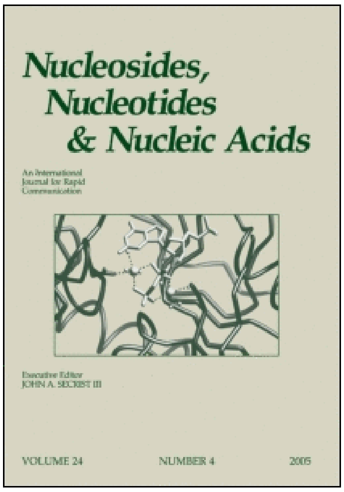

Nucleosides, Nucleotides and Nucleic Acids

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USE OF CYCLOHEXENE EPOXIDES IN THE PREPARATION OF CARBOCYCLIC NUCLEOSIDES

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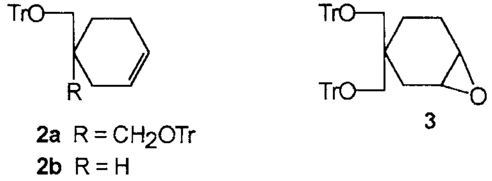
Abstract[2] : A simple route towards 4,4-dihydroxymethyl-cyclohexane nucleosides has been developed. The structure of 4,4-dihydroxymethyl-1-(thymin-1-yl)-cyclohexane was confirmed by X-ray analysis. The synthesized compounds were inactive against all viruses tested.

Recently it was shown that 1,5-anhydrohexitol nucleosides have remarkable antiviral properties[3], the most promising of this series being the 5-iodouracil[3] and the 5-ethyluracil[4] congeners. Both compounds are highly active anti-herpes agents without toxicity to the host cells. These data stimulated us to synthesize other nucleoside analogues with a six membered ring structure. Here we present the synthesis of 4,4-dihydroxymethyl substituted cyclohexane nucleosides. A literature analysis reveals that only a few cyclohexane nucleosides have been prepared, as compared with more than 300 papers dealing with cyclopentane nucleosides[5,6]. This article deals with the preparation of a series of cyclohexane nucleosides having two hydroxymethyl groups in the 4'-position (**1a-c**). Cyclohexane adenosine nucleosides were prepared for the first time by H. Schaeffer and coworker. They prepared the 1,2-, 1,3- and 1,4- substituted compounds[7-9] together with a 2'-deoxyadenosine[10] analogue. Both substituents were positioned either in cis[7,8], or trans[7-9]. Their synthetic scheme invariably started with the synthesis of the corresponding amino alcohol followed by construction of the purine heterocycle. Recently, the 1,2-trans derivative was prepared by direct alkylation of thymine with cyclohexene oxide[11]. The 1,3-substituted guanine derivatives were synthesized by Michael addition of 6-chloroguanine to cyclohexenone, followed by reduction and separation of the cis and trans isomers[12]. In a recent report[11], several related examples of Michael addition were described. Carbocyclic β-D-glucopyranosyladeninewas prepared utilizing also this type of addition reaction for the introduction of the base moiety[13]. Finally, some adenine cyclohexane derivatives were obtained using a palladium catalyzed reaction of adenine with epoxides[14].



SCHEME 1

Synthesis

For the preparation of the carbocyclic nucleoside analogues (**1a-c**) we decided to use **2a** as starting material, in the hope that the bulky trityl group will provide some regioselectivity during the alkylation step.

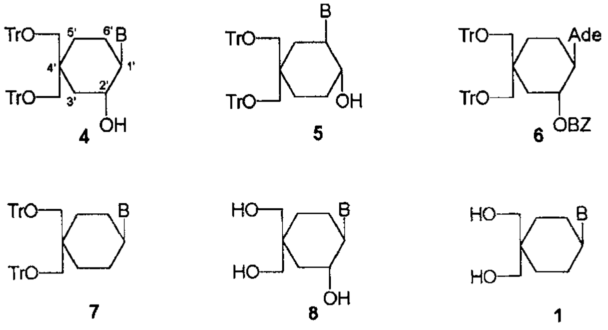
Epoxidation of **2a**[15] gave **3** in 95% yield (Scheme 1). When using **2b** as starting material an intractable mixture of trans and cis isomers were formed in a ratio of 1:1 to 2:3. The epoxidation of **2b** was examined in different solvents (benzene, toluene, ether, chloroform, dichloroethane and methylene chloride) at different temperatures. The yields are over 90% in each solvent. The purity and ratio of the isomers were determined by ^{1}H and ^{13}C NMR spectra. The cleanest reaction was observed in aromatic solvents such as benzene and toluene. We were not able to separate this mixture on a preparative scale using silica gel column chromatography or crystallization. It should be mentioned that in the case of cyclopentene derivatives epoxidation is much more stereoselective[16].

Opening of the epoxide **3** with the sodium salt of adenine necessitates severe conditions (boiling for 5-7 h in dimethylformamide). A complex mixture was obtained which could be separated by silica gel chromatography. The main products, **4a** and **5a**, were obtained in 28% combined yield in a ratio 4:1 (Scheme 2). The reaction of the epoxide **3** with heterocyclic bases in the presence of DBU under reflux temperature in DMF was even less regioselective (**4a**:**5a** = 3:1), and the yield was lower.

The alkylation of thymine gave the same regioselectivity and the yield was higher (56%). It was not possible to separate these isomers by silica gel column chromatography, but fortunately the main isomer could be isolated by direct crystallization. It should be mentioned that the starting epoxide is extremely stable. Even after boiling for several hours some of the starting compound **3** remained unchanged.

^{1}H NMR-spectra of racemic **4** and **5** are very complicated due to the presence of five CH$_2$ groups with diastereotopic protons. The structure of the prepared compounds was confirmed by ^{13}C NMR and mass spectra. Additional information was obtained from the 2'-O-benzoyl derivative **6** (Table 1).

In order to obtain further structure proof, racemic **4a,b** were converted to the symmetrical derivatives **7a,b** using standard deoxygenation procedure[17] (phenoxythiocarbonyl chloride;



a. B = Ade
b. B = Thy
c. B = Cyt
c'. B = NbzCyt

SCHEME 2

TABLE 1 : ^{13}C NMR Chemical shifts.

Compound	4a	6	7a	4b	7b	8a	1a	8b	1b
solvent :	(CDCl3)	(CDCl3)	(CDCl3)	(DMSO)	(CDCl3)	(DMSO)	(DMSO)	(D2O)	(D2O)
heterocyclic base									
C-2	152.64	152.74	152.59	151.31	150.94	152.03	152.28	153.19	152.72
C-4	149.84	150.00	149.36	163.74	163.67	149.86	149.34	166.88	164.85
C-5	118.73	119.51	119.30	108.67	110.31	119.52	119.27	111.64	111.25
C-6	154.86	155.29	155.21	136.70	135.91	156.10	156.18	139.96	139.98
C-8	137.88	138.19	137.83	-	-	140.85	139.46	-	-
Me-5	-	-	-	12.39	12.43	-	-	11.86	11.83
cyclohexane residue									
C-1'	60.59	56.52	53.00	60.99	53.30	61.46	54.39	66.80	56.22
C-2'	68.62	72.15	29.55	68.55	29.60	66.30	28.14	66.80	28.20
			and		and		and		and
C-3'	38.92	36.10	27.89	38.44	26.02	38.21	27.48	37.03	26.00
C-4'	40.65	40.88	37.98	40.93	37.82	41.30	38.40	41.21	38.18
C-5'/C-6'	26.92	26.62	27.89	24.97	26.02	26.64	27.48	25.13	26.00
	and	and	and	and	and	and	and	and	and
	28.82	28.53	29.55	28.70	29.60	27.57	28.14	27.43	28.20
2 x CH_2O	68.62	68.61	68.95	68.55	69.00	67.65	67.91	68.07	68.70
	and	and	and	and	and	and	and	and	and
	61.31	61.36	60.30	64.78	60.22	61.87	60.70	61.97	61.49

Characteristic signals of trityl group in **4a,b**, **6** and **7a,b** : 143.99, 128.81, 127.67, 126.77 and 86.15.

AIBN, Bu_3SnH) in high yields. This conversion results in the simplification of the spectra : the two doublets for each CH_2OTr group in **4** are converted in two singlets in the ^1H NMR spectra of 7 and in identical chemical shifts of 2'-C and 6'-C, 3'-C and 5'-C (Table 1) in the ^{13}C NMR spectra. This conversion confirmed the structure of the whole series of synthesized compounds. Additional proof was obtained by determination of the X-ray structure of **1b**.

Detritylation of **4a-b** and **7a-b** was performed in two steps. The compounds were first heated with 90% aqueous acetic acid. Under these conditions up to 30% of the nucleosides were converted into their O-acetylated derivatives. Deacetylation was accomplished by ammonia in methanol to give crystalline **8a-b** and **1a-b** in high yield.

Alkylation of N^4-benzoylcytosine yielded a 3:1 mixture of the benzoylated **4c'** and **5c'**. Numerous attempts to separate this mixture or to separate mixtures of the debenzoylated compounds itself (**4c** and **5c** or **8c** and its regiosiomer) using silica gel chromatography and crystallization techniques failed.

In conclusion, the obtained results demonstrate that cyclohexane epoxides may be used as starting material for the synthesis of carbocyclic nucleosides. The straightforward synthetic procedure, however, is limited by the difficult problems encountered during the separation of the obtained regioisomers.

Conformational studies

$C_{13}H_{20}N_2O_4.1/2H_2O$, $M_r = 277.32$, colourless crystals, monoclinic, *I*2, $a = 13.531$ (3), $b = 10.4305$ (10), $c = 19.205$ (4) Å, $\beta = 90.515$ (13)°, $V = 2710.4$ (9) Å^3, $Z = 8$, $D_c = 1.359$, Stoe STADI4 computer controlled four-circle diffractometer, graphite-monochromated Mo K_α-radiation, $\lambda = 0.71069$, $\mu = 0.103$ mm-1, $F(000) = 1192$, $T = 292$ K, crystal size : 0.7 x 0.3 x 0.3 mm, $1.83 \leq 0 \leq 27.51$, $-2 \leq h \leq 9$, $-2 \leq k \leq 13$, $-24 \leq \iota \leq 24$, reflections collected : 4353, independent reflections : 2765 ($R_{int} = 0.0214$), absorption corrections by the method of North[18], structure solved using *SIR92*[19], full-matric least-squares refinement on F^2 using *SHELXL-93*[20], G.O.F. on $F^2 = 1.039$, final R [$I>2\sigma(I)$] = 0.034, extinction coefficient : 0.0057 (2), largest diff. peak and hole : 0.149 and -0.147 e.Å^{-3}, geometry calculations using PARST[21].

Biological activity

Compounds **1a**, **1b**, **8a**, **8b** and the mixture of **8c** and its regioisomer were tested against human immunodeficiency virus (HIV-1, HIV-2), herpes simplex virus [HSV-1, (thymidine kinase deficient) TK^- HSV-1, HSV-2], vaccinia virus, vesicular stomatitis virus, Coxsackie virus B4, polio virus-1, parainfluenza-3 virus, reovirus-1, Sindbis virus and Semliki forest virus. All compounds were found to be inactive at the highest concentration tested (400 µg/mL). No cytotoxicity was observed in Vero cell cultures, Hela cell cultures and E_6SM cell cultures at concentrations up to 400 µg/mL.

TABLE 2 : Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3). U(eq) defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(e)
Molecule A	6973 (2)	2987 (2)	2884 (1)	30 (1)
C1'	5900 (2)	3319 (2)	3016 (1)	41 (1)
C2'	5434 (2)	3815 (2)	2359 (1)	43 (1)
C3'	5508 (2)	3000 (2)	1721 (1)	28 (1)
C4'	6590 (2)	2611 (2)	1639 (1)	35 (1)
C5'	7066 (2)	2050 (2)	2284 (1)	32 (1)
C6'	4846 (2)	1859(2)	1818 (1)	37 (1)
C7'	4952 (1)	871 (2)	1296 (1)	48 (1)
O7'	5168 (2)	3729 (2)	1067 (1)	49 (1)
C8'	4161 (1)	4128 (2)	1138 (1)	57 (1)
O8'	7515 (1)	2520 (2)	3525 (1)	31 (1)
N1	8237 (2)	3252 (2)	3808 (1)	35 (1)
C2	8520 (1)	4238 (1)	3548 (1)	50 (1)
O2	8639 (1)	2805 (2)	4414 (1)	37 (1)
N3	8395 (2)	1670 (2)	4756 (1)	32 (1)
C4	8824 (1)	1365 (2)	5304 (1)	47 (1)
O4	7635 (2)	925 (2)	4432 (1)	31 (1)
C5M	7270 (2)	-303 (2)	4770 (1)	31 (1)
C6	7203 (2)	1378 (2)	3841 (1)	31 (1)
O1	5000	1186 (2)	5000	39 (1)
Molecule B				
C1'	3019 (2)	-2094 (2)	2115 (1)	34 (1)
C2'	2939 (2)	-1139 (2)	2711 (1)	35 (1)
C3'	3423 (2)	-1737 (2)	3367 (1)	37 (1)
C4'	4503 (2)	-2119 (2)	3271 (1)	27 (1)
C5'	4610 (2)	-3003 (2)	2616 (1)	36 (1)
C6'	4073 (2)	-2419 (2)	1964 (1)	42 (1)
C7'	5173 (2)	-941 (2)	3195 (1)	30 (1)
O7'	5077 (1)	-13 (2)	3717 (1)	45 (1)
C8'	4822 (2)	-2896 (2)	3910 (1)	43 (1)
O8'	5825 (2)	-3228 (2)	3866 (1)	71 (1)
N1	2509 (2)	-1640 (2)	1481 (1)	31 (1)
C2	1766 (2)	-2384 (2)	1200 (1)	32 (1)

(continued)

TABLE 2. Continued.

O2	1489 (1)	-3408 (2)	1467 (1)	50 (1)
N3	1351 (2)	-1907 (2)	592 (1)	36 (1)
C4	1598 (2)	-806 (2)	241 (1)	36 (1)
O4	1176 (1)	-554 (2)	-302 (1)	50 (1)
C5	2397 (2)	-68 (2)	567 (1)	31 (1)
C5M	2694 (2)	1122 (3)	213 (1)	54 (1)
C6	2779 (2)	-539 (2)	1154 (1)	35 (1)
O2	5000	-364 (2)	0 (1)	43 (1)

The chair puckering for the sequence C1'-C2'-C3'-C4'-C5'-C6' can be described using the method of Cremer and Pople[22] with phase angles ϕ_2 = -23 (2), 27 (3) and θ_2 = 5.92 (2), 5.27 (2)° and a total puckering amplitude Q_t = 0.544 (2), 0.507 (2) Å for molecule A and B, respectively.

The crystal packing is determined by hydrogen bonds summarised in Table 3.

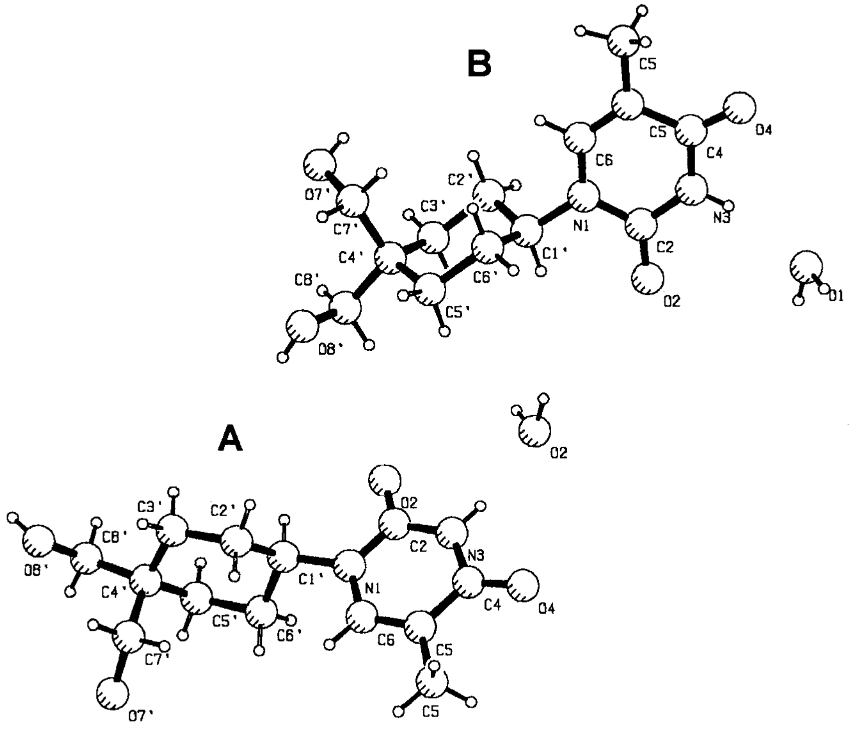
The orientation of the base moiety with respect to the cyclohexyl part is described by the C6'-C1'-N1-C2 torsion angle : -112.0 (2)° for molecule A and -121.6 (2)° for molecule B. Ohter important torsion angles are : C3'-C4'-C7'-O7' and C3'-C4'-C8'-O8' : -170.6 (2), -59.8 (2)° for molecule A and 51.8 (2), -177.3 (2)° for molecule B.

TABLE 3 : Geometry of hydrogen bonds with e.s.d.'s in parentheses (distances in Å, angles in °).

A B C	AB	BC	AC	ABC
O2WB-H2W...O7'A(i)	.920 (2)	2.804(2)	1.941(2)	155.3(1)
N3A-H3...O2WB(ii)	.860(2)	2.875(2)	2.025(2)	169.6(2)
O8'A-H8'...O4A(iii)	.820(16)	2.865(2)	2.050(16)	172.7(13)
O1WA-H1W...O7'B(iv)	.897(2)	2.766(2)	1.931(2)	153.9(11)
N3B-H3A...O1WA(v)	.860(3)	2.924(2)	2.084(2)	165.4(2)
O8'B-H8'5...O4B(vi)	.820(2)	2.941(3)	2.157(22)	159.9(15)

Equivalent positions :

(i)	x, y, z
(ii)	x+1/2, y+1/2, z+1/2
(iiii)	x-1/2, y+1/2, z-1/2
(iv)	-x+1, y, -z+1
(v)	x-1/2, y-1/2, z-1/2
(vi)	x+1/2, y-1/2, z+1/2



A PLUTON[23] view showing the numbering scheme of the two molecules.

Experimental

Melting points were determined with a Buchi-Tottoli apparatus and are uncorrected. Ultraviolet spectra were recorded with a Philips PU8740 UV/VIS scanning spectrophotometer. The NMR spectra were determined with a Gemini 200 NMR Spectrometer. Liquid secondary ion mass spectra (LSIMS) were obtained using a Kratos Concept 1H mass spectrometer. Column chromatography was performed on Janssen Chimica silica gel (0.06-0.20 mm) in methylene chloride - methanol mixtures.

4,4-Ditrityloxymethylcyclohexene (2a) A mixture of 4,4-dihydroxymethylcyclohexene (7.1 g, 50 mmol) and trityl chloride (30.7 g, 110 mmol) in 100 ml of dry pyridine was kept at 20°C for 7 days. After the usual work up procedure, silica gel column chromatographic purification and crystallization from a mixture of methylene chloride and hexane, compound **2a** was obtained in 68% yield (21.4 g). M.p. 140-141°C. LSIMS: M+Na 649. ^{1}H NMR ($CDCl_3$): 7.45-7.10 (m, 30H, 2Tr), 5.35 (m, 2H, HC=CH), 3.26 (d, 2H, J = 8.4 Hz, C$\underline{H}$HOTr), 3.14 (d, 2H, J = 8.4 Hz, CH$\underline{H}$OTr), 1.91 (m, 2H, CH_2), 1.50 (m, 4H, 2 CH_2). ^{13}C NMR ($CDCl_3$): 144.2, 128.8, 127.8, 127.5, 127.1, 126.9, 126.6 and 85.9 (2 Tr), 125.8 and 124.4 (HC=CH), 65.3 (2 CH_2OTr), 37.5 (C-4), 30.5 (C-3), 26.7 (C-6), 21.3 (C-5). Anal. Calc. for $C_{46}H_{42}O_2$: C 88.14; H 6.75. Found: C 88.44; H 6.89.

4-Trityloxymethylcyclohexene (2b). Analogous tritylation of 4-hydroxymethylcyclohexene yielded **2b** as an oil which slowly crystallized on standing (95%). LSIMS: M+Na 377. ^{1}H NMR ($CDCl_3$): 7.50-7.15 (m, 15H, Tr), 5.67 (m, 2H, HC=CH), 2.96 (d, 2H, J = 6.2 Hz, CH_2OTr), 2.20-1.20 (m, 6H, 3 CH_2). ^{13}C NMR ($CDCl_3$): 144.3, 128.6, 127.5, 126.7, and 86.0 (Tr), 126.9 and 126.1 (HC=CH), 68.0 (CH_2OTr), 34.4 (C-4), 28.8 (C-3), 25.8 (C-6), 24.7 (C-5).

4,4-Ditrityloxymethyl-1,2-epoxycyclohexane (3). To a cold (0°C) solution of **2a** (10.4g, 16.6 mmol) in 120 ml of dry methylene chloride was added 3.44g (20 mmol) of m-chloroperbenzoic acid and the mixture was stirred at 0°C for 2.5 h. After usual work up and crystallization from a mixture of methylene chloride and hexane **3** was obtained in 95% yield (10.12 g). M.p. 187-188°C. LSIMS: M+Na 665. ^{1}H NMR ($CDCl_3$): 7.48-7.10 (m, 30H, 2 Tr), 3.17 (brs, 2H, CH_2OTr), 3.15 (d, 1H, J = 8.4 Hz, $C\underline{H}HOTr$), 3.02 (d, 1H, J = 8.4 Hz, $CH\underline{H}OTr$), 2.72 (m, 2H, H-1,2), 1.90-1.05 (m, 6H, 3 CH_2). ^{13}C NMR ($CDCl_3$): 144.0, 143.9, 128.7, 127.8, 127.5, 127.1, 126.7, 86.1 and 86.0 (2 Tr), 67.9 (CH_2OTr), 62.3 (CH_2OTr), 51.7 (C-2), 50.0 (C-1), 36.0 (C-4), 29.0 (C-3), 22.7 (C-6), 19.8 (C-5). Anal. Calc. for $C_{46}H_{42}O_3$: C 85.95; H 6.59. Found: C 86.03; H 6.68.

Epoxidation of 4-trityloxymethylcyclohexene. Analogous epoxidation of **2b** in either benzene, or toluene, or ether, or chloroform, or dichloroethane or methylene chloride gave a mixture of trans and cis isomers. Yields 95-98%. The ratio of products was determined from ^{13}C NMR spectra in $CDCl_3$: 67.8 and 67.4 (CH_2OTr), 52.8 and 52.5; 51.8 and 51.3 (C-1; C-2).

4,4-Ditrityloxymethyl-1-(adenin-9-yl)-2-hydroxymethylcyclohexane (4a) and 5,5-ditrityloxymethyl-1-(adenin-9-yl)-2-hydroxymethylcyclohexane (5a).

A. To a stirred suspension of dry adenine (0.675 g, 5 mmol) in dry DMF (10 ml) 60% NaH in oil (0.2 g, 5 mmol) was added. The mixture was stirred for 1 h at 100°C. After addition of the epoxide **3** (2.35 g, 3.66 mmol), the mixture was refluxed for 5 h, evaporated to dryness, extracted with methylene chloride, and the organic layer was washed with water and dried. The residue was purified by column chromatography on silica gel (50 g). Elution with methylene chloride gave starting compound **3** (0.8 g, 1,23 mmol, 34%). Further elution with 3% methanol in methylene chloride gave a mixture of **4a** and **5a** (0.8 g, 28%). The ratio of the products was determined from ^{1}H NMR spectra in $CDCl_3$: 8.10 (s, 0.8H, H-8), and 8.00 (s, 0.2H, H-8). This mixture was separated on a column of silica gel (50 g) in 2.5% methanol in methylene chloride (a mixed fraction was chromatographed a second time under the same conditions) to give **4a** as a foam. Yield 0.67 g (24%). TLC: Rf 0.47 (methylene chloride - methanol 9:1). LSIMS: M+H 778. ^{1}H NMR ($CDCl_3$): 8.10 (s, 1H, H-8), 7.55-7.20 (m, 31H, H-2, 2 Tr), 5.80 (brs, 2H, NH_2, exchangeable with D_2O), 4.12 (m, 1H, H-2'), 3.55 (m, 1H, H-1'), 3.54 (d, 1H, J = 9.6 Hz, $C\underline{H}HOTr$), 3.43 (d, 1H, J = 9.6 Hz, $CH\underline{H}OTr$), 3.39 (d, 1H, J = 8.4 Hz, CHHOTr), 3.21 (d, 1H, J = 8.4 Hz, CHHOTr), 2.40-1.20 (m, 6H, 3 CH_2). Further elution with the same solvent gave **5a** as a foam : yield 0.15 g (5%). TLC: Rf 0.43 (methylene chloride - methanol 9:1). ^{1}H NMR ($CDCl_3$): 8.00 (s, 1H, H-8), 7.52-7.10 (m, 31H, H-2, 2 Tr), 5.91 (brs, 2H, NH_2, exchanged with D_2O), 4.40 (m, 1H, H-2'), 4.18 (m, 1H, H-1'), 3.53 (d, 1H, J = 9.1 Hz, CHHOTr), 3.41 (d, 1H, J = 9.1 Hz, CHHOTr), 3.30 (d, 1H, J = 8.6 Hz, CHHOTr), 3.18 (d, 1H, J = 8.6 Hz, CHHOTr), 2.10-1.07 (m, 6H, 3 CH_2).

B. A mixture of adenine (0.945 g, 7 mmol), 1.8-diazabicyclo[5.4.0]undece-7ene (DBU) (1.05 ml, 7 mmol) and **3** (3.80 g, 5.92 mmol) in 15 ml of dry DMF was refluxed for 5 hr. The same work-up as in method A gave starting epoxide **3** (500 mg, 13%), **4a** (700 mg, 15%) and **5a** (140 mg, 3%).

4,4-Ditrityloxymethyl-1-(thymin-1-yl)-2-hydroxymethylcyclohexane (4b). Analogous alkylation of thymine (504 mg, 4 mmol) with **3** (1.94 g, 3.02 mmol) in the presence of DBU (0.60 ml, 4 mmol) in 7 ml of DMF gave after separation on silica gel the starting epoxide **3** (0.50 g, 26%) and a mixture of **4b** and **5b** (1.30 g, 56%). The ratio (3:1) between **4b** and **5b** was determined from the ^{1}H NMR spectra in DMSO-d_6: 1.92 (d, 2.25H, Me-5), and 1.85 (d, 0.75H, Me-5). The mixture of isomers was dissolved in a minimal volume of methylene chloride and hexane was added to slight turbidity. The open flask was kept at room temperature for 3 days. The precipitate was filtered and dried to give **4b** : yield 0.75 g (32%). M.p. 269-270°C (softening at 178-180°C). LSIMS: M+Na 791. ^{1}H NMR (DMSO-d_6): 11.24 (brs, 1H, NH, exchanged with D_2O), 7.62-7.15 (m, 30H, 2 Tr), 6.60 (brs, 1H, H-6), 4.83 (d, 1H, J = 4.7 Hz, HO-2', exchangeable with D_2O), 4.00 (m, 2H, H-1', H-2'), 3.41 (d, 1H, J = 7.8 Hz, CHHOTr), 3.31 (d, 1H, J = 7.8 Hz, CHHOTr), 3.23 (d, 1H, J = 7.9 Hz, CHHOTr), 3.12 (d, 1H, J = 7.9 Hz, CHHOTr), 1.92 (d, 3H, J = 1.2 Hz, Me-5), 2.25-1.12 (m, 6H, 3 CH_2). Anal. Calc. for $C_{51}H_{48}N_2O_5$ x 0.5 H_2O: C 78.74; H 6.35; N 3.60. Found: C 78.91; H 6.34; N 3.59.

4,4-Ditrityloxymethyl-1-(adenin-9-yl)-2-benzoyloxymethylcyclohexane (6). A mixture of **4a** (110 mg, 0.14 mmol), benzoyl cyanide (22 mg, 0.17 mmol) and triethylamine (0.024 ml, 0.17 mmol) in dry dioxane (2 ml) was kept at 20°C for 2.5 h and evaporated to dryness. The residue was purified by silica gel column chromatography. Yield: 120 mg, 97% as a foam. LSIMS: M+H 882. ^{1}H NMR ($CDCl_3$): 8.22 (s, 1H, H-8), 7.70-7.19 (m, 36H, H-2, 2 Tr, Bz), 5.64 (brs, 2H, NH_2, exchangeable with D_2O), 5.05 (m, 1H, H-2'), 4.56 (m, 1H, H-1'), 3.61 (d, 1H, J = 9.5 Hz, CHHOTr), 3.40 (d, 1H, J = 9.5 Hz, CHHOTr), 3.37 (d, 1H, J = 8.5 Hz, CHHOTr), 3.22 (d, 1H, J = 8.5 Hz, CHHOTr), 2.50-1.23 (m, 6H, 3 CH_2).

4,4-Ditrityloxymethyl-1-(adenin-9-yl)-cyclohexane (7a). To a stirred suspension of **4a** (800 mg, 1.03 mmol) and 4,4-dimethylaminopyridine (244 mg, 2 mmol) in dry methylene chloride (10 ml), phenoxythiocarbonyl chloride (0.184 ml, 1.34 mmol) was added at 20°C. The mixture was kept at 20°C for 1 h, water (10 ml) and methylene chloride (10 ml) were added, the organic layer was separated, dried and evaporated. The residue (900 mg) was evaporated with dry toluene (10 ml), 2,2'-azobis(2-methylpropionitrile) (30 mg), dry toluene (20 ml) and tri-n-butyltin hydride (0.538 ml, 2 mmol) were added under nitrogen. The mixture was refluxed for 2 h, evaporated and the residue was purified using silica gel column chromatography. Yield: 700 mg, 89% as a foam. LSIMS: M+H 762. ^{1}H NMR ($CDCl_3$): 8.29 (s, 1H, H-8), 7.55-7.20 (m, 31H, H-2, 2Tr), 5.69 (brs, 2H, NH_2, exchangeable with D_2O), 4.24 (m, 1H, H-1'), 3.46 (s, 2H, CH_2OTr), 3.21 (s, 2H, CH_2OTr), 2.00-0.98 (m, 8H, 4 CH_2).

4,4-Ditrityloxymethyl-1-(thymin-1-yl)-cyclohexane (7b). Starting from **4b**, **7b** was synthesized as described for the preparation of **7a** from **4a**. Yield: 85% as a foam. LSIMS: M+Na 775. ^{1}H NMR ($CDCl_3$): 9.52 (brs, 1H, NH, exchangeable with D_2O), 7.55-7.12 (m, 30H, 2 Tr), 6.32 (brs, 1H, H-6), 4.27 (m, 1H, H-1'), 3.40 (s, 2H, CH_2OTr), 3.19 (s, 2H, CH_2OTr), 1.93 (d, 3H, J = 1.2 Hz, Me-5), 2.00-0.65 (m, 8H, 4 CH_2).

4,4-Dihydroxymethyl-1-(adenin-9-yl)-2-hydroxymethylcyclohexane (8a). A solution of **4a** (500 mg, 0.643 mmol) in 90% acetic acid was heated at 100°C for 3 h, evaporated to dryness and co-evaporated with iso-propanol (5 x 10 ml). The residue was dissolved in saturated ammonia in methanol (10 ml), the solution was kept at 20°C for 16 h and evaporated to dryness. The residue was dissolved in a mixture of water (10 ml) and methylene chloride (10 ml), the water layer was separated, washed with methylene chloride (3 x 10 ml) and evaporated. The product crystallized slowly at 0°C on addition of acetone. Yield: 155 mg, 82%. M.p. 257-258°C. UV(pH 7): λ_{max} 262 nm (ε 13700), (pH 1): λ_{max} 260 nm (ε 13200). LSIMS: ($C_{13}H_{18}N_5O_3$ + H). Calc. 294.1566. Found 294.1581. ^{1}H NMR (DMSO-d_6): 8.25 (s, 1H, H-8), 8.20 (s, 1H, H-2), 7.22 (brs, 2H, NH_2, exchangeable with D_2O), 4.81 (d, 1H, J = 5.4 Hz, HO-2', exchangeable with D_2O), 4.57 (brs, 2H, CH_2OH, exchanged with D_2O), 4.40-4.05 (m, 2H, H-1', H-2'), 3.63 (brs, 2H, CH_2OH), 3.33 (brs, 2H, CH_2OH), 2.55-1.25 (m, 6H, 3 CH_2). Anal. Calc. for $C_{13}H_{18}N_5O_3$ x 0.25 H_2O: C 52.43; H 6.60; N 23.51. Found: C 52.80; H 6.59; N 23.19.

4,4-Dihydroxymethyl-1-(thymin-1-yl)-2-hydroxymethylcyclohexane (8b). Starting from **4b**, **8b** was obtained in the same way as described for the synthesis of **8a** from **4a**. Yield: 87%. M.p. 221-222°C. UV(pH 7): λ_{max} 274 nm (ε 9300), (pH 13): λ_{max} 271 nm (ε 7100). LSIMS: ($C_{13}H_{20}N_2O_5$ + H). Calc. 285.1450. Found 285.1452. ^{1}H NMR (D_2O): 7.46 (q, 1H, J = 1.2 Hz, H-6), 4.00 (m, 2H, H-1', H-2'), 3.57 (s, 2H, CH_2OH), 3.35 (s, 2H, CH_2OH), 1.81 (d, 3H, J = 1.2 Hz, Me-5), 1.95-1.15 (m, 6H, 3 CH_2). Anal. Calc. for $C_{13}H_{20}N_2O_5$ x 0.5 H_2O: C 53.23; H 7.22; N 9.55. Found: C 53.24; H 7.35; N 9.45.

4,4-Dihydroxymethyl-1-(adenin-9-yl)-cyclohexane (1a). Compound **1a** was prepared from **7a** as described for the synthesis of **8a**. Yield: 80%. M.p. 251-252°C. UV(pH 7): λ_{max} 263 nm (ε 13600), (pH 1): λ_{max} 262 nm (ε 13300). LSIMS: ($C_{13}H_{19}N_5O_2$ + H). Calc. 278.1617. Found 278.1600. ^{1}H NMR (DMSO-d_6): 8.25 (s, 1H, H-8), 8.12 (s, 1H, H-2), 7.17 (brs, 2H, NH_2, exchangeable with D_2O), 4.81 (d, 1H, J = 5.4 Hz, HO-2', exchanged with D_2O), 4.45 (brs, 2H, CH_2OH, exchangeable with D_2O), 4.25 (m, 1H, H-1'), 3.54 (brs, 2H, CH_2OH), 3.22 (brs, 2H, CH_2OH), 2.15-1.20 (m, 8H, 4 CH_2). Anal. Calc. for $C_{13}H_{19}N_5O_2$ x 0.3 H_2O: C 55.23; H 6.99; N 24.77. Found: C 55.36; H 6.89; N 24.47.

4,4-Dihydroxymethyl-1-(thymin-1-yl)-cyclohexane (1b). Compound **1b** was prepared from **7b** as described for the synthesis of **8a**. Yield: 84%. M.p. 225-226°C. UV(pH 7): λ_{max} 274 nm (ε 9200), (pH 13): λ_{max} 272 nm (ε 7000). LSIMS: ($C_{13}H_{20}N_2O_4$ + H). Calc. 269.1501. Found 269.1517.

^{1}H NMR (D_2O): 7.41 (q, 1H, J = 1.2 Hz, H-6), 4.20 (m, 1H, H-1'), 3.59 (s, 2H, CH_2OH), 3.30 (s, 2H, CH_2OH), 1.78 (d, 3H, J = 1.2 Hz, Me-5), 1.72-1.15 (m, 6H, 4 CH_2). Anal. Calc. for $C_{13}H_{20}N_2O_4$ x 0.7 H2O: C 55.58; H 7.68; N 9.97. Found: C 55.90; H 7.68; N 9.84.

Alkylation of N^4-benzoylcytosine. Alkylation of N^4-benzoylcytosine (860 mg, 4 mmol) with **3** (1.93 g, 3.0 mmols) in the presence of DBU (0.60 ml, 4 mmol) in 7 ml of DMF gave the starting epoxide **3** (0.50 g, 26%) and a mixture of 4,4-ditrityloxymethyl-1-(N^4-benzoylcytosin-1-yl)-2-hydroxymethylcyclohexane **4c'** and 5,5-ditrityloxymethyl-1-(N^4-benzoyl-cytosin-1-yl)-2-hydroxymethyl-cyclohexane **5c'** as foams (0.95 g, 37%). The ratio of benzoylated **4c'** and **5c'** was determined from ^{1}H NMR spectra in $CDCl_3$: 7.94 (d, 0.75H, J = 7.0 Hz, H-6) and 7.88 (d, 0.25H, J = 7.0 Hz, H-6). Attempts to separate this mixture using methylene chloride - methanol; toluene - ethyl acetate; toluene - ethyl acetate - methanol as eluent failed. LSIMS: M+H 858.

After debenzoylation with ammonia in methanol a mixture of regioisomers **4c** and **5c** was obtained which could not be separated in the solvents mentioned above. The ratio of products were determined from the ^{1}H NMR spectra $CDCl_3$: 6.87 (d, 0.25H, J = 7.0 Hz, H-6), 6.42 (d, 0.75H, J = 6.9 Hz, H-6), 5.71 (d, 0.75H, J = 6.9 Hz, H-5) and 5.62 (d, 0.25H, J = 7.0 Hz, H-5).

After detritylation a mixture of **8c** and its regioisomer was obtained as a foam. The ratio (3:1) was determined from ^{1}H NMR spectra in D_2O: 7.53 (d, 1H, J = 7.3 Hz, H-6), 5.89 (d, 0.25H, J = 7.3 Hz, H-5), 5.88 (d, 0.75H, J = 7.3 Hz, H-5), 4.00 (m, 2H, H-1', H-2'), 3.56 (s, 0.5H, CH_2OH), 3.51 (s, 1.5H, CH_2OH), 3.29 (s, 1.5H, CH_2OH), 3.26 (s, 0.5H, CH_2OH), 1.92-1.10 (m, 6H, 3 CH_2). UV(pH 7): λ_{max} 276 nm (ε 8600), (pH 1): λ_{max} 284 nm (ε 12300). LSIMS: M+H 270.

References

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2. This paper is dedicated to the memory of Prof. N. Prileschajew.
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