



(86) Date de dépôt PCT/PCT Filing Date: 2006/07/07
(87) Date publication PCT/PCT Publication Date: 2007/01/18
(85) Entrée phase nationale/National Entry: 2008/01/03
(86) N° demande PCT/PCT Application No.: US 2006/026292
(87) N° publication PCT/PCT Publication No.: 2007/008563
(30) Priorité/Priority: 2005/07/08 (US60/697,726)

(51) Cl.Int./Int.Cl. *A61K 47/48* (2006.01)
(71) Demandeur/Applicant:
ELAN PHARMACEUTICALS, INC., US
(72) Inventeurs/Inventors:
KONRADI, ANDREI W., US;
SMITH, JENIFER L., US;
DAPPEN, MICHAEL S., US;
SEMKO, CHRISTOPHER M., US
(74) Agent: OSLER, HOSKIN & HARCOURT LLP

(54) Titre : PREPARATION DE CONJUGUES POLYMERES DE COMPOSES THERAPEUTIQUES, DE COMPOSES
DESTINES A UN USAGE AGRICOLE ET DE COMPOSES D'ADDITIF ALIMENTAIRE
(54) Title: PREPARATION OF POLYMER CONJUGATES OF THERAPEUTIC, AGRICULTURAL, AND FOOD ADDITIVE
COMPOUNDS

(57) **Abrégé/Abstract:**

Disclosed is a process for preparing polymer conjugates of agricultural, therapeutic, and food additive compounds using Mitsunobu conditions.



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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
18 January 2007 (18.01.2007)

PCT

(10) International Publication Number
WO 2007/008563 A2

(51) International Patent Classification:
A61K 47/48 (2006.01)

(21) International Application Number:
PCT/US2006/026292

(22) International Filing Date: 7 July 2006 (07.07.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/697,726 8 July 2005 (08.07.2005) US

(71) Applicant (for all designated States except US): **ELAN PHARMACEUTICALS, INC.** [US/US]; 800 Gateway Blvd., South San Francisco, CA 94080 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **KONRADI, Andrei, W.** [US/US]; 30 Victoria Road, Burlingame, CA 94010 (US). **SMITH, Jenifer, L.** [US/US]; 512 Cortesti Avenue, Douth San Francisco, CA 94080 (US). **DAPPEN, Michael, S.** [US/US]; 740 Vernal Way, Redwood City, CA 94062 (US). **SEMKO, Christopher, M.** [US/US]; 4493 Norris Road, Fremont, CA 94536 (US).

(74) Agent: **SARUSSI, Steven, J.**; MCDONNELL BOEHNEN HULBERT & BERGHOFF LLP, 300 South Wacker Drive, Suite 3200, Chicago, IL 60606 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

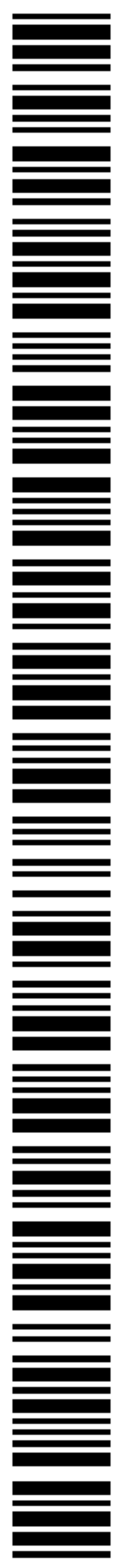
Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PREPARATION OF POLYMER CONJUGATES OF THERAPEUTIC, AGRICULTURAL, AND FOOD ADDITIVE COMPOUNDS

(57) Abstract: Disclosed is a process for preparing polymer conjugates of agricultural, therapeutic, and food additive compounds using Mitsunobu conditions.



WO 2007/008563 A2

PREPARATION OF POLYMER CONJUGATES OF THERAPEUTIC, AGRICULTURAL, AND FOOD ADDITIVE COMPOUNDS

BACKGROUND OF THE INVENTION

5 Field of the Invention

This invention relates to processes for making polymer conjugates of therapeutic, agricultural, and food additive compounds. More specifically, the invention relates to processes employing Mitsunobu reaction conditions to prepare conjugates for use in treating various mammalian, particularly human, diseases and disorders as well as in agriculture and
10 as food additives. In certain aspects, the invention relates to using Mitsunobu conditions with an alcohol, particularly an alcohol-containing polymer, and an amine to form the desired conjugate.

State of the Art

15 Attachment of biologically active compounds to polymers has received significant attention and has become a common method to control various characteristics, e.g., biodistribution, pharmacokinetics, and toxicity, of such compounds. A frequent choice of polymer for use in making polymer conjugates of biologically active compounds is polyethylene glycol (PEG). It is widely used as a covalent modifier of both small and large biologically active molecules. For
20 discussions of such conjugates, see, Eur. Polym. J. 19, No. 12, pages. 1177-1183 (Zaplinsky et al., 1983) et al., Journal of Controlled Release 10 (1989) 145-154 (Veronese et al., 1989), and Advanced Drug Delivery Reviews, 16, 157-182 (Zaplinsky, 1995).

It has recently been discovered that polymer conjugates of, for example, $\alpha_4\beta_1$ (VLA-4) antagonists have greatly improved serum half-life. These polymer compounds can be
25 prepared using various synthetic methods, including carboxamide formation by reacting an ester of the active molecule with a polymer amine, carbamate formation between an amine of the active molecule and a polymer chloroformate, or carbamate formation between an isocyanate of the active molecule and a polymer alcohol. The overall yields from these methods are typically less than desirable, often involving multiple steps and purification
30 means. It is therefore necessary to design a process which isolates a conjugate of a VLA-4 inhibitor in quantitative or near quantitative yields.

The importance of such polymer conjugates indicates that there is a need for convenient and efficient syntheses of such materials.

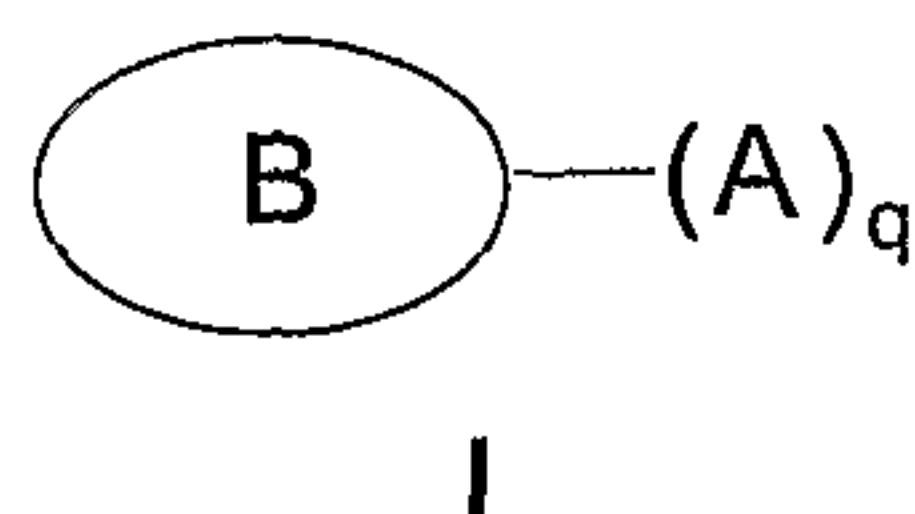
SUMMARY OF THE INVENTION

This invention provides an improved synthesis of polymer conjugates of agricultural, therapeutic, and food additive compounds. The process of this invention produces the final conjugate products in excellent yields. In a preferred aspect, the invention provides a process for the preparation of conjugates by condensing polymeric alcohols with amines using Mitsunobu reaction conditions. In another preferred aspect, the invention provides a process for the preparation of conjugates by condensing polymeric amines with alcohols using Mitsunobu reaction conditions.

In one aspect, the invention provides a process for the preparation of conjugates of active compounds, comprising the steps of: a) reacting a polymeric alcohol with a nucleophile in the presence of a trivalent phosphine and an appropriate azodicarbonyl compound, e.g., diethylazodicarboxylate or azodicarbonyldipiperidide, to form the conjugate; and b) isolating the conjugate.

In a specific aspect, the active compounds and the resulting conjugates exhibit VLA-4 antagonistic properties.

In another aspect, the invention provides a process for the preparation of conjugates of formula I below:



B is a bio-compatible polymer moiety;

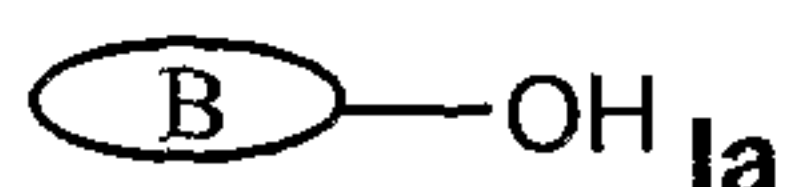
q is from about 1 to about 100;

A at each occurrence is independently a compound having biological or agricultural activity or

A is a food additive compound.

This process comprises

a) reacting polymeric alcohol of formula Ia



with a nucleophile of formula H-Nu

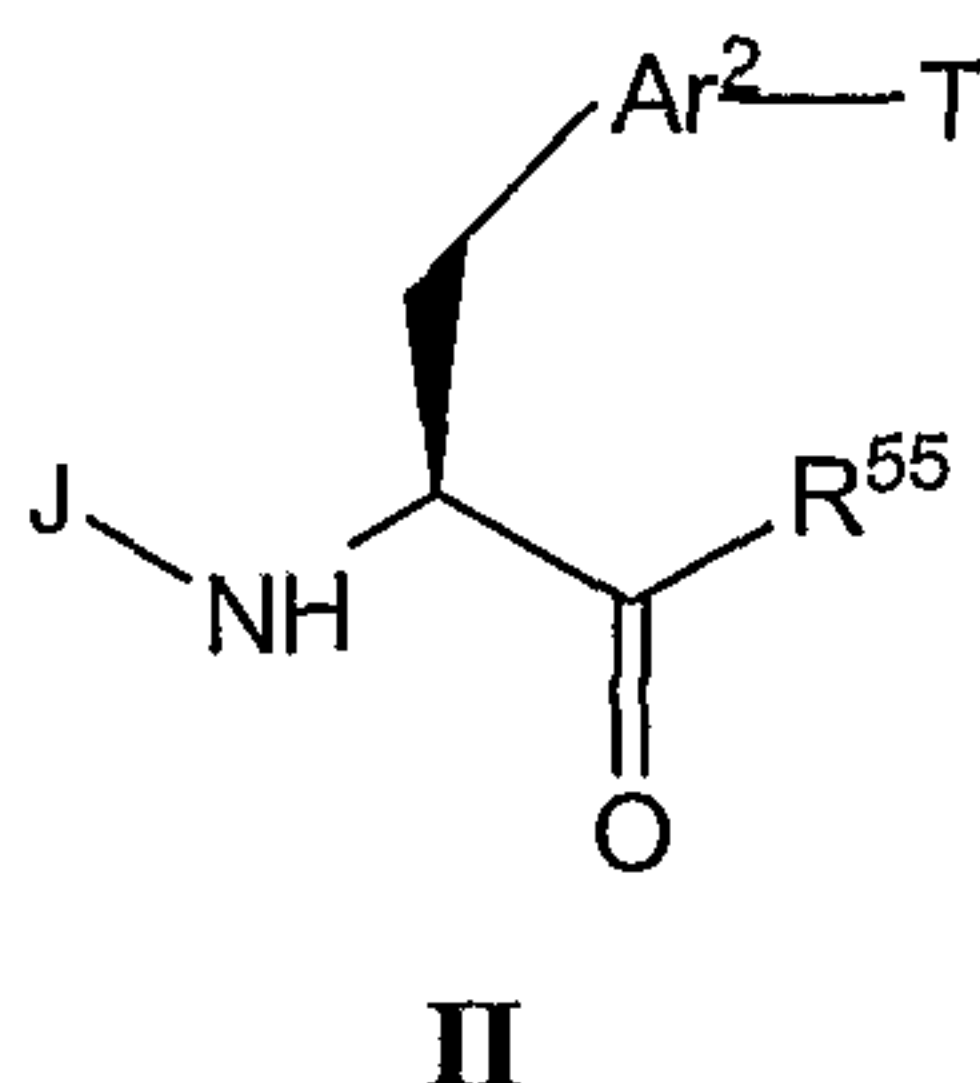
in the presence of a trivalent phosphine and an appropriately substituted azodicarbonyl compound to form the compound of formula I, where Nu is a radical corresponding to formula A above and the H is an acidic hydrogen on Nu; and

b) isolating the compound of formula I.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, this invention provides processes for the preparation of conjugates of agricultural, therapeutic, and food additive compounds (hereinafter "active compounds"). The conjugates comprise one or more polymer moieties covalently attached to one or more active compounds where the resulting conjugates have the same type of activity as the active compound. In one aspect, the active compounds and resulting conjugates are compounds that inhibit leukocyte adhesion and, in particular, leukocyte adhesion mediated, at least in part, by α_4 integrins.

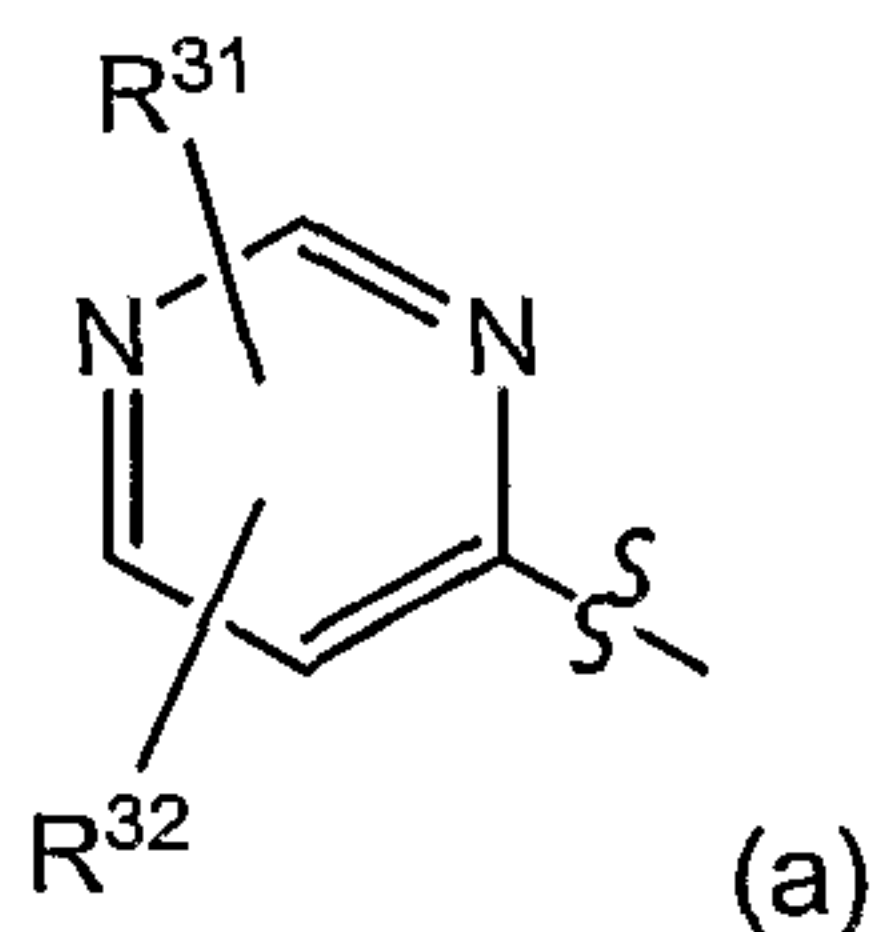
In a preferred aspect, the A group in the conjugates of Formula I can be represented by formula II



wherein

J is selected from:

a) a group of formula (a):



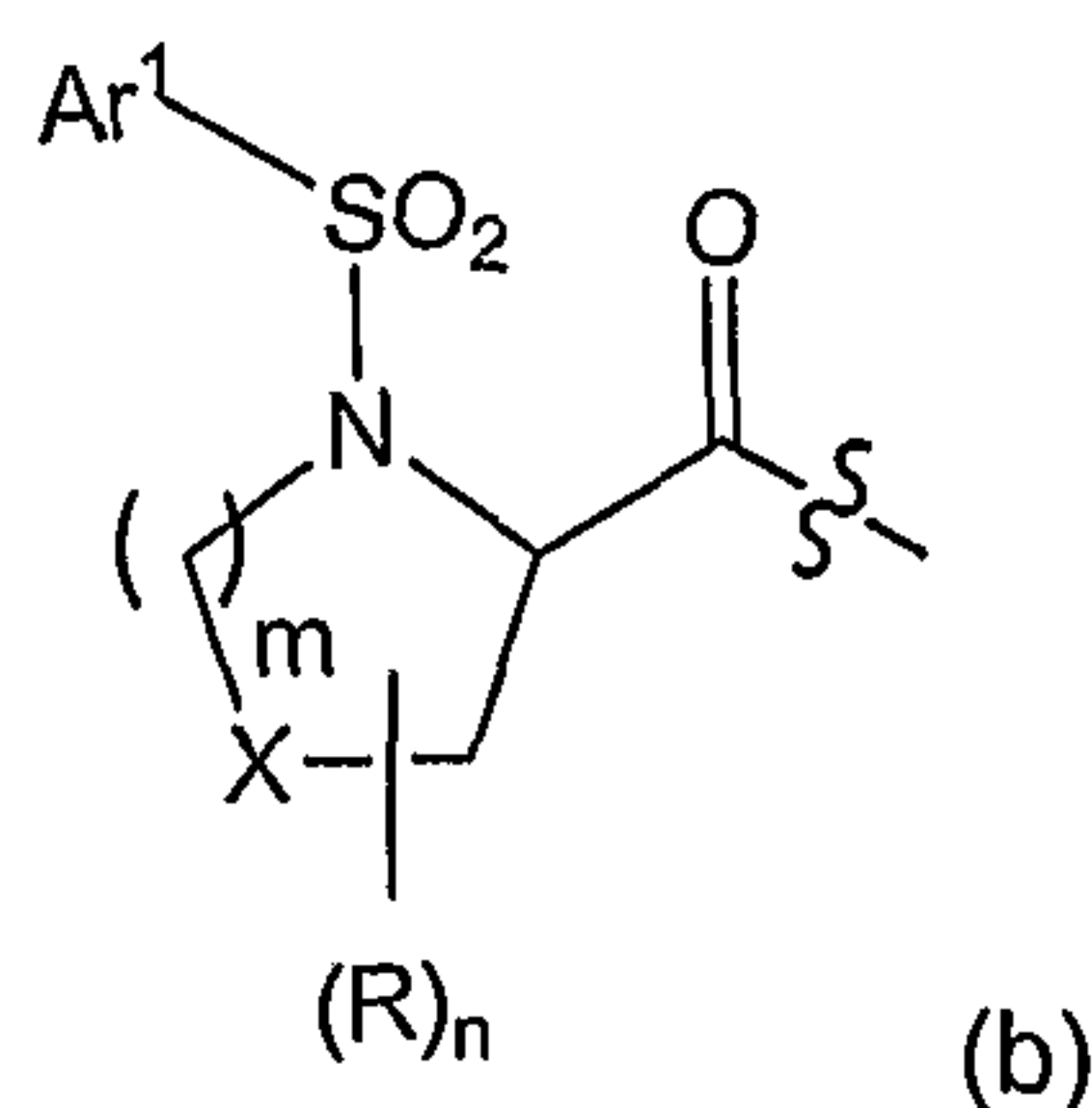
wherein R^{31} is a covalent bond to the polymer moiety which optionally comprises a linker, or R^{31} is $-H$, $R^{31'}$, $-NH_2$, $-NHR^{31'}$ or $-N(R^{31'})_2$, $-NC_3-C_6$ cyclic, $-OR^{31'}$, $-SR^{31'}$, wherein each $R^{31'}$ is independently an optionally substituted straight or branched C_1-C_6 alkyl, optionally substituted C_3-C_6 cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl,

and R^{32} is a covalent bond to the polymer moiety which optionally comprises a linker, or R^{32} is $-H$, $-NO_2$, haloalkyl or the group $-N(MR^{41})R^{42}$ wherein M is a covalent bond, $-C(O)-$ or $-SO_2-$, R^{41} is $R^{41'}$, $N(R^{41'})_2$, or $-OR^{41'}$,

wherein each $R^{41'}$ is independently hydrogen, an optionally substituted straight or branched C_1-C_6 alkyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclic or an optionally substituted heteroaryl, wherein optional substitutions are halide, C_1-C_6 alkyl, or $-OC_1-C_6$ alkyl,

and R^{42} is hydrogen or $R^{41'}$; and

b) a group of formula (b):



wherein R is selected from the group consisting of a covalent bond to the polymer moiety, amino, substituted amino, alkyl and substituted alkyl wherein each amino, substituted amino, alkyl and substituted alkyl is optionally covalently bound to the polymer moiety wherein, in each case, the polymer moiety optionally comprises a linker which covalently links the polymer moiety;

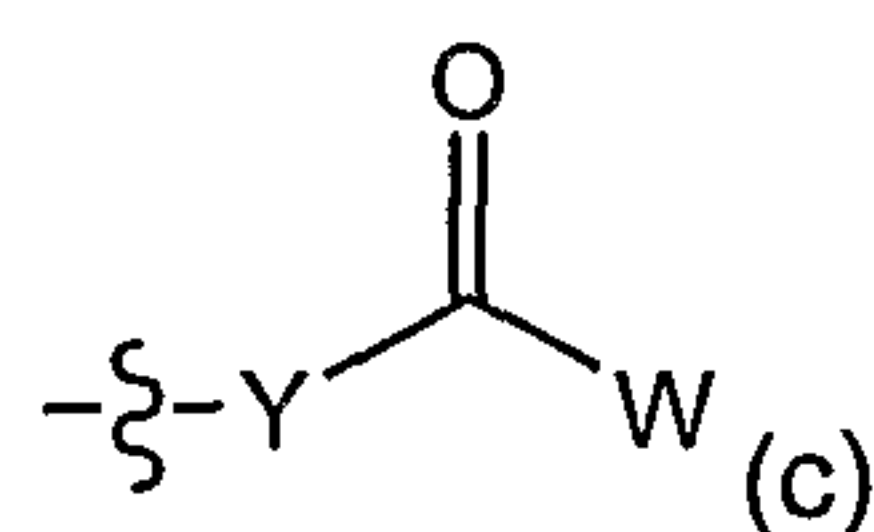
Ar^1 is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl wherein each of aryl, substituted aryl, heteroaryl and substituted heteroaryl is optionally covalently bound to the polymer moiety wherein the polymer moiety optionally comprises a linker which covalently links the polymer moiety to Ar^1 ;

Ar^2 is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl wherein each of aryl, substituted aryl, heteroaryl and substituted heteroaryl is optionally covalently bound to the polymer moiety wherein the polymer moiety optionally comprises a linker which covalently links the polymer moiety to Ar^2 ;

X is selected from the group consisting of $-NR^1-$, $-O-$, $-S-$, $-SO-$, $-SO_2$ and optionally substituted $-CH_2-$ where R^1 is selected from the group consisting of hydrogen and alkyl;

T is selected from:

a) a group of formula (c)

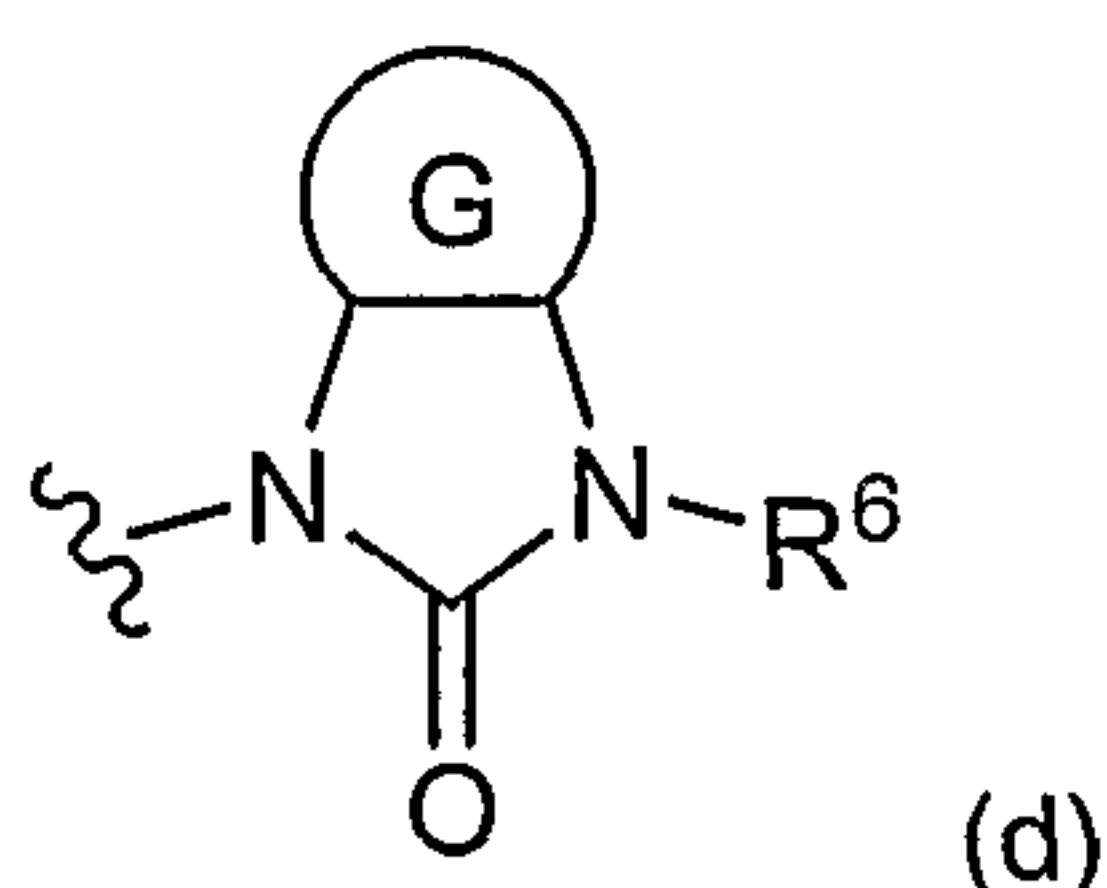


wherein Y is selected from the group consisting of $-O-$ and $-NR^1-$ wherein R^1 is selected from the group consisting of hydrogen and alkyl;

W is selected from the group consisting of a covalent bond to a polymer moiety which optionally comprises a linker and $-NR^2R^3$ wherein R^2 and R^3 are independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, and where R^2 and R^3 , together with the nitrogen atom bound thereto, form a

heterocyclic ring or a substituted heterocyclic ring wherein each of alkyl, substituted alkyl, heterocyclic and substituted heterocyclic is optionally covalently bound to a polymer moiety which further optionally comprises a linker;

- 5 m is an integer equal to 0, 1 or 2;
 n is an integer equal to 0, 1 or 2; and
 b) a group of formula (d)



- 10 wherein G is an optionally substituted aryl or optionally substituted heteroaryl 5 or 6 membered ring containing 0 to 3 nitrogens, wherein said aryl or heteroaryl optionally further comprises a covalent bond to a polymer moiety which optionally comprises a linker;

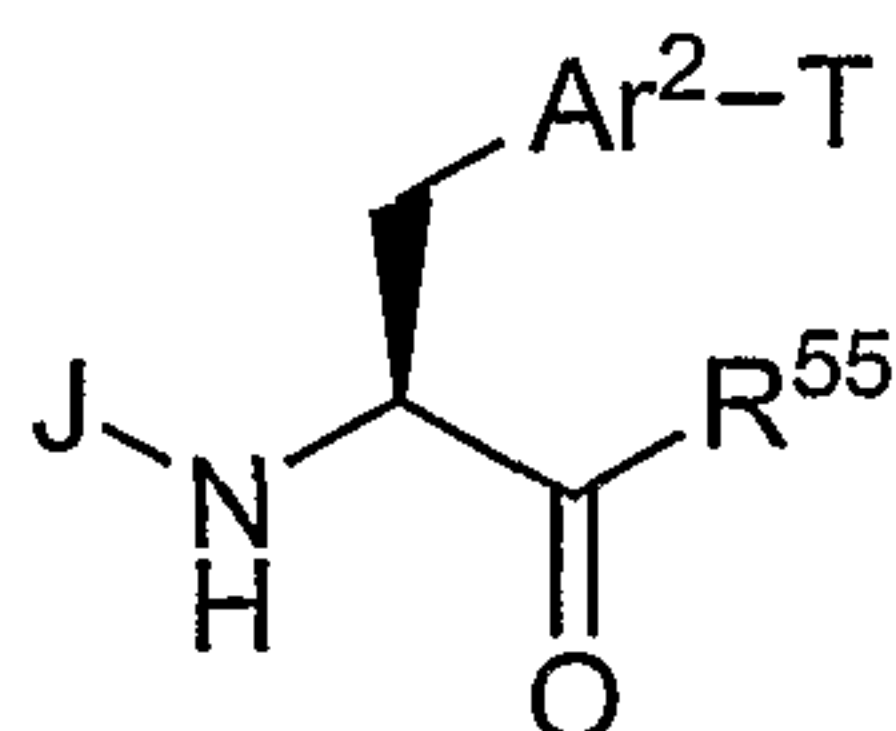
R^6 is a covalent bond to a polymer moiety which optionally comprises a linker, or R^6 is -H;

- 15 R^{55} is selected from the group consisting of alkoxy, substituted alkoxy, cycloalkoxy, substituted cycloalkoxy, aryloxy and substituted aryloxy, and -OH;
 provided that:

- A. at least one of J, Ar^1 , Ar^2 , and T contains a covalent bond to the polymer moiety;
 20 B. when J is covalently bound to the polymer moiety, n is one and X is not -O-, -S-, -SO-, or -SO₂-; and
 C. when X is -O-, then m is two.

- 25 In a preferred embodiment, only one of J, Ar^1 , Ar^2 , and T contains a covalent bond to a polymer moiety.

The compound H-Nu employed in the conjugation reaction is a compound containing an acidic hydrogen covalently bonded to Nu. Preferred compounds of formula H-Nu can be represented by the formula II.1



II.1

wherein

J and Ar² carry the same definitions as set forth for Formula II;

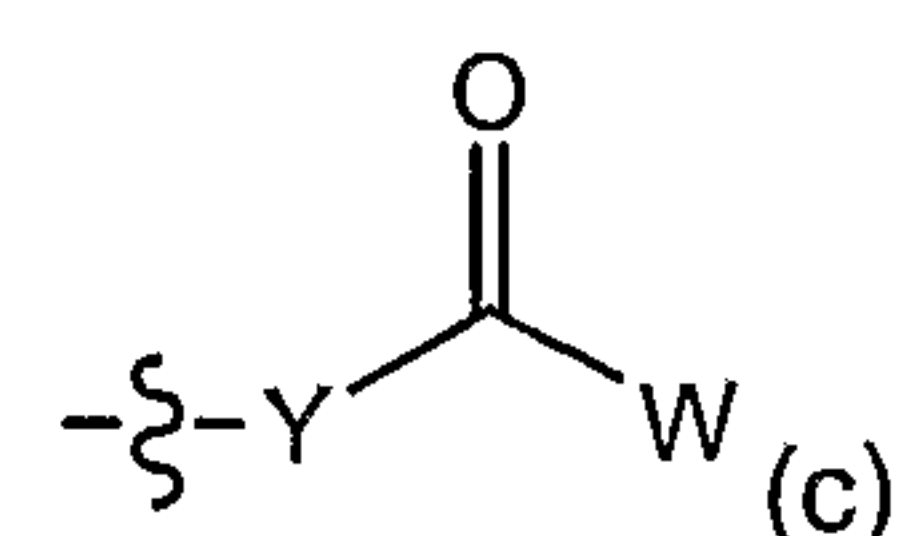
T is a group carrying an acidic hydrogen; and

5 R⁵⁶ is a acid protecting group.

Suitable T groups include heterocyclic groups, imides, phenols, phosphoric mono- and diesters, carboxylic acids, hydroxamates, thiols, thioamides, β-keto esters, and 1,3-diketones,

10

A preferred T group is a group of formula (c)

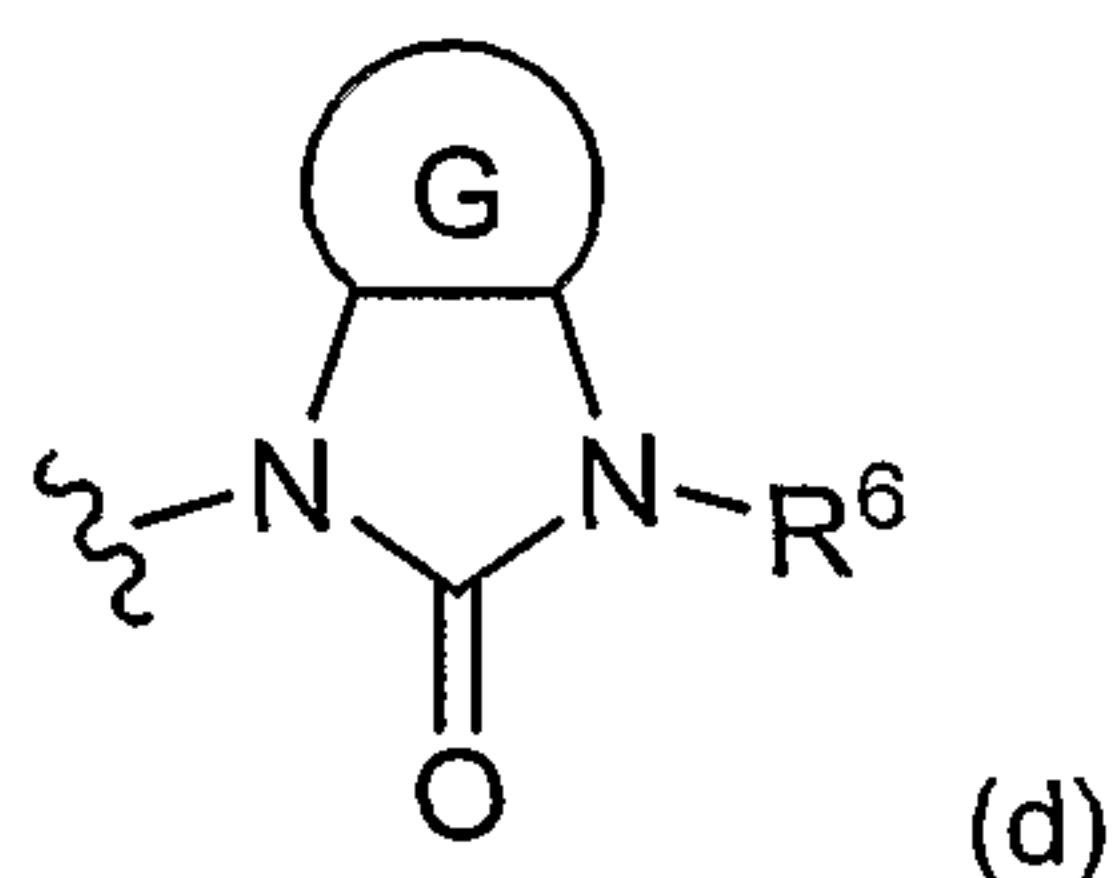


wherein Y is selected from the group consisting of -O- and -NR¹- wherein R¹ is selected from the group consisting of hydrogen and alkyl;

15

W is a group containing an acidic hydrogen, preferably on a nitrogen atom adjacent a carbonyl, where the W group optionally connected to Y(CO) by a linker.

Another preferred T group is a group of formula (d)

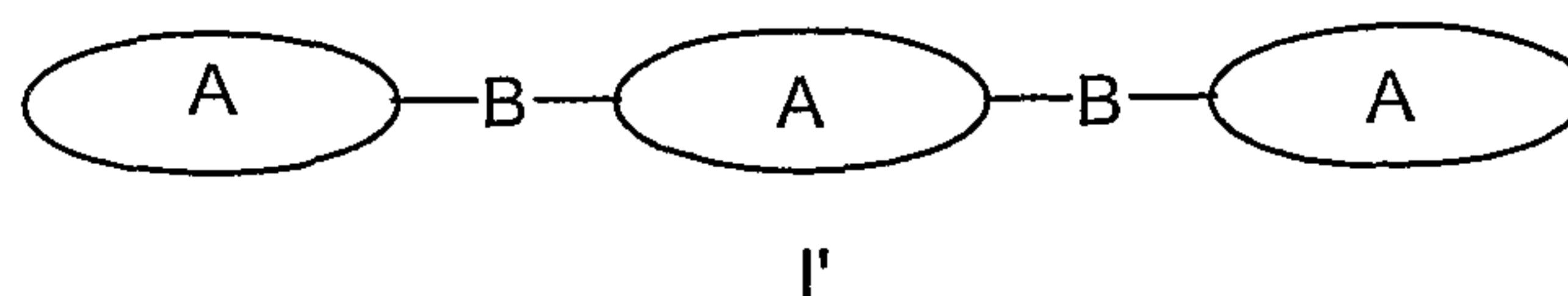


20

wherein G is an optionally substituted aryl or optionally substituted heteroaryl 5 or 6 membered ring containing 0 to 3 nitrogens; and R⁶ is hydrogen.

25

It should be understood that the value for q is calculated based on the ratio of the number of polymer moieties and the number of A-moieties. In other words, when q is 1.5, this contemplates, for example, the following formula I':



30

Preferred conjugates of formula I prepared by the invention include those of formula Ia below:

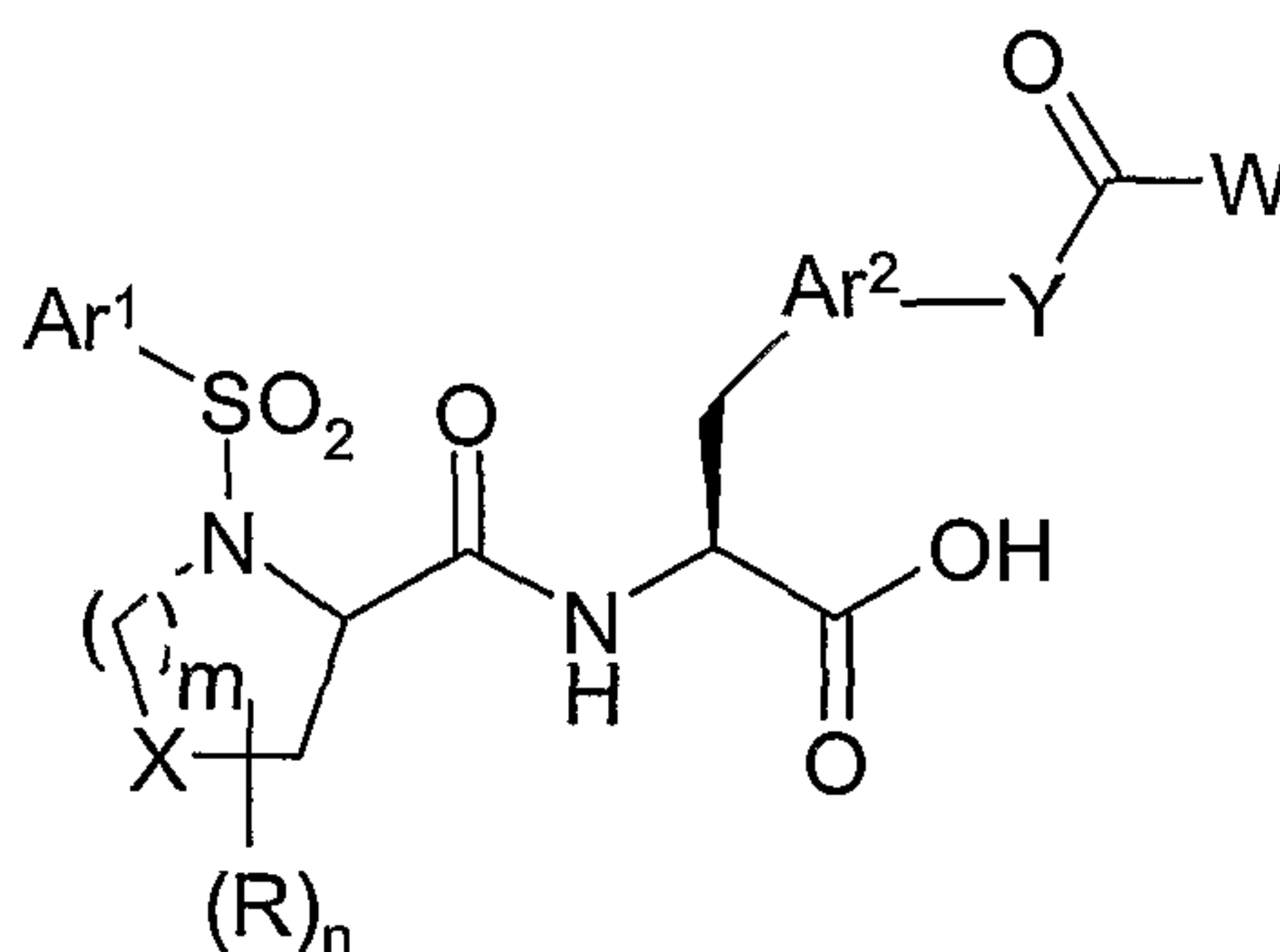


and pharmaceutically acceptable salts thereof, wherein

B is a polymer moiety optionally covalently attached to a carrier;

q is from about 1 to about 100;

10 **A** at each occurrence is independently a compound of formula IIa



IIa

wherein

15 **R** is selected from the group consisting of a covalent bond to the polymer moiety, amino, substituted amino, alkyl and substituted alkyl wherein each amino, substituted amino, alkyl and substituted alkyl is optionally covalently bound to the polymer moiety wherein, in each case, the polymer moiety optionally comprises a linker which covalently links the polymer moiety;

20 **Ar¹** is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl wherein each of aryl, substituted aryl, heteroaryl and substituted heteroaryl is optionally covalently bound to the polymer moiety wherein the polymer moiety optionally comprises a linker which covalently links the polymer moiety to **Ar¹**;

25 **Ar²** is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl wherein each of aryl, substituted aryl, heteroaryl and substituted heteroaryl is optionally covalently bound to the polymer moiety wherein the polymer moiety optionally comprises a linker which covalently links the polymer moiety to **Ar²**;

X is selected from the group consisting of -NR¹-, -O-, -S-, -SO-, -SO₂ and optionally substituted -CH₂- where R¹ is selected from the group consisting of hydrogen and alkyl;

5 Y is selected from the group consisting of -O- and -NR¹- wherein R¹ is selected from the group consisting of hydrogen and alkyl;

W is selected from the group consisting of a covalent bond to a polymer moiety which optionally comprises a linker and -NR²R³ wherein R² and R³ are independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, and where R² and R³, together with the nitrogen atom bound thereto, form a heterocyclic ring or a substituted heterocyclic ring wherein each of alkyl, substituted alkyl, heterocyclic and substituted heterocyclic is optionally covalently bound to a polymer moiety which further optionally comprises a linker;

m is an integer equal to 0, 1 or 2;

n is an integer equal to 0, 1 or 2; and

15 pharmaceutically acceptable salts thereof;
provided that:

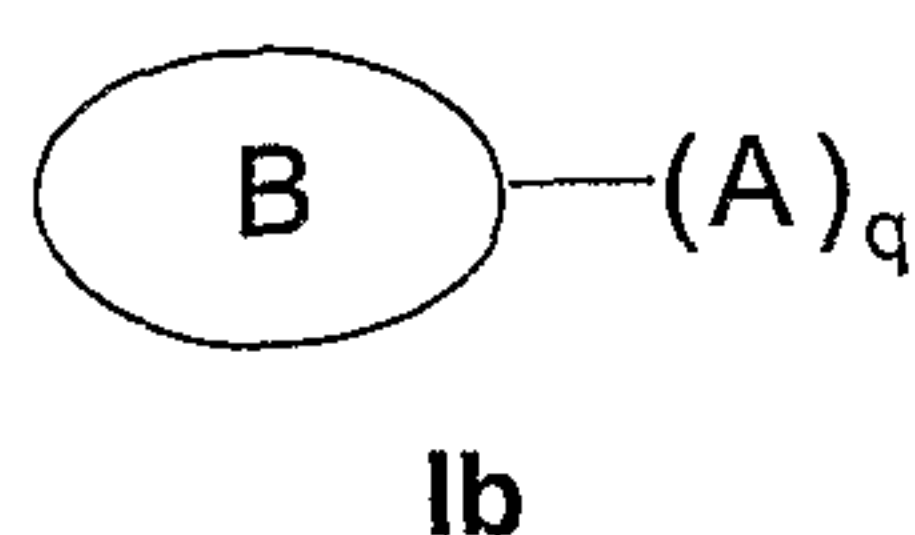
A. at least one of R, Ar¹, Ar², W and -NR²R³ contain a covalent bond to the polymer moiety;

B. when R is covalently bound to the polymer moiety, *n* is one and X is not -O-,
20 -S-, -SO-, or -SO₂;

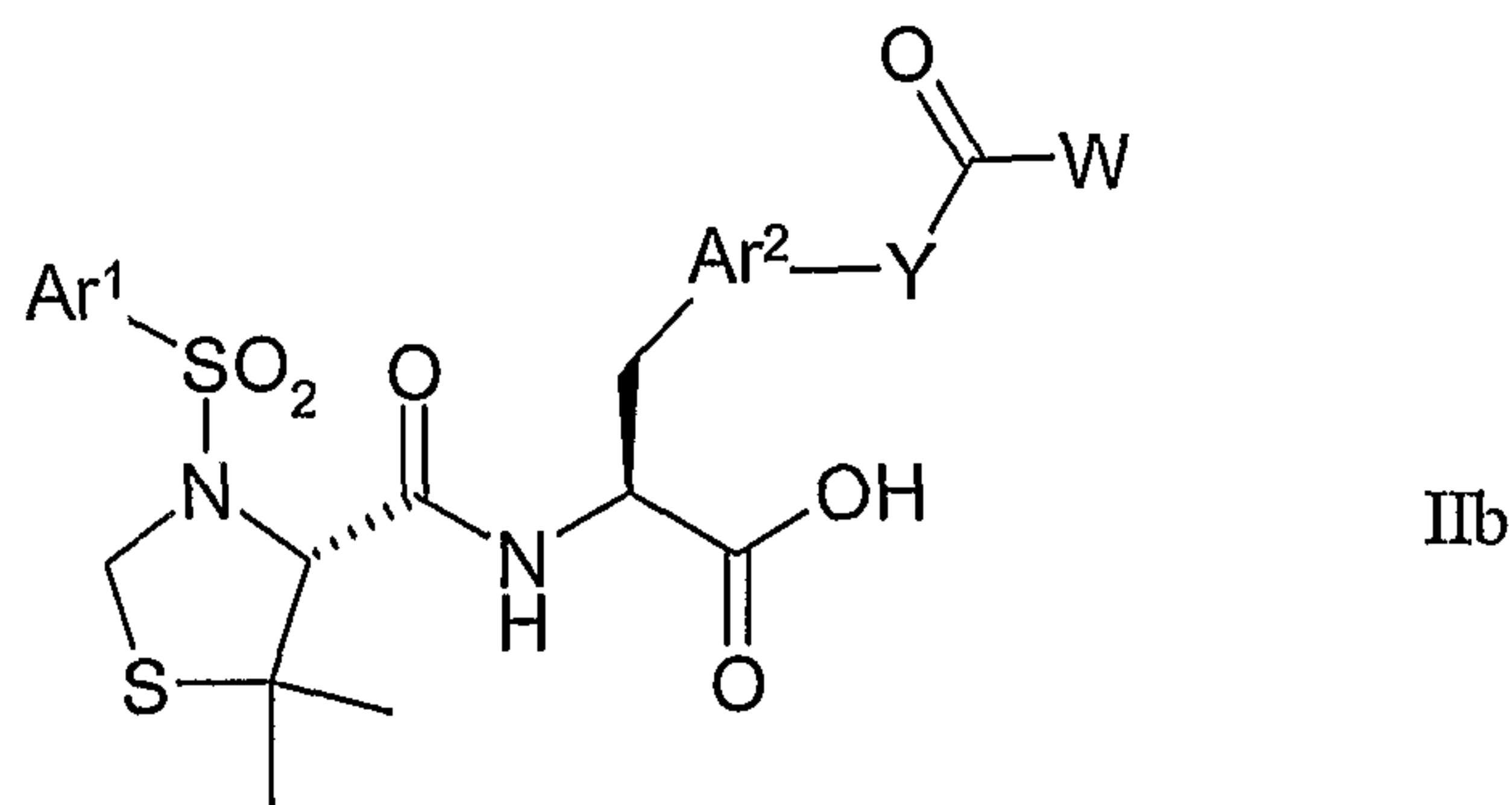
C. when X is -O- or -NR¹-, then *m* is two; and

D. the conjugate of formula Ia has a molecular weight of no more than 100,000.

Preferred conjugates of formula I prepared by the invention include those of formula Ib
25 below:



30 wherein each **A** is independently a compound of formula **IIb** below:



and wherein q is about 1 to about 100;

B is a polymer moiety optionally covalently attached to a carrier;

5 Ar^1 is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl wherein each of aryl, substituted aryl, heteroaryl and substituted heteroaryl is optionally covalently bound to a polymer moiety wherein the PEG moiety optionally comprises a linker which covalently links the PEG moiety to Ar^1 ;

10 Ar^2 is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl wherein each of aryl, substituted aryl, heteroaryl and substituted heteroaryl is optionally covalently bound to a polymer moiety wherein the polymer moiety optionally comprises a linker which covalently links the polymer moiety to Ar^2 ;

Y is selected from the group consisting of -O- and -NR¹- wherein R¹ is selected from the group consisting of hydrogen and alkyl;

15 W is selected from the group consisting of a covalent bond to a polymer moiety which optionally comprises a linker and -NR²R³ wherein R² and R³ are independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, and where R² and R³, together with the nitrogen atom bound thereto, form a heterocyclic ring or a substituted heterocyclic ring wherein each of alkyl, substituted alkyl, heterocyclic and substituted heterocyclic is optionally

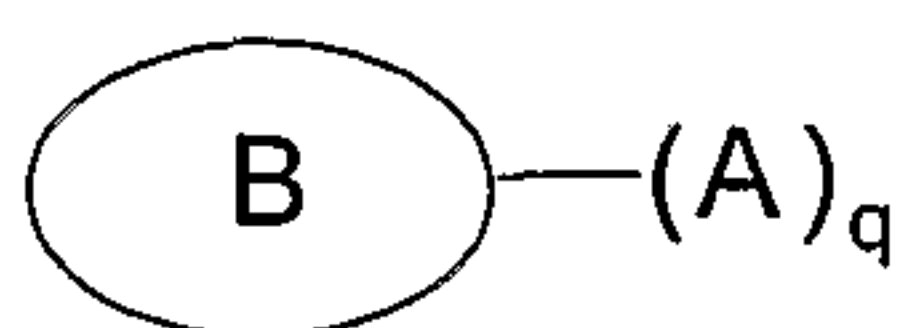
20 covalently bound to the polymer moiety which further optionally comprises a linker;

provided that at least one of Ar^1 , Ar^2 , W and -NR²R³ is covalently bound to a polymer moiety which optionally comprises a linker;

and further provided that the conjugate of formula Ib has a molecular weight of no more than 100,000.

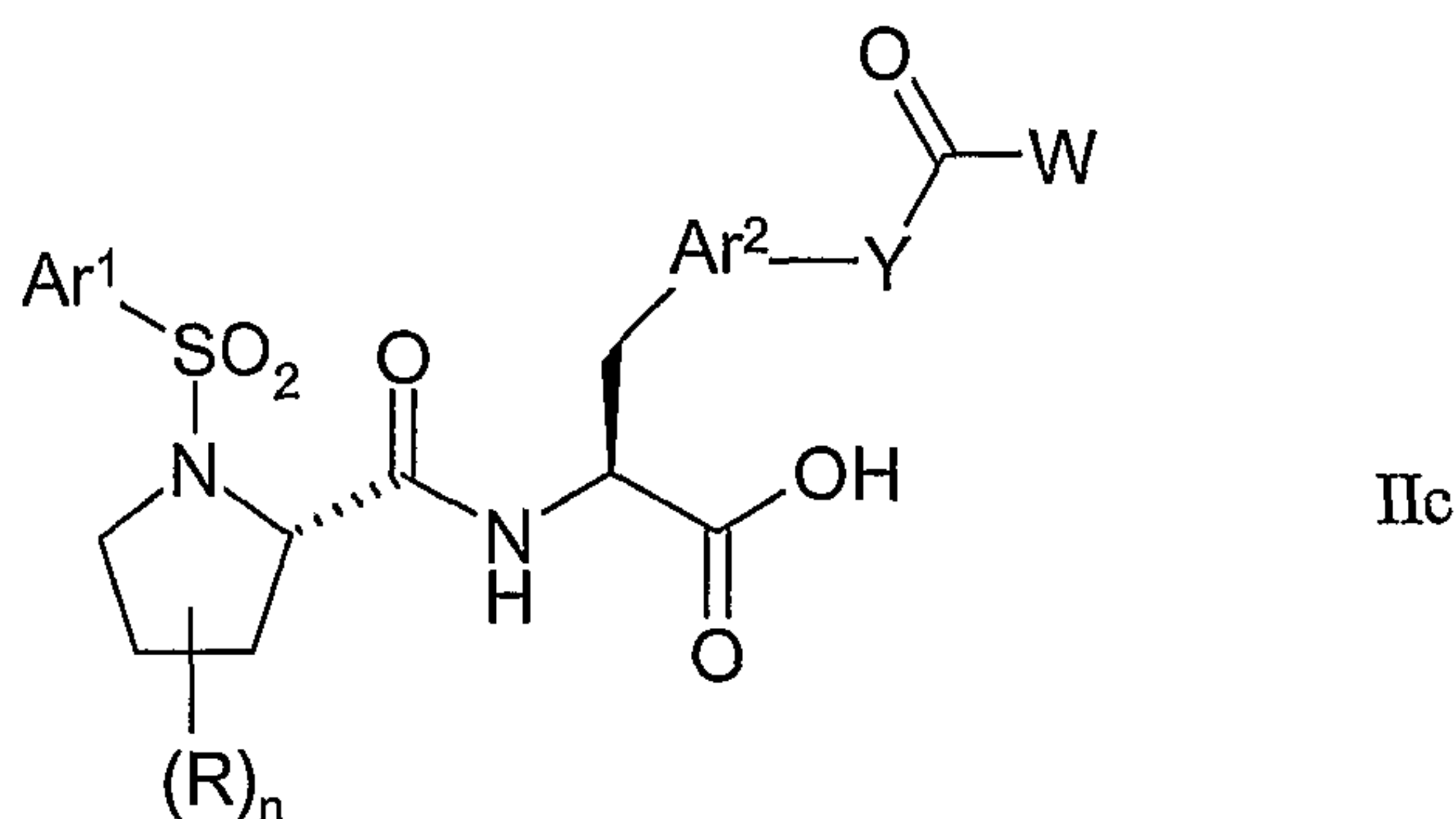
25

Preferred conjugates of formula I formed by the process of the invention include those of formula Ic below:



Ic

wherein each A is independently a compound of formula IIc below:



5

and wherein q is about 1 to about 100;

B is a polymer moiety optionally covalently attached to a carrier;

R is selected from the group consisting of a covalent bond to a polymer moiety,
 10 amino, substituted amino, alkyl and substituted alkyl wherein each amino, substituted amino, alkyl and substituted alkyl is optionally covalently bound to the polymer moiety wherein, in each case, the polymer moiety optionally comprises a linker which covalently links the polymer moiety;

Ar¹ is selected from the group consisting of aryl, substituted aryl, heteroaryl and
 15 substituted heteroaryl wherein each of aryl, substituted aryl, heteroaryl and substituted heteroaryl is optionally covalently bound to a polymer moiety wherein the polymer moiety optionally comprises a linker which covalently links the polymer moiety to Ar¹;

Ar² is selected from the group consisting of aryl, substituted aryl, heteroaryl and
 20 substituted heteroaryl wherein each of aryl, substituted aryl, heteroaryl and substituted heteroaryl is optionally covalently bound to a polymer moiety wherein the polymer moiety optionally comprises a linker which covalently links the polymer moiety to Ar²;

Y is selected from the group consisting of -O- and -NR¹- wherein R¹ is selected from the group consisting of hydrogen and alkyl;

W is selected from the group consisting of a covalent bond to a polymer moiety which
 25 optionally comprises a linker and -NR²R³ where R² and R³, together with the nitrogen atom bound thereto, form a heterocyclic ring or a substituted heterocyclic ring wherein each of alkyl, substituted alkyl, heterocyclic and substituted heterocyclic is optionally covalently bound to a polymer moiety which further optionally comprises a linker;

n is an integer equal to 0, 1 or 2; and

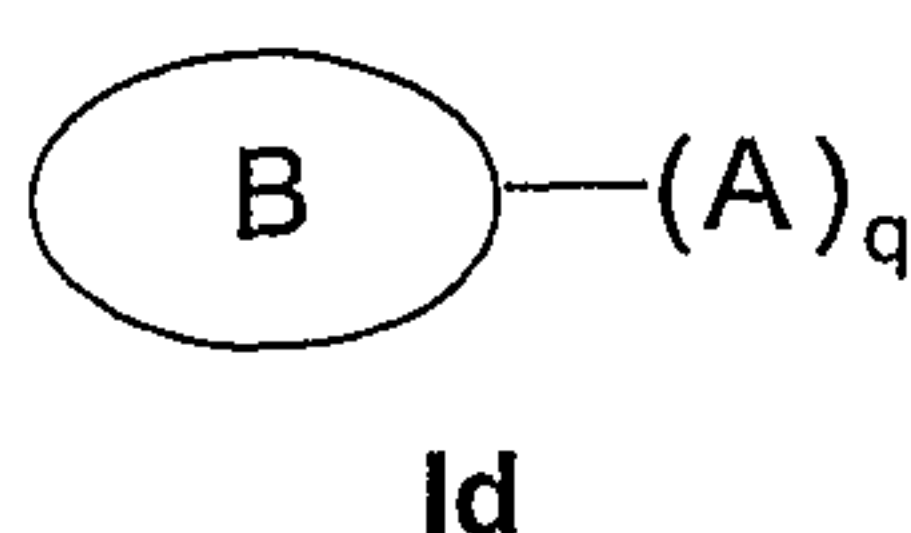
30 pharmaceutically acceptable salts thereof;

provided that at least one of R, Ar¹, Ar², W and -NR²R³ is covalently bound to a polymer moiety which optionally comprises a linker;

and further provided that the conjugate of formula Ic has a molecular weight of no more than 100,000.

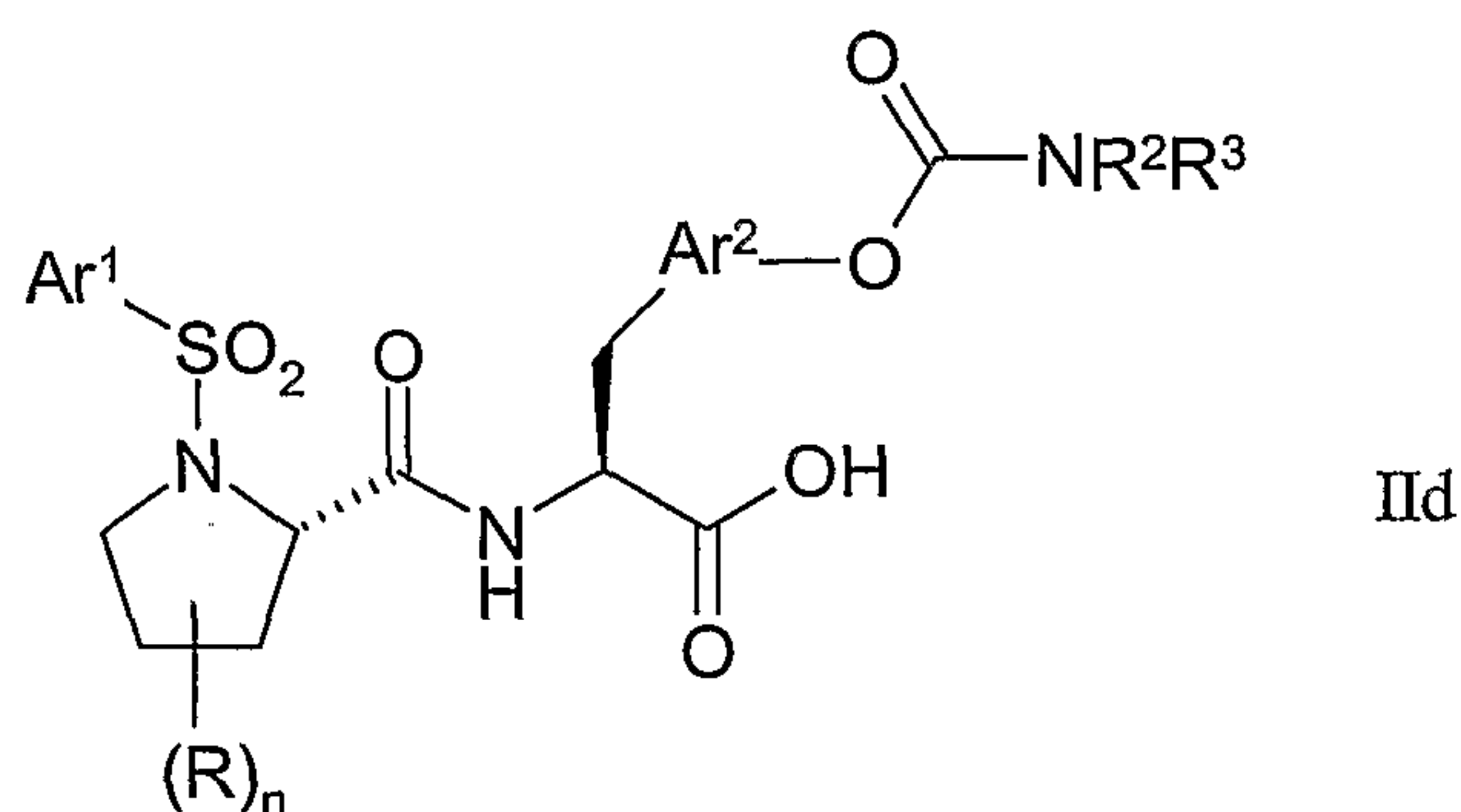
5

Preferred conjugates of formula I include those of formula Id below:



10

wherein each A is independently a compound of formula IId below:



15

and wherein *q* is about 1 to about 100;

B is a polymer moiety optionally covalently attached to a carrier;

20

R is selected from the group consisting of a covalent bond to a polymer moiety, amino, substituted amino, alkyl and substituted alkyl wherein each amino, substituted amino, alkyl and substituted alkyl is optionally covalently bound to a polymer moiety wherein, in each case, the polymer moiety optionally comprises a linker which covalently links the polymer moiety;

25

Ar¹ is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl wherein each of aryl, substituted aryl, heteroaryl and substituted heteroaryl is optionally covalently bound to a polymer moiety wherein the polymer moiety optionally comprises a linker which covalently links the polymer moiety to Ar¹;

Ar² is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl wherein each of aryl, substituted aryl, heteroaryl and substituted heteroaryl is optionally covalently bound to a polymer moiety wherein the polymer moiety optionally comprises a linker which covalently links the polymer moiety to Ar²;

R^2 and R^3 are independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, and where R^2 and R^3 , together with the nitrogen atom bound thereto, form a heterocyclic ring or a substituted heterocyclic ring wherein each of alkyl, substituted alkyl, heterocyclic and substituted heterocyclic is optionally covalently bound to a polymer moiety

5 which further optionally comprises a linker;

n is an integer equal to 0, 1 or 2; and

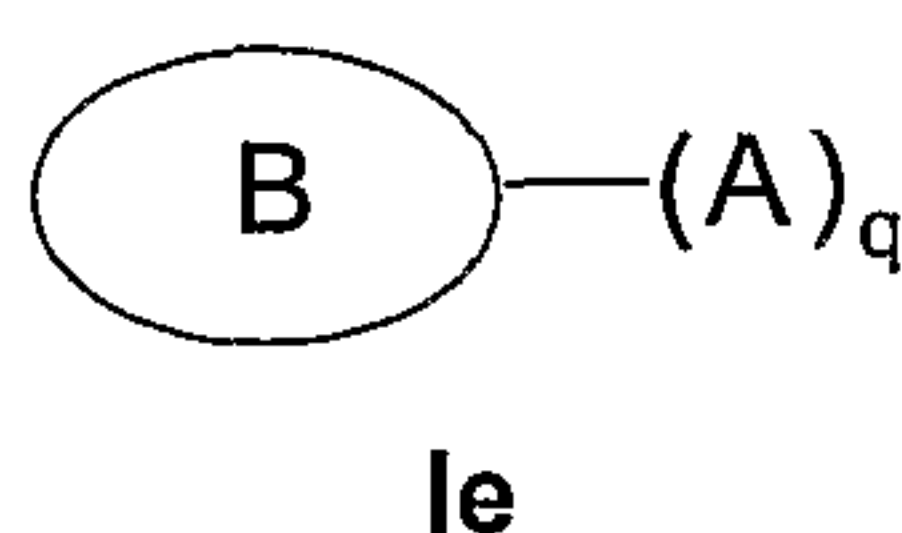
pharmaceutically acceptable salts thereof;

provided that at least one of R , Ar^1 , Ar^2 , and $-NR^2R^3$ is covalently bound to a polymer which optionally comprises a linker;

10 and further provided that the conjugate of formula **Id** has a molecular weight of no more than 100,000.

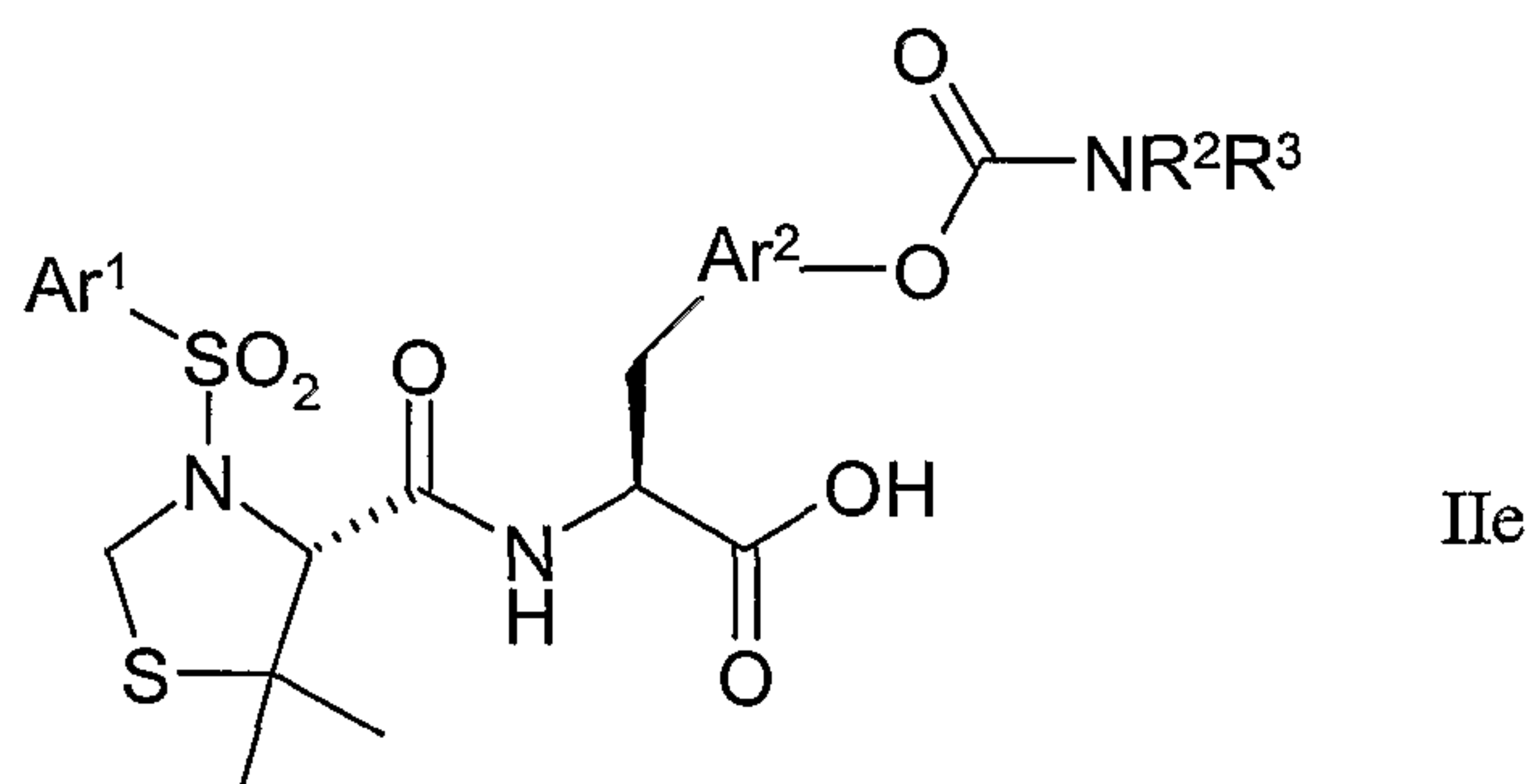
Preferred conjugates of formula **I** include those of formula **Ie** below:

15



wherein each A is independently a compound of formula **Ile** below:

20



and wherein q is about 1 to about 100;

B is a polymer moiety optionally covalently attached to a carrier;

25

Ar^1 is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl wherein each of aryl, substituted aryl, heteroaryl and substituted heteroaryl is optionally covalently bound to a polymer moiety wherein the polymer moiety optionally comprises a linker which covalently links the polymer moiety to Ar^1 ;

Ar² is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl wherein each of aryl, substituted aryl, heteroaryl and substituted heteroaryl is optionally covalently bound to a polymer moiety wherein the polymer moiety optionally comprises a linker which covalently links the polymer moiety to Ar²;

5 R² and R³ are independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, and where R² and R³, together with the nitrogen atom bound thereto, form a heterocyclic ring or a substituted heterocyclic ring wherein each of alkyl, substituted alkyl, heterocyclic and substituted heterocyclic is optionally covalently bound to a polymer moiety which further optionally comprises a linker; and

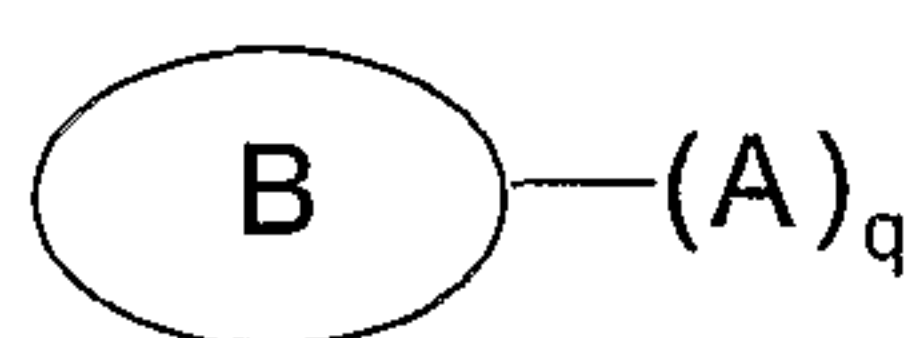
10 pharmaceutically acceptable salts thereof;

provided that at least one of Ar¹, Ar² and -NR²R³ is covalently bound to a polymer moiety which optionally comprises a linker;

and further provided that the conjugate of formula **Ie** has a molecular weight of no more than 100,000.

15

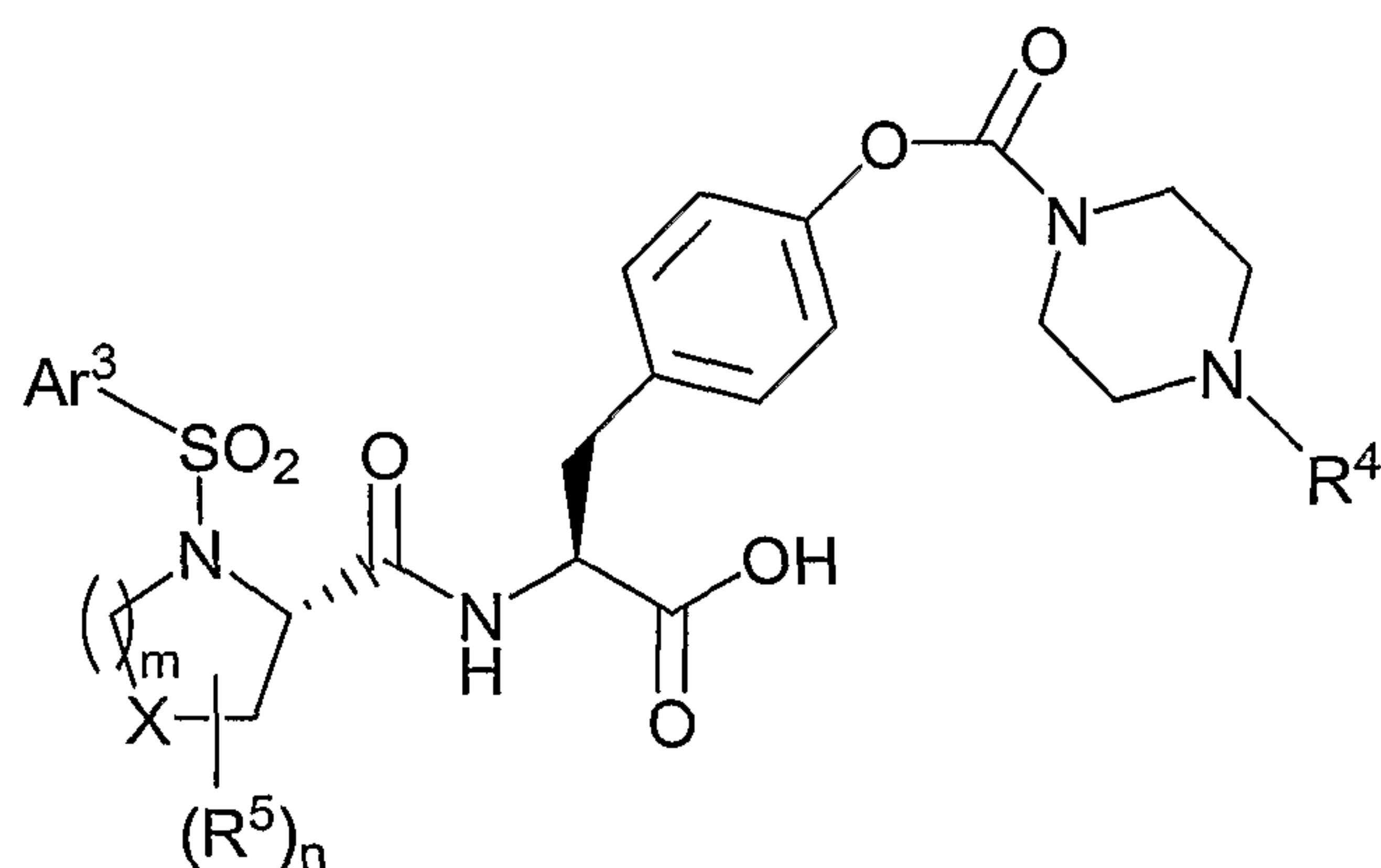
Preferred conjugates of formula **I** include those of formula **If** below:



If

20

wherein each A is independently a compound of formula **IIf** below:



IIf

25

and wherein q is about 1 to about 100;

B is a polymer moiety optionally covalently attached to a carrier;

R⁴ is covalently bound to a polymer moiety which optionally comprises a linker;

R^5 is selected from the group consisting of alkyl and substituted alkyl;

Ar^3 is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl;

X is selected from the group consisting of $-NR^1$ -, $-O$ -, $-S$ -, $-SO$ -, $-SO_2$ and optionally substituted $-CH_2$ - where R^1 is selected from the group consisting of hydrogen and alkyl;

m is an integer equal to 0, 1 or 2;

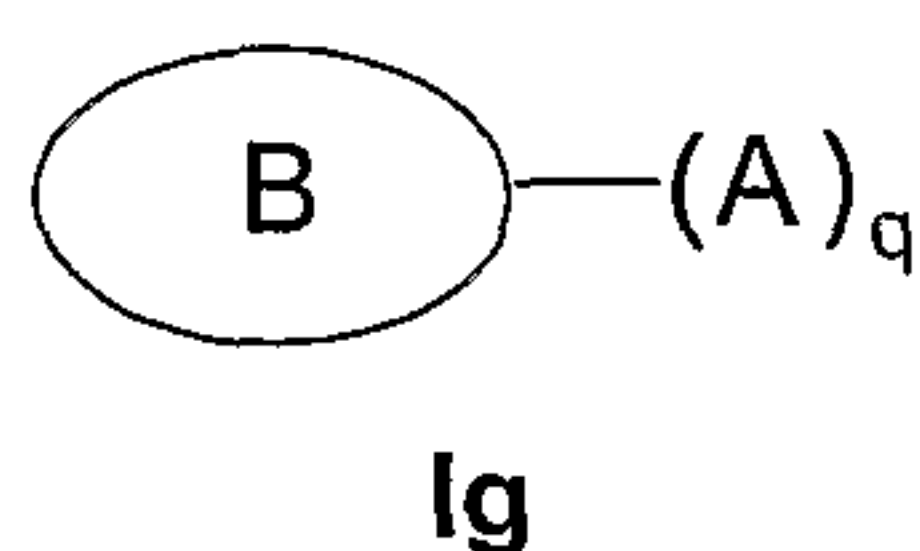
n is an integer equal to 0, 1 or 2; and

pharmaceutically acceptable salts thereof;

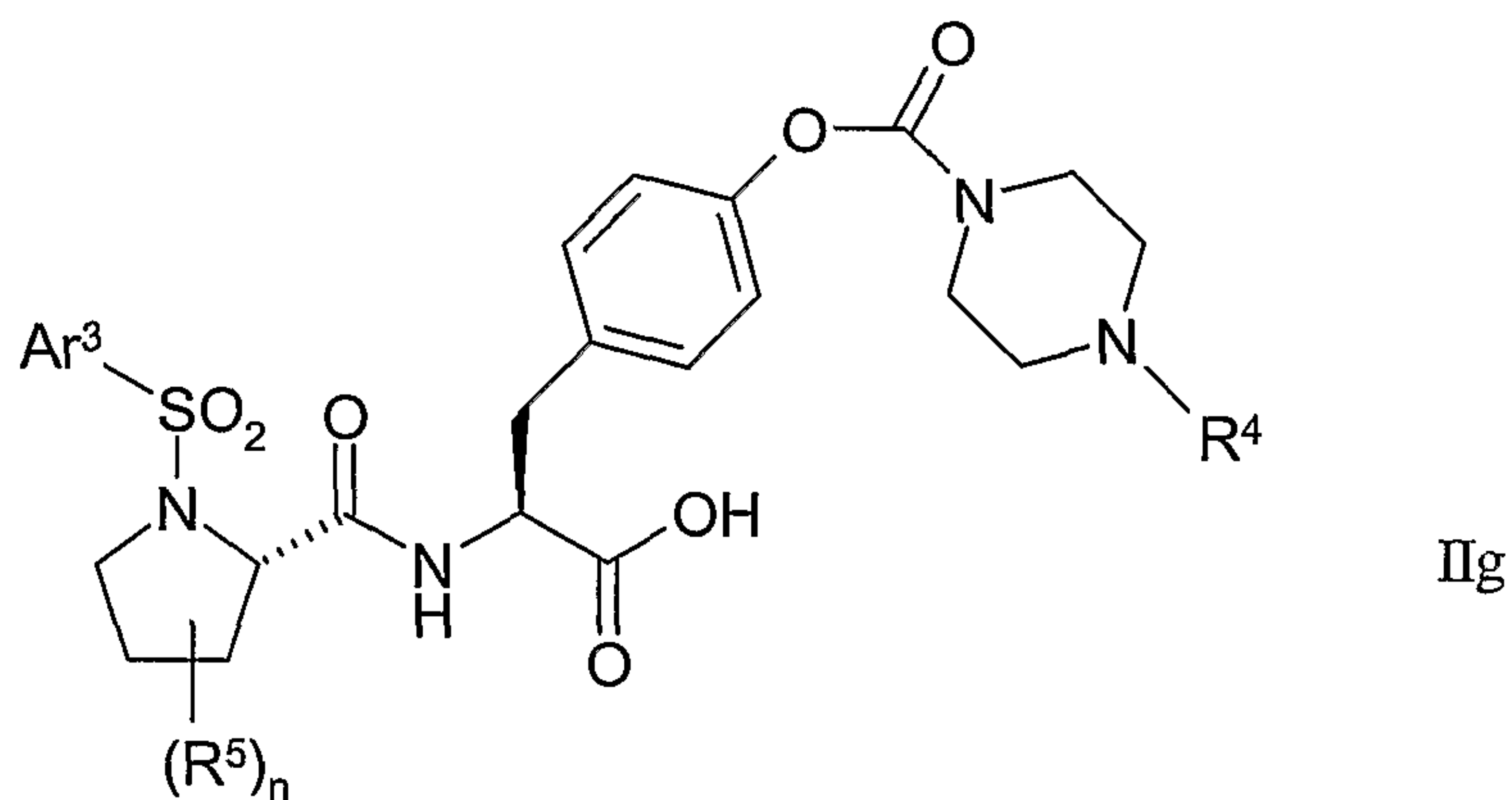
provided that:

- 10 A. when R is covalently bound to the polymer moiety, n is one and X is not $-O$ -, $-S$ -, $-SO$ -, or $-SO_2$;
- B. when X is $-O$ - or $-NR^1$ -, then m is two; and
- C. the conjugate of formula **If** has a molecular weight of no more than 100,000.

15 Preferred conjugates of formula **I** include those of formula **Ig** below:



20 wherein each A is independently a compound of formula **Ilg** below:



and wherein q is about 1 to about 100;

25 **B** is a polymer moiety optionally covalently attached to a carrier;

R^4 is covalently bound to a polymer moiety which optionally comprises a linker;

R^5 is selected from the group consisting of alkyl and substituted alkyl;

Ar^3 is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl;

n is an integer equal to 0, 1 or 2; and

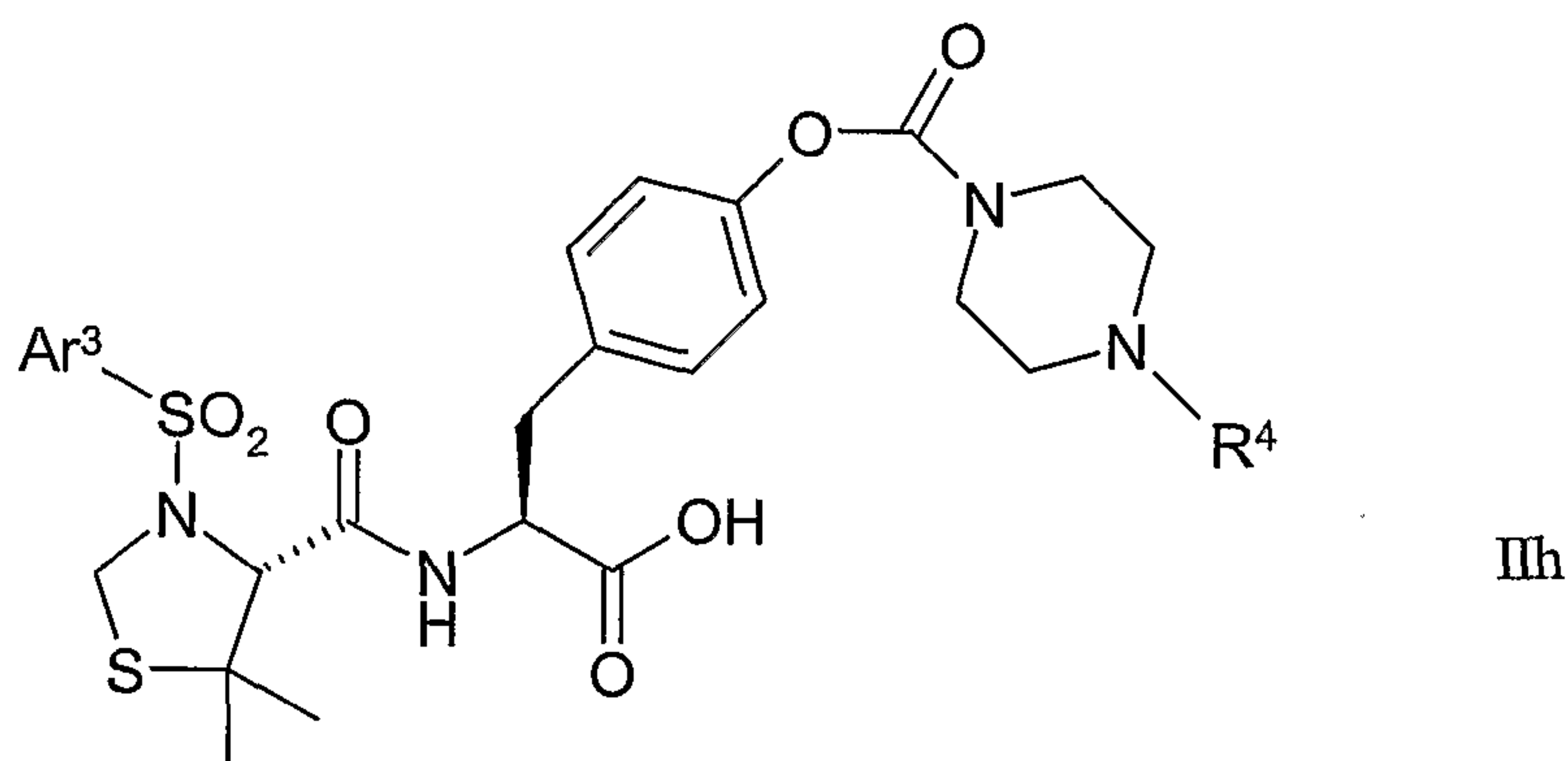
pharmaceutically acceptable salts thereof;

5 provided that the conjugate of formula **Ig** has a molecular weight of no more than 100,000.

Preferred conjugates of formula **I** include those of formula **Ih** below:



wherein each A is independently a compound of formula **IIh** below:



15 and wherein q is about 1 to about 100;

R^4 is covalently bound to a polymer moiety which optionally comprises a linker;

Ar^3 is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl;

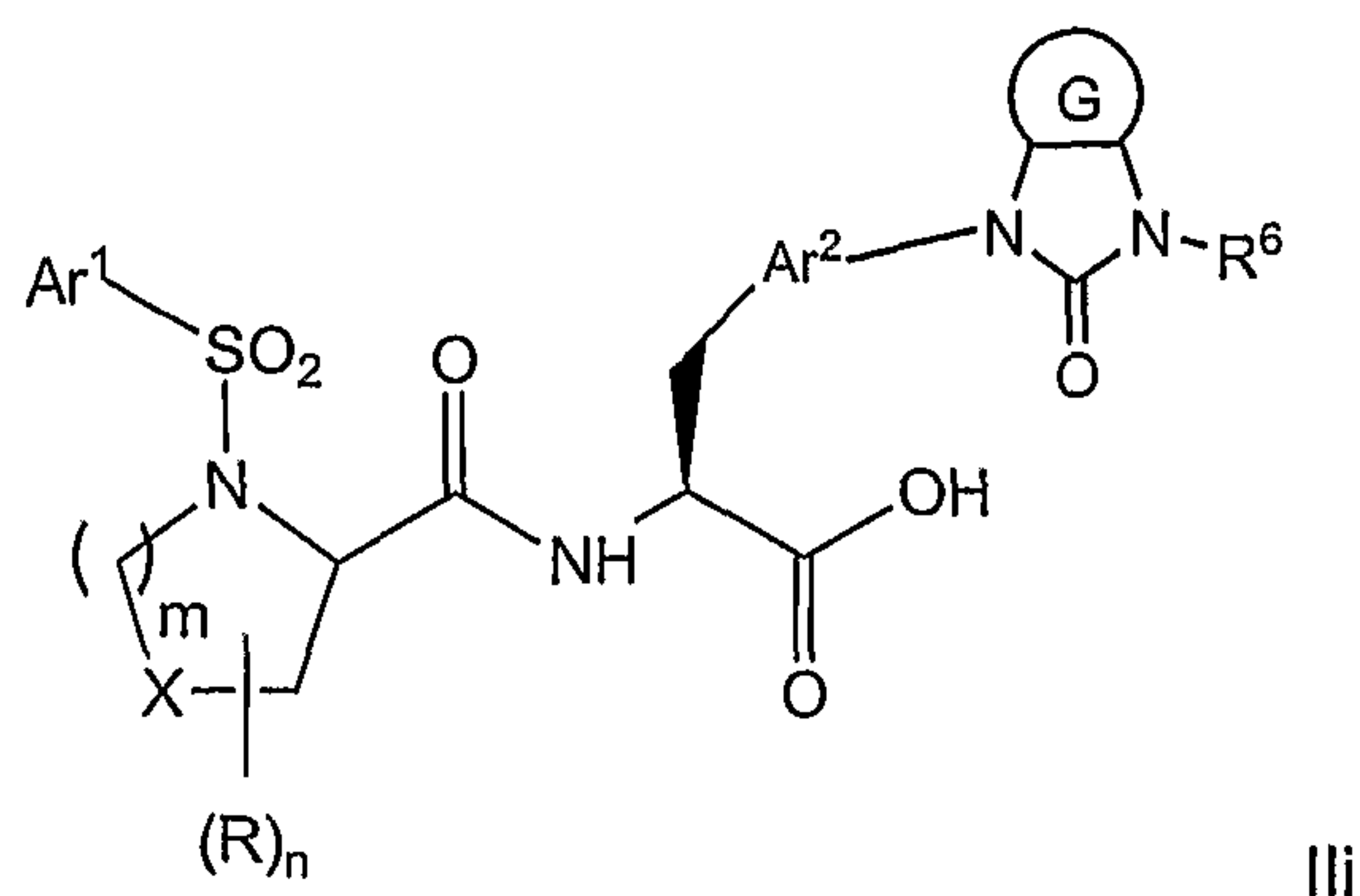
20 pharmaceutically acceptable salts thereof;

provided that the conjugate of formula **Ih** has a molecular weight of no more than 100,000.

Preferred conjugates of formula **I** include those of formula **Ii** below:



wherein each A is independently a compound of formula **IIi** below:



or a pharmaceutically acceptable salt thereof,

and provided that the conjugate of formula II has a molecular weight of no more than 100,000.

5

Preferably, when Ar¹ is not bound to a polymer moiety, Ar¹ in formulae IIa -IIe and Ar³ in formulae IIf-IIh is selected from the group consisting of:

- phenyl,
- 4-methylphenyl,
- 10 4-*t*-butylphenyl,
- 2,4,6-trimethylphenyl,
- 2-fluorophenyl,
- 3-fluorophenyl,
- 4-fluorophenyl,
- 15 2,4-difluorophenyl,
- 3,4-difluorophenyl,
- 3,5-difluorophenyl,
- 2-chlorophenyl,
- 3-chlorophenyl,
- 20 4-chlorophenyl,
- 3,4-dichlorophenyl,
- 3,5-dichlorophenyl,
- 3-chloro-4-fluorophenyl,
- 4-bromophenyl,
- 25 2-methoxyphenyl,
- 3-methoxyphenyl,
- 4-methoxyphenyl,
- 3,4-dimethoxyphenyl,
- 4-*t*-butoxyphenyl,

- 4-(3'-dimethylamino-*n*-propoxy)-phenyl,
2-carboxyphenyl,
2-(methoxycarbonyl)phenyl,
4-(H₂NC(O)-)phenyl,
5 4-(H₂NC(S)-)phenyl,
4-cyanophenyl,
4-trifluoromethylphenyl,
4-trifluoromethoxyphenyl,
3,5-di-(trifluoromethyl)phenyl,
10 4-nitrophenyl,
4-aminophenyl,
4-(CH₃C(O)NH-)phenyl,
4-(phenylNHC(O)NH-)phenyl,
4-amidinophenyl,
15 4-methylamidinophenyl,
4-[CH₃SC(=NH)-]phenyl,
4-chloro-3-[H₂NS(O)₂-]phenyl,
1-naphthyl,
2-naphthyl,
20 pyridin-2-yl,
pyridin-3-yl,
pyridin-4-yl,
pyrimidin-2-yl,
quinolin-8-yl,
25 2-(trifluoroacetyl)-1,2,3,4-tetrahydroisoquinolin-7-yl,
2-thienyl,
5-chloro-2-thienyl,
2,5-dichloro-4-thienyl,
1-*N*-methylimidazol-4-yl,
30 1-*N*-methylpyrazol-3-yl,
1-*N*-methylpyrazol-4-yl,
1-*N*-butylpyrazol-4-yl,
1-*N*-methyl-3-methyl-5-chloropyrazol-4-yl,
1-*N*-methyl-5-methyl-3-chloropyrazol-4-yl,
35 2-thiazolyl and
5-methyl-1,3,4-thiadiazol-2-yl.

Preferably, when **A** is of the formulae IIa, IIb, IIc, IId, and IIe, and Ar¹ is bound to a polymer moiety, then Ar¹ is of the formula:



wherein

Ar¹ is selected from the group consisting of aryl, substituted aryl, heteroaryl, and substituted heteroaryl,

10 Z is selected from the group consisting of a covalent bond, a linking group of from 1 to 40 atoms, -O-, and -NR⁹-, where R⁹ is selected from the group consisting of hydrogen and alkyl,

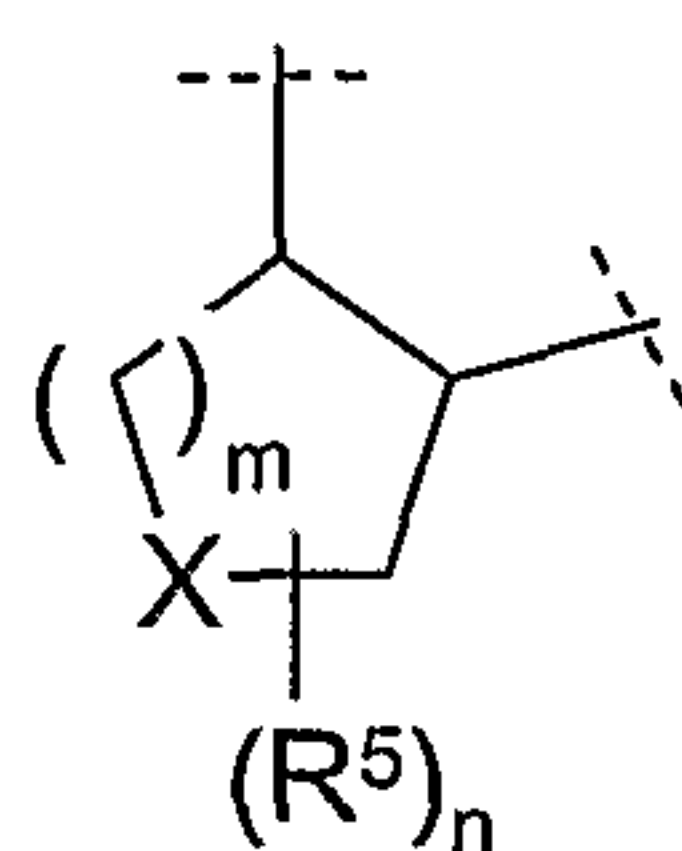
R⁷ is selected from the group consisting of hydrogen and methyl;

15 R⁸ is selected from the group consisting of **A**, -(L)_w-polymer carrier, hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and -CH₂CHR⁷NR¹⁰R¹¹ where R⁷ is as defined above, R¹⁰ and R¹¹ are independently selected from the group consisting of hydrogen and alkyl, **A** is represented by any of formulae IIa through IIh above, L is a linking group of from 1 to 40 atoms and w is zero or one: and

p is an integer of from about 100 to 2200, preferably from about 200-1360.

20

When **A** is of the Formulae IIa or IIc, and R is not bound to a polymer moiety, the substituent of the following formula:

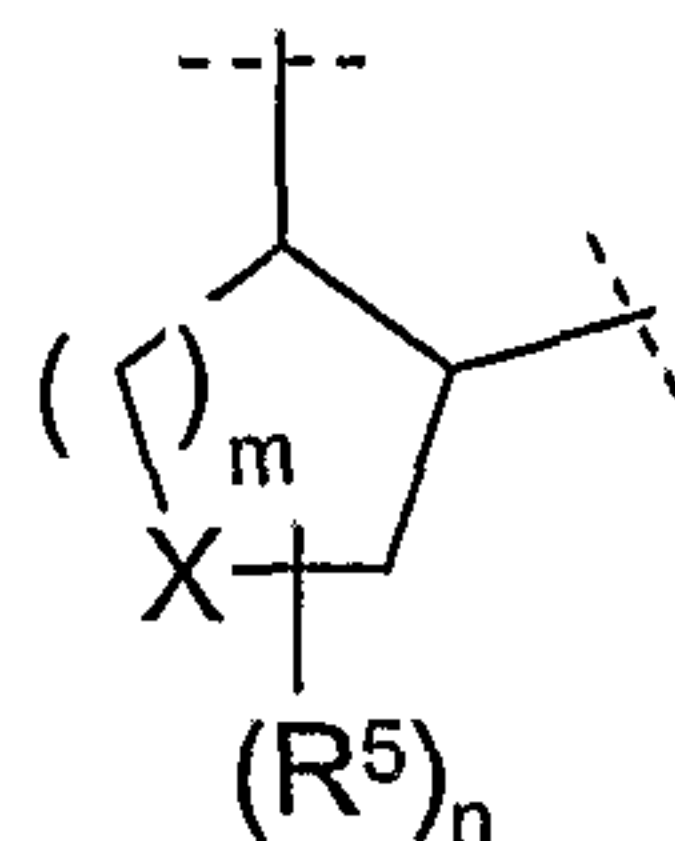


25

where R⁵, X, m and n are as defined above, is preferably selected from the group consisting of azetidiny, thiazolidiny, piperidiny, piperaziny, morpholino, thiomorpholino, pyrrolidiny, 4-hydroxypyrrolidiny, 4-oxopyrrolidiny, 4-fluoropyrrolidiny, 4,4-difluoropyrrolidiny, 4-(thiomorpholin-4-ylC(O)O-)pyrrolidiny, 4-[CH₃S(O)₂O-]pyrrolidiny, 3-phenylpyrrolidiny, 3-thiophenylpyrrolidiny, 4-amino-pyrrolidiny, 3-methoxypyrrolidiny, 4,4-dimethylpyrrolidiny, 4-N-Cbz-piperaziny, 4-[CH₃S(O)₂-]piperaziny, 5,5-dimethylthiazolidin-4-yl, 1,1-dioxo-thiazolidiny, 1,1-dioxo-5,5-dimethylthiazolidin-2-yl and 1,1-dioxothiomorpholino.

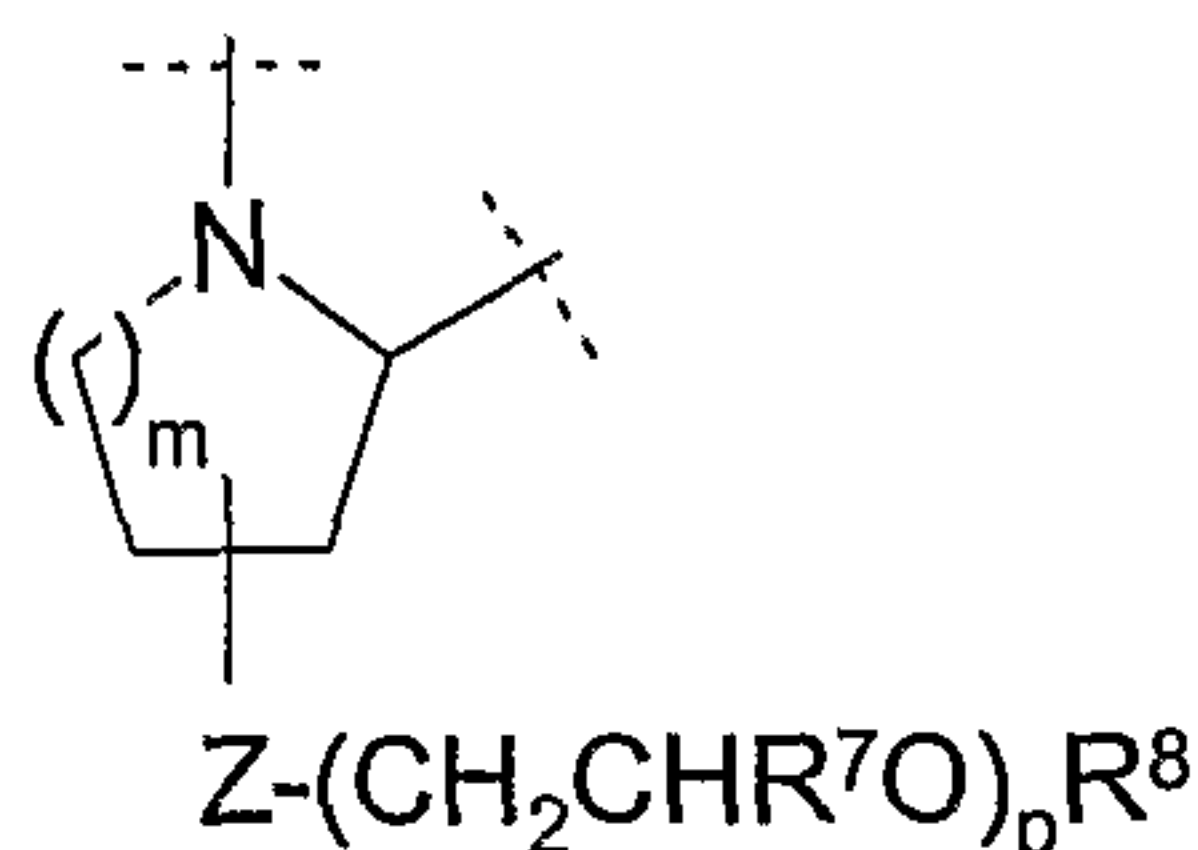
30

Preferably, when **A** is of the formulae IIa and the substituent of the formula:



5

is bound to the PEG moiety, then preferably the substituent is of the formula:



wherein

m is an integer equal to zero, one or two;

10 Z is selected from the group consisting of a covalent bond, a linking group of from 1 to 40 atoms, $-O-$, and $-NR^9-$, where R^9 is selected from the group consisting of hydrogen and alkyl,

R^7 is selected from the group consisting of hydrogen and methyl;

15 R^8 is selected from the group consisting of **A**, $-(L)_w$ -polymer carrier, hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and $-\text{CH}_2\text{CHR}^7\text{NR}^{10}\text{R}^{11}$ where R^7 is as defined above, R^{10} and R^{11} are independently selected from the group consisting of hydrogen and alkyl, **A** is represented by any of formulae IIa through IIh above, L is a linking group of from 1 to 40 atoms and w is zero or one: and

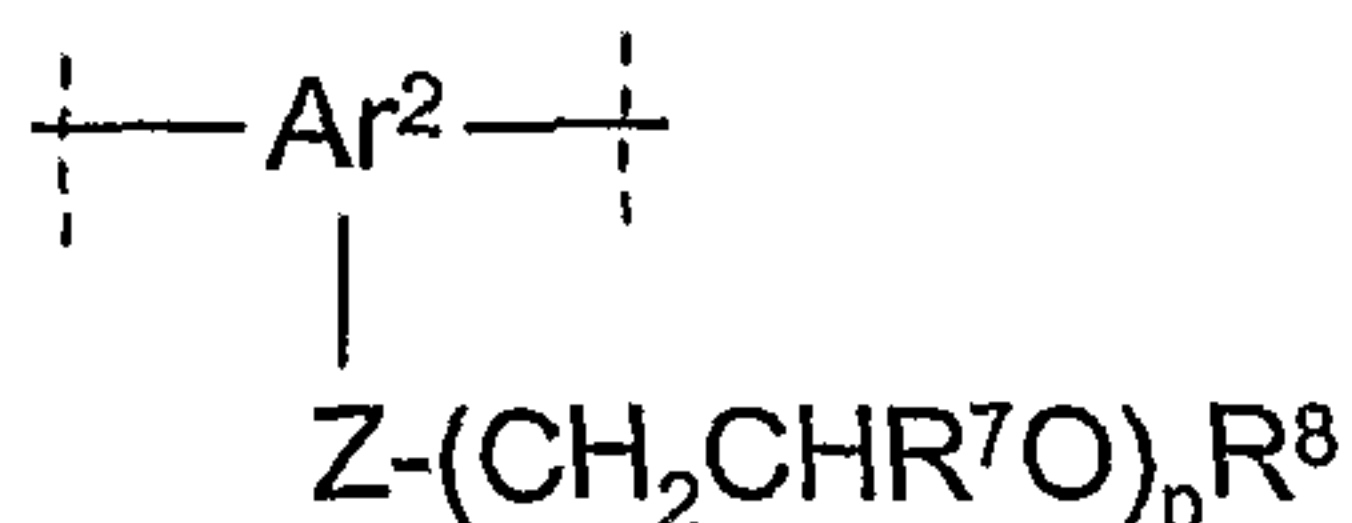
p is an integer of from about 100 to 2200, preferably from about 200-1360.

20

When **A** is of the formula IIa, IIb, IIc, IId, IIe and when Ar^2 is not bound to a polymer moiety, then preferably Ar^2 is selected from the group consisting of phenyl, substituted phenyl, 2-pyridinyl, 3-pyridinyl, 4-pyridinyl, and 4-pyridin-2-onyl.

25

When **A** is of the formula IIa, IIb, IIc, IId, IIe and when Ar^2 is bound to a polymer moiety, then Ar^2 is preferably represented by the formula:



where Ar^2 is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl;

5 Z is selected from the group consisting of a covalent bond, a linking group of from 1 to 40 atoms, -O-, and -NR⁹-, where R⁹ is selected from the group consisting of hydrogen and alkyl,

R⁷ is selected from the group consisting of hydrogen and methyl;

10 R⁸ is selected from the group consisting of A, -(L)_w-polymer carrier, hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and -CH₂CHR⁷NR¹⁰R¹¹ where R⁷ is as defined above, R¹⁰ and R¹¹ are independently selected from the group consisting of hydrogen and alkyl, A is represented by any of formulae IIa through IIh above, L is a linking group of from 1 to 40 atoms and w is zero or one: and

p is an integer of from about 100 to 2200, preferably from about 200-1360.

15

In one preferred embodiment of conjugates prepared by the inventive process, -YC(O)W is -OC(O)NR²R³.

20 When A is of the formulae IIa, IIb, or IIc, -YC(O)W is -OC(O)NR²R³ and neither R² nor R³ are not bound to a polymer moiety, then preferably -OC(O)NR²R³ is selected from the group consisting of:

(CH₃)₂NC(O)O-,
 (piperidin-1-yl)-C(O)O-,
 (piperidin-4-yl)-C(O)O-,
 25 (1-methylpiperidin-4-yl)-C(O)O-,
 (4-hydroxypiperidin-1-yl)-C(O)O-,
 (4-formyloxypiperidin-1-yl)-C(O)O-,
 (4-ethoxycarbonylpiperidin-1-yl)-C(O)O-,
 (4-carboxypiperidin-1-yl)-C(O)O-,
 30 (3-hydroxymethylpiperidin-1-yl)-C(O)O-,
 (4-hydroxymethylpiperidin-1-yl)-C(O)O-,
 (4-phenyl-1-Boc-piperidin-4-yl)-C(O)O-,
 (4-piperidon-1-yl ethylene ketal)-C(O)O-,
 (piperazin-4-yl)-C(O)O-,

- (1-Boc-piperazin-4-yl)-C(O)O-,
 (4-methylpiperazin-1-yl)-C(O)O-,
 (4-methylhomopiperazin-1-yl)-C(O)O-,
 (4-(2-hydroxyethyl)piperazin-1-yl)-C(O)O-,
 5 (4-phenylpiperazin-1-yl)-C(O)O-,
 (4-(pyridin-2-yl)piperazin-1-yl)-C(O)O-,
 (4-(4-trifluoromethylpyridin-2-yl)piperazin-1-yl)-C(O)O-,
 (4-(pyrimidin-2-yl)piperazin-1-yl)-C(O)O-,
 (4-acetyl piperazin-1-yl)-C(O)O-,
 10 (4-(phenyl-C(O)-)piperazin-1-yl)-C(O)O-,
 (4-(pyridin-4'-yl-C(O)-)piperazin-1-yl)-C(O)O-,
 (4-(phenyl-NHC(O)-)piperazin-1-yl)-C(O)O-,
 (4-(phenyl-NHC(S)-)piperazin-1-yl)-C(O)O-,
 (4-methanesulfonylpiperazin-1-yl)-C(O)O-,
 15 (4-trifluoromethanesulfonylpiperazin-1-yl)-C(O)O-,
 (morpholin-4-yl)-C(O)O-,
 (thiomorpholin-4-yl)-C(O)O-,
 (thiomorpholin-4'-yl sulfone)-C(O)O-,
 (pyrrolidin-1-yl)-C(O)O-,
 20 (2-methylpyrrolidin-1-yl)-C(O)O-,
 (2-(methoxycarbonyl)pyrrolidin-1-yl)-C(O)O-,
 (2-(hydroxymethyl)pyrrolidin-1-yl)-C(O)O-,
 (2-(N,N-dimethylamino)ethyl)(CH₃)NC(O)O-,
 (2-(N-methyl-N-toluene-4-sulfonylamino)ethyl)(CH₃)N-C(O)O-,
 25 (2-(morpholin-4-yl)ethyl)(CH₃)NC(O)O-,
 (2-(hydroxy)ethyl)(CH₃)NC(O)O-,
 bis(2-(hydroxy)ethyl)NC(O)O-,
 (2-(formyloxy)ethyl)(CH₃)NC(O)O-,
 (CH₃OC(O)CH₂)HNC(O)O-, and
 30 2-(phenylNHC(O)O-)ethyl-]HNC(O)O-.

When A is of the formulae IIa, IIb, or IIc, -YC(O)W is -OC(O)NR²R³ and R² and/or R³ are/is bound to the PEG moiety, the PEG moiety is preferably represented by the formula:



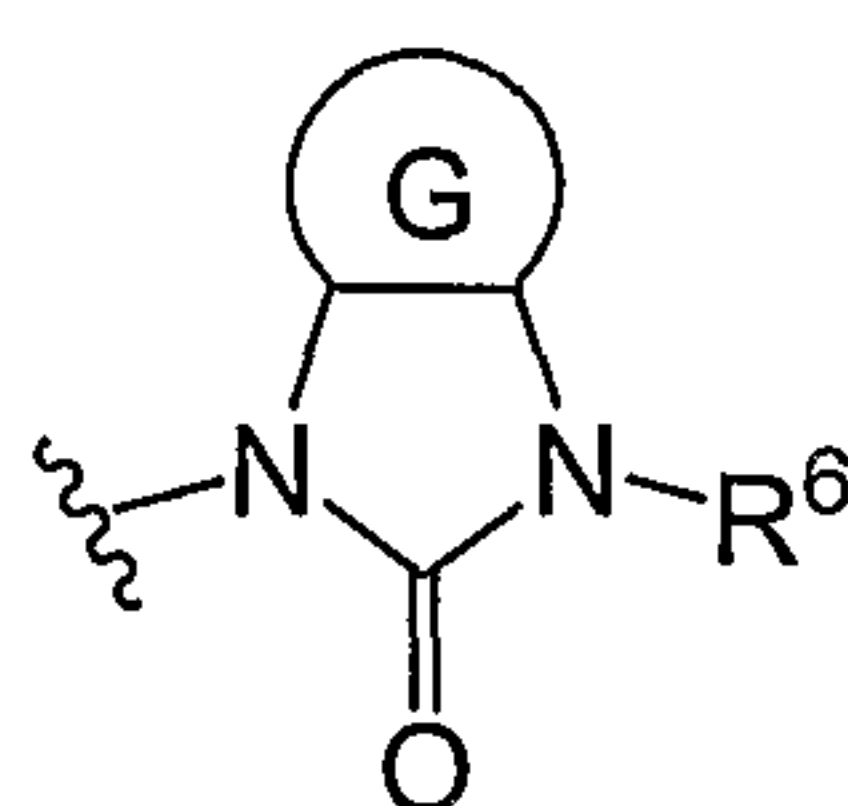
Z' is selected from the group consisting of a covalent bond and a linking group of from 1 to 40 atoms;

R⁷ is selected from the group consisting of hydrogen and methyl;

R⁸ is selected from the group consisting of A, -(L)_w-polymer carrier, hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and -CH₂CHR⁷NR¹⁰R¹¹ where R⁷ is as defined above, R¹⁰ and R¹¹ are independently selected from the group consisting of hydrogen and alkyl, A is represented by formula II above, L is a linking group of from 1 to 40 atoms and w is zero or one: and

p is an integer of from about 100 to 2200.

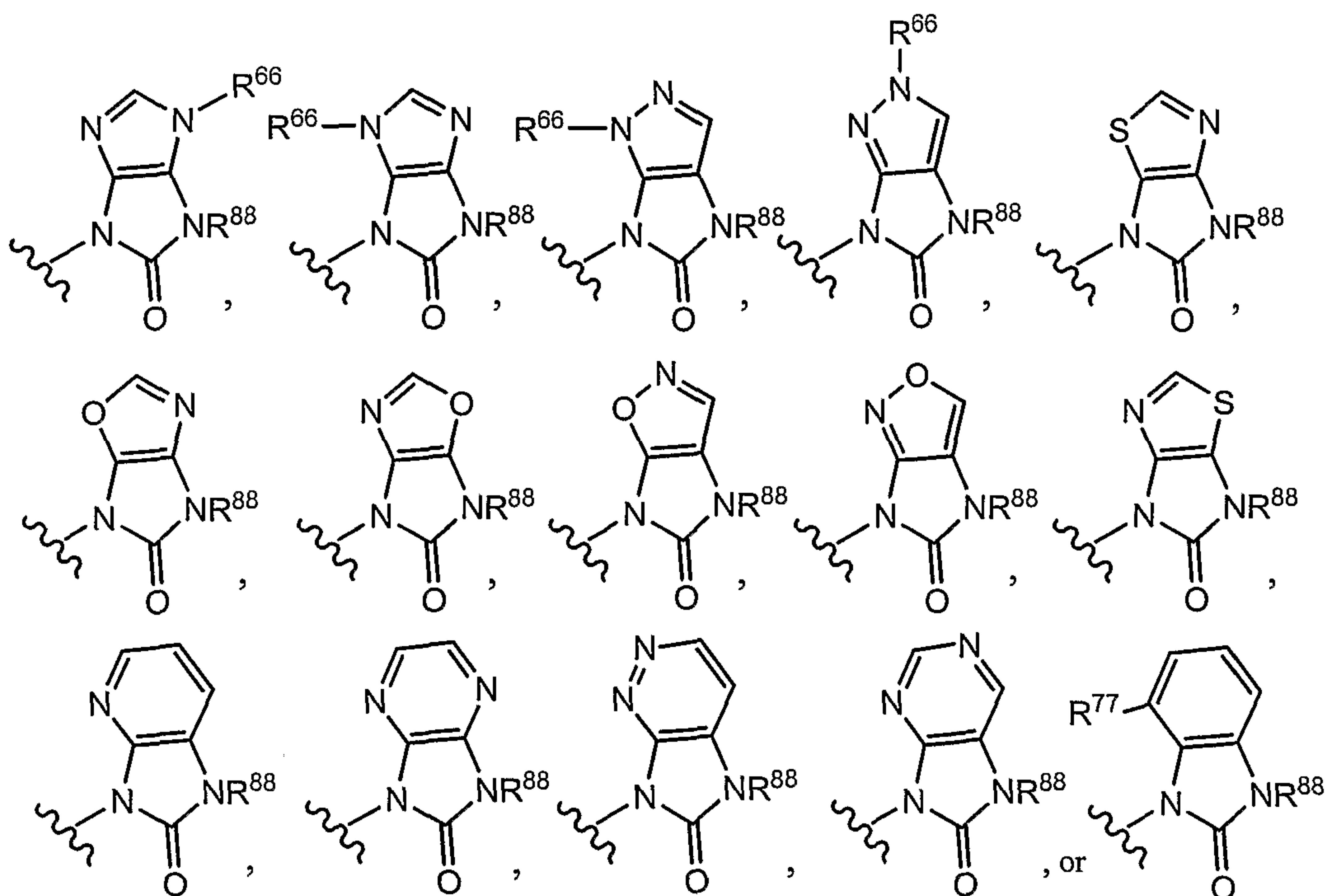
Preferred T groups in Formula II.1 have formula (d) below.



where R⁶ is hydrogen.

Preferred (d) groups include those shown below in Table D.

Table D



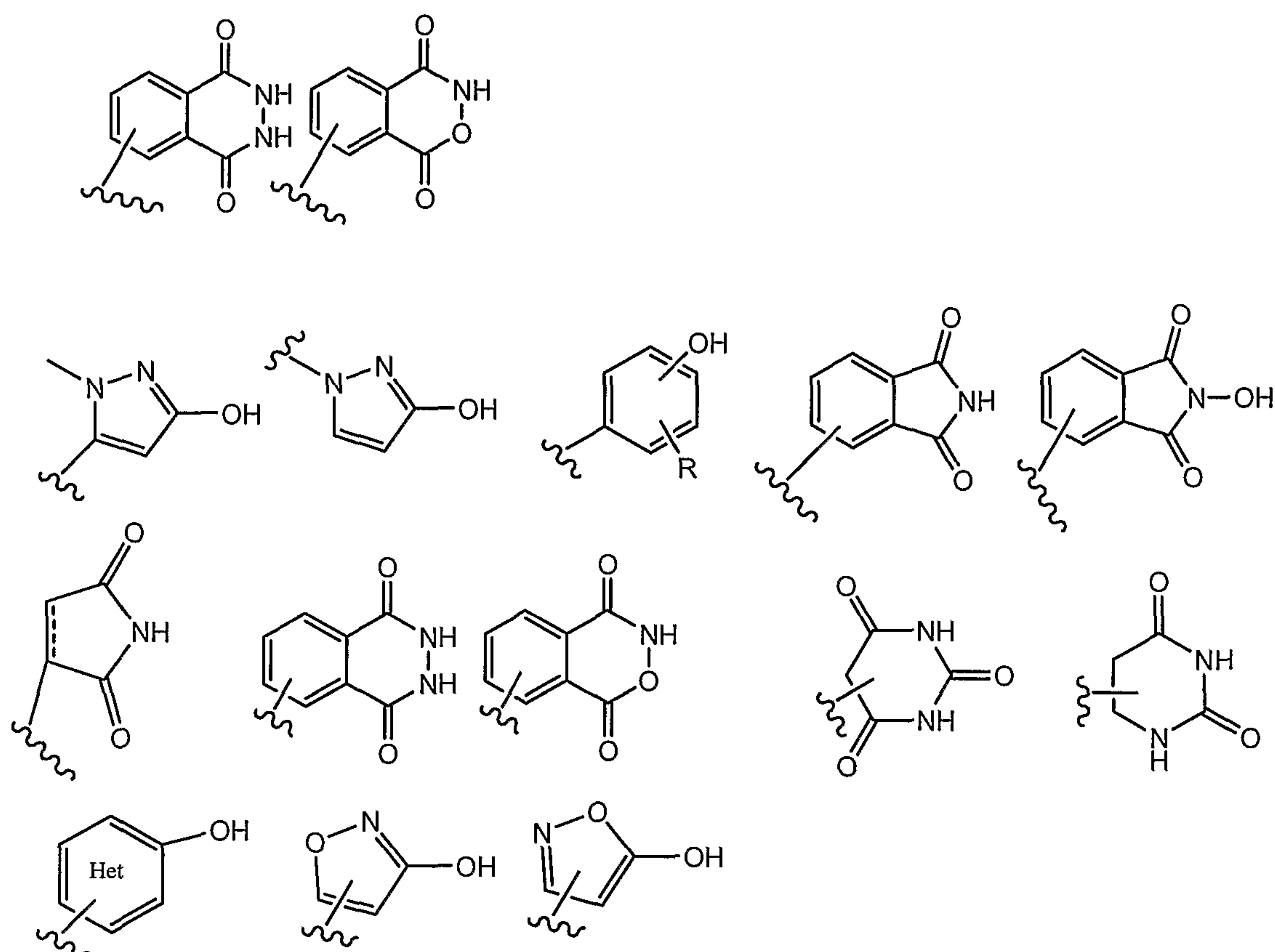
wherein

R⁶⁶ is a covalent bond to a polymer moiety which optionally comprises a linker, or

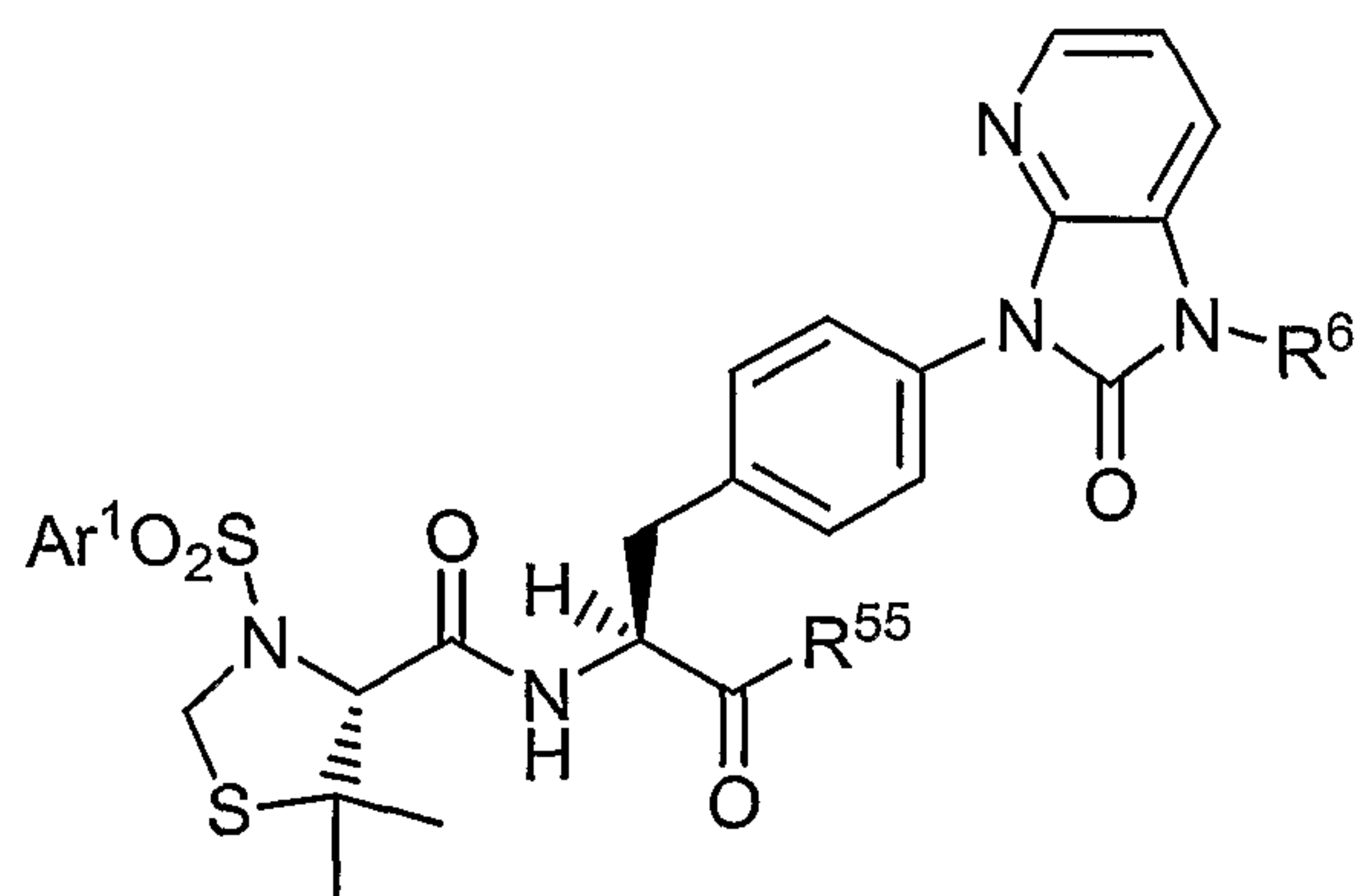
R^{88} is hydrogen.

Other (d) groups useful in the invention include those shown in Table D1.

Table D1



10 Other preferred compounds of formula II.1 for use in the processes of the invention
have formula II.1-a:



II.1-a

and pharmaceutically acceptable salts thereof, wherein

R^{55} is an acid protecting group, preferably C_1 - C_6 alkoxy, more preferably C_2 - C_4 alkoxy;

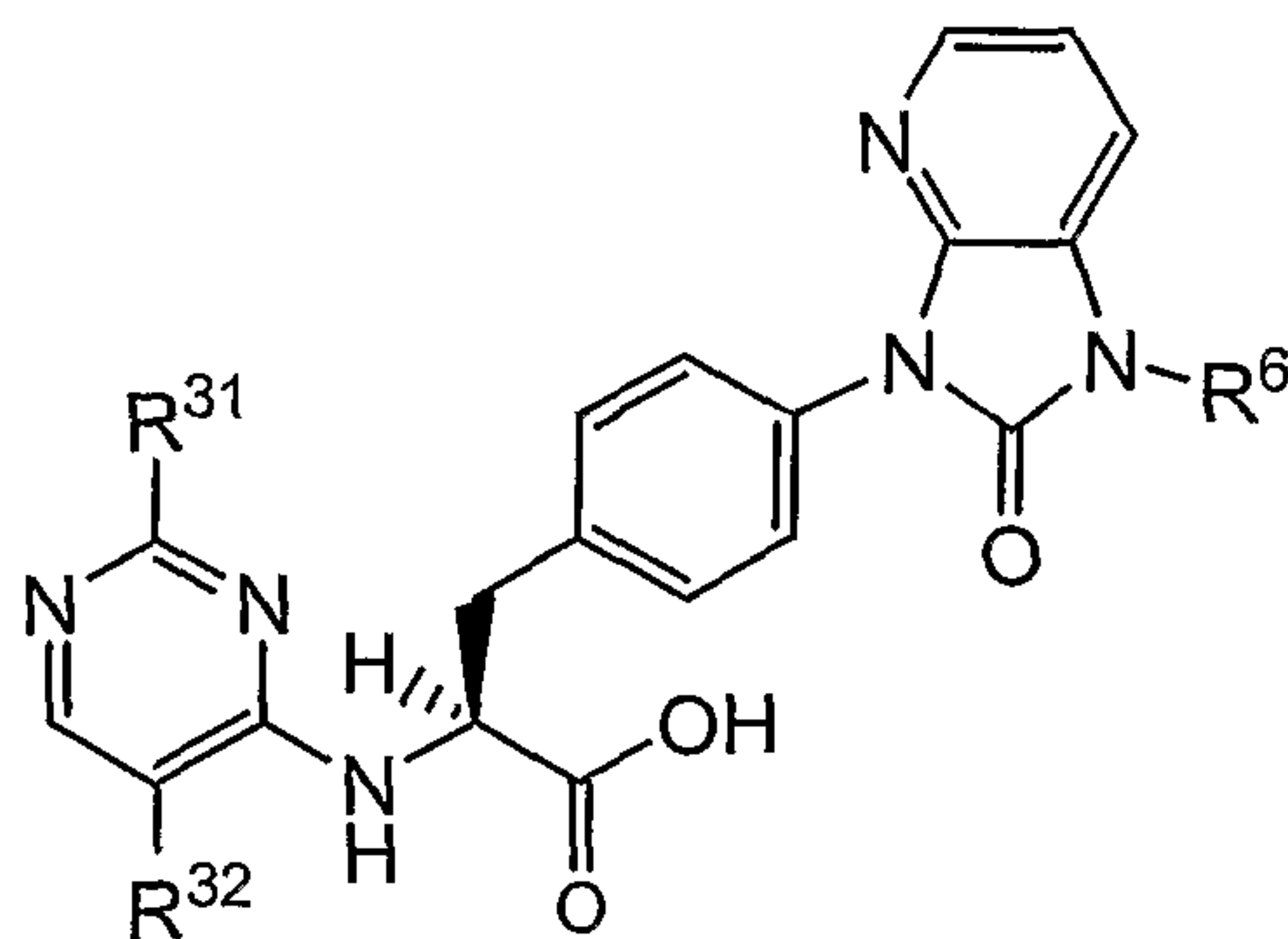
Ar^1 is selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl,

5 cycloalkyl, substituted cycloalkyl, heterocyclic, substituted heterocyclic, heteroaryl and substituted heteroaryl; and

R^6 is hydrogen.

Preferred compounds of Formula II.1-a include those wherein Ar^1 is phenyl or a 5- or 6-membered heteroaryl group having at least one nitrogen atom, each of which is optionally substituted with halogen, hydroxy, C_1 - C_6 alkoxy, C_1 - C_6 alkyl, nitro, trifluoromethyl, amino, 10 mono- or di(C_1 - C_6)alkylamino, amino(C_1 - C_6)alkyl, C_2 - C_6 acyl, C_2 - C_6 acylamino, or amino(C_1 - C_6)acyl. Ar^1 is pyridyl optionally substituted with halogen, hydroxy, C_1 - C_6 alkyl, C_1 - C_6 alkoxy, nitro, trifluoromethyl, amino, mono- or di(C_1 - C_6)alkylamino, amino(C_1 - C_6)alkyl, C_2 - C_6 acyl, C_2 - C_6 acylamino, or amino(C_1 - C_6)acyl. Particularly preferred compounds of Formula II.1-a 15 include those where Ar^1 is pyridyl optionally substituted with C_1 - C_6 alkyl, hydroxy, halogen, C_1 - C_6 alkoxy, nitro, trifluoromethyl, amino, or mono- or di(C_1 - C_6)alkylamino.

Still other preferred compounds of formula II.1 are also those of the formula II.2-a:



II.2-a

20 and pharmaceutically acceptable salts thereof, wherein

R^{55} is an acid protecting group, preferably C_1 - C_6 alkoxy, more preferably C_2 - C_4 alkoxy;

R^6 is hydrogen.

Preferred compounds of Formula II.2-a include those where R^{31} is amino or mono- or di(C_1 - C_6)alkylamino; and R^{32} is $-H$, $-NO_2$ or haloalkyl, more preferably trifluoromethylmethyl.

25 Still other preferred compounds of Formula II.2-a are those where

R^{31} is amino or mono- or di(C_1 - C_6)alkylamino; and

R^{32} is $-N(MR^{41})R^{42}$; where M is $-SO_2-$ or $-CO-$;

R^{41} is C_1 - C_6 alkyl optionally substituted with halogen, hydroxy, C_1 - C_6 alkoxy, amino, or mono- or di(C_1 - C_6)alkylamino; or

30 phenyl or a 5- or 6-membered heteroaryl containing at least one nitrogen, each of

which is optionally substituted with halogen, hydroxy, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₃-C₇ cycloalkyl, amino, nitro, trifluoromethyl, or mono- or di(C₁-C₆)alkylamino; and

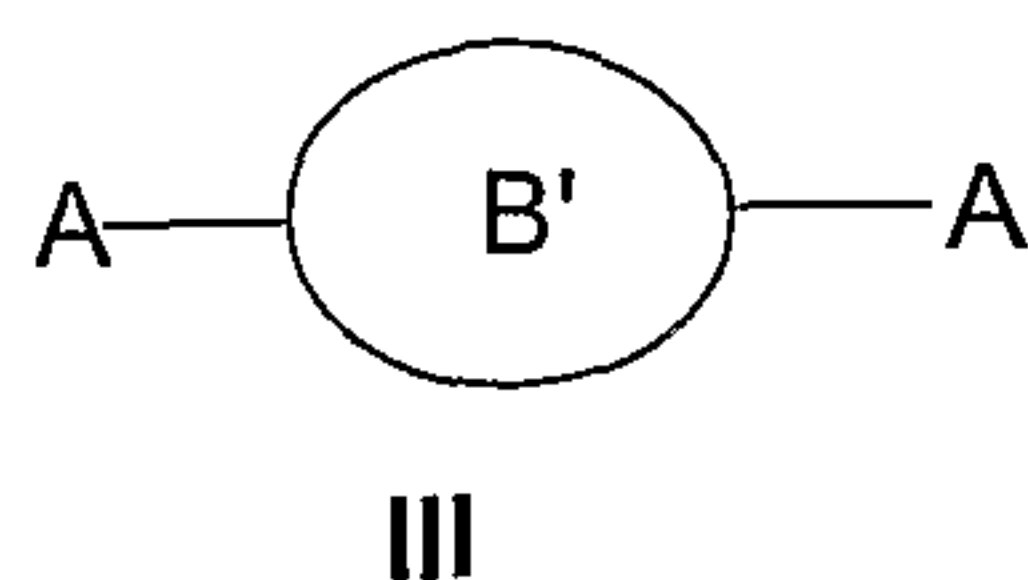
R⁴² is hydrogen, C₁-C₆alkyl, or C₃-C₇cycloalkyl.

5 Further preferred compounds of formula II.2-a include those wherein

R⁴¹ groups within Formula II.2-a are C₁-C₄ alkyl optionally substituted with halogen, hydroxy, C₁-C₆ alkoxy, amino, or mono- or di(C₁-C₆)alkylamino; or pyridyl or pyrimidinyl, each of which is optionally substituted with halogen, hydroxy, C₁-C₃ alkyl, C₁-C₃ alkoxy, amino, or mono- or di(C₁-C₄)alkylamino; and

10 R⁴² is hydrogen, C₁-C₄alkyl, or C₃-C₇cycloalkyl.

In one example, the conjugates of this invention are divalent and are represented by formula III:

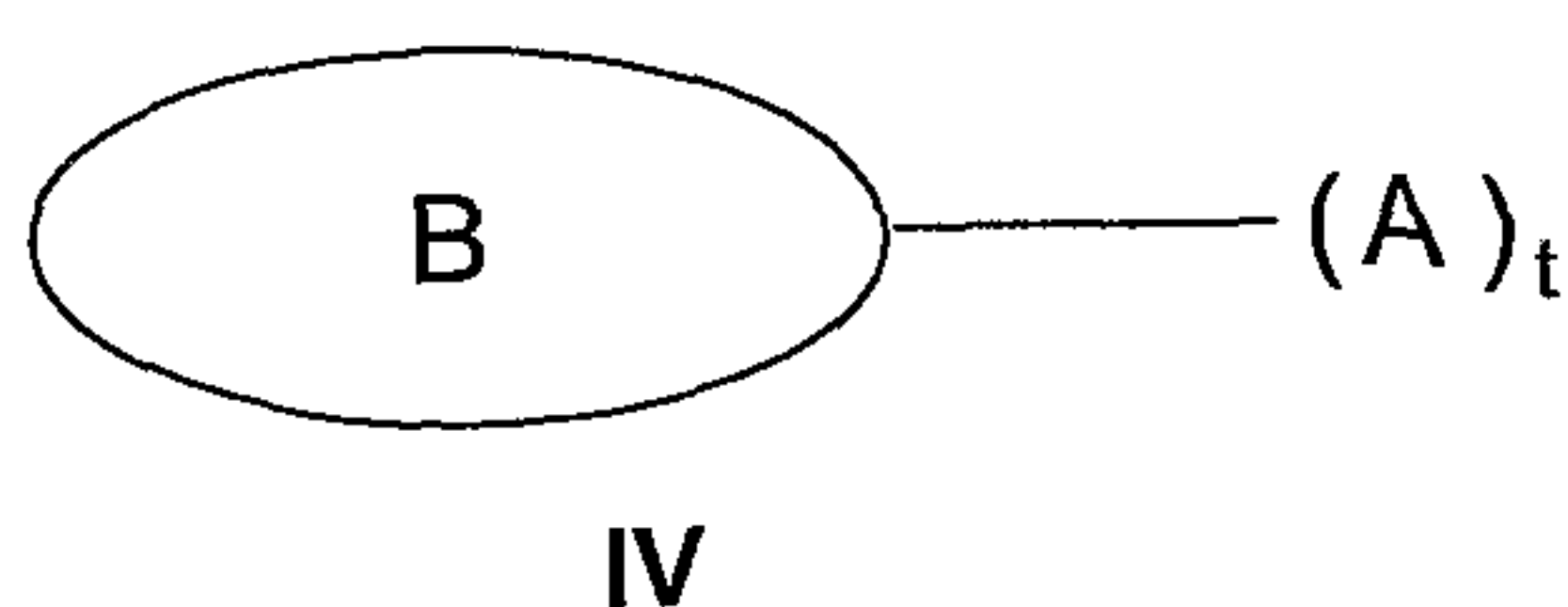


15

where each A is independently as defined above and B' is -Z'-(CH₂CHR⁷O)_p-Z'- where each Z' is independently a covalent bond or a linking group, R⁷ is hydrogen or methyl and p is an integer of from about 100 to 1360.

20

In another example, the conjugates of this invention are trivalent to decavalent and are preferably represented by formula IV:



25

where each A is independently as defined above and t is an integer from 3 to 10.

30

The process of the instant invention employs the Mitsunobu reaction, a condensation reaction of alcohols in the presence of a triaryl- or trialkylphosphine and an appropriate azodicarboxylate. In preferred reactions, polymer alcohols, e.g, polyethylene glycol, are reacted with nucleophiles in the presence of a triaryl- or trialkylphosphine and the diazo reagent to afford the conjugates. Nucleophiles are compounds having an acidic hydrogen, i.e., a moiety suitable to donate electrons. The bond formed by reacting a polymeric alcohol with a nucleophile can be diverse, such as, for example, carbon-oxygen bond formation,

carbon-nitrogen bond formation, carbon-sulfur bond formation, carbon-halogen bond formation, and carbon-carbon bond formation.

Thus, non-derivatized active compounds can have a wide variety of functional groups that can react with the polymer alcohols. Examples include carboxylic acids, alcohols, β -ketoesters, amines, thiols, alkyl halides, acyl halides, β -diketones and the like. Other examples of nucleophiles that can be utilized in the process of the present invention can be found in *Organic Reactions*, **1992**, 42, 335-656, which is incorporated herein by reference in its entirety.

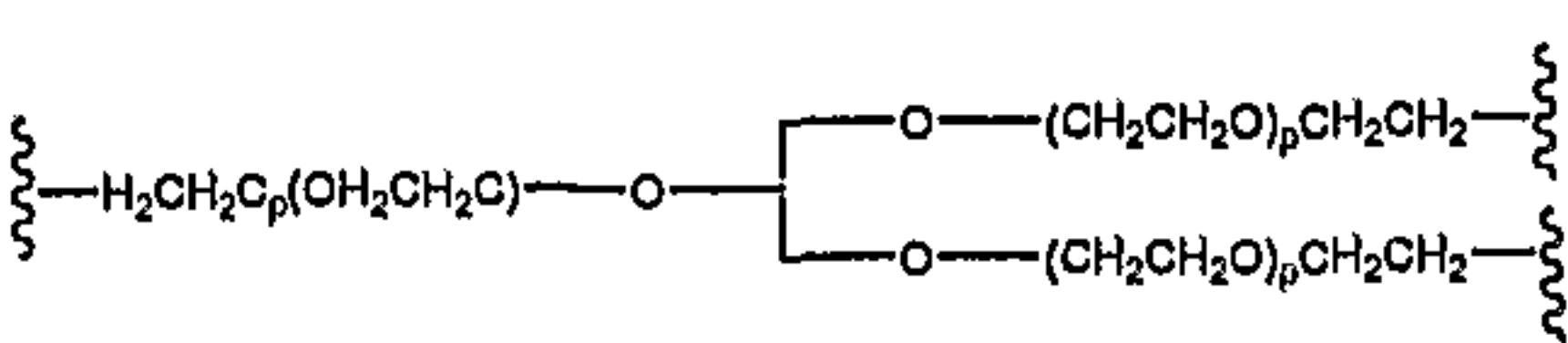
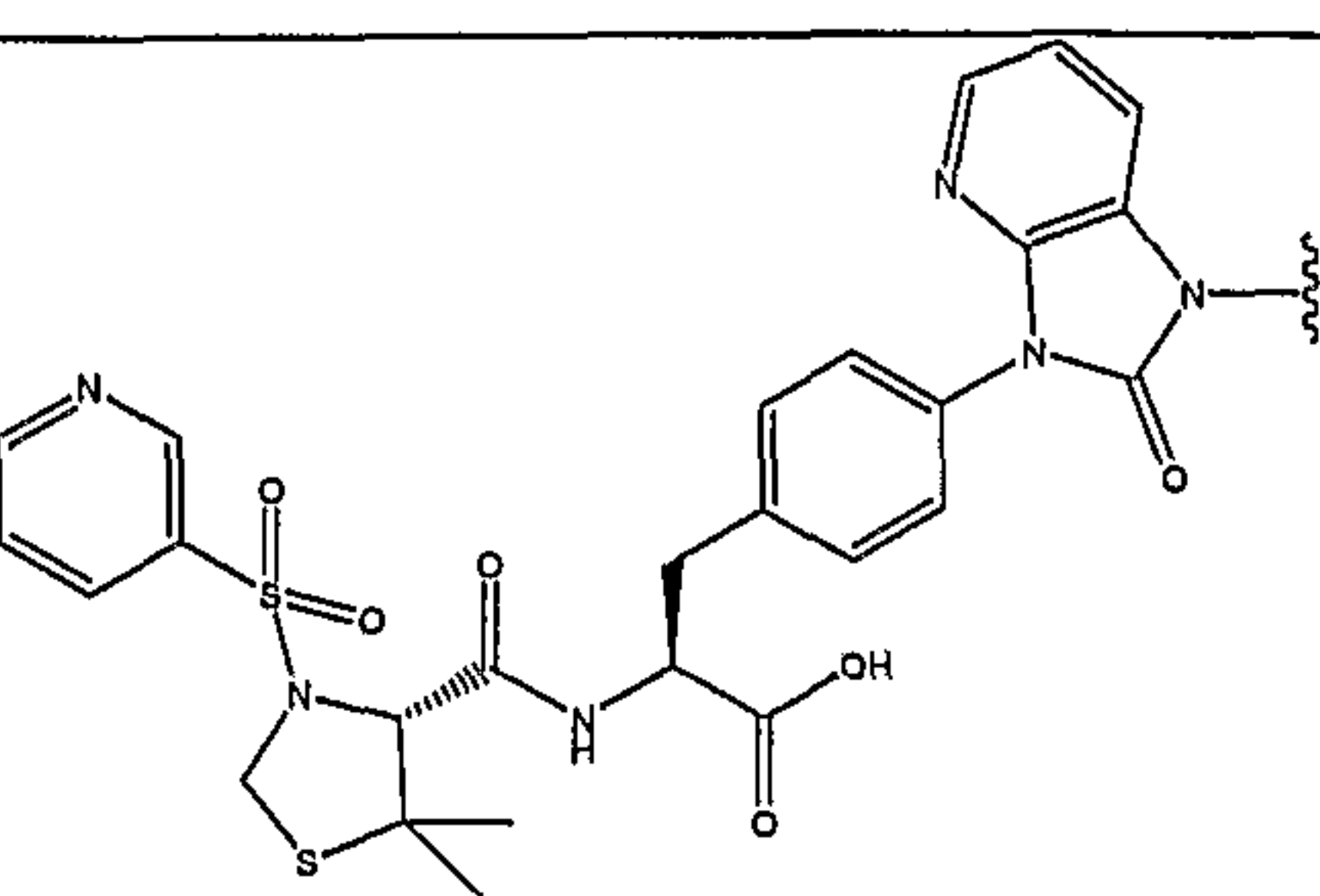
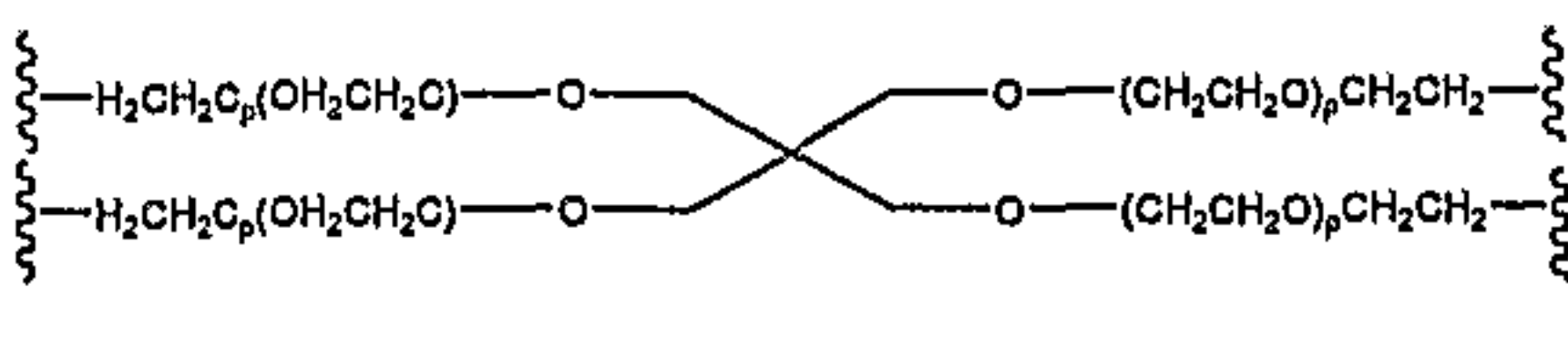
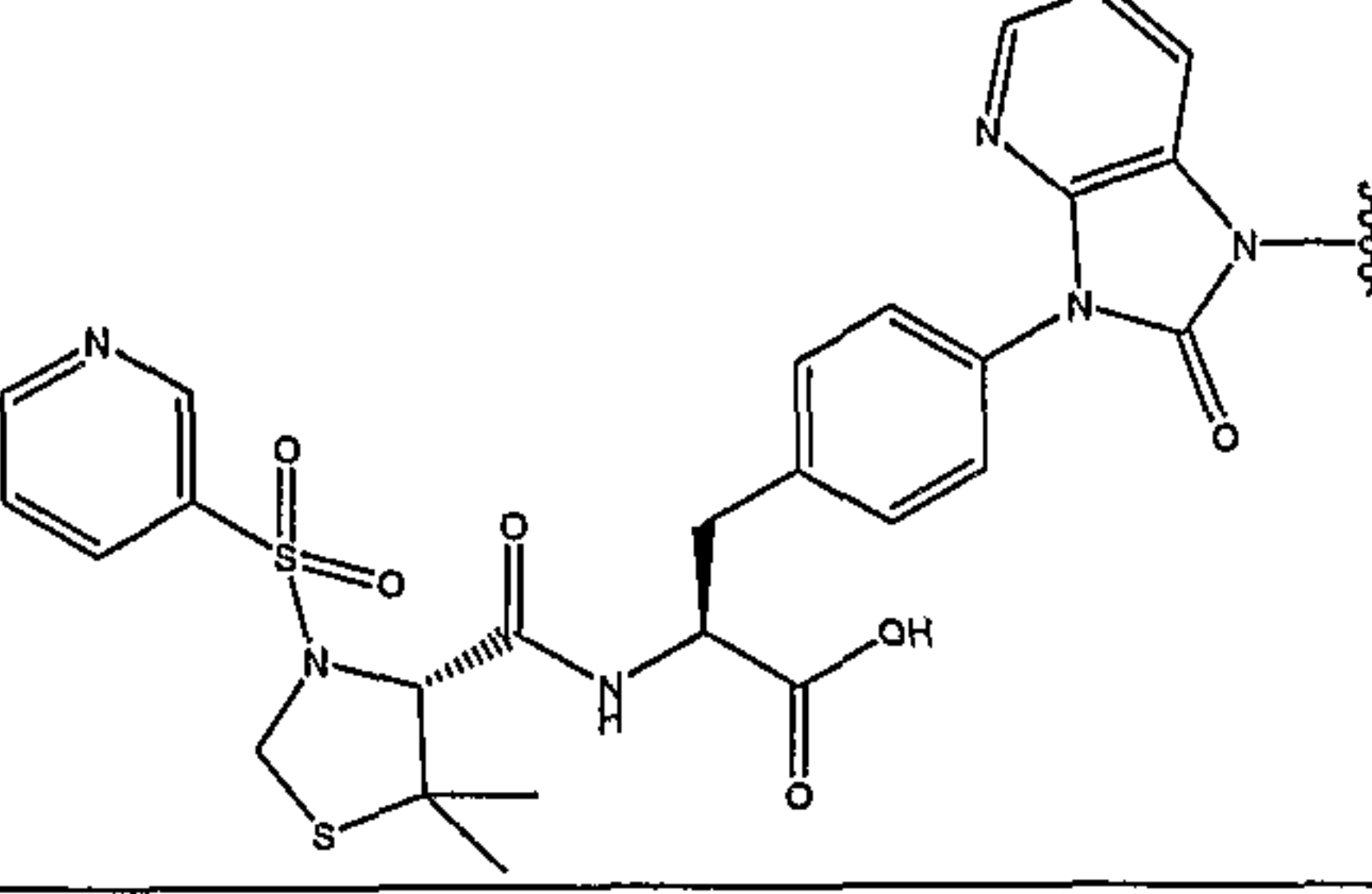
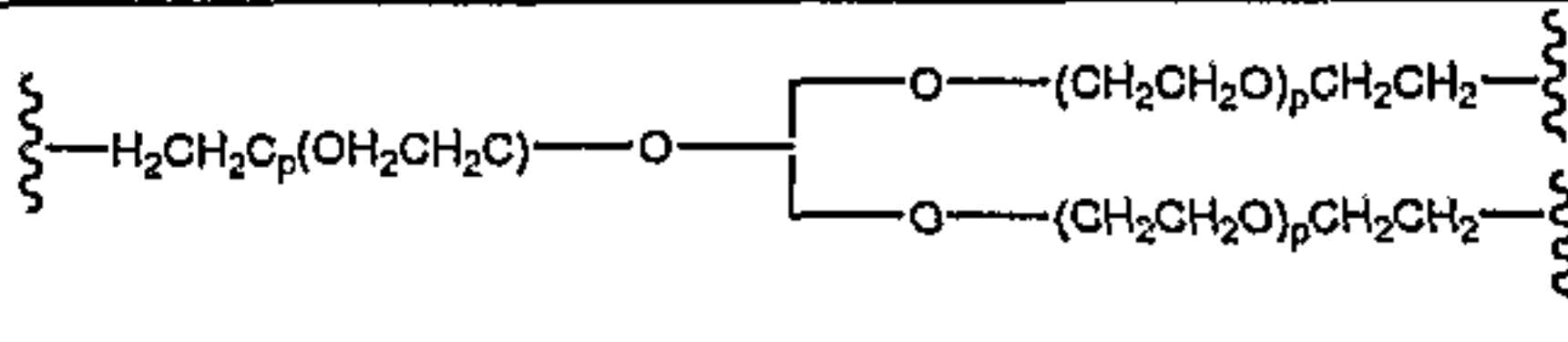
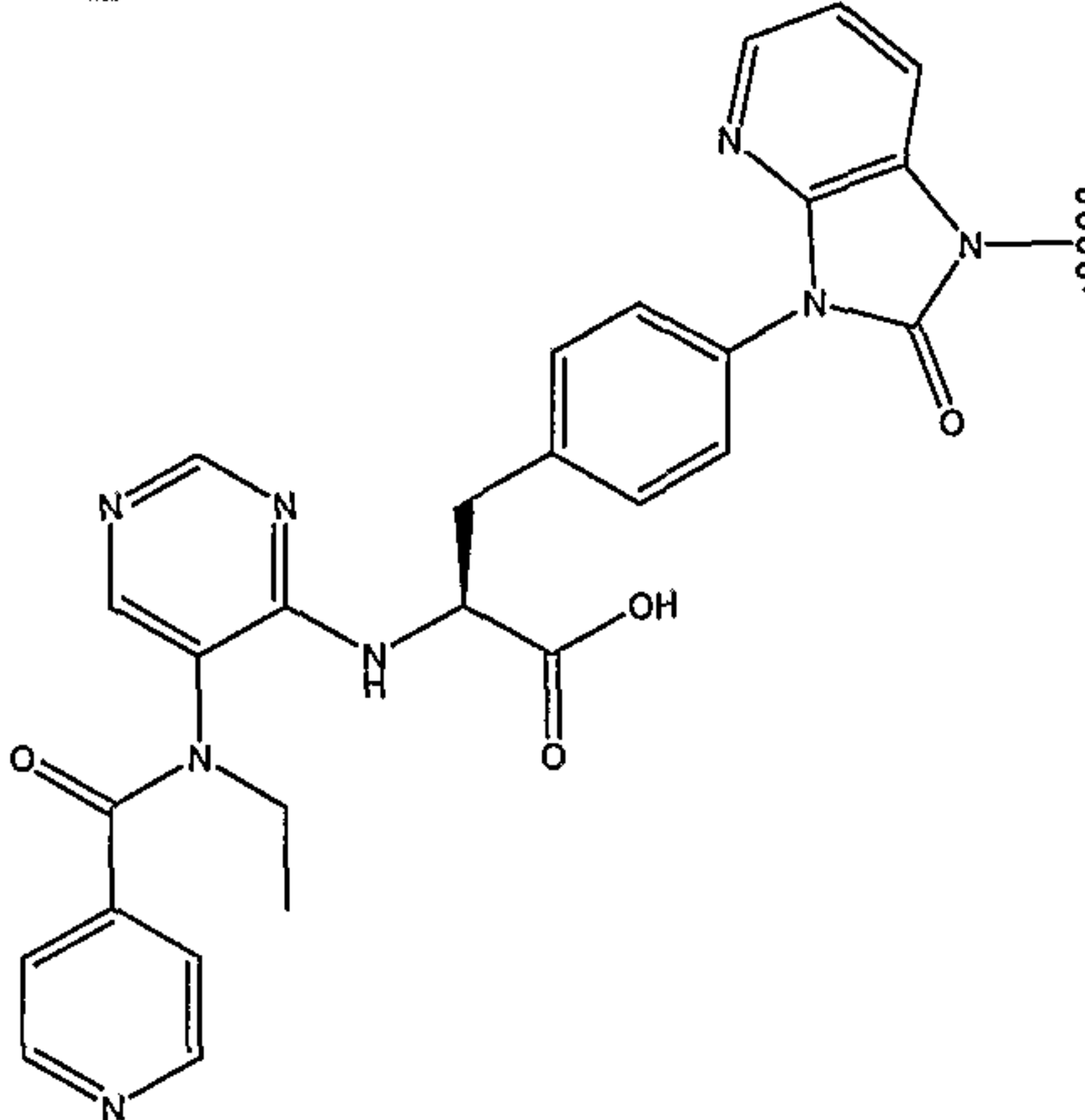
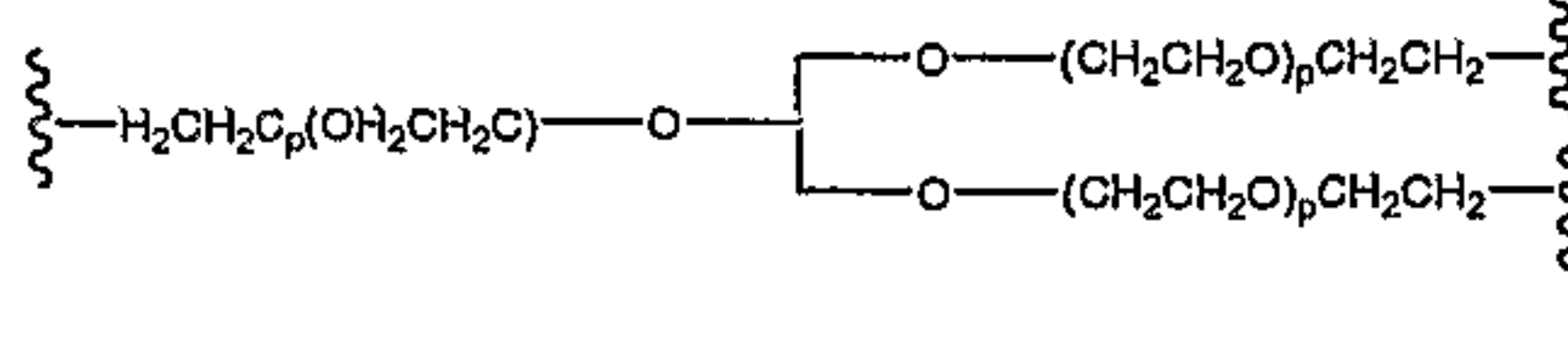
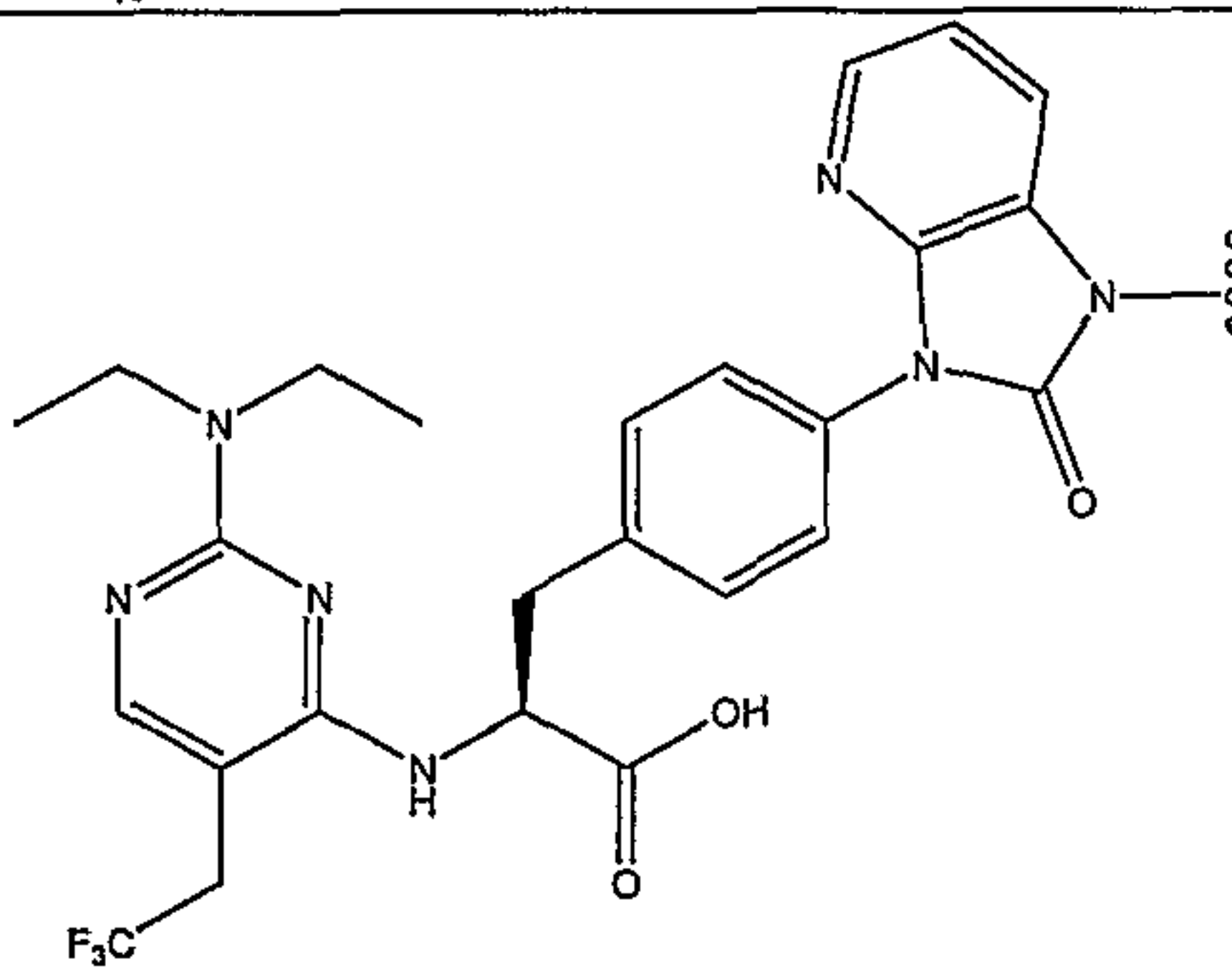
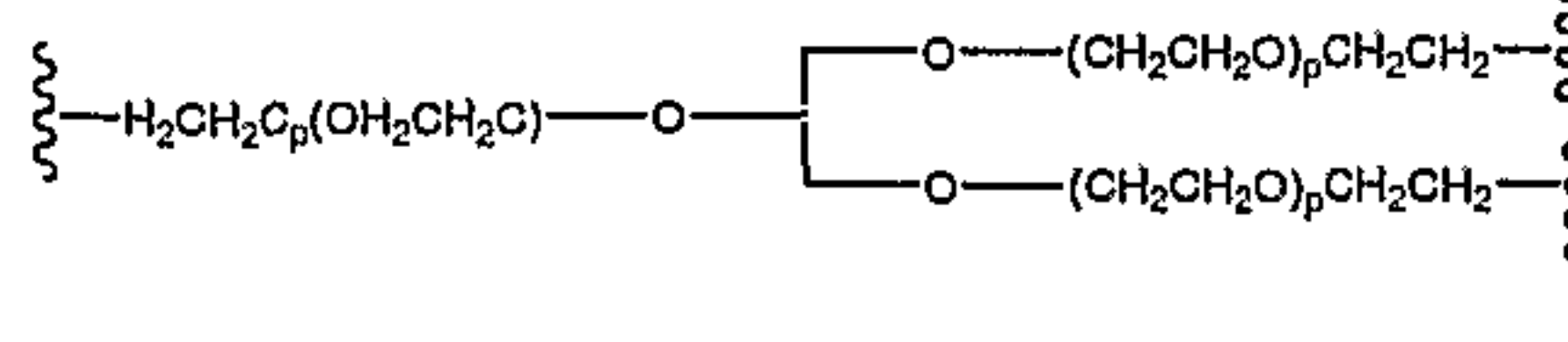
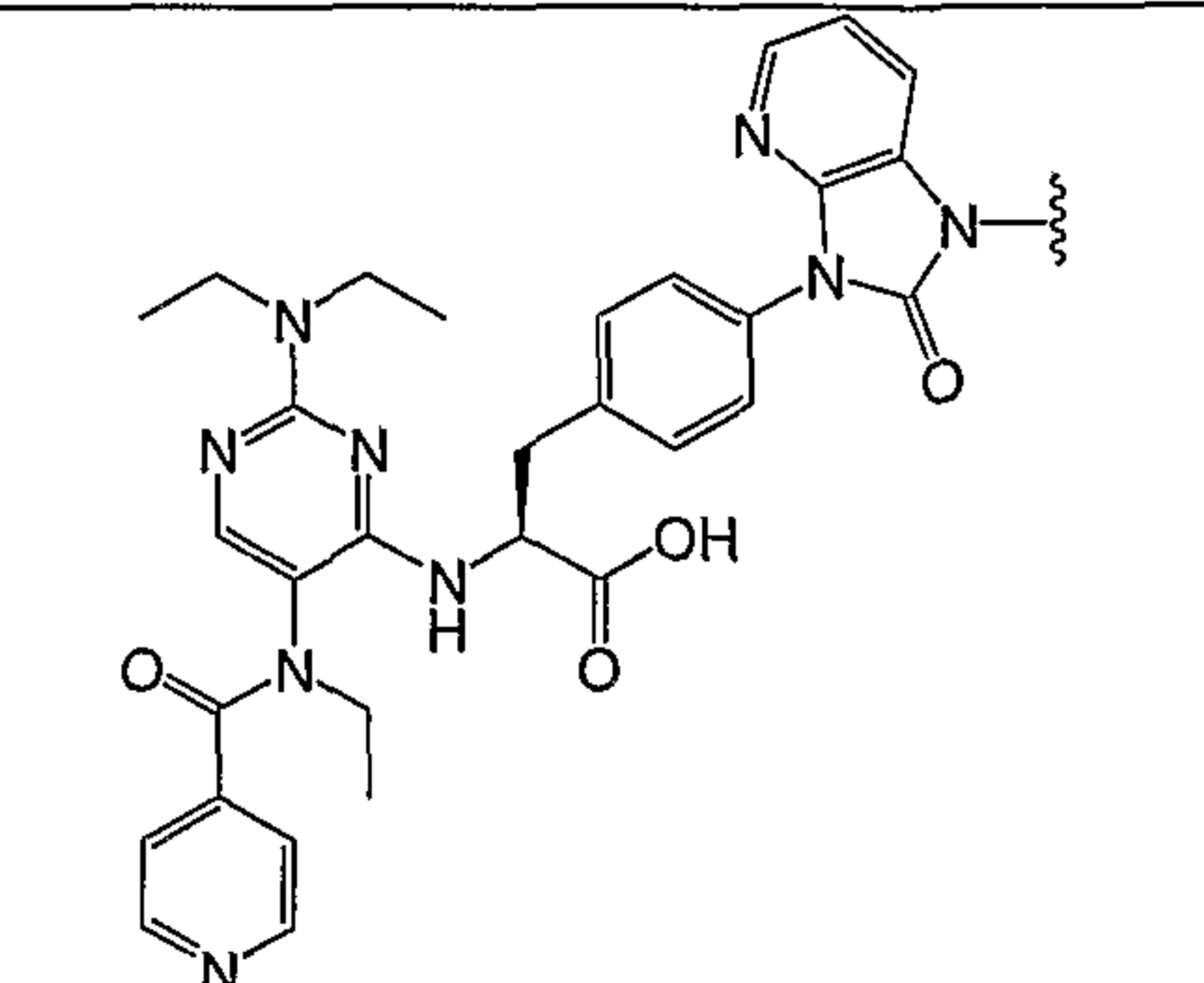
Examples of triaryl- or trialkylphosphines are described in *Synthesis*, **2003**, 3, 317-334; *Tetrahedron Lett.*, **1998**, 39, 7787; *Chem. Commun.* **1997**, 759; and *Nucleosides Nucleotides*, **1999**, 18, 727, all incorporated herein by reference and include triphenylphosphine, trimethylphosphine, triethylphosphine, tributylphosphine, and 1,2-bis-(diphenylphosphino)ethane. The phosphines can also be polymer supported or water soluble. A preferred triarylphosphine is triphenylphosphine.

The diazo reagents are generally esters or amides of azodicarboxylic acids, and include those found in *Synthesis*, **2003**, 3, 317-334; *Tetrahedron Lett.*, **1999**, 40, 7359; and *Bull. Chem. Soc. Jpn.*, **1984**, 57, 2675. Specific examples of such diazo compounds are diethyldiazocarboxylate, diisopropylazodicarboxylate, 4-methyl-1,2,4-triazolidine-3,5-dione, N,N,N',N'-tetramethylazodicarboxamide, azodicarboxylic acid dipiperidide, bis(N-4-methylpiperazin-1-yl)azodicarboxamide, dimorpholinoazodicarboxamide and di-tert-butyl azodicarboxylate.

Representative conjugates prepared by the process of the instant invention, including pharmaceutically acceptable salts thereof, are set forth in the following table:

TABLE I



Comp.	B	A
A		
B		
C		
D		
E		

--	--	--

where in each of the structures the sum of all p's is from 100 to 2200, preferably from about 200-1360.

5 Definitions

As used herein, "alkyl" refers to monovalent saturated aliphatic hydrocarbyl groups having from 1 to 5 carbon atoms and more preferably 1 to 3 carbon atoms. This term is exemplified by groups such as methyl, ethyl, n-propyl, iso-propyl, n-butyl, t-butyl, n-pentyl and the like.

10 "Substituted alkyl" refers to an alkyl group having from 1 to 3, and preferably 1 to 2, substituents selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aryl, substituted aryl, aryloxy, substituted aryloxy, cyano, halogen, hydroxyl, nitro, carboxyl, carboxyl esters, cycloalkyl, substituted cycloalkyl, spirocycloalkyl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted
15 heterocyclic.

"Alkylene" refers to divalent saturated aliphatic hydrocarbyl groups preferably having from 1 to 5 and more preferably 1 to 3 carbon atoms which are either straight-chained or branched. This term is exemplified by groups such as methylene (-CH₂-), ethylene (-CH₂CH₂-), n-propylene (-CH₂CH₂CH₂-), iso-propylene (-CH₂CH(CH₃)-) and the like.

20 "Alkoxy" refers to the group "alkyl-O-" which includes, by way of example, methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, t-butoxy, sec-butoxy, n-pentoxy and the like.

"Substituted alkoxy" refers to the group "substituted alkyl-O-".

"Acyl" refers to the groups H-C(O)-, alkyl-C(O)-, substituted alkyl-C(O)-, alkenyl-C(O)-, substituted alkenyl-C(O)-, alkynyl-C(O)-, substituted alkynyl-C(O)-, cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, aryl-C(O)-, substituted aryl-C(O)-, heteroaryl-C(O)-, substituted heteroaryl-C(O)-, heterocyclic-C(O)-, and substituted heterocyclic-C(O)-, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic are as defined herein.

30 "Aminoacyl" refers to the group -C(O)NR¹⁰R¹⁰ where each R¹⁰ is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic and where each R¹⁰ is joined to form together with the nitrogen atom a heterocyclic or substituted heterocyclic ring
35 wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl,

cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic are as defined herein.

"Acyloxy" refers to the groups alkyl-C(O)O-, substituted alkyl-C(O)O-, alkenyl-C(O)O-, substituted alkenyl-C(O)O-, alkynyl-C(O)O-, substituted alkynyl-C(O)O-, aryl-C(O)O-, substituted aryl-C(O)O-, cycloalkyl-C(O)O-, substituted cycloalkyl-C(O)O-, heteroaryl-C(O)O-, substituted heteroaryl-C(O)O-, heterocyclic-C(O)O-, and substituted heterocyclic-C(O)O- wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic are as defined herein.

"Alkenyl" refers to alkenyl groups having from 2 to 6 carbon atoms and preferably 2 to 4 carbon atoms and having at least 1 and preferably from 1 to 2 sites of alkenyl unsaturation. Such groups are exemplified by vinyl, allyl, but-3-en-1-yl, and the like.

"Substituted alkenyl" refers to alkenyl groups having from 1 to 3 substituents, and preferably 1 to 2 substituents, selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aryl, substituted aryl, aryloxy, substituted aryloxy, cyano, halogen, hydroxyl, nitro, carboxyl, carboxyl esters, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic with the proviso that any hydroxyl substitution is not attached to a vinyl (unsaturated) carbon atom.

"Alkynyl" refers to alkynyl groups having from 2 to 6 carbon atoms and preferably 2 to 3 carbon atoms and having at least 1 and preferably from 1 to 2 sites of alkynyl unsaturation.

"Substituted alkynyl" refers to alkynyl groups having from 1 to 3 substituents, and preferably 1 to 2 substituents, selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aryl, substituted aryl, aryloxy, substituted aryloxy, cyano, halogen, hydroxyl, nitro, carboxyl, carboxyl esters, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic.

"Amino" refers to the group -NH₂.

"Cyano" refers to the group -CN.

"Substituted amino" refers to the group -NR'R'' where R' and R'' are independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic and where R' and R'' are joined, together with the nitrogen bound thereto to form a heterocyclic or substituted heterocyclic group provided that R' and R'' are both not hydrogen. When R' is hydrogen and R'' is alkyl, the substituted amino group is sometimes referred to herein as alkylamino. When

R' and R'' are alkyl, the substituted amino group is sometimes referred to herein as dialkylamino. When referring to a monosubstituted amino, it is meant that either R' or R'' is hydrogen but not both. When referring to a disubstituted amino, it is meant that neither R' or R'' is hydrogen.

5 "Aminoacyl" refers to the groups $-NR^{11}C(O)alkyl$, $-NR^{11}C(O)substituted\ alkyl$, $-NR^{11}C(O)cycloalkyl$, $-NR^{11}C(O)substituted\ cycloalkyl$, $-NR^{11}C(O)alkenyl$, $-NR^{11}C(O)substituted\ alkenyl$, $-NR^{11}C(O)alkynyl$, $-NR^{11}C(O)substituted\ alkynyl$, $-NR^{11}C(O)aryl$, $-NR^{11}C(O)substituted\ aryl$, $-NR^{11}C(O)heteroaryl$, $-NR^{11}C(O)substituted\ heteroaryl$, $-NR^{11}C(O)heterocyclic$, and $-NR^{11}C(O)substituted\ heterocyclic$ where R^{11} is hydrogen or alkyl
10 and wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic are as defined herein.

"Nitro" refers to the group $-NO_2$.

"Aryl" or "Ar" refers to a monovalent aromatic carbocyclic group of from 6 to 14 carbon
15 atoms having a single ring (e.g., phenyl) or multiple condensed rings (e.g., naphthyl or anthryl) which condensed rings may or may not be aromatic (e.g., 2-benzoxazolinone, 2H-1,4-benzoxazin-3(4H)-one-7-yl, and the like) provided that the point of attachment is at an aromatic carbon atom. Preferred aryls include phenyl and naphthyl.

"Substituted aryl" refers to aryl groups which are substituted with from 1 to 3
20 substituents, and preferably 1 to 2 substituents, selected from the group consisting of hydroxy, acyl, acylamino, acyloxy, alkyl, substituted alkyl, alkoxy, substituted alkoxy, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, amino, substituted amino, aminoacyl, aryl, substituted aryl, aryloxy, substituted aryloxy, carboxyl, carboxyl esters, cyano, thiol, thioalkyl, substituted thioalkyl, thioaryl, substituted thioaryl, thioheteroaryl, substituted thioheteroaryl,
25 thiocycloalkyl, substituted thiocycloalkyl, thioheterocyclic, substituted thioheterocyclic, cycloalkyl, substituted cycloalkyl, halo, nitro, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, amino sulfonyl (NH_2-SO_2-), and substituted amino sulfonyl.

30 "Aryloxy" refers to the group aryl-O- that includes, by way of example, phenoxy, naphthoxy, and the like.

"Substituted aryloxy" refers to substituted aryl-O- groups.

"Carboxyl" refers to $-COOH$ or salts thereof.

"Carboxyl ester" refers to the groups $-C(O)O-alkyl$, $-C(O)O-substituted\ alkyl$,
35 $-C(O)-aryl$, and $-C(O)O-substituted\ aryl$ wherein alkyl, substituted alkyl, aryl and substituted aryl are as defined herein.

"Cycloalkyl" refers to cyclic alkyl groups of from 3 to 10 carbon atoms having single or multiple cyclic rings including, by way of example, adamantyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl and the like.

5 "Cycloalkenyl" refers to cyclic alkenyl groups of from 4 to 10 carbon atoms having single or multiple cyclic rings and further having at least 1 and preferably from 1 to 2 internal sites of ethylenic or vinyl ($>C=C<$) unsaturation.

"Substituted cycloalkyl" and "substituted cycloalkenyl" refers to an cycloalkyl or cycloalkenyl group, having from 1 to 5 substituents selected from the group consisting of oxo ($=O$), thioxo ($=S$), alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, substituted
10 amino, aminoacyl, aryl, substituted aryl, aryloxy, substituted aryloxy, cyano, halogen, hydroxyl, nitro, carboxyl, carboxyl esters, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic.

"Cycloalkoxy" refers to -O-cycloalkyl groups.

"Substituted cycloalkoxy" refers to -O-substituted cycloalkyl groups.

15 "Halo" or "halogen" refers to fluoro, chloro, bromo and iodo and preferably is fluoro or chloro.

"Hydroxy" refers to the group -OH.

"Heteroaryl" refers to an aromatic group of from 1 to 10 carbon atoms and 1 to 4 heteroatoms selected from the group consisting of oxygen, nitrogen and sulfur within the ring.
20 Such heteroaryl groups can have a single ring (e.g., pyridinyl or furyl) or multiple condensed rings (e.g., indolizinyl or benzothienyl) wherein the condensed rings may or may not be aromatic and/or contain a heteroatom provided that the point of attachment is through an atom of the aromatic heteroaryl group. Preferred heteroaryls include pyridinyl, pyrrolyl, indolyl, thiophenyl, and furanyl.

25 "Substituted heteroaryl" refers to heteroaryl groups that are substituted with from 1 to 3 substituents selected from the same group of substituents defined for substituted aryl.

"Heteroaryloxy" refers to the group -O-heteroaryl and "substituted heteroaryloxy" refers to the group -O-substituted heteroaryl.

30 "Heterocycle" or "heterocyclic" or "heterocycloalkyl" or "heterocyclyl" refers to a saturated or unsaturated group having a single ring or multiple condensed rings, from 1 to 10 carbon atoms and from 1 to 4 hetero atoms selected from the group consisting of nitrogen, sulfur or oxygen within the ring wherein, in fused ring systems, one or more the rings can be cycloalkyl, aryl or heteroaryl provided that the point of attachment is through the heterocyclic
35 ring.

"Substituted heterocyclic" or "substituted heterocycloalkyl" or "substituted heterocyclyl" refers to heterocyclyl groups that are substituted with from 1 to 3 of the same substituents as defined for substituted cycloalkyl.

Examples of heterocyclyls and heteroaryls include, but are not limited to, azetidine, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, dihydroindole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, phthalimide, 1,2,3,4-tetrahydro-isoquinoline, 4,5,6,7-tetrahydrobenzo[b]thiophene, thiazole, thiazolidine, thiophene, benzo[b]thiophene, morpholinyl, thiomorpholinyl (also referred to as thiamorpholinyl), piperidinyl, pyrrolidine, tetrahydrofuranyl, and the like.

"Thiol" refers to the group -SH.

"Thioalkyl" or "alkylthioether" or "thioalkoxy" refers to the group -S-alkyl.

"Substituted thioalkyl" or "substituted alkylthioether" or "substituted thioalkoxy" refers to the group -S-substituted alkyl.

"Thioaryl" refers to the group -S-aryl, where aryl is defined above.

"Substituted thioaryl" refers to the group -S-substituted aryl, where substituted aryl is defined above.

"Thioheteroaryl" refers to the group -S-heteroaryl, where heteroaryl is as defined above.

"Substituted thioheteroaryl" refers to the group -S-substituted heteroaryl, where substituted thioheteroaryl is defined above.

"Thioheterocyclic" refers to the group -S-heterocyclic and "substituted thioheterocyclic" refers to the group -S-substituted heterocyclic, where heterocyclic and substituted heterocyclic.

"Heterocyclyloxy" refers to the group heterocyclyl-O- and "substituted heterocyclyl-O-" refers to the group substituted heterocyclyl-O- where heterocyclyl and substituted heterocyclyl are as defined above.

"Thiocycloalkyl" refers to the group -S-cycloalkyl and "substituted thiocycloalkyl" refers to the group -S-substituted cycloalkyl, where cycloalkyl and substituted cycloalkyl are as defined above.

The terms "compound" and "active compound" are used to refer to the VLA-4 antagonist portion of a conjugate of the invention or to a VLA-4 antagonist as it exists prior to conjugation to a polymer.

The terms "Linker", "linking group" or "linker of from 1 to 40 atoms" refer to a group or groups that (1) covalently links the polymer to the active compound and/or (2) covalently link the polyalkylene oxide moieties of a polymer one to another. Within any particular conjugate, the linker connecting the polyalkylene oxide moieties of a polymer together, and the linker bonding a polymer to an active compound may be the same or different (i.e., may have the same or different chemical structures). Representative functional group linkages, of which a linking group may have one or more, are amides, ethers, carbamates, thiocarbamates, ureas, thioureas, amino groups, carbonyl groups, alkoxy groups, etc. The linker may be homogenous or heterogeneous in its atom content (e.g., linkers containing only carbon atoms or linkers containing carbon atoms as well as one or more heteroatoms present on the linker. Preferably, the linker contains 1 to 25 carbon atoms and 0 to 15 heteroatoms selected from oxygen, NR^{22} , sulfur, $-\text{S}(\text{O})-$ and $-\text{S}(\text{O})_2-$, where R^{22} is as defined above. The linker may also be chiral or achiral, linear, branched or cyclic.

Intervening between the functional group linkages or bonds within the linker, the linker may further contain spacer groups including, but not limited to, spacers selected from alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, and combinations thereof. The spacer may be homogenous or heterogeneous in its atom content (e.g., spacers containing only carbon atoms or spacers containing carbon atoms as well as one or more heteroatoms present on the spacer. Preferably, the spacer contains 1 to 25 carbon atoms and 0 to 15 heteroatoms selected from oxygen, NR^{22} , sulfur, $-\text{S}(\text{O})-$ and $-\text{S}(\text{O})_2-$, where R^{22} is as defined above. The spacer may also be chiral or achiral, linear, branched or cyclic.

Non-limiting examples of spacers are straight or branched alkylene chains, phenylene, biphenylene, etc. rings, all of which are capable of carrying one or more than one functional group capable of forming a linkage with the active compound and one or more polyalkylene oxide moieties. One particular example of a polyfunctional linker-spacer group is lysine, which may link any of the active compounds to two polymer moieties via the two amino groups substituted on a C_4 alkylene chain. Other non-limiting examples include p-aminobenzoic acid and 3,5-diaminobenzoic acid which have 2 and 3 functional groups respectively available for linkage formation. Other such polyfunctional linkage plus spacer groups can be readily envisaged by one of skill in the art.

The term "polymer" refers to biocompatible, water-soluble, substantially non-immunogenic, polymers which are capable of being coupled to more than one VLA-4 antagonists of formula II. Preferably the polymer is non-ionic and biocompatible as measured by lack of toxicity at the doses used. Inclusive of such polymers are multiple copies of polymers coupled to a carrier.

Examples of suitable polymers include, but are not limited to: polyoxyalkylene polymers such as polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), polyacrylamide (PAAm), polydimethylacrylamide (PDAAm), polyvinyl alcohol (PVA), dextran, poly (L-glutamic acid) (PGA), styrene maleic anhydride (SMA), poly-N-(2-hydroxypropyl) methacrylamide (HPMA), polydivinylether maleic anhydride (DIVEMA) (Kameda, Y. et al., Biomaterials 25: 3259-3266, 2004; Thanou, M. et al, Current Opinion in Investigational Drugs 4(6): 701-709, 2003; Veronese, F.M., et al., Il Farmaco 54: 497-516, 1999).

Preferred polymers are polyoxyalkylenes. By "polyoxyalkylenes" is meant macromolecules that include at least one polyalkylene oxide portion that is optionally covalently bonded to one or more additional polyalkylene oxides, wherein the polyalkylene oxides are the same or different. Non-limiting examples include polyethylene glycol (PEG), polypropylene glycol (PPG), polyisopropylene glycol (PIPG), PEG-PEG, PEG-PPG, PPG-PIPG, and the like. Also included within the definition of polyoxyalkylenes are macromolecules wherein the polyalkylene oxide portions are optionally connected to each other by a linker. Illustrative examples are PEG-linker-PEG, PEG-linker-PIPG, and the like. More specific examples include the commercially available poly[di(ethylene glycol)adipates, poly[di(ethylene glycol)phthalate diols, and the like. Other examples are block copolymers of oxyalkylene, polyethylene glycol, polypropylene glycol, and polyoxyethylenated polyol units.

At at least one of its termini, the polymer is covalently attached to non-polymer substituted compound of Formula II optionally through a linker using the process of the instant invention providing for covalent linkage of the polymer to the non-polymer substituted compound of Formula II.

When a linker is employed, the linker is covalently bonded to at least one of the polymer termini which, in turn, is covalently attached to the otherwise, non-polymer substituted compound of Formula II. It is understood, of course, that if the appropriate substituents are found on the non-polymer substituted compound of Formula II then the optional linker may not be needed as there can be direct linkage of the polymer to the non-polymer substituted compound of Formula II.

Preferred linkers include, by way of example, the following -O-, -NR²²-, -NR²²C(O)O-, -OC(O)NR²²-, -NR²²C(O)-, -C(O)NR²²-, -NR²²C(O)NR²²-, -alkylene-NR²²C(O)O-, -alkylene-NR²²C(O)NR²²-, -alkylene-OC(O)NR²²-, -alkylene-NR²²-, -alkylene-O-, -alkylene-NR²²C(O)-, -alkylene-C(O)NR²²-, -NR³C(O)O-alkylene-, -NR²²C(O)NR²²-alkylene-, -OC(O)NR²²-alkylene-, -NR²²-alkylene-, -O-alkylene-, -NR²²C(O)-alkylene-, -C(O)NR²²-alkylene-, -alkylene-NR²²C(O)O-alkylene-, -alkylene-NR³C(O)NR²²-alkylene-, -alkylene-OC(O)NR²²-alkylene-, -alkylene-NR²²-alkylene-, alkylene-O-alkylene-, -alkylene-NR²²C(O)-alkylene-, -C(O)NR²²-alkylene-, and



where

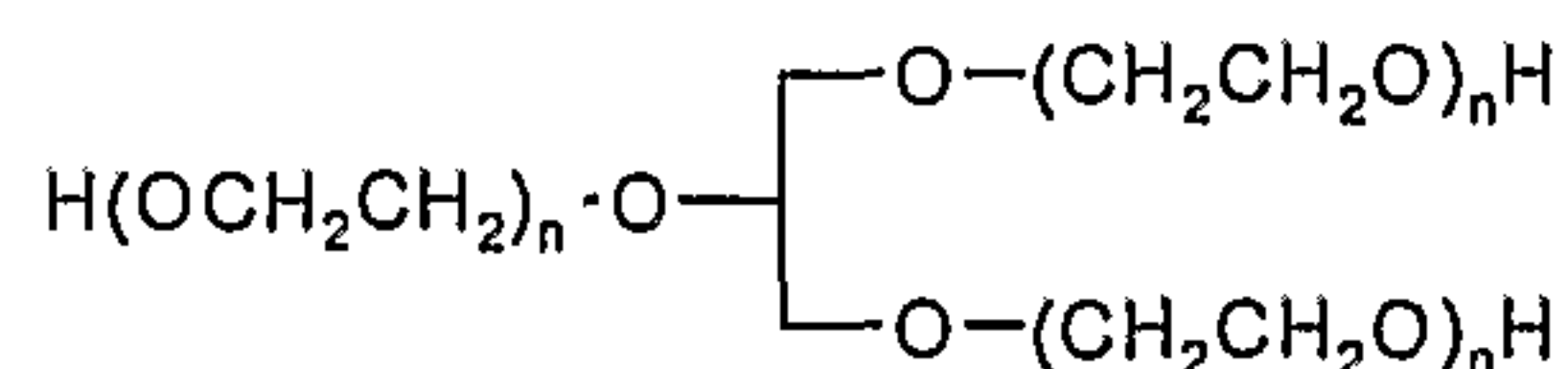
$\textcircled{\text{C}}$ is selected from the group consisting of aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic, and D and E are independently selected from the group consisting of a bond, -O-, CO, -NR²²-, -NR²²C(O)O-, -OC(O)NR²²-, -NR²²C(O)-, -C(O)NR²²-, -NR²²C(O)NR²²-, -alkylene-NR²²C(O)O-, -alkylene-NR²²C(O)NR²²-, -alkylene-OC(O)NR²²-, -alkylene-NR²²-, -alkylene-O-, -alkylene-NR²²C(O)-, -alkylene-C(O)NR²²-, -NR²²C(O)O-alkylene-, -NR²²C(O)NR²²-alkylene-, -OC(O)NR²²-alkylene-, -NR²²-alkylene-, -O-alkylene-, -NR²²C(O)-alkylene-, -C(O)NR²²-alkylene-, -alkylene-NR²²C(O)O-alkylene-, -alkylene-NR²²C(O)NR²²-alkylene-, -alkylene-OC(O)NR²²-alkylene-, -alkylene-NR²²-alkylene-, -alkylene-O-alkylene-, -alkylene-NR²²C(O)-alkylene-, and -C(O)NR²²-alkylene-, where R²² is as defined above.

Preferred alkylene groups in the above linkers include C₁-C₁₅ alkylene groups, more preferably C₁-C₆ alkylene groups, and most preferably C₁-C₃ alkylene groups. Preferred heterocyclic groups include piperazinyl, piperidinyl, homopiperazinyl, homopiperidinyl, pyrrolidinyl, and imidazolidinyl.

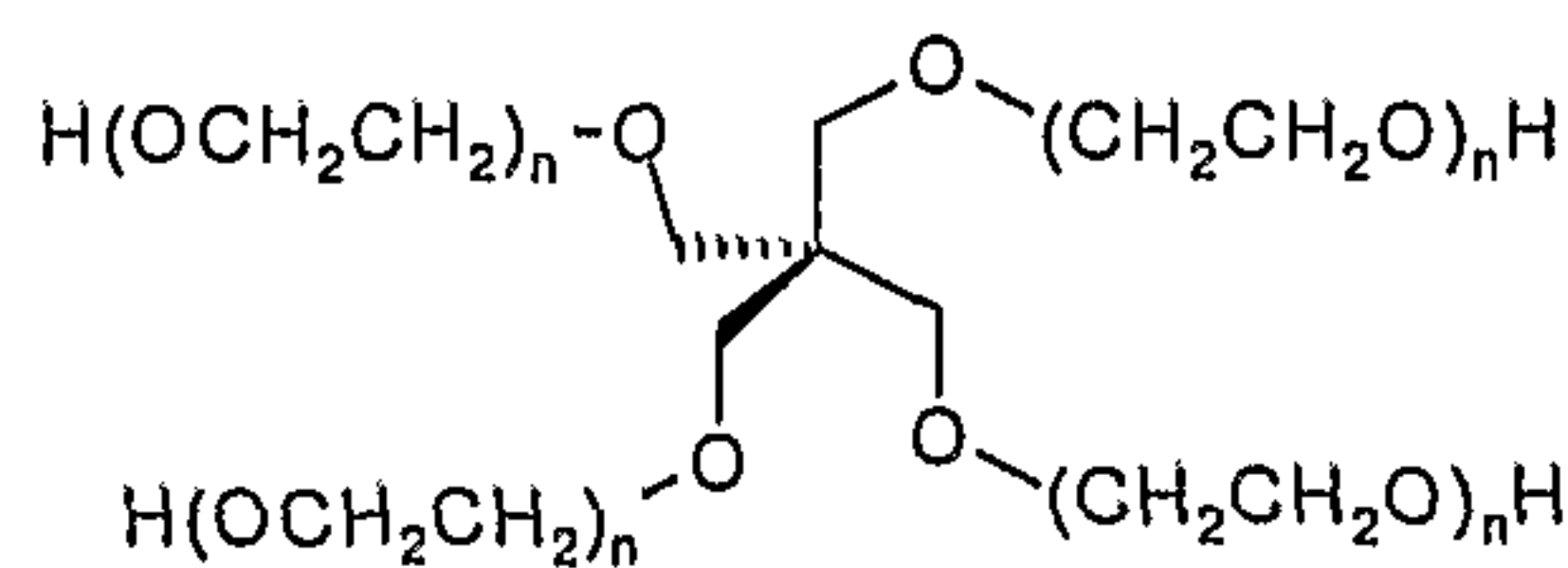
The conjugates of the invention may be incorporated into a carrier which may have from 1 to 19 additional conjugates bound thereto. The term "carrier" refers to an optional component or scaffold to which multiple conjugates can be bound and which when incorporated into the conjugates of this invention do not impart either substantial immunogenicity or toxicity. Such carriers are preferably mono- to decavalent materials containing multiple functionalities for polymer attachment. The functionalities can be homogeneous or heterogeneous; although homogeneous functionalities are preferred.

Examples of commercially available carriers comprising homogeneous functionalities include, by way of example only, catechol, resorcinol, 1,2-phenylenediamine, 1,3-phenylenediamine, 1,4-phenylenediamine; phthalic acid, isophthalic acid, 1,3-propanediol, glycerol, 1,2,4-benzenetriol, pentaerythritol, glucose (in its pyranose form), 1,3-phenylene diisocyanate, 1,4-phenylene diisocyanate, isophthalaldehyde, phthalaldehyde, 1,3-cyclopentanediol, ethylene diamine, ethylenediamine tetraacetic acid, and the like.

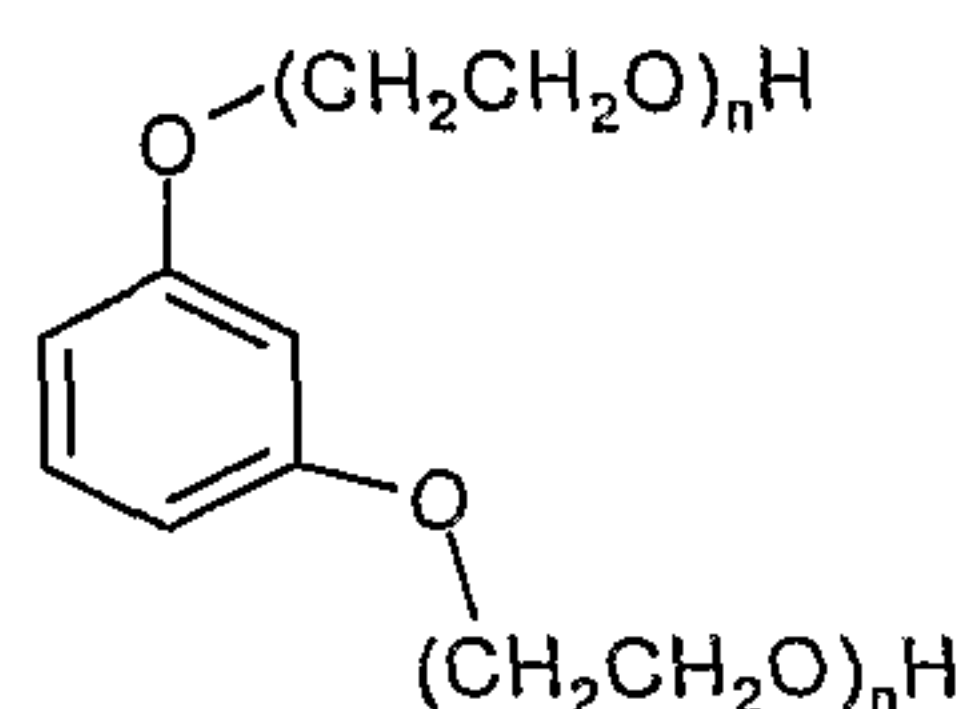
Representative structures useful as carriers or scaffolds include the following (where PEG is used for representative purposes only):



tri-PEG-glycerol



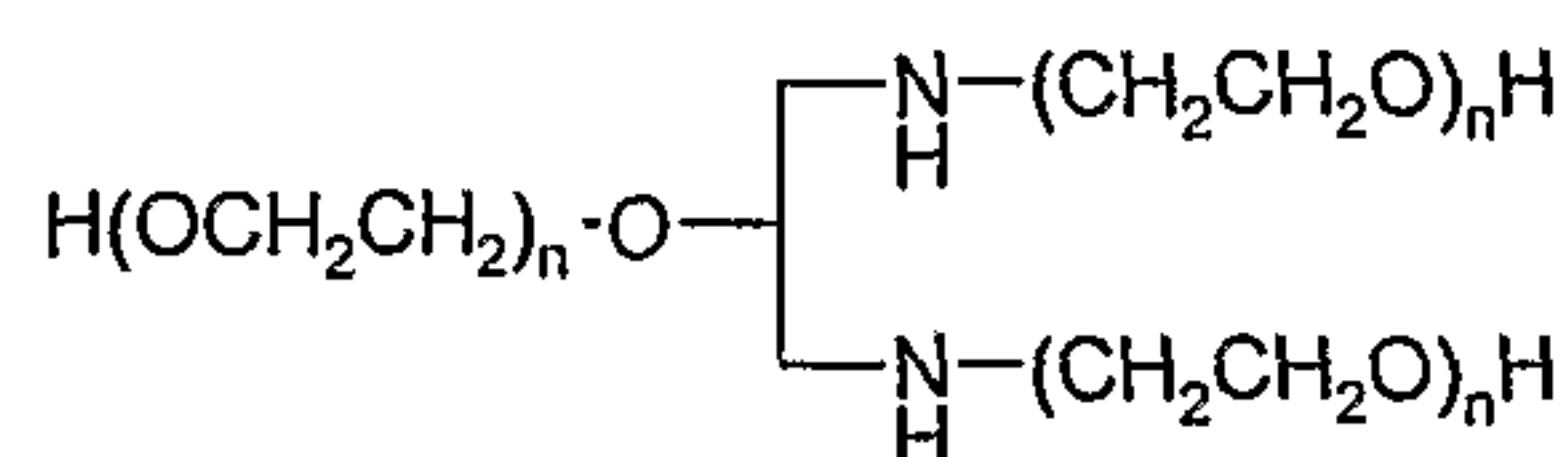
tetra-PEG-pentaerythritol



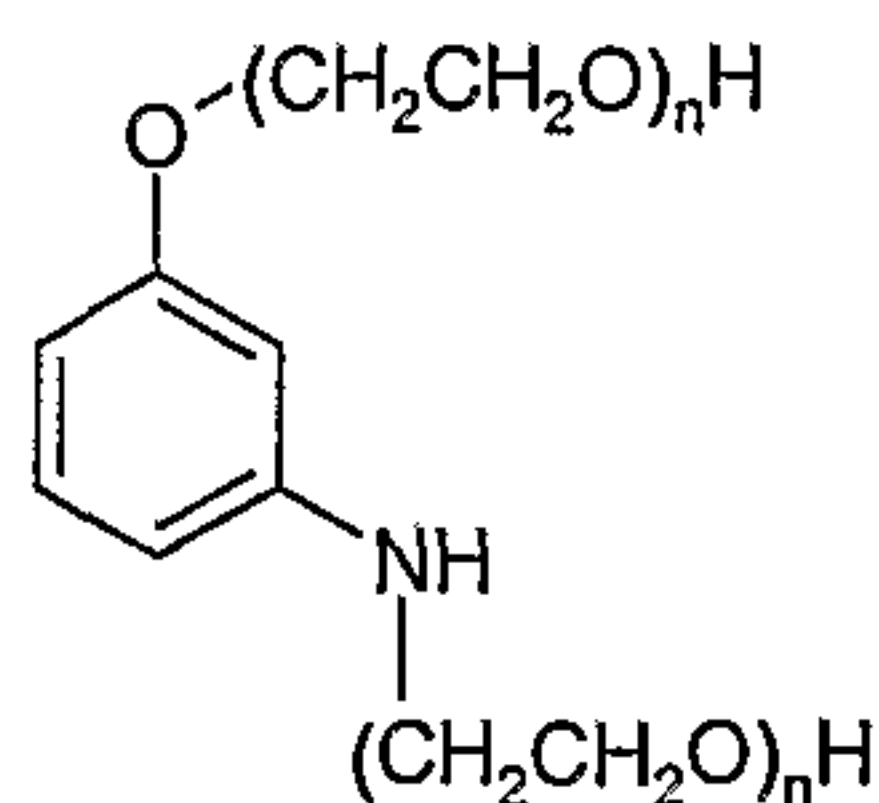
di-PEG resorcinol

Examples of commercially available carriers comprising heterogeneous functionalities include, by the way of example only, 6-hydroxycaproic acid, amino acids, salicylic acid, 3- or 4-aminosalicylic acid, 1,3-diamino-2-hydroxypropane, 2-aminoethanol, 3-aminopropanol, glucosamine, sialic acid, amino acids, and the like.

Representative structures arising from polymer attachment using such carriers or scaffolds include the following (where PEG is used for representative purposes only):

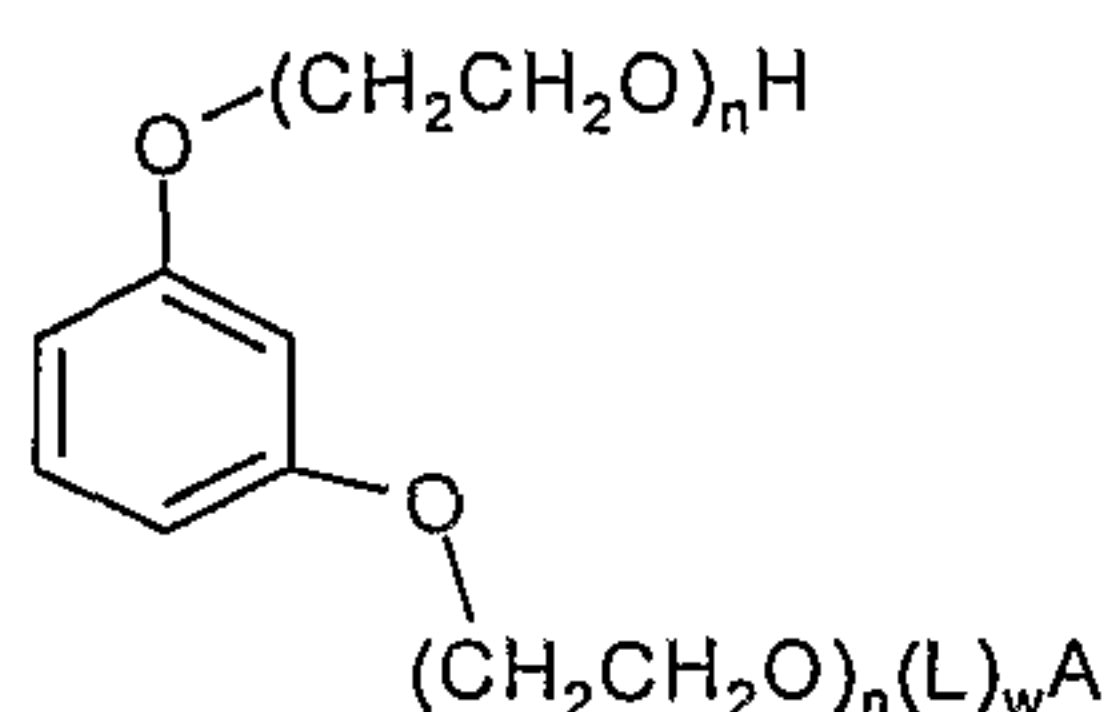
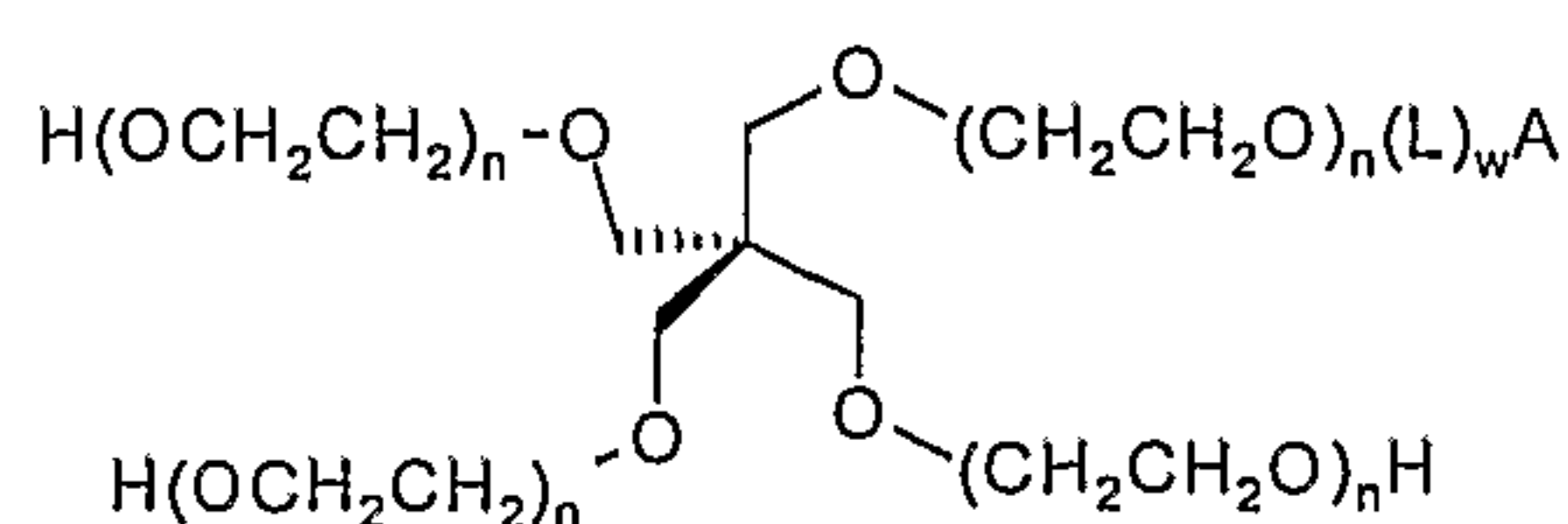
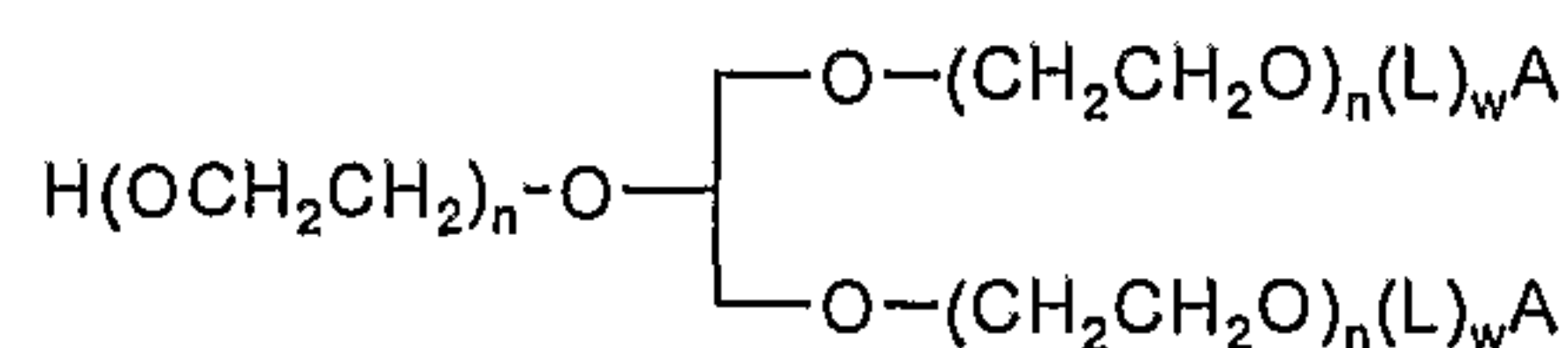


tri-PEG-1,3-diamino-2-hydroxypropane



di-PEG 3-aminophenol

The carrier can optionally contain one or more copies of **A** bound to the carrier optionally through a linker provided that there is at least one further functional group which can bind to a further copy of **A**. For example, each of the following structures is deemed a carrier or scaffold because there is at least one additional functional group for binding of an additional **A** substituent:



where **L**, **w** and **A** are as defined above.

The term “oxyalkylene” refers to $-\text{OCH}_2\text{CHR}^d-$ where R^d is alkyl. Polymerized oxyalkylenes are referred to as polyoxyalkylenes, polyalkylene oxides or polyalkylene glycols, non-limiting examples of which include PEG, poly propylene glycol, polybutylene glycol, polyisopropylene glycol, and the like.

Such polymers are optionally mono-capped with a substituent preferably selected from alkyl, aryl, substituted alkyl, substituted aryl and a carrier as described above. Inclusive of such polymers are those diamino capped polyoxyalkylene polymers which are known in the art as Jeffamines[®]. Still further, such polymers can optionally contain one or more non-oxyalkylene units such as the commercially available poly[di(ethylene glycol)adipates, poly[di(ethylene glycol)phthalate diols, and the like. Also included are block copolymers of oxyalkylene, polyethylene glycol, polypropylene glycol, and polyoxyethylenated polyol units.

Polyoxyalkylenes, such as PEG, are usually provided as a water soluble, waxy solid. Generally, as the polymer's molecular weight increases, its viscosity and freezing point also

increase. Commercial preparations are usually characterized by the "average molecular weight" of the constituent polymers.

Typically, the average molecular weight of the total amount of polymer arising from single or multiple polymer moieties in the conjugates of the invention is between about 100 to 100,000; preferably from about 10,000 to 80,000; more preferably from about 20,000 to about 70,000.

Similarly, other suitable polymers such polyvinylpyrrolidone (PVP), polyacrylamide (PAAm), polydimethylacrylamide (PDAAm), polyvinyl alcohol (PVA), dextran, poly (L-glutamic acid) (PGA), styrene maleic anhydride (SMA), poly-N-(2-hydroxypropyl) methacrylamide (HPMA), polydivinylether maleic anhydride (DIVEMA) are well known in the art and have molecular weights of from about 100 to 100,000; preferably from about 10,000 to 80,000; more preferably from about 20,000 to about 70,000.

"Pharmaceutically acceptable salt" refers to salts which retain the biological effectiveness and properties of the compounds of this invention and which are not biologically or otherwise undesirable. In many cases, the compounds of this invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

Pharmaceutically-acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl) amines, tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl amines, substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted cycloalkyl amines, cycloalkenyl amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines, disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines, triheterocyclic amines, mixed di- and tri-amines where at least two of the substituents on the amine are different and are selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl, heterocyclic, and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic or heteroaryl group.

Examples of suitable amines include, by way of example only, isopropylamine,

trimethyl amine, diethyl amine, tri(iso-propyl) amine, tri(n-propyl) amine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like. It

5 should also be understood that other carboxylic acid derivatives would be useful in the practice of this invention, for example, carboxylic acid amides, including carboxamides, lower alkyl carboxamides, dialkyl carboxamides, and the like.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, 10 sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluene-sulfonic acid, salicylic acid, and the like.

15 The term "pharmaceutically-acceptable cation" refers to the cation of a pharmaceutically-acceptable salt.

It is understood that in all substituted groups defined herein, polymers arrived at by defining substituents with further substituents to themselves (e.g., substituted aryl having a substituted aryl group as a substituent which is itself substituted with a substituted aryl group, 20 etc.) are not intended for inclusion herein. In such cases, the maximum number of such substituents is three. That is to say that each of the above definitions is constrained by a limitation that, for example, substituted aryl groups are limited to -substituted aryl-(substituted aryl)-(substituted aryl).

Similarly, it is understood that the above definitions are not intended to include 25 impermissible substitution patterns (e.g., methyl substituted with 5 fluoro groups or a hydroxyl group alpha to ethenyl or acetylenic unsaturation). Such impermissible substitution patterns are well known to the skilled artisan.

Compound Preparation

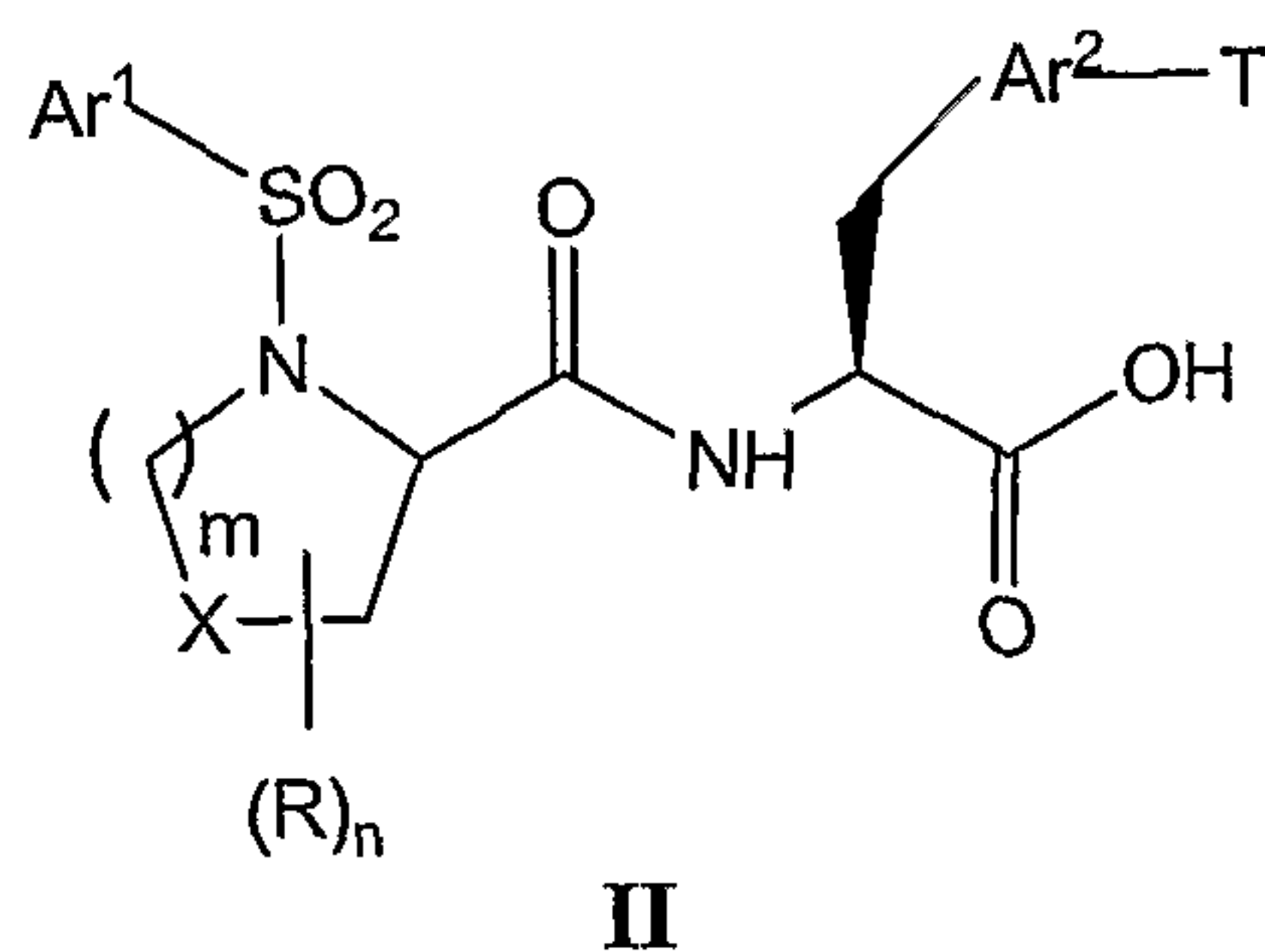
30 The starting active compounds employed in the process of this invention can be prepared from readily available starting materials using known procedures and readily available starting materials or, where the starting material is not known or commercially available, such materials can readily be prepared using literature procedures. It will be appreciated that where typical or preferred process conditions (i.e., reaction temperatures, 35 times, mole ratios of reactants, solvents, pressures, etc.) are given, other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the

particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

Additionally, as will be apparent to those skilled in the art, conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. Suitable protecting groups for various functional groups as well as suitable conditions for protecting and deprotecting particular functional groups are well known in the art. For example, numerous protecting groups are described in T. W. Greene and G. M. Wuts, *Protecting Groups in Organic Synthesis*, Second Edition, Wiley, New York, 1991, and references cited therein.

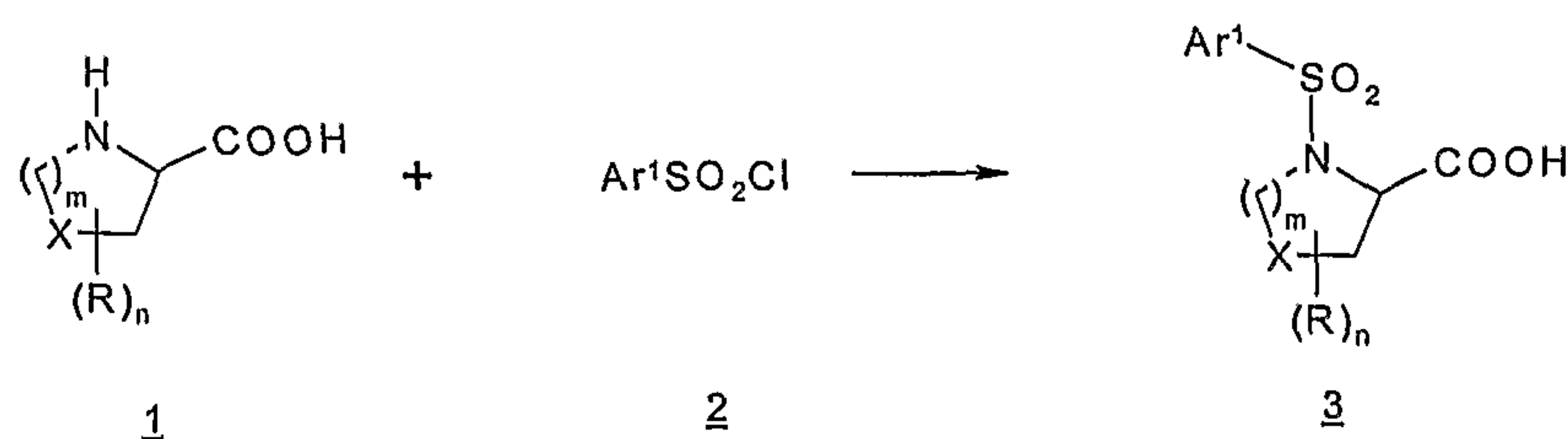
Furthermore, the compounds of this invention will typically contain one or more chiral centers. Accordingly, if desired, such compounds can be prepared or isolated as pure stereoisomers, *i.e.*, as individual enantiomers or diastereomers, or as stereoisomer-enriched mixtures. All such stereoisomers (and enriched mixtures) are included within the scope of this invention, unless otherwise indicated. Pure stereoisomers (or enriched mixtures) may be prepared using, for example, optically active starting materials or stereoselective reagents well-known in the art. Alternatively, racemic mixtures of such compounds can be separated using, for example, chiral column chromatography, chiral resolving agents and the like.

Preferred conjugates prepared according to this invention comprise a polymer moiety/optional carrier containing about 1 to about 100 substituents of Formula II:



Specifically, the polymer moiety can be bound through a covalent bond to the Ar¹ substituent, the R substituent, the Ar² substituent and/or in the T substituent wherein the polymer moiety is either directly attached or is attached via a linker. In turn, the polymer moiety may optionally be bound to a carrier having multiple copies of the polymer attached thereto.

Compounds of Formula II can be prepared by first coupling a heterocyclic amino acid, 1, with an appropriate aryl sulfonyl chloride as illustrated in Scheme 1 below:



Scheme 1

where R, Ar¹, X, *m* and *n* are as defined above.

Specifically, in Scheme 1 above, heterocyclic amino acid, 1, is combined with a stoichiometric equivalent or excess amount (preferably from about 1.1 to about 2 equivalents) of arylsulfonyl halide, 2, in a suitable inert diluent such as dichloromethane and the like. Generally, the reaction is conducted at a temperature ranging from about -70°C to about 40°C until the reaction is substantially complete, which typically occurs within 1 to 24 hours. Preferably, the reaction is conducted in the presence of a suitable base to scavenge the acid generated during the reaction. Suitable bases include, by way of example, tertiary amines, such as triethylamine, diisopropylethylamine, N-methyl-morpholine and the like. Alternatively, the reaction can be conducted under Schotten-Baumann-type conditions using an aqueous alkali solution such as an aqueous solution of sodium hydroxide, an aqueous phosphate solution buffered to pH 7.4, and the like. The resulting product, 3, can be recovered by conventional methods, such as chromatography, filtration, evaporation, crystallization, and the like or, alternatively, used in the next step without purification and/or isolation.

Heterocyclic amino acids, 1, employed in the above reaction are either known compounds or compounds that can be prepared from known compounds by conventional synthetic procedures. Examples of suitable amino acids for use in this reaction include, but are not limited to, L-proline, *trans*-4-hydroxyl-L-proline, *cis*-4-hydroxyl-L-proline, *trans*-3-phenyl-L-proline, *cis*-3-phenyl-L-proline, L-(2-methyl)proline, L-pipecolinic acid, L-azetidine-2-carboxylic acid, L-thiazolidine-4-carboxylic acid, L-(5,5-dimethyl)thiazolidine-4-carboxylic acid, L-thiamorpholine-3-carboxylic acid. If desired, the corresponding carboxylic acid esters of the amino acids, 1, such as the methyl esters, ethyl esters, *t*-butyl esters, and the like, can be employed in the above reaction with the arylsulfonyl chloride. Subsequent hydrolysis of the ester group to the carboxylic acid using conventional reagents and conditions, *i.e.*, treatment with an alkali metal hydroxide in an inert diluent such as methanol/water, then provides the N-sulfonyl amino acid, 3.

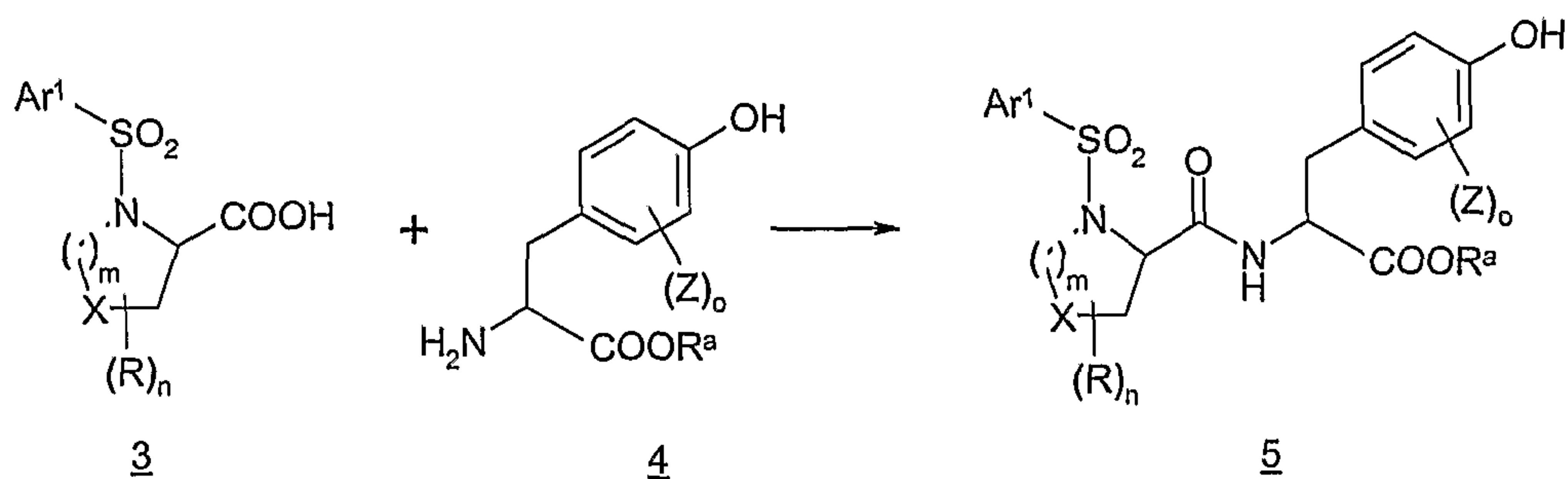
Similarly, the arylsulfonyl chlorides, 2, employed in the above reaction are either known compounds or compounds that can be prepared from known compounds by conventional synthetic procedures. Such compounds are typically prepared from the corresponding sulfonic acid, *i.e.*, from compounds of the formula Ar¹SO₃H where Ar¹ is as

defined above, using phosphorous trichloride and phosphorous pentachloride. This reaction is generally conducted by contacting the sulfonic acid with about 2 to 5 molar equivalents of phosphorous trichloride and phosphorous pentachloride, either neat or in an inert solvent, such as dichloromethane, at temperature in the range of about 0°C to about 80°C for about 1
 5 to about 48 hours to afford the sulfonyl chloride. Alternatively, the arylsulfonyl chlorides, 2, can be prepared from the corresponding thiol compound, *i.e.*, from compounds of the Ar¹-SH where Ar¹ is as defined herein, by treating the thiol with chlorine (Cl₂) and water under conventional reaction conditions.

Alternatively, arylsulfonyl chlorides, 2, employed in the above reaction may be
 10 prepared by chlorosulfonylation of substituted benzene or heterocycloalkyl group using Cl-SO₃H.

Examples of arylsulfonyl chlorides suitable for use in this invention include, but are not limited to, benzenesulfonyl chloride, 1-naphthalenesulfonyl chloride, 2-naphthalenesulfonyl chloride, p-toluenesulfonyl chloride, o-toluenesulfonyl chloride, 4-acetamidobenzenesulfonyl
 15 chloride, 4-*tert*-butylbenzenesulfonyl chloride, 4-bromobenzenesulfonyl chloride, 2-carboxybenzenesulfonyl chloride, 4-cyanobenzenesulfonyl chloride, 3,4-dichlorobenzenesulfonyl chloride, 3,5-dichlorobenzenesulfonyl chloride, 3,4-dimethoxybenzenesulfonyl chloride, 3,5-ditrifluoromethylbenzenesulfonyl chloride, 4-fluorobenzenesulfonyl chloride, 4-methoxybenzenesulfonyl chloride, 2-
 20 methoxycarbonylbenzenesulfonyl chloride, 4-methylamido-benzenesulfonyl chloride, 4-nitrobenzenesulfonyl chloride, 4-trifluoromethyl-benzenesulfonyl chloride, 4-trifluoromethoxybenzenesulfonyl chloride, 2,4,6-trimethylbenzenesulfonyl chloride, 2-thiophenesulfonyl chloride, 5-chloro-2-thiophenesulfonyl chloride, 2,5-dichloro-4-thiophenesulfonyl chloride, 2-thiazolesulfonyl chloride, 2-methyl-4-thiazolesulfonyl chloride, 1-
 25 methyl-4-imidazolesulfonyl chloride, 1-methyl-4-pyrazolesulfonyl chloride, 5-chloro-1,3-dimethyl-4-pyrazolesulfonyl chloride, 3-pyridinesulfonyl chloride, 2-pyrimidinesulfonyl chloride and the like. If desired, a sulfonyl fluoride, sulfonyl bromide or sulfonic acid anhydride may be used in place of the sulfonyl chloride in the above reaction to form the N-sulfonyl amino acid, 3.

30 The N-arylsulfonyl amino acid, 3, is then coupled to commercially available tyrosine esters as shown in Scheme 2 below:



Scheme 2

where R, Ar¹, X, *m* and *n* are as defined above, R^a is hydrogen or alkyl but preferably is an alkyl group such as *t*-butyl, Z represents optional substitution on the aryl ring and *o* is zero, one or two.

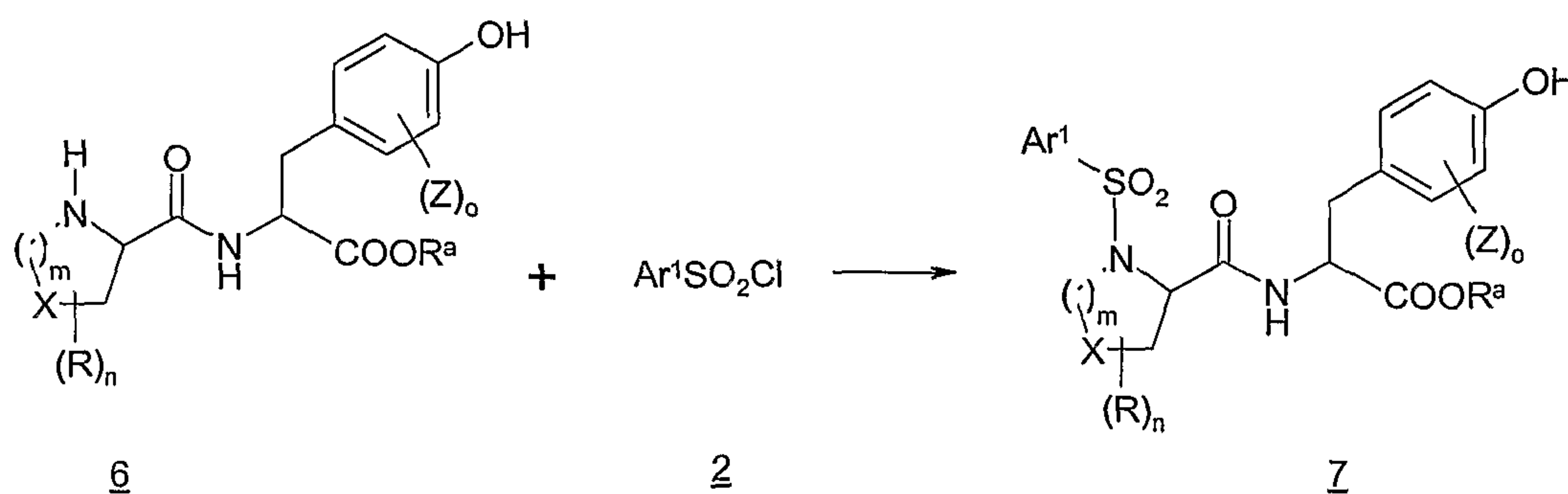
5 This coupling reaction is typically conducted using well-known coupling reagents such as carbodiimides, BOP reagent (benzotriazol-1-yloxy-tris(dimethylamino)-phosphonium hexafluorophosphonate) and the like. Suitable carbodiimides include, by way of example, dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and the like. If desired, polymer supported forms of carbodiimide coupling reagents may also be
 10 used including, for example, those described in *Tetrahedron Letters*, 34(48), 7685 (1993). Additionally, well-known coupling promoters, such as N-hydroxysuccinimide, 1-hydroxybenzotriazole and the like, may be used to facilitate the coupling reaction.

This coupling reaction is typically conducted by contacting the N-sulfonylamino acid, 3, with about 1 to about 2 equivalents of the coupling reagent and at least one equivalent,
 15 preferably about 1 to about 1.2 equivalents, of tyrosine derivative, 4, in an inert diluent, such as dichloromethane, chloroform, acetonitrile, tetrahydrofuran, N,N-dimethylformamide and the like. Generally, this reaction is conducted at a temperature ranging from about 0°C to about 37°C for about 12 to about 24 hours. Upon completion of the reaction, the compound 5 is recovered by conventional methods including neutralization, evaporation, extraction,
 20 precipitation, chromatography, filtration, and the like.

Alternatively, the N-sulfonyl amino acid, 3, can be converted into an acid halide which is then coupled with compound, 4, to provide compound 5. The acid halide can be prepared by contacting compound 3 with an inorganic acid halide, such as thionyl chloride, phosphorous trichloride, phosphorous tribromide or phosphorous pentachloride, or preferably,
 25 with oxalyl chloride under conventional conditions. Generally, this reaction is conducted using about 1 to 5 molar equivalents of the inorganic acid halide or oxalyl chloride, either neat or in an inert solvent, such as dichloromethane or carbon tetrachloride, at temperature in the range of about 0°C to about 80°C for about 1 to about 48 hours. A catalyst, such as DMF, may also be used in this reaction.

The acid halide of N-sulfonyl amino acid, 3, is then contacted with at least one equivalent, preferably about 1.1 to about 1.5 equivalents, of the tyrosine derivative, 4, in an inert diluent, such as dichloromethane, at a temperature ranging from about -70°C to about 40°C for about 1 to about 24 hours. Preferably, this reaction is conducted in the presence of a suitable base to scavenge the acid generated during the reaction. Suitable bases include, by way of example, tertiary amines, such as triethylamine, diisopropylethylamine, N-methylmorpholine and the like. Alternatively, the reaction can be conducted under Schotten-Baumann-type conditions using aqueous alkali, such as sodium hydroxide and the like. Upon completion of the reaction, compound 5 is recovered by conventional methods including neutralization, evaporation, extraction, precipitation, chromatography, filtration, and the like.

Alternatively, compound 5 can be prepared by first forming a diamino acid derivative and then coupling the diamino acid to the arylsulfonyl halide, 2, as shown in scheme 3 below:



Scheme 3

where R, R^a, Ar¹, X, Z, *m*, *n* and *o* are as defined above.

The diamino acid, 6, can be readily prepared by coupling amino acid, 1, with amino acid, 4, using conventional amino acid coupling techniques and reagents, such as carbodiimides, BOP reagent and the like, as described above. Diamino acid, 6, can then be sulfonated using sulfonyl chloride, 2, and using the synthetic procedures described above to provide compound 7.

The tyrosine derivatives, 4, employed in the above reactions are either known compounds or compounds that can be prepared from known compounds by conventional synthetic procedures. For example, tyrosine derivatives, 4, suitable for use in the above reactions include, but are not limited to, L-tyrosine methyl ester, L-tyrosine *t*-butyl ester, L-3,5-diiodotyrosine methyl ester, L-3-iodotyrosine methyl ester, β-(4-hydroxy-naphth-1-yl)-L-alanine methyl ester, β-(6-hydroxy-naphth-2-yl)-L-alanine methyl ester, and the like. If desired, of course, other esters or amides of the above-described compounds may also be employed.

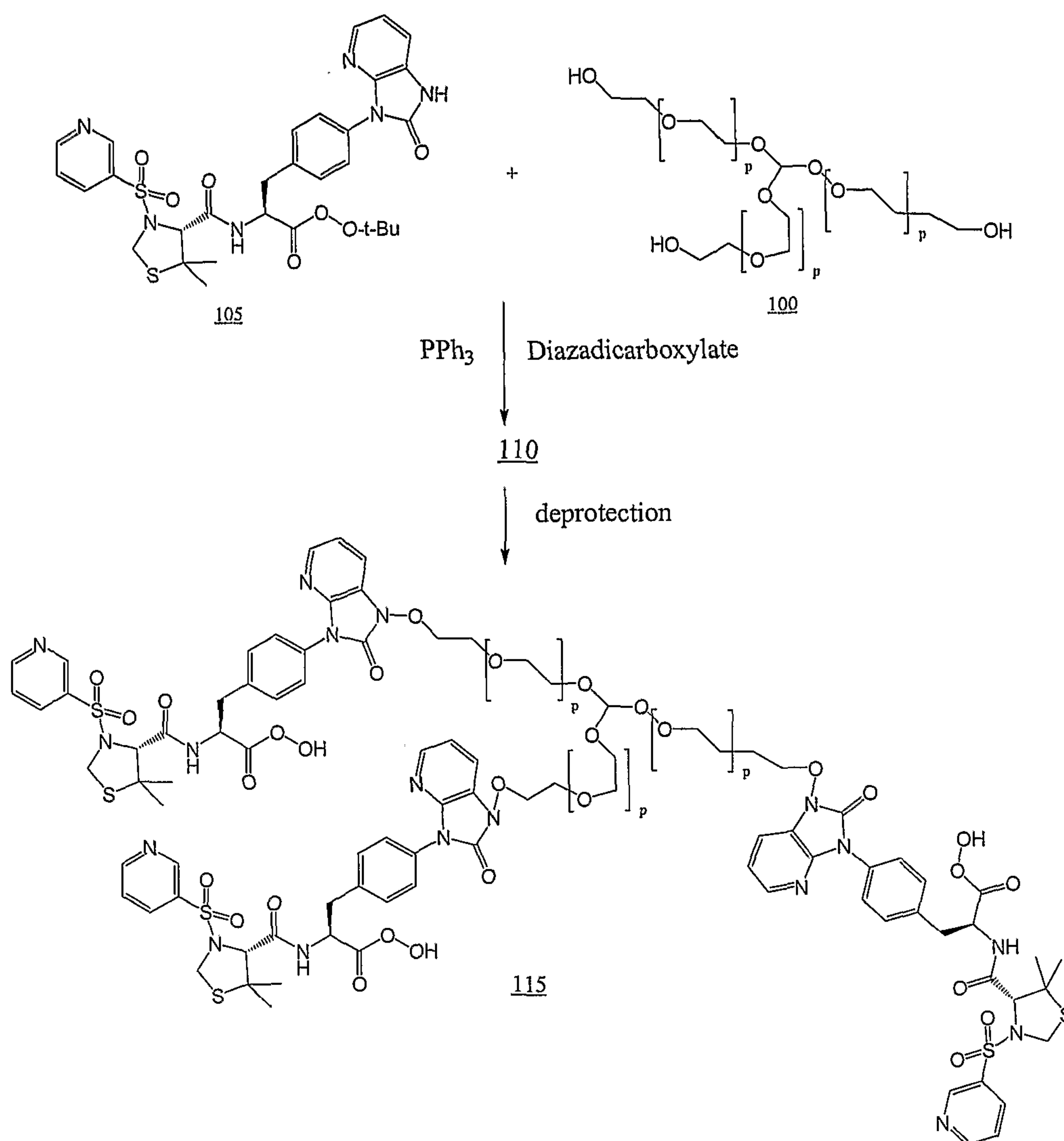
The N-arylsulfonyl-heterocyclic amino acid-tyrosine derivative, Z, can be used as a starting point to attach a polymer moiety at the Ar² group by employing the process of the invention.

5 Amine moieties located on other portions of the molecule can be employed in the manner described above to covalently link a polymer group to the molecule. For example, amines located on Ar¹, on the heterocyclic amino acid or on Ar² can be similarly derivatized to provide for PEG substitution using the process of the invention. The amine moieties can be included in these substituents during synthesis and appropriately protected as necessary. Alternatively, amine precursors can be employed.

10 Further, the amino substitution can be incorporated into the heterocyclic amino acid functionality and then derivatized to include a polymer moiety. For example, the heterocyclic amino acid functionality can be 2-carboxypiperazine depicted in U.S. Patent No. 6,489,300. Alternatively, commercially available 3- or 4-hydroxyproline can be oxidized to the corresponding ketone and then reductively aminated with ammonia in the presence of sodium
15 cyanoborohydride to form the corresponding amine moiety. Still further, 4-cyanoproline can be reduced to provide for a substituted alkyl group of the formula -CH₂NH₂ which can be derivatized through the amine.

Still further, the amine moiety can be incorporated into the Ar² functionality. Preferably, the amine moiety is present as an amine precursor such as a nitro or cyano group
20 bound to Ar².

The non-derivatized compounds of Formula IIa-IIh are subsequently coupled to a polymer by employing the process of the invention. Scheme 4 depicts an embodiment of the invention:



Scheme 4

In Scheme 9, a PEG alcohol **100** is treated with a nucleophile **105** in the presence of a phosphine, here PPh_3 , and a diazodicarboxylate, e.g., diisopropylazodicarboxylate, to form the protected ester **110**. The ester is then hydrolyzed to form the desired conjugate **115**.

The reaction occurs under mild and essentially neutral conditions. The reaction is preferably carried out in at least one suitable solvent. Examples include halogenated solvents such as methylene chloride, aromatic solvents such as benzene or toluene, or ether solvents such as tetrahydrofuran and diethylether. Other suitable solvents include ethylacetate, acetonitrile, and DMF. Most preferably, a chlorinated solvent or an ether solvent is employed. In a most preferred embodiment, the solvent is methylene chloride or tetrahydrofuran.

Reaction temperature is typically in the range of about -100 to 100°C , preferably in the

range of about -20 to 50°C, and even more preferably from about 0° to about room temperature. In a particularly preferred embodiment, reaction temperatures are from between about -10 to 10°C.

The reaction time is in the range of about 5 minutes to about 100 hours, preferably in the range of from between about 30 minutes to about 50 hours. More preferably, the reaction proceeds to completion of from between about 45 minutes to about 10 hours.

As mentioned above, examples of triaryl- or trialkylphosphines include triphenylphosphine, trimethylphosphine, triethylphosphine, tributylphosphine, and 1,2-bis-(diphenylphosphino)ethane. The phosphines can also be polymer supported or water soluble. A preferred triarylphosphine is triphenylphosphine.

Examples of diazo compounds are diethyldiazocarboxylate, diisopropylazodicarboxylate, 4-methyl-1,2,4-triazolidine-3,5-dione, N,N,N',N'-tetramethylazodicarboxamide, azodicarboxylic acid dipiperidide, bis(N-4 -methylpiperazin-1-yl)azodicarboxamide, dimorpholinoazodicarboxamide and di-tert-butyl azodicarboxylate.

It is understood that other suitable polymeric alcohols could be used in place of PEG and that one of ordinary skill in the art would readily be able to modify the reaction schemes below to incorporate such other polymers. In some cases, the PEG moiety can be directly introduced onto the Ar² group and, in other cases, the PEG moiety can be introduced by linkage through a linker moiety.

Other polymers suitable for conjugation to a compound of formula II include, without limitation, polyvinylpyrrolidone (PVP), polyacrylamide (PAAm), polydimethylacrylamide (PDAAm), polyvinyl alcohol (PVA), dextran, poly (L-glutamic acid) (PGA), styrene maleic anhydride (SMA), poly-N-(2-hydroxypropyl) methacrylamide (HPMA), polydivinylether maleic anhydride (DIVEMA). By way of example, PVP, PAAm and PDAAm may be functionalized by introduction of co-monomers during radical polymerization. PVA and dextran each contain primary hydroxyl (OH) groups suitable for conjugation. Methods for synthesis of these biopolymers and for conjugating them to biological materials are well known in the art (see, for example, published U.S. Patent Application 20040043030; U.S. Patent 5,177,059; U.S. Patent 6,716,821; U.S. Patent 5,824,701; U.S. Patent 6,664,331; U.S. Patent 5,880,131; Kameda, Y. et al., Biomaterials 25: 3259-3266, 2004; Thanou, M. et al, Current Opinion in Investigational Drugs 4(6): 701-709, 2003; Veronese, F.M., et al., Il Farmaco 54: 497-516, 1999, all of which are incorporated herein in their entireties).

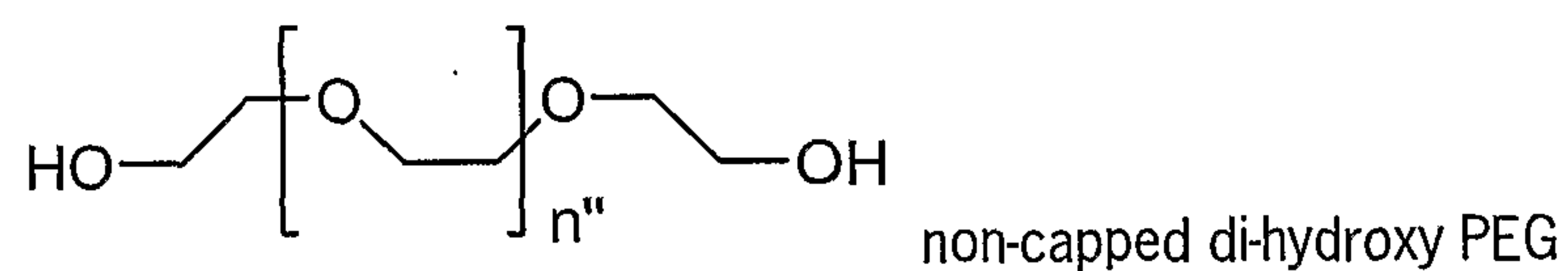
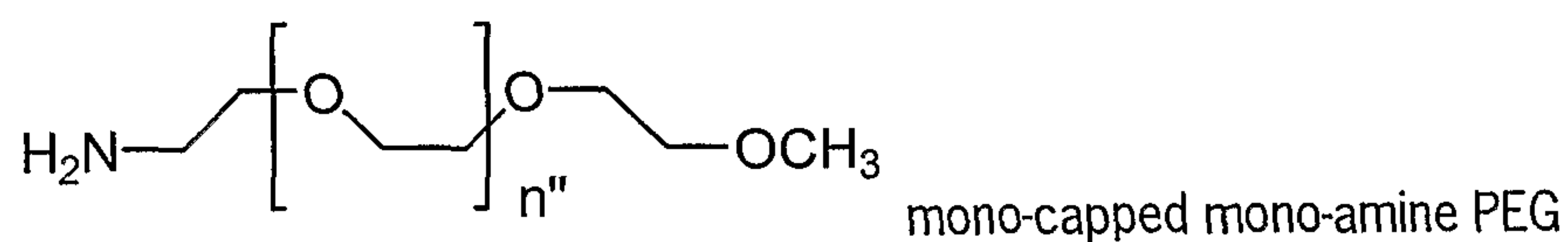
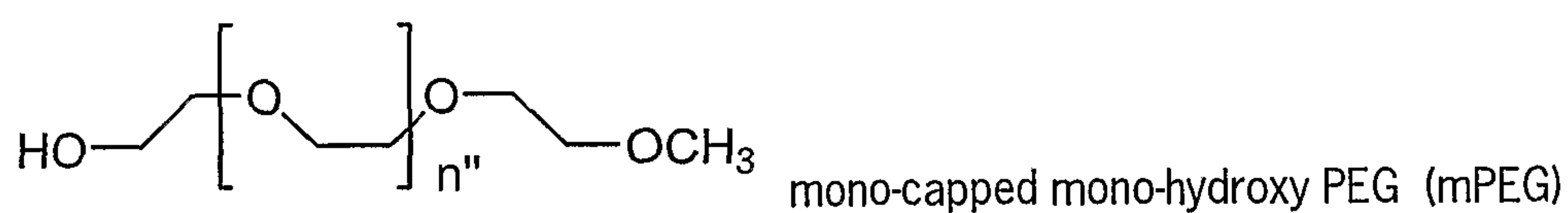
Representative polymers suitable for use in the invention include:

HO(alkylene-O) _{pp} R ^{bb}	mono-capped mono-hydroxy PEG (mPEG)
--	-------------------------------------

$H_2N(alkylene-O)_{pp}R^{bb}$	mono-capped mono-amino PEG
$HO(alkylene-O)_{pp}R-OH$	non-capped di-hydroxy PEG
$H_2N(alkylene-O)_{pp}R-OH$	non-capped mono-amino PEG
$HO(alkylene-O)_{pp}R^{bb}$	branched mono-hydroxy PEG
$HO(alkylene-O)_{pp}R^{bb}$	dendrimeric mono-hydroxy PEG

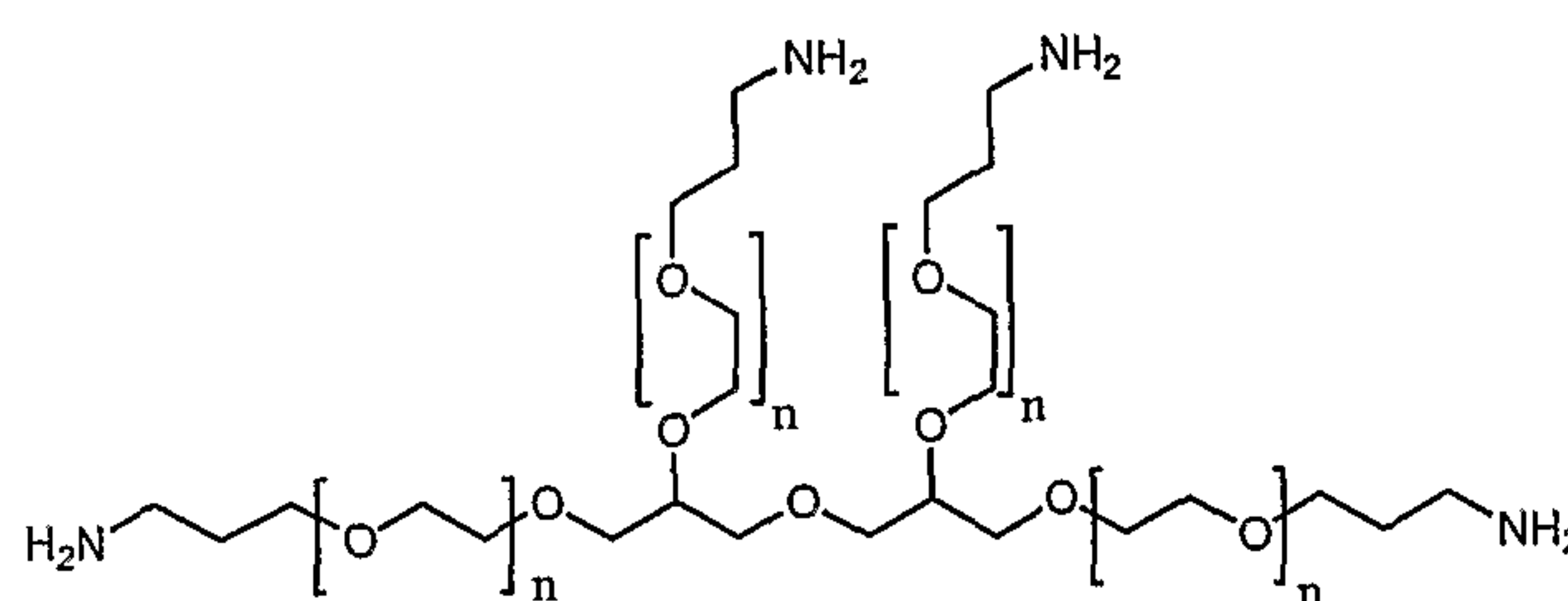
where pp and alkylene are as defined herein and R^{bb} is preferably selected from the group consisting of alkyl, substituted alkyl, aryl and substituted aryl.

5 Other suitable polymers are shown below:



Branched PEGs:

PEG Reagents available from NOF (20 kDa 4-arm)

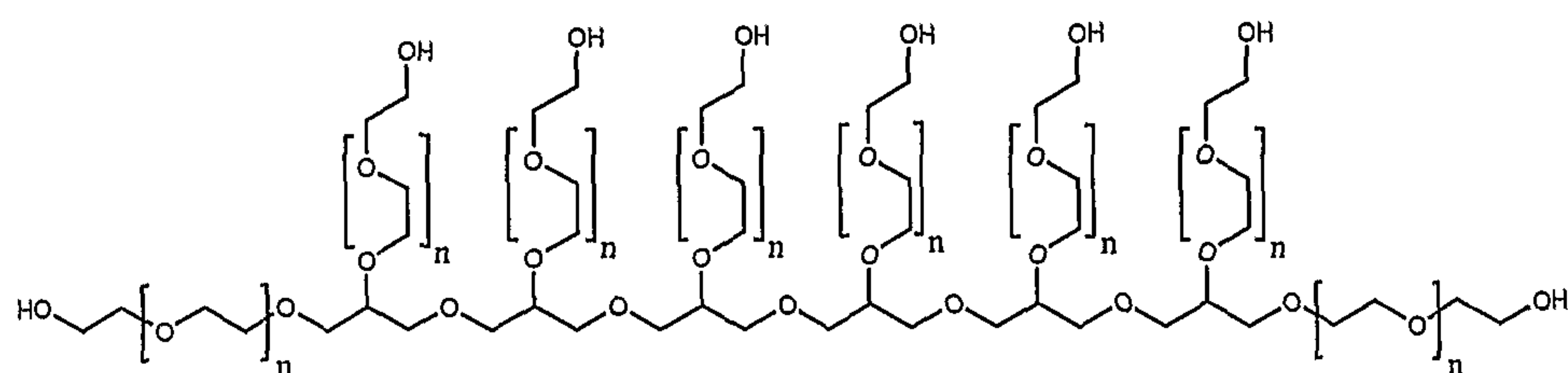


20 kDa 4-arm PEG tetra-amine

Diglycerine core

Cat # Sunbright DG-200PA

PEG Reagents available from Nektar (40 kDa 8-arm)

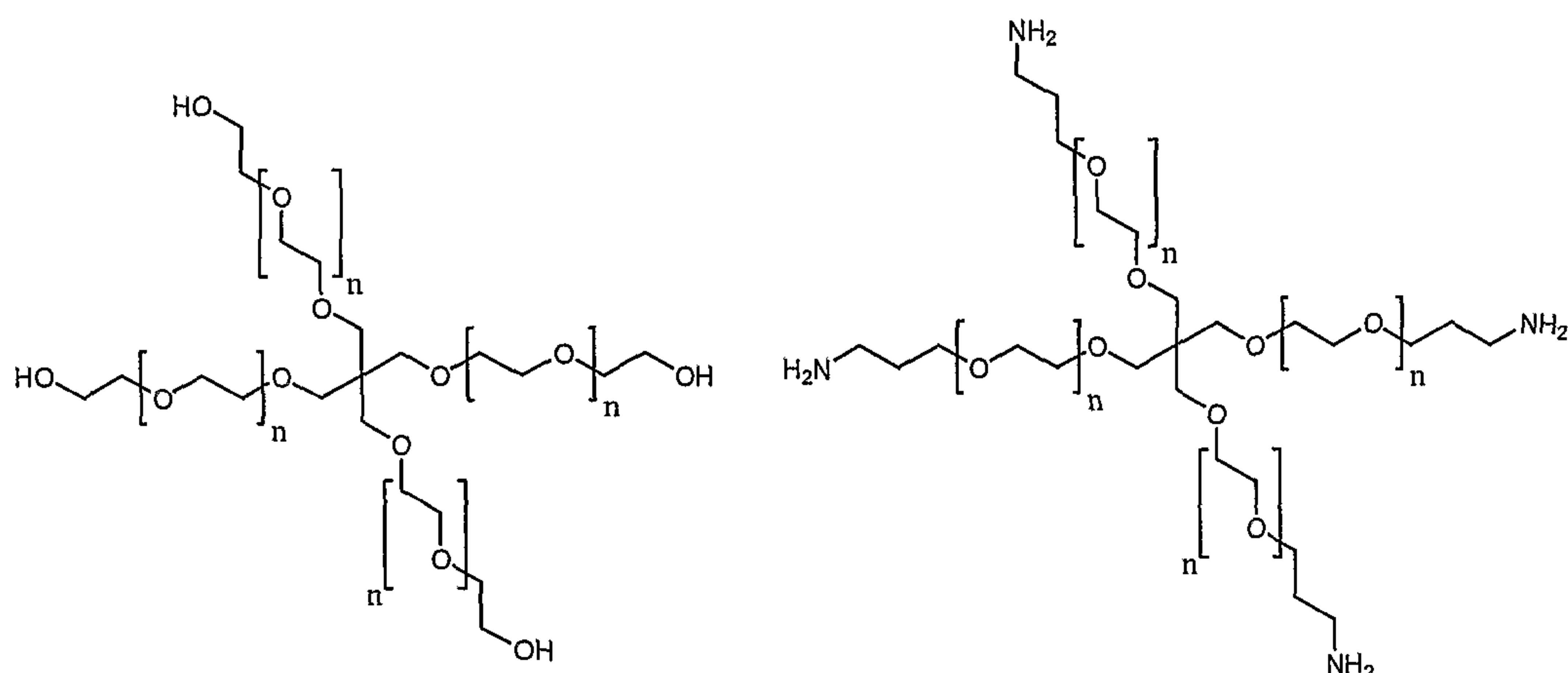
40 kDa 8-arm PEG

Hexaglycerine core

Cat # 0J000T08

Dendrimeric PEGs:

PEG Reagents available from NOF (40 kDa 4-arm)

40 kDa 4-arm PEG alcohol

pentaerythritol core

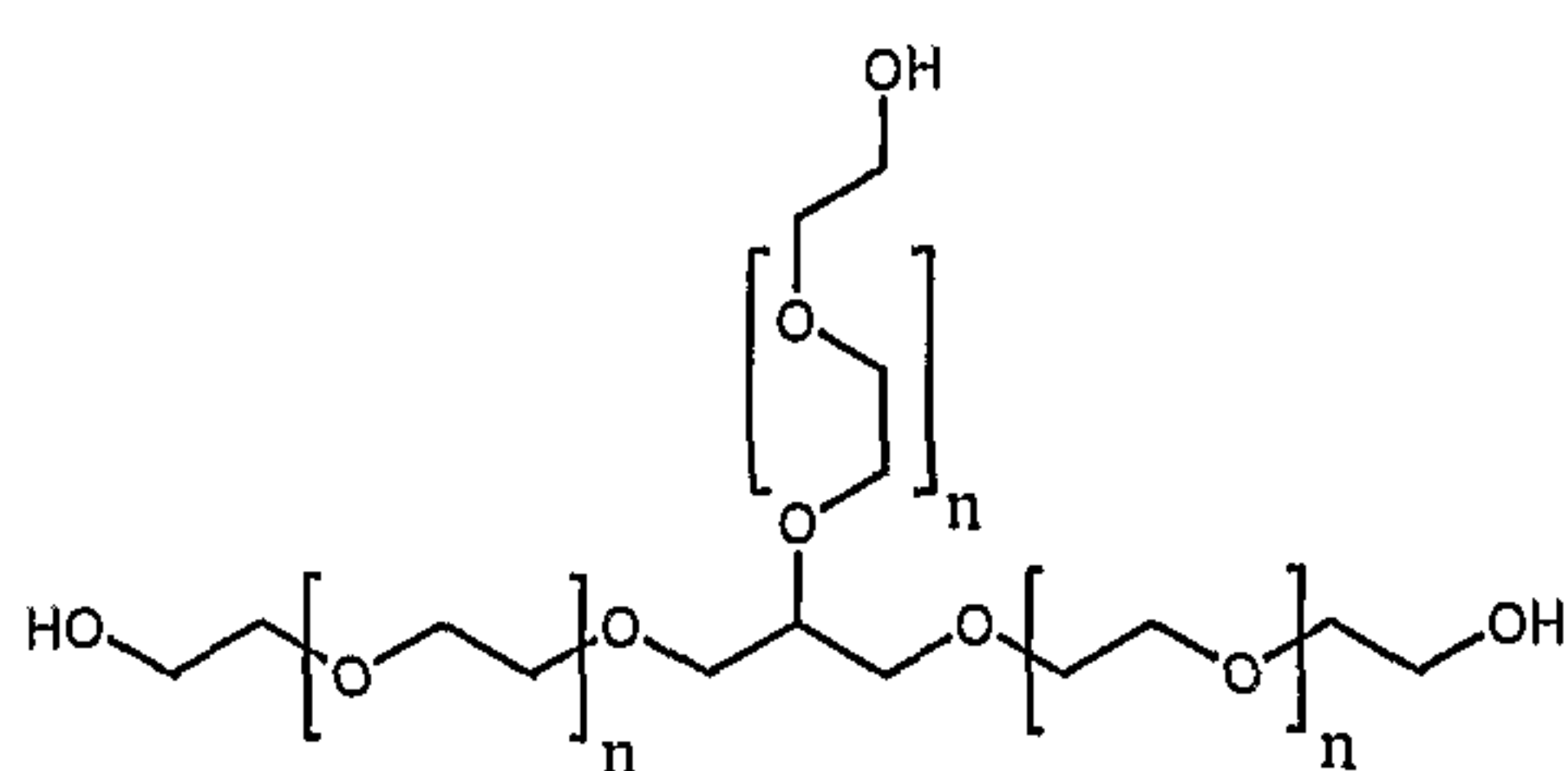
cat. # Sunbright PTE-40000

40 kDa 4-arm PEG tetra-amine

pentaerythritol core

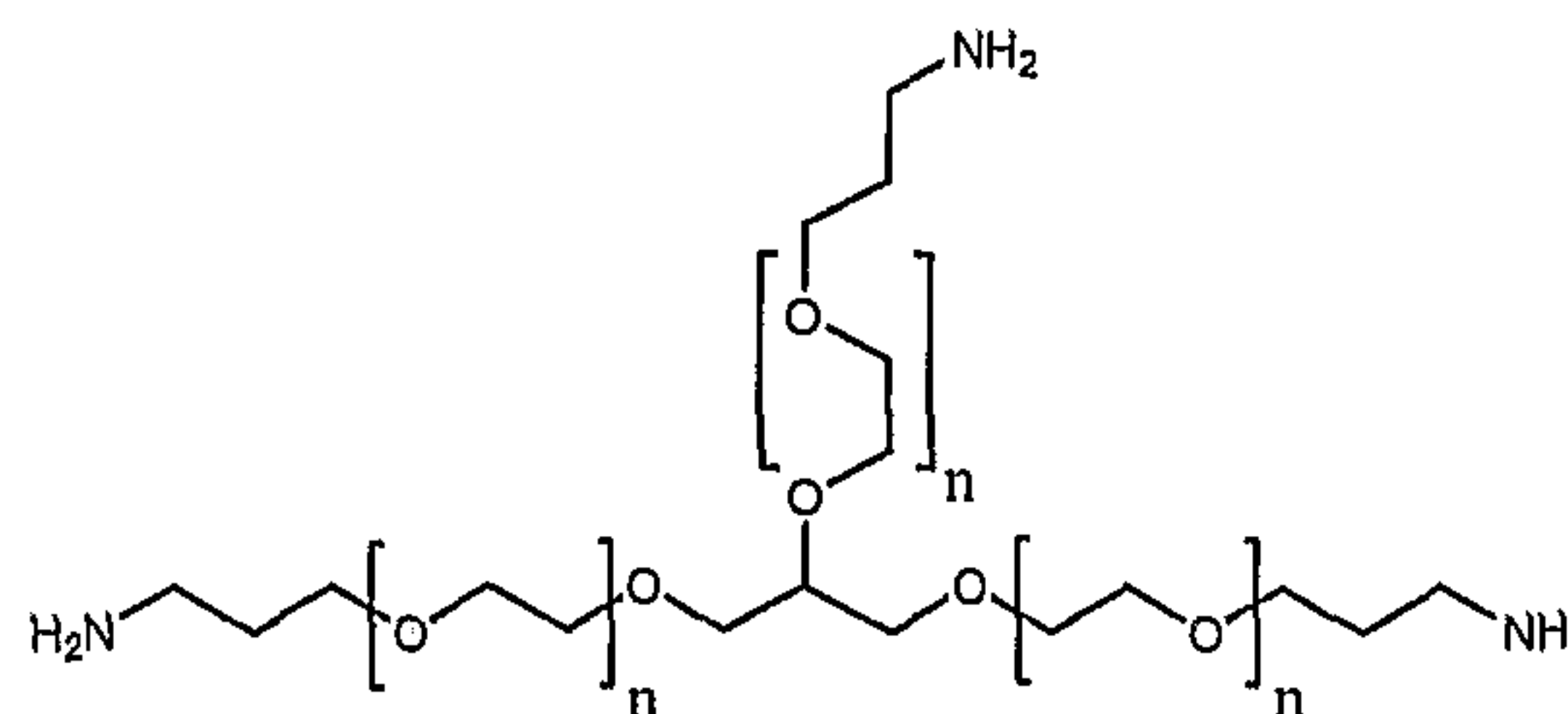
cat. # Sunbright PTE-400PA

PEG Reagents available from NOF (40 kDa 3-arm)

40 kDa 3-arm PEG

glycerin core

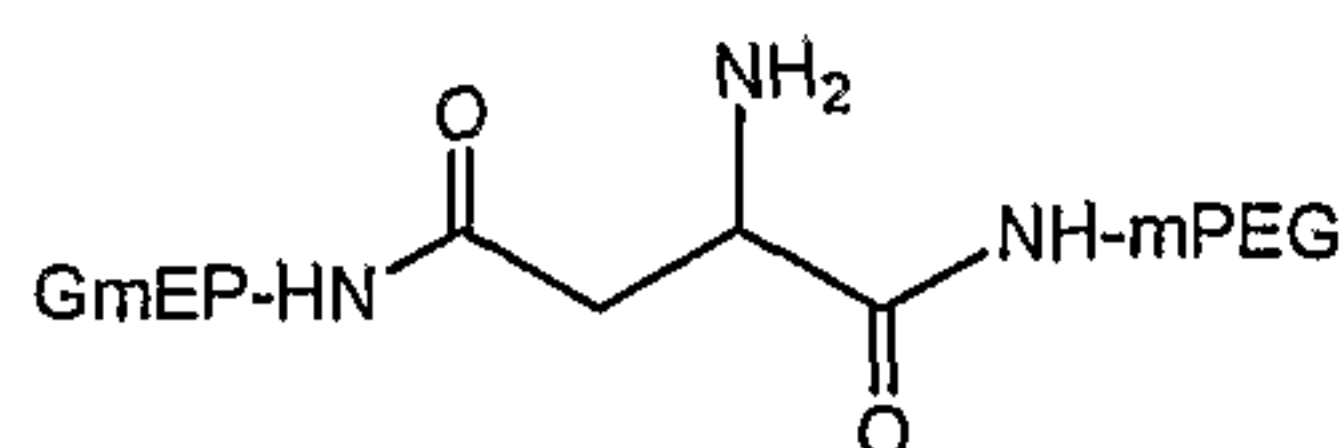
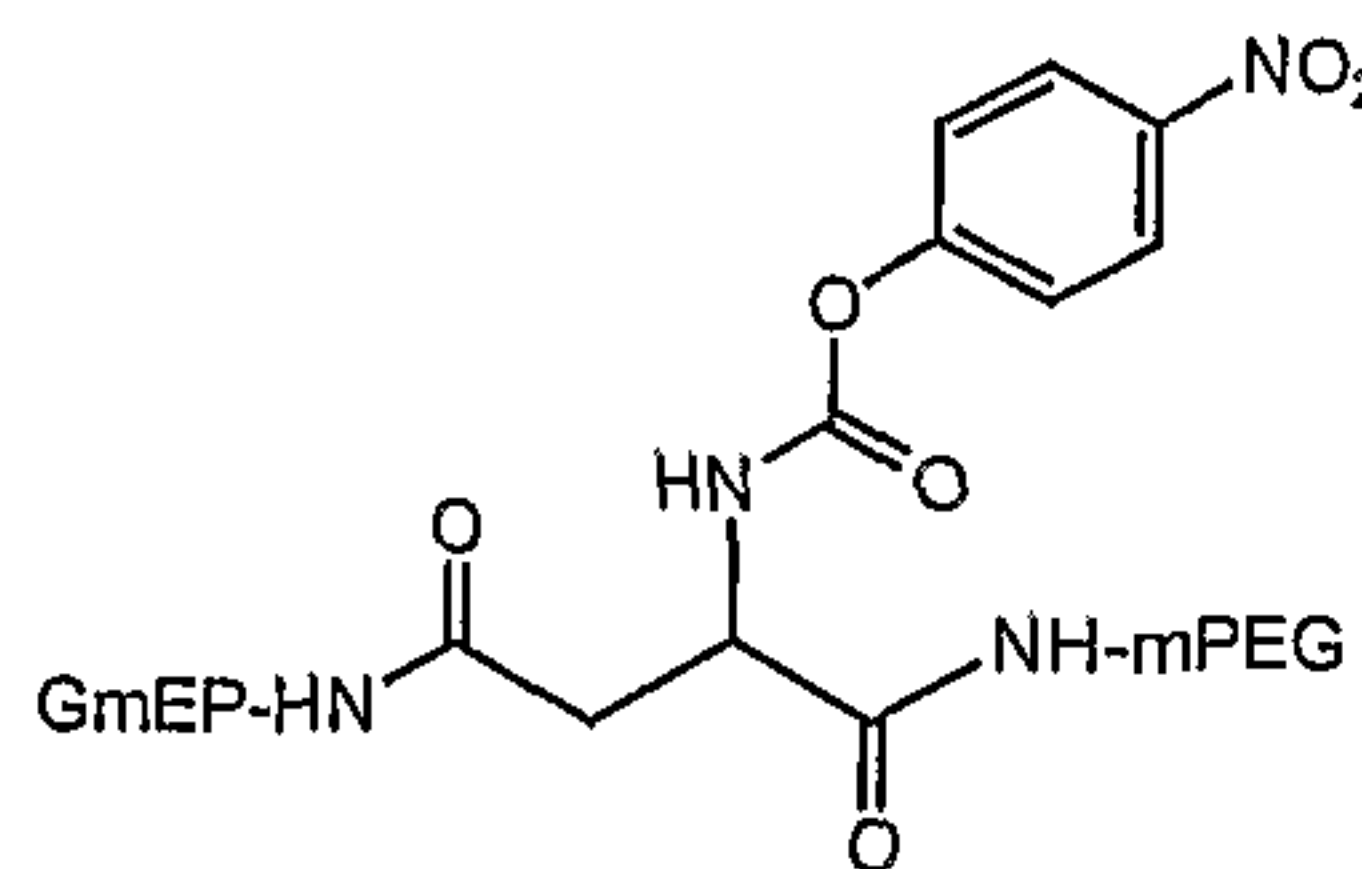
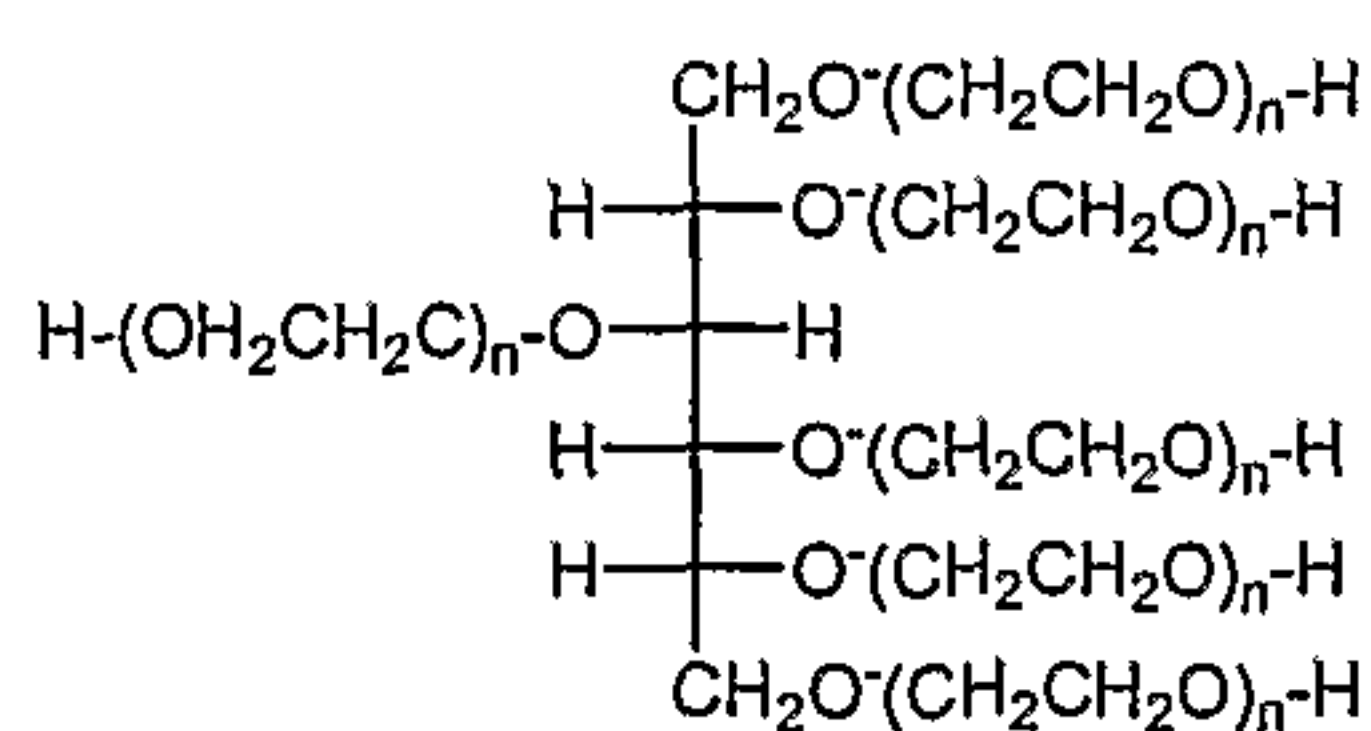
cat # Sunbright GL-40000

40 kDa 3-arm PEG tri-amine

glycerin core

cat # Sunbright GL-400PA

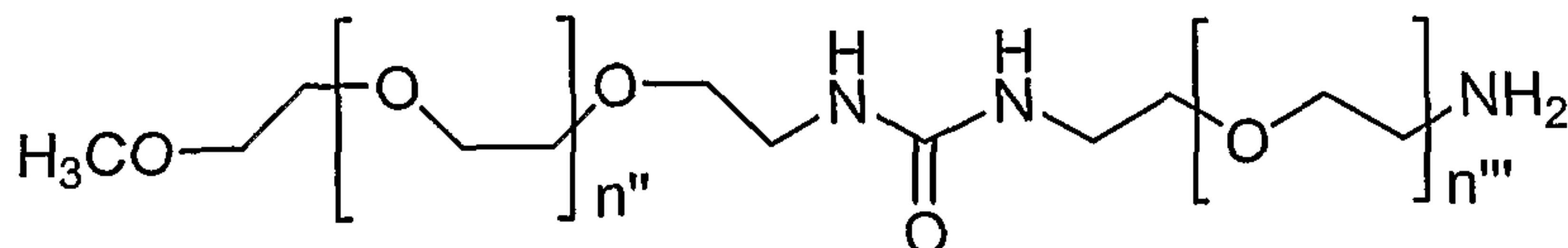
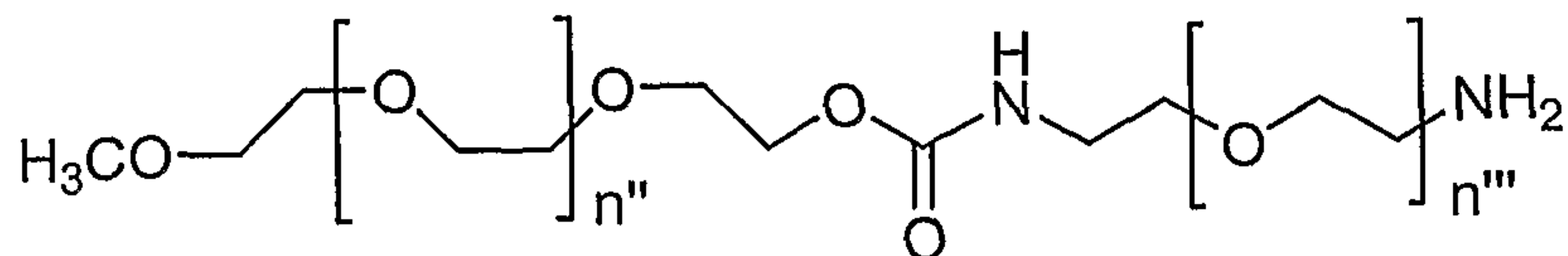
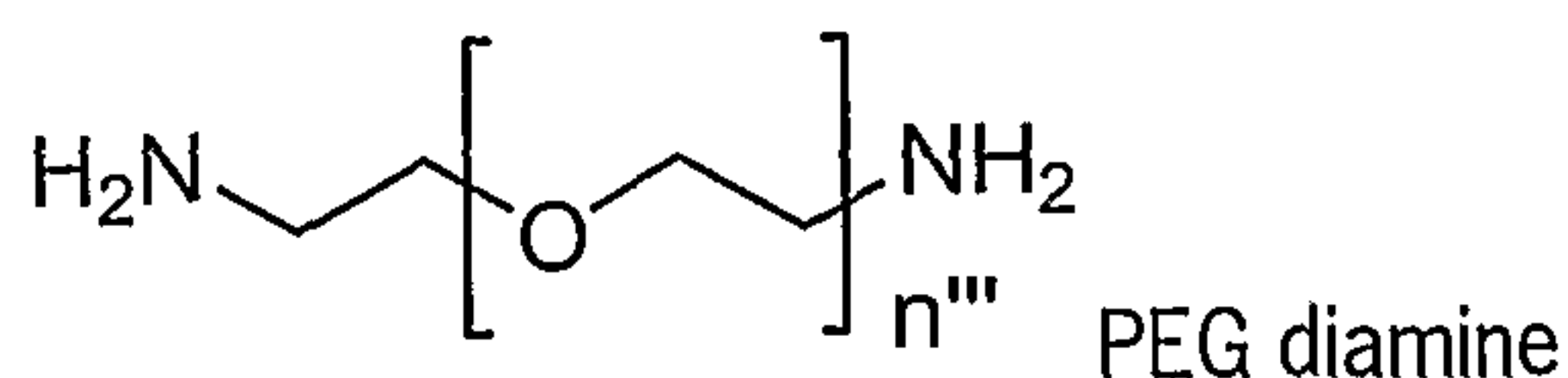
PEG Reagents available from SunBio (40 and 20 kDa)

Y-PEG Series (Aspartic acid core)Y-PEG amine (40 kDa)
Cat# PYAM-40Y-PEG nitrophenyl carbamate (40 kDa)
Cat# PYNPC-406-Arm Series (Sorbitol Core)40 kDa 6-arm PEG
custom product

Lower molecular weights available in the sorbitol 6-arm series include 10, 15 and 20 kDa. Derivatives other than the alcohol include the 6-arm amine.

For example, the 10 kDa 6-arm amine (cat # P6AM-10) could be converted to a 40 kDa 6-arm hexa-amine (with Nektars 5 kDa BocNH-PEG-NHS ester) and then conjugated to a small molecule.

- 5 These PEG polymers may be further modified by extending the chains with PEG diamines through an appropriate linker, for example a carbamate (urethane) or a urea.



5

A variety of nucleophilic compounds having an acidic hydrogen can be used in the process of the invention. Such compounds can be biologically active compounds, i.e., therapeutic compounds (pharmaceuticals) and agricultural chemicals (pesticides, herbicides, and plant growth stimulators such as fertilizers), or food additive compounds. Examples of groups having an acidic nitrogen that can be incorporated into such compounds are shown above; see the structures set forth in Tables D and D1.

Pharmaceutical Formulations

When employed as pharmaceuticals, the conjugates of this invention are usually administered in the form of pharmaceutical compositions. These conjugates can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, sublingual, ophthalmic, or inhalation including administration by nasal or oral inhalation. Preferred administration routes include subcutaneous, intravenous, and inhalation. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one conjugate.

The invention also provides pharmaceutical compositions comprising a conjugate according to the invention, e.g., a conjugate of Formula I, in combination with a separate compound which is an $\alpha_4\beta_7$ inhibitor. Such compositions also comprise a pharmaceutically acceptable carrier or excipient and may be administered as discussed elsewhere herein.

This invention also includes pharmaceutical compositions which contain, as the active ingredient, one or more of the conjugate of formula I together with pharmaceutically acceptable carriers. In making the compositions of this invention, the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier

which can be in, sterile injectable solutions, and sterile packaged powders. For subcutaneous administration, a simple carrier may comprise a sterile solution of water, Na_2HPO_4 , NaH_2PO_4 , and NaCl , in proportions that provide an isotonic and physiologically acceptable pH, also known as PBS or phosphate-buffered saline. Other options are known to those of skill in the art and include mixed solvent systems that can affect the rate of absorption and total exposure. These options include mixed solvent systems containing glycerin, Polyethylene glycol 400, and cottonseed oil. Also of potential use are ethanol, N,N'-dimethylacetamide, propylene glycol and benzyl alcohol all of which may be used to manipulate permeability enhancement and hypertonicity.

In preparing a formulation, it may be necessary to mill the active compound to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

Administration of therapeutic agents by subcutaneous or intravenous formulation is well known in the pharmaceutical industry. A subcutaneous or intravenous formulation should possess certain qualities aside from being just a composition in which the therapeutic agent is soluble. For example, the formulation should promote the overall stability of the active ingredient(s), also, the manufacture of the formulation should be cost effective. All of these factors ultimately determine the overall success and usefulness of an intravenous formulation.

Other accessory additives that may be included in pharmaceutical formulations of compounds of the present invention as follow: solvents: ethanol, glycerol, propylene glycol; stabilizers: EDTA (ethylene diamine tetraacetic acid), citric acid; antimicrobial preservatives: benzyl alcohol, methyl paraben, propyl paraben; buffering agents: citric acid/sodium citrate, potassium hydrogen tartrate, sodium hydrogen tartrate, acetic acid/sodium acetate, maleic

acid/sodium maleate, sodium hydrogen phthalate, phosphoric acid/potassium dihydrogen phosphate, phosphoric acid/disodium hydrogen phosphate; and tonicity modifiers: sodium chloride, mannitol, dextrose.

5 The presence of a buffer is necessary to maintain the aqueous pH in the range of from about 4 to about 8 and more preferably in a range of from about 4 to about 6. The buffer system is generally a mixture of a weak acid and a soluble salt thereof, e.g., sodium citrate/citric acid; or the monocation or dication salt of a dibasic acid, e.g., potassium hydrogen tartrate; sodium hydrogen tartrate, phosphoric acid/potassium dihydrogen phosphate, and phosphoric acid/disodium hydrogen phosphate.

10 The amount of buffer system used is dependent on (1) the desired pH; and (2) the amount of drug. Generally, the amount of buffer used is in a 0.5:1 to 50:1 mole ratio of buffer:alendronate (where the moles of buffer are taken as the combined moles of the buffer ingredients, e.g., sodium citrate and citric acid) of formulation to maintain a pH in the range of 4 to 8 and generally, a 1:1 to 10:1 mole ratio of buffer (combined) to drug present is used.

15 A useful buffer in the invention is sodium citrate/citric acid in the range of 5 to 50 mg per ml of sodium citrate to 1 to 15 mg per ml of citric acid, sufficient to maintain an aqueous pH of 4-6 of the composition.

20 The buffer agent may also be present to prevent the precipitation of the drug through soluble metal complex formation with dissolved metal ions, e.g., Ca, Mg, Fe, Al, Ba, which may leach out of glass containers or rubber stoppers or be present in ordinary tap water. The agent may act as a competitive complexing agent with the drug and produce a soluble metal complex leading to the presence of undesirable particulates.

25 In addition, the presence of an agent, e.g., sodium chloride in an amount of about of 1-8 mg/ml, to adjust the tonicity to the same value of human blood may be required to avoid the swelling or shrinkage of erythrocytes upon administration of the intravenous formulation leading to undesirable side effects such as nausea or diarrhea and possibly to associated blood disorders. In general, the tonicity of the formulation matches that of human blood which is in the range of 282 to 288 mOsm/kg, and in general is 285 mOsm/kg, which is equivalent to the osmotic pressure corresponding to a 0.9% solution of sodium chloride.

30 The intravenous formulation can be administered by direct intravenous injection, i.v. bolus, or can be administered by infusion by addition to an appropriate infusion solution such as 0.9% sodium chloride injection or other compatible infusion solution.

35 The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 100 mg, more usually about 10 to about 30 mg, of the active ingredient. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined

quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

The conjugate is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It, will be understood, however, that the amount of the conjugate actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above containing from, for example, 0.1 to about 500 mg of the active ingredient of the present invention.

The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described *supra*. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically

acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be breathed directly from the nebulizing device or the nebulizing device may be attached to a face masks tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner. For inhalation or insufflation administration, it is preferred that the total molecular weight of the conjugate is between about 10,000 Daltons and 70,000 Daltons, more preferably between about 20,000 Daltons and 45,000 Daltons.

10 Polymer conjugates

Compounds of this invention as formulated and administered are polymer conjugates. Polymer conjugates are anticipated to provide benefits over non-conjugated polymers, such as improved solubility and *in vivo* stability.

As such, single polymer molecule may be employed for conjugation with the compounds of the present invention, although it is also contemplated that more than one polymer molecule can be attached as well, typically through a carrier. The conjugated compounds of the present invention may find utility in both *in vivo* as well as non-*in vivo* applications. Additionally, it will be recognized that the conjugating polymer may utilize any other groups, moieties, or other conjugated species, as appropriate to the end use application. As an example, it may be advantageous in some applications to functionalize the polymer to render it reactive and enable it to conjugate to a compound of formula II and to enhance various properties or characteristics of the overall conjugated material. Accordingly, the polymer may contain any functionality, repeating groups, linkages, or other constituent structures which do not preclude the efficacy of the conjugated compounds of the present invention for its intended purpose.

Illustrative polymers that are usefully employed to achieve these desirable characteristics are described *supra*, as well as in PCT WO 01/54690 (to Zheng *et al.*) incorporated by reference herein in its entirety. The polymer may be coupled to the compounds of the present invention (preferably via a linker moiety) to form stable bonds that are not significantly cleavable by human enzymes. Generally, for a bond to be not 'significantly' cleavable requires that no more than about 20% of the bonds connecting the polymer and the compounds of the present invention to which the polymer is linked, are cleaved within a 24 hour period, as measured by standard techniques in the art including, but not limited to, high pressure liquid chromatography (HPLC).

Generally, the compounds of this invention contain at least about 2 compounds of formula II bound to a polymer. The final amount is a balance between maximizing the extent of the reaction while minimizing non-specific modifications of the product and, at the same

time, defining chemistries that will maintain optimum activity, while at the same time optimizing the half-life of the compounds of the present invention. Preferably, at least about 50% of the biological activity of the compounds of the present invention is retained, and most preferably 100% is retained.

5 As noted above in the preferred practice of the present invention, polyalkylene glycol residues of C₂-C₄ alkyl polyalkylene glycols, preferably polyethylene glycol (PEG), or poly(oxy)alkylene glycol residues of such glycols are advantageously incorporated in the polymer systems of interest. Thus, the polymer to which the compounds of the present invention are attached may be a homopolymer of polyethylene glycol (PEG) or is a
10 polyoxyethylated polyol, provided in all cases that the polymer is soluble in water at room temperature. Non-limiting examples of such polymers include polyalkylene oxide homopolymers such as PEG or polypropylene glycols, polyoxyethylenated glycols, copolymers thereof and block copolymers thereof, provided that the water solubility of the *block copolymer* is maintained.

15 Examples of polyoxyethylated polyols include, but are not limited to, polyoxyethylated glycerol, polyoxyethylated sorbitol, polyoxyethylated glucose, or the like. The glycerol backbone of polyoxyethylated glycerol is the same backbone occurring naturally in, for example, animals and humans in mono-, di-, and triglycerides. Therefore, this branching would not necessarily be seen as a foreign agent in the body.

20 Those of ordinary skill in the art will recognize that the foregoing list is merely illustrative and that all polymer materials having the qualities described herein are contemplated. The polymer need not have any particular molecular weight, but it is preferred that the molecular weight be between about 100 and 100,000, preferably from about 10,000 to 80,000; more preferably from about 20,000 to about 70,000. In particular, sizes of 20,000 or more are most
25 effective at preventing loss of the product due to filtration in the kidneys.

 By PEG derivative is meant a polyethylene glycol polymer in which one or both of the terminal hydroxyl groups found in polyethylene glycol itself has been modified. Examples of suitable modifications include replacing one or both hydroxyl group(s) with alternative functional groups, which may be protected or unprotected, with low molecular weight ligands,
30 or with another macromolecule or polymer. Modification of the terminal hydroxyl groups in the polyethylene glycol may be achieved by reacting the polyethylene glycol with compounds comprising complementary reactive functional groups, including functional groups which are able to undergo a reaction with the hydroxyl groups in polyethylene glycol. The PEG derivatives of the compounds of this invention may contain one or more polyethylene glycol
35 (PEG) substituents covalently attached thereto by a linking group.

The following formulation examples illustrate the pharmaceutical compositions of the present invention.

Formulation Example 1

5 Hard gelatin capsules containing the following ingredients are prepared:

Ingredient	Quantity (mg/capsule)
Active Ingredient	30.0
Starch	305.0
Magnesium stearate	5.0

The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

10

Formulation Example 2

A tablet formula is prepared using the ingredients below:

Ingredient	Quantity (mg/tablet)
Active Ingredient	25.0
Cellulose, microcrystalline	200.0
Colloidal silicon dioxide	10.0
Stearic acid	5.0

15 The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Example 3

A dry powder inhaler formulation is prepared containing the following components:

Ingredient	Weight %
Active Ingredient	5
Lactose	95

20

The active ingredient is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

Formulation Example 4

5 Tablets, each containing 30 mg of active ingredient, are prepared as follows:

Ingredient	Quantity (mg/tablet)
Active Ingredient	30.0 mg
Starch	45.0 mg
Microcrystalline cellulose	35.0 mg
Polyvinylpyrrolidone (as 10% solution in sterile water)	4.0 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1.0 mg
Total	120 mg

10 The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 50°C to 60°C and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a
15 tablet machine to yield tablets each weighing 120 mg.

Formulation Example 5

Capsules, each containing 40 mg of medicament are made as follows:

Ingredient	Quantity (mg/capsule)
Active Ingredient	40.0 mg
Starch	109.0 mg
Magnesium stearate	1.0 mg

Formulation Example 8

	Quantity
Ingredient	(mg/capsule)
Active Ingredient	15.0 mg
Starch	407.0 mg
Magnesium stearate	3.0 mg
Total	425.0 mg

- 5 The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 425.0 mg quantities.

Formulation Example 9

A subcutaneous formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	50 mg.mL mg
Phosphate buffered saline	1.0 ml

10

Formulation Example 10

A topical formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	1-10 g
Emulsifying Wax	30 g
Liquid Paraffin	20 g
White Soft Paraffin	to 100 g

- 15 The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

Formulation Example 11

- 20 An intravenous formulation may be prepared as follows:

Ingredient	Quantity
------------	----------

Active Ingredient	250 mg
Isotonic saline	100 ml

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, *e.g.*, U.S. Patent 5,023,252, issued June 11, 1991, herein incorporated by reference. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Frequently, it will be desirable or necessary to introduce the pharmaceutical composition to the brain, either directly or indirectly. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system used for the transport of biological factors to specific anatomical regions of the body is described in U.S. Patent 5,011,472 which is herein incorporated by reference.

Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions which can transiently open the blood-brain barrier.

Other suitable formulations for use in the present invention can be found in Remington's Pharmaceutical Sciences, Mace Publishing Company, Philadelphia, PA, 17th ed. (1985).

As noted above, the compounds described herein are suitable for use in a variety of drug delivery systems described above. Additionally, in order to enhance the *in vivo* serum half-life of the administered compound, the compounds may be encapsulated, introduced into the lumen of liposomes, prepared as a colloid, or other conventional techniques may be employed which provide an extended serum half-life of the compounds. A variety of methods are available for preparing liposomes, as described in, *e.g.*, Szoka, et al., U.S. Patent Nos. 4,235,871, 4,501,728 and 4,837,028 each of which is incorporated herein by reference.

Utility

The conjugates of this invention are VLA-4 antagonists. Some also have at least a partial affinity for alpha4 beta7 integrins. The conjugates provide enhanced in vivo retention as compared to the non-conjugated compounds. The improved retention of the conjugate
5 within the body results in lower required dosages of the drug, which in turn results in fewer side effects and reduced likelihood of toxicity. In addition, the drug formulation may be administered less frequently to the patient while achieving a similar or improved therapeutic effect.

The conjugates of this invention have improved inhibition, in vivo, of adhesion of
10 leukocytes to endothelial cells mediated by inhibition of alpha4 beta1 or alpha4 beta7 binding to cellular receptors such as VCAM-1, fibronectin and MadCAM. Preferably, the conjugates of this invention can be used, e.g., by infusion, or by subcutaneous injection or oral administration, for the treatment of diseases mediated by alpha4 beta1 or alpha4 beta7 or, in general terms, leucocyte adhesion. The conjugates of the invention can be used to treat a
15 variety of inflammatory brain disorders, especially central nervous system disorders in which the endothelium/leukocyte adhesion mechanism results in destruction to otherwise healthy brain tissue. Thus, the conjugates of the invention can be used for, e.g., the treatment of experimental autoimmune encephalomyelitis (EAE), multiple sclerosis (MS), meningitis, and encephalitis.

The conjugates of the invention can also be used to treat disorders and diseases due
20 to tissue damage in other organ systems, i.e., where tissue damage also occurs via an adhesion mechanism resulting in migration or activation of leukocytes. Examples of such diseases in mammalian patients are inflammatory diseases such as asthma, Alzheimer's disease, atherosclerosis, AIDS dementia, diabetes (including acute juvenile onset diabetes),
25 inflammatory bowel disease (including ulcerative colitis and Crohn's disease), rheumatoid arthritis, tissue transplantation rejection, tumor metastasis, stroke, and other cerebral traumas, nephritis, retinitis, atopic dermatitis, psoriasis, myocardial ischemia and acute leukocyte-mediated lung injury such as that which occurs in adult respiratory distress syndrome.

Still other disease conditions which may be treated using conjugates of the invention
30 include erythema nodosum, allergic conjunctivitis, optic neuritis, uveitis, allergic rhinitis, ankylosing spondylitis, psoriatic arthritis, vasculitis, Reiter's syndrome, systemic lupus erythematosus, progressive systemic sclerosis, polymyositis, dermatomyositis, Wegner's granulomatosis, aortitis, sarcoidosis, lymphocytopenia, temporal arteritis, pericarditis,
35 myocarditis, congestive heart failure, polyarteritis nodosa, hypersensitivity syndromes, allergy, hypereosinophilic syndromes, Churg-Strauss syndrome, chronic obstructive pulmonary

disease, hypersensitivity pneumonitis, chronic active hepatitis, interstitial cystitis, autoimmune endocrine failure, primary biliary cirrhosis, autoimmune aplastic anemia, chronic persistent hepatitis and thyroiditis.

The invention also provides methods for treating a disease state caused or
5 exacerbated at least in part by alpha 4 integrin-mediated leukocyte binding in a patient, which methods comprise co-administration of an effective amount of a conjugate of the invention, e.g., a conjugate of Formula I, and an effective amount of a separate compound which is an $\alpha_4\beta_7$ inhibitor. The co-administration can be carried out simultaneously or sequentially. For example, administration of the conjugate of the invention can precede administration of the
10 $\alpha_4\beta_7$ inhibitor by minutes or hours. Alternatively, the $\alpha_4\beta_7$ inhibitor can be administered prior to the conjugate of the invention.

Appropriate in vivo models for demonstrating efficacy in treating inflammatory responses include EAE (experimental autoimmune encephalomyelitis) in mice, rats, guinea pigs or primates, as well as other inflammatory models dependent upon α_4 integrins.

15 Inflammatory bowel disease is a collective term for two similar diseases referred to as Crohn's disease and ulcerative colitis. Crohn's disease is an idiopathic, chronic ulceroinflammatory disease characterized by sharply delimited and typically transmural involvement of all layers of the bowel wall by a granulomatous inflammatory reaction. Any segment of the gastrointestinal tract, from the mouth to the anus, may be
20 involved, although the disease most commonly affects the terminal ileum and/or colon. Ulcerative colitis is an inflammatory response limited largely to the colonic mucosa and submucosa. Lymphocytes and macrophages are numerous in lesions of inflammatory bowel disease and may contribute to inflammatory injury.

Asthma is a disease characterized by increased responsiveness of the
25 tracheobronchial tree to various stimuli potentiating paroxysmal constriction of the bronchial airways. The stimuli cause release of various mediators of inflammation from IgE-coated mast cells including histamine, eosinophilic and neutrophilic chemotactic factors, leukotrienes, prostaglandin and platelet activating factor. Release of these factors recruits basophils, eosinophils and neutrophils, which cause inflammatory injury.

30 Atherosclerosis is a disease of arteries (e.g., coronary, carotid, aorta and iliac). The basic lesion, the atheroma, consists of a raised focal plaque within the intima, having a core of lipid and a covering fibrous cap. Atheromas compromise arterial blood flow and weaken affected arteries. Myocardial and cerebral infarcts are a major consequence of this disease. Macrophages and leukocytes are recruited to atheromas and contribute to inflammatory
35 injury.

Rheumatoid arthritis is a chronic, relapsing inflammatory disease that primarily causes

impairment and destruction of joints. Rheumatoid arthritis usually first affects the small joints of the hands and feet but then may involve the wrists, elbows, ankles and knees. The arthritis results from interaction of synovial cells with leukocytes that infiltrate from the circulation into the synovial lining of the joints. See e.g., Paul, Immunology (3d ed., Raven Press, 1993).

5 Another indication for the conjugates of this invention is in treatment of organ or graft rejection mediated by VLA-4. Over recent years there has been a considerable improvement in the efficiency of surgical techniques for transplanting tissues and organs such as skin, kidney, liver, heart, lung, pancreas and bone marrow. Perhaps the principal outstanding problem is the lack of satisfactory agents for inducing immunotolerance in the recipient to the
10 transplanted allograft or organ. When allogeneic cells or organs are transplanted into a host (i.e., the donor and donee are different individuals from the same species), the host immune system is likely to mount an immune response to foreign antigens in the transplant (host-versus-graft disease) leading to destruction of the transplanted tissue. CD8⁺ cells, CD4 cells and monocytes are all involved in the rejection of transplant tissues. Conjugates of this
15 invention which bind to alpha-4 integrin are useful, inter alia, to block alloantigen-induced immune responses in the donee thereby preventing such cells from participating in the destruction of the transplanted tissue or organ. See, e.g., Paul et al., Transplant International 9, 420-425 (1996); Georczynski et al., Immunology 87, 573-580 (1996); Georczynski et al., Transplant. Immunol. 3, 55-61 (1995); Yang et al., Transplantation 60, 71-76 (1995);
20 Anderson et al., APMIS 102, 23-27 (1994).

A related use for conjugates of this invention which bind to VLA-4 is in modulating the immune response involved in "graft versus host" disease (GVHD). See e.g., Schlegel et al., J. Immunol. 155, 3856-3865 (1995). GVHD is a potentially fatal disease that occurs when immunologically competent cells are transferred to an allogeneic recipient. In this situation,
25 the donor's immunocompetent cells may attack tissues in the recipient. Tissues of the skin, gut epithelia and liver are frequent targets and may be destroyed during the course of GVHD. The disease presents an especially severe problem when immune tissue is being transplanted, such as in bone marrow transplantation; but less severe GVHD has also been reported in other cases as well, including heart and liver transplants. The therapeutic agents
30 of the present invention are used, inter alia, to block activation of the donor T-cells thereby interfering with their ability to lyse target cells in the host.

The formulations of the present invention are especially useful in the treatment of multiple sclerosis, rheumatoid arthritis and asthma.

A further use of the conjugates of this invention is inhibiting tumor metastasis.
35 Several tumor cells have been reported to express VLA-4 and compounds which bind VLA-4 block adhesion of such cells to endothelial cells. Steinback et al., Urol. Res. 23, 175-83

(1995); Orosz et al., Int. J. Cancer 60, 867-71 (1995); Freedman et al., Leuk. Lymphoma 13, 47-52 (1994); Okahara et al., Cancer Res. 54, 3233-6 (1994).

Compounds having the desired biological activity may be modified as necessary to provide desired properties such as improved pharmacological properties (e.g., in vivo stability, bio-availability), or the ability to be detected in diagnostic applications. Stability can be assayed in a variety of ways such as by measuring the half-life of the proteins during incubation with peptidases or human plasma or serum. A number of such protein stability assays have been described (see, e.g., Verhoef et al., Eur. J. Drug Metab. Pharmacokinet., 1990, 15(2):83-93).

A further use of the conjugates of this invention is in treating multiple sclerosis. Multiple sclerosis is a progressive neurological autoimmune disease that affects an estimated 250,000 to 350,000 people in the United States. Multiple sclerosis is thought to be the result of a specific autoimmune reaction in which certain leukocytes attack and initiate the destruction of myelin, the insulating sheath covering nerve fibers. In an animal model for multiple sclerosis, murine monoclonal antibodies directed against VLA-4 have been shown to block the adhesion of leukocytes to the endothelium, and thus prevent inflammation of the central nervous system and subsequent paralysis in the animals¹⁶.

Pharmaceutical compositions of the invention are suitable for use in a variety of drug delivery systems. Suitable formulations for use in the present invention are found in Remington's Pharmaceutical Sciences, Mace Publishing Company, Philadelphia, PA, 17th ed. (1985).

The amount administered to the patient will vary depending upon what is being administered, the purpose of the administration, such as prophylaxis or therapy, the state of the patient, the manner of administration, and the like. In therapeutic applications, compositions are administered to a patient already suffering from a disease in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications.

An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on the disease condition being treated as well as by the judgment of the attending clinician depending upon factors such as the severity of the inflammation, the age, weight and general condition of the patient, and the like.

The compositions administered to a patient are in the form of pharmaceutical compositions described above. These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration.

The therapeutic dosage of the conjugates of the present invention will vary according

to, for example, the particular use for which the treatment is made, the manner of administration of the conjugate, the health and condition of the patient, and the judgment of the prescribing physician. For example, for intravenous administration, the dose will typically be in the range of about 20 μg to about 2000 μg per kilogram body weight, preferably about 20 μg to about 500 μg , more preferably about 100 μg to about 300 μg per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.1 μg to 1 mg per kilogram body weight. Effective doses can be extrapolated from dose-response curves derived from in vitro or animal model test systems.

Conjugates of this invention are also capable of binding or antagonizing the actions of $\alpha_6\beta_1$, $\alpha_9\beta_1$, $\alpha_4\beta_7$, $\alpha_d\beta_2$, $\alpha_e\beta_7$ integrins (although $\alpha_4\beta_1$ and $\alpha_9\beta_1$ are preferred in this invention). Accordingly, conjugates of this invention are also useful for preventing or reversing the symptoms, disorders or diseases induced by the binding of these integrins to their respective ligands.

For example, International Publication Number WO 98/53817, published December 3, 1998 (the disclosure of which is incorporated herein by reference in its entirety) and references cited therein describe disorders mediated by $\alpha_4\beta_7$. This reference also describes an assay for determining antagonism of $\alpha_4\beta_7$ dependent binding to VCAM-Ig fusion protein.

Additionally, compounds that bind $\alpha_d\beta_2$ and $\alpha_e\beta_7$ integrins are particularly useful for the treatment of asthma and related lung diseases. See, for example, M. H. Grayson et al., *J. Exp. Med.* **1998**, 188(11) 2187-2191. Compounds that bind $\alpha_e\beta_7$ integrin are also useful for the treatment of systemic lupus erythematosus (see, for example, M. Pang et al., *Arthritis Rheum.* **1998**, 41(8), 1456-1463); Crohn's disease, ulcerative colitis and inflammatory bowel disease (IBD) (see, for example, D. Elewaut et al., *Scand J. Gastroenterol* **1998**, 33(7) 743-748); Sjogren's syndrome (see, for example, U. Kroneld et al., *Scand J. Gastroenterol* **1998**, 27(3), 215-218); and rheumatoid arthritis (see, for example, *Scand J. Gastroenterol* **1996**, 44(3), 293-298). And compounds that bind $\alpha_6\beta_1$ may be useful in preventing fertilization (see, for example, H. Chen et al., *Chem. Biol.* **1999**, 6, 1-10).

In another aspect of the invention, the conjugates and compositions described herein can be used to inhibit immune cell migration from the bloodstream to the central nervous system in the instance of, for example, multiple sclerosis, or to areas which result in inflammatory-induced destruction of the myelin. Preferably, these reagents inhibit immune cell migration in a manner that inhibits demyelination and that further may promote remyelination. The reagents may also prevent demyelination and promote remyelination of the central nervous system for congenital metabolic disorders in which infiltrating immune cells affect the development myelin sheath, mainly in the CNS. The reagents preferably also reduce paralysis when administered to a subject with paralysis induced by a demyelinating

disease or condition.

Inflammatory diseases that are included for treatment by the compositions, conjugates and methods disclosed herein include generally conditions relating to demyelination.

Histologically, myelin abnormalities are either demyelinating or dysmyelinating.

5 Demyelination implies the destruction of myelin. Dysmyelination refers to defective formation or maintenance of myelin resulting from dysfunction of the oligodendrocytes. Preferably, the compositions and methods disclosed herein are contemplated to treat diseases and conditions relating to demyelination and aid with remyelination. Additional diseases or conditions contemplated for treatment include meningitis, encephalitis, and spinal cord
10 injuries and conditions generally which induce demyelination as a result of an inflammatory response. The conjugates, compositions and methods disclosed herein are not directed towards diseases and conditions wherein there is, for example, a genetic defect leading to improper myelin formation, e.g., dysmyelination.

The compositions, conjugates and cocktails disclosed herein are contemplated for
15 use in treating conditions and diseases associated with demyelination. Diseases and conditions involving demyelination include, but are not limited to, multiple sclerosis, congenital metabolic disorders (e.g., phenylketonuria, Tay-Sachs disease, Niemann-Pick disease, Gaucher's disease, Hurler's syndrome, Krabbe's disease and other leukodystrophies), neuropathies with abnormal myelination (e.g., Guillain Barré, chronic immune demyelinating
20 polyneuropathy (CIDP), multifocal CIDP, anti-MAG syndrome, GALOP syndrome, anti-sulfatide antibody syndrome, anti-GM2 antibody syndrome, POEMS syndrome, perineuritis, IgM anti-GD1b antibody syndrome), drug related demyelination (e.g., caused by the administration of chloroquine, FK506, perhexiline, procainamide, and zimeldine), other hereditary demyelinating conditions (e.g., carbohydrate-deficient glycoprotein, Cockayne's
25 syndrome, congenital hypomyelinating, congenital muscular dystrophy, Farber's disease, Marinesco-Sjögren syndrome, metachromatic leukodystrophy, Pelizaeus-Merzbacher disease, Refsum disease, prion related conditions, and Salla disease) and other demyelinating conditions (e.g., meningitis, encephalitis or spinal cord injury) or diseases.

There are various disease models that can be used to study these diseases in vivo.
30 For example, animal models include but are not limited to:

Table III

Disease Model	Species
EAE	Mouse, rat, guinea pig
Myelin-oligodendrocyte glycoprotein (MOG) induced EAE	Rat
TNF- α transgenic model of demyelination	Mouse

Multiple Sclerosis

The most common demyelinating disease is multiple sclerosis, but many other metabolic and inflammatory disorders result in deficient or abnormal myelination. MS is a chronic neurologic disease, which appears in early adulthood and progresses to a significant disability in most cases. There are approximately 350,000 cases of MS in the United States alone. Outside of trauma, MS is the most frequent cause of neurologic disability in early to middle adulthood.

The cause of MS is yet to be determined. MS is characterized by chronic inflammation, demyelination and gliosis (scarring). Demyelination may result in either negative or positive effects on axonal conduction. Positive conduction abnormalities include slowed axonal conduction, variable conduction block that occurs in the presence of high-but not low-frequency trains of impulses or complete conduction block. Positive conduction abnormalities include ectopic impulse generation, spontaneously or following mechanical stress and abnormal "cross-talk" between demyelinated axons.

T cells reactive against myelin proteins, either myelin basic protein (MBP) or myelin proteolipid protein (PLP) have been observed to mediate CNS inflammation in experimental allergic encephalomyelitis. Patients have also been observed as having elevated levels of CNS immunoglobulin (Ig). It is further possible that some of the tissue damage observed in MS is mediated by cytokine products of activated T cells, macrophages or astrocytes.

Today, 80% patients diagnosed with MS live 20 years after onset of illness. Therapies for managing MS include: (1) treatment aimed at modification of the disease course, including treatment of acute exacerbation and directed to long-term suppression of the disease; (2) treatment of the symptoms of MS; (3) prevention and treatment of medical complications; and (4) management of secondary personal and social problems.

The onset of MS may be dramatic or so mild as to not cause a patient to seek medical attention. The most common symptoms include weakness in one or more limbs, visual blurring due to optic neuritis, sensory disturbances, diplopia and ataxia. The course of disease may be stratified into three general categories: (1) relapsing MS, (2) chronic progressive MS, and (3) inactive MS. Relapsing MS is characterized by recurrent attacks of neurologic dysfunction. MS attacks generally evolve over days to weeks and may be followed by complete, partial or no recovery. Recovery from attacks generally occurs within weeks to several months from the peak of symptoms, although rarely some recovery may continue for 2 or more years.

Chronic progressive MS results in gradually progressive worsening without periods of stabilization or remission. This form develops in patients with a prior history of relapsing MS,

although in 20% of patients, no relapses can be recalled. Acute relapses also may occur during the progressive course.

A third form is inactive MS. Inactive MS is characterized by fixed neurologic deficits of variable magnitude. Most patients with inactive MS have an earlier history of relapsing MS.

5 Disease course is also dependent on the age of the patient. For example, favourable prognostic factors include early onset (excluding childhood), a relapsing course and little residual disability 5 years after onset. By contrast, poor prognosis is associated with a late age of onset (*i.e.*, age 40 or older) and a progressive course. These variables are interdependent, since chronic progressive MS tends to begin at a later age than relapsing MS.

10 Disability from chronic progressive MS is usually due to progressive paraplegia or quadriplegia (paralysis) in patients. In one aspect of the invention, patients will preferably be treated when the patient is in remission rather than in a relapsing stage of the disease.

Short-term use of either adrenocorticotrophic hormone or oral corticosteroids (*e.g.*, oral prednisone or intravenous methylprednisolone) is the only specific therapeutic measure for
15 treating patients with acute exacerbation of MS.

Newer therapies for MS include treating the patient with interferon beta-1b, interferon beta-1a, and Copaxone® (formerly known as copolymer 1). These three drugs have been shown to significantly reduce the relapse rate of the disease. These drugs are self-administered intramuscularly or subcutaneously.

20 However, none of the current treatment modalities inhibit demyelination, let alone promotes or allows spontaneous remyelination or reduces paralysis. One aspect of the invention contemplates treating MS with agents disclosed herein either alone or in combination with other standard treatment modalities.

25 Congenital Metabolic Disorders

Congenital metabolic disorders include phenylketonuria (PKU) and other aminoacidurias, Tay-Sachs disease, Niemann-Pick disease, Gaucher's disease, Hurler's syndrome, Krabbe's disease and other leukodystrophies that impact the developing sheath as described more fully below.

30 PKU is an inherited error of metabolism caused by a deficiency in the enzyme phenylalanine hydroxylase. Loss of this enzyme results in mental retardation, organ damage, unusual posture and can, in cases of maternal PKU, severely compromise pregnancy. A model for studying PKU has been discovered in mice. Preferably infants identified with PKU are sustained on a phenylalanine free or lowered diet. An aspect of the invention would be to
35 combine such diets with the conjugates and compositions disclosed herein to prevent demyelination and remyelinate cells damaged due to PKU.

Classical Tay-Sachs disease appears in the subject at about age 6 months and will eventually result in the death of the subject by age 5 years. The disease is due to the lack of the enzyme, hexoaminidase A (hex A), which is necessary for degrading certain fatty substances in the brain and nerve cells. The substances in the absence of the enzyme
5 accumulate and lead to the destruction of nerve cells. Another form of hex A enzyme deficiency occurs later in life and is referred to as juvenile, chronic and adult onset forms of hex A deficiency. Symptoms are similar to those that characterize classical Tay-Sachs disease. There is also an adult onset form of the enzyme deficiency. Currently there is no cure or treatment for the disease/deficiency, only the preventative measure of *in utero* testing
10 of the fetus for the disease. Thus, the conjugates and compositions disclosed herein may be useful in ameliorating or preventing the destruction of nerve cells in such patients.

Niemann-Pick disease falls into three categories: the acute infantile form, Type B is a less common, chronic, non-neurological form, and Type C is a biochemically and genetically distinct form of the disease. In a normal individual, cellular cholesterol is imported into
15 lysosomes for processing, after which it is released. Cells taken from subjects with Niemann-Pick have been shown to be defective in releasing cholesterol from lysosomes. This leads to an excessive build-up of cholesterol inside lysosomes, causing processing errors. NPC1 was found to have known sterol-sensing regions similar to those in other proteins, which suggests it plays a role in regulating cholesterol traffic. No successful therapies have been identified
20 for Types A and C forms of Neumann-Pick. For Type C, patients are recommended to follow a low-cholesterol diet. Thus, the conjugates and compositions disclosed herein may be useful in ameliorating or preventing the destruction of the cells.

Gaucher's disease is an inherited illness caused by a gene mutation. Normally, this gene is responsible for an enzyme called glucocerebrosidase that the body needs to break
25 down the fat, glucocerebroside. In patients with Gaucher's disease, the body is not able to properly produce this enzyme and the fat cannot be broken down. Like Tay-Sachs disease, Gaucher's disease is considerably more common in the descendants of Jewish people from Eastern Europe (Ashkenazi), although individuals from any ethnic group may be affected. Among the Ashkenazi Jewish population, Gaucher's disease is the most common genetic
30 disorder, with an incidence of approximately 1 in 450 persons. In the general public, Gaucher's disease affects approximately 1 in 100,000 persons.

In 1991, enzyme replacement therapy became available as the first effective treatment for Gaucher's disease. The treatment consists of a modified form of the glucocerebrosidase enzyme given intravenously. It is contemplated that the compositions
35 and conjugates disclosed herein can be used alone or more preferably in combination with glycocerebrosidase administration to treat the disease in an afflicted subject.

Hurler's syndrome, also known as mucopolysaccharidosis type I, is a class of overlapping diseases. These genetic diseases share in common the cellular accumulation of mucopolysaccharides in fibroblasts. The diseases are genetically distinguishable. Fibroblast and bone marrow transplantation does not seem to be helpful, thus conjugates and compositions useful in ameliorating disease severity and progression are needed. The conjugates and compositions disclosed herein may be administered to a subject to ameliorate disease progression and/or severity.

Krabbe's disease (also known as Globoid cell leukodystrophy) is an autosomal recessive condition resulting from galactosylceramidase (or galactocerebrosidase) deficiency, a lysosomal enzyme that catabolises a major lipid component of myelin. Incidence in France is an estimated 1:150,000 births. The disease leads to demyelination of the central and peripheral nervous system. Onset generally occurs during the first year of life and the condition is rapidly progressive, but juvenile, adolescent or adult onset forms have also been reported, with a more variable rate of progression. Diagnosis is established from enzyme assay (galactosylceramidase deficiency). There are several natural animal models (mouse, dog, monkey). Krabbe's disease, like all leukodystrophies, has no known cures or effective treatments. One embodiment of the instant invention is to use the compositions and conjugates disclosed herein to treat or ameliorate Krabbe's disease and other leukodystrophies.

Leukodystrophies are a group of genetically determined progressive disorders that affect the brain, spinal cord and peripheral nerves. They include adrenoleukodystrophy (ALD), adrenomyeloneuropathy (AMN), Aicardi-Goutiers syndrome, Alexander's disease, CACH (*i.e.*, childhood ataxia with central nervous system hypomyelination or vanishing white matter disease), CADASIL (*i.e.*, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), Canavan disease (spongy degeneration), Cerebrotendinous Xanthomatosis (CTX), Krabbe's disease (discussed above), metachromatic leukodystrophy (MLD), neonatal adrenoleukodystrophy, ovarioleukodystrophy syndrome, Pelizaeus-Merzbacher disease (X-linked spastic paraplegia), Refsum disease, van der Knaap syndrome (vacuulating leukodystrophy with subcortical cysts) and Zellweger syndrome. None of the diseases have effective treatments let alone cures. Consequently, means of treating or ameliorating the symptoms of the disease, such as by using the compositions and conjugates disclosed herein, is needed.

Neuropathies with Abnormal Myelination

A variety of chronic immune polyneuropathies exist which result in demyelination in the patient. The age of onset for the conditions varies by condition. Standard treatments for

these diseases exist and could be combined with the compositions and conjugates disclosed herein. Alternatively, the compositions and conjugates disclosed can be used alone. Existing standard therapies include the following:

Table IV

Neuropathy	Clinical Features	Treatment
Chronic Immune Demyelinating Polyneuropathy (CIDP)	Onset between 1-80 years. Characterized by weakness, sensory loss, and nerve hypertrophy.	T-cell immunosuppression with prednisone, cyclosporine A or methotrexate, HIG, plasma exchange
Multifocal CIDP	Onset between 28 to 58 years and characterized by asymmetric weakness, sensory loss with a course that is slowly progressive or relapsing-remitting.	T cell immunosuppression with prednisone Human immunoglobulin (HIG)
Multifocal Motor Neuropathy (MMN)	Onset ranges from 25 to 70 years, with twice as many men as women. Features include weakness, muscle atrophy, fasciculations, and cramps which are progressive over 1-30 years.	HIG B cell immunosuppression with plasma exchange cyclophosphamide, Rituxan
Neuropathy with IgM binding to Myelin-Associated Glycoprotein (MAG)	Onset is usually over age 50 and is characterized by sensory loss (100%), weakness, gait disorder, tremor which is all slowly progressive.	B-cell immunosuppression plasma exchange cyclophosphamide Rituxan α -interferon cladribine or fludarabine prednisone
GALOP Syndrome (<u>G</u> ait disorder, <u>A</u> utoantibody, <u>L</u> ate-age, <u>O</u> nset, <u>P</u> olyneuropathy)	A gait disorder with polyneuropathy	HIG Plasma exchange cyclophosphamide
POEMS Syndrome (<u>P</u> olyneuropathy, <u>O</u> rganomegaly, <u>E</u> ndocrinopathy, <u>M</u> -Protein and <u>S</u> kin changes) also known as Crow-Fukase Syndrome and Takatsuki disease	Onset occurs between 27 and 80 years with weakness, sensory loss, reduced or absent tendon reflexes, skin disorders and other features.	Osteosclerotic lesions are treated with irradiation. Widespread lesions with chemotherapy (Melfalan and prednisone).

5

Drug and Radiation Induced Demyelination

Certain drugs and radiation can induce demyelination in subjects. Drugs that are responsible for demyelination include but are not limited to chloroquine, FK506, perhexiline, procainamide, and zimeldine.

10

Radiation also can induce demyelination. Central nervous system (CNS) toxicity due to radiation is believed to be caused by (1) damage to vessel structures, (2) deletion of oligodendrocyte-2 astrocyte progenitors and mature oligodendrocytes, (3) deletion of neural stem cell populations in the hippocampus, cerebellum and cortex, and generalized alterations of cytokine expression. Most radiation damage results from radiotherapies administered during the treatment of certain cancers. See for review Belka *et al.*, 2001 *Br. J. Cancer* 85: 1233-9. However, radiation exposure may also be an issue for astronauts (Hopewell, 1994 *Adv. Space Res.* 14: 433-42) as well as in the event of exposure to radioactive substances.

Patients who have received drugs or been exposed accidentally or intentionally to radiation may experience a benefit by administered one of the conjugates or compositions disclosed herein to prevent demyelination or to promote remyelination.

Conditions Involving Demyelination

Additional inherited syndromes/diseases that result in demyelination include Cockayne's syndrome, congenital hypomyelinating, Farber's disease, metachromatic leukodystrophy, Pelizaeus-Merzbacher disease, Refsum, prion related conditions and Salla disease.

Cockayne's syndrome (CS) is a rare inherited disorder in which people are sensitive to sunlight, have short stature and have the appearance of premature aging. In the classical form of Cockayne's syndrome (Type I), the symptoms are progressive and typically become apparent after the age of one year. An early onset or congenital form of Cockayne's syndrome (Type II) is apparent at birth. Interestingly, unlike other DNA repair diseases, Cockayne's syndrome is not linked to cancer. CS is a multi-system disorder that causes both profound growth failure of the soma and brain and progressive cachexia, retinal, cochlear, and neurologic degeneration, with a leukodystrophy and demyelinating neuropathy without an increase in cancer. After exposure to UV (e.g., sunlight), subjects with Cockayne's syndrome can no longer perform transcription-coupled repair. Two genes defective in Cockayne's syndrome, CSA and CSB, have been identified so far. The CSA gene is found on chromosome 5. Both genes code for proteins that interact with components of the transcriptional machinery and with DNA repair proteins.

To date, no cures or effective treatments for patients with this disease have been identified. Thus, one aspect of the invention is treatment of this disease with the conjugates and compositions disclosed herein.

Congenital hypomyelination has several names including congenital dysmyelinating neuropathy, congenital hypomyelinating polyneuropathy, congenital hypomyelination (Onion Bulb) polyneuropathy, congenital hypomyelination neuropathy, congenital neuropathy caused by hypomyelination, hypomyelination neuropathy and CHN. Hereditary peripheral

neuropathies, among the most common genetic disorders in humans, are a complex, clinically and genetically heterogeneous group of disorders that produce progressive deterioration of the peripheral nerves. Congenital hypomyelination is one of a group of disorders. This group includes hereditary neuropathy with liability to pressure palsies,
5 Charcot-Marie-Tooth disease, Dejerine-Sottas syndrome, and congenital hypomyelinating neuropathy. There are no known cures or effective treatments for any of these disorders.

Farber's disease has several names including: Farber lipogranulomatosis, ceramidase deficiency, acid ceramidase deficiency, AC deficiency, N-laurylsphingosine deacylase deficiency, and N-acylsphingosine amidohydrolase. As certain names reveal, the
10 disease occurs due to a deficiency of acid ceramidase (also known as N-acylsphingosine amidohydrolase, ASAH). The lack of the enzyme results in an accumulation of non-sulfonated acid mucopolysaccharide in the neurons and glial cells. Patients with the disease usually die before the age of 2 years.

Metachromatic leukodystrophy (MLD) is a genetic disorder caused by a deficiency of
15 the enzyme arylsulfatase A. It is one of a group of genetic disorders called the leukodystrophies that affect growth of the myelin sheath. There are three forms of MLD: late infantile, juvenile, and adult. In the late infantile form, which is the most common, onset of symptoms begins between ages 6 months and 2 years. The infant is usually normal at birth, but eventually loses previously gained abilities. Symptoms include hypotonia (low muscle
20 tone), speech abnormalities, loss of mental abilities, blindness, rigidity (*i.e.*, uncontrolled muscle tightness), convulsions, impaired swallowing, paralysis, and dementia. Symptoms of the juvenile form begin between ages 4 and 14, and include impaired school performance, mental deterioration, ataxia, seizures, and dementia. In the adult form, symptoms, which begin after age 16, may include impaired concentration, depression, psychiatric disturbances,
25 ataxia, tremor, and dementia. Seizures may occur in the adult form, but are less common than in the other forms. In all three forms mental deterioration is usually the first sign.

Peliszaeus-Merzbacher disease (also known as perinatal sudanophilic leukodystrophy) is an X-linked genetic disorder that causes an abnormality of a proteolipid protein. The abnormality results in an infant's death typically before the age of one year.

30 There are no known treatments or cures for the disease.

Refsum disease (also referred to as phytanic acid oxidase deficiency, heredopathia atactica polyneuritiformis or hereditary motor and sensory neuropathy IV, HMSN IV) is caused by mutations in the gene, which encodes phytanoyl-CoA hydroxylase (PAHX or PHYH). The major clinical features are retinitis pigmentosa, chronic polyneuropathy and cerebellar signs.
35 Phytanic acid, an unusual branched chain fatty acid (3,7,11,15-tetramethyl-hexadecanoic acid) accumulates in the tissues and body fluids of patients with the disease and is unable to

be metabolised due to the lack of PAHX. Plasmapheresis performed once or twice monthly effectively removes the acid from the body and permits liberalization of dietary restrictions limiting phytanic acid intake.

Prion related conditions include Gerstmann-Straussler disease (GSD), Creutzfeldt-Jakob disease (CJD), familial fatal insomnia and aberrant isoforms of the prion protein can act as infectious agents in these disorders as well as in kuru and scrapie (a disease found in sheep). The term prion derives from "protein infectious agent" (Prusiner, *Science* 216: 136-44, 1982). There is a proteolytic cleavage of the prion related protein (PRP) which results in an amyloidogenic peptide that polymerises into insoluble fibrils.

Salla disease and other types of sialurias are diseases involving problems with sialic acid storage. They are autosomal recessive neurodegenerative disorders that may present as a severe infantile form (*i.e.*, ISSD) or as a slowly progressive adult form that is prevalent in Finland (*i.e.*, Salla disease). The main symptoms are hypotonia, cerebellar ataxia and mental retardation. These conditions and diseases are also contemplated for palliative or ameliorating treatments.

Other conditions that result in demyelination include post-infectious encephalitis (also known as acute disseminated encephalomyelitis, ADEM), meningitis and injuries to the spinal cord. The compositions and conjugates disclosed herein are also contemplated for use in treating these other demyelinating conditions.

The following synthetic and biological examples are offered to illustrate this invention and are not to be construed in any way as limiting the scope of this invention. Unless otherwise stated, all temperatures are in degrees Celsius.

EXAMPLES

In the examples below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning.

ACN	=	acetonitrile
bs	=	broad singlet
d	=	Doublet
dd	=	doublet of doublets
Et ₃ N	=	triethylamine
g	=	Grams
h and hr	=	Hour
HPLC	=	High performance (or pressure) liquid chromatography

kg	=	kilogram
kDa	=	kilodalton
L	=	Liter
m	=	multiplet
M	=	Molar
mg	=	milligram
min	=	Minute
mL	=	milliliter
mm	=	millimeter
mM	=	millimolar
mmol	=	millimol
s	=	Singlet
sat.	=	saturated
t	=	Triplet
TFA	=	trifluoroacetic acid
TLC or tlc	=	thin layer chromatography
Ts	=	Tosyl
μL	=	microliter
μg	=	microgram
μm	=	micron or micrometer

General Methods: Proton (^1H) and carbon (^{13}C) nuclear magnetic resonance spectra (NMR) were obtained using a Gemini 2000 or Bruker Avance 300 spectrometer. The presence of the polyethylene glycol (PEG) protons can be detected by a large, broad singlet at 3.6 ppm. The integration of this signal can vary depending on the size of the PEG moiety. Presence of the conjugated VLA-4 antagonist can also be detected in the ^1H NMR spectra of conjugates. Thin layer chromatography was performed on pre-coated sheets of silica 60 F₂₅₄ (EMD 15341-1) or pre-coated MKC18F silica 60 Å (Whatman 4803-110). Mass spectrometry was performed on an Agilent mass spectrometer (LC/MSD VL) in positive ion single quad mode.

HPLC Methods for PEG products and PEG conjugates:

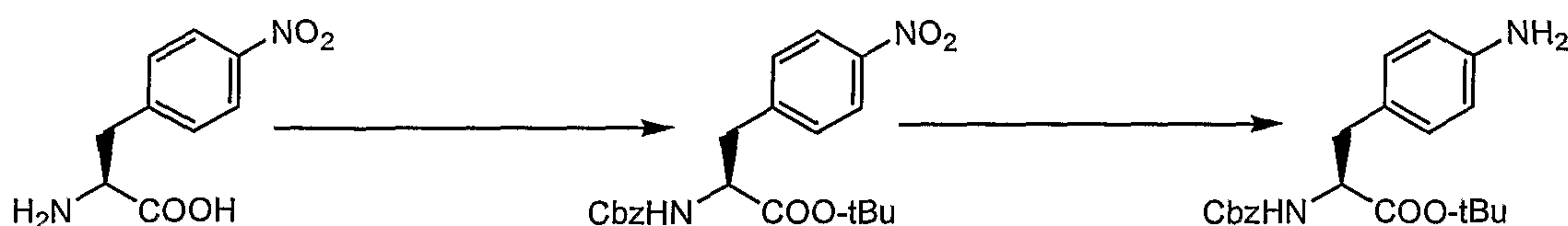
Preparative reverse phase HPLC was performed using a Varian Prep Star (Model SD-1) module with a Varian UV detector set at 210 nm. **Method A:** Samples of PEG products and PEG conjugates were purified using reverse phase HPLC on a Vydac C18, 300 Å pore size column (250 mm x 21.2 mm), typically using a gradient of 35-50% ACN + 0.1% TFA in 100 min at 20 mL/min. **Method B:** Samples of PEG products and conjugates were purified

using reverse phase HPLC on a Vydac C18, 300 Å pore size column (250 mm x 50 mm), typically using a gradient of 35-50% ACN + 0.1% TFA in 100 min at 60 mL/min.

Method C: The purity of PEG products and conjugates was confirmed via reverse phase analytical HPLC using an Agilent Series 1100 Quaternary system equipped with a Waters Symmetry 300 Å pore size, 3.5µ C18 column (150 mm x 4.6 mm), using a gradient of 40-50% ACN w/ 0.1% TFA at a flow rate of 1.5 mL/min. and coupled to an Agilent 1100 variable wavelength detector set at 210 nm and a Sedex 75 evaporative light scattering detector (40°C, gain=5)

PEG Reagents: PEG starting materials were acquired through NOF Corporation (Yebisu Garden Place Tower, 20-3 Ebisu 4-chome, Shibuya-ku, Tokyo 150-6019) or Nektar Therapeutics (150 Industrial Road, San Carlos, CA 94070) as follows: 40 kDa 4-arm PEG alcohol (NOF Cat. Sunbright PTE-40000); 40 kDa 3-arm PEG alcohol (NOF Cat. Sunbright GL-400).

Example 1



Sodium hydroxide (10 g, 0.25 m) is dissolved in water (300 ml). To this solution 4-nitrophenylalanine (50.3 g, 0.22 m) is added and stirred until complete dissolution. To the resulting solution the sodium carbonate (28.8 g, 0.26 m) is added and stirred suspension is cooled in an ice bath to +8°C. Benzyl chloroformate (44.7 g, 0.26 m) is added dropwise with vigorous stirring, maintaining internal temperature in +6° to +9°C range. The mixture is stirred at +6°C for additional 1 hr, transferred to the separatory funnel and washed with ether (2 x 150 ml). Aqueous phase is placed in a large Erlenmeyer flask (2L) and is cautiously acidified with dil. aq. HCl to pH=2 and extracted with ethyl acetate (4 x 500 ml). The combined extracts are washed with water and dried with MgSO₄. The solution is filtered and filtrate evaporated, residue is dissolved in ethyl acetate (150 ml) and diluted with hexane (500 ml). Crystalline material is filtered off and rinsed with cold solvent, air dried to give Cbz-4-nitrophenylalanine, 75 g (99.5% yield). ¹H-NMR, DMSO-d₆, (δ): 12.85 (bs, 1H), 8.12 (d, 2H, J=9Hz), 7.52 (d, 2H, J=9Hz), 7.30 (m, 5H), 4.95 (s, 2H), 4.28 (m, 1H), 3.32 (bs, 1H), 3.10 (m, 2H). ¹³C-NMR (δ): 173.1, 156.3, 146.6, 137.3, 130.8, 128.5, 128.0, 127.8, 123.5, 65.6, 55.1, 36.6. MS (m/z): 367.1 [M+23].

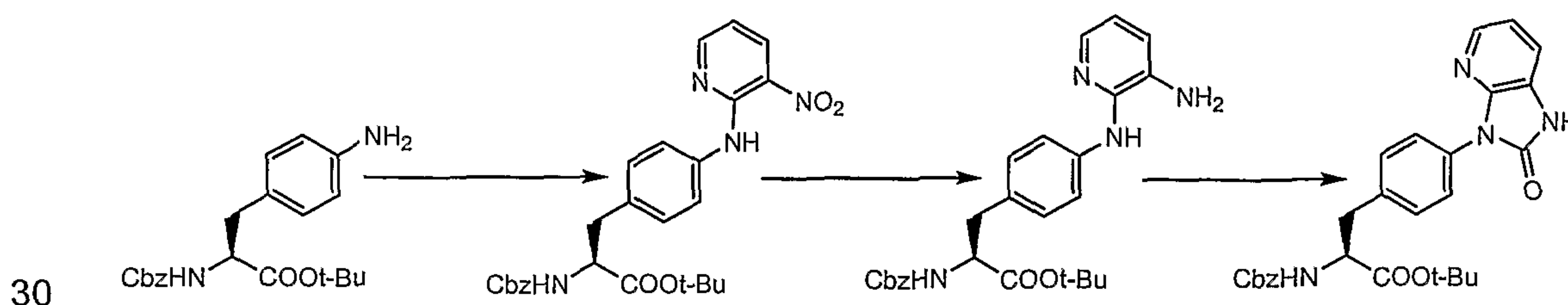
The Cbz-4-nitrophenylalanine (75 g, 0.22 m) is dissolved in dioxane (300 ml). The

resulted stirred solution is cooled in Dry Ice bath to -20°C (internal). The liquefied isobutylene (approx. 290 ml) is added followed by conc. sulfuric acid (35 ml) added in three equal portions, 30 min apart. The addition of acid is a very exothermic process, accompanied by substantial degree of polymerization. Efficient mechanical stirring is essential at this stage.

- 5 Resulted mixture is stirred for 20 hr, allowing to warm up to ambient temperature then is cautiously poured into sat. aq. sodium carbonate solution (2L) and diluted with ethyl acetate (600 ml). Organic layer is separated and aqueous layer is extracted with ethyl acetate (2 x 200 ml). Combined extracts are washed with water and dried with sodium sulfate. The solution is filtered and evaporated to dryness. The residue is taken up in ethyl acetate/hexane mixture (500 ml; 1:1) and filtered through plug of silica gel (ca. 2x2 in). The silica is rinsed with an additional amount of the same solvent (2 L total) and the filtrates are evaporated to give fully protected 4-nitrophenylalanine as a viscous oil, 73 g (83% after two steps). ¹H-NMR, CDCl₃, (δ): 8.12 (d, 2H, J=8.4Hz), 7.36 (m, 7H), 5.35 (m, 1H), 5.10 (m, 2H), 4.57 (m, 1H), 3.31 (m, 2H), 1.43 (s, 9H). ¹³C-NMR, CDCl₃, (δ): 169.7, 155.3, 146.9, 143.9, 136.0, 130.2, 128.4, 128.2, 128.0, 123.3, 82.9, 66.9, 54.7, 38.2, 31.4, 27.8, 13.9. MS (m/z): 423.1 [M+23].

- 15 Protected 4-nitrophenylalanine (73 g, 0.18 m) is dissolved in ethanol (500 ml) and platinum oxide catalyst (1.5 g) is added. The resulting solution is vigorously stirred in hydrogen atmosphere (50-60 psi) at ambient temperature until further hydrogen adsorption ceased (3 hr). The catalyst is filtered off and the filtrate is evaporated to dryness, the residue is taken up in ethyl acetate (200 ml) and filtered through plug of silica gel (2x2 in) using ethyl acetate-hexane mixture (3:2, 2L) to rinse silica. The filtrate is concentrated to approx. 200 ml and hexane (500 ml) is added. The crystalline product is filtered off, rinsed with cold solvent and air-dried. Yield – 56 g, 84%. ¹H-NMR, CDCl₃, (δ): 7.30 (bs, 5H), 6.92 (d, 2H, J=8.1Hz), 6.58 (d, 2H, J=8.1Hz), 5.21 (m, 1H), 5.10 (d, 2H, J=2.1Hz), 4.46 (m, 1H), 3.59 (bs, 2H), 2.97 (s, 2H, J=5.4Hz), 1.42 (s, 9H). ¹³C-NMR, CDCl₃, (δ): 170.6, 145.1, 136.3, 130.2, 128.3, 127.9, 125.6, 115.0, 81.9, 66.6, 55.2, 37.4, 27.8 MS (m/z): 393.1 [M+23].

Example 2



The product of Example 1, 4-aminophenylalanine, (20 g, 0.054 m) was dissolved in

ethanol (200 ml) and treated with Hunig's base (21 g, 0.162 m, 3 eq) and 2-chloro-3-nitropyridine (10.3 g, 0.65 m, 1.2 eq). Resulted solution was stirred under nitrogen atmosphere and heated to reflux for 24 hr. LC analysis indicated presence of small amount of unreacted amine. The small additional amount of chloronitropyridine (1.1 g, 0.13 eq) was
5 added and reflux continued for another 24 hr. Reaction mixture was cooled and evaporated to dryness. Residue was dissolved in ethyl acetate (600 ml) and obtained solution was washed with water (1 x 200 ml), dil. aq. citric acid (0.2 N, 2 x 200 ml), brine (1 x 200 ml) and dried with sodium sulfate. Solids were filtered off and filtrate evaporated to give 37 g of deep-red oil, containing expected product contaminated with excess of chloronitropyridine. Impure product
10 was purified by flash chromatography (Biotage 75L system) eluting with ethyl acetate:hexane (3:17) mixture. Fractions containing pure product were combined and evaporated to give deep-red, viscous oil, 26 g (99%). ¹H-NMR, CDCl₃, (δ): 10.10 (s, 1H), 8.49 (m, 2H), 7.57 (d, 2H, J=9Hz), 7.35 (bs, 5H), 7.19 (d, 2H, J=9Hz), 6.84 (m, 1H), 5.30 (m, 1H), 5.13 (d, 2H, J=3Hz), 4.57 (m, 1H), 3.11 (m, 2H), 1.45 (s, 9H). ¹³C-NMR, CDCl₃, (δ): 170.4, 155.5, 155.1,
15 150.0, 136.7, 136.3, 135.4, 132.4, 129.9, 128.5, 128.3, 128.0, 127.9, 122.2, 113.7, 82.2, 66.7, 55.1, 37.7, 27.8, 20.9. MS (m/z): 493.1 [M+1], 515.1 [M+23].

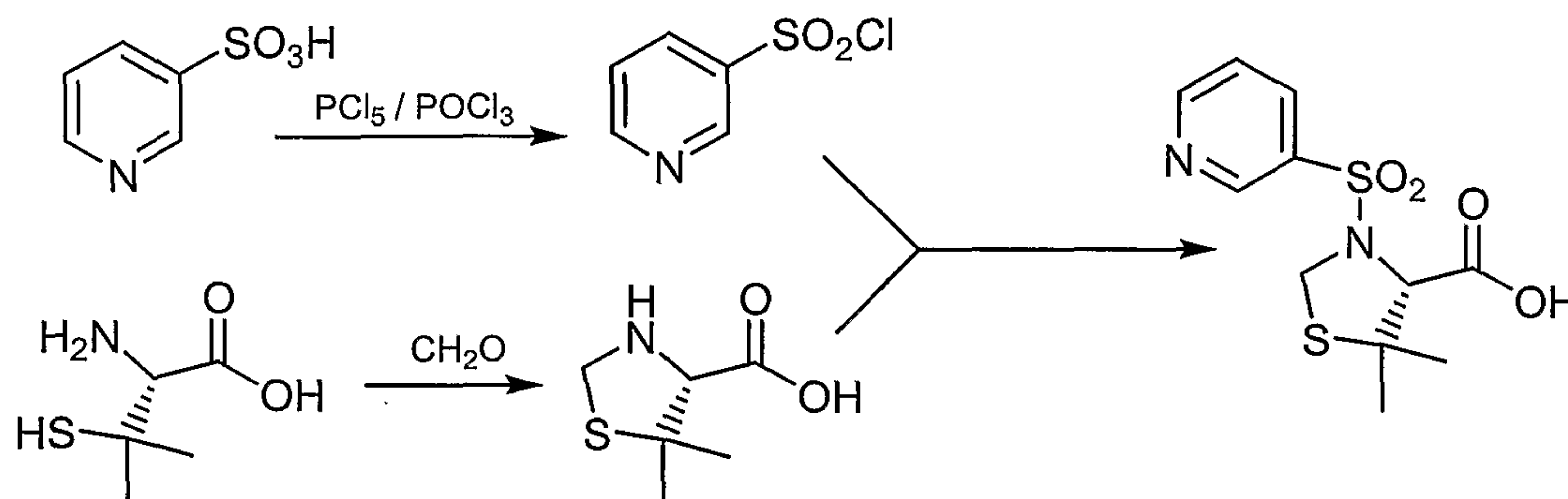
The red nitro compound (26 g, 0.054 m) was dissolved in THF (350 ml) and platinum oxide catalyst (1.35 g) was added. Resulted mixture was vigorously stirred under hydrogen atmosphere (50-60 psi) until hydrogen adsorption ceased (2 hr). Catalyst was filtered off and
20 filtrate evaporated to dryness. Residue was dissolved in ethyl acetate (100 ml) and diluted with hexane (50 ml) till beginning of crystallization. Mixture was further diluted with ethyl acetate/hexane (1:1) mixture (300 ml) and was left standing in refrigerator for 3 hr. Crystalline solids were filtered off, rinsed with cold solvent and air-dried to give product, 23 g, 94%. ¹H-NMR, CDCl₃, (δ): 7.81 (dd, 1H, J1=1.5Hz, J2=4.8Hz), 7.33 (bs, 5H), 7.17 (d, 2H, J=8.4Hz),
25 7.03 (d, 2H, J=8.4Hz), 6.96 (dd, 1H, J1=1.5Hz, J2=7.5Hz), 6.75 (dd, 1H, J1=5.0Hz, J2=7.7Hz), 6.22 (s, 1H), 5.31 (m, 1H), 5.09 (bs, 2H), 4.50 (m, 1H), 3.41 (bs, 2H), 3.02 (m, 2H), 1.43 (s, 9H). ¹³C-NMR, CDCl₃, (δ): 170.6, 155.6, 145.5, 140.21, 138.8, 136.3, 130.8, 129.9, 128.5, 128.3, 127.9, 123.4, 118.2, 117.0, 82.0, 66.6, 55.2, 37.4, 27.9. MS (m/z): 407.1 [M-56], 463.1 [M+1], 485.1 [M+23].

The aminopyridine (19 g, 0.041 m) was suspended in dichloromethane (200 ml) and CDI (12 g, 0.074 m, 1.8 eq) was added. Resulted mixture was stirred at ambient temperature for 20 hr. Reaction mixture was washed with sat. aq. bicarbonate (2 x 100 ml), brine (1 x 100 ml) and dried with sodium sulfate. Solids were filtered off and filtrate evaporated to dryness. Residue was dissolved in ethyl acetate (hot, 300 ml) and set to crystallize. Crystalline product
35 was filtered off, rinsed with cold ethyl acetate and air-dried to give 19.9 g, 81% of the imidazolone. ¹H-NMR, CDCl₃, (δ): 10.63 (s, 1H), 8.06 (d, 1H, J=3Hz), 7.66 (d, 2H, J=9Hz),

7.32 (m, 8H), 7.05 (m, 1H), 5.36 (m, 1H), 5.13 (s, 2H), 4.59 (m, 1H), 3.17 (m, 2H), 1.45 (s, 9H). ^{13}C -NMR, CDCl_3 , (δ): 170.4, 155.6, 154.3, 143.8, 141.0, 136.2, 135.8, 131.8, 130.2, 128.3, 128.0, 125.9, 122.2, 118.3, 116.0, 82.4, 66.8, 55.0, 37.7, 27.8. MS (m/z): 433.1 [$M-56$], 489.2 [$M+1$], 511.2 [$M+23$].

5

10 Example 3



Pyridine-3-sulfonic acid (125 g, 0.78 m) was placed in a 1L, 3-necked flask equipped with mechanical stirrer, reflux condenser, thermometer and nitrogen inlet. Next, the phosphorus pentachloride (250 g, 1.19 m, 1.5 eq) was added, followed immediately by the phosphorus oxychloride (330ml, 3.8 m, 4.5 eq). The contents of flask were initially stirred at ambient temperature for 30 min, then brought slowly to gentle reflux (internal temp. approx. 110°C) over the next hour, kept at this temperature for approx. 3.5 hr then allowed over the next 12 hr to cool back to ambient temperature. Gas evolution was observed during this time.

The volatiles were stripped under reduced pressure (at 12 mmHg/40°C) and yellow semi-solid residue was diluted with DCM (1L). The slurry was poured slowly into the stirred, ice-cold sat. aq. bicarbonate, maintaining pH=7. Gas evolution was observed. The organic layer was separated and aqueous layer was back-extracted with DCM. The combined extracts were washed with cold sat. aq. bicarbonate, brine and dried with magnesium sulfate. The solids were filtered off and filtrate evaporated, leaving pyridine-3-sulfonyl chloride as a pale yellow, oily liquid, 123 g (93% pure; 88% theory). ^1H -NMR, CDCl_3 , (δ): 9.26 (d, 1H), 8.98 (dd, 1H), 8.34 (m, 1H), 7.62 (m, 1H). ^{13}C -NMR, CDCl_3 , (δ): 155.3, 147.4, 140.9, 134.6, 124.2.

MS (m/z): 178.0 [$M+1$].

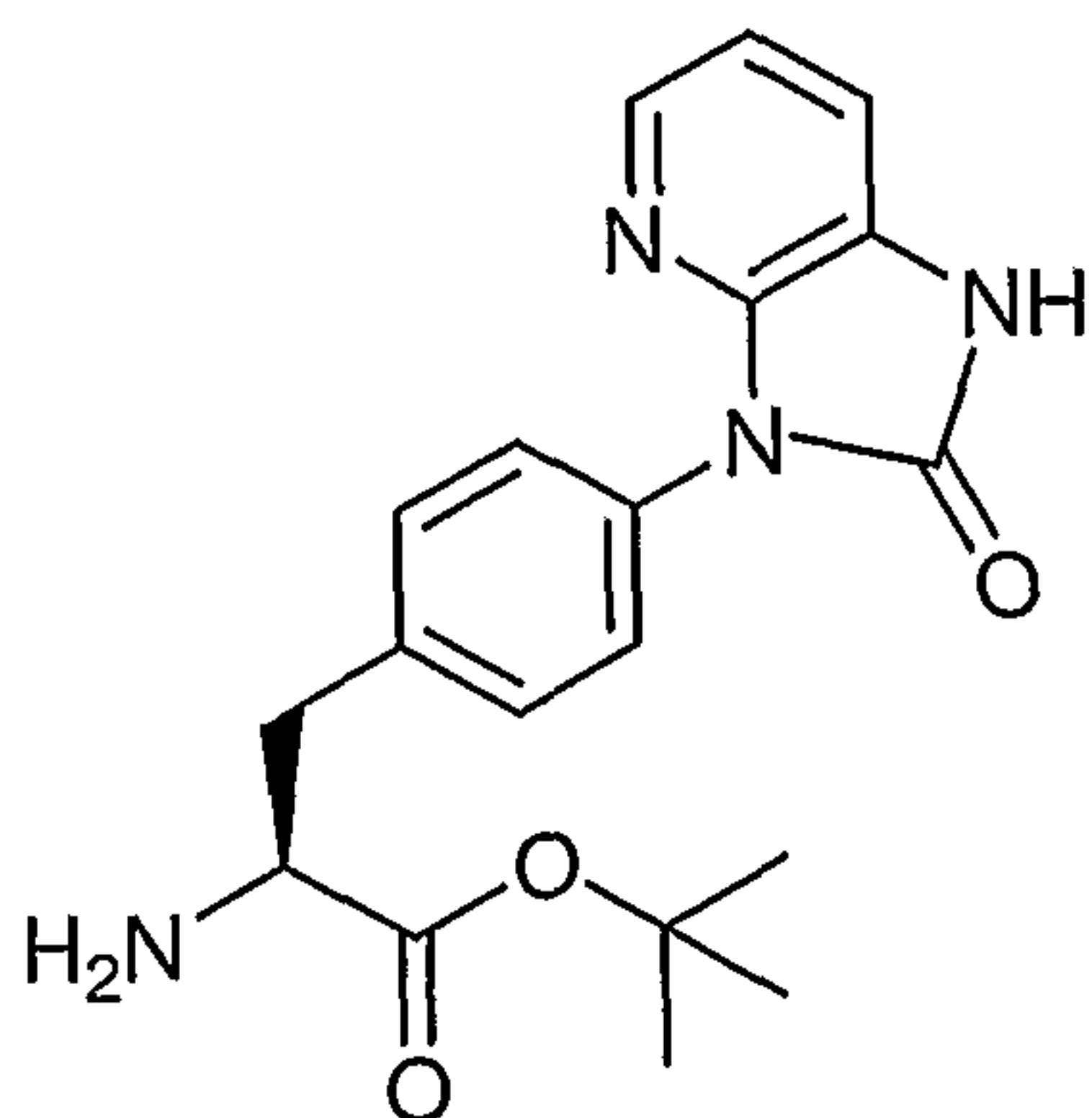
L-penicillamine (150 g, 1.0 m) was dissolved with stirring in DI water (1500 ml), cooled

in ice-bath to +8°C and treated with formalin (150 ml, 37% aq.). The reaction mixture was stirred at +8°C for 2 hr, then cooling bath was removed and stirring continued for 12 hr. The clear solution was concentrated under reduced pressure (14 mmHg/50°) leaving white residue. The solids were re-suspended, then dissolved in hot MeOH (2500 ml) and left
5 standing at ambient temperature for 12 hr. The white, fluffy precipitate was filtered off and rinsed with cold methanol. The filtrate was concentrated and set to crystallize again. The collected precipitate was combined with the first crop and dried in vacuum oven for 24 hr at 55°C at 45 mmHg. The yield of (R)-5,5-dimethylthiazolidine-4-carboxylic acid was 138 g (>99% pure; 86% theory). ¹H-NMR, DMSO-d₆, (δ): 4.25 (d, 1H), 4.05 (d, 1H), 3.33 (s, 1H),
10 1.57 (s, 3H), 1.19 (s, 3H). ¹³C-NMR, DMSO-d₆, (δ): 170.8, 74.4, 57.6, 51.8, 28.9, 27.9. MS (m/z): 162.3 [M+1].

In a 4L reactor equipped with mechanical stirrer and thermometer, a buffer solution was prepared from potassium monobasic phosphate (43 g, 0.31 m) and potassium dibasic phosphate (188.7 g, 1.08 m) in DI water (2L). The (R)-5,5-dimethylthiazolidine-4-carboxylic
15 acid (107 g, 0.675 m) was added and stirred until complete dissolution. The solution was cooled in an ice-bath to +8°C. A separately prepared solution of pyridine-3-sulfonyl chloride (124 g, 0.695 m) in DCM (125 ml) was added dropwise to the reactor with vigorous stirring over the 1 hr. The pH of reaction mixture was monitored and after 4 hr, found to be pH=5 and adjusted to pH=6 by addition of solid bicarbonate. The mixture was allowed to warm up to
20 ambient temperature over 18 hr. The pH was adjusted to 2 with dil. aq. sulfuric acid, stirred for 1 hr and precipitated yellow solids were filtered off, rinsed with water to neutral. The solid cake was transferred into 2L Erlenmeyer flask, suspended in DCM (500 ml) with occasional swirling for 5 min and filtered off again. The filter cake was washed with DCM and air-dried. The yield of the title compound, (R)-5,5-dimethyl-3-(pyridin-3-ylsulfonyl)thiazolidine-4-
25 carboxylic acid was 148.9 g (98% pure; 73% theory). ¹H-NMR, DMSO-d₆, (δ): 9.05 (d, 1H), 8.89 (m, 1H), 8.32 (m, 1H), 7.69 (m, 1H), 4.68 (q, 2H), 4.14 (s, 1H), 1.35 (s, 3H), 1.29 (s, 3H). ¹³C-NMR, DMSO-d₆, (δ): 170.0, 154.3, 147.9, 135.8, 134.1, 124.8, 72.6, 54.3, 50.2, 29.4, 25.0. MS (m/z): 303.2 [M+1].

30

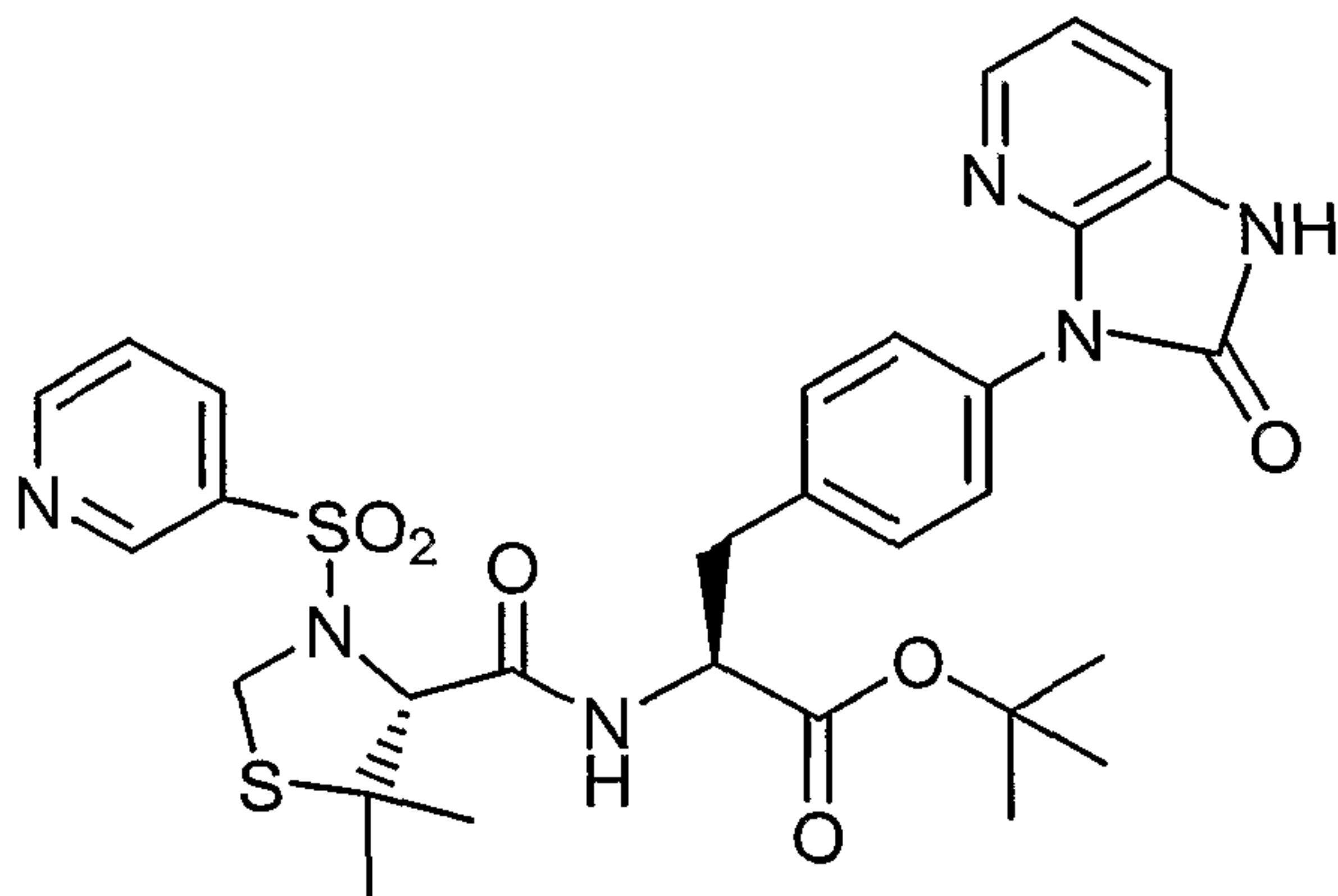
Example 4



The product of Example 2 (52 g, 0.106 m) was slurried in MeOH (450 ml), hydrogenation catalyst (8.7 g, 5% Pd/C, Degussa) was added and the mixture was stirred under the hydrogen atmosphere (60 psi) until further absorption ceased (ca. 2 hrs). THF (150 ml) was added to dissolve precipitated solids and the solution was filtered through plug of Celite, using DCM to rinse the filter. The filtrate was evaporated to dryness, re-dissolved in DCM (300 ml) and stripped again. This operation was repeated twice. The foamy solids were kept under high vacuum for 3 hrs. The yield of title compound was 38.3 g (101% of theory).

¹H-NMR, CDCl₃, (δ): 8.08 (m, 1H), 7.56 (AB q, 4H), 7.37 (m, 1H), 7.06 (m, 1H), 3.68 (m, 1H), 2.03 (m, 2H), 1.49 (s, 9H). ¹³C-NMR, CDCl₃, (δ): 173.8, 154.6, 143.9, 141.0, 137.4, 131.5, 130.2, 126.1, 122.3, 118.0, 116.1, 81.4, 56.0, 40.6, 27.9. MS (m/z): 299.3 [M-56], 355.4 [M+1], 377.4 [M+23].

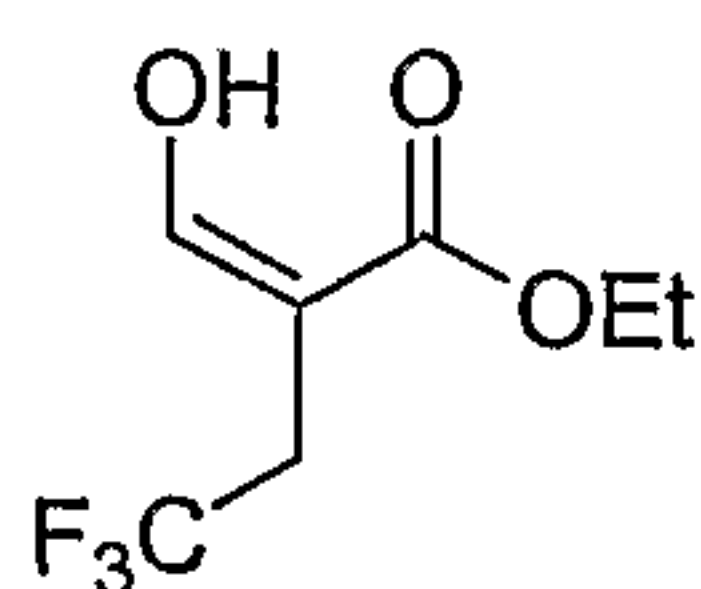
Example 5



The product of Example 4 (38.3 g, assume 0.106 m) was dissolved in DCM (500 ml) and treated successively with: N-methylmorpholine (27 g, 30 ml, 0.266 m; 2.5 eq), HOBt (17.3 g, 0.128 m, ; 1.2 eq), and the product of Example 3 (33.8 g, 0.112 m; 1.06 eq). The resulting non-homogenous solution was cooled in an ice-bath to +4°C and treated with EDC (22.5 g,

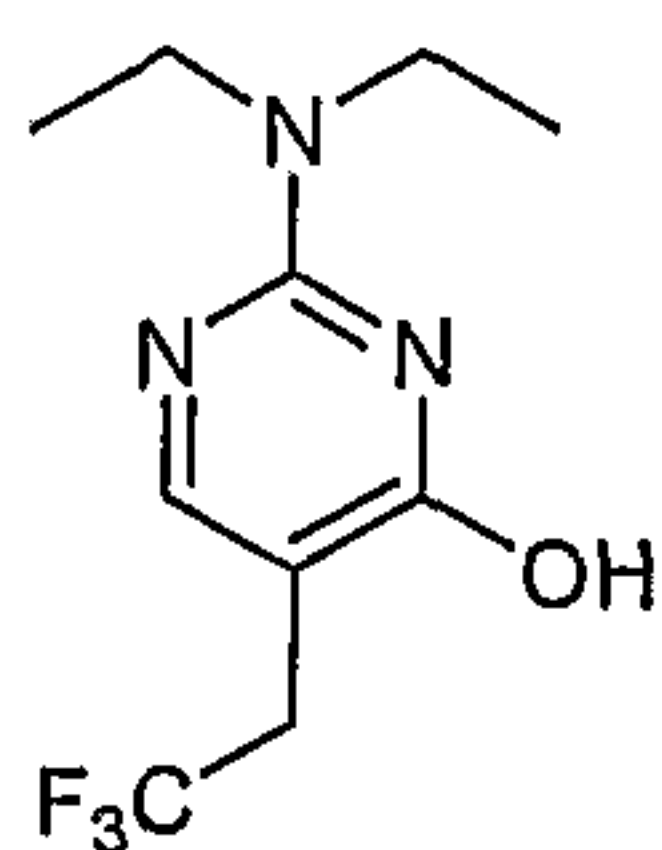
0.117 m; 1.1 eq) in one portion. The reaction mixture was stirred, allowing it to warm up to ambient temperature over the next 4 hr and then for 18 hr more. The solvent was stripped and residue dissolved in ethyl acetate (1.2L), washed with sat. aq. bicarbonate (2 x 250 ml), water (250 ml), brine (300 ml) and dried with magnesium sulfate. The solution was filtered and evaporated to dryness, leaving a light orange, viscous oil, 76 g (>>100%). The crude product was purified by flash chromatography on silica gel (Biotage 75L, in ethyl-acetate/methanol (3%) mixture. Fractions, containing pure product, were combined and evaporated to give 54 g of the title compound (yield 83%). ¹H-NMR, CDCl₃, (δ): 10.37 (s, 1H), 9.11 (s, 1H), 8.87 (m, 1H), 8.19 (m, 1H), 8.05 (m, 1H), 7.56 (AB q, 4H), 7.52 (m, 1H), 7.36 (m, 1H), 7.06 (m, 2H), 4.83 (m, 1H), 4.58 (AB a, 2H), 3.96 (s, 1H), 3.19 (m, 2H), 1.49 (s, 9H), 1.22 (s, 3H), 1.18 (s, 3H). ¹³C-NMR, CDCl₃, (δ): 169.7, 167.6, 153.9, 148.4, 143.8, 140.9, 135.8, 135.6, 132.9, 131.9, 130.2, 125.9, 123.8, 122.1, 118.0, 115.9, 82.8, 73.6, 60.3, 54.8, 53.7, 50.6, 37.8, 29.1, 27.8, 23.9, 14.1. MS (m/z): 583.3[M-56], 639.4 [M+1], 661.3 [M+23].

Example 6



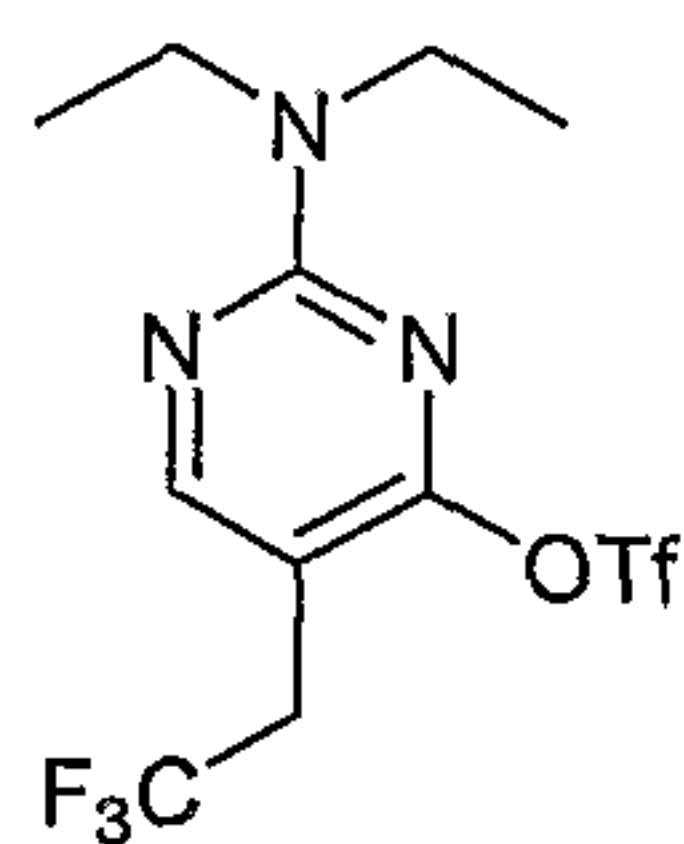
To an ice chilled solution of ethyl trifluorobutyrate (15 g, 89 mmol) and ethyl formate (36 mL, 444 mmol) in THF (200 mL) under N₂ was added a solution of 1 M KOtBu in THF (107 mmol, 107 mL) over a 25-minute period. After 15 minutes the ice bath was removed and the reaction mixture was stirred one hour at room temperature. Additional ethyl formate (18 mL, 222 mmol) was then added and the reaction mixture was stirred overnight. The reaction mixture was concentrated and the residue partitioned between cold ether (100 mL) and cold water (300 mL). The pH of the aqueous phase was adjusted to 2 with concentrated HCl. The product was extracted with dichloromethane (1 x 100 mL, 45 x 75 mL) and the combined organic extracts were washed with brine (1 x 100 mL), dried (MgSO₄), filtered, and concentrated to yield the title compound as thick oil which solidified upon standing, 10.2 g (58.5%). MS (m/z) = 198 (M + H)⁺.

Example 7



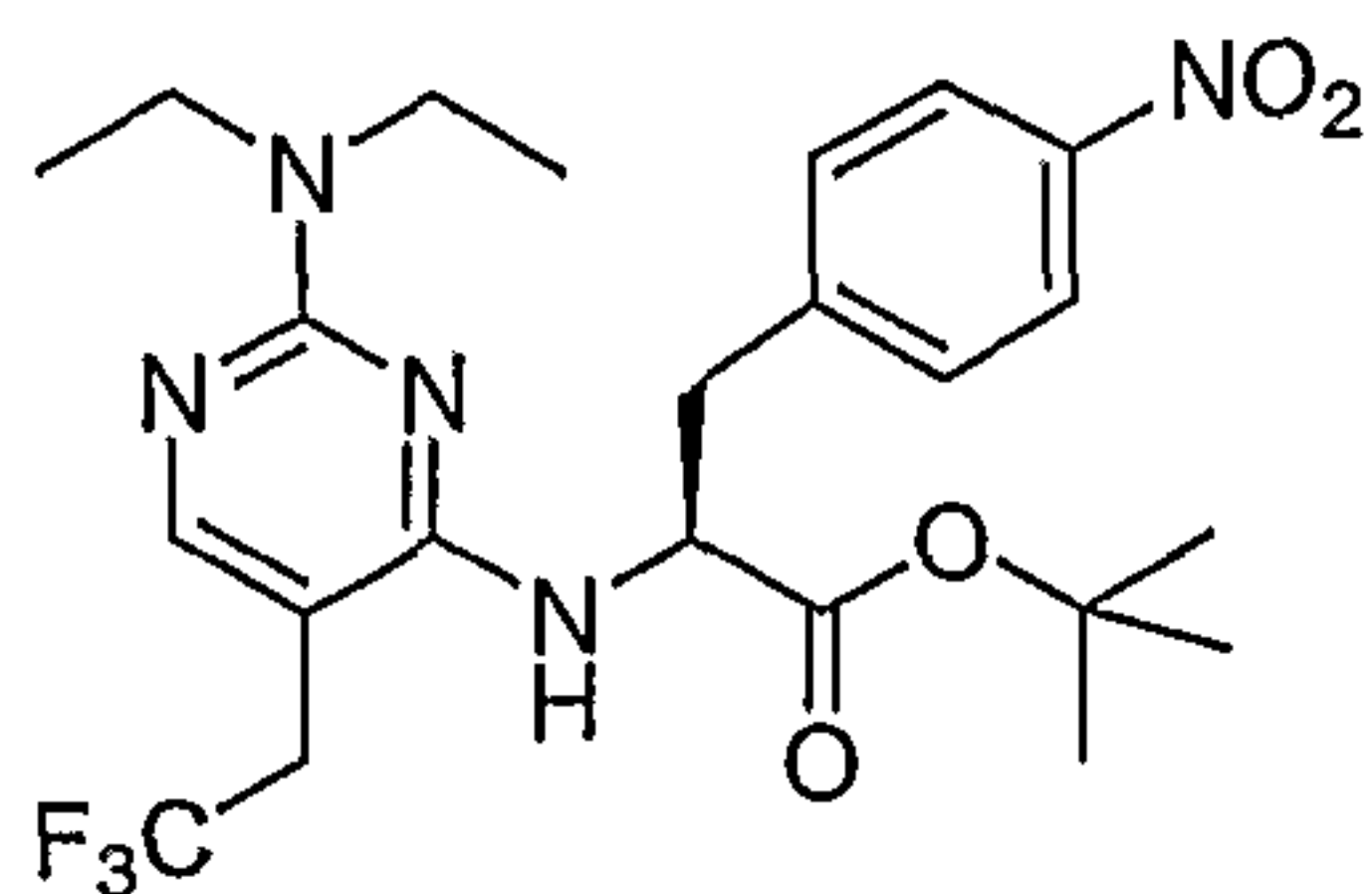
To a solution of the product of Example 6 (10 g, 51 mmol) and diethylguanidine sulfate (8.3g, 25.2 mmol) in EtOH (60 mL) under N₂, was added NaOEt, 21% solution in EtOH (20.7 mL, 55.5 mmol) over a 10-minute period. The reaction mixture was then heated at reflux for 5 hours. The heterogeneous solution was cooled and poured into cold water (100 mL) to give a homogenous solution. The pH of the solution was adjusted to approximately 3.5 with conc. HCl and 1 N HCl. A solid precipitated from solution, which was collected by filtration. The light tan solid was washed with water and air-dried, yielding 2.9 g, (23%) of the title compound. MS (m/z) = 250 (M + H)⁺. ¹H NMR (300 MHz, CD₃OD) δ 7.65 (br s, 1H), 3.55 (q, 4H), 3.30 (q, 2H), 1.25 (t, 6H).

Example 8



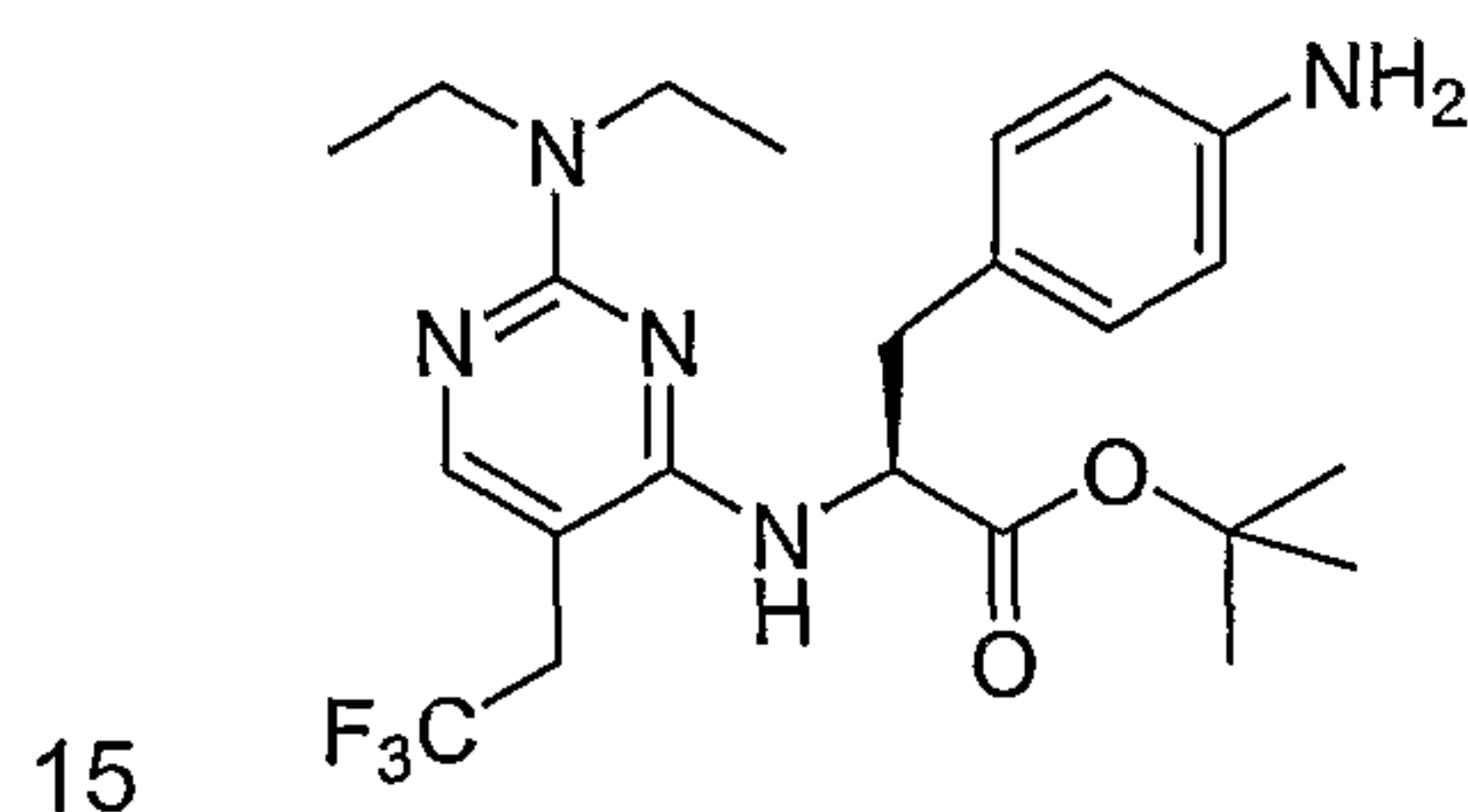
A flask was charged with the product of Example 7 (2.0 g, 8.02 mmol), DIEA (1.5 mL, 8.83 mmol), DMAP (.98 g, 0.8 mmol), and dichloromethane (30 mL). The mixture was cooled to 0°C and trifluoroacetic anhydride (1.5 mL, 8.83 mmol) was added. The reaction became homogeneous and was stirred at 0°C for 3 hours. The mixture was quenched with sat. NaHCO₃ and extracted with dichloromethane. The organic phase was washed with 0.2 N citric acid, dried over Na₂SO₄, filtered, and concentrated *in vacuo* to yield 2.87 g (94%) of the title compound as a brown solid. ¹H NMR (300 MHz, CDCl₃) δ 8.28 (s, 1H), 3.65-3.52 (m, 4H), 3.29-3.19 (q, 2H), 1.22-1.17 (t, 6H).

Example 9



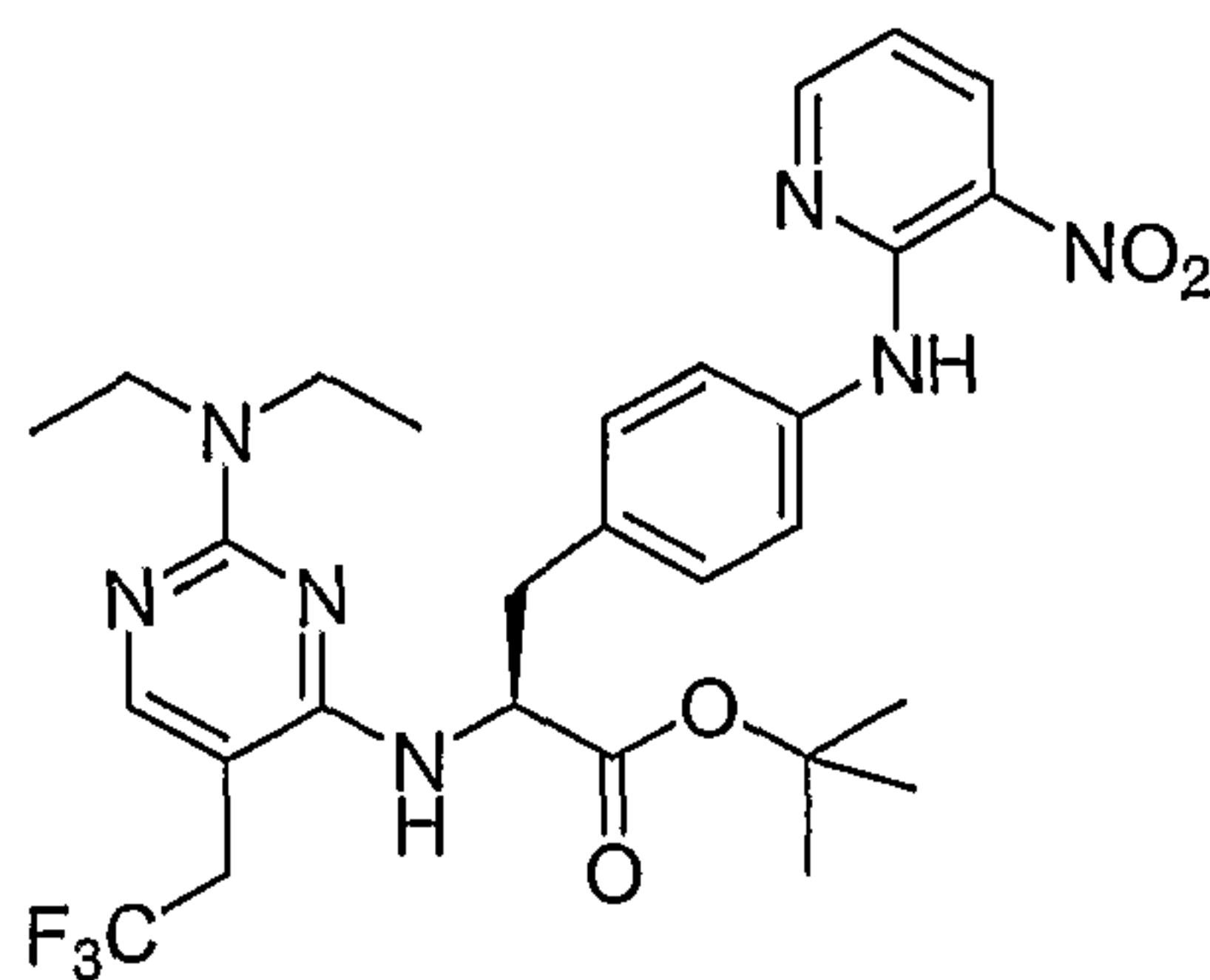
A solution of the product of Example 8 (1.3g, 3.5 mmol), H-Phe(p-NO₂)OtBu (1.1 g, 4.2 mmol), and DIEA (0.9 mL, 5.3 mmol) in CH₃CN (14 mL) under N₂ was heated to reflux overnight. The next day additional H-Phe(p-NO₂)OtBu (0.8 g, 3 mmol) was added and reflux
 5 was continued for 3 days. The reaction mixture was then cooled and concentrated. The residue taken-up in EtOAc (50 mL) and the organic portion washed with 0.5 N KHSO₄ (3 x 50 mL), water (1 x 50 mL), brine (1 x 10 mL), dried (MgSO₄), filtered and concentrated to a brownish gum. The crude material was purified by flash chromatography (5:1 hexanes/EtOAc) to yield 640 mg (38%) of the title compound as a golden gum. TLC: 3:1
 10 hexanes/EtOAc, R_f = 0.30, MS (m/z) = 498 (M+H)⁺, ¹H NMR, (300 MHz, CDCl₃) δ 8.19 (d, 2H), 7.80 (s, 1H), 7.25 (d, 2H), 5.19 (br d, 1H), 4.95 (q, 1H), 3.70 – 3.50 (m, 4 H), 3.45 – 3.25 (m, 2 H), 3.10 (q, 2H), 1.40 (s, 9 H), 1.05 (t, 6 H).

Example 10



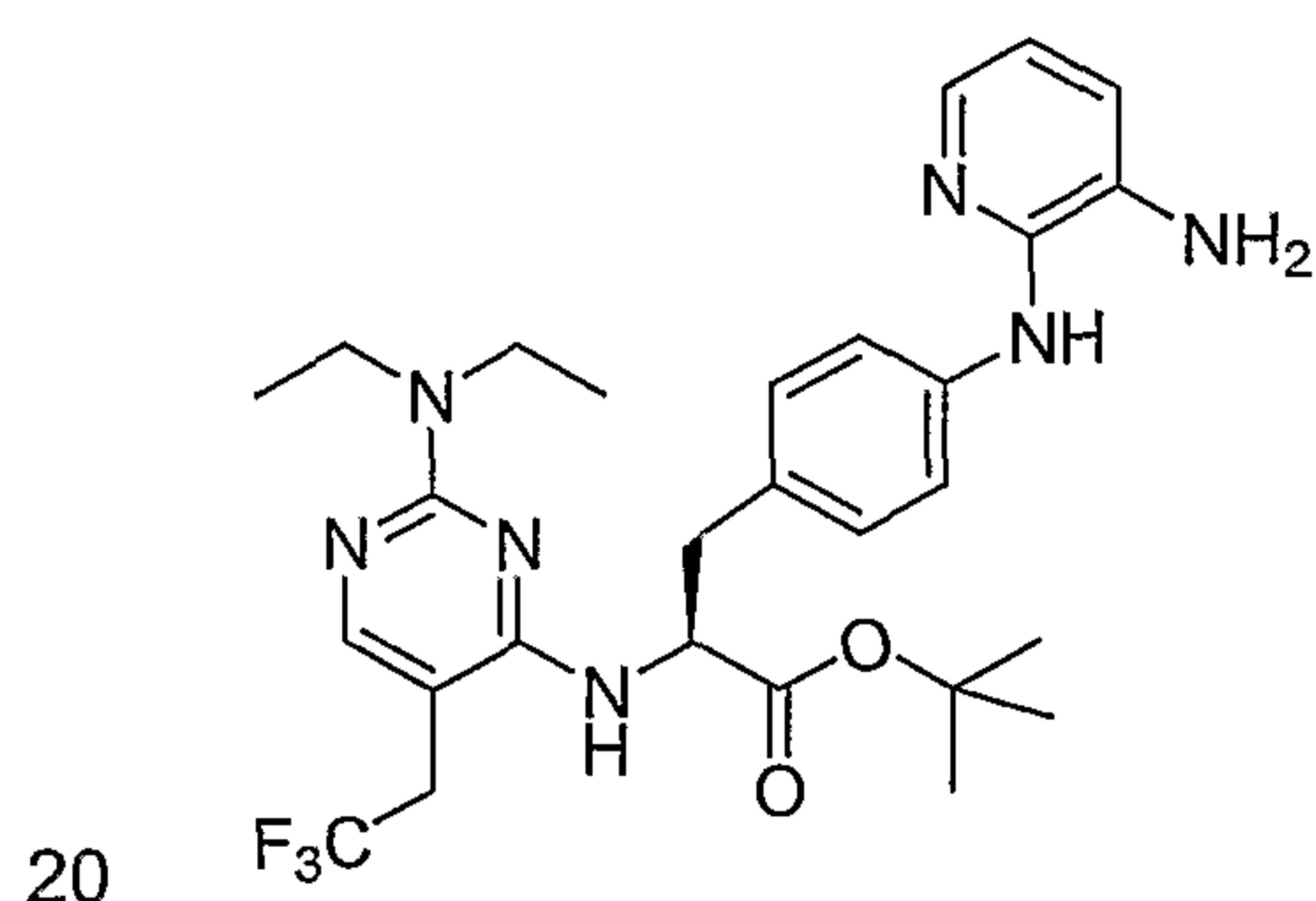
The product of Example 9 (635 mg, 1.27 mmol) was dissolved in absolute EtOH (5 mL) to which was added 35 mg of Pd/C, 10 wt%. The reaction was subjected to hydrogenation (45 psi H₂) for 2.5 hours at which time 50 mgs of Pd/C, 10 wt % was added
 20 and the reaction mixture again subjected to hydrogenation (45 psi H₂) overnight. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated to give 452 mg (76%) of the title compound. MS (m/z) = 468 (M+H)⁺, ¹H NMR (300 MHz, CDCl₃) δ 7.75 (s, 1 H), 6.90 (d, 2 H), 6.60 (d, 2 H), 5.05 (br d, 1 H), 4.80 (q, 1 H), 3.70 – 3.45 (m, 6 H), 3.10 – 2.90 (m, 4 H), 1.40 (s, 9 H), 1.15 (t, 6H).

Example 11



A solution of the product of Example 10 (598mg, 1.28mmol), 2-chloro-3-nitropyridine (243 mg, 1.53 mmol), and DIEA (0.67 mL, 3.83 mmol) in EtOH (5 mL) under N₂ was heated at reflux. The next day the reaction was cooled and additional 2-chloro-3-nitropyridine (40 mg, 0.25 mmol) and DIEA (0.11 mL, 0.60 mmol) was added and the reaction was heated at reflux for one day. The reaction mixture was then concentrated and the residue taken-up in EtOAc (20 mL). The organic phase was washed with water (2 x 20 mL). The combined aqueous washes were back extracted with EtOAc (2 x 10 mL). The combined organic extracts were washed with 0.2 N citric acid (3 x 20 mL), water (1 x 10 mL), sat. NaHCO₃ (3 x 20 mL), brine (1 x 10 mL), dried (MgSO₄), filtered and stripped to an orange gum. The crude product was purified by flash chromatography eluting with 4:1 hexanes/EtOAc (*R_f* = 0.14) to yield 610 mg (81%) of the title compound as a red oil. MS (*m/z*) = 590 (*M*+H)⁺, ¹H NMR (300 MHz, CDCl₃) δ 10.10 (s, 1H), 8.55 (d, 1H), 8.50 (m, 1H), 7.79 (s, 1H), 7.75 (d, 2H), 7.15 (d, 2H), 6.80 (q, 1H), 5.10 (br d, 1H), 4.90 (m, 1H), 3.70 – 3.45 (m, 4H), 3.25 (m, 2H), 3.10 (q, 2H), 1.40 (s, 9H), 1.10 (t, 6H).

Example 12

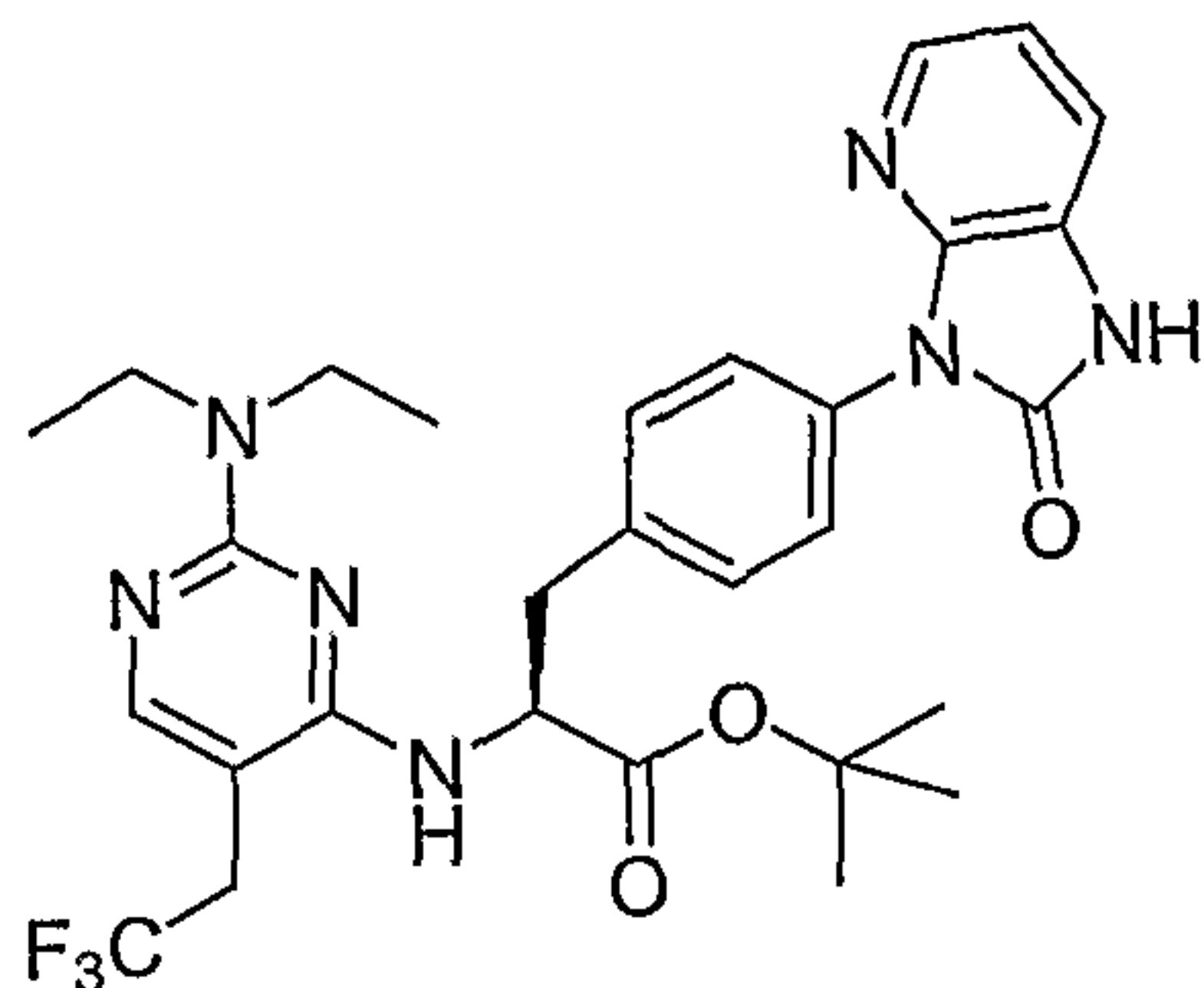


To a solution of the product of Example 11 (610 mg, 1.03 mmol) in absolute EtOH (5 mL) was added 60 mg of Pd/C, 10 wt%. The mixture was subjected to hydrogenation (45 psi H₂) overnight. The next day the reaction mix was filtered through Celite and the filtrate

concentrated to give 500 mg (87%) of the title compound. MS (m/z) = 560 ($M+H$)⁺, ¹H NMR (300 MHz, CDCl₃) δ 7.85 (d, 2H), 7.80 (s, 1H), 7.20 (d, 2H), 7.05 (d, 2H), 7.00 (d, 1H), 7.75 (m, 1H), 6.20 (br s, 1H), 5.15 (br s, 1H), 4.85 (m, 1H), 3.75 – 3.45 (m, 4H), 3.40 (br s, 2H), 3.15 (m, 2H), 3.05 (q, 2H), 1.40 (s, 9H), 1.15 (t, 6H).

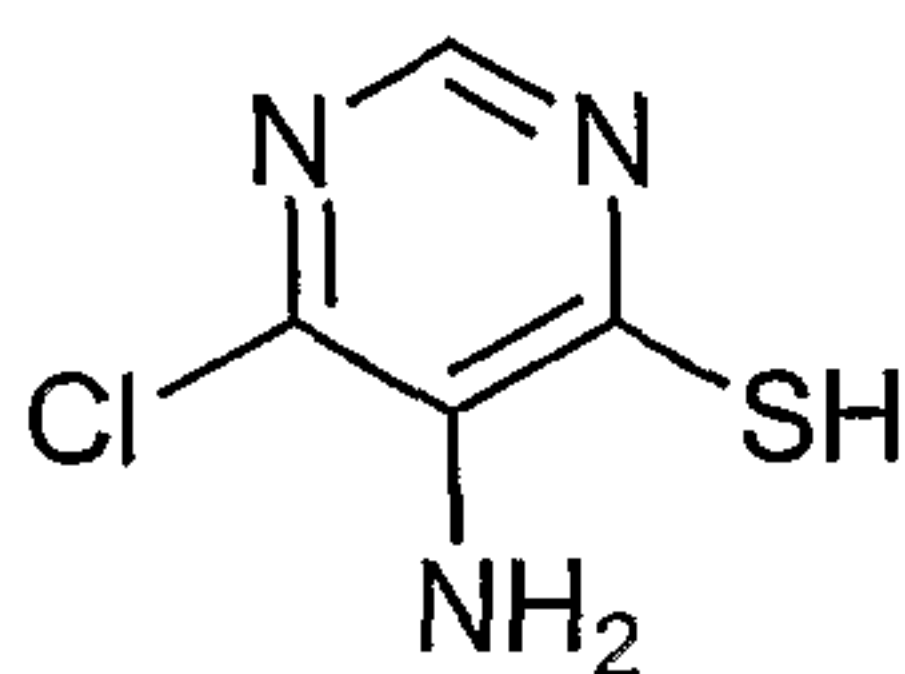
5

Example 13



A solution of the product of Example 12 (141 mg, 0.250 mmol) and CDI (62 mg, 0.378 mmol) in CH₂Cl₂ (3 mL) was stirred overnight. The next day additional CDI (30 mg, 0.185 mmol) was added and the reaction was stirred another day. The reaction mixture was then concentrated and taken-up in EtOAc (10mL) and the organic portion washed with 0.2 N citric acid (3 x 5 mL), water (1 x 5 mL), sat. NaHCO₃ (3 x 5 mL), brine (1 x 5 mL), dried (MgSO₄), filtered and concentrated to yield 69 mg (47%) the title compound as a foam which was used without further purification. MS (m/z) = 586 ($M+H$)⁺, ¹H NMR (300 MHz, CDCl₃) δ 8.20 (br s, 1H), 8.05 (d, 1H), 7.80 (s, 1H), 7.65 (d, 2H), 7.90 (m, 3H), 7.05 (m, 1H), 5.15 (br d, 1H), 4.95 (m, 1H), 3.70 – 3.45 (m, 4H), 3.25 (app d, 2H), 3.10 (q, 2H), 1.40 (s, 9H), 1.15 (t, 6H).

20 Example 14



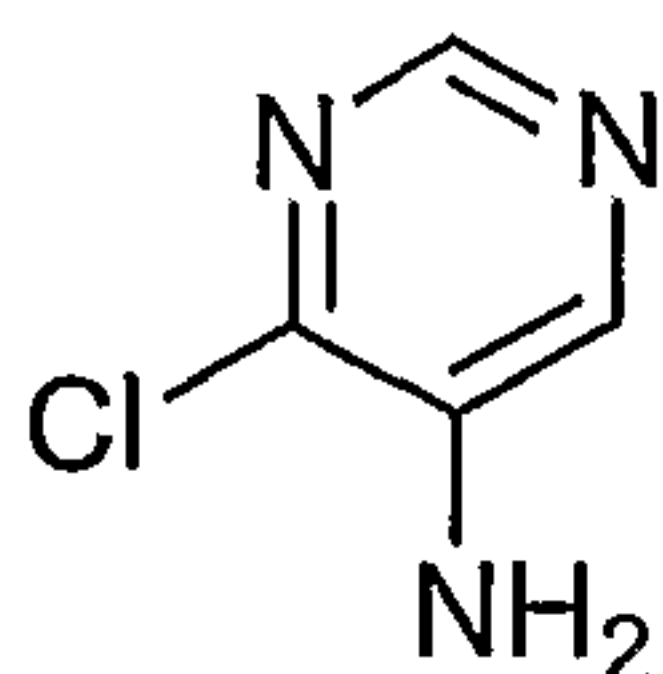
To a solution of 4,6-dichloro-5-aminopyrimidine (5.0g, 30.7 mmol) in DMSO (30 mL) was added Na₂S·9H₂O (7.4 g, 30.8 mmol). The mixture was stirred at room temperature overnight. Water (40 mL) was then added to the mixture and the solution evaporated under reduced pressure to approximately 6 mL. To this solution was added conc. HCl (0.5 mL) and water to precipitate the product. The solution was filtered and the orange solid was washed

25

with water and dried to afford 4.3 g (86%) of the title compound. ^1H NMR (300 MHz, DMSO- d_6) δ 5.84 (2 H, s), 7.79 (1 H, s), 14.37 (1H, br s); MS(m/z): $\text{MH}^+ = 162$.

Example 15

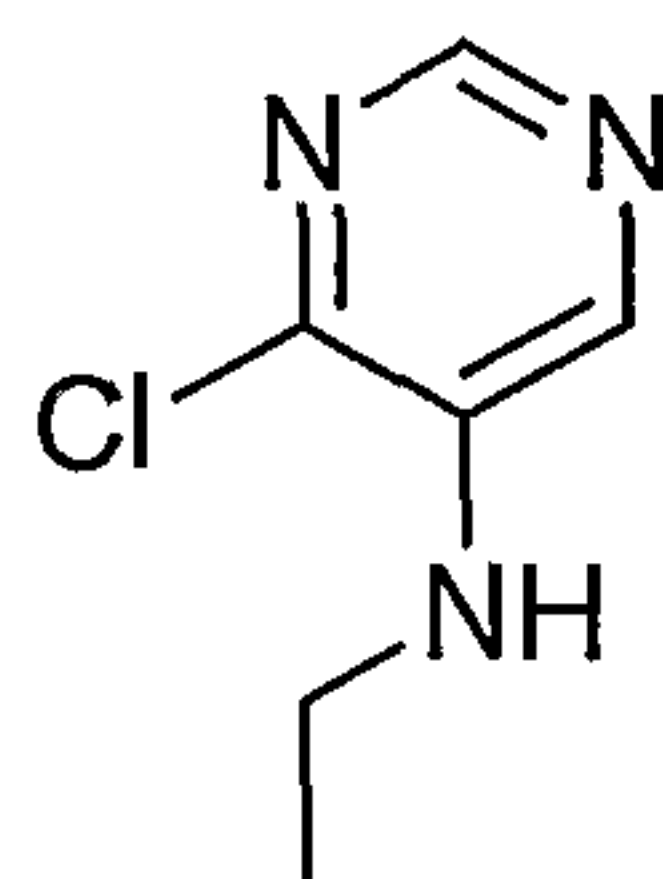
5



To the product of Example 14 (4.3 g, 26 mmol) dissolved in conc. NH_4OH (4 mL) was added EtOH (40 mL). To this solution, Raney Nickel (excess) was added in portions. The reaction was stirred at room temperature overnight and then heated at 80°C for 2 hrs.

10 The mixture was filtered through Celite and the filtrate concentrated. The crude product was purified by flash chromatography on silica using EtOAc/hexanes to afford 1.6 g (47%) of the title compound as a yellow solid. ^1H NMR (300 MHz, DMSO- d_6) δ 5.90 (2 H, s), 8.20 (2 H, s); MS(m/z) $\text{MH}^+ = 130$.

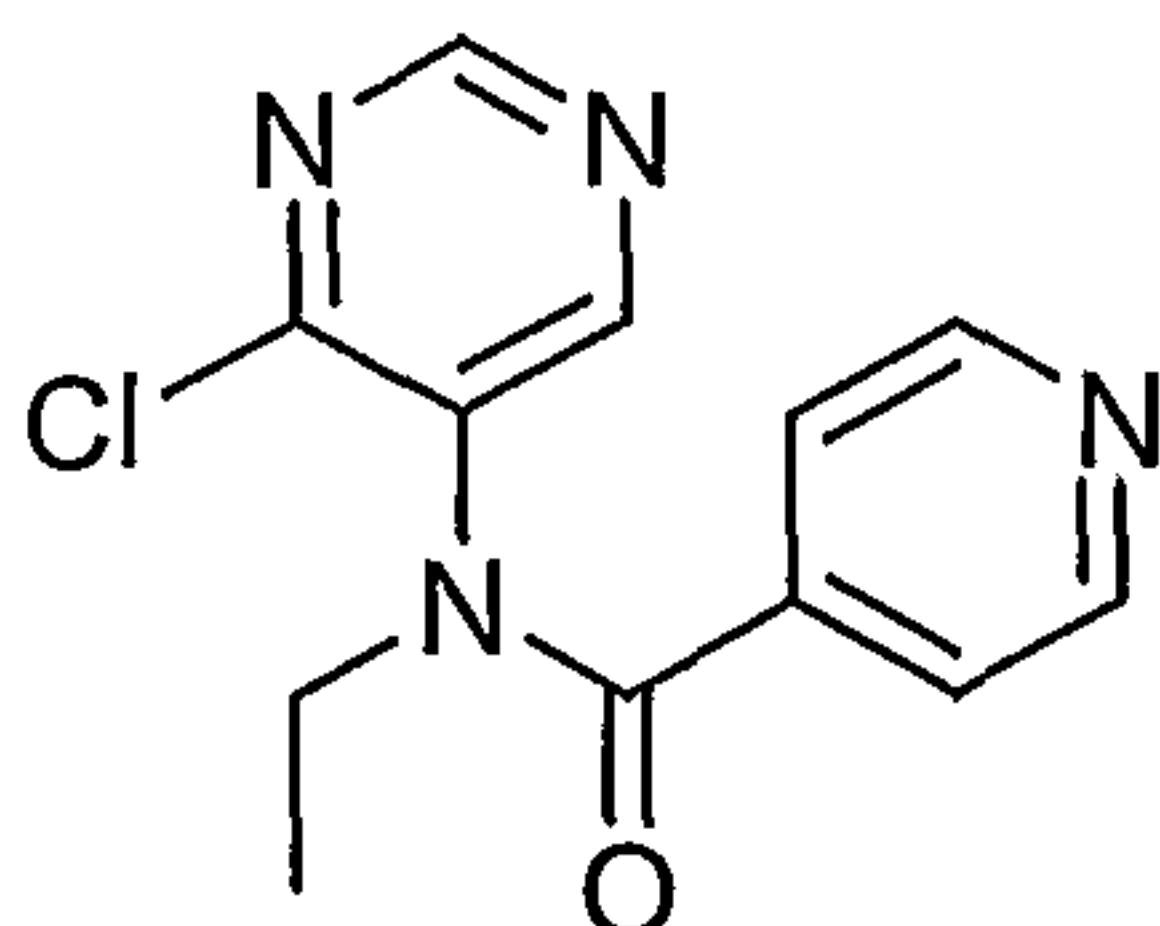
15 Example 16



To the product of Example 15 (0.51 g, 3.9 mmol) in MeOH (20 mL) and HOAc (0.5 mL) was added CH_3CHO (0.52 mL, 9.2 mmol). Then NaBH_3CN (590 mg, 9.2 mmol) was added in one portion. The reaction was stirred at room temperature overnight and additional HOAc, CH_3CHO , and NaBH_3CN were added. The reaction was stirred overnight, concentrated, and the residue was taken up in EtOAc and sat. NaHCO_3 . The separated aqueous layer was back extracted with EtOAc. The combined organic layer was dried and concentrated to a residue. The residue was dissolved in MeOH and treated with HOAc, CH_3CHO and NaBH_3CN as described above. Following the work up procedure described above the crude product was purified by flash chromatography on silica using EtOAc/hexanes, to afford 0.35g (57%) of the title compound as a yellow oil. ^1H NMR (300 MHz, CDCl_3) δ 1.35 (3 H, q, $J = 12$ Hz), 3.29 (2 H, m), 4.21 (1H, bs), 8.04 (1 H, s), 8.36 (1H, s); MS(m/z): $\text{MH}^+ = 158$.

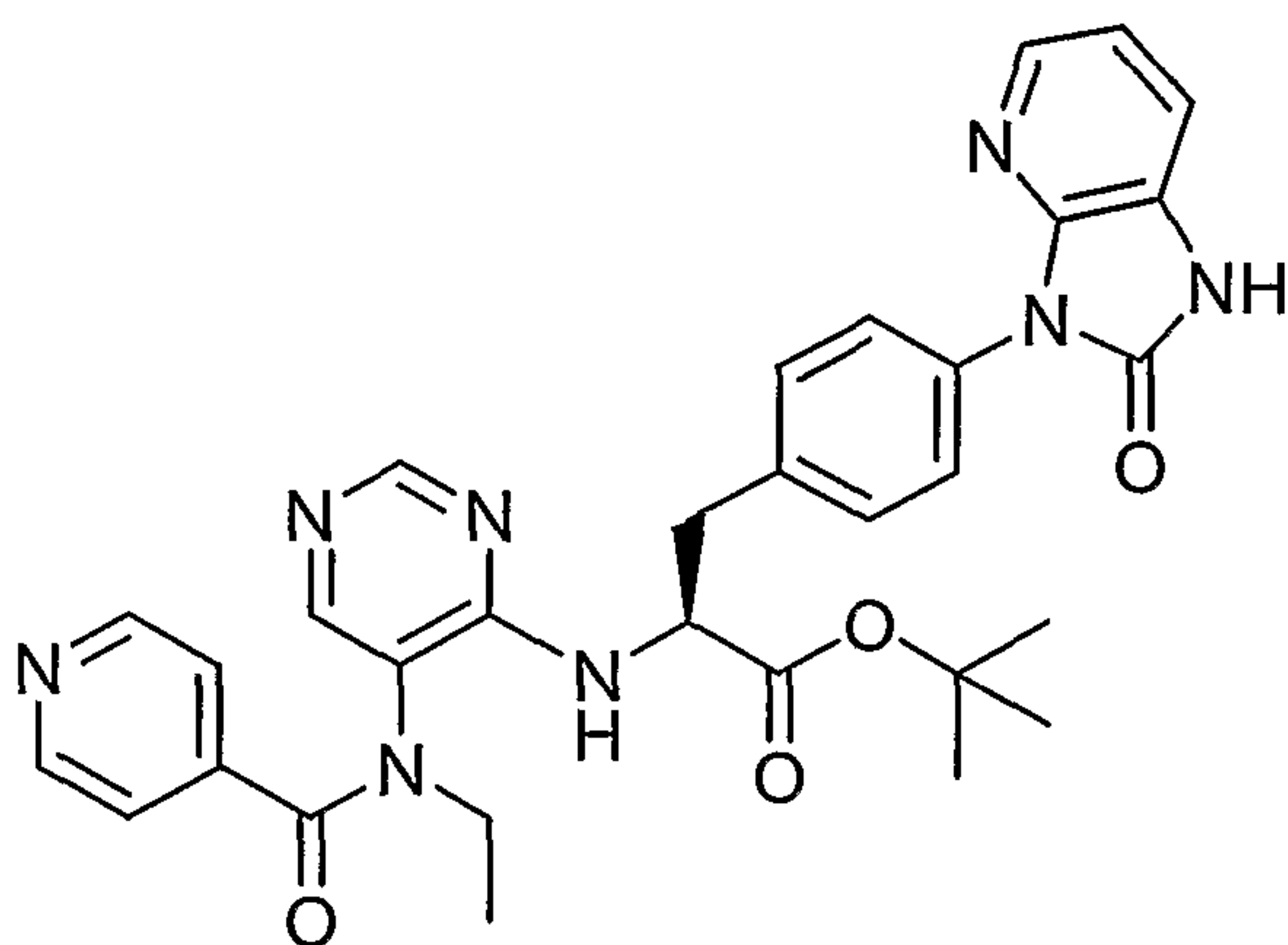
30

Example 17



- 5 To the product of Example 16 (70 mg, 0.45 mmol) dissolved in DMF (1 mL) was added TEA (93 μ L) and isonicotinoyl chloride (0.12 g, 0.67 mmol). The reaction mixture was stirred at room temperature for 2 days and then partitioned between EtOAc and sat. NaHCO_3 . The separated aqueous layer was back extracted with EtOAc. The combined organic layer was dried and concentrated to give 67 mg (57%) of the title compound which was used
- 10 without further purification. ^1H NMR (300 MHz, CDCl_3) δ 1.26 (3 H), 3.65-3.69 (1 H), 4.21 (1H), 7.17 (2 H), 8.43 (1H), 8.54 (2 H), 8.86 (1 H) Note: ^1H NMR shows evidence of rotamers as demonstrated of broadness of all peaks; MS(m/z): $\text{MH}^+ = 263$.

Example 18

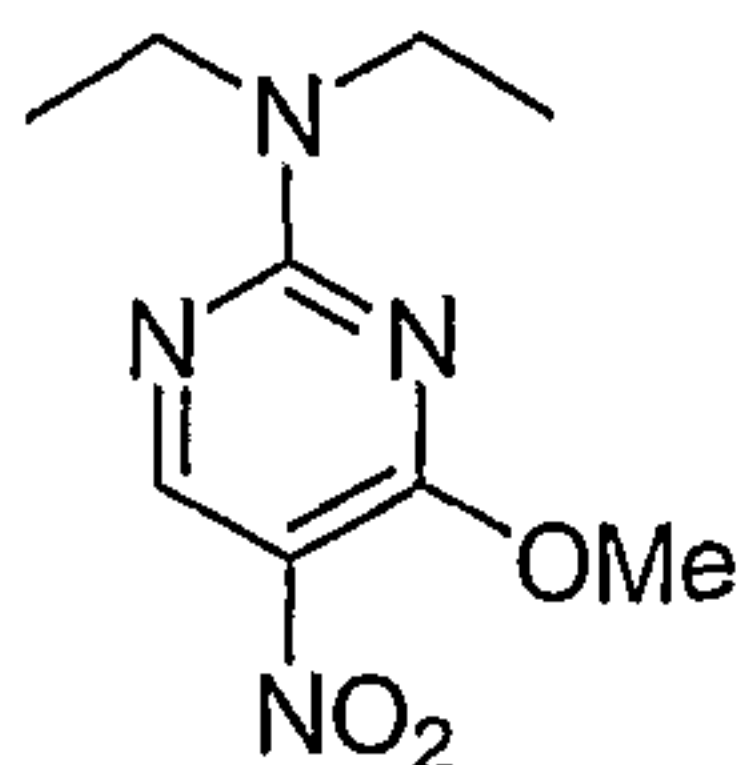


15

- To a solution of the product of Example 17 (0.11 g, 0.42 mmol) and the product of Example 8 (0.135 g, 0.38 mmol) in IPA (2.5 ml) was added DIEA (0.35 ml, 1.9 mmol). The reaction mixture was stirred in a sealed tube at 130°C for 2 days. The crude mixture was
- 20 concentrated and the oil was purified by flash column chromatography with a solvent gradient of 0-10% MeOH in CH_2Cl_2 to yield the title compound as an oil. ^1H NMR (300 MHz, CDCl_3) δ 1.16 (1.2 H, m), 1.26-1.31 (1.8 H, m), 1.50-1.53 (9 H, d, $J = 9$ Hz), 3.0 (1 H, m), 3.2 (0.8 H, m), 3.36 (1.2 H, m), 4.12-4.18 (1.2 H, m), 4.96-5.10 (.8 H, m), 5.80-5.95 (1 H, m), 6.93-6.96 (1 H, m), 7.07 (1 H, m), 7.31-7.45 (5 H, m), 7.66-7.75 (3 H, m), 8.06 (1 H, m), 8.44-8.51 (2 H, m);

HPLC/MS: single peak at 1.29 min, $MH^+ = 581$.

Example 19

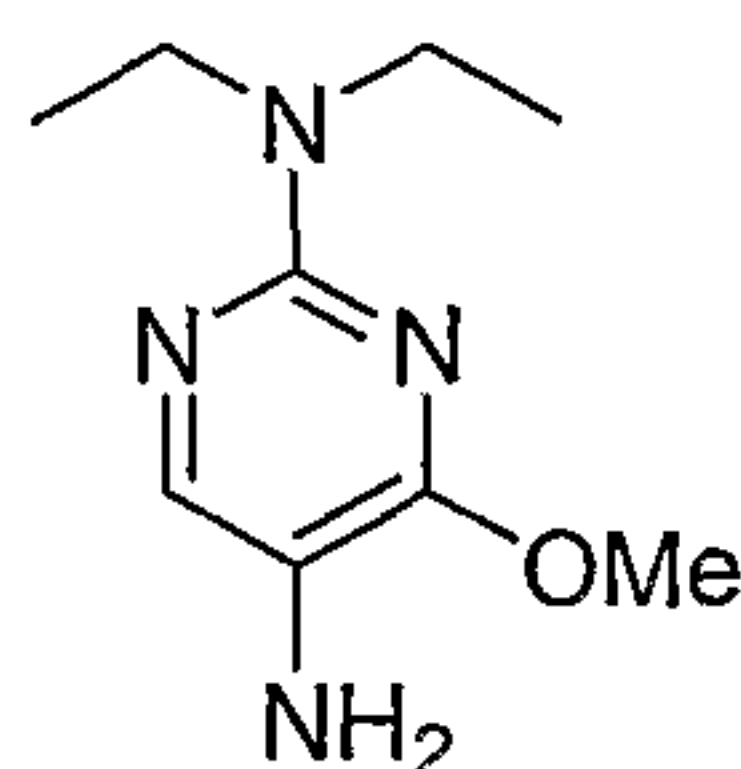


5

To 2,4-dichloro-5-nitropyrimidine (2.0 g, 10.3 mmol) in MeOH (7 mL) at 0°C under N_2 was added NaOMe (0.5 M in MeOH, 25 mL) dropwise. After the addition was completed, the reaction mixture was stirred at 0°C for 15 min. Then diethylamine (5 mL) was added and the mixture was stirred at rt overnight. The reaction mixture was concentrated and the residue was partitioned between EtOAc and H_2O . The organic layer was dried and concentrated to a residue which was purified by flash chromatography on silica using EtOAc/Hexanes, to afford the title compounds as an off white solid (1.1 g, 4.9 mmol, 47% yield). ^1H NMR (300 MHz, $CDCl_3$) δ 1.26 (6H, t, $J = 6.6$ Hz), 3.70 (4 H, m), 4.08 (3 H, s), 9.01 (1H, s); HPLC/MS: $MH^+ = 227$.

15

Example 20

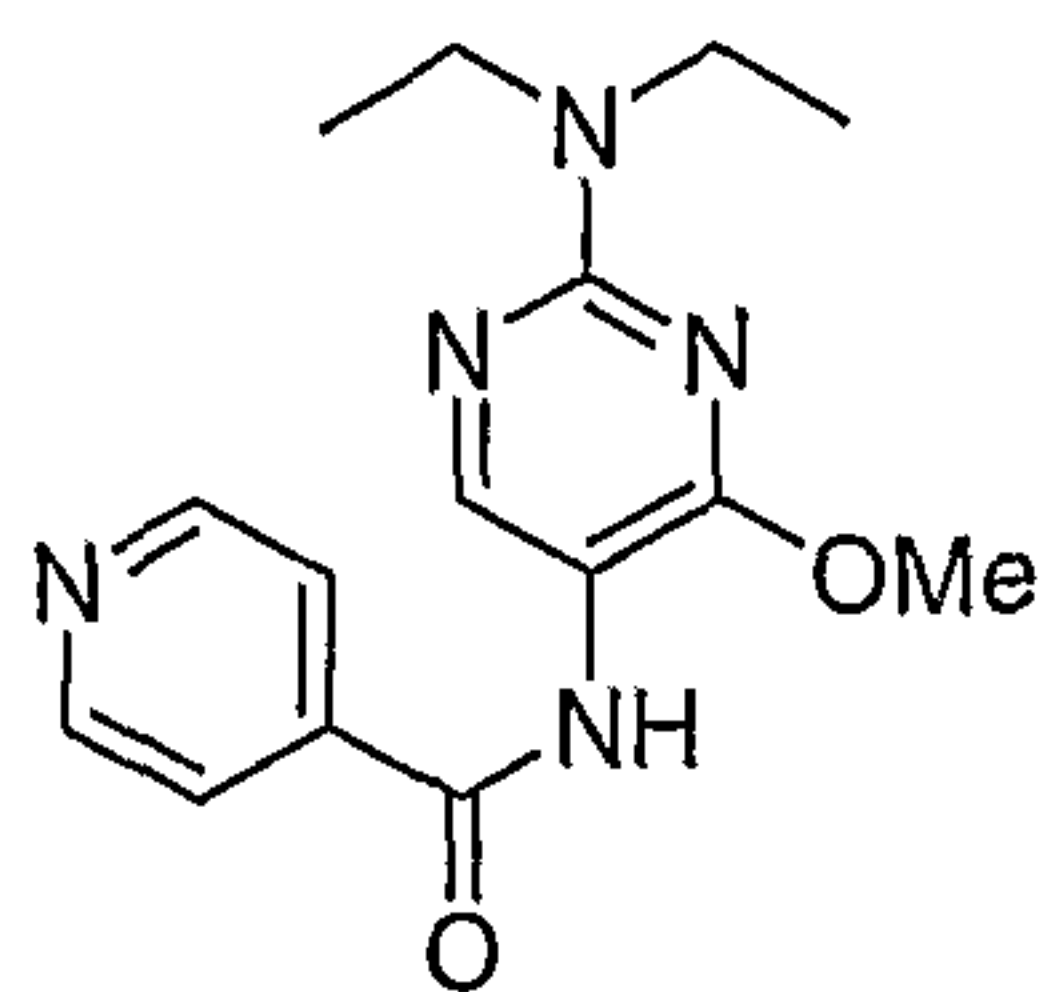


20

The product of Example 19 (1.1g, 4.9 mmol) in MeOH/EtOAc (1:1, 20 mL) was reduced with Pd/C (5% degussa, 0.5g) and H_2 (50 psi) in a Parr shaker overnight. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to afford the title compound as a solid (0.85g, 4.3 mmol, 88.5% yield). ^1H NMR (300 MHz, $CDCl_3$) δ 1.18 (6H, t, $J = 6.9$ Hz), 3.03 (2 H, br), 3.57 (6H, t, $J = 6.9$ Hz), 3.96 (3H, s), 7.71 (1H, s); HPLC/MS: $MH^+ = 197$.

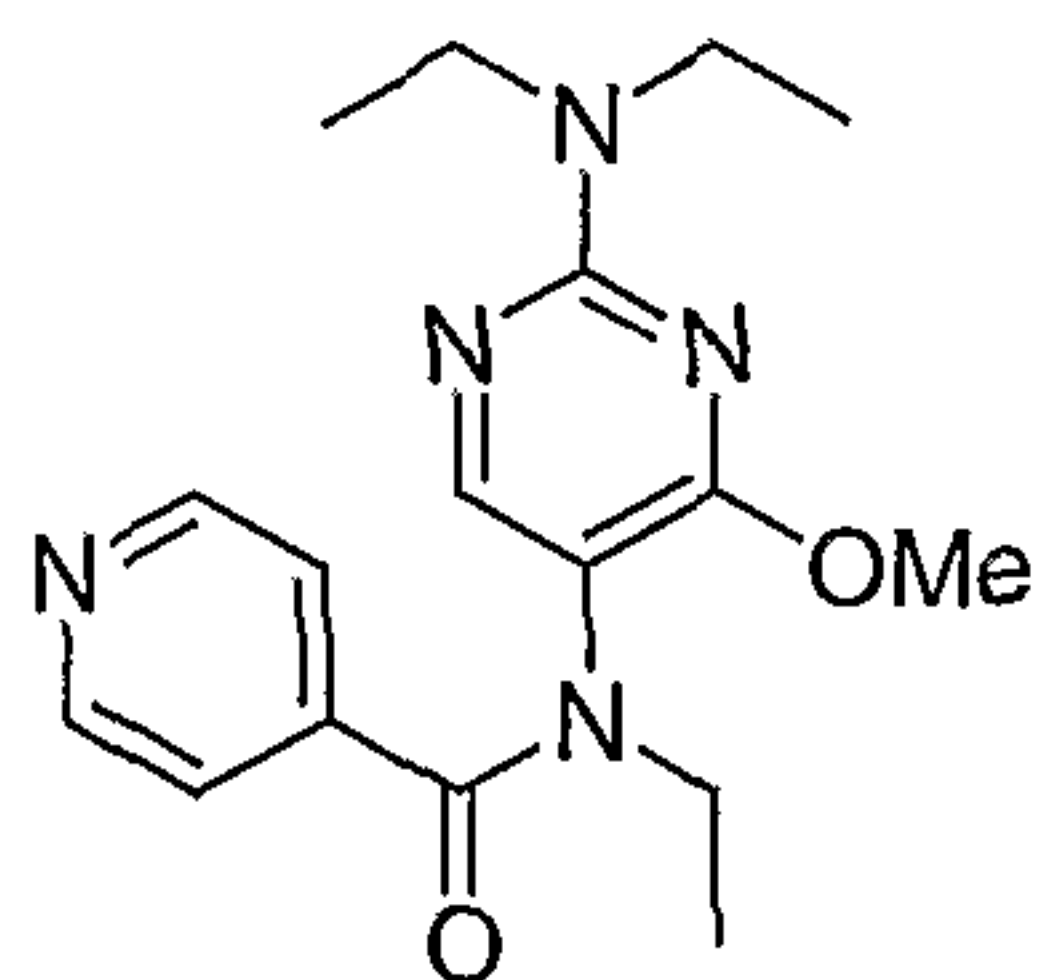
25

Example 21



To the product of Example 20 (0.85g, 4.3 mmol) in CH₂Cl₂ (15 mL) and TEA (1.4 mL, 10 mmol) was added isonicotinyl chloride HCl salt (1.13g, 6.3 mmol). After 15 min, TLC
 5 showed no starting material. The mixture was extracted between EtOAc and sat. NaHCO₃. The aqueous layer was washed with EtOAc twice. The combined organic layers were washed with sat. NaHCO₃ and brine. It was dried over MgSO₄ and filtered. The filtrate was concentrated to give the title compound as a brown solid (1.3g, 4.3 mmol, 100% yield). ¹H
 NMR (300 MHz, CDCl₃) δ 1.20 (6H, t, J = 6.9 Hz), 3.60 (4 H, q, J = 6.9 Hz), 3.96 (3 H, s), 7.72
 10 (2H, d, J = 6.0 Hz), 7.75 (1H, bs), 8.80 (2H, d, J = 6.0 Hz), 8.89 (1H, s); HPLC/MS: MH⁺ = 302.

Example 22

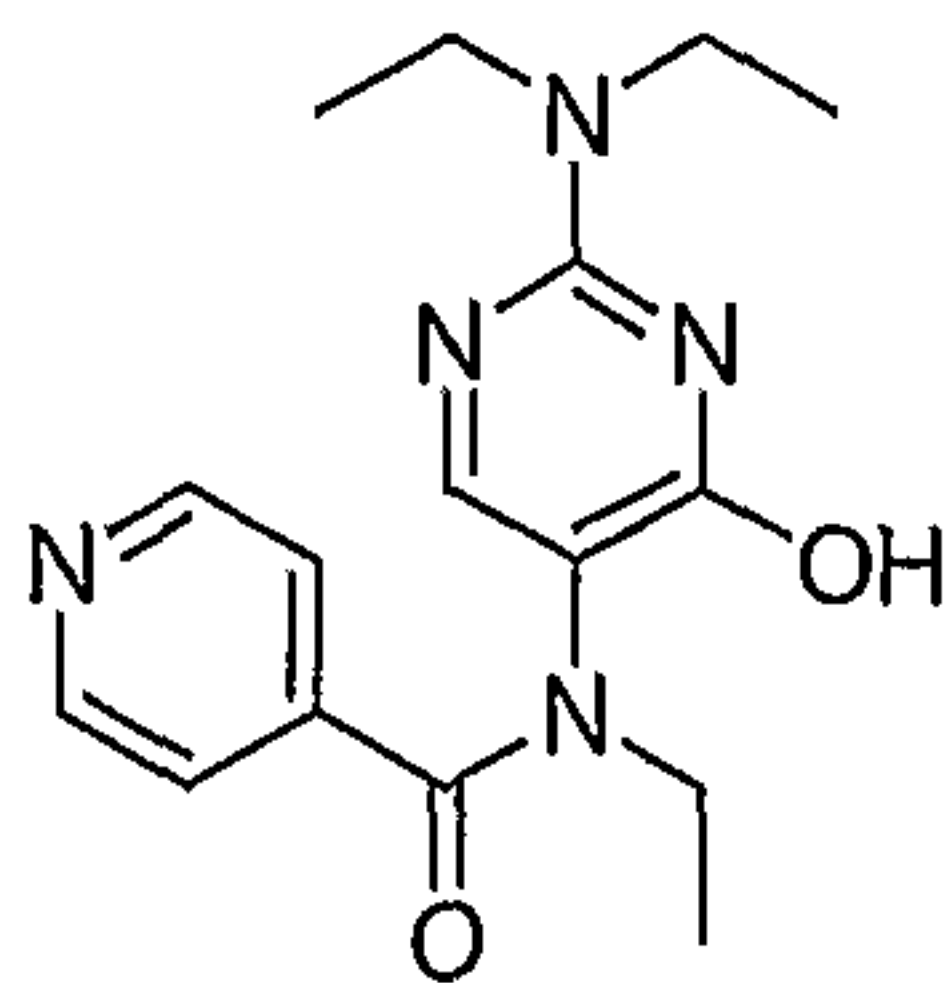


15

To the product of Example 21 (100 mg, 0.33 mmol) in THF (1 mL) was added KOtBu (1M in THF, 0.5 mL) slowly followed by EtI (40 μL, 0.5 mmol). The reaction mixture was stirred at rt overnight. TLC showed the disappearance of the starting material. The mixture
 20 was partitioned between EtOAc and H₂O. The aqueous layer was washed with EtOAc. The combined organic layers were washed with sat. NaHCO₃ and brine. It was dried and concentrated to give the title compound (90 mg, 0.27 mmol, 83%) that was used without further purification. ¹H NMR (300 MHz, CDCl₃) δ 1.10 (9H, m), 3.47 (5 H, m), 3.92 (1 H, m),
 7.14 (2H, d, J = 6.0 Hz), 7.78 (1H, bs), 8.44 (2H, d, J = 6.0 Hz); HPLC/MS: MH⁺ = 330.

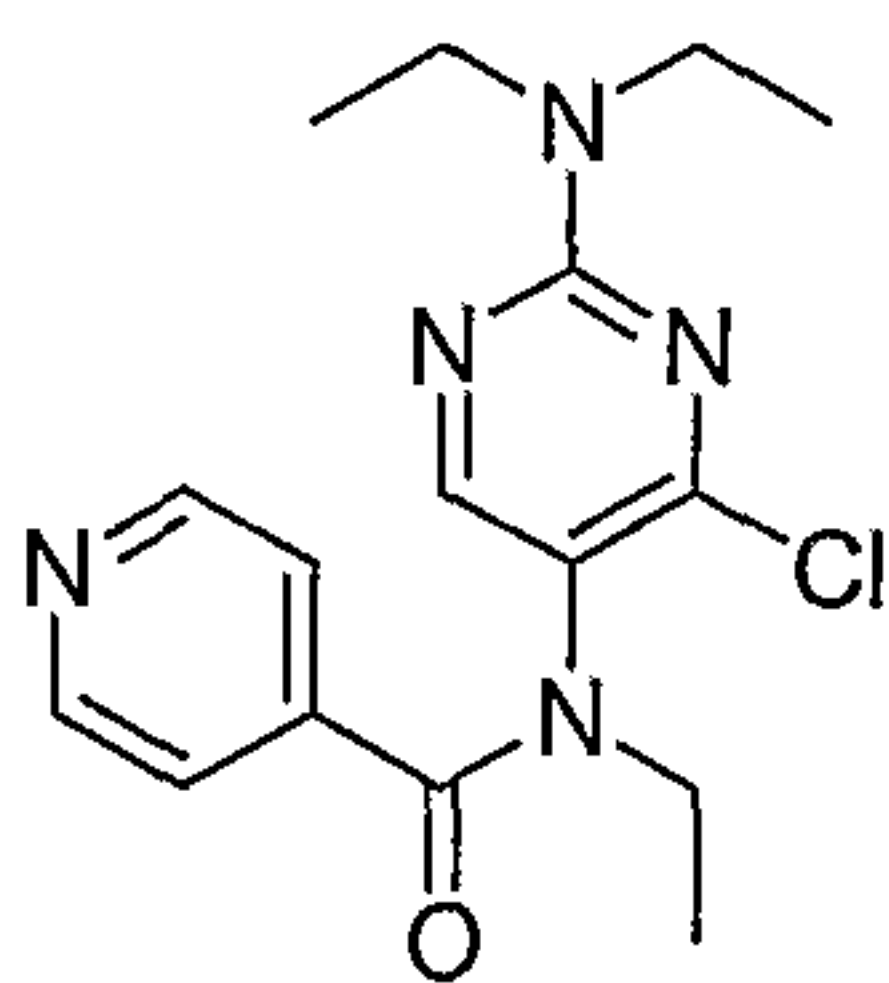
25

Example 23



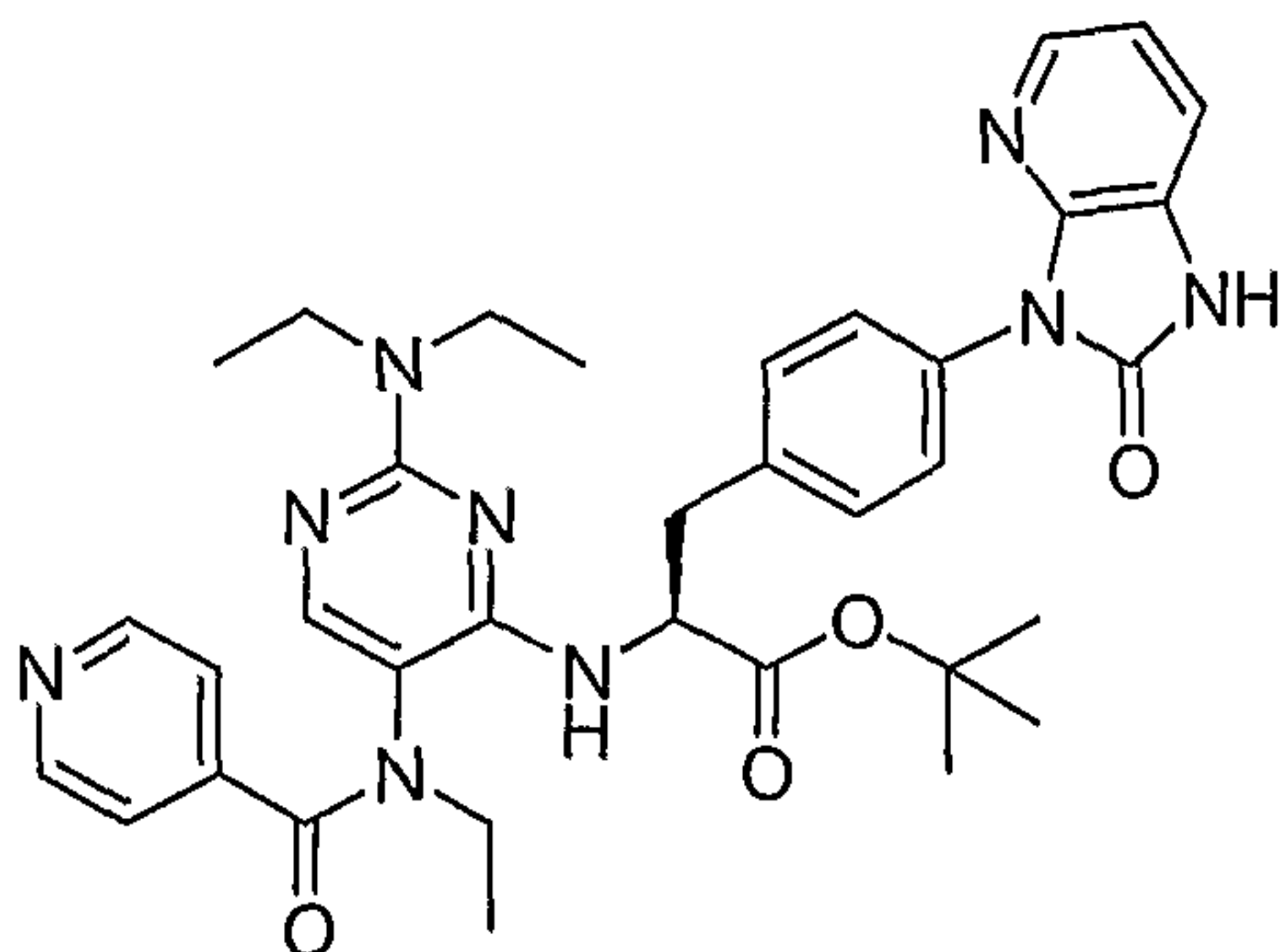
To the product of Example 22 (200 mg, 0.61 mmol) in DMF (4 mL) was added EtSNa (66 mg, 0.79 mmol) and the reaction mixture was heated at 100 C for 1 hr. LC/MS showed
 5 starting material still present. Another portion of NaSEt (66 mg, 0.79 mmol) was added and the reaction heated for another 2 hr. LC/MS showed product only. DMF was removed under reduced pressure and H₂O (10 mL) was added followed by conc. HCl (0.132 mL). Evaporating of the solvent left a residue. It was dissolved in EtOH and filtered. The filtrate was concentrated to to yield the title compound (190 mg, 100%) that was used without further
 10 purification. ¹H NMR (300 MHz, CD₃OD) δ 1.24 (9H, m), 3.60 (4 H, m), 3.60-4.00 (2 H, br), 8.12 (3H, d, *J* = 5.7 Hz), 8.92 (2H, d, *J* = 5.7 Hz); HPLC/MS: MH⁺ = 316.

Example 24



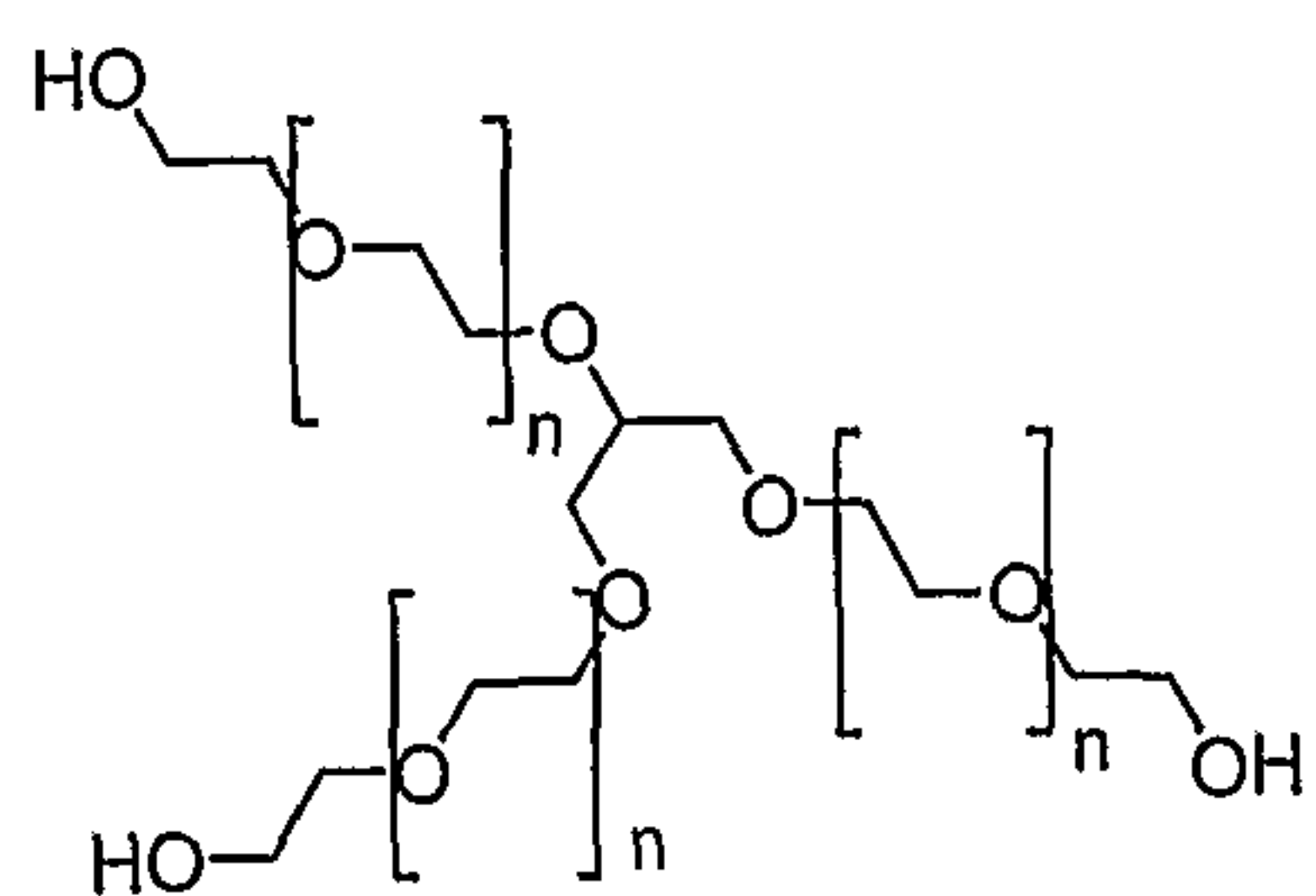
To the product of Example 23 (70 mg, 0.22 mmol) in POCl₃ (3 mL) at rt was added diethylaniline (30 μ L). The reaction mixture was heated to 100 C for 30 min. Then it was concentrated. The residue was partitioned between EtOAc and H₂O. The organic layer was washed with H₂O twice. Then it was dried and concentrated to give the title compound (50
 15 mg, 0.15 mmol, 68%) and used for the next reaction without further purification. HPLC/MS: MH⁺ = 334

Example 25

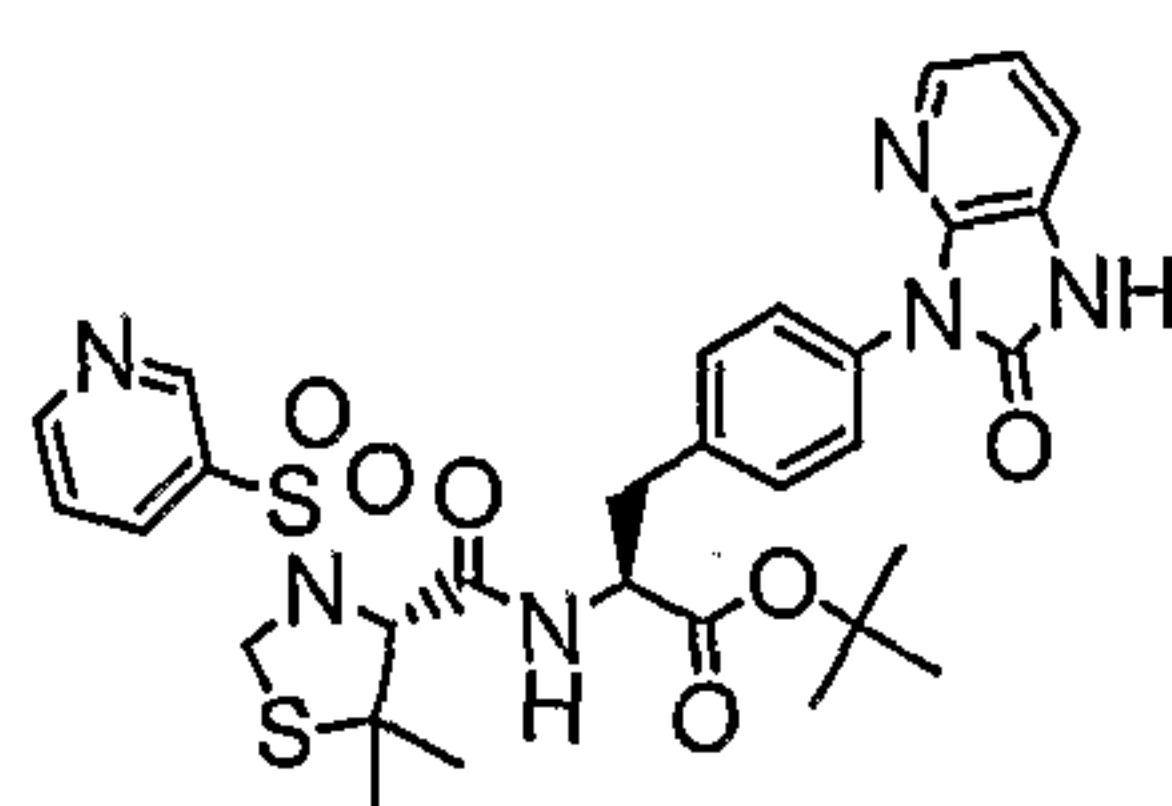


To a solution of the product of Example 24 (50 mg, 0.15 mmol) and the product of Example 8 (60 mg, 0.17 mmol) in IPA (0.75 mL) was added DIEA (0.15 mL, 0.8 mmol). The reaction mixture was stirred in a sealed tube at 130 degrees for 7 days. The crude mixture
5 was concentrated and the residue was purified by preparative HPLC and silica gel flash chromatography to yield an off white solid (10 mg). ¹H NMR (300 MHz, CDCl₃) δ 1.10-1.30 (9H, m), 1.48 (4.5H, s), 1.51 (4.5H, s), 2.80-3.38 (3H, m), 3.53 (4H, m), 4.05-4.30 (1H, m), 4.83 (0.5H, m), 4.96 (0.5H, m), 5.15-5.50 (1H, m), 6.95-7.10 (2H, m), 7.25-7.50 (5H, m), 7.69 (0.5H, d, *J* = 8.4 Hz), 7.76 (0.5H, d, *J* = 8.4 Hz), 8.08 (1H, d, *J* = 5.1 Hz), 8.51 (2H, m), 8.83
10 (0.5H, br), 8.95 (0.5H, br);

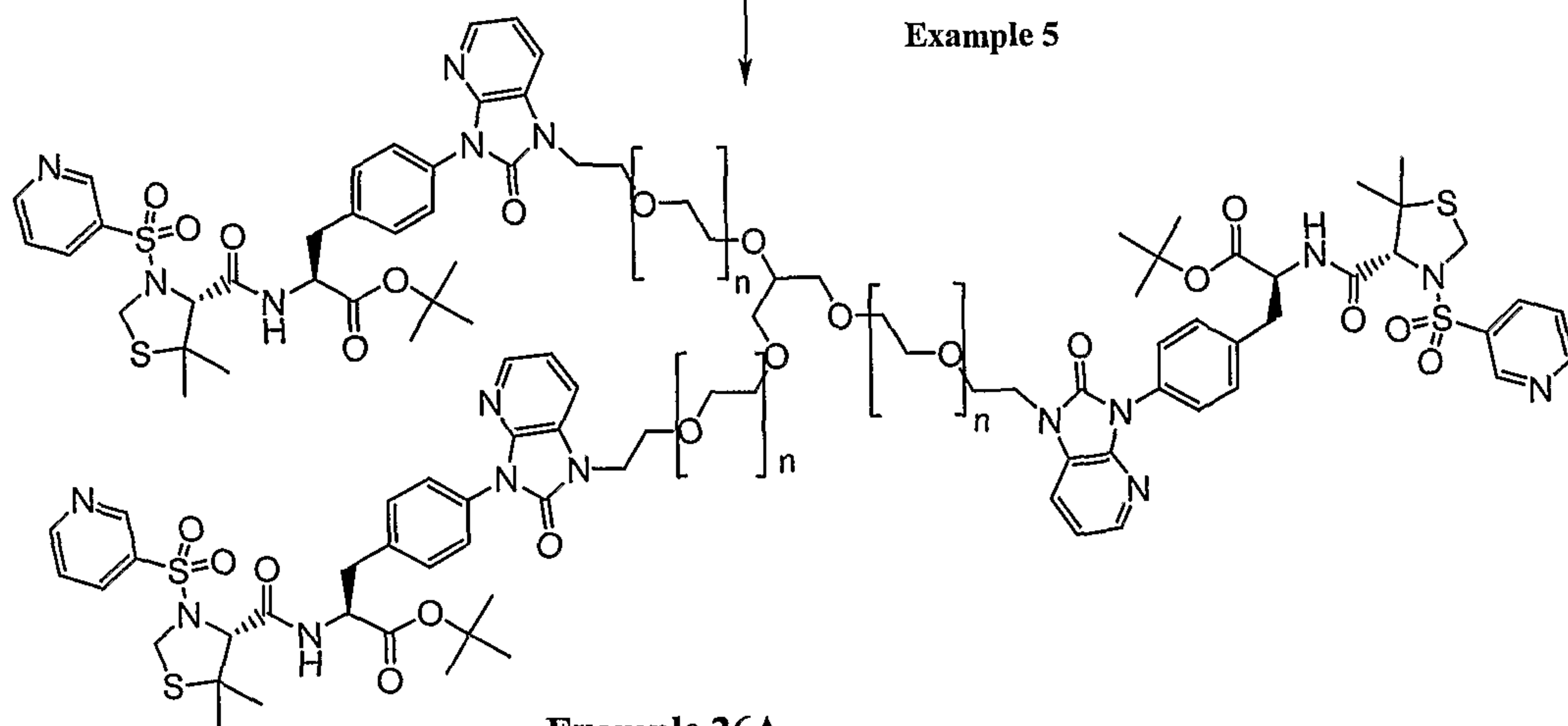
HPLC/MS: MH⁺ = 652.

Example 26**Procedure A**

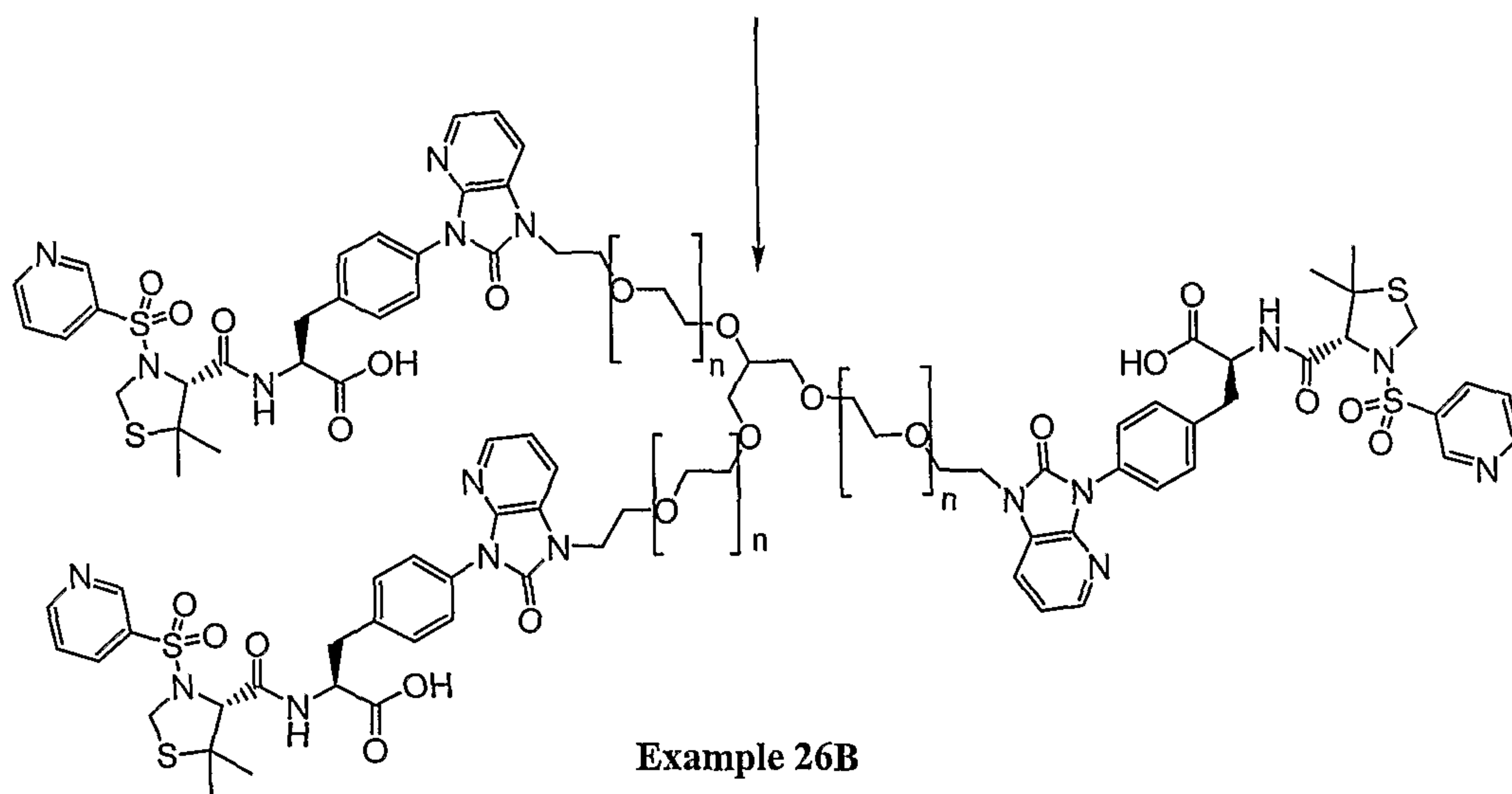
40 kDa 3-arm PEG alcohol
NOF Corporation



Example 5



Example 26A



Example 26B

Example 26A The 40 kDa 3-arm PEG alcohol (0.25 g, 0.00625 mmol), the product from Example 5 (0.04 g, 0.056 mmol), and triphenylphosphine (0.025 g, 0.094 mmol) were dried by azeotropic distillation from toluene (5 mL). Half of the volume was distilled over (2.5 mL), and the mixture was cooled to room temperature. CH₂Cl₂ (0.5 mL) was added to make the reaction homogeneous. Diethylazodicarboxylate (0.015 mL, 0.094 mmol) was added drop-wise and the reaction stirred for 48 hours. HPLC Method C showed the complete disappearance of the starting PEG alcohol. The reaction was concentrated *in vacuo* to yield the **t-butyl ester** Example 26A as a white solid.

10 **Example 26B**

Example 26A (0.2 g, 0.005 mmol) was dissolved in formic acid (3 mL) and heated at 40°C for 24 hours. The reaction was concentrated *in vacuo* and was purified according to HPLC Method A to yield 0.1 g (48%) of Example 26B as a white solid. HPLC Method C determined the conjugate to be >99% pure (retention time= 8.1 minutes). ¹H NMR (CDCl₃) δ 9.08 (bs, 3H), 8.84 (bs, 3H), 8.18-8.16 (d, 3H), 8.02-8.00 (d, 3H), 7.67-7.61 (m, 6H), 7.47-7.38 (m, 9H), 7.08-7.04 (m, 3H), 6.91 (m, 3H), 4.88 (m, 3H), 4.62-4.49 (dd, 6H), 4.13 (m, 6H), 3.64 (bs, 5919H PEG), 3.23 (m, 6H), 1.25-1.24 (d, 18H).

Procedure B

20 A mixture of GL400 Sunbright PEG (50.0 g, NOF lot# M4N594), the product from Example 5 (7.19 g, 9 eq vs. GL400, 3 eq/hydroxyl), and triphenylphosphine (2.95 g, 9 eq) was taken up in toluene (300 mL) and distilled to azeotropically remove water. The mixture was cooled to room temperature and an additional amount of the remaining toluene was removed via rotary evaporation. The mixture was re-dissolved in dry dichloromethane (180 mL) and cooled in an ice bath. Diisopropylazodicarboxylate (DIAD, 2.27 g, 2.17 mL, 9 eq) was added via syringe drive over 1 hour. The mixture was stirred in the ice bath 1.5 h whereupon HPLC analysis indicated complete conversion to Example 26A.

30 The viscous reaction mixture was slowly added via a narrow-necked funnel with stirring to a mixture of 75:25 MTBE/IPA (4.0 L) and allowed to stir 1 hour. The precipitate was collected via vacuum filtration, washed with MTBE (200 mL), and dried under vacuum to give purified Example 26A (51.5 g.).

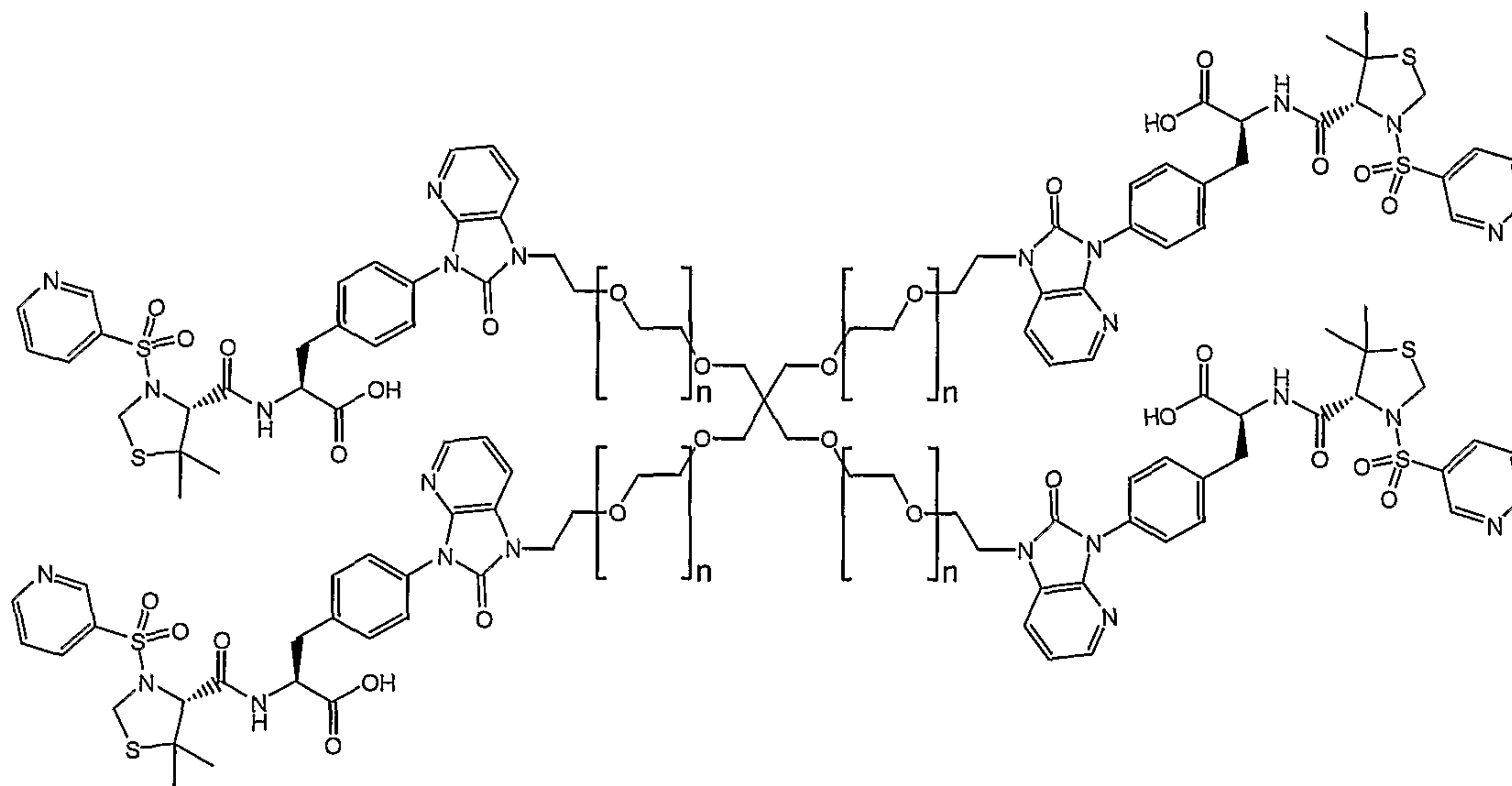
35 Example 26A (51.4 g) was taken up into formic acid (250 mL) and heated at near reflux for 1.0 h whereupon HPLC analysis indicated the deprotection was complete. The mixture was cooled to room temp and a portion of the formic acid (-100mL) was removed by rotary evaporation. The mixture was diluted with methylene chloride (50 mL). The viscous reaction mixture was then added with stirring to a mixture of 75:25 MTBE/IPA (4.0 L) and allowed to stir

45 min (Note 4). The precipitate was collected via vacuum filtration, washed with MTBE (300 ml) and dried under vacuum (< 1 Torr, 24 h) to yield Example 26B (50.0 g).

This material was taken up in methylene chloride (400 mL) and filtered through a sintered-glass Buchner funnel ("polish filtration"). The filtrate was concentrated via rotary evaporation to a volume of ~ 200 mL and slowly added with stirring to a 75:25 mixture of MTBE/IPA (4.0L). The precipitate was collected via vacuum filtration, washed with MTBE (300 ml) and dried under vacuum (< 1 Torr, 3d) to yield Example 26B (48.8 g, ~94%).

10 Similar methods were used to synthesize the following conjugates:

Example 27

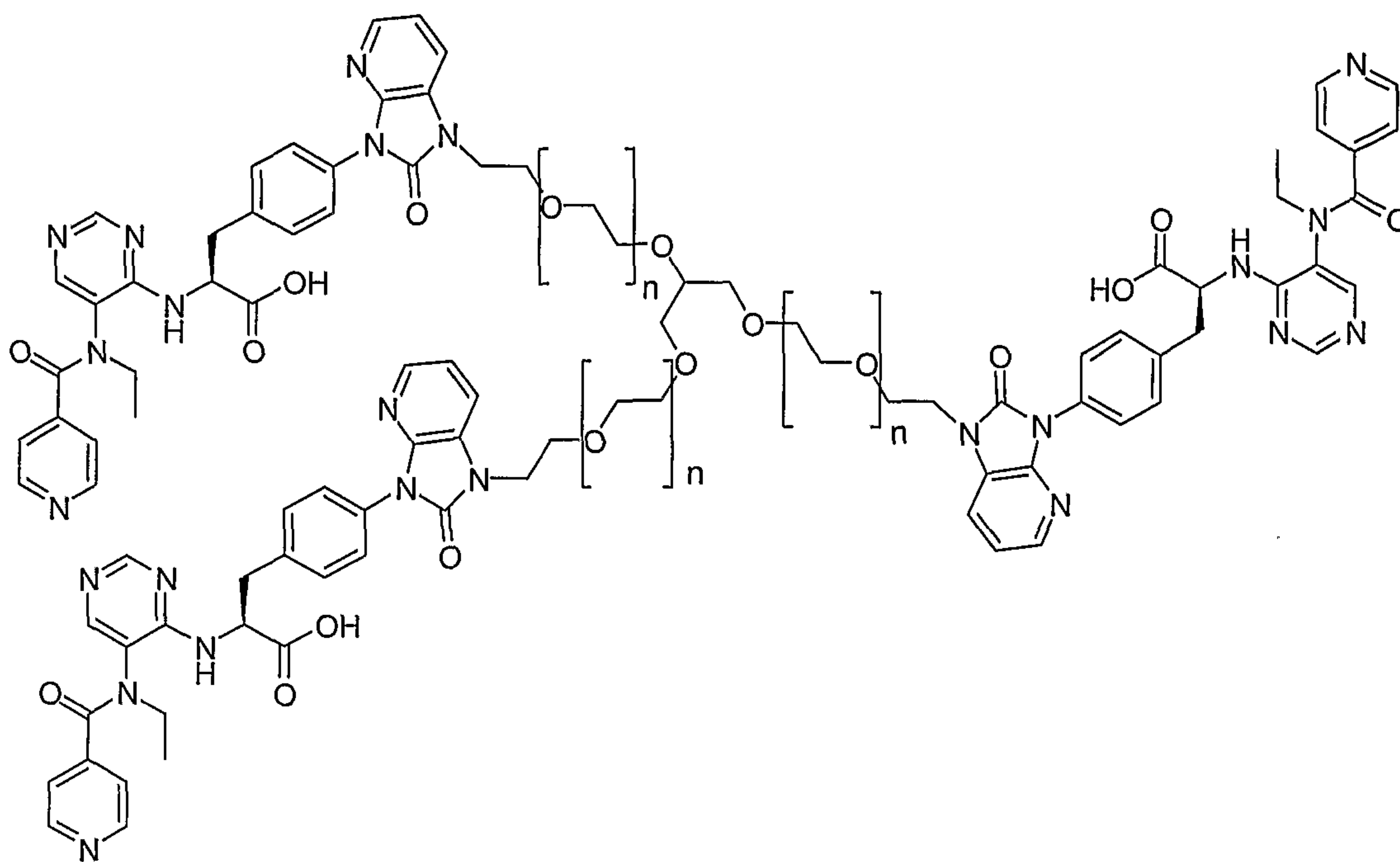


Example 27

40 kDa 4-arm PEG alcohol was coupled to the product of Example 5 and deprotected to final product using similar methods as with Example 26. The product was purified according to HPLC Method A. HPLC Method C determined the conjugate to be >95% pure (retention time= 7.5-8.1 minutes). ¹H NMR (CDCl₃) δ 9.08 (bs, 4H), 8.84 (bs, 4H), 8.18-8.16 (d, 4H), 8.02-8.00 (d, 4H), 7.67-7.61 (m, 8H), 7.47-7.38 (m, 12H), 7.08-7.04 (m, 4H), 6.91 (m, 4H), 4.88 (m, 4H), 4.62-4.49 (dd, 8H), 4.13 (m, 8H), 3.64 (bs, 10101H PEG), 3.23 (m, 8H), 1.25-1.24 (d, 24H).

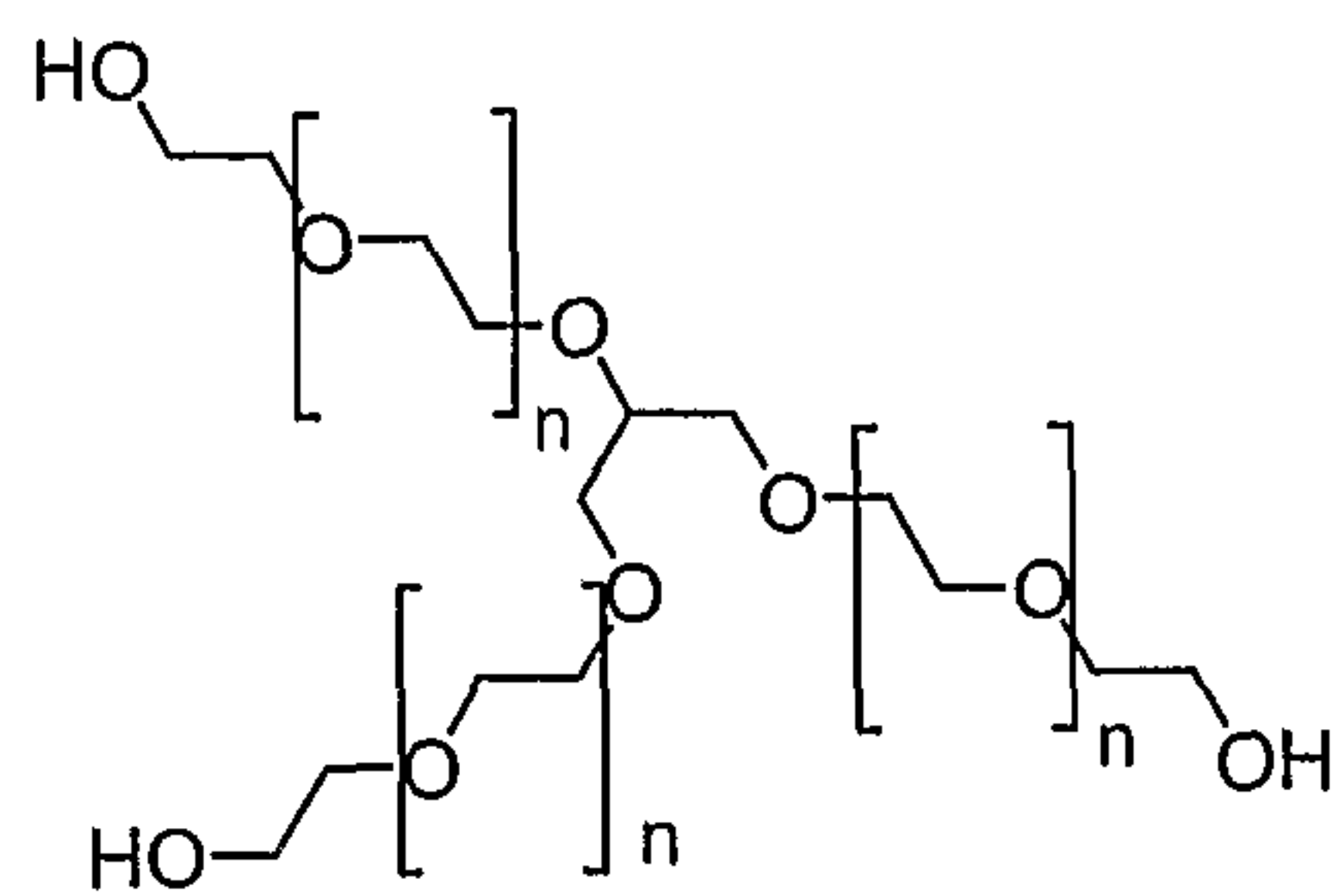
20

Example 28

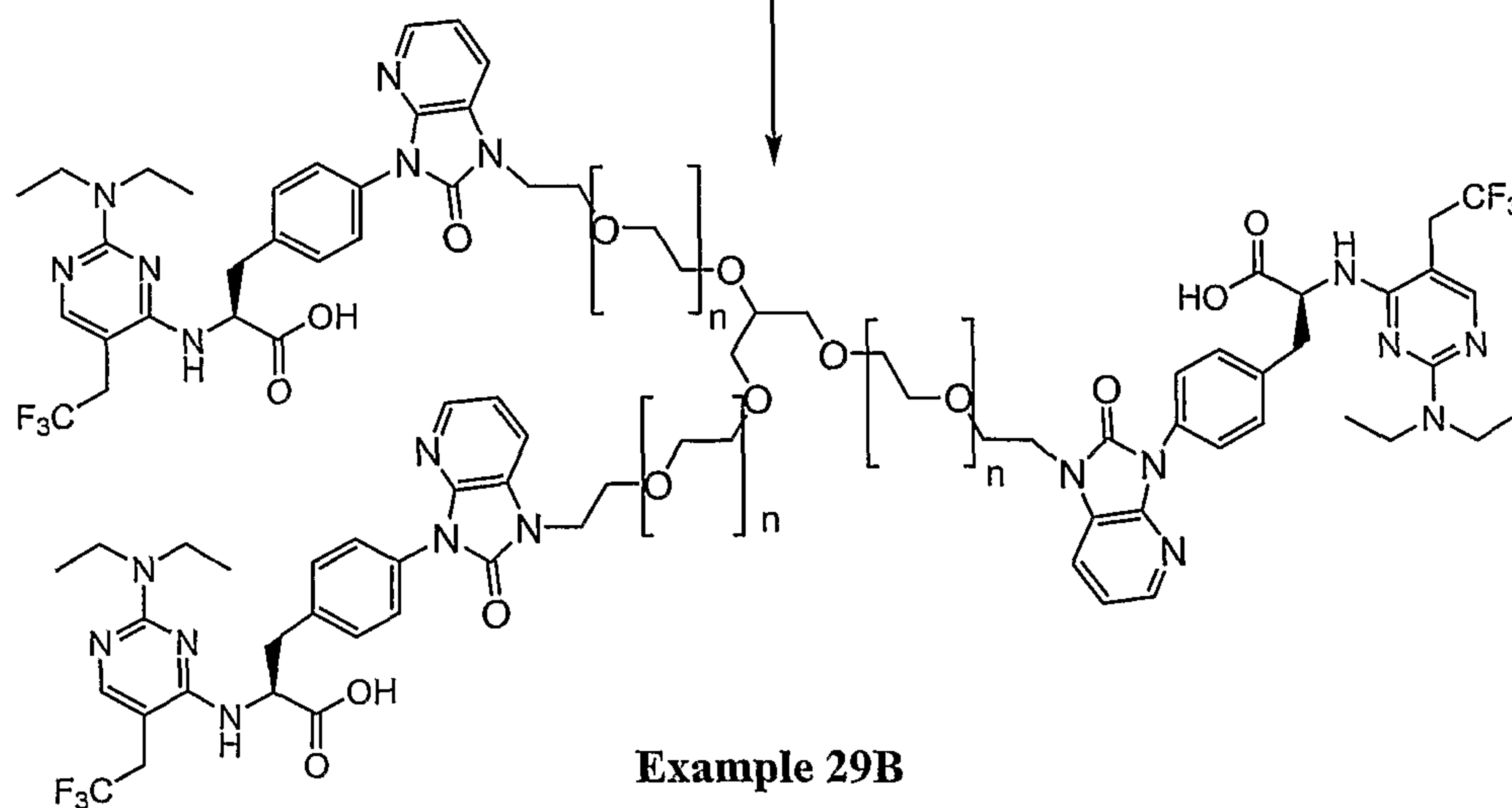
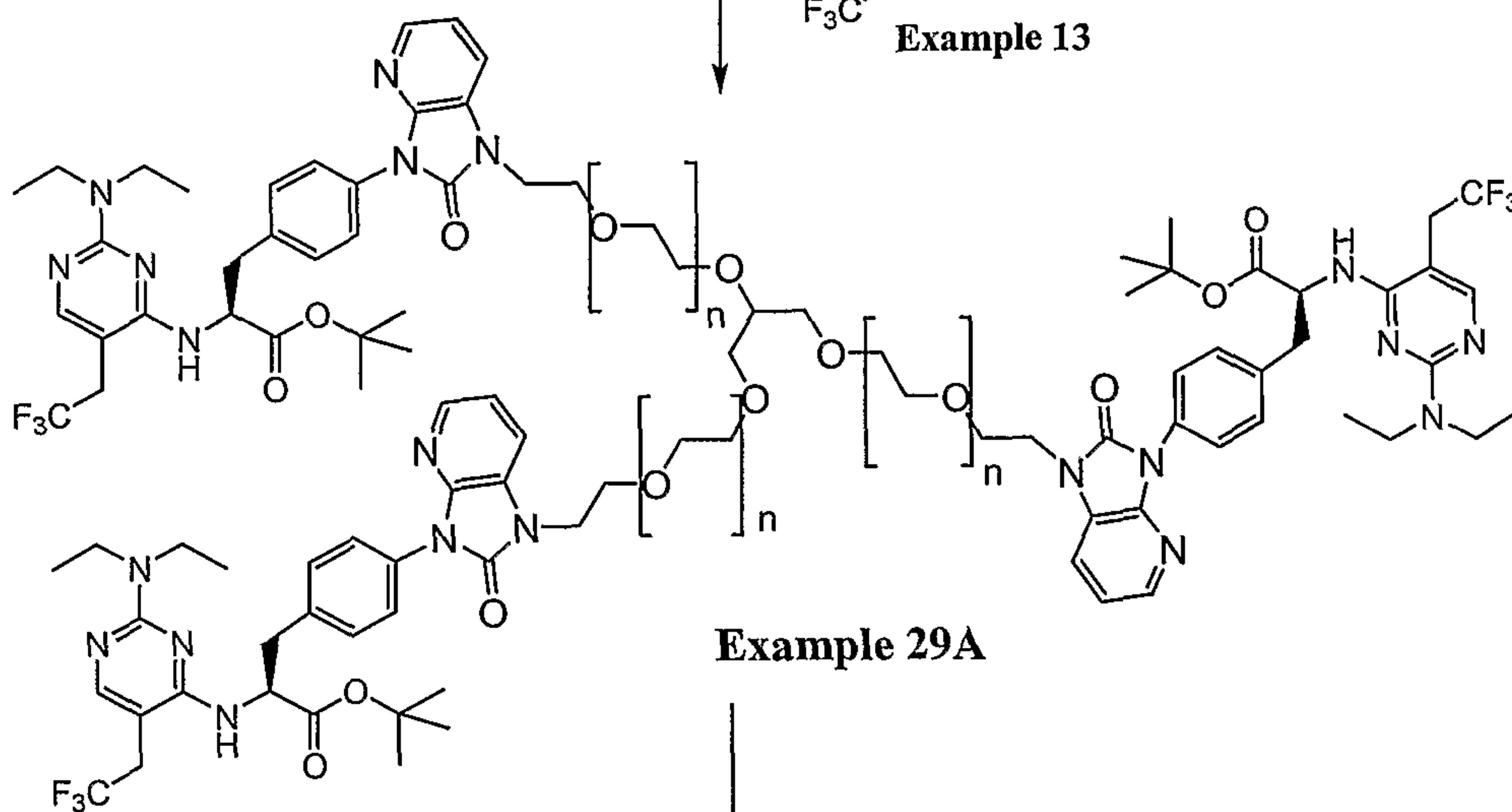
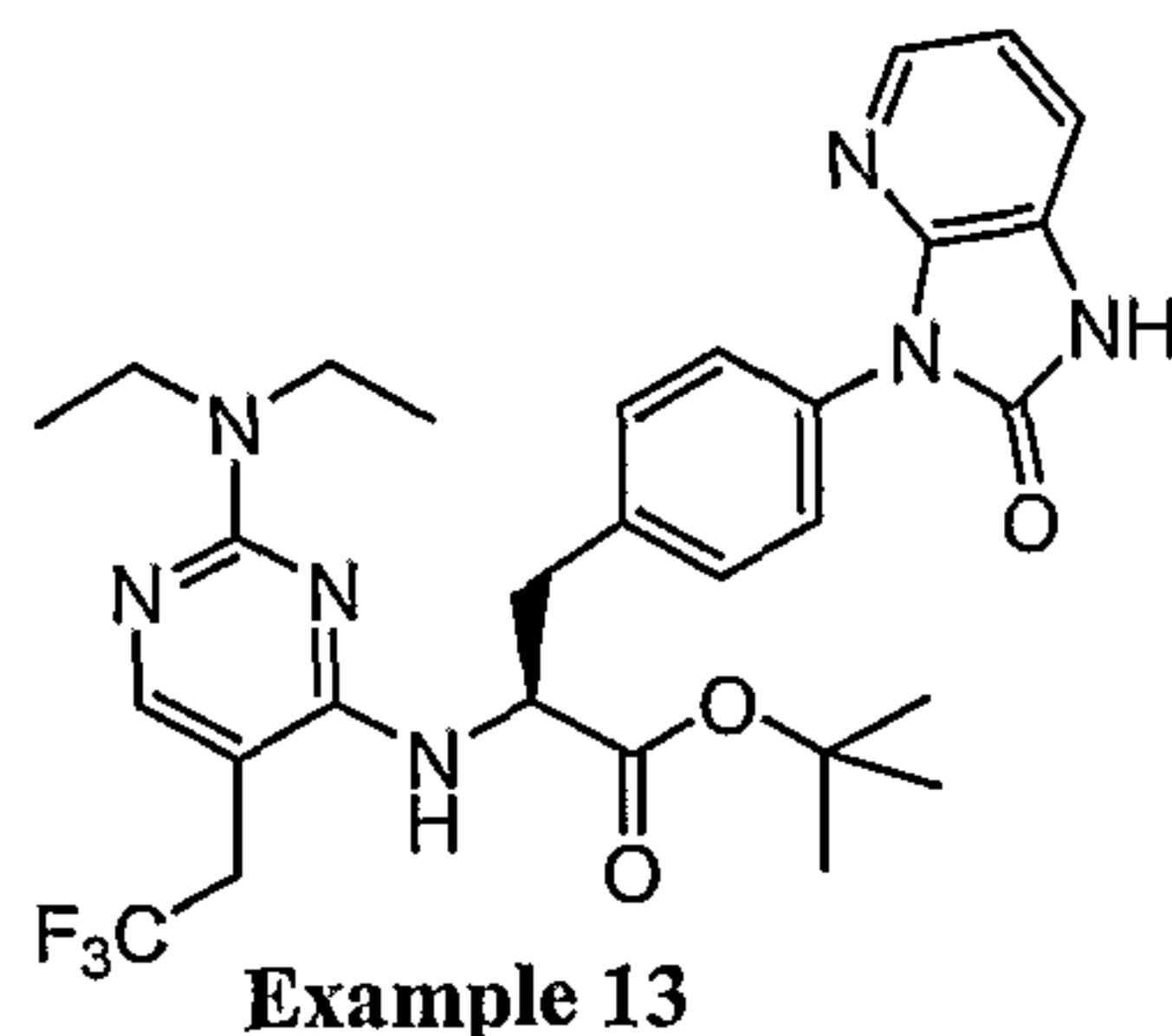


Example 28.

- 40 kDa 3-arm PEG alcohol** was coupled to the t-butyl ester product from Example 18 (shown below) and deprotected to final product using methods similar to those of Example 26.
- 5 The product was purified according to HPLC Method A. HPLC Method C determined the conjugate to be >95% pure (retention time= 7.3 minutes). ¹H NMR (CDCl₃) δ 8.66 (bs, 3H), 8.44 (bs, 3H), 8.04-8.02 (d, 3H), 7.75- 7.30 (m, 24H), 7.10-7.06 (m, 3H), 6.93 (s, 3H), 5.60-5.50 (m, 3H), 4.15 (m, 6H), 3.66 (bs, 4270H PEG), 3.00 (m, 3H), 3.40-3.20 (m, 6H), 1.27 (d, 9H).

Example 29

40 kDa 3-arm PEG alcohol
NOF Corporation



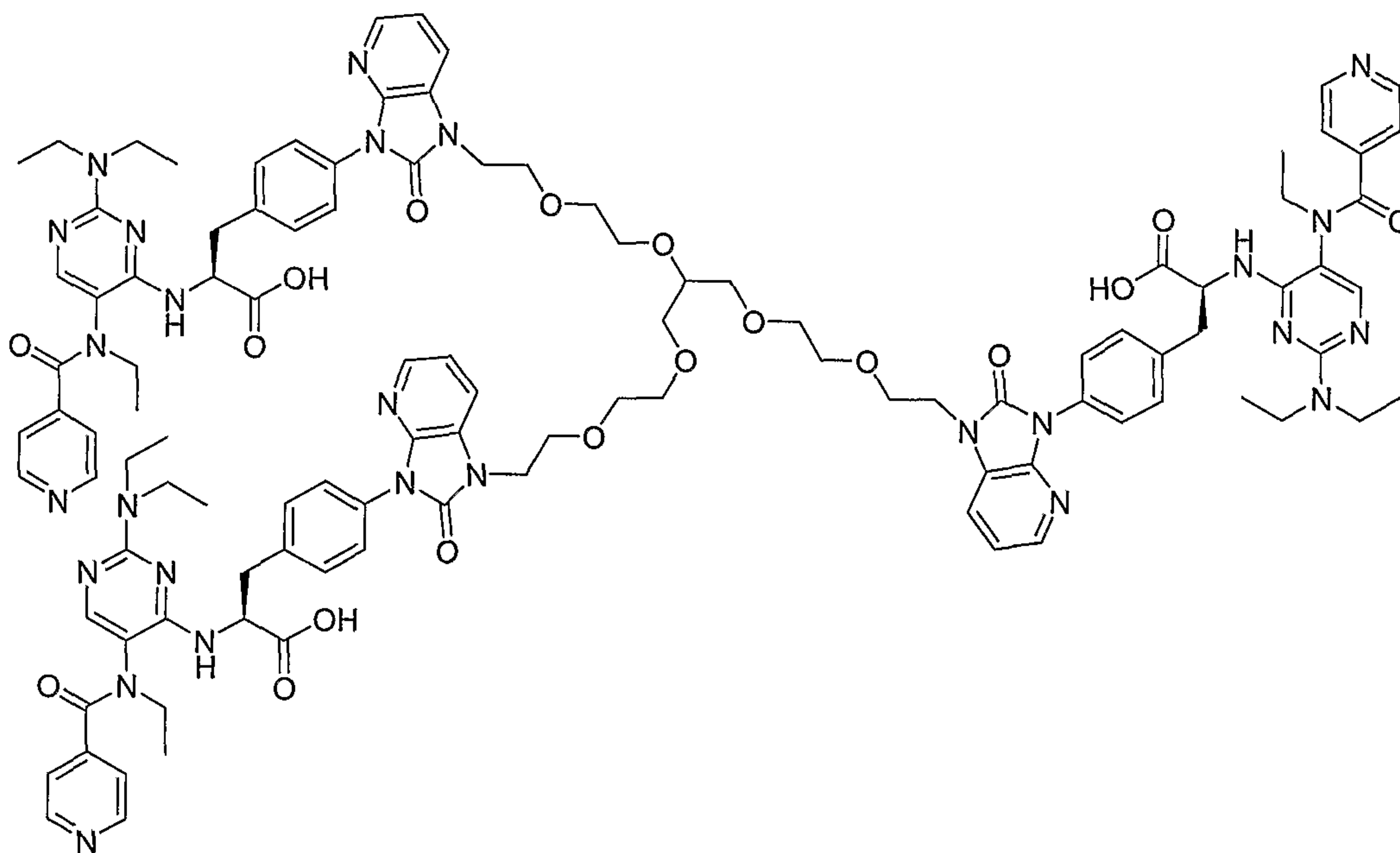
Example 29

Example 29A

The **40 kDa 3-arm PEG alcohol** (0.00625 mmol), the product from Example 13 (0.056 mmol), and triphenylphosphine (0.094 mmol) are dried by azeotropic distillation from toluene (5 mL). Half of the volume is distilled over (2.5 mL), and the mixture is cooled to room temperature. CH₂Cl₂ (0.5 mL) is added to make the reaction homogeneous. Diethylazodicarboxylate (0.094 mmol) is added drop-wise and the reaction stirred for 48 hours. The reaction is concentrated *in vacuo* to yield the **t-butyl ester** Example 29A.

Example 29B

Example 29A (0.005 mmol) is dissolved in formic acid (3 mL) and heated at 40°C for 24 hours. The reaction is concentrated *in vacuo* and is purified according to HPLC Method A to yield Example 29B.

Example 30

Example 30

40 kDa 3-arm PEG alcohol is coupled to the t-butyl ester product from Example 25 and deprotected to final product using similar methods as with Example 26. The product is purified according to HPLC Method A.

References

The following publications, patents and patent applications are cited in this application as superscript numbers:

- 1 Hemler and Takada, European Patent Application Publication No. 330,506, published
5 August 30, 1989
- 2 Elices, et al., Cell, 60:577-584 (1990)
- 3 Springer, Nature, 346:425-434 (1990)
- 4 Osborn, Cell, 62:3-6 (1990)
- 5 Vedder, et al., Surgery, 106:509 (1989)
- 10 6 Pretolani, et al., J. Exp. Med., 180:795 (1994)
- 7 Abraham, et al., J. Clin. Invest., 93:776 (1994)
- 8 Mulligan, et al., J. Immunology, 150:2407 (1993)
- 9 Cybulsky, et al., Science, 251:788 (1991)
- 10 Li, et al., Arterioscler. Thromb., 13:197 (1993)
- 15 11 Sasseville, et al., Am. J. Path., 144:27 (1994)
- 12 Yang, et al., Proc. Nat. Acad. Science (USA), 90:10494 (1993)
- 13 Burkly, et al., Diabetes, 43:529 (1994)
- 14 Baron, et al., J. Clin. Invest., 93:1700 (1994)
- 15 Hamann, et al., J. Immunology, 152:3238 (1994)
- 20 16 Yednock, et al., Nature, 356:63 (1992)
- 17 Baron, et al., J. Exp. Med., 177:57 (1993)
- 18 van Dinther-Janssen, et al., J. Immunology, 147:4207 (1991)
- 19 van Dinther-Janssen, et al., Annals. Rheumatic Dis., 52:672 (1993)
- 20 Elices, et al., J. Clin. Invest., 93:405 (1994)
- 25 21 Postigo, et al., J. Clin. Invest., 89:1445 (1991)
- 22 Paul, et al., Transpl. Proceed., 25:813 (1993)
- 23 Okarhara, et al., Can. Res., 54:3233 (1994)
- 24 Paavonen, et al., Int. J. Can., 58:298 (1994)
- 25 Schadendorf, et al., J. Path., 170:429 (1993)
- 30 26 Bao, et al., Diff., 52:239 (1993)
- 27 Lauri, et al., British J. Cancer, 68:862 (1993)
- 28 Kawaguchi, et al., Japanese J. Cancer Res., 83:1304 (1992)
- 29 Kogan, et al., U.S. Patent No. 5,510,332, issued April 23, 1996
- 30 International Patent Appl. Publication No. WO 96/01644
- 35 31 Thorsett, et al., U.S. Patent No. 6,489,300, issued December 3, 2002 and Konradi, et al.,
U.S. Patent No. 66,492,372, issued December 10, 2002.

All of the above publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

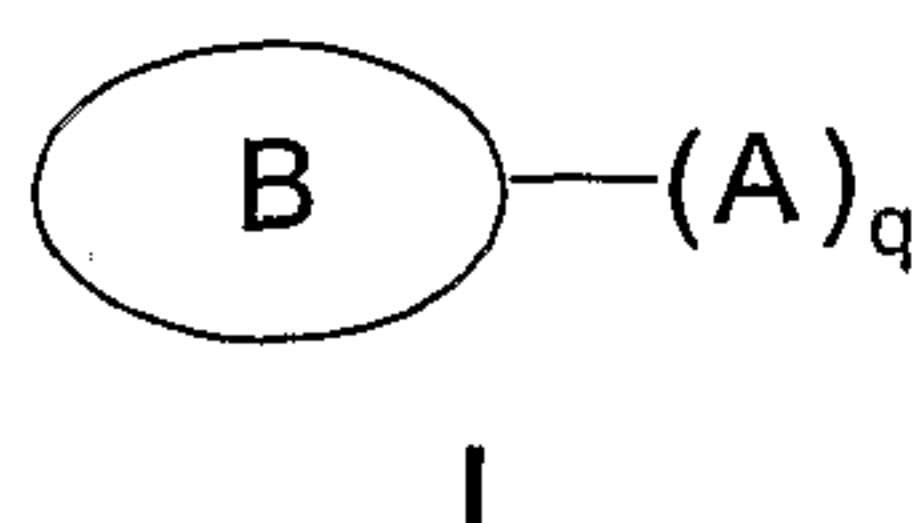
WHAT IS CLAIMED IS:

1. A process for the preparation of conjugates of active compounds comprising:
 a) reacting a polymeric alcohol with a nucleophilic active compound having an acidic
 hydrogen under Mitsunobu conditions; and

5 b) isolating the conjugate.

2. A process according to claim 1, where the conditions comprise a
 trivalent phosphine and an azodicarbonyl compound.

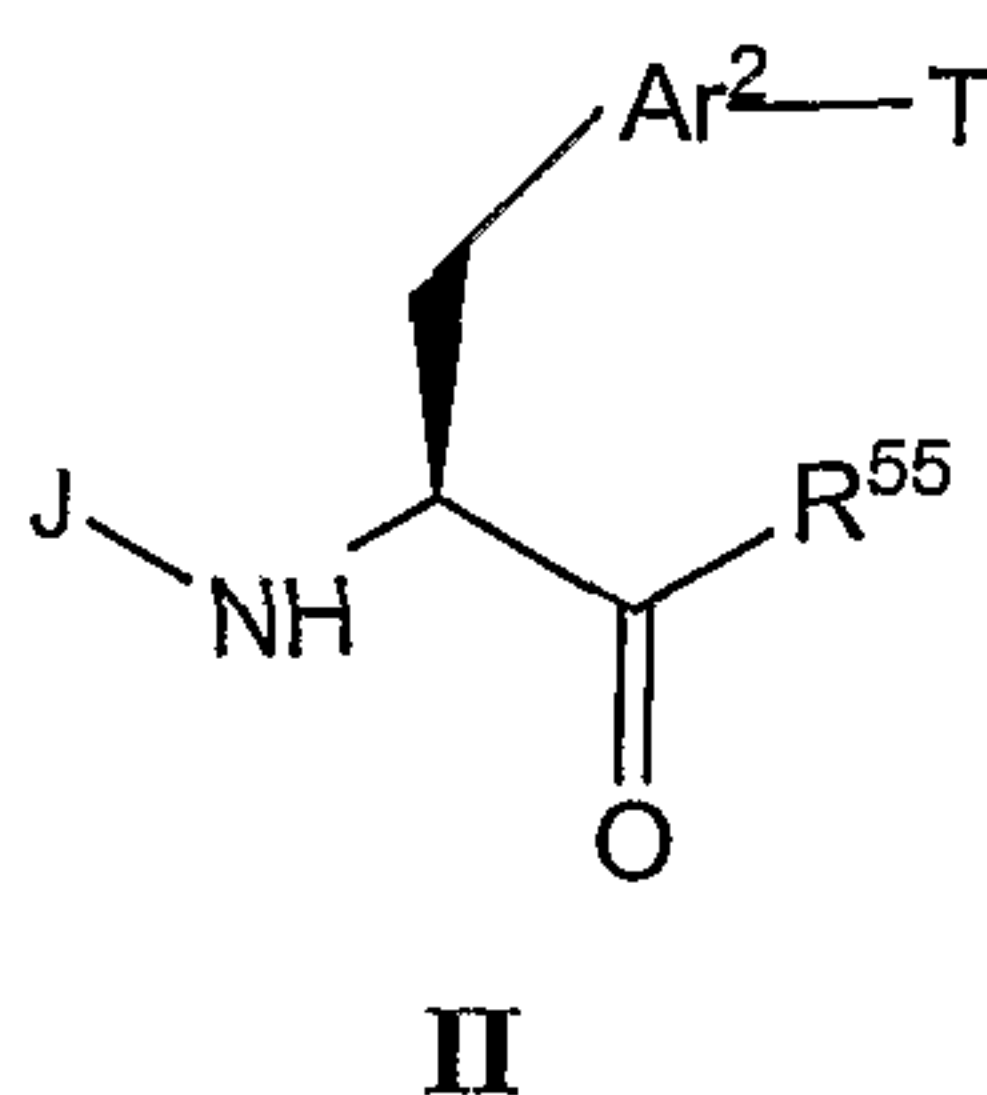
10 3. A process for the preparing a conjugate of the formula I:



B is a bio-compatible polymer moiety optionally covalently attached to a branched-arm hub
 molecule;

15 q is from about 1 to about 100;

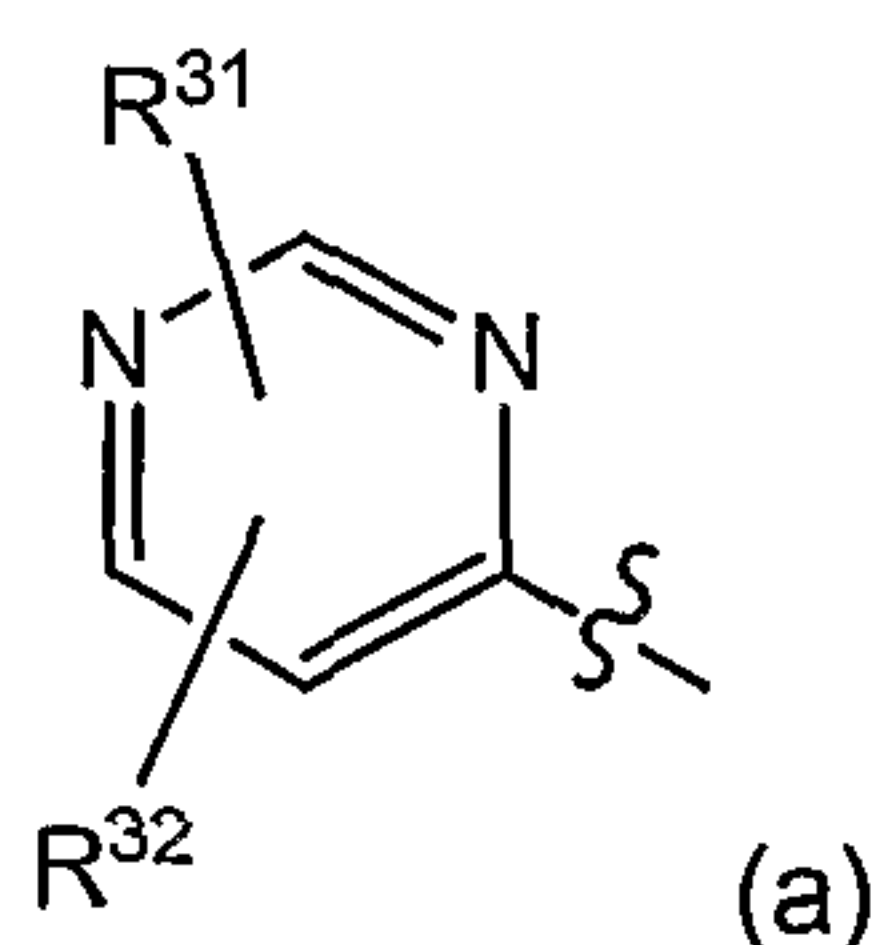
A at each occurrence is independently a compound of formula II



or a pharmaceutically acceptable salt thereof, wherein

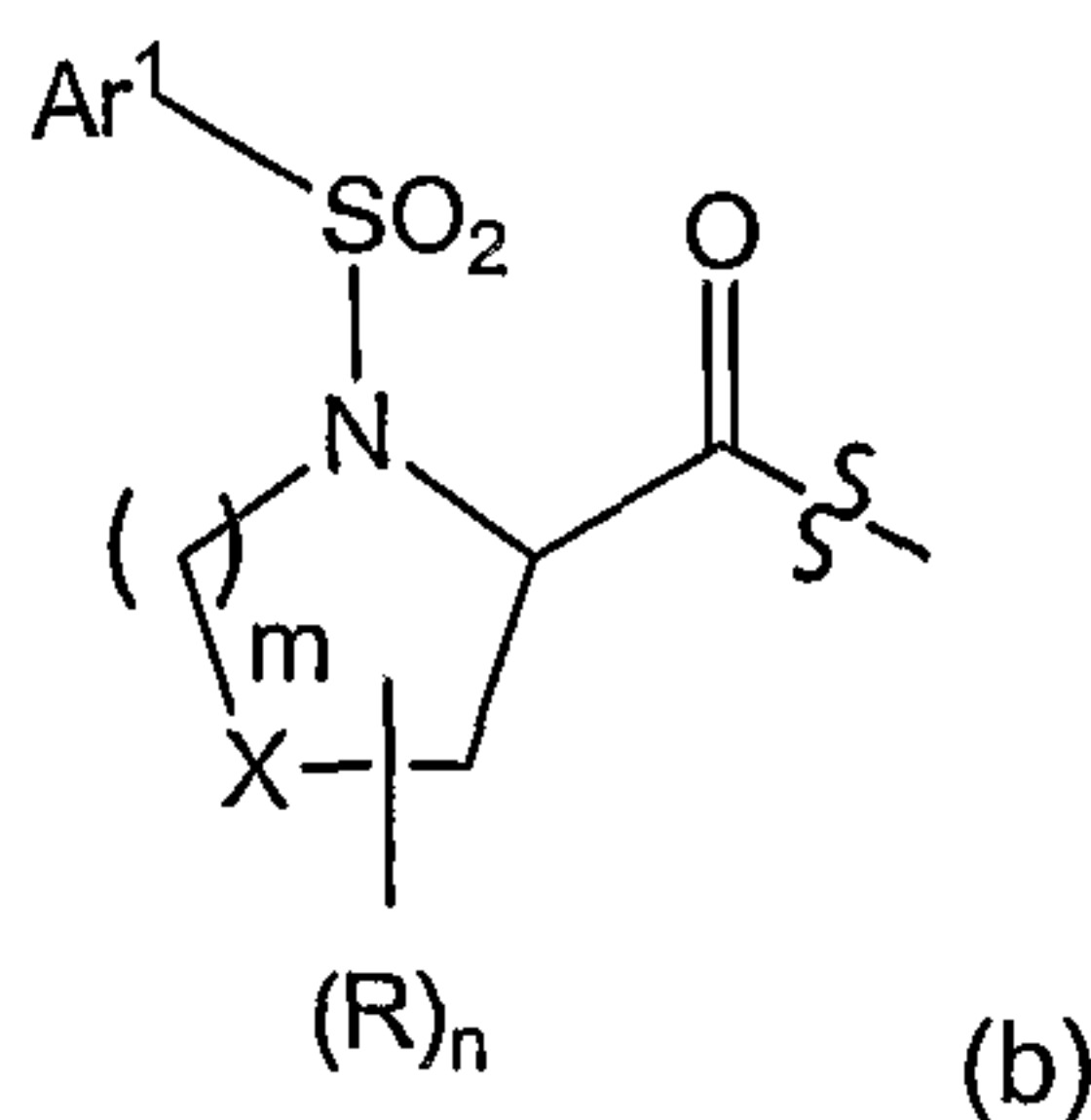
J is selected from:

20 a) a group of formula (a):



wherein R^{31} is a covalent bond to the polymer moiety which optionally comprises a linker,
 or R^{31} is $-H$, $R^{31'}$, $-NH_2$, $-NHR^{31'}$ or $-N(R^{31'})_2$, $-NC_3-C_6$ cyclic, $-OR^{31'}$, $-SR^{31'}$,
 wherein each $R^{31'}$ is independently an optionally substituted straight or branched
 25 C_1-C_6 alkyl, optionally substituted C_3-C_6 cycloalkyl, optionally substituted aryl,
 optionally substituted heteroaryl,

and R^{32} is a covalent bond to the polymer moiety which optionally comprises a linker, or
 R^{32} is $-H$, $-NO_2$, haloalkyl or the group $-N(MR^{41})R^{42}$ wherein M is a covalent bond,
 $-C(O)-$ or $-SO_2-$, R^{41} is $R^{41'}$, $N(R^{41'})_2$, or $-OR^{41'}$,
 wherein each $R^{41'}$ is independently hydrogen, an optionally substituted straight or
 branched C_1-C_6 alkyl, optionally substituted cycloalkyl, optionally substituted aryl,
 optionally substituted heterocyclic or an optionally substituted heteroaryl, wherein
 optional substitutions are halide, C_1-C_6 alkyl, or $-OC_1-C_6$ alkyl,
 and R^{42} is hydrogen or $R^{41'}$; and
 b) a group of formula (b):



wherein R is selected from the group consisting of a covalent bond to the polymer moiety, amino, hydroxyl, substituted amino, alkyl, alkyloxy, aryloxy, heteroaryloxy, heterocyclyloxy, thiol, arylthio, heteroarylthio, heterocyclylthio and substituted alkyl wherein each amino, substituted amino, alkyl and substituted alkyl is optionally covalently bound to the polymer moiety wherein, in each case, the polymer moiety optionally comprises a linker which covalently links the polymer moiety;

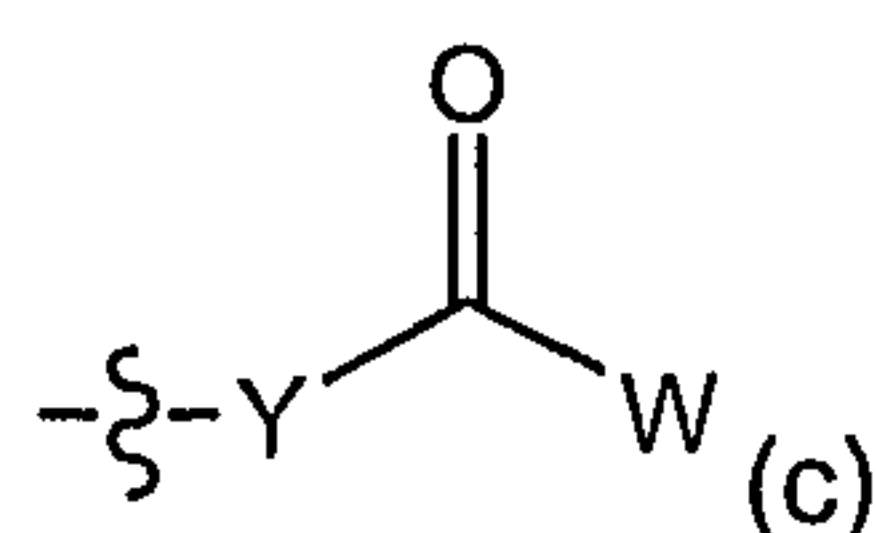
Ar^1 is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl wherein each of aryl, substituted aryl, heteroaryl and substituted heteroaryl is optionally covalently bound to the polymer moiety wherein the polymer moiety optionally comprises a linker which covalently links the polymer moiety to Ar^1 ;

Ar^2 is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl wherein each of aryl, substituted aryl, heteroaryl and substituted heteroaryl is optionally covalently bound to the polymer moiety wherein the polymer moiety optionally comprises a linker which covalently links the polymer moiety to Ar^2 ;

X is selected from the group consisting of $-NR^1-$, $-O-$, $-S-$, $-SO-$, $-SO_2$ and optionally substituted $-CH_2-$ where R^1 is selected from the group consisting of hydrogen and alkyl;

T is selected from:

a) a group of formula (c)



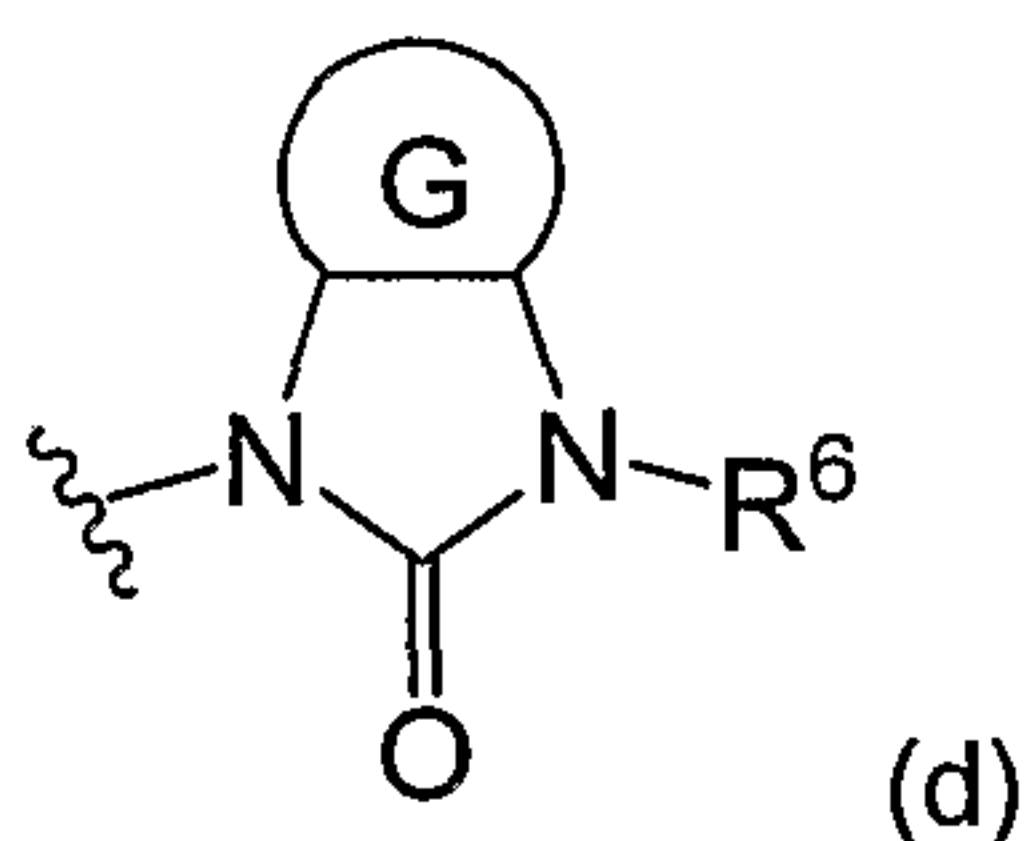
wherein Y is selected from the group consisting of -O- and -NR¹- wherein R¹ is selected from the group consisting of hydrogen and alkyl;

5 W is selected from the group consisting of a covalent bond to a polymer moiety which optionally comprises a linker and -NR²R³ wherein R² and R³ are independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, and where R² and R³, together with the nitrogen atom bound thereto, form a heterocyclic ring or a substituted heterocyclic ring wherein each of alkyl, substituted alkyl, heterocyclic and substituted heterocyclic is optionally covalently bound to a polymer moiety which further optionally comprises a linker;

m is an integer equal to 0, 1 or 2;

n is an integer equal to 0, 1 or 2; and

15 b) a group of formula (d)



wherein G is an optionally substituted aryl or optionally substituted heteroaryl 5 or 6 membered ring containing 0 to 3 nitrogens, wherein said aryl or heteroaryl optionally further comprises a covalent bond to a polymer moiety which optionally comprises a linker;

R⁶ is a covalent bond to a polymer moiety which optionally comprises a linker, or R⁶ is -H, alkyl, substituted alkyl, or -CH₂C(O)R¹⁷, wherein R¹⁷ is -OH, -OR¹⁸, or -NHR¹⁸, wherein R¹⁸ is alkyl, substituted alkyl, aryl or substituted aryl;

25 R⁵⁵ is selected from the group consisting of amino, substituted amino, alkoxy, substituted alkoxy, cycloalkoxy, substituted cycloalkoxy, aryloxy and substituted aryloxy, and -OH; provided that:

A. at least one of R, Ar¹, Ar², and T contains a covalent bond to the polymer moiety;

30 B. when R is covalently bound to the polymer moiety, *n* is one and X is not -O-, -S-, -SO-, or -SO₂-;

- C. when X is -O- or -NR¹-, then *m* is two; and
 D. the conjugate of formula I has a molecular weight of no more than 100,000;

comprising the steps of:

- c) adding a polymeric alcohol of formula Ia

5



wherein B is as described above, to a nucleophile of formula H-Nu in the presence of a trivalent phosphine of the formula PR₃ and a dialkyl azodicarboxylate to form the compound of formula I, wherein Nu is a radical of formula A described above; and isolating the compound of formula I.

10

4. The process according to Claim 1, wherein only one of J, Ar², and T contains a covalent bond to a polymer moiety.

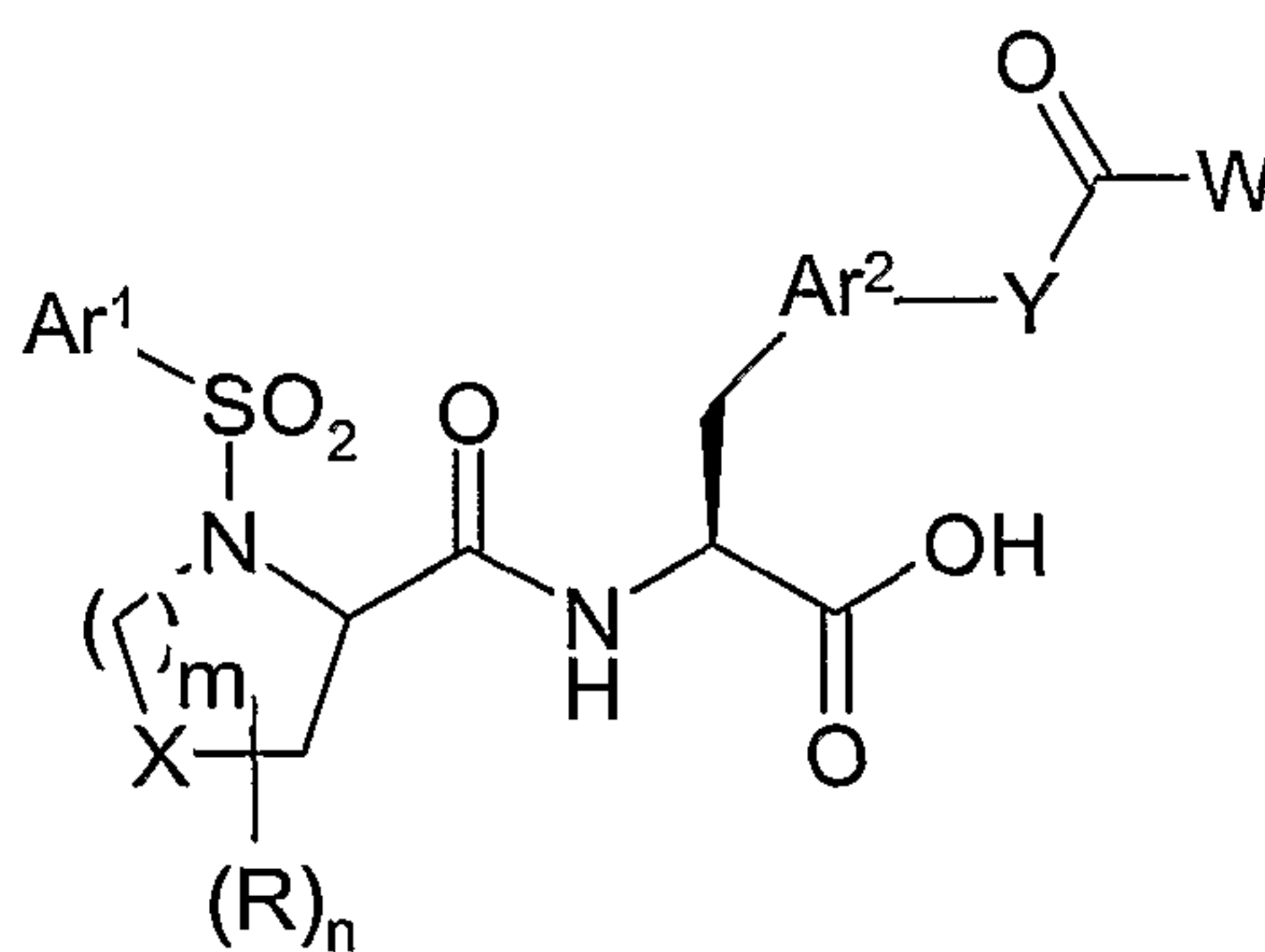
5. The process according to Claim 1, wherein, *q* is an integer of from 1 to about 20.

15

6. The process according to Claim 1, wherein *q* is an integer of from 1 to about 8.

7. The process according to Claim 1, wherein A at each occurrence is independently a compound of formula IIa

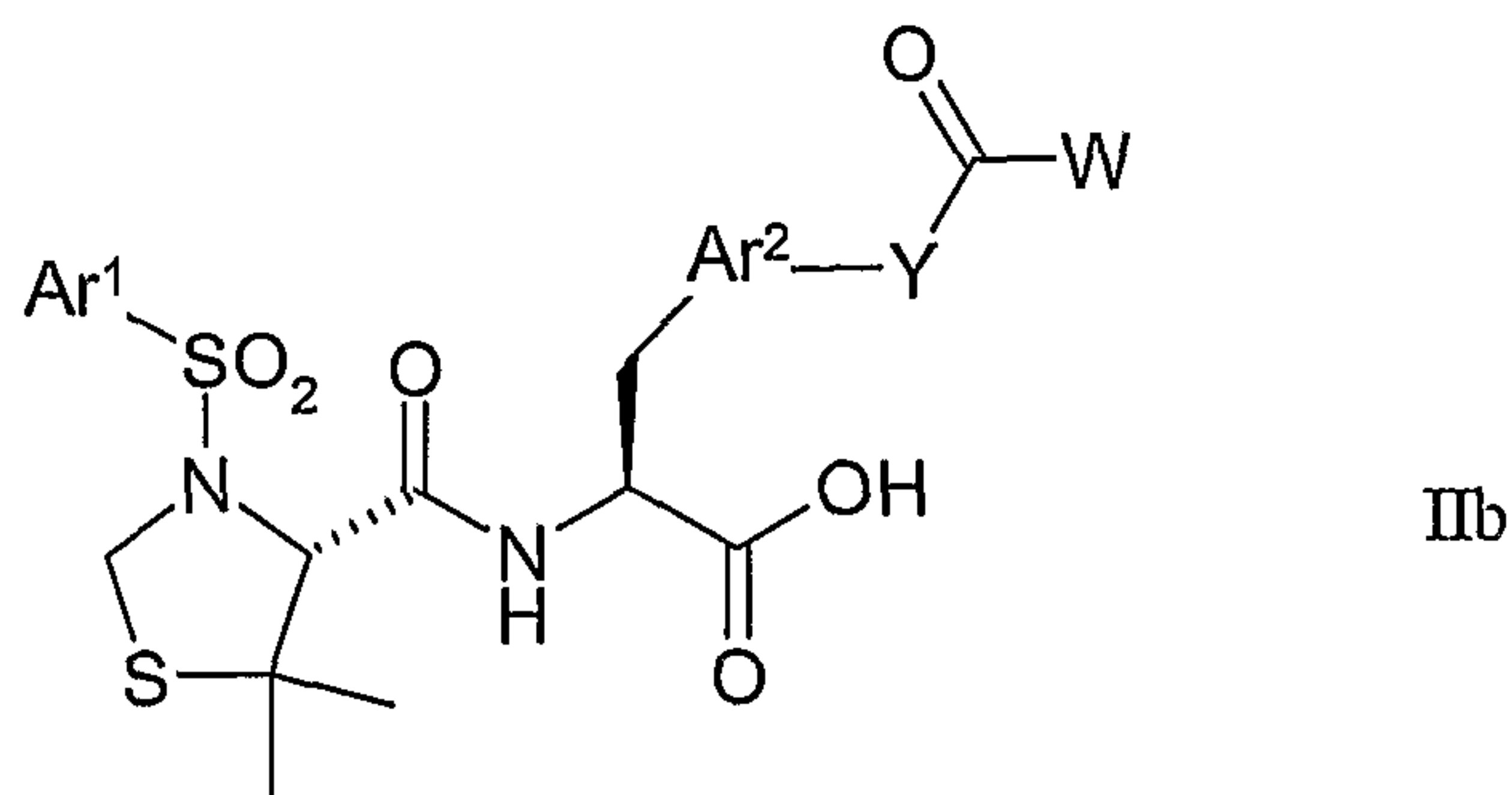
20



IIa

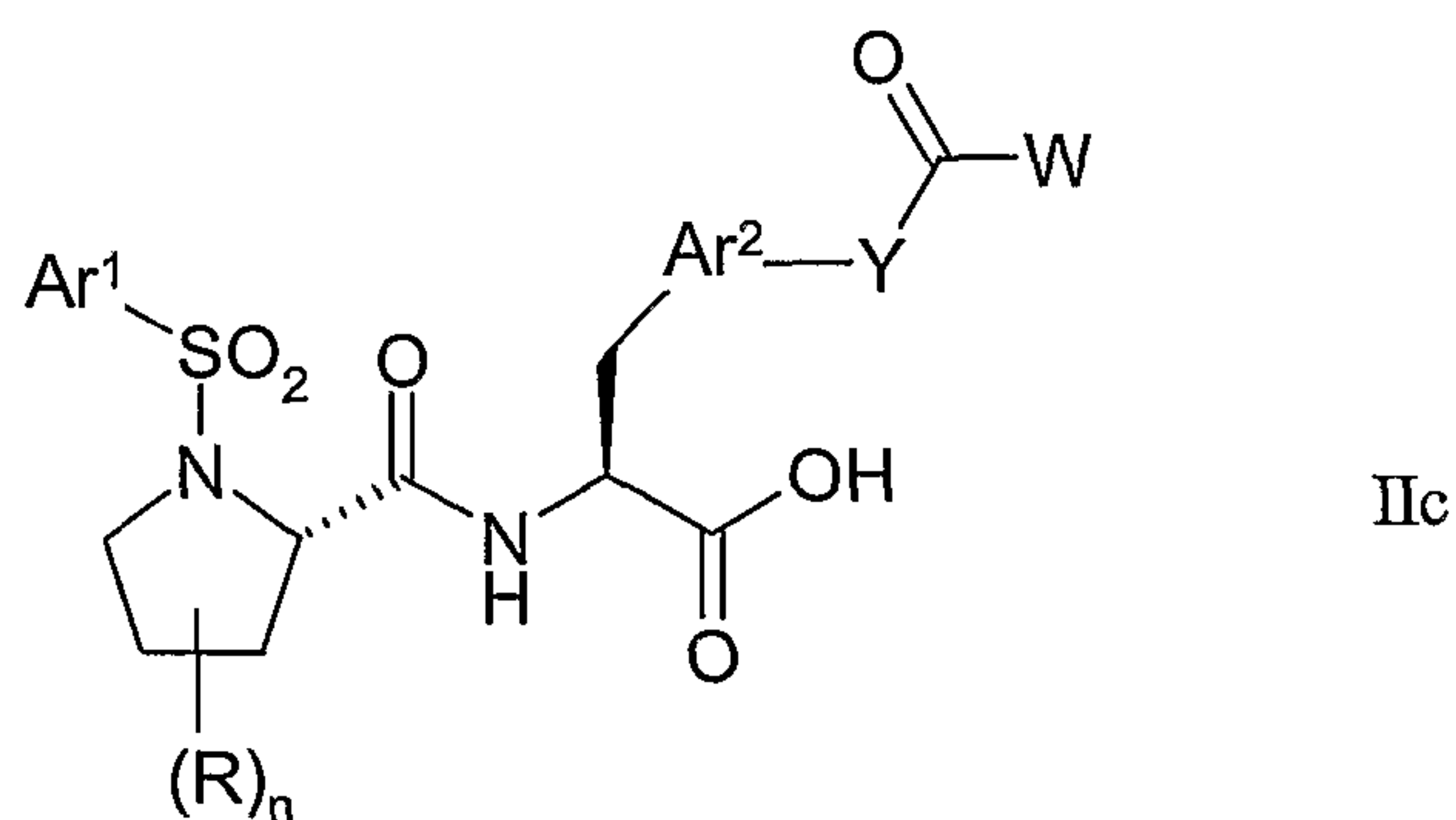
8. The process according to Claim 1, wherein A at each occurrence is independently a compound of formula IIb:

25

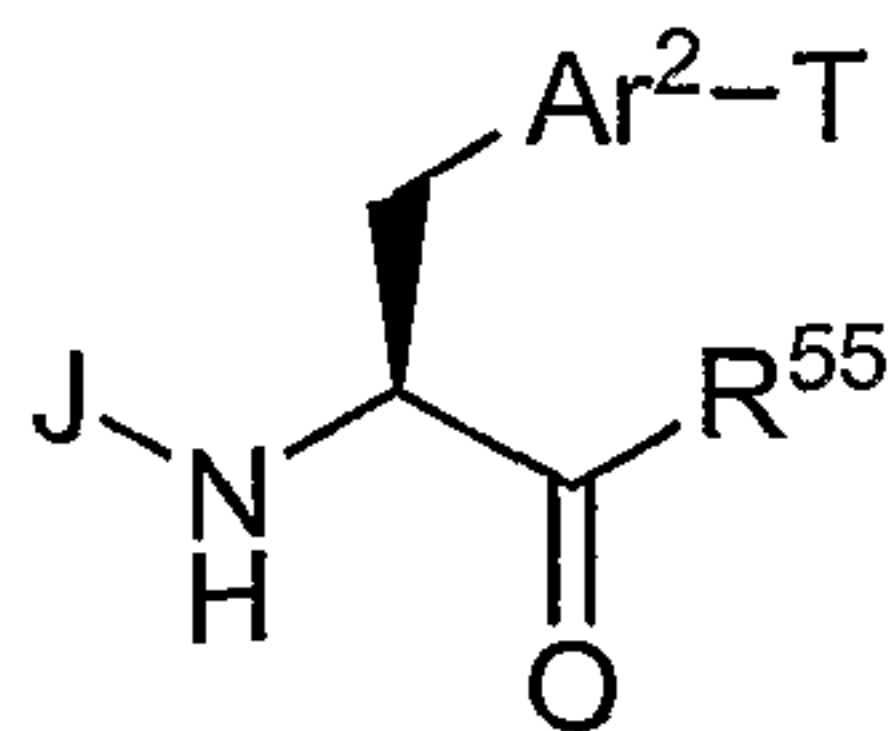


9. The process according to Claim 1, wherein
A at each occurrence is independently a compound of IIc:

5



10. The process of Claim 1, wherein the nucleophilic active compound is a
compound of the formula

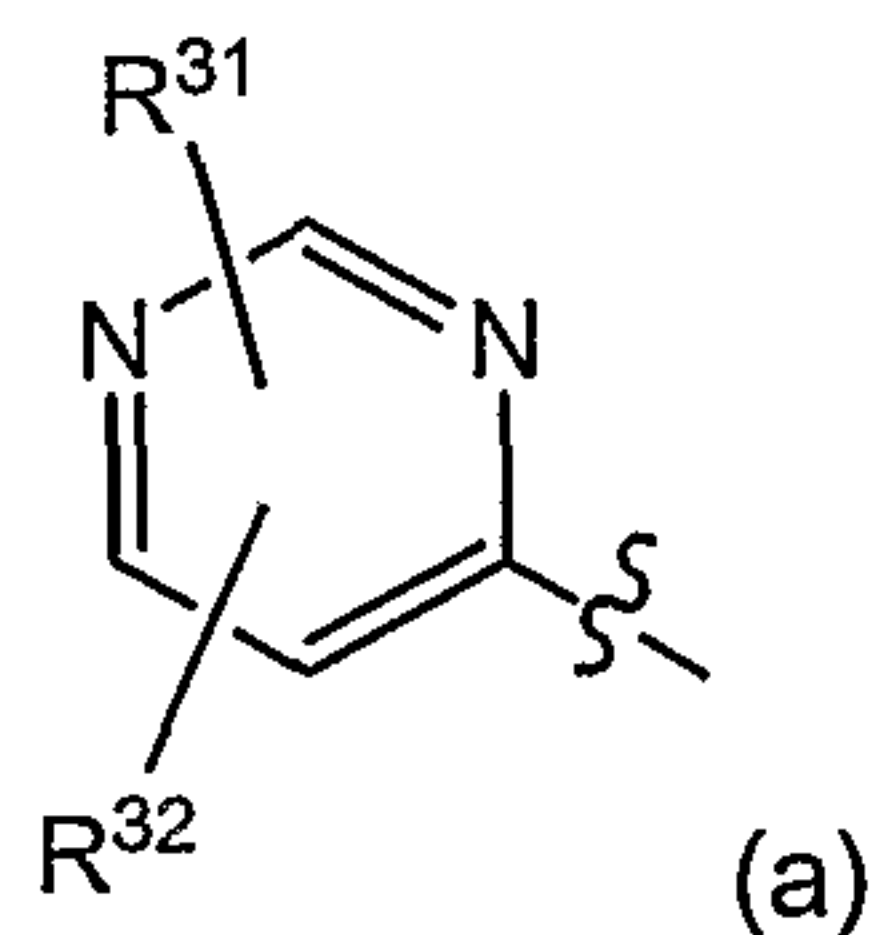


10

wherein

J is selected from:

a) a group of formula (a):



15

wherein R^{31} is a covalent bond to the polymer moiety which optionally comprises a linker,
or R^{31} is $-H$, $R^{31'}$, $-NH_2$, $-NHR^{31'}$ or $-N(R^{31'})_2$, $-NC_3-C_6cyclic$, $-OR^{31'}$, $-SR^{31'}$,

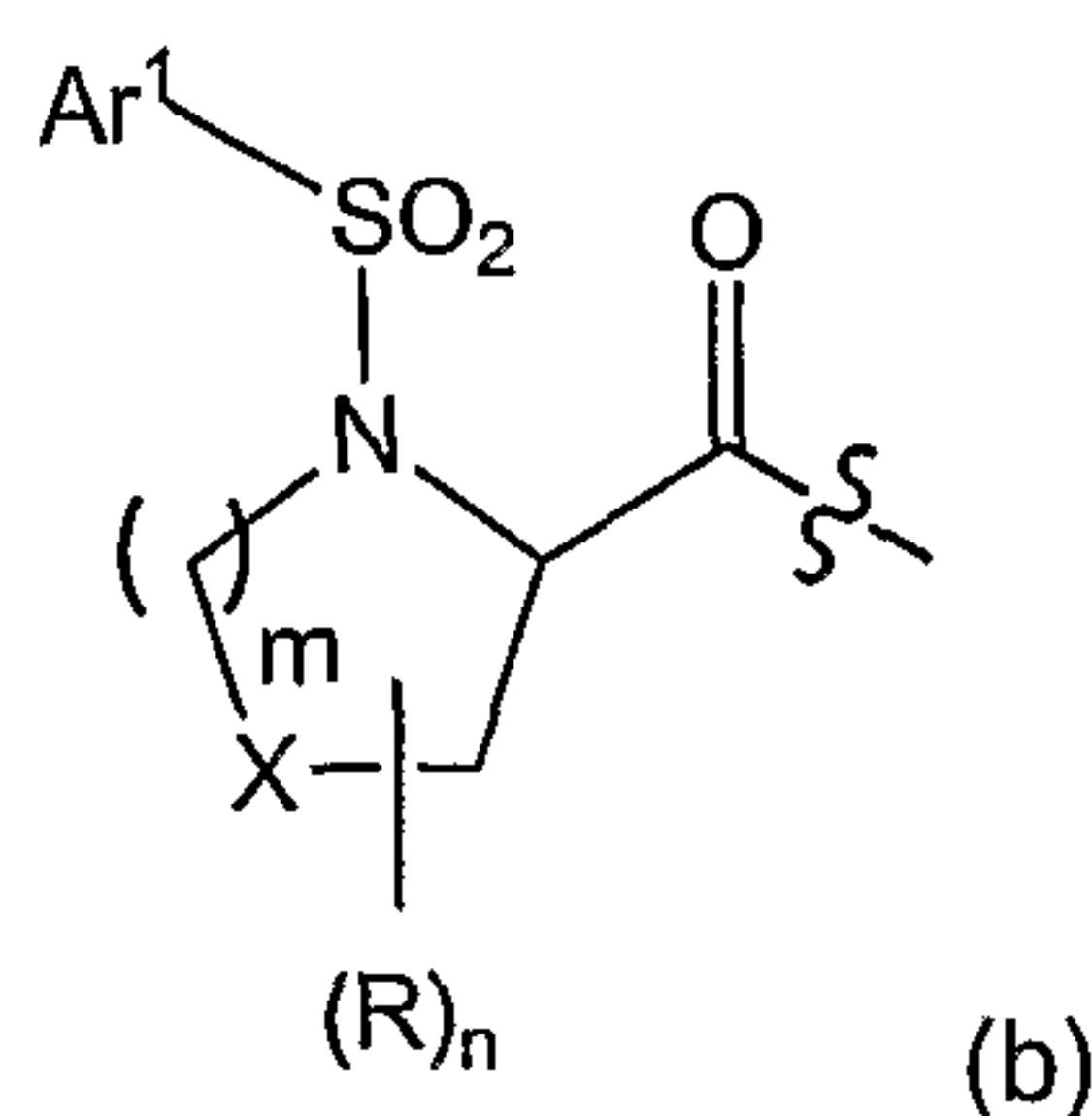
wherein each R^{31} is independently an optionally substituted straight or branched C_1 - C_6 alkyl, optionally substituted C_3 - C_6 cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl,

and R^{32} is a covalent bond to the polymer moiety which optionally comprises a linker, or
 R^{32} is $-H$, $-NO_2$, haloalkyl or the group $-N(MR^{41})R^{42}$ wherein M is a covalent bond,
 $-C(O)-$ or $-SO_2-$, R^{41} is $R^{41'}$, $N(R^{41'})_2$, or $-OR^{41'}$,

wherein each $R^{41'}$ is independently hydrogen, an optionally substituted straight or branched C_1 - C_6 alkyl, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclic or an optionally substituted heteroaryl, wherein optional substitutions are halide, C_1 - C_6 alkyl, or $-OC_1$ - C_6 alkyl,

and R^{42} is hydrogen or $R^{41'}$; and

b) a group of formula (b):



wherein R is selected from the group consisting of a covalent bond to the polymer moiety, amino, hydroxyl, substituted amino, alkyl, alkyloxy, aryloxy, heteroaryloxy, heterocyclyloxy, thiol, arylthio, heteroarylthio, heterocyclylthio and substituted alkyl wherein each amino, substituted amino, alkyl and substituted alkyl is optionally covalently bound to the polymer moiety wherein, in each case, the polymer moiety optionally comprises a linker which covalently links the polymer moiety;

Ar^1 is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl wherein each of aryl, substituted aryl, heteroaryl and substituted heteroaryl is optionally covalently bound to the polymer moiety wherein the polymer moiety optionally comprises a linker which covalently links the polymer moiety to Ar^1 ;

Ar^2 is selected from the group consisting of aryl, substituted aryl, heteroaryl and substituted heteroaryl wherein each of aryl, substituted aryl, heteroaryl and substituted heteroaryl is optionally covalently bound to the polymer moiety wherein the polymer moiety optionally comprises a linker which covalently links the polymer moiety to Ar^2 ;

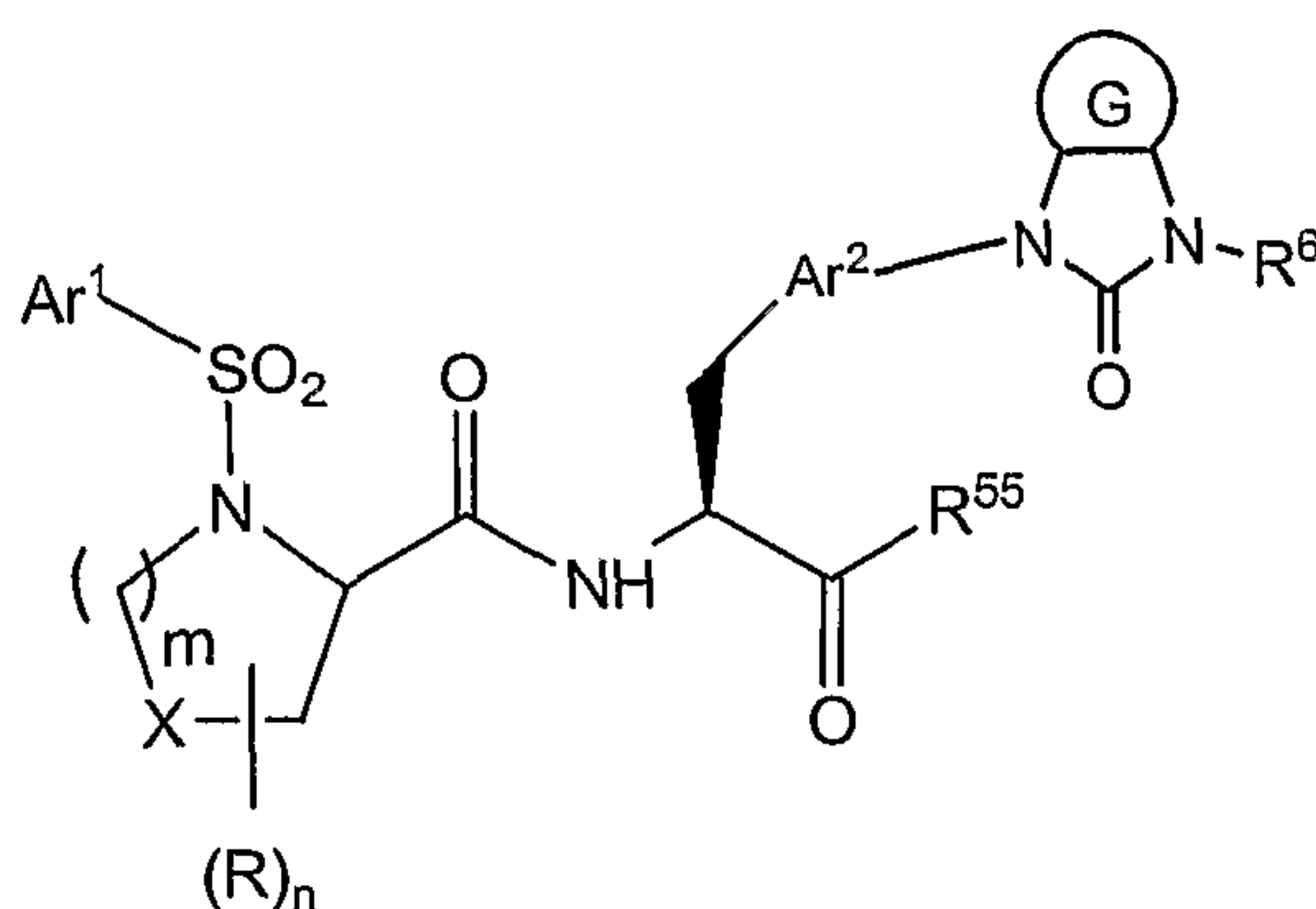
X is selected from the group consisting of $-\text{NR}^1-$, $-\text{O}-$, $-\text{S}-$, $-\text{SO}-$, $-\text{SO}_2$ and optionally substituted $-\text{CH}_2-$ where R^1 is selected from the group consisting of hydrogen and alkyl;

T is a group carrying an acidic hydrogen; and

5 R^{55} is a acid protecting group.

10

11. The of Claim 10, the nucleophilic active compound has the formula:



where R^{55} is an acid protecting group.

15

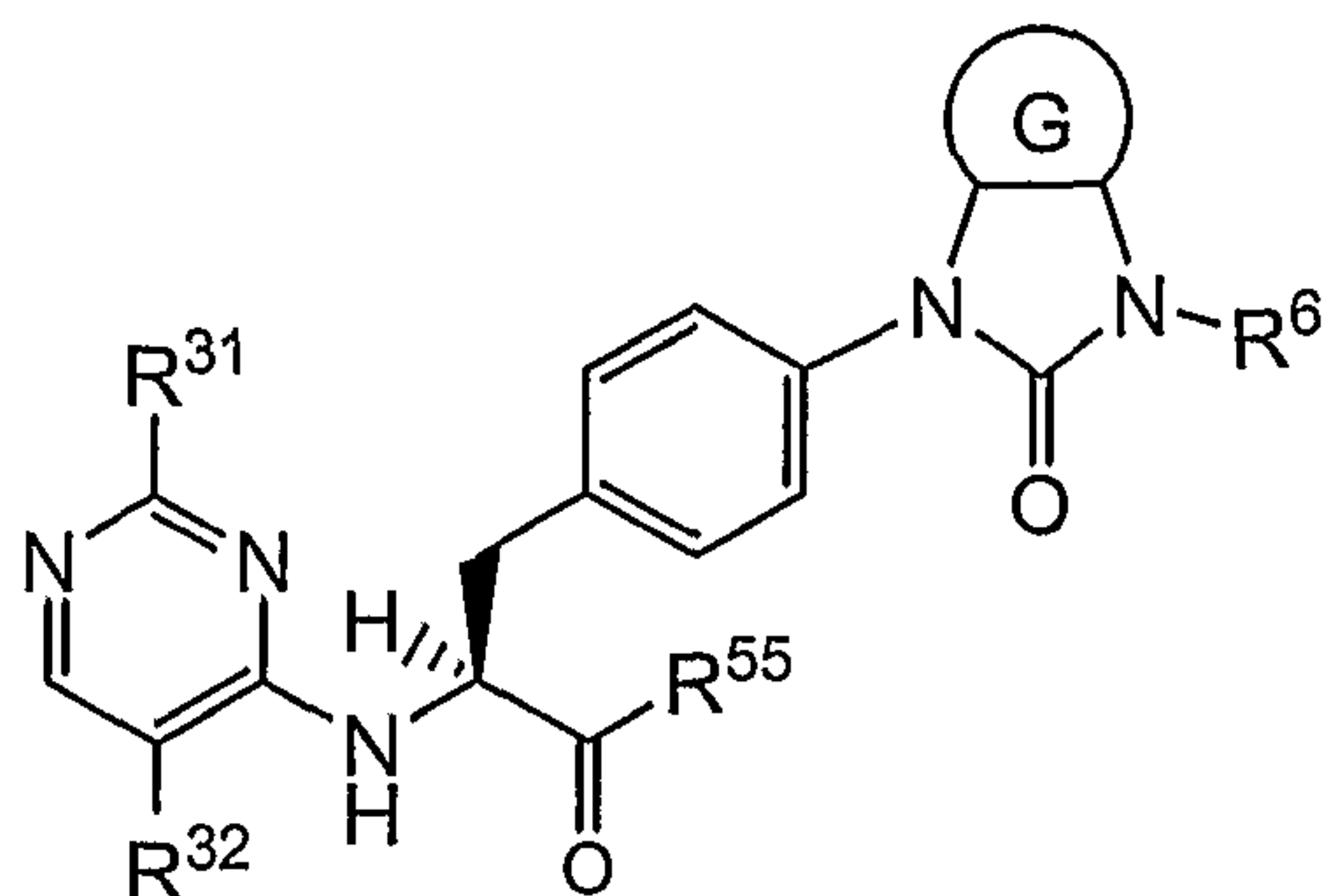
12. A process according to claim 11, where R^{55} is C_1 - C_6 alkoxy.

13. The process according to Claim 11 wherein m is 1, X is S, and R at each occurrence is independently selected from hydroxyl, alkyloxy, alkyl, or a covalent bond to the polymer moiety.

20

14. The process according to claim 12 wherein n is 2, and R at both occurrences is methyl.

15. The process of Claim 1, wherein the nucleophilic active compound has the
25 formula:

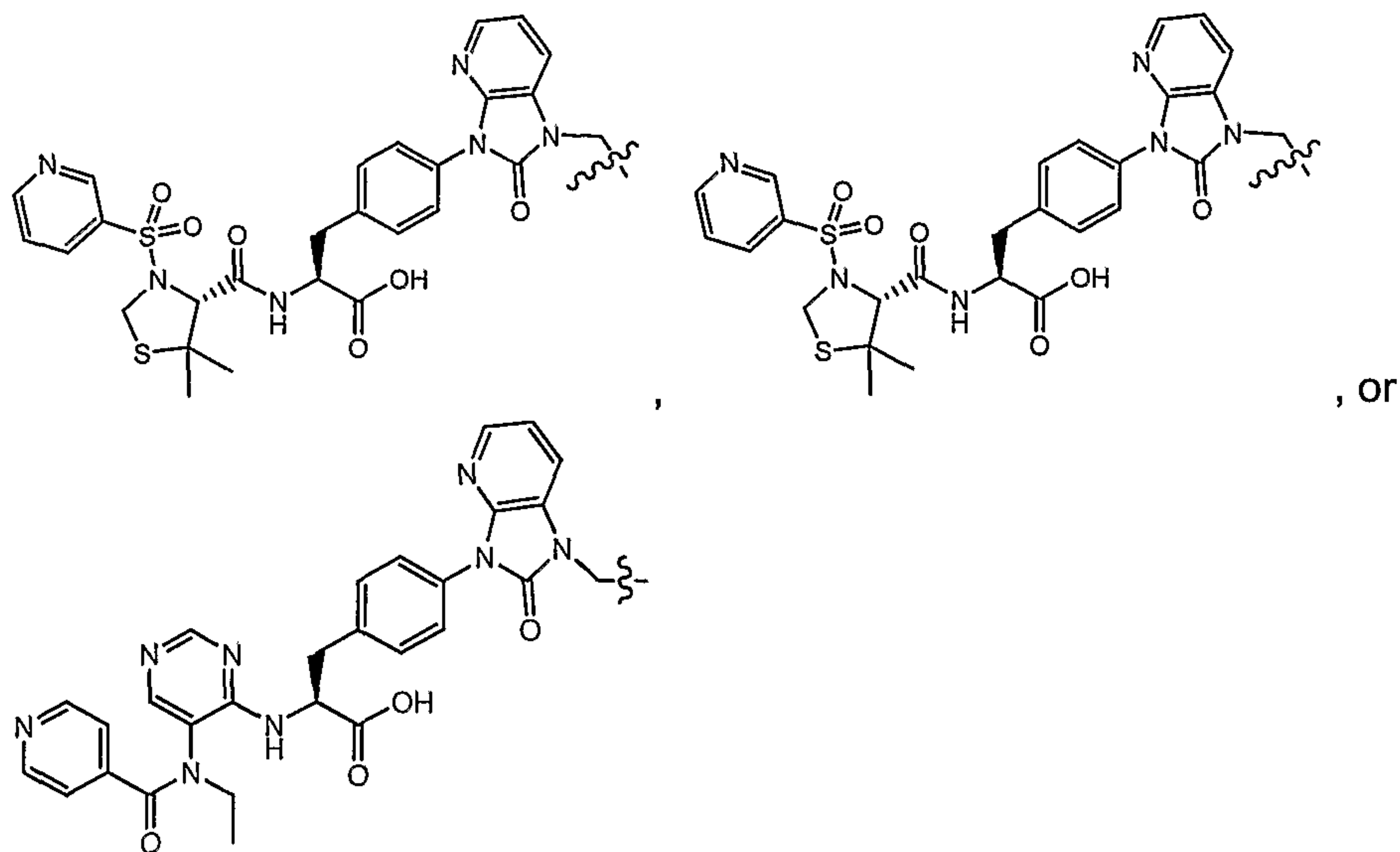


where R^{55} is an acid protecting group.

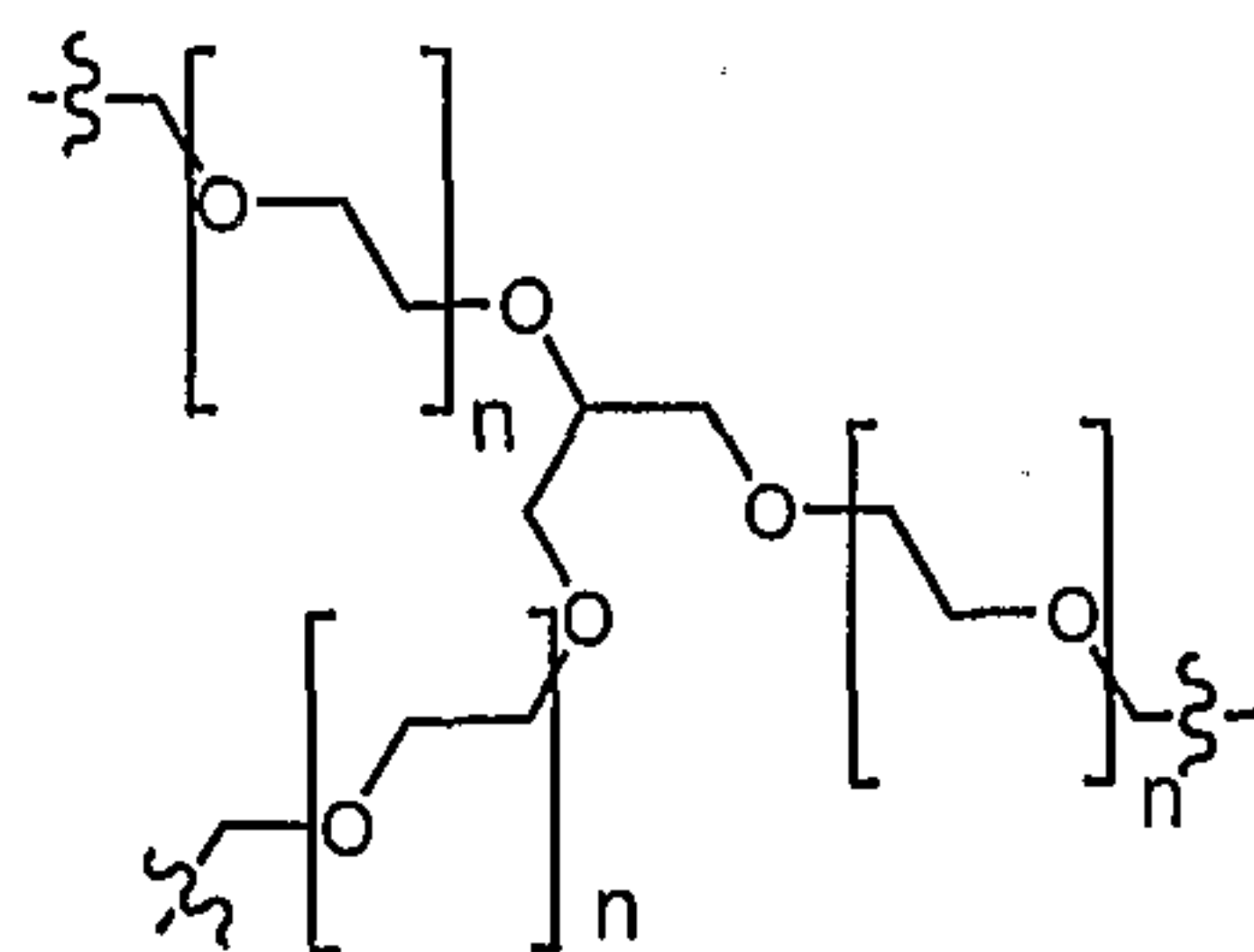
5 16. A process according to claim 15, where R^{55} is C_1 - C_6 alkoxy.

17. The process according to claim 15 wherein G is pyridinyl, R31 is hydrogen or dialkylamino, and R32 is sulfonamide, amide, or urea.

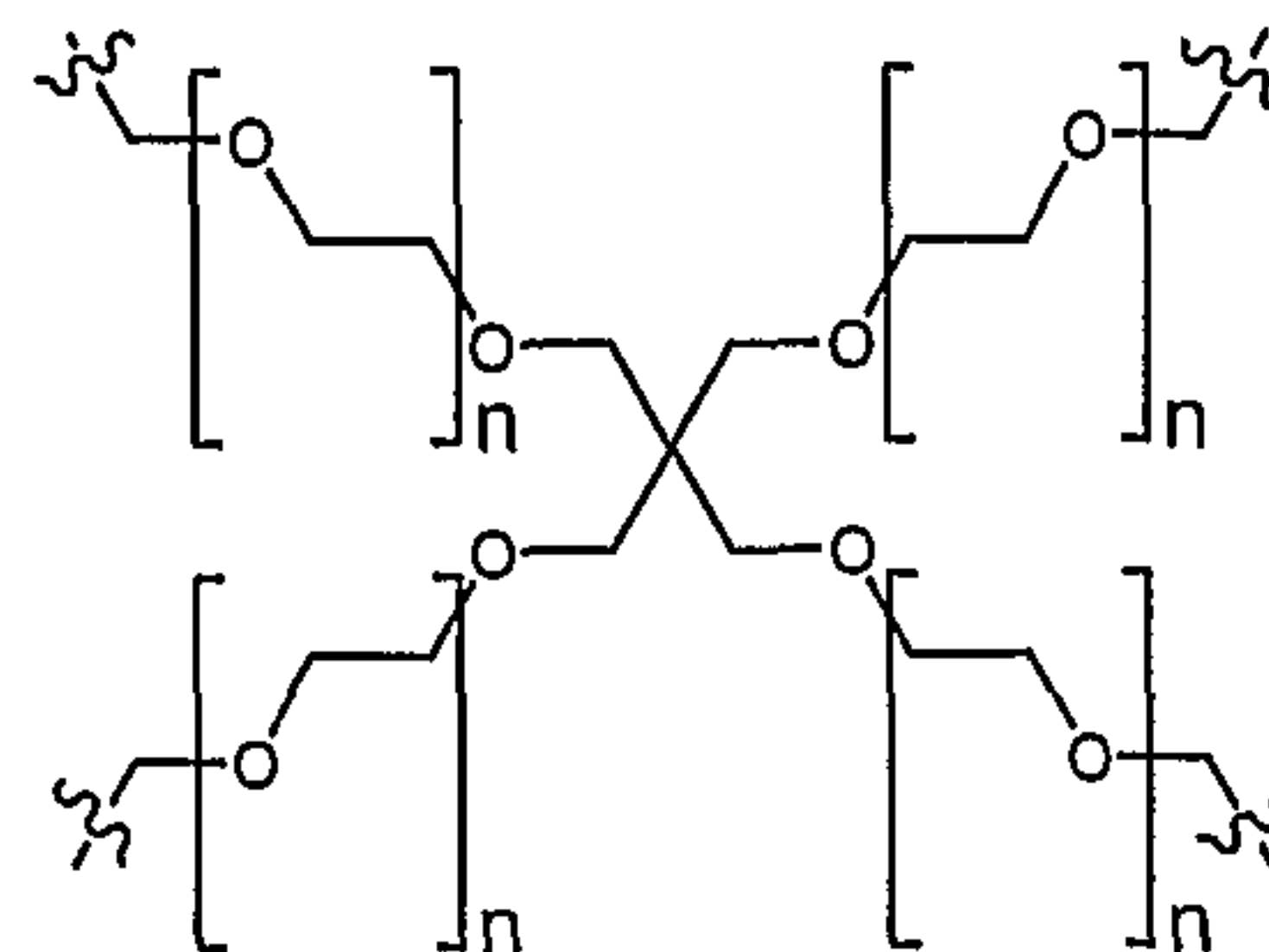
10 18. A process according to Claim 1, wherein the nucleophilic active compound is:



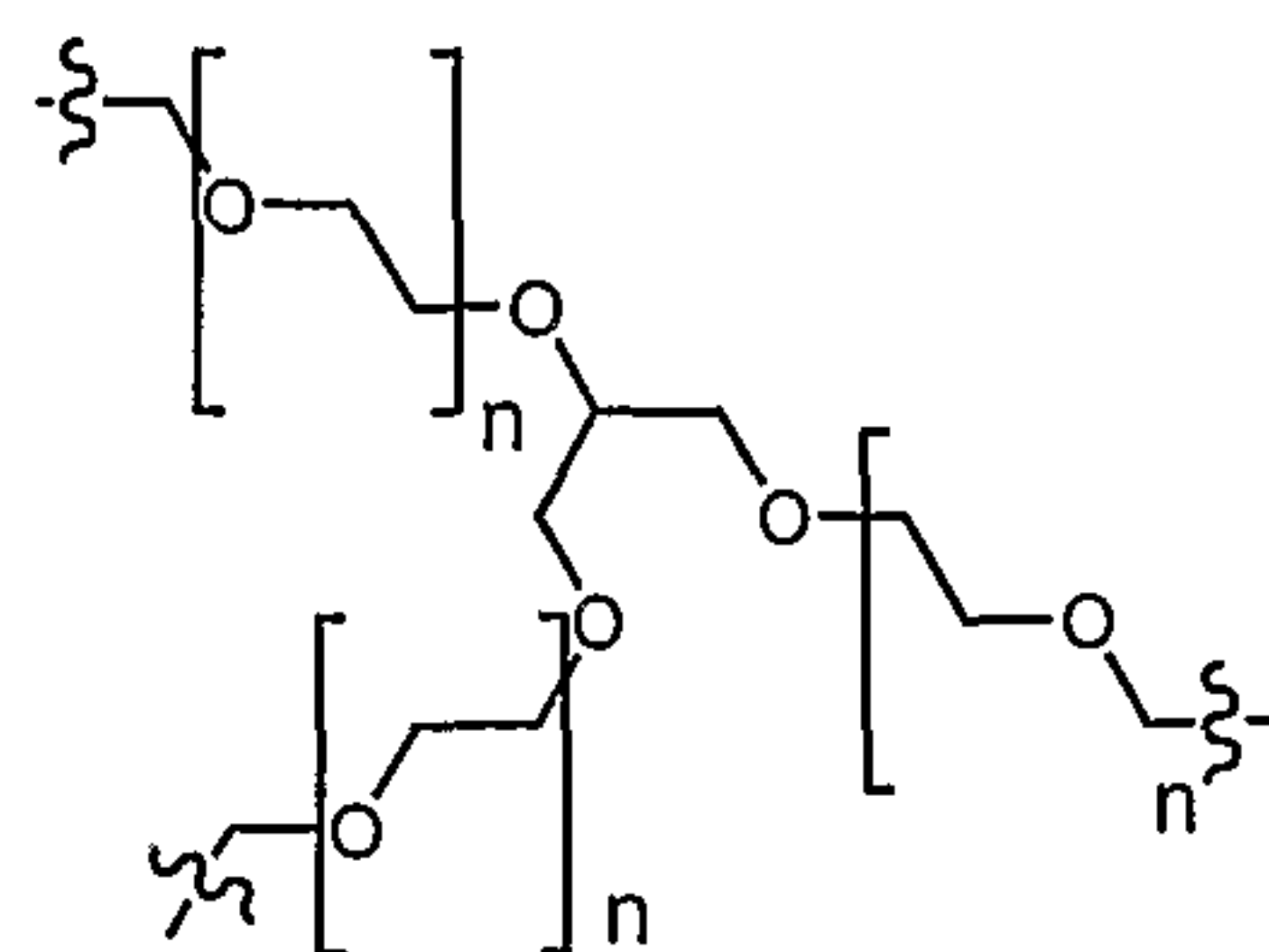
15 19. A process according to Claim 1, wherein the polymer alcohol is



(total Mw of conjugate is about 41,500),



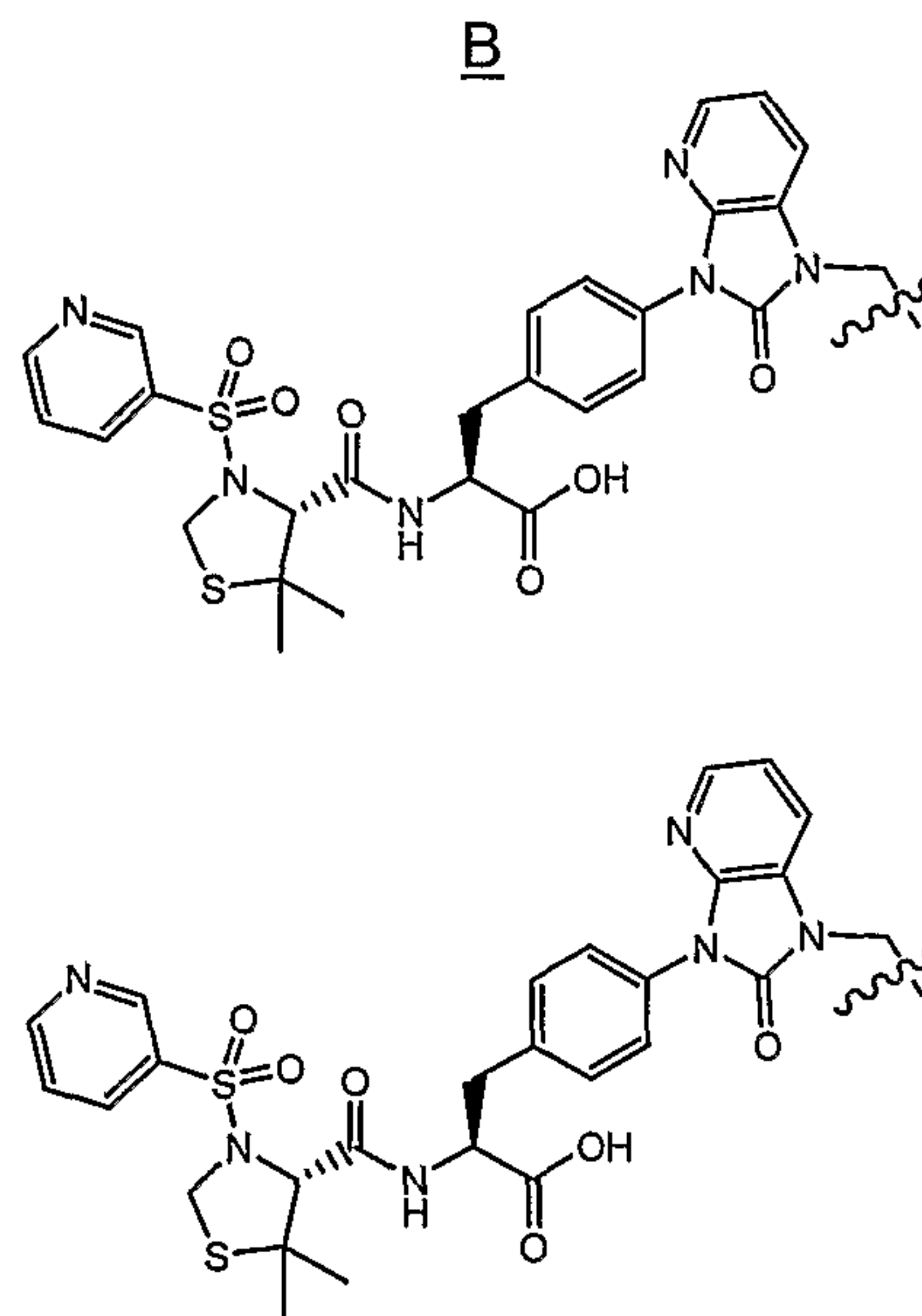
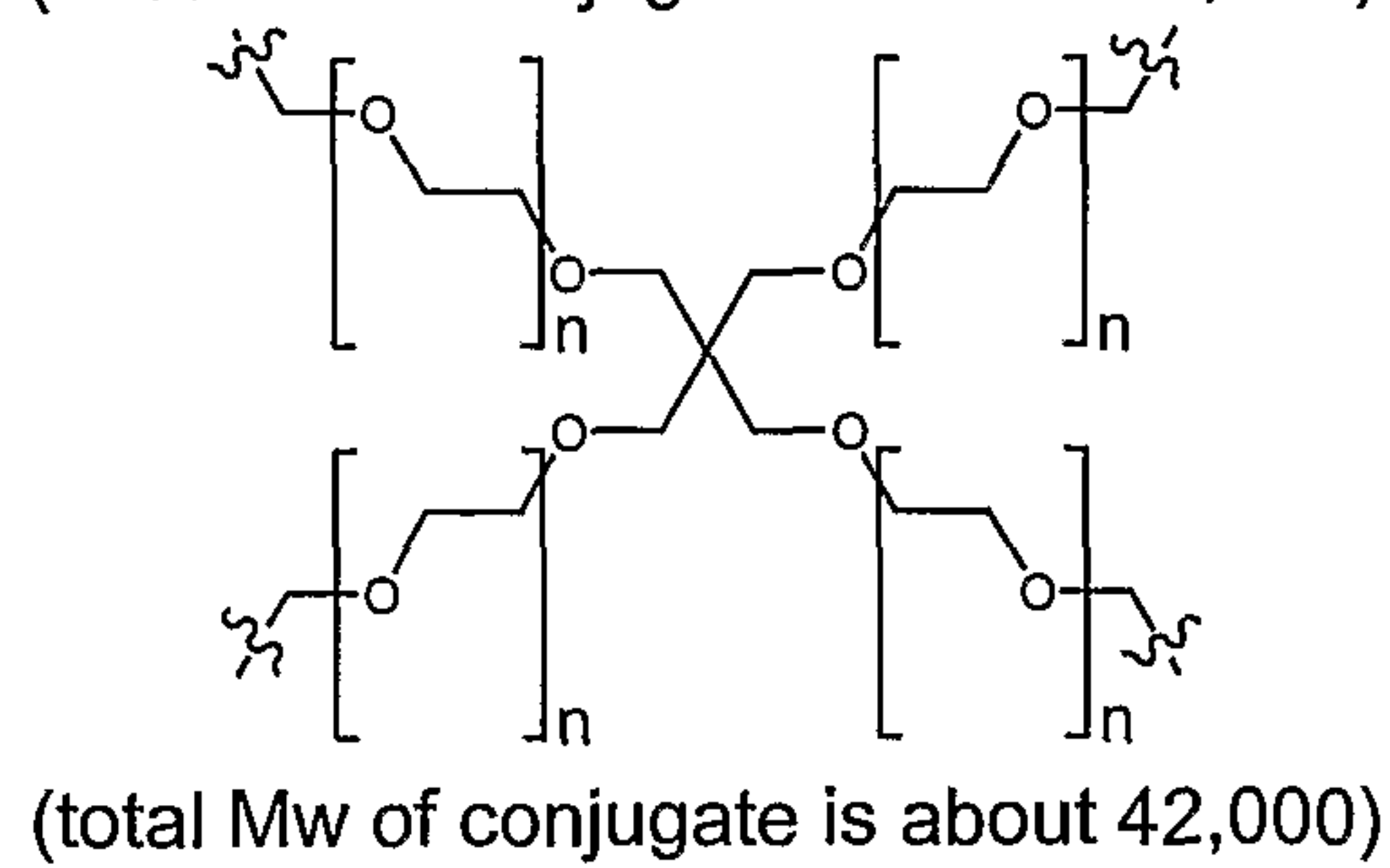
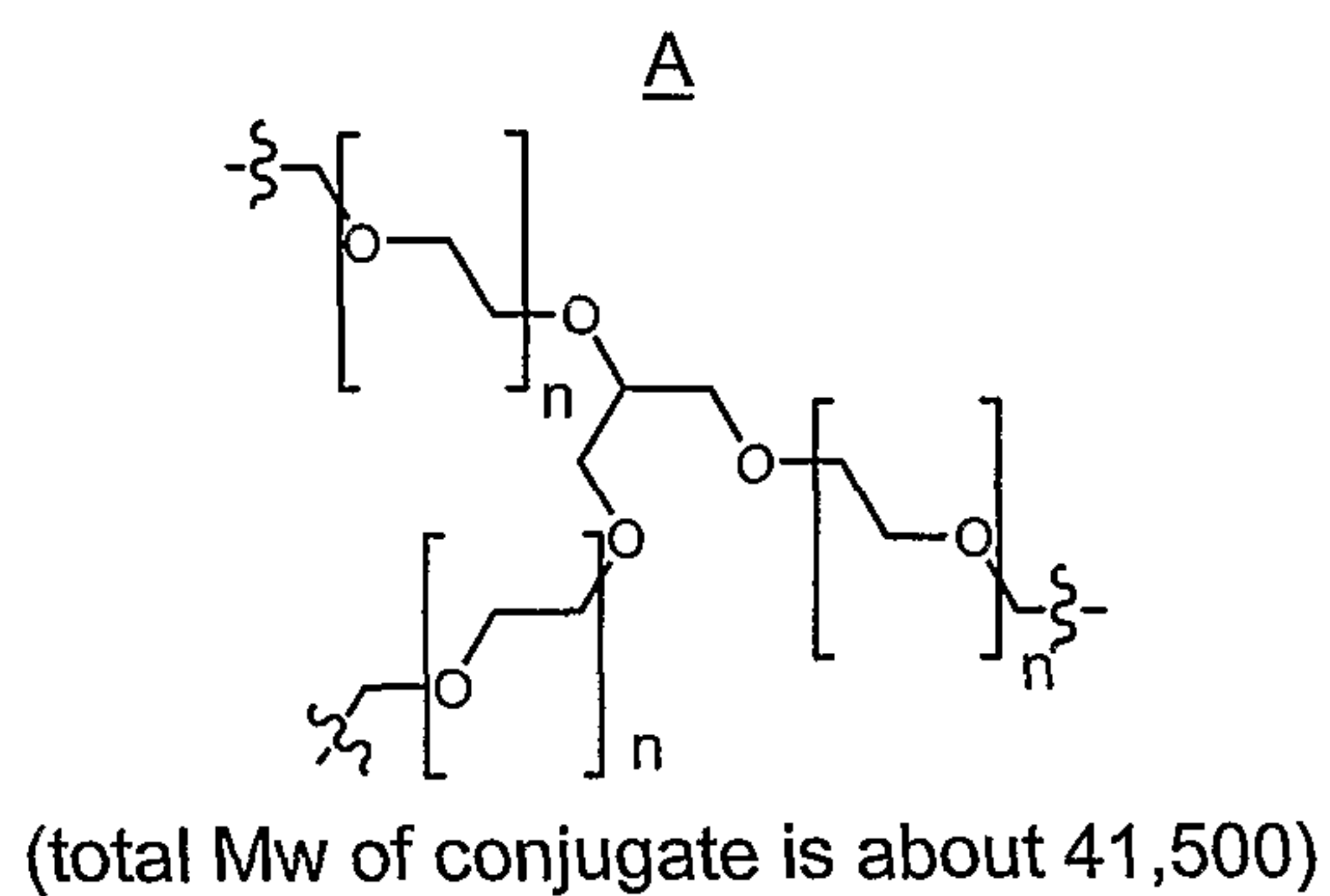
(total Mw of conjugate is about 42,000), or

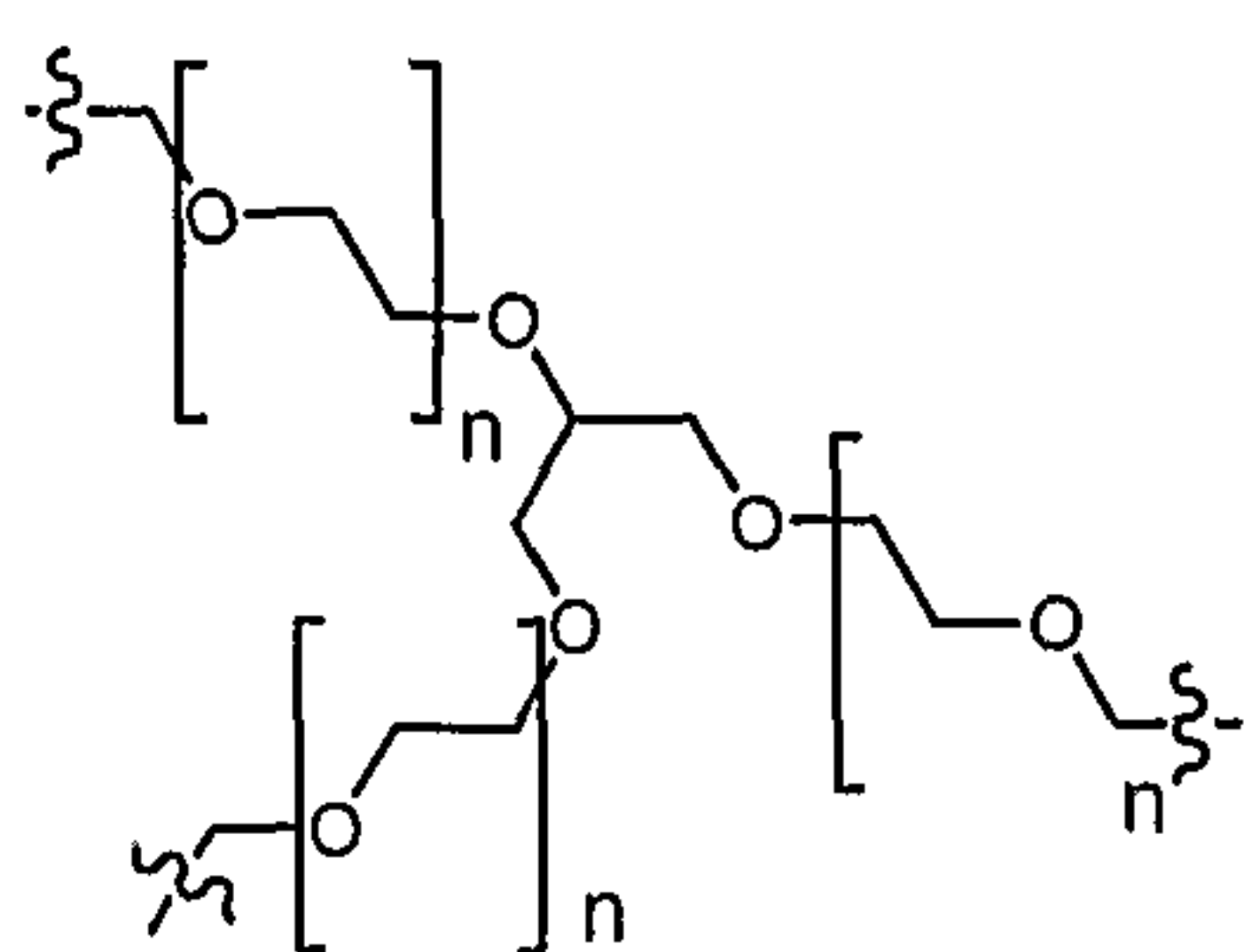


(total Mw of conjugate is about 41,500).

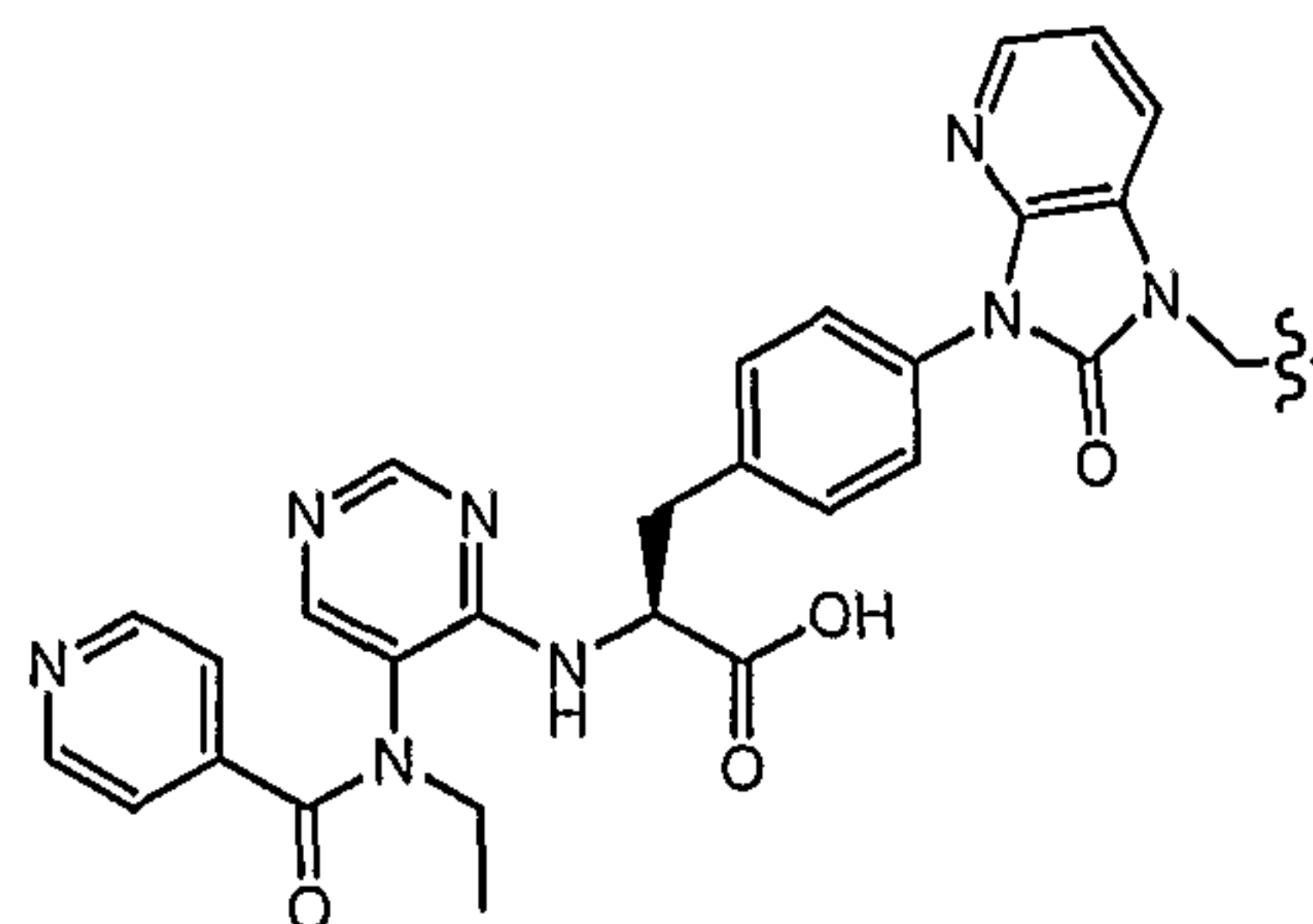
20. A process according to Claim 1, wherein the polymer alcohol is selected from the group in column A and the nucleophilic active compound is selected from the group in column B:

5





(total Mw of conjugate is about 41,500)



21. The process according to Claim 1 wherein the polymeric alcohol is added to the nucleophile of formula H-Nu in the presence of the trivalent phosphine and the dialkyl azodicarboxylate in at least one solvent.

5

22. The process according to Claim 21 wherein the dialkyl azodicarboxylate is selected from the group consisting of diethyldiazocarboxylate, diisopropylazodicarboxylate, 4-methyl-1,2,4-triazolidine-3,5-dione, N,N,N',N'-tetramethylazodicarboxamide, azodicarboxylic acid dipiperidide, bis(N-4-methylpiperazin-1-yl)azodicarboxamide, dimorpholinoazodicarboxamide and di-tert-butyl azodicarboxylate.

10

23. The process according to Claim 22 wherein the dialkyl azodicarboxylate is selected from the group consisting of diethyldiazocarboxylate, diisopropylazodicarboxylate, 4-methyl-1,2,4-triazolidine-3,5-dione, N,N,N',N'-tetramethylazodicarboxamide, azodicarboxylic acid dipiperidide and di-tert-butyl azodicarboxylate.

15

24. The process according to Claim 21 wherein the solvent is a chlorinated solvent or an ether solvent.

20

25. The process according to Claim 24 wherein the solvent is a dichloromethane or tetrahydrofuran.

26. The process according to Claim 21 wherein the trivalent phosphine is selected from the group consisting of triphenylphosphine, trimethylphosphine, triethylphosphine, tributylphosphine 1,2-bis-(diphenylphosphino)ethane, a polymer bound phosphine and a water soluble phosphine.

25

27. The process according to Claim 26 wherein the trivalent phosphine is triphenylphosphine.

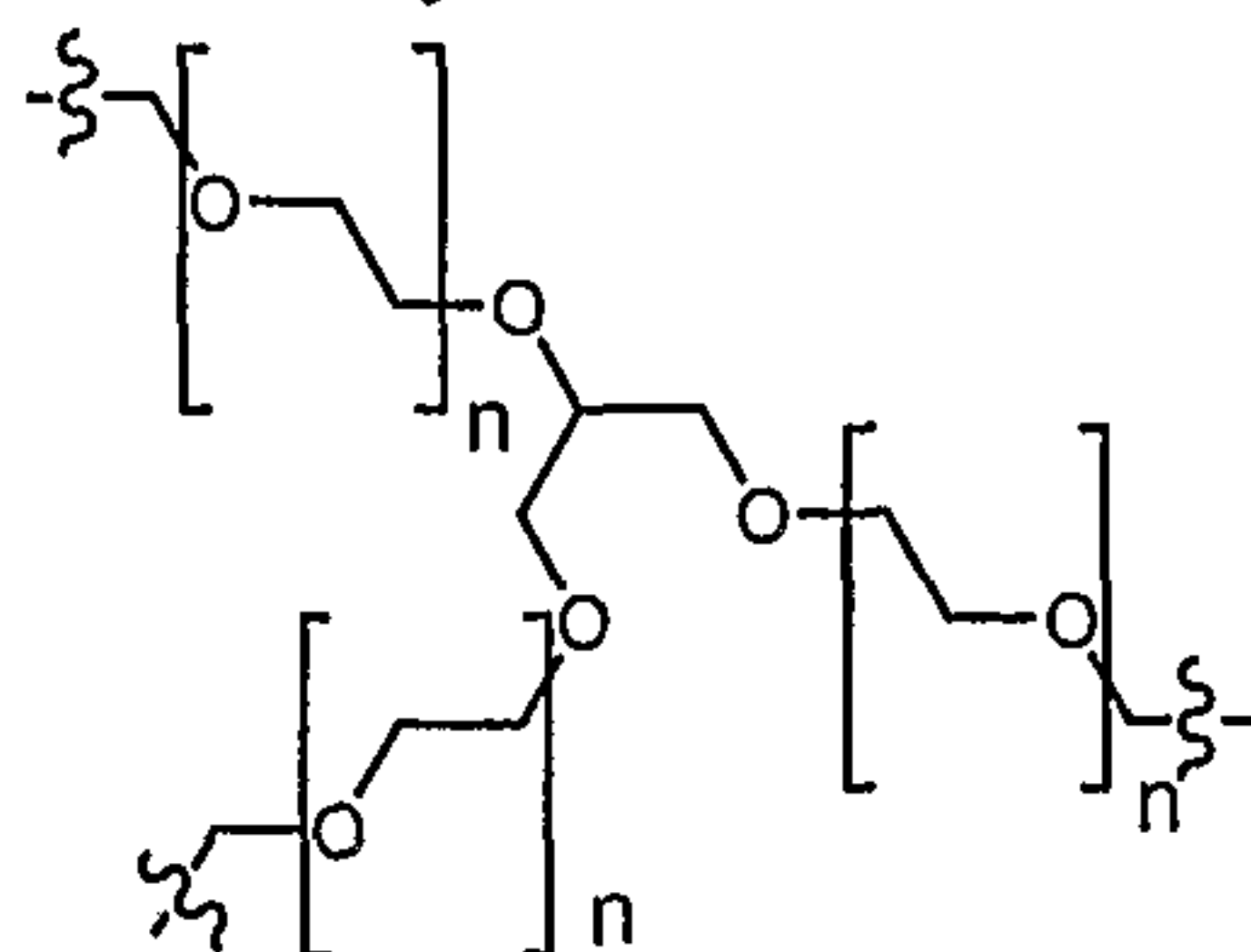
30

28. A process according to Claim 1, wherein the conjugate is prepared using polymer alcohol/nucleophilic active compound combination 1, 2, or 3:

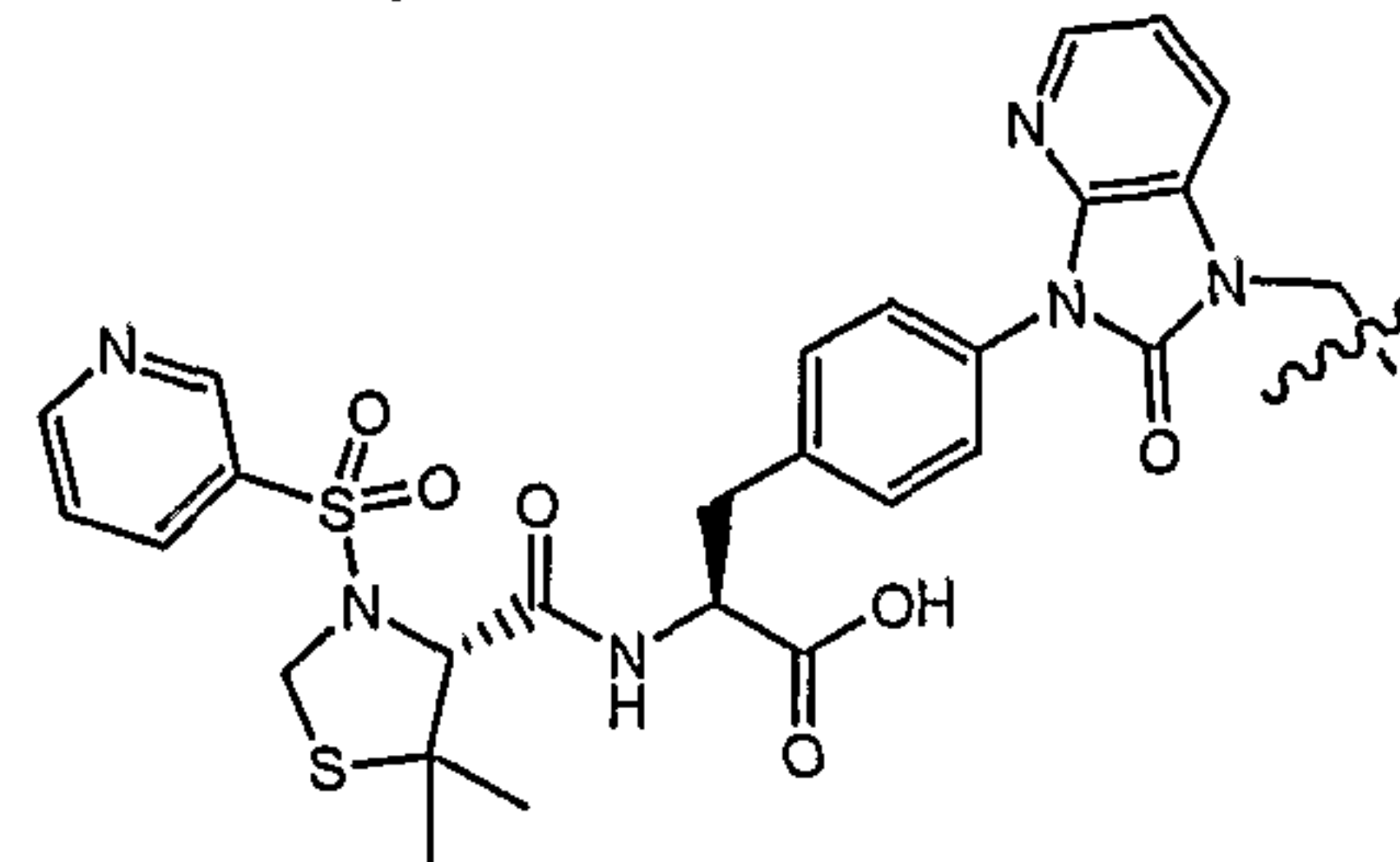
Combination

1

Polymer Alcohol

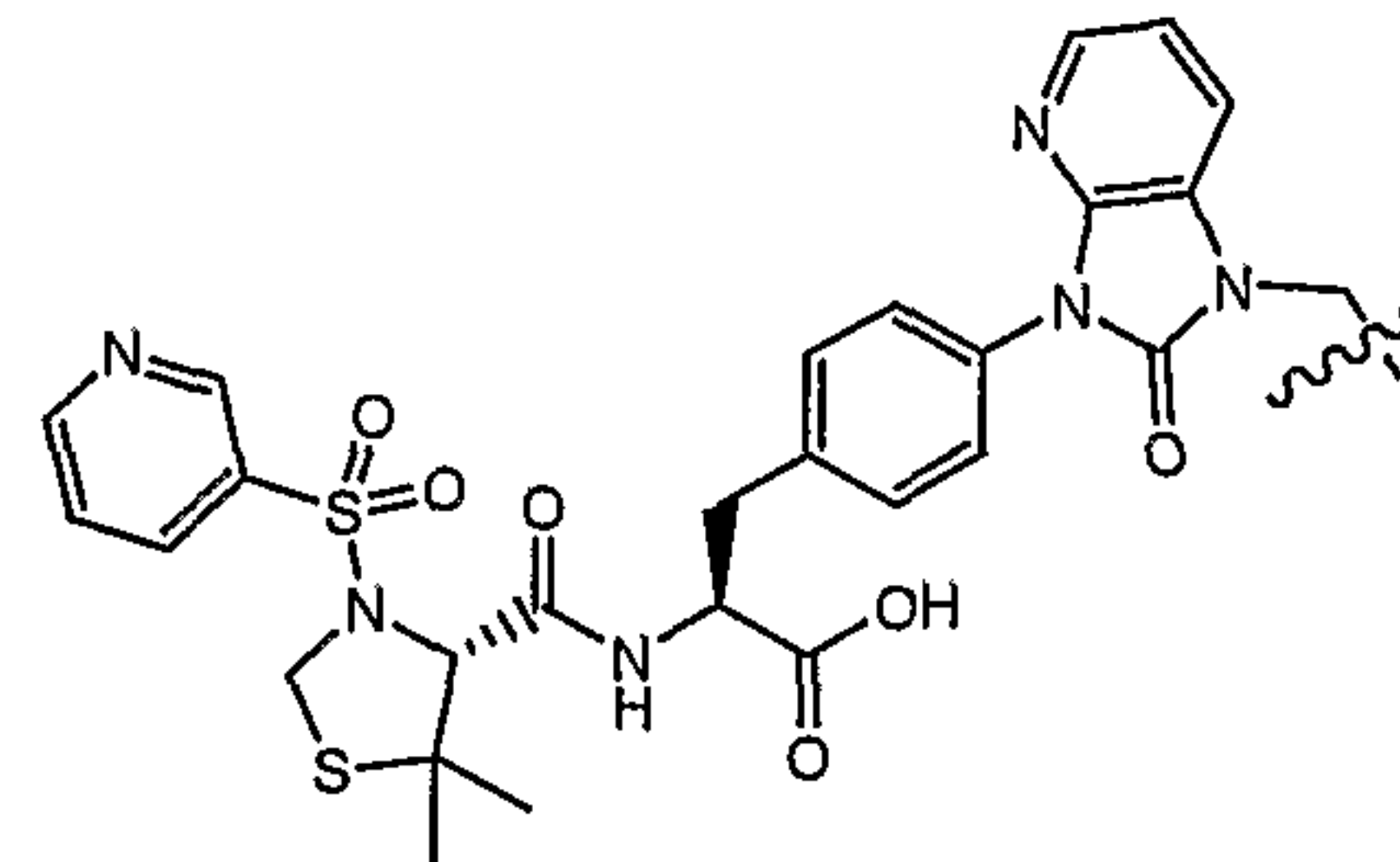
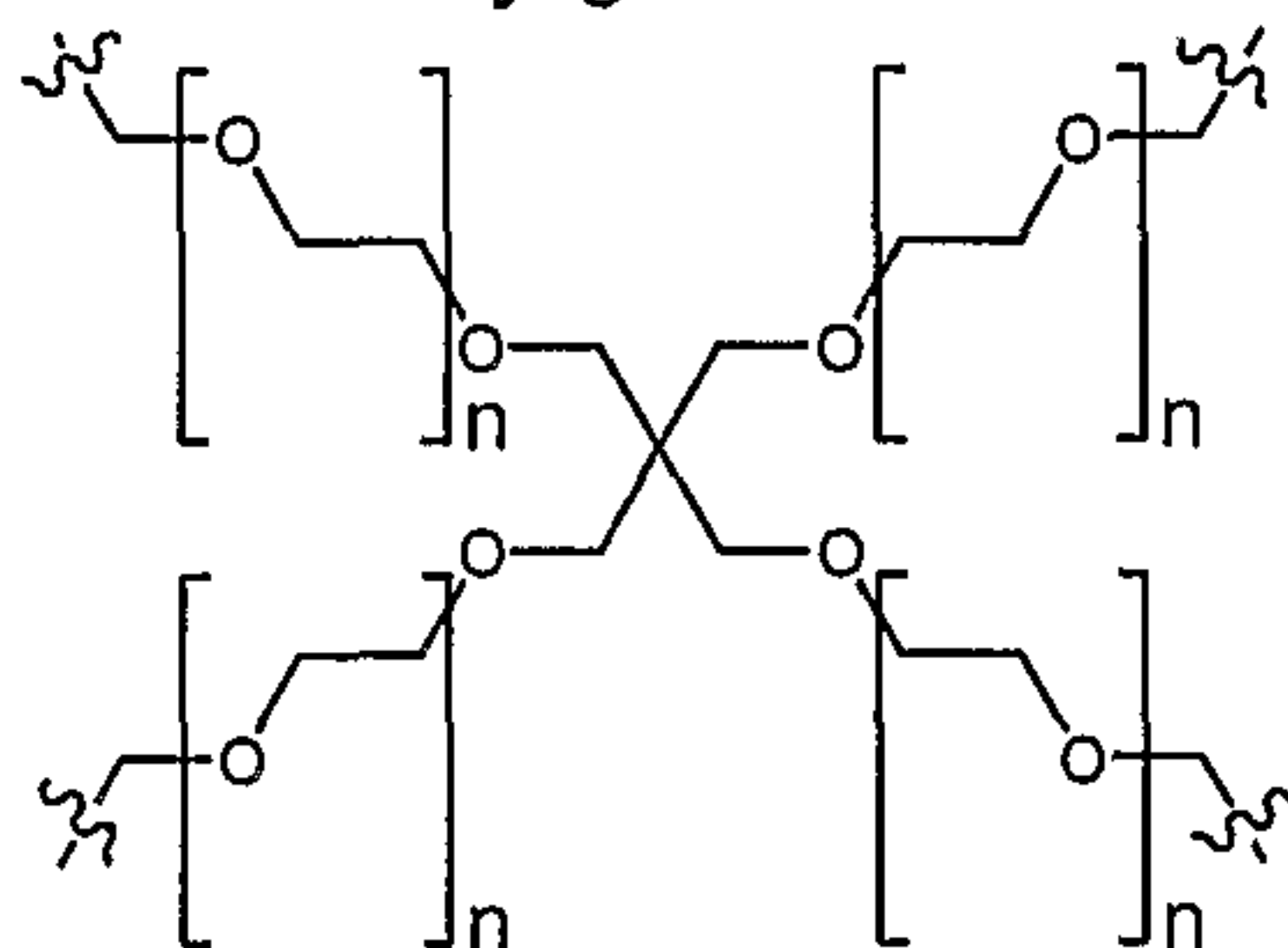


Nucleophilic Active Compound



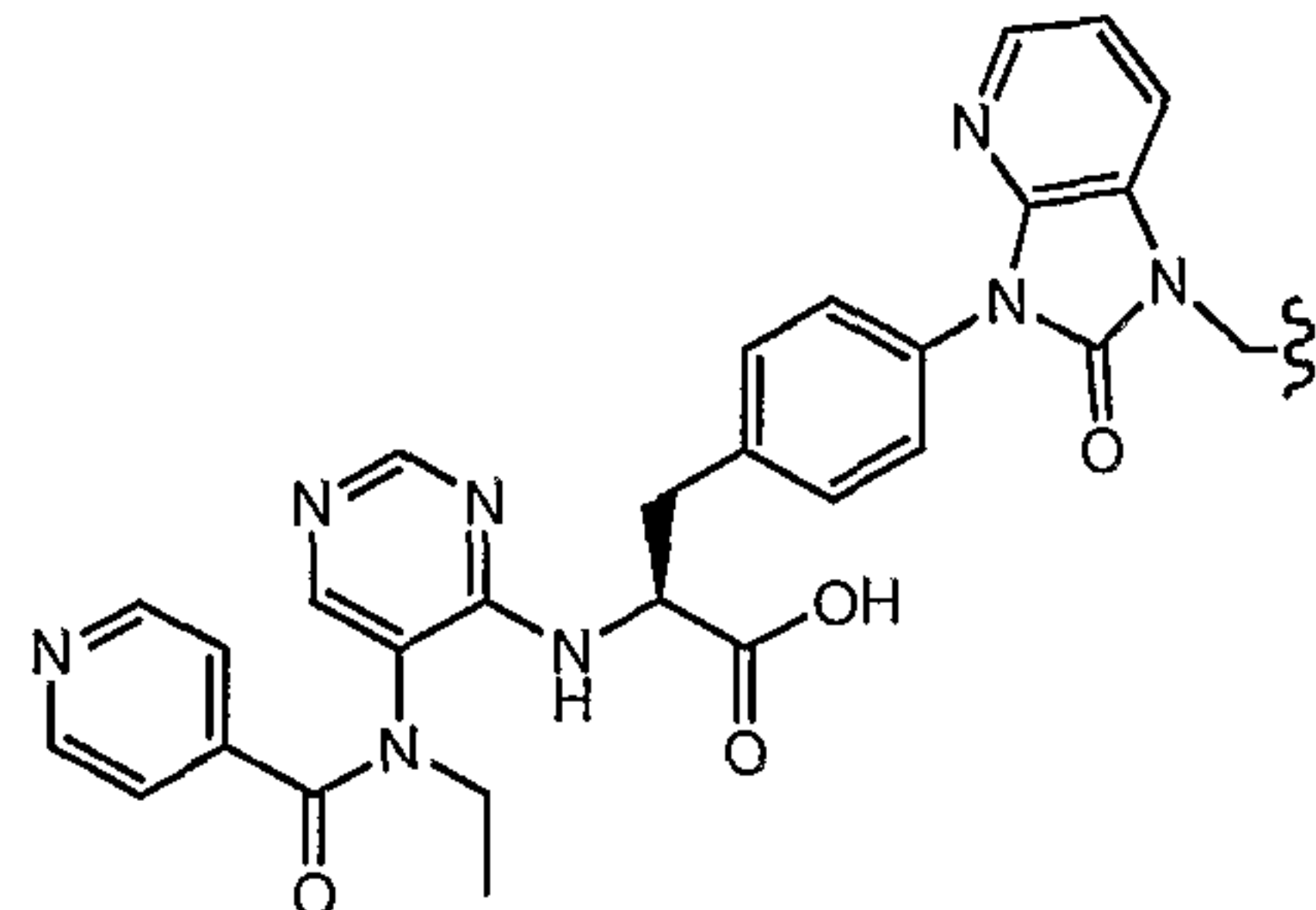
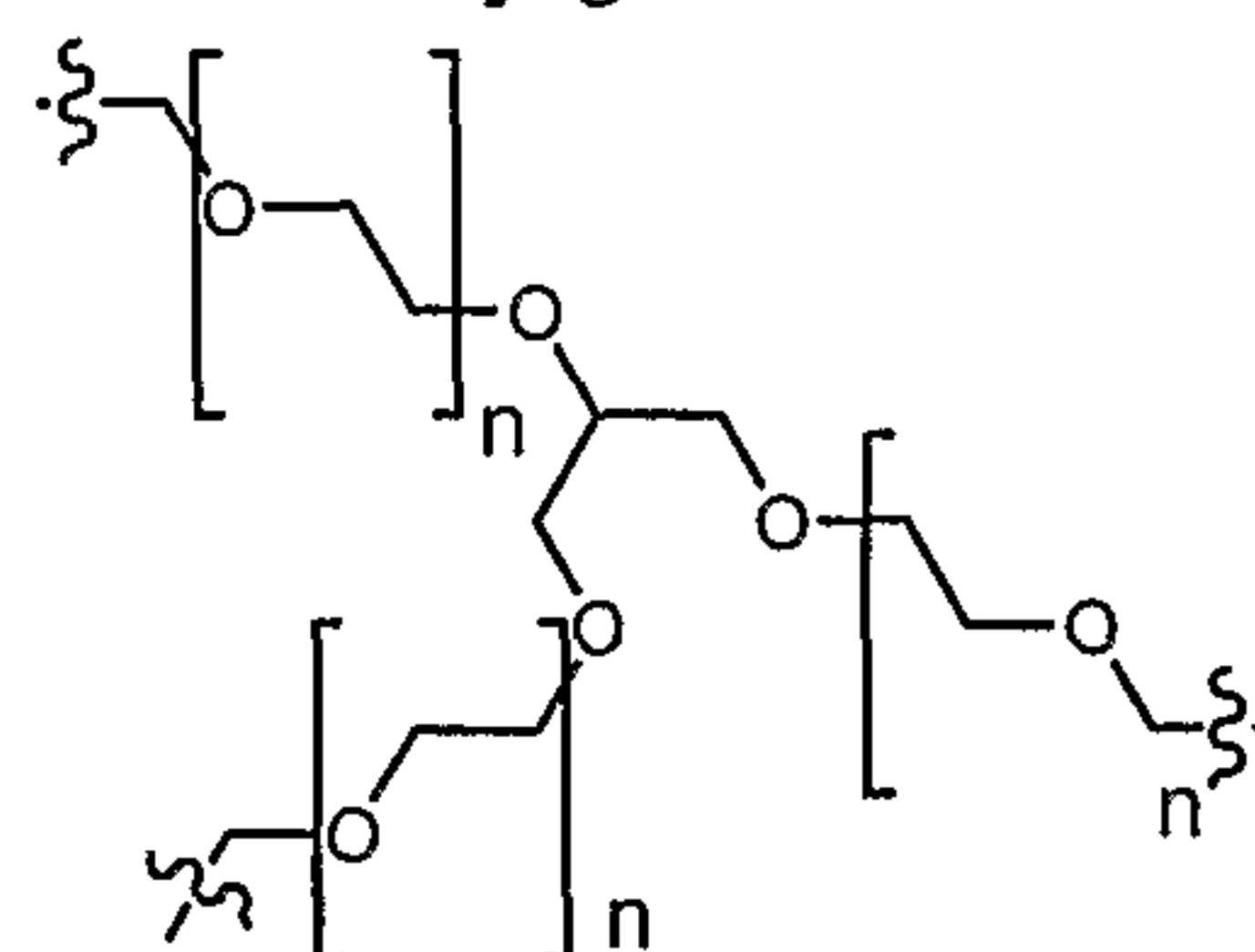
(total Mw of conjugate is about 41,500)

2



(total Mw of conjugate is about 42,000)

3



(total Mw of conjugate is about 41,500)