

(19) World Intellectual Property
Organization
International Bureau



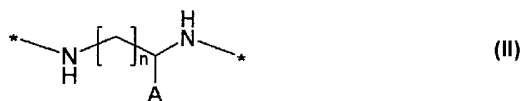
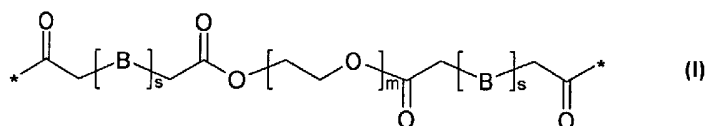
(43) International Publication Date
19 February 2004 (19.02.2004)

PCT

(10) International Publication Number
WO 2004/014973 A2

- (51) International Patent Classification⁷: **C08G**
- (74) Agent: **GILL JENNINGS & EVERY**; Broadgate House,
7 Eldon Street, London EC2M 7LH (GB).
- (21) International Application Number:
PCT/GB2003/003522
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC,
SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA,
UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (22) International Filing Date: 13 August 2003 (13.08.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
- | | | |
|-----------|-----------------------------|----|
| 0218831.6 | 13 August 2002 (13.08.2002) | GB |
| 0218827.4 | 13 August 2002 (13.08.2002) | GB |
| 0218830.8 | 13 August 2002 (13.08.2002) | GB |
| 0218829.0 | 13 August 2002 (13.08.2002) | GB |
| 0218828.2 | 13 August 2002 (13.08.2002) | GB |
| 0306445.8 | 20 March 2003 (20.03.2003) | GB |
- (84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicant (*for all designated States except US*): **SIRUS
PHARMACEUTICALS LTD** [GB/GB]; Babraham Hall,
Babraham, Cambridge CB2 4AT (GB).
- (72) Inventor; and
- (75) Inventor/Applicant (*for US only*): **CLARK, Jonathan**
[GB/GB]; Sirus Pharmaceuticals Ltd, Babraham Hall,
Babraham, Cambridge CB2 4AT (GB).
- Published:**
— *without international search report and to be republished
upon receipt of that report*
- For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: BIODEGRADABLE POLYMER



(57) Abstract: A polymer comprises units of formulae (I) and (II) wherein B, s, m, n and A are as defined in the specification. There are also provided process for preparing them and their use in therapy, *e.g.* when A includes a therapeutic agent.

Biodegradable Polymer

Field of the Invention

The present invention relates to biodegradable polymers and conjugates thereof, processes for preparing them, and their use in therapy.

5 Background to Invention

A number of polymer systems have been developed for use as carrier agents for drug delivery. Most polymeric drug delivery systems rely on encapsulation of payload, e.g. drug, during synthesis and thereafter release of drug through erosion of the polymer leading to exposure of free drug, by the processes of diffusion
10 through the polymer matrix, or a combination of both. There are fewer examples of delivery systems that covalently attach the drug to the polymer itself; these are usually based on derivatised dextran, N-(2-hydroxypropyl)methacrylamide (HMPA), activated polyethylene glycol (PEG) or similar materials. These systems have a number of limitations. Materials such as PEG and dextran are often used because
15 they can impart water-solubility on the drug-polymer conjugate, where the drug can be for example a small chemical entity, peptide or protein. In the case of dextran, where the drug can in theory be added along the entire length of the polymer chain, there comes a point where if more drug is added, phase transitions occur and the polymer separates from water in an aqueous environment. It is therefore often hard
20 to fully load a dextran molecule and retain water solubility. PEG is a particularly useful polymer for evading clearance via the reticuloendothelial system (RES). However, drug can only be added to PEG at the ends of the PEG chain which results in very low drug to PEG ratios. This limitation can to some extent be overcome by using branched PEG, but even then the ratio of drug to PEG is limited
25 to the number of branches.

With all these systems there is a compromise, particularly relevant when the payload is hydrophobic, between drug loading and final polymer size, wherein the maximum drug loading ratio is dictated by the intrinsic chemical properties of the payload, and the final payload capacity for the polymer is dictated by the final
30 polymer size. For non-biodegradable systems, this is particularly problematic because bio-acceptability and safety parameters are linked to the ability of the body to safely clear itself of the polymeric carrier system. In this regard, physiological parameters that relate to tissue permeability and renal clearance become major factors.

35 With most known polymer systems, the drug is added to the polymer system as one of the final steps. The polymers are often not fully loaded and the drug to polymer ratio is variable and poorly defined. This method also can lead to

entrapment of the drug in the polymer matrix as well as bound to the polymer itself. Where the drug is a potent compound this can lead to potential toxicity due either to uncertainty in the amount of drug bound to the polymer or potential burst effects as the free compound in the polymer matrix rapidly diffuses out. To avoid these
5 problems it may be necessary to assay the bound and free drug content carefully, which inevitably adds to the regulatory issues and cost of production, particularly if the level of detection for free compound required is very low.

Water-soluble polymers constructed from lysine or other diamines and PEG activated with phosgene or similar agent (e.g. as succinimidyl carbonate) are known
10 (J.Kohn, *Macromolecules*, 1992, 25, 4476-4484; J.Kohn, *Bioconjugate Chem.*, 1993, 4, 54-62; J.Kohn, *J.Bioactive and Compatible Polymers*, 1994, 9, 239-251). These polymers have only urethane bonds in the polymer backbone. The urethane-based polymers are not biodegradable and the therapeutic entities and linkers are added after the polymer has been synthesised. Although the number of potential anchor
15 points for drug is strictly defined in these systems, the drug loading is dictated by the efficiency of activation and coupling through these anchor points, which gives rise to potentially variable loading and unreacted intermediates.

With many of the known polymer systems, particularly the PEG, dextran and HMPA polymers, the molecular weight has to be kept below the renal clearance
20 threshold of about 50 kDa and preferably below ca. < 30 kDa for PEG so that they can be removed from the systemic circulation. This is because they are not biodegradable, and even if the molecular weight is kept below 50 kDa, they can still accumulate in various tissues and remain for extended periods of time because they are not biodegradable. Indeed, this phenomenon is usefully exploited in the
25 enhanced, yet passive, targeted delivery and retention of polymeric antineoplastic drug systems by tumour tissues. Polymer-conjugate systems constructed entirely from amino acid derivatives such as aspartic or glutamic acid are biodegradable, but do not convey the water solubility required, particularly when fully loaded, that the polymers of this invention are designed to provide. Dextrin polymers are also
30 potentially biodegradable, however, once high levels of derivatisation are achieved they can lose their biodegradable properties and additionally they can suffer the solubility problems which occur with dextran, particularly when hydrophobic compounds are added to the polymer backbone.

The limitations described above mean that, in general, polymeric conjugates
35 of drugs are generally only used to treat life-threatening or very serious conditions, for which these limitations are acceptable. (For reviews on polymer drug conjugate systems, see R.Duncan, S.Dimitrijevic and E.G.Evagorou, *S.T.P. Pharma Sciences*

1996, 6(4), 237-263; R.Duncan, Anti-Cancer Drugs 1992, 3, 175-210; S.Brocchini, R.Duncan, Encyclopedia of Controlled Drug Delivery, John Wiley & Sons, Inc. 1999, Vol 2, 786-815).

WO98/00454 discloses copolymers of the polyesteramide or
5 polyesterurethane type which are based on symmetrical crystalline diamide-diols, diamide-di-acids or diurethane-diols as a first monomer co-polymerised with a second monomer selected from alkane-di-acids, alkane-di-acid chlorides, alkane-diols, poly(alkane ether) diols, hydroxyl acids, diisocyanates and combinations thereof.

10 WO01/23457 discloses a modification to the polymers of WO98/00454, which incorporates a polyalkylene glycol component into the copolymer; this apparently results in faster polymer degradation. These polymers have been designed primarily for use in biodegradable plastics and therefore have properties, such as mechanical strength, which are irrelevant to therapeutic use.

15 WO02/18477 discloses certain co-polymers formed from functionalised alpha-amino acids which are susceptible to catalytic hydrolysis by hydrolases.

Zalipsky (1995) Adv Drug Deliv Reviews 16, 157-182, has reviewed certain polymer conjugates based on polyethylene glycol.

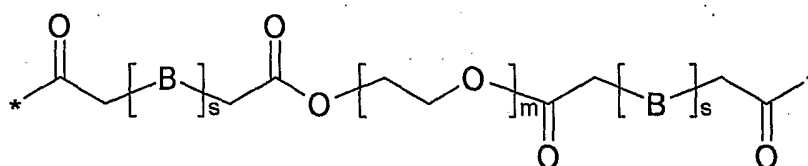
WO96/2374, WO02/065988 and US 6395266 describe conjugates of
20 polyethylene glycol bearing terminal drug moieties.

Summary of the Invention

The present invention provides an enhanced, generally water-soluble polymer comprising safe, low molecular weight components, which is also fully biodegradable down to these components. The drug entity may be added to one of
25 the monomer components prior to polymerisation which conveys the added advantage that free drug cannot become trapped in the polymer matrix unless it is deliberately added during or after polymerisation. The polymer system of this invention allows the addition of hydrophobic drugs with reduced risk of the solubility problems seen with other polymer systems while still allowing high levels of drug
30 loading. Thus, an additional feature of this invention is the inherent flexibility to design the polymer size and drug loading ratios for optimal solubility, for example, whilst being able to exploit useful characteristics of larger polymer systems, such as tissue, organ or plasma retention, if desirable, without the inherent safety issues associated with toxic accumulation of a non-biodegradable system. The loading of
35 drug is highly controllable at the polymerisation step and other components or drugs can additionally be added to the polymerisation step to become additionally incorporated into the polymer.

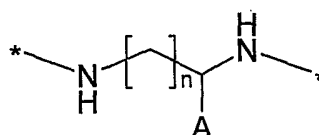
More generally, an object of the present invention is to provide a versatile polymer capable of degradation in physiological aqueous environments with or without assistance from enzymes, which are non-toxic, confer desirable biological properties onto therapeutically active substances with which they are conjugated,
 5 and are susceptible to ready and economic synthesis.

As a first aspect of the invention, a polymer comprises units of formulae (I) and (II):



(I)

10 and



(II)

wherein B is selected from oxygen, sulphur, alkyl, alkyl ether, alkyl thioether, hydroxyl alkyl and alkyl aryl;

s independently represents 0 or an integer of 1 to 100;

m is an integer of 1 to 1000;

n is an integer of 1 to 100; and

A is a functional group optionally conjugated to a further component.

Polymers of the present invention may comprise one or more different monomer units (I) and one or more different monomer units (II). For example the
 20 units (I) and (II) may contain different A and B groups.

Polymers of the present invention may comprise one or more different units A optionally with one or more different further components attached to A. Typically, the polymers are linear, but they may also be cyclic or branched. The exact structure will be determined by the reaction conditions used and the ratio of diacid to diamine
 25 used during the polymerisation step.

Functional group A is adapted to provide a point of attachment for further components. Functional group A may include carboxyl, amino, amido, thio and hydroxyl groups as the point of attachment for further components.

The further components which may be conjugated to the polymers of the invention include drugs, e.g. small molecules, peptides, proteins, saccharides, groups that modify the properties of the polymer, such as water-solubility, and cell targeting agents. Each of the further components may be conjugated to the functional group A via a linker. Drugs may be included as such or as pro-drugs (e.g. esters of drugs with acid or drugs bearing hydroxyl groups).

Thus, according to another aspect of the invention, a conjugate is of a polymer as described above and, linked via the group A, optionally via a linker, a drug, protein, peptide, saccharide, or a group that modifies the properties of the polymer, e.g. a group providing the conjugate with a degree of water-solubility that is higher or lower than that of the polymer.

The polymers according to the invention are generally water-soluble, although they may not be water soluble at the very large molecular weights, depending on the nature of the further components. Sometimes they form gels. Water soluble polymers are preferred.

Polymers of the current invention differ from urethane-based polymers because the ester and amide bonds of the current invention impart biodegradability to the polymer backbone itself and the current synthesis allows precise and strict drug to polymer ratios without the potential for unreacted intermediates. It also retains the advantages of PEG polymers, namely low immunogenicity, solubility and enhanced retention times (low RES clearance).

Description of Preferred Embodiments

As used herein, the term "alkyl" means a straight or branched chain alkyl group of up to 8 carbon atoms. Examples are methyl and ethyl. "Alkyl ether" i.e. alkoxy may be interpreted accordingly. Examples are methoxy and ethoxy. "Alkyl thioether" i.e. alkylthio may also be interpreted accordingly. Examples are methylthio and ethylthio.

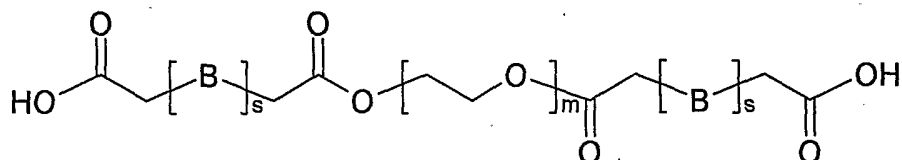
Halogen means F, Cl, Br or I.

"Aryl" means any aromatic group including heteroaromatic groups, e.g. containing up to three heteroatoms selected from N, O and S, monocyclic or bicyclic, having up to 12, e.g. 5 to 10, ring atoms. Examples are thienyl, phenyl and naphthyl. Aryl groups may optionally be substituted e.g. with one or more groups selected from hydroxy, C₁₋₄ alkyl, halogen and C₁₋₄ alkoxy, but are preferably unsubstituted. A preferred aryl group is phenyl. "Alkyl aryl" may also be interpreted accordingly. Examples include methylphenyl.

Example of acyl include alkyl carbonyl especially wherein alkyl represents methyl or ethyl (i.e. wherein acyl represents acetyl or propanoyl).

A polymer of the invention may be prepared by methods that are generally known. A typical example includes the polymerisation of a di-acid and a di-amine. Certain monomers and other materials used in such processes may be new.

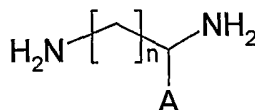
The polymers of the invention may be preferably prepared by a process
5 comprises co-polymerising one or more first monomers (I'):



(I')

or an analogue derived from a branched PEG,
or an activated derivative thereof;

10 with one or more second monomers (II'):



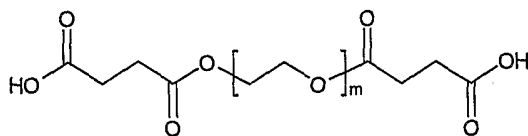
(II').

The invention also provides co-polymers obtainable by and obtained by said process.

Preferably the two carboxylic acid moieties of the di-acid monomer (I') are
15 activated. Suitable activating groups will be well known to a skilled person. For example, they may suitably be activated by treatment with N-hydroxysuccinimide.

In polymers of the invention, examples of B include O and (CH₂)₁₋₃, e.g. CH₂. However, preferably s represents 0.

By way of example, a diacid of the formula
20



may be polymerised with a di-amine containing substituents. A typical example of a
25 di-amine is lysine. The di-acid will typically have a range of values for m, the exact range mixture affecting the physical properties of the polymer produced. In one embodiment of this invention, the average molecular weight of the PEG unit is 1500, which corresponds to an average value for m of 34. In other embodiments of this invention the PEG unit can have, but is not limited to, an average molecular weight

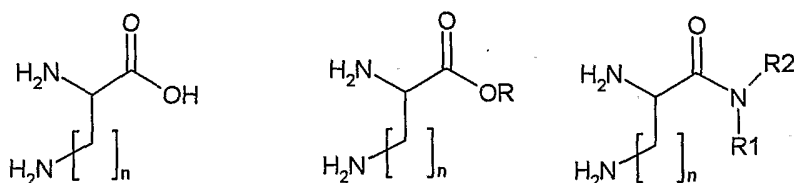
of 200, 400, 600, 800, 900, 2000, 3000 and 4000 which corresponds to average values of m of 4.5, 9, 13.6, 18, 20.5, 45.5, 68 and 91.

The diacid component used in the polymerisation can be selected from a range of diacids made from different batches of PEG with different average values of m . Additionally, branched PEG can also be used, but in this case the amount of di-amine used in the polymerisation step is adjusted to take account of the additional acid groups introduced by the additional PEG chains.

Branched PEG's, which are commercially available, are generally prepared by incorporating a cross-linking monomer into the polymerisation mixture. An example of a suitable cross-linking monomer is glycerol.

For example, a simple branched PEG is of formula $\text{CH}_2[(\text{OCH}_2\text{CH}_2)_m\text{OH}]\text{CH}[(\text{OCH}_2\text{CH}_2)_m\text{OH}]\text{CH}_2[(\text{OCH}_2\text{CH}_2)_m\text{OH}]$.

Example di-amine components which can be used in this polymerisation are



20

wherein n is typically 1 or an integer of up to 10, and

R , R^1 and R^2 are typically selected from hydrogen, alkyl, aryl, alkyl ether, amino acid, peptide, linker and therapeutic agent.

The first example above mentioned may also suitably be reduced thereby forming a CH_2OH moiety to which further components may be added (e.g. acids through ester connections).

In one preferred embodiment of this invention di-amine is a derivative of lysine, where the two amines of the lysine become part of the polymer backbone and the acid group of the lysine has been added to a therapeutic entity (or other component), preferably through a linker such as 5-amino valeric acid. There may also be additional elements in the linker between the therapeutic and the polymer chain such as a hemiacetal group, amino acid or peptide.

Another di-amine of interest is the derivative of lysine in which the -COOH moiety has been reduced to $\text{-CH}_2\text{OH}$ which then forms a point of attachment for further components, especially carboxylic acids.

A typical procedure for the preparation of the polymer of this invention
5 comprises prior activation of the di-acid component as an acid chloride, acid bromide, acid fluoride, or as an active ester such as a N-hydroxysuccinimide. Alternatively, the diacid can be activated *in situ* using reagents commonly used for the preparation of amide bonds in peptide synthesis. Further, the may be carried out by heating the di-acid and di-amine components together to dehydrate the material
10 to effect polymerisation. The preferred method for this invention is to activate the diacid prior to use, so that the activated material can be purified and stored for use at a later stage. The preferred activation method is to form the N-hydroxysuccinimide ester from N-hydroxysuccinimide, di-isopropylcarbodiimide and the diacid in dichloromethane. The activated di-acid can then be reacted with
15 diamine in the ratio of one diacid to one di-amine to provide the polymer of this invention. By controlling the exact ratio of di-acid to di-amine, different molecular weights can be achieved. It is probable that by limiting the di-amine ratio to less than one to one of di-acid, that the material will contain cyclic material. The molecular weight can also be controlled by varying the polymerisation conditions, such as
20 temperature, time, concentration and by the addition of components which can stop the polymerisation, such as water, mono-amine, alcohols and alkoxide. By the addition of branching units, such as a tri-amine, the molecular weight can be increased dramatically. In these cases, the ratio of di-acid to di-amine must be adjusted to take into account the addition of the branching agent, which in the case
25 of a tri-amine branching unit would reduce the amount of di-amine required. The aim in this case is to keep the total amine content (di-amine plus tri-amine) the same as with the di-amine alone.

If an excess of activated di-acid is used, then the termini of the polymer chains will have activated acid groups at the ends. Alternatively additional activated
30 di-acid can be added at the end of the bulk polymerisation to achieve a similar result, but generally of a polymer with higher molecular weight. The termini can then be reacted with further components, such as cell-targeting agents, proteins, peptides, saccharides, polysaccharides or cross-linking reagents such as tri-amines.

Preferably, the polymer contains amine equivalents to acid equivalents in a ratio of 1:1 or (1:1)+1 or (1:1)-1 to take account of the fact that the termini of the polymer may be formed from di-acid monomer or the di-amine monomer or one may be di-acid monomer and the other may be di-amine monomer. When the monomers
5 are straight chain then this ratio will be the ratio of monomers (I') to (II'). However when cross-linking components are used (whether acid or amine) then a correction will need to be applied accordingly.

In some cases the polymers are preferably straight-chain. In other cases they are preferably cross linked. The polymer may also be cyclic (in which case the
10 ratio would be 1:1). In order to make it more likely that one of the monomers forms the termini then an excess of that monomer can be used.

The termini of the polymer may be derivatised (capped), e.g. as an acid terminus with an alcohol (to form an ester) or an amine (to form an amide) and/or an amine terminus with an acid (to form an amide).

15 Advantageously the polymer may be capped with a substance capable of usefully modifying the properties of the polymer. Example substances are described later in the specification for polymer property modifying agent (variable K). For example if the polymer has at least one acid terminus then the terminus may be reacted with a substance bearing amine groups e.g. a protein with surface lysine
20 residues. Examples include lectins such a wheat germ agglutinin. It may be necessary to activate the acid termini to facilitate reaction e.g. by reaction with N-hydroxysuccinimide.

Peptides as well as proteins may also conveniently be used as capping groups, and may readily be attached when the terminus is the amine or the acid.

25 Other capping groups of particular interest include saccharides especially mono and disaccharides.

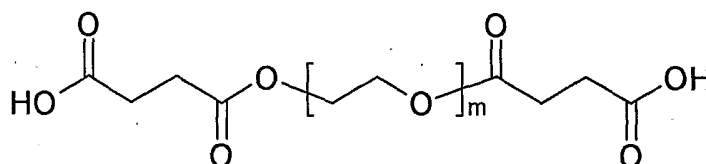
Preferably the polymer contains up to 10,000 especially up to 1000 units of each monomer. Preferably the polymer contains at least 5, more preferably at least 10 units of each monomer. Most preferably the number of units is 10-30 especially
30 15-20.

The molecular weight of polymers of the invention will be typically in the range 6 kDa to 2000 kDa, preferably 15 kDa to 250 kDa, excluding the contribution of any further components conjugated to a functional group A or any terminal capping groups. The total molecular weight of the polymer (including further

components and/or capping groups) will be typically in the range 10 kDa to 2500 kDa, preferably 25 kDa to 300 kDa.

Preferably, s represents 0. Thus monomer (I') is preferably a compound of formula:

5

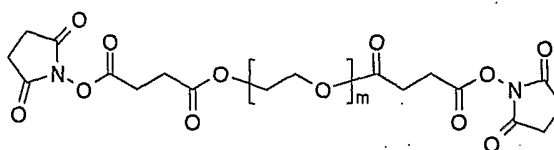


or an analogue derived from a branched PEG;

wherein m is as defined for formula (I), or an activated derivative thereof.

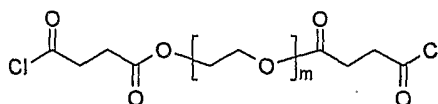
These monomers are particularly favoured since they are capable of degradation to PEG and succinic acid products, which are physiologically benign.

10 The preferred activated derivative is a compound of formula:



An alternative activated derivative is a compound of formula:

15



Preferably m represents an average integer of 20-100, especially 30-40.

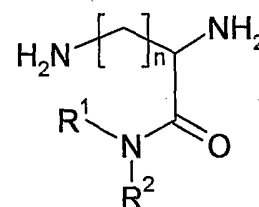
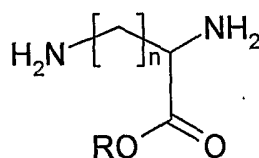
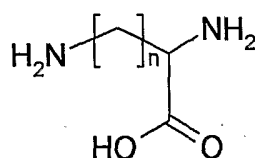
The carboxylic acid groups of monomer (I') are preferably activated. Such
20 monomers can be prepared by treating a polyethylene glycol (PEG) with succinic anhydride under standard conditions. For example the reagents may be mixed in the presence of dimethylaminopyridine (DMAP) in an inert solvent such as dichloromethane (DCM). A suitable PEG is PEG 1500 (average molecular weight 1500) which results in an average value m of 34. PEGs for use in preparing the
25 polymers of the invention are commercially available.

A feature of the present invention, and an example of its versatility, is that specific monomers, particularly those of monomer (II') may be chosen with a view to determining properties of the polymer.

n preferably represents 1 to 10, more preferably 3-6, particularly 4.

- 5 For example, functional groups A include COOH, COOR and CONR¹R² wherein R, R¹ and R² are typically selected from hydrogen, alkyl, aryl, alkyl ether, amino acid, peptide, linker and therapeutic agent.

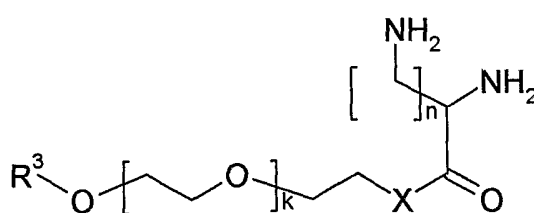
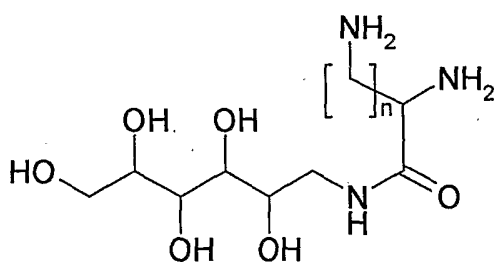
Specific examples of monomer (II') include the following:



- 10 Thus suitable di-amine monomers (II') include diaminopropionic acid, ornithine and lysine derivatives. They can be used as free carboxylic acids or may be connected to saccharide derivatives such as glucamine or alternatively to polyethylene glycols to modulate water-solubility, as described below

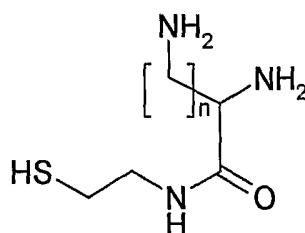
Further examples of monomers (II') include the following:

15

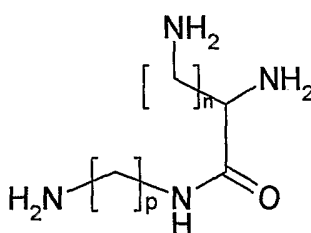


wherein X may be oxygen, sulphur or nitrogen (NR e.g. NH), R³ is typically as defined above for R, and k is an integer from 1 to 100.

- 20 A further example of a di-amine monomer (II') include a free thiol which when incorporated within a polymer allows the attachment of biological agents which may be therapeutic or may serve the function of targeting the polymer to a particular tissue, cellular compartment or biological process. An example of such a group is the following:



Additionally, tri-functional groups such as tri-amines can be added to increase cross-linking, e.g. compounds of the formula:



5 wherein preferably n represents 1 to 10, more preferably 3-6, especially 4 and p represents 1 to 10, more preferably 3-6, especially 4.

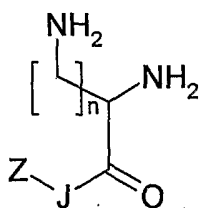
Cross-linking may have a significant effect on polymer properties which would be understood by those skilled in the art of polymer chemistry. Solubility and molecular weight in particular may be altered. The degree of cross-linking also has
10 an impact on biodegradability which would also be understood by someone skilled in the art of polymer therapeutics and delivery systems.

These monomers can all readily be derived from the corresponding amino acid. In view of its versatility and availability, the preferred amino acid precursor for the di-amine monomer (II') is lysine.

15 As indicated above the copolymers of the invention are optionally linked to further components; such components preferably comprise a therapeutic component, e.g. a drug. The therapeutic component may be connected to the di-amine unit directly or through a linker to aid the release of the therapeutic agent or to modulate the physical or biological properties of the material, or to aid synthesis.
20 In a preferred embodiment of the invention, the therapeutic compound is linked to the monomer (II') prior to polymerisation. Thus in a preferred embodiment the monomer (II') comprises the group -A-J-Z, wherein A is a functional group, J is an optional linker and Z is a drug moiety.

Therapeutic agents can be attached to the polymer through functional groups which can be incorporated into the polymer in a similar way to the thiol or through the di-amine component. These include amine, carboxylic and hydroxyl groups.

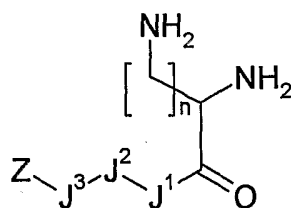
- 5 The functional group A preferably comprises a carbonyl moiety, i.e. it is derived from a carboxy group, and optional linker J such that a preferred monomer (II') is a compound of the formula:



wherein J is an optional linker and Z is a therapeutic agent. J therefore represents a
10 linker or a bond but preferably J represents a linker. When J represents a linker it preferably represents the group J¹-J²-J³. Preferably n represents an integer of 1 to 10, especially 3 to 6 particularly 4.

Suitable linkers include amino acids, peptides or a chain such as 6-aminohexanoic acid, 5-aminopentanoic acid, 4-aminobutanoic acid and 3-
15 aminopropanoic acid. 5-Aminopentanoic acid is a particularly preferred linker.

More preferably monomer (II'') is a compound of formula:



(II'')

The invention also provides polymers obtainable and obtained by such a
20 process.

Z is a therapeutic agent which contains a functional group which allows it to be connected to the di-amine via linker J¹-J²-J³ if present. Preferably therapeutic agent Z contains a free amino or, more preferably, a hydroxyl as functional group. When linker J is absent, i.e. J¹-J²-J³ is a bond, and A comprises a carbonyl group,

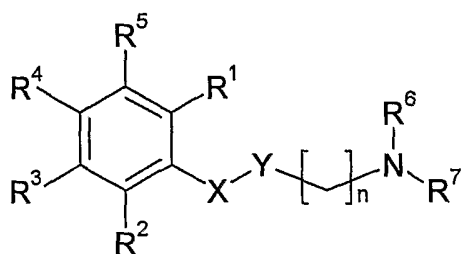
therapeutic agent Z may then be released from the polymer by hydrolysis of the ester connection.

J¹ preferably represents a sulphur, oxygen or an amino group (e.g. NH or NMe, preferably NH), preferably oxygen or an amino group, especially an amino group. J² preferably represents a spacer group. J³ preferably represents a carbonyl group. This permits Z to be released from the polymer by hydrolysis of the amido or more preferably the ester connection between J³ and Z. Spacer group J² may represent an alkylene group, e.g. a C₁₋₁₀alkylene group e.g. (CH₂)₃₋₆. The preferred linker J¹-J²-J³ is -NH(CH₂)₄CO-.

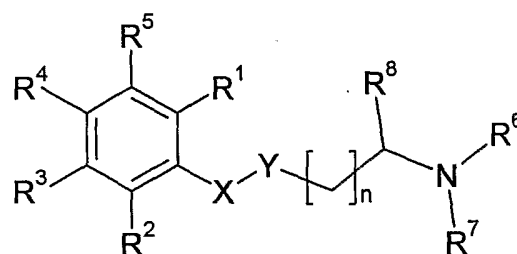
10 Z may be selected from a wide range of therapeutic substance whose properties may benefit from being incorporated into a biodegradable polymer. Z may also be a pro-drug which is converted into the active species after cleavage from the polymer. Z may be a protected substance.

15 Examples of other linkers that may usefully be used include those described in US 6,214,345.

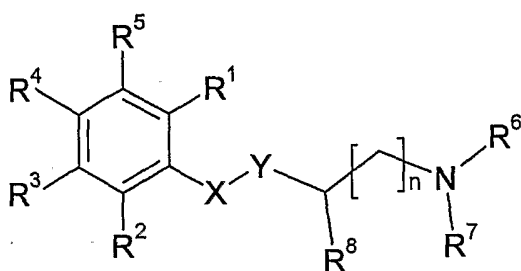
For example, Z may be a local anaesthetic substance. Example compounds are defined by the following chemical formulae:



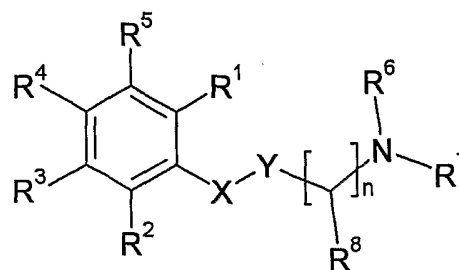
(III)



(IV)



(V)



(VI)

wherein:

R^1 and R^2 are independently selected from hydrogen, halogen, alkyl and alkyl ether groups;

5 X is $C=O$ and Y is NR , or X is NR and Y is $C=O$, or X is $C=O$ and Y is O , and R is selected from hydrogen, halogen, hydroxyl, alkyl, aryl and acyl;

R^6 and R^7 are independently selected from alkyl, aryl and alkylaryl groups; and

R^8 is selected from hydrogen, halogen, hydroxyl, alkyl, aryl; or

10 R^7 and R^8 may be joined, typically through a chain of carbon atoms and, optionally, heteroatoms, to form a ring 5, 6, 7 or 8 atoms in size;

n is 0, 1, 2, 3, 4 or 5; and

R^3 , R^4 and R^5 are each independently selected from hydrogen, hydroxyl, halogen, alkyl, aryl, hydroxyalkyl, hydroxyaryl, aminoalkyl and aminoaryl, with the
15 proviso that at least one of R^3 , R^4 and R^5 is a hydroxyl moiety connected to the polymer through a covalent bond.

Preferably R^6 represents alkyl especially methyl or ethyl. Preferably R^1 represents alkyl especially methyl. Preferably R^2 represents alkyl especially methyl. Preferably those groups of R^3 , R^4 and R^5 that do not represent hydroxyl represent
20 hydrogen. Preferably X represents NH and Y represents $C=O$.

More preferably R^3 represents hydroxy and R^4 and R^5 represent H .

In structure (III), preferably n represents 1, 2 or 3, particularly 1 or 2, especially 1. Preferably R^8 represents alkyl especially methyl. Preferably R^7 represents alkyl especially ethyl. Preferably R^6 represents ethyl.

25 The preferred structure is that of 3- or 4-hydroxy-lidocaine, especially 3-hydroxy-lidocaine.

In structure (IV), preferably n represents 0, 1 or 2, especially 0 or 1, particularly 0. Preferably R^6 represents methyl or ethyl, especially ethyl. Preferably R^7 and R^8 are joined and represent an alkylene chain especially $(CH_2)_4$.

30 Alternatively, R^7 and R^8 may independently represent alkyl, especially ethyl.

The preferred structure is that of 3- or 4-hydroxy-bupivacaine, especially 3-hydroxy-bupivacaine. 3- or 4-hydroxy-mepivacaine, especially 3-hydroxy-mepivacaine is also of interest. 3- or 4-hydroxy-etidocaine, especially 3-hydroxy-etidocaine, is also of interest.

In structure (V), preferably n represents 0, 1 or 2, especially 0 or 1, particularly 0.

Preferably R^8 represents alkyl, especially methyl or ethyl. Preferably R^7 represents alkyl, especially methyl or ethyl.

- 5 In structure (VI), preferably n represents 1 or 2. Preferably R^8 represents alkyl, especially methyl or ethyl, particularly methyl. Preferably R^7 represents alkyl, especially methyl or ethyl.

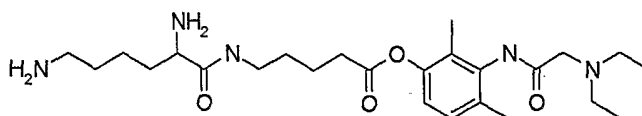
The drug is preferably conjugated to the polymer through a hydroxy substituent, thus for example 3- or 4-hydroxy-lidocaine is conjugated through the 3-
10 or 4-hydroxy substituent.

The above mentioned compounds may be prepared by methods analogous to those described in US 5,849,737 (Chaplan et al).

When local anaesthetic substances are incorporated into polymers according to the invention their properties may be modified in a beneficial way, for example,
15 the therapeutic window of the substance may be widened or the duration of action may be lengthened.

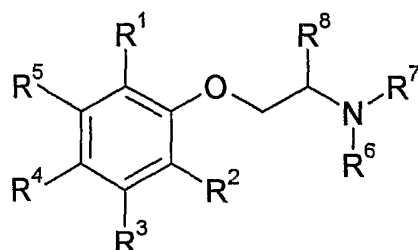
One substance of particular interest is 3-hydroxylidocaine. According to US Patent 5,849,737, 3-hydroxylidocaine is useful in the treatment of neuropathic pain. However the use of this substance for this purpose is difficult because of the risk of
20 overdose resulting in both central and cardiovascular side effects. Because of this potential risk, compounds such as local anaesthetics with similar mechanisms of action are often administered by infusion, rather than as a bolus. When incorporated into the polymers of the invention the risk of harmful effect is lessened since the body is not exposed to either high local or high rapidly-achieved systemic
25 concentrations of free drug as the release of the drug from the polymer is extended over many minutes or hours. 3-hydroxylidocaine is preferably incorporated into polymers of the invention by attaching the 3-hydroxy group to the di-amine of monomer (II') preferably through a linker as just described.

The preferred di-amine monomer unit required for the polymerisation would
30 have the following structure:



Another group of local anaesthetic compounds which Z may represent is defined by the following formula:

5



wherein:

R^1 and R^2 are independently selected from hydrogen, halogen, alkyl and
10 alkyl ether groups;

R^6 and R^7 are independently selected from hydrogen, hydroxyl, alkyl, aryl
and alkylaryl groups; and

R^8 is selected from hydrogen, halogen, hydroxyl, alkyl, aryl and alkylaryl;

or

15 R^7 and R^8 may be joined, typically through a carbon chain, to form a ring 5, 6,
7 or 8 atoms in size, which may contain heteroatoms; and

R^3 , R^4 and R^5 are each independently selected from hydrogen, hydroxyl,
halogen, alkyl, aryl, hydroxyalkyl, hydroxyaryl, aminoalkyl or aminoaryl, with the
proviso that at least one of R^3 , R^4 and R^5 is hydroxyl through which it is linked to the
20 polymer by a covalent bond.

Preferably R^1 represents alkyl, especially methyl. Preferably R^2 represents
alkyl, especially methyl. Preferably R^6 represents alkyl, especially ethyl.
Preferably R^7 represents alkyl, especially ethyl. Preferably R^8 represents alkyl,
especially methyl. Preferably those R^3 , R^4 , R^5 groups that do not represent OH
25 represent hydrogen.

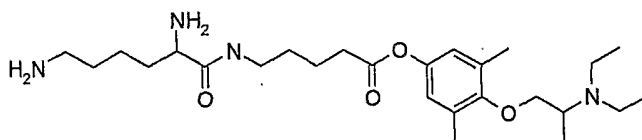
Substances within this formula of particular interest are those in which:

R^6 and R^7 represent alkyl, especially ethyl, R^3 and R^5 represent hydrogen, R^8
represents alkyl, especially methyl, R^1 and R^2 represent alkyl, especially methyl, and
 R^4 represents hydroxyl; or R^6 and R^7 represent alkyl, especially ethyl, R^5 and R^4

represent hydrogen, R^8 represents alkyl, especially methyl, R^1 and R^2 represent alkyl, especially methyl, and R^3 represents hydroxyl. The preferred compounds are 3-hydroxy-N,N-diethylmexiletine and 4-hydroxy-N,N-diethylmexiletine.

The above mentioned compounds may be prepared by processes generally known *per se*. For example secondary and tertiary amines can be prepared by alkylating primary amines. Hydroxyphenyl derivatives can be derived from the corresponding nitrophenyl derivatives by successive reduction and treatment with nitrite. Nitrophenyl derivatives may be obtained by nitrating the corresponding unnitrated aromatic compound. The corresponding unnitrated aromatic compounds may be prepared for example by reference to methods described in US 3,954,872 (Koppe et al) and US 3,659,019 (Koppe et al). For example the compound may be assembled by reacting a phenol derivative with a compound of formula $\text{Hal-CH}_2\text{-CHR}^8\text{-NR}^6\text{R}^7$ or a protected derivative thereof wherein Hal represents halogen (or other leaving group). Alternatively the order of steps may be reversed, e.g. the nitration of the aromatic ring may be performed before the phenol derivative is reacted with the compound of formula $\text{Hal-CH}_2\text{-CHR}^8\text{-NR}^6\text{R}^7$.

In one embodiment the preferred di-amine monomer unit required for the polymerisation would have the following structure:

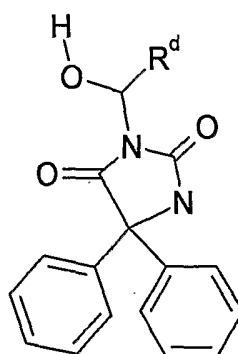


20

Polymers according to the invention are also capable of increasing the water-solubility of drugs which have limited solubility in aqueous environments, for example in the acid environment of the stomach. An example of such a drug is phenytoin. Phenytoin has been used in the treatment of epilepsy for many years and may additionally have uses in the treatment of pain through its sodium channel-blocking activity. When administered, patient plasma levels have to be monitored regularly because of variable bioavailability. One factor contributing to this variability is that phenytoin is very insoluble. Phenytoin is usually administered as a sodium salt to improve solubility, however on contact with the acidic environment of the stomach it becomes protonated and is believed to aggregate into lumps. The dissolution of these lumps is the variable contributing to the variable plasma levels.

Phenytoin can be incorporated into a polymer according to the invention. Such polymers are water-soluble and largely soluble in the acid environment of the stomach, whereupon it undergoes controlled degradation to release free phenytoin. Thereby the undesirable precipitation of phenytoin which occurs when the free drug is administered is substantially mitigated.

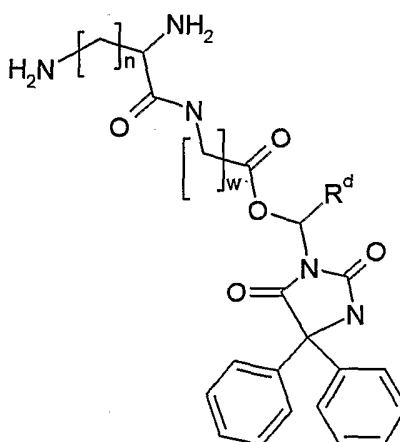
Preferably phenytoin is connected to the polymer as a pro-drug which releases phenytoin when itself released from the polymer. Preferred pro-drugs of phenytoin are hemiacetals formed with formaldehyde and higher aldehydes at the N2 nitrogen position. The preferred pro-drug is given by the following formula:



10

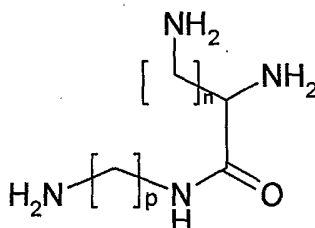
in which R^d represents hydrogen, alkyl, aryl and alkylaryl e.g., of up to 12 or 20 carbon atoms, and is preferably hydrogen, and which is linked to the polymer by a covalent bond through the hydroxyl moiety.

Thus preferred conjugates of phenytoin may be prepared using a di-amine of formula (II') given above in which Z represents a pro-drug of phenytoin, such as the one just mentioned. The preferred monomer (II') for phenytoin is given below:



in which R^d is as defined above but preferably represents hydrogen and w and n independently represent an integer of 1 to 10, preferably 3 to 5, especially 4.

In monomer (I'), m is preferably 4 to 70. The number of repeating units is 5 preferably 5 to 30; or up to 10,000. In some preparations a cross-linker is added to attain higher molecular weights. The preferred cross-linker has the formula:



wherein n is preferably 3-6, especially 4, and p is 1-10, preferably 3-6, especially 4.

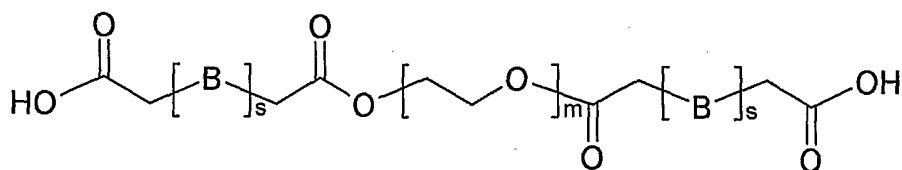
Other examples of therapeutic agents include agents capable of causing cell 10 death which may have particular application in the treatment of cancers. Examples of such agents include toxins.

It will be understood that polymers according to the invention may be prepared in which more than one monomer (I') (which different monomers may, for example, differ in chain length m) may be reacted with more than one monomer (II') 15 (which different monomers may, for example, differ in values for n and nature of (I)).

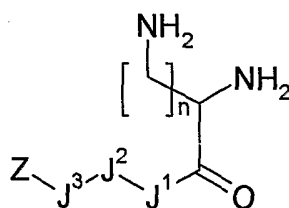
Typically monomer (I') comprises a dispersion of chain length m based on the dispersion of the polyethylene glycol from which it will have been derived.

For certain purposes it will be most suitable to use a single monomer (II'). However a further aspect of the invention provides the formation of multi-functional 20 polymers in which different functional groups A are incorporated through use of two or more monomers (II'). For example, therapeutic agent Z could be different for different second monomers (i.e. the polymer would comprise more than one therapeutic agent) if combination therapy were desired.

According to one embodiment of this aspect of the invention, there is 25 provided a process for preparing a polymer according to the invention which comprises co-polymerising one or more first monomers (I')

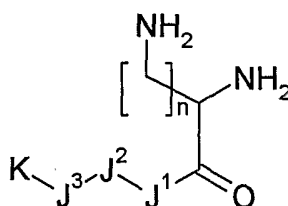


or an analogue derived from a branched PEG,
 or an activated derivative thereof;
 with mixture of one or more second monomers (II''')



5

and one or more second monomers (II''')



10

wherein J^1 , J^2 and J^3 are as defined above, Z represents a therapeutic agent or a pro-drug thereof and K represents a polymer property modifying agent, or precursor thereof.

We also provide polymers obtainable and obtained by such a process.

15 The ratio of monomers (II''') to (II''') will vary depending on the desired properties of the polymer. However they may, typically, be employed in a ratio of 1:10 to 10:1, eg 1:3 to 3:1.

In one embodiment, the polymer property-modifying agent is a targeting agent. Such a targeting agent K will be an agent capable of directing or aiding
 20 direction of the polymer to the target for the therapeutic agent. Examples of targeting agents include cell adhesion moieties. Such substances can assist with intracellular delivery. Of particular interest in the context of the present invention are

targeting agents which can direct the polymer to neuronal cells, for example a neuronal cell adhesion moiety, e.g. a sensory nerve adhesion moiety. Particular examples of nerve adhesion moieties include: antibodies and in particular those which have affinity for nerve cell membranes, lectins such as lectins derived from
5 vertebrates, mammals or humans or other lectins such as plant lectins, and in particular wheat germ agglutinin, hormone receptor ligands, cytokines, growth factors, such as nerve growth factor, epidermal growth factor and insulin-related growth factors, neuropeptides such as endorphins, vasoactive intestinal polypeptide, calcitonin, cholecystokinin, substance P, somatostatin, neuropeptide Y, fragments of
10 neurotrophic viruses such as viral coat proteins of herpes simplex virus, polio virus, rabies virus or fragments thereof, bacterial toxins and in particular non-toxic fragments thereof such as cholera toxin B chain and tetanus toxin fragment C, or fragments thereof.

The use of peptides and proteins as targeting agent K is of particular
15 interest.

The use of saccharides especially mono and di-saccharides is also of particular interest.

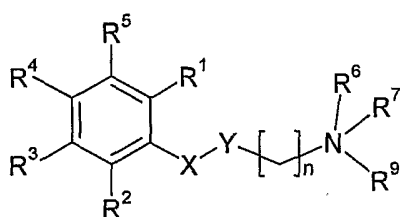
If the group K is a precursor of a polymer property modifying agent, then it may be converted to the polymer behaviour-modifying agent in one or more further
20 synthetic steps. For example K may be a reactive group (or such a group in protected form) which may be reacted with a targeting agent, or other polymer property modifying agent after the polymer has been formed.

In one particular embodiment of this aspect of the invention, the targeting agent targets the therapeutic agent to a neuronal cell and the therapeutic agent is a
25 pain relieving agent such as an anaesthetic. Example anaesthetic substances are given above. In this aspect of the invention it may be particularly advantageous to use a local anaesthetic agent which is a quaternary salt. Quaternary salts of substances such as lidocaine are known to have certain improved properties relative to the tertiary amine parent substance, such as longer duration of action. However
30 the quaternary salts suffer from the disadvantage that they are not readily taken up by cells, thereby greatly reducing their therapeutic usefulness. Polymers of the present invention incorporating a targeting agent and a quaternary salt of an anaesthetic as therapeutic agent allow the benefits of the quaternary salt to be

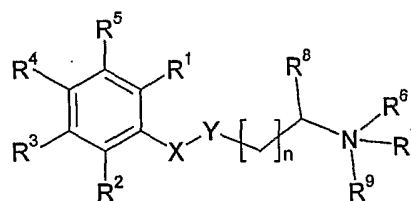
achieved whilst at the same time mitigating the disadvantage through greatly improved cellular entry.

Example anaesthetic substances are of the formulae

5

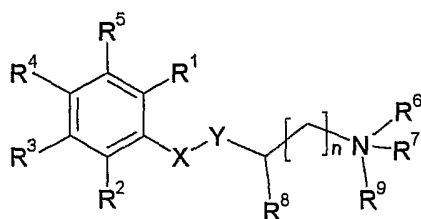


(VII)

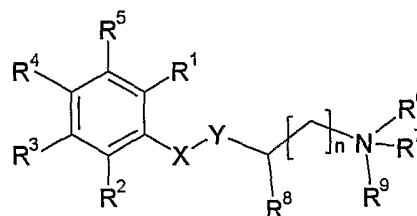


(VIII)

10



(IX)



(X)

15

in which each quaternary nitrogen atom bears a positive charge and is accompanied by X^o as a counter-anion;

X, Y, R¹, R², R³, R⁴, R⁵, R⁶, R⁷ and R⁸ and preferences therefor are as defined above for formulae III-VI;

in addition, X may represent O and Y may represent CH₂;

R⁹ represents alkyl, aryl or alkylaryl; and which substances are connected to the polymer by a covalent bond through the hydroxyl moiety that R³, R⁴ or R⁵ may represent.

Of particular interest are compounds of structure (VI) wherein X represents O and Y represents CH₂, R¹ and R² independently represent alkyl especially methyl, n represents 1, R⁸ represents alkyl, especially methyl, R⁶ and R⁷ independently represent alkyl, especially methyl or ethyl, particularly ethyl, one of R³, R⁴ and R⁵ represents hydroxyl and the other groups represent hydrogen.

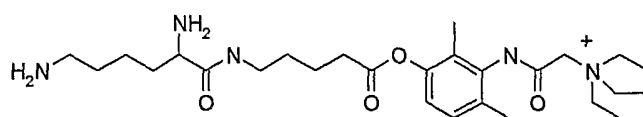
Preferably R⁹ represents alkyl, particularly methyl or ethyl, especially ethyl.

Example compounds include 3-hydroxy-N,N,N-triethylmexiletine, 4-hydroxy-N,N,N-triethylmexiletine and N-ethyl-3-hydroxylicocaine quaternary compounds.

Example counter-anions include pharmaceutically acceptable counter-ions such as halide ions, e.g. iodide.

The quaternary compounds may be prepared from the corresponding tertiary compounds by conventional methods, e.g. by treatment with R⁹X^c, especially when X^c represents iodide.

A preferred di-amine monomer unit required for the polymerisation has the following structure:



accompanied by a counterion.

An advantageous feature of the polymers of the present invention is that synthesis is ready and efficient. As described above, components such as therapeutically active agents, targeting agents and the like may be incorporated into the polymer by incorporating such components into monomer (II'). Alternatively it may be preferred to incorporate a precursor of the component into the monomer, and hence into the polymer, and then convert the precursor to the component after polymerisation. In this connection, precursors include derivatives such as protected derivatives and other chemical intermediates.

A further aspect of the invention is polymers which incorporate therapeutic substances as herein described for use in therapy. We also provide pharmaceutical compositions comprising a polymer incorporating a therapeutic substance as herein described together with a pharmaceutically acceptable diluent or carrier. In general, the therapeutic substance will have a known therapeutic function.

Polymers incorporating therapeutic substances according to the present invention may be administered in therapy by whatever route of administration and in whatever presentation may be deemed most suitable.

For example, formulations for parenteral injection may comprise a polymer
5 according to the present invention dissolved in an aqueous carrier. The aqueous carrier may include conventional excipients such as buffers, preservatives and the like.

For example, formulations for oral administration include capsules and tablets. These may comprise a polymer according to the present invention together
10 with conventional excipients such as diluents, dispersing aids and the like.

Anaesthetic compositions may be administered parenterally, by injection, for example subcutaneously, intercostally, intramuscularly, intravenously or by epidural or spinal injection. Alternatively, the compositions may be formulated for topical administration and in particular for administration to a mucosal surface such as by
15 oral, rectal, vaginal or nasal administration. The compositions may also be administered intradermally, for example using a needleless injection device. Injectable anaesthetic compositions may be administered as liquid solutions or suspensions. Solid forms suitable for solution or suspension in liquid prior to injection may also be prepared. The preparation may be emulsified.

20 Suitable diluents or carriers for use in compositions according to the invention will be known to those skilled in the art. For example diluents and carriers for anaesthetic compositions suitably include water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, compositions may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH
25 buffering agents and antibacterial agents (e.g. thimerosal).

The compositions are administered in a manner compatible with the dosage required and in such amount as will be therapeutically or prophylactically effective. The quantity to be administered, which is generally in the range 5pg/kg to 10g/kg, preferably 250µg/kg to 30mg/kg per dose, depends on a number of factors. These
30 include the subject to be treated, and the degree of therapeutic or prophylactic activity desired and, if applicable, the size of the area to be treated. Precise amounts of active ingredient required to be administered may depend on the judgment of the practitioner and may be peculiar to each subject.

Depending on the therapeutic substance and the condition being treated, compositions of the present invention may also potentially be administered by the ocular, intraocular, intrathecal and intraarticular routes.

Further aspects of the invention include the use of polymers incorporating
5 therapeutic agents according to the invention in the manufacture of a medicament for the treatment of a human disease or condition and a method of treatment of a human disease or condition which comprises administering to a patient a polymer incorporating a therapeutic agent according to the invention. For example when the therapeutic agent is an anaesthetic the condition would be pain, e.g. neuropathic
10 pain. When the agent is phenytoin, the condition would be epilepsy or pain.

During the synthesis of the polymers of the invention, labile functional groups in the intermediate compounds, e.g. hydroxy, carboxy and amino groups, may if desired or necessary, be protected. A comprehensive discussion of the ways in which various labile functional groups may be protected and methods for cleaving
15 the resulting protected derivatives is given in for example *Protective Groups in Organic Chemistry*, T.W. Greene and P.G.M. Wuts, (Wiley-Interscience, New York, 2nd edition, 1991).

The chemical substances described herein may contain chiral centres and are provided in the form of purified enantiomers and diastereomers or as mixtures
20 thereof (e.g. as racemic mixtures).

The polymers and conjugates of the invention have the advantage that they may be more biodegradable, more readily or predicably degraded in aqueous environments, may provide more rapid, less rapid or predictable release of drug, may be more readily prepared, may be more reproducibly prepared, provide more
25 predictable or otherwise better pharmacokinetics for a given route of administration, may be less toxic, provide better sustained release properties or may have other desirable properties as compared with known substances.

All documents referred to herein, including patents and patent applications, are incorporated herein in their entirety by reference.

30 Abbreviations:

WGA	Wheat Germ Agglutinin	DCM	dichloromethane	Boc	t-butyloxycarbonyl
DIC	Diisopropylcarbodiimide	CBZ	benzyloxycarbonyl		
NHS	N-hydroxy-succinimide	NMM	N-methylmorpholine		
DMF	dimethylformamide	DMAP	dimethylaminopyridine	Ac	acetyl

PEG	polyethylene glycol	Me	methyl	Et	ethyl
Su	NHS active ester	TFA	trifluoroacetic acid	Suc	succinyl
GPC	Gel permeation chromatography				

5 Description of the Drawings

Figure 1 shows analytical data for a 3-hydroxy-lidocaine polymer conjugate as per Example 1. Figure 1A shows the polymer with atom assignments, Figure 1B shows the NMR spectrum and Figure 1C shows a GPC spectrum.

Figure 2 shows the NMR spectrum for a phenytoin polymer conjugate 10 (Example 4).

Figure 3 shows a comparison of the effect on paw withdrawal latency of the 3-hydroxylidocaine polymer conjugate (Example 1) with free 3-hydroxylidocaine and control.

Figure 4 shows a linear plot of the plasma concentrations of 3- 15 hydroxylidocaine following intravenous administration of 3-hydroxylidocaine or 3-hydroxylidocaine polymer conjugate (Example 1)

Figure 5 shows a linear plot of the plasma concentrations of 3-hydroxylidocaine following intramuscular administration of 3-hydroxylidocaine or 3-hydroxylidocaine polymer conjugate (Example 1)

20 Figure 6 shows a log/linear plot of the plasma concentrations of 3-hydroxylidocaine following intravenous administration of 3-hydroxylidocaine or 3-hydroxylidocaine polymer conjugate (Example 1)

Figure 7 shows a log/linear plot of the plasma concentrations of 3-hydroxylidocaine following intramuscular administration of 3-hydroxylidocaine or 3- 25 hydroxylidocaine polymer conjugate (Example 1)

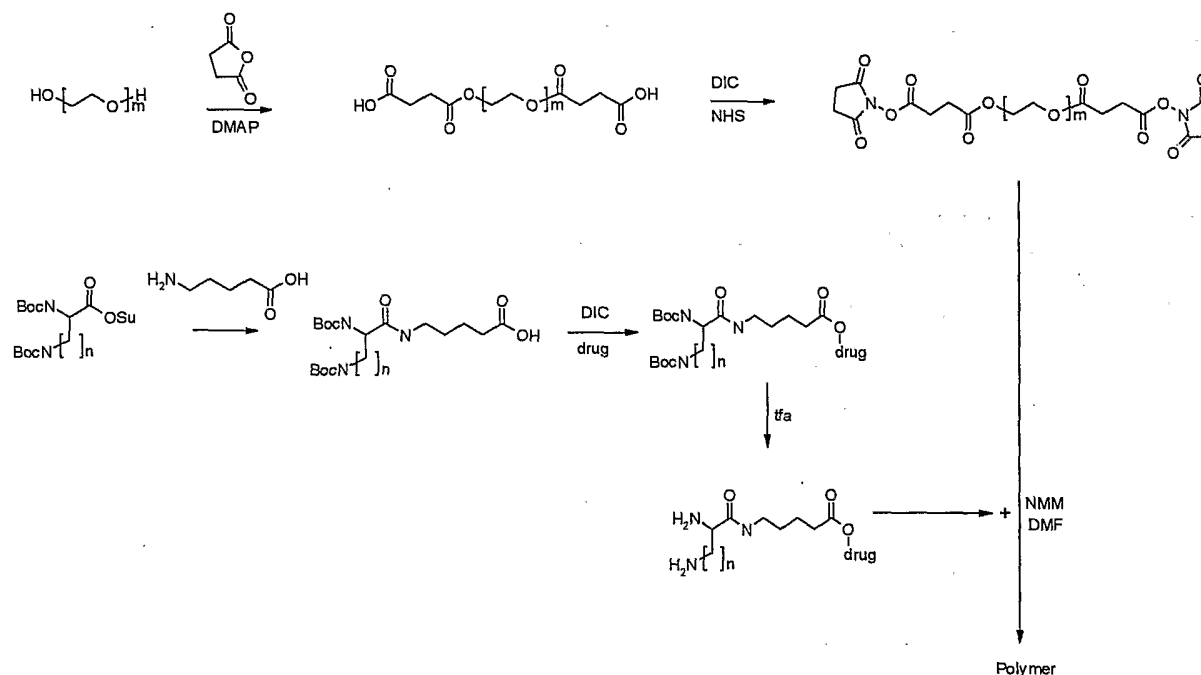
Figure 8 shows a linear plot of the plasma concentration of phenytoin with time following administration of phenytoin sodium or phenytoin polymer conjugate (Example 4)

Figure 9 shows a log/linear plot of the plasma concentration of phenytoin 30 with time following administration of phenytoin sodium or phenytoin polymer conjugate (Example 4)

Figure 10 shows a plot of percentage release of 3-hydroxylidocaine from 3-hydroxylidocaine polymer conjugate (Example 1) against time under two aqueous conditions.

Figure 11 shows photographs of the solutions of free phenytoin (labelled -) and phenytoin polymer conjugate (labelled +) which are described in Example 11.

The invention will now be illustrated by reference to the following Examples. Processes for the production of polymers according to the invention are illustrated 5 by reference to the following flow chart



10 Analytical methods

^{13}C NMR spectra were obtained on a Bruker Analytik GmbH Avance 500 machine. Spectra were run with samples dissolved in CDCl_3 .

The GPC method was as follows: Viscotek GMPWXL guard and two GMPWXL columns were used. The solvent system was [(10% acetonitrile 90% water) 0.1% formic acid] used at 0.8ml/min, with a run time of 35 min. The system was then allowed to re-equilibrate for 15 min. before the next run. The samples were made to 10mg/ml in water and 100uL was injected. The detector used was a Viscotek Triple detector, model 301 TDA and PEO 25.8k and 80k were used as standards to check the machine.

Example 1Conjugate of polymer with 3-hydroxylidocaine(a) Preparation of poly(ethylene glycol)-bis succinic acid

Polyethylene glycol (Mw = 1500, 119 g), succinic anhydride (24.0 g, 237.6 mmol, 3.0 eq) and DMAP (1.0 g, 8.20 mmol, 0.1 eq) were dissolved in DCM (300 mL). The solution was then heated to reflux and left at reflux for 48h. The precipitate that formed was then filtered off and the filtrate concentrated to give a white solid, the crude di-acid product. Purification via precipitation and reverse phase chromatography yielded the PEG di-acid compound.

10 (b) Preparation of N-hydroxysuccinimide activated bis (succinic acid) poly(ethylene glycol) ester

The bis (succinic acid) poly(ethylene glycol) ester (1.3 g), DIC (362 μ L, 3 eq), DMAP (1 mg, 0.01 eq) and N-hydroxysuccinimide (266 mg, 3.0 eq) were dissolved in dry DCM (10 mL) and stirred overnight. The reaction was then concentrated down and acetonitrile added. The solution was filtered, concentrated and precipitated to yield bis (succinic acid N-hydroxysuccinimide ester) poly(ethylene glycol) ester.

(c) Preparation of Boc-lysine(Boc)-5-aminovaleric acid

5-Aminovaleric acid monohydrochloride (693 mg, 4.5 mmol, 1.0 eq) was dissolved in water (15 mL) and MeCN (10 mL). Sodium carbonate (478 mg, 4.5 mmol, 1.0 eq) was added as a solution in water (5 mL), followed by Boc-Lys(Boc)OSu (2.0 g, 4.5 mmol, 1.0 eq) as a solution in MeCN (20 mL). The reaction was then left to stir overnight. The reaction mixture was concentrated and water added before being extracted with ethyl acetate (3 x 50 mL). The combined organic layers were then washed with 0.01N HCl (2 x 50 mL), brine, dried over MgSO₄ and concentrated. Purification by normal phase chromatography yielded the product (1.6g, 80%).

(d) Boc-lysine(Boc)-(5-aminovaleric acid) coupling to 3-hydroxy lidocaine

Boc-lysine(Boc)-5-aminovaleric acid (1.63 g, 3.66 mmol) was dissolved in dry DCM (16 mL). To this was added DIC (573 μ L, 3.66 mmol, 1.0 eq) and DMAP (58 mg, 0.48 mmol, 0.13 eq). The now cloudy solution was left to stir for 15 min. 3-Hydroxylidocaine lidocaine (1.01 g, 4.03 mmol, 1.1 eq) was added as a solution in DCM. This was then left to stir overnight. The solvent was evaporated, the residue dissolved in ethyl acetate and washed with water. The organic layer was then

washed with a weak base solution, dried and evaporated to dryness. Purification via reverse phase chromatography yielded the product (pale yellow solid, 1.85 g, 75%).

(e) Deprotection of BocLys(Boc)-(5-aminovalerate)-3-hydroxy-lidocaine ester

The BocLys(Boc)-(5-aminovalerate)-3-hydroxy-lidocaine ester (0.20 g, 0.29 mmol) was dissolved in TFA:water (95:5, 10 mL) and stirred at room temperature for 30 min. The solvents were evaporated, the residue taken up in water (15 mL) and freeze dried to give the deprotected material.

(f) Polymerisation of Lys-(5-aminovalerate)-3-hydroxy-lidocaine ester with bis (succinic acid N-hydroxysuccinimide ester) poly(ethylene glycol) ester

10 The Lys-(5-aminovalerate)-3-hydroxy-lidocaine ester (0.12 g, 0.15 mmol) and bis (succinic acid N-hydroxysuccinimide ester) poly(ethylene glycol) ester (0.28 g, 0.15 mmol) were dissolved in DMF (400 μ L) and treated with NMM (64 μ L, 0.58 mmol). The oil was left to stand overnight, then precipitated from ether. The resulting polymer was dried on high vac line for 30 min.

15 ^{13}C NMR and GPC analytical data on the product are shown in Figure 1.

Typical GPC data are as follows: M_n = 17.5k, M_w = 27.0k, M_z = 40.2k, M_p = 21.8k, P_d = 1.54, IV_w = 0.4127, dn/dc set to 0.147, calculated concentration was 10.38mg/ml. The small peak to the left of the main peak represents an impurity (N-methylmorpholine hydrochloride). This can be removed by dissolving the polymer in
20 toluene, filtering the polymer conjugate solution to remove the impurity, and then re-precipitating the polymer conjugate with ether.

Example 2

Conjugate of polymer with 4-hydroxy-N,N-diethylmexiletine

25 (a) Preparation of N,N-diethyl mexiletine

Mexiletine monohydrochloride (0.3g, 1.4 mmol) was dissolved in AcOH (18 mL) and the solution was heated at 55°C. Sodium borohydride (1.3g, 34 mmol) was added in small portions. The reaction was heated at 55°C for three days then cooled and poured into ice. The pH was adjusted to 12 and the aqueous layer was
30 extracted with DCM. Combined organic extracts were washed with brine, dried over MgSO_4 and concentrated. Purification by normal phase chromatography yielded the product (0.2g, 62%).

(b) Nitration of N,N-diethyl mexiletine

N,N-Diethyl mexiletine (0.2g, 0.85 mmol) was dissolved in a cooled solution of water (1 mL) and concentrated sulphuric acid (3.6 mL). The resulting solution was cooled to 0°C and a solution of 1M nitric acid (0.85 mL, 0.85 mmol) was added drop wise. The cooled reaction mixture was stirred for 1h then poured into ice. The pH was adjusted to 12 and the aqueous layer was extracted with DCM. Combined organic extracts were washed with brine, dried over MgSO₄ and concentrated. Purification by normal phase chromatography yielded the product (0.19g, 80%).

(c) Hydrogenation of nitrated N,N-diethyl mexiletine

10 5% Pd/C (20 mg) was suspended in EtOH (0.5 mL) under nitrogen. The nitro compound (0.17g, 0.63 mmol) prepared above was added as a solution in EtOH (1.5 mL). The resulting reaction mixture was flushed with hydrogen, stirred overnight then filtered through celite. The filtrate was concentrated then purified by normal phase chromatography to afford the product (0.11g, 70%).

15 (d) Preparation of 4-hydroxy-N,N-diethyl mexiletine

The aniline prepared above (95 mg, 0.38 mmol) was dissolved in a cooled solution of water (1.7 mL) and 2M sulphuric acid (0.5 mL). The resulting solution was cooled to 0°C and sodium nitrite (30mg, 0.42 mmol) was added slowly as a cooled solution in water (0.7 mL). The resulting solution was allowed to warm up to room temperature and stirred for three days. After cooling, the pH was adjusted to 7-8 and the aqueous layer was extracted with EtOAc. Combined organic extracts were washed with brine, dried over MgSO₄ and concentrated. Purification by normal phase chromatography yielded the product.

(e) Preparation of poly(ethylene glycol)-bis succinic acid

25 This was prepared as for Example 1 part (a).

(f) Preparation of N-hydroxysuccinimide activated bis (succinic acid) poly(ethylene glycol) ester

This was prepared as for Example 1 part (b).

(g) Preparation of Boc-lysine(Boc)-5-aminovaleric acid

30 This was prepared as for Example 1 part (c).

(h) Boc-lysine (Boc)-5-aminovaleric acid coupling to 4-hydroxy-N,N-diethyl mexiletine ester

Boc-Lys(Boc)-5-aminovaleric acid (3.66 mmol) was dissolved in dry DCM (16 mL). To this was added DIC (573 μ L, 3.66 mmol, 1.0 eq) and DMAP (58 mg, 0.48

mmol, 0.13 eq). The now cloudy solution was left to stir for 15 min. Hydroxy-N,N-diethyl mexiletine ester (4.03 mmol, 1.1 eq) was added as a solution in DCM. This was then left to stir overnight. The solvent was evaporated, the residue dissolved in ethyl acetate and washed with water. The organic layer was then washed with a weak base solution, dried and evaporated to dryness. Purification via reverse phase chromatography yielded the product (pale yellow solid, 75%).

10 (i) Deprotection of BocLys(Boc)-(5-aminovalerate)-4-hydroxy-N,N-diethyl mexiletine ester

The BocLys(Boc)-(5-amiovalerate)-hydroxy-N,N-diethyl mexiletine ester (0.29 mmol) was dissolved in TFA:water (95:5, 10 mL) and stirred at room temperature for 30 min. The solvents were evaporated, the residue taken up in water (15 mL) and freeze dried to give the deprotected material.

15 (j) Polymerisation of Lys-(5-aminovalerate)-4-hydroxy-N,N-diethyl mexiletine ester with bis (succinic acid N-hydroxysuccinimide ester) poly(ethylene glycol) ester

The Lys-(5-aminovalerate)-4-hydroxy-N,N-diethyl mexiletine ester (0.15 mmol) and bis (succinic acid N-hydroxysuccinimide ester) poly(ethylene glycol) ester (0.28g, 0.15 mmol) were dissolved in DMF (400 μ L) and treated with NMM (64 μ L, 20 0.58 mmol). The oil was left to stand overnight, then precipitated from ether. The resulting polymer was dried on high vac line for 30 min.

Example 3

Conjugate with quarternary 3-hydroxylicocaine ethyl iodide salt

25 (a) Preparation of poly(ethylene glycol)-bis succinic acid

This was prepared as for Example 1, part (a).

(b) Preparation of N-hydroxysuccinimide-activated bis(succinic acid) poly(ethylene glycol) ester

This was prepared as for Example 1, part (b).

30 (c) Preparation of CBZLys(CBZ)-5-aminovaleric acid

The CBZLys(CBZ)OH (1.50g, 3.62 mmol) was stirred in dioxane (60 mL), treated with NHS (0.42g, 3.63 mmol) and chilled in ice. DIC (0.57 mL, 3.62 mmol) was added and, after stirring for 2h, the solution was filtered. 5-Aminovaleric acid (0.47g, 4.04 mmol) was dissolved in dioxane (30 mL) and treated with sodium

carbonate (8mL, 1M), followed by the active ester solution. The solution was stirred overnight at room temperature, filtered and evaporated. The residue was taken up in citric acid (50 mL) and ethyl acetate (50 mL). The aqueous layer was further extracted with ethyl acetate (2 x 50 mL). The organic extracts were combined, washed with citric acid (50 mL), dried (MgSO₄) and evaporated to give an oil. The oil was purified by flash chromatography (SiO₂, 7:2:1, toluene:ethyl acetate: acetic acid).

(d) Preparation of Quaternary 3-Hydroxylidocaine ethyl iodide salt

10 3-Hydroxylidocaine (0.10g, 0.4 mmol) was dissolved in DMF (1 mL) and treated with ethyl iodide (96 μ L, 1.2 mmol). The solution was heated to 60°C overnight. The solvents were evaporated and the residue purified by reverse phase chromatography to give the quaternary salt.

(e) Coupling of quaternary 3-hydroxylidocaine to CBZLys(CBZ)-5-aminovaleric acid

15 The CBZLys(CBZ)-5-aminovaleric acid (0.23g, 0.45 mmol) was suspended in DCM and stirred at room temperature. The DIC (74 μ L, 0.47 mmol) and DMAP (6.4 mg, 0.05 mmol) were added and stirring continued for 10 min. The quaternary salt (0.18g, 0.45 mmol), dissolved in DCM:DMF (3 ml, 4:1), was added and stirring continued for 1 week. The solvent was evaporated to leave a crude mixture of both
20 un-reacted starting materials and the product.

(f) Polymerisation of Lys-(5-aminovalerate)-3-hydroxy-quaternary lidocaine ester with bis (succinic acid N-hydroxysuccinimide ester) poly(ethylene glycol) ester

The Lys-(5-aminovalerate)-3-hydroxy-quaternary lidocaine ester (0.15 mmol) and bis (succinic acid N-hydroxysuccinimide ester) poly(ethylene glycol) ester (0.15
25 mmol) were dissolved in DMF (400 μ L) and treated with NMM (64 μ L, 0.58 mmol). The oil was left to stand overnight, then precipitated from ether. The resulting polymer was dried in high vacuum line for 30 min.

Example 4

30 Conjugate with hydroxymethylphenytoin

(a) Preparation of poly(ethylene glycol)-bis succinic acid

This was prepared as for Example 1, step (a).

(b) Preparation of N-hydroxysuccinimide-activated bis (succinic acid) poly(ethylene glycol) ester

The is was prepared as for Example 1, step (b).

(c) Preparation of Boc-lysine(Boc)-5-aminovaleric acid

This was prepared as for Example 1, step (c).

(d) Preparation of N-2-(hydroxymethyl)-phenytoin

- 5 Sodium hydroxide (53mg, 1.31 mmol, 0.033 eq) and formaldehyde (37% soln, 10 mL) were dissolved in ethanol (20 mL) and left to stir for 1h or until the NaOH had dissolved. Phenytoin (10g, 39.64 mmol) was then added portion wise. The solution was then left to stir at room temperature for about 1h. Water was added and the reaction mixture filtered through a sintered funnel. The product was
10 air dried to leave a white powder (8.8g, 79%).

(e) N-2-(hydroxymethyl)-phenytoin coupling to Boc-lysine(Boc)-5-aminovaleric acid

- Boc-Lys-(Boc)-5-aminovaleric acid (5.48g, 12.30 mmol), 1.0 eq), DIC (1.93 mL, 12.30 mmol, 1.0 eq) and DMAP (150 mg, 1.23 mmol, 0.1 eq) were dissolved in *dry* dioxane (50 mL) and stirred for 15-20 min at room temperature. N-2-
15 (hydroxymethyl)-phenytoin was added to the now cloudy solution as a solution in *dry* dioxane. The reaction was then allowed to stir at room temperature overnight. The reaction mixture was evaporated to dryness and the residue partitioned between ethyl acetate and water. The organic layer was removed and the aqueous layer further extracted with ethyl acetate (2 x 60 mL). The combined organic extracts
20 were washed with 0.2M NaHCO₃ dried (MgSO₄) and evaporated to dryness. Crude material; pale clear yellow oil (foamed under high vacuum) 8.3g, 95%, TLC (60% EtOAc: PhMe, ninhydrin stain) 3 spots, R_f: 0.10 purple, 0.36 UV/brown (product), 0.63 faint UV. Purification was carried out via normal phase chromatography (50% EtOAc: PhMe to 60% EtOAc: PhMe) to give a white foam 5.4g, 61%.

25 (f) Deprotection of BocLys(Boc)-(5-aminovalerate)-N-(2)-(hydroxymethyl)-phenytoin

The BocLys(Boc)-(5-aminovalerate)-N-2-(hydroxymethyl)-phenytoin (0.5g) was dissolved in TFA (10 mL) and stirred at room temperature for 30 min. The solvents were evaporated, the residue taken up in water (15 mL) and freeze dried to give the deprotected material.

30 (g) Polymerisation of Lys-(5-aminovalerate)-N-2-(hydroxymethyl)-phenytoin ester with bis (succinic acid N-hydroxysuccinimide ester) poly(ethylene glycol) ester

The Lys-linker-N-2-(hydroxymethyl)-phenytoin (0.15 mmol) and bis (succinic acid N-hydroxysuccinimide ester) poly(ethylene glycol) ester (0.15 mmol) were dissolved in DMF (400 µL) and treated with NMM (64 µL, 0.58 mmol). The oil was

left to stand overnight, then precipitated from ether. The resulting polymer was dried on high vac line for 30 min.

A typical ^{13}C NMR trace of the product is shown in Figure 2.

5 Example 5

Conjugate with 3-hydroxylidocaine and wheatgerm antigen (WGA)

(a) Preparation of poly(ethylene glycol)-bis succinic acid

This was prepared as for Example 1, part (a).

(b) Preparation of N-hydroxysuccinimide activated bis (succinic acid) poly(ethylene

glycol) ester

This was prepared as for Example 1, part (b).

(c) Preparation of Boc-lysine(Boc)-5-aminovaleric acid

This was prepared as for Example 1, part (c).

(d) Boc-lysine (Boc)-5-aminovaleric acid coupling to 3-hydroxy lidocaine

15 Boc-lysine (Boc)-5-aminovaleric acid (1.63g, 3.66 mmol) was dissolved in dry DCM (16 mL). To this was added DIC (573 μL , 3.66 mmol, 1.0 eq) and DMAP (58 mg, 0.48 mmol, 0.13 eq). The now cloudy solution was left to stir for 15 min. 3-Hydroxylidocaine lidocaine (1.01g, 4.03 mmol, 1.1 eq) was added as a solution in DCM. This was then left to stir overnight. The solvent was evaporated, the residue
20 dissolved in ethyl acetate and washed with water. The organic layer was then washed with a weak base solution, dried and evaporated to dryness. Purification via reverse phase chromatography yielded the product (pale yellow solid, 1.85g, 75%).

(e) Deprotection of BocLys(Boc) -(5-aminovalerate)-3-hydroxy-lidocaine ester

The BocLys(Boc)-(5-aminovalerate)-3-hydroxy-lidocaine ester (0.20g, 0.29
25 mmol) was dissolved in TFA:water (95:5, 10 mL) and stirred at room temperature for 30 min. The solvents were evaporated, the residue taken up in water (15 mL) and freeze dried to give the deprotected material.

(f) Polymerisation of Lys-(5-aminovalerate)-3-hydroxy-lidocaine ester with bis (succinic acid N-hydroxysuccinimide ester) poly(ethylene glycol) ester

30 The Lys-linker-3-hydroxy-lidocaine ester (0.15 mmol) and bis (succinic acid N-hydroxysuccinimide ester) poly(ethylene glycol) ester (0.165 mmol) were dissolved in DMF (400 μL) and treated with NMM (64 μL , 0.58 mmol). The oil was left to stand overnight, then precipitated from ether. The resulting polymer was dried on high vac line for 30 min.

(g) Coupling of Wheat Germ (WGA) to PEG-3-hydroxy-lidocaine ester polymer

WGA (45mg, 0.92 eq) was dissolved in water (3mL) and the pH adjusted to 7.6 with 0.1N NaOH. This was then added to a flask containing the PEG-lidocaine polymer (product of step (f)) (75mg, 1.0 eq). The solution was allowed to dissolve before being freeze-dried overnight. Water (7 mL) was added followed by lysine hydrochloride (5mg) to quench excess NHS active ester present. After being swirled for a few min the solution was then freeze-dried again.

Example 6

10 For syntheses described herein it may be advantageous to employ a cross-linking agent,

Example of the synthesis of a crosslinking agent

Preparation of Boc-Lys(Boc)-CONH-butyl-NHBoc

N-Boc-1,4-diaminobutane monohydrochloride (430 mg, 2.25 mmol, 1.0 eq) was dissolved in water (15 mL) and MeCN (10mL). Sodium carbonate (120 mg, 1.12 mmol, 0.5 eq) was added as a solution in water (5 mL), followed by Boc-Lys(Boc)OSu (1.0 g, 2.25 mmol, 1.0 eq) as a solution in MeCN (20 mL). The reaction was then left to stir overnight. The reaction mixture was concentrated and water added before being extracted with DCM (3 x 50 mL). The combined organic layers were then washed with 0.01N HCl (2 x 50 mL), brine, dried over MgSO₄ and concentrated. Purification by normal phase chromatography yielded the product (white foam, 1.0 g, 86%).

Deprotection of Boc-Lys(Boc)-butyl-NHBoc

Method as for deprotection of BOC-Lys(BOC)-(5-aminovalerate)-3-hydroxy-lidocaine ester.

Biological Data

Example 7

Test of paw withdrawal latency for 3-hydroxylidocaine polymer conjugate

30 *Subjects and Surgical Procedures*

Male Harlan Sprague-Dawley rats were used (150-250g). Rats were housed 4-5 per cage and provided with food and water ad libitum with a 12hr/ 12hr day/night cycle.

To induce neuropathy, rats were anaesthetised with halothane/oxygen mix and the common sciatic nerve was exposed at the level of the mid thigh through the biceps femoris in a method similar to that reported (Bennett & Xie, Pain (1988) 33:87-107). The sciatic nerve was freed of connective/adhering tissue and loosely
5 ligated three times with 4-0 G chromic cat gut suture approximately 1mm apart. The wound is then sutured. The chronic constriction injury (CCI) model in rats is associated with hyperalgesia, allodynia and spontaneous pain and constitutes a model for peripheral neuropathic pain in humans.

10

Behavioural Observations

Animals were tested pre-operatively for baseline paw withdrawal latency (PWL) responses to thermal stimulation using a Ugo Basile Hargreaves apparatus (Hargreaves, et al., (1988) Pain 32:77-88) which consisted of 3 separated holding
15 boxes, each approximately 25 cm by 20 cm placed over a glass floor elevated to accommodate a mobile infrared radiant heat source underneath. Animals were given 10 minutes to acclimatise and explore their testing environment before the radiant heat source was aimed at the plantar surface of the hind paw. On appreciation of a noxious stimulation the animal withdraw the hind paw which interrupts the reflected
20 light to the photocell and stops the timer. Three latency measurements were recorded on each hind paw. Following chronic constriction injury, as outlined above, animals evolved hyperalgesic state on the injured side (left) over 5-7 days at which point the separation of paw withdrawal latency between ipsilateral ("ips") and contralateral ("con") sides stabilised. Test materials were administered once the
25 PWL on the injured side stabilised.

The antihyperalgesic effect of free 3OH-lidocaine given via the intraperitoneal route was evaluated in the CCI model during chronic dosing over 4 consecutive days. The test material was made up to a stock concentration of 4.5 mg/ml in distilled water. On the first treatment day the animals were administered an
30 equivalent of 15 mg/kg and thereafter received 7.5 mg/kg on the subsequent 3 days treatment. Likewise, gabapentin was administered chronically over 4 days by the intraperitoneal route at a dose of 50 mg/kg per day. Testing of thermal hyperalgesia was performed approximately 45 minutes post dose. 3OH-lidocaine in a polymer according to the present invention (as per Example 1) was given as a single dose

intramuscularly at 120 mg/kg. This dose of polymer delivers an equivalent of 20 mg/kg of 3-hydroxylidocaine.

Administration of gabapentin on day 1 of treatment produced an increase in PWL indicating a suppression of the thermal hyperalgesic state on the injured paw (Figure 3). No corresponding effect was seen in the non-injured PWL response. The measured antihyperalgesic effect on subsequent treatment days was less pronounced. Free 3-hydroxylidocaine produced a consistent antihyperalgesic effect throughout treatment which was lost on cessation of treatment. Treatment with a single dose of polymer 3-hydroxylidocaine provided an antihyperalgesic effect similar to that produced by 15 mg/kg free 3-hydroxylidocaine i.p. on day 1 of treatment and a sustained antihyperalgesic effect for the next 4 days.

Data showing a comparison of the effect on paw withdrawal latency of the 3-hydroxylidocaine polymer conjugate of the present invention (Example 1) when measured on the isp and con sides with free 3-hydroxylidocaine and saline control are shown in Figure 3.

Example 8

Pharmacological study of 3-hydroxylidocaine polymer conjugate

Male Sprague-Dawley rats, supplied by Charles River UK Ltd, were used for pharmacodynamic studies. On the day of study the animals were in the weight range 254-283g and approximately 8 weeks old.

The test materials were dissolved in distilled water at the highest concentration required. These were 12.5 mg/ml for 3-hydroxylidocaine and 60 mg/ml for a polymer conjugate of the present invention containing 3-hydroxylidocaine (as per Example 1). For the free drug, the hydrogen chloride salt of 3-hydroxylidocaine was used and allowance was made for this in preparation of doses.

These stocks were used as prepared for intramuscular administration. For intravenous administration appropriate dilutions were made using distilled water to give solutions of 1 and 10 mg/ml for 3-hydroxylidocaine and the polymer conjugate, respectively.

Four groups of three male rats were dosed as indicated below:

Group No.	Treatment	Dose (mg/kg)	Route

1	3-hydroxylidocaine	2	i.v.
2	3-hydroxylidocaine	25	i.m.
3	Polymer conjugate	20	i.v.
4	Polymer conjugate	120	i.m.

The doses were administered as a single bolus dose at a constant dose volume of 2 mL/kg into a tail vein not used for blood sampling or (for i.m.) in the rear leg muscle. Doses of 3-hydroxylidocaine are expressed as free base.

- 5 Following dosing, serial blood samples (approximately 0.3ml) were obtained from each rat via a cannula which had been previously inserted into a lateral tail vein not used for dosing. Blood samples were taken into individual heparinised containers at the time points shown.

Following collection, blood samples were centrifuged and plasma retained
10 and stored at -20°C for bioanalysis of plasma 3-hydroxylidocaine using an LC-MS/MS methodology.

Liquid chromatography was carried out using a C8, 5micrometer, 30 x 4.6 mm analytical column. Mobile phase consisted of 10 mM ammonium acetate and methanol. The MS/MS conditions were; Ionisation mode, electrospray in +ve ion
15 mode; m/z 251.3 → m/z 86.3 (collision energy 27 eV). No unusual clinical signs were observed in any of the intravenously or intramuscularly dosed animals.

Linear plots of the plasma concentrations of 3-hydroxylidocaine following intravenous administration and intramuscular administration of 3-hydroxylidocaine or 3-hydroxylidocaine polymer conjugate are shown in Figures 4 and 5 respectively.
20 Log/Linear plots of the plasma concentrations of 3-hydroxylidocaine following intravenous administration and intramuscular administration of 3-hydroxylidocaine or 3-hydroxylidocaine polymer conjugate are shown in Figures 6 and 7 respectively. 3-OH is the code for the free 3-hydroxylidocaine HCl salt sample and SGX355 is the code for the 3-hydroxylidocaine polymer conjugate (Example 1) sample.

- 25 Following intravenous administration of 3-hydroxylidocaine the mean maximum plasma concentrations at 5 minutes post-dose was 892 ng/mL. The plasma concentrations then declined rapidly in a mono exponential fashion with no 3-hydroxylidocaine being detected in the plasma after 60 minutes post-dose. The calculated half life for this elimination was 0.2 hours.

In the animals dosed intramuscularly with 3-hydroxylidocaine C_{max} was seen at 30 minutes post-dose (mean value 2110 ng/mL). Plasma concentrations of 3-hydroxylidocaine again declined in a monoexponential fashion with no 3-hydroxylidocaine being detected in the plasma after 4 hours post-dose. The
5 calculated half life for this elimination was 0.6 hours.

Following intravenous administration of polymer the maximum plasma concentrations of 3-hydroxylidocaine were seen at 5 minutes post-dose (mean value 3322 ng/mL). The plasma concentrations then declined in a mono exponential fashion with low levels of 3-hydroxylidocaine being detected in the plasma at 6
10 hours but none at 12 hours post-dose.

In the rats treated intramuscularly with polymer conjugate the maximum concentrations of 3-hydroxylidocaine were seen at 2 hours post-dose (mean value 701 ng/mL). Plasma concentrations of 3-hydroxylidocaine again declined with low levels of 3-hydroxylidocaine being detected in the plasma at 12 hours post-dose but
15 none at 24 hours. The calculated half life for this elimination was 2.9 hours.

These data show that administration of the polymer conjugate increases the elimination half-life of 3-hydroxylidocaine compared with that seen following administration of the free base. As a result, 3-hydroxylidocaine was present in the plasma for a much longer period than seen following administration of the free base,
20 6 hours as opposed to 60 minutes and 12 as opposed to 4 hours in the intravenously and intramuscularly dosed animals, respectively.

Example 9

Pharmokinetic study on phenytoin polymer conjugate

25 A pharmacokinetic study was designed to compare plasma concentration versus time data for phenytoin following single oral dose of phenytoin as a polymer conjugate of the present invention with that of phenytoin sodium free drug given by the oral route.

12 rats (HSdBrHan:WI strain; male) were divided into two cohorts (n=6).
30 Each animal in Group 1 received a single oral dose of phenytoin polymer conjugate according to the present invention (as per Example 4), whilst animals of Group 2 received a single oral dose of phenytoin sodium. The animals were fasted for 14 hours prior to dosing. Doses were administered using a rubber catheter and

disposable syringe (oral gavage). The dose volume was 10ml/kg, adjusted according to bodyweight recorded on day of dosing.

Both the phenytoin polymer conjugate and phenytoin sodium were dissolved in distilled water and appeared as clear solutions. Each animal received an 5 equivalent phenytoin dose of 20 mg/kg, which equated to 181.8 mg/kg and 21.8 mg/kg of phenytoin polymer conjugate and free phenytoin sodium, respectively.

Blood samples were obtained at the following time points relative to dosing:

0, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after dosing

Three animals from each group were sampled alternately at each of the time 10 points detailed above. Approximately 0.25ml blood samples were obtained from the lateral tail vein of each animal and placed into tubes containing lithium heparin anticoagulant. Blood samples were maintained on ice before plasma was separated by centrifugation (1500g; 10 mins). The plasma was then frozen awaiting 15 liquid extraction procedure using 0.5% acetic acid (final). Reverse phase chromatography was carried out using a mobile phase of 0.1% formic acid in water: 0.1% formic acid in acetonitrile (25:75, v/v). The analyte was ionized using the TubolonSpray™ interface operating in positive mode. Methetoin was used as internal standard. The characteristic ion dissociation transitions m/z 253 $\rightarrow$ 182 20 (phenytoin) and m/z 219 $\rightarrow$ 159 (methetoin) were monitored via tandem MS.

The plasma concentration with time is shown in linear plot and and log/linear plot in Figures 8 and 9 respectively.

This study demonstrated that it is feasible to deliver phenytoin systemically using the polymer technology according to the invention via the oral route. 25 Administration of the polymer conjugate resulted in peak plasma levels of 164ng/ml at 1 h with concentrations in excess of 100ng/ml sustained for 12 h compared with a peak concentration of 1880 ng/ml for free phenytoin at 1 h which had declined rapidly to 40 ng/ml at 12 h. Plasma concentrations with time appear to be zero order over 12 h using polymer delivery.

30

Example 10

Drug release from 3-hydroxylidocaine polymer conjugate

A polymer conjugate according to the present invention, wherein the polymer contained 3-hydroxylidocaine (as per Example 1), was used to examine stability in

various solutions. The polymer remains stable as a lyophilised powder. A plot of percentage release of 3-hydroxylidocaine from 3-hydroxylidocaine polymer conjugate against time is shown in Figure 10. When dissolved in distilled water or phosphate buffered saline solution (PBS), pH7.5, at 40 µg/ml concentration, less than 2% 3-hydroxylidocaine was released into the solution after 48 hours, with approximately 5% drug released after 6 days.

In a basic solution (Tris buffer, pH 9.5), 20% drug was released after 15 hours, 35% after 40 hours and 60% after 6 days. In plasma serum, all 3-hydroxylidocaine was released from polymer after approximately 24 h. Pre-heat treated plasma gave rise to a significantly reduced rate of release of drug from polymer. Thus, breakdown of polymer to produce free drug is catalysed by both enzymatic and chemical processes.

It was observed that in plasma obtained from animals that had been dosed intramuscularly with 3-hydroxylidocaine polymer the 3-hydroxylidocaine measured was dependent on downstream plasma handling. There appeared a discrepancy in the measured compound in those plasmas frozen immediately following in vivo sampling and analysed and those left at room temperature for, as example, >3 hours prior to bioanalysis. Moreover, the 3-hydroxylidocaine measured in samples left to incubate at room temperature was significantly greater than those maintained frozen. This observation would strongly suggest that plasma obtained from an intramuscularly dosed animal contained a mobile depot of polymer-bound 3-hydroxylidocaine as well as circulating free 3-hydroxylidocaine. Approximately 15% of the total 3-hydroxylidocaine present in the plasma is free during the first 6 hours post dose.

25

Example 11

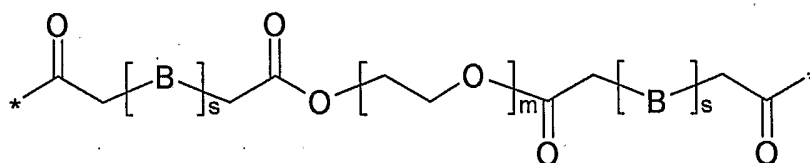
Aqueous solubility of phenytoin conjugate

Samples of free phenytoin (5mg) and phenytoin polymer conjugate (Example 4) (10mg) were dissolved in 0.5ml water. The samples were adjusted to pH2, pH7 and pH12.

In Figure 11, it can be seen that the polymeric bound material is clearly soluble across the pH range and that the free compound is insoluble below pH12.

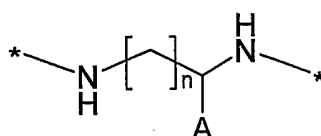
Claims

1. A polymer comprising units of formulae (I) and (II):



(I)

5 and



(II)

wherein B is selected from oxygen, sulphur, alkyl, alkyl ether, alkyl thioether, hydroxyalkyl and alkyl aryl ;

each s independently represents 0 or an integer of 1 to 100;

10 m is 0 or an integer of 1 to 1000;

n is 0 or an integer of 1 to 100; and

A is a functional group optionally conjugated to a further component.

2. A polymer according to claim 1 which contains at least 5 units of each monomer.

15 3. A polymer according to claim 1 or claim 2 which contains up to 10,000 units of each monomer.

4. A polymer according to any one of claims 1 to 3 wherein s is 0.

5. A polymer according to any one of claims 1 to 4 wherein m is an integer of 20-100.

20 6. A polymer according to any one of claims 1 to 5 wherein n is an integer of 1 to 10.

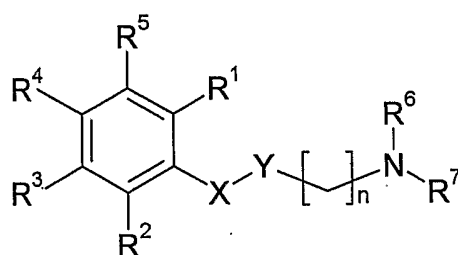
7. A polymer according to claim 6 wherein n is 4.

8. A polymer according to any one of the preceding claims wherein A incorporates a moiety selected from carboxy, amino, amido, thio and hydroxyl

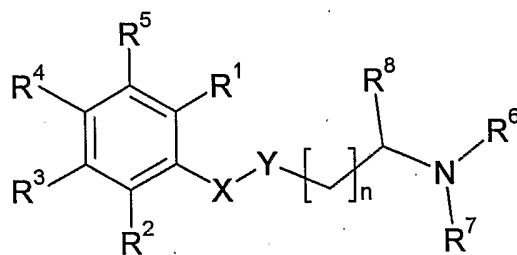
25 groups as a point of attachment for further components.

9. A polymer according to any one of the preceding claims wherein A includes a targeting agent in at least one subunit.

10. A polymer according to claim 9 wherein the targeting agent is connected to the polymer by means of a linker.
11. A polymer according to any one of the preceding claims wherein A includes Z, i.e. a drug or pro-drug, in at least one subunit.
- 5 12. A polymer according to claim 11 wherein Z is a compound of formula:

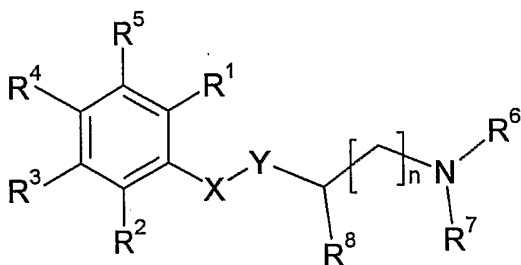


(III)

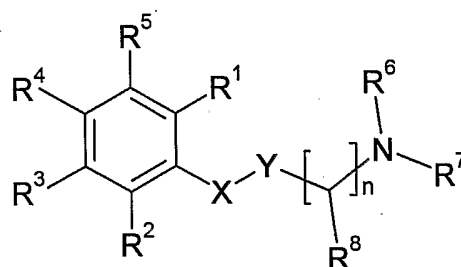


(IV)

10



(V)



(VI)

15 wherein:

R^1 and R^2 are independently selected from hydrogen, halogen, alkyl and alkyl ether groups;

X is C=O and Y is NR, or X is NR and Y is C=O, or X is C=O and Y is O, and R is selected from hydrogen, halogen, hydroxyl, alkyl, aryl and acyl;

20 R^6 and R^7 are independently selected from alkyl, aryl and alkylaryl groups; and

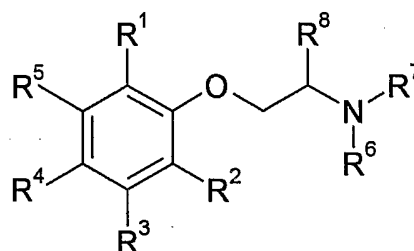
R^8 is selected from hydrogen, halogen, hydroxyl, alkyl, aryl ; or

R^7 and R^8 may be joined, typically through a chain of carbon atoms and, optionally, heteroatoms, to form a ring 5, 6, 7 or 8 atoms in size;

n is 0, 1, 2, 3, 4 or 5; and

R^3 , R^4 and R^5 are each independently selected from hydrogen, hydroxyl, halogen, alkyl, aryl, hydroxyalkyl, hydroxyaryl, aminoalkyl and aminoaryl, with the proviso that at least one of R^3 , R^4 and R^5 is a hydroxyl moiety connected to the polymer through a covalent bond.

13. A polymer according to claim 11 wherein Z is a compound of formula:



wherein:

10 R^1 and R^2 are independently selected from hydrogen, halogen, alkyl and alkyl ether groups;

R^6 and R^7 are independently selected from hydrogen, hydroxyl, alkyl, aryl and alkylaryl groups; and

R^8 is selected from hydrogen, halogen, hydroxyl, alkyl, aryl and alkylaryl;

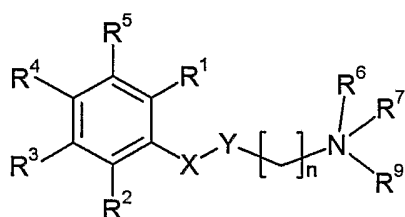
15 or

R^7 and R^8 may be joined, typically through a carbon chain, to form a ring 5, 6, 7 or 8 atoms in size, which may contain heteroatoms; and

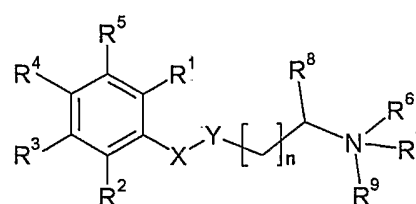
R^3 , R^4 and R^5 are each independently selected from hydrogen, hydroxyl, halogen, alkyl, aryl, hydroxyalkyl, hydroxyaryl, aminoalkyl or aminoaryl, with the proviso that at least one of R^3 , R^4 and R^5 is hydroxyl through which it is linked by a covalent bond to the polymer.

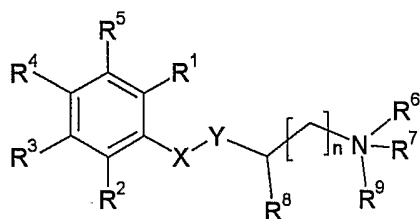
14. A polymer according to claim 11 wherein Z is a compound of formula:

(VII)

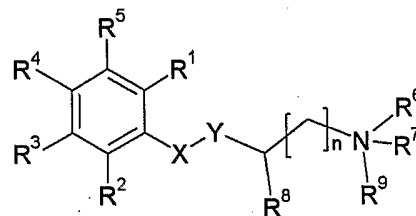


(VIII)





(IX)



(X)

in which each quaternary nitrogen atom bears a positive charge and is accompanied by $X^{\ominus}$ as a counter-anion;

- 5 R^1 and R^2 are independently selected from hydrogen, halogen, alkyl and alkyl ether groups;

X is $C=O$ and Y is NR , or X is NR and Y is $C=O$, or X is $C=O$ and Y is O , and R is selected from hydrogen, halogen, hydroxyl, alkyl, aryl and acyl;

in addition, X may represent O and Y may represent CH_2 ;

- 10 R^6 and R^7 are independently selected from alkyl, aryl and alkylaryl groups; and

R^8 is selected from hydrogen, halogen, hydroxyl, alkyl, aryl ; or

R^7 and R^8 may be joined, typically through a chain of carbon atoms and, optionally, heteroatoms, to form a ring 5, 6, 7 or 8 atoms in size;

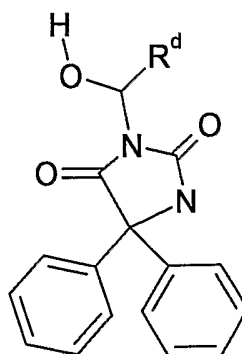
- 15 R^9 represents alkyl, aryl or alkylaryl;

n is 0, 1, 2, 3, 4 or 5; and

R^3 , R^4 and R^5 are each independently selected from hydrogen, hydroxyl, halogen, alkyl, aryl, hydroxyalkyl, hydroxyaryl, aminoalkyl and aminoaryl, with the proviso that at least one of R^3 , R^4 and R^5 is a hydroxyl moiety through which the

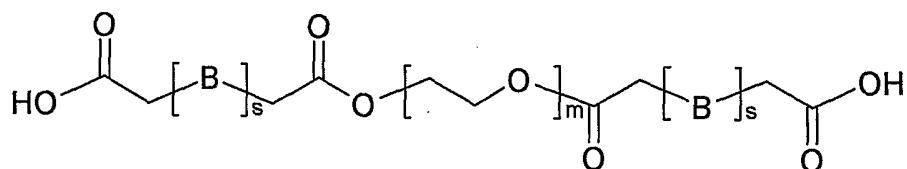
- 20 compound is connected to the polymer through a covalent bond.

15. A polymer according to claim 11 wherein Z is a compound of formula

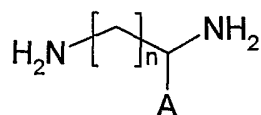


in which R^d represents hydrogen, alkyl, aryl and alkylaryl and is linked to the polymer by a covalent bond through the hydroxyl group.

16. A polymer according to claim 11 wherein Z is phenytoin or a pro-drug thereof.
17. A polymer according to claim 11 wherein Z is an anaesthetic substance.
18. A polymer according to any one of claims 11 to 17 wherein Z is connected to the polymer by means of a linker.
19. A process for preparing a polymer according to any one of the preceding claims which comprises co-polymerising one or more first monomers (I'):



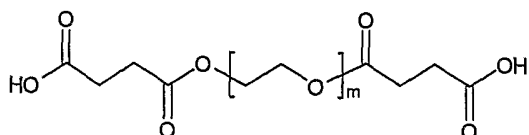
- or an analogue derived from a branched PEG,
- 15 or an activated derivative thereof;
- with one or more second monomers (II'):



wherein A, B, m, n and s are as defined in any one of the preceding claims.

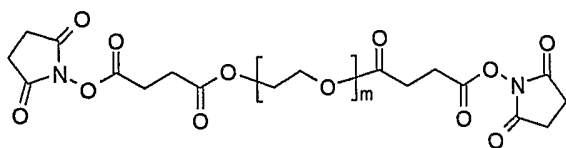
20. A process according to claim 19 wherein the one or more first monomers (I') is a compound of formula:

20

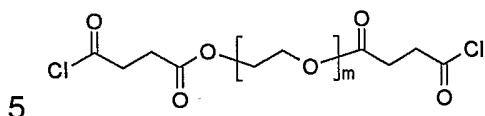


- 25 or an analogue derived from a branched PEG,
- or an activated derivative thereof.

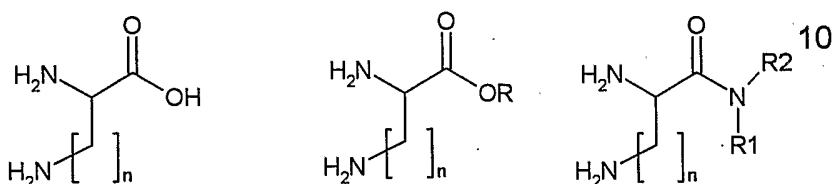
21. A process according to claim 20 wherein the one or more first monomers (I') is selected from



and



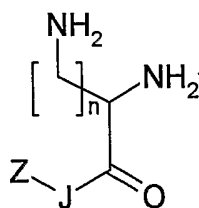
22. A process according to any one of claims 19 to 21 wherein the one or more second monomers (II') is selected from



15 wherein n is an integer of 1 to 10, and

R, R¹ and R² are selected from hydrogen, alkyl, aryl, alkyl ether, amino acid, peptide, linker and therapeutic agent.

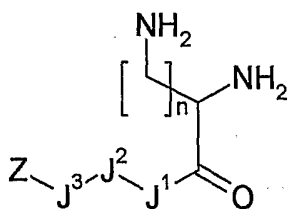
23. A process according to any one of claims 19 to 22 wherein a second monomer (II') is a compound of formula:



20

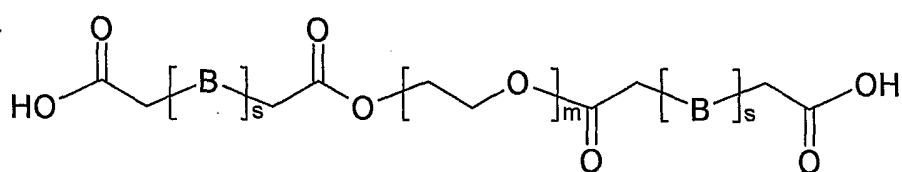
wherein J represents a bond or a linker and Z represents a therapeutic agent or a pro-drug or a protected derivative thereof.

24. A process according to claim 23 wherein a second monomer (II') is a compound of formula



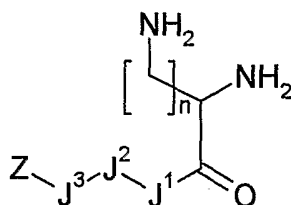
wherein J^1 - J^2 - J^3 defines a linker group.

25. A process according to claim 19 which comprises co-polymerising one or more first monomers (I'):

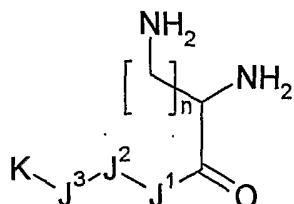


5

or an analogue derived from a branched PEG,
or an activated derivative thereof,
with a mixture of one or more monomers (II''):



10 wherein J^1 - J^2 - J^3 defines a linker group and Z represents a therapeutic agent or a pro-drug or a protected derivative thereof
and one or more second monomers (II''')



wherein J^1 - J^2 - J^3 defines a linker group,

and K represents a polymer property modifying agent or a protected derivative thereof.

26. A process according to claim 25 wherein K represents a targeting agent.

27. A process according to any one of claims 24 to 26 wherein J¹ represents
5 sulphur, oxygen or an amino group.

28. A process according to claim 27 wherein J¹ is an amino group.

29. A process according to any one of claims 24 to 28 wherein J² represents a spacer group.

30. A process according to claim 29 wherein J² represents an alkylene group.

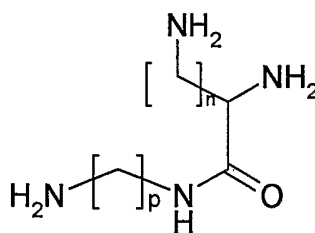
10 31. A process according to any one of claims 24 to 30 wherein J³ represents a carbonyl group.

32. A process according to any one of claims 24 to 31 wherein Z is connected to J³ by means of a free amino or hydroxyl group.

33. A process according to claim 32 wherein Z is connected to J³ by means of a
15 free hydroxyl group.

34. A process according to any one of claims 19 to 33 wherein the polymerisation involves use of a cross-linking di-amine monomer.

35. A process according to claim 34 wherein the cross-linking di-amine monomer is a monomer of formula:



20

wherein p represents 1 to 10.

36. A polymer obtainable by a process according to any one of claims 19 to 35.

37. A polymer obtained by the process of any one of claims 19 to 35.

25 38. A polymer according to any one of claims 1 to 18, 36 and 37 wherein one or both ends of the polymer are capped.

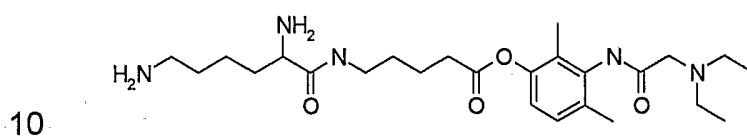
39. A polymer according to claim 38 wherein one or both ends of the polymer are capped with a polymer property-modifying agent.

40. A polymer according to claim 37 wherein the polymer property-modifying agent is a targeting agent.

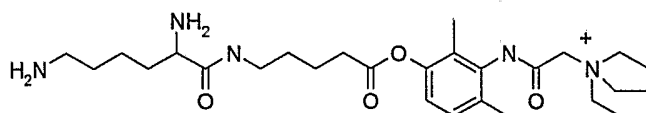
41. A pharmaceutical composition comprising a polymer according to any one of claims 11 to 18 and 36 to 41 together with a pharmaceutically acceptable diluent or carrier.

42. A polymer according to any one of claims 11 to 18 and 36 to 40 for use in therapy.

43. A compound of formula

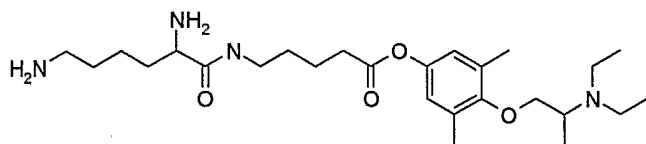


44. A compound of formula

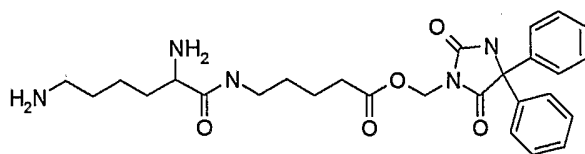


accompanied by a counterion.

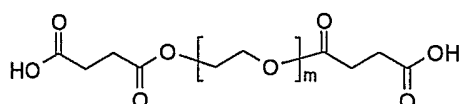
15 45. A compound of formula



46. A compound of formula

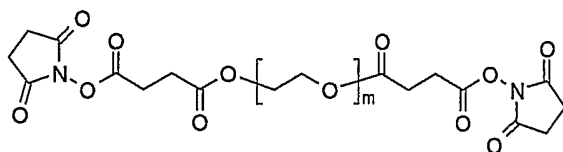


20 47. A compound of formula



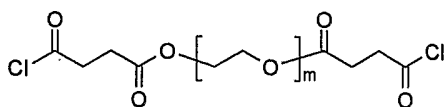
wherein m represents 20 to 100;
or an activated derivative thereof.

48. A compound according to claim 47 of formula



wherein m represents 20 to 100.

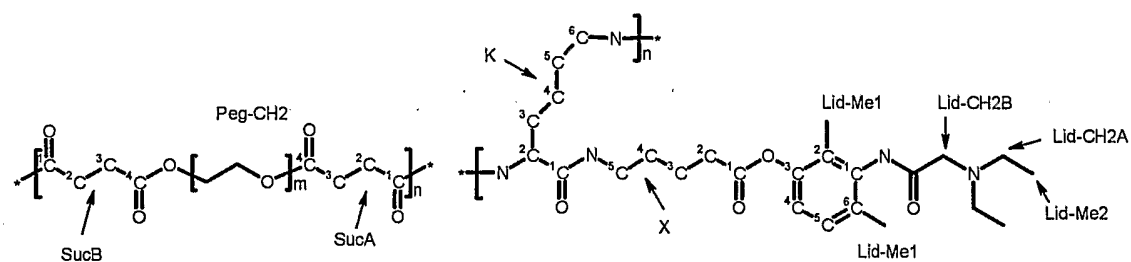
5 49. A compound according to claim 48 of formula



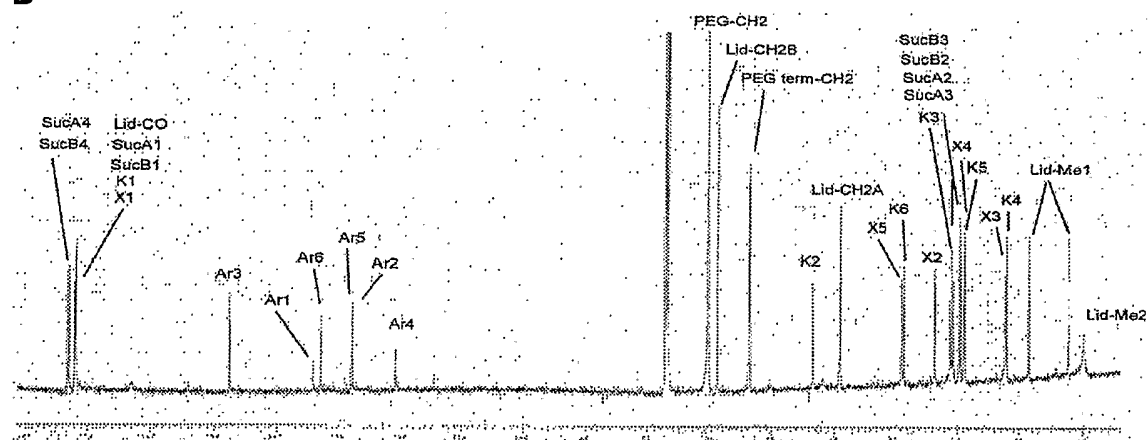
wherein m represents 20 to 100.

Figure 1

A



B



C

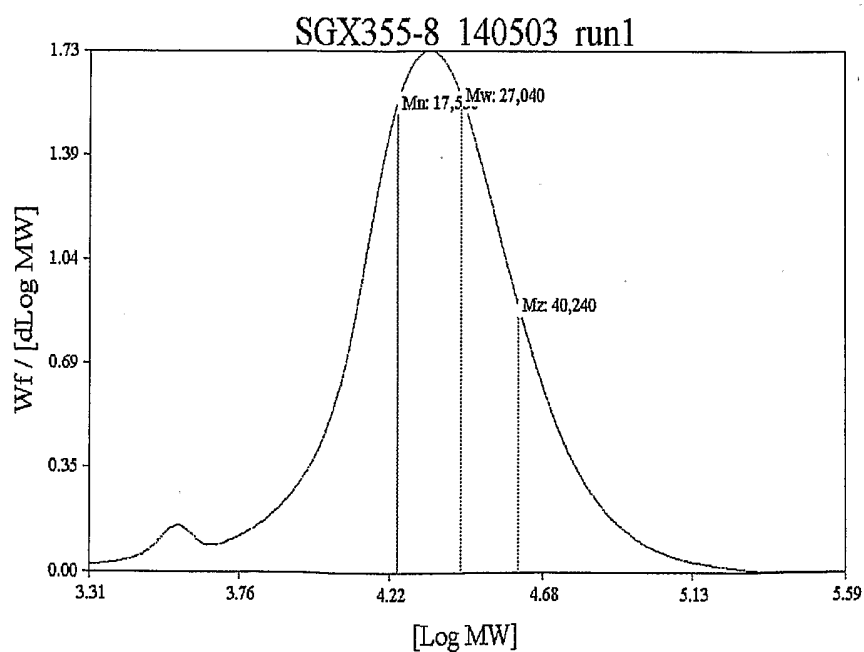


Figure 2

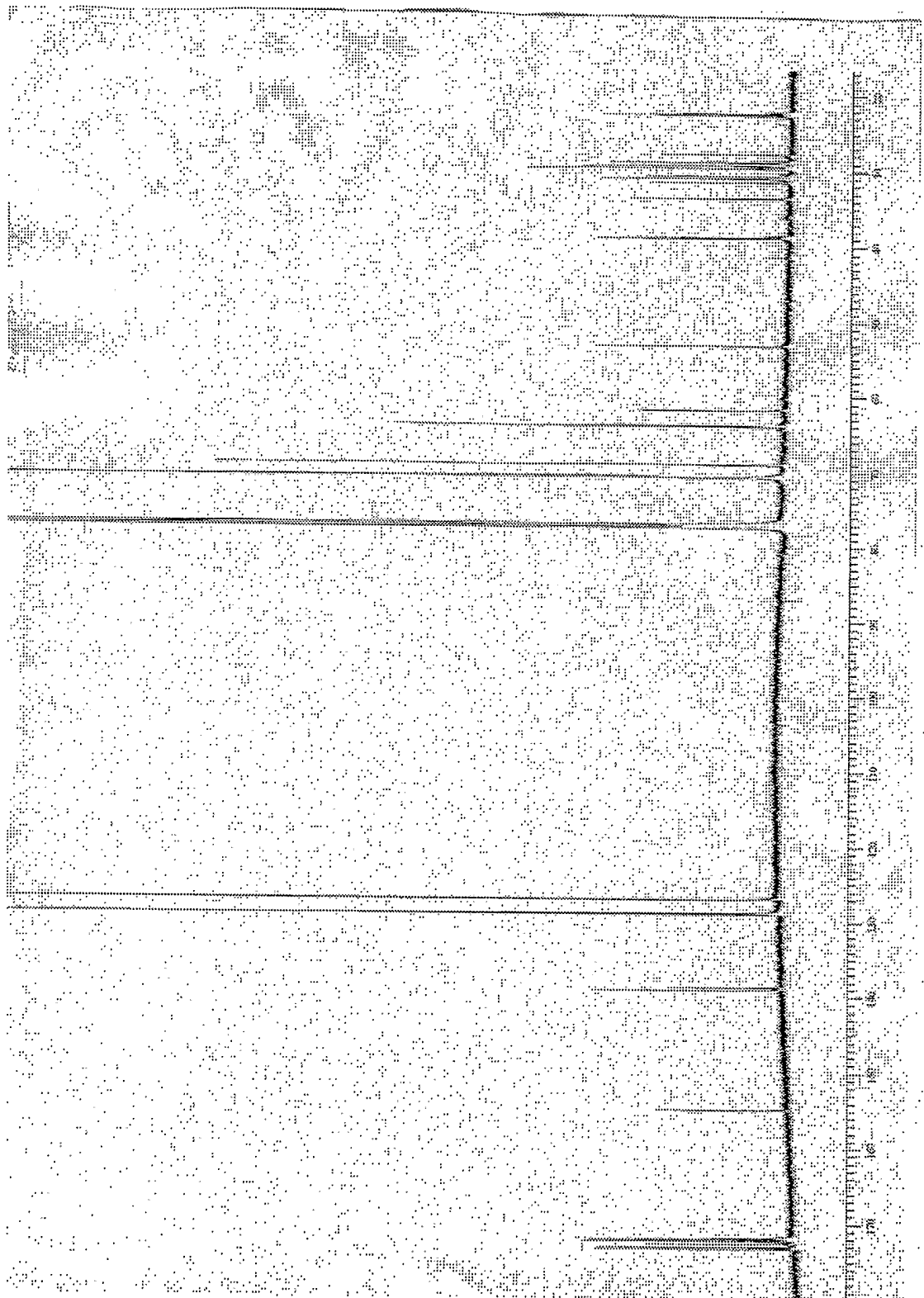


Figure 3

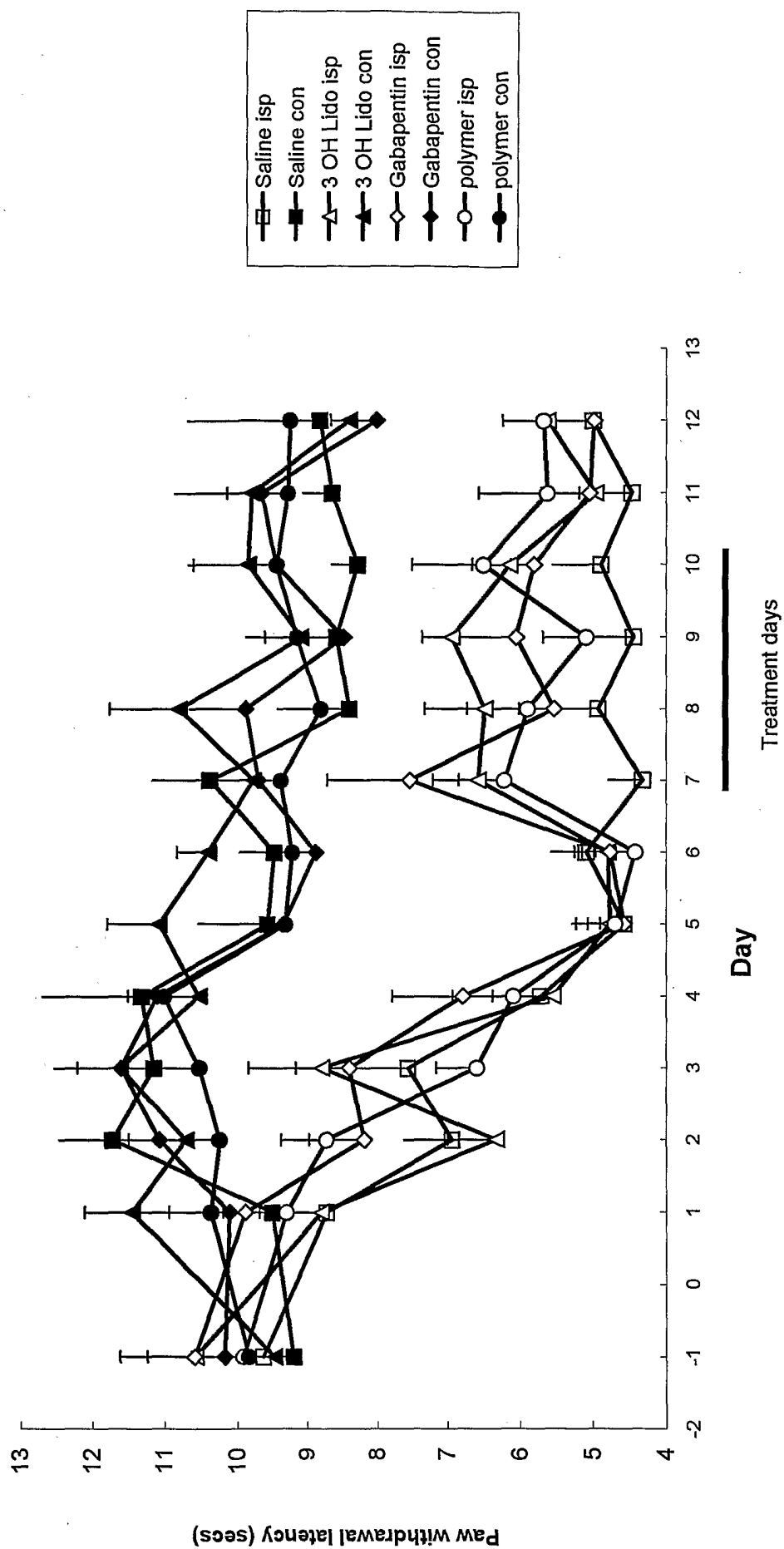
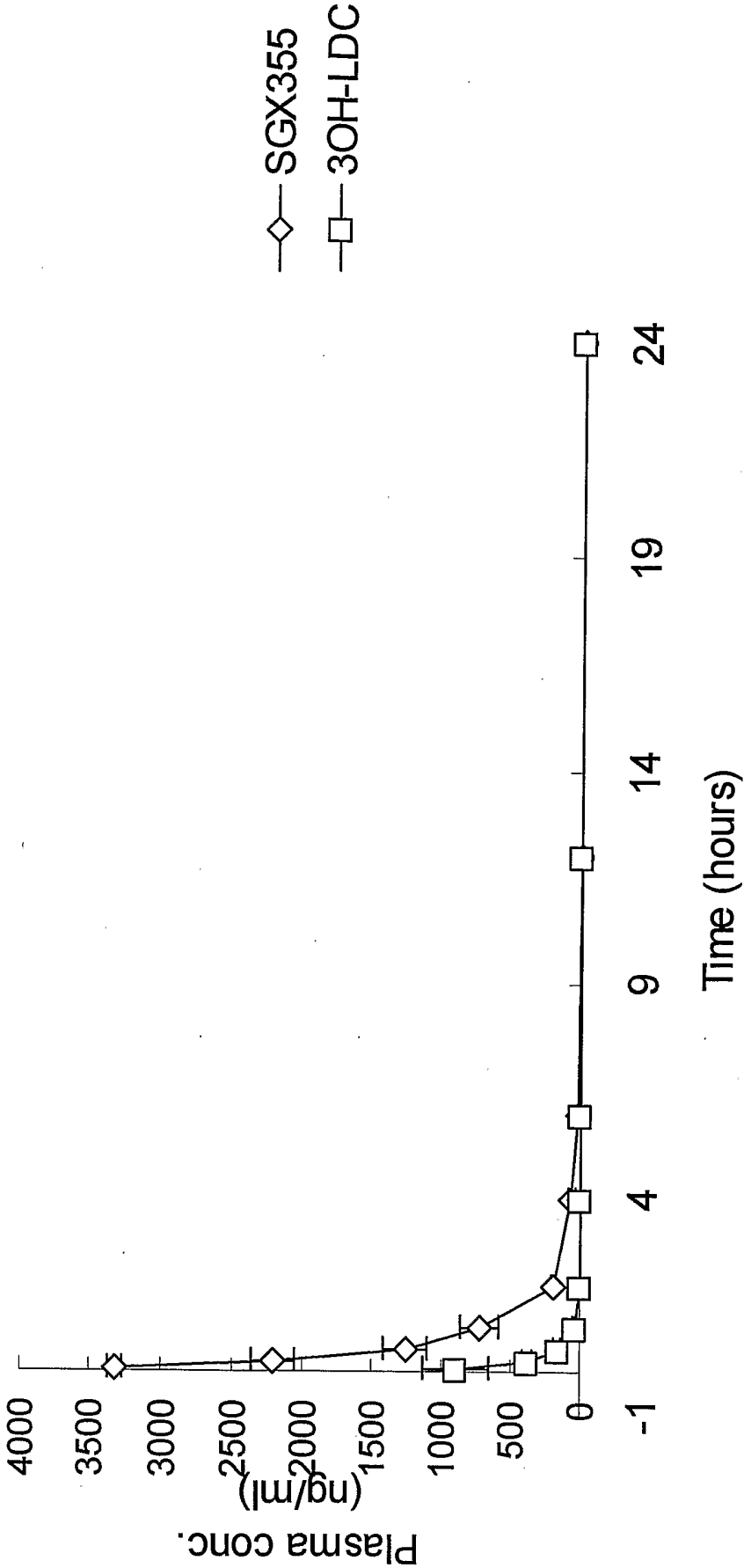


Figure 4



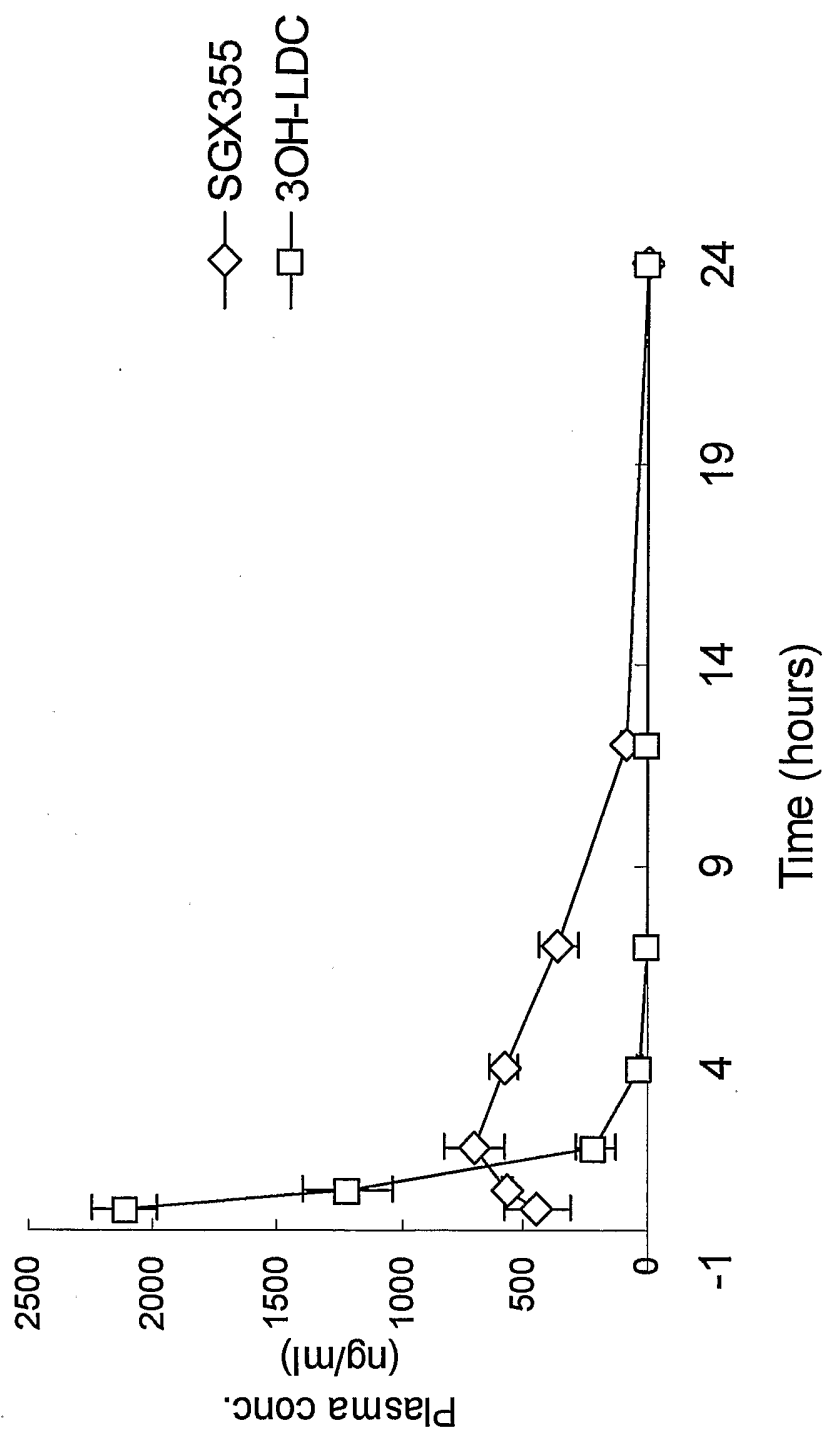


Figure 5

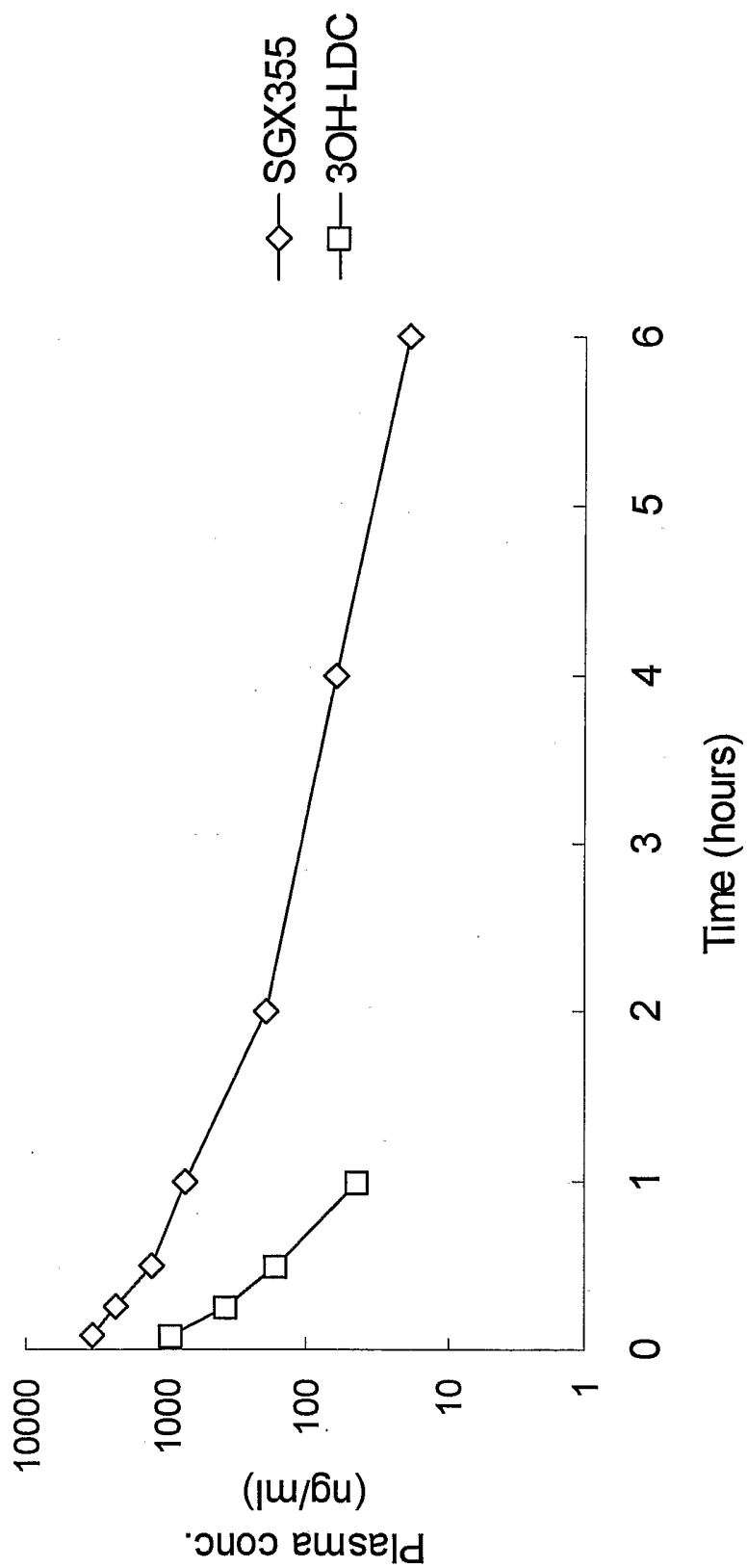


Figure 6

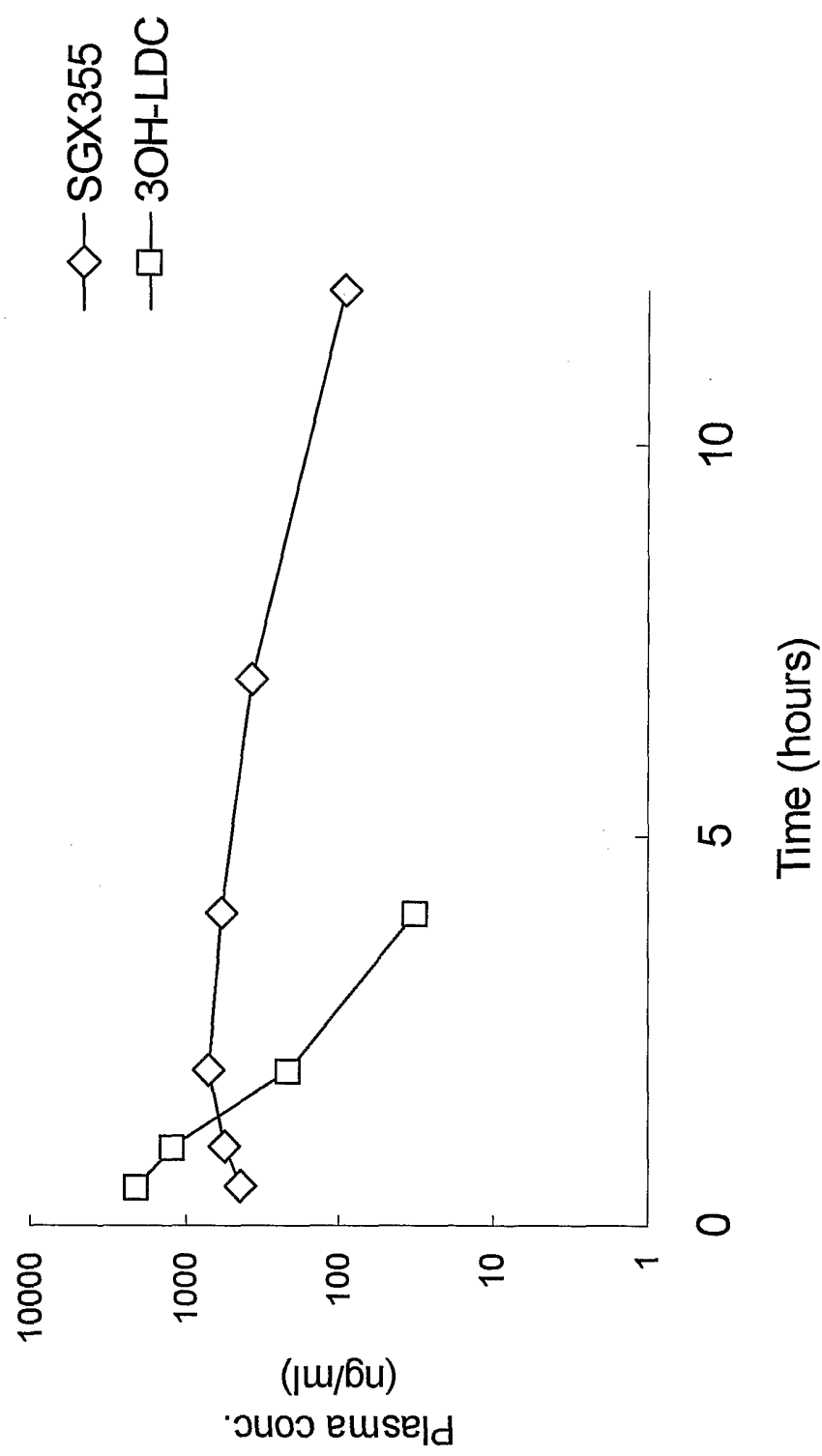


Figure 7

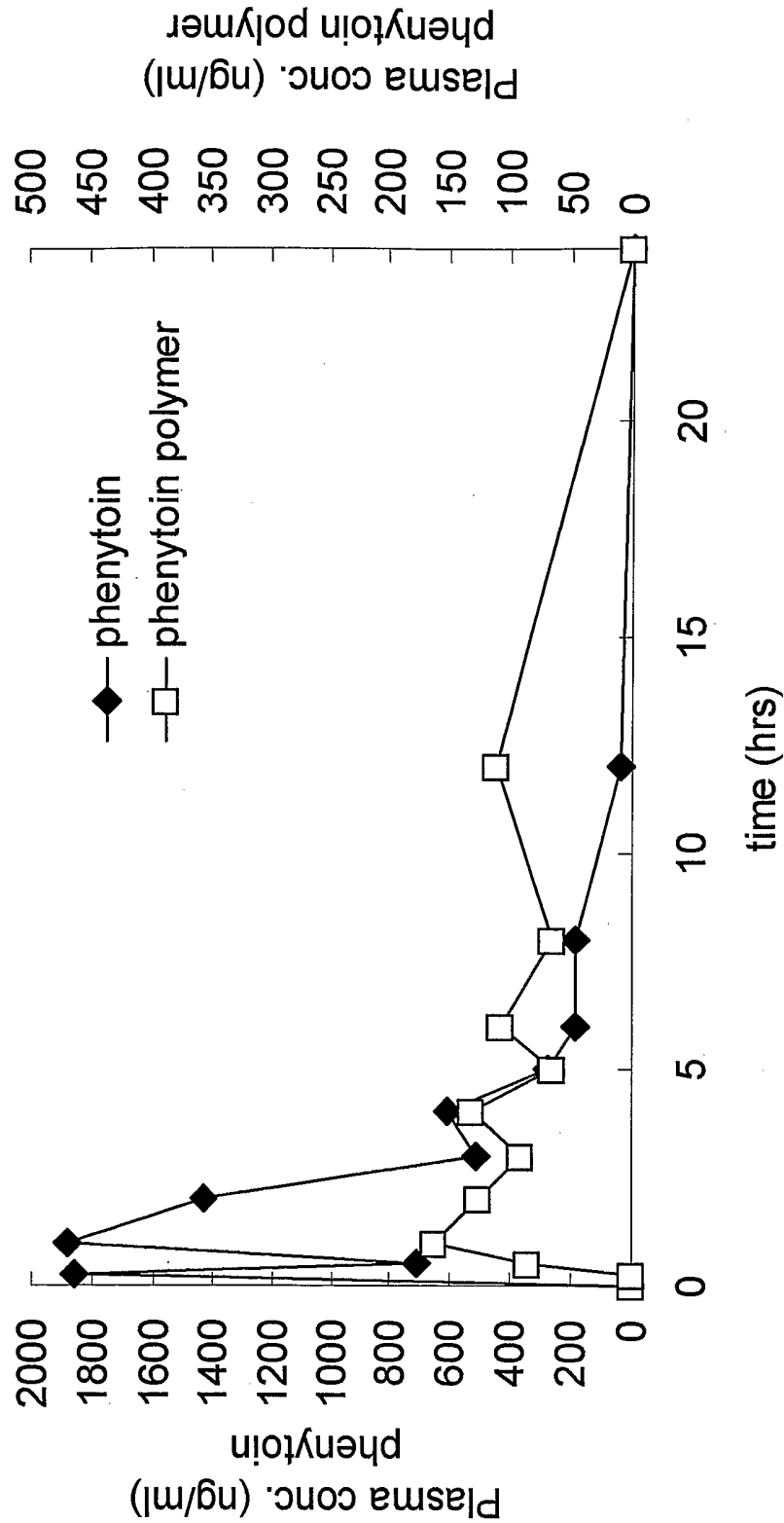
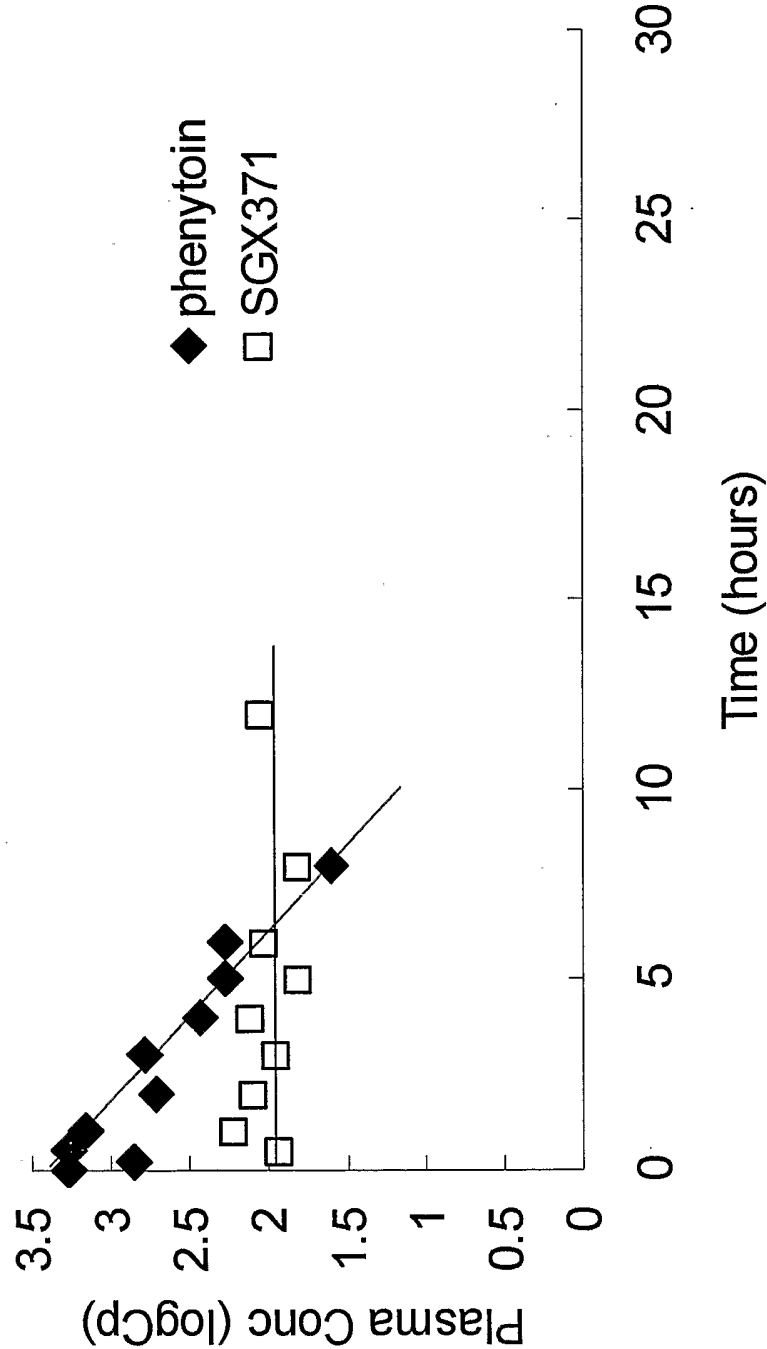


Figure 8

Figure 9



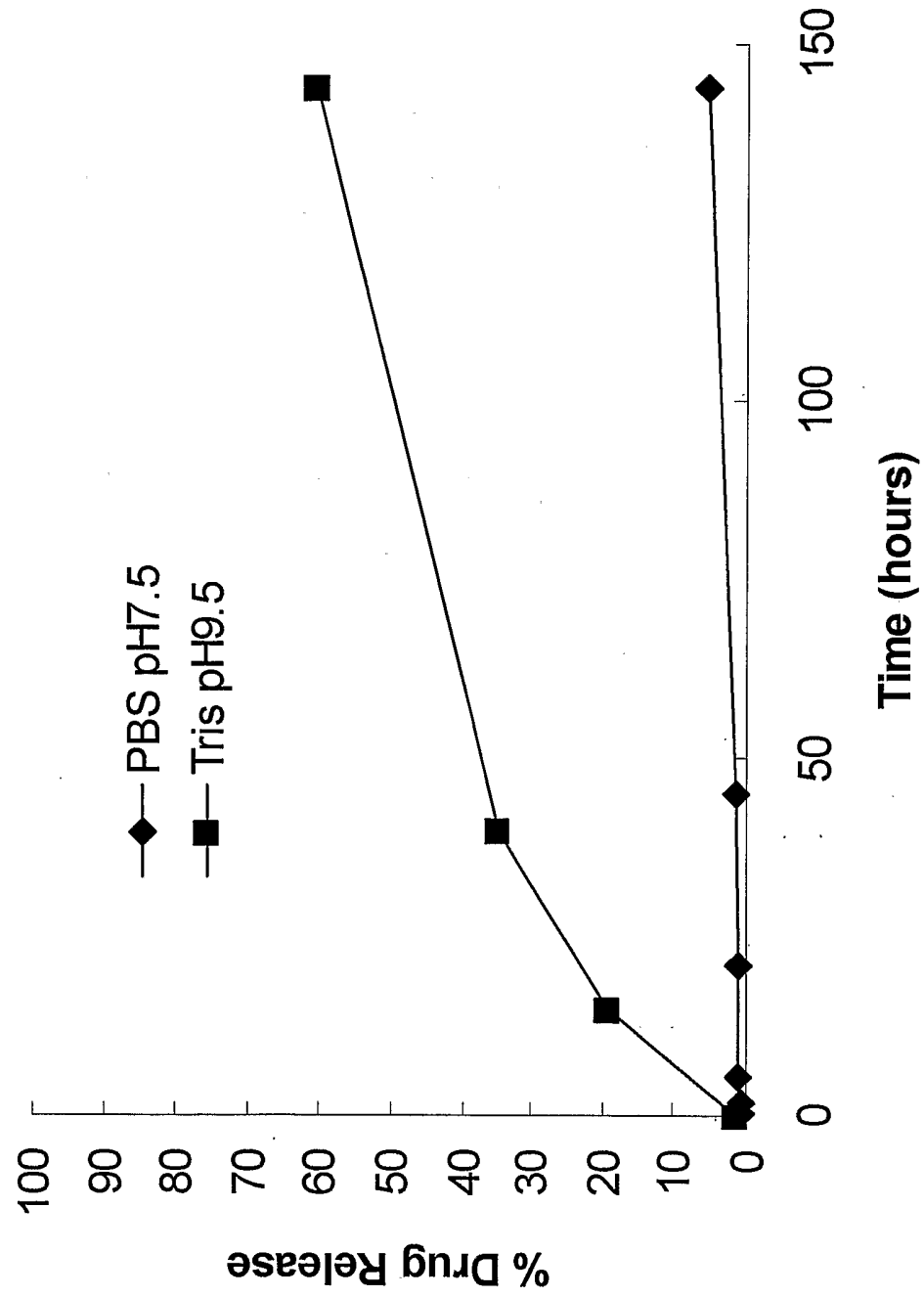


Figure 10

Figure 11

