

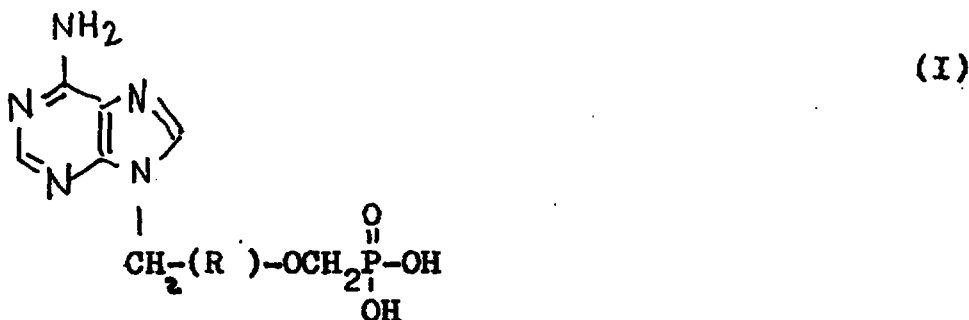
Description

This invention relates to new 9-(phosphonylmethoxyalkyl)adenines as well as their preparation and utilization.

Phosphonylmethyl ethers of alcohols (O-substituted hydroxymethanephosphonic acids) are analogues of esters of these alcohols with phosphoric acid, differing from the latter in having a chemically and enzymatically stable ether linkage. Since phosphoric acid esters, e.g. nucleotides, phosphoglyceric acid, sugar phosphates etc., are of great importance for metabolic processes in the living matter, such analogues may also be biologically active. The said compounds can be prepared e.g. by reaction of alcohols with chloromethanephosphonic acid or its esters (E.N. Walsh, T.M. Beck, A.D.F. Toy: J. Amer. Chem. Soc. 78, 4455 (1956)) or by reaction of formals with phosphorus trichloride (U.S. 2 500 022) or, in case of derivatives of 1,2-diols, by reaction of these diols with chloromethanephosphonyl dichloride and subsequent alkaline hydrolysis (PV 88-83). Another method, applicable also to monohydric alcohols, is reaction of a sodium alkoxide with an ester of p-toluenesulfonyloxymethanephosphonic acid; this reaction has been used in the preparation of 5'-O-phosphonylmethyl derivatives of nucleosides (A. Holý, I. Rosenberg: Collect. Czech. Chem. Commun. 47, 3447 (1982)).

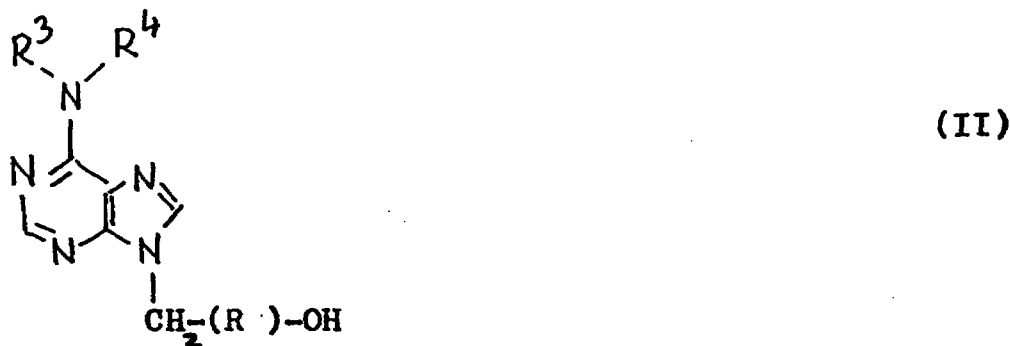
9-Alkyladenines containing one or more hydroxy groups in the alkyl chain behave as analogues of the metabolite adenosine and exhibit various biological activities (e.g. antiviral, chemosterilizing etc., see Czech. Author's Certificate N° 199093, 199094, 199095, PV 377-83, PV 7380-83, PV 970-84). Therefore, phosphonylmethyl ethers of these compounds can be regarded as so-called acyclic analogues of adenine nucleotides. Some of these compounds show also a chemosterilizing effect in insects (Czech. Author's Certificate N° 233 665).

This invention relates to 9-(phosphonylmethoxyalkyl)adenines of the general formula I



wherein R is a CH₂, CH(CH₃), CH₂ CH₂ or CH(OCH₃) CH₂ group and the salts thereof with alkali metals, ammonia or amines.

Further, the invention relates to the method of preparing compounds of the general formula I, characterized in that 9-hydroxyalkyladenines of the general formula II

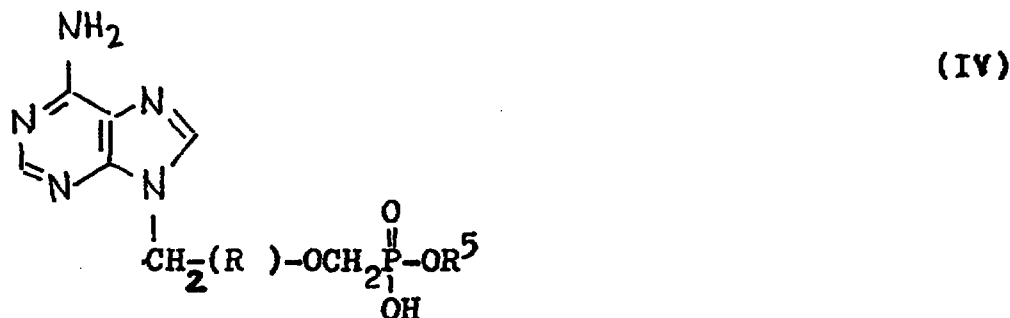


wherein R³ is a benzoyl group, R⁴ is a hydrogen atom or a benzoyl group, or both R³ and R⁴ together are a dimethylaminomethylene group, and R is as defined above, are brought into a reaction with 1 to 2 equivalents (relative to compound II) of sodium hydride in a dipolar aprotic solvent, preferably dimethylfor-

amide, and with 1 to 2 molar equivalents of an ester of p-toluenesulfonyloxymethanephosphonic acid of the general formula III,



wherein R^5 is a methyl or ethyl group, at temperatures 0°C to 100°C , whereupon the mixture is worked up in an alkaline aqueous or aqueous-alcoholic medium and products of the general formula IV



wherein R and R^5 have the same signification as in the formula I and III, are isolated by chromatography, preferably on an ion-exchanging resin or hydrophobized silica gel, treated with a solution of trimethyliodosilane in dimethylformamide at temperatures 0°C to 50°C , and the compounds of the general formula I are isolated by chromatography, preferably on an ion-exchanging resin or hydrophobized silica gel.

The starting compounds of the general formula II are accessible by reactions of suitably activated alcohols (e.g. tosyl or mesyl derivatives) or alkyl halides with salts of adenine, usually in dimethylformamide (see e.g. A. Holý: Collect. Czech. Chem. Commun. 43, 3444 (1978); 44, 593 (1979); 43, 3103 (1978); 43, 2054 (1978)).

The method of preparing the compounds according to this invention is based on formation of sodium salts of 9-hydroxyalkyladenine of the general formula II which contain an isolated hydroxy group. In order to prevent reaction of sodium hydride, used in the preparation of the salts, with other functionalities in the molecule, particularly those bonded to the heterocyclic adenine base, it is necessary to protect them with aroyl (benzoyl) groups or preferably with the N-dimethylaminomethylene group which can be easily introduced by reaction with the so-called dimethylformamide acetals (dialkoxymethyldimethylamines). The salts of thus-protected starting compounds of the formula II are prepared by addition of an equivalent amount or a slight excess of sodium hydride to a solution of compounds II in a solvent which does not react with sodium hydride, preferably dimethylformamide.

These salts of compounds of the general formula II are then condensed with tosylates of the general formula III which are easily accessible from diesters or triesters of phosphoric acid (Czech. Author's Certificate N° 220713, 220714). The compounds of the formula III are used in a slight excess relative to compounds of the formula II in order to eliminate possible side reactions. The condensation is carried out at room or slightly elevated temperature under strictly anhydrous conditions.

The reaction mixtures are worked up simply by dilution with water. The arising alkaline medium removes the protecting groups (aroyl or dimethylaminomethylene) together with one of the two groups bonded to the phosphonic acid by ester bonds. The arising monophosphonates of the general formula IV are not hydrolyzed further and can be easily isolated from the mixture, preferably by deionization of the adenine derivatives on strongly acidic ion-exchangers. Compounds of the general formula IV, obtained by desalting, are purified by chromatography, e.g. on an anion-exchanging resin or octadecyl-silica gel.

In the reaction with trimethyliodosilane, dried compounds of the formula IV are dissolved in dimethylformamide and mixed with the reagent (or its solution in dimethylformamide). The amount of the reagent taken is at least twice of that calculated for the number of equivalents of compound IV (taking into account all the hydroxy or amino groups present in its molecule). (Trimethyliodosilane can be also prepared in situ by

reaction of trimethylchlorosilane with sodium, lithium or potassium iodides in dimethylformamide.) The reaction of compounds of the formula IV with trimethyliodosilane is carried out under anhydrous conditions, the reaction time being usually 18-24 hours at room temperature. The reaction mixture is then decomposed by addition of a neutral or weakly alkaline buffer, e.g. the volatile triethylammonium hydrogen carbonate, and compounds of the general formula I are desalted, preferably using a medium acidic cation-exchanging resin from which, after removal of the salts, they are eluted with a volatile base such as aqueous ammonia. Compounds of the formula I are purified by chromatography on anion-exchanging resins in neutral or acidic medium, or on octadecyl-silica gel.

Compounds of the general formula I and V can be stored as free acids or their salts, prepared either by exact neutralization of the free acids or conversion of their ammonium salts into alkali metal salts using cation-exchanging resins in the appropriate form. The advantage of the last-mentioned salts (sodium and lithium salts) is their good solubility in water.

The method of preparing compounds of the formula I according to the invention can be used also for preparation of individual isomers of phosphonylmethoxyalkyladenines derived from di- or trihydroxyalkyladenines, i.e. when the mentioned preparation of these compounds by reaction with chloromethanephosphonyl chloride followed by alkaline hydrolysis (Czech Author's Certificate N° 233 665) leads to a mixture of isomers which have to be separated. In such cases the reaction is performed with a dihydroxy- or trihydroxyalkyladenine, protected on the adenine ring with a benzoyl or dimethylaminomethylene group, and with suitable alkali-stable groups, such as an acetal grouping or a benzyl group, on all the side-chain hydroxy groups except the one which shall react according to this invention. Isolated hydroxy groups can be protected preferably with tetrahydropyran-2-yl or 1-ethoxyethyl group, cis-diol groupings as isopropylidene or ethoxymethylene derivatives. Also other groups, resistant to sodium hydride, such as substituted silyl groups (tert-butyldimethylsilyl) or groups of the benzyl or trityl type, can be used. After the reaction with trimethyliodosilane, the crude compounds of the general formula I are stripped of the mentioned protected groups using a suitable procedure, such as acid hydrolysis, hydrogenolysis in an acid medium, or treatment with fluorides.

The compounds of the general formula I which are the subject of this invention, are important active components of antiviral drugs. An example of such compound is 9-phosphonylmethoxyethyladenine which exhibits a specific activity against DNA-viruses and Moloney sarcoma disclosed in (EP-A-0205826 of the same effective date).

The following Examples, together with the Table, illustrate the preparation and use of the new compounds of the general formula I according to this invention.

Example 1

9-(2-Phosphonylmethoxyethyl)adenine

Sodium hydride (0.48 g; 20 mmol) is added to a solution of 9-(2-hydroxyethyl)-N⁶-benzoyladenine (2.83 g; 10 mmol) in dimethylformamide and the mixture is stirred under exclusion of moisture (calcium chloride protecting tube) at room temperature for 20 minutes. After addition of dimethyl p-toluenesulfonyloxymethanephosphonate (2.95 g; 10 mmol) the mixture is stirred in a stoppered bottle for 48 hours at room temperature. Water (100 ml) is added and the mixture is set aside at room temperature for 15 hours. Dowex 50 X 8 (H⁺ form) is added until the mixture has acid reaction and the suspension is applied on a column of the same ion-exchanging resin (200 ml). The column is washed with water until the eluate is no longer acidic and does not absorb at 260 nm, and then with dilute (1:10, vol/vol) ammonia. Fractions, absorbing at 260 nm, are taken down at 40°C/2 kPa, and the residue is dissolved in water (10 ml) and adjusted to pH 9-10 with ammonia. This solution is applied on a column of Sephadex A-25 (HCO₃⁻ form; 100 ml) and the column is washed with water until the eluate no longer absorbs at 260 nm. Then the material is eluted by a linear gradient of triethylammonium hydrogen carbonate (prepared from 1 litre of water and 1 litre of 0.2 mol l⁻¹ of the mentioned buffer). The fractions, containing the principal UV-absorbing portion of the eluate, are pooled and taken down at 40°C/2 kPa. The residue is twice coevaporated with ethanol (50 ml) and the obtained compound of the formula IV (triethylammonium salt) is dried over phosphorus pentoxide at room temperature and 13 Pa for 24 hours. Yield, 70%.

This material is dissolved in dimethylformamide (70 ml) and trimethyliodosilane (12 g; 60 mmol) is added at 0°C with magnetic stirring. After stirring in a stoppered flask at room temperature overnight, 2 mol l⁻¹ triethylammonium hydrogen carbonate (90 ml) is added and the mixture is heated to 60°C for 3 hours. Water (700 ml) is added, the mixture is extracted three times with chloroform (100 ml), the aqueous phase is evaporated at 40°C/2 kPa and the residue is coevaporated with ethanol (3 x 100 ml). The residue is

again deionized on a column of Dowex 50 X 8 (H^+ form; 200 ml) as described above. The crude compound I in water (20 ml) is adjusted to pH 9-10 with ammonia, and applied on a column of Dowex 1 X 2 (100 ml; acetate form). After washing with water, the column is eluted with a linear gradient of acetic acid (made from 1 litre of water and 1 litre of 1 mol l^{-1} acetic acid). The fractions of the main UV-absorbing portion of the eluate are combined, evaporated at 40°C/2 kPa, and the acetic acid is removed by repeated evaporation with water (3 x 50 ml). The residue is mixed with ethanol (5 ml) and then with ether (100 ml) and the crystalline product is filtered, washed with ether and dried in vacuo, affording 1.15 g (60% based on compound of the formula IV) of 9-(2-phosphonylmethoxyethyl)adenine, not melting up to 260°C. For $C_8H_{12}N_5O_4$ (273.3) calculated: 35.16% C, 4.43% H, 25.63% N, 11.36% P; found: 34.84% C, 4.50% H, 25.33% N, 11.40% P.

The characteristics of this compound are given in Table 1 under N° 1. According to Example 1, also 9-(3-phosphonylmethoxypropyl)adenine (N° 3) was prepared.

Example 2

9-(3-Phosphonylmethoxy-2-methoxypropyl)adenine

Dimethylformamide dimethylacetal (15 ml) is added to a suspension of 9-(3-hydroxy-2-methoxypropyl)adenine (1.12 g; 5 mmol) in dimethylformamide (25 ml). The mixture is stirred at room temperature in a stoppered flask for 15 hours and then evaporated at 40°C/13 Pa. After addition of 50% aqueous pyridine (50 ml) and solid carbon dioxide (50 g), the mixture is stirred for 30 minutes, again evaporated at 40°C/13 Pa, dried by repeated coevaporation with pyridine (3 x 50 ml) and then with dimethylformamide (25 ml) under the same conditions. This crude N⁶-dimethylaminomethylene derivative is dissolved in dimethylformamide (50 ml) and sodium hydride (0.24 g; 10 mmol) is added. The subsequent reaction and work-up procedure is the same as described in Example 1 and affords 0.80 g (50%) of free 9-(3-phosphonylmethoxy-2-methoxypropyl)adenine, not melting up to 260°C. For $C_{10}H_{16}N_5O_5P$ (317.3) calculated: 37.85% C, 5.08% H, 22.07% N, 9.78% P; found: 37.50% C, 5.24% H, 22.15% N, 9.54% P. The characteristics of this compound (N° 4) are given in Table 1.

According to Example 2 was also prepared 9-(2-phosphonylmethoxypropyl)adenine (N° 2).

Example 3

Primary rabbit kidney cells grown in Eagle's essential medium are infected for 1 hour with $10^{4.5}$ PFU/0.5 ml (PFU denotes a plaque formation unit) of herpes simplex virus, type 1 (KOS strain). Then the medium is replaced by a solution of compound N° 1 (in Table 1), in Eagle's essential medium (concentration, 100 μ g/l/ml medium). After incubation for 48 hours at 37°C, the yield of the virus is determined by plaque formation in the PRK cells. A control experiment is carried out in the same manner but the culture is incubated only in Eagle's essential medium. Under these conditions the virus titre decreases 14 500 times ($\Delta \log \text{PFU/ml} = 3.16$).

Example 4

Primary rabbit kidney cells in Petri dishes are infected with herpes simplex virus, type 2 (G-strain), in a dose hundred times larger than that necessary for inducing 50% of the cytopathic effect of the virus. After 1 hour the cells are incubated with increasingly concentrated solutions of the compounds in Eagle's essential medium for 24 hours at 37°C. The cytopathic effect of the virus is determined as described in "Tissue culture", Pergamon Press, New York 1973, p. 510. Under these conditions, compound, N° 1 (in Table 1), shows a 50% inhibition of the cytopathic effect of HSV-2 virus in concentration 7 μ g/ml.

Table 1 Characteristics of the compounds according to the invention

N°		IV		I	
		Yield %	R _P ^a	Yield %	R _{UP} ^b
1	CH ₂	70	0.45	60	0.11
2	CH(CH ₃)	75	0.50	80	0.18
3	CH ₂ CH ₂	72	0.50	67	0.18
4	CH(OCH ₃)CH ₂	78	0.55	64	0.25
					0.82
					0.80
					0.80
					0.78

^a Paper chromatography in the system 2-propanol - conc. aqueous ammonia - water (7:1:2); ^b paper electrophoresis (20 V/cm) in 0.1 mol l⁻¹ triethylammonium hydrogen carbonate

Claims

1. A compound of the general formula I



Table 1 Characteristics of the compounds according to the invention

N°		IV		I	
		Yield. %	R _F ^a	Yield %	R _F ^b
1	CH ₂	70	0.45	50	0.11
2	CH(CH ₃)	75	0.50	80	0.18
3	CH ₂ CH ₂	72	0.50	57	0.18
4	CH(OCH ₃)CH ₂	78	0.55	64	0.25
					0.82
					0.80
					0.80
					0.78

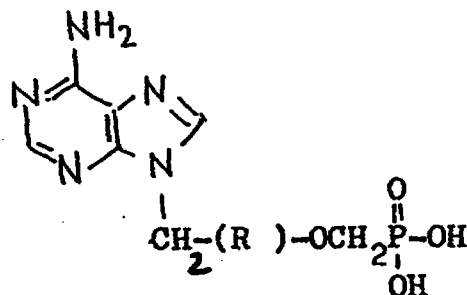
^a Paper chromatography in the system 2-propanol - conc. aqueous ammonia - water (7:1:2); ^b paper electrophoresis (20 V/cm) in 0.1 mol l⁻¹ triethylammonium hydrogen carbonate

Claims

1. A compound of the general formula I

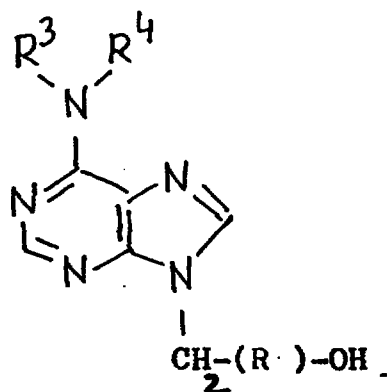


(I)



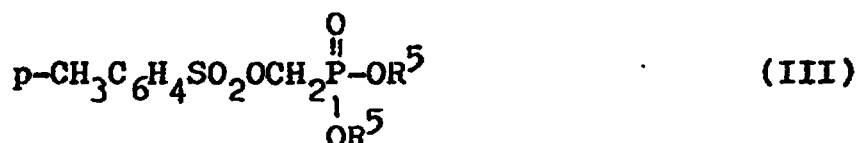
in which R is a CH₂, CH(CH₃), CH₂CH₂ or CH (OCH₃)CH₂ group, or an alkali metal, ammonia or amine salt thereof.

2. A compound according to claim 1, in which R is CH₂, CH₂CH₂ or CH(CH₃).
3. A method of preparing compounds of the general formula I according to claim 1, characterized in that 9-hydroxyalkyladenines of the general formula II



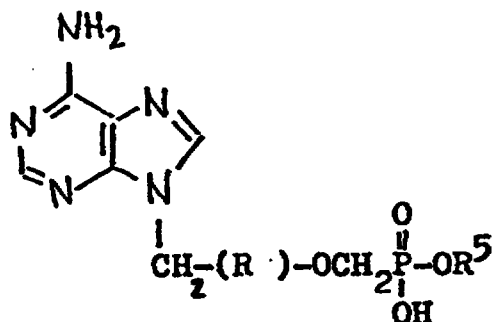
(II)

wherein R is as defined in claim 1, R³ is a benzoyl group R⁴ is a hydrogen atom or a benzoyl group, or both R³ and R⁴ together are a dimethylaminomethylene group, are brought into a reaction with 1 to 2 equivalents (relative to compound II) of sodium hydride in a dipolar aprotic solvent, preferably dimethylformamide, and with 1 to 2 molar equivalents of an ester of p-toluenesulfonyloxymethanephosphonic acid of the general formula III



(III)

wherein R⁵ is a methyl or ethyl group, at temperatures 0°C to 100°C, whereupon the mixture is worked up in an alkaline aqueous or aqueous-alcoholic medium and products of the general formula IV



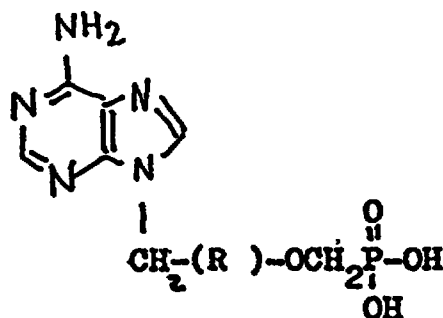
(IV)

wherein R and R⁵ have the same signification as in the formulae I and III, are isolated by chromatography, preferably on an ion-exchanging resin or hydrophobized silica gel, treated with a solution of trimethyliodosilane in dimethylformamide at temperatures 0°C to 50°C, and the compounds of the general formula I are isolated by chromatography, preferably on an ion-exchanging resin or hydrophobized silica gel.

4. Use of a compound according to claim 1 or 2 for the manufacture of an anti-viral medicament.

Revendications

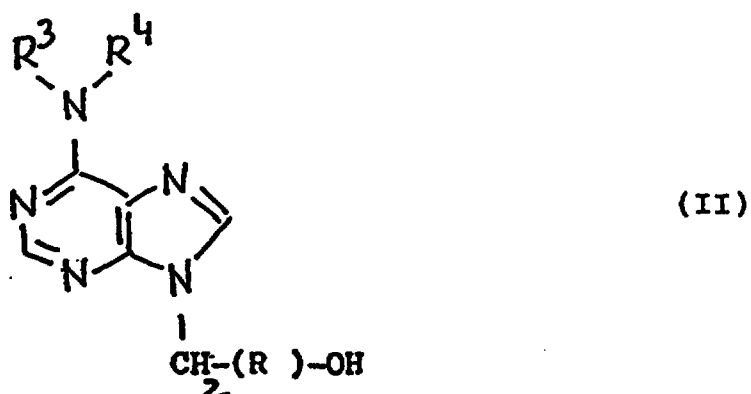
1. Composé répondant à la formule générale I



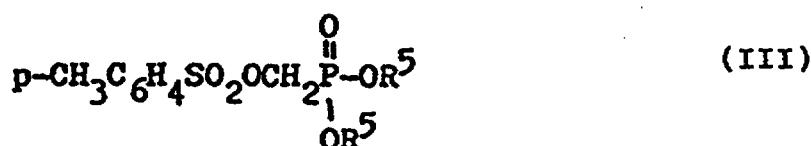
(I)

dans laquelle R représente un groupe CH₂, CH(CH₃), CH₂CH₂ ou CH(OCH₃)CH₂, ou un sel de métal alcalin, d'ammoniaque ou d'amine de celui-ci.

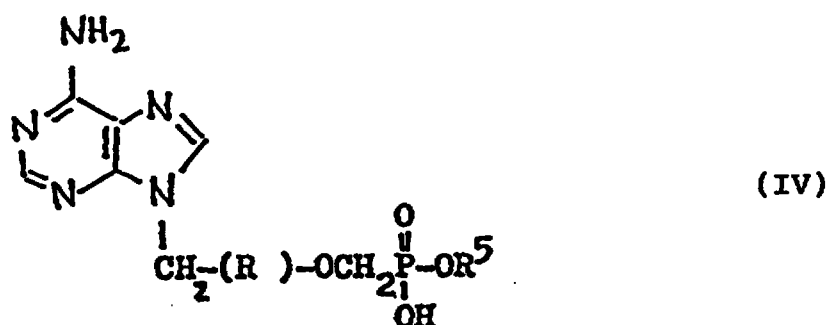
2. Composé selon la revendication 1, dans lequel R représente CH₂, CH₂CH₂ ou CH(CH₃).
3. Procédé de préparation des composés de formule générale I selon la revendication 1, caractérisé en ce qu'on fait réagir des 9-hydroxyalcoyladénines répondant à la formule générale II



dans laquelle R est défini comme dans la revendication 1, R³ représente un groupe benzyle, R⁴ représente un atome d'hydrogène ou un groupe benzyle, ou R³ et R⁴ forment ensemble un groupe diméthylaminométhylène, avec 1 à 2 équivalents (par rapport au composé II) d'hydrure de sodium dans un solvant aprotique dipolaire, de préférence du diméthylformamide, et avec 1 à 2 équivalents en moles d'un ester de l'acide p-toluènesulfonyloxyméthane phosphonique répondant à la formule générale III



dans laquelle R⁵ représente un groupe méthyle ou éthyle, à une température comprise entre 0 et 100 °C, après quoi on reprend le mélange dans un milieu aqueux alcalin ou aqueux alcoolique, et on isole par chromatographie, de préférence sur une résine échangeuse d'ions ou sur du gel de silice rendu hydrophobe, les produits répondant à la formule générale IV

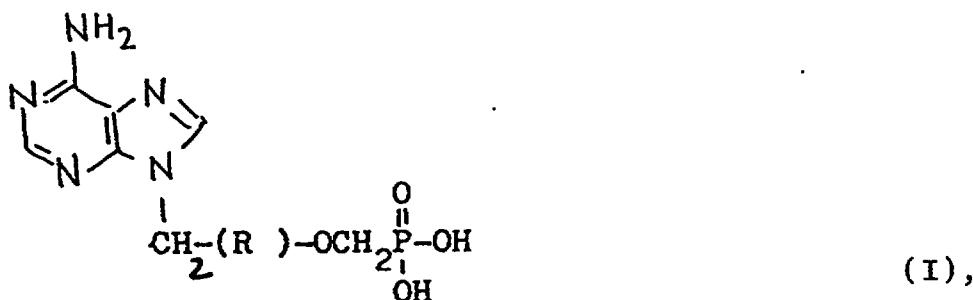


dans laquelle R et R⁵ ont la même signification que dans les formules I et III, on les traite avec une solution de triméthylsilylène dans du diméthylformamide à une température de 0 à 50 °C, et on isole les composés de formule générale I par chromatographie, de préférence sur une résine échangeuse d'ions ou sur du gel de silice rendu hydrophobe.

4. Utilisation du composé selon la revendication 1 ou 2, pour la fabrication d'un médicament antiviral.

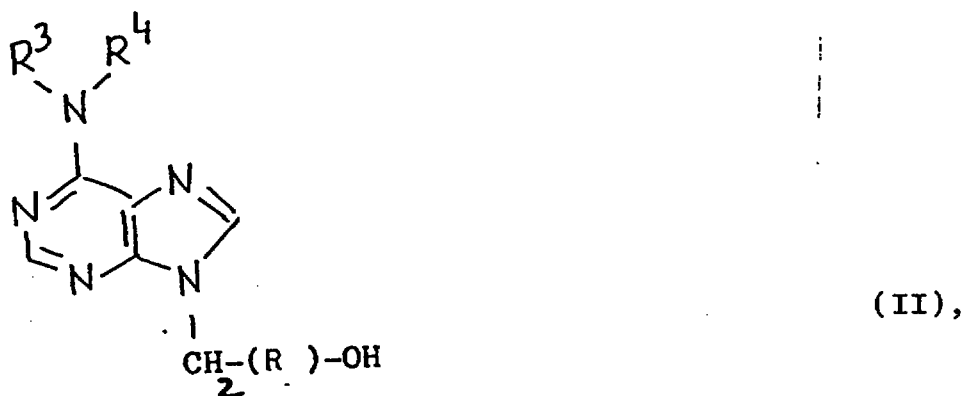
Patentansprüche

1. Verbindung der allgemeinen Formel I



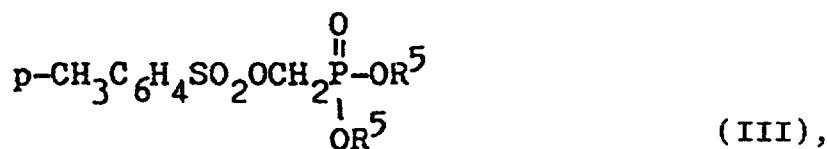
worin R eine CH_2 -, $\text{CH}(\text{CH}_3)$ -, CH_2CH_2 - oder $\text{CH}(\text{OCH}_3)\text{CH}_2$ -Gruppe ist, oder ihr Salz mit Alkalimetallen, Ammoniak oder Aminen.

2. Verbindung nach Anspruch 1, worin R = CH_2 , CH_2CH_2 oder $\text{CH}(\text{CH}_3)$ ist.
3. Verfahren zur Herstellung von Verbindungen der allgemeinen Formel I nach Anspruch 1, dadurch gekennzeichnet, daß 9-(Hydroxyalk)adenine der allgemeinen Formel II

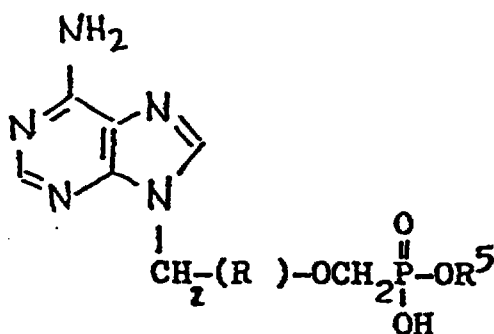


worin R dasselbe bedeutet wie in Anspruch 1, R^3 eine Benzoylgruppe, R^4 ein Wasserstoffatom oder eine Benzoylgruppe oder R^3 und R^4 zusammen eine Dimethylaminomethylengruppe sind,

in einem dipolaren aprotischen Lösungsmittel, vorzugsweise Dimethylformamid, mit 1 bis 2 Äquivalenten (bezogen auf Verbindung II) Natriumhydrid und mit 1 bis 2 molaren Äquivalenten eines Esters der p-Toluolsulfonyloxymethanphosphonsäure der allgemeinen Formel III



worin R^5 eine Methyl- oder Ethylgruppe ist, bei Temperaturen zwischen 0°C und 100°C umgesetzt werden, worauf das Gemisch in einem alkalischen wässrigen oder wäßrig-alkoholischen Medium aufgearbeitet wird, und daß die Produkte der allgemeinen Formel IV



(IV),

worin R und R⁵ die gleiche Bedeutung besitzen wie in den Formeln I und III, durch Chromatographie, vorzugsweise an einem Ionenaustauscherharz oder hydrophobisierten Silicagel, isoliert werden, mit einer Lösung von Trimethyljodsilan in Dimethylformamid bei Temperaturen zwischen 0 °C bis 50 °C behandelt werden und daß die Verbindungen der allgemeinen Formel I durch Chromatographie, vorzugsweise an einem Ionenaustauscherharz oder hydrophobisierten Silicagel, isoliert werden.

4. Verwendung einer Verbindung nach Anspruch 1 oder 2 für die Herstellung eines antiviralen Medikaments.