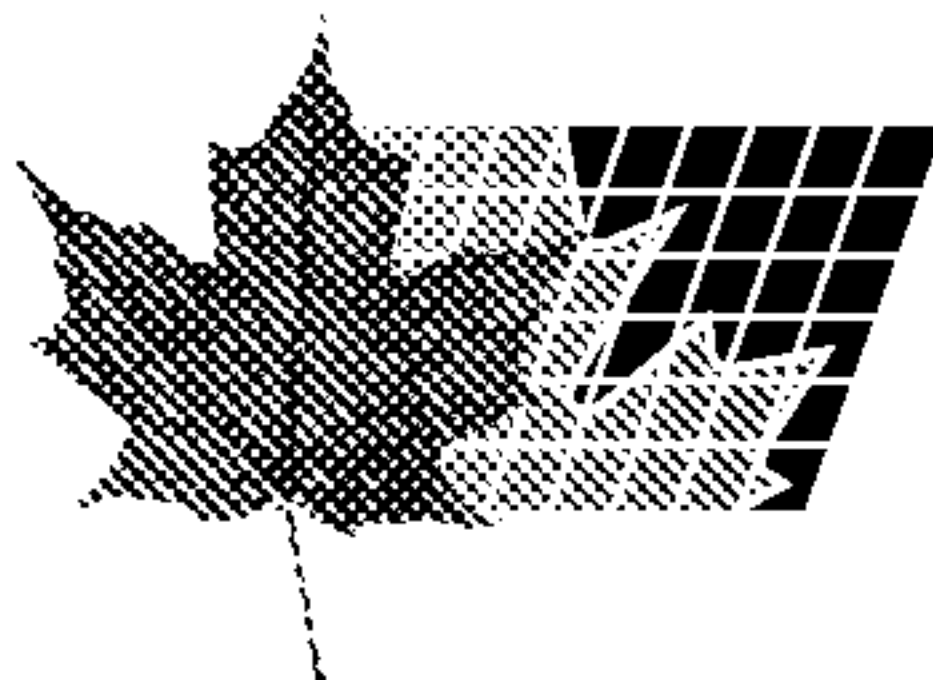




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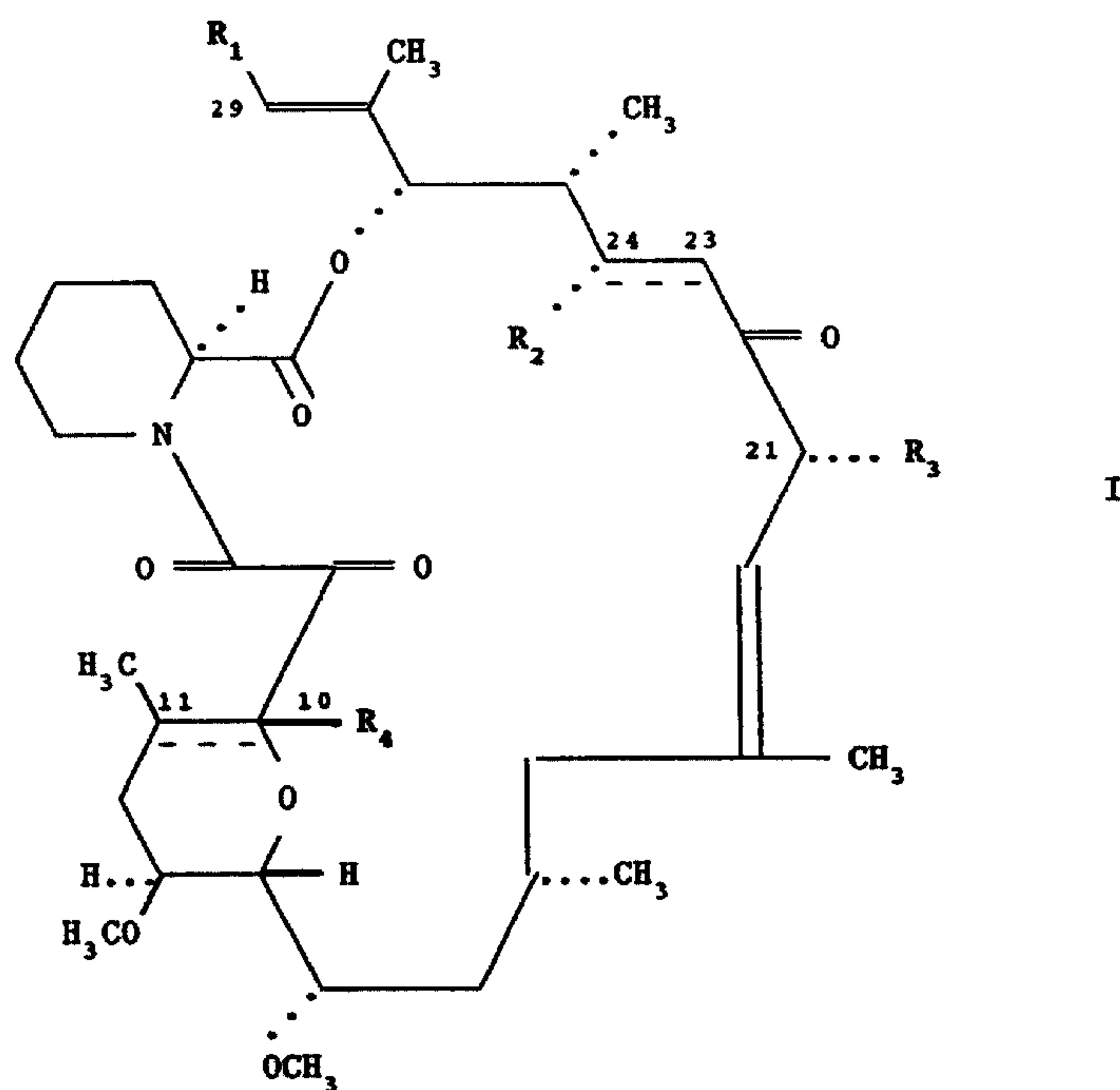
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(54) **COMPOSES TRICYCLIQUES CONTENANT DES
HETEROATOMES**

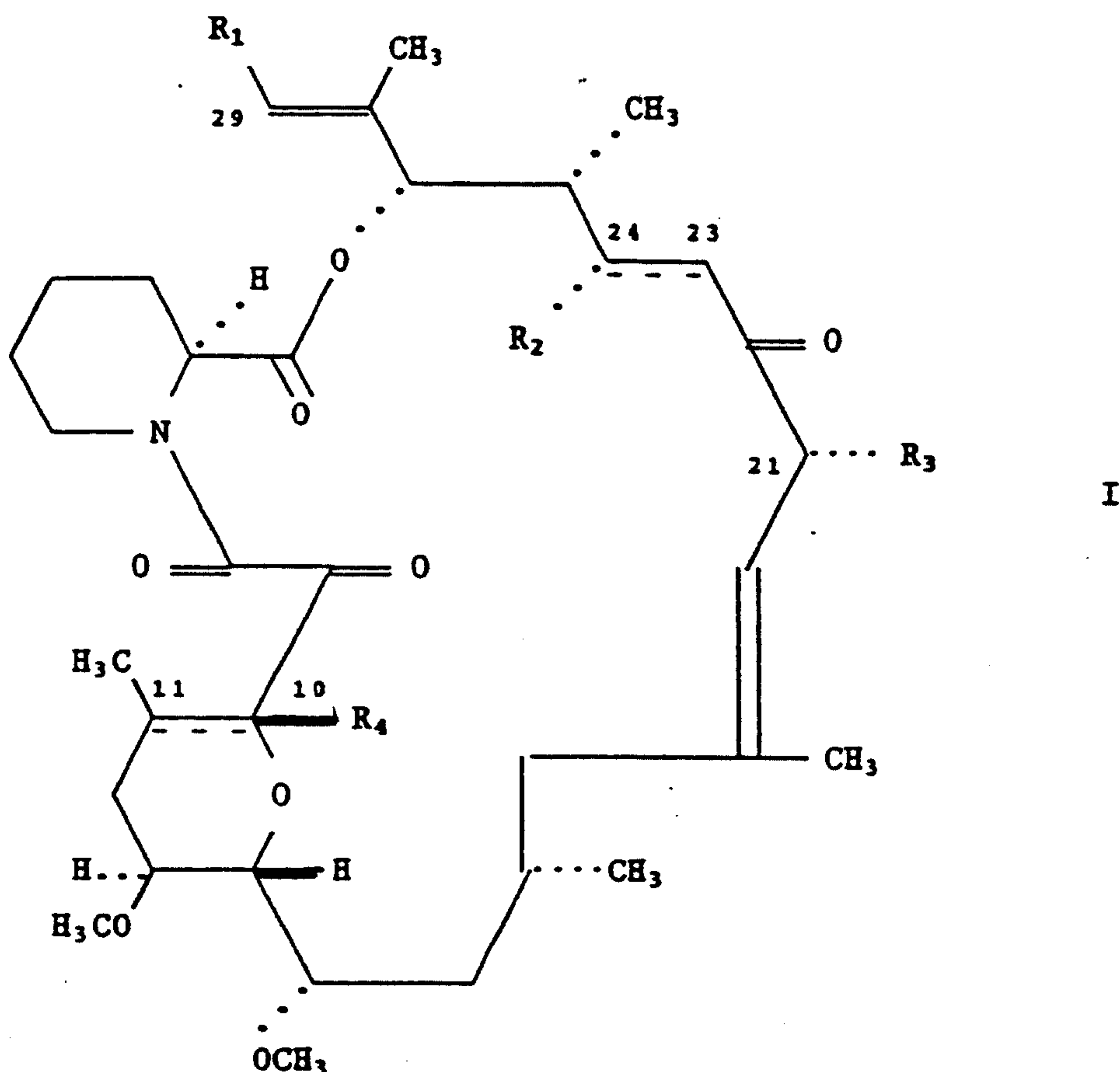
(54) **HETEROATOMS-CONTAINING TRICYCLIC COMPOUNDS**



(57) The invention concerns the compounds of formula I (see formula I) wherein the substituents have various significances. They are prepared by several processes including epimerizing replacement, treatment with cyanogen bromide or thiophosgene, treatment with an acid having a non-nucleophilic anion, deprotection and protection. They possess interesting pharmacological activity as antiinflammatory immunosuppressant, antiproliferative and chemotherapeutic drug resistance reversing agents.

Abstract

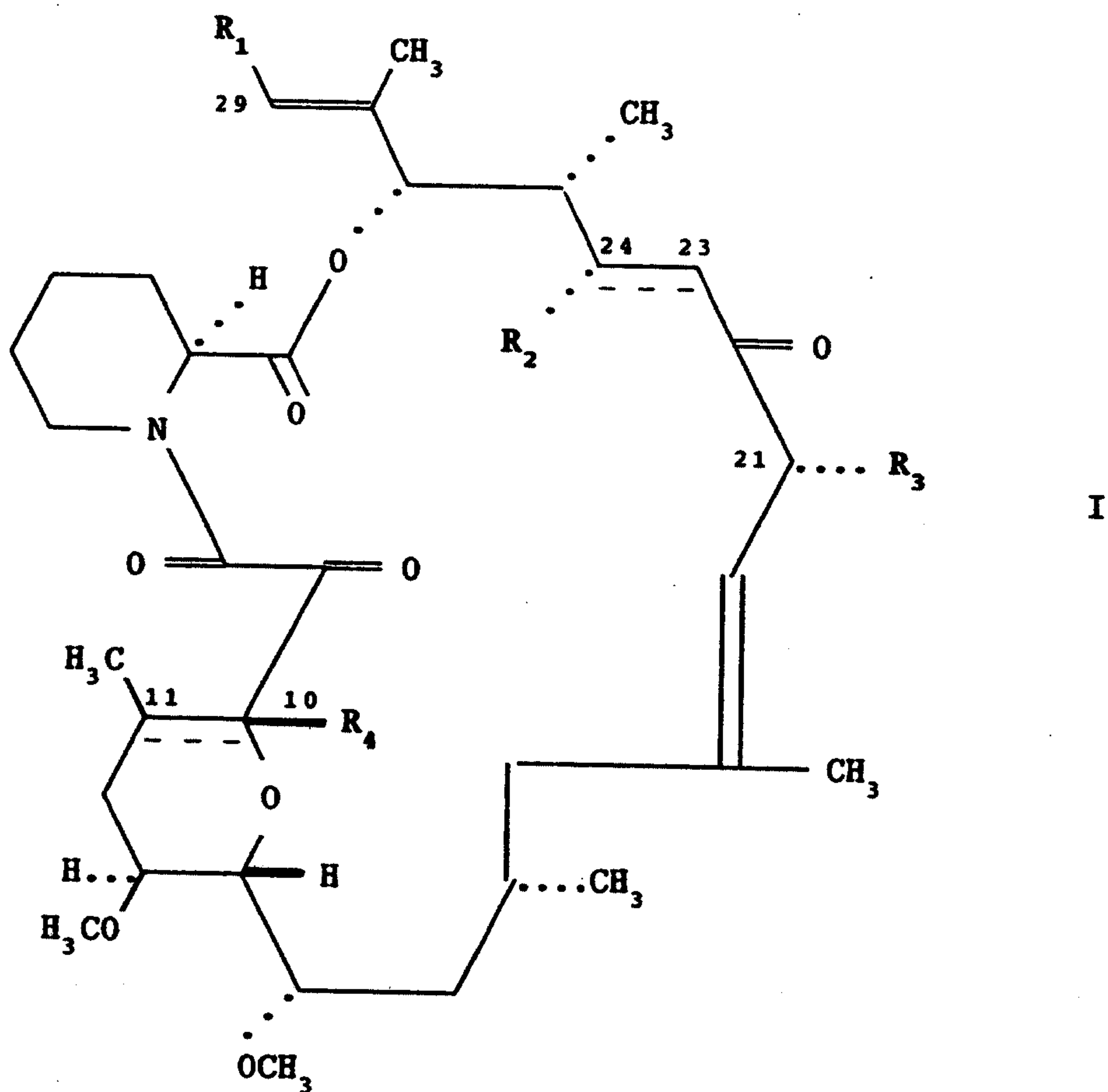
The invention concerns the compounds of formula I



wherein the substituents have various significances. They are prepared by several processes including epimerizing replacement, treatment with cyanogen bromide or thiophosgene, treatment with an acid having a non-nucleophilic anion, deprotection and protection. They possess interesting pharmacological activity as antiinflammatory immunosuppressant, antiproliferative and chemotherapeutic drug resistance reversing agents.

HETEROATOMS-CONTAINING TRICYCLIC COMPOUNDS

The invention relates to the field of macrolides. It concerns the compounds of formula I



wherein

either R_1 is a group (a) of formula

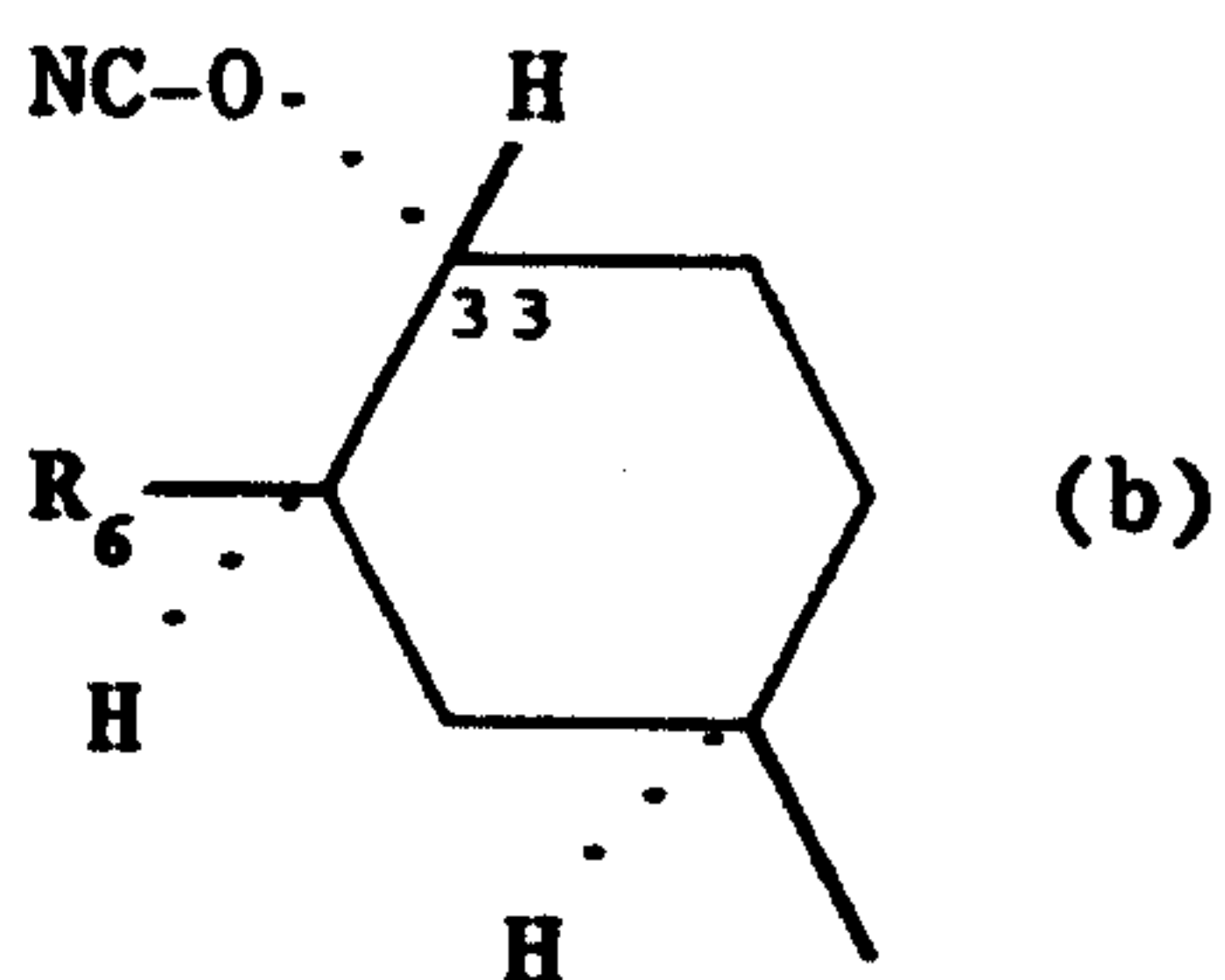


wherein R_5 is chloro, bromo, iodo or azido and
 R_6 is hydroxy or methoxy;

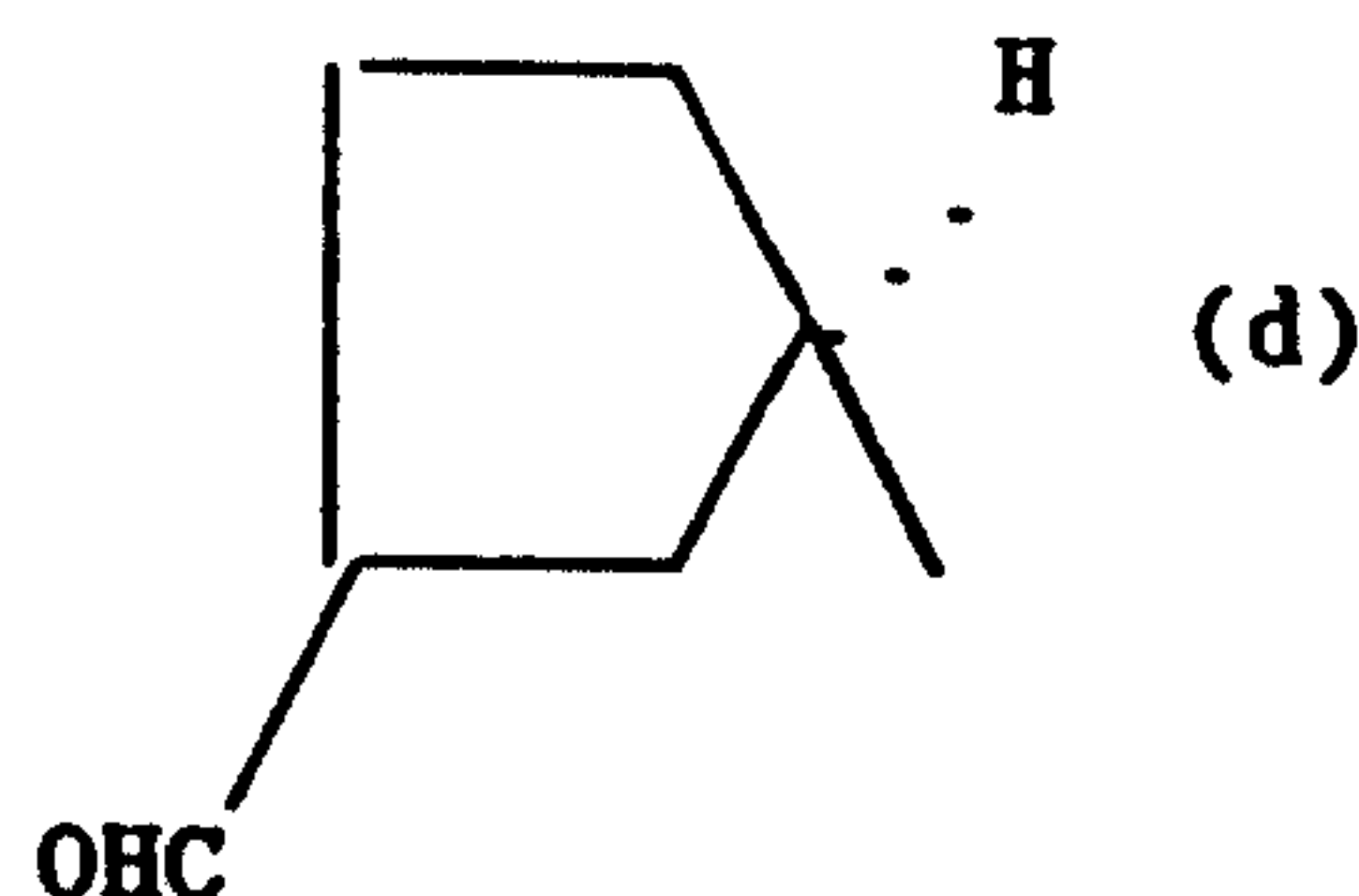
R_2 is oxo and there is a single bond in 23,24 position; optionally protected hydroxy and there is a single or a double bond in 23,24 position; or absent and there is a double bond in 23,24 position; and

R_4 is hydroxy and there is a single bond in 10,11 position; or absent and there is a double bond in 10,11 position;

or R_1 is a group (b) or (d) of formula



and



wherein R_6 is as defined above;

R_2 is as defined above; and

R_4 is hydroxy and there is a single bond in 10,11 position; and
 R_3 is methyl, ethyl, n-propyl or allyl;

in free form and, where such forms exist, in salt form,

hereinafter referred to as "the compounds of the invention".

As is evident from formula I and the definition of the substituents when there is a single bond in 10,11 position the carbon atom to which the methyl group in 11 position is attached has the β -configuration and there is a hydrogen atom with the α -configuration attached to the carbon atom in 11 position; when there is a double bond in 10,11 position this methyl

group lies in the plane of the paper and there is no hydrogen atom in 11 position. When R_2 is oxo no hydrogen atom is attached to the carbon atom in 24 position.

R_1 preferably is a group (d). R_2 preferably is unprotected hydroxy and there is a single bond in 23,24 position. R_3 preferably is ethyl or allyl. R_4 preferably is hydroxy. R_5 preferably is chloro. R_6 preferably is methoxy.

Protected hydroxy preferably is hydroxy protected by a conventional hydroxy-protecting group such as formyl, tert-butoxycarbonyl, or trialkylsilyl; it especially is tert-butyldimethylsilyloxy.

A compound of the invention preferably is in free form. It preferably is in unprotected form.

A subgroup of compounds of the invention is the compounds Ip_1 , i.e. the compounds of formula I wherein

R_1 is a group (a) wherein R_6 is methoxy and either R_5 is chloro or bromo and

R_4 is hydroxy and there is a single bond in 10,11 position or R_5 is azido and

R_4 is hydroxy and there is a single bond in 10,11 position or absent and there is a double bond in 10,11 position;

R_2 is optionally protected hydroxy and there is a single or a double bond in 23,24 position; and

R_3 is as defined above under formula I;

in free form and, where such forms exist, in salt form.

A further subgroup of compounds of the invention is the compounds Ip_3 , i.e. the compounds of formula I wherein

R_1 is a group (b) wherein R_6 is methoxy,

R_2 is optionally protected hydroxy and there is a single bond in 23,24 position; or absent and there is a double bond in 23,24 position;

R_4 is hydroxy and there is a single bond in 10,11 position; and

R_3 is as defined above under formula I;

in free form and, where such forms exist, in salt form.

A further subgroup of compounds of the invention is the compounds Ip₄, i.e. the compounds of formula I wherein

R₁ is a group (d),

R₂ is optionally protected hydroxy and there is a single bond in 23,24 position; or absent and there is a double bond in 23,24 position;

R₄ is hydroxy and there is a single bond in 10,11 position; and

R₃ is as defined above under formula I;

in free form and, where such forms exist, in salt form.

A preferred subgroup of compounds of the invention is the compounds of formula I wherein

R₁ is a group (a) wherein R₅ is as defined above under formula I and R₆ is methoxy;

R₂ is optionally protected hydroxy and there is a single bond in 23,24 position;

R₄ is hydroxy and there is a single bond in 10,11 position; or absent and there is a double bond in 10,11 position; and

R₃ is ethyl or allyl.

A further preferred group of compounds of the invention is the compounds of formula I wherein

R₁ is a group (b) wherein R₆ is methoxy;

R₂ is optionally protected hydroxy and there is a single bond in 23,24 position; or absent and there is a double bond in 23,24 position;

R₄ is hydroxy and there is a single bond in 10,11 position; and

R₃ is ethyl or allyl.

A further preferred subgroup of compounds of the invention is the compounds of formula I wherein

R₁ is a group (d),

R₂ is optionally protected hydroxy and there is a single bond in 23,24 position; or absent and there is a double bond in 23,24 position;

R₄ is hydroxy and there is a single bond in 10,11 position; and

R₃ is ethyl or allyl.

A further subgroup of compounds of the invention is the compounds Iq, i.e. the compounds of formula I wherein

either R_1 is a group (a) wherein R_5 is chloro, bromo, iodo or azido and R_6 is hydroxy or methoxy,

R_2 is oxo and there is a single bond in 23,24 position; optionally protected hydroxy and there is a single or a double bond in 23,24 position; or absent and there is a double bond in 23,24 position; and

R_4 is hydroxy and there is a single bond in 10,11 position; or absent and there is a double bond in 10,11 position;

or R_1 is a group (b) or (d) wherein R_6 is hydroxy or methoxy;

R_2 is as defined above for this subgroup; and

R_4 is hydroxy and there is a single bond in 10,11 position; and

R_3 is methyl, ethyl, n-propyl or allyl,

in free form and, where such forms exist, in salt form.

A further subgroup of compounds of the invention is the compounds Ir, i.e. the compounds of formula I wherein

either R_1 is a group (a) as defined above under formula I; and

R_2 and R_4 have the significance indicated above under group (a);

or R_1 is a group (b) or (d) as defined above under formula I; and

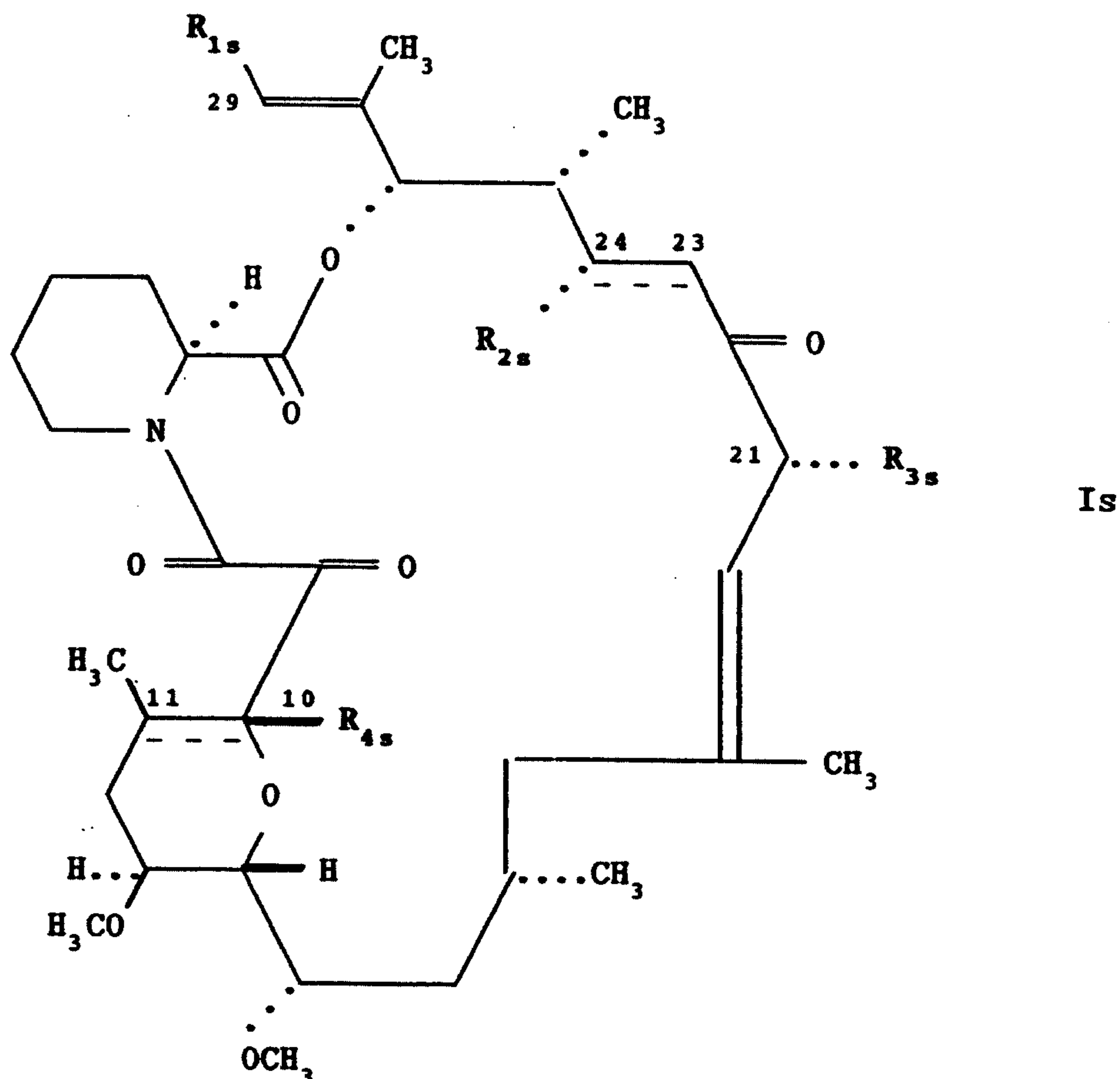
R_2 and R_4 have the significance indicated above under groups (b) and (d); and

R_3 is as defined above under formula I;

in free form and, where such forms exist, in salt form.

In a subgroup of compounds Ir R_2 is other than absent.

A further subgroup of compounds of the invention is the compounds of formula Is



wherein

either R_{1s} is a group (a) wherein R_5 is chloro, bromo, iodo or azido and R_6 is methoxy;

R_{2s} is hydroxy optionally protected by tert-butyldimethylsilyloxy and there is a single bond in 23,24 position; and

R_{4s} is hydroxy and there is a single bond in 10,11 position; or absent and there is a double bond in 10,11 position;

or R_{1s} is a group (b) wherein R_6 is methoxy, or a group (d);

R_{2s} is hydroxy optionally protected by tert-butyldimethylsilyloxy and there is a single bond in 23,24 position; or absent and there is a double bond in 23,24 position; and

R_{4s} is hydroxy and there is a single bond in 10,11 position; and

R_{3s} is ethyl or allyl,

in free form and, where such forms exist, in salt form.

A compound of the invention can be obtained by a process comprising

a) for the preparation of a compound of formula I wherein

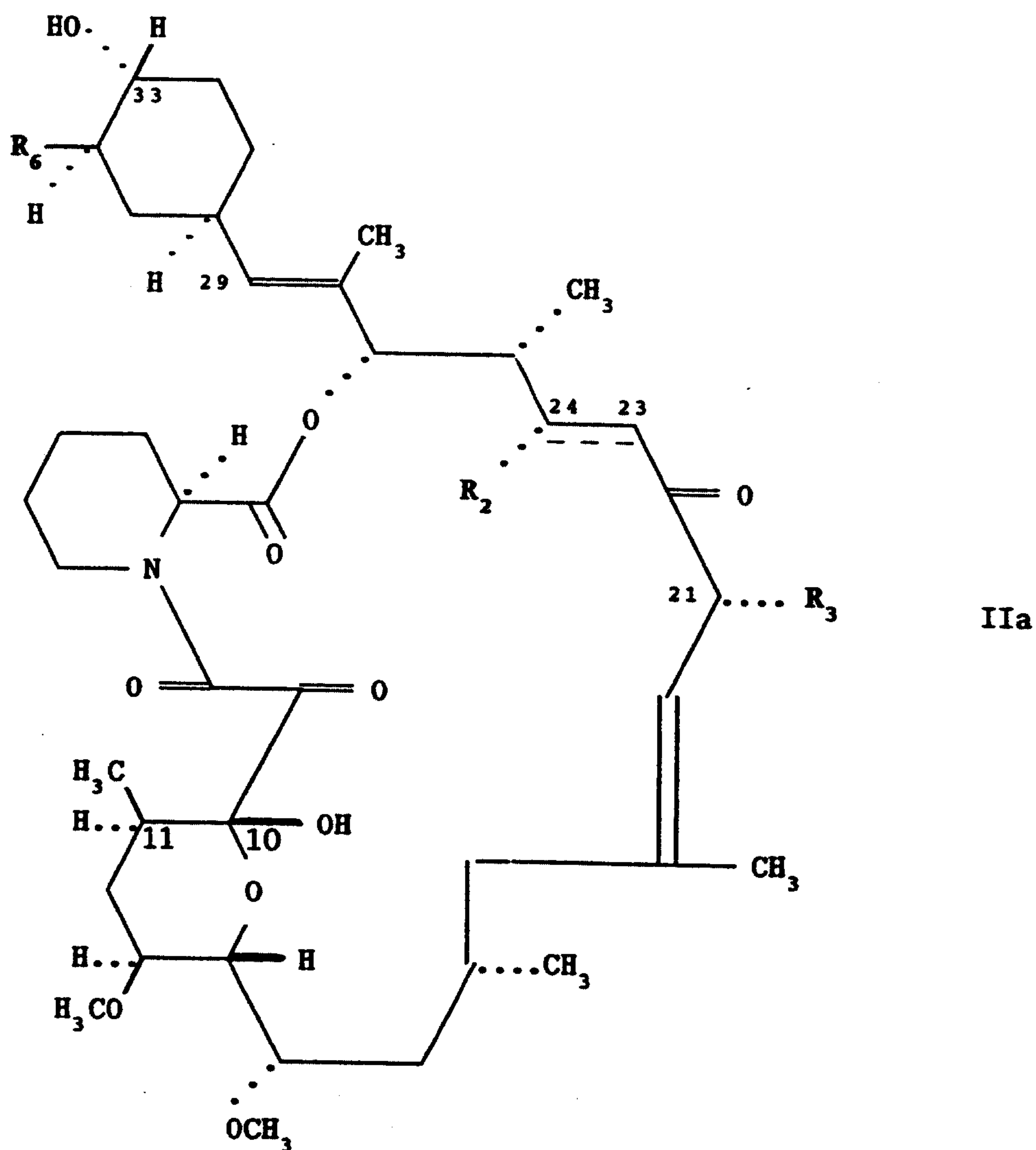
R_1 is a group (a) as defined above under formula I,

R_2 and R_3 are as defined above under formula I and

R_4 is hydroxy

(i.e. a compound Ia),

replacing under simultaneous epimerization the hydroxy group by chlorine, bromine, iodine or azido in a corresponding compound having unprotected hydroxy in 33 position (i.e. a compound IIa, of formula IIa



wherein R_2 and R_3 are as defined above under formula I and R_6 is hydroxy or methoxy);

b) for the preparation of a compound of formula I wherein

R_1 is a group (b) as defined above under formula I,

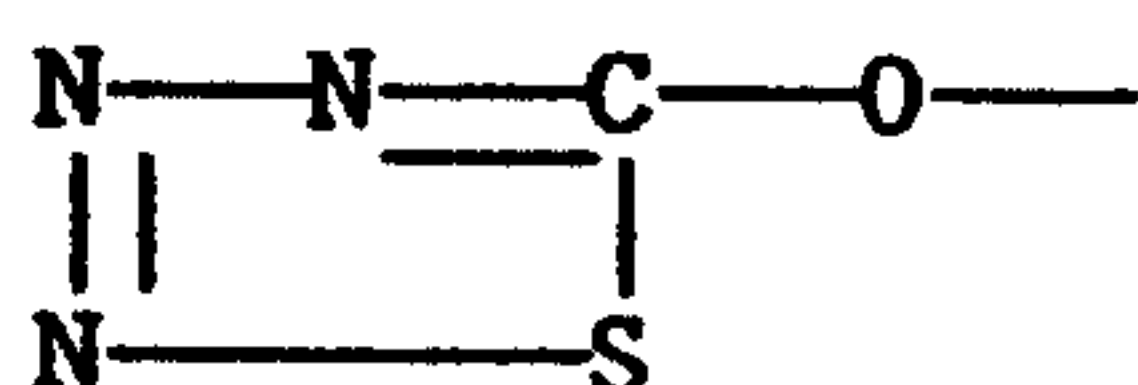
R_2 and R_3 are as defined above under formula I and

R_4 is hydroxy

(i.e. a compound Ib),

treating a corresponding compound IIa with cyanogen bromide in the presence of a base or

treating a corresponding compound IIa with thiophosgene, reacting the resultant product with an inorganic azide and allowing the resultant unstable intermediate having a group



in 33 position (i.e. a compound IIb)

to decompose to a corresponding compound Ib;

c) for the preparation of a compound of formula I wherein

R_1 is a group (d) as defined above under formula I,

R_2 and R_3 are as defined above under formula I and

R_4 is hydroxy

(i.e. a compound Ic),

treating a corresponding compound Ib with an acid having a non-nucleophilic anion; and

- when a resultant compound of formula I has a protected hydroxy and/or a protected amino group,

optionally splitting off the protecting group(s) to give a corresponding compound of formula I having one or more unprotected hydroxy and/or unprotected amino group(s)

(i.e. a compound Ij),

whereby when R_1 is a group (a), a water molecule may be

simultaneously split off and a compound of formula I is obtained wherein

R_1 is a group (a) as defined above under formula I,

R_2 is unprotected hydroxy and there is a single or double bond in 23,24 position; and

R_4 is absent and there is a double bond in 10,11 position (i.e. a compound Ii); or

- optionally protecting an unprotected hydroxy and/or unprotected amino group in a resultant compound of formula I as appropriate to give a corresponding compound of formula I having one or more protected hydroxy and/or protected amino groups(s) (i.e. a compound Ik), and recovering the resultant compound of formula I in free form and, where such forms exist, in salt form.

The process variants of the invention can be effected in a manner analogous to known procedures.

Process variant a) is a substitution reaction under simultaneous epimerization. It is preferably effected in an inert solvent such as tetrahydrofuran or toluene. Preferably for the substitution by halogen the reaction is effected with tetrachloro-, tetrabromo- or tetraiodomethane in the presence of triphenylphosphine, and for the substitution by azido with azodicarboxylic acid ester, preferably diethyl ester, and hydrazoic acid. A hydroxy group in 24 position may be in protected form. As protecting group known hydroxy protecting groups such as tert-butyldimethylsilyl may be used. A protecting group may subsequently be split off in accordance with known procedures, e.g. with hydrofluoric acid in acetonitrile. Upon deprotection a water molecule may, depending on the reaction conditions chosen, simultaneously be split off in position 10,11 and a double bond formed. The individual compounds can be separated from such a resultant mixture in conventional manner, e.g. chromatographically.

Compounds Ia may be further processed by e.g. oxidation or dehydration to corresponding compounds wherein R_4 is absent; for example, oxidation of compounds Ia wherein R_2 is hydroxy leads to corresponding compounds wherein R_4 is absent and R_2 is oxo.

Process variant b) is a cyanidation reaction. It preferably is effected in an inert solvent such as a chlorinated hydrocarbon, e.g. dichloromethane. The temperature preferably is about room temperature. The base is e.g. 4-dimethylaminopyridine.

A compound of formula I obtained accordance to process variants a) and b) above may be isolated from the reaction mixture and purified in accordance with known methods. When R_2 is hydroxy and there is a single bond in 23,24 position a water molecule may be simultaneously split off.

A corresponding mixture of compounds Ib is obtained wherein either R_2 is hydroxy and there is a single bond in 23,24 position or R_2 is absent and there is a double bond in 23,24 position. The individual compounds can be separated from such a resultant mixture in conventional manner, e.g. chromatographically.

The second procedure according to process variant b) is effected by reaction with thiophosgene, preferably in the presence of an acid scavenger such as 4-dimethylaminopyridine. Preferably an inert solvent such as acetonitrile is used. The temperature preferably is about room temperature. The subsequent reaction with an inorganic azide is preferably effected with sodium azide. The resultant compounds IIb are unstable and decompose already at room temperature to compounds Ib, under splitting off of nitrogen and sulfur. This reaction step preferably is effected in an inert solvent such as an aromatic hydrocarbon, e.g. benzene. Temperature preferably is elevated, e.g. about 50°C.

In process variant c) a ring contraction takes place. Protecting groups which are present may be simultaneously split off. Preferably an inert solvent such as acetonitrile is used. Preferably hydrofluoric acid is used as acid having a non-nucleophilic anion. Temperature preferably is about room temperature.

The optional deprotection process variant may also be effect in conventional manner. For splitting off of e.g. tert-butyldimethylsilyl it is effected by treatment with e.g. hydrofluoric acid in a solvent such as acetonitrile. Depending on the reaction conditions selected (duration, temperature, etc.) the splitting can be steered in such a manner that either all or only some protecting group are removed. Partial deprotection is particularly indicated where a definite hydroxy group is to be subsequently reacted in a later reaction.

The optional protection step variant may also be effected in conventional manner along similar lines.

Thus for subsequent reactions involving a hydroxy group, particularly a hydroxy group in position 24 and/or 33, selective protection of only one of the two free hydroxy groups or selective deprotection of only one of the two protected hydroxy groups may be effected in such a manner that reaction occurs only at the desired position. Mixtures of end products may be obtained thereby; such mixtures can be separated in conventional manner,

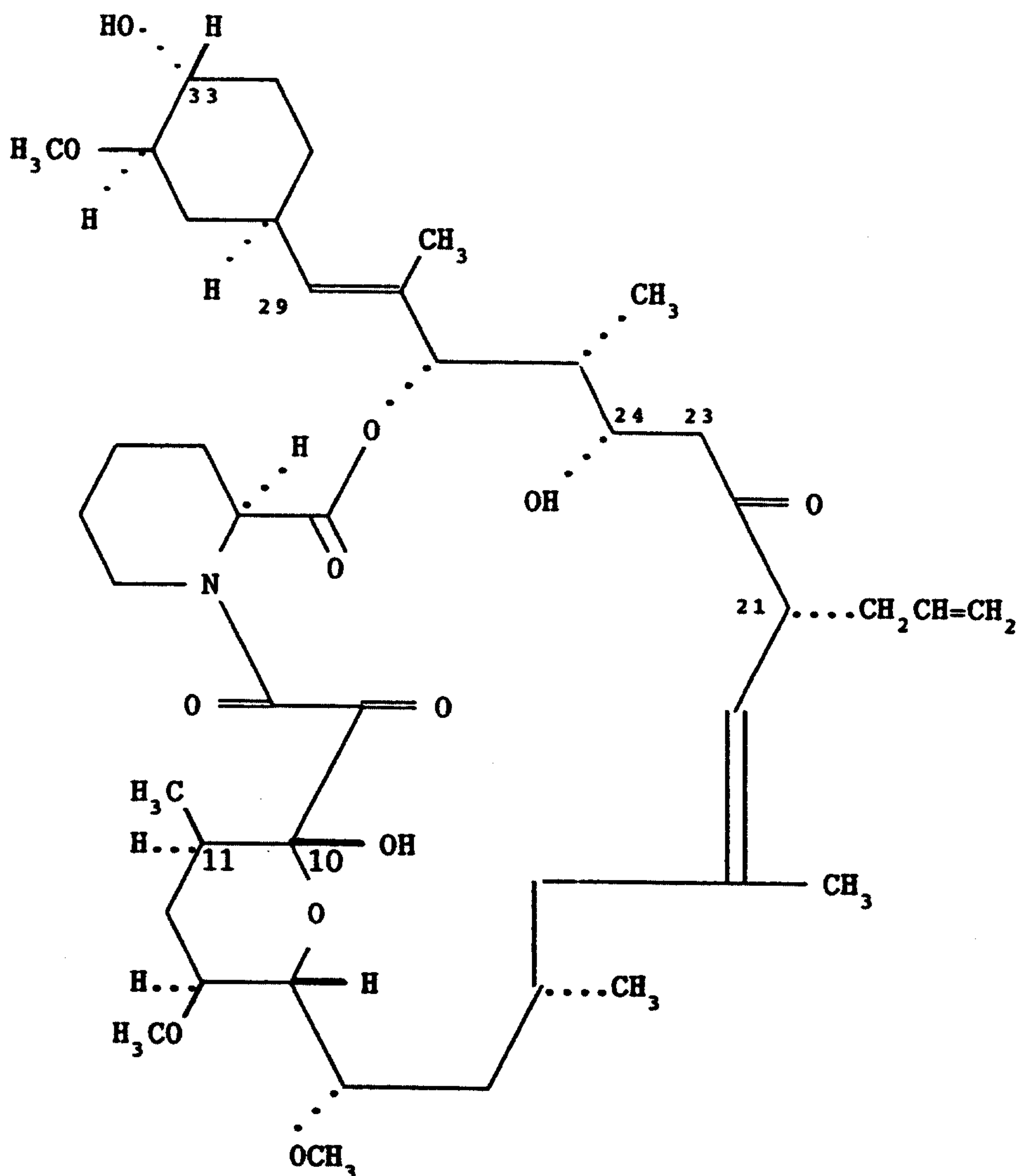
e.g. chromatographically. Resultant end products still containing protecting groups can be subsequently deprotected. Reaction conditions may alternatively be selected such that simultaneously with or immediately after reaction the protecting groups are removed (one pot process).

The compounds of formula I may be isolated and purified from the reaction mixture in conventional manner.

Insofar as their preparation is not specifically described herein, e.g. in the Examples, the compounds used as starting materials are known or can be obtained in conventional manner from known compounds, e.g. starting from appropriate Streptomyces strains such as Streptomyces tsukubaensis No. 9993 described in e.g. Fujisawa EP 184162. Samples can be obtained from the Fermentation Research Institute, Tsukuba, Ibaraki 305, Japan under provisions of the Budapest Treaty under deposit No. FERM BP-927. This strain has been redeposited on April 27, 1989 e.g. as disclosed in Sandoz EP 356399, with the Agricultural Research Culture Collection International Depository, Peoria, Illinois 61604, USA under the provisions of the Budapest Treaty under deposit No. NRRL 18488.

The following Examples illustrate the invention and are not limitative. All temperatures are in degrees Centigrade. All NMR spectra are in CDCl_3 , ppm. The abbreviations have the following meanings:

BOC: tert-butoxycarbonyl;
 cfr: colourless foamy resin;
 db: double bond;
 Et: ethyl;
 FK 506: the compound of formula



i.e. 17 α -allyl-1 β ,14 α -dihydroxy-12-[2'-(4''(R)-hydroxy-3''(R)-methoxycyclohex-1''(R)-yl)-1'-methyl-trans-vinyl]-23 α ,25 β -dimethoxy-13 α ,19,21 α ,27 β -tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0']octacos-18-trans-ene-2,3,10,16-tetraone (according to the atom numbering in EP 184162; however, in the Examples the atom numbering of formula I is used throughout);

FR 520: as FK 506, but with ...CH₂CH₃ (ethyl) in place of allyl in position 21 in the formula;

iBuoyloxy: isobutanoyloxy [(H₃C)₂CHCOO-];
iPr: isopropyl;

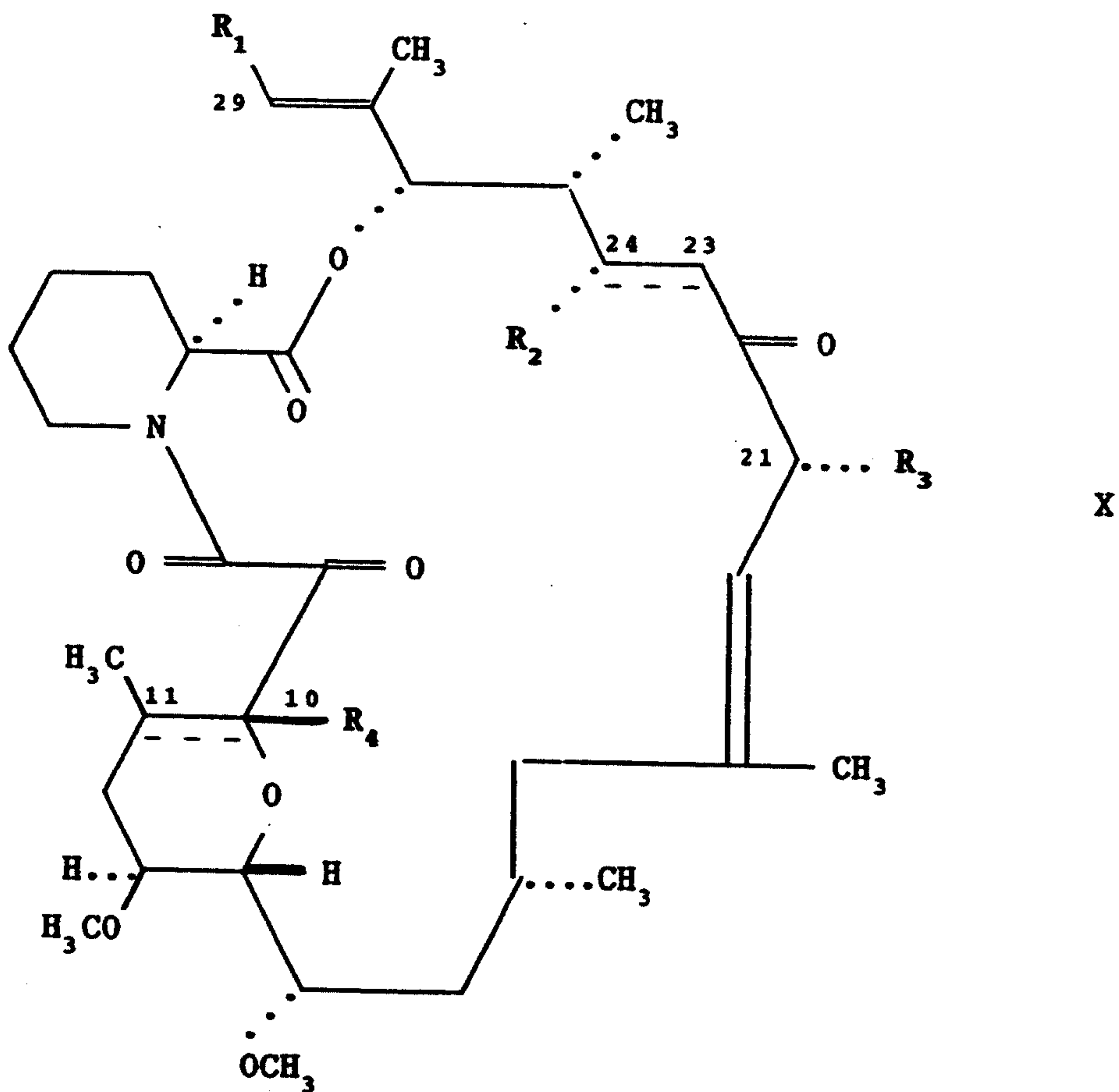
na: not applicable;
N₃: azido;

OMe (or MeO): methoxy;
OtBDMS: tert-butyldimethylsilyloxy;

sb: **single bond;**

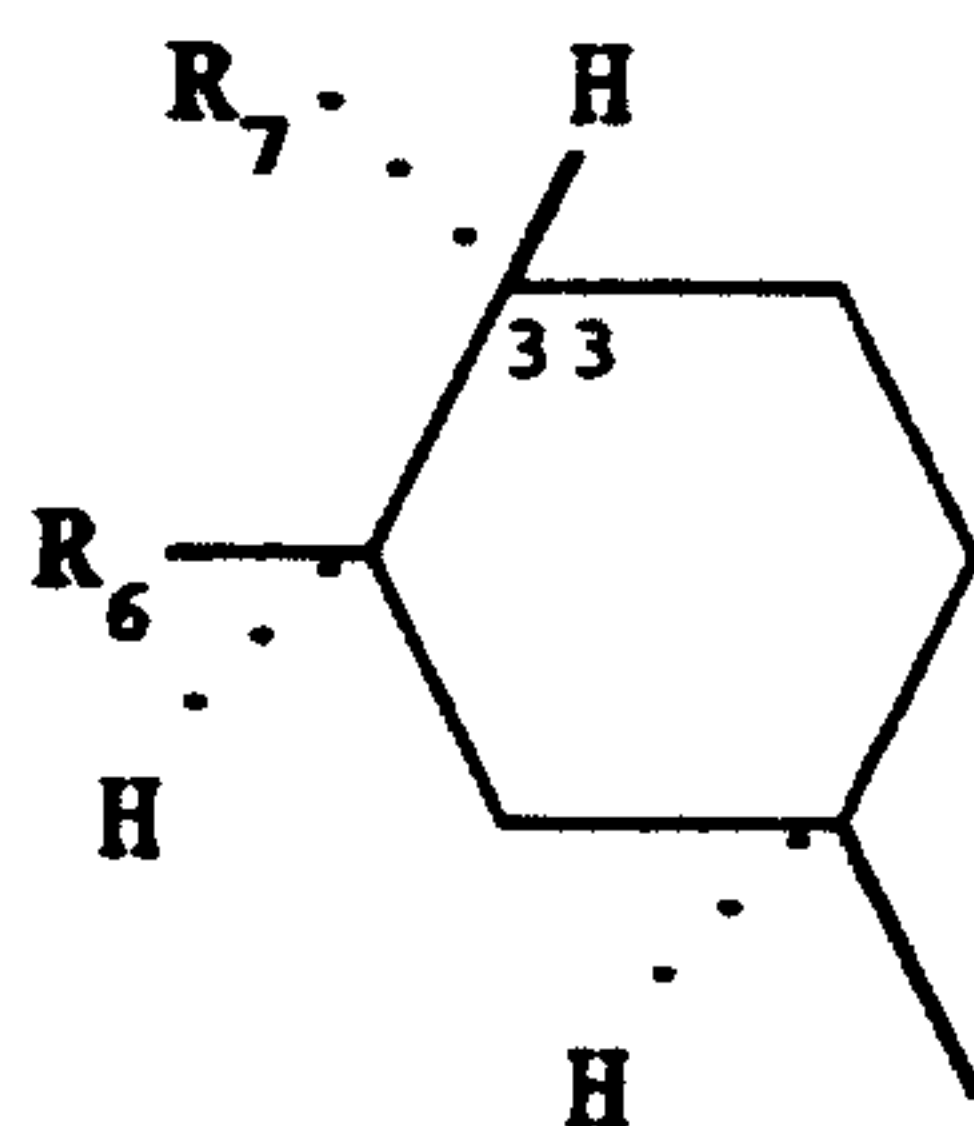
tBu: **tert-butyl.**

Examples 15 to 63 illustrate the preparation of compounds no longer encompassed by the scope of the present invention but useful as intermediates for the preparation of compounds of formula I. They are of formula X



wherein

R_1 is a group (c) of formula



(c)

wherein R_6 is as defined above and

R_7 is oxo; optionally protected hydroxy; methoxy; methylthiomethoxy; isobutanoyloxy; aminooxalyloxy; $R_8R_9CHCOO-$ wherein R_8 is optionally protected hydroxy or optionally protected amino and R_9 is hydrogen or methyl; or p-tolyloxythiocarbonyloxy;

R_2 is oxo and there is a single bond in 23,24 position; absent and there is a double bond in 23,24 position; or is optionally protected hydroxy, methoxy, methylthiomethoxy, isobutanoyloxy, aminooxalyloxy or $R_8R_9CHCOO-$ wherein R_8 and R_9 are as defined above, and there is a single or a double bond in 23,24 position;

whereby for that group (c)

1) when R_7 is oxo, unprotected hydroxy or methoxy

then R_2 is other than absent and other than unprotected hydroxy or methoxy, and

there is a single bond in 23,24 position;

2) when R_6 is methoxy and R_7 is methylthiomethoxy

then R_2 is other than absent and other than unprotected hydroxy;

3) when R_6 is methoxy and R_7 is protected hydroxy

then R_2 is other than optionally protected hydroxy; and

4) when R_6 is hydroxy

then R_7 is other than optionally protected hydroxy; and

R_4 is hydroxy and there is a single bond in 10,11 position; and

R_3 is as defined above,

in free form and, where such forms exist, in salt form.

Example 1: 24-tert-Butyldimethylsilyloxy-33-epi-33-chloro-FK506

[Formula I: R_1 = a group (a) wherein R_5 = chloro, R_6 = OMe; R_2 = OtBDMS, single bond in 23,24 position; R_3 = allyl; R_4 = OH, single bond in 10,11 position]

[Process variant a), replacement with epimerization]

0.092 g 24-tert-butyldimethylsilyloxy-FK506 is heated for 15 hours under refluxing with 0.037 g triphenylphosphine in 4 ml of tetrachloromethane. The solvent is evaporated to dryness under reduced pressure and the residue is purified by column chromatography over silicagel using a mixture of hexane and acetic acid ethyl ester (2:1) as the eluant. The title compound is obtained (colourless foam):

¹H-NMR: about 2:3 mixture of conformers:
main conformer: 4.56 (m, $w_{1/2}$ = 7 Hz, H-33).

The starting material is obtained as follows:

a) 20 g FK 506 is dissolved in 400 ml of dry dimethylformamide, 5.08 g imidazole and 11.25 g tert-butyldimethylchlorosilane is added in portions and the mixture is stirred for 110 hours at room temperature. The reaction mixture is diluted with acetic acid ethyl ester and washed five times with water. The organic phase is dried over sodium sulfate and the solvent distilled off under reduced pressure. The resultant crude product is purified by chromatography over silicagel using hexane/acetic acid ethyl ester 3:1 as the eluant. 24,33-Bis-(tert-butyldimethylsilyloxy)-FK 506 is obtained:

¹³C-NMR: main conformer: 69.7 (C-24); 75.1 (C-33); 84.1 (C-32); 164.6 (C-8); 168.9 (C-1); 196.4 (C-9); 209.3 (C-22);
minor conformer: 70.9 (C-24); 75.3 (C-33); 84.1 (C-32); 165.8 (C-8); 168.2 (C-1); 191.2 (C-9); 210.0 (C-22);

b) 0.5 g 24,33-bis-(tert-butyldimethylsilyloxy)-FK506 is dissolved at 0° under stirring into a mixture of 10 ml of acetonitrile and 0.5 ml of 40 % hydrofluoric acid. After 2 hours at that temperature the reaction medium is diluted with dichloromethane. The solution is successively washed with saturated aqueous sodium bicarbonate solution and water and the organic phase is dried over sodium sulfate, and the solvent is evaporated under

reduced pressure. The resultant residue is purified by column chromatography over silicagel (eluant: dichloromethane/methanol 9:1).

24-tert-Butyldimethylsilyloxy-FK 506 is obtained as a colourless foam:

¹³C-NMR: main conformer: 69.7 (C-24); 73.6 (C-33); 84.1 (C-32); 164.6 (C-8); 168.9 (C-1); 196.4 (C-9); 209.2 (C-22);
minor conformer: 70.7 (C-24); 73.6 (C-33); 84.2 (C-32); 165.8 (C-8); 168.2 (C-1); 191.4 (C-9); 209.2 (C-22).

Example 2: 24-tert-Butyldimethylsilyloxy-33-epi-33-azido-FK506

[Formula I: R_1 = a group (a) wherein R_5 = azido, R_6 = OMe; R_2 = OtBDMS, single bond in 23,24 position; R_3 = allyl; R_4 = OH, single bond in 10,11 position]

[Process variant a)]

To a solution of 0.092 g 24-tert-butyldimethylsilyloxy-FK506 and 0.08 g triphenylphosphine in 2 ml of dry tetrahydrofuran is added at 0° 0.047 ml of azodicarboxylic acid diethyl ester, followed by 0.15 ml of a 2 M solution of hydrazoic acid in toluene. The solution is brought to room temperature and stirred for 18 hours. The solvent is evaporated to dryness under reduced pressure and the residue purified as described above under Example 1. The title compound is obtained (colourless foam):

¹H-NMR: 4.07 (m, H-33).

The following compounds of formula I are obtained in analogous manner in accordance with process variant a):

Analogous					Physicochemical					
Example to	R ₁	R ₅	R ₆	R ₇	R ₂	Position R ₃ 23,24	R ₄	Position 10,11	characterization data	
No.	Ex. No.									
3	1 ¹⁾	(a)	Cl	OMe	na	OtBDMS	sb	Et	OH	NMR [*]
4	1 ¹⁾	(a)	Br	OMe	na	OtBDMS	sb	Et	OH	
5	1 ¹⁾	(a)	Br	OMe	na	OtBDMS	sb	allyl	OH	
6	2 ¹⁾	(a)	N ₃	OMe	na	OtBDMS	sb	Et	OH	
6a	1 ¹⁾	(a)	I ₃	OMe	na	OtBDMS	sb	Et	OH	NMR [*]

* NMR: Example 3: ¹H-NMR: 4.56 (m, H-33);

Example 6a: ¹³C-NMR: mixture of conformers: 210.33 (C-22); 168.91 (C-1); 164.59 (C-8); 123.64 (C-20); 78.90 (C-32); 25.81 (tBu).

¹⁾ The starting material is obtained from FR 520 in a manner analogous to 24-tert-butyltrimethylsilyloxy-FK 506 (see Example 1):

a) 24,33-bis-(tert-butyltrimethylsilyloxy)-FR 520: ¹H-NMR: about 2:1 mixture of 2 conformers:

main conformer: 4.42 (m, H-2); 4.41 (db, 13 Hz, H-6 eq.); 4.05 (txt, J=1.5 Hz and 6 Hz, H-24); 3.80 (dxd, J=1.5 Hz and 10 Hz, H-14); 2.95 (dxdxd, J=4 Hz, 8 Hz and 11 Hz, H-32);

minor conformer: 4.25 (q, J=5 Hz, H-24); 3.94 (dxd, J=2 Hz and 10 Hz, H-14); 2.95 (dxdxd, J=4 Hz, 8 Hz and 11 Hz, H-32);

b) 24-tert-butyltrimethylsilyloxy-FR 520: ¹H-NMR: about 2:1 mixture of 2 conformers:

main conformer: 4.44 (b, H-2); 4.42 (db, J=13 Hz, H-6 eq.); 4.05 (dxt, J=1.5 Hz and 6 Hz, H-24); 3.81 (dxd, J=1.5 Hz and 10 Hz, H-14); 3.01 (dxdxd, J=4 Hz, 8 Hz and 11 Hz, H-32);

minor conformer: 4.24 (H-24); 3.94 (dxd, J=2 Hz and 10 Hz, H-14); 3.01 (dxdxd, J=4 Hz, 8 Hz and 11 Hz, H-32).

Example 7: 24-tert-Butyldimethylsilyloxy-33-cyanoxy-FR 520

[Formula I: R_1 = a group (b) wherein R_6 = OMe; R_2 = OtBDMS, single bond in 23,24 position; R_3 = Et; R_4 = OH, single bond in 10,11 position]

[Process variant b), treatment with cyanogen bromide]

A solution of 2 g 24-tert-butyldimethylsilyloxy-FR 520 and 0.94 g 4-dimethylaminopyridine in 100 ml of dichloromethane is rapidly reacted at room temperature with a solution of 0.4 g cyanogen bromide in 15 ml of dichloromethane and the mixture is stirred at room temperature for 20 minutes. The mixture is filtered over silicagel (eluant: n-hexane/acetic acid ethyl ester) and the solvent is removed from the relevant fraction under reduced pressure. The title compound is obtained as a colourless foamy resin:

¹H-NMR: mixture of conformers: 4.3 (m; H-33).

Example 8: 24-tert-Butyldimethylsilyloxy-33-cyanoxy-FR 520

[Formula I: as for Example 7]

[Process variant b), treatment with thiophosgene and sodium azide]

A solution of 2 g 24-tert-butyldimethylsilyloxy-FR 520 and 2 g 4-dimethylaminopyridine in 50 ml of acetonitrile is carefully reacted with 0.4 ml of thiophosgen and the mixture stirred for 3 hours at room temperature. The reaction mixture is poured onto a well-stirred mixture consisting of 150 ml of acetic acid ethyl ester, 40 ml of saturated aqueous sodium chloride solution and 50 ml of 2 N sodium azide solution, vigorous stirring is continued for 5 minutes and the organic phase is separated. The organic phase is then successively washed with water, 1 N hydrochloric acid solution, water, and saturated aqueous sodium chloride solution, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue is taken up in about 100 ml of benzene and heated at 30-40° for 2 hours. The benzene is removed under reduced pressure and the title compound is recovered from the residue as a colourless foamy resin by column chromatography over silicagel (eluant: n-hexane / acetic acid ethyl ester):

¹H-NMR: see Example 7.

The following compounds of formula I are obtained in analogous manner in accordance with process variant b):

Analogous				Physicochemical						
Example No.	to Ex. No.	R ₁	R ₅	R ₆	R ₇	R ₂	Position R ₃ 23,24	R ₄	Position 10,11	characterization data
9	7,8	(b)	na	OMe	na	OTBDMS	sb	allyl	sb	NMR*
10a ₁	7,8	(b)	na	OMe	na	OH	sb	Et	sb	NMR*
10b ₂	7,8	(b)	na	OMe	na	absent	db	Et	sb	NMR
11a ₂	7,8	(b)	na	OMe	na	OH	sb	allyl	sb	
11b	7,8	(b)	na	OMe	na	absent	db	allyl	sb	

Example 12: 29-Des-(4-hydroxy-3-methoxycyclohexyl)-29-(3-formylcyclopentyl)-FR 520

[Formula I: R_1 = a group (d); R_2 = OH, single bond in 23,24 position;
 R_3 = Et; R_4 = OH, single bond in 10,11 position]

[Process variant c), treatment with a non-nucleophilic anion]

0.5 g 24-tert-butyldimethylsilyloxy-33-cyanoxy-FK 520 (compound of Examples 7 and 8) or 33-cyanoxy-FR 520 (compound of Example 10a) is dissolved into a mixture of 50 ml of acetonitrile and 2 ml of 40 % wt. aqueous hydrofluoric acid and the mixture is stirred for 2.5 hours at room temperature. The reaction mixture is then distributed between acetic acid ethyl ester and saturated aqueous sodium bicarbonate solution, the aqueous phase is discarded and the organic phase is washed with saturated aqueous sodium chloride solution, dried over sodium sulfate, filtered and concentrated under reduced pressure. The title compound is obtained as a colourless foamy resin from the residue by column chromatography over silicagel (eluant: n-hexane / acetic acid ethyl ester):

¹H-NMR: mixture of conformers: 9.64 (d, J=2 Hz, CHO); 2.87 (m, H-32);
2.67 (m, H-30).

The following compounds of formula I are obtained in analogous manner in accordance with process variant c):

Analogous		Physicochemical								
Example No.	to Ex. No.	R ₁	R ₅	R ₆	R ₇	R ₂	Position R ₃ 23,24	R ₄	Position 10,11	characterization data
13	12 ¹⁾	(d)	na	na	na	OH	sb	allyl	OH	NMR*
14	12 ²⁾	(d)	na	na	na	absent	db	Et	OH	NMR
* NMR: Example 13: ¹H-NMR: mixture of conformers: 9.65 (d, J=2 Hz, CHO); 2.86 (m, H-32); 2.15 (dxdxd, J=12.5 Hz and 7.5 Hz and 5 Hz, H-31a); 1.45 (dxt, J=12.5 and 9 Hz, H-31b); 2.67 (m, H-30); Example 14: ¹H-NMR: about 5:3 mixture of conformers: 9.66 (d, J=2 Hz, CHO); 6.83 (dxd, J=15 and 5 Hz) resp. 6.77 (dxd, J=15 and 7.5 Hz) H-24; 6.19 (dxd, J=15 and 1.5 Hz) resp. 6.30 (dxd, J=15 and 1 Hz) H-23;										
1) Starting from the compound of Example 9 or 11a; 2) Starting from the compound of Example 10b.										

- Example 15:** a) 24-tert-Butyldimethylsilyloxy-33-oxo-FK 506
and
b) 24-tert-Butyldimethylsilyloxy-33-methylthiomethoxy
FK 506

[Formula X: R_1 = a group (c) wherein R_6 = OMe, R_7 = oxo and, respectively, methylthiomethoxy; R_2 = OtBDMS, single bond in 23,24 position; R_3 = allyl; R_4 = OH, single bond in 10,11 position]

1 g 24-tert-Butyldimethylsilyloxy-FK 506 is dissolved at room temperature into a mixture of 20 ml of acetic anhydride and 30 ml of dimethylsulfoxide and stirring is effected for 5 hours at room temperature. The reaction mixture is poured onto a mixture of acetic acid ethyl ester and potassium carbonate solution, stirred for 20 minutes, the phases are separated and the organic phase is repeatedly washed with water, dried over sodium sulfate, filtered and concentrated under reduced pressure. Following column chromatographic fractionation of the residue over silicagel (eluant: acetic acid ethyl ester / n-hexane 2:1) the title compounds are obtained as colourless foamy resins.

The following compounds of formula X are obtained in analogous manner as colourless foamy resins:

Analogous		R ₁	R ₅	R ₆	R ₇	R ₂	Position R ₃ 23,24	R ₄	Position 10,11
Example No.	Ex. No.								
16a	15 ¹⁾	(c)	na	OMe	OtBDMS	OCH ₂ SCH ₃	sb	allyl	sb
16b	15 ²⁾	(c)	na	OMe	OtBDMS	oxo	sb	allyl	sb
16c	15 ²⁾	(c)	na	OMe	oxo	OtBDMS	sb	Bt	sb
16d	15 ³⁾	(c)	na	OMe	OtBDMS	oxo	sb	Bt	sb

- 1) Starting from 33-tert-butylidimethylsilyloxy-FK 506 (compound of Example 16 in EP 184162);
 eluant: toluene / acetic acid ethyl ester 9:1;
 2) Starting from 24-tert-butylidimethylsilyloxy-FR 520;
 3) Starting from 33-tert-butylidimethylsilyloxy-FR 520 (DOS 39 38 754);

Example 17: 33-p-Tolyloxythiocarbonyloxy-FK 506

[Formula X: R_1 = a group (c) wherein R_6 = OMe, R_7 = p-tolyloxythiocarbonyloxy; R_2 = OH, single bond in 23,24 position; R_3 = allyl; R_4 = OH, single bond in 10,11 position]

A solution of 2 g FK 506 in 70 ml of acetonitrile is successively reacted with 0.46 g 4-dimethylaminopyridine and 1.8 g p-tolyloxythiocarbonyl chloride and the mixture is stirred for 15 hours at room temperature. The reaction mixture is then diluted with acetic acid ethyl ester and successively washed with saturated aqueous sodium bicarbonate solution, 0.5 N hydrochloric acid and water, dried over sodium sulfate, filtered and concentrated under reduced pressure. The title compound is isolated from the residue as a light yellow foamy resin by column chromatography over silicagel (eluant: acetic acid ethyl ester / n-hexane 1:1).

Example 18: 33-Aminomethylcarbonyloxy- Δ^{23} -FK 506

[Formula X: R_1 = a group (c) wherein R_6 = OMe, R_7 = $R_8R_9CHCOO-$ (R_8 = amino; R_9 = H); R_2 = absent, double bond in 23,24 position; R_3 = allyl; R_4 = OH, single bond in 10,11 position]

2 g N-BOC-glycine, 1 g dicyclohexylcarbodiimide, 0.5 g Δ^{23} -FK 506 (second compound of Example 17 in EP 184162) and 1 g 4-dimethylaminopyridine are successively taken up at room temperature in 70 ml of acetonitrile and the mixture is stirred for 20 minutes at room temperature. The reaction mixture is filtered, the filtrate diluted with acetic acid ethyl ester and successively washed with 1 N hydrochloric acid, aqueous sodium bicarbonate solution and water, the organic phase is dried over sodium sulfate, filtered, concentrated, and the residue is taken up in 50 ml of acetonitrile.

In order to split off the protecting group 0.5 g p-toluenesulfonic acid monohydrate is added and the mixture heated to refluxing for 5 minutes, the solution is cooled off, diluted with acetic acid ethyl ester, washed to neutrality with water, the organic phase is dried over sodium sulfate and concentrated. From the residue the title compound is obtained as a colourless foamy resin after column chromatography over silicagel (eluant: acetic acid ethyl ester / methanol 20:3).

Example 19: 24-tert-Butyldimethylsilyloxy-FR 520-
 33-[(tert-butyldimethylsilyloxy)-(S)-lactate]

[Formula X: R_1 = a group (c) wherein R_6 = OMe, R_7 = $R_8R_9CHCOO-$
 (R_8 = OtBDMS, R_9 = Me, S-configuration);
 R_2 = OtBDMS, single bond in 23,24 position; R_3 = Et;
 R_4 = OH, single bond in 10,11 position]

To a solution of 450 mg 24-tert-butyldimethylsilyloxy-FR 520 and 120 mg tert-butyldimethylsilyloxy-(S)-lactic acid in 10 ml of dichloromethane are added at room temperature 120 mg N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride and 23 mg dimethylaminopyridine. After 60 hours the reaction mixture is diluted with acetic acid ethyl ester, washed successively with 0.5 N hydrochloric acid and then water, dried over sodium sulfate, filtered, and the solvent is evaporated under reduced pressure. The residue is chromatographed over silicagel (eluant: n-hexane / acetic acid ethyl ester 2:1). The title compound is obtained as a colourless foam.

Example 20: FK 506-33-glycolate

[Formula X: R_1 = a group (c) wherein R_6 = OMe, R_7 = $R_8R_9CHCOO-$ (R_8 = OH,
 R_9 = H); R_2 = OH, single bond in 23,24 position; R_3 = allyl;
 R_4 = OH, single bond in 10,11 position]

To a solution of 300 mg tert-butyldimethylsilyloxymethylcarboxylic acid in 5 ml of dichloromethane are added under stirring at 0° 0.67 ml of oxalyl chloride and one drop of dimethylformamide. The mixture is brought to room temperature and is stirred for 1 hour. The reaction mixture is concentrated under reduced pressure. The residue is taken up in 5 ml of dichloromethane and this solution is added dropwise at 0° to a solution of 600 mg FK 506, 0.28 ml triethylamine and a catalytic quantity of 4-dimethylaminopyridine. After 18 hours stirring at 0° the solution is diluted with acetic acid ethyl ester, successively washed with 0.1 N hydrochloric acid and water, the organic phase is dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue is taken up in 20 ml of acetonitrile, reacted with 0.5 ml of 40 % wt. aqueous

hydrofluoric acid and stirred for 20 minutes at room temperature. The mixture is diluted with acetic acid ethyl ester, washed with saturated aqueous sodium hydrogen carbonate solution, dried over sodium sulfate, filtered and concentrated under reduced pressure. The title compound is obtained as a colourless foamy resin from the residue by chromatography over silicagel (eluant: n-hexane / acetic acid ethyl ester).

The following compounds of formula X are obtained in analogous manner as a colourless foamy resin:

Analogous		R ₁	R ₅	R ₆	R ₇	R ₂	Position R ₃ 23,24	R ₄	Position 10,11
Example No.	Ex. No.								
21	17 to 20	(c)	na	OMe	BOC-NHCH ₂ COO-	OtBDMS	sb	allyl	sb
22	17 to 20	(c)	na	OMe	tBDMS-OCH ₂ COO-	OtBDMS	sb	allyl	sb
23	17 to 20	(c)	na	OMe	BOC-NHCH ₂ COO-	OtBDMS	sb	Et	sb
24	17 to 20	(c)	na	OMe	tBDMS-OCH ₂ COO-	OtBDMS	sb	Et	sb
25a	17 to 20	(c)	na	OMe	BOC-NHCH ₂ COO-	OH	sb	allyl	sb
25b	17 to 20	(c)	na	OMe	OH	BOC-NHCH ₂ COO-	sb	allyl	sb
25c	17 to 20	(c)	na	OMe	BOC-NHCH ₂ COO-	BOC-NHCH ₂ COO-	sb	allyl	sb
25d	17,19,20	(c)	na	OMe	BOC-NHCH ₂ COO-	absent	db	allyl	sb
26a	17 to 20	(c)	na	OMe	BOC-NHCH ₂ COO-	OH	sb	Et	sb
26b	17 to 20	(c)	na	OMe	OH	BOC-NHCH ₂ COO-	sb	Et	sb
26c	17 to 20	(c)	na	OMe	BOC-NHCH ₂ COO-	BOC-NHCH ₂ COO-	sb	Et	sb
26d	17 to 20	(c)	na	OMe	BOC-NHCH ₂ COO-	absent	db	Et	sb
27a	17 to 20	(c)	na	OMe	NH ₂ COCOO-	OH	sb	allyl	sb
27b	17 to 20	(c)	na	OMe	NH ₂ COCOO-	NH ₂ COCOO-	sb	allyl	sb
27c	17 to 20	(c)	na	OMe	NH ₂ COCOO-	absent	db	allyl	sb
28a	17 to 20	(c)	na	OMe	NH ₂ COCOO-	OH	sb	Et	sb
28b	17 to 20	(c)	na	OMe	NH ₂ COCOO-	NH ₂ COCOO-	sb	Et	sb
28c	17 to 20	(c)	na	OMe	NH ₂ COCOO-	absent	db	Et	sb
29	17 to 20	(c)	na	OMe	NH ₂ COCOO-	OtBDMS	sb	Et	sb
30	17 to 20	(c)	na	OMe	NH ₂ COCOO-	OtBDMS	sb	allyl	sb
31	17 to 20	(c)	na	OMe	p-tolyloxy- thiocarbonyloxy	OH	sb	Et	sb
32	17 to 20	(c)	na	OMe	HOCH ₂ COO-	OH	sb	Et	sb
33	17 to 20	(c)	na	OMe	tBDMS-OCH ₂ (CH ₃)COO-(S)	OH	sb	Et	sb
34	17 to 20	(c)	na	OMe	iBuoyloxy	OtBDMS sb	Et	OH	sb
35	17 to 20	(c)	na	OMe	iBuoyloxy	OtBDMS sb	allyl	OH	sb
36	17 to 20	(c)	na	OMe	iBuoyloxy	OH	sb	allyl	sb
37	17 to 20	(c)	na	OMe	iBuoyloxy	OH	sb	Et	sb
38	17,18,20	(c)	na	OMe	tBDMS-OCH ₂ (CH ₃)COO-(S)	OtBDMS	sb	Et	sb

Example 39: 24-tert-Butyldimethylsilyloxy-33-aminooxalyloxy-FK 506

[Formula X: R_1 = a group (c) wherein R_6 = OMe, R_7 = aminooxalyloxy;
 R_2 = OtBDMS, single bond in 23,24 position; R_3 = allyl;
 R_4 = OH, single bond in 10,11 position]

A solution of 24,33-bis-(tert-butyldimethylsilyloxy)-FK 506 in 70 ml of acetonitrile is reacted at 0° to 5° with 1 ml of oxalyl chloride and stirred at 0 to 5° for 40 minutes. The reaction mixture is stirred with a mixture of acetic acid ethyl ester and saturated aqueous ammonia solution, any precipitate formed is sucked off, the phases are separated, the organic phase is washed successively with 1 N hydrochloric acid and then water, dried over sodium sulfate, filtered and concentrated under reduced pressure. From the residue the title compound is obtained as a colourless foamy resin following column chromatography over silicagel (eluant: n-hexane / acetic acid ethyl ester 1:1).

The following compounds of formula X are obtained in analogous manner as a colourless foamy resin:

Analogous									
Example No.	to Ex. No.	R ₁	R ₅	R ₆	R ₇	R ₂	Position R ₃ 23,24	R ₄	Position 10,11
40	39 ¹⁾	(c)	na	OMe	NH ₂ COCOCOO-	NH ₂ COCOCOO-	sb	allyl	sb
41	39,40	(c)	na	OMe	NH ₂ COCOCOO-	OH ² BDMS	sb	Et	sb
42	39,40	(c)	na	OMe	NH ₂ COCOCOO-	NH ₂ COCOCOO-	sb	Et	sb

¹⁾ Stirring is effected for 1 hour at room temperature; column chromatography is effected using an eluant gradient of 3:1 to 1:3.

Example 43: 24-Methoxy-33-tert-butyldimethylsilyloxy-FK 506

[Formula X: R_1 = a group (c) wherein R_6 = OMe, R_7 = OtBDMS; R_2 = OMe, single bond in 23,24 position; R_3 = allyl; R_4 = OH, single bond in 10,11 position]

1 g 33-tert-butyldimethylsilyloxy-FK 506 is dissolved into a mixture of 50 ml of dichloromethane and 0.04 ml of borotrifluoride etherate previously cooled to 0° to 5°. A solution of 20 ml of an approximately 1 N solution of diazomethane in methylene chloride is then added dropwise in such a manner that the yellow coloration of the solution which initially forms persists for as shortly as possible. The reaction mixture is diluted with acetic acid ethyl ester, successively washed with saturated aqueous sodium hydrogen carbonate solution and water, dried over sodium sulfate, filtered and the solvent is removed under reduced pressure. The title compound is obtained as a colourless foamy resin from the residue following column chromatographic purification over silicagel (eluant: acetic acid ethyl ester / n-hexane).

The following compounds of formula X are obtained in analogous manner as a colourless foamy resin:

Analogous							
Example No.	to Ex. No.	R ₁	R ₅	R ₆	R ₇	R ₂	Position 23,24
							R ₃
							R ₄
							Position 10,11
44	43 ¹⁾	(c)	na	OMe	OMe	OtBDMS	sb
							allyl
							OH
							sb

1) Starting from 24-tert-butyltrimethylsilyloxy-FK 506.

Example 45: 24-tert-Butyldimethylsilyloxy-33-oxo-FR 520

[Formula X: R_1 = a group (c) wherein R_6 = OMe, R_7 = oxo; R_2 = OtBDMS, single bond in 23,24 position; R_3 = Et; R_4 = OH, single bond in 10,11 position]

2 g 24-tert-butyldimethylsilyloxy-FR 520 and 1 g N-methylmorpholin-N-oxide are dissolved in 100 ml of methylene chloride, reacted with 5 g molecular sieve (Molsieb 4A) and the mixture is stirred for 15 minutes at room temperature. 0.15 g tetrapropylammonium perruthenate is added and stirring is continued for 3 more hours at room temperature. The mixture is concentrated, the residue is taken up in acetic acid ethyl ester and the solution successively washed with saturated aqueous sodium hydrogen sulfite solution, saturated aqueous sodium chloride and saturated aqueous copper sulfate solution, the organic phase is dried over sodium sulfate, filtered and concentrated under reduced pressure. The title compound is obtained from the residue following column chromatography over silicagel (eluant: n-hexane / acetic acid ethyl ester).

The following compounds of formula X are obtained in analogous manner as a colourless foamy resin:

Analogous		R ₁	R ₅	R ₆	R ₇	R ₂	Position 23,24	R ₃	R ₄	Position 10,11
Example No.	Ex. No.									
46	45 ¹⁾	(c)	na	OMe	OtBDMS	oxo	sb	Et	OH	sb
46a	45 ²⁾	(c)	na	OMe	oxo	OtBDMS	sb	allyl	OH	sb
46b	45	(c)	na	OMe	OtBDMS	oxo	sb	allyl	OH	sb

- ¹⁾ Starting from 33-tert-butyltrimethylsilyloxy-FR 520 (DOS 39 38 754);
²⁾ Starting from 24-tert-butyltrimethylsilyloxy-FK 506.

Example 47: 24-Oxo-FK 506

[Formula X: R_1 = a group (c) wherein R_6 = OMe, R_7 = OH; R_2 = oxo, single bond in 23,24 position; R_3 = allyl; R_4 = OH, single bond in 10,11 position]

3.6 g 24-oxo-33-tert-butyldimethylsilyloxy-FK506 (compound of Example 16b) is dissolved at room temperature into a mixture of 110 ml of acetonitrile and 3 ml of 40 % wt. aqueous hydrofluoric acid and the mixture is stirred at room temperature for 45 minutes. The reaction mixture is diluted with acetic acid ethyl ester, washed successively with saturated aqueous sodium bicarbonate solution and then water, dried over sodium sulfate, filtered and concentrated under reduced pressure. The title compound is obtained as a colourless foamy resin following chromatographic purification of the residue over silicagel (eluant: acetic acid ethyl ester / n-hexane 3:2).

The following compounds of formula X are obtained in analogous manner as a colourless foamy resin:

Analogous		R ₁	R ₅	R ₆	R ₇	R ₂	Position 23,24	R ₃	R ₄	Position 10,11
Example No.	Ex. No.									
48	47 ¹⁾	(c)	na	OMe	NH ₂ CH ₂ COO-	OH	sb	allyl	OH	sb
49	47 ²⁾	(c)	na	OMe	NH ₂ CH ₂ COO-	absent	db	allyl	OH	sb
50	47 ³⁾	(c)	na	OMe	NH ₂ CH ₂ OH	oxo	sb	Et	OH	sb
51	47 ⁴⁾	(c)	na	OMe	NH ₂ CH ₂ COO-	OH	sb	Et	OH	sb
52	47 ⁵⁾	(c)	na	OMe	NH ₂ CH ₂ COO-	absent	db	Et	OH	sb
53	47 ⁶⁾	(c)	na	OMe	HOCH ₂ COO-	OH	sb	allyl	OH	sb
54	47 ⁷⁾	(c)	na	OMe	HOCH ₂ COO-	OH	sb	Et	OH	sb
55	47 ⁸⁾	(c)	na	OMe	HOCH ₂ (CH ₃)COO-	OH	sb	Et	OH	sb
	47 ⁹⁾				(S)					
56	47 ¹⁰⁾	(c)	na	OMe	OH	NH ₂ CH ₂ COO-	sb	Et	OH	sb
57	47 ¹¹⁾	(c)	na	OMe	NH ₂ CH ₂ COO-	NH ₂ CH ₂ COO-	sb	Et	OH	sb
58	47 ¹²⁾	(c)	na	OMe	NH ₂ CH ₂ COO-	NH ₂ CH ₂ COO-	sb	allyl	OH	sb
59	47 ¹³⁾	(c)	na	OMe	NH ₂ CH ₂ OH	NH ₂ CH ₂ COO-	sb	allyl	OH	sb
60	47 ¹⁴⁾	(c)	na	OMe	NH ₂ CH ₂ COO-	OH	sb	allyl	OH	sb
61	47 ¹⁵⁾	(c)	na	OMe	NH ₂ CH ₂ COO-	OH	sb	Et	OH	sb
62	47 ¹⁶⁾	(c)	na	OMe	iBuoyloxy	OH	sb	Et	OH	sb
63	47	(c)	na	OMe	iBuoyloxy	OH	sb	allyl	OH	sb

The following compounds of formula I are obtained in analogous manner in accordance with process variant deprotection:

Example No.	to Ex. No.	Analogous					R ₂	Position 23,24	R ₃	R ₄	Position 10,11	Physicochemical characterization data
		R ₁	R ₅	R ₆	R ₇							
64	47 ¹⁷⁾	(a) I		OMe	na		OH	sb	Et	OH	sb	cfr; **
65a	47 ¹⁸⁾	(a) Cl		OMe	na		OH	sb	allyl	OH	sb	cfr; NMR *
65b	47 ¹⁸⁾	(a) Cl		OMe	na		OH	sb	allyl	absent	db	cfr *
66a	47 ¹⁹⁾	(a) Cl		OMe	na		OH	sb	Et	OH	sb	cfr; NMR *
66b	47 ²⁰⁾	(a) Cl		OMe	na		OH	sb	Et	absent	db	cfr; NMR *
67a	47 ²⁰⁾	(a) Br		OMe	na		OH	sb	Et	OH	sb	cfr; NMR *
67b	47 ²¹⁾	(a) Br		OMe	na		OH	sb	Et	absent	db	cfr *
68a	47 ²¹⁾	(a) N ₃		OMe	na		OH	sb	allyl	OH	sb	cfr; NMR *
68b	47 ²²⁾	(a) N ₃		OMe	na		OH	sb	allyl	absent	db	cfr; NMR *
69a	47 ²²⁾	(a) Br		OMe	na		OH	sb	allyl	OH	sb	cfr
69b	47 ²³⁾	(a) Br		OMe	na		OH	sb	allyl	absent	db	cfr *
70a	47 ²³⁾	(a) N ₃		OMe	na		OH	sb	Et	OH	sb	cfr; NMR *
70b	47	(a) N ₃		OMe	na		OH	sb	Et	absent	db	cfr

- 1) Starting from the compound of Example 25a;
- 2) Starting from the compound of Example 25d;
- 3) Starting from the compound of Example 46 (=16d);
- 4) Starting from the compound of Example 23;
- 5) Starting from the compound of Example 26d;
- 6) Starting from the compound of Example 22;
- 7) Starting from the compound of Example 24;
- 8) Starting from the compound of Example 19 or of Example 33;
- 9) Starting from the compound of Example 26b;

- 10) Starting from the compound of Example 26c;
- 11) Starting from the compound of Example 25c;
- 12) Starting from the compound of Example 25b;
- 13) Starting from the compound of Example 25a;
- 14) Starting from the compound of Example 26a;
- 15) Starting from the compound of Example 34;
- 16) Starting from the compound of Example 35;
- 17) Starting from the compound of Example 6a;
- 18) Starting from the compound of Example 1;
- 19) Starting from the compound of Example 3;
- 20) Starting from the compound of Example 4;
- 21) Starting from the compound of Example 2;
- 22) Starting from the compound of Example 5;
- 23) Starting from the compound of Example 6;

- * NMR: Example 48: ¹H-NMR: about 2:1 mixture of conformers:
 5.33 and 5.20 (d/d, J=1 Hz and 1 Hz, H-26); 4.84 (dxdd, J=5 Hz, 9.5 Hz and 11 Hz, H-33); 3.44 (s, 2H, O=C-CH₂-N-); 3.22 (dxdd, J=5 Hz, 9.5 Hz and 11 Hz, H-32);
- Example 49: ¹H-NMR: see Example 18;
- Example 50: ¹H-NMR: mixture of conformers:
 5.8 and 5.6 (s/s, H-23); 5.69 (H-26); 4.38 (d, J=13 Hz, H-6e); 4.19 (t, H-2); 3.80 (dx, J=9 Hz and 2 Hz, H-14);
- Example 51: ¹H-NMR: about 2:1 mixture of conformers:
 5.34 (d, J=2 Hz, H-26); 4.75 (dxdd, J=5 Hz, 9 Hz and 10 Hz, H-33); 4.61 (db, J=4 Hz, H-2); 4.44 (db, J=13 Hz, H-6e); 3.45 (s, -CH₂-N);

Example 53:	¹ H-NMR:	see Example 20;
Example 54:	¹ H-NMR:	see Example 32;
Example 55:	¹ H-NMR:	mixture of conformers: main conformer: 1.23 (d, J=7 Hz); 4.30 [dq, J ₁ =5 Hz, J ₂ =7 Hz, -COCH(CH ₃)OH]; 4.44 (d, br, J=13 Hz, H-6e); 4.61 (d, br, J=4 Hz); 4.78 (ddd, J ₁ =5 Hz, J ₂ =5 Hz, J ₃ =11 Hz, H-33); 5.34 (H-26);
Example 60:	¹ H-NMR:	see Example 48;
Example 61:	¹ H-NMR:	see Example 51;
Example 62:	¹ H-NMR:	see Example 37;
Example 65a:	¹ H-NMR ¹³ C-NMR:	4.59 (m, H-33); about 2:3 mixture of conformers: main conformer: 59.1 (C-33); 79.2 (C-32); 97.5 (C-10); 116.4 (C-38); 123.0 (C-20); 135.6 (C-37); 138.4 (C-19); 164.6 (C-8); 168.9 (C-1); 196.4 (C-9); 209.4 (C-22);
Example 66a:	¹ H-NMR:	4.56 (m, H-33);
Example 66b:	¹ H-NMR: ¹³ C-NMR:	2.09 (s, 11-CH ₃); 4.5 (bm, H-33); about 2:1 mixture of conformers: main conformer: 56.2 (C-33); 80.6 (C-32); 116.4 (C-38); 122.9 (C-20); 124.8 (C-11); 129.5 (C-29); 131.9 (C-28); 135.8 (C-37); 140.0 (C-19); 142.9 (C-10); 166.7 (C-8); 168.7 (C-1); 188.0 (C-9); 212.4 (C-22); minor conformer: 56.1 (C-33); 80.6 (C-32); 116.5 (C-38); 123.6 (C-20); 126.4 (C-11); 128.5 (C-29); 131.8 (C-28); 135.6 (C-37); 137.4 (C-19); 144.1 (C-10); 166.5 (C-8); 169.5 (C-1); 184.8 (C-9); 213.3 (C-22);
Example 67a:	¹ H-NMR:	4.44 (d, J=13 Hz, H-6 eq.); 4.60 (d, J=4 Hz, H-2); 4.70 (sb, H-33);
Example 68a:	¹ H-NMR:	4.07 (m, w _{1/2} = 8 Hz, H-33);
Example 68b:	¹ H-NMR:	about 2:1 mixture of conformers: 4.06 (m, H-33); 2.09 and 1.94 (2s, 11-CH ₃);
Example 70a:	¹ H-NMR:	about 5:4 mixture of conformers: 5.60 resp. 5.79 (s resp. s, H-23); 5.70 resp. 5.66 (d, J=3 Hz resp. d, J=3 Hz, H-26); 4.38 (d, J=13 Hz, H-6e); 4.15 (t, H-2); 3.80 (dx d, J=9 Hz and 2 Hz, H-14).
** Iodine analysis: theor.: 14.06 %; found: 13.57 %.		

The compounds of Examples 10a, 11a, 12 and 13 may be prepared in analogous manner according to process variant deprotection.

Example 71: 24-tert-Butyldimethylsilyloxy-29-des-(4-hydroxy-3-methyl-cyclohexyl)-29-(3-formylcyclopentyl)-FR 520

[Formula I: R_1 = a group (d); R_2 = OtBDMS, single bond in 23,24 position;
 R_3 = Et; R_4 = OH, single bond in 10,11 position]

[Process variant protection]

A solution of 1.2 g 29-des-(4-hydroxy-3-methylcyclohexyl)-29-(3-formylcyclopentyl)-FR 520 (compound of Example 12), 1.5 g tert-butyl-dimethylsilyl chloride and 0.8 g imidazole in 20 ml of dry dimethylformamide is stirred for 15 hours at room temperature and thereafter partitioned between 1 N hydrochloric acid solution and acetic acid ethyl ester. The organic phase is separated, washed with saturated aqueous sodium chloride solution, dried over sodium sulfate, filtered and concentrated under reduced pressure. The title compound is obtained from the residue as a colourless foamy resin following column chromatography over silicagel (eluant: n-hexane / acetic acid ethyl ester):

¹H-NMR: mixture of rotamers: 9.65 (d, J = 2 Hz, CHO); 5.39 (d, J = 9 Hz, H-29); 5.01 (d, J = 7.5 Hz, H-26); 4.81 (d, J = 10 Hz, H-20); 3.82 (dxd, J = 9/2 Hz, H-24).

The compounds of Examples 1 to 9 may be prepared in analogous manner according to process variant protection.

The compounds of the invention possess pharmacological activity. They are indicated for use as pharmaceuticals. In particular they possess antiinflammatory, and immunosuppressant and antiproliferative activity.

Antiinflammatory activity may e.g. be determined in the following test methods:

1. Oxazolone allergic contact dermatitis in the mouse in vivo upon topical application: the test method is as described in F.M. Dietrich and R. Hess, Int. Arch. Allergy 38 (1970) 246-259. The compounds elicit in this test an activity between about 15 % and about 68 % upon topical administration at a concentration of about 0.01 %.
2. DNFB allergy (swine): the test method is as described in e.g. EP 315978. Topical application of a 1.2 % formulation of the compounds repeated twice results in from about 36 % to about 40 % inhibition of the inflammatory reaction.

Immunosuppressant and antiproliferative activity may e.g. be determined in the following test methods:

1. Proliferative response of lymphocytes to allogeneic stimulation in the mixed lymphocyte reaction (MLR) in vitro: T. Meo, "The MLR in the Mouse", Immunological Methods, L. Lefkovits and B. Pernis, Eds., Academic Press, N.Y. (1979), 227-239.

The compounds elicit in this test (IC_{50}) suppression of mixed lymphocytes at a dosage of from about $< 0.0008 \mu\text{g/ml}$ to about $0.09 \mu\text{g/ml}$.

2. Inhibition of the primary humoral immune response to sheep erythrocytes in vitro: the test method is as described in R.I. Mishell and R.W. Dutton, Science 153 (1966) 1004-1006; R.I. Mishell and R.W. Dutton, J. Exp. Med. 126 (1967) 423-442.

The compounds are active in this test with an IC_{50} of from about $0.0024 \mu\text{g/ml}$ to about $0.32 \mu\text{g/ml}$.

3. Inhibition of proliferation of human keratinocytes: the test method is as described in e.g. EP 315978.

The compounds are active in this test at concentrations of from about $1 \mu\text{g/ml}$ to about $10 \mu\text{g/ml}$ resulting in an inhibition of from about 30 % to about 90 %.

The compounds of the invention in free form and where such forms exist in pharmaceutically acceptable salt form are therefore indicated as antiinflammatory and as immunosuppressant and antiproliferative agents for

use in the prevention and treatment of inflammatory conditions and of conditions requiring immunosuppression, such as

a) the prevention and treatment of

- resistance in situations of organ or tissue transplantation, e.g. of heart, kidney, liver, bone marrow and skin,
- graft-versus-host disease, such as following bone marrow grafts,
- autoimmune diseases such as rheumatoid arthritis, systemic Lupus erythematosus, Hashimoto's thyroiditis, multiple sclerosis, Myasthenia gravis, diabetes type I and uveitis,
- cutaneous manifestations of immunologically-mediated illnesses;

b) the treatment of inflammatory and hyperproliferative skin

diseases, such as psoriasis, atopic dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous Pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus and acne; and

c) Alopecia areata.

The compounds may be administered systemically or topically.

For these indications the appropriate dosage will, of course, vary depending upon, for example, the host, the mode of administration and the nature and severity of the condition being treated. However, in general, satisfactory results are indicated to be obtained systemically at daily dosages of from about 0.15 mg/kg to about 1.5 mg/kg animal body weight. An indicated daily dosage in the larger mammal is in the range from about 0.01 mg to about 100 mg, conveniently administered, for example, in divided doses up to four times a day or in retard form.

For topical use satisfactory results are obtained with local administration of a 1-3 % concentration of active substance several times daily, e.g. 2 to 5 times daily. Examples of indicated galenical forms are lotions, gels and creams.

The compounds of the invention may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets or capsules, or topically, e.g. in the form of lotions, gels or creams.

Pharmaceutical compositions comprising a compound of the invention in association with at least one pharmaceutical acceptable carrier or diluent may be manufactured in conventional manner by mixing with a

pharmaceutically acceptable carrier or diluent. Unit dosage forms contain, for example, from about 0.0025 mg to about 50 mg of active substance.

Topical administration is e.g. to the skin. A further form of topical administration is to the eye, for the treatment of immune-mediated conditions of the eye, such as: auto-immune diseases, e.g. uveitis, keratoplasty and chronic keratitis; allergic conditions, e.g. vernal conjunctivitis; inflammatory conditions and corneal transplants, by the topical administration to the eye surface of a compound of the invention in a pharmaceutically acceptable ophthalmic vehicle.

The ophthalmic vehicle is such that the compound is maintained in contact with the ocular surface for a sufficient time period to allow the compound to penetrate the corneal and internal regions of the eye, e.g. the anterior chamber, posterior chamber, vitreous body, aqueous humor, vitreous humor, cornea, iris/ciliary, lens, choroid/retina and sclera.

The pharmaceutically acceptable ophthalmic vehicle may be e.g. an ointment, vegetable oil, or an encapsulating material.

Whilst the antiinflammatory and immunosuppressant and antiproliferative activity is the main activity of the compounds of the invention they also possesses some degree of activity in increasing sensitivity to, or in increasing the efficacy of, chemotherapeutic drug therapy.

This activity may e.g. be determined according to the test methods described in EP 360760.

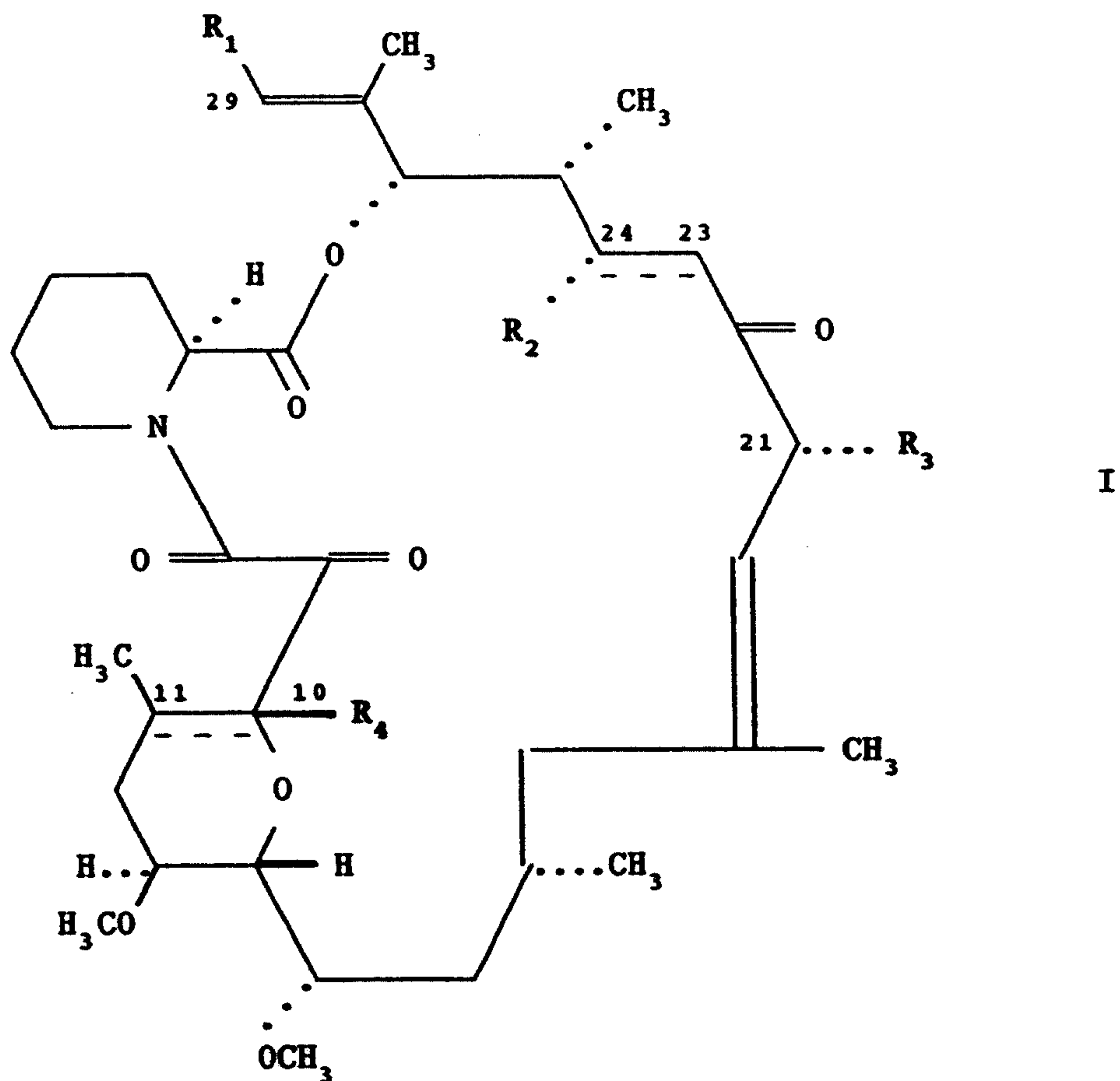
The compounds of the invention are therefore indicated for use in reversing chemotherapeutic drug resistance of varying types, e.g. acquired or innate, or in increasing sensitivity to administered drug therapy, e.g. as a means of reducing regular chemotherapeutic dosage levels, for example in the case of anti-neoplastic or cytostatic drug therapy, as a means of decreasing overall drug toxicity and, more especially, as a means of reversing or reducing resistance, including both inherent and acquired resistance, to chemotherapy.

Preferred in the above indications are the following compounds of the invention:

- 29-des-(4-hydroxy-3-methoxycyclohexyl)-29-(3-formylcyclopentyl)-FR 520 (compound of Example 12); and
- 33-epi-33-chloro-FR 520 (compound of Example 66a).

CLAIMS

1. A compound of formula I



wherein

either R_1 is a group (a) of formula



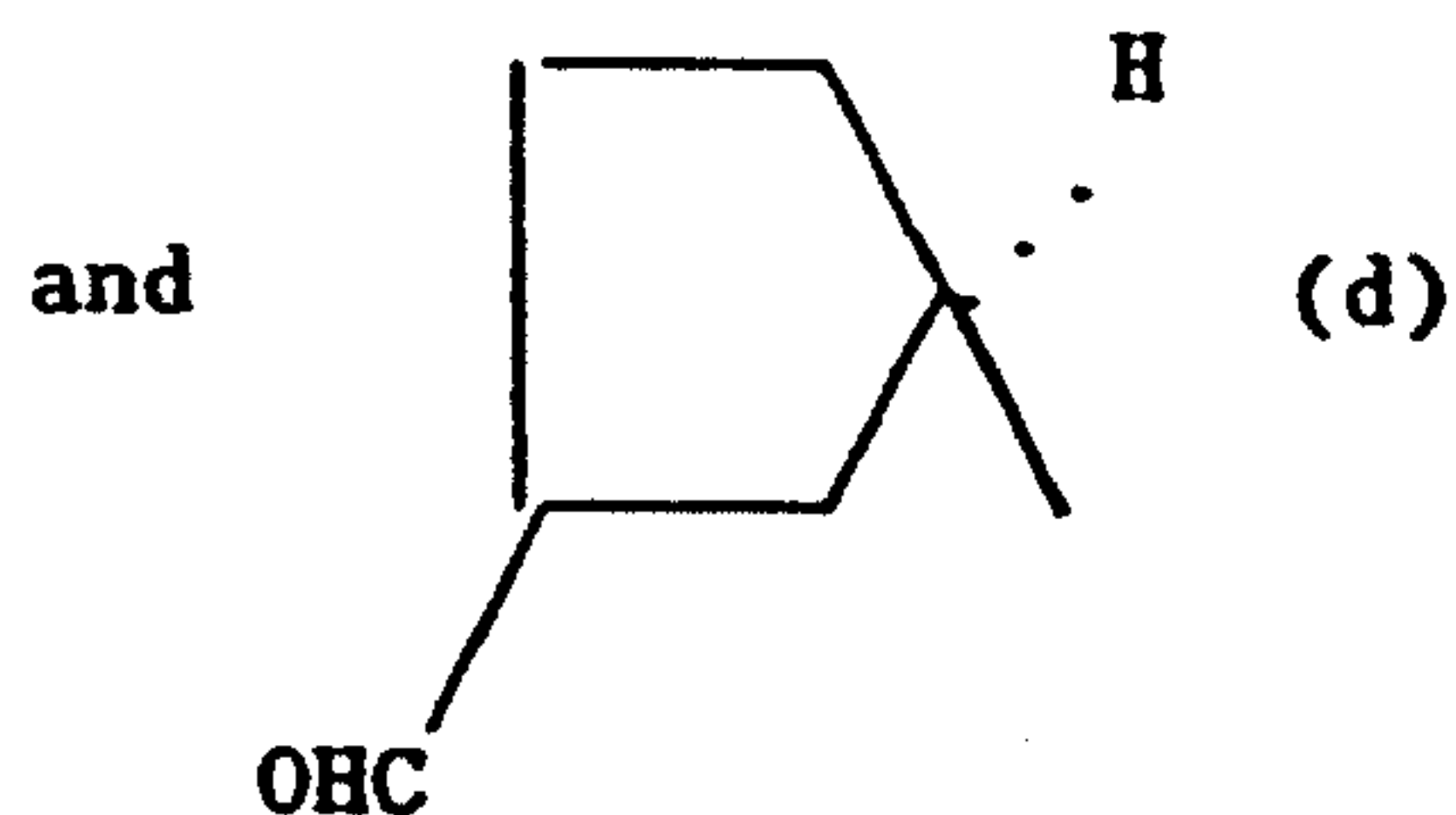
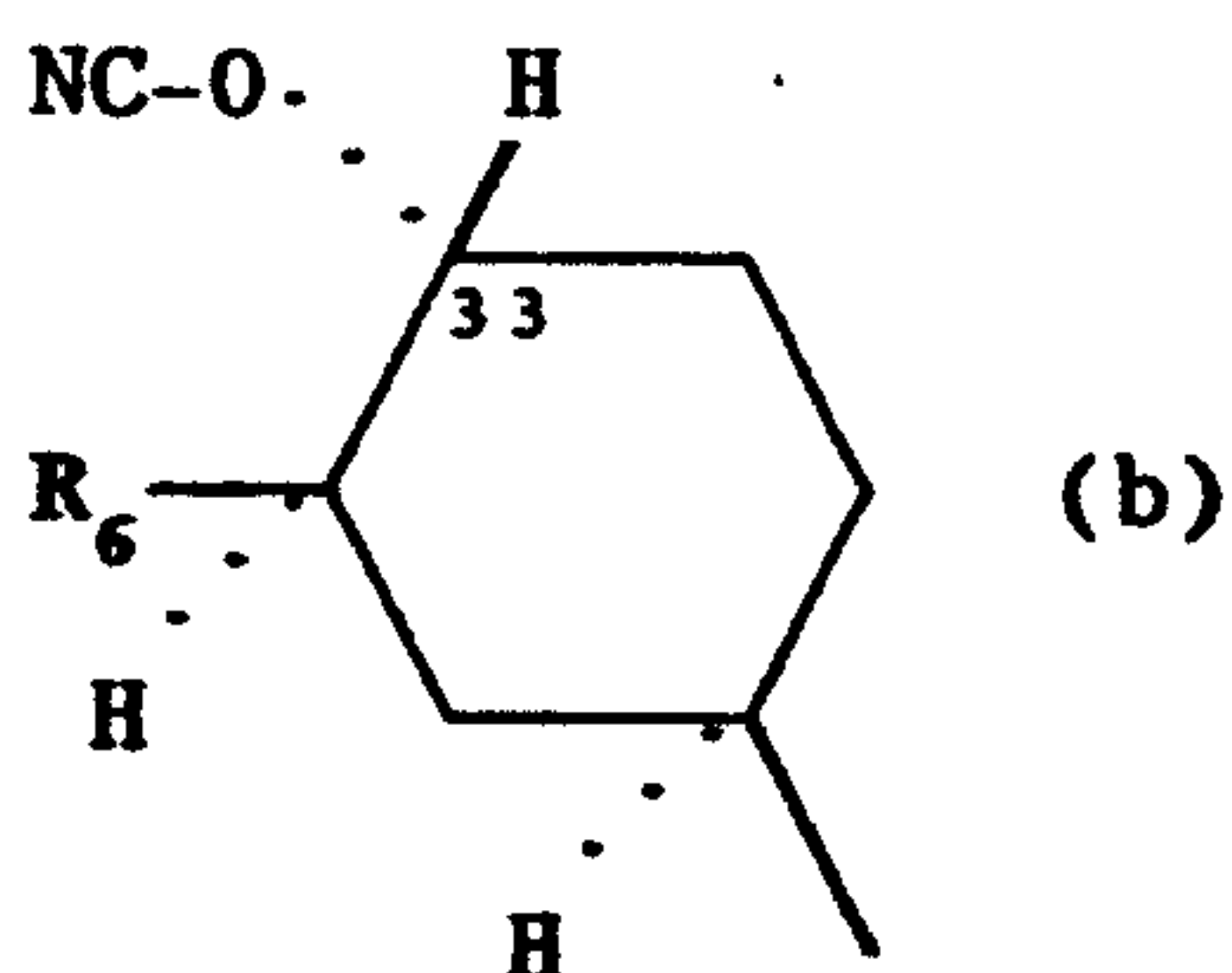
wherein R_5 is chloro, bromo, iodo or azido and

R_6 is hydroxy or methoxy;

R_2 is oxo and there is a single bond in 23,24 position; optionally protected hydroxy and there is a single or a double bond in 23,24 position; or absent and there is a double bond in 23,24 position; and

R_4 is hydroxy and there is a single bond in 10,11 position; or absent and there is a double bond in 10,11 position;

or R_1 is a group (b) or (d) of formula



wherein R_6 is as defined above;

R_2 is as defined above; and

R_4 is hydroxy and there is a single bond in 10,11 position; and

R_3 is methyl, ethyl, n-propyl or allyl;

in free form or, where such forms exist, in salt form.

2. A compound according to claim 1 which is a compound Ip_1 ,
of formula I wherein

R_1 is a group (a) wherein R_6 is methoxy and

either R_5 is chloro or bromo and

R_4 is hydroxy and there is a single bond in 10,11 position

or R_5 is azido and

R_4 is hydroxy and there is a single bond in 10,11 position or absent
and there is a double bond in 10,11 position;

R_2 is optionally protected hydroxy and there is a single or a double bond in 23,24 position; and

R_3 is as defined in claim 1;

in free form or, where such forms exist, in salt form.

3. A compound according to claim 1 which is a compound Ip_3 ,
of formula I wherein

R_1 is a group (b) wherein R_6 is methoxy,

R_2 is optionally protected hydroxy and there is a single bond in 23,24 position; or absent and there is a double bond in 23,24 position;

R_4 is hydroxy and there is a single bond in 10,11 position; and

R_3 is as defined in claim 1;

in free form or, where such forms exist, in salt form.

4. A compound according to claim 1 which is a compound Ip_4 ,
of formula I wherein

R_1 is a group (d),

R_2 is optionally protected hydroxy and there is a single bond in 23,24 position; or absent and there is a double bond in 23,24 position;

R_4 is hydroxy and there is a single bond in 10,11 position; and

R_3 is as defined in claim 1;

in free form or, where such forms exist, in salt form.

5. A compound of formula I according to claim 1 wherein R_1 is a group (d); R_2 is hydroxy and there is a single bond in 23,24 position; R_3 is ethyl; and R_4 is hydroxy and there is a single bond in 10,11 position; which is 29-des-(4-hydroxy-3-methoxycyclohexyl)-29-(3-formylcyclopentyl)-FR 520.

6. A compound of formula I according to claim 1 wherein R_1 is a group (a) wherein R_5 is chloro and R_6 is methoxy; R_2 is hydroxy and there is a single bond in 23,24 position; R_3 is ethyl; and R_4 is hydroxy and there is a single bond in 10,11 position; which is 33-epi-33-chloro-FR 520.

7. A process for the preparation of a compound according to claim 1 comprising

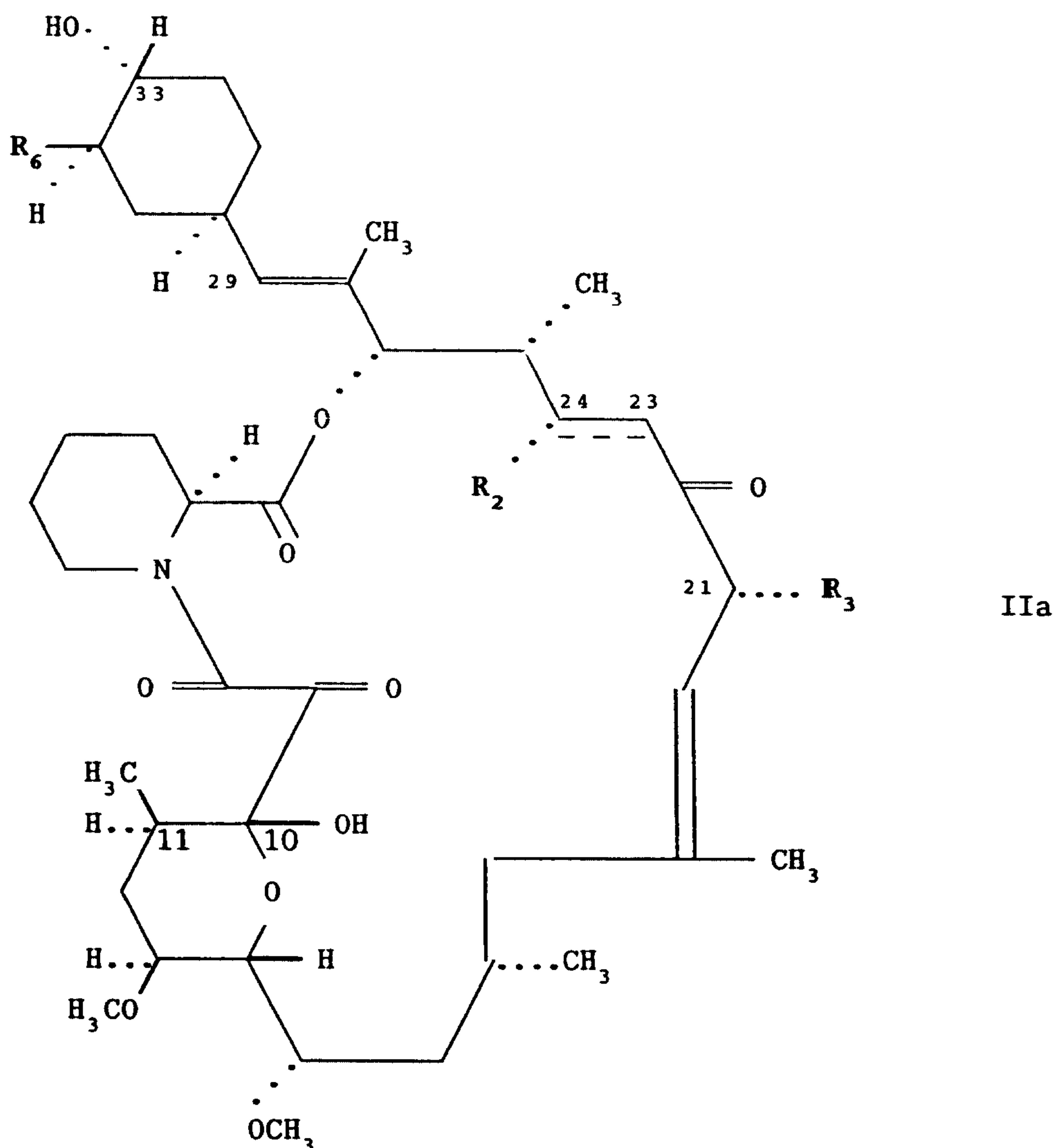
a) for the preparation of a compound of formula I wherein

R_1 is a group (a) as defined in claim 1,

R_2 and R_3 are as defined in claim 1 and

R_4 is hydroxy,

replacing under simultaneous epimerization the hydroxy group by chlorine, bromine, iodine or azido in a corresponding compound having unprotected hydroxy in 33 position of formula IIa



wherein R_2 and R_3 are as defined in claim 1 under formula I and R_6 is hydroxy or methoxy);

b) for the preparation of a compound of formula I wherein

R_1 is a group (b) as defined in claim 1,

R_2 and R_3 are as defined in claim 1 and

R_4 is hydroxy,

treating a corresponding compound IIa with cyanogen bromide in the presence of a base or

treating a corresponding compound IIa with thiophosgene, reacting the resultant product with an inorganic azide and allowing the resultant unstable intermediate having a group



to decompose to a corresponding compound Ib;

c) for the preparation of a compound of formula I wherein

R_1 is a group (d) as defined in claim 1,

R_2 and R_3 are as defined in claim 1 and

R_4 is hydroxy,

treating a corresponding compound Ib with an acid having a non-nucleophilic anion; and

- when a resultant compound of formula I has a protected hydroxy and/or a protected amino group,

optionally splitting off the protecting group(s) to give a corresponding compound of formula I having one or more unprotected hydroxy and/or unprotected amino group(s),

whereby when R_1 is a group (a), a water molecule may be

simultaneously split off and a compound of formula I is obtained wherein

R_1 is a group (a) as defined in claim 1,

R_2 is unprotected hydroxy and there is a single or double bond in 23,24 position; and

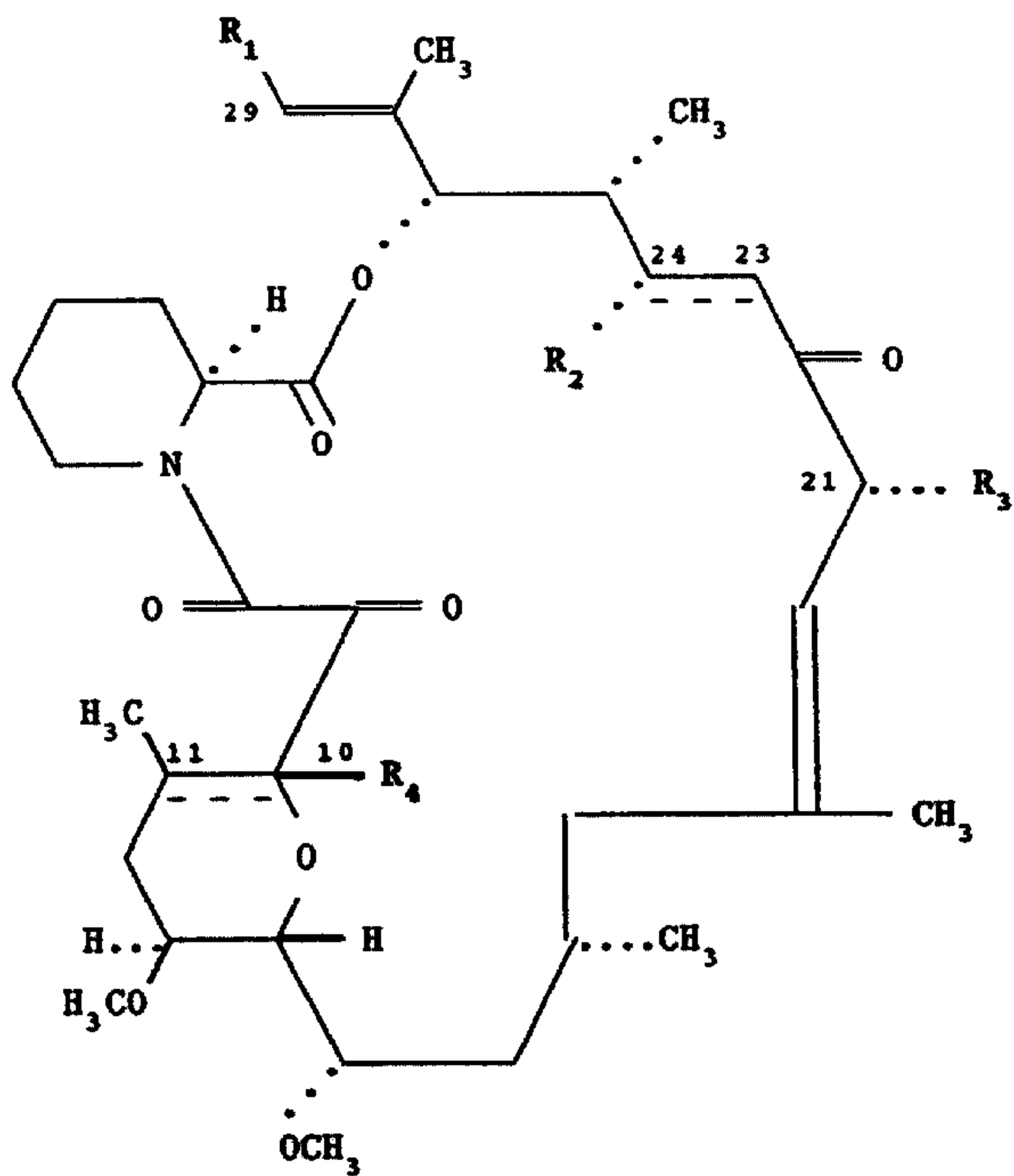
R_4 is absent and there is a double bond in 10,11 position;

or

- optionally protecting an unprotected hydroxy and/or unprotected amino group in a resultant compound of formula I as appropriate to give a corresponding compound of formula I having one or more protected hydroxy and/or protected amino groups(s);

and recovering the resultant compound of formula I in free form and, where such forms exist, in salt form.

8. A pharmaceutical composition comprising a compound according to any one of claims 1 to 6 in free form or, where such forms exist, in pharmaceutically acceptable salt form, together with a pharmaceutically acceptable carrier or diluent.



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