Monatshefte für Chemie 124, 37–53 (1993)

Monatshefte für Chemie
Chemical Monthly

Printed in Austria

Convergent Synthesis of 2′,3′-Dideoxy-3′-methylthio and 2′,3′-Dideoxy-3′-mercapto Nucleosides and their Disulfide Analogues – Potential Anti-HIV Agents

Kim L. Dueholm[1], **Youssef L. Aly**[1,†], **Per T. Jørgensen**[1], **Ahmed A. El-Barbary**[1,†], **Erik B. Pedersen**[1,*], and **Claus Nielsen**[2]

[1] Department of Chemistry, Odense University, DK-5230 Odense M, Denmark
[2] Retrovirus Laboratory, Department of Virology, Statens Seruminstitut, DK-2300 Copenhagen, Denmark

Summary. The iodide **4**(α) or **7** synthesized in three steps from 2-deoxy-*D*-ribose **1**, has been subjected to a number of nucleophilic substitution reactions producing the 3-benzoylthio-, 3-methylthio- and the 3-thiocyanato-2,3-dideoxy-*D-erythro*-pentofuranosides **8**, **13** and **15**, respectively, in addition to the disulfide **17** of their 3-mercapto analogue. Subjecting the thiobenzoate **8** to the Friedel-Crafts catalyzed silyl Hilbert Johnson reaction in conjunction with the silylated nucleobases of uracil, thymine and N^4-isobutyrylcytosine **9a–c** resulted in the isolation of the 2′,3′-dideoxy-3′-mercapto nucleosides **11** and their disulfides **12** subsequent to deprotection. The 2,3-dideoxy-3-methylthio-pentofuranoside **13** afforded both anomers of the 2′,3′-dideoxy-3′-methylthio nucleosides **19** and **20** under similar conditions. The first known example of a coupling directly on a 2,3-didehydro-2,3-dideoxyfuranose is presented. 2′,3′-Dideoxy-3′-mercaptocytidine showed protection against HIV-1 in MT-4 cells with $ED_{50} = 20\,\mu M$.

Keywords. Nucleosides, 2′,3′-dideoxy-3′-mercapto; Nucleosides, 2′,3′-dideoxy-3′-methylthio; Nucleosides, 2′,3′-didehydro-2′,3′-dideoxy; Disulfide, bis(2′,3′-dideoxy-nucleosid-3′-yl).

Konvergente Synthese von 2′,3′-Didesoxy-3′-methylthio und 2′,3′-Didesoxy-3′-mercapto-Nucleosiden und ihren Disulfid-Analogen – Potentielle Anti-HIV – Agentien

Zusammenfassung. Die in drei Stufen aus 2-Desoxy-*D*-ribose hergestellten Jodide **4**(α) bzw. **7** wurden einer Reihe von nucleophilen Substitutionsreaktionen unterzogen, wobei die 3-Benzoylthio-, 3-Methylthio- und 3-Thiocyanato-2,3-didesoxy-*D-erythro*-pentofuranoside **8**, **13** und **15** zusätzlich zum Disulfid **17** ihrer 3-Mercapto-Analogen entstanden. Bei der Friedel-Crafts-katalysierten Silyl-Hilbert-Johnson Reaktion des Thiobenzoats **8** in Verbindung mit den silylierten Nucleobasen Uracil, Thymin und N^4-Isobutyrylcytosin **9a–c** entstanden nach der Schutzgruppenentfernung die 2′,3′-Didesoxy-3′-mercapto-Nucleoside **11** und ihre Disulfide **12**. Unter ähnlichen Bedingungen ergaben die 2′,3′-Didesoxy-3′-methylthiopentofuranoside **13** beide Anomere der 2′,3′-Didesoxy-3′-methylthionucleoside

† On leave from Tanta University, Tanta, Egypt.

19 und **20**. Es wird das erste Beispiel einer direkten Kopplung 2,3-Didehydro-2,3-didesoxyfuranose vorgestellt. 2′,3′-Didesoxy-3′-mercaptocytidin zeigte Schutzwirkung gegenüber HIV-1 in MT-4 Zellen mit $ED_{50} = 20\,\mu M$.

Introduction

Since the human immunodeficiency virus (HIV) was found to be the causative agent of AIDS [1, 2], the interest in 2′,3′-dideoxy nucleosides has been extensive. This interest has been spurred by the selectivity with which 3′-azido-3′-deoxythymidine (*AZT*) inhibits the replication of HIV, at the present time making it the most successful drug to be employed in the treatment of patients with AIDS [3–5]. The 2′,3′-dideoxy nucleosides interact with the target enzyme – the retroviral reverse transcriptase – by competing with the natural substrate and/or by causing chain termination subsequent to incorporation into the DNA [6].

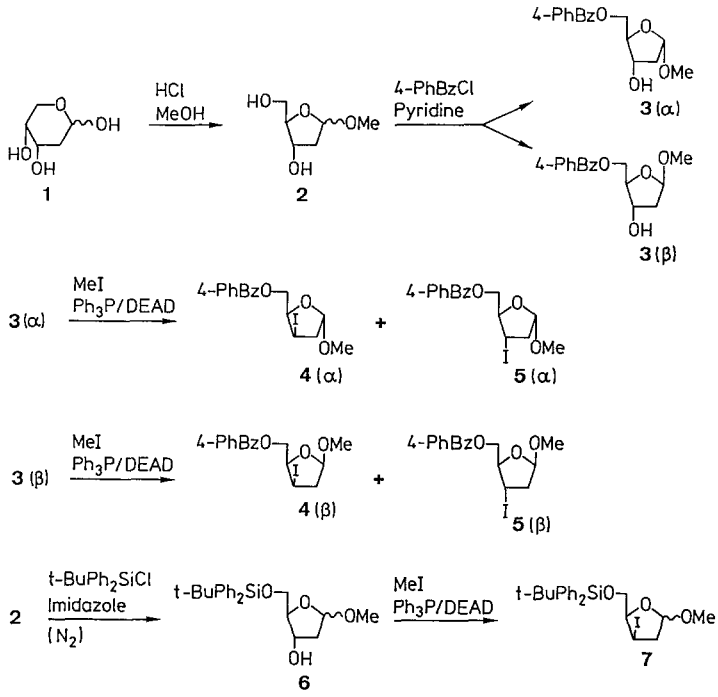
We found it interesting to synthesize various novel 2′,3′-dideoxy nucleosides with substituents in the 3′-position different from azido, yet retaining some of the characteristics of this group. The mercapto and the methylthio groups were substituents of particular interest, since their steric bulk is comparable to that of azido (as expressed by their molar refractivity values [7]: OH 2.85; SH 9.22; N_3 10.22; S*Me* 13.82) and so is their hydrophobicity (lipophilic π_x values [7]: OH −0.67; SH 0.39; N_3 0.46; S*Me* 0.61). In addition these substituents are electronically similar to azido (polar $\mathcal{F}$ values [7]: S*Me* 0.20; SH 0.28; OH 0.29; N_3 0.30).

This paper describes the convergent syntheses of 2,3-dideoxy-3-mercapto-β-*D-erthro*-pentofuranosyl nucleosides **11**, their disulfides **12**, 2,3-dideoxy-3-methylthio-β-*D-erythro*-pentofuranosyl nucleosides **19** and the α-anomers **20** of the last-mentioned.

Results

The conversion of 2-deoxy-*D*-ribose (**1**) to 5-O-protected methyl 2-deoxy-*D-erythro*-pentofuranoside **3** and **6** has been described thoroughly in the literature. Following a glycosidation with hydrochloric acid in methanol with concomitant ring contraction to the pentofuranoside [8–13], the primary hydroxy group was selectively protected. Treatment with 4-biphenylcarbonyl chloride in dry pyridine [13] afforded **3**(α) and **3**(β) which were separated by chromatography whereas treatment with *tert*-butyldiphenylchlorosilane in N,N-dimethylformamide (*DMF*) in the presence of imidazole [14, 15] allowed **6** to be isolated. Performing the latter reaction under nitrogen allowed the overall yield to be increased somewhat (to 84%).

Subjecting **3** and **6** to methyl iodide in the presence of triphenylphosphine and diethyl azodicarboxylate (*DEAD*) in dry toluene [15–17] afforded the iodides **4**, **5** and **7**. Heating at 80 °C instead of 110 °C [15] resulted in an increased yield of the mixture of **4**(α) and **5**(α) (82%). It was not possible to separate the mixture by chromatography on a silica gel column. Pure **4**(α) was obtained as white needles by fractional crystallization in 35% yield based on **3**(α), whereas a pure sample of **5**(α) was isolated by preparative thin layer chromatography on silica gel. **4**(β) and **5**(β) were separated by chromatography on silica gel column in 35% and 38% yield, respectively.



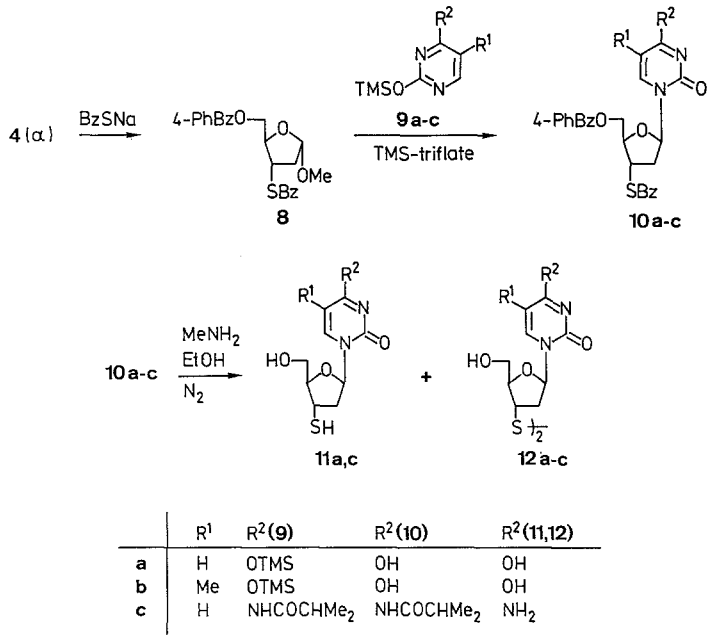
Scheme 1

Structure elucidation – i.e. assignment of configuration of **4** and **5** – is given in the discussion. Compounds **4**(α) and **7** were subjected to a number of nucleophilic substitution reactions (Schemes 2 and 3).

The benzoylthio group was introduced in the 3-position by reacting with sodium thiobenzoate in dry *DMF*, following a procedure that Cosstick and Vyle [18] have devised for the introduction of thiobenzoate in a 2′-deoxy-3′-O-methanesulphonyl-*xylo*-thymidine derivative. Methyl 3-benzoylthio-2,3-dideoxy-5-O-(4-phenylbenzoyl)-α-*D-erythro*-pentofuranoside **8** was obtained in 71% yield.

Silylation of the nucleobase in order to obtain **9** was accomplished according to standard procedures [19, 20] by refluxing the nucleobase in 1,1,1,3,3,3-hexamethyldisilazane (*HMDS*) in the presence of catalytic amounts of ammonium sulfate. When working with very small amounts, the problem of desilylation of part of the base due to contact with atmospheric moisture is frequently encountered. Therefore the standard procedure was modified by performing all operations under nitrogen. Instead of cytosine itself, N^4-isobutyrylcytosine was used (as its silylated nucleobase **9c**) due to its greater solubility in organic solvents [21].

Condensation of the thiobenzoate **8** and the silylated nucleobase **9** was carried out according to the Friedel-Crafts catalyzed [22] silyl Hilbert Johnson reaction modified by Vorbrüggen et al. [20]. The reaction was performed in dry acetonitrile in the presence of trimethylsilyl trifluoromethanesulfonate (*TMS*-triflate) producing **10** in 54–70% yield (Scheme 2). Their α-anomers were not observed. The protected nucleosides **10** were deblocked through treatment with methylamine in absolute ethanol. This reaction was performed under nitrogen to prevent oxidation of the deprotected product to the corresponding disulfide by atmospheric oxygen, as has



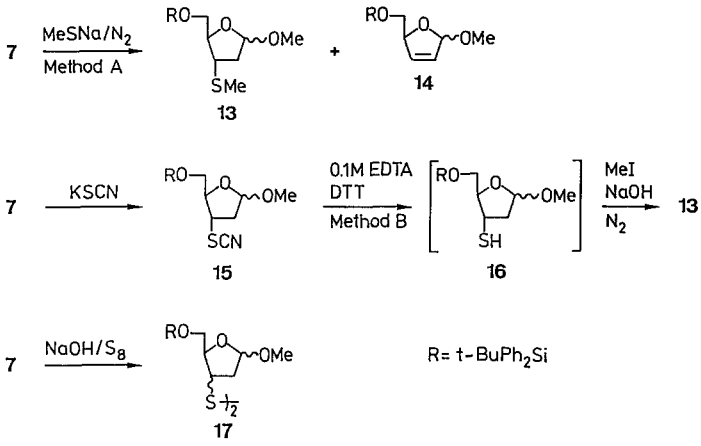
	R^1	R^2(9)	R^2(10)	R^2(11,12)
a	H	OTMS	OH	OH
b	Me	OTMS	OH	OH
c	H	$NHCOCHMe_2$	$NHCOCHMe_2$	NH_2

Scheme 2

been observed by Herdewijn et al. [23]. However, this precaution was insufficient, as the disulfide **12** was obtained along with the expected product **11**. The ratio between the two products varied from predominantly **11** in the case of **11c** to entirely **12** in the case of **12b** – presumably dependent upon the amount of oxygen that entered the system. Compounds **11** and **12** were separated by silica column chromatography (Scheme 2).

The 2,3-dideoxy-3-methylthio-*D-erythro*-pentofuranoside **13** was obtained via two alternative routes. According to the more direct procedure (Method A) **7** is simply exposed to sodium methanethiolate in absolute ethanol under reflux. However, this treatment resulted in a relatively low yield (36%), partially due to the fact that the competing elimination consumed much of the iodide (20%) as methyl 2,3-didehydro-2,3-dideoxy-5-O-(*tert*-butyldiphenylsilyl)-*D-glycero*-pentofuranoside **14** was formed. Therefore an alternative route was warranted.

Inspired by the transformations that Lin et al. [24] had made with 3'-azido-2',3'-dideoxy-5-thiocyanatouridine in order to obtain the corresponding 5-methylthio derivative, we set out to prepare the 2,3-dideoxy-3-thiocyanato-*D-erythro*-pentofuranoside **15**. Simply heating **7** in dry *DMF* in the presence of an excess of potassium thiocyanate yielded 56% of **15** (Scheme 3). The characteristic band at 2157 cm^{-1} in the IR spectrum confirms that this is the thiocyanate and not the isothiocyanate. In two steps **13** was synthesized from the thiocyanate **15** (Method B). The first step comprised the reduction of the thiocyanate function by 1,4-dithiothreitol (*DTT*) in a 0.1 *M* solution of ethylenediaminetetraacetic acid (*EDTA*) to give the thiol **16**. The second step was the alkylation of the thiol by methyl iodide in the presence of sodium hydroxide. This reaction was carried out under nitrogen to prevent loss of the thiol caused by oxidation to the corresponding disulfide by atmospheric oxygen. The transformation of **15** to give **13** proceeded with a yield of 32%. Method A is therefore

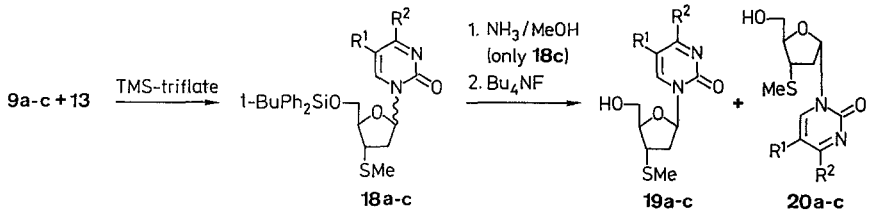


Scheme 3

considered the better of the two procedures in terms of yield but also because of its simplicity.

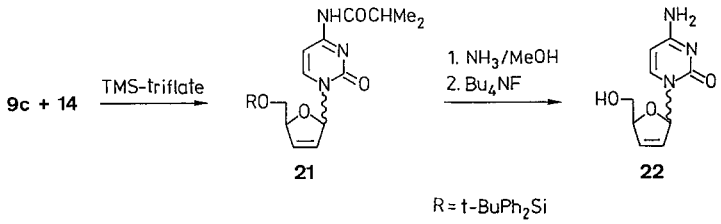
Heating a 1/1 mixture of sodium hydroxide and elemental sulfur in 96% ethanol for one hour [25] produced a deep green turbid solution, indicative of the presence of sodium disulfide. Reacting the iodide **7** with this reagent afforded several isomers **17** (inseparable by column chromatography) – the disulfides of methyl 2,3-dideoxy-3-mercapto-5-O-(*tert*-butyldiphenylsilyl)-*D*-pentofuranoside – in 57% yield (Scheme 3).

The coupling of **13** with the silylated nucleobase **9** resulted in modest yields of the anomeric mixture of the protected nucleosides **18** (Scheme 4). The coupled product might be isolated, but isolation is by no means a prerequisite for the success of the ensuing deprotection as was demonstrated with the direct deprotection of the thymine derivative **18b**. The silyl protection group was removed through treatment with a 1.1 *M* dry tetrahydrofuran solution of tetrabutylammonium fluoride [14]. In the case of **18c**, a preliminary deprotection of the amino group of the base moiety was necessary. This was accomplished by subjecting **18c** to methanolic ammonia. The nucleosides – obtained in good yields with α/β ratios close to 1.5 – were separated into their individual anomers **19** and **20** by HPLC (Scheme 4).



	R^1	R^2 (18)	R^2 (19, 20)
a	H	OH	OH
b	Me	OH	OH
c	H	$NHCOCHMe_2$	NH_2

Scheme 4



Scheme 5

As was mentioned above, the synthesis of the 2,3-dideoxy-3-methylthio-*D-erythro*-pentofuranoside **13** was accompanied by a side reaction producing the 2,3-didehydro compound **14** (Scheme 3). Subjecting a mixture of all four anomers of these compound (**13/14** = 41/59) or the α-anomer of **14** to condensation with silylated N^4-isobutyrylcytosine resulted in the anomeric mixture of the coupled product **21** in 44% and 49% yield, respectively (Scheme 5). This constitutes the first known example of a successful coupling on a 2,3-didehydro-2,3-dideoxy-furanoside. Deprotection with methanolic ammonia and subsequently with tetrabutylammonium fluoride yielded an inseparable (even by means of HPLC) ~1/1 anomeric mixture of the coupled product **22** in 43% yield.

Discussion

The 3-iodo *threo*-furanoside **4** is the expected compound from reaction of the 3-hydroxy *erythro*-furanoside **3** in a Mitsunobu reaction [26]. Formation of the corresponding 3-iodo *erythro*-furanoside **5** is easily explained assuming a neighbouring group participation of the carbonyl group from the 5-O-(4-biphenylcarbonyl) protecting group. In this way a bicyclic intermediate is formed with the carbonyl oxygen attached to C-3 allowing iodide to attack from the α-face of the sugar at C-3 with formation of **5**. An attack of iodide at C-5 of the bicyclic intermediate with formation of methyl 3-O-(4-biphenylcarbonyl)-2,5-dideoxy-5-iodo-*D-threo*-pentofuranoside should be disproved. The CH_2I group in such a product is expected to show a characteristic triplet at ca. 10 ppm in the undecoupled ^{13}C-NMR spectrum. However, we did not isolate any product with such a characteristic coupling pattern. Instead, we correctly observed the doublets of C-3 in the undecoupled ^{13}C-NMR spectra of the 3-iodo pentofuranosides **4** and **5** at 26 ppm and 15 ppm, respectively, for each of the pure anomers. The protons in the ^{1}H-NMR spectra were easily assigned using 2D-NMR and a downfield shift for H-3 and an upfield shift of H-4 were noticed for the *threo* derivative **4** when compared with the corresponding protons in the *erythro* derivative **5**.

The configuration of **4**(α) was deduced from NOE spectra. On irradiation of the H-1 proton, an NOE of 6% is observed for the resonance (2.73 ppm) of one of the H-2 protons but 0% for the resonance (2.61 ppm) of the other one. Accordingly, the former is assigned to the H-2β and the latter to the H-2α proton. As seen from Table 1, this assignment is confirmed by a large NOE in H-1 on irradiation of H-2β, and a small one on irradiation of H-2α. The *threo* configuration of **4**(α) was assigned on irradiation of the H-2α and H-2β protons; an NOE of 21% and 6%, respectively, is observed for the resonance of the H-3 proton. Accordingly, the H-3 proton is

Table 1. NOE's (%) with significance for structural assignment of compounds **4** and **5** [**4**(α), **5**(α) in *DMSO-d_6*; **4**(β) and **5**(β) in $CDCl_3$]

NOE	Irradiated proton	Compound			
		4(α)	**5**(α)	**4**(β)	**5**(β)
H-1	H-2α	5	0.5	8	9
H-1	H-2β	21	12	3	4
H-1	OCH_3	6			
H-1	H-3		0.7	0.5	
H-2α	H-1			5	
H-2α	H-2β	19	30	16	9
H-2α	H-3	5		5	0.3
H-2β	H-1	6	6	1	
H-2β	H-2α	40	25	19	14
H-2β	H-3		5	0.8	5
OCH_3	H-1	3	3	2	3
OCH_3	H-2α	0.3			
H-3	H-2α	21		7	2
H-3	H-2β	6	9	2	9
H-3	H-4	13		6	
H-4	H-2α	4			
H-4	H-3	12		4	
H-4	H-5	10		5	
H-5	H-4	2		2	

much closer to the H-2α proton than to the H-2β proton which confirmed the *threo* configuration of **4**(α). The assignment of the configurations of the compounds **4**(β), **5**(α) and **5**(β) was similarly performed, see Table 1.

The direct synthesis of **13** from the iodide **7** provides an interesting example of the influence of the solvent on the rate of the reaction. The incapability of the dipolar aprotic solvent *DMF* to solvate anions such as methanethiolate causes the rate of the reaction to be greatly increased compared to the rate in ethanol. In *DMF* the reaction is complete in less than 5 min even during cooling, whereas in ethanol 3 hours of reflux is necessary to drive the reaction to completion. However, ethanol is preferred to *DMF* as the ratio of substitution product to elimination product is more favorable in the former.

The reaction of the thiocyanate **15** with *DTT*, to give the thiol **16**, deserves comment. *DTT* apparently has two purposes: 1) It reduces the thiocyanate function [27], and 2) it reduces any disulfide that might be produced by oxidation of the desired thiol **16** [28]. Both actions are driven by the fact that *DTT* is oxidized to a stable six membered ring – an intramolecular disulfide. The *EDTA* solution acts as a buffer, thereby providing a *pH* value (7.8) favorable to the oxidation of *DTT* [28].

There is an interesting contrast between the couplings with the carbohydrate **8** compared to those with **13**. An anomeric mixture, like those obtained with **13**, is expected when employing a 2-deoxy-furanose in the coupling reaction. The lack of

a stabilizing and bulky group in the 2-position renders the intermediate carbocation susceptible to base attack at both the α- and the β-face of the ring. However, with **8** only the β-anomer is isolated. This observation might be explained as being a result of anchimeric assistance provided by the benzoylthio group. A six membered ring is formed, if the carbonyl oxygen lends assistance to the positive center and thereby blocks the access to the α-face. The size of the sulfur atom makes such a ring formation more plausible than for the corresponding benzoate, for which no reports on such a highly selective formation of the β-anomer have occurred.

Since a large number of isomers is produced in the synthesis of the disulfide **17**, coupling of this compound with a silylated nucleobase seemed to present a problem and all attempts failed. Coupling is further complicated by the combination of the hygroscopic nature of **17** and the moisture sensitivity of the silylated nucleobase.

It also seemed natural to attempt coupling on the thiocyanate **15**, since one step (parallel with the step from **15** to **16**) would allow the 5′-O-protected analogues of **11** to be obtained, whereas an additional step (parallel with the step from **16** to **13**) would allow compounds like **19** and **20** to be attained subsequent to deprotection. However, also in this instance all attempts failed.

Structure elucidation – i.e. assignment of configuration – of the products **11**, **19** and **20** was accomplished by comparison with the corresponding 3′-ethylthio and 3′-benzylthio derivatives that we have synthesized previously [29]. The structure of these compounds was elucidated with the help of 2D-NMR and NOE spectra. The chemical shifts of H-5′ and of H-4′ (in particular of the latter) indicate the configuration of C-1′. If the H-4′ is *syn* to the base moiety, it will appear at a lower field than if it is *anti* to the base moiety due to a larger deshielding. The same relationship holds for H-5′ [30]. These considerations add up to the α-anomer having H-4′ at a lower field and H-5′ at a higher field than is the case for the β-anomer. The data for **11** are also consistent with those reported for 5′-O-monomethoxytrityl-3′-thiothymidine by Cosstick and Vyle [18]. Since that compound was prepared according to a linear approach from thymidine, there is no question about its configuration at C-1′. In a similar manner the configurations of **19** and **20** were confirmed by Mansuri et al. [31], as these authors prepared **19b**.

Assignment of the disulfide structure to **12** was based on the following facts: 1) The melting point of **12b** is in agreement with that reported by Miller and Fox [32]; 2) The ^{13}C-NMR values for C-3′ of **12** are close to those of the disulfide **17**; 3) The ^{1}H-NMR data are consistent with those reported by Cosstick and Vyle [18] for the disulfide of 5′-O-monomethoxytrityl-3′-thiothymidine.

Numerous examples of the instability of 2,3-didehydro-2,3-dideoxy-furanose derivatives similar to **14** are found in the literature. Hildesheim et al. [33] reported that the β-anomer of the 5-O-benzoyl analogue of **14** is unstable to mild treatment with acid, but when pure is reasonable stable at room temperature and may be used as a synthetic intermediate. The 5-O-(triphenylmethyl) analogue of **14** was claimed to be highly unstable to traces of acid by Köll and Deyhim [34]. Even silica column chromatographic work up was sufficient to partly decompose this compound. Chu et al. [35] considered the possibility of a total synthetic approach to 3′-deoxy-2′,3′-didehydrothymidine (*D4T*) using the 1-O-acetyl analogue of **14** unlikely, due to the high instability of this compound. In our laboratory failure to couple silylated thymine and the 5-O-(4-methylbenzoyl) analogue of **14** as a synthetic approach to

D4T has also been observed [36]. Common to all of these 2,3-didehydro-2,3-dideoxy-furanose derivatives is the fact that they are readily converted to the furfuryl alcohol derivative by the elimination of acetic acid or methanol when subjected to acid.

As a mixture of **13** and the 2,3-unsaturated **14** is produced when the iodide **7** is treated with methanethiolate, and as this mixture is difficult to separate by silica chromatography when starting with an anomeric mixture of **7**, it was decided to subject the mixture to coupling conditions, in view of the observations mentioned above. The furfuryl alcohol derivative should be very easy to separate from the coupled product by silica chromatography. However, surprisingly we isolated the 2′,3′-didehydro-2′,3′-dideoxy nucleoside subsequent to a direct deprotection of the coupled product. Repeating the reaction with both the mixture and with the α-anomer of **14**, and isolating the product immediately after coupling, confirmed that the 2,3-didehydro-2,3-dideoxy-furanose derivative indeed was capable of condensating with the silylated base, although the expected furfuryl alcohol derivative was also formed. The α-anomer of **14** can be obtained by separating the anomeric mixture of **6** or of **7** and then performing the rest of the reactions leading to α-anomers of **13** and **14**, which are separable. In view of the claimed instability of 2,3-didehydro-2,3-dideoxy-furanoses, it is noteworthy that the sample of **14** subjected to coupling had been stored – although cold and in the dark – in an ether solution for more than three months.

One explanation that might be given of why coupling may be accomplished with **14** is that the ratio of Lewis acid to silylated nucleobase is important. In the conventional base coupling reaction [20, 37] an excess of the acid is used, whereas the ratio did not exceed 1 in any of the successful attempts of coupling on **14** in this work. One possible consequence is that coupling favorably competes with elimination (giving the furfuryl alcohol derivative) due to a combination of reduced acidity of the reaction mixture and an increased fraction of free nucleophilic silylated nucleobase (due to less complexation with the Lewis acid). Another possible consequence of the lower acidity is a reduced risk of destruction of coupled product that might be important in the conventional base coupling reaction.

The ^{1}H-NMR data of **22** are consistent with those reported by Horwitz et al. [38] for the β-anomer (recorded in D_2O).

The finding that a 2,3-didehydro-2,3-dideoxy-furanose may be coupled has one important implication: If the carbohydrate employed in the coupling has been formed in a step of the synthetic route – as in the case of **13** – that additionally yields a 2,3-unsaturated furanose, any contamination with the latter may in turn lead to contamination of the coupled product with the corresponding 2′,3′-unsaturated nucleoside and consequently to false indications of biological activity, as 2′,3′-didehydro-2′,3′-dideoxy nucleosides are known to inhibit HIV [39].

Antiviral Studies

The nucleosides **19a–c** and **20a–c** 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 the test compound. The 3′-mercapto

Table 2. Anti-HIV activity

Compd.	ED_{50}[a] (μM)
11c	20
12b	>100
DDC	3
AZT	0.04

[a] Effective dose of compound, achieving 50% reduction of HIV antigen production in cultures of MT-4 cells

nucleoside **11c** and the disulfides **12b, c** showed some cytoxicity at 100 μM but no activity against HSV-1 at subtoxic concentrations. The compounds **19a–c** and **20a–c** were also devoid on any activity against HIV-1 (strain HTLV-IIIB) in MT-4 cells. MT-4 cells were 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 quantitated by HIV antigen detection ELISA [40]. The 3′-methylthio nucleoside **19b** has already been reported inactive against HIV (LAV strain) in CEM cells by Mansuri et al. [31] and we also observed the inactivity against HIV-1 of the corresponding 3′-ethylthio analogues of 2′,3′-dideoxyuridine and 2′,3-dideoxycytidine [29]. In continuation of the report from Yuzhakov et al. [41] finding 3′-mercapto-3′-deoxythymidine to suppress HIV viruses as efficiently as *AZT*, we can now report that the corresponding 2′,3′-dideoxy-3′-mercaptocytidine **11c** showed protection against HIV-1 in MT-4 cells with $ED_{50} = 20\ \mu M$ (Table 2) and 30% inhibition of cell growth of uninfected MT-4 cells at 400 μM. The 3′-mercapto nucleoside **11a** and its corresponding disulfide **12a** were tested as a mixture but without showing activity against HIV-1. Neither did we observe any activity of the disulfides **12b, c** against HIV-1 in MT-4 cells although this has previously been observed for **12b** in MT-4 cells at 16 μM, but with cell toxicity at 34 μM [23].

Experimental Part

The ^{13}C- and ^{1}H-NMR spectra were recorded with a Bruker 250 FT NMR spectrometer. Mass spectra were recorded and peakmatchings determined on a Varian MAT 311A spectrometer. Microanalyses were carried out at Novo Nordisk Microanalytical Laboratory A/S, Novo Allé, DK-2880 Bagsvaerd. The silica used in the column chromatographic separations was: Merck 9385, 230–400 mesh (0.040–0.063 mm). The C,H,I-analysis for **4**(α), the C,H-analysis for **17**, and the high resolution mass spectral data (M^+) for **19a, b, c, 20a, b, c** and **22** agreed very well with the proposed molecular masses.

5-O-Protected Methyl 2,3-Dideoxy-3-iodo-D-threo-pentofuranoside [**4**(α), **4**(β), **7**] *and Methyl 2,3-Dideoxy-3-iodo-5-O-(4-phenylbenzoyl)-D-erythro-pentofuranoside* [**5**(α), **5**(β)]

To a mixture of **3**(α), **3**(β) or an anomeric mixture of **6** (15.0 mmol) and triphenylphosphine (4.30 g, 16.4 mmol) in dry toluene (50 ml) is added diethyl azodicarboxylate (*DEAD*, 2.86 g, 16.4 mmol),

dissolved in dry toluene (5 ml), dropwise at −10 °C during stirring. Stirring is maintained for 0.5 h at −10 °C. Methyl iodide (10 ml, 161 mmol in the case of **4**(α) and **4**(β) and 7 ml, 112 mmol in the case of **7**) is added dropwise to the reaction mixture at −10 °C. 15 min after the addition has been completed, the reaction mixture is heated at 80 °C for 1.5–3 h, during which time the reaction mixture changes from yellow to red. The solvent is evaporated and the residue chromatographed on a silica column ($CHCl_3$/petroleum ether (65–70 °C), 7/3, *v/v* in the case of **4**(α), **4**(β) and petroleum ether (65–70 °C)/ether, 4/1, *v/v* in the case of **7**) to give a mixture of **4**(α) and **5**(α) is an approximate ratio of 1:1 (5.41 g, 82%). **4**(α) was isolated as white needles by fractional crystallization from petroleum ether (65–70 °C) (2.27 g, 35%, m.p. 78–79 °C). Pure sample of **5**(α) was obtained by preparative TLC on silica gel (petroleum ether (65–70 °C/ether, 7:1 *v/v*) as a yellowish syrup. **4**(β) was isolated as white needles (2.3 g, 35%, m.p. 74 °C) and **5**(β) as a white solid (2.5 g, 38%, m.p. 45 °C). **7** was isolated as a clear oil (4.8 g, 64%) and had NMR spectra identical with those previously reported [15].

Methyl 2,3-Dideoxy-3-iodo-5-O-(4-phenylbenzoyl)-α-D-threo-pentofuranoside [**4**(α); $C_{19}H_{19}IO_4$]

FAB MS (3-nitrobenzylalcohol): *m/z* (%) = 439 ($M + H^+$, 6); 407 (60); 209 (16); 181 (100); 136 (32); 81 (41); 77 (16). ^{1}H-NMR (*DMSO-d$_6$/TMS*): δ = 2.61 (ddd, 1 H, H-2α); 2.73 (ddd, 1 H, H-2β); 3.32 (s, 3 H, OCH_3); 3.79 (dt, 1 H, H-4); 4.35 (dd, 1 H, H-5′); 4.43 (dd, 1 H, H-5); 4.75 (dt, 1 H, H-3); 5.25 (dd, 1 H, H-1); 7.43–8.08 (m, 9 H, Biphenyl). $J(1, 2\alpha)$ = 4.0 Hz; $J(1, 2\beta)$ = 5.2 Hz; $J(2\alpha, 2\beta)$ = 14.9 Hz; $J(2\alpha, 3)$ = 6.4 Hz; $J(2\beta, 3)$ = 3.7 Hz; $J(3, 4)$ = 4.6 Hz; $J(4, 5')$ = 4.5 Hz; $J(4, 5)$ = 6.1 Hz; $J(5, 5')$ = 11.2 Hz. ^{13}C-NMR (*DMSO-d$_6$/TMS*): δ = 26.47 (d, C-3); 45.36 (t, C-2); 54.92 (q, OCH_3); 69.42 (t, C-5); 76.45 (d, C-4); 104.32 (d, C-1); 128.86, 128.00, 128.29, 128.95, 129.77, 138.68, 144.80 (Biphenyl); 165.05 (C=O).

Methyl 2,3-Dideoxy-3-iodo-5-O-(4-phenylbenzoyl)-α-D-erthro-pentofuranoside [**5**(α)]

FAB MS (3-nitrobenzylalcohol): *m/z* (%) = 439 ($M + H^+$, 8); 307 (38); 289 (22); 181 (25); 136 (78); 77 (31). ^{1}H-NMR (*DMSO-d$_6$/TMS*): δ = 2.15 (ddd, 1 H, H-2α); 2.95 (ddd, 1 H, H-2β); 3.30 (s, 3 H, OCH_3); 4.12 (m, 1 H, H-3); 4.37–4.60 (m, 3 H, H-4, H-5); 5.11 (dd, 1 H, H-1); 7.44–8.09 (m, 9 H, Biphenyl). $J(1, 2\alpha)$ = 2.8 Hz; $J(1, 2\beta)$ = 5.5 Hz; $J(2\alpha, 2\beta)$ = 14.4 Hz; $J(2\alpha, 3)$ = 7.5 Hz; $J(2\beta, 3)$ = 9.2 Hz. ^{13}C-NMR (*DMSO-d$_6$/TMS*): δ = 15.02 (d, C-3); 44.09 (t, C-2); 54.72 (q, OCH_3); 62.55 (t, C-5); 83.11 (d, C-4); 104.36 (d, C-1); 126.88, 128.02, 128.32, 129.89, 138.70, 144.81 (Biphenyl); 165.16 (C=O).

Methyl 2,3-Dideoxy-3-iodo-5-O-(4-phenylbenzoyl)-β-D-threo-pentofuranoside [**4**(β)]

FAB MS (3-nitrobenzylalcohol): *m/z* (%) = 359 (16); 289 (22); 181 (62); 81 (100). ^{1}H-NMR ($CDCl_3$/*TMS*): δ = 2.52 (ddd, 1 H, H-2β); 2.85 (ddd, 1 H, H-2α); 3.40 (s, 3 H, OCH_3); 4.12 (dd, 1 H, H-4); 4.34 (dt, 1 H, H-3); 4.53 (dd, 1 H, H-5′); 4.53 (dd, 1 H, H-5); 5.13 (dd, 1 H, H-1); 7.39–8.17 (m, 9 H, Biphenyl). $J(1,2\alpha)$ = 5.8 Hz; $J(1, 2\beta)$ = 2.7 Hz; $J(2\alpha, 2\beta)$ = 14.3 Hz; $J(2\alpha, 3)$ = 8.1 Hz; $J(2\beta, 3)$ = 6.1 Hz; $J(3, 4)$ = 6.2 Hz; $J(4, 5')$ = 4.8 Hz; $J(4, 5)$ = 6.8 Hz; $J(5, 5')$ = 11.6 Hz. ^{13}C-NMR ($CDCl_3$/*TMS*): δ = 17.03 (d, C-3); 44.17 (t, C-2); 55.46 (q, OCH_3); 69.39 (t, C-5); 78.46 (d, C-4); 105.19 (d, C-1); 126.94, 127.15, 128.03, 128.63, 128.80, 130.16, 139.89, 144.66 (Biphenyl); 165.90 (C=O).

Methyl 2,3-Dideoxy-3-iodo-5-O-(4-phenylbenzoyl)-β-D-erythro-pentofuranoside [**5**(β)]

FAB MS (3-nitrobenzylalcohol): *m/z* (%) = 359 (3); 278 (16); 181 (58); 81 (100); 77 (17). ^{1}H-NMR ($CDCl_3$/*TMS*): δ = 2.44 (ddd, 1 H, H-2β); 2.59 (dd, 1 H, H-2α); 3.30 (s, 3 H, OCH_3); 4.3–4.6 (m, 4 H, H-3, H-4, H-5); 4.90 (d, 1 H, H-1); 7.34–8.17 (m, 9 H, Biphenyl). $J(1, 2\beta)$ = 4.8 Hz; $J(2\alpha, 2\beta)$ = 13.0 Hz; $J(2\alpha, 3)$ = 7.0 Hz; $J(2\beta, 3)$ = 7.0 Hz. ^{13}C-NMR ($CDCl_3$/*TMS*): δ = 14.54 (d, C-3); 45.84 (t, C-2); 54.73 (q, OCH_3); 63.58 (t, C-5); 86.68 (d, C-4); 104.60 (d, C-1); 123.86, 127.02, 128.15, 128.56, 128.90, 130.29, 139.91, 145.82 (Biphenyl); 166.07 (C=O).

Methyl 3-Benzoylthio-2,3-dideoxy-5-O-(4-phenylbenzoyl)-α-D-erythro-pentofuranoside (**8**)

A solution of **4**(α) (4.38 g, 10 mmol) and sodium thiobenzoate [18] (6.24 g, 39 mmol) in dry *DMF* (60 ml) is stirred at 75 °C for 3.5 h. After cooling to room temperature, CH_2Cl_2 (300 ml) is added and the mixture is extracted with saturated aqueous $NaHCO_3$ (2 × 300 ml) and with saturated aqueous NaCl (2 × 200 ml). The organic phase is dried over sodium sulfate. The solvent is removed under reduced pressure and the residue is chromatographed on a silica column ($CHCl_3$) to give the title compound as a pale brown gum (3.20 g, 71%). ^{1}H-NMR ($CDCl_3/TMS$): $\delta = 2.08$ (ddd, 1 H, H-2); 2.76 (ddd, 1 H, H-2'); 4.22 (ddd, 1 H, H-3); 4.37–4.48 (m, 1 H, H-4); 4.52 (dd, 1 H, H-5); 4.67 (dd, 1 H, H-5'); 5.18 (dd, 1 H, H-1'); 7.41–8.17 (m, 14 H, Biphenyl, Phenyl). $J(1,2) = 1.3$ Hz; $J(1,2') = 5.0$ Hz; $J(2,2') = 14.0$ Hz; $J(2,3) = 4.4$ Hz; $J(2',3) = 10$ Hz; $J(3,4) = 6.2$ Hz; $J(4,5) = 4.7$ Hz; $J(4,5') = 3.3$ Hz, $J(5,5') = 11.8$ Hz. ^{13}C-NMR ($CDCl_3/TMS$): $\delta = 40.09, 40.58$ (C-2 and C-3); 55.35 (OMe); 65.29 (C-5); 82.45 (C-4); 105.34 (C-1); 127.57, 127.70, 127.79, 127.84, 128.68, 129.19, 129.45, 130.77, 134.18, 137.04, 140.53, 146.32 (aryl); 166.79 (C=O); 192.08 (SC=O).

2,3-Dideoxy-3-mercapto-β-D-erythro-pentofuranosyl nucleosides (**11**) *and their Disulfides* (**12**)

Under an atmosphere of nitrogen the appropriate silylated pyrimidine **9** (5.5 mmol) is dissolved in dry acetonitrile (30 ml) and the thiobenzoate **8** (2.20 g, 5.0 mmol) dissolved in dry acetonitrile (20 ml) is added. The mixture is cooled to −25 °C and *TMS*-triflate (1.27 ml, 6.6 mmol) dissolved in dry acetonitrile (10 ml) is added dropwise during stirring. The reaction mixture is stirred for 5–6 h at −25 °C under nitrogen. CH_2Cl_2 (150 ml) is added to the reaction mixture and this solution is extracted with icecold saturated aqueous $NaHCO_3$ (3 × 100 ml) and cold water (3 × 100 ml). The organic phase is dried over sodium sulfate and subsequently evaporated under reduced pressure. The residue is chromatographed on a silica column ($CH_2Cl_2/MeOH$, 98/2 (**10a** and **10c**) or 95/5 (**10b**), *v/v*) to give **10a–c** (in 64%, 54% and 70% yield, respectively). The protected nucleoside **10** (0.8 mmol) is added to 33% methylamine is abs. *Et*OH (60 ml) and stirred at room temperature under nitrogen for 2–3 days. The solvent is removed under reduced pressure and the residue is chromatographed on a silica column ($CH_2Cl_2/MeOH$, 95/5 (**11a, 12a**), 97/3 (**12b**) or 7/3 (**11c, 12c**), *v/v*) to give the nucleosides **11a, c** as gums and the disulfides **12a–c**. **12a** was only isolated as a (ca. 1/1) mixture together with **11a**.

11a: Yield 13 mg (7%). ^{1}H-NMR (*DMSO-d*$_6$/*TMS*): $\delta = 2.36$ (m, 1 H, H-2'); 2.50 (m, 1 H, H-2'); 3.41 (m, 1 H, H-3'); 3.75 (m, 3 H, H-4', H-5'); 5.66 (d, 1 H, H-5); 6.10 (dd, 1 H, H-1'); 8.01 (d, 1 H, H-6). $J(1',2') = 3$ Hz and 7 Hz; $J(5,6) = 8$ Hz. ^{13}C-NMR (*DMSO-d*$_6$/*TMS*): $\delta = 33.70, 42.00$ (C-2' and C-3'); 60.74 (C-5'); 83.93, 88.82 (C-1' and C-4'); 101.64 (C-5); 140.60 (C-6); 150.45 (C-2); 163.21 (C-4).

11c: Yield 180 mg (44%). ^{1}H-NMR (*DMSO-d*$_6$/*TMS*): $\delta = 2.28$ (m, 2 H, H-2'); 3.41 (m, 2 H, H-3', H-5'); 3.69 (m, 2H, H-4', H-5'); 4.35 (br, 1 H, SH); 5.10 (br, 1 H, OH); 5.69 (d, 1 H, H-5); 6.01 (dd, 1 H, H-1'); 7.11 (br, 2 H, NH_2); 7.91 (d, 1 H, H-6). $J(1',2') = 4$ Hz and 6 Hz; $J(5,6) = 7$ Hz. ^{13}C-NMR (*DMSO-d*$_6$/*TMS*): $\delta = 33.44, 42.51$ (C-2' and C-3'); 59.09 (C-5'); 84.11, 88.48 (C-1' and C-4'); 93.30 (C-5); 140.82 (C-6); 154.90 (C-2); 165.50 (C-4).

12a: As a mixture with **11a**. ^{1}H-NMR (*DMSO-d*$_6$/*TMS*): $\delta = 2.54$ (m, 4 H, H-2'); 3.76 (m, 6 H, H-3', H-5'); 3.99 (m, 2 H, H-4'); 5.29 (br, 2 H, OH); 5.71 (d, 2 H, H-5); 6.14 (m, 2 H, H-1'); 8.00 (d, 2 H, H-6); 11.38 (br, 2 H, NH). $J(5,6) = 8$ Hz. ^{13}C-NMR (*DMSO-d*$_6$/*TMS*): $\delta = 37.41$ (C-2'); 46.36 (C-3'); 60.80 (C-5'); 84.18, 85.25 (C-1' and C-4'); 101.65 (C-5); 140.65 (C-6); 150.45 (C-2); 163.29 (C-4).

12b: Yield 176 mg (47%), m.p. 239 °C (lit. [32]: 239–241.5 °C). ^{1}H-NMR (*DMSO-d*$_6$/*TMS*): $\delta = 1.78$ (s, 6H, *Me*); 2.46 (m, 4 H, H-2'); 3.70 (m, 6 H, H-3', H-5'); 3.90 (m, 2 H, H-4'); 5.24 (br, 2 H, OH); 6.10 (t, 2 H, H-1'); 7.78 (s, 2 H, H-6); 11.31 (br, 2 H, NH). $J(1',2') = 6$ Hz. ^{13}C-NMR (*DMSO-d*$_6$/*TMS*): $\delta = 12.09$ (*Me*); 36.99 (C-2'); 46.24 (C-3'); 60.63 (C-5'); 83.37, 84.86 (C-1' and C-4'); 109.07 (C-5); 136.00 (C-6); 150.25 (C-2); 163.59 (C-4).

12c: Yield 60 mg (15%). ^{1}H-NMR (*DMSO-d*$_6$/*TMS*): $\delta = 2.28$ (m, 2 H, H-2'); 2.44 (m, 2H, H-2'); 3.65 (m, 6 H, H-3', H-5'); 3.91 (m, 2 H, H-4'); 5.18 (br, 2 H, OH); 5.72 (d, 2 H, H-5); 6.08 (t, 2 H, H-1'); 7.14 (br, 4 H, NH_2); 7.88 (d, 2 H, H-6). $J(1',2') = 6$ Hz; $J(5,6) = 7$ Hz. ^{13}C-NMR (*DMSO-d*$_6$/*TMS*):

δ = 37.87 (C-2'); 46.30 (C-3'); 60.77 (C-5'); 84.51, 84.93 (C-1' and C-4'); 93.69 (C-5); 140.83 (C-6); 154.89 (C-2); 165.51 (C-4).

Methyl 2,3-Dideoxy-3-methylthio-5-O-(tert-butyldiphenylsilyl)-D-erythro-pentofuranoside (**13**)

Method A:
To a solution of sodium methanethiolate (3.20 g, 45 mmol) in abs. *Et*OH (500 ml) is added the α-anomer of **7** (15.13 g, 30 mmol) dissolved in abs. *Et*OH (200 ml) and the reaction mixture is refluxed for 3 h under nitrogen. After cooling to room temperature, the solvent is removed under reduced pressure. The residue is dissolved in ether (350 ml) and extracted with water (3 × 200 ml). The ether phase is dried over sodium sulfate and evaporated. The residue is chromatographed on a silica column (petroleum ether (65–70 °C)/ether, 95/5, *v/v*) to give the α-anomer of **13** (4.53 g, 36%) and methyl 2,3-didehydro-2,3-dideoxy-5-O-(*tert*-butyldiphenylsilyl)-α-*D-glycero*-pentofuranoside **14** (2.30 g, 20%) as pale yellow oils.

Method B:
"0.1 *M EDTA* solution": Ethylenediaminetetraacetic acid (*EDTA*, 14.61 g, 50 mmol) is suspended in water (350 ml). Sodium hydroxide (2 *M*) is added until the *EDTA* has dissolved completely, and *pH* is adjusted to 7.8 by the dropwise addition of sodium hydroxide (4 *M*) or hydrogen chloride (4 *M*). Water is added to a total volume of 500 ml and if necessary *pH* is again adjusted to 7.8. The thiocyanate **15** (2.50 g, 5.9 mmol) is dissolved in *Me*OH (300 ml) and the "0.1 *M EDTA* solution" (300 ml) is added. 1,4-Dithiothreitol (*DTT*, 2.98 g, 19 mmol) is added to the reaction mixture during stirring. The pale purple, turbid reaction mixture is stirred at room temperature for 45 h and then evaporated under reduced pressure. The residue is suspended in *Me*OH and filtered through silica which is washed with an additional amount of *Me*OH. This solution of the thiol **16** is diluted with *Me*OH to a total volume of 450 ml. Under an atmosphere of nitrogen 0.1 *M* NaOH (118 ml) and methyl iodide (20.00 g, 141 mmol) are added and the reaction mixture is stirred at room temperature for 1.5 h. The solvents are removed under reduced pressure and the residue is chromatographed on a silica column (petroleum ether (65–70 °C)/ether, 9/1, *v/v*) to yield the anomeric mixture of **13** (0.77 g, 32% based on **15**).

13 (α-anomer): MS: *m/z* (%) = 359 (6); 311 (7); 256 (38); 199 (38); 71 (100); 61 (44), 57 (17). ^{1}H-NMR ($CDCl_3$/*TMS*): δ = 1.06 (s, 9 H, Me_3C); 1.87–2.07 (m, 4 H, H-2, *Me*S); 2.48–2.59 (m, 1 H, H-2); 3.11–3.20 (m, 1 H, H-3); 3.38 (s, 3 H, *Me*O); 3.77 (dd, 1 H, H-5); 3.87 (dd, 1 H, H-5'); 3.98–4.03 (m, 1 H, H-4); 5.06 (dd, 1 H, H-1); 7.40–7.72 (m, 10 H, aryl). $J(1,2')$ = 5.4 Hz; $J(1,2)$ = 2.0 Hz; $J(4,5')$ = 3.1 Hz; $J(4,5)$ = 4.2 Hz; $J(5,5')$ = 11.3 Hz. ^{13}C-NMR ($CDCl_3$/*TMS*): δ = 14.08 (*Me*S); 19.22 (Me_3**C**); 26.77 (***Me***$_3$C); 39.81, 42.78 (C-2 and C-3); 54.84 (*Me*O); 64.06 (C-5); 83.58 (C-4); 104.55 (C-1); 127.54, 129.56, 135.56, 135.63 (aryl).

14 (α-anomer): MS: *m/z* (%) = 311 (6); 243 (47); 213 (37); 135 (40); 71 (100); 57 (14); 41 (37). ^{1}H-NMR ($CDCl_3$/*TMS*): δ = 1.05 (s, 9 H, Me_3C); 3.37 (s, 3 H, *Me*O); 3.67 (dd, 1 H, H-5); 3.79 (dd, 1 H, H-5'); 4.9–5.0 (m, 1 H, H-4); 5.78–5.85 (m, 2 H, H-2, H-3); 6.22 (d, 1 H, H-1); 7.37–7.68 (m, 9 H, aryl). $J(1,2)$ = 6.0 Hz; $J(4,5')$ = 4.5 Hz; $J(4,5)$ = 5.6 Hz; $J(5,5')$ = 10.3 Hz. ^{13}C-NMR ($CDCl_3$/*TMS*): δ = 19.15 (Me_3C); 26.70 (Me_3C); 53.69 (*Me*O); 65.97 (C-5); 85.88 (C-4); 109.22 (C-1); 127.00 (C-2); 127.54, 129.55, 129.57, 133.41 (aryl); 133.76 (C-3); 135.50 (aryl).

Methyl 2,3-Dideoxy-5-O-(tert-butyldiphenylsilyl)-3-thiocyanato-D-erythro-pentofuranoside (**15**)

To a solution of **7** (12.35 g, 25 mmol) in dry *DMF* (100 ml) is added potassium thiocyanate (11.97 g, 125 mmol) and the reaction mixture is heated during stirring at 80 °C for 7 h. After cooling to room temperature, the reaction mixture is poured into water (500 ml) and extracted with CH_2Cl_2 (3 × 300 ml). The organic phase is dried over sodium sulfate and evaporated under reduced pressure. The residue is chromatographed on a silica column (petroleum ether (65–70 °C)/ether, 95/5, *v/v*) to give the title compound as a clear oil (6.00 g, 56%). MS: *m/z*(%) = 370 (20); 311 (23); 243 (88); 213 (81); 199 (66); 81 (37); 71 (100); 57 (6). IR (cm^{-1}): 2157 m (SCN). ^{1}H-NMR ($CDCl_3$/*TMS*):

δ = 1.07–1.08 (m, 18 H, Me_3C); 2.06–2.67 (m, 4 H, H-2); 3.27 (s, 3 H, *Me*O); 3.35 (s, 3 H, *Me*O); 3.73–3.86 (m, 6 H, H-3, H-5); 4.05–4.21 (m, 2 H, H-4); 5.04–5.11 (m, 2 H, H-1); 7.37–7.69 (m, 20 H, aryl). ^{13}C-NMR ($CDCl_3$/*TMS*): δ = 19.17 (Me_3**C**); 26.73 (***Me***$_3$C); 40.06, 40.43, 44.01, 44.71 (C-2 and C-3); 54.80 (*Me*O); 63.63, 64.15 (C-5); 84.40 (C-4); 104.06, 104.31 (C-1); 127.73, 127.77, 129.79, 129.85, 132.91, 135.17, 135.56 (aryl).

The Disulfide of Methyl 2,3-Dideoxy-3-mercapto-5-O-(tert-butyldiphenylsilyl)-D-pentofuranoside [**17**; $C_{44}H_{58}O_6S_2Si_2 \cdot 2H_2O$]

Finely ground sodium hydroxide pellets (1.09 g, 27 mmol) are dissolved in 96% *Et*OH (150 ml) and elemental sulfur (0.87 g, 27 mmol) is added during stirring. This suspension is heated at 78 °C for 1 h, during which time the suspension changes from yellow to brown to deep green. After cooling to room temperature, **7** (1.35 g, 2.7 mmol) dissolved in 96% *Et*OH (40 ml) is added and heating at 78 °C continued for 1 h. Subsequent to cooling to room temperature, the solvent is evaporated under reduced pressure. The residue is dissolved in water (150 ml) and extracted with ether (4 × 300 ml). The organic phase is dried over sodium sulfate and evaporated under reduced pressure. The residue is chromatographed on a silica column (petroleum ether (65–70 °C)/ether, 9/1, *v/v*) to give **17** (0.62 g, 57%) as a clear oil. MS: *m/z* (%) = 802 (1, M^+); 745 (9); 337 (35); 311 (94); 269 (39); 243 (56); 199 (49); 135 (54); 81 (34); 71 (100). ^{1}H-NMR ($CDCl_3$/*TMS*): δ = 1.05–1.07 (m, 18 H, Me_3C); 2.05–2.58 (m, 4 H, H-2); 3.28–3.35 (m, 6 H, *Me*O); 3.67–3.90 (m, 6 H, H-3 and H-5); 4.14–4.23 (m, 2 H, H-4); 5.02–5.09 (m, 2H, H-1); 7.37–7.70 (m, 20 H, aryl). ^{13}C-NMR ($CDCl_3$/*TMS*): δ = 19.19 (Me_3C); 26.47, 26.76 (Me_3C); 39.42, 39.55, 39.90, 40.00 (C-2); 48.02, 48.15, 48.23, 48.31, 48.61, 48.68, 48.84, 48.92 (C-3); 54.65, 54.75 (*Me*O); 64.14, 64.26, 64.77, 64.94 (C-5); 84.15, 84.34, 84.45, 84.65 (C-4); 104.46, 104.57 (C-1); 127.59, 129.59, 133.25, 134.69, 135.53, 135.59 (aryl).

1-(2,3-Dideoxy-3-methylthio-β-D-erythro-pentofuranosyl)uracil [**19a**; $C_{10}H_{14}N_2O_4S$ (MS)] *and its α-anomer* [**20a**; $C_{10}H_{14}N_2O_4S$ (MS)]

Dry acetonitrile (30 ml) is added to the oil of silylated uracil (1.74 g, 6.8 mmol) under nitrogen, and the α-anomer of the carbohydrate **13** (1.62 g, 3.9 mmol) dissolved in dry acetonitrile (20 ml) is added dropwise during stirring at temperatures below −20 °C. *TMS*-triflate (0.38 ml, 1.9 mmol) dissolved in dry acetonitrile (10 ml) is added dropwise to the reaction mixture. The latter operation is repeated after 35 min and after 2.25 h (in order to make the total amount of *TMS*-triflate added: 1.14 ml, 5.7 mmol). The reaction mixture is stirred below −20 °C for a total of 4 h. The reaction mixture is dissolved in CH_2Cl_2 (200 ml) and extracted with icecold saturated aqueous $NaHCO_3$ (500 ml) which is extracted with additional CH_2Cl_2 (3 × 100 ml). The organic phases are collected and extracted with cold water (4 × 250 ml) until neutral (very crucial). The organic phase is dried over sodium sulfate and subsequently evaporated under reduced pressure. The residue is chromatographed on a silica column (petroleum ether (65–70 °C)/ether, 9/1, *v/v* to isolate the anomerized and otherwise unreacted **13**, CH_2Cl_2 until **18a** appears and finally CH_2Cl_2/*Me*OH, 97/3, *v/v*) to isolate **18a** (0.36 g, 20%) as a pale yellow foam. The protected nucleoside **18a** (0.36 g, 0.8 mmol) is dissolved in dry tetrahydrofuran (4.8 ml), a 1.1 *M* dry tetrahydrofuran solution of tetrabutylammonium fluoride (0.87 ml, 0.96 mmol Bu_4NF) is added and the reaction mixture is stirred at room temperature for 1.5 h. The reaction mixture is evaporated under reduced pressure and the residue is chromatographed on a silica column (CH_2Cl_2/*Me*OH, 97/3, *v/v*) to yield a mixture of **19a** and **20a** (168 mg, 85%, α/β = 1.3) as an oil. This mixture is subjected to HPLC (H_2O/*Et*OH, 96/4, *v/v*) to afford the pure anomers as glassy solids.

19a: ^{1}H-NMR (*DMSO-d*$_6$/*TMS*): δ = 2.12 (s, 3 H, S*Me*); 2.35 (m, 2 H, H-2′); 3.33 (q (partly obscured), H-3′); 3.58 (dd, 1 H, H-5′); 3.71 (dd, 1 H, H-5′); 3.80 (ddd, 1 H, H-4′); 5.60 (d, 1 H, H-5); 6.04 (dd, 1 H, H-1′); 7.95 (d, 1 H, H-6). J(1′, 2′) = 7 Hz and 5 Hz; J(3′, 4′) = 7 Hz; J(4′, 5′) = 3 Hz, downfield H-5′; J(4′, 5′) = 3 Hz, upfield H-5′; J(5′, 5′) = 12 Hz; J(5, 6) = 8 Hz. ^{13}C-NMR (*DMSO-d*$_6$/*TMS*): δ = 13.16 (S*Me*); 38.13, 41.45 (C-2′ and C-3′); 60.41 (C-5′); 83.85, 85.37 (C-1′ and C-4′); 101.25 (C-5); 140.46 (C-6); 150.30 (C-2); 163.09 (C-4).

20a: ^{1}H-NMR (*DMSO-d_6/TMS*): $\delta = 2.01$ (ddd, 1 H, H-2'); 2.11 (s, 3H, S*Me*); 2.75 (ddd, 1 H, H-2'); 3.30 (q (partly obscured), H-3'); 3.44 (dd, 1 H, H-5'); 3.58 (dd, 1 H, H-5'); 4.15 (ddd, 1 H, H-4'); 5.63 (d, 1 H, H-5) 6.01 (t, 1 H, H-1'); 7.73 (d, 1 H, H-6). $J(1',2') = 6$ Hz; $J(2',2') = 14$ Hz; $J(2',3') = 8$ Hz; $J(3',4') = 7$ Hz; $J(4',5') = 3$ Hz, downfield H-5'; $J(4',5') = 4$ Hz, upfield H-5'; $J(5',5') = 12$ Hz; $J(5,6) = 8$ Hz. ^{13}C-NMR (*DMSO-d_6/TMS*): $\delta = 13.67$ (S*Me*); 38.19, 42.73 (C-2' and C-3'); 61.63 (C-5'); 85.46, 85.74 (C-1' and C-4'); 101.45 (C-5); 140.82 (C-6); 150.40 (C-2); 163.35 (C-4).

1-(2,3-Dideoxy-3-methylthio-β-D-erythro-pentofuranosyl)thymine [**19b**; $C_{11}H_{16}N_2O_4S$ (MS)] *and its α-Anomer* [**20b**; $C_{11}H_{16}N_2O_4S$ (MS)]

These compounds are prepared in analogy with **19a** and **20a** from silylated thymine (1.08 g, 4.0 mmol) and an anomeric mixture of the carbohydrate **13** (0.95 g, 2.3 mmol) in the presence of *TMS*-triflate (0.22 ml, 1.1 mmol). Twice the amount of *TMS*-triflate is added after 35 min (in order to make the total amount of *TMS*-triflate added: 0.66 ml, 3.3 mmol). The reaction time is 3 h. No chromatographic work up is performed prior to deprotection. Deprotection of the residue obtained above, dissolved in dry tetrahydrofuran (14.3 ml), is carried out with a 1.1 *M* dry tetrahydrofuran solution of tetrabutylammonium fluoride (2.59 ml, 2.9 mmol Bu_4NF, based on the carbohydrate **13**). The reaction time is 1 h. The raw material is chromatographed on a silica column (CH_2Cl_2/*Me*OH, 97/3, *v/v*) to yield a mixture of **19b** and **20b** (260 mg, 42% based on the carbohydrate **13**, $\alpha/\beta = 1.4$) as an oil. This mixture is subjected to HPLC (H_2O/*Et*OH, 92/8, *v/v*) to afford the pure anomers as glassy solids.

19b: ^{1}H-NMR (CD_3OD/*TMS*): $\delta = 1.87$ (d, 3 H, *Me*); 2.16 (s, 3 H, S*Me*); 2.42 (m, 2 H, H-2'); 3.36 (q (partly obscured), H-3'); 3.77 (dd, 1 H, H-5'); 3.85 (m, 1 H, H-4'); 3.92 (dd, 1 H, H-5'); 6.12 (dd, 1 H, H-1'); 7.92 (q, 1 H, H-6). $J(1',2') = 7$ Hz and 5 Hz; $J(2',3') = 8$ Hz; $J(3',4') = 8$ Hz; $J(4',5') = 2$ Hz, downfield H-5'; $J(4',5') = 3$ Hz, upfield H-5'; $J(5',5') = 12$ Hz; $J(6,Me) = 1$ Hz. ^{13}C-NMR (CD_3OD/*TMS*): $\delta = 12.42$ (*Me*); 14.03 (S*Me*); 40.37, 43.13 (C-2' and C-3');62.07 (C-5'); 86.14, 87.35 (C-1' and C-4'); 138.27 (C-6).

20b: ^{1}H-NMR (CD_3OD/*TMS*): $\delta = 1.89$ (d, 3 H, *Me*); 2.09 (ddd, 1 H, H-2'); 2.16 (s, 1 H, S*Me*); 2.86 (ddd, 1 H, H-2'); 3.32 (m, H-3'); 3.61 (dd, 1 H, H-5'); 3.77 (dd, 1 H, H-5'); 4.22 (ddd, 1 H, H-4'); 6.09 (t, 1 H, H-1'); 7.58 (q, 1 H, H-6). $J(1',2') = 6$ Hz; $J(2',2') = 14$ Hz; $J(2',3') = 8$ Hz; $J(3',4') = 7$ Hz; $J(4',5') = 3$ Hz, downfield H-5'; $J(4',5') = 4$ Hz, upfield H-5'; $J(5',5') = 12$ Hz; $J(6,Me) = 1$ Hz. ^{13}C-NMR (CD_3OD/*TMS*): $\delta = 12.70$ (*Me*); 14.83 (S*Me*); 40.63, 44.90 (C-2' and C-3'); 63.64 (C-5'); 87.99 (C-1', C-4'); 111.59 (C-5); 138.24 (C-6); 152.64 (C-2); 166.85 (C-4).

1-(2,3-Dideoxy-3-methylthio-β-D-erythro-pentofuranosyl)cytosine [**19c**; $C_{10}H_{15}N_3O_3S$ (MS)] *and its α-Anomer* [20c; $C_{10}H_{15}N_3O_3S$ (MS)]

These compounds are prepared in analogy with **19a** and **20a** from silylated N^4-isobutyrylcytosine (0.72 g, 2.9 mmol) and an anomeric mixture of the carbohydrate **13** (0.68 g, 1.6 mmol) in the presence of *TMS*-triflate (0.16 ml, 0.8 mmol). The same amount of *TMS*-triflate is added after 1.5 h (in order to make the total amount of *TMS*-triflate added: 0.32 ml, 1.6 mmol). The reaction time is 4 h at −20 °C and then 17 h at room temperature. Subsequent to aqueous work up, the residue is chromatographed on a silica column (first with CH_2Cl_2 to isolate the unreacted **13** and then CH_2Cl_2/*Me*OH, 97/3, *v/v*) to isolate **18c** (0.26 g, 28%) as a light brown gum.

The protected nucleoside **18c** (0.21 g, 0.4 mmol) is dissolved in ammonia saturated methanol (20 ml) and the reaction mixture is stirred in a closed flask at room temperature for 21 h. The reaction mixture is evaporated under reduced pressure. The residue is dissolved in dry tetrahydrofuran (5 ml), a 1.1 *M* dry tetrahydrofuran solution of tetrabutylammonium fluoride (0.42 ml, 0.5 mmol Bu_4NF) is added and the reaction mixture is stirred at room temperature for 1.5 h. The reaction mixture is evaporated under reduced pressure and the residue is chromatographed on a silica column (CH_2Cl_2/*Me*OH, 9/1, *v/v*) to yield a mixture of **19c** and **20c** (91 mg, 95%, $\alpha/\beta = 1.6$) as an oil. This mixture is subjected to HPLC (H_2O/*Et*OH, 96/4, *v/v*) to afford the pure anomers as glassy solids.

19c: ^{1}H-NMR (CD_3OD/TMS): $\delta = 2.25$ (s, 3 H, S*Me*); 2.52 (m, 2 H, H-2′); 3.40 (m, H-3′); 3.86 (dd, 1 H, H-5′); 3.99 (m, 2 H, H-4′, H-5′); 5.97 (d, 1 H, H-5); 6.18 (dd, 1 H, H-1′); 8.22 (d, 1 H, H-6). $J(1',2') = 6$ Hz and 4 Hz; $J(4',5') = 4$ Hz, upfield H-5′; $J(5',5') = 13$ Hz; $J(5,6) = 8$ Hz. ^{13}C-NMR (CD_3OD/TMS): $\delta = 13.96$ (S*Me*); 41.13, 42.74 (C-2′ and C-3′); 61.92 (C-5′); 87.24, 87.52 (C-1′ and C-4′); 95.52 (C-5); 142.73 (C-6); 158.22 (C-2).

20c: ^{1}H-NMR (CD_3OD/TMS): $\delta = 2.13$ (ddd, 1 H, H-2′); 2.23 (s, 3 H, S*Me*); 3.03 (ddd, 1 H, H-2′); 3.41 (m, H-3′); 3.71 (dd, 1 H, H-5′); 3.86 (dd, 1 H, H-5′); 4.33 (ddd, 1 H, H-4′); 6.01 (d, 1 H, H-5); 6.15 (dd, 1 H, H-1′); 7.87 (d, 1 H, H-6). $J(1',2') = 6$ Hz and 5 Hz; $J(2',2') = 14$ Hz; $J(2',3') = 8$ Hz; $J(3',4') = 8$ Hz; $J(4',5') = 3$ Hz, downfield H-5′; $J(4',5') = 4$ Hz, upfield H-5′; $J(5',5') = 12$ Hz; $J(5,6) = 7$ Hz. ^{13}C-NMR (CD_3OD/TMS): $\delta = 14.63$ (S*Me*); 41.07, 44.67 (C-2′ and C-3′); 63.51 (C-5′); 88.12, 88.81 (C-1′ and C-4′); 95.63 (C-5); 142.26 (C-6); 158.26 (C-2); 167.80 (C-4).

1-(2,3-Didehydro-2,3-dideoxy-D-glycero-pentofuranosyl)cytosine [**22**; $C_9H_{11}N_3O_3$ (MS)]

Dry acetonitrile (30 ml) is added to the oil of silylated N^4-isobutyrylcytosine (1.37 g, 5.4 mmol) under nitrogen, and the α-anomer of the carbohydrate **14** (1.14 g, 3.1 mmol) dissolved in dry acetonitrile (20 ml) is added dropwise during stirring at temperatures below −20 °C. *TMS*-triflate (0.30 ml, 1.5 mmol) dissolved in dry acetonitrile (10 ml) is added dropwise to the reaction mixture. The latter operation is repeated after 70 min (in order to make the total amount of *TMS*-triflate added: 0.60 ml, 3.0 mmol). The reaction mixture is stirred below −20 °C for a total of 2.5 h. The reaction mixture is dissolved in CH_2Cl_2 (200 ml) and extracted with ice-cold saturated aqueous $NaHCO_3$ (500 ml) which is extracted with additional CH_2Cl_2 (3 × 100 ml). The organic phases are collected and extracted with cold water (4 × 250 ml) until neutral (very crucial). The organic phase is dried over sodium sulfate and subsequently evaporated under reduced pressure. The residue might either be deprotected directly or chromatographed on a silica column (first with CH_2Cl_2 to isolate the furfuryl alcohol derivative and then $CH_2Cl_2/MeOH$, 97/3, *v/v*) to isolate **21** (0.78 g, 49%) as a pale yellow foam. The protected nucleoside **21** (0.78 g, 1.5 mmol) is dissolved in ammonia saturated methanol (75 ml) and the reaction mixture is stirred in a closed flask at room temperature for 16 h. The reaction mixture is evaporated under reduced pressure. The residue is dissolved in dry tetrahydrofuran (10 ml), a 1.1 *M* dry tetrahydrofuran solution of tetrabutylammonium fluoride (1.78 ml, 2.0 mmol Bu_4NF) is added and the reaction mixture is stirred at room temperature for 1.5 h. The reaction mixture is evaporated under reduced pressure. The residue is chromatographed on a silica column ($CH_2Cl_2/MeOH$, 9/1, *v/v*) and further purified by means of HPLC ($H_2O/EtOH$, 99/1, *v/v*) to give the title compound **22** (134 mg, 43%, $\alpha/\beta \cong 1/1$) as a glassy solid.

^{1}H-NMR ($DMSO\text{-}d_6/TMS$): $\delta = 3.46$–3.63 (m, 4 H, H-5′); 4.76 (m, 1 H, H-4′); 5.00 (m, 1 H, H-4′); 5.70 (d, 1 H, H-5); 5.73 (d, 1 H, H-5); 5.90 (m, 2 H, H-2′); 6.35 (m, 2 H, H-3′); 6.90 (m, 2 H, H-1′); 7.18 (br, 4 H, NH_2); 7.28 (d, 1 H, αH-6); 7.68 (d, 1 H, βH-6). $J(5,6) = 7$ Hz. ^{13}C-NMR ($DMSO\text{-}d_6/TMS$): $\delta = 62.59$, 63.31 (C-5′); 87.01, 87.39 (C-4′); 89.72, 90.19 (C-1′); 94.02, 94.44 (C-5); 126.58, 126.65 (C-2′); 133.91, 133.96 (C-3′); 140.81, 141.47 (C-6); 155.19, 155.34 (C-2); 165.57 (C-4).

References

[1] Barré-Sinoussi F., Chermann J. C., Rey F., Nugeyre M. T., Chamaret S., Gruest J., Dauguet C., Axler-Blin C., Vézinet-Brun F., Rouzioux C., Rozenbaum W., Montagnier L. (1983) Science **220**: 868

[2] Gallo R. C., Salahuddin S. Z., Popovic M., Shearer G. M., Kaplan M., Haynes B. F., Palker T. J., Redfield R., Oleske J., Safai B., White G., Foster P., Markham P. D. (1984) Science **224**: 500

[3] Mitsuya H., Weinhold K. J., Furman P. A., St. Clair M. H., Lehrman S. N., Gallo R. C., Bolognesi D., Barry D. W., Broder S. (1985) Proc. Natl. Acad. Sci. USA **82**: 7096

[4] Mitsuya H., Broder S. (1987) Nature **325**: 773

[5] Ono K., Ogasawara M., Iwata Y., Nakane H., Fujii T., Sawai K., Saneyoshi M. (1986) Biochem. Biophys. Res. Commun. **140**: 498
[6] De Clercq E. (1990) TiPS **11**: 198 and references cited therein
[7] Hansch C., Leo A. (1979) Ch. VI Cluster Analysis and the Design of Congener Sets, Substituent Constants for Correlation Analysis in Chemistry and Biology. Wiley USA, p. 49
[8] Fischer E. (1893) Ber. Dtsch. Chem. Ges. **26**: 2400
[9] Fischer E. (1895) Ber. Dtsch. Chem. Ges. **28**: 1145
[10] Hoffer M. (1960) Chem. Ber. **93**: 2777
[11] Fox J. J., Yung N. C., Wempen I., Hoffer M. (1961) J. Am. Chem. Soc. **83**: 4066
[12] Deriaz R. E., Overend W. G., Stacey M., Wiggins L. F. (1949) J. Chem. Soc.: 2836
[13] Motawia M. S., Pedersen E. B. (1990) Liebigs Ann. Chem.: 599
[14] Fleet G. W. J., Son J. C., Derome A. E. (1988) Tetrahedron **44**: 625
[15] Hansen P., Pedersen E. B. (1990) Acta Chem. Scand. **44**: 522
[16] Kunz H., Schmidt P. (1979) Tetrahedron Lett. **23**: 2123
[17] Kunz H., Schmidt P. (1979) Chem. Ber. **112**: 3886
[18] Cosstick R., Vyle J. S. (1990) Nucleic Acids Res. **18**: 829
[19] Wittenburg E. (1964) Z. Chem. **4**: 303
[20] Vorbrüggen H., Krolikiewicz K., Bennua B. (1981) Chem. Ber. **114**: 1234
[21] Sigiura Y., Furuya S., Furukawa Y. (1988) Chem. Pharm. Bull. **36**: 3253
[22] Niedballa U., Vorbrüggen H. (1974) J. Org. Chem. **39**: 3654
[23] Herdewijn P., Balzarini J., Baba M., Pauwels R., Van Aerschot A., Janssen G., De Clercq E. (1988) J. Med. Chem. **31**: 2040
[24] Lin T.-S., Guo J.-Y., Schinazi R. F., Chu C. K., Xiang J.-N., Prusoff W. H. (1988) J. Med. Chem. **31**: 336
[25] Chorbadjiev S., Roumian C., Markov P. (1977) J. Prakt. Chem. **319**: 1036
[26] Mitsunobu O. (1981) Synthesis: 1
[27] Nagamachi T., Fourrey J.-L., Torrence P. F., Waters J. A., Witkop B. (1974) J. Med. Chem. **17**: 403
[28] Cleland W. W. (1964) Biochemistry **3**: 480
[29] Dueholm K. L., Motawia M. S., Pedersen E. B., Nielsen C. M., Lundt I. (1992) Arch. Pharm. (Weinheim) **325**: 597
[30] Okabe M., Sun R.-C., Tam S. Y.-K., Todaro L. J., Coffen D. L. (1988) J. Org. Chem. **53**: 4780
[31] Mansuri M. M., Wos J. A., Martin J. C. (1989) Nucleosides Nucleotides **8**: 1463
[32] Miller N., Fox J. J. (1964) J. Org. Chem. **29**: 1772
[33] Hildesheim J., Cléophax J., Géro S. D. (1967) Tetrahedron Lett. **18**: 1685
[34] Köll P., Deyhim S. (1978) Chem. Ber. **111**: 2913
[35] Chu C. K., Babu J. R., Beach J. W., Ahn S. K., Huang H., Jeong L. S., Lee S. J. (1990) J. Org. Chem. **55**: 1418
[36] Abdel-Megied A. E.-S., Pedersen E. B., Nielsen C. M. (1991) Synthesis: 313
[37] Vorbrüggen H., Höfle G. (1981) Chem. Ber. **114**: 1256
[38] Horwitz J. P., Chua J., Noel M., Donatti J. T. (1967) J. Org. Chem. **32**: 817
[39] Greengrass C. W., Hoople D. W. T., Street S. D. A., Hamilton F., Marriott M. S., Bordner J., Dalgleish A. G., Mitsuya H., Broder S. (1989) J. Med. Chem. **32**: 618 and references cited therein
[40] Nielsen C. M., Bygbjerg I. C., Vestergaard B. F. (1987) Lancet I: 566
[41] Yuzhakov A. A., Chidzhavadze Z. G., Bibilashvilli R. Sh., Kraevskii A. A., Galegov G. A., Korneeva M. N., Nosik D. N., Kilesso T. Yu. (1991) Biorg. Khim. **17**: 504; (1991) Chem. Abstr. **115**: 84923g

Received February 24, 1992. Accepted March 24, 1992