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(54) Title: METHODS OF ACTIVATION OF SULFATASES AND METHODS AND COMPOSITIONS OF USING THE SAME

(57) Abstract: The present invention is directed to a methods of producing activated sulfatase proteins. Methods and compositions of using such activated compositions in the intervention of a variety of disorders are disclosed.



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**METHODS OF ACTIVATION OF SULFATASES AND METHODS AND
COMPOSITIONS OF USING THE SAME**

BACKGROUND

Field of the Invention

5 The present invention is generally directed to methods and compositions for producing activated enzymes for enzyme replacement therapy in lysosomal storage disease.

Background of the Related Art

10 Enzyme replacement therapy relies on recombinant protein production for the preparation of therapeutically effective protein compositions for the treatment of a variety of disorders. There are at least six lysosomal storage diseases caused by deficiencies in a related family of mammalian sulfatase enzymes. Enzyme replacement therapy has been employed for the treatment of such disorders (Kakkis et al., N Engl J Med 344, 182-8, 2001). Unfortunately, however, a key problem in the
15 manufacture of lysosomal sulfatases is low yield.

The Chinese hamster ovary cell line CHO-K1 is a common cell line used in biopharmaceutical manufacturing and it previously has been employed to establish clones expressing a number of the sulfatases. Despite optimization of the
20 expression vector, careful clonal selection and, in one case, DHFR amplification, cell lines that produce a high yield of the recombinant protein have been particularly elusive and have not been obtained (Bielicki et al., Biochem J 311 (Pt 1), 333-9, 1995; Bielicki et al., Biochem J 289 (Pt 1), 241-6, 1993; Bielicki et al., Biochem J 329 (Pt 1), 145-50, 1998; Litjens et al., Biochem J 327 (Pt 1), 89-94, 1997). Over-expression
25 of arylsulfatase B (ASB) from recombinant retrovirus in deficient human fibroblasts has been reported to occur only at the expense of endogenous sulfatase expression levels (Anson et al., Biochem J 294 (Pt 3), 657-62, 1993). Overexpression of arylsulfatase A (ASA) also causes measurable decreases in endogenous sulfatases (Ohashi et al., Gene Ther 2, 363-8, 1995). Therefore, recombinant production of
30 enzymes is hindered by endogenous limitation on the total sulfatase output of the mammalian cell line being used for such production.

A biosynthetic limitation specific to sulfatases has been described in the literature. All sulfatases share a unique post-translational modification (Rommerskirch et al., Proc. Nat'l Acad. Sci., USA 89, 2561-5, 1992). A single active site cysteine is converted into a hydrated formylglycine (von Figura et al., Bioessays 20, 505-10, 1998). The converted residue is situated within a highly conserved peptide sequence that is required for the activation reaction to occur (Dierks et al., Embo J 18, 2084-91, 1999). Conversion is strictly required for sulfatase activity and occurs shortly after translocation of nascent sulfatase into the ER of eukaryotes (Dierks et al., FEBS Lett 423, 61-5, 1998).

Cysteine conversion to formylglycine proceeds by sequential oxidation and hydrolysis reactions. The oxidation step is enzyme catalyzed, and hence is saturable (Schmidt et al., Cell 82, 271-8, 1995). The hydrolysis step releases H₂S, which is toxic. Since the cell normally expresses sulfatase enzymes at relatively low levels, during recombinant enzyme production where large quantities of the enzyme are produced, the conversion step is overwhelmed by large amounts of recombinant enzyme. Saturation of the sulfatase activation process also is thought to have detrimental effects on enzyme yield and on the health/viability of the overexpressing line.

Saturation of the sulfatase activation pathway in the ER will initially result in a failure to convert the active site cysteine to formylglycine in newly translocated sulfatase. Failure to perform this conversion will result in an additional cysteine within the sulfatase sequence that is not accounted for in the normal folding pathway. The introduction of additional cysteines into proteins leads to misfolding as a result of inappropriate disulfide bonds (Wallis and Cheng, J Immunol 163, 4953-9, 1999; Simon et al., J Biol Chem 277, 5404-10, 2002; Pomponio et al., Hum Genet 99, 506-12, 1997). The degree of misfolding likely depends on the particular sulfatase in question. Of note, the overexpression of iduronate 2-sulfatase (I2S) and N-acetylgalactosamine 6-sulfatase (GALNS) results in secretion of large amounts of folded, unconverted enzyme while the overexpression of ASB does not. In the latter case, the likely accumulation of misfolded sulfatase within the ER could stress the cell through the unfolded protein and the ER overload responses (Cudna and Dickson, Biotechnol. Bioeng., 81:56-65, 2003; Psachen, J. Cereb. Blood Flow Metab., 23:773-9, 2003). Subsequent retrotranslocation and accumulation of misfolded sulfatase in

the cytoplasm could, in turn, tax the proteosome system and result in aggresomes, which are toxic to the cell (Garcia-Mata et al., Traffic 3:388-96, 2002). These effects will select against cell lines that over-express ASB.

The enzyme responsible for active-site cysteine conversion, SUMF1, has recently been cloned and characterized (Cosma et al., Cell, 113:445-56, 2003; Dierks et al., Cell 113:435-44, 2003). Transient co-transfection of SUMF1 with I2S and GALNS has been reported to increase yields of active enzyme by 5 and 50-fold, respectively. By relieving saturation of the sulfatase conversion step, co-expression of SUMF1 with sulfatase enzymes promises to remove the constraint that low sulfatase yield has had on efforts to treat sulfatase deficiencies by ERT (Baenziger, Cell 113:421-422, 2003). However, co-expression of sulfatase processing enzymes with the sulfatases may have other attendant problems relating to toxicity associated with the conversion of cysteine to formylglycine.

More particularly, limits on the capacity of the sulfatase modification machinery may be an adaptation to H₂S toxicity. Even at a modest sulfatase expression rate of 1pg/cell/day, the cell will be burdened with a build-up of H₂S that would reach intracellular concentrations of at least 30 µM over a 24-hour interval. For example, at an approximate molecular weight of 60kD, 1 pg of sulfatase is equivalent to 3.0×10^{-17} moles of sulfatase expressed per cell per day. The same number of moles of H₂S will be generated if all of the sulfatase is activated. At an average cell volume of 1×10^{-12} L, a concentration of 30 µM H₂S will accumulate over the entire volume of the cell in 24 hours in the absence of removal mechanisms or diffusion out of the cell. Considering the ER alone, the concentration would be higher. Concentrations of H₂S ten-fold lower than this have been shown to be toxic to cells (Nicholson et al., J Toxicol Environ Health A, 54(6): 491-507, 1998). The cell must work to keep the H₂S concentrations low in order to survive. A direct relation between sulfatase expression and toxicity could automatically prevent survival of overexpressing clones. Increasing the capacity of a cell to activate mammalian sulfatases, for example by co-expression of sulfatases with the modification enzyme, should only exacerbate H₂S toxicity. Efforts to reconstitute the activation pathways of prokaryotic sulfatases, in which an active site serine residue is converted to formylglycine, in mammalian cells have failed to date, despite the absence of H₂S byproduct in the conversion of serine to formylglycine.

Thus, there remains a need for alternative approaches to the expression of active sulfatases for use in enzyme replacement therapy.

SUMMARY OF THE INVENTION

5 The present application is based in part on the discovery that serine mutants of sulfatases that comprise a serine residue instead of a cysteine residue at the active site can be activated using a vanadate-dependent photoreaction which converts the active site serine of these mutant sulfatases to a formylglycine residue thereby rendering the mutants catalytically active. The photoreaction involves conversion of
10 the active site serine to a formylglycine. Therefore, particular embodiments of the present invention are directed to methods of converting recombinant sulfatase protein to a catalytically active sulfatase enzyme by contacting a preparation that comprises the protein with a photoactivatable redox agent, such as for example, vanadate and irradiating the preparation with light in the range of between 300 nm and 400 nm
15 wavelength. The sulfatase may be any sulfatase that comprises a serine residue instead of an active site cysteine residue. As such it is contemplated that the sulfatase may be a eukaryotic sulfatase protein, or a prokaryotic sulfatase protein. In preferred embodiments the protein is a mammalian sulfatase, and more particularly, it is a human sulfatase protein.

20 In specific embodiments, the recombinant sulfatase protein is a mutant mammalian arylsulfatase protein in which the active site cysteine residue has been replaced by a serine residue. More particularly, the active-site serine in the mutant mammalian arylsulfatase is converted to formylglycine by incubating the mammalian arylsulfatase with vanadate in the presence of an energy source of 300 nm to 400 nm
25 wavelength or a laser energy source. Preferably, the preparation is contacted with the redox agent prior to the irradiating step, however, the preparation may be irradiated prior to contacting with the vanadate as long as the contacting with the vanadate ions is sufficient to produce an activation of the sulfatase enzyme. In specific embodiments, the preparation is subjected to a cyclic irradiation wherein the sample is
30 subjected to a period of irradiation followed by a period of incubation in a dark environment, and preferably the cyclic irradiation is repeated, 2, 3, 4, 5, or more times to achieve an optimal photoactivation of the sulfatase. The time for the period of

irradiation and the time period for incubation in the dark is not limiting and may be selected from any convenient time that is sufficient to activate at least 10% of the mutant sulfatase as compared to the absence of the activation by irradiation and vanadate. An exemplary time for irradiating is a 30-minute period of irradiation ("light period of irradiation cycle") followed by a 20 minute period of incubation in the dark ("dark period of irradiation cycle"). However it should be understood that the light period of the irradiation cycle may be 10 minutes, 15 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 60 minutes, 90 minutes, 120 minutes or longer. The dark period of the irradiation cycle may be 5 minutes, 10 minutes, 15 minutes, 20 minutes 30 minutes, 40 minutes, 50 minutes, 60 minutes, 90 minutes, 120 minutes or longer. Any combination of light and dark period may be used

The sulfatase may be any sulfatase that comprises an active site serine residue instead of an active site cysteine residue. It is particularly contemplated that the arylsulfatase is selected from the group consisting of arylsulfatase A, arylsulfatase B, Arylsulfatase C, arylsulfatase D, arylsulfatase E, arylsulfatase F, arylsulfatase G, Sulfatase 1, Sulfatase 2, galactosamine (N-acetyl)-6-sulfate sulfatase, iduronate 2 sulfatase, N-sulfoglucosamine sulfohydrolase, glucosamine (N-acetyl)-6-sulfatase and arylsulfatase A cerebroside. In specific embodiments, the arylsulfatase is an arylsulfatase B comprising a C→S mutation at a position corresponding to amino acid position number 91 of SEQ ID NO:4. In other embodiments, the arylsulfatase is an arylsulfatase A comprising a C→S mutation at a position corresponding to amino acid position number 91 of SEQ ID NO:2. In other preferred embodiments, the sulfatase is a protein sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39 or variants thereof that retain sulfatase activity.

In other aspects of the invention, the method uses an arylsulfatase protein that is a mutant human arylsulfatase B protein obtained from a CHO cell line according to a method comprising the steps of transfecting CHO cells with an expression construct comprising a nucleic acid encoding an arylsulfatase an arylsulfatase B comprising a C→S mutation at a position corresponding to amino acid position number 91 of SEQ ID NO:4; selecting cells transfected with the

vector; culturing the selected cells under conditions to permit the secretion of the mutant human arylsulfatase B protein (ASB) protein into the culture medium of the cells; and isolating mutant human ASB protein from the culture medium. Preferably, the CHO cell line is a CHO-K1 cell line. In other embodiments, the CHO cell line is a CHO-S cell line.

In further aspects of the invention, the method uses an arylsulfatase protein that is a mutant human arylsulfatase A protein obtained from a CHO cell line according to a method comprising transfecting CHO cells with an expression construct comprising a nucleic acid encoding an arylsulfatase A (ASA) comprising a C→S mutation at a position corresponding to amino acid position number 91 of SEQ ID NO:2; selecting cells transfected with the vector; culturing the selected cells under conditions to permit the secretion of the mutant ASA protein into the culture medium of the cells; and isolating the mutant ASA protein from the culture medium. Preferably, the CHO cell line is a CHO-K1 cell line. In other embodiments, the CHO cell line is a CHO-S cell line.

Recombinant CHO-S cell lines that are transfected with expression constructs that encode mutant ASA and ASB proteins form another aspect of the invention.

Also contemplated herein are methods of preparing a pharmaceutically acceptable composition comprising a catalytically active arylsulfatase comprising contacting a preparation comprising recombinant arylsulfatase protein with a photoactivatable redox agent composition, irradiating the preparation with light in the range of between 300 nm and 400 nm wavelength, and formulating the preparation in a pharmaceutically acceptable carrier, diluent or excipient.

Additional aspects of the invention describe methods of preparing a pharmaceutically acceptable composition comprising a catalytically active arylsulfatase comprising contacting a preparation comprising recombinant arylsulfatase protein with a vanadate composition, irradiating the preparation with light in the range of between 300 nm and 400 nm wavelength, and formulating the preparation in a pharmaceutically acceptable carrier, diluent or excipient.

Also contemplated herein are pharmaceutical compositions comprising catalytically active arylsulfatase proteins prepared according to the method discussed

above and described in further detail herein below, and a pharmaceutically acceptable carrier, diluent or excipient.

Other features and advantages of the invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred
5 embodiments of the invention, are given by way of illustration only, because various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

10 **BRIEF DESCRIPTION OF THE DRAWINGS**

The following drawings form part of the present specification and are included to further illustrate aspects of the present invention. The invention may be better understood by reference to the drawings in combination with the detailed description of the specific embodiments presented herein.

15 Figure 1. ClustalW alignment of mammalian sulfatases showing the alignment of the active site amino acid residues. The conserved cysteine residue is shown at residue 50, the proline residue is shown at residue 52, and the arginine residue is shown at residue 54 of the ClustalW alignment.

20 Figure 2A. Specific productivity of stably transfected pools of cells expressing either ASB or ASB C91S at 450 $\mu\text{g/mL}$ G418 and 900 $\mu\text{g/mL}$ G418. Cells were grown in duplicate T-25 flasks. Medium was conditioned for 48 hours prior to harvest. Levels of secreted sulfatase in the conditioned medium were assayed by ELISA. Cell numbers were measured with a Coulter counter.

25 Figure 2B. Western blots of secreted and intracellular protein preparations from pools of stably transfected CHO cells expressing ASA or ASA C69S. Equal amounts of protein were loaded in each well as determined by Bradford assay. Duplicate flasks of cells expressing ASA or ASA C69S were compared. Representative samples are shown.

30 Figure 2C shows normalized NPT and sulfatase transcript ratios obtained from pools of stably transfected cells selected at either 450 $\mu\text{g/mL}$ G418 or 900 $\mu\text{g/mL}$ G418 and expressing different sulfatase proteins. RNA was isolated and

transcribed as described in Example 1. Sulfatase and NPT transcript levels were normalized to the ribosomal S14 internal standard.

Figure 2D shows the ratio of secreted protein per transcript for pools of stably transfected cells expressing either ASB or ASB C91S. Protein levels were measured by ELISA and normalized to cell number measured with a Coulter counter; transcript levels were measured by RT-QPCR and normalized to the ribosomal S14 internal standard.

Figure 3A shows the dependence of the photo-conversion of ASB C91S on vanadate concentration and time-of-irradiation. For this experiment, samples of ASB C91S (10 μ L, 0.5 mg/mL) in 50 mM NaOAc pH 6.5, 100 mM NaCl were added to a 96-well polystyrene microtiter plate. Vanadate from a 100 mM stock in water at pH 10 was added to each well in order to achieve final concentrations of 10, 40 and 400 μ M. maintained at 20°C as described in Example 1. The plate was then irradiated for 120 minutes as described. Sample aliquots (2 μ L) were removed at 60, 90 and 120 minutes and assayed as described.

Figure 3B shows photo-conversion of ASB C91S concomitant with photo-inactivation of native ASB by vanadate and light. For this experiment, ASB C91S (0.5 mg/mL) and native ASB (0.3 mg/mL) in 50 mM NaOAc pH 6.5, 100 mM NaCl were irradiated with 2 x 5W Hg Ace glass lamps. Protein samples were irradiated in 1.5 mL polypropylene microcentrifuge tubes at 20°C as described in the Example 1. A 2 mM sodium metavanadate stock in 20 mM Tris pH 9.6 was diluted into the protein solutions to a final concentration of 8 μ M (2 μ L to X). Following each irradiation interval (30 minutes), 5 μ L aliquots were removed and placed on ice. Upon completion of irradiation, all samples were assayed at once. UVA intensity measurements were made at 1, 15, 25 and 40 minutes during the course of the experiment with a probe fixed at the same distance from the source as the samples. Irradiation intensity had a mean and standard deviation of $90 \pm 5 \mu$ W/cm.

Figure 3C shows activation of ASBC91S with vanadate and light. ASB C91S was converted to active enzyme using the conditions specified in Example 1, including 100 mM Tris-acetate pH 8 and 10-500 molar equivalents of vanadate. To measure the extent of activation, activity values for ASB C91S obtained using different activation conditions were divided by activity values for the same amount of

wild-type ASB obtained using the same conditions, but without irradiation (irradiation of wild-type ASB in the absence of vanadate has no effect on the activity of the enzyme, data not shown). Closed circles and dotted line represents activation at 500 μ M vanadate. Closed diamonds and solid line represents activation at 10 μ M vanadate.

Figure 3D shows the LC/MS profile of peptides derived from ASB and from ASB treated with vanadate and light. To test the hypothesis that vanadate modifications of ASB and ASBC91S would be restricted to the active site, ASB was treated exhaustively with vanadate and light and then compared to untreated ASB by tryptic mapping using LC/MS.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Mammalian sulfatases are involved in the degradation of sulfated substrate such as mucopolysaccharides, cerebroside sulfates and sulfated steroids. Key to the catalytic activity of all of sulfatases is the presence of a C α -formylglycine residue, which is post-translationally generated by the sequential oxidation and hydrolysis of cysteine in the endoplasmic reticulum. In the absence of this post-translational modification, the sulfatases are inactive and cause multiple sulfatase deficiency (MSD), a rare, yet fatal lysosomal storage disease. The importance of sulfatases in human metabolism is further underscored by the identification of at least eight other distinct human monogenic diseases caused by deficiencies in individual sulfatase activities (Hopwood and Ballabio, 2001). The treatment of each of these disorders requires enzyme replacement therapy with an enzyme that is active. To date, there remains a need for methods and compositions for producing sulfatases that are properly post-translationally modified (i.e., at least possess an active site formylglycine residue) for use in such enzyme replacement therapies.

In the present application, there are provided in vitro approaches to the production of active sulfatases. Previous work has shown that sulfatase mutants in which a serine has been used to replace the active site cysteine (*i.e.*, a C $\rightarrow$ S mutation) are expressed at levels that are at least comparable to those of the corresponding native enzymes (Daniele and Di Natale, FEBS Lett 505, 445-823, 2001; Brooks et al., Biochem J 307 (Pt 2), 457-63, 1995; Recksiek et al., J Biol Chem 273, 6096-103,

1998; Millat et al., *Biochem J* 326 (Pt 1), 243-7, 1997). A serine mutant protein might be expected in some cases to be expressed more efficiently than native enzyme since it will not be prone to misfolding as a result of inappropriate disulfide formation that may result in a related enzyme that contains the native cysteine residues. For the same reason, overexpression of the serine mutant would be less likely to be detrimental to the overexpressing cell because the conversion of the serine to formylglycine would not produce H₂S. Importantly, serine mutants of ASB and ASA have been shown to be virtually indistinguishable from their respective native counterparts by epitope analysis and X-ray diffraction (Brooks et al., *Biochem J* 307 (Pt 2), 457-63, 1995, 27, 28). The present application meets the challenge of identifying an in vitro process that converts serine to formylglycine in a secreted serine-mutant protein.

In the present application, it is shown that serine mutants of sulfatases may be prepared and are expressed in recombinant cell lines at much higher levels than the native enzymes under identical conditions. Further the data and details presented below demonstrate an in vitro sulfatase processing method in which vanadate and light can be used to convert the serine mutant of sulfatases to catalytically active sulfatases as demonstrated by the conversion of ASB (C91S) to catalytically active ASB. Vanadate-dependent photo-conversion of sulfatase mutants represents a novel method of sulfatase production. Methods and compositions for exploiting this finding in the production of members of the sulfatase family for enzyme replacement therapy are provided.

IN VITRO SULFATASE CONVERSION REACTION

It was reasoned that an in vitro sulfatase conversion reaction would ideally target a single amino acid, the active site serine. The binding specificity of the sulfatase active site can be exploited to achieve this end. A wide variety of small oxo-anionic species bind and inhibit sulfatases, presumably by competing for the sulfate-binding site. Vanadate has been demonstrated to have a particularly high affinity for wild-type sulfatases (Dibbelt, and Kuss, *Biol Chem Hoppe Seyler* 372, 173-85, 1991; Bond et al., *Structure* 5, 277-89, 1997). The crystal structure of ASB complexed to vanadate has been solved showing that a single vanadate occupies the active site

sulfate-binding pocket (Daniele and Di Natale, FEBS Lett 505, 445-823, 2001).

Vanadate is a mimic of phosphate and sulfate, but vanadium has redox chemistry that phosphorus and sulfur do not have. In particular, vanadate (V^{+5}) can undergo reduction to vanadyl (V^{+4}) in the presence of suitable electron sources. This reaction is facilitated by light. When vanadate is bound to protein, the electron sources for vanadate reduction are amino acid side-chains.

Specific side-chain oxidations mediated by vanadate and light have been previously described for a few proteins (Muhlrad and Ringel, Met Ions Biol Syst 31, 211-30, 1995). To date, these reactions have only been described for phosphate-binding proteins, and there has been no indication that such a reaction is applicable to other proteins. In the best characterized case, that of myosin, a serine residue within the ATP-binding pocket is converted to a formylglycine as a result of its proximity to vanadate (Cremo et al., Biochemistry 27, 8415-20, 1988; Cremo et al., J Biol Chem 264, 6608-11, 1989; Cremo et al., Biochemistry 29, 7982-90, 1990; Cremo et al., Methods Enzymol 196, 442-9, 1991; Grammer et al., Biochemistry 27, 8408-15, 1988; Grammer et al., Biochemistry 35, 15582-92, 1996).

The present application for the first time demonstrates that serine mutants of mammalian sulfatases can be activated using a vanadate-dependent photoreaction, which converts the active site serine of these sulfatases to a formylglycine residue thereby rendering the mutants catalytically active. The photoreaction involves conversion of the active site serine to a formylglycine.

In preferred embodiments, the sulfatase preparation is contacted with the a photoactivatable redox agent and ultraviolet light after the sulfatase preparation has been purified from a recombinant cell line, however, it is contemplated that the vanadate-dependent photoreaction may take place while the sulfatase preparation is still in the culture medium. As used herein "photoactivatable redox agent" is used to mean an agent that catalyzes a serine to formylglycine change when contacted with a sulfatase in the presence of an energy source from light in the wavelength of 300 nm to about 400 nm or a laser source. In preferred embodiments this agent is a vanadate composition. In exemplary embodiments, it is contemplated that the CHO cell culture from which the recombinant protein is to be isolated is irradiated with U.V. light source of greater than 300 nm and supplied with a source of vanadium ions.

Vanadate compositions that may be supplied to the sulfatase include but are not limited to ammonium polyvanadate, sodium ammonium vanadate, potassium orthovanadate, potassium metavanadate, potassium vanadate/borate-solution, lithium vanadate, sodium metavanadate, ammonium metavanadate, 5 vanadium tetroxide, vanadium pentoxide, hexavanadium tridekoxide vanadylacetylacetonate, vanadyl sulphate, vanadium triacetylacetonate, vanadyl oxalate-solution, vanadium trichloride or any other readily available source of vanadium. The concentration of vanadium used in the methods of the invention may vary. For example, the concentration of vanadium in a solution of sulfatase being 10 activated may range from about 0.01 mM vanadium to about 10 mM. In certain embodiments, the vanadate is present at concentration of less than 0.01mM. For example, the vanadate is present at concentrations of 0.01 μ M to 0.01mM. In particular embodiments, the sulfatase mutant and the vanadate are present in equimolar quantities. The reaction is a catalytic reaction and as such, it is 15 contemplated that the vanadate may be present at concentration that is less than the concentration of the sulfatase. In the catalytic reaction, the vanadyl reaction product is re-oxidized to vanadate in the presence of atmospheric oxygen or other oxidizing agent. In other embodiments, it is contemplated that the sulfatase mutant may be contacted with an excess concentration of vanadate and excess vanadate may be 20 removed from the sulfatase mutant-vanadate complex.

Thus, in a given preparation the vanadium ions may be present at a concentration of 0.01 μ M, 0.05 μ M, 0.10 μ M, 0.2 μ M, 0.4 μ M, 0.5 μ M, 0.6 μ M, 0.7 μ M, 0.8 μ M, 0.9 μ M, 1.0 μ M, 2 μ M, 4 μ M, 5 μ M, 6 μ M, 7, 8 μ M, 9 μ M, 0.01 mM; 0.02 mM; 0.04 mM; 0.05 mM; 0.10 mM; 0.15 mM, 0.2 mM, 0.25 mM, 0.30 mM, 25 0.35 mM, 0.40 mM, 0.45 mM, 0.50 mM, 0.55 mM, 0.60 mM, 0.65 mM, 0.70 mM, 0.75 mM, 0.80 mM, 0.85 mM, 0.90 mM, 1.0 mM, 1.25 mM, 1.50 mM, 1.75 mM, 2.0 mM, 2.25 mM, 2.50 mM, 2.75 mM, 3.00 mM, 3.25 mM, 3.50 mM, 3.75 mM, 4.0 mM, 4.25 mM, 4.50 mM, 4.75 mM, 5.0 mM, 5.25 mM, 5.50 mM, 5.75 mM, 6.0 mM, 6.25 mM, 6.50 mM, 6.75 mM, 7.0 mM, 7.25 mM, 7.5 mM, 7.75 mM, 8.0 mM, 8.25 30 mM, 8.5 mM, 8.75 mM, 9.0 mM, 9.25 mM, 9.50 mM, 9.75 mM, 10.0 mM. Of course, it should be understood that these are merely exemplary concentrations of vanadate to be used in the methods described herein and those of skill in the art may vary the concentration of the ions and still produce results commensurate in scope

with the methods of the invention. Ranges of vanadate concentrations between any of the above figures are expressly contemplated.

Sources of UV light are well known to those of skill in the art as are methods of irradiating compositions. In the present invention a UV light source
5 having a wavelength of from about 300nm to about 400 nm is used to irradiate sulfatases. The irradiation may be by way of a constant source of light source. Alternatively, the compositions may be irradiated periodically using intermittent periods of irradiation interspersed with periods of full spectrum light or periods of darkness. In specific embodiments, it is important to exclude wavelengths of
10 irradiation that are below 300nm (i.e., >300nm) as such wavelengths may cause damage to aromatic amino acids. Light of less than 300nm may be filtered with materials that strongly absorb light of less than 300nm. An alternative irradiation source is laser light treatment. A protocol for pulsed laser photolysis could be very useful in efficiently inducing the photo-dependent activation former and then allowing
15 the light-independent activation to occur while the laser is off, followed by another burst of laser irradiation etc. Finally, the problem of destructive oxidation of the already activated sulfatase might be addressed if the complex between activated sulfatase and vanadate (which probably results in inactivation upon photolysis) and the complex of sulfatase mutant and vanadate (which is required for activation) may
20 be partly or wholly differentiable by their absorption spectra. The interval irradiation and darkness may be optimized such that it is sufficient to activate at least 10%, more preferably 20%, even more preferably 30%, even more preferably 40%, 50%, 60%, 75%, 80%, 85%, 95%, or more of the mutant sulfatase as compared to the absence of the activation by irradiation and vanadate. Such an interval will preferably be
25 optimized to determine the time at which cleavage or degradation of the protein occurs so as to produce an upper limit to the time period of irradiation as that period at which measurable protein degradation or degradation of enzyme activity is observed. An exemplary time period for irradiation is 30 minutes, followed by a dark period of 20 minutes. This irradiation cycle may be repeated 2, 3, 4, 5, or more times.
30 Another exemplary time period of irradiation is 120 minutes. It should be understood that any time period of 10, 20, 30, 45, 60, 75, 90, 120 minutes (of light, optionally followed by a similar period of incubation in the dark) or any multiple of any such time period may be used and optimized for a given sulfatase. Thus, it is contemplated

that the in optimization protocols, the skill artisan will subject the mutant sulfatase to varying concentrations of vanadate and varying wavelengths of light and varying time intervals of irradiation. Preferably, each of these parameters will be varied separately such that initially, a first parameter, e.g., vanadate concentration is optimized, followed by optimization of a second parameter e.g., optimization of the wavelength of energy source of irradiation, followed by optimization of interval of time of irradiation. Those of skill in the art will readily be able to optimize these features by determining the enzyme activity of the sulfatase under the different conditions

10 METHODS OF PREPARING SULFATASES

In the present invention, mutant sulfatases will be prepared and used for a variety of methods and formulations. Methods of preparing such enzymes may involve the production of the proteins using automated peptide synthesis methods or by recombinant expression. Automated peptide synthesis methods are useful for producing short peptide fragments e.g., fragment of 50 amino acids in length whereas recombinant techniques are preferred where a longer protein sequence is to be generated. General principles for making proteins are well known to those of skill in the art. Methods and compositions for the preparation and purification of sulfatases are further described below.

20

A. Sulfatase Protein Sequences

Given the above finding, it is contemplated that recombinant sulfatases may be processed in vitro to produce an active enzyme containing a formylglycine residue at the active site. The formylglycine residue is generated from the sulfatase having a serine residue at the active site. To date, seventeen sulfatases have been identified in humans (Ferrante et al., 2002; Morimoto-Tomita et al., 2002; http://www.mad-cow.org/00/annotation_frames/tools/genbrow/sulfatases/sulfatases.html). These include, arylsulfatases, e.g., arylsulfatase A, B, C, D, E, F, G, sulfatase 1, sulfatase 2, iduronate 2 sulfatase, galactosamine (N-acetyl)-6-sulfate sulfatase, N-sulfoglucosamine sulfohydrolase, and glucosamine (N-acetyl)-6-sulfatase. Homologs of these proteins from various mammalian sources are known to those of skill in the

30

art. A ClustalW alignment of mammalian sulfatases showing the alignment of the active site cysteinyl residues is presented in Figure 1.

The following table provides the active site cysteine residue and the GenBank Acc. No. of various exemplary mammalian sulfatases. Given that the nucleic acid sequences of these sulfatases are known to those of skill in the art, and that the active site cysteine residue (identified in table below) may be mutated to serine residue using site-directed mutagenesis, those of skill in the art will readily be able to produce recombinant such sulfatases that comprise an active site serine rather than an active site cysteine. Such recombinant proteins will serve as substrates for the photoreaction methods of the present invention in which a combination of vanadate and a light source such as a source of light of from about 300 nm to about 400 nm or a pulsating laser light source is employed to render the sulfatases active by conversion of the active site serine residue to a formylglycine residue.

Table of sulfatase sequences and mutants containing active site mutations

Sulfatase	GenBank ID No.	SEQ ID NO:	Active Site Mutation	SEQ ID NO:
arylsulfatase A	BC014210	SEQ ID NO:2	C69S	SEQ ID NO:27
arylsulfatase B	J05225	SEQ ID NO:4	C91S	SEQ ID NO:28
Arylsulfatase C	NM_000351	SEQ ID NO:6	C65S	SEQ ID NO:29
arylsulfatase D	NM_001669	SEQ ID NO:8	C89S	SEQ ID NO:30
arylsulfatase E	NM_000047	SEQ ID NO:10	C86S	SEQ ID NO:31
arylsulfatase F	NM_004042	SEQ ID NO:12	C78S	SEQ ID NO:32
arylsulfatase G	BC012375	SEQ ID NO:14	C84S	SEQ ID NO:33
Sulfatase 1	NM_015170	SEQ ID NO:16	C87S	SEQ ID NO:34
Sulfatase 2	NM_198596	SEQ ID NO:18	C88S	SEQ ID NO:35
galactosamine (N-acetyl)-6-sulfate sulfatase	NM_000512	SEQ ID NO:20	C79S	SEQ ID NO:36
iduronate 2 sulfatase	NM_000202	SEQ ID NO:22	C84S	SEQ ID NO:37

N-sulfoglucosamine sulfohydrolase	NM_000199	SEQ ID NO:24	C70S	SEQ ID NO:38
glucosamine (N-acetyl)-6-sulfatase	NM_002076	SEQ ID NO:26	C91S	SEQ ID NO:39

The active sulfatases may be further treated to ensure that the formylglycine is stabilized. Formylglycine is an aldehyde. There are specific chemical reagents (e.g., hydrazines, hydroxylamines, bis-alcohols and others) that react reversibly with aldehydes that may be used to "trap" the activated enzyme in an activated form and prevent further photo-oxidation. The active site also contains a metal ion (Ca^{2+} or Mg^{2+}) also in the active site of sulfatases. Therefore, different metal ions may also be employed to stabilize the formylglycine by making the formylglycine less reactive. These metal ions could then be substituted with Ca or Mg after the activation reaction is finished (by dialysis for example) to reconstitute the active enzyme.

The sequences of other sulfatases also may be similarly modified. In particular, those of skill in the art may desire to modify the active sites of sulfatases from other mammalian sources (see e.g., clustal alignment in Figure 1 showing sequences from other sources including rat; bovine; feline; chicken and the like).

B. Automated Solid-Phase Peptide Synthesis

Automated syntheses are typically performed in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and are used in accordance with known protocols. See, for example, Stewart and Young, Solid Phase Peptide Synthesis, 2d. ed., Pierce Chemical Co., (1984); Tam et al., J. Am. Chem. Soc., 105:6442, (1983); Merrifield, Science, 232: 341-347, (1986); and Barany and Merrifield, The Peptides, Gross and Meienhofer, eds, Academic Press, New York, 1-284, (1979); Fields, (1997) Solid-Phase Peptide Synthesis. Academic Press, San Diego.); Andersson et al., Large-scale synthesis of peptides. Biopolymers (Pept. Sci.), 55, 227-250 (2000); Burgess et al., DiSSiMiL: Diverse Small Size Mini-Libraries applied to simple and rapid epitope mapping of a monoclonal antibody. J. Pept. Res., 57, 68-76, (2001); Peptides for the

New Millennium, Fields, J.P. Tam & G. Barany (Eds.), Kluwer Academic Publisher, Dordrecht. Numerous other documents teaching solid phase synthesis of peptides are known to those of skill in the art and may be used to synthesize epitope arrays from any given protein or peptide.

5 For example, the peptides are synthesized by solid-phase technology employing an exemplary peptide synthesizer such as a Model 433A from Applied Biosystems Inc. This instrument combines the Fmoc chemistry with the HBTU activation to perform solid-phase peptide synthesis. Synthesis starts with the C-terminal amino acid. Amino acids are then added one at a time till the N-terminus is
10 reached. Three steps are repeated each time an amino acid is added. Initially, there is deprotection of the N-terminal amino acid of the peptide bound to the resin. The second step involves activation and addition of the next amino acid and the third step involves deprotection of the new N-terminal amino acid. In between each step there are washing steps. This type of synthesizer is capable of monitoring the deprotection
15 and coupling steps.

 At the end of the synthesis the protected peptide and the resin are collected, the peptide is then cleaved from the resin and the side-chain protection groups are removed from the peptide. Both the cleavage and deprotection reactions are typically carried out in the presence of 90% TFA, 5% thioanisole and 2.5%
20 ethanedithiol. After the peptide is separated from the resin, e.g., by filtration through glass wool, the peptide is precipitated in the presence of MTBE (methyl t-butyl ether). Diethyl ether is used in the case of very hydrophobic peptides. The peptide is then washed a plurality of times with MTBE in order to remove the protection groups and to neutralize any residual acidity. The purity of the peptide is further monitored by
25 mass spectrometry and in some case by amino acid analysis and sequencing.

 The peptides also may be modified, and such modifications may be carried out on the synthesizer with very minor interventions. An amide could be added at the C-terminus of the peptide. An acetyl group could be added to the N-terminus. Biotin, stearate and other modifications could also be added to the N-
30 terminus.

 The purity of any given peptide, generated through automated peptide synthesis or through recombinant methods, is typically determined using reverse

phase HPLC analysis. Chemical authenticity of each peptide is established by any method well known to those of skill in the art. In certain embodiments, the authenticity is established by mass spectrometry. Additionally, the peptides also are quantified using amino acid analysis in which microwave hydrolyses are conducted.

- 5 In one aspect, such analyses use a microwave oven such as the CEM Corporation's MDS 2000 microwave oven. The peptide (approximately 2 μ g protein) is contacted with e.g., 6 N HCl (Pierce Constant Boiling e.g., about 4 ml) with approximately 0.5% (volume to volume) phenol (Mallinckrodt). Prior to the hydrolysis, the samples are alternately evacuated and flushed with N₂. The protein hydrolysis is conducted
- 10 using a two-stage process. During the first stage, the peptides are subjected to a reaction temperature of about 100°C and held at that temperature for 1 minute. Immediately after this step, the temperature is increased to 150°C and held at that temperature for about 25 minutes. After cooling, the samples are dried and amino acid from the hydrolysed peptides samples are derivatized using 6-aminoquinolyl-N-
- 15 hydroxysuccinimidyl carbamate to yield stable ureas that fluoresce at 395 nm (Waters AccQ Tag Chemistry Package). In certain aspects, the samples are analyzed by reverse phase HPLC and quantification is achieved using an enhanced integrator. Such conditions may readily be adapted for large scale production and/or for purification of other peptides.

20 C. Recombinant Protein Production.

- As an alternative to automated peptide synthesis, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes a peptide of the invention is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression as
- 25 described herein below. Recombinant methods are especially preferred for producing longer polypeptides. In particularly preferred embodiments, the arylsulfatases of any of sequences of SEQ ID NO:27-SEQ ID NO:39 are prepared using recombinant techniques.

- A variety of expression vector/host systems may be utilized to contain
- 30 and express the arylsulfatase proteins. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus);

plant cell systems transfected with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (e.g., Ti or pBR322 plasmid); or animal cell systems. Mammalian cells that are useful in recombinant protein productions include but are not limited to VERO
5 cells, HeLa cells, Chinese hamster ovary (CHO) cell lines, COS cells (such as COS-7), W138, BHK, HepG2, 3T3, RIN, MDCK, A549, PC12, K562 and 293 cells.

As described below, in particularly preferred embodiments, the arylsulfatases are prepared in a CHO cell line such as a CHO K1 cell line. In particularly preferred embodiments, the recombinant sulfatases may be produced from
10 a stable transfected CHO-K1 cell line prepared according to methods outlined for the preparation of the CSL4S-342 cell line (Crawley, J.Clin.Invest. 99:651-662, 1997; Peters, et al. J. Biol. Chem. 265:3374-3381). In addition to CHO cells, other mammalian host systems for the expression of recombinant proteins also are well known to those of skill in the art. Host cell strains may be chosen for a particular
15 ability to process the expressed protein or produce certain post-translation modifications that will be useful in providing protein activity. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing, which cleaves a "prepro" form of the protein, may also be important for
20 correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, 293, WI38, and the like have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein.

It is preferable that the transformed cells are used for long-term, high-
25 yield protein production and as such stable expression is desirable. Once such cells are transformed with vectors that contain selectable markers along with the desired expression cassette, the cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The selectable marker is designed to confer resistance to selection and its presence allows growth and recovery of cells,
30 which successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell.

A number of selection systems may be used to recover the cells that have been transformed for recombinant protein production. Such selection systems include, but are not limited to, HSV thymidine kinase, hypoxanthine-guanine phosphoribosyltransferase and adenine phosphoribosyltransferase genes, in tk-, hgp^rt- or ap^rt- cells, respectively. Also, anti-metabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate; gpt, which confers resistance to mycophenolic acid; neo, which confers resistance to the aminoglycoside G418; also which confers resistance to chlorsulfuron; and hyg^r, which confers resistance to hygromycin. Additional selectable genes that may be useful include trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine. Markers that give a visual indication for identification of transformants include anthocyanins, β -glucuronidase and its substrate, GUS, and luciferase and its substrate, luciferin.

15 **D. Expression Constructs for Recombinant Protein Production**

In the recombinant production of arylsulfatases described herein, expression vectors comprising polynucleotide molecules that encode those arylsulfatases. For example, the wild-type proteins of SEQ ID NO:2, SEQ ID NO:4; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:10; SEQ ID NO:12; SEQ ID NO:14; SEQ ID NO:16; SEQ ID NO:18; SEQ ID NO:20; SEQ ID NO:22; SEQ ID NO:24; and SEQ ID NO:26, are encoded by nucleic acid sequence of SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:13; SEQ ID NO:15; SEQ ID NO:17; SEQ ID NO:19; SEQ ID NO:21; SEQ ID NO:23; SEQ and ID NO:25, respectively. Methods of mutating these nucleic acid sequences in order that proteins of SEQ ID NO:27-39 are encoded are well known to those of skill in the art and are discussed in further detail below.

Methods of preparing expression vectors, as well as producing host cells transformed with such vectors, are well known to those skilled in the art. The polynucleotide molecules used in such an endeavor may be joined to a vector, which generally includes a selectable marker and an origin of replication, for propagation in a host. These elements of the expression constructs are well known to those of skill in the art. Generally, the expression vectors include DNA encoding the given protein

being operably linked to suitable transcriptional or translational regulatory sequences, such as those derived from a mammalian, microbial, viral, or insect gene. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, mRNA ribosomal binding sites, and appropriate sequences, which control
5 transcription and translation.

In preferred embodiments, an expression vector is prepared to transfer a cDNA that encodes an arylsulfatase into a suitable cell or cell line for expression thereof. In one particularly preferred embodiment, the cDNA is transfected into a Chinese hamster ovary cell, such as the CHO-K1 cell line. In yet other preferred
10 embodiments, the production procedure comprises the following steps: (a) growing cells transfected with a DNA encoding all or a biologically active fragment or mutant of any of the sulfatases of SEQ ID NO:27; SEQ ID NO:28; SEQ ID NO:28; SEQ ID NO:29; SEQ ID NO:30; SEQ ID NO:31; SEQ ID NO:32; SEQ ID NO:33; SEQ ID NO:34; SEQ ID NO:35; SEQ ID NO:36; SEQ ID NO:37; SEQ ID NO:38 or SEQ ID
15 NO:39 in a suitable growth medium to an appropriate density, (b) introducing the transfected cells into a bioreactor, (c) supplying a suitable growth medium to the bioreactor, (d) harvesting said medium containing the recombinant enzyme, and (e) substantially removing the transfected cells from the harvest medium.

The terms "expression vector," "expression construct " or "expression
20 cassette " are used interchangeably throughout this specification and are meant to include any type of genetic construct containing a nucleic acid coding for a gene product in which part or all of the nucleic acid encoding sequence is capable of being transcribed.

The expression construct may further comprise a selectable marker that
25 allows for the detection of the expression of a peptide or polypeptide. Usually the inclusion of a drug selection marker aids in cloning and in the selection of transformants, for example, neomycin, puromycin, hygromycin, DHFR, zeocin and histidinol. Alternatively, enzymes such as herpes simplex virus thymidine kinase (tk) (eukaryotic), b-galactosidase, luciferase, or chloramphenicol acetyltransferase (CAT)
30 (prokaryotic) may be employed. Immunologic markers also can be employed. For example, epitope tags such as the FLAG system (IBI, New Haven, CT), HA and the 6xHis system (Qiagen, Chatsworth, CA) may be employed. Additionally, glutathione S-transferase (GST) system (Pharmacia, Piscataway, NJ), or the maltose binding

protein system (NEB, Beverley, MA) also may be used. The selectable marker employed is not believed to be important, so long as it is capable of being expressed simultaneously with the nucleic acid encoding a gene product. Further examples of selectable markers are well known to one of skill in the art.

5 Expression requires that appropriate signals be provided in the vectors, such as enhancers/promoters from both viral and mammalian sources that may be used to drive expression of the nucleic acids of interest in host cells. Usually, the nucleic acid being expressed is under transcriptional control of a promoter. A "promoter" refers to a DNA sequence recognized by the synthetic machinery of the
10 cell, or introduced synthetic machinery, required to initiate the specific transcription of a gene. Nucleotide sequences are operably linked when the regulatory sequence functionally relates to the DNA encoding the peptide substrate or the fusion polypeptide. Thus, a promoter nucleotide sequence is operably linked to a given DNA sequence if the promoter nucleotide sequence directs the transcription of the
15 sequence. Similarly, the phrase "under transcriptional control" means that the promoter is in the correct location and orientation in relation to the nucleic acid to control RNA polymerase initiation and expression of the gene.

Any promoter that will drive the expression of the nucleic acid may be used. The particular promoter employed to control the expression of a nucleic acid
20 sequence of interest is not believed to be important, so long as it is capable of directing the expression of the nucleic acid in the targeted cell. Thus, where a human cell is targeted, it is preferable to position the nucleic acid coding region adjacent to and under the control of a promoter that is capable of being expressed in a human cell. Generally speaking, such a promoter might include either a human or viral promoter.
25 Common promoters include, e.g., the human cytomegalovirus (CMV) immediate early gene promoter, the SV40 early promoter, the Rous sarcoma virus long terminal repeat, β -actin, rat insulin promoter, the phosphoglycerol kinase promoter and glyceraldehyde-3-phosphate dehydrogenase promoter, all of which are promoters well known and readily available to those of skill in the art, can be used to obtain high-
30 level expression of the coding sequence of interest. The use of other viral or mammalian cellular or bacterial phage promoters, which are well-known in the art to achieve expression of a coding sequence of interest, is contemplated as well, provided that the levels of expression are sufficient for a given purpose. By employing a

promoter with well known properties, the level and pattern of expression of the protein of interest following transfection or transformation can be optimized. Inducible promoters also may be used.

Another regulatory element that is used in protein expression is an enhancer. These are genetic elements that increase transcription from a promoter located at a distant position on the same molecule of DNA. Where an expression construct employs a cDNA insert, one will typically desire to include a polyadenylation signal sequence to effect proper polyadenylation of the gene transcript. Any polyadenylation signal sequence recognized by cells of the selected transgenic animal species is suitable for the practice of the invention, such as human or bovine growth hormone and SV40 polyadenylation signals.

Also contemplated as an element of the expression cassette is a terminator. These elements can serve to enhance message levels and to minimize read through from the cassette into other sequences. The termination region which is employed primarily will be one selected for convenience, since termination regions for the applications such as those contemplated by the present invention appear to be relatively interchangeable. The termination region may be native with the transcriptional initiation, may be native to the DNA sequence of interest, or may be derived for another source.

20

E. Site-Specific Mutagenesis

Site-specific mutagenesis will be useful in the present invention to create the C→S mutants that are described herein above in Table 1. This technique employs specific mutagenesis of the underlying DNA (that encodes the amino acid sequence that is targeted for modification). Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences that encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Typically, a primer of about 17 to 25 nucleotides in length is preferred, with about 5 to 10 residues on both sides of the junction of the sequence being altered. Those of skill in the art have previously used site-directed mutagenesis for the

preparation of arylsulfatase mutants (Dierks et al., EMBO Journal., 18(8):2084-2091, 1999) to show that in an arylsulfatase active site the sequence CTPSR (SEQ ID NO:56) is a conserved sequence and the cysteine, proline, and arginine residues are key residues within the motif for formylglycine formation. Thus, the C→S mutants prepared herein should preferably retain the proline and arginine residues. Recksiek et al. (J. Biol. Chem., 273(11):6096-6103, 1998) teach exemplary methods for preparing C69S arylsulfatase A mutants and C91S arylsulfatase B mutants. These teachings are incorporated herein by reference as providing a teaching of art recognized methods for producing C→S mutants.

Site-directed mutagenesis typically employs a bacteriophage vector that exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phage vectors are commercially available and their use is generally well known to those skilled in the art. Double stranded plasmids also are routinely employed in site directed mutagenesis, which eliminates the step of transferring the gene of interest from a phage to a plasmid.

In general, site-directed mutagenesis is performed by first obtaining a single-stranded vector, or melting of two strands of a double stranded vector, which includes within its sequence a DNA sequence encoding the desired protein. An oligonucleotide primer bearing the desired mutated sequence is synthetically prepared. For example, the codons that encode the active site mutations shown in Table 1 are mutated into serine codons by PCT methods using non-coding mutagenesis primers. Such a primer is then annealed with the single-stranded DNA preparation, taking into account the degree of mismatch when selecting hybridization (annealing) conditions, and subjected to DNA polymerizing enzymes such as E. coli polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as E. coli cells, and clones are selected that include recombinant vectors bearing the mutated sequence arrangement.

Of course, the above described approach for site-directed mutagenesis is not the only method of generating potentially useful mutant peptide species and as

such is not meant to be limiting. Exemplary expression constructs and primers are discussed in the Examples herein below.

PROTEIN PRODUCTION AND PURIFICATION

5 The present invention is directed to the production of serine mutant recombinant sulfatase proteins and the subsequent in vitro activation of these mutants. The proteins are prepared from CHO cells transfected with expression constructs by growing the transfected cells in a suitable growth medium to an appropriate density, introducing the transfected cells into a bioreactor, supplying a suitable growth
10 medium to the bioreactor, harvesting the medium containing the recombinant enzyme, and substantially removing the transfected cells from the harvest medium. Exemplary such methods for the production of recombinant arylsulfatase B are provided in U.S. Patent Application No. 10/704,365, filed November 7, 2003 published as U.S. patent publication number _____ (specifically incorporated herein by reference in its
15 entirety).

A. Cell Culture

 In certain embodiments, an exemplary suitable medium for the growth of the transfected cells is a JRH Excell 302 medium supplemented with L-glutamine, glucose and hypoxanthine/thymidine, optionally with or without G418. In one
20 exemplary medium, the JRH Excell 302 medium is further supplemented with folic acid, serine, and asparagine, and there is no G418 present in the medium. This medium has been shown to provide a higher purity precursor recombinant arylsulfatase B as compared medium supplemented with G418 but not with folic acid, serine, and asparagine (see U.S. Patent Application No. 10/704,365). The CHO cells
25 expressing any of the recombinant sulfatases described herein may be grown in such a medium. Preferably, the cells are grown to a cell density of about 1×10^7 cells/ml resulting in 10-40 mg/ml of active enzyme.

 In preferred embodiments, the cells are grown in a bioreactor. Preferably, the transfected cells are grown in a bioreactor for about 5 to 15 days.
30 More preferably, it is about 9 days. Preferably, the transfected cells are grown in a bioreactor using a perfusion-based process with collections continuing up to 35 days. More preferably, the transfected cells are grown in a bioreactor using a perfusion-

based process with collections continuing up to 45 days. Even more preferably, the transfected cells are grown in a bioreactor using a perfusion-based process with collections continuing up to 60 days. Even much more preferably, the transfected cells are grown in a bioreactor using a perfusion-based process with collections continuing up to 90 days. For a detailed discussion of considerations involved in selecting bioreactors for large-scale cell growth see e.g., WO International Application No. PCT US03/26831 (WO 2004/020971, incorporated herein by reference).

Thus, it is contemplated that the transfected cells may be grown as either a suspension culture or an anchorage-dependent culture in a suitable bioreactors. The bioreactors should preferably have high volume-specific culture surface area in order to achieve high cell density and high yield of recombinant product. Microcarrier cell culture in stirred tank bioreactor provides very high volume-specific culture surface area and has been used for the production of viral vaccines (Griffiths, J. B., In "Animal Cell Biotechnology", vol. 3, p179-220, (Eds. Spier, R. E. and Griffiths, J. B.), Academic Press, London. (1986)). The multiplate Cellcube™ cell culture system manufactured by Corning-Costar also offers a very high volume-specific culture surface area. Cells grow on both sides of the culture plates hermetically sealed together in the shape of a compact cube. Unlike stirred tank bioreactors, the Cellcube™ culture unit is disposable. This is very desirable at the early stage production of clinical product because of the reduced capital expenditure, quality control and quality assurance costs associated with disposable systems.

As an alternative to stirred-tank bioreactors, wave-induced agitation for fluid mixing and oxygenation of cell cultures in inflated plastic bags was pioneered in 1995 and is gaining popularity as a method for use in large-scale cell culture. This concept was developed into the Wave Bioreactor, which was initially introduced in 1998. In this apparatus, an inflated, sterile bag is partially filled with liquid cultivation media and cells, and placed on rocking mechanism that moves the bag to and fro thereby inducing a wave-like motion to the liquid contained therein. This motion ensures cell suspension, bulk mixing, and oxygen transfer from the liquid surface to the respiring cells without damaging shear forces or foam generation. Air is passed through the bag to provide oxygen, and sweep out evolved carbon dioxide.

The specially designed bags used in this system are optimized to induce wave motion in the culture media. The wave motion provides nutrient mixing and oxygenation to support more than 1×10^7 cells/ml. Therefore, cells can grow to a much higher density than that obtainable with rollers or spinners. This type of bioreactor is described in
5 further detail in U.S. Patent No. 6,190,913. Such bioreactors are used for perfusion and the scale up to culture volumes over 500 liters.

In most large scale bioreactors are operated through batch or fed-batch processes because such methods are the most straightforward to operate and scale up. However, continuous processes based on chemostat or perfusion principles are
10 available. A batch process is a closed system in which a typical growth profile is seen. A lag phase is followed by exponential, stationary and decline phases. In such a system, the environment is continuously changing as nutrients are depleted and metabolites accumulate. This makes analysis of factors influencing cell growth and productivity, and hence optimization of the process, a complex task. Productivity of a
15 batch process may be increased by controlled feeding of key nutrients to prolong the growth cycle. Such a fed-batch process is still a closed system because cells, products and waste products are not removed.

In what is still a closed system, perfusion of fresh medium through the culture can be achieved by retaining the cells with a variety of devices (e.g., fine mesh
20 spin filter, hollow fiber or flat plate membrane filters, settling tubes). Spin filter cultures can produce cell densities of approximately 5×10^7 cells/ml. A true open system and the simplest perfusion process is the chemostat in which there is an inflow of medium and an outflow of cells and products. Culture medium is fed to the reactor at a predetermined and constant rate, which maintains the dilution rate of the culture
25 at a value less than the maximum specific growth rate of the cells (to prevent washout of the cell mass from the reactor). Culture fluid containing cells and cell products and byproducts is removed at the same rate.

Roller-bottles may be used to anchorage-dependent non-perfused attachment cell culture. Fully automated robots are available that can handle
30 thousands of roller bottles per day, thus eliminating the risk of contamination and inconsistency associated with the otherwise required intense human handling. With frequent media changes, roller bottle cultures can achieve cell densities of close to 0.5

$\times 10^6$ cells/cm² (corresponding to approximately 10^9 cells/bottle or almost 10^7 cells/ml of culture media).

Microcarriers also may be used in large scale anchorage-dependent culture processes. Here, the transfected cells are propagated on the surface of small solid particles suspended in the growth medium by slow agitation. Cells attach to the microcarriers and grow gradually to confluency on the microcarrier surface. Microcarrier cultures offer a high surface-to-volume ratio (variable by changing the carrier concentration) which leads to high cell density yields and a potential for obtaining highly concentrated cell products. Cell yields are up to $1-2 \times 10^7$ cells/ml when cultures are propagated in a perfused reactor mode. Further, the cells can be propagated in one unit process vessels instead of using many small low-productivity vessels (i.e., flasks or dishes), thereby resulting in a more efficient use of nutrient and culture medium. Other advantages of a microcarrier system are discussed in WO 2004/020971.

In addition, it is contemplated that the CHO cells may be cultured using microencapsulation. The cells are retained inside a semipermeable hydrogel membrane. A porous membrane is formed around the cells permitting the exchange of nutrients, gases, and metabolic products with the bulk medium surrounding the capsule. Several methods have been developed that are gentle, rapid and non-toxic and where the resulting membrane is sufficiently porous and strong to sustain the growing cell mass throughout the term of the culture. These methods are all based on soluble alginate gelled by droplet contact with a calcium-containing solution. (Lim, U.S. Pat. No. 4,352,883, Oct. 5, 1982, incorporated herein by reference,) describes cells concentrated in an approximately 1% solution of sodium alginate which are forced through a small orifice, forming droplets, and breaking free into an approximately 1% calcium chloride solution. The droplets are then cast in a layer of polyamino acid that ionically bonds to the surface alginate. Finally the alginate is reliquefied by treating the droplet in a chelating agent to remove the calcium ions. Other methods use cells in a calcium solution to be dropped into a alginate solution, thus creating a hollow alginate sphere. A similar approach involves cells in a chitosan solution dropped into alginate, also creating hollow spheres.

Microencapsulated cells are easily propagated in stirred tank reactors and, with beads sizes in the range of 150-1500 μ m in diameter, are easily retained in a

perfused reactor using a fine-meshed screen. The ratio of capsule volume to total media volume can be maintained from as dense as 1:2 to 1:10. With intracapsular cell densities of up to 10^8 , the effective cell density in the culture is $1-5 \times 10^7$.

The advantages of microencapsulation over other processes include the protection from the deleterious effects of shear stresses which occur from sparging and agitation, the ability to easily retain beads for the purpose of using perfused systems, scale up is relatively straightforward and the ability to use the beads for implantation.

In an exemplary batch-fed process for the large-scale growth of the recombinant cells, the cells are initially inoculated into T-75 flask with 25mL of JRH Exell 302 medium supplemented with 4 mM L-glutamine, 4.5 g/L glucose and 10mg/L hypoxanthine/thymidine; further supplemented with folic acid, serine and asparagine (no G418). These cells are cultured in this medium for approximately three days or until cell density of approximately 1×10^{10} cell is achieved. Once such a cell density is reached the cells may be transferred to a 250 mL spinner flask and cultured in a volume of 175 mL of supplemented medium (no G418), this cell culture is allowed to grow to an exponential phase (approx. 3 days). The cells are then transferred to a 1 L flask and grown in a 800 mL medium supplemented (no G418). After 1 to 2 days of growth, the cells are transferred to an 8 L spinner flask and grown in a volume of 4L medium for 1 to 2 days. The 4 liters of cells are then divided into two 8 L flasks and grown for a further 1-2 days in 5.5 L volumes of medium in each 8 L flask. The cells from the two 8 L flasks are harvested, resuspended in 7 mL medium and used to inoculate a 110 L bioreactor. The cells are grown in 100 L of medium for a period of approx. 9 days. At the end of this growth period, the supernatant from the bioreactor is pumped into 100 L bags and refrigerated overnight. The cells are then removed from the harvested medium by filtration through a 10 μ m membrane cartridge followed by 1 μ m and 0.2 μ m cartridges. Since the cells have been allowed to settle overnight the final 5 to 10% of the harvest medium is discarded prior to filtration.

The development of a perfused packed-bed reactor using a bed matrix of a non-woven fabric has provided a means for maintaining a perfusion culture at densities exceeding 10^8 cells/ml of the bed volume (CelliGen™, New Brunswick Scientific, Edison, N.J.; Wang et al., Cytotechnology, 9:41-49 (1992); Wang et al., In:

Animal Cell Technology: Basic & Applied Aspects, S. Kaminogawa et al., (eds), vol. 5, pp463-469, Kluwer Academic Publishers, Netherlands (1993); Wang et al., Proceeding of the Japanese Society for Animal Cell Technology (1994)). In certain embodiments, the CHO cells are grown in a perfused attachment system in which
5 there is a continuous flow of medium at a steady rate, through or over a population of cells (of a physiological nutrient solution). More particularly, the transfected cells are grown in a cell culture process that is a perfusion-based process with collections continuing for up to 35 or more days with a collection rate of approximately 400 L per day from one 110 L bioreactor. Preferably, the collection rate is approximately 800 L
10 per day from one 110 L bioreactor.

The following table provides a comparison between an exemplary batch-fed process and a perfusion process for the production of recombinant sulfatase proteins from the transfected CHO cells.

Table: Summary Description of the Differences between the Fed Batch and Perfusion Processes

Production Step	Description (Batch)	Description (Perfusion)
CELL CULTURE	One batch is the result of one production run with two 110 L bioreactors.	Change: One batch is the result of one production run with one 110 L bioreactor
Thawing of WCB vial	One vial for each production batch. Tests: Expected cell viability > 95% Expected $\geq 1 \times 10^6$ cells recovered	Change: Cell Viability: > 90% Viability of > 90% on thaw has proven to yield successful runs and product that meets specifications
T75 cm ² flasks	Approximately 5×10^6 cells plated into 1 flask. Length of step: ~ 3 days Tests: Expected cell viability > 90% Expected $\geq 2 \times 10^7$ cells recovered	No Change
250 mL Spinner Flask	Cells from T75 is split onto a 250 mL spinner flask. Length of step: ~ 2 days Tests: Expected cell viability > 90% Expected $\geq 2 \times 10^8$ cells recovered	No Change
2 x 250 mL Spinner Flask	Cells from 1 x 250 mL spinner flask are split into two 250 mL spinner flask. Length of step: ~ 3 days Tests: Expected cell viability > 90% Expected $\geq 4 \times 10^8$ cells recovered	No Change
2 x 3 L Spinner Flask	Cells from 2 x 250 mL spinner flasks are split into two 3 L spinner flask Length of step: ~ 4 days Tests: Expected cell viability > 90% Expected $\geq 4.8 \times 10^9$ cells recovered	No Change
2 x 8 L Spinner Flask	Cells from 2 x 3 L spinner flasks are split into two 8 L spinner flask.	No Change

	Length of step: ~ 2 days Tests: Expected cell viability > 90% Expected $\geq 1.6 \times 10^{10}$ cells recovered	
Inoculation of culture flask	Inoculum of $\geq 0.8 \times 10^{10}$ cells are added to each of two bioreactors containing 32 L culture medium each. Culture monitored with PC-interfaced control system.	Change: Inoculum of $\geq 1.6 \times 10^{10}$ cells added to one bioreactor containing 64 L culture medium. (Reflects the use of one bioreactor versus two.)
Production	Growth: 3 days of growth until culture reaches density of $> 3.2 \times 10^{10}$ cells. Vertical Split: Culture media added to final volume of 95 L. Harvesting: Supernatant was harvested at day 11 or when cell viability fell below 70%. Supernatant is filtered and stored.	Changes: Growth/Transition/Harvesting: When cell density reaches $> 8 \times 10^{10}$ cells/mL, perfusion is started. The perfusion rate is gradually increased to 5 vessel volumes per day based on glucose levels. When the cell density is $> 1.28 \times 10^{12}$ the pH set point is adjusted to 7.35. Once increases in perfusion rates cease, glucose level is maintained by reducing cell density with a cell bleed. Harvested supernatant is collected and filtered.
Termination	Run is terminated at harvest.	Change: Run is terminated after 35 or more days is reached, or activity falls below 2 mg/L for three consecutive days or after adequate harvested supernatant is collected.

The inoculum preparation for scale-up process is the same for the fed batch and perfusion processes. In one embodiment, the recombinant cell culture is

initiated by thawing a single vial from the Working Cell Bank and transferring its contents (approximately 1 mL) to approximately 25 mL of EX-CELL 302 Medium (Modified w/ L-Glutamine, No Phenol Red) in a T75 cm² cell culture flask. In each expansion step, the cell culture is incubated until a viable cell count of approximately
5 0.8 x 10⁶ cells/mL is achieved. Each cell expansion step is monitored for cell growth (cell density) and viability (via trypan blue exclusion). All additions of EX-CELL 302 Medium (Modified w/ L-Glutamine, No Phenol Red) medium and cell transfers are preformed aseptically in a laminar flow hood. The cell culture is expanded sequentially from the T75 cm² flask to a 250 mL spinner flask, to two 250 mL
10 spinner flasks, to two 3 L spinner flasks, and finally to two 8 L spinner flasks. The entire scale-up process lasts approximately 14 days. When the two 8 L spinner flasks are at a density of at least 1.0 x 10⁶ cells/mL, the flasks are used to seed one 110 L bioreactor.

In specific embodiments, it is preferred that the bioreactor operations
15 for recombinant sulfatase expression or production or manufacture utilize a perfusion-based cell culture process. Preferably, the bioreactor, using the perfusion process, can control cell densities up to as high as 37 million cells per mL; compared to 4-5 million cells per mL using the fed batch process. Typically, the perfusion-based process runs longer (35 days) than the fed batch process (11 to 12 days) and produces a greater
20 volume of harvested cell culture fluid (approximately 400 L/day at a perfusion rate of 5 vessel volumes per day) compared to the fed batch process (190 L/run). Preferably, harvesting is performed up to 35 days for a total collection of approximately 8400 L of supernatant.

Throughout scaled-up production procedure from either the fed-batch
25 or the perfusion procedure, cell counts and viability determinations may be made. After the final removal of the cells from the harvested medium in either procedure, the cells are assessed for quality control purposes. The end of production cells are evaluated for genetic stability, identity, sterility and adventitious agent contamination per ICH guideline. Preferably, EPC results, obtained from a 35-day long bioreactor
30 run, AC60108, produced under cGMP conditions, show no growth or negative results or no detection of the presence of bacteria and fungi, mycoplasma, adventitious viral contaminants, murine viruses, or like contaminants or particles.

B. Purification of Protein Product from Cell Culture

It will be desirable to purify the recombinant arylsulfatase proteins from the supernatant of the cell culture. Such purification may employ purification techniques that are well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the cellular milieu to polypeptide and non-polypeptide fractions. Having separated the recombinant proteins from other CHO and other culture derived proteins, the sulfatase proteins of interest may be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). Analytical methods particularly suited to the preparation of a pure peptide are ion-exchange chromatography, exclusion chromatography; polyacrylamide gel electrophoresis; isoelectric focusing. Particularly efficient methods of purifying peptides include fast protein liquid chromatography (FPLC) and high performance liquid chromatography (HPLC).

Certain aspects of the present invention concern the purification, and in particular embodiments, the substantial purification, of an encoded polypeptide, protein or peptide. The term "purified polypeptide, protein or peptide" as used herein, is intended to refer to a composition, isolated from other components, wherein the polypeptide, protein or peptide is purified to any degree relative to its naturally-obtainable state. A purified polypeptide, protein or peptide therefore also refers to a polypeptide, protein or peptide, free from the environment in which it may naturally occur.

Generally, "purified" will refer to a polypeptide, protein or peptide composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term "substantially purified" is used, this designation will refer to a composition in which the polypeptide, protein or peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or more of the proteins in the composition. The quality of the recombinant enzyme is key to patients. In preferred embodiments, the recombinant enzyme is substantially pure to a purity level greater than 90%, preferably greater than 91%, greater than 92%, greater than 93%, greater than 94%. More preferably, the recombinant protein is at least 95% and does not contain

contaminants from the CHO, such as CHO proteins. In particularly preferred embodiments, the recombinant protein is at least 96%, at least 97%, at least 98%, at least 99% pure. Preferably the protein is 99.2% pure, 99.4% pure, 99.5% pure, 99.6% pure, 99.8% pure or 100% pure.

5 Various techniques suitable for use in protein purification will be well known to those of skill in the art. These include, for example, precipitation with ammonium sulphate, PEG, antibodies and the like or by heat denaturation, followed by centrifugation; chromatography steps such as ion exchange, gel filtration, reverse phase, hydroxylapatite and affinity chromatography; isoelectric focusing; gel
10 electrophoresis; and combinations of such and other techniques. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified polypeptide, protein or peptide.

15 In exemplary embodiments, the proteins are purified using a combination of chromatography techniques. The cellular milieu is separated from the supernatant to prevent fouling of the columns. Preferably, the growth medium containing the recombinant enzyme is passed through an ultrafiltration and diafiltration step. In one method, the filtered solution is passed through a DEAE
20 Sephacel chromatography column, then a Blue Sepharose chromatography column, then a Cu⁺⁺ Chelating Sepharose chromatography column, and then a Phenyl Sepharose chromatography column. Such a four step column chromatography including using a DEAE Sepharose, a Blue Sepharose, a Cu⁺⁺ Chelating Sepharose and a Phenyl Sepharose chromatography column sequentially results in especially
25 highly purified recombinant enzyme. Those of skill in the art appreciate that one or more chromatography steps may be omitted or substituted or the order of the steps altered within the scope of the present invention. In other preferred embodiments, the eluent from the final chromatography column is ultrafiltered/diafiltered, and an appropriate step is performed to remove any remaining viruses. Finally, appropriate
30 sterilizing steps may be performed as desired.

 In a preferred embodiment, the ultrafiltration/diafiltration step is performed with a sodium phosphate solution of about 10 mM and with a sodium chloride solution of about 100 mM at a pH of about 7.3. In another embodiment, the

DEAE Sepharose chromatography step is performed at a pH of about 7.3 wherein the elute solution is adjusted with an appropriate buffer, preferably a sodium chloride and sodium phosphate buffer. In additional preferred embodiments, the Blue Sepharose chromatography step is performed at a pH of about 5.5 wherein the elute solution is adjusted with an appropriate buffer, preferably a sodium chloride and sodium acetate buffer. Also, in preferred embodiments, the Cu⁺⁺ Chelating Sepharose chromatography step is performed with an elution buffer including sodium chloride and sodium acetate. In especially preferred embodiments, a second ultrafiltration/diafiltration step is performed on the eluate from the chromatography runs wherein the recombinant enzyme is concentrated to a concentration of about 1 mg/ml in a formulation buffer such as a sodium chloride and sodium phosphate buffer to a pH of about 5.5 to 6.0, most preferably to a pH of 5.8. Phosphate buffer is a preferred buffer used in the process because phosphate buffer prevents critical degradation and improves the stability of the enzyme.

More particularly, in step 1, the initial purification of the recombinant sulfatases comprises an ultrafiltration/diafiltration step in which filtered harvest fluid (e.g., the filtered supernatant from the bulk cell culture described above) is concentrated ten fold and then diafiltered with 5 volumes of 10 mM Sodium Phosphate, 100 mM NaCl, pH 7.3 using a tangential flow filtration (TFF) system. In step 2, the diafiltrate is then subjected to a first chromatographic separation on a DEAE Sepharose FF (flow through) column. Prior to loading, the DEAE sepharose column is prewashed and equilibrated: it is prewashed with buffer 1 (0.1 N NaOH) and then buffer 2 (100 mM NaPO₄ pH 7.3). The column is then equilibrated with an equilibration buffer (100 mM NaCl, 10 mM NaPO₄, pH 7.3). Once the column has been equilibrated, the product from the ultrafiltration/diafiltration step is loaded onto the column and washed (wash buffer: 100 mM NaCl, 10mM NaPO₄, pH 7.3). Once washed, the protein is eluted from the column with a strip buffer (1 M NaCl, 10 mM NaPO₄, pH 7.3). The column is then sanitized (sanitization buffer: 0.5 N NaOH) and stored (storage buffer: 0.1 N NaOH).

Meanwhile, in step 3, the protein eluted from the DEAE Sepharose FF column in step 2 is applied to a second chromatographic separation on a Blue Sepharose FF. This column is prewashed with 0.1 N NaOH, followed by a washing step with distilled water and a third prewash with 1 M NaAc, pH 5.5. This washed

column is equilibrated with 150 mM NaCl, 20 mM NaAc, pH 5.5, before the DEAE Sepharose FF column flow through is loaded. After the protein has been loaded onto the column, the loaded column is washed (wash buffer: 150 mM NaCl, 20 mM NaAc, pH 5.5) and the protein is eluted (elution buffer: 500 mM NaCl, 20 mM NaAc, pH 5.5). After elution, the Blue Sepharose column is regenerated (regeneration buffer: 1 M NaCl, 20 mM NaAc, pH 5.5); sanitized (sanitization buffer: 0.1 N NaOH, 0.5-2 hours) and stored (storage buffer: 500 mM NaCl, 20 mM NaAc, pH 5.5, 20% ETOH).

The eluted protein from the Blue Sepharose column in step 3 is subjected to a fourth chromatographic separation in which the eluate from step 3 is subjected to metal ion chelation chromatography on a Cu⁺⁺ Chelating Sepharose FF chromatography column. Prior to application of the eluate from step 3, the Cu⁺⁺ column is sanitized with 0.1 N NaOH, and washed with water. The sepharose column is then charged with metal ions using a solution of 0.1 M Copper Sulfate and equilibrated in an appropriate buffer (20 mM NaAc, 0.5 M NaCl, 10% Glycerol, pH 6.0). Once the Cu⁺⁺ column is thus prepared, the eluate from step 3 is loaded onto the column and washed with an initial wash buffer (20 mM NaAc, 0.5 M NaCl, 10% Glycerol, pH 6.0), the loaded column is then washed with a second wash buffer (20 mM NaAc, 1 M NaCl, 10% Glycerol, pH 4.0), followed by a third washing step (20 mM NaAc, 1 M NaCl, 10% Glycerol, pH 3.8). After washing, the protein is eluted (elution buffer: 20 mM NaAc, 1 M NaCl, 10% Glycerol, pH 3.6). The column is then stripped of any remaining metal ions using a strip buffer (50 mM EDTA, 1 M NaCl); sanitized (sanitization Buffer: 0.5 N NaOH, 0.5-2 hours) and stored (storage buffer: 0.1 N NaOH).

In a fifth step, the eluted proteins from the Cu⁺⁺ column are subjected to a fourth chromatographic separation in which the eluate is applied to a Phenyl Sepharose HP column. Prior to loading of the eluate from step 4, the phenyl sepharose HP column is prewashed with 0.1 N NaOH followed by distilled water and then equilibrated (equilibration buffer: 3 M NaCl, 20 mM NaAc, pH 4.5). The Cu⁺⁺ Chelating Sepharose eluate is then loaded onto the column and washed initially with buffer 1 (3.0 M NaCl, 20 mM NaAc, pH 4.5) and then with buffer 2 (1.5 M NaCl, 20 mM NaAc, pH 4.5). After washing, the proteins are eluted (elution buffer: 1.0 M NaCl, 20 mM, NaAc, pH 4.5). The column is then stripped (strip buffer: 0 M NaCl,

20 mM NaAc, pH 4.5), sanitized (sanitization Buffer: 0.5 N NaOH) and stored (storage Buffer: 0.1 N NaOH).

In step 6, the recombinant protein is concentrated and diafiltered to a final concentration of 1.5 mg/ml in formulation buffer (150 mM NaCl, 10 mM NaPO₄, pH 5.8) using a TFF system. If necessary, this preparation may be diluted in formulation buffer to a concentration of 1.0mg/ml. The preparation is then sterilized and the viral load reduced. Typically, the sterilized bulk drug substance can be sterilized through a 0.04 micron or preferably a 2 micron filter in a class 100 laminar flow hood into Type 1 glass vials. The vials may be filled to a final volume of about 5mL using a semi-automatic liquid filling machine. The vials may then be manually stoppered, sealed and labeled. In specific embodiments, the vials contain 1mg/mL of the sulfatase along with excipients sodium phosphate, monobasic, 1 H₂O (9mM), sodium phosphate, dibasic, 7 H₂O (1mM) and sodium chloride (150mM).

It is expressly contemplated that the vanadate-dependent photoreaction may be carried out at any stage during this purification process. Preferably, the photoreaction is carried out immediately prior to placing the sulfatase drug product into the vials.

Another preferred method of purifying recombinant sulfatase protein comprises: (a) obtaining a fluid containing the sulfatase; (b) contacting the fluid with a Cibracon blue dye interaction chromatography resin; (c) contacting the fluid with a copper chelation chromatography resin; (d) contacting the fluid with a phenyl hydrophobic interaction chromatography resin; and (e) recovering the sulfatase protein. Preferable, steps (b), (c) and (d) are performed sequentially. This method requires no more than three chromatography steps or columns. In order to obtain highly purified sulfatase no further chromatography steps or columns are required. Thus, the method does not comprise the fluid contacting a DEAE Sepharose resin. The recovered recombinant protein preferably has a purity of at least equal to or greater than 95%. The overall recovery yield can be at least about 40-60%.

Preferably, step (b) comprises passing the fluid through a Cibracon blue dye interaction chromatography column. More preferably, the Cibracon blue dye interaction chromatography column is a Blue Sepharose 6 Fast Flow column. Preferably, step (c) comprises passing the fluid through a copper chelation

chromatography column. More preferably, the copper chelation chromatography column is a Chelating Sepharose Fast Flow column. Preferably, step (d) comprises passing the fluid through a phenyl hydrophobic interaction chromatography column. More preferably, the phenyl hydrophobic interaction chromatography column is a Phenyl Sepharose 6 Fast Flow High Sub column. Preferably, the temporal sequence of steps (b), (c) and (d) is step (b), step (c) and step (d).

The recovering step can comprise ultrafiltration and/or diafiltration of the fluid. The recovering can comprise filtering the fluid to remove DNA and/or filtering the fluid to remove virus. The filtering, for removing virus, can comprise passing said fluid through a 0.02 μm filter.

The purity of the recombinant sulfatase is typically measured or determined using reverse-phase high performance liquid chromatography ("RP-HPLC"), which separates proteins based on differences in hydrophobicity. In a preferred method, a C4 column (Phenomenex Jupiter) is used as the stationary phase and a gradient of water:acetonitrile as the mobile phase. The protein samples are initially injected onto the column in water; under these conditions, all proteins will bind to the column. A gradually increasing concentration of acetonitrile is then infused through the column. This acetonitrile gradient increases the hydrophobicity of the mobile phase, to the point where individual proteins become soluble in the mobile phase and elute from the column. These elution times are accurately reproducible for each individual protein in a mixture. Proteins are detected as peaks on a chromatogram by ultraviolet absorbance at 210 nm. The areas of each peak are calculated, and the sample purity can be calculated as the ratio of the recombinant sulfatase protein peak area to the total area of all peaks in the chromatogram. RP-HPLC is a proven high-resolution, reproducible method of determining the purity of recombinant sulfatases such as recombinant human arylsulfatase B. ELISA also may be used to determine the purity of the proteins.

METHODS OF TESTING ACTIVATED SULFATASES

The sulfatases produced and purified herein may be tested for their activity in vitro and efficacy in vivo.

A. In vitro assays

In vitro assays for determining the efficacy of any of the sulfatases are well known to those of skill in the art (see Cosma et al., Cell 113:445-456, 2003). Such assays typically are enzymes assays that determine the cleavage of sulfate esters from a variety of substrates including e.g., glycosaminoglycans, sulfolipids and steroid sulfates (Parenti et al., 1997; Hopwood and Ballabio, 2001). Exemplary such assays for arylsulfatases A, B, C and E are provided in Cosma et al., (Cell 113:445-456, 2003) another exemplary assays for a C69S arylsulfatase mutant protein activity is provided in Recksiek et al., J. Biol. Chem., 273(11):6096-6103, 1998). Additional assays are exemplified in Example 1.

Such assays typically incubate the protein composition in the presence of a substrate to be cleaved. The cleavage of the substrate is then monitored using spectrophotometry or fluorometry. Alternatively, the substrate may be one, which is radiolabeled, and as such the detection of the cleavage products may be achieved through detection of the radioactive label. In certain embodiments, the cleavage products may be separated using chromatography, e.g., thin layer chromatography, HPLC and the like.

The assays may be varied depending on the particular protein and substrate being tested.

B. In vivo efficacy

As discussed above, the sulfatases are involved in a number of different lysosomal storage disorders. The following table provides examples of such diseases and the associated enzymes.

<u>Lysosomal Storage Disease</u>	<u>Protein deficiency</u>
Mucopolysaccharidosis type II Hunter syndrome	Iduronate-2-sulfatase
Mucopolysaccharidosis type IIIA Sanfilippo syndrome	Heparan-N-sulfatase
Mucopolysaccharidosis type IIID Sanfilippo syndrome sulfatase	N-Acetylglucosamine 6-
Mucopolysaccharidosis type IVA Morquio syndrome	Galactose 6-sulfatase
Mucopolysaccharidosis type VI sulfatase	N-Acetylgalactosamine 4-

Metachromatic leukodystrophy

Arylsulfatase A

Multiple sulfatase deficiency

Multiple sulfatases

5 The recombinant activated protein of the present invention will be tested in vivo in animal models and in a clinical setting.

Canine and feline models for diseases such as MPS VI and MPS I have been developed. (See detailed examples in U.S. Patent Application No. 10/704,365, filed November 7, 2003; see also Jezyk, Science 198:834-36 1977; Haskins, et al., Am. J. Pathol. 101:657-674 (1980); Haskins, et al., J. Am. Vet. Med. Assoc. 182:983-10 985 (1983); Konde, et al., Vet. Radiol. 28:223-228 (1987; Konde, et al., Vet. Radiol. 28:223-228 (1987). For example, such models may be treated with a dosage of a given enzyme ranging from about 0.1 to about 10mg/kg, more preferably between about 1 mg/kg and about 5 mg/kg and more particularly at about 2 mg/kg and clinical parameters of the disease are monitored. Studies also may be performed to compare 15 enzyme distribution, clearance of tissue glycosaminoglycan storage, sulfatide storage and decrease of urinary glycosaminoglycans or sulfatide levels after bolus and slow (2 hour) infusion. Other symptoms that may be monitored include facial dysmorphism, Diffuse corneal clouding, epiphyseal dysplasia, Subluxations, Pectus excavatum, reduced body weight, reduced spine flexibility, hind limb gait, hind limb paralysis, 20 and the like. Other biochemical/morphological determinations indicate that by 35 days, organs of untreated cats have maximal storage of glycosaminoglycans in tissues (Crawley, et al., J. Clin. Invest. 99:651-662 (1997)). Urinary glycosaminoglycan levels are elevated at birth in both normal and MPS VI cats but after approximately 40 days, normal cats have decreased levels. Any alleviation of these symptoms will be 25 useful in the present invention. In preferred embodiments, the recombinant enzyme should produce an improvement in urinary sulfatides, body weight/growth, bone morphometry and clearance of stored material (i.e., the material that is stored in the lysosomal disorder associated with the particular enzyme deficiency) from several tissues relative to these parameters in the absence of the treatment. The dosage ranges 30 and efficacy parameters identified from such animal studies may be used for the development of clinical protocols in humans

Moreover, many related enzymes are already in human clinical trials and as such, the protocols used in those clinical trials will be effective in testing the compositions of the present invention. Typically, the clinical development program for the recombinant sulfatase will comprise an initial open-label clinical trial that will provide an assessment of weekly infusions of the enzyme for safety, pharmacokinetics, and initial response of both surrogate and defined clinical endpoints. Such evaluation will be conducted for a minimum of three months to collect sufficient safety information for 5 evaluable patients. At this time, should the initial dose of 1 mg/kg not produce a reasonable reduction in excess urinary glycosaminoglycans or produce a significant direct clinical benefit, the dose will be doubled and maintained for an additional three months to establish safety and to evaluate further efficacy.

In this manner it may be demonstrated that periodic e.g., weekly, biweekly or monthly infusions of the recombinant mutant sulfatase in patients with the disorder in which the enzyme is lacking will be effective in the treatment of the disorder. Measurements of safety will include adverse events, immune response and allergic reactions (complement activation, antibody formation to recombinant enzyme), complete clinical chemistry panel (kidney and liver function), urinalysis, and CBC with differential.

In addition, the efficacy of the enzyme replacement therapy may be evaluated by monitoring changes in several parameters known to be affected in patients with the given lysosomal storage disease. These include for example, a timed walk test or stair climb (as a measure of exercise tolerance), full pulmonary function (PFT) evaluation, reduction in levels of urinary sulfatides or other storage products produced due to lack of specific enzyme involved and hepatomegaly (as measures of kidney, CNS and liver storage of sulfatides or other storage products produces due to the specific enzyme deficiency), growth velocity, joint range of motion, Children's Health Assessment Questionnaire (CHAQ), visual acuity, cardiac function, sleeping studies, and two different global assessments; one performed by the investigator, one performed by the patient/caregiver. A second secondary objective is to determine pharmacokinetic parameters of infused drug in the circulation, and general distribution and half-life of intracellular enzyme using leukocytes and buccal tissue as

sources of tissue. It is anticipated that these measures will help relate dose to clinical response based on the levels of enzyme delivered to the lysosomes of cells.

Typically, the subjects will be admitted for a two week baseline evaluation that will include a medical history and physical exam, psychological testing, endurance testing (treadmill), a standard set of clinical laboratory tests (CBC, Panel 20, CH50, UA), a MRI or CAT scan of the body (liver and spleen volumetric determination, bone and bone marrow evaluation, and lymph node and tonsillar size), a cardiology evaluation (echocardiogram, EKG, CXR), an airway evaluation (pulmonary function tests), a sleep study to evaluate for obstructive events during sleep, a joint restriction analysis (range of motion will be measured at the elbows and interphalangeal joints), a LP with CNS pressure, and biochemical studies (buccal sulfatase activity on two occasions, leukocyte sulfatase activity on two occasions, urinary sulfatide on three occasions, serum generation for ELISA of anti-sulfatase antibodies and 24 hour urine for creatinine clearance). In addition to the above evaluations, each patient will be photographed and videotaped performing some physical movements such as attempting to raise their hands over their heads and walking. Patients will be titrated with antihistamines such that pretreatment with these agents could be effectively employed prior to infusion of enzyme. An exemplary proposed human dose of 1 mg/kg (50 U/kg) or 2mg/kg (100U/kg) will be administered weekly by i.v. infusion over 4 hours. The patient will remain in the hospital for the first two weeks, followed by short stays for the next four weeks. Treatment for the final six weeks will be conducted at a facility close to the patient's home. Patients will return to the hospital for a complete evaluation at three months. Should dose escalation to higher doses be required, the patients will follow the same schedule outlined above for the first twelve weeks. Under either scenario, a complete evaluation will also occur at 6 months from the time of entering the trial. Safety will be monitored throughout the trial. Patients completing the trial will be continued on therapy following an extended protocol for as long as safety and efficacy conditions warrant it until BLA approval.

A single patient will be enrolled at the onset of the trial, with two additional patients one month later, and two more patients two weeks later barring any unforeseen complications related to treatment. Additional patients will be admitted

should any of the enrolled patients become critically ill, or if a child is in need of an acute clinical procedure for life threatening or harmful conditions.

The patient may be male or female, aged five years or older with a documented diagnosis of the lysosomal storage disease to be treated confirmed by measurable clinical signs and symptoms of that disease, and supported by a diminished fibroblast or leukocyte enzyme activity level of the sulfatase enzyme in question. Female patients of childbearing potential must have a negative pregnancy test (urine β -hCG) just prior to each dosing and must be advised to use a medically accepted method of contraception throughout the study. A patient will be excluded from this study if the patient has previously undergone bone marrow transplantation; is pregnant or lactating; has received an investigational drug within 30 days prior to study enrollment; or has a medical condition, serious intercurrent illness, or other extenuating circumstance that may significantly decrease study compliance.

Patients will receive the recombinant sulfatase enzyme at a dose of e.g., 1 mg/kg (~50 U/kg) for the first 3 months of the study. In the event that excess urine sulfatides are not decreased by a reasonable amount and no clinical benefit is observed, the dose will be doubled. Dose escalation will occur only after all 5 patients have undergone 3 months of therapy. This recombinant enzyme dosage form will be administered intravenously over approximately a four-hour period once weekly for a minimum of 12 consecutive weeks. A peripheral intravenous catheter will be placed in the cephalic or other appropriate vein and an infusion of saline begun at 30 cc/hr. The patient will be premedicated with up to 1.25 mg/kg of diphenylhydramine intravenously based on titration experiments completed prior to the trial. The recombinant enzyme is diluted into 100 cc of normal saline supplemented with 1 mg/ml human albumin. The diluted enzyme is then infused at 1 mg/kg (about 50 units per kg) over a 4 hour period with cardiorespiratory and pulse oximeter monitoring. The patients are monitored clinically as well as for any adverse reaction to the infusion. If any unusual symptoms are observed, including but not limited to malaise, shortness of breath, hypoxemia, hypotension, tachycardia, nausea, chills, fever, and abdominal pain, the infusion will be stopped immediately. Based on clinical symptoms and signs, an additional dose of diphenylhydramine, oxygen by mask, a bolus of intravenous fluids or other appropriate clinical interventions such as steroid treatment may be administered. If an acute reaction does occur, an assessment

for the consumption of complement in the serum will be tested. A second i.v. site will be used for the sampling required for pharmacokinetic analysis.

The data from any given patient will be considered evaluable as long as no more than two non-sequential infusions are missed during the 12 weeks of therapy. The initial, midpoint and final evaluations must be completed.

The enzyme therapy is determined to be safe if no significant acute reactions occur that cannot be prevented by altering the rate of administration of the enzyme, or acute antihistamine or steroid use. The longer-term administration of the enzyme will be determined to be safe if no significant abnormalities are observed in the clinical examinations, clinical labs, or other appropriate studies. The presence of antibodies or complement activation will not by themselves be considered unsafe, but such antibodies will require monitoring by ELISA, and by clinical assessments of possible immune complex disease.

Improvements in the surrogate and clinical endpoints are expected as a result of delivery of enzyme and removal of glycosaminoglycan storage from the body. Dose escalation can be performed if mean excess levels urinary glycosaminoglycans, sulfatides and other products of the lysosomal storage disease are not reduced by a reasonable amount over three months and no significant clinical benefit is observed at 3 months. Improvements should include improved airway index or resolution of sleep apnea, improved joint mobility, and increased endurance.

In specific embodiments, the enzyme replacement therapy will advantageously result in normalization of liver volume and urinary glycosaminoglycan excretion, reduction in spleen size and apnea/hypopnea events, increase in height and growth velocity in prepubertal subjects, increase in shoulder flexion and elbow and knee extension, and reduction in tricuspid regurgitation or pulmonic regurgitation. For methods of monitoring such effects, those of skill in the art are specifically referred to Example 5 of U.S. Patent No. 6,585,971 for assessing such effects.

In specific embodiments, the therapeutic administering of the recombinant sulfatase involves administration of human recombinant enzyme, which reduces lysosomal storage in cells of the individual having the lysosomal storage disease. In such embodiments, the composition being delivered comprises about 1 mg

recombinant enzyme/20 kg of body weight of the mammal being treated for the disorder.

METHODS OF ENHANCING UPTAKE OF SULFATASES

5 In the methods of the present invention, it may be preferable to ensure that the enzyme being administered to the subject is one, which comprises a moiety that may be readily taken up by a high affinity uptake receptor on the surface of a cell. For example, such a receptor may be the mannose-6-phosphate receptor and the enzyme may be modified to comprise up to about an average of about at least 20%
10 bis-phosphorylated oligosaccharides per enzyme. In other embodiments, the enzyme may comprise 10%, 15%, 18%, 20%, 25%, 30%, 35%, 40%, 45% bis-phosphorylated oligosaccharides per enzyme. While such bis-phosphorylated oligosaccharides may be naturally present on the enzyme, it should be noted that the enzymes may be modified to possess such oligosaccharides. For example, those of skill in the art are
15 aware of enzymes, which are capable of catalyzing the transfer of N-acetylglucosamine-L-phosphate from UDP-GlcNAc to the 6' position of α -1,2-linked mannoses on lysosomal enzymes. Methods and compositions for producing and using such enzymes are described by, for example, Canfield et al. in U.S. Patent No. 6,537,785, and U.S. Patent No. 6,534,300, each incorporated herein by reference.

20 In other embodiments, the lysosomal enzymes for use in the present invention may be conjugated to a RAP and RAP polypeptides, which selectively bind to LRP receptors such as megalin that may be present on cells. As such, these RAP or other megalin ligand molecules will serve to increase the transport of the lysosomal enzyme across the blood brain barrier and/or deliver agents to lysosomes of cells
25 within the CNS. Methods and compositions for preparing enzyme compositions that comprise RAP moieties attached thereto are described in detail in U.S. Patent Application No. 10/206,448, filed on July 25, 2002 and in U.S. Patent Application No. 10/600,862, filed June 20, 2003, each incorporated herein by reference.

30 In still a further embodiment, those of skill in the art may employ a delivery of the enzyme conjugated to melanotransferrin (p97) as described in e.g., U.S. Patent No. 6,455,494 and U.S. Patent No. 5,981,194, each incorporated herein by reference. Of course the above agents that enhance the delivery and/or uptake of

therapeutic agents are merely exemplary and those of skill in the art will be aware of other receptors, ligands or other agents that may be used in a similar context to deliver a therapeutic agent across the brain-CSF interface, transcytosis across an endothelial layer or even the BBB.

5

PHARMACEUTICAL COMPOSITIONS

Pharmaceutical compositions for administration according to the present invention can comprise the recombinant enzymes described herein either alone or e.g., conjugated to moiety that facilitates the transcytosis of the sulfatase enzyme. The pharmaceutical compositions also may include additional therapeutic agents for the treatment of the given disease being treated. Regardless of whether the active component of the pharmaceutical composition is an enzyme alone, an enzyme conjugated to an uptake moiety, or the combination of either or both of these former entities with yet another therapeutic composition, each of these preparations is in some aspects provided in a pharmaceutically acceptable form optionally combined with a pharmaceutically acceptable carrier. These compositions are administered by any methods that achieve their intended purposes. Individualized amounts and regimens for the administration of the compositions for the treatment of the given disorder are determined readily by those with ordinary skill in the art using assays that are used for the diagnosis of the disorder and determining the level of effect a given therapeutic intervention produces.

It is understood that the suitable dose of a composition according to the present invention will depend upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. However, the dosage is tailored to the individual subject, as is understood and determinable by one of skill in the art, without undue experimentation. This typically involves adjustment of a standard dose, e.g., reduction of the dose if the patient has a low body weight.

The total dose of therapeutic recombinant sulfatase enzyme may be administered in multiple doses or in a single dose. In certain embodiments, the compositions are administered alone, in other embodiments the compositions are

administered in conjunction with other therapeutics directed to the disease or directed to other symptoms thereof.

In some aspects, the compositions of the invention are formulated into suitable pharmaceutical compositions, i.e., in a form appropriate for in vivo applications in the therapeutic intervention of a given disease. Generally, this will entail preparing compositions that are essentially free of pyrogens, as well as other impurities that could be harmful to humans or animals. In some aspects, the compositions are prepared for administration directly to the lung. These formulations are for oral administration via an inhalant, however, other routes of administration are contemplated (e.g. injection and the like). An inhaler device is any device useful in the administration of the inhalable medicament. Examples of inhaler devices include nebulizers, metered dose inhalers, dry powder inhalers, intermittent positive pressure breathing apparatuses, humidifiers, bubble environments, oxygen chambers, oxygen masks and artificial respirators.

In other embodiments, the enzyme is administered by introduction into the central nervous system of the subject, *e.g.*, into the cerebrospinal fluid of the subject. In certain aspects of the invention, the enzyme is introduced intrathecally, *e.g.*, into the lumbar area, or the cisterna magna or intraventricularly into a cerebral ventricle space. Those of skill in the art are aware of devices that may be used to effect intrathecal administration of a therapeutic composition. For example, the therapy may be given using an Ommaya reservoir, which is in common use for intrathecally administering drugs for meningeal carcinomatosis (Lancet 2: 983-84, 1963). More specifically, in this method, a ventricular tube is inserted through a hole formed in the anterior horn and is connected to an Ommaya reservoir installed under the scalp, and the reservoir is subcutaneously punctured to intrathecally deliver the particular enzyme being replaced, which is injected into the reservoir. Other devices for intrathecal administration of therapeutic compositions to an individual are described in U.S. Patent No. 6,217,552, incorporated herein by reference.

Alternatively, the drug may be intrathecally given, for example, by a single injection, or continuous infusion. It should be understood that the dosage treatment may be in the form of a single dose administration or multiple doses.

As used herein, the term "intrathecal administration" is intended to include delivering a pharmaceutical composition directly into the cerebrospinal fluid

of a subject, by techniques including lateral cerebroventricular injection through a burrhole or cisternal or lumbar puncture or the like (described in Lazorthes *et al.* *Advances in Drug Delivery Systems and Applications in Neurosurgery*, 143-192 and Omayya *et al.*, *Cancer Drug Delivery*, 1: 169-179, the contents of which are

5 incorporated herein by reference). The term "lumbar region" is intended to include the area between the third and fourth lumbar (lower back) vertebrae and, more inclusively, the L2-S1 region of the spine. The term "cisterna magna" is intended to include access to the space around and below the cerebellum via the opening between the skull and the top of the spine. The term "cerebral ventricle" is intended to include

10 the cavities in the brain that are continuous with the central canal of the spinal cord. Administration of a pharmaceutical composition in accordance with the present invention to any of the above mentioned sites can be achieved by direct injection of the composition or by the use of infusion pumps. For injection, the composition of the invention can be formulated in liquid solutions, preferably in physiologically

15 compatible buffers such as Hank's solution, Ringer's solution or phosphate buffer. In addition, the enzyme may be formulated in solid form and re-dissolved or suspended immediately prior to use. Lyophilized forms are also included. The injection can be, for example, in the form of a bolus injection or continuous infusion (e.g., using infusion pumps) of the enzyme.

20 In another embodiment of the method of the invention, the pharmaceutically acceptable formulation provides sustained delivery, e.g., "slow release" of the enzyme or other pharmaceutical composition used in the present invention, to a subject for at least one, two, three, four weeks or longer periods of time after the pharmaceutically acceptable formulation is administered to the subject.

25 As used herein, the term "sustained delivery" is intended to include continual delivery of a pharmaceutical composition of the invention in vivo over a period of time following administration, preferably at least several days, a week or several weeks. Sustained delivery of the composition can be demonstrated by, for example, the continued therapeutic effect of the enzyme over time (e.g., sustained

30 delivery of the enzyme can be demonstrated by continued reduced amount of storage granules in the subject). Alternatively, sustained delivery of the enzyme may be demonstrated by detecting the presence of the enzyme in vivo over time.

The pharmaceutical formulation used in the method of the invention contains a therapeutically effective amount of an enzyme for use in enzyme replacement therapy of a lysosome storage disease. Such a therapeutically effective amount is any amount effective, at dosages and for periods of time necessary, to achieve the desired result. In preferred embodiments, the compositions comprises a therapeutically effective amount of a sulphatase such as arylsulfatase B. A therapeutically effective amount of a sulfatase may vary according to factors such as the disease state, age, and weight of the subject, and the ability of the enzyme (alone or in combination with one or more other agents) to elicit a desired response in the subject. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition are outweighed by the therapeutically beneficial effects. A non-limiting range for a therapeutically effective concentration of enzyme is 0.001 μg enzyme/ml to about 150 μg enzyme/ml. It is to be noted that dosage values may vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the enzyme replacement therapy and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed invention.

One will generally desire to employ appropriate salts and buffers to render the compositions stable and allow for uptake of the compositions at the target site. Generally, the pharmaceutical compositions of the invention are provided in lyophilized form to be reconstituted prior to administration. Alternatively, the pharmaceutical compositions may be formulated into tablet form. Buffers and solutions for the reconstitution of the pharmaceutical compositions may be provided along with the pharmaceutical formulation to produce aqueous compositions of the present invention for administration. Such aqueous compositions will comprise an effective amount of each of the therapeutic agents being used, dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. Such compositions also are referred to as inocula. The phrase "pharmaceutically or pharmacologically acceptable" refer to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As

used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any
5 conventional media or agent is incompatible with the therapeutic compositions, its use in therapeutic compositions is contemplated. Supplementary active ingredients also are incorporated into the compositions.

Methods of formulating the therapeutic enzymes for therapeutic administration also are known to those of skill in the art. Administration of these
10 compositions according to the present invention will be via any common route so long as the target tissue is available via that route. Most commonly, these compositions are formulated for oral administration, such as by an inhalant. However, other conventional routes of administration, e.g., by subcutaneous, intravenous, intradermal, intramuscular, intramammary, intraperitoneal, intrathecal, intraocular, retrobulbar,
15 intrapulmonary (e.g., term release), aerosol, sublingual, nasal, anal, vaginal, or transdermal delivery, or by surgical implantation at a particular site also is used particularly when oral administration is problematic. The treatment may consist of a single dose or a plurality of doses over a period of time.

In certain embodiments, the active compounds are prepared for
20 administration as solutions of free base or pharmacologically acceptable salts in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions also are prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

25 The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the
30 contaminating action of microorganisms, such as bacteria and fungi. In some aspects, the carrier is a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity is

maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

The prevention of the action of microorganisms is brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions is brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization.

Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients also are incorporated into the compositions.

In some aspects, the compositions of the present invention are formulated in a neutral or salt form. Pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids; or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups also are derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like. For parenteral
5 administration in an aqueous solution, for example, the solution is suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration.

"Unit dose" is defined as a discrete amount of a therapeutic
10 composition dispersed in a suitable carrier. In certain embodiment, parenteral administration of the therapeutic compounds is carried out with an initial bolus followed by continuous infusion to maintain therapeutic circulating levels of drug product. Those of ordinary skill in the art will readily optimize effective dosages and administration regimens as determined by good medical practice and the clinical
15 condition of the individual patient.

The frequency of dosing will depend on the pharmacokinetic parameters of the agents and the routes of administration. The optimal pharmaceutical formulation will be determined by one of skill in the art depending on the route of administration and the desired dosage. Such formulations may influence
20 the physical state, stability, rate of in vivo release and rate of in vivo clearance of the administered agents. Depending on the route of administration, a suitable dose is calculated according to body weight, body surface areas or organ size. The availability of animal models is particularly useful in facilitating a determination of appropriate dosages of a given therapeutic. Further refinement of the calculations
25 necessary to determine the appropriate treatment dose is routinely made by those of ordinary skill in the art without undue experimentation, especially in light of the dosage information and assays disclosed herein as well as the pharmacokinetic data observed in animals or human clinical trials.

Typically, appropriate dosages are ascertained through the use of
30 established assays for determining blood levels in conjunction with relevant dose response data. The final dosage regimen will be determined by the attending physician, considering factors which modify the action of drugs, e.g., the drug's specific activity, severity of the damage and the responsiveness of the patient, the age,

condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. As studies are conducted, further information will emerge regarding appropriate dosage levels and duration of treatment for specific diseases and conditions.

5 It will be appreciated that the pharmaceutical compositions and treatment methods of the invention are useful in fields of human medicine and veterinary medicine. Thus the subject to be treated is a mammal, such as a human or other mammalian animal. For veterinary purposes, subjects include for example, farm animals including cows, sheep, pigs, horses and goats, companion animals such as
10 dogs and cats, exotic and/or zoo animals, laboratory animals including mice rats, rabbits, guinea pigs and hamsters; and poultry such as chickens, turkey ducks and geese.

 The present invention also contemplated kits for use in the treatment of various disorders. Such kits include at least a first composition comprising the
15 sulfatases described above in a pharmaceutically acceptable carrier. Another component is a second therapeutic agent for the treatment of the disorder along with suitable container and vehicles for administrations of the therapeutic compositions. The kits may additionally comprise solutions or buffers for effecting the delivery of the first and second compositions. The kits may further comprise catheters, syringes
20 or other delivering devices for the delivery of one or more of the compositions used in the methods of the invention. The kits may further comprise instructions containing administration protocols for the therapeutic regimens.

 Another kit may comprise instructions for specific routes of administration e.g., intrathecal administration of the therapeutic compositions of the
25 present invention, in addition to the therapeutic compositions. In certain embodiments, the kits of the invention may comprise catheters or other devices for the intrathecal administration of the enzyme replacement therapy that are preloaded with the therapeutic compositions of the present invention. For example, catheters preloaded with 0.001 mg, 0.005 mg, 0.01 mg, 0.015 mg, 0.02 mg, 0.03 mg, 0.04 mg,
30 0.05 mg, 0.06 mg, 0.07 mg, 0.08 mg, 0.09 mg, 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, or 1.0 mg or more of a given recombinant sulfatase of the present invention, e.g., a sulfatase having a sequence of SEQ ID NO:27; SEQ ID NO:28; SEQ ID NO:28; SEQ ID NO:29; SEQ ID NO:30; SEQ ID

NO:31; SEQ ID NO:32; SEQ ID NO:33; SEQ ID NO:34; SEQ ID NO:35; SEQ ID NO:36; SEQ ID NO:37; SEQ ID NO:38 or SEQ ID NO:39, or biologically active fragments or mutants thereof that have a formylglycine residue at the active site in a pharmaceutically acceptable formulation are specifically contemplated. Exemplary
5 catheters may be single use catheters that can be discarded after one use. Alternatively, the preloaded catheters may be refillable and presented in kits that have appropriate amounts of the enzyme for refilling such catheters.

EXAMPLES

10 The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of
15 skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1

Materials and Methods

20 Sulfatases, such as e.g., arylsulfatase B (ASB) and arylsulfatase A (ASA) are subject to a unique post-translational modification that is obligate for their function. The modification reaction, conversion of a cysteine to a formylglycine, becomes saturated when these enzymes are over-expressed. The present application shows that the modification the mutants of ASB and ASA in which the modified
25 cysteine is substituted with a serine are expressed much more efficiently than the native enzymes under identical conditions. The purified ASB mutant can then be converted to catalytically active ASB using vanadate and light, thereby obviating the need for the preparation of expression constructs and transfected cells that are transfected with both the sulfatase and a sulfatase processing enzyme, such as SUMF1
30 (Cosma et al., Cell 113:445-456, 2003). The present example provides the materials and methods that were employed to elucidate these findings.

Materials

The BP15 rabbit polyclonal anti-ASB antibody and G192 sheep polyclonal anti-ASB antibody were raised against native recombinant human enzymes. Rabbit polyclonal anti-ASA were obtained from UCLA. Antibodies were
5 purified over protein G columns prior to use. PCR primers were synthesized by Qiagen. SybrGreen QPCR reagents were purchased from Roche Biochemicals. Highly purified recombinant human ASB was manufactured at BioMarin Pharmaceutical, Inc.

Preparation of vanadate solutions

10 A 100 mM stock solution of sodium metavanadate (Sigma, S6383) was prepared as described previously (Goodno, C. C. (1982) Methods Enzymol 85 Pt B, 116-23).

ASB Activity Assays

Samples were diluted 1000-fold in assay buffer (50 mM NaOAc pH
15 5.6). Aliquots of the dilutions (5 μ L) were combined with 150 μ L of 5 mM 4-MU-sulfate substrate (4-MUS, Sigma) in assay buffer and incubated at 37°C for 20 minutes in black Costar flat-bottom plates. Reactions were terminated with 150 μ L of 350 mM glycine, 440 mM sodium carbonate pH 10.7. Fluorescence was measured with a micro-plate fluorimeter (Molecular Devices) using an excitation wavelength of
20 366 nm and an emission wavelength of 446 nm. All samples were run in duplicate. One unit of enzyme activity is defined as 1 μ mole of 4-methylumbelliferone (4-MU) produced per minute at 37°C. For quantitation of ASB using the activity assay described, a specific activity for ASB of 67 U/mg was used. Total protein was measured by Bradford assay (BioRad) using bovine serum albumin as the standard.

25 ASB ELISA

ASB native and mutant proteins were quantified by sandwich ELISA with the polyclonal BP15 antibody (ASB). Detecting antibody was conjugated to HRP. A standard curve was constructed with dilutions of purified ASB.

30 ASA Western Blotting

For Western blotting, proteins were separated by reducing SDS-PAGE, electroblotted to PVDF membranes, and blocked for 1 hour at room temperature with blocking buffer containing 2% (w/v) dry milk powder in Tris-buffered saline (TBS, 20 mM Tris-HCl, 150 mM NaCl, pH 7.5) with 0.05% (v/v) NP-40. ASA antigen was
 5 detected with anti-rhASA antiserum diluted 25,000-fold into blocking buffer. Bound primary antibodies were then detected with affinity-purified alkaline phosphatase-conjugated goat secondary antibody (Promega) diluted 5,000-fold in blocking buffer. Blots were developed with Western Blue colorimetric substrate (Promega), rinsed with distilled water and scanned.

10

Protein Mass Measurements

Protein concentrations for purified ASB and ASB C91S were calculated from A_{280} values and theoretical extinction coefficients based on amino acid composition. The latter were obtained using ProtParam software. By this
 15 method of determination, both ASB and ASB C91S had extinctions coefficients of 2 mL mg⁻¹ cm⁻¹.

QPCR

RNA was extracted using Qiagen RNeasy™ reagents and reverse
 20 transcribed with Superscript II+ (Invitrogen) and an oligo (T)25VN primer using manufacturer's protocols. cDNA was assayed using a Roche LightCycler™ with SybrGreen™ reagents. Primers specific for ASB (QASBF: 5'-A G A C T T T G G C A G G G G G T A A T (SEQ ID NO:40), QASBR: 5'-C A G C C A G T C A G A G A T G T G G A-3' (SEQ ID NO:41), ASA (ASAF: 5'-C G C C C C T T C C T G T A C T A-3' (SEQ ID NO:42), ASAR: 5'-C A G G T C C A T T G T C T G C A G T G-3' (SEQ ID NO:43)), NPT (MutSeqF: 5'-G C C G G C C A C A G T C G A T G A A T C C -3' (SEQ ID NO:44), MutSeqR: 5'-C T T G T C G A T C A G G A T G A T C T G G -3' (SEQ ID NO:45)) and the hamster ribosomal S14 protein (CHOS14F: 5'-G A A A C T A T C T G C C G G G T G A C-3' (SEQ ID NO:46),
 25 CHOS14R: 5'-T T T G A T A T G C A G G G C A G T G A-3' (SEQ ID NO:47))
 30 were used. The primers do not differentiate between native and mutant sequences.

ASB C91S expression construct

A human ASB cDNA was amplified from human liver cDNA (BD Biosciences) by high-stringency PCR using Stratagene PfuTurbo™ polymerase and the primers ASBF1: 5'-G C G A T A G G T A C C G C C A T G G G T C C G C G
5 C G G C G C G G C G A G C -3' (SEQ ID NO:48) and ASBR2: 5'-G C G A T A C T C G A G C C C T G A A A T C C T A C A T C C A A G G G -3' (SEQ ID NO:49). The cDNA was sequenced and found to be identical to that previously described. The cDNA was then reamplified with a different 5'-primer to decrease the GC bias at the 5'-end without changing the amino acid sequence (ASBatF: 5'-G C G
10 A T A G G T A C C G C C A T G G G T C C T A G A G G A G C T G C T T C T T T G C C T C G A G G A C C C G G A C C T C G G C G G C T G C T C C T C -3') (SEQ ID NO:50). The engineered cDNA was digested with KpnI and XhoI and ligated into pcDNA3.1(+) (Invitrogen) to give pcDNA3.1-ASB. The 5'-modified ASB was used to make both the native and C91S proteins. The pcDNA3.1-ASB
15 plasmid was then cut with AatII and the vector portion recircularized in order to remove the 5'-182 base pairs of the ASB cDNA. The deleted vector was then cut with PstI and the vector portion again recircularized in order to remove the 3'-760 base pairs of the cDNA. The truncated ASB construct was then cut a third time with EagI and XcmI, treated with alkaline phosphatase, gel purified and ligated to a
20 double-stranded oligonucleotide containing the C91S mutation (ASBmut3: 5'-P G G C C G G C G G G G T G C T C C T G G A C A A C T A C T A C A C G C A G C C G C T G A G C A C G C C G T C G C G G A G C C A G C T G C (SEQ ID NO:51), ASBmut4: 5'-P C A G C T G G C T C C G C G A C G G C G T G C T C A G C G G C T G C G T G T A G T A G T T G T C C A G G A G C A C C C C G
25 C C -3') (SEQ ID NO:52). The mutation introduces an ApaLI site into the ASB coding sequence. The remaining, mutagenized, portion of ASB, flanked by AatII and PstI sites was then used to reconstruct a full-length ASB C91S cDNA by ligation with KpnI/AatII and Pst/XbaI fragments from pcDNA3.1 (+)-ASB into pcDNA 3.1 (+). The reconstructed cDNA was sequenced and found to be identical to the published
30 sequence except for the engineered changes described.

ASA C69S expression construct

A human ASA cDNA was amplified from human liver cDNA (BD Biosciences) by high-stringency PCR using Stratagene PfuTurbo™ polymerase and the primers AryAF1: 5'- G C G A T A G G T A C C G C C A T G G G G G C A C C
 5 G C G G T C C C T C C T C -3' (SEQ ID NO:53) and AryAR: 5'- G C G A T A C T C G A G T C A G G C A T G G G G A T C T G G G C A A T G -3' (SEQ ID NO:54). The amplified cDNA was cut with KpnI and XhoI for ligation into pcDNA3.1 (+). The cDNA was sequenced and found to be identical to that previously described. The ASA C69S was created in the same vector using the
 10 Promega Altered Sites II™ kit and a single mutagenic primer (ASAsr 5' – P C G G C C G G T C A G G A G G G C G G C C C T A G A G G G T G T G C T C A G A G A C A C A G G C A C – 3') (SEQ ID NO:55).

Transfection and selection of pools

15 The expression constructs, along with empty vector, were linearized and gel purified. CHO-K1 cells were seeded at 2×10^5 cells/well in Corning Cell-Well™ 6-well polystyrene plates and allowed to grow overnight. Cells were transfected with Bio-Rad Cytofectene™ reagent using manufacturer protocols. Cells were selected in UltraCHO (Cambrex) medium supplemented with 2.5% dialyzed
 20 fetal bovine serum, 2 mM glutamine, 50 µg/mL gentamycin, 2.5 µg/mL amphotericin and either 450 or 900 µg/mL G418. Resistant colonies were evident within a week. All transfections yielded hundreds of colonies. Mock transfection dishes had no colonies. After 12 days, resistant pools of colonies for each of the construct types were pooled, expanded and frozen.

25

Comparison of expression of stably-transfected pools

Pooled cells for each plasmid type and selection level were seeded in duplicate T-25 flasks at 1.2×10^4 cells/cm² in UltraCHO medium supplemented with 2.5% dialyzed fetal bovine serum, 2 mM glutamine, 50 µg/mL gentamycin, 2.5
 30 µg/mL amphotericin and 450 or 900 µg/mL G418. After 24 hours of incubation, cell layers were rinsed with Hank's buffered salt solution and fed with seeding medium

from which serum had been omitted. Flasks were incubated for 48 hours at 37°C and 5% CO₂. Supernatant was then removed and stored at 4°C. Cells were trypsinized and counted with a Coulter counter. Cell pellets were washed with Dulbecco's phosphate-buffered saline (DPBS) and stored at -80°C. Cell lysates were prepared by
5 subjecting DPBS-resuspended cell pellets to three cycles of freeze-thaw.

Production of ASB C91S

The ASB C91S sequence was cloned into a second vector, pBMPCINt1. The pBMPCINt 1-ASB C91S construct was linearized with SalI, gel
10 purified and used to transfect CHO-S (Invitrogen) cells as described above. Cells were selected with 450 µg/mL G418. A total of 11 clones were picked and screened for expression of ASB by ELISA with the BP15 antibody. Clone 7 had the highest volumetric productivity of ASB C91S (1 µg/mL) with an associated specific productivity under these conditions of 3 pg/cell/day. Clone 7 was used for production
15 of ASB C91S. Cells were seeded onto Cytopore 1™ beads in a 250 mL Corning spinner flask with Excell 302 medium (JRH) supplemented with 2.5% dialyzed fetal bovine serum, 2 mM glutamine, 50 µg/mL gentamycin, 2.5 µg/mL amphotericin and 450 µg/mL G418. Serum was gradually reduced to 0% over a two-week period. Medium was harvested every two days, filtered through a 0.2 µm PES sterile filter
20 unit and stored at 4°C.

Purification of ASB C91S

ASB C91S was purified on an anti-ASB (G192) affinity column prepared by coupling the antibody to NHS-sepharose beads. Harvest medium was
25 loaded onto the column. Unbound proteins were eluted with Dulbecco's phosphate-buffered saline. ASB C91S was eluted with 100 mM Glycine-HCl pH 2.25. Fractions were collected into tubes containing Tris-HCl, bringing the pH of the eluate immediately up to 6.5. The purified material from the affinity column was buffer exchanged and concentrated in 50 mM NaOAc pH 6.5 with a Millipore Centricon
30 Plus-20 (8,000 NMWL). Purified protein was stored at 4°C.

Photochemical reactions

Photochemical reactions were carried out in an Ace Glass Photochemical safety cabinet (7836-20). Two UVP Pen-Ray 5.5 Watt lamps (90-0012-02) were used as light sources. The lamps were powered by a UVP Photochemical UV power supply (99-0055-01) and housed in an Ace Glass jacketed immersion well (7892-45). Lamp temperature was held at 20°C with a 1:1 mixture of Flag™ anti-freeze and tap water, which was pumped through the immersion well jacket at 16 liters per minute. Coolant temperature was maintained with a recirculating chiller. Samples in microfuge tubes were floated in a water bath in a 500 mL jacketed beaker. The beaker temperature was controlled with a second recirculating chiller. The lamp was positioned immediately below the samples. UVB radiation was removed with the anti-freeze mixture (which absorbs strongly at 280 nm) and with polystyrene plates. Irradiation dose was measured with an Oriel Goldilux™ Smartmeter Photometer using calibrated UVA (365 nm) and UVB (280 nm) probes. All samples were irradiated in 1.5 mL polypropylene microfuge tubes (USA Scientific 1415-2600) or Corning clear polystyrene 96-well plates.

Optimized conditions for activation of ASB C91S with vanadate and light

A 10 µM solution of affinity-purified ASB C91S or ASB in 50 mM sodium acetate pH 5.6, 150 mM NaCl was diluted 10-fold into 100 mM Tris-acetate pH 8.0. Protein solutions were then supplemented with 10 µM or 500 µM sodium metavanadate from a 100 mM stock solution (prepared as described above). The ASB C91S solution was then placed in a 1 cm-path length quartz cuvette and irradiated at a light intensity of 3.7 mW/cm² measured at 350 nm. Excitation wavelengths were restricted to the 250-450 nm range using appropriate cut-off filters. Irradiation was performed for intervals from 10 to 240 seconds after which samples were placed on ice. For activity assays, samples were then diluted 800-fold in 50 mM sodium acetate pH 5.6 supplemented with 0.05% Tween 20 and 0.01 mg/mL BSA, and then serially diluted 2-fold five times. Activity assays were carried out as described above. Activity values in the linear dilution range were used for further calculations.

LC/MS analysis of photolyzed ASB

Wild-type ASB (0.2 µg/mL, 200 µL) was combined with 10 mM vanadate in 50 mM sodium acetate pH 5.6, 150 mM NaCl. The protein solution was irradiated for 2 minutes with 250-450 nm light at an intensity of 3.7 mW/cm². Total
5 inactivation was confirmed by activity assay as described above. Protein was precipitated by the addition of 470 µL of acetone and pelleted by centrifugation. The protein pellet was then washed with acetone and allowed to air-dry prior to resuspension in 10 µL of 8 M urea. The resolubilized protein (10 µL) was then combined with an equal volume of 0.5 M Tris-HCl pH 8.2, 1 µL of 0.1 M CaCl₂, 74
10 µL of water and 5 µL of trypsin (0.5 µg/µL). Digestion was allowed to proceed at room temperature for 16 hours. Disulfide bonds were reduced by the addition of DTT to 2 mM and incubation for an additional 30 minutes at 37°C. All reactions were quenched by the addition of 2 µL formic acid. Digested peptides were resolved and analyzed with a Hewlett-Packard 1100 LC/MS with an Agilent 1100 series
15 LC/electrospray MSD (Phenomenex column C18 RP; P/N 00G-4396-B0; 250 x 2mm).

Example 2

Expression of Recombinant proteins in CHO cells

20 The present Example provides details of CHO cell production, transcript levels, protein productivity and protein processing efficiency. The methods described in Example 1 were used to generate the results described herein.

CHO cells expressing ASB, ASB C91S, ASA and ASA C69S were created in order to ascertain the level to which sulfatase conversion affects expression.
25 All sequences were expressed in identical vectors. All vectors contained the neomycin phosphotransferase gene (NPT) in the same expression cassette to allow selection on G418. Complete native and mutant expression vectors differed by a single base pair. Cells in replicate 6-well plates were transfected at the same time with identical amounts of different plasmids using the same reagents. Transfected cells
30 were selected at 450 and at 900 µg/mL G418 with the thought that the latter level would enforce the requirement for high expression of NPT and the immediately adjacent sulfatase. Large pools of G418-resistant colonies were used to eliminate

clonal effects. Identical numbers of cells for each pool of stable transfected cells were used in the productivity studies. Sulfatase productivity per cell for each stably transfected pool was determined by ELISA and qualitatively confirmed by Western blot. Transcript number was quantitated for both sulfatase and NPT by RT-QPCR assay. Transcript levels were normalized to an internal control, the hamster ribosomal S14 protein transcript, also assayed by RT-QPCR. Normalization of protein productivity to S14 transcript values as a proxy for cell number did not change the relative relationships among the samples. The data indicate at least three differences in the expression behavior of the native and serine mutant sulfatases: Serine mutant protein is expressed at higher levels than native enzyme on both cell and transcript normalized bases; overexpression of native enzyme appears to be toxic in a way that serine mutant expression is not; and, in a possibly related observation, serine mutant transcript appears to be converted to secreted protein more efficiently than native transcript.

15 **Protein productivity**

Measuring the quantity of secreted sulfatase protein by ELISA, it was evident that pools of stably transfected cells expressing ASB C91S protein did so at significantly higher levels than cells expressing ASB (Figure 1, Panel A). At the 400 $\mu\text{g/mL}$ G418 selection level, ASB C91S was present in conditioned medium at levels 5-fold greater than for ASB. This difference became even more pronounced at the 900 $\mu\text{g/mL}$ G418 concentration, where ASB C91S was secreted to a level 29-fold greater than ASB. Western blotting with an anti-ASA antibody demonstrated that ASA C69S levels in conditioned medium were also significantly higher than those of native ASA. A conservative estimate of Western blot band intensities for ASA and ASA C69S at a selection level of 900 $\mu\text{g/mL}$ G418 suggests that the mutant ASA is secreted to a level at least 10-fold that of native (Figure 1, Panel B). Strikingly, levels of secreted ASB (ELISA) and ASA (Western blot) both dropped upon selection at higher G418 levels. This result is consistent with toxicity associated with overexpression of native ASB and ASA.

30

Transcript Levels

NPT transcript levels increased with increasing levels G418 selection. More surprising was the observation that native ASB and ASA transcript levels in the same pools did not increase (Figure 1, Panel C). In agreement with the levels of
5 secreted protein, transcript levels for the native enzymes actually went down in cells surviving at 900 $\mu\text{g/mL}$ G418. This result suggests that transcription of the native sulfatases and transcription of NPT have become uncoupled in cells surviving at higher G418 concentrations. That uncoupling would be required for survival is indicative, again, of some kind of toxicity associated with expression of native ASB
10 and ASA. In contrast to the native transcripts, ASB C91S and ASA C69S transcript levels increased in parallel with NPT from the 450 to the 900 $\mu\text{g/mL}$ G418 level. If it is toxicity that constrains increases in native enzyme expression with increasing G418 selection, then serine mutant expression does not seem to result in the same toxicity.

Protein Processing Efficiency

15 The quantity of secreted protein derived per transcript was calculated from the cell-normalized protein and S14-normalized transcript levels. Significantly more ASB C91S protein was derived from similar amounts of transcript relative to native ASB at both G418 selection levels (Figure 1, Panel D). This result is consistent with protein mass being lost to misfolding and subsequent degradation in
20 the case of the native sulfatases.

The data from the pool experiments is consistent with enhanced expression of serine-mutant sulfatases relative to native sulfatases. Reduced productivity of the native enzymes may be a result of toxicity and decreased protein-processing efficiency. It is likely that the former is a result of the latter, whereby
25 decreases in protein processing efficiency leads to stresses on the cell that compromise the ability to survive under strong antibiotic selection.

Example 3

A vanadate-dependent photoreaction to produce activated sulfatases

The inventors determined that a vanadate-dependent photoreaction can
30 be used to convert the active site serine of sulfatases to a formylglycine and render the sulfatase protein catalytically active. The present example demonstrates this phenomenon using ASB C91S.

Affinity-purified ASB C91S was irradiated with near UV light in the presence of 2, 20, 40 and 400 μ M sodium metavanadate in 50 mM NaOAc pH 6.5, 100 mM NaCl. Light below 300 nm was removed by placing appropriate absorptive materials between the light source and the samples (see Example 1). Samples were
5 subjected to cycles of irradiation consisting of 30 minutes of light followed by 20 minutes in the dark on ice. Treated samples were then assayed for activity against the 4-MUS after dilution of the vanadate to concentrations below those necessary to inhibit the sulfatase.

ASB C91S was converted to catalytically active enzyme by the above-
10 described procedure. Maximal activation was achieved at the highest vanadate concentration (Figure 3A). No activation was observed by irradiation in the absence of vanadate or with vanadate in the absence of irradiation. Increases in the amount of activated enzyme were observed through the last time point. Activation was reproducible over a number of experiments. Assuming that the entire mutant enzyme
15 population in the sample was activatable, conversion efficiencies ranged from 20-30%.

Without being bound to any theory of mechanism of action, the present invention for the first time demonstrates that there may be an association between vanadate and a serine side-chain that leads to light-catalyzed two-electron transfer
20 from the serine C β to vanadium. This reaction would explain the observed serine-mutant sulfatase conversion. The oxovanadium (V^{+3}) generated as a by-product would be expected to com-proportionate in presence of vanadate (V^{+5}) to form two moles of vanadyl (V^{+4}). Vanadyl slowly oxidizes back to vanadate in the presence of oxygen. The proximity of vanadate to the convertible serine residue leaves open the
25 possibility of other redox mechanisms that do not require covalent association between vanadate and serine. In particular, hydrogen abstraction from C β by photo-excited vanadate might lead to di-radical intermediates that can be resolved in a manner leading to conversion. The native form of ASB (i.e., the form that contains an active site cysteine rather than serine) is inactivated by light and vanadate.

30 Further, there does not seem to be cleavage of the protein backbone as has been observed for other proteins. Cleavage at the oxidized serine in myosin is believed to occur by enolization of the resulting formylglycine through deprotonation

of the backbone C α . A second vanadate-dependent photo-oxidation step can then occur leading to addition of molecular oxygen to the backbone C α and eventual cleavage of the myosin peptide chain. Enolization of the aldehyde in the context of the sulfatase active site is less likely since the protein is expected to strongly stabilize
5 the hydrated keto form of the side-chain.

The present example clearly demonstrates the vanadate dependent photoreactive activation of ASB. The active site of sulfatase enzymes is highly conserved among both eukaryotic and prokaryotic sulfatases. Therefore, it is predicted that light-dependent oxidation of serine mutants will be generally applicable
10 to all members of the sulfatase family and will therefore be useful in the activation of prokaryotic sulfatases as well as eukaryotic sulfatases.

Conditions for the activation of ASB C91S were further optimized for pH and buffer composition (Figure 3C). To measure the extent of activation, activity values for ASB C91S obtained using different activation conditions were divided by
15 activity values for the same amount of wild-type ASB obtained using the same conditions, but without irradiation (irradiation of wild-type ASB in the absence of vanadate has no effect on the activity of the enzyme, data not shown). Using the conditions specified in Example 1, including 100 mM Tris-acetate pH 8 and 10-500 molar equivalents of vanadate, 60-65% of the ASB C91S was converted to active
20 enzyme.

To test the hypothesis that vanadate modifications of ASB and ASBC91S would be restricted to the active site, ASB was treated exhaustively with vanadate and light and then compared to untreated ASB by tryptic mapping using LC/MS. Careful analysis revealed that only the active-site peptide was altered by the
25 treatment described (Figure 3D). The alteration indicated a single amino acid substitution at the active site of ASB (only the mobility of active site peptide changes). The substitution is consistent with the conversion of the active site formylglycine to carboxylglycine. These results demonstrate that modification of ASB and ASB C91S with vanadate and light is likely to be specific to the active site
30 and will not significantly affect other residues in the protein.

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present

disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

The references cited herein throughout, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are all specifically incorporated herein by reference.

CLAIMS

What is Claimed Is:

1. A method of converting a recombinant sulfatase protein to a catalytically active sulfatase enzyme comprising:
 - a. contacting a preparation comprising said protein with a photoactivatable redox agent, and
 - b. irradiating said preparation with light in the range of between 300 nm and 400nm wavelength.
2. The method of claim 1, wherein said sulfatase is a eukaryotic sulfatase protein.
3. The method of claim 1, wherein said sulfatase is a prokaryotic sulfatase protein.
4. The method of claim 1, wherein said sulfatase is a human sulfatase protein.
5. The method of claim 1, wherein said photoactivatable redox agent composition comprises vanadium.
6. The method of claim 1, wherein said recombinant sulfatase protein is a mutant mammalian arylsulfatase protein in which the active site cysteine residue has been replaced by a serine residue.
7. The method of claim 6, wherein said active-site serine in the mutant mammalian arylsulfatase is converted to formylglycine by incubating said mammalian arylsulfatase with vanadate.

8. The method of claim 1, wherein said preparation is contacted with said vanadate prior to said irradiating step.

9. The method of claim 1, wherein said preparation is irradiated prior to contacting with said vanadate.

10. The method of claim 1, wherein said preparation is subjected to a cycle of irradiation wherein said sample is subjected to a period of irradiation followed by a period of incubation in a dark environment.

11. The method of claim 10, wherein said cycle is repeated.

12. The method of claim 6, wherein said arylsulfatase is selected from the group consisting of arylsulfatase A, arylsulfatase B, Arylsulfatase C, arylsulfatase D, arylsulfatase E, arylsulfatase F, arylsulfatase G, Sulfatase 1, Sulfatase 2, galactosamine (N-acetyl)-6-sulfate sulfatase, iduronate 2 sulfatase, N-sulfoglucosamine sulfohydrolase, glucosamine (N-acetyl)-6-sulfatase and arylsulfatase A cerebroside.

13. The method of claim 12, wherein said arylsulfatase is an arylsulfatase B comprising a C→S mutation at a position corresponding to amino acid position number 91 of SEQ ID NO:4.

14. The method of claim 12, wherein said arylsulfatase is an arylsulfatase A comprising a C→S mutation at a position corresponding to amino acid position number 91 of SEQ ID NO:2.

15. The method of claim 12, wherein said sulfatase is a protein sequence comprising an amino acid sequence selected from the group consisting of

SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39 or variants thereof that retain sulfatase activity.

16. The method of claim 1, wherein said arylsulfatase protein is a mutant human arylsulfatase B protein obtained from a CHO cell line according to a method comprising:

- a. transfecting CHO cells with an expression construct comprising a nucleic acid encoding an arylsulfatase of claim 13;
- b. selecting cells transfected with said vector;
- c. culturing said selected cells under conditions to permit the secretion of said mutant human arylsulfatase B protein (ASB) protein into the culture medium of said cells;
- d. isolating mutant human ASB protein from said culture medium.

17. The method of claim 16, wherein said CHO cell line is a CHO-K1 cell line.

18. The method of claim 16, wherein said CHO cell line is a CHO-S cell line.

19. The method of claim 1, wherein said arylsulfatase protein is a mutant human arylsulfatase A protein obtained from a CHO cell line according to a method comprising:

- a. transfecting CHO cells with an expression construct comprising a nucleic acid encoding an arylsulfatase A (ASA) of claim 14;
- b. selecting cells transfected with said vector;

- c. culturing said selected cells under conditions to permit the secretion of said mutant ASA protein into the culture medium of said cells; and
 - d. isolating said mutant ASA protein from said culture medium.
20. The method of claim 19, wherein said CHO cell line is a CHO-K1 cell line.
21. The method of claim 19, wherein said CHO cell line is a CHO-S cell line.
22. A recombinant CHO-S cell line transfect with an expression construct that encodes ASB C91S.
23. A method of preparing a pharmaceutically acceptable composition comprising a catalytically active arylsulfatase comprising:
- a. contacting a preparation comprising recombinant arylsulfatase protein with a photoactivatable redox agent composition,
 - b. irradiating said preparation with light in the range of between 300 nm and 400 nm wavelength, and
 - c. formulating said preparation in a pharmaceutically acceptable carrier, diluent or excipient.
24. A method of preparing a pharmaceutically acceptable composition comprising a catalytically active arylsulfatase comprising:
- a. contacting a preparation comprising recombinant arylsulfatase protein with a vanadate composition,
 - b. irradiating said preparation with light in the range of between 300 nm and 400 nm wavelength, and

c. formulating said preparation in a pharmaceutically acceptable carrier, diluent or excipient.

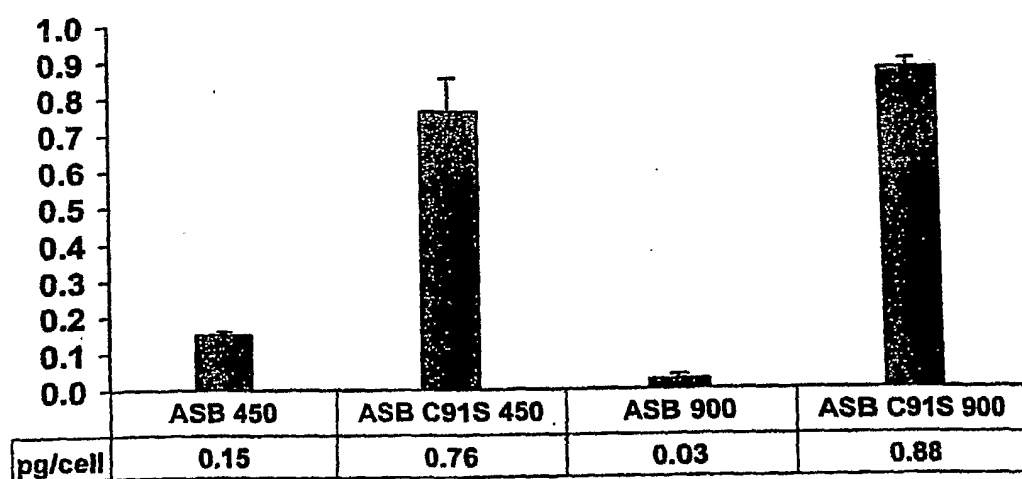
25. A pharmaceutical composition comprising a catalytically active arylsulfatase protein prepared according to the method of any of claims 1-17, and a pharmaceutically acceptable carrier, diluent or excipient.

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Figure 2A



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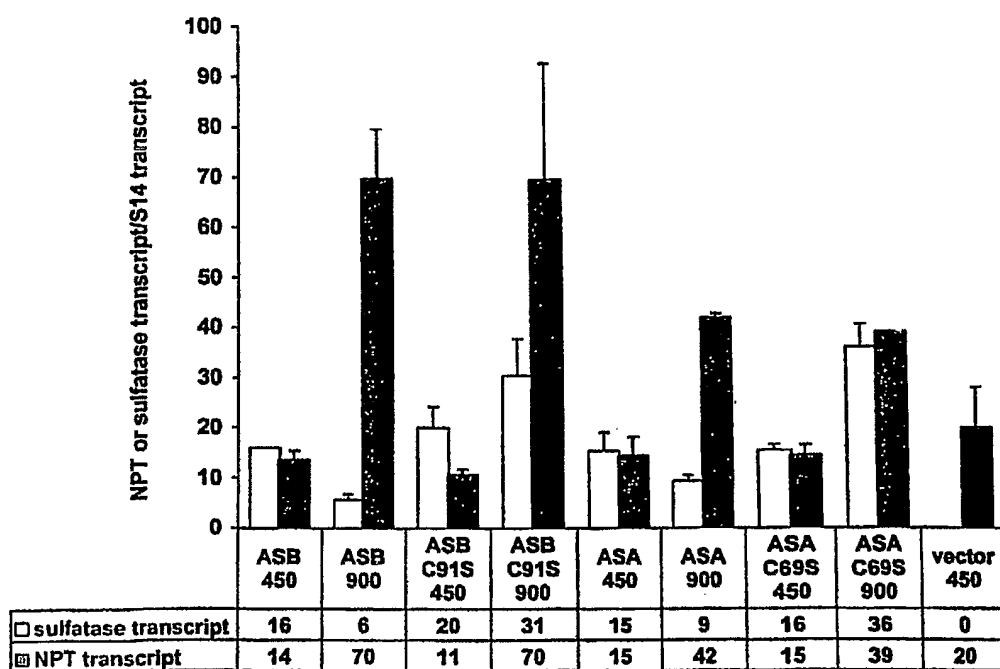
Figure 2B



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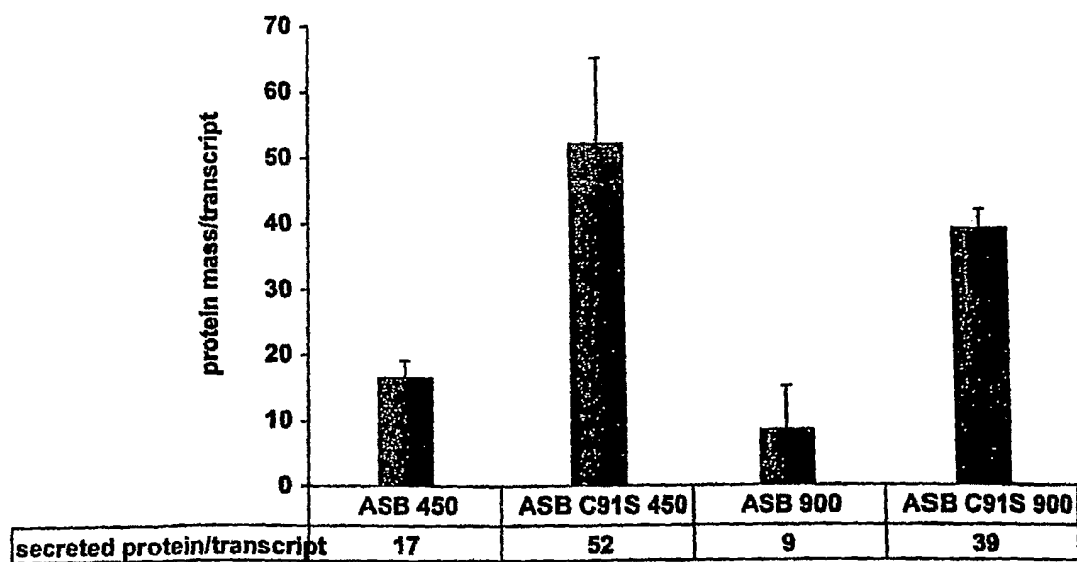
Figure 2C



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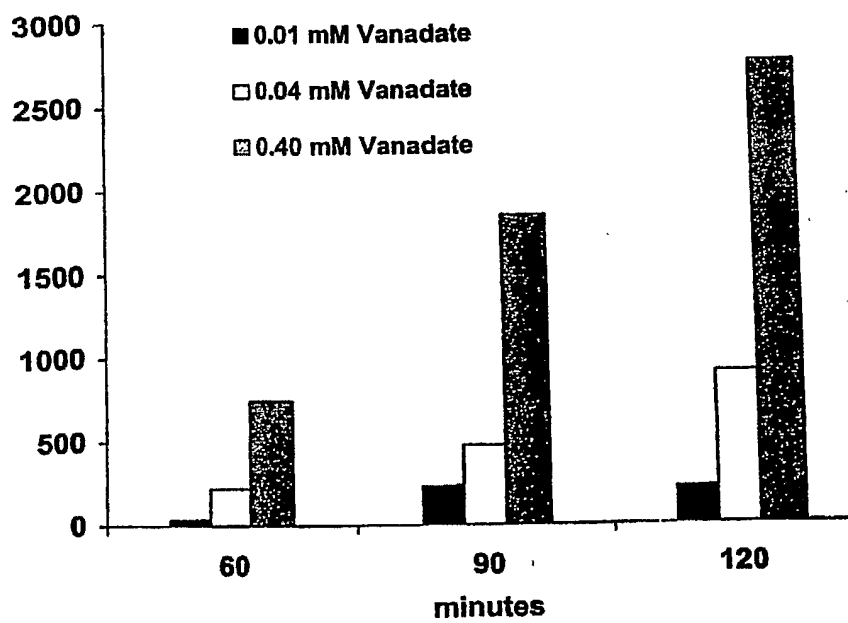
Figure 2D



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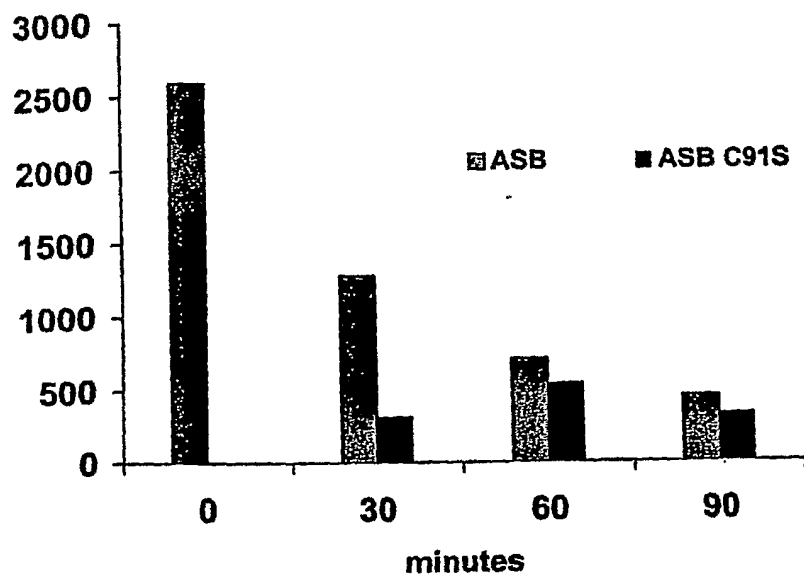
Figure 3A



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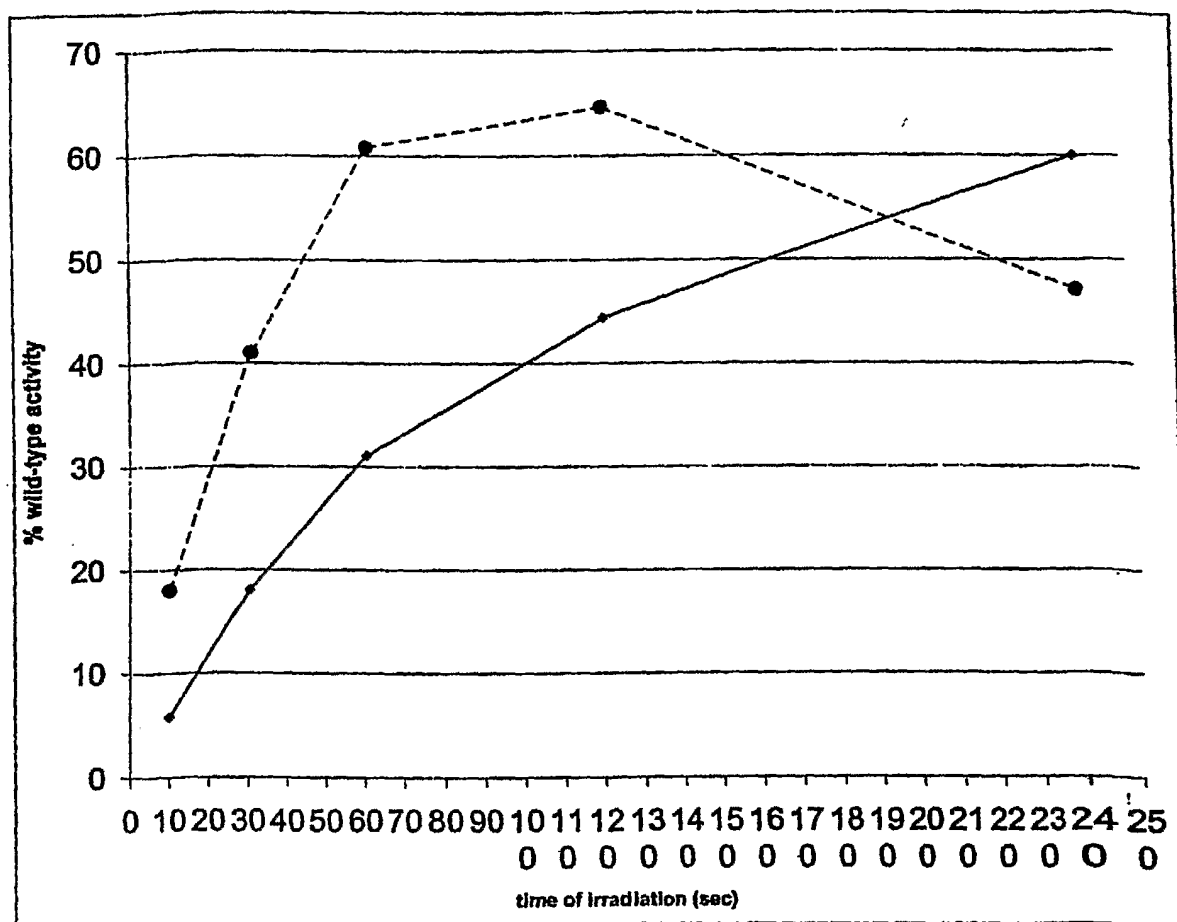
Figure 3B



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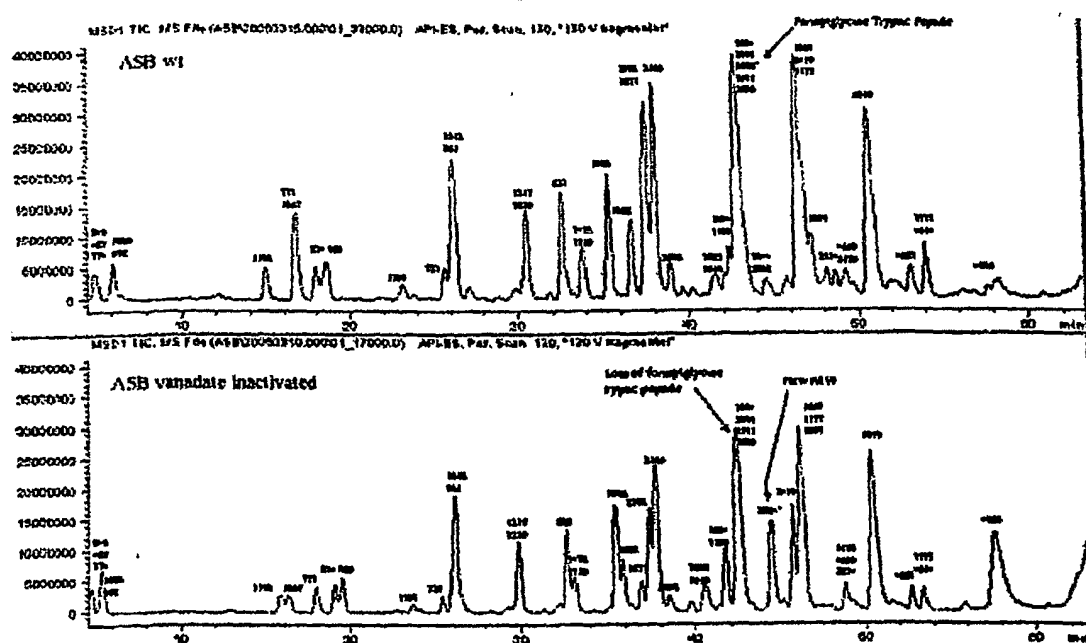
Figure 3C



SUBSTITUTE SHEET (RULE 26)

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Figure 3D



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Gly Pro Glu Gly Ala Phe Leu Pro Pro His Gln Gly Phe His Arg Phe
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Leu Gly Ile Pro Tyr Ser His Asp Gln Gly Pro Cys Gln Asn Leu Thr
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Cys Phe Pro Pro Ala Thr Pro Cys Asp Gly Gly Cys Asp Gln Gly Leu
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Val Pro Ile Pro Leu Leu Ala Asn Leu Ser Val Glu Ala Gln Pro Pro
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Trp Leu Pro Gly Leu Glu Ala Arg Tyr Met Ala Phe Ala His Asp Leu
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Met Ala Asp Ala Gln Arg Gln Asp Arg Pro Phe Phe Leu Tyr Tyr Ala
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Ser His His Thr His Tyr Pro Gln Phe Ser Gly Gln Ser Phe Ala Glu
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Arg Ser Gly Arg Gly Pro Phe Gly Asp Ser Leu Met Glu Leu Asp Ala
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Ala Val Gly Thr Leu Met Thr Ala Ile Gly Asp Leu Gly Leu Leu Glu
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Glu Thr Leu Val Ile Phe Thr Ala Asp Asn Gly Pro Glu Thr Met Arg
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Met Ser Arg Gly Gly Cys Ser Gly Leu Leu Arg Cys Gly Lys Gly Thr
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Thr Tyr Glu Gly Gly Val Arg Glu Pro Ala Leu Ala Phe Trp Pro Gly
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His Ile Ala Pro Gly Val Thr His Glu Leu Ala Ser Ser Leu Asp Leu
 325 330 335

Leu Pro Thr Leu Ala Ala Leu Ala Gly Ala Pro Leu Pro Asn Val Thr
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Leu Asp Gly Phe Asp Leu Ser Pro Leu Leu Leu Gly Thr Gly Lys Ser
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Pro Arg Gln Ser Leu Phe Phe Tyr Pro Ser Tyr Pro Asp Glu Val Arg
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Gly Val Phe Ala Val Arg Ser Gly Lys Tyr Lys Ala His Phe Phe Thr
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Gln Gly Ser Ala His Ser Asp Thr Thr Ala Asp Pro Ala Cys His Ala
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Ser Ser Ser Leu Thr Ala His Glu Pro Pro Leu Leu Tyr Asp Leu Ser
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Lys Asp Pro Gly Glu Asn Tyr Asn Leu Leu Gly Gly Val Ala Gly Ala
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Thr Pro Glu Val Leu Gln Ala Leu Lys Gln Leu Gln Leu Leu Lys Ala
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Gln Leu Asp Ala Ala Val Thr Phe Gly Pro Ser Gln Val Ala Arg Gly
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Ala Pro Pro Gly Ser Gly Ala Gly Ala Ser Arg Pro Pro His Leu Val	35	40	45
Phe Leu Leu Ala Asp Asp Leu Gly Trp Asn Asp Val Gly Phe His Gly	50	55	60
Ser Arg Ile Arg Thr Pro His Leu Asp Ala Leu Ala Ala Gly Gly Val	65	70	75
Leu Leu Asp Asn Tyr Tyr Thr Gln Pro Leu Cys Thr Pro Ser Arg Ser	85	90	95
Gln Leu Leu Thr Gly Arg Tyr Gln Ile Arg Thr Gly Leu Gln His Gln	100	105	110
Ile Ile Trp Pro Cys Gln Pro Ser Cys Val Pro Leu Asp Glu Lys Leu	115	120	125
Leu Pro Gln Leu Leu Lys Glu Ala Gly Tyr Thr Thr His Met Val Gly	130	135	140
Lys Trp His Leu Gly Met Tyr Arg Lys Glu Cys Leu Pro Thr Arg Arg	145	150	155
Gly Phe Asp Thr Tyr Phe Gly Tyr Leu Leu Gly Ser Glu Asp Tyr Tyr	165	170	175
Ser His Glu Arg Cys Thr Leu Ile Asp Ala Leu Asn Val Thr Arg Cys	180	185	190
Ala Leu Asp Phe Arg Asp Gly Glu Glu Val Ala Thr Gly Tyr Lys Asn	195	200	205
Met Tyr Ser Thr Asn Ile Phe Thr Lys Arg Ala Ile Ala Leu Ile Thr	210	215	220
Asn His Pro Pro Glu Lys Pro Leu Phe Leu Tyr Leu Ala Leu Gln Ser	225	230	235
Val His Glu Pro Leu Gln Val Pro Glu Glu Tyr Leu Lys Pro Tyr Asp	245	250	255

Phe Ile Gln Asp Lys Asn Arg His His Tyr Ala Gly Met Val Ser Leu
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Met Asp Glu Ala Val Gly Asn Val Thr Ala Ala Leu Lys Ser Ser Gly
 275 280 285

Leu Trp Asn Asn Thr Val Phe Ile Phe Ser Thr Asp Asn Gly Gly Gln
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Thr Leu Ala Gly Gly Asn Asn Trp Pro Leu Arg Gly Arg Lys Trp Ser
 305 310 315 320

Leu Trp Glu Gly Gly Val Arg Gly Val Gly Phe Val Ala Ser Pro Leu
 325 330 335

Leu Lys Gln Lys Gly Val Lys Asn Arg Glu Leu Ile His Ile Ser Asp
 340 345 350

Trp Leu Pro Thr Leu Val Lys Leu Ala Arg Gly His Thr Asn Gly Thr
 355 360 365

Lys Pro Leu Asp Gly Phe Asp Val Trp Lys Thr Ile Ser Glu Gly Ser
 370 375 380

Pro Ser Pro Arg Ile Glu Leu Leu His Asn Ile Asp Pro Asn Phe Val
 385 390 395 400

Asp Ser Ser Pro Cys Pro Arg Asn Ser Met Ala Pro Ala Lys Asp Asp
 405 410 415

Ser Ser Leu Pro Glu Tyr Ser Ala Phe Asn Thr Ser Val His Ala Ala
 420 425 430

Ile Arg His Gly Asn Trp Lys Leu Leu Thr Gly Tyr Pro Gly Cys Gly
 435 440 445

Tyr Trp Phe Pro Pro Pro Ser Gln Tyr Asn Val Ser Glu Ile Pro Ser
 450 455 460

Ser Asp Pro Pro Thr Lys Thr Leu Trp Leu Phe Asp Ile Asp Arg Asp
 465 470 475 480

Pro Glu Glu Arg His Asp Leu Ser Arg Glu Tyr Pro His Ile Val Thr
 485 490 495

Lys Leu Leu Ser Arg Leu Gln Phe Tyr His Lys His Ser Val Pro Val
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Tyr Phe Pro Ala Gln Asp Pro Arg Cys Asp Pro Lys Ala Thr Gly Val
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Trp Gly Pro Trp Met
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Trp Glu Ala Glu Ser His Ala Ala Ser Arg Pro Asn Ile Ile Leu Val
          20          25          30

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Met Ala Asp Asp Leu Gly Ile Gly Asp Pro Gly Cys Tyr Gly Asn Lys
          35          40          45

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Thr Ile Arg Thr Pro Asn Ile Asp Arg Leu Ala Ser Gly Gly Val Lys
50          55          60

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Leu Thr Gln His Leu Ala Ala Ser Pro Leu Cys Thr Pro Ser Arg Ala
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Ala Phe Met Thr Gly Arg Tyr Pro Val Arg Ser Gly Met Ala Ser Trp
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Ser Arg Thr Gly Val Phe Leu Phe Thr Ala Ser Ser Gly Gly Leu Pro
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Thr Asp Glu Ile Thr Phe Ala Lys Leu Leu Lys Asp Gln Gly Tyr Ser
115 120 125

Thr Ala Leu Ile Gly Lys Trp His Leu Gly Met Ser Cys His Ser Lys
130 135 140

Thr Asp Phe Cys His His Pro Leu His His Gly Phe Asn Tyr Phe Tyr
145 150 155 160

Gly Ile Ser Leu Thr Asn Leu Arg Asp Cys Lys Pro Gly Glu Gly Ser
165 170 175

Val Phe Thr Thr Gly Phe Lys Arg Leu Val Phe Leu Pro Leu Gln Ile
180 185 190

Val Gly Val Thr Leu Leu Thr Leu Ala Ala Leu Asn Cys Leu Gly Leu
195 200 205

Leu His Val Pro Leu Gly Val Phe Phe Ser Leu Leu Phe Leu Ala Ala
210 215 220

Leu Ile Leu Thr Leu Phe Leu Gly Phe Leu His Tyr Phe Arg Pro Leu
225 230 235 240

Asn Cys Phe Met Met Arg Asn Tyr Glu Ile Ile Gln Gln Pro Met Ser
245 250 255

Tyr Asp Asn Leu Thr Gln Arg Leu Thr Val Glu Ala Ala Gln Phe Ile
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Gln Arg Asn Thr Glu Thr Pro Phe Leu Leu Val Leu Ser Tyr Leu His
275 280 285

Val His Thr Ala Leu Phe Ser Ser Lys Asp Phe Ala Gly Lys Ser Gln
290 295 300

His Gly Val Tyr Gly Asp Ala Val Glu Glu Met Asp Trp Ser Val Gly
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Gln Ile Leu Asn Leu Leu Asp Glu Leu Arg Leu Ala Asn Asp Thr Leu
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Ile Tyr Phe Thr Ser Asp Gln Gly Ala His Val Glu Glu Val Ser Ser
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Lys Gly Glu Ile His Gly Gly Ser Asn Gly Ile Tyr Lys Gly Gly Lys
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Pro Arg Val Ile Gln Ala Gly Gln Lys Ile Asp Glu Pro Thr Ser Asn
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Glu Asp Arg Ile Ile Asp Gly Arg Asp Leu Met Pro Leu Leu Glu Gly
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Tyr Leu Asn Ala Val Arg Trp His Pro Gln Asn Ser Thr Ser Ile Trp
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Lys Ala Phe Phe Phe Thr Pro Asn Phe Asn Pro Val Gly Ser Asn Gly
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Cys Phe Ala Thr His Val Cys Phe Cys Phe Gly Ser Tyr Val Thr His
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Val Met Gln Glu Ala Ala Asp Arg His Thr Gln Thr Leu Pro Glu Val
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Pro Asp Gln Phe Ser Trp Asn Asn Phe Leu Trp Lys Pro Trp Leu Gln
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Pro Lys Thr Ala Asn Ala Phe Lys Pro Asn Ile Leu Leu Ile Met Ala
 35 40 45

Asp Asp Leu Gly Thr Gly Asp Leu Gly Cys Tyr Gly Asn Asn Thr Leu
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Arg Thr Pro Asn Ile Asp Gln Leu Ala Glu Glu Gly Val Arg Leu Thr
 65 70 75 80

Gln His Leu Ala Ala Ala Pro Leu Cys Thr Pro Ser Arg Ala Ala Phe
 85 90 95

Leu Thr Gly Arg His Ser Phe Arg Ser Gly Met Asp Ala Ser Asn Gly
 100 105 110

Tyr Arg Ala Leu Gln Trp Asn Ala Gly Ser Gly Gly Leu Pro Glu Asn
 115 120 125

Glu Thr Thr Phe Ala Arg Ile Leu Gln Gln His Gly Tyr Ala Thr Gly
 130 135 140

Leu Ile Gly Lys Trp His Gln Gly Val Asn Cys Ala Ser Arg Gly Asp
 145 150 155 160

His Cys His His Pro Leu Asn His Gly Phe Asp Tyr Phe Tyr Gly Met
 165 170 175

Pro Phe Thr Leu Thr Asn Asp Cys Asp Pro Gly Arg Pro Pro Glu Val
 180 185 190

Asp Ala Ala Leu Arg Ala Gln Leu Trp Gly Tyr Thr Gln Phe Leu Ala
195 200 205

Leu Gly Ile Leu Thr Leu Ala Ala Gly Gln Thr Cys Gly Phe Phe Ser
210 215 220

Val Ser Ala Arg Ala Val Thr Gly Met Ala Gly Val Gly Cys Leu Phe
225 230 235 240

Phe Ile Ser Trp Tyr Ser Ser Phe Gly Phe Val Arg Arg Trp Asn Cys
245 250 255

Ile Leu Met Arg Asn His Asp Val Thr Glu Gln Pro Met Val Leu Glu
260 265 270

Lys Thr Ala Ser Leu Met Leu Lys Glu Ala Val Ser Tyr Ile Glu Arg
275 280 285

His Lys His Gly Pro Phe Leu Leu Phe Leu Ser Leu Leu His Val His
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Ile Pro Leu Val Thr Thr Ser Ala Phe Leu Gly Lys Ser Gln His Gly
305 310 315 320

Leu Tyr Gly Asp Asn Val Glu Glu Met Asp Trp Leu Ile Gly Lys Val
325 330 335

Leu Asn Ala Ile Glu Asp Asn Gly Leu Lys Asn Ser Thr Phe Thr Tyr
340 345 350

Phe Thr Ser Asp His Gly Gly His Leu Glu Ala Arg Asp Gly His Ser
355 360 365

Gln Leu Gly Gly Trp Asn Gly Ile Tyr Lys Gly Gly Lys Gly Met Gly
370 375 380

Gly Trp Glu Gly Gly Ile Arg Val Pro Gly Ile Phe His Trp Pro Gly
385 390 395 400

Val Leu Pro Ala Gly Arg Val Ile Gly Glu Pro Thr Ser Leu Met Asp
405 410 415

Val Phe Pro Thr Val Val Gln Leu Val Gly Gly Glu Val Pro Gln Asp
420 425 430

Arg Val Ile Asp Gly His Ser Leu Val Pro Leu Leu Gln Gly Ala Glu
435 440 445

Ala Arg Ser Ala His Glu Phe Leu Phe His Tyr Cys Gly Gln His Leu
450 455 460

His Ala Ala Arg Trp His Gln Lys Asp Ser Gly Ser Val Trp Lys Val
465 470 475 480

His Tyr Thr Thr Pro Gln Phe His Pro Glu Glu Arg Gly Leu Leu Thr
485 490 495

Ala Glu Ala Ser Ala His Ala Glu Trp Gly Gly Val Thr His His Arg
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Pro Pro Leu Leu Phe Asp Leu Ser Arg Asp Pro Ser Glu Ala Arg Pro
515 520 525

Leu Thr Pro Asp Ser Glu Pro Leu Tyr His Ala Val Ile Ala Arg Val
530 535 540

Gly Ala Ala Val Ser Glu His Arg Gln Thr Leu Ser Pro Val Pro Gln
545 550 555 560

Gln Phe Ser Met Ser Asn Ile Leu Trp Lys Pro Trp Leu Gln Pro Cys
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Cys Gly His Phe Pro Phe Cys Ser Cys His Glu Asp Gly Asp Gly Thr
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<212> DNA
<213> Homo sapiens

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<210> 10

<211> 589

<212> PRT

<213> Homo sapiens

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Ile Ser Ala Ser Arg Pro Asn Ile Leu Leu Leu Met Ala Asp Asp Leu
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 65 70 75 80
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 Arg Tyr Pro Val Arg Ser Gly Met Val Ser Ser Ile Gly Tyr Arg Val
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 Leu Gln Trp Thr Gly Ala Ser Gly Gly Leu Pro Thr Asn Glu Thr Thr
 115 120 125
 Phe Ala Lys Ile Leu Lys Glu Lys Gly Tyr Ala Thr Gly Leu Ile Gly
 130 135 140
 Lys Trp His Leu Gly Leu Asn Cys Glu Ser Ala Ser Asp His Cys His
 145 150 155 160
 His Pro Leu His His Gly Phe Glu His Phe Tyr Gly Met Pro Phe Ser
 165 170 175
 Leu Met Gly Asp Cys Ala Arg Trp Glu Leu Ser Glu Lys Arg Val Asn
 180 185 190
 Leu Glu Gln Lys Leu Asn Phe Leu Phe Gln Val Leu Ala Leu Val Ala
 195 200 205
 Leu Thr Leu Val Ala Gly Lys Leu Thr His Leu Ile Pro Val Ser Trp
 210 215 220
 Met Pro Val Ile Trp Ser Ala Leu Ser Ala Val Leu Leu Leu Ala Ser
 225 230 235 240
 Ser Tyr Phe Val Gly Ala Leu Ile Val His Ala Asp Cys Phe Leu Met
 245 250 255
 Arg Asn His Thr Ile Thr Glu Gln Pro Met Cys Phe Gln Arg Thr Thr
 260 265 270
 Pro Leu Ile Leu Gln Glu Val Ala Ser Phe Leu Lys Arg Asn Lys His
 275 280 285
 Gly Pro Phe Leu Leu Phe Val Ser Phe Leu His Val His Ile Pro Leu
 290 295 300

Ile Thr Met Glu Asn Phe Leu Gly Lys Ser Leu His Gly Leu Tyr Gly
 305 310 315 320

Asp Asn Val Glu Glu Met Asp Trp Met Val Gly Arg Ile Leu Asp Thr
 325 330 335

Leu Asp Val Glu Gly Leu Ser Asn Ser Thr Leu Ile Tyr Phe Thr Ser
 340 345 350

Asp His Gly Gly Ser Leu Glu Asn Gln Leu Gly Asn Thr Gln Tyr Gly
 355 360 365

Gly Trp Asn Gly Ile Tyr Lys Gly Gly Lys Gly Met Gly Gly Trp Glu
 370 375 380

Gly Gly Ile Arg Val Pro Gly Ile Phe Arg Trp Pro Gly Val Leu Pro
 385 390 395 400

Ala Gly Arg Val Ile Gly Glu Pro Thr Ser Leu Met Asp Val Phe Pro
 405 410 415

Thr Val Val Arg Leu Ala Gly Gly Glu Val Pro Gln Asp Arg Val Ile
 420 425 430

Asp Gly Gln Asp Leu Leu Pro Leu Leu Leu Gly Thr Ala Gln His Ser
 435 440 445

Asp His Glu Phe Leu Met His Tyr Cys Glu Arg Phe Leu His Ala Ala
 450 455 460

Arg Trp His Gln Arg Asp Arg Gly Thr Met Trp Lys Val His Phe Val
 465 470 475 480

Thr Pro Val Phe Gln Pro Glu Gly Ala Gly Ala Cys Tyr Gly Arg Lys
 485 490 495

Val Cys Pro Cys Phe Gly Glu Lys Val Val His His Asp Pro Pro Leu
 500 505 510

Leu Phe Asp Leu Ser Arg Asp Pro Ser Glu Thr His Ile Leu Thr Pro
 515 520 525

Ala Ser Glu Pro Val Phe Tyr Gln Val Met Glu Arg Val Gln Gln Ala
 530 535 540

Val Trp Glu His Gln Arg Thr Leu Ser Pro Val Pro Leu Gln Leu Asp
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Arg Leu Gly Asn Ile Trp Arg Pro Trp Leu Gln Pro Cys Cys Gly Pro
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Phe Pro Leu Cys Trp Cys Leu Arg Glu Asp Asp Pro Gln
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 <211> 2012
 <212> DNA
 <213> Homo sapiens

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<210> 12
<211> 590
<212> PRT
<213> Homo sapiens

<400> 12

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Val Leu Ile Met Val Asp Asp Leu Gly Ile Gly Asp Leu Gly Cys Tyr
35 40 45

Gly Asn Asp Thr Met Arg Thr Pro His Ile Asp Arg Leu Ala Arg Glu
50 55 60

Gly Val Arg Leu Thr Gln His Ile Ser Ala Ala Ser Leu Cys Ser Pro
65 70 75 80

Ser Arg Ser Ala Phe Leu Thr Gly Arg Tyr Pro Ile Arg Ser Gly Met
85 90 95

Val Ser Ser Gly Asn Arg Arg Val Ile Gln Asn Leu Ala Val Pro Ala
100 105 110

Gly Leu Pro Leu Asn Glu Thr Thr Leu Ala Ala Leu Leu Lys Lys Gln
115 120 125

Gly Tyr Ser Thr Gly Leu Ile Gly Lys Trp His Gln Gly Leu Asn Cys
130 135 140

Asp Ser Arg Ser Asp Gln Cys His His Pro Tyr Asn Tyr Gly Phe Asp

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Tyr Tyr Tyr Gly Met Pro Phe Thr Leu Val Asp Ser Cys Trp Pro Asp						
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Pro Ser Arg Asn Thr Glu Leu Ala Phe Glu Ser Gln Leu Trp Leu Cys						
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Val Gln Leu Val Ala Ile Ala Ile Leu Thr Leu Thr Phe Gly Lys Leu						
	195		200			205
Ser Gly Trp Val Ser Val Pro Trp Leu Leu Ile Phe Ser Met Ile Leu						
	210		215		220	
Phe Ile Phe Leu Leu Gly Tyr Ala Trp Phe Ser Ser His Thr Ser Pro						
225		230		235		240
Leu Tyr Trp Asp Cys Leu Leu Met Arg Gly His Glu Ile Thr Glu Gln						
	245		250			255
Pro Met Lys Ala Glu Arg Ala Gly Ser Ile Met Val Lys Glu Ala Ile						
	260		265			270
Ser Phe Leu Glu Arg His Ser Lys Glu Thr Phe Leu Leu Phe Phe Ser						
	275		280			285
Phe Leu His Val His Thr Pro Leu Pro Thr Thr Asp Asp Phe Thr Gly						
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Thr Ser Lys His Gly Leu Tyr Gly Asp Asn Val Glu Glu Met Asp Ser						
305		310		315		320
Met Val Gly Lys Ile Leu Asp Ala Ile Asp Asp Phe Gly Leu Arg Asn						
	325		330			335
Asn Thr Leu Val Tyr Phe Thr Ser Asp His Gly Gly His Leu Glu Ala						
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Arg Arg Gly His Ala Gln Leu Gly Gly Trp Asn Gly Ile Tyr Lys Gly						
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Gly Lys Gly Met Gly Gly Trp Glu Gly Gly Ile Arg Val Pro Gly Ile						
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Val Arg Trp Pro Gly Lys Val Pro Ala Gly Arg Leu Ile Lys Glu Pro						
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Thr Ser Leu Met Asp Ile Leu Pro Thr Val Ala Ser Val Ser Gly Gly
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Ser Leu Pro Gln Asp Arg Val Ile Asp Gly Arg Asp Leu Met Pro Leu
420 425 430

Leu Gln Gly Asn Val Arg His Ser Glu His Glu Phe Leu Phe His Tyr
435 440 445

Cys Gly Ser Tyr Leu His Ala Val Arg Trp Ile Pro Lys Asp Asp Ser
450 455 460

Gly Ser Val Trp Lys Ala His Tyr Val Thr Pro Val Phe Gln Pro Pro
465 470 475 480

Ala Ser Gly Gly Cys Tyr Val Thr Ser Leu Cys Arg Cys Phe Gly Glu
485 490 495

Gln Val Thr Tyr His Asn Pro Pro Leu Leu Phe Asp Leu Ser Arg Asp
500 505 510

Pro Ser Glu Ser Thr Pro Leu Thr Pro Ala Thr Glu Pro Leu His Asp
515 520 525

Phe Val Ile Lys Lys Val Ala Asn Ala Leu Lys Glu His Gln Glu Thr
530 535 540

Ile Val Pro Val Thr Tyr Gln Leu Ser Glu Leu Asn Gln Gly Arg Thr
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Trp Leu Lys Pro Cys Cys Gly Val Phe Pro Phe Cys Leu Cys Asp Lys
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<211> 2028

<212> DNA

<213> Homo sapiens

<400> 13

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<210> 14
<211> 525
<212> PRT
<213> Homo sapiens

<400> 14

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35 40 45

Gly Asp Leu Gly Ala Asn Trp Ala Glu Thr Lys Asp Thr Ala Asn Leu
50 55 60

Asp Lys Met Ala Ser Glu Gly Met Arg Phe Val Asp Phe His Ala Ala
65 70 75 80

Ala Ser Thr Cys Ser Pro Ser Arg Ala Ser Leu Leu Thr Gly Arg Leu
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Tyr Ser His Asp Met Gly Cys Thr Asp Thr Pro Gly Tyr Asn His Pro
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Met Tyr Pro His Arg Pro Val Met Met Val Ile Ser His Ala Ala Pro
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His Gly Pro Glu Asp Ser Ala Pro Gln Phe Ser Lys Leu Tyr Pro Asn
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Lys His Trp Ile Met Gln Tyr Thr Gly Pro Met Leu Pro Ile His Met
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Ile Gly Gln Phe Gly Leu Val Lys Gly Lys Ser Met Pro Tyr Asp Phe
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Val Leu Lys Leu Leu Asp Pro Glu Lys Pro Gly Asn Arg Phe Arg Thr
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Arg Tyr Gln Thr Ala Cys Glu Gln Pro Gly Gln Lys Trp Gln Cys Ile
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Pro Thr Thr Val Arg Val Thr His Lys Cys Phe Ile Leu Pro Asn Asp
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755 760 765

Thr Ser Ser Asn Asn Asn Thr Tyr Trp Cys Leu Arg Thr Val Asn Glu
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Val Glu Arg Gly Ile Leu Asn Gln Leu His Val Gln Leu Met Glu Leu
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Arg Ser Cys Gln Gly Tyr Lys Gln Cys Asn Pro Arg Pro Lys Asn Leu
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 Val Leu Thr Asp Asp Gln Asp Val Glu Leu Gly Ser Met Gln Val Met
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 Asn Lys Thr Arg Arg Ile Met Glu Gln Gly Gly Ala His Phe Ile Asn
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 Ala Phe Val Thr Thr Pro Met Cys Cys Pro Ser Arg Ser Ser Ile Leu
 85 90 95
 Thr Gly Lys Tyr Val His Asn His Asn Thr Tyr Thr Asn Asn Glu Asn
 100 105 110
 Cys Ser Ser Pro Ser Trp Gln Ala Gln His Glu Ser Arg Thr Phe Ala
 115 120 125
 Val Tyr Leu Asn Ser Thr Gly Tyr Arg Thr Ala Phe Phe Gly Lys Tyr
 130 135 140
 Leu Asn Glu Tyr Asn Gly Ser Tyr Val Pro Pro Gly Trp Lys Glu Trp
 145 150 155 160
 Val Gly Leu Leu Lys Asn Ser Arg Phe Tyr Asn Tyr Thr Leu Cys Arg
 165 170 175
 Asn Gly Val Lys Glu Lys His Gly Ser Asp Tyr Ser Lys Asp Tyr Leu
 180 185 190
 Thr Asp Leu Ile Thr Asn Asp Ser Val Ser Phe Phe Arg Thr Ser Lys
 195 200 205
 Lys Met Tyr Pro His Arg Pro Val Leu Met Val Ile Ser His Ala Ala
 210 215 220
 Pro His Gly Pro Glu Asp Ser Ala Pro Gln Tyr Ser Arg Leu Phe Pro
 225 230 235 240
 Asn Ala Ser Gln His Ile Thr Pro Ser Tyr Asn Tyr Ala Pro Asn Pro
 245 250 255

Asp Lys His Trp Ile Met Arg Tyr Thr Gly Pro Met Lys Pro Ile His
 260 265 270

Met Glu Phe Thr Asn Met Leu Gln Arg Lys Arg Leu Gln Thr Leu Met
 275 280 285

Ser Val Asp Asp Ser Met Glu Thr Ile Tyr Asn Met Leu Val Glu Thr
 290 295 300

Gly Glu Leu Asp Asn Thr Tyr Ile Val Tyr Thr Ala Asp His Gly Tyr
 305 310 315 320

His Ile Gly Gln Phe Gly Leu Val Lys Gly Lys Ser Met Pro Tyr Glu
 325 330 335

Phe Asp Ile Arg Val Pro Phe Tyr Val Arg Gly Pro Asn Val Glu Ala
 340 345 350

Gly Cys Leu Asn Pro His Ile Val Leu Asn Ile Asp Leu Ala Pro Thr
 355 360 365

Ile Leu Asp Ile Ala Gly Leu Asp Ile Pro Ala Asp Met Asp Gly Lys
 370 375 380

Ser Ile Leu Lys Leu Leu Asp Thr Glu Arg Pro Val Asn Arg Phe His
 385 390 395 400

Leu Lys Lys Lys Met Arg Val Trp Arg Asp Ser Phe Leu Val Glu Arg
 405 410 415

Gly Lys Leu Leu His Lys Arg Asp Asn Asp Lys Val Asp Ala Gln Glu
 420 425 430

Glu Asn Phe Leu Pro Lys Tyr Gln Arg Val Lys Asp Leu Cys Gln Arg
 435 440 445

Ala Glu Tyr Gln Thr Ala Cys Glu Gln Leu Gly Gln Lys Trp Gln Cys
 450 455 460

Val Glu Asp Ala Thr Gly Lys Leu Lys Leu His Lys Cys Lys Gly Pro
 465 470 475 480

Met Arg Leu Gly Gly Ser Arg Ala Leu Ser Asn Leu Val Pro Lys Tyr
 485 490 495

Tyr Gly Gln Gly Ser Glu Ala Cys Thr Cys Asp Ser Gly Asp Tyr Lys
 500 505 510

Leu Ser Leu Ala Gly Arg Arg Lys Lys Leu Phe Lys Lys Lys Tyr Lys
515 520 525

Ala Ser Tyr Val Arg Ser Arg Ser Ile Arg Ser Val Ala Ile Glu Val
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Asp Gly Arg Val Tyr His Val Gly Leu Gly Asp Ala Ala Gln Pro Arg
545 550 555 560

Asn Leu Thr Lys Arg His Trp Pro Gly Ala Pro Glu Asp Gln Asp Asp
565 570 575

Lys Asp Gly Gly Asp Phe Ser Gly Thr Gly Gly Leu Pro Asp Tyr Ser
580 585 590

Ala Ala Asn Pro Ile Lys Val Thr His Arg Cys Tyr Ile Leu Glu Asn
595 600 605

Asp Thr Val Gln Cys Asp Leu Asp Leu Tyr Lys Ser Leu Gln Ala Trp
610 615 620

Lys Asp His Lys Leu His Ile Asp His Glu Ile Glu Thr Leu Gln Asn
625 630 635 640

Lys Ile Lys Asn Leu Arg Glu Val Arg Gly His Leu Lys Lys Lys Arg
645 650 655

Pro Glu Glu Cys Asp Cys His Lys Ile Ser Tyr His Thr Gln His Lys
660 665 670

Gly Arg Leu Lys His Arg Gly Ser Ser Leu His Pro Phe Arg Lys Gly
675 680 685

Leu Gln Glu Lys Asp Lys Val Trp Leu Leu Arg Glu Gln Lys Arg Lys
690 695 700

Lys Lys Leu Arg Lys Leu Leu Lys Arg Leu Gln Asn Asn Asp Thr Cys
705 710 715 720

Ser Met Pro Gly Leu Thr Cys Phe Thr His Asp Asn Gln His Trp Gln
725 730 735

Thr Ala Pro Phe Trp Thr Leu Gly Pro Phe Cys Ala Cys Thr Ser Ala
740 745 750

Asn Asn Asn Thr Tyr Trp Cys Met Arg Thr Ile Asn Glu Thr His Asn
755 760 765

Phe Leu Phe Cys Glu Phe Ala Thr Gly Phe Leu Glu Tyr Phe Asp Leu
 770 775 780

Asn Thr Asp Pro Tyr Gln Leu Met Asn Ala Val Asn Thr Leu Asp Arg
 785 790 795 800

Asp Val Leu Asn Gln Leu His Val Gln Leu Met Glu Leu Arg Ser Cys
 805 810 815

Lys Gly Tyr Lys Gln Cys Asn Pro Arg Thr Arg Asn Met Asp Leu Gly
 820 825 830

Leu Lys Asp Gly Gly Ser Tyr Glu Gln Tyr Arg Gly Gln Leu Trp Glu
 835 840 845

Gly Trp Glu Gly
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 <211> 2390
 <212> DNA
 <213> Homo sapiens

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<210> 20

<211> 522

<212> PRT

<213> Homo sapiens

<400> 20

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Glu Gly Leu Leu Phe Pro Asn Phe Tyr Ser Ala Asn Pro Leu Cys Ser		
65	70	75
Pro Ser Arg Ala Ala Leu Leu Thr Gly Arg Leu Pro Ile Arg Asn Gly		
85	90	95
Phe Tyr Thr Thr Asn Ala His Ala Arg Asn Ala Tyr Thr Pro Gln Glu		
100	105	110
Ile Val Gly Gly Ile Pro Asp Ser Glu Gln Leu Leu Pro Glu Leu Leu		
115	120	125
Lys Lys Ala Gly Tyr Val Ser Lys Ile Val Gly Lys Trp His Leu Gly		
130	135	140
His Arg Pro Gln Phe His Pro Leu Lys His Gly Phe Asp Glu Trp Phe		
145	150	155
Gly Ser Pro Asn Cys His Phe Gly Pro Tyr Asp Asn Lys Ala Arg Pro		
165	170	175
Asn Ile Pro Val Tyr Arg Asp Trp Glu Met Val Gly Arg Tyr Tyr Glu		
180	185	190
Glu Phe Pro Ile Asn Leu Lys Thr Gly Glu Ala Asn Leu Thr Gln Ile		
195	200	205
Tyr Leu Gln Glu Ala Leu Asp Phe Ile Lys Arg Gln Ala Arg His His		
210	215	220
Pro Phe Phe Leu Tyr Trp Ala Val Asp Ala Thr His Ala Pro Val Tyr		
225	230	235
Ala Ser Lys Pro Phe Leu Gly Thr Ser Gln Arg Gly Arg Tyr Gly Asp		
245	250	255
Ala Val Arg Glu Ile Asp Asp Ser Ile Gly Lys Ile Leu Glu Leu Leu		
260	265	270
Gln Asp Leu His Val Ala Asp Asn Thr Phe Val Phe Phe Thr Ser Asp		
275	280	285

Asn Gly Ala Ala Leu Ile Ser Ala Pro Glu Gln Gly Gly Ser Asn Gly
 290 295 300

Pro Phe Leu Cys Gly Lys Gln Thr Thr Phe Glu Gly Gly Met Arg Glu
 305 310 315 320

Pro Ala Leu Ala Trp Trp Pro Gly His Val Thr Ala Gly Gln Val Ser
 325 330 335

His Gln Leu Gly Ser Ile Met Asp Leu Phe Thr Thr Ser Leu Ala Leu
 340 345 350

Ala Gly Leu Thr Pro Pro Ser Asp Arg Ala Ile Asp Gly Leu Asn Leu
 355 360 365

Leu Pro Thr Leu Leu Gln Gly Arg Leu Met Asp Arg Pro Ile Phe Tyr
 370 375 380

Tyr Arg Gly Asp Thr Leu Met Ala Ala Thr Leu Gly Gln His Lys Ala
 385 390 395 400

His Phe Trp Thr Trp Thr Asn Ser Trp Glu Asn Phe Arg Gln Gly Ile
 405 410 415

Asp Phe Cys Pro Gly Gln Asn Val Ser Gly Val Thr Thr His Asn Leu
 420 425 430

Glu Asp His Thr Lys Leu Pro Leu Ile Phe His Leu Gly Arg Asp Pro
 435 440 445

Gly Glu Arg Phe Pro Leu Ser Phe Ala Ser Ala Glu Tyr Gln Glu Ala
 450 455 460

Leu Ser Arg Ile Thr Ser Val Val Gln Gln His Gln Glu Ala Leu Val
 465 470 475 480

Pro Ala Gln Pro Gln Leu Asn Val Cys Asn Trp Ala Val Met Asn Trp
 485 490 495

Ala Pro Pro Gly Cys Glu Lys Leu Gly Lys Cys Leu Thr Pro Pro Glu
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Ser Ile Pro Lys Lys Cys Leu Trp Ser His
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<210> 21
 <211> 2504
 <212> DNA

<213> Homo sapiens

<400> 21

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<210> 22
 <211> 550
 <212> PRT
 <213> Homo sapiens

<400> 22

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Thr Thr Asp Ala Leu Asn Val Leu Leu Ile Ile Val Asp Asp Leu Arg
35 40 45

Pro Ser Leu Gly Cys Tyr Gly Asp Lys Leu Val Arg Ser Pro Asn Ile
50 55 60

Asp Gln Leu Ala Ser His Ser Leu Leu Phe Gln Asn Ala Phe Ala Gln
65 70 75 80

Gln Ala Val Cys Ala Pro Ser Arg Val Ser Phe Leu Thr Gly Arg Arg
85 90 95

Pro Asp Thr Thr Arg Leu Tyr Asp Phe Asn Ser Tyr Trp Arg Val His
100 105 110

Ala Gly Asn Phe Ser Thr Ile Pro Gln Tyr Phe Lys Glu Asn Gly Tyr
115 120 125

Val Thr Met Ser Val Gly Lys Val Phe His Pro Gly Ile Ser Ser Asn
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 His Thr Asp Asp Ser Pro Tyr Ser Trp Ser Phe Pro Pro Tyr His Pro
 145 150 155 160
 Ser Ser Glu Lys Tyr Glu Asn Thr Lys Thr Cys Arg Gly Pro Asp Gly
 165 170 175
 Glu Leu His Ala Asn Leu Leu Cys Pro Val Asp Val Leu Asp Val Pro
 180 185 190
 Glu Gly Thr Leu Pro Asp Lys Gln Ser Thr Glu Gln Ala Ile Gln Leu
 195 200 205
 Leu Glu Lys Met Lys Thr Ser Ala Ser Pro Phe Phe Leu Ala Val Gly
 210 215 220
 Tyr His Lys Pro His Ile Pro Phe Arg Tyr Pro Lys Glu Phe Gln Lys
 225 230 235 240
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 Asp Gly Leu Pro Pro Val Ala Tyr Asn Pro Trp Met Asp Ile Arg Gln
 260 265 270
 Arg Glu Asp Val Gln Ala Leu Asn Ile Ser Val Pro Tyr Gly Pro Ile
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 Pro Val Asp Phe Gln Arg Lys Ile Arg Gln Ser Tyr Phe Ala Ser Val
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 Ser Tyr Leu Asp Thr Gln Val Gly Arg Leu Leu Ser Ala Leu Asp Asp
 305 310 315 320
 Leu Gln Leu Ala Asn Ser Thr Ile Ile Ala Phe Thr Ser Asp His Gly
 325 330 335
 Trp Ala Leu Gly Glu His Gly Glu Trp Ala Lys Tyr Ser Asn Phe Asp
 340 345 350
 Val Ala Thr His Val Pro Leu Ile Phe Tyr Val Pro Gly Arg Thr Ala
 355 360 365
 Ser Leu Pro Glu Ala Gly Glu Lys Leu Phe Pro Tyr Leu Asp Pro Phe
 370 375 380

Asp Ser Ala Ser Gln Leu Met Glu Pro Gly Arg Gln Ser Met Asp Leu
385 390 395 400

Val Glu Leu Val Ser Leu Phe Pro Thr Leu Ala Gly Leu Ala Gly Leu
405 410 415

Gln Val Pro Pro Arg Cys Pro Val Pro Ser Phe His Val Glu Leu Cys
420 425 430

Arg Glu Gly Lys Asn Leu Leu Lys His Phe Arg Phe Arg Asp Leu Glu
435 440 445

Glu Asp Pro Tyr Leu Pro Gly Asn Pro Arg Glu Leu Ile Ala Tyr Ser
450 455 460

Gln Tyr Pro Arg Pro Ser Asp Ile Pro Gln Trp Asn Ser Asp Lys Pro
465 470 475 480

Ser Leu Lys Asp Ile Lys Ile Met Gly Tyr Ser Ile Arg Thr Ile Asp
485 490 495

Tyr Arg Tyr Thr Val Trp Val Gly Phe Asn Pro Asp Glu Phe Leu Ala
500 505 510

Asn Phe Ser Asp Ile His Ala Gly Glu Leu Tyr Phe Val Asp Ser Asp
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Pro Leu Gln Asp His Asn Met Tyr Asn Asp Ser Gln Gly Gly Asp Leu
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Phe Gln Leu Leu Met Pro
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<212> DNA
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<210> 24
<211> 502
<212> PRT
<213> Homo sapiens
<400> 24

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Met Ser Cys Pro Val Pro Ala Cys Cys Ala Leu Leu Leu Val Leu Gly
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Leu Cys Arg Ala Arg Pro Arg Asn Ala Leu Leu Leu Leu Ala Asp Asp
          20          25          30

```

```

Gly Gly Phe Glu Ser Gly Ala Tyr Asn Asn Ser Ala Ile Ala Thr Pro
          35          40          45

```

```

His Leu Asp Ala Leu Ala Arg Arg Ser Leu Leu Phe Arg Asn Ala Phe
          50          55          60

```

```

Thr Ser Val Ser Ser Cys Ser Pro Ser Arg Ala Ser Leu Leu Thr Gly
65          70          75          80

```

```

Leu Pro Gln His Gln Asn Gly Met Tyr Gly Leu His Gln Asp Val His
          85          90          95

```

```

His Phe Asn Ser Phe Asp Lys Val Arg Ser Leu Pro Leu Leu Leu Ser
          100          105          110

```

```

Gln Ala Gly Val Arg Thr Gly Ile Ile Gly Lys Lys His Val Gly Pro
          115          120          125

```

```

Glu Thr Val Tyr Pro Phe Asp Phe Ala Tyr Thr Glu Glu Asn Gly Ser
          130          135          140

```

```

Val Leu Gln Val Gly Arg Asn Ile Thr Arg Ile Lys Leu Leu Val Arg
          145          150          155          160

```

```

Lys Phe Leu Gln Thr Gln Asp Asp Arg Pro Phe Phe Leu Tyr Val Ala

```

- 50 -

Ala Gly Gln Pro Thr Gly Trp Tyr Lys Asp Leu Arg His Tyr Tyr Tyr
 420 425 430

Arg Ala Arg Trp Glu Leu Tyr Asp Arg Ser Arg Asp Pro His Glu Thr
 435 440 445

Gln Asn Leu Ala Thr Asp Pro Arg Phe Ala Gln Leu Leu Glu Met Leu
 450 455 460

Arg Asp Gln Leu Ala Lys Trp Gln Trp Glu Thr His Asp Pro Trp Val
 465 470 475 480

Cys Ala Pro Asp Gly Val Leu Glu Glu Lys Leu Ser Pro Gln Cys Gln
 485 490 495

Pro Leu His Asn Glu Leu
 500

<210> 25

<211> 5130

<212> DNA

<213> Homo sapiens

<400> 25

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cgtcccgctcc cggggtcacc cgggagcctg tccgctatgc ggctcctgcc tctagcccca	180
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gtgggtgctgc tcctcacgga cgaccaggac gaagtgtctg gcggcatgac accgctaaag	360
aaaaccaaag ctctcatcgg agagatgggg atgacttttt ccagtgttta tgtgccaagt	420
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gttgtgaaca aactcttga ggggaactgc agtagtaagt cctggcagaa gatccaagaa	540
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cagaatgtct ttgcaccaag aaacaagaac ttcaacatcc atggaacgaa caagcactgg	960
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<210> 26
<211> 552
<212> PRT
<213> Homo sapiens

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```

<400> 26

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Met Arg Leu Leu Pro Leu Ala Pro Gly Arg Leu Arg Arg Gly Ser Pro
1 5 10 15

```

```

Arg His Leu Pro Ser Cys Ser Pro Ala Leu Leu Leu Leu Val Leu Gly
20 25 30

```

```

Gly Cys Leu Gly Val Phe Gly Val Ala Ala Gly Thr Arg Arg Pro Asn
35 40 45

```

```

Val Val Leu Leu Leu Thr Asp Asp Gln Asp Glu Val Leu Gly Gly Met
50 55 60

```

```

Thr Pro Leu Lys Lys Thr Lys Ala Leu Ile Gly Glu Met Gly Met Thr
65 70 75 80

```

```

Phe Ser Ser Ala Tyr Val Pro Ser Ala Leu Cys Cys Pro Ser Arg Ala
85 90 95

```

```

Ser Ile Leu Thr Gly Lys Tyr Pro His Asn His His Val Val Asn Asn
100 105 110

```

```

Thr Leu Glu Gly Asn Cys Ser Ser Lys Ser Trp Gln Lys Ile Gln Glu
115 120 125

```

```

Pro Asn Thr Phe Pro Ala Ile Leu Arg Ser Met Cys Gly Tyr Gln Thr
130 135 140

```

```

Phe Phe Ala Gly Lys Tyr Leu Asn Glu Tyr Gly Ala Pro Asp Ala Gly
145 150 155 160

```

```

Gly Leu Glu His Val Pro Leu Gly Trp Ser Tyr Trp Tyr Ala Leu Glu
165 170 175

```

```

Lys Asn Ser Lys Tyr Tyr Asn Tyr Thr Leu Ser Ile Asn Gly Lys Ala

```

180	185	190
Arg Lys His Gly Glu Asn Tyr Ser Val Asp Tyr Leu Thr Asp Val Leu		
195	200	205
Ala Asn Val Ser Leu Asp Phe Leu Asp Tyr Lys Ser Asn Phe Glu Pro		
210	215	220
Phe Phe Met Met Ile Ala Thr Pro Ala Pro His Ser Pro Trp Thr Ala		
225	230	235
Ala Pro Gln Tyr Gln Lys Ala Phe Gln Asn Val Phe Ala Pro Arg Asn		
245	250	255
Lys Asn Phe Asn Ile His Gly Thr Asn Lys His Trp Leu Ile Arg Gln		
260	265	270
Ala Lys Thr Pro Met Thr Asn Ser Ser Ile Gln Phe Leu Asp Asn Ala		
275	280	285
Phe Arg Lys Arg Trp Gln Thr Leu Leu Ser Val Asp Asp Leu Val Glu		
290	295	300
Lys Leu Val Lys Arg Leu Glu Phe Thr Gly Glu Leu Asn Asn Thr Tyr		
305	310	315
Ile Phe Tyr Thr Ser Asp Asn Gly Tyr His Thr Gly Gln Phe Ser Leu		
325	330	335
Pro Ile Asp Lys Arg Gln Leu Tyr Glu Phe Asp Ile Lys Val Pro Leu		
340	345	350
Leu Val Arg Gly Pro Gly Ile Lys Pro Asn Gln Thr Ser Lys Met Leu		
355	360	365
Val Ala Asn Ile Asp Leu Gly Pro Thr Ile Leu Asp Ile Ala Gly Tyr		
370	375	380
Asp Leu Asn Lys Thr Gln Met Asp Gly Met Ser Leu Leu Pro Ile Leu		
385	390	395
Arg Gly Ala Ser Asn Leu Thr Trp Arg Ser Asp Val Leu Val Glu Tyr		
405	410	415
Gln Gly Glu Gly Arg Asn Val Thr Asp Pro Thr Cys Pro Ser Leu Ser		
420	425	430

Pro Gly Val Ser Gln Cys Phe Pro Asp Cys Val Cys Glu Asp Ala Tyr
 435 440 445

Asn Asn Thr Tyr Ala Cys Val Arg Thr Met Ser Ala Leu Trp Asn Leu
 450 455 460

Gln Tyr Cys Glu Phe Asp Asp Gln Glu Val Phe Val Glu Val Tyr Asn
 465 470 475 480

Leu Thr Ala Asp Pro Asp Gln Ile Thr Asn Ile Ala Lys Thr Ile Asp
 485 490 495

Pro Glu Leu Leu Gly Lys Met Asn Tyr Arg Leu Met Met Leu Gln Ser
 500 505 510

Cys Ser Gly Pro Thr Cys Arg Thr Pro Gly Val Phe Asp Pro Gly Tyr
 515 520 525

Arg Phe Asp Pro Arg Leu Met Phe Ser Asn Arg Gly Ser Val Arg Thr
 530 535 540

Arg Arg Phe Ser Lys His Leu Leu
 545 550

<210> 27
 <211> 507
 <212> PRT
 <213> Homo sapiens

<400> 27

Met Gly Ala Pro Arg Ser Leu Leu Leu Ala Leu Ala Ala Gly Leu Ala
 1 5 10 15

Val Ala Arg Pro Pro Asn Ile Val Leu Ile Phe Ala Asp Asp Leu Gly
 20 25 30

Tyr Gly Asp Leu Gly Cys Tyr Gly His Pro Ser Ser Thr Thr Pro Asn
 35 40 45

Leu Asp Gln Leu Ala Ala Gly Gly Leu Arg Phe Thr Asp Phe Tyr Val
 50 55 60

Pro Val Ser Leu Ser Thr Pro Ser Arg Ala Ala Leu Leu Thr Gly Arg
 65 70 75 80

Leu Pro Val Arg Met Gly Met Tyr Pro Gly Val Leu Val Pro Ser Ser
 85 90 95

Arg Gly Gly Leu Pro Leu Glu Glu Val Thr Val Ala Glu Val Leu Ala
 100 105 110

Ala Arg Gly Tyr Leu Thr Gly Met Ala Gly Lys Trp His Leu Gly Val
 115 120 125

Gly Pro Glu Gly Ala Phe Leu Pro Pro His Gln Gly Phe His Arg Phe
 130 135 140

Leu Gly Ile Pro Tyr Ser His Asp Gln Gly Pro Cys Gln Asn Leu Thr
 145 150 155 160

Cys Phe Pro Pro Ala Thr Pro Cys Asp Gly Gly Cys Asp Gln Gly Leu
 165 170 175

Val Pro Ile Pro Leu Leu Ala Asn Leu Ser Val Glu Ala Gln Pro Pro
 180 185 190

Trp Leu Pro Gly Leu Glu Ala Arg Tyr Met Ala Phe Ala His Asp Leu
 195 200 205

Met Ala Asp Ala Gln Arg Gln Asp Arg Pro Phe Phe Leu Tyr Tyr Ala
 210 215 220

Ser His His Thr His Tyr Pro Gln Phe Ser Gly Gln Ser Phe Ala Glu
 225 230 235 240

Arg Ser Gly Arg Gly Pro Phe Gly Asp Ser Leu Met Glu Leu Asp Ala
 245 250 255

Ala Val Gly Thr Leu Met Thr Ala Ile Gly Asp Leu Gly Leu Leu Glu
 260 265 270

Glu Thr Leu Val Ile Phe Thr Ala Asp Asn Gly Pro Glu Thr Met Arg
 275 280 285

Met Ser Arg Gly Gly Cys Ser Gly Leu Leu Arg Cys Gly Lys Gly Thr
 290 295 300

Thr Tyr Glu Gly Gly Val Arg Glu Pro Ala Leu Ala Phe Trp Pro Gly
 305 310 315 320

His Ile Ala Pro Gly Val Thr His Glu Leu Ala Ser Ser Leu Asp Leu
 325 330 335

Leu Pro Thr Leu Ala Ala Leu Ala Gly Ala Pro Leu Pro Asn Val Thr
 340 345 350

Leu Asp Gly Phe Asp Leu Ser Pro Leu Leu Leu Gly Thr Gly Lys Ser
 355 360 365

Pro Arg Gln Ser Leu Phe Phe Tyr Pro Ser Tyr Pro Asp Glu Val Arg
 370 375 380

Gly Val Phe Ala Val Arg Ser Gly Lys Tyr Lys Ala His Phe Phe Thr
 385 390 395 400

Gln Gly Ser Ala His Ser Asp Thr Thr Ala Asp Pro Ala Cys His Ala
 405 410 415

Ser Ser Ser Leu Thr Ala His Glu Pro Pro Leu Leu Tyr Asp Leu Ser
 420 425 430

Lys Asp Pro Gly Glu Asn Tyr Asn Leu Leu Gly Gly Val Ala Gly Ala
 435 440 445

Thr Pro Glu Val Leu Gln Ala Leu Lys Gln Leu Gln Leu Leu Lys Ala
 450 455 460

Gln Leu Asp Ala Ala Val Thr Phe Gly Pro Ser Gln Val Ala Arg Gly
 465 470 475 480

Glu Asp Pro Ala Leu Gln Ile Cys Cys His Pro Gly Cys Thr Pro Arg
 485 490 495

Pro Ala Cys Cys His Cys Pro Asp Pro His Ala
 500 505

<210> 28
 <211> 533
 <212> PRT
 <213> Homo sapiens

<400> 28

Met Gly Pro Arg Gly Ala Ala Ser Leu Pro Arg Gly Pro Gly Pro Arg
 1 5 10 15

Arg Leu Leu Leu Pro Val Val Leu Pro Leu Leu Leu Leu Leu Leu
 20 25 30

Ala Pro Pro Gly Ser Gly Ala Gly Ala Ser Arg Pro Pro His Leu Val
 35 40 45

Phe Leu Leu Ala Asp Asp Leu Gly Trp Asn Asp Val Gly Phe His Gly
 50 55 60

Ser Arg Ile Arg Thr Pro His Leu Asp Ala Leu Ala Ala Gly Gly Val
 65 70 75 80
 Leu Leu Asp Asn Tyr Tyr Thr Gln Pro Leu Ser Thr Pro Ser Arg Ser
 85 90 95
 Gln Leu Leu Thr Gly Arg Tyr Gln Ile Arg Thr Gly Leu Gln His Gln
 100 105 110
 Ile Ile Trp Pro Cys Gln Pro Ser Cys Val Pro Leu Asp Glu Lys Leu
 115 120 125
 Leu Pro Gln Leu Leu Lys Glu Ala Gly Tyr Thr Thr His Met Val Gly
 130 135 140
 Lys Trp His Leu Gly Met Tyr Arg Lys Glu Cys Leu Pro Thr Arg Arg
 145 150 155 160
 Gly Phe Asp Thr Tyr Phe Gly Tyr Leu Leu Gly Ser Glu Asp Tyr Tyr
 165 170 175
 Ser His Glu Arg Cys Thr Leu Ile Asp Ala Leu Asn Val Thr Arg Cys
 180 185 190
 Ala Leu Asp Phe Arg Asp Gly Glu Glu Val Ala Thr Gly Tyr Lys Asn
 195 200 205
 Met Tyr Ser Thr Asn Ile Phe Thr Lys Arg Ala Ile Ala Leu Ile Thr
 210 215 220
 Asn His Pro Pro Glu Lys Pro Leu Phe Leu Tyr Leu Ala Leu Gln Ser
 225 230 235 240
 Val His Glu Pro Leu Gln Val Pro Glu Glu Tyr Leu Lys Pro Tyr Asp
 245 250 255
 Phe Ile Gln Asp Lys Asn Arg His His Tyr Ala Gly Met Val Ser Leu
 260 265 270
 Met Asp Glu Ala Val Gly Asn Val Thr Ala Ala Leu Lys Ser Ser Gly
 275 280 285
 Leu Trp Asn Asn Thr Val Phe Ile Phe Ser Thr Asp Asn Gly Gly Gln
 290 295 300
 Thr Leu Ala Gly Gly Asn Asn Trp Pro Leu Arg Gly Arg Lys Trp Ser
 305 310 315 320

Leu Trp Glu Gly Gly Val Arg Gly Val Gly Phe Val Ala Ser Pro Leu
 325 330 335

Leu Lys Gln Lys Gly Val Lys Asn Arg Glu Leu Ile His Ile Ser Asp
 340 345 350

Trp Leu Pro Thr Leu Val Lys Leu Ala Arg Gly His Thr Asn Gly Thr
 355 360 365

Lys Pro Leu Asp Gly Phe Asp Val Trp Lys Thr Ile Ser Glu Gly Ser
 370 375 380

Pro Ser Pro Arg Ile Glu Leu Leu His Asn Ile Asp Pro Asn Phe Val
 385 390 395 400

Asp Ser Ser Pro Cys Pro Arg Asn Ser Met Ala Pro Ala Lys Asp Asp
 405 410 415

Ser Ser Leu Pro Glu Tyr Ser Ala Phe Asn Thr Ser Val His Ala Ala
 420 425 430

Ile Arg His Gly Asn Trp Lys Leu Leu Thr Gly Tyr Pro Gly Cys Gly
 435 440 445

Tyr Trp Phe Pro Pro Pro Ser Gln Tyr Asn Val Ser Glu Ile Pro Ser
 450 455 460

Ser Asp Pro Pro Thr Lys Thr Leu Trp Leu Phe Asp Ile Asp Arg Asp
 465 470 475 480

Pro Glu Glu Arg His Asp Leu Ser Arg Glu Tyr Pro His Ile Val Thr
 485 490 495

Lys Leu Leu Ser Arg Leu Gln Phe Tyr His Lys His Ser Val Pro Val
 500 505 510

Tyr Phe Pro Ala Gln Asp Pro Arg Cys Asp Pro Lys Ala Thr Gly Val
 515 520 525

Trp Gly Pro Trp Met
 530

<210> 29
 <211> 583
 <212> PRT
 <213> Homo sapiens

<400> 29

Met Pro Leu Arg Lys Met Lys Ile Pro Phe Leu Leu Leu Phe Phe Leu
 1 5 10 15
 Trp Glu Ala Glu Ser His Glu Ala Ser Arg Pro Asn Ile Ile Leu Val
 20 25 30
 Met Ala Asp Asp Leu Gly Ile Gly Asp Pro Gly Cys Tyr Gly Asn Lys
 35 40 45
 Thr Ile Arg Thr Pro Asn Ile Asp Arg Leu Ala Ser Gly Gly Val Lys
 50 55 60
 Leu Thr Gln His Leu Ala Ala Ser Pro Leu Ser Thr Pro Ser Arg Ala
 65 70 75 80
 Ala Phe Met Thr Gly Arg Tyr Pro Val Arg Ser Gly Met Ala Ser Trp
 85 90 95
 Ser Arg Thr Gly Val Phe Leu Phe Thr Ala Ser Ser Gly Gly Leu Pro
 100 105 110
 Thr Asp Glu Ile Thr Phe Ala Lys Leu Leu Lys Asp Gln Gly Tyr Ser
 115 120 125
 Thr Ala Leu Ile Gly Lys Trp His Leu Gly Met Ser Cys His Ser Lys
 130 135 140
 Thr Asp Phe Cys His His Pro Leu His His Gly Phe Asn Tyr Phe Tyr
 145 150 155 160
 Gly Ile Ser Leu Thr Asn Leu Arg Asp Cys Lys Pro Gly Glu Gly Ser
 165 170 175
 Val Phe Thr Thr Gly Phe Lys Arg Leu Val Phe Leu Pro Leu Gln Ile
 180 185 190
 Val Gly Val Thr Leu Leu Thr Leu Ala Ala Leu Asn Cys Leu Gly Leu
 195 200 205
 Leu His Val Pro Leu Gly Val Phe Phe Ser Leu Leu Phe Leu Ala Ala
 210 215 220
 Leu Ile Leu Thr Leu Phe Leu Gly Phe Leu His Tyr Phe Arg Pro Leu
 225 230 235 240
 Asn Cys Phe Met Met Arg Asn Tyr Glu Ile Ile Gln Gln Pro Met Ser
 245 250 255

Tyr Asp Asn Leu Thr Gln Arg Leu Thr Val Glu Ala Ala Gln Phe Ile
 260 265 270

Gln Arg Asn Thr Glu Thr Pro Phe Leu Leu Val Leu Ser Tyr Leu His
 275 280 285

Val His Thr Ala Leu Phe Ser Ser Lys Asp Phe Ala Gly Lys Ser Gln
 290 295 300

His Gly Val Tyr Gly Asp Ala Val Glu Glu Met Asp Trp Ser Val Gly
 305 310 315 320

Gln Ile Leu Asn Leu Leu Asp Glu Leu Arg Leu Ala Asn Asp Thr Leu
 325 330 335

Ile Tyr Phe Thr Ser Asp Gln Gly Ala His Val Glu Glu Val Ser Ser
 340 345 350

Lys Gly Glu Ile His Gly Gly Ser Asn Gly Ile Tyr Lys Gly Gly Lys
 355 360 365

Ala Asn Asn Trp Glu Gly Gly Ile Arg Val Pro Gly Ile Leu Arg Trp
 370 375 380

Pro Arg Val Ile Gln Ala Gly Gln Lys Ile Asp Glu Pro Thr Ser Asn
 385 390 395 400

Met Asp Ile Phe Pro Thr Val Ala Lys Leu Ala Gly Ala Pro Leu Pro
 405 410 415

Glu Asp Arg Ile Ile Asp Gly Arg Asp Leu Met Pro Leu Leu Glu Gly
 420 425 430

Lys Ser Gln Arg Ser Asp His Glu Phe Leu Phe His Tyr Cys Asn Ala
 435 440 445

Tyr Leu Asn Ala Val Arg Trp His Pro Gln Asn Ser Thr Ser Ile Trp
 450 455 460

Lys Ala Phe Phe Phe Thr Pro Asn Phe Asn Pro Val Gly Ser Asn Gly
 465 470 475 480

Cys Phe Ala Thr His Val Cys Phe Cys Phe Gly Ser Tyr Val Thr His
 485 490 495

His Asp Pro Pro Leu Leu Phe Asp Ile Ser Lys Asp Pro Arg Glu Arg
 500 505 510

Asn Pro Leu Thr Pro Ala Ser Glu Pro Arg Phe Tyr Glu Ile Leu Lys
515 520 525

Val Met Gln Glu Ala Ala Asp Arg His Thr Gln Thr Leu Pro Glu Val
530 535 540

Pro Asp Gln Phe Ser Trp Asn Asn Phe Leu Trp Lys Pro Trp Leu Gln
545 550 555 560

Leu Cys Cys Pro Ser Thr Gly Leu Ser Cys Gln Cys Asp Arg Glu Lys
565 570 575

Gln Asp Lys Arg Leu Ser Arg
580

<210> 30
<211> 593
<212> PRT
<213> Homo sapiens

<400> 30

Met Arg Ser Ala Ala Arg Arg Gly Arg Ala Ala Pro Ala Ala Arg Asp
1 5 10 15

Ser Leu Pro Val Leu Leu Phe Leu Cys Leu Leu Leu Lys Thr Cys Glu
20 25 30

Pro Lys Thr Ala Asn Ala Phe Lys Pro Asn Ile Leu Leu Ile Met Ala
35 40 45

Asp Asp Leu Gly Thr Gly Asp Leu Gly Cys Tyr Gly Asn Asn Thr Leu
50 55 60

Arg Thr Pro Asn Ile Asp Gln Leu Ala Glu Glu Gly Val Arg Leu Thr
65 70 75 80

Gln His Leu Ala Ala Ala Pro Leu Ser Thr Pro Ser Arg Ala Ala Phe
85 90 95

Leu Thr Gly Arg His Ser Phe Arg Ser Gly Met Asp Ala Ser Asn Gly
100 105 110

Tyr Arg Ala Leu Gln Trp Asn Ala Gly Ser Gly Gly Leu Pro Glu Asn
115 120 125

Glu Thr Thr Phe Ala Arg Ile Leu Gln Gln His Gly Tyr Ala Thr Gly
130 135 140

Leu Ile Gly Lys Trp His Gln Gly Val Asn Cys Ala Ser Arg Gly Asp
145 150 155 160

His Cys His His Pro Leu Asn His Gly Phe Asp Tyr Phe Tyr Gly Met
165 170 175

Pro Phe Thr Leu Thr Asn Asp Cys Asp Pro Gly Arg Pro Pro Glu Val
180 185 190

Asp Ala Ala Leu Arg Ala Gln Leu Trp Gly Tyr Thr Gln Phe Leu Ala
195 200 205

Leu Gly Ile Leu Thr Leu Ala Ala Gly Gln Thr Cys Gly Phe Phe Ser
210 215 220

Val Ser Ala Arg Ala Val Thr Gly Met Ala Gly Val Gly Cys Leu Phe
225 230 235 240

Phe Ile Ser Trp Tyr Ser Ser Phe Gly Phe Val Arg Arg Trp Asn Cys
245 250 255

Ile Leu Met Arg Asn His Asp Val Thr Glu Gln Pro Met Val Leu Glu
260 265 270

Lys Thr Ala Ser Leu Met Leu Lys Glu Ala Val Ser Tyr Ile Glu Arg
275 280 285

His Lys His Gly Pro Phe Leu Leu Phe Leu Ser Leu Leu His Val His
290 295 300

Ile Pro Leu Val Thr Thr Ser Ala Phe Leu Gly Lys Ser Gln His Gly
305 310 315 320

Leu Tyr Gly Asp Asn Val Glu Glu Met Asp Trp Leu Ile Gly Lys Val
325 330 335

Leu Asn Ala Ile Glu Asp Asn Gly Leu Lys Asn Ser Thr Phe Thr Tyr
340 345 350

Phe Thr Ser Asp His Gly Gly His Leu Glu Ala Arg Asp Gly His Ser
355 360 365

Gln Leu Gly Gly Trp Asn Gly Ile Tyr Lys Gly Gly Lys Gly Met Gly
370 375 380

Gly Trp Glu Gly Gly Ile Arg Val Pro Gly Ile Phe His Trp Pro Gly
385 390 395 400

Val Leu Pro Ala Gly Arg Val Ile Gly Glu Pro Thr Ser Leu Met Asp
405 410 415

Val Phe Pro Thr Val Val Gln Leu Val Gly Gly Glu Val Pro Gln Asp
420 425 430

Arg Val Ile Asp Gly His Ser Leu Val Pro Leu Leu Gln Gly Ala Glu
435 440 445

Ala Arg Ser Ala His Glu Phe Leu Phe His Tyr Cys Gly Gln His Leu
450 455 460

His Ala Ala Arg Trp His Gln Lys Asp Ser Gly Ser Val Trp Lys Val
465 470 475 480

His Tyr Thr Thr Pro Gln Phe His Pro Glu Glu Arg Gly Leu Leu Thr
485 490 495

Ala Glu Ala Ser Ala His Ala Glu Trp Gly Gly Val Thr His His Arg
500 505 510

Pro Pro Leu Leu Phe Asp Leu Ser Arg Asp Pro Ser Glu Ala Arg Pro
515 520 525

Leu Thr Pro Asp Ser Glu Pro Leu Tyr His Ala Val Ile Ala Arg Val
530 535 540

Gly Ala Ala Val Ser Glu His Arg Gln Thr Leu Ser Pro Val Pro Gln
545 550 555 560

Gln Phe Ser Met Ser Asn Ile Leu Trp Lys Pro Trp Leu Gln Pro Cys
565 570 575

Cys Gly His Phe Pro Phe Cys Ser Cys His Glu Asp Gly Asp Gly Thr
580 585 590

Pro

<210> 31
<211> 589
<212> PRT
<213> Homo sapiens

<400> 31

Met Leu His Leu His His Ser Cys Leu Cys Phe Arg Ser Trp Leu Pro
1 5 10 15

Ala Met Leu Ala Val Leu Leu Ser Leu Ala Pro Ser Ala Ser Ser Asp
 20 25 30
 Ile Ser Ala Ser Arg Pro Asn Ile Leu Leu Leu Met Ala Asp Asp Leu
 35 40 45
 Gly Ile Gly Asp Ile Gly Cys Tyr Gly Asn Asn Thr Met Arg Thr Pro
 50 55 60
 Asn Ile Asp Arg Leu Ala Glu Asp Gly Val Lys Leu Thr Gln His Ile
 65 70 75 80
 Ser Ala Ala Ser Leu Ser Thr Pro Ser Arg Ala Ala Phe Leu Thr Gly
 85 90 95
 Arg Tyr Pro Val Arg Ser Gly Met Val Ser Ser Ile Gly Tyr Arg Val
 100 105 110
 Leu Gln Trp Thr Gly Ala Ser Gly Gly Leu Pro Thr Asn Glu Thr Thr
 115 120 125
 Phe Ala Lys Ile Leu Lys Glu Lys Gly Tyr Ala Thr Gly Leu Ile Gly
 130 135 140
 Lys Trp His Leu Gly Leu Asn Cys Glu Ser Ala Ser Asp His Cys His
 145 150 155 160
 His Pro Leu His His Gly Phe Glu His Phe Tyr Gly Met Pro Phe Ser
 165 170 175
 Leu Met Gly Asp Cys Ala Arg Trp Glu Leu Ser Glu Lys Arg Val Asn
 180 185 190
 Leu Glu Gln Lys Leu Asn Phe Leu Phe Gln Val Leu Ala Leu Val Ala
 195 200 205
 Leu Thr Leu Val Ala Gly Lys Leu Thr His Leu Ile Pro Val Ser Trp
 210 215 220
 Met Pro Val Ile Trp Ser Ala Leu Ser Ala Val Leu Leu Leu Ala Ser
 225 230 235 240
 Ser Tyr Phe Val Gly Ala Leu Ile Val His Ala Asp Cys Phe Leu Met
 245 250 255
 Arg Asn His Thr Ile Thr Glu Gln Pro Met Cys Phe Gln Arg Thr Thr
 260 265 270

Pro Leu Ile Leu Gln Glu Val Ala Ser Phe Leu Lys Arg Asn Lys His
275 280 285

Gly Pro Phe Leu Leu Phe Val Ser Phe Leu His Val His Ile Pro Leu
290 295 300

Ile Thr Met Glu Asn Phe Leu Gly Lys Ser Leu His Gly Leu Tyr Gly
305 310 315 320

Asp Asn Val Glu Glu Met Asp Trp Met Val Gly Arg Ile Leu Asp Thr
325 330 335

Leu Asp Val Glu Gly Leu Ser Asn Ser Thr Leu Ile Tyr Phe Thr Ser
340 345 350

Asp His Gly Gly Ser Leu Glu Asn Gln Leu Gly Asn Thr Gln Tyr Gly
355 360 365

Gly Trp Asn Gly Ile Tyr Lys Gly Gly Lys Gly Met Gly Gly Trp Glu
370 375 380

Gly Gly Ile Arg Val Pro Gly Ile Phe Arg Trp Pro Gly Val Leu Pro
385 390 395 400

Ala Gly Arg Val Ile Gly Glu Pro Thr Ser Leu Met Asp Val Phe Pro
405 410 415

Thr Val Val Arg Leu Ala Gly Gly Glu Val Pro Gln Asp Arg Val Ile
420 425 430

Asp Gly Gln Asp Leu Leu Pro Leu Leu Leu Gly Thr Ala Gln His Ser
435 440 445

Asp His Glu Phe Leu Met His Tyr Cys Glu Arg Phe Leu His Ala Ala
450 455 460

Arg Trp His Gln Arg Asp Arg Gly Thr Met Trp Lys Val His Phe Val
465 470 475 480

Thr Pro Val Phe Gln Pro Glu Gly Ala Gly Ala Cys Tyr Gly Arg Lys
485 490 495

Val Cys Pro Cys Phe Gly Glu Lys Val Val His His Asp Pro Pro Leu
500 505 510

Leu Phe Asp Leu Ser Arg Asp Pro Ser Glu Thr His Ile Leu Thr Pro
515 520 525

Ala Ser Glu Pro Val Phe Tyr Gln Val Met Glu Arg Val Gln Gln Ala
 530 535 540

Val Trp Glu His Gln Arg Thr Leu Ser Pro Val Pro Leu Gln Leu Asp
 545 550 555 560

Arg Leu Gly Asn Ile Trp Arg Pro Trp Leu Gln Pro Cys Cys Gly Pro
 565 570 575

Phe Pro Leu Cys Trp Cys Leu Arg Glu Asp Asp Pro Gln
 580 585

<210> 32
 <211> 590
 <212> PRT
 <213> Homo sapiens

<400> 32

Met Arg Pro Arg Arg Pro Leu Val Phe Met Ser Leu Val Cys Ala Leu
 1 5 10 15

Leu Asn Thr Cys Gln Ala His Arg Val His Asp Asp Lys Pro Asn Ile
 20 25 30

Val Leu Ile Met Val Asp Asp Leu Gly Ile Gly Asp Leu Gly Cys Tyr
 35 40 45

Gly Asn Asp Thr Met Arg Thr Pro His Ile Asp Arg Leu Ala Arg Glu
 50 55 60

Gly Val Arg Leu Thr Gln His Ile Ser Ala Ala Ser Leu Ser Ser Pro
 65 70 75 80

Ser Arg Ser Ala Phe Leu Thr Gly Arg Tyr Pro Ile Arg Ser Gly Met
 85 90 95

Val Ser Ser Gly Asn Arg Arg Val Ile Gln Asn Leu Ala Val Pro Ala
 100 105 110

Gly Leu Pro Leu Asn Glu Thr Thr Leu Ala Ala Leu Leu Lys Lys Gln
 115 120 125

Gly Tyr Ser Thr Gly Leu Ile Gly Lys Trp His Gln Gly Leu Asn Cys
 130 135 140

Asp Ser Arg Ser Asp Gln Cys His His Pro Tyr Asn Tyr Gly Phe Asp
 145 150 155 160

Tyr Tyr Tyr Gly Met Pro Phe Thr Leu Val Asp Ser Cys Trp Pro Asp
 165 170 175

Pro Ser Arg Asn Thr Glu Leu Ala Phe Glu Ser Gln Leu Trp Leu Cys
 180 185 190

Val Gln Leu Val Ala Ile Ala Ile Leu Thr Leu Thr Phe Gly Lys Leu
 195 200 205

Ser Gly Trp Val Ser Val Pro Trp Leu Leu Ile Phe Ser Met Ile Leu
 210 215 220

Phe Ile Phe Leu Leu Gly Tyr Ala Trp Phe Ser Ser His Thr Ser Pro
 225 230 235 240

Leu Tyr Trp Asp Cys Leu Leu Met Arg Gly His Glu Ile Thr Glu Gln
 245 250 255

Pro Met Lys Ala Glu Arg Ala Gly Ser Ile Met Val Lys Glu Ala Ile
 260 265 270

Ser Phe Leu Glu Arg His Ser Lys Glu Thr Phe Leu Leu Phe Phe Ser
 275 280 285

Phe Leu His Val His Thr Pro Leu Pro Thr Thr Asp Asp Phe Thr Gly
 290 295 300

Thr Ser Lys His Gly Leu Tyr Gly Asp Asn Val Glu Glu Met Asp Ser
 305 310 315 320

Met Val Gly Lys Ile Leu Asp Ala Ile Asp Asp Phe Gly Leu Arg Asn
 325 330 335

Asn Thr Leu Val Tyr Phe Thr Ser Asp His Gly Gly His Leu Glu Ala
 340 345 350

Arg Arg Gly His Ala Gln Leu Gly Gly Trp Asn Gly Ile Tyr Lys Gly
 355 360 365

Gly Lys Gly Met Gly Gly Trp Glu Gly Gly Ile Arg Val Pro Gly Ile
 370 375 380

Val Arg Trp Pro Gly Lys Val Pro Ala Gly Arg Leu Ile Lys Glu Pro
 385 390 395 400

Thr Ser Leu Met Asp Ile Leu Pro Thr Val Ala Ser Val Ser Gly Gly
 405 410 415

Ser Leu Pro Gln Asp Arg Val Ile Asp Gly Arg Asp Leu Met Pro Leu
 420 425 430

Leu Gln Gly Asn Val Arg His Ser Glu His Glu Phe Leu Phe His Tyr
 435 440 445

Cys Gly Ser Tyr Leu His Ala Val Arg Trp Ile Pro Lys Asp Asp Ser
 450 455 460

Gly Ser Val Trp Lys Ala His Tyr Val Thr Pro Val Phe Gln Pro Pro
 465 470 475 480

Ala Ser Gly Gly Cys Tyr Val Thr Ser Leu Cys Arg Cys Phe Gly Glu
 485 490 495

Gln Val Thr Tyr His Asn Pro Pro Leu Leu Phe Asp Leu Ser Arg Asp
 500 505 510

Pro Ser Glu Ser Thr Pro Leu Thr Pro Ala Thr Glu Pro Leu His Asp
 515 520 525

Phe Val Ile Lys Lys Val Ala Asn Ala Leu Lys Glu His Gln Glu Thr
 530 535 540

Ile Val Pro Val Thr Tyr Gln Leu Ser Glu Leu Asn Gln Gly Arg Thr
 545 550 555 560

Trp Leu Lys Pro Cys Cys Gly Val Phe Pro Phe Cys Leu Cys Asp Lys
 565 570 575

Glu Glu Glu Val Ser Gln Pro Arg Gly Pro Asn Glu Lys Arg
 580 585 590

<210> 33
 <211> 525
 <212> PRT
 <213> Homo sapiens

<400> 33

Met Gly Trp Leu Phe Leu Lys Val Leu Leu Ala Gly Val Ser Phe Ser
 1 5 10 15

Gly Phe Leu Tyr Pro Leu Val Asp Phe Cys Ile Ser Gly Lys Thr Arg
 20 25 30

Gly Gln Lys Pro Asn Phe Val Ile Ile Leu Ala Asp Asp Met Gly Trp
 35 40 45

Gly Asp Leu Gly Ala Asn Trp Ala Glu Thr Lys Asp Thr Ala Asn Leu
50 55 60

Asp Lys Met Ala Ser Glu Gly Met Arg Phe Val Asp Phe His Ala Ala
65 70 75 80

Ala Ser Thr Ser Ser Pro Ser Arg Ala Ser Leu Leu Thr Gly Arg Leu
85 90 95

Gly Leu Arg Asn Gly Val Thr Arg Asn Phe Ala Val Thr Ser Val Gly
100 105 110

Gly Leu Pro Leu Asn Glu Thr Thr Leu Ala Glu Val Leu Gln Gln Ala
115 120 125

Gly Tyr Val Thr Gly Ile Ile Gly Lys Trp His Leu Gly His His Gly
130 135 140

Ser Tyr His Pro Asn Phe Arg Gly Phe Asp Tyr Tyr Phe Gly Ile Pro
145 150 155 160

Tyr Ser His Asp Met Gly Cys Thr Asp Thr Pro Gly Tyr Asn His Pro
165 170 175

Pro Cys Pro Ala Cys Pro Gln Gly Asp Gly Pro Ser Arg Asn Leu Gln
180 185 190

Arg Asp Cys Tyr Thr Asp Val Ala Leu Pro Leu Tyr Glu Asn Leu Asn
195 200 205

Ile Val Glu Gln Pro Val Asn Leu Ser Ser Leu Ala Gln Lys Tyr Ala
210 215 220

Glu Lys Ala Thr Gln Phe Ile Gln Arg Ala Ser Thr Ser Gly Arg Pro
225 230 235 240

Phe Leu Leu Tyr Val Ala Leu Ala His Met His Val Pro Leu Pro Val
245 250 255

Thr Gln Leu Pro Ala Ala Pro Arg Gly Arg Ser Leu Tyr Gly Ala Gly
260 265 270

Leu Trp Glu Met Asp Ser Leu Val Gly Gln Ile Lys Asp Lys Val Asp
275 280 285

His Thr Val Lys Glu Asn Thr Phe Leu Trp Phe Thr Gly Asp Asn Gly
290 295 300

Pro Trp Ala Gln Lys Cys Glu Leu Ala Gly Ser Val Gly Pro Phe Thr
305 310 315 320

Gly Phe Trp Gln Thr Arg Gln Gly Gly Ser Pro Ala Lys Gln Thr Thr
325 330 335

Trp Glu Gly Gly His Arg Val Pro Ala Leu Ala Tyr Trp Pro Gly Arg
340 345 350

Val Pro Val Asn Val Thr Ser Thr Ala Leu Leu Ser Val Leu Asp Ile
355 360 365

Phe Pro Thr Val Val Ala Leu Ala Gln Ala Ser Leu Pro Gln Gly Arg
370 375 380

Arg Phe Asp Gly Val Asp Val Ser Glu Val Leu Phe Gly Arg Ser Gln
385 390 395 400

Pro Gly His Arg Val Leu Phe His Pro Asn Ser Gly Ala Ala Gly Glu
405 410 415

Phe Gly Ala Leu Gln Thr Val Arg Leu Glu Arg Tyr Lys Ala Phe Tyr
420 425 430

Ile Thr Gly Gly Ala Arg Ala Cys Asp Gly Ser Thr Gly Pro Glu Leu
435 440 445

Gln His Lys Phe Pro Leu Ile Phe Asn Leu Glu Asp Asp Thr Ala Glu
450 455 460

Ala Val Pro Leu Glu Arg Gly Gly Ala Glu Tyr Gln Ala Val Leu Pro
465 470 475 480

Glu Val Arg Lys Val Leu Ala Asp Val Leu Gln Asp Ile Ala Asn Asp
485 490 495

Asn Ile Ser Ser Ala Asp Tyr Thr Gln Asp Pro Ser Val Thr Pro Cys
500 505 510

Cys Asn Pro Tyr Gln Ile Ala Cys Arg Cys Gln Ala Ala
515 520 525

<210> 34
<211> 871
<212> PRT
<213> Homo sapiens

<400> 34

Met Lys Tyr Ser Cys Cys Ala Leu Val Leu Ala Val Leu Gly Thr Glu
 1 5 10 15
 Leu Leu Gly Ser Leu Cys Ser Thr Val Arg Ser Pro Arg Phe Arg Gly
 20 25 30
 Arg Ile Gln Gln Glu Arg Lys Asn Ile Arg Pro Asn Ile Ile Leu Val
 35 40 45
 Pro Thr Asp Asp Gln Asp Val Glu Leu Gly Ser Leu Gln Val Met Asn
 50 55 60
 Lys Thr Arg Lys Ile Met Glu His Gly Gly Ala Thr Phe Ile Asn Ala
 65 70 75 80
 Phe Val Thr Thr Pro Met Ser Cys Pro Ser Arg Ser Ser Met Leu Thr
 85 90 95
 Gly Lys Tyr Val His Asn His Asn Val Tyr Thr Asn Asn Glu Asn Cys
 100 105 110
 Ser Ser Pro Ser Trp Gln Ala Met His Glu Pro Arg Thr Phe Ala Val
 115 120 125
 Tyr Leu Asn Asn Thr Gly Tyr Arg Thr Ala Phe Phe Gly Lys Tyr Leu
 130 135 140
 Asn Glu Tyr Asn Gly Ser Tyr Ile Pro Pro Gly Trp Arg Glu Trp Leu
 145 150 155 160
 Gly Leu Ile Lys Asn Ser Arg Phe Tyr Asn Tyr Thr Val Cys Arg Asn
 165 170 175
 Gly Ile Lys Glu Lys His Gly Phe Asp Tyr Ala Lys Asp Tyr Phe Thr
 180 185 190
 Asp Leu Ile Thr Asn Glu Ser Ile Asn Tyr Phe Lys Met Ser Lys Arg
 195 200 205
 Met Tyr Pro His Arg Pro Val Met Met Val Ile Ser His Ala Ala Pro
 210 215 220
 His Gly Pro Glu Asp Ser Ala Pro Gln Phe Ser Lys Leu Tyr Pro Asn
 225 230 235 240
 Ala Ser Gln His Ile Thr Pro Ser Tyr Asn Tyr Ala Pro Asn Met Asp
 245 250 255

Lys His Trp Ile Met Gln Tyr Thr Gly Pro Met Leu Pro Ile His Met
260 265 270

Glu Phe Thr Asn Ile Leu Gln Arg Lys Arg Leu Gln Thr Leu Met Ser
275 280 285

Val Asp Asp Ser Val Glu Arg Leu Tyr Asn Met Leu Val Glu Thr Gly
290 295 300

Glu Leu Glu Asn Thr Tyr Ile Ile Tyr Thr Ala Asp His Gly Tyr His
305 310 315 320

Ile Gly Gln Phe Gly Leu Val Lys Gly Lys Ser Met Pro Tyr Asp Phe
325 330 335

Asp Ile Arg Val Pro Phe Phe Ile Arg Gly Pro Ser Val Glu Pro Gly
340 345 350

Ser Ile Val Pro Gln Ile Val Leu Asn Ile Asp Leu Ala Pro Thr Ile
355 360 365

Leu Asp Ile Ala Gly Leu Asp Thr Pro Pro Asp Val Asp Gly Lys Ser
370 375 380

Val Leu Lys Leu Leu Asp Pro Glu Lys Pro Gly Asn Arg Phe Arg Thr
385 390 395 400

Asn Lys Lys Ala Lys Ile Trp Arg Asp Thr Phe Leu Val Glu Arg Gly
405 410 415

Lys Phe Leu Arg Lys Lys Glu Glu Ser Ser Lys Asn Ile Gln Gln Ser
420 425 430

Asn His Leu Pro Lys Tyr Glu Arg Val Lys Glu Leu Cys Gln Gln Ala
435 440 445

Arg Tyr Gln Thr Ala Cys Glu Gln Pro Gly Gln Lys Trp Gln Cys Ile
450 455 460

Glu Asp Thr Ser Gly Lys Leu Arg Ile His Lys Cys Lys Gly Pro Ser
465 470 475 480

Asp Leu Leu Thr Val Arg Gln Ser Thr Arg Asn Leu Tyr Ala Arg Gly
485 490 495

Phe His Asp Lys Asp Lys Glu Cys Ser Cys Arg Glu Ser Gly Tyr Arg
500 505 510

Ala Ser Arg Ser Gln Arg Lys Ser Gln Arg Gln Phe Leu Arg Asn Gln
515 520 525

Gly Thr Pro Lys Tyr Lys Pro Arg Phe Val His Thr Arg Gln Thr Arg
530 535 540

Ser Leu Ser Val Glu Phe Glu Gly Glu Ile Tyr Asp Ile Asn Leu Glu
545 550 555 560

Glu Glu Glu Glu Leu Gln Val Leu Gln Pro Arg Asn Ile Ala Lys Arg
565 570 575

His Asp Glu Gly His Lys Gly Pro Arg Asp Leu Gln Ala Ser Ser Gly
580 585 590

Gly Asn Arg Gly Arg Met Leu Ala Asp Ser Ser Asn Ala Val Gly Pro
595 600 605

Pro Thr Thr Val Arg Val Thr His Lys Cys Phe Ile Leu Pro Asn Asp
610 615 620

Ser Ile His Cys Glu Arg Glu Leu Tyr Gln Ser Ala Arg Ala Trp Lys
625 630 635 640

Asp His Lys Ala Tyr Ile Asp Lys Glu Ile Glu Ala Leu Gln Asp Lys
645 650 655

Ile Lys Asn Leu Arg Glu Val Arg Gly His Leu Lys Arg Arg Lys Pro
660 665 670

Glu Glu Cys Ser Cys Ser Lys Gln Ser Tyr Tyr Asn Lys Glu Lys Gly
675 680 685

Val Lys Lys Gln Glu Lys Leu Lys Ser His Leu His Pro Phe Lys Glu
690 695 700

Ala Ala Gln Glu Val Asp Ser Lys Leu Gln Leu Phe Lys Glu Asn Asn
705 710 715 720

Arg Arg Arg Lys Lys Glu Arg Lys Glu Lys Arg Arg Gln Arg Lys Gly
725 730 735

Glu Glu Cys Ser Leu Pro Gly Leu Thr Cys Phe Thr His Asp Asn Asn
740 745 750

His Trp Gln Thr Ala Pro Phe Trp Asn Leu Gly Ser Phe Cys Ala Cys
755 760 765

Thr Ser Ser Asn Asn Asn Thr Tyr Trp Cys Leu Arg Thr Val Asn Glu
770 775 780

Thr His Asn Phe Leu Phe Cys Glu Phe Ala Thr Gly Phe Leu Glu Tyr
785 790 795 800

Phe Asp Met Asn Thr Asp Pro Tyr Gln Leu Thr Asn Thr Val His Thr
805 810 815

Val Glu Arg Gly Ile Leu Asn Gln Leu His Val Gln Leu Met Glu Leu
820 825 830

Arg Ser Cys Gln Gly Tyr Lys Gln Cys Asn Pro Arg Pro Lys Asn Leu
835 840 845

Asp Val Gly Asn Lys Asp Gly Gly Ser Tyr Asp Leu His Arg Gly Gln
850 855 860

Leu Trp Asp Gly Trp Glu Gly
865 870

<210> 35
<211> 852
<212> PRT
<213> Homo sapiens

<400> 35

Met Gly Pro Pro Ser Leu Val Leu Cys Leu Leu Ser Ala Thr Val Phe
1 5 10 15

Ser Leu Leu Gly Gly Ser Ser Ala Phe Leu Ser His His Arg Leu Lys
20 25 30

Gly Arg Phe Gln Arg Asp Arg Arg Asn Ile Arg Pro Asn Ile Ile Leu
35 40 45

Val Leu Thr Asp Asp Gln Asp Val Glu Leu Gly Ser Met Gln Val Met
50 55 60

Asn Lys Thr Arg Arg Ile Met Glu Gln Gly Gly Ala His Phe Ile Asn
65 70 75 80

Ala Phe Val Thr Thr Pro Met Ser Cys Pro Ser Arg Ser Ser Ile Leu
85 90 95

Thr Gly Lys Tyr Val His Asn His Asn Thr Tyr Thr Asn Asn Glu Asn
100 105 110

Cys Ser Ser Pro Ser Trp Gln Ala Gln His Glu Ser Arg Thr Phe Ala
 115 120 125

Val Tyr Leu Asn Ser Thr Gly Tyr Arg Thr Ala Phe Phe Gly Lys Tyr
 130 135 140

Leu Asn Glu Tyr Asn Gly Ser Tyr Val Pro Pro Gly Trp Lys Glu Trp
 145 150 155 160

Val Gly Leu Leu Lys Asn Ser Arg Phe Tyr Asn Tyr Thr Leu Cys Arg
 165 170 175

Asn Gly Val Lys Glu Lys His Gly Ser Asp Tyr Ser Lys Asp Tyr Leu
 180 185 190

Thr Asp Leu Ile Thr Asn Asp Ser Val Ser Phe Phe Arg Thr Ser Lys
 195 200 205

Lys Met Tyr Pro His Arg Pro Val Leu Met Val Ile Ser His Ala Ala
 210 215 220

Pro His Gly Pro Glu Asp Ser Ala Pro Gln Tyr Ser Arg Leu Phe Pro
 225 230 235 240

Asn Ala Ser Gln His Ile Thr Pro Ser Tyr Asn Tyr Ala Pro Asn Pro
 245 250 255

Asp Lys His Trp Ile Met Arg Tyr Thr Gly Pro Met Lys Pro Ile His
 260 265 270

Met Glu Phe Thr Asn Met Leu Gln Arg Lys Arg Leu Gln Thr Leu Met
 275 280 285

Ser Val Asp Asp Ser Met Glu Thr Ile Tyr Asn Met Leu Val Glu Thr
 290 295 300

Gly Glu Leu Asp Asn Thr Tyr Ile Val Tyr Thr Ala Asp His Gly Tyr
 305 310 315 320

His Ile Gly Gln Phe Gly Leu Val Lys Gly Lys Ser Met Pro Tyr Glu
 325 330 335

Phe Asp Ile Arg Val Pro Phe Tyr Val Arg Gly Pro Asn Val Glu Ala
 340 345 350

Gly Cys Leu Asn Pro His Ile Val Leu Asn Ile Asp Leu Ala Pro Thr
 355 360 365

Ile Leu Asp Ile Ala Gly Leu Asp Ile Pro Ala Asp Met Asp Gly Lys
370 375 380

Ser Ile Leu Lys Leu Leu Asp Thr Glu Arg Pro Val Asn Arg Phe His
385 390 395 400

Leu Lys Lys Lys Met Arg Val Trp Arg Asp Ser Phe Leu Val Glu Arg
405 410 415

Gly Lys Leu Leu His Lys Arg Asp Asn Asp Lys Val Asp Ala Gln Glu
420 425 430

Glu Asn Phe Leu Pro Lys Tyr Gln Arg Val Lys Asp Leu Cys Gln Arg
435 440 445

Ala Glu Tyr Gln Thr Ala Cys Glu Gln Leu Gly Gln Lys Trp Gln Cys
450 455 460

Val Glu Asp Ala Thr Gly Lys Leu Lys Leu His Lys Cys Lys Gly Pro
465 470 475 480

Met Arg Leu Gly Gly Ser Arg Ala Leu Ser Asn Leu Val Pro Lys Tyr
485 490 495

Tyr Gly Gln Gly Ser Glu Ala Cys Thr Cys Asp Ser Gly Asp Tyr Lys
500 505 510

Leu Ser Leu Ala Gly Arg Arg Lys Lys Leu Phe Lys Lys Lys Tyr Lys
515 520 525

Ala Ser Tyr Val Arg Ser Arg Ser Ile Arg Ser Val Ala Ile Glu Val
530 535 540

Asp Gly Arg Val Tyr His Val Gly Leu Gly Asp Ala Ala Gln Pro Arg
545 550 555 560

Asn Leu Thr Lys Arg His Trp Pro Gly Ala Pro Glu Asp Gln Asp Asp
565 570 575

Lys Asp Gly Gly Asp Phe Ser Gly Thr Gly Gly Leu Pro Asp Tyr Ser
580 585 590

Ala Ala Asn Pro Ile Lys Val Thr His Arg Cys Tyr Ile Leu Glu Asn
595 600 605

Asp Thr Val Gln Cys Asp Leu Asp Leu Tyr Lys Ser Leu Gln Ala Trp
610 615 620

Lys Asp His Lys Leu His Ile Asp His Glu Ile Glu Thr Leu Gln Asn
625 630 635 640

Lys Ile Lys Asn Leu Arg Glu Val Arg Gly His Leu Lys Lys Lys Arg
645 650 655

Pro Glu Glu Cys Asp Cys His Lys Ile Ser Tyr His Thr Gln His Lys
660 665 670

Gly Arg Leu Lys His Arg Gly Ser Ser Leu His Pro Phe Arg Lys Gly
675 680 685

Leu Gln Glu Lys Asp Lys Val Trp Leu Leu Arg Glu Gln Lys Arg Lys
690 695 700

Lys Lys Leu Arg Lys Leu Leu Lys Arg Leu Gln Asn Asn Asp Thr Cys
705 710 715 720

Ser Met Pro Gly Leu Thr Cys Phe Thr His Asp Asn Gln His Trp Gln
725 730 735

Thr Ala Pro Phe Trp Thr Leu Gly Pro Phe Cys Ala Cys Thr Ser Ala
740 745 750

Asn Asn Asn Thr Tyr Trp Cys Met Arg Thr Ile Asn Glu Thr His Asn
755 760 765

Phe Leu Phe Cys Glu Phe Ala Thr Gly Phe Leu Glu Tyr Phe Asp Leu
770 775 780

Asn Thr Asp Pro Tyr Gln Leu Met Asn Ala Val Asn Thr Leu Asp Arg
785 790 795 800

Asp Val Leu Asn Gln Leu His Val Gln Leu Met Glu Leu Arg Ser Cys
805 810 815

Lys Gly Tyr Lys Gln Cys Asn Pro Arg Thr Arg Asn Met Asp Leu Gly
820 825 830

Leu Lys Asp Gly Gly Ser Tyr Glu Gln Tyr Arg Gly Gln Leu Trp Glu
835 840 845

Gly Trp Glu Gly
850

<210> 36
<211> 522
<212> PRT

<213> Homo sapiens

<400> 36

Met Ala Ala Val Val Ala Ala Thr Arg Trp Trp Gln Leu Leu Leu Val
 1 5 10 15

Leu Ser Ala Ala Gly Met Gly Ala Ser Gly Ala Pro Gln Pro Pro Asn
 20 25 30

Ile Leu Leu Leu Leu Met Asp Asp Met Gly Trp Gly Asp Leu Gly Val
 35 40 45

Tyr Gly Glu Pro Ser Arg Glu Thr Pro Asn Leu Asp Arg Met Ala Ala
 50 55 60

Glu Gly Leu Leu Phe Pro Asn Phe Tyr Ser Ala Asn Pro Leu Ser Ser
 65 70 75 80

Pro Ser Arg Ala Ala Leu Leu Thr Gly Arg Leu Pro Ile Arg Asn Gly
 85 90 95

Phe Tyr Thr Thr Asn Ala His Ala Arg Asn Ala Tyr Thr Pro Gln Glu
 100 105 110

Ile Val Gly Gly Ile Pro Asp Ser Glu Gln Leu Leu Pro Glu Leu Leu
 115 120 125

Lys Lys Ala Gly Tyr Val Ser Lys Ile Val Gly Lys Trp His Leu Gly
 130 135 140

His Arg Pro Gln Phe His Pro Leu Lys His Gly Phe Asp Glu Trp Phe
 145 150 155 160

Gly Ser Pro Asn Cys His Phe Gly Pro Tyr Asp Asn Lys Ala Arg Pro
 165 170 175

Asn Ile Pro Val Tyr Arg Asp Trp Glu Met Val Gly Arg Tyr Tyr Glu
 180 185 190

Glu Phe Pro Ile Asn Leu Lys Thr Gly Glu Ala Asn Leu Thr Gln Ile
 195 200 205

Tyr Leu Gln Glu Ala Leu Asp Phe Ile Lys Arg Gln Ala Arg His His
 210 215 220

Pro Phe Phe Leu Tyr Trp Ala Val Asp Ala Thr His Ala Pro Val Tyr
 225 230 235 240

Ala Ser Lys Pro Phe Leu Gly Thr Ser Gln Arg Gly Arg Tyr Gly Asp
245 250 255

Ala Val Arg Glu Ile Asp Asp Ser Ile Gly Lys Ile Leu Glu Leu Leu
260 265 270

Gln Asp Leu His Val Ala Asp Asn Thr Phe Val Phe Phe Thr Ser Asp
275 280 285

Asn Gly Ala Ala Leu Ile Ser Ala Pro Glu Gln Gly Gly Ser Asn Gly
290 295 300

Pro Phe Leu Cys Gly Lys Gln Thr Thr Phe Glu Gly Gly Met Arg Glu
305 310 315 320

Pro Ala Leu Ala Trp Trp Pro Gly His Val Thr Ala Gly Gln Val Ser
325 330 335

His Gln Leu Gly Ser Ile Met Asp Leu Phe Thr Thr Ser Leu Ala Leu
340 345 350

Ala Gly Leu Thr Pro Pro Ser Asp Arg Ala Ile Asp Gly Leu Asn Leu
355 360 365

Leu Pro Thr Leu Leu Gln Gly Arg Leu Met Asp Arg Pro Ile Phe Tyr
370 375 380

Tyr Arg Gly Asp Thr Leu Met Ala Ala Thr Leu Gly Gln His Lys Ala
385 390 395 400

His Phe Trp Thr Trp Thr Asn Ser Trp Glu Asn Phe Arg Gln Gly Ile
405 410 415

Asp Phe Cys Pro Gly Gln Asn Val Ser Gly Val Thr Thr His Asn Leu
420 425 430

Glu Asp His Thr Lys Leu Pro Leu Ile Phe His Leu Gly Arg Asp Pro
435 440 445

Gly Glu Arg Phe Pro Leu Ser Phe Ala Ser Ala Glu Tyr Gln Glu Ala
450 455 460

Leu Ser Arg Ile Thr Ser Val Val Gln Gln His Gln Glu Ala Leu Val
465 470 475 480

Pro Ala Gln Pro Gln Leu Asn Val Cys Asn Trp Ala Val Met Asn Trp
485 490 495

Ala Pro Pro Gly Cys Glu Lys Leu Gly Lys Cys Leu Thr Pro Pro Glu
 500 505 510

Ser Ile Pro Lys Lys Cys Leu Trp Ser His
 515 520

<210> 37
 <211> 522
 <212> PRT
 <213> Homo sapiens

<400> 37

Met Ala Ala Val Val Ala Ala Thr Arg Trp Trp Gln Leu Leu Leu Val
 1 5 10 15

Leu Ser Ala Ala Gly Met Gly Ala Ser Gly Ala Pro Gln Pro Pro Asn
 20 25 30

Ile Leu Leu Leu Leu Met Asp Asp Met Gly Trp Gly Asp Leu Gly Val
 35 40 45

Tyr Gly Glu Pro Ser Arg Glu Thr Pro Asn Leu Asp Arg Met Ala Ala
 50 55 60

Glu Gly Leu Leu Phe Pro Asn Phe Tyr Ser Ala Asn Pro Leu Ser Ser
 65 70 75 80

Pro Ser Arg Ala Ala Leu Leu Thr Gly Arg Leu Pro Ile Arg Asn Gly
 85 90 95

Phe Tyr Thr Thr Asn Ala His Ala Arg Asn Ala Tyr Thr Pro Gln Glu
 100 105 110

Ile Val Gly Gly Ile Pro Asp Ser Glu Gln Leu Leu Pro Glu Leu Leu
 115 120 125

Lys Lys Ala Gly Tyr Val Ser Lys Ile Val Gly Lys Trp His Leu Gly
 130 135 140

His Arg Pro Gln Phe His Pro Leu Lys His Gly Phe Asp Glu Trp Phe
 145 150 155 160

Gly Ser Pro Asn Cys His Phe Gly Pro Tyr Asp Asn Lys Ala Arg Pro
 165 170 175

Asn Ile Pro Val Tyr Arg Asp Trp Glu Met Val Gly Arg Tyr Tyr Glu
 180 185 190

Glu Phe Pro Ile Asn Leu Lys Thr Gly Glu Ala Asn Leu Thr Gln Ile
 195 200 205

Tyr Leu Gln Glu Ala Leu Asp Phe Ile Lys Arg Gln Ala Arg His His
 210 215 220

Pro Phe Phe Leu Tyr Trp Ala Val Asp Ala Thr His Ala Pro Val Tyr
 225 230 235 240

Ala Ser Lys Pro Phe Leu Gly Thr Ser Gln Arg Gly Arg Tyr Gly Asp
 245 250 255

Ala Val Arg Glu Ile Asp Asp Ser Ile Gly Lys Ile Leu Glu Leu Leu
 260 265 270

Gln Asp Leu His Val Ala Asp Asn Thr Phe Val Phe Phe Thr Ser Asp
 275 280 285

Asn Gly Ala Ala Leu Ile Ser Ala Pro Glu Gln Gly Gly Ser Asn Gly
 290 295 300

Pro Phe Leu Cys Gly Lys Gln Thr Thr Phe Glu Gly Gly Met Arg Glu
 305 310 315 320

Pro Ala Leu Ala Trp Trp Pro Gly His Val Thr Ala Gly Gln Val Ser
 325 330 335

His Gln Leu Gly Ser Ile Met Asp Leu Phe Thr Thr Ser Leu Ala Leu
 340 345 350

Ala Gly Leu Thr Pro Pro Ser Asp Arg Ala Ile Asp Gly Leu Asn Leu
 355 360 365

Leu Pro Thr Leu Leu Gln Gly Arg Leu Met Asp Arg Pro Ile Phe Tyr
 370 375 380

Tyr Arg Gly Asp Thr Leu Met Ala Ala Thr Leu Gly Gln His Lys Ala
 385 390 395 400

His Phe Trp Thr Trp Thr Asn Ser Trp Glu Asn Phe Arg Gln Gly Ile
 405 410 415

Asp Phe Cys Pro Gly Gln Asn Val Ser Gly Val Thr Thr His Asn Leu
 420 425 430

Glu Asp His Thr Lys Leu Pro Leu Ile Phe His Leu Gly Arg Asp Pro
 435 440 445

Gly Glu Arg Phe Pro Leu Ser Phe Ala Ser Ala Glu Tyr Gln Glu Ala
 450 455 460

Leu Ser Arg Ile Thr Ser Val Val Gln Gln His Gln Glu Ala Leu Val
 465 470 475 480

Pro Ala Gln Pro Gln Leu Asn Val Cys Asn Trp Ala Val Met Asn Trp
 485 490 495

Ala Pro Pro Gly Cys Glu Lys Leu Gly Lys Cys Leu Thr Pro Pro Glu
 500 505 510

Ser Ile Pro Lys Lys Cys Leu Trp Ser His
 515 520

<210> 38
 <211> 502
 <212> PRT
 <213> Homo sapiens

<400> 38

Met Ser Cys Pro Val Pro Ala Cys Cys Ala Leu Leu Leu Val Leu Gly
 1 5 10 15

Leu Cys Arg Ala Arg Pro Arg Asn Ala Leu Leu Leu Leu Ala Asp Asp
 20 25 30

Gly Gly Phe Glu Ser Gly Ala Tyr Asn Asn Ser Ala Ile Ala Thr Pro
 35 40 45

His Leu Asp Ala Leu Ala Arg Arg Ser Leu Leu Phe Arg Asn Ala Phe
 50 55 60

Thr Ser Val Ser Ser Ser Ser Pro Ser Arg Ala Ser Leu Leu Thr Gly
 65 70 75 80

Leu Pro Gln His Gln Asn Gly Met Tyr Gly Leu His Gln Asp Val His
 85 90 95

His Phe Asn Ser Phe Asp Lys Val Arg Ser Leu Pro Leu Leu Leu Ser
 100 105 110

Gln Ala Gly Val Arg Thr Gly Ile Ile Gly Lys Lys His Val Gly Pro
 115 120 125

Glu Thr Val Tyr Pro Phe Asp Phe Ala Tyr Thr Glu Glu Asn Gly Ser
 130 135 140

Val Leu Gln Val Gly Arg Asn Ile Thr Arg Ile Lys Leu Leu Val Arg
 145 150 155 160

Lys Phe Leu Gln Thr Gln Asp Asp Arg Pro Phe Phe Leu Tyr Val Ala
 165 170 175

Phe His Asp Pro His Arg Cys Gly His Ser Gln Pro Gln Tyr Gly Thr
 180 185 190

Phe Cys Glu Lys Phe Gly Asn Gly Glu Ser Gly Met Gly Arg Ile Pro
 195 200 205

Asp Trp Thr Pro Gln Ala Tyr Asp Pro Leu Asp Val Leu Val Pro Tyr
 210 215 220

Phe Val Pro Asn Thr Pro Ala Ala Arg Ala Asp Leu Ala Ala Gln Tyr
 225 230 235 240

Thr Thr Val Gly Arg Met Asp Gln Gly Val Gly Leu Val Leu Gln Glu
 245 250 255

Leu Arg Asp Ala Gly Val Leu Asn Asp Thr Leu Val Ile Phe Thr Ser
 260 265 270

Asp Asn Gly Ile Pro Phe Pro Ser Gly Arg Thr Asn Leu Tyr Trp Pro
 275 280 285

Gly Thr Ala Glu Pro Leu Leu Val Ser Ser Pro Glu His Pro Lys Arg
 290 295 300

Trp Gly Gln Val Ser Glu Ala Tyr Val Ser Leu Leu Asp Leu Thr Pro
 305 310 315 320

Thr Ile Leu Asp Trp Phe Ser Ile Pro Tyr Pro Ser Tyr Ala Ile Phe
 325 330 335

Gly Ser Lys Thr Ile His Leu Thr Gly Arg Ser Leu Leu Pro Ala Leu
 340 345 350

Glu Ala Glu Pro Leu Trp Ala Thr Val Phe Gly Ser Gln Ser His His
 355 360 365

Glu Val Thr Met Ser Tyr Pro Met Arg Ser Val Gln His Arg His Phe
 370 375 380

Arg Leu Val His Asn Leu Asn Phe Lys Met Pro Phe Pro Ile Asp Gln
 385 390 395 400

Asp Phe Tyr Val Ser Pro Thr Phe Gln Asp Leu Leu Asn Arg Thr Thr
 405 410 415

Ala Gly Gln Pro Thr Gly Trp Tyr Lys Asp Leu Arg His Tyr Tyr Tyr
 420 425 430

Arg Ala Arg Trp Glu Leu Tyr Asp Arg Ser Arg Asp Pro His Glu Thr
 435 440 445

Gln Asn Leu Ala Thr Asp Pro Arg Phe Ala Gln Leu Leu Glu Met Leu
 450 455 460

Arg Asp Gln Leu Ala Lys Trp Gln Trp Glu Thr His Asp Pro Trp Val
 465 470 475 480

Cys Ala Pro Asp Gly Val Leu Glu Glu Lys Leu Ser Pro Gln Cys Gln
 485 490 495

Pro Leu His Asn Glu Leu
 500

<210> 39
 <211> 552
 <212> PRT
 <213> Homo sapiens

<400> 39

Met Arg Leu Leu Pro Leu Ala Pro Gly Arg Leu Arg Arg Gly Ser Pro
 1 5 10 15

Arg His Leu Pro Ser Cys Ser Pro Ala Leu Leu Leu Leu Val Leu Gly
 20 25 30

Gly Cys Leu Gly Val Phe Gly Val Ala Ala Gly Thr Arg Arg Pro Asn
 35 40 45

Val Val Leu Leu Leu Thr Asp Asp Gln Asp Glu Val Leu Gly Gly Met
 50 55 60

Thr Pro Leu Lys Lys Thr Lys Ala Leu Ile Gly Glu Met Gly Met Thr
 65 70 75 80

Phe Ser Ser Ala Tyr Val Pro Ser Ala Leu Ser Cys Pro Ser Arg Ala
 85 90 95

Ser Ile Leu Thr Gly Lys Tyr Pro His Asn His His Val Val Asn Asn
 100 105 110

Thr Leu Glu Gly Asn Cys Ser Ser Lys Ser Trp Gln Lys Ile Gln Glu
 115 120 125

Pro Asn Thr Phe Pro Ala Ile Leu Arg Ser Met Cys Gly Tyr Gln Thr
 130 135 140

Phe Phe Ala Gly Lys Tyr Leu Asn Glu Tyr Gly Ala Pro Asp Ala Gly
 145 150 155 160

Gly Leu Glu His Val Pro Leu Gly Trp Ser Tyr Trp Tyr Ala Leu Glu
 165 170 175

Lys Asn Ser Lys Tyr Tyr Asn Tyr Thr Leu Ser Ile Asn Gly Lys Ala
 180 185 190

Arg Lys His Gly Glu Asn Tyr Ser Val Asp Tyr Leu Thr Asp Val Leu
 195 200 205

Ala Asn Val Ser Leu Asp Phe Leu Asp Tyr Lys Ser Asn Phe Glu Pro
 210 215 220

Phe Phe Met Met Ile Ala Thr Pro Ala Pro His Ser Pro Trp Thr Ala
 225 230 235 240

Ala Pro Gln Tyr Gln Lys Ala Phe Gln Asn Val Phe Ala Pro Arg Asn
 245 250 255

Lys Asn Phe Asn Ile His Gly Thr Asn Lys His Trp Leu Ile Arg Gln
 260 265 270

Ala Lys Thr Pro Met Thr Asn Ser Ser Ile Gln Phe Leu Asp Asn Ala
 275 280 285

Phe Arg Lys Arg Trp Gln Thr Leu Leu Ser Val Asp Asp Leu Val Glu
 290 295 300

Lys Leu Val Lys Arg Leu Glu Phe Thr Gly Glu Leu Asn Asn Thr Tyr
 305 310 315 320

Ile Phe Tyr Thr Ser Asp Asn Gly Tyr His Thr Gly Gln Phe Ser Leu
 325 330 335

Pro Ile Asp Lys Arg Gln Leu Tyr Glu Phe Asp Ile Lys Val Pro Leu
 340 345 350

Leu Val Arg Gly Pro Gly Ile Lys Pro Asn Gln Thr Ser Lys Met Leu
 355 360 365

Val Ala Asn Ile Asp Leu Gly Pro Thr Ile Leu Asp Ile Ala Gly Tyr
 370 375 380

Asp Leu Asn Lys Thr Gln Met Asp Gly Met Ser Leu Leu Pro Ile Leu
 385 390 395 400

Arg Gly Ala Ser Asn Leu Thr Trp Arg Ser Asp Val Leu Val Glu Tyr
 405 410 415

Gln Gly Glu Gly Arg Asn Val Thr Asp Pro Thr Cys Pro Ser Leu Ser
 420 425 430

Pro Gly Val Ser Gln Cys Phe Pro Asp Cys Val Cys Glu Asp Ala Tyr
 435 440 445

Asn Asn Thr Tyr Ala Cys Val Arg Thr Met Ser Ala Leu Trp Asn Leu
 450 455 460

Gln Tyr Cys Glu Phe Asp Asp Gln Glu Val Phe Val Glu Val Tyr Asn
 465 470 475 480

Leu Thr Ala Asp Pro Asp Gln Ile Thr Asn Ile Ala Lys Thr Ile Asp
 485 490 495

Pro Glu Leu Leu Gly Lys Met Asn Tyr Arg Leu Met Met Leu Gln Ser
 500 505 510

Cys Ser Gly Pro Thr Cys Arg Thr Pro Gly Val Phe Asp Pro Gly Tyr
 515 520 525

Arg Phe Asp Pro Arg Leu Met Phe Ser Asn Arg Gly Ser Val Arg Thr
 530 535 540

Arg Arg Phe Ser Lys His Leu Leu
 545 550

<210> 40
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic primer

<400> 40
 agactttggc agggggtaat

20

<210> 41
 <211> 20
 <212> DNA

<213> Artificial sequence

<220>

<223> Synthetic primer

<400> 41

cagccagtca gagatgtgga

20

<210> 42

<211> 17

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic primer

<400> 42

cgccccttcc tgtacta

17

<210> 43

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic primer

<400> 43

caggtccatt gtctgcagtg

20

<210> 44

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic primer

<400> 44

gccggccaca gtcgatgaat cc

22

<210> 45

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic primer

<400> 45

cttgtcgatc aggatgatct gg

22

<210> 46

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic primer

<400> 46

gaaactatct gccgggtgac

20

<210> 47

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic primer

<400> 47

tttgatatgc agggcagtga

20

<210> 48

<211> 39

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic primer

<400> 48

gcgataggta ccgccatggg tccgcgcggc gcggcgagc

39

<210> 49

<211> 35

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic primer

<400> 49

gcgatactcg agccctgaaa tcctacatcc aaggg

35

<210> 50

<211> 75

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic primer

<400> 50

gcgataggta ccgccatggg tcctagagga gctgcttctt tgcctcgagg acccgacct

60

cggcggctgc tctc

75

<210> 51

<211> 68

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic primer

<220>
 <221> modified_base
 <222> (1)..(1)
 <223> p

<400> 51
 ggccggcggg gtgctcctgg acaactacta cacgcagccg ctgagcacgc cgtcgcggag 60
 ccagctgc 68

<210> 52
 <211> 63
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic primer

<220>
 <221> modified_base
 <222> (1)..(1)
 <223> p

<400> 52
 cagctggctc cgcgacggcg tgctcagcgg ctgcgtgtag tagttgtcca ggagcacccc 60
 gcc 63

<210> 53
 <211> 26
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic primer

<220>
 <221> modified_base
 <222> (1)..(1)
 <223> p

<400> 53
 gcctgtgtct ctgagcacac cctcta 26

<210> 54
 <211> 36
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic primer

<400> 54
 gcgatactcg agtcaggcat ggggatctgg gcaatg 36

<210> 55

<211> 50
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic primer

<220>
<221> modified_base
<222> (1)..(1)
<223> p

<400> 55
cggccggtca ggagggcggc cctagagggt gtgctcagag acacaggcac

50

<210> 56
<211> 5
<212> PRT
<213> Homo sapiens

<220>
<223> arylsulfatase active site

<400> 56

Cys Thr Pro Ser Arg
1 5