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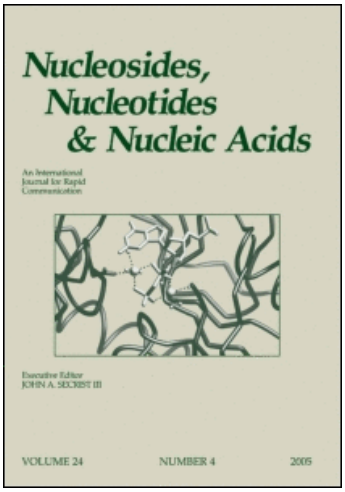

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G. J. Koomen[a]; L. M. Provoost[a]; D. A. H. van Maarschalkerwaart[a]; N. P. Willard[a]
[a] Organic Chemistry Laboratory, University of Amsterdam, WS, The Netherlands

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SYNTHESIS OF AN ACYCLIC ANALOGUE OF AZIDOTHYMIDINE.

G.J. Koomen, L.M. Provoost, D.A.H. van Maarschalkerwaart and N.P. Willard.*

Organic Chemistry Laboratory, University of Amsterdam,
Nieuwe Achtergracht 129, 1018 WS, The Netherlands.

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Abstract: *As possible anti-Aids drugs, hydroxyl- and azido-substituted acyclothymidine derivatives, were prepared via coupling of thymine with suitably substituted bromobutane derivatives.*

During a long period of time, our laboratory has been involved in the synthesis of nucleoside analogues in which the (deoxy) ribose unit has been replaced by a non cyclic system, mimicking the carbohydrate residue. As part of these studies we published the synthesis of 9-(4-hydroxy-3-hydroxymethylbutyl) substituted purines and pyrimidines already in 1972[1]. An important advantage of this acyclic system is the absence of the amino acetal oxygen atom which improves the chemical and enzymatic stability. Furthermore the lack of chirality of the system simplifies the synthesis. The corresponding guanine derivative **1** showed strong anti-herpes activity and has been developed further by Beecham Pharmaceuticals as an antiviral drug[2]. It has been proven by ^{13}C labelling, that in the virus infected cell the viral thymidine kinase phosphorylates one hydroxyl function, i.e. the pro S hydroxymethyl group[3]. Since then many derivatives have been reported by different research groups[4].

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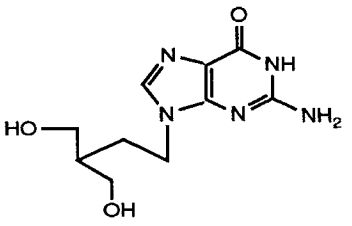

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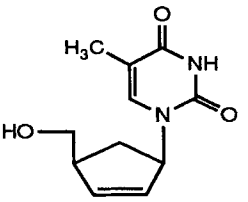

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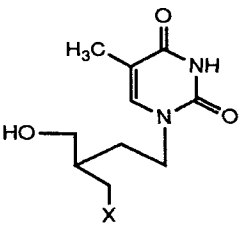

3a X = OH
3b X = N_3

Several nucleoside analogues have been reported that show anti-AIDS activity due to the absence of a hydroxyl group at the corresponding 3' position of the ribose moiety. These compounds are inhibitors of reverse transcriptase and/or can act as chain terminators in polynucleotide synthesis. In earlier work, we combined the stability of carba systems described above with chain terminating properties resulting in the synthesis of the carbocyclic nucleoside analogue **2** [5]. In this paper we describe the synthesis and anti-HIV activity of acyclo nucleoside analogues **3a** and **3b**. In the latter compound one of the hydroxyl groups is converted into an azidofunction in order to obtain an acyclic analogue of AZT.

For the synthesis of thymine derivatives several reaction paths can be followed. Construction of the thymine ring via the Shaw synthesis [6] requires the availability of the corresponding amine **4c**. In view of earlier work we decide to prepare the target compounds via direct alkylation of thymine under basic conditions.

The starting material **4b** for the synthesis was obtained from diethyl malonate and 1-bromoacetaldehyde-dimethylacetal followed by reduction of both ester functions of the condensation product with $LiAlH_4$ and protection of the resulting diol as benzylethers. After hydrolysis of the acetal, the resulting aldehyde was once again reduced to obtain **4a.** Reaction with triphenylphosphine and CBr_4 afforded the bromide **4b** [7] in 74% yield.

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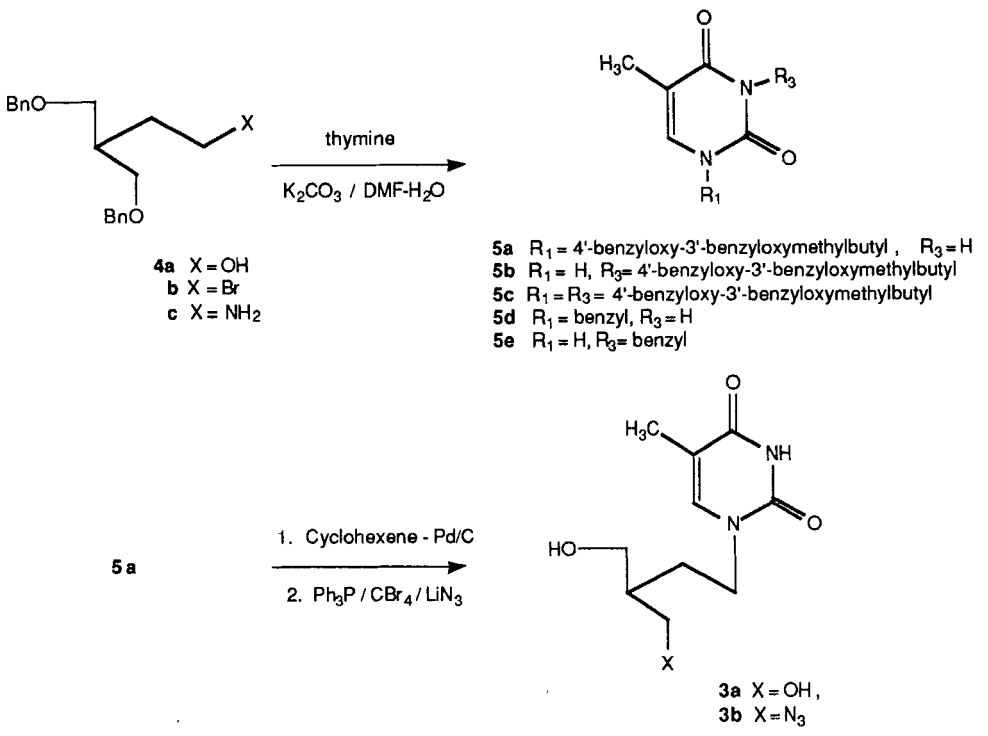

The poor solubility of thymine in most organic solvents was a source of problems in the alkylation. In order to realize the alkylation in acceptable yields, excess of base (potassium carbonate) and thymine was necessary. Even under these conditions, using DMF as a solvent, the formation of excess dialkylated product **5c** (43 %) was observed. Apparently the mono alkylated product is far better soluble in pure DMF than thymine, leading easily to the dialkylated product. Addition of water to the DMF results in better solubility of thymine, which results in the formation of product **5 a** in better yields. Optimal results were obtained with 5 eq of thymine and potassium carbonate in a 2 : 1 mixture of DMF/water, leading to the isolation of the desired product **5a** in 36 % and to the dialkylated product **5c** in 12 % yield. The rest of the reaction mixture consisted of the N-3 substituted product **5b** (9%), N-1- and N-3 benzyl thymidine **5d** (11%)and **5e** (1%). The N-1,N-3 disubstituted product **5c** could be removed easily by column chromatography due to the lower polarity . Separation of the mono-alkylated products **5a** and **5b** was considerably more laborious.[8)]

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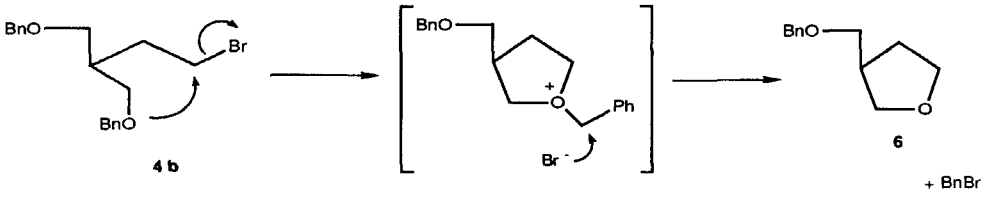
Application of Mitsunobu conditions did not reduce the problems but resulted in a lower overall yield. In order to prevent N-3 substitution by steric hindrance, reactions were carried out with the bis-trimethylsilyl ether of thymine. This did not result in the production of **5a**, but provided information on the formation of the benzyl substituted thymines **5d** and **5e** isolated from previous reactions. Since the reactivity of the silylether was found considerably lower than that of the anion of thymine, the reaction mixture had to be heated for two days. No reaction was observed using toluene as a solvent, but in DMF the tetrahydrofuran derivative **6** was obtained in 52 % yield. The higher polarity of the solvent stimulates the cyclization reaction outlined below in which besides **6** one equivalent of benzyl bromide is formed.



In the reactions with thymine and potassium carbonate described above apparently this reaction also takes place, followed by alkylation of thymine at N-1 and N-3 by the benzyl bromide formed.

The protective benzyl groups could be easily removed by catalytic reduction using palladium carbon 10% with cyclohexene as hydrogen donor (yield 86%) [9]. The diol **3a** was purified by column chromatography [10]. In order to convert **3a** into the acyclo AZT analogue, the compound was treated with triphenylphosphine and lithium azide in DMF. The reaction was started by adding an excess of carbon tetrabromide. After column chromatography **3b** was obtained in low yield (11 %) as a racemate [11].

ANTI -HIV ACTIVITY

The acyclo derivatives **3a** and **3b** were tested for anti-HIV activity on MT-4 cells (3.10^4 /well), infected with HIV-1 (100 $CCID_{50}$ / well). Antiviral activity and cytotox-

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icity were determined after five days. Both compounds were found inactive in doses up to 625 μM.

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7. 4-benzyloxy-3-benzyloxymethyl-1-bromobutane **4b**

   To a solution of 6.5 g CBr_4 (20 mmol) and 2.5 g 4-benzyloxy-3-benzyloxy methylbutanol **4a** (8.4 mmol) in dry diethyl ether under a nitrogen atmos phere is added dropwise a solution of 5.31 g triphenylphosphine in 20 mL dry diethyl ether. After refluxing the solution during 20 hrs the reaction was

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quenched with 5 mL methanol. The reaction mixture was stirred at rt for 1 hr, the suspension filtered and the residue washed twice with diethyl ether. After evaporation under reduced pressure the residue is kept for 25 hrs *in vacuo* to remove the last traces of bromoform. The residue is chromato graphed yielding 2.3 g **4b** as a colourless oil.
4b: ^{1}H NMR ($CDCl_3$, 60 mHz): δ 7.3(m, 10 H, arom.), 4.5 (s, 4H, CH_2Ar.), 3.7-3.3 (m. 6H, CH_2Br+ CH_2OBn), 2.3-1.9 (m, 3H, CH-CH_2).

8. 1-(4'-benzyloxy-3'-benzyloxymethylbutyl)-5-methyl-2,4-(1H,3H)-pyrimidine dione **5a**.

To a suspension of 0.78 g thymine (6.6 mmol) in 50 mL water/DMF 1:1 is added a solution of 0.47 g **4b** (1.3 mmol) and 0.86 g K_2CO_3 (6.2 mmol) in 25 mL DMF. This mixture is stirred during 5 days at rt (mechanical stir rer). Concentration *in vacuo* of the reaction mixture and extraction with dichloromethane and water gave after drying ($MgSO_4$) the crude product. This was chromatographed (PE/EtOAc, 1/1, v/v), yielding 0.19 g **5a** (0.47 mmol, 36%), 0.048 g **5b** (0.12 mmol, 9%), 0.053 g **5c** (0.078 mmol, 12 %), 0.029 g **5d** (0.14 mmol, 11%), 0.003 g **5e** (0.013 mmol, 1%).
The regioisomerism of compounds **5a** and **5b** was established via the dif ferences in the chemical shifts of protons H-1'. In the N-3 alkylated product **5b** the H-1' protons absorb at lower field than in the N-1 alkylated product **5a** due to the elctronwithdrawing properties of the two adjacent carbonyl functionalities. Furthermore is via synthetic evidence established that the observed NMR properties of the N-1 alkylated derivative **5a** are in accord with those reported in the doctorate thesis of W.F.A. Grose, University of Amsterdam, 1971, who constructed the thymine moiety on a suitable amine via a Shaw synthesis with the appropriate acyl isocyanate.

5a: oil , ^{1}H NMR ($CDCl_3$, 200 mHz): δ 8.85 (s, 1H, NH), 7.3 (m, 10H, ar om.), 6.95 (s, 1H, H-6), 4.49 (s, 4H, CH_2Ar.), 3.78 (t, 2H, J=7.4, H-1'), 3.5 (m, 4H, CH_2O), 1.99 - 1.73 (m, 3H, H-3' + H-2'), 1.83 (s, 3H, Me).

5b: oil , ^{1}H NMR ($CDCl_3$ 200 mHz): δ 9.69 (d, 1H, J=4.6, NH), 7.3 (m, 10H,arom.), 6.95 (s, 1H, H-6), 4.50 (s, 4H, CH_2Ar.), 4.00 (t, 2H, J=7.4, H-1'), 3.5 (m, 4H,CH_2O), 1.99 - 1.73 (m, 3H, H-3' + H-2'), 1.89 (s, 3H, Me).

5c: oil, ^{1}H NMR ($CDCl_3$ 250 mHz): δ 7.3 (m, 20H, arom.), 6.88 (s, 1H, H-6), 4.49 (s, 8H, CH_2Ar.), 4.04 (t, J=7.4, 2H, CH_2N-3), 3.77 (t, 2H, J=7.4, CH_2N-1), 3,5 (m, 8H, CH_2O), 1.99-1.70 (m, 6H, CH_2CH), 1.84 (s, 3H,Me).

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5d: oil, ^{1}H NMR ($CDCl_3$ 200 mHz): δ 9.4 (bs, 1H, NH), 6.95 (s, 1H, H-6), 4.89 (s, 2H, CH_2Ar.), 1.83 (d, 3H, J=1.0, Me).

5e: oil, ^{1}H NMR ($CDCl_3$ 200 mHz): δ 9.97 (bs, 1H, NH),6.95 (s, 1H, H-6), 5.13 (s, 2H, CH_2Ar.), 1.90 (d, 3H, J=0.9, Me).

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10. 1-(4'-hydroxy-3'-hydroxymethylbutyl)-5-methyl-2,4-(1H,3H)-pyrimidinedione **3a**.

A suspension of 0.219 g dibenzylderivative **5a** (0.54 mmol) and 0.129 g 10% Pd/C and 4 mL cyclohexene (59 mmol) in 8 mL EtOH is refluxed dur ing 25 hrs.The reaction mixture is filtered over hi-flo. The residue is washed twice with EtOH (60%). The filtrates are combined and concentrat ed *in vacuo*. The crude product is chromatographed (10% MeOH in EtOAc and 20% MeOH in EtOAc), yielding 0.102 g diol **3a** (0.46 mmol, 86%) as a colourless oil.

3a: oil, IR (KBr): 3400 (OH,NH), 1700, 1675 (C=O).^{1}H NMR (d-6 DMSO 250 mHz): δ 11.16 (bs, 1H, NH), 7.53 (s, 1H, H-6), 4.44 (t, 2H, J=5.0, OH), 3.69 (m, 2H, H-1'), 3.60 (m,CH_2O + DMSO), 1.77 (s, 3H, CH_3), 1.60-1.45 (m, 3H, H-2' + H-3'). (OH and NH exchangeable for D).

11. 1-(4'-azido-3'-hydrxymethylbutyl)-2,4-(1H,3H)-pyrimidinedione **3b**.

To a mixture of 0.127 g diol **3a** (0.58 mmol), 0.316 g PPh_3 (1,2 mmol) and 0.298 g LiN_3 (6.1 mmol) in 3 mL DMF is added 3.9 g CBr_4 (12 mmol). Af ter stirring the mixture at rt for 4 hrs the reaction mixture is concentrated *in vacuo*. The residue was chromatographed (5% EtOH in CH_2Cl_2), yielding 0.016 g azidoderivative **3b** (0.065 mmol, 12%) as a colorless oil.

3b: oil, IR ($CHCl_3$): 3400 (OH),3200 (NH), 2100 (N_3). ^{1}H NMR ($CHCl_3$, 250 mHz): δ 9.95 (bs, 1H, NH), 7.05 (d, 1H, J=1.1, H-6), 3.78 (t, 2H, J=7.4 H-1'), 3.65 (m, 2H, CH_2OH), 3.38 (m,2H, CH_2N-3), 3.13 (bs, 1H, OH), 1.89, (d, J=0.9, 3H, CH_3), 1.68 (m, 3H, H-2' + H-3').

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