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(71) Applicants *(for all designated States except US)*: **MEDIVIR UK Ltd** [GB/GB]; Chesterford Research Park, Little Chesterford Essex CB10 1XL (GB). **PEPTIMMUNE, Inc** [US/US]; 64 Sydney St Cambridge, MA 02139 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **HARDICK, David** [GB/GB]; Medivir UK Ltd, Chesterford Research Park, Little Chesterford Essex CB10 1XL (GB). **TOZER, Matt** [GB/GB]; Medivir UK Ltd, Chesterford Research Park, Little Chesterford Essex CB10 1XL (GB). **CANFIELD, Julie** [GB/GB]; Medivir UK Ltd, Chesterford Research Park, Little Chesterford Essex CB10 1XL (GB). **WILSON, Michelle** [GB/GB]; Medivir UK Ltd, Chesterford Research Park, Little Chesterford Essex CB10 1XL (GB). **RAE, Alastair** [GB/GB]; Medivir AB, Chesterford Research Park, Little Chesterford Essex CB10 1XL (GB). **FALLON, Philip** [GB/GB]; Medivir AB, Chesterford Research Park, Little Chesterford Essex CB10 1XL (GB). **CLASSON, Bjorn** [SE/SE]; Medivir AB, Lunastigen 7, S-141 Huddinge (SE). **LINDQUIST, Charlotta** [SE/SE]; Medivir AB, Lunastigen 7, S-141 Huddinge (SE). **AYESA, Susana** [ES/SE]; Medivir AB, Lunastigen 7, S-141 Huddinge (SE).

(74) Agent: **TEUTEN, Andrew**; Sagittarius IPC Limited, Taylor House 39 High St, Marlow Buckinghamshire SL7 1AF (GB).

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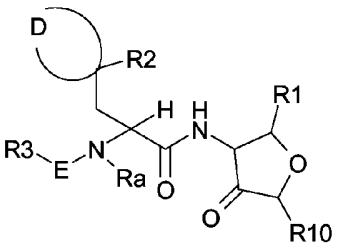
(54) Title: CATHEPSIN S INHIBITORS



(I)

(57) Abstract: Compounds of the formula (I) where R^1 is C_1-C_4 straight or branched alkyl, optionally substituted with up to three substituents selected from halo and hydroxy; R^2 is halo, hydroxy, methyloxy, or C_1-C_2 alkyl, which alkyl is optionally substituted with up to three halogens or an hydroxy or a methyloxy; D is - C_3-C_7 alkylene-, thereby defining a cycloalkyl ring; E is -C(=O)-, -S(=O)$_m$-, -NRdS(=O)$_m$-, -NRaC(=O)-, -OC(=O)-, R^3 is an optionally substituted carbocyclic or heterocyclic ring R^{10} is H, ORc, SRc or together with the gem H is =O or $(ORc)_2$; Ra is independently selected from H, C_1-C_4 alkyl; have utility in the inhibition of cathepsin S and are thus useful pharmaceuticals against disorders such as autoimmune disorders and chronic pain.

WO 2006/064286 A1

Cathepsin S Inhibitors

Technical Field

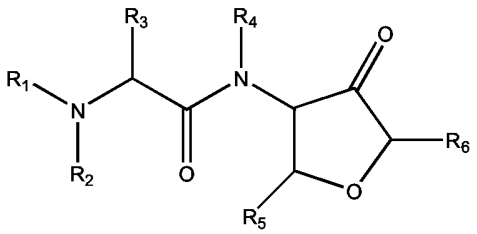
This invention relates to inhibitors of cathepsin S, and their use in methods of treatment for disorders involving cathepsin S such as autoimmune, allergy and chronic pain conditions.

Background to the invention and prior art

The papain superfamily of cysteine proteases are widely distributed in diverse species including mammals, invertebrates, protozoa, plants and bacteria. A number of mammalian cathepsin enzymes, including cathepsins B, F, H, K, L, O, S, and W, have been ascribed to this superfamily, and inappropriate regulation of their activity has been implicated in a number of metabolic disorders including arthritis, muscular dystrophy, inflammation, glomerulonephritis and tumour invasion. Pathogenic cathepsin like enzymes include the bacterial gingipains, the malarial falcipains I, II, III et seq and cysteine proteases from Pneumocystis carinii, Trypanosoma cruzei and brucei, Crithidia fusiculata, Schistosoma spp.

In WO 97/40066, the use of inhibitors against Cathepsin S is described. The inhibition of this enzyme is suggested to prevent or treat disease caused by protease activity. Cathepsin S is a highly active cysteine protease belonging to the papain superfamily. Its primary structure is 57%, 41% and 45% homologous with that of the human cathepsin L and H and plant cysteine proteases papain respectively, although only 31% homologous with cathepsin B. It is found mainly in B cells, dendritic cells and macrophages and this limited occurrence suggests the potential involvement of this enzyme in the pathogenesis of degenerative disease. Moreover, it has been found that destruction of Ii by proteolysis is required for MHC class II molecules to bind antigenic peptides, and for transport of the resulting complex to the cell surface. Furthermore, it has been found that Cathepsin S is essential in B cells for effective Ii proteolysis necessary to render class II molecules competent for binding peptides. Therefore, the inhibition of this enzyme may be useful in modulating class II-restricted immune response (WO 97/40066). Other disorders in which cathepsin S is implicated are asthma, chronic obstructive pulmonary disease, endometriosis and chronic pain.

International patent application no WO00/69855 describes cathepsin S inhibitors of the formula:



(II)

wherein:

R^1 = R', R'C(O) , R' C(S), R' SO2 , R' OC(O), R' NHC(O)

wherein R' is a monocyclic ring;

R^2, R^4 = H, C_{1-7}-alkyl, C_{3-7}-cycloalkyl;

R^3 = C_{1-7}-alkyl, C_{3-7}-cycloalkyl, Ar-C_{1-7}-alkyl;

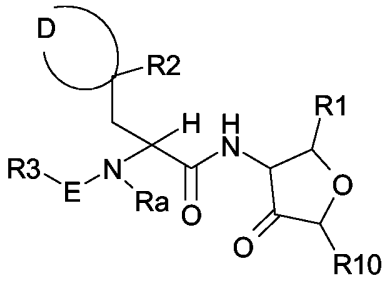
R^5 = C_{1-7}-alkyl, Halogen, Ar-C_{1-7}-alkyl, C_{1-3}-alkyl-CONR'",

R^6 = H, C_{1-7}-alkyl, Ar-C_{1-7}-alkyl, C_{1-3}-alkyl-SO_2-R^{ix},

C_{1-3}-alkyl-C(O)-NHR^{ix} or CH_2XAr,

The R^3 groups specifically disclosed in WO00/69855 are branched chain alkyl moieties such as n-butyl, t-butyl, 3-(2,2-dimethylpropyl), 4-(2-methylbutyl), 4-(3,3-dimethylbutyl), 4-(3,3-dimethyl-2-methylbutyl), 4-(3-methyl-2-methylbutyl), or 5-(2-methyl-3-methylpentyl). Page 27, line 13 of WO00/69855 discloses the compound morpholine-4-carboxylic acid [3,3-dimethyl-1S-(2-ethyl-4-oxo-tetrahydrofuran-3-ylcarbamoyl)butyl]amide.

In accordance with the invention, there is provided novel compounds of the formula I



where

R^1 is C_1-C_4 straight or branched alkyl, optionally substituted with up to three substituents selected from halo and hydroxy;

R^2 is halo, hydroxy, methyloxy, or C_1-C_2 alkyl, which alkyl is optionally substituted with up to three halogens or an hydroxy or a methyloxy;

D is -C_3-C_7 alkylene-, thereby defining a cycloalkyl ring;

E is -C(=O)-, -S(=O)$_m$-, -NRaS(=O)$_m$-, -NRaC(=O)-, -OC(=O)-,

R^3 is a carbocyclic ring selected from C_3-C_6 cycloalkyl, C_5-C_6 cycloalkenyl or phenyl, or a heterocyclic ring I selected from azepanyl, pyrrolidinyl, piperidinyl, morpholinyl, thiomorpholinyl, piperazinyl, indolinyl, pyranyl, tetrahydropyranyl, tetrahydrothiopyranyl, thiopyranyl, furanyl, tetrahydrofuranyl, thienyl, pyrrolyl, oxazolyl, isoxazolyl, thiazolyl, imidazolyl, pyridinyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazolyl, tetrazolyl, pyrazolyl, indolyl, which ring is optionally substituted with up to 3 substituents independently selected from R^4; or

R^4 is independently selected from halo, oxo, nitrile, nitro, C_1-C_4 alkyl, -NRaRb, NH_2CO-, X-R^5, X-O-R^5, X-O-C(=O)R^5, X-C(=O)NRaR^5, X-NRaC(=O)R^{5a}, X-NRdSO$_m$$R^{5a}$, X-SO$_mNRdR^5$, X-S(=O)$_m$$R^5$, X-C(=O)O$R^5$, X-NRaC(=O)O$R^5$; or a pair of R^4 together define a 5 or 6 membered nitrogen-containing ring fused to R^3, optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkyloxy, oxo, hydroxy, halo, NRaRb,;

R^5 is H, C_1-C_4 alkyl, C_3-C_6 cycloalkyl, pyrrolidinyl, piperidinyl, morpholinyl, thiomorpholinyl, piperazinyl, indolinyl, pyranyl, thiopyranyl, furanyl, thienyl, pyrrolyl, oxazolyl, isoxazolyl, thiazolyl, imidazolyl, pyridinyl, pyrimidinyl, pyrazinyl, indolyl, phenyl, benzyl, any of which is optionally substituted with R^6;

R^{5a} is R^5 or -NRaRb;

R^6 is independently selected from hydroxy, -NH_2, NHC_1-C_3alkyl, N(C_1-C_3alkyl)$_2$, nitro, cyano, carboxy, oxo, C_1-C_4 alkyl, C_1-C_4-alkoxy, C_1-C_4 alkanoyl, carbamoyl;

Ra and Rb are independently selected from H, C_1-C_4 alkyl and acetyl, or Ra, Rb and the N atom to which they both are joined form a ring selected from morpholine, piperazine, piperidine, pyrrolidine;

R^{10} is H, ORc, SRc or together with the gem H is =O;

Ra and Rb are independently selected from H, C_1-C_4 alkyl and acetyl, or Ra, Rb and the N atom to which they both are joined form a ring selected from morpholine, piperazine, piperidine, pyrrolidine

Rc is H, C_1-C_4 alkyl, C_0-C_3alkylcarbocyclyl;

Rd is H, C_1-C_4 alkyl, C(=O)C_1-C_4 alkyl, C_0-C_3alkylcarbocyclyl;

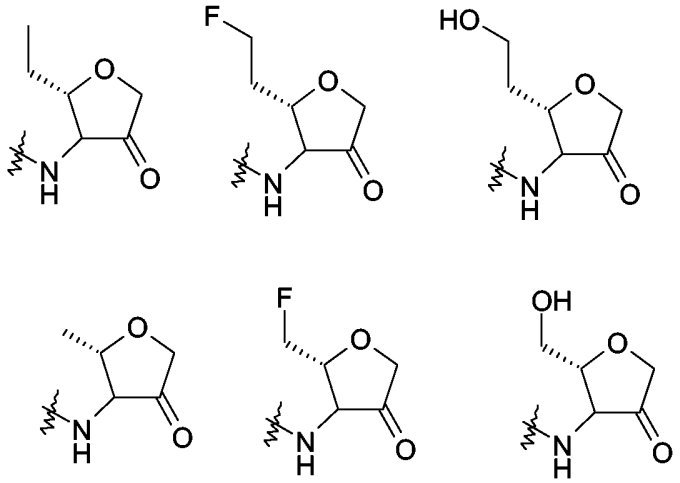
X is independently a bond or C_1-C_4 alkylenyl;

m is independently 0,1 or 2;

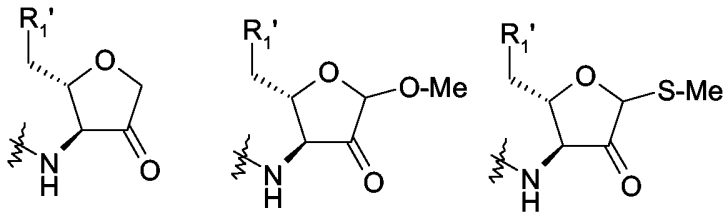
and pharmaceutically acceptable salts thereof.

Preferred values for R^1 include ethyl, 2-fluoroethyl or 2-hydroxyethyl, and methyl, fluoromethyl and hydroxyethyl, especially ethyl and methyl. Preferably the stereochemistry at the C-4 and C-5 positions of the furanone ring (ie those from which R^1 and the backbone nitrogen extends) is enantiomerically pure, or at least 85%, for example at least 90% or more preferably at least 95% enantiomerically pure 4S, 5S configuration.

Preferred P1 groups (as defined below) therefore include:

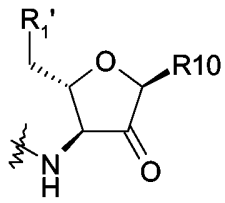


R^{10} is conveniently H or a C_1-C_4 alkyl ether or C_1-C_4 alkylthioether such as methyloxy, ethyloxy, methylthio- or ethylthio or the corresponding ketals. Embodiments of P1 groups thus include:



especially where $R^{1'}$ is H or $-CH_3$.

Where R^{10} is other than H, it is currently preferred that the stereochemistry at the ring carbon atom which bears R^{10} comprises at least 85%, for example at least 90% preferably at least 95% and more preferably 100% enantiomerically pure alpha configuration:



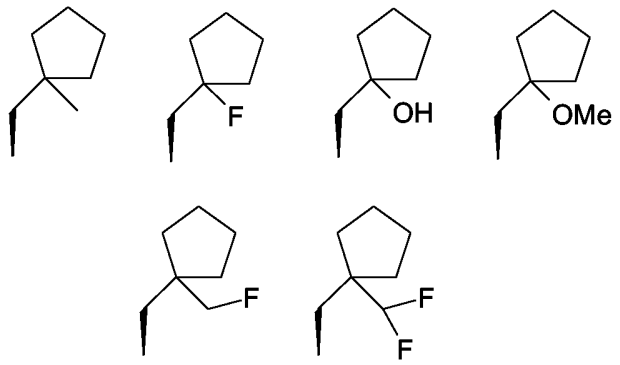
but both alpha and beta configurations produce cathepsin S-active compounds.

D is conveniently pentylene, thereby defining a cyclohexyl ring, or propylene, thereby defining a cyclobutyl ring, but more preferably D is butylene, thereby defining a cyclopentyl ring.

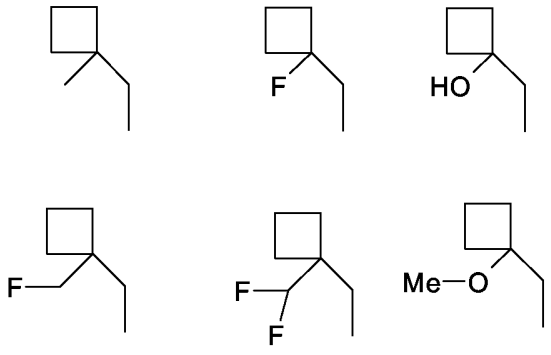
Embodiments of R^2 include a halogen such as fluoro, fluoromethyl, difluoromethyl or trifluoromethyl, and most preferably methyl.

The side chain comprising D and R^2, ie the P2 group (as defined below) may be in the R or S configuration, or a racemate thereof. Preferably, however, the P2 group is substantially, for example greater than 90% and most preferably greater than 95% in the S stereoconfiguration, that is reflecting that of an L-amino acid.

Preferred side chains thus include:



Other embodiments include:



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Currently favoured values for E include -O-C(=O)-, $-S(=O)_2-$ and especially -C(=O)-.

Returning now to other values of E, typical values for R^3 include:
unsubstituted or substituted furanyl, especially furan-2-yl or furan-3- yl, or alkyl substituted furanyl such as 2-methylfuran-3-yl, 2,4-dimethylfuran-3-yl, or aryl substituted furanyl, even more especially 5-phenylfuran-2-yl, 5-(2-chlorophenyl)furan-2-yl, 5-(3chlorophenyl)furan-2-yl, 5-(4-chlorophenyl)furan-2- yl, 5-(4-fluorophenyl)furan-2-yl, 5-(4hydroxyphenyl)furan-2-yl, 5-(3-trifluoromethylphenyl)furan-2-yl, 5-(4-trifluoromethylphenyl)furan-2-yl, 5-(3-

trifluoromethylphenyl)furan-2-yl, 5-(4-methylphenyl)furan-2-yl, 5-(4-acetylphenyl)furan-2-yl, or 5-trifluoromethylfuran-2-yl;

unsubstituted or substituted tetrahydrofuranyl, particularly tetrahydrofuran-2-yl or tetrahydrofuran-3-yl;

unsubstituted or substituted morpholinyl;

unsubstituteted or substituted pyrrolyl, particularly pyrrol-2-yl;

unsubstituted or substituted piperazinyl, particularly piperazin-1- yl or 4-alkylpiperazinyl, e.g., 4-methylpipeperazin-1-yl;

unsubstituted or substituted pyrazolyl, particularly IH-pyrazol-2-yl, 1H-pyrazol-4- yl, 1- or 2-methyl-2H-pyrazol-2-yl or 1- or 2-methyl-2H-pyrazol-3-yl;

unsubstituted or substituted isoxazolyl, particularly isoxazol-5-yl, 3-methylisoxazol-4-yl, 5-methylisoxazol-3-yl, 5-methylisoxazol-4-yl, or 3,5-dimethylisoxazol4-yl;

unsubstituted or substituted thiazolyl, particularly thiazol-2-yl, 2-methylthiazol-2-yl, 2,4-dimethylthiazol-5-yl, or 4-methyl-2-phenylthiazol-5-yl;

unsubstituted or substituted pyrazolyl, particularly alkyl-substituted pyrazolyl including 2-methyl-2H-pyrazolyl;

unsubstituted or aryl-substituted triazolyl, particularly phenyl-substituted triazoles including 3-phenyl-3H-[1,2,3]triazol-3-yl;

unsubstituted or substituted pyrazinyl, particularly pyrazin-2-yl and 5-methylpyrazin-2-yl;

unsubstituted or substituted imidazolyl, particularly 1-H-imidazol-2-yl, 1-methyl-1H-imidazol-4-yl or 1-methyl-lH-imidazol-2-yl;

thiophenyl, especially thiophene-3-yl and thiophen-2-yl, more especially heterocycle or aryl substituted C_0-C_6alkylthiophenyl, particularly 5-pyridin-2-ylthiophen-2-yl, more especially C_l-C_6alkylthiophenyl, particularly 5-methylthiophenyl or 3-methylthiophen-2-yl; more especially C_1-C_6alkoxythiophenyl, particularly 3-ethoxythiophen-2-yl;

phenyl, especially alkyl-substituted phenyl, halogen-substituted phenyl, trihaloalkylsubstituted phenyl, alkoxy-substituted phenyl, or acetoxy-substituted phenyl, especially 4-methylphenyl, 3-chlorophenyl, 4-chlorophenyl, 3-trifluoromethylphenyl, 4-trifluoromethylphenyl, 2-chlorophenyl, 4-fluorophenyl, 4-hydroxyphenyl, or 4-acetylphenyl;

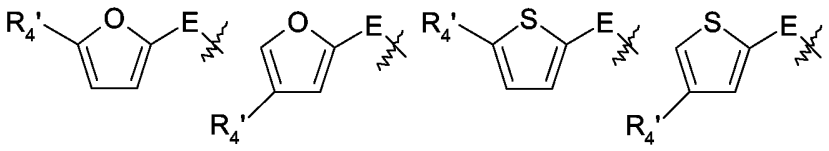
unsubstituted or substituted pyridinyl, particularly pyridine-2-yl;

unsubstituted or substituted cyclobutyl or cyclopentyl.

Preferred values for R^3 include optionally substituted thienyl, pyrazinyl, pyridyl, pyrrolyl, and especially furyl or morpholinyl.

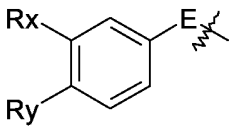
Favoured values for R^3 include fur-3-yl, thien-3-yl, pyrazin-2-yl, pyrid-4-yl, pyrrol-2-yl and especially N-morpholino.

Other preferred embodiments of R^3, for example when E is (=O), include optionally substituted, fur-2-yl or thien-2yl:



where $R^{4'}$ is H, halo (such as F or Cl), OC_1-C_4 alkyl (such as methoxy), C(=O)NRaRb (for example dimethylcarbamoyl), $NRaC(=O)C_1$-C_4 alkyl such as NHC(=O)Me, ureas, such as NRaC(=O)NRaRb,(for example –$NHC(=O)NHCH_3$, $NHC(=O)N(CH_3)_2$ or NHC(=O)NRrRr, where RrRr define a cyclic amine such as pyrrolidine, morpholine, piperidine, piperazine or N-methylpiperazine), and carbamates such as –$NRaC(=O)OC_1$-C_4 alkyl such as NHC(=O)OMe.

A further preferred value for R^3 is phenyl particularly phenyl substituted as follows:



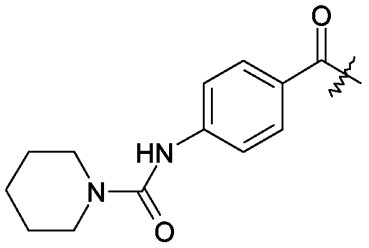
where

Ry is -NHC(=O)-Me, -NHC(=O)OMe, F, especially OH and NHAc

and Rx is H, OMe, F, Cl, CN, CF_3, Me.

Other embodiments include those wherein Ry is halomethyl such as CF_3 or CF_2 or an hydroxylated methyl group, such as $HOCH_2$ or $HO(CH_2)_2)C$-, any of these preferences being optionally further substituted with an R^4 group such as Rx.

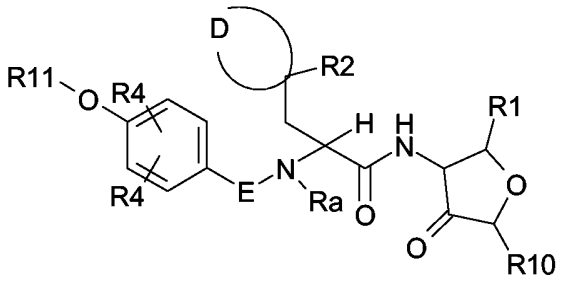
An alternative embodiment for R3 comprises phenyl which is substituted with a urea, such as a cyclic urea:



Other urea substituent include NRaC(=O)NRaRb, (for example –NHC(=O)NHCH$_3$, NHC(=O)N(CH$_3$)$_2$ or NHC(=O)NRrRr, where RrRr define a cyclic amine such as pyrrolidine, morpholine, piperidine, piperazine or N-methylpiperazine).

Other favoured substituents to a phenyl R^3 include 3,5-dichloro, 3,5-difluoro, 3-fluoro-5-cyano, 3-cyano, 4-NHAc-3-Me, 4-NHAC-6-Me, 4-NHAc-3,5-diMe and the like.

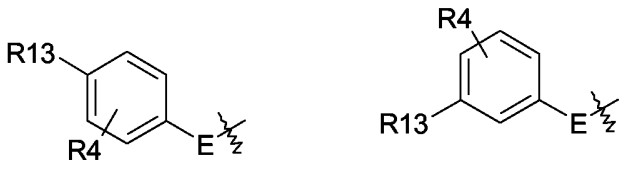
A favoured aspect of the invention thus comprises compounds of the formula:



where R^1, R^2, D, Rc, E and R^4 are as defined above and R^{11} is H, R^{12} or -C(=O)R^{12} where R^{12} is independently H, C$_1$-C$_6$-alkyl which is optionally substituted with R^6, C$_0$-C$_3$alkylcarbocyclyl or C$_0$-C$_3$alkylheterocyclyl. R^{12} typically comprises a pharmaceutically acceptable ether or ester prodrug which is hydrolysed in vivo to release the parent phenol. Currently favoured values of this aspect include those wherein R^1 is Me or Et, R^{10} is H, R^2 is Me, D is butylene, Ra is H and E is C(=O).

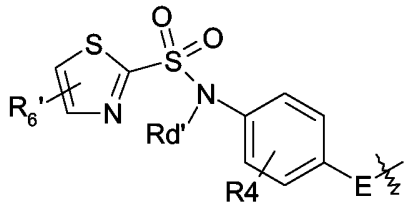
Favoured variants of the aspect of the invention in the immediately preceding paragraph include those wherein R^4 is at the 3, or the 3 and 5 positions of the phenyl ring. Representative values include R^4 as halo, such as 3-fluoro, 3,5-difluoro, 3-chloro or 3,5-dichloro. Alternative R^4 values include fluorinated methyl such as trifluormethyl, for example 3-trifluoromethyl, C$_1$-C$_3$alkyloxy, such as methyloxy for example 3-methoxy, or 3,5-dimethoxy, or -C(=O)C$_1$-C$_3$ alkyl such as acetyl, for example 3-acetyl. Additional favoured R^4 values include one or more C$_1$-C$_4$ alkyl, such as methyl, ethyl, i-propyl or t-butyl. Representative values for this aspect of the invention thus include 5-methyl, 5-ethyl, 5-i-propyl, 5-t-butyl, 6-methyl, 5-methyl-3-fluoro.

Additional favoured substituents to R^3, for example on a phenyl R^3, include sulphonamides. Accordingly, a favoured aspect of the invention comprises compounds of the formula I, wherein R^3 has the partial structure:

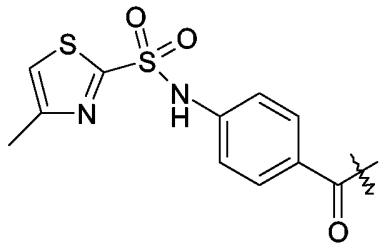


where E is as defined above, preferably -C(=O)- and R^{13} is -NRdSO$_m$R^{5a}, where R^{5a} is R^5 as defined above, preferably C_1-C_4 alkyl, such as methyl, ethyl or i-propyl or t-butyl; halogenated C_1-C_4 alkyl such as trifluoromethyl; C_3-C_6 cycloalkyl, such as cyclopropyl or cyclohexyl; or phenyl or benzyl, any of which is optionally substituted with R^6. Alternatively R^{5a} may be NRaRb as defined above including cyclic amines, such as -NHMe or -N(Me)$_2$, or piperazine, N-methyl piperazine, pyrrolidine, piperidine or morpholine.

Further preferred values for R^5 include heteroaryl rings such as pyrrolyl, oxazolyl, isoxazolyl, imidazolyl, pyridinyl, pyrimidinyl, pyrazinyl or indolyl, especially thiazolyl, any of which is substituted with R^6 groups such as C_1-C_4 alkyl. A currently favoured sulphonamide has the partial structure:



where E and R4 are as defined above, Rd' is Me or preferably H, and $R^{6'}$ is H or methyl, especially at the ring position, adjacent the N for example:



.

Other representative values of R^5 thus include F_3C-S(=O)$_2$-NH, cyclopropyl-S(=O)$_2$NH, Me-S(=O)$_2$)NH-, Et-S(=O)$_2$NH-, i-Pr-S(=O)$_2$NH-, Ph-S(=O)$_2$NH-; MeNH-S(=O)$_2$NH; (Me)$_2$S(=O)$_2$NH- and the like.

Alternatively the sulphonamide may have the other orientation -S(=O)$_m$NRdR^5 where R^5 as defined above, preferably C_1-C_4 alkyl, such as methyl, ethyl or i-propyl or t-butyl; halogenated

C_1-C_4 alkyl such as trifluoromethyl; C_3-C_6 cycloalkyl, such as cyclopropyl or cyclohexyl; or phenyl or benzyl, any of which is optionally substituted with R^6. Alternatively R^5 together with Rd defines a 3-6 membered N-containing ring such as azidine, pyrrolidine, pyridine, piperidine, morpholine, piperazine or N-methylpiperizine.

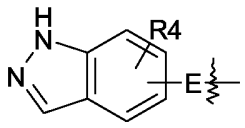
Representative values of sulphonamide thus include MeNH-S(=O)$_2$-, (Me)$_2$N-S(=O)$_2$- and the like.

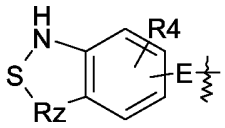
As depicted above, a sulphonamide substituted phenyl is optionally substituted with an additional substituent R^4, typically, but not invariably, in the 4 position if the sulphonamide is in the 3 position and vice versa. Representative R^4 groups thus include halo such as chloro or fluoro, C_1-C_4 alkyl such as methyl (including 2-methyl) and C_1-C_4alkoxy such as methoxy.

Rd is typically H or an acyl moiety such as -C(=O)C_1-C_4 alkyl or optionally substituted benzoyl. Representative values for Rc thus include H, acetyl, pivaloyl or benzoyl. For sulphonamides in the orientation –S(=O)mNRdR^5, Rd is conveniently C_1-C_4 alkyl or together with R^5 defines a 3-6 membered N-containing ring such as azidine, pyrrolidine, pyridine, piperidine, morpholine, piperazine or N-methylpiperizine.

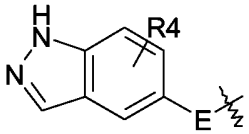
In either orientation, m is typically 1 (sulphenamide) or preferably 2 (sulphonamide).

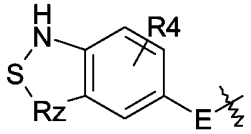
An alternative phenyl-based R^3 value is phenyl substituted with a pair of R^4 groups which together constitute a nitrogen containing chain of 3 or 4 atoms thereby defining a ring fused to the phenyl such as:



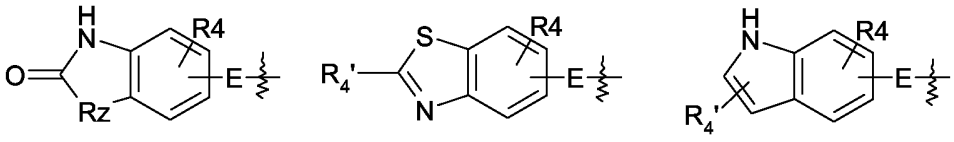


where R^4 and E are as defined above, Rz is CH, NH or O and the S atom is optionally oxidised to >S=O or preferably >S(=O)$_2$. Ring nitrogens are optionally substituted with C_1-C_4 alkyl (such as methyl, ethyl or t-butyl) , or C(=O)C_1-C_4 alkyl (such as acetyl). Typically R^4 is methyl or especially H. Preferably the linkage to E is para to a nitrogen in the fused ring:

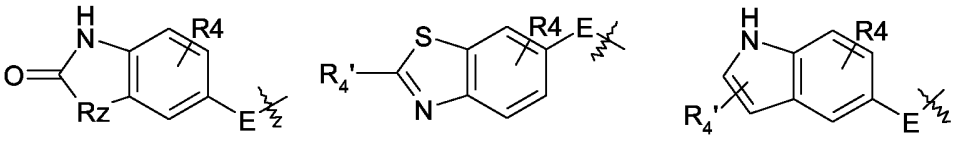




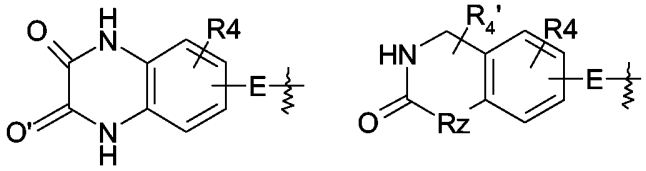
Other fused rings for constituting a nitrogen containing ring fused to a phenyl R^3 include



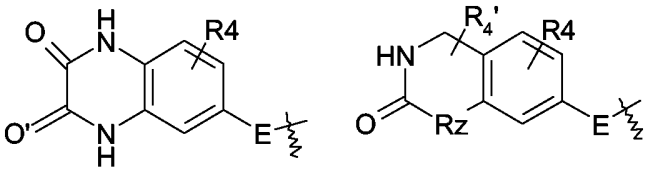
where Rz is CH; NH or O, especially O and preferably NH, $R^{4'}$ is H, C_1-C_4 alkyl, NH_2, NHC_1-C_4alkyl (such as methylamide), $N(C_1$-C_4alkyl$)_2$ such as dimethylamide), $NHC(=O)C_1$-C_4alkyl (such as acetamide). Ring nitrogens are optionally substituted with C_1-C_4 alkyl (such as methyl, ethyl or t-butyl) , or $C(=O)C_1$-C_4 alkyl (such as acetyl). Typically R^4 is methyl or especially H. Preferably the linkage to E is para to a nitrogen in the fused ring.



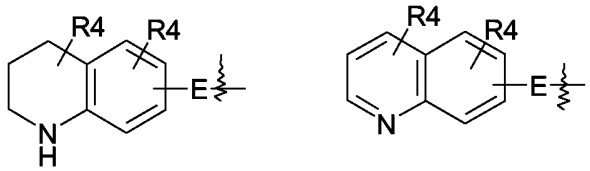
Still further fused rings for R^3 include variants wherein the fused nitrogen-containing ring defines a saturated or unsaturated 6 membered heterocycle, such as:



where Rz is NH, O or CH, especially O and preferably NH, R^4 and $R^{4'}$ are optional substituents as defined above, preferably H, and O' is absent (ie 2 hydrogen atoms) or keto. Preferably the linkage to E is para to a nitrogen in the fused ring.



Still further fused rings for R^3 include variants wherein the fused nitrogen containing ring defines an optionally substituted quinoline, isoquinoline, tetrohydroquinoline or tetrahydroisoquinoline moiety, such as



especially wherein R^4 and $R^{4'}$ are H and the linkage to E is para to the nitrogen in the fused ring.

Other favoured R^3 groups include pyrimidyl, such as 2-pyrimidyl, for example 5-OH-pyrimid-2-yl; or pyridyl, such as pyrid-4-yl, for example O→pyrid-4yl; or pyrid-3-yl, for example 6-hydroxy-pyrid-3-yl.

A further aspect of the invention comprises a method employing the compounds of the invention for the treatment of diseases caused by aberrant expression or activation of cathepsin, ie diseases or conditions alleviated or modified by inhibition of cathepsin S, preferably without substantial concomitant inhibition of other members of the papain superfamily.

Examples of such diseases or conditions include those enumerated in WO 97/40066, such as autoimmune diseases, allergies, such as asthma and hayfever, multiple sclerosis, rheumatoid arthritis and the like. A further example is the treatment of endometriasis, and especially chronic pain, as disclosed in WO0320287. The invention further provides the use of the compounds of formula IV in therapy and in the manufacture of a medicament for the treatment of diseases or conditions alleviated or moderated by inhibition of cathepsin S.

In one series of embodiments, the methods are employed to treat mammals, particularly humans at risk of, or afflicted with, autoimmune disease. By autoimmunity is meant the phenomenon in which the host's immune response is turned against its own constituent parts, resulting in pathology. Many human autoimmune diseases are associated with certain class II MHC-complexes. This association occurs because the structures recognized by T cells, the cells that cause autoimmunity, are complexes comprised of class II MHC molecules and antigenic peptides. Autoimmune disease can result when T cells react with the host's class II MHC molecules when complexed with peptides derived from the host's own gene products. If these class II MHC/antigenic peptide complexes are inhibited from being formed, the autoimmune response is reduced or suppressed. Any autoimmune disease in which class II MHC/antigenic complexes play a role may be treated according to the methods of the present invention.

Such autoimmune diseases include, e.g., juvenile onset diabetes (insulin dependent), multiple sclerosis, pemphigus vulgaris, Graves' disease, myasthenia gravis, systemic lupus erythematosus, rheumatoid arthritis and Hashimoto's thyroiditis.

In another series of embodiments, the methods are employed to treat mammals, particularly humans, at risk of, or afflicted with, allergic responses. By "allergic response" is meant the phenomenon in which the host's immune response to a particular antigen is unnecessary or disproportionate, resulting in pathology. Allergies are well known in the art, and the term "allergic response" is used herein in accordance with standard usage in the medical field.

Examples of allergies include, but are not limited to, allergies to pollen, "ragweed," shellfish, domestic animals (e.g., cats and dogs), bee venom, house dust mite allergens and the like. Another particularly contemplated allergic response is that which causes asthma. Allergic responses may occur, in man, because T cells recognize particular class II MHC/antigenic peptide complexes. If these class II MHC/antigenic peptide complexes are inhibited from being formed, the allergic response is reduced or suppressed. Any allergic response in which class II MHC/antigenic peptide complexes play a role may be treated according to the methods of the present invention Immunosuppression by the methods of the present invention will typically be a prophylactic or therapeutic treatment for severe or life-threatening allergic responses, as may arise during asthmatic attacks or anaphylactic shock.

In another series of embodiments, the methods are employed to treat mammals, particularly humans, which have undergone, or are about to undergo, an organ transplant or tissue graft. In tissue transplantation (e.g., kidney, lung, liver, heart) or skin grafting, when there is a mismatch between the class II MHC genotypes (HLA types) of the donor and recipient, there may be a severe "allogeneic" immune response against the donor tissues which results from the presence of non-self or allogeneic class II MHC molecules presenting antigenic peptides on the surface of donor cells. To the extent that this response is dependent upon the formation of class II MHC/antigenic peptide complexes, inhibition of cathepsin S may suppress this response and mitigate the tissue rejection. An inhibitor of cathepsin S can be used alone or in conjunction with other therapeutic agents, e.g., as an adjunct to cyclosporin A and/or antilymphocyte gamma globulin, to achieve immunosuppression and promote graft survival. Preferably, administration is accomplished by systemic application to the host before and/or after surgery. Alternatively or in addition, perfusion of the donor organ or tissue, either prior or subsequent to transplantation or grafting, may be effective.

The above embodiments have been illustrated with an MHC class II mechanism but the invention is not limited to this mechanism of action. Suppression of cathepsin S as a treatment of COPD or chronic pain may not, for example, involve MHC class II at all.

Assays for the assessment of cathepisn S inhibitors in the treatment of chronic pain, including neuropathic or inflammatory pain are as described in WO 03 20287.

Currently preferred indications treatable in accordance with the present invention include:
Psoriasis;
Autoimmune indications, including idiopathic thrombocytopenic purpura (ITP), rheumatoid arthritis (RA), multiple schlerosis (MS), myasthenia gravis (MG), Sjögrens syndrome, Grave's disease and systemic lupus erythematosis (SLE);
Non-automimmune indications include allergic rhinitis, asthma, artherosclerosis, chronic obstructive pulmonary disease (COPD) and chronic pain.

The compounds of the invention can form salts which form an additional aspect of the invention. Appropriate pharmaceutically acceptable salts of the compounds of the invention include salts of organic acids, especially carboxylic acids, including but not limited to acetate, trifluoroacetate, lactate, gluconate, citrate, tartrate, maleate, malate, pantothenate, isethionate, adipate, alginate, aspartate, benzoate, butyrate, digluconate, cyclopentanate, glucoheptanate, glycerophosphate, oxalate, heptanoate, hexanoate, fumarate, nicotinate, palmoate, pectinate, 3-phenylpropionate, picrate, pivalate, propionate, tartrate, lactobionate, pivolate, camphorate, undecanoate and succinate, organic sulphonic acids such as methanesulphonate, ethanesulphonate, 2-hydroxyethane sulphonate, camphorsulphonate, 2-naphthalenesulphonate, benzenesulphonate, p-chlorobenzenesulphonate and p-toluenesulphonate; and inorganic acids such as hydrochloride, hydrobromide, hydroiodide, sulphate, bisulphate, hemisulphate, thiocyanate, persulphate, phosphoric and sulphonic acids. The compounds of the invention may in some cases be isolated as the hydrate.

Prodrugs

The compounds of the invention include a number of handles such as OH, NH or COOH groups to which conventional prodrug moieties can be applied. Prodrugs are typically hydrolysed in vivo to release the parent compound in the plasma, liver or intestinal wall. Favoured prodrugs are esters of hydroxyl groups such as a phenolic hydroxyl group at R^3, or amine functions such as an R^4 sulphonamide amine function. Preferred pharmaceutically acceptable esters include those derived from C_1-C_6 carboxylic acids such as acetyl or pivaloyl or optionally substituted benzoic acid esters, preferably unsubstituted or substituted with R^6.

Favoured sulphonamide prodrugs include aminoacyls derived from C_1-C_6 carboxylic acids such as acetyl or pivaloyl or optionally substituted benzoic acid esters, preferably unsubsbstituted or substituted with R^6.

C_0-C_3alkylcarbocyclyl comprises C_0-C_3alkylaryl and C_0-C_3alkylC_3-C_7cycloalkyl. 'C_0-C_3alkylaryl' as applied herein is meant to include an aryl moiety such as a phenyl, naphthyl or phenyl fused to a C_3-C_7cycloalkyl (for example indanyl), which aryl is directly bonded (i.e. C_0) or through an intermediate methylene, ethylene, or propylene group. 'C_0-C_3alkylC_3-C_7cycloalkyl' as applied herein is meant to include a C_3-C_7cycloalkyl group such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl, which cycloalkyl is directly bonded (i.e. C_0alkyl) or through an intermediate methylene, ethylene, propylene or isopropylene group. The cycloalkyl group may contain an unsaturated bond.

Unless otherwise indicated the aryl or cycloalkyl group is optionally substituted with 1-3 substituents selected from halo, hydroxy, nitro, cyano, carboxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_1-C_6alkoxyC_1-C_6alkyl, C_1-C_6alkanoyl, amino, azido, oxo, mercapto, nitro, or C_0-C_3alkylR^3.

While it is possible for the active agent to be administered alone, it is preferable to present it as part of a pharmaceutical formulation. Such a formulation will comprise the above defined active agent together with one or more acceptable carriers/excipients and optionally other therapeutic ingredients. The carrier(s) must be acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient.

The formulations include those suitable for rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration, but preferably the formulation is an orally administered formulation. The formulations may conveniently be presented in unit dosage form, e.g. tablets and sustained release capsules, and may be prepared by any methods well known in the art of pharmacy.

Such methods include the step of bringing into association the above defined active agent with the carrier. In general, the formulations are prepared by uniformly and intimately bringing into association the active agent with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product. The invention extends to methods for preparing a pharmaceutical composition comprising bringing a compound of Formula IV or its pharmaceutically acceptable salt in conjunction or association with a pharmaceutically acceptable carrier or vehicle. If the manufacture of pharmaceutical formulations involves intimate mixing of pharmaceutical excipients and the active ingredient in salt form, then it is often preferred to use excipients which are non-basic in nature, i.e. either acidic or neutral.

Formulations for oral administration in the present invention may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active agent; as a powder or granules; as a solution or a suspension of the active agent in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water in oil liquid emulsion and as a bolus etc.

With regard to compositions for oral administration (e.g. tablets and capsules), the term suitable carrier includes vehicles such as common excipients e.g. binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, polyvinylpyrrolidone (Povidone), methylcellulose, ethylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, sucrose and starch; fillers and carriers, for example corn starch, gelatin, lactose, sucrose, microcrystalline cellulose, kaolin, mannitol, dicalcium phosphate, sodium chloride and alginic acid; and lubricants such as magnesium stearate, sodium stearate and other metallic stearates, glycerol stearate stearic acid, silicone fluid, talc waxes, oils and colloidal silica. Flavouring agents such as peppermint, oil of wintergreen, cherry flavouring or the like can also be used. It may be desirable to add a colouring agent to make the dosage form readily identifiable. Tablets may also be coated by methods well known in the art.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active agent in a free flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active agent.

Other formulations suitable for oral administration include lozenges comprising the active agent in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active agent in an inert base such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active agent in a suitable liquid carrier.

As with all pharmaceuticals, the appropriate dosage for the compounds or formulations of the invention will depend upon the indication, the severity of the disease, the size and metabolic vigour and the patient, the mode of administration and is readily determined by conventional animal trials. Dosages providing intracellular (for inhibition of physiological proteases of the papain superamily) concentrations of the order 0.01-100 uM, more preferably 0.01-10 uM, such as 0.1-5 uM are typically desirable and achievable.

Synthesis of the compounds of the present invention can be performed by different chemical strategies in solution or solid phase or a combination of both. The compounds are typically prepared as building blocks reflecting the P1, P2 and P3 moieties of the end product inhibitor. Without in any way wishing to be bound by theory, or the ascription of tentative binding modes for specific variables, the notional concepts P1, P2 and P3 as used herein are provided for convenience only and have substantially their conventional Schlecter & Berger meanings and denote those portions of the inhibitor believed to fill the S1, S2, and S3 subsites respectively of the enzyme, where S1 is adjacent the cleavage site and S3 remote from the cleavage site. Compounds defined by Formula I are intended to be within the scope of the invention, regardless of binding mode.

Broadly speaking the P1 building block will be an N-protected C-5-substituted furan-3-onamine, P2 will be an N-protected amino acid in which the side chain comprises the D-containing saturated ring and branched alkyl linker, whereas P3 typically comprises a capping group such as a substituted, heteroaroyl or aroyl moiety.

The suitably protected individual building blocks can first be prepared and subsequently coupled together i.e. P2+P1→ P2-P1. Alternatively, precursors of the building blocks can be coupled together and modified at a later stage of the synthesis of the inhibitor sequence. Further building blocks, precursors of building blocks or prefabricated bigger fragments of the desired structure, can then be coupled to the growing chain, e.g. R^3-E-P2*+ P1→ R^3-E-P2-P1 or R^3-E*+P2-P1→ R^3-E-P2-P1, where * denotes an activated form.

Coupling between two amino acids, an amino acid and a peptide, or two peptide fragments can be carried out using standard coupling procedures such as the azide method, mixed carbonic-carboxylic acid anhydride (isobutyl chloroformate) method, carbodiimide (dicyclohexylcarbodiimide, diisopropylcarbodiimide, or water-soluble carbodiimide) method, active ester (pnitrophenyl ester, N-hydroxysuccinic imido ester) method, Woodward reagent K-method, carbonyldiimidazole method, phosphorus reagents or oxidation-reduction methods. Some of these methods (especially the carbodiimide method) can be enhanced by adding 1-hydroxybenzotriazole or 4-DMAP. These coupling reactions can be performed in either solution (liquid phase) or solid phase.

More explicitly, the coupling step involves the dehydrative coupling of a free carboxyl of one reactant with the free amino group of the other reactant in the present of a coupling agent to form a linking amide bond. Descriptions of such coupling agents are found in general textbooks on peptide chemistry, for example, M. Bodanszky, "Peptide Chemistry", 2nd rev ed., Springer-

Verlag, Berlin, Germany, (1993) hereafter simply referred to as Bodanszky, the contents of which are hereby incorporated by reference. Examples of suitable coupling agents are N,N'-dicyclohexylcarbodiimide, 1-hydroxybenzotriazole in the presence of N,N'-dicyclohexylcarbodiimide or N-ethyl-N'- [(3dimethylamino) propyl] carbodiimide. A practical and useful coupling agent is the commercially available (benzotriazol-1-yloxy) tris- (dimethylamino) phosphonium hexafluorophosphate, either by itself or in the present of 1-hydroxybenzotriazole or 4-DMAP. Another practical and useful coupling agent is commercially available 2-(lH-benzotriazol-1-yl)-N, N, N',N'- tetramethyluronium tetrafluoroborate. Still another practical and useful coupling agent is commercially available 0-(7-azabenzotrizol-1-yl)-N, N,N', N'-tetramethyluronium hexafluorophosphate.

The coupling reaction is conducted in an inert solvent, e. g. dichloromethane, acetonitrile or dimethylformamide. An excess of a tertiary amine, e. g. diisopropylethylamine, N-methylmorpholine, N-methylpyrrolidine or 4-DMAP is added to maintain the reaction mixture at a pH of about 8. The reaction temperature usually ranges between 0 °C and 50 °C and the reaction time usually ranges between 15 min and 24 h.

The functional groups of the constituent non-natural amino acids generally must be protected during the coupling reactions to avoid formation of undesired bonds. The protecting groups that can be used are listed in Greene, "Protective Groups in Organic Chemistry", John Wiley & Sons, New York (1981) and "The Peptides: Analysis, Synthesis, Biology", Vol. 3, Academic Press, New York (1981), hereafter referred to simply as Greene, the disclosures of which are hereby incorporated by reference.

The alpha-carboxyl group of the C-terminal residue is usually protected as an ester that can be cleaved to give the carboxylic acid. Protecting groups that can be used include 1) alkyl esters such as methyl, trimethylsilyl and t.butyl, 2) aralkyl esters such as benzyl and substituted benzyl, or 3) esters that can be cleaved by mild base or mild reductive means such as trichloroethyl and phenacyl esters.

The alpha-amino group of each amino acid to be coupled is typically be protected. Any protecting group known in the art can be used. Examples of such groups include: 1) acyl groups such as formyl, trifluoroacetyl, phthalyl, and p-toluenesulfonyl; 2) aromatic carbamate groups such as benzyloxycarbonyl (Cbz or Z) and substituted bensyloxycarbonyls, and 9-fluorenylmethyloxycarbonyl (Fmoc); 3) aliphatic carbamate groups such as tertbutyloxycarbonyl (Boc), ethoxycarbonyl, diisopropylmethoxycarbonyl, and allyloxycarbonyl; 4) cyclic alkyl carbamate groups such as cyclopentyloxycarbonyl and adamantyloxycarbonyl; 5) alkyl groups

such as triphenylmethyl and benzyl; 6) trialkylsilyl such as trimethylsilyl; and 7) thiol containing groups such asphenylthiocarbonyl anddithiasuccinoyl. The preferred alpha-amino protecting group is either Boc or Fmoc. Many amino acid derivatives suitably protected for peptide synthesis are commercially available.

The alpha-amino protecting group is typically cleaved prior to the next coupling step. When the Boc group is used, the methods of choice are trifluoroacetic acid, neat or in dichloromethane, or HCl in dioxane or in ethyl acetate. The resulting ammonium salt is then neutralized either prior to the coupling or in situ with basic solutions such as aqueous buffers, or tertiary amines in dichloromethane or acetonitrile or dimethylformamide. When the Fmoc group is used, the reagents of choice are piperidine or substituted piperidine in dimethylformamide, but any secondary amine can be used. The deprotection is carried out at a temperature between 0 °C and room temperature usually 20-22 °C.

Any of the natural or non-natural amino acids having side chain functionalities will typically be protected during the preparation of the peptide using any of the above described groups. Those skilled in the art will appreciate that the selection and use of appropriate protecting groups for these side chain functionalities depend upon the amino acid and presence of other protecting groups in the peptide. In the selection of such protecting groups it is desirable that the group is not removed during the deprotection and coupling of the alpha-amino group.

For example, when Boc is used as the alpha-amino protecting group, the following side chain protecting groups are suitable: p-toluenesulfonyl (tosyl) moieties can be used to protect the amino side chain of amino acids such as Lys and Arg; acetamidomethyl, benzyl (Bn), or tert-butylsulfonyl moities can be used to protect the sulfide containing side chain of cysteine; benzyl (Bn) ethers can be used to protect the hydroxy containing side chains of serine, threonine or hydroxyproline; and benzyl esters can be used to protect the carboxy containing side chains of aspartic acid and glutamic acid.

When Fmoc is chosen for the alpha-amine protection, usually tert. butyl based protecting groups are acceptable. For instance, Boc can be used for lysine and arginine, tert.butyl ether for serine, threonine and hydroxyproline, and tert-butyl ester for aspartic acid and glutamic acid. Triphenylmethyl (Trityl) moiety can be used to protect the sulfide containing side chain of cysteine.

Once the inhibitor sequence is completed any protecting groups are removed in whatever

manner is dictated by the choice of protecting groups. These procedures are well known to those skilled in the art.

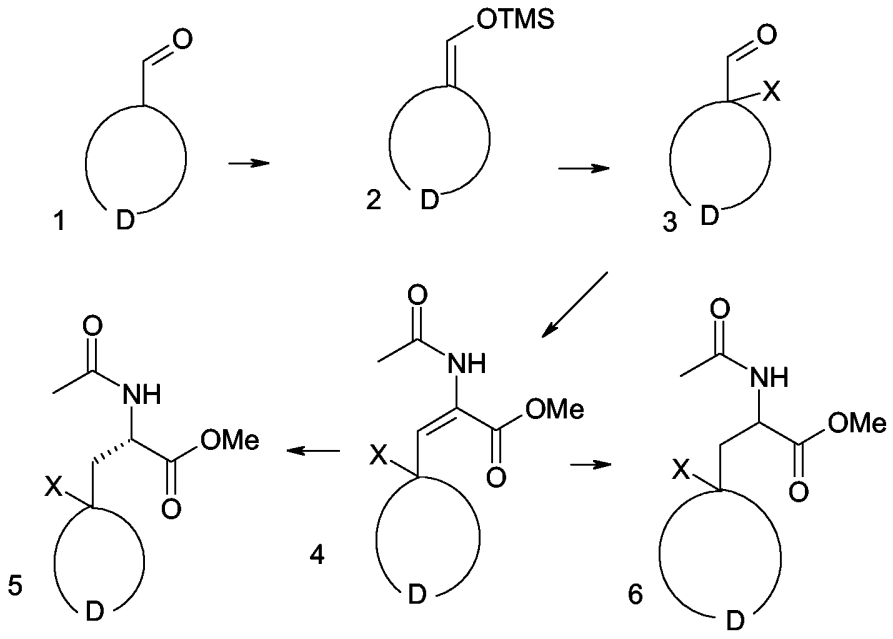
Preparation of P1 building blocks

The preparation and manipulation of C-5 substituted furanone amines is extensively described in WO0069055 and WO05/082876, whose disclosures are respectively incorporated herein.

The P1 building block may be elongated with the P2 amino acid (or the ready formed P3-P2 intermediate) while the P1 is in the furanone form. Alternatively elongation with P2/P3 may take place on a furanol which is subsequently oxidised by Dess Martin chemistry in an organic solvent such as DCM.

Preparation of P2 building blocks

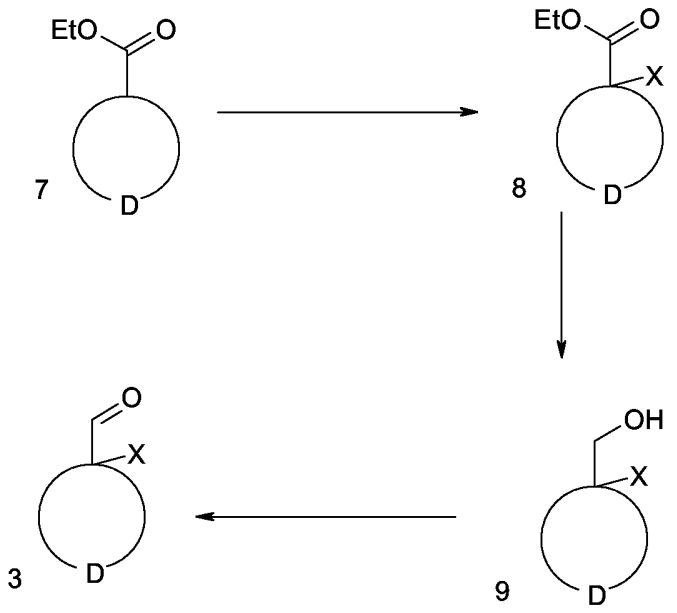
Scheme 1



Scheme 1 depicts that a suitable aldehyde 1, such as cyclopentylaldehyde, can be derivatised into the silyl enol ether 2 using, for example, N-Methyl-N-trimethylsilylacetamide in DMF at room temperature. 2 can then act as a suitable precursor for a number of variations of X. For example, by using a suitable alkyl halide in the presence of fluoride anion, X can represent suitable alkyl groups. By employing suitable electrophilic fluorinating agents, such as Selectfluor™ in a solvent such as DMF or acetonitrile, X can represent fluorine. Other electrophilic halogenating reagents, such as N-chlorosuccinimide in the presence of fluoride anion will give derivatives where X is chlorine. Conversion of 3 to 4 can be achieved with N-

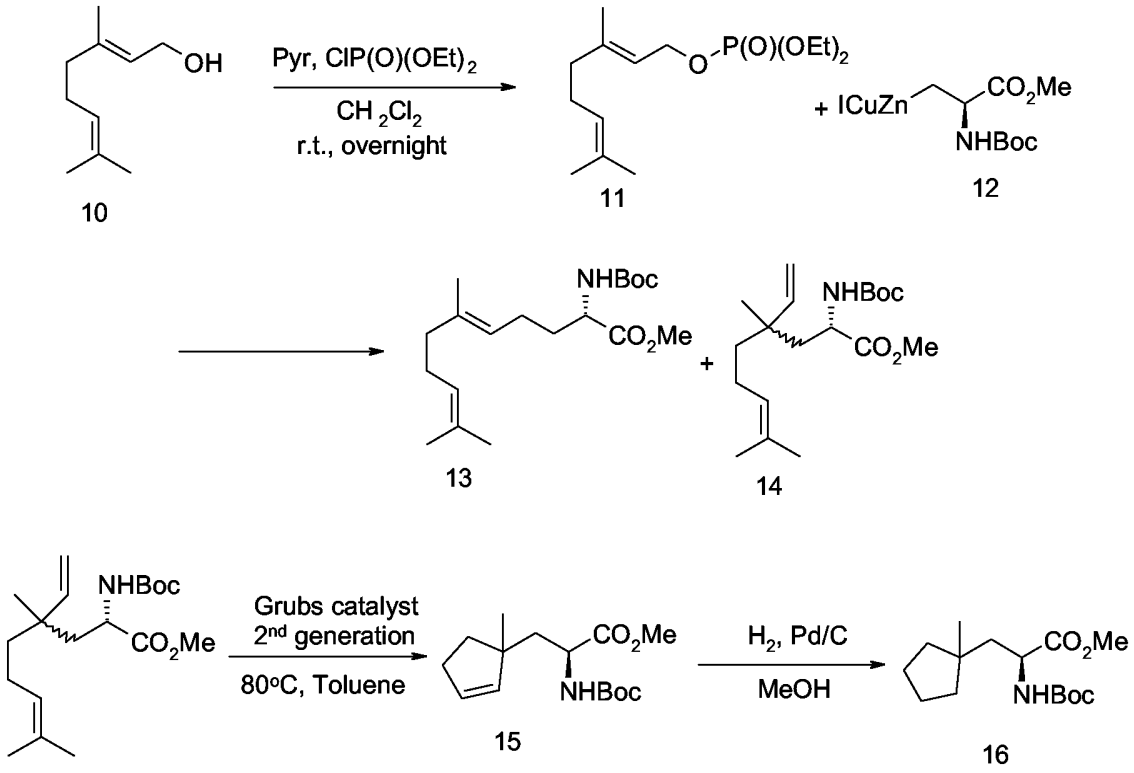
acetamidophosphonoglycine trimethyl ester in the presence of a suitable base such as potassium tert-butoxide in a solvent such as THF at 0 ^{0}C. Alternatively preparation of 4 can be achieved by following the protocol outlined by Schollkopf et al in *Liebigs Ann. Chemie* 1981 1469-1475. The conversion of 4 into the chiral amino acid 5 can be achieved with a chiral catalyst, such as [EtDuPHOS-Rh (COD)]+ in a solvent such as methanol under hydrogen pressure of between 1 and 5 bar. Alternatively, 4 can be converted into the achiral amino acid 6 using a non-chiral catalyst such as that based on a palladium or rhodium-containing species e.g. Wilkinson's catalyst. Resolution of the amino acid will then follow one of many well documented methods, such as enzymatic hydrolysis of the ester, or separation of the racemates by chiral-HPLC.

Scheme 2



Scheme 2 depicts that the preparation of 3 can also be achieved from direct reaction of a suitable lithium enolate with a suitable electrophilic reagent, such as an alkyl halide. Therefore, treatment of 7 with LDA in THF at -78 °C followed by quenching of the resultant anion affords 8. The ester group of 8 can be reduced, for example with lithium aluminium hydride to the corresponding alcohol 9. Compound 3 is then prepared by oxidation of the alcohol with a suitable oxidant, such as pyridinium chlorochromate in DCM at room temperature.

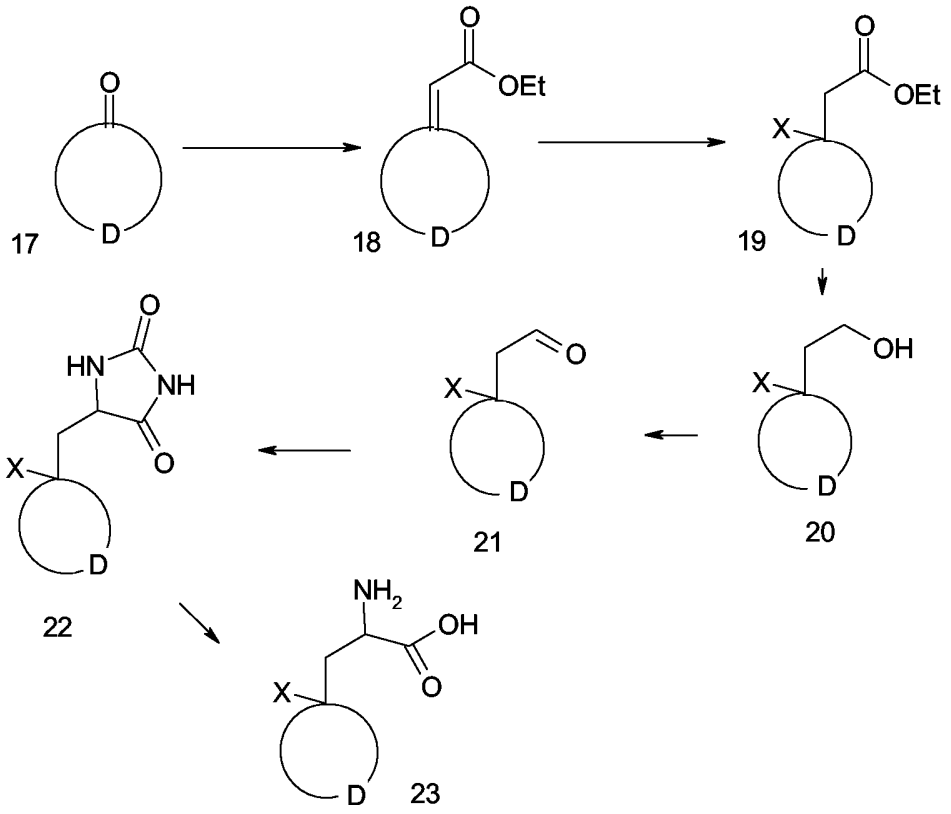
Scheme 3



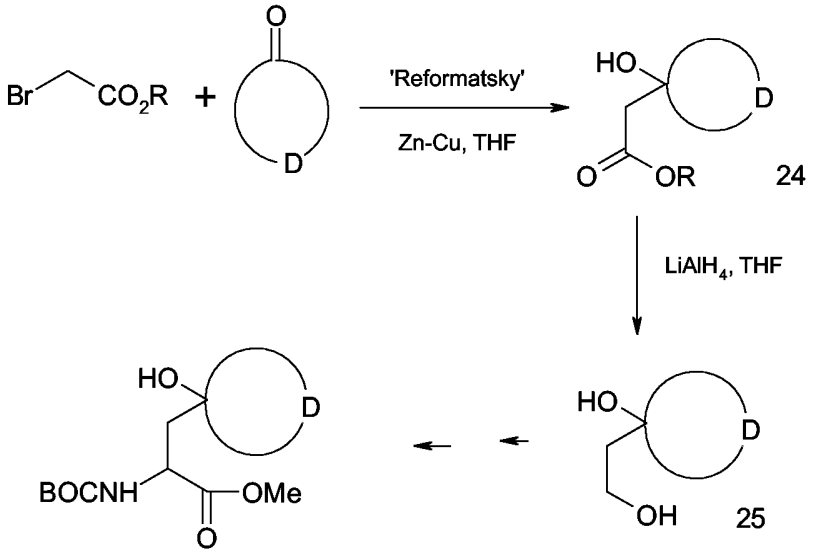
Scheme 3 shows an alternative synthesis to prepare the C5 alkylene amino acid 16 in homochiral form. Geraniol 10 is converted to the phosphate 11 with diethyl chlorophosphonate and then reacted with a homochiral zinc/copper couple of alanine 12. Compound 13 is obtained from Sn2 reaction of the zinc/copper couple, whilst compound 14 is obtained from the alternative Sn2' mechanism. Ring-closing metathesis reaction of 14 using for example Grubb's catalyst gives the methylcyclopentene derivative 15. Atmospheric pressure hydrogenation of the methylcyclopentene double bond can be achieved with a palladium catalyst in a solvent like methanol to afford the amino acid 16. Enantioselectivity is in excess of 95%.

An alternative method to prepare substituted cycloalkyl alanines of the type shown in Scheme 4, below, is to take a suitable cycloalkanone 17 and treat this with triethylphosphonoacetate in THF at 0 °C with potassium tert-butoxide as base. The resultant eneoate 18 can be treated with a variey of nucleophiles, such as organocuprates, substituted amines and substituted thiols to generate compounds of the type shown in 19. Treatment of compounds such as 19 in the manner described for 38 in Scheme 8 below gives the racemic substituted cycloalkyl alanine derivatives. Resolution of the amino acid will then follow one of many well documented methods, such as enzymatic hydrolysis of the ester or separation of the racemates by chiral-HPLC.

Scheme 4



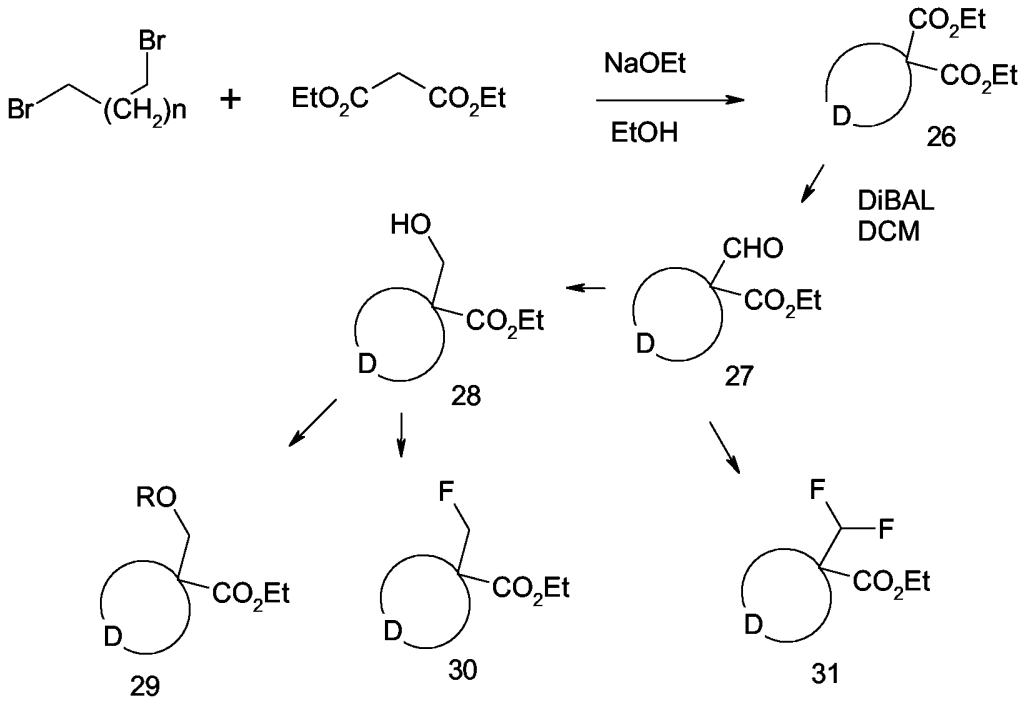
Scheme 5



An alternative for substitution at R^2 involves the method shown in Scheme 5. An appropriate cycloalkanone is treated with a zinc/copper couple of alpha-bromomethylacetate in a solvent such as THF at reflux. The hydroxyl group in 24 can be left underivatised and the ester can be

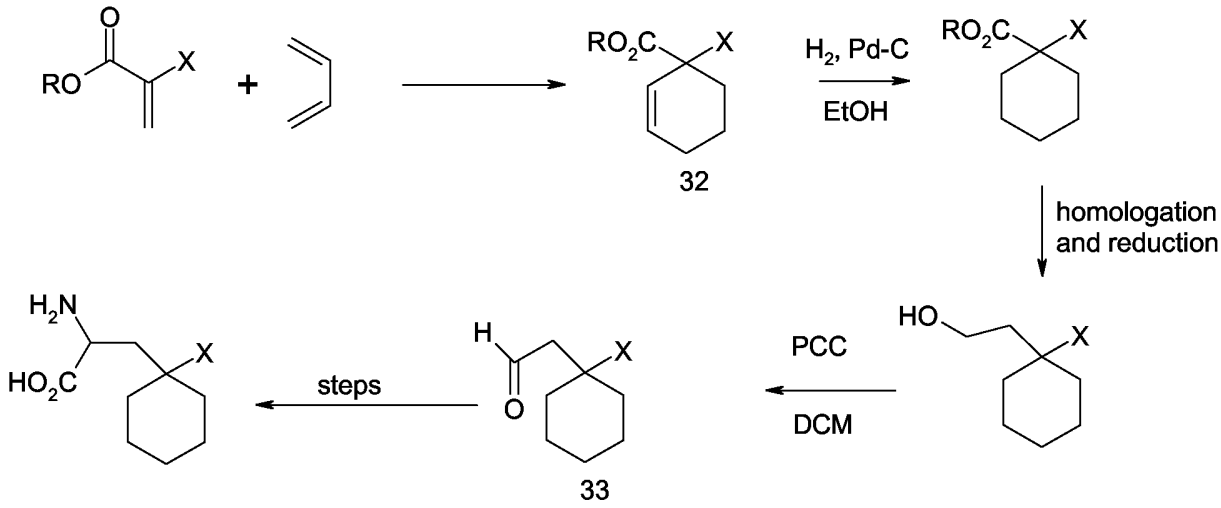
reduced to the primary alcohol with a reducing agent such as lithium aluminium hydride. A compound such as 25 can be treated in the same way as compound 20 to afford the desired substituted cycloalkyl alanine. Alternatively, the hydroxyl group in 24 can be derivatised to form the alkyloxy at R^2, using a reagent such as sodium hydride and an alkyl halide in THF at room temperature or reflux. The same procedure for the synthesis of the achiral amino acid would then apply.

Scheme 6



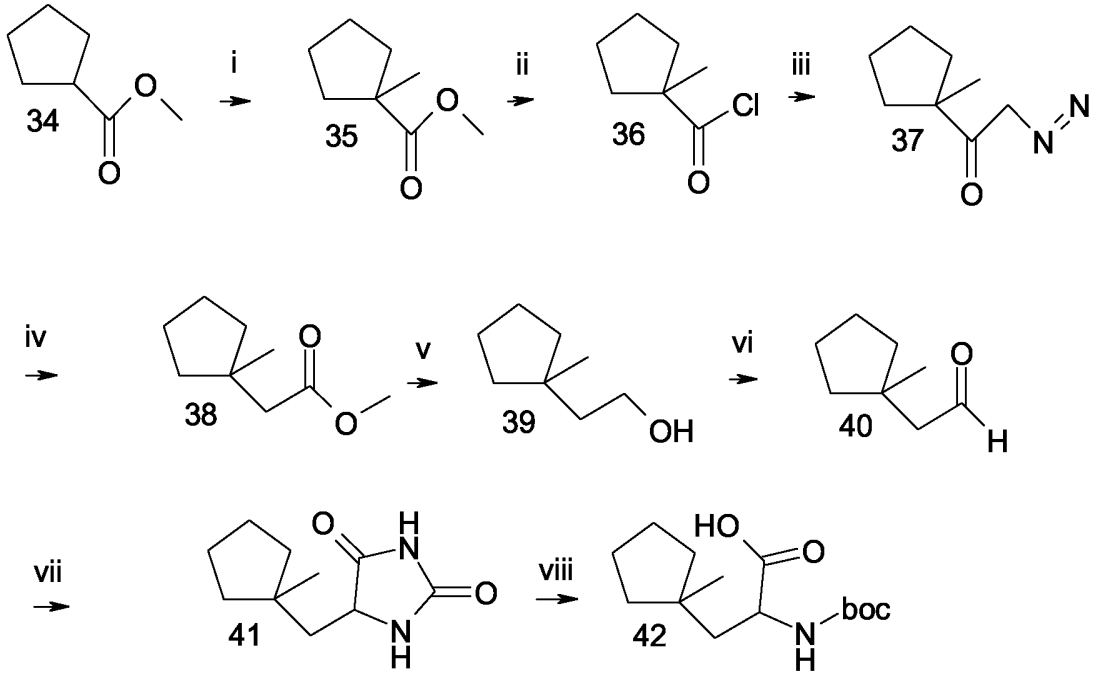
A suitable dibromoalkane can be reacted with diethylmalonate with, for example, sodium ethoxide in ethanol to afford the diester 26. This can be converted into the ester/aldehyde 27 in a number of ways, for example with diisobutylaluminium hydride in dichloromethane at -78 deg C. This aldehyde serves as a useful precursor for a number of derivatives. The methylene alcohol 28 can be prepared by reduction of the aldehyde with sodium borohydride in a solvent such as ethanol; the alkyloxy methylene 29 is produced by alkylation of the methylene alcohol 28 with an alkyl halide and a suitable base such as sodium hydride; the methylene fluoride 30 is produced by fluorination of 28 with a suitable fluorinating agent such as DAST or Deoxyfluor. These reagents can also be employed to give the difluorinated compound 31 from the aldehyde 27. Substituted cycloalkyl esters, typified by compound 31, can be used to prepare the appropriate substituted cycloalkyl alanines 33 in an analogous manner to that outlined in Scheme 8.

Scheme 7



Appropriately substituted cyclohexylalanines can be prepared as in Scheme 7. Diels-Alder reaction of 1,3-butadiene with appropriately substituted dieneophiles can afford the cyclohexene derivative 32. Reduction of the cyclohexene double bond and manipulation of the ester moiety to the aldehyde 33, as shown in the scheme, provides the precursor to the substituted cyclohexyl alanine amino acids. These final steps can be achieved using the chemistry outlined in Scheme 8.

Scheme 8



Scheme 8 depicts the synthesis of a methylcyclopentylalanine building block. Commercially available methyl cyclopentane carboxylate 34 is methylated with LDA and iodomethane (i, BuLi,

diisopropylamine, MeI) to give 35. Hydrolysis of the ester with LiOH followed by treatment with oxalyl chloride (ii, LiOH, oxalylchloride) gives acid chloride 36. Wolff rearrangement with diazomethane (iii, CH_2N_2, Et_3N) and silver benzoate (iv, silver benzoate, Et_3N, MeOH) gives the ester 38. Reduction of the ester (v, $LiAlH_4$) followed by Dess-Martin periodinane oxidation (vi) gives the aldehyde 40. The aldehyde is treated with KCN and ammonium carbonate (vii, KCN, $(NH_4)_2CO_3$, HCl), followed by hydrolysis with NaOH and protection of the free amine as its Boc carbamate to give racemic amino acid 16. The enantiomers are separated by conventional chromatographic techniques such as chiral HPLC, before or after coupling to the P1 and/or P3 building blocks. Although the scheme has been illustrated with a cyclopentane variant, the methodology is applicable to other variants of D.

Elongation with P3.

Compounds wherein E is carbonyl are readily prepared by conventional peptide chemistry, from the corresponding (optionally substituted) R3-carboxylic acid. For example the N-protected P2-P1 intermediate is treated with 4M HCl /dioxan and the carboxy protected R3 acid is added together with a coupling mixture such as HBTU/HOBT/DMF/NMM. A substantial number of (substituted) R3 carboxylic acids are commercially available or readily converted from commercially available synthons.

Sulphonamide derivatives i.e. E = $S(=O)_2$- can be prepared by reaction of the amino group of the P2 amino acid with a suitable sulfonyl chloride in a solvent such as dichloromethane in the presence of a suitable base such as triethylamine or dimethylaminopyridine. For example the N-protected P1-P2 intermediate is treated with 4M HCl in dioxan. An optionally substituted R^3-SO_2Cl is added with Et_3N and a catalytic amount of DMAP.

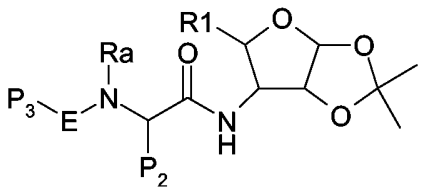
Urethane compounds i.e. E is -OC(=O)- can be formed for example by reaction of an R_3 alcohol with the isocyanate of the P2 amino acid. The isocyanate, or equivalent reactive intermediate, can be formed by reaction of the amino group of the P2-amino acid with phosgene, or with dinitrophenylcarbonate in the presence of a suitable base, e.g. triethylamine. Alternatively they can be formed by reaction of the amino group of the P2 amino acid with a suitable chloroformate, e.g. benzylchloroformate.

Sulphamide derivatives i.e. E = -NRaS(=O)$_2$- can be prepared by reacting a suitable R_3 amine in a sulphonyl chloride solvent followed by reaction of the formed sulfamoyl chloride derivative with the amino group of the above mentioned R_4 amino acid in a solvent such as dichloromethane in the presence of a suitable base such as triethylamine.

Urea derivatives i.e. E = -NRa-C(=O)- can be prepared by reaction of the corresponding R3 isocyanate with the N-protected amide of the P2-P1 intermediate, typically in an inert organic solvent such as N,N-dimethyl formamide. Conversely, the R3 amine is reacted with the isocyanate of the P2-P1 intermediate under similar conditions. Alternatively the N-protected R3-amine, and N-protected P2-P1 amine are together reacted with $L_1C(=O)L_2$, where L_1 and L_2 are good leaving groups in an inert organic solvent such as N,N-dimethyl formamide, tetrahydrofuran, ethyl acetate or benzene, as shown in J Org Chem 56, 891 (1991). The time, temperature and sequence of addition used depends on the reactivity of the individual reagents.

A special case of a urea derivative are compounds wherein R^3 represents an unsaturated ring such as morpholine, piperazine or piperidine which is N-bonded to E as carbonyl. Such compounds are readily prepared, for example by treating the N-protected P2-P1 intermediate with 4M HCl/dioxane, adding the R3-chloride, for example morpholinyl carbonyl chloride, together with TEA in DCM.

Compounds wherein R^{10} is an hydroxyl, ether, ester or ketone are typically prepared by coupling P1 to P2 & P3 as the 2,3 isopropylidinyl protected building block, such as:



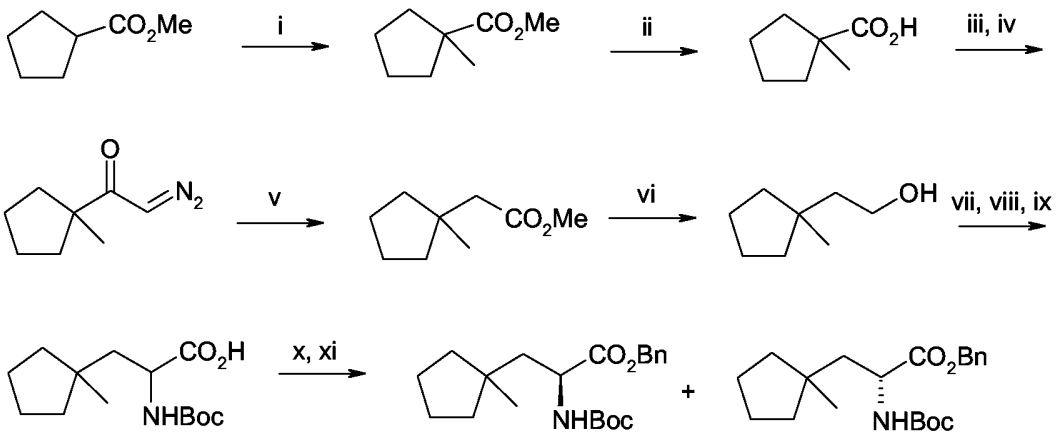
Preparation of the 2,3, P1 building block for a variety of is shown in WO00/69055 and WO05/082876, the content of which is incorporated by reference. For example, acid catalysed removal of the diisopropylidene protecting group, such as with HCl in a suitable solvent such as methanol, produces the alcohol acetal (denoted C1) and the hydroxyl at C2. This hydroxyl can be oxidized to the ketone as described in WO0069055 and WO05/082876 using, for example, Dess-Martin periodination. The alpha anomer at R^{10} is isolated by preparative HPLC or flash chromatography. Appropriate choice of alcohol and reaction conditions produces other acetal groups as defined by R10 in the invention which can undergo oxidation of the hydroxyl functionality at C2. Suitable protection of the C2 hydroxy function will allow manipulation of the alcohol acetal to the hemi-acetal form, which in turn can be oxidized to the lactone. Preparation of the thioacetal from the hemi-acetal can be achieved by methods as described in *Liebigs Ann Chem* 1993 p1211-1218 or *Journal of Organic Chemistry* 1986 p4802-4806.

Various aspects of the invention will now be described by way of example only with reference to the accompanying Examples.

Example 1

Preparation of 1-methylcyclobutylalanine P2 bulding block.

(R)- and (S)- N-Boc-(1-methylcyclopentyl)-alanine benzyl ester



(i) LDA, MeI, -78°C, (ii) LiOH, MeOH, (iii) oxalyl chloride, DMF, (iv) Diazomethane, (v) AgOBz, MeOH, (vi) $LiAlH_4$, (vii) Dess – Martin periodinane, (viii) KCN, $(NH_4)_2CO_3$, (ix) NaOH then Boc_2O, (x) BnBr, Cs_2CO_3, (xi) Chiral HPLC.

a) 1-Methylcyclopentanecarboxylic acid methyl ester

A solution of cyclopentanecarboxylic acid methyl ester (78 mmol) in THF was added to a solution of freshly prepared LDA (78 mmol) in THF at -78°C over 5 minutes. The mixture was warmed to 0°C and stirred for 30 minutes at which point it was re-cooled to -78°C. A solution of methyl iodide (78 mmol) in THF was then added and the mixture was allowed to come to room temperature and stirred overnight. NH_4Cl (sat. aq.) was added then the mixture was extracted with TBME. The organics were dried ($MgSO_4$) then concentrated in vacuo to give a yellow oil. Column chromatography (silica gel, 1 – 15% EtOAc in heptane) gave the product as a clear oil (42 mmol, 54%).

b) 1-Methylcyclopentanecarboxylic acid

NaOH (88 mmol) was added to a solution of 1-methyl-cyclopentanecarboxylic acid methyl ester (88 mmol) in MeOH and the mixture was stirred overnight. The resulting solution was then

acidified with conc. HCl to pH 2. Water and EtOAc were then added and the organics were separated. The aqueous phase was extracted with EtOAc. The combined organics were dried ($MgSO_4$) then concentrated in vacuo to give a yellow oil which was used with no further purification (74 mmol, 84%).

c) 1-Methylcyclopentane diazoketone

Oxalyl chloride (89 mmol) was added to a solution of 1-methyl-cyclopentanecarboxylic acid (74 mmol) in DCM at 0°C. This was followed by a few drops of DMF. The mixture was stirred overnight then the solvents were removed in vacuo to give a pale brown semi-solid which was dissolved 1:1 THF:MeCN. Triethylamine (96 mmol) was added followed by diazomethane (222 mmol) in diethyl ether. The mixture was stirred overnight then the solvents were removed in vacuo. The residue was dissolved in TBME. The organics were washed (water then $NaHCO_3$ (sat. aq.)), dried ($MgSO_4$) then concentrated in vacuo to give a yellow oil which was used with no further purification (66.2 mmol, 90%).

d) (1-Methylcyclopentyl)acetic acid methyl ester

Silver benzoate (11.2 mmol) was added to a solution of 1-methylcyclopentane diazoketone (22.4 mmol) in methanol containing triethylamine (56 mmol). Gas was seen to be evolved from the mixture which also darkened to black. After 1 hour the mixture was filtered through silica and the filtrate was concentrated in vacuo to give a dark oil. Column chromatography (silica gel, 1 – 10% EtOAc in heptane) gave the product as a clear oil (10.9 mmol, 49%).

e) (1-Methylcyclopentyl)ethanol

$LiAlH_4$ (99.3 mmol) was added portionwise to a solution of (1-methylcyclopentyl)acetic acid methyl ester (66.2 mmol) in THF at 0°C. The mixture was allowed to warm to room temperature and stirring was continued for 1.5 hours. Ether was added and the mixture cooled to 0°C. 3.8 ml of water was added followed by 3.8 ml of 15% aqueous NaOH solution then 11.4 ml of water. The mixture was warmed to room temperature and stirred for 15 minutes. Anhydrous $MgSO_4$ was added and stirring was continued for a further 15 minutes. The mixture was filtered and the filtrate was concentrated in vacuo. Distillation (76°C @ 17 mmHg) gave the product as a clear oil (40.7 mmol, 62%).

f) N-Boc-(1-methylcyclopentyl)-alanine

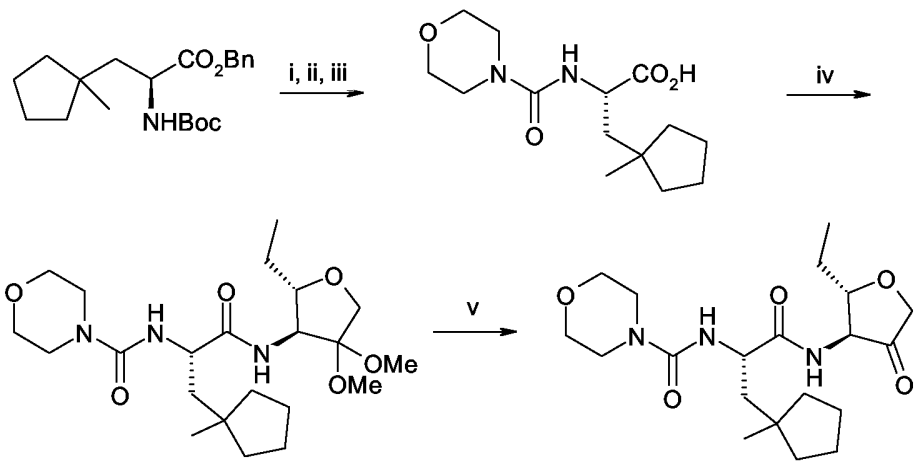
Dess–Martin periodinane (11.30 mmol) was added to a solution of (1-methylcyclopentyl)ethanol (9.48 mmol) in DCM. The mixture was stirred for 2 hours then diluted with DCM. 1:1 1M aqueous sodium thiosulfate : saturated aqueous sodium carbonate solution was added and the mixture stirred for 1 hour. The organics were separated, dried ($MgSO_4$) then concentrated in vacuo to give a yellow oil. This was dissolved in 1:1 ethanol:water then KCN (11.30 mmol) and $(NH_4)_2CO_3$ (33.20 mmol) were added. The mixture was heated at 60°C overnight. The ethanol was removed in vacuo and concentrated HCl was added to pH=1. A solid (hydantoin) was formed which was isolated by filtration then dried under vacuum (2.83 mmol, 30%). The solid was dissolved in NaOH (0.7M aqueous) and heated at reflux overnight. The mixture was concentrated to 1/3 original volume and a solution of Boc_2O (3.40 mmol) in THF was added. The mixture was stirred overnight then washed with TBME. The aqueous layer was cooled in an ice bath and 10% aqueous $KHSO_4$ was added dropwise to pH = 2. The resulting suspension was extracted with $CHCl_3$. The organics were separated, dried ($MgSO_4$) then concentrated in vacuo to give a white solid (1.48 mmol, 50% based on yield of hydantoin).

g) (R)- and (S)- N-Boc-(1-methylcyclopentyl)-alanine benzyl ester

Benzyl bromide (3.25 mmol) was added to a solution of N-Boc-(1-methylcyclopentyl) alanine (2.96 mmol) in DMF containing caesium carbonate (5.92 mmol). The mixture was heated at reflux overnight then cooled to 0°C and filtered. The filtrate was concentrated in vacuo to give a yellow gum (0.75 mmol, 25%). This was separated on a Chiralpak AD column using an isocratic eluent of 3% 2-propanol in *iso*-hexane to give the (R)- and (S)- esters as clear gums.

Example 2

2-[3-Ethyl-3-(2-methoxy-ethyl)-ureido]-N-(2-ethyl-4-oxo-tetrahydro-furan-3-yl)-3-(1-methyl-cyclopentyl)-propionamide



(i) 4M HCl, (ii) 4-morpholinecarbonyl chloride, TEA, in DCM (iii) H_2, 10% Pd/C, (iv) amino ketal, WSC.HCl, HOBt, (v) HCl, dioxane, water.

a) 3-(1-Methyl-cyclopentyl)-2-(S)-[(morpholine-4-carbonyl)-amino]-propionic acid

4M HCl in dioxane was added to (S)-N-Boc-(1-methylcyclopentyl)-alanine benzyl ester and the mixture was stirred for 30 minutes. The solvents were removed in vacuo to give a brown powder. This was dissolved in DCM and cooled to 0°C. Triethylamine then 4-morpholine carbonyl chloride were added and the mixture was stirred for 2 hours prior to being washed with 1M aqueous HCl solution. The organics were dried ($MgSO_4$) then concentrated in vacuo to give a yellow oil. This was dissolved in EtOAc and 2% AcOH (v/v) was added followed by 10% Pd/carbon (catalytic amount). The reaction was stirred under an atmosphere of hydrogen gas overnight. The mixture was filtered and the filtrate concentrated to give the crude acid which was used directly in the next step.

b) N-(S)-(2-(S)-Ethyl-4,4-dimethoxy-tetrahydro-furan-3-yl)-2-(S)-[3-ethyl-3-(2-methoxyethyl)-ureido]-3-(1-methyl-cyclopentyl)-propionamide

WSC.HCl (0.20 mmol) and HOBt (0.20 mmol) were added to a solution of 3-(1-methyl-cyclopentyl)-2-(S)-[(morpholine-4-carbonyl)-amino]-propionic acid (0.18 mmol) in DCM and stirred for 5 minutes. A solution of 1-(S)-2-(S)-ethyl-4,4-dimethoxy-tetrahydro-furan-3-ylamine (0.18 mmol, 1M in DCM) was then added and the mixture was stirred overnight. The mixture was diluted with EtOAc then washed (1M citric acid solution then saturated aqueous $NaHCO_3$), dried (Na_2SO_4) then concentrated in vacuo to give a yellow oil. This was purified by column (silica gel, 5% methanol in DCM) to give the title compound (0.095 mmol, 53%).

c) 2-[3-Ethyl-3-(2-methoxy-ethyl)-ureido]-N-(2-ethyl-4-oxo-tetrahydro-furan-3-yl)-3-(1-methyl-cyclopentyl)-propionamide

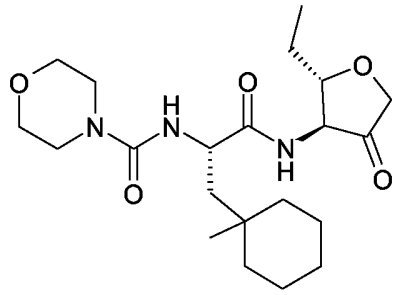
N-(S)-(2-(S)-Ethyl-4,4-dimethoxy-tetrahydro-furan-3-yl)-2-(S)-[3-ethyl-3-(2-methoxyethyl)-ureido]-3-(1-methyl-cyclopentyl)-propionamide (0.095 mmol) was dissolved in a mixture of 4M HCl in dioxane : water (1:1) and stirred for 1 hour. The solvents were removed in vacuo to give the title compound (0.03 mmol, 32%).

^{1}H NMR ($\delta CDCl_3$) 6.99 - 7.03 (m, 1H), 4.70 (d, 1H), 4.36 - 4.42 (m, 1H), 4.05 - 4.21 (m, 2H), 3.95 - 4.03 (m, 1H), 3.85 - 3.92 (m, 1H), 3.65 - 3.75 (m, 4H), 3.30 - 3.42 (m, 4H), 2.05 - 2.12 (m, 1H), 1.52 - 1.88 (m, 8H), 1.35 - 1.42 (m, 4H), 1.03 (t, 3H), 0.97 (s, 3H),

ESMS m/z 418 (MNa^+, 100%), 396 (MH^+, 45%).

Example 3

2-[3-Ethyl-3-(2-methoxy-ethyl)-ureido]-N-(2-ethyl-4-oxo-tetrahydro-furan-3-yl)-3-(1-methyl-cyclohexyl)-propionamide



Using the route outlined in Examples 1 & 2 (excepting steps i and ii in the scheme in Example 1), 1-methylcyclohexyl carboxylic acid was converted into the title compound.

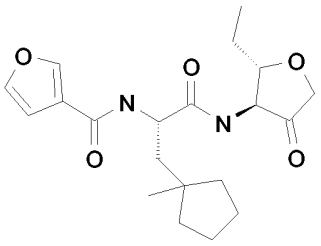
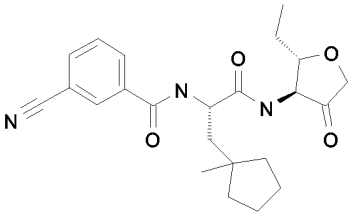
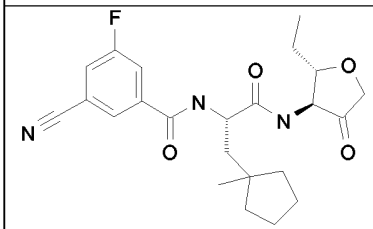
^1H NMR ($\delta CDCl_3$) 6.99 (d, 1H), 4.65 (d, 1H), 4.38 - 4.42 (m, 1H), 4.05 - 4.21 (m, 2H), 3.95 - 4.03 (m, 1H), 3.80 - 3.90 (m, 5H), 3.25 - 3.40 (m, 4H), 1.95 - 2.00 (m, 1H), 1.70 - 1.85 (m, 2H), 1.23 - 1.60 (m, 10H), 1.05 (t, 3H), 0.95 (s, 3H),

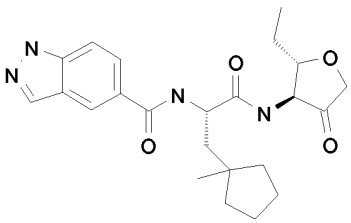
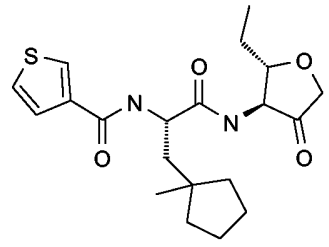
ESMS m/z 432 (MNa^+, 30%), 410 (MH^+, 100%).

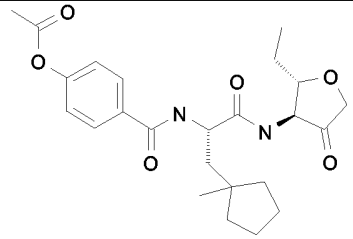
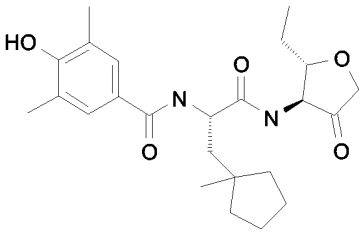
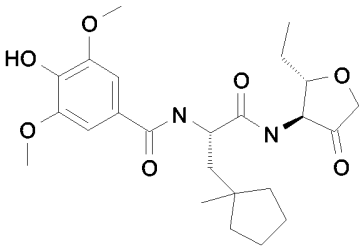
Example 4

The compounds in the table immediately below were prepared analogously to the method disclosed in Example 13.

Example 4.1 Furan-3-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide	
	HPLC retention time of 5.00 min using Synergy Max RP 80 μm 50x4.6mm column, 10→90% 6 min gradient of solution B (solution A = 0.1% TFA in water and solution B = 10% A in acetonitrile) at flow rate of 2ml/min.

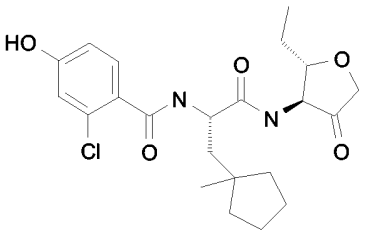
	^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.39 (s, 1H), 6.76 (br.d, *J* = 7.0, 1H), 6.53 (s, 1H), 6.04 (br.d, *J* = 8.5, 1H), 4.61-4.57 (m, 1H), 4.13 (d, *J* = 17.0, 1H), 3.99 (d, *J* = 17.0, 1H), 3.91-3.86 (m, 1H), 3.83-3.79 (m, 1H), 2.09 (dd, *J* = 4.5 and 14.5, 1H), 1.78-1.33 (m, 11H), 0.94 (t, *J* = 7.0, 3H), 0.92 (s, 3H). Electrospray mass spectroscopy using an acetonitrile / 10mM ammonium formate buffer : *m/z* 399 (40, MNa^+), 377 (100, MH^+).
Example 4.2 3-Cyano-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-benzamide	
	HPLC retention time of 5.31 min. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.96 (d, *J* = 8.0, 1H), 7.81 (d, *J* = 8.0, 1H), 7.59 (t, *J* = 8.0, 1H), 6.64-6.59 (br.m, 2H), 4.74-4.69 (m, 1H), 4.22 (d, *J* = 17.0, 1H), 4.06 (d, *J* = 17.0, 1H), 3.99-3.87 (m, 2H), 2.15 (dd, *J* = 4.5 and 14.5, 1H), 1.86-1.38 (m, 11H), 1.02 (t, *J* = 7.0, 3H), 1.01 (s, 3H). Mass spectroscopy : *m/z* 434 (30, MNa^+), 412 (100, MH^+).
Example 4.3 3-Cyano-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-5-fluoro-benzamide	
	HPLC retention time of 5.56 min. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.74-7.71 (m, 1H), 7.53-7.50 (m, 1H), 6.62 (br.d, *J* = 8.0, 1H), 6.50 (br.d, *J* = 6.0, 1H), 4.72-4.68 (m, 1H), 4.23 (d, *J* = 17.0, 1H), 4.05 (d, *J* = 17.0, 1H), 3.95-3.89 (m, 2H), 2.14 (dd, *J* = 4.5 and 14.5, 1H), 1.86-1.39 (m, 11H), 1.03 (t, *J* = 7.0, 3H), 1.01 (s, 3H).

	Mass spectroscopy : *m/z* 452 (20, MNa^+), 430 (100, MH^+).
Example 4.4 1H-Indazole-5-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide	
	HPLC retention time of 4.68 min. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 8.16 (s, 1H), 7.80 (d, *J* = 8.5, 1H), 7.52 (d, *J* = 8.5, 1H), 7.14 (br.s, 1H), 6.74 (br.s, 1H), 4.81-4.76 (m, 1H), 4.22 (d, *J* = 17.0, 1H), 4.08 (d, *J* = 17.0, 1H), 4.03-3.99 (m, 1H), 3.91-3.86 (m, 1H), 2.18 (dd, *J* = 4.5 and 14.5, 1H), 1.86-1.41 (m, 11H), 1.02 (s, 3H), 1.01 (t, *J* = 7.0, 3H). Mass spectroscopy : *m/z* 449 (25, MNa^+), 427 (75, MH^+).
Example 4.5 Thiophene-3-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide	
	HPLC retention time of 5.14 min. Mass spectroscopy : *m/z* 415 (100, MNa^+), 393 (40, MH^+).
Example 4.6 Acetic acid 4-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethylcarbamoyl]-phenyl ester	

	HPLC retention time of 5.24 min. Mass spectroscopy : *m/z* 467 (100, MNa^+), 445 (30, MH^+).
Example 4.7 *N*-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-3,5-dimethyl-benzamide	
	HPLC retention time of 5.11 min. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.41 (s, 2H), 6.97 (br.d, , *J* = 7.5, 1H), 6.35 (br.d, *J* = 8.0, 1H), 4.74-4.68 (m, 1H), 4.19 (d, *J* = 17.0, 1H), 4.16 (d, *J* = 17.0, 1H), 3.98-3.93 (m, 1H), 3.89-3.84 (m, 1H), 2.26 (s, 6H), 2.19 (dd, *J* = 4.5 and 14.5, 1H), 1.84-1.41 (m, 11H), 1.00 (t, *J* = 7.0, 3H), 0.99 (s, 3H). Mass spectroscopy: *m/z* 453 (100, MNa^+), 431 (20, MH^+).
Example 4.8 *N*-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-3,5-dimethoxy-benzamide	
	HPLC retention time of 4.79 min. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.03 (s, 2H), 6.94 (br.d, , *J* = 7.5, 1H), 6.55 (br.d, *J* = 8.0, 1H), 4.76-4.71 (m, 1H), 4.21 (d, *J* = 17.0, 1H), 4.06 (d, *J* = 17.0, 1H), 3.99-3.95 (m, 1H), 3.94 (s, 6H), 3.90-3.86 (m, 1H), 2.16 (dd, *J* = 5.0 and 14.5, 1H), 1.84-1.39 (m, 11H), 1.00 (t, *J* = 7.5, 3H), 0.99 (s, 3H). Mass spectroscopy : *m/z* 485 (40, MNa^+), 463 (100, MH^+).

Example 4.9

2-Chloro-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-benzamide

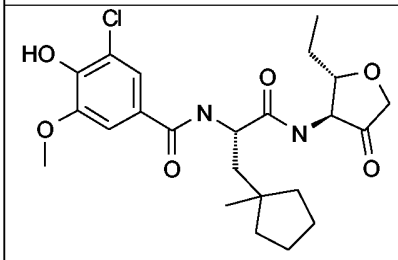


HPLC retention time of 4.94 min.

Mass spectroscopy : *m/z* 459 (60, MNa^+), 437 (100, MH^+).

Example 4.10

3-Chloro-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-5-methoxy-benzamide

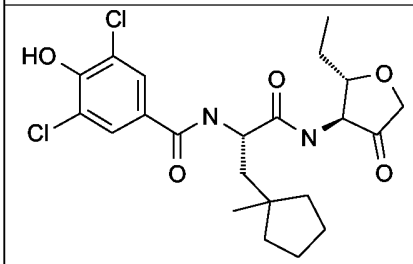


HPLC retention time of 5.21 min.

Mass spectroscopy : *m/z* 467 (100, MH^+).

Example 4.11

3,5-Dichloro-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-benzamide



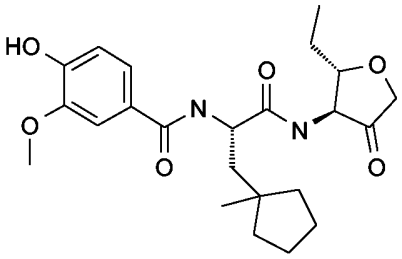
HPLC retention time of 5.43 min.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.70 (s, 2H), 6.44 (br.d, , *J* = 8.0, 1H), 4.70-4.65 (m, 1H), 4.21 (d, *J* = 17.0, 1H), 4.06 (d, *J* = 17.0, 1H), 3.98-3.87 (m, 2H), 2.15 (dd, *J* = 5.0 and 14.5, 1H), 1.87-1.37 (m, 11H), 1.02 (t, *J* = 7.5, 3H), 1.00 (s, 3H).

Mass spectroscopy : *m/z* 493 (20, MNa^+), 471 (100, MH^+).

Example 4.12

N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-3-methoxy-benzamide



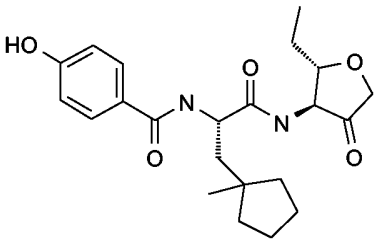
HPLC retention time of 4.79 min.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.40 (s, 1H), 7.24 (d, *J* = 8.5, 1H), 6.94 (d, *J* = 8.5, 1H), 6.88 (br.d, , *J* = 8.0, 1H), 6.39 (br.d, , *J* = 8.5, 1H), 4.73-4.68 (m, 1H), 4.20 (d, *J* = 17.0, 1H), 4.06 (d, *J* = 17.0, 1H), 3.97-3.85 (m, 2H), 3.95 (s, 3H), 2.19 (dd, *J* = 5.0 and 14.5, 1H), 1.85-1.38 (m, 11H), 1.00 (t, *J* = 7.5, 3H), 0.99 (s, 3H).

Mass spectroscopy : *m/z* 455 (20, MNa^+), 433 (100, MH^+).

Example 4.13

N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-benzamide



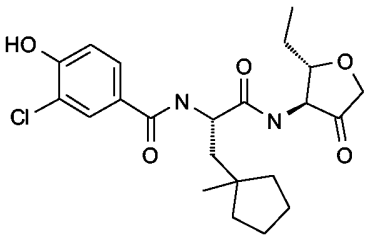
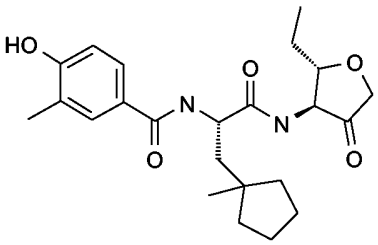
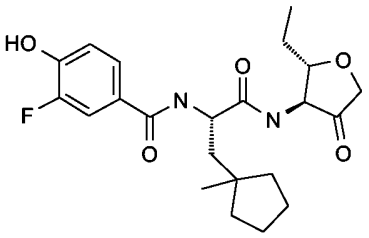
HPLC retention time of 4.70 min.

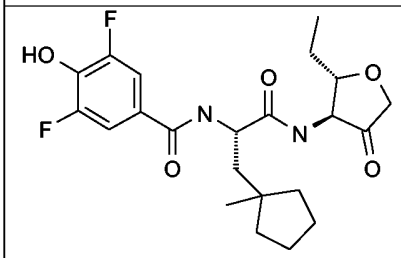
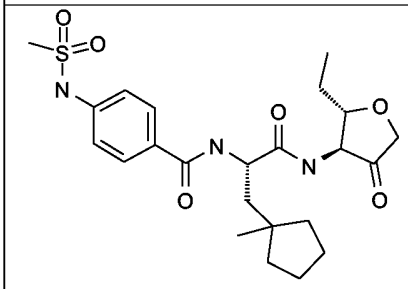
^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.56 (d, *J* = 8.5, 2H), 6.76 (d, *J* = 8.5, 2H), 6.55 (d, *J* = 8.5, 1H), 4.75-4.69 (m, 1H), 4.19 (d, *J* = 17.0, 1H), 4.08 (d, *J* = 17.0, 1H), 4.01-3.96 (m, 1H), 3.89-3.84 (m, 1H), 2.10 (dd, *J* = 4.5 and 14.5, 1H), 1.79-1.40 (m, 11H), 1.00 (s, 3H), 0.99 (t, *J* = 7.5, 3H).

Mass spectroscopy : *m/z* 425 (20, MNa^+), 403 (100, MH^+).

Example 4.14

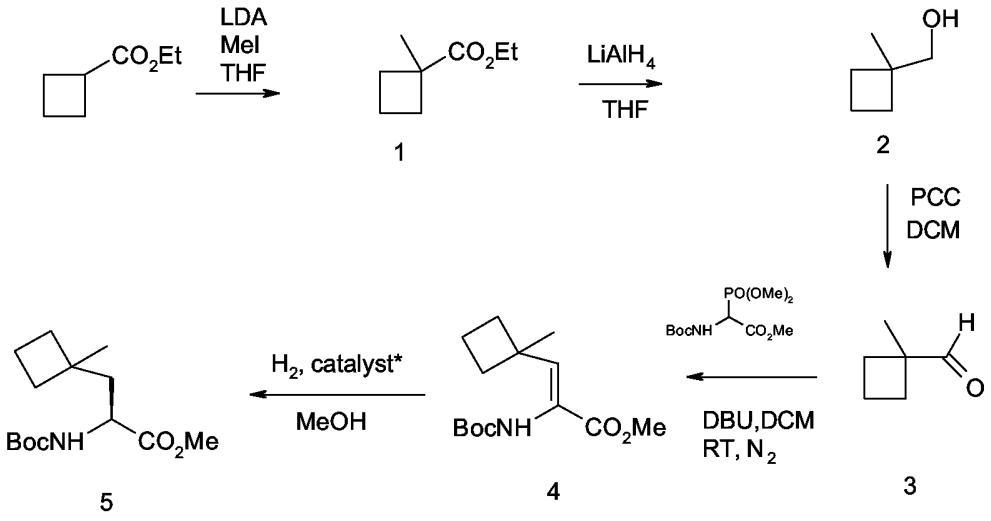
3-Chloro-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-benzamide

	HPLC retention time of 5.10 min. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.49 (d, *J* = 8.5, 1H), 6.98 (d, *J* = 8.5, 1H), 6.87 (br.s, 1H), 6.43 (br.d, *J* = 8.0, 1H), 4.66-4.60 (m, 1H), 4.13 (d, *J* = 17.0, 1H), 3.99 (d, *J* = 17.0, 1H), 3.92-3.85 (m, 1H), 3.83-3.79 (m, 1H), 2.08 (dd, *J* = 4.5 and 14.5, 1H), 1.79-1.31 (m, 11H), 0.94 (t, *J* = 7.5, 3H), 0.93 (s, 3H). Mass spectroscopy : *m/z* 437 (100, MH^+).
Example 4.15 *N*-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-3-methyl-benzamide	
	HPLC retention time of 4.94 min. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.52 (br.s, 1H), 7.45 (br.d, *J* = 9.0, 1H), 6.76 (br.d, *J* = 9.0, 1H), 6.42 (br.d, *J* = 8.0, 1H), 4.74-4.68 (m, 1H), 4.19 (d, *J* = 17.0, 1H), 4.06 (d, *J* = 17.0, 1H), 3.98-3.92 (m, 1H), 3.89-3.84 (m, 1H), 2.26 (s, 3H), 2.16 (dd, *J* = 4.0 and 10.0, 1H), 1.83-1.40 (m, 11H), 1.00 (t, *J* = 7.5, 3H), 1.00 (s, 3H). Mass spectroscopy : *m/z* 439 (20, MNa^+), 417 (100, MH^+).
Example 4.16 *N*-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-3-fluoro-4-hydroxy-benzamide	
	HPLC retention time of 4.89 min. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.51-7.49 (m, 1H), 7.41-7.38 (m, 1H), 7.00-6.96 (m, 1H), 6.48 (br.d, *J* = 8.0, 1H), 4.72-4.66 (m, 1H), 4.20 (d, *J* = 17.0, 1H), 4.07 (d, *J* = 17.0, 1H), 4.00-3.95 (m, 1H), 3.88-3.84 (m, 1H), 2.12 (dd, *J* = 4.5 and 14.5, 1H), 1.85-1.39 (m, 11H), 1.01 (t, *J* = 7.5, 3H), 1.00 (s, 3H). Mass spectroscopy : *m/z* 443 (40, MNa^+), 421 (100,

	MH^+).
Example 4.17 *N*-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-3,5-difluoro-4-hydroxy-benzamide	
	HPLC retention time of 5.02 min. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.35-7.32 (m, 2H), 6.71 (br.s, 1H), 6.40 (br.s, 1H), 4.69-4.64 (m, 1H), 4.22 (d, *J* = 17.0, 1H), 4.06 (d, *J* = 17.0, 1H), 3.96-3.87 (m, 2H), 2.14 (dd, *J* = 4.5 and 14.5, 1H), 1.85-1.40 (m, 11H), 1.02 (t, *J* = 7.5, 3H), 1.00 (s, 3H). Mass spectroscopy : *m/z* 461 (50, MNa^+), 439 (100, MH^+).
Example 4.18 *N*-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-methanesulfonylamino-benzamide	
	HPLC retention time of 4.77 min. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.97 (br.s, 1H), 7.68 (d, *J* = 8.5, 2H), 7.41 (br.s, 1H), 7.18 (d, *J* = 8.5, 2H), 7.02 (br.s, 1H), 4.79-4.74 (m, 1H), 4.18 (d, *J* = 17.0, 1H), 4.08 (d, *J* = 17.0, 1H), 4.07-4.01 (m, 1H), 3.82 (t, *J* = 8.5, 1H), 3.08 (s, 3H), 2.05 (dd, *J* = 4.5 and 14.5, 1H), 1.86-1.40 (m, 11H), 1.00 (s, 3H), 0.99 (t, *J* = 7.5, 3H). Mass spectroscopy : *m/z* 480 (100, MH^+).

Example 5

<u>Preparation of 1-methylcyclobutylalanine building block</u>



*(+)-1,2-bis(2S,5S)-diethylphosphonolanbenzene (cyclooctadiene)rhodium (I)triflate

1-Methyl-cyclobutanecarboxylic acid ethyl ester 1 was prepared from ethyl cyclobutanecarboxylate by the method described in J. Am. Chem. Soc., Vol. 103 No.2 1981 436-442.

1-Methyl-cyclobutanecarboxylic acid ethyl ester 1 (1eq) was stirred under a nitrogen atmosphere at 0°C in anhydrous THF. To this solution was added portionwise lithium aluminium hydride (1.5eq) and the suspension was stirred at room temperature for 3 hours. The reaction mixture was cooled on ice, treated with 1M HCl (aq) and stirred at 0°C 20 minutes. The solution was passed through a pad of celite and the filtrate extracted into diethyl ether. The organic phases were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give (1-methyl-cyclobutyl)-methanol, 2.

Pyridinium chlorochromate (1.25eq) and the same weight of celite were taken up as a suspension in anhydrous dichloromethane. To this was added dropwise a solution of compound 2 (1eq) in anhydrous dichloromethane and the resulting heterogeneous mixture was stirred at room temperature for 3 hours. The reaction mixture was passed through a pad of silica, eluting with 19:1 isohexanes: ethyl acetate to give 1-methylcyclobutanecarboxaldehyde, 3.

Compound 3 (1eq) was dissolved with stirring in anhydrous dichloromethane, and to this was added Boc-phosphoglycine trimethyl ester (0.5eq) and 1,8-diazabicyclo[5.4.0]undec-7-ene (1.2eq). The resulting solution was stirred at ambient temperature under nitrogen overnight. The reaction mixture was partitioned between dichloromethane and successively 1M HCl (aq), sat. $NaHCO_3$ (aq) and sat. NaCl (aq). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting oil was purified by flash column chromatography, eluting

with 1%methanol in dichloromethane to give 2-tert-butoxycarbonylamino-3-(1-methyl-cyclobutyl)-acrylic acid methyl ester, 4.

Compound 4 was dissolved in anhydrous methanol and degassed with nitrogen. (+)-1,2-bis (2S,5S)-diethylphosphonolanbenzene (cyclooctadiene)rhodium (I) triflate was added and degassing was continued for a further 10 minutes. The reaction was shaken under a hydrogen atmosphere (4 bar) for 48 hours. The solution was concentrated. *in vacuo* and purified by flash chromatography, eluting with dichloromethane, to give 2S-tert-butoxycarbonylamino-3-(1-methyl-cyclobutyl)-propionic acid methyl ester, 5.

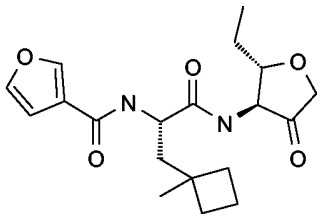
HPLC retention time 5.88min (monitored at 215 and 254 nm)
HPLC using Synergy Max RP 80 μm 50x4.6mm column, 10→90% 6 min gradient of solution B (solution A = 0.1% TFA in water and solution B = 10% A in acetonitrile) at flow rate of 2ml/min.

MS $[M+H]^+$ 272.08 (20%) $[M\text{-}Boc+H]^+$ 172.06 (100%)
Electrospray ionisation, eluting with acetonitrile / ammonium formate buffer.

^1H NMR (400 MHz, $CDCl_3$) δ 4.89-4.79 (1H, m) 4.33-4.27 (1H, m) 3.71 (3H, s) 1.98-1.62 (8H, m) 1.42 (9H, s) 1.22 (3H, s)

Example 6

Furan-3-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydrofuran-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide

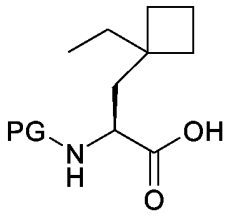


The title compound was prepared analogously to example 2 employing the P2 building block of Example 5.

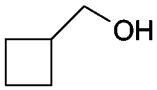
MS $[M+H]^+$ 363.02 (70%) $[M+Na]^+$ 385.03 (100%)
Electrospray ionisation, eluting with acetonitrile / ammonium formate buffer.

Example 7

Preparation of 1-ethylcyclobutylalanine P2 Building Block



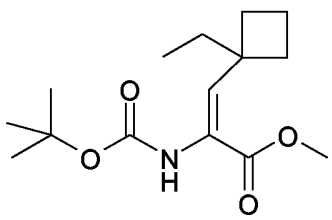
1-Hydroxymethyl-1-ethyl cyclobutane



^{n}-Butyl lithium (1.6 M (hexanes), 100 mmol, 62.5 ml, 2 eq.) was added dropwise to a solution of $^{i}Pr_2NH$ (100 mmol, 14.13 ml, 2 eq.) in THF (100 ml) at 0°C. This was stirred for 15 minutes then cyclobutane carboxylic acid (50 mmol, 4.78 ml, 1 eq.) was added dropwise at 0°C. The solution was stirred for 1.5 hours. EtI (50 mmol, 4.08 ml, 1 eq.) was added dropwise and the solution was stirred overnight, allowing it to come to room temperature. HCl (2M, 150 ml) was added and the resulting mixture was extracted with EtOAc. The organics were dried ($MgSO_4$) then concentrated *in vacuo* to give the crude product as a semi-solid (6.98g). This was dissolved in THF (70 ml), cooled in an ice-bath and $LiAlH_4$ (75 mmol, 2.84g) was added portionwise. This was stirred at room temperature for 3 hours then cooled in an ice-bath and diluted with diethyl ether. Water (2.84 ml), 10% NaOH(aq) (2.84 ml) and further water (8.5 ml) were added sequentially. The mixture was stirred for 15 minutes then $MgSO_4$ was added. After a further 30 minutes stirring, the mixture was filtered and the organics evaporated to give a clear oil. Distillation (68 – 70C at 10 mmHg) gave the product as a clear oil (4.24 g, 74%).

^{1}H NMR (400 MHz) (δ, $CDCl_3$) 3.50 (s, 2H), 1.65 – 1.85 (m, 6H), 1.49 (q, *J* 7.0, 2H), 0.82 (t, *J* 7.0, 3H).

2-tert-Butoxycarbonylamino-3-(1-ethyl-cyclobutyl)-acrylic acid methyl ester

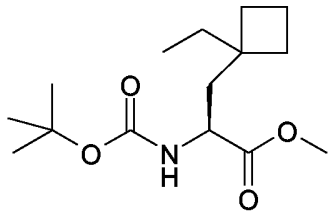


A solution of DMSO (15 mmol, 0.57g, 3 eq.)) in DCM (2.5 ml) was added dropwise to a solution of oxalyl chloride (7.5 mmol, 0.65 ml, 1.5 eq.) in DCM (10 ml) keeping the temperature at less than -70°C. 1-Hydroxymethyl-1-ethyl cyclobutane (5 mmol, 0.57 g) in DCM (2.5 ml) was added after 10 minutes again keeping the temperature less than -70°C. The mixture was then warmed to -50°C for 30 minutes and then re-cooled to -78°C. Triethylamine (26.5 mmol, 3.69 ml, 5.3 eq) was then added (keeping the temperature less than -70°C) and the mixture was then stirred for 1 hour during which time it was allowed to warm to 0°C. The mixture was quenched with NH_4Cl solution : water (1:1). The organics were isolated, dried ($MgSO_4$) then concentrated to give the crude aldehyde. This was dissolved in DCM (2.5 ml) and added dropwise to a solution of phosphonate (5.5 mmol, 1.63 g, 1.1 eq.) in DCM (2.5 ml) to which DBU (11.00 mmol, 1.64 ml, 2.2 eq.) had been added at 0°C and had been stirred for 10 minutes. The mixture was stirred overnight at room temperature then washed (cold 1M HCl solution then $NaHCO_3$ solution), dried (Na_2SO_4) and concentrated *in vacuo* to give a yellow solid. The was purified by silica column (isohexane → 10% EtOAc in ihexane) to give the product as a white solid (0.42g, 30%).

^{1}H NMR (400 MHz) (δ, $CDCl_3$) 6.44 (s, 1H), 3.76 (s, 3H), 2.14 – 2.24 (m, 2H), 1.90 – 1.98 (m, 3H), 1.78 – 1.83 (m, 1H), 1.73 (q, *J* 7.5 2H), 1.45 (s, 9H), 0.86 (t, *J* 7.5, 3H)

ESMS m/z(%) 306 (MNa^+, 100), 284 (MH^+, 65), 184 (MH-Boc$^+$, 80).

N-*tert*Butoxycarbonyl-(ethyl)cyclobutyl alanine methyl ester

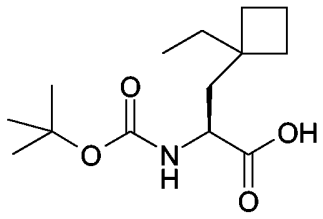


(S, S)-Et-DuPhos (0.0148 mmol, 10.69 mg, 1 mol%) was added to a solution of 2-tert-Butoxycarbonylamino-3-(1-ethyl-cyclobutyl)-acrylic acid methyl ester in methanol (10 ml). The solution was agitated under 4 atm. pressure of hydrogen for 72 hours. The solvents were then evaporated and the residue passed through silica (eluting with DCM) to give the product as a clear gum (0.35g, 82%).

^{1}H NMR (400 MHz) (δ, $CDCl_3$) 4.84 (br d, 1H), 4.18 – 4.30 (m, 1H), 3.71 (s, 3H), 1.58 – 1.92(m, 10H), 1.43 (s, 9H), 0.84 (t, *J* 7.5, 3H),

CIMS m/z(%) 306 (MNa^+, 100), 286 (MH^+, 80).

N-*tert*Butoxycarbonyl-(ethyl)cyclobutyl alanine



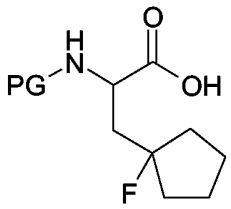
IMLiOH (aq) (1.81 mmol, 1.81 ml, 1.5 eq.) was added to a solution of N-*tert*Butoxycarbonyl-(ethyl)cyclobutyl alanine methyl ester (1.21 mmol, 0.35g) in THF (10 ml) at 0°C. This was stirred overnight then diluted with water and extracted with EtOAc. The organics were dried ($MgSO_4$) and concentrated to give the product as clear oil (0.32g, 97%).

^{1}H NMR (400 MHz) (δ, $CDCl_3$) 4.84 (br d, 1H), 4.22 – 4.30 (m, 1H), 1.54 – 2.02 (m, 10H), 1.44 (s, 9H), 0.84 (t, *J* 7.5, 3H)

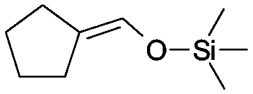
Although this example has been illustrated with a Boc protecting group it will be apparent that other conventional N-protecting groups such as those described above in Greene, including Fmoc, CBz etc will be amenable to this route and/or the Boc group can be removed and replced with an alternative N-protecting groups using conventional protecting group manipulations.

Example 8

Preparation of 1-fluorocyclobutylalanine P2 Building Block



Cyclopentylidenemethoxy-trimethyl-silane

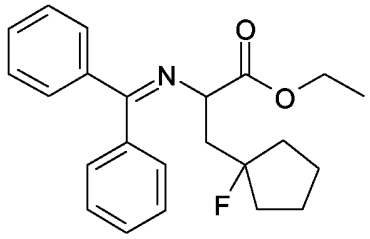


A solution of cyclopentane carboxaldehyde (129.5 mmol, 13.18g) in DCM (100 ml) was added to a solution of trimethylsilyl trifluoromethanesulfonate (155.4 mmol, 28.09 ml, 1.2 eq.) and

diisopropylethylamine (155.4 mmol, 27.54 ml, 1.2 eq.) in DCM (700 ml) at 0°C. The mixture was stirred for 1.5 hours then concentrated *in vacuo*. The residue was suspended in isohexane then filtered. The organics were washed (water), dried (Na_2SO_4) then concentrated *in vacuo* to give a pale brown oil. This was distilled (53 – 54 °C at 9 mmHg) to give a clear oil (13.21g, 60%).

^{1}H NMR (400 MHz) (δ, $CDCl_3$) 6.17 (m, 1H), 2.21 – 2.26 (m, 2H), 2.13 – 2.18 (m, 2H), 1.55 – 1.67 (m, 4H), 0.17 (s, 9H).

2-(Benzhydrylidene-amino)-3-(1-fluoro-cyclopentyl)-propionic acid ethyl ester



Selectfluor (19.36 mmol, 6.85g, 1.1 eq.) was added to a solution of cyclopentylidenemethoxy-trimethyl-silane (17.6 mmol, 3.00g) in DMF (50 ml) at 0°C. The was allowed to warm to room temperature and stirred for 1.5 hours. The mixture was diluted with water then extracted with isohexane. The organics were dried ($MgSO_4$) and carefully concentrated to give a pale yellow oil. This was dissolved in THF (20 ml) and a solution of $LiBH_4$ (2M (THF), 21.12 mmol, 10.56 ml, 1.2 eq.) was added dropwise at 0°C. The mixture was stirred for 30 minutes. Water was added and the mixture stirred for a further 10 minutes. The mixture was extracted with diethyl ether. The organics were dried ($MgSO_4$) and the solvent removed by distillation (36°C at 750 mmHg) to give a yellow oil (17.6 mmol, 2.08g). This was dissolved in DCM (10 ml) and triethylamine (19.4 mmol, 2.70 ml, 1.1 eq.) was added. The solution was cooled to -20°C and trifluoromethanesulfonic anhydride (19.4 mmol, 3.21 ml, 1.1 eq.) was added dropwise over 5 minutes. The solution was warmed to 0°C and stirred for 1 hour then poured onto ice. The organics were washed (cold 1M HCl solution then 10% Na_2CO_3), dried ($MgSO_4$) was concentrated by distillation (38°C at 750 mmHg) to give a black solution which was shown to be the triflate by ^{1}H and ^{19}F NMR. This was added to a solution in which potassium *tert*butoxide (1M (THF), 19.4 mmol, 19.4 ml) had been added to glycine imine ethyl ester (17.6 mmol, 4.70g, 1 eq.) dissolved in DMF (25 ml) and stirred for 20 minutes at 0°C. The resulting mixture was stirred at room temperature for 5 hours at room temperature then poured into a diethyl ether : saturated ammonium chloride solution mixture. The organics were washed (water then brine), dried ($MgSO_4$) and concentrated *in vacuo* to give a brown oil. This was purified by silica

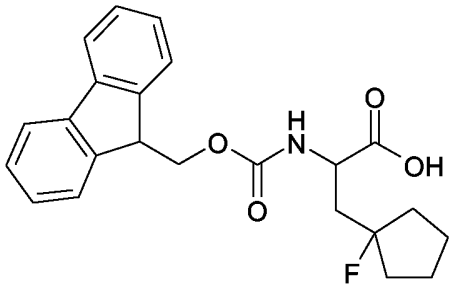
chromatography (1% EtOAc in isohexane → 33% EtOAc in isohexane) to give a yellow solid (0.61g, 9% (4 steps)).

^{1}H NMR (400 MHz) (δ, $CDCl_3$) 7.14 – 7.69 (m, 10H), 4.36 – 4.41 (m, 1H), 4.13 – 4.21 (m, 2H), 2.58 – 2.68 (m, 1H), 2.21 – 2.34 (m, 1H), 1.45 – 2.00 (m, 8H), 1.22 – 1.30 (m, 3H).

^{19}F NMR (376 MHz) (δ, $CDCl_3$) -144.19 – -144.67 (m)

CIMS m/z (%) 368 (MH^+, 100).

N-(Fluorenylmethoxycarbonyl)-(fluoro)cyclopentyl alanine



2MNaOH (aq), 3.30 mmol, 1.65 ml, 2 eq.) was added to a solution of 2-(benzhydrylidene-amino)-3-(1-fluoro-cyclopentyl)-propionic acid ethyl ester (1.65 mmol, 0.608g) in dioxane at 0°C. The mixture was allowed to warm to room temperature then stirred overnight. 1M HCl (aq) was then added to pH 0.5 and the mixture was stirred for 6 hours then extracted with diethyl ether. The aqueous phase was neutralised with 1M NaOH(aq) solution. Dioxane (5 ml) then 10%Na_2CO_3 (aq), 4.12 mmol, 4.25 ml, 2.5 eq.) were added and the mixture was cooled in an ice bath. Fluorenylmethylchloroformate (1.65 mmol, 0.43g, 1 eq.) was then added portionwise with stirring over 30 minutes. The ice bath was removed and the mixture stirred overnight and was acidifed to pH 3 with 1M HCl(aq). The aqueous layer was evaporated and the residual solid washed with EtOAc. The organics were concentrated *in vacuo* to give the product as a cream solid (0.54g, 81%).

^{1}H NMR (400 MHz) (δ, $CDCl_3$) 7.25 – 7.81 (m, 8H), 4.31 – 4.42 (m, 2H), 4.19 – 4.24 (m, 1H), 2.32 -2.43 (m, 1H), 1.54 – 2.12 (m, 9H).

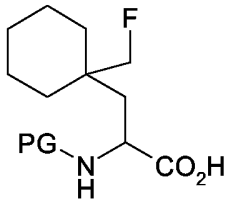
^{19}F NMR (376 MHz) (δ, $CDCl_3$) -145.25 – -145.74 (m)

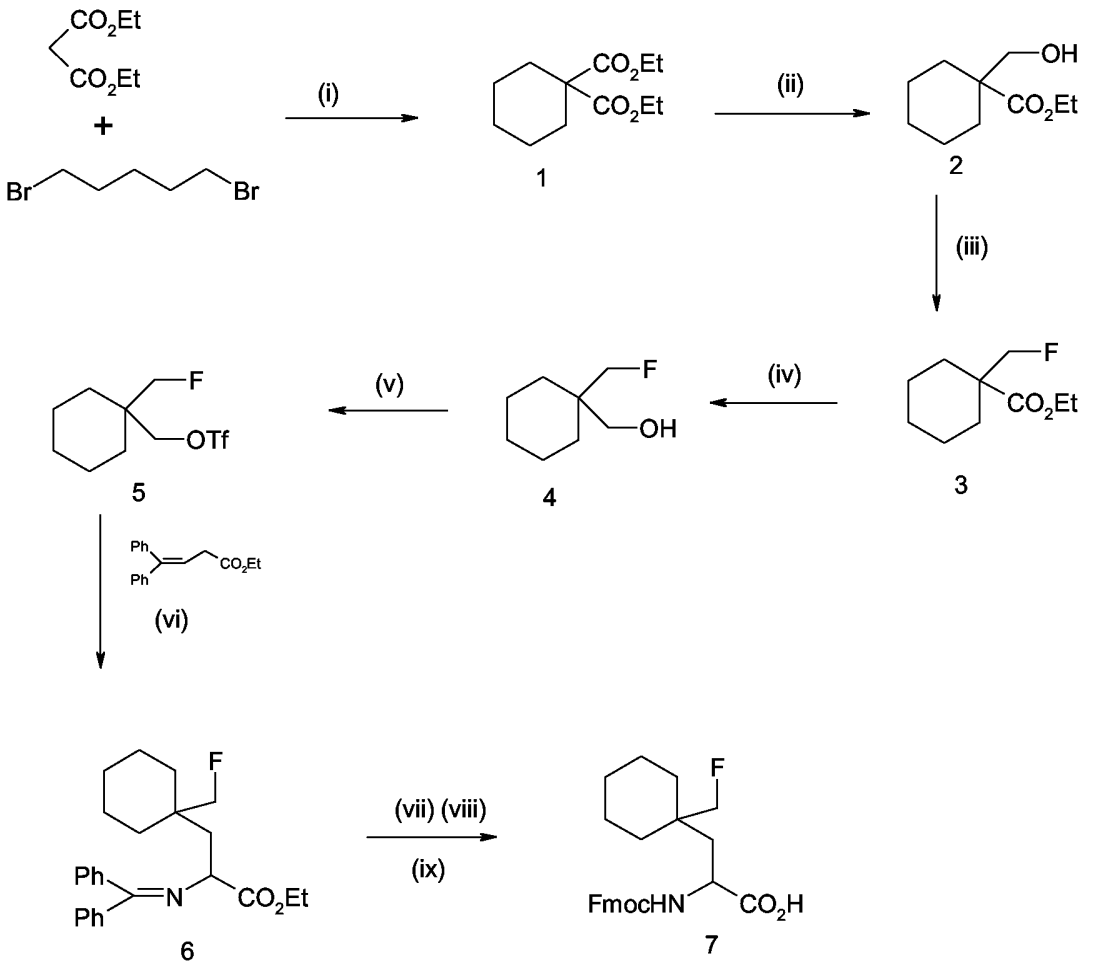
CIMS m/z 378 (MH^+, 45%)

Although this example has been illustrated with an Fmoc protecting group it will be apparent that other conventional N-protecting groups such as those described above in Greene, including Boc, CBz etc, will be amenable to this route and/or the Fmoc group can be removed and replaced with another N-protecting group using conventional protecting group manipulations. As with other non-natural amino acids, the L and D diastereomers are separated by conventional chiral HPLC, or as described in Advanced Organic Chemistry: 3rd Edition: author J March, pp 104-107 including for example the formation of diastereomeric derivatives having convenient optically active auxiliary species followed by separation and then cleavage of the auxiliary species.

Example 9

Preparation of 1-fluoromethylcyclohexylalanine P2 building block





(i) NaOEt, EtOH, 80°C, (ii) $LiAl(O^tBu)_3H$, THF, reflux, (iii) Deoxofluor, (iv) $LiAlH_4$, THF 0°C, (v) Tf_2O, Et_3N, DCM (vi) KO^tBu, DMF, (vii) NaOH (aq, 1,4-dioxan, (viii) HCl(aq), (ix) FmocCl Na_2CO_3, 1,4-dioxan

Cyclohexane-1,1-dicarboxylic acid diethyl ester,1, was prepared in accordance with JACS 43, 1921, 1368 from diethyl malonate and 1,5-dibromopentane.

Cyclohexane-1,1-dicarboxylic acid diethyl ester, 1, was taken up in anhydrous THF under nitrogen at room temperature. This was treated with $LiAl(O^tBu)_3H$ (2.5eq) portionwise before refluxing overnight. The reaction mixture was cooled in an ice-bath and treated carefully with 10% $KHSO_4$(aq) and allowed to stir for 10 minutes. The resulting precipitate was removed by vacuum filtration and the mother liquors were partitioned between EtOAc and brine. The organic phases were combined, dried over MgSO4, filtered and concentrated in vacuo to give a mobile oil. This was purified by flash column chromatography to give 1-hydroxymethyl-cyclohexanecarboxylic acid ethyl ester, 2 as a colourless oil (51%).

1-Hydroxymethyl-cyclohexanecarboxylic acid ethyl ester, 2 was taken up in [bis (2-methoxyethyl) amino] sulphur trifluoride and heated overnight at 70°C. The reaction was then allowed to cool to 0°C and carefully treated with saturated $NaHCO_3$ (aq) dropwise. This was stirred at room temperature for 30 minutes. The mixture was washed twice with DCM and the combined organics were washed with saturated $NaHCO_3$ (aq) and brine, dried over MgSO4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography to give 1-fluoromethyl-cyclohexane carboxylic acid ethyl ester, 3, as a colourless oil (34%)

1-Fluoromethyl-cyclohexane carboxylic acid ethyl ester, 3, was dissolved in anhydrous THF and cooled under a nitrogen atmosphere to 0°C. This was treated portionwise with $LiAlH_4$ (2eq) and warmed to room temperature for 4 hours. After this time the reaction was cooled to 0°C and carefully treated with 2M HCl(aq) and stirred for 20 minutes. The reaction was filtered through a pad of celite and the pad was washed with diethyl ether. The collected solution was partitioned between brine and diethyl ether. The combined organic phases were dried over MgSO4, filtered and concentrated in vacuo to give an oil. This was purified by flash chromatography to give (1-fluoromethyl-cyclohexyl) methanol, 4, as a colourless oil (67%).

(1-Fluoromethyl-cyclohexyl) methanol, 4, was dissolved in anhydrous DCM under N_2 and cooled to -20°C. NEt_3 (1.1eq) was added and the reaction was stirred for 5 minutes. This was then treated dropwise with triflic anhydride (1.1eq) and the solution was stirred at 0°C for 1hour. The reaction mixture was poured onto ice and the organics were washed with 1M HCl (aq), saturated $NaHCO_3$ (aq) and brine, then dried over MgSO4, filtered and concentrated in vacuo, to give trifluoro-methanesulfonic acid-1-fluoromethyl-cyclohexylmethyl ester, 5, as an amber oil which was used immediately without further purification in the next step.

N-(Diphenylmethylene)glycine ethyl ester was dissolved in DMF and under a nitrogen atmosphere was cooled to 0°C. This was treated with KO^tBu (1.1eq) and stirred for 20 minutes. To this solution was added trifluoro-methanesulfonic acid-1-fluoromethyl-cyclohexylmethyl ester, 5 dropwise. The reaction mixture was stirred at room temperature under nitrogen overnight then poured into a 1:1 mixture of diethyl ether : NH_4Cl (aq). The phases were separated and the aqueous phase was washed twice with diethyl ether. The organic phases were combined and washed several times with brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The resulting residue was purified by flash column chromatography to give 2-(benzhydrylidene-amino)-3-(1fluoromethyl-cyclohexyl) propionic acid ethyl ester, 6 (28%).

2- (Benzhydrylidene-amino)-3-(1-fluoromethyl-cyclohexyl)propionic acid ethyl ester, 6, was taken up in 1,4-dioxan and treated with 2 M NaOH (aq) (2eq) with stirring. After the starting

material had been consumed (tlc), the reaction mixture was acidified by addition of 2M HCl (aq) and stirred overnight at room temperature. The solution was concentrated in vacuo and the residue was partitioned between TBME and water. The pH of the aqueous phase was adjusted to pH7 by careful addition of 2M NaOH (aq) prior to lyophilisation. The resulting residue was then suspended in 10% Na_2CO_3 (aq) and dioxan until a homogeneous solution was obtained. Fmoc chloride was added portion wise to the ice-cooled solution over 12 hrs and this was allowed to stir at room temperature overnight. The reaction mixture was washed with TBME and the resulting aqueous was acidified with 2M HCl (aq) and allowed to freeze dry. The resulting solid was triturated with methanol and the mother liquors were collected by filtration and concentrated in vacuo. To remove last traces of salts the resulting solids were partitioned between water and ethyl acetate. The organics were dried over MgSO4, filtered and concentrated in vacuo to give 2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-(10fluoromethyl-cyclohexyl)-propionic acid ethyl ester, compound 7.

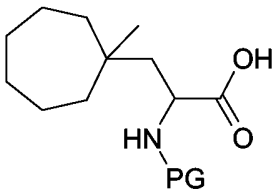
HPLC retention time 6.00min (monitored at 215 and 254 nm)
HPLC using Synergy Max RP 80 μm 50x4.6mm column, 10→90% 6 min gradient of solution B (solution A = 0.1% TFA in water and solution B = 10% A in acetonitrile) at flow rate of 2ml/min.

MS $[M+H]^+$ 426.26 $[M=Na]^+$ 449.25
Electrospray ionisation, eluting with acetonitrile / ammonium formate buffer.

Although this example has been illustrated with a Boc protecting group it will be apparent that other conventional N-protecting groups such as those described above in Greene, such as Fmoc, CBz etc will be amenable to this route and/or the Boc group can be removed and replaced with a conventional N-protecting group using conventional protecting group manipulations. The diastereomers are isolated by conventional amino acid chiral HPLC.

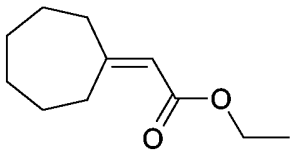
Example 10

Preparation of 1-methylcycloheptylalanine P2 Building Block



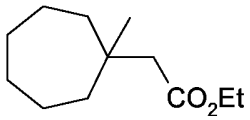
Scheme 4 above provides an effective route to this building block as follows:

Step a)



Potassium *tert*-butoxide (10.5 g, 0.09 mol) was suspended in anhydrous THF (200 ml) and the solution was cooled in an ice-bath. Ethyl phosphonoacetate (17.6 ml, 0.09 mol) was dissolved in anhydrous THF (30 ml) and added dropwise to the cooled solution. After the addition was complete, cycloheptanone (10 g, 0.09 mol) was dissolved in anhydrous THF (40 ml) and added dropwise to the ylide. The reaction was allowed to reach room temperature and stirring was continued overnight. The reaction was concentrated *in vacuo* and the residue was taken up in diethyl ether and extracted with water and saturated brine. The organic fraction was then dried (Na_2SO_4), filtered and concentrated. Purification on silica gel eluting with heptane only gave the product as a clear oil (9 g, 55 %).

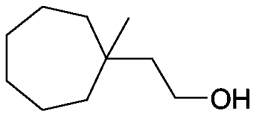
Step b.



As reference: *Tetrahedron Lett.* 2003, **44**, 4265.

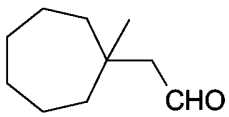
Copper (I) iodide (2.090 g, 10.98 mmol) was suspended in dry diethyl ether (5 ml) and stirred at 0 ^{0}C under argon. A 1.4M solution of methyl lithium in diethyl ether (15.7ml, 21.96 mmol) was slowly added until the initial yellow colour turned white. The reaction was stirred for a further 10 min before the solvent was thoroughly removed in vacuo at 0°C. Ice-cooled dry dichloromethane (30 ml) was added to the residue under argon and this solution was then further cooled to -78°C. Ice-cooled trimethylsilyl chloride (1.38 ml, 10.98 mmol) and cycloheptylidene-acetic acid ethyl ester (1 g, 5.49 mmol) were added. The solution was stirred at -78°C for a further 3 hrs and allowed to warm-up to 0°C prior to being quenched with saturated ammonium chloride solution and ammonium hydroxide (1:1). The organic layer was separated, dried (Na_2SO_4) and the solvent was removed in *vacuo* to afford the product (856 mg, 79 %), without need for further purification.

Step c.



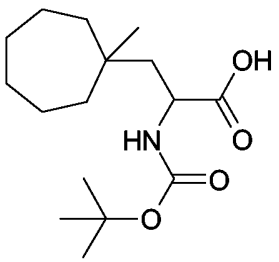
(1-Methyl-cycloheptyl)-acetic acid ethyl ester (500 mg, 2.53 mmol) was slowly added to a stirring 1M solution of lithium aluminium hydride in tetrahydrofuran (5.1 ml) at room temperature. The solution was heated to reflux for 2hrs until complete by TLC. 1M NaOH (aq) was then added dropwise to the ice-cooled solution, until the salts precipitated. The solution was filtered through celite and extracted with ethyl acetate. The combined organics were dried ($MgSO_4$) and the solvent was removed in *vacuo* to afford the product (352 mg, 89 %), without need for further purification.

Step d.



2-(1-Methyl-cycloheptyl)-ethanol (352 mg, 2.26 mmol) was dissolved in dry dichloromethane and stirred at room temperature while Dess-Martin periodinane (959 mg, 2.26 mmol) was added. The solution was stirred for a further 4 hrs when TLC indicated the reaction had gone to completion. Then 10 % sodium thiosulphate solution and saturated sodium bicarbonate solution (1:1) were added and the solution was stirred at room temperature for 15 mins. After filtration, the organic layer was separated, dried ($MgSO_4$) and the solvent was removed in *vacuo* to afford the product (340 mg, 98 %). This material was used immediately in the next step.

Step e.



Freshly prepared (1-methyl-cycloheptyl)-acetaldehyde (1.980 g, 12.86 mmol) was dissolved in ethanol (25 ml) and water (25 ml) and stirred at room temperature while potassium cyanide (921 mg, 14.14 mmol) and ammonium carbonate (3.331 g, 34.67 mmol) were added. The solution

was then heated to 60°C for 24 hrs. The next day, the ethanol was removed in *vacuo* to aid the precipitation of the hydantoin intermediate, 5-(1-methyl-cycloheptylmethyl)-imidazolidine-2,4-dione, which was subsequently removed by filtration and dried in *vacuo*, (1.919 g, 67 %).

^{1}H-NMR (400 MHz, DMSO) δ 10.60 (br.s, 1H), 7.80 (br.s, 1H), 4.00-3.90 (m, 1H), 1.80-1.20 (m, 14H), 0.90 (s, 3H).

5-(1-Methyl-cycloheptylmethyl)-imidazolidine-2,4-dione (1.919 g, 8.57 mmol) was dissolved in 0.7 M sodium hydroxide solution (40 ml) and stirred at 100°C overnight. The following day, the solution was cooled to room temperature and the volume was reduced in *vacuo* by half. The di-*tert*-butyl dicarbonate (2.054 g, 9.42 mmol) in tetrahydrofuran (25 ml) was added and the solution was stirred at room temperature for 48 hrs. The solution was then acidified to pH 3 using 1 M potassium hydrogen sulphate solution and extracted with ethyl acetate. The combined organics were dried ($MgSO_4$) and the solvent was removed in *vacuo*. Purification by column chromatography (isohexane: ethyl acetate; 1:1) afforded the product.

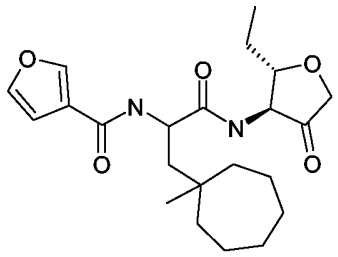
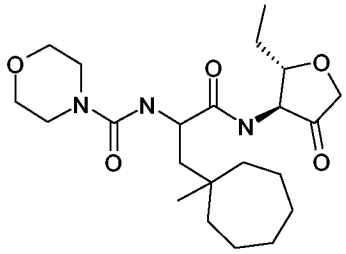
Although the example has been illustrated with a Boc protecting group it will be apparent that other conventional N protecting groups such as those described above in Greene will be amenable to this route and/or the Boc group can be removed and replaced by another conventional N-protecting group such as Fmoc or CBz using conventional protecting group manipulation.

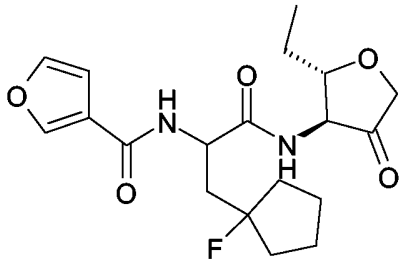
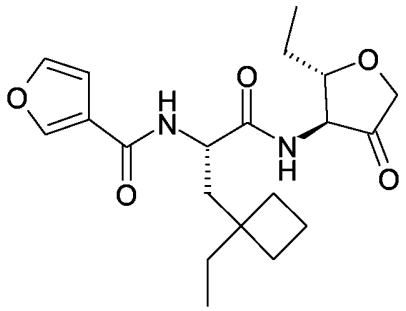
Example 11

Solution phase preparation of inhibitors

The compounds in the table immediately below were prepared analogously to the method disclosed in Example 13 using the appropriate commercially available P3 acid, and the respective P2 building block of Example 6, 7 or 10:

Example 11.1 Furan-3-carboxylic acid [(1*R*,*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cycloheptyl)-ethyl]-amide

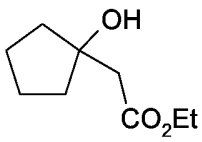
	Data is given for a mixture of diastereoisomers (1:1 ratio). HPLC retention time of 5.67 min. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.18 (br.d, *J* = 6.0, 0.5H), 7.98-7.96 (m, 0.5H), 7.92-7.91 (m, 0.5H), 7.71 (br.d, *J* = 8.0, 0.5H), 7.61 (br.d, *J* = 7.5, 0.5H), 7.40 (app.t, *J* = 1.5, 0.5H), 7.38 (app.t, *J* = 1.5, 0.5H), 7.30 (br.d, *J* = 8.0, 0.5H), 6.69-6.67 (m, 0.5H), 6.65-6.63 (m, 0.5H), 4.80-4.70 (m, 1H), 4.20-3.80 (m, 4H), 1.90-1.64 (m, 4H), 1.50-1.25 (m, 12H), 1.02 (t, *J* = 7.0, 1.5H), 0.97 (t, *J* = 7.0, 1.5H), 0.91 (s, 1.5H), 0.89 (s, 1.5H). Mass spectroscopy: *m/z* 810 (15, $2MH^+$), 427 (20, MNa^+), 405 (100, MH^+).
Example 11.2 Morpholine-4-carboxylic acid [(1*R*,*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cycloheptyl)-ethyl]-amide	
	Data is given for a mixture of diastereoisomers (1:1 ratio). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.15 (br.d, *J* = 6.0, 1H), 4.73 (br.d, *J* = 8.0, 1H), 4.36-4.26 (m, 1H), 4.16-3.80 (m, 4H), 3.66-3.58 (m, 4H), 3.35-3.22 (m, 4H), 1.93-1.65 (m, 4H), 1.50-1.28 (m, 12H), 0.96 (t, *J* = 7.0, 3H), 0.84 (s, 3H). Mass spectroscopy: *m/z* 424 (100, MH^+).
Example 11.3 Furan-3-carboxylic acid [1-(R,S)-(2-(S)-ethyl-4-oxo-tetrahydro-furan-3-(S)-ylcarbamoyl)-2-(1-fluoro-cyclopentyl)-ethyl]-amide	

	^{1}H NMR (400 MHz) (δCDCl$_3$) 7.89 (s, 1H), 7.39 (s, 1H), 7.00 – 7.07 (m, 1H), 6.52 – 6.60 (m, 2H), 4.63 – 4.70 (m, 0.5H), 4.10 – 4.18 (m, 1H), 3.73 – 4.05 (m, 3H), 2.22 – 2.34 (m, 2.5H), 1.83 – 2.06 (m, 1H), 1.34 – 1.80 (m, 10.5H), 0.96 (t, *J* 7.5, 3H) ^{19}F NMR (376 MHz) (δCDCl$_3$) -138.84 ESMS m/z (%) 403 MNa$^+$ (50%), 381 MH$^+$ (100), 361 M-F$^+$.
Example 11.4 Furan-3-carboxylic acid [2-(1-ethyl-cyclobutyl)-1-(S)-(2-(S)-ethyl-4-oxo-tetrahydro-furan-3-(S)-ylcarbamoyl)-ethyl]-amide	
	^{1}H NMR (400 MHz) (δCDCl$_3$) 7.93 – 7.94 (m, 1H), 7.45 (t, *J* 2, 1H), 6.76, br d, 1H), 6.58 – 6.59 (m, 1H), 6.13 (br d, 1H), 4.53 – 4.59 (m, 1H), 4.21 (d, *J* 17, 1H), 4.21 (d, *J* 17, 1H), 4.06, 3.92 – 3.98 (m, 1H), 3.84 – 3.89 (m, 1H), 2.12 (dd, *J* 14.5, 5, 1H), 1.69 – 1.90 (m, 9H), 1.02 (t, *J* 7.5, 3H), 0.85 (t, *J* 7.5, 3H), ESMS (%) 377 (MH$^+$, 50)

Example 12

Preparation of 1-Hydroxycyclopentyl-(D,L)-alanine P2 Building block

Step a) 1-(1-hydroxy-cyclopropyl)ethanoic acid, ethyl ester

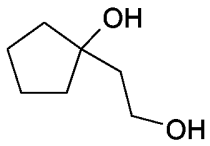


See scheme 5 in conjunction with *Tetrahedron*, 1994, **50**, 11709.

Preparation of the Zn-Cu couple : To a vigorously stirring and hot (nearly refluxing) solution of copper(II) acetate (750 mg, 4.13 mmol) in glacial acetic acid (100 ml) was added granulated zinc (7.5 g, 114.73 mmol). The solution was stirred for 10 min. The solution was then allowed to cool to room temperature and decanted. The residue was washed with diethyl ether (6 x 50 ml) and the washes were repeatedly decanted off.

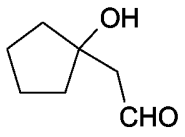
Experimental: The freshly prepared Zn-Cu couple was suspended in dry diethyl ether (50 ml) and vigorously stirred at room temperature. Ethylbromoacetate (7.08 ml, 63.85 mmol) was very carefully added until the reaction was initiated (this may take a little gentle heating). Once this careful addition had been completed the process was repeated for the addition of cyclopentanone (4.51 ml, 51.06 mmol). The mixture was then heated to 40°C for 12 hrs. The cooled solution was acidified with 5M sulphuric acid solution and extracted with diethyl ether. The combined organics were dried (Na_2SO_4) and the solvent was removed in *vacuo*. Purification by column chromatography (isohexane: ethyl acetate; 10:1) afforded the product.

Step b)



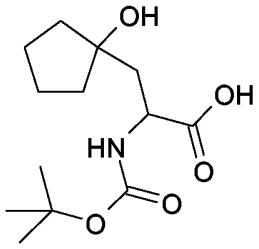
The title product is prepared analogously to Example 10, step c) from 1-(1-hydroxy-cyclopropyl)ethanoic acid, ethyl ester

Step c)



The title product is prepared analogously to Example 10 step d) from 1-(1-hydroxy-cyclopentyl)ethanol.

Step d)2-*tert*-Butoxycarbonylamino-(3*R*,*S*)-(1-hydroxy-cyclopentyl)-propionic acid



The title compound is prepared from the material of step c) above using the same procedure as Example 10, step e).

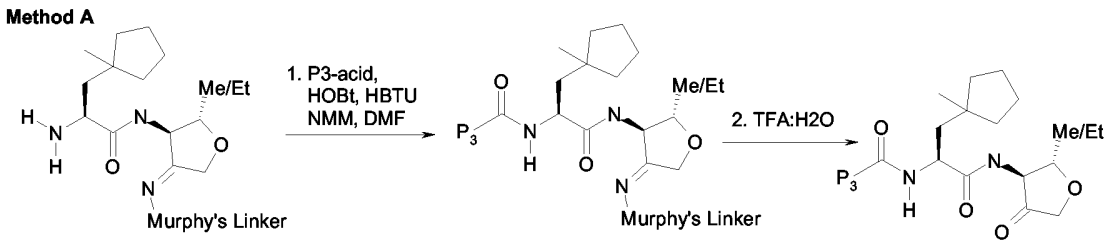
Although this example has been illustrated with a Boc protecting group it will be apparent that other conventional N-protecting groups such as those described above in Greene, such as Fmoc, CBz etc will be amenable to this route and/or the Boc group can be removed and replaced with a conventional N-protecting group using conventional protecting group manipulations. As is conventional with non-natural amino acids, the D and L diasteromers are isolated by chiral HPLC.

Example 13

Preparation on solid phase

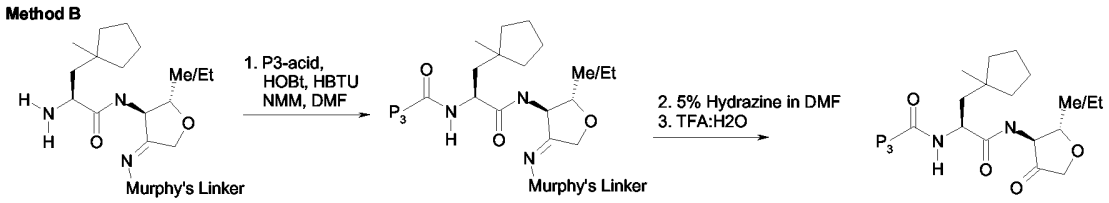
Immobilisation of a P1 buliding block, such as those prepared in WO05/82876, onto a resin via Murphy's linker proceeds as described in Scheme 7 of WO00/69855 and its accompanying text. The Fmoc-protected 5-substituted furan-4-amine is de-protected, extended with the P2 building block of the invention, such as those described at examples 1 and 5, using conventional peptide activation and coupling reagents such as HOBt/HBTU/DMF, as described in WO00/69855.

Method A: General Synthesis of P3 amides using coupling on solid phase



Method B: Synthesis of phenol P3s using solid phase coupling

(includes a hydrazine wash)

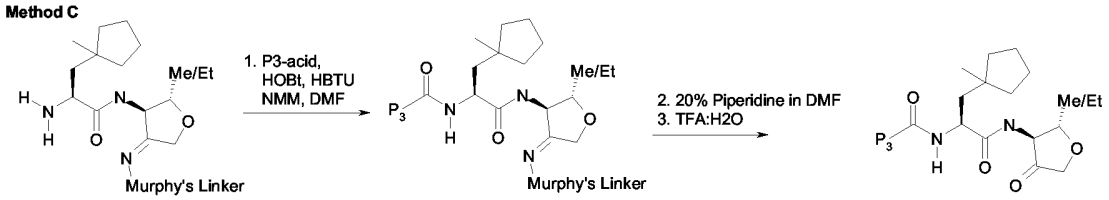


After coupling, the resin was suspended in a 5% solution of hydrazine in DMF for 1h. The mixture was filtered, and the resin washed with DMF. The hydrazine treatment and DMF wash was then repeated.

(Resin cleaved as standard)

Method C: Synthesis of aniline P3s using solid phase coupling

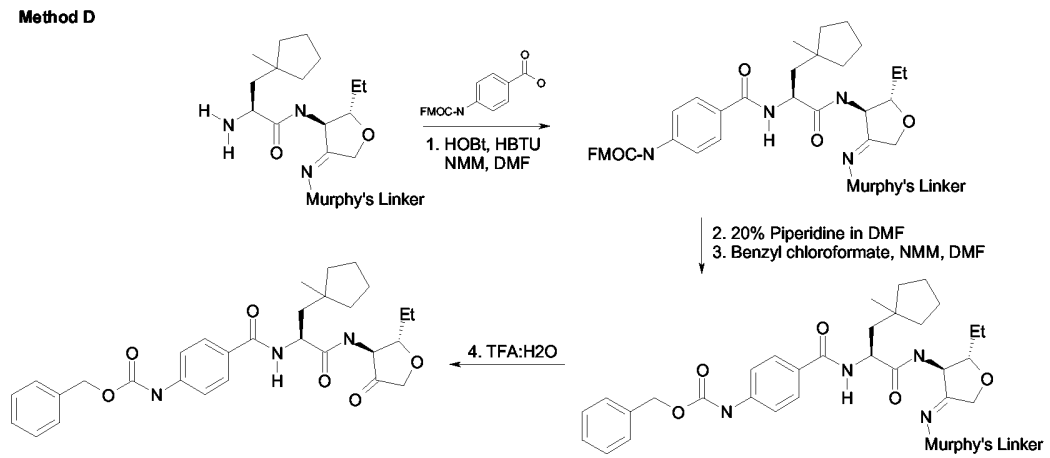
(includes a piperidine treatment to remove FMOC protecting groups)



After coupling, the resin was suspended in a 20% solution of piperidine in DMF for 0.5h. The mixture was filtered, and the resin washed with DMF. The piperidine treatment and DMF wash was then repeated.

(Resin cleaved as standard)

Method D: Alternative synthesis of aniline P3s.

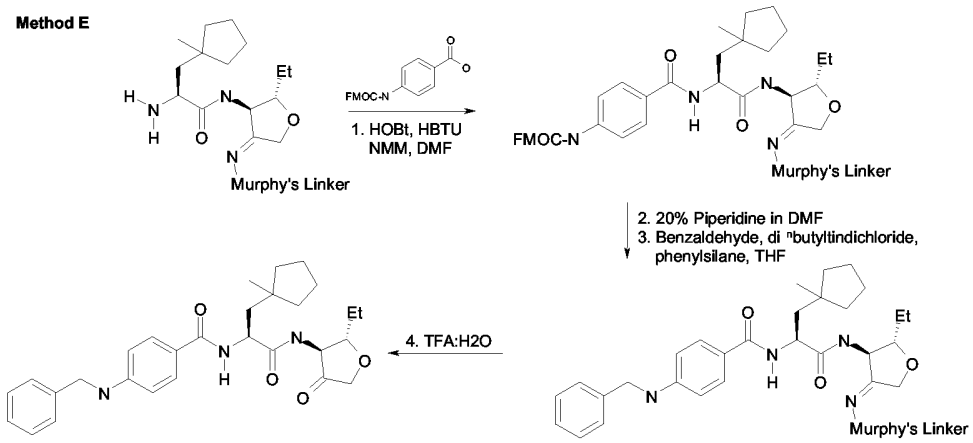


After coupling of 4-FMOC-aminobenzoic acid, the resin was suspended in a 20% solution of piperidine in DMF for 0.5h. The mixture was filtered, and the resin washed with DMF. The piperidine treatment and DMF wash was then repeated.

The resin was suspended in a solution of benzyl chloroformate and *N*-methyl morpholine in DMF, filtered and the residue washed with 1:1 water: DMF, DMF, THF, DCM and MTBE.

(Resin cleaved as standard)

Method E: Alternative synthesis of anilines



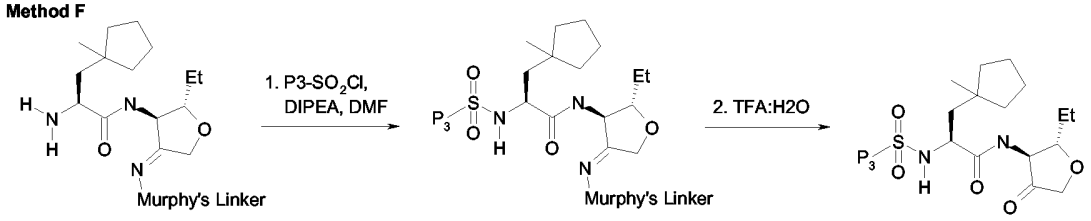
After coupling of 4-FMOC-aminobenzoic acid, the resin was suspended in a 20% solution of piperidine in DMF for 0.5h. The mixture was filtered, and the resin washed with DMF. The piperidine treatment and DMF wash was then repeated.

The resin was suspended in a solution of benzylaldehyde in DMF, and a solution of dibutyltin dichloride in THF was added. After 10 minutes, phenyl silane was added and the mixture was shaken overnight. The mixture was filtered and the residue washed with DMF, THF, DCM and MTBE.

(Resin cleaved as standard)

Method F: General Synthesis of P3 sulfonamides on solid phase

Method F



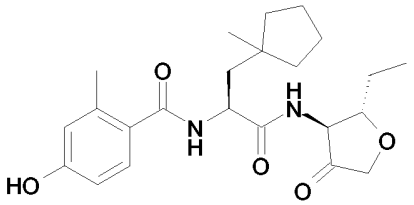
To a suspension of P2-P1 on resin in DMF, was added diisopropylethylamine and sulfonyl chloride. After 16h, the mixture was filtered and resin was washed with DMF, THF, DCM and MTBE.

(Resin cleaved as standard)

Although methods A-F have been illustrated with methyl or ethyl as R^1, and 1-methyl-cyclopentyl-L-Ala as P2, it will be apparent that corresponding methodology, in conjunction with conventional protection of hydroxyl groups, will be applicable to other P1 and P2 building blocks. Similarly, methods A-F are not limited to the specified classes of P3, but are widely applicable to other species of R^3, optionally in conjunction with conventional protection of amine, hydroxyl and carboxyl groups.

The synthesis of P3 building blocks which were not commercially available is described at the foot of the table.

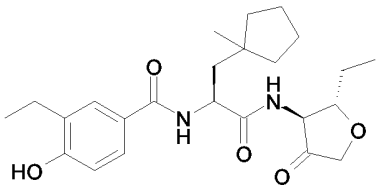
Example 14.1 *N*-[(1*S*) -((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-2-methyl-benzamide By following the procedure described in Method B, the title compound was prepared in 45% yield from P2-P1 on resin and 4-hydroxy-2-methyl-benzoic acid

	MS/ES: *m/z* 417 (100%, MH^+)

Example 14.2

3-Ethyl-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-benzamide

By following the procedure described in Method B, the title compound was prepared in 44% yield from P2-P1 on resin and 2-ethyl-4-hydroxy-benzoic acid.

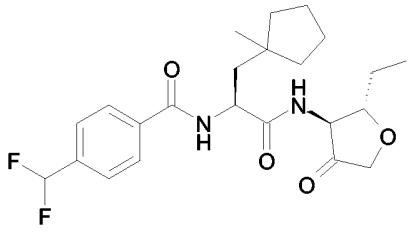


MS/ES: *m/z* 431 (100%, MH^+)

Example 14.3

4-Difluoromethyl-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-benzamide

By following the procedure described in Method B, the title compound was prepared in 38% yield from P2-P1 on resin and 4-difluoromethyl-benzoic acid.

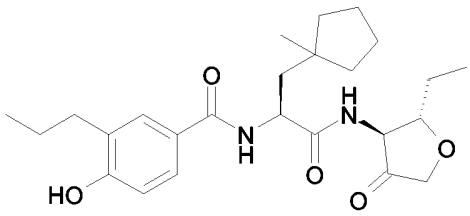
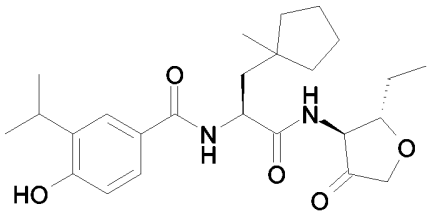
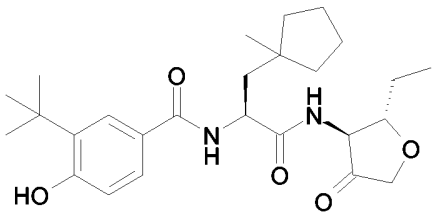


MS/ES: *m/z* 437 (100%, MH^+)

Example 14.4

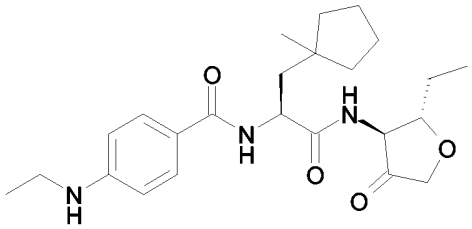
N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-3-propyl-benzamide

By following the procedure described in Method B, the title compound was prepared in 35% yield from P2-P1 on resin and 4-hydroxy-3-propyl-benzoic acid.

	MS/ES: *m/z* 445 (100%, MH^+)
Example 14.5 *N*-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-3-isopropyl-benzamide By following the procedure described in Method B, the title compound was prepared in 42% yield from P2-P1 on resin and 4-hydroxy-3-isopropyl-benzoic acid.	
	MS/ES: *m/z* 445 (100%, MH^+)
Example 14.6 3-*tert*-Butyl-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-benzamide By following the procedure described in Method B, the title compound was prepared in 11% yield from P2-P1 on resin and 3-*tert*-butyl-4-hydroxy-benzoic acid.	
	MS/ES: *m/z* 459 (90%, MH^+), 381 (100%), 330 (42%, M-$C_6H_{10}NO_2$)
Example 14.7 4-Ethylamino-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-	

cyclopentyl)-ethyl]-benzamide

By following the procedure described in Method C, the title compound was prepared in 41% yield from P2-P1 on resin and 4-[ethyl-(9H-fluoren-9-ylmethoxycarbonyl)-amino]-benzoic acid.

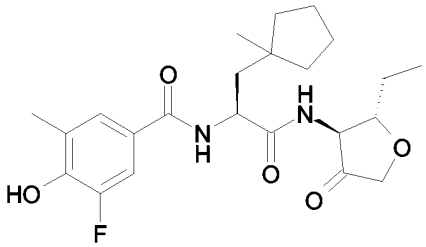


MS/ES: *m/z* 430 (100%, MH^+)

Example 14.8

N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-3-fluoro-4-hydroxy-5-methyl-benzamide

By following the procedure described in Method B, the title compound was prepared in 23% yield from P2-P1 on resin and 3-fluoro-4-hydroxy-5-methylbenzoic acid.

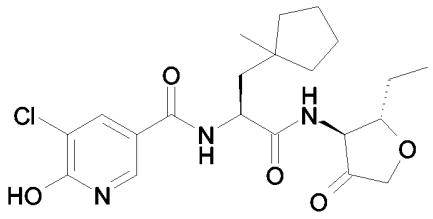


MS/ES: *m/z* 435 (100%, MH^+)

Example 14.9

5-Chloro-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-6-hydroxy-nicotinamide

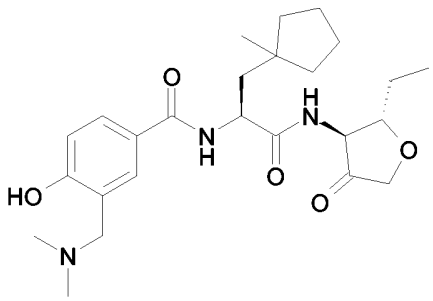
By following the procedure described in Method B, the title compound was prepared in 25% yield from P2-P1 on resin and 5-chloro-6-hydroxynicotinic acid.

	MS/ES: *m/z* 438 (100%, MH^+)

Example 14.10

3-Dimethylaminomethyl-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-benzamide

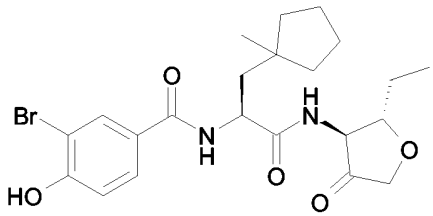
By following the procedure described in Method B, the title compound was prepared in 26% yield from P2-P1 on resin and 3-dimethylaminomethyl-4-hydroxy-benzoic acid.

	MS/ES: *m/z* 460 (100%, MH^+)

Example 14.11

3-Bromo-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-benzamide

By following the procedure described in Method B, the title compound was prepared in 25% yield from P2-P1 on resin and 3-bromo-4-hydroxy-benzoic acid.

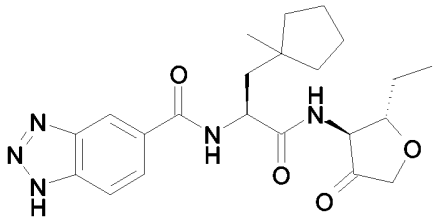
	MS/ES: *m/z* 481 and 483 (100%, MH^+)

Example 14.12

1H-Benzotriazole-5-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-

ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide

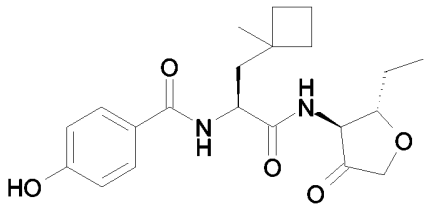
By following the procedure described in Method A, the title compound was prepared in 37% yield from P2-P1 on resin and 1H-1,2,3-benzotriazole-5-carboxylic acid.

	MS/ES: *m/z* 428 (85%, MH^+), 299 (100%, M-$C_6H_{10}NO_2$)

Example 14.13

N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclobutyl)-ethyl]-4-hydroxy-benzamide

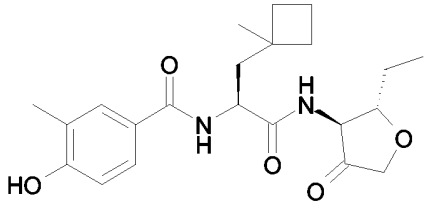
By following the procedure described in Method B, the title compound was prepared in 18% yield from P2-P1 on resin and 4-hydroxybenzoic acid.

	MS/ES: *m/z* 389 (62%, MH^+), 260 (100%, M-$C_6H_{10}NO_2$)

Example 14.14

N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclobutyl)-ethyl]-4-hydroxy-3-methyl-benzamide

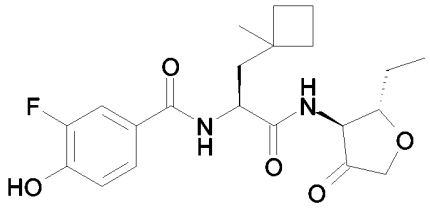
By following the procedure described in Method B, the title compound was prepared in 16% yield from P2-P1 on resin and 4-hydroxy-3-methyl-benzoic acid.

	MS/ES: *m/z* 403 (64%, MH^+), 274 (100%, M-$C_6H_{10}NO_2$)

Example 14.15

N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclobutyl)-ethyl]-3-fluoro-4-hydroxy-benzamide

By following the procedure described in Method B, the title compound was prepared in 20% yield from P2-P1 on resin and 3-fluoro-4-hydroxy-benzoic acid.

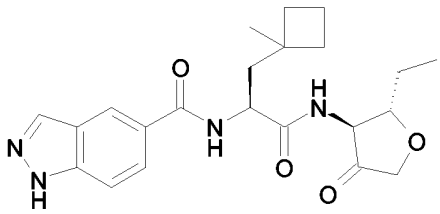


MS/ES: *m/z* 407 (79%, MH^+), 278 (100%, M-$C_6H_{10}NO_2$)

Example 14.16

1H-Indazole-5-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclobutyl)-ethyl]-amide

By following the procedure described in Method B, the title compound was prepared in 20% yield from P2-P1 on resin and 1H-indazole-5-carboxylic acid.

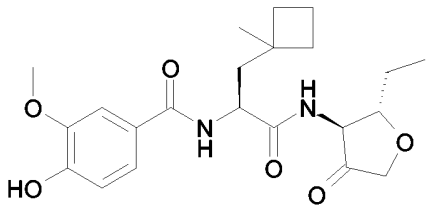


MS/ES: *m/z* 413 (54%, MH^+), 284 (100%, M-$C_6H_{10}NO_2$)

Example 14.17

N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclobutyl)-ethyl]-4-hydroxy-3-methoxy-benzamide

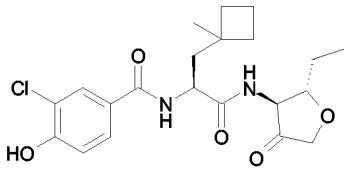
By following the procedure described in Method B, the title compound was prepared in 20% yield from P2-P1 on resin and 4-hydroxy-3-methoxy-benzoic acid.

	MS/ES: *m/z* 419 (75%, MH^+), 290 (100%, M-$C_6H_{10}NO_2$)

Example 14.18

3-Chloro-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclobutyl)-ethyl]-4-hydroxy-benzamide

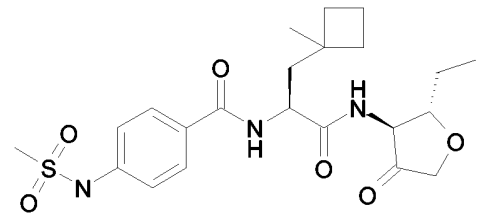
By following the procedure described in Method B, the title compound was prepared in 20% yield from P2-P1 on resin and 3-chloro-4-hydroxy-benzoic acid.

	MS/ES: *m/z* 423 (100%, MH^+)

Example 14.19

N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclobutyl)-ethyl]-4-methanesulfonylamino-benzamide

By following the procedure described in Method B, the title compound was prepared in 20% yield from P2-P1 on resin and 4-[(methylsulfonyl)amino]benzoic acid.

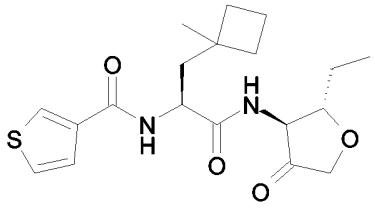
	MS/ES: *m/z* 466 (100%, MH^+)

Example 14.20

Thiophene-3-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclobutyl)-ethyl]-amide

By following the procedure described in Method A, the title compound was prepared in 16%

yield from P2-P1 on resin and thiophene-3-carboxylic acid.

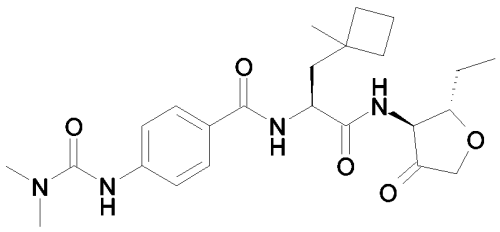


MS/ES: *m/z* 379 (94 %, MH^+), 250 (100%, M-$C_6H_{10}NO_2$)

Example 14.21

4-(3,3-Dimethyl-ureido)-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclobutyl)-ethyl]-benzamide

By following the procedure described in Method A, the title compound was prepared in 16% yield from P2-P1 on resin and 4-{[(dimethylamino)carbonyl]amino}benzoic acid.

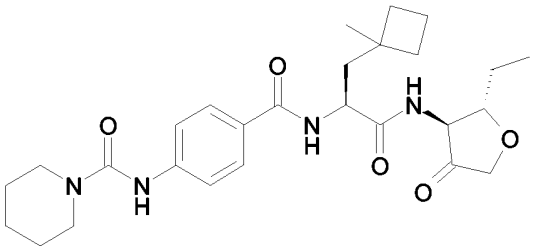


MS/ES: *m/z* 459 (100%, MH^+)

Example 14.22

Piperidine-1-carboxylic acid {4-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclobutyl)-ethylcarbamoyl]-phenyl}-amide

By following the procedure described in Method A, the title compound was prepared in 12% yield from P2-P1 on resin and 4-[(piperidin-1-ylcarbonyl)amino]benzoic acid.

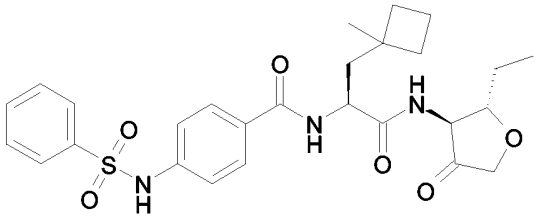


MS/ES: *m/z* 499 (100%, MH^+)

Example 14.23

4-Benzenesulfonylamino-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclobutyl)-ethyl]-benzamide

By following the procedure described in Method A, the title compound was prepared in 19% yield from P2-P1 on resin and 4-[(phenylsulfonyl)amino]benzoic acid.

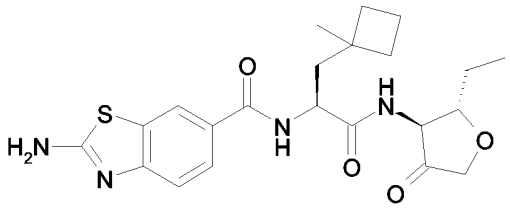


MS/ES: *m/z* 528 (100%, MH^+)

Example 14.24

2-Amino-benzothiazole-6-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclobutyl)-ethyl]-amide

By following the procedure described in Method A, the title compound was prepared in 21% yield from P2-P1 on resin and 2-[(tert-butoxycarbonyl)amino]-1,3-benzothiazole-6-carboxylic acid.



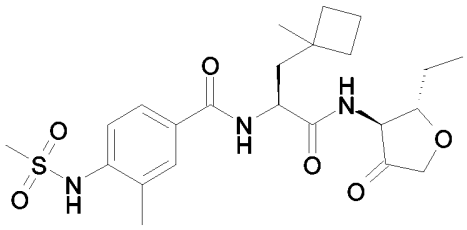
^{1}H-NMR (400 MHz, CD_3CN) δ 8.18-8.19 (m, 1H), 7.82-7.85 (m, 1H), 7.46-7.48 (m, 1H), 7.30-7.32 (m, 1H), 7.14-7.16 (m, 1H), 4.58-4.64 (m, 1H), 3.95-4.15 (m, 3H), 3.85-3.89 (m, 1H), 3.60 (s, 3H), 1.88-2.06 (m, 4H), 1.60-1.86 (m, 6H), 1.21 (3H, s), 0.99 (t, J=7.4, 3H)
MS/ES: *m/z* 445 (87 %, MH^+)

Example 14.25

N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclobutyl)-ethyl]-

4-methanesulfonylamino-3-methyl-benzamide

By following the procedure described in Method A, the title compound was prepared in 24% yield from P2-P1 on resin and 3-methyl-4-[(methylsulfonyl)amino]benzoic acid.

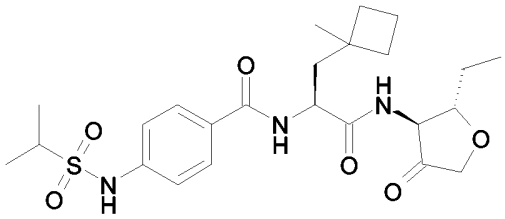


MS/ES: *m/z* 480 (100%, MH^+)

Example 14.26

N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclobutyl)-ethyl]-4-(propane-2-sulfonylamino)-benzamide

By following the procedure described in Method A, the title compound was prepared in 23% yield from P2-P1 on resin and 4-[(isopropylsulfonyl)amino]benzoic acid.

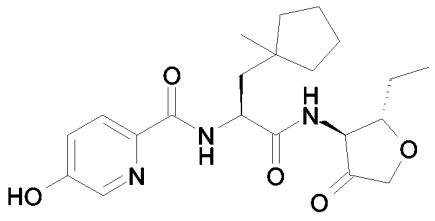


MS/ES: *m/z* 494 (100%, MH^+)

Example 14.27

5-Hydroxy-pyridine-2-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide

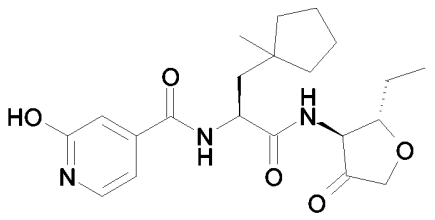
By following the procedure described in Method B, the title compound was prepared in 44% yield from P2-P1 on resin and 5-hydroxypyridine-2-carboxylic acid.

	MS/ES: *m/z* 404 (100%, MH^+)

Example 14.28

N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-2-hydroxy-isonicotinamide

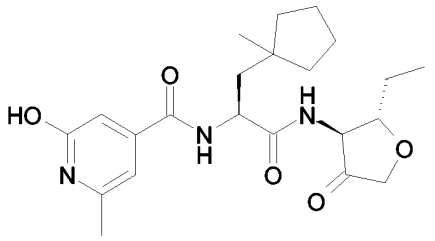
By following the procedure described in Method B, the title compound was prepared in 41% yield from P2-P1 on resin and 2-hydroxyisonicotinic acid.

	MS/ES: *m/z* 404 (100%, MH^+)

Example 14.29

N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-2-hydroxy-6-methyl-isonicotinamide

By following the procedure described in Method B, the title compound was prepared in 37% yield from P2-P1 on resin and 2-hydroxy-6-methylisonicotinic acid.

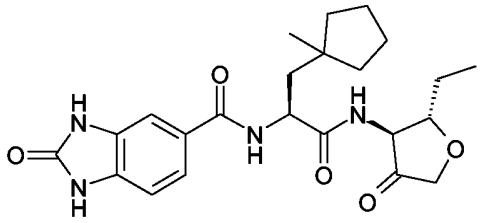
	MS/ES: *m/z* 418 (100%, MH^+)

Example 14.30

2-Oxo-2,3-dihydro-1H-benzoimidazole-5-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-

furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide

By following the procedure described in Method A, the title compound was prepared in 39% yield from P2-P1 on resin and 2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylic acid.

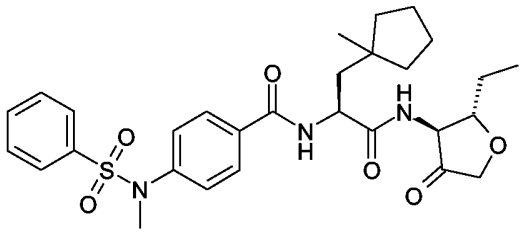


MS/ES: *m/z* 443 (100%, MH^+)

Example 14.31

4-(Benzenesulfonyl-methyl-amino)-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-benzamide

By following the procedure described in Method A, the title compound was prepared in 49% yield from P2-P1 on resin and lithium 4-[methyl(phenylsulfonyl)amino]benzoate.

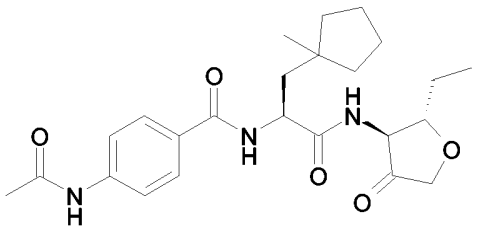


MS/ES: *m/z* 556 (100%, MH^+)

Example 14.32

4-Acetylamino-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-benzamide

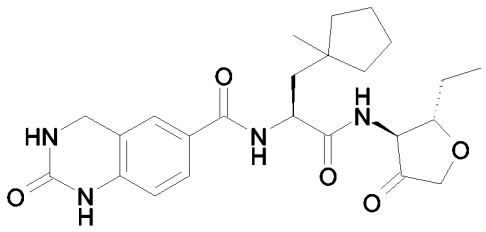
By following the procedure described in Method A, the title compound was prepared in 48% yield from P2-P1 on resin and 4-(acetylamino)benzoic acid.

	MS/ES: *m/z* 444 (100%, MH^+)

Example 14.33

2-Oxo-1,2,3,4-tetrahydro-quinazoline-6-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide

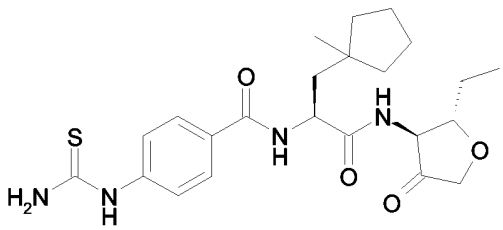
By following the procedure described in Method A, the title compound was prepared in 35% yield from P2-P1 on resin and 2-oxo-1,2,3,4-tetrahydroquinazoline-6-carboxylic acid.

	MS/ES: *m/z* 457 (100%, MH^+)

Example 14.34

N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-thioureido-benzamide

By following the procedure described in Method A, the title compound was prepared in 18% yield from P2-P1 on resin and 4-[(aminocarbonothioyl)amino]benzoic acid.

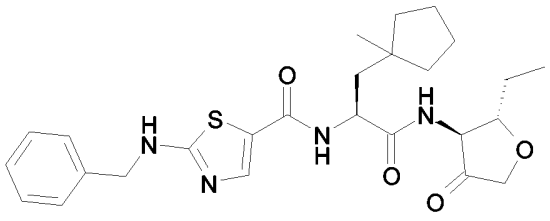
	MS/ES: *m/z* 461 (100%, MH^+)

Example 14.35

2-Benzylamino-thiazole-5-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-

ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide

By following the procedure described in Method A, the title compound was prepared in 39% yield from P2-P1 on resin and 2-(benzylamino)-1,3-thiazole-5-carboxylic acid.

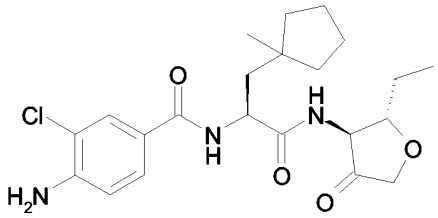


MS/ES: *m/z* 499 (100%, MH^+)

Example 14.36

4-Amino-3-chloro-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-benzamide

By following the procedure described in Method A, the title compound was prepared in 10% yield from P2-P1 on resin and 4-amino-3-chlorobenzoic acid.

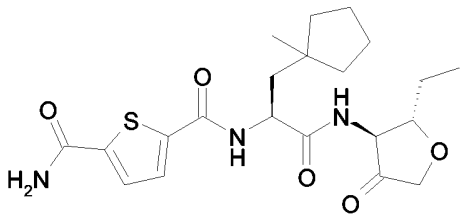


MS/ES: *m/z* 436 (100%, MH^+)

Example 14.37

Thiophene-2,5-dicarboxylic acid 2-amide 5-{[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide}

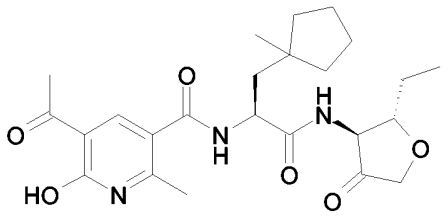
By following the procedure described in Method A, the title compound was prepared in 33% yield from P2-P1 on resin and 5-(aminocarbonyl)thiophene-2-carboxylic acid.

	^{1}H-NMR (400 MHz, CD_3CN) δ 7.60 (d J=4.1, 1H), 7.55 (d J=4.1, 1H), 7.38 (d J=8.3, 1H), 7.17 (d J=7.7, 1H), 4.57-4.62 (m, 1H), 3.94-4.14 (m, 3H), 3.83-3.88 (m, 1H), 1.99-2.03 (m, 1H), 1.60-1.82 (m, 7H), 1.33-1.52 (m, 4H), 0.97-1.00 (m, 6H) MS/ES: *m/z* 436 (100%, MH^+)

Example 14.38

5-Acetyl-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-6-hydroxy-2-methyl-nicotinamide

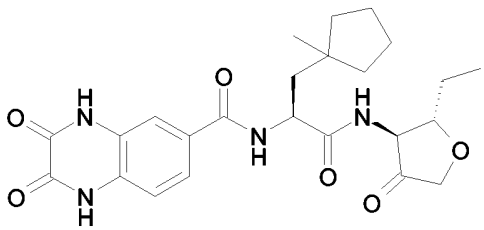
By following the procedure described in Method A, the title compound was prepared in 10% yield from P2-P1 on resin and 5-acetyl-6-hydroxy-2-methylnicotinic acid.

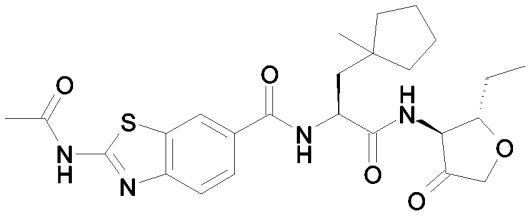
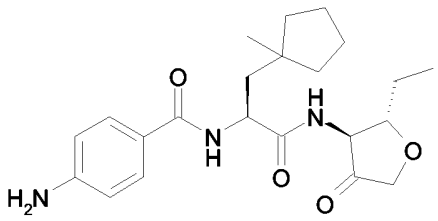
	MS/ES: *m/z* 460 (100%,)

Example 14.39

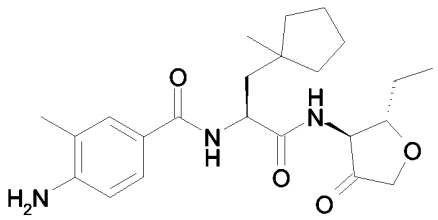
2,3-Dioxo-1,2,3,4-tetrahydro-quinoxaline-6-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide

By following the procedure described in Method A, the title compound was prepared in 61% yield from P2-P1 on resin and 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-carboxylic acid.

	^{1}H-NMR (400 MHz, CD_3OD) δ 8.42 (d J=8.4, 1H), 7.61-7.64 (m, 2H), 7.19-7.22 (m, 1H), 4.69-4.75 (m, 1H), 3.95-4.20 (m, 3H), 3.63 (s, 3H), 1.99-2.03 (m, 1H), 1.60-1.89 (m, 7H), 1.37-1.57 (m, 4H), 1.04 (s,

	3H), 1.01 (t, J=7.5, 3H) MS/ES: *m/z* 471 (37 %, MH^+)
Example 14.40 2-Acetylamino-benzothiazole-6-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide By following the procedure described in Method A, the title compound was prepared in 46% yield from P2-P1 on resin and 2-(acetylamino)-1,3-benzothiazole-6-carboxylic acid.	
	MS/ES: *m/z* 501 (100%, MH^+)
Example 14.41 4-Amino-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-benzamide By following the procedure described in Method C, the title compound was prepared in 29% yield from P2-P1 on resin and 4-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}benzoic acid.	
	MS/ES: *m/z* 402 (100%, MH^+)
Example 14.42 4-Amino-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-3-methyl-benzamide By following the procedure described in Method C, the title compound was prepared in	

28% yield from P2-P1 on resin and 4-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}-3-methylbenzoic acid.

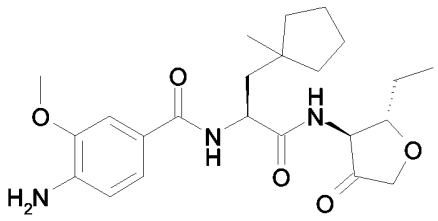


MS/ES: *m/z* 416 (100%, MH^+)

Example 14.43

4-Amino-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-3-methoxy-benzamide

By following the procedure described in Method C, the title compound was prepared in 9% yield from P2-P1 on resin and 4-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}-3-methoxybenzoic acid.

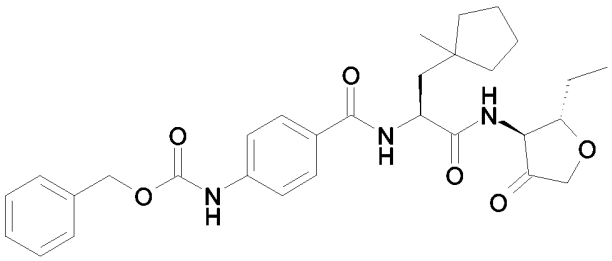


MS/ES: *m/z* 432 (100%, MH^+)

Example 14.44

{4-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethylcarbamoyl]-phenyl}-carbamic acid benzyl ester

By following the procedure described in Method D, the title compound was prepared in 5% yield from P2-P1 on resin, 4-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}-benzoic acid and benzyl chloroformate.

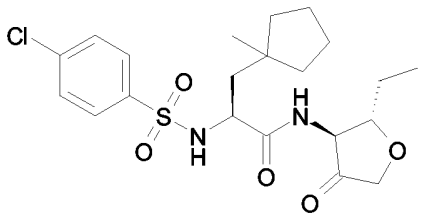
	^{1}H-NMR (400 MHz, CD_3OD) δ 9.62 (s, 1H), 8.29 (d J=8.4, 1H), 7.80 (d J=8.7, 2H), 7.55 (d J=8.4, 2H), 7.31-7.43 (m, 5H), 5.19 (s, 2H), 4.68-4.73 (m, 1H), 3.94-4.18 (m, 3H), 3.63 (s, 2H), 1.99-2.03 (m, 1H), 1.62-1.86 (m, 7H), 1.37-1.52 (m, 4H), 1.03 (s, 3H), 1.00 (t, J=7.5, 3H) MS/ES: *m/z* 536 (37 %, MH^+)

Example 14.44

(*2S*)-(4-Chloro-benzenesulfonylamino)-*N*-((*2S*)-ethyl-4-oxo-tetrahydro-furan-(*3S*)-yl)-3-(1-methyl-cyclopentyl)-propionamide

By following the procedure described in Method A, the title compound was obtained as a side-product in 8% yield from P2-P1 on resin and a sample containing 4-chlorobenzenesulfonic acid.

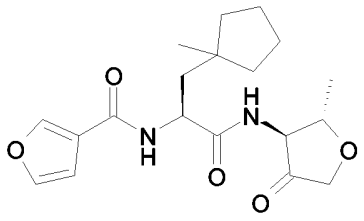
Sample could also be made *via* general procedure given in Method F.

	MS/ES: *m/z* 457 (100%, MH^+)

Example 14.45

Furan-3-carboxylic acid [2-(1-methyl-cyclopentyl)-(1*S*)-((2*S*)-methyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-ethyl]-amide

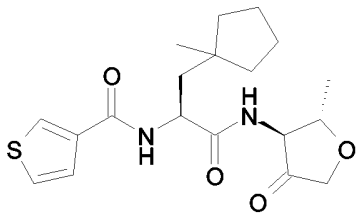
By following the procedure described in Method A, the title compound was prepared in 77% yield from P2-P1 on resin and 3-furoic acid.

	MS/ES: *m/z* 363 (100%, MH^+)

Example 14.46

Thiophene-3-carboxylic acid [2-(1-methyl-cyclopentyl)- (1*S*)-((2*S*)-methyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-ethyl]-amide

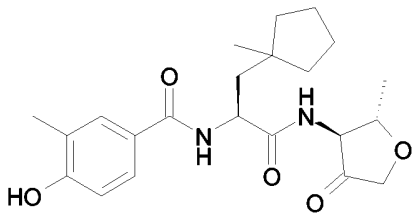
By following the procedure described in Method A, the title compound was prepared in 59% yield from P2-P1 on resin and thiophene-3-carboxylic acid.

	MS/ES: *m/z* 379 (100%, MH^+)

Example 14.47

4-Hydroxy-3-methyl-*N*-[2-(1-methyl-cyclopentyl)- (1*S*)-((2*S*)-methyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-ethyl]-benzamide

By following the procedure described in Method B, the title compound was prepared in 64% yield from P2-P1 on resin and 4-hydroxy-3-methylbenzoic acid.

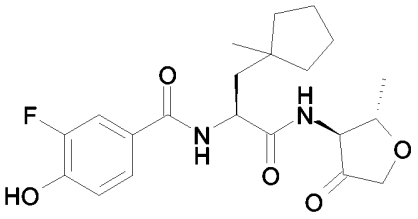
	MS/ES: *m/z* 403 (100%, MH^+)

Example 14.48

3-Fluoro-4-hydroxy-*N*-[2-(1-methyl-cyclopentyl)- (1*S*)-((2*S*)-methyl-4-oxo-tetrahydro-furan-

(3*S*)-ylcarbamoyl)-ethyl]-benzamide

By following the procedure described in Method B, the title compound was prepared in 67% yield from P2-P1 on resin and 3-fluoro-4-hydroxybenzoic acid.

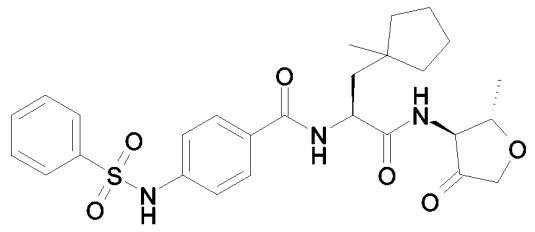


^{1}H-NMR (400 MHz, CD_3CN) δ 7.61-7.64 (m, 1H), 7.54-7.57 (m, 1H), 7.11-7.16 (m, 2H), 7.02-7.07 (m, 1H), 4.58-4.63 (m, 1H), 3.96-4.14 (m, 3H), 3.74-3.79 (m, 1H), 1.99-2.04 (m, 1H), 1.75-1.81 (m, 1H), 1.61-1.67 (m, 4H), 1.41-1.50 (m, 3H), 1.32-1.38 (m, 4H), 0.99 (s, 3H)
MS/ES: *m/z* 407 (100%, MH^+)

Example 14.49

4-Benzenesulfonylamino-*N*-[2-(1-methyl-cyclopentyl)- (1*S*)-((2*S*)-methyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-ethyl]-benzamide

By following the procedure described in Method A, the title compound was prepared in 76% yield from P2-P1 on resin and 4-[(phenylsulfonyl)amino]benzoic acid.

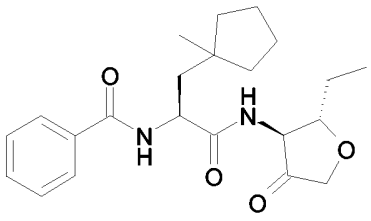


MS/ES: *m/z* 528 (100%, MH^+)

Example 14.50

N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-benzamide

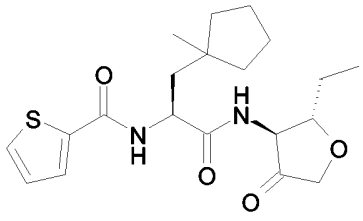
By following the procedure described in Method A, the title compound was prepared in 55% yield from P2-P1 on resin and benzoic acid.

	^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.74-7.77 (m, 2H), 7.51-7.55 (m, 1H), 7.42-7.46 (m, 2H), 7.14 (d J=7.5, 1H), 6.60 (d J=8.3, 1H), 4.74-4.79 (m, 1H), 4.02-4.20 (m, 2H), 3.92-3.97 (m, 1H), 3.84-3.88 (m, 1H), 2.14-2.19 (m, 1H), 1.59-1.83 (m, 7H), 1.43 (t J 6.4, 4H), 0.97-1.01 (m, 6H) MS/ES: *m/z* 387 (100%, MH^+)

Example 14.51

Thiophene-2-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide

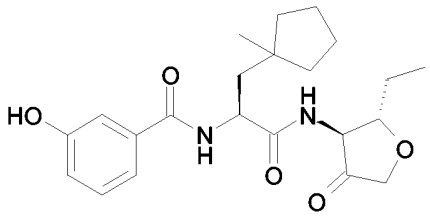
By following the procedure described in Method A, the title compound was prepared in 55% yield from P2-P1 on resin and thiophene-2-carboxylic acid.

	^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.50-7.53 (m, 2H), 7.26-7.28 (m, 1H), 7.07-7.09 (m, 1H), 6.64 (d J 8.0, 1H), 4.70-4.75 (m, 1H), 4.04-4.19 (m, 2H), 3.94-3.99 (m, 1H), 3.85-3.89 (m, 1H), 2.08-2.13 (m, 1H), 1.58-1.84 (m, 7H), 1.41-1.44 (m, 4H), 0.98-1.02 (m, 6H) MS/ES: *m/z* 393 (100%, MH^+)

Example 14.52

N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-3-hydroxy-benzamide

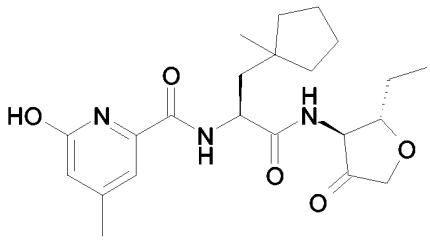
By following the procedure described in Method B, the title compound was prepared in 48% yield from P2-P1 on resin and 3-hydroxybenzoic acid.

	^{1}H-NMR (400 MHz, CD_3CN) δ 7.29-7.33 (m, 3H), 7.19 (d J=8, 1H), 7.14 (d J=8, 1H), 6.98-7.03 (m, 1H), 4.58-4.63 (m, 1H), 3.94-4.14 (m, 3H), 3.83-3.87 (m, 1H), 1.99-2.03 (m, 1H), 1.59-1.81 (m, 7H), 1.33-1.51 (m, 4H), 0.96-1.00 (m, 6H) MS/ES: *m/z* 403 (100%, MH^+)

Example 14.53

6-Hydroxy-4-methyl-pyridine-2-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide

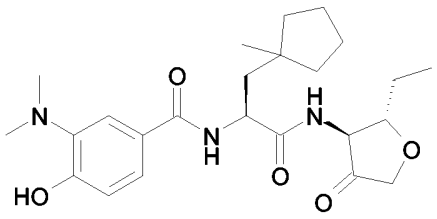
By following the procedure described in Method B, the title compound was prepared in 47% yield from P2-P1 on resin and 6-hydroxy-4-methylpyridine-2-carboxylic acid.

	^{1}H-NMR (400 MHz, CD3CN) δ 7.29-7.33 (m, 3H), 7.19 (d J=8, 1H), 7.14 (d J=8, 1H), 6.98-7.03 (m, 1H), 4.58-4.63 (m, 1H), 3.94-4.14 (m, 3H), 3.83-3.87 (m, 1H), 1.99-2.03 (m, 1H), 1.59-1.81 (m, 7H), 1.33-1.51 (m, 4H), 0.96-1.00 (m, 6H) MS/ES: *m/z* 418 (100%, MH^+)

Example 14.54

3-Dimethylamino-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-benzamide

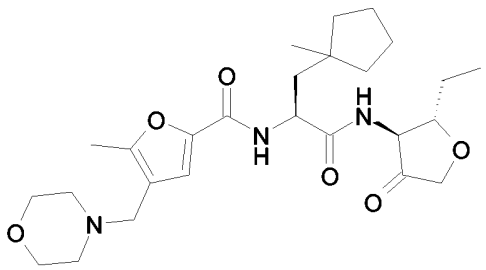
By following the procedure described in Method B, the title compound was prepared in 28% yield from P2-P1 on resin and 3-dimethylamino-4-hydroxybenzoic acid.

	MS/ES: *m/z* 446 (100%, MH^+)

Example 14.55

5-Methyl-4-morpholin-4-ylmethyl-furan-2-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide

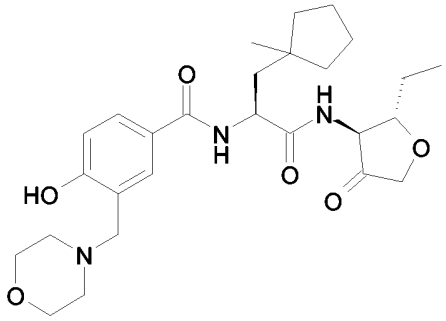
By following the procedure described in Method A, the title compound was prepared in 56% yield from P2-P1 on resin and 5-methyl-4-(morpholin-4-ylmethyl)-2-furoic acid.

	MS/ES: *m/z* 490 (100%, MH^+)

Example 14.56

N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-hydroxy-3-morpholin-4-ylmethyl-benzamide

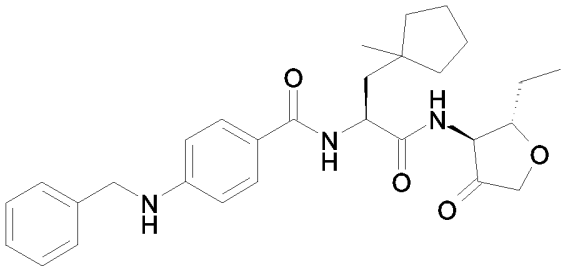
By following the procedure described in Method B, the title compound was prepared in 35% yield from P2-P1 on resin and 4-hydroxy-3-(morpholin-4-ylmethyl)benzoic acid.

	MS/ES: *m/z* 502 (100%, MH^+)

Example 14.57

4-Benzylamino-*N*-[(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-benzamide

By following the procedure described in Method E, the title compound was prepared in 27% yield from P2-P1 on resin, 4-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}benzoic acid and benzaldehyde.

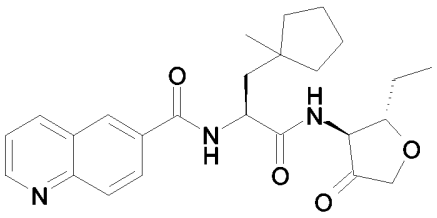


MS/ES: *m/z* 492 (100%, MH^+)

Example 14.58

Quinoline-6-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide

By following the procedure described in Method A, the title compound was prepared in 54% yield from P2-P1 on resin and quinoline-6-carboxylic acid.



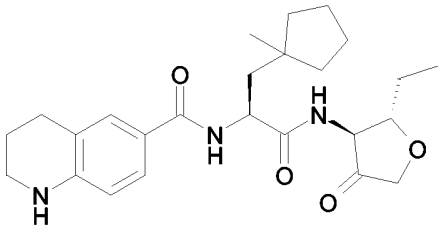
^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.28 (s, 1H), 8.77 (s, 1H), 8.40 (br s, 1H), 8.24 (br s, 1H), 8.06 (s, 2H), 7.83-7.88 (m, 2H), 4.85-4.89 (m, 1H), 4.11-4.28 (m, 3H), 3.82 (t, 1H, J=8.4), 1.80-1.98 (m, 4H), 1.64(s, 4H), 1.48-1.55 (m, 3H), 1.36-140 (m, 1H), 1.25 (s, 1H), 1.11 (t, 3H, *J* = 7.4), 1.02 (s, 3H)
MS/ES: *m/z* 438 (100%, MH^+)

Example 14.59

1,2,3,4-Tetrahydro-quinoline-6-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-

(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide

By following the procedure described in Method C, the title compound was prepared in 49% yield from P2-P1 on resin and 1-[(9H-fluoren-9-ylmethoxy)carbonyl]-1,2,3,4-tetrahydroquinoline-6-carboxylic acid.

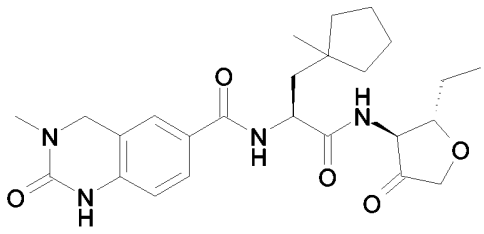


MS/ES: *m/z* 442 (100%, MH^+)

Example 14.60

3-Methyl-2-oxo-1,2,3,4-tetrahydro-quinazoline-6-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide

By following the procedure described in Method A, the title compound was prepared in 37% yield from P2-P1 on resin and 3-methyl-2-oxo-1,2,3,4-tetrahydroquinazoline-6-carboxylic acid.

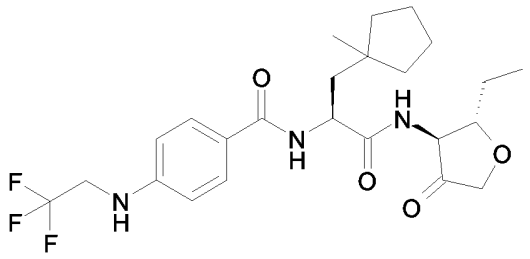
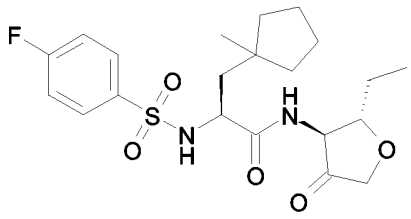
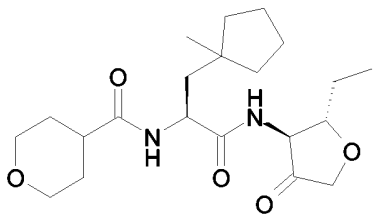


MS/ES: *m/z* 471 (100%, MH^+)

Example 14.61

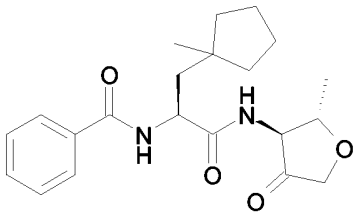
N-[(1*S*)-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-(2,2,2-trifluoro-ethylamino)-benzamide

By following the procedure described in Method A, the title compound was prepared in 51% yield from P2-P1 on resin and 4-[(2,2,2-trifluoroethyl)amino]benzoic acid.

	MS/ES: *m/z* 484 (100%, MH^+)
Example 14.62 *N*-((2*S*)-Ethyl-4-oxo-tetrahydro-furan-(3*S*)-yl)-(2*S*)-(4-fluoro-benzenesulfonylamino)-3-(1-methyl-cyclopentyl)-propionamide By following the procedure described in Method F, the title compound was prepared in 42% yield from P2-P1 on resin and 4-fluorobenzenesulfonyl chloride.	
	MS/ES: *m/z* 441 (100%, MH^+)
Example 14.63 Tetrahydro-pyran-4-carboxylic acid [(1*S*)-((2*S*)-ethyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide By following the procedure described in Method A, the title compound was prepared in 54% yield from P2-P1 on resin and tetrahydro-2*H*-pyran-4-carboxylic acid.	
	MS/ES: *m/z* 395 (100%, MH^+)
Example 14.64 *N*-[2-(1-Methyl-cyclopentyl)- (1*S*)-((2*S*)-methyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-	

ethyl]-benzamide

By following the procedure described in Method A, the title compound was prepared in 31% yield from P2-P1 on resin and benzoic acid.

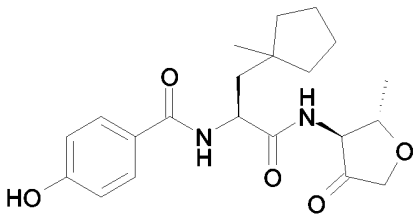


MS/ES: *m/z* 373 (100% ,MH^+)

Example 14.65

4-Hydroxy-*N*-[2-(1-methyl-cyclopentyl)- (1*S*)-((2*S*)-methyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-ethyl]-benzamide

By following the procedure described in Method B, the title compound was prepared in 56% yield from P2-P1 on resin and 4-hydroxybenzoic acid.

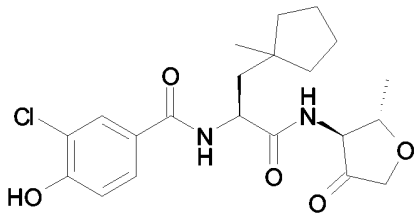


MS/ES: *m/z* 389 (100%, MH^+)

Example 14.66

3-Chloro-4-hydroxy-*N*-[2-(1-methyl-cyclopentyl)- (1*S*)-((2*S*)-methyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-ethyl]-benzamide

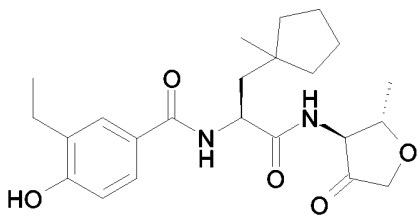
By following the procedure described in Method B, the title compound was prepared in 59% yield from P2-P1 on resin and 3-chloro-4-hydroxybenzoic acid.

	MS/ES: *m/z* 423 (100%, MH^+)

Example 14.67

3-Ethyl-4-hydroxy-*N*-[2-(1-methyl-cyclopentyl)- (1*S*)-((2*S*)-methyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-ethyl]-benzamide

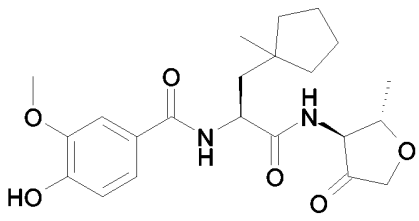
By following the procedure described in Method B, the title compound was prepared in 36% yield from P2-P1 on resin and 3-ethyl-4-hydroxybenzoic acid.

	MS/ES: *m/z* 417 (100%, MH^+)

Example 14.68

4-Hydroxy-3-methoxy-*N*-[2-(1-methyl-cyclopentyl)- (1*S*)-((2*S*)-methyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-ethyl]-benzamide

By following the procedure described in Method B, the title compound was prepared in 41% yield from P2-P1 on resin and 4-hydroxy-3-methoxybenzoic acid.

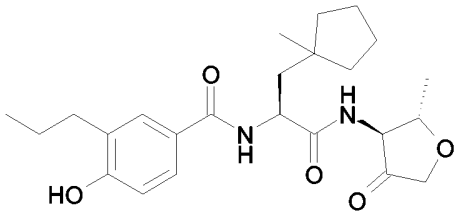
	MS/ES: *m/z* 419 (100%, MH^+)

Example 14.69

4-Hydroxy-*N*-[2-(1-methyl-cyclopentyl)- (1*S*)-((2*S*)-methyl-4-oxo-tetrahydro-furan-(3*S*)-

ylcarbamoyl)-ethyl]-3-propyl-benzamide

By following the procedure described in Method B, the title compound was prepared in 29% yield from P2-P1 on resin and 4-hydroxy-3-propylbenzoic acid.

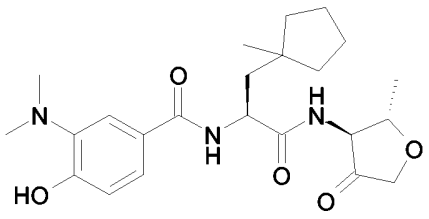


MS/ES: *m/z* 431 (100%, MH^+)

Example 14.70

3-Dimethylamino-4-hydroxy-*N*-[2-(1-methyl-cyclopentyl)- (1*S*)-((2*S*)-methyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-ethyl]-benzamide

By following the procedure described in Method B, the title compound was prepared in 34% yield from P2-P1 on resin and 3-dimethylamino-4-hydroxybenzoic acid.

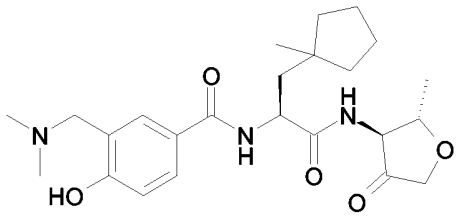
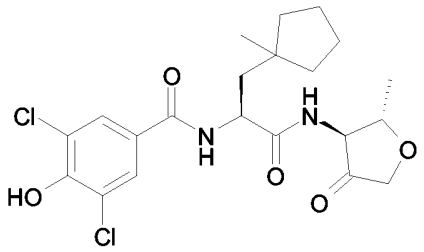
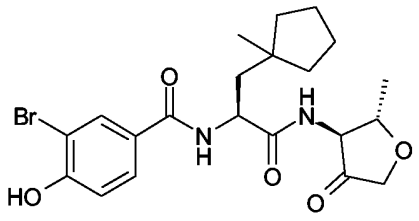


MS/ES: *m/z* 432 (100%, MH^+)

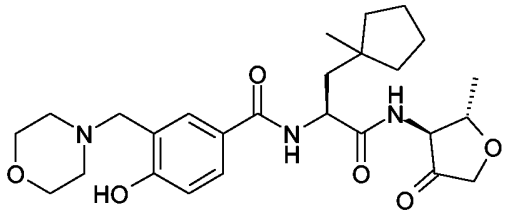
Example 14.71

3-Dimethylaminomethyl-4-hydroxy-*N*-[2-(1-methyl-cyclopentyl)- (1*S*)-((2*S*)-methyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-ethyl]-benzamide

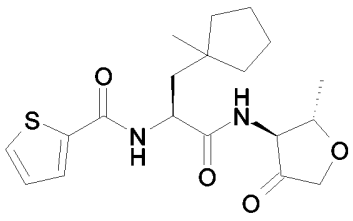
By following the procedure described in Method B, the title compound was prepared in 27% yield from P2-P1 on resin and 3-dimethylaminomethyl-4-hydroxy-benzoic acid.

	MS/ES: *m/z* 446 (100%, MH^+)
Example 14.73 3,5-Dichloro-4-hydroxy-*N*-[2-(1-methyl-cyclopentyl)- (1*S*)-((2*S*)-methyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-ethyl]-benzamide By following the procedure described in Method B, the title compound was prepared in 17% yield from P2-P1 on resin and 3-dichloro-4-hydroxybenzoic acid.	
	MS/ES: *m/z* 457 (100%, MH^+)
Example 14.74 3-Bromo-4-hydroxy-*N*-[2-(1-methyl-cyclopentyl)- (1*S*)-((2*S*)-methyl-4-oxo-tetrahydro-furan-(3*S*)-ylcarbamoyl)-ethyl]-benzamide By following the procedure described in Method B, the title compound was prepared in 30% yield from P2-P1 on resin and 3-bromo-4-hydroxybenzoic acid.	
	MS/ES: *m/z* 467 and 469 (100%, MH^+)
Example 14.75 4-Hydroxy-*N*-[2-(1-methyl-cyclopentyl)- (1*S*)-((2*S*)-methyl-4-oxo-tetrahydro-furan-(3*S*)-	

<table>
<tr><td colspan="2">ylcarbamoyl)-ethyl]-3-morpholin-4-ylmethyl-benzamide

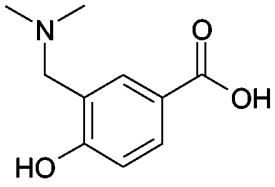
By following the procedure described in Method B, the title compound was prepared in 39% yield from P2-P1 on resin and 4-hydroxy-3-(morpholin-4-ylmethyl)benzoic acid.</td></tr>
<tr><td>  </td><td>MS/ES: m/z 488 (100%, MH^+)</td></tr>
<tr><td colspan="2">Example 14.76

Thiophene-2-carboxylic acid [2-(1-methyl-cyclopentyl)- (1S)-((2S)-methyl-4-oxo-tetrahydro-furan-(3S)-ylcarbamoyl)-ethyl]-amide

By following the procedure described in Method B, the title compound was prepared in 29% yield from P2-P1 on resin and thiophene-2-carboxylic acid.</td></tr>
<tr><td>  </td><td>MS/ES: m/z 379 (100%, MH^+)</td></tr>
</table>

Example 14 Continued: Non-commercial P3 Building Blocks

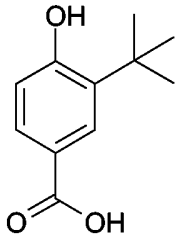
3-Dimethylaminomethyl-4-hydroxy-benzoic acid



A solution of formaldehyde (37% (water), 11.28 mmol, 0.914g, 0.94 eq.) was added to a solution of ethyl 4-hydroxybenzoate (12.00 mmol, 2g, 1 eq.) and dimethylamine (40% (water),

12.75 mmol, 1.43g, 1.06 eq.) in water (5 ml). The solution turned cloudy and was stirred overnight by which time it had cleared. The mixture was then heated to 90°C for 2 hours during which time it turned orange. This was poured into water and extracted with EtOAc. The organics were dried ($MgSO_4$) and concentrated *in vacuo* to give a viscous orange oil (2.57 g, 96%). This was dissolved in wet methanol (20 ml) and NaOH (23 mmol, 0.92 g, 2 eq.) was added. The mixture was heated at reflux for 2 hours then it was cooled and acidified (1M HCl(aq), pH 5). The acidic mixture was lyophilized and the resulting solid extracted with methanol. The solvents were evaporated to give the product as a white solid (1.47g, 61%).

3-tert-Butyl-4-hydroxy-benzoic acid



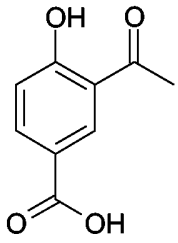
MeI (66.6 mmol, 4.14 ml, 2 eq.) and K_2CO_3 (59.9 mmol, 8.28g, 1.8 eq.) were added to a solution of 2-*tert*butyl phenol (33.3 mmol, 5g) in DMF (60 ml) and the mixture heated at 80C for 24 hours. The mixture was cooled, diluted with diethyl ether then washed with water. The organics were dried ($MgSO_4$) and the solvents removed *in vacuo* to give a yellow oil. This was purified by silica column (isohexane → 10% EtOAc in isohexane) to give the product as a pale yellow oil (1.79g, 33%).

This oil was dissolved in acetonitrile (55ml) and N-bromo-succinimide (10.9 mmol, 1.94g, 1eq.) was added. The mixture was stirred overnight then the solvent was removed *in vacuo*. The residue was partitioned between water and EtOAc. The solvents were evaporated to give the product as a yellow oil (1.79g, 61%).

A portion of this product (4.11 mmol, 1.00 g) was dissolved in THF (5 ml) and added dropwise to a suspension of magnesium (8.22 mmol, 0.20g, 2 eq.) in THF (5 ml) containing 1 crystal of iodine. This mixture was heated at reflux for 1 hour and then allowed to cool to room temperature whereupon it was poured onto vigorously stirred solid CO_2. This was stirred until the mixture came to room temperature. 1MHCl (aq) (10 ml) was added and the organics separated. The aqueous layer was extracted with diethyl ether. The solvents were evaporated to give the product as a pale brown solid (0.37 g, 43%). This was dissolved in DCM (20 ml) and BBr_3 (20 mmol, 5g, 11 eq.) was added. The mixture was stirred for 3 days whilst being

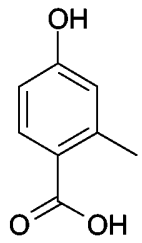
monitored by HPLC. The mixture was treated with HCl solution (0.1M) then filtered. The aqueous layer was evaporated then dissolved in methanol. The solvent was evaporated. The dissolution/evaporation protocol was repeated a further 3 times and gave the pure product as a white solid (0.04 g, 12%).

3-Acetyl-4-hydroxy-benzoic acid



$Mg(ClO_4)_2$ (0.602 mmol, 0.134g, 2 mol%) was added to a solution of ethyl 4-hydroxybenzoate (30.1 mmol, 5g) in Ac_2O (45.15 mmol, 4.25 ml) and the mixture was stirred overnight. This was diluted with DCM then washed with water. The organics were dried ($MgSO_4$) and the solvent evaporated to give a clear oil. This was azeotroped twice with toluene to give the product as a clear oil (5.73g, 92%). This was mixed with $AlCl_3$ (82.5 mmol, 10.99g, 3 eq.) and KCl (28.9 mmol, 2.15g, 1.05 eq.) and heated to 150°C for 1.5 hours during which time a dark foam was formed. This was cooled in an ice bath and ice cold 2MHCl (aq) (100 ml) was added. The solution was stirred for 5 minutes then ethanol (20 ml) was added. This was heated at reflux for 45 minutes then cooled in an ice bath. The solid formed was collected by filtration then purified by recrystallisation from THF/EtOH to give the product as a cream solid (1.30g, 26%).

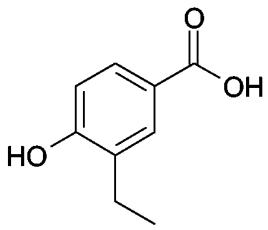
4-Hydroxy-2-methyl-benzoic acid



BBr_3 (20 mmol, 5g, 10 eq.) was added to a solution of 4-methoxy-2-methyl benzoic acid (2 mmol, 0.332g) in DCM (20 ml) and the mixture was stirred under argon until HPLC indicated no starting material remained. HCl (0.1 M, 20 ml) was added and the mixture was filtered. The aqueous layer was evaporated then dissolved in methanol. The solvent was evaporated. The

dissolution/evaporation protocol was repeated a further 3 times and gave the pure product as a yellow solid (0.24g, 80%).

3-Ethyl-4-hydroxy-benzoic acid



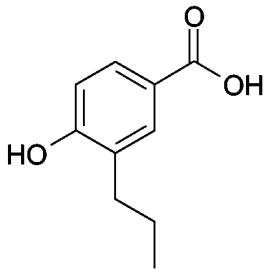
As reference: *J. Am. Chem. Soc.*, 1984, **106**, 174.

A sodium hydroxide solution (5 ml, 20 % w/v) was added to β-cyclodextrin (371 mg, 0.33 mmol) and copper powder (26 mg, 0.41 mmol). Then 2-ethyl phenol (0.48 ml, 4.09 mmol) was added followed by the dropwise addition of carbon tetrachloride (0.77 ml, 7.98 mmol). The reaction was stirred at 80°C under nitrogen for 6 hrs. It was then allowed to cool to room temperature, ethyl acetate was added (10 ml) and the solution was acidified using 1MHCl (aq). The solution was extracted with more ethyl acetate and the combined organics were dried ($MgSO_4$) and the solvent was removed in *vacuo*. Purification by column chromatography (isohexane: ethyl acetate; 1:1) afforded the product (204 mg, 30 %).

HPLC retention time of 3.70 min.

Mass spectroscopy: *m/z* 166 (100, MH^+).

4-Hydroxy-3-propyl-benzoic acid

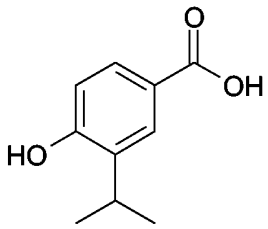


Prepared *via* the same method as above.

HPLC retention time of 4.13 min.

Mass spectroscopy: *m/z* 181 (100, MH^+).

4-Hydroxy-3-isopropyl-benzoic acid

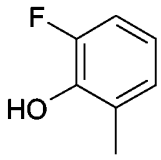


Prepared *via* the same method as above.

HPLC retention time of 4.03 min.

Mass spectroscopy: *m/z* 181 (100, MH^+).

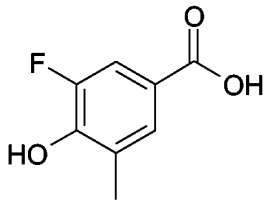
2-Fluoro-6-methyl-phenol



3-Fluorosalicylaldehyde (117 mg, 0.83 mmol) was dissolved in dry ethyl acetate (15 ml) and Pd/C (12mg, 10 % w/w) was added. The solution was vigorously stirred at room temperature under a hydrogen atmosphere for 6 hrs. Filtration through celite and removal of the ethyl acetate under *vacuo* afforded the product (70 mg, 67 %) without need for further purification.

HPLC retention time of 4.09 min.

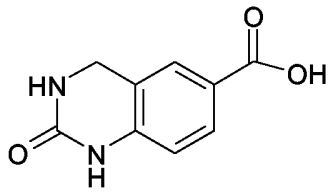
3-Fluoro-4-hydroxy-5-methyl-benzoic acid



Prepared *via* the same method outlined previously.

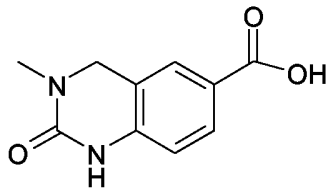
HPLC retention time of 3.17 min.

2-Oxo-1,2,3,4-tetrahydro-quinazoline-6-carboxylic acid



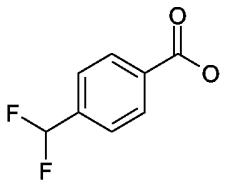
Prepared as described in Eur. J. Org. Chem. 22 2000 3755-62.

3- Methyl -2-oxo-1,2,3,4-tetrahydro-quinazoline-6-carboxylic acid



Prepared as described in Chem. Pharm. Bull. 36 (6) 2253-2258.

4-(Difluoromethyl)benzoic acid

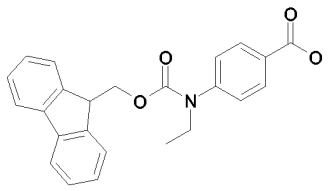


4-Cyanobenzaldehyde (655 mg, 5 mmol) was dissolved in [bis(2-methoxyethyl)amino]sulfur trifluoride (2 ml) – exothermic! The reaction was then heated to 60°C and monitored by HPLC for the disappearance of starting aldehyde (typically 24 to 36 h). After this time, DCM (20 ml) was added and the reaction was poured onto ice. The organic fraction was separated off, dried (Na_2SO_4), filtered and concentrated. Purified on silica (5 % ethyl acetate / iso-hexane) to give 4-(difluoromethyl)benzonitrile as a clear, colourless oil, 345 mg, 45%.

4-(Difluoromethyl) benzonitrile (600 mg, 3.9 mmol) was heated to reflux in 2M aqueous sodium hydroxide solution (20 ml) for 2h. During this time the initial suspension of starting material dissolved. The reaction was cooled and acidified with aqueous 2M HCl solution to give a white

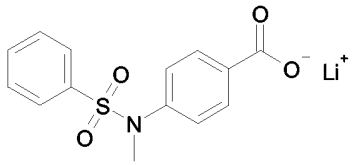
precipitate. This was collected by filtration, washed with water and then dried *in vacuo*. 4-(Difluoromethyl) benzoic acid was obtained as a white powder, 543 mg

4-{ethyl[(9H-fluoren-9-ylmethoxy)carbonyl]amino}benzoic acid



To a mixture of 4-ethylaminobenzoic acid (1.0g, 6.05mmol) in 1,4-dioxane (12ml) and 0.5M aqueous sodium hydroxide solution (12ml) was added 9-fluorenylmethoxycarbonyl chloride (1.72g, 6.66mmol). The mixture was partitioned between 1M HCl (aq) and dichloromethane. The aqueous layer was extracted with dichloromethane and the combined organic extracts were washed with water, brine and dried over sodium sulfate. The solvent was removed and the crude product purified by flash column chromatography on silica to give 4-{ethyl[(9H-fluoren-9-ylmethoxy)carbonyl]amino}benzoic acid as a cream solid.

Lithium 4-[methyl(phenylsulfonyl)amino]benzoate

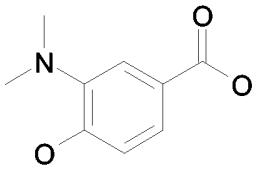


To a mixture of methyl 4-(methylamino)benzoate (1 mmol), 4-dimethylaminopyridine (2 mg), and diisopropylethylamine (1.1 mmol) in acetonitrile (3 ml) was added benzenesulfonyl chloride (1.1 mmol). The reaction mixture was stirred for 16h, and the mixture concentrated by nitrogen stream. The crude product was partitioned between 1M HCl (aq) and ethyl acetate. The aqueous layer was extracted with ethyl acetate and the combined organic extracts were washed with 1M HCl (aq), water, brine and dried over sodium sulfate. The solvent was removed and the crude product was purified by flash column chromatography on silica to give methyl 4-[methyl(phenylsulfonyl)amino]benzoate as a white solid (249 mg, 81%).

Methyl 4-[methyl(phenylsulfonyl)amino]benzoate (0.80 mmol) was dissolved in 1,4-dioxane (5 ml) and 1M LiOH (aq) (0.80mmol) and water (1 ml) were added. After stirring 16h, the sample was concentrated under vacuum, the residue dissolved in 1:1 water-acetonitrile and the mixture

lyophilized to give lithium 4-[methyl(phenylsulfonyl)amino]benzoate as an off-white solid (238mg, 100%).

3-(dimethylamino)-4-hydroxybenzoic acid

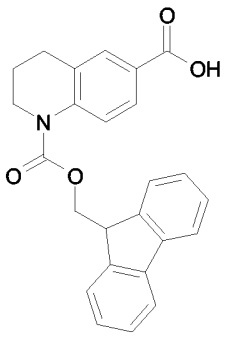


3-Amino-4-hydroxybenzoic acid (459mg, 3 mmol) was dissolved in methanol (12ml) and toluene (36ml) was added. A 2.0M solution of (trimethylsilyl)diazomethane in hexanes (1.5ml, 3.0 mmol) was added dropwise and the mixture stirred for 0.5h. The reaction mixture was concentrated under vacuum and the residue purified by flash column chromatography on silica to afford methyl 3-amino-4-hydroxybenzoate as a pink solid (254mg, 51%).

A buffer solution at pH 5.5 was prepared by the addition of acetic acid to a 1M aqueous sodium acetate solution. Methyl 3-amino-4-hydroxybenzoate (254mg, 1.5 mmol) was dissolved in a mixture of buffer (1ml) and methanol (2ml). Formaldehyde solution (37% by weight in water; 0.75ml, 10mmol) was added, the mixture stirred for 15 minutes, and then sodium cyanoborohydride (283mg, 4.5mmol) was added portionwise. The reaction mixture was stirred for an additional 0.5h and then concentrated. The residual oil was partitioned between water and ethyl acetate. The aqueous layer was extracted with ethyl acetate and the combined organic extracts were washed with water, brine and dried over sodium sulfate. The solvent was removed and the crude product was purified by flash column chromatography on silica to give methyl 3-dimethylamino-4-hydroxybenzoate as a yellow gum (213mg, 73%).

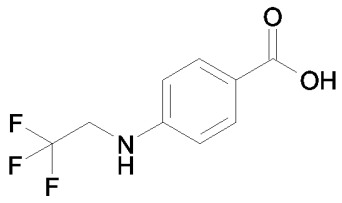
To a solution of methyl 3-dimethylamino-4-hydroxybenzoate (210mg, 1.1 mmol) in 1,4-dioxane (2ml) was added 1M LiOH (aq) (4mmol). After stirring for 40h, the mixture was acidified to pH 2 by addition of 1M HCl (aq). The mixture was partitioned between water and ethyl acetate, and the aqueous layer was lyophilized to give a mixture of sodium chloride and 3-dimethylamino-4-hydroxybenzoic acid as a brown semi-solid (378mg). The crude material was used in the subsequent reaction.

1-[(9H-fluoren-9-ylmethoxy)carbonyl]-1,2,3,4-tetrahydroquinoline-6-carboxylic acid



To a mixture of 1,2,3,4-tetrahydroquinoline-6-carboxylic acid (1.03g, 5.8mmol) in 1,4-dioxane (12ml) and 0.5M aqueous sodium hydroxide solution (12ml) was added 9-fluorenylmethoxycarbonyl chloride (1.68g, 6.5mmol). The mixture was partitioned between 1M HCl (aq) and dichloromethane. The aqueous layer was extracted with dichloromethane and the combined organic extracts were washed with water then brine and dried over sodium sulfate. The solvent was removed in vacuo and the crude product purified by flash column chromatography on silica to give 1-[(9H-fluoren-9-ylmethoxy)carbonyl]-1,2,3,4-tetrahydroquinoline-6-carboxylic acid as a white solid (2.0g; 86%).

4-[(2,2,2-trifluoroethyl)amino]benzoic acid



To a suspension of methyl-4-aminobenzoate (302mg, 2.0mmol) in dichloromethane (3ml) was added trifluoroacetic anhydride (0.31ml, 2.2mmol). The mixture was stirred for 1hour and then partitioned between 1M $NaHCO_3$ (aq) and dichloromethane. The aqueous layer was further extracted with dichloromethane, and the combined organic extracts washed with water then brine and dried over sodium sulfate. The solvent was removed to give methyl 4-[(trifluoroacetyl)amino]benzoate as a white solid (525mg, 100%).

To a stirred solution of methyl 4-[(trifluoroacetyl)amino]benzoate (124mg, 0.5mmol) in an. THF(1ml) at 0°C under an argon atmosphere was added borane-dimethyl sulfide complex (57mg, 0.75mmol) and the mixture heated at reflux for 2h. The mixture was allowed to cool, methanol (approx. 0.1ml) was added dropwise until effervescence ceased. The mixture was partitioned between water and 1:1 ethyl acetate-MTBE. The aqueous layer was extracted three times with ethyl acetate-MTBE, and the combined organic extracts washed with water, brine

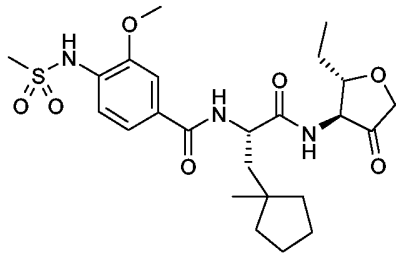
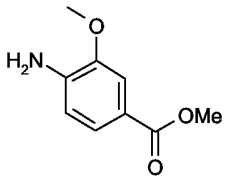
and dried over sodium sulfate. The solvent was removed and the crude product was purified by flash column chromatography on silica to give methyl 4-[(2,2,2-trifluoroethyl)amino]benzoate as a white crystalline solid (47mg, 32%).

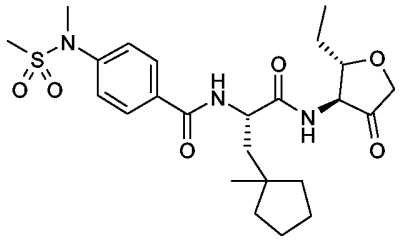
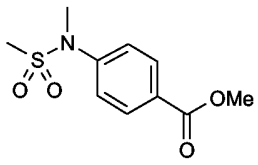
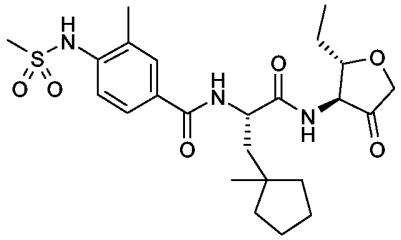
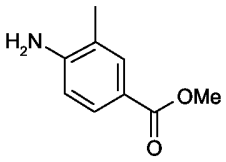
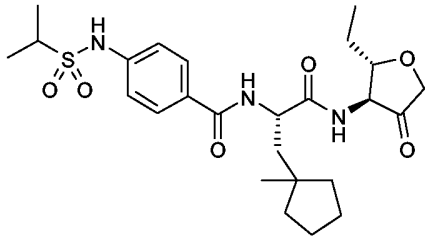
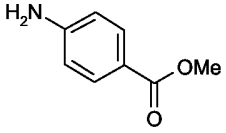
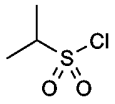
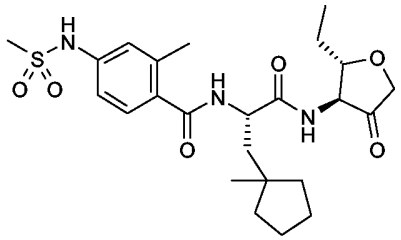
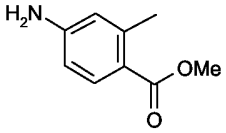
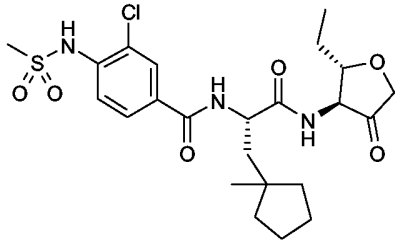
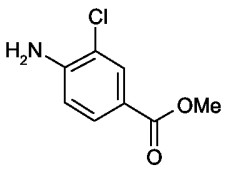
To a solution of methyl 4-[(2,2,2-trifluoroethyl)amino]benzoate (130mg, 0.56 mmol) in 1,4-dioxane (2ml) was added 1M LiOH (aq) (0.61mmol). After stirring at 40C for 4h, further 1M LiOH (aq) (0.30mmol) was added. The mixture was stirred at room temperature until all ester was hydrolysed. The mixture was concentrated and the residue partitioned between 1M HCl (aq) and ethyl acetate. The aqueous layer was further extracted with ethyl acetate and the combined organic extracts were washed with water, brine and dried over sodium sulfate. The solvent was removed to give 4-[(2,2,2-trifluoroethyl)amino]benzoic acid as an off-white solid (119mg, 97%).

Example 15

Solid phase synthesis

Compounds depicted in the table below were synthesized on solid phase by successive coupling of the indicated P3 substituent (if present) to the depicted P3 building block, hydrolysis of the ester protecting group (as necessary) and coupling to the P1-P2 building block. Typical coupling conditions and the construction of P3 building blocks not available from commercial sources appears below the table.

Structure	P3 building block	P3 sub-stituent	MS data $[M+H]^+$
 Example 15.1			510.2

 Example 15.2			494.2
 Example 15.3			494.2
 Example 15.4			508.2
 Example 15.5			494.2
			514.2

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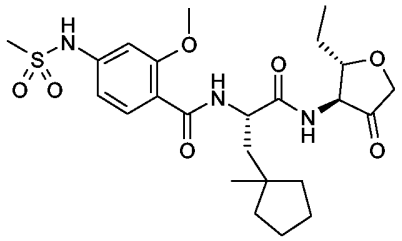
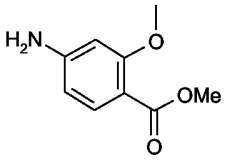
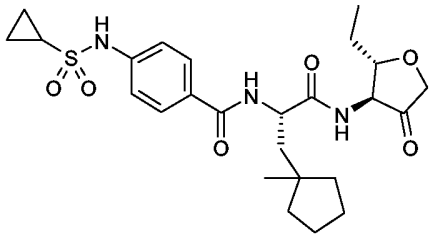
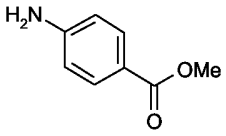
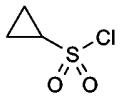
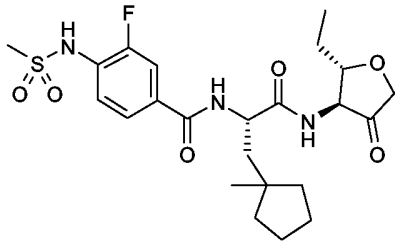
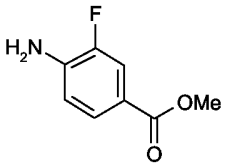
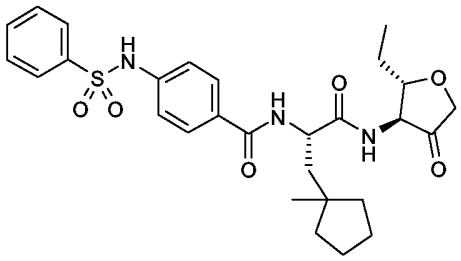
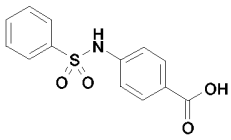
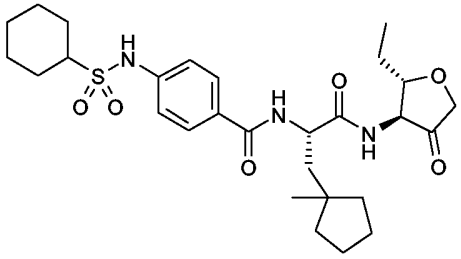
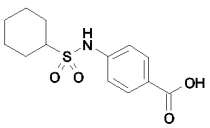
 Example 15.7			510.2
 Example 15.8			506.2
 Example 15.9			498.2
 Example 15.10			542.2
			548.3

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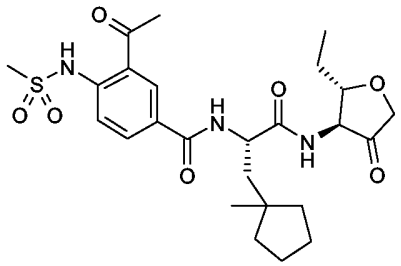
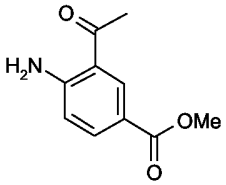
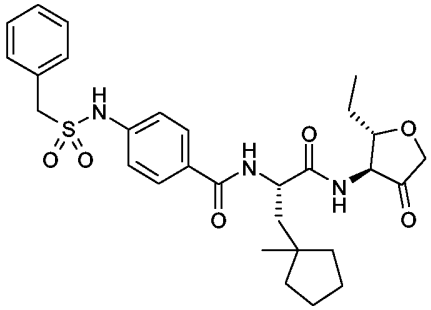
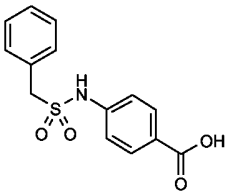
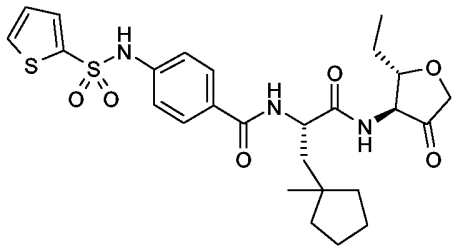
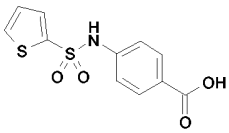
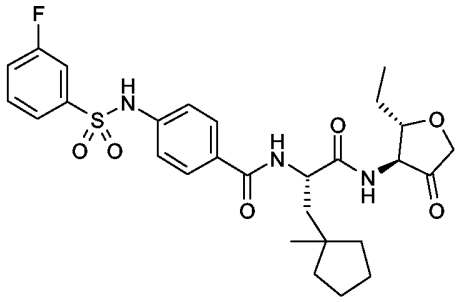
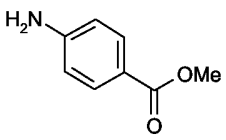
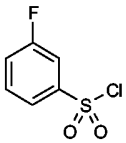
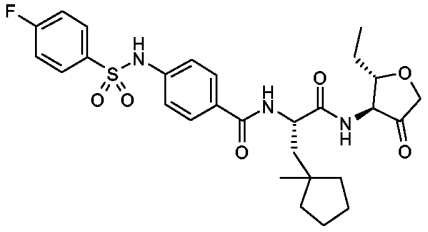
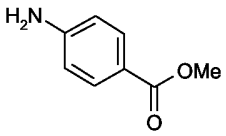
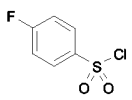
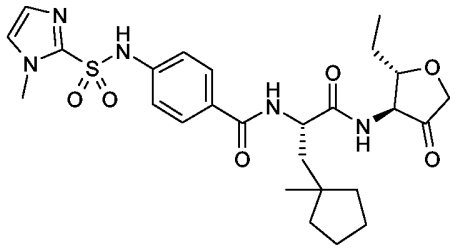
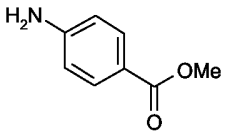
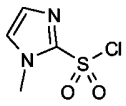
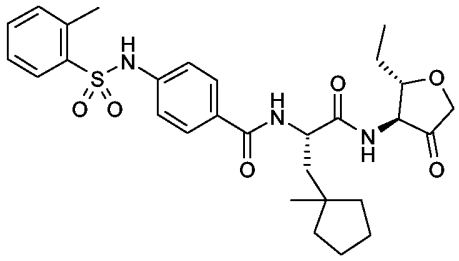
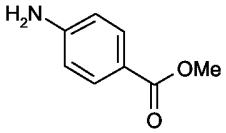
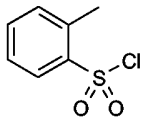
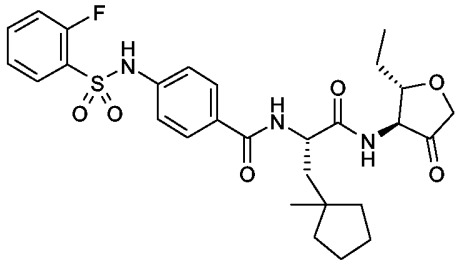
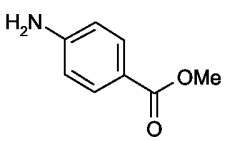
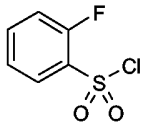
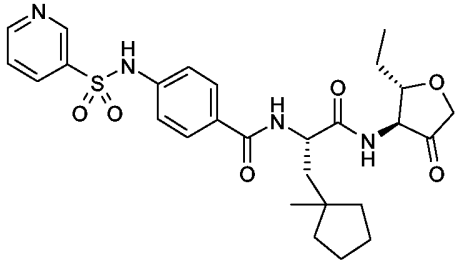
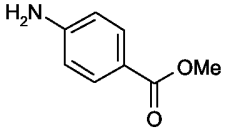
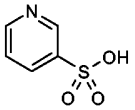
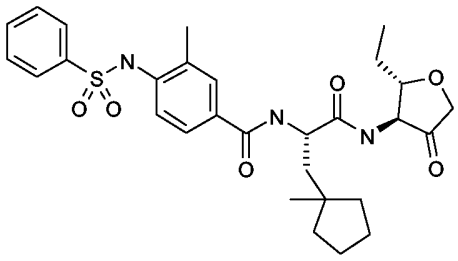
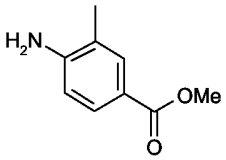
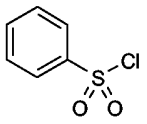
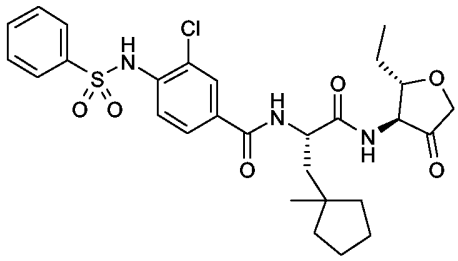
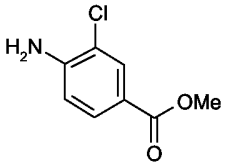
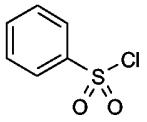
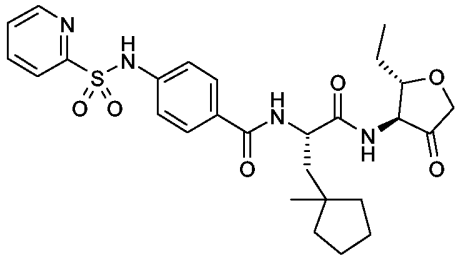
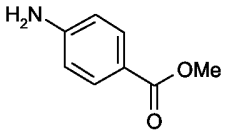
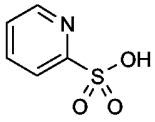
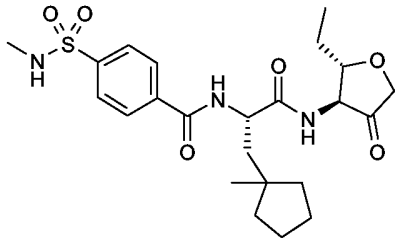
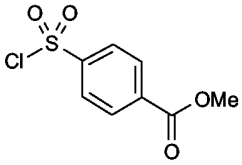
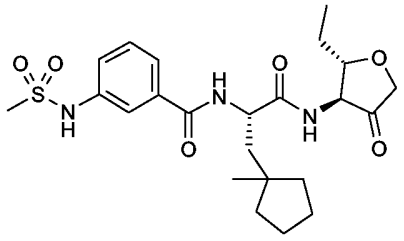
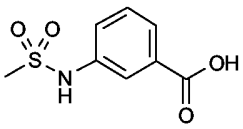
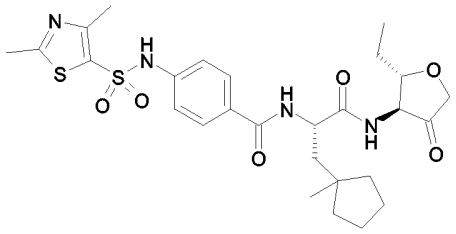
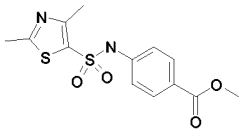
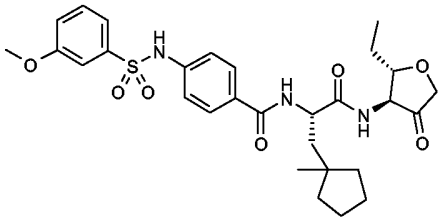
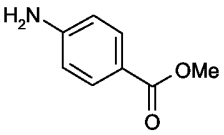
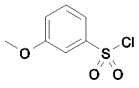
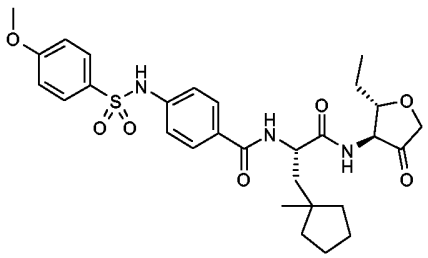
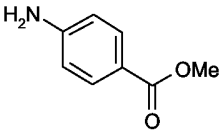
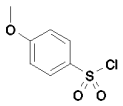
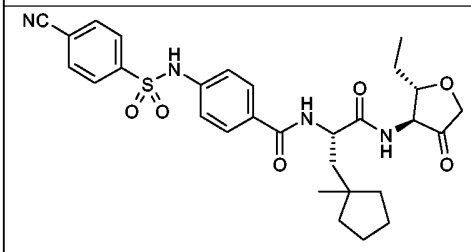
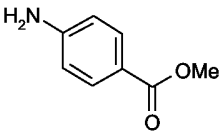
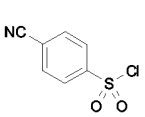
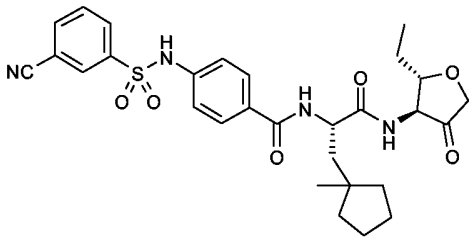
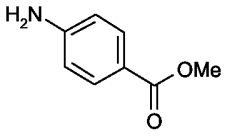
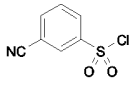
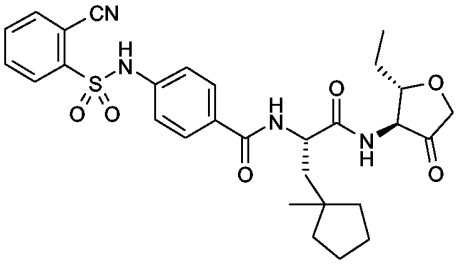
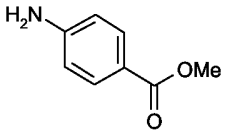
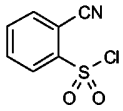
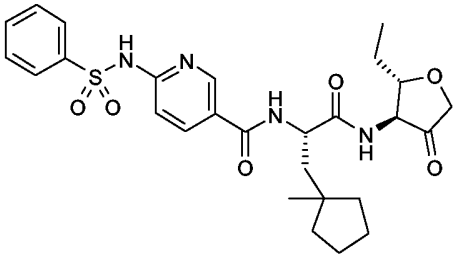
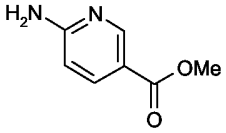
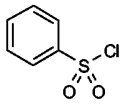
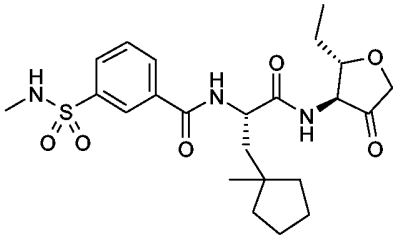
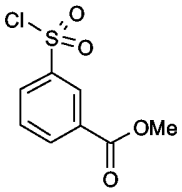
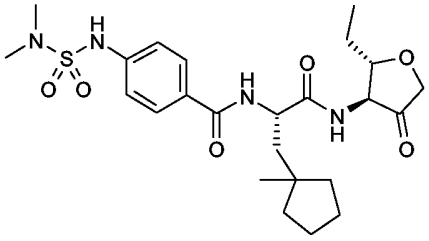
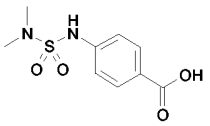
 Example 15.12			522.2
 Example 15.13			556.2
 Example 15.14			548.2
 Example 15.15			560.2

 Example 15.16			560.2
 Example 15.17			546.2
 Example 15.18			556.2
 Example 15.19			560.2
			543.2

Example 15.20			
 Example 15.21			556.2
 Example 15.22			576.2
 Example 15.23			543.2
 Example 15.24			480.2

 Example 15.25			480.2
 Example 15.26			577.2
 Example 15.27			572.2
 Example 15.29			572.2
 Example 15.29			567.2

 Example 15.30			567.2
 Example 15.31			567.2
 Example 15.32			543.2
 Example 15.33			480.2
			509.2

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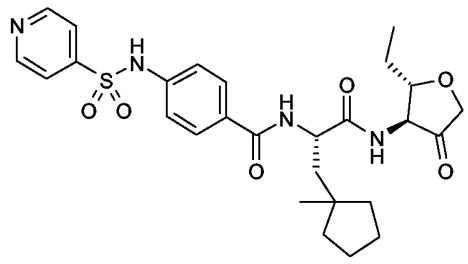
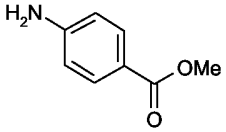
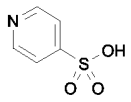
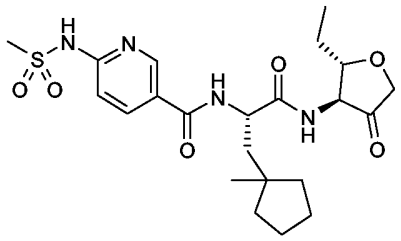
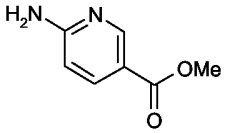
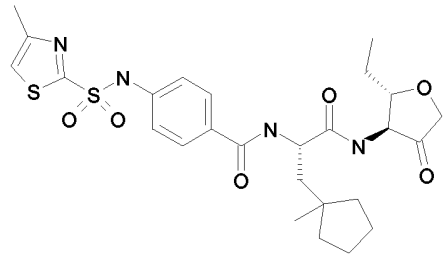
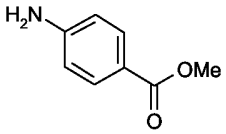
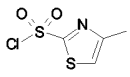
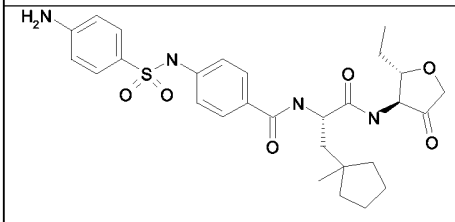
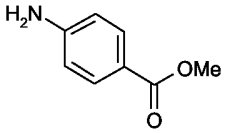
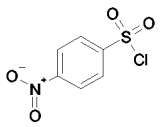
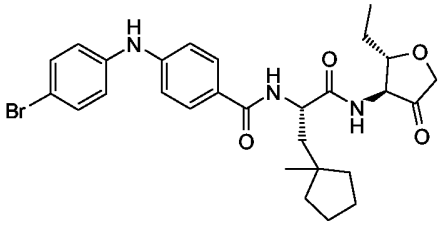
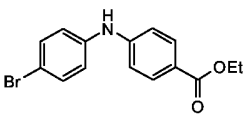
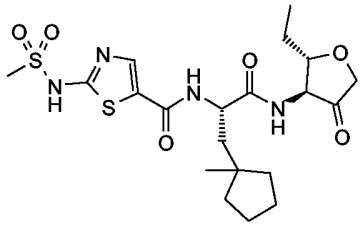
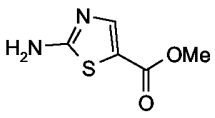
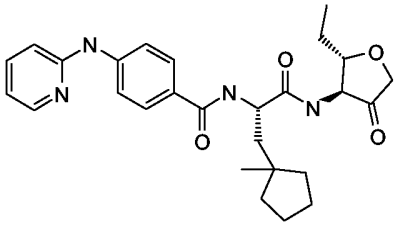
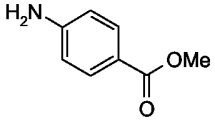
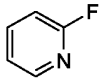
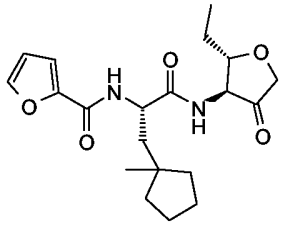
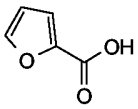
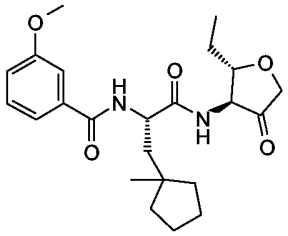
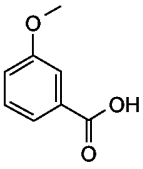
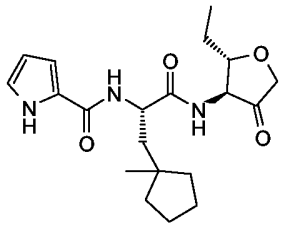
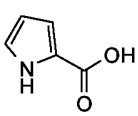
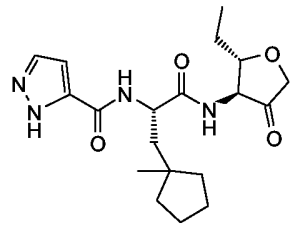
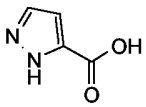
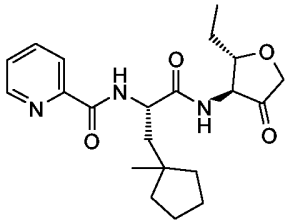
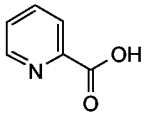
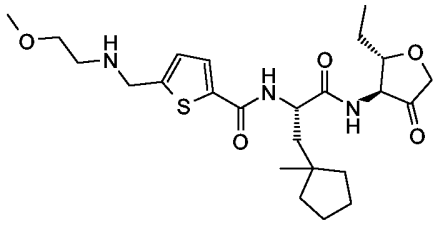
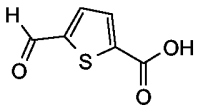
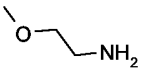
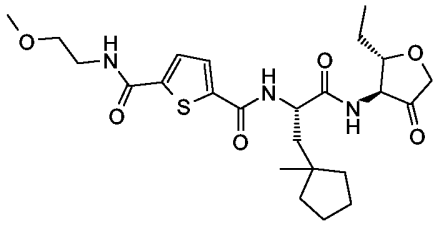
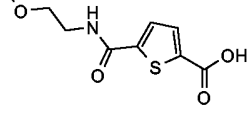
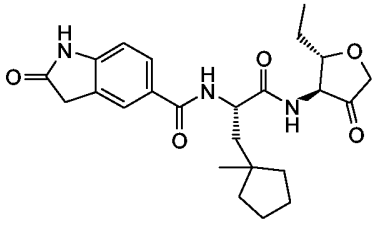
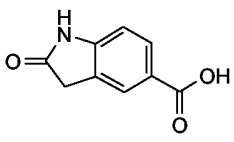
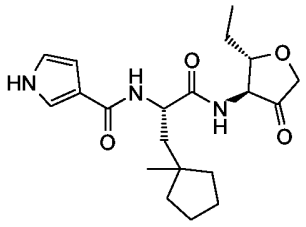
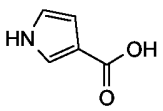
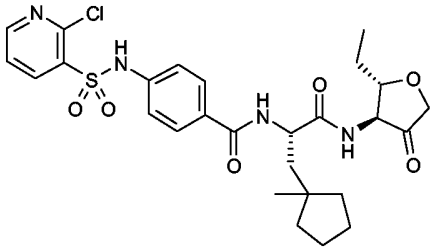
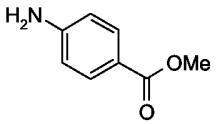
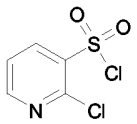
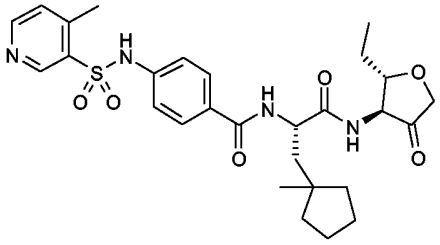
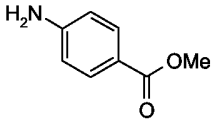
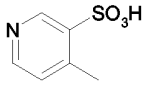
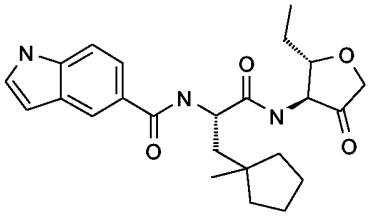
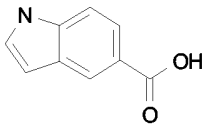
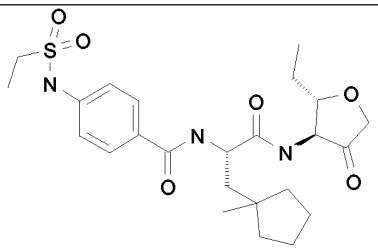
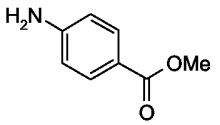
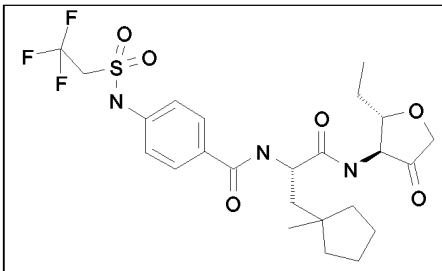
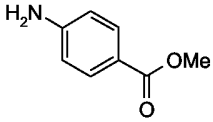
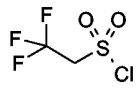
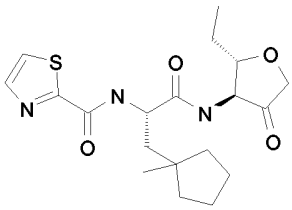
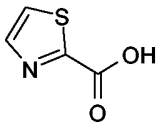
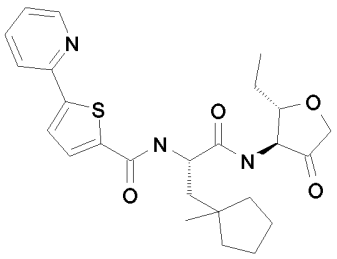
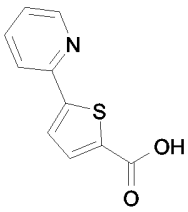
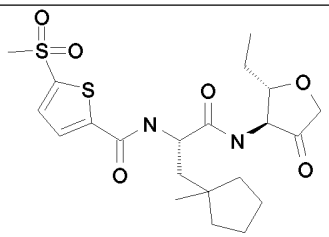
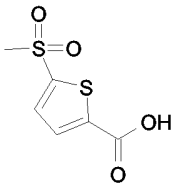
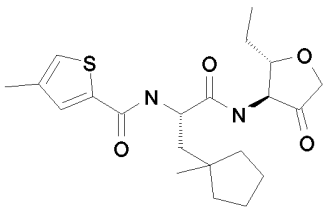
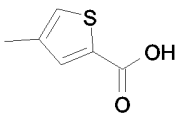
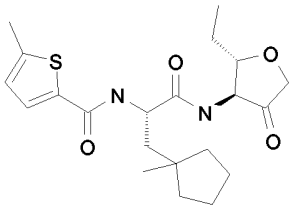
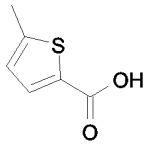
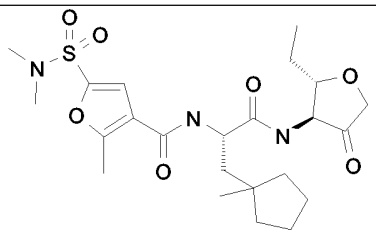
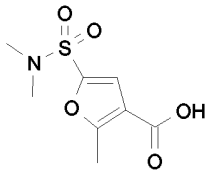
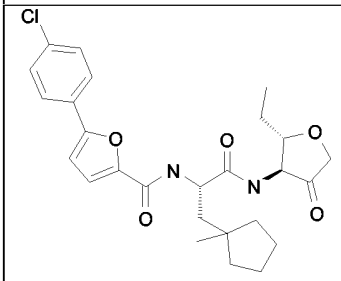
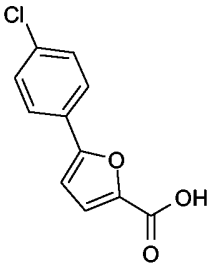
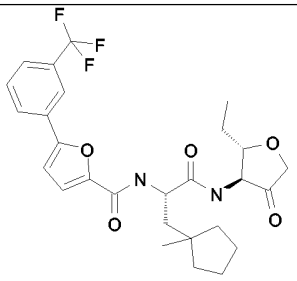
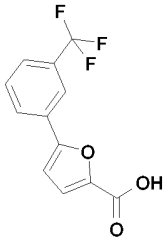
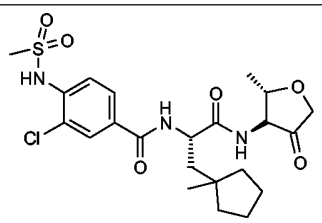
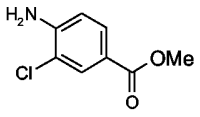
 Example 15.35			543.2
 Example 15.36			481.2
 Example 15.37			563.2
 Example 15.38			557.2
			557.2

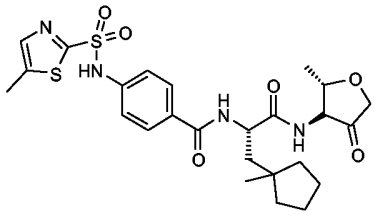
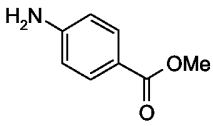
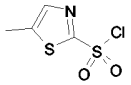
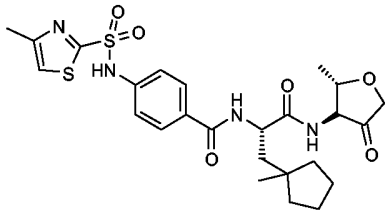
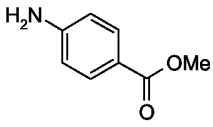
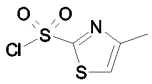
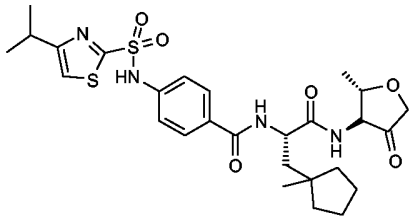
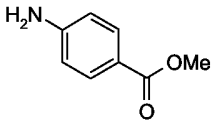
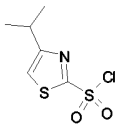
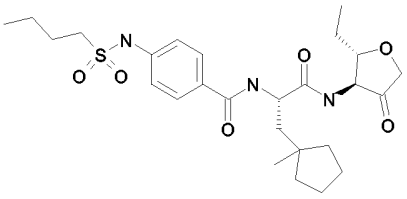
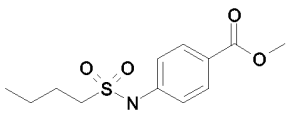
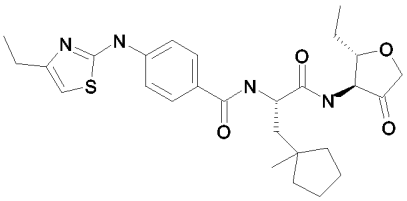
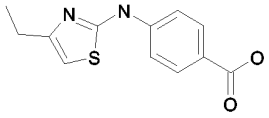
Example 15.39			
 Example 15.40			487.2
 Example 15.41			479.3
 Example 15.42			377.2
 Example 15.43			417.2
			376.2

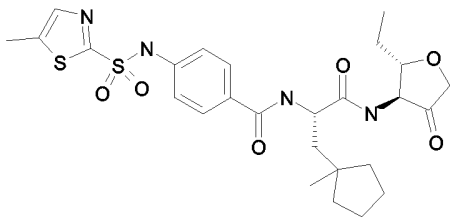
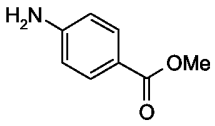
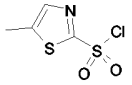
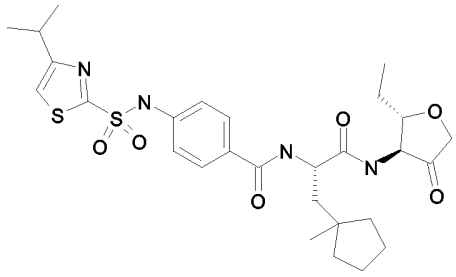
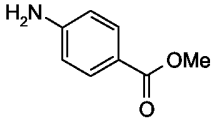
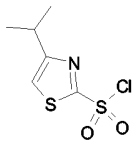
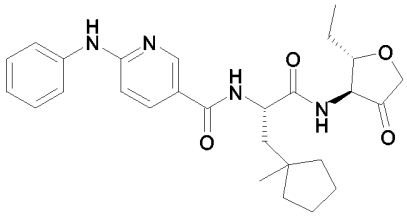
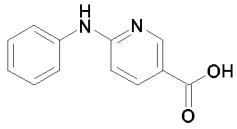
Example 15.44			
 Example 15.45			377.2
 Example 15.46			388.2
 Example 15.47			480.2
 Example 15.48			494.2
 Example 15.49			442.2

 Example 15.50			376.2
 Example 15.51			577.2
 Example 15.52			557.2
 Example 15.53			426.2
 Example 15.54			494.2

 Example 15.55			548.2
 Example 15.56			394.2
 Example 15.57			470.2
 Example 15.58			471.2
 Example 15.59			407.2

 Example 15.60			407.2
 Example 15.61			498.2
 Example 15.62			487.2
 Example 15.63			521.2
 Example 15.64			500.2

 Example 15.65			549.2
 Example 15.66			549.2
 Example 15.67			577.2
 Example 15.68			522.3
 Example 15.69			513.2

 Example 15.70			563.2
 Example 15.71			591.2
 Example 15.72			479.3

Example 15 Continued: Reaction conditions and non-commercial building blocks

Solid phase synthesis of compounds in the table above was carried out using methodology as described in Example 13.

After Fmoc removal the P3 acids were introduced using standard coupling conditions which are exemplified by the following procedure for Example 15.65:

4-(5-Methyl-Thiazole-2-sulfonamino)-benzoic acid (39 mg, 0.13 mmol), HOBt (19 mg, 0.12 mmol), HBTU (45 mg, 0.12mmol) and NMM (25µl, 0.24 mmol) were added to the resin bound P1-P2 building block (170mg, 0.025mmol) in DMF (6mL). The reaction was stirred for 16hrs. After filtration of the resin and washing with DCM and MeOH, the title compound was obtained when cleaved from the resin with 95% TFA in water. After concentration the product was purified on HPLC and freeze dried. The product was characterized by HPLC-MS and NMR.

N-[1-(2-Ethyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-methanesulfonylamino-3-methoxy-benzamide (Example 15.1)

Methanesulfonyl chloride (615 uL) was added to a solution of 4-Amino-3-methoxy-benzoic acid methyl ester (1 g) in dichloromethane (20 mL) and pyridine (1.5 mL) and a catalytic amount of DMAP. After 1-16 hrs the mixture was concentrated to near dryness and the product crystallized from added ethanol. This product was hydrolyzed in 2.5 M LiOH (5 mL), THF (14 mL), MeOH (7 mL) in a microwave oven at 110 deg C for 30 min. After cooling, the solution was acidified with aq. HCl and extracted with ethyl acetate, dried with Na_2SO_4 and concentrated to dryness. The remaining powder was used for coupling to the resin bound P1-P2 building block (described above). The title compound was obtained when cleaved from the resin with 95% TFA in water. After concentration the product was purified on HPLC and freeze dried. The product was characterized by HPLC-MS and NMR.

For the synthesis of Examples 15.33, 15.4, 15.6, 15.7, 15.8, 15.9, 15.15, 15.16, 15.17, 15.18, 15.19, 15.22, 15.24, 15.27, 15.28, 15.32, 15.33, 15.36, 15.40, 15.51, 15.54, 15.64, 15.70 and 15.71 the same procedure as in Example 15.1 was followed.

For the synthesis of Examples 15.10, 15.11, 15.13, 15.14, 15.25, 15.34, 15.39, 15.42, 15.43, 15.44, 15.45, 15.46, 15.49, 15.50, 15.53, 15.56, 15.57, 15.58, 15.59, 15.60, 15.61, 15.62, 15.63 and 15.72 the P3 building block came from commercial sources. The rest of the synthesis followed the procedure from Example 15.1.

The P3 building blocks of Examples 15.2, 15.5, 15.12, 15.26, 15.37, 15.48, 15.68, 15.69, 15.71 and the P3 substituents for examples 15.35, 15.41, 15.65, 15.66, 15.67, 15.70, 15.71 were synthesized according to procedures presented below. Subsequent synthesis followed the procedure from Example 15.1.

4-(Methanesulfonyl-methyl-amino)-benzoic acid methyl ester (P3 building block for Example 15.2)

A mixture of 4-Methanesulfonylamino-benzoic acid methyl ester (0.5 g), methyl iodide (0.4 mL) and potassium carbonate (0.9 g) in acetonitrile (10 mL) was kept in a microwave oven at 120 deg C for 10 min. The cooled mixture was filtered and concentrated to dryness. The remains were precipitated from added DCM and the solid was collected, dried in a vacuum and used in the next step.

4-Amino-2-methyl-benzoic acid methyl ester (P3 building block for Example 15.5)

4-Acetylamino-2-methyl-benzoic acid methyl ester was kept in conc. HCl/MeOH 1:1 in a microwave oven at 70 deg C for 2 hrs. After cooling the solid was collected, dried in a vacuum and used in the next step.

3-Acetyl-4-amino-benzoic acid methyl ester (P3 building block for Example 15.12)

5-Amino-furan-2-carboxylic acid methyl ester (0.42 g, 3.0 mmol) were mixed together with methyl vinyl ketone (10 mL) in benzene and heated at reflux for 1h. Evaporation of solvents were followed by flash chromatography using DCM / MeOH (95:5) as eluent to yield 44% (278 mg. 1.31 mmol) of 5-Acetyl-4-amino-1-hydroxy-cyclohexa-2,4-dienecarboxylic acid methyl ester. This compound were mixed with BF_3 OEt_2 ((284 mg, 2.0 mmol) in benzene (15 mL) and refluxed for 0.5 h. The reaction mixture was quenched with $NaHCO_3$ (sat) and extracted with dichloromethane. A precipitation formed in the organic phase was collected and confirmed to be the product by characterization with LC-MS and ^1H NMR. Yield: 127 mg (50%).

N-[1-(2-Ethyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-(pyridine-3-sulfonylamino)-benzamide (Example 15.20)

Trifluoromethane sulfonic anhydride (380uL) was added to polymer supported tiriphenylphosphine oxide (1g) in dichloromethane (15mL). After 1hrs the mixture was cooled to 0 deg C and a solution of pyridine 3-sulfonic acid (360 mg) as pyridine salt in DCM (4mL) was added. After 30 min. 4-Methanesulfonylamino-benzoic acid methyl ester (318 mg) in dichloromethane (4mL) was added. The mixture was shaken at 25 deg C for 16 hrs. The resin was filtered off and the filtrate concentrated to dryness. The crude was purified by silica column chromatography. Subsequent synthesis was done according to the procedure in Example 1.

For the synthesis of Examples 15.23, 15.35, and 15.52 the same procedure as in Example 15.20 was followed.

4-Benzenesulfonylamino-N-[1-(2-ethyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-3-methyl-benzamide (Example 15. 21)

For synthesis of P3 cap see procedure for P3 building block for Example 15.68. Subsequent synthesis was done according to Example 15.1.

4-(2,4-Dimethyl-thiazole-5-sulfonylamino)-benzoic acid methyl ester (P3 building block for Example 15.26)

See procedure for P3 cap Example 15.68.

4-(4-Cyano-benzenesulfonylamino)-N-[1-(2-ethyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-benzamide (Example 15.29)

Example 15.29 was synthesized via solid phase synthesis methodology. First coupling of the 4-Amino-benzoic acid to the P1-P2 building block was done followed by washing as described in WO00/69055. Secondly 4-Cyano-benzenesulfonyl chloride (53.2 mg, 0.26 mmol) and a catalytic amount DMAP dissolved in pyridine (2 mL) and DCM (4 mL) was added to the P1-P2 building block (220 mg, 0.053 mmol). The reaction was left on agitation at room temperature over weekend. Cleavage from resin was done by addition of 95% TFA (aq, 6 mL) and agitation for 0.5 h. Toluene (3 mL) was added after filtration from resin, followed by evaporation. Subsequent purification and characterization was done according to the procedure in Example 15.1.

For the synthesis of Examples 15.30 and 15.31 the same procedure as in Example 15.29 was followed.

Pyridine 4-sulfonic acid (P3 substituent for Example 15.35)

4-Mercaptopyridine (500 mg) was dissolved in glacial acetic acid (18mL), followed by the addition of 35% hydrogen peroxide (6mL). The solution was warmed at 80 deg C for 90 min. and then concentrated to dryness. The product was re-crystallized from methanol:water, dried in a vacuo and used in the next step.

Subsequent synthesis to P3 building block was done according to the procedure in Example 20.

N-[1-(2-Ethyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-(4-methyl-thiazole-2-sulfonylamino)-benzamide (P3 substituent for Example 15.37)

4-methyl-1,3-thiazole (2.0 g, 20.2 mmol) was dissolved in methyl-*t*-butyl ether (46 mL) and the solution was cooled to 0°C. Addition of isopropyl magnesium chloride (10.1 mL, 2.0 M) was done drop wise at 0°C. The mixture was then heated to 40°C and sulfur dioxide in dimethoxymethane (4.2 mL, 6.0 M) was added drop wise and the reaction was then left at this temperature for 1h. After cooling the reaction mixture to 0°C, *N*-chlorosuccinimide (4.05 g, 30.3 mmol) was added, and the reaction were kept at 0°C for 45 min. After addition of HCl (0.2 M, aq, 50 mL) at 0°C the reaction was left to warm up to ambient temperature for 2 hrs and extracted with methyl-*t*-butyl ether. The organic phase was washed with of HCl (0.2 M, aq),

water and brine then dried over Na_2SO_4, filtered and evaporated to yield 2.33 g (59%) of product.

4-(4-Amino-benzenesulfonylamino)-N-[1-(2-ethyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-benzamide (Example 15.38)

Example 15.38 was synthesized as described in Example 15.1 by successive coupling of the P3 substituent to the P3 building block, hydrolysis of the ester and coupling to the P1-P2 building block. After cleavage from P1-P2 resin, reduction of nitro group was done by dissolving 4-(4-Nitro-benzenesulfonylamino)-N-[1-(2-ethyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-benzamide (13.9 mg, 23.4 µmol) in MeOH (3mL) and degassing the solution with N_2 gas. A catalytic amount of palladium on carbon was then added to the reaction solution and a H_2 atmosphere was connected. After 2 hrs, filtration through celite was done, with MeOH as eluent, to yield 11.8 mg (91%) of product after concentration.

N-[1-(2-Ethyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-(pyridin-2-ylamino)benzamide (Example 15.41)

4-Aminobenzoic acid methyl ester (1 g, 6.6 mmol), 2-fluoropyridine (1.28 g, 13.2 mmol) and potassium carbonate (1.83 g, 13.2 mmol) in DMF was heated in a microwave oven at 200°C for 20 min. The residue was extracted with dichloromethane and water. The organic layer was dried and concentrated, purified on a SiO2 column (Toluene-EtOAc 8:2) to give 4-(Pyridin-2-ylamino)-benzoic acid methyl ester.

Subsequent synthesis was done according to procedure in Example 15.1.

5-[(2-Methoxy-ethylamino)-methyl]-thiophene-2-carboxylic acid [1-(2-ethyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-amide (Example 15.47)

5-Formyl-thiophene-2-carboxylic acid was coupled to the resin as described in example 1. The resin was swollen in DCM-trimethylortoformate 1:1 and 4 equiv. of methoxyethylamine was added. After 4 hrs of agitation the resin was washed with DCM and MeOH (2X) and the resin was dried. To this resin in DCM-MeOH-HOAc 2:2:1 borane-pyridine complex was added. After 16 hrs of agitation the resin was washed and cleaved and purified as described in Example 15.1.

5-(2-Methoxy-ethylcarbamoyl)-thiophene-2-carboxylic acid (P3 building block for Example 15.48)

5-Formyl-thiophene-2-carboxylic acid (740 mg, 4.7 mmol), methoxyethylamine (412 µL, 4.7 mmol), HATU (4.5 g 11.8 mmol), ethyldiisopropylamine (4 ml) and DMF (2 m) in DCM (20 ml) was stirred for 2 hrs. The mixture was extracted with DCM and aq. bicarbonate. The organic layer was dried and concentrated. This residue was oxidized in t-BuOH (4ml) by the addition of sodium phosphate buffer (0.5M, 2 ml) and aq. KMnO4 (1M, 2ml). After 10 min. addition of sat. aq. Na2SO4 (2ml) was done and pH was adjusted to 3 with aq. HCl. Extracted with EtOAc. Organic layer was dried and concentrated.

N-[1-(2-Ethyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-2-(1-methyl-cyclopentyl)-ethyl]-4-(2,2,2-trifluoro-ethanesulfonylamino)-benzamide (Example 15.55)

4-Aminobenzoic acid (16.5mg, 0.12 mmol) was used for coupling to the resin bound P1-P2 building block (described above). 2,2,2, trifluoromethane sulfonyl chloride (21.1µl, 0.192 mmol), Pyridine (31µl, 0.384 mmol), and catalytic amount of DMAP were added to the resin in DCM (6mL) and stirred for 16 hrs. After filtration of the resin and washing with DCM and MeOH, the title compound was obtained when cleaved from the resin with 95% TFA in water. After concentration the product was purified on HPLC and freeze dried. The product was characterized by HPLC-MS. Yield: 7.3 mg (44%).

4-(5-Methyl-Thiazole-2-sulfonamino)-benzoic acid (P3 substituent for Example 15.65)

See procedure for P3 substituent Example 15.70

4-(4-Methyl-Thiazole-2-sulfonamino)-benzoic acid (P3 substituent for Example 15.66)

See procedure for P3 substituent Example 15.37

4-(4-Isopropyl-Thiazole-2-sulfonamino)-benzoic acid (P3 substituent for Example 15.67)

See procedure for P3 substituent Example 15.71

4-(Butane-1-sulfonylamino)-benzoic acid methyl ester (P3 building block for Example 15.68)

4-Amino-benzoic acid methyl ester (1.0 g, 6.62 mmol) and a catalytic amount of DMAP were dissolved in pyridine (0.5 mL) and DCM (15 mL). The reaction mixture was cooled to 0 °C and butylsulfonyl chloride (1.01 mL, 6.62 mmol) was added via a syringe. The reaction was left stirring and allowed to warm up to room temperature over night. Evaporation of solvents was followed by addition of DCM and the organic phase was washed with HCl (aq, 1 M), water and

brine. After being dried over Na_2SO_4, the organic phase was filtered and evaporated to yield 1.34 g (74%) of 4-(butane-1-sulfonylamino)-benzoic acid methyl ester.

Subsequent hydrolysis of 4-(butane-1-sulfonylamino)-benzoic acid methyl ester was done according to the procedure in Example 15.1.

4-(4-Ethyl-thiazol-2-ylamino)-benzoic acid (P3 building block for Example 15.69)

4-Thioureido-benzoic acid ethyl ester (0.52 g, 2.23 mmol) and 1-Bromo-butan-2-one (0.25 mL, 2.45 mmol) was mixed in dioxane (4 mL) and microwave heated at 110 °C for 15 min. To the precipitated crystals were added DCM and $NaHCO_3$. The organic phase was after separation washed with water and brine, dried over Na_2SO_4, filtered and evaporated to yield 0.58 g (93%) of 4-(4-Ethyl-thiazol-2-ylamino)-benzoic acid ethyl ester. Subsequent hydrolysis of 4-(4-Ethyl-thiazol-2-ylamino)-benzoic acid ethyl ester was done according to the procedure in Example 15.1.

5-Methyl-thiazole-2-sulfonyl chloride (P3 substituent for Example 15.70)

5-methyl-1,3-thiazole (1.0 g, 10.1 mmol) was dissolved in methyl-*t*-butyl ether (25 mL) and the solution was cooled to 0°C. Addition of isopropyl magnesium chloride (10.1 mL, 2.0 M) was done drop wise at 0°C. The mixture was then heated to 40°C and sulfur dioxide in dimethoxyethane (1.64 mL, 7.7 M) was added drop wise and the reaction was then left at this temperature for 45 min. After cooling the reaction mixture to 0°C, *N*-chlorosuccinimide (2.02 g, 15.2 mmol) was added and the reaction were kept at 0°C for 1 h. After addition of HCl (aq, 0.2 M, 25 mL) at 0°C the reaction was left to warm up to ambient temperature for 2 hrs and extracted with methyl-*t*-butyl ether. The organic phase was washed with of HCl (aq, 0.2 M), water and brine then dried over Na_2SO_4, filtered and evaporated to yield 1.82 g (91%) of product.

4-Isopropyl-thiazole-2-sulfonyl chloride (P3 substituent for Example 15.71)

1-Bromo-3-methyl-butan-2-one (1.15 g, 6.95 mmol) and thioformamide (0.43 g, 6.95 mmol) were dissolved in dioxane (10 mL) and heated in microwave at 110 °C for 15 min. Dichloromethane and $NaHCO_3$ was added and after separation the organic phase was washed with NaOH (aq, 1 M) and water. Back-extracted water phase with dichloromethane. The combined organic phases were washed with water and brine, dried over Na_2SO_4, filtered and evaporated to yield 0.82g (70%) of 4- isopropyl-1,3-thiazole.

4-Isopropyl-1,3-thiazole (0.62 g, 4.9 mmol) was dissolved in methyl-*t*-butyl ether (15 mL) and the solution was cooled to 0°C. Addition of isopropyl magnesium chloride (2.9 mL, 2.0 M) was done drop wise at 0°C. The mixture was then heated to 40°C and sulfur dioxide in dimethoxyethane (0.79 mL, 7.7 M) was added drop wise and the reaction was then left at this temperature for 45 min. After cooling the reaction mixture to 0°C, *N*-chlorosuccinimide (0.97 g, 7.3 mmol) was added, and the reaction were kept at 0°C for 1 h. After addition of HCl (aq, 0.2 M, 10 mL) at 0°C the reaction was left to warm up to ambient temperature for 2 hrs and extracted with methyl-*t*-butylether. The organic phase was washed with of HCl (aq, 0.2 M), water and brine then dried over Na_2SO_4, filtered and evaporated to yield 0.92 g (84%) of product.

Biological Example 1

Cathepsin S Ki determination

The assay uses baculovirus-expressed human cathepsin S and the boc-Val-Leu-Lys-AMC fluorescent substrate available from Bachem in a 384 well plate format, in which 7 test compounds can be tested in parallel with a positive control comprising a known cathepsin S inhibitor comparator.

Substrate dilutions

280µl/well of 12.5% DMSO are added to rows B – H of two columns of a 96 deep well polypropylene plate. 70µl/well of substrate is added to row A. 2 x 250µl/well of assay buffer (100mM Na phosphate, 100mM NaCl, pH 6.5) is added to row A, mixed, and double diluted down the plate to row H.

Inhibitor dilutions.

100µl/well of assay buffer is added to columns 2-5 and 7-12 of 4 rows of a 96 well V bottom polypropylene plate. 200µl/well of assay buffer is added to columns 1 and 6.

The first test compound prepared in DMSO is added to column 1 of the top row, typically at a volume to provide between 10 and 30 times the initially determined rough K_i. The rough Ki is calculated from a preliminary run in which 10 µl/well of 1mM boc-VLK-AMC (1/10 dilution of 10 mM stock in DMSO diluted into assay buffer) is dispensed to rows B to H and 20 µl/well to row A of a 96 well Microfluor ™ plate. 2 µl of each 10mM test compound is added to a separate well on row A, columns 1-10. Add 90 µl assay buffer containing 1mM DTT and 2 nM cathepsin S to

each well of rows B-H and180 µl to row A.Mix row A using a multichannel pipette and double dilute to row G. Mix row H and read in the fluorescent spectrophotometer. The readings are Prism data fitted to the competitive inhibition equation, setting S = 100µM and K_M = 100µM to obtain an estimate of the K_i, up to a maximum of 100µM.

The second test compound is added to column 6 of the top row, the third to column 1 of the second row etc. Add 1µl of comparator to column 6 of the bottom row. Mix column 1 and double dilute to column 5. Mix column 6 and double dilute to column 10.

Using an 8-channel multistepping pipette set to 5 x 10µl, distribute 10µl/well of substrate to the 384 well assay plate. Distribute the first column of the substrate dilution plate to all columns of the assay plate starting at row A. The tip spacing of the multichannel pipette will correctly skip alternate rows. Distribute the second column to all columns starting at row B.

Using a 12-channel multistepping pipette set to 4 x 10µl, distribute 10µl/well of inhibitor to the 384 well assay plate. Distribute the first row of the inhibitor dilution plate to alternate rows of the assay plate starting at A1. The tip spacing of the multichannel pipette will correctly skip alternate columns. Similarly, distribute the second, third and fourth rows to alternate rows and columns starting at A2, B1 and B2 respectively.

Mix 20ml assay buffer and 20µl 1M DTT. Add sufficient cathepsin S to give 2nM final concentration.

Using the a distributor such as a Multidrop 384, add 30µl/well to all wells of the assay plate and read in fluorescent spectrophotomoter such as an Ascent.

Fluorescent readings, (excitation and emission wavelengths 390nm and 460nm respectively, set using bandpass filters) reflecting the extent of enzyme cleavage of the fluorescent substrate, notwithstanding the inhibitor, are linear rate fitted for each well.

Fitted rates for all wells for each inhibitor are fitted to the competitive inhibition equation using SigmaPlot 2000 to determine V, Km and Ki values. The majority of the compounds illustrated above provide Ki values of 100 nM or less in this assay.

Biological Example 2

Cathepsin K Ki

The procedure of Biological Example 1 with the following amendments is used for the determination of Ki for cathepsin K.

The enzyme is E coli expressed human cathepsin K. The substrate is H-D-Ala-Leu-Lys-AMC from Bachem. The assay buffer is 100 mM Na phosphate, 1mM EDTA, 0.1% PEG 4000, pH 6.5. The DMSO stock (see substrate dilutions) is diluted to 10% in assay buffer . 56 ul of substrate is added to row A and 2 x 256 ul of buffer is added to row A. The final cathepsin K concentration is 0.5 nM. The majority of compounds illustrated above provide selectivities over cathepsin K of at least 10-100 fold.

Biological Example 3

Cathepsin L Ki

The procedure of Biological Example 1 with the following amendments is used for the determination of Ki for cathepsin L.

The enzyme is commercially available human cathepsin L (for example Calbiochem). The substrate is H-D-Val-Leu-Lys-AMC available from Bahcem. The assay buffer is 100mM sodium acetate 1mM EDTA, pH5.5) The DMSO stock (10mM in 100%DMSO) is diluted to 10% in assay buffer. Enzyme is prepared at 5nM concentration in assay buffer plus 1mM dithiothreitol just before use. 2ul of 10mM inhbitor made up in 100% DMSO is dispensed into row A. 10ul of 50 uM substrate (=1/200 dilution of 10mM stock in DMSO,diluted in assay buffer.) The majority of the compounds illustrated above provide selectivity over cathepsin L of at least 10-100 fold.

Biological Example 4

Permeability

This example measures transport of inhibitors through the cells of the human gastroenteric canal. The assay uses the well known Caco-2 cells with a passage number between 40 and 60.

Apical to basolateral transport

Generally every compound will be tested in 2-4 wells. The basolateral and the apical wells will contain 1.5 mL and 0.4 mL transport buffer (TB), respectively, and the standard concentration of the tested substances is 10 μM. Furthermore all test solutions and buffers will contain 1% DMSO. Prior to the experiment the transport plates are pre-coated with culture medium

containing 10% serum for 30 minutes to avoid nonspecific binding to plastic material. After 21 to 28 days in culture on filter supports the cells are ready for permeability experiments.

Transport plate no 1 comprises 3 rows of 4 wells each. Row 1 is denoted Wash, row 2 "30 minutes" and row 3 "60 minutes". Transport plate no 2 comprises 3 rows of 4 wells, one denoted row 4 "90 minutes", row 5 "120 minutes and the remaining row unassigned.

The culture medium from the apical wells is removed and the inserts are transferred to a wash row (No. 1) in a transport plate (plate no.1) out of 2 plates without inserts, which have already been prepared with 1.5 mL transport buffer (HBSS, 25 mM HEPES, pH 7.4) in rows 1 to 5. In A→B screening the TB in basolateral well also contains 1% Bovine Serum Albumin.

0.5 mL transport buffer (HBSS, 25 mM MES, pH 6.5) is added to the inserts and the cell monolayers equilibrated in the transport buffer system for 30 minutes at 37 °C in a polymix shaker. After being equilibrated to the buffer system the Transepithelial electrical resistance value (TEER) is measured in each well by an EVOM chop stick instrument. The TEER values are usually between 400 to 1000 Ω per well (depends on passage number used).

The transport buffer (TB, pH 6.5) is removed from the apical side and the insert is transferred to the 30 minutes row (No. 2) and fresh 425 μL TB (pH 6.5), including the test substance is added to the apical (donor) well. The plates are incubated in a polymix shaker at 37°C with a low shaking velocity of approximately 150 to 300 rpm.

After 30 minutes incubation in row 2 the inserts will be moved to new pre-warmed basolateral (receiver) wells every 30 minutes; row 3 (60 minutes), 4 (90 minutes) and 5 (120 minutes).

25 μL samples will be taken from the apical solution after ~2 minutes and at the end of the experiment. These samples represent donor samples from the start and the end of the experiment.

300 μL will be taken from the basolateral (receiver) wells at each scheduled time point and the post value of TEER is measured at the end the experiment. To all collected samples acetonitrile will be added to a final concentration of 50% in the samples. The collected samples will be stored at -20°C until analysis by HPLC or LC-MS.

Basolateral to apical transport

Generally every compound will be tested in 2-4 wells. The basolateral and the apical wells will contain 1.55 mL and 0.4 mL TB, respectively, and the standard concentration of the tested substances is 10 μM. Furthermore all test solutions and buffers will contain 1% DMSO. Prior to the experiment the transport plates are precoated with culture medium containing 10% serum for 30 minutes to avoid nonspecific binding to plastic material.

After 21 to 28 days in culture on filter supports the cells are ready for permeability experiments. The culture medium from the apical wells are removed and the inserts are transferred to a wash row (No.1) in a new plate without inserts (Transport plate).

The transport plate comprises 3 rows of 4 wells. Row 1 is denoted "wash" and row 3 is the "experimental row". The transport plate has previously been prepared with 1.5 mL TB (pH 7.4) in wash row No. 1 and with 1.55 mL TB (pH 7.4), including the test substance, in experimental row No. 3 (donor side).

0.5 mL transport buffer (HBSS, 25 mM MES, pH 6.5) is added to the inserts in row No. 1 and the cell monolayers are equilibrated in the transport buffer system for 30 minutes, 37 °C in a polymix shaker. After being equilibrated to the buffer system the TEER value is measured in each well by an EVOM chop stick instrument.

The transport buffer (TB, pH 6.5) is removed from the apical side and the insert is transferred to row 3 and 400 μL fresh TB, pH 6.5 is added to the inserts. After 30 minutes 250 μL is withdrawn from the apical (receiver) well and replaced by fresh transport buffer. Thereafter 250 μL samples will be withdrawn and replaced by fresh transport buffer every 30 minutes until the end of the experiment at 120 minutes, and finally a post value of TEER is measured at the end of the experiment. A 25 μL samples will be taken from the basolateral (donor) compartment after ~2 minutes and at the end of the experiment. These samples represent donor samples from the start and the end of the experiment.

To all collected samples acetonitrile will be added to a final concentration of 50% in the samples. The collected samples will be stored at -20°C until analysis by HPLC or LC-MS.

Calculation

Determination of the cumulative fraction absorbed, FA_{cum}, versus time. FA_{cum} is calculated from:

$$FA_{cum} = \sum \frac{C_{RI}}{C_{DI}}$$

Where C_{Ri} is the receiver concentration at the end of the interval i and C_{Di} is the donor concentration at the beginning of interval i. A linear relationship should be obtained.

The determination of permeability coefficients (P_{app}, cm/s) are calculated from:

$$P_{app} = \frac{(k \cdot V_R)}{(A \cdot 60)}$$

where k is the transport rate (min^{-1}) defined as the slope obtained by linear regression of cumulative fraction absorbed (FA_{cum}) as a function of time (min), V_R is the volume in the receiver chamber (mL), and A is the area of the filter (cm^2).

Reference compounds

Category of absorption in man	Markers	% absorption in man
PASSIVE TRANSPORT		
Low (0-20%)	Mannitol Methotrexate	16 20
Moderate (21-75%)	Acyclovir	30
High (76-100%)	Propranolol Caffeine	90 100
ACTIVE TRANSPORT		
Amino acid transporter	L-Phenylalanine	100
ACTIVE EFFLUX		
PGP-MDR1	Digoxin	30

Biological Example 5

Cellular cathepsin S K_i

This example describes procedures for assessing potency of cathepsin S inhibitors on inhibition of in vitro T cell activation by determining concentration of the compound required for reducing

50% of the IL-2 secretion in T cells stimulated with compound-treated antigen presenting cells in an antigen presentation assay using the 19.3 cells and the 9001 cells as the effector cells and the antigen presenting cells, respectively. 19.3 cells are murine T cell hybridomas recognizing type II collagen (260-272) in the context of HLA-DR1, and 9001 is an EBV-transformed human B cell line expressing homozygous DR1 molecule. The 9001 cells will be pre-treated with varying concentration of the compounds for 1 hour and then incubated with the T cells in the presence of collagen at a final concentration of 0.1 mg/ml. The cultures will be incubated overnight at 37°C with 5% CO_2 and amount of IL-2 in the supernatant determined with ELISA. The IC_{50}-IL-2 values representing the concentration of compounds at which secretion of IL-2 from the T cells is reduced by 50% will be determined by regression analysis

Major histocompatibility complex (MHC) class II molecules bind peptides generated by degradation of endocytosed antigens and display them as MHC class II-peptide complexes at the cell surface for recognition by CD4+ T cells. MHC class II molecules are assembled with the assistance of invariant chain (Ii) in the endoplasmic reticulum (ER) and transported to an endocytic compartment where Ii undergoes rapid degradation by endosomal and lysosomal proteases. A peptide fragment of Ii, CLIP (class II-associated Invariant chain Peptides) remains bound in the class II peptide binding groove, until removed by the chaperone molecule H-2M in mouse or HLA-DM in humans. This allows peptides derived from proteolytic degradation of foreign and self proteins to bind class II molecules and subsequently to be presented to T cells in the context of MHC molecules. In dendritic cells and B cells, cathepsin S is required for complete invariant chain processing and CLIP generation. Inactivating cathepsin S with inhibitors will impair MHC class II peptide loading and formation of stable MHC/peptide complexes leading to reduced antigen presentation and T cell activation.

To assess the potency of the cathepsin S inhibitors, an antigen presentation assay uses a collagen specific,HLA-DR1 restricted mouse T cell hybridoma (19.3) as effector cells, human EBV-transformed B cells (9001) as antigen presenting cells (APC), and mIL-2 ELISA as the read-out system. Inhibition of Cathepsin S with specific inhibitors will impair the processing and presentation of collagen in APCs which in turn reduces the activation of the T cells. The extent of inhibition on T cells is measured by the degree of reduction in IL-2 secretion. IC_{50}-IL-2 represents the concentration of compounds at which secretion of IL-2 from the T cells is reduced by 50% in the antigen presentation assay.

MATERIALS

Cathepsin S inhibitors

Compounds will be dissolved in DMSO to a final concentration of 10 mM, aliquotted, and stored at -80 C until used.

Cells

All the cells will be cultured in DMEM medium (Invitrogen, cat #11995-065) supplemented with 10% fetal bovine serum (Hyclone, cat #SH30070.03), 100 U/ml penicillin, 100 ug/ml streptomycin and 2 mM L-glutamine (Invitrogen, cat #10378-016).

T cell: 19.3, murine DR1 transgenic T cell hybridomas, DR1 restricted, Type II collagen 260-272 specific

Antigen presentation cells (APCs): 9001, EBV-transformed human B cells expressing homozygous DR1

Antigen

Type II collagen from chicken sternal cartilage (Sigma, cat. # C-9301) will be dissolved in PBS at 1 mg/ml and stored in aliquots at -80 C.

EQUIPMENT

Tissue culture incubator (Forma Scientific, model. #3120)
Sorvall centrifuge (Sorvall RC-3B)
Plate washer
Plate-reader (Tecan, Spectra shell, cat. #20-074)

PROCEDURES

Antigen presentation assay

1. Two-fold serial dilutions of the compounds, starting at 400uM in AIMV medium, will be transferred to a 96-well round-bottom microtiter plate at a volume of 50ul/well.

2. Antigen-presenting cells will be washed and resuspended in AIMV medium to a density of $0.8x10^6$/ml, and then added to the plates at a volume of 50ul/well, giving the number of cells per well as 40,000.

3. The APC's will be pretreated with compounds for 1 hour at 37C with 5% CO_2.

4. The T cells will be washed and resuspended in AIMV to a density of $0.8x10^6$/ml.

5. The antigen will be diluted to a 4X concentration in AIMV and mixed 1 to 1 with the T cells.

6. The T cells/antigen mixture will then be added to the assay plates at a volume of 100ul/well.

6. The plates will be incubated overnight at 37C with 5% CO_2.

7. Supernatant will be carefully removed from each well and measured for amount of IL-2 with ELISA.

IL-2 ELISA

Mouse IL-2 ELISA kits will be purchased from Pharmingen (Mouse IL-2 OptEIA set, #2614KI). The ELISA will be performed per manufacturer's instruction.

1. Anti-mIL-2 antibodies will be diluted in carbonate buffer to a final concentration of 2 ug/ml, transferred to an ELISA plate (Costar) at 100 ul/well and then incubated overnight at 4 degreesC.

2. The ELISA plates will be washed 4 times with PBS/0.5% FBS containing 0.05% Tween 20 (wash buffer).

3. The plates will be blocked with the blocking buffer, 10% FBS (fetal bovine serum, Hyclone) for 2 hrs at room temperature (RT) and then washed 4 times with wash buffer.

4. 100 µL of supernatants from each well of the assay plates will be transferred to the ELISA plate and incubated for 2 hrs at RT.

5. After washing 4 times, the plate will be incubated for 1 hr at RT with a mixture of a biotinylated anti-mIL2 antibody and avidin-HRP prepared in blocking buffer.

6. Following 8 washes with wash buffer, the substrate (TMB) will be added to the plate and incubated at RT for 15-30 minutes until the color develops.

7. Color development will be terminated by the addition of 2N sulfuric acid.

8. The plates will be measured at 450 nm with an ELISA plate reader (Spectra, Tecan).

9. A set of purified recombinant mIL-2 with known concentration will be prepared from the stock solution (provided in the kit) with the blocking buffer and assayed in each plate to provide a standard curve for quantification of IL-2.

DETERMINATION OF IC50-IL-2 OF CATHEPSIN S INHIBITORS

The potency of each compound will be measured by the IC_{50} value derived from this assay. IC_{50} represents the concentration of compound at which secretion of IL-2 from the T cells is reduced by 50%.

The absorbance at 450 nm from each well will be converted into amount of IL-2 (pg/ml) using the Winselect software (Tecan) based on the standard curve generated from in-plate standards of purified recombinant mIL-2. Means and standard deviations will be calculated from triplicates with Excel.

The average amounts of IL-2 (pg/ml) from triplicates of both the test and the control wells (received comparable amount of DMSO) will be used to generate the percent inhibition using the following formula.

$$\text{Percent Inhibition} = \frac{\text{average of control wells} - \text{average of test wells}}{\text{average of control wells}} \times 100$$

A dose response curve will be generated by plotting the percent inhibition versus concentration of the compound and the IC_{50}-IL-2 value will be calculated with regression analysis.

DR-1 transgenic T cell hybridoma has been prepared by E. Rosloniec, University of Tennessee.

Following controls are included and analyzed as appropriate:

T + APCs, without antigen, without compound treatment, for background signal. We usually get negligible amounts of IL-2 form these wells, and usually don't perform background subtraction.

T + APCs, with anti-CD3/CD28, with compounds, for toxicity associated with compounds.

T + APCs, with antigen, with DMSO (comparable to those received compounds), for toxicity associated with DMSO and for calculation of percent of inhibition.

Biological Example 6

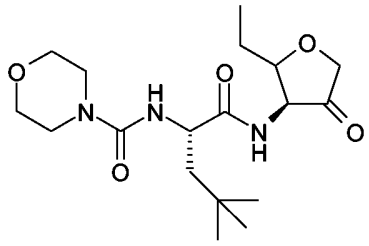
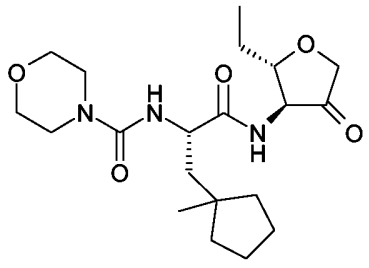
Human Liver Micrososomes

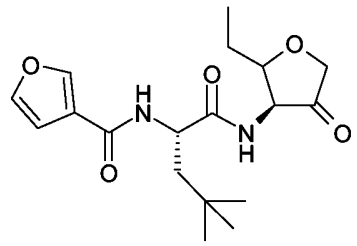
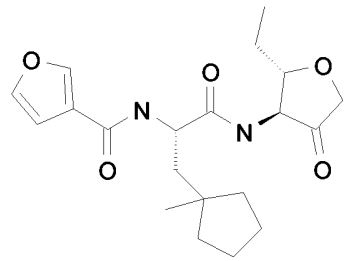
Metabolic stability is determined by commercially available human liver microsome assays, such as XEN 042, assayed in accordace with manufacturer's recommendations.

Biological Example 7

Comparative Trial

Compounds of the invention and the closest prior art were assayed in the assays above and produced the results tabulated below.

	Prior art Cat S K_i 88 nM Cat K 3.7 uM (selectivity 42 fold) Caco-2. 1.2 x 10^{-6} cm/sec
	Example 2 Cat S K_i 10.8 nM Cat K 2.61 uM (selectivity 240 fold) Caco-2. 5.8 x 10^{-6} cm/sec

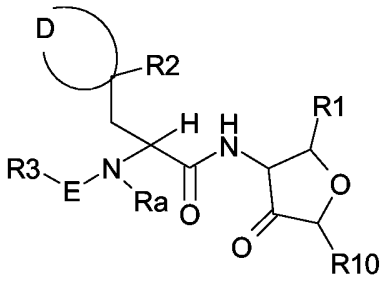
	Prior art Cat S K_i 48.7 nM Cat K 0.5 uM (selectivity 11 fold) Cat L 15.6 uM (selectivity 348 fold)
	Example 4.1 Cat S K_i 2.3 nM Cat K 1.82 uM (selectivity 791 fold) Cat L 57.6 uM (selectivity 25043 fold)

All references referred to in this application, including patent and patent applications, are incorporated herein by reference to the fullest extent possible.

Throughout the specification and the claims which follow, unless the context requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be understood to imply the inclusion of a stated integer, step, group of integers or group of steps but not to the exclusion of any other integer, step, group of integers or group of steps.

Claims

1. A compound of the formula I,



where

R^1 is C_1-C_4 straight or branched alkyl, optionally substituted with up to three substituents selected from halo and hydroxy;

R^2 is halo, hydroxy, methyloxy, or C_1-C_2 alkyl, which alkyl is optionally substituted with up to three halogens or an hydroxy or a methyloxy;

D is -C_3-C_7 alkylene-, thereby defining a cycloalkyl ring;

E is -C(=O)-, -$S(=O)_m$-, -NRdS(=O)$_m$-, -NRaC(=O)-, -OC(=O)-,

R^3 is a carbocyclic ring selected from C_3-C_6 cycloalkyl, C_5-C_6 cyclohexenyl or phenyl, or a heterocyclic ring selected from azepanyl, pyrrolidinyl, piperidinyl, morpholinyl, thiomorpholinyl, piperazinyl, indolinyl, pyranyl, tetrahydropyranyl, tetrahydrothiopyranyl, thiopyranyl, furanyl, tetrahydrofuranyl, thienyl, pyrrolyl, oxazolyl, isoxazolyl, thiazolyl, imidazolyl, pyridinyl, pyrimidinyl, pyrazinyl, pyridazinyl, tetrazolyl, pyrazolyl, indolyl, which ring is optionally substituted with up to 3 substituents independently selected from R^4;;

R^4 is independently selected from halo, oxo, nitrile, nitro, C_1-C_4 alkyl, -NRaRb, NH_2CO-, X-R^5, X-O-R^5, X-O-C(=O)R^5, X-C(=O)R^5, X-C(=O)NRaR^5, X-NRaC(=O)R^5, X- NRdSO_mR^{5a}, X-SO_mNRdR^5, X-$S(=O)_mR^5$, X-C(=O)OR^5, X-NRaC(=O)OR^5; or a pair of R^4 together define a 5 or 6 membered, nitrogen-containing ring fused to R^3, optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkyloxy, oxo, hydroxy, halo, NRaRb,

R^5 is H, C_1-C_4 alkyl, C_3-C_6 cycloalkyl, pyrrolidinyl, piperidinyl, morpholinyl, thiomorpholinyl, piperazinyl, indolinyl, pyranyl, thiopyranyl, furanyl, thienyl, pyrrolyl, oxazolyl, isoxazolyl, thiazolyl, imidazolyl, pyridinyl, pyrimidinyl, pyrazinyl, indolyl, phenyl, benzyl, any of which is optionally substituted with R^6;

R^{5a} is R^5 or –NRaRb;

R^6 is independently selected from hydroxy, -NH_2, NHC_1-C_3alkyl, N(C_1-C_3alkyl)$_2$, nitro, cyano, carboxy, oxo, C_1-C_4 alkyl, C_1-C_4-alkoxy, C_1-C_4 alkanoyl, carbamoyl;

R^{10} is H, ORc, SRc or together with the gem H is =O or (ORc)$_2$;

Ra is independently selected from H, C_1-C_4 alkyl;
Rb is H, C_1-C_4 alkyl or acetyl, or Ra, Rb and the N atom to which they both are joined form a ring selected from morpholine, piperazine, piperidine, pyrrolidine;
Rc is H, C_1-C_4 alkyl, C_0-C_3alkylcarbocyclyl;
Rd is H, C_1-C_4 alkyl, C(=O)C_1-C_4 alkyl, C_0-C_3alkylcarbocyclyl;
X is independently a bond or C_1-C_4 alkylenyl;
m is independently 0,1 or 2;
and pharmaceutically acceptable salts thereof.

2. A compound according to claim 1, wherein R^1 is ethyl, 2-fluoroethyl or 2-hydroxyethyl.

3. A compound according to claim 1, wherein R^1 is methyl.

4. A compound according to claim 1, wherein R^2 is methyl.

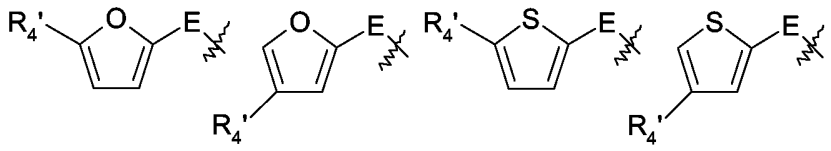
5. A compound according to claim 4, wherein D is butylene, thereby defining a cyclopentyl ring.

6. A compound according to any of claims 1-5, wherein Ra and/or Rb and/or Rc and/or Rd are H.

7. A compound according to any of claims 1-6, wherein E is -C(=O)-.

8. A compound according to claim 7, wherein R^3 is optionally substituted furyl, thienyl, pyrazinyl, pyridyl, pyrrolyl or morpholinyl.

9. A compound according to claim 8 wherein R3-E- is one of the partial structures:



where $R^{4'}$ is H, halo, OC_1-C_4 alkyl, C(=O)NRaRb, NRaC(=O)C_1-C_4 alkyl, NRaC(=O)NRaRb or -NRaC(=O)OC_1-C_4 alkyl. NHC(=O)OMe.

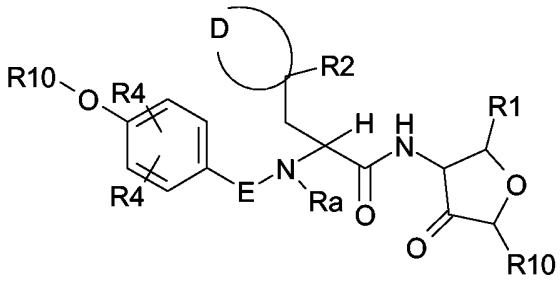
10. A compound according to claim 9, wherein R4′ is fluoro, methoxy, dimethylcarbamoyl, NHC(=O)Me, –NHC(=O)$NHCH_3$, NHC(=O)$N(CH3)_2$, NHC(=O)OMe or NHC(=O)NRrRr,where

RrRr define a cyclic amine selected from pyrrolidine, morpholine, piperidine, piperazine or N-methylpiperazine.

11. A compound according to claim 1, wherein R^3 is phenyl,

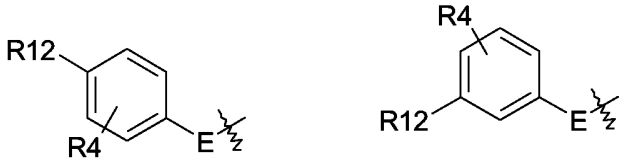
12. A compound according to claim 11, wherein the phenyl is substituted with m-fluoro, p-fluoro, p-hydroxy, p-hydroxy-m-chloro, p-hydroxy-m-fluoro, p-hydroxy-m-methoxy, p-hydroxy-m-methyl, bis-p-chloro-p-hydroxy, m-cyano, p-acetamido or p-pyrimid-2-yl.

13. A compound according to claim 11, with the formula:



where R^1, R^2, D, Ra, E and R^4 are as defined above and R^{10} is H, R^{11} or -C(=O)R^{11} where R^{11} is independently H, C_1-C_6-alkyl which is optionally substituted with R^6, C_0-C_3alkylcarbocyclyl or C_0-C_3alkylheterocyclyl.

14. A compound according to claim 1, wherein R^3 comprises the partial structure:

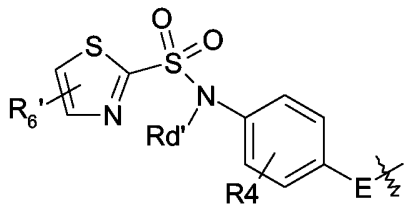


where R^{12} is –NRdS$O_m$$R^{5a}$ and E, R^4, R^{5a}, Rd′ are as defined in claim 1.

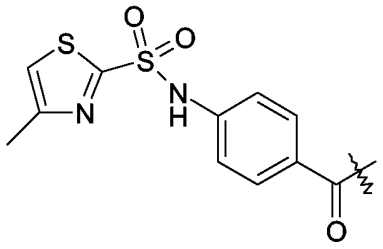
15. A compound according to claim 14 wherein E is –(C=O)-.

16. A compound according to claim 15, wherein R^5 is C_1-C_4 alkyl, such as methyl, ethyl or i-propyl or t-butyl; halogenated C_1-C_4 alkyl such as trifluoromethyl; C_3-C_6 cycloalkyl, such as cyclopropyl or cyclohexyl; or phenyl or benzyl, any of which is optionally substituted with R^6.

17. A compound according to claim 15, with the partial structure:



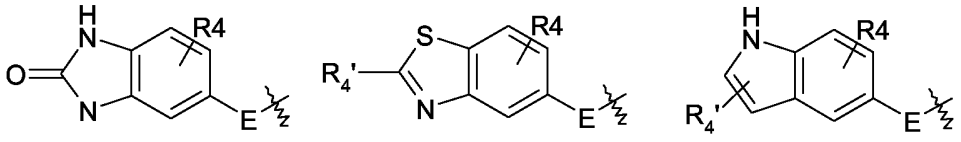
where E and R^4 are as defined above, Rd' is Me or preferably H, and $R^{6'}$ is H or methyl, especially with the partial structure:



18. A compound according to claim 1, wherein R^3 has the partial structure:

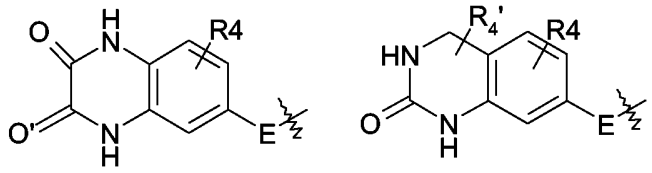
where R^4 and E are as defined above, Rz is CH, NH or O and the S atom is optionally oxidised to >S=O or preferably $>S(=O)_2$.

19. A compound according to claim 1, wherein R^3 has the partial structure:



where $R^{4'}$ is H, C_1-C_4 alkyl, NH_2, NHC_1-C_4alkyl (such as methylamide), $N(C_1$-C_4alkyl$)_2$ such as dimethylamide), $NHC(=O)C_1$-C_4alkyl (such as acetamide); ring nitrogens are optionally substituted with C_1-C_4 alkyl (such as methyl, ethyl or t-butyl) , or $C(=O)C_1$-C_4 alkyl (such as acetyl); and R^4 is as defined in claim 1.

20. A compound according to claim 1, wherein a pair of R^4 define a nitrogen containing ring fused to R^3 with the partial structure:



where

$R^{4'}$ is H, C_1-C_4 alkyl, NH_2, NHC_1-C_4alkyl (such as methylamide), $N(C_1$-C_4alkyl$)_2$ such as dimethylamide), $NHC(=O)C_1$-C_4alkyl (such as acetamide); ring nitrogens are optionally substituted with C_1-C_4 alkyl (such as methyl, ethyl or t-butyl) , or $C(=O)C_1$-C_4 alkyl (such as acetyl);

R^4 is as defined in claim 1;

O' is absent (ie 2 hydrogen atoms) or keto.

21. A compound according to claim 1, wherein R^{10} is H.

22. A pharmaceutical composition comprising a compound as claimed in any of claims 1-21 and a pharmaceutically acceptable carrier or vehicle therefor.

23. Use of a compound as claimed in any of claims 1 to 21 in the manufacture of a medicament for the treatment or prophylaxis of disorders caused by aberrant cathepsin S expression or activation.

24 Use according to claim 23, wherein the disorder is an autoimmune disorder such as MS, RA, juvenile diabetes or asthma.

25. Use according to claim 23, wherein the disorder is chronic pain.

26. A method for the treatment or prophylaxis of disorders caused by aberrant cathepsin S expression or activation comprising the administration of an effective amount of a compound as defined in any of claims 1-21 to an individual suffering from or threatened with the disorder.

27 A method according to claim 26, wherein the disorder is an autoimmune disorder such as MS, RA, juvenile diabetes or asthma.

28. A method according to claim 26, wherein the disorder is chronic pain or psoriasis.

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2005/050243

A. CLASSIFICATION OF SUBJECT MATTER
C07D307/32 C07D307/68 C07D409/12 C07D417/12 A61K31/34
A61K31/416 A61K31/381 A61K31/427 A61P37/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/057249 A (INCENTA LIMITED; QUIBELL, MARTIN) 25 July 2002 (2002-07-25) page 7, line 20 - page 10, line 16; table 2	1-28
A	WO 00/69855 A (MEDIVIR UK LIMITED; PEPTIMMUNE, INC; QUIBELL, MARTIN; TAYLOR, STEVEN) 23 November 2000 (2000-11-23) cited in the application page 2, line 7 - page 3, line 16	1-28
A	WO 03/024924 A (AVENTIS PHARMACEUTICALS INC; CELERA, AN APPLERA CORPORATION BUSINESS;) 27 March 2003 (2003-03-27) page 1, line 6 - page 6, line 12	1-28

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 February 2006	01/03/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Usuelli, A

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2005/050243

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	WO 2005/082876 A (MEDIVIR UK LTD; PEPTIMMUNE, INC; MIAH, SOYFUR; NILSSON, MAGNUS; WAHLIN) 9 September 2005 (2005-09-09) page 1, line 1 - page 3, line 6	1-28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2005/050243

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 26-28 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compounds.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2005/050243

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			PT	1178986 T	30-07-2004
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			CN	1553892 A	08-12-2004
			EP	1436255 A1	14-07-2004
			HR	20040249 A2	30-04-2005
			JP	2005504078 T	10-02-2005
			NO	20040996 A	12-05-2004
			ZA	200401882 A	18-04-2005
WO 2005082876	A	09-09-2005	NONE		