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(57) **Abrégé/Abstract:**

The invention relates to PNA derivatives that carry one or more phosphoryl groups at the C terminus or at the C and N terminus of the PNA backbone, said phosphoryl groups optionally carrying one or more marker groups, or groups for cross-linking, or groups that promote the intracellular uptake, or groups that improve the binding affinity of the PNA derivative to nucleic acids. The invention further relates to a method for producing the above PNA derivatives and to the use thereof as a medicament or diagnostic agent.



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(54) Title: POLYAMIDE NUCLEIC ACID DERIVATIVES, AGENTS AND METHODS FOR PRODUCING THEM

(54) Bezeichnung: POLYAMIDNUKLEINSÄURE-DERIVATE, MITTEL UND VERFAHREN ZUR IHRER HERSTELLUNG

(57) Abstract: The invention relates to PNA derivatives that carry one or more phosphoryl groups at the C terminus or at the C and N terminus of the PNA backbone, said phosphoryl groups optionally carrying one or more marker groups, or groups for cross-linking, or groups that promote the intracellular uptake, or groups that improve the binding affinity of the PNA derivative to nucleic acids. The invention further relates to a method for producing the above PNA derivatives and to the use thereof as a medicament or diagnostic agent.

(57) Zusammenfassung: Gegenstand der vorliegenden Erfindung sind PNA-Derivate, die am C-Terminus oder C- und N-Terminus des PNA-Rückgrates einen oder mehrere Phosphoryl-Reste tragen, die gegebenenfalls eine oder mehrere Markergruppen, oder Gruppen zur Quervernetzung, oder Gruppen, die die intrazelluläre Aufnahme begünstigen, oder Gruppen, die die Bindungsaffinität des PNA-Derivats an Nukleinsäuren steigern, tragen. Gegenstand der Erfindung ist ferner ein Verfahren zur Herstellung der oben genannten PNA-Derivate und ihre Anwendung als Arzneimittel oder Diagnostikum.

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Description:

Polyamide nucleic acid derivatives, and agents and processes for preparing them

5

The present invention relates to carboxy-terminally and carboxy/amino-terminally phosphorylated polyamide nucleic acid (PNA) derivatives having improved properties, to their use and to agents and processes for preparing them.

10

Polyamide nucleic acids, also termed peptide nucleic acids (PNA), bind to complementary target sequences (DNA or RNA) with a higher affinity than do natural oligonucleotides and, furthermore, have the advantage, as compared with natural DNA, that they are very stable in serum. PNA were originally described as unnatural nucleic acid analogs in which the entire sugar-phosphate backbone is replaced with N-(2-aminoethyl)glycine units (M. Egholm et al. (1991) Science 254, 1497-1500; WO 92/20702; M. Egholm et al. Nature (1993) 365, 566-568; P. Nielsen, (1994) Bioconjugate Chem. 5, 3-7; E. Uhlmann et al. (1998) Angewandte Chemie Int. Ed. Engl. 37, 2796-2823). The bases employed are the nucleobases which occur naturally and are customary in nucleotide chemistry or else nucleobases which do not occur naturally, or their prodrug forms, that is precursors which are only converted into the free base by biotransformation in the body. In addition to this, PNAs have been described in which not all the positions in the backbone carry base residues (Greiner et al. (1999) Helv. Chim Acta 82, 2151), and in which aminoethylglycine is replaced by more complex units (Uhlmann et al. (1998) Angewandte Chem. Int. Ed. 37, 2796; Falkiewicz (1999) Biochim. Pol., 46, 509-529).

The fact that the PNA backbone does not have any net charge is an important feature of this class of substances and has far-reaching consequences. The fact that PNA binds to complementary DNA and RNA even at low salt concentration (e.g. Peptide Nucleic Acids: Protocols and Applications;

30

Peter E. Nielsen and Michael Egholm (Edit.) Horizon Scientific Press, 1999, 3), with the Watson-Crick base pairing rules being obeyed, is ascribed to the neutral character of the PNA and the decrease in charge repulsion which is associated therewith. For this reason, PNA can, in principle, be used for numerous applications in which natural oligonucleotides or oligonucleotide derivatives would otherwise be employed. However, in addition to this, because of the unique binding properties, a large number of applications which are not possible with natural oligonucleotides also ensue (see, for example: Peptide Nucleic Acids: Protocols and Applications; Peter E. Nielsen and Michael Egholm (Edit.) Horizon Scientific Press, 1999). For example, a strand invasion of double- stranded DNA has been observed when using PNA.

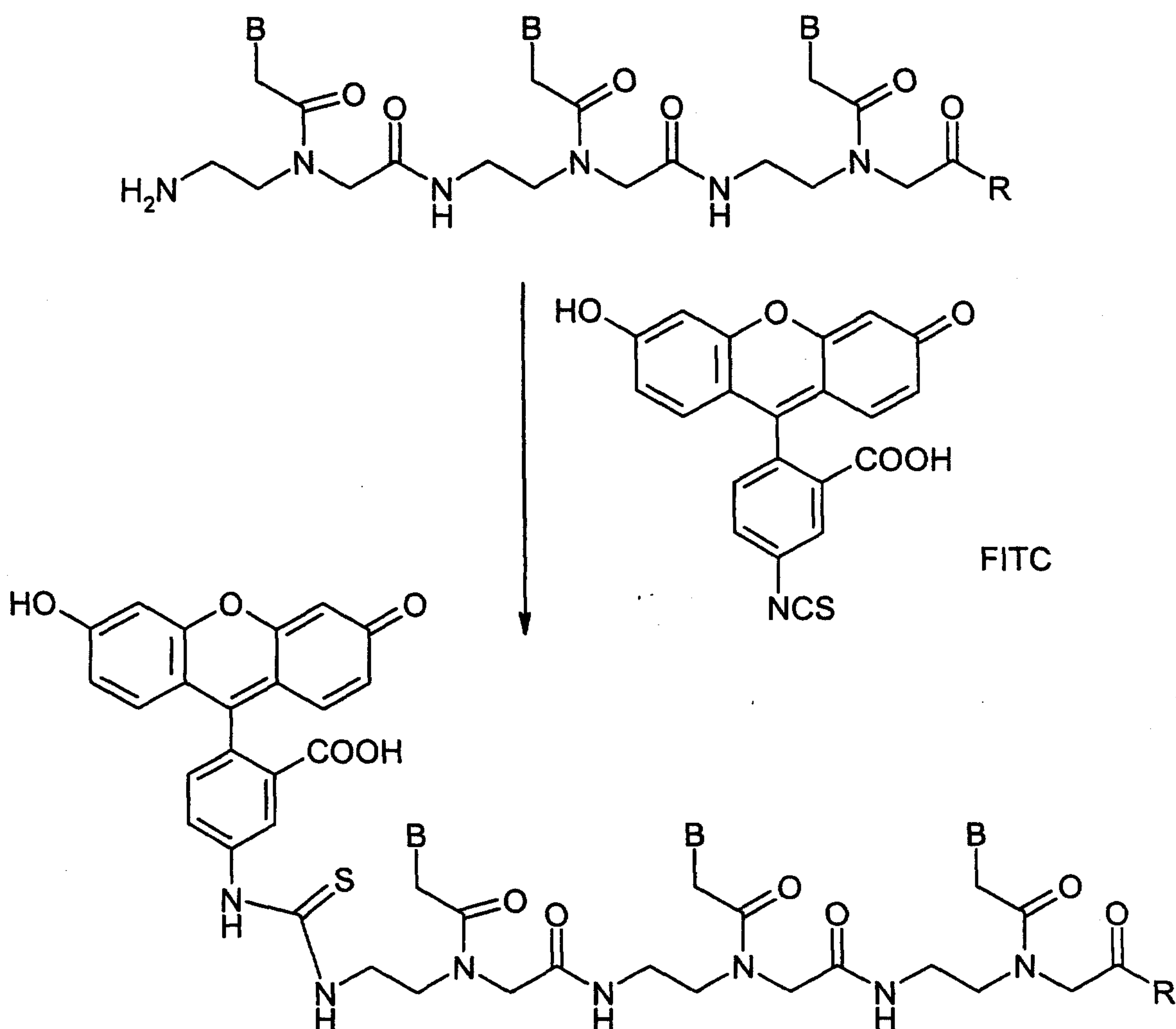
Typical examples of applications for PNA include its use for inhibiting gene expression by binding, in a sequence-specific manner, to cellular DNA or RNA. "Antisense agents" are short, single-stranded nucleic acid derivatives which bind, by means of Watson-Crick base pairing, to a complementary mRNA whose translation into the corresponding protein is to be inhibited (Uhlmann and Peyman (1990) Chem. Rev. 90, 543; Larsen et al. (1999) Biochem. Biophys. Acta 1489, 159). "Anti-gene agents" bind, by way of Hoogsteen base pairing, in the major groove of the DNA double helix with the formation of a triple helix, resulting in transcription of the genes being inhibited in a sequence-specific manner (Praseuth et al. (1999) Biochem. Biophys. Acta 1489, 181). Gene expression can also be specifically inhibited by so-called decoy oligomers, which mimic the regions for binding transcription factors. By treating with decoy agents, particular transcription factors can be captured in a sequence-specific manner and activation of transcription thereby prevented (Mischiati et al. (1999) J. Biol. Chem. 274, 33114). Another group of oligonucleotide derivatives which act intracellularly, i.e. the chimeraplasts, are used for specific gene proof-reading (Cole-Strauss et al. (1996) Science 273, 1386-1389).

PNAs can, therefore, be used as pharmaceuticals and/or diagnostic agents or for producing pharmaceuticals and/or diagnostic agents.

For example, after having been labeled with biotin, fluorescein or other labels, PNA can be used, for diagnostic purposes and in molecular biology, as a specific hybridization probe. Four methods have so far been described in the literature for introducing the labeling groups (Oerum et al. (1999), in *Peptide Nucleic Acids: Protocols and Applications*, pages 81-86; Lohse et al. (1997) *Bioconjugate Chem.* 8, 503). The first method is based on labeling the free (deprotected) PNA after it has been synthesized in solution. In this method, the aminotermminus of the PNA is reacted with an activated carboxylic acid or an isothiocyanate. However, additional lysine residues are frequently introduced into the PNA, with these residues then being reacted with fluorescein isothiocyanate (FITC).

In the second method, the protected PNA is modified at its aminotermminus with activated carboxylic acid derivatives or isothiocyanates while it is still on the solid phase. This method is only suitable for labeling groups which are stable under the conditions which pertain during deprotection of the PNA and during its cleavage from the support. The reactive reagents which are preferably used in both cases are isothiocyanates (P. Wittung et al., (1995) *FEBS Lett.* 375, 27) and activated carboxylic acids, such as N-hydroxysuccinimide esters (NHS) (Oerum et al., 1999). A disadvantage of the reaction using the NHS derivatives is that it is frequently only accomplished with poor yields. For this reason, 8-amino-3,6-dioxaoctanoic acid is frequently condensed, as a linker or spacer, between the PNA and the labeling group (Oerum et al., 1999). Both linkages are effected by way of amide bonds or thiourea bonds, which, as such, are, however, more likely to lead to insolubility. Alternatively, the carboxylic acids are caused to react using activators which are customary in peptide chemistry, such as HBTU, TBTU or HATU.

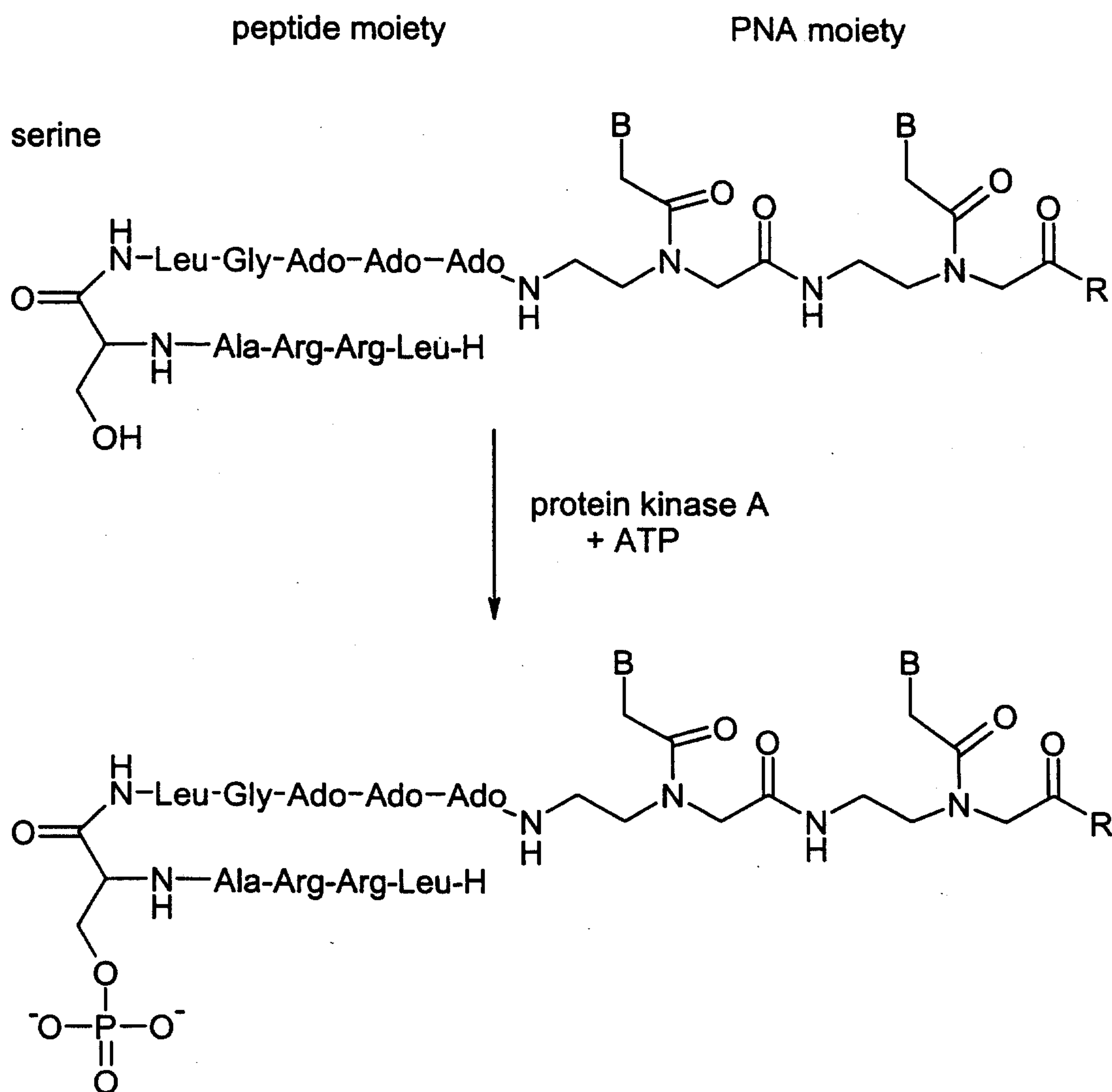
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5 In a third method, fluorescein-conjugated monomers are used during the synthesis of the PNA on the solid phase, with the fluorescence labeling being effected by way of an amide bond (Lohse et al. (1997) Bioconjugate Chem. 8, 503), which once again leads to conjugates which are relatively difficult to dissolve.

10 A fourth method uses PNA peptide conjugates in which the peptide moiety forms a substrate for a protein kinase (Koch et al. (1995) Tetrahedron Lett. 36, 6933). In this way, therefore, it is not the PNA moiety which is modified; rather, the serine residue in the peptide segment is phosphorylated enzymically. When this method is used, therefore, it is only possible to introduce radioactive

phosphate, and not, for example, any fluorescein or biotin, into the peptide segment of the PNA-peptide conjugate.



It is known that PNA tends to aggregate in aqueous solution, that is under physiological conditions as well. PNA is therefore poorly soluble in aqueous buffer and is then unavailable for hybridizing to complementary sequences. Furthermore, PNA has a high affinity for various materials such as Sephadex® (from Pharmacia), Bond Elut® (from Varian) or various HPLC chromatograph materials which are used in purifying oligomers, which means that PNA can frequently only be isolated in poor yields. It is therefore necessary to conjugate PNA with lysine or other positively charged amino acids (by way of the

C terminus) (Egholm et al (1992) J. Am. Chem. Soc. 114, 1895). Guanine-rich PNA sequences have a very particular tendency to aggregate, for which reason the advice is not to use such PNA (see "Guidelines for sequence design of PNA oligomers" in Peptide Nucleic Acids: Protocols and Applications (1999) pages 5 253-255). For example, relatively long fluorescein-labeled PNA oligomers are particularly difficult to dissolve, with the addition of an organic solvent and heating to 50°C being recommended.

It is particularly difficult to purify the poorly soluble lipophilic PNA derivatives.

10 Several peaks which are due to PNA aggregates are frequently detected in the HPLC. The technique of polyacrylamide (PAA) gel electrophoresis, which is frequently employed for purifying and separating nucleic acids, cannot be used for these PNA derivatives.

15 In the methods of derivatizing PNA which are described above, the labeling group is always introduced by forming an amide bond or a thioamide bond, with PNA derivatives being formed which are relatively difficult to dissolve. Poorly soluble PNA derivatives are formed, in particular, when lipophilic residues, such as fluorescein, are introduced. The insertion markers at the two ends of the

20 PNA is technically even more difficult and generally leads to even poorer solubility. In addition, no efficient method for simultaneously derivatizing PNA at the amino and carboxy termini, in particular by means of solid phase synthesis, has been described. Since, furthermore, the labeling reactions frequently proceed with poor yields, an object to be achieved consists in making available

25 novel PNA derivatives which it should be possible to prepare in high yields, which should exhibit advantageous properties, such as improved solubility, improved binding behavior and better cellular uptake, and which, in addition, make it possible to use efficient methods for purifying the PNA oligomers.

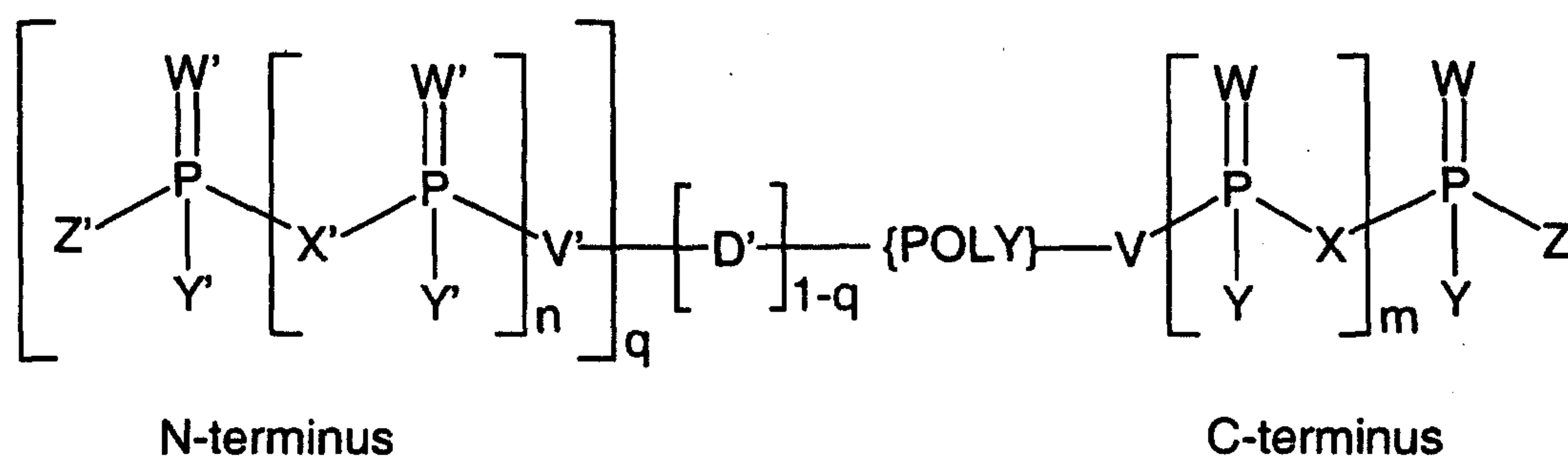
According to the invention, this object is achieved by making available PNA

30 derivatives which carry one or more phosphoryl radicals at the C terminus or at the C and N termini of the PNA backbone, with thiophosphoryl radicals and iminophosphoryl radicals also being included in addition to oxophosphoryl

radicals, and with at least one of the phosphoryl radicals carrying one or more deprotonatable groups, preferably hydroxyl groups or mercapto groups. The phosphoryl radicals are linked to the PNA backbone by way of an oxygen-phosphorus bond, sulfur-phosphorus bond or nitrogen-phosphorus bond, either
 5 directly or by way of a spacer, with it being possible for the spacer to be, for example, an alkanoylamide, a poly(alkoxy)carboxamide or an amino acid. Examples of phosphoryl radicals are phosphate, phosphonate, thiophosphate, phosphoamidate and substituted phosphoryl radicals, with substituted
 10 phosphoryl radicals carrying, where appropriate, one or more labeling groups, or groups for crosslinking, or groups which promote intracellular uptake, or groups which increase the binding affinity of the PNA derivative for nucleic acids.

In this connection, labeling groups (labels) are understood as being groups which enable the chemical or biological activity of the PNA derivatives to be
 15 assessed qualitatively or quantitatively, for example biotin or fluorescein. Crosslinking is understood as being the formation of intramolecular or intermolecular bonds between spatially adjacent functionalities. An example of a group for crosslinking is the psoralen group.

20 Invention preferably relates to PNA derivatives of the formula I



Formula I

where

25 q is 0 or 1,

- D' is hydroxyl, mercapto, amino, alkylamino or acylamino, preferably acetylamino,
- 5 V is, independently of each other, oxygen, sulfur, NR_1 ,
- V' is, independently of each other, oxygen, sulfur, NR_1 , a group $\text{U}-(\text{CR}_3\text{R}_4)_{\text{U}'}-\text{C}(\text{O})-\text{NH}$ or a group $\text{U}-(\text{CH}_2\text{CH}_2\text{O})_{\text{U}'}-\text{CH}_2-\text{C}(\text{O})-\text{NH}$,
- 10 U is, independently of each other, oxygen, sulfur or NH ,
- u' is, independently of each other, from 1 to 10, preferably from 1 to 4, particularly preferably 1,
- 15 W and W' are, independently of each other, oxygen, sulfur or NR_1 ,
- Y and Y' are, independently of each other, hydroxyl, mercapto, oxyanion, thioate or NR_1R_2 ,
- 20 X and X' are independently of each other, a group $\text{U}-(\text{C}_2-\text{C}_{22}\text{-alkanediyl})-\text{U}$ or a group $\text{U}-(\text{CH}_2\text{CH}_2-\text{O})_{\text{U}'}-$,
 or are a labeling group or a group for crosslinking, or a group which promotes intracellular uptake, or a group which increases the binding affinity of the PNA derivative for nucleic acids, for
 25 example a bifunctional fluorescein, rhodamine, TAMRA, biotin, pyrene, dinitrophenyl, cholesteryl, acridine, adamantyl, vitamin E, cyanine dye, Dabcyl, Edans, lexitropsin, psoralen, BODIPY, ROX, R6G or digoxigenin radical,

Z and Z' are, independently of each other, hydroxyl, mercapto, oxyanion, thioate or NR_1R_2 , $\text{C}_1\text{-C}_{22}\text{-alkyl}$, $\text{C}_1\text{-C}_8\text{-arylalkyl}$, $\text{C}_1\text{-C}_{22}\text{-alkyl-U}$, $\text{C}_1\text{-C}_8\text{-arylalkyl-U}$, hydroxy- $\text{C}_1\text{-C}_{18}\text{-U}$, aminoalkyl-U or mercaptoalkyl-U,

5 or a group of the formula $\text{R}_7(\text{CH}_2\text{CH}_2\text{-O})_m$, where R_7 is hydroxyl, amino or $\text{C}_1\text{-C}_{22}\text{-alkoxy}$, and m is from 1 to 100, preferably from 2 to 10,

10 or are a labeling group, or a group for crosslinking, or a group which promotes intracellular uptake, or a group which increases the binding affinity of the PNA derivative for nucleic acids, for example a monofunctional or bifunctional fluorescein, rhodamine, TAMRA, biotin, pyrene, dinitrophenyl, cholesteryl, acridine, adamantyl, vitamin E, cyanine dye, Dabcyl, Edans, lexitropsin, psoralen, BODIPY, ROX, R6G or digoxigenin radical,

15

R_1 and R_2 are, independently of each other, a radical consisting of hydrogen or $\text{C}_1\text{-C}_6\text{-alkyl}$, preferably hydrogen,

20 R_3 and R_4 are, independently of each other, a radical consisting of hydrogen or $\text{C}_1\text{-C}_6\text{-alkyl}$, or the radical of an amino acid side chain, preferably hydrogen, it being possible for adjacent radicals R_3 and R_4 in V' to also form a $\text{C}_5\text{-C}_8\text{-cycloalkyl}$ ring,

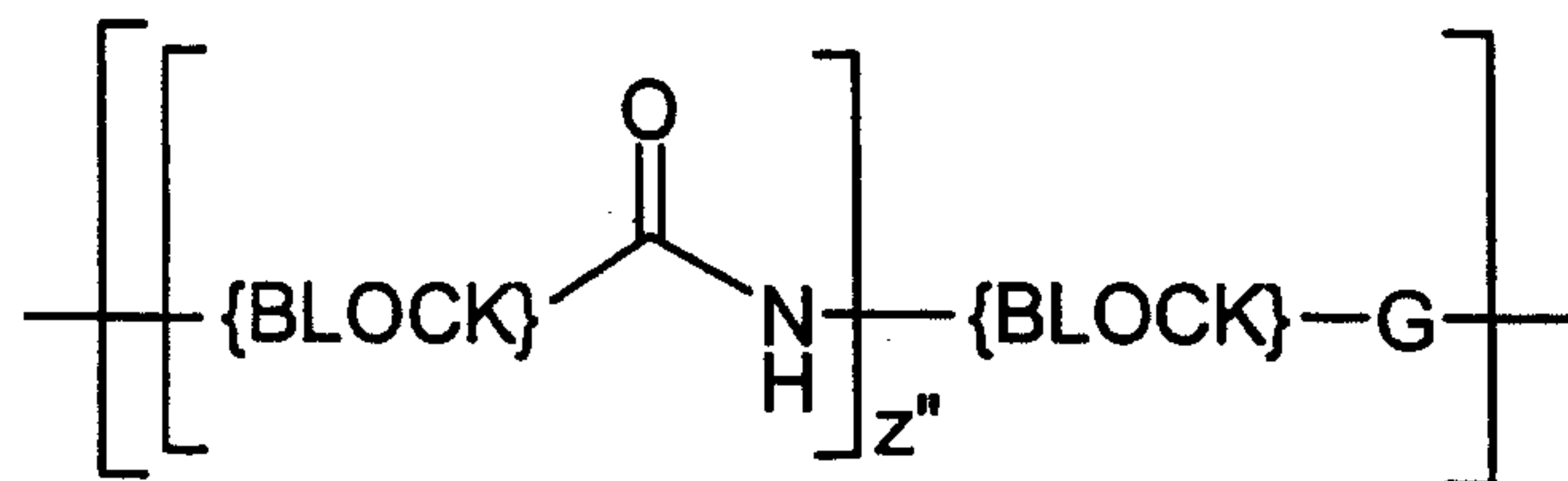
25 n is from 0 to 10, preferably from 0 to 3,

m is from 0 to 10, preferably from 0 to 3,

with the proviso that at least one Y, Y' or Z, Z' radical is hydroxyl, mercapto, oxyanion or thioate.

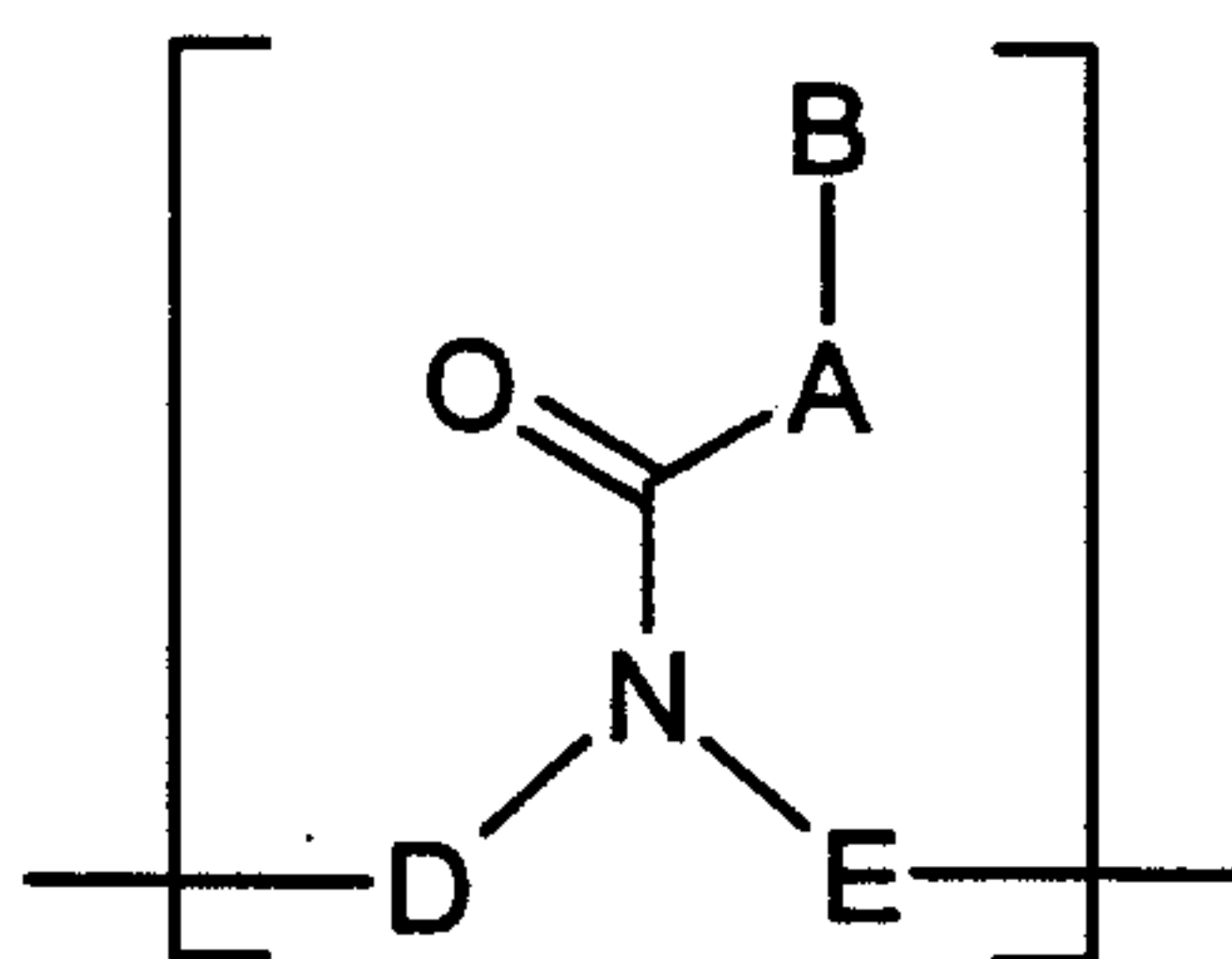
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The group {POLY} is described by the formula II.



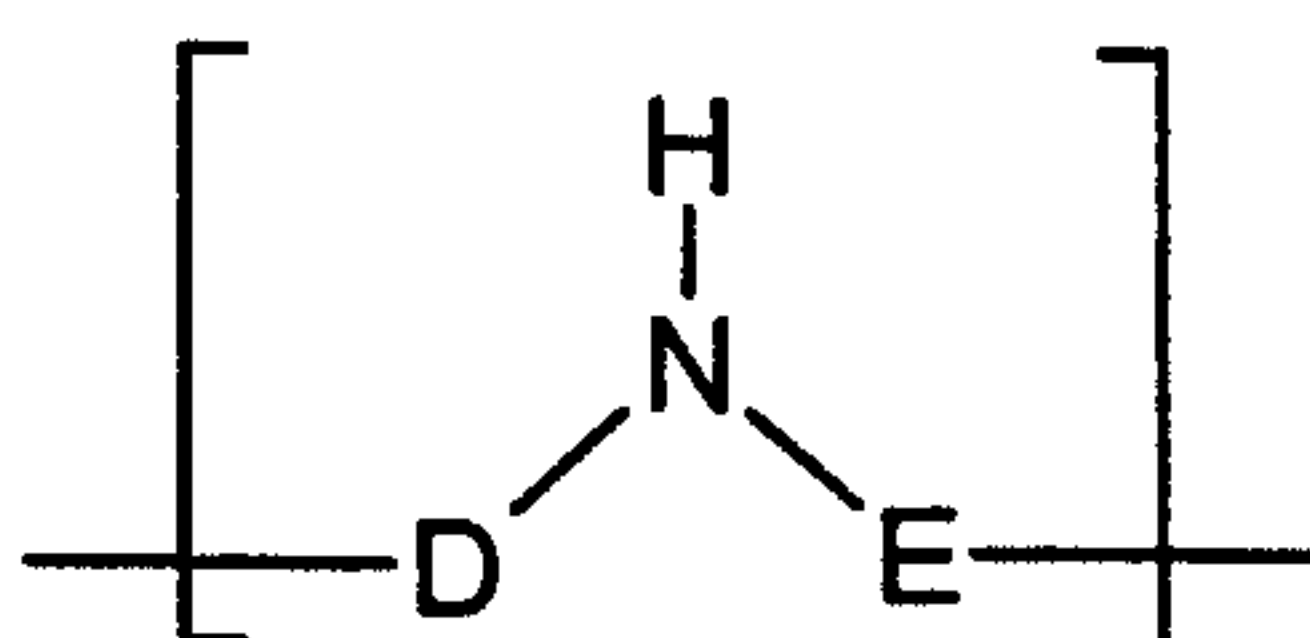
Formula II

where {BLOCK} is, independently of each other, a group selected from
5 formula IIIA,



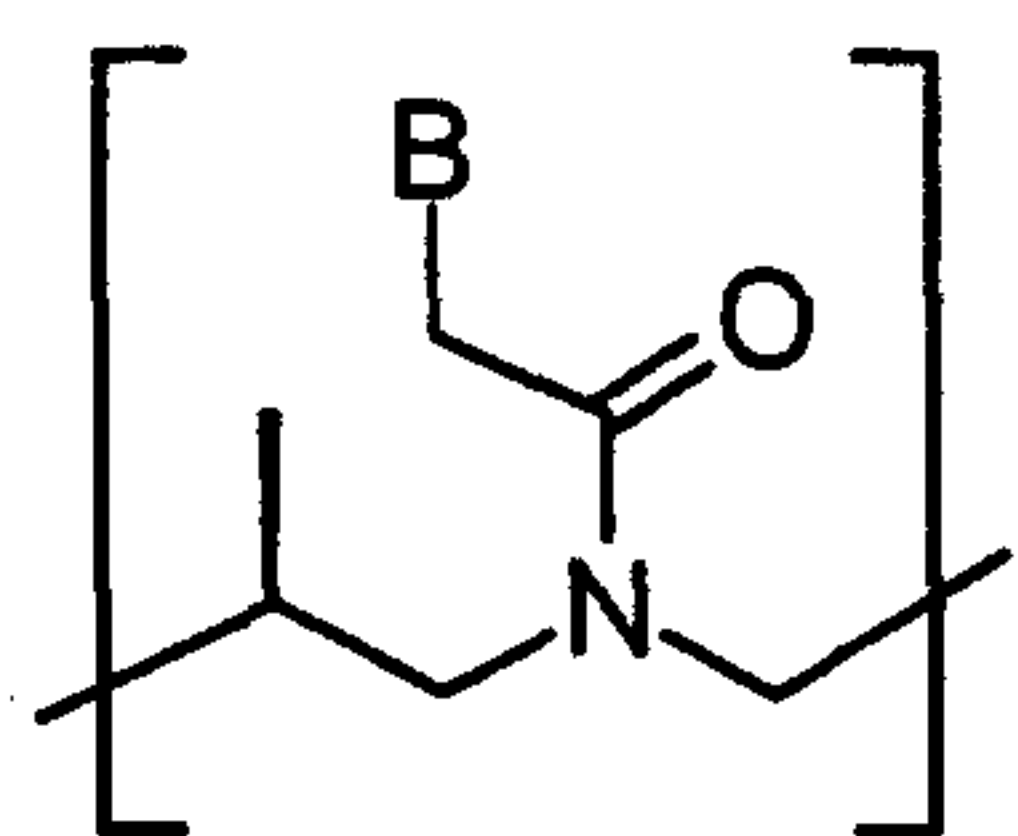
Formula IIIA

10 or from formula IIIB (Greiner et al. (1999) *Helv. Chim Acta* 82, 2151),

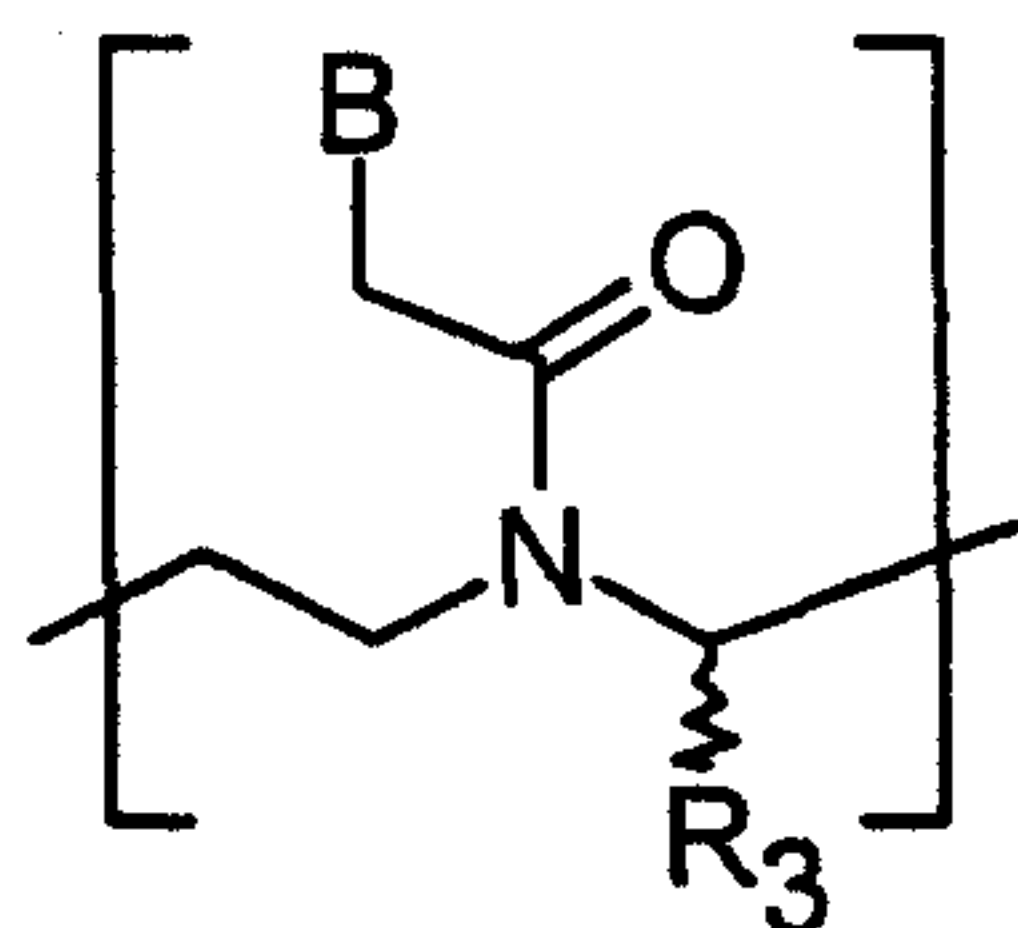


Formula IIIB

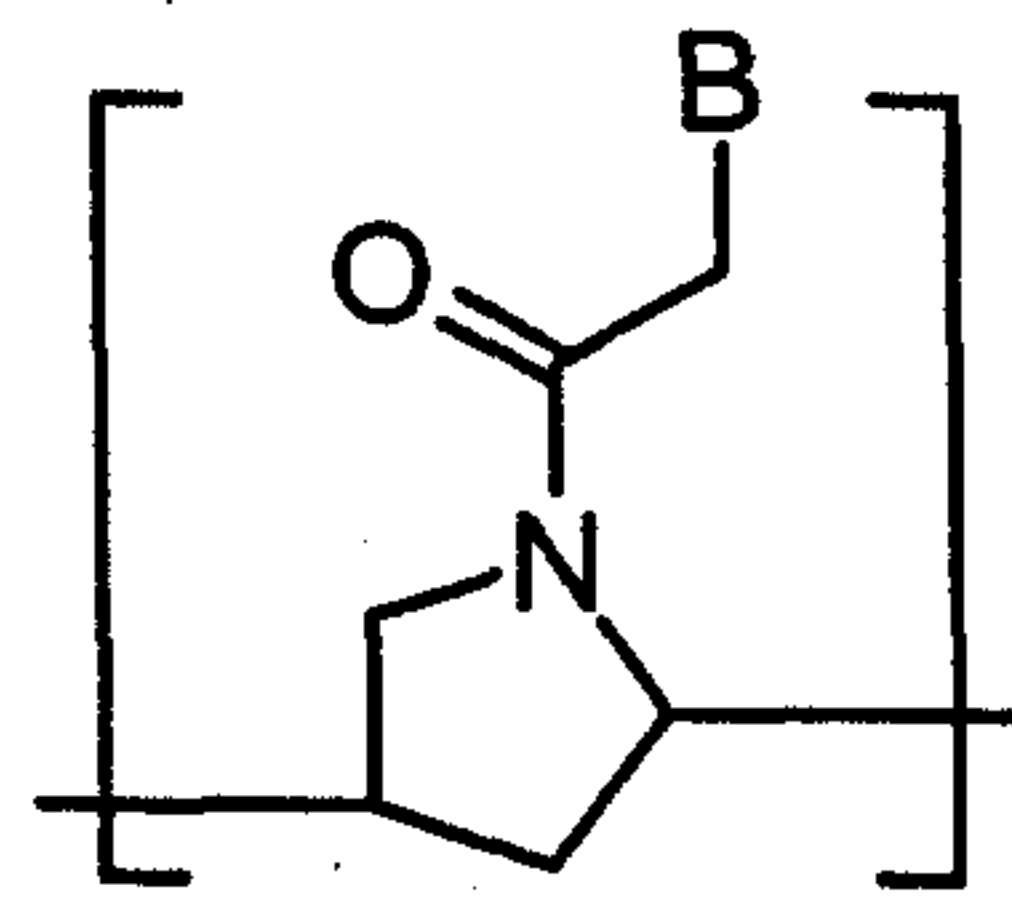
15 or from the formulae IV A to IV G (Uhlmann et al. (1998) *Angewandte Chem. Int. Ed.* 37, 2796; Falkiewicz (1999) *Biochim. Pol.*, 46, 509-529),



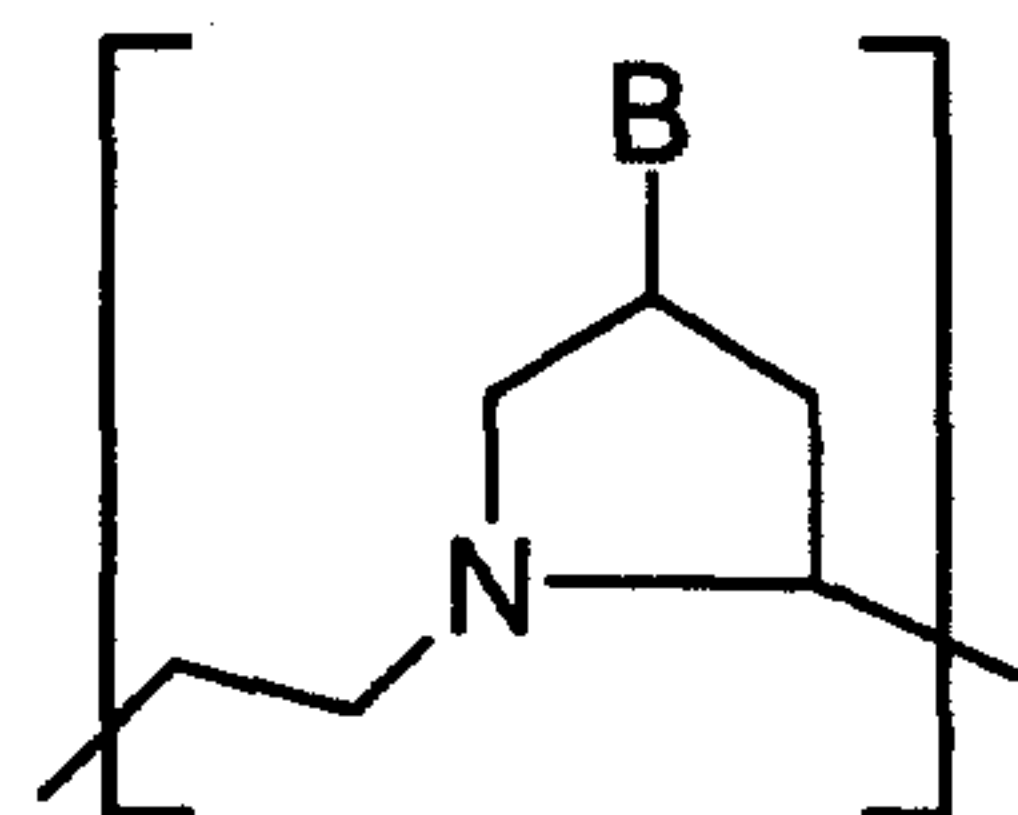
Formula IV A



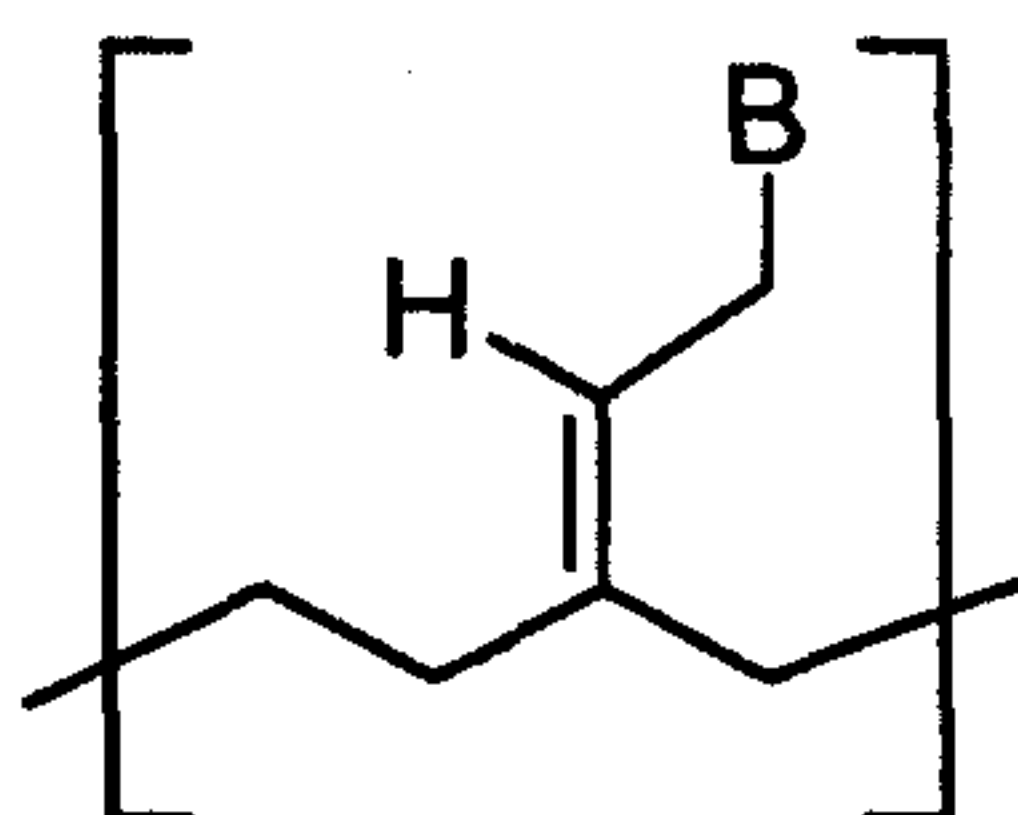
Formula IV B



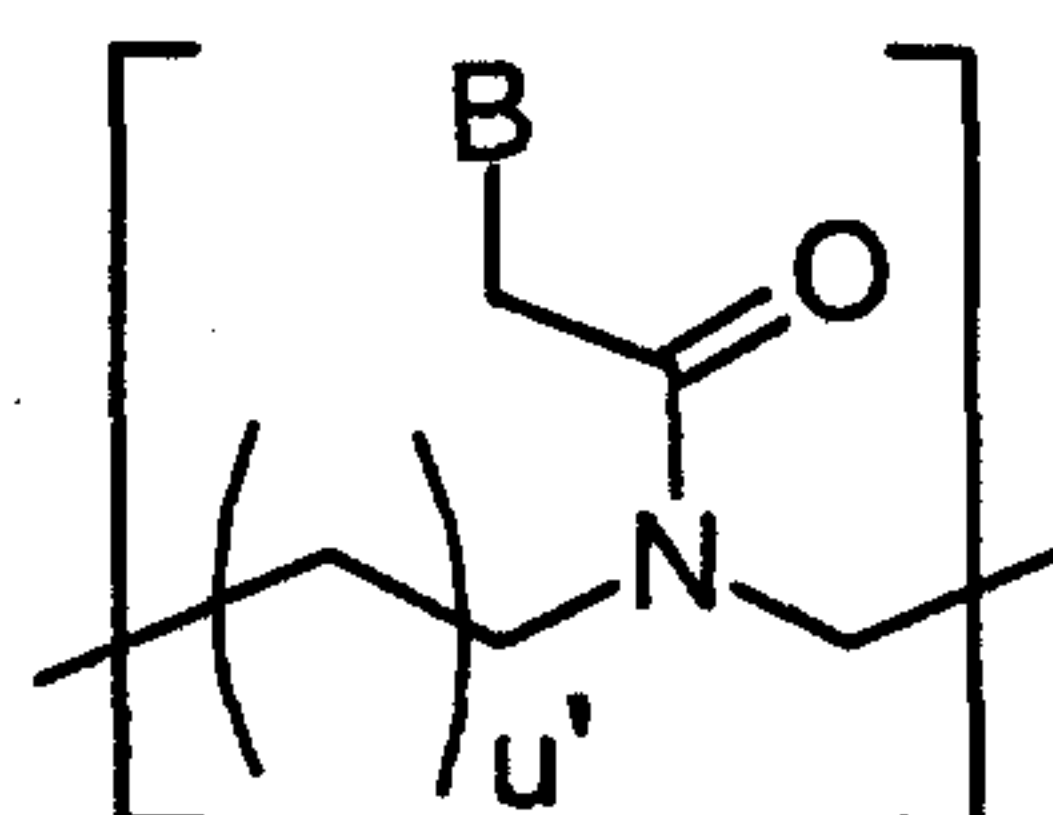
Formula IV C



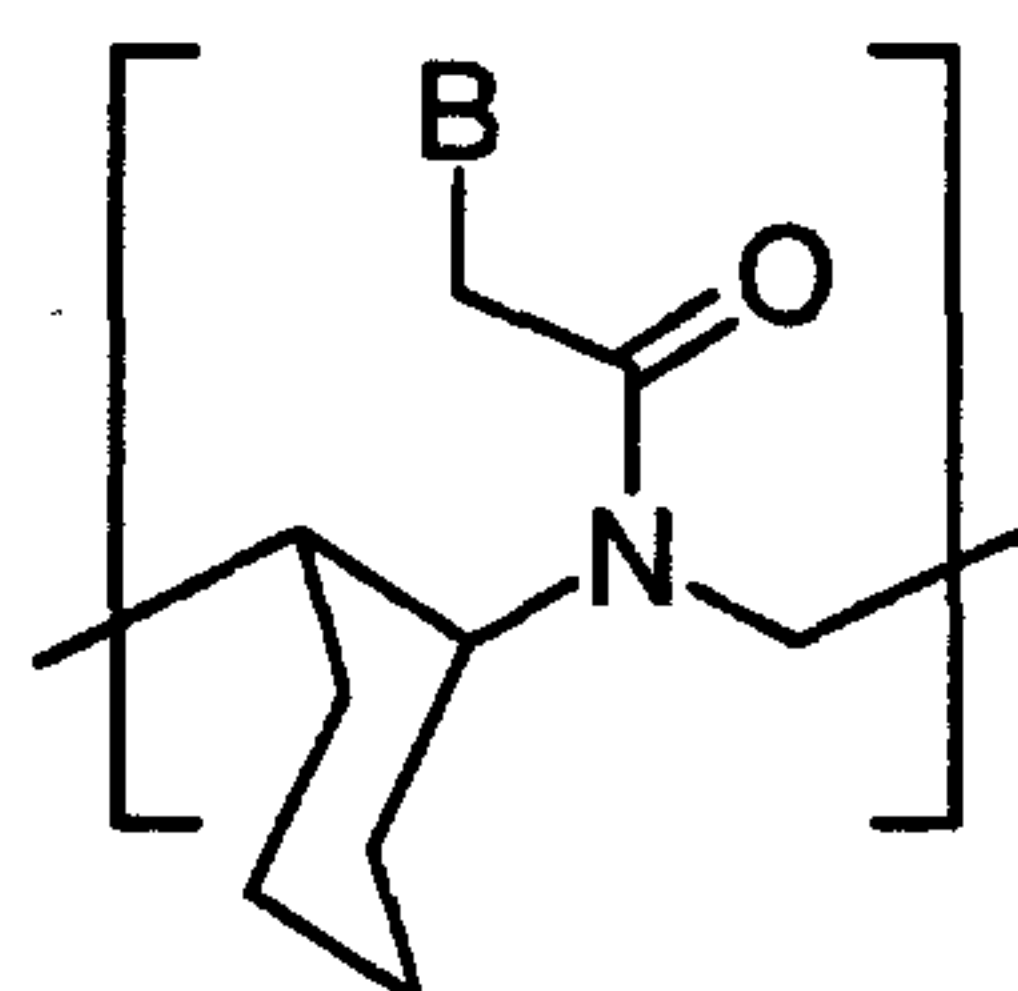
Formula IV D



Formula IV E



Formula IV F



Formula IV G

where each {BLOCK} building block can be different,

5 and where it furthermore applies that

Z'' is from 0 to 100, preferably 1-20, particularly preferably 4-15,

G is selected from the groups $(CR_5R_6)_{u'}$, $C(O)NH-(CR_1R_2)_{t'}$ or $C(O)NH-(CH_2CH_2O)_{u'}-CH_2CH_2$, where u' has the abovementioned meaning and
 10 t' is from 2 to 10, preferably 6,

A is, independently of each other, a group $(CR_1R_2)_s$, where s is from 1 to 3, preferably 1,

- B is, independently of each other, either an aromatic radical, which can also possess heteroaromatic character, or hydrogen, or hydroxyl or C₁-C₁₈-alkyl,
or a nucleobase which occurs naturally, and is customary in nucleotide chemistry, or which does not occur naturally, or its prodrug form,
5 with the proviso that at least one B radical is a nucleobase,
- D is, independently of each other, a group (CR₃R₄)_t, where t is from 2 to 10, preferably from 2 to 4, particularly preferably 2,
10
- E is, independently of each other, a group (CR₅R₆)_{u'}, where adjacent R₅ and R₆ radicals can also form a C₅- to C₈-cycloalkyl ring or a spiro compound,
- 15 R₅ and R₆ are, independently of each other, a radical consisting of hydrogen or C₁-C₆-alkyl, or the radical of an amino acid side chain, preferably hydrogen,
- and where u', R₁, R₂, R₃ and R₄ have the same meaning as described above,
20
- as well as physiologically tolerated salts of the PNA derivatives of the formula I. Physiologically tolerated salts are described, inter alia, in Remingtons Pharmaceutical Science (1985) Mack Publishing Company, Easton, PA, USA, page 1418. Preference is given to ammonium salts, trialkylammonium salts,
25 alkali metal salts (such as sodium salts and potassium salts) and alkaline earth metal salts (such as magnesium salts and calcium salts). Particular preference is given to sodium salts
- A surprising, positive effect which was found was that the introduction of a
30 phosphoryl radical, for example as phosphate or else in the form of a lipophilic derivatization (e.g. as a hexadecyl phosphodiester) increases the affinity of the

PNA for complementary DNA or RNA. This effect was unexpected since the strong bonding of PNA to complementary DNA or RNA was attributed to the neutral character of the PNA and the reduced charge repulsion which was associated therewith (e.g. Peptide Nucleic Acids: Protocols and Applications; 5 Peter E. Nielsen and Michael Egholm (Edit.) Horizon Scientific Press, 1999, 3).

The biotin was introduced particularly efficiently by way of a phosphoryl radical. When used as hybridization probes, the biotinylated PNA of the formula I (X, X', Z and/or Z' = biotin radical) displayed better binding properties and fewer 10 spurious, nonspecific background effects than did corresponding biotinylated DNA probes.

In contrast to the uncharged PNA, the PNA derivatives of the formula I according to the invention can also migrate in an electric field, thereby making it 15 possible to microlocate them and concentrate them on immobilized complementary nucleic acid derivatives. In the case of the polyanionic oligonucleotides, this method using the electrical field has already been described for rapidly determining base mismatches (Sosnowski et al. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 1119).

20

The hydroxy or mercapto substituents of the phosphoryl radicals of the DNA derivatives according to the invention can be deprotonated in a pH range of from 4.5 to 14, preferably of from 6.5 to 12, particularly preferably of from 6.5 to 9. The property of the ionizability of the phosphoryl radicals can advantageously 25 be exploited for purifying the compounds of the formula I. On the one hand, the compounds of the formula I can be purified by electrophoresis, for example polyacrylamide gel electrophoresis. On the other hand, it is also possible to purify them using anion exchangers. In this case, the desired products are preferably eluted by using a salt gradient, for example a sodium chloride 30 gradient, or a pH gradient. The PNA derivatives of the formula I according to the invention can be particularly simply and efficiently purified using anion exchangers. It was found that the uncharged byproducts are not retarded on the

anion exchanger whereas the charged product adhered to the column. After washing with water, it was possible to isolate the desired product in pure form using acetic acid or a sodium chloride solution. The anion exchangers employed are preferably strong anion exchangers or mixed-mode phases, such as ®Oasis
5 MAX (Waters GmbH, Eschborn).

It was furthermore found that the compounds of the formula I according to the invention are in general more readily soluble in aqueous medium than are the corresponding PNA oligomers which do not possess the phosphoryl radical.
10 This makes itself very particularly apparent, in the form of a greatly improved solubility in aqueous medium, in the case of the lipophilic derivatives, such as the fluorescein derivatives or the hexadecyl derivatives.

The invention relates, in particular, to PNA derivatives in which A and E are
15 CH₂. The invention furthermore relates, in particular, to PNA derivatives in which D substituents are (CH₂)₂. Preference is also given to PNA derivatives of the formula I in which W and W' are oxo, furthermore those in which Y and Y' are hydroxyl or oxyanion, and those in which V and V' are oxy.

20 Examples of natural bases are adenine, cytosine, 5-methylcytosine, guanine, thymine and uracil. Examples of unnatural bases are purine, 2,6-diaminopurine, N⁴N⁴-ethanocytosine, N⁶N⁶-ethano-2,6-diaminopurine, 5-(C₃-C₆)-alkynyluracil, 5-(C₃-C₆)-alkynylcytosine, 5-(1-propargylamino)uracil, 5-(1-propargylamino)cytosine, phenoxazine, 9-aminoethoxyphenoxazine,
25 5-fluorouracil or pseudoisocytosine, 5-(hydroxymethyl)uracil, 5-aminouracil, pseudouracil, dihydrouracil, 5-(C₁-C₆)-alkyluracil, 5-(C₁-C₆)-alkylcytosine, 5-(C₂-C₆)-alkenylcytosine, 5-fluorocytosine, 5-chlorouracil, 5-chlorocytosine, 5-bromouracil, 5-bromocytosine, 7-deazaadenine, 7-deazaguanine, 8-azapurine, and 7-deaza-7-substituted purines.

In the case of PNA derivatives which only carry a phosphoryl radical at the C terminus (and for which q is 0), the N terminus can be linked to a peptide sequence. Suitable peptide sequences are preferably those which optimize the organ distribution or the cellular location of the PNA, such as transportan,
5 insulin-like growth factor, nuclear localization signals or other carrier sequences (Larsen et al. (1999) Biochim. Biophys. Acta 159-166). The peptide can also be used as an affinity tag, as, for example, a (His)₆ chain.

The present invention enables the X, X', Z and Z' radicals to be varied broadly
10 (Examples are given in Figures 1a, 1b, 2a, 2b, 3a and 3b) and thereby makes it possible to introduce different specific functional features into the PNA.

A preferred embodiment of Z or Z' is a C₁- to C₂₂-alkyl radical. Preference is also given to C₁- to C₂₂-alkoxy radicals, in particular C₁₆-alkoxy radicals. Other
15 preferred radicals are hydroxy (C₁-C₁₈-alkoxy) radicals, in particular HO(CH₂)₃₋₁₂O. Preference is also given to aminoalkoxy radicals, in particular 6-aminohexoxy and 5-aminopentoxo radicals. Preference is also given to radicals of the formula R₇(CH₂CH₂-O)_m, where R₇ is hydroxyl, amino or C₁-C₂₂-alkoxy, preferably, however, hydroxyl, and m is from 0 to 100, preferably
20 from 2 to 10. Particular preference is given to HO(CH₂CH₂-O)₂, HO(CH₂CH₂-O)₆ and H₂N-(CH₂CH₂-O)₂. Other preferred examples of Z or Z' include mercaptoalkoxy radicals, in particular 6-mercaptohexyloxy.

In another preferred embodiment, Z or Z' comprises a fluorescent group, such
25 as fluorescein, rhodamine, TAMRA or a cyanine dye. Preferred fluorescent groups can be found in Figures 1a to 3b. Very particular preference is also given to Z being biotin. Other preferred groups for Z include Dabcyl, psoralen, acridine, DNP and cholesterol (Figures 1b and 2b), BODIPY, ROX or R6G

radicals (Su-Chun Hung et al. (1998) Analytical Biochemistry 255, 32-38) and digoxigenin (Tarrason et al., Methods in Enzyology (1999) Vol. 313, 257-268).

In addition to this, Z or Z' can be a group consisting of a monofunctional or a
 5 bifunctional fluorescein, rhodamine, TAMRA, biotin, pyrene, dinitrophenyl, cholesteryl, acridine, adamantyl, vitamin E, cyanine dye, Dabcyl, Edans, lexitropsin, or psoralen radical. Monofunctional end groups are listed by way of example in Figures 1a, 1b, 2a and 3a, while bifunctional bridging groups are listed in Figures 2b and 3b. In another preferred embodiment, n and/or m,
 10 independently of each other, are 0, i.e. the PNA moiety carries in each case only one phosphoryl radical on the N and/or on the C terminus.

A preferred embodiment of X or X' is U-(C₂-C₂₂-alkanediyl)-U, in particular O-(C₂-C₂₂-alkanediyl)-O, particularly preferably O-(CH₂)₂₋₆-O. Another preferred
 15 embodiment of X or X' is a group of the formula U-(CH₂CH₂-O)_u', where u' is from 1 to 10, preferably from 1 to 6, and where U is preferably oxy. In a further preferred embodiment, X or X' comprises a fluorescent group such as fluorescein, rhodamine, TAMRA or a cyanine dye, for example Cy3® (from Amersham Pharmacia Biotech). Preferred bifunctional groups can be found in
 20 Figures 2a and 3a. Very particular preference is also given to X or X' being biotin. Other groups which are preferred are Dabcyl, psoralen, acridine, DNP, cholesterol, BODIPY, lexitropsin, digoxigenin, ROX and R6G radicals.

The different radicals for X, X', Z and Z' in formula I can fulfill different functions.
 25 The fluorescein radicals have far-reaching applications in DNA sequencing and signal amplification or as markers for determining the cellular uptake of PNA. The cyanine dye radicals (Cy3® and Cy5®) give a substantially more intense and longer-lasting fluorescence signal than does fluorescein itself. The psoralen radical is employed for crosslinking with complementary nucleic acids. The
 30 acridine radical is an effective intercalator and can thereby augment the binding affinity of the PNA. Biotin, acridine and psoralen derivatives can also be used

for antisense experiments. In addition, hexadecyloxy and cholesterol derivatives can be used for increasing the ability of the PNA to traverse membranes. DNP-labeled compounds of the formula I can be detected using anti-DNP antibodies. Aminoalkoxy radicals can be used for coupling on other groups, for
5 example lexitropsin (cf. Example 17; PNA-16). In a similar way, mercaptoalkoxy groups can also be used for further derivatization.

The invention furthermore relates to the use of the PNA derivatives of the formula I as pharmaceuticals. These pharmaceuticals can be used for
10 preventing and/or treating diseases which are accompanied by the expression or overexpression of particular genes. The invention furthermore relates to the use of PNA derivatives as diagnostic agents. These diagnostic agents can be used for diagnosing the abovementioned diseases at an early stage. When being employed as pharmaceuticals or diagnostic agents, the PNA
15 derivatives of the formula I can be used as antisense agents, anti-gene agents, decoy agents and chimeraplast agents, depending on their sequence.

The PNA derivatives according to the invention are used, in particular, for producing pharmaceuticals for treating diseases in which defined genes are the
20 cause, or are involved, as a result of their overexpression.

These pharmaceuticals can, for example, be used for treating diseases which are provoked by viruses, for example by CMV, HIV, HSV-1, HSV-2, influenza, VSV, hepatitis B or papilloma viruses, with the corresponding virus sequence
25 being the target.

Antisense PNA derivatives according to the invention which are active against these targets have, for example, the following base sequences.

30 a) against CMV, e.g.

SEQ ID NO. 1 5'-G C G T T T G C T C T T C T T G C G-3'

b) against HIV, e.g.

SEQ ID NO. 2 5'-A C A C C C A A T T C T G A A A A T G G -3'

5 SEQ ID NO. 3 5'-A G G T C C C T G T T C G G G C G C C A -3'

c) against HSV-1, e.g.

SEQ ID NO. 4 5'-G C G G G G C T C C A T G G G G G T C G -3'

10

Such pharmaceuticals are also suitable, for example, for treating cancer. In this connection, it is preferably possible to use sequences which are directed against targets which are responsible for the carcinogenesis or the growth of a cancer, in particular by inhibiting the telomerase (E. Matthes et al. (1999)

15 Nucleic Acids Res. 27, 1152). Additional targets of this nature are:

1) Nuclear oncoproteins, such as c-myc, N-myc, c-myb, c-fos, c-fos/jun, PCNA, p120,

20 2) Cytoplasmic/membrane-associated oncoproteins, such as EJ-ras, c-Ha-ras, N-ras, rrg, bcl-2, cdc-2, c-raf-1, c-mos, c-src, c-abl, c-ets,

3) Cell receptors, such as EGF receptor, Her-2, c-erbA, VEGF receptor (KDR-1), retinoid receptors, protein kinase regulatory subunit, c-fms, Tie-2, c-raf-1
25 kinase, PKC-alpha, and protein kinase A (R1 alpha),

4) Cytokines, growth factors and extracellular matrix, such as CSF-1, IL-6, IL-1a, IL-1b, IL-2, IL-4, IL-6, IL-8, bFGF, VEGF, myeloblastin and fibronectin.

30 Antisense PNA derivatives which are active against such targets have, for example, the following base sequences:

a) against c-Ha-ras, e.g.

SEQ ID NO. 5 5'-CAGCTGCAACCCAGC-3'
SEQ ID NO. 6 5'-TATTCCGTCAT-3'
5 SEQ ID NO. 7 5'-TTCCGTCATCGCTCCTCAGGGG-3'

b) bFGF, e.g.

10 SEQ ID NO. 8 5'-GGCTGCCATGGTCCC-3'

c) c-myc, e.g.

15 SEQ ID NO. 9 5'-GGCTGCTGGAGCGGGGCACAC-3'
SEQ ID NO. 10 5'-AACGTTGAGGGGGCAT-3'

d) c-myb, e.g.

SEQ ID NO. 11 5'-GTGCCGGGGTCTTCGGGC-3'

20 e) c-fos, e.g.

SEQ ID NO. 12 5'-CGAGAACATCATCGTGG-3'
SEQ ID NO. 13 5'-GGAGAACATCATGGTCGAAAG-3'
SEQ ID NO. 14 5'-CCCGAGAACATCATGGTCGAAAG-3'
25 SEQ ID NO. 15 5'-GGGGAAAGCCCGGCAAGGGG-3'

f) p120, e.g.

30 SEQ ID NO. 16 5'-CACCCGCCTTGGCCTCCAC-3'

g) EGF receptor, e.g.

SEQ ID NO. 17 5'-G G G A C T C C G G C G C A G C G C -3'

SEQ ID NO. 18 5'-G G C A A A C T T T C T T T T C C T C C -3'

h) p53 tumor suppressor, e.g.

5

SEQ ID NO. 19 5'-G G G A A G G A G G A G G A T G A G G -3'

SEQ ID NO. 20 5'-G G C A G T C A T C C A G C T T C G G A G -3'

i) bcl-2, e.g.

10 SEQ ID NO. 21 5'-T C T C C C A G C G T G C G C C A T -3'

k) VEGF, e.g.

SEQ ID NO. 22 5'-G C G C T G A T A G A C A T C C A T G -3'

15 SEQ ID NO. 23 5'-G G A G G C C C G A C C -3'

SEQ ID NO. 24 5'-G G T T T C G G A G G C -3'

SEQ ID NO. 25 5'-T G G T G G A G G T A G -3'

SEQ ID NO. 26 5'-G C A T G G T G G A G G -3'

SEQ ID NO. 27 5'-T T G G C A T G G T G G -3'

20 SEQ ID NO. 28 5'-G C C T G G G A C C A C -3'

SEQ ID NO. 29 5'-C A G C C T G G G A C C -3'

SEQ ID NO. 30 5'-T G C A G C C T G G G A -3'

SEQ ID NO. 31 5'-G T G C A G C C T G G G -3'

SEQ ID NO. 32 5'-G G T G C A G C C T G G -3'

25 SEQ ID NO. 33 5'-A T G G G T G C A G C C -3'

SEQ ID NO. 34 5'-G G C T T G A A G A T G -3'

SEQ ID NO. 35 5'-G C A G C C C C C G C A -3'

SEQ ID NO. 36 5'-G C A G C A G C C C C C -3'

30 l) c-raf kinase, e.g.

SEQ ID NO. 37 5'-T C C C G C C T G T G A C A T G C A T T -3'

m) PKC-alpha, e.g.

SEQ ID NO. 38 5'-G T T C T C G C T G G T G A G T T T C A-3'

5

n) protein kinase A, e.g.

SEQ ID NO. 39 5'-G C G T G C C T C C T C A C T G G C-3'

10 Pharmaceuticals which comprise PNA derivatives of formula I are furthermore
suitable, for example, for treating diseases which are effected by integrins or
cell-cell adhesion receptors, for example by VLA-4, VLA-2, ICAM, VCAM or
ELAM.

15 Antisense PNA derivatives which are active against such targets have, for
example, the following base sequences:

a) VLA-4, e.g.

SEQ ID NO. 40 5'-G C A G T A A G C A T C C A T A T C-3'

20

b) ICAM-1, e.g.

SEQ ID NO. 41 5'-G C C C A A G C T G G C A T C C G T C A-3'

SEQ ID NO. 42 5'-C C C C C A C C A C T T C C C C T C T C-3'

25 SEQ ID NO. 43 5'-C T C C C C C A C C A C T T C C C C T C-3'

SEQ ID NO. 44 5'-G C T G G G A G C C A T A G C G A G G-3'

c) ELAM-1, e.g.

30 SEQ ID NO. 45 5'-A C T G C T G C C T C T T G T C T C A G G-3'

SEQ ID NO. 46 5'-C A A T C A A T G A C T T C A A G A G T T C-3'

d) Integrin alpha(V), e.g.

SEQ ID NO. 47 5'-G C G G C G G A A A G C C A T C G -3'

- 5 Pharmaceuticals which comprise PNA derivatives of the formula I are also suitable, for example, for preventing restenosis. In this connection, it is possible to use PNA sequences which are directed against targets which are responsible for proliferation or migration. Examples of such targets are:
- 10 1) Nuclear transactivator proteins and cyclins, such as c-myc, c-myb, c-fos, c-fos/jun, cyclins and cdc2 kinase
- 2) Mitogens or growth factors, such as PDGF, bFGF, VEGF, EGF, HB-EGF and TGF- β .
- 15 3) Cell receptors, such as bFGF receptor, EGF receptor and PDGF receptor.

Antisense PNA derivatives which are active against such targets have, for example, the following base sequences:

20

a) c-myb, e.g.

SEQ ID NO. 48 5'-G T G T C G G G G T C T C C G G G C-3'

25

b) c-myc, e.g.

SEQ ID NO. 49 5'-C A C G T T G A G G G G C A T-3'

c) cdc2 kinase, e.g.

30

SEQ ID NO. 50 5'-G T C T T C C A T A G T T A C T C A-3'

d) PCNA (proliferating cell nuclear antigen of rat), e.g.

SEQ ID NO. 51 5'-G A T C A G G C G T G C C T C A A A-3'

- 5 PNA derivatives can likewise be used for treating vitiligo and other depigmentation diseases or depigmentation disturbances (e.g. of the skin, the hair and the eyes), such as albinism and psoriasis, or for treating asthma, with expression of the adenosine A1 receptor, the adenosine A3 receptor or the bradykinin receptor, or of IL-13, being inhibited using suitable antisense agents.
- 10 An example of such a base sequence is:

SEQ ID NO. 52 5'-G A T G G A G G G C G G C A T G G C G G G-3'

- Pharmaceuticals which comprise a PNA derivative of the formula I can be used,
- 15 for example, in the form of pharmaceutical preparations which can be administered orally, for example in the form of tablets, coated tablets, hard or soft gelatin capsules, solutions, emulsions or suspensions. They can also be administered rectally, e.g. in the form of suppositories, or parenterally, e.g. in the form of solutions for injection. In order to produce pharmaceutical
- 20 preparations, these compounds can be processed in therapeutically inert organic and inorganic excipients. Examples of such excipients for tablets, coated tablets and hard gelatin capsules are lactose, cornstarch or derivatives thereof, tallow and stearic acid or salts thereof. Suitable excipients for preparing solutions are water, polyols, sucrose, invert sugar and glucose. Suitable
- 25 excipients for injection solutions are water, alcohols, polyols, glycerol and vegetable oils. Suitable excipients for suppositories are vegetable oils and hydrogenated oils, waxes, fats and semiliquid polyols. The pharmaceutical preparations can also comprise preservatives, solvents, stabilizers, wetting agents, emulsifiers, sweeteners, dyes, flavorants, salts for altering the osmotic
- 30 pressure, buffers, coating agents, antioxidants and, where appropriate, other therapeutic active compounds.

- Preferred administration forms are topical applications, local applications, for example using a catheter or by inhalation, injections or infusions and oral administration. For injection, the PNA derivatives of the formula I are formulated in a liquid solution, preferably in a physiologically acceptable buffer, such as
- 5 Hank's solution or Ringer's solution. However, the oligonucleotides can also be formulated in solid form and dissolved or suspended before use. The doses which are preferred for systemic administration are from about 0.01 mg/kg to about 50 mg/kg of bodyweight and per day.
- 10 The invention furthermore relates to pharmaceutical preparations which comprise PNA derivatives of the formula I and/or their physiologically tolerated salts in addition to pharmaceutically unobjectionable excipients and/or additives. The PNA derivatives of the formula I and/or their physiologically tolerated salts can be administered to animals, preferably to mammals, and in particular to
- 15 humans, as pharmaceuticals, on their own, in mixtures with each other, or in the form of pharmaceutical preparations which permit topical, percutaneous, parenteral or enteral use and which comprise, as the active constituent, an effective dose of at least one PNA derivative together with customary, pharmaceutically unobjectionable excipients and additives. The preparations
- 20 normally comprise from about 0.1 to 90% by weight of the therapeutically active compound. A topical application, for example in the form of ointments, lotions or tinctures, emulsions or suspensions, is preferred for treating skin diseases.
- The pharmaceutical preparations are produced in a manner known per se (e. g.
- 25 Remingtons Pharmaceutical Sciences, Mack Publ. Co., Easton, PA.), with pharmaceutically inert inorganic and/or organic excipients being used. It is possible, for example, to use lactose, cornstarch and/or derivatives thereof, tallow, stearic acid and/or its salts, etc., for producing pills, tablets, coated tablets and hard gelatin capsules. Examples of excipients for soft gelatin
- 30 capsules and/or suppositories are fats, waxes, semisolid and liquid polyols, natural and/or hydrogenated oils, etc. Suitable excipients for producing solutions and/or syrups are, for example, water, sucrose, invert sugar, glucose, polyols,

etc. Suitable excipients for producing solutions for injection are water, alcohols, glycerol, polyols, vegetable oils, etc. Suitable excipients for microcapsules, implants and/or rods are copolymers consisting of glycolic acid and lactic acid. Liposome formulations which are known to the skilled person (N. Weiner, Drug Develop Ind Pharm 15 (1989) 1523; "Liposome Dermatics, Springer Verlag 5 1992), for example HVJ liposomes (Hayashi, Gene Therapy 3 (1996) 878) are also suitable. Dermal application can also be effected, for example, using ionophoretic methods and/or using electroporation.

10 In addition to the active compounds and excipients, a pharmaceutical preparation can also contain additives, such as fillers, extenders, disintegrants, binders, glidants, wetting agents, stabilizers, emulsifiers, preservatives, sweeteners, dyes, flavorants or aromatizing agents, thickeners, diluents and buffering substances, and, furthermore, solvents and/or solubilizing agents 15 and/or agents for achieving a sustained release effect, and also salts for altering the osmotic pressure, coating agents and/or antioxidants. They can also comprise two or more different PNA derivatives of the formula I and/or their physiologically tolerated salts and also, furthermore, in addition to at least one PNA derivative of the formula I, one or more different therapeutically active 20 substances. The dose can vary within wide limits and is to be adjusted to the individual circumstances in each individual case.

The invention furthermore relates to the use of PNA derivatives of the formula I as diagnostic agents, in particular as aids in DNA diagnosis and in molecular 25 biology (see, for example: Peptide Nucleic Acids: Protocols and Applications; Peter E. Nielsen and Michael Egholm (Edit.) Horizon Scientific Press, 1999). In DNA diagnosis, gene probes, also termed DNA probes or hybridization probes, play an important role in the sequence-specific detection of particular genes. In general, a gene probe consists of a recognition sequence and one or more 30 suitable labeling groups (labels). The specificity with which a target sequence in an analytical sample is identified by means of hybridization with a complementary gene probe is determined by the recognition sequence and its

chemical structure. This technique can also be applied to PNA. As compared with oligonucleotides having a natural structure, PNA has the advantage that it has a higher affinity for the target sequence and a greater ability to discriminate between bases.

5

The use of the compounds of the formula I therefore also relates to in-situ hybridization and fluorescence in-situ hybridization (FISH). In-situ hybridization can also be used, for example, for detecting microorganisms and viruses (Just et al. (1998) J. Vir. Method. 73, 163-174). Another application of the compounds
10 of the invention relates to detecting and quantifying nucleic acids. For this, use is preferably also made of array technology (Strother et al. J. Am. Chem. Soc. (2000) 122, 1205-1209; Niemeyer et al., Angew. Chem. (1999) 111, 3039-3043; Pirrung (1997) Chem. Rev. 97, 473-488), which provides high sample throughput and a high degree of sensitivity. In this case, the PNA probes are
15 fixed on a suitable support or PNA chip. To achieve this, PNA can be synthesized as described in the examples and subsequently fixed onto the support or PNA chip. Alternatively, the PNA can be prepared directly on the support. Another application is the use of the compounds of the formula I as biosensors for detecting nucleic acids (Wang et al (1996) J. Am. Chem. Soc.
20 118, 7667). The use of PNA of the formula I possessing an affinity label, such as histidyl-PNA, is another application for purifying nucleic acids (Oerum et al. (1999), in Peptide Nucleic Acids: Protocols and Applications).

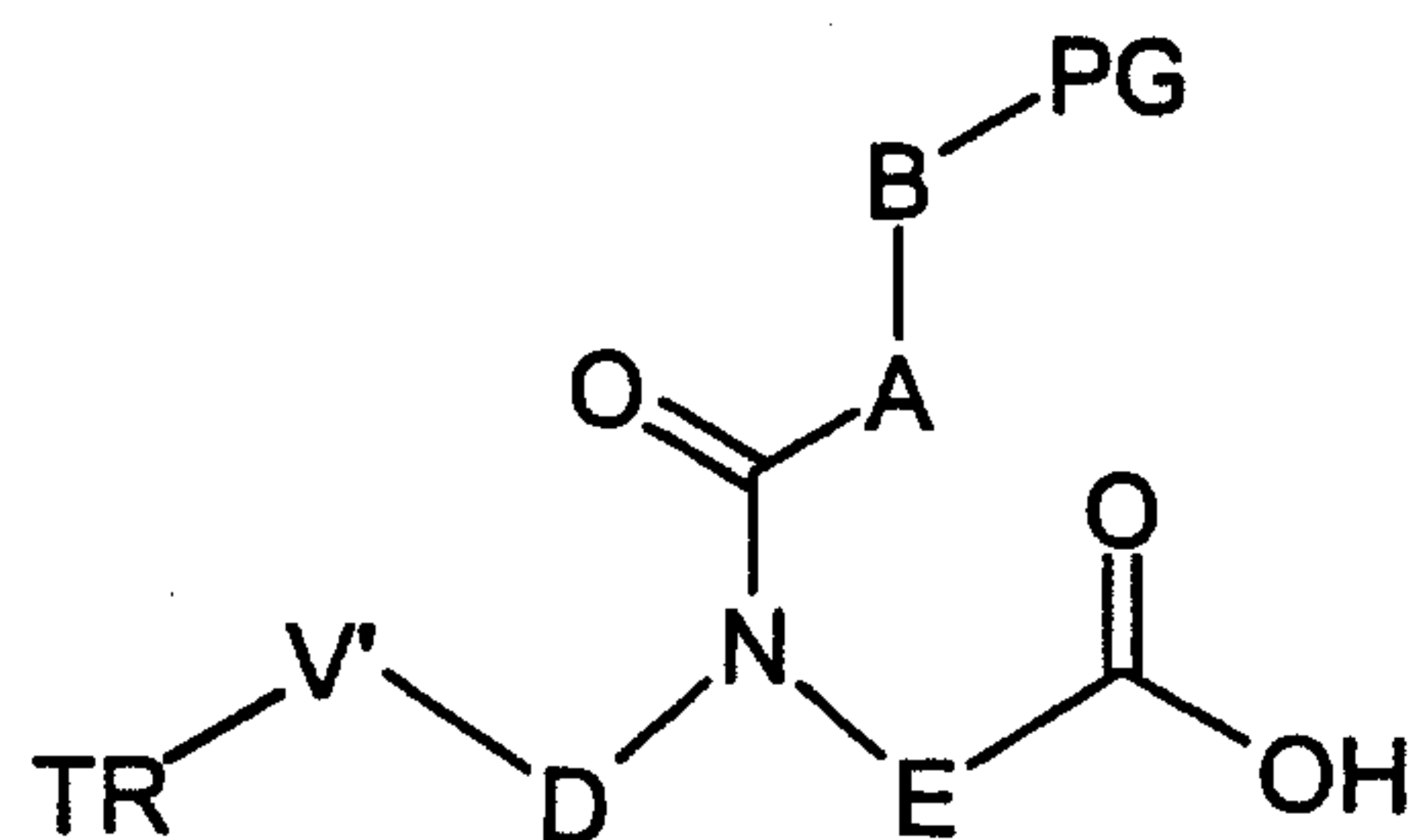
The two phosphoryl radicals at the amino terminus and the carboxy terminus
25 can fulfill different functions. For example the amino terminus can be substituted lipophilically in order to increase the cell uptake, with a fluorescein residue being located at the carboxy terminus for the purpose of detecting the improved cell uptake (cf. PNA-6 in Example 7).

30 The doubly derivatized compounds of the formula I are also suitable for use as so-called "molecular beacons" (Li et al. (2000) Angew. Chemie 112, 1091-1094), which only emit a fluorescence signal in association with binding to a

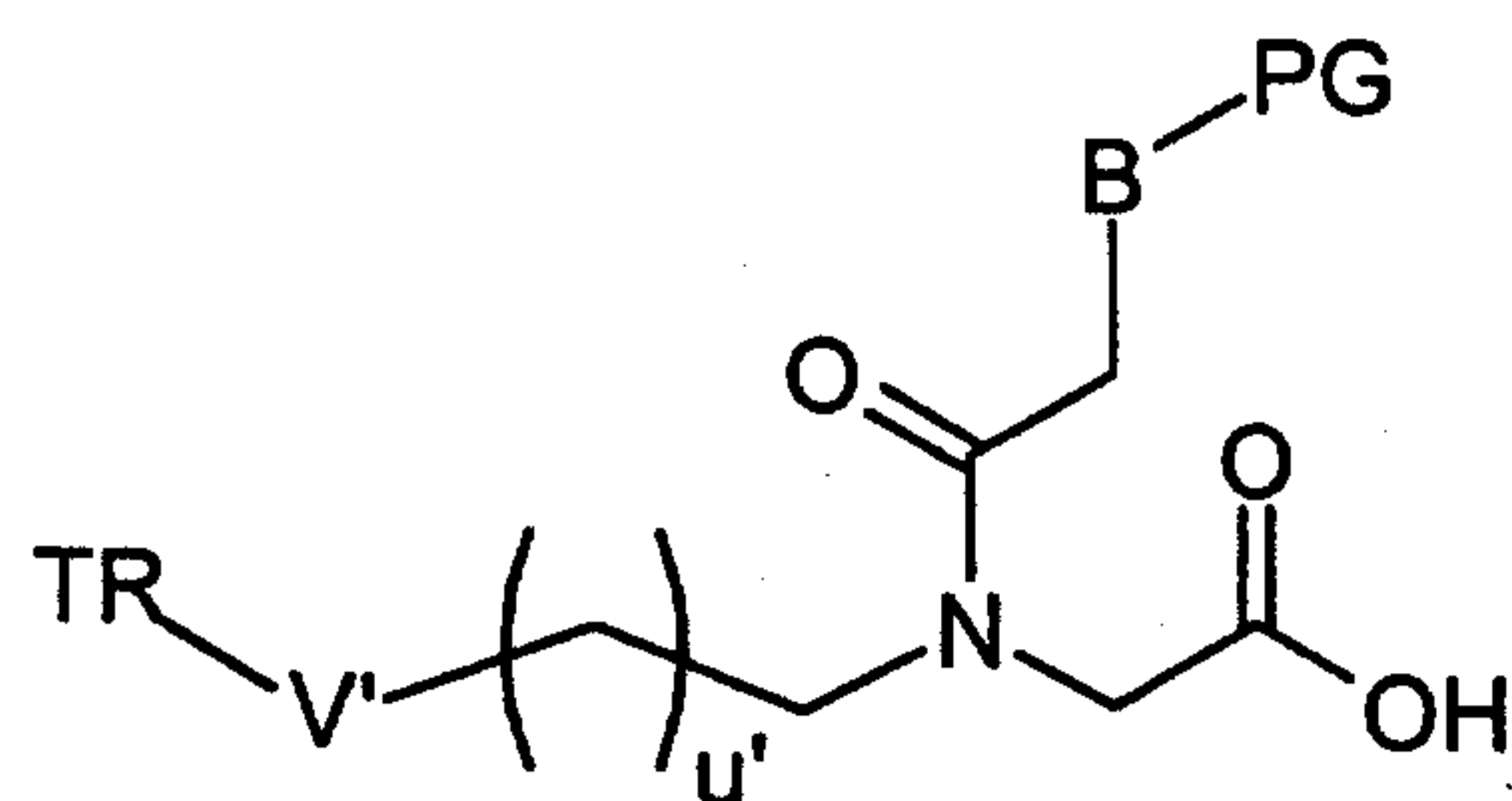
complementary nucleic acid. In these beacons, one end of the PNA, for example the amino terminus, is provided with a fluorescence label whereas the other end, for example the carboxy terminus, is provided with a quencher. The opposite case, in which the N terminus carries a quencher and the C terminus carries a fluorescence label, is also possible. This results in the fluorescence signal being suppressed as long as the doubly labeled PNA derivative does not bind to a complementary nucleic acid. It is only in association with binding that the fluorescence residue (e.g. Edans) and the quencher (e.g. Dabcyl) become spatially separated from each other, resulting in a fluorescence signal being emitted (Sokol et al. (1998) Proc. Natl. Acad. Sci. 95, 11538).

The PNA backbone is synthesized using the methods described in the literature, for example using the tert-butyloxycarbonyl(BOC), 9-fluorenylmethoxycarbonyl (Fmoc) or monomethoxytrityl (Mmt) protecting group strategy (Peptide Nucleic Acids: Protocols and Applications; Peter E. Nielsen and Michael Egholm (Edit.) Horizon Scientific Press, 1999). Preference is given to using the Mmt protecting group for temporarily protecting the amino function of the aminoethylglycine and base-labile protecting groups on the heterocyclic nucleobases (D. Will et al. (1995) Tetrahedron 51, 12069; Breipohl et al. (1997) Tetrahedron 53, 14671-14686). Examples of monomeric building blocks are compounds of the formulae V to V D, with A, B, D, E, u' and V' having the above meaning. PG is an amino-protecting group such as benzoyl, anisoyl-, isobutyryl-, acetyl- or tert-butylbenzoyl (Breipohl et al. (1997) Tetrahedron 53, 14671-14686). TR is an acid-labile protecting group such as dimethoxytrityl (Dmt) (for V' = O and S) or Mmt (for V' = NH).

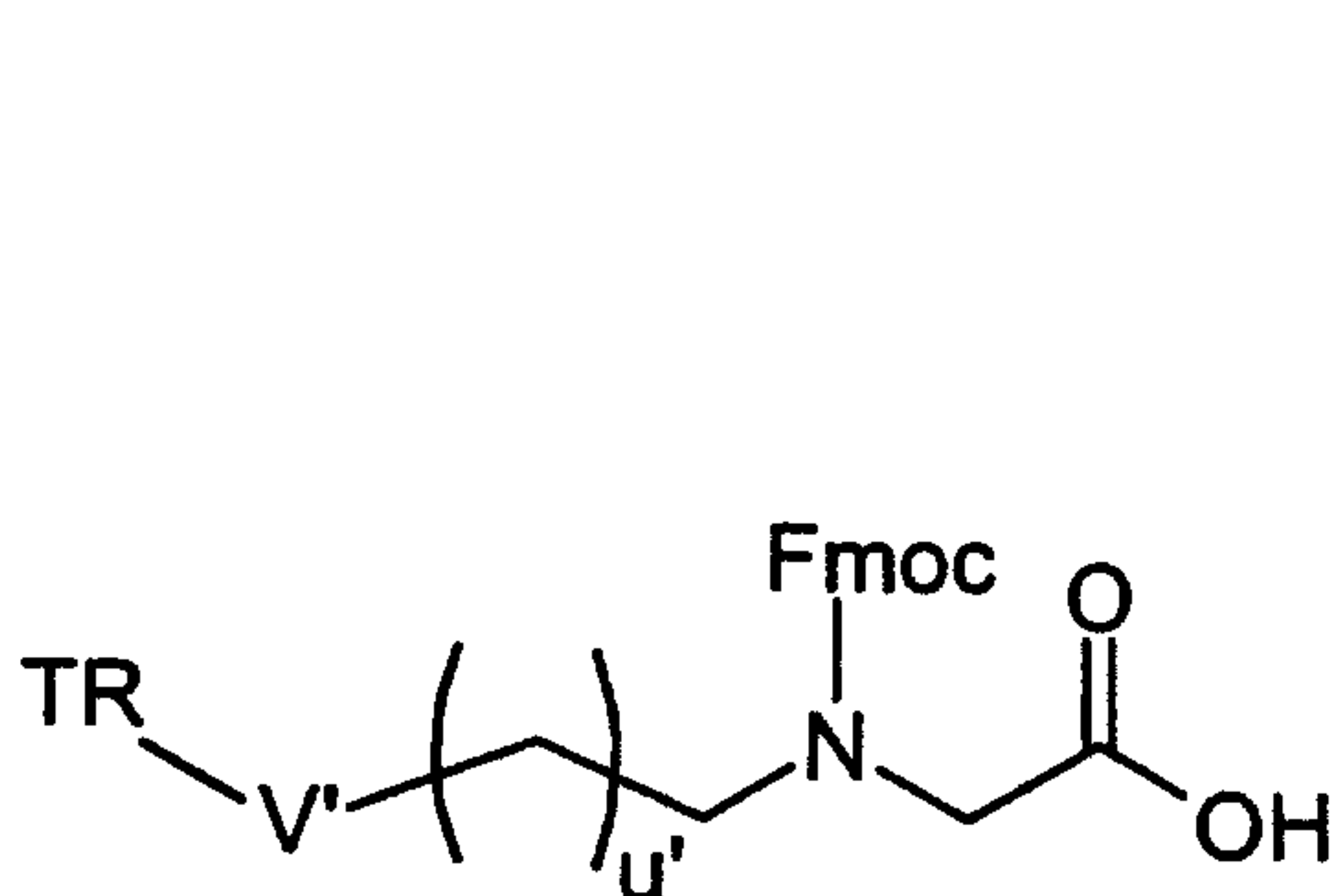
28



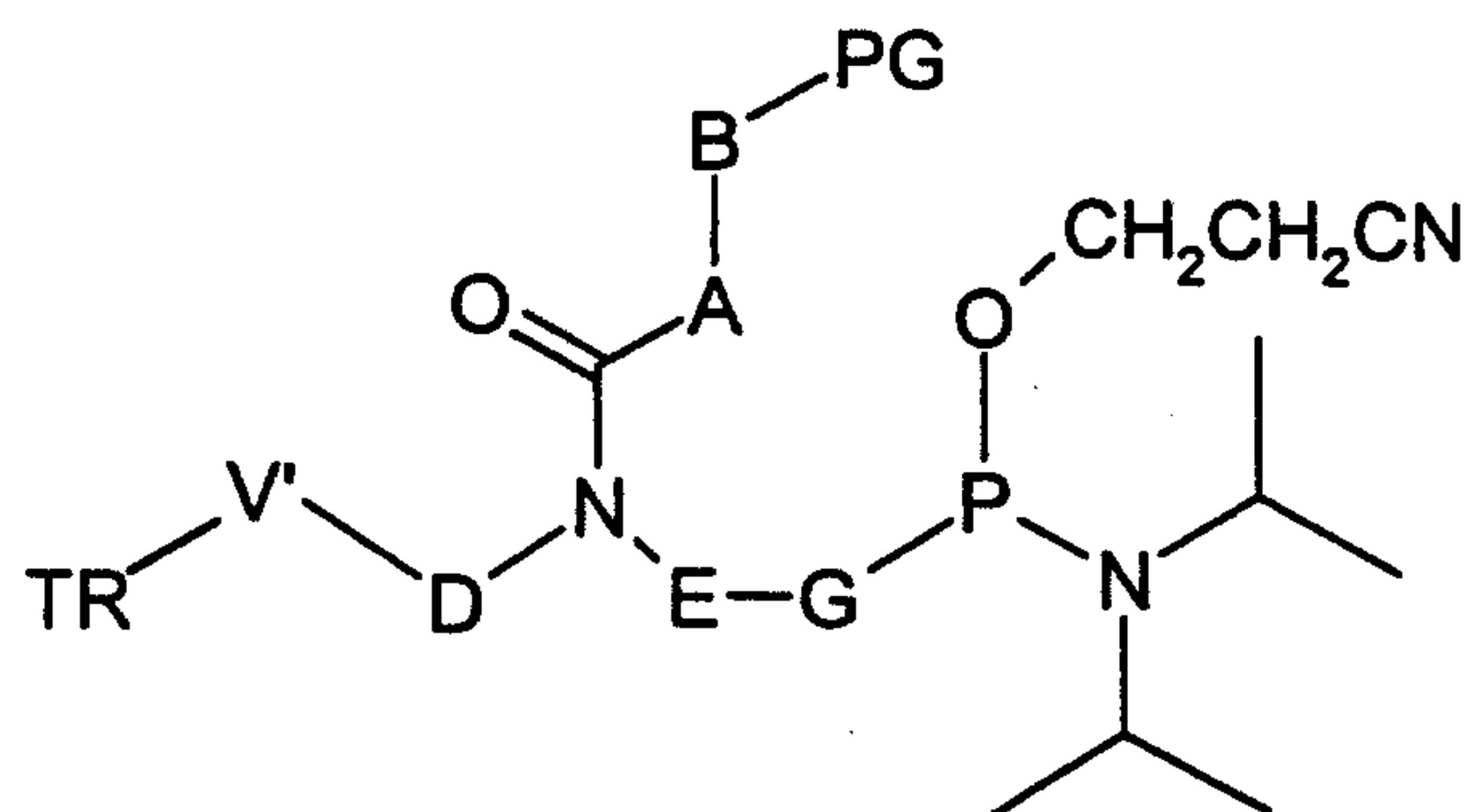
Formula V



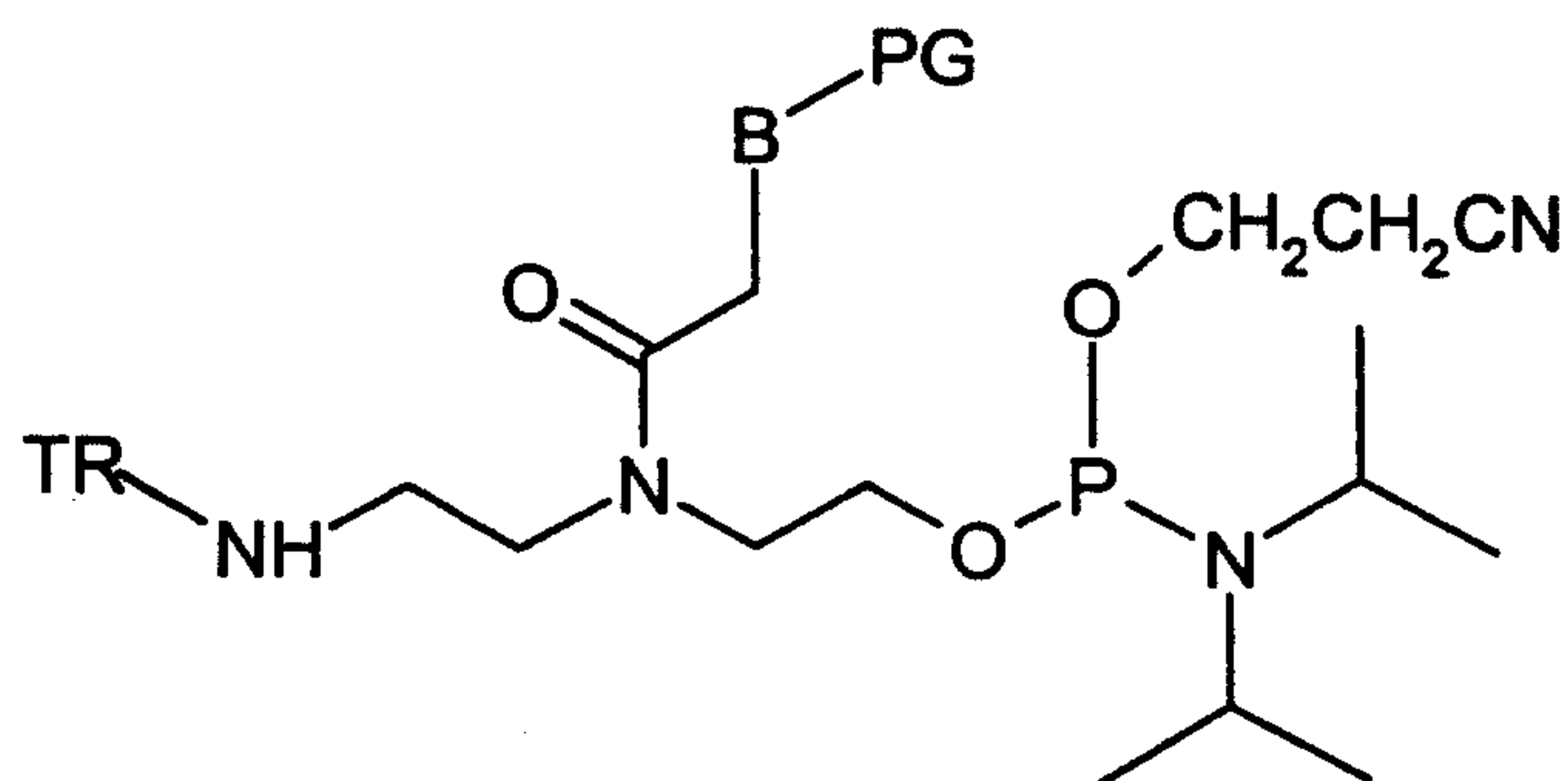
Formula V A



Formula V B



Formula V C



Formula V D

After the PNA backbone has been constructed, the free amino function of the N terminus can be reacted directly with an appropriate phosphorylating reagent, for example to give the corresponding phosphoramidate ($V' = NR_1$ in formula I).

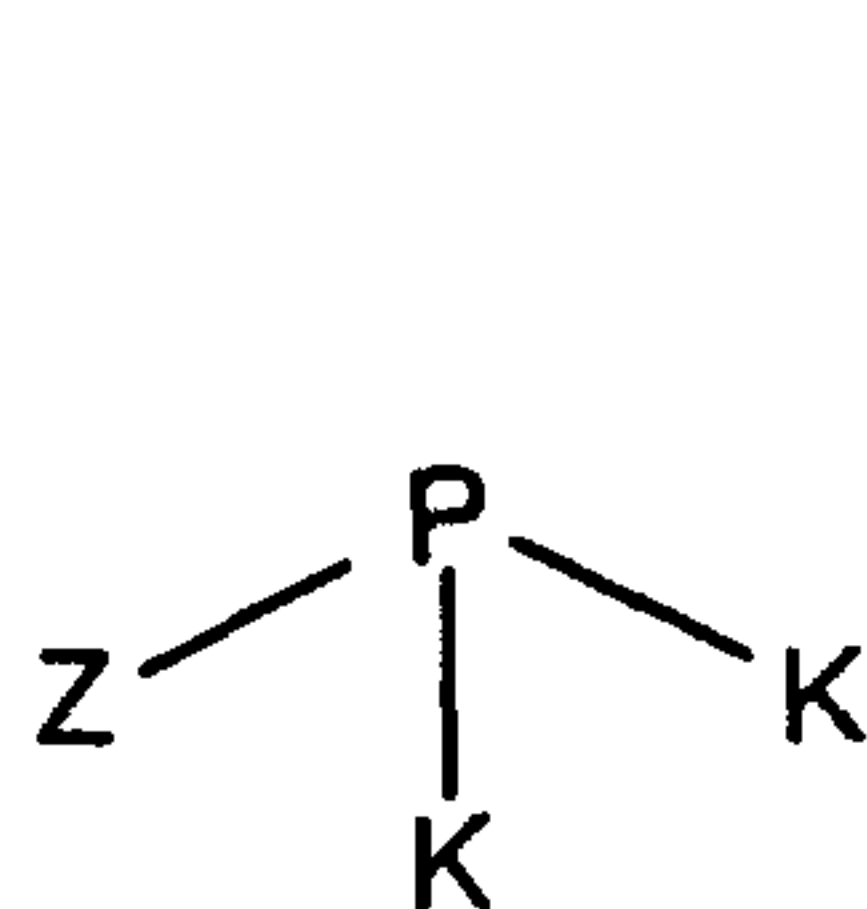
The phosphoryl radicals can be introduced using the reagents which are customarily employed in nucleotide chemistry. There are a large number of phosphorylating reagents available which can be used for preparing the compounds of the formula I. A selection of the reagents is shown in Figures 4a

to 4d, with the invention not, however, being restricted to these special derivatives. Appropriately modified supports, in particular CPG supports for solid phase synthesis, are used for the carboxy-terminal modification. Examples of such supports are listed in Figure 6.

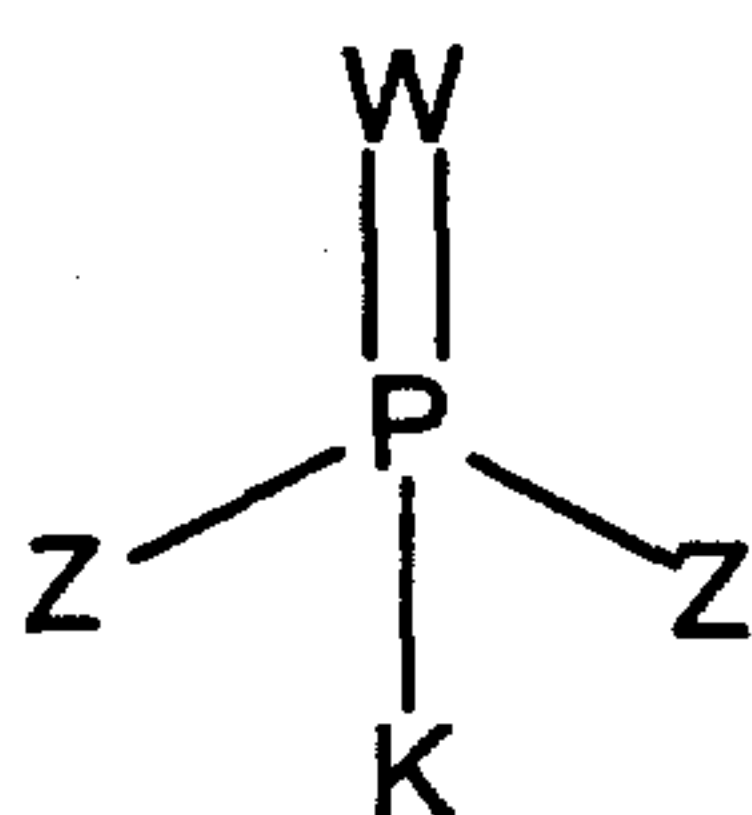
5

The phosphorylating reagents employed can be the reagents which are customary in nucleotide chemistry (Glen Research Corporation, Sterling, VA 20164, U.S.A.; Figures 4a to 4d) and which react, for example, in accordance with the phosphoramidite method, the H-phosphonate method or the
 10 phosphotriester method (E. Sonveaux (1986) Bioorganic Chemistry 14, 274; S.L. Beaucage and R.P. Iyer (1993) Tetrahedron 49, 1925; E. Uhlmann and A. Peyman (1990) Chem. Rev. 90, 543). The wide variety of possible modifications is determined by the large number of known phosphorylating reagents and appropriately derivatized supports, in particular of controlled pore glass (CPG)
 15 supports. ®TentaGel (from Rapp Polymers GmbH, Tübingen) and aminomethylpolystyrene are also preferably used as solid supports.

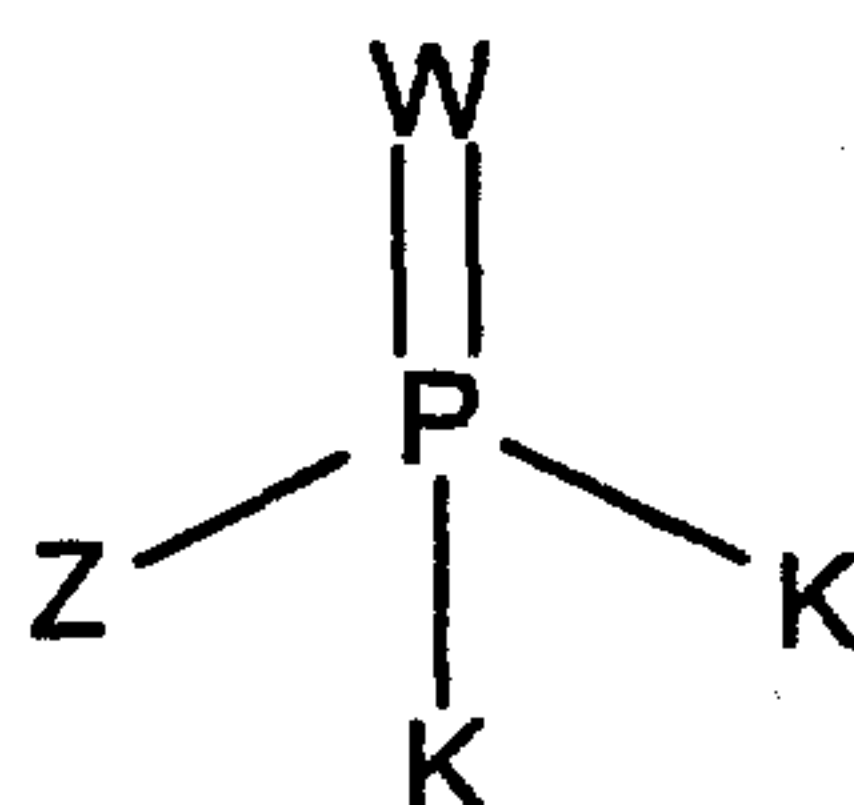
While, in principle, all the reagents which are known in nucleotide chemistry are suitable for introducing the phosphoryl function, those which are particularly
 20 suitable are the following reagents of the formulae VI A, VI B, VI C and VI D



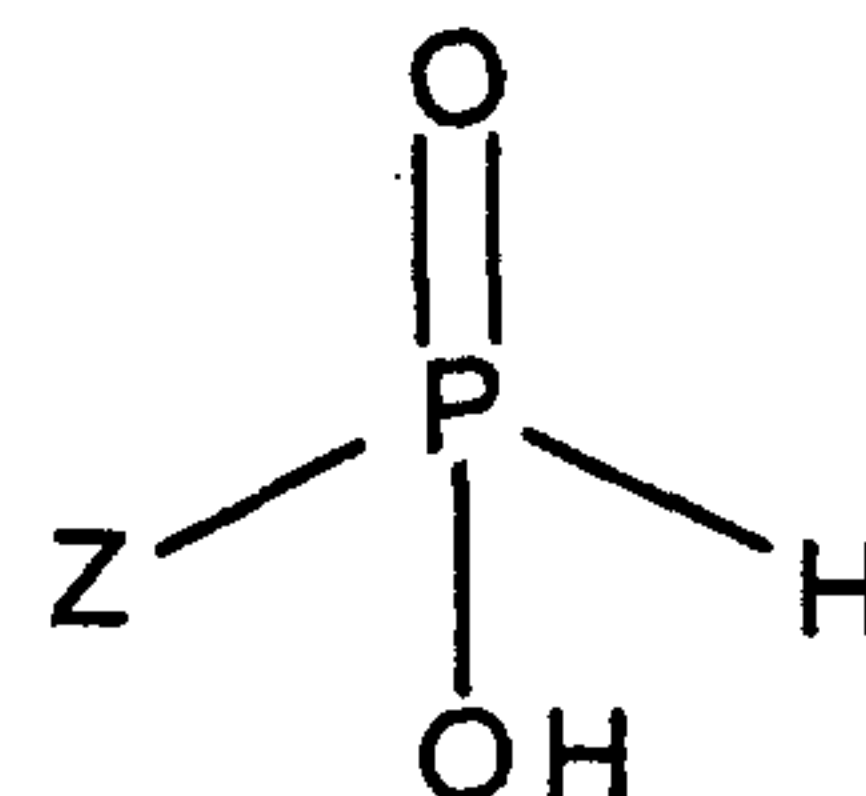
Formula VI A



Formula VI B



Formula VI C



Formula VI D

25 where K is halogen, preferably Cl, triazolyl, imidazolyl or dialkylamino, W can have the abovementioned meaning or the meaning of W', and Z can have the abovementioned meaning or the meaning of X, X' or Z', with reactive groups being appropriately protected.

For example, the hydroxyl groups of the fluorescein-phosphoramidite 3 (Figure 4a) are protected by esterifying with pivalic acid.

5 The compounds of the formula VI are only to be regarded as being examples of such reagents, which react, where appropriate, in the added presence of other auxiliary reagents such as bases, acids or condensing reagents. Particular preference is given to the reagents of the formula VI A, which react in accordance with the phosphoramidite method (Beaucage and Iyer, 1993). These reagents are reacted as the phosphorus (III) compound and
10 subsequently oxidized. If, for example, the oxidation is carried out using iodine/water/pyridine or tert-butyl hydroperoxide, the phosphoryl derivatives ($W = O$) are then obtained. If, on the other hand, the oxidation is carried out using elemental sulfur or Beaucage reagent, the corresponding thiophosphoryl compound ($W = S$) is then obtained.

15 Among the reagents (Figures 4a to 4d), are also to be found "bifunctional reagents" which, because of the possession of a second function, which is initially protected, can be caused to react several times. The phosphoramidites 4, 6 and 8 to 13 are examples of such bifunctional reagents. In this connection,
20 it can be a matter of the multiple conjugation of a reagent or else of successive reaction with different reagents. Thus, for example, the fluorescein-phosphoramidite 3 can only be caused to react once. By comparison, the fluorescein-phosphoramidite 4 possesses a Dmt group-protected hydroxyl function which can be reacted once again with a phosphorylating reagent after
25 the Dmt group has been eliminated. In this way, it is possible to introduce one and the same group or else different groups several times. PNA-6 is an example of a multiple conjugation at the carboxy terminus and an additional modification at the amino terminus. The fluoroscein and the amino linker were firstly synthesized successively at the carboxy terminus. After the PNA moiety
30 had been synthesized, a hydroxyethylglycine-t building block was coupled on in the last cycle, with this building block being reacted with C16-phosphorylating reagent 7. PNA-1 and PNA-2 are compounds of the formula I which are only

modified with a phosphoryl radical at the carboxy terminus ($q = 0$). This substance class is likewise novel and part of the subject-matter of the invention. Figures 5a and 5b show some examples of compound types for the N-terminal modification of the compounds of the formula I. Compound type A is obtained
5 by reacting the terminal hydroxyl group of the PNA with the phosphorylation reagent 1. Compound type B is obtained by reacting the terminal amino group of the PNA with the biotin-phosphoramidite 5. Compound type C is obtained by successively reacting the PNA having a terminal hydroxyl group with the spacer-18 phosphoramidite 9, amino modifier-5 phosphoramidite 12 and lexitropsin.
10 Compound type D is obtained by successively reacting the PNA having a terminal hydroxyl group with the spacer-9 phosphoramidite 8 and the cyanine-3 phosphoramidite 10. Compound type E is obtained by successively reacting the PNA having a terminal hydroxyl group with the bifunctional fluorescein-phosphoramidite 4, the spacer-9 phosphoramidite 8, and the C16-phosphorylating reagent 7. The steps which additionally have to be carried out, such as oxidation and protecting group elimination are described in the examples.

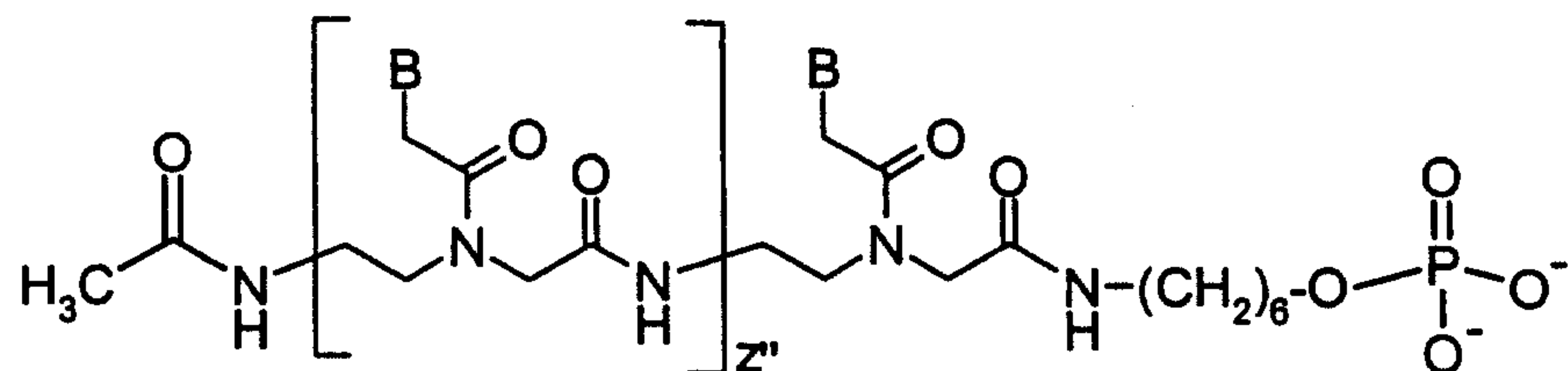
An example of a carboxy-terminal modification of PNA obtained using a
20 phosphoramidite of the formula V D is depicted in Figure 7. In this case, the starting material is a bishydroxyethylsulfone support I (Figure 6), which, after the Dmt group has been eliminated with 3% trichloroacetic acid, is reacted with the phosphoramidite of the formula V D using tetrazole as catalyst. After oxidizing with iodine water, the amino-terminal Mmt group is eliminated with 3%
25 trichloroacetic acid and the PNA moiety is then synthesized using methods known from the literature, for example using the Mmt method which is explained below. An alternative method for the carboxy-terminal modification uses CPG supports which are modified in accordance with the radical to be introduced, and consequently contain the fluorescein radical, for example (Figure 8). This
30 method will be explained using the example of a PNA derivative which is modified amino-terminally with a hexadecyl phosphate radical and carboxy-terminally with a fluorescein phosphate. The fluorescein support 3 (Figure 6) is

first of all detritylated with trichloroacetic acid and then condensed with the amino modifier C6 phosphoramidite 13 (Figure 4d) using tetrazole. After oxidizing with iodine water and eliminating the Mmt group, the PNA moiety can be synthesized using customary methods. In the last cycle, a hydroxyethyl-glycine-based PNA building block (formula V A, $u' = 2$, $V' = \text{oxygen}$) is coupled on, with this building block being reacted as shown in Figure 9 after eliminating the Dmt protecting group using the C16 phosphorylating reagent 7. The doubly modified PNA derivative is obtained after eliminating all the protecting groups and cleaving from the CPG support.

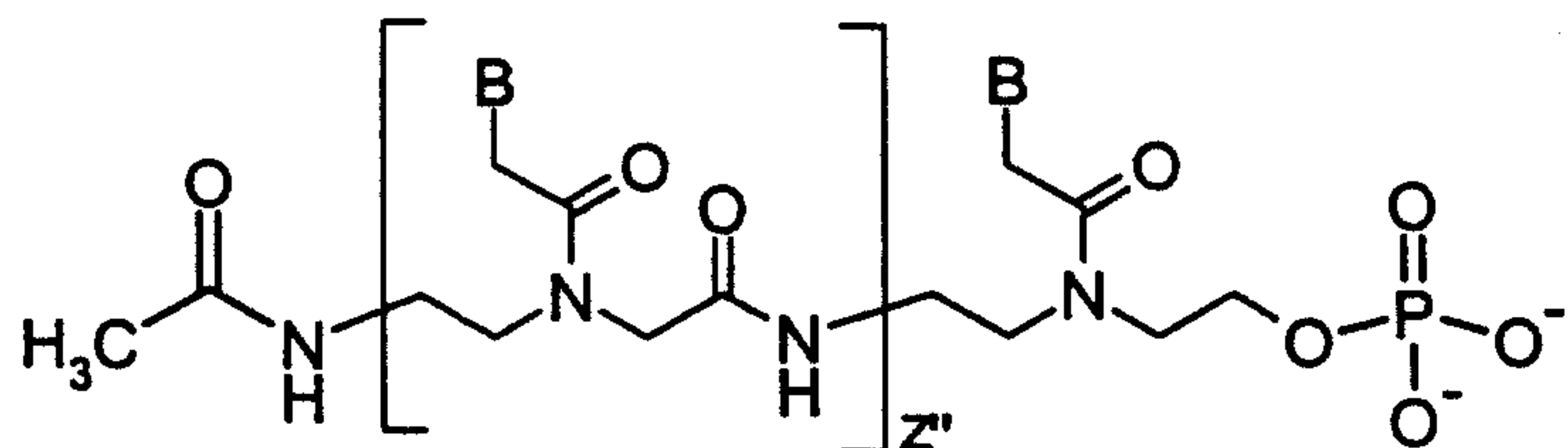
Examples:

The preparation of the following compounds is described by way of example:

5 PNA-1:

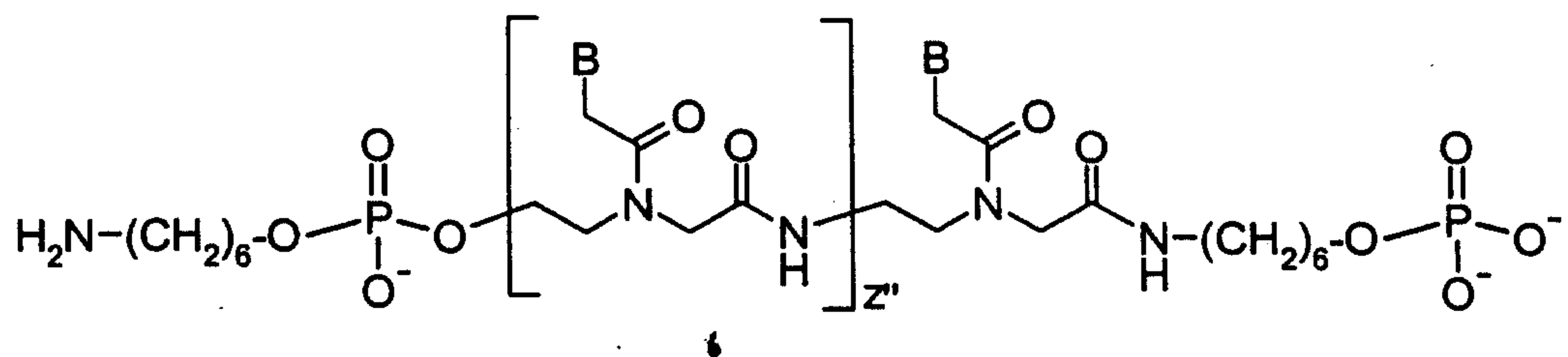


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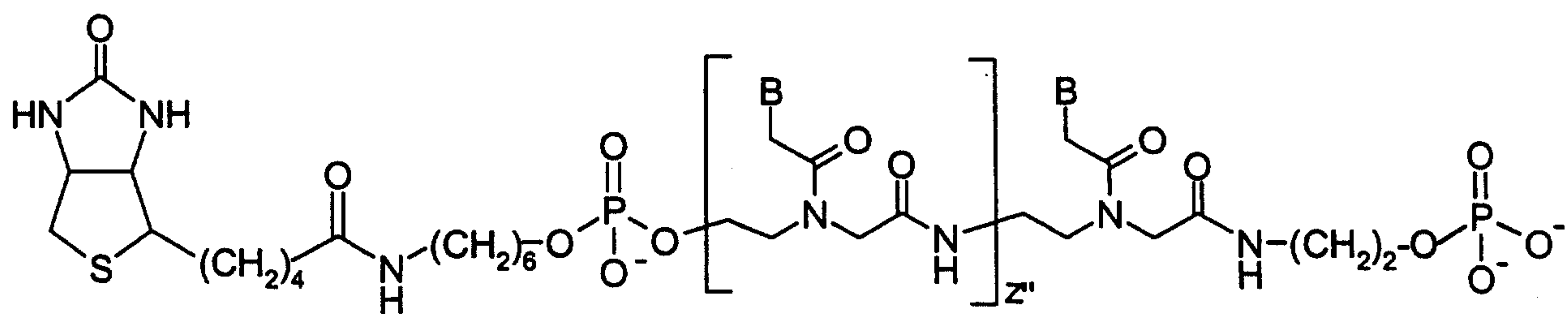
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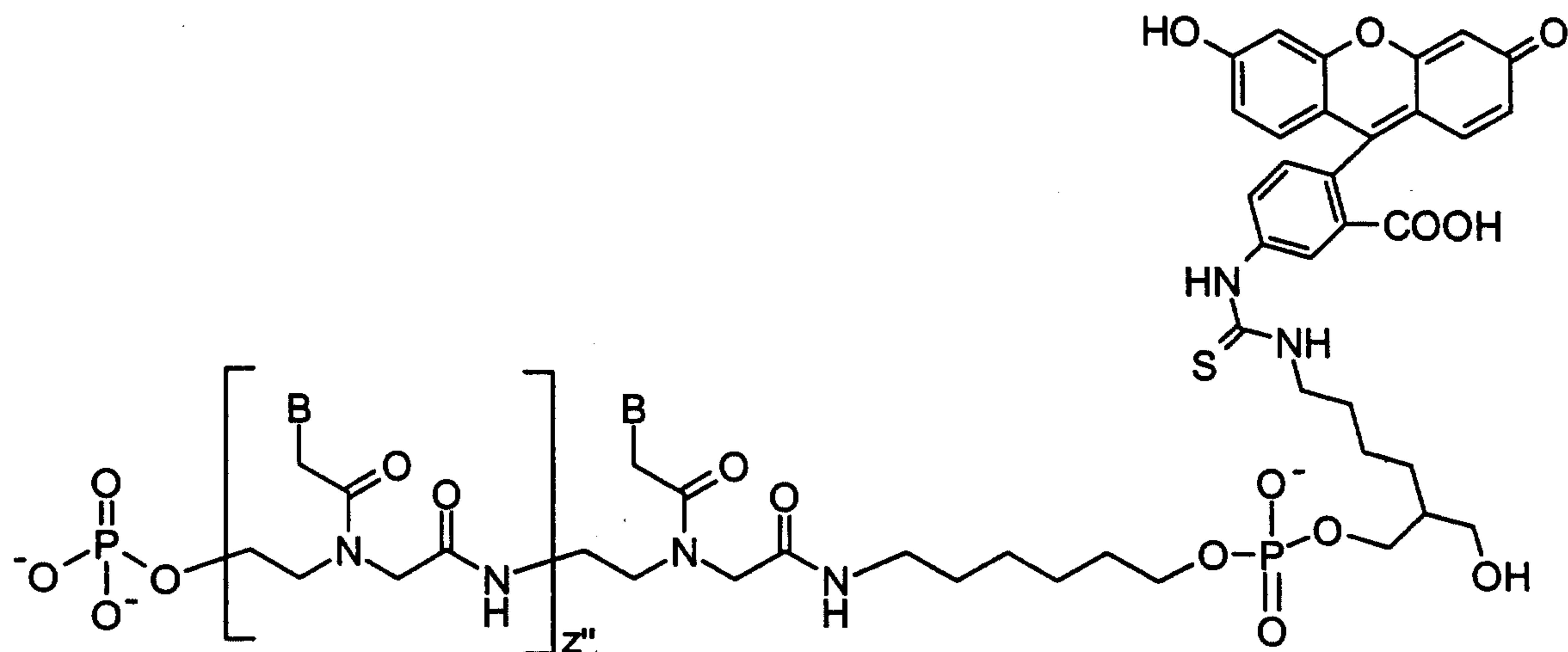


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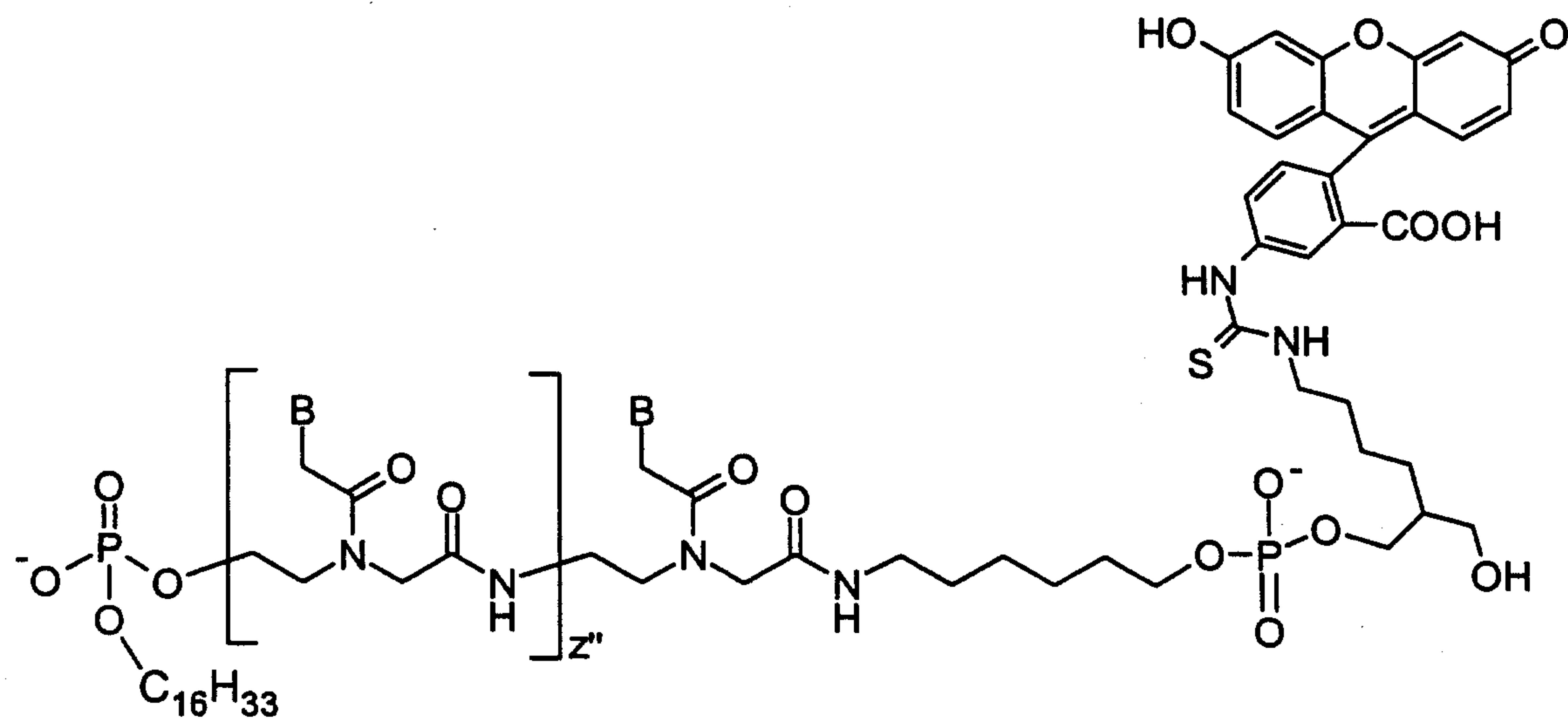
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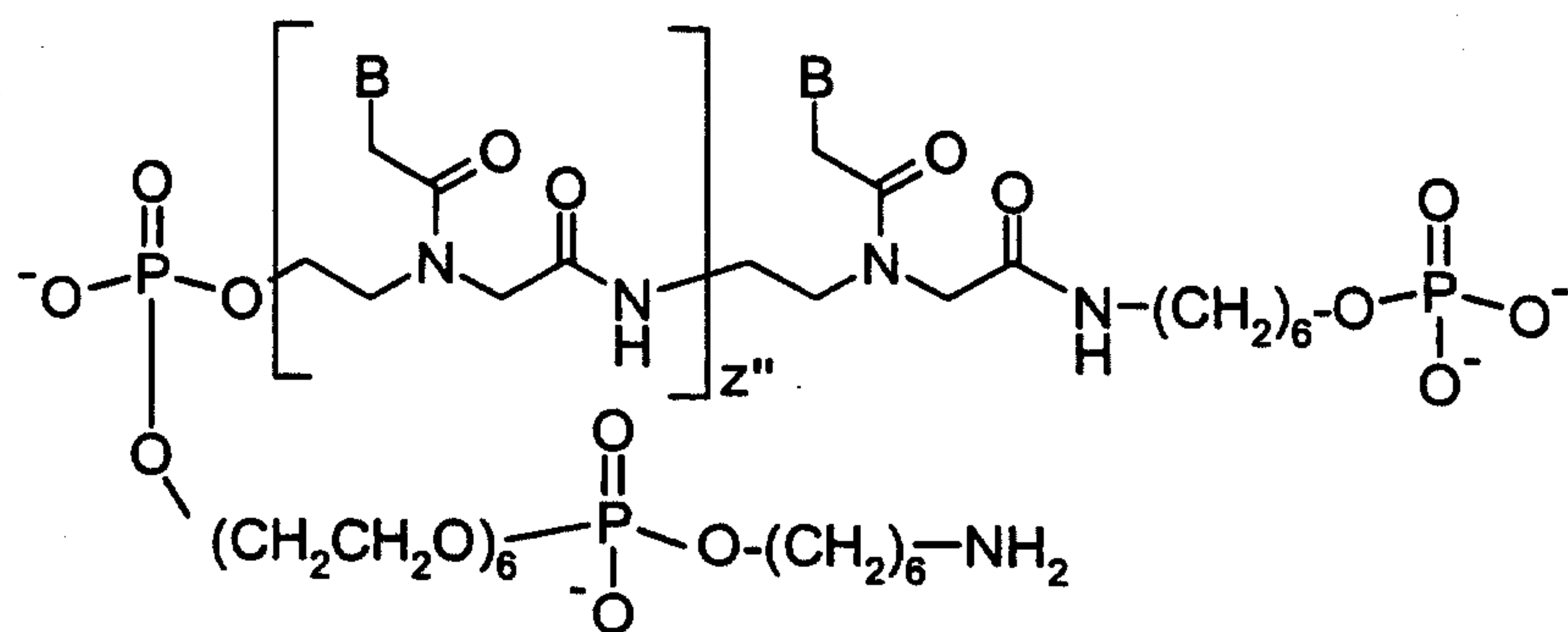


PNA-6:



5

PNA-7:



10 where the sequences of the 13 bases are in each case described by SEQ ID NO. 53, and z" in each case is 10:

SEQ ID NO. 53 5'-T A T T C C G T C A T-3' (PNA-1 to PNA-7)

Example 1: Synthesizing the PNA chain

5

The following reagents were used for preparing the PNA moiety:

1. Phosphoramidite reagent (0.1 M in acetonitrile (ACN))
2. Mmt-PNA monomers and/or Dmt-oeg-PNA monomers (0.2 M in DMF:ACN (1:1; v:v))
- 10 3. Anhydrous ACN (≤ 30 ppm of water)
4. Trichloroacetic acid (3%) in dichloromethane (DCM)
5. Acetic anhydride, 2,6-lutidine in THF (1:1:8; v:v:v); (Cap A)
6. N-Methylimidazole (16%) in THF; (Cap B)
7. Iodine solution (0.05 M) in THF, water, pyridine (7:2:1; v:v:v)
- 15 8. Washing solution (THF, water, pyridine (7:2:1; v:v:v))
9. Tetrazole (0.3 M) in ACN
10. HBTU; 0.2 M in DMF:ACN (1:1; v:v)
11. DIPEA; 0.2 M in DMF:ACN (1:1; v:v)
12. DMF ($> 99.5\%$)
- 20 13. Solid phase support: aminopropyl-CPG (550 Å) loaded with Mmt-amino-hex-1-yl hemisuccinate (for PNA-hexylamides).

The Mmt/acyl-protected or Dmt/acyl-protected oeg monomers were prepared as has already been described (Breipohl et al. (1997) Tetrahedron 53, 14671-14686). The loading of aminopropyl-CPG with the Mmt-amino-hex-1-yl hemisuccinate has likewise already been described (Will et al. (1995) Tetrahedron 51, 12069-12082). The derivatized CPG supports are commercially available (Glen Research Corporation, Sterling, VA 20164, U.S.A.). The PNA syntheses were in general carried out on a scale of from 2 to 5 μmol .

30

The following cycle was used for the PNA synthesis:

1. Step of washing with ACN
2. Deprotecting the Mmt group or the Dmt group by treating with 3% TCA in DCM; 110 sec.
3. Step of washing with DMF/ACN (1:1)
- 5 4. Neutralizing with DIPEA in DMF/ACN (1:1)
5. Coupling on the monomeric building block by preactivating (15 min) with HBTU/DIPEA/PNA monomer (ratio 1:1:1; total volume 450 μ l) charging the solid phase and coupling (45 min)
6. Step of washing with ACN
- 10 7. Capping with acetic anhydride/N-methylimidazole
8. Step of washing with ACN
9. New cycle

Example 2: Synthesizing acetyl-tat tcc gtc at-aminohexyl-p (PNA-1)

15

The Dmt protecting group is first of all eliminated from the bishydroxyethylsulfonfyl support 1 (1 μ mol, Figure 6) by treating with 3% trichloroacetic acid. The free hydroxyl function is then reacted with the amino modifier C6 phosphoramidite 13 (Figure 4d) using tetrazole as catalyst. The reaction employs an excess of the phosphorylating reagent 13 (approx. 25-fold), as an 0.3 M solution in acetonitrile/tetrahydrofuran (1:1; v:v), and the tetrazole (approx. 50-fold; 0.5 M in acetonitrile). After the condensation has taken place, oxidation is effected using an iodine solution (0.05 M in tetrahydrofuran/water, pyridine (7:2:1; v:v:v)). After that, the PNA moiety is prepared by solid phase synthesis as described in Example 1. In the last cycle, the free amino function is acetylated by treating with the capping reagent. This prevents the PNA from being degraded amino-terminally during deprotection with conc. ammonia. Finally, the PNA is cleaved from the support, and the protecting groups are removed at the same time, by treating with conc. ammonia at 50°C overnight.

30 103 OD (260) of the desired crude product are obtained, with this crude product being purified by preparative polyacrylamide (PAA) gel electrophoresis. The desired product band is eluted with 0.2 M triethylammonium bicarbonate buffer

and desalted through a Bond-Elute C 18 column (1 g). 23.3 OD are obtained. The product was analyzed by negative ion mass spectrometry, which confirmed the calculated mass (calc. 3166.2; found 3166.8).

5 Example 3: Synthesizing acetyl-tat tcc gtc at(eo)-p (PNA-2)

The preparation is effected, in a 1 μ mol synthesis, in an analogous manner to that described in Example 2. After the Dmt protecting group has been eliminated from the support 1 (Figure 6), the free hydroxyl function is reacted
10 with the phosphoramidite of the formula V D using tetrazole as catalyst. The reaction employs an excess of the phosphoramidite (approx. 20-fold), as a 0.1 M solution in acetonitrile/tetrahydrofuran (1:1; v:v) and the tetrazole (approx. 50-fold; 0.5 M in acetonitrile). After the condensation has taken place, oxidation is effected using an iodine solution (0.05 M in tetrahydrofuran/water, pyridine
15 (7:2:1; v:v:v)). 50 OD of crude product are obtained after cleaving with ammonia. 45 OD of this crude product are purified by electrophoresis through a 15% PAA gel. 13.2 OD of product, having a molecular weight of 3052.9 (calc. 3052.9), are obtained.

20 Example 4: Synthesizing aminohexyl-p-t(oeg) at tcc gtc at-aminohexyl-p (PNA-3)

The preparation is effected, in a 1 μ mol synthesis, in an analogous manner to that described in Example 2. However, after the carboxy terminus and the PNA
25 moiety had been synthesized, a hydroxyethylglycine-based building block having thiamine as the nucleobase (oegT) is coupled on in the last cycle. After the Dmt group has been eliminated, the free hydroxyl function is coupled to the amino modifier C6 phosphoramidite 13 (Figure 4d) using tetrazole as catalyst and subsequently oxidized with iodine water. The oligomer is cleaved from the
30 support, and all the base-labile protecting groups are removed at the same time, by treating with conc. ammonia at 50°C. The terminal Mmt protecting group is then removed by treating with 80% acetic acid. 130 OD of the crude

product are obtained, with this group product being purified by gel electrophoresis. 22.5 OD of product, having a molecular weight of 3303.8 (calc. 3305.0), are obtained.

5 Example 5: Synthesizing biotin-p-t(oeg) at tcc gtc at-aminohexyl-p (PNA-4)

The preparation is effected, in a 0.5 μ mol synthesis, in an analogous manner to that described in Example 2. However, after synthesizing the carboxy terminus and the PNA moiety, a hydroxyethylglycine-based building block having
10 thiamine as the nucleobase (oegT) is coupled on in the last cycle. After eliminating the Dmt group, the free hydroxyl function is coupled to the biotin phosphoramidite 5 (Figure 4b) using tetrazole as catalyst and subsequently oxidized with iodine water and detritylated with trichloroacetic acid. The oligomer is cleaved from the support, and all the protecting groups are removed at the
15 same time, by treating with conc. ammonia at 50°C. 37 OD of the crude product are obtained, with this crude product being purified by gel electrophoresis. 22.5 OD are obtained.

20 Example 6: Synthesizing p-t(oeg) at tcc gtc at-aminohexyl-p-fluorescein (PNA-5)

The synthesis is effected in analogy with Example 2 proceeding from the fluorescein-support 3 (Figure 6a and 8). The Dmt protecting group is eliminated from the fluorescein-support 3 by treating with 3% trichloroacetic acid. The free hydroxyl function is then reacted with the amino modifier C6
25 phosphoramidite 13 (4d) using tetrazole as catalyst. After condensation has taken place, oxidation is effected using an iodine solution (0.05 M in tetrahydrofuran/water, pyridine (7:2:1; v:v:v)). After that, the PNA moiety is prepared by solid phase synthesis as described in Example 1. A hydroxyethylglycine-based building block having thiamine as nucleobase
30 ((t)oeg) is coupled on in the last cycle. After eliminating the Dmt group, the free hydroxyl function is coupled to the phosphorylating reagent 1 (Figure a) using tetrazole as catalyst and subsequently oxidized with iodine water. Finally, the

PNA is cleaved from the support, and the protecting groups are removed at the same time, by treating with conc. ammonia at 50°C overnight. 61 OD (260) of the crude product are obtained; with this crude product being purified by preparative polyacrylamide (PAA) gel electrophoresis. The desired product
5 band is eluted with 0.2M triethylammonium bicarbonate buffer and desalted through a Bond-Elut C18 column (1 g). 5.6 OD are obtained. The product was analyzed by negative ion mass spectroscopy, which showed the calculated mass (calc. 3709.5; found 3706.3).

10 Example 7: Synthesizing C16-p-t(oeg) at tcc gtc at-aminohexyl-p-fluorescein (PNA-6)

The synthesis is effected in analogy with Example 6 starting from 1 µmol of fluorescein support 3 (Figures 6a and 8). A hydroxyethylglycine-based building
15 block having thiamine as the nucleobase ((t)oeg) was coupled on in the last cycle. However, after eliminating the Dmt group, the free hydroxyl function is coupled to the C16 phosphorylating reagent 7 (Figure 4c) using tetrazole as catalyst and subsequently oxidized with iodine water. Finally, the PNA is eliminated from the support, and the protecting groups are removed at the same
20 time, by treating with conc. ammonia at 50°C overnight. 61 OD (260) of the desired crude product are obtained, with this crude product being purified by preparative polyacrylamide (PAA) gel electrophoresis. The desired product band is eluted with 0.2M triethylammonium bicarbonate buffer and desalted through a Bond-Elut C18 column (1 g). 4.6 OD are obtained. The product was
25 analyzed by negative ion mass spectrometry, which showed the calculated mass (calc. 3934, found 3931).

Example 8: Determining the melting temperatures

30 The melting temperatures were determined using an HP 8452A diode-array spectrophotometer, an HP 89090A Peltier element and HP Temperature Control Software Rev. B5.1 (from Hewlett Packard). Measurement takes place

in 0.5°C/min steps in 140 mM KCl, 10 mM sodium dihydrogen phosphate, 0.1 mM EDTA (pH 7.4) as the buffer. The oligomer concentration is from 0.5 to 1 OD₂₆₀ per ml.

- 5 Surprisingly, the doubly phosphoryl-modified PNA-5 and PNA-6 derivatives having two or three negative charges exhibited an equally good or better degree of binding towards complementary DNA and RNA than did the uncharged PNA (reference substance).

PNA derivative		T _m (DNA)	T _m (RNA)
Reference	Ac-HN-tat tcc gtc at-hex	41.9°C	56.6°C
PNA-5	p-t(oeg) at tcc gtc at-aminohexyl-p-fluorescein	41.8 °C	56.9 °C
PNA-6	C16-p-t(oeg) at tcc gtc at-aminohexyl-p-fluorescein	44.1 °C	56.9 °C

10

Example 9: Determining cell uptake after fluorescence labelling

COS cells are allowed to grow to confluence in Dulbecco's MEM, which has been supplemented with 10% FCS, in 5 cm Petri dishes. The cells are washed
 15 twice with serum-free DMEM. An area of approx. 1 cm² is scratched out in the middle of the Petri dish using a sterile needle. The PNA solution (10 µM) under investigation is applied in this area. The dish is incubated at 37°C under a CO₂ atmosphere. After 2, 4 and 16 hours, the cells are examined by fluorescence microscopy. For this, the cells are washed four times with serum-free DMEM,
 20 covered with a cover slip and evaluated under the fluorescence microscope or by phase contrast. PNA-5 and PNA-6 were examined by fluorescence microscopy.

In this connection, it was found that the hexadecyl-PNA derivative (PNA-6) was
 25 taken up more efficiently into the cells than the PNA with no hexadecyl radical.

Example 10: Inhibiting cell proliferation with PNA-6

The sequence of PNA-6 is directed against the translation start of the Ha-ras mRNA. REH cells (human pre-B leukemia cells, DSM ACC 22) or A549 tumor
5 cells were cultured, at 37°C and under 5% CO₂, in OptiMEM (Gibco BRL) containing 10% fetal calf serum (FCS, GIBCO-BRL). The cell density for the assay was approx. 1×10^6 /ml. The PNA-6 (10 µM) was incubated with the cells in 24-well plates. After incubating at 37°C and under 5% CO₂ for 96 hours, the cell density was determined. Mean values for the cell density were determined
10 from 3 individual wells at a given PNA concentration. It was found that PNA-13 inhibits proliferation of the REH cells. After > 4 days of incubation, the inhibition brought about by PNA-6 is greater than that brought about by a corresponding phosphorothioate oligonucleotide.

15 **Example 11: Synthesizing aminohexyl-p-spacer18-p-t(oeg) at tcc gtc at-aminohexyl-p (PNA-7)**

The preparation is effected, in a 1 µmol synthesis, in an analogous manner to that described in Example 2. However, after the carboxy terminus and the PNA
20 moiety had been synthesized, a hydroxyethylglycine-based building block having thymine as the nucleobase (oegT) is coupled on in the last cycle. After eliminating the Dmt group, the free hydroxyl function is coupled to the spacer 18 phosphoramidite (Figure 4c) and, after detritylating once again, to the amino
25 modifier C6 phosphoramidite 13 (Figure 4d) using tetrazole as catalyst and subsequently oxidized with iodine water. The oligomer is cleaved from the support, and all the base-labile protecting groups are removed at the same time, by treating with conc. ammonia at 50°C. The terminal Mmt protecting group is then removed by treating with 80% acetic acid. 57 OD of the crude
30 product are obtained, with this crude product being purified by gel electrophoresis. 7.4 OD of product, which exhibits the expected molecular weight of 3647.5 (calc. 3648.5) in the mass spectrum, are obtained.

List of abbreviations:

ACN	Acetonitrile
BOC	tert-butyloxycarbonyl
<u>C</u> , <u>c</u>	pseudo-iso-cytosine
COS	CV1 origin SV 40
CPG	controlled pore glass
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DMEM	Dulbecco's MEM
DMF	Dimethylformamide
Dmt	Dimethoxytrityl
DNA	deoxyribonucleic acid
DNP	Dinitroaryl
FITC	fluorescein isothiocyanate
Fmoc	Fluorenylmethoxycarbonyl
HATU	O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HBTU	O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
hex	-NH-(CH ₂) ₆ -OH
MEM	modified Eagle's minimal essential medium
Mmt	Monomethoxytrityl
OD	optical density
oeg	N-(2-hydroxyethyl)glycine
PAA	Polyacrylamide
PG	protecting group
PNA	polyamide nucleic acid
RNA	ribonucleic acid
TBTU	O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TCA	trichloroacetic acid

THF Tetrahydrofuran
TR acid-labile protecting group

Figures 1a, 1b, 2b and 3b show examples of terminal Z and Z' radicals.

Figures 2a and 3a show examples of bridging X and X' radicals.

5

Figures 4a, 4b, 4c and 4d show examples of phosphorylating reagents.

Figures 5a and 5b show examples of single (A, B) and multiple (C to E) derivatization of PNA at the N terminus.

10

Figure 6 shows examples of support-bound reagents for the solid phase synthesis.

Figures 7, 8 and 9 show examples of synthesizing C- and N-terminally modified
15 PNA.

44

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12

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<210> 28

<211> 12

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12

<210> 29

<211> 12

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12

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18

<210> 40

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<210> 41

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<210> 42

<211> 20

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<210> 43

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<400> 43
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21

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15

<210> 50
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18

57

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<400> 52
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21

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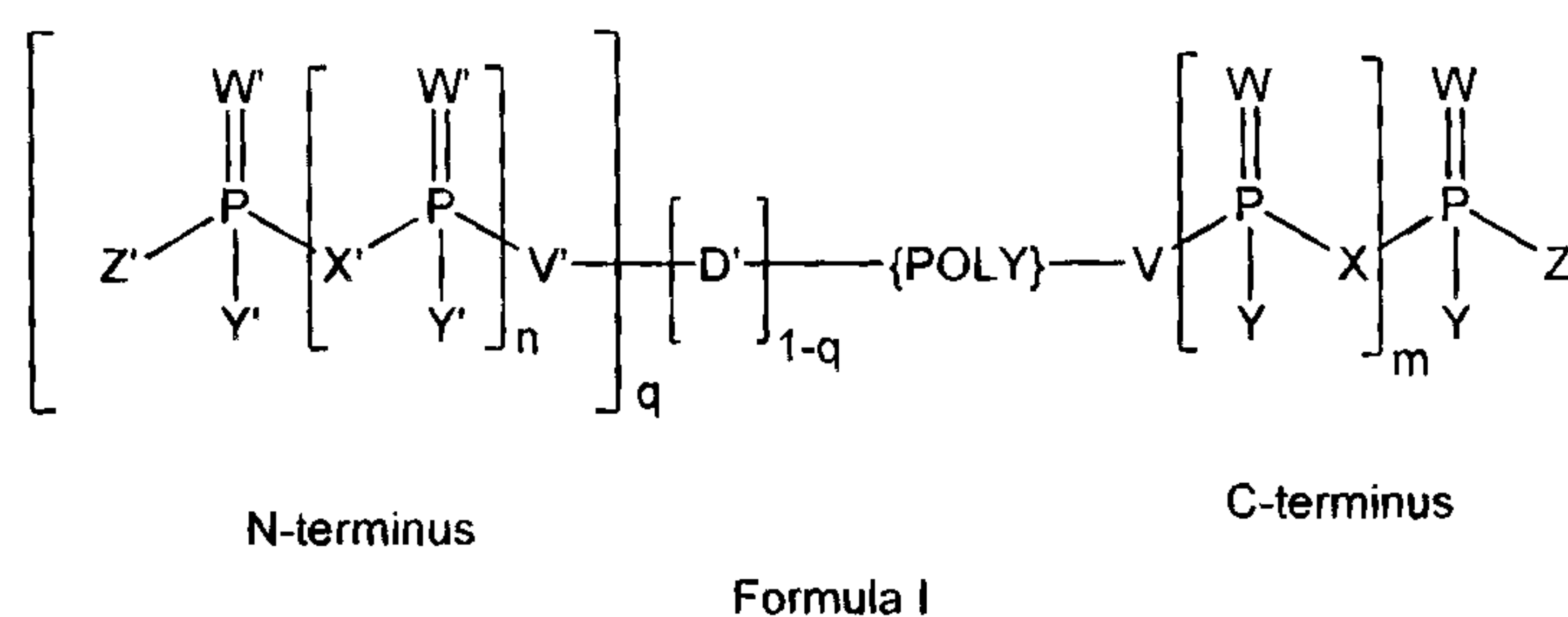
<400> 53
 tattccgtca t

11

Claims:

1. A PNA derivative which carries one or more phosphoryl radicals at the C terminus or at the C and N termini of the PNA backbone, wherein the one or more phosphoryl radicals are selected from oxo-, thio-, and imino-phosphoryl radicals, and wherein at least one of the phosphoryl radicals carries one or more deprotonatable groups, and the phosphoryl radicals are linked to the PNA backbone by way of an oxygen-phosphorus bond, a sulfur-phosphorus bond or a nitrogen-phosphorus bond, either directly or by way of a spacer.
5
2. A PNA derivative as claimed in claim 1, wherein the one or more deprotonatable groups are hydroxyl groups or mercapto groups.
10
3. A PNA derivative as claimed in claim 1 or 2, wherein the spacer is an alkanoylamide, a poly(alkoxy)carboxamide or an amino acid, and wherein at least one of the phosphoryl radicals carries one or more hydroxyl or mercapto groups, which is/are deprotonatable in a pH range of from 4.5 to 14, and wherein, furthermore, the phosphoryl radical is a phosphate, a phosphonate, a thiophosphate, a phosphoamidate or a substituted phosphoryl radical, and wherein substituted phosphoryl radicals optionally carry, one or more labeling groups, or groups for crosslinking, or groups which promote intracellular uptake, or groups which increase the binding affinity of the PNA derivative for nucleic acids.
15
20
4. A PNA derivative as claimed in claim 3, wherein the pH range is from 6.5 to 12.
25
5. A PNA derivative as claimed in claim 4, wherein the pH range is from 6.5 to 9.
30

6. A PNA derivative of the formula I



where

q is 0 or 1,

D' is hydroxyl, mercapto, amino, alkylamino or acylamino,

V is, independently of each other, oxygen, sulfur or NR₁,

10 V' is, independently of each other, oxygen, sulfur, NR₁, a group U-(CR₃R₄)_u'-C(O)-NH or a group U-(CH₂CH₂O)_u'-CH₂-C(O)-NH,

U is, independently of each other, oxygen, sulfur or NH,

15 u' is, independently of each other, from 1 to 10,

W and W' are, independently of each other, oxygen, sulfur or NR₁,

20 Y and Y' are, independently of each other, hydroxyl, mercapto, oxyanion, thioate or NR₁R₂,

X and X' are, independently of each other, a group U-(C₂-C₂₂-alkanediyl)-U or

a group $U-(CH_2CH_2-O)_u'$,

25 or are a labeling group or a group for crosslinking, or a group which promotes intracellular uptake, or a group which increases the binding affinity of the PNA derivative for nucleic acids,

Z and Z' are, independently of each other, hydroxyl, mercapto, oxyanion, thioate

5 or NR_1R_2 , $\text{C}_1\text{-C}_{22}\text{-alkyl}$, $\text{C}_1\text{-C}_8\text{-arylalkyl}$, $\text{C}_1\text{-C}_{22}\text{-alkyl-U}$, $\text{C}_1\text{-C}_8\text{-arylalkyl-U}$, hydroxy- $\text{C}_1\text{-C}_{18}\text{-U}$, aminoalkyl-U, mercaptoalkyl-U, or a group of the formula $\text{R}_7(\text{CH}_2\text{CH}_2\text{-O})_p$, where R_7 is hydroxyl, amino or $\text{C}_1\text{-C}_{22}\text{-alkoxy}$, and p is from 1 to 100, or are a labeling group, or a group for crosslinking, or a group which promotes intracellular uptake, or a group which increases the binding affinity of the PNA derivative for nucleic acids,

10

R_1 and R_2 are, independently of each other, a radical consisting of hydrogen

or $\text{C}_1\text{-C}_6\text{-alkyl}$,

15

R_3 and R_4 are, independently of each other, a radical consisting of hydrogen

or $\text{C}_1\text{-C}_6\text{-alkyl}$, or the radical of an amino acid side chain, it being possible for adjacent radicals R_3 and R_4 in V' to also form a $\text{C}_5\text{-C}_8\text{-cycloalkyl}$ ring,

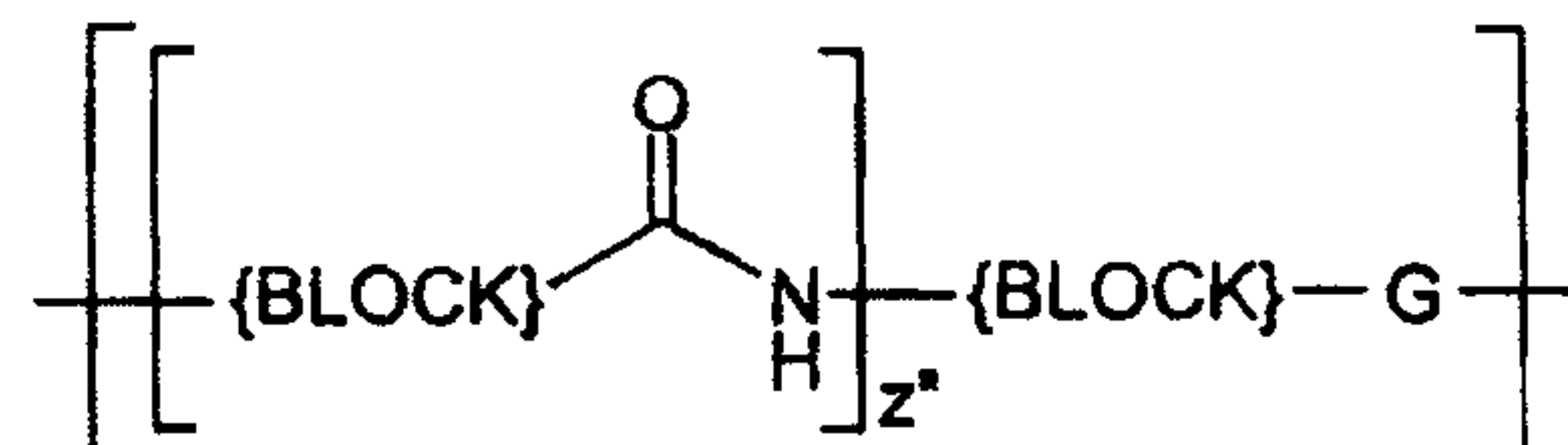
20

n is from 0 to 10,

m is from 0 to 10,

25

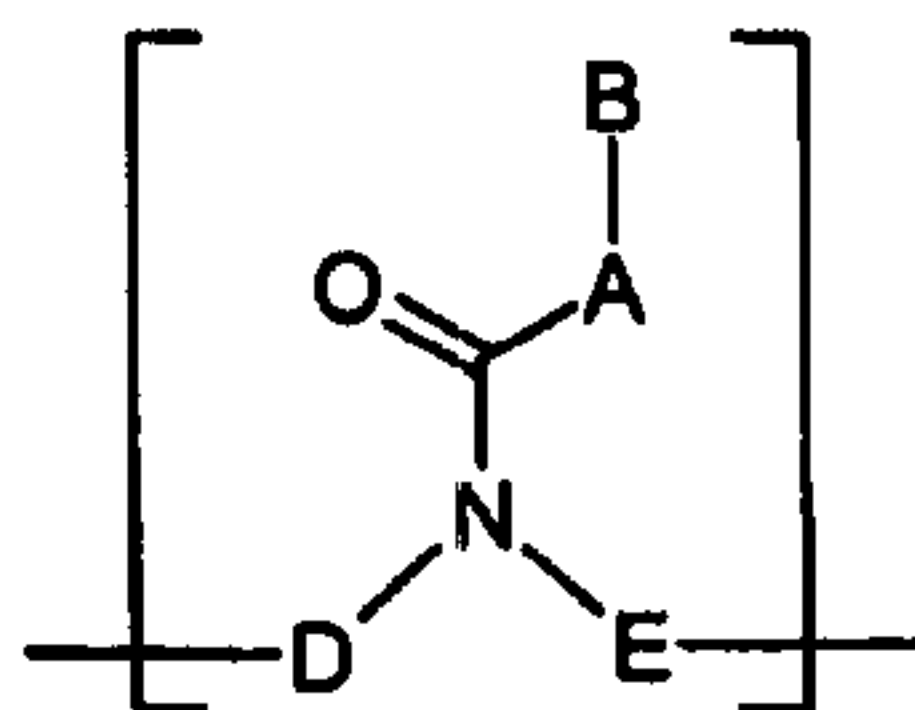
and where {POLY} is described by the formula II



Formula II

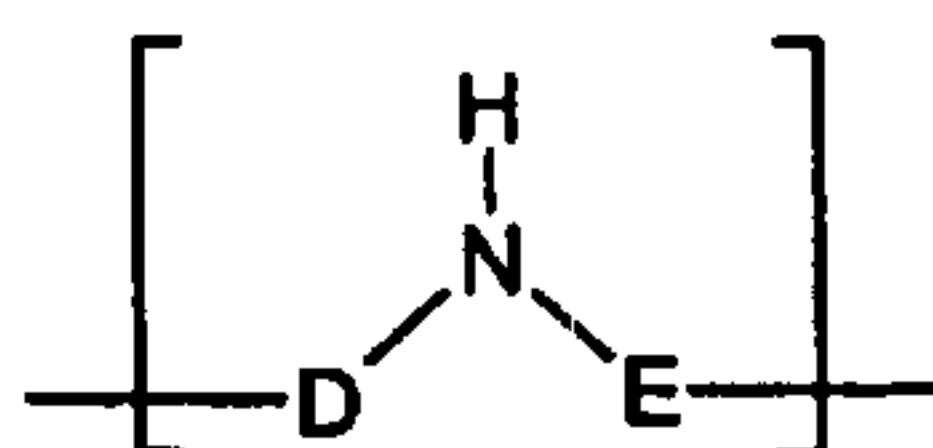
61

where {BLOCK} is also, independently of each other, a group selected from formula IIIA,



Formula IIIA

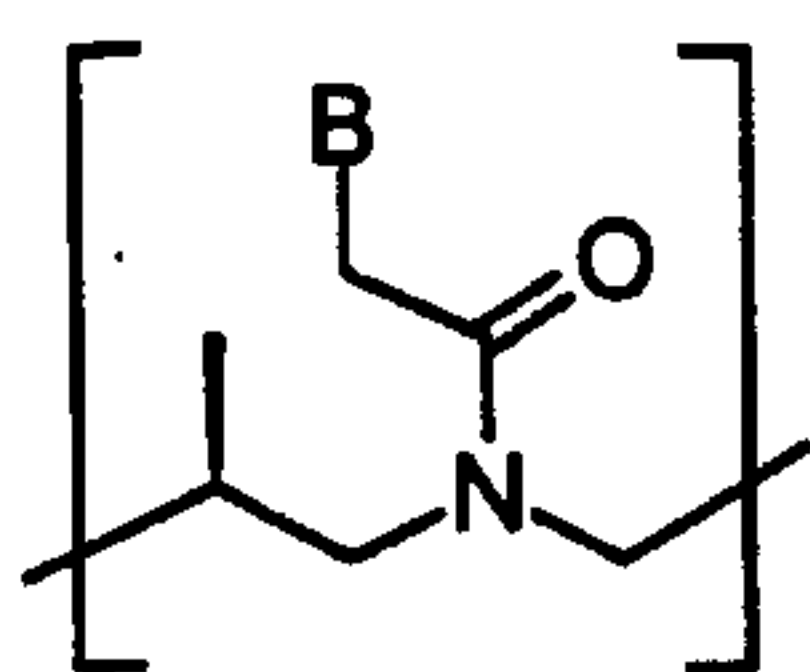
or from fomula IIIB,



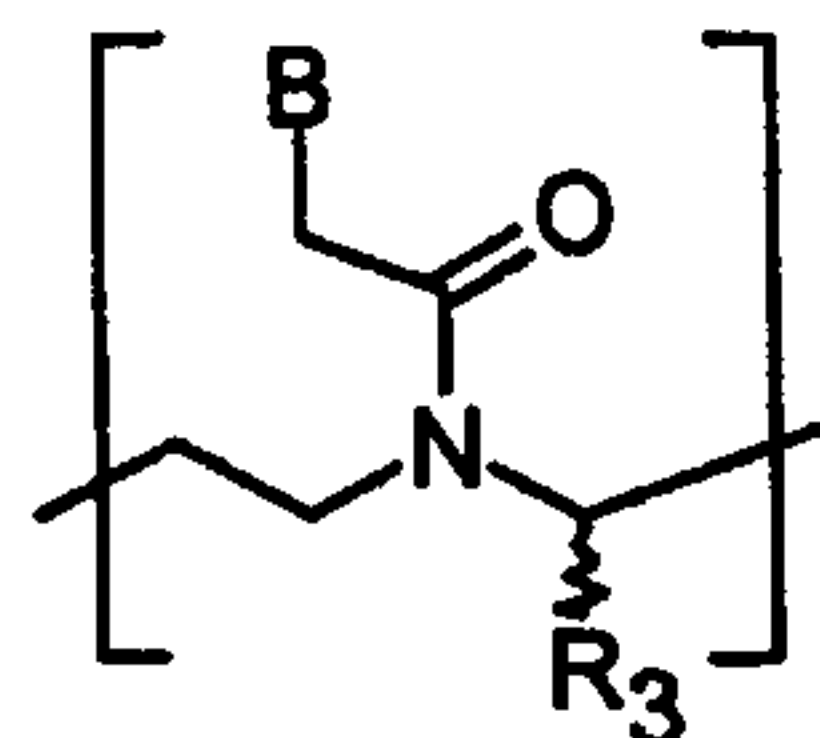
Formula IIIB

5

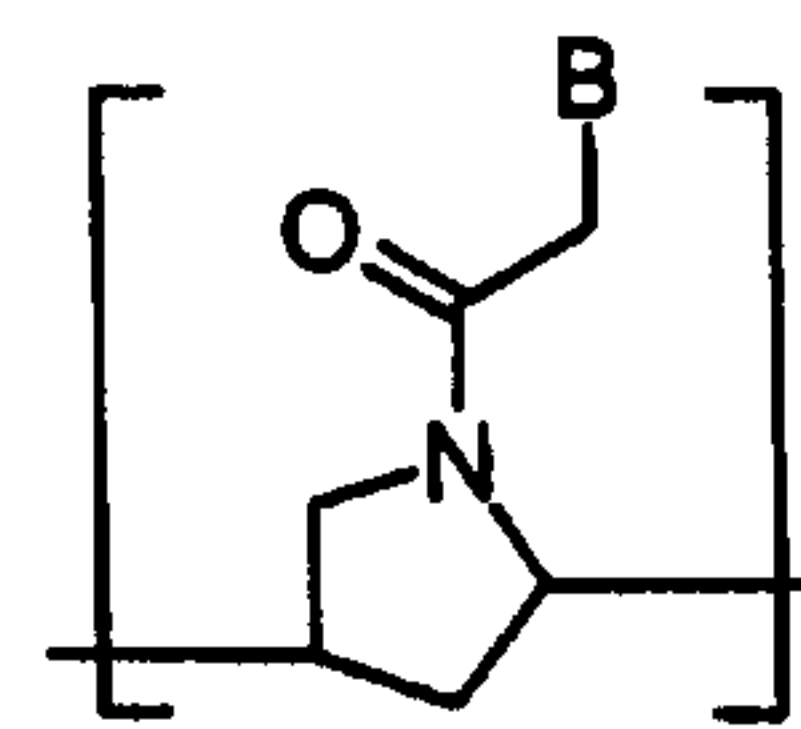
or from the formulae IV A to IV G,



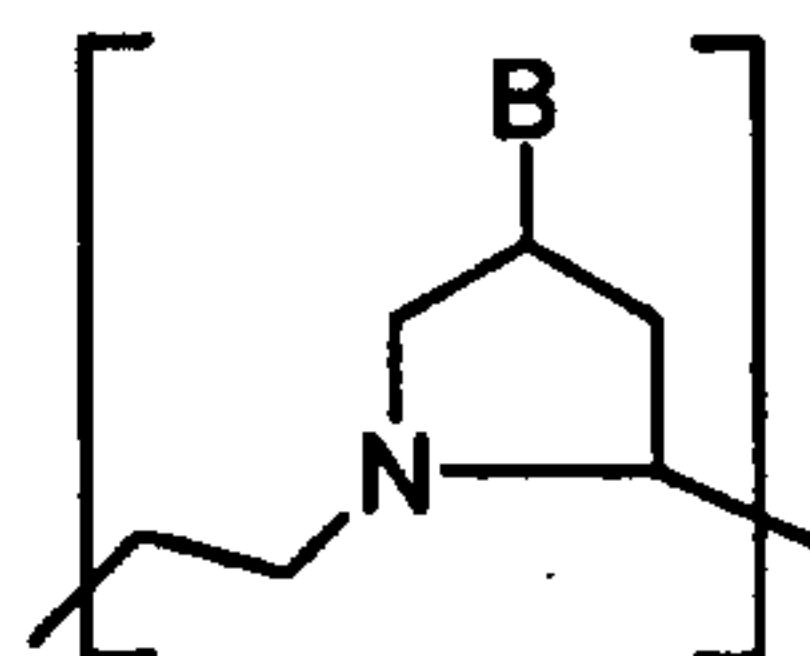
Formula IV A



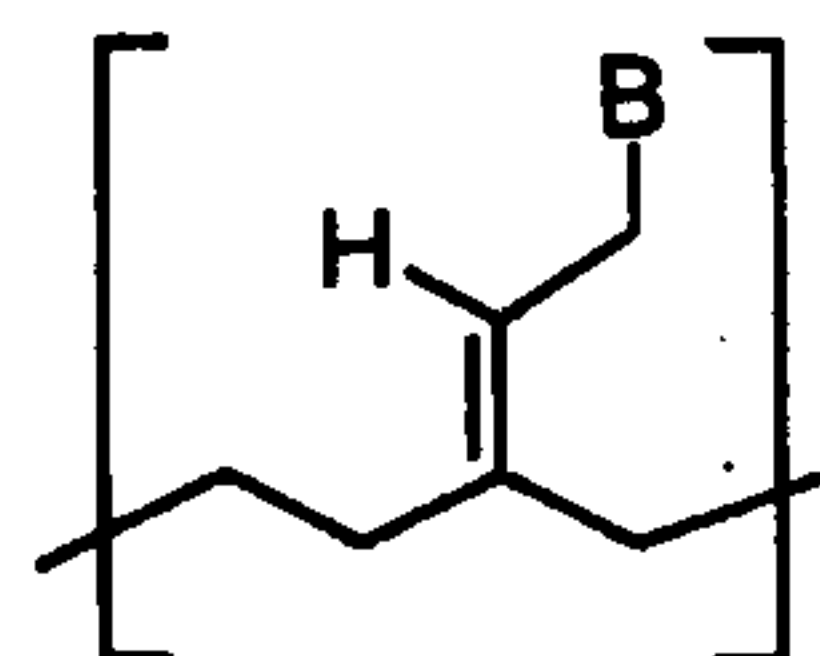
Formula IV B



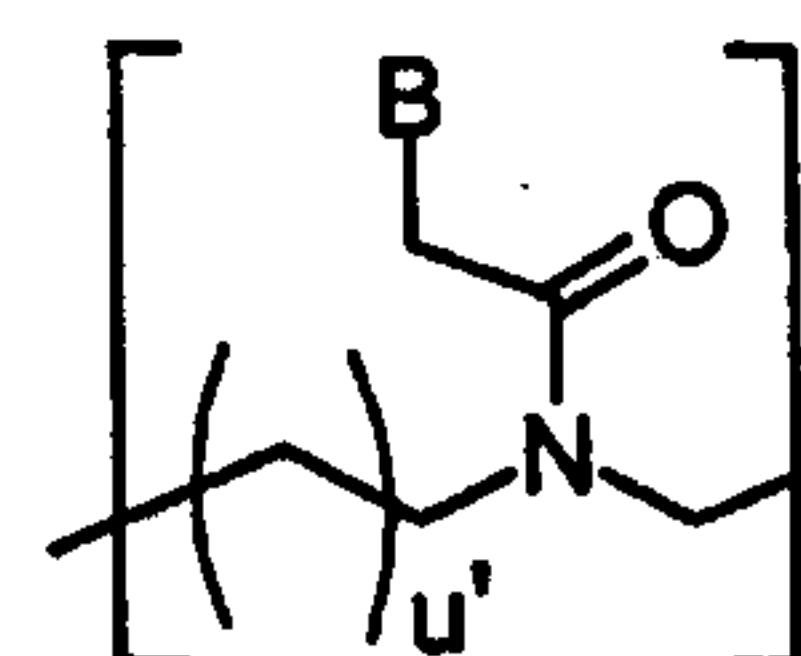
Formula IV C



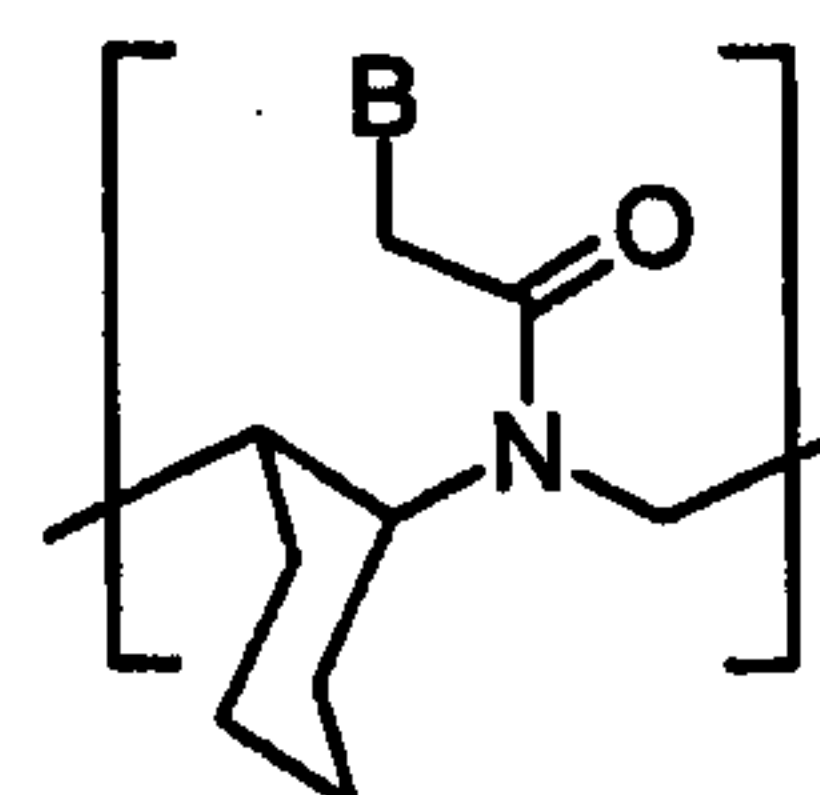
Formula IV D



Formula IV E



Formula IV F



Formula IV G

10

where each {BLOCK} building block can be different,

and it furthermore applies that

z'' is from 0 to 100,

G is selected from the groups $(\text{CR}_5\text{R}_6)_{u'}$, $\text{C}(\text{O})\text{NH}-(\text{CR}_1\text{R}_2)_t$ or $\text{C}(\text{O})\text{NH}-(\text{CH}_2\text{CH}_2\text{O})_{u'}-\text{CH}_2\text{CH}_2$, where t' is from 2 to 10,

5 A is, independently of each other, a group $(\text{CR}_1\text{R}_2)_s$, where s is from 1 to 3,

10 B is, independently of each other, either an aromatic radical, which can also possess heteroaromatic character, or hydrogen, or hydroxyl or $\text{C}_1\text{-C}_{18}$ -alkyl, or a nucleobase which occurs naturally, and is customary in nucleotide chemistry, or which does not occur naturally, or its prodrug form,

15 D is, independently of each other, a group $(\text{CR}_3\text{R}_4)_t$, where t is from 2 to 10,

20 E is, independently of each other, a group $(\text{CR}_5\text{R}_6)_{u'}$, where adjacent R_5 and R_6 radicals can also form a $\text{C}_5\text{-C}_8$ -cycloalkyl ring or a spiro compound,

R_5 and R_6 are, independently of each other, a radical consisting of hydrogen or $\text{C}_1\text{-C}_6$ -alkyl or the radical of an amino acid side chain,

25 and where u' , R_1 , R_2 , R_3 and R_4 have the same meaning as described above,

30 as well as physiologically tolerated salts of the PNA derivative of the formula I,

with the provisos that at least one Y , Y' , Z or Z' radical is hydroxyl, mercapto, oxyanion or thioate, and that at least one B radical is a nucleobase.

7. A PNA derivative as claimed in claim 6, wherein u' is from 1 to 4.
8. A PNA derivative as claimed in claim 7, wherein u' is 1.
- 5 9. A PNA derivative as claimed in claim 6, wherein the group which increases the binding affinity of the PNA derivative for nucleic acids in the definition of X and X' is a bifunctional fluorescein, rhodamine, TAMRA, biotin, pyrene, dinitrophenyl, cholesteryl, acridine, adamantyl, vitamin E, cyanin dye, Dabcyl, Edans, lexitropsin, psoralen, BODIPY, ROX, R6G, or digoxienin radical.
10
10. A PNA derivative as claimed in claim 6, wherein m in the definition of Z and Z' is from 2 to 10.
- 15 11. A PNA derivative as claimed in claim 6, wherein the group which increases the binding affinity of the PNA derivative for nucleic acids in the definition of Z and Z' is a monofunctional or bifunctional fluorescein, rhodamine, TAMRA, biotin, pyrene, dinitrophenyl, cholesteryl, acridine, adamantyl, vitamin E, cyanin dye, Dabcyl, Edans, lexitropsin, psoralen, BODIPY, ROX, R6G, or digoxienin radical.
20
12. A PNA derivative as claimed in claim 6, wherein R₁ and R₂ are hydrogen.
13. A PNA derivative as claimed in claim 6, wherein R₃ and R₄ are hydrogen.
25
14. A PNA derivative as claimed in claim 6, wherein n is from 0 to 3.
15. A PNA derivative as claimed in claim 6, wherein m is from 0 to 3.
- 30 16. A PNA derivative as claimed in claim 6, wherein z'' is from 1 to 20.
17. A PNA derivative as claimed in claim 16, wherein z'' is from 4 to 15.

18. A PNA derivative as claimed in claim 6, wherein s is 1.
19. A PNA derivative as claimed in claim 6, wherein t is from 2 to 4.
- 5 20. A PNA derivative as claimed in claim 19, wherein t is 2.
21. A PNA derivative as claimed in claim 6, wherein R₅ and R₆ are hydrogen.
22. A PNA derivative as claimed in claim 6, wherein at least one Y, Y', Z or Z'
10 radical in formula I is oxyanion or thioate in a pH range from 4.5 to 14.
23. A PNA derivative as claimed in claim 22, wherein the pH range is from 6.5 to 12.
- 15 24. A PNA derivative as claimed in claim 23, wherein the pH range is from 6.5 to 9.
25. A PNA derivative as claimed in any one of claims 6-24, wherein n and m are, independently of each other, 0.
- 20 26. A PNA derivative as claimed in any one of claims 6-25, wherein q is 1.
27. A PNA derivative as claimed in any one of claims 6-26, wherein W and W' are oxo.
- 25 28. A PNA derivative as claimed in any one of claims 6-27, wherein Y and Y' are hydroxyl or oxyanion.
- 30 29. A PNA derivative as claimed in any one of claims 6-28, wherein V and V' are oxy.

30. A PNA derivative as claimed in any one of claims 6 to 29, wherein X and X' are, independently of each other, a group U-(C₂-C₂₂-alkanediyl)-U or a group U-(CH₂CH₂-O)_{u'}, where u' is from 1 to 6.
- 5 31. A PNA derivative as claimed in claim 30, wherein U-(C₂-C₂₂-alkanediyl)-U is O-(C₂-C₂₂-alkanediyl)-O.
32. A PNA derivative as claimed in claim 30, wherein U-(C₂-C₂₂-alkanediyl)-U is O-(CH₂)₆-O.
- 10 33. A PNA derivative as claimed in claim 30, wherein U-(CH₂CH₂-O)_{u'} is O-(CH₂CH₂-O).
- 15 34. A PNA derivative as claimed in one of claims 6-33 wherein X, X', Z and Z' are selected, independently of each other, from the group fluorescein, rhodamine, TAMRA cyanine dye, biotin, dabcyI, psoralen, acridine, DNP, cholesterol, vitamin E, dabcyI, edans, lexitropsin, psoralen, BODIPY, ROX, R6G and digoxigenin.
- 20 35. A PNA derivative as claimed in any one of claims 6-34, wherein X, X', Z and Z' are selected, independently of each other, from the group monophosphate, biotin derivative and fluorescein derivative.
- 25 36. A PNA derivative as claimed in any one of claims 6-34, wherein Z is a fluorescence label and Z' is a quencher.
37. A PNA derivative as claimed in any one of claims 6-34, wherein Z is a quencher and Z' is a fluorescence label.
- 30 38. A PNA derivative as claimed in any one of claims 6-34, wherein Z and Z' are, independently of each other, a C₁-C₂₂-alkyl radical, or a C₁-C₂₂-U radical,

or hydroxy-C₁-C₁₈-U,

or an aminoalkyl-U radical,

or a group of the formula R₇-(CH₂CH₂-O)_m, where R₇ is OH or NH₂ and m is from 1 to 6, or a mercaptoalkyl-V radical.

5

39. A PNA derivative as claimed in claim 38, wherein the C₁-C₂₂-U radical is a C₁-C₂₂-alkoxy radical.

10

40. A PNA derivative as claimed in claim 39, wherein the C₁-C₂₂-alkoxy radical is C₁₆-alkoxy.

41. A PNA derivative as claimed in claim 38, wherein the hydroxy-C₁-C₁₈-U radical is a hydroxy-C₁-C₁₈-O radical.

15

42. A PNA derivative as claimed in claim 41, wherein the hydroxy-C₁-C₁₈-O radical is HO-(CH₂)₃₋₁₂-O.

43. A PNA derivative as claimed in claim 38, wherein the aminoalkyl-U radical is an aminoalkoxy radical.

20

44. A PNA derivative as claimed in claim 43, wherein the aminoalkoxy radical is 6-aminohexoxy or 5-aminohexoxy.

25

45. A PNA derivative as claimed in claim 38, wherein the R₇-(CH₂CH₂-O)_m is HO-(CH₂CH₂-O)₂, HO-(CH₂CH₂-O)₆ or H₂N-(CH₂CH₂-O)₂.

46. A PNA derivative as claimed in claim 38, wherein the mercapto-V radical is a mercaptoalkoxy radical.

30

47. A PNA derivative as claimed in claim 46, wherein the mercaptoalkoxy radical is 6-mercaptohexyloxy.

48. A PNA derivative as claimed in any one of claims 6 or 22-25, wherein q is 0.
49. A PNA derivative as claimed in claim 16, wherein D' is acylamino.
- 5 50. The PNA derivative as claimed in claim 49, wherein D' is acetylamino.
51. A PNA derivative as claimed in one of claims 6-50, wherein D is (CH₂)_t.
52. The PNA derivative as claimed in claim 51, wherein D is (CH₂)₂.
- 10 53. A PNA derivative as claimed in any one of claims 6-52, wherein A, E and G are CH₂.
- 15 54. A PNA derivative as claimed in one of claims 6-53, wherein B is adenine, cytosine, 5-methylcytosine, guanine, thymine and uracil, or is purine, 2,6-diaminopurine, N⁴N⁴-ethanocytosine, N⁶N⁶-ethano-2,6-diaminopurine, 5-(C₃-C₆)-alkynyluracil, 5-(C₃-C₆)-alkynyl-cytosine, 5-(1-propargylamino)uracil, 5-(1-propargylamino)cytosine, phenoxazine, 9-aminoethoxyphenoxazine, 5-fluorouracil or pseudoisocytosine, 5-(hydroxymethyl)uracil, 5-aminouracil, pseudouracil, dihydrouracil, 5-(C₁-C₆)-alkyluracil, 5-(C₁-C₆)-alkyl-cytosine, 5-(C₂-C₆)-alkenylcytosine, 5-fluorocytosine, 5-chlorouracil, 5-chlorocytosine, 5-bromouracil, 5-bromocytosine, 7-deazaadenine, 7-deazaguanine, 8-azapurine, or a 7-deaza-7-substituted purine.
- 20 55. A PNA derivative as claimed in any one of claims 6-54, wherein {POLY} comprises a nucleotide base sequence that binds parts of tumor suppressor genes, oncogenes or telomerases, or their transcription products.
- 25 56. A PNA derivative as claimed in claim 55, wherein the base sequence of the PNA moiety is directed against the translation start of HA-ras mRNA.
- 30

57. The use of a PNA derivative as claimed in any one of claims 1-36 or 38-56 for producing a pharmaceutical for tumor therapy.
58. A PNA derivative as claimed in any one of claims 1-56 for use as a
5 diagnostic agent.
59. The use of a PNA derivative as claimed in any one of claims 1-56 for detecting microorganisms and/or viruses.
- 10 60. The use of a PNA derivative as claimed in any one of claims 1-56 for detecting and/or quantifying nucleic acids.
- 15 61. The use of a PNA derivative as claimed in any one of claims 1-56 as a detection reagent for in-situ hybridization or fluorescence in-situ hybridization.
62. The use of a PNA derivative as claimed in any one of claims 1-56 as an antisense agent, anti-gene agent, decoy agent or chimeraplasty agent.
- 20 63. The use of a PNA derivative as claimed in claim 37 or 38 as a molecular beacon.
64. A detection reagent, comprising a PNA derivative as claimed in any one of claims 1-56.
- 25 65. A PNA chip, comprising a PNA derivative as claimed in any one of claims 1-56.
- 30 66. A biosensor, comprising a PNA derivative as claimed in any one of claims 1-56.

67. A pharmaceutical composition, comprising a PNA derivative as claimed in any one of claims 1-36 or 39-56 and a pharmacologically tolerated additive and/or excipient.
- 5 68. An antisense agent, anti-gene agent, decoy agent or chimeraplasty agent, comprising a PNA derivative as claimed in any one of claims 1-56.
69. A process for preparing a PNA derivative of the formula I as defined in claim 6, wherein
- 10 a) the C terminus of an amidonucleic acid is linked to a solid phase-bound phosphorylating reagent, or an amidonucleic acid which is phosphorylated C-terminally is bound to a solid support,
- b) the backbone of the PNA oligomer is extended sequentially by coupling with amidonucleic acid monomers,
- 15 c) optimally, the N-terminus is reacted with a phosphorylating reagent.
70. The process as claimed in claim 69, wherein the RNA is prepared using the t-butyloxycarbonyl (BOC), 9-fluorenylmethoxycarbonyl (Fmoc) or monomethoxytrityl (Mmt) protecting groups.
- 20 71. The process as claimed in claim 69 or 70, wherein the PNA is prepared using solid supports.
72. The process as claimed in claim 71, wherein CPG®, tentagel® or aminomethylpolystyrene is used as the solid support.
- 25 73. A process for producing a pharmaceutical, wherein
- a) a PNA derivative as claimed in any one of claims 1-36 or 39-56 is prepared, and
- 30 b) further pharmacologically tolerated additives and/or excipients are optionally added to it.

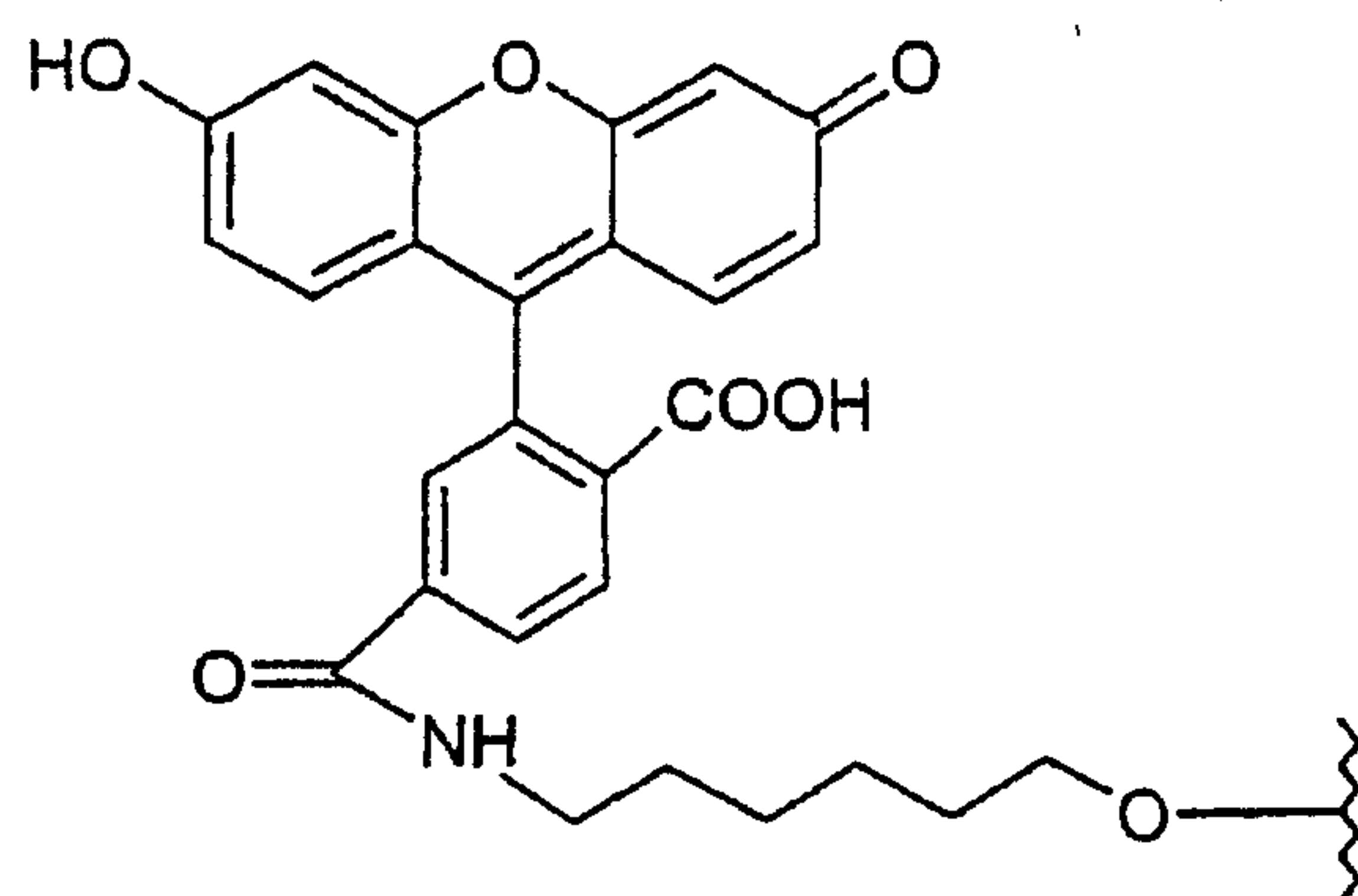
74. A process for producing a PNA chip, wherein a PNA derivative as claimed in any one of claims 1-56 is either firstly prepared and then fixed onto a solid support, or the PNA derivative is prepared directly on the support.
- 5 75. A process for preparing a PNA derivative of the formula I as claimed in any one of claims 69-72, wherein, in addition, the PNA is purified by means of chromatography or electrophoresis while exploiting the acid character of the phosphorus radical.
- 10 76. The process as claimed in claim 75, wherein the PNA derivative is purified by means of chromatography using a basic stationary phase and a gradient of an acid or salt-containing eluent.
- 15 77. The process as claimed in claim 76, wherein the stationary phase is an anion exchanger or a mixed-mode phase.

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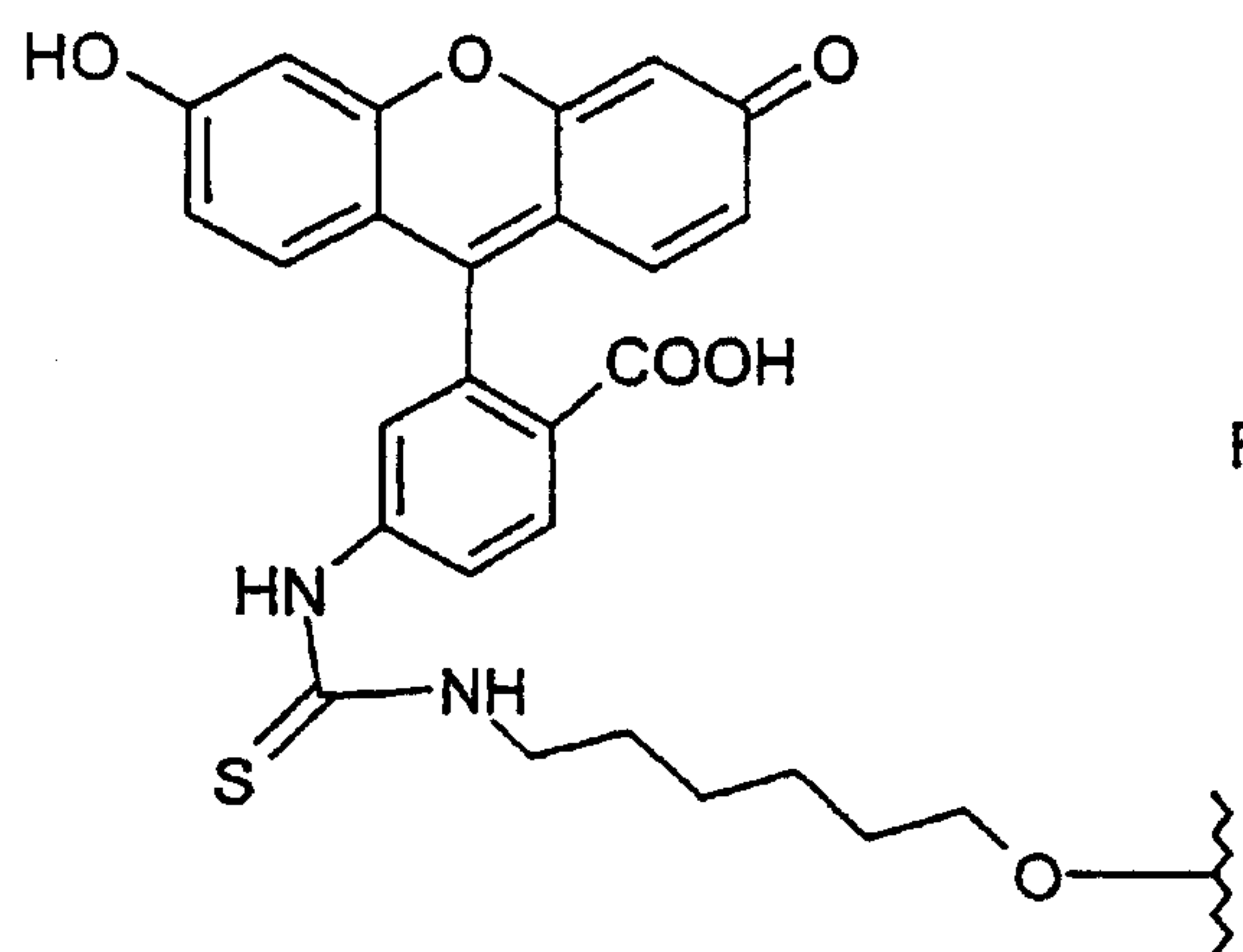
PCT/EP01/04030

Fig. 1a:

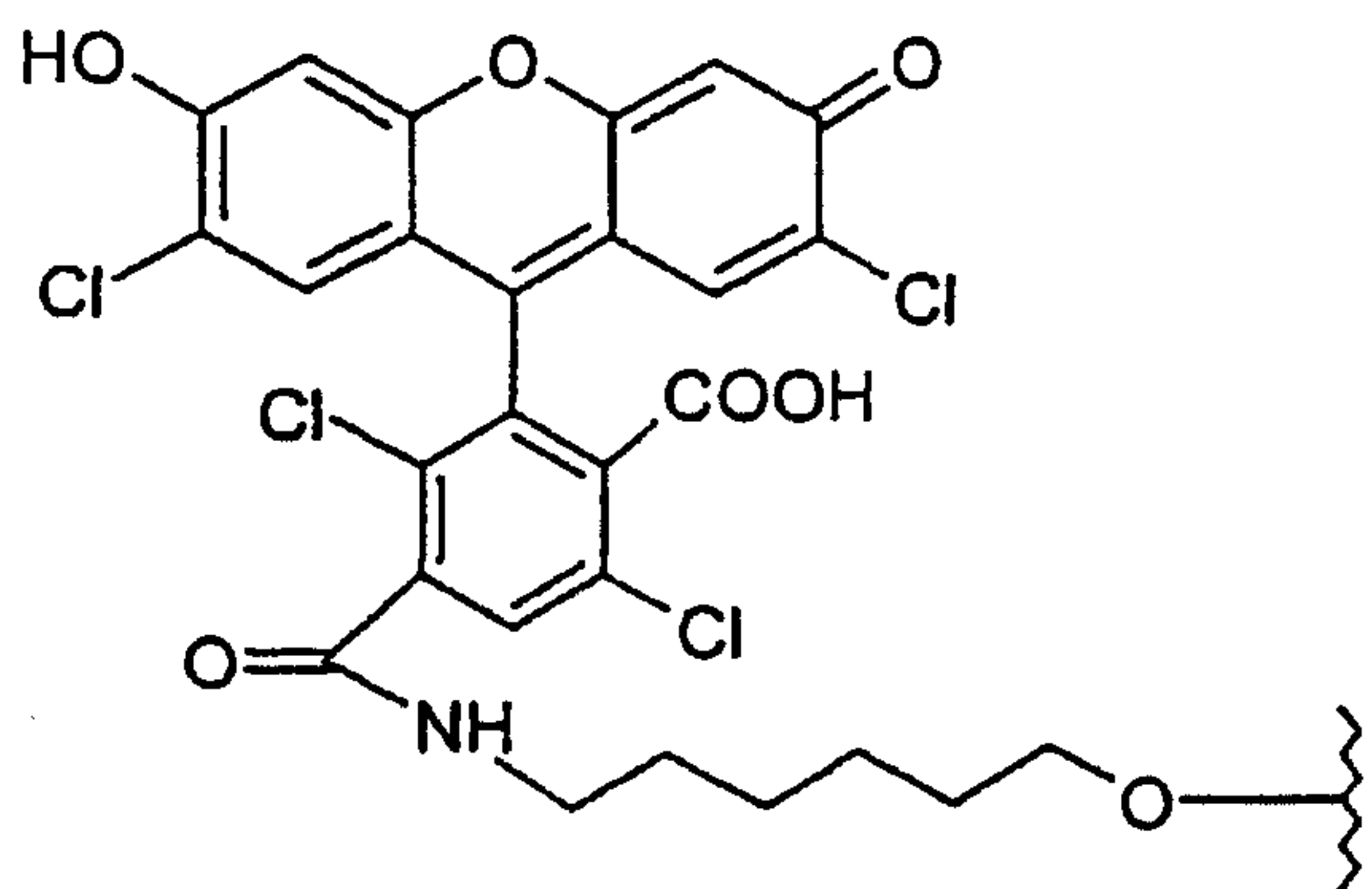
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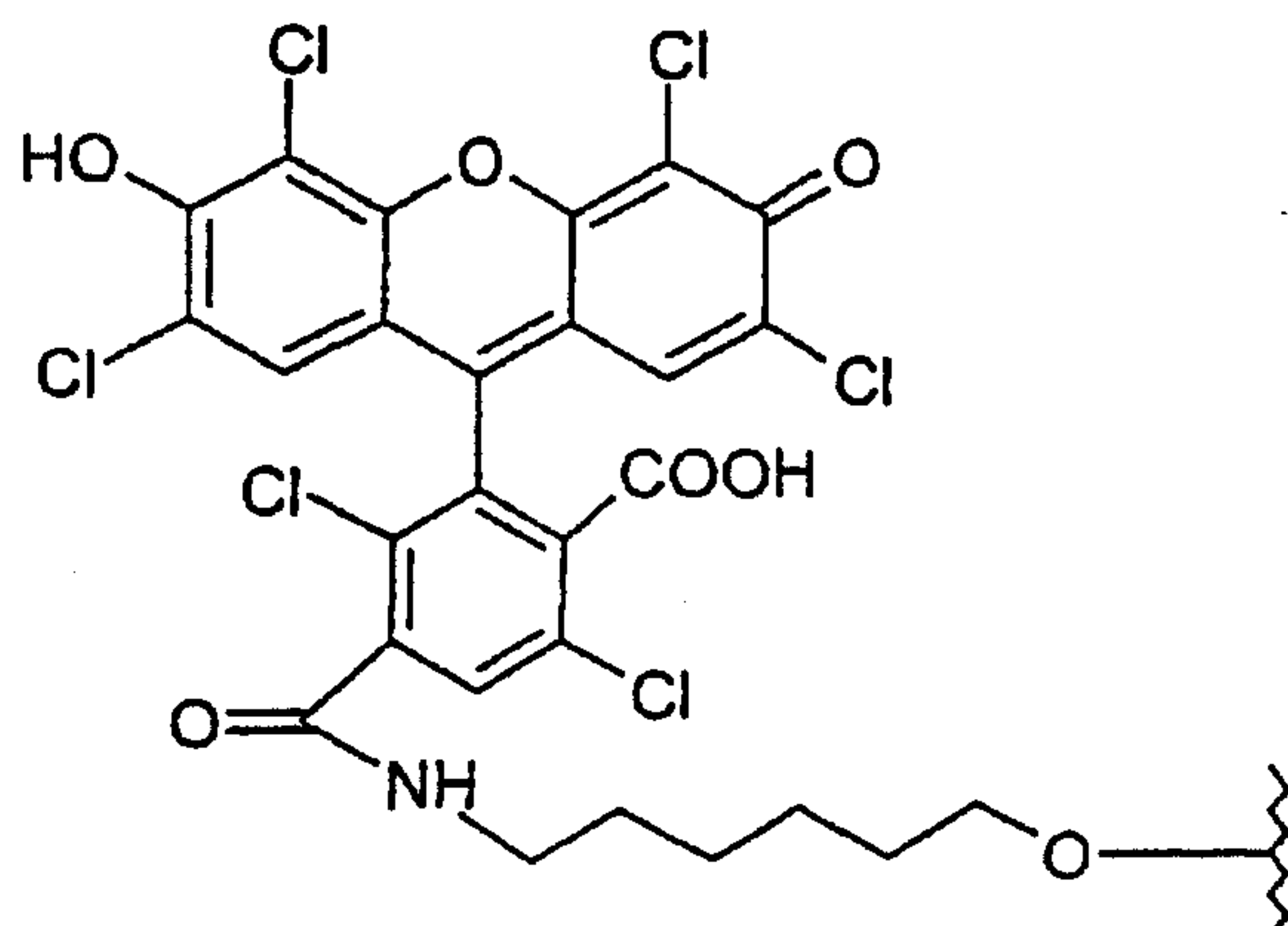
Fluorescein (amide)



Fluorescein (thiourea)



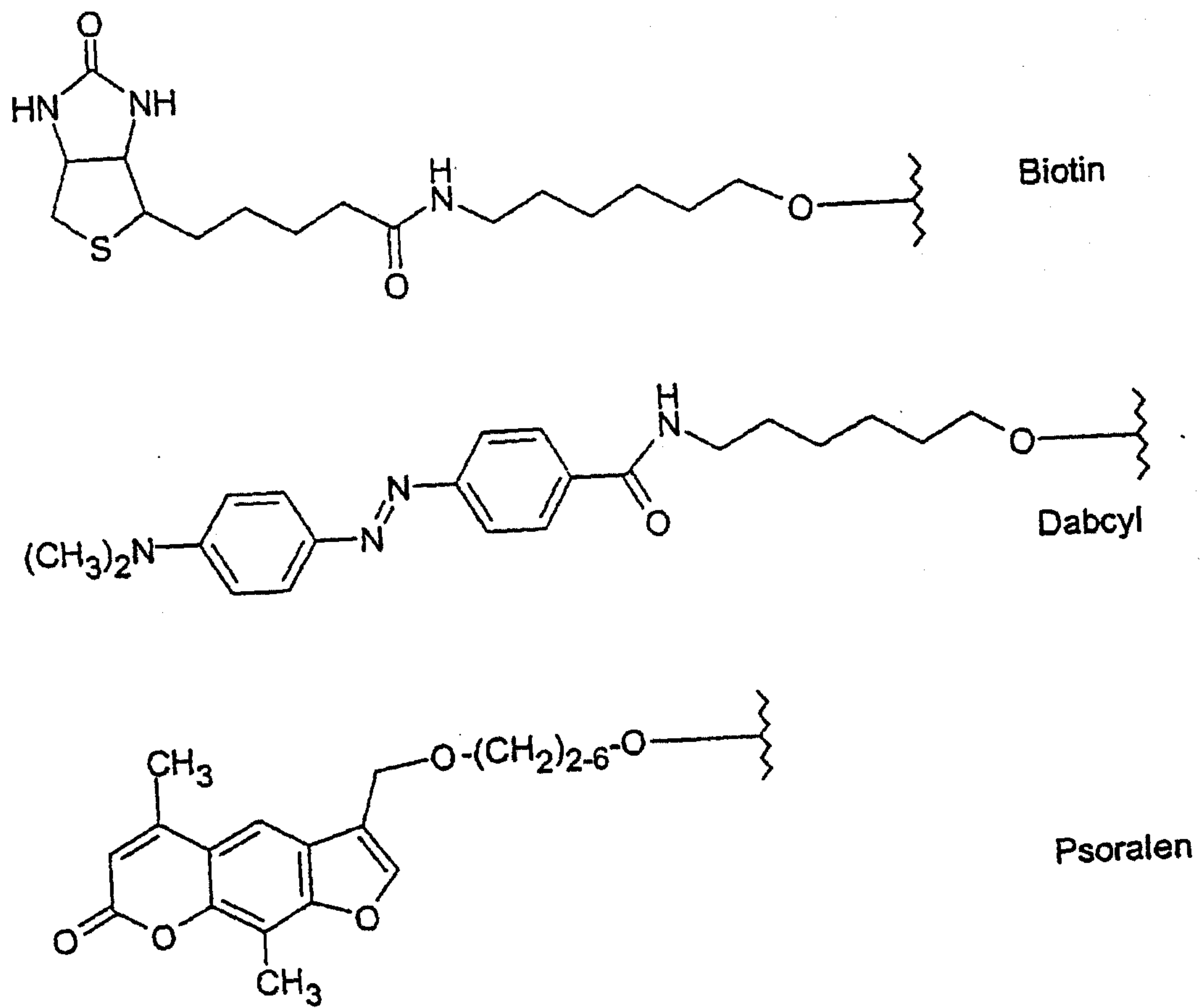
Tetrachlorofluorescein



Hexachlorofluorescein

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Fig. 1b:



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Fig. 2a:

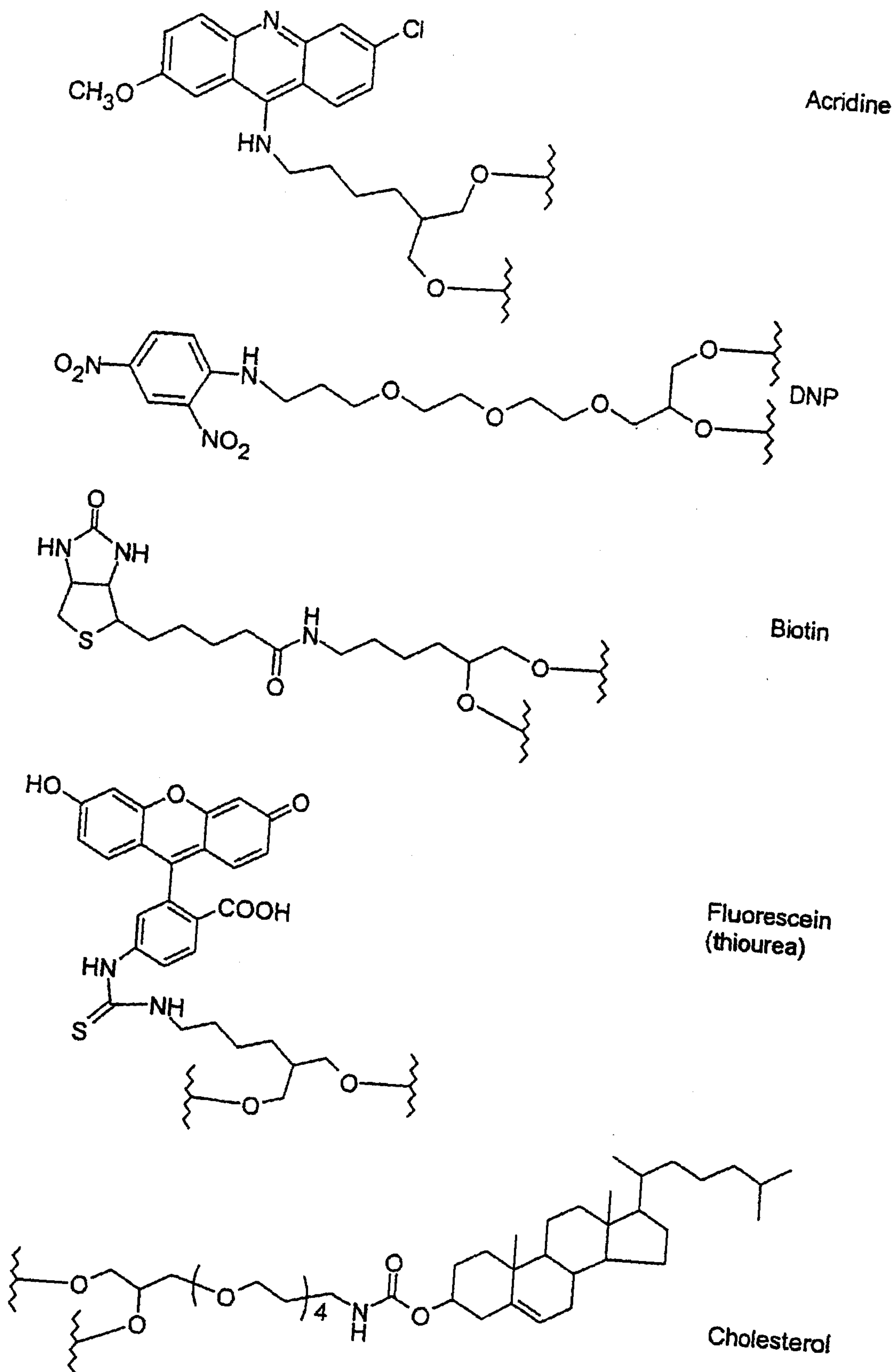
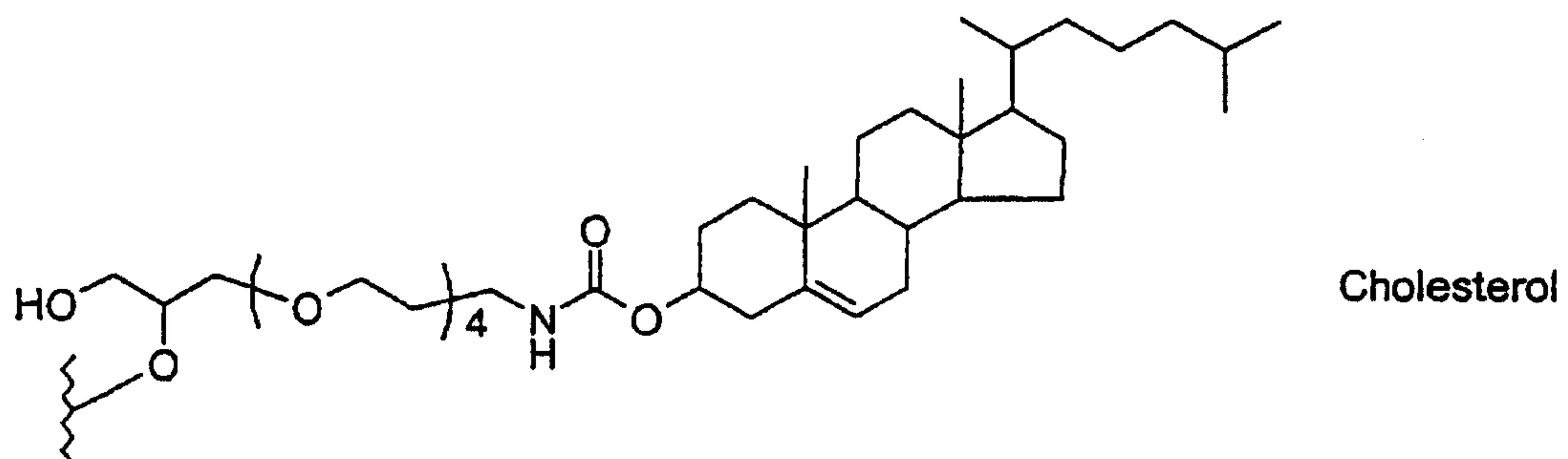
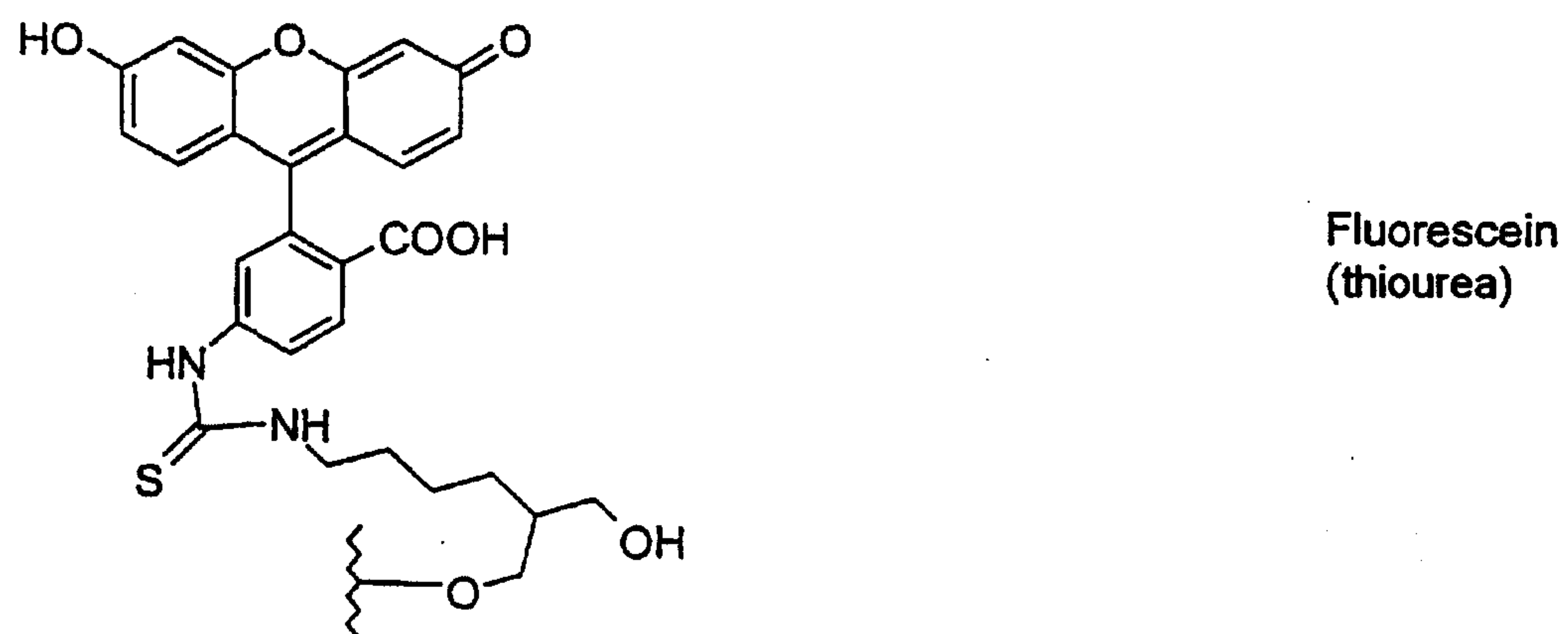
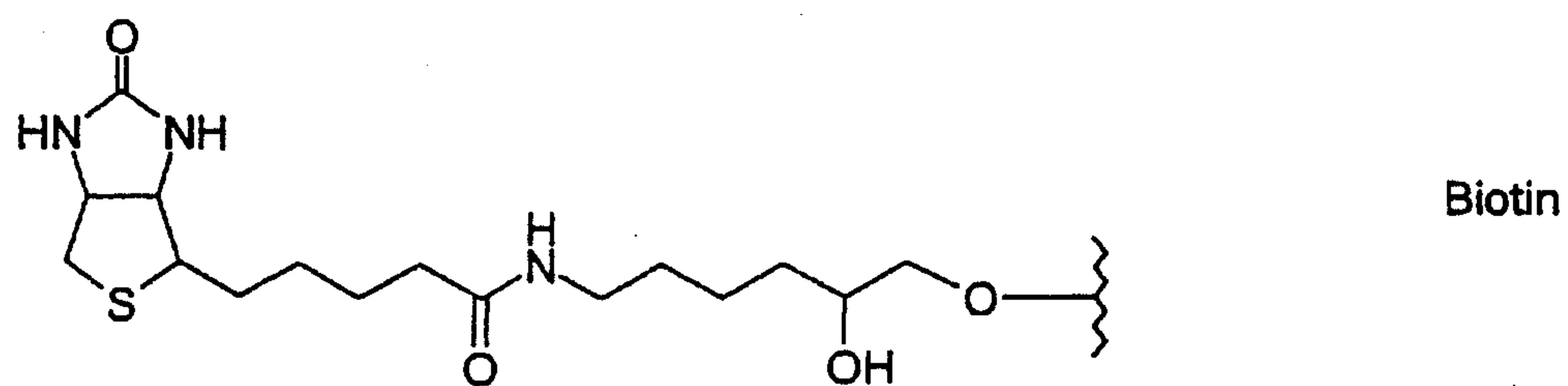
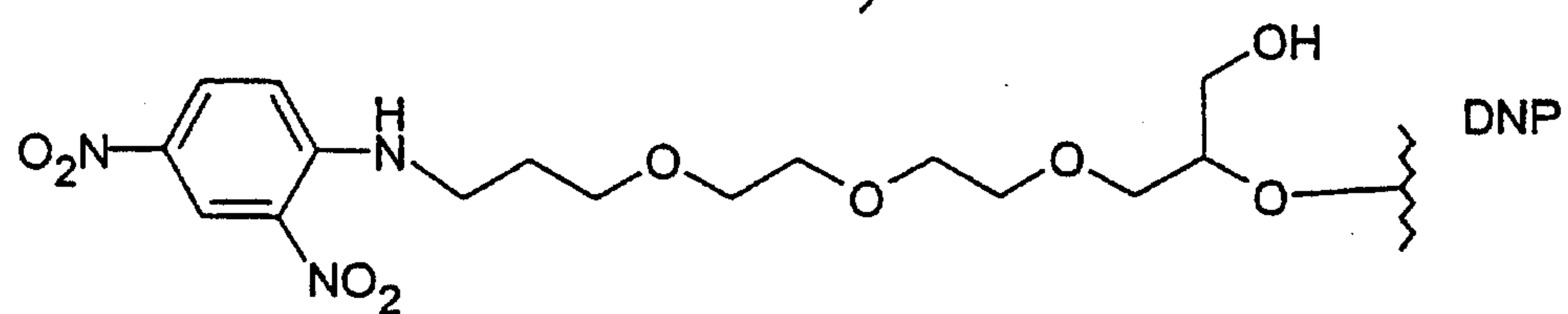
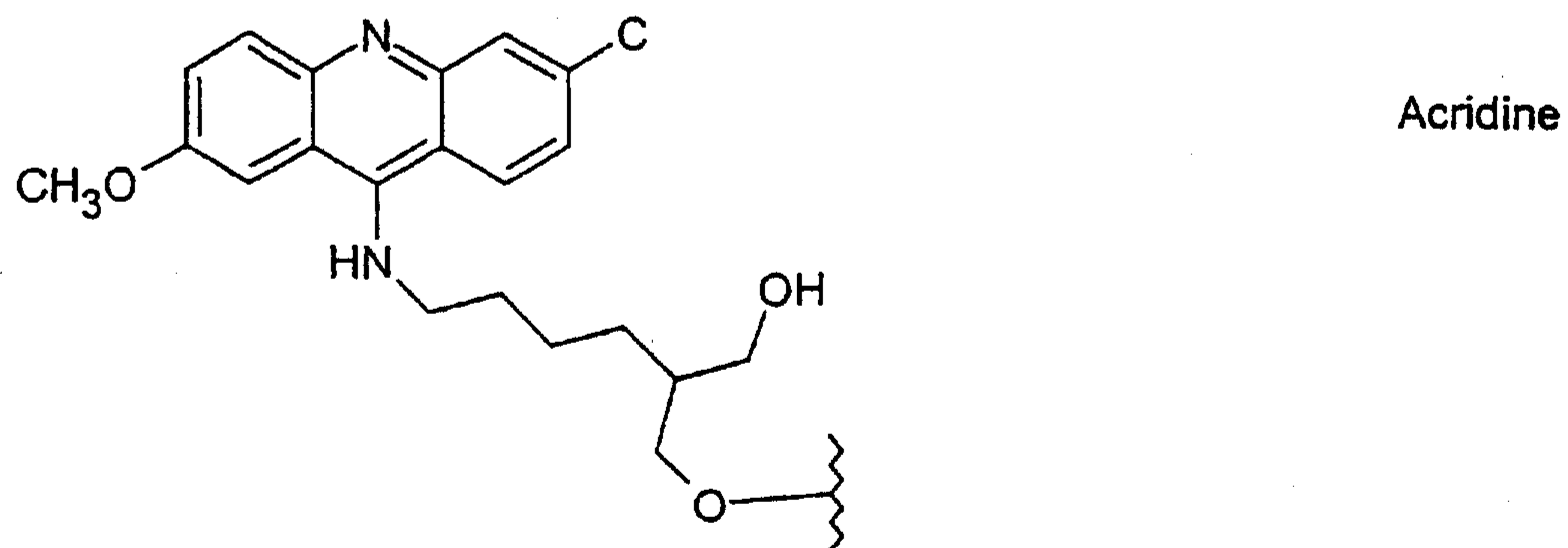
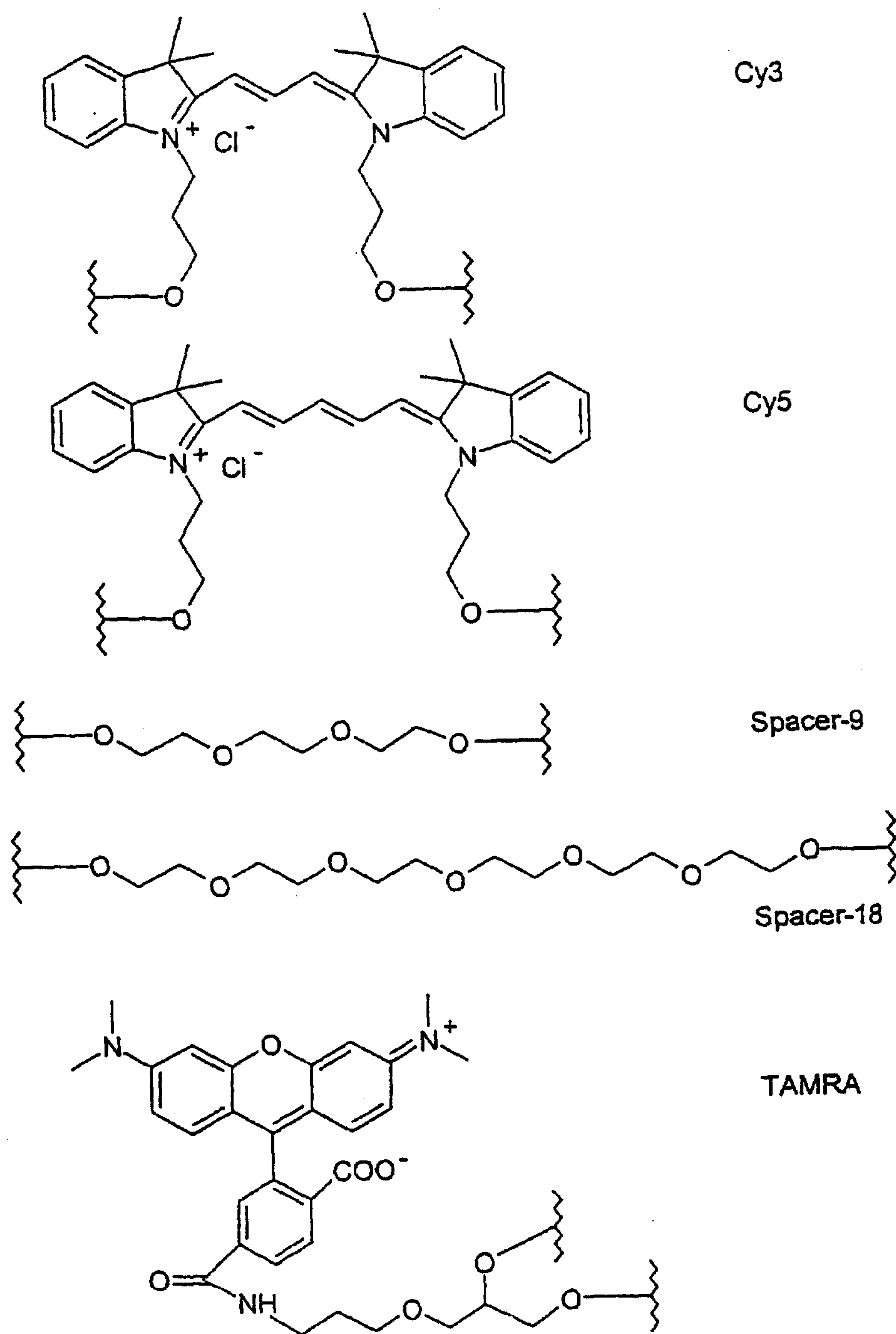


Fig. 2b:



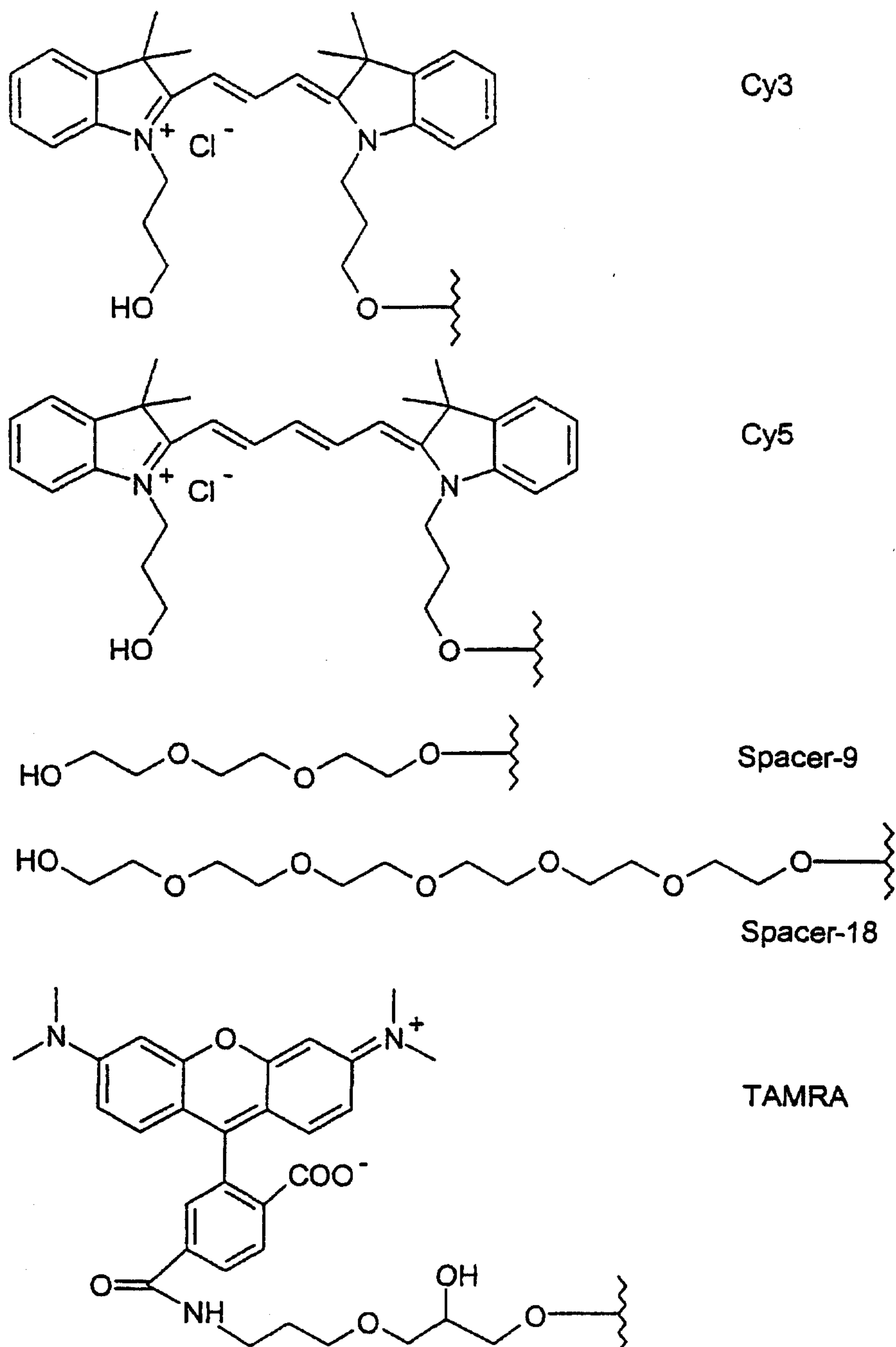
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Fig. 3a:



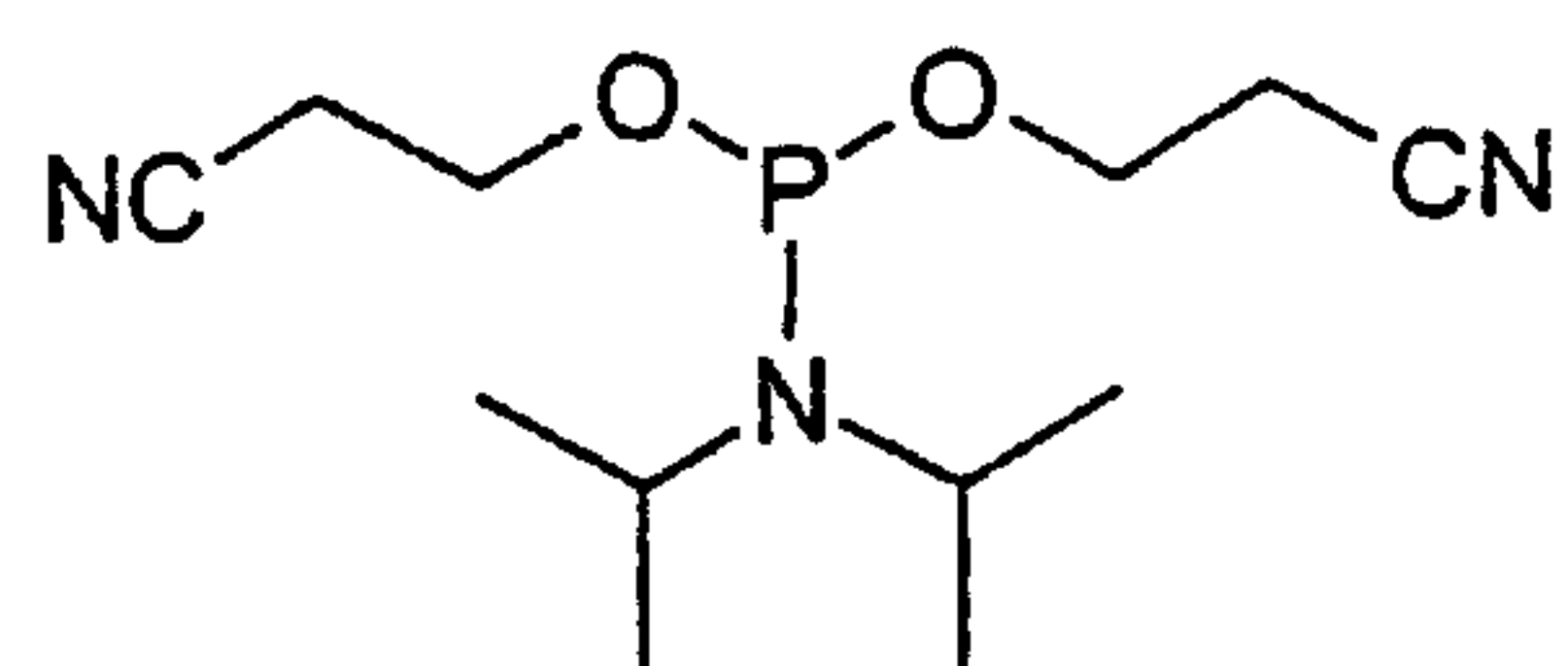
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Fig. 3b:

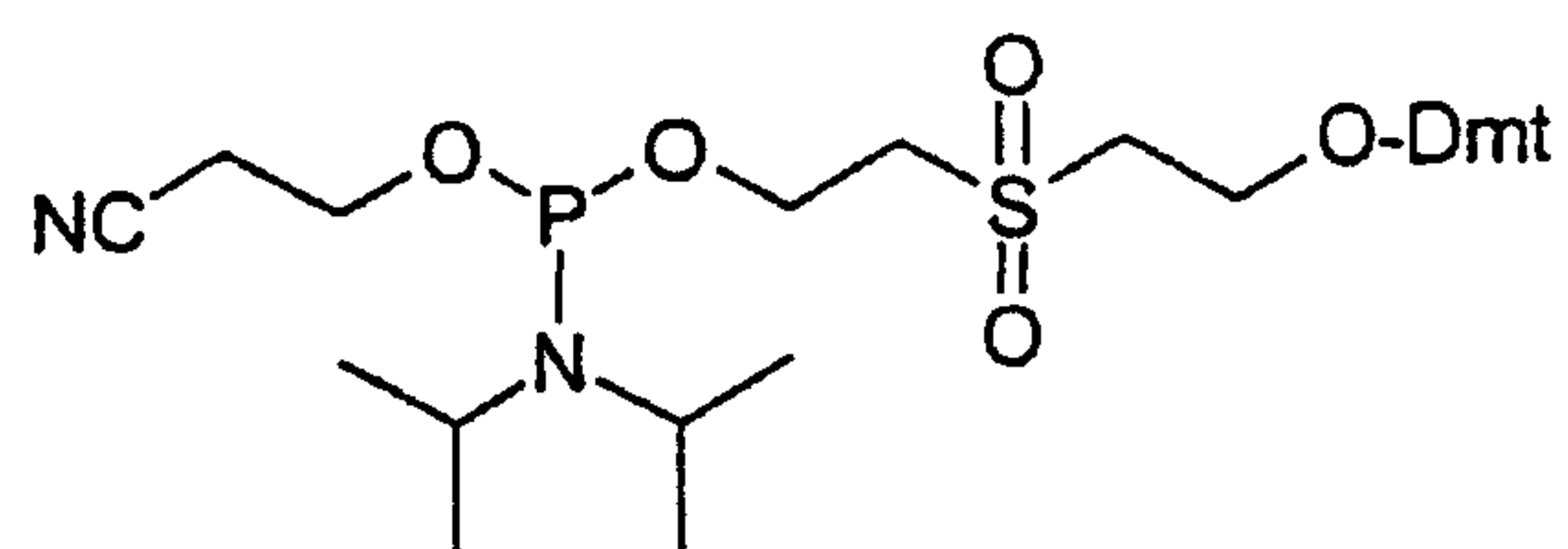


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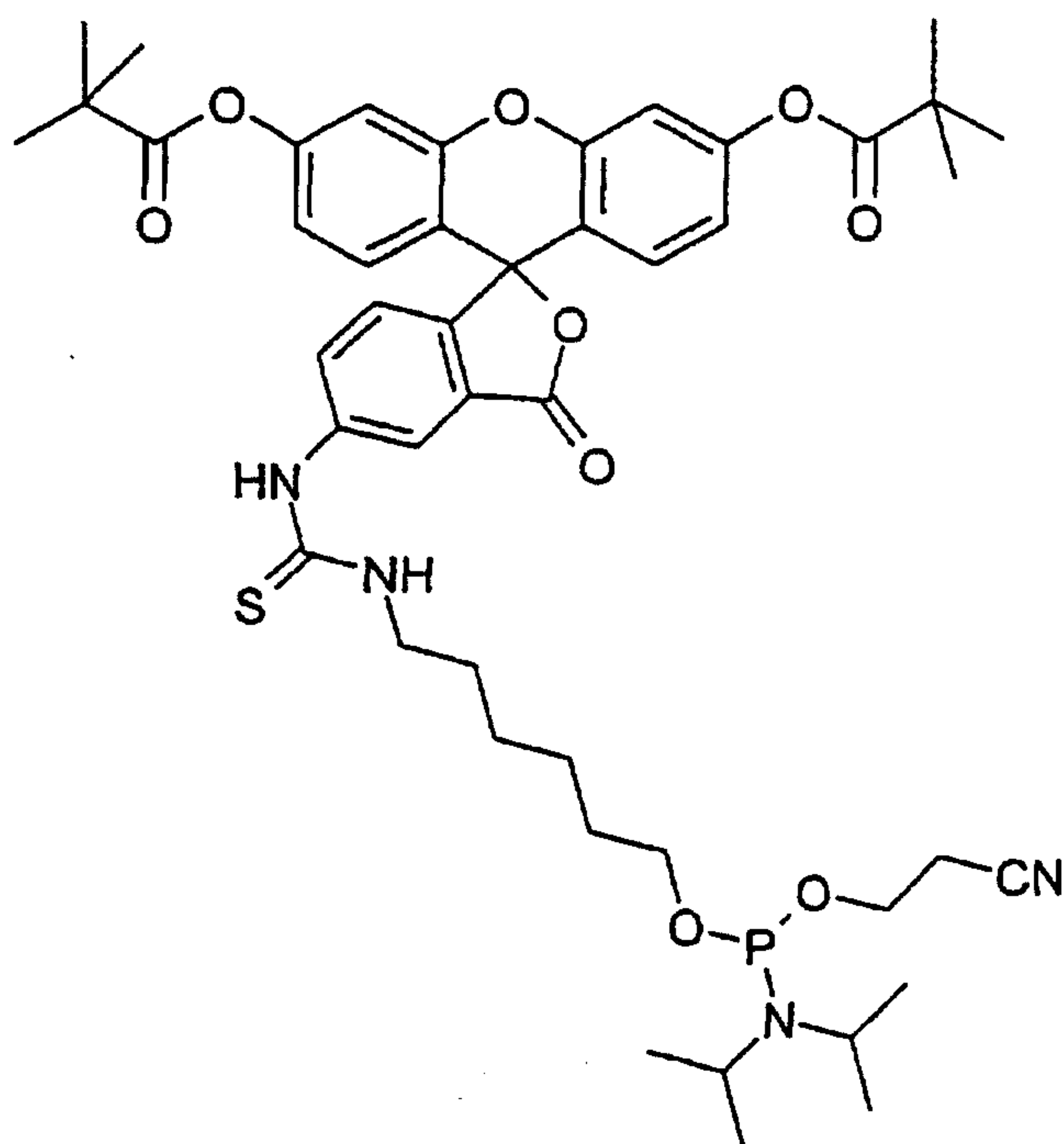
Fig. 4a:



Phosphorylating reagent 1

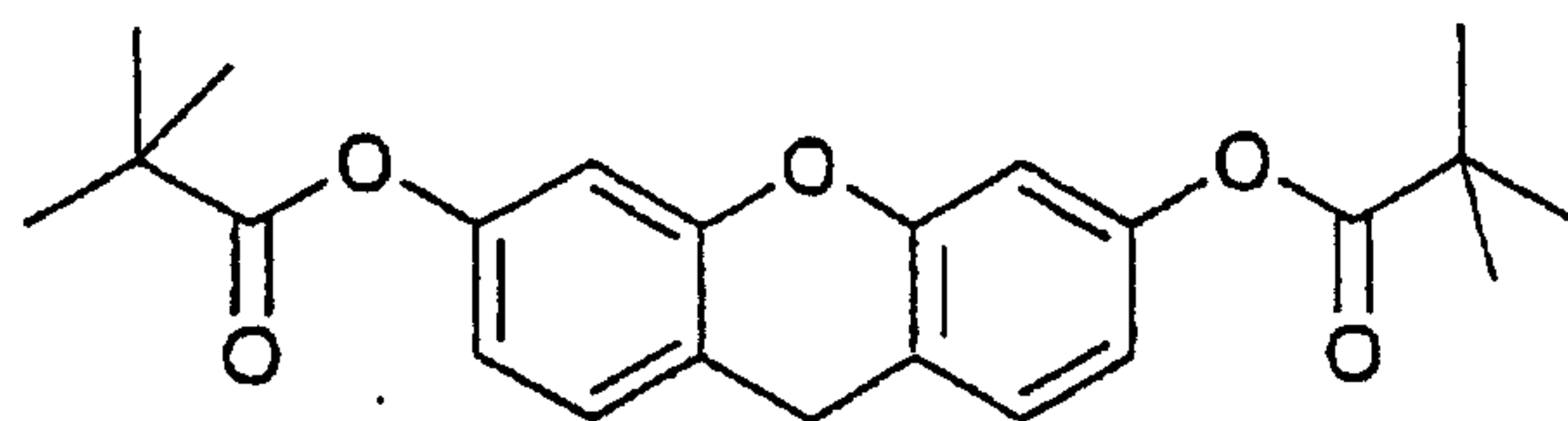


Phosphorylating reagent 2

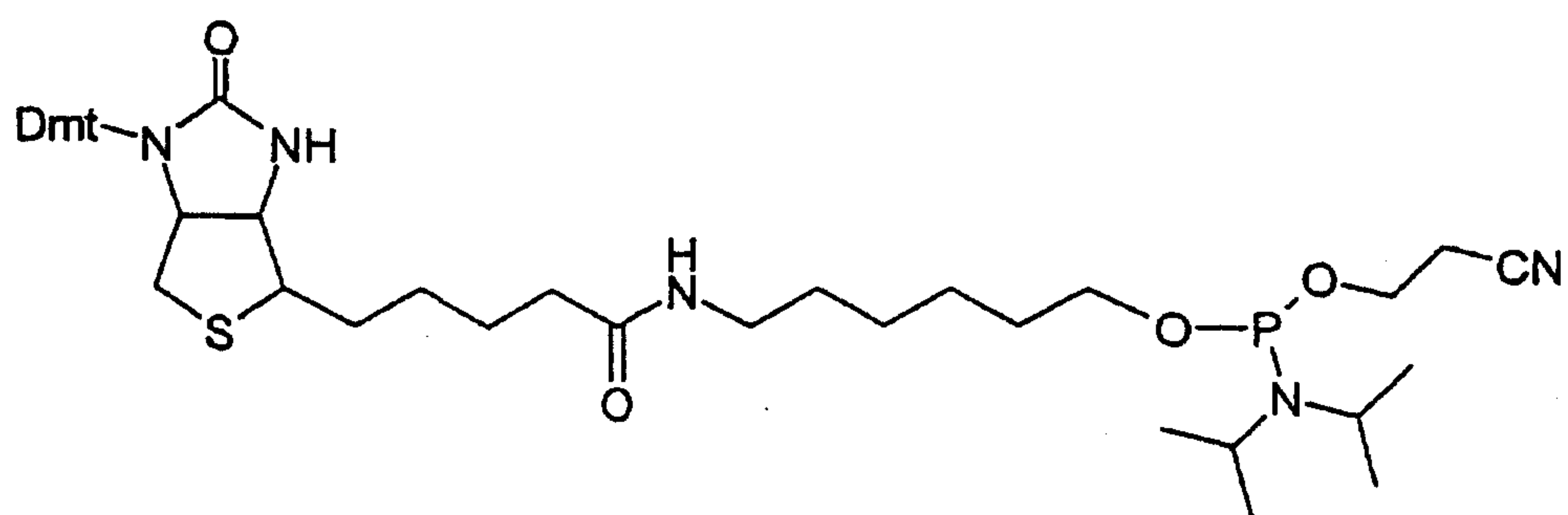
Fluorescein phosphoramidite 3
(monofunctional)

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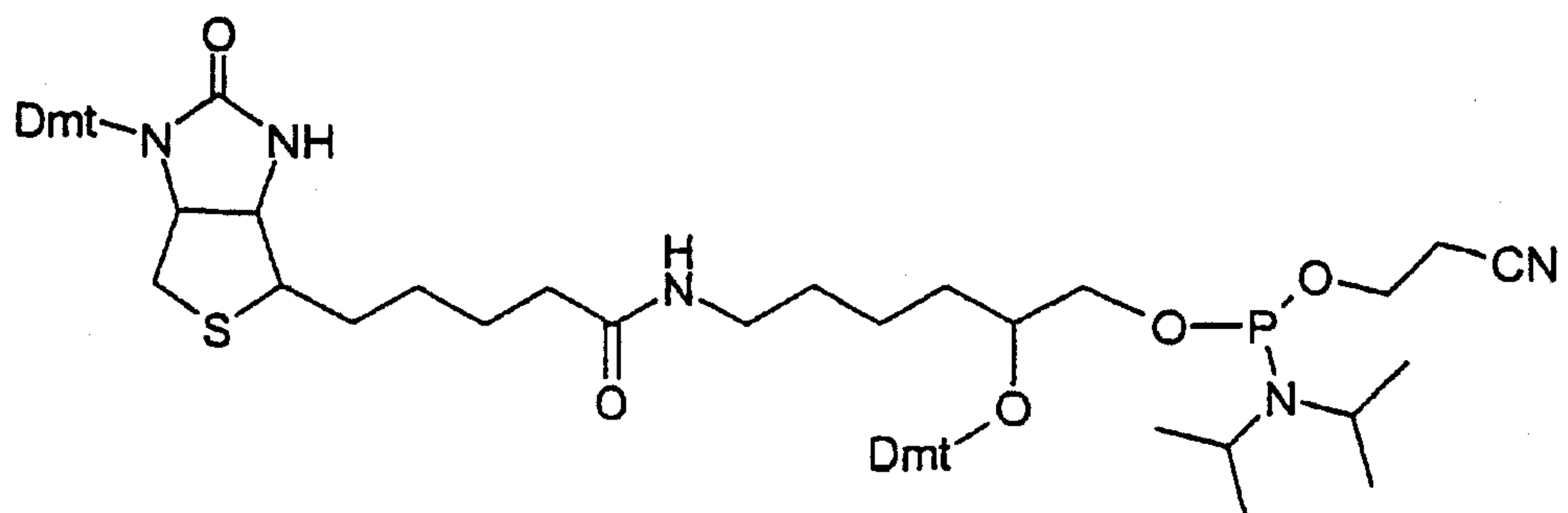
Fig. 4b:



Fluorescein phosphoramidite 4
(bifunctional)



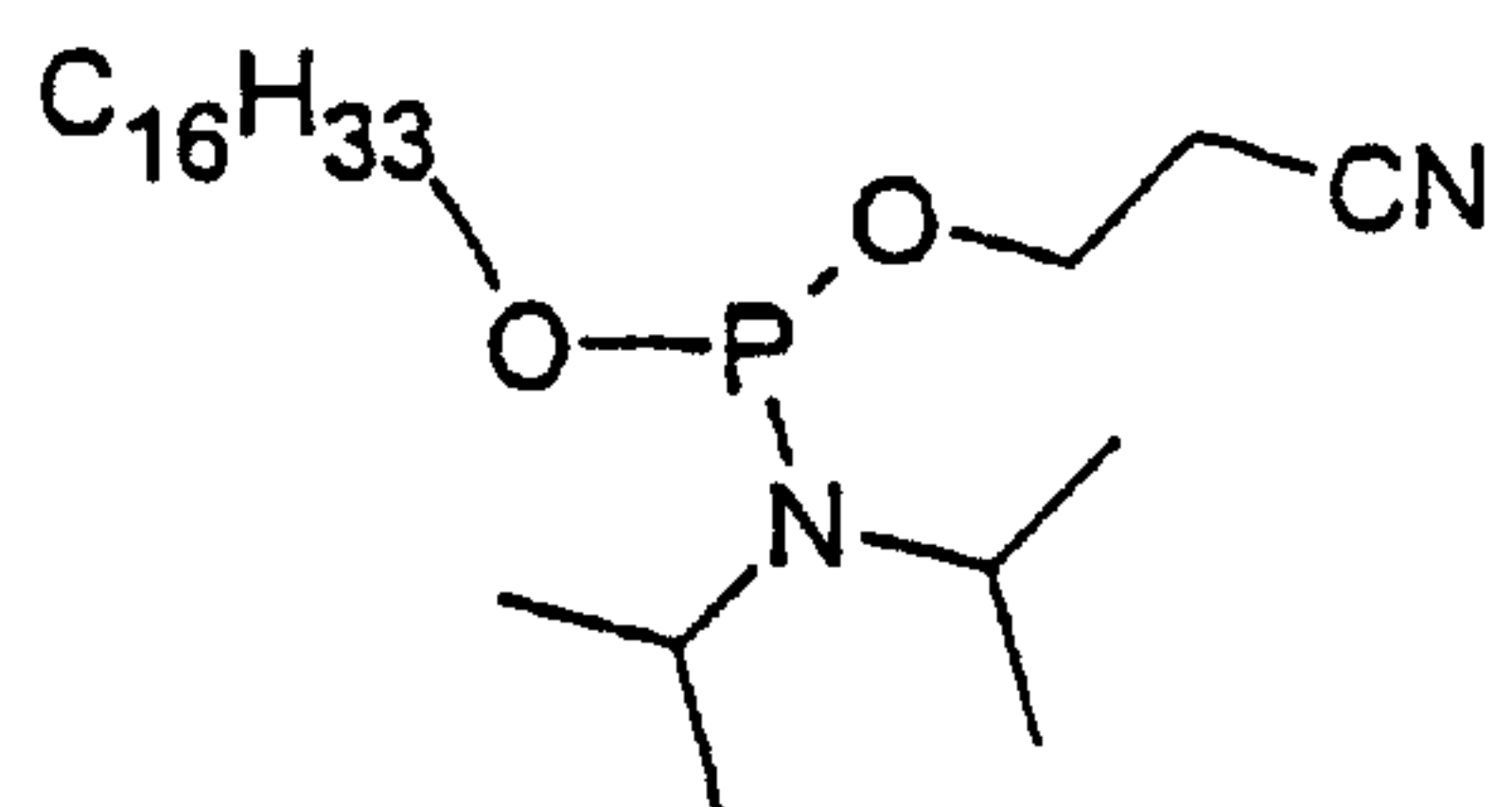
Biotin phosphoramidite 5 (monofunctional)



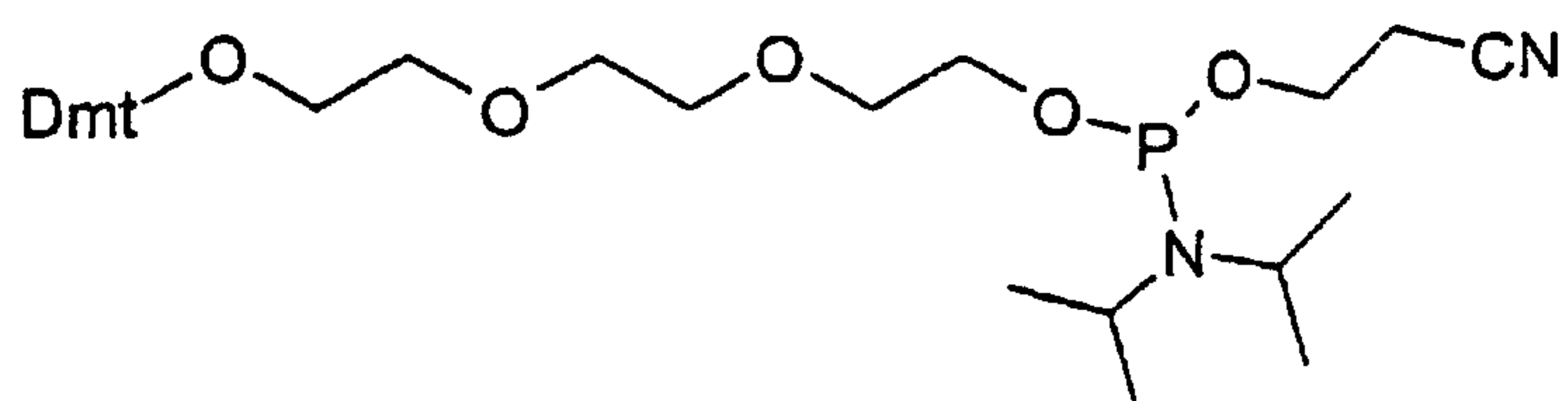
Biotin phosphoramidite 6 (bifunctional)

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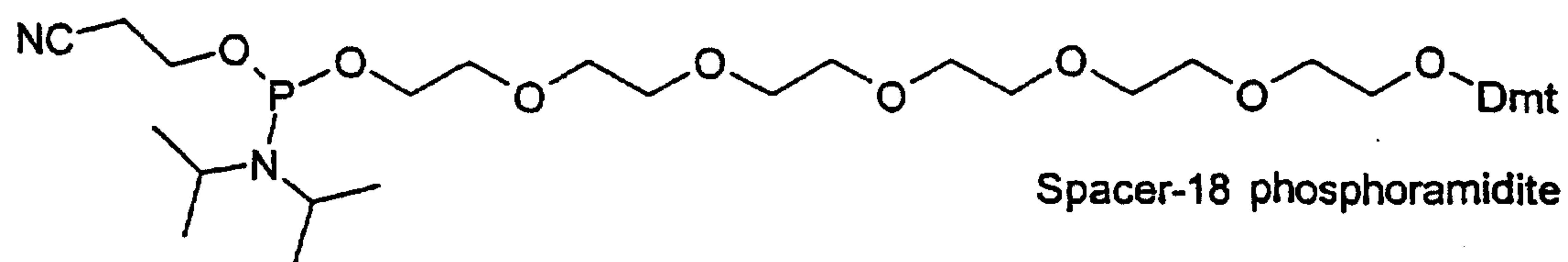
Fig. 4c:



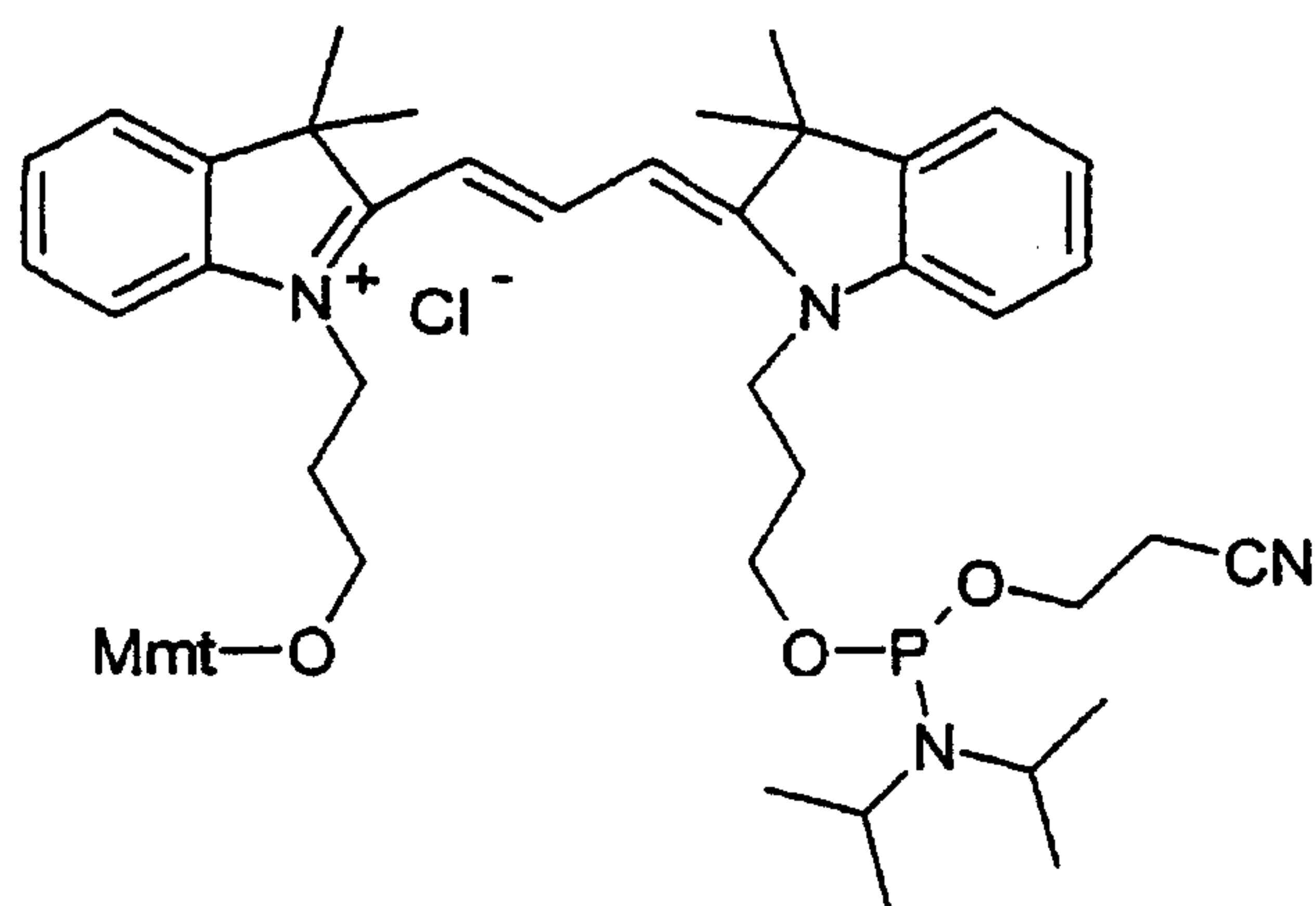
C16-phosphorylating reagent 7



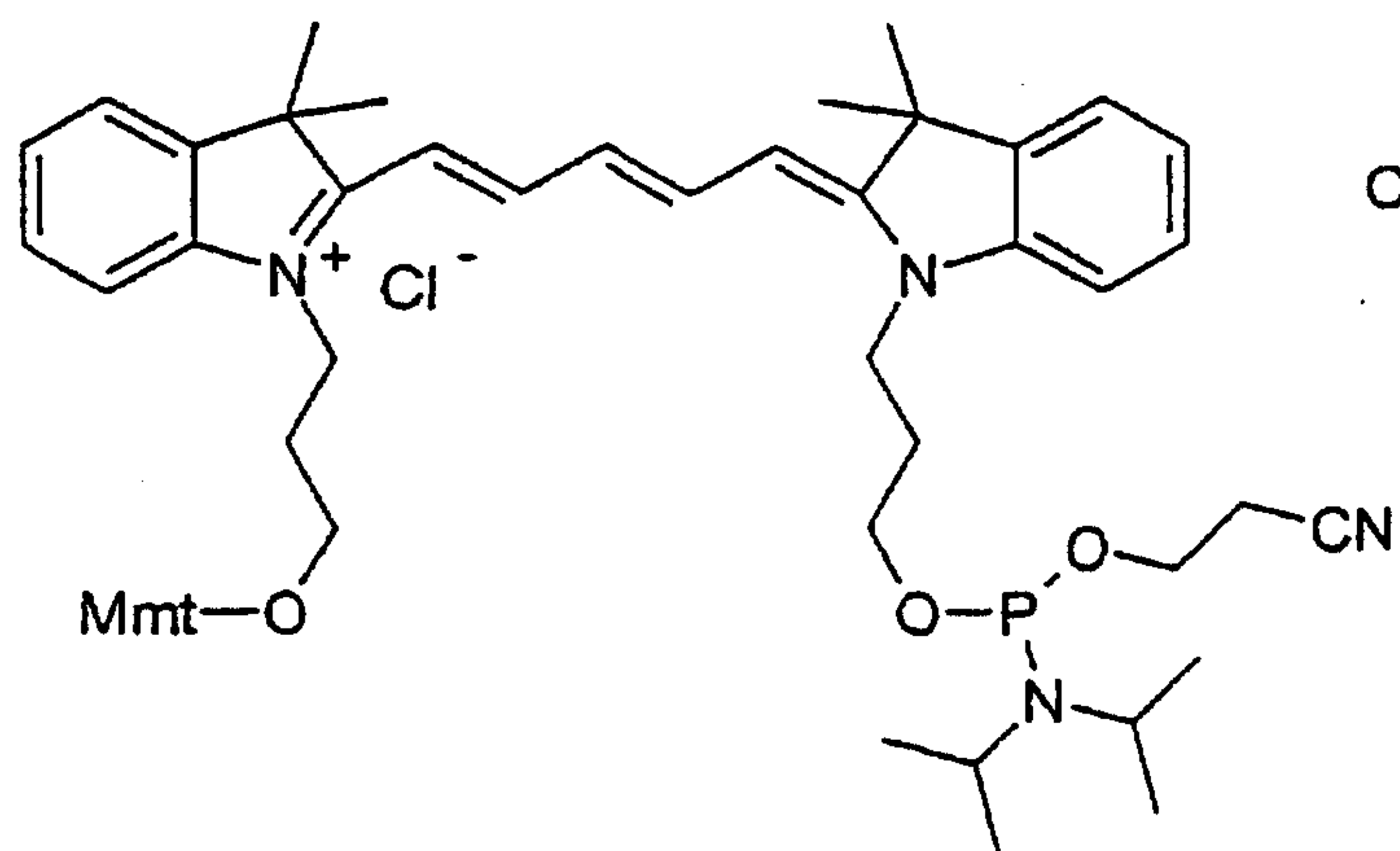
Spacer-9 phosphoramidite 8



Spacer-18 phosphoramidite 9



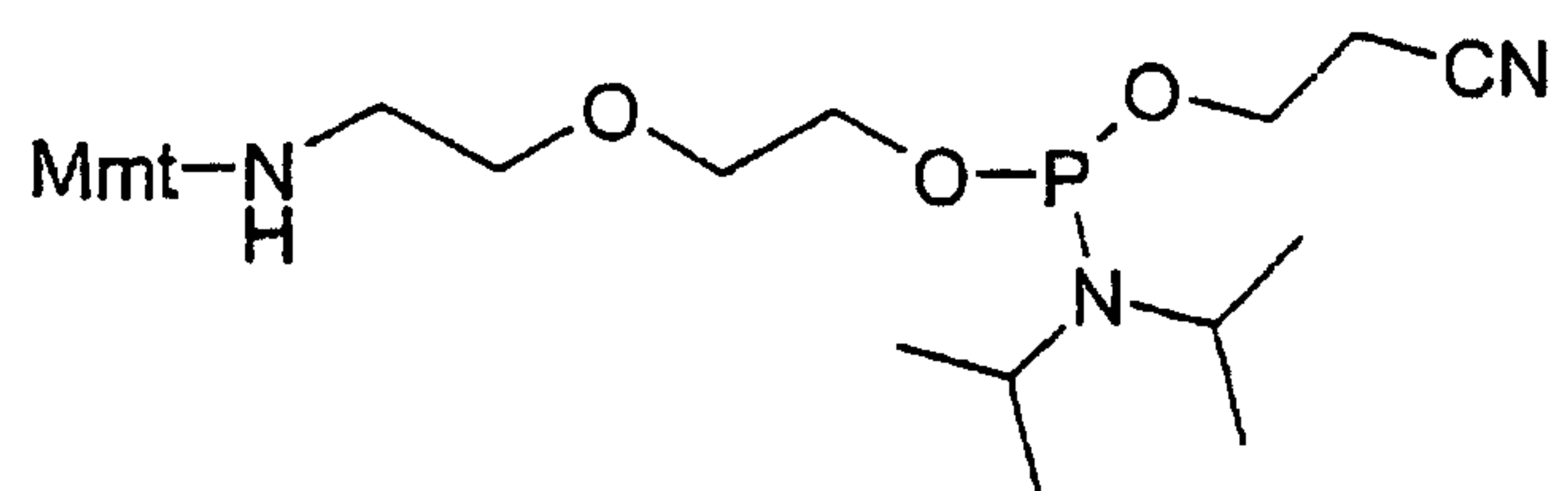
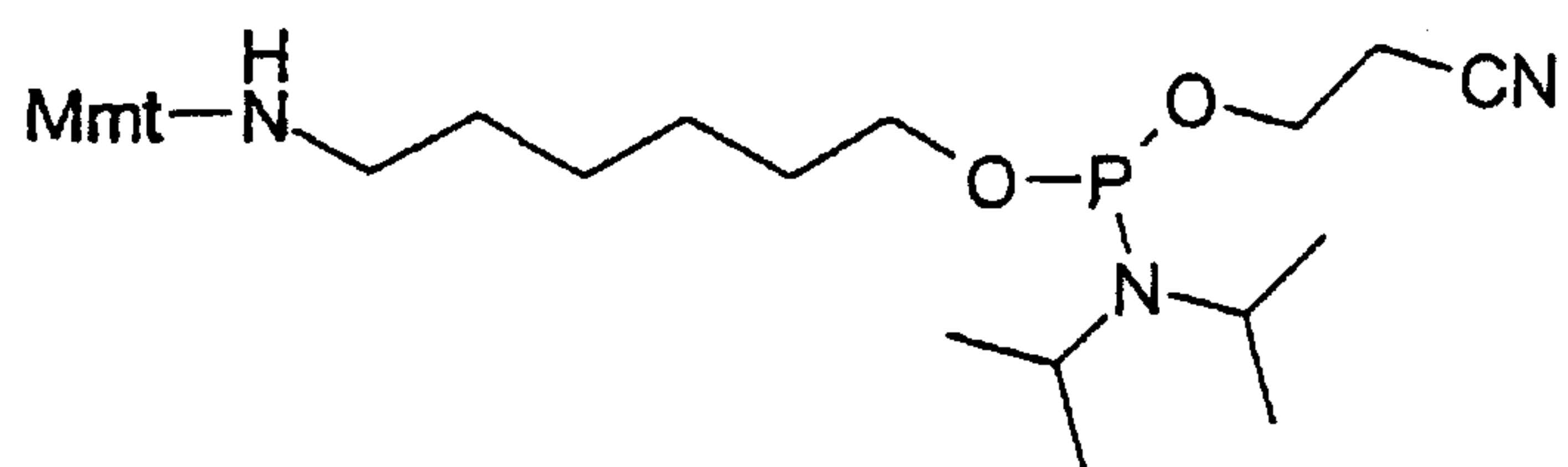
Cyanine-3 phosphoramidite 10



Cyanine-5 phosphoramidite 11

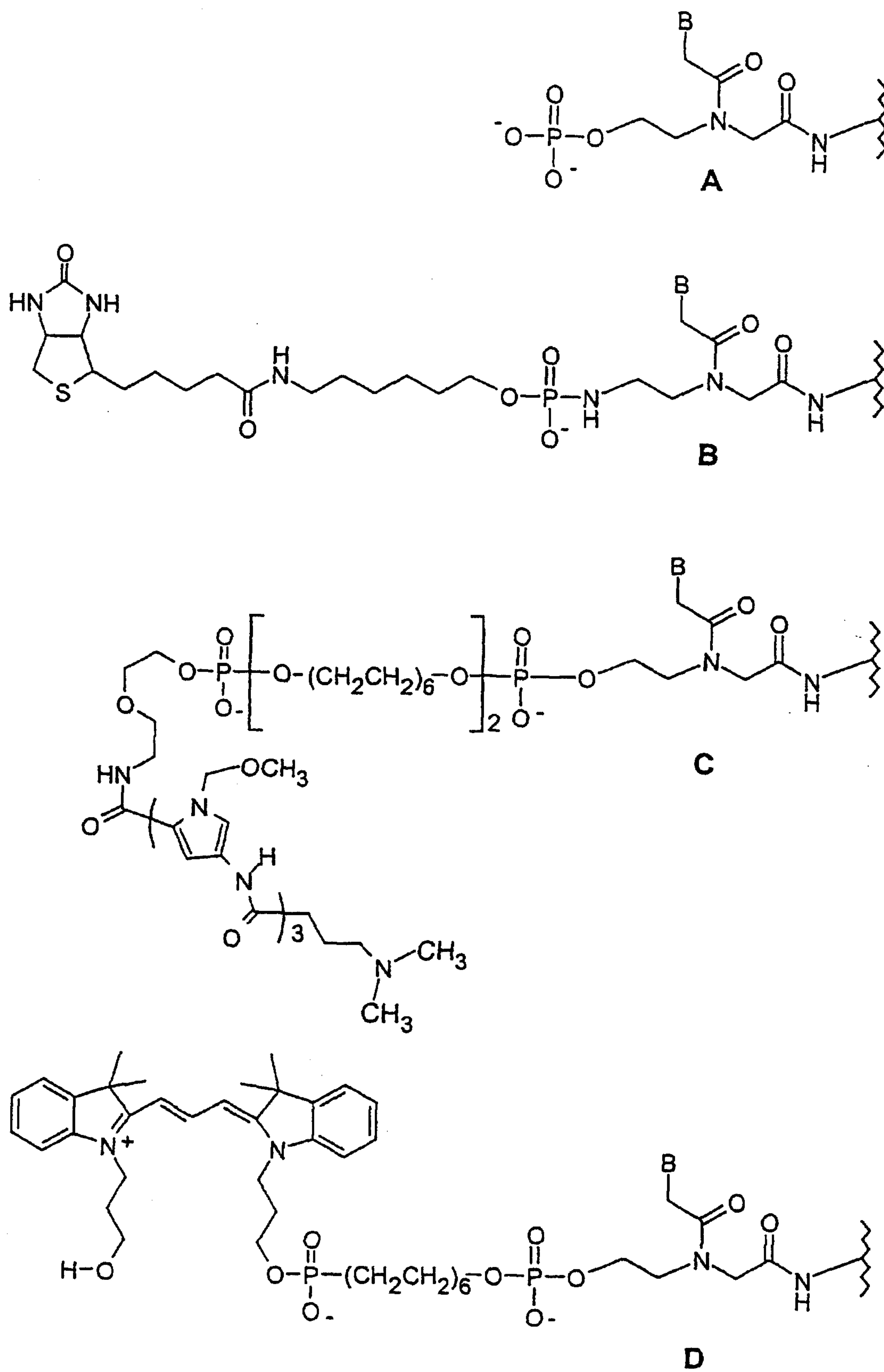
Fig. 4d:

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Aminomodifier-5
phosphoramidite 12Aminomodifier-C6
phosphoramidite 13

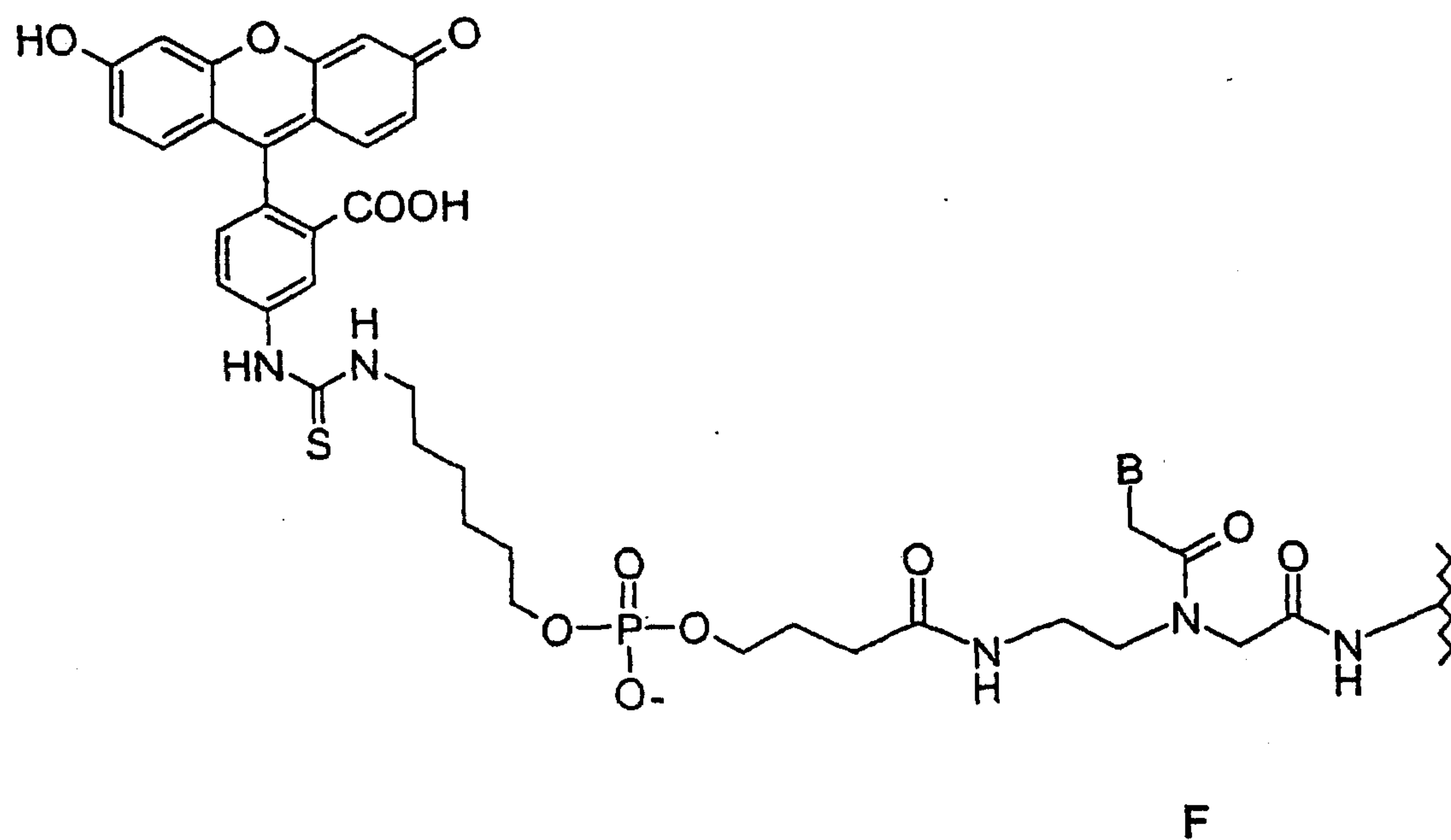
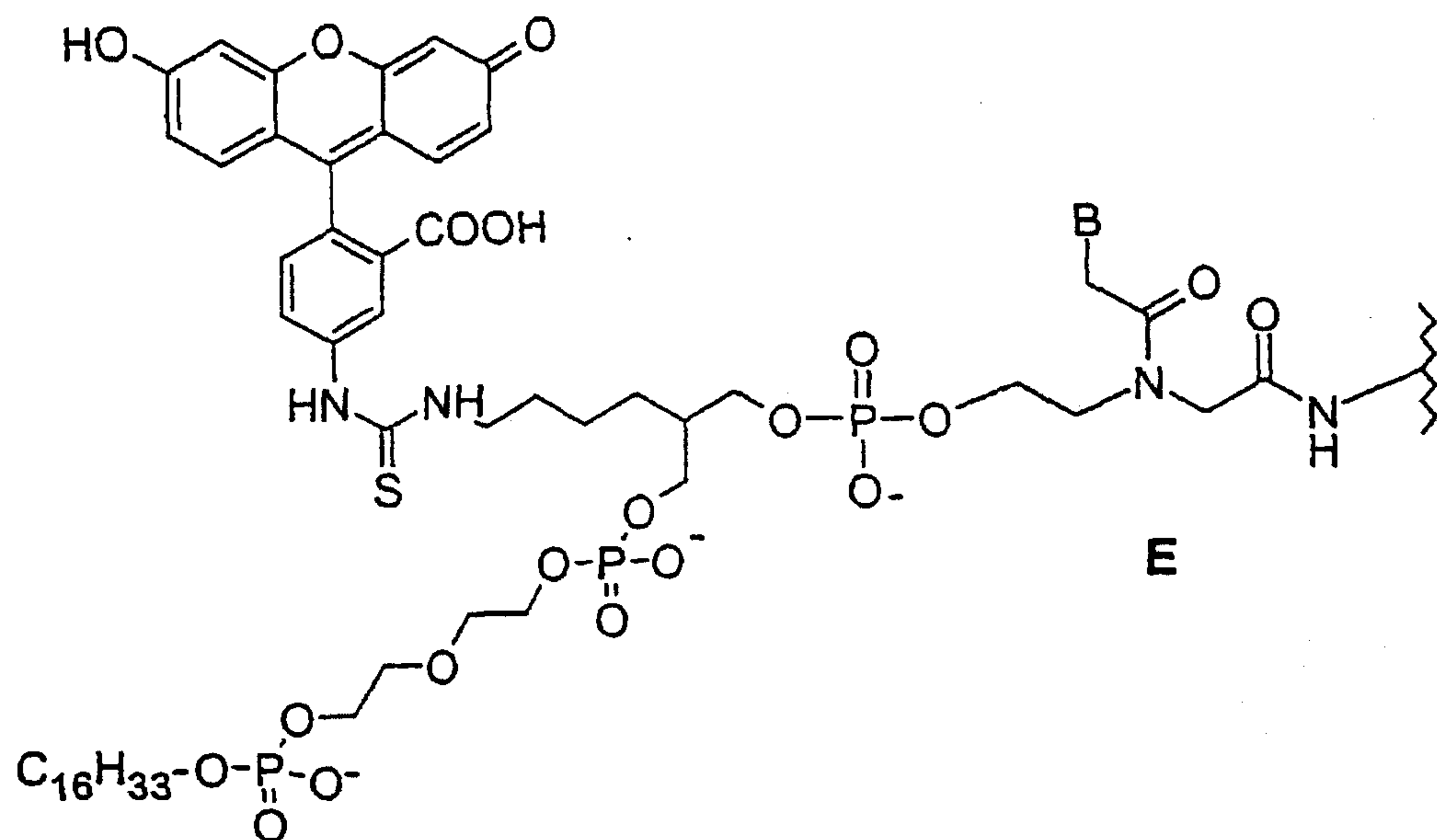
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Fig. 5a:



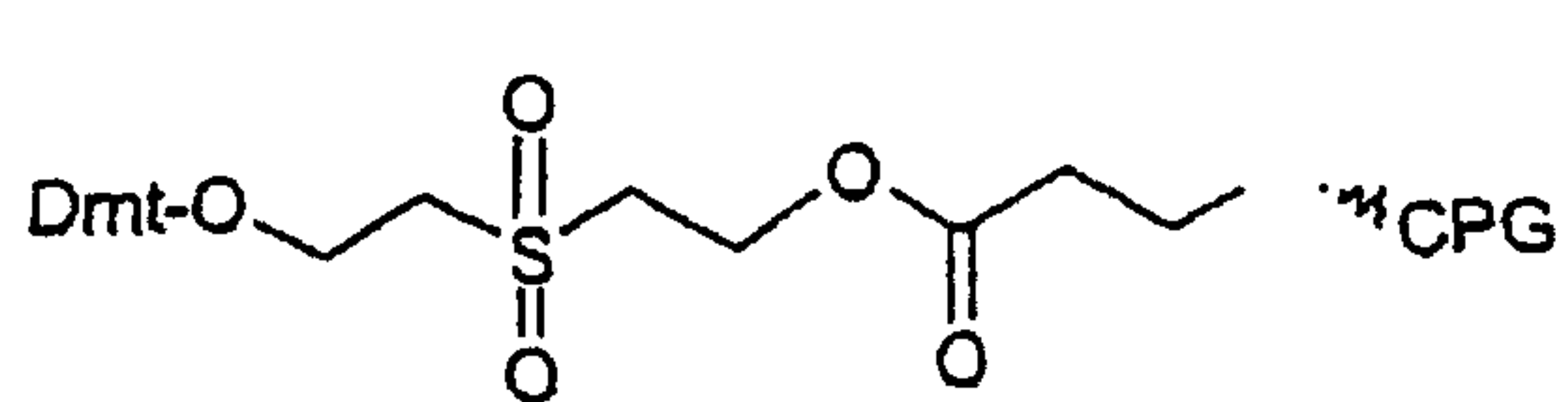
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Fig. 5b:

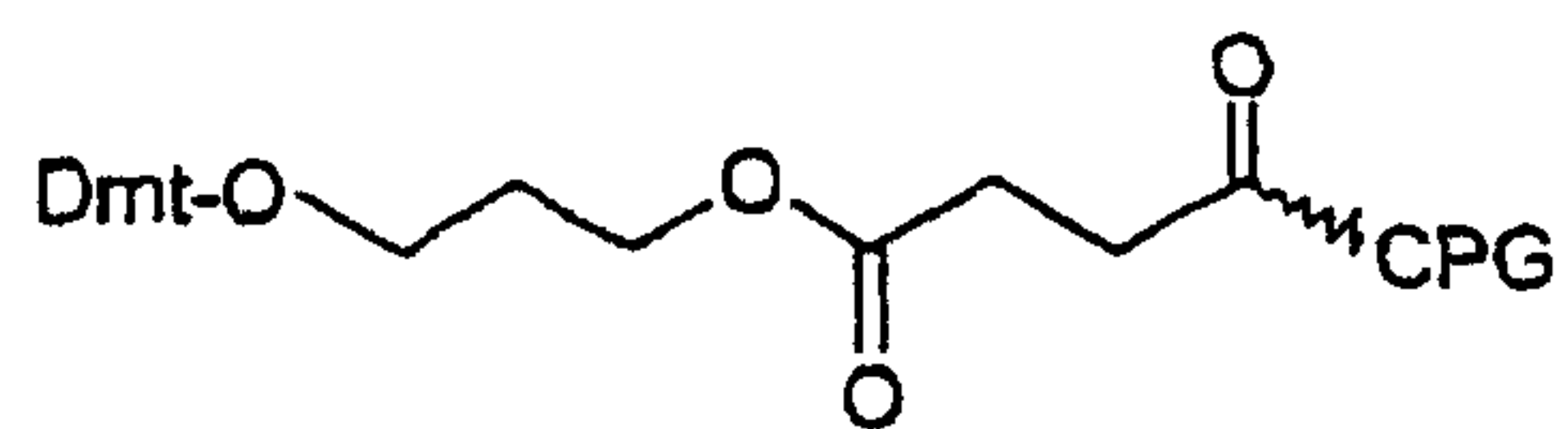


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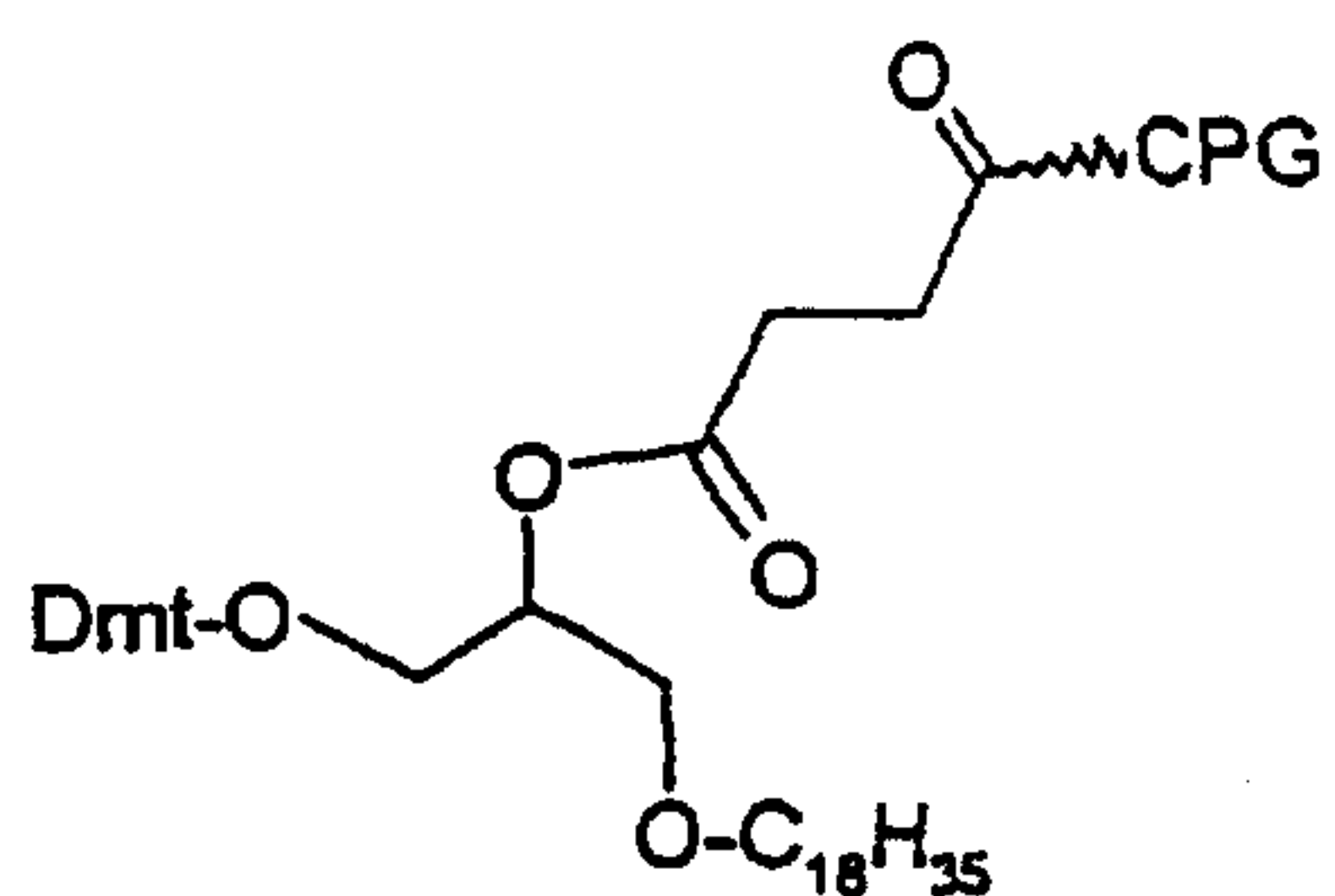
Fig. 6:



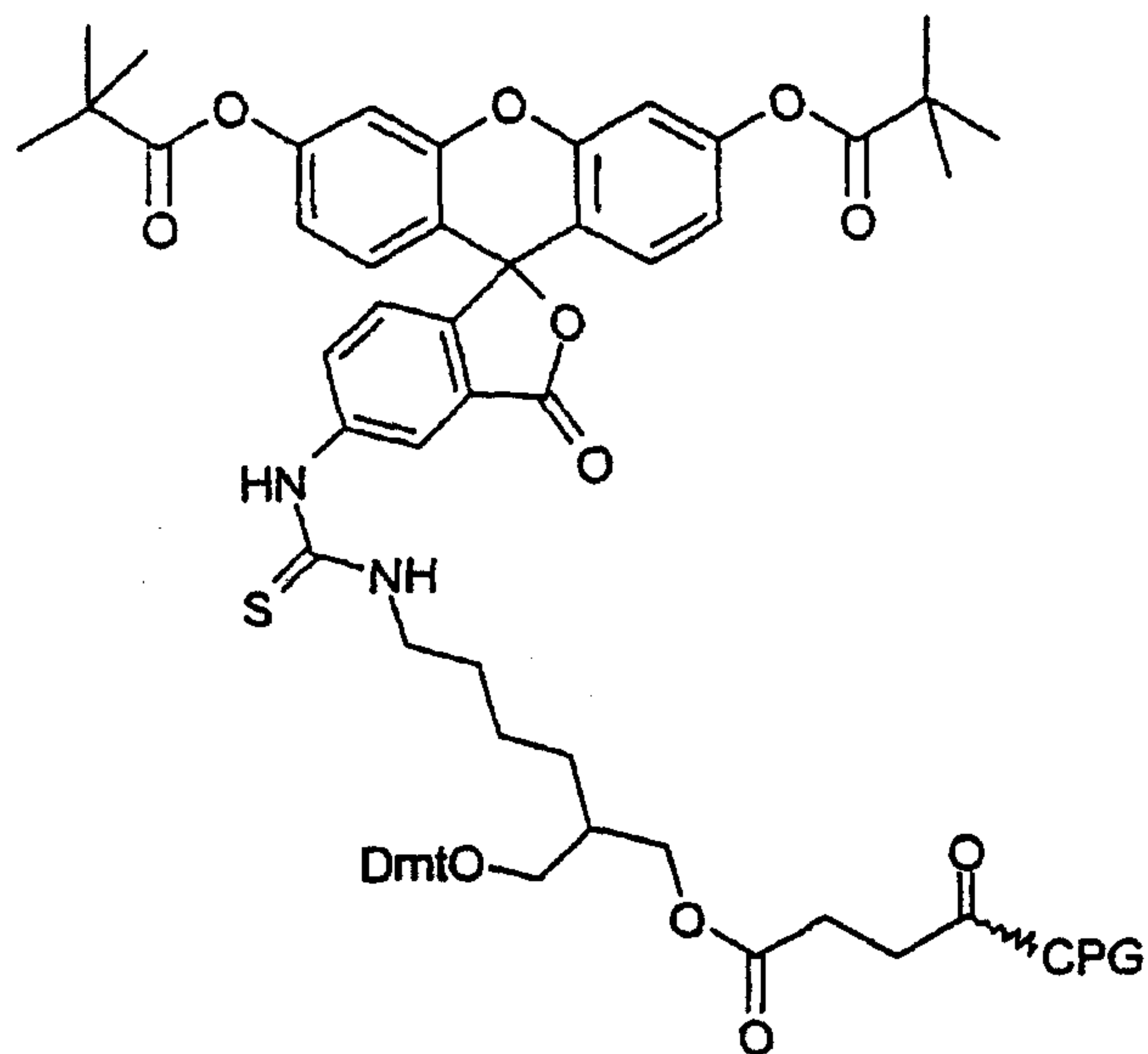
Bishydroxyethylsulfonyl support 1



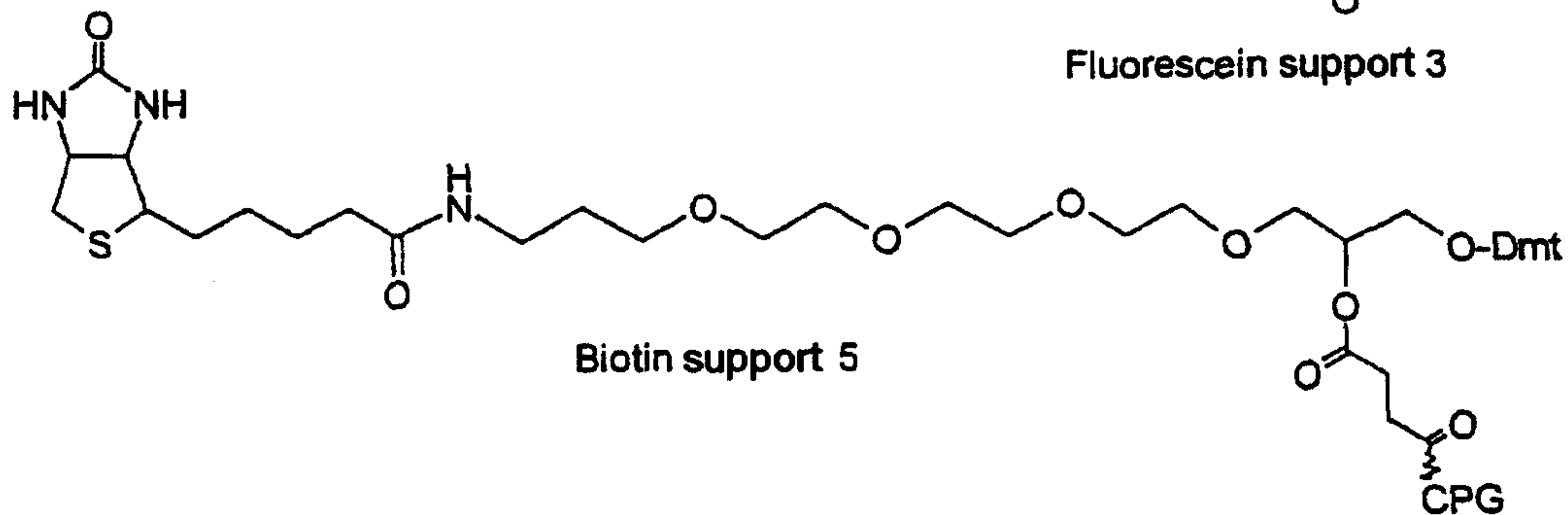
3'-Spacer-C3 support 2



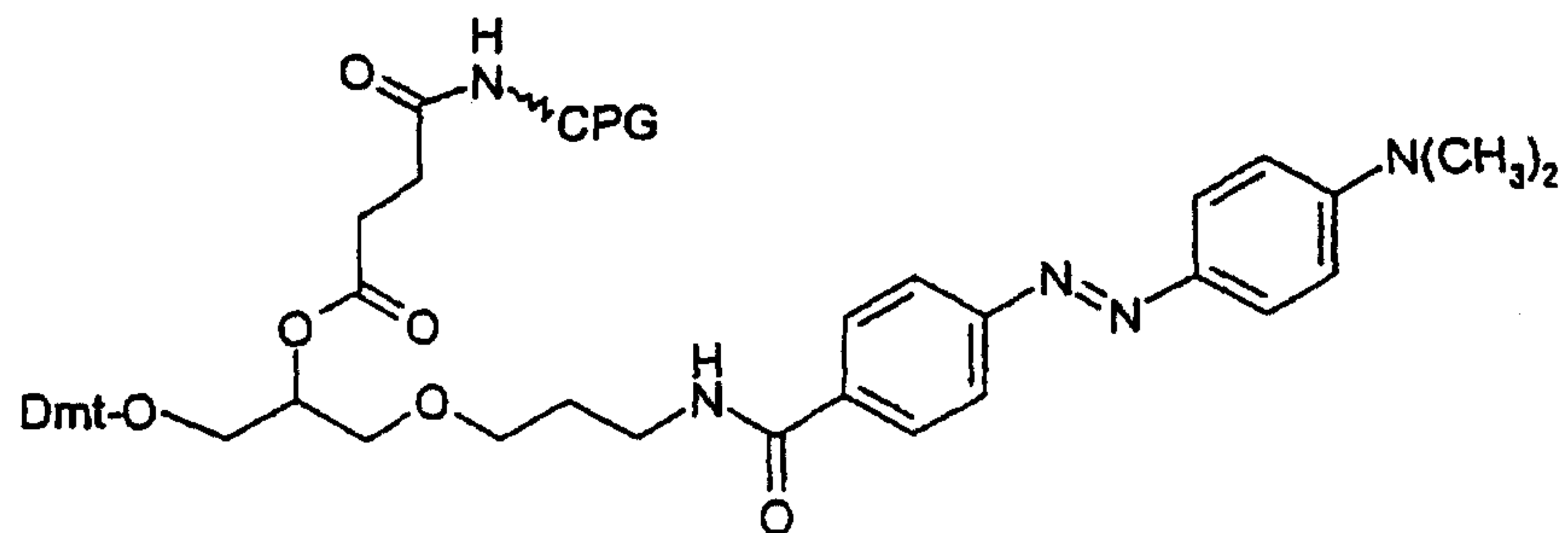
3'-Batyl support 4



Fluorescein support 3



Biotin support 5



DABCYL support 6

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Fig. 7:

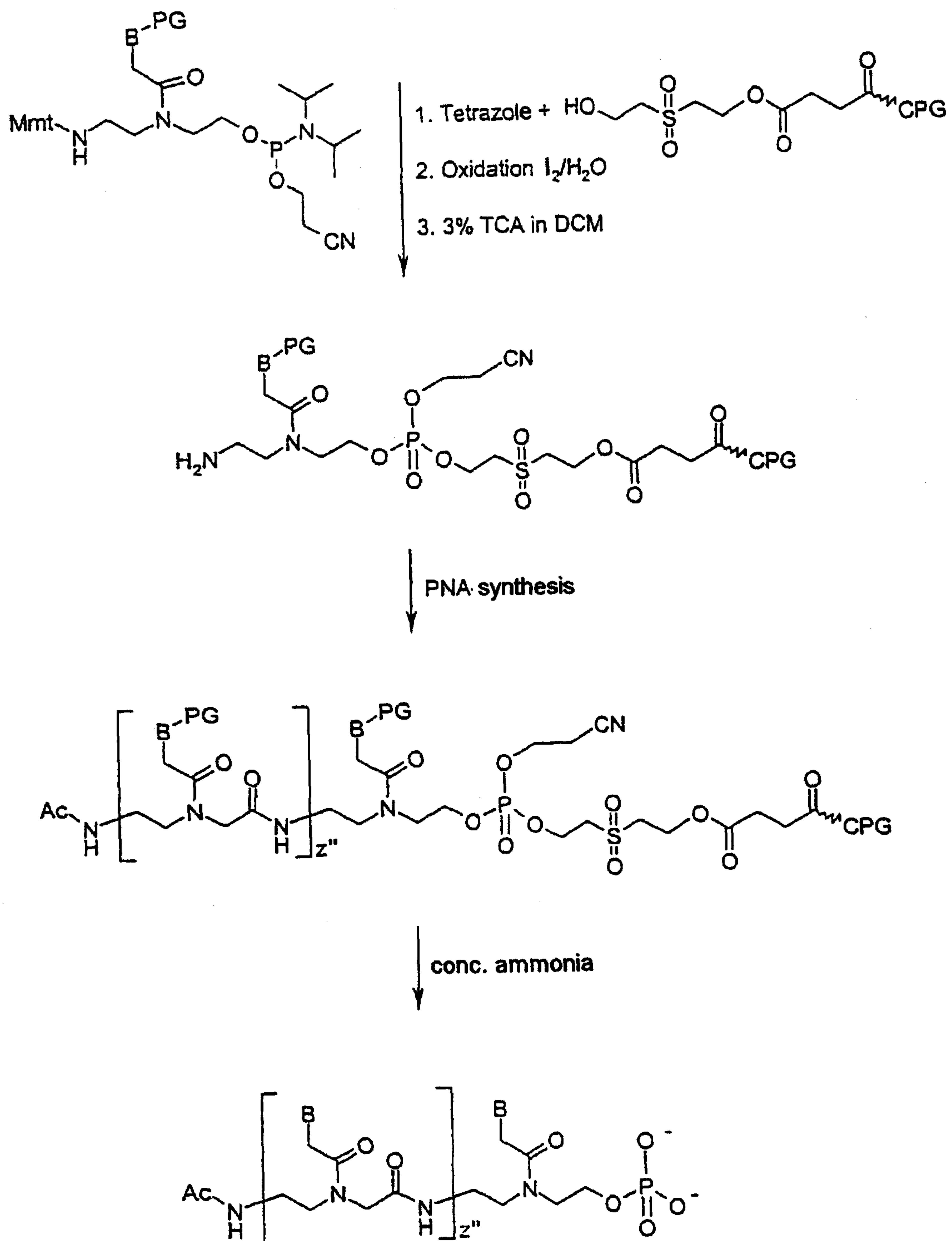


Fig. 8:

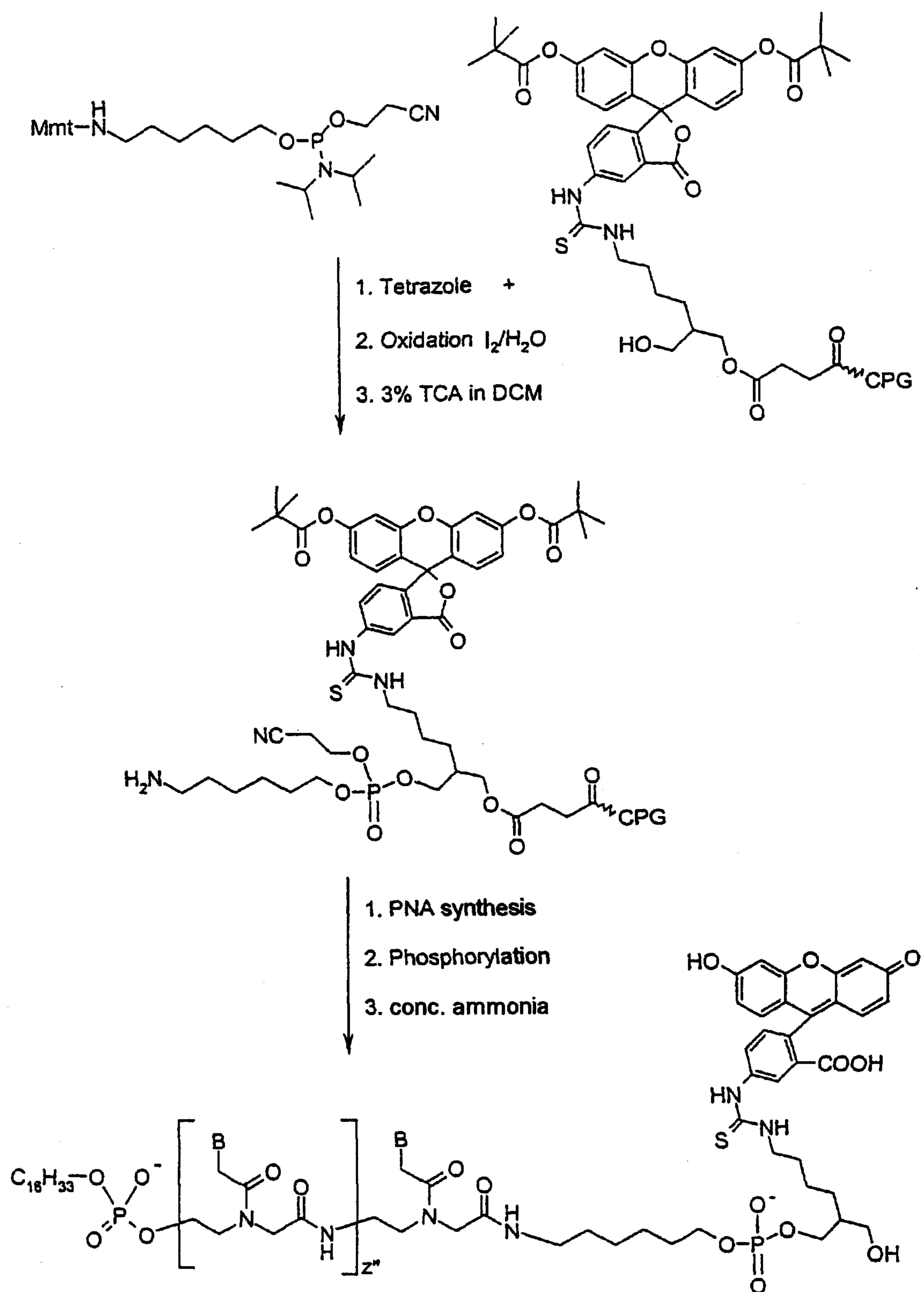


Fig. 9:

