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(54) Titre : SYNTHESE D'OLIGONUCLEOTIDES EN PHASE SOLIDE

(54) Title: SOLID PHASE SYNTHESIS OF OLIGONUCLEOTIDES

(57) **Abrégé/Abstract:**

The invention therefore relates to a process for preparing oligonucleotides by means of solid phase synthesis by a) sequentially synthesizing the nucleotides on a solid support in accordance with known methods, with exocyclic amino groups which are present on the nucleobases being protected by a cyclic diacyl group and with it being possible to eliminate any phosphate protecting groups which are present with strong, nonnucleophilic bases, b) deprotecting the oligonucleotides which are bound to the solid support, and c) cleaving the deprotected oligonucleotides from the solid support in accordance with known methods, which comprises deprotecting the oligonucleotides, which are bound to the solid support, in the presence of a strong, nonnucleophilic base in a suitable organic solvent.

Abstract of the disclosure

Solid phase synthesis of oligonucleotides

The invention therefore relates to a process for preparing oligonucleotides by means of solid phase synthesis by

- a) sequentially synthesizing the nucleotides on a solid support in accordance with known methods, with exocyclic amino groups which are present on the nucleobases being protected by a cyclic diacyl group and with it being possible to eliminate any phosphate protecting groups which are present with strong, nonnucleophilic bases,
- b) deprotecting the oligonucleotides which are bound to the solid support, and
- c) cleaving the deprotected oligonucleotides from the solid support in accordance with known methods,

which comprises deprotecting the oligonucleotides, which are bound to the solid support, in the presence of a strong, nonnucleophilic base in a suitable organic solvent.

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Dr. MBA/St

Description**5 Solid phase synthesis of oligonucleotides**

The chemical polycondensation of mononucleotides is an important method for preparing deoxyribonucleic acid (DNA) or ribonucleic acid (RNA).

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Oligonucleotides are used to an increasing extent as inhibitors of gene expression (J. F. Milligan, M. D. Matteucci and J. C. Martin, *J. Med. Chem.* 36 (1993) 1923; E. Uhlmann and A. Peyman, *Chemical Reviews* 90 (1990) 543) or as ribozymes (e.g. D. Castanotto, J. J. Rossi, J. O. Deshler, *Critical Rev. Eukar. Gene Expr.* 2 (1992) 331), or in diagnosis as DNA probes (e.g. Beck und Köster, *Anal. Chem.* 62 (1990) 2258). There is therefore a great need for suitable methods for synthesizing such compounds.

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The state of the art with regard to oligonucleotide synthesis is reviewed in E. Sonveaux, *Bioorg. Chem.* 14 (1986) 274; E. Uhlmann and A. Peyman, *Chemical Reviews* 90 (1990) 543, Beaucage and Iyer *Tetrahedron* 49 (1993) 10441-10488.

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A basic problem in the chemical synthesis of DNA or RNA is that of finding suitable protecting groups for the amino and hydroxyl groups of the nucleoside bases and the sugar residues. On the one hand, these protecting groups have to be stable under the conditions of the polycondensation reaction, i.e. during the reaction, and, on the other hand, they have to be sufficiently labile to enable them to be removed again at the end of the reaction without recleaving the phosphodiester bond [H. G. Khorana; *Pure Appl. Chem.* 17 (1968) 349].

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Current practice in DNA synthesis essentially provides for three steps:

(a) sequential synthesis of the variously protected nucleotides on a solid support; (b) cleavage of the synthesized oligonucleotides from the support; (c) deprotection of the oligonucleotides. While the synthesis of the oligonucleotide on the solid

support takes place very rapidly - approximately one hour is required for a 20mer - and cleavage from the support is also complete within an hour, the final deprotection of the oligonucleotide remains a problem. Standard oligonucleotide synthesis (e.g. M. Reddy, N. B. Hanna, F. Farooqui, WO 95/24413) provides for a treatment of approx. 6 h at 55 °C with conc. NH₃ when the established benzoyl, for dA and dC, and butyryl for dG, protecting groups are employed. A whole series of protecting groups which are more sensitive to ammoniacal aminolysis than are the conventionally protected nucleotide derivatives have recently been proposed for speeding up this latter step (M. Reddy et al., see above; Beaucage and Iyer, see above). These protecting groups comprise, for example, the phenoxyacetal group (Schulhof et al., Nucl. Acids Res. 15 (1987) 397; the dimethylformamidine group (Vu et al., Tetrahedron Lett. 31 (1990) 7269, or the tert-butylphenoxyacetyl group (Sinha et al., Biochimie 75 (1993) 13, or phenylacetyl protecting groups as described in Reddy et al. - see above. While the deprotecting time at 55°C is reduced to 15-60 minutes when these ammonia-labile protecting groups are employed, their use also suffers from disadvantages: in the first place, the lability of these groups also leads to instability toward the DNA synthesis conditions, for example during the capping step (Beaucage and Iyer, see above). Phenoxyacetyl protecting groups, for example, reduce the solubility of the nucleotide derivatives so that solvent mixtures have to be employed.

An additional criterion for the use of protecting groups for the exocyclic amino functions of the nucleobases is the purity of the resulting products. In the case of deprotecting procedures which use ammonia, and which are carried out after or during cleavage from the support, a mixture of oligonucleotide and eliminated protecting groups is always obtained. The oligonucleotide has then to be cleaned up in additional purification steps. Protecting groups which can be eliminated while the oligonucleotide is still on the solid support, without the oligonucleotide being cleaved from the latter, are more advantageous. An example of such a protecting group is the para-nitrophenylethyloxycarbonyl protecting group, which can be removed with DBU while the oligonucleotide is still on the support. Subsequent cleavage of the oligonucleotide from the support with ammonia yields

oligonucleotide which is already pure (F. Himmelsbach et al., Tetrahedron 40 (1984) 59).

5 A further criterion for the usability of protecting groups for the exocyclic amino functions of the nucleobases is the stability toward acid conditions as employed, as a rule, in each reaction cycle for eliminating the 5'-hydroxyl protecting group, for example 2% dichloroacetic acid in dichloromethane. These conditions lead, in particular in the case of deoxyadenosine, to a not insubstantial degree of depurination. Cyclic diacyl groups, such as phthaloyl or succinoyl groups, were
10 found, when used as protection for the exocyclic amino function, to be particularly stable toward depurination conditions (Kume et al., Tetrahedron Lett. 23 (1982) 4365; Nucleic Acids Res. 12 (1984) 8525; Nucleic Acids Res. Symp. Ser. 11 (1982) 26; Chemistry Letters 1983, 1597). However, these groups were also deprotected with ammonia (Kume et al., see above). An example of other cyclic diacyl groups is
15 the naphthaloyl group (Dikshit et al., Can. J. Chem. 66 (1988) 2989, which is likewise stable toward depurination and was also removed with ammonia.

It has now been found, surprisingly, that these cyclic diacyl groups are not only particularly stable toward depurination conditions but can be readily eliminated with
20 a strong, nonnucleophilic base such as DBU. This is all the more astonishing since some phthaloyl groups were introduced using DBU - although this was at relatively low DBU concentrations (Kamaike et al., Tetrahedron Lett. 36 (1995) 91). Owing to the high stability toward depurination of the nucleobases which are protected with cyclic diacyl groups, and the possibility of readily removing these groups before
25 cleaving the oligonucleotide from the support, these cyclic diacyl groups are ideally suitable for use as protecting groups for preparing oligonucleotides.

The invention therefore relates to a process for preparing oligonucleotides by means of solid phase synthesis by

- 30 a) sequentially synthesizing the nucleotides on a solid support in accordance with known methods, with exocyclic amino groups which are present on the nucleobases being protected by a cyclic diacyl group and with it being possible to eliminate any phosphate protecting groups which are present with

strong, nonnucleophilic bases,

- b) deprotecting the oligonucleotides which are bound to the solid support, and
- c) cleaving the deprotected oligonucleotides from the solid support in accordance with known methods,

5 which comprises deprotecting the oligonucleotides which are bound to the solid support in the presence of a strong, nonnucleophilic base in a suitable organic solvent such as acetonitrile, pyridine or N-methylimidazole.

10 Strong, nonnucleophilic bases, such as DBU (diazabicyclo[5.4.0]undec-7-ene), DABCO (diazabicyclo-[2.2.2]octane), DBN (diazabicyclo-[4.3.0]non-5-ene), ethyldiisopropylamine, triethylamine, N-ethylmorpholine, DMAP (dimethylaminopyridine) or lutidine or uncharged, peralkylated polyaminophosphazene bases (R. Schwesinger, Angew. Chem. 99 (1987) 1212) are known to the skilled person. The strong, nonnucleophilic base DBU is preferred.

15 Preferably, the deprotection is effected in the presence of an 0.1 to 5 M solution of DBU at from 0 to 70°C for from 0.1 to 16 h, particularly preferably in the presence of an 0.3 to 3 M solution of DBU at from 10 to 40°C for from 0.1 to 2 h, very particularly preferably in the presence of an 0.5 to 2.5 M solution of DBU at from 20
20 to 30°C for from 0.2 to 1.5 h.

The term "oligonucleotides" quite generally encompasses polydeoxyribonucleotides which contain modified and/or unmodified 2'-deoxyribose building blocks (DNA); polyribonucleotides which contain modified and/or unmodified ribose building blocks
25 (RNA); and also other polynucleotides which are synthesized from N-glycosides or C-glycosides of modified and/or unmodified purine or pyrimidine bases, where the phosphate bridges of the polydeoxyribonucleotides, polyribonucleotides or polynucleotides can also exhibit modifications or be replaced with other structures, with the oligonucleotides possessing at least one base having an exocyclic amino
30 group.

Examples of these modifications, which are introduced using methods which are known per se, are:

a) **Modifications of the phosphate bridge**

The following may be mentioned by way of example: phosphorothioates, phosphorodithioates, methylphosphonates, phosphoramidates, boranophosphates, methyl phosphates, ethyl phosphates and phenyl-phosphonates. Preferred modifications of the phosphate bridge are phosphorothioates, phosphorodithioates and methylphosphonates.

b) **Replacement of the phosphate bridge**

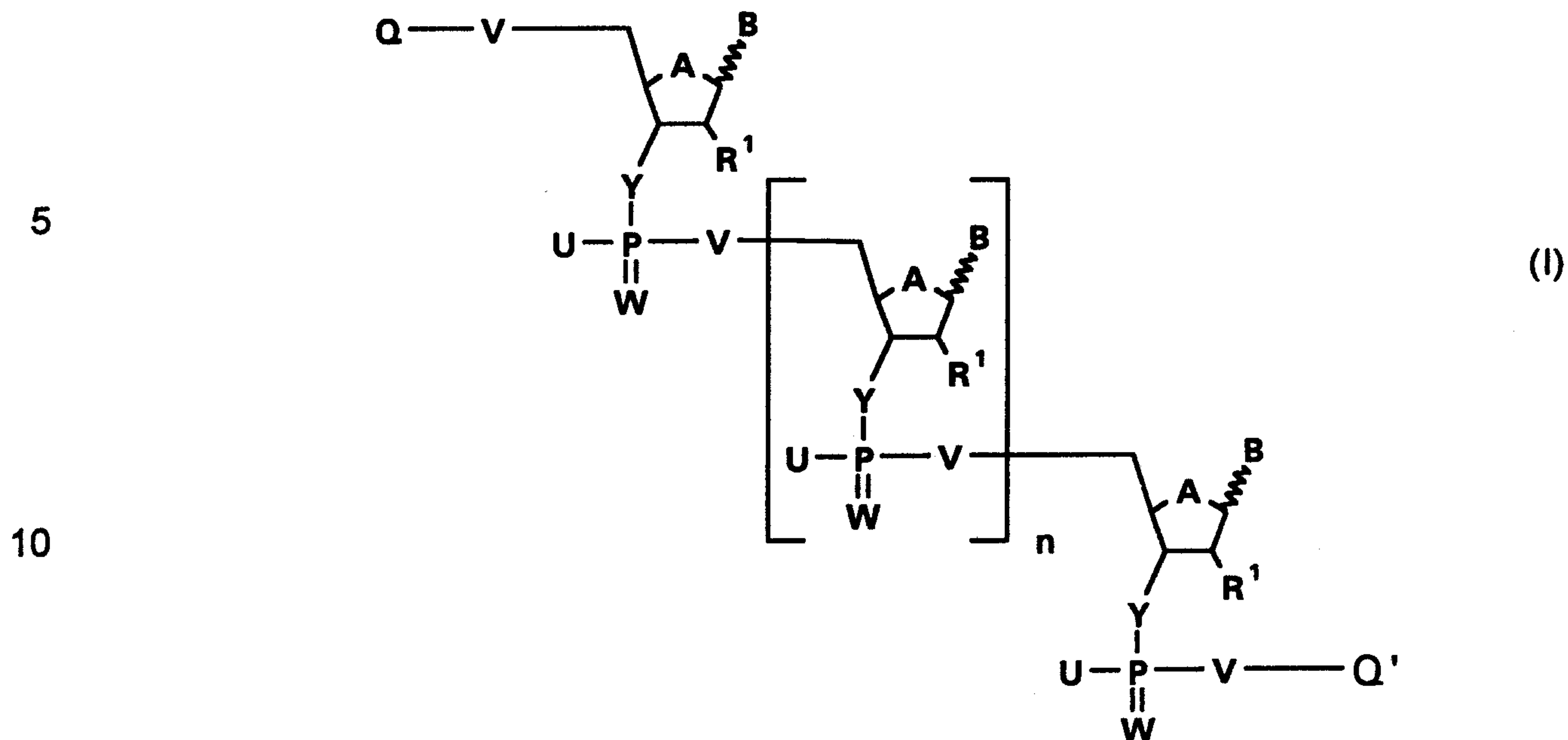
The following may be mentioned by way of example: replacement with acetamide, formacetal, 3'-thioformacetal, methylhydroxylamine, oxime, methylenedimethylhydrazo, dimethylsulfone and silyl groups. Preference is given to replacement with acetamide, formacetals and 3'-thioformacetals.

c) **Modifications of the sugar**

The following may be mentioned by way of example: α -anomeric sugars, 2'-O-methylribose, O-butylribose, 2'-O-allylribose, 2'-fluoro-2'-deoxyribose, 2'-amino-2'-deoxyribose, α -arabinofuranose and carbocyclic sugar analogs. Modification with 2'-O-methylribose and 2'-O-n-butylribose is preferred. Those modifications are very particularly preferred in which the 2' and 3' carbon atoms of the O-ribose are linked by way of a double bond and in each case carry a hydrogen atom as substituent.

The novel process is consequently suitable, for example, for preparing compounds of the formula I

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15 in which

R_1 is hydrogen, hydroxyl, C_1 - C_{18} -alkoxy, which is optionally substituted one to three times by hydroxyl or C_1 - C_4 -alkoxy, C_1 - C_4 -alkyl-O- $(CH_2CH_2O)_s$, in which s is a number from 1 to 3; O-allyl, halogen, azido or amino;

20 1.

A is, independently of each other, oxy, thioxy or methylene;

W is, independently of each other, oxo, thioxo or selenoxo;

25 V is, independently of each other, oxy, sulfanediyl or imino;

Y is, independently of each other, oxy, sulfanediyl, imino or methylene;

30 B is a base which is customary in nucleotide chemistry, for example natural bases, such as adenine, cytosine, guanine, uracil and thymine, or unnatural bases, such as purine, 2,6-diaminopurine, 7-deazaadenine, 7-deazaguanine, 7-deaza-7- C_1 - C_3 -alkynyladenine, 7-deaza-7- C_1 - C_3 -alkynylguanine, N_4N_4 -ethanocytosine, N_6N_6 -ethano-2,6-diaminopurine, pseudoisocytosine, 5-

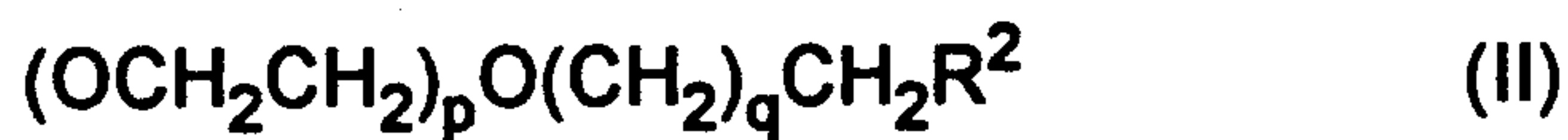
C₂-C₆-alkyneuracil, 5-C₂-C₆-alkynecytosine, preferably 5-propyneuracil, 5-propynecytosine, 5-hexyneuracil, 5-hexynecytosine, or 5-fluorocytosine, 5-fluorouracil, 5-hydroxymethyluracil or 5-bromocytosine; with at least one B being a base which possesses an exocyclic amino group;

5

n is an integer from 1 to 100;

U is hydroxyl, mercapto, BH₃, SeH, C₁-C₁₈-alkoxy, preferably C₁-C₆-alkoxy, C₁-C₁₈-alkyl, preferably C₁-C₆-alkyl, C₆-C₂₀-aryl, (C₆-C₁₄)-aryl-(C₁-C₈)-alkyl, NHR³, NR³R⁴ or a radical of the formula II

10



in which

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R³ is C₁-C₁₈-alkyl, preferably C₁-C₈-alkyl, C₆-C₂₀-aryl, (C₆-C₁₄)-aryl-(C₁-C₈)-alkyl, or -(CH₂)_c-[NH(CH₂)_d]_d-NR⁵R⁵, in which c is an integer from 2 to 6 and d is an integer from 0 to 6;

20

R⁵ is, independently of each other, hydrogen, C₁-C₆-alkyl or C₁-C₄-alkoxy-C₁-C₆-alkyl, preferably methoxyethyl;

25

R⁴ is C₁-C₁₈-alkyl, preferably C₁-C₈-alkyl and particularly preferably C₁-C₄-alkyl, C₆-C₂₀-aryl or (C₆-C₁₀)-aryl-(C₁-C₈)-alkyl, or, in the case of NR³R⁴, is, together with R³ and the nitrogen atom carrying them, a 5-6-membered heterocyclic ring which can additionally contain a further heteroatom from the group O, S and N;

30

p is an integer from 1 to 100, preferably from 3 to 10;

q is an integer from 0 to 22, preferably from 0 to 15;

R² is hydrogen or a functional group such as hydroxyl, amino, NHR₆,

COOH, CONH₂, COOR₇ or halogen, in which

R⁶ is C₁-C₄-alkyl, and

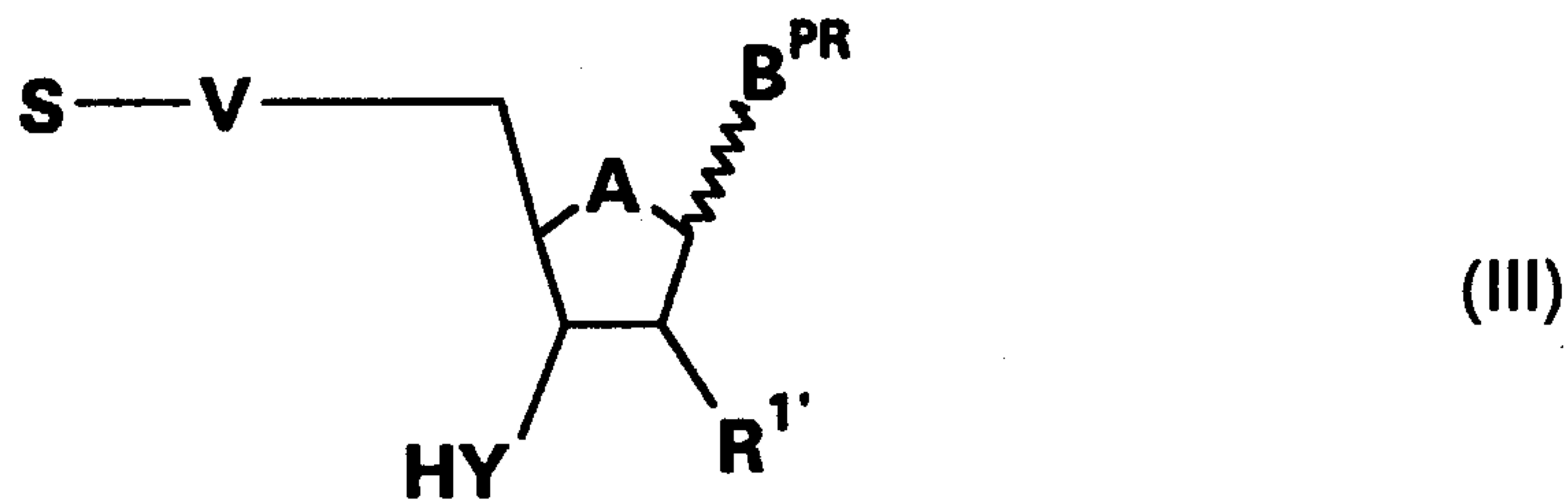
5 R⁷ is C₁-C₄-alkyl, preferably methyl;

Q and Q' are, independently of each other, hydrogen or conjugates which have a favorable effect on the properties of antisense oligonucleotides or of triple helix-forming oligonucleotides (for example cell penetration, degradation by nucleases, affinity for target RNA/DNA or pharmacokinetics), or are used as the label for a DNA probe, or, in association with the hybridization of the oligonucleotide analog to the target nucleic acid, attack the latter while binding or cross-linking, such as, for example conjugates with polylysine, with intercalators such as pyrene, acridine, phenazine or phenanthridine, with fluorescent compounds such as fluorescein, with cross-linkers such as psoralene or azidoproflavine, with lipophilic molecules such as (C₁₂-C₂₀)-alkyl, with lipids such as rac-1,2-dihexadecylglycerol, with steroids such as cholesterol or testosterone, with vitamins such as vitamin E, with polyethylene glycol or oligoethylene glycol, with (C₁₂-C₁₈)-alkyl phosphate diesters, or with-O-CH₂-CH(OH)-O-(C₁₂-C₁₈)-alkyl; or, particularly preferably, are conjugates with lipophilic molecules such as (C₁₂-C₂₀)-alkyl, with steroids such as cholesterol or testosterone, with polyethylene glycol or oligoethylene glycol, with vitamin E, with intercalators such as pyrene, with (C₁₄-C₁₈)-alkyl phosphate diesters, or with -O-CH₂-CH(OH)-O-(C₁₂-C₁₆)-alkyl;

25 R¹ and the adjacent phosphoryl radical can be located either in the 2' and 3' positions or, conversely, in the 3' and 2' positions, with it being possible for each nucleotide to be present in its D or L configuration and the base B to be located in the alpha or beta position, with it also being possible for the oligonucleotide to contain 3'-3' or 5'-5' inversions (Ch. Chaix et al., Bioorg. & Med. Letters 6 (1996) 827); wherein

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(a) a compound of the formula III



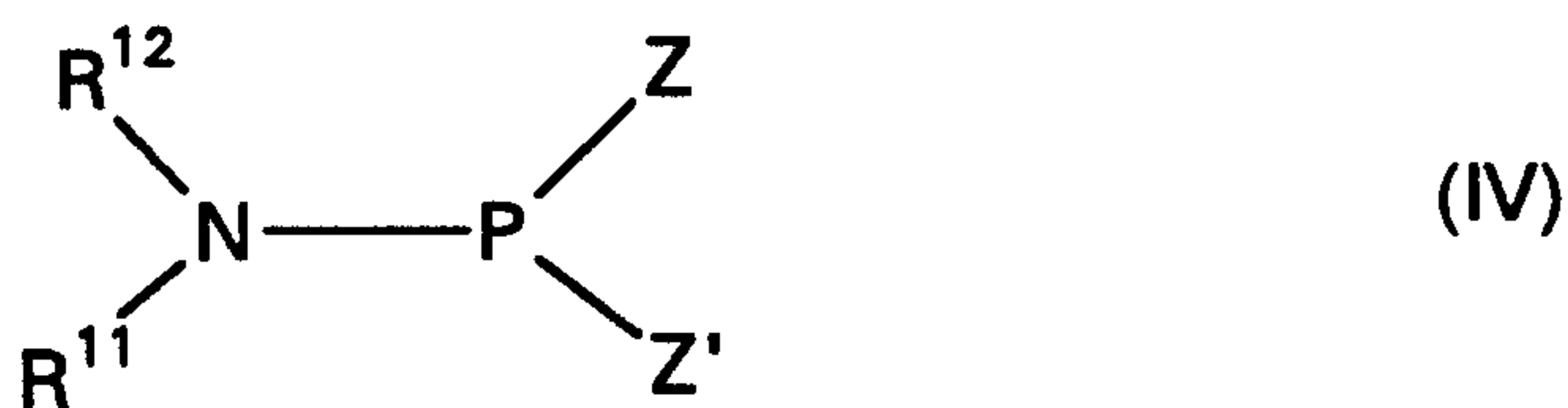
in which A, Y and V are defined as above, and

10 R^{1'} is defined as R¹ where, when R¹ is hydroxyl or amino, R^{1'} is a correspondingly protected group;

15 S is a 5' protecting group which can be eliminated under acid conditions, for example dimethoxytrityl, monomethoxytrityl, trityl or pixyl, preferably dimethoxytrityl and monomethoxytrityl;

B^{PR} is a natural or unnatural nucleobase in which any exocyclic amino groups which may be present are protected by a cyclic diacyl group,

20 is reacted, in accordance with known methods, with a compound of the formula IV



in which

30 Z' is OR¹³ or C₁-C₁₈-alkyl, C₁-C₁₈-alkoxy, C₆-C₂₀-aryl, C₆-C₁₄-aryl-C₁-C₈-alkyl, preferably OR¹³, C₁-C₆-alkyl, C₁-C₆-alkoxy, C₆-C₂₀-aryl, or C₆-C₁₄-aryl-C₁-C₈-alkyl, particularly preferably OR¹³;

R¹¹ and R¹² are identical or different and are

C₁-C₈-alkyl, preferably isopropyl, or C₅-C₁₂-cycloalkyl, preferably up to C₈, benzyl or phenyl or, together with the nitrogen atom to which they are

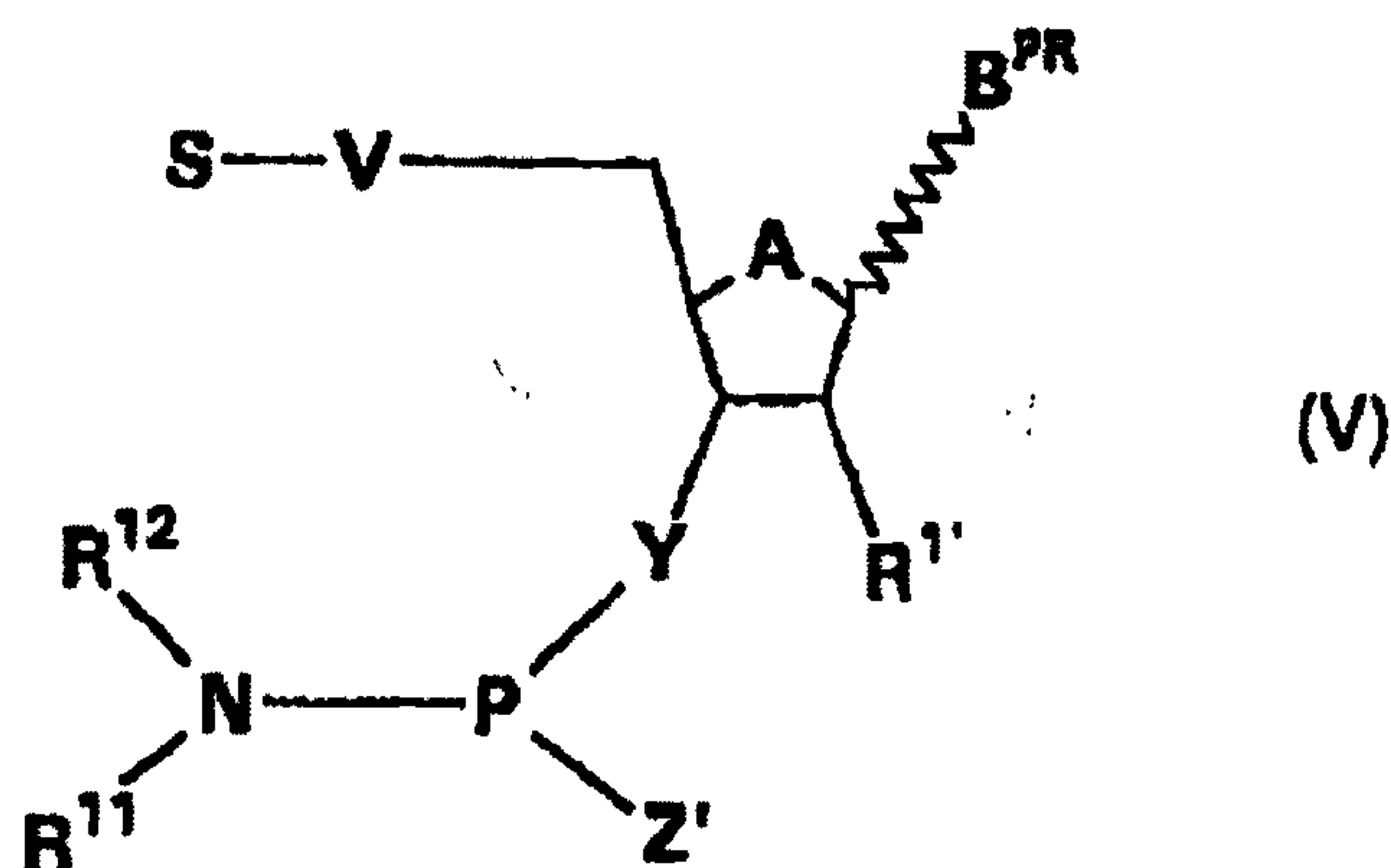
bonded, a saturated or unsaturated heterocyclic ring having, where appropriate, additional heteroatoms selected from O, S and N, for example morpholine, and substituents, such OC(O)O-C₁-C₄-alkyl esters; and

5 R¹³ is a protecting group which can be eliminated with strong, nonnucleophilic bases,

Z is chlorine or bromine or a radical of the formula NR¹¹R¹², where R¹¹ and R¹² are defined as above;

10

in the presence of a base, preferably pyridine or a mixture of tetrahydrofuran (THF), dioxane, dichloromethane (DCM), chloroform, and/or acetonitrile with a C₁-C₄-trialkylamine, preferably trimethylamine, triethylamine or diisopropylethylamine, or, when Z is a radical of the formula NR¹¹R¹², then in the presence of a compound of
 15 the formula [HNR¹⁴R¹⁵R¹⁶]⁽⁺⁾ A⁽⁻⁾, where R¹⁴, R¹⁵ and R¹⁶ are identical or different and are a C₁-C₄-alkyl group and A is fluorine, chlorine or bromine, in particular chlorine, or tetrazole or 5-(C₁-C₄-alkylthio)-1H-tetrazole, or 5-(C₆-C₁₂)-aryl-1H-tetrazole or other activators, such as pyridine hydrochloride, preferably in the presence of tetrazole or pyridine hydrochloride, to form a compound of the formula V

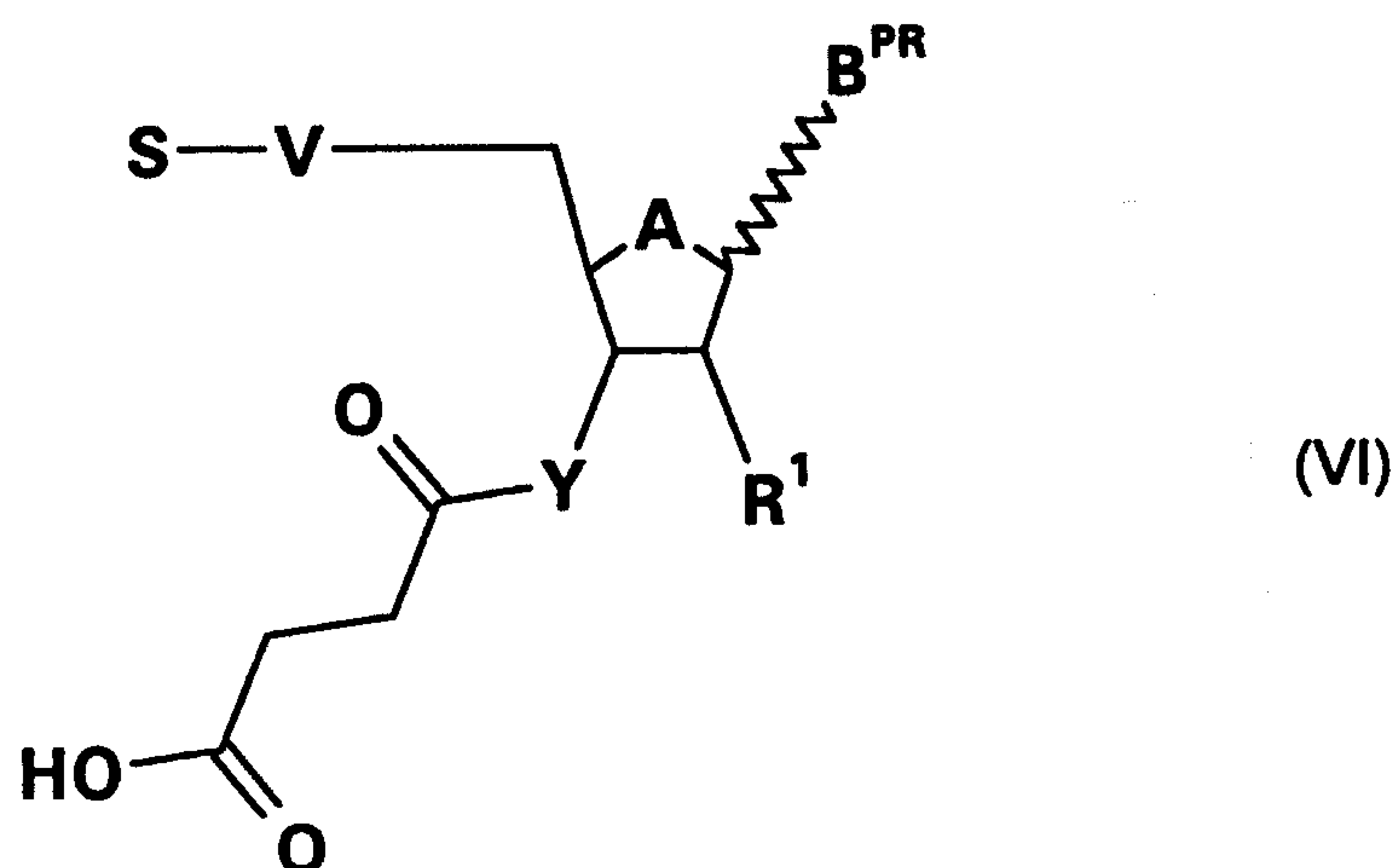


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in which S, V, Y, A, B^{PR}, Z', R¹, R¹¹ and R¹² are as defined above;

(b) compounds of the formula III are reacted, in accordance with known methods, with from 1 to 10 equivalents, preferably with from 1 to 2 equivalents, of a linker,
 25 such as succinic anhydride, in a suitable organic solvent, for example methylene

chloride, where appropriate after adding a catalyst, for example 4-dimethylaminopyridine, to give a compound of the formula VI



where S, V, Y, A, B^{PR} and R¹ are defined as above, and subsequently worked up in accordance with known methods, such as extraction, crystallization and chromatography, with the succinic acid radical in the 3' position serving as the linker to the polymer support which is employed in the synthesis and it being possible also to use other linkers, such as those described in Sonveaux [Bioorg. Chem. 14 (1986) 274], as an alternative to the succinic acid linker;

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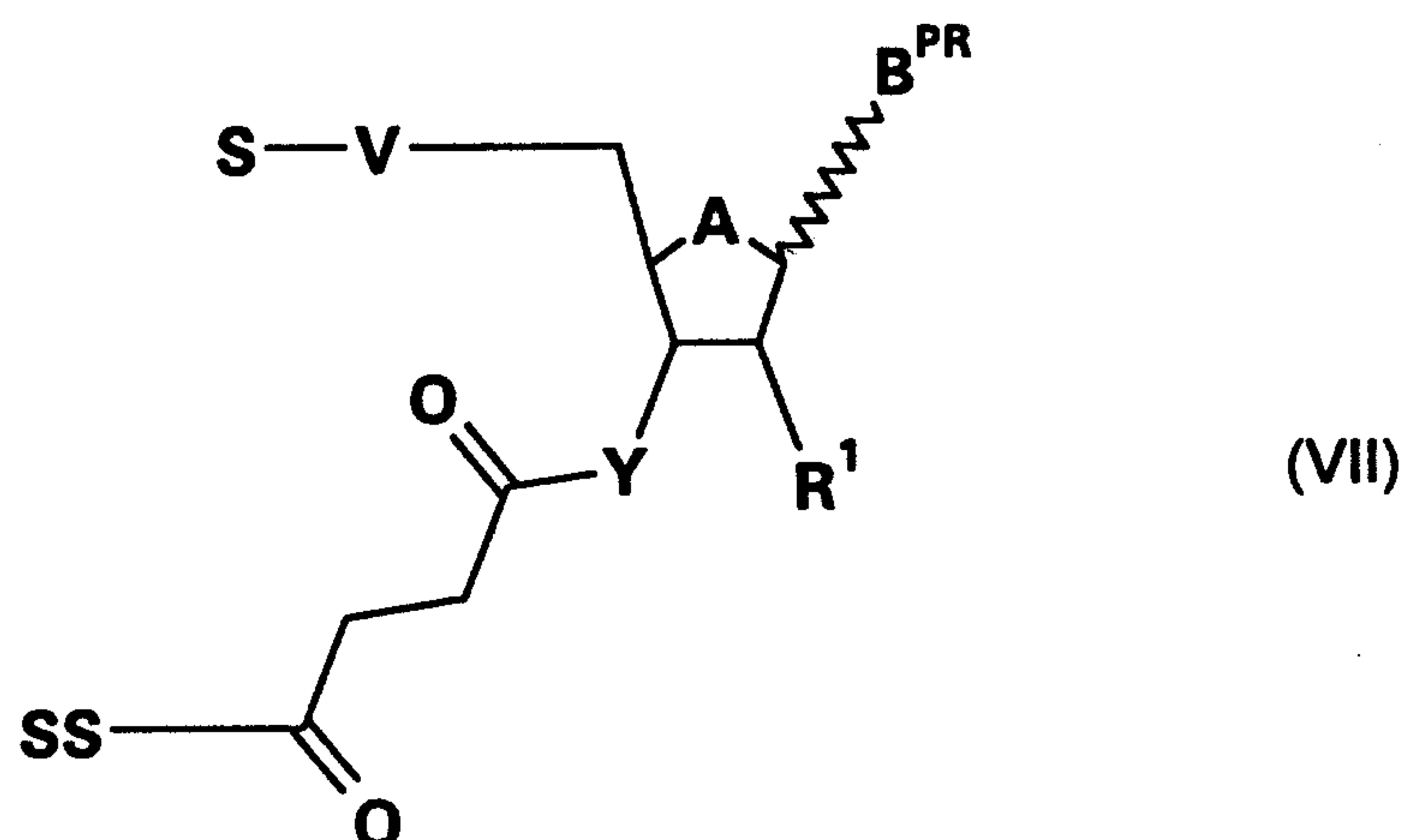
(c) the compound of the formula VI is coupled, in accordance with known methods, to a solid support SS, such as CPG® (CPG = controlled pore glass) or tentagel®, preferably DBU-stable supports such as "long-chain methylaminoalkyl-CPG" [Stengele, Tetrahedron Lett. 1990, 31, 2549], for example by reacting with DCC and p-nitrophenol in a suitable solvent, or by reacting with TOTU (O-[(ethoxycarbonyl)cyanomethyleneamino]-N,N,N',N'-tetramethyluronium tetrafluoroborate: W. König, G. Breipohl, P. Pokorny, M. Birkner, Proceedings of the 21st European Peptide Symposium 1990, E. Giralt, D. Andreu, Eds., ESCOM, Leiden, p. 143.), where appropriate with the addition of a suitable base such as N-methylmorpholine, N-ethylmorpholine or ethyldiisopropylamine or triethylamine, in a suitable solvent [as described, for example, in M.J. Gait, Oligonucleotide Synthesis - a practical approach, IRL Press, 1984], with compounds of the formula VII being obtained;

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15 (d) the 5' protecting group is eliminated from VII in accordance with known methods, for example by treating with 1 - 4% dichloroacetic or trichloroacetic acid in methylene chloride or chloroform;

20 (e) the resulting compound is reacted with a compound of the formula V in a suitable organic solvent, preferably acetonitrile, in the presence of a compound of the formula $[HNR^{14}R^{15}R^{16}]^{(+)} A^{(-)}$, which is defined as above, or tetrazole, 5-(C₁-C₄-alkylthio)-1H-tetrazole or 5-(C₆-C₁₂)-aryl-1H-tetrazole, or other activators such as pyridine hydrochloride, preferably in the presence of tetrazole or pyridine hydrochloride,

25 (f) the resulting compound is oxidized in accordance with known methods, for example by reacting with iodine in the presence of aqueous pyridine, lutidine or collidine, where appropriate also in the presence of additional organic solvents such as tetrahydrofuran, or, for example, by reacting with tert-butyl hydroperoxide in tetrahydrofuran, or by reacting with N,N,N',N'-tetraethylthiuram disulfide in acetonitrile, or, for example, by reacting with iodine in the presence of alkylamine or arylamine, where the different oxidation methods, which are known to the skilled

30 person and which are used for preparing natural and modified oligonucleotides, and are summarized, for example, in Beaucage and Iyer [Tetrahedron 49 (1993) 6123] and also Uhlmann and Peyman [Chem. Rev. 90 (1990) 543], where the oxidation is preferably carried out by reacting with iodine in the presence of aqueous pyridine,

lutidine or collidine, where appropriate also in the presence of additional organic solvents such as tetrahydrofuran;

5 (f) unreacted compounds from step (d) are, where appropriate, deactivated by means of a capping step, for example by reacting with acetic anhydride-lutidine-N-methylimidazole in THF;

10 (g) the reaction steps d-f are repeated until the desired chain length has been obtained;

(h) the compound which has been obtained in this way is deprotected by treating with a 0.1 to 5 M solution of DBU in a suitable organic solvent such as acetonitrile, pyridine or N-methylimidazole at from 0 to 70°C for from 0.1 to 16 h, preferably with an 0.3 to 3 M solution at from 10 to 40°C for from 0.1 to 2 h, particularly preferably with an 0.5 to 2.5 M solution between 20 and 30 °C for from 0.2 to 1.5 h;

20 (i) the oligonucleotide is cleaved from the support in accordance with known methods, for example with NH_3 at 20-30 °C, and the compounds of the formula I are obtained by lyophilizing the ammoniacal solution.

25 Protecting groups which can be eliminated by strong, nonnucleophilic bases are those protecting groups which are eliminated by treatment with strong, nonnucleophilic bases with the elimination as a rule taking place by means of β -elimination. Examples of these protecting groups are 4-nitrophenylethyl, 2-cyanoethyl, dansylsulfonylethyl, aryylethyl, and arylsulfonylethyl, where phenyl can, where appropriate, be substituted, once or more than once, by chlorine, bromine, CN, NO_2 , or F, preferably 4-nitrophenylethyl or 2-cyanoethyl.

30 Compounds of the formula VII can also be synthesized by succinoylating the solid support and subsequently condensing-on compounds of the formula III.

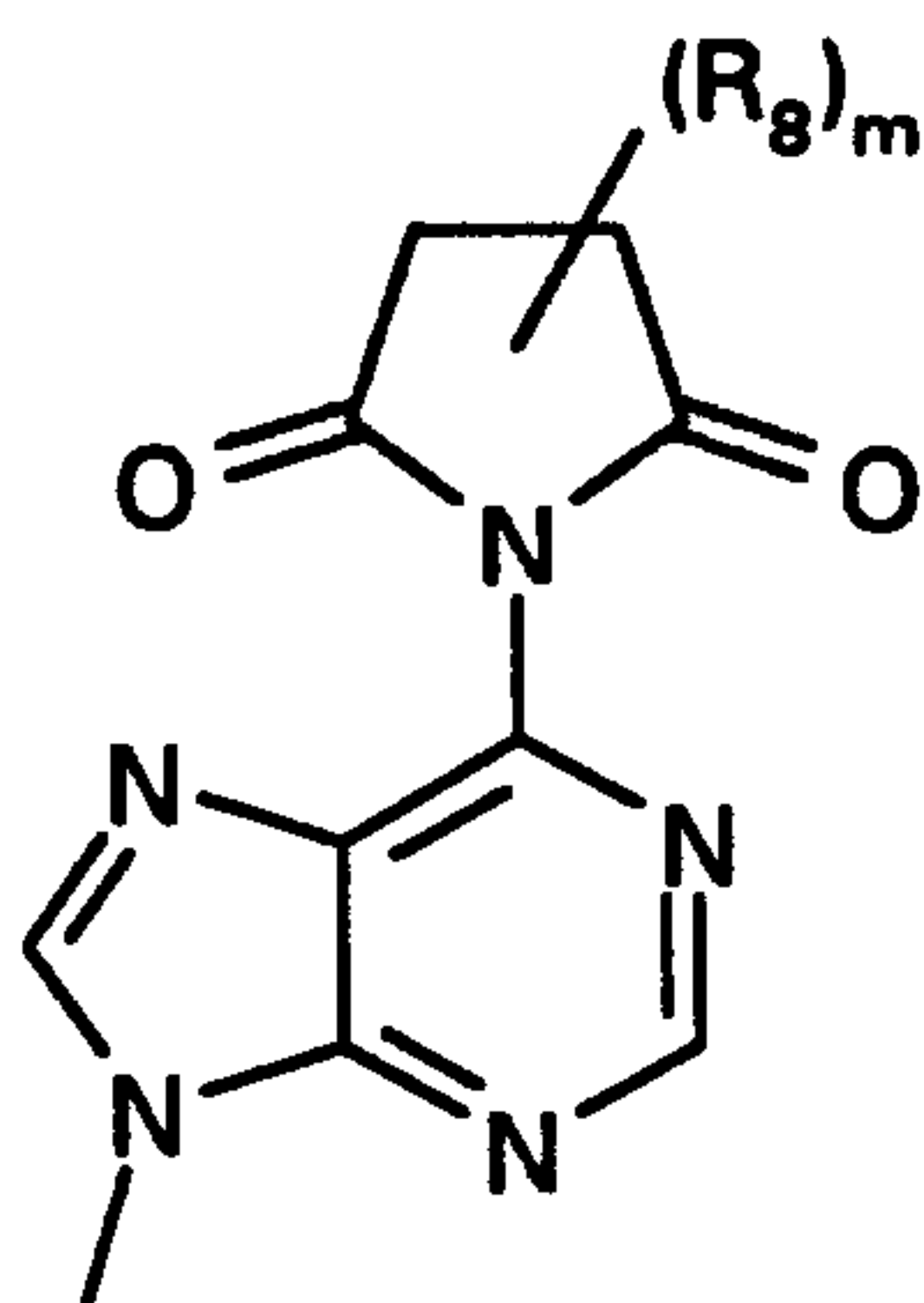
The groups Q and Q' are introduced, where appropriate, using methods which are

known to the skilled person (see, for example, Uhlmann & Peyman, Chem. Rev. 90 (1990) 543; M. Manoharan in "Antisense Research and Applications", Crooke and Lebleu, Eds., CRC Press, Boca Raton, 1993, Chapter 17, pp. 303 ff. and EP-A 0 552 766).

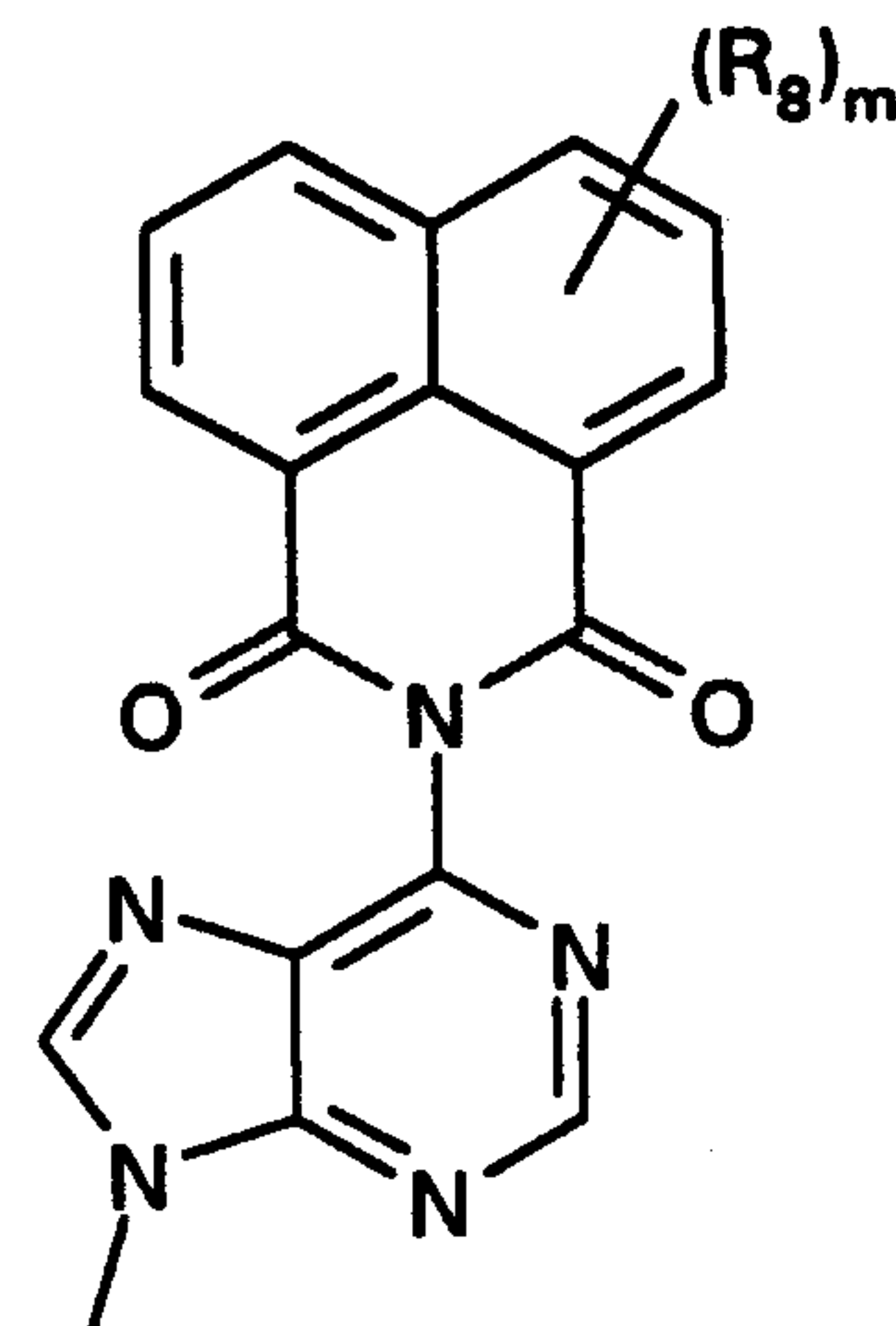
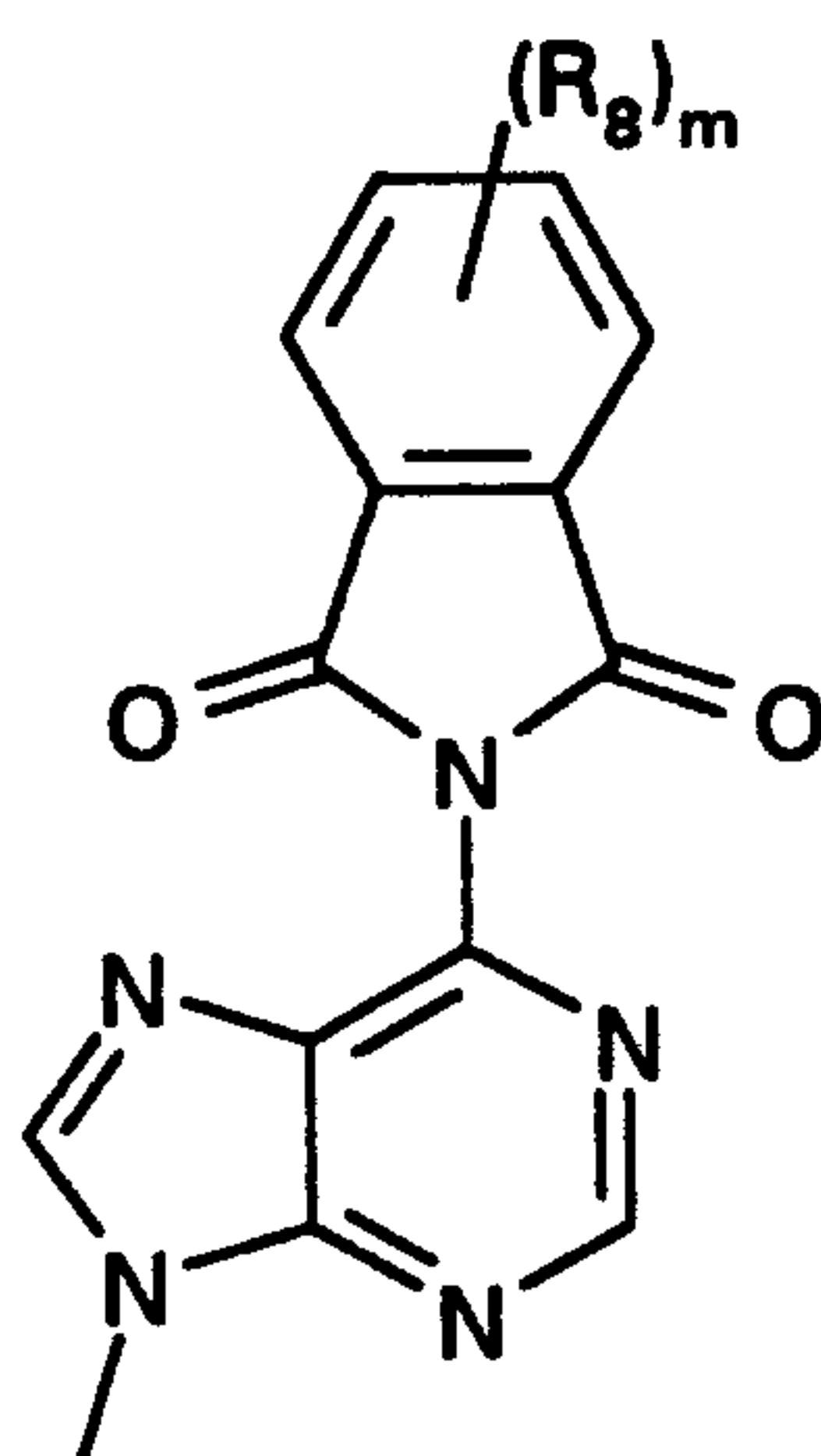
- 5 Protecting groups for R^1 , when R^1 is OH or amino, and the synthesis of correspondingly derivatized nucleoside building blocks, are known to the skilled person (such as, for example, the dimethyl-tert-butylsilyl group or the 1-(2-chloro-4-methylphenyl)-4-methoxy-4-piperidinyl group for R^1 is hydroxyl or the acetyl group for R^1 is amino) and are described, for example, in Uhlmann and Peyman Chem. Rev. 90 (1990) 543; Beaucage and Iyer Tetrahedron 48 (1993) 2223 or C. Hendrix et al., Nucl Acids Res. 26 (1995) 51.

Examples of B^{PR} are

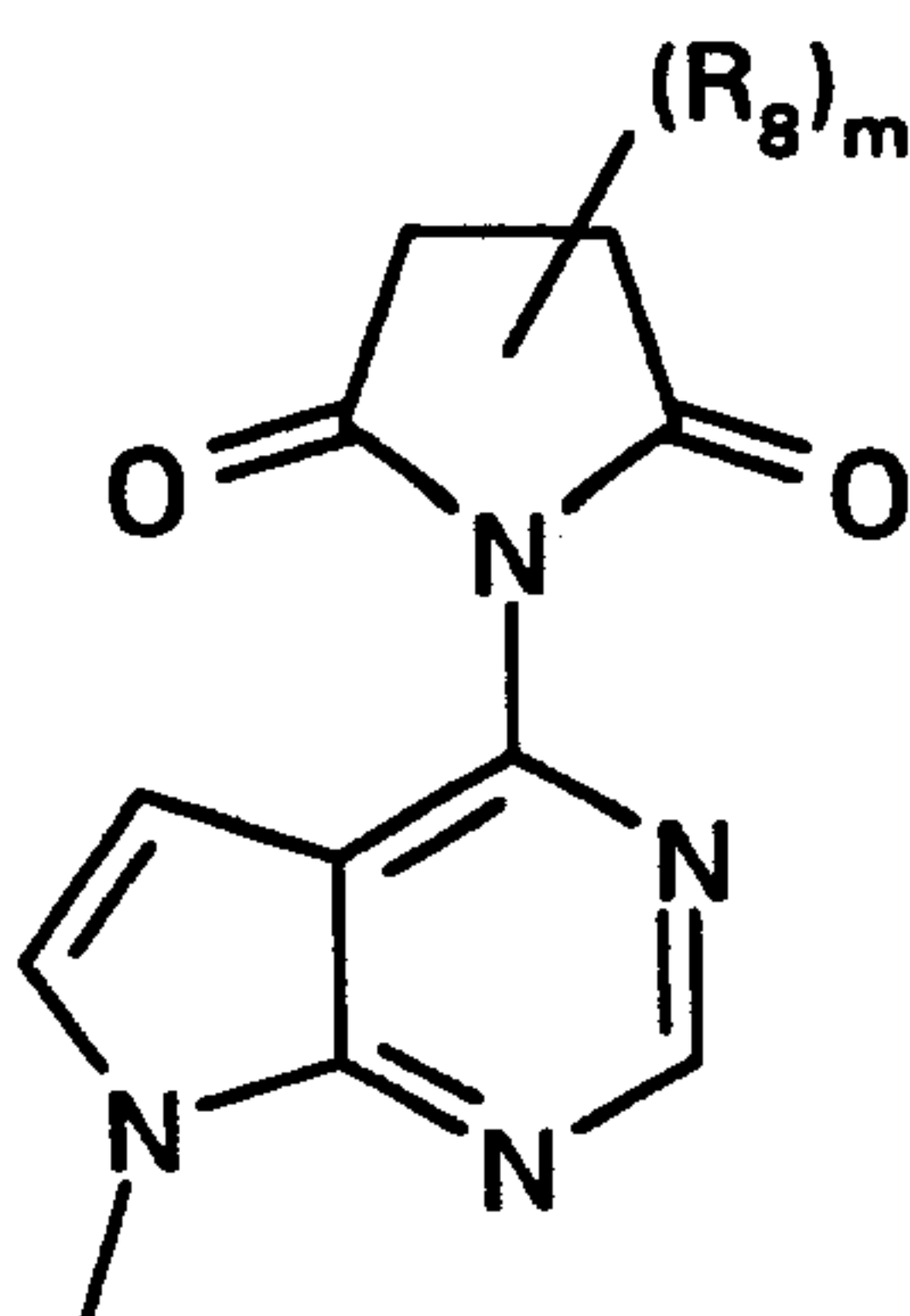
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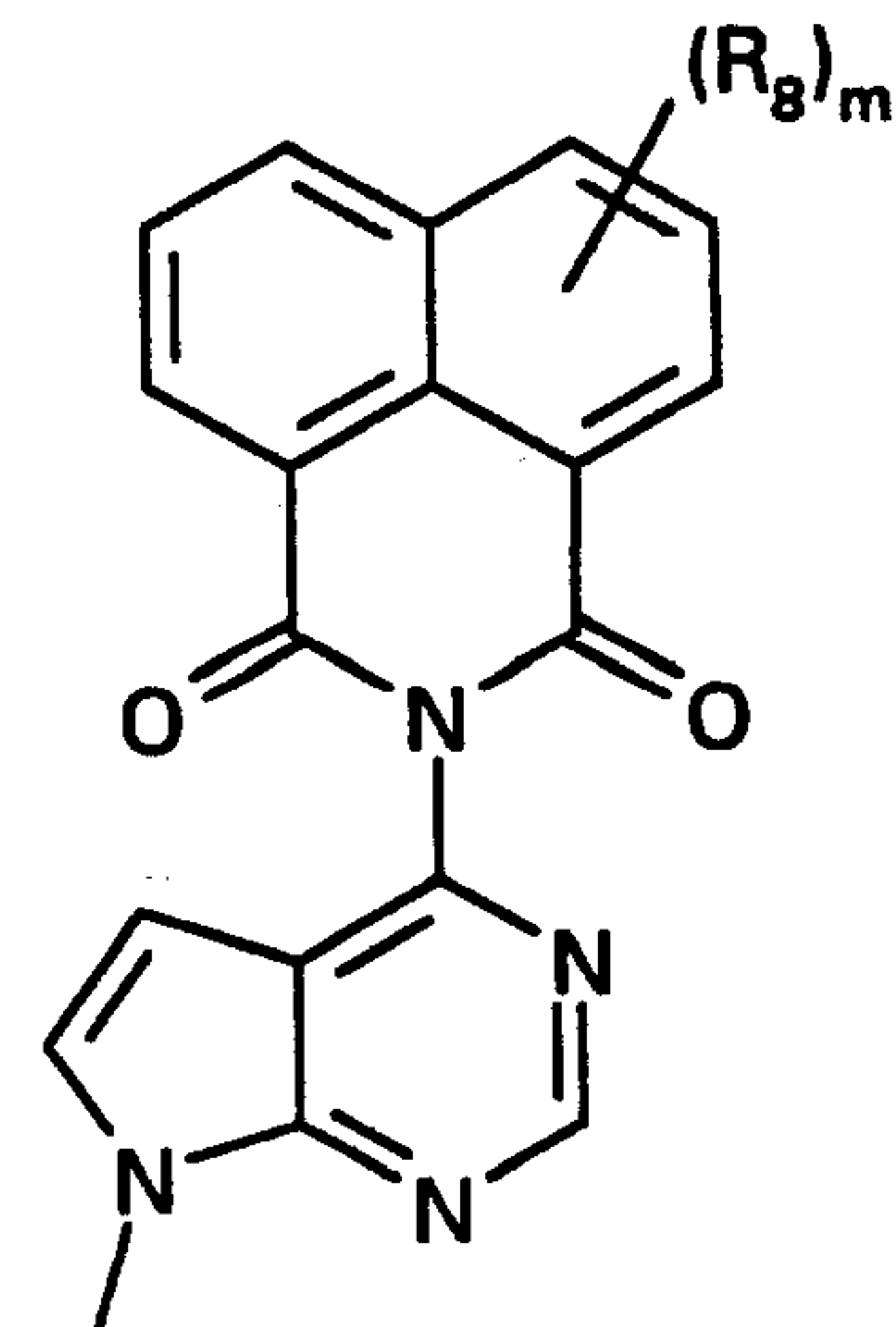
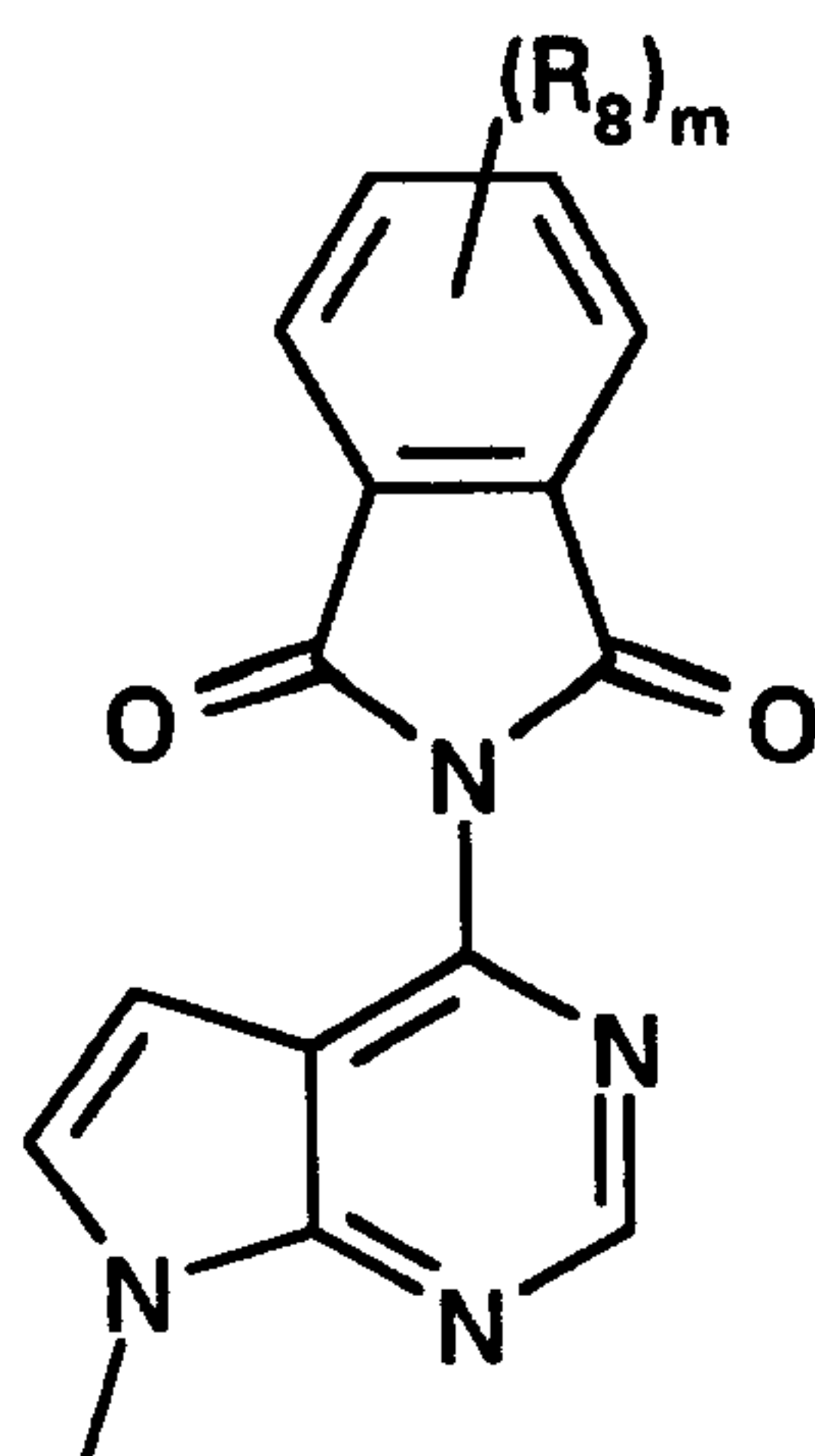
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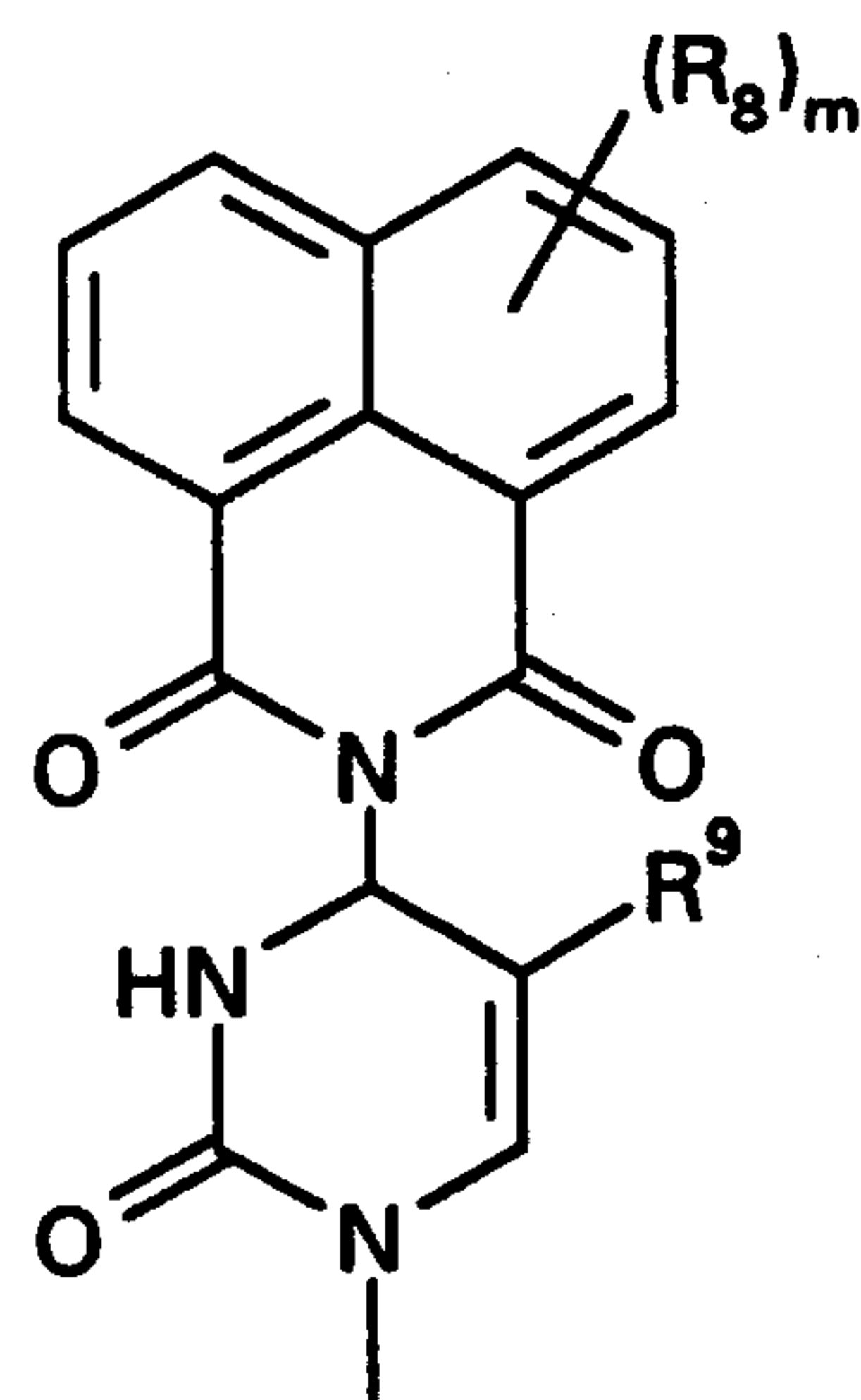
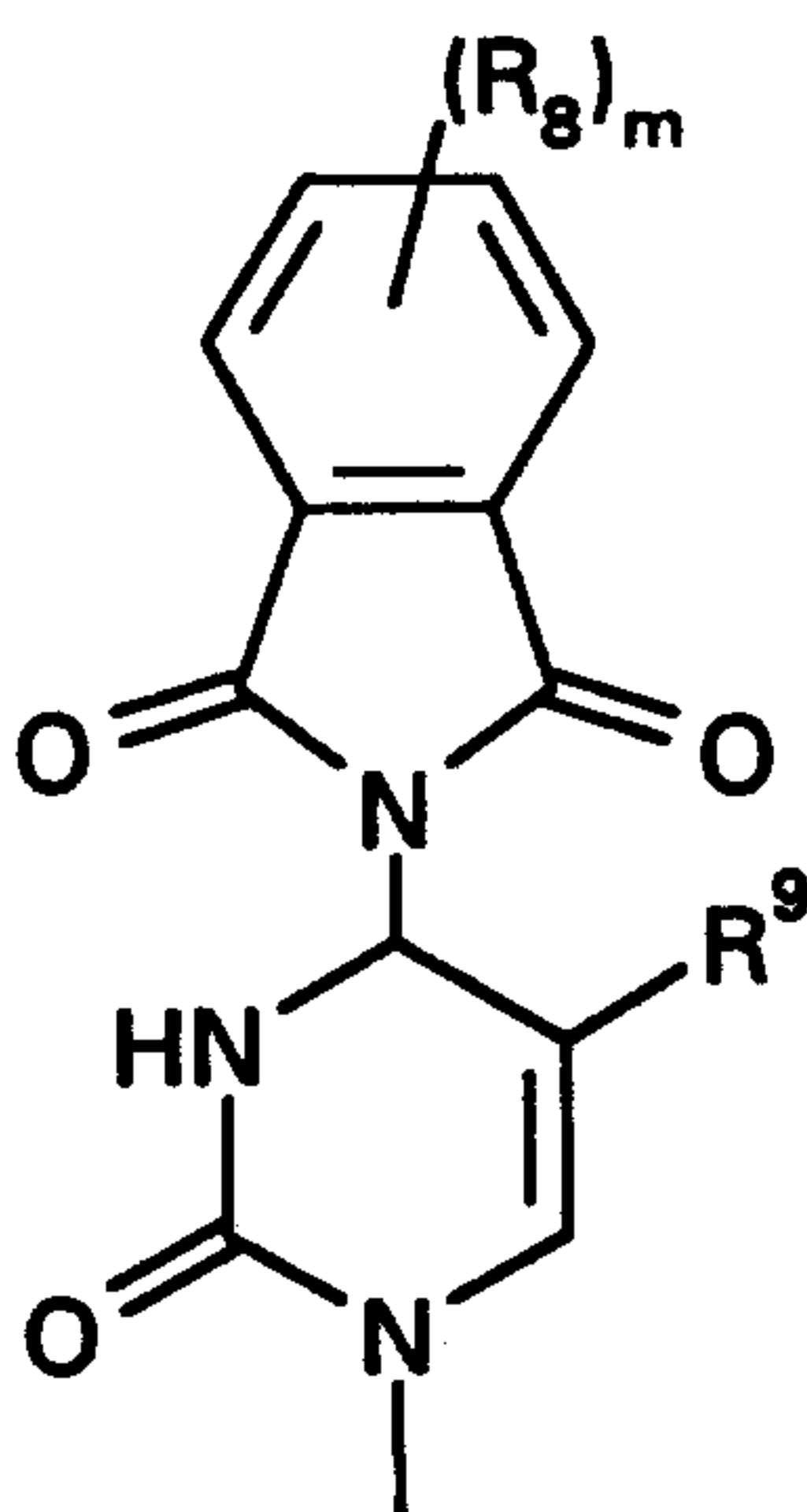
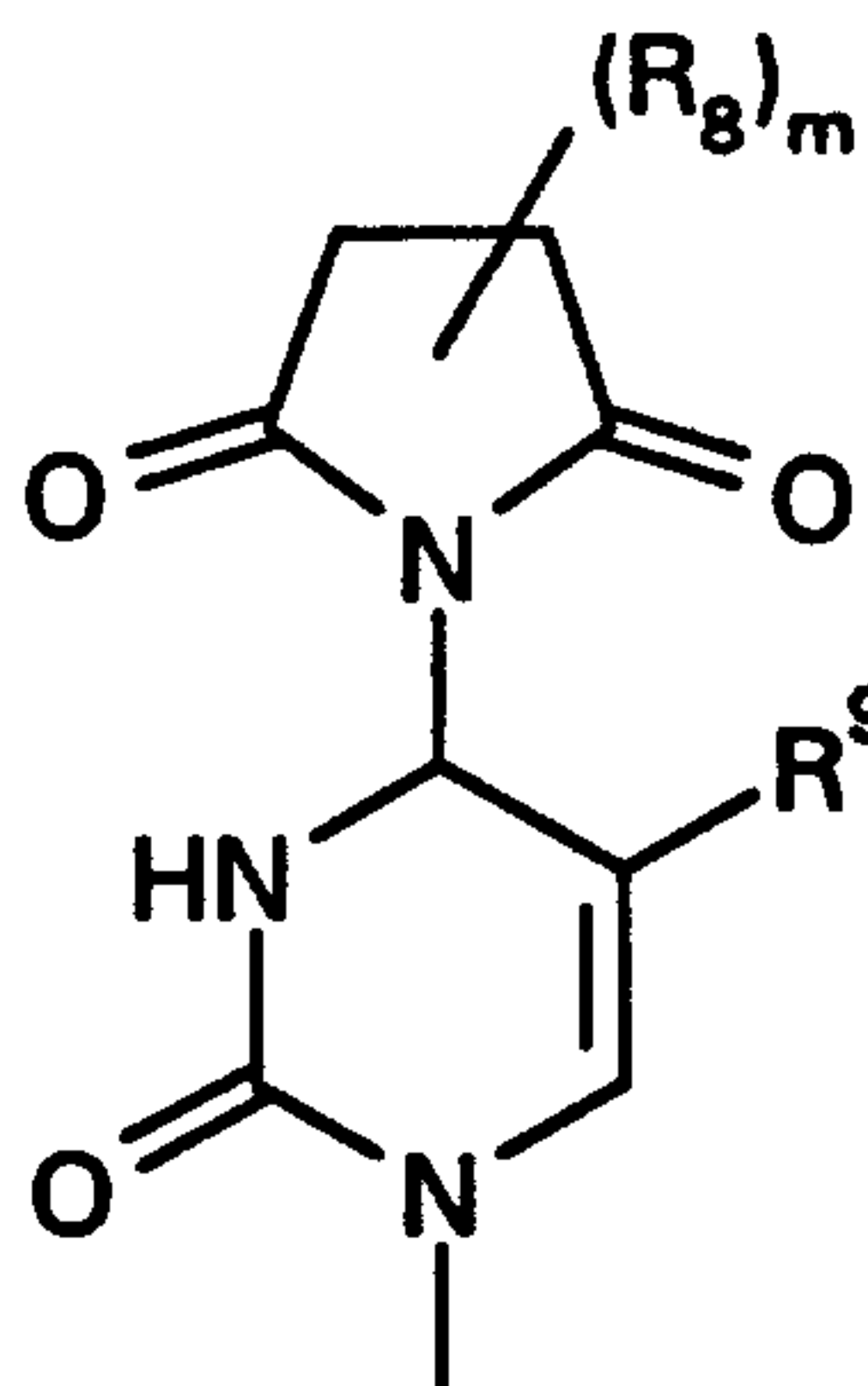


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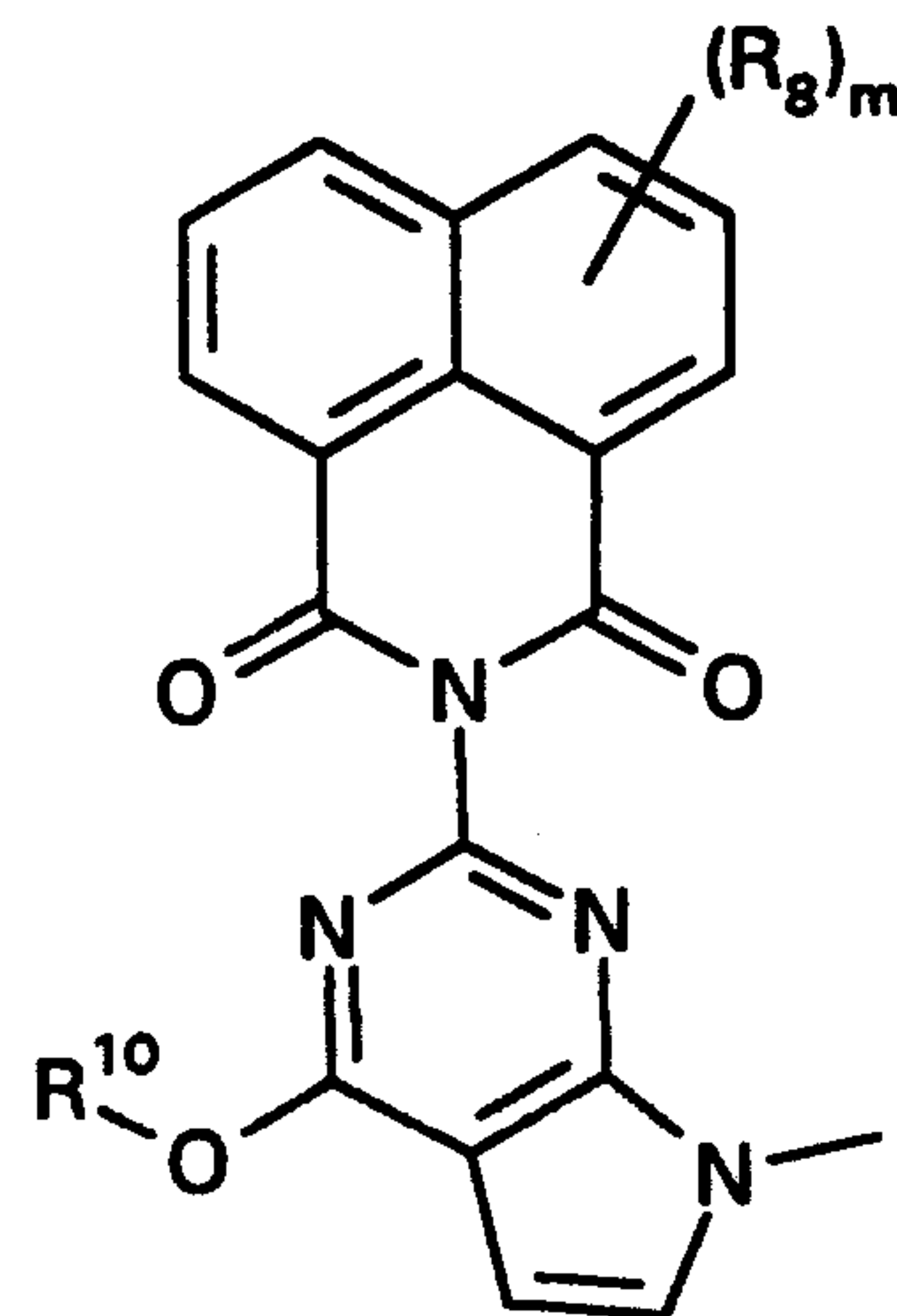
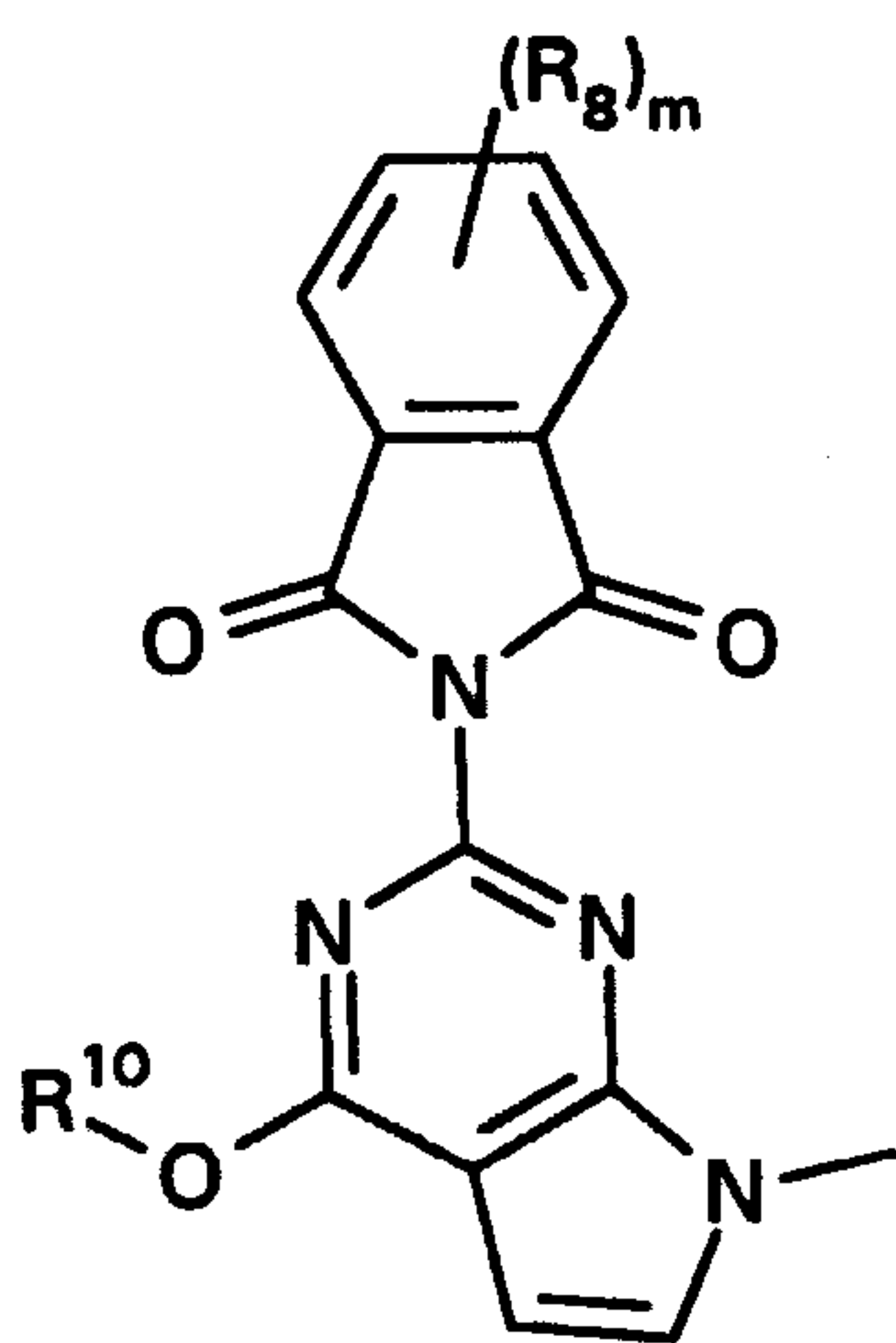
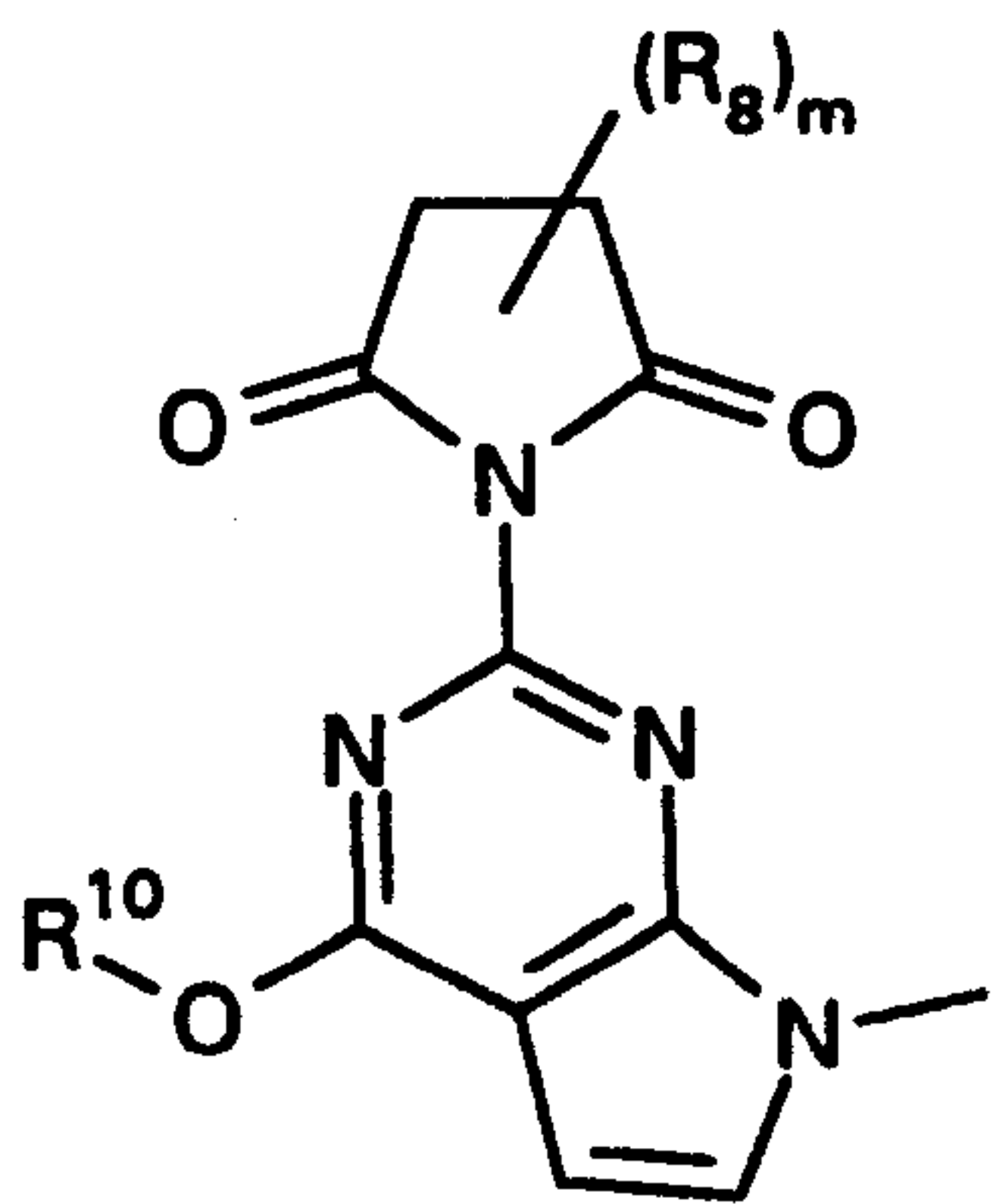


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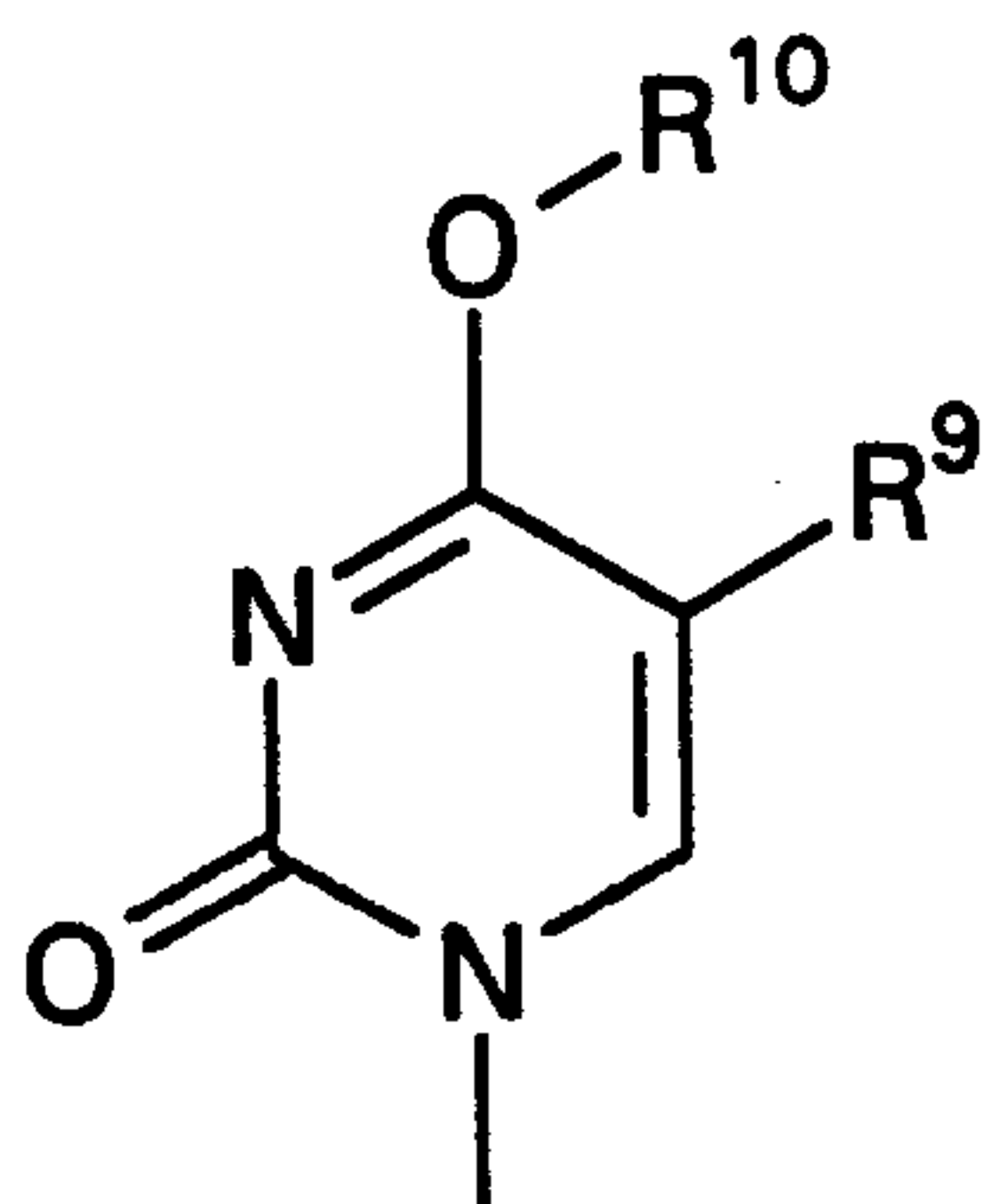


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25



in which

m is a number from zero to four, preferably zero, and

30

R⁸ is, independently of each other, hydrogen, fluorine, chlorine, bromine, nitro, C₁-C₄-alkyl, C₁-C₄-alkoxy or CN,

R^9 is, independently of each other, hydrogen, C_1 - C_6 -alkyl, C_2 - C_6 -(1-alkyne), preferably 1-propynyl and 1-hexynyl or fluorine; and

R^{10} is, independently of each other, hydrogen or a β -eliminatable protecting group such as para-nitrophenylethyl or phenylsulfonylethyl,

with the introduction into nucleosides of cyclic diacyl protecting groups and the 5' protecting group S being known to the skilled person, see, for example, Kume et al., Tetrahedron Lett. 23 (1982) 4365; Nucleic Acids Res. 12 (1984) 8525; Nucleic Acids Res. Symp. Ser. 11 (1982) 26; Chemistry Letters 1983, 1597 or Dikshit et al., Can. J. Chem. 66 (1988) 2989 or Kamaike et al., Tetrahedron Lett. 36 (1995) 91. Modified nucleoside building blocks can be protected in an analogous manner. The R^{10} protecting groups are likewise introduced, prior to introducing the cyclic diacyl groups, using known methods, for example in accordance with F. Himmelsbach et al., Tetrahedron 40 (1984) 59 or Beaucage and Iyer Tetrahedron 49 (1993) 6123; Beaucage and Iyer Tetrahedron 48 (1993) 2223.

Preference is given to the novel process for preparing compounds of the formula I in which

R^1 is hydrogen, hydroxyl, C_1 - C_4 -alkoxy or fluorine;

A is oxy;

W is oxo or thioxo;

V is oxy;

Y is oxy;

B is, independently of each other, adenine, cytosine, guanine, uracil, thymine, 5-propyneuracil and 5-propynecytosine, 5-hexyneuracil or 5-hexynecytosine, where at least one B is a base which possesses an exocyclic amino group,

n is an integer from 5 to 40;

U is hydroxyl, mercapto, C₁-C₆-alkoxy, C₁-C₆-alkyl, NR³R⁴ or NHR³, in which

5 R³ is C₁-C₈-alkyl or methoxyethyl;

R⁴ is C₁-C₈-alkyl, C₆-C₂₀-aryl or (C₆-C₁₀)-aryl-(C₁-C₈)-alkyl, or, in the case of NR³R⁴, is, together with R³ and the nitrogen atom carrying them, a 5-6-membered heterocyclic ring which can additionally contain a further heteroatom from the group O, S and N; and

10

Q and Q' are, independently of each other, hydrogen.

Particular preference is given to the novel process for preparing compounds of the formula I in which

15

R¹ is hydrogen

A is oxy;

20

W is oxo or thioxo;

V is oxy;

25

Y is oxy;

B is adenine, cytosine, guanine, uracil, thymine, 5-propyneuracil and 5-propynecytosine, 5-hexyneuracil or 5-hexynecytosine; where at least one B is a base which possesses an exocyclic amino group;

30

n is preferably from 5 to 30;

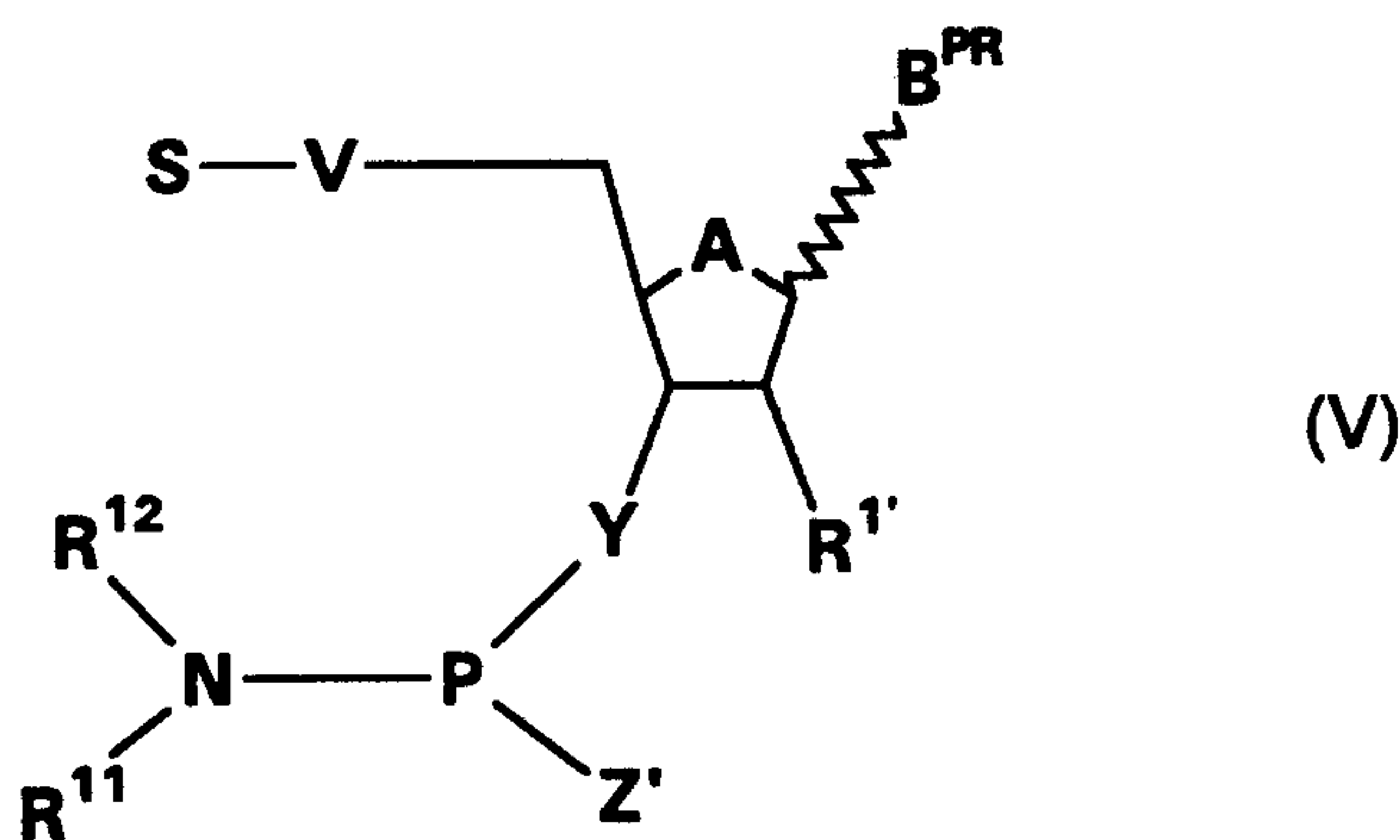
U is hydroxyl or C₁-C₆-alkyl, and

Q and Q' are hydrogen.

The oligonucleotides which are prepared using the novel process can be employed in many different ways, for example as inhibitors of gene expression, as ribozymes
5 or as probes in diagnosis (in particular as DNA probes), or, in a general manner, as aids in molecular biology.

The invention furthermore relates to a compound of the formula V

10



15

in which

20 $R^{1'}$ is, independently of each other, hydrogen, C_1 - C_{18} -alkoxy, where appropriate substituted one to three times by hydroxyl, or C_1 - C_4 -alkoxy, C_1 - C_4 -alkyl-O- $(CH_2CH_2O)_{1-3}$, O-allyl, fluorine, chlorine, azido or a protected hydroxyl or amino group;

25 A is oxy, thioxy or methylene;

V is oxy, sulfanediyl or imino;

Y is oxy, sulfanediyl, imino or methylene;

30

S is a 5' protecting group which can be eliminated under acid conditions, for example dimethoxytrityl, monomethoxytrityl, trityl or pixyl, preferably dimethoxytrityl and monomethoxytrityl;

B^{PR} is a natural or unnatural nucleobase having an exocyclic amino group, with the exocyclic amino groups being protected by a cyclic diacyl group;

Z' is OR^{13} or C_1-C_{18} -alkyl, C_1-C_{18} -alkoxy, C_6-C_{20} -aryl, or C_6-C_{14} -aryl- C_1-C_8 -alkyl;

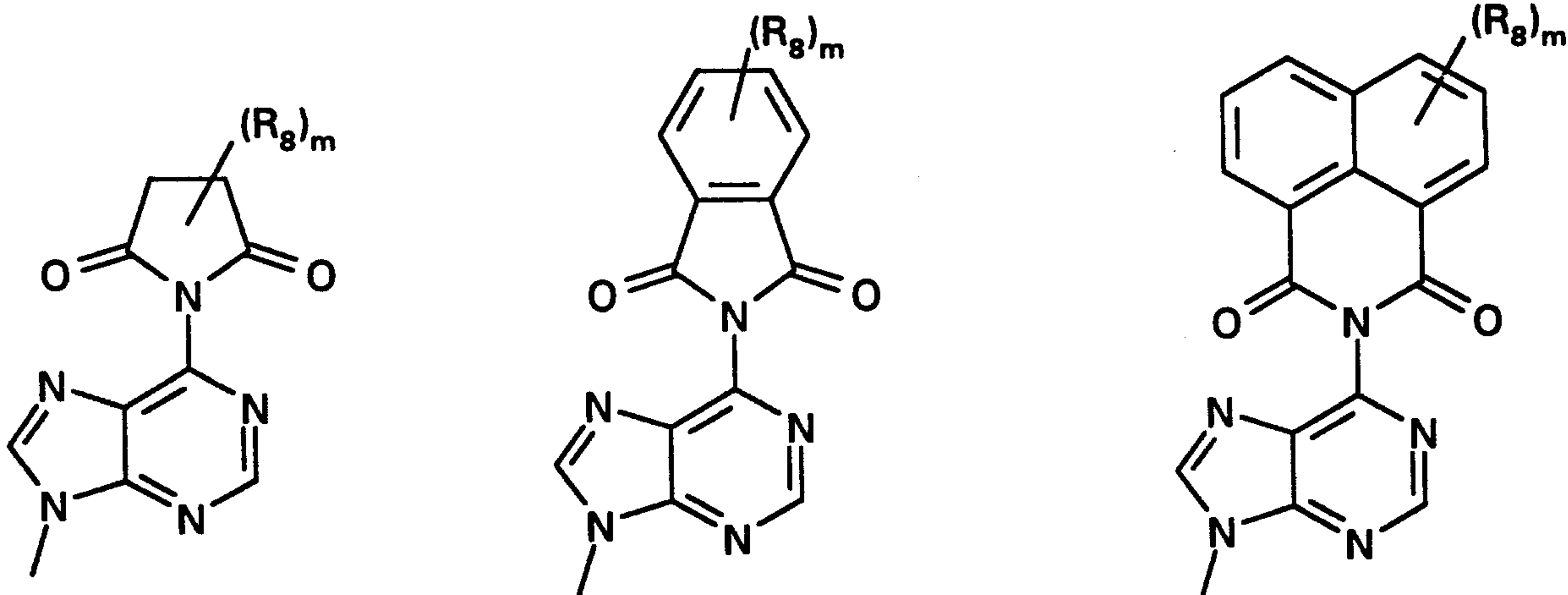
R^{11} and R^{12} are identical or different and are

C_1-C_8 -alkyl, preferably isopropyl, or C_5-C_{12} -cycloalkyl, preferably up to C_8 , benzyl or phenyl, or, together with the nitrogen atom to which they are bonded, are a saturated or unsaturated heterocyclic ring having, where appropriate, additional heteroatoms, for example morpholine, and substituents, such as $OC(O)O-C_1-C_4$ -alkyl esters; and

R^{13} is para-nitrophenylethyl or 2-cyanoethyl.

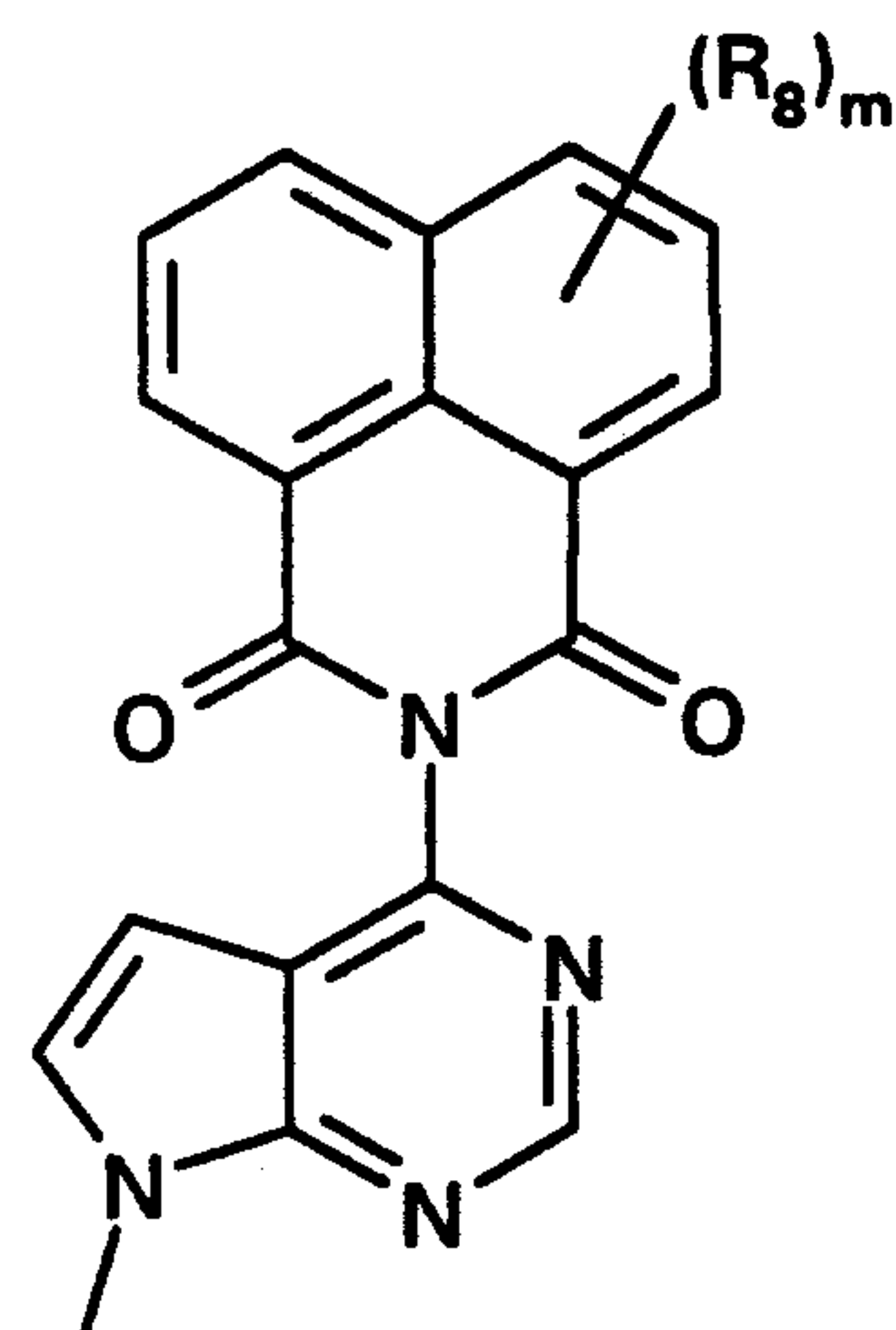
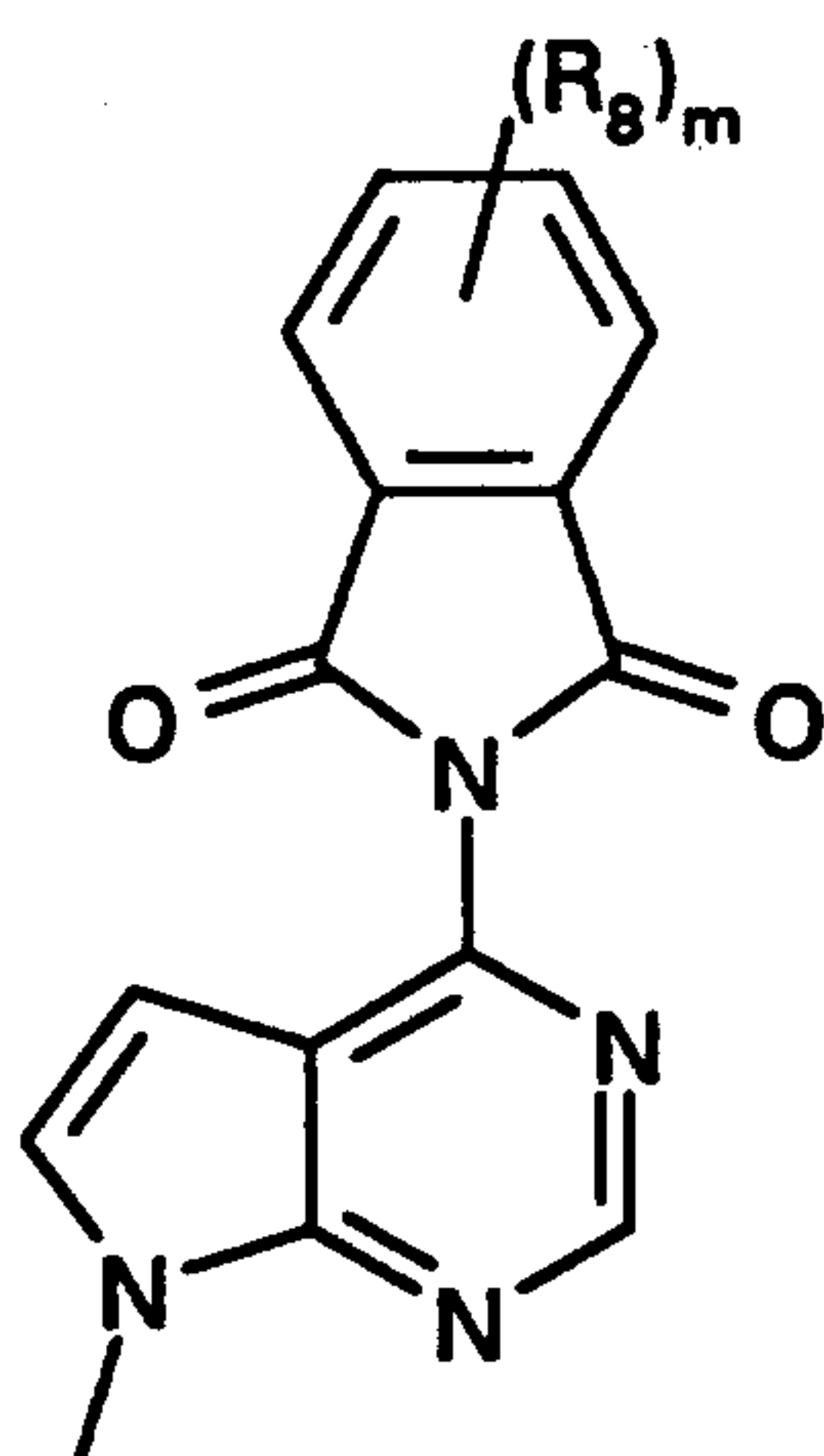
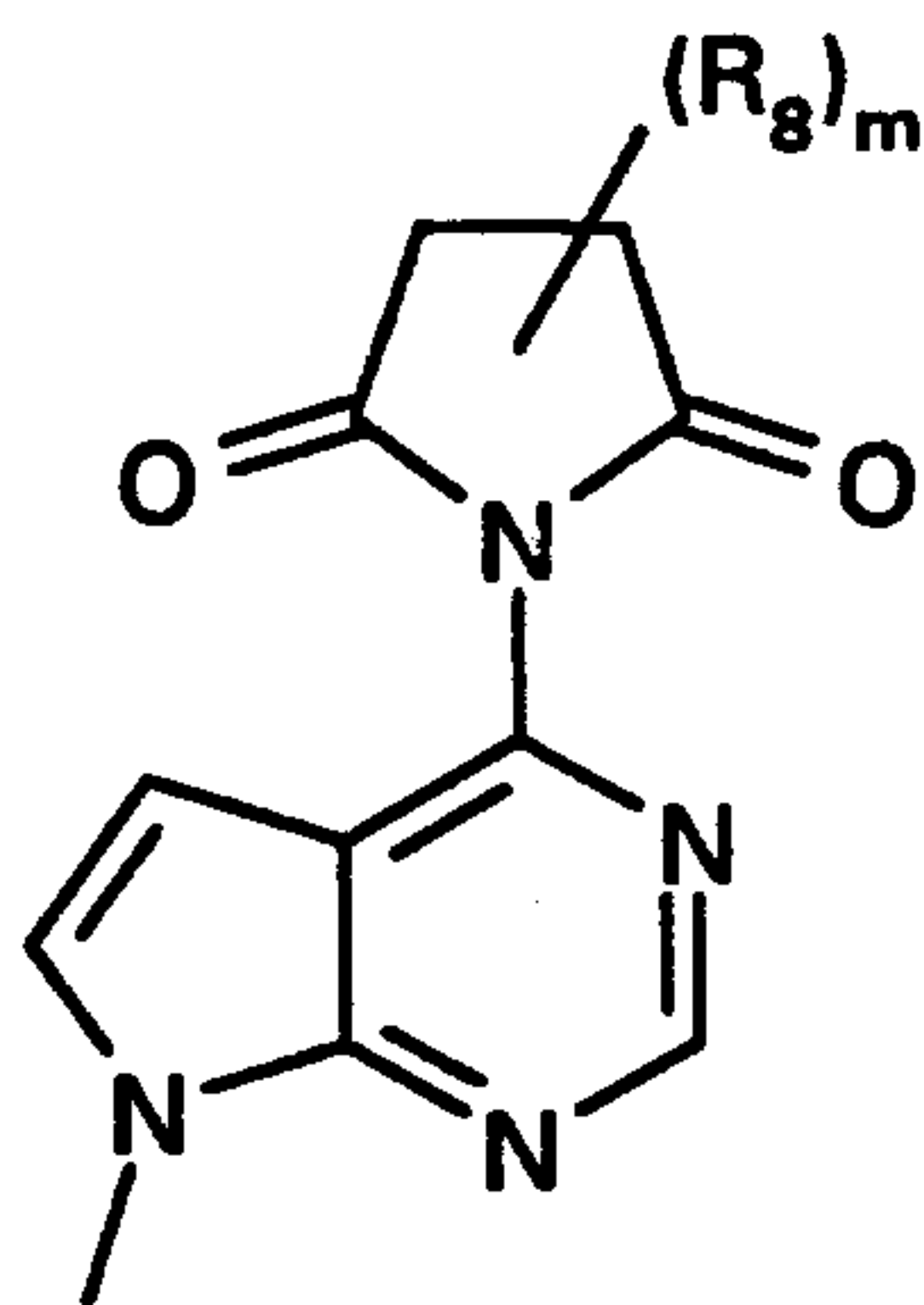
Preference is given to compounds of the formula V in which

B^{PR} is

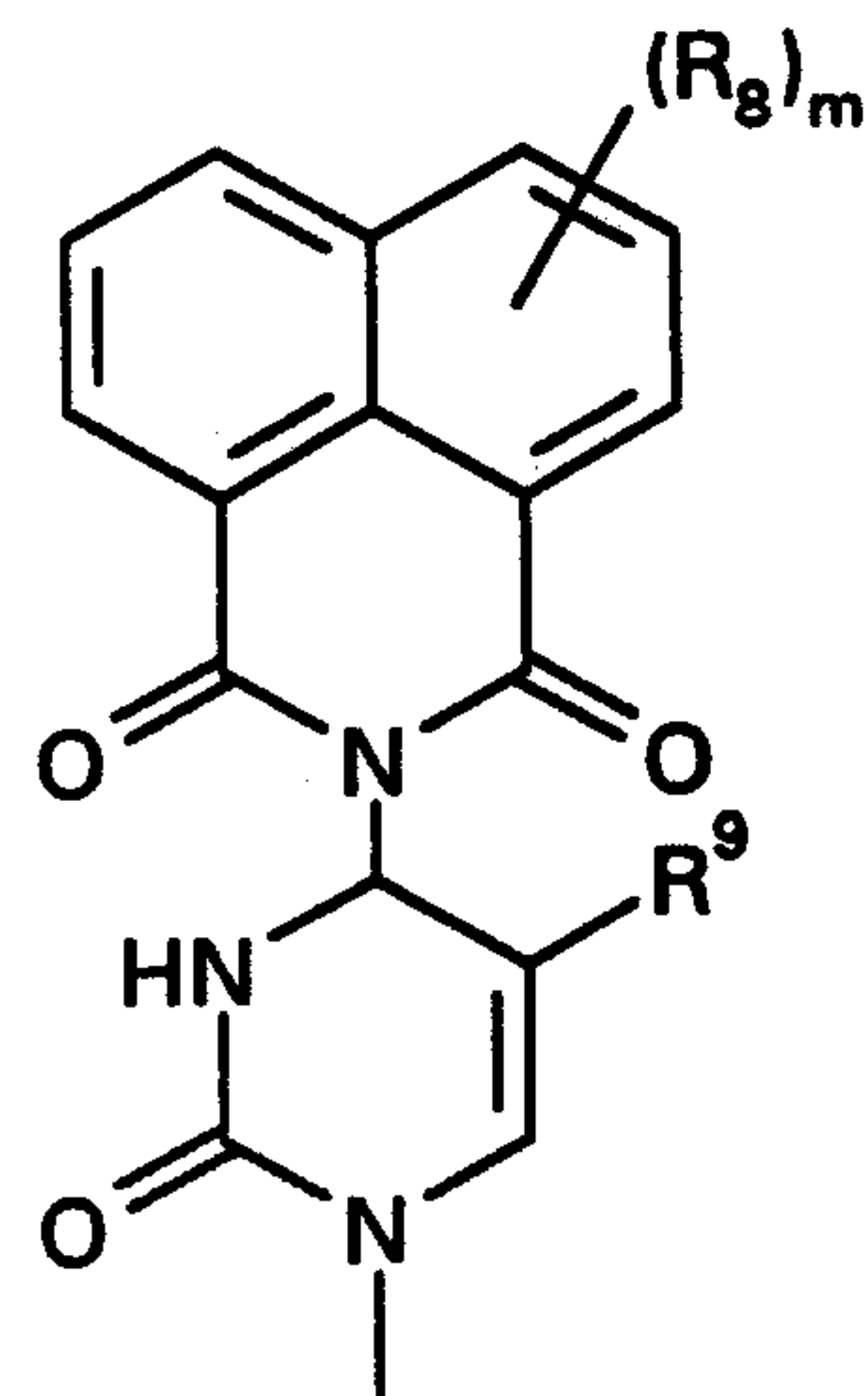
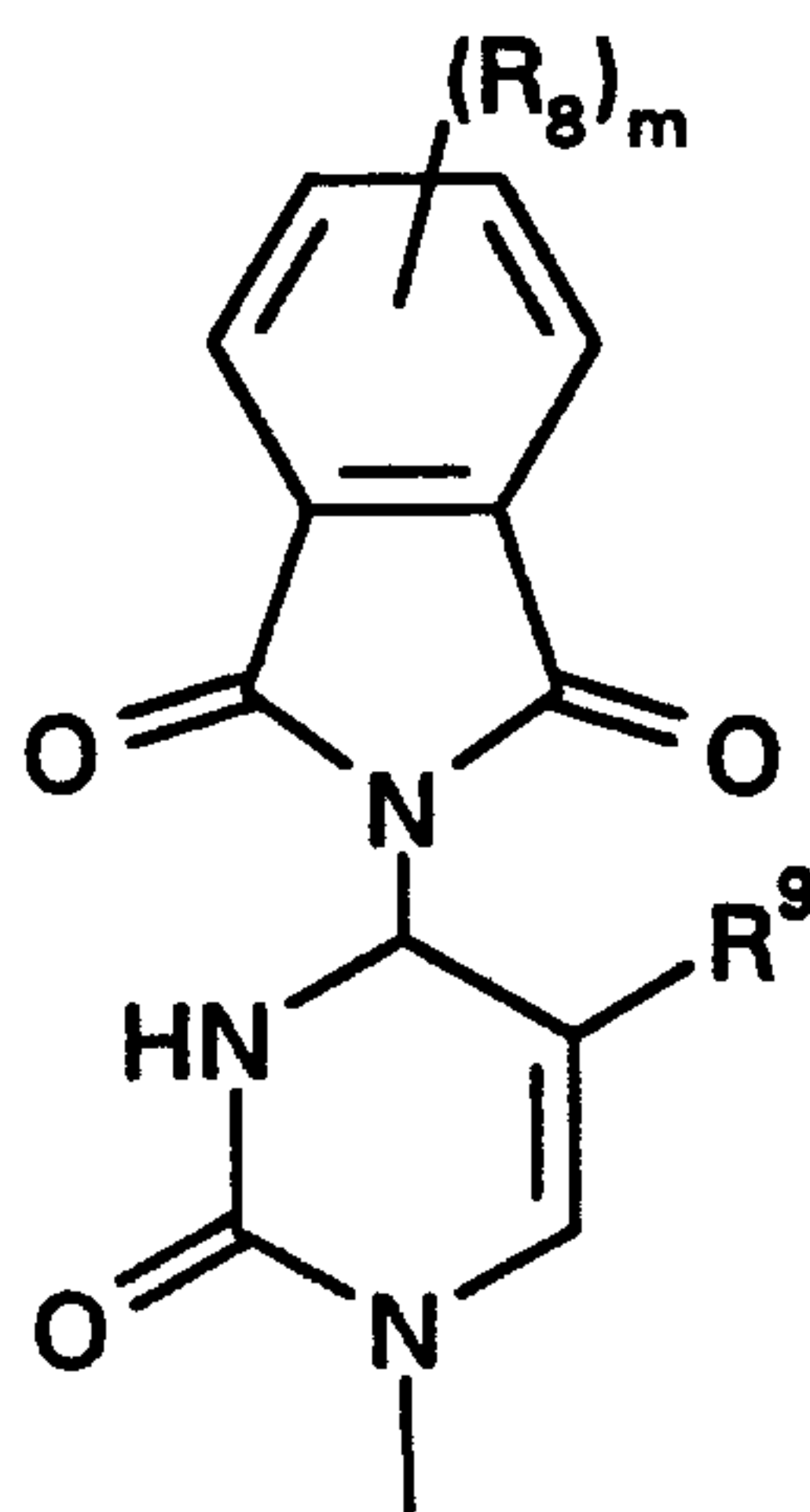
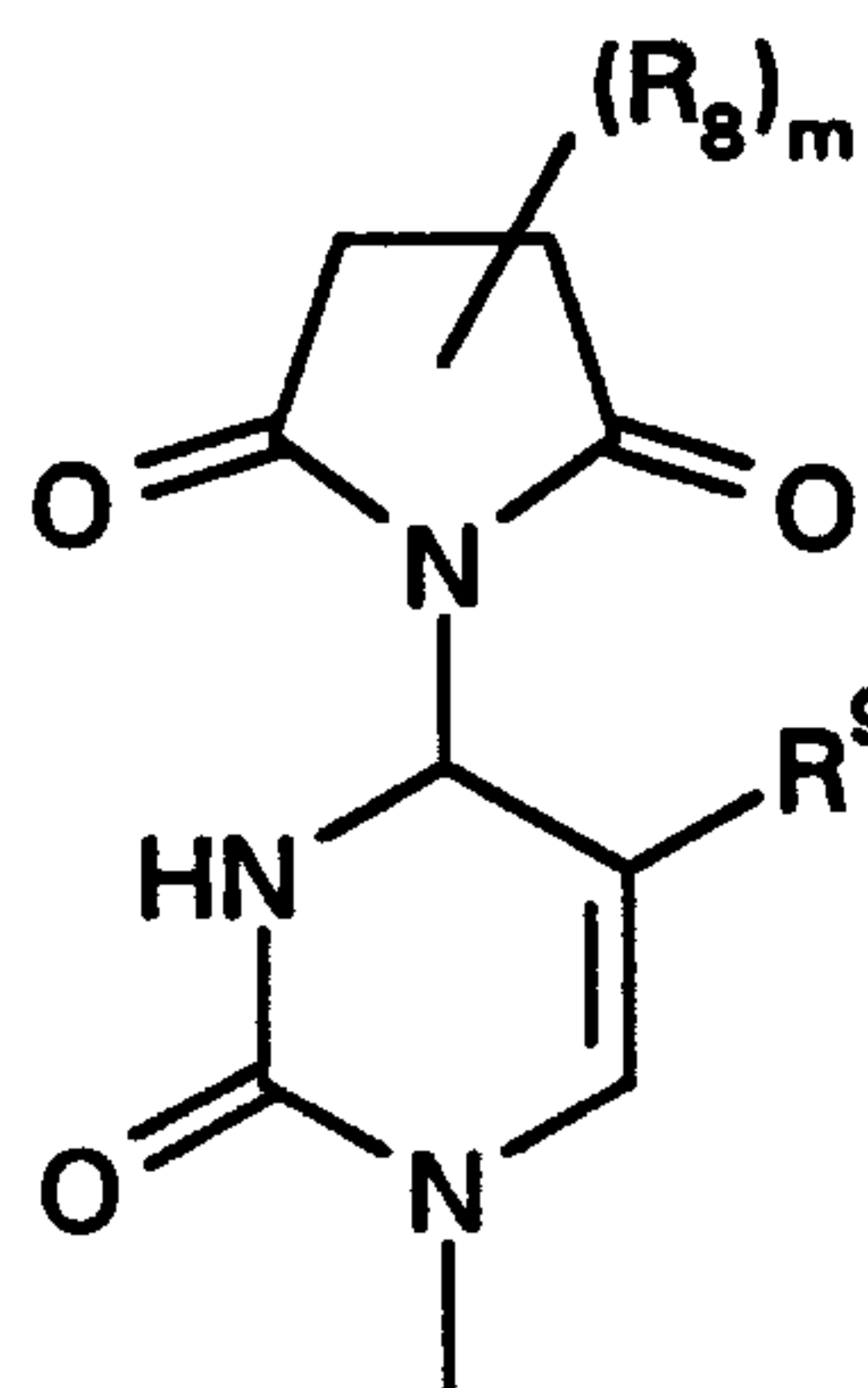


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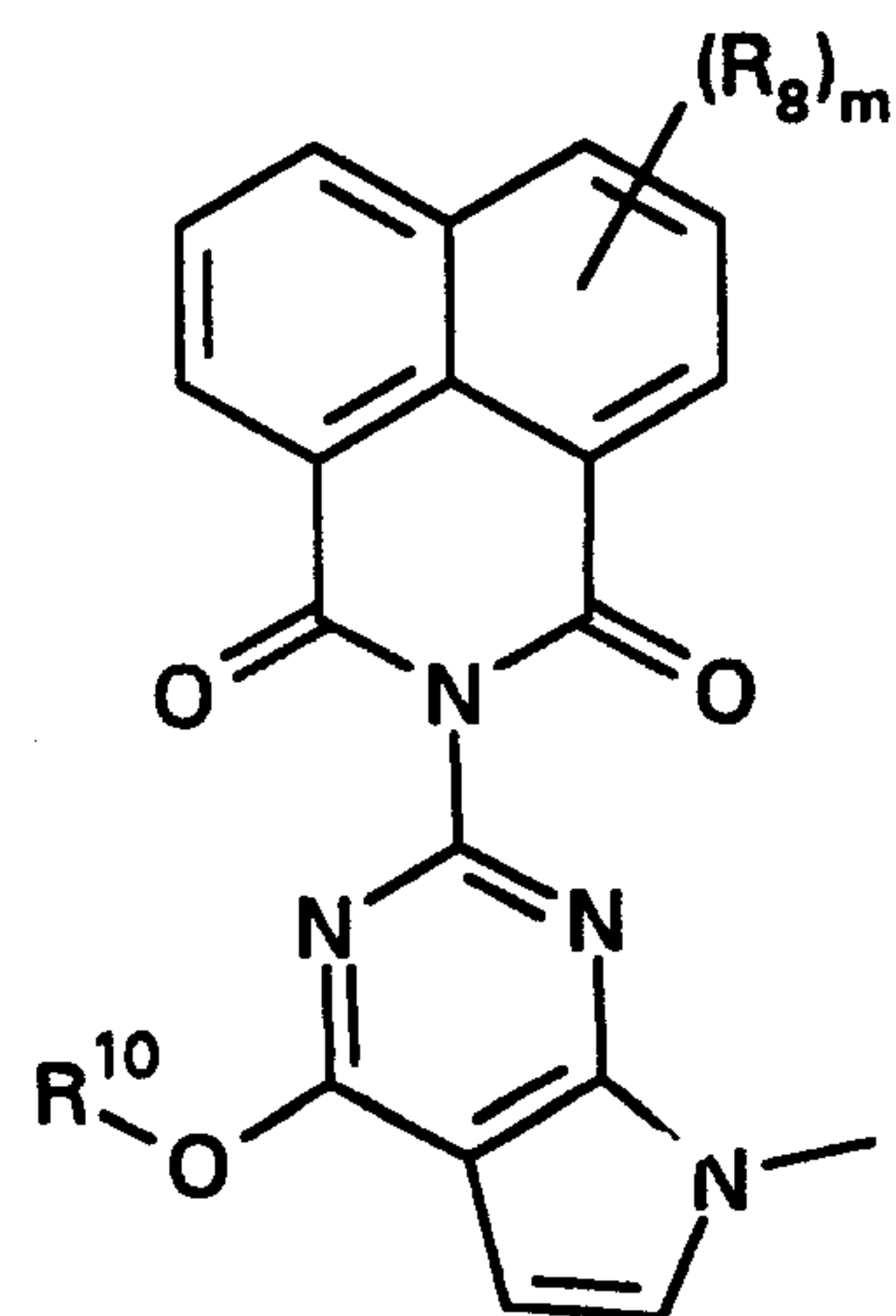
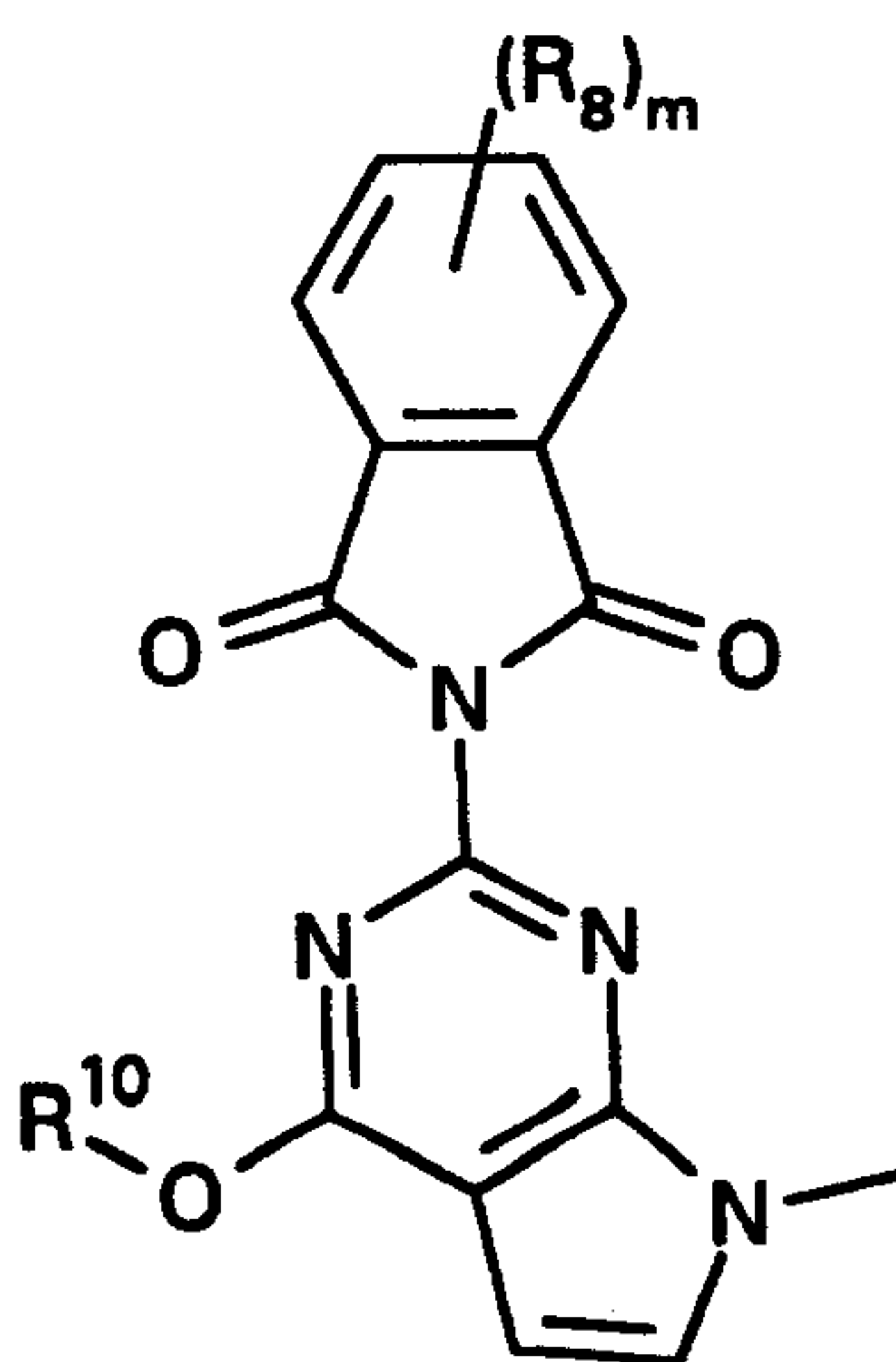
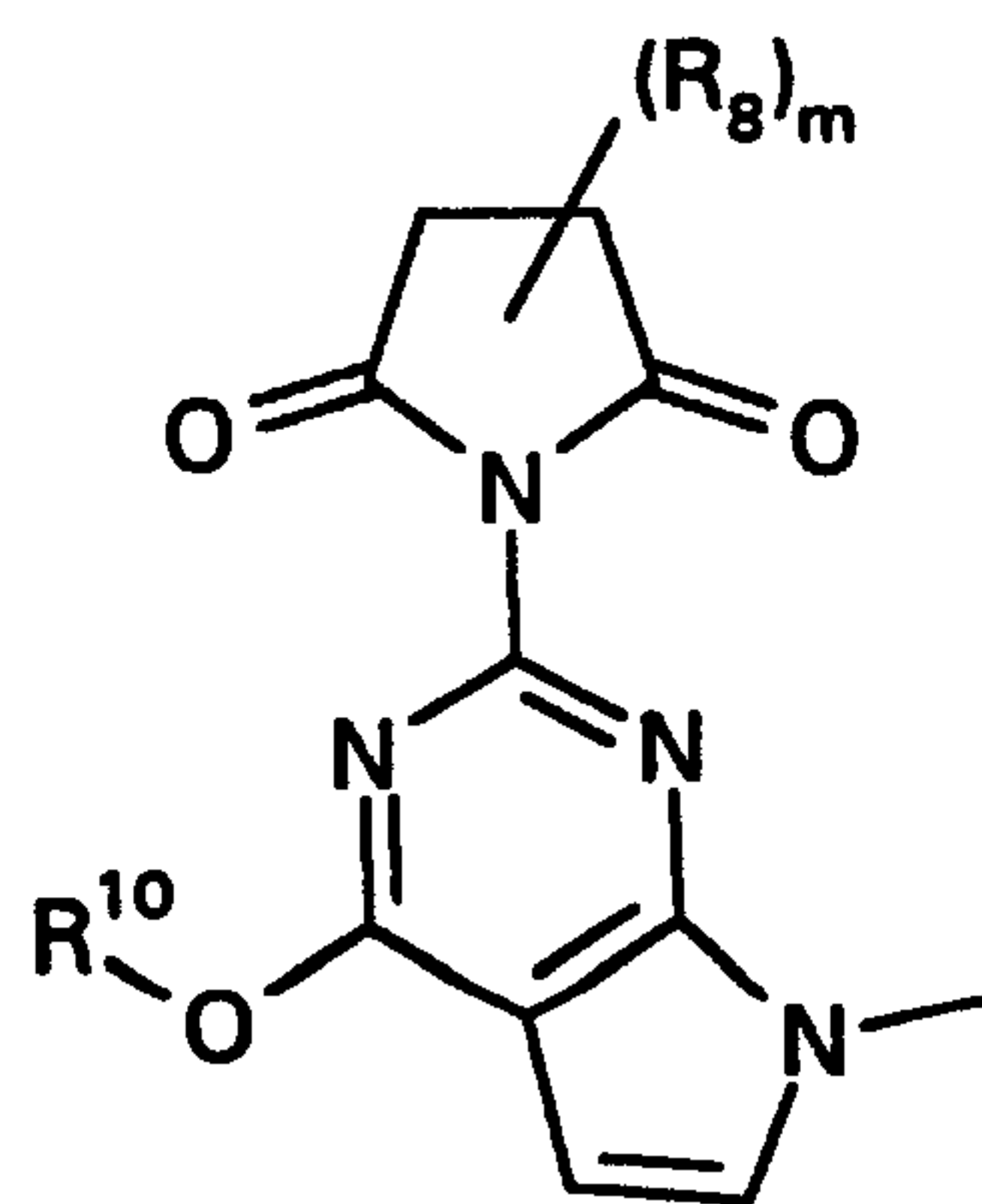
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in which

m is a number from zero to four, preferably zero; and

R^8 is, independently of each other, hydrogen, fluorine, chlorine, bromine, nitro, C_1-C_4 -alkyl, C_1-C_4 -alkoxy, or CN; and R^9 and R^{10} are as defined above.

5 Particular preference is given to compounds of formula V in which

R^{11} is hydrogen, a protected hydroxyl group, C_1-C_4 -alkoxy or fluorine,

A is oxy;

10

V is oxy;

Y is oxy;

15

Z' is OR^{13} , C_1-C_6 -alkyl, C_1-C_6 -alkoxy, C_6-C_{20} -aryl, or C_6-C_{14} -aryl- C_1-C_8 -alkyl, preferably OR^{13} .

The compounds of the formula V are important intermediates for the preparation, according to the invention, of the compounds of the formula I.

20

Examples:

Example 1

25 5'-O-Dimethoxytrityl- N^6 -phthaloyl-2'-O-deoxyadenosine-3'-O-(N,N-diisopropyl[2-(4-nitrophenyl)ethyl])phosphitamide

1.1 N^6 -Phthaloyl-2'-O-deoxyadenosine

30 5 g of 2'-O-desoxyadenosine (20 mmol) are first of all coevaporated in absolute pyridine and then dissolved in 80 ml of pyridine; 6 ml of chlorotrimethylsilane (50 mmol) are added and the whole is stirred at room temperature (RT) for 30 min. 4 ml of phthaloyl chloride (28 mmol) are then added. After 2 hr, hydrolysis takes place

using ice water and the mixture is subsequently stirred for 10 min. It is diluted with 150 ml of AcOEt and extracted four times with 100 ml of a saturated solution of sodium chloride; the aqueous phase is then back-extracted four times with 50 ml of AcOEt. For purification, the residue is taken up in 50 ml of dichloromethane and precipitation takes place from 1000 ml of petroleum ether. 7.37 g (18.5 mmol ; 93 %) of an ocher-colored solid are obtained.

1.2 5'-O-Dimethoxytrityl-N⁶-phthaloyl-2'-O-deoxyadenosine (Literature: Akiko Kume, Mitsuo Sekine, Tsujiaki Hata, Tetrahedron Letters 23(42), 4365-4368 (1982))

4 g of N⁶-phthaloyl-2'-O-deoxyadenosine (10 mmol) are coevaporated with absolute pyridine, then dissolved in 50 ml of absolute pyridine and 50 ml of dichloromethane with 20 mg of N,N-dimethylaminopyridine (0.16 mmol) and 4 Å molecular sieve are stirred, for 2.5 hr, with 2.72 g of 4,4-dimethoxytrityl chloride (8 mmol). After stripping off the solvent, the residue is dissolved in 100 ml of dichloromethane and this solution is extracted with 100 ml of a solution of sodium hydrogen carbonate. The combined organic phases are dried over magnesium sulfate. They are then filtered and subjected to rotary evaporation and the residue is coevaporated with toluene. It is then chromatographed on silica gel (100 g) using a toluene/ AcOEt gradient (44-80 % AcOEt). 3.12 g (4.56 mmol; 46 %) of a white foam result.

1.3 5'-O-Dimethoxytrityl-N⁶-phthaloyl-2'-O-deoxyadenosine-3'-O-(N,N-diisopropyl[2-(4-nitrophenyl)ethyl])phosphitamide

400 mg of 5'-O-dimethoxytrityl-N⁶-phthaloyl-2'-deoxyadenosine (0.58 mmol) are dissolved in 5 ml of acetonitrile, and 313 mg of bis(N,N-diisopropylamino)-2-(4-nitrophenyl)ethoxyphosphane (0.79 mmol) and 20 mg tetrazole (0.29 mmol) are added. After stirring for 1 hr, the mixture is subjected to rotary evaporation under a protective gas and the residue is dissolved in 50 ml of dichloromethane; this solution is extracted with 50 ml of a saturated solution of sodium hydrogen carbonate. After having been dried over MgSO₄, it is subjected to rotary evaporation. The residue is chromatographed on silica gel (6 g of SiO₂) using toluene/AcOEt (1:1), and 380 mg (0.39 mmol; 67 %) of a white foam are obtained.

TLC (silica gel): $R_f = 0.79$ (toluene/AcOEt 1:9); $^1\text{H-NMR}$ (DMSO, 250 MHz): 8.91 (s, 1H, H-C(2)), 8.80 (s, 1H, H-C(8)), 8.07 (m, 6H, 4H pth, 2 H o to NO_2), 7.46 (d, 2H, 2H m to NO_2), 7.16 (m, 9H, DMTr), 6.80 (m, 4H, o to OCH_3), 6.51 (t, 1H, H-C(1')), 4.75 (m, 1H, H-C(3')), 4.13 (m, 1H, H-C(4')), 3.75 (m, 8H, CH_2O , 2x O- CH^3), 3.50 (m, 2H, H-C(5'), H-C(5'')), 3.20 (m, 6H, CH_2 -phenyl, 2x N-CH, H-C(2'), H-C(2'')), 1.01 (m, 12 H, 2x $\text{C}(\text{CH}_3)_2$); $^{31}\text{P-NMR}$ (DMSO, 161.7 MHz) : 1s 147.50; $\text{C}_{53}\text{H}_{54}\text{N}_7\text{O}_{10}\text{P}_1$ (980.04); calc.: C 64.95, H 5.55, N 10.00; found: C 64.15, H 5.53, N 9.72

10 Example 2:

5'-O-Dimethoxytrityl- N^6 -phthaloyl-2'-O-deoxyadenosine-3'-O-(β -cyanoethyl-N,N-diisopropyl)phosphitamide

15 500 mg of 5'-O-dimethoxytrityl- N^6 -phthaloyl-2'-deoxyadenosine (Example 1.2) (0.73 mmol) are dissolved in 5 ml of acetonitrile, and 280 mg of bis-(N,N-diisopropylamino)-2-cyanoethoxyphosphane (0.92 mmol) and 26 mg of tetrazole (0.37 mmol) are added. After the mixture has been stirred for 1.5 hr under a protective gas, it is extracted with 70 ml of dichloromethane and 60 ml of a solution
20 of sodium hydrogen carbonate. After having been dried over MgSO_4 , it is subjected to rotary evaporation. The residue is chromatographed on silica gel (9 g of SiO_2) in a toluene/AcOEt gradient (40-50 % AcOEt), and 430 mg (0.49 mmol; 67 %) of a white foam are obtained.

TLC (silica gel): $R_f = 0.33/0.42$ (toluene/AcOEt 1:1); $^1\text{H-NMR}$ (DMSO, 250 MHz):
25 8.92 (s, 1H, H-C(2)), 8.82 (s, 1H, H-C(8)), 8.09 (m, 4H, pth), 7.33 (m, 2H, DMTr), 7.21 (m, 7H, DMTr), 6.82 (m, 4H, o to OCH_3), 6.55 (m, 1H, H-C(1')), 4.82 (m, 1H, H-C(3')), 4.27 (m, 1H, H-C(4')), 3.65 (m, 10H, CH_2O , 2x O- CH_3 , H-C(5'), H-C(5'')), 3.20 (m, 4H, 2x N-CH, H-C(2'), H-C(2'')), 1.10 (m, 12 H, 2x $\text{C}(\text{CH}_3)_2$); $^{31}\text{P-NMR}$ (DMSO, 161.7 MHz) : 2s 148.51/147.99 ; $\text{C}_{48}\text{H}_{50}\text{N}_7\text{O}_8\text{P}_1$ (883.95); calc.: C 65.22, H 5.70, N 11.09 ; found: C 64.94, H 5.78, N 10.87
30

Example 3:

5'-O-Dimethoxytrityl-N⁶-phthaloyl-2'-O-deoxycytidine-3'-O-(β-cyanoethyl-N,N-diisopropyl)phosphitamide

5

3.1 N⁶-Phthaloyl-2'-O-deoxycytidine

The synthesis is effected in analogy with Example 1.1: 5.8 g of 2'-O-deoxycytidine (22 mmol) are stirred in 80 ml of pyridine together with 7 ml of chlorotrimethylsilane (55 mmol) and, after 1 hr, 4.4 ml of phthaloyl chloride (33 mmol) in 10 ml of dry dioxane are added dropwise within a period of 60 min. 50 mg of DMAP are added and the reaction is stopped with water after 45 min; the mixture is then stirred for a further 15 min. The mixture is extracted with 10 % pyridine in dichloromethane and 100 ml of water. The organic phase is washed with 100 ml of water and the aqueous phase is back-extracted twice with 50 ml of 10 % pyridine in dichloromethane on each occasion. After drying and rotary evaporation, and coevaporation of the residue with toluene, the residue is slurried in dichloromethane and filtered off with suction. 5.07 g (14.2 mmol; 64 %) of an ocher-colored powder result.

TLC (silica gel): R_f = 0.10 (toluene/AcOEt/MeOH 5:4:1); ¹H-NMR (DMSO, 250 MHz): 8.63 (d, 1H, H-C(6)), 8.01 (m, 4H, pth), 6.69 (d, 1H, H-C(5)), 6.10 (t, 1H, H-C(1')), 5.30 (d, 1H, HO-C(3')), 5.11 (t, 1H, HO-C(5')), 4.23 (m, 1H, H-C(3')), 3.91 (m, 1H, H-C(4')), 3.63 (m, 2H, H-C(5'), H-C(5'')), 2.38 (m, 1H, H-C(2')), 2.14 (m, 1H, H-C(2'')); UV (ACN): λ_{max} (nm) / log ε: [331/3.68] , 316/3.80 , [306/3.79] , 218/4.51; C₁₇H₁₅N₃O₆ (357.32) ; calc.: C 57.14, H 4.23, N 11.76 ; found: C 57.21, H 4.45, N 11.66

25

3.2 5'-O-Dimethoxytrityl-N⁶-phthaloyl-2'-O-deoxycytidine

The synthesis is effected in analogy with Example 1.2: 3 g of N⁶-phthaloyl-2'-O-deoxycytidine (8.39 mmol) in 60 ml of pyridine together with 50 mg of N,N-dimethylaminopyridine (0.41 mmol) and 4 Å molecular sieve are stirred for 2 hr with 3.13 g of 4,4-dimethoxytrityl chloride (9.23 mmol). After extracting with 100 ml of dichloromethane and 100 ml of a solution of sodium hydrogen carbonate,

30

chromatography takes place on silica gel (75 g) using a toluene/AcOEt gradient (33-75 % AcOEt). 3.35 g (5.10 mmol; 61 %) of a white foam result.

TLC (silica gel): $R_f = 0.39$ (toluene/AcOEt/MeOH 5:4:1); $^1\text{H-NMR}$ (DMSO, 250 MHz): 8.42 (d, 1H, H-C(6)), 7.99 (m, 4H, pth), 7.30 (m, 9H, DMTr), 6.92 (m, 4H, o to OCH₃), 6.53 (d, 1H, H-C(5)), 6.11 (t, 1H, H-C(1')), 5.40 (d, 1H, HO-C(3')), 4.31 (m, 1H, H-C(3')), 4.01 (m, 1H, H-C(4')), 3.73 (s, 6H, 2x O-CH₃), 3.33 (m, 2H, H-C(5'), H-C(5'')), 2.40 (m, 1H, H-C(2')), 2.20 (m, 1H, H-C(2'')); UV (ACN): λ_{max} (nm) / log ϵ : [331/3.68] , 317/3.84 , [306/3.83] , 283/3.72 , [275/3.69] , 232/4.65; C₃₈H₃₃N₃O₈ (659.70) ; calc.: C 69.19, H 5.04, N 6.37 ; found: C 69.29, H 5.35, N 6.17

10

3.3 5'-O-Dimethoxytrityl-N⁶-phthaloyl-2'-O-deoxycytidine-3'-O-(β -cyanoethyl-N,N-diisopropyl)phosphitamide

15

The synthesis is effected in analogy with Example 2: 660 mg of 5'-O-dimethoxytrityl-N⁶-phthaloyl-2'-deoxycytidine (1 mmol) are dissolved in 7 ml of acetonitrile, and 362 mg of bis-(N,N-diisopropylamino)-2-cyanoethoxyphosphane (1.2 mmol) and 35 mg of tetrazole (0.5 mmol) are added. After 1.5 hr, extraction takes place with 100 ml of dichloromethane and 60 ml of a solution of sodium hydrogen carbonate.

20

Chromatography takes place on silica gel (8 g of SiO₂) in a toluene/AcOEt gradient (40-50 % AcOEt), and 620 mg (0.72 mmol; 72 %) of a white foam are obtained.

25

TLC (silica gel) : $R_f = 0.47/0.51$ (toluene/AcOEt 1:6); $^1\text{H-NMR}$ (DMSO, 250 MHz): 8.45 (m, 1H, H-C(6)), 7.99 (m, 4H, pth), 7.30 (m, 9H, DMTr), 6.91 (m, 4H, o to OCH₃), 6.55 (d, 1H, H-C(5)), 6.15 (m, 1H, H-C(1')), 4.52 (m, 1H, H-C(3')), 4.17 (m, 1H, H-C(4')), 3.72 (s, 6H, 2x O-CH₃), 3.60 (m, 6H, H-C(5'), H-C(5'')), CH₂O, 2x N-CH), 2.70 (2t, 2H, CH₂CN), 2.60 (m, 1H, H-C(2')), 2.40 (m, 1H, H-C(2'')), 0.97-1.19 (m, 12 H, 2x C(CH₃)₂)

$^{31}\text{P-NMR}$ (DMSO, 161.7 MHz) : 2s 148.63/148.38 ; C₄₇H₅₀N₅O₈P₁ (859.91); calc.: C 65.65, H 5.86, N 8.14 ; found: C 64.16, H 6.04, N 8.23

30

Example 4:

5'-O-Dimethoxytrityl-O⁶-[2-(4-nitrophenyl)ethyl]-N²-phthaloyl-2'-deoxyguanosine-3'-
O-(cyanoethyl-N,N-diisopropyl)phosphitamide

5

4.1 O⁶-[2-(4-Nitrophenyl)ethyl]-N²-phthaloyl-2'-deoxyguanosine

The synthesis is effected in analogy with Example 1.1

10 Variant A: Elimination of the trimethylsilyl groups with pyridine/water

833 mg of O⁶-[2-(4-nitrophenyl)ethyl]-2'-deoxyguanosine (2 mmol) are stirred together with 0.63 ml of chlorotrimethylsilane (5 mmol) in 15 ml of pyridine and, after 30 min, 0.43 ml of phthaloyl chloride (3 mmol) in 5 ml of dry dioxane are added dropwise within a period of 15 min. After 2 hr, the reaction is stopped with water and the mixture is then stirred for a further 15 min. The mixture is extracted twice with 50 ml of a solution of sodium hydrogen carbonate and 50 ml of dichloromethane, and the aqueous phases are extracted once again with 50 ml of dichloromethane. The organic phase is dried over MgSO₄, filtered and concentrated. The residue is purified on a silica gel column (25 g of SiO₂) by means of flash chromatography in a toluene/AcOEt (5:4)-MeOH gradient (0-5 % MeOH). 0.56 g (1.02 mmol; 51 %) of a slightly yellowish foam is obtained.

15
20

Variant B: Elimination of the trimethylsilyl groups with ammonium fluoride

25

833 mg of O⁶-[2-(4-nitrophenyl)ethyl]-2'-deoxyguanosine (2 mmol) are stirred together with 0.63 ml of chlorotrimethylsilane (5 mmol) in 15 ml of pyridine and, after 30 min, 0.57 ml of phthaloyl chloride (4 mmol) in 2 ml of dry dioxane is added dropwise within a period of 5 min. After 2 hr, the mixture is subjected to rotary evaporation and the residue is coevaporated with toluene and then treated with 160 mg of ammonium fluoride (4.3 mmol) in 20 ml of MeOH. The mixture is stirred for 3 min and then extracted twice with 100 ml of a solution of sodium hydrogen carbonate and 100 ml of dichloromethane, and the aqueous phases are extracted

30

once again with 50 ml of dichloromethane. Purification is effected on a silica gel column (25 g of SiO₂) by means of flash chromatography in a toluene/AcOEt (5:4)-MeOH gradient (0-5 % MeOH). 0.45 g (0.82 mmol; 41 %) of a slightly yellowish foam is obtained.

5

Variant C: Elimination of the trimethylsilyl groups with tetrabutylammonium fluoride

833 mg of O⁶-[2-(4-nitrophenyl)ethyl]-2'-deoxyguanosine (2 mmol) are stirred together with 0.7 ml of chlorotrimethylsilane (5.5 mmol) in 15 ml of pyridine and, after 30 min, 0.55 ml of phthaloyl chloride (3.8 mmol) in 3 ml of dry dioxane is added dropwise within a period of 10 min. After 2 hr, 1.26 g of tetrabutylammonium fluoride trihydrate (4 mmol) are added and the mixture is stirred for 5 min. It is subsequently extracted twice with 100 ml of a solution of sodium hydrogen carbonate and 100 ml of dichloromethane, and the aqueous phases are extracted once again with 50 ml of dichloromethane. Purification is effected on a silica gel column (25 g of SiO₂) by means of flash chromatography in a toluene/AcOEt (5:4)-MeOH gradient (0-5 % MeOH). 0.39 g (0.71 mmol; 36 %) of a slightly yellowish foam is obtained.

TLC (silica gel): R_f = 0.42 (chloroform/methanol 9:1); ¹H-NMR (DMSO, 250 MHz): 8.71 (s, 1H, H-C(8)), 8.14 (d, 2H, 2H o to NO₂), 8.00 (m, 4H, pth), 7.61 (d, 2H, 2H m to NO₂), 6.39 (t, 1H, H-C(1')), 5.32 (d, 1H, HO-C(3')), 4.91 (t, 1H, HO-C(5')), 4.83 (t, 2H, CH₂O), 4.40 (m, 1H, H-C(3')), 3.85 (m, 1H, H-C(4')), 3.51-3.58 (m, 2H, H-C(5'), H-C(5'')), 3.32 (t, 2H, CH₂-phenyl), 2.74 (m, 1H, H-C(2')), 2.32 (m, 1H, H-C(2'')); UV (ACN): λ_{max} (nm) / log ε: 262/4.34 , 219/4.68; C₂₆H₂₂N₆O₈ (546.50) ; calc: C 57.14, H 4.06, N 15.38 ; found: C 57.27, H 4.37, N 15.11

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4.2 5'-O-Dimethoxytrityl-O⁶-[2-(4-nitrophenyl)ethyl]-N²-phthaloyl-2'-deoxyguanosine

Synthesis in analogy with Example 1.2: 990 mg of O⁶-[2-(4-nitro-phenyl)ethyl]-N²-phthaloyl-2'-deoxyguanosine (1.81 mmol) are dissolved in 25 ml of pyridine, 674 mg of 4,4-dimethoxytrityl chloride (1.99 mmol) are added and the mixture is stirred for 3 hr. It is extracted with 100 ml of AcOEt and 100 ml of a saturated solution of sodium hydrogen carbonate. Chromatography subsequently takes place on silica gel (25 g of SiO₂) in a toluene/AcOEt gradient (20-66 % AcOEt), and 1.00 g (1.18

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mmol; 67 %) of a white foam is obtained.

TLC (silica gel) : $R_f = 0.57$ (toluene/AcOEt 1:6); $^1\text{H-NMR}$ (DMSO, 250 MHz): 8.59 (s, 1H, H-C(8)), 8.13 (d, 2H, 2H o to NO_2), 8.00 (m, 4H, pth), 7.60 (d, 2H, 2H m to NO_2), 7.10-7.26 (m, 9H, DMTr), 6.68 (m, 4H, o to OCH_3), 6.43 (m, 1H, H-C(1')), 5.36 (d, 1H, HO-C(3')), 4.81 (t, 2H, CH_2O), 4.46 (m, 1H, H-C(3')), 3.95 (m, 1H, H-C(4')), 3.67 (s, 6H, 2x O-CH_3), 3.32 (t, 2H, CH_2 -phenyl), 3.05-3.25 (m, 2H, H-C(5'), H-C(5'')), 2.85 (m, 1H, H-C(2')), 2.38 (m, 1H, H-C(2'')); UV (ACN): λ_{max} (nm) / log ϵ : 262/4.38, 218/4.78

$\text{C}_{47}\text{H}_{40}\text{N}_6\text{O}_{10}$ (848.87) ; calc.: C 66.50, H 4.75, N 9.90 ; found: C 66.65, H 4.90, N 9.52

4.3 5'-O-Dimethoxytrityl- O^6 -[2-(4-nitrophenyl)ethyl]- N^2 -phthaloyl-2'-deoxyguanosine-3'-O-(β -cyanoethyl-N,N-diisopropyl)phosphitamide

15 Synthesis in analogy with Example 1.3, but using pyridine hydrochloride instead of tetrazole; yield: 87%.

TLC (silica gel): $R_f = 0.74/0.82$ (toluene/AcOEt 1:6); $^1\text{H-NMR}$ (DMSO, 250 MHz): 8.63 (s, 1H, H-C(8)), 8.13 (s, 2H, 2H o to NO_2), 8.00 (m, 4H, pth), 7.61 (s, 2H, 2H m to NO_2), 7.08-7.24 (m, 9H, DMTr), 6.63-6.71 (m, 4H, DMTr), 6.45 (m, 1H, H-C(1')), 4.80 (m, 3H, H-C(3'), CH_2O), 4.10 (m, 1H, H-C(4')), 3.66 and 3.67 (2s, 6H, 2x OCH_3), 2.95-3.59 (m, 10 H, $\text{NC-CH}_2\text{O}$, H-C(5'), H-C(5'')), 2x N-CH, CH_2CN , CH_2 -phenyl) 2.72 (m, 1H, H-C(2')), 2.61 (m, 1H, H-C(2'')), 0.90-1.20 (m, 12 H, 2x $\text{C}(\text{CH}_3)_2$); $^{31}\text{P-NMR}$ (DMSO, 161.7 MHz) : 2s 148.55/148.05 ; $\text{C}_{56}\text{N}_{17}\text{O}_{11}\text{P}_1$ (1049.09) ; calc.: C 64.11, H 5.48, N 10.68 ; found: C 63.92, N 10.68, H 5.47

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Example 5:

5'-O-Dimethoxytrityl- O^6 -[2-(phenylsulfonyl)ethyl]- N^2 -phthaloyl-2'-deoxyguanosine-3'-O-(cyanoethyl-N,N-diisopropyl)phosphitamide

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5.1 O^6 -[2-(Phenylsulfonyl)ethyl]- N^2 -phthaloyl-2'-deoxyguanosine

The synthesis is effected in analogy with Example 1.1

Variant A: Elimination of the trimethylsilyl groups with pyridine/water

300 mg of O⁶-[2-(Phenylsulfonyl)ethyl]-2'-deoxyguanosine (0.69 mmol) are stirred together with 0.22 ml of chlorotrimethylsilane (1.7 mmol) in 10 ml of pyridine and, after 30 min, 0.14 ml of phthaloyl chloride (0.97 mmol) in 2 ml of dry dioxane is added dropwise within a period of 7 min. After 1 hr, the reaction is stopped with water and the mixture is then stirred for a further 15 min. It is extracted twice with 25 ml of water and 25 ml of dichloromethane, and the aqueous phases are extracted once again with 25 ml of dichloromethane. Purification is effected on a silica gel column (7 g of SiO₂) by means of flash chromatography in a petroleum ether/acetone gradient (50-66 % acetone). 0.14 g (0.24 mmol; 36 %) of a virtually colorless foam is obtained.

Variant B: Elimination of the trimethylsilyl groups with ammonium fluoride

0.87 g of O⁶-[2-(Phenylsulfonyl)ethyl]-2'-deoxyguanosine (2 mmol) is stirred together with 0.56 ml of chlorotrimethylsilane (4.4 mmol) in 15 ml of dry acetonitrile and 1.03 ml of pyridine and, after 30 min, 0.29 ml of phthaloyl chloride (2 mmol) is added dropwise. After 35 min, the mixture is subjected to rotary evaporation and the residue is coevaporated with toluene and treated with 160 mg of ammonium fluoride (4.3 mmol) in 25 ml of MeOH. This mixture is stirred for 3 min and extracted twice with 100 ml of a solution of sodium hydrogen carbonate and 100 ml of dichloromethane, and the aqueous phases are extracted once again with 50 ml of dichloromethane. Purification is effected on a silica gel column (25 g of SiO₂) by means of flash chromatography in a petroleum ether/acetone gradient (30-75 % acetone). 0.45 g (0.79 mmol; 40 %) of a slightly yellowish foam is obtained.

TLC (silica gel): R_f = 0.27 (petroleum ether/acetone 1:2); ¹H-NMR (DMSO, 250 MHz): 8.65 (s, 1H, H-C(8)), 7.96-8.7 (m, 4H, pth), 7.82 (m, 2H, o to SO₂), 7.36-7.49 (m, 3H, 2H m to SO₂, 1H p to SO₂), 6.37 (t, 1H, H-C(1')), 5.32 (d, 1H, HO-C(3')), 4.92 (t, 1H, HO-C(5')), 4.80 (t, 2H, CH₂O), 4.41 (m, 1H, H-C(3')), 4.12 (t, 2H, CH₂SO₂), 3.86 (m, 1H, H-C(4')), 3.45-3.65 (m, 2H, H-C(5'), H-C(5'')), 2.72 (m, 1H, H-C(2')), 2.33 (m, 1H, H-C(2'')); UV (ACN): λ_{max} (nm) / log ε: [294/3.50] , 259/4.26 , 219/4.73; C₂₆H₂₃N₅O₈S₁ x H₂O (583.58) calc.: C 53,51; H 4,32; N 12,00; found:

C 53,95; H 4,45; N 11,72

5.2 5'-O-Dimethoxytrityl-O⁶-[2-(phenylsulfonyl)ethyl]-N²-phthaloyl-2'-deoxyguanosine

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Synthesis in analogy with Example 1.2: 560 mg of O⁶-[2-(phenylsulfonyl)ethyl]-N⁶-phthaloyl-2'-deoxyguanosine (1 mmol) in 20 ml of pyridine are stirred for 16 hr together with 373 mg of 4,4-dimethoxytrityl chloride (1.1 mmol). The mixture is extracted with 100 ml of dichloromethane and 100 ml of a saturated solution of sodium hydrogen carbonate. Chromatography subsequently takes place on silica gel (20 g of SiO₂) in a toluene/AcOEt gradient (30-80 % AcOEt), and 570 mg (0.66 mmol; 66 %) of a slightly yellowish foam are obtained.

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TLC (silica gel): R_f = 0.39 (toluene/AcOEt/MeOH 5:4:1); ¹H-NMR (DMSO, 250 MHz): 8.55 (s, 1H, H-C(8)), 8.01-8.07 (m, 4H, pth), 7.82 (m, 2H, 2H o to SO₂), 7.32-7.38 (m, 3H, 2H m to SO₂, 1H p to SO₂), 7.05-7.27 (m, 9H, DMTr), 6.67-6.73 (m, 4H, o to OCH₃), 6.41 (m, 1H, H-C(1')), 5.37 (d, 1H, HO-C(3')), 4.79 (t, 2H, CH₂O), 4.46 (m, 1H, H-C(3')), 4.10 (t, 2H, CH₂SO₂), 3.96 (m, 1H, H-C(4')), 3.68 (s, 6H, 2x OCH₃), 3.09-3.25 (m, 2H, H-C(5'), H-C(5'')), 2.81 (m, 1H, H-C(2')), 2.35 (m, 1H, H-C(2'')); UV (ACN): λ_{max} (nm)/ log ε: 261/4.26, 217/4.80; C₄₇H₄₁N₅O₁₀S₁ (867.93); calc.: C 65.04, H 4.76, N 8.07; found: C 64.97, H 4.82, N 7.88

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5.3 5'-O-Dimethoxytrityl-O⁶-[2-(phenylsulfonyl)ethyl]-N²-phthaloyl-2'-deoxyguanosine-3'-O-(cyanoethyl-N,N-diisopropyl)phosphitamide

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Synthesis in analogy with Example 2: 470 mg of 5'-O-dimethoxytrityl-O⁶-[2-(phenylsulfonyl)ethyl]-N²-phthaloyl-2'-deoxyguanosine (0.54 mmol) are dissolved in 8 ml of acetonitrile, and 196 mg of bis(N,N-diisopropylamino)-2-cyanoethoxyphosphane (0.65 mmol) and 0.54 ml of an 0.5 M solution of pyridinium chloride are added. After 3 hr, a further 150 mg of phosphane and 0.27 ml of pyridinium chloride solution are added and the mixture is stirred for a further 3.5 hr. It is extracted with 50 ml of dichloromethane and 50 ml of a solution of sodium hydrogen carbonate. Chromatography is carried out in a toluene/ethyl acetate gradient (33-50 % AcOEt), and 420 mg (0.39 mmol; 73 %) of a white foam are

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obtained.

TLC (silica gel): $R_f = 0.49/0.60$ (petroleum ether/AcOEt/triethylamine 1:9:1); $^1\text{H-NMR}$ (DMSO, 250 MHz): 8.57 (2s, 1H, H-C(8)), 7.98-8.03 (m, 4H, pth), 7.81 (m, 2H, 2H o to SO_2), 7.31-7.40 (m, 3H, 2H m to SO_2 , 1H o to SO_2), 7.08-7.25 (m, 9H, DMTr), 6.39-6.73 (m, 4H, DMTr), 6.43 (m, 1H, H-C(1')), 4.78 (m, 3H, H-C(3')), CH_2O), 4.10 (m, 3H, CH_2SO_2 , H-C(4')), 3.66 and 3.67 (2s, 6H, 2x OCH_3), 3.30-3.60 (m, 4 H, NC- CH_2O , 2x N-CH), 3.23 (m, 2H, H-C(5'), H-C(5'')), 3.00 (m, 1H, H-C(2')), 2.62 and 2.73 (2t, 2H, CH_2CN), 2.50 (m, 1H, H-C(2'')), 0.86-1.19 (m, 12 H, 2x $\text{C}(\text{CH}_3)_2$); $^{31}\text{P-NMR}$ (DMSO, 161.7 MHz) : 2s 148.54/148.06 ; $\text{C}_{56}\text{H}_{58}\text{N}_7\text{O}_{11}\text{P}_1\text{S}_1$ (1068.15) ; calc.: C 62.97, H 5.47, N 9.18; found: C 62.25, N 5.65, H 8.82

Example 6:

5'-O-Dimethoxytrityl- N^6 -phthaloyl-3'-O-succinoyl-2'-O-deoxyadenosine

342 mg of 5'-O-dimethoxytrityl- N^6 -phthaloyl-2'-O-deoxyadenosine (Example 1.2) (0.5 mmol) are dissolved in 10 ml of absolute dichloromethane, and 79 mg of 4,4-dimethylaminopyridine (0.65 mmol) and 100 mg of succinic anhydride (1 mmol) are added. After 17 hr, the mixture is diluted with 50 ml of dichloromethane and extracted with 30 ml of a saturated solution of sodium hydrogen carbonate and then 10 % citric acid. After drying the organic phase over MgSO_4 and subjecting it to rotary evaporation, and drying the residue under high vacuum, 390 mg (0.49 mmol; 98 %) of a white foam are obtained.

TLC (silica gel): $R_f = 0.17$ (toluene//AcOEt/MeOH 5:4:1); $^1\text{H-NMR}$ (DMSO, 250 MHz): 12.30 (s, 1H, COOH), 8.91 (s, 1H, H-C(2)), 8.82 (s, 1H, H-C(8)), 8.06 (m, 4H, pth), 7.17 (m, 9H, DMTr), 6.83 (m, 4H, o to OCH_3), 6.56 (t, 1H, H-C(1')), 5.45 (m, 1H, H-C(3')), 4.25 (m, 1H, H-C(4')), 3.70 (s, 6H, 2x O-CH_3), 3.33 (m, 2H, H-C(5'), H-C(5'')), 2.58 (m, 6H, H-C(2'), H-C(2''), CH_2CH_2); UV (ACN): λ_{max} (nm) / log ϵ : [300/3.60] , 271/4.17 , [220/4.72]; $\text{C}_{43}\text{H}_{37}\text{N}_5\text{O}_{10} \times \text{H}_2\text{O}$ (801.82) ; calc.: C 64.41, H 4.90, N 8.73 ; found: C 64.47, H 4.98, N 8.74

Example 7:

5'-O-Dimethoxytrityl-O⁶-[2-(4-nitrophenyl)ethyl]-N²-phthaloyl-3'-O-succinoyl-2'-O-deoxyguanosine

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212 mg of 5'-O-Dimethoxytrityl-O⁶-[2-(4-nitrophenyl)ethyl]-N²-phthaloyl-2'-deoxyguanosine (0.25 mmol) are dissolved in 5 ml of absolute dichloromethane, and 50 mg of succinic anhydride (0.5 mmol) and 40 mg of 4,4-dimethylaminopyridine (0.32 mmol) are added. After the mixture has been stirred for 24 hr, it is diluted with 60 ml of dichloromethane and this mixture is extracted with 40 ml of a saturated solution of sodium hydrogen carbonate and then with 40 ml 10 % citric acid solution. After the organic phase has been dried over magnesium sulfate, it is subjected to rotary evaporation and the residue is dried under high vacuum. 210 mg (0.22 mmol ; 88 %) of a slightly yellowish foam are obtained.

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15 ¹H-NMR (DMSO, 250 MHz): 12.27 (s, 1H, COOH), 8.61 (s, 1H, H-C(8)), 8.14 (d, 2H, 2H o to NO₂), 7.96-8.05 (m, 4H, pth), 7.61 (d, 2H, 2H m to NO₂), 7.05-7.27 (m, 9H, DMTr), 6.65-6.71 (m, 4H, o to OCH₃), 6.44 (t, 1H, H-C(1')), 5.36 (m, 1H, H-C(3')), 4.82 (t, 2H, OCH₂), 4.14 (m, 1H, H-C(4')), 3.67 (s, 6H, 2x O-CH₃), 3.16-3.42 (m, 4H, H-C(5'), H-C(5''), CH₂-phenyl), 2.45-2.60 (m, 6H, H-C(2'), H-C(2''), CH₂CH₂); UV (ACN): λ_{max} (nm) / log ε: 262/4.37 , 216/4.78.

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Example 8:

8.1 Support-derivatization of long-chain methylaminoalkyl (LCMAA) -CPG:

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1 g of 1000 Å or 1400 Å CPG material is dried for 1.5 hours under high vacuum. 1 g of carbonyldiimidazole (12.4 mmol) is dissolved in 20 ml of dry dichloromethane, and the dried CPG support material is added and the whole is thoroughly mixed on a vibrator for 6 hours. The overlying solution is decanted off and digestion is carried out three times with dry dichloromethane. This is followed by taking up in 15 ml of dry dichloromethane, after which 1 ml of 1,6-bis(methylamino)hexane (5.8 mmol) is added. After 3 hours on the vibrator, the supernatant is decanted off and this is followed by filtering off with suction and washing consecutively with pyridine, DMF,

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methanol, acetone and diethyl ether. Drying then takes place under high vacuum.

8.2 Loading LCMAA-CPG with phthaloyl-protected succinates:

5 400 mg of LCMAA-CPG are shaken with 7 mg TOTU (O-
 [(ethoxycarbonyl)cyanomethyleneamino]-N,N,N',N'-tetramethyluronium tetra-
 fluoroborates: W. König, G. Breipohl, P. Pokorny, M. Birkner, Proceedings of the
 21st European Peptide Symposium 1990, E. Giralt, D. Andreu, Eds., ESCOM,
 Leiden, p. 143) (21 μmol), 3 μl of N-methylmorpholine (27 μmol) and 23 μmol of
 10 nucleoside succinate for 2 hours in 5 ml of dry acetonitrile. Filtering off with suction
 takes place and the filter residue is washed consecutively with DMF, methanol,
 acetone and diethyl ether.

Capping :

15 The nucleoside-loaded support is shaken for 0.5 hours with 10 mg of DMAP, 0.5 ml
 of acetic anhydride and 10 ml of pyridine. Filtering off with suction then takes place
 and the filter residue is washed consecutively with DMF, methanol, acetone and
 diethyl ether. In the case of 5'-O-dimethoxytrityl-O⁶-[2-(4-nitrophenyl)ethyl]-N₂-
 phthaloyl-3'-O-succinyl-2'-deoxyguanosine, capping is effected using a mixture
 composed of 0.5 ml of N-methylimidazole, 0.5 ml of acetic anhydride and 5 ml of
 20 pyridine.

The following loadings are obtained:

| Support | Succinate | Loading [$\mu\text{mol/g}$] |
|---------|------------------------|-------------------------------|
| 1000 Å | dA ^{pth} | 8.9 |
| 1400 Å | dA ^{pth} | 12.5 |
| 1400 Å | dGP ^{pth/npe} | 10.9 |

8.3 Derivatizing LCMAA-CPG with succinic anhydride:

30 500 mg of 1400 Å LCMAA-CPG are shaken for 24 hours in 4 ml of dry pyridine
 together with 12 mg of DMAP (0.1 mmol) and 400 mg of succinic anhydride. Filtering
 off with suction takes place and the filter residue is washed with pyridine and

dichloromethane.

Loading the succinyl-LCMAA-CPG with phthaloyl-protected 5'-O-DMTr-nucleosides:

5 3 ml of dry pyridine and 12 μ l of triethylamine are added to 150 mg of succinylated LCMAA-CPG, 1.8 mg of DMAP (0.015 mmol), 29 mg of 1-(3-dimethyl-aminopropyl)ethylcarbodiimide (0.15 mmol) and 0.015 mmol of 5'-O-DMTr-protected phthaloyl compound. The mixture is shaken for 24 hours, after which 20 mg of pentachlorophenol (0.07 mmol) are added and the whole is shaken for a further
10 23 hours. 0.75 ml of piperidine is then added and filtering off with suction takes place immediately after 5 minutes and the filter residue is washed consecutively with dichloromethane and diethyl ether.

Capping : the nucleoside-loaded support is shaken for 1.5 hours with a mixture composed of 0.5 ml of N-methylimidazole, 0.5 ml of acetic anhydride and 5 ml of
15 pyridine and subsequently washed with DMF, methanol, acetone and diethyl ether.

The following loadings are obtained:

| Support | 5'-O-DMTr-nucleoside | Loading [μ mol/g] |
|---------|-----------------------|------------------------|
| 1400 Å | dC ^{pth} | 2.2 |
| 1400 Å | dG ^{pth/npe} | 3 |

Example 9:

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Oligonucleotide synthesis

Unmodified oligonucleotides were synthesized on an automated DNA synthesizer (Applied Biosystems Model 392) using the standard phosphoramidite synthesis
30 cycle. The phthaloyl strategy makes possible either use of the water-containing oxidation mixture iodine/pyridine/THF/water or water-free oxidation using tert-butyl hydroperoxide in acetonitrile. The oxidation times are 15 seconds for iodine and 90

seconds for tert-butyl hydroperoxide. After the synthesis cycles have been completed, the protecting groups are eliminated, while the oligonucleotide is still on the support, with DBU in N-methylimidazole or acetonitrile (see below) (DBU: 1,8-diazobicyclo[6.4.0]undec-7-ene) over a period of from 15 minutes to 12 h. The oligonucleotide, which is now free of all protecting groups, is then released from the support by treating with concentrated aqueous ammonia (25 %). The oligonucleotide is now present in highly pure form in ammoniacal solution and is obtained by lyophilizing the ammoniacal solution. In the phthaloyl strategy, in contrast to the acyl strategy, there is no necessity for an additional purification by HPLC or PAGE for the purpose of removing the protecting groups.

The following sequences were synthesized using the phosphoramidites described in Examples 1-5 and the derivatized solid supports described in Example 8. In the individual sequences, the conditions described for eliminating the protecting groups were varied successfully.

| Sequence | Ox | Phthaloyl elimination | | | |
|----------------------------------|----|-----------------------|------|--------|----------------------------|
| | | 12 hr | 1 hr | 0.5 hr | other times |
| 5'-A ₅ | B | (1) | (1) | | 3 hr ⁽¹⁾ |
| 5'-A ₆ | I | (1) | | | |
| 5'-A ₇ | I | (1) | | | |
| 5'-A ₈ | B | | | | 25 min ⁽¹⁾ |
| 5'-A ₉ | B | (1) | | | 15 min/3 hr ⁽¹⁾ |
| 5'-A ₁₅ | B | (1) | | (1) | |
| 5'-A ₂₀ | B | (1) | | | |
| 5'-CCC CCC CT | B | (2) | | | 25 min ⁽¹⁾ |
| 5'-CCC CAT TAT | B | | | | 25 min ⁽¹⁾ |
| 5'-AAT CCTA | B | | | | 12 min ⁽¹⁾ |
| 5'-ATT CCTA | B | | | | 12 min ⁽¹⁾ |
| 5'-ATT TAA TTT AAT TTAA | B | (1) | | | |
| 5'-GCC TCT GAA CCT CTT CAG CA | I | (1) | | (3) | |
| 5'-TTA CTA ATC AGA ATG TCT CTC A | I | (1) | (3) | (3) | |
| 5'-GTT GGG TCC GAA TAT TTC AGA A | B | (1) | | (3) | |

Abbreviations: oxidizing agents (Ox): I = iodine; B = tert-butyl hydroperoxide;
 phthaloyl elimination: (1) = 1 M DBU/ACN; (2) = 2 M DBU/ACN; (3) = 2 M DBU in N-
 methylimidazole

5 The following sequences were synthesized using the phosphoramidites described in
 Examples 1-5 and the solid supports described in Example 8. In this case, a solution
 of 0.5 M pyridine hydrochloride in acetonitrile was employed as the activator instead
 of tetrazole, with condensation times of from 12 to 30 seconds. In the individual
 sequences, the conditions described for eliminating the protecting groups were
 10 varied successfully.

| Sequence | Ox | Co | Phthaloyl elimination | | |
|----------------------------------|----|----|-----------------------|------|--------|
| | | | 12 hr | 1 hr | 0.5 hr |
| 5'-A ₆ | B | 12 | (1) | | |
| 5'-GGG t | I | 30 | (1) | | (2) |
| 5'-GCC TCT GAA CCT CTT CAG CA | I | 24 | (1) | | |
| 5'-TTA CTA ATC AGA ATG TCT CTC A | I | 24 | (1) | (3) | (3) |
| 5'-GTT GGG TCC GAA TAT TTC AAG A | I | 24 | (1) | | (3) |
| 5'-GCT GCA TG | I | 24 | | | (3) |
| 5'-CCT CCA ATC TAG | I | 30 | (1) | (3) | |
| 5'-TGT AGT AGT GGT | I | 30 | (1) | (3) | |
| 5'-GTT ATT | I | 30 | (1) | (3) | |
| 5'-TGA CGT TAT T | I | 30 | (1) | (3) | |

Abbreviations: oxidizing agents (Ox): I = iodine ; B = tert-butyl hydroperoxide;
 Condensation time (Co): in seconds; phthaloyl elimination: (1) = 1 M DBU/ACN ; (2)
 = 2 M DBU/ACN; (3) = 2 M DBU in N-methylimidazole

30 The high purity of the oligonucleotides (>95%) was confirmed by HPLC.

HPLC : Gradient:

(reversed phase ; RP 18) ; flow rate 1 ml/min

Solution A: 0.1 N TEAAc pH 7

Solution B : 0.1 N TEAAc: AcCN 1:1

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| Step | Min | Vol. % | Vol. % |
|------|-----|--------|--------|
| 1 | 0 | 95 | 5 |
| 2 | 2 | 95 | 5 |
| 3 | 32 | 60 | 40 |
| 4 | 45 | 0 | 100 |
| 5 | 50 | 95 | 5 |
| 6 | 55 | 95 | 5 |

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Patent Claims:

1. A process for preparing oligonucleotides by means of solid phase synthesis by

5 a) sequentially synthesizing the nucleotides on a solid support in accordance with known methods, with exocyclic amino groups which are present on the nucleobases being protected by a cyclic diacyl group and with it being possible to eliminate any phosphate protecting groups which are present with strong, nonnucleophilic bases,

10 b) deprotecting the oligonucleotides which are bound to the solid support, and

c) cleaving the deprotected oligonucleotides from the solid support,

which comprises deprotecting the oligonucleotides, which are bound to the solid support, in the presence of a strong, nonnucleophilic base in a suitable organic solvent.

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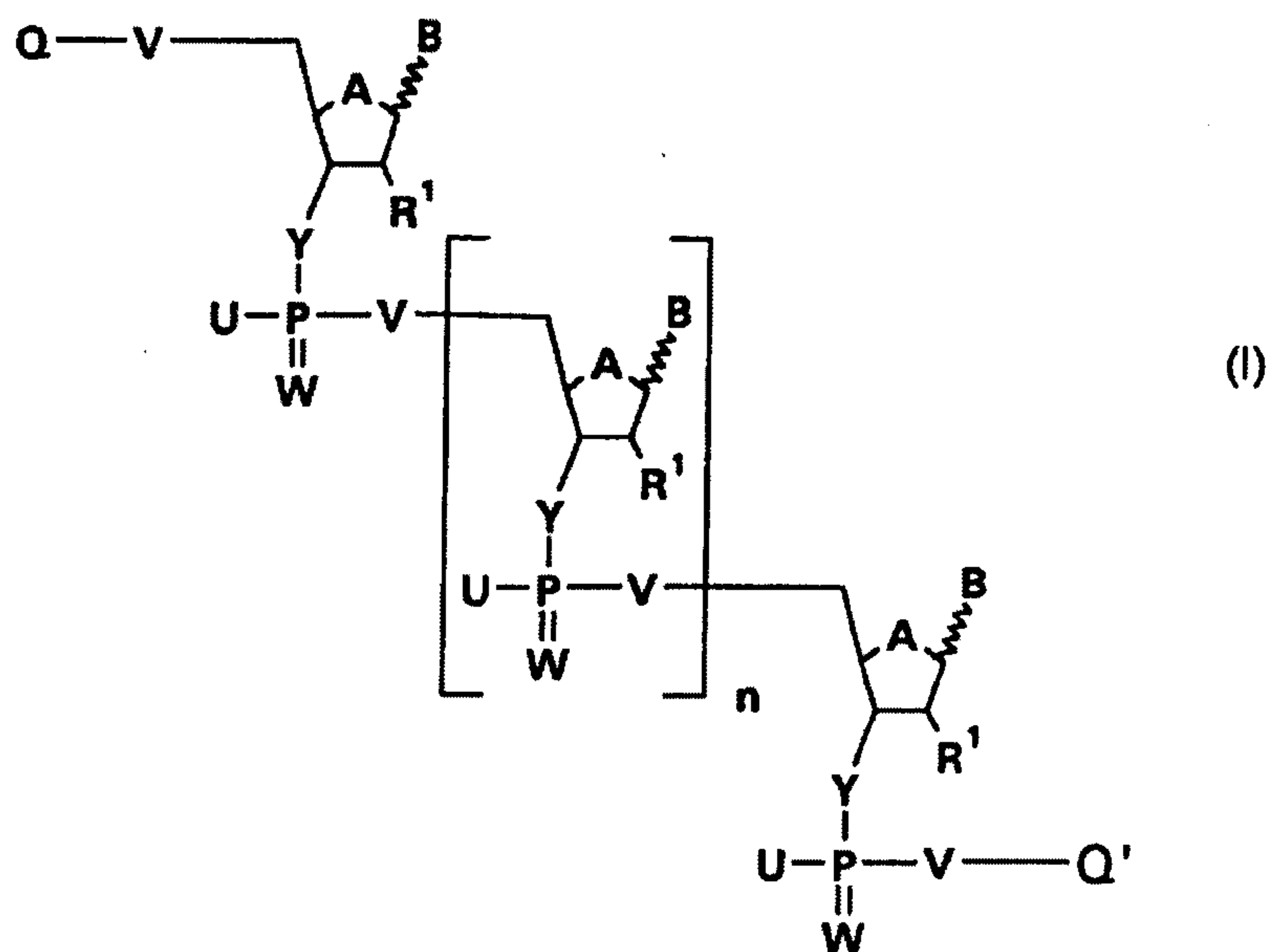
2. The process as claimed in claim 1, wherein the oligonucleotides, which are bound to the solid support, are deprotected, at from 0 to 70°C and for from 0.1 to 16 h, with an 0.1 to 5 M solution of diazabicyclo[5.4.0]undec-7ene (DBU).

20 3. The process as claimed in claim 1 or 2, wherein the oligonucleotides, which are bound to the solid support, are deprotected, at from 10 to 40°C and for from 0.1 to 2 h, with an 0.3 to 3 M solution of DBU.

25 4. The process as claimed in any one of claims 1 to 3, wherein the oligonucleotides, which are bound to the solid support, are deprotected, at from 20 to 30°C and for from 0.2 to 1.5 h, with an 0.5 to 2.5 M solution of DBU.

5. The process as claimed in any one of claims 1 to 4 for preparing a compound of the formula I

30



in which

- 5 R^1 is, independently at each occurrence, hydrogen, hydroxyl, C_1 - C_{18} -alkoxy, O-allyl, halogen, azido or amino, wherein C_1 - C_{18} -alkoxy is optionally substituted one to three times by hydroxyl, C_1 - C_4 -alkoxy, or C_1 - C_4 -alkyl-O-(CH_2CH_2O)_s, in which s is a number from 1-3;
- 10 A is, independently at each occurrence, oxy, thioxy or methylene;
- W is, independently at each occurrence, oxo, thioxo or selenoxo;
- V is, independently at each occurrence, oxy, sulfanediyl or imino;
- 15 Y is, independently at each occurrence, oxy, sulfanediyl, imino or methylene;
- B is a base which is customary in nucleotide chemistry, with at least one B being a base which possesses an exocyclic amino group;

n is an integer from 1 to 100;

U is, independently at each occurrence, hydroxyl, mercapto, BH₃, SeH, C₁-C₁₈-alkoxy, C₆-C₂₀-aryl, (C₆-C₁₄)-aryl-(C₁-C₈)-alkyl, NHR³, NR³R⁴ or a radical of the formula II



in which

R³ is C₁-C₁₈-alkyl, C₆-C₂₀-aryl, (C₆-C₁₄)-aryl-(C₁-C₈)-alkyl, or $-(\text{CH}_2)_c-[\text{NH}(\text{CH}_2)_c]_d-\text{NR}^5\text{R}^5$, in which c is an integer from 2 to 6 and d is an integer from 0 to 6;

R⁵ is, independently at each occurrence, hydrogen, C₁-C₆-alkyl or C₁-C₄-alkoxy-C₁-C₆-alkyl;

R⁴ is C₁-C₁₈-alkyl, C₆-C₂₀-aryl or (C₆-C₁₀)-aryl-(C₁-C₈)-alkyl, or, in the case of NR³R⁴, is, together with R³ and the nitrogen atom carrying them, a 5-6-membered heterocyclic ring which can additionally contain a further heteroatom selected from the group O, S and N;

p is an integer from 1 to 100;

q is an integer from 0 to 22;

R² is hydrogen, hydroxyl, amino, NHR⁶, COOH, CONH₂, COOR⁷ or halogen, in which

R⁶ is C₁-C₄-alkyl, and

R⁷ is C₁-C₄-alkyl;

Q and Q' are, independently of each other, hydrogen or conjugates wherein the conjugate has a favorable effect on the properties of antisense oligonucleotides or of triple helix-forming oligonucleotides, or are used as the label for a DNA probe, or, in association with hybridization of an oligonucleotide analog to the target nucleic acid, attack the latter while binding or cross-linking;

where

R¹ and the adjacent phosphoryl radical can be located either in the 2' and 3' positions or, conversely, in the 3' and 2' positions;

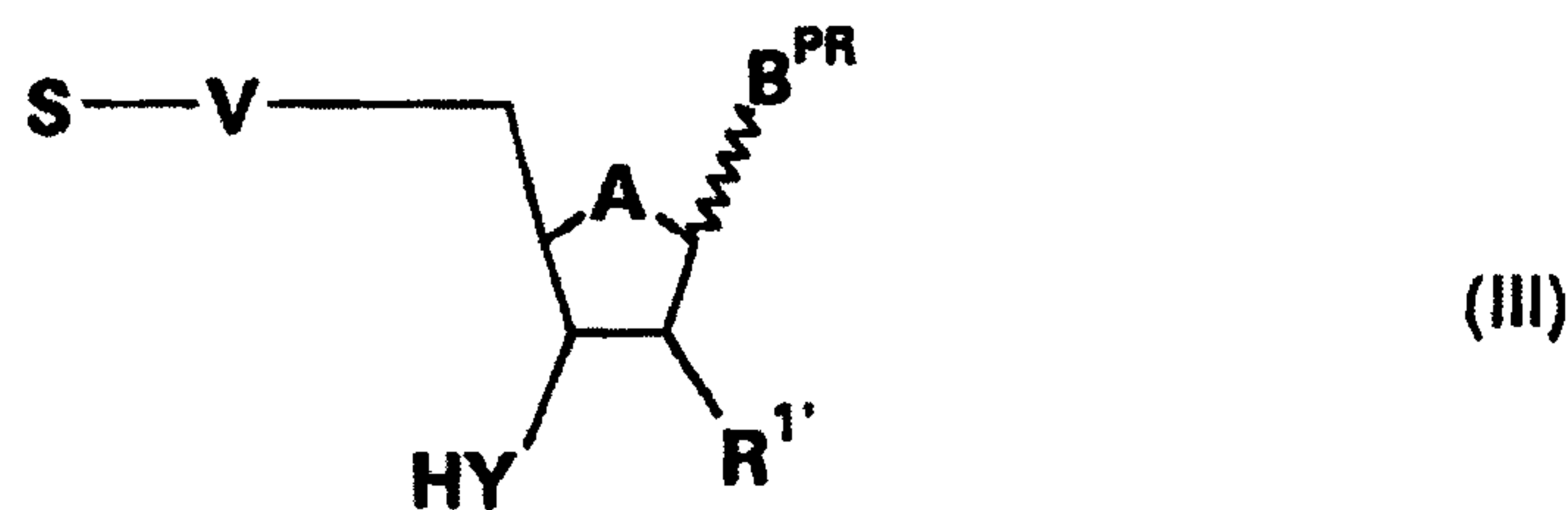
each nucleotide can be present in its D or L configuration;

the base B can be located in the alpha or beta position, the oligonucleotide optionally contains 3'-3'- or 5'-5' inversions,

wherein

15

(a) a compound of the formula III



20 in which A, Y and V are defined as above, and

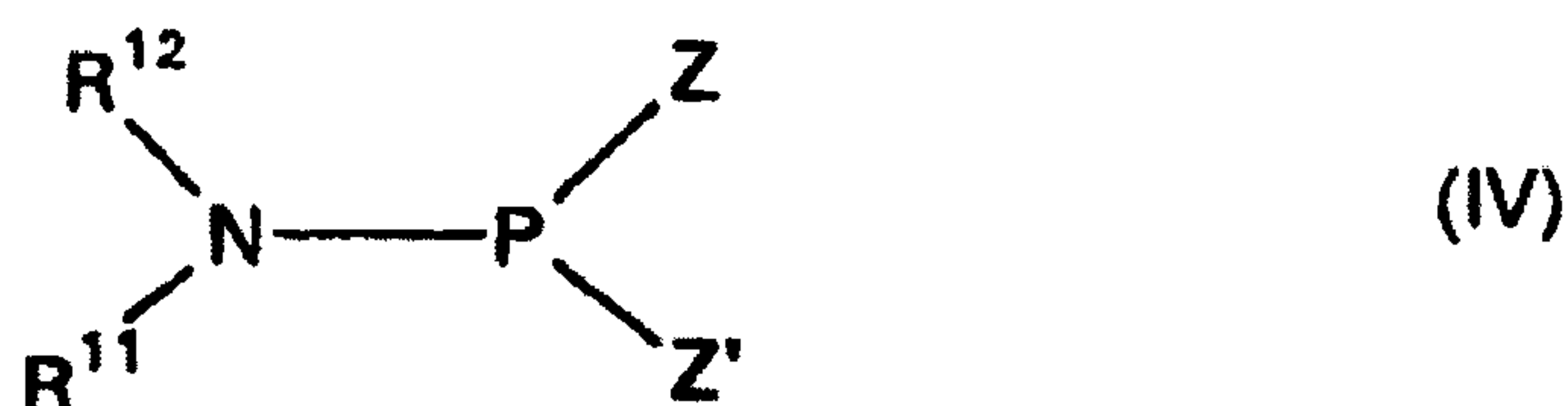
R^{1'} is defined as R¹ except that, when R¹ is hydroxyl or amino, R^{1'} is a correspondingly protected group;

25 S is a 5' protecting group which can be eliminated under acid conditions;

B^{PR} is a natural or unnatural nucleobase in which any exocyclic amino groups which may be present are protected by a cyclic diacyl group;

is reacted, in accordance with known methods, with a compound of the formula IV

5



in which

Z' is OR^{13} or C_1-C_{18} -alkyl, C_1-C_{18} -alkoxy, C_6-C_{20} -aryl or C_6-C_{14} -aryl- C_1-C_8 -alkyl;

10

R^{11} and R^{12} are identical or different and are

C_1-C_8 -alkyl, or C_5-C_{12} -cycloalkyl, together with the nitrogen atom to which they are bonded or form a saturated or unsaturated heterocyclic ring optionally having additional heteroatoms selected from O, S and N and substituents selected from $OC(O)OC_1-C_4$ -alkyl esters;

15

R^{13} is a protecting group which can be eliminated with strong, nonnucleophilic bases,

Z is chlorine or bromine or a radical of the formula $NR^{11}R^{12}$, where R^{11} and R^{12} are defined as above;

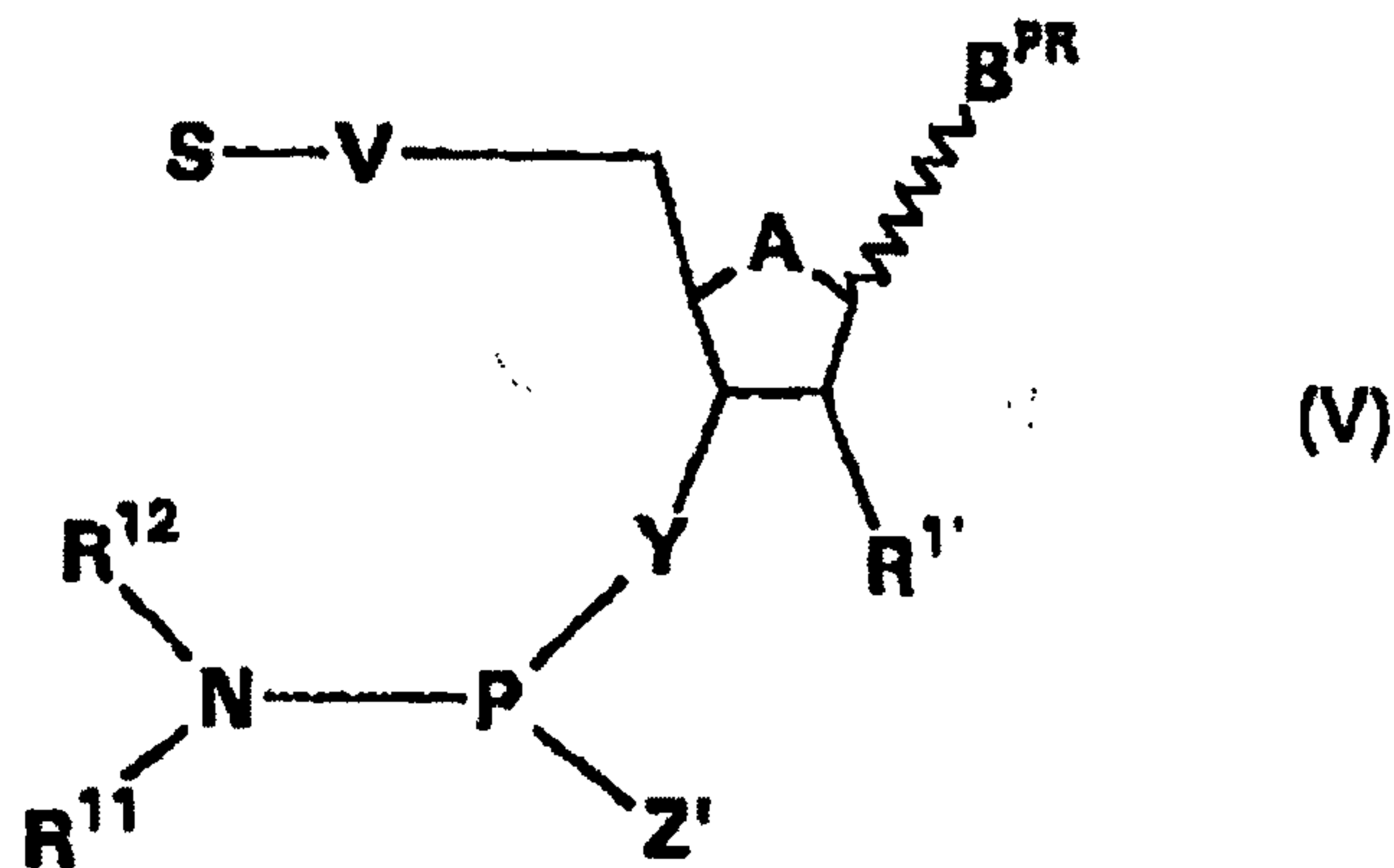
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in the presence of a base, or, when Z is a radical of the formula $NR^{11}R^{12}$, then in the presence of a compound of the formula $[HNR^{14}R^{15}R^{16}]^{(+)} A^{(-)}$,

25

where R^{14} , R^{15} and R^{16} are identical or different and are a C_1-C_4 -alkyl group and A is fluorine, chlorine or bromine;

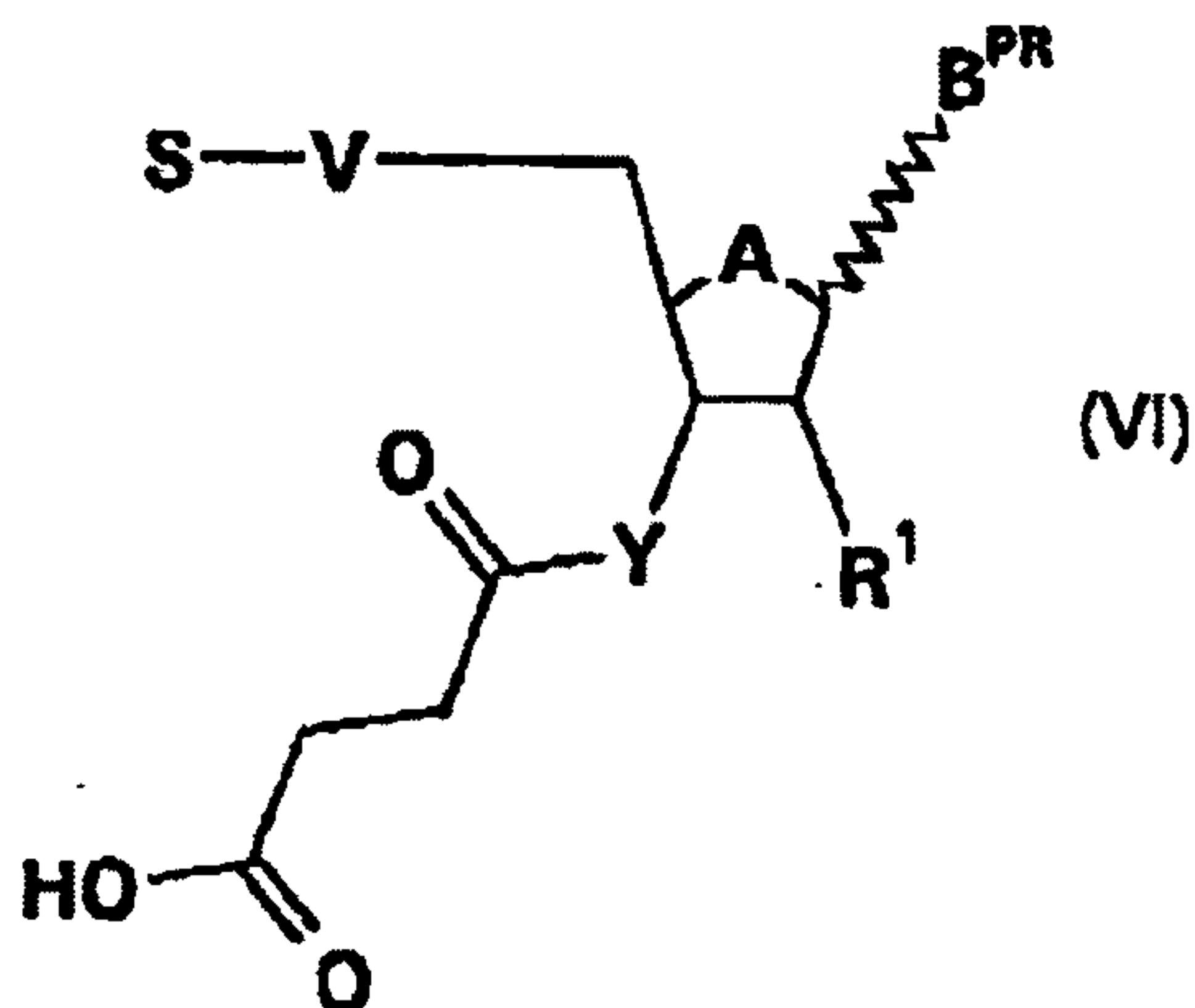
or tetrazole or 5-(C_1-C_4 -alkylthio)-1H-tetrazole, to form a compound of the formula V



in which S, V, Y, A, B^{PR}, Z', R¹, R¹¹ and R¹² are as defined above;

5

- (b) compounds of the formula III are reacted, in accordance with known methods, with from 1 to 10 equivalents, of a linker in a suitable organic solvent, optionally after adding a catalyst, to give a compound of the formula VI

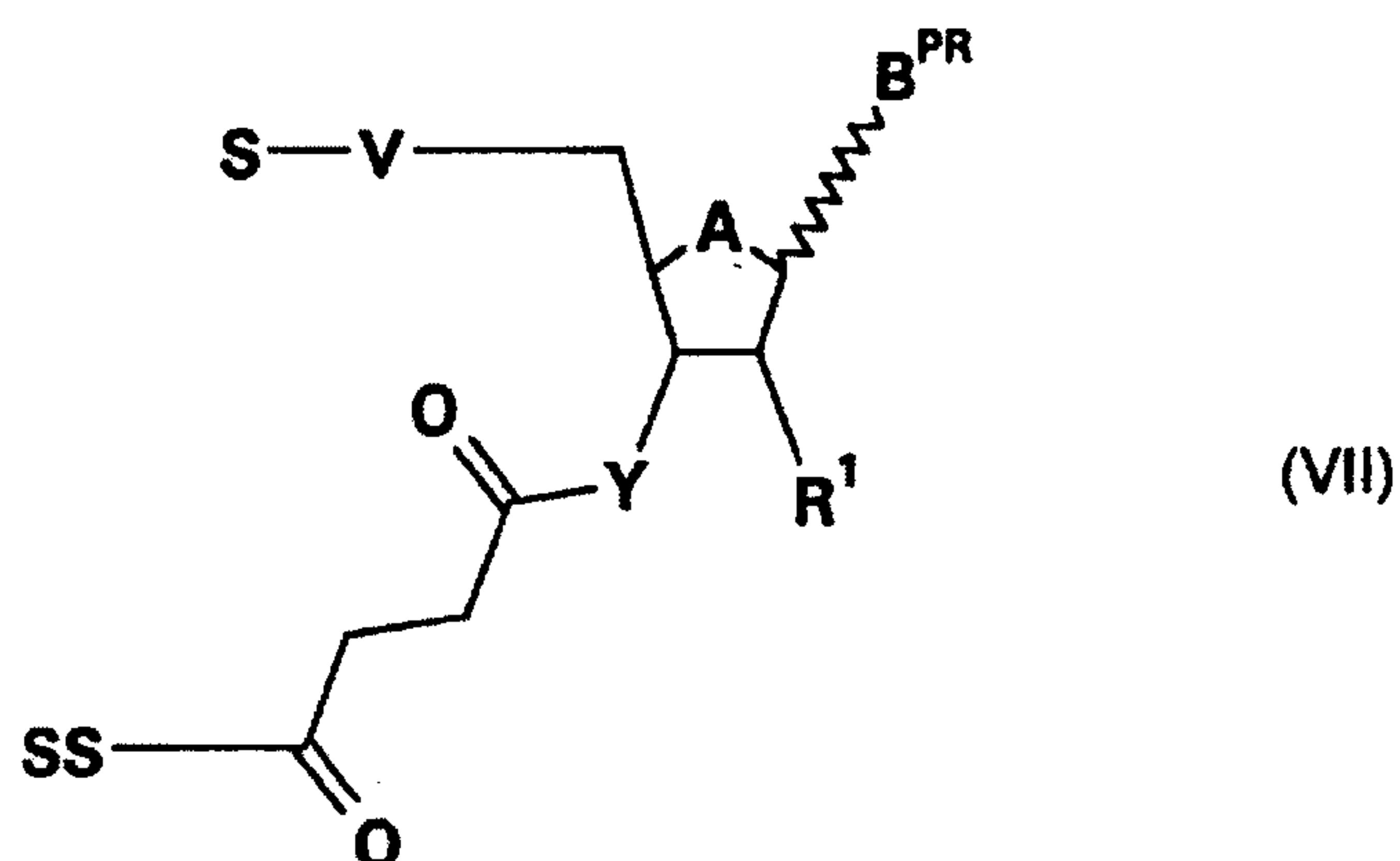


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where S, V, Y, A, B^{PR}, and R¹ are defined as above, and subsequently purified by extraction, crystallization or chromatography, with the succinic acid residue in the 3' position serving as the linker to a polymer support which is employed in the synthesis;

15

- (c) the compound of the formula VI is coupled, in accordance with known methods, to a solid support SS by reacting in a suitable solvent, with compounds of formula VII being obtained;



5

- (d) the 5' protecting group is eliminated from VII in accordance with known methods;
- 10 (e) the resulting compound is reacted with a compound of the formula V in a suitable organic solvent in the presence of a compound of the formula $[\text{HNR}^{14}\text{R}^{15}\text{R}^{16}]^{(+)} \text{A}^{(-)}$, which is defined as above, or tetrazole, 5-(C₁-C₄-alkylthio)-1H-tetrazole or 5-(C₆-C₁₂-aryl)-1H-tetrazole, and
- 15 (f) the resulting compound is oxidized in accordance with known methods, optionally also in the presence of additional organic solvents,
- (g) the reaction steps d – f are repeated until the desired chain length has been obtained;
- 20 (h) the compound which has been obtained in this way is deprotected by treating with diazabicyclo[5.4.0]undec-7ene (DBU) in a suitable organic solvent; and

(i) the oligonucleotide is cleaved from the support in accordance with known methods.

5 6. The process of claim 5 wherein U is independently at each occurrence C₁-C₆ alkoxy or C₁-C₁₈ alkyl.

7. The process of claim 6 wherein U is C₁-C₆ alkyl.

8. The process of claim 5 wherein R³ is C₁-C₈ alkyl.

10

9. The process of claim 5 wherein R⁵ is methoxyethyl.

10. The process of claim 5 wherein R⁴ is C₁-C₈ alkyl.

15

11. The process of claim 10 wherein R⁴ is C₁-C₄ alkyl.

12. The process of claim 5 wherein p is from 3 to 10.

13. The process of claim 5 wherein q is from 0 to 15.

20

14. The process of claim 5 wherein R⁷ is methyl.

15. The process of claim 5 wherein Z' is OR¹³, C₁-C₆-alkyl, C₁-C₆-alkoxy, C₆-C₂₀-aryl or C₆-C₁₄-aryl-C₁-C₈-alkyl.

25

16. The process of claim 15 wherein Z' is OR¹³.

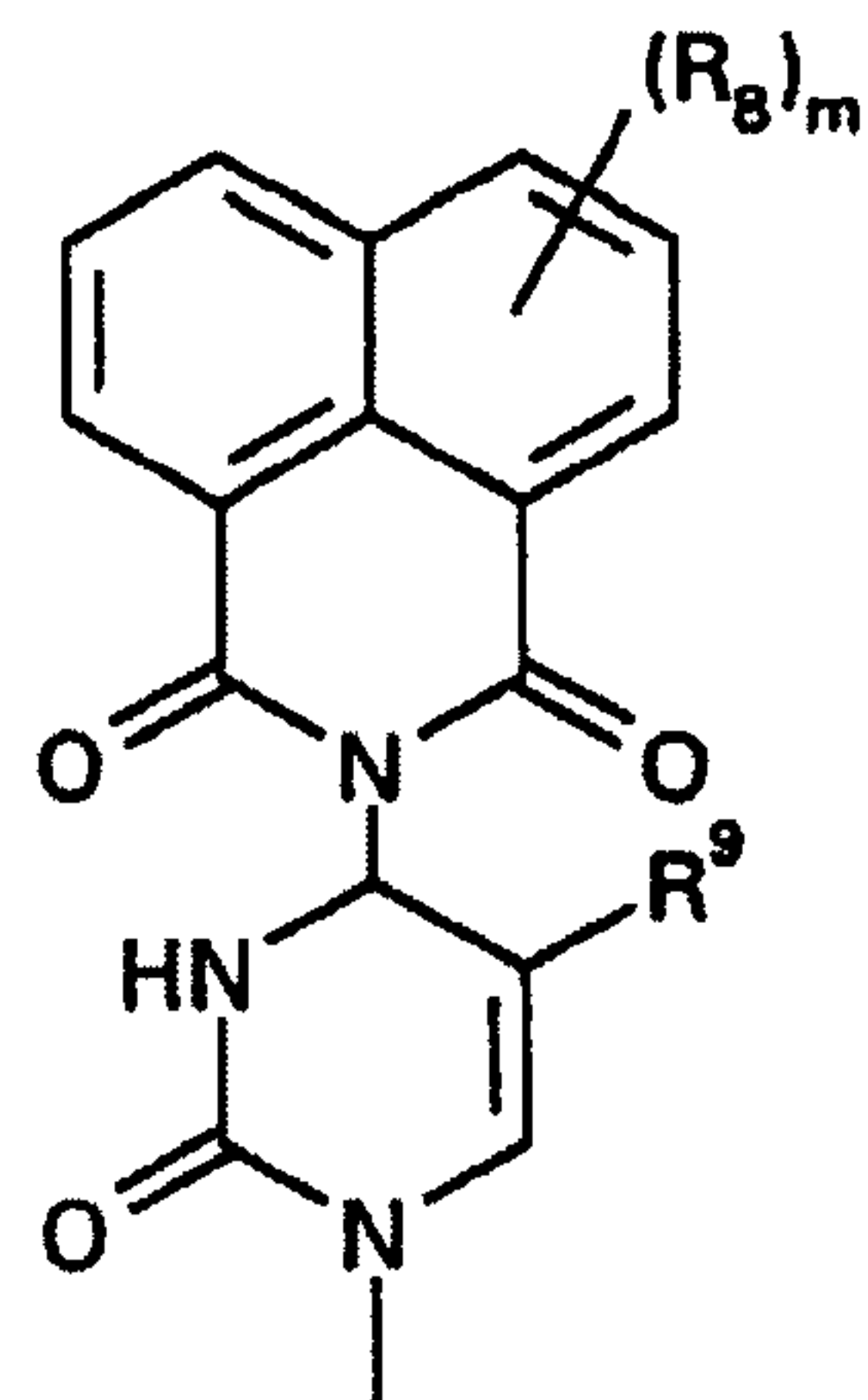
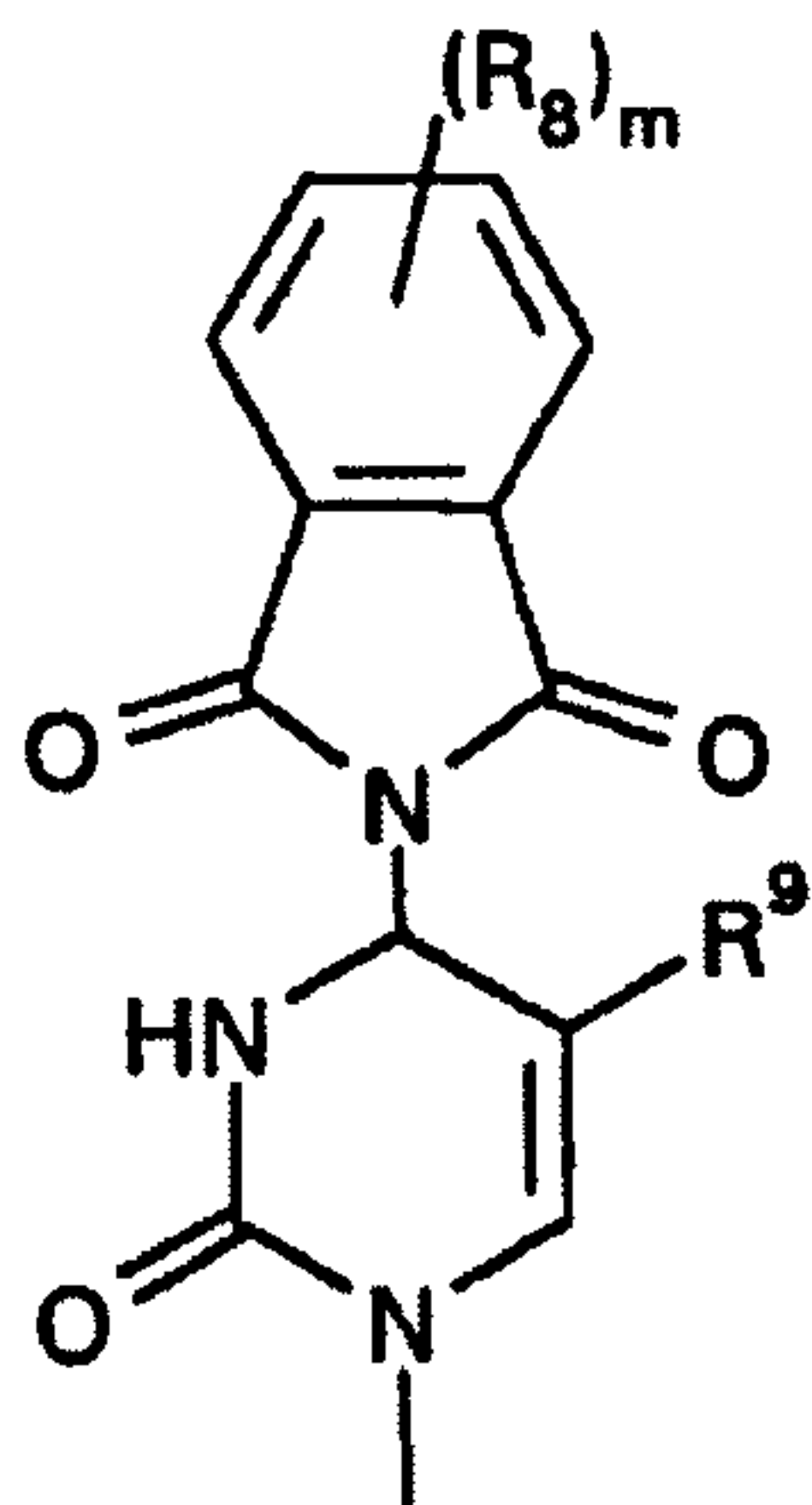
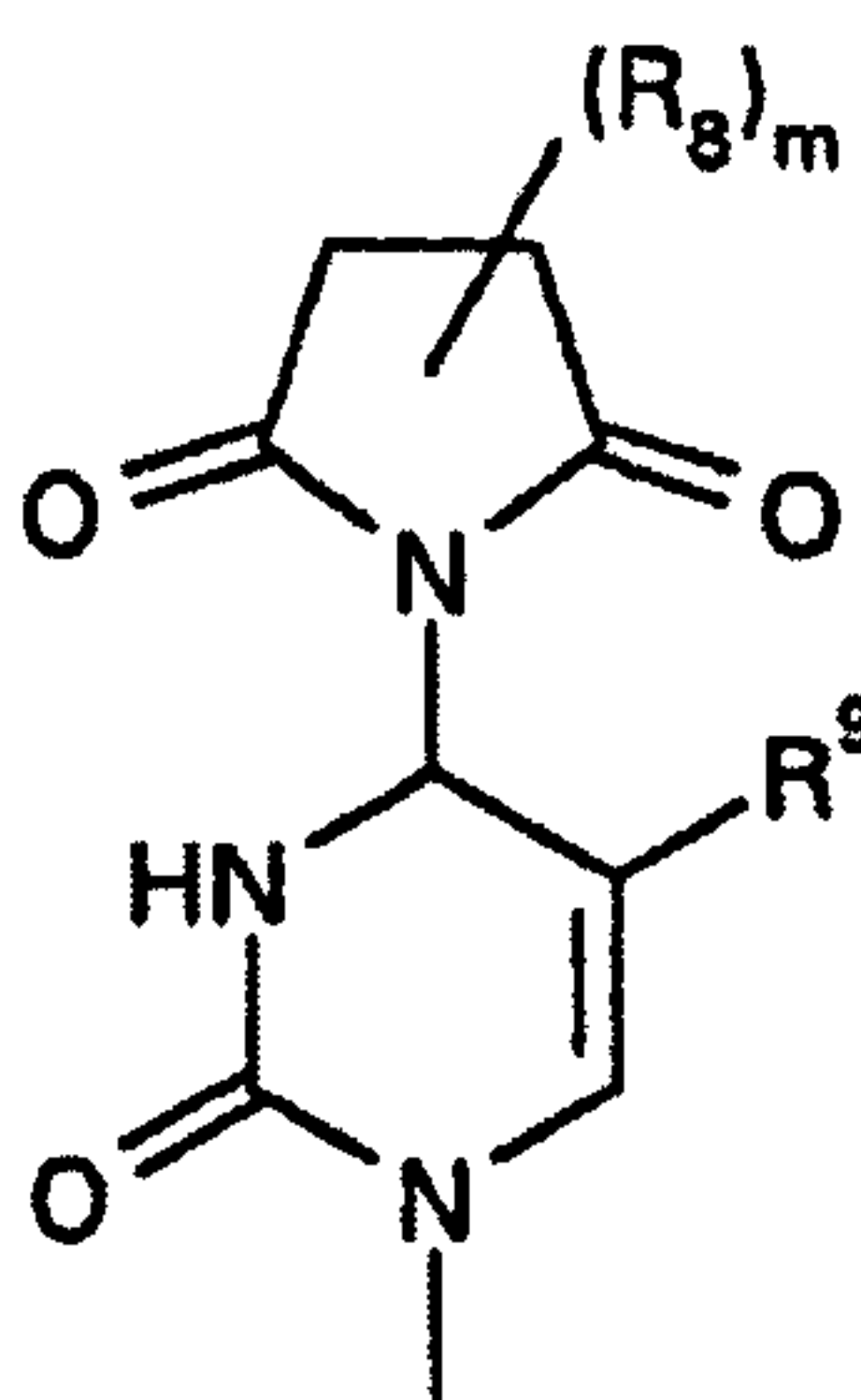
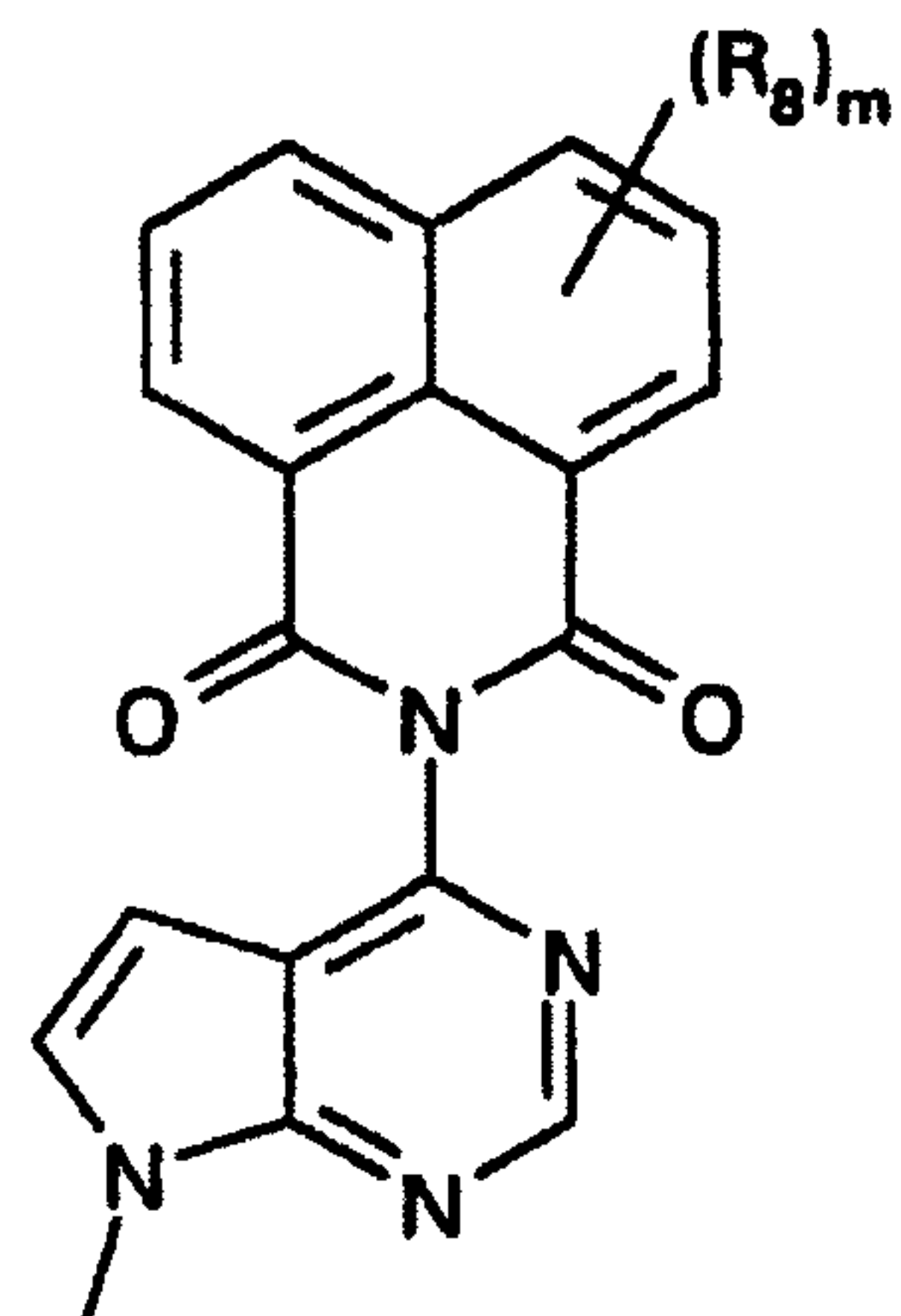
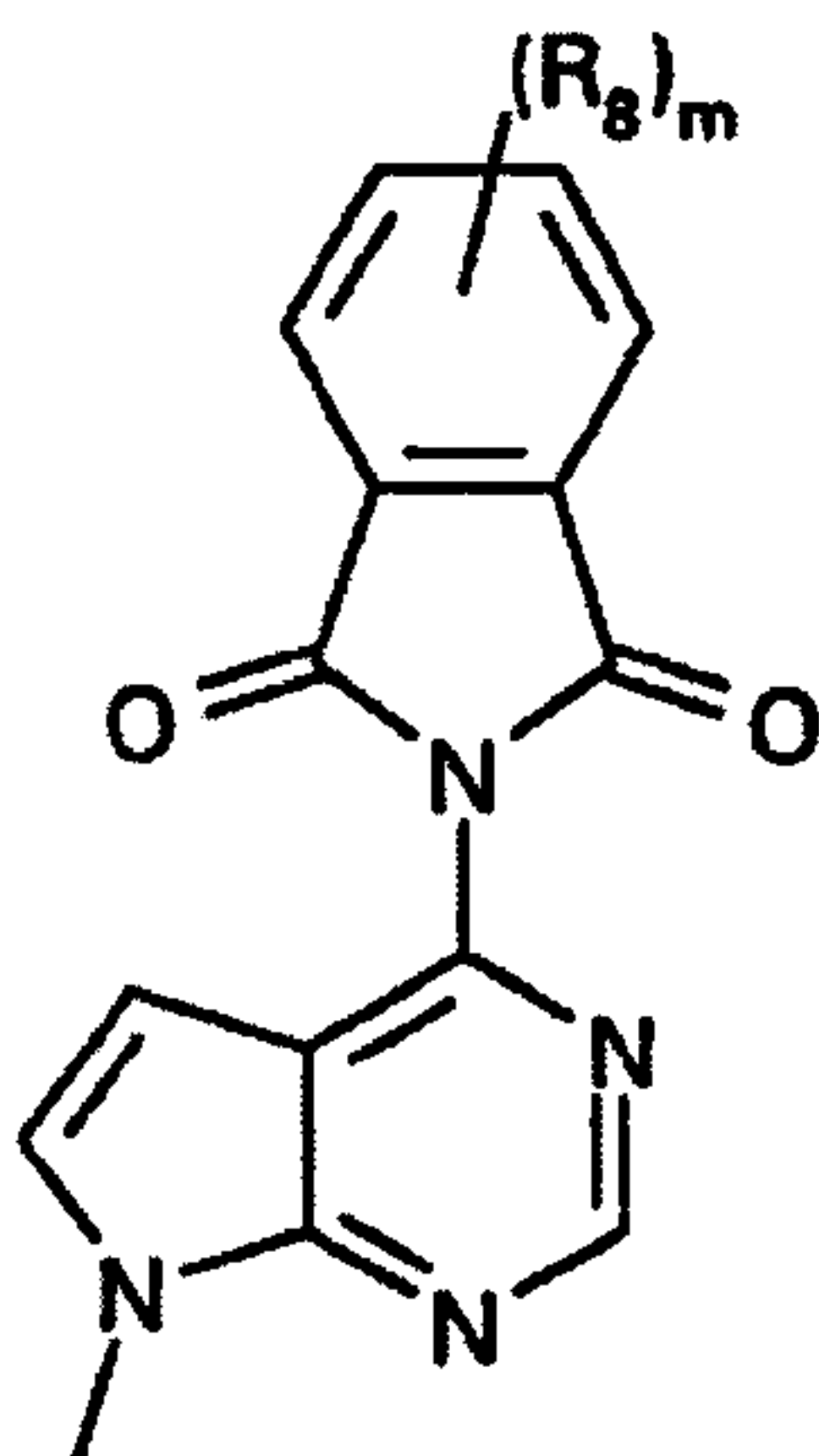
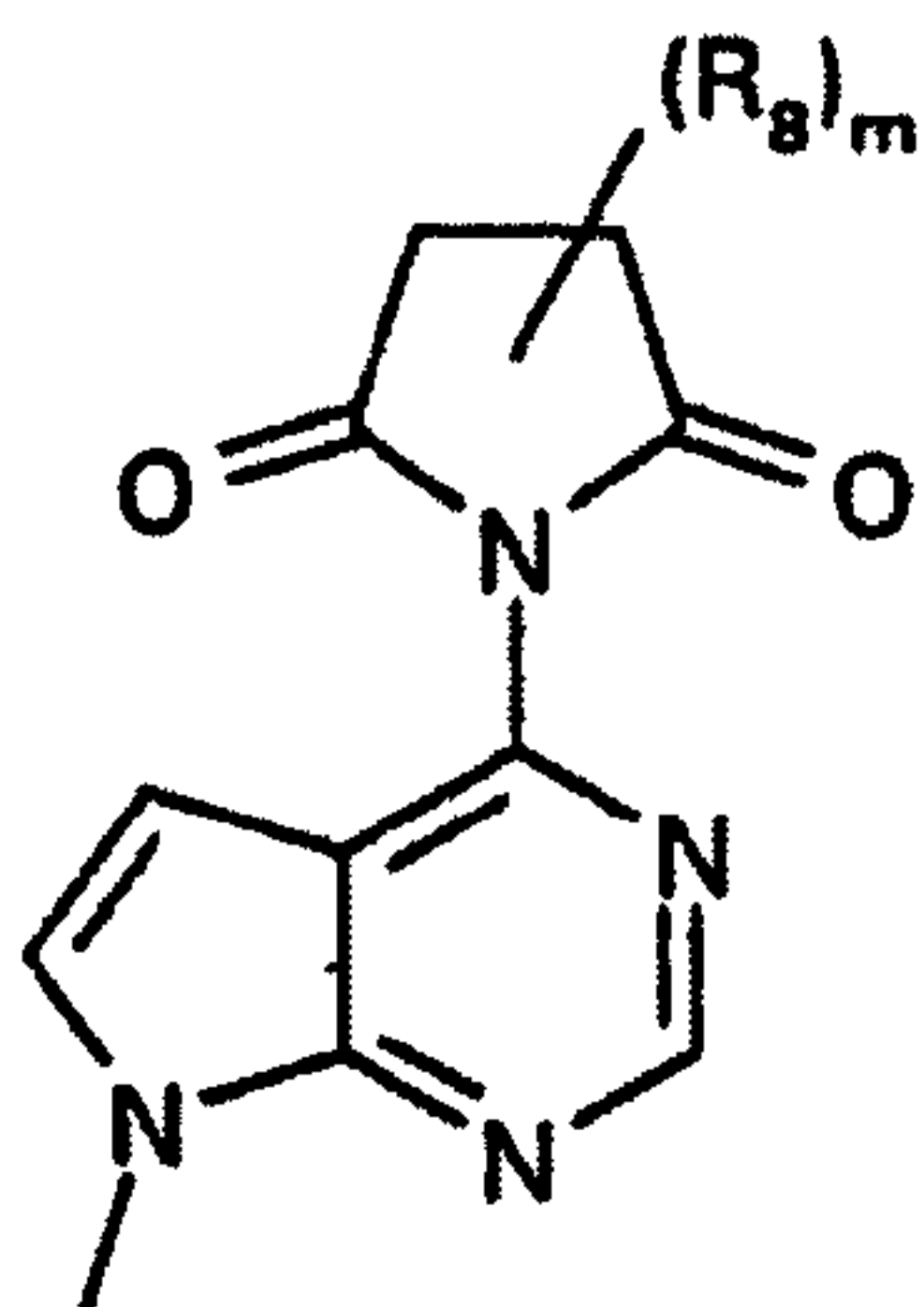
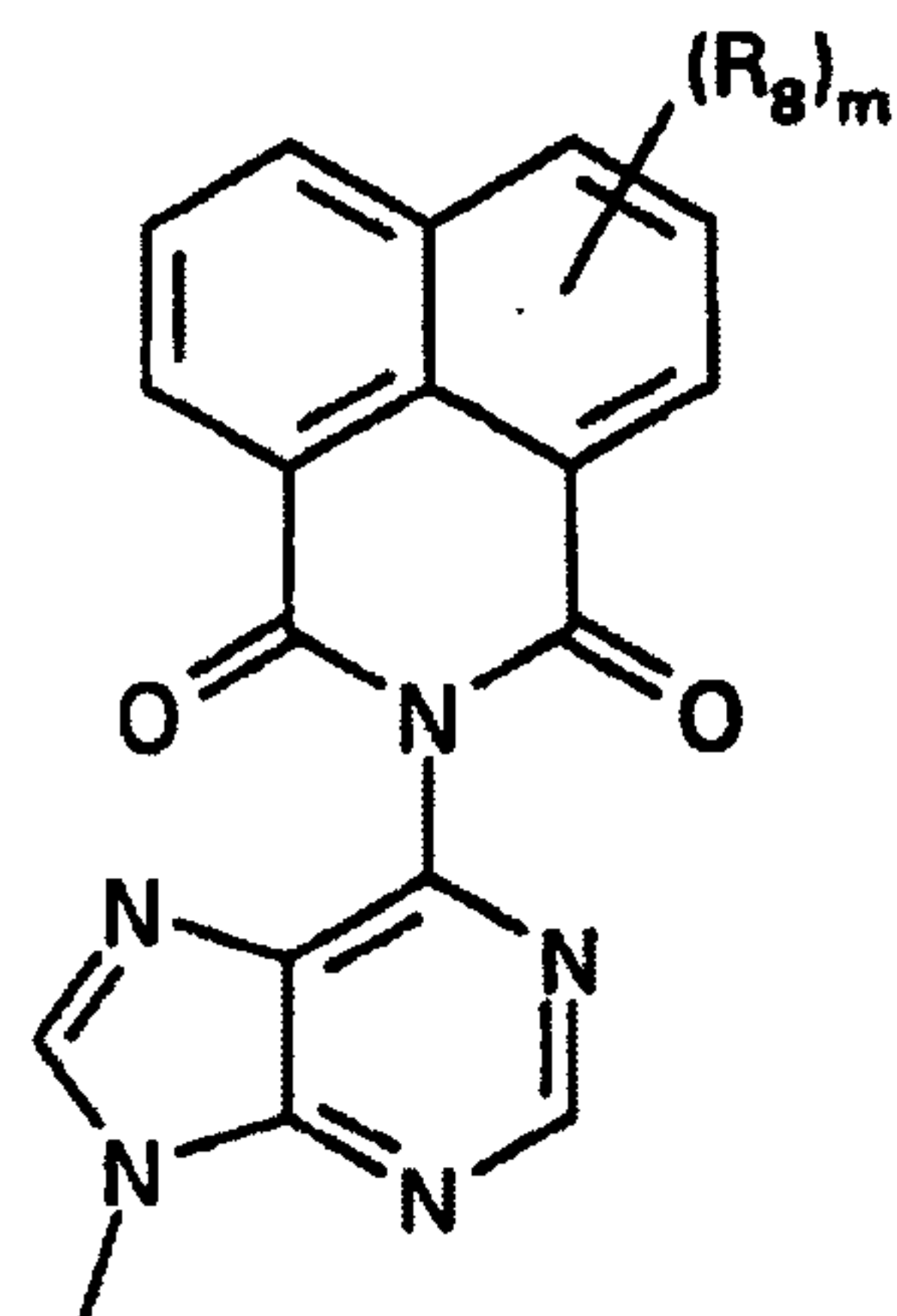
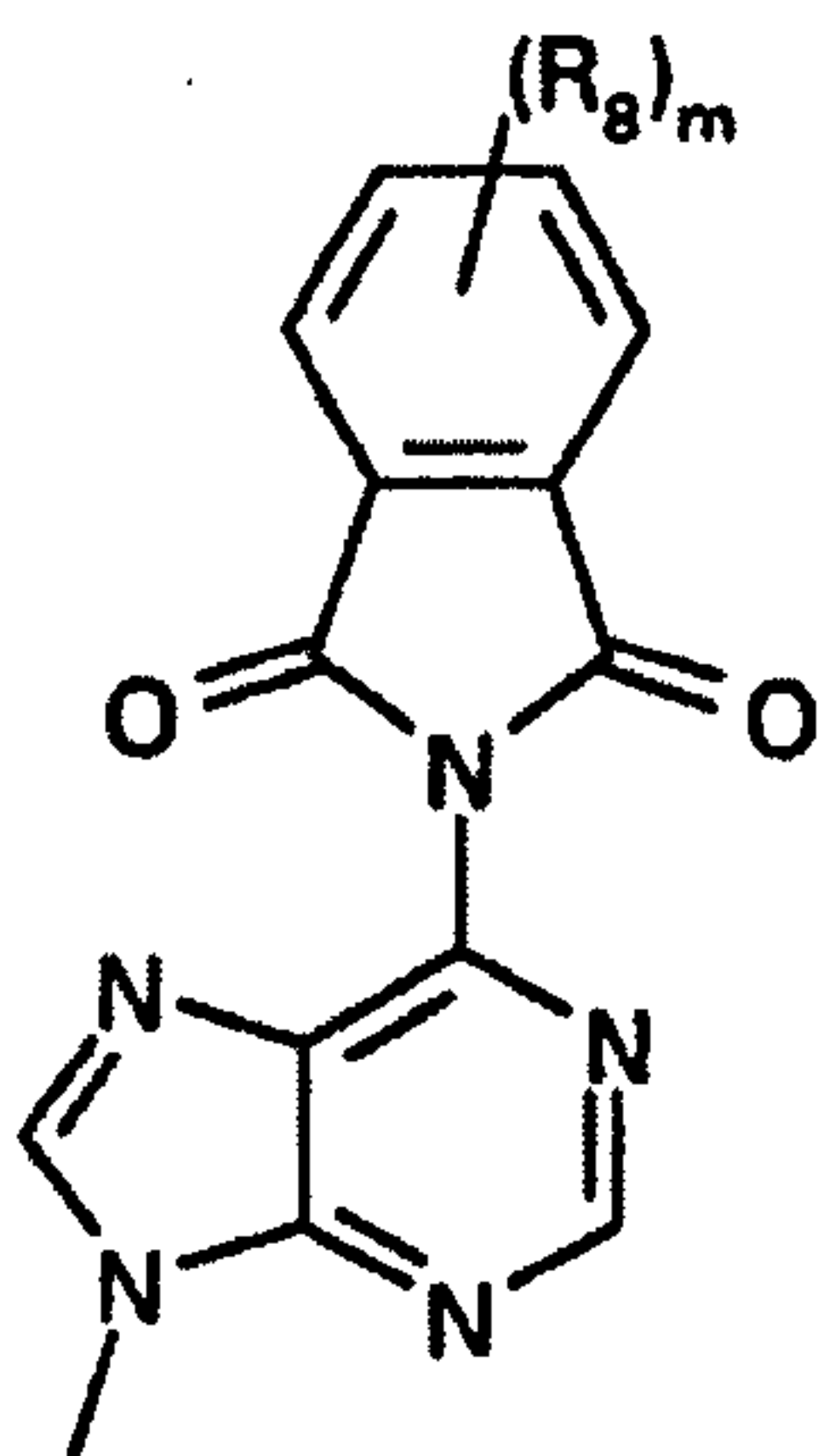
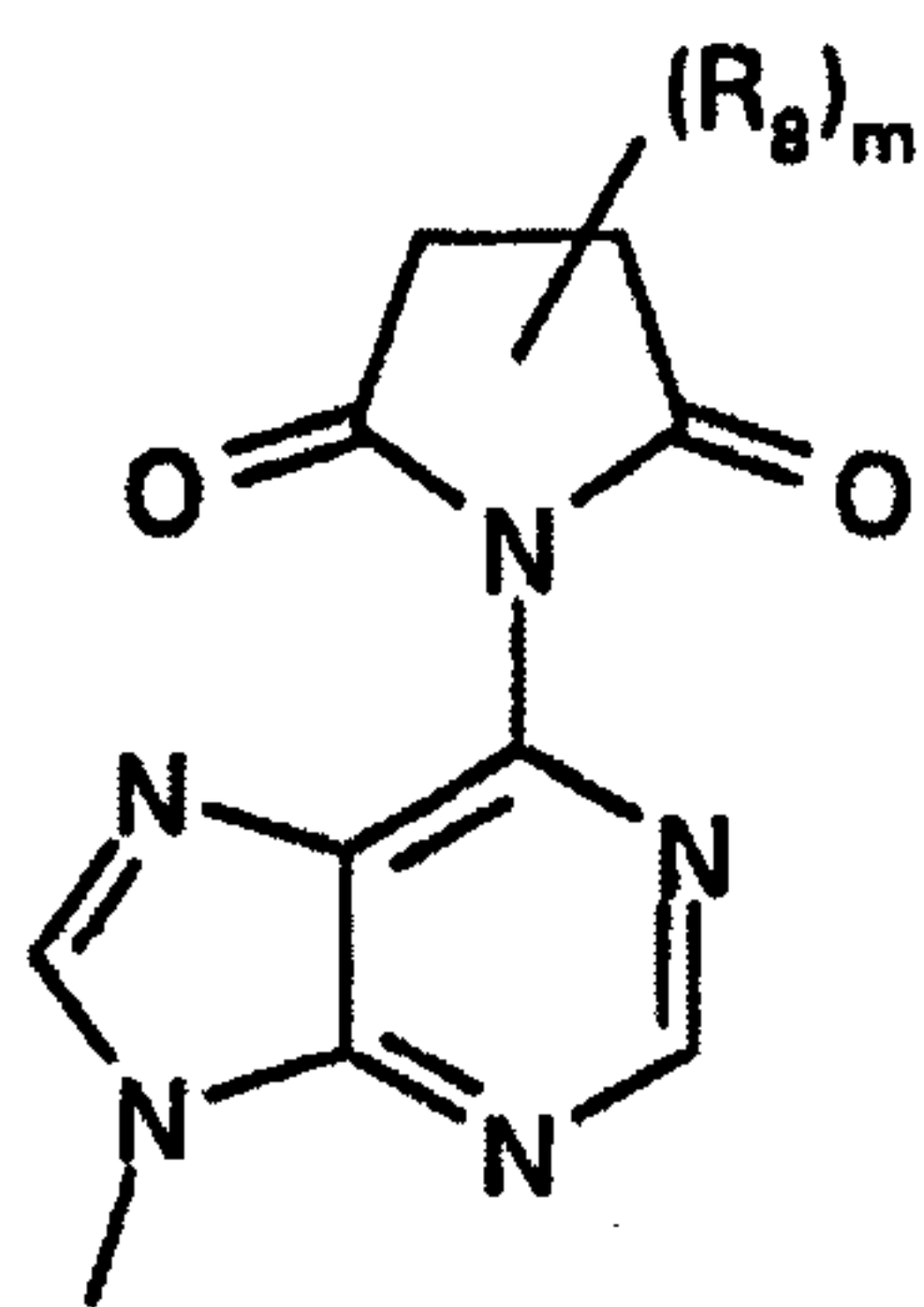
17. The process of claim 5 wherein R¹¹ and R¹² are identical or different and are selected from isopropyl, C₅-C₈-cycloalkyl, benzyl and phenyl.

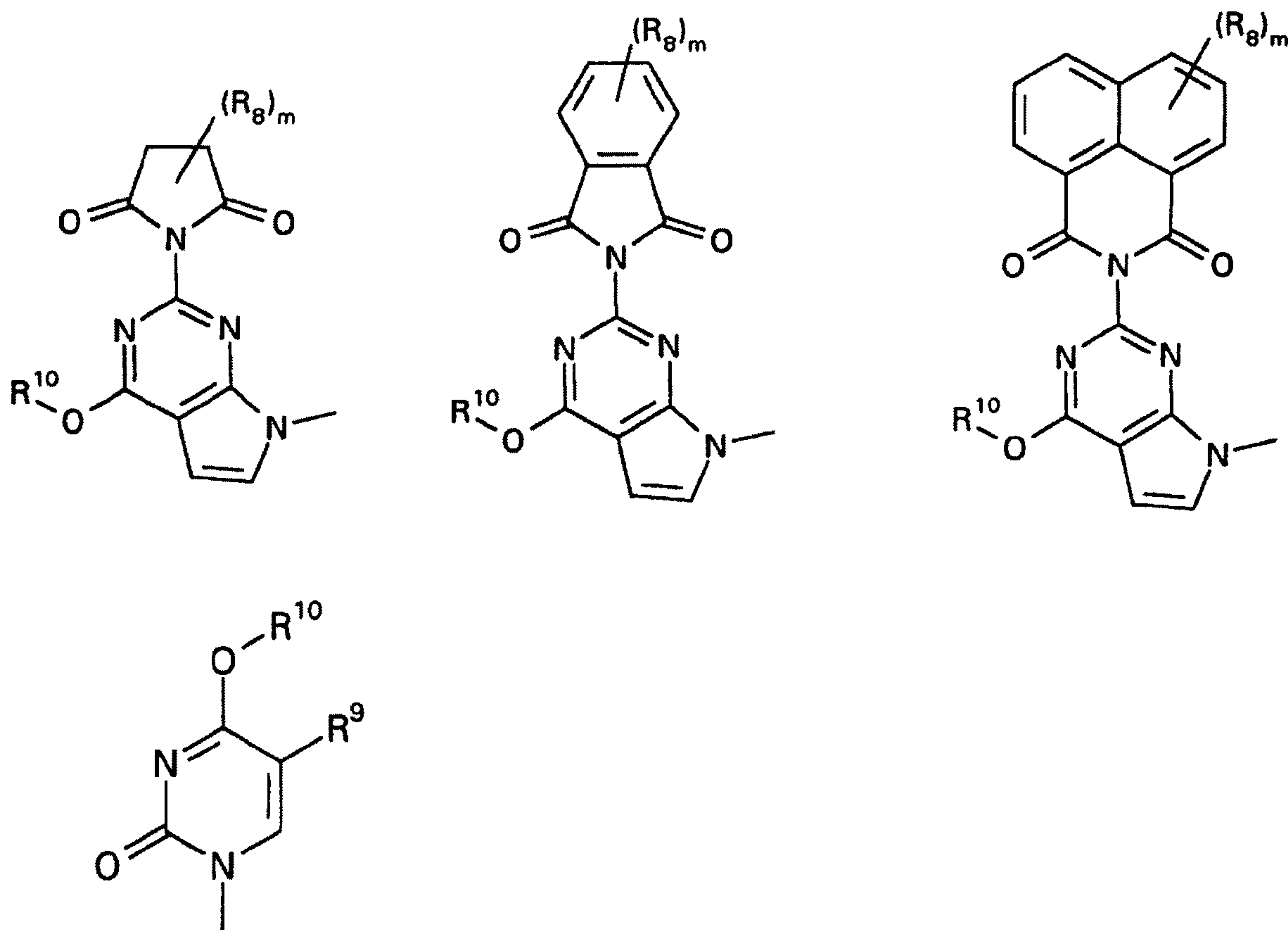
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18. The process of claim 5 wherein the base present in step (a) is pyridine or a mixture of one or more of tetrahydrofuran (THF), dioxane, dichloromethane (DCM), chloroform and acetonitrile with a C₁-C₄-trialkylamine.
- 5 19. The process of claim 18 wherein the C₁-C₄-trialkylamine is trimethylamine, triethylamine or diisopropylamine.
20. The process of claim 5 wherein R¹¹ and R¹² together with the nitrogen to which they are bonded form morpholine, optionally substituted with OC(O)O-C₁-C₄ alkyl esters.
- 10
21. The process of claim 5 wherein A is chlorine.
22. The process of claim 5 wherein step (a) is in the presence of tetrazole or pyridine hydrochloride.
- 15
23. The process of claim 5 wherein in step (b) the compounds of formula III are reacted with 1 to 2 equivalents of a linker.
- 20 24. The process of claim 5 wherein in the reaction in step (e) the organic solvent is acetonitrile.
25. The process of claim 5 wherein the reaction in step (e) is carried out in the presence of tetrazole or pyridine hydrochloride.
- 25
26. The process as claimed in claim 5, wherein unreacted compounds from step (d) are deactivated by means of a capping step.
27. The process as claimed in any one of claims 5 to 26, wherein, in compounds of the formulae (III), (V), (VI) and (VII), B^{PR} is, independently at each occurrence,
- 30

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in which

m is a number from zero to four,

5

R^8 is, independently at each occurrence, hydrogen, fluorine, chlorine, bromine, nitro, C_1 - C_4 -alkyl, C_1 - C_4 -alkoxy or CN,

10

R^9 is, independently at each occurrence, hydrogen, C_1 - C_6 -alkyl or C_2 - C_6 -(1-alkyne), and

R^{10} is, independently at each occurrence, hydrogen or a β -eliminatable protecting group.

15 28. The process of claim 27 wherein m is zero.

29. The process of claim 27, wherein R⁹ is 1-propynyl, 1-hexynyl or fluorine.

30. The process as claimed in any one of claims 5 to 29 for preparing a compound of the formula I in which

5

R¹ is hydrogen, hydroxyl, C₁-C₄-alkoxy or fluorine;

A is oxy;

10 W is oxo or thioxo;

V is oxy;

Y is oxy;

15

B is, independently at each occurrence, adenine, cytosine, guanine, uracil, thymine, 5-propyneuracil, 5-propynecytosine, 5-hexyneuracil or 5-hexynecytosine;

20 n is an integer from 5 to 40;

U is hydroxyl, mercapto, C₁-C₆-alkoxy, C₁-C₆-alkyl, NR³R⁴ or NHR³, in which

R³ is C₁-C₈-alkyl or methoxyethyl

25

R⁴ is C₁-C₈-alkyl, C₆-C₂₀-aryl or (C₆-C₁₀)-aryl-(C₁-C₈)-alkyl, or, in the case of NR³R⁴, is, together with R³ and the nitrogen atom carrying them, a 5-6-membered heterocyclic ring which can additionally contain a further heteroatom selected from the group O, S and N,

30

Q and Q' are hydrogen.

31. The process as claimed in claim 30, wherein

R¹ is hydrogen

5

n is an integer from 5 to 30

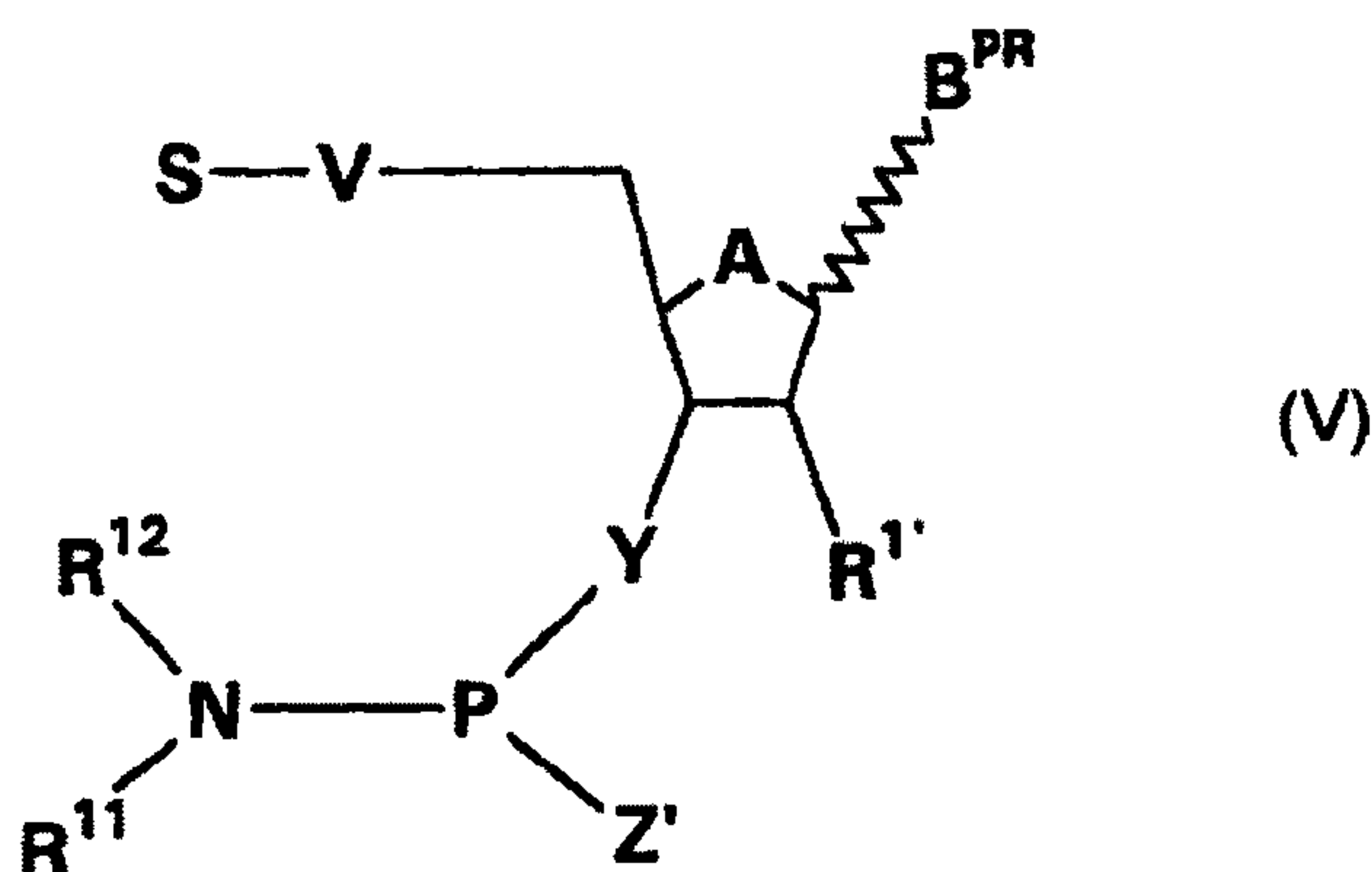
U is hydroxyl or C₁-C₆-alkyl

10 Q and Q' are hydrogen

and the remaining variables are defined as in claim 27.

32. A compound of the formula V

15



in which

20 R¹ is, independently at each occurrence, hydrogen, C₁-C₁₈-alkoxy, optionally substituted one to three times by hydroxyl, or C₁-C₄-alkoxy, C₁-C₄-alkyl-O-(CH₂CH₂O)₁₋₃, O-allyl, fluorine, chlorine, azido or a protected hydroxyl group or amino group;

A is oxy, thioxy or methylene;

V is oxy, sulfanediyl or imino;

5 Y is oxy, sulfanediyl, imino or methylene;

S is a 5' protecting group which can be eliminated under acid conditions;

10 B^{PR} is a natural or unnatural nucleobase having an exocyclic amino group, with the exocyclic amino groups being protected by a cyclic diacyl group;

Z' is OR¹³, C₁-C₁₈-alkyl, C₁-C₁₈-alkoxy, C₆-C₂₀-aryl or C₆-C₁₄-aryl-C₁-C₈-alkyl;

R¹¹ and R¹² are identical or different and are

15

C₁-C₈-alkyl or C₅-C₁₂-cycloalkyl, or, together with the nitrogen atom to which they are bonded, form a saturated or unsaturated heterocyclic ring optionally having additional heteroatoms selected from O, S and N and substituents selected from OC(O)O-C₁-C₄-alkyl esters; and

20

R¹³ is para-nitrophenylethyl or 2-cyanoethyl.

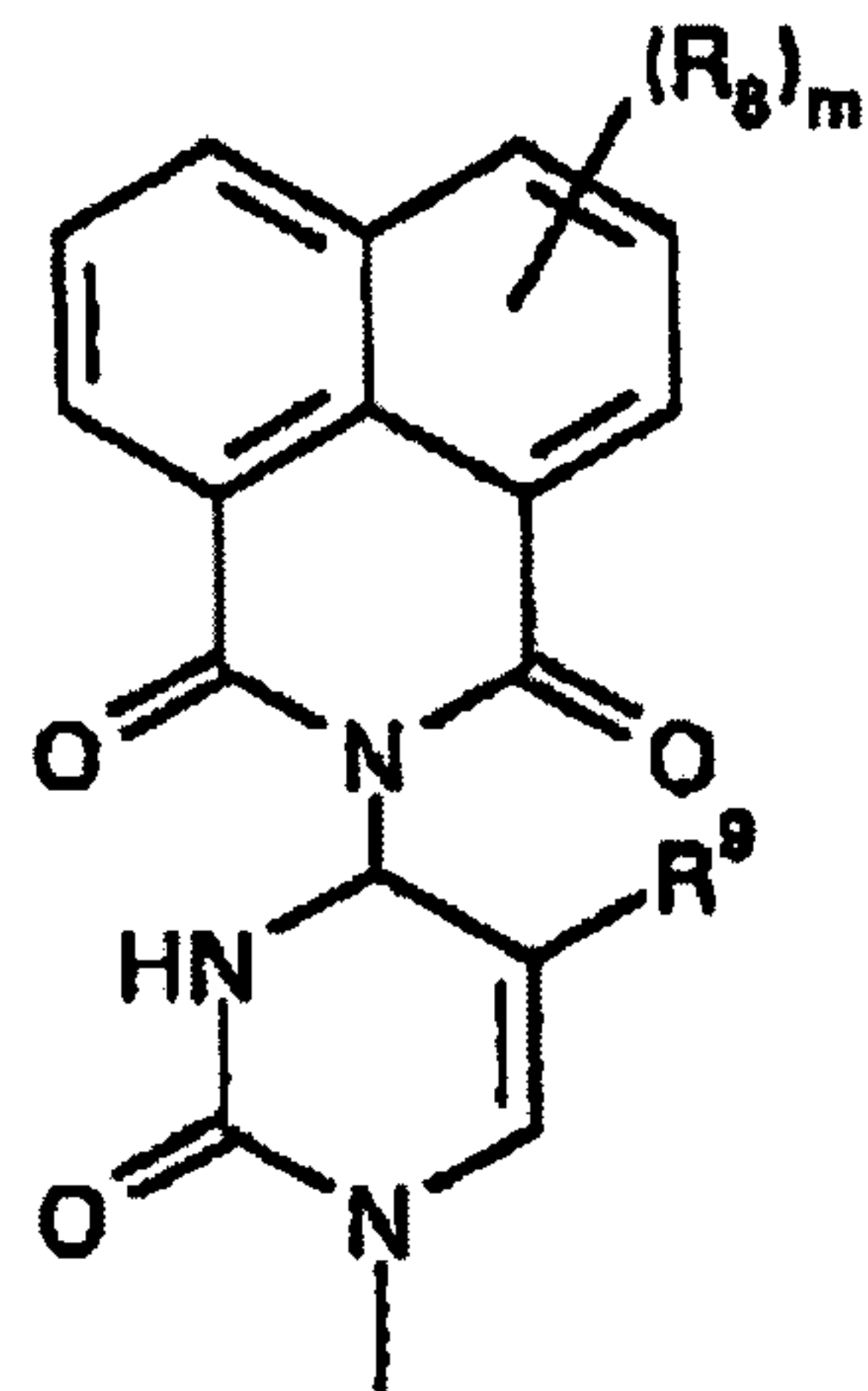
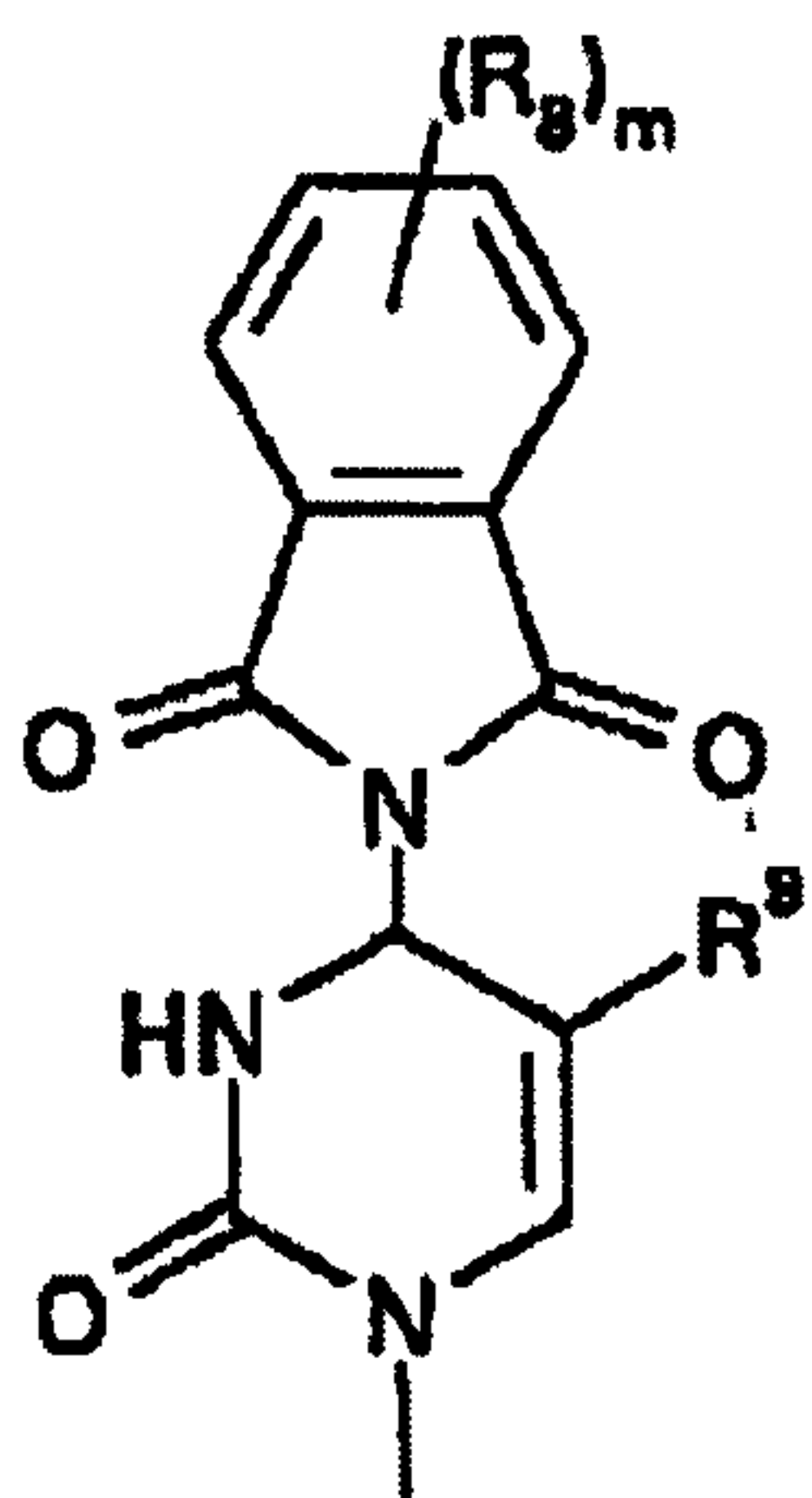
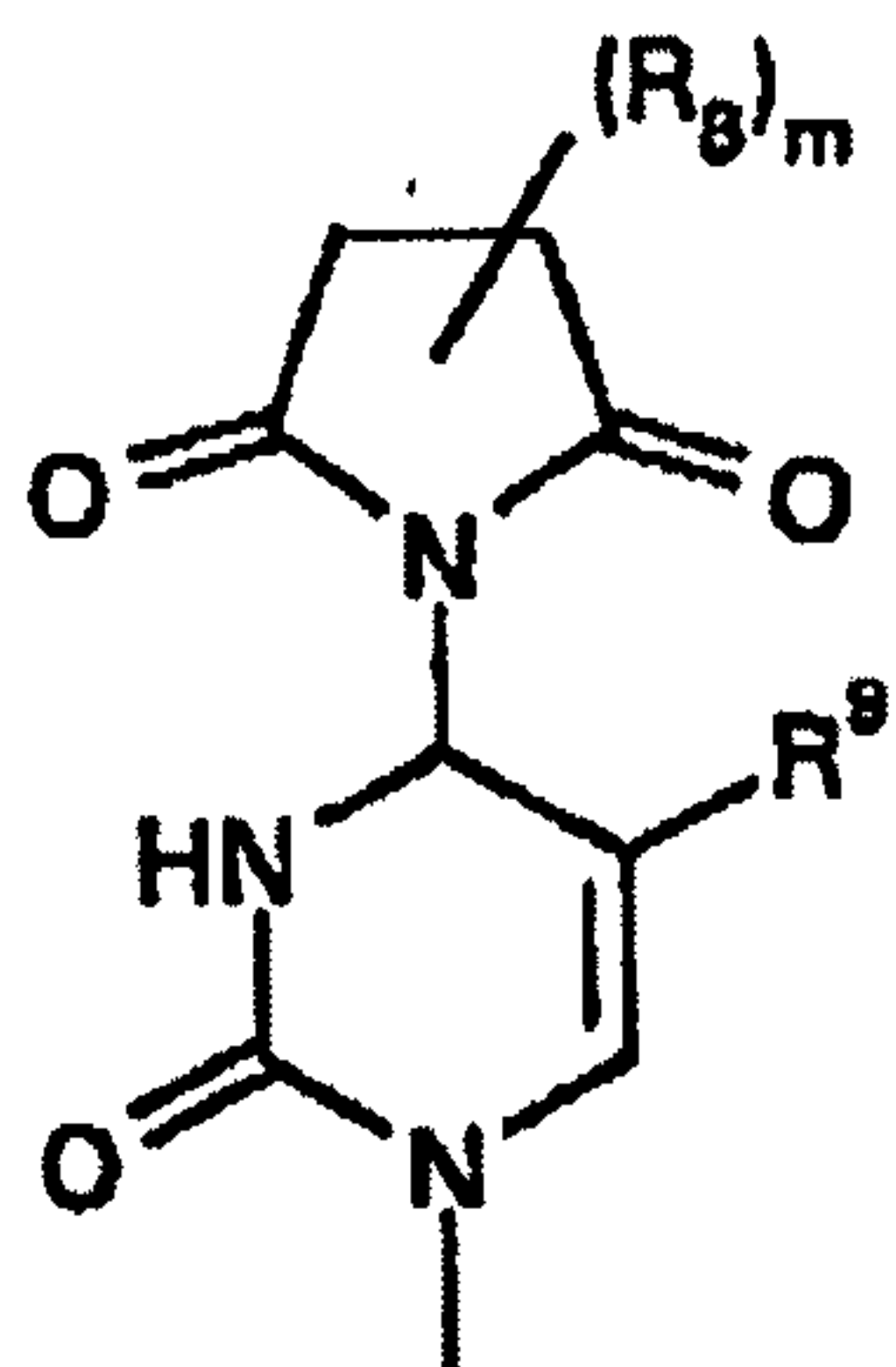
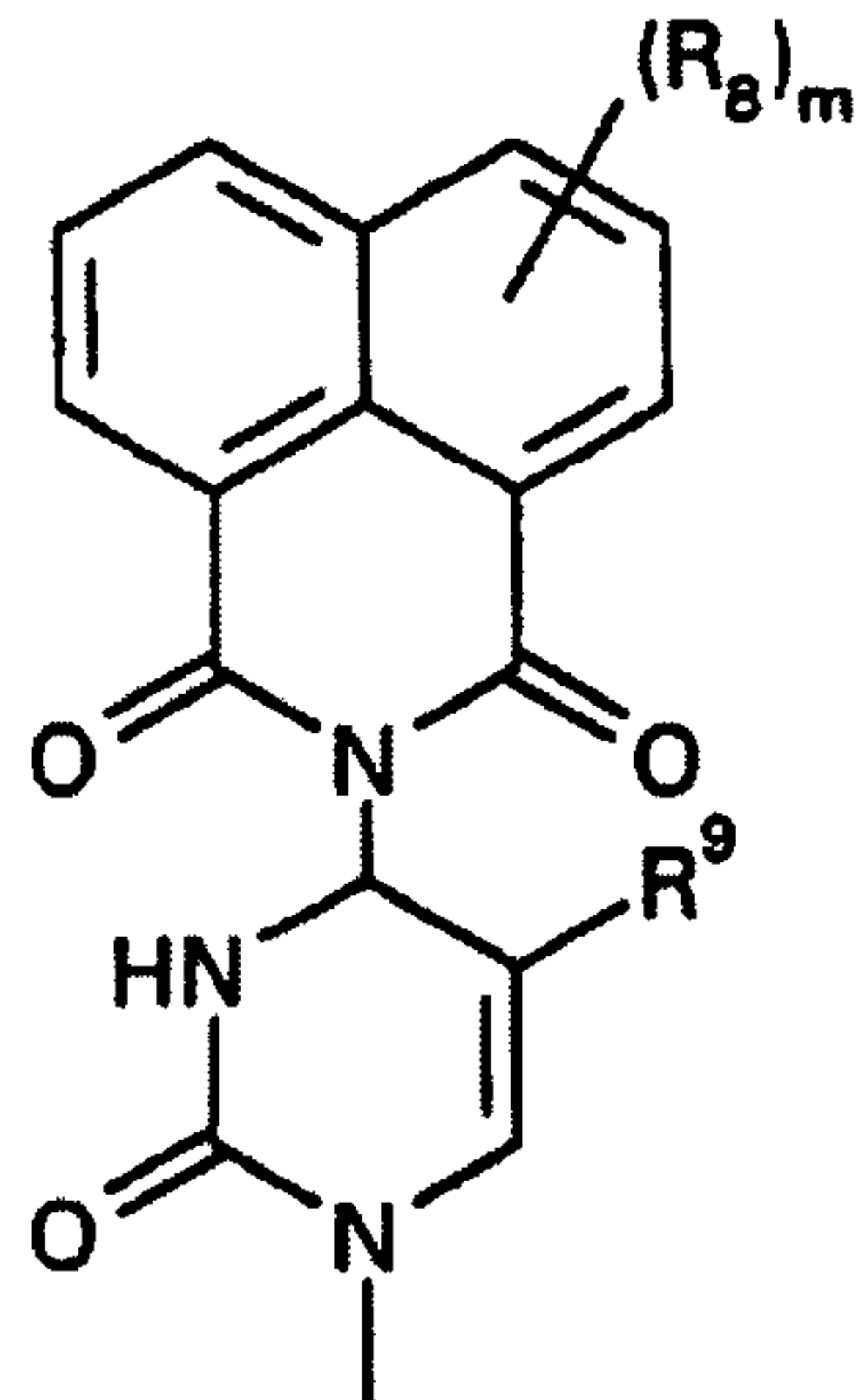
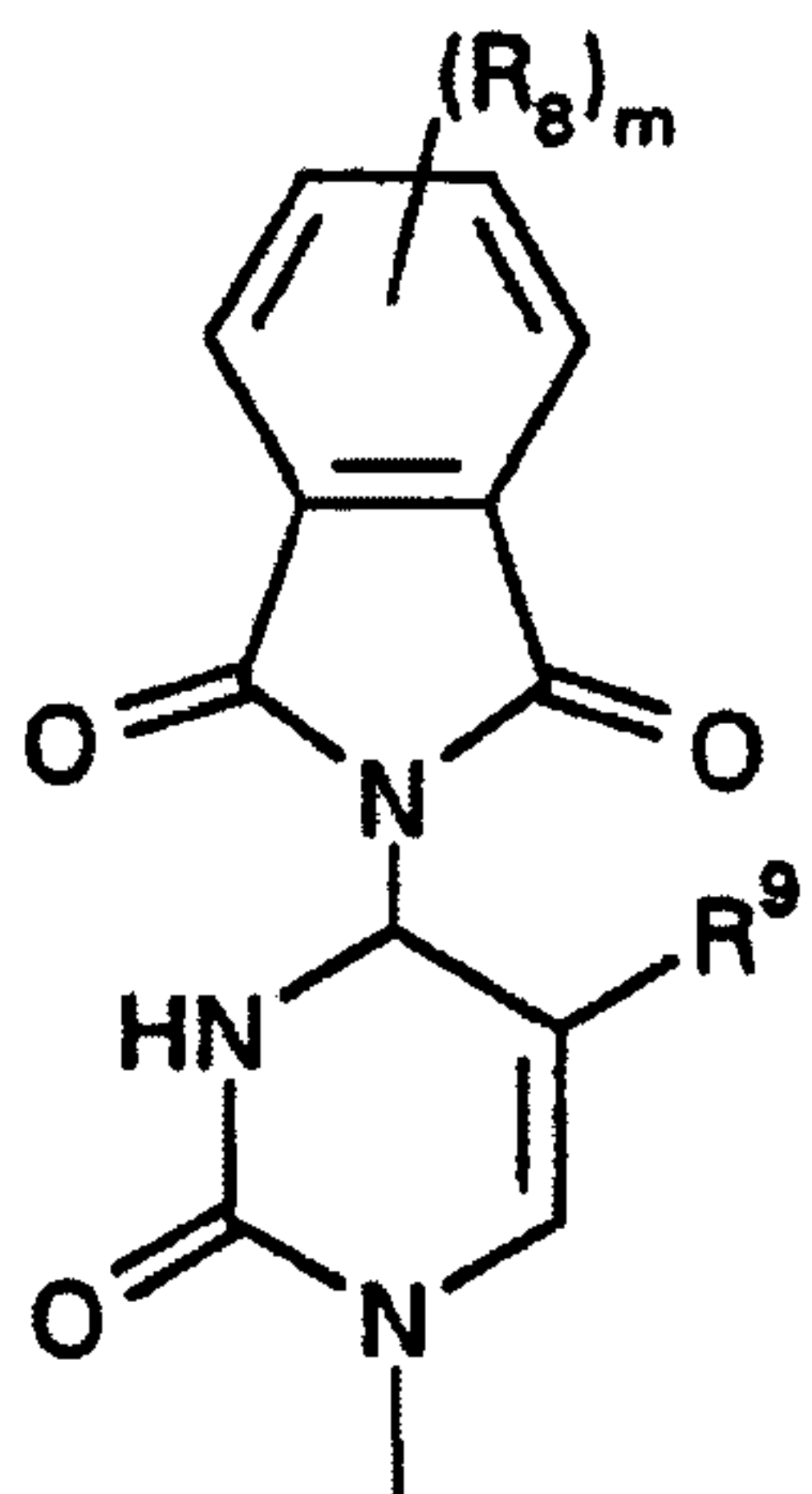
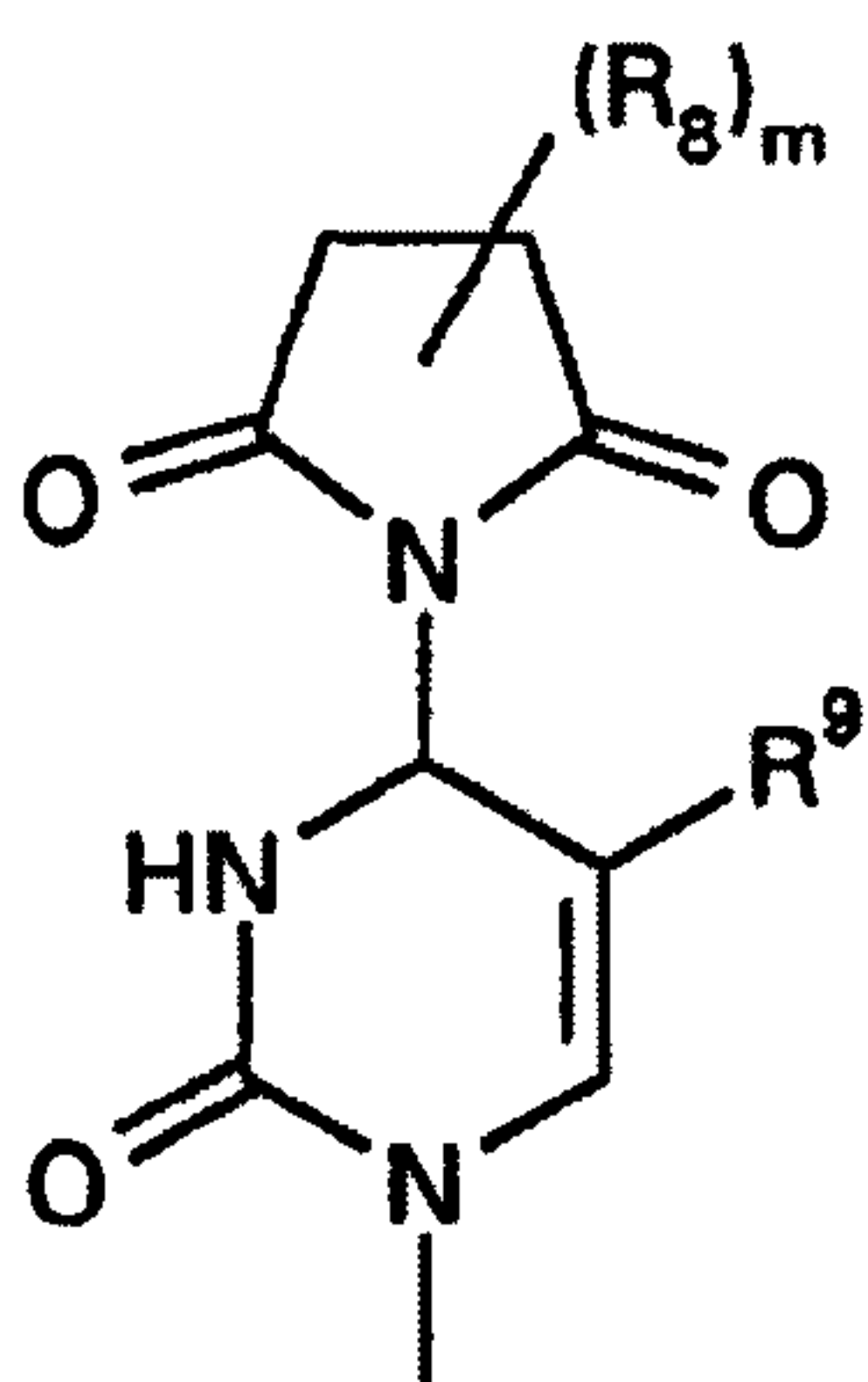
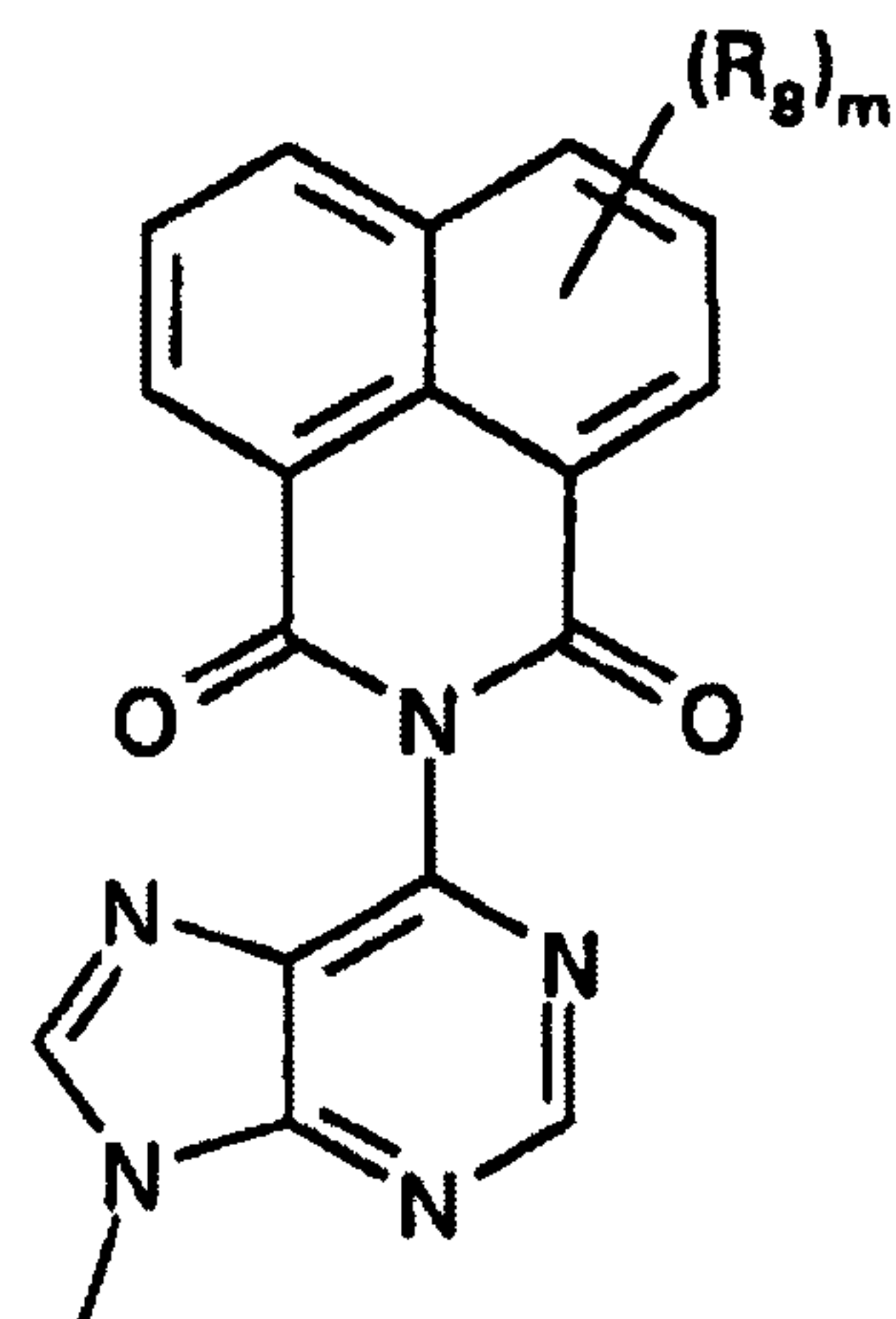
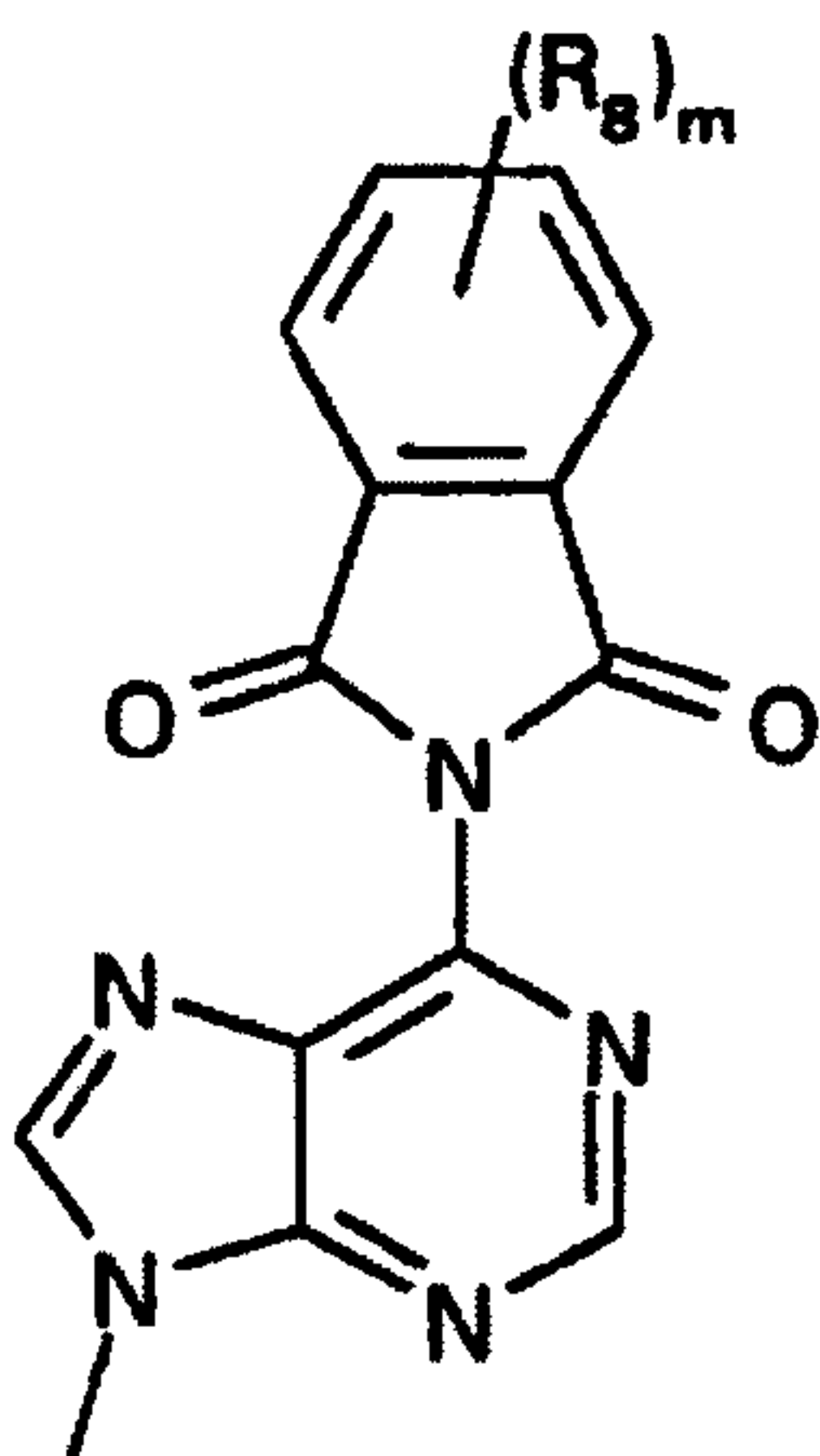
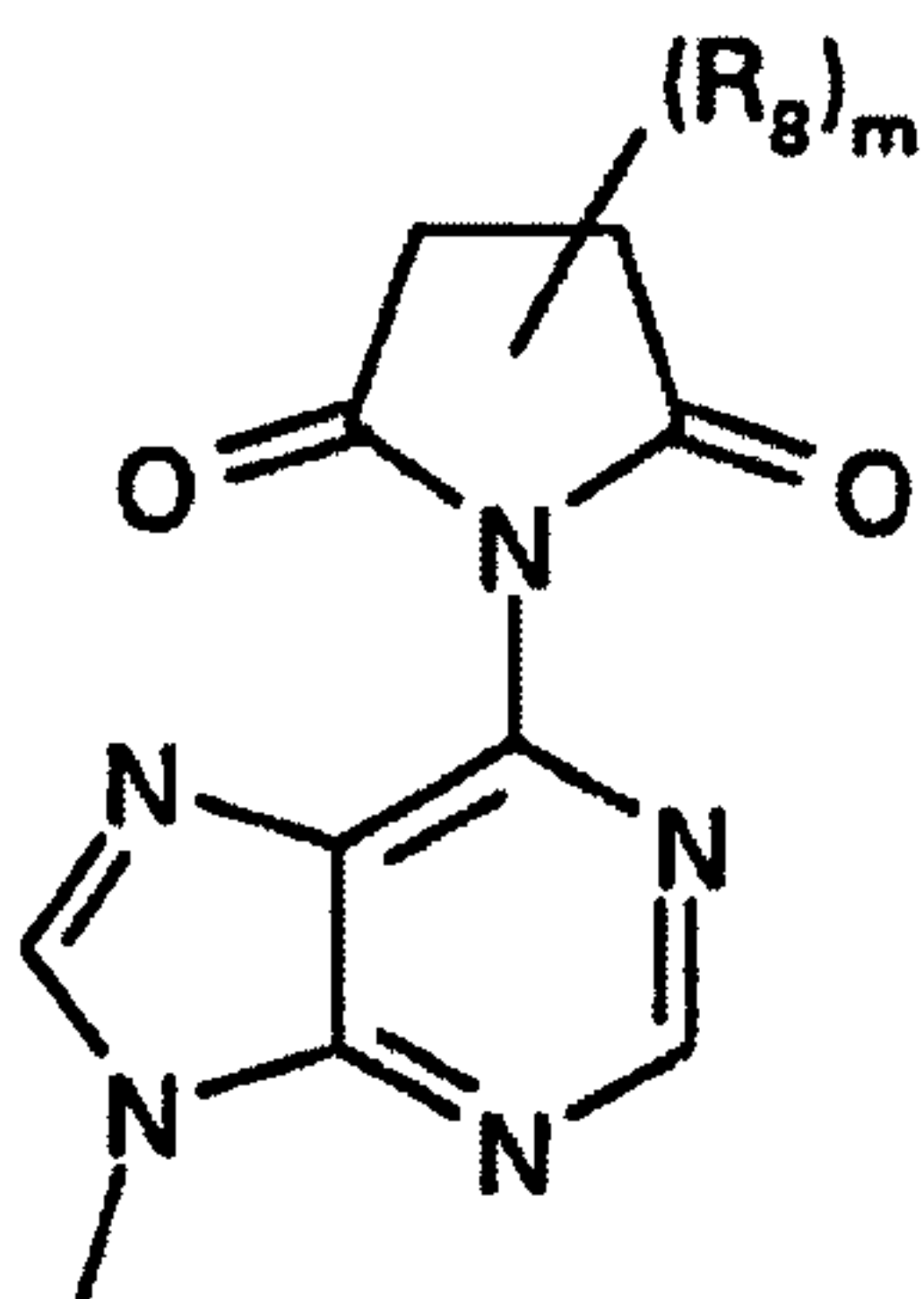
33. The compound of claim 32 wherein R¹¹ and R¹² are identical or different and are selected from isopropyl, C₅-C₈-cycloalkyl, benzyl or phenyl.

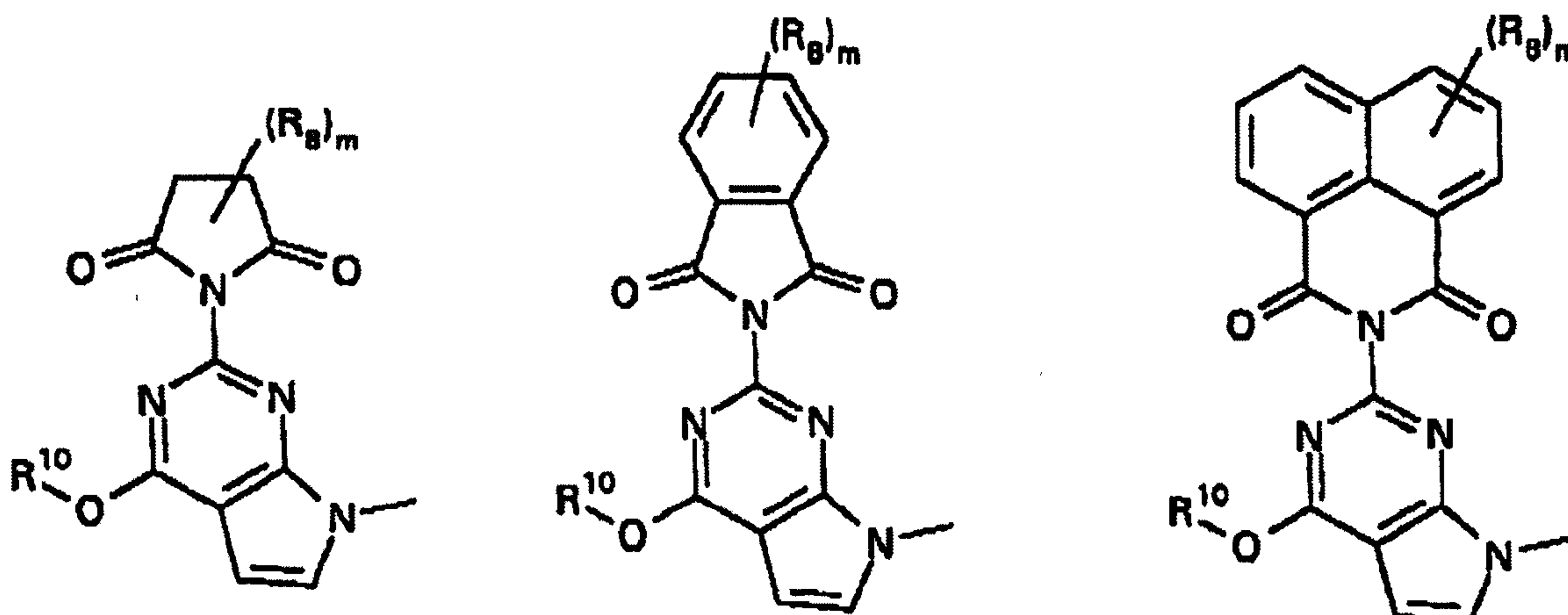
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34. The compound of claim 32 wherein R¹¹ and R¹² together with the nitrogen to which they are bonded form morpholine optionally substituted with OC(O)O-C₁-C₄-alkyl esters.

30

35. A compound of the formula V as claimed in any one of claims 32 to 34, in which B^{PR} is





in which

m is a number from zero to four; and

5

R^8 is, independently at each occurrence, hydrogen, fluorine, chlorine, bromine, nitro, C_1 - C_4 -alkyl, C_1 - C_4 -alkoxy or CN, and R^9 and R^{10} are as defined in claim 27.

10 36. The compound of claim 35 wherein m is zero.

37. A compound of the formula V as claimed in claim 35 or 36, in which

R^{11} is hydrogen, a protected hydroxyl group, C_1 - C_4 -alkoxy or fluorine;

15

A is oxy;

V is oxy;

20

Y is oxy;

Z' is OR^{13} , C_1 - C_6 -alkyl, C_1 - C_6 -alkoxy, C_6 - C_{20} -aryl or C_6 - C_{14} -aryl- C_1 - C_8 -alkyl.

38. The compound of claim 37 wherein Z' is OR¹³.