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19th August 2014

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Ref: Patent Application No. **10158/DELNP/2007**

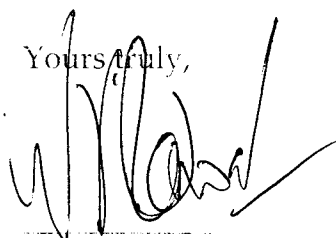Sub: Filing of Written Submission u/s 25(1) of the Act for Pre-Grant Opposition.

Sir,

With regards to the captioned subject and on behalf of our clients, M/s. Indian Pharmaceutical Alliance, a society registered under the Societies Registration Act, Written Submission (In duplicate) U/s 25(1) of the Patent Act for opposing the Grant of Patent Application No. **10158/DELNP/2007** and also Please find attached herewith FORM 7A (Duplicate).
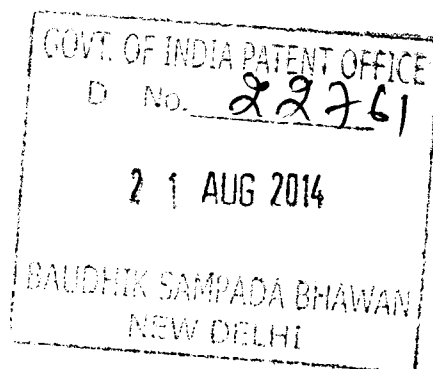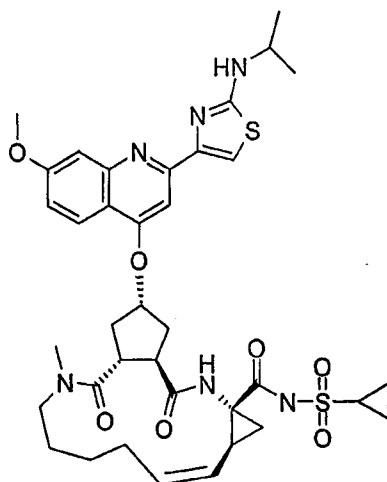
Kindly take them on record and oblige.

Thanking You,

Yours truly,

Nayan Rawal
Constituted Attorney for
the Opponents

GOVT. OF INDIA PATENT OFFICE
D No. 22761

2 1 AUG 2014

BAUDHIK SAMPADA BHAWAN
NEW DELHI

Cyclopropanesulphonic acid {17-[2-(2-isopropylamino-thiazol-4-yl)-7-methoxy-
quionlin-4-yloxy]-13-methyl-2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0*4,6*]octadec-7-
ene-4-carbonyl} amide (111)

5   The acid 110 (20 mg, 0.15 mmol), DMAP (28 mg, 0.225 mmol) and EDAC (58 mg,
       0.3 mmol) was dissolved in DMF (1,5 mL). The reaction mixture was stirred overnight
       at R.T. whereafter cyclopropylsulphonamide (91 mg, 1.125 mmol) and DBU (114 μL,
       0.75 mmol) was added. After stirring at RT overnight the reaction mixture was added
       to 5% citric acid and extracted three times with chloroform. The organic phase was
10   dried over sodium sulphate and evaporated. The afforded residue was purified by
       preparative HPLC to give the title product (5.6 mg) (yield 24%).

Assays

       The compounds of the invention are conveniently assayed for activity against the NS3
15   protease of flavivirus such as HCV using conventional in vitro (enzyme) assays or cell
       culture assays.

       A useful assay is the Bartenshlager replicon assay disclosed in EP 1043399. An
       alternative replicon assay is described in WO 03064416.
20

       A convenient enzyme assay involving the inhibition of full-length hepatitis C NS3 is
       essentially as described in Poliakov, 2002 Prot Expression & Purification 25 363 371.

Briefly, the hydrolysis of a depsipeptide substrate, Ac-
DED(Edans)EEAbuψ[COO]ASK(Dabcyl)-NH$_2$ (AnaSpec, San José, USA), is
measured spectrofluorometrically in the presence of a peptide cofactor,
KKGSVVIVGRIVLSGK, as described by Landro, 1997 Biochem 36 9340-9348. The

5   enzyme (1 nM) is incubated in a buffer such as 50 mM HEPES, pH 7.5, 10 mM DTT,
40% glycerol, 0.1% n-octyl-β-D-glucoside, with 25 μM cofactor and inhibitor at say 30
°C for 10 min, whereupon the reaction is initiated by addition of substrate, typically 0.5
μM substrate. Inhibitors are typically dissolved in DMSO, sonicated for 30 s and
vortexed. The solutions are generally stored at -20°C between measurements.

10

An alternative enzyme assay is described in WO 0399316 and employs an HCV
NS3/4A protease complex FRET peptide assay. The purpose of this in vitro assay is
to measure the inhibition of HCV NS3 protease complexes, derived from the BMS,
H77C or J416S strains, as described below, by compounds of the present invention.

15   This assay provides an indication of how effective compounds of the present
invention would be in inhibiting HCV proteolytic activity.

Serum is taken from an HCV-infected patient. An engineered full-length cDNA
template of the HCV genome (BMS strain) was constructed from DNA fragments

20   obtained by reverse transcription-PCR (RT-PCR) of serum RNA and using primers
selected on the basis of homology between other genotype la strains. From the
determination of the entire genome sequence, a genotype I a was assigned to the
HCV isolate according to the classification of Simmonds et al. (See P Simmonds, KA
Rose, S Graham, SW Chan, F McOmish, BC Dow, EA Follett, PL Yap and H

25   Marsden, J.Clin. Microbiol., 31(6), 1493-1503 (1993)). The amino acid sequence of
the nonstructural region, NS2-5B, was shown to be >97% identical to HCV genotype
la (H77C) and 87% identical to genotype lb (J4L6S). The infectious clones, H77C (I a
genotype) and J4L6S (I b genotype) can be obtained from R. Purcell (NIH) and the
sequences are published in Genbank (AAB67036, see Yanagi,M., Purcell,R.H.,

30   Emerson,S.U. and Bukh. Proc. Natl. Acad. Sci. U.S.A. 94 (16) 8738-8743 (1997);

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AF054247, see Yanagi,M., St Claire,M., Shapiro,M., Emerson,S.U., Purcell,R.H. and
Bukhj, Virology 244 (1), 161 (1998)).

The BMS, H77C and J4L6S strains are conventional for production of recombinant
NS3/4A protease complexes. DNA encoding the recombinant HCV NS3/4A protease
complex (amino acids 1027 to 1711) for these strains were manipulated as described
by P. Gallinari et al. (see Gallinari P, Paolini C, Brennan D, Nardi C, Steinkuhler C,
De Francesco R. Biochemistry. 38(17):562032, (1999)). Briefly, a three-lysine
solubilizing tail was added at the 3'-end of the 3 0 NS4A coding region. The cysteine
in the P1 position of the NS4A-NS4B cleavage site (amino acid 1711) was changed to
a glycine to avoid the proteolytic cleavage of the lysine tag. Furthermore, a cysteine
to serine mutation can be introduced by PCR at amino acid position 1454 to prevent
the autolytic cleavage in the NS3 helicase domain. The variant DNA fragment can be
cloned in the pET21b bacterial expression vector (Novagen) and the NS3/4A complex
can be expressed in Escherichia coli strain BL21 (DE3) (Invitrogen) following the
protocol described by P. Gallinari et al. (see Gallinari P, Brennan D, Nardi C, Brunetti
M, Tomei L, Steinkuhler C, De Francesco R., J Virol. 72(8):6758-69 (1998)) with
modifications. Briefly, NS3/4A expression can be induced with 0.5mM Isopropyl beta-
D thiogalactopyranoside (IPTG) for 22hr at 20'C. A typical fermentation (l0 l) yields
approximately 80g of wet cell paste. The cells are resuspended in lysis buffer (l0
mL/g) consisting of 25mM N-(2Hydroxyethyl)Piperazine-N'-(2-Ethane Sulfonic acid)
(HEPES), pH7.5, 20% glycerol, 5OOmM Sodium Chloride (NaCl), 0.5% Triton-X100, I
ug/mL lysozyme, 5mM Magnesium Chloride (MgCl2), I ug/mL Dnasel, 5mM beta-
Mercaptoethanol (BME), Protease inhibitor - Ethylenediamine Tetraacetic acid
(EDTA) free (Roche), homogenized and incubated for 20 mins at VC. The
homogenate is sonicated and clarified by ultra-centrifugation at 235000 g for 1 hr at
4'C.

Imidazole is added to the supernatant to a final concentration of 15mM and the pH
adjusted to 8. The crude protein extract is loaded on a Nickel Nitrilotriacetic acid (Ni-
NTA) column pre-equilibrated with buffer B (25n-tM 2 0 HEPES, pH8 20% glycerol,

5OOmM NaCl, 0.5% Triton-XIOO, 15mM imidazole, 5mM BME). The sample is loaded at a flow rate of lmL/min. The column is washed with 15 column volumes of buffer C (same as buffer B except with 0.2% Triton-X100). The protein is eluted with 5 column volumes of buffer D (same as buffer C except with 200mM imidazole).

5

NS3/4A protease complex-containing fractions are pooled and loaded on a desalting column Superdex-S200 pre-equilibrated with buffer D (25MM HEPES, pH7.5, 20% ` glycerol, 3OOmM NaCl, 0.2% Triton-XIOO, lOmM BME). Sample is loaded at a flow rate of lmUmin. NS3/4A protease complex3 0 containing fractions are pooled and

10 concentrated to approximately 0.5mg/mL. The purity of the NS3/4A protease complexes, derived from the BMS, H77C and J4L6S strains, are typically judged to be greater than 90% by SDS-PAGE and mass spectrometry analyses.

The enzyme is generally stored at -80'C, thawed on ice and diluted prior to use in

15 assay buffer. The substrate used for the NS3/4A protease assay, is conveniently RET S 1 (Resonance Energy Transfer Depsipeptide Substrate; AnaSpec, Inc. cat # 22991)(FRET peptide), described by Taliani et al. in Anal. Biochem. 240(2):6067 (1996). The sequence of this peptide is loosely based on the NS4A/NS4B natural cleavage site except there is an ester linkage rather than an amide bond at the

20 cleavage site. The peptide substrate is incubated with one of the three recombinant NS3/4A complexes, in the absence or presence of a compound of the present invention, and the formation of fluorescent reaction product was followed in real time using a Cytofluor Series 4000. Useful reagents are as follow: HEPES and Glycerol (Ultrapure) can be obtained from GIBCO-BRL. Dimethyl Sulfoxide (DMSO) is

25 obtained from Sigma. Beta-Mercaptoethanol is obtained from Bio Rad.

Assay buffer: 50m.M HEPES, pH7.5; 0. 15M NaCl; 0. I% Triton; 15 % Glycerol; 10mM BME. Substrate: 2 uM final concentration (from a 2mM stock 2 0 solution in DMSO stored at -20'C). HCV NS3/4A type la (Ib), 2-3 nM final concentration (from a 5uM

30 stock solution in 25mM HEPES, pH7.5, 20% glycerol, 300m.M NaCl, 0.2% Triton-X100, 10mM BME). For compounds with potencies approaching the assay limit, the

assay can be made more sensitive by adding 50 ug/mL BSA to the assay buffer and/or reducing the end protease concentration to 300 pM.

The assay is conveniently performed in a 96-well polystyrene black plate from Falcon.

5   Each well contains 25ul NS3/4A protease complex in assay buffer, 50ul of a compound of the present invention in 10% DMSO/assay buffer and 25ul substrate in assay buffer. A control (no compound) is also prepared on the same assay plate. The enzyme complex is mixed with compound or control solution, typically for 1 min before initiating the enzymatic reaction by the addition of substrate. The assay plate is

10   generally read immediately using a spectrophotometer such as a Cytofluor Series 4000 (Perspective Biosysterns). The instrument is conveniently set to read an emission of 340nm and excitation of 490nm at 25'C. Reactions are generally followed for approximately 15 minutes.

15   The percent inhibition can be calculated with the following equation.

$$100.- [(dF_{inh}/dF_{con})X100]$$

where dF is the change in fluorescence over the linear range of the curve. A nonlinear curve fit is applied to the inhibition-concentration data, and the 50% effective concentration (IC_{50}) is calculated by the use software such as Excel XI-fit software

20   using the equation:

$$y=A+((B-A)/(1+((C/x)^{\wedge}D))).$$

Enzyme assays conveniently utilize a fluorescence resonance energy transfer (FRET) principle to generate a spectroscopic response to an HCV NS3 serine protease catalyzed NS4A/4B cleavage event. The activity is typically measured in a

25   continuous fluorometric assay using an excitation wavelength of 355 nm and emission wavelength of 500 nm. The initial velocity may be determined from 10 minutes continuous reading of increased fluorescence intensities as a result of the NS3 protease catalyzed cleavage event.

30   An alternative enzyme assay can be carried out as follows:

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Materials

Recombinant HCV NS3 full length enzyme can be prepared as shown in Poliakov et al Protein Expression & purification 25 (2002) 363-371. The NS4A cofactor conveniently has an amino acid sequence of KKGSVVIVGRIVLSGK (commercially available), generally prepared as a 10 mM stock solution in DMSO. The FRET-substrate (Ac-Asp-Glu-Asp(EDANS)-Glu-Glu-Abu-ψ-[COO)Ala-Ser-Lys(DABCYL)-NH2, MW1548.60 can be purchased from AnaSpec RET S1, CA. USA) and is typically prepared as a 1.61 mM stock solution in DMSO. Aliquots (50μl/tube) should be wrapped with aluminum foil to protect from direct light and stored in -20 °C.

Reference compound-1, N-1725 with a sequence of AcAsp-D-Gla-Leu-Ile-Cha-Cys, MW 830.95 may be purchased from BACHEM, Switzerland and is generally prepare as a 2 mM stock solution in DMSO and stored in aliquots in -20°C.

1M HEPES buffer may be purchased from Invitrogen Corporation, storage at 20°C.

Glycerol may be purchased from Sigma, 99% purity.

CHAPS, 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate: may be purchased from Research Organics, Cleveland, OH44125, USA. MW614.90

DTT, DL-Dithiothreitol (Cleland Reagent: DL-DTT) 99% purity, MW.154.2 Storage: +4°C.

DMSO may be purchased from SDS, 13124 Peypin, France. 99.5% purity.

TRIS, ultra pure (TRIS-(hydroxymethylaminomethane), may be purchased from ICN Biomedicals Inc.

Sodium Chloride, may be obtained from KEBOlab AB.

N-dodecyl-β-D-maltoside, minimum 98%, may be purchased from Sigma, storage -20°C.

Equipment

Microtiter plates (white cliniplate, ThermoLab Systems cat no. 9502890

Eppendorf pipettes

Biohit pipette, multi dosing

Ascent fluorimeter, filterpair ex 355nm, em500 nm

Method

Experimental procedure:

10 mM stock solutions of the compounds are made in DMSO. The stock solutions are

5   stored in room temperature while testing and placed in -20°C at long-time storage.

Assay buffer A:

50 mM HEPES buffer, pH=7.5, 40% Glycerol, 0.1% CHAPS

Storage: room temperature.

10 mM DTT (stored in aliquots at -20°C and added fresh at each experiment)

10   Assay buffer B:

25 mM TRIS pH7.5, 0.15 M NaCl, 10% glycerol, 0.05% n-dodecyl-β-D-maltoside

5mM DTT (stored in aliquots at -20°C and added fresh at each experiment)

Assay sequence:

15   Preparation of reaction buffer (for one plate, 100 reactions)(buffer A)

1.   Prepare 9500µl assay buffer (HEPES, pH=7.5, 40% glycerol and 0.1% CHAPS

in de ionized water. Add DTT giving a final concentration of 10mM (freshly prepared

for every run).

2.   Thaw rapidly the NS3 protease

20   3.   Add 13.6 µl NS3 protease and 13.6 µl NS4A peptide and mix properly. Leave

the mixture for 15 minutes in room temperature.

4.   Place the enzyme stock solution back into liquid nitrogen or -80°C. as soon as

possible.

25   Preparation of reaction buffer (for one plate, 100 reactions)(buffer B)

5.   Prepare 9500µl assay buffer (TRIS, pH=7.5, 0.15 M NaCl, 0.5 mM EDTA, 10%

glycerol and 0.05% n-dodecyl β-D-maltoside in de ionized water. Add DTT giving a

final concentration of 5mM (freshly prepared for every run).

6.   Thaw the NS3 protease rapidly.

30   7.   Add 27.2 µl NS3 protease and 13.6 µl NS4A peptide and mix properly. Leave

the mixture for 15 minutes in room temperature.

8.   Place the enzyme stock solution back into liquid nitrogen or -80°C as soon as possible.

Preparation of inhibitor/reference compound

Make a dilution series of the inhibitors in DMSO to 100x the final concentrations 10, 1, 0.1, 0.01 and 0.001 µM. The final DMSO concentration in 100µl total reaction volume is 1%.

Make a dilution series of the reference compound, N-1725 in DMSO to 100x the final concentrations 120, 60, 30, 15, 7.5 and 3.75 nM.

Eight enzyme control wells are needed for every run.

Blank wells contain 95µL buffer (without NS3 PR), 1µL DMSO and 5µL substrate.

Preparation of FRET substrate

Dilute the substrate stock solution (1.61 mM) with assay buffer to 40 µM working solution. Avoid exposure to light.

Assay sequence

Use 96-well cliniplate, the total assay volume per well is 100µl.

1.   Add 95µL of assay buffer to each well

2.   Add 1µl inhibitor/reference compound

3.   Pre incubate for 30 minutes at room temperature

4.   Start the reaction by adding 5µL 40 µM substrate solution (final concentration 2µM)

5.   Read continuously for 20 minutes at ex=355nm and em=500nm, monitoring the increased fluorescence per minute.

6.   Plot the progression curve (within linear range, 8 – 10 time points) and determine the slope as an initial velocity with respect to each individual inhibitor concentration.

7.   Calculate %inhibition with respect to enzyme control.

Treatment of Results

The result is expressed as %inhibition at a certain concentration (screen) or as a Ki value in nM or μM.

Calculation of % inhibition: The initial velocity is determined from 10 minutes continuous reading of increased fluorescence intensities as a result of the NS3 protease catalyzed cleavage event. The change in slope for the inhibitor compared to the enzyme control gives the % inhibition at a certain concentration.

Calculation of Ki: All inhibitors are treated as if they follow the rules of competitive inhibition. The IC_{50} value is calculated from the inhibition values of a series of inhibitor concentrations. The calculated value is used in the following equation:

$K_i = IC_{50}/(1+S/Km)$

Plotting of the graph is done by help of two calculation programs: Grafit and Graphpad

Various compounds of the invention exemplified above displayed IC_{50}s in the range 1nM to 6.9 micromolar and ED_{50}s in the sub-micromolar to micromolar range in the above assays.

Drug escape resistance pattern and rate

Replicon cultures in microtitre plates can be used to determine resistance development rates and to select out drug escape mutants. The compounds being tested are added at concentrations around their ED_{50} using, say, 8 duplicates per concentration. After the appropriate replicon incubation period the protease activity in the supernatant or lysed cells is measured.

The following procedure is followed at subsequent passages of the cultures. Virus produced at the concentration of test compound showing > 50% of the protease activity of untreated infected cells (SIC, Starting Inhibitory Concentration) are passaged to fresh replicon cultures. An aliquot, say, 15μl supernatent from each of the eight duplicates are transferred to replicon cells without the test compound

(control) and to cells with test compound at the same concentration, and additionally two respectively fivefold higher concentrations. (See the table below)

When the viral component of replicon propagation (for example as measured by HCV protease activity) is permitted at the highest non-toxic concentration (5 - 40 μM), 2-4 parallel wells are collected and expanded to give material for sequence analysis and cross-wise resistance.

Key:

<u>Viral growth permitted</u>

Virus production inhibited

Pass 1	Pass 2	Pass 3	Pass 4	Pass 5
				125 x SIC
			125 x SIC	*25 x SIC* →
			25 x SIC	5 x SIC
		25 x SIC	*5 x SIC* →	No compound
	25 x SIC	*5 x SIC* →	No compound	
	5 x SIC	SIC		
	SIC →	No compound		
SIC →	No compound			

Alternative methods for assessing activity on drug escape mutants include the preparation of mutant enzyme bearing the distinctive mutation for use in standard Ki determinations as shown above. For example WO 04/039970 describes constructions allowing access to HCV proteases bearing the 155, 156 and/or 168 drug escape mutants arising from the selective pressure of BILN-2061 and VX-950. Susch constructs can then be engineered into replicon vectors in place of the wild type protease, thereby allowing ready assessment in a cellular assay, of whether a given compound is active against a give drug escape mutant.

P450 metabolism

The metabolism of compounds of the invention through the main isoforms of the human cytochrome system P450 are conveniently determined in baculovirus infected insect cells transfected with human cytochrome P450 cDNA (supersomes) Gentest

5 Corp. Woburn USA.

The test compounds at concentrations 0.5, 5 and 50 μM are incubated in duplicate in the presence of supersomes overexpressing various cytochrome P450 isoforms, including CYP1A2 + P450 reductase, CYP2A6 + P450 reductase, CYP2C9-Arg 144 + P450 reductase, CYP2C19 + P450 reductase, CYP2D6-Val 374 + P450 reductase

10 and CYP3A4 + P 450 reductase. Incubates contain a fixed concentration of cytochrome P450 (eg 50 pmoles) and are conducted over 1 hour. The involvement of a given isoform in the metabolism of the test compound is determined by UV HPLC chromatographically measuring the disappearance of parent compound.

Claims

1.   A compound of the formula VI:
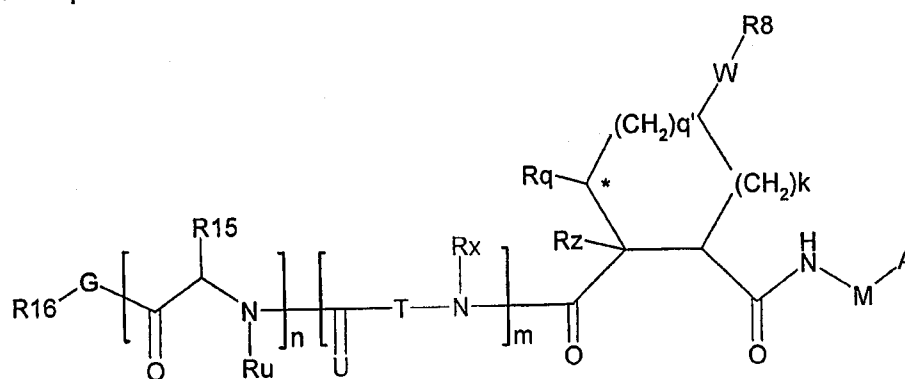
VI

5   wherein

A is $C(=O)OR^1$, $C(=O)NHSO_2R^2$, $C(=O)NHR^3$, or $CR^4R^{4'}$ wherein;

R^1 is hydrogen, C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl;

R^2 is C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl;

R^3 is C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl, -OC_1-C_6alkyl,   -OC_0-

10   C_3alkylcarbocyclyl, -OC_0-C_3alkylheterocyclyl;

R^4 is halo, amino, or OH; or R^4 and $R^{4'}$ together are =O;

$R^{4'}$ is C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl;

wherein R^2, R^3, and $R^{4'}$ are each optionally substituted from 1 to 3 substituents

independently selected from the group consisting of halo, oxo, nitrile, azido, nitro, C_1-

15   C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl, $NH_2C(=O)$-, Y-NRaRb, Y-O-

R_b, Y-C(=O)Rb, Y-(C=O)NRaRb, Y-NRaC(=O)Rb, Y-NHSO$_p$Rb, Y-S(=O)$_p$Rb, Y-

S(=O)$_p$NRaRb, Y-C(=O)Orb and Y-NRaC(=O)ORb;

Y is independently a bond or C_1-C_3alkylene;

Ra is independently H or C_1-C_3alkyl;

20   Rb is independently H, C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl or C_0-C_3alkylheterocyclyl;

p is independently 1 or 2;

M is $CR^7R^{7'}$ or NRu;

Ru is H or C_1-C_3alkyl;

R^7 is C_1-C_6alkyl, C_0-C_3alkylC_3-C_7cycloalkyl, or C_2-C_6alkenyl, any of which is optionally substituted with 1-3 halo atoms, or an amino, –SH or C_0-C_3alkylcycloalkyl group; or R^7 is J;

$R^{7'}$ is H or taken together with R^7 forms a C_3-C_6cycloalkyl ring optionally substituted
5   with $R^{7'a}$ wherein;

$R^{7'a}$ is C_1-C_6alkyl, C_3-C_5cycloalkyl, C_2-C_6alkenyl any of which may be optionally substituted with halo; or $R^{7'a}$ is J;

q' is 0 or 1 and k is 0 to 3;

Rz is H, or together with the asterisked carbon forms an olefinic bond;
10   Rq is H or C_1-C_6alkyl;

W is -CH_2-, -O-, -OC(=O)H-, -OC(=O)-, -S-, –NH-, -NRa, -$NHSO_2$-, -NHC(=O)NH- or -NHC(=O)-, -NHC(=S)NH- or a bond;

R^8 is a ring system containing 1 or 2 saturated, partially saturated or unsaturated rings each of which has 4-7 ring atoms and each of which has 0 to 4 hetero atoms
15   selected from S, O and N, the ring system being optionally spaced from W by a C_1-C_3alkyl group; or R^8 is C_1-C_6alkyl; any of which R^8 groups can be optionally mono, di, or tri substituted with R^9, wherein

R^9 is independently selected from the group consisting of halo, oxo, nitrile, azido, nitro, C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl, NH_2C(=O)-, Y-
20   NRaRb, Y-O-Rb, Y-C(=O)Rb, Y-(C=O)NRaRb, Y-NRaC(=O)Rb, Y-$NHSO_p$Rb, Y-$S(=O)_p$Rb, Y-$S(=O)_p$NRaRb, Y-C(=O)ORb and Y-NRaC(=O)ORb; wherein said carbocyclyl or heterocyclyl moeity is optionally substituted with R^{10}; wherein

R^{10} is C_1-C_6alkyl, C_3-C_7cycloalkyl, C_1-C_6alkoxy, amino, sulfonyl, (C_1-C_3 alkyl)sulfonyl, NO_2, OH, SH, halo, haloalkyl, carboxyl, amido;
25   Rx is H or C_1-C_5 alkyl; or Rx is J;

T is -CHR^{11}- or -NRd-, where Rd is H, C_1-C_3alkyl or Rd is J;

R^{11} is H or R^{11} is C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl, any of which can be substituted with 1 to 3 substituents independently selected from the group consisting of halo, oxo, nitrile, azido, nitro, C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl,
30   C_0-C_3alkylheterocyclyl, NH_2CO-, Y-NRaRb, Y-O-Rb, Y-C(=O)Rb, Y-(C=O)NRaRb, Y-

NRaC(=O)Rb, Y-NHSO$_p$Rb, Y-S(=O)$_p$Rb, Y-S(=O)$_p$NRaRb, Y-C(=O)ORb, Y-NRaC(=O)ORb; or R^{11} is J;

J, if present, is a single 3 to 10-membered saturated or partially unsaturated alkylene chain extending from the R^7/R$^{7'}$ cycloalkyl, or from the carbon atom to which R^7 is

5   attached to one of Rd, Rj, Rx, Ry or R^{11} to form a macrocycle, which chain is optionally interrupted by one to three heteroatoms independently selected from: -O-, -S- or -NR^{12}-,and wherein 0 to 3 carbon atoms in the chain are optionally substituted with R^{14}; wherein;

R^{12} is H, C$_1$-C$_6$ alkyl, C$_3$-C$_6$cycloalkyl, or COR^{13};

10   R^{13} is C$_1$-C$_6$alkyl, C$_0$-C$_3$alkylcarbocyclyl, C$_0$-C$_3$alkylheterocyclyl;

R^{14} is independently selected from H, C$_1$-C$_6$alkyl, C$_1$-C$_6$haloalkyl, C$_1$-C$_6$alkoxy, hydroxy, halo, amino, oxo, thio, or C$_1$-C$_6$ thioalkyl;

m is 0 or 1; n is 0 or 1;

U is O or is absent;

15   R^{15} is H, C$_1$-C$_6$alkyl, C$_0$-C$_3$alkylcarbocyclyl, C$_0$-C$_3$alkylheterocyclyl, any of which can be substituted with 1 to 3 substituents independently selected from the group consisting of halo, oxo, nitrile, azido, nitro, C$_1$-C$_6$ alkyl, C$_0$-C$_3$alkylheterocyclyl, C$_0$-C$_3$alkylcarbocyclyl, NH$_2$C(=O)-, Y-NRaRb, Y-O-Rb, Y-C(=O)Rb, Y-(C=O)NRaRb, Y-NRaC(=O)Rb, Y-NHSO$_p$Rb, Y-S(=O)$_p$Rb, Y-S(=O)$_p$NRaRb, Y-C(=O)ORb and Y-

20   NRaC(=O)ORb;

G is -O-, -NRy-, -NRjNRj-;

Ry is H, C$_1$-C$_3$ alkyl; or Ry is J;

one Rj is H and the other Rj is H or J;

R^{16} is H; or R^{16} is C$_1$-C$_6$alkyl, C$_0$-C$_3$alkylcarbocyclyl, C$_0$-C$_3$alkylheterocyclyl, any of

25   which can be substituted with halo, oxo, nitrile, azido, nitro, C$_1$-C$_6$alkyl, C$_0$-C$_3$alkylcarbocyclyl, C$_0$-C$_3$alkylheterocyclyl, NH$_2$CO-, Y-NRaRb, Y-O-Rb, Y-C(=O)Rb, Y-(C=O)NRaRb, Y-NRaC(=O)Rb, Y-NHSO$_p$Rb, Y-S(=O)$_p$Rb, Y-S(=O)$_p$NRaRb, Y-C(=O)ORb, Y-NRaC(=O)ORb;

or a pharmaceutically acceptable salt or prodrug thereof.

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2.   A compound according to claim 1, where M is CR^{7}R$^{7'}$.

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3.   A compound according to claim 1, with the partial structure:
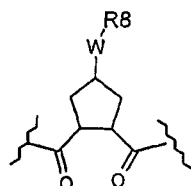
4.   A compound according to claim 1, with the partial structure
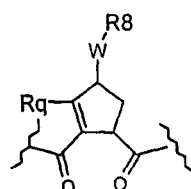
5.   A compound according to claim 4, wherein Rq is C_1-C_3 alkyl, preferably methyl.

6.   A compound according to claim 1, wherein m is 0 and n is 0.

7.   A compound according to claim 6, wherein G is –NRy- or –NRjNRj-.

8.   A compound according to claim 7, where Ry or one of the Rj groups is J, thereby defining a macrocyclic compound.

9.   A compound according to claim 7, wherein R^{16} is H, C_1-C_6alkyl or C_3-C_6 cycloalkyl.

10.   A compound according to claim 1, wherein m is 1.

11.   A compound according to claim 10, wherein U is O.

12.   A compound according to claim 10, wherein T is CR^{11}.

13.   A compound according to claim 12, wherein R^{11} is C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylaryl or C_0-C_3alkylheteroaryl, any of which is optionally

substituted with halo, amino, C_1-C_6alkoxy, C_1-C_6thioalkyl, COOR^{14}, carboxyl, (C_1-C_6alkoxy)carbonyl, aryl, heteroaryl or heterocyclyl; or especially substituted with hydroxyl or COOR^{14}.

5   14.   A compound according to claim 13, wherein R^{11} is tert-butyl, iso-butyl, cyclohexyl, phenylethyl, 2,2-dimetyl-propyl, cyclohexylmethyl, phenylmethyl, 2-pyridylmethyl, 4-hydroxy-phenylmethyl, or carboxylpropyl, especially tert-butyl, iso-butyl, or cyclohexyl.

10   15.   A compound according to claim 10, wherein one of Rd, Rx or R^{11} is J, thereby defining a macrocyclic compound.

16.   A compound according to claim 10, wherein n is 1.

15   17.   A compound according to claim 16, wherein R^{15} is C_1-C_6alkyl or C_0-C_3alkylcarbocyclyl, either of which is optionally substituted.

18.   A compound according to claim 17, wherein R^{15} is cyclohexyl, cyclohexylmethyl, tert-butyl, iso-propyl, or iso-butyl.

20

19.   A compound according to claim 10, wherein G is NRy or NRjNRj, where Ry or one Rj is H or methyl and the other is H.

20.   A compound according to claim 19, wherein R^{16} is H, C_1-C_6alkyl or a 5 or 6
25   membered heterocycle, especially morpholine, piperidine or piperazine.

21.   A compound according to claim 10, wherein R^{16} is C_1-C_6alkyl, C_0-C_3alkylheterocyclyl, C_0-C_3alkylcarbocyclyl, any of which is optionally substituted with hydroxy, halo, amino, or C_1-C_6alkoxy.

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22.   A compound according to claim 21, wherein R^{16} is 2-indanol, indanyl, 2-hydroxy-1-phenyl-ethyl, 2-thiophenemethyl, cyclohexylmethyl, 2,3-methylenedioxybenzyl, cyclohexyl, benzyl, 2-pyridylmethyl, cyclobutyl, iso-butyl, n-propyl, or 4-methoxyphenylethyl.

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23.   A compound according to claim 1, wherein W is -OC(=O)-, -NRa-, $-NHS(O)_2$-or -NHC(=O)-; or especially –OC(=O)NH- or –NH.

24.   A compound according to claim 1, wherein W is –S-, a bond or especially –O-.

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25.   A compound according to claim 23 or 24 wherein R^8 is optionally substituted C_0-C_3alkylcarbocyclyl or optionally substituted C_0-C_3-alkylheterocyclyl.

26.   A compound according to claim 25, wherein the C_0-C_3 alkyl moiety is

15   methylene or preferably a bond.

27   A compound according to claim 26 wherein R^8 is C_0-C_3alkylaryl, or C_0-C_3alkylheteroaryl, either of which is optionally mono, di, or tri substituted with R^9, wherein;

20   R^9 is C_1-C_6 alkyl, C_1-C_6alkoxy, NO_2, OH, halo, trifluoromethyl, amino or amido optionally mono- or di-substituted with C_1-C_6alkyl, C_0-C_3alkylaryl, C_0-C_3alkylheteroaryl, carboxyl, aryl or heteroaryl being optionally substituted with R^{10}; wherein

R^{10} is C_1-C_6alkyl, C_3-C_7cycloalkyl, C_1-C_6alkoxy, amino optionally mono- or di-

25   substituted with C_1-C_6alkyl, C_1-C_3 alkyl amide, sulfonylC_1-C_3alkyl, NO_2, OH, halo, trifluoromethyl, carboxyl, or heteroaryl.

28.   A compound according to claim 27 wherein R^9 is C_1-C_6 alkyl, C_1-C_6alkoxy, amino, di-(C_1-C_3 alkyl)amino, C_1-C_3alkylamide, aryl or heteroaryl, the aryl or

30   heteroaryl being optionally substituted with R^{10}; wherein

R^{10} is C_1-C_6alkyl, C_3-C_7cycloalkyl, C_1-C_6alkoxy, amino, mono- or di-C_1-C_3 alkylamino, amido, C_1-C_3 alkylamide, halo, trifluoromethyl, or heteroaryl.

29.   A compound according to claim 28, wherein, R^{10} is C_1-C_6alkyl, C_1-C_6alkoxy, amino optionally mono- or di substituted with C_1-C_3 alkyl, amido, C_1-C_3-alkylamide, halo, or heteroaryl.

30.   A compound according to claim 29 wherein R^{10} is methyl, ethyl, isopropyl , tert-butyl, methoxy, chloro, amino optionally mono- or di substituted with C_1-C_3 alkyl, amido, C_1-C_3alkylamide, or C_1-C_3alkyl thiazolyl.

31   A compound according to claim 26, wherein R^8 is 1-naphthylmethyl, 2-naphthylmethyl, benzyl, 1-naphthyl, 2-naphthyl, or quinolinyl any of which is unsubstituted, mono, or disubstituted with R^9 as defined.

32   A compound according to claim 31 wherein R^8 is 1-naphthylmethyl, or quinolinyl any of which is unsubstituted, mono, or disubstituted with R^9 as defined.

33   A compound according to claim 32 wherein R^8 is:
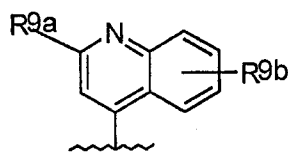
wherein R^{9a} is C_1-C_6 alkyl; C_1-C_6alkoxy; thioC_1-C_3alkyl; amino optionally substituted with C_1-C_6alkyl; C_0-C_3alkylaryl; or C_0-C_3alkylheteroaryl, C_0-C_3alkylheterocyclyl, said aryl, heteroaryl or heterocycle being optionally substituted with R^{10} wherein R^{10} is C_1-C_6alkyl, C_0-C_3alkylC_3-C_7cycloalkyl, C_1-C_6alkoxy, amino optionally mono- or di-substituted with C_1-C_6alkyl, amido, C_1-C_3alkyl amide; and

R^{9b} is C_1-C_6 alkyl, C_1-C_6-alkoxy, amino, di(C_1-C_3alkyl)amino, (C_1-C_3alkyl) amide, NO_2, OH, halo, trifluoromethyl, carboxyl.

34   A compound according to claim 33, wherein R^{9a} is aryl or heteroaryl, either of which is optionally substituted with R^{10} as defined.

35   A compound according to 34, wherein R^{9a} is selected from the group consisted of:
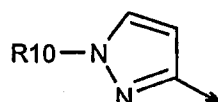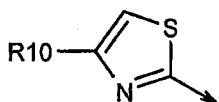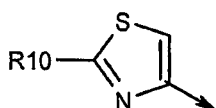
wherein R^{10} is H, C_1-C_6alkyl, or C_0-C_3alkylcycloalkyl, amino optionally mono- or di-substituted with C_1-C_6alkyl, amido, (C_1-C_3alkyl)amide.

36.   A compound according to claim 34, wherein R^{9a} is optionally substituted phenyl, preferably phenyl substituted with C_1-C_6alkyl; C_1-C_6alkoxy; or halo.

37.   A compound according to claim 33, wherein R^8 is:
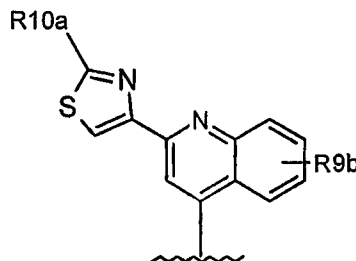
wherein R^{10a} is H, C_1-C_6alkyl, or C_0-C_3alkylcarbocyclyl, amino optionally mono- or di-substituted with C_1-C_6alkyl, amido, (C_1-C_3 alkyl)amide, heteroaryl or heterocyclyl; and R^{9b} is C_1-C_6 alkyl, C_1-C_6-alkoxy, amino, di(C_1-C_3 alkyl)amino, (C_1-C_3 alkyl)amide, NO_2, OH, halo, trifluoromethyl, or carboxyl.

38.   A compound according to any claim 33, wherein R^{9b} is C_1-C_6-alkoxy, preferably methoxy.

39.   A compound according to claim 1, wherein A is C(=O)NHSO$_2$R^2.

40.   A compound according to claim 39, wherein R^2 is optionally substituted C_1-C_6 alkyl, preferably methyl.

41.   A compound according to claim 39, wherein R^2 is optionally substituted C_3-C_7cycloalkyl, preferably cyclopropyl.

42.   A compound according to claim 39, wherein R^2 is optionally substituted C_0-C_6alkylaryl, preferably optionally substituted phenyl.

43.   A compound according to claim 1, wherein A is $C(=O)OR^1$.

44.   A compound according to claim 43, wherein R^1 is H or C_1-C_6 alkyl, preferably hydrogen, methyl, ethyl, or tert-butyl.

45.   A compound according to claim 2, wherein $R^{7'}$ is H and R^7 is n-ethyl, cyclopropylmethyl, cyclobutylmethyl or mercaptomethyl, preferably n-propyl or 2,2-difluoroethyl.

46.   A compound according to claim 2, wherein R^7 and $R^{7'}$ together define a spiro-cyclopropyl or spiro-cyclobutyl ring, both optionally mono or di-substituted with $R^{7'a}$ wherein;
$R^{7'a}$ is C_1-C_6 alkyl, C_3-C_5cycloalkyl, or C_2-C_6 alkenyl, any of which is optionally substituted with halo; or R^{7a} is J.

47.   A compound according to claim 47 wherein the ring is a spiro-cyclopropyl ring substituted with $R^{7'a}$ wherein;
$R^{7'a}$ is ethyl, vinyl, cyclopropyl, 1- or 2-bromoethyl, 1-or 2-fluoroethyl, 2-bromovinyl or 2-fluorethyl.

48.   A compound according to claim 2, wherein R^7 is J and $R^{7'}$ is H.

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49.   A compound according to claim 1, wherein J is a 3 to 8-membered saturated or unsaturated alkylene chain optionally containing one to two heteroatoms independently selected from: -O-, -S- or -NR^{12}- , wherein R^{12} is H, C$_1$-C$_6$ alkyl, such as methyl, or -C(=O)C$_1$-C$_6$ alkyl, such as acetyl.

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50.   A compound according to claim 49, wherein J is a 4 to 7-membered saturated or unsaturated, all carbon alkylene chain.

51.   A compound according to claim 49, wherein J is saturated or mono-
10   unsaturated.

52.   A compound according to claim 49, wherein J is dimensioned to provide a macrocycle of 14 or 15 ring atoms.

15   53.   A pharmaceutical composition comprising a compound as defined in claim 1 and a pharmaceutically acceptable carrier therefor.

54.   A pharmaceutical composition according to claim 53, further comprising an additional HCV antiviral, selected from nucleoside analogue polymerase inhibitors,
20   protease inhibitors, ribavirin and interferon.

55.   Use of a compound as defined in claim 1 in therapy.

56.   Use of a compound as defined in claim 1 in the manufacture of a medicament
25   for the prophylaxis or treatment of flavivirus infections, including HCV.

57.   A method for treatment or prophylaxis of flavivirus infection such as HCV comprising the administration of an effective amount of a compound as defined in claim 1 to an individual afflicted or at risk of such infection.

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NMR Structural Characterization of Peptide Inhibitors Bound to the Hepatitis C Virus NS3 Protease: Design of a New P2 Substituent

Nathalie Goudreau,*,† Dale R. Cameron,†,‡ Pierre Bonneau,§ Vida Gorys,† Céline Plouffe,§ Martin Poirier,† Daniel Lamarre,§ and Montse Llinas-Brunet†

Departments of Chemistry and Biological Sciences, Boehringer Ingelheim (Canada) Ltd., Research & Development, 2100 Cunard Street, Laval, Québec, Canada H7S 2G5

A comparative NMR conformational analysis of three distinct tetrapeptide inhibitors of the Hepatitis C NS3 protease that differ at the 4-aryloxy-substituted P2 proline position was undertaken. Specifically, transferred nuclear Overhauser effect experiments in combination with restrained systematic conformational searches were used to characterize the orientation of the P2 aryl substituents of these inhibitors when bound to the NS3 protease. Differences between free and bound conformations were also investigated. Analysis of the results allowed the design of a new P2 aromatic substituent, which significantly increased the potency of our inhibitors. The bound conformation of a specific competitive inhibitor having this novel P2 substituent is also described, along with a model of this inhibitor bound to the NS3 protease. This NS3 protease/inhibitor complex model also supports a hypothetical stabilization role for the P2 residue of the substrates and/or inhibitors and further elucidates the subtle details of the binding of the P2 residue of substrate-based inhibitors.

Introduction

Chronic Hepatitis C virus (HCV) infection is a major health problem that leads to cirrhosis, hepatocellular carcinoma, and liver failure in a substantial number of infected individuals, now estimated to be more than 170 million worldwide.[1] For many patients, the current therapies, which involve treatment with interferon α alone or in combination with the nucleoside analogue ribavirin, are not highly effective and result in severe side effects.[2] There is consequently an urgent need for the development of novel, specific, and more efficacious treatment strategies against this viral infection.

HCV is a small, enveloped virus containing a single positive strand RNA genome of approximately 9.6 kb. The genome carries a single long open-reading frame, which encodes a polyprotein of about 3000 amino acids. The HCV polyprotein, which is composed of structural proteins in its N-terminal portion and of nonstructural (NS) proteins in the remainder, is cleaved co- and posttranslationally by cellular and viral proteinases into at least 10 different products.[3]

The most intensively studied and best characterized target in the HCV genome is the NS3 serine protease, which is located within the N-terminal one-third (180 amino acids) of the NS3 protein.[4] This protease is responsible for the maturation of the viral polyprotein through cleavage at the NS3/4A, NS4A/B, NS4B/5A, and NS5A/B junctions. The first cleavage (NS3/4A) occurs rapidly and in cis, while the remaining three others occur in trans. Although the NS3 protease has proteolytic activity on its own in vitro, the presence of the

NS4A protein, which acts as a cofactor, was shown to be essential for the cleavage of the NS4A/B and NS4B/5A junctions, whereas its presence enhances the cleavage efficiency at the NS5A/B junction.[5] The NS4A activator is a 54 residue polypeptide that forms a noncovalent complex with the NS3 protease. The crystal and the NMR solution structures of the uncomplexed NS3 protease domain have already been reported.[6,7] Both of these studies revealed that this enzyme adopts a canonical chymotrypsin-like fold consisting of N-terminal and C-terminal β-barrel domains, with the catalytic triad residues His57, Asp81, and Ser139 located in a deep cleft at the interface between these two domains. In addition, two X-ray structures of the NS3 protease in complex with a synthetic NS4A 12 residue cofactor peptide show that the NS4A peptide intercalates within the N-terminal β-sheet domain of the enzyme, resulting in a more ordered structure.[8,9] The combined structural data suggest that binding of the NS4A primarily affects the fold of the N-terminal domain of the NS3 protease, which corresponds to the S′ binding site of the substrates (as defined by Schechter and Berger nomenclature).[10]

The NS3 protease was shown to be essential for HCV replication and infection in the chimpanzee model, providing direct validation of this enzyme as a target for the development of new antiviral drugs.[11] In this respect, we and others have reported that N-terminal cleavage products of peptide substrates (S binding site) are competitive inhibitors of the NS3 protease activity.[12,13] This finding has served as the basis for designing substrate-based inhibitors, and our efforts in this area focused on improving the potency and on decreasing the peptidic character of these inhibitors. Starting with the hexapeptide Ac-DDIVPNva-OH as our original lead (IC$_{50}$ = 150 μM), we found that a 400-fold increase in potency could be obtained by the introduction of a (4*R*)-

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† Department of Chemistry.
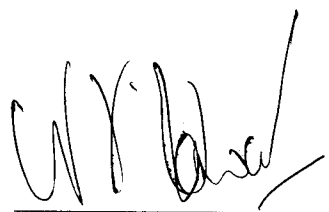‡ Present address: Micrologix Biotech Inc., BC Research Complex, 3650 Wesbrook Mall, Vancouver, BC, V6S 2L2.
§ Department of Biological Sciences.

FORM 7A

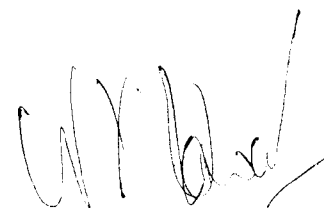## THE PATENTS ACT, 1970
(39 OF 1970)

AND

THE PATENTS RULES, 2003

REPRESENTATION FOR OPPOSITION TO GRANT OF PATENT
[see rule 55]

We, Indian Pharmaceutical Alliance, hereby give representation by way of opposition to the Grant of patent in respect of Application No.**10158/DELNP/2007** Dated **28/12/2007** made by **TIBOTEC PHARMACEUTICALS AND MEDIVIR AB** and published on **20/06/2008** on the grounds of Filing of Written Submission u/s 25(1) of the Act for Pre-Grant Opposition.

Our address for service in India is:-

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> nayanrawal@gmail.com

Nayan Rawal
Constituted Attorney for
the Opponents
IN/PA 654

Dated- 19th August 2014

To
The Conrtroller of patents,
The patent office,
at Delhi

FORM 7A

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Nayan Rawal
Constituted Attorney for
the Opponents
IN/PA 654

Dated- 19th August 2014

To
The Conrtroller of patents,
The patent office,
at Delhi

GENERAL POWER OF ATTORNEY

We, **Indian Pharmaceutical Alliance**, a Society registered under the Societies Registration Act having its mailing address as follows **C/o VISION CONSULTING GROUP, 201 Darvesh Chambers, 743 P D Hinduja Road, Khar (W), Mumbai 400 052** hereby authorize Mr. Nayan J. Rawal, Patent Agent (Agent No.654), having its Office at L-303, Panchsheel Gardens, Mahavir Nagar, Kandivli West, Mumbai 400 067, to act as our Agents and Attorneys for various Pre-Grant and Post-Grant Opposition, rectification and any other proceedings under the Patents (Amendment) Act, 1970 in respect of various Patents filed in India.

We request that all notices, requisitions and communications may be sent to the said Agents at the following address:-

Vision Consulting Group, 201 Darvesh Chambers, 743 P D Hinduja Road, Khar (W), Mumbai 400 052.

We hereby revoke all previous authorizations, if any; we hereby ratify all acts done by the said Agents

Dated this 19th day of April 2010

For Indian Pharmaceutical Alliance

D G Shah
Secretary General

IN THE MATTER OF THE PATENTS ACT, 1970

and

IN THE MATTER OF THE PATENT RULES, 2003
(as amended by the Patents (Amendment) Rules 2006)

and

IN THE MATTER OF INDIAN PATENT APPLICATION NO. 10158/DELNP/2007 FILED BY TIBOTECH PHARMACEUTICAL AND MEDIVIR AB

..........the Applicants

and

IN THE MATTER OF A REPRESENTATION BY WAY OF AN OPPOSITION UNDER SECTION 25(1) AND RULE 55 THERETO BY INDIAN PHARMACEUTICALS ALLIANCE

..........the Opponents

REPRESENTATION BY WAY OF OPPOSITION U/S 25(1)

1.0   It is respectfully submitted on behalf of Indian Pharmaceutical Alliance, that a pre-grant Opposition under Section 25(1) of the Patents Act, 1970 and rule 55(1) of the Patents Rules, 2003 (as amended by the Patents (Amendment) Rules 2006), is hereby presented by the "Opponents" against Indian Patent Application No. **10158/DELNP/2007** (hereinafter also referred to as the "Opposed Application") in the name of **TIBOTECH PHARMACEUTICAL AND MEDIVIR AB** (hereinafter referred to as the "Applicants").

It is respectfully submitted:

2.0   The Opponents are an associations of person registered under the SOCIETIES REGISTRATION ACT, XXI OF 1860 in the name and style of "INDIAN PHARMACEUTICAL ALLIANCE" having its registered office 115/11, GROUND FLOOR, WORLD TRADE CENTRE, BABAR ROAD, CONNAUGHT PLACE, NEW DELHI – 110001, the main object of as follows:-

(a)   To support the development of international and regional policies, which seek to ensure, access to medical care for all customers.

(b)   To promote balanced and generic friendly intellectual property rights in the pharmaceuticals sector to ensure that timely access to markets is guaranteed for new and generic pharmaceutical products.

(c)   To promote the global harmonization relating to generic products.

(d)   To support the right of all governments to regulate their own pricing, substitution, prescribing and reimbursement policies.

(e)   To suggest measures for enhancing pharmaceutical research in India, both in the areas of basic as well as applied research.

(f)   To interact with the environmental protection agencies to evolve uniform standards of environmental protection measures across the country and ensure implementation of the same.

(g)   To suggest measures to strengthen the pharmaceutical pricing framework that ensures an equitable pricing system for industry and consumers.

(h)   One of the further object of the society is to promote cause of generic pharmaceutical industry and to provide support for the development of competition on the off Patent pharmaceutical sector and to prepare position papers for representing India at international for a to highlight the problems face by generic pharmaceutical companies in international market. It also aims at strengthening regulatory agencies for patenting registration and quality assurance of drugs and pharmaceuticals by providing gaudiness to government and international organization in improving the regulatory and legal expertise relating to registration and marketing of drugs and pharmaceutical. It also further aims at interacting with the regulatory authorities to streamline the guidelines for clinical trials and bio-equivalence studies, to ensure expeditious registration of new as well as existing drugs.

2.1 The opposed patent application pertains to certain macrocyclic compound having inhibitory activity on the replication of the Hepatitis C virus, including specifically the compound known as Simeprevir.

2.2 Although a representation of Opposition can be made by "any person", "in writing" under Section 25(1) of The Patents Act, 1970; however, the Opponents interest in opposing this application is, substantial and real. The Opponents, therefore, have *locus standi* in opposing this application.

2.3 It is respectfully submitted that the Opposed Application entitled **"MACROCYCLIC INHIBITORS OF HEPATITIS C VIRUS"** has been filed on December 28, 2007 and published in the Official Journal of the Indian Patent office on June 20, 2008. The specification of Opposed Application is attached herewith as **Document 4 (D4)**.

2.4 The Opponents are filing this Representation by way of Opposition against Indian Patent Application No. 10158/DELNP/2007 (the Opposed Application), along with documentary evidence and facts in support thereof.

2.5   In this representation by way of opposition, the following grounds enumerated in Section 25 (1) of The Patents Act, 1970 are relied upon (hereinafter referred to as the "Act"):

(a) that the applicant for the patent or the person under or through whom he claims, wrongfully obtained the invention or any part thereof from him or from a person under or through whom he claims;

(b) that the invention so far as claimed in any claim of complete specification has been published before the priority date of the claim –

i) in any specification filed in pursuance of an application for a patent made in India on or after the 1ˢᵗ day of January, 1912; or

ii) in India or elsewhere, in any other document

Provided that the ground specified in sub-clause (ii) shall not be available where such publication does not constitute an anticipation of the invention by virtue of sub-section (2) or sub-section (3) of section 29;

(c) that the invention so far as claimed in any claim of the complete specification is claimed in a claim of a complete specification published on or after the priority date of the applicant's claim and filed in pursuance of an application for a patent in India, being a claim of which the priority date is earlier than that of the applicant's claim;

(d) that the invention so far as claimed in any claim of the complete specification was publicly known or publicly used in India before the priority date of the claim.

Explanation:- For the purpose of this clause, an invention relating to a process for which a patent is claimed shall be deemed to have been publicly known or publicly used in India before the priority date of the claim if a product made by that process had already been imported into India before that date expect where such importation has been for the purpose of reasonable trial or experiment only;

(e) That the invention so far as claimed in any claim of the complete specification is obvious and clearly does not involve any inventive step having regard to the matter published as mentioned in clause (b) or having regard what was used in India before the priority date of the applicant's claim;

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(f) that the subject matter of any claim of the complete specification is not invention within the meaning of this Act, or is not patentable under this Act;

(g) that the complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed;

(h) that the applicant has failed to disclose to the Controller the information required by section 8 or has furnished the information which in any material particular was false to his knowledge;

(i) that in the case of convention application, the application was not made within twelve months from the date of the first application for protection for the invention made in a convention country by the applicant or a person from whom he derives title;

(j) that the complete specification does not disclose or wrongly mentions the source or geographical origin of biological material used for the invention;

(k) That the invention so far as claimed in any claim of the complete specification is anticipated having regard to the knowledge, oral or otherwise, available within any local or indigenous community in India or elsewhere;

The present *Representation By Way Of Opposition U/S 25(1)* takes into consideration the following documents:

Document 1 [D1] – WO2005073195 Published on 11th August 2005.

Document 2 [D2] – J. Med. Chem., (2004), 47, 123-132.

Document 3 [D3] – J. Med. Chem., (2004), 47, 6584-6594.

Document 4 [D4] – Specification of Opposed Application.

Annexure-I – Bristol-Myers Squibb Company v.Teva Pharmaceuticals USA, INC. (US Case Decision).

Since the earliest priority date of the opposed patent application is July 29, 2005, it is apparent that all documents listed above were publicly available before the claimed priority date of the opposed patent application and thus represent prior art according to Section 25 (1) (a), (b), (c) and (d) of The Patents Act, 1970.

3.0 THE ALLEGED INVENTION AND THE CLAIMED SUBJECT MATTER OF THE OPPOSED PATENT APPLICATION

Preliminary Remarks

1. The opposed patent application at present is having 26 claims (amended claim as on 3 October 2013 as obtained from the IPO website). Claims 1 is independent claim and other claims are dependent on claim 1.

2. The opposed patent application pertains to certain macrocyclic compound having inhibitory activity on the replication of the Hepatitis C virus, including specificallythe compound known as Simeprevir.

The Applicant's claimed invention in '10158/DELNP/2007' may be summarised as follows:

a) Claim 1 relates to a compound of Formula (1) and the salts, N-oxide and stereoisomers thereof covering a broader group of compounds (Markush claim).

b) Claim 2 relates to the compound of claim 1 but is narrower in its coverage wherein various possible functional groups mentioned in the general Formula 1 are described and claim formula (I-c), (I-d) and (I-e).

c) Claims 3-16 relate to various compounds resulting from different combinations of various functional groups covered by the general formulas in Claims 1 and 2.

d) Claim 17 relate to specific compound resulting from different combinations of various functional groups covered by the general formulas in Claims 1 and 2.

e) Claim 18 relate to specific compound as claimed in claim 17 is white solid.

f) Claim 19-21 relate to specific compound resulting from different combinations of various functional groups covered by the general formulas in Claims 1 and 2.

g) Claim 22-23relate to specific compound as claimed in claim1-21 is other than N-oxide.

h) Claim 24 relates to pharmaceutical composition of compound as claimed in claim 1-23

i) Claim 25 relates to a method of use of compound as claimed in claim 1-23 as inhibiting HCV replication.

j) Claim 26 relates to a method for preparing a compound of formula (I).

It is Opponents humble submission that none of the claims contained in the INDIAN PATENT APPLICATION No. 10158/DELNP/2007 is novel or inventive or is otherwise patentable under the Act. The Opposed Application does not fulfill the patentability criteria under the Act. The subject matter of the claims lack novelty and inventive step over the prior art. The Opposed Application also does not sufficiently and clearly describes the alleged invention for it to be carried out by a person skilled in the art. These submissions will be elaborated while dealing with the specific grounds of opposition hereunder.

4.0   ANTICIPATION BY PRIOR PUBLICATION – SECTION 25 (1) (b)

4.1   Claim 1 lacks Novelty vis-à-vis WO 2005073195 (D1 to Medivir AB)

Claim 1 of the opposed patent is for a compound:

A compound having the formula

(I)

7
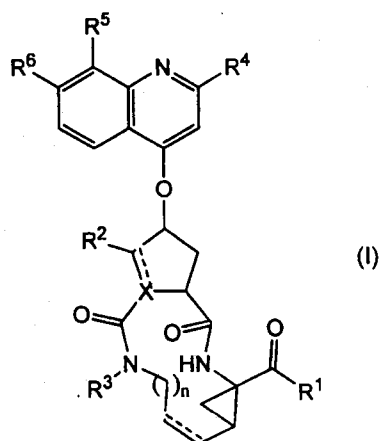
Wherein

X isCH;

R^5 is C_1-C_6 alkyl;

R^6 is C_1- C_6 alkoxy;

R^4 is Het;

R^3 is C_1-C_6 alkyl;

R^2 is hydrogen;

R^1 is –$NHSO_2R_8$;

R^8 is C_3-C_7 cycloalkyl;

Claim 1 and its specific compound in claim 17.

17. A compound as claimed in claim 1 wherein the compound of formula (I) is:
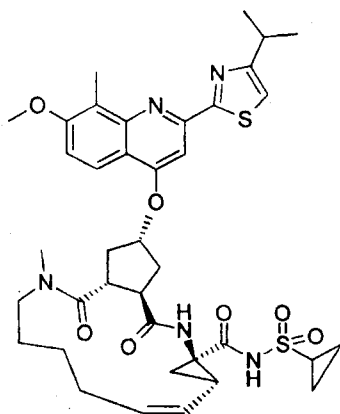
The alleged application is not novel in the light of WO2005073195 (D1) which was published on 1 1August2005 (prior publication, earlier than the filing date of the opposed application) and is therefore citable under Section 25(1)(b) of the Act Opponent would like to draw the attention of Examiner/ Controller that-

D1 is an international application of Medivir AB and discloses compounds of Formula VI, or their pharmaceutically salts or pro-drug thereof, and the use of such compounds as inhibitor of NS3 serine protease of the Flavivirus HCV and to method for their use in the treatment of prophylaxis and HCV. Moreover, the patent application also discloses that the macrocyclic structure within formula VI is a favored embodiment of the invention (See page 11 of D1).

R15

G

R16

N

O

Ru n

U

T

Rx

N

Jm

O

Rz

Rq *

(CH₂)q'

W

R8

(CH₂)k

N H

O

M

A

VI
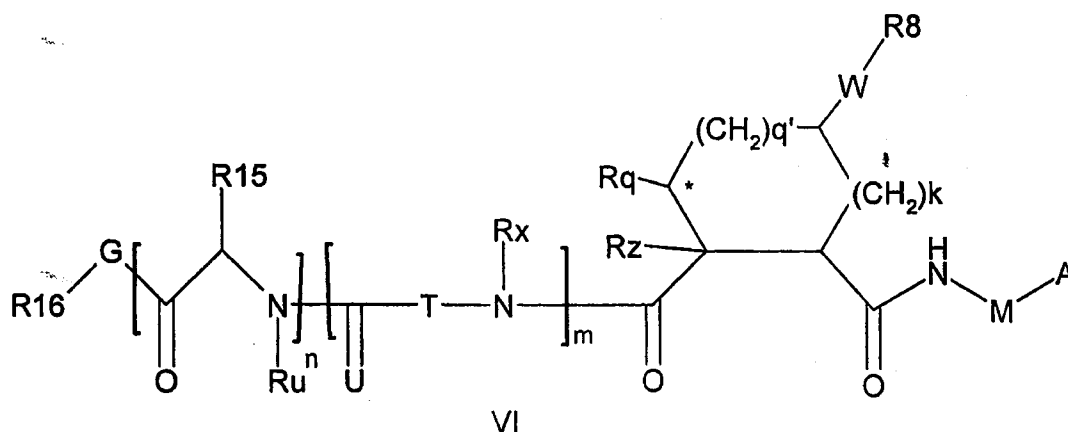
More specifically, document D1 disclosed a chemical compound having formula (VIhe) including its favored definition of R8: (see page 14 of D1)
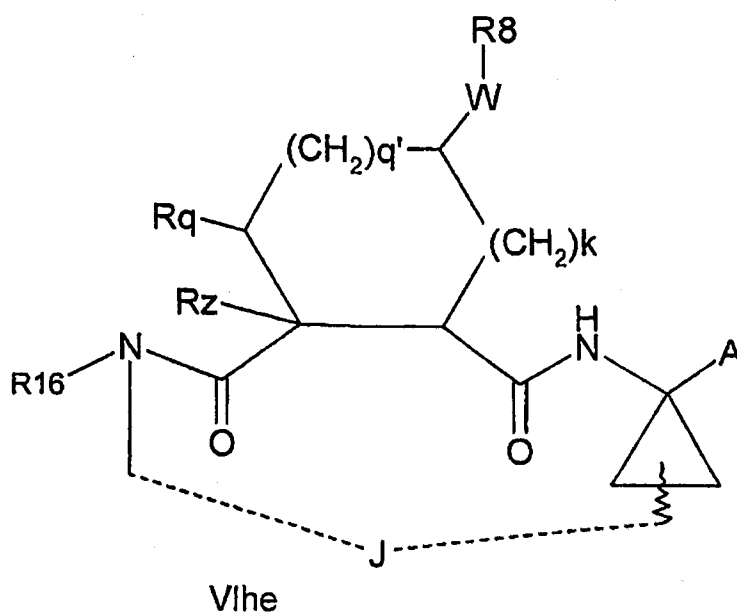
R8

W

(CH₂)q'

Rq

(CH₂)k

Rz

R16

N

O

N H

O

A

J

VIhe

Favoured embodiments of R8 include 1-naphthylmethyl, 2-naphthylmethyl, benzyl, 1-naphthyl, 2-naphthyl, or quinolinyl, any of which is unsubstituted, or mono-or disubstituted with R9 as defined, in particular 1-naphthylmethyl, or quinolinyl unsubstituted, mono, or **disubstituted with R9 as defined.**

Wherein **R9 is C1-C6 alkyl, C1-C6alkoxy**, NO_2, OH, halo, trifluoromethyl, amino or amido (such as amino or amido optionally mono-or di-substituted with C1-C6alkyl), C0-C3alkylaryl, C0-C3alkylheteroaryl, carboxyl, aryl or heteroaryl being optionally substituted with R10; wherein R10 is C1-C6alkyl, C3-C7cycloalkyl, C1-C6alkoxy, amino (such as amino mono-or di- substituted with C1-C6alkyl), amido (such as Cl-C3 alkyl amide), sulfonylC1-C3alkyl, NO2, OH, halo, trifluoromethyl, carboxyl, or heteroaryl.

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A currently preferred R8 is:

wherein R9a is **C1-C6alkyl ; C1-C6alkoxy**; thioC1-C3alkyl ; amino optionally substituted with C1-C6alkyl; C0-C3alkylaryl; or C0-C3alkylheteroaryl, C0-C3alkylheterocyclyl, said aryl, heteroaryl or heterocycle being optionally substituted with R10 wherein R10 is C1-C6alkyl, C3-C7cycloalkyl, C1-C6alkoxy, amino, amido, heteroaryl or heterocyclyl ; and R9b is C1-C6alkyl, C1-C6alkoxy, amino, amido, NO_2, OH, halo, trifluoromethyl, carboxyl.

Convenient R9a include aryl or heteroaryl, all optionally substituted with R10 as defined, especially where R9a is selected from the group consisted of:

An alternative preferred R8 is:
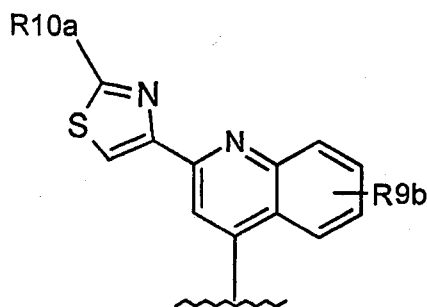
wherein R10a is H, **C1-C6alkyl** or C0-C3alkyl-C3-C6cycloalkyl, amino (such as amino optionally mono-or di-substituted with C1-C6alkyl), amido (such as as-NHC(O)C1-C6alkyl or C(=O)NHC1-C3alkyl or C(=O)(C1-C3alkyl) 2), heteroaryl or heterocyclyl ; and **R9b is C1-C6 alkyl, C1-C6-alkoxy**, amino optionally **mono-or di-substituted with** C1-C6alkyl, amido (such as as-NHC(O)C1-C6alkyl or C(=O)NHC1-C3alkyl or C(=O)N(C1-C3alkyl) 2), NO_2, OH, halo, trifluoromethyl, or carboxyl.

A particularly preferred configuration for A is C(=O)NHSO2R2, especially where R2 is optionally substituted C1-C6alkyl, preferably methyl, or optionally substituted C3-C7cycloalkyl, preferably cyclopropyl, or optionally substituted C0-C6alkylaryl, preferably optionally substituted phenyl. Appropriate substituents appear in the definitions section below.

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Accordingly, the compounds of claims 1 and 17 of the opposed application is directly disclosed in document D1, if the definition of W, R^q, R^z, R^8, A, J, R^{16} and 'k' given in D1 are considered as is provided below:

'Wherein W is O;

R^q and R^z are H;

R^{16} is CH_3;

J is hexylene;

K is 0;

A is $C(=O)NHSO_2R_2$; where R_2 is optionally substituted C3-C7cycloalkyl, preferably cyclopropyl;

R^8 is ;
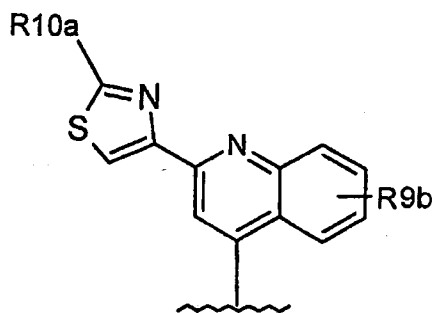
Wherein R10a is C1-C6alkyl and; **R9b is C1-C6 alkyl, C1-C6-alkoxy** optionally **mono-or di-substituted.**

Hence, the disclosure & claim to a compound according to claims 1 and 17 of the opposed patent application is found in the document D1.

This means that compound of formula (I) of claim 1 and compound of formula (I-b) of claim 17 are anticipated by D1. Hence compound of formula (I) of claim 1 and compound of formula (I-b) of claim 17are not novel and is clearly anticipated by D1.

4.2 **Dependent Claim 24 lacks Novelty vis-à-vis WO2005073195 (D1,Medivir AB)**

Claim 24 A pharmaceutical compositions comprising a carrier, and as active ingredient an ant virally effective amount of compound as claimed in anyone of the claim 1-23.

Claims 24 of opposed patent application, which is for pharmaceutical composition of compound of claim 1 with a pharmaceutical acceptable medium is anticipated by D1.

Claim 53 and 54 of Document D1

53. A pharmaceutical composition comprising a compound as defined in claim 1 and a pharmaceutically acceptable carrier therefor.

54. A pharmaceutical composition according to claim 53, further comprising an additional HCV antiviral, selected from nucleoside analogue polymerase inhibitors, protease inhibitors, ribavirin and interferon.

As the discussion above already demonstrates the disclosure and claims made in D1 include the structure of the compound claimed in claims 1 & 17. Additionally claim 53 and 54 disclosed pharmaceutical composition of the same compound, which is claimed in claims 24 of the opposed application. Hence, claim 24 is anticipated by D1.

4.3 Dependent Claim 26 process claim lacks novelty vis-à-vis WO2005073195 (D1 to Medivir AB)

Claim 26 of the opposed patent application is;

26. A process for preparing a compound as claimed in any of claims 1 - 23, wherein said process comprises:

(a) preparing a compound of formula (I) wherein the bond between C_7 and C_8 is a double bond, which is a compound of formula (I-i), by forming a double bond between C_7 and C_8, in particular via an olefin metathesis reaction, with concomitant cyclization to the macrocycle as outlined in the following reaction scheme:

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(1a) → (I-i)
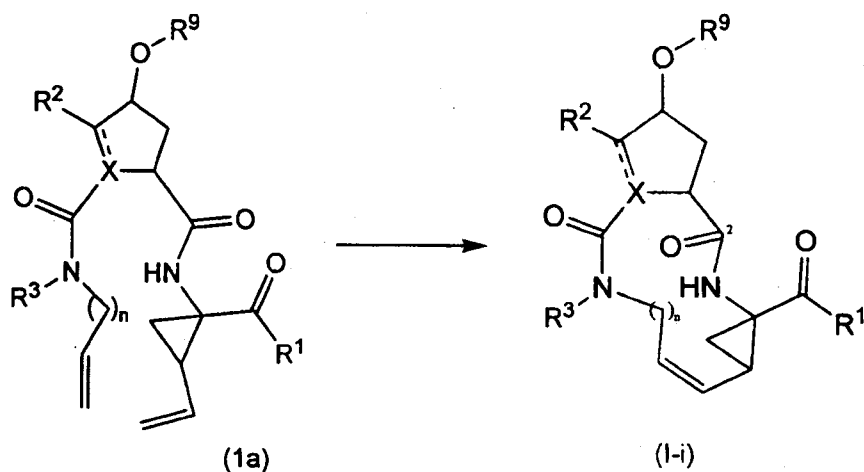
wherein in the above and following reaction schemes R^9 represents a radical

;
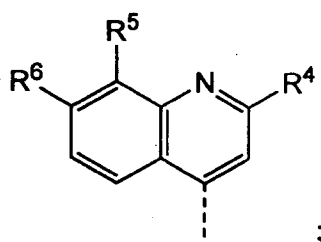
(b) converting a compound of formula (I-i) to a compound of formula (I) wherein the link between C7 and C8 in the macrocycle is a single bond, i.e. a compound of formula (I-j):

(I-j)
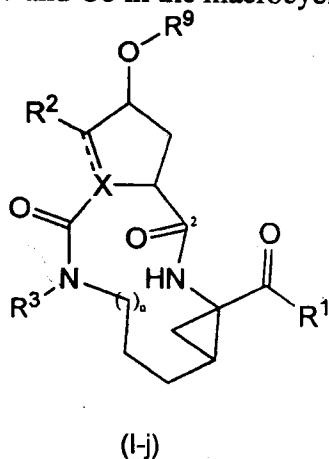
by a reduction of the C7-C8 double bond in the compounds of formula (I-j);

(c) preparing a compound of formula (I) wherein R^1 represents $-NHSO_2R^8$, said compounds being represented by formula (I-k-1), by forming an amide bond between a intermediate (2a) and an sulfonylamine (2b), or preparing a compound of formula (I) wherein R^1 represents $-OR^7$, i.e. a compound (I-k-2), by forming an ester bond between

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an intermediate (2a) and an alcohol (2c) as outlined in the following scheme wherein G represents a group:

(a),

G-COOH   + H_2N-SO_2R^8 $\longrightarrow$

(2a)   (2b)

(I-k-1)

G-COOH   + HOR^7 $\longrightarrow$

(2a)   (2c)

(I-k-2)
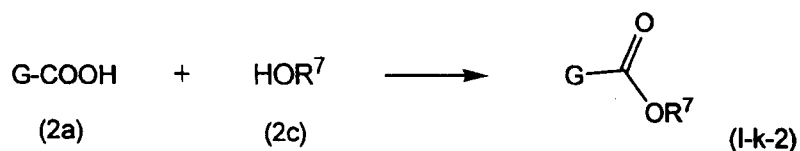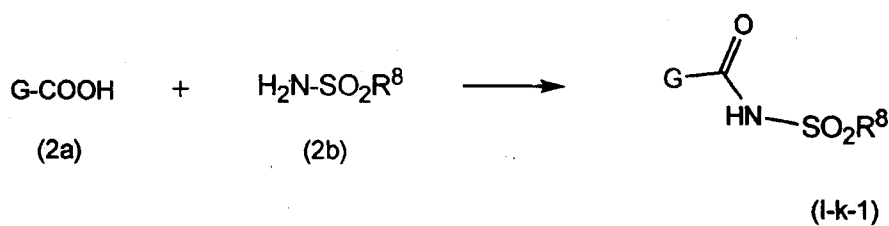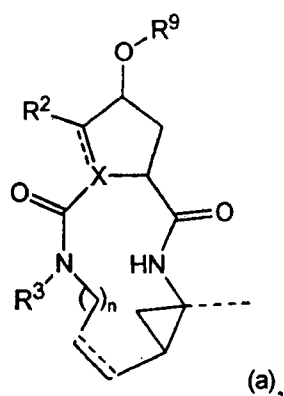
(d) preparing a compound of formula (I) wherein R^3 is hydrogen, said compound being represented by (I-l), from a corresponding nitrogen-protected intermediate (3a), wherein PG represents a nitrogen protecting group:

(3a)   (I-l)
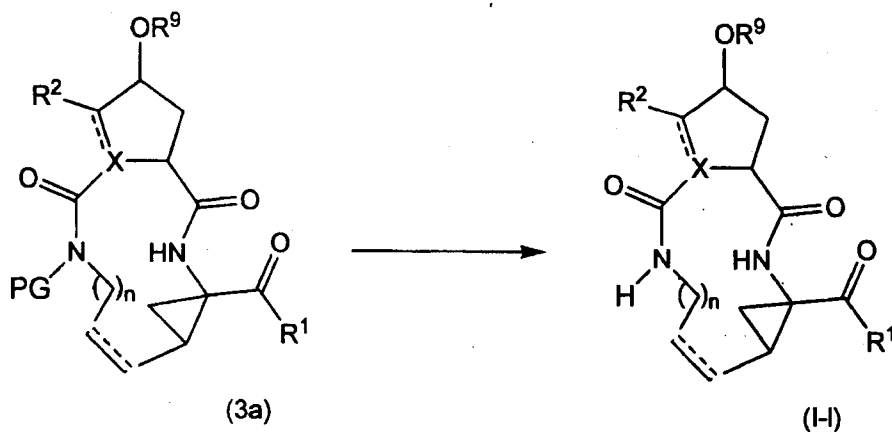
(e) reacting an intermediate (4a) with intermediate (4b) as outlined in the following reaction scheme:

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(4a) → Y-R⁹ (4b) → (I)

$$\text{(4a)} \quad \xrightarrow{\text{Y-R}^9 \quad \text{(4b)}} \quad \text{(I)}$$
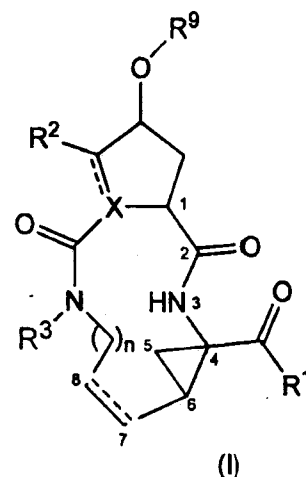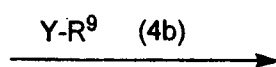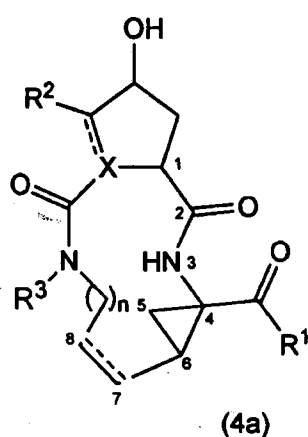
wherein Y in (4b) represents hydroxy or a leaving group; and where Y represents hydroxy the reaction of (4a) with (4b) is a Mitsunobu reaction; and where Y represemts a leaving group the reaction of (4a) with (4b) is a substitution reaction;

(f) converting compounds of formula (I) into each other by a functional group transformation reaction; or

(g) preparing a salt form by reacting the free form of a compound of formula (I) with an acid or a base.

Process disclosed in document D1

- Macrocycle formation via olefin metathesis reaction was disclosed in document D1 (page 55, line 6). (Corresponding to step-a of the claim 26 of the opposed application).

- Reduction of C7-C8 double bond was disclosed in document D1 (page 55, line 12). (Corresponding to step-b of the claim 26 of the opposed application).

- Introduce the sulphonamide group was disclosed in document D1 (page 40, line 10). (Corresponding to step-cof the claim 26 of the opposed application).

- Deprotection of amino group was disclosed in document D1 (page 50, Scheme 13). (Corresponding to step-d of the claim 26 of the opposed application).

- That the R9-substituent of the carbocycle can be introducedby Mitsunobu reactionwas disclosed in document D1 (page 45, line 14). (Corresponding to step-e of the claim 26 of the opposed application).

- Functional group transformation and salt formation was disclosed in document D1 (page 05, line 13). (Corresponding to steps-f and g of the claim 26 of the opposed application).

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Therefore, it can be seen that all the process steps of the claim 26 of the opposed application was disclosed in D1 and also the compounds of claim 1 and its dependant claims up to claim 23 of the opposed application is directly disclosed in document D1, when the definition of W, R^q, R^z, R^8, A, J, R^{16} and 'k' given in D1 are considered as is provided below:

Wherein W is O;

R^q and R^z are H;

R^{16} is CH3;

J is hexylene;

K is 0;

A is C(=O)NHSO2R2; where R2 is optionally substituted C3-C7cycloalkyl, preferably cyclopropyl; R^8 is;
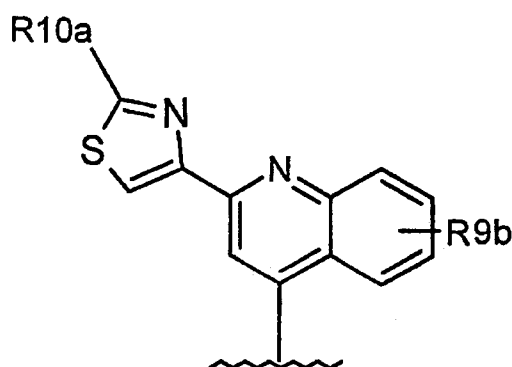
Wherein R10a is C1-C6alkyl and; **R9b is C1-C6 alkyl, C1-C6-alkoxy** optionally **mono-or di-substituted.**

Hence, the disclosure of the compound according to claim 1 and dependant claim compounds of the opposed patent application is found in the document D1. And also the process for the preparation of compound 1 and its dependant claim up to claim 23 as claimed in claim 26 of the opposed patent application is anticipated by discloser of the D1. Therefore, claim 26 is not novel over D1.

5.0 OBVIOUSNESS AND LACK OF INVENTIVE STEP – SECTION 25 (1) (e)

According to Indian patent Act, Section 2 (ja), "inventive step" means a feature of an invention that involves technical advance as compared to the existing knowledge or having economic significance or both and that makes the invention not obvious to a person skilled in the art;

Without prejudice to what has been submitted in the paragraphs above, it is respectfully submitted that the alleged invention as described and claimed in the opposed specification lacks in inventiveness and is obvious to a person skilled in the art. In other words, even if it is assumed without admitting that the alleged invention is novel, it is still obvious and lacks in inventive step in view of the teachings contained in the following documents:

As the discussion above already demonstrates, the disclosure and claims made in D1 include the structure of the compound of formula I and its dependent claims of the opposed patent application. D1 also teaches the use of compound of formula I as claimed in claim 1and its dependent claims compounds use as a medicament and for inhibiting HCV replication.

Even if it is considered for the sake of argument that D1 does not teach the specific compounds claimed in claims 1 & its dependant claims up to 23 of the opposed patent application, one has to determine whether making of such a compounds from those known in the art involves an inventive step or not.

5.1 Claim 1 and dependant claim 17 is obvious over D1

Claim 1 of the opposed patent application claims compound of formula (I) and claim 17 is for specific compound of formula (I-b) dependant on claim 1 are obvious over the D1 for the following reasons.

WO2005073195, which has an international filing date of 28 the January 2005, prior to the priority date of the opposed application.

At the time the invention was made, a compound according to instant claim 1 or claim 17 would have been obvious to one of the ordinary skill in the art, given the teaching of D1. On page 139 of D1, the compound having the structure corresponding to the following structure is disclosed (Example No-104).
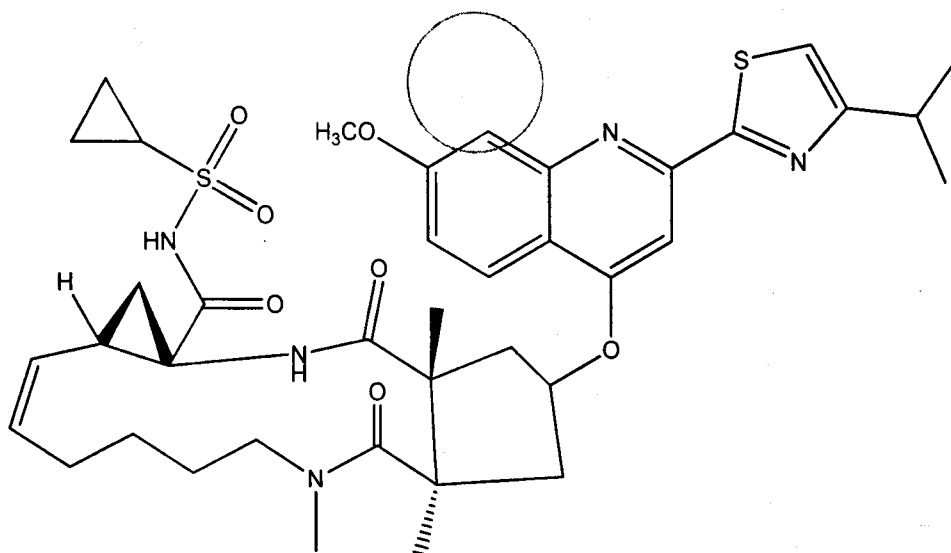
Compound of Example No 104 of D1
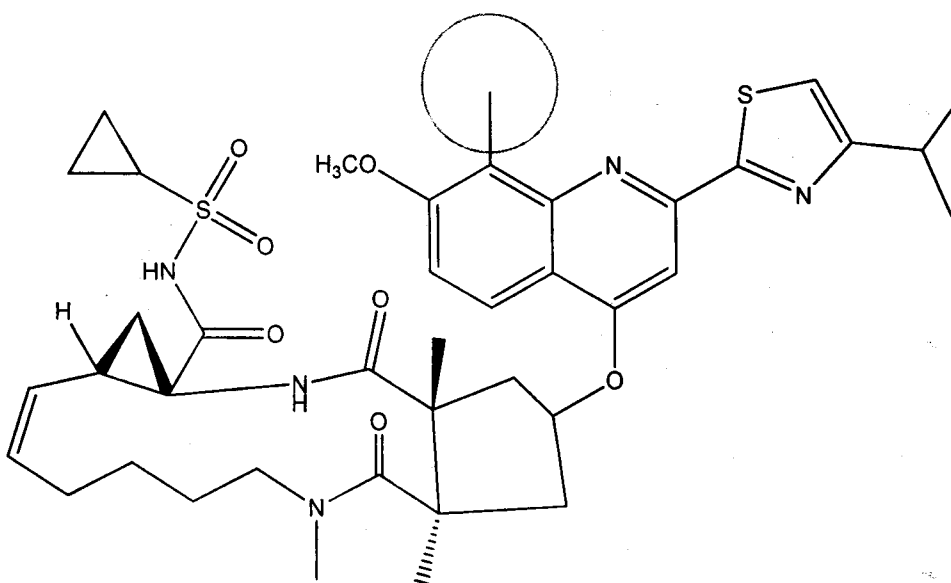
Compound claimed in claim 17

This compound differs from the compounds according to opposed claim 17 only in the omission of a methyl group at the 8-position of the quinolone ring system in the shown structure

Moreover, during the European prosecution of WO2005073195 (D1) the applicant, who is one of the applicants of the opposed application have submitted the inhibition activity data which is given below.

i.e.
m = 0, n = 0
k= 1, q' = 0
$R^7R^{7'}$ = cyclopropyl subst. with J
A = C(=O)A'
G = NRj
    Rj = J
J = 6-membered alkenylene chain
R^{16} = CH_3 unless otherwise indicated

W-R^8	A'		
	OH		
	Inhibition activity		
		Ki = 0.39 nM	
			Ki = 0.1nM

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Table 1 shows the HCV NS-3 serine protease inhibition activity of macrocyclic compounds with variation in the variables A, W, R8 and R16.It can be seen that the introduction of methyl substitution at 8-position of quinolone ring give higher potency.

From the above data a skilled person is aware that introduction of a methyl group at 8-position of quinolone ring will increase the efficacy of the compounds, at least there is a guidance available in D1. Therefore, one skilled in the art, starting from the compound (Example No: 104) of D1, if he wishes to make a more potent drug compound he will be motivated to prepare a compound with introduction of methyl group at 8-position of quinolone ring as seen from the inhibition activity data, and arrive at the present invention without undue burden. At least it will be obvious for him to try such modifications. Once he prepares the compound, he will find that the compound is effective in treatment of HCV. Hence, the compounds of claims 1 &17 of the opposed patent application is clearly obvious over the teachings of D1.

From the above, it becomes evident that in absence of any superior technical effect, the subject-matter of the opposed claims 1 and 8involves no inventive step. One could arrive at alleged application without the aid of any inventive genius but purely by routine skill and hence the compounds claimed in the present application is clearly obvious and lacks in inventive step.

5.2   Claim 1 and dependant claim 17 are obvious over D1 and general art D2

The document D2 disclosed that P2 residues of the substrates and/or inhibitors play a role in the stabilization of the catalytic His-Asp hydrogen bond, by shielding the corresponding region of the protease from solvent (Page 128, para 3). While in the opposed application the P2 residue, which design for better activity as NS3 protease inhibitor is shown in below structure with red highlighted part.

P2 residue as disclosed in opposed patent application
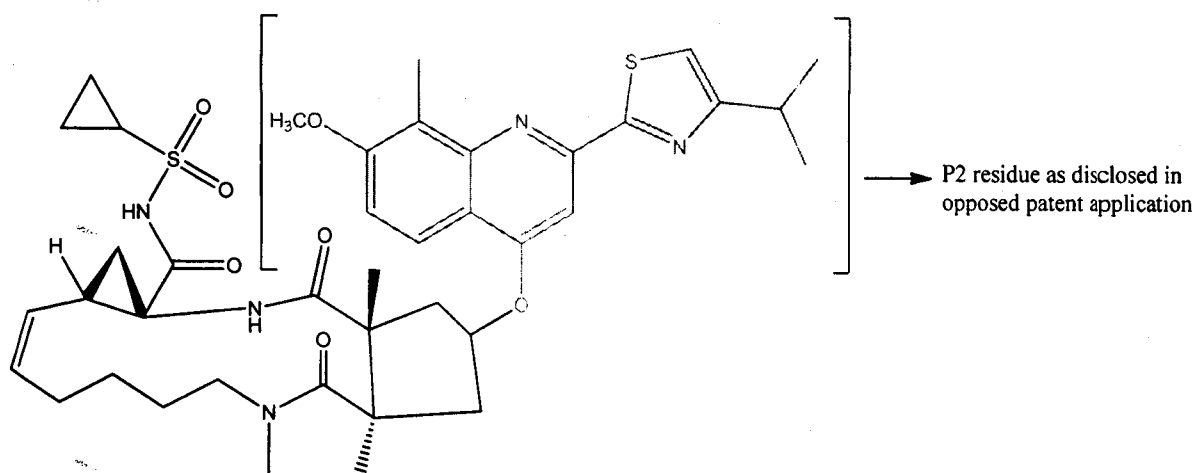
The said document also disclosed design of an improved P2 substitution by exploring the role of P2 residue in NS3 protease inhibitor (page 125, para 3).

A notable proposition from the NMR study was that the P2 residue of the substrates and/or inhibitors may stabilize the catalytic His-Asp hydrogen bond by shielding the corresponding region of the protease from solvent (page 124, para 2).The said document finally concluded that 'our SAR studies revealed a significant gain in inhibitor potency by introducing different hydrophobic substituents of their P2 substitution can be accommodated and are beneficial at this position.

P2 residue as disclosed in document D1.
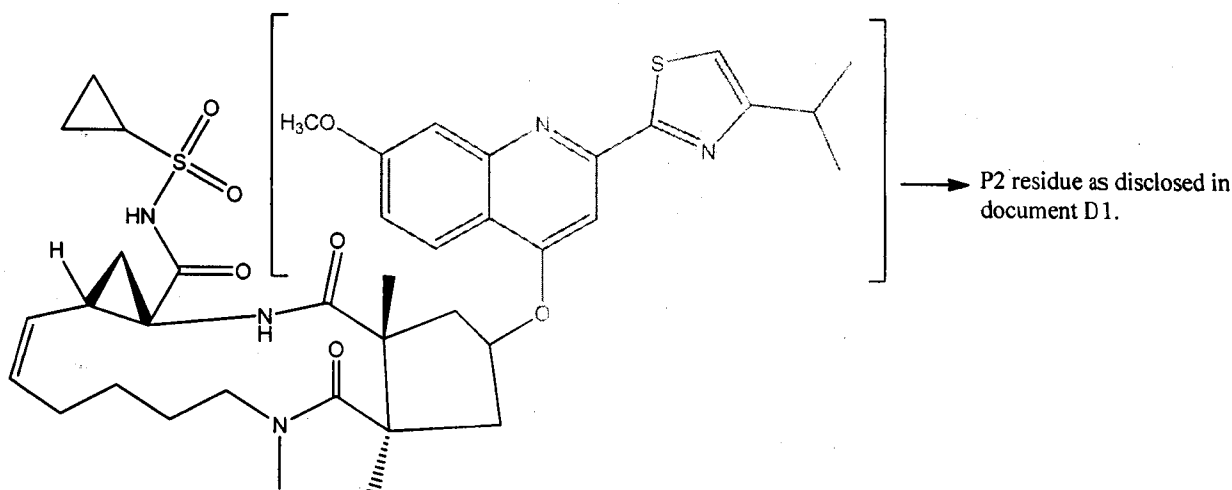
The compound as claimed in claim 1 and 17 in the opposed patent application is having the HCV inhibitor activity, while the compound (Example no-104) of the document D1 also shows the HCV inhibitor activity. Moreover, one skilled in the art, starting from the compound (Example no-104 of D1 and wishes to make a more potent drug, will use the knowledge of D2 i.e. hydrophobic substituents can be accommodated and are beneficial Methyl group is

hydrophobic in nature (see the reference http://telstar.ote.cmu.edu/biology/supplements/ functional-group/)and being a small sized substituent which is routinely used, will attempt to introduce a methyl substitution as taught in D2 and arrive at the present invention.

The present application is rendered obvious in light of D2 when read in combination of D1.

5.3 Claim 1 and dependant claim 17 are obvious over D1 and general art D3

Document D3 discloses the combination of the best binding residues in a tetrapeptide series that resulted in extremely potent inhibitors that bind exquisitely well to this enzyme. A substantial increase in potency was obtained with the introduction of a 7-methoxy-2-phenyl-4-quinolinoxy moiety at the P2 residue.The increase in potency allowed for the further truncation and led to the identification of tripeptide inhibitors. Structure activity relationship studies on this inhibitor series led to the identification of potential of becoming antiviral agents for the treatment of HCV infections(Page 6584, abstract).

Moreover the document D3 also shown that the addition of a methoxy group at the **C7-position** of the 4-quinolinoxy moiety provided a **10-fold increase** in their potencies(page 6587, para 2).The document also disclosed the effect of the systematic introduction of a methoxy group at the different positions of the quinoline ring B. Introduction of a methoxy group at the **C5-position** of the quinoline moiety was highly **detrimental** to potency. This significant loss in potency might be due to an unfavorable interaction between the C5-methoxy group and the rest of the inhibitor, which would destabilize the bioactive conformation, or to a steric clash between the C5-methoxy group and the enzyme side chain of Ala156(page 6587, para 3). Introduction of a methoxy group at the **C6-position** of the quinoline moiety was tolerated, resulting in a 2-**fold loss** in potency. Interestingly, the introduction of either a **C7- or C8-methoxy** substituent showed an approximate **5-fold increase** in the potency of the corresponding inhibitors and that this due to increase in potency might be attributed to a favorable interaction between the guanidine moiety of the protease Arg155 side chain and the C7- or C8-methoxy quinoline unit (page 6587, Colum 2).

The document also discloses the evaluation of different groups on the quinolone ring and their effect on the potency. The document D3also shows that introduction of electron withdrawing group such as –CF3, -OCF3 and –COOH at 7-position of quinolone ring resulted in decrease in the potency of such compounds (Page 6588, para 2).

Finally, the document D3 teaches which group is suitable at which position of quinoline ring to increase the potency. More specifically this document suggests7 and 8-position of quinolone ring is preferred and further non-electron withdrawing group at these positions is suitable for higher potency.

The teachings of D3 would motivate him to try a –CH3 substituent at 8-position of quinoline ring. There is a clear suggestion, motivation to introduce or at least attempt to introduce the -CH3 substituent at 8-position of the compound as disclosed in D1 (i.e compound of Example No.104).

Thus, the compounds of the Opposed patent application which has a -CH3 substituent at 8-positionwhen compared to the compound no. 104 disclosed in D1is rendered obvious from the teachings of D1, in combination with D3.

It is worth be mentioning in this context that decision of United state of appeal for the federal circuit case (BMS Vs Teva) (Annexure I) concerns a drug for the treatment of hepatitis B. After a four-day bench trial, the United States District Court for the District of Delaware found claim 8 of U.S. Patent No. 5,206,244 ('244 patent)invalid as obvious. Claim 8 of the '244 patent is directed to anucleoside analog composed of two regions: a carbocyclicring and a guanine base. Claim 8 covers one such compound, entecavir. BMS markets entecavir as a treatment forhepatitis B.

Entecavir is a modified version of the natural nucleoside 2'-deoxyguanosine (deoxyguanosine). Entecavir is structurally identical to deoxyguanosine except for one difference: it has a carbon-carbon double bond as an exocyclic methylene group) at the 5' position of the carbocyclic ring where deoxyguanosine has an oxygen atom.

The chemical structures of entecavir and deoxyguanosine are illustrated below:

entecavir   deoxyguanosine
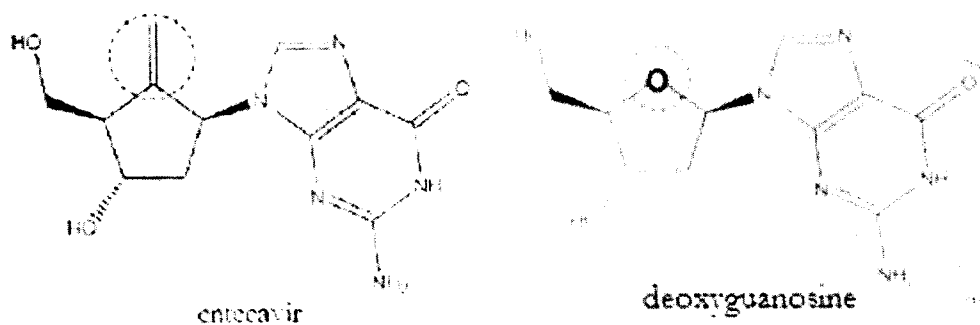
Teva's obviousness argument focused on the selection of 2'-CDGas a lead compound from the prior art.2'-CDG is a potent antiviral carbocyclic nucleoside analogue that is structurally similar to the natural nucleosidedeoxyguanosine, differing only in that it replaces an oxygen atom with a carbon atom at the 5' position.

The earliest priority date for the '244 patent is the date that BMS filed the application, October 18, 1990. 2'-CDG's synthesis was first published in the *Journal of Medicinal Chemistry* in 1984 by Dr. Y. Fulmer Shealy (the Shealy reference) of the Southern Research Institute (the SRI). The Shealy reference taught that 2'-CDG exhibited better *in vitro* antiviral activity against the herpes virusthan the FDA-approved best-selling drug at the time, Ara-A. Dr. Shealy obtained a patent for 2'-CDG and other related compounds, stating that they were useful in the treatment of viral infections. Subsequent research on 2'-CDG by Dr. Shealy showed *in vivo* activity against herpes viruses.

In 1989, Dr. J.A. Montgomery of SRI published an article summarizing the state of antiviral research at the time and reported that 2'-CDG was far the most promising antiviral against herpes. The Montgomery reference also taught that 2'-CDG was five to six times more potent than one of the leading drugs on the market, acyclovir. Teva's expert, Dr. Heath cock, stated that the Montgomery reference was a "lamp post that really illuminated2'-CDG as ... a very exciting lead compound to work from," and other chemists, during the relevant time period, were using 2'-CDG as a lead compound. Also in 1989, Peter M. Price and other researchers published the results of testing 2'-CDG against hepatitis (the Price reference). The Price reference disclosed that 2'-CDG showed "excellent activity" against the hepatitis B virus "with as little as 25 ng of 2'-CDG per ml" resulting in the "almost complete disappearance of replicating" hepatitis

The Price reference disclosed that the substitution of an exocyclic methylene group (carbon to carbon double bond) for the oxygen atom at the 5' position on the carbocyclic ring on the nucleoside lead to a nucleoside analogue with significantly superior antiviral properties.

The exocyclic substitution of methylene group is the same Modification at the same location made to 2'-CDG to form entecavir.

2'-CDG

entecavir

Madhavan compound 30
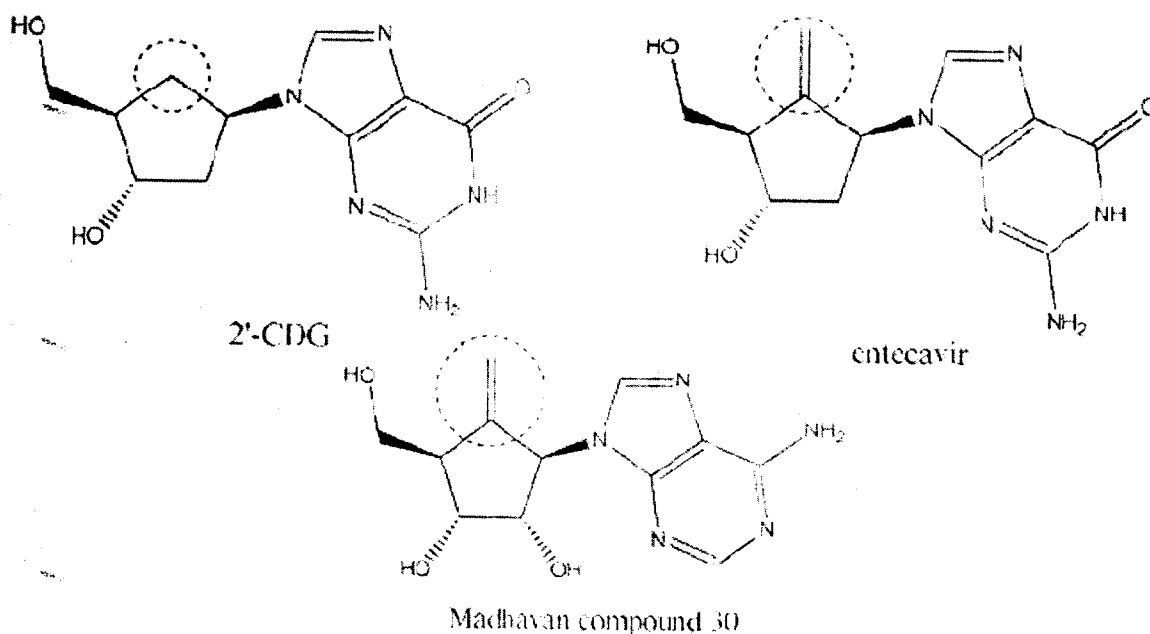
Based on the record, the court's finding that the modification required totrans form 2'-CDG into the structurally similar entecaviris a minor one: the addition of a single carbon atom to form an exocyclic methylene with the already-present carbon atom at the 5' position of the carbocyclic ring of 2'-CDG to create entecavir. Upon selecting2'-CDG as the lead compound, the steps of deciding which bond to modify and how to modify that bond "equate to asmall, finite number of changes to try to arrive at the lead compound." Further, the skilled artisan's reasonable expectationof success is measured "as of the date of the invention."The Madhavan reference's teachings ofimproved potency with an exocyclic methylene at the 5'position supports the expectation that the same substitutionto 2'-CDG would reasonably lead to similar properties.

As explained above, 2'-CDG and entecavir are very structurally similar, and it is well settled that structurally similar compounds often have similar properties.

Based on above finding after a four-day bench trial, the United States District Court for the District of Delaware found claim 8 of U.S. Patent No. 5,206,244 ('244 patent)invalid as obvious.

Now, comparing with the present case, the opposed patent application claim 1 and its dependent claim 17 claims the Hepatitis C drug Simeprevir. As mentioned above the closest analogue to the compounds disclosed and claimed in the opposed patent application are certain compounds, such as Example no-104 of D1. Simeprevir is structurally identical to the compound disclosed in Example no-104 except for one difference - it has a methyl group at the 8 position of the quinoline ring where the compound of example no-104 has a hydrogen atom.

The earliest priority date for the opposed patent is, July, 2005. Example no-104 was first published in the *D1* in Jan 2004 by the same applicant. There are two other prior art (D2 and D3) available before the priority date of the opposed patent application, which suggested that the substitution of methyl group at the 8 position on the quinoline ring lead to an analogue with significantly superior antiviral properties as discussed above in part6.2 and 6.3.

If one follows the decision of United State Court of Appeal for the Federal Circuit in BMS Vs Teva on Entecavir, discussed above, the present application which presents a case similar to the one decided therein. Therefore, following the judgement of the said Court, the present application should also be considered as obvious.
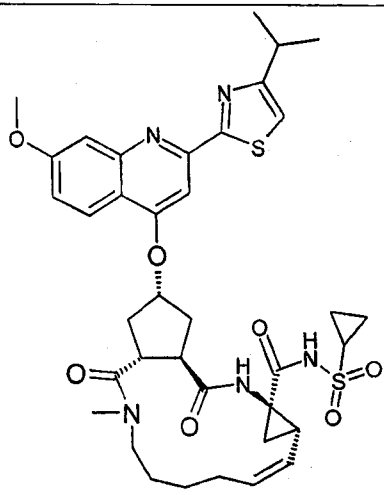
6.0   NOT PROVIDING ANY COMPARATIVE DATA

Furthermore the compounds of the alleged invention possess anti HCV properties (claim 25 of the opposed application). The document D1 also discloses inhibitory activity on the replication of the Hepatitis C virus. Therefore claimed compound possess the same anti HCV activity. Document D1 clearly state at several places that anti HCV properties are expected of the compound disclosed therein. Therefore the alleged invention is a blatant attempt to again claim compounds already disclosed in the prior art and create monopoly over these compounds, which are already in the public domain.

Additionally, the invention as claimed in the opposed application does not provide any comparative data with the closest prior art to overcome the problem of obviousness by showing either superior or surprising effects. Also the opposed application does not provide any technical advancement over the

existing knowledge in the same field. Therefore, in the absence of any comparative data showing the compounds of claim 1 and claim 17 are better in some way to the known compounds of D1 there is no technical advancement over the prior art. During the European prosecution of WO2005073195 the applicant which is one of the applicants of the opposed application also submitted the inhibition activity data for the compound (Example No.104), which is the closest prior art for the opposed application. The data provided during prosecution is below;

Activity data of compound of Example No.104;

Compound	Structure	Inhibition activity
Closest prior art (Example No.104)		Ki =0.39 nM

Activity data of compound No.47; of the opposed application (Simeprevir):

Compound	Structure	Inhibition activity
Compound No.47 of the Opposed application		Ki =0.00050 μM

From the data it is clear that no enhancement in the activity of compound 47 of the opposed patent application as compared to the closest prior art compound. Data shows inhibition activity of the closest prior art compound is 0.39 nM (0.00039 µM) and the activity of compound 47 of the opposed patent application is (0.0005 µM), which shows no technical advancement over the prior art.

As per the Indian Patent Act, patent cannot be granted for compounds in absence of clear proof of beneficial effects or technical improvements if the compounds are per se obvious.

Therefore, from the above it is clear that the claimed compounds do not possess any surprising properties compared to the properties of the closest prior art. Hence, the opposed patent application directed to certain compounds of claims 1 and dependant claim 17 and their use are obvious and does not involve inventive merit and therefore do not qualify as a patentable invention as per the provisions of the Indian Patent Act .

7.0 LACK OF CLARITY & INSUFFICIENCY of DESCRIPTION – SECTION 25 (1) (g)

Without prejudice to what is submitted above, it is submitted that the Opposed Application does not contain sufficient information to enable the person skilled in the art to carry out the alleged invention over the entire scope as claimed.

According to section 12(1) (a) read with section 10(4) of the Indian Patent Act during the examination the learned Controller considers whether the complete specification fully and particularly describes the invention.

Whereas, according to section 25(1) (g), in opposition proceedings the Learned controller decides whether the complete specification sufficiently and clearly describes the invention or the method by which it is to be performed.

It is frequently stressed that the monopoly of a patent may only be awarded for subject matter which is re-workable by the skilled person within the entire claimed range. Subject matter which is not accessible based on the provided teaching and by using ordinary skill is to be excluded from the patent protection.

7.1 **Insufficient Disclosure of the Subject-Matter of Claim 2 and claim 9.**

Claim 24. A pharmaceutical composition comprising a carrier, and as active ingredient an ant virally effective amount of compound as claimed in anyone of the claim 1-23.

Claim 24 is directed to the pharmaceutical composition of the compound which is claimed in claims 1- 23. Claim 24 is not supported by the body of the specification. The specification does not give definition of pharmaceutically acceptable carrier and also not defined excipient and diluent. Also there is no suggestion in the opposed patent how the specific excipient, carrier and diluent is to be used for the specific compounds of claims 1-23 and how it is to be produced. Hence, claim 24 lacks sufficient disclosure for obtaining the composition of compounds claimed in claim 1-23. Therefore, a person skilled in the art will not be able to make the specific composition of compounds claimed in claims 1-23 from the teaching of the opposed application.

8.0 **FAILURE TO DISCLOSE DETAILS OF CORRESPONDING FOREIGN APPLICATIONS - SECTION 25(1)(H)**

It is respectfully prayed that the Learned Controller should check whether the Applicant of the opposed application has dutifully informed the status of their co-pending applications, their prosecutions in other convention countries as required under Section 8(1)(a) of the Patents Act, 1970 as amended by the Patents (Amendment) Act, 2005.

It is also respectfully prayed that the Learned Controller should also check whether the Applicant of the opposed application has dutifully informed the status of every other application relating to the same or substantially the same invention, if any, filed in any country outside India subsequently to the filing of the statement referred to in the Section 8(1)(a) as required under Section 8(1)(b) of the Patents Act, 1970 as amended by the Patents (Amendment) Act, 2005.

9.0   **DEPENDENT CLAIMS**

Claim 2-26 are dependent on claim 1. In the view of above discussions it is evident that claim 1 is not fulfilling the patentability criteria as per the provisions of the Indian Patent Act 1970 as amended by the Patents (Amendment) Act, 2005, therefore dependent claims also should not be allowed.

10.0   The Opponents further submits that the Claims 1-26 as contained in the opposed Indian Patent Application ·No. 10158/DELNP/2007 are neither novel nor inventive under the Act. The Opposed Application also does not sufficiently and clearly describe the alleged invention for it to be carried out by a person skilled in the art.

11.0   In view of the aforementioned submissions, it is respectfully submitted that the Opposed Application lacks clarity and sufficiency, i.e. the description of the Opposed Application does not enable a person reasonably skilled in the art to achieve the results of the present invention as claimed in the present application, without inventive merit.

12.0   Accordingly, it is respectfully submitted that the Opposed Application does not contain sufficient information to enable the person skilled in the art to perform the invention disclosed in opposed Indian Patent Application No. 10158/DELNP/2007. Therefore, this ground of opposition has been established and the entire Opposed Application ought to be rejected on this ground alone.
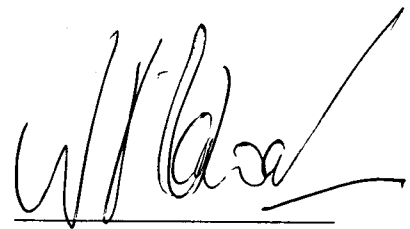
CONCLUSION

13.0 In view of the submissions presented above, we humbly pray that:

    i)   the Indian Patent Application No. 10158/DELNP/2007 be dismissed in *toto*;

    ii)   the opponent be granted leave to file further evidence;

    iii)   the copy of reply statement and evidence filed by the applicant in the response to this opposition be made available to the opponent;

    iv)   the opponent be permitted to file further response and evidence to the reply or evidence produced by the applicant;

    v)   the opponent be granted leave to make further submissions in case the applicant makes any amendments in the claims;

    vi)   any other relief as the Learned Controller may deem fit be awarded in favor of the Opponents.

As a matter of precaution, we request the Learned Controller to grant us an oral hearing before disposing of this representation.

Dated this the 19th day of August 2014

Nayan J. Rawal
Constituted Attorney
for the Opponent
IPA no. 654

31

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(54) Title: HCV NS-3 SERINE PROTEASE INHIBITORS
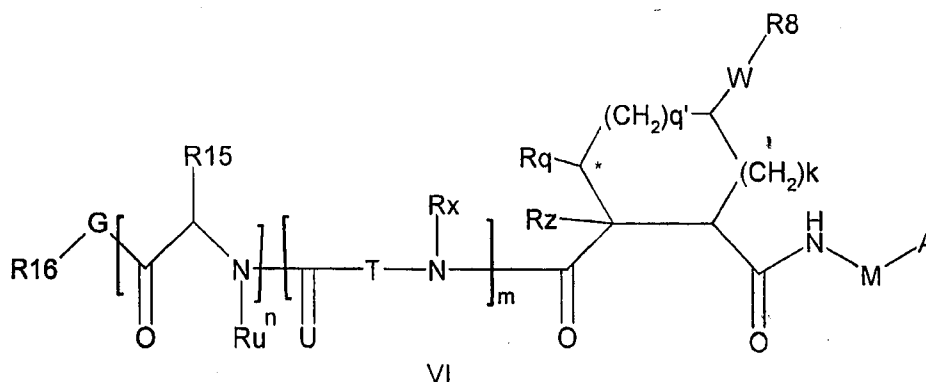
(57) Abstract: Peptidomimetic compounds are described which inhibit the NS3 protease of the hepatitis C virus (HCV). The com-
pounds have the formula where the variable definitions are as provided in the specification. The compounds comprise a carbocyclic
P2 unit in conjunction with a novel linkage to those portions of the inhibitor more distal to the nominal cleavage site of the native
substrate, which linkage reverses the orientation of peptidic bonds on the distal side relative to those proximal to the cleavage site.

HCV NS-3 Serine Protease Inhibitors

Technical Field

This invention relates to novel inhibitors of the NS3 serine protease of the flavivirus
HCV and to methods for their use in the treatment or prophylaxis of HCV.

5

Background Art

The NS3 serine protease of HCV is a multifunctional protein which contains a serine
protease domain and a RNA helicase domain. The protease cofactor NS4A, which is
a relatively small protein, is absolutely required for enhanced serine protease activity.

10   The NS3 serine protease is essential in the viral lifecycle. From analysis of the
substrate binding site as revealed by X-ray crystal structure, it has been shown that
the binding site of the NS3 protease is remarkably shallow and solvent exposed
making small molecule inhibitor design a challenge.

15   It is believed that two HCV protease inhibitors have entered clinical trials, namely
Boehringer Ingelheim's BILN-2061 disclosed in WO 0059929 and Vertex' VX-950
disclosed in WO 0387092. A number of similar peptidomimetic HCV protease
inhibitors have also been proposed in the academic and patent literature. Common
for the vast majority of such prior art peptidomimetics is the presence of an L-proline

20   derivative at the P2 position of the inhibitor and interacting with the S2 subsite of the
HCV protease enzyme. In the case of BILN-2061, the L-proline is 4-substituted with a
quinoline ether, whereas VX-950 has a carboyclic ring fused to the L-proline ring.
Most peptidomimetics additionally comprise additional L-amino acid derivatives
peptide bonded at the P3 position, with many proposed inhibitors also including

25   additional L-amino acid derivatives extending into P4, P5 and P6.

It has already become apparent that the sustained administration of BILN-2061 or
VX-950 selects HCV mutants which are resistant to the respective drug, so called
drug escape mutants. These drug escape mutants have characteristic mutations in

30   the HCV protease genome, notably D168V, D168Y and/or A165S. Treatment
paradigms for HCV will thus have to resemble HIV treatment, where drug escape

mutations also arise readily. Accordingly, additional drugs with different resistance
patterns will consistently be required to provide failing patients with treatment options,
and combination therapy with multiple drugs is likely to be the norm in the future,
even for first line treatment.

5

Experience with HIV drugs, and HIV protease inhibitors in particular, has further
emphasized that sub-optimal pharmacokinetics and complex dosage regimes quickly
result in inadvertent compliance failures. This in turn means that the 24 hour trough
concentration (minimum plasma concentration) for the respective drugs in an HIV

10   regime frequently falls below the IC_{90} or ED_{90} threshold for large parts of the day. It is
considered that a 24 hour trough level of at least the IC_{50}, and more realistically, the
IC_{90} or ED_{90} is essential to slow down the development of drug escape mutants and
achieving the necessary pharmacokinetics and drug metabolism to allow such trough
levels provides a stringent challenge to drug design. The strongly peptidomimetic

15   nature of prior art HCV protease inhibitors, with multiple peptide bonds in native
configurations poses pharmacokinetic hurdles to effective dosage regimes.

Brief description of the invention

20   In accordance with a first aspect of the invention, there are provided compounds of
the formula VI:
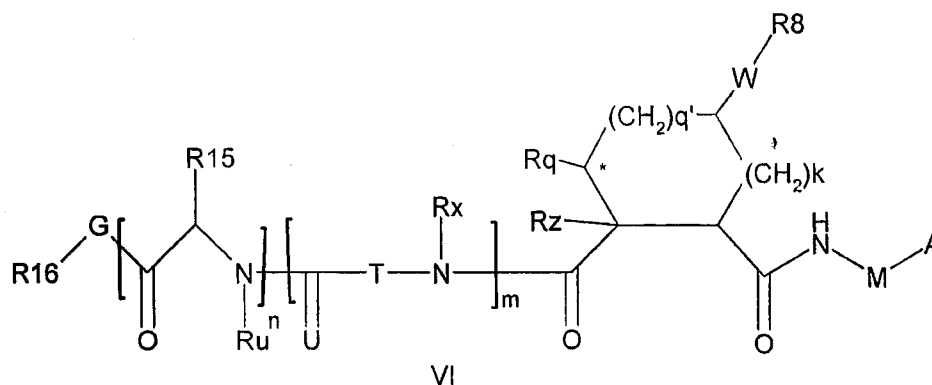
VI

wherein
A is $C(=O)OR^1$, $C(=O)NHSO_2R^2$, $C(=O)NHR^3$, or $CR^4R^{4'}$ wherein;

R^1 is hydrogen, C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl;

R^2 is C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl;

R^3 is C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl, $-OC_1$-C_6alkyl, $-OC_0$-C_3alkylcarbocyclyl, $-OC_0$-C_3alkylheterocyclyl;

R^4 is halo, amino, or OH; or R^4 and $R^{4'}$ are =O;

$R^{4'}$ is C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl; wherein

R^2, R^3, and $R^{4'}$ are each optionally substituted from 1 to 3 substituents independently selected from the group consisting of times with halo, oxo, nitrile, azido, nitro, C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl, $NH_2C(=O)$-, Y-NRaRb, Y-O-Rb, Y-C(=O)Rb, Y-(C=O)NRaRb, Y-NRaC(=O)Rb, Y-NHSO$_p$Rb, Y-S(=O)$_p$Rb, Y-S(=O)$_p$NRaRb, Y-C(=O)Orb and Y-NRaC(=O)ORb;

Y is independently a bond or C_1-C_3alkylene;

Ra is independently H or C_1-C_3alkyl;

Rb is independently H, C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl or C_0-C_3alkylheterocyclyl;

p is independently 1 or 2;

M is $CR^7R^{7'}$ or NRu;

Ru is H or C_1-C_3alkyl;

R^7 is C_1-C_6alkyl, C_0-C_3alkylC_3-C_7cycloalkyl, or C_2-C_6alkenyl, any of which is optionally substituted with 1-3 halo atoms, or an amino,–SH or C_0-C_3alkylcycloalkyl group, or R^7 is J;

$R^{7'}$ is H or taken together with R^7 forms a C_3-C_6cycloalkyl ring optionally substituted with $R^{7'a}$ wherein;

$R^{7'a}$ is C_1-C_6alkyl, C_3-C_5cycloalkyl, C_2-C_6alkenyl any of which may be optionally substituted with halo; or $R^{7'a}$ is J;

q' is 0 or 1 and k is 0 to 3;

Rz is H, or together with the asterisked carbon forms an olefinic bond;

Rq is H or C_1-C_6alkyl;

W is -CH$_2$-, -O-, -OC(=O)H-, -OC(=O)-, -S-, –NH-, -NRa, -NHSO$_2$-, -NHC(=O)NH- or -NHC(=O)-, -NHC(=S)NH- or a bond;

R^8 is a ring system containing 1 or 2 saturated, partially saturated or unsaturated rings each of which has 4-7 ring atoms and each of which has 0 to 4 hetero atoms

selected from S, O and N, the ring system being optionally spaced from W by a C_1-C_3alkyl group; or R^8 is C_1-C_6alkyl; any of which R^8 groups can be optionally mono, di, or tri substituted with R^9, wherein

R^9 is independently selected from the group consisting of halo, oxo, nitrile, azido,

5   nitro, C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl, NH_2CO-, Y-NRaRb, Y-O-Rb, Y-C(=O)Rb, Y-(C=O)NRaRb, Y-NRaC(=O)Rb, Y-NHSO$_p$Rb, Y-S(=O)$_p$Rb, Y-S(=O)$_p$NRaRb, Y-C(=O)Orb and Y-NRaC(=O)ORb; wherein said carbocyclyl or heterocyclyl moiety is optionally substituted with R^{10}; wherein

R^{10} is C_1-C_6alkyl, C_3-C_7cycloalkyl, C_1-C_6alkoxy, amino, sulfonyl, (C_1-C_3 alkyl)sulfonyl,

10   NO_2, OH, SH, halo, haloalkyl, carboxyl, amido,

Rx is H or C_1-C_5 alkyl; or Rx is J;

T is -CHR^{11}- or -NRd-, where Rd is H, C_1-C_3alkyl; or Rd is J;

R^{11} is H, C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl, any of which can be substituted with halo, oxo, nitrile, azido, nitro, C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl,

15   C_0-C_3alkylheterocyclyl, NH_2CO-, Y-NRaRb, Y-O-Rb, Y-C(=O)Rb, Y-(C=O)NRaRb, Y-NRaC(=O)Rb, Y-NHSO$_p$Rb, Y-S(=O)$_p$Rb, Y-S(=O)$_p$NRaRb, Y-C(=O)ORb, Y-NRaC(=O)ORb; or R^{11} is J;

J, if present, is a single 3 to 10-membered saturated or partially unsaturated alkylene chain extending from the $R^7/R^{7'}$ cycloalkyl, or from the carbon atom to which R^7, is

20   attached to one of Rd, Rj, Rx, Ry or R^{11} to form a macrocycle, which chain is optionally interrupted by one to three heteroatoms independently selected from: -O-, -S- or -NR^{12}-,and wherein 0 to 3 carbon atoms in the chain are optionally substituted with R^{14}; wherein;

R^{12} is H, C_1-C_6 alkyl, C_3-C_6cycloalkyl, or C(=O)R^{13};

25   R^{13} is C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl;

R^{14} is independently selected from the group consisting of H, C_1-C_6alkyl, C_1-C_6haloalkyl, C_1-C_6alkoxy, hydroxy, halo, amino, oxo, thio and C_1-C_6 thioalkyl;

m is 0 or 1; n is 0 or 1;

U is =O or is absent;

30   R^{15} is H, C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl, any of which can be substituted with halo, oxo, nitrile, azido, nitro, C_1-C_6 alkyl, C_0-C_3alkylheterocyclyl,

C_0-C_3alkylcarbocyclyl, NH_2CO-, Y-NRaRb, Y-O-Rb, Y-C(=O)Rb, Y-(C=O)NRaRb, Y-NRaC(=O)Rb, Y-NHS(=O)$_p$Rb, Y-S(=O)$_p$Rb, Y-S(=O)$_p$NRaRb, Y-C(=O)ORb, Y-NRaC(=O)ORb;

G is -O-, -NRy-, -NRjNRj-;

5   Ry is H, C_1-C_3 alkyl; or Ry is J;

one Rj is H and the other Rj is H or J;

R^{16} is H; or R^{16} is C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl, any of which can be substituted with halo, oxo, nitrile, azido, nitro, C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl, NH_2CO-, Y-NRaRb, Y-O-Rb, Y-C(=O)Rb,

10   Y-(C=O)NRaRb, Y-NRaC(=O)Rb, Y-NHSO$_p$Rb, Y-S(=O)$_p$Rb, Y-S(=O)$_p$NRaRb, Y-C(=O)ORb, Y-NRaC(=O)ORb;

or a pharmaceutically acceptable salt or prodrug thereof.

Without in any way wishing to be bound by theory, or the ascription of tentative

15   binding modes for specific variables, the notional concepts P1, P2, P3 and P4 as used herein are provided for convenience only and have substantially their conventional meanings, as illustrated by Schechter & Berger, (1976) Biochem Biophys Res Comm 27 157-162, and denote those portions of the inhibitor believed to fill the S1, S2, S3 and S4 subsites respectively of the enzyme, where S1 is adjacent

20   the cleavage site and S4 remote from the cleavage site. Regardless of binding mode, the components defined by Formula VI etc are intended to be within the scope of the invention. For example it is expected that capping group R^{16}-G may interact with the S3 and S4 subsites especially when m and/or n is 0.

25   The various embodiments of the present invention can be notionally represented as R^{16}-G-P4-P3- P2-P1, wherein P3 and/or P4 may be absent. P1, P3 and P4 each represents a building block constituted of a derivative of a natural or unnatural amino acid, P2 is a substituted carbocyclic residue and G-R^{16} is a capping group. The building blocks are typically linked together by amide bonds.which are reversed

30   relative to each other on each side of the P2 building block in the compounds of the invention.

Additional aspects of the invention include a pharmaceutical composition comprising
a compound of the invention as defined above and a pharmaceutically acceptable
carrier or diluent therefor.

5

The compounds and compositions of the invention have utility in methods of medical
treatment or prophylaxis of HCV infections in humans. Accordingly, a further aspect
of the invention is the use of a compound as defined above in therapy such as the
manufacture of a medicament for the prophylaxis or treatment of flavivirus infections
10   in humans or animals. Exemplary flavivirus include BVDV, dengue and especially
HCV.

In the compounds of the invention the amide bond linking the P2 and P3 together is
reversed relative to the amide bond linking the P1 and P2, i.e. the amino acid
15   derivatives, P1 and P3, on each side of the P2 scaffold are both coupled through their
amino functions to the acid groups on each side of the P2 scaffold. This means that
the side chains of the P3 and P4 (including the R^{16} cap to the extent this interacts with
S3 or S4) point in the opposite direction compared to in a native peptide substrate.
Another consequence of the reversed P3 and P4 amino acids is that the side chains
20   of these amino acids are displaced one atom outwardly relative to a native peptide
substrate.

Change of direction of the P3 and P4 side chains in this fashion would be expected to
favour non-natural D stereochemistries for the pocket filling groups (eg side chains) of
25   P3 and/or P4 and/or R^{16}. Indeed, such compounds are typically highly active and
within the scope of the invention. However, it has been surprisingly found that even
compounds of the invention bearing L-amino acid side chains at P3 and/or P4 exhibit
good activity, notwithstanding that the respective entity must approach the S3 or S4
pocket from a different angle relative to a native peptide substrate. Accordingly L-
30   stereochemistry at R^{11} and/or R^{15} and/or the corresponding configuration at R^{16} to
mimic L stereochemistry represents a favoured aspect of the invention.

The different angle of approach to the S3 and/or S4 pockets also has implications for
the ability of the compounds of the invention to avoid resistance patterns exhibited by
prior art HCV protease inhibitors which hitherto have all had a conventional peptide
5   backbone of natural or non-natural L-amino acid residues. As with the reverse
transcriptase of HIV which is notorious for quickly generating drug escape mutants
under the slective pressure of antiviral therapy, the RNA dependent RNA polymerase
NS5A of HCV has a very poor proof reading capacity. This in turn means that the
HCV polymerase is highly error prone and it is likely that characteristic resistance
10   patterns will arise when HCV antivirals are administered over long periods. Even
before launch, it is apparent that BILN 2061 with a substantially peptidic backbone
(albeit macrocyclised) and Vertex' NS3 protease inhibitor VX-950 with a linear peptide
backbone at P3 and P4 quickly give rise to characteristic resistance mutations at
positions 155, 156 or 168 of the NS3 protease (Lin et al J Biol Chem 2004
15   279(17):17808-17).

A preferred group of compounds of the invention comprises those wherein P1
represents a hydrazine derivative, that is M is NRu where Ru is typically H or C_1-
C_3alkyl. Compounds wherein M is $CR^7R^{7'}$ constitute a further preferred aspect of the
20   invention.

Preferred embodiments wherein M is $CR^7R^{7'}$ in formulae VI include formulae VIA
below:

VIA
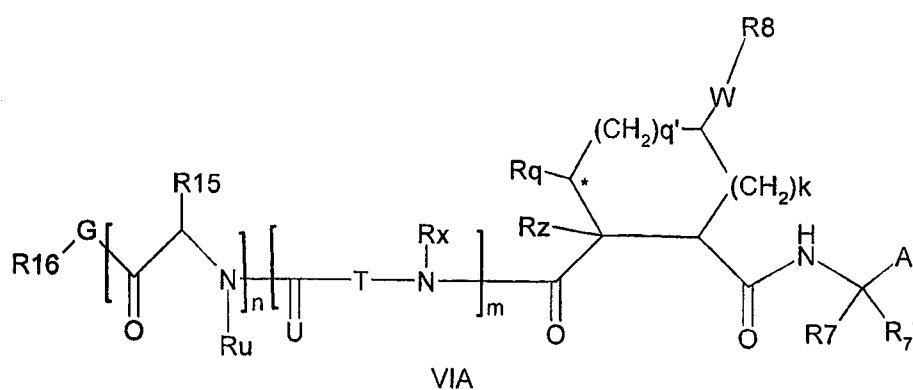
Preferred values for q' and k in formula VI include 1:1, 1:2, 1:3, 2:2, 2:3, more preferably 0:2 and 0:0; and most preferably 0:1, in which case preferred compounds have one of the partial structures:

VIa   VIb   VIc
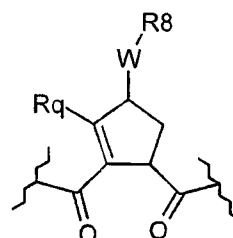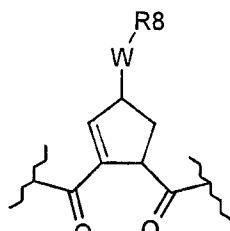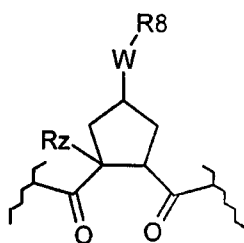
especially where Rz is H or Rq is H or methyl.

Compounds of the invention may comprise both a P3 and a P4 function, viz m and n are each 1. Favoured embodiments within Formula VI comprising both a P3 and P4 function include formula VIda-VIdb below:
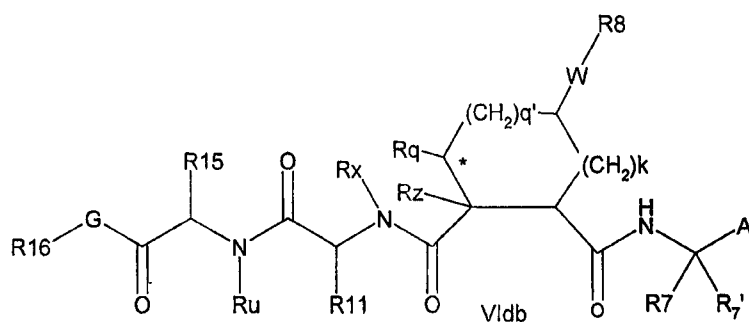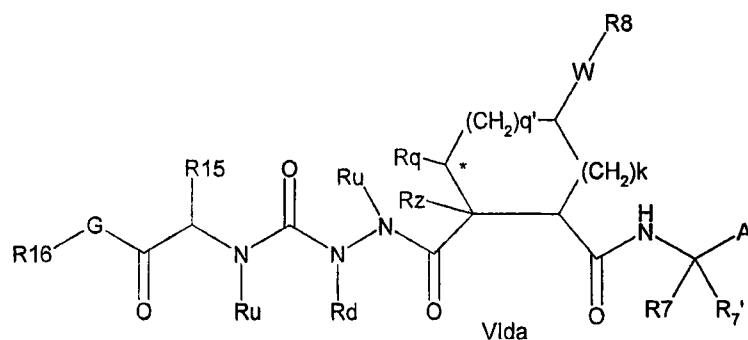
Vlda

Vldb

Alternative embodiments include the structures corresponding to Vlda, and Vldb
wherein M is NRu.

Alternative configurations of the compounds of the invention comprise a P3, but no
P4 function, viz m is 1 and n is zero. Favoured embodiments within Formula VI
comprising a P3, but no P4 include formula Vlea-Vleb below:
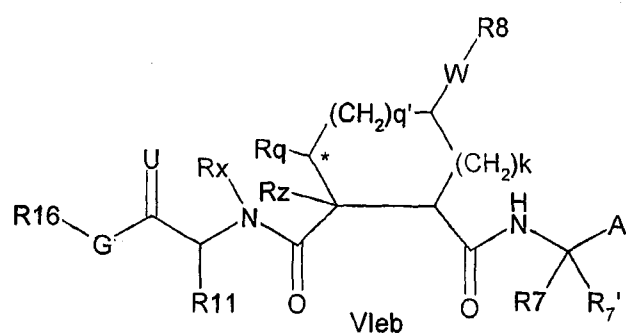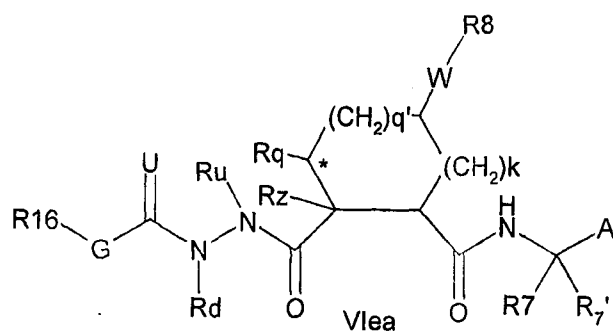
VIea

VIeb

Alternative embodiments include the structures corresponding to VIea and VIeb wherein M is NRu.

Still further alternative configurations of the compounds of the invention include those where m and n are zero and thus groups R^{16}-G abut P2, but as mentioned above, the capping group R16-G may interact favourably with S3 and/or S4.

Favoured embodiments within Formula VI wherein m and n are zero include those of formula VIfa below:
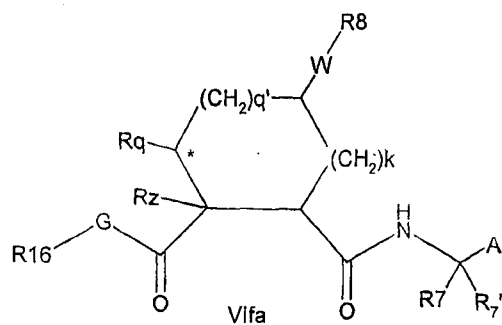
VIfa

Alternative embodiments include the structures corresponding to VIfa, wherein M is NRu.

The compounds of the invention may comprise linear molecules, as depicted above.
Alternatively, in embodiments wherein R^7 and $R^{7'}$ together define a spiro cycloalkyl group, such as spiro-cyclopropyl, the compounds of the invention may be configured as macrocycles, wherein a linking group J extends between one of Rj, Rx, Ry, Rd or R^{11} of Formula VI. Alternatively the macrocycle J may extend from the carbon adjacent R^7 to one of Rj, Rx, Ry, Rd or Ru.

Favoured embodiments of such macrocyclic structures within formula VI wherein m is 0 and n is 1 include those of the formula VIga- VIgc below:
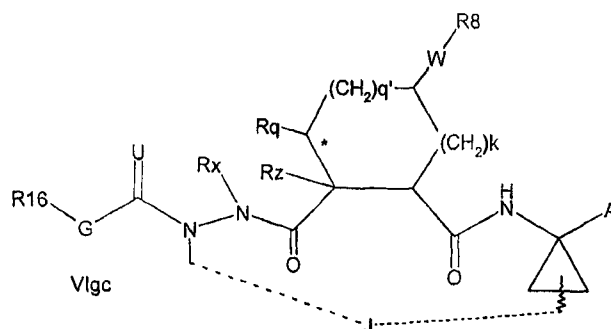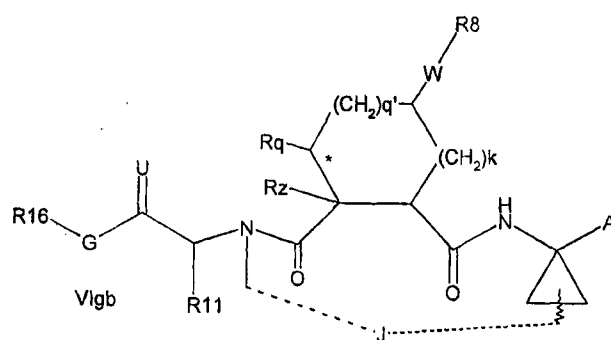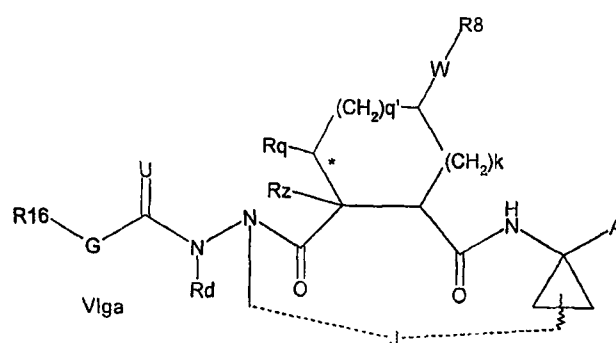
VIga

VIgb

VIgc

The corresponding structures wherein the J chain bonds to the carbon adjacent R⁷ are also favoured.

Favoured embodiments of macrocyclic structures within formula VI comprising both a P3 and P4 functions, i.e. wherein both m and n are 1, include those of the formula VIha- VIhc below:
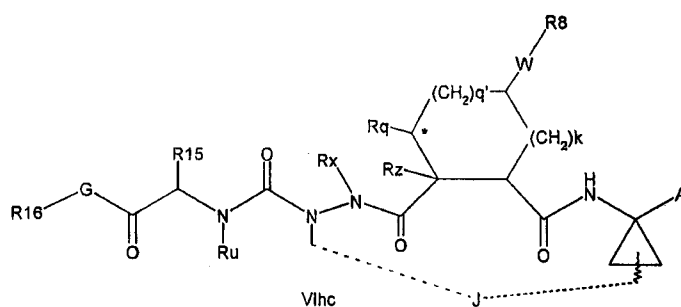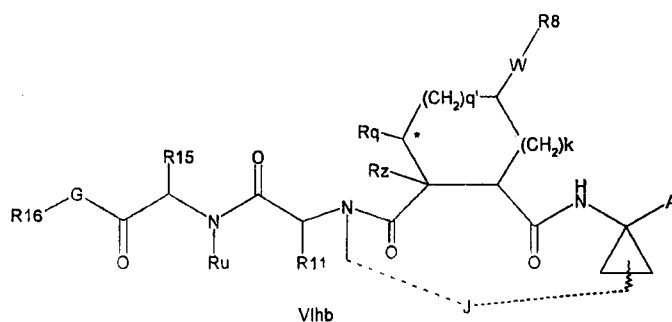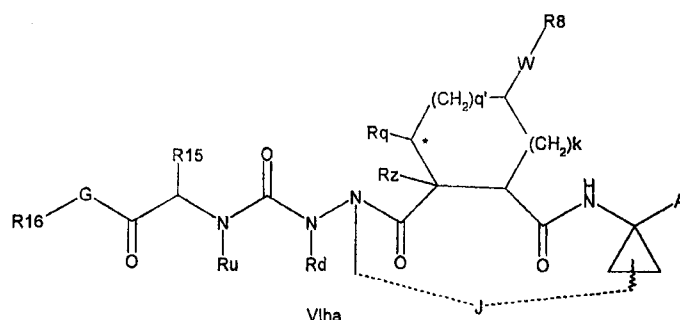
13

Vlha

Vlhb

Vlhc

The corresponding structures wherein the J chain bonds to the carbon adjacent R^7 are also favoured.

5   Favoured macrocyclic structures within Formula VI, wherein both of the P3 and P4 functions are absent, i.e. wherein m and n are each 0, include those of the formulae Vlhe-Vlhf below.
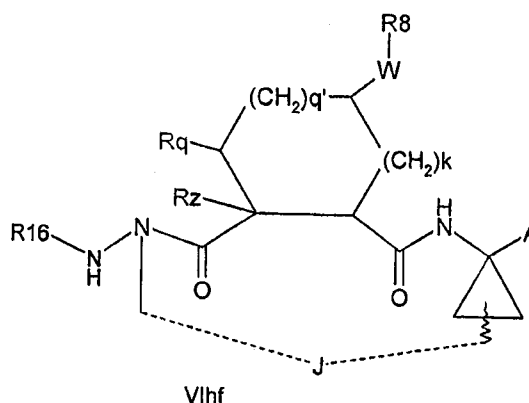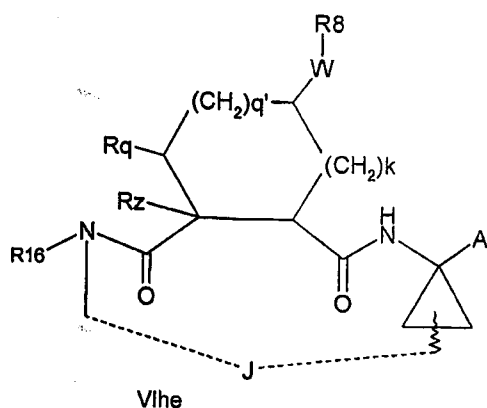
VIhe   VIhf

The corresponding structures wherein the J chain bonds to the carbon adjacent R[7]
are also favoured.

5   In general, in the optionally macrocyclic structures such as those illustrated above,
     linker J is a 3 to 10 chain atom, preferably 4 to 7 chain atom, such as 5 or 6 chain
     atom, saturated or partially unsaturated alkylene chain, that is an alkylene chain
     bearing 1 to 3 unsaturated bonds between adjacent carbons, typically one
     unsaturation. The length of the chain will, of course, depend on whether J extends

10   from Rd, Rj, Rx, Ry, R^{11} or the carbon adjacent R^7. Suitable chains are described in
     detail in WO 00/59929. Typically J will be dimensioned to provide a macrocycle of 13
     to 16 ring atoms (including those atoms in the P1, P2 and if present P3 groups
     contributing to the ring). Conveniently J is dimensioned to provide a macrocycle of 14
     or 15 ring atoms.

15

     Conveniently, the J chain contains one or two heteroatoms selected from: O, S, NH,
     NC_1-C_6 alkyl or N-C(=O)C_1-C_6alkyl. More preferably, the J chain optionally contains
     one heteroatom selected from: NH, or N-C(=O)C_1-C_6alkyl, most preferably N(Ac).
     Most preferably, the chain containing a nitrogen atom is saturated. In an alternative

20   embodiment, J contains one heteroatom selected from O or S. The chain may be
     substituted with R^{14}, such as H or methyl.

Typically the J linker structure is saturated. Alternatively, J contains 1 to 3, preferably one double bond, typically spaced one carbon from the cycloalkyl R^7 function, if present. The double bond may be cis or trans.

5   Representative examples of J thus include pentylene, hexylene, heptylene, any of which are substituted with C_1-C_6alkyl, C_1-C_6haloalkyl, C_1-C_6alkoxy, hydroxyl, halo, amino, oxo, thio or C_1-C_6thioalkyl; penten-3-yl, hexen-4-yl, hepten-5-yl, where 3, 4 or 5 refers to a double bond between carbon atoms 3 and 4, 4 and 5 etc.

10   Convenient R^7 and $R^{7'}$ groups include those wherein $R^{7'}$ is H and R^7 is n-ethyl, n-propyl, cyclopropyl, cyclopropylmethyl, cyclobutyl, cyclobutylmethyl, 2,2-difluoroethyl, or mercaptomethyl. Preferred embodiments include those wherein R^7 is n-propyl or 2,2-difluoroethyl.

15   Alternative favoured configurations for R^7 and $R^{7'}$ include those wherein $R^{7'}$ is H and R^7 is C_3-C_7 cycloalkyl or C_1-C_3alkylC_3-C_7cycloalkyl.

     Still further favoured configurations for R^7 and $R^{7'}$ include those wherein $R^{7'}$ is H and R^7 is J.

. 20

     Alternatively, R^7 and $R^{7'}$ together define a spiro-cycloalkyl function, such as a spiro-cyclobutyl ring, and more preferably a spiro-cyclopropyl ring. "Spiro" in this context simply means that the cycloalkyl ring shares a single carbon atom with the peptidic backbone of the compound. The ring is substituted or unsubstituted. Preferred

25   substituents include mono or di-substitutions with $R^{7'a}$ wherein $R^{7'a}$ is C_1-C_6 alkyl, C_3-C_5cycloalkyl, or C_2-C_6 alkenyl, any of which is optionally substituted with halo.

     Alternatively the substituent may be a J linker as described above. Currently preferred stereochemistries for a spiro-cyclopropyl ring are defined below.

30

Particularly preferred substituents include $R^{7'a}$ as ethyl, vinyl, cyclopropyl (ie a spiro-cyclopropyl substituent to the "spiro" cycloalkyl ring of $R^7/R^{7'}$), 1- or 2-bromoethyl, 1-or 2-fluoroethyl, 2-bromovinyl or 2-fluorethyl.

5   In one embodiment of the invention A is $-CR^4R^{4'}$ as illustrated in detail in PCT/EP03/10595, the contents of which are incorporated by reference.

Convenient $R^{4'}$ groups thus include C_1-C_6alkyl, such as methyl, ethyl, propyl, ethenyl and $-CHCHCH_3$. Alternative preferred $R^{4'}$ groups include aryl or heteroaryl such as

10   optionally substituted phenyl, pyridyl, thiazolyl or benzimidazolyl or C_1-C_3alkylaryl or C_1-C_3alkylheteroaryl, where the alkyl moiety is methyl, ethyl, propyl, ethenyl and $-$CHCHCH$_3$. Preferred aryl moieties include optionally substituted phenyl, benzothiazole and benzimidazole.

15   Favoured R^4 groups include $-NH_2$, fluoro or chloro. Alternative preferred R^4 groups include $-OH$ and especially $=O$.

An alternative embodiment for A is $C(=O)NHR^3$, where R^3 is optionally substituted C_0-C_3alkylaryl, C_0-C_3alkylheteroaryl, OC_0-C_3alkylaryl or OC_0-C_3alkylheteroaryl.

20   Appropriate susbstituents appear in the definitions section below.

An alternative favoured configuration for A is $C(=O)OR^1$, especially where R^1 is C_1-C_6alkyl, such as methyl, ethyl, or tert-butyl and most preferably hydrogen.

25   A particularly preferred configuration for A is $C(=O)NHSO_2R^2$, especially where R^2 is optionally substituted C_1-C_6alkyl, preferably methyl, or optionally substituted C_3-C_7cycloalkyl, preferably cyclopropyl, or optionally substituted C_0-C_6alkylaryl, preferably optionally substituted phenyl. Appropriate substituents appear in the definitions section below.

30

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Substituent -W-R8 on the cyclic P2 group can employ any of the proline substituents which are extensively described in WO 00/59929, WO 00/09543, WO 00/09558, WO 99/07734, WO 99/07733, WO 02/60926, WO03/35060, WO 03/53349, WO03/064416, W=03/66103, WO03/064455, WO03/064456, WO03/62265,

5   WO03/062228, WO03/87092, WO 03/99274, WO03/99316, WO03/99274, ··
WO04/03670, , WO04/032827, WO04/037855, WO04/43339, WO04/92161,
WO04/72243, 5WO04/93798. WO04/93915, WO04/94452, WO04/101505,
WO04/101602, WO04/103996, WO04113365 and the like.

10   Favoured W functions include W as -OC(=O)NH-, -OC(=O)-, -NH-, $-NR^8-$, -NHS(O)$_2$-
or -NHC(=O)-, especially –OC(=O)NH- or –NH-. Favoured R^8 groups for such W
functions include optionally substituted C_0-C_3alkylcarbocyclyl or C_0-
C_3alkylheterocyclyl, including those described in WO0009543, WO0009558 and WO
00/174768. For example ester substituents, -W-R^8, on the cyclic P2 group, include

15   those disclosed in WO 01/74768 such as C_1-C_6alkanoyloxy, C_0-C_3alkylaryloyloxy,
particularly (optionally substituted) benzoyloxy or C_0-C_3alkylheterocicloyloxy,
especially
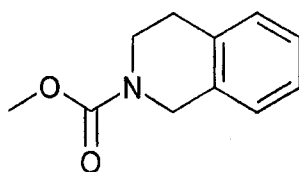
This publication also describes alternative –W-R^8 possibilities for example C_1-C_6alkyl,

20   such as ethyl, isopropyl, C_0-C_3alkylcarbocyclyl such as cyclohexyl, 2,2-difluoroethyl, -
C(=O)NRc, where Rc is C_1-C_6alkyl, C_0-C_3alkylcyclopropyl, C_0-C_3alkylaryl or C_0-
C_3alkylheterocyclyl.

Currently preferred W functions include -S- and especially -O-. Convenient values for

25   R^8 in such embodiments include C_0-C_3alkylaryl, or C_0-C_3alkylheteroaryl either of
which is optionally mono, di, or tri substituted with R^9, wherein;
R^9 is C_1-C_6 alkyl, C_1-C_6alkoxy, NO$_2$, OH, halo, trifluoromethyl, amino or amido
(suchas amino or amido optionally mono- or di-substituted with C_1-C_6alkyl), C_0-

C_3alkylaryl, C_0-C_3alkylheteroaryl, carboxyl, aryl or heteroaryl being optionally substituted with R^{10}; wherein

R^{10} is C_1-C_6alkyl, C_3-C_7cycloalkyl, C_1-C_6alkoxy, amino (such as amino mono- or di-substituted with C_1-C_6alkyl), amido (such as C_1-C_3 alkyl amide), sulfonylC_1-C_3alkyl,

5   NO_2, OH, halo, trifluoromethyl, carboxyl, or heteroaryl.

Typically, the C_0-C_3 alkyl component of R^8 as C_0-C_3alkylaryl, or C_0-C_3alkylheteroaryl is methyl and especially absent, ie C_0. The aryl or heteroaryl component is as extensively illustrated in the definition section below.

10

Preferred R^9 include C_1-C_6 alkyl, C_1-C_6alkoxy, amino (such as di-(C_1-C_3 alkyl)amino), amide (such as as $-NHC(O)C_1$-C_6alkyl or $C(=O)NHC_1$-C_3alkyl), aryl or heteroaryl, the aryl or heteroaryl moiety being optionally substituted with R^{10}; wherein

R^{10} is C_1-C_6alkyl, C_3-C_7cycloalkyl, C_1-C_6alkoxy, amino (such as mono- or di-C_1-C_3

15   alkylamino), amido (such as as $-NHC(O)C_1$-C_3alkyl or $C(=O)NHC_1$-C_6alkyl), halo, trifluoromethyl, or heteroaryl.

Preferred R^{10} include C_1-C_6alkyl, C_1-C_6alkoxy, amino, amido (such as as $-NHC(O)C_1$-C_6alkyl or $C(=O)NHC_1$-C_6alkyl) halo, or heteroaryl.

20

Particularly preferred R^{10} include methyl, ethyl, isopropyl, tert.butyl, methoxy, chloro, amino, amido (such as as $-NHC(O)C_1$-C_3alkyl or $C(=O)NHC_1$-C_6alkyl), or C_1-C_3alkyl thiazole.

25   Favoured embodiments of R^8 include 1-naphthylmethyl, 2-naphthylmethyl, benzyl, 1-naphthyl, 2-naphthyl, or quinolinyl, any opf which is unsubstituted, or mono- or disubstituted with R^9 as defined, in particular 1-naphthylmethyl, or quinolinyl unsubstituted, mono, or disubstituted with R^9 as defined.

30   A currently preferred R^8 is:
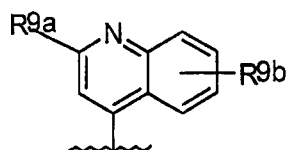
wherein R^{9a} is C_1-C_6alkyl; C_1-C_6alkoxy; thioC_1-C_3alkyl; amino optionally substituted with C_1-C_6alkyl; C_0-C_3alkylaryl; or C_0-C_3alkylheteroaryl, C_0-C_3alkylheterocyclyl, said aryl, heteroaryl or heterocycle being optionally substituted with R^{10} wherein

5   R^{10} is C_1-C_6alkyl, C_3-C_7cycloalkyl, C_1-C_6alkoxy, amino, amido, heteroaryl or heterocyclyl; and

R^{9b} is C_1-C_6alkyl, C_1-C_6alkoxy, amino, amido, NO_2, OH, halo, trifluoromethyl, carboxyl.

10   Convenient R^{9a} include aryl or heteroaryl, all optionally substituted with R^{10} as defined, especially where R^{9a} is selected from the group consisted of:
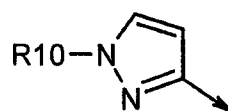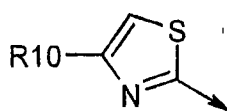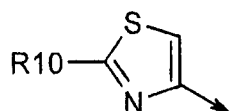
wherein R^{10} is H, C_1-C_6alkyl, or C_0-C_3alkyl-C_3-C_6cycloalkyl, amino (sch as amino mono- or di-substituted with C_1-C_6alkyl), amido (such as as –NHC(O)C_1-C_6alkyl or

15   C(=O)NHC_1-C_6alkyl) heteroaryl or heterocyclyl.

R^{9a} is conveniently phenyl and thus R^8 is:
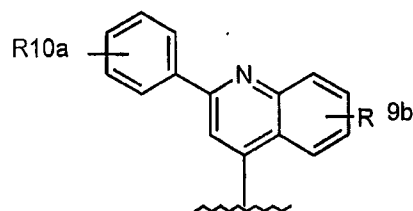
wherein R^{10a} is H, C_1-C_6alkyl; C_1-C_6alkoxy; or halo; and R^{9b} is C_1-C_6 alkyl, C_1-C_6-

20   alkoxy, amino (such as C_1-C_3alkylamino), amido (such as as –NHC(O)C_1-C_6alkyl or C(=O)NHC_1-C_3alkyl), NO_2, OH, halo, trifluoromethyl or carboxyl.
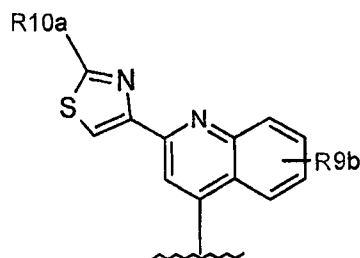
An alternative preferred R^8 is:

wherein R^{10a} is H, C_1-C_6alkyl, or C_0-C_3alkyl-C_3-C_6cycloalkyl, amino (such as amino optionally mono- or di-substituted with C_1-C_6alkyl), amido (such as as –NHC(O)C_1-
5   C_6alkyl or C(=O)NHC_1-C_3alkyl or C(=O)N(C_1-C_3alkyl)$_2$), heteroaryl or heterocyclyl; and R^{9b} is C_1-C_6 alkyl, C_1-C_6-alkoxy, amino optionally mono- or di-substituted with C_1-C_6alkyl, amido (such as as –NHC(O)C_1-C_6alkyl or C(=O)NHC_1-C_3alkyl or C(=O)N(C_1-C_3alkyl)$_2$), NO$_2$, OH, halo, trifluoromethyl, or carboxyl.

10   In the immediately above described embodiments R^{9b} is conveniently C_1-C_6-alkoxy, preferably methoxy.

A further R^8 group, for example when W is an ether has the formula
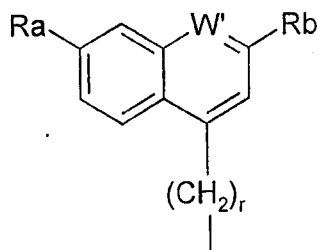
15   where W' is N or CH, r is 0 or 1, Ra' is H, C_1-C_6 alkyl, C_0-C_3alkylcycloalkyl, C_1-C_6alkyloxy, hydroxy or amine and Rb' is H, halo, C_1-C_6alkyl, C_0-C_3alkylcycloalkyl, C_1-C_6alkyloxy, C_1-C_6thioalkyl, cycloalkylC_0-C_3alkyloxy, C_1-C_3alkyloxyC_1-C_3alkyl, C_0-C_3alkylaryl or C_0-C_3alkylheterocyclyl. A particularly preferred ether substituent is 7-methoxy-2-phenyl-quinolin-4-yl oxy.
20

When W is a bond then R^8 is preferably a substituted or unsubstituted heterocyclic ring system as described in WO2004/072243 or WO2004/113665.

Representative examples of R^8 when W is a bond include the following aromatics which may optionally be substituted: 1*H*-pyrrole, 1*H*-imidazole, 1*H*-pyrazole, furan, thiophene, oxazole, thiazole, isoxazole, isothiazole, pyridine, pyndazine, pyrimidine,

5   pyrazine, phthalazine, quinoxaline, quinazoline, quinoline, cinnoline, 1*H*-pyrrolo[2,3]-*b*]pyridine, 1*H*-indole, 1*H*-benzoimidazole, 1*H*-indazole, 7*H*-purine, benzothiazole, benzooxazole, 1*H*-imidazo[4, 5-*c*]pyridine, 1*H*-imidazo[4,5-*b*]pyridine, 1, 3-dihydro-benzoimidazol-2-one, 1, 3-dihydro-benzoimidazol-2-thione, 2, 3-dihydro-1*H*-indole, 1,3-dihydro-indol-2-one, 1*H*-indole-2,3-dione, 1, 3-dihydro-benzoimidazole-2-one, 1*H*,

10   1*H*-pyrrolo [2, 3-*c*]pyridine, benzofuran, benzo[*b*]thiophene, benzo[*d*]isoxazole, benzo[*d*]isothiazole, 1H-quinotin-2-one, 1*H*-quinolin-4-one, 1*H*-quinazolin-4-one, 9*H*-carbazole, 1*H*-quinazolin-2-one.

Additional representative examples of R^8 when W is a bond, include the following

15   non-aromatics, which may be optionally subsbstituted: aziridine, azetidine, pyrrolidine, 4,5-dihydro-1*H*-pyrazole, pyrazolidine, imidazolidin-2-one, imidazolidine-2-thione, pyrrolidin-2-one, pyrolidine-2,5-dione, piperidine-2,6-dione, piperidin-2-one, piperazine-2,6-dione, piperazin-2-one, piperazine, morpholine, thiomorpholine-1,1-dioxide, pyrazolidin-3-one, imidazolidine-2,4-dione, piperidine, tetrahydrofuran,

20   tetrahydropyran, [1,4]dioxane, 1,2,3,6-tetrahydropyridine.

Preferred values for R^8 when W is a bond, include tetrazole and derivatives thereof. The tetrazole moiety is linked to the cyclic P2 scaffold and optionally substituted as shown below:
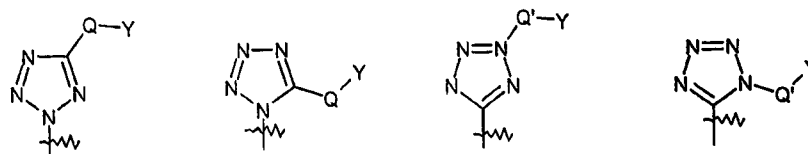

25

wherein Q* is selected from the group consisting of absent, -CH₂-, -O-, -NH-, -N(R^{1*})-, -S-, -S(=O)₂- and –(C=O)-; Q* is selected from the group consisting of: absent, -CH₂-and -NH; Y* is selected from the group consisting of: H, C_1-C_6alkyl, C_0-C_3aryl, C_0-

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C_3heterocyclyl and R^{1*} is selected from the group consisting of: H, C_1-C_6alkyl, carbocyclyl, C_0-C_3aryl, C_0-C_3heterocyclyl.

Representative examples of substituted tetrazoles are as described in table 1 of WO2004/072243 and the structures following immediately after, or WO2004/113665.

Further preferred values for R^8 when W is a bond, include triazole and derivatives thereof. The triazole moiety is linked to the cyclic P2 scaffold and optionally substituted as shown below:
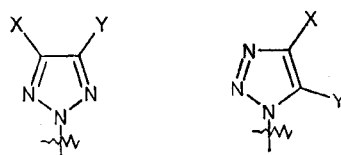
wherein X* and Y* are independently selected from the group consisting of: H, halogen, C_1-C_6alkyl, C_0-C_3carbocyclyl, -CH_2-amino, -CH_2-arylamino, -CH_2-diarylamino, -(C=O)-amino, -(C=O)-arylamino, -(C=O)-diarylamino, C_0-C_3aryl, C_0-C_3heterocyclyl or alternatively, X* and Y* taken together with the carbon atoms to which they are attached, form a cyclic moiety selected from the group consisting of aryl and heteroaryl.

Representative examples of substituted triazoles are as described in table 2 of WO2004/072243 and the structures following immediately after, or WO2004/113665.

Further preferred values for R^8 when W is a bond, include pyridazinone and derivatives thereof. The pyridazinone moiety is linked to the cyclic P2 scaffold and optionally substituted as shown below:
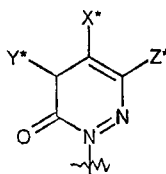
wherein X*, Y* and Z* are independently selected from the group consisting of: H, N₃, halogen, C_1-C_6alkyl, carbocyclyl, amino, C_0-C_3aryl, -S-aryl, -O-aryl, -NH-aryl, diarylamino, diheteroarylamino, C_0-C_3heterocyclyl, -S-heteroaryl, -O-heteroaryl, NH-heteroaryl or, alternatively, X and Y or Y and Z taken together with the carbon atoms

5   to which they are attached, form an aryl or heteroaryl cyclic moiety.

Representative examples of substituted pyridazinones are as described in table 3 of WO2004/072243 and the structures following immediately after or WO2004/113665.

10   Preferred P3 groups, i.e. when m is 1 resemble natural or unnatural amino acids, especially aliphatic amino acids, such as L-valyl, L-leucyl, L- isoleucyl or L- t-leucyl. Further preferred P3 groups, as shown in WO 02/01898 include C_0-C_3alkylcycloalkylalanine, especially cyclohexylalanine, optionally substituted with CO_2Rg, where Rg is H, is C_1-C_6alkyl, C_0-C_3alkylaryl, C_0-C_3alkylheterocyclyl, C_0-

15   C_3alkylcycloalkyl or amine; or N-acetylpiperidine or tetrahydropyran. Preferred R^{11} groups thus include C_1-C_6alkyl, C_0-C_3alkylcarbocyclyl for example C_0-C_3alkylC_3-C_7cycloalkylyl, C_0-C_3alkylaryl or C_0-C_3alkylheteroaryl, any of which is optionally substituted with hydroxy, halo, amino, C_1-C_6alkoxy, C_1-C_6thioalkyl, $C(=O)OR^{14}$, carboxyl, (C_1-C_6alkoxy)carbonyl, aryl, heteroaryl or heterocyclyl, especially where the

· 20   substituent is hydroxy or $C(=O)OR^{14}$.

Particularly preferred R^{11} include tert-butyl, iso-butyl, cyclohexyl, phenylethyl, 2,2-dimethyl-propyl, cyclohexylmethyl, phenylmethyl, 2-pyridylmethyl, 4-hydroxy-phenylmethyl, or carboxylpropyl. The most preferred R^{11} values are currently tert-

25   butyl, isobutyl, or cyclohexyl.

An embodiment of the invention include compounds wherein P4 is absent (ie n is 0) and wherein the P3 function lacks a carbonyl, ie U is absent. Representative substructures include those of formula Ii below:
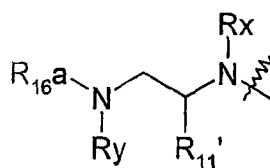
Ii

wherein Rx and Ry are as defined above, preferably H,

$R^{11'}$ is C_1-C_6 alkyl, preferably C_3-C_5 branched alkyl such as the side chains of L-valyl, L-leucyl, L-isoleucyl, L-t-leucyl; or C_0-C_2alkylC_3-C_7 cycloalkyl such as cyclohexyl or cyclohexylmethyl;

R^{16a} is –Rba, -S(=O)pRba, -C(=O)Rba;

Rba is C_1-C_6 alkyl, C_0-C_3alkylheterocyclyl, C_0-C_3alkylcarbocyclyl.

Alternatively, compounds of partial structure Ii may be macrocyclised between an appropriate value of R^7 and one of Rx, Ry or $R^{11'}$.

Representative embodiments of P3 groups which lack a carboxy function (ie variable U is absent) include those of formula VIia-VIid below:

VIia

VIib
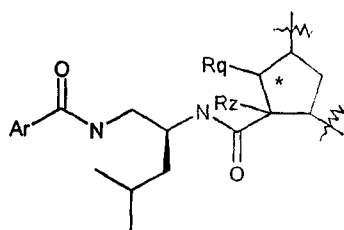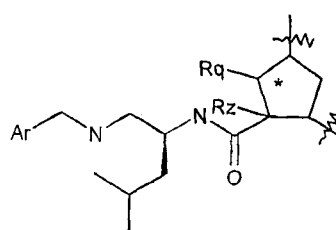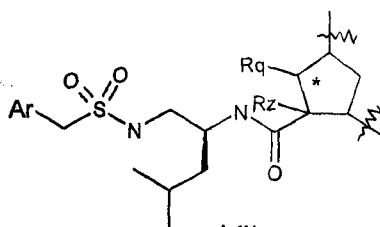
VIic
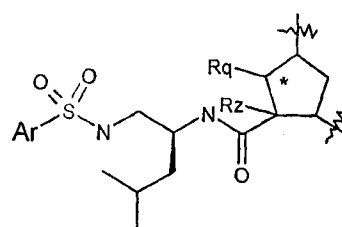
VIid

where Ar is carbocyclyl or heterocyclyl, especially aryl or heteroaryl, any of which is optionally substituted with R^9. Although the partial structures of Formulae VIia - VIid have been illustrated in the context of a compound wherin k is 1 and q' is 0, it will be

apparent that such configurations of Formula VIi apply also to other values of q' and k. Similarly, although the partial structures of formulae VIic and VIid show an R^{11} group corresponding to leucine, it will be apparent that these configurations will be applicable to other R^{11} groups, especially those resembling the side chains of natural

5   or unnatural L-amino acids, for example t-butyl alanine/t.leucine.

R^{15} in those compounds of the invention wherein n is 1, is preferably optionally substituted C_1-C_6alkyl or C_0-C_3alkylcarbocyclyl for example C_0-C_3alkylC_3-C_7cycloalkyl, any of which may be optionally substituted. Preferred P4 groups are typically

10   analogues of natural or unnatural amino acids, especially aliphatic amino acids such as L-valyl, L-leucyl, L-isoleucyl, L-t-leucyl or L-cyclohexylalanine and thus favoured R^{15} groups include cyclohexyl, cyclohexylmethyl, tert-butyl, iso-propyl, or iso-butyl.

Preferred G values include --NRy-, especially wherein Ry is methyl or preferably H, or

15   hydrazine.

A further preferred G value is O thereby defining an ester with the carbonyl of P4 (if present) or the carbonyl of P3 (if present) or an ether in the case of variants wherein group U is absent. Conventional pharmaceutically acceptable ethers or esters

20   capping groups for R^{16} include C_1-C_6alkyl (especially methyl or t-butyl), C_0-C_3alkylheterocyclyl (especially pyridyl, benzimidazolyl, piperidyl, morpholinyl, piperazinyl) or C_0-C_3alkylcarbocyclyl (especially phenyl, benzyl, indanyl) any of which is optionally substituted with hydroxy, halo, amino, or C_1-C_6alkoxy.

25   Favoured compounds of the invention can comprise a hydrazine functionality, for example where T is -NRd- and m is 1; with n being zero or 1. Alternatively, especially where m is zero, G can be -NRjNRj- such as -NHNH-. Compounds will generally not comprise a hydrazine at both G and T. Preferred hydrazines within Formula VI, wherein m and n are zero include compounds of the partial structures VIja- VIjb

30   below:
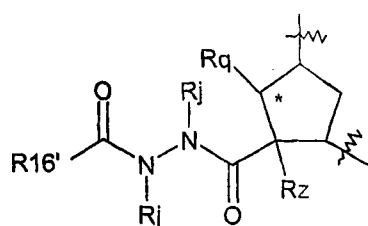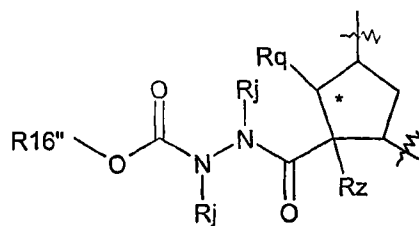
VIja   VIjb

R$^{16'}$ in formulae VIja and VIjb can be regarded as an alkyl (or C$_1$-C$_3$alkylheterocyclyl or C$_1$-C$_3$alkylcarbocyclyl) wherein the first alkyl carbon is substituted with an oxo group
5   to define the keto function and R$^{16'}$ is the remainder of the alkyl, alkylheterocyclyl or alkylcarbocyclyl moiety. Formula VIjb depicts a variant where R^{16} is a methylene group whose carbon is substituted with an oxo substituent and also with -ORb, where Rb is as defined above, typically, C$_1$-C$_6$alkyl, such as t-butyl, C$_0$-C$_3$alkylheterocyclyl such as pyridyl, or C$_0$-C$_3$alkylcarbocyclyl, such as benzyl or phenyl, any of which is
10   optionally substituted as defined above. Compounds of partial structures VIja and VIjb can be linear molecules as shown (both Rj are H), or preferably one of the depicted Rj groups can be macrocyclised via J to an appropriate R^7 group.

Alternative hydrazines of Formula VI where m is 1 include those of partial structures
15   VIjc and VIjd below:
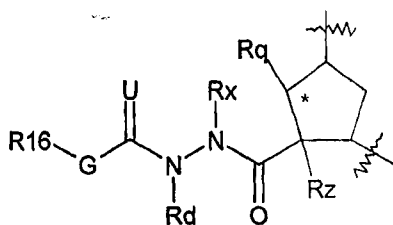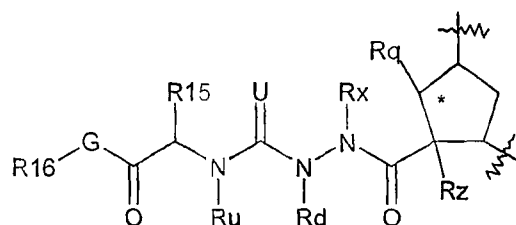
VIjc   VIjd

where G, R^{15}, R^{16}, Rx, Rd, Rq, Rz,and Ru are as defined for formula VI above. Compounds of partial structures VIjc and VIjd can be linear molecules as shown (both Rx and Rd are H), or preferably one of the depicted Rx or Rd groups can be
20   macrocyclised via J to an appropriate R^7 group.

Although formulae VIja-VIjd are depicted with a five membered carbocyclic ring as P2 scaffold, it will be apparent that this aspect of the invention is equally adapted to other configurations of q' and k. Favoured embodiments within formula VIja-VIjd include

5   those wherein Rq and Rz are H, or those wherein Rz is an olefinic bond and Rq is C_1-C_3alkyl.

Alternative hydrazine-like configuration are found when G is amino, and m and n are 0, and R^{16} is an N-linked unsaturated heterocycle as defined below, for example

10 pyridyl or pyrimidyl or a saturated heterocycle as defined below, such as piperazinyl, piperidinyl and especially morpholinyl., Examples of such embodiments include those of the formulae VIjc and VIjd:

VIjc   VIjd
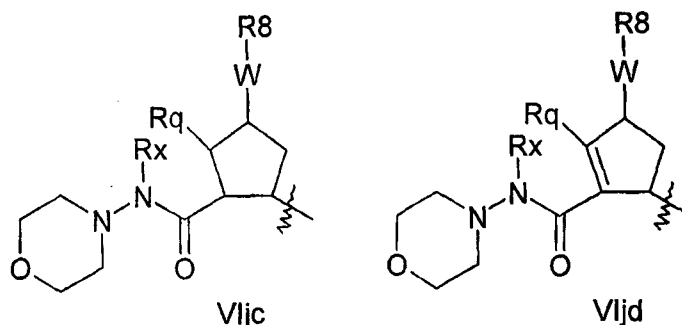
Compounds of partial structures VIjc and VIjd can be linear molecules as shown or

15 preferably Rx can be macrocyclised via J to an appropriate R^7 group. Although these partial structures are depicted with a five membered ring as the P2 scaffold, it will be readily apparent that this configuration extends to other values of q' and k. Similarly these configurations will be applicable to other N-linked heterocycles as R^{16}.

20 Returning now to Formulae VI in general, favoured R^{16} groups for the compounds of the invention include 2-indanol, indanyl, 2-hydroxy-1-phenyl-ethyl, 2-thiophenemethyl, cyclohexylmethyl, 2,3-methylenedioxybenzyl, cyclohexyl, phenyl, benzyl, 2-pyridylmethyl, cyclobutyl, iso-butyl, n-propyl, methyl, or 4-methoxyphenylethyl.

Currently preferred R^{16} groups include 2-indanol, indan, 2-hydroxy-1-phenyl-ethyl, 2-thiophenemethyl, 2,3-methylenedioxybenzyl, or cyclohexylmethyl.

5   Unnatural amino acids include L-amino acids wherein the side chain is not one of the 20 naturally occurring amino acids. Examples of non-natural amino acids include L-beta-methylsulfonylmethylalanine, L-cyclohexylalanine, L-tertiary-leucine, L-norleucine, L-norvaline, L-ornithine, L-sarcosine, L-citurline, L-homophenylalanine, L-homoserine, L-beta-(1-napthyl)alanine, L-beta-(2-napthyl)alanine etc. Non natural amino acids also include the D-amino acids corresponding to the 20 natural amino

10   acids and D-amino acids bearing other side chains, such as those listed above.

'C_1-C_6alkyl' (also abbreviated as C_1-C_6alk, or used in compound expressions such as C_1-C_6alkyloxy etc) as applied herein is meant to include straight and branched chain aliphatic carbon chains such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, t-

15   butyl, pentyl, isopentyl, hexyl, heptyl and any simple isomers thereof. The alkyl group may have an unsaturated bond. Additionally, any C atom in C_1-C_6alkyl may optionally be substituted by one, two or where valency permits three halogens and/or substituted or the alkylene chain interrupted by a heteroatom S, O, NH. If the heteroatom is located at a chain terminus then it is appropriately substituted with one

20   or 2 hydrogen atoms. C_1-C_4alkyl and C_1-C_5alkyl have the corresponding meaning to C_1-C_6alkyl adjusted as necessary for the carbon number.

'C_1-C_3alkyl' as applied herein includes methyl, ethyl, propyl, isopropyl, cyclopropyl, any of which may be optionally substituted or heteroatom interupted as described in

25   the paragraph above or in the case of C_2 or C_3, bear an unsaturated bond such as CH_2=CH.

"C_1-C_3 alkylene" as applied herein describes a divalent C1-C3alkyldiyl moiety, including propylene, ethylene and especially methylene. The typically longer alkylene

30   chains for J may comprise 1 to 3 unsaturations and/or interruptions with heteroatoms as defined above.

'Amino' includes NH_2, NHC_1-C_6alkyl or $N(C_1$-C_6-$alkyl)_2$, especially C_1-C_3 alkyl variants

'Amido' includes $C(=O)NH_2$ and alkylamido such as $C(=O)NHC_1$-C_6alkyl, $C(=O)N(C_1$-

5    $C_6alkyl)_2$ especially $C(=O)NHC_1$-C_3alkyl, $C(=O)N(C_1$-$C_3alkyl)_2$ or -$NH(C=O)C_1$-C_6alkyl,

for example -$NHC(=O)CHC(CH_3)_3$, including -$NH(C=O)C_1$-C_3alkyl.

'Halo' or halogen as applied herein is meant to include F, Cl, Br, I, particularly chloro

and preferably fluoro.

10

'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 methyl, ethyl, propyl, or isopropyl

group as defined for C_1-$C_3alkylene$ above. Unless otherwise indicated the aryl and/or

15   its fused cycloalkyl moiety 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 C_0-$C_3alkylcarbocyclyl$, C_0-

$C_3alkylheterocyclyl$. "Aryl" has the corresponding meaning, i.e. where the C_0-C_3alkyl

linkage is absent.

20

'C_0-$C_3alkylC_3C_7cycloalkyl$' 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 methyl, ethyl or

proyl group as defined for C_1-$C_3alkylene$ above. The cycloalkyl group may contain an

25   unsaturated bond. Unless otherwise indicated the cycloalkyl moiety 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 C_0-$C_3alkylcarbocyclyl$, C_0-$C_3alkylheterocyclyl$.

30   'C_0-$C_3alkylcarbocyclyl$' as applied herein is meant to include C_0-$C_3alkylaryl$ and C_0-

$C_3alkylC_3$-$C_7cycloalkyl$. 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, C_0-C_3alkylcarbocyclyl and/or C_0-C_3alkylheterocyclyl. "Carbocyclyl" has the corresponding meaning, i.e. where the C_0-C_3alkyl linkage is

5   absent

'C_0-C_3alkylheterocycylyl' as applied herein is meant to include a monocyclic, saturated or unsaturated, heteroatom-containing ring such as piperidinyl, morpholinyl, piperazinyl, pyrazolyl, imidazolyl, oxazolyl, isoxazolyl, thiazinolyl, isothiazinolyl,

10   thiazolyl, oxadiazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, tetrazolyl, furanyl, thienyl, pyridyl, pyrimidyl, pyridazinyl, pyrazolyl, or any of such groups fused to a phenyl ring, such as quinolinyl, benzimidazolyl, benzoxazolyl, benzisoxazolyl, benzothiazinolyl, benzisothiazinolyl, benzothiazolyl, benzoxadiazolyl, benzo-1,2,3-triazolyl, benzo-1,2,4-triazolyl, benzotetrazolyl, benzofuranyl, benzothienyl, benzopyridyl,

15   benzopyrimidyl, benzopyridazinyl, benzopyrazolyl etc, which ring is bonded directly i.e. (C_0), or through an intermediate methyl, ethyl, propyl, or isopropyl group as defined for C_1-C_3alkylene above. Any such non-saturated rings having an aromatic character may be referred to as heteroaryl herein. Unless otherwise indicated the hetero ring and/or its fused phenyl moeity is optionally substituted with 1-3

20   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, C_0-C_3alkylcarbocyclyl, C_0-C_3alkylheterocyclyl. "Heterocyclyl" and "Heteroaryl" have the corresponding meaning, i.e. where the C_0-C_3alkyl linkage is absent.

25   Typically heterocycyl and carbocyclyl moieties within the scope of the above definitions are thus a monocyclic ring with 5 or especially 6 ring atoms, or a bicyclic ring structure comprising a 6 membered ring fused to a 4, 5 or 6 membered ring.

Typical such groups include C_3-C_8cycloalkyl, phenyl, benzyl, tetrahydronaphthyl,

30   indenyl, indanyl, heterocyclyl such as from azepanyl, azocanyl, 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, benzofuranyl, benzothienyl, benzimidazolyl, benzthiazolyl, benzoxazolyl, benzisoxazolyl, quinolinyl,
5   tetrahydroquinolinyl, isoquinolinyl, tetrahydroisoquinolinyl, quinazolinyl, tetrahydroquinazolinyl and quinoxalinyl, any of which may be optionally substituted as defined herein.

The saturated heterocycle moiety thus includes radicals such as pyrrolinyl,
10   pyrrolidinyl, pyrazolinyl, pyrazolidinyl, piperidinyl, morpholinyl, thiomorpholinyl, pyranyl, thiopyranyl, piperazinyl, indolinyl, azetidinyl, tetrahydropyranyl, tetrahydrothiopyranyl, tetrahydrofuranyl, hexahydropyrimidinyl, hexahydropyridazinyl, 1,4,5,6-tetrahydropyrimidinylamine, dihydro-oxazolyl, 1,2-thiazinanyl-1,1-dioxide, 1,2,6-thiadiazinanyl-1,1-dioxide, isothiazolidinyl-1,1 -dioxide and imidazolidinyl-2,4-
15   dione, whereas the unsaturated heterocycle include radicals with an aromatic character such as furanyl, thienyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, tetrazolyl, thiadiazolyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, indolizinyl, indolyl, isoindolyl. In each case the heterocycle may be condensed with a phenyl ring to form a bicyclic ring system.
20

Synthesis

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 suitably protected individual building blocks can first be prepared and subsequently coupled
25   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^{16}-G-P3+C(=O)-P2-P1→ R^{16}-G-P3-C(=O)-P2-P1 or R^{16}-G-P4-P3+C(=O)-P2-
30   P1→ R^{16}-G-P4-P3-C(=O)-P2-P1.

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
5   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
10   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
15   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-
20   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-(IH-
benzotriazol-1-yl)-N, N, N',N'- tetramethyluronium tetrafluoroborate. Still another
25   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.
30   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 amino acids generally must be protected
5   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.
10

The α-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
15   reductive means such as trichloroethyl and phenacyl esters.

The α-amino group of each amino acid to be coupled is typically 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
20   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
25   triphenylmethyl and benzyl; 6) trialkylsilyl such as trimethylsilyl; and 7) thiol containing
groups such asphenylthiocarbonyl anddithiasuccinoyl. The preferred α-amino
protecting group is either Boc or Fmoc. Many amino acid derivatives suitably
protected for peptide synthesis are commercially available.

30   The α-amino protecting group is 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

5   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.

10   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

15   such protecting groups it is desirable that the group is not removed during the deprotection and coupling of the α-amino group.

For example, when Boc is used as the α-amino protecting group, the following side chain protecting groups are suitable: p-toluenesulfonyl (tosyl) moieties can be used to

20   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

25   acid.

When Fmoc is chosen for the α-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

30   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.

Introduction of the P2 substituent

The R^8 group can be coupled to the P2 scaffold at any convenient stage of the synthesis of compounds according to the present invention. One approach is to first couple the R^8 group to the P2 scaffold and subsequently add the other desired building blocks, i.e. P1 and optionally P3 and P4. Another approach is to couple the P1, P2 and if present P3 and P4 moieties using an unsubstituted P2 scaffold and add the R^8 group afterwards.

Compounds wherein W is O and R^8 is alkyl, C_0-C_3alkylcarbocycylyl, C_0-C_3alkylheterocycylyl can be prepared according to the procedure described by E. M. Smith et al. (J. Med. Chem. (1988), 31, 875-885), as depicted in Scheme 1, which illustrates the technique with a saturated P2 scaffold wherein q' is 0 and k is 1.

1a   1b
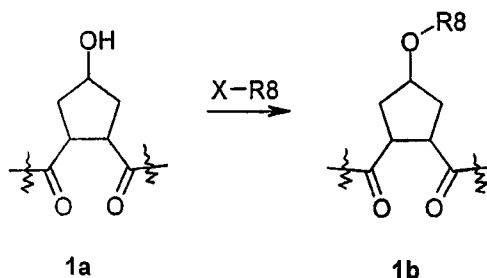
Scheme 1

Treatment of a compound containing an unsubstituted P2 scaffold (1a), which ca be prepared as described herein below with a base such as sodium hydride or potassium t.butoxide in a solvent like dimethylformamide followed by reaction of the resulting alkoxide with an alkylating agent, R^8-X, wherein X is a suitable leaving group such as a halide, mesylate, triflate or tosylate, provides the desired substituted derivative (1b).

Alternatively, if X is OH or SH, the P2 substituent can be introduced via a Mitsunobu reaction by reacting the hydroxy group of compound 1a with the desired alcohol or thiol in the presence of triphenylphosphine and an activating agent like diethyl

5   azodicarboxylate (DEAD), diisopropyl azodicarboxylate (DIAD) or the like. (Mitsunobu, 1981, Synthesis, January, 1-28; Rano et al., Tetrahedron Lett., 1995, 36, 22, 3779-3792; Krchnak et al., Tetrahedron Lett., 1995, 36, 5, 6193-6196; Richter et al., Tetrahedron Lett., 1994, 35, 27, 4705-4706).

10   Alcohol (1a) can alternatively be treated with phosgene thus providing the corresponding chloroformate which upon reaction with an amine, R^8NH_2, in the presence of a base like sodium hydrogen carbonate or triethylamine, provides carbamates i.e. W is -OC(=O)NH-, whereas reaction of alcohol (1a) with an acylating agent, R^8-CO-X, like an acid anhydride or acid halide for instance the acid chloride, to

15   provide esters, i.e. W is -OC(=O)-.

Various alcohols R^8-OH, and alkylating agents R^8-X are described in WO 00/09543 and WO00/59929. An example of the synthesis wherein R^8 is a substituted quinoline derivative is shown in Scheme 2.

Scheme 2

20
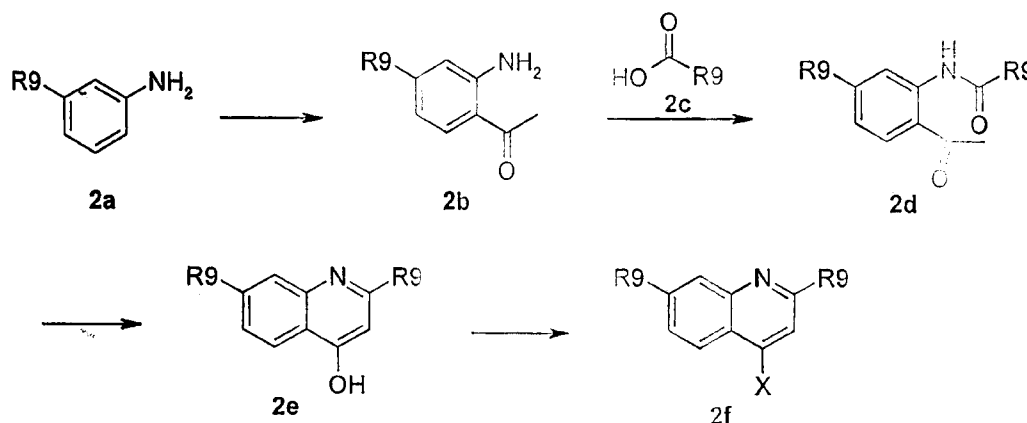
Friedel-Craft acylation of a suitable substituted aniline (2a), available either
commercially or in the literature, using an acylating agent like acetyl chloride or the
like in the presence of boron trichloride and aluminium trichloride in a solvent like
dichloromethane provides (2b). Coupling of (2b) to a heterocyclic carboxylic acid (2c)

5   under basic conditions, such as in pyridine, in the presence of an activating agent for
the carboxylate group, for instance POCl$_3$, followed by ring closure and dehydration
under basic conditions like potassium tert-butoxide in tert-butanol provides quinoline
derivative (2e). Quinoline derivative (2e) can be coupled in a Mitsunobu reaction to an
alcohol as described above, or the hydroxy group can be displaced by a suitable

10 leaving group such as a halide like chloride, bromide or iodide, by treatment of
quinoline (2e) with an appropriate halogenating agent for example phosphoryl
chloride or the like.

A variety of carboxylic acids with the general structure (2c) can be used in Scheme 2.

15 These acids are available either commercially or in the literature. An example of the
preparation of 2-(substituted)-amino-carboxy-aminothiazole derivatives, following the
procedure by Berdikhina et al. Chem. Heterocycl. Compd. (Engl. Transl.) (1991), 427-
433, is shown in scheme 3 below.
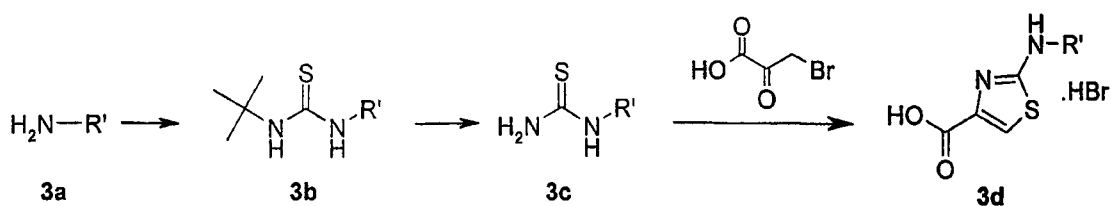
20 Scheme 3

Thiourea (3c) with different alkyl substituents R' can be formed by reaction of the
appropriate amine (3a) with tert-butylisothiocyanate in the presence of a base like
diisopropylethylamine in a solvent like dichloromethane followed by removal of the
tert- butyl group under acidic conditions. Subsequent condensation of thiourea

25 derivative (3c) with 3-bromopyruvic acid provides the acid (3d).

P2 building blocks wherein the R^8 substituent is attached via an amine, amide, urea or sulphonamide, can be prepared from amino substituted carbocycles achieved for example by transforming the hydroxy group of the corresponding hydroxy derivative into an azide group for example by transforming the hydroxy group into a suitable

5   leaving group such as a mesylate or halogen like chloride, followed by substitution of the leaving group with azide or by the use of an azide transfer agent like diphenylphosphoryl azide (DPPA). Reduction of the azide by catalytic hydrogenation or any other suitable reduction method provides the amine. The amino derivative can be reacted in a displacement reaction with an alkylating agent of the general formula

10   R^8-X wherein R^8 and X are as described for scheme 1, to form P2 building blocks for use in the preparation of compounds of general formula VI, wherein W is –NH-. Reaction of the amino substituted carbocycle with an acid of the general formula R^8-COOH under standard amide coupling conditions provides compounds wherein the R^8 substituent is linked via an amide bond, whereas reaction of the amino substituted

15   carbocycle with an appropriate derivative of sulphonic acid, R^8-S(O)$_2$-X where X is a leaving group for example chloride, in the presence of a base, provides sulphonamides. Compounds wherein the linkage between the cyclic scaffold and the R^8 substituent is constituted of a urea group can for example be achieved by treatment of amino substituted carbocycle with phosgene to afford the corresponding

20   chlorocarbamate followed by reaction with the desired amine. Alternatively, the amino substituted carbocycle can be reacted with the carbamoyl chloride or isocyanate of the desired R^8 substituent for the formation of the urea linkage. It will be apparent that corresponding reactions will be available for P2 groups with other ring sizes and substitution pattern.

25

Compounds wherein a heterocyclic R^8 group is attached directly to the cyclic P2 scaffold i.e. W is a bond in general formula VI, can be prepared for example by using a replacement reaction wherein a suitable leaving group such as halide a or a mesylate or the like on the P2 scaffold is replaced by the desired R^8 group such as a

30   heterocyclic group. Alternatively the R^8 group can be introduced by way of a

Mitsunobu reaction wherein the hydroxy group of the P2 precursor is reacted with a nitrogen atom in the heterocyclic R^8 group.

Rq, Rz and * are as descibed above
W is a bond
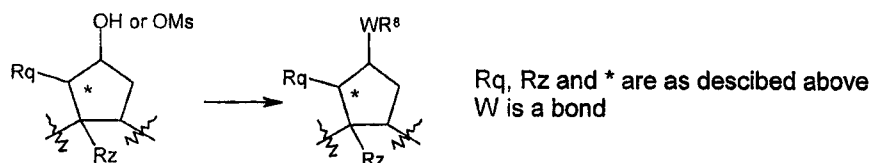
Compounds wherein a tetrazole derivative is attached to one of its ring carbons are conveniently prepared by building up the tetrazole moiety directly on the P2 precursor. This can be achieved for instance by transforming the hydroxy group of the P2 precursor into a cyano group followed by reaction with an azide reagent like sodium azide. Triazole derivatives can also be built up directly on the P2 precursor for example by transforming the hydroxy group of the P2 precursor into an azide group followed by a 3+2 cycloaddition reaction of the afforded azide and a suitable alkyne derivative.

Structurally diverse tetrazoles for use in the above described substitution or Mitsunobu reactions can be prepared by reacting commercially available nitrile compounds with sodium azide. Triazole derivatives can be prepared by reaction of an alkyne compound and trimethylsilyl azide. Useful alkyne compounds are available either commercially or they can be prepared for instance according to the Sonogashira reaction i.e. reaction of a primary alkyne, an aryl halide and triethylamine in the presence of $PdCl_2(PPh)_3$ and CuI as described for example in A. Elangovan, Y.-H. Wang, T.-I. Ho, *Org. Lett.*, 2003, 5, 1841-1844. The heterocyclic substituent can also be modified when attached to the P2 building block either before or after coupling of the P2 building block to the other building blocks.

These methods and further alternatives for the preparation of compounds wherein W is a bond and R^8 is an optionally substituted heterocycle are extensively described in WO2004/072243.

40

Compounds having an alternative ring size and/or position of the W-R^8 substituent of the carbocyclic derivative in scheme 1 may also be used in the preparation of compounds according to the present invention.

5   **Synthesis and introduction of P1 building blocks.**

The amino acids used in the preparation of P1 fragments are available either commercially or in the literature, see for example WO 00/09543 and WO00/59929 from Boehringer-Ingelheim or US2004/0048802 from BMS.

10 . Scheme 4 shows an example of the preparation of a sulphonamide derivative to be used as a P1 building block, and the subsequent coupling to a P2 building block.

4a   4b   4c

4d   4e
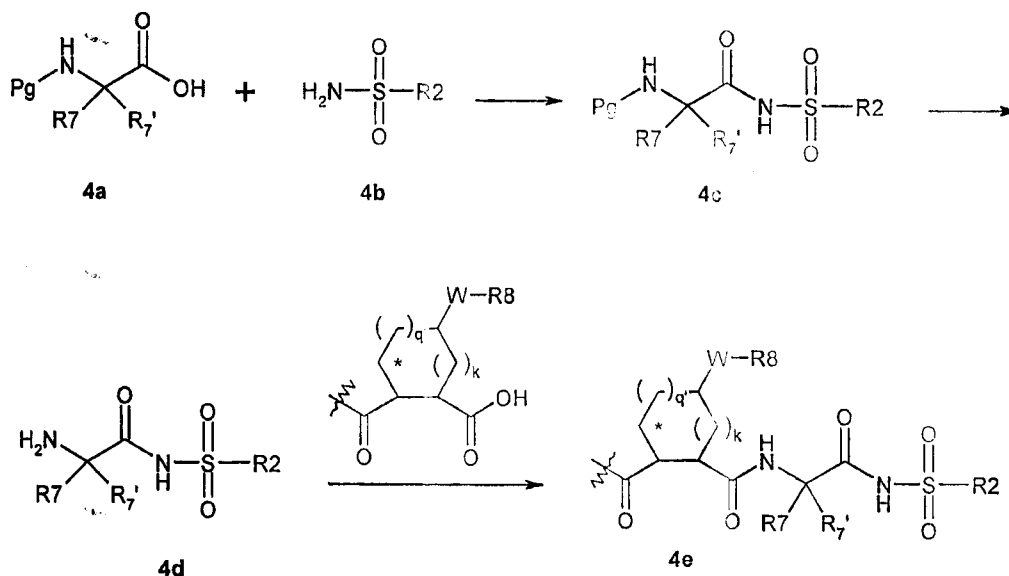
Scheme 6

The sulphonamide group can be introduced on a suitably protected amino acid (**4a**)

15   by treatment of the amino acid with a coupling agent, for example N,N'-carbonyldiimidazole (CDI) or the like, in a solvent like THF followed by reaction with the desired sulphonamide (6b) in the presence of a strong base such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Alternatively the amino acid can be treated with the desired sulphonamide (4b) in the presence of a base like diisopropyl

20   ethylamine followed by treatment with a coupling agent like PyBOP® to effect the

introduction of the sulphonamide group. Removal of the amino protecting group by standard methods and subsequent coupling to a P2 building block, prepared as described below, using standard methods for amide bond formation, like using a coupling agent such as O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) in the presence of a base such as diisopropylamine in a solvent like dimethylformamide, gives 4e. Alternatively, the sulphonamide group can be introduced at a later stage of the synthesis, for example as the last step. In this case an amino acid with the reversed protection pattern, i.e. with an unprotected amino function and protected acid function, is coupled the acid function of the P2 building block using standard peptide coupling conditions for example as described above. Removal of the acid protection group, using the appropriate conditions for the present protection group, followed by coupling of the sulphonamide as described above then yields compound 4e .

P1 building blocks for the preparation of compounds according to general formula VI wherein A is an ester or an amide can be prepared by reacting amino acid (4a) with the appropriate amine or alcohol respectively under standard conditions for amide or ester formation. Compounds according to general formula I wherein A is $CR^4R^{4'}$ can be prepared by coupling of the appropriate P1 building block to the P2 building block as described in Oscarsson et al Bioorg Med Chem 2003 11(13) 2955-2963 and PCT/EP03/10595 filed 23.09.2003, the contents of which are incorporated by reference.

Compounds comprising an azapeptide P1 residue, i.e. M is NRu in general formula VI can be prepared by using a suitable P1 aza-amino acyl moiety in the coupling to the P2 fragment. The preparation of aza-amino acyl moieties is described by M. D. Bailey et al. in J. Med. Chem., 47, (2004), 3788-3799, and an example is shown in scheme 5.

5a + (chloroformate) → **5b**

R1' is as defined for R1 but is not H

5c + H$_2$N—S(O)$_2$—R2 → TFA → Coupling of a P2 fragment → **5d**
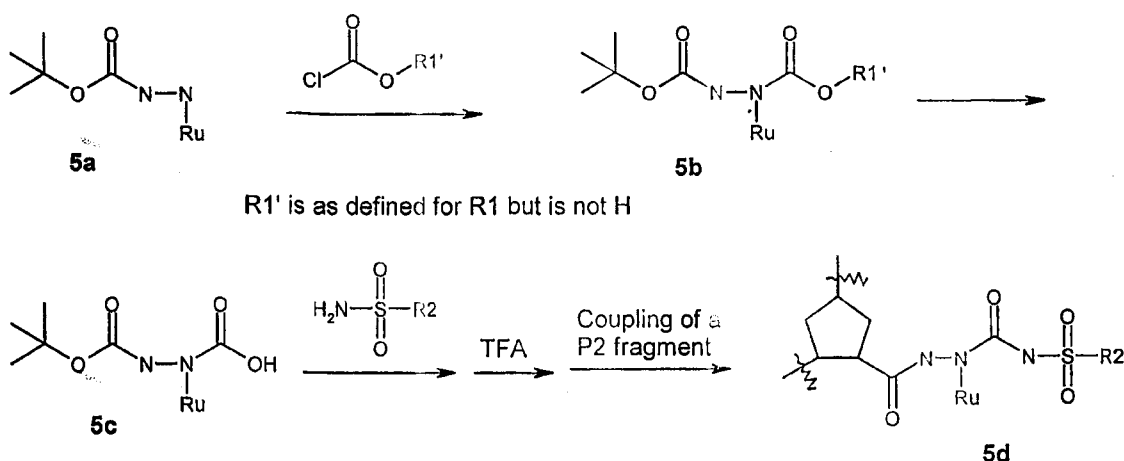
Scheme 5

Incorporation of the appropriate N-linked side chain, Ru, on commercially available tert-butylhydrazine can be performed for example by a reductive amination reaction

5   with the appropriate aldehyde or ketone as described in scheme 19 below which produces the N-alkylated carbazate (5a). Condensation of 5a with a desired chloroformate in the presence of a base like triethylamine or diisopropylethylamine in a solvent like THF provides 5b. The R1' moiety can then optionally be removed using the appropriate conditions depending on the specific R1', such as catalytic

10   hydrogenation for R1' being benzyl, which gives the corresponding acids. Subsequent reaction of the afforded acid with a desired sulphonamide derivative as described in scheme 4 yields sulphonamide capped building blocks. Alternatively, reaction of carbazate 5a with an isocyanate, R3-N=C=O, provides building blocks for the preparation of compounds according to general formula VI wherein M is NRu and A is

15   CONHR^3.

Synthesis of capped P3 and P4-P3 building blocks

The building blocks R^{16}-G-P3 and R^{16}-G-P4-P3 can be prepared as generally depicted in scheme 6.

43
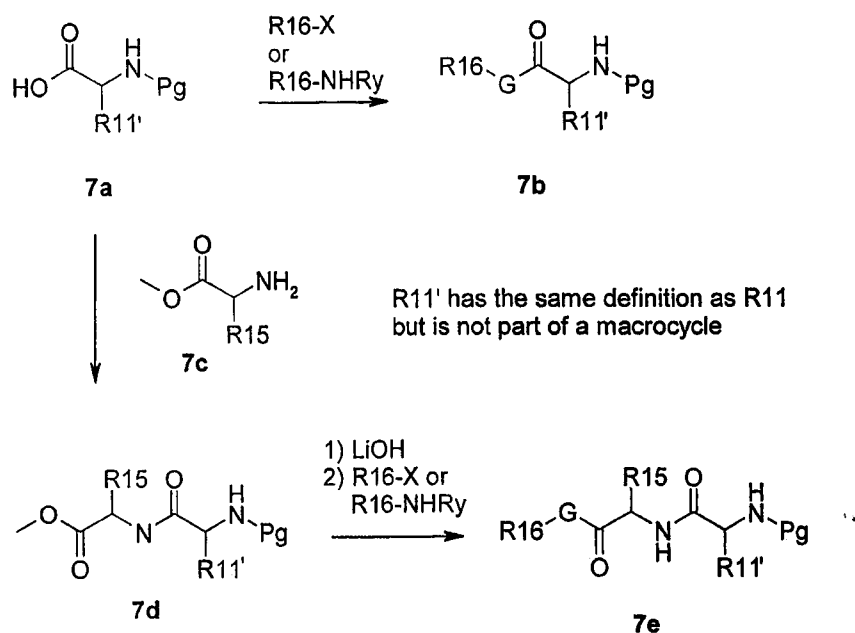
Scheme 6

A suitable N-protected amino acid (6a) can be coupled with an amino capping group
(R^{16}-NHRy) using standard peptide coupling conditions like with coupling agents such
5   as HATU, DCC, HOBt or the like in the presence of a base such as DIEA or DMAP in
a solvent like dichloromethane, chloroform or dimethylformamide or a mixture thereof
and ester formation conditions like providing amides i.e. G is NHRy (6b). Alternatively,
reaction of amino acid (6a) with a compound of general formula R^{16}-X where R^{16} is as
defined above and X is a leaving group such as a halide, in the presence of a base
10   like cesium carbonate or silver (I) oxide provides esters, i.e. G is O (6b). On the other
hand, amino acid (6a) can be coupled to a second, suitably O-protected, amino acid
(6d) using standard peptide coupling conditions as described above, providing (6e).
Displacement of the ester group with a suitable capping group (6b) provides fragment
(6f) useful for the preparation of compounds according to the present invention
15   wherein m and n are 1.

When G is N-Ry, the capped P3 or P2 building block can also be prepared on solid
support as exemplified in Scheme 7.
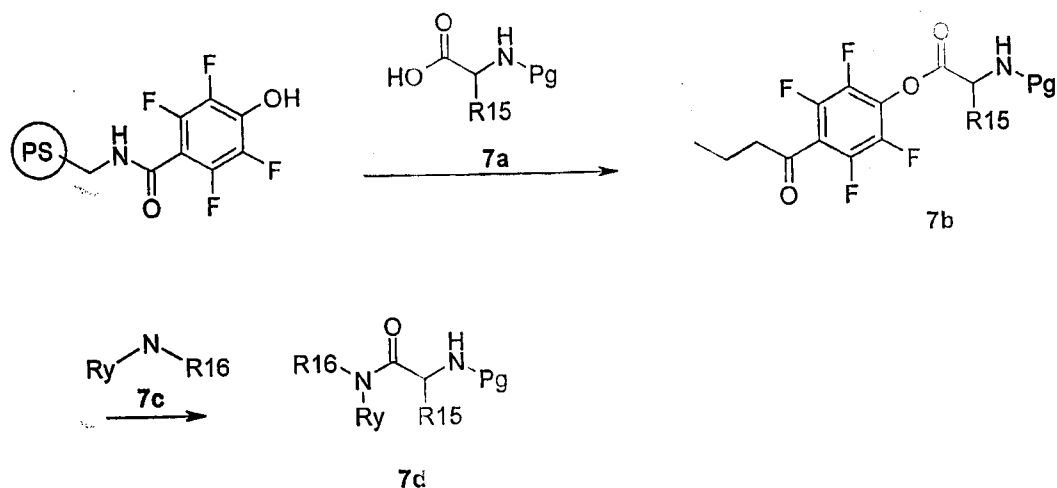
Scheme 7

An appropriate N-protected, for example Boc protected, amino acid (7a) can be
immobilized on a solid support, here exemplified by Agronaut resin PS-TFP, by

5   reacting the amino acid with the desired solid support in the presence of coupling
reagent like N,N'-diisopropylcarbodiimide and a base like DMAP in a solvent like
dichloromethane and dimethylformamide. The immobilized amino acid (7b) can then
be cleaved from the support with a suitable capping group (7c) thus giving fragments
(7d) useful for the preparation of compounds according to the present invention

10   wherein m or n is 1. Optionally the amino protecting group can be removed followed
by coupling of an appropriate amino acid using standard methods thus providing
fragments useful for the preparation of compounds according to the present invention
wherein m and n are 1.

15   Preparation and incorporation of P2 building blocks
A typical route to compounds containing a 5 membered saturated P2 scaffold is
shown in Scheme 8.
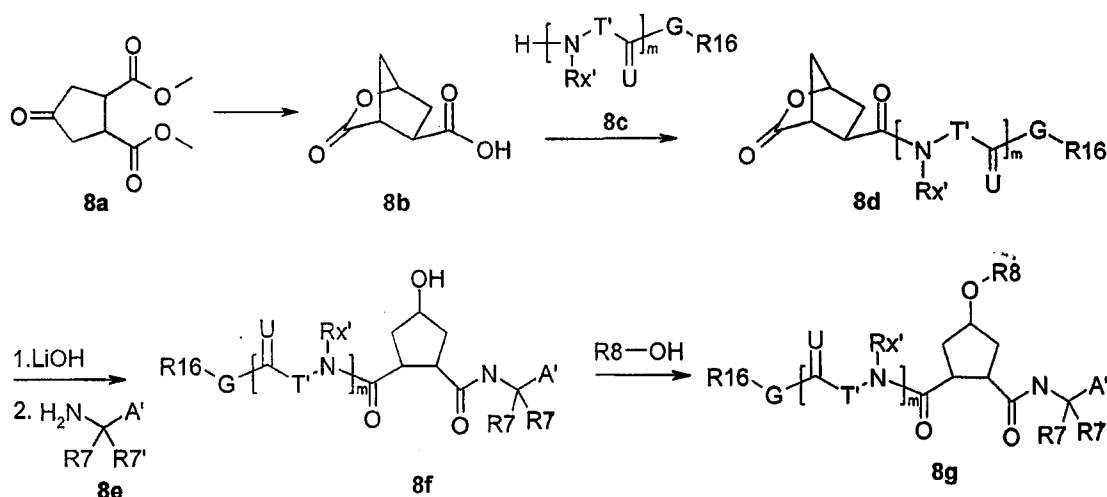
Rx' and T' have the same definitions as Rx and T respectively but are not part of a macrocycle.
A' is a protected carboxylic acid, substituted amide or sulphone amide or CR4R4'.

Scheme 8

The cyclic scaffold (8b) can be prepared, for example, from 3,4-
bis(methoxycarbonyl)cyclopentanone (8a), as described by Rosenquist et al. in Acta

5   Chem. Scand. 46 (1992) 1127-1129 by reduction of the keto group with a reduction
agent like sodium borohydride in a solvent like methanol followed by hydrolysis of the
esters and finally ring closure in acetic anhydride in the presence of pyridine. The
provided bicyclic acid (8b) can then be coupled to the amine function of the desired
P3 fragment (8c), P3-P4 fragment or capping group R^{16}-NHRy, using conventional

10   peptide coupling conditions like with HATU and diisopropyl amine in a solvent like
dimethyl formamide, giving (8d). Lactone opening of (8d) with for example lithium
hydroxide provides the acid which subsequently can be coupled to the amino group of
a P1 building block or a precursor of a desired P1 fragment (8e), using conventional
peptide coupling conditions. The R^8-substituent of the carbocycle can be introduced

15   for example by a Mitsunobu reaction with the appropriate alcohol as described above
or by any other suitable method previously described. When R^7, $R^{7'}$ and A' contains
functional groups, these are optionally suitably protected by methods recognized by
persons skilled in the art, se for example Bodanzky or Greene cited above.

Scheme 9 shows an alternative route towards compounds of formula VI containing a saturated P2 scaffold, where the building blocks are introduced in the reversed order, i.e. the P1 fragment is introduced before the capping group, P3 or P3-P4 building block.
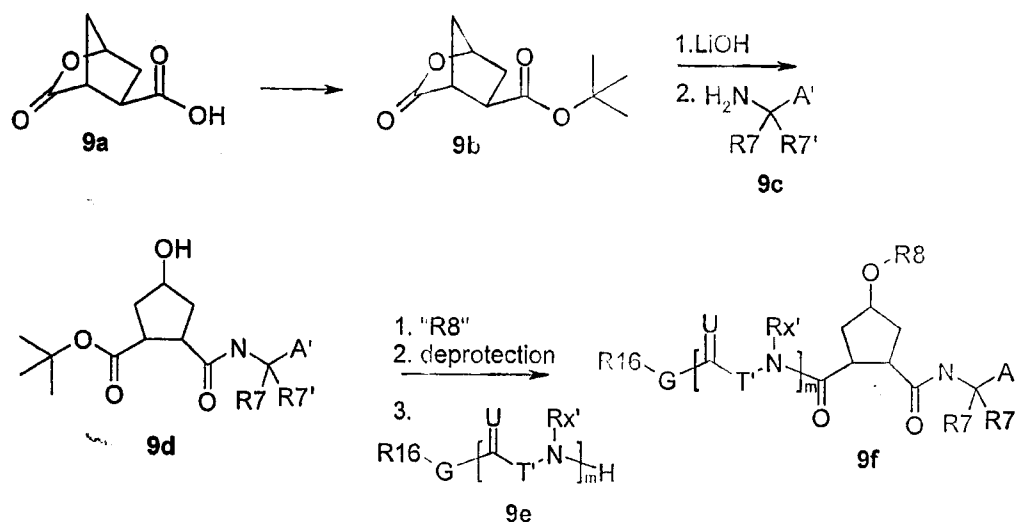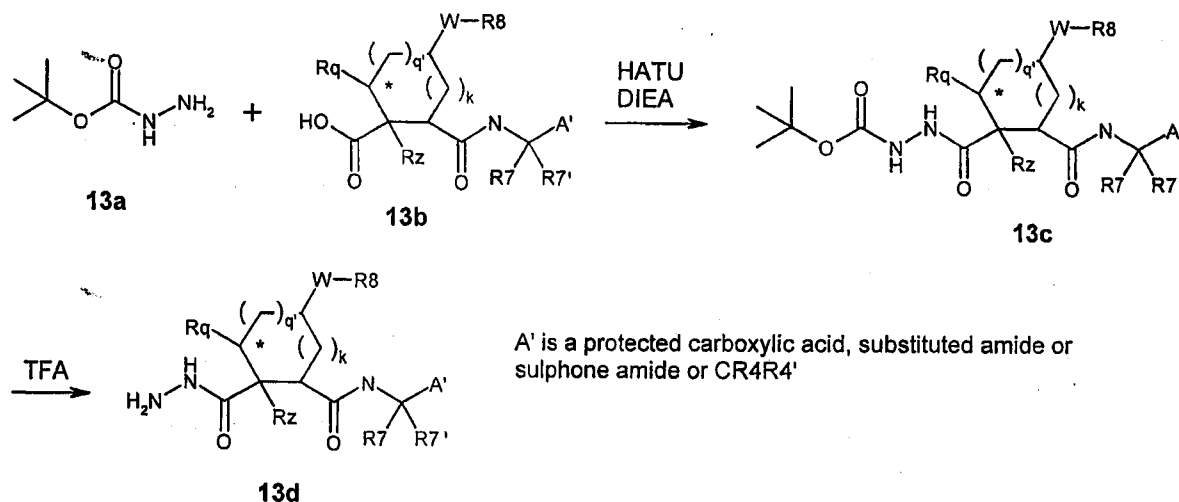

Rx' and T' have the same definitions as Rx and T respectively but are not part of a macrocycle.
A' is a protected carboxylic acid, substituted amide or sulphone amide or CR4R4'.

Scheme 9

Protection of the acid group of (9a) for example as the tert- butyl ester by treatment with di-tert- butyl dicarbonate in the presence of a base like dimethylaminopyridine and triethylamine in a solvent like dichloromethane provides ester (9b). Lactone opening and coupling of a P1 building block (9c) as described in scheme 13 or directly by the amine group of the P1 fragment provides (9d). Introduction of R^8- substituent as described above followed by removal of the acid protection group by subjecting the ester to acidic conditions like trifluoroacetic acid and triethylsilane in a solvent like methylene chloride and finally coupling of the P3 building block (9e), P3- P4 building block or capping group R^{16}-NHRv, as described above provides (9f). When R^7, $R^{7'}$ and A' contain functional groups these are optionally suitably protected by methods recognized by persons skilled in the art, see for example Bodanzky or Greene cited above.

An unsaturated P2 scaffold to be used in the preparation of compounds of formula VI
can be prepared as illustrated with cyclopentene below.

The cyclopentene scaffold is typically prepared as described in scheme 10.

15a   15b   15c
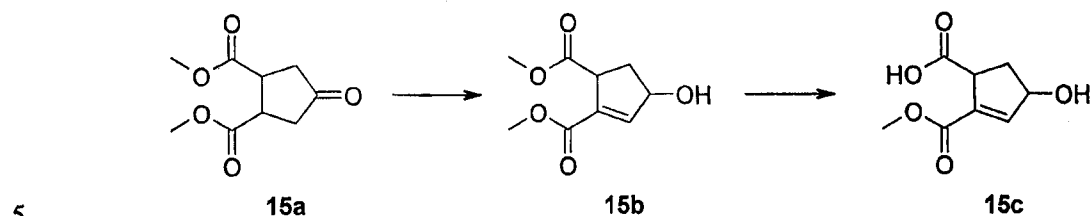
Scheme 10

A bromination-elemination reaction of 3,4-bis(methoxycarbonyl)cyclopentanone (10a)
as described by Dolby et al. in J. Org. Chem. 36 (1971) 1277-1285 followed by
reduction of the keto functionality with a reduction agent like sodium borohydride
provides the unsaturated hydroxy compound (10b). Selective ester hydrolysis using
for example lithium hydroxide in a solvent like a mixture of dioxane and water
provides hydroxy substituted monoester derivative (10c).

An unsaturated P2 building scaffold wherein Rq is other than hydrogen, such as a
methylated cyclopentene scaffold can be prepared as shown in scheme 11.

48
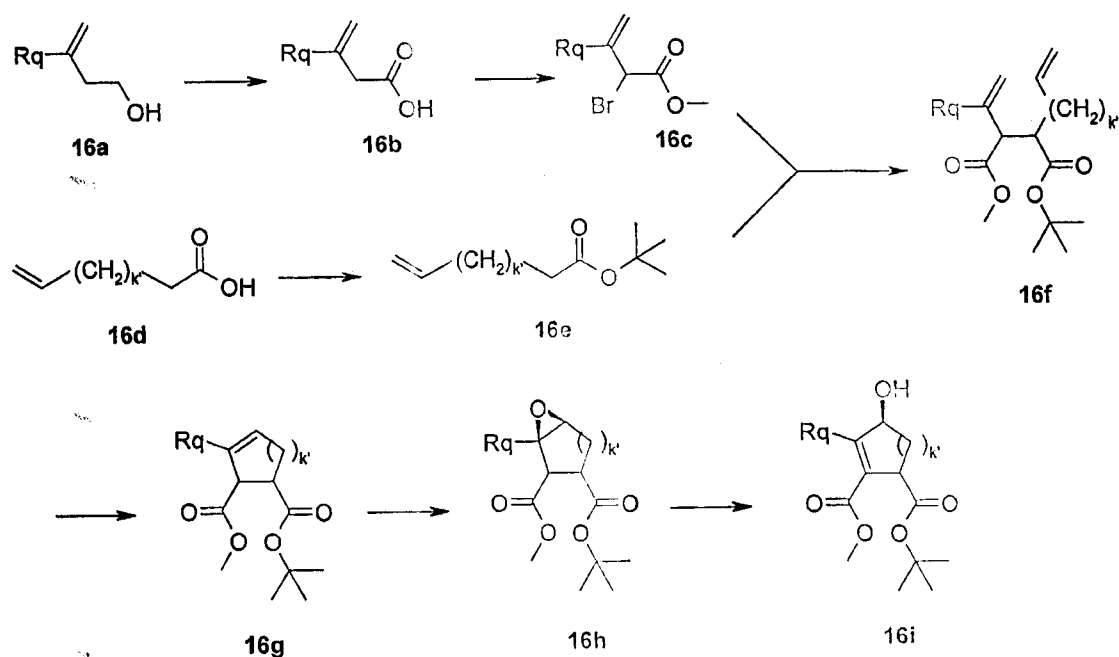
Scheme 11

Oxidation of commercially available 3-methyl-3-buten-1-ol (11a) by the use of an oxidation agent like pyridinium chlorochromate followed by treatment with acetyl

5   chloride, bromine and methanol provides the α-bromo ester (11c). The afforded ester (11c) can then be reacted with the enolate (11e), achieved for example by treatment of the corresponding tert-butyl ester with a base such as lithium diisopropyl amide in a solvent like tetrahydrofuran, to give the alkylated compound (11f). The tert-butyl ester (11e) can be prepared by treatment of the corresponding commercially available acid

10   (11d) where k' is 1 to 3 with di-tert-butyl dicarbonate in the presence of a base like dimethylaminopyridine. Cyclisation of (11f) by an olefin metathesis reaction performed as described above provides cyclopentene derivative (11g). Stereoselective epoxidation of (11g) can be carried out using the Jacobsen asymmetric epoxidation method to furnish the epoxide (11h). Finally, addition of a base like DBN (1,5-

15   diazabicyclo-[4.3.0]non-5-ene) yields the alcohol (11i). Optionally the double bond of compound (11i) can be reduced for example by catalytic hydrogenation using a catalyst like palladium on carbon which provides the corresponding saturated compound.

The afforded cyclic scaffolds can then be used, as described above, to complete the inhibitor sequence. An example is shown in scheme 12.
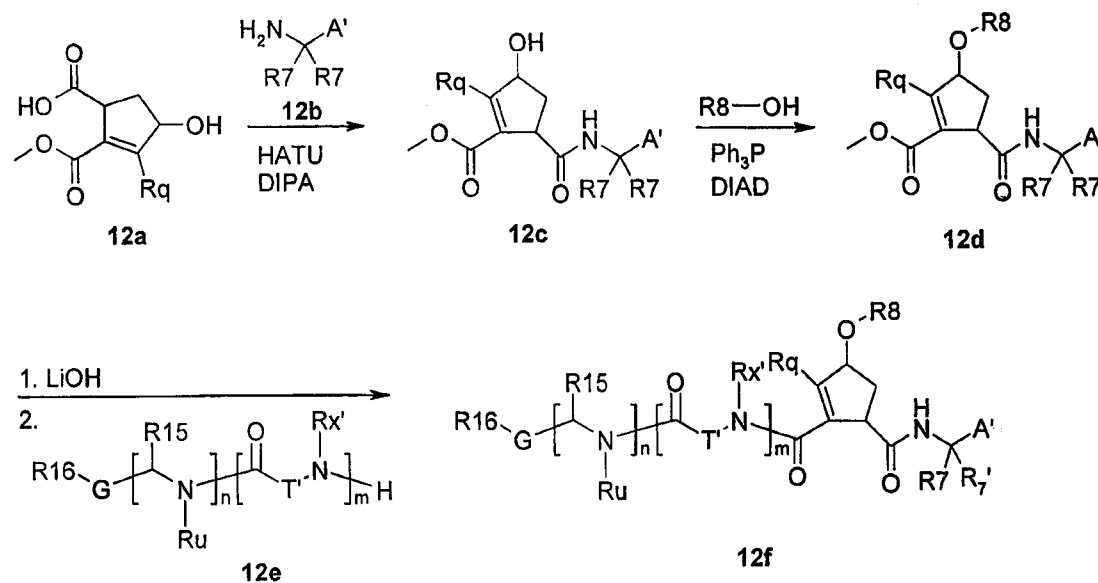
Rx' and T' have the same definitions as Rx and T respectively but are not part of a macrocycle.
A' is a protected carboxylic acid, substituted amide or sulphone amide or CR4R4'.

Scheme 12

5   The amino group of a P1-building block or a suitable precursor thereof (12b) can be coupled to the acid of the cyclopentene derivative (12a) using standard amide coupling conditions such as using HATU in the presence of a base like diisopropyl phenylamine or the like, followed by introduction of the R^8-substituent for example by Mitsunobu conditions as described above to provide (12d). Hydrolysis of the

10 remaining ester and subsequent amide coupling of a desired P3 or P3-P4 building block (12e) optionally followed by manipulations of the P1 part provides cyclopentene containing compounds (12f) according to general formula VI. When R^7, $R^{7'}$ and A' contain functional groups, these are optionally suitably protected by methods recognized by persons skilled in the art, see for example Bodanzky or Greene cited

15 above.

Compounds having a hydrazine containing capping group attached directly to the P2 moiety, i.e. P3 and P4 are absent and G is NRjNRj, can be prepared as depicted in Scheme 13

13a + **13b** → **13c**

A' is a protected carboxylic acid, substituted amide or sulphone amide or CR4R4'

13d

5   Scheme 13

Reaction of tert-butyl carbazate (13a), optionally alkyl substituted on one or both nitrgogens, with the acid (13b) under peptide coupling conditions like with HATU and DIEA in a solvent like DMF provides 9Ac . Optional removal of the boc group by standard procedures like acidic treatment with for example TFA in a suitable solvent

10 such as dichloromethane, provides the hydrazine containing derivative (13d). Alternatively, any appropriate hydrazine derivative, such as morpholin-1-ylamine, piperidin-1-ylamine or the like can be linked to the acid (13b) instead of the tert-butyl carbazate derivative.

15 The achieved compound can then be further extended by cuopling of a P3 or P4-P3 building block to the primary amine of compound 13d for example as shown in scheme 14.
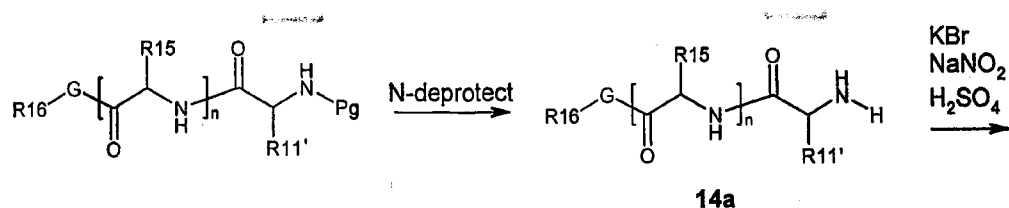
prepared as described in scheme 7 or 8
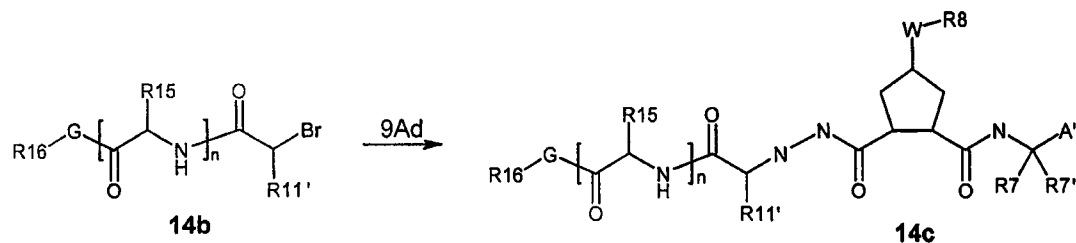
R11' has the same definition as R11 but is not part of a macrocycle.
A' is a protected carboxylic acid, substituted amide or sulphone amide or CR4R4'

Scheme 14

Treatment of the α-amino compound (14a) with sodium nitrite, potassium bromide and sulphuric acid (Yang *et al.* J. Org. Chem. (2001), 66, 7303-7312) provides the corresponding α-bromo compound (14b) which upon reaction with the above described derivative (13d) provides the hydrazine containing derivative (14c).

Compounds lacking a carboxy group in the P3 unit can be prepared as illustrated in Scheme 15 exemplified with a cyclopentane derivative as P2 scaffold. .
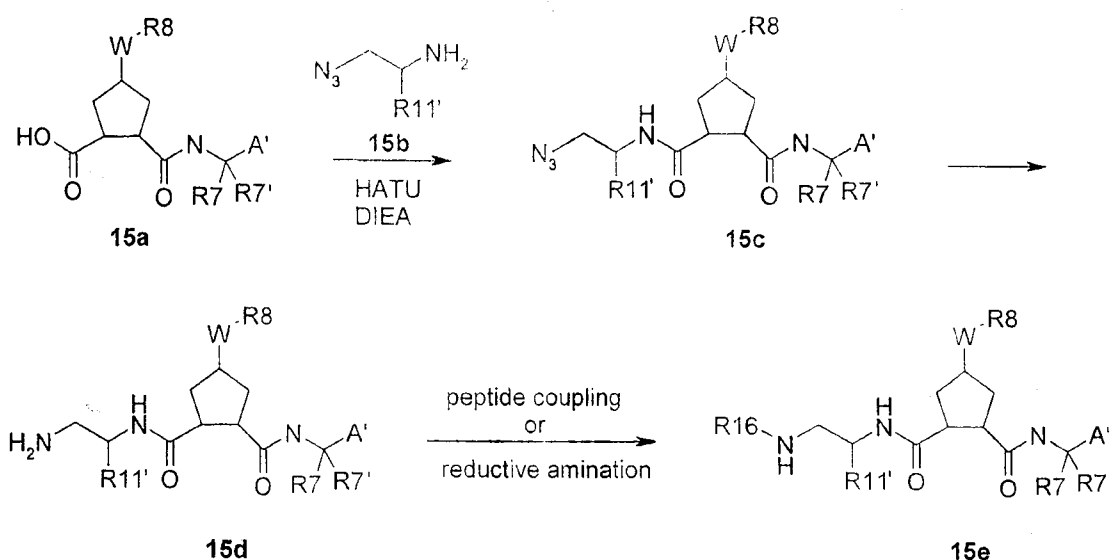
R11' has the same definition as R11but is not part of a macrocycle.
A' is a protected carboxylic acid, substituted amide or sulphone amide or CR4R4'.

Scheme 15

The acid (15a) can be coupled to an amino azide derivative (15b), prepared by
methods known from the literature using standard peptide coupling conditions to give

5   the amide derivative (15c). Reduction of the azide function for example by polymer
bound triphenyl phosphine in a solvent like methanol or any other suitable reduction
method provides intermediate (15d) which subsequently can be reacted with an acid
under peptide coupling conditions or with an amine in a reductive amination reaction
providing amides and secondary amines respectively.

10

Scheme 16 shows an alternative route towards compounds lacking a carboxy group
in the P3 unit
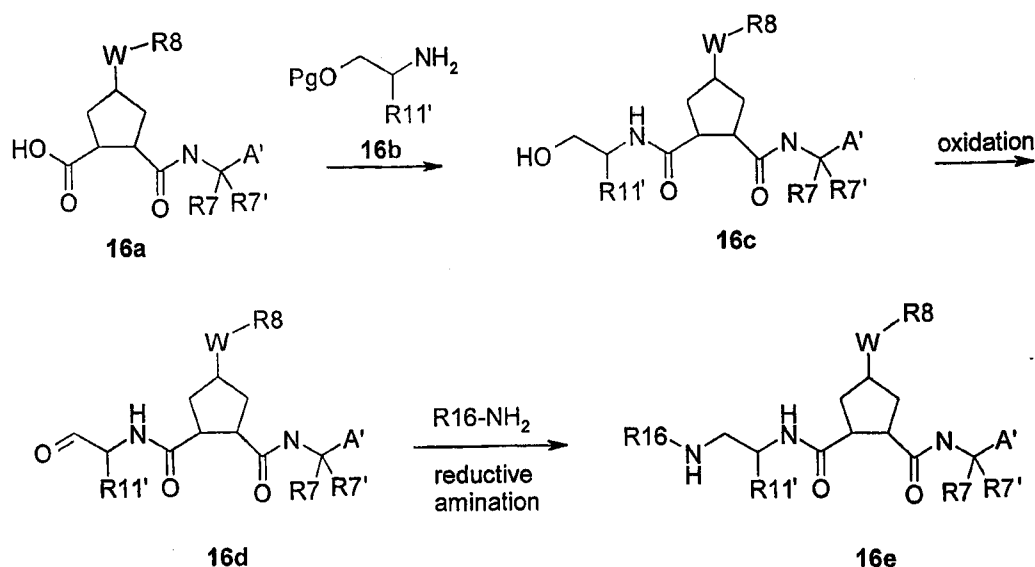
R11' has the same definition as R11 but is not part of a macrocycle.
A' is a protected carboxylic acid, substituted amide or sulphone amide or CR4R4'.

Scheme 16

Instead of using the azide derivative (15b) in scheme 15 the corresponding, optionally protected, hydroxy derivative (16b) can be used in the coupling with the acid (16a)

5　　and thus introducing a primary alcohol. The alcohol (16c) can then, after optional deprotection, be oxidized with a suitable oxidizing agent like for example Dess-Martin periodinane to form the corresponding aldehyde. Reaction of the aldehyde with a desired amine in a reductive amination reaction using a reagent like for example polystyrene bound cyanoborohydride in a solvent like THF provides amine derivatives

10　　(16e).

Alternatively alcohol (16c) can be reacted with a suitable acylating or alkylating agent under the appropriate conditions to provide ester and ether compounds respectively, i.e. G is O in general formula VI.

15

Subsequent reaction of the formed alcohol with a suitable acylating or alkylating agent using the appropriate conditions provides the ester and ether compounds respectively, i.e. G is O in general formula VI.

Although Scheme 15 and 16 have been described with reference to a cyclopentane derivative i.e. q' is 0 and k is 1 in Formula VI, it will be readily apparent that the corresponding methodology is applicable for other compounds of the Formula VI.

5   When R^7, $R^{7'}$ and A' contains functional groups, these are suitably protected by methods recognized by persons skilled in the art, see for example Bodanzky or Greene cited above.

Formation of macrocyclic compounds

10   Compounds according to the present invention wherein an alkylene chain extending from the $R^7/R^{7'}$ cycloalkyl to Rx, Rd or R^{11} thus forming a macrocycle, can be prepared as described below. Suitable P1, P2 and P3 building blocks, or precursors thereof, are coupled together using the strategies described above, followed by a ring-closing reaction (macrocyclization). The substituent $W-R^8$ of the P2 building block

15   can be incorporated via a Mitsunobu reaction as described above, before or after formation of the macrocycle or the assembly can be done with the required substituted proline analogue or carbocycle. For macrocyclic structures extending from the $R^7/R^{7'}$ cycloalkyl to R^{11}, P3 amino acids containing the appropriate side chain can be prepared as described in WO00/59929.

20

A typical route to macrocyclic compounds is shown in Scheme 17 which illustrates the technique applied to a compound having a spiro-cyclopropyl P1, where the macrocycle incorporates the P3 side chain.

17   +   **17b**   →   **17c**

→   **17d**   n = 1, 2, 3, 4, 5, 6, 7
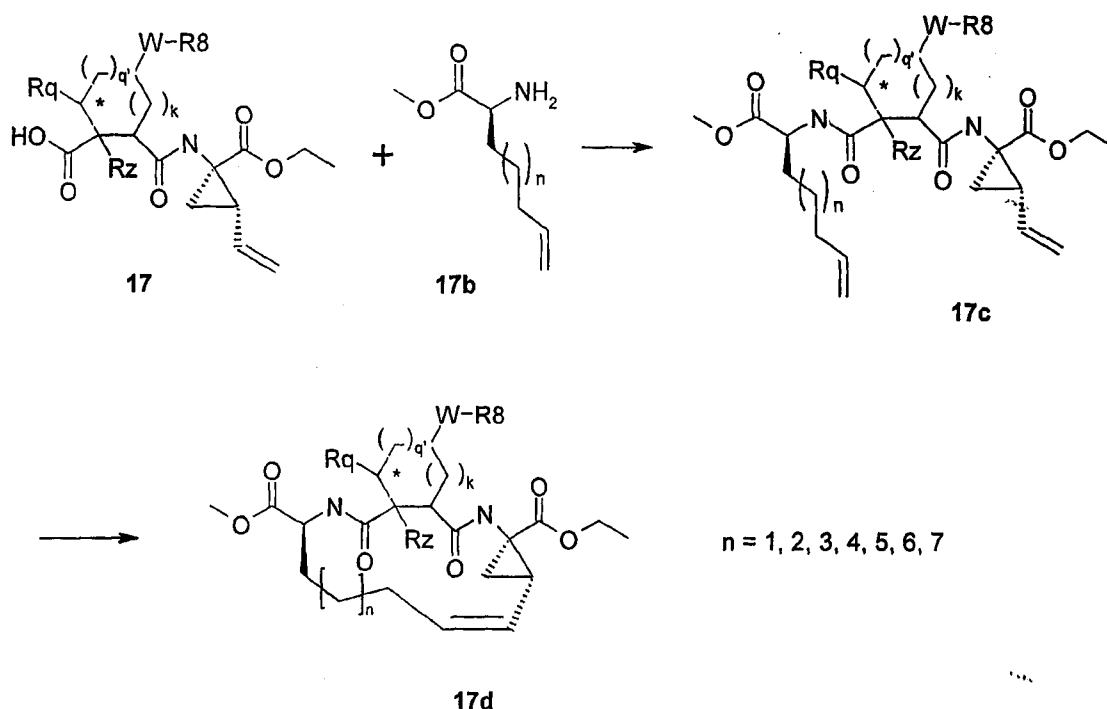
Scheme 17

Coupling of acid derivative (17a) with the appropriate, acid protected, amino acid

5   (17b) using standard peptide coupling conditions as described above provides (17c).
Formation of the macrocycle can then be carried out via an olefin metathesis reaction
using a Ru-based catalyst such as the one reported by Miller, S.J., Blackwell, H.E.;
Grubbs, R.H. J. Am. Chem. Soc. 118, (1996), 9606-9614, Kingsbury, J. S., Harrity, J.
P. A., Bonitatebus, P. J., Hoveyda, A. H., J. Am. Chem. Soc. 121, (1999), 791-799

10   and Huang et al., J. Am. Chem. Soc. 121, (1999), 2674-2678. It will also be
recognized that catalysts containing other transition metals such as Mo can be used
for this reaction. Optionally the double bond is reduced and/or the ethyl ester is
hydrolysed by standard hydrogenation and/or hydrolysation methods respectively well
known in the art. Alternatively the methyl ester can be selectively hydrolysed followed

15   by coupling of a R^{16}-G-P4 building block by standard peptide coupling conditions. The
macrocyclisation step described in Scheme 17 can also be applied to the
corresponding carbocyclic analogues described above. When the linker contains a

56

nitrogen atom the ring closure can be carried out by reductive amination as described in WO00/59929.

Macrocyclic compounds without the cyclopropyl moiety in the P1 part, i.e the
5   macrocyclic ring extends directly from the peptidic backbone at the carbon adjacent R⁷, can be prepared using the methods described herein. An example wherein a 5 membered cycloalkyl derivative is used as the P2 scaffold is shown in scheme 18.

A' is a protected carboxylic acid, substituted amide or sulfon amide.
n is 1, 2, 3, 4 or 5
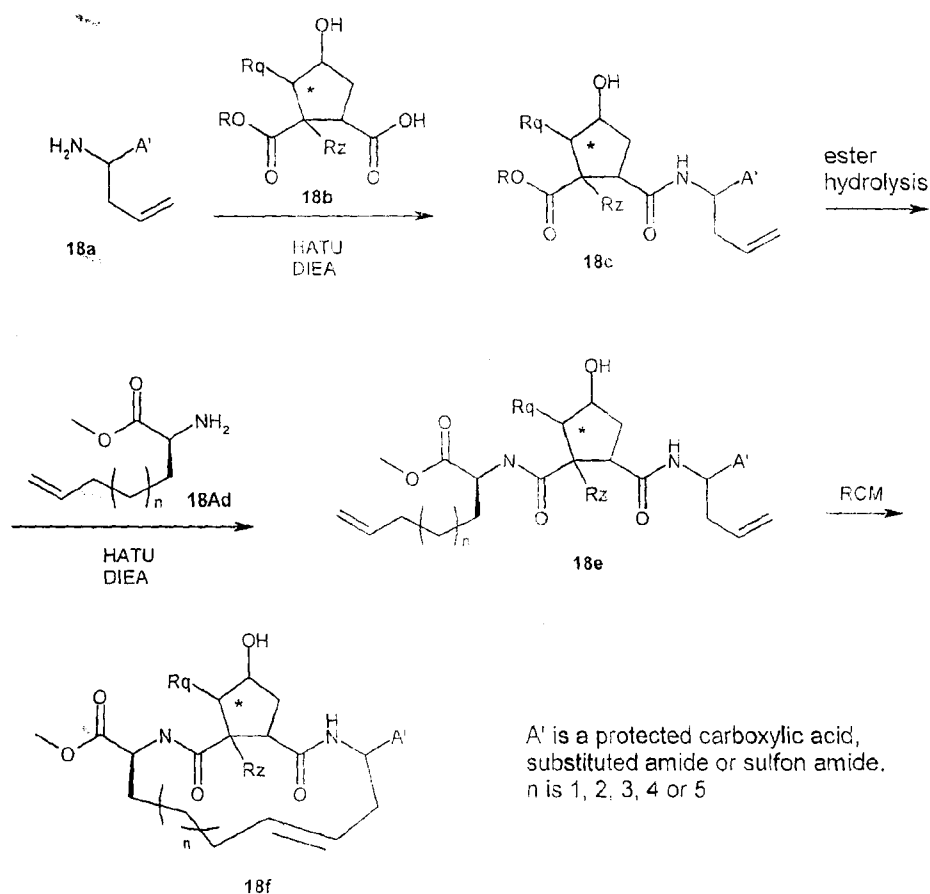
Scheme 18

10 Coupling of a suitable allylglycine derivative (18a), to the acid function of the P2 scaffold (18b) using standard peptide coupling conditions yields the amide derivative (18c). Hydrolysis of the ester group followed by a peptide coupling reaction with the olefin substituted amino acid (18Ad) provides the amide compound (18e). A ring

57

closing metathesis reaction is then effected by using for example Hoveyda-Grubbs catalyst which gives the macrocyclic compound (18f).

Even though scheme 18 shows the synthetic sequence using a P2 scaffold with an unsubstituted hydroxy group, it will be apparent that the R8 substituent can be

5   introduced at any convenient stage of the synthesis for example as described in scheme 9 and 10 or it can be introduced after the metathesis reaction, i.e. on compound 18f, using any of the methods described herein.

Building blocks to be used in the preparation of compounds wherein the macrocycle

10   extends from the amide nitrogen in the P3 fragment i.e Rx is J in general formula VI, or in the preparation of compounds wherein the P3 and P4 fragments are absent, i.e. m and n are 0 and G is NRj in general formula VI, can typically be prepared as outlined in scheme 18B.

18Ba   DIAD
              Ph₃P   18Bb   n is 1, 2, 3, 4, 5, 6, 7, 8   18Bc
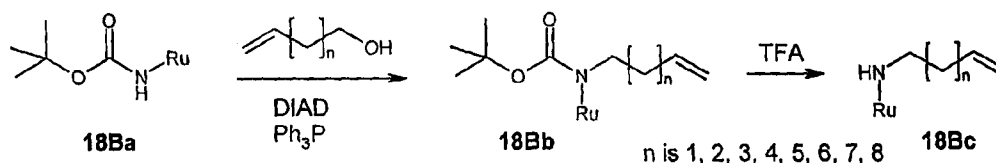
15   Scheme 18B

Carbamate 18Ba, which is commercially available or is readily prepared for instance by reaction of the desired alkyl amine with di-tert-butyl dicarbonate, can be reacted with an appropriate ω–unsaturated alcohol under Mitsunobu conditions to provide the alkylated carbamate (18Bb). Subjection of 18Bb to acidic conditions like for example

20   treatment with trifluoroacetic acid in a solvent like dichloromethane gives the free amine (18Bc) which can be linked to a P2 fragment using any of the previously described strategies.

Macrocyclic structures containing a hydrazine group i.e. T is NRd or m and n are 0

25   and G is NRjNRj, in general formula VI, can be prepared by linking a suitably N-alkylated carbazate derivative to the P2 fragment. Alkylated carbazate derivatives can be prepared, for example, as described in Scheme 19.
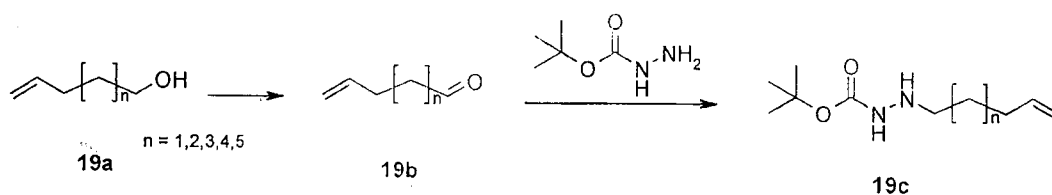
Scheme 19

Oxidation of the appropriate alcohol (19a) effected by a suitable oxidation method like
for example with N-methyl morpholine oxide and tetrapropylammonium perruthenate
5   in a solvent like dichloromethane provides aldehyde (19b). Reductive alkylation of
tert-butyl carbazate with the afforded aldehyde gives the desired N-alkylated building
block (19c). Alternatively, any desired hydrazine derivative such as morpholin-1-
ylamine, piperidin-1-ylamine or the like can be used instead of tert-butyl carbazate in
the reaction with aldehyde 19b.

10

Scheme 20 illustrates synthetic sequences to building blocks suitable for the
preparation of compounds wherein the "outer" nitrogen of the hydrazine group is
alkylated, either with an ω-unsaturated alkyl chain appropriate for subsequent
macrocycle formation or with any other suitable alkyl group.
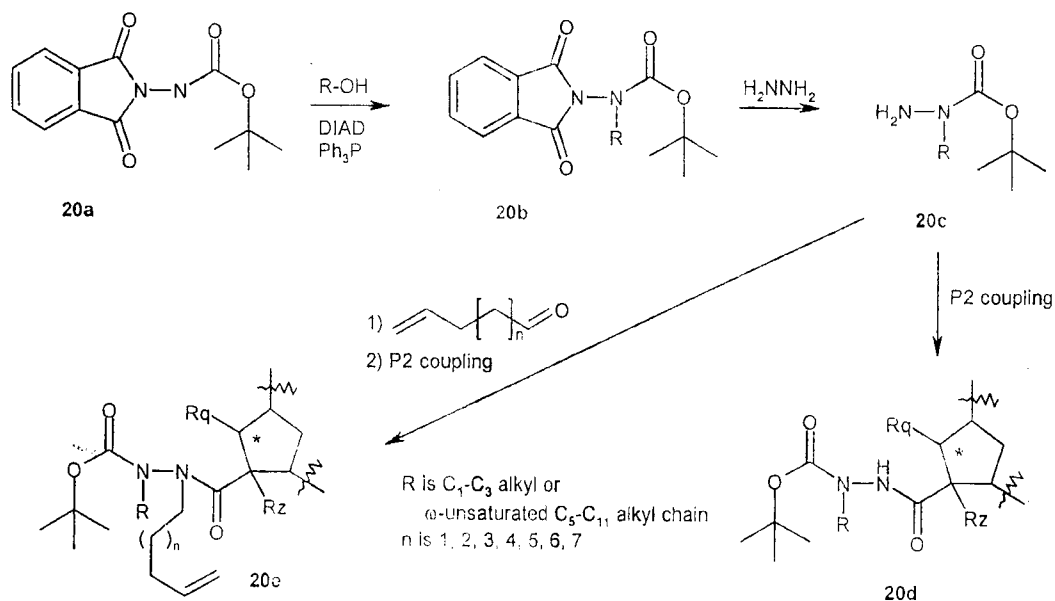
15

Scheme 20

Reaction of a suitably protected hydrazine derivative, for example (1,3-dioxo-1,3-dihydro-isonidol-2-yl)-carbamic acid tert-butyl ester (20a), which can easily be prepared by a person skilled in the art, with a desired alcohol, R-OH, under Mitsunobu conditions provides N-alkylated hydrazine compound (20b). Removal of

5   the phtalimido group effected by treatment with hydrazine or a derivative thereof like hydrazine hydrate or hydrazine acetate provides the carbazate (20c). The afforded primary amine can then either be be coupled to any desired P2 fragment using any of the methods previously described to give 20d or alternatively it can be further alkylated using for example the reductive amination method described in scheme 19

10   followed by coupling to a P2 fragment as previously described to give 20e .

Scheme 21 exemplifies the coupling of a hydrazine containing P3 building block to a cyclopentane scaffold followed by macrocyclisation.
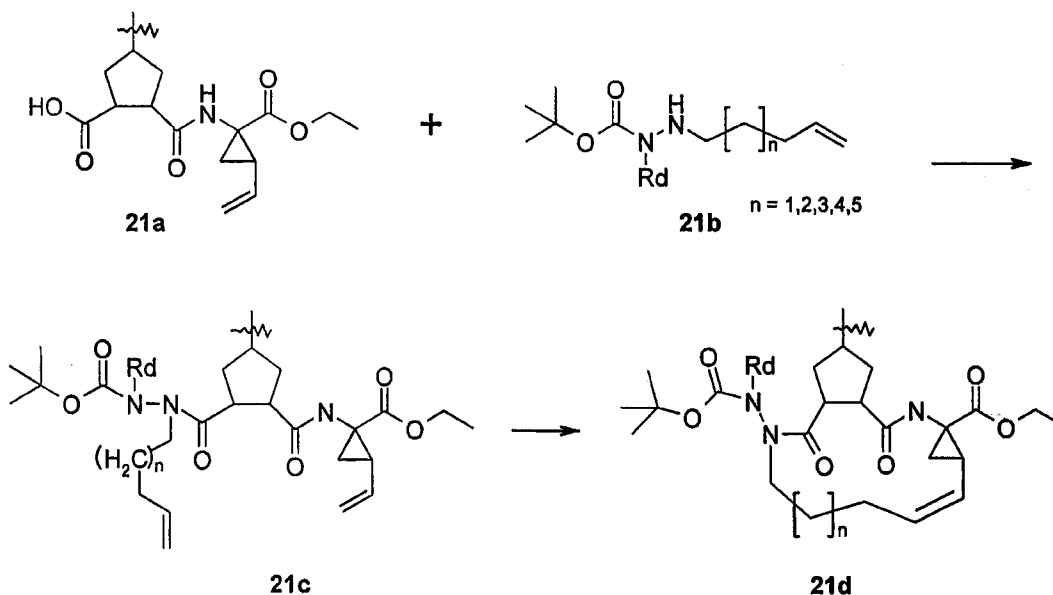
15   Scheme 21

Coupling of the carbazate derivative (21b) to the P2-P1 building block (21a) using standard peptide coupling conditions provides intermediate (21c). Ring closure of (21c) by an olefin metathesis reaction as described in scheme 18 gives the macrocyclic compound (21d).

20

The term "N-protecting group" or "N-protected" as used herein refers to those groups intended to protect the N-terminus of an amino acid or peptide or to protect an amino group against undesirable reactions during synthetic procedures. Commonly used N-protecting groups are disclosed in Greene, "Protective Groups in Organic Synthesis"

5   (John Wiley & Sons, New York, 1981), which is hereby incorporated by reference. N-protecting groups include acyl groups such as formyl, acetyl, propionyl, pivaloyl, t-butylacetyl, 2-chloroacetyl, 2-bromoacetyl, trifluoroacetyl, trichloroacetyl, phthalyl, o-nitrophenoxyacetyl, α-chlorobutyryl, benzoyl, 4-chlorobenzoyl, 4-bromobenzoyl, 4-nitrobenzoyl, and the like; sulfonyl groups such as benzenesulfonyl, p-toluenesulfonyl,

10   and the like, carbamate forming groups such as benzyloxycarbonyl, p-chlorobenzyl-oxycarbonyl, p-methoxybenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, 2-nitrobenzyl-oxycarbonyl, p-bromobenzyloxycarbonyl, 3,4-dimethoxybenzyloxy-carbonyl, 4-methoxybenzyloxycarbonyl, 2-nitro-4,5-dimethoxybenzyloxycarbonyl, 3,4,5-trimethoxybenzyloxycarbonyl, 1-(p-biphenylyl)-1-methylethoxycarbonyl,

15   α,α-dimethyl-3,5-dimethoxybenzyloxycarbonyl, benzhydryloxycarbonyl, t-butoxycarbonyl, diisopropylmethoxycarbonyl, isopropyloxycarbonyl, ethoxycarbonyl, methoxycarbonyl, allyloxycarbonyl, 2,2,2-trichloroethoxycarbonyl, phenoxycarbonyl, 4-nitrophenoxycarbonyl, fluorenyl-9-methoxycarbonyl, cyclopentyloxycarbonyl, adamantyloxycarbonyl, cyclohexyloxycarbonyl, phenylthiocarbonyl, and the like; alkyl

20   gropus such as benzyl, triphenylmethyl, benzyloxymethyl and the like; and silyl groups such as trimethylsilyl and the like. Favoured N-protecting groups include Fmoc, formyl, acetyl, benzoyl, pivaloyl, t-butylacetyl, phenylsulfonyl, benzyl, t-butoxycarbonyl (BOC) and benzyloxycarbonyl (Cbz).

25   Hydroxy protecting group as used herein refers to a substituent which protects hydroxyl groups against undesirable reactions during synthetic procedures such as those O-protecting groups disclosed in Greene, "Protective Groups In Organic Synthesis," (John Wiley & Sons, New York (1981)). Hydroxy protecting groups comprise substituted methyl ethers, for example, methoxymethyl, benzyloxymethyl, 2-

30   methoxyethoxymethyl, 2-(trimethylsilyl)ethoxymethyl, t-butyl and other lower alkyl

ethers, such as isopropyl, ethyl and especially methyl, benzyl and triphenylmethyl; tetrahydropyranyl ethers; substituted ethyl ethers, for example, 2,2,2-trichloroethyl; silyl ethers, for example, trimethylsilyl, t-butyldimethylsilyl and t-butyldiphenylsilyl; and esters prepared by reacting the hydroxyl group with a carboxylic acid, for example,

5   acetate, propionate, benzoate and the like.

In treating conditions caused by flavivirus such as HCV, the compounds of formula VI are typically administered in an amount to achieve a plasma level of around 100 to 5000 nM, such as 300 to 2000 nM. This corresponds to a dosage rate, depending on

10   the bioavailability of the formulation, of the order 0.01 to 10 mg/kg/day, preferably 0.1 to 2 mg/kg/day. A typical dosage rate for a normal adult will be around 0.05 to 5 g per day, preferably 0.1 to 2 g such as 500-750 mg, in one to four dosage units per day. As with all pharmaceuticals, dosage rates will vary with the size and metabolic condition of the patient as well as the severity of the infection and may need to be

15   adjusted for concomitant medications.

As is good prescribing practice with antiviral therapy, the compounds of formula I are typically coadministered with other HCV therapies to avoid the generation of drug escape mutants. Examples of such additional HCV antiviral therapies include

20   ribavirin, interferons, including pegylated interferons. Additionally a number of nucleoside analogues and protease inhibitors are in clinical or preclinical development and will be amenable to co-administration with the compounds of the invention.

25   Accordingly a further aspect of the invention provides a composition comprising a compound of formula I and at least one further HCV antiviral in a common dosage unit, such as any of the dosage forms described below, but especially an orally administered tablet, or capsule or a liquid suspension or solution for oral or injection use. A further aspect of the invention provides a method for the treatment or

30   prophylaxis of flavivirus infection, such as HCV, comprising the sequential or simultaneous administration of a compound of formula I and at least one further HCV

antiviral. A related aspect of the invention provides a patient pack comprising a first pharmaceutical composition, preferably in unit dosage form, of the compound of formula I and a second pharmaceutical composition of a second HCV antiviral, typically also in unit dosage form and generally in a separate container within the

5   patient pack. A patient pack will conveniently also be provided with instructions printed on the package or a container therein, or on a package insert, for the simultaneous or sequential administration of the respective pharmaceutical compositions.

10   Many HCV patients are co-infected, or prone to superinfection, with other infectious diseases. Accordingly, a further aspect of the invention provides combination therapies comprising the compound of the invention co-formulated in the same dosage unit or co-packaged with at least one further anti-infective pharmaceutical. The compound of the invention and the at least one further antinfective are

15   administered simultaneously or sequentially, typically at doses corresponding to the monotherapy dose for the agent concerned. However, certain antifectives can induce a synergistic response, allowing one or both of the active ingredients to be administered at a lower dose that the corresponding monotherapy. For example in drugs prone to rapid metabolism by Cyp3A4, co-dosing with the HIV protease

20   inhibitor ritonavir can allow lower dosage regimes to be administered.

Typical coinfections or superinfections with HCV include hepatitis B virus or HIV. Accordingly the compound of the invention is advantageously co-administered (either in the same dosage unit, co-packaged or separately prescibed dosage unit) with at

25   least one HIV antiviral and/or at least one HBV antiviral.

Representative HIV antivirals include NRTI such as alovudine (FLT), zudovudine (AZT, ZDV), stavudine (d4T, Zerit), zalcitabine (ddC), didanosine (ddl, Videx), abacavir, (ABC, Ziagen), lamivudine (3TC, Epivir), emtricitabine (FTC, Emtriva),

30   racevir (racemic FTC), adefovir (ADV), entacavir (BMS 200475), alovudine (FLT), tenofovir disoproxil fumarate (TNF, Viread), amdoxavir (DAPD), D-d4FC (DPC-817), -

dOTC (Shire SPD754), elvucitabine (Achillion ACH-126443), BCH 10681 (Shire)
SPD-756, racivir, D-FDOC, GS7340, INK-20 (thioether phospholipid AZT, Kucera),
2'3'-dideoxy-3'-fluoroguanosine (FLG) & its prodrgus such as MIV-210, reverset (RVT,
D-D4FC, Pharmasset DPC-817) .

5

Representative NNRTI include delavirdine (Rescriptor), efavirenz (DMP-266,
Sustiva), nevirapine (BIRG-587, Viramune), (+)calanolide A and B (Advanced Life
Sciences), capravirine (AG1549f S-1153; Pfizer), GW-695634 (GW-8248; GSK), MIV-
150 (Medivir), MV026048 (R-1495; Medivir AB/Roche), NV-05 2 2 (Idenix Pharm.), R-
10   278474 (Johnson & Johnson), RS-1588 (Idenix Pharm.), TMC-120/125 (Johnson &
Johnson), TMC-125 (R-165335; Johnson & Johnson), UC-781 (Biosyn Inc.) and
YM215389 (Yamanoushi).

Representative HIV protease inhibitors include PA-457 (Panacos), KPC-2 (Kucera
15   Pharm.), 5 HGTV-43 (Enzo Biochem), amprenavir (VX-478, Agenerase), atazanavir
(Reyataz), indinavir sulfate (MK-639, Crixivan), Lexiva (fosamprenavir calcium, GW -
433908 or 908, VX-175), ritonavir (Norvir), lopinavir + ritonavir (ABT-378, Kaletra),
tipranavir, nelfinavir mesylate (Viracept), saquinavir (Invirase, Fortovase), AG1776
(JE-2147, KNI-764; Nippon Mining Holdings), AG-1859 (Pfizer), DPC-681/684 (BMS),
20   GS224338; Gilead Sciences), KNI-272 (Nippon Mining Holdings), Nar-DG-35
(Narhex), P(PL)-100 (P-1946; Procyon Biopharma), P-1946 (Procyon Biopharma), R-
944 (Hoffmann-LaRoche), RO-0334649 (Hoffmann-LaRoche), TMC-114 (Johnson &
Johnson), VX-385 (GW640385; GSK/Vertex), VX-478 (Vertex/GSK).

25   Other HIV antivirals include entry inhibitors, including fusion inhibitors, inhibitors of the
CD4 receptor, inhibitors of the CCR5 co-receptor and inhibitors of the CXCR4
coreceptor, or a pharmaceutically acceptable salt or prodrug thereof. Examples of
entry inhibitors are AMD-070 (AMD11070; AnorMed), BlockAide/CR (ADVENTRX
Pharm.), BMS 806 (BMS-378806; BMS), Enfurvirtide (T-20, R698, Fuzeon),
30   KRH1636 (Kureha Pharmaceuticals), ONO-4128 (GW-873140, AK-602, E-913; ONO
Pharmaceuticals), Pro-140 (Progenics Pharm), PRO542 (Progenics Pharm.), SCH-D

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(SCH-417690; Schering-Plough), T-1249 (R724; Roche/Trimeris), TAK-220 (Takeda Chem. Ind.), TNX-355 (Tanox) and UK-427,857 (Pfizer). Examples of integrase inhibitors are L-870810 (Merck & Co.), c-2507 (Merck & Co.) and S(RSC)-1838 (shionogi/GSK).

5

Examples of HBV antivirals include adefovir dipivoxil (Hepsera), and especially lamivudine and 2'3'-dideoxy-3'-fluoroguanosine (FLG) & its prodrugs such as MIV-210, the 5'-O-valyl-L-lactyl prodrug of FLG. These latter HBV antivirals are particularly convenient as they are also active against HIV.

10

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 or excipients and optionally other therapeutic ingredients. The carrier(s) must be

15   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

20   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.

25   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

30   bringing a compound of Formula VI 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
5   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.

10   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
15   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, stearic acid,
glycerol stearate, 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.
20   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
25   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
30   provide slow or controlled release of the active agent.

66

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.

5

The compounds of formula VI can form salts which form an additional aspect of the invention. Appropriate pharmaceutically acceptable salts of the compounds of formula I include salts of organic acids, especially carboxylic acids, including but not limited to acetate, trifluoroacetate, lactate, gluconate, citrate, tartrate, maleate, malate,

10   pantothenate, isethionate, adipate, alginate, aspartate, benzoate, butyrate, digluconate, cyclopentanate, glucoheptanate, glycerophosphate, oxalate, heptanoate, hexanoate, fumarate, nicotinate, palmoate, pectinate, 3-phenylpropionate, picrate, pivalate, proprionate, tartrate, lactobionate, pivolate, camphorate, undecanoate and succinate, organic sulphonic acids such as methanesulphonate, ethanesulphonate,

15   2-hydroxyethane sulphonate, camphorsulphonate, 2-napthalenesulphonate, benzenesulphonate, p-chlorobenzenesulphonate and p-toluenesulphonate; and inorganic acids such as hydrochloride, hydrobromide, hydroiodide, sulphate, bisulphate, hemisulphate, thiocyanate, persulphate, phosphoric and sulphonic acids. The invention further extends to salts of the compounds of formula I which may or

20   may not be pharmaceutically acceptable, but which are useful as synthetic intermediates, the salt moiety being displaced or replaced as necessary.

The invention includes prodrugs of the compounds of formula I. Prodrugs of the compounds of formula VI are those compounds which following administration to a

25   patient release a compound of the formula VI in vivo generally following hydrolysis in the gut, liver or plasma. Typical prodrugs are pharmaceutically acceptable ethers and especially esters (including phosphate esters) of hydroxy functions, pharmaceutically acceptable amides or carbamates of amine functions or pharmaceutically acceptable esters of carboxy functions. Preferred pharmaceutically acceptable esters include

30   alkyl esters, including acetyl, ethanoyl, butyryl, t-butyryl, stearyl and pivaloyl, phosphate esters and sulphonic esters (ie those derived from RSO_2OH, where R is

lower alkyl or aryl). Pharmaceutically acceptable esters include lower alkyl ethers and the ethers disclosed in WO00/47561, especially methoxyaminoacyl and ethoxyaminoacyl.

5   The compounds of the invention have various steric centres and the invention extends to racemates and enantiomers at each of these steric centres.

Typically, the stereochemistry of the groups corresponding to the P3 and P4 side chains (ie R^{15} and/or R^{11}) will correspond to an L-amino acid configuration, although

10   the invention also extends to D-isomers at one or both of these centres. It is noteworthy that the L configuration is active notwithstanding that the nature of the E moiety means that P3 and P4 are typically translated one atom relative to a conventional polypeptide and the fact that reversal of a peptide residue, as envisaged for P3 and P4 then pitches the amine acid side chain to the opposite side compared

15   to a conventional peptide substrate.

The stereochemistry of the backbone component of the cyclic P2 group (i.e. spanning the carbonyl of the P1 amide bond and the carbonyl extending of P3 will typically correspond to L-proline. The stereochemistry of the P2 ring atom to which W is

20   bonded is typically as shown:
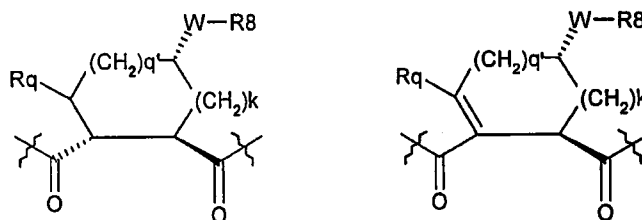
In compounds of the invention wherein R^7 and $R^{7'}$ together define a spiroalkyl group, such a spiro-cycloalkyl will typically comprise an R^{7a} substituent on the spiro-cyclopropyl ring which is is orientated syn to A:
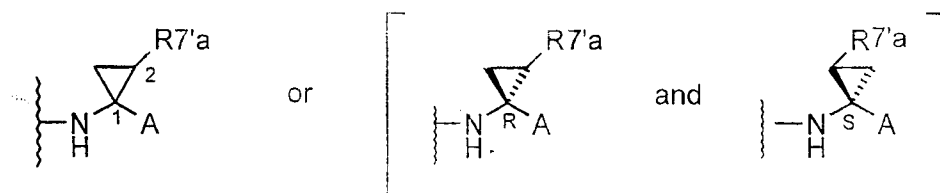
or anti to A:
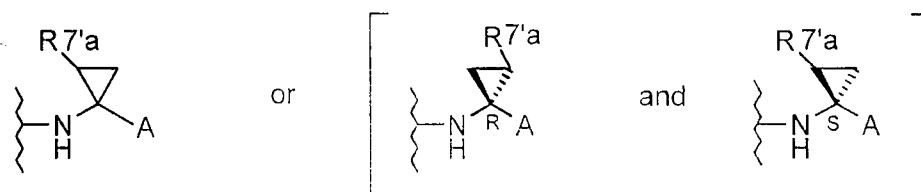
Conveniently, the spiro carbon of such a spiro-cyclopropyl ring has the R
configuration:
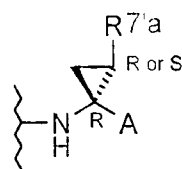
Conveniently an $R^{7'a}$ substituent on a spiro-cyclopropyl ring adjacent to A is in a syn
orientation in the following absolute configuration:
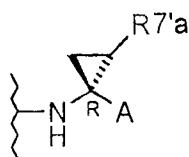
Particularly preferred variants have R^{7a} include ethyl, hence the asymmetric carbon
atoms at position 1 and 2 have the R, R configuration. Alternative preferred $R^{7'a}$
include vinyl, hence the asymmetric carbon atoms at position 1 and 2 have the R, S
configuration.

Where the compound of the invention is a macrocycle comprising a J group, J is
preferably a diastereomer represented by partial structures (i) or (ii):

J syn to the amide (i)   J syn to A (ii)
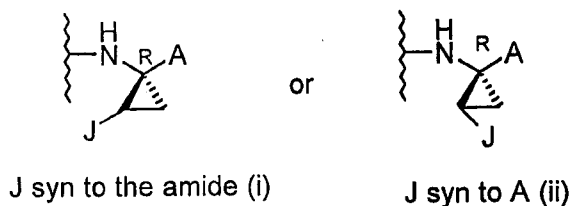
especially where J is syn to A.

Detailed Description of the Embodiments

5   Various embodiments of the invention will now be described by way of illustration only
     with reference to the following non-limiting examples.

Example 1
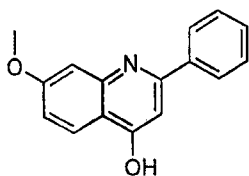
10   7-Methoxy-2-phenyl-quinolin-4-ol (1)

     To a stirred round bottled flask with toluene (100 mL) ethyl benzoyl acetate (18.7 g,
     97 mmol) and *m*-anisidine (12 g, 97 mmol) was added. 4 M HCl in dioxane (0.5 mL)
     was added and the reaction mixture was refluxed for 6 h (140 °C). The mixture was
     co-evaporated with toluene. To the crude mixture diphenyl ether (50 mL) was added

15   and the mixture was heated to 280 °C for 2 h. When the theoretical amount ethanol (6
     mL) was collected in a Dean Stark trap the heating was stopped and the mixture was
     cooled to rt. The crude mixture was dissolved in CH_2Cl_2 (100 mL) and stirred for 30
     min. The formed precipitate was filtered off and dried which gave 1 (4.12 g, 16.4
     mmol, 17 %): pale yellow powder.

20   ^{1}H (300 MHz, DMSO-D$_6$): δ 3.8 (s, 3H), 6.24 (s, 1H), 6.88-6.96 (dd, 1H, J = 9.07 Hz, J
     = 2.47 Hz), 7.19 (d, 1H, J=2.19 Hz), 7.56 (t, 3H, J = 2.19 Hz), 7.8 (dd, 2H, J = 7.14
     Hz, J = 2.19 Hz), 8.0 (d, 1H, J = 9.06 Hz); ^{13}C (75.5 MHz, DMSO-D$_6$): δ 55.3, 99.6,
     106.9, 113.1, 119.1, 126.4, 127.5, 128.8, 130.2, 134.1, 142.2, 149.4, 161.8, 176.4.

Example 2
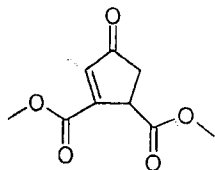
(Rac)-4-oxocyclopent-2-ene-1, 2-dicarboxylic acid dimethyl ester (2)

(1R, 2S)-4-oxo-cyclopentane-1, 2-dicarboxylic acid dimethyl ester (4.8 g, 23.8 mmol)

5   and CuBr₂ (11.9 g, 53.2 mmol) were dissolved in dry THF (70 mL) and the mixture
was refluxed for two hours at 90 °C. The formed CuBr was filtrated off and the organic
phase was concentrated. CaCO₃ (2.7 g, 27.2 mmol) and DMF (70 mL) were added
and the mixture was held at 100 °C for one hour. The dark brown mixture was poured
over ice (35 g) and the formed precipitate was filtrated off. The aqueous layer was

10 extracted with ethyl acetate (1 x 300mL + 3 x 150 mL). The organic phases were
dried, filtrated and concentrated. Purification by flash chromatography (toluene/EtOAc
9:1) gave 2 (2.1 g, 45 %) as yellow crystals

Example 3
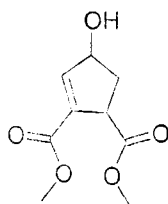
15 ((1S,4R) & (1R,4S))-4-hydroxy-cyclopent-2-ene-1,2-dicarboxylic acid dimethyl ester
(3)

To a cold solution (-30 °C) of 2 (3.18 g, 16.1 mmol) dissolved in MeOH (23 mL),
NaBH₄ (0.66 g, 17.5 mmol) was added. After nine minutes the excess of NaBH₄ was
destroyed by adding brine (80 mL). The mixture was concentrated and extracted with

20 ethyl acetate (4 x 80 mL). The organic phases were dried, filtrated and concentrated
and gave 3 (3.0 g, 92 %) as a yellow oil.

Example 4
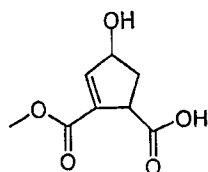
(1S,4R) & (1R,4S)-4-hydroxy-cyclopent-2-ene-1,2-dicarboxylic acid 2-methyl ester (4)

To an ice-cold solution of 3 (3.4 g, 22 mmol) dissolved in dioxane and water (1:1,

110mL), LiOH (0.52 g, 22 mmol) was added. After two and a half hours the mixture

5   was co-evaporated with toluene and methanol. Purification by flash chromatography

(toluene/Ethyl acetate 3:1 + 1 % HOAc) gave the title compound (1.0 g, 27 %) as

yellow-white crystals.

^{1}H-NMR (300 MHz, CD$_3$OD): δ 1.78-1.89 (m, 1H), 2.70-2.84 (m, 1H), 3.56-3.71 (m,

1H), 3.76 (s, 3H), 4.81-4.90 (m, 1H), 6.76-6.81 (m, 1H); ^{13}C-NMR (75.5 MHz, CDCl$_3$):

10   δ 38.0, 48.0, 52.4, 75.7, 137.0, 146.2, 165.0 178.4.

Example 5
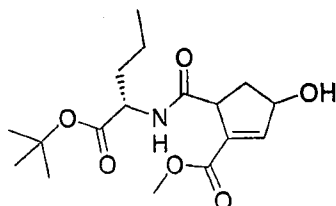
((3S,5R) & (3R,5S))-5-((S)-1-tert-Butoxycarbonyl-butylcarbamoyl)-3-hydroxy-

15   cyclopent-1-enecarboxylic acid methyl (5)

To an ice cooled solution of 4 (0.20 g, 1.1 mmol) and 2-amino-pentanoic acid

tert.butyl ester (0.24 g, 1.4 mmol) in DMF (7 mL), DIPEA (0.18 g, 1.4 mmol) and

HATU (0.53 g, 1.4 mmol) were added. After two hours the solution was concentrated

and purified using column chromatography (toluene/ethyl acetate 3:1). This gave the

20   title compound as a yellow oil (0.22 g, 63 %).

^{1}H-NMR (300 MHz, CDCl$_3$): δ 0.84-0.96 (m, 3H), 1.14-1.39 (m, 2H), [(1.44 & 1.49) s,

9H], 1.50-1.60 (m, 1H), 1.61-1.85 (m, 1H), 1.97-2.10 (m, 1H), 2.11-2.28 (m, 1H), 3.57-

3.68 (m, 1H), [(3.73 & 3.76) s, 3H], 4.30-4.50 (m, 1H), 4.63-4.73 (m, 1H), 6.80-6.95

(m, 1H), 6.95-7.00 (m, 1H).

Example 6

((3*S*,5*R*) & (3*R*,5*S*))-5-((*S*)-1-*tert*-Butoxycarbonyl-propylcarbamoyl)-3-hydroxy-

5   cyclopent-1-enecarboxylic acid methyl ester (6)

Reaction of 4 (141 mg, 76 mmol) according to the method described for the

preparation of 5 using L-2-amino-N-butyric acid tert.butyl ester instead of 2-amino-

pentanoic acid tert.butyl ester gave the title compound as a slightly yellow oil (171

mg, 69 %).

10   ^{1}H-NMR (300 MHz, CDCl$_3$): δ 0.89-0.98 (m, 3H), [(1.42 & 1.44) s, 9H], 1.60-1.78 (m,

1H), 1.79-1.95 (m, 1H), 1.99-2.11 (m, 1H), 2.18-2.30 (m, 1H), 3.58-3.65 (m, 1H), [3.75

& 3.78) s, 3 H], 4.22-4.39 (m, 1H), 4.61-4.66 (m, 1H), 6.77-6.90 (m, 1H), 6.91-6.92

(m, 1H).

15   ## Example 7
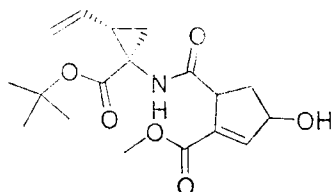
((3*S*,5*R*) & (3*R*,5*S*))-5-((1*R*,2*S*)-1-*tert*-Butoxycarbonyl-2-vinyl-cyclopropylcarbamoyl)-

3-hydroxy-cyclopent-1-enecarboxylic acid methyl ester (7)

Reaction of 4 (50 mg, 37 mmol) according to the method described for the

preparation of 5 using (1R, 2S)-1-amino-2-vinyl-cyclopropane carboxylic acid

20   *tert*.butyl ester instead of 2-amino-pentanoic acid *tert*.butyl ester provided the title

compound as a slightly yellow oil (50 mg, 38 %).

^{1}H-NMR (300 MHz, CDCl$_3$): δ [(1.38 & 1.42) s, 9H], 1.75-1.83 (m, 1H), 2.00-2.21 (m,

3H), 3.55-3.63 (m, 1H), [(3.77 & 3.82) s, 3H], 4.20-4.38 (m, 1H), 4.65-4.80 (m, 1H),

5.13-5.20 (m, 1H), 5.22-5.38 (m, 1H), 5.60-5.82 (m, 1H), 6.95-6.96 (m, 2H).

## Example 8
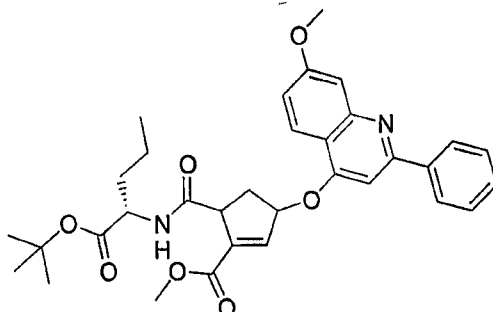
((3*R*,5*R*) & (3*S*,5*S*))-5-((*S*)-1-tert-Butoxycarbonyl-butylcarbamoyl)-3-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-1-enecarboxylic acid methyl ester (8)

5   To an ice cooled solution of 5 (0.23 g, 0.67 mmol) in dry THF, 7-methoxy-2-phenyl-quinolin-4-ol (0.22 g, 0.88 mmol) and triphenylphosphine (0.23 g, 0.88 mmol) were added. Then DIAD (0.19 g, 0.92 mmol) was dissolved in THF (2 mL) and added dropwise to the solution. After one hour the mixture was concentrated and purified using flash chromatography (toluene/ethyl acetate 3:1). This gave the title compound

10   as a white powder (0.30 g, 77 %).

^{1}H-NMR (300 MHz, CDCl$_3$): δ 0,88-1.00 (m, 3H), 1.18-1.43 (m, 2H), [(1.45 & 1.50) s, 9H], 1.53-1.65 (m, 1H), 1.66-1.85 (m, 1H), 2.29-2.43 (m, 1H), 3.10-3.25 (m, 1H), [(3.79 & 3.83) s, 3H], 3.97 (s, 3H), 4.05-4.20 (m, 1H), 4.38-4.50 (m, 1H), 6.03-6.13 (m, 1H), 6.65-6.90 (m, 1H), 7.04-7.18 (m, 3H), 7.40-7.56 (m, 4H), 8.00-8.12 (m, 3H).

15

Example 9
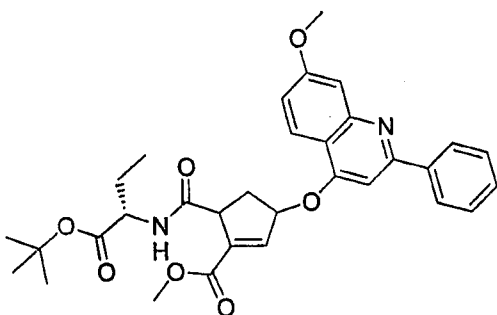
((3*R*,5*R*) & (3*S*,5*S*))--5-((*S*)-1-*tert*-Butoxycarbonyl-propylcarbamoyl)-3-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-1-enecarboxylic acid methyl ester (9)

Reaction of 6 (132 mg, 40 mmol) according to the method described for the
preparation of 8 gave the title compound as a yellow oil (137 mg, 61 %).
^{1}H-NMR (300 MHz, CDCl$_3$): δ 0.83-0.98 (m, 3H), [(1.42 & 1.44) s, 9H], 1.65-1.78 (m,
1H), 1.80-1.97 (m, 1H), 2.30-2.40 (m, 1H), 3.05-3.20 (m, 1H), [(3.78 & 3.80) s, 3H],
5   3.94 (s, 3H), 3.95-4.01 (m, 1H), 4.38-4.44 (s, 1H), 6.05-6.15 (m, 1H), 6.80-6.94 (m,
1H), 7.02-7.15 (m, 3H), 7.38-7.55 (m, 4H), 7.97-8.18 (m, 3H).

Example 10
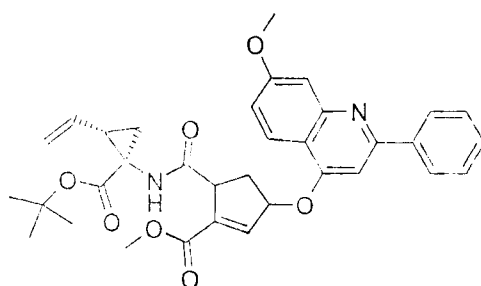
10   ((3R,5R) & (3S,5S))-5-((1R,2S)-1-*tert*-Butoxycarbonyl-2-vinyl-cyclopropylcarbamoyl)-
3-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-1-enecarboxylic acid methyl ester
(10)
Reaction of 7 (41 mg, 116 mmol) according to the method described for the
preparation of 8 provided the title compound as a yellow oil.
15   ^{1}H-NMR (300 MHz, CDCl$_3$): δ 1.52-1.57 (m, 1H), 1.58 (m, 9H), 1.80-1.83 (m, 1H),
2.00-2.17 (m, 1H), 2.20-2.38 (m, 1H), 3.20-3.37 (m, 1H), 3.80 (s, 3H), 3.81-3-3.98 (m,
1H), 3.99 (s, 3H), 5.12-5.20 (m, 1H), 5.22-5.40 (m, 1H), 5.63-5.80 (m, 1H), 6.05-6-20
(m, 1H), 7.00-7.21 (m, 4H), 7.40-7.58 (m, 4H), 8.02-8.18 (m, 3H).

20   Example 11
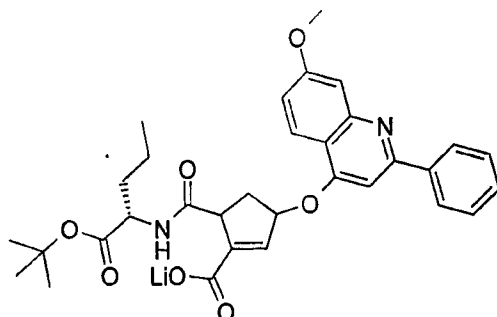
((3*R*,5*R*) & (3*S*,5*S*))-5-((*S*)-1-tert-Butoxycarbonyl-butylcarbamoyl)-3-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-1-enecarboxylic acid (11)

The methyl ester 8 (0.35 g, 0.61 mmol) was dissolved in dioxane/water (1:1, 7mL)
and LiOH (0.031 g, 1.3 mmol) was added. The reaction was stirred over night and
then co-concentrated. This gave the lithium salt of 11 (0.32 g, 90 %) as a brown
powder.

Example 12
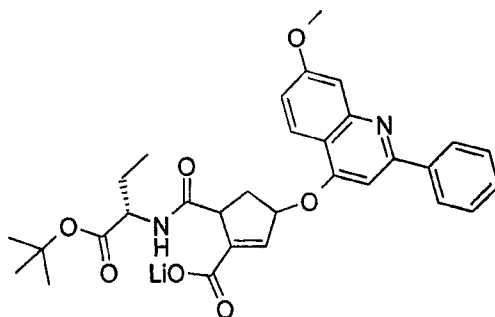
((3*R*,5*R*) & (3*S*,5*S*))-5-((*S*)-1-*tert*-Butoxycarbonyl-propylcarbamoyl)-3-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-1-enecarboxylic acid (12)

Reaction of 9 (225 mg, 40 mmol) according to the method described for the
preparation of 11 provided the title compound as a yellow salt (157 mg, 72 %).
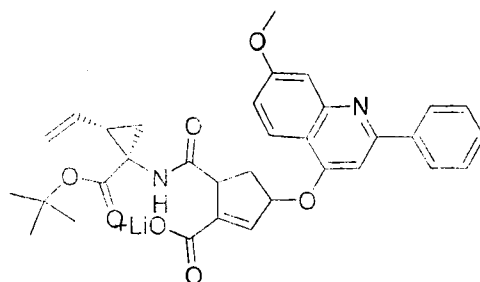
Example 13

((3R,5R) & (3S,5S))-5-((1R,2S)-1-*tert*-Butoxycarbonyl-2-vinyl-cyclopropylcarbamoyl)-3-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-1-enecarboxylic acid (13)

Reaction of 10 (35 mg, 59 mmol) according to the method described for the
preparation of 11 (33 mg, 97 %) provided the title compound as a yellow salt.

Example 14
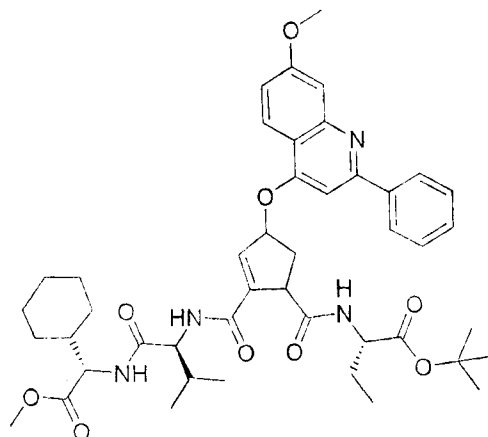
(S)-2-{[(((1S,4S) & (1R,4R))-2-{(S)-1-[((S)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2-methyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-butyric acid *tert*-butyl ester (14)

The acid 12 (38.4 mg, 0.070 mmol) and (2-amino-3-methyl-butyrylamino)-cyclohexyl acetic acid methyl ester (26.6 mg, 0.098 mmol) were dissolved in DMF (1.5 mL) and cooled in an ice-bath. DIPEA (17.1 µL, 0.098 mmol) and HATU (37.4 mg, 0.098 mmol) were added. After ninety minutes the mixture was co-concentrated with toluene and methanol and then purified by flash column chromatography (toluene/ethyl acetate 6:1). Further purification was performed on HPLC (90 % MeOH

+ 0.2 % TEA). The diastereomeric mixture 14 was concentrated and gave a slightly yellow oil (20.6 mg, 37 %). After lyophilisation 14 was collected as a white powder. ^{1}H-NMR (300 MHz, CDCl$_3$): δ 0.93-1.02 (m, 9H), 1.03-1.25 (m, 4H), 1.44 (s, 9H), 1.65-1.86 (m, 9H), 2.05-2.10 (m, 1H), 2.22-2.40 (m, 1H), 3.05-3.20 (m, 1H), 3.77 (s, 3H), 3.98 (s, 3H), 4.18-4.22 (m, 1H), 4.38-4.60 (m, 3H), 6.01-6.10 (m, 1H), 6.61-6.70 (m, 2H), 6.80-6.85 (m, 1H), 7.05-7.18 (m, 2H), 7.40-7.58 (m, 5H), 8.00-8.13 (m, 3H). ^{13}C-NMR (75.5 MHz, CDCl$_3$): δ 9.7, 18.4, 19.2, [25.9 & 26.1], [28.2 & 28.5], 29.6, 32.0, 37.3, 41.0, 46.2, 50.7, 52.4, 54.4, 55.8, 57.2, 58.5, 82.0, 82.8, 98.4, 110.2, 118.4, 120.1, 123.2, 127.9, 128.2, 128.9, 129.5, 131.2, 135.1, 135.2, 142.7, 144.2, 161.6, 164.3, 164.7, 170.9, 171.4, 172.4. MALDI-TOF m/z 821.56 [(M +Na)$^+$ calcd for C$_{45}$H$_{58}$N$_4$NaO$_9$$^+$ 821.41].

Example 15

15   (S)-2-{[((1R,4R) & (1S,4S))-2-{(R)-1-[((R)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2-methyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-butyric acid tert-butyl ester (15)
Reaction of 12 (20 mg, 37 mmol) according to the method described for the preparation of 14 using (2-amino-3-methyl-butyrylamino)-(R)-cyclohexyl acetic acid
20   methyl ester instead of (2-amino-3-methyl-butyrylamino)-(S)-cyclohexyl acetic acid methyl ester, gave the title compound (19 mg, 66 %) as a white powder.

^{1}H-NMR (300 MHz, CDCl$_3$): δ 0.91-0.98 (m, 3H), 0.99-1.10 (m, 6H), 1.11-1.38 (m, 4H), [(1.43 & 1.45) s, 9H], 1-45-1.94 (m, 9H), 2.05-2.18 (m, 1H), 2.22-2.40 (m, 1H), 3.16-3.24 (m, 1H), 3.77 (s, 3H), 3.98 (s, 3H), 4.04-4.18 (m, 1H), 4.36-4.57 (m, 3H), 6.00-6.08 (m, 1H), 6.13-6.21 (m, 1H), 6.62-6.70 (m, 1H), 6.81-6.85 (m, 1H), 7.05-7.18
5 (m, 3H), 7.41-7.57 (m, 4H), 8.02-8.13 (m, 3H). ^{13}C-NMR (75.5 MHz, CDCl$_3$): δ 9.3, 18.2, 19.0, [25.5 & 25.9], [28.0 & 28.3], 29.4, 31.4, 32.1, 35.7, 40.7, 50.4, 52.2, 54.2, 55.5, 57.0, 58.2, 81.8, 82.4, 98.2, 107.5, 115.0, 118.1, 122.9, 127.6, 128.7, 128.8, 128.9, 129.2, 135.1, 140.4, 142.2, 151.4, 161.3, 163.9, 170.4, 170.9, 171.2, 172.0. MALDI-TOF m/z 821.60 [(M +Na)$^+$ calcd for C$_{45}$H$_{58}$N$_4$NaO$_9$$^+$ 821.41].
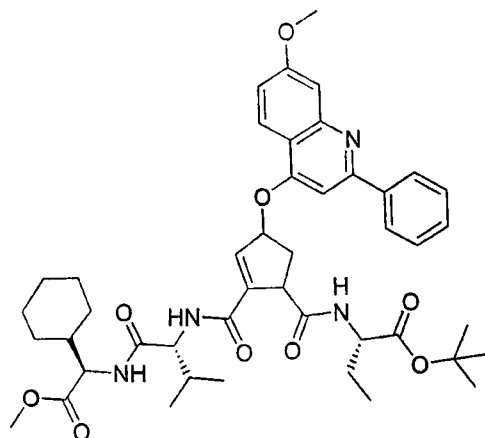
10

Example 16
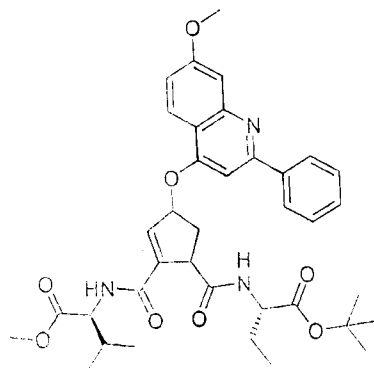
(S)-2-{[((3R,5R) & (3S,5S))-5-((S)-1-tert-Butoxycarbonyl-propylcarbamoyl)-3-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-1-enecarbonyl]-amino}-3-methyl-butyric
15 acid methyl ester (16)

Reaction of 12 (24 mg, 44 mmol) according to the method described for the preparation of 14 using D-valine methyl ester instead of (2-amino-3-methyl-butyrylamino)cyclohexyl acetic acid methyl ester, gave the title compound (27 mg, 97 %) as a white powder.
20 ^{1}H-NMR (300 MHz, CDCl$_3$): δ 0.82-0.99 (m, 9H), [(1.42 & 1.44) s, 9H] 1.65-1.95 (m, 2H), 2.18-2.25 (m, 1H), 2.26-2.40 (m, 1H), 3.20-3.25 (m, 1H), 3.75 (s, 3H), 3.97 (s, 3H), 4.15-4.19 (m, 1H), 4.36-4.43 (m, 1H), 4.64-4.75 (m, 1H), 6.03-6.15 (m, 1H), 6.80-6.85 (m, 2H), 7.10-7.20 (m, 3H), 7.42-7.58 (m, 4H), 8.0-8.10 (m, 3H). ^{13}C-NMR (75.5 MHz, CDCl$_3$): δ 9.7, [18.2 & 19.1], 25.7, [28.1 & 28.2], 32.0, 35.6, 50.4, 52.4,

54.5, 55.7, 57.6, 81.7, 82.7, 98.4, 107.7, 115.2, 118.4, 123.2, 127.8, 129.0, 129.2, 129.5, 134.8, 135.0, 140.4, 142.5, 151.6, 159.6, [161.1 & 161.5], 164.6, 171.1, 172.2. MALDI-TOF m/z 682.51[(M +Na)$^+$ calcd for $C_{37}H_{45}N_3NaO_8^+$ 682.31].
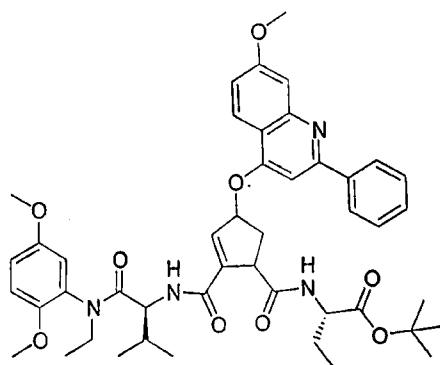
Example 17

(S)-2-{[((1R,4R) & (1S,4S))-2-{(S)-1-[(2,5-Dimethoxy-phenyl)-ethyl-carbamoyl]-2-methyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-butyric acid *tert*-butyl ester (17)

Compound 17 (28.6 mg, 59 %) was prepared from 12 (33 mg, 60 mmol) according to the method for the preparation of 14 using 2-amino-N-(2,5-dimethoxy-phenyl)-N-ethyl-3-methyl butyramide instead of (2-amino-3-methyl-butyrylamino)-cyclohexyl acetic acid methyl ester. This gave the title compound as a white powder.

^{1}H-NMR (300 MHz, CDCl$_3$): δ 0.75-0.95 (m, 9H) 1.05-1.18 (m, 3H), [(1.42 & 1.44) s, 9H],1.60-1.95 (m, 3H), 2.20-2.40 (m, 1H), 3.20-3.34 (m, 1H), 3.60-3.80 (m, 2H), [3.62-3.65 (m, 3H)], [3.79-3.82 (m, 3H)], 3.98 (s, 3H), 4.02-4-18 (m, 1H), 4.30-4.44 (m, 2H), 6.05-6.18 (m, 1H), 6.60-6.63 (m, 1H), 6.77-6.80 (m, 2H), 6.85-6.93 (m, 2H), 7.12-7.20 (m, 2H), 7.35-7.60 (m, 5H), 8.02-8.20 (m, 3H). ^{13}C-NMR (75.5 MHz, CDCl$_3$): δ [9.6 & 9.7], [12.5 & 12.8], [17.1 & 17.5], [19.4 & 19.5], 25.6, [28.0 & 28.1], 32.4, 35.8, 43.0, 44.3, [50.2 & 50.3], 54.3, [54.8 & 55.0 & 55.2 & 55.5], [55.6 & 55.7 & 55.9 & 56.0], 81.7, 82.8, 98.4, 106.9, [112.4 & 112.5],113.7, 115.0, 115.2, 115.9, 116.3, 118.4, [123.0 & 123.1], [127.7 & 127.8], 128.8, 128.9, 129.5, 130.1, [134.1 & 134.2], 142.6, 149.1, 149.4, 153.4, 158.9,[161.4 & 161.6], [163.2 & 163.5], 170.9, [171.3 & 171.5], 172.3. MALDI-TOF m/z 831.62 [(M +Na)$^+$ calcd for $C_{46}H_{56}N_4NaO_9^+$ 831.39].
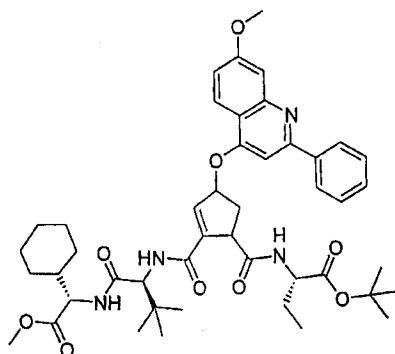
Example 18

(S)-2-{[((1R,4R) &(1S,4S))-2-{(S)-1-[((S)-Cyclohexyl-methoxycarbonyl-methyl)-
carbamoyl]-2,2-dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-
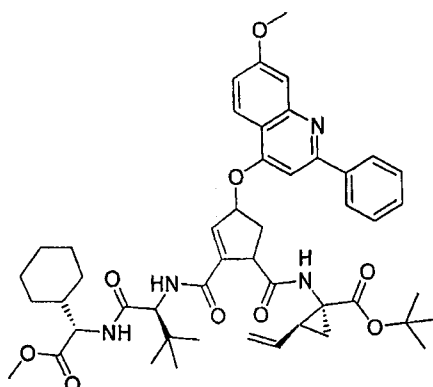cyclopent-2-enecarbonyl]-amino}-butyric acid tert-butyl ester (18)

Compound 18 (16.1 mg, 26 %) was prepared from 12 (43.2 mg, 0.077 mmol)
according to the method for the preparation of 14 using (2-amino-3,3-dimethyl-
butyrylamino)-cyclohexyl-acetic acid methyl ester instead of (2-amino-3-methyl-
butyrylamino)-cyclohexyl acetic acid methyl ester. Flash column chromatography was
performed in toluene/ethyl acetate 3:1 instead of 6:1: This gave the title compound as
a white powder.

^{1}H-NMR (300 MHz, CDCl$_3$): δ 0.77-0.83 (m, 3H), [(0.92 & 0.93) s, 9H] 0.94-1.20 (m, 4
H), [(1.36 & 1.38) s, 9H], 1.42-1.76 (m, 8H), 2.20-2.38 (m, 1H), 2.81-2.96 (m, 1H),
3.20-3.22 (m, 1H), 2.78 (s, 3H), [(3.83 & 3.85) s, 3H], 3.97-4.02 (m, 1H), 4.17-4.21
(m, 1H), 4.22-4.37 (m, 2H), 5.85-5.97 (m, 1H), [6.76-6.78 (m, 0.5H)], [6.80-6.82 (m,
0.5H)], 6.98-7.05 (m, 3H), 7.23-7.41 (m, 6H), 7.82-7.99 (m, 3H). ^{13}C-NMR (75.5 MHz,
CDCl$_3$): δ [9.4 & 9.5], [25.4 & 25.5], 25.8, [26.5 & 26.6], [27.9 & 28.0], [28.4 & 28.5],
29.3, [35.4 & 35.7], [36.0 & 36.4], [40.5 & 40.7], [50.2 & 50.5], [52.1 & 52.2],[54.1 &
54.3], 55.5, [57.0 & 57.3], [60.4 & 60.7], [81.8 & 82.0], [82.4 & 82.5] 98.1, 107.5,
115.0, 118.1, 123.0, 127.5, 128.7, 128.8, 129.2, 134.9, 135.8, 141.9, 142.5, 151.3,
159.4, [160.9 & 161.3], [163.7 & 163.9], [169.9 & 170.0] [170.0 & 171.3], [172.5 &
172.4]. MALDI-TOF m/z 835.68 [(M +Na)$^+$ calcd for C$_{46}$H$_{60}$N$_4$NaO$_9^+$ 835.43].

Example 19
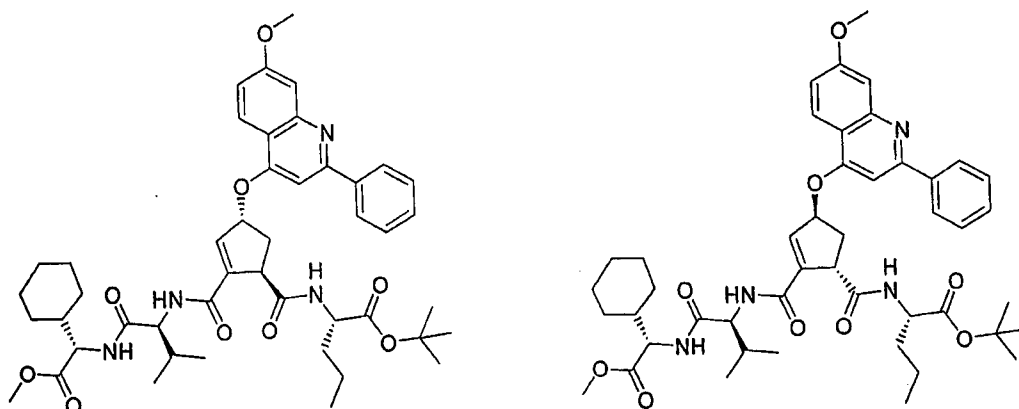
(S)-2-{[(1R,4R)-2-{(S)-1-[((S)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2-

5   methyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-

enecarbonyl]-amino}-pentanoic acid tert-butyl ester (19a) and

(S)-2-{[(1S,4S)-2-{(S)-1-[((S)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2-

methyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-

enecarbonyl]-amino}-pentanoic acid tert-butyl ester (19b)

10   The acid 11 (0.051 g, 0.087 mmol) and (2-amino-3-methyl-butyrylamino)-cyclohexyl-

acetic acid methyl ester (0.054 g, 0.21 mmol) were dissolved in DMF (1.5 mL) and

cooled in an ice-bath. DIPEA (16 mg, 0.12 mmol) and HATU (47 mg, 0.13 mmol)

were added. After two and a half hours the mixture was co-concentrated with toluene

and methanol and then purified by flash column chromatography (toluene/ethyl

15   acetate 3:1). Further purification was performed on HPLC (90 % MeOH + 0.2 % TEA).

This gave after co-concentration the two diastereomers 19a (9.4 mg, 13 %) and 19b

(5.3 mg, 7 %) as slightly yellow syrups. After lyophilisation 19a and 19b were

collected as white powders:

^{1}H-NMR (300 MHz, CDCl$_3$): δ 0.86-0.93 (m, 3H), 0.94-1.00 (m, 6H), 1.00-1.41 (m,

20   7H), 1.46 (s, 9H), 1.50-1.88 (m, 8H), 2.05-2.20 (m, 1H), 2.20-2.37 (m, 1H), 3.12-3.25

(m, 1H), 3.73 (s, 3H), 3.97 (s, 3H), 4.05-4.20 (m, 1H), 4.40-4.55 (m, 3H), 6.02-6.18

(m, 1H), 6.30 (d, J = 8.52 Hz, 1H), 6.63 (s, 1H), 6.76 (d, J = 8.51 Hz, 1H), 7.06-7.16

(m, 2H), 7.42-7.56 (m, 5H), 8.00-8.12 (m, 3H); ^{13}C-NMR (75.5 MHz, CD$_3$OD): δ 14.0,

18.4, 19.3, 26.1, 28.3, 28.5, 29.7, 31.9, 34.9, 36.0, 41.0, 50.7, 52.4, 53.3, 55.7, 57.2, 58.6, 82.0, 82.7, 98.4, 105.7, 107.7, 115.2, 118.4, 123.2, 125.3, 127.9, 129.0, 129.1, 135.1, 138.0, 142.4, 151.6, 159.4, 161.6, 164.3, 170.7, 171.2, 172.3. 19b: ^{1}H-NMR (300 MHz, CDCl$_3$): δ 0.90-1.04 (m, 9H), 1.04-1.43 (m, 7H), 1.47 (s, 9H), 1.50-1.87 (m, 8H), 2.10-2.27 (m, 1H), 2.33-2.45 (m, 1H), 3.10-3.20 (m, 1H), 3.73 (s, 3H), 3.96 (s, 3H), 4.02-4.10 (m, 1H), 4.36-4.53 (m, 3H), 6.00-6.16 (m, 1H), 6.30 (d, J = 8.52 Hz, 1H), 6.73 (s, 1H), 6.86 (d, J = 7.96 Hz, 1H), 7.08-7.16 (m, 2H), 7.36-7.56 (m, 5H), 8.03-8.11 (m, 3H). ^{13}C-NMR (75.5 MHz, CD$_3$OD): δ 14.0, 18.6, 19.2, 26.1, 28.2, 28.7, 29.7, 34.5, 36.1, 36.6, 40.8, 50.5, 52.4, 53.4, 55.7, 57.3, 59.1, 64.8, 82.3, 98.4, 105.8, 107.8, 115.3, 118.4, 123.2, 127.8, 129.0, 129.4, 135.2, 142.2, 144.9, 151.0, 151.6, 159.2, 164.3, 164.3, 170.2, 171.6, 171.9

Example 20
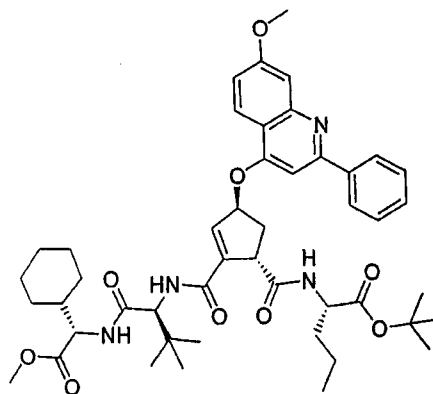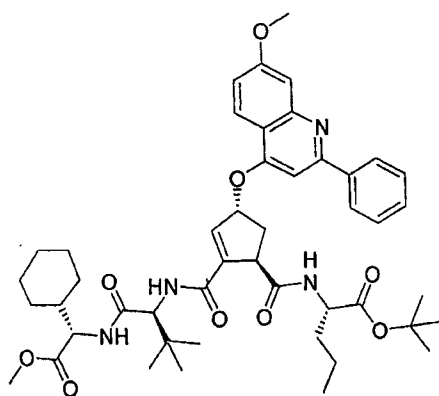
(S)-2-{[(1R,4R)-2-{(R)-1-[((S)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2,2-dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-pentanoic acid tert-butyl ester (20a) and
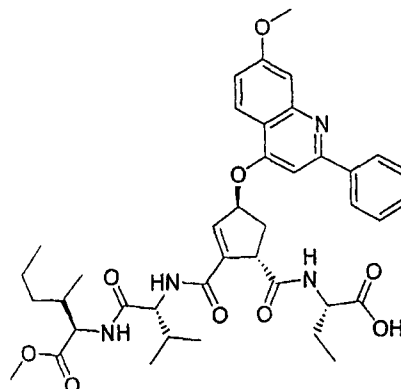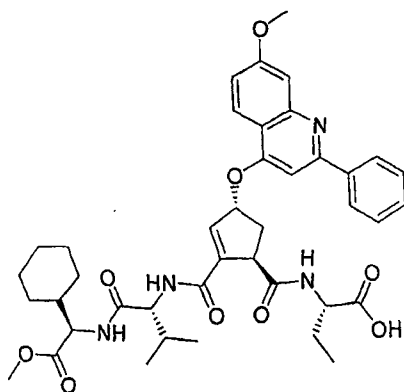(S)-2-{[(1S,4S)-2-{(R)-1-[((S)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2,2-dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-pentanoic acid tert-butyl ester (20b)

Method A: The carboxylic acid 11 (57 mg, 0.10 mmol) was dissolved in warm (50 °C) dry THF (2 mL). (2-Amino-3,3-dimethyl-butyrylamino)-cyclohexyl-acetic acid methyl ester (50 mg, 0.12 mmol), DIPEA (30 mg, 0.23 mmol), DCC (25 mg, 0.12 mmol) and HOBt (17 mg, 13 mmol) were added. After two hours the mixture was concentrated

and added to a short column (toluene/Ethyl acetate 1:3 + 3 % AcOH). Then it was further purified on HPLC using 90 % MeOH + 0.2 % TEA. The diastereomeric products were not separated. After HPLC the solution was co-concentrated with toluene and methanol to give 20 (28 mg, 34%).

5   Method B: To an ice-cold solution of 11 (60 mg, 0.10 mmol) and (2-amino-3,3-dimethyl-butyrylamino)-cyclohexyl-acetic acid methyl ester (42 mg, 0.15 mmol) DIPEA (19 mg, 0.15 mmol) and HATU (62 mg, 0.16 mmol) were added. After two and a half hours the mixture was concentrated and purified using column chromatography. (toluene/Ethyl acetate 3:1). The diastereomeric mixture was

10   separated using HPLC (90 % MeOH + 0.2 % TEA). This gave 20a (6 mg, 6 %) and 20b (9 mg, 10%).

20a: ^{1}H-NMR (300 MHz, CDCl$_3$): δ 0.82-0.90 (m, 3H), 1.01 (s, 9H), 1.05-1.40 (m, 7H), 1.46 (s, 9H), 1.50-1.80 (m, 8H), 2.20-2.35 (m, 1H), 3.07-3.25 (m, 1H), 3.73 (s, 3H), 3.97 (s, 3H), 4.11 (d, J = 7.96 Hz, 1H), 4.38-4.52 (m, 3H), 6.03-6.12 (m, 1H), 6.24 (d,

15   J = 8.79 Hz, 1H), 6.63 (s, 1H), 6.82 (d, J = 9.06 Hz, 1H), 7.07-7.27 (m, 2H), 7.36 (d, J = 7.96 Hz, 1H), 7.41-7.55 (m, 4H), 8.01-8.10 (m, 3H); ^{13}C-NMR (75.5 MHz, CD$_3$OD): δ 14.0, 18.8, 26.1, 26.8, 28.2, 28.6, 29.6, 34.9, 35.6, 36.2, 40.9, 50.7, 52.4, 53.3, 55.7, 57.3, 60.8, 82.0, 82.7, 98.4, 105.2, 107.7, 115.2, 118.4, 123.2, 127.9, 129.0, 129.4, 131.1, 135.1, 138.4, 142.4, 153.3, 159.6, 161.6, 164.2, 170.1, 171.3, 172.2.

20   20b: ^{1}H-NMR (300 MHz, CDCl$_3$): δ 0.90-0.98 (m, 3H), 1.04 (s, 9H), 1.08-1.40 (m, 7H), 1.44 (s, 9H), 1.55-1.90 (m, 8H), 2.20-2.38 (m, 1H), 3.10-3.22 (m, 1H), 3.73 (s, 3H), 3.97 (s, 3H), 4.02-4.15 (m, 1H), 4.35-4.48 (m, 3H), 6.00-6.08 (m, 1H), 6.72 (s, 1H), 6.90 (d, J = 9.06 Hz, 1H), 7.09-7.20 (m, 3H), 7.44-7.55 (m, 5H), 8.03-8.11 (m, 3H).

25   Example 21

84

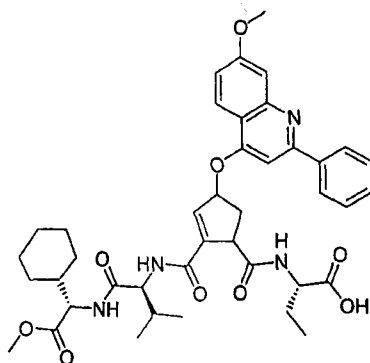(1R,2S)-1-{[((1R,4R) & (1S,4S))-2-{(S)-1-[((S)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2,2-dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid *tert*-butyl ester (21)

The acid 13 (35 mg, 0.060 mmol) and (2-amino-3,3-dimethyl-butyrylamino)-cyclohexyl-acetic acid methyl ester (22 mg, 0.080 mmol) were dissolved in dry THF (1.5 mL) and warmed to 50 °C. HOBt (11 mg, 0.080 mmol) and DCC (31 mg, 0.15 mmol) were added. After one hour the mixture was co-concentrated with toluene and methanol and then purified by flash column chromatography (toluene/ethyl acetate 1:1). Further purification was performed on HPLC (80 % MeOH + 0.2 % TEA. The diastereomeric mixture 21 was concentrated and gave a slightly yellow oil (26.4 mg, 53 %). After lyophilisation 21 was collected as a white powder.

^{1}H-NMR (300 MHz, CDCl$_3$): δ [(0.98 & 1.00), s, 9H], 1.01-1.38 (m, 5H), [(1.39 & 1.40) s, 9H], 1.52-1.63 (m, 4H), 1.65-1.80 (m, 4H), 1.90-2.05 (m, 1H), 2.20-2.40 (m, 1H), 3.02-3.20 (m, 1H), [(3.66 & 3.67) s, 3H), 3.98 (s, 3H), 3.99-4.02 (m, 1H), 4.30-4.45 (m, 2H), 5.05-5.11 (m, 1H), 5.20-5.30 (m, 1H), 5.60-5.81 (m, 1H), 6.03-6.17 (m, 1H), 6.77-6.82 (m, 1H), 6.95-7.22 (m, 5H), 7.40-7.50 (m, 4H), 8.01-8.10 (m, 3H). ^{13}C-NMR (75.5 MHz, CDCl$_3$): δ 22.3, [25.7 & 25.8], [26.4 & 26.5], [28.0 & 28.4] 29.2, 32.7, 33.3, [35.3 & 35.4], 36.0, [40.2 & 40.3], 40.7, 52.0, 55.4, [57.2 & 57.4] [60.4 & 60.5], [87.6 & 87.7], [82.3 & 82.5], 98.4, 107.0, 114.9, [117.4 & 117.5], 118.1, 122.9, 127.6, 128.6,128.9, 129.2, [133.6 & 133.8], 135.9, 136.9, 140.1, [141.4 & 141.6], 151.1, 159.6, [160.9 & 161.3], [164.2 & 164.6], 168.9, 170.3, [172.1 & 172.6]. MALDI-TOF m/z 859.77 [(M +Na)$^+$ calcd for C$_{48}$H$_{60}$N$_4$NaO$_9$$^+$ 859.43].

Example 22

(S)-2-{[(1R,4R)-2-{(R)-1-[((S)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2,2-
dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-
enecarbonyl]-amino}-pentanoic acid (22a) and

(S)-2-{[(1S,4S)-2-{(R)-1-[((S)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2,2-
dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-
enecarbonyl]-amino}-pentanoic acid (22b)

The tert.butyl ester 20 (28 mg, 0.034 mmol), TES (8.7 mg, 0.075 mmol), DCM (1 mL)
and TFA (1 mL) were mixed in a round bottomed flask. Two hours later the mixture
was concentrated and the diastereomers were separated on HPLC using 65 % MeOH
+ 0.2 % TEA as mobile phase. This gave 22a (15 mg, 55 %) and 22b (12 mg, 45 %)
as slightly yellow syrups. After lyophilisation the title compounds were collected as
white powders.

22a: $[\alpha]^{22}D$ + 155.8; ^{1}H-NMR (300 MHz, CD$_3$OD): δ 0.90-0.97 (m, 3H), 1.03 (s, 9H),
1.05-1.50 (m, 7H), 1.50-1.80 (m, 8H), 2.43-2.55 (m, 1H), 2.77-2.90 (m, 1H), 3.68 (s,
3H), 3.96 (s, 3H), 4.20-4.30 (m, 2H), 4.31-4.40 (m, 1H), 4.45-4.50 (m, 1H), 6.03-6.11
(m, 1H), 6.98 (s, 1H), 7.12-7.19 (m, 1H), 7.36 (s, 1H), 7.41 (d, J = 2.2 Hz, 1H), 7.50-
7.60 (m, 3H), 8.03-8.10 (m, 3H): ^{13}C-NMR (75.5 MHz, CD$_3$OD): δ 13.1, 19.1, 26.1,
28.7, 28.9, 29.5, 34.3, 34.8, 35.9, 40.1, 50.8, 51.2, 54.8, 55.0, 57.9, 60.7, 83.5, 99.1,
106.0, 115.2, 118.2, 123.3, 127.8, 128.0, 128.7, 128.8, 129.7, 135.2, 139.8, 143.7,
150.6, 160.1, 162.2, 165.2, 171.7, 172.2, 173.4. 22b: $[\alpha]^{22}D$ -72,3; ^{1}H-NMR (300 MHz,

CD3OD): δ 0.90-0.97 (m, 3H), 1.02 (s, 9H), 1.07-1.35 (m, 7H), 1.53-1.90 (m, 8H), 2.46-2.61 (m, 1H), 2.76-2.88 (m, 1H), 3.69 (s, 3H), 3.96 (s, 3H), 4.15-4.35 (m, 2H), 4.37-4.41 (m, 1H), 4.42-4.47 (m, 1H), 6.02-6.12 (m, 1H), 7.02 (s, 1H), 7.16 (dd, J = 2.47, 9.34 Hz, 1H), 7.32 (s, 1H), 7.40 (d, J = 2.47 Hz, 1H), 7.48-7.58 (m, 3H), 8.03-8.12 (m, 3H); ^{13}C-NMR (75.5 MHz, CD3OD): δ 13.0, 18.8, 25.9, 26.0, 28.8, 29.4, 34.2, 34.8, 36.3, 39.9, 48.8, 50.5, 51.1, 54.8, 57.9, 60.5, 82.8, 99.0, 106.0, 115.1, 118.2, 123.1, 127.8, 127.9, 128.7, 129.0, 129.5, 136.7, 139.8, 142.8, 150.6, 160.1, 162.0, 162.2, 164.7, 172.1, 173.5.

Example 23

(S)-2-{[(1R,4R)-2-{(R)-1-[((R)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2-methyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-butyric acid (23a) and (S)-2-{[(1S,4S)-2-{(R)-1-[((R)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2-methyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-butyric acid (23b)
Compound 23a (6.6 mg, 50 %) and compound 23b (1.3 mg, 10 %) were prepared from 15 (14 mg, 0.018 mmol) according to the method for the preparation of 22a and 22b. This gave the title compounds as white powders.

23a: ^{1}H-NMR (300 MHz, CD3OD): 0.88-1.02 (m, 9H), 1.02-1.40 (m, 7H), 1.55-1.97 (m, 6H), 2.01-2.10 (m, 1H), 2.38-2.52 (m, 1H), 2.88-3.00 (m, 1H), 3.77 (s, 3H), 3.98 (s, 3H), 4.08-4.20 (m, 1H), 4.22-4.40 (m, 3H). 6.03-6.18 (m, 1H), 6.86-6.99 (m, 1H), 7.08-7.20 (m, 1H), 7.23 (s, 1H), 7.40-7.43 (m, 1H), 7.45-7.70 (m, 3H), 8.02-8.20 (m,

3H). ^{13}C-NMR (75.5 MHz, CD$_3$OD): δ 9.0, 17.6, 18.2, 24.5, 25.3, 28.1, 28.8, 30.9, 35.4, 39.4, 49.6, 51.1, 54.7, 57.2, 58.0, 82.4, 98.5, 105.5, 114.5, 117.7, 122.7, 127.2, 127.3, 128.2, 129.0, 135.6, 136.4, 141.7, 149.9, 159.5, 161.2, 161.4, 164.0, 171.0, 171.7, 172.4. 23b: ^{1}H-NMR (300 MHz, CD$_3$OD): δ 0.9-1.20 (m, 9H), 1.21-1.53 (m, 7H), 1.55-1.93 (m, 6H), 2.05-2.20 (m, 1H), 2.41-2.50 (m, 1H), 2.96-3-05 (m, 1H), 3.77 (s, 3H), 4.00 (s, 3H), 4.05-4.40 (m, 4H), 6.05-6.18 (m, 1H), 6.90-6.95 (m, 1H), 7.05-7.22 (m, 2H), 7.50-7.65 (m, 4H), 8.01-8.16 (m, 3H).

<u>Example 24</u>

(S)-2-{[((1R,4R) & (1S,4S))-2-{(S)-1-[((S)-Carboxy-cyclohexyl-methyl)-carbamoyl]-2-methyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-butyric acid (24)

The tert.butyl ester 14 (13.4 mg, 0.017 mmol), TES (4.83 mg, 0.042 mmol), DCM (2 mL) and TFA (2 mL) were mixed in a round bottomed flask. One hour later the mixture was concentrated and purified by HPLC using 65 % MeOH + 0.2 % TEA as mobile phase. This gave 24 (4.3 mg, 34 %) as a slightly yellow syrup. After lyophilisation 24 was collected as a white powder.

^{1}H-NMR (300 MHz, CD$_3$OD): δ 0.91-0.99 (m, 9H), 1.00-1.28 (m, 4H), 1.55-1.78 (m, 9H), 1.92-1.95 (m, 1H), 2.00-2.05 (m, 1H), 2.93-3.01 (m, 1H), 3.75 (s, 3H), 3.97 (s, 3H), 4.10-4.40 (m, 4H), 6.05-6.15(m, 1H), 6.88-6.94 (m, 1H), 7.05-7.10 (m, 2H), 7.41-7.43 (m, 1H), 7.44-7.55 (m, 2H), 8.62-8.68 (m, 1H), 8.69-8.79 (m, 1H), 7.97-8.05 (m, 2H). ^{13}C-NMR (75.5 MHz, CD$_3$OD): δ 9.2, 18.5, 25.5, [29.0 & 29.2], [30.0 & 30.5], 35.3, 37.7, 39.7, 46.2, 50.0, [51.4 & 51.5], 53.6, 55.1, 57.1, 58.4, 83.1, 98.9, 104.9,

114.6, 118.3, 123.0, 123.4, 127.5, 128.4, 128.5, 129.7, 135.0, 142.1, 145.7, 146.2, 159.2, 161.9, 164.3, 171.5, 171.9, 172.2. MALDI-TOF *m/z* 791.27 [(M +K)$^+$ calcd for C$_{42}$H$_{48}$KN$_4$O$_9$$^+$ 791.31].

5   Example 25
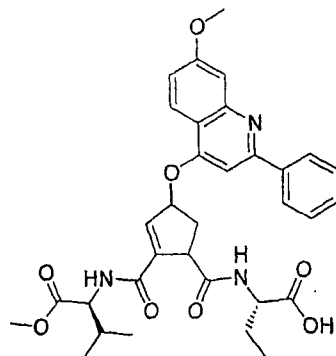
(*S*)-2-{[((3*R*,5*R*) & (3*S*,5*S*))-5-((*S*)-1-Carboxy-propylcarbamoyl)-3-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-1-enecarbonyl]-amino}-3-methyl-butyric acid methyl ester (25)

10   Compound 25 (8.0 mg, 60 %) was prepared from 16 (13.8 mg, 0.022 mmol) according to the method for the preparation of 24 which gave the title compound as a white powder.

^{1}H-NMR (300 MHz, CD$_3$OD): δ 0.83-1.02 (m, 9H), 1.68-1.80 (m, 1H), 1.82-2.02 (m, 1H), 2.10-2.22 (m, 1H), 2.40-2.60 (m, 1H), 2.81-2.95 (m, 1H), 3.75 (s, 3H), 4.00 (s, 15   3H), 4.18-4.22 (m, 1H), 4.27-4.40 (m, 2H), 6.05-6.12 (m, 1H), 6.99-7.02 (m, 1H), 7.16-7.21 (m, 1H), 7.38 (s, 1H), 7.40-7.43 (m, 1H), 7.48-7.61 (m, 3H), 7.98-8.12 (m, 3H).

Example 26
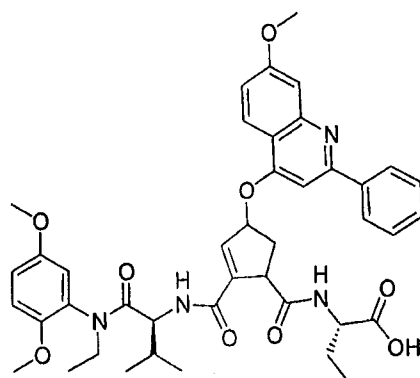
(S)-2-{[((1R,4R) &(1S,4S))-2-{(S)-1-[(2,5-Dimethoxy-phenyl)-ethyl-carbamoyl]-2-methyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-butyric acid (26)

Compound 26 (5.7 mg, 36 %) was prepared from 17 (16.7 mg, 0.021 mmol) according to the method for the preparation of 24 which gave the title compound as a white powder.

^{1}H-NMR (300 MHz, CD$_3$OD): δ 0.75-0.81 (m, 6H), 0.82-0.98 (m, 3H), 1.00-1.10 (m, 3H), 1.60-2.00 (m, 3H), 2.40-2.56 (m, 1H), 2.80-2.88 (m, 1H), 3.18-3.24 (m, 1H), 3.40-3.46 (m, 1H), [3.67-3.80 (m, 6H)], 3.97 (s, 3H), 4.10-4.20 (m, 1H), 4.21-4.40 (m, 2H), 6.02-6.17(m, 1H), 6.75-6.82 (m, 1H), 6.84-7.01 (m, 3H), 7.10-7.20 (m, 1H), 7.30-7.37 (m, 1H), 7.40-7.43 (m, 1H), 7.50-7.60 (m, 3H), 8.00-8.17 (m, 3H). ^{13}C-NMR (75.5 MHz, CD$_3$OD): δ 9.6, [11.8 & 12.0], [17.2 & 17.4], 18.9, 25.0, 32.3, 35.7, 43.3, 44.2, [50.3 & 50.5], [54.5 & 54.8 & 54.9 & 55.0], [55.1 & 55.2 & 55.3 & 56.0] ,58.7, 83.6, 99.3, 105.5, [112.5 & 112.7], 114.3, [15.1 & 115.2], 115.7, 116.1, 118.4, [123.3 & 123.4], 125.2, [128.0 & 128.1, 128.8, 129.1, 129.8, [135.1 & 135.3], 139.2, [143.3 & 144.4], 149.2, [149.6 & 149.9], 153.8, 159.9, 162.4, [163.9 & 164.5], 172.1, 172.8, [173.6 & 173.7]. MALDI-TOF m/z 775.30 [(M +Na)$^+$ calcd for C$_{42}$H$_{48}$N$_4$NaO$_9{}^+$ 775.33].
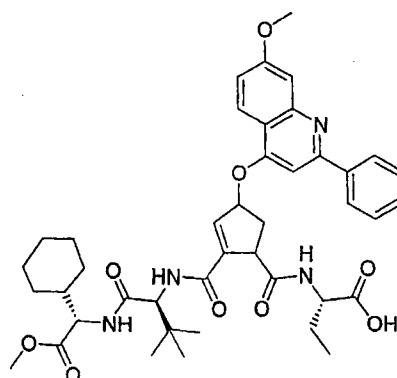
Example 27

(S)-2-{[(((1R,4R) &(1S,4S))-2-{(S)-1-[(((S)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2,2-dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-butyric acid (27)

5   Compound 27 (6.0 mg, 72 %) was prepared from 18 (8.6 mg, 0.011 mmol) according to the method for the preparation of 24. Purification by HPLC (60 % methanol + 0.2 % TEA) gave the title compound as a white powder.

^{1}H-NMR (300 MHz, CD$_3$OD): δ 0.88-0.95 (m, 3H), 0.96 (s, 9H), 0.97-1.24 (m, 4H), 1.57-1.62 (m, 3H), 1.58-1.78 (m, 4H), 1.79-1.99 (m, 1H), 2.35-2.44 (m, 2H), 2.85-2.98

10   (m, 1H), [(3.67 & 3.69) s, 3H], 3.94 (s, 3H), 4.10-4.20 (m, 1H), 4.30-4.40 (m, 3H), 6.00-6.09 (m, 1H), [6.80-6.82 (m, 0.5H)] [6.85-6.87 (m, 0.5H)], 7.05-7.19 (m, 2H), 7.38-7.55 (m, 4H), 7.95-8.07 (m, 3H). ^{13}C-NMR (75.5 MHz, CD$_3$OD): δ [9.1 & 9.2], [24.7 & 24.9], [25.4 & 25.5], [25.9 & 26.0], [28.3 & 28.4], 28.9, [34.8 & 34.9], [35.6 & 35.9], [39.6 & 39.7], [49.9 & 50.1], [51.4 & 51.2], [53.9 & 54.0] 55.0, [57.2 & 57.4],

15   60.0, [82.1 & 82.5], 98.6, 106.2, 114.7, 117.8, 122.7, 127.5, 127.7, [128.4 & 128.5], 129.1, 135.3, 136.3, 141.6, 142.0, 150.5, 159.8, [161.0 & 161.3] [164.0 & 164.1], [171.6 & 171.9], [172.2 & 172.3], [173,0 & 173.2].MALDI-TOF m/z 779.43 [(M +Na)$^+$ calcd for C$_{42}$H$_{52}$N$_4$NaO$_9^+$ 779.36].

20   Example 28
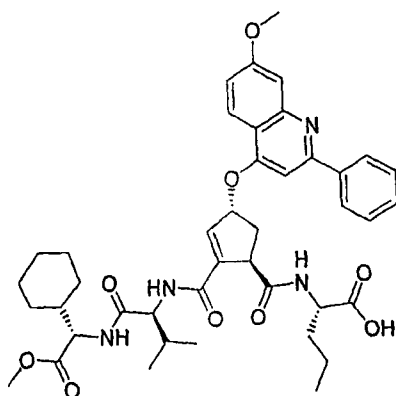
(S)-2-{[(1R,4R)-2-{(S)-1-[((S)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2-
methyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-
enecarbonyl]-amino}-pentanoic acid tert-butyl ester (28)

5   The tert.butyl ester 19a (7.6 mg, 0.0094 mmol) and TES (2.4 mg, 0.021 mmol) were
dissolved in DCM (1 mL) and the mixture was cooled in an ice-bath. TFA (1 mL) was
added. After two hours the mixture was concentrated and purified on HPLC using 60
% MeOH + 0.2 % TEA as mobile phase. This gave 28 (6.1 mg, 86 %) as a slightly
yellow syrup. After lyophilisation the title compound was collected as white powder.

10   ^{1}H-NMR (300 MHz, CD$_3$OD + CDCl3 (1:1)): δ 0.90-1.00 (m, 9H), 1.00-1.30 (m, 7H),
1.50-1.90 (m, 8H), 2.00-2.10 (m, 1H), 2.40-2.50 (m, 1H), 2.85-2.98 (m, 1H), 3.65-3.72
(s, 3H), 3.99 (s, 3H), 4.15-4.22 (m, 1H), 4.24-4.35 (m, 2H), 4.38-4.44 (m, 1H), 6.10-
6.20 (m, 1H), 6.95-6.96 (m, 1H), 7.16-7.23 (m, 1H), 7.31 (s, 1H), 7.42 (d, J = 2.47 Hz,
1H), 7.53-7.72 (m, 3H), 7.97-8.16 (m, 3H); ^{13}C-NMR (75.5 MHz, CD$_3$OD + CDCl$_3$ 1:1):

15   δ 13.5, 18.3, 19.0, 26.0, 29.0, 29.7, 31.0, 34.1, 35.8, 40.2, 51.9, 55.9, 57.7, 58.9,
63.5, 68.4, 84.0, 99.6, 104.8, 105.7, 115.1, 119.0, 123.7, 128.1, 128.9, 129.1, 130.4,
131.3, 135.3, 138.0, 142.9, 159.5, 162.8, 164.8, 172.2, 172.2, 172.4

Example 29
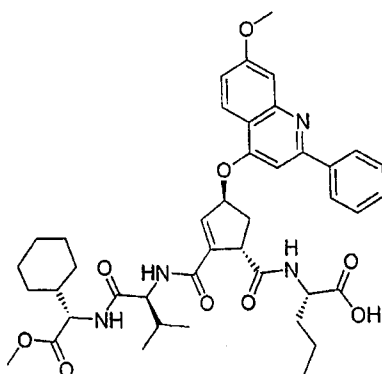
92

(S)-2-{[(1S,4S)-2-{(S)-1-[((S)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2-
methyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-
enecarbonyl]-amino}-pentanoic acid tert-butyl ester (29)

Compound 29 (1.3 mg, 26 %) was prepared from 19b (5.3 mg, 0.065 mmol)
according to the method for the preparation of 28. This gave the title compound as a
white powder.

^{1}H-NMR (300 MHz, CD3OD): δ 0.85-1.00 (m, 9H), 1.00-1.23 (m, 7H), 1.50-1.78 (m,
8H), 2.05-2.23 (m, 1H), 2.50-2.66 (m, 1H), 2.70-2.85 (m, 1H), 3.69 (s, 3H), 3.92 (s,
3H), 4.02-4.16 (m, 1H), 4.20-4.25 (m, 1H), 4.35-4.40 (m, 2H), 6.09 (m, 1H), 7.00 (s,
1H), 7.12-7.18 (dd, J = 2.47, 2.19 Hz, 1H), 7.30 (s, 1H), 7.40 (d, J = 2.42 Hz, 1H),
7.48-7.74 (m, 3H), 8.03-8.10 (m, 3H); ^{13}C-NMR (75.5 MHz, CDCl3): δ 11.7, 16.5,
17.0, 24.4, 27.2, 27.9, 29.0, 29.1 37.5, 41.8, 49.7, 50.5, 53.3, 56.3, 63.5, 66.5, 81.0,
100.3, 101.0, 105.7, 113.6, 121.6, 126.3, 127.1, 127.9, 130.1, 131.4, 135.6, 138.7,
141.1, 150.4, 160.2, 160.5, 165.3, 173.0, 173.6, 173.7

Example 30
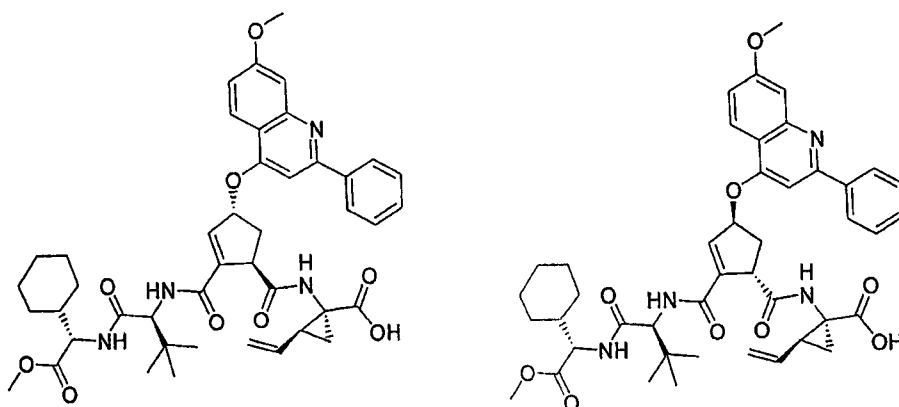
(1R,2S)-1-{[(1R,4R)-2-{(S)-1-[((S)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-
2,2-dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-
enecarbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid (30a) and

1R,2S)-1-{[(1S,4S)-2-{(S)-1-[((S)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-
2,2-dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-
enecarbonyl]-amino}-2-vinyl-cyclopropane-carboxylic acid (30b)

Compound 30a (6.3 mg, 49 %) and compound 30b (5.6 mg, 43 %) were synthesized
from 21 (13.8 mg, 0.0016 mmol) according to the method of the preparation of 22a
and 22b. 30a and 30b: White powder.

30a: ^{1}H-NMR (300 MHz, CD$_3$OD): δ 1.02 (s, 9H), 1.03-1.43 (m, 5H), 1.61-1.95 (m,
8H), 2.11-2.21 (m, 1H), 2.43-2.58 (m, 1H), 2.97-3.04 (m, 1H), 3,78 (s, 3H), 4.01 (s,
3H), 4.02-4.17 (m, 1H), 4.25-4.40 (m, 2H), 5.10-5-20 (m, 1H), 5.27-5.40 (m, 1H),
6.77-6.94 (m, 1H), 6.10-6.20 (m, 1H), 6.97 (s, 1H), 7.18 (dd, J = 2.5, 9.2 Hz, 1H), 7.22
(s, 1H), 7.46 (d, J = 2.5 Hz, 1H), 7.52-7.65 (m, 3H), 8.00-8.18 (m, 3H). ^{13}C-NMR (75.5
MHz, CD$_3$OD): δ 13.5, 25.3, 25.7, 28.3, 28.7, 29.0, 32.8, 34.6, 35.3, 39.3, 49.7, 51.1,
54.6, 57.2, 59.8, 82.1, 98.4, 105.8, 114.5, 116.3, 117.6, 122.6, 127.2, 128.1, 128.2,
128.8, 130.2, 133.7, 136.0, 139.5, 141.5, 150.3, 159.7, 161.0, 161.2, 163,4, 171.6,
172.5. MALDI-TOF m/z 803.56 [(M +Na)$^+$ calcd for C$_{44}$H$_{52}$N$_4$NaO$_9$$^+$ 803.36]. 30b: ^{1}H-
NMR (300 MHz, CD$_3$OD): δ 1.03 (s, 9H), 1.04-1.42 (m, 5H), 2.60-2.90 (m, 8H), 2.17-
2.22 (m, 1H), 2.40-2.55 (m, 1H), 2.96-3.10 (m, 1H), 3.77 (s, 3H), 4.01 (s, 3H), 4.05-
4.16 (m, 1H), 4.30-4.40 (m, 2H), 5.15-5.20 (m, 1H), 5.25-5.40 (m, 1H), 5.78-5.95 (m,
1H), 6.10-6.20 (m, 1H), 6.98 (s, 1H), 7.17 (dd, J = 2.5, 9.1 Hz, 1H), 7.26 (s, 1H), 7.46

(d, J = 2.5 Hz, 1H), 7.50-7.65 (m, 3H), 8.03-8.28 (m, 3H). ^{13}C-NMR (75.5 MHz, CD$_3$OD): δ 13.7, 26.0, 26.3, 28.8, 29.4, 29.6, 34.0, 35.2, 35.8, 40.1, 50.6, 51.7, 55.3, 57.8, 60.6, 83.0, 99.1, 106.3, 115.2, 117.0, 118.3, 123.2, 127.9, 128.0, 128.8, 129.6, 130.6, 134.4, 136.1, 140.0, 142.5, 150.8, 160.3, 161.8, 162.0, 165.7, 172.3, 173.0

Example 31
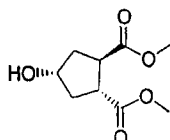
trans-(3*R*,4*R*)-Bis(methoxycarbonyl)cyclopentanol (31)

Sodium borohydride (1.11 g, 0.029 mol) was added to a stirred solution of (1R, 2S)-4-oxo-cyclopentane1,2-dicarboxylic acid dimethyl ester (4.88 g, 0.0244 mol) in methanol (300 mL) at 0 °C. After 1 h the reaction was quenched with 90 mL brine, concentrated and extracted with ethyl acetate. The organic phases were pooled, dried, filtered and concentrated. The crude product was purified by flash column chromatography (toluene/ethyl acetate 1:1) to give 31 (3.73 g, 76%) as a yellow oil.

Example 32
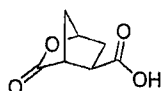
3-Oxo-2-oxa-bicyclo[2.2.1]heptane-5-carboxylic acid (32)

Sodium hydroxide (1M, 74 mL, 0.074 mol) was added to a stirred solution of 31 (3.73 g, 0.018 mol) in methanol (105 mL) at room temperature. After 4 h, the reaction mixture was neutralized with 3M HCl, evaporated and co-evaporated with toluene several times. Pyridine (75 mL) and Ac$_2$O (53 mL) were added and the reaction mixture was allowed to shake overnight at room temperature. The mixture was then co-evaporated with toluene and purified by flash column chromatography (ethyl acetate + 1% acetic acid) to give 32 (2.51 g, 88%) as a yellow oil.

Example 33
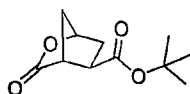
3-Oxo-2-oxa-bicyclo[2.2.1]heptane-5-carboxylic acid *tert*-butyl ester (33)

DMAP (14 mg, 0.115 mmol) and Boc₂O (252 mg, 1.44 mmol) was added to a stirred

5   solution of 32 (180 mg, 1.15 mmol) in 2 mL CH₂Cl₂ under inert argon atmosphere at 0

°C. The reaction was allowed to warm to room temperature and was stirred overnight.

The reaction mixture was concentrated and the crude product was purified by flash

column chromatography (toluene/ethyl acetate gradient 15:1, 9:1, 6:1, 4:1, 2:1) to

give 33 (124 mg, 51%) as white crystals.

10   ¹H-NMR (300 MHz, CD₃OD) δ 1.45 (s, 9H), 1.90 (d, *J* = 11.0 Hz, 1H), 2.10-2.19 (m,

3H), 2.76-2.83 (m, 1H), 3.10 (s, 1H), 4.99 (s, 1H); ¹³C-NMR (75.5 MHz, CD₃OD) δ

27.1, 33.0, 37.7, 40.8, 46.1, 81.1, 81.6, 172.0, 177.7.

<u>Example 34</u>

15
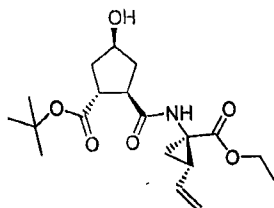
(1*R*,2*R*,4*S*)-2-((1*R*,2*S*)-1-Ethoxycarbonyl-2-vinyl-cyclopropylcarbamoyl)-4-hydroxy-

cyclopentanecarboxylic acid *tert*-butyl ester (34)

Compound 33 (56 mg, 0.264 mmol) was dissolved in dioxane/ water 1:1 (5 mL) and

the mixture was cooled to 0 °C. 1 M lithium hydroxide (0.52 mL, 0.520 mmol) was

20   added and the mixture was stirred at 0 °C for 45 minutes, after which the mixture was

neutralized with 1M hydrochloric acid and evaporated and coevaporated with toluene.

The residue was dissolved in DMF (5 mL) and (1*R*,2*S*)-1-amino-2-vinylcyclopropane

carboxylic acid ethyl ester hydrochloride (60 mg, 0.313 mmol) and

diisopropylethylamine (DIEA) (138 □L, 0.792 mmol) were added and the solution was

25   cooled to 0 °C. HATU (120 mg, 0.316 mmol) was added and the mixture was stirred

for 0.5 h at 0 °C and for an additional 2 h at room temperature. The mixture was then

evaporated and extracted with EtOAc, washed with brine, dried, filtered and concentrated. Purification by flash column chromatography (toluene/ EtOAc 1:1) provided compound 34 (86 mg, 89 %) as a colorless oil.

5 Example 35
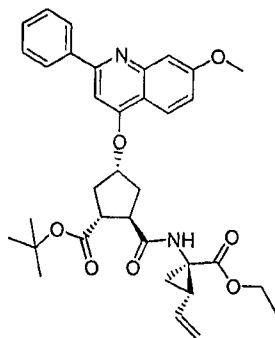
(1R,2R,4R)-2-((1R,2S)-1-Ethoxycarbonyl-2-vinyl-cyclopropylcarbamoyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopentanecarboxylic acid tert-butyl ester (35)

Compound 34 (73 mg, 0.199 mmol) was dissolved in dry THF (4 mL) and 2-phenyl-7-

10 methoxy-4-quinolinol (86 mg, 0.342 mmol) and triphenylphosphine (141 mg, 0.538 mmol) were added. The mixture was cooled to 0°C and DIAD (0.567 mmol) dissolved in 1 mL THF was added dropwise. The mixture was stirred for 48 h at room temperature. The solvent was evaporated and the crude product was purified by flash column chromatography gradient elution (toluene/ EtOAc 9:1, 6:1, 4:1) to give

15 compound 35 (81 mg, 68 %).

Example 36
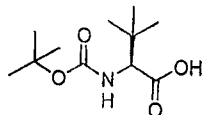
Boc-L-tert-leucine-OH (36)

20 Triethylamine (890 uL, 6.40 mmol) was added dropwise to a stirred solution of L-tert-leucine (300 mg, 2.29 mmol) and di-tert-butyl dicarbonate (599 mg, 2.74 mmol) in dioxane/ water 1:1 (8 mL) and the solution was stirred overnight. The mixture was extracted with petroleum ether (2×) and the aqueous phase was cooled to 0 °C and

carefully acidified to pH 3 by slow addition of 4M $NaHSO_4 \cdot H_2O$. The acidified water phase was extracted with EtOAc (3×) and the combined organic phases were washed with brine (2×) and was then dried, filtered and concentrated to give compound 36 (522 mg, 99 %) as a colorless powder. No further purification was needed.

5   ^{1}H-NMR (300 MHz, CD_3OD) δ 0.99 (s, 9H), 1.44 (s, 9H), 3.96 (s, 1H); ^{13}C-NMR (75.5 MHz, CD_3OD) δ 27.1, 28.7, 34.9, 68.0, 80.5, 157.8, 174.7.

Example 37
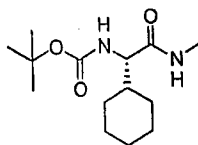
10   ((S)-Cyclohexyl-methylcarbamoyl-methyl)-carbamic acid *tert*-butyl ester (37)
Boc-Chg-OH (387 mg, 1.50 mmol) was coupled to methylamine hydrochloride (111 mg, 1.65 mmol) using the same HATU coupling conditions as in the synthesis of compound 34. The crude product was extracted with EtOAc, washed with brine and concentrated. Purification by flash column chromatography (EtOAc) provided

15   compound 37 (307 mg, 76 %) as a colorless solid.
^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.91-1.13 (m, 2H), 1.14-1.31 (m, 3H), 1.44 (s, 9H), 1.61-1.80 (m, 6H), 2.80 (d, J = 4.7 Hz, 3H), 3.91 (dd, J = 7.1, 9.1 Hz, 1H), 5.23 (b, 1H), 6.52 (bs, 1H); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 25.9, 26.0, 26.1, 28.3, 28.5, 29.6, 40.5, 59.5, 79.7, 155.9, 172.4.

20

Example 38
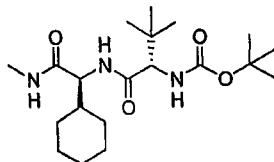
{(S)-1-[((S)-Cyclohexyl-methylcarbamoyl-methyl)-carbamoyl]-2,2-dimethyl-propyl}-carbamic acid *tert*-butyl ester (38)

To a solution of compound 37 (98 mg, 0.362 mmol) in methylene chloride (3 mL)
were added triethylsilane (115 mL, 0.742 mmol) and TFA (3 mL). The mixture was
stirred for 2 h at room temperature and was then evaporated and coevaporated with
toluene. The deprotected amine was dissolved in DMF (5 mL) and coupled to

5   compound 36 (84 mg, 0.363 mmol) using the same HATU coupling conditions as in
the synthesis of 34. The crude product was extracted with EtOAc, washed with brine,
dried, filtered and concentrated. Purification by flash column chromatography
(toluene/ EtOAc 1:1) provided compound 38 (128 mg, 92 %) as a colorless solid.

10   ^{1}H-NMR (300 MHz, CDCl$_3$) δ 0.99 (s, 9H), 1.02-1.30 (m, 5H), 1.44 (s, 9H), 1.58-1.77
(m, 4H), 1.78-1.89 (m, 2H), 2.79 (d, J = 4.7 Hz, 3H), 4.11 (d, J = 9.3 Hz, 1H), 4.33
(app. t, J = 8.5 Hz, 1H), 5.65 (b, 1H), 7.25 (b, 1H), 7.39 (b, 1H); ^{13}C-NMR (75.5 MHz,
CDCl$_3$) δ 25.9, 25.9, 26.0, 26.2, 26.8, 28.4, 29.0, 29.7, 34.5, 39.7, 58.4, 62.4, 79.4,
156.0, 171.4, 171.8.

15

Example 39
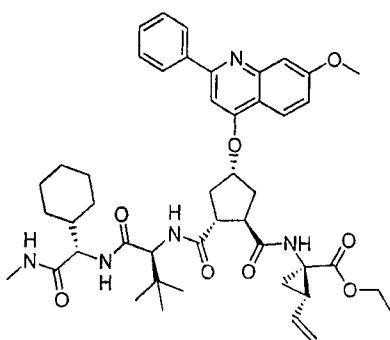
(1R,2S)-1-{[(1R,2R,4S)-2-{(S)-1-[(((S)-Cyclohexyl-methylcarbamoyl-methyl)-
carbamoyl]-2,2-dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-
20   cyclopentanecarbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid ethyl ester (39)
To a solution of compound 35 (30 mg, 0.050 mmol) in methylene chloride (1.5 mL)
were added triethylsilane (21 □L, 0.132 mmol) and TFA (1.5 mL). The mixture was
stirred for 2 h at room temperature and was then evaporated and coevaporated with
toluene. The amine 38 (1.3 eq) was deprotected in the same manner as compound
25   35 and was then coupled to deprotected compound 35 using the same HATU

coupling conditions as in the synthesis of 34. The crude product was extracted with EtOAc, washed with brine, dried, filtered and concentrated. Purification using HPLC (MeOH/ water 9:1 + 0.2% triethylamine) provided compound 39 (30 mg, 74%) as a colorless solid.

^{1}H-NMR (300 MHz, CD$_3$OD) δ 0.81-1.14 (m, 4H), 0.99 (s, overlapped, 9H), 1.21 (t, J = 7.1 Hz, 3H), 1.35-1.51 (m, 4H), 1.52-1.65 (m, 3H), 1.66-1.72 (m, 2H), 2.03-2.20 (m, 2H), 2.24-2.39 (m, 1H), 2.46-2.56 (m, 1H), 2.66 (s, 3H), 2.72-2.85 (m, 1H), 3.39-3.48 (m, 2H), 3.90 (s, 3H), 4.03-4.15 (m, 3H), 4.44 (s, 1H), 5.09 (dd, J = 1.9, 10.3 Hz, 1H), 5.19-5.27 (m, 1H), 5.25 (dd, overlapped, 1H), 5.79 (ddd, J = 8.8, 10.3, 17.2 Hz, 1H), 6.99 (s, 1H), 7.07 (dd, J = 2.5, 9.1, Hz, 1H), 7.29 (d, J = 2.5 Hz, 1H), 7.43-7.52 (m, 3H), 7.86-7.98 (m, 2H), 8.05 (d, J = 9.3 Hz, 1H); ^{13}C-NMR (75.5 MHz, CD$_3$OD) δ 14.7, 23.4, 26.0, 26.9, 27.1, 27.3, 30.1, 30.7, 35.0, 35.4, 38.3, 38.8, 40.9, 41.0, 47.9, 55.9, 59.6, 62.0, 62.4, 79.8, 99.9, 107.3, 116.4, 118.0, 119.1, 124.4, 128.9, 129.8, 130.5, 135.3, 141.3, 152.1, 161.1, 162.4, 163.0, 171.6, 172.5, 173.7, 175.2, 176.8. Maldi-TOF-spectrum: (M+H)$^+$ calcd: 810.4, found: 810.5; (M+Na)$^+$ calcd: 832.4, found: 832.4; (M+K)$^+$ calcd: 848.5, found: 848.4.
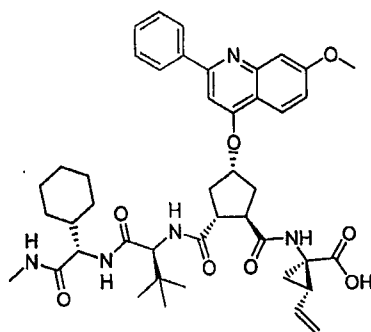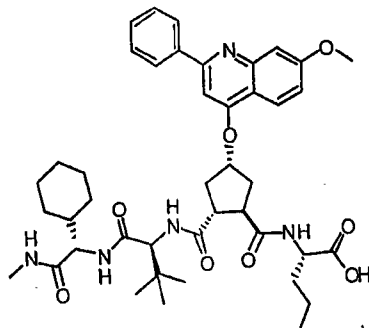
Example 40

(1R,2S)-1-{[(1R,2R,4S)-2-{(S)-1-[((S)-Cyclohexyl-methylcarbamoyl-methyl)-carbamoyl]-2,2-dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopentanecarbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid (40)

To a solution of compound 39 (20 mg, 0.025 mmol) in THF/MeOH/water 2:1:1 (2 mL) at 0 °C was added 1M LiOH (175 uL, 0.175 mmol) and the solution was allowed to

attain room temperature and was stirred for 48 h. The solution was acidified to pH 3
with 1M HCl and was then evaporated and coevaporated with toluene. The crude
product was purified by HPLC (MeOH/ water 6:4 + 0.5% TFA followed by MeOH/
water 4:1 + 0.2% TFA) to give compound 40 (13 mg, 67 %) as a colorless solid.

5 ^{1}H-NMR (300 MHz, CD$_3$OD) δ 0.82-0.98 (m, 1H), 1.01 (s, 9H), 1.05-1.26 (m, 3H),
1.34-1.43 (m, 1H), 1.49-1.77 (m, 8H), 2.10-2.21 (m, 1H), 2.28-2.42 (m, 2H), 2.50-2.61
(m, 1H), 2.64 (s, 3H), 2.68-2.81 (m, 1H), 3.36-3.45 (m, 2H), 4.04-4.11 (m, 1H), 4.06
(s, overlapped, 3H), 4.27 (d, J = 8.8 Hz, 1H), 5.10 (dd, J = 1.8, 10.3 Hz, 1H), 5.28 (dd,
J = 1.8, 17.2 Hz, 1H), 5.59-5.68 (m, 1H), 5.82 (ddd, J = 9.1, 10.3, 17.2 Hz, 1H), 7.44
10 (dd, J = 2.5, 11.8 Hz, 1H), 7.50 (s, 1H), 7.53 (d, J = 2.5 Hz, 1H), 7.69-7.78 (m, 3H),
8.02-8.07 (m, 2H), 8.39 (d, J = 9.3 Hz, 1H); ^{13}C-NMR (75.5 MHz, CD$_3$OD) δ 23.5,
26.0, 26.9, 27.2, 27.3, 30.0, 30.7, 34.7, 35.3, 37.0, 38.7, 41.0, 41.3, 47.4, 56.9, 59.4,
62.7, 83.9, 100.4, 102.2, 116.2, 117.7, 121.7, 126.7, 129.8, 130.8, 133.4, 133.9,
135.6, 143.5, 158.0, 166.6, 168.6, 172.5, 173.4, 173.6, 175.4, 176.4. Maldi-TOF-
15 spectrum: (M+H)$^+$ calcd: 782.4, found: 782.2; (M+Na)$^+$ calcd: 804.4, found: 804.2;
(M+K)$^+$ calcd: 820.5, found: 820.2.

Example 41
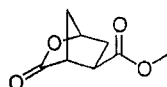
20 3-Oxo-2-oxa-bicyclo[2.2.1]heptane-5-carboxylic acid methyl ester (41)
Compound 32 (1.014 g, 6.50 mmol) was dissolved in acetone (35 mL) before methyl
iodide (13.68 g, 96.4 mmol) and silver(I)oxide (1.61 g, 6.95 mmol) were added. After
stirring for 3h the mixture was filtered through celite and the filtrate was evaporated
before purification by flash column chromatography (toluene/ethyl acetate 4:1) was
25 performed yielding the methyl ester 41 (702 mg, 64 %) as white crystals.
^{1}H-NMR (300 MHz, CDCl$_3$): δ 1.96 (d, J = 10.7 Hz, 1H), 2.21-2.25 (m, 3H), 2.91-2.95
(m, 1H), 3.16 (s, 1H), 3.75 (s, 3H), 4.98 (app. s, 1H).

Example 42
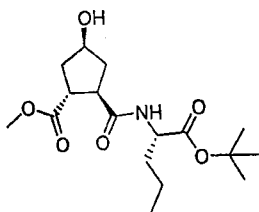
(1*R*,2*R*,4*S*)-2-((*S*)-1-*tert*-Butoxycarbonyl-butylcarbamoyl)-4-hydroxy-
cyclopentanecarboxylic acid methyl ester (42)

Compound 41 (263 mg, 1.55 mmol) and H-Nva-O*t*Bu (420 mg, 2.42 mmol) were
5   dissolved in dry THF (20 mL). DIEA (530 uL, 3.04 mmol) and 2-hydroxypyridine (260
mg, 2.73 mmol) were added and the mixture was refluxed for five days. The solvent
was evaporated and the crude product was purified by flash column chromatography
(toluene/ EtOAc 1:2) to give 42 (510 mg, 96%).

10   Example 43
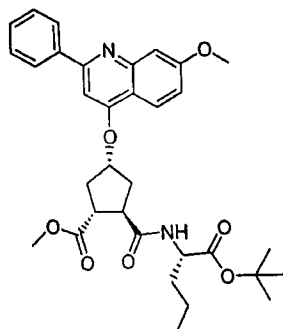
(1*R*,2*R*,4*R*)-2-((*S*)-1-*tert*-Butoxycarbonyl-butylcarbamoyl)-4-(7-methoxy-2-phenyl-
quinolin-4-yloxy)-cyclopentanecarboxylic acid methyl ester (43)

Compound 42 (249 mg, 0.725 mmol), 2-phenyl-7-methoxy-4-quinolinol (310 mg, 1.23
15   mmol) and PPh₃ (580 mg, 2.21 mmol) were dissolved in dry THF and the temperature
was lowered to 0°C. DIAD (435 uL. 2.21 mmol) dissolved in 2 mL dry THF, was
added to the mixture during five minutes. After two hours the temperature was raised
to room temperature and the solution was stirred overnight. Evaporation and
purification by flash column chromatography (toluene/ EtOAc gradient 6:1 to 4:1)
20   gave 43 (324 mg, 78%).

Example 44
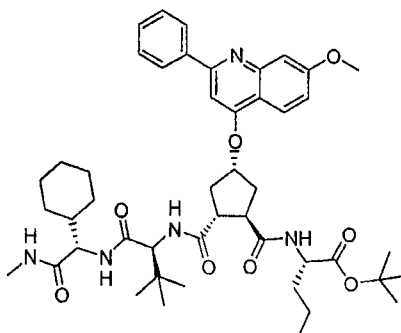
(*S*)-2-{[(1*R*,2*R*,4*S*)-2-{(*S*)-1-[((*S*)-Cyclohexyl-methylcarbamoyl-methyl)-carbamoyl]-
2,2-dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-
5   cyclopentanecarbonyl]-amino}-pentanoic acid *tert*-butyl ester (44)

Compound 43 (38 mg, 0.066 mmol) was dissolved in dioxane/ water 1:1 (4 mL) and
the solution was cooled to 0 °C and 1 M LiOH (132 ul, 0.132 mmol) was added. The
temperature was raised to room temperature and the solution was stirred for 2 hours
after which it was neutralized by addition of 1M HCl and evaporated and
10   coevaporated with toluene. The residue and deprotected amine 38 (1.1 eq) was
dissolved in DMF and coupled using the standard HATU coupling conditions as in the
synthesis of compound 34. The crude product was extracted with EtOAc, washed
with brine, dried, filtered and concentrated. Purification with HPLC (MeOH/ water 9:1
+ 0.2% TEA) provided compound 44 (44 mg, 81 %) as a colorless solid.
15   ^{1}H-NMR (CDCl$_3$, 300 MHz) rotamers (5:1) δ 0.79 (t, *J* = 7.3 Hz, 3H), 0.85-1.19 (m,
3H), 0.93 (s, overlapped, 9H), 1.20-1.35 (m, 2H), 1.39 (s, 1.5 H), 1.43 (s, 7.5 H), 1.54-
1.79 (m, 6H), 2.06-2.28 (m, 3H), 2.39-2.51 (m, 2H), 2.66-2.78 (m, 1H), 2.74 (d,
overlapped, *J* = 4.7 Hz, 3H), 3.42-3.68 (m, 2H), 3.84 (s, 2.5 H), 3.88 (s, 0.5 H), 4.19
(t, *J* = 8.9 Hz, 1H), 4.39-4.59 (m, 1H), 4.68 (d, *J* = 9.6 Hz, 1H), 5.04-5.14 (m, 1H),
20   6.77 (s, 1H), 6.88-7.06 (m, 2H), 7.26-7.47 (m, 6H), 7.53 (b, 1H), 7.85-7.97 (m, 3H);
^{13}C-NMR (75.5 MHz, CDCl$_3$) δ 13.7, 18.7, 25.6, 25.7, 26.0, 26.7, 28.0, 28.9, 29,7,
34.5, 34.7, 37.7, 38.0, 39.2, 46.6, 47.7, 52.7, 55.3, 58.5, 60.3, 77.9, 81.7, 98.0, 107.4,
115.0, 117.9, 122.8, 127.4, 128.6, 129.0, 140.2, 151.2, 158.9, 160.6, 161.1, 170.9,
171.6, 171.8, 172.7, 173.3. Maldi-TOF-spectrum: (M+H)$^+$ calcd: 828.5, found: 828.6;
25   (M+Na)$^+$ calcd: 850.5, found: 850.6; (M+K)$^+$ calcd: 866.6, found: 866.6.

Example 45

(S)-2-{[(1R,2R,4S)-2-{(S)-1-[((S)-Cyclohexyl-methylcarbamoyl-methyl)-carbamoyl]-
2,2-dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-
cyclopentanecarbonyl]-amino}-pentanoic acid (45)

Compound 44 (21 mg, 0.025 mmol) was dissolved in CH_2Cl_2 (1.5 mL) and
triethylsilane (10 uL, 0.063 mmol) and TFA (1.5 mL) were added. The solution was
stirred for 2 hours at room temperature after which the solvents were evaporated and
co-evaporated with toluene to provide compound 45 (20 mg, 100 %) as a colorless
solid.

^{1}H-NMR (300 MHz, CD$_3$OD) δ 0.93 (t, overlapped, 3H), 0.98 (s, 9H), 0.99-1.25 (m,
4H), 1.30-1.49 (m, 3H), 1.50-1.90 (m, 8H), 2.25-2.39 (m, 2H), 2.54-2.62 (m, 1H), 2.64
(s, 3H), 2.72-2.87 (m, 1H), 3.34-3.57 (m, 3H), 4.02-4.13 (m, 1H), 4.06 (s, overlapped,
3H), 4.27-4.36 (m, 1H), 4.37-4.47 (m, 1H), 5.57-5.66 (m, 1H), 7.45 (dd, J = 2.3, 9.2
Hz, 1H), 7.48 (s, 1H), 7.54 (d, J = 2.2 Hz, 1H), 7.69-7.79 (m, 3H), 8.01-8.07 (m, 2H),
8.42 (d, J = 9.3 Hz, 1H); ^{13}C-NMR (75.5 MHz, CD$_3$OD) δ 14.0, 20.2, 26.0, 26.9, 27.2,
30.1, 30.7, 34.6, 35.3, 37.2, 39.1, 41.2, 47.7, 53.7, 56.9, 59.4, 59.5, 62.5, 83.7, 100.4,
101.3, 102.2, 116.2, 121.7, 126.7, 129.8, 130.8, 133.3, 133.9, 143.5, 157.9, 166.6,
168.5, 172.5, 173.6, 175.3, 175.4, 175.5.

Maldi-TOF-spectrum: (M+H)$^+$ calcd: 772.4, found: 772.6; (M+Na)$^+$ calcd: 794.4, found:
794.6; (M+K)$^+$ calcd: 810.5, found: 810.6.

Example 46
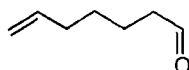
Hept-6-enal (46)

To a solution of hept-6-en-1-ol (1 mL, 7.44 mmol) and N-methylmorpholine N-oxide
(1.308 g, 11.17 mmol) in DCM (17 mL) was added ground molecular sieves (3.5 g, 4
5   Å). The mixture was stirred for 10 min at room temperature under nitrogen
atmosphere before tetrapropylammonium perruthenate (TPAP) (131 mg, 0.37 mmol)
was added. After stirring for additional 2.5 h the solution was filtered through celite.
The solvent was then carefully evaporated and the remaining liquid was purified by
flash column chromatography (DCM) to give the volatile aldehyde 46 (620 mg, 74%)
10 as an oil.

Example 47
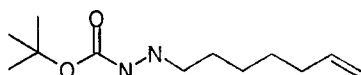
N'-Hept-6-en-(E)-ylidene-hydrazinecarboxylic acid tert-butyl ester (47)

15 To a solution of 46 (68 mg, 0.610 mmol) and tert-butyl carbazate (81 mg, 0.613
mmol) in MeOH (5 mL) was added ground molecular sieves (115 mg, 3Å). The
mixture was stirred for 3 h after which it was filtered through celite and evaporated.
The residue was dissolved in dry THF (3 mL) and AcOH (3mL). NaBH$_3$CN (95 mg,
1.51 mmol) was added and the solution was stirred over night. The reaction mixture
20 was diluted with saturated NaHCO$_3$ solution (6 mL) and EtOAc (6 mL). The organic
phase was washed with brine, saturated NaHCO$_3$, brine, dried over MgSO$_4$ and
evaporated. The cyanoborane adduct was hydrolyzed by treatment with MeOH (3
mL) and 2 M NaOH (1.9 mL). The mixture was stirred for 2 h and the MeOH was
evaporated. H$_2$O (5 mL) and DCM (5 mL) were added and the water phase was
25 extracted three times with DCM. The combined organic phases were dried and
evaporated. Purification by flash column chromatography (toluene/ethyl acetate 9:1
with 1 % triethylamine and toluene/ethyl acetate 6:1 with 1 % triethylamine) provided
47 (85 mg, 61 %) as an oil.

Example 48
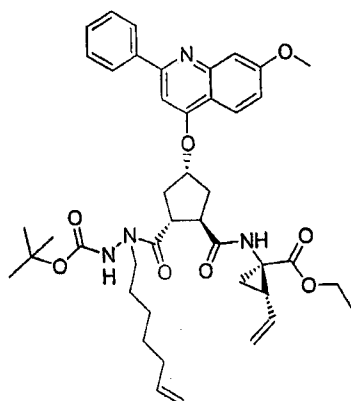
(1*R*,2*S*)-1-{[(1*R*,2*R*,4*R*)-2-(*N'*-*tert*-Butoxycarbonyl-*N*-hept-6-enyl-hydrazinocarbonyl)-
4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopentanecarbonyl]-amino}-2-vinyl-
cyclopropanecarboxylic acid ethyl ester (48)

Scaffold molecule 35 (135 mg, 0.225 mmol) and triethylsilane (71 μL, 0.447 mmol)
was dissolved in DCM (2 mL) after which trifluoroacetic acid (TFA) (2 mL) was added.
The mixture was stirred for 2 h and thereafter co-evaporated with toluene in order to
remove the TFA. The residue was dissolved in DMF (3 mL) and 47 (60 mg, 0.263
mmol) and DIEA (118 μL, 0.677 mmol) were added. The temperature was lowered to
0° C and the coupling reagent *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-
tetramethyluronium hexafluorophosphate (HATU) (94 mg, 0.247 mmol) was added.
The cold solution was allowed to stir for half an hour and then for additional 16 h in
room temperature. The solvent was removed by heating the reaction flask in a water
bath under diminished pressure. The residue was thereafter dissolved in ethyl acetate
and the organic phase was washed three times with brine, dried, filtered and
evaporated. Purification by HPLC (MeOH/H₂O 90:10 with 0.2 % triethylamine) gave
48 (140 mg, 82 %) as an oil.

¹H-NMR (300 MHz, CDCl₃, 40° C): δ 1.22 (t, *J* = 7.1 Hz, 3H), 1.28-1.42 (m, 6H), 1.46
(s, 9H), 1.52-1.62 (m, 2H), 1.82-1.91 (m, 1H), 1.96-2.16 (m, 3H), 2.18-2.34 (m, 2H),
2.42-2.56 (m, 1H), 2.58-2.72 (m, 1H), 3.42 (app. bs, 3H), 3.66-3.84 (m, 1H), 3.92 (s,
3H), 4.15 (q, *J* = 7.1 Hz, 2H), 4.88-5.02 (m, 2H), 5.07-5.18 (m, 2H), 5.20-5.32 (m, 1H),
5.63-5.84 (m, 2H), 6.62 (bs, 1H), 6.94 (s, 1H), 7.09 (dd, *J* = 2.6, 9.2 Hz, 1H), 7.36-

7.51 (m, 4H), 7.99-8.10 (m, 3H); ^{13}C-NMR (75.5 MHz, CDCl$_3$): δ 14.3, 23.0, **26.4**, **26.6**, **28.3**, **28.6**, **33.2**, **33.5**, **35.6**, **37.6**, **40.6**, **44.7**, **47.1**, **48.6**, 55.5, 61.5, 81.9, **98.4**, 107.9, 114.5, 115.6, 118.1, 123.2, 127.6, 128.3, 128.7, 129.1, 133.5, 138.7, **140.7**, **151.5**, **154.5**, **159.2**, **160.9**, **161.5**, **170.5**, **174.2**, **176.3**.
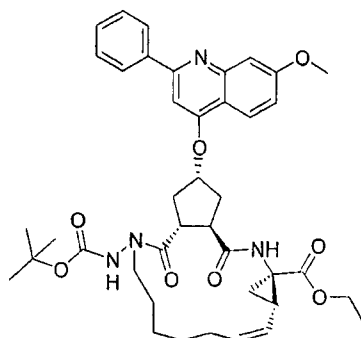
Example 49

(Z)-(1R,4R,6S,16R,18R)-14-*tert*-Butoxycarbonylamino-18-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,15-dioxo-3,14-diaza-tricyclo[14.3.0.0^{4,6}]nonadec-7-ene-4-carboxylic acid ethyl ester (49)

A solution of 48 (158 mg, 0.209 mmol) in dry DCM (25 mL) was bubbled with argon for 5 min. To the stirred solution under argon atmosphere was then added a solution of Hoveyda-Grubbs catalyst 2nd generation (11 mg, 0.018 mmol) in dry DCM (5 mL). The mixture was stirred at reflux under argon atmosphere for 16 h. The solvent was evaporated and purification by HPLC (MeOH/H$_2$O 90:10 with 0.2 % triethylamine) yielded 49 (107 mg, 70 %) as a colorless solid.

^{1}H-NMR (300 MHz, CD$_3$OD): δ 1.03-1.22 (m, 1H), 1.28 (t, J = 7.1 Hz, 3H), **1.32-1.44** **(m, 4H)**, 1.49 (s, 9H), 1.55-1.73 (m, 2H), 1.81-1.91 (m, 1H), 2.04-2.28 (m, 3H), **2.30-2.52 (m, 3H)**, 2.53-2.70 (m, 1H), 2.86-3.00 (m, 1H), 3.34-3.44 (m, 1H), **3.46-3.62 (m, 1H)**, 3.95 (s, 3H), 4.19 (q, J = 7.1 Hz, 2H), 4.32-4.48 (m, 1H), 5.20-5.33 (m, 1H), **5.34 (bs, 1H)**, 5.58-5.70 (m, 1H), 7.10 (s, 1H), 7.14 (dd, J = 2.5, 9.1 Hz, 1H), 7.39 (d, J = 2.5 Hz, 1H), 7.45-7.55 (m, 3H), 8.00 (d, J = 8.0 Hz, 2H), 8.17 (d, J = 9.3 Hz, 1H); ^{13}C-NMR (75.5 MHz, CD$_3$OD): δ 14.6, 23.4, 27.5, 27.7, 28.0, 28.5, 30.7, 36.1, **38.1**, **42.5**, 45.6, 56.0, 62.7, 79.9, 82.8, 100.2, 107.4, 116.6, 119.1, 124.5, 126.5, 128.9, **129.8**,

130.5, 135.8, 141.5, 152.2, 156.4, 161.3, 162.5, 163.1, 171.9, 175.8, 179.0. MALDI-TOF-spectrum: $(M+H)^+$ calcd: 727.4, found: 727.5.

Example 50
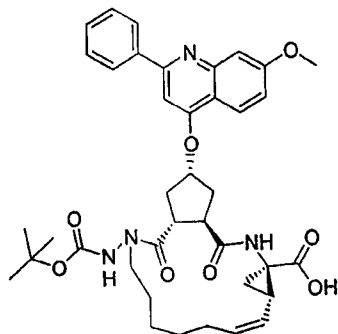
(Z)-(1R,4R,6S,16R,18R)-14-*tert*-Butoxycarbonylamino-18-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,15-dioxo-3,14-diaza-tricyclo[14.3.0.0^{4,6}]nonadec-7-ene-4-carboxylic acid (50)

To a solution of 49 (27 mg, 0.037 mmol) in THF/MeOH/H_2O 2:1:1 (5 mL) was added 1 M LiOH (300 μL, 0.300 mmol). The solution was stirred for 24 h at room temperature and finally for one hour at reflux. After acidification to pH 3-4 with 1 M HCl and evaporation the residue was purified by HPLC (MeOH/H_2O 80:20 and MeOH/H_2O 90:10) providing 50 (12 mg, 46 %) as a colorless solid.

^{1}H-NMR (300 MHz, CD_3OD): δ 1.06-1.24 (m, 1H), 1.26-1.42 (m, 3H), 1.48 (s, 9H), 1.52-1.73 (m, 3H), 1.80-1.90 (m, 1H), 2.02-2.15 (m, 1H), 2.15-2.40 (m, 4H), 2.43-2.54 (m, 1H), 2.54-2.68 (m, 1H), 2.88-3.00 (m, 1H), 3.35-3.48 (m, 1H), 3.49-3.66 (m, 1H), 3.96 (s, 3H), 4.32-4.48 (m, 1H), 5.25-5.42 (m, 2H), 5.56-5.68 (m, 1H), 7.14 (s, 1H), 7.17 (dd, J = 2.5, 9.1 Hz, 1H), 7.40 (d, J = 2.2 Hz, 1H), 7.46-7.58 (m, 3H), 8.00 (d, J = 8.0 Hz, 2H), 8.19 (d, J = 9.1 Hz, 1H); ^{13}C-NMR (75.5 MHz, CD_3OD): δ 23.6, 26.8, 27.8, 28.3, 28.5, 30.5, 35.8, 38.1, 43.0, 45.5, 56.0, 80.2, 82.7, 100.4, 106.9, 116.6, 119.2, 124.7, 127.4, 129.0, 129.8, 130.7, 134.8, 140.9, 151.6, 156.5, 161.1, 163.0, 163.4, 173.8, 175.7, 179.3.

Example 51

108
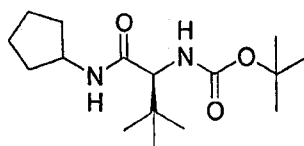
((S)-1-Cyclopentylcarbamoyl-2,2-dimethyl-propyl)-carbamic acid *tert*-butyl ester (51)

To a cold solution of 36 (133 mg, 0.575 mmol), cyclopentylamine (64 µL, 0.648 mmol)

and DIEA (301 µL, 1.73 mmol) in DMF (3 mL) was added the coupling reagent HATU

5 (240 mg, 0.631 mmol). The mixture was stirred for half an hour and for additional two

hours at room temperature. The solvent was removed by heating the reaction flask in

a water bath under diminished pressure and the residue was dissolved in ethyl

acetate, after which the organic phase was washed three times with brine, dried,

filtered and evaporated. Purification by flash column chromatography (toluene/ethyl

10 acetate 4:1) provided 51 (140 mg, 82 %) as colorless crystals. .

^{1}H-NMR (300 MHz, CDCl$_3$): δ 0.95 (s, 9H), 1.28-1.48 (m, overlapped, 2H), 1.40 (s,

9H), 1.49-1.71 (m, 4H), 1.86-2.01 (m, 2H), 3.76 (b, 1H), 4.09-4.23 (m, 1H), 5.32 (b,

1H), 5.91 (b, 1H); ^{13}C-NMR (75.5 MHz, CDCl$_3$): δ 23.6, 23.7, 26.5, 28.3, 32.6, 33.1,

34.5, 51.0, 62.2, 79.4, 155.9, 170.3.
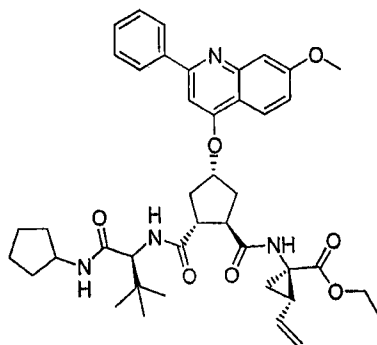
15

Example 52

(1R,2S)-1-{[(1R,2R,4S)-2-((S)-1-Cyclopentylcarbamoyl-2,2-dimethyl-

propylcarbamoyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopentanecarbonyl]-

20 amino}-2-vinyl-cyclopropanecarboxylic acid ethyl ester (52)

Compound 51 (298 mg, 0.048 mmol) and 35 (16 mg, 0.054 mmol) was deprotected

and coupled according to the method for the preparation of 39. Purification by HPLC

(MeOH/H$_2$O 90:10 with 0.2 % triethylamine) gave 52 (22 mg, 63 %) as a colorless solid.

^{1}H-NMR (CDCl$_3$, 300 MHz): δ 0.97 (s, 9H), 1.21 (t, J = 7.1 Hz, 3H), 1.26-1.37 (m, 1H), 1.38-1.46 (m, 2H), 1.48-1.58 (m, 4H), 1.78-1.85 (m, 1H), 1.86-2.02 (m, 3H), 2.03-2.19 (m, 1H), 2.28-2.40 (m, 2H), 2.41-2.54 (m, 1H), 2.64-2.78 (m, 1H), 3.10-3.24 (m, 1H), 3.30-3.44 (m, 1H), 3.95 (s, 3H), 4.04-4.21 (m, 3H), 5.12 (dd, J = 1.7, 10.3 Hz, 1H), 5.14-5.22 (m, 1H), 5.28 (dd, J = 1.7, 17.0 Hz, 1H), 5.59 (b, 1H), 5.75 (ddd, J = 8.8, 10.3, 17.0 Hz, 1H), 6.66-6.82 (m, 2H), 6.99 (s, 1H), 7.09 (dd, J = 2.5, 9.1 Hz, 1H), 7.41-7.55 (m, 4H), 7.99-8.09 (m, 3H); ^{13}C-NMR (75.5 MHz, CDCl$_3$): δ 14.3, 22.9, 23.6, 23.6, 26.7, 32.7, 33.2, 33.7, 34.8, 35.9, 36.6, 40.2, 46.4, 47.5, 51.3, 55.5, 61.1, 61.4, 78.0, 98.4, 107.1, 115.2, 117.9, 118.2, 123.1, 127.6, 128.8, 129.3, 133.5, 159.1, 161.4, 169.4, 169.9, 173.1, 174.0. MALDI-TOF-spectrum: (M+H)$^+$ calcd: 725.4, found: 725.6; (M+Na)$^+$ calcd: 747.4, found: 747.6; (M+K)$^+$ calcd: 763.3, found: 763.5.
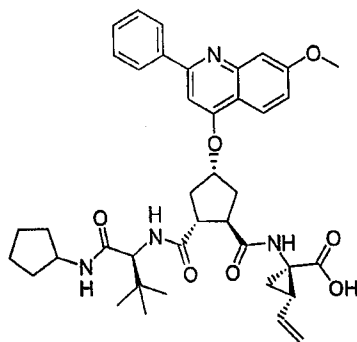
Example 53

(1R,2S)-1-{[(1R,2R,4S)-2-((S)-1-Cyclopentylcarbamoyl-2,2-dimethyl-propylcarbamoyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopentanecarbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid (53)

To a solution of 52 (14 mg, 0.019 mmol) in dioxane/H$_2$O 1:1: (4 mL) was added 1 M LiOH (115 µL, 0.115 mmol). The solution was stirred for 24 h at room temperature. Thereafter an additional portion of LiOH (75 µL, 0.075 mmol) was added and the solution was stirred for another 24 h. After acidification to approximately pH 3 with 1

M HCl and co-evaporation with toluene the residue was purified by HPLC (MeOH/H₂O

70:30 with 0.2 % TFA) yielding 53 (8 mg, 60 %) as a colorless solid.

^{1}H-NMR (300 MHz, CD₃OD): δ 0.98 (s, 9H), 1.28-1.48 (m, 3H), 1.49-1.76 (m, 5H),

1.78-1.94 (m, 2H), 2.10-2.24 (m, 1H), 2.26-2.45 (m, 2H), 2.50-2.62 (m, 1H), 2.66-2.79

(m, 1H), 3.35-3.48 (m, 2H), 3.94-4.03 (m, 1H), 4.06 (s, 3H), 4.16-4.24 (m, 1H), 5.10

(dd, J = 1.8, 10.3 Hz, 1H), 5.29 (dd, J = 1.8, 17.2 Hz, 1H), 5.62 (b, 1H), 5.82 (ddd, J =

9.1, 10.3, 17.2 Hz, 1H), 7.43 (dd, J = 2.5, 9.3 Hz, 1H), 7.50 (s, 1H), 7.50-7.69 (dd,

overlapped, 1H), 7.67-7.80 (m, 3H), 8.01-8.11 (m, 2H), 8.39 (d, J = 9.3 Hz, 1H); ^{13}C-

NMR (75.5 MHz, CD₃OD): δ 24.7, 24.7, 27.3, 33.1, 33.6, 34.7, 35.4, 36.9, 38.7, 41.0,

47.4, 52.3, 56.9, 62.3, 83.9, 100.4, 102.3, 116.2, 117.7, 121.6, 126.7, 129.8, 130.8,

133.4, 133.8, 135.6, 143.5, 158.0, 166.5, 168.6, 171.9, 173.4, 175.2, 176.4. MALDI-

TOF-spectrum: (M+H)$^+$ calcd: 697.4, found: 697.3; (M+Na)$^+$ calcd: 718.7, found:

719.3; (M+K)$^+$ calcd: 735.3, found: 735.3.

15   Example 54
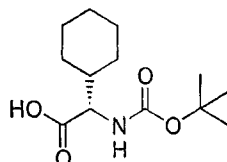
(S)-tert-Butoxycarbonylamino-cyclohexyl-acetic acid methyl ester (54)

To a solution of Boc-Chg-OH (53 mg, 0.206 mmol) in acetone (3 mL) were added

methyl iodide (195 µL, 3.1 mmol) and silver (I) oxide (53 mg, 0.229 mmol). The

20   mixture was allowed to stir over night in a reaction flask that was covered with

aluminium foil. Thereafter the solution was filtered through celite and evaporated.

Purification by flash column chromatography (toluene/ethyl acetate 15:1) provided

methyl ester 54 (56 mg, 100 %) as a colorless oil.

^{1}H-NMR (300 MHz, CDCl₃): δ 1.00-1.34 (m, 5H), 1.44 (s, 9H), 1.54-1.82 (m, 6H), 3.73

25   (s, 3H), 4.20 (dd, J = 2.8, 5.0 Hz, 1H), 5.05 (bs, 1H); ^{13}C-NMR (75.5 MHz, CDCl₃): δ

26.0, 28.2, 28.3, 29.5, 41.1, 52.0, 58.3, 79.7, 155.6, 172.9.

Example 55
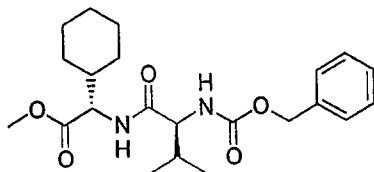
(S)-((S)-2-Benzyloxycarbonylamino-3-methyl-butyrylamino)-cyclohexyl-acetic acid
methyl ester (55)

Compound 54 (93 mg, 0.343 mmol) was deprotected and coupled to Z-Val-OH (95
mg, 0.378 mmol) according to the method for the preparation of 39. Flash column
chromatography (toluene/ethyl acetate 4:1) gave 55 (131 mg, 94 %) as a colorless
solid.

^{1}H-NMR (300 MHz, CDCl$_3$): δ 0.92-1.30 (m, 11H), 1.54-1.88 (m, 6H), 2.02-2.18 (m,
1H), 3.72 (s, 3H), 4.05-4.18 (m, 1H), 4.52 (dd, J = 3.0, 5.5 Hz, 1H), 5.12 (s, 2H), 5.49
(bs, 1H), 6.52 (bs, 1H), 7.34 (s, 5H); ^{13}C-NMR (75.5 MHz, CDCl$_3$): δ 17.8, 19.0, 25.8,
28.2, 29.3, 31.2, 40.5, 51.9, 56.8, 60.0, 66.8, 127.7, 127.9, 128.1, 128.3, 136.2,
156.3, 171.3, 172.2.

Example 56
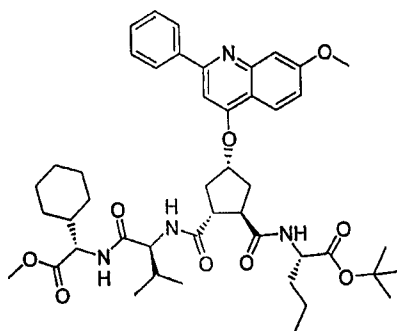
(S)-2-{[(1R,2R,4S)-2-{(S)-1-[((S)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2-
methyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-
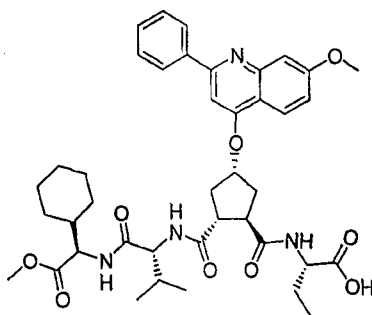cyclopentanecarbonyl]-amino}-pentanoic acid tert-butyl ester (56)

To a solution of 55 (40 mg, 0.099 mmol) in ethanol (95%) (7.5 mL) was added
palladium on active carbon (10 %, 40 mg) and the mixture was hydrogenated under
pressure at room temperature for 2 h. The mixture was filtered through celite and
evaporated. Compound 43 (38 mg, 0.083 mmol) was dissolved in dioxane/H$_2$O 1:1 (3

mL) and the mixture was cooled to 0° C before 1 M LiOH (140 µL, 0.140 mmol) was
added to the stirred solution. After 1h the mixture was neutralized with 1 M
hydrochloric acid and the solvent was evaporated and co-evaporated with toluene.
The residue was coupled to deprotected 55 using the same HATU coupling

5   conditions as in the synthesis of compound 48. Purification by HPLC (MeOH/H$_2$O
90:10 with 0.2 % triethylamine) gave 56 (56 mg, 88 %) as a colorless solid.
^{1}H-NMR (300 MHz, CDCl$_3$): δ 0.82-0.96 (m, 9H), 0.82-1.22 (m, overlapped, 6H), 1.23-
1.40 (m, 2H), 1.44 (s, 9H), 1.50-1.69 (m, 4H), 1.71-1.87 (m, 2H), 1.95-2.06 (m, 1H),
2.07-2.22 (m, 1H), 2.28-2.54 (m, 3H), 2.60-2.75 (m, 1H), 3.08-3.28 (m, 1H), 3.30-3.49

10 (m, 1H), 3.70 (s, 3H), 3.94 (s, 3H), 4.28-4.38 (m, 1H), 4.41-4.57 (m, 2H), 5.17 (b, 1H),
6.54-6.70 (m, 2H), 6.74 (b, 1H), 6.95 (s, 1H), 7.09 (dd, J = 2.5, 9.1 Hz, 1H), 7.39-7.55
(m, 5H), 7.98-8.10 (m, 3H); ^{13}C-NMR (75.5 MHz, CDCl$_3$): δ 13.7, 18.1, 18.6, 19.2,
25.9, 28.0, 28.2, 29.6, 30.7, 34.6, 36.5, 37.6, 40.8, 47.4, 47.5, 52.1, 52.8, 55.5, 56.8,
58.9, 77.8, 82.0, 98.3, 107.5, 115.3, 118.1, 123.1, 127.5, 128.7, 129.1, 140.5, 151.4,

15 159.2, 160.7, 161.3, 171.0, 171.5, 172.3, 172.8, 173.0. MALDI-TOF-spectrum:
(M+H)$^+$ calcd: 815.5, found: 815.7; (M+Na)$^+$ calcd: 837.4, found: 837.6; (M+K)$^+$ calcd:
853.4, found: 853.6.

Example 57
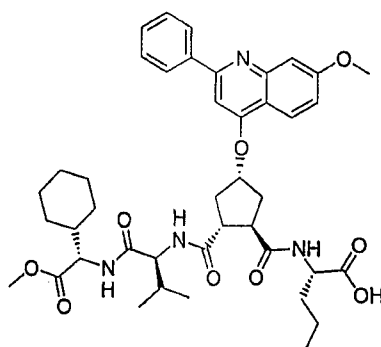
20

(S)-2-{[(1R,2R,4S)-2-{(S)-1-[((S)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2-
methyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-
cyclopentanecarbonyl]-amino}-pentanoic acid (57)

Tert.butyl ester 56 (28 mg, 0.034 mmol) and triethylsilane (14 μL, 0.088 mmol) was dissolved in DCM (2 mL) after which trifluoroacetic acid (2 mL) was added and the mixture was stirred for 2 h. Co-evaporation with toluene gave 57 (26 mg, 100 %) as a colorless solid.

5   ^{1}H-NMR (300 MHz, CD$_3$OD): δ 0.86-1.00 (m, 9H), 1.01-1.24 (m, 4H), 1.36-1.46 (m, 2H), 1.48-1.75 (m, 8H), 1.70-1.89 (m, overlapped, 1H), 1.96-2.12 (m, 1H), 2.22-2.40 (m, overlapped, 2H), 2.49-2.64 (m, 1H), 2.72-2.91 (m, 1H), 3.26-3.40 (m, overlapped, 1H), 3.50-3.68 (m, overlapped, 1H), 3.62 (s, 3H), 4.05 (s, 3H), 4.09-4.17 (m, 1H), 4.17-4.25 (m, 1H), 4.35-4.45 (m, 1H), 5.62 (b, 1H), 7.44 (dd, J = 2.2, 9.3 Hz, 1H), 7.49

10   (s, 1H), 7.53 (d, J = 2.2 Hz, 1H), 7.65-7.78 (m, 3H), 7.98-8.06 (m, 2H), 8.41 (dd, J = 2.8, 9.3 Hz, 1H); ^{13}C-NMR (CD$_3$OD, 75.5 MHz): δ 13.9, 18.8, 19.7, 20.2, 27.0, 29.7, 30.5, 31.8, 34.6, 37.7, 38.9, 41.1, 47.8, 52.3, 53.6, 56.9, 58.8, 58.9, 60.3, 83.8, 100.4, 102.2, 116.2, 121.6, 126.7, 129.8, 130.8, 133.3, 133.8, 143.5, 157.9, 166.5, 168.5, 173.3, 173.9, 175.5, 175.5, 175.6. MALDI-TOF-spectrum: (M+H)$^+$ calcd: 759.4, found:

15   759.7; (M+Na)$^+$ calcd: 781.4, found: 781.7; (M+K)$^+$ calcd: 797.4, found: 797.7.
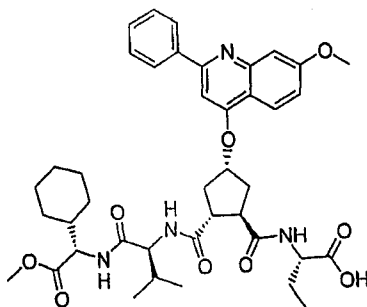
Example 58

(S)-2-{[(1R,2R,4S)-2-{(S)-1-[((S)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2-
20   methyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-
cyclopentanecarbonyl]-amino}-butyric acid (58)
The procedure described in example 42 was followed but with the use of L-2-amino-N-butyric acid tert.butyl ester instead of H-Nva-OtBu. The afforded compound was then reacted as described in example 43 which gave (1R,2R,4R)-2-((S)-1-tert-

25   butoxycarbonyl-propylcarbamoyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-

cyclopentanecarboxylic acid methyl ester. Coupling of this compound with 55 as described in example 56 followed by esterhydrolysis as described in example 57 gave 58 as a colourless solid.

^{1}H-NMR (300 MHz, CD$_3$OD): δ 0.82-0.99 (m, 9H), 0.82-1.40 (m, overlapped, 6H),
1.48-1.78 (m, 6H), 1.80-1.95 (m, 1H), 1.97-2.12 (m, 1H), 2.22-2.40 (m, overlapped, 2H), 2.51-2.64 (m, 1H), 2.71-2.90 (m, 1H), 3.16-3.39 (m, overlapped, 1H), 3.49-3.59 (m, 1H), 3.63 (s, 3H), 3.95 (s, 3H), 4.12-4.23 (m, 2H), 4.28-4.38 (m, 1H), 5.31 (b, 1H), 7.43 (dd, J = 2.2, 9.3 Hz, 1H), 7.47 (s, 1H), 7.51 (s, 1H), 7.66-7.89 (m, 3H), 7.99-8.07 (m, 2H), 8.42 (d, J = 9.1 Hz, 1H); ^{13}C-NMR (75.5 MHz, CD$_3$OD): δ 10.7, 18.8, 19.7, 25.8, 27.0, 27.0, 29.7, 30.5, 31.8, 37.7, 38.9, 41.2, 47.9, 52.3, 55.3, 56.9, 58.8, 60.6, 83.6, 100.7, 102.2, 116.3, 121.5, 126.7, 129.8, 130.8, 133.7, 133.8, 143.9, 158.2, 166.4, 168.3, 173.3, 173.8, 175.2, 175.5, 175.6. MALDI-TOF-spectrum: (M+H)$^+$ calcd: 745.4, found: 744.9; (M+Na)$^+$ calcd: 767.4, found: 766.9; (M+K)$^+$ calcd: 783.5, found: 782.9.

Example 59

(S)-2-{[(1R,2R,4S)-2-{(R)-1-[((R)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2-methyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopentanecarbonyl]-amino}-butyric acid (59)

The procedure described in example 54 was followed but with the use of Boc-D-cyclohexylglycine instead of Boc-L-cyclohexylglycine. The afforded compound was then reacted as described in example 55 followed by coupling with (1R,2R,4R)-2-((S)-1-tert-Butoxycarbonyl-pentylcarbamoyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopentanecarboxylic acid methyl ester as described in example 56. Removal of the

ester group as described in example 57 gave compound 59 as a colourless solid.
^{1}H-NMR (CD$_3$OD, 300 MHz): δ 0.82-1.02 (m, 9H), 1.04-1.42 (m, 6H), 1.52-1.80 (m,
6H), 1.80-1.96 (m, overlapped, 1H), 2.00-2.14 (m, 1H), 2.29-2.46 (m, 2H), 2.51-2.65
(m, 1H), 2.68-2.84 (m, 1H), 3.24-3.39 (m, overlapped, 1H), 3.47-3.60 (m, 1H), 3.67 (s,
3H), 4.07 (s, 3H), 4.18-4.27 (m, 2H), 4.28-4.38 (m, 1H), 5.64 (app. bs, 1H), 7.44 (d, J
= 2.3, 6.9 Hz, 1H), 7.42 (s, 2H), 7.67-7.81 (m, 3H), 8.04 (d, J = 7.8 Hz, 2H), 8.41 (d, J
= 9.1 Hz, 1H); ^{13}C-NMR (CD$_3$OD, 75.5 MHz): δ 10.8, 18.5, 19.6, 25.7, 27.1, 27.1,
30.1, 30.6, 31.9, 37.3, 38.2, 41.1, 47.8, 52.3, 55.4, 56.9, 59.0, 59.1, 60.2, 83.8, 100.5,
102.2, 116.3, 121.6, 126.8, 129.8, 130.8, 133.6, 133.8, 143.7, 158.1, 166.5, 168.5,
173.4, 173.8, 175.4, 175.7, 175.7. MALDI-TOF-spectrum: (M+H)$^+$ calcd: 745.4, found:
745.4; (M+Na)$^+$ calcd: 767.4, found: 767.4; (M+K)$^+$ calcd: 783.5, found: 783.3.

Example 60
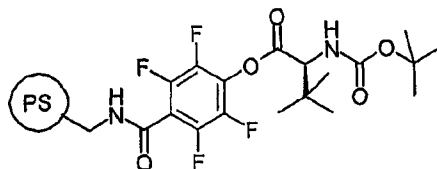
Resin bound 2-tert.butoxycarbonylamino-3,3-dimetylbutyric acid (60)

To Argonaut resin PS-TFP (1.38 mmol/g, 10 g) and 2-tert-butoxycarbonylamino-3,3-
dimethyl-butyric acid (4.5 g, 20.7mmol) was added dichloromethane (40 mL) and
DMF (10 mL). To this mixture was added DMAP (1 g, 8.28 mmol) and then DIC (9.5
mL, 60.7 mmol). After 3 hrs of agitation at RT the resin was filtered and washed
successively with DMF, THF, DCM, THF, DCM and ether and then dried in a vacuum.

Example 61
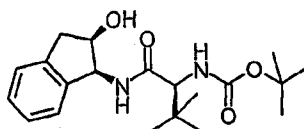
[1-(2-Hydroxy-indan-1-ylcarbamoyl)-2,2-dimethyl-propyl]-carbamic acid tert.butyl ester
(61)

To a portion of 60 (200 mg) in DCM aminoindanol (0.14 mmol) was added. The
mixture was agitated for 2 hrs. The liquid was filtered of and the resin washed with
2xDCM. The combined liquids were combined and concentrated to dryness to afford
the title compound (20.5 mg, 0.055 mmol) Purity >95% by HPLC. M+H$^+$ 363.15.

5   ^{13}C NMR δ_C (100 MHz; CDCl$_3$; Me$_4$Si) 27.0, 28.5, 34.2, 39. 8, 50.8, 57.9, 68.2, 73.7,
124.8, 125.6, 127.4, 128.5, 140.4, 171.6. ^{1}H NMR δ_H (400 MHz; CDCl$_3$; Me$_4$Si) 1.07
(9H, s, CCH$_3$), 1.44 (9H, s, OCCH$_3$), 2.93 (1H, dd, J_{gem}16.4 Hz, $J_{3,2}$ 2.3 Hz, CH$_2$), 3.15
(1H, dd, J_{gem}16.4 Hz, $J_{3,2}$ 5.2 Hz, CH$_2$),

10   Example 62
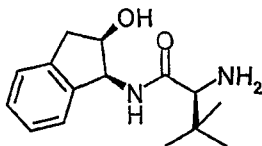
2-Amino-*N*-(2-hydroxy-indan-1-yl)-3,3-dimethyl butyramide (62)
Compound 61 was kept in DCM-TFA 2:1 (2 mL) for 60 min at RT. The solution was
co-evaporated with toluene to dryness.

15

Example 63
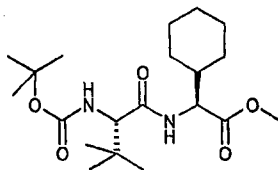
(2-*tert*-Butoxycarbonylamino-3,3-dimethyl-butyrylamino)-cyclohexyl-acetic acid methyl
ester (63)

20   To a solution of 2-*tert*.butoxycarbonylamino-3,3-dimethyl butyric acid (500 mg, 2.16
mmol), Amino-cyclohexyl-acetic acid methyl ester (444 mg, 2.59 mmol) and HATU (2
g, 5.40 mmol) in DMF (20 mL) was added diisopropylethylamine (1.88 mL, 10.8
mmol). The solution was stirred for 1 hrs at r.t. and diluted with dichloromethane (40
mL). This solution was washed with aqueous. NaHCO$_3$ (sat.) and water (x2), dried

25   and concentrated. The product was >95 % pure. M+H$^+$ 385.4.

Example 64
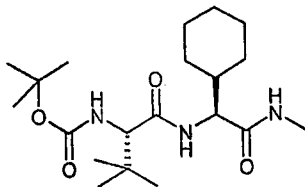
{1-[(Cyclohexyl-methylcarbamoyl-methyl)-carbamoyl]-2,2-dimethyl-propyl}-carbamic

5   acid *tert*-butyl ester (64)

To compound 63 in EtOH-THF 1:2 was added a large excess of methylamine (30% in water) and left at rt. for 2 weeks. The solution was concentrated to dryness and the residue subjected to a short silica gel column eluted with 2% MeOH in dichloromethane to give a pure (>95%) product M+H$^+$ 384.5.

10

Example 65
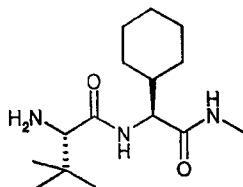
2-Amino-*N*-(cyclohexyl-methylcarbamoyl-methyl)-3,3-dimethyl-butyramide (65)

Compound 64 was kept in dichloromethane-trifuoroacetic acid 2:1 for 1 h at rt and

15   concentrated to dryness. The residue was dried in a vacuum for 16 hrs. Reversed phase C18 HPLC showed >95% purity M+H$^+$ 283.1.

Example 66
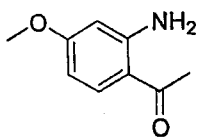
20   1-(2-Amino-4-methoxyphenyl)ethanone (66)

m-Anisidine (10.0 g, 82 mmol) was dissolved in CH_2Cl_2 (50 mL), and the solution was cooled to -50 °C. BCl_3 (1 M in CH_2Cl_2, 82 mL, 82 mmol) was added slowly during 20

min, after which the mixture was stirred at -50 °C for 30 min, followed by sequential addition of AcCl (6.0 mL, 84 mmol) and AlCl$_3$ (11 g, 82 mmol). The mixture was stirred at -50 °C for 1 h and was then allowed to assume rt. After stirring at rt overnight, the solution was heated at 40 °C for 4 h, after which the mixture was

5   poured over ice. The aqueous mixture was made alkaline with 10 % NaOH (w/v) and extracted with EtOAc (4 x 200 mL). The combined organic phases were washed with brine, dried (MgSO$_4$), and evaporated to give a black solid, which was purified by flash column chromatography (ether/CH$_2$Cl$_2$ 20:80). The resulting solid was recrystallized from ether/hexane to give compound 93 as shiny tan leaflets (5.6 g, 42

10 %).

Example 67

N-(tert-Butyl)-N'-isopropylthiourea (67)

To a solution of tert-butylisothiocyanate (5.0 mL, 39 mmol) in CH$_2$Cl$_2$ (200 mL) were

15 added isopropylamine (4.0 mL, 47 mmol) and diisopropylethylamine (DIEA) (6.8 mL, 39 mmol), and the mixture was stirred at rt for 2h. The reaction mixture was diluted with EtOAc, washed with 10 % citric acid (2x), saturated NaHCO$_3$ (2x), H$_2$O (2x), and brine (1x). The organic layer was dried (MgSO$_4$) and evaporated to yield the title compound (3.3 g, 52 %) as a white solid which was used without further purification.

20

Example 68

N-Isopropylthiourea (68)

Compound 67 (3.3 g, 20 mmol) was dissolved in conc. HCl (45 mL) and the solution was refluxed for 40 min. The mixture was allowed to cool to rt and then cooled in an

25 ice bath and basified to pH 9.5 with solid and saturated NaHCO$_3$, after which the product was extracted into EtOAc (3x). The combined organic phases were washed with H$_2$O (2x) and brine (1x), dried (MgSO$_4$), and evaporated to yield the crude title compound (2.1 g, 90 %) which was used without further purification.

30 Example 69
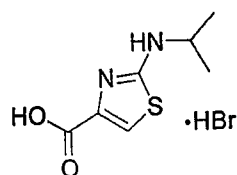
2-(Isopropylamino)-1,3-thiazole-4-carboxylic acid hydrobromide (69)

A suspension of compound 68 (2.1 g, 18 mmol) and 3-bromopyruvic acid (3.0 g, 18 mmol) in dioxane (180 mL) was heated to 80°C. Upon reaching 80 °C the mixture became clear, and soon thereafter the product started to precipitate as a white solid. After 2 h of heating, the reaction mixture was cooled to rt and the precipitate was filtered off and collected. This yielded pure title product (4.4 g, 94 %).

Example 70
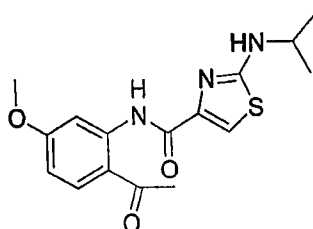
N-(2-Acetyl-5-methoxyphenyl)-2-(isopropylamino)-1,3-thiazole-4-carboxamide (70)

A mixture of compound 69 (4.4 g, 16.5 mmol) and the aniline derivative 66 (2.75 g, 16.5 mmol) in pyridine (140 mL) was cooled to -30 °C (upon cooling, the clear solution became partially a suspension). $POCl_3$ (3.3 mL, 35 mmol) was added slowly over a 5 min period. The mixture was stirred at -30 °C for 1 h, and was then allowed to assume rt. After stirring at rt for 1.5 h the reaction mixture was poured over ice, and the pH was adjusted to about 9-10 using solid and saturated $NaHCO_3$. The crude product was extracted into CH_2Cl_2 (3x) and the combined organic phases were dried ($MgSO_4$) and evaporated. The crude dark-beige solid was purified by flash column chromatography (hexane/EtOAc 55:45) to give compound 70 (5.6 g, 76 %) as a pale yellow solid.

Example 71

120
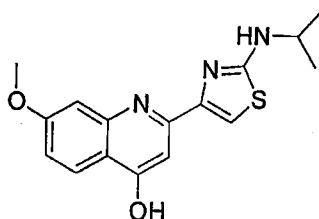
2-[2-(Isopropylamino)-1,3-thiazol-4-yl]-7-methoxyquinolin-4-ol (71)

A solution of t.BuOK (2.42 g, 21 mmol) in anhydrous t.BuOH (40 mL) was heated to reflux. Compound 70 (1.8 g, 5.4 mmol) was added portion-wise over a 5 min period, and the dark red solution formed was stirred at reflux for an additional 20 min. The mixture was cooled to rt, and HCl (4 M in dioxane, 8.0 mL, 32 mmol) was added, after which the reaction mixture was concentrated under vacuum. In order to assure that all of the HCl and dioxane were removed, the crude product was re-dissolved in CH_2Cl_2 twice and thoroughly evaporated to obtain the slightly impure HCl salt of compound 71 (1.62 g) as a brown solid. The product was dissolved in CH_2Cl_2 and washed with saturated $NaHCO_3$, after which the aqueous phase was extracted several times with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$) and evaporated to give compound 71 (1.38 g, 81 %) as a light brown solid (> 95 % pure according to HPLC tests). ^{1}H-NMR (MeOH-d_4, 400 MHz): δ 1.30 (d, J = 6.0 Hz, 6H), 3.93 (s, 3H), 3.95-4.07 (m, 1H), 6.73 (s, 1H), 6.99 (dd, J = 2.4, 9.2 Hz, 1H), 7.26 (d, J = 2.4 Hz, 1H), 7.37 (s, 1H), 8.10 (d, J = 9.2 Hz, 1H).

Example 72
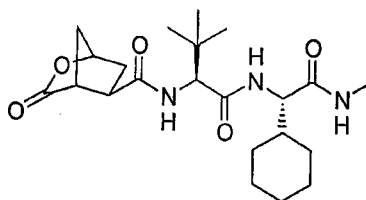
(1R,4R,5R)-N-[(1S)-1-[[[(1S)-1-Cyclohexyl-2-(methylamino)-2-oxoethyl]amino]carbonyl]-2,2-dimethylpropyl]-3-oxo-2-oxabicyclo[2.2.1]heptane-5-carboxamide (72)

To a solution of compound 32 (53 mg, 0.34 mmol) in DMF (9 mL) was added compound 65 (80 mg, 0.28 mmol) and DIEA (290 ☐L, 1.66 mmol). The solution was cooled to 0 °C and HATU (127 mg, 0.33 mmol) was added. After stirring at 0 °C for 1 h and at rt for 1 h the solvent was evaporated, and the crude product was purified by

5   flash column chromatography (EtOAc/toluene 2:1) to give compound 72 (110 mg, 92 %) as a white solid.

Example 73
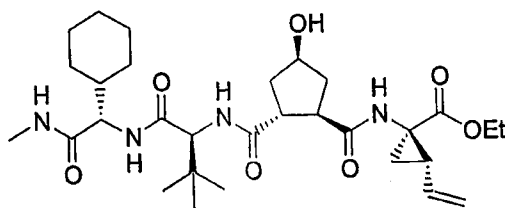
10   (1R)-1-[[[(1R,2R,4R)-2-[[[(1S)-1-[[[(1S)-1-Cyclohexyl-2-(methylamino)-2-oxoethyl]amino]carbonyl]-2,2-dimethylpropyl]amino]carbonyl]-4-hydroxycyclopentyl]carbonyl]amino]-2-ethenyl-cyclopropanecarboxylic acid ethyl ester (73)

Compound 72 (60 mg, 0.14 mmol) was dissolved in dioxane (3.5 mL) and H₂O (2.5

15   mL) and the solution was cooled to 0 °C. LiOH (1 M, 280 ☐L, 0.28 mmol) was added dropwise during 5 min, after which the reaction mixture was stirred at 0 °C for 40 min. The pH was adjusted to 7 using 1 M HCl, and the solvents were evaporated. The residue was suspended in DMF (5 mL) and 1-amino-2-vinyl-cyclopropanecarboxylic acid ethyl ester (32 mg, 0.17 mmol), and DIEA (146 ☐L, 0.84 mmol) were added.

20   After cooling to 0 °C HATU (64 mg, 0.17 mmol) was added and the mixture was stirred at 0 °C for 1 h and at rt for 1 h. The solvent was evaporated and the product was purified using flash column chromatography (EtOAc/MeOH 9:1) to give compound 73 (67 mg, 82 %) as a white solid.

25   Example 74
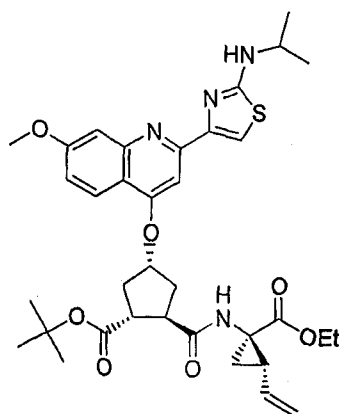
tert-Butyl (1*R*,2*R*,4*R*)-2-[[[(1*R*)-1-(ethoxycarbonyl)-2-vinylcyclopropyl]amino]carbonyl]-
4-[[2-[2-(isopropylamino)-1,3-thiazol-4-yl]-7-methoxyquinolin-4-
yl]oxy]cyclopentanecarboxylate (74)

The title compound was prepared according to the procedure described in example
76 method A but with the use of compound 34 instead of compound 73. (Note: 4
equivalents of Ph₃P and DIAD were used. Chromatography eluent: Toluene/EtOAc
1:1.)
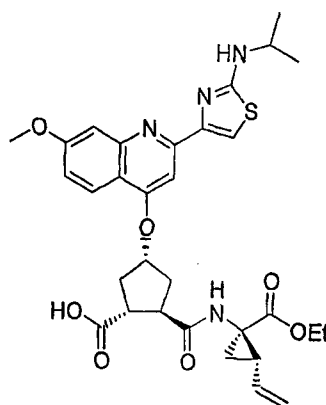
Example 75

(1*R*,2*R*,4*R*)-2-[[[(1*R*)-1-(Ethoxycarbonyl)-2-vinylcyclopropyl]amino]carbonyl]-4-[[2-[2-
(isopropylamino)-1,3-thiazol-4-yl]-7-methoxyquinolin-4-yl]oxy]cyclopentanecarboxylic
acid (75)

To a solution of compound 74 (20 mg, 30 umol) in CH₂Cl₂ (2 mL) was added TFA (2
mL) and Et₃SiH (10 uL, 63 umol). After 2 h the volatiles were evaporated and the

product was used without any purification step. Compound 75: 18 mg, quant. as a white solid.

Example 76
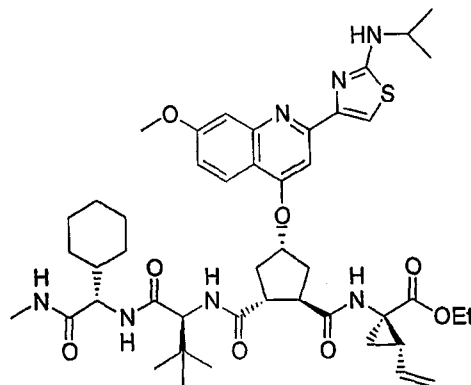
5

(1*R*)-1-[[[(1*R*,2*R*,4*S*)-2-[[[(1*S*)-1-[[[(1*S*)-1-Cyclohexyl-2-(methylamino)-2-oxoethyl]amino]carbonyl]-2,2-dimethylpropyl]amino]carbonyl]-4-[[7-methoxy-2-[2-[(1-methylethyl)amino]-4-thiazolyl]-4-quinolinyl]oxy]cyclopentyl]carbonyl]amino]-2-ethenyl-cyclopropanecarboxylic acid ethyl ester (76)

10   Method A: To a solution of compound 73 (59 mg, 0.10 mmol) in dry THF (4 mL) was added the quinoline 71 (49 mg, 0.16 mmol) and Ph₃P (65 mg, 0.25 mmol). After cooling to 0 °C DIAD (50 uL, 0.25 mmol) was added dropwise during 5 min. The solution was stirred at 0 °C for 1 h and at rt for 48 h. The solvent was evaporated and the remainder was purified using flash column chromatography (CHCl₃/2 M NH₃ in

15   MeOH 95:5) to give compound 76 (9 mg, 10 %) as a white solid.

Method B: Compound 75 was coupled to compound 65 according to the procedure in example 72 which gave the title compound (82%).

Example 77
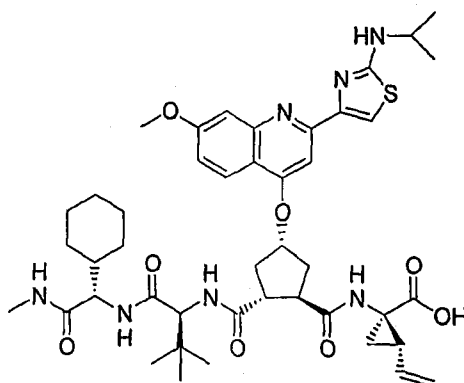
(1*R*)-1-[[[(1*R*,2*R*,4*S*)-2-[[[(1*S*)-1-[[[(1*S*)-1-Cyclohexyl-2-(methylamino)-2-
oxoethyl]amino]carbonyl]-2,2-dimethylpropyl]amino]carbonyl]-4-[[7-methoxy-2-[2-[(1-
methylethyl)amino]-4-thiazolyl]-4-quinolinyl]oxy]cyclopentyl]carbonyl]amino]-2-
ethenyl-cyclopropanecarboxylic acid (77)

Compound 76 (8 mg, 9 μmol) was dissolved in a mixture of MeOH (150 μL) and THF
(100 uL). A solution of LiOH (1 mg, 42 μmol) in H_2O (25□L) was added and the
mixture was stirred at 50 °C overnight. The solution was neutralized with HOAc and
evaporated. The residue was suspended in CH_2Cl_2 and washed with H_2O. The
organic phase was evaporated to give the title compound (8 mg, quant.) as a white
solid.

^{1}H-NMR (MeOH-*d₄*, 400 MHz) (mixture of rotamers): δ 0.60-1.33 (m, 21H), 1.35-1.73
(m, 12H), 1.90-2.42 (m, 2H), 2.51-2.75 (m, 6H), 3.20-3.38 (m, 1H), 3.85 (s, 3H), 3.95-
4.28 (m, 1H), 4.91-5.02 (m, 1H), 5.12-5.23 (m, 1H), 5.64-5.83 (m, 1H), 7.01-7.11 (m,
1H), 7.25-7.40 (m, 1H), 7.42-7.57 (m, 1H), 7.85-8.08 (m, 1H).

Example 78
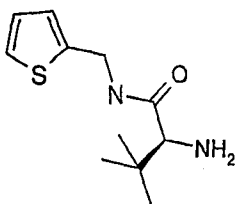
2-Amino-3,3-dimethyl-N-thiophen-2-yl-methyl-butyramide (78)

The title compound was prepared as described in example 61 but with the use of
thiophene-2-methylamine instead of aminoindanole followed by removal of the Boc
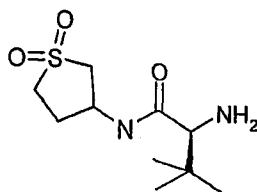group as described in example 62.

5   Example 79
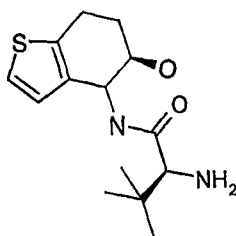
2-Amino-N-(6-hydroxy-4,5,6,7-tetrahydro-benzo[b]thiophen-5-yl)-3,3-dimethyl-
butyramide (79)

The title compound was prepared as described in example 61 but with the use of 2-

10   amino-4,5,6,7-tetrahydro-benzo[b]thiophen-5-ol instead of aminoindanole followed by
removal of the Boc group as described in example 62.

Example 80
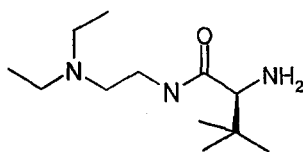
15   2-Amino-N-(2-diethylamino-ethyl)-3,3-dimethyl-butyramide (80)

The title compound was prepared as described in example 61 but with the use of
N,N-diethylethylenediamine instead of aminoindanole followed by removal of the Boc
group as described in example 62.

20   Example 81
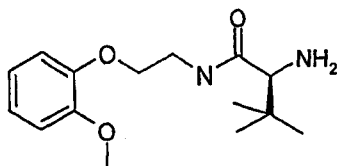
2-Amino-N-[2-(2-methoxy-phenoxy)-ethyl]-3,3-dimethyl-butyramide (81)

The title compound was prepared as described in example 61 but with the use of 2-methoxyphenoxyethylamine instead of aminoindanole followed by removal of the Boc group as described in example 62.

5

Example 82
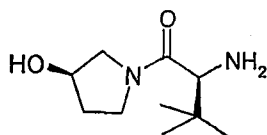
2-Amino-1-(3-hydroxy-pyrrolidin-1-yl)-3,3-dimethyl-butan-1-one (82)

The title compound was prepared as described in example 61 but with the use of (R)-10   3-pyrrolidinone instead of aminoindanole followed by removal of the Boc group as described in example 62.

Example 83

15   2-Amino-N-(1,1-dioxo-tetrahydro-1-thiophen-3-yl)-3,3-dimethyl-butyramide (83)

The title compound was prepared as described in example 61 but with the use of 2-methoxyphenoxyethylamine instead of aminoindanole followed by removal of the Boc group as described in example 62.

20   Example 84
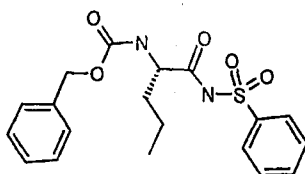
Carbamic acid, [(1*S*)-1-[[(phenylsulfonyl)amino]carbonyl]butyl]-, phenylmethyl ester
(84)

To a stirred solution of Z-Nva-OH (150 mg, 0.59 mmol) in THF (6 mL), CDI (400 mg,
2.4 mmol) was added. The slurry was agitated for 30 min at RT followed by the

5   addition of DBU (200 uL, 1.3 mmol) and a solution of benzenesulfonamide (250 mg,
1.59 mmol) in THF(2 mL). The mixture was stirred at 60 °C for 48 hrs followed by
concentration to dryness. The residue was dissolved in MeOH and subjected to
HPLC purification to give the title compound (118.5 mg, 0.304 mmol). Purity > 95% by
HPLC. M-H$^+$ 389.0, +Na 412.96.

10

Example 85
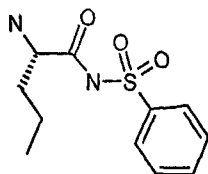
(2S)-2-Amino-N-(phenylsulphonyl)pentanamide (85)

Compound 84 was dissolved in MeOH (5 mL) followed by the addition of Pd/C and

15   subjected to hydrogenation for 2 hrs. The slurry was filtered through celite, washed
with MeOH and concentrated to dryness to give the title compound. Yield 100%.
M+H$^+$ 257.3.

Example 86

20

128
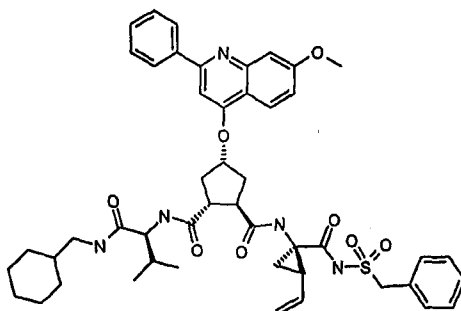
4-(7-Methoxy-2-phenyl-quinolin-4-yloxy)-cyclopentane-1,2-dicarboxylic acid 1-{[1-
(cyclohexylmethyl-carbamoyl)-2-methyl-propyl]-amide} 2-[(1-
phenylmethanesulfonylaminocarbonyl-2-vinyl-cyclopropyl)-amide] (86)

N-(tert-Butoxycarbonyl)-L-valine was attached to Argonaut resin PS-TFP as

5   described in example 60 followed by reaction with cyclohexanemethylamine as
described in example 61 and removal of the Boc group as described in example 62.
The afforded amine was used in a coupling reaction with compound 35 as described
in example 39 followed by hydrolysis of the ethyl ester as described in example 40
which gave 1-{[2-[1-(cyclohexylmethyl-carbamoyl)-2-methyl-propylcarbamoyl]-4-(7-

10   methoxy-2-phenyl-quinolin-4-yloxy)-cyclopentanecarbonyl]-amino}-2-vinyl-
cyclopropanecarboxylic acid. The afforded acid was then treated as described in
example 94 but using toluenesulphonamide instead of cyclopropylsulphonamide
which gave the title compound. Yield 6%. Purity > 95% by HPLC. M+H$^+$ 864.32.

15   Example 87
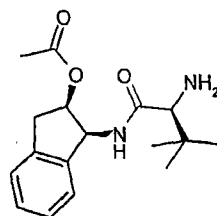
Acetic acid (1S,2R)-1-((2S)-2-amino-3,3-dimethyl-butyrylamino)-indan-2-yl ester (87)
A solution of compound 61 (4g) was kept in pyridine-acetic anhydride 2:1 for 30 min.
DCM was added and the solution was washed with citric acid (aq) and NaHCO$_3$ (aq).

20   The organic layer was concentrated to dryness which gave the acetylated product
>90% pure by HPLC. The afforded compound was then kept in a solution of 30% TFA
in DCM for 1.5 hrs and then concentrated to dryness. Co-evaporation twice from
toluene gave the title product >90% pure by HPLC.

25   Example 88

129
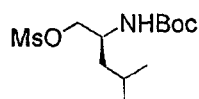
(2S)-Methanesulphonic acid 2-tert.butoxycarbonylamino-4-methyl-pentyl ester (88)

To a solution of ((1S)-1-hydroxymethyl-3-methyl-butyl)-carbamic acid tert-butyl ester (25 g, 115 mmol) in dichloromethane (500 mL) cooled by an ice-water bath was successively added diisopropylethylamine (35.7 g, 276 mmol) and methanesulphonyl chloride (15.81 g, 138 mmol). The resulting solution was stirred over night during which time the mixture was allowed to gradually warm up to ambient temperature. The mixture was washed successively with water, 10 % citric acid (aq), water and saturated $NaHCO_3$ (aq), then dried with Na_2SO_4 and concentrated to a brown solid (32.6 g, 96 %) which was used in the next reaction without further purification.

Example 89
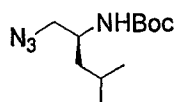
ii) ((1S)-1-Azidomethyl-3-methyl-butyl)-carbamic acid tert.butyl ester (89)

The mesylate from example 88 (32.6 g, 110 mmol) was treated with sodium azide (21.45 g, 330 mmol) in DMF at 80 °C for 24 hrs. The solvent was evaporated, the residue was taken up in DCM, filtered and washed with saturated $NaHCO_3$ (aq). The solution was dried with Na_2SO_4 and concentrated to a brown oil which was purified by flash chromatography using a gradient of ethyl acetate and hexane to afford the title compound as a white solid (19.55 g, 73 %).

Example 90
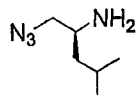
(1S)-1-Azidomethyl-3-methyl-butylamine (90)

((1S)-1-Azidomethyl-3-methyl-butyl)-carbamic acid tert-butyl ester (9.64 g, 39.78 mmol) was treated with TFA (30 mL) in DCM (150 mL) for 3 hrs, the mixture was evaporated under reduced pressure and the residue was dissolved in ethyl acetate and washed with aqueous 1 M K_2CO_3, dried with Na_2SO_4 and concentrated to a yellow liquid (4.55 g, 80 %).

Example 91
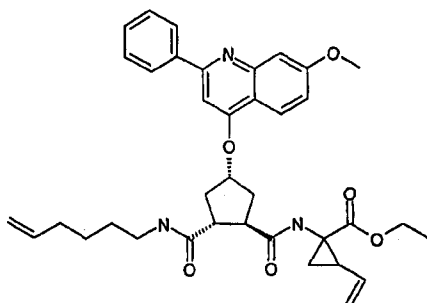
1-{[2-Hex-5-enylcarbamoyl-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-

5   cyclopentanecarbonyl]-amino}-2-vinyl-cycloprpanecarboxylic acid ethyl ester (91)

The tert.butyl ester of compound 35 was removed by treatment with triethylsilane as

described in Example 39. The afforded acid (724 mg, 1.33 mmol), hex-5-enylamine

hydrochloride (271 mg, 2 mmol) and diisopropylethylamine (1.85 ml, 10.65 mmol)

was dissolved in DMF (20 ml) and cooled to 0°C. After 30 min. HATU (608 mg, 1.6

10   mmol) was added and the flask was removed from the ice-bath. The reaction was

followed with LC-MS. After 3 h the reaction mixture was extracted between EtOAc

(100 ml) and aqueous sodium hydrogencarbonate (15 ml). The EtOAc-phase was

dried over magnesium sulphate, evaporated and purified by chromatography on silica

gel (25% EtOAc in hexane → 50 % EtOAc in hexane) to give the pure title product
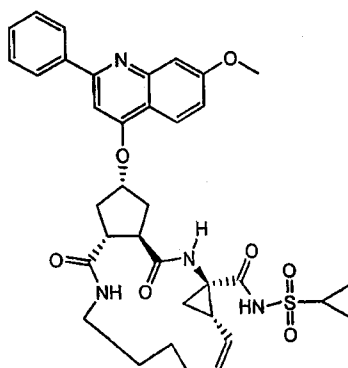
15   (726 mg, 87%). MS (M +H$^+$): 525.8

Example 92
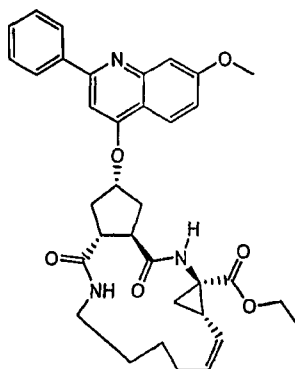
17-(7-Methoxy-2-phenyl-quinolin-4-yloxy)-2,14-dioxo-3,13-diaza-
tricyclo[13.3.0.0*4,6*]octadec-7-ene-4-carboxylic acid ethyl ester (92)

Compound 91 (363 mg, 0.58 mmol) was dissolved in degassed dichloromethane (100
ml). Hoveyda-Grubbs catalyst 2nd generation (26 mg, 0.041 mmol) was added and

5   the mixture was refluxed under argon atmosphere overnight. The reaction mixture
was evaporated on silica and purified by silica gel chromatography (50 % EtOAc in
hexane → 70 % EtOAc in hexane) to give the pure title product (111 mg, 32%). MS
(M +H⁺): 597.7

10   Example 93
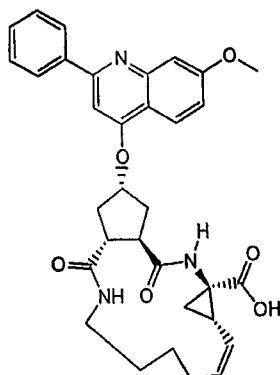
17-(7-Methoxy-2-phenyl-quinolin-4-yloxy)-2,14-dioxo-3,13-diaza-
tricyclo[13.3.0.0*4,6*]octadec-7-ene-4-carboxylic acid (93)

Compound 92 (95 mg, 0.159 mmol) was dissolved in tetrahydrofuran (10 ml),

15   methanol (5 ml) and water (4 ml) Lithium hydroxide (40 mg, 1.67 mmol) was
dissolved in water (1 ml) and added. The reaction mixture was heated to 65˚C. After 3
h the reaction mixture was cooled, acidified with aqueous HCl (pH=5), evaporated on
silica and purified by silica gel chromatography (10 % MeOH in dichloromethane →
15 % MeOH in dichloromethane) to give the pure title product (65 mg, 72 %). MS (M

20   +H⁺): 569.8

Example 94

132

Cyclopropanesulphonic acid [17-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,14-dioxo-
3,13-diaza-tricyclo[13.3.0.0*4,6*octadec-7-ene-4-carbonyl]-amide (94)

Compound 93 (65 mg, 0.12 mmol), DMAP (21 mg, 0.17 mmol) and EDAC (44 mg,
0.23 mmol) was dissolved in DMF (0.2 ml). The reaction mixture was stirred for 5 h at
R.T. whereafter cyclopropylsulfonamide (69 mg, 0.57 mmol) and DBU (80 μl, 0.57
mmol) was added. After stirring at R.T overnight the reaction mixture was extracted
between EtOAc (80 ml) and aqueous citric acid (10 %, 2 x 15 ml). The organic phase
was dried over MgSO₄, evaporated on silica and purified twice by chromatography on
silica gel (5 % MeOH in dichloromethane → 15 % MeOH in dichloromethane) which
gave a syrup. This syrup was dissolved in a small volume acetonitrile and precipitated
with ethyl ether to give the pure title product (19 mg, 23 %). MS (M +H⁺): 673.2
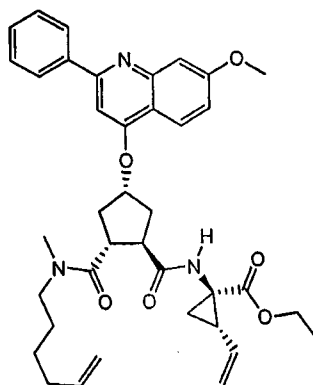
Example 95

1-{[2-Hex-5-enyl-methyl-carbamoyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-
cyclopentanecarbonyl]-amino-2-vinyl-cyclopropanecarboxylic acid ethyl ester (95)

The tert.butyl ester of compound 35 was removed according to the procedure described in Example 39. The afforded acid (850 mg, 1.56 mmol), N -methyl hex-5-enylamine hydrochloride (380 mg, 2,5 mmol) and diisopropylethylamine (2,3 ml, 13,4 mmol) was dissolved in DMF (60 mL) and cooled to 0°C. After 30 min. HATU (0,76 mg, 2,0 mmol) was added and the flask was removed from the ice-bath. The reaction was followed with TLC. After 2 h the reaction mixture was added to 5% citric acid and extracted three times with ethyl acetate. The organic phase was dried over sodium sulphate and evaporated under reduced pressure. The crude product was purified by silica gel chromatography which gave the title product (820 mg, 82%.
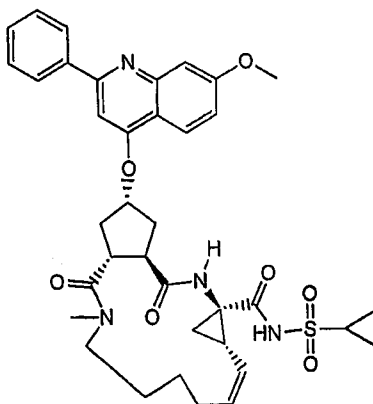
Example 96
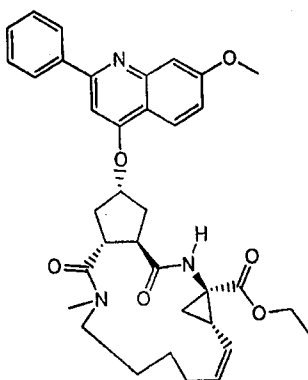
17-(7-Methoxy-2-phenyl-quinolin-4-yloxy)-13-methyl-2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0*4,6*]octadec-7-ene-4-carboxylic acid ethyl ester (96)

Compound 95 (648 mg, 1,01 mmol) was dissolved in degassed dichloroethane (500 mL). Hoveyda-Grubbs catalyst 2:nd generation (35 mg, 0.055 mmol) was added and the mixture was refluxed under argon atmosphere overnight. The reaction mixture was evaporated on silica and purified by chromatography on silica gel (30 % EtOAc in toluene → 50 % EtOAc in toluene) to give the pure title product (230 mg mg, 37%).

MS (M +H⁺): 612.8

Example 97
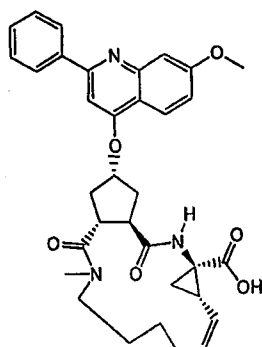
17-(7-Methoxy-2-phenyl-quinolin-4-yloxy)-13-methyl-2,14-dioxo-3,13-diaza-
tricyclo[13.3.0.0*4,6*]octadec-7-ene-4-carboxylic acid ethyl ester (97)

Compound 96 (260 mg, 0.42 mmol) was dissolved in 1,4-dioxan (20 mL), 1.0 M

5   Lithium hydroxide (6.0 ml) was added and the mixture was stirred at room
temperature overnight and then for six hours at 60°C. The mixture was added to 5%
citric acid and extracted 3 times with ethyl acetate. The organic phase was dried over
sodium sulphate and evaporated under reduced pressure. The crude product was
purified by silica gel chromatography with DCM and 5% MeOH

10   which gave the title product (130mg, 53%). MS (M + H) : 584,7

Example 98

15   Cyclopropanesulphonic acid [17-(7-methoxy-2-phenyl-quinolin-4-yloxy)-13-methyl-
2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0*4,6*octadec-7-ene-4-carbonyl]-amide (98)

Compound 97 (58,3 mg, 0.1 mmol), DMAP (18,3 mg, 0.15 mmol) and EDAC (38,7 mg, 0.2 mmol) was dissolved in DMF (1,0 mL). The reaction mixture was stirred overnight at R.T. whereafter cyclopropylsulphonamide (60,5 mg, 0.5 mmol) and DBU (76 µg, 0.5 mmol) was added. After stirring at R.T overnight the reaction mixture was

5   added to 5% citric acid and extracted three times with ethyl acetate. The organic phase was dried over sodium sulphate and evaporated .The afforded residue was purified two times by silica gel chromatography which gave the title product (20 mg). MS (M + H) 687,8.

10   Example 99
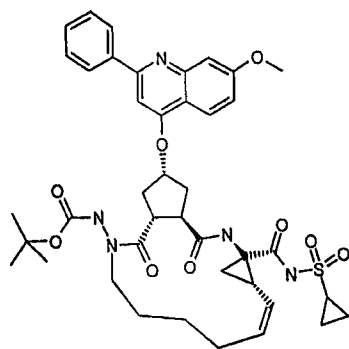
[4-Cyclopropanesulphonylaminocarbonyl-17-(7-methoxy-phenyl-quinolin-4-yloxy)-2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0*4,6*]octadec-7-en-13-yl]-carbamic acid tert.butyl ester (99)

15   N'-Hex-5-en-(E)-ylidene-hydrazinecarboxylic acid tert.butyl ester was prepared according to the procedure described in Example 46 and 47 but starting from hex-5-en-ol instead of hept-6-en-ol. Compound 35 was treated as described in Example 48 but using the above described N'-Hex-5-en-(E)-ylidene-hydrazinecarboxylic acid tert.butyl ester instead of the corresponding hept-6-en derivative followed by

20   macrocyclisation as described in Example 49 and hydrolysis of the ethyl ester as described in Example 50 gave the acid. The afforded acid (58 mg, 0.0846 mmol) was dissolved in dry DMF (7 mL) and DIEA was added drop wise during one minute. The solution was stirred at room temperature for 1h prior to the addition of a solution containing cyclopropylsulfonamide (41 mg, 0.338 mmol), DMAP (41.3mg, 0.338

136

mmol) and DBU (50 µL, 0.338 mmol) in dry DMF (1.5 mL). The solution was stirred at
room temperature for 5 days. The solution was diluted with EtOAc (50 mL) and
washed with sat. NaHCO₃. The aqueous phase was extracted with DCM. The
combined organic layers were dried, concentrated and subjected to purification by
5   HPLC, which gave the title compound as a white solid (14.3 mg, 0.018 mmol), Purity
by HPLC > 95%, M+H⁺ 788.3.

Example 100
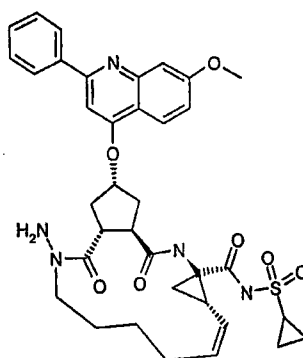
10   Cyclopropanesulphonic acid[13-amino-17-(7-methoxy-2-phenyl-quinolin-4-yloxy)-
2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0*4,6*]octadec-7-ene-4-carbonyl]-amide
trifluoroacetic acid salt (100)
Compound 99 (2.4 mg, 0.00304mmol) was kept in TFA-DCM 1:2 (3mL) at room
temperature for 60 min. Toluene (3 mL) was added. The sample was co-evaporated
15   to dryness to afford the title compound (2.1 mg, 0.0026 mmol) Purity by HPLC >95%.
M+H⁺ 688.3.

Example 101
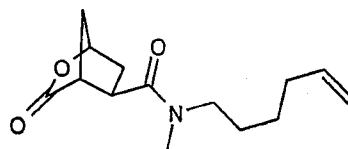
20   3-Oxo-2-oxa-bicyclo[2.2.1]heptane-5-carboxylic acid hex-5-enyl-methylamide (101)
To HATU (2.17 g, 5.7 mmol) and N-methyl hex-5-enylamine hydrochloride (6.47
mmol) in 5 mL DMF, under argon in an ice bath, were added *1R,4R,5R*-3-oxo-2-oxa-

bicyclo[2.2.1]heptane-5-carboxylic acid (835.6 mg, 5.35 mmol) in 11 mL DMF followed by DIEA (2.80 mL, 16 mmol). After stirring for 40 min, the mixture was stirred at rt for 5 h. The solvent was evaporated, the residue dissolved in EtOAc (70 mL) and washed with saturated NaHCO₃ (10 mL). The aqueous phase was extracted

5   with EtOAc (2 x 25 mL). The organic phases were combined, washed with saturated NaCl (20 mL), dried over Na₂SO₄, and evaporated. Flash column chromatography (150 g silica gel, 2/1 EtOAc – petroleum ether (PE), TLC detection by aqueous KMnO4, Rf 0.55 in 4/1 EtOAc – PE) gave the compound as a yellow oil (1.01 g, 75%).

10   Example 102
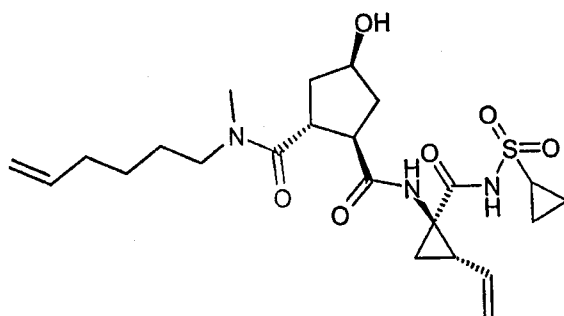
4-Hydroxycyclopentane-1,2-dicarboxylic acid 1-[(1-cyclopropanesulphonylaminocarbonyl-2-vinylcyclopropyl)-amide] 2-(hex-5-enyl-methylamide (102)

15   LiOH solution (0.15M, 53 mL, 8 mmol) was added to the lactone amide 101 (996 mg, 3.96 mmol) in an ice bath and stirred for 1 h. The mixture was acidified to pH 2 – 3 with 1N HCl and evaporated, co-evaporated with toluene several times, and dried under vacuum overnight. (1R,2S)-cyclopropanesulfonic acid (1- amino-2-vinyl-cyclopropanecarbonyl)amide hydrochloride (4.21 mmol) and HATU (1.78 g, 4.68

20   mmol) were added. The mixture was cooled in an ice bath under argon, DMF (25 mL) and then DIEA (2.0 mL, 11.5 mmol) were added. After stirring for 30 min, the mxture was stirred at rt for 3 h. After evaporation of solvent, the residue was dissolved in EtOAc (120 mL), washed successively with 0.5 N HCl (20 mL) and saturated NaCl (2 x 20 mL), and dried over Na₂SO₄. Flash column chromatography (200g YMC silica

25   gel, 2 - 4% MeOH in CH₂Cl₂ gave white solids (1.25 g, 66%).
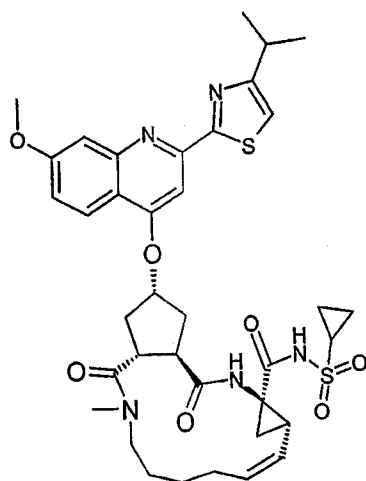
Example 103
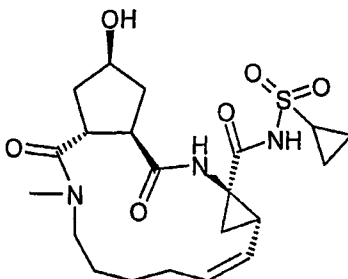
Cyclopropanesulphonic acid (17-hydroxy-13-methyl-2,14-dioxo-3,13-
diazatricyclo[13.3.0.0*4,6*]octadec-7-ene-4-carbonyl)-amide (103)

The cyclopentanol 102 (52.0 mg, 0.108 mmol) was dissolved in 19 mL 1,2-
dichloroethane (bubbled with argon prior to use). The Hoveyda-Grubbs 2nd generation
catalyst (6.62 mg, 10 mole %) was dissolved in DCE (2 x 0.5 mL) and added. The
green solution was bubbled with Ar for 1 min. Aliquots (4 mL each) were transferred
into five 2 to 5-mL microwave tubes. To the last tube was added 0.8 mL rinsing with
solvent. Each tube was heated by microwave (rt to 160 °C in 5 min). All aliquots were
combined and the solvent evaporated. Flash column chromatography (silica gel, 3 –
7% MeOH in CH_2Cl_2) gave 24.39 mg solids (Rf 0.28 in 10% MeOH - CH_2Cl_2 with two
spots). The solids were combined with a 9.66-mg sample and subjected to a second
chromatography (2 – 8% MeOH in EtOAc) to give cream solids (23 mg) with 80% of
the desired compound (26% yield).

Example 104

Cyclopropanesulphonic acid {17-[2-(4-isopropylthiazol-2-yl)-7-methoxyquinolin-4-yloxy]-13-methyl-2,14-dioxo-3,13-diazatricyclo[13.3.0.0*4,6*]octadec-7-ene-4-carbonyl)-amide (104)

5   DIAD (22 uL, 0.11 mmol) was added to a mixture of the metathesis product 103 (23 mg), 2-(4-isopropyl-1,3-thiazol-2-yl)-7-methoxyquinolin-4-ol (24 mg, 0.08 mmol), and PPh$_3$ (30 mg, 0.11 mmol) in 1 mL dry THF, in an ice bath. The mixture was stirred at rt overnight and then evaporated. The residue (1.2 mL of a 1.5-mL MeCN solution) was purified by prep-HPLC (Hypercarb 7 uL100 x 21.2 mm, 40% to 99% aqueous

10   MeCN in 10 min) to give 3.18 mg MV062308 as cream solids (13 % yield).

1H NMR (DMSO-d6) δ ppm: major rotamer 0.99 (m, 2H), 1.11 (m, 2H), 1.20 – 1.30 (m, 2H), 1.37 and 1.38 (2d, J = 7.0 Hz, 6H), 1.46 – 1.58 (m, 2H), 1.70 (m, 1H), 1.85 (m, 1H), 1.90 (dd, J = 8.5, 6.0 Hz, 1H), 2.06 (br, 1H), 2.26 (m, 1H), 2.38 (m, 1H), 2.52 – 2.62 (m, 3H), 2.90 – 2.97 (m, 2H), 3.06 (s, 3H), 3.21 (m, 1H), 3.40 – 3.56 (m, 2H)

15   3.97 (s, 3H), 4.60 (m, 1H), 5.04 (m, 1H), 5.41 (br, 1H), 5.66 (m, 1H), 7.16 (m), 7.58 (br), 8.02 (m), 10.92 (s, 1H)
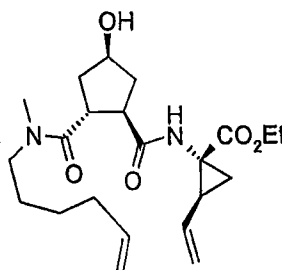

Example 105

140
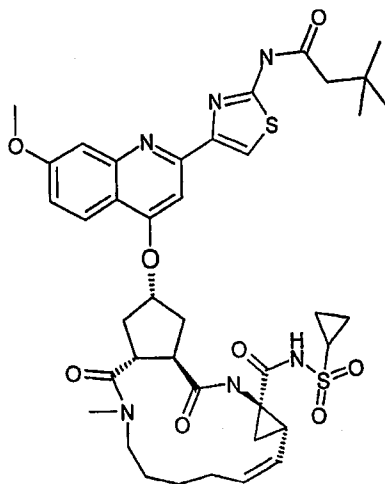
N-{4-[4-(4-Cyclopropanesulphonylaminocarbonyl-13-methyl-2,14-dioxo-3,13-diaza-
tricyclo[13.3.0.0*4,6]octadec-7-en-17-yloxy)-7-methoxy-quinoli-2-yl]-thiazol-2-yl}-
3,3dimethylbutyramide (105)

5   Treatment of compound 103 with 4-hydroxy-7-methoxy-2-[2-(2,2-
dimethylbutanoyl)aminothiazol-4-yl]quinoline as described in Example 104 gave the
title compound.

LCMS: retention time 2.30 min gradient 30% - 80% B in 3 min (flow: 0.8 mL/ min, UV
220 nm, ACE C8 3 x 50 mm; mobile phase A 10 mM NH₄Ac in 90% H₂O, B 10 mM

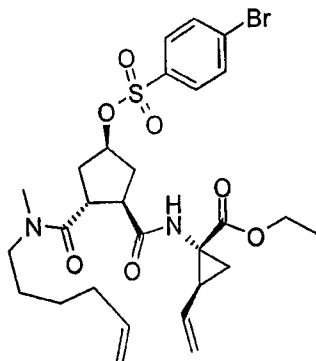10   NH₄Ac in 90% ACN), (M+1)⁺ = 807.

Example 106

1-{[2-(Hex-5-enyl-methyl-carbamoyl)-4-hydroxy-cyclopentanecarbonyl]-amino}-2-
15   vinyl-cyclopropanecarboxylic acid ethyl ester (106)

Reaction of compound 101 as described in example 102 but using 1-amino-2-
vinylcycloprpanecarboxylic acid ethyl ester instead of (1R,2S)-cyclopropanesulfonic

acid (1- amino-2-vinyl-cyclopropanecarbonyl)amide hydrochloride gave the title compound.

Example 107

1-{[4-(4-Bromo-benzensulphonyloxy-2-(hex-5-enyl-methyl-carbamoyl)-cyclopentanecarbonyl]-amino}2-vinyl-cyclopropanecarboxylic acid ethyl ester (107)

Compound 106 (115 mg, 0.286 mmol) was dissolved in toluene 5 ml and dichloromethane 1 ml. DABCO (2.2.2 –diazobicyclooctane) (96 mg, 0.857 mmol, 3 eq.) was added to the solution, followed by addition of BsCl (109 mg, 0.428 mmol, 1.5 eq). The reaction was stirred at room temperature overnight, diluted with toluene (+10% ethyl acetate), washed with saturated sodium bicarbonate, brine, dried over sodium sulphate and evaporated. The desired product was obtained by column chromatography (eluent EtOAc) R_f 0.25). Conversion 80%. Yield 106 mg.
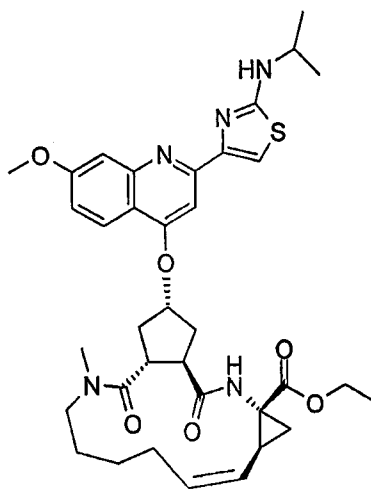
Example 108
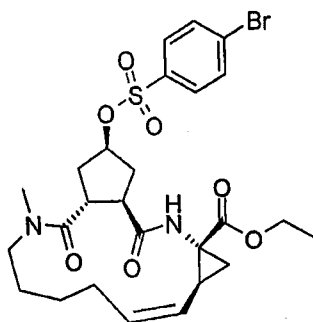
17-(4-Bromo-benzensulphonyloxy)-13-methyl-2,14-dioxo-3,13-diaza-
tricyclo[13.3.0.0*4,6*]octadec-7-ene-4-carboxylic acid ethyl ester (108)

Compound 107 (106 mg, 0.169 mmol) was dissolved in dichloromethane (40 ml) and
degassed by bubbling nitrogen through the solution for 20 min. Hoveyda – Grubbs
5   catalyst 1st generation (10 mg, 0.017 mmol, 10 mol %) was then added and the
mixture was refluxed under nitrogen atmosphere overnight. The reaction mixture was
then cooled down to room temperature and MP-TMT palladium scavenger (approx
100 mg) was added and stirred for 2.5 h. The scavenger was removed by filtration
and washed with 50 ml of dichloromethane. The solution obtained was concentrated
10   by rotary evaporation. The crude was purified by column chromatography (EtOAc) to
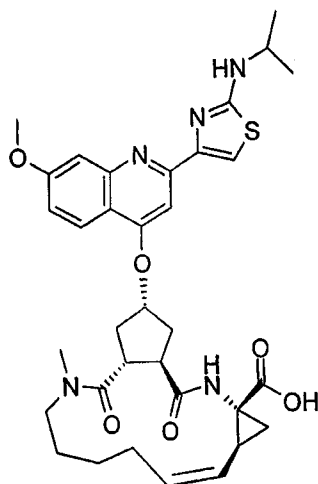give 61 mg of product. Yield 60%.

Example 109

15   17-[2-(2-Isopropylamino-thiazol-4-yl)-7-methoxy-quionlin-4-yloxy]-13-methyl-2,14-
dioxo-3,13-diaza-tricyclo[13.3.0.0*4,6*]octadec-7-ene-4-carboxylic acid ethyl ester
(109)

2-(Isopropylamino-thiazol-4-yl)-7-methoxy-quinolin-4-ol (220 mg, 0.7 mmol) (prepared
as described in WO 00/59929) was dissolved in 7 ml of NMP (N-methyl
20   pyrrolidinone), one spoon of Cs₂CO₃ was added, stirred at 60°C for 1.5h. Then
compound 108 (150 mg, 0.24 mmol) was added. The reaction mixture was stirred at
80°C overnight. Was diluted with chloroform and washed with sodium bicarbonate,

brine. Water phases were back-extracted with chloroform. The combined organic layers were dried over sodium sulphate and evaporated. The crude product was purified by preparative HPLC (Gilson) (MeOH-H$_2$O, 65%) to give 21 mg of product (yield 13%) as well as 12 mg of isomer.

5

Example 110

17-[2-(2-Isopropylamino-thiazol-4-yl)-7-methoxy-quionlin-4-yloxy]-13-methyl-2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0*4,6*]octadec-7-ene-4-carboxylic acid (110)

10   To the solution of the ester 109 (21 mg, 0.031 mmol) in a mixture of THF (0.2 ml) and methanol (0.3 ml) was added solution of LiOH (4 mg, 0.17 mmol) in 0.15 ml water. The resulting mixture was stirred at 60 °C for 3.5h. After cooling to room temperature, acetic acid was added (30 eq). The mixture was co-evaporated with toluene. The residue was distributed between chloroform and water, the water phase was

15   extracted with chloroform 3 times, the organic phases were combined, dried over sodium sulphate and evaporated which gave 20 mg of pure product (yield 99%).

Example 111