	SER	A	295	-12.635	-0.810	32.064	1.00	22.62	B	C
ATOM	2344	CB	SER	A	295	-12.911	-1.389	33.404	1.00	27.72	B	C
ATOM	2345	OG	SER	A	295	-14.323	-1.538	33.562	1.00	30.37	B	O
ATOM	2346	C	SER	A	295	-11.135	-0.512	31.917	1.00	21.89	B	C
ATOM	2347	O	SER	A	295	-10.685	0.643	32.079	1.00	23.28	B	O
ATOM	2348	N	LEU	A	296	-10.342	-1.545	31.610	1.00	22.03	B	N
ATOM	2349	CA	LEU	A	296	-8.872	-1.432	31.467	1.00	20.37	B	C
ATOM	2350	CB	LEU	A	296	-8.132	-1.949	32.701	1.00	23.56	B	C
ATOM	2351	CG	LEU	A	296	-6.610	-2.012	32.536	1.00	25.61	B	C
ATOM	2352	CD1	LEU	A	296	-6.004	-0.614	32.327	1.00	26.52	B	C
ATOM	2353	CD2	LEU	A	296	-5.992	-2.736	33.714	1.00	27.62	B	C
ATOM	2354	C	LEU	A	296	-8.428	-2.200	30.249	1.00	21.73	B	C
ATOM	2355	O	LEU	A	296	-8.667	-3.424	30.182	1.00	21.74	B	O
ATOM	2356	N	LEU	A	297	-7.758	-1.525	29.337	1.00	20.47	B	N
ATOM	2357	CA	LEU	A	297	-7.109	-2.098	28.170	1.00	18.39	B	C
ATOM	2358	CB	LEU	A	297	-7.573	-1.460	26.867	1.00	19.43	B	C
ATOM	2359	CG	LEU	A	297	-6.873	-1.925	25.585	1.00	21.95	B	C
ATOM	2360	CD1	LEU	A	297	-7.062	-3.417	25.324	1.00	24.31	B	C
ATOM	2361	CD2	LEU	A	297	-7.334	-1.157	24.350	1.00	21.57	B	C
ATOM	2362	C	LEU	A	297	-5.628	-1.863	28.389	1.00	18.27	B	C
ATOM	2363	O	LEU	A	297	-5.190	-0.722	28.539	1.00	18.82	B	O
ATOM	2364	N	PHE	A	298	-4.840	-2.932	28.379	1.00	18.28	B	N
ATOM	2365	CA	PHE	A	298	-3.385	-2.909	28.557	1.00	16.84	B	C
ATOM	2366	CB	PHE	A	298	-2.957	-3.675	29.818	1.00	16.63	B	C
ATOM	2367	CG	PHE	A	298	-1.478	-3.746	30.075	1.00	15.76	B	C
ATOM	2368	CD1	PHE	A	298	-0.652	-4.681	29.496	1.00	17.11	B	C
ATOM	2369	CE1	PHE	A	298	0.696	-4.725	29.790	1.00	17.18	B	C
ATOM	2370	CZ	PHE	A	298	1.253	-3.870	30.697	1.00	15.91	B	C
ATOM	2371	CD2	PHE	A	298	-0.901	-2.884	31.014	1.00	15.77	B	C
ATOM	2372	CE2	PHE	A	298	0.444	-2.941	31.285	1.00	15.46	B	C
ATOM	2373	C	PHE	A	298	-2.779	-3.414	27.280	1.00	18.10	B	C
ATOM	2374	O	PHE	A	298	-3.031	-4.551	26.908	1.00	17.86	B	O
ATOM	2375	N	ILE	A	299	-1.839	-2.678	26.688	1.00	16.86	B	N
ATOM	2376	CA	ILE	A	299	-1.181	-3.019	25.429	1.00	16.91	B	C

Fig. 5 (cont.)

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ATOM	2377	CB	ILE	A	299	-1.507	-1.966	24.340	1.00	18.01	B	C
ATOM	2378	CG1	ILE	A	299	-3.012	-1.842	24.132	1.00	18.37	B	C
ATOM	2379	CG2	ILE	A	299	-0.770	-2.235	23.054	1.00	19.72	B	C
ATOM	2380	CD1	ILE	A	299	-3.460	-0.503	23.602	1.00	20.39	B	C
ATOM	2381	C	ILE	A	299	0.310	-3.112	25.687	1.00	17.49	B	C
ATOM	2382	O	ILE	A	299	0.924	-2.108	26.038	1.00	17.60	B	O
ATOM	2383	N	ALA	A	300	0.903	-4.289	25.449	1.00	16.14	B	N
ATOM	2384	CA	ALA	A	300	2.335	-4.536	25.651	1.00	14.95	B	C
ATOM	2385	CB	ALA	A	300	2.518	-5.909	26.303	1.00	15.95	B	C
ATOM	2386	C	ALA	A	300	3.095	-4.420	24.342	1.00	16.31	B	C
ATOM	2387	O	ALA	A	300	2.986	-5.312	23.473	1.00	16.07	B	O
ATOM	2388	N	GLY	A	301	3.824	-3.314	24.216	1.00	16.06	B	N
ATOM	2389	CA	GLY	A	301	4.644	-2.982	23.052	1.00	15.19	B	C
ATOM	2390	C	GLY	A	301	6.061	-2.657	23.375	1.00	15.90	B	C
ATOM	2391	O	GLY	A	301	6.683	-3.243	24.287	1.00	15.52	B	O
ATOM	2392	N	HIS	A	302	6.578	-1.599	22.728	1.00	16.09	B	N
ATOM	2393	CA	HIS	A	302	8.007	-1.324	22.658	1.00	15.26	B	C
ATOM	2394	CB	HIS	A	302	8.540	-1.664	21.263	1.00	16.83	B	C
ATOM	2395	CG	HIS	A	302	8.185	-3.046	20.812	1.00	18.38	B	C
ATOM	2396	ND1	HIS	A	302	8.899	-4.145	21.175	1.00	21.45	B	N
ATOM	2397	CE1	HIS	A	302	8.275	-5.192	20.596	1.00	20.28	B	C
ATOM	2398	NE2	HIS	A	302	7.207	-4.813	19.959	1.00	19.50	B	N
ATOM	2399	CD2	HIS	A	302	7.133	-3.447	20.088	1.00	19.47	B	C
ATOM	2400	C	HIS	A	302	8.268	0.143	23.040	1.00	15.85	B	C
ATOM	2401	O	HIS	A	302	7.324	0.964	22.985	1.00	17.20	B	O
ATOM	2402	N	ASP	A	303	9.490	0.415	23.308	1.00	14.97	B	N
ATOM	2403	CA	ASP	A	303	9.915	1.831	23.487	1.00	16.28	B	C
ATOM	2404	CB	ASP	A	303	11.368	1.919	23.834	1.00	16.35	B	C
ATOM	2405	CG	ASP	A	303	12.353	0.987	23.112	1.00	19.57	B	C
ATOM	2406	OD1	ASP	A	303	12.024	0.539	21.958	1.00	21.32	B	O
ATOM	2407	OD2	ASP	A	303	13.486	0.855	23.636	1.00	18.98	B	O
ATOM	2408	C	ASP	A	303	9.567	2.647	22.253	1.00	16.93	B	C
ATOM	2409	O	ASP	A	303	9.208	3.837	22.432	1.00	17.86	B	O
ATOM	2410	N	THR	A	304	9.703	2.103	21.048	1.00	16.23	B	N
ATOM	2411	CA	THR	A	304	9.408	2.876	19.827	1.00	16.50	B	C
ATOM	2412	CB	THR	A	304	9.682	2.119	18.522	1.00	18.35	B	C
ATOM	2413	OG1	THR	A	304	8.868	0.956	18.488	1.00	20.59	B	O
ATOM	2414	CG2	THR	A	304	11.127	1.748	18.449	1.00	18.90	B	C
ATOM	2415	C	THR	A	304	7.973	3.305	19.856	1.00	17.36	B	C
ATOM	2416	O	THR	A	304	7.673	4.439	19.410	1.00	17.49	B	O
ATOM	2417	N	ASN	A	305	7.068	2.496	20.350	1.00	16.06	B	N
ATOM	2418	CA	ASN	A	305	5.651	2.833	20.405	1.00	16.54	B	C
ATOM	2419	CB	ASN	A	305	4.803	1.617	20.788	1.00	17.34	B	C
ATOM	2420	CG	ASN	A	305	4.989	0.439	19.873	1.00	18.40	B	C
ATOM	2421	OD1	ASN	A	305	5.317	-0.666	20.329	1.00	19.42	B	O
ATOM	2422	ND2	ASN	A	305	4.771	0.610	18.576	1.00	18.32	B	N
ATOM	2423	C	ASN	A	305	5.396	4.005	21.350	1.00	18.23	B	C
ATOM	2424	O	ASN	A	305	4.569	4.880	21.044	1.00	18.55	B	O
ATOM	2425	N	LEU	A	306	6.007	3.961	22.512	1.00	17.16	B	N
ATOM	2426	CA	LEU	A	306	5.848	5.042	23.503	1.00	17.63	B	C
ATOM	2427	CB	LEU	A	306	6.660	4.745	24.750	1.00	17.16	B	C
ATOM	2428	CG	LEU	A	306	6.264	3.522	25.576	1.00	16.52	B	C
ATOM	2429	CD1	LEU	A	306	7.167	3.411	26.768	1.00	18.41	B	C
ATOM	2430	CD2	LEU	A	306	4.842	3.577	25.996	1.00	18.01	B	C
ATOM	2431	C	LEU	A	306	6.355	6.328	22.861	1.00	16.48	B	C
ATOM	2432	O	LEU	A	306	5.717	7.396	23.019	1.00	16.64	B	O
ATOM	2433	N	ALA	A	307	7.508	6.280	22.227	1.00	16.33	B	N
ATOM	2434	CA	ALA	A	307	8.087	7.476	21.557	1.00	16.42	B	C

Fig. 5 (cont.)

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ATOM	2435	CB	ALA	A	307	9.484	7.226	21.124	1.00	16.43	B	C
ATOM	2436	C	ALA	A	307	7.183	7.948	20.418	1.00	17.82	B	C
ATOM	2437	O	ALA	A	307	6.986	9.182	20.287	1.00	17.54	B	O
ATOM	2438	N	ASN	A	308	6.615	7.080	19.622	1.00	17.76	B	N
ATOM	2439	CA	ASN	A	308	5.749	7.518	18.486	1.00	17.57	B	C
ATOM	2440	CB	ASN	A	308	5.264	6.332	17.652	1.00	19.21	B	C
ATOM	2441	CG	ASN	A	308	6.332	5.574	16.894	1.00	18.70	B	C
ATOM	2442	OD1	ASN	A	308	6.156	4.377	16.650	1.00	18.85	B	O
ATOM	2443	ND2	ASN	A	308	7.462	6.156	16.629	1.00	17.70	B	N
ATOM	2444	C	ASN	A	308	4.528	8.191	19.087	1.00	18.65	B	C
ATOM	2445	O	ASN	A	308	4.048	9.220	18.575	1.00	18.81	B	O
ATOM	2446	N	LEU	A	309	3.911	7.651	20.155	1.00	17.64	B	N
ATOM	2447	CA	LEU	A	309	2.721	8.281	20.753	1.00	17.53	B	C
ATOM	2448	CB	LEU	A	309	2.142	7.377	21.857	1.00	18.45	B	C
ATOM	2449	CG	LEU	A	309	1.398	6.174	21.289	1.00	18.61	B	C
ATOM	2450	CD1	LEU	A	309	1.169	5.114	22.397	1.00	19.77	B	C
ATOM	2451	CD2	LEU	A	309	0.063	6.555	20.684	1.00	19.13	B	C
ATOM	2452	C	LEU	A	309	3.148	9.626	21.328	1.00	17.47	B	C
ATOM	2453	O	LEU	A	309	2.376	10.640	21.158	1.00	18.85	B	O
ATOM	2454	N	SER	A	310	4.302	9.705	21.977	1.00	16.96	B	N
ATOM	2455	CA	SER	A	310	4.827	10.973	22.522	1.00	17.84	B	C
ATOM	2456	CB	SER	A	310	6.138	10.760	23.184	1.00	17.56	B	C
ATOM	2457	OG	SER	A	310	6.750	11.964	23.511	1.00	20.95	B	O
ATOM	2458	C	SER	A	310	4.920	12.017	21.398	1.00	18.41	B	C
ATOM	2459	O	SER	A	310	4.486	13.187	21.563	1.00	18.76	B	O
ATOM	2460	N	GLY	A	311	5.545	11.661	20.310	1.00	18.59	B	N
ATOM	2461	CA	GLY	A	311	5.789	12.616	19.191	1.00	17.59	B	C
ATOM	2462	C	GLY	A	311	4.503	13.000	18.525	1.00	20.52	B	C
ATOM	2463	O	GLY	A	311	4.323	14.221	18.197	1.00	21.93	B	O
ATOM	2464	N	ALA	A	312	3.587	12.101	18.278	1.00	18.55	B	N
ATOM	2465	CA	ALA	A	312	2.307	12.401	17.601	1.00	19.28	B	C
ATOM	2466	CB	ALA	A	312	1.559	11.147	17.270	1.00	19.10	B	C
ATOM	2467	C	ALA	A	312	1.477	13.346	18.469	1.00	21.73	B	C
ATOM	2468	O	ALA	A	312	0.779	14.228	17.950	1.00	21.76	B	O
ATOM	2469	N	LEU	A	313	1.411	13.143	19.784	1.00	19.75	B	N
ATOM	2470	CA	LEU	A	313	0.557	13.905	20.725	1.00	19.42	B	C
ATOM	2471	CB	LEU	A	313	0.030	12.918	21.779	1.00	20.51	B	C
ATOM	2472	CG	LEU	A	313	-0.968	11.908	21.230	1.00	19.98	B	C
ATOM	2473	CD1	LEU	A	313	-1.234	10.803	22.251	1.00	23.20	B	C
ATOM	2474	CD2	LEU	A	313	-2.258	12.565	20.798	1.00	21.43	B	C
ATOM	2475	C	LEU	A	313	1.276	15.086	21.349	1.00	20.26	B	C
ATOM	2476	O	LEU	A	313	0.674	15.674	22.295	1.00	23.30	B	O
ATOM	2477	N	ASP	A	314	2.526	15.300	21.016	1.00	19.32	B	N
ATOM	2478	CA	ASP	A	314	3.359	16.382	21.604	1.00	21.54	B	C
ATOM	2479	CB	ASP	A	314	2.787	17.783	21.304	1.00	24.91	B	C
ATOM	2480	CG	ASP	A	314	2.650	18.074	19.828	1.00	33.43	B	C
ATOM	2481	OD1	ASP	A	314	3.672	17.993	19.162	1.00	38.37	B	O
ATOM	2482	OD2	ASP	A	314	1.511	18.364	19.364	1.00	37.43	B	O
ATOM	2483	C	ASP	A	314	3.459	16.270	23.133	1.00	24.12	B	C
ATOM	2484	O	ASP	A	314	3.444	17.280	23.851	1.00	25.74	B	O
ATOM	2485	N	LEU	A	315	3.685	15.057	23.624	1.00	20.93	B	N
ATOM	2486	CA	LEU	A	315	3.881	14.757	25.052	1.00	21.19	B	C
ATOM	2487	CB	LEU	A	315	3.335	13.393	25.420	1.00	21.79	B	C
ATOM	2488	CG	LEU	A	315	1.866	13.141	25.162	1.00	23.97	B	C
ATOM	2489	CD1	LEU	A	315	1.570	11.680	25.384	1.00	22.25	B	C
ATOM	2490	CD2	LEU	A	315	0.978	14.042	26.010	1.00	27.60	B	C
ATOM	2491	C	LEU	A	315	5.361	14.789	25.341	1.00	22.56	B	C
ATOM	2492	O	LEU	A	315	6.157	13.944	24.866	1.00	24.88	B	O

Fig. 5 (cont.)

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ATOM	2493	N	AASN	A	316	5.744	15.720	26.182	0.50	18.91	B	N
ATOM	2494	N	BASN	A	316	5.756	15.730	26.173	0.50	19.59	B	N
ATOM	2495	CA	AASN	A	316	7.151	15.938	26.550	0.50	18.35	B	C
ATOM	2496	CA	BASN	A	316	7.173	15.900	26.540	0.50	20.05	B	C
ATOM	2497	CB	AASN	A	316	7.480	17.418	26.373	0.50	18.63	B	C
ATOM	2498	CB	BASN	A	316	7.654	17.318	26.239	0.50	23.43	B	C
ATOM	2499	CG	AASN	A	316	7.198	17.841	24.947	0.50	21.02	B	C
ATOM	2500	CG	BASN	A	316	8.108	17.359	24.802	0.50	30.31	B	C
ATOM	2501	OD1	AASN	A	316	7.769	17.259	23.998	0.50	27.35	B	O
ATOM	2502	OD1	BASN	A	316	7.309	17.627	23.900	0.50	35.76	B	O
ATOM	2503	ND2	AASN	A	316	6.364	18.861	24.776	0.50	21.36	B	N
ATOM	2504	ND2	BASN	A	316	9.319	16.899	24.569	0.50	31.04	B	N
ATOM	2505	C	AASN	A	316	7.375	15.542	28.001	0.50	18.50	B	C
ATOM	2506	C	BASN	A	316	7.383	15.549	28.002	0.50	19.16	B	C
ATOM	2507	O	AASN	A	316	6.547	15.920	28.846	0.50	20.54	B	O
ATOM	2508	O	BASN	A	316	6.559	15.944	28.842	0.50	20.69	B	O
ATOM	2509	N	TRP	A	317	8.476	14.829	28.310	1.00	18.19	B	N
ATOM	2510	CA	TRP	A	317	8.762	14.456	29.700	1.00	17.20	B	C
ATOM	2511	CB	TRP	A	317	7.847	13.284	30.164	1.00	17.17	B	C
ATOM	2512	CG	TRP	A	317	8.139	11.937	29.531	1.00	18.05	B	C
ATOM	2513	CD1	TRP	A	317	9.199	11.126	29.827	1.00	16.98	B	C
ATOM	2514	NE1	TRP	A	317	9.068	9.960	29.087	1.00	16.86	B	N
ATOM	2515	CE2	TRP	A	317	7.967	10.008	28.302	1.00	16.06	B	C
ATOM	2516	CD2	TRP	A	317	7.387	11.303	28.497	1.00	16.87	B	C
ATOM	2517	CE3	TRP	A	317	6.194	11.580	27.831	1.00	17.67	B	C
ATOM	2518	CZ3	TRP	A	317	5.688	10.699	26.919	1.00	16.99	B	C
ATOM	2519	CH2	TRP	A	317	6.291	9.457	26.720	1.00	16.50	B	C
ATOM	2520	CZ2	TRP	A	317	7.448	9.124	27.357	1.00	17.32	B	C
ATOM	2521	C	TRP	A	317	10.213	14.137	29.938	1.00	16.82	B	C
ATOM	2522	O	TRP	A	317	10.939	13.721	29.019	1.00	18.14	B	O
ATOM	2523	N	SER	A	318	10.519	14.227	31.219	1.00	16.54	B	N
ATOM	2524	CA	SER	A	318	11.715	13.656	31.812	1.00	17.40	B	C
ATOM	2525	CB	SER	A	318	12.709	14.750	32.164	1.00	18.48	B	C
ATOM	2526	OG	SER	A	318	13.842	14.218	32.740	1.00	20.74	B	O
ATOM	2527	C	SER	A	318	11.231	12.931	33.059	1.00	18.32	B	C
ATOM	2528	O	SER	A	318	10.244	13.374	33.704	1.00	18.00	B	O
ATOM	2529	N	LEU	A	319	11.782	11.748	33.340	1.00	17.18	B	N
ATOM	2530	CA	LEU	A	319	11.308	10.906	34.457	1.00	16.50	B	C
ATOM	2531	CB	LEU	A	319	11.199	9.448	33.975	1.00	16.33	B	C
ATOM	2532	CG	LEU	A	319	10.339	9.217	32.769	1.00	15.86	B	C
ATOM	2533	CD1	LEU	A	319	10.329	7.709	32.411	1.00	16.54	B	C
ATOM	2534	CD2	LEU	A	319	8.900	9.673	33.007	1.00	17.09	B	C
ATOM	2535	C	LEU	A	319	12.285	10.930	35.601	1.00	17.73	B	C
ATOM	2536	O	LEU	A	319	13.415	10.469	35.509	1.00	17.15	B	O
ATOM	2537	N	PRO	A	320	11.901	11.525	36.746	1.00	17.65	B	N
ATOM	2538	CA	PRO	A	320	12.830	11.577	37.870	1.00	18.21	B	C
ATOM	2539	CB	PRO	A	320	12.037	12.308	38.969	1.00	22.07	B	C
ATOM	2540	CG	PRO	A	320	11.053	13.145	38.187	1.00	20.81	B	C
ATOM	2541	CD	PRO	A	320	10.676	12.319	36.957	1.00	19.61	B	C
ATOM	2542	C	PRO	A	320	13.323	10.160	38.266	1.00	16.92	B	C
ATOM	2543	O	PRO	A	320	12.509	9.263	38.403	1.00	18.72	B	O
ATOM	2544	N	GLY	A	321	14.622	10.029	38.405	1.00	18.79	B	N
ATOM	2545	CA	GLY	A	321	15.190	8.751	38.859	1.00	17.76	B	C
ATOM	2546	C	GLY	A	321	15.243	7.723	37.755	1.00	18.68	B	C
ATOM	2547	O	GLY	A	321	15.750	6.611	38.024	1.00	18.82	B	O
ATOM	2548	N	GLN	A	322	14.883	8.070	36.529	1.00	17.69	B	N
ATOM	2549	CA	GLN	A	322	14.866	7.048	35.441	1.00	16.75	B	C
ATOM	2550	CB	GLN	A	322	13.468	6.559	35.238	1.00	16.13	B	C

Fig. 5 (cont.)

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ATOM	2551	CG	GLN	A	322	13.316	5.561	34.084	1.00	15.99	B	C
ATOM	2552	CD	GLN	A	322	14.360	4.468	34.105	1.00	16.40	B	C
ATOM	2553	OE1	GLN	A	322	15.352	4.495	33.402	1.00	16.25	B	O
ATOM	2554	NE2	GLN	A	322	14.097	3.481	34.988	1.00	16.00	B	N
ATOM	2555	C	GLN	A	322	15.497	7.670	34.211	1.00	16.71	B	C
ATOM	2556	O	GLN	A	322	14.812	8.374	33.445	1.00	17.78	B	O
ATOM	2557	N	PRO	A	323	16.780	7.414	33.918	1.00	16.53	B	N
ATOM	2558	CA	PRO	A	323	17.426	8.077	32.796	1.00	16.67	B	C
ATOM	2559	CB	PRO	A	323	18.910	7.714	32.882	1.00	18.92	B	C
ATOM	2560	CG	PRO	A	323	19.067	6.889	34.112	1.00	24.65	B	C
ATOM	2561	CD	PRO	A	323	17.704	6.601	34.702	1.00	18.91	B	C
ATOM	2562	C	PRO	A	323	16.890	7.783	31.407	1.00	17.03	B	C
ATOM	2563	O	PRO	A	323	17.090	8.541	30.493	1.00	17.70	B	O
ATOM	2564	N	ASP	A	324	16.272	6.596	31.192	1.00	15.38	B	N
ATOM	2565	CA	ASP	A	324	15.683	6.249	29.898	1.00	15.15	B	C
ATOM	2566	CB	ASP	A	324	15.691	4.719	29.696	1.00	15.48	B	C
ATOM	2567	CG	ASP	A	324	15.177	4.263	28.363	1.00	17.33	B	C
ATOM	2568	OD1	ASP	A	324	14.645	5.017	27.543	1.00	16.21	B	O
ATOM	2569	OD2	ASP	A	324	15.195	2.978	28.186	1.00	17.25	B	O
ATOM	2570	C	ASP	A	324	14.280	6.852	29.862	1.00	15.56	B	C
ATOM	2571	O	ASP	A	324	13.450	6.577	30.703	1.00	16.03	B	O
ATOM	2572	N	ASN	A	325	14.012	7.693	28.860	1.00	15.39	B	N
ATOM	2573	CA	ASN	A	325	12.673	8.284	28.734	1.00	15.97	B	C
ATOM	2574	CB	ASN	A	325	12.624	9.341	27.634	1.00	17.79	B	C
ATOM	2575	CG	ASN	A	325	13.401	10.597	27.949	1.00	18.55	B	C
ATOM	2576	OD1	ASN	A	325	13.664	10.912	29.092	1.00	18.07	B	O
ATOM	2577	ND2	ASN	A	325	13.742	11.289	26.895	1.00	20.10	B	N
ATOM	2578	C	ASN	A	325	11.584	7.265	28.411	1.00	15.27	B	C
ATOM	2579	O	ASN	A	325	10.422	7.509	28.624	1.00	16.48	B	O
ATOM	2580	N	THR	A	326	11.992	6.102	27.893	1.00	15.47	B	N
ATOM	2581	CA	THR	A	326	11.043	5.045	27.480	1.00	15.18	B	C
ATOM	2582	CB	THR	A	326	10.960	4.919	25.930	1.00	15.22	B	C
ATOM	2583	OG1	THR	A	326	12.258	4.855	25.394	1.00	16.58	B	O
ATOM	2584	CG2	THR	A	326	10.240	6.105	25.357	1.00	17.29	B	C
ATOM	2585	C	THR	A	326	11.507	3.756	28.184	1.00	15.44	B	C
ATOM	2586	O	THR	A	326	11.948	2.800	27.516	1.00	15.65	B	O
ATOM	2587	N	PRO	A	327	11.478	3.700	29.532	1.00	15.28	B	N
ATOM	2588	CA	PRO	A	327	12.140	2.657	30.290	1.00	15.10	B	C
ATOM	2589	CB	PRO	A	327	12.135	3.188	31.733	1.00	15.72	B	C
ATOM	2590	CG	PRO	A	327	10.880	4.037	31.799	1.00	15.45	B	C
ATOM	2591	CD	PRO	A	327	10.823	4.678	30.401	1.00	15.09	B	C
ATOM	2592	C	PRO	A	327	11.377	1.328	30.267	1.00	13.72	B	C
ATOM	2593	O	PRO	A	327	10.197	1.297	29.990	1.00	14.62	B	O
ATOM	2594	N	PRO	A	328	12.106	0.242	30.674	1.00	15.03	B	N
ATOM	2595	CA	PRO	A	328	11.458	-1.080	30.732	1.00	15.00	B	C
ATOM	2596	CB	PRO	A	328	12.545	-1.977	31.275	1.00	16.22	B	C
ATOM	2597	CG	PRO	A	328	13.828	-1.315	30.927	1.00	17.57	B	C
ATOM	2598	CD	PRO	A	328	13.539	0.195	30.948	1.00	14.87	B	C
ATOM	2599	C	PRO	A	328	10.211	-1.024	31.599	1.00	15.29	B	C
ATOM	2600	O	PRO	A	328	10.320	-0.506	32.737	1.00	15.52	B	O
ATOM	2601	N	GLY	A	329	9.101	-1.550	31.138	1.00	13.96	B	N
ATOM	2602	CA	GLY	A	329	7.844	-1.526	31.874	1.00	15.67	B	C
ATOM	2603	C	GLY	A	329	7.164	-0.174	31.989	1.00	15.47	B	C
ATOM	2604	O	GLY	A	329	6.089	-0.079	32.576	1.00	15.93	B	O
ATOM	2605	N	GLY	A	330	7.761	0.859	31.392	1.00	14.83	B	N
ATOM	2606	CA	GLY	A	330	7.107	2.172	31.465	1.00	15.33	B	C
ATOM	2607	C	GLY	A	330	5.772	2.199	30.754	1.00	15.74	B	C
ATOM	2608	O	GLY	A	330	5.609	1.599	29.667	1.00	14.95	B	O

Fig. 5 (cont.)

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ATOM	2609	N	GLU	A	331	4.812	2.952	31.304	1.00	14.66	B	N
ATOM	2610	CA	GLU	A	331	3.428	2.958	30.896	1.00	15.06	B	C
ATOM	2611	CB	GLU	A	331	2.571	2.428	32.040	1.00	16.93	B	C
ATOM	2612	CG	GLU	A	331	2.824	0.990	32.391	1.00	16.75	B	C
ATOM	2613	CD	GLU	A	331	1.973	0.445	33.515	1.00	17.95	B	C
ATOM	2614	OE1	GLU	A	331	1.102	1.149	34.015	1.00	18.92	B	O
ATOM	2615	OE2	GLU	A	331	2.139	-0.809	33.859	1.00	17.27	B	O
ATOM	2616	C	GLU	A	331	2.970	4.383	30.547	1.00	16.37	B	C
ATOM	2617	O	GLU	A	331	3.010	5.282	31.413	1.00	17.94	B	O
ATOM	2618	N	ALEU	A	332	2.471	4.539	29.314	0.50	15.69	B	N
ATOM	2619	N	BLEU	A	332	2.521	4.553	29.332	0.50	16.37	B	N
ATOM	2620	CA	ALEU	A	332	1.605	5.700	28.964	0.50	15.36	B	C
ATOM	2621	CA	BLEU	A	332	1.767	5.782	29.058	0.50	16.43	B	C
ATOM	2622	CB	ALEU	A	332	1.599	5.974	27.460	0.50	15.38	B	C
ATOM	2623	CB	BLEU	A	332	2.107	6.129	27.613	0.50	17.26	B	C
ATOM	2624	CG	ALEU	A	332	2.660	6.972	26.997	0.50	17.39	B	C
ATOM	2625	CG	BLEU	A	332	1.419	7.389	27.135	0.50	18.39	B	C
ATOM	2626	CD1	ALEU	A	332	2.556	7.190	25.506	0.50	17.14	B	C
ATOM	2627	CD1	BLEU	A	332	-0.046	7.250	27.393	0.50	25.45	B	C
ATOM	2628	CD2	ALEU	A	332	2.551	8.321	27.705	0.50	17.06	B	C
ATOM	2629	CD2	BLEU	A	332	1.917	8.633	27.850	0.50	19.91	B	C
ATOM	2630	C	ALEU	A	332	0.209	5.363	29.404	0.50	16.24	B	C
ATOM	2631	C	BLEU	A	332	0.298	5.460	29.338	0.50	16.61	B	C
ATOM	2632	O	ALEU	A	332	-0.377	4.407	28.892	0.50	17.59	B	O
ATOM	2633	O	BLEU	A	332	-0.215	4.527	28.727	0.50	17.49	B	O
ATOM	2634	N	VAL	A	333	-0.346	6.193	30.262	1.00	17.24	B	N
ATOM	2635	CA	VAL	A	333	-1.657	5.875	30.842	1.00	18.05	B	C
ATOM	2636	CB	VAL	A	333	-1.567	5.798	32.367	1.00	18.22	B	C
ATOM	2637	CG1	VAL	A	333	-2.892	5.400	32.959	1.00	20.88	B	C
ATOM	2638	CG2	VAL	A	333	-0.432	4.866	32.819	1.00	18.83	B	C
ATOM	2639	C	VAL	A	333	-2.649	6.943	30.423	1.00	17.39	B	C
ATOM	2640	O	VAL	A	333	-2.396	8.135	30.747	1.00	19.31	B	O
ATOM	2641	N	PHE	A	334	-3.699	6.520	29.783	1.00	16.84	B	N
ATOM	2642	CA	PHE	A	334	-4.789	7.413	29.301	1.00	17.69	B	C
ATOM	2643	CB	PHE	A	334	-5.125	7.189	27.841	1.00	19.01	B	C
ATOM	2644	CG	PHE	A	334	-3.979	7.451	26.913	1.00	18.89	B	C
ATOM	2645	CD1	PHE	A	334	-3.020	6.502	26.641	1.00	17.33	B	C
ATOM	2646	CE1	PHE	A	334	-1.982	6.789	25.764	1.00	18.63	B	C
ATOM	2647	CZ	PHE	A	334	-1.874	8.029	25.195	1.00	20.26	B	C
ATOM	2648	CD2	PHE	A	334	-3.859	8.688	26.316	1.00	20.36	B	C
ATOM	2649	CE2	PHE	A	334	-2.844	8.953	25.437	1.00	20.30	B	C
ATOM	2650	C	PHE	A	334	-6.011	7.090	30.148	1.00	19.57	B	C
ATOM	2651	O	PHE	A	334	-6.542	5.962	30.064	1.00	19.83	B	O
ATOM	2652	N	GLU	A	335	-6.430	8.058	30.948	1.00	22.41	B	N
ATOM	2653	CA	GLU	A	335	-7.582	7.901	31.851	1.00	21.51	B	C
ATOM	2654	CB	GLU	A	335	-7.238	8.304	33.258	1.00	22.44	B	C
ATOM	2655	CG	GLU	A	335	-6.210	7.441	33.973	1.00	30.06	B	C
ATOM	2656	CD	GLU	A	335	-5.523	8.171	35.117	1.00	37.25	B	C
ATOM	2657	OE1	GLU	A	335	-5.766	9.383	35.313	1.00	40.45	B	O
ATOM	2658	OE2	GLU	A	335	-4.705	7.522	35.798	1.00	50.58	B	O
ATOM	2659	C	GLU	A	335	-8.772	8.738	31.395	1.00	22.52	B	C
ATOM	2660	O	GLU	A	335	-8.579	9.962	31.203	1.00	22.93	B	O
ATOM	2661	N	LYS	A	336	-9.927	8.106	31.256	1.00	24.49	B	N
ATOM	2662	CA	LYS	A	336	-11.222	8.787	31.002	1.00	21.22	B	C
ATOM	2663	CB	LYS	A	336	-12.123	7.976	30.103	1.00	22.65	B	C
ATOM	2664	CG	LYS	A	336	-13.488	8.585	29.869	1.00	24.58	B	C
ATOM	2665	CD	LYS	A	336	-14.374	7.694	29.082	1.00	28.09	B	C
ATOM	2666	CE	LYS	A	336	-15.775	8.229	28.970	1.00	35.25	B	C

Fig. 5 (cont.)

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ATOM	2667	NZ	LYS	A	336	-16.537	7.381	28.031	1.00	40.46	B	N
ATOM	2668	C	LYS	A	336	-11.918	9.051	32.322	1.00	22.10	B	C
ATOM	2669	O	LYS	A	336	-12.251	8.118	33.048	1.00	21.83	B	O
ATOM	2670	N	TRP	A	337	-12.080	10.343	32.594	1.00	22.06	B	N
ATOM	2671	CA	TRP	A	337	-12.711	10.872	33.807	1.00	22.31	B	C
ATOM	2672	CB	TRP	A	337	-11.812	11.847	34.559	1.00	24.24	B	C
ATOM	2673	CG	TRP	A	337	-10.562	11.276	35.143	1.00	21.96	B	C
ATOM	2674	CD1	TRP	A	337	-9.340	11.210	34.548	1.00	24.24	B	C
ATOM	2675	NE1	TRP	A	337	-8.414	10.751	35.438	1.00	25.79	B	N
ATOM	2676	CE2	TRP	A	337	-9.036	10.401	36.599	1.00	27.12	B	C
ATOM	2677	CD2	TRP	A	337	-10.400	10.759	36.469	1.00	24.64	B	C
ATOM	2678	CE3	TRP	A	337	-11.251	10.564	37.565	1.00	28.32	B	C
ATOM	2679	CZ3	TRP	A	337	-10.741	10.012	38.718	1.00	32.16	B	C
ATOM	2680	CH2	TRP	A	337	-9.385	9.688	38.827	1.00	30.53	B	C
ATOM	2681	CZ2	TRP	A	337	-8.520	9.878	37.782	1.00	30.32	B	C
ATOM	2682	C	TRP	A	337	-14.038	11.533	33.462	1.00	23.88	B	C
ATOM	2683	O	TRP	A	337	-14.083	12.342	32.529	1.00	24.74	B	O
ATOM	2684	N	LYS	A	338	-15.069	11.220	34.244	1.00	24.62	B	N
ATOM	2685	CA	LYS	A	338	-16.383	11.910	34.106	1.00	24.45	B	C
ATOM	2686	CB	LYS	A	338	-17.534	10.907	34.163	1.00	27.26	B	C
ATOM	2687	CG	LYS	A	338	-18.918	11.558	34.011	1.00	31.83	B	C
ATOM	2688	CD	LYS	A	338	-20.037	10.673	33.489	1.00	39.55	B	C
ATOM	2689	CE	LYS	A	338	-20.338	10.864	32.018	1.00	44.51	B	C
ATOM	2690	NZ	LYS	A	338	-19.610	9.867	31.192	1.00	56.49	B	N
ATOM	2691	C	LYS	A	338	-16.475	12.986	35.167	1.00	24.76	B	C
ATOM	2692	O	LYS	A	338	-16.285	12.660	36.352	1.00	26.46	B	O
ATOM	2693	N	ARG	A	339	-16.761	14.230	34.743	1.00	24.06	B	N
ATOM	2694	CA	ARG	A	339	-17.053	15.316	35.716	1.00	27.27	B	C
ATOM	2695	CB	ARG	A	339	-16.616	16.678	35.170	1.00	28.86	B	C
ATOM	2696	CG	ARG	A	339	-17.072	17.863	36.014	1.00	33.24	B	C
ATOM	2697	CD	ARG	A	339	-17.226	19.111	35.119	1.00	39.64	B	C
ATOM	2698	NE	ARG	A	339	-15.941	19.710	35.009	1.00	39.87	B	N
ATOM	2699	CZ	ARG	A	339	-15.535	20.657	34.151	1.00	35.71	B	C
ATOM	2700	NH1	ARG	A	339	-16.301	21.134	33.189	1.00	34.61	B	N
ATOM	2701	NH2	ARG	A	339	-14.296	21.086	34.274	1.00	39.43	B	N
ATOM	2702	C	ARG	A	339	-18.556	15.253	36.018	1.00	25.66	B	C
ATOM	2703	O	ARG	A	339	-19.350	15.433	35.056	1.00	28.63	B	O
ATOM	2704	N	THR	A	340	-18.930	14.852	37.238	1.00	30.31	B	N
ATOM	2705	CA	THR	A	340	-20.320	14.452	37.579	1.00	30.30	B	C
ATOM	2706	CB	THR	A	340	-20.356	13.556	38.822	1.00	32.71	B	C
ATOM	2707	OG1	THR	A	340	-19.630	14.214	39.843	1.00	36.55	B	O
ATOM	2708	CG2	THR	A	340	-19.698	12.222	38.554	1.00	35.47	B	C
ATOM	2709	C	THR	A	340	-21.252	15.682	37.660	1.00	34.40	B	C
ATOM	2710	O	THR	A	340	-22.429	15.512	37.331	1.00	39.32	B	O
ATOM	2711	N	THR	A	341	-20.766	16.879	37.953	1.00	36.28	B	N
ATOM	2712	CA	SER	A	341	-21.652	18.089	37.930	1.00	35.06	B	C
ATOM	2713	CB	SER	A	341	-20.900	19.285	38.327	1.00	38.32	B	C
ATOM	2714	OG	SER	A	341	-19.880	19.552	37.382	1.00	41.13	B	O
ATOM	2715	C	SER	A	341	-22.325	18.289	36.556	1.00	40.42	B	C
ATOM	2716	O	SER	A	341	-23.582	18.433	36.497	1.00	37.14	B	O
ATOM	2717	N	ASP	A	342	-21.553	18.306	35.463	1.00	34.29	B	N
ATOM	2718	CA	ASP	A	342	-22.099	18.689	34.136	1.00	37.27	B	C
ATOM	2719	CB	ASP	A	342	-21.410	19.961	33.619	1.00	39.31	B	C
ATOM	2720	CG	ASP	A	342	-19.911	19.829	33.446	1.00	42.15	B	C
ATOM	2721	OD1	ASP	A	342	-19.410	18.712	33.686	1.00	35.99	B	O
ATOM	2722	OD2	ASP	A	342	-19.244	20.830	33.090	1.00	38.92	B	O
ATOM	2723	C	ASP	A	342	-22.058	17.470	33.220	1.00	34.29	B	C
ATOM	2724	O	ASP	A	342	-22.421	17.625	32.050	1.00	36.13	B	O

Fig. 5 (cont.)

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ATOM	2725	N	ASN	A	343	-21.670	16.283	33.729	1.00	36.03	B	N
ATOM	2726	CA	ASN	A	343	-21.695	15.049	32.898	1.00	38.73	B	C
ATOM	2727	CB	ASN	A	343	-23.105	14.711	32.405	1.00	49.42	B	C
ATOM	2728	CG	ASN	A	343	-23.362	13.228	32.475	1.00	54.84	B	C
ATOM	2729	OD1	ASN	A	343	-23.368	12.664	33.567	1.00	63.56	B	O
ATOM	2730	ND2	ASN	A	343	-23.547	12.595	31.332	1.00	64.38	B	N
ATOM	2731	C	ASN	A	343	-20.771	15.234	31.685	1.00	36.68	B	C
ATOM	2732	O	ASN	A	343	-21.089	14.746	30.587	1.00	39.47	B	O
ATOM	2733	N	THR	A	344	-19.618	15.866	31.879	1.00	30.12	B	N
ATOM	2734	CA	THR	A	344	-18.631	16.046	30.786	1.00	26.24	B	C
ATOM	2735	CB	THR	A	344	-18.124	17.493	30.732	1.00	25.05	B	C
ATOM	2736	OG1	THR	A	344	-17.554	17.892	31.974	1.00	25.23	B	O
ATOM	2737	CG2	THR	A	344	-19.223	18.451	30.291	1.00	28.45	B	C
ATOM	2738	C	THR	A	344	-17.489	15.030	30.980	1.00	25.63	B	C
ATOM	2739	O	THR	A	344	-17.150	14.714	32.114	1.00	24.15	B	O
ATOM	2740	N	ASP	A	345	-16.874	14.642	29.879	1.00	25.15	B	N
ATOM	2741	CA	ASP	A	345	-15.784	13.640	29.930	1.00	23.43	B	C
ATOM	2742	CB	ASP	A	345	-16.119	12.475	29.038	1.00	26.84	B	C
ATOM	2743	CG	ASP	A	345	-17.397	11.756	29.444	1.00	31.44	B	C
ATOM	2744	OD1	ASP	A	345	-17.519	11.430	30.603	1.00	34.31	B	O
ATOM	2745	OD2	ASP	A	345	-18.215	11.511	28.559	1.00	39.90	B	O
ATOM	2746	C	ASP	A	345	-14.458	14.303	29.581	1.00	22.26	B	C
ATOM	2747	O	ASP	A	345	-14.383	15.089	28.639	1.00	22.13	B	O
ATOM	2748	N	TRP	A	346	-13.403	13.780	30.178	1.00	21.69	B	N
ATOM	2749	CA	TRP	A	346	-12.051	14.370	30.152	1.00	21.52	B	C
ATOM	2750	CB	TRP	A	346	-11.843	15.221	31.398	1.00	21.39	B	C
ATOM	2751	CG	TRP	A	346	-12.886	16.294	31.471	1.00	22.93	B	C
ATOM	2752	CD1	TRP	A	346	-14.068	16.229	32.151	1.00	23.76	B	C
ATOM	2753	NE1	TRP	A	346	-14.770	17.396	31.936	1.00	25.13	B	N
ATOM	2754	CE2	TRP	A	346	-14.044	18.225	31.127	1.00	23.86	B	C
ATOM	2755	CD2	TRP	A	346	-12.832	17.584	30.822	1.00	24.38	B	C
ATOM	2756	CE3	TRP	A	346	-11.897	18.229	29.995	1.00	24.68	B	C
ATOM	2757	CZ3	TRP	A	346	-12.173	19.516	29.569	1.00	26.16	B	C
ATOM	2758	CH2	TRP	A	346	-13.395	20.118	29.873	1.00	25.30	B	C
ATOM	2759	CZ2	TRP	A	346	-14.333	19.521	30.678	1.00	25.95	B	C
ATOM	2760	C	TRP	A	346	-11.032	13.237	30.046	1.00	22.07	B	C
ATOM	2761	O	TRP	A	346	-11.309	12.145	30.558	1.00	22.47	B	O
ATOM	2762	N	VAL	A	347	-9.880	13.542	29.483	1.00	21.39	B	N
ATOM	2763	CA	VAL	A	347	-8.780	12.555	29.329	1.00	20.88	B	C
ATOM	2764	CB	VAL	A	347	-8.473	12.281	27.864	1.00	21.80	B	C
ATOM	2765	CG1	VAL	A	347	-7.298	11.320	27.774	1.00	23.79	B	C
ATOM	2766	CG2	VAL	A	347	-9.664	11.742	27.128	1.00	24.94	B	C
ATOM	2767	C	VAL	A	347	-7.569	13.094	30.065	1.00	21.99	B	C
ATOM	2768	O	VAL	A	347	-7.079	14.237	29.778	1.00	21.91	B	O
ATOM	2769	N	GLN	A	348	-7.055	12.321	30.993	1.00	22.92	B	N
ATOM	2770	CA	GLN	A	348	-5.801	12.631	31.691	1.00	21.70	B	C
ATOM	2771	CB	GLN	A	348	-5.957	12.518	33.180	1.00	22.98	B	C
ATOM	2772	CG	GLN	A	348	-4.679	12.880	33.898	1.00	26.42	B	C
ATOM	2773	CD	GLN	A	348	-4.984	13.396	35.270	1.00	33.74	B	C
ATOM	2774	OE1	GLN	A	348	-5.384	12.623	36.129	1.00	35.76	B	O
ATOM	2775	NE2	GLN	A	348	-4.884	14.700	35.448	1.00	34.83	B	N
ATOM	2776	C	GLN	A	348	-4.735	11.677	31.192	1.00	21.66	B	C
ATOM	2777	O	GLN	A	348	-5.044	10.488	31.018	1.00	22.49	B	O
ATOM	2778	N	VAL	A	349	-3.570	12.189	30.949	1.00	19.76	B	N
ATOM	2779	CA	VAL	A	349	-2.415	11.378	30.453	1.00	19.13	B	C
ATOM	2780	CB	VAL	A	349	-1.965	11.816	29.048	1.00	20.92	B	C
ATOM	2781	CG1	VAL	A	349	-0.915	10.876	28.460	1.00	23.30	B	C
ATOM	2782	CG2	VAL	A	349	-3.137	11.930	28.090	1.00	23.40	B	C

Fig. 5 (cont.)

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ATOM	2783	C	VAL	A	349	-1.312	11.474	31.488	1.00	19.38	B	C
ATOM	2784	O	VAL	A	349	-1.015	12.550	32.041	1.00	19.81	B	O
ATOM	2785	N	ASER	A	350	-0.709	10.321	31.803	0.50	19.25	B	N
ATOM	2786	N	BSER	A	350	-0.703	10.325	31.796	0.50	18.28	B	N
ATOM	2787	CA	ASER	A	350	0.388	10.200	32.783	0.50	19.08	B	C
ATOM	2788	CA	BSER	A	350	0.429	10.250	32.735	0.50	17.35	B	C
ATOM	2789	CB	ASER	A	350	-0.106	9.778	34.126	0.50	21.35	B	C
ATOM	2790	CB	BSER	A	350	-0.010	9.979	34.144	0.50	17.62	B	C
ATOM	2791	OG	ASER	A	350	-1.175	10.599	34.546	0.50	23.35	B	O
ATOM	2792	OG	BSER	A	350	-0.820	8.810	34.215	0.50	17.13	B	O
ATOM	2793	C	ASER	A	350	1.404	9.175	32.288	0.50	17.12	B	C
ATOM	2794	C	BSER	A	350	1.415	9.179	32.274	0.50	16.36	B	C
ATOM	2795	O	ASER	A	350	1.053	8.353	31.448	0.50	17.52	B	O
ATOM	2796	O	BSER	A	350	1.077	8.361	31.420	0.50	17.12	B	O
ATOM	2797	N	PHE	A	351	2.609	9.278	32.801	1.00	16.96	B	N
ATOM	2798	CA	PHE	A	351	3.662	8.254	32.578	1.00	16.57	B	C
ATOM	2799	CB	PHE	A	351	4.932	8.839	31.976	1.00	15.47	B	C
ATOM	2800	CG	PHE	A	351	5.722	7.770	31.262	1.00	16.59	B	C
ATOM	2801	CD1	PHE	A	351	6.607	6.954	31.939	1.00	16.26	B	C
ATOM	2802	CE1	PHE	A	351	7.258	5.906	31.285	1.00	15.30	B	C
ATOM	2803	CZ	PHE	A	351	7.052	5.666	29.963	1.00	16.09	B	C
ATOM	2804	CD2	PHE	A	351	5.472	7.460	29.938	1.00	17.52	B	C
ATOM	2805	CE2	PHE	A	351	6.131	6.427	29.297	1.00	16.90	B	C
ATOM	2806	C	PHE	A	351	3.942	7.599	33.913	1.00	17.58	B	C
ATOM	2807	O	PHE	A	351	4.360	8.249	34.851	1.00	17.55	B	O
ATOM	2808	N	VAL	A	352	3.709	6.270	33.976	1.00	16.95	B	N
ATOM	2809	CA	VAL	A	352	3.883	5.469	35.214	1.00	17.51	B	C
ATOM	2810	CB	VAL	A	352	2.626	4.709	35.571	1.00	16.94	B	C
ATOM	2811	CG1	VAL	A	352	2.950	3.736	36.723	1.00	19.47	B	C
ATOM	2812	CG2	VAL	A	352	1.529	5.702	35.976	1.00	19.87	B	C
ATOM	2813	C	VAL	A	352	5.098	4.574	35.021	1.00	17.01	B	C
ATOM	2814	O	VAL	A	352	5.231	3.953	33.957	1.00	17.85	B	O
ATOM	2815	N	TYR	A	353	6.007	4.539	35.963	1.00	16.30	B	N
ATOM	2816	CA	TYR	A	353	7.301	3.878	35.765	1.00	17.33	B	C
ATOM	2817	CB	TYR	A	353	8.208	4.793	34.953	1.00	17.49	B	C
ATOM	2818	CG	TYR	A	353	8.676	6.004	35.695	1.00	16.35	B	C
ATOM	2819	CD1	TYR	A	353	7.836	7.074	35.902	1.00	16.74	B	C
ATOM	2820	CE1	TYR	A	353	8.228	8.158	36.676	1.00	18.24	B	C
ATOM	2821	CZ	TYR	A	353	9.487	8.210	37.220	1.00	17.25	B	C
ATOM	2822	OH	TYR	A	353	9.888	9.283	37.991	1.00	19.82	B	O
ATOM	2823	CE2	TYR	A	353	10.357	7.146	37.028	1.00	17.63	B	C
ATOM	2824	CD2	TYR	A	353	9.930	6.083	36.244	1.00	16.28	B	C
ATOM	2825	C	TYR	A	353	7.945	3.538	37.100	1.00	17.72	B	C
ATOM	2826	O	TYR	A	353	7.444	4.019	38.172	1.00	18.14	B	O
ATOM	2827	N	GLN	A	354	9.009	2.759	37.039	1.00	16.58	B	N
ATOM	2828	CA	GLN	A	354	9.903	2.568	38.178	1.00	16.41	B	C
ATOM	2829	CB	GLN	A	354	10.144	1.056	38.315	1.00	17.64	B	C
ATOM	2830	CG	GLN	A	354	8.898	0.327	38.802	1.00	17.79	B	C
ATOM	2831	CD	GLN	A	354	8.721	-1.036	38.165	1.00	18.24	B	C
ATOM	2832	OE1	GLN	A	354	8.740	-1.153	36.920	1.00	19.69	B	O
ATOM	2833	NE2	GLN	A	354	8.371	-2.018	39.002	1.00	19.45	B	N
ATOM	2834	C	GLN	A	354	11.187	3.312	37.965	1.00	15.26	B	C
ATOM	2835	O	GLN	A	354	11.792	3.298	36.899	1.00	16.12	B	O
ATOM	2836	N	THR	A	355	11.677	3.928	39.041	1.00	17.12	B	N
ATOM	2837	CA	THR	A	355	13.009	4.508	39.027	1.00	16.94	B	C
ATOM	2838	CB	THR	A	355	13.351	5.253	40.325	1.00	18.61	B	C
ATOM	2839	OG1	THR	A	355	13.448	4.284	41.380	1.00	20.07	B	O
ATOM	2840	CG2	THR	A	355	12.377	6.354	40.656	1.00	18.83	B	C

Fig. 5 (cont.)

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ATOM	2841	C	THR	A	355	14.058	3.415	38.836	1.00	16.20	B	C
ATOM	2842	O	THR	A	355	13.787	2.192	39.110	1.00	18.27	B	O
ATOM	2843	N	LEU	A	356	15.194	3.773	38.324	1.00	17.17	B	N
ATOM	2844	CA	LEU	A	356	16.239	2.783	38.037	1.00	19.22	B	C
ATOM	2845	CB	LEU	A	356	17.427	3.465	37.374	1.00	18.37	B	C
ATOM	2846	CG	LEU	A	356	18.530	2.545	36.871	1.00	19.21	B	C
ATOM	2847	CD1	LEU	A	356	17.991	1.443	35.971	1.00	19.58	B	C
ATOM	2848	CD2	LEU	A	356	19.612	3.337	36.167	1.00	20.34	B	C
ATOM	2849	C	LEU	A	356	16.630	2.089	39.348	1.00	20.96	B	C
ATOM	2850	O	LEU	A	356	16.839	0.874	39.346	1.00	19.94	B	O
ATOM	2851	N	ARG	A	357	16.700	2.831	40.433	1.00	20.92	B	N
ATOM	2852	CA	ARG	A	357	17.001	2.211	41.744	1.00	21.97	B	C
ATOM	2853	CB	ARG	A	357	17.168	3.352	42.747	1.00	27.09	B	C
ATOM	2854	CG	ARG	A	357	17.467	2.825	44.144	1.00	32.94	B	C
ATOM	2855	CD	ARG	A	357	18.956	2.727	44.340	1.00	42.99	B	C
ATOM	2856	NE	ARG	A	357	19.463	1.368	44.267	1.00	55.08	B	N
ATOM	2857	CZ	ARG	A	357	20.599	1.007	43.676	1.00	56.61	B	C
ATOM	2858	NH1	ARG	A	357	21.345	1.904	43.054	1.00	54.58	B	N
ATOM	2859	NH2	ARG	A	357	20.971	-0.262	43.701	1.00	59.97	B	N
ATOM	2860	C	ARG	A	357	15.886	1.278	42.192	1.00	22.41	B	C
ATOM	2861	O	ARG	A	357	16.230	0.186	42.732	1.00	22.44	B	O
ATOM	2862	N	ASP	A	358	14.608	1.584	41.997	1.00	20.61	B	N
ATOM	2863	CA	ASP	A	358	13.493	0.694	42.362	1.00	20.74	B	C
ATOM	2864	CB	ASP	A	358	12.160	1.423	42.287	1.00	22.91	B	C
ATOM	2865	CG	ASP	A	358	11.888	2.298	43.525	1.00	24.26	B	C
ATOM	2866	OD1	ASP	A	358	12.745	2.272	44.483	1.00	26.29	B	O
ATOM	2867	OD2	ASP	A	358	10.803	2.878	43.566	1.00	27.45	B	O
ATOM	2868	C	ASP	A	358	13.547	-0.579	41.506	1.00	21.63	B	C
ATOM	2869	O	ASP	A	358	12.960	-1.626	41.931	1.00	22.40	B	O
ATOM	2870	N	MET	A	359	14.052	-0.487	40.266	1.00	18.94	B	N
ATOM	2871	CA	MET	A	359	14.201	-1.720	39.468	1.00	19.32	B	C
ATOM	2872	CB	MET	A	359	14.518	-1.326	38.020	1.00	20.32	B	C
ATOM	2873	CG	MET	A	359	13.362	-0.672	37.302	1.00	19.98	B	C
ATOM	2874	SD	MET	A	359	13.850	-0.119	35.625	1.00	20.05	B	S
ATOM	2875	CE	MET	A	359	12.292	0.450	34.993	1.00	21.34	B	C
ATOM	2876	C	MET	A	359	15.330	-2.546	40.068	1.00	20.98	B	C
ATOM	2877	O	MET	A	359	15.133	-3.758	40.277	1.00	21.04	B	O
ATOM	2878	N	ARG	A	360	16.477	-1.962	40.294	1.00	19.55	B	N
ATOM	2879	CA	ARG	A	360	17.682	-2.696	40.735	1.00	21.52	B	C
ATOM	2880	CB	ARG	A	360	18.855	-1.734	40.843	1.00	21.48	B	C
ATOM	2881	CG	ARG	A	360	20.098	-2.488	41.297	1.00	25.76	B	C
ATOM	2882	CD	ARG	A	360	21.312	-1.777	40.874	1.00	26.28	B	C
ATOM	2883	NE	ARG	A	360	22.541	-2.483	41.233	1.00	31.25	B	N
ATOM	2884	CZ	ARG	A	360	23.180	-3.396	40.479	1.00	27.91	B	C
ATOM	2885	NH1	ARG	A	360	22.807	-3.675	39.249	1.00	25.80	B	N
ATOM	2886	NH2	ARG	A	360	24.292	-3.942	40.927	1.00	33.13	B	N
ATOM	2887	C	ARG	A	360	17.336	-3.346	42.091	1.00	23.50	B	C
ATOM	2888	O	ARG	A	360	17.694	-4.553	42.307	1.00	24.88	B	O
ATOM	2889	N	ASP	A	361	16.653	-2.637	42.971	1.00	23.23	B	N
ATOM	2890	CA	ASP	A	361	16.285	-3.130	44.329	1.00	25.02	B	C
ATOM	2891	CB	ASP	A	361	16.085	-1.940	45.265	1.00	28.72	B	C
ATOM	2892	CG	ASP	A	361	17.321	-1.127	45.562	1.00	33.07	B	C
ATOM	2893	OD1	ASP	A	361	18.456	-1.607	45.312	1.00	35.24	B	O
ATOM	2894	OD2	ASP	A	361	17.128	0.023	46.071	1.00	41.97	B	O
ATOM	2895	C	ASP	A	361	15.034	-4.029	44.313	1.00	30.46	B	C
ATOM	2896	O	ASP	A	361	14.701	-4.542	45.382	1.00	29.89	B	O
ATOM	2897	N	ILE	A	362	14.302	-4.228	43.197	1.00	27.58	B	N
ATOM	2898	CA	ILE	A	362	12.953	-4.903	43.126	1.00	27.50	B	C

Fig. 5 (cont.)

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ATOM	2899	CB	ILE	A	362	12.991	-6.461	43.053	1.00	27.48	B	C
ATOM	2900	CG1	ILE	A	362	14.170	-6.848	42.184	1.00	30.29	B	C
ATOM	2901	CG2	ILE	A	362	11.700	-7.041	42.490	1.00	30.49	B	C
ATOM	2902	CD1	ILE	A	362	13.905	-6.745	40.702	1.00	34.10	B	C
ATOM	2903	C	ILE	A	362	12.081	-4.403	44.271	1.00	26.27	B	C
ATOM	2904	O	ILE	A	362	11.523	-5.184	45.043	1.00	28.51	B	O
ATOM	2905	N	GLN	A	363	11.847	-3.094	44.291	1.00	25.69	B	N
ATOM	2906	CA	GLN	A	363	11.112	-2.427	45.378	1.00	28.87	B	C
ATOM	2907	CB	GLN	A	363	11.378	-0.936	45.307	1.00	30.35	B	C
ATOM	2908	CG	GLN	A	363	10.393	-0.124	46.124	1.00	33.00	B	C
ATOM	2909	CD	GLN	A	363	10.647	-0.345	47.591	1.00	37.15	B	C
ATOM	2910	OE1	GLN	A	363	11.774	-0.226	48.059	1.00	35.56	B	O
ATOM	2911	NE2	GLN	A	363	9.594	-0.687	48.314	1.00	35.70	B	N
ATOM	2912	C	GLN	A	363	9.628	-2.716	45.214	1.00	29.46	B	C
ATOM	2913	O	GLN	A	363	9.028	-2.320	44.202	1.00	26.09	B	O
ATOM	2914	N	PRO	A	364	8.946	-3.335	46.218	1.00	30.38	B	N
ATOM	2915	CA	PRO	A	364	7.503	-3.539	46.176	1.00	30.50	B	C
ATOM	2916	CB	PRO	A	364	7.179	-4.238	47.515	1.00	33.81	B	C
ATOM	2917	CG	PRO	A	364	8.476	-4.851	47.929	1.00	38.54	B	C
ATOM	2918	CD	PRO	A	364	9.535	-3.881	47.459	1.00	35.40	B	C
ATOM	2919	C	PRO	A	364	6.792	-2.188	46.073	1.00	31.56	B	C
ATOM	2920	O	PRO	A	364	7.233	-1.196	46.676	1.00	29.88	B	O
ATOM	2921	N	LEU	A	365	5.696	-2.159	45.302	1.00	31.14	B	N
ATOM	2922	CA	LEU	A	365	4.894	-0.938	45.071	1.00	31.26	B	C
ATOM	2923	CB	LEU	A	365	4.785	-0.746	43.549	1.00	30.26	B	C
ATOM	2924	CG	LEU	A	365	6.141	-0.668	42.851	1.00	29.51	B	C
ATOM	2925	CD1	LEU	A	365	5.974	-0.535	41.334	1.00	26.40	B	C
ATOM	2926	CD2	LEU	A	365	6.958	0.500	43.367	1.00	27.73	B	C
ATOM	2927	C	LEU	A	365	3.527	-1.075	45.744	1.00	31.92	B	C
ATOM	2928	O	LEU	A	365	2.942	-2.161	45.688	1.00	36.85	B	O
ATOM	2929	N	SER	A	366	3.040	0.009	46.331	1.00	32.82	B	N
ATOM	2930	CA	SER	A	366	1.762	0.074	47.078	1.00	34.03	B	C
ATOM	2931	CB	SER	A	366	2.043	-0.297	48.523	1.00	36.45	B	C
ATOM	2932	OG	SER	A	366	2.813	0.717	49.136	1.00	40.16	B	O
ATOM	2933	C	SER	A	366	1.210	1.494	46.943	1.00	35.67	B	C
ATOM	2934	O	SER	A	366	1.930	2.359	46.361	1.00	36.28	B	O
ATOM	2935	N	LEU	A	367	0.028	1.798	47.488	1.00	35.52	B	N
ATOM	2936	CA	LEU	A	367	-0.463	3.209	47.443	1.00	41.28	B	C
ATOM	2937	CB	LEU	A	367	-1.940	3.303	47.844	1.00	40.37	B	C
ATOM	2938	CG	LEU	A	367	-2.902	2.439	47.030	1.00	43.19	B	C
ATOM	2939	CD1	LEU	A	367	-4.292	2.481	47.642	1.00	43.08	B	C
ATOM	2940	CD2	LEU	A	367	-2.942	2.873	45.571	1.00	42.86	B	C
ATOM	2941	C	LEU	A	367	0.445	4.073	48.342	1.00	44.36	B	C
ATOM	2942	O	LEU	A	367	0.586	5.268	48.025	1.00	43.86	B	O
ATOM	2943	N	GLU	A	368	1.059	3.468	49.369	1.00	49.44	B	N
ATOM	2944	CA	GLU	A	368	2.013	4.098	50.332	1.00	58.52	B	C
ATOM	2945	CB	GLU	A	368	2.208	3.203	51.566	1.00	66.96	B	C
ATOM	2946	CG	GLU	A	368	0.930	2.897	52.332	1.00	73.32	B	C
ATOM	2947	CD	GLU	A	368	0.022	4.092	52.564	1.00	85.37	B	C
ATOM	2948	OE1	GLU	A	368	0.542	5.173	52.932	1.00	92.25	B	O
ATOM	2949	OE2	GLU	A	368	-1.204	3.943	52.356	1.00	93.13	B	O
ATOM	2950	C	GLU	A	368	3.387	4.340	49.687	1.00	55.00	B	C
ATOM	2951	O	GLU	A	368	3.997	5.383	49.980	1.00	50.38	B	O
ATOM	2952	N	LYS	A	369	3.881	3.384	48.894	1.00	52.24	B	N
ATOM	2953	CA	LYS	A	369	5.191	3.445	48.191	1.00	52.07	B	C
ATOM	2954	CB	LYS	A	369	6.123	2.338	48.703	1.00	62.62	B	C
ATOM	2955	CG	LYS	A	369	5.914	1.973	50.170	1.00	76.15	B	C
ATOM	2956	CD	LYS	A	369	6.977	1.078	50.784	1.00	80.58	B	C

Fig. 5 (cont.)

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ATOM	2957	CE	LYS	A	369	6.454	0.314	51.987	1.00	81.43	B	C
ATOM	2958	NZ	LYS	A	369	7.553	-0.171	52.856	1.00	84.93	B	N
ATOM	2959	C	LYS	A	369	4.894	3.309	46.698	1.00	37.41	B	C
ATOM	2960	O	LYS	A	369	5.070	2.234	46.137	1.00	35.00	B	O
ATOM	2961	N	PRO	A	370	4.364	4.381	46.058	1.00	38.27	B	N
ATOM	2962	CA	PRO	A	370	3.823	4.270	44.706	1.00	31.85	B	C
ATOM	2963	CB	PRO	A	370	2.993	5.545	44.527	1.00	33.39	B	C
ATOM	2964	CG	PRO	A	370	3.712	6.554	45.421	1.00	38.62	B	C
ATOM	2965	CD	PRO	A	370	4.223	5.746	46.596	1.00	39.11	B	C
ATOM	2966	C	PRO	A	370	4.909	4.136	43.637	1.00	29.73	B	C
ATOM	2967	O	PRO	A	370	6.048	4.503	43.844	1.00	30.98	B	O
ATOM	2968	N	ALA	A	371	4.491	3.589	42.501	1.00	26.08	B	N
ATOM	2969	CA	ALA	A	371	5.305	3.724	41.277	1.00	24.14	B	C
ATOM	2970	CB	ALA	A	371	4.557	3.168	40.139	1.00	24.59	B	C
ATOM	2971	C	ALA	A	371	5.595	5.215	41.080	1.00	23.68	B	C
ATOM	2972	O	ALA	A	371	4.809	6.106	41.522	1.00	24.23	B	O
ATOM	2973	N	GLY	A	372	6.604	5.498	40.286	1.00	20.97	B	N
ATOM	2974	CA	GLY	A	372	6.780	6.869	39.792	1.00	21.28	B	C
ATOM	2975	C	GLY	A	372	5.654	7.243	38.861	1.00	21.18	B	C
ATOM	2976	O	GLY	A	372	5.073	6.404	38.139	1.00	18.75	B	O
ATOM	2977	N	LYS	A	373	5.274	8.523	38.836	1.00	22.93	B	N
ATOM	2978	CA	LYS	A	373	4.161	8.956	37.994	1.00	21.09	B	C
ATOM	2979	CB	LYS	A	373	2.841	8.838	38.714	1.00	24.90	B	C
ATOM	2980	CG	LYS	A	373	1.651	9.225	37.867	1.00	27.08	B	C
ATOM	2981	CD	LYS	A	373	0.384	8.751	38.488	1.00	30.00	B	C
ATOM	2982	CE	LYS	A	373	-0.864	9.301	37.880	1.00	37.51	B	C
ATOM	2983	NZ	LYS	A	373	-1.998	8.956	38.765	1.00	37.26	B	N
ATOM	2984	C	LYS	A	373	4.429	10.407	37.607	1.00	24.18	B	C
ATOM	2985	O	LYS	A	373	4.777	11.242	38.524	1.00	29.33	B	O
ATOM	2986	N	VAL	A	374	4.453	10.669	36.326	1.00	20.30	B	N
ATOM	2987	CA	VAL	A	374	4.658	12.056	35.827	1.00	19.77	B	C
ATOM	2988	CB	VAL	A	374	5.880	12.103	34.889	1.00	21.41	B	C
ATOM	2989	CG1	VAL	A	374	5.953	13.442	34.150	1.00	24.13	B	C
ATOM	2990	CG2	VAL	A	374	7.160	11.824	35.619	1.00	22.46	B	C
ATOM	2991	C	VAL	A	374	3.369	12.434	35.119	1.00	22.53	B	C
ATOM	2992	O	VAL	A	374	2.885	11.771	34.200	1.00	20.93	B	O
ATOM	2993	N	ASP	A	375	2.844	13.614	35.492	1.00	22.77	B	N
ATOM	2994	CA	ASP	A	375	1.652	14.167	34.830	1.00	25.10	B	C
ATOM	2995	CB	ASP	A	375	1.109	15.341	35.682	1.00	31.58	B	C
ATOM	2996	CG	ASP	A	375	-0.383	15.694	35.607	1.00	47.73	B	C
ATOM	2997	OD1	ASP	A	375	-0.937	15.894	34.494	1.00	43.48	B	O
ATOM	2998	OD2	ASP	A	375	-1.001	15.814	36.703	1.00	69.61	B	O
ATOM	2999	C	ASP	A	375	2.061	14.702	33.454	1.00	23.05	B	C
ATOM	3000	O	ASP	A	375	3.101	15.378	33.375	1.00	26.54	B	O
ATOM	3001	N	ALEU	A	376	1.370	14.316	32.428	0.50	20.82	B	N
ATOM	3002	N	BLEU	A	376	1.331	14.377	32.379	0.50	20.28	B	N
ATOM	3003	CA	ALEU	A	376	1.661	14.819	31.091	0.50	20.38	B	C
ATOM	3004	CA	BLEU	A	376	1.631	14.682	30.943	0.50	19.85	B	C
ATOM	3005	CB	ALEU	A	376	1.727	13.663	30.102	0.50	19.55	B	C
ATOM	3006	CB	BLEU	A	376	1.716	13.419	30.083	0.50	20.59	B	C
ATOM	3007	CG	ALEU	A	376	2.751	12.586	30.476	0.50	19.32	B	C
ATOM	3008	CG	BLEU	A	376	2.846	12.429	30.373	0.50	20.12	B	C
ATOM	3009	CD1	ALEU	A	376	2.816	11.492	29.435	0.50	19.57	B	C
ATOM	3010	CD1	BLEU	A	376	2.835	11.274	29.388	0.50	21.20	B	C
ATOM	3011	CD2	ALEU	A	376	4.126	13.193	30.711	0.50	19.11	B	C
ATOM	3012	CD2	BLEU	A	376	4.208	13.099	30.368	0.50	20.77	B	C
ATOM	3013	C	ALEU	A	376	0.552	15.818	30.783	0.50	25.37	B	C
ATOM	3014	C	BLEU	A	376	0.529	15.567	30.318	0.50	21.85	B	C

Fig. 5 (cont.)

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ATOM	3015	O	ALEU	A	376	-0.494	15.760	31.442	0.50	22.62	B	O
ATOM	3016	O	BLEU	A	376	-0.592	15.110	30.154	0.50	20.64	B	O
ATOM	3017	N	LYS	A	377	0.881	16.793	29.938	1.00	25.17	B	N
ATOM	3018	CA	LYS	A	377	-0.125	17.704	29.357	1.00	25.69	B	C
ATOM	3019	CB	LYS	A	377	0.192	19.166	29.651	1.00	29.60	B	C
ATOM	3020	CG	LYS	A	377	0.434	19.501	31.100	1.00	33.80	B	C
ATOM	3021	CD	LYS	A	377	-0.766	19.365	31.958	1.00	37.01	B	C
ATOM	3022	CE	LYS	A	377	-0.393	19.796	33.355	1.00	41.84	B	C
ATOM	3023	NZ	LYS	A	377	-1.256	19.125	34.344	1.00	46.68	B	N
ATOM	3024	C	LYS	A	377	-0.105	17.532	27.861	1.00	24.72	B	C
ATOM	3025	O	LYS	A	377	0.967	17.565	27.187	1.00	25.32	B	O
ATOM	3026	N	LEU	A	378	-1.278	17.395	27.304	1.00	26.82	B	N
ATOM	3027	CA	LEU	A	378	-1.486	17.463	25.860	1.00	28.00	B	C
ATOM	3028	CB	LEU	A	378	-2.656	16.550	25.482	1.00	31.83	B	C
ATOM	3029	CG	LEU	A	378	-2.269	15.189	24.943	1.00	34.63	B	C
ATOM	3030	CD1	LEU	A	378	-3.451	14.393	24.449	1.00	36.32	B	C
ATOM	3031	CD2	LEU	A	378	-1.278	15.351	23.838	1.00	42.30	B	C
ATOM	3032	C	LEU	A	378	-1.804	18.914	25.518	1.00	32.07	B	C
ATOM	3033	O	LEU	A	378	-2.993	19.254	25.596	1.00	30.90	B	O
ATOM	3034	N	ILE	A	379	-0.806	19.676	25.053	1.00	37.84	B	N
ATOM	3035	CA	ILE	A	379	-0.968	21.119	24.691	1.00	42.67	B	C
ATOM	3036	CB	ILE	A	379	0.374	21.859	24.479	1.00	51.82	B	C
ATOM	3037	CG1	ILE	A	379	1.366	21.659	25.628	1.00	55.73	B	C
ATOM	3038	CG2	ILE	A	379	0.118	23.346	24.270	1.00	52.33	B	C
ATOM	3039	CD1	ILE	A	379	2.817	21.905	25.242	1.00	57.06	B	C
ATOM	3040	C	ILE	A	379	-1.874	21.260	23.461	1.00	37.18	B	C
ATOM	3041	O	ILE	A	379	-2.583	22.258	23.413	1.00	42.18	B	O
ATOM	3042	N	ALA	A	380	-1.926	20.330	22.503	1.00	31.98	B	N
ATOM	3043	CA	ALA	A	380	-2.586	20.524	21.196	1.00	35.89	B	C
ATOM	3044	CB	ALA	A	380	-2.021	19.616	20.117	1.00	42.28	B	C
ATOM	3045	C	ALA	A	380	-4.104	20.369	21.242	1.00	33.84	B	C
ATOM	3046	O	ALA	A	380	-4.742	20.650	20.238	1.00	39.21	B	O
ATOM	3047	N	ACYS	A	381	-4.728	19.796	22.268	0.50	34.04	B	N
ATOM	3048	N	BCYS	A	381	-4.646	19.991	22.407	0.50	34.87	B	N
ATOM	3049	CA	ACYS	A	381	-6.205	19.677	22.182	0.50	30.59	B	C
ATOM	3050	CA	BCYS	A	381	-6.102	19.800	22.626	0.50	31.64	B	C
ATOM	3051	CB	ACYS	A	381	-6.808	18.610	23.081	0.50	27.32	B	C
ATOM	3052	CB	BCYS	A	381	-6.336	19.061	23.937	0.50	26.77	B	C
ATOM	3053	SG	ACYS	A	381	-6.433	18.967	24.801	0.50	26.92	B	S
ATOM	3054	SG	BCYS	A	381	-6.226	17.264	23.780	0.50	36.50	B	S
ATOM	3055	C	ACYS	A	381	-6.802	21.056	22.495	0.50	29.03	B	C
ATOM	3056	C	BCYS	A	381	-6.820	21.159	22.601	0.50	28.09	B	C
ATOM	3057	O	ACYS	A	381	-6.221	21.806	23.302	0.50	33.35	B	O
ATOM	3058	O	BCYS	A	381	-6.314	22.050	23.296	0.50	35.54	B	O
ATOM	3059	N	GLU	A	382	-7.949	21.266	21.875	1.00	31.70	B	N
ATOM	3060	CA	GLU	A	382	-8.687	22.562	21.763	1.00	33.50	B	C
ATOM	3061	CB	GLU	A	382	-9.665	22.458	20.594	1.00	39.93	B	C
ATOM	3062	CG	GLU	A	382	-8.925	22.281	19.265	1.00	49.34	B	C
ATOM	3063	CD	GLU	A	382	-9.495	22.928	18.016	1.00	57.32	B	C
ATOM	3064	OE1	GLU	A	382	-8.713	23.124	17.052	1.00	62.20	B	O
ATOM	3065	OE2	GLU	A	382	-10.717	23.196	17.987	1.00	65.17	B	O
ATOM	3066	C	GLU	A	382	-9.361	22.921	23.095	1.00	38.49	B	C
ATOM	3067	O	GLU	A	382	-9.341	24.104	23.430	1.00	40.86	B	O
ATOM	3068	N	GLU	A	383	-9.846	21.951	23.872	1.00	28.98	B	N
ATOM	3069	CA	GLU	A	383	-10.672	22.200	25.084	1.00	29.31	B	C
ATOM	3070	CB	GLU	A	383	-12.079	21.615	24.901	1.00	32.00	B	C
ATOM	3071	CG	GLU	A	383	-12.839	21.986	23.607	1.00	37.11	B	C
ATOM	3072	CD	GLU	A	383	-14.109	21.200	23.226	1.00	41.13	B	C

Fig. 5 (cont.)

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ATOM	3073	OE1	GLU	A	383	-14.324	20.040	23.712	1.00	36.48	B	O
ATOM	3074	OE2	GLU	A	383	-14.939	21.740	22.412	1.00	39.25	B	O
ATOM	3075	C	GLU	A	383	-9.967	21.515	26.257	1.00	28.95	B	C
ATOM	3076	O	GLU	A	383	-9.767	20.293	26.195	1.00	28.22	B	O
ATOM	3077	N	LYS	A	384	-9.520	22.234	27.258	1.00	26.94	B	N
ATOM	3078	CA	LYS	A	384	-8.861	21.666	28.452	1.00	27.38	B	C
ATOM	3079	CB	LYS	A	384	-7.401	22.098	28.593	1.00	31.54	B	C
ATOM	3080	CG	LYS	A	384	-6.492	21.617	27.479	1.00	37.81	B	C
ATOM	3081	CD	LYS	A	384	-5.482	22.670	27.112	1.00	42.63	B	C
ATOM	3082	CE	LYS	A	384	-4.326	22.152	26.300	1.00	45.00	B	C
ATOM	3083	NZ	LYS	A	384	-3.737	23.286	25.566	1.00	48.49	B	N
ATOM	3084	C	LYS	A	384	-9.639	22.085	29.683	1.00	29.15	B	C
ATOM	3085	O	LYS	A	384	-10.221	23.189	29.668	1.00	31.92	B	O
ATOM	3086	N	ASN	A	385	-9.575	21.303	30.749	1.00	28.65	B	N
ATOM	3087	CA	ASN	A	385	-10.120	21.725	32.062	1.00	26.25	B	C
ATOM	3088	CB	ASN	A	385	-10.863	20.583	32.743	1.00	25.02	B	C
ATOM	3089	CG	ASN	A	385	-9.963	19.470	33.234	1.00	26.15	B	C
ATOM	3090	OD1	ASN	A	385	-8.724	19.569	33.296	1.00	25.85	B	O
ATOM	3091	ND2	ASN	A	385	-10.630	18.385	33.611	1.00	28.30	B	N
ATOM	3092	C	ASN	A	385	-9.008	22.375	32.881	1.00	25.18	B	C
ATOM	3093	O	ASN	A	385	-7.875	22.522	32.387	1.00	25.81	B	O
ATOM	3094	N	SER	A	386	-9.278	22.713	34.136	1.00	26.74	B	N
ATOM	3095	CA	SER	A	386	-8.352	23.451	35.013	1.00	29.85	B	C
ATOM	3096	CB	SER	A	386	-9.065	23.921	36.256	1.00	33.18	B	C
ATOM	3097	OG	SER	A	386	-8.970	22.945	37.285	1.00	37.55	B	O
ATOM	3098	C	SER	A	386	-7.141	22.588	35.353	1.00	32.17	B	C
ATOM	3099	O	SER	A	386	-6.065	23.141	35.566	1.00	29.39	B	O
ATOM	3100	N	GLN	A	387	-7.289	21.246	35.323	1.00	30.09	B	N
ATOM	3101	CA	GLN	A	387	-6.155	20.321	35.591	1.00	29.72	B	C
ATOM	3102	CB	GLN	A	387	-6.678	19.047	36.241	1.00	35.37	B	C
ATOM	3103	CG	GLN	A	387	-7.339	19.312	37.585	1.00	40.24	B	C
ATOM	3104	CD	GLN	A	387	-8.195	18.136	37.967	1.00	47.85	B	C
ATOM	3105	OE1	GLN	A	387	-9.386	18.078	37.651	1.00	51.93	B	O
ATOM	3106	NE2	GLN	A	387	-7.556	17.151	38.577	1.00	59.07	B	N
ATOM	3107	C	GLN	A	387	-5.391	19.996	34.312	1.00	26.98	B	C
ATOM	3108	O	GLN	A	387	-4.451	19.213	34.394	1.00	30.15	B	O
ATOM	3109	N	GLY	A	388	-5.749	20.581	33.176	1.00	23.30	B	N
ATOM	3110	CA	GLY	A	388	-5.052	20.402	31.897	1.00	26.00	B	C
ATOM	3111	C	GLY	A	388	-5.442	19.123	31.215	1.00	24.59	B	C
ATOM	3112	O	GLY	A	388	-4.841	18.790	30.200	1.00	24.74	B	O
ATOM	3113	N	MET	A	389	-6.521	18.478	31.662	1.00	23.36	B	N
ATOM	3114	CA	MET	A	389	-7.062	17.325	30.936	1.00	21.73	B	C
ATOM	3115	CB	MET	A	389	-8.110	16.614	31.785	1.00	22.73	B	C
ATOM	3116	CG	MET	A	389	-7.645	16.164	33.082	1.00	22.83	B	C
ATOM	3117	SD	MET	A	389	-8.984	15.133	33.869	1.00	26.03	B	S
ATOM	3118	CE	MET	A	389	-8.331	14.880	35.513	1.00	29.93	B	C
ATOM	3119	C	MET	A	389	-7.773	17.821	29.680	1.00	24.40	B	C
ATOM	3120	O	MET	A	389	-8.476	18.863	29.715	1.00	24.18	B	O
ATOM	3121	N	ACYS	A	390	-7.590	17.154	28.554	0.50	22.26	B	N
ATOM	3122	N	BCYS	A	390	-7.630	17.095	28.617	0.50	24.28	B	N
ATOM	3123	CA	ACYS	A	390	-8.344	17.403	27.308	0.50	21.52	B	C
ATOM	3124	CA	BCYS	A	390	-8.305	17.323	27.344	0.50	24.37	B	C
ATOM	3125	CB	ACYS	A	390	-7.805	16.651	26.102	0.50	23.03	B	C
ATOM	3126	CB	BCYS	A	390	-7.539	16.469	26.367	0.50	30.04	B	C
ATOM	3127	SG	ACYS	A	390	-6.109	17.085	25.637	0.50	23.88	B	S
ATOM	3128	SG	BCYS	A	390	-7.999	16.846	24.677	0.50	35.42	B	S
ATOM	3129	C	ACYS	A	390	-9.800	16.947	27.446	0.50	20.91	B	C
ATOM	3130	C	BCYS	A	390	-9.789	16.911	27.409	0.50	22.63	B	C

Fig. 5 (cont.)

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ATOM	3131	O	ACYS	A	390	-10.061	15.839	27.944	0.50	21.45	B	O
ATOM	3132	O	BCYS	A	390	-10.069	15.809	27.895	0.50	22.57	B	O
ATOM	3133	N	SER	A	391	-10.754	17.686	26.884	1.00	22.68	B	N
ATOM	3134	CA	SER	A	391	-12.124	17.156	26.749	1.00	22.53	B	C
ATOM	3135	CB	SER	A	391	-13.093	18.152	26.120	1.00	24.35	B	C
ATOM	3136	OG	SER	A	391	-12.849	18.328	24.714	1.00	27.41	B	O
ATOM	3137	C	SER	A	391	-12.088	15.871	25.912	1.00	22.93	B	C
ATOM	3138	O	SER	A	391	-11.286	15.764	24.963	1.00	22.12	B	O
ATOM	3139	N	LEU	A	392	-12.943	14.886	26.204	1.00	23.43	B	N
ATOM	3140	CA	LEU	A	392	-12.940	13.628	25.450	1.00	23.80	B	C
ATOM	3141	CB	LEU	A	392	-14.020	12.696	26.002	1.00	26.58	B	C
ATOM	3142	CG	LEU	A	392	-14.075	11.314	25.338	1.00	28.13	B	C
ATOM	3143	CD1	LEU	A	392	-12.824	10.510	25.669	1.00	30.97	B	C
ATOM	3144	CD2	LEU	A	392	-15.357	10.555	25.697	1.00	31.67	B	C
ATOM	3145	C	LEU	A	392	-13.171	13.943	23.978	1.00	24.44	B	C
ATOM	3146	O	LEU	A	392	-12.493	13.380	23.090	1.00	26.22	B	O
ATOM	3147	N	LYS	A	393	-14.156	14.790	23.650	1.00	25.61	B	N
ATOM	3148	CA	LYS	A	393	-14.459	15.041	22.224	1.00	27.16	B	C
ATOM	3149	CB	LYS	A	393	-15.802	15.783	22.125	1.00	31.03	B	C
ATOM	3150	CG	LYS	A	393	-17.012	14.958	22.556	1.00	38.60	B	C
ATOM	3151	CD	LYS	A	393	-18.407	15.499	22.152	1.00	47.03	B	C
ATOM	3152	CE	LYS	A	393	-18.552	17.006	22.239	1.00	55.44	B	C
ATOM	3153	NZ	LYS	A	393	-19.741	17.486	21.490	1.00	62.06	B	N
ATOM	3154	C	LYS	A	393	-13.287	15.769	21.558	1.00	24.91	B	C
ATOM	3155	O	LYS	A	393	-13.032	15.421	20.392	1.00	28.59	B	O
ATOM	3156	N	SER	A	394	-12.570	16.680	22.219	1.00	25.06	B	N
ATOM	3157	CA	SER	A	394	-11.424	17.397	21.609	1.00	26.34	B	C
ATOM	3158	CB	SER	A	394	-11.118	18.710	22.198	1.00	31.89	B	C
ATOM	3159	OG	SER	A	394	-10.298	18.632	23.321	1.00	33.52	B	O
ATOM	3160	C	SER	A	394	-10.201	16.474	21.527	1.00	27.20	B	C
ATOM	3161	O	SER	A	394	-9.472	16.569	20.558	1.00	24.28	B	O
ATOM	3162	N	PHE	A	395	-10.035	15.524	22.453	1.00	24.08	B	N
ATOM	3163	CA	PHE	A	395	-8.985	14.477	22.286	1.00	23.08	B	C
ATOM	3164	CB	PHE	A	395	-8.932	13.682	23.573	1.00	22.06	B	C
ATOM	3165	CG	PHE	A	395	-8.005	12.489	23.579	1.00	21.21	B	C
ATOM	3166	CD1	PHE	A	395	-6.669	12.680	23.839	1.00	24.21	B	C
ATOM	3167	CE1	PHE	A	395	-5.811	11.584	23.913	1.00	23.23	B	C
ATOM	3168	CZ	PHE	A	395	-6.303	10.343	23.683	1.00	21.40	B	C
ATOM	3169	CD2	PHE	A	395	-8.478	11.215	23.362	1.00	25.57	B	C
ATOM	3170	CE2	PHE	A	395	-7.616	10.124	23.399	1.00	24.10	B	C
ATOM	3171	C	PHE	A	395	-9.269	13.657	21.026	1.00	22.50	B	C
ATOM	3172	O	PHE	A	395	-8.348	13.383	20.206	1.00	22.37	B	O
ATOM	3173	N	SER	A	396	-10.500	13.244	20.825	1.00	23.85	B	N
ATOM	3174	CA	SER	A	396	-10.915	12.406	19.680	1.00	23.75	B	C
ATOM	3175	CB	SER	A	396	-12.296	11.866	19.861	1.00	26.15	B	C
ATOM	3176	OG	SER	A	396	-12.595	10.947	18.844	1.00	29.23	B	O
ATOM	3177	C	SER	A	396	-10.728	13.216	18.392	1.00	26.49	B	C
ATOM	3178	O	SER	A	396	-10.161	12.699	17.387	1.00	25.78	B	O
ATOM	3179	N	ARG	A	397	-11.074	14.505	18.441	1.00	27.05	B	N
ATOM	3180	CA	ARG	A	397	-10.904	15.351	17.227	1.00	30.25	B	C
ATOM	3181	CB	ARG	A	397	-11.650	16.674	17.403	1.00	36.60	B	C
ATOM	3182	CG	ARG	A	397	-13.170	16.531	17.337	1.00	47.03	B	C
ATOM	3183	CD	ARG	A	397	-13.877	17.875	17.478	1.00	55.08	B	C
ATOM	3184	NE	ARG	A	397	-12.916	18.881	17.042	1.00	60.38	B	N
ATOM	3185	CZ	ARG	A	397	-12.174	19.655	17.837	1.00	61.10	B	C
ATOM	3186	NH1	ARG	A	397	-12.323	19.642	19.153	1.00	65.91	B	N
ATOM	3187	NH2	ARG	A	397	-11.289	20.469	17.292	1.00	58.64	B	N
ATOM	3188	C	ARG	A	397	-9.419	15.508	16.909	1.00	27.57	B	C

Fig. 5 (cont.)

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ATOM	3189	O	ARG	A	397	-9.052	15.440	15.693	1.00	28.12	B	O
ATOM	3190	N	LEU	A	398	-8.565	15.677	17.908	1.00	24.64	B	N
ATOM	3191	CA	LEU	A	398	-7.093	15.756	17.707	1.00	24.12	B	C
ATOM	3192	CB	LEU	A	398	-6.389	15.999	19.041	1.00	26.55	B	C
ATOM	3193	CG	LEU	A	398	-4.871	15.972	19.018	1.00	28.32	B	C
ATOM	3194	CD1	LEU	A	398	-4.313	17.079	18.142	1.00	31.35	B	C
ATOM	3195	CD2	LEU	A	398	-4.283	16.088	20.419	1.00	29.95	B	C
ATOM	3196	C	LEU	A	398	-6.591	14.476	17.010	1.00	24.80	B	C
ATOM	3197	O	LEU	A	398	-5.807	14.554	16.036	1.00	25.02	B	O
ATOM	3198	N	ILE	A	399	-6.951	13.309	17.508	1.00	24.19	B	N
ATOM	3199	CA	ILE	A	399	-6.570	12.031	16.854	1.00	23.73	B	C
ATOM	3200	CB	ILE	A	399	-7.083	10.830	17.668	1.00	24.61	B	C
ATOM	3201	CG1	ILE	A	399	-6.264	10.747	18.955	1.00	24.75	B	C
ATOM	3202	CG2	ILE	A	399	-6.976	9.551	16.867	1.00	24.92	B	C
ATOM	3203	CD1	ILE	A	399	-6.742	9.712	19.975	1.00	26.56	B	C
ATOM	3204	C	ILE	A	399	-7.030	12.048	15.394	1.00	25.30	B	C
ATOM	3205	O	ILE	A	399	-6.237	11.606	14.507	1.00	25.26	B	O
ATOM	3206	N	LYS	A	400	-8.284	12.451	15.137	1.00	27.19	B	N
ATOM	3207	CA	LYS	A	400	-8.816	12.444	13.739	1.00	29.61	B	C
ATOM	3208	CB	LYS	A	400	-10.236	12.999	13.658	1.00	36.36	B	C
ATOM	3209	CG	LYS	A	400	-11.294	12.169	14.339	1.00	46.59	B	C
ATOM	3210	CD	LYS	A	400	-11.456	10.778	13.744	1.00	53.91	B	C
ATOM	3211	CE	LYS	A	400	-11.121	9.700	14.740	1.00	55.39	B	C
ATOM	3212	NZ	LYS	A	400	-11.678	10.037	16.080	1.00	50.62	B	N
ATOM	3213	C	LYS	A	400	-7.930	13.314	12.855	1.00	26.35	B	C
ATOM	3214	O	LYS	A	400	-7.708	12.944	11.675	1.00	28.80	B	O
ATOM	3215	N	GLU	A	401	-7.460	14.430	13.371	1.00	28.47	B	N
ATOM	3216	CA	GLU	A	401	-6.617	15.397	12.623	1.00	31.95	B	C
ATOM	3217	CB	GLU	A	401	-6.614	16.724	13.382	1.00	36.95	B	C
ATOM	3218	CG	GLU	A	401	-5.490	17.682	13.054	1.00	47.91	B	C
ATOM	3219	CD	GLU	A	401	-5.548	18.886	13.990	1.00	58.43	B	C
ATOM	3220	OE1	GLU	A	401	-6.689	19.372	14.260	1.00	62.50	B	O
ATOM	3221	OE2	GLU	A	401	-4.479	19.290	14.515	1.00	61.24	B	O
ATOM	3222	C	GLU	A	401	-5.223	14.816	12.360	1.00	33.03	B	C
ATOM	3223	O	GLU	A	401	-4.709	15.030	11.239	1.00	35.99	B	O
ATOM	3224	N	ILE	A	402	-4.600	14.117	13.317	1.00	27.00	B	N
ATOM	3225	CA	ILE	A	402	-3.148	13.780	13.213	1.00	25.64	B	C
ATOM	3226	CB	ILE	A	402	-2.399	13.941	14.543	1.00	27.41	B	C
ATOM	3227	CG1	ILE	A	402	-2.856	12.877	15.553	1.00	27.91	B	C
ATOM	3228	CG2	ILE	A	402	-2.535	15.367	15.055	1.00	29.82	B	C
ATOM	3229	CD1	ILE	A	402	-2.201	12.957	16.941	1.00	31.33	B	C
ATOM	3230	C	ILE	A	402	-2.948	12.375	12.648	1.00	23.96	B	C
ATOM	3231	O	ILE	A	402	-1.889	12.160	12.125	1.00	25.88	B	O
ATOM	3232	N	ARG	A	403	-3.947	11.513	12.685	1.00	24.21	B	N
ATOM	3233	CA	ARG	A	403	-3.810	10.113	12.209	1.00	23.94	B	C
ATOM	3234	CB	ARG	A	403	-5.040	9.293	12.569	1.00	25.09	B	C
ATOM	3235	CG	ARG	A	403	-6.333	9.736	11.885	1.00	24.99	B	C
ATOM	3236	CD	ARG	A	403	-7.525	8.982	12.390	1.00	26.39	B	C
ATOM	3237	NE	ARG	A	403	-7.485	7.549	12.087	1.00	26.41	B	N
ATOM	3238	CZ	ARG	A	403	-7.939	6.982	10.973	1.00	27.56	B	C
ATOM	3239	NH1	ARG	A	403	-8.450	7.730	9.997	1.00	30.13	B	N
ATOM	3240	NH2	ARG	A	403	-7.893	5.686	10.791	1.00	27.67	B	N
ATOM	3241	C	ARG	A	403	-3.596	10.084	10.693	1.00	25.92	B	C
ATOM	3242	O	ARG	A	403	-3.981	11.050	9.968	1.00	27.01	B	O
ATOM	3243	N	VAL	A	404	-3.018	9.001	10.209	1.00	23.88	B	N
ATOM	3244	CA	VAL	A	404	-2.707	8.827	8.769	1.00	23.27	B	C
ATOM	3245	CB	VAL	A	404	-1.207	8.580	8.602	1.00	24.45	B	C
ATOM	3246	CG1	VAL	A	404	-0.848	8.318	7.155	1.00	24.23	B	C

Fig. 5 (cont.)

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ATOM	3247	CG2	VAL	A	404	-0.385	9.704	9.138	1.00	23.43	B	C
ATOM	3248	C	VAL	A	404	-3.536	7.644	8.310	1.00	26.29	B	C
ATOM	3249	O	VAL	A	404	-3.233	6.507	8.669	1.00	24.13	B	O
ATOM	3250	N	PRO	A	405	-4.639	7.860	7.553	1.00	23.90	B	N
ATOM	3251	CA	PRO	A	405	-5.603	6.817	7.282	1.00	26.07	B	C
ATOM	3252	CB	PRO	A	405	-6.651	7.490	6.359	1.00	28.37	B	C
ATOM	3253	CG	PRO	A	405	-6.366	8.960	6.393	1.00	33.29	B	C
ATOM	3254	CD	PRO	A	405	-5.080	9.204	7.134	1.00	27.65	B	C
ATOM	3255	C	PRO	A	405	-4.989	5.570	6.629	1.00	23.70	B	C
ATOM	3256	O	PRO	A	405	-5.500	4.482	6.859	1.00	27.47	B	O
ATOM	3257	N	GLU	A	406	-3.973	5.760	5.803	1.00	26.12	B	N
ATOM	3258	CA	GLU	A	406	-3.285	4.687	5.042	1.00	26.73	B	C
ATOM	3259	CB	GLU	A	406	-2.259	5.282	4.060	1.00	28.27	B	C
ATOM	3260	CG	GLU	A	406	-2.884	6.183	2.972	1.00	34.74	B	C
ATOM	3261	CD	GLU	A	406	-3.180	7.635	3.346	1.00	38.21	B	C
ATOM	3262	OE1	GLU	A	406	-2.717	8.072	4.370	1.00	33.43	B	O
ATOM	3263	OE2	GLU	A	406	-3.871	8.358	2.554	1.00	42.13	B	O
ATOM	3264	C	GLU	A	406	-2.570	3.740	6.027	1.00	27.19	B	C
ATOM	3265	O	GLU	A	406	-2.281	2.559	5.697	1.00	25.65	B	O
ATOM	3266	N	CYS	A	407	-2.341	4.226	7.254	1.00	23.60	B	N
ATOM	3267	CA	CYS	A	407	-1.591	3.462	8.289	1.00	24.48	B	C
ATOM	3268	CB	CYS	A	407	-0.486	4.331	8.858	1.00	22.76	B	C
ATOM	3269	SG	CYS	A	407	0.786	4.693	7.625	1.00	25.56	B	S
ATOM	3270	C	CYS	A	407	-2.523	2.893	9.337	1.00	23.71	B	C
ATOM	3271	O	CYS	A	407	-2.002	2.331	10.342	1.00	24.80	B	O
ATOM	3272	N	ALA	A	408	-3.836	2.961	9.144	1.00	24.46	B	N
ATOM	3273	CA	ALA	A	408	-4.827	2.266	9.970	1.00	24.35	B	C
ATOM	3274	CB	ALA	A	408	-6.209	2.794	9.731	1.00	26.57	B	C
ATOM	3275	C	ALA	A	408	-4.775	0.769	9.677	1.00	27.52	B	C
ATOM	3276	O	ALA	A	408	-4.509	0.360	8.534	1.00	28.11	B	O
ATOM	3277	N	VAL	A	409	-5.045	-0.031	10.701	1.00	27.60	B	N
ATOM	3278	CA	VAL	A	409	-5.177	-1.504	10.599	1.00	29.06	B	C
ATOM	3279	CB	VAL	A	409	-4.448	-2.198	11.763	1.00	29.87	B	C
ATOM	3280	CG1	VAL	A	409	-2.973	-1.928	11.682	1.00	27.47	B	C
ATOM	3281	CG2	VAL	A	409	-4.978	-1.825	13.131	1.00	31.86	B	C
ATOM	3282	C	VAL	A	409	-6.659	-1.876	10.581	1.00	35.68	B	C
ATOM	3283	O	VAL	A	409	-7.609	-1.091	10.730	1.00	40.47	B	O
ATOM	3284	OXT	VAL	A	409	-6.876	-3.047	10.439	1.00	37.84		O
TER												

Fig. 5 (cont.)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2021/072591

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2021/072591

A. CLASSIFICATION OF SUBJECT MATTER INV. C12N9/16 C12N15/82 A23K10/30 A23K20/147 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C12N C11C A23K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, WPI Data, EMBL		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/117396 A2 (NOVOZYMES AS [DK]; SKOV LARS KOBBEROEE [DK] ET AL.) 29 September 2011 (2011-09-29) cited in the application the whole document table 2 examples 4-7	1-6,8-27
A	WO 2019/228441 A1 (NANJING BESTZYME BIO ENG CO LTD [CN]) 5 December 2019 (2019-12-05) the whole document ----- -/-	1-27
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 26 November 2021		Date of mailing of the international search report 10/12/2021
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Bilang, Jürg

INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TAN HAO ET AL: "Enhancing the Thermal Resistance of a Novel Acidobacteria-Derived Phytase by Engineering of Disulfide Bridges", JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY, vol. 26, no. 10, 28 October 2016 (2016-10-28), pages 1717-1722, XP55866457, ISSN: 1017-7825, DOI: 10.4014/jmb.1604.04051 Retrieved from the Internet: URL:http://dx.doi.org/10.4014/jmb.1604.04051 51> the whole document -----	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2021/072591

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