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Synthesis and Investigation of Antiviral Activity of 3′-O-(Aminoalkyl)-thymidines and their Quaternary Ammonium Salts

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Summary. Alkylation of 5′-O-tritylthymidine with dialkylaminoalkyl chlorides in the presence of sodium hydride yields 3′-O-dialkylaminoalkyl-5′-O-tritylthymidine derivatives **2** which were treated with an excess of iodomethane to afford the corresponding quaternary ammonium derivatives **3**. Deprotected nucleosides **4** and **6** were obtained by refluxing **3** and **2**, respectively, in 80% acetic acid. When the compounds **2**–**4** and **6** were investigated for activity against HSV and HIV, the trityl derivatives **2a** and **2c** were found active against HSV.

Keywords. Nucleoside synthesis; Alkylation; 3′-O-dialkylaminoalkylthymidine; 3′-O-dialkylammoniumalkylthymidine; HIV-1; Herpes simplex virus.

Synthese und Untersuchung der antiviralen Aktivität von 3′-O-(Aminoalkyl)-thymidinen und ihrer quartären Ammoniumsalze

Zusammenfassung. Alkylierung von 5′-O-Tritylthymidin mit Dialkylaminoalkylchloriden in Gegenwart von Natriumhydrid ergab 3′-O-Dialkylaminoalkyl-5′-O-tritylthymidinderivate **2**, die mit einem Überschuß an Iodmethan zu den entsprechenden quartären Ammoniumderivaten **3** umgesetzt wurden. Die entschützten Nucleoside **4** und **6** wurden durch Kochen von **3** bzw. **2** in 80%iger Essigsäure unter Rückfluß erhalten. Bei der Untersuchung von **2**–**4** und **6** im Hinblick auf ihre Wirksamkeit gegen HSV und HIV wurde bei den Tritylderivaten **2a** und **2c** Aktivität gegen HSV festgestellt.

Introduction

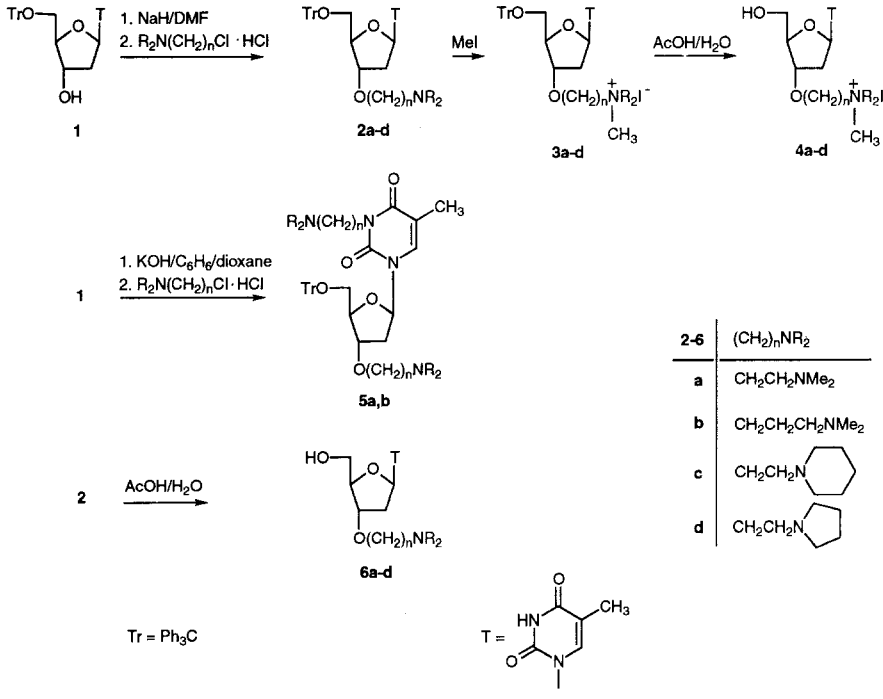
Shortly after the retrovirus human immunodeficiency virus (HIV) [1] was identified as the cause of AIDS [2, 3], 3′-azido-3′-deoxythymidine (*AZT*), originally synthesized by Horwitz et al. [4], was shown to be effective against HIV [5]. Many nucleosides analogues with anti-HIV activity have been synthesized and their structure-antiviral relationships have been reviewed [6, 7]. Since cholin is an essential constituent of the cell membrane phospholipids, we found it of interest to synthesize a series of nucleosides containing side chains similar to choline at the 3′-position of β-thymidine assuming this similarity to facilitate the transport of the

nucleosides through the cell membrane. The linear substituent at the 3′-position, should then ensure chain termination during the reverse transcriptase if incorporation of the nucleoside took place. By increasing the number of carbons it is easy to enhance the lipophilicity of these compounds which could be an important factor for the transport through the cell membrane.

Results and Discussion

In this investigation the 5′-hydroxyl group of thymidine was protected by tritylation as described by Horwitz et al. [8]. 5′-O-Tritylthymidine (**1**) was alkylated in the presence of a large excess of sodium hydride with N-(2-chloroethyl)-dimethylamine, N-(3-chloropropyl)-dimethylamine, N-(3-chloropropyl)-piperidine, and N-(2-chloroethyl)-pyrrolidine, all used as their hydrochlorides. Alkylation with N-(2-chloroethyl)-morpholine did not succeed which may be due to degradation of the morpholine ring during the reaction conditions.

First it was attempted to carry out the alkylation according to the procedure of Griffin et al. [9]. 5′-O-tritylthymidine was treated with the alkylating agent in the presence of potassium hydroxide in a refluxing mixture of benzene and dioxane. It was observed during the alkylation that the bisalkylated compounds **5a, b** were formed along with the monoalkylated compounds **2a, b**. The percentage of the dialkylated compounds was gradually increasing during the reaction until it became the single product after 20 h. To overcome this problem we decided to use sodium hydride because of its powerful basicity which has been useful [10] in similar alkylation reactions of 5′-O-tritylthymidine. In this way compounds **2a–d** were



Scheme 1

prepared in 25–30% yield using dry N,N-dimethylformamide as solvent. The reaction was stopped at the first appearance of the bisalkylated compound on TLC. In agreement with a report from Nyilas et al. [11] the ^{13}C NMR spectra of compound **2** showed a downfield shift of approximately 9 ppm of the C-3′ resonances relative to those of the parent compound **1**. ^{13}C NMR spectra of dialkylated compounds **5** showed a double set of peaks due to the two alkyl groups, with a large difference in shifts only for the carbon attached to N-3 (*ca.* 39 ppm) and the carbon attached to 3′-O (*ca.* 67 ppm).

The monoalkylated compounds **2a–d** could be easily deprotected by refluxing in 80% acetic acid for 10 min. These compounds were isolated as their acetate salts in 60–70% yield. The quaternary ammonium compounds **3a–d** were prepared in a spontaneous reaction of **2a–d** with an excess of methyl iodide at room temperature. The quaternization was deduced from the ^{13}C NMR spectrum, exhibiting a 3–9 ppm downfield shift of all carbons directly attached to the quaternized nitrogen [12]. Trityl groups were split off from **3a–d** again by refluxing in 80% acetic acid for 10 min to give the deblocked compounds **4a–d** in 76–83% yield.

The deproctected nucleosides **4a–d** and **6a–d** did not show any significant activity at 100 μ*M* against Herpes Simplex Virus, type 1 (HSV-1), strain McIntyre, when tested in a continuous cell line from rabbit cornea (SIRC) which was maintained in Eagle's MEM containing 1% fetal calf serum (FCS) and test compound. Instead, we found active compounds against HSV-1 among the protected compounds **2a–d**. A significant selectivity, when compared with toxicity, was found for the compounds **2a** and **2c**, but lower than the one found for acyclovir. The corresponding tritylated quaternary ammonium salts **3a–d** were toxic at 100 μ*M* and without any activity against HSV-1 at 10 μ*M*.

Table 1. Antiviral activity of 3′-O-(2-Dialkylaminoalkyl)-5′-O-tritylthymidines **2a–d** against HSV-1 in SIRC cells

Compound	ED_{50}[a], μ*M*	CD_{50}[b], μ*M*
2a	3	27
2b	2	4
2c	3	22
2d	3	5
Acyclovir	0.5	>50

[a] Effective dose of compound, achieving 50% protection of SIRC against HSV-1

[b] Cytotoxic dose of compound, required to reduce viability of normal uninfected SIRC cells by 50%

The compounds **4a–d** and **6a–d** were also devoid of any activity against HIV-1 (strain HTLV-IIIB) in MT-4 cells when incubated with virus, washed and added in a proportion of 1:10 to uninfected MT-4 cells which had been preincubated in test compound containing culture medium (RPM 1640 containing 10% FCS) for 2 h. The MT-4 cells were maintained in culture medium likewise containing the test

compound. Expression of HIV in culture medium was quantized by HIV antigen detection ELISA [13]. The trityl derivatives **2a–d** and **3a–d** showed cytotoxicity against MT-4 cells at 100 μ*M*, but not significant activity was observed against HIV-1 at 10 μ*M*.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 250 spectrometer. EI and FAB mass spectra were recorded on a Varian MAT 311A spectrometer.

Monoalkylation of 5′-O-tritylthymidine (**1**) *to give* **2a–d**. *General procedure*

To an ice cold solution of *5′-O-tritylthymidine* (**1**, 2.9 g, 6 mmol) in 30 ml of dry N,N-dimethylformamide was added portion-wise 60% oil-immersed sodium hydride (2.4 g, 60 mmol). The resulting mixture was stirred at room temperature for 1 h. The appropriate dialkylaminoalkyl chloride hydrochloride (36 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature until silica gel TLC with CH_2Cl_2/CH_3OH (9:1) showed the first appearance of the undesired dialkylated compound at a lower R_f value than the one for the monoalkylated compound. The reaction was stopped (3–10 hours) by destroying exess of sodium hydride with methanol at 0 °C; this was followed by evaporation of the solvents under reduced pressure at 1 torr. The residue was mixed with 200 ml of water and then extracted with methylene chloride. The methylene chloride phase was dried over Na_2SO_4, evaporated and chromatographed on silica gel (120 g, 0.04–0.063 mm) with CH_3OH (2–7%) in CH_2Cl_2 to give **2** in 25–30% yield.

3′-O-(2-Dimethylaminoethyl)-5′-O-tritylthymidine [**2a**; $C_{33}H_{37}N_3O_5 \cdot 1/2H_2O$]

Reaction time: 3 h; m.p.: 75–78 °C; yield: 1.02 g (25%); ^{1}H NMR ($CDCl_3$): $d = 1.48$ (3H, s, CH_3), 2.08–2.26 (8H, m, 2′-H and $(CH_3)_2N$), 2.44–2.50 (2H, m, CH_2N), 3.29–3.45 (4H, m, CH_2O and 5′-H), 3.95–4.20 (2H, m, 4′-H and 3′-H), 6.10–6.30 (1H, broad s, 1′-H), 7.26–7.50 (15H, m, arom), 7.55 (1H, s, 6-H); ^{13}C NMR ($CDCl_3$): $\delta = 11.71$ (CH_3), 37.68 (C-2′), 45.58 ($(CH_3)_2N$), 58.44 (CH_2N), 63.70 (C-5′), 67.38 (CH_2O), 79.65 (C-3′), 83.70 (C-1′), 84.63 (CPh_3), 87.26 (C-4′), 111.00 (C-5), 127.22, 127.80, 128.47 (arom) 135.25 (C-6), 143.23 (arom), 150.47 (C-2), 163.93 (C-4); MS (EI): m/z (%) = 312 (M^+-CPh_3, 2.1).

(3′-O-Dimethylaminopropyl)-5′-O-tritylthymidine [**2b**; $C_{34}H_{39}N_3O_5 \cdot 3/4H_2O$]

Reaction time: 10 h; m.p.: 80–82 °C; yield: 1.02 g (30%); ^{1}H NMR ($CDCl_3$): $\delta = 1.49$ (3H, s, CH_3), 1.75–1.88 (2H, m, CH_2), 2.10–2.25 (2H, m, 2′-H), 2.35 (6H, s, $(CH_3)_2N$), 2.40–2.58 (2H, m, CH_2N), 3.25–3.51 (4H, m, CH_2O and 5′-H), 4.10–4.20 (2H, m, 3′-H and 4′-H), 6.32 (1 H, dd, $J = 8.1$, 5.7 Hz, 1′-H), 7.25–7.43 (15H, m, arom), 7.56 (1H, s, 6-H); ^{13}C NMR ($CDCl_3$): $\delta = 11.69$ (CH_3), 26.87 (CH_2), 37.66 (C-2′), 44.54 ($(CH_3)_2N$), 56.00 (CH_2N), 63.84 (C-5′), 67.09 (CH_2O), 79.67 (C-3′), 83.77 (C-1′), 84.58 (CPh_3), 87.27 (C-4′), 111.03 (C-5), 127.24, 127.83, 128.47 (arom), 135.26 (C-6), 143.17 (arom), 150.47 (C-2), 163.92 (C-4); MS (EI): m/z (%) = 569 (M^+, 0.2), 326 (1.2).

3′-O-(2-Piperidinoethyl)-5′-O-tritylthymidine (**2c**)

Reaction time: 3 h; yield 0.89 g (25%); ^{1}H NMR ($CDCl_3$): $\delta = 1.42$–1.45 (2H, m, CH_2), 1.49 (3H, s, CH_3), 1.52–1.67 (4H, m, CH_2), 2.10–2.25 (2H, m, 2′-H), 2.46–2.50 (4H, t, $J = 5$ Hz, NCH_2), 2.55–2.60 (2H, t, $J = 5.9$ Hz, CH_2N), 3.34–3.59 (4H, m, CH_2O and 5′-H), 4.10–4.25 (2H, m, 3′-H and 4′-H), 6.28–6.32 (1H, m, 1′-H). 7.25–7.42 (15 H, m, arom), 7.55 (1H, s, 6-H); ^{13}C NMR ($CDCl_3$): $\delta = 11.74$ (CH_3), 23.84 (CH_2), 25.45 (2 × CH_2), 37.79 (C-2′), 54.89 (2 × CH_2), 58.16 (CH_2N), 63.85 (C-5′), 67.09

(CH_2O), 79.84 (C-3′), 83.81 (C-1′), 84.69 (CPh_3), 87.35 (C-4′), 111.03 (C-5), 127.32, 127.91, 128.56 (arom), 135.35 (C-6), 143.26 (arom), 150.24 (C-2), 163.65 (C-4).

3′-O-(2-Pyrrolidinoethyl)-5′-O-tritylthymidine [**2d**; $C_{35}H_{39}N_3O_5 \cdot 3/2H_2O$]

Reaction time: 5 h; m.p.: 125–127 °C; yield: 1.01 g (28%); ^{1}H NMR ($CDCl_3$): $\delta = 1.49$ (3H, s, CH_3), 1.80–1.90 (4H, m, CH_2), 2.10–2.50 (2H, m, 2′-H), 2.70–2.88 (6H, m, $(CH_2)_3N$), 3.30–3.55 (2H, m, 5′-H), 3.58–3.72 (2H, m, CH_2O), 4.10–4.22 (2H, m, 3′-H and 4′-H) 6.33 (1H, t, $J = 6$ Hz, 1′-H), 7.20–7.40 (15H, m, arom), 7.55 (1H, s, 6-H); ^{13}C NMR ($CDCl_3$): $\delta = 11.71$ (CH_3), 23.27 ($2 \times CH_2$), 37.63 (C-2′), 54.45 ($2 \times CH_2$), 55.18 (CH_2N), 63.86 (C-5′), 67.34 (CH_2O), 80.04 (C-3′), 83.69 (C-1′), 84.65 (CPh_3), 87.86 (C-4′), 111.09 (C-5), 127.29, 127.89, 128.52 (arom), 135.29 (C-6), 143.19 (arom), 150.37 (C-2), 163.80 (C-4); MS (EI): m/z (%) = 388 (M^+, CPh_3, 0.2).

Preparation of the quaternary ammonium nucleosides **3a–d**. *General procedure*

The tertiary amino compound (**2a–d**, 0.7 mmol) was dissolved in an excess of pure methyl iodide (2 ml). After few minutes of shaking a precipate was formed. The reaction mixture was left at room temprature for 3 h. The excess of methyl iodide was evaporated under reduced pressure and the solid washed several times with ether to give **3** in 76–80% yield.

3′-O-(2-Trimethylammoniumethyl)-5′-O-tritylthymidine iodide [**3a**; $C_{34}H_{40}IN_3O \cdot 3/2H_2O$]

M.p.: 130–132 °C; yield 390 mg (80%); ^{1}H NMR ($CDCl_3$): $\delta = 1.46$ (3H, s, CH_3), 2.15–2.60 (2H, m, 2′-H), 3.37 (9H, s, $(CH_3)_3N$), 3.80–4.10 (4H, m, CH_2O and 5′-H), 4.11 (1H, broad s, 4′-H), 4.27 (1H, broad s, 3′-H), 6.20 (1H, t, $J = 6$ Hz, 1′-H), 7.20–7.39 (15H, m, arom), 7.51 (1H, s, 6-H), 10.03 (1H, s, NH); ^{13}C NMR ($CDCl_3$): $\delta = 11.76$ (CH_3), 37.19 (C-2′), 54.86 ($(CH_3)_3N$), 63.21 (CH_2N), 63.78 (C-5′), 65.90 (CH_2O), 80.72 (C-3′), 83.52 (C-1′), 84.53 (CPh_3), 87.42 (C-4′), 111.37 (C-5), 127.36, 127.98, 128.49 (arom), 135.42 (C-6), 143.03 (arom), 150.75 (C-2), 163.93 (C-4); MS (FAB): m/z (%) = 570 (M^+, 100).

3′-O-(3-Trimethylammoniumpropyl)-5′-O-tritylthymidine iodide [**3b**; $C_{35}H_{42}IN_3O_7 \cdot 3/2H_2O$]

M.p.: 120–123 °C; yield: 385 mg (78%) ^{1}H NMR ($CDCl_3$): $\delta = 1.46$ (3H, s, CH_3), 2.10–2.16 (2H, m, CH_2) 2.20–2.50 (2H, m, 2′-H), 3.29 (9H, s, $(CH_3)_3N$), 3.43–3.48 (2H, m, CH_2N), 3.59–3.80 (4H, m, CH_2O and 5′-H), 4.05–4.20 (2H, m, 3′-H and 4′-H), 6.21 (1H, broad s, 1′-H), 7.27–7.37 (15H, m, arom), 7.54 (1H, s, 6-H), 10.03 (1H, s, NH); ^{13}C NMR ($CDCl_3$): $\delta = 11.75$ (CH_3), 29.44 (CH_2), 37.30 (C-2′), 54.04 ($(CH_3)_3N$), 63.50 (C-5′), 64.80 (CH_2N), 65.40 (CH_2O), 80.23 (C-3′), 83.59 (C-1′), 84.46 (CPh_3), 87.31 (C-4′), 111.24 (C-5), 127.29, 127.93, 128.45 (arom), 135.50 (C-6), 143.06 (arom), 150.73 (C-2), 163.99 (C-4); MS (FAB): m/z (%) = 584 (M^+, 100).

3′-O-[2-(N-Methylpiperidinium)ethyl]-5′-O-tritylthymidine iodide (**3c**)

Yield: 392 mg (76%); ^{1}H NMR ($CDCl_3$): $\delta = 1.51$ (3H, s, CH_3), 1.70–1.95 (6H, m, $3 \times CH_2$), 2.20–2.35, 2.48–2.60 (2H, $2 \times$ m, 2′-H), 3.34–4.15 (14H, m, CH_3N, $(CH_2)_3N$, CH_2O, 5′-H, 4′-H), 4.25–4.32 (1H, m, 3′-H), 6.30 (1H, t, $J = 5.2$ Hz, 1′-H), 7.22–7.40 (15H, m, arom), 7.50 (1H, s, 6-H), 9.45 (1H, s, NH); ^{13}C NMR ($CDCl_3$): $\delta = 11.75$ (CH_3), 20.08, ($2 \times CH_2$), 20.42 (CH_2), 37.19 (C-2′), 49.63 (CH_3N), 62.26, 62.89 ($(CH_2)_3N$), 63.79 (C-5′), 65.94 (CH_2O), 80.77 (C-3′), 83.52 (C-1′), 84.54 (CPh_3), 87.49 (C-4′), 111.41 (C-5), 127.37, 127.98, 128.52 (arom), 135.36 (C-6), 143.04 (arom), 150.55 (C-2), 163.67 (C-4).

3′-O-[2-(N-Methylpyrrolidinium)ethyl]-5′-O-tritylthymidine iodide (**3d**)

M.p.: 133–136 °C; yield: 385 mg (76%); ^{1}H NMR ($CDCl_3$): $\delta = 1.52$ (3H, s, CH_3), 2.10–2.35 (5H, m, $2 \times CH_2$, 2′-H), 2.50–2.65 (1H, m, 2′-H), 3.27 (3H, s, CH_3N) 3.30–3.52 (2H, m, 5′-H), 3.65–4.10 (9H, m,

CH_2O, $(CH_2)_3N$, 4′-H), 4.18–4.30 (1H, m, 3′-H), 6.22 (1H, t, $J = 6.0$ Hz, 1′-H), 7.25–7.38 (15H, m, arom), 7.50 (1H, s, 6-H), 9.35 (1H, s, NH); ^{13}C NMR ($CDCl_3$): $\delta = 11.85$ (CH_3), 21.57 (2 × CH_2), 37.20 (C-2′), 49.68 (CH_3N), 63.76 (C-5′), 65.88 (CH_2O), 65.93 ($(CH_2)_3N$), 80.94 (C-3′), 83.63 (C-1′), 84.66 (CPh_3), 87.90 (C-4′), 111.57 (C-5), 127.48, 128.07, 128.60 (arom), 135.32 (C-5), 143.09 (arom), 150.63 (C-2), 163.50 (C-4); MS (FAB): m/z (%) = 596 (M^+, 100).

Deprotection of the quaternary ammonium nucleosides **3** *to produce* **4**. *General procedure*

Compounds **3** were deprotected by refluxing in aqueous 80% acetic acid (2 ml) for 10 min. On standing, triphenylmethanol precipitated. After filtration and evaporation of water and acetic acid, the residue was washed several times with diethyl ether and then with ethanol to give **4** in 76–83% yield.

3′-O-(2-Trimethylammoniumethyl)thymidine iodide [**4a**; $C_{15}H_{26}IN_3O_5 \cdot 3/2H_2O$]

Yield: 151 mg (83%); ^{1}H NMR (D_2O): $\delta = 1.90$ (3H, s, CH_3), 2.30–2.56 (2H, m, 2′-H), 3.21 (9H, s, $(CH_3)_3N$), 3.65 (2H, t, $J = 4.9$ Hz, CH_2N), 3.78–3.81 (2H, m, CH_2O), 3.99–4.01 (2H, m, 5′-H), 4.16–4.20 (1H, m, 4′-H), 4.27–4.31 (1H, m, 3′-H), 6.24 (1H, t, $J = 6.3$ Hz, 1′-H), 7.63 (1H, s, 6-H); ^{13}C NMR (D_2O): $\delta = 12.43$ (CH_3), 36.71 (C-2′), 54.95 ($(CH_3)_3N$), 62.35 (C-5′), 63.75 (CH_2O), 66.57 (CH_2N), 80.46 (C-3′), 85.38 (C-1′), 86.07 (C-4′), 112.29 (C-5), 138.14 (C-6), 152.39 (C-2); MS (FAB): m/z (%) = 328 (M^+, 100).

3′-O-(3-Trimethylammoniumpropyl)thymidine iodide [**4b**; $C_{16}H_{28}IN_3O_5$]

M.p.: 204–206 °C; yield: 142 mg (76%); ^{1}H NMR (D_2O): $\delta = 1.90$ (CH_3), 2.09–2.18 (2H, m, CH_2), 2.27–2.53 (2H, m, 2′-H), 3.16 (9H, s, $(CH_3)_3N$), 3.44–3.50 (2H, m, CH_2N), 3.66–3.72 (2H, m, CH_2O), 3.75–3.85 (2H, m, 5′-H) 4.11–4.16 (1H, q, $J = 4$ Hz), 4.22–4.27 (1H, dt, $J = 6.1$, 3.0 Hz, 3′-H), 6.23 (1H, t, $J = 7.0$, 1′-H), 7.64 (1H, s, 6-H); ^{13}C NMR (D_2O): $\delta = 12.53$ (CH_3), 23.89 (CH_2), 36.99 (C-2′), 53.93 ($(CH_3)_3N$), 62.39 (C-3′), 65.07 (CH_2O), 66.64 (CH_2N), 80.03 (C-3′), 85.46 (C-1′), 86.00 (C-4′), 112.20 (C-5), 138.20 (C-6), 153.52 (C-2), 160.0 (C-4); MS (FAB): m/z (%) = 342 (M^+).

3′-O-[2-(N-Methylpiperidinium)ethyl]thymidine iodide [**4c**; $C_{18}H_{30}IN_3O_5 \cdot 5/2H_2O$]

Yield: 158 mg (80%); ^{1}H NMR (D_2O): $\delta = 1.64$–1.70 (2H, m, CH_2), 1.88 (3H, s, CH_3), 1.91–1.92 (4H, m, 2 × CH_2), 2.25–2.59 (2H, m, 2′-H), 3.10 (3H, s, NCH_3), 3.30–4.49 (12H, m, $(CH_2)_3N$, OCH_2, 5′-H, 4′-H, 3′-H), 6.23 (1H, t, $J = 6.4$ Hz, 1′-H), 7.62 (1H, s, 6-H); ^{13}C NMR (D_2O): $\delta = 12.36$ (CH_3), 20.43 (2 × CH_2), 21.26 (CH_2), 36.59 (C-2′), 49.86 (CH_3N), 62.32 (C-5′), 63.17 ($(CH_2)_3N$), 66.06 (CH_2O), 80.45 (C-3′), 85.38 (C-1′), 86.07 (C-4′), 112.38 (C-5), 138.14 (C-6), 152.50 (C-2), 167.22 (C-4); MS (FAB): m/z (%) = 368 (M^+).

3′-O-[2-(N-Methylpyrrolidinium)ethyl]thymidine [**4d**; $C_{17}H_{28}IN_3O_5 \cdot 2.75H_2O$]

Yield: 148 mg (77%); ^{1}H NMR (D_2O): $\delta = 1.89$ (3H, s, CH_3), 2.10–2.24 (4H, m, 2 × CH_2), 2.31–2.56 (2H, m, 2′-H), 3.35–3.61 (9H, m, CH_3N and $(CH_2)_3N$), 3.64–3.68 (2H, m, 5′-H), 3.74–3.87 (2H, m, CH_2O), 4.01–4.19 (1H, m, 4′-H), 4.29–4.37 (1H, m, 3′-H), 6.24 (1H, t, $J = 7.3$ Hz, 1′-H), 7.65 (1H, s, 6-H); ^{13}C NMR (D_2O): $\delta = 12.2$ (CH_3), 22.0 (2 × CH_2), 36.8 (C-2′), 49.3 (CH_3N), 62.7 (C-5′), 63.9 ($(CH_2)_3N$), 66.2 (CH_2O), 80.1 (C-3′), 85.2 (C-1′), 86.2 (C-4′), 112.2 (C-5), 138.5 (C-6), 152.0 (C-2); MS (FAB): m/z (%) = 354 (M^+, 100).

3,3′-O-Bisalkylation of 5′-O-tritylthymidine to give **5a**, **b**. *General procedure*

5′-*O*-Tritylthymidine (**1**, 2.99, 6 mmol) and powdered potassium hydroxide (8.7 g, 156 mmol) were suspended in a mixture of benzene (30 ml) and dioxan (10 ml). The mixture was refluxed for one hour

with virogous stirring. The appropriate dialkylaminoalkyl chloride hydrochloride (36 mmol) was added at room temperature. The mixture was refluxed for 20 h. The solvents were evaporated under reduced pressure. The residue was mixed with 200 ml of water and then extracted with methylene chloride. The organic layer was dried over Na_2SO_4, evaporated and chromatographed on silica gel (120 g, 0.04–0.063 mm) with CH_3OH (2–10%) in CH_2Cl_2 to give **5** in 50–55% yields.

3,3′-O-Bis(2-dimethylaminoethyl)-5′-O-tritylthymidine (**5a**)

M.p.: 126–129 °C; yield: 1.9 g (50%); ^{1}H NMR ($CDCl_3$): $\delta = 1.52$ (3H, s, CH_3), 2.12–2.22 (2H, m, 2′-H), 2.26, 2.32 (12H, 2 × s, 2(CH_3)$_2$N), 2.51, 2.57 (6H, 2 × t, $J = 5.7$, 7.5 Hz, 2 × CH_2N), 3.30–3.54 (4H, m, CH_2N and 5′-H), 4.06–4.20 (2H, m, OCH_2, 3′-H and 4′-H), 6.36 (1H, dd, $J = 6.0$, 7.6 Hz, 1′-H), 7.25–7.43 (15H, m, arom), 7.57 (1H, s, 6-H); ^{13}C NMR ($CDCl_3$): $\delta = 12.45$ (CH_3), 37.69 (C-2′), 38.81 (NCH_2), 45.38, 45.65 (2 × (CH_3)$_2$N), 56.25, 58.58 (2 × CH_2N), 63.63 (C-5′), 67.44 (CH_2O), 79.61 (C-3′), 83.64 (C-1′), 85.28 (CPh_3), 87.20 (C-4′), 110.05 (C-5), 127.36, 127.79, 127.92 (arom), 133.40 (C-6), 143.18 (arom), 150.70 (C-2), 163.26 (C-4).

3,3′-O-Bis(3-dimethylaminopropyl)-5′-O-tritylthymidine (**5b**)

M.p.: 135–137 °C; yield 2.2 g (55%); ^{1}H NMR ($CDCl_3$): $\delta = 1.53$ (3H, s, CH_3) 1.67–1.85 (4H, m, 2 × CH_2), 1.93–2.51 (6H, m, 2′-H, 2 × CH_2N), 2.19, 2.22 (12H, 2 × s, 2 × (CH_3)$_2$N), 3.35–3.51 (4H, m, CH_2N and 5′-H), 4.02–4.17 (4H, m, CH_2O, 4′-H and 3′-H), 6.34–6.39 (1H, dd, $J = 5.8$, 7.7 Hz, 1′-H), 7.25–7.44 (15H, m, arom), 7.56 (1H, s, 6-H); ^{13}C NMR ($CDCl_3$): $\delta = 12.45$ (CH_3), 25.46, 27.78 (2 × CH_2), 37.83 (C-2′), 39.48 (CH_2N), 45.17, 45.32 (2 × (CH_3)$_2$N), 56.22, 57.01 (2 × CH_2N), 63.78 (C-5′), 67.48 (CH_2O), 79.38 (C-3′), 83.76 (C-1′), 85.27 (CPh_3), 87.21 (C-4′), 110.07 (C-5), 127.19, 127.80, 128.48 (arom), 133.25 (C-6), 143.23 (arom), 150.67 (C-2), 163.23 (C-4).

Deprotection of the nucleosides **2a–d** *to give* **6a–d**. *General procedure*

The protected monoalkylated compounds (**2a–d**, 0.5 mmol) were refluxed for 10 minutes with aqueous 80% acetic acid (2 ml). The acetic acid solution was then left at room temperature for several hours. Precipitated triphenylmethanol was filtered off and washed with cold aqueous 80% acetic acid (1 ml). The combined filtrates were poured into ice-water (30 ml). Water and acetic acid were evaporated under reduced pressure. The residue was chromatographed on silica gel (10 g, 0.04–0.063 mm) with CH_3OH in CH_2Cl_2 (5–15%) to give **5** in 60–70% yield as acetates.

3′-O-(2-Dimethylaminoethyl)thymidine, acetic acid salt (**6a**)

Yield: 116 mg (62%); ^{1}H NMR ($CDCl_3$): $\delta = 1.88$ (3H, s, CH_3), 1.92 (3H, s, Ac), 2.15–2.48 (2H, m, 2′-H), 2.70 (6H, s, (CH_3)$_2$N), 3.10–3.15 (2H, m, CH_2N), 3.70–3.85 (4H, m, CH_2O and 5′-H), 4.08, 4.24 (2H, 2 × m, 3′-H, 4′-H), 4.90 (1H, s, OH), 6.21 (1H, t, $J = 5.2$, 1′-H) 7.8 (1H, s, 6-H); ^{13}C NMR (CD_3OD): $\delta = 12.75$ (CH_3), 24.13 (Ac), 38.28 (C-2′), 44.78 ((CH_3)$_2$N), 58.84 (CH_2N), 63.42 (C-5′), 65.73 (CH_2O), 81.81 (C-3′), 86.56 (C-1′), 86.62 (C-4′), 111.94 (C-5), 138.24 (C-6), 152.63 (C-2), 166.00 (C-4), 180.30 (Ac); MS (EI): m/z (%) = 313 (M^+, 0.05); HRMS: calcd. for $C_{14}H_{23}N_3O_5$: 313.1638, found: 313.1625.

3′-O-(3-Dimethylaminopropyl)thymidine, acetic acid salt (**6b**)

Yield: 128 mg (66%); ^{1}H NMR (CD_3OD): $\delta = 1.88$ (3H, s, CH_3), 1.92 (3H, s, Ac), 2.10–2.40 (2H, m, 2′-H), 2.62 (6H, s, (CH_3)$_2$N), 2.86–2.92 (2H, m, CH_2N), 3.55–3.59 (2H, m, CH_2O), 3.71–3.82 (2H, m, 5′-H), 4.03 (1H, q, $J = 3.3$ Hz, 4′-H), 4.14–4.17 (1H, m, 3′-H), 6.21 (1H, dd, $J = 6.0$, 7.9 Hz, 1′-H), 7.78 (1H, s, 6-H); ^{13}C NMR (CD_3OD): $\delta = 12.74$ (CH_3), 24.28 (Ac), 27.46 (CH_2), 38.47 (C-2′), 44.65 ((CH_3)$_2$N), 55.83 (CH_2N), 63.51 (C-5′), 67.98 (CH_2O), 81.32 (C-3′), 86.60 (C-1′), 86.82 (C-4′), 111.98 (C-5), 138.30 (C-6), 152.69 (C-2), 166 (C-4), 181 (Ac); MS (EI): m/z (%) = 327 (M^+, 1.1).

3'-O-(2-Piperidinoethyl)thymidine, acetic acid salt [**6c**; $C_{19}H_{31}N_3O_7 \cdot 7/4H_2O$]

Yield: 124 mg (60%); ^{1}H NMR (CD_3OD): $\delta = 1.55$–1.61 (2H, m, CH_2), 1.71–1.78 (7H, m, CH_3 and $2 \times CH_2$), 1.88 (3H, s, Ac), 2.23–2.53 (2H, m, 2'-H), 2.87–3.11 (6H, m, $(CH_2)_3N$), 3.72–3.84 (4H, m, CH_2O and 5'-H), 4.12–4.16 (1H, m, 4'-H), 4.21–4.25 (1H, m, 3'-H), 6.25 (1H, t, $J = 6.5$ Hz, 1'-H), 7.58 (1H, s, 6-H); ^{13}C NMR (CD_3OD): $\delta = 11.74$ (CH_3), 23.81 (CH_2), 24.29 (Ac), 25.15 ($2 \times CH_2$), 38.31 (C-2'), 55.34 ($2 \times CH_2$) 58.43 (CH_2N), 63.45 (C-5'), 65.77 (CH_2O), 81.78 (C-3'), 86.56 (C-1'), 86.64 (C-4'), 111.95 (C-5), 138.26 (C-6), 152.63 (C-2), 166 (C-4), 186.60 (Ac); MS (EI): m/z (%) = 353 (M^+, 0.8).

3'-O-(2-Pyrrolidinoethyl)thymidine, acetic acid salt (**6d**)

Yield: 140 mg (70%); ^{1}H NMR (CD_3OD): $\delta = 1.88$ (3H, s, CH_3), 1.92 (3H, s, Ac), 1.95–2.03 (4H, m, $2 \times CH_2$), 2.14–2.46 (2H, m, 2'-H), 3.15–3.31 (6H, m, $(CH_2)_3N$), 3.71–3.83 (4H, m, CH_2O and 5'-H), 4.08 (1H, q, $J = 3.0$, 4'-H), 4.20–4.25 (1H, m, 3'-H), 6.23 (1H, dd, $J = 5.9$, 8.0, 1'-H), 7.78 (1H, s, 6-H); ^{13}C NMR (CD_3OD): $\delta = 12.75$ (CH_3), 24.33 ($2 \times CH_2$ and Ac), 38.30 (C-2'), 55.79 ($2 \times CH_2$), 56.19 (CH_2N), 63.46 (C-5'), 66.68 (CH_2O), 81.86 (C-3'), 86.60 (C-1'), 86.60 (C-4'), 111.97 (C-5), 138.28 (C-6), 152.66 (C-2), 166.59 (C-4), 180.68 (Ac); MS (EI): m/z (%) = 339 (M^+, 0.3).

Microanalyses

	Found:			Calcd:		
	C	H	N	C	H	N
3a $C_{33}H_{37}N_3O_5 \cdot 1/2H_2O$	69.63	6.87	7.24	70.19	6.78	7.44
3b $C_{34}H_{39}N_3O_5 \cdot 3/4H_2O$	70.07	6.91	7.08	70.02	6.99	7.20
3d $C_{35}H_{39}N_3O_5 \cdot 3/4H_2O$	68.93	6.70	7.01	69.06	6.95	6.90
5c $C_{19}H_{31}N_3O_7 \cdot 7/4H_2O$	51.54	7.70	8.91	51.28	7.81	9.44
6a $C_{34}H_{40}IN_3O_5 \cdot 3/2H_2O$	55.96	5.68	5.81	56.35	5.98	5.79
6b $C_{35}H_{42}IN_3O_5 \cdot 3/2H_2O$	56.78	6.04	5.61	56.78	6.14	5.69
7a $C_{15}H_{26}IN_3O_5 \cdot 3/2H_2O$	37.11	6.19	8.62	37.35	6.06	8.71
7b $C_{16}H_{28}IN_3O_5$	40.74	6.14	8.74	40.95	6.01	8.93
7c $C_{18}H_{30}IN_3O_5 \cdot 5/2H_2O$	39.61	6.45	7.09	39.28	6.41	7.63
7d $C_{17}H_{28}IN_3O_5 \cdot 2\,3/4H_2O$	38.99	6.13	8.12	38.46	6.35	7.91

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