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AN EFFICIENT APPROACH TO THE SYNTHESIS OF THYMIDINE DERIVATIVES CONTAINING PHOSPHATE-ISOSTERIC METHYLENE ACETAL LINKAGES

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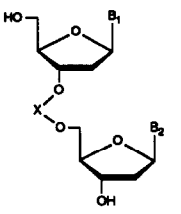
Abstract. Iodonium ion promoted condensation of properly protected 3'-*O*-methylthiomethyl or 3'-*O*-(4-penten-1-oxymethyl)-thymidine with 3'-*O*-methoxyacetyl-thymidine, was explored. A judicious choice of the iodonium source and protecting groups led to an efficient preparation of thymidine dimers having internucleosidic-(3'-5')-methylene bonds. The latter procedure was utilized towards the synthesis, in solution and on a solid support, of DNA-fragments containing one or more T-CH_2-T dimers. Further, 5'-*O*-methylthiomethyl-3'-*O*-methoxyacetyl-*N* ^{3}-benzoyl-thymidine proved to be a suitable donor for the introduction of 5'-*O*-methylene acetal-linkages between 2,3,4,6-tetra-*O*-benzyl-D-glucose, benzyl *N*-benzyloxycarbonyl-L-serine and dibenzyl phosphate.

Introduction

Phosphate esters and anhydrides are common structural elements of biomolecules[1]. For instance, the genetic material DNA and RNA consists of nucleosides interlinked by phosphodiester bonds. In addition, analogous phosphate functions are present in teichoic acids[2], nucleopeptides[3] and (glyco)phospholipids[4]. Moreover, phosphoric acid anhydrides such as UDP-D-glucose[5] and nucleoside triphosphates[6] are key-intermediates in the synthesis of biopolymers. To deepen the insight in the structure-activity relation of the above phosphate esters and to be able to influence biochemical processes, much effort has been devoted to the chemical synthesis of phosphate derivatives. As a result, a plethora of effective phosphorylation procedures have now become available[7,8]. In the last decade, however, interest to prepare phosphate-modified analogues has increased dramatically. The reason for this is that the modified congeners may not only be more resistant to degradation by enzymes but also, due to a decrease of the highly charged nature of the native phosphate groups, facilitate the cellular uptake. Interest in this direction, particularly in the field of the nucleic acids chemistry, has been enhanced by the finding that oligonucleotides comprising a nucleotide sequence complementary to a specific gene or RNA messenger were potentially promising antisense inhibitors of gene function and expression[9-11]. The inhibitory effect may be ascribed to a specific binding of antisense molecules to messenger RNAs (sense molecules) as DNA-RNA complexes, thus infringing the translation process. The latter phenomenon suggests a potential application of synthetic DNA-fragments as anti-viral and anti-cancer drugs. To prevent rapid hydrolysis by DNAses, much research has been focussed on the synthesis of antisense DNA containing charged (*e.g.* **1b,c**) or non-charged (*e.g.* **1d-f**) modified phosphate bonds[12-21]. All these modifications

Figure 1



1a X = $P(O)O^-$
1b X = $P(S)S^-$
1c X = $P(S)O^-$
1d X = P(O)Me
1e X = P(O)Oalkyl
1f X = $P(O)N(alkyl)_2$
1g X = C=O
1h X = $Si(CHMe_2)_2$
1i X = CH_2

fulfil more or less the requirement that they are resistant towards DNAses. However, all the phosphate alterations, apart from the charged phosphorodithioate one (*i.e.*, **1b**), introduce additional stereoisomerism, the effect of which on the stability of the proposed DNA-RNA duplexes is still a subject of debate. Furthermore, the synthesis of DNA fragments in which the phosphate-diester was replaced by a carbonate (*e.g.* **1g**)[22] or a *bis*(isopropyl)silyl-di-oxy (*e.g.* **1h**)[23] linkage have been reported. The intrinsic base-lability of the carbonate function is, however, a serious drawback. On the other hand, the silyl modification embodies a rather bulky and highly lipophilic center which makes duplex formation less effective.

With respect to the anti-sense concept, we[24], and independently also Matteucci[25], reported that the replacement of the native phosphodiester bond by an isosteric methylene acetal linkage would afford DNA-fragments containing enzymatically stable, achiral and non-charged internucleosidic bonds.

We now report in full that iodonium ion assisted activation of 3′-*O*-methylthiomethylene and 4-pentenyl-1-oxymethylene acetals of thymidine with 5′-*O*-unprotected thymidine is an efficient route to the formation of (3′-5′)-methylene-linked thymidine dimers which, in turn, could be applied to the preparation of several modified DNA-fragments. Further, the same methodology proved also to be suitable for the introduction of 5′-*O*-methylene acetal-linkages between thymidine and 2,3,4,6-tetra-*O*-benzyl-D-glucose, benzyl *N*-benzyloxycarbonyl-L-serine and dibenzyl phosphate.

Results and discussion

Recently, we introduced an efficient method towards the synthesis of oligosaccharides involving iodonium ion mediated condensation of ethyl 1-thio-glycosides with hydroxylic acceptors[26,27]. We anticipated that activation of methylthiomethylene (MTM) acetals, due to the structural resemblance with thioglycosides, would afford methylene acetals in the presence of appropriate acceptor molecules. In addition, similar acetals may be obtained starting from *n*-pentenyloxymethylene (POM) acetals, resembling *n*-pentenylglycosides, which are also amenable to activation with iodonium ions[28,29]. On the other hand, Van Boeckel *et al.* prepared[30] methylene acetal analogues of heparin by utilizing a methylene fluoride precursor. The application of the latter approach seemed to us less attractive for sensitive compounds such as nucleosides.

In this study we primarily explored in detail the introduction of (3′-5′)-internucleosidic methylene

linkages of thymidine dimers starting from the appropriate MTM and POM precursors. To this end, the repective methylthiomethylene and pentenyloxymethylene acetals **3** and **4** were prepared. Attempts to synthesize donor **3** by treating **2** with methylthiomethyl chloride, in the presence of sodium hydride[25,31], were not successful. However, the reaction of **2** with methylsulphide and benzoyl peroxide[32] afforded donor **3** in 60% yield. The yield of the latter reaction could be increased to 75% by performing the same transformation in the presence of 2,6-lutidine. The corresponding donor **4** was easily accessible (65% yield) by reacting **2** with pentenyloxymethyl chloride[33] and *N,N*-diisopropylethylamine (DIPEA). Acceptor molecule **6** was obtained by acylation of **2** with methoxyacetic anhydride in pyridine, followed by acid treatment of the resulting derivative **5**.

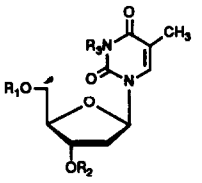
In a first attempt (*Table 1*, entry *1*) to prepare the methylene dimer **7**, iodonium dicollidine triflate (IDCT)[34] was added to a solution of MTM-donor **3** and acceptor **6**. Unfortunately, after 2 h at ambient temperature, only a trace of dimer **7** could be detected. On the other hand, reaction of POM donor **4** with acceptor **6** (entry *2*), in the presence of IDCT, furnished **7** in 15% yield.

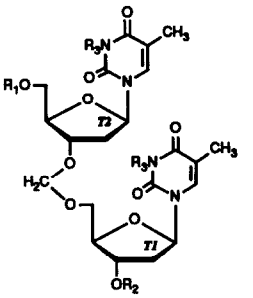
In the course of our investigations, Matteucci reported[25] that similar methylene acetals could be obtained in 45% yield, by activating **3** with *N*-bromosuccinimide in the presence of 2,6-di-*tert*.butylpyridine. However, execution (entry *3*) of the above condensation of **3** with **6** using *N*-bromosuccinimide (NBS) as the thiophilic promoter, resulted in a complex mixture from which dimer **7** could be isolated in only 11% yield.

The unfavourable outcome of the above transformations may be due to the fact that the thymine moiety is not inert towards the iodonium ion-assisted activation. For example, it has been documented[35] that pyrimidine nucleosides react with *N*-halosuccinimides to give the corresponding 5′-*O*-6-cyclo-5,5-dihalogeno-5,6-dihydropyrimidine nucleosides. Further, the susceptibility of *N*-3 of thymidine residues towards alkylating reagents has been described[36]. For these reasons protection of *N*-3 of thymine may be essential in precluding the above side-reactions.

Among the several protecting groups proposed for the thymine imido function, the benzoyl group appeared to us the most attractive[37]. Accordingly, treatment of **5** with benzoyl chloride and DIPEA gave **8**. Selective saponification of the MAc group in **8** could be effected with a catalytic amount of potassium *tert*-butoxide in dichloromethane-methanol to give **9** in 85% overall yield (based on **5**). Reaction of **9** with either dimethylsulphide/benzoylperoxide/lutidine or POM-Cl/DIPEA furnished **10** (70%) or **11** (80%), respectively. Acceptor molecule **12** was obtained by deblocking of the DMTr-group in **8** by acid hydrolysis.

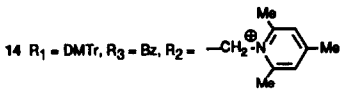
Unfortunately, IDCT-assisted condensation of MTM-donor **10** with **12** (entry *4*), in the presence of IDCT, gave only a trace of the target dimer **13**. The thiophilic promoter is apparently inable to activate MTM acetals effectively. We next studied the possibility to apply *N*-iodosuccinimide (NIS) as the activating agent[27,38]. To this end (entry *5*), excess NIS (6 eq.) was added to a solution of MTM-donor **10** and acceptor **12** and the reaction mixture was stirred for 72 h at 20°C. Work-up and purification of the reaction mixture gave dimer **13** in 33% yield. However, the main product isolated from the reaction mixture proved to be the unexpected succinimide derivative **15** (50% yield). In contrast, (entry *6*), IDCT-mediated reaction of POM-donor **11** with acceptor **12** proceeded rapidly to give dimer **13** in



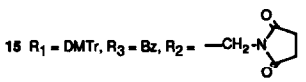


2 R_1 = DMTr, R_2 = R_3 = H
3 R_1 = DMTr, R_2 = MTM, R_3 = H
4 R_1 = DMTr, R_2 = POM, R_3 = H
5 R_1 = DMTr, R_2 = Mac, R_3 = H
6 R_1 = R_3 = H, R_2 = MAc
8 R_1 = DMTr, R_2 = MAc, R_3 = Bz
9 R_1 = DMTr, R_2 = H, R_3 = Bz
10 R_1 = DMTr, R_2 = MTM, R_3 = Bz
11 R_1 = DMTr, R_2 = POM, R_3 = Bz
12 R_1 = H, R_2 = MAc, R_3 = Bz
16 R_1 = H, R_2 = MTM, R_3 = Bz
17 R_1 = Lev, R_2 = MTM, R_3 = Bz
18 R_1 = H, R_2 = POM, R_3 = Bz
19 R_1 = Lev, R_2 = POM, R_3 = Bz
21 R_1 = R_3 = H, R_2 = MTM
22 R_1 = Lev, R_2 = MTM, R_3 = H

7 R_1 = DMTr, R_2 = MAc, R_3 = H
13 R_1 = DMTr, R_2 = MAc, R_3 = Bz
20 R_1 = Lev, R_2 = MAc, R_3 = Bz
23 R_1 = Lev, R_2 = MAc, R_3 = H
24 R_1 = R_2 = R_3 = H



14 R_1 = DMTr, R_3 = Bz, R_2 =



15 R_1 = DMTr, R_3 = Bz, R_2 =

MAc = $-C(O)CH_2OCH_3$ *POM* = $-CH_2O(CH_2)_2CH{=}CH_2$
MTM = $-CH_2SCH_3$ *Lev* = $-C(O)CH_2CH_2C(O)CH_3$

Table 1. Results on the preparation of thymidine dimers containing internucleosidic (3'-5')-methylene linkages

Entry	Donor	Acceptor	Promoter	Product	Time	Yield (%)
1	3	6	IDCT	7	2 h	Trace
2	4	6	IDCT	7	30 min	15
3	3	6	NBS[a]	7	30 min	11
4	10	12	IDCT	13	2 h	Trace
5	10	12	NIS	13	72 h	32
6	11	12	IDCT	13	30 min	55
7	11	12	NIS	13	72 h	80
8	17	12	NIS-TfOH	20	1 min	85
9	19	12	NIS-TfOH	20	10 min	44
10	22	6	NIS-TfOH	23	1 min	84

a) Condensation was performed in the presence of 2,6-di-tert. butyl pyridine

55% yield. In this case, the concomitant formation of a polar side product was observed, which is presumably the charged collidine derivative **14**. Fortunately, condensation of **11** with **12** (entry *7*) but using NIS, instead of IDCT, furnished predominantly dimer **13** (isolated in 80% yield), together with a small amount (~10%) of side-product **15**. The above results show that a combined use of the POM-derivative **11** and the promoter NIS is quite effective for the formation of an internucleosidic methylene linkage between thymidines. In addition, the application of benzoyl protection of the thymine base

seems to be adequate in preventing side reactions.

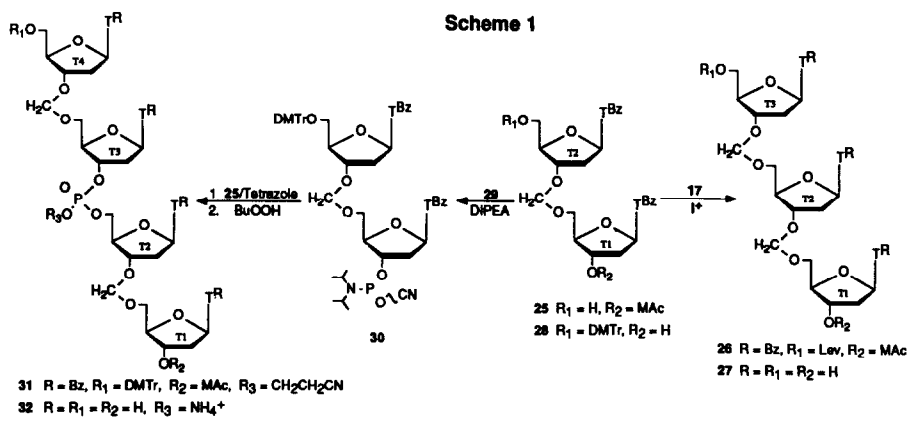
In an attempt to shorten the reaction time, we now focused our attention on the application of the recently developed powerful promoter NIS-*cat*.trifluoromethanesulphonic acid (TfOH)[27,29]. In view of the acidic nature of this activator, the acid labile DMTr-group in **10** and **11** was replaced by the levulinoyl group. Hence, donors **17** and **19** were prepared by acid treatment of **10** and **11** and subsequent acylation (levulinic anhydride/pyridine/*N*-methylimidazole)[39] of the respective derivatives **16** and **18**. Addition of NIS (1 eq.) and TfOH (0.15 eq.) to a cooled (0°C) mixture of MTM-donor **17** (1 eq.) and acceptor **12** (0.8 eq.) resulted (entry *8*) in the instant disappearance of both reactants. Work-up and purification afforded dimer **20** in 85% yield. Similarly (entry *9*), condensation of POM-derivative **19** with **12** furnished **20**, albeit in a much lower yield (44%). These results show that NIS-*cat*.TfOH mediated activation of the MTM-function in **17** proceeds with a high degree of efficiency.

We also exercised the NIS-*cat*.TfOH-mediated reaction of donor **22** with acceptor **6**, both components having *N*-3 unprotected thymine residues. Derivative **22** was conveniently accessible by acid hydrolysis of **3** and subsequent acylation of resulting **21** with levulinic anhydride. Condensation (entry *10*) of **22** with **6**, under the agency of NIS-*cat*.TfOH, furnished almost instantaneously, and in a surprisingly high yield, dimer **23**. The latter result indicates that protection of the thymine imido function is not a prerequisite in the NIS-*cat*.TfOH mediated activation of the MTM-group.

The identity of dimers **7**, **13**, **20** and **23** as well as fully deprotected **24** was ascertained by ^{1}H and ^{13}C NMR spectroscopy. In addition, dimer **24** was, as expected, completely resistant towards the exonucleolytic action of the enzymes snake venom and spleen phosphodiesterase.

Elongation of dimer **20** to give dimethylene-trimer **26** was readily accomplished as follows. Removal (NH_2NH_2/pyridine/acetic acid/ethylacetate, 1 min)[40] of the levulinoyl group from **20** afforded **25**. Coupling of the latter with **17** in the presence of NIS-*cat*.TfOH furnished **26** in a yield of 85%. Complete deblocking of **26** by ammonolysis gave homogeneous **27** in 91% yield.

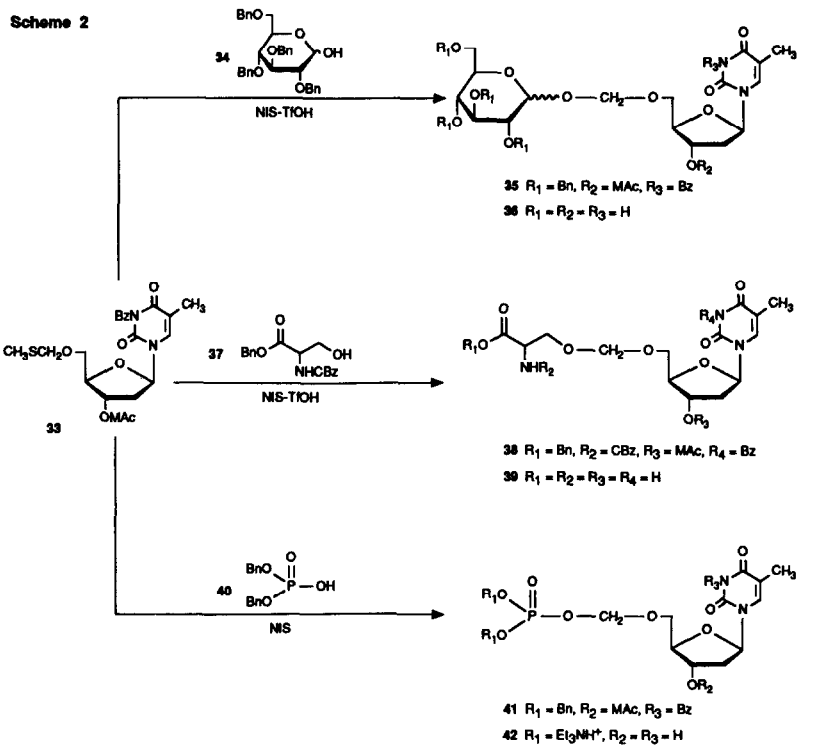
Scheme 1



The application of dimers **13** and **25** was further illustrated (see *Scheme 1*) by the succesful as-

semblage of tetramer **31** having one phosphodiester and two methylene linkages. Thus, phosphitylation of **28**, prepared by selective removal of the 3′-*O*-MAc from **13** (catalytic potassium *tert.*-butoxide in 1:1 dichloromethane-methanol), with 2-cyanoethoxy(N,N-diisopropylamino)chlorophosphine (**29**)[41], in the presence of DIPEA, gave the amidite **30** (yield 85%, based on **28**). 1-*H*-Tetrazole-mediated condensation[42] of the latter with **25**, and subsequent oxidation (tBuOOH)[43] of the intermediate phosphite-triester, gave fully protected tetramer **31**. The latter was completely deblocked by acid hydrolysis of the dimethoxytrityl (R_1) group, followed by ammonolysis of the base labile (R_2, R_3 and R) groups, to give homogeneous **32** in an overall yield of 75% (based on **25**).

The easy accessibility of dimer **30** also enabled us to prepare the decamer d-G*p*C*p*G*p*T*p*T-CH_2-T*p*T*p*G*p*C*p*G, containing one methylene-TT dimer [T-CH_2-T], by a conventional phosphitetriester protocol[44] and using an automated DNA-synthesizer (*Gene Assembler*, Pharmacia). In this respect, it is of interest to note that elongation of the immobilized tetramer TGCG at the 5′-end with dimer **30** proceeded with a coupling efficiency of 95%. High-resolution (600 MHz) ^{1}H NMR spectroscopic data of the modified decamer were in full accord with the proposed structure[45]. Preliminary experiments also indicated that the earlier mentioned modified decamer formed a stable duplex (T_M = 330°K), as gauged by high-field NMR spectroscopy[45], with the complementary decamer d-C*p*G*p*C*p*A*p*A*p*A*p*A*p*C*p*G*p*C.



Other interesting applications of the NIS-*cat*.TfOH mediated activation of methylthiomethylene acetals are outlined in *Scheme 2*. Thus, iodonium ion promoted condensation of 5′-*O*-methylthiomethyl-3′-*O*-methoxyacetyl-N^3-benzoyl-thymidine (**33**), readily obtained from **12** and dimethylsulphide/benzoylperoxide, with 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (**34**, α/β 8:1) furnished dimer **35** (91% yield, α/β 8:1). Deblocking of **35** by hydrogenolysis (H_2, Pd-C), followed by ammonolysis and purification gave **36**. Further, coupling of benzyl *N*-benzyloxycarbonyl-L-serine (**37**) with thymidine derivative **33** afforded **38**, which, after deblocking, yielded modified[46] nucleopeptide **39** in 86% yield. Finally, NIS-promoted reaction of **33** with dibenzyl phosphate provided the pyrophosphate isostere **41**. Derivative **41** was found to be highly labile and did not survive chromatographic purification. Nevertheless, ^{1}H and ^{13}C NMR spectroscopy of the crude product confirmed the identity of **41**. It was established that direct ammonolysis of **41** resulted, as evidenced by the exclusive formation of thymidine, in the complete hydrolysis of the methylene-phosphate linkage. Fortunately, hydrogenolysis, and subsequent ammonolysis of **41** afforded predominantly **42**, together with a minor amount (~15%) of thymidine. Purification then furnished homogeneous **42** in 65% yield.

In conclusion, the results presented in this paper show that DNA fragments having an internucleosidic-(3′→5′)-methylene acetal linkage between thymidine units can be prepared conveniently *via* iodonium ion-activatable precursors. In particular, the combined use of a MTM-donor and the promoter NIS-*cat*.TfOH proved to be highly effective in the formation of the required methylene linkage. At present, we are studying in detail the duplex formation between DNA-fragments containing several T-CH_2-T units and non-altered complementary DNA-fragments and, further, the feasibility to introduce an internucleosidic methylene acetal linkage between other d-nucleosides. In addition, the biological activity of the phosphate isosteres **36**, **39**, **42** and analogues thereof is currently under investigation and will be published in due course.

Acknowledgments

We wish to thank Prof. Dr. C. Altona and Dr. J. M. Pikkemaat for valuable discussions and Drs. C. Erkelens for recording the ^{1}H and ^{13}C NMR spectra.

Experimental

General methods and materials

Pyridine was dried by refluxing with CaH_2 (5g/L) and then distilled. Dichloromethane, 1,2 dichloroethane and toluene were distilled from P_2O_5. *N,N*-Dimethylformamide was stirred with CaH_2 at room temperature and distilled under reduced pressure. Diethyl ether and tetrahydrofuran were distilled from $LiAlH_4$. Pyridine and *N,N*-dimethylformamide were stored over molecular sieves 4Å (Aldrich), toluene, diethyl ether and tetrahydrofuran over sodium wire and dichloromethane and 1,2-dichloroethane were stored over alumina. *N*-Iodosuccinimide, trifluoromethanesulphonic acid and dibenzyl phosphate were purchased from Aldrich. Reactions were performed at ambient

temperature unless noted otherwise. Column chromatography was performed on columns of silica gel 60 (Merck 70-230 mesh). Gel filtration was performed on Sephadex LH-20 (Pharmacia). TLC was conducted on DC Fertigfolien (Schleicher & Schüll F1500 LS254). Compounds were detected by charring with 20% sulfuric acid in methanol. Optical rotations were determined with a Perkin-Elmer Model 241 polarimeter, for solutions in $CHCl_3$ at 22° unless stated otherwise. NMR spectra were recorded with a Jeol JNM-FX200 (^{13}C, ^1H and ^{31}P at 50.1, 200 and 80.7 MHz, respectively) or a Bruker WM-300 spectrometer equipped with an Aspect-2000 computer (^1H, 300 MHz). ^1H and ^{13}C chemical shifts are given in ppm (δ) relative to TMS as internal standard and ^{31}P-chemical shifts in ppm (δ) to 85% H_3PO_4 as external standard.

5'-O-Dimethoxytrityl-3'-O-methylthiomethyl-thymidine (**3**). - Benzoyl peroxide (0.97 g, 4 mmol) was added, within 30 min, to a cooled (0°C) and stirred solution of *5'-O*-dimethoxytrityl-thymidine (**2**) (0.54 g, 1 mmol), dimethylsulphide (0.73 mL, 10 mmol) and 2,6-lutidine (0.11 g, 1 mmol) in acetonitrile (10 mL). After 2 h, the reaction mixture was concentrated, dissolved in dichloromethane, extracted with water (2x 20 mL) and aq. $NaHCO_3$ (0.9M, 20 mL), dried ($MgSO_4$) and concentrated once more. The residue was chromatographed on silica gel with 7:3 dichloromethane-hexane to give **3** (0.45 g, 75%). ^1H NMR data ($CDCl_3$): δ 9.58 ((s, 1 H, H-3); 6.61-6.82 (m, 14 H, *H*-arom.); 6.35 (dd, 1 H, H-1', $J_{1,2a}$ 5.9 Hz, $J_{1,2b}$ 7.7 Hz); 4.67 (m, 1 H, H-3'); 4.59 (AB, 2 H, OCH_2S); 4.11 (m, 1 H, H-4'); 3.78 (s, 6 H, OCH_3); 3.52-3.30 (m, 2 H, H-5'); 2.55-2.09 (m, 2 H, H-2'); 2.06 (s, 3 H, SCH_3); 1.49 (s, 3 H, CH_3-thymine). ^{13}C NMR data ($CDCl_3$): δ 164.0 (C-2); 150.4 (C-4); 158.6-113.2 (*C*-arom.); 111.2 (C-5); 86.8 (C_{quat} DMTr); 84.7, 83.8 (C-1', C-4'); 75.8 (C-3'); 73.5 (OCH_2S); 63.3 (C-5'); 55.2 (OCH_3); 37.9 (C-2'); 13.7 (SCH_3); 11.8 (CH_3-thymine).

5'-O-Dimethoxytrityl-3'-O-(4-pentenyloxymethyl)-thymidine (**4**). - To a solution of **2** (0.54 g, 1 mmol) in 1,2-dichloroethane (5 mL) was added diisopropylethylamine (0.52 mL, 3 mmol) and pentenyloxymethyl chloride (0.27 g, 2 mmol). The resulting mixture was stirred for 4 h at 50°C, diluted with dichloromethane (20 mL), extracted with aq. $NaHCO_3$ (0.9M, 2x 20 mL), dried ($MgSO_4$) and concentrated. Purification of the remaining oil by silica gel chromatography (eluens dichloromethane-hexane 4:1) afforded **4** (0.42 g, 65%). ^{13}C NMR data ($CDCl_3$): δ 163.4 (C-2); 150.7 (C-4); 158.7-113.0 (*C*-arom.); 137.8 (*C*-4, POM); 115.0 (C-5, POM); 111.2 (C-5, thymine); 94.5 (OCH_2O); 86.8 (C_{quat} DMTr); 85.1, 84.4 (C-1', C-4'); 76.5 (C-3'); 67.7 (C-1, POM); 63.3 (C-5'); 55.2 (OCH_3); 38.7 (C-2'); 30.1, 28.7 (C-2, C-3, POM); 11.7 (CH_3, thymine).

3'-O-Methoxyacetyl-thymidine (**6**). - A mixture of **2** (0.54 g, 1 mmol) and methoxyacetic anhydride (0.32 g, 2 mmol) in pyridine (5 mL) was stirred for 2 h at 20°C. Water (0.5 mL) was added and the reaction mixture was concentrated. The oily residue was dissolved in dichloromethane, extracted with water (20 mL) and aq. $NaHCO_3$ (0.9M, 2x 20 mL), dried ($MgSO_4$), concentrated and coevaporated with toluene (2x 20 mL). The remaining **5** was redissolved in acetic acid (20 mL) and heated at 50°C. Water (2 mL) was added and the resulting mixture was stirred for 30 min. The mixture was concentrated and coevaporated with ethanol (3x 20 mL) and toluene (20 mL). Purification by silica gel chromatography yielded **6** (0.25 g, 79%). ^1H NMR data ($CDCl_3$): δ 9.94 (s, 1 H, H-3); 7.63 (s, 1 H, H-6); 6.28 (dd, 1 H, H-1', $J_{1,2a}$~$J_{1,2b}$ 7.1 Hz); 5.47 (m, 1 H, H-3'); 4.12 (m, 1 H, H-4'); 4.09 (s, 2 H, CH_2-MAc); 3.93 (m, 2 H, H-5'); 3.46 (s, 3 H, OCH_3); 2.43 (m, 2 H, H-2'); 1.90 (s, 3 H, CH_3-thymine). ^{13}C NMR data ($CDCl_3$): δ 169.6 (*C*=O, MAc); 164.2 (C-2); 150.6 (C-4); 136.4 (C-6); 111.2 (C-5); 85.6, 85.0 (C-1', C-4'); 75.3 (C-3'); 69.5 (CH_2, MAc); 62.2 (C-5'); 59.3 (OCH_3); 37.2 (C-2'); 12.4 (CH_3, thymine).

Preparation of dimer **7**.

a) from MTM-donor **3**. - To a stirred mixture of **3** (180 mg, 0.3 mmol), **6** (79 mg, 0.25 mmol), 2,6-di-*tert*.butyl-

pyridine (115 mg, 0.6 mmol) and powdered molecular sieves (5Å) in 1,2-dichloroethane (5 mL) was added *N*-bromosuccinimide (80 mg, 0.45 mmol) and stirring was continued for 30 min. The reaction mixture was filtered, diluted with dichloromethane and extracted with aq. $Na_2S_2O_3$ (1M, 10 mL) and aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated. The residue was applied on a column of Sephadex LH-20 and eluted with 1:1 dichloromethane-methanol. The first fraction was collected and concentrated. Further purification was achieved by chromatography on silica gel with 95:5 dichloromethane-acetone to give **7** (22 mg, 11%). ^{1}H NMR data ($CDCl_3$): δ 9.12, 9.04 (2x s, 2 H, 2x H-3); 7.60-6.80 (m, 15 H, *H*-arom.); 6.40-6.29 (m, 2 H, 2x H-2′); 5.20 (m, 1 H, H-3′, *T1*); 4.78 (AB, 2 H, OC*H*$_2$O); 4.41 (m, 1 H, H-3′, *T2*); 4.18, 4.13 (2x m, 2 H, 2x H-4′); 4.06 (s, 2 H, C*H*$_2$, MAc); 3.79 (OC*H*$_3$, DMTr); 3.57-3.30 (m, 4 H, 2x H-5′); 3.44 (s, 3 H, OC*H*$_3$, Mac); 2.50-2.16 (m, 4 H, 2x H-2′); 1.89, 1.46 (2x C*H*$_3$, thymine). ^{13}C NMR data ($CDCl_3$): δ 169.5 (*C*=O, Mac); 163.7, 163.5 (2x C-2); 158.7-113.2 (*C*-arom.); 111.4 (bs, 2x C-5); 95.1 (O*C*H$_2$O); 84.8, 84.7, 84.4, 83.3 (2x C-1′, 2x C-4′); 79.0 (C-3′, *T2*); 75.0 (C-3′, *T1*); 69.5 (*C*H$_2$, MAc); 68.2 (C-5′, *T1*); 63.6 (C-5′, *T2*); 59.3 (OCH$_3$, MAc); 55.2 (O*C*H$_3$, DMTr); 38.5 (C-2′, *T2*); 37.3 (C-2′, *T1*); 12.7, 11.8 (2x *C*H$_3$, thymine).

b) from POM-donor 4. - IDCT (207 mg, 0.4 mmol) was added to a stirred mixture of **4** (180 mg, 0.3 mmol), **6** (79 mg, 0.25 mmol) and powdered molecular sieves (5Å) in 1,2-dichloroethane (5 mL). After 30 min, the reaction mixture was filtered, and processed as described above to give **7** (29 mg, 15%).

Anal. Calcd. for $C_{45}H_{50}N_4O_{14}$: C 62.1, H 5.8; found: C 62.3, H 5.8%.

5′-O-Dimethoxytrityl-N^3-benzoyl-thymidine (**9**). - Benzoyl chloride (1.75 mL, 10 mmol) was added to a solution of **6** (6.16 g, 10 mmol) and DIPEA (3.5 mL, 20 mmol) in pyridine (40 mL). After 1 h, the dark reaction mixture was diluted with dichloromethane (100 mL) and extracted with water (50 mL) and aq. $NaHCO_3$ (0.9M, 2x 50 mL), dried ($MgSO_4$) and concentrated. The residue, containing crude **8**, was redissolved in 1:1 dichloromethane-methanol (50 mL) and treated with sodium methoxide (~15 mg). After 20 min, the reaction mixture was diluted with dichloromethane and extracted with water (20 mL) and aq. NaCl (1.5M, 20 mL), dried ($MgSO_4$) and concentrated. Purification on silica gel afforded **9** (5.5 g, 85%) as a foam. ^{1}H NMR data ($CDCl_3$): δ 7.91-6.80 (m, 19 H, *H*-arom.); 6.34 (dd, 1 H, H-1′, $J_{1,2a}$~$J_{1,2b}$ 6.8 Hz); 4.52 (m, 1 H, H-3′); 4.00 (m, 1 H, H-4′); 3.76 (s, 6 H, OC*H*$_3$, DMTr); 3.48-3.29 (m, 2 H, H-5′); 2.33 (m, 2 H, H-2′); 1.43 (s, 3 H, C*H*$_3$, thymine). ^{13}C NMR data ($CDCl_3$): δ 169.0 (*C*=O, Bz); 162.8 (C-2); 149.2 (C-4); 158.6-113.2 (*C*-arom.); 110.9 (C-5); 86.8 (C_{quat}, DMTr); 86.3, 84.9 (C-1′, C-4′); 71.9 (C-3′); 63.4 (C-5); 55.1 (O*C*H$_3$, DMTr); 41.0 (C-2′); 11.6 (*C*H$_3$, thymine).

5′-O-Dimethoxytrityl-3′-O-methylthiomethyl-N^3-benzoyl-thymidine (**10**). - Compound **9** (1.3 g, 2 mmol) was dissolved in acetonitrile (10 mL), dimethylsulphide (1.46 mL, 20 mmol) and 2,6-lutidine (0.22g, 2 mmol) whereupon benzoyl peroxide (2 g, 8 mmol) was added. Work-up and purification as described for the preparation of **3** afforded **10** (1.0 g, 71%). ^{1}H NMR data ($CDCl_3$): δ 8.0-6.8 (m, 14 H, *H*-arom.); 6.34 (dd, 1 H, H-1′, $J_{1,2a}$~$J_{1,2b}$ 6.2 Hz); 4.72 (m, 1 H, H-3′); 4.58 (AB, 2 H, OC*H*$_2$S); 4.13 (dd, 1 H, H-3′); 3.79 (s, 6 H, OC*H*$_3$, DMTr); 3.52 (dd, 1 H, H-5a′, $J_{5a,4}$ 3.1 Hz, $J_{5a,5b}$ 10.7 Hz); 3.36 (dd, 1 H, H-5b′, $J_{5b,4}$ 2.6 Hz, $J_{5b,5a}$ 10.8 Hz); 2.55-2.08 (m, 2 H, H-2′); 2.05 (s, 3 H, SC*H*$_3$); 1.49 (s, 3 H, C*H*$_3$, thymine). ^{13}C NMR data ($CDCl_3$): δ 158.7- 113.2 (*C*-arom.); 111.2 (C-5); 85.0, 84.1 (C-1′, C-4′); 75.7 (C-3′); 73.6 (OCH$_2$S); 63.2 (C-5′); 55.2 (O*C*H$_3$); 38.1 (C-2′); 13.7 (S*C*H$_3$); 11.8 (*C*H$_3$, thymine).

5′-O-Dimethoxytrityl-3′-O-(4-pentenyloxymethyl)-N^3-benzoyl-thymidine (**11**). - A mixture of **9** (1.3 g, 2 mmol), pentenyloxymethyl chloride (0.54 g, 4 mmol) and DIPEA (0.87 mL, 5 mmol) was heated for 3 h at 50°C. Processing of the reaction mixture, as described for the preparation of **4**, furnished **11** (1.2 g, 80%). ^{1}H NMR data ($CDCl_3$): δ 8.0-6.8 (m, 18 H, *H*-arom.); 6.35 (dd, 1 H, H-1′, $J_{1,2a}$~$J_{1,2b}$ 6.2 Hz); 5.75 (m, 1 H, H-4, POM); 5.02 (m, 2 H, H-5, POM); 4.66 (AB, 2 H, OC*H*$_2$O); 4.53 (m, 1 H, H-3′); 4.15 (m, 1 H, H-4′); 3.79 (s, 6 H, OC*H*$_3$, DMTr); 3.62-3.30

(m, 4 H, H-5′, H-1 POM); 2.60-2.28 (m, 2 H, H-2′); 2.07, 1.64 (2x m, H-2, H-3 POM); 1.46 (s, 3 H, C*H*$_3$, thymine). ^{13}C NMR data ($CDCl_3$): δ 162.8 (C-2); 158.7-113.2 (*C*-arom.); 137.8 (C-4, POM); 114.9 (C-5, POM); 111.1 (C-5); 94.3 (OCH_2O); 86.9 (C_{quat}, DMTr); 85.0, 84.4 (C-1′, C-4′); 76.5 (C-3′); 67.6 (C-1, POM); 63.3 (C-5′); 55.2 (OCH_3); 38.6 (C-2′); 30.1, 28.7 (C-2, C-3, POM); 11.7 (*C*H$_3$, thymine).

3′-O-Methoxyacetyl-N^3-benzoyl-thymidine (**12**). - Compound **8** (1.3 g, 2 mmol) was dissolved in 90% aq. acetic acid (15 mL) and heated at 50°C for 30 min. The reaction mixture was diluted with dichloromethane (20 mL), extracted with water (2x 20 mL) and aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica gel with 98:2 dichloromethane-acetone to give **12** (0.71 g, 85%). ^{1}H NMR data ($CDCl_3$): δ 8.0-7.4 (m, 6 H, *H*-arom.); 6.28 (dd, 1 H, H-1′, $J_{1,2a}$~$J_{1,2b}$ 7.2 Hz); 5.43 (m, 1 H, H-3′); 4.11 (m, 1 H, H-4′); 4.05 (s, 2 H, C*H*$_2$, MAc); 3.89 (m, 2 H, H-5′); 3.44 (s, 3 H, OC*H*$_3$, MAc); 2.43 (m, 2 H, H-2′); 1.93 (s, 3 H, C*H*$_3$, thymine). ^{13}C NMR data ($CDCl_3$): δ 169.9 (*C*=O, MAc); 168.8 (*C*=O, Bz); 162.7 (C-2); 149.3 (C-4); 136.1-128.9 (*C*-arom.); 111.2 (C-5); 85.6, 85.1 (C-1′, C-4′); 75.3 (C-3′); 69.5 (*C*H$_2$, MAc); 62.3 (C-5′); 59.4 (OCH_3, MAc); 37.4 (C-2′); 12.5 (*C*H$_3$, thymine).

Preparation of dimer ***13***.

a) From donor ***10***. - To a mixture containing **10** (0.35 g, 0.5 mmol), **12** (0.17 g, 0.4 mmol) and powdered molecular sieves (4Å) in 1,2-dichloroethane (7 mL) was added NIS (675 mg, 3 mmol) in three equal portions with an interval of 24 h. After stirring for 72 h, the reaction mixture was filtered, diluted with dichloromethane, washed with aq. $Na_2S_2O_3$ (1M, 2x 20 mL) and aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated. The residue was applied on a column of Sephadex LH-20 and eluted with 1:1 dichloromethane-methanol. The first fraction was concentrated to yield **13** (140 mg, 33%). ^{1}H NMR data ($CDCl_3$): δ 8.0-6.8 (m, 25 H, *H*-arom.); 6.38 (dd, 1 H, H-1′, $J_{1,2a}$~$J_{1,2b}$ 5.1 Hz); 6.29 (dd, 1 H, H-1′, $J_{1,2a}$~$J_{1,2b}$ 5.9 Hz); 5.25 (m, 1 H, H-3′, *T1*); 4.79 (AB, 2 H, OC*H*$_2$O); 4.48 (m, 1 H, H-3′, *T2*); 4.23, 4.14 (2x m, 2x H-4′); 4.00 (s, 2 H, C*H*$_2$, MAc); 3.78 (s, 6 H, OC*H*$_3$, DMTr); 3.80-3.45 (m, 4 H, 2x H-5′); 3.39 (s, 3 H, OC*H*$_3$, MAc); 2.80-2.20 (m, 4 H, 2x H-2′); 1.89, 1.49 (2x C*H*$_3$, thymine). ^{13}C NMR data ($CDCl_3$): δ 169.5 (*C*=O, MAc); 168.9, 168.8 (2x *C*=O, Bz); 158.6-113.1 (*C*-arom.); 111.0, 110.9 (2x C-5); 94.8 (OCH_2O); 86.9 (C_{quat}, DMTr); 84.8, 84.7, 84.4, 83.2 (2x C-1′, 2x C-4′); 78.7 (C-3′, *T2*); 74.7 (C-3′, *T1*); 69.3 (*C*H$_2$, MAc); 68.0 (C-5′, *T1*); 63.4 (C-5′, *T2*); 59.2 (OCH$_3$, MAc); 55.0 (OCH_3, DMTr); 38.4 (C-2′, *T2*); 37.1 (C-2′, *T1*); 12.5, 11.6 (2x *C*H$_3$, thymine).

Concentration of the second fraction furnished **15** (94 mg, 50% based on **10**). ^{1}H NMR data ($CDCl_3$): δ 8.0-6.8 (m, 19 H, *H*-arom.); 6.32 (dd, 1 H, H-1′, $J_{1,2a}$~$J_{1,2b}$ 6.2 Hz); 4.92 (s, 2 H, OCH_2N); 4.56 (m, 1 H, H-3′); 4.14 (m, 1 H, H-4′); 3.82 (s, 6 H, OC*H*$_3$, DMTr); 3.43 (m,2 H, H-5′); 2.68 (s, 4 H, C*H*$_2$, succinimide); 2.40 (m, 2 H, H-2′); 1.48 (s, 3 H, C*H*$_3$, thymine). ^{13}C NMR data ($CDCl_3$): δ 176.5 (*C*=O, succinimide); 158.6-113.2 (*C*-arom.); 111.0 (C-5); 84.7 (C_{quat}, DMTr); 84.7, 83.8 (C-1′, C-4′); 78.8 (C-3′); 66.3 (OCH$_2$N); 63.2 (C-5′); 55.1 (OCH$_3$, DMTr); 38.2 (C-2′); 28.0 (*C*H$_2$, succinimide); 11.7 (*C*H$_3$, thymine).

Anal. Calcd. for $C_{43}H_{41}N_3O_{11}$: C 68.0, H 5.4; found: C 67.8, H 5.5%.

b) From donor ***11*** *and NIS*. - A solution of **11** (0.37 g, 0.5 mmol) and **12** (0.17 g, 0.4 mmol) was treated with NIS (675 mg, 3 mmol) and processed as described above to provide **13** (0.34 g, 80%).

Anal. Calcd. for $C_{59}H_{58}N_4O_{16}$: C 65.7, H 5.4; found: C 65.9, H 5.2%.

c) From donor ***11*** *and IDCT*. - IDCT (0.31 g, 0.6 mmol) was added to a mixture of **11** (0.37 g, 0.5 mmol), **12** (0.17 g, 0.4 mmol) and powdered molecular sieves (5Å) in 1,2-dichloroethane (7 mL). After 30 min, the reaction mixture was filtered. The filtrate was diluted with dichloromethane, washed successively with aq. $Na_2S_2O_3$ (1M, 2x 20 mL) and aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated. Purification on silica gel (eluens: 97:3 dichloromethane-acetone) afforded **13** (0.24 g, 55%).

5'-O-Levulinoyl-3'-O-methylthiomethyl-N ^{3}-benzoyl-thymidine (**17**). - A solution of **10** (0.71 g, 1 mmol) in 90% aq. acetic acid (15 mL) was heated at 50°C for 30 min. The reaction mixture was taken up in dichloromethane, extracted with water (2x 20 mL) and aq. $NaHCO_3$ (0.9M, 20 mL), dried ($MgSO_4$) and concentrated. Purification by silica gel chromatography (eluens: 98:2 dichloromethane-acetone) gave **16** (365 mg, 0.9 mmol). Compound **16** was dissolved in pyridine (5 mL) whereupon levulinic anhydride (1M in dioxane, 2 mL) and *N*-methylimidazole (50µL) were added. After 2 h, water (1 mL) was added and the reaction mixture was concentrated. The residue was redissolved in dichloromethane, washed successively with water (20 mL) and aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated once more. The remaining oil was chromatographed on silica gel with 4:1 dichloromethane-hexane to give **17** (0.38 g, 84%). ^{1}H NMR data ($CDCl_3$): δ 7.94-7.45 (m, 6 H, *H*-arom.); 6.23 (dd, 1 H, H-1', $J_{1,2a}$~$J_{1,2b}$ 6.8 Hz); 4.62 (AB, 2 H, OC*H*$_2$S); 4.46-4.20 (m, 4 H, H-3', H-4', H-5'); 2.84-2.40 (m, 6 H, H-2', 2x C*H*$_2$-Lev); 2.18, 2.12 (2x s, 6 H, SC*H*$_3$, C*H*$_3$-Lev); 1.95 (s, 3 H, C*H*$_3$, thymine). ^{13}C NMR data ($CDCl_3$): δ 206.2 (Cγ, Lev); 172.1 (*C*=O, Lev); 168.7 (*C*=O, Bz); 162.4 (C-2); 148.9 (C-4); 135.1-128.8 (*C*-arom.); 110.8 (C-5); 85.1, 82.0 (C-1', C-4'); 77.5 (C-3'); 73.7 ((OCH$_2$S); 63.3 (C-5'); 37.5, 37.1 (C-2', *C*H$_2$β Lev); 29.4 (*C*H$_3$, Lev); 27.5 (*C*H$_2$α, Lev); 13.5 (*C*H$_3$S); 12.3 (*C*H$_3$, thymine).

5'-O-Levulinoyl-3'-O-(4-pentenyloxymethyl)-N ^{3}-benzoyl-thymidine (**18**). - Compound **11** (0.75 g, 1 mmol) was dissolved in 90% acetic acid and heated at 50°C for 30 min. Work-up as described above for the preparation of **16**, gave **18** (0.40 g, 0.89 mmol) which was redissolved in pyridine (5 mL) and treated with levulinic anhydride (1M in dioxane, 2 mL) and *N*-methylimidazole (50µL). Work-up and purification was effected similarly as described for the preparation of **17**, to give **19** (0.41 g, 86%). ^{13}C NMR data ($CDCl_3$): δ 206.3 (Cγ, Lev); 172.1 (*C*=O, Lev); 168.7 (*C*=O, Bz); 162.5 (C-2); 148.9 (C-4); 137.6 (C-4, POM); 135.1-128.9 (*C*-arom.); 114.7 (C-5, POM); 110.8 (C-6); 94.5 (OCH$_2$O); 85.1, 82.4 (C-1', C-4'); 76.1 (C-3'); 67.5 (C-1, POM); 63.4 (C-5'); 38.0 (C-2'); 37.5 (*C*H$_2$β, Lev); 30.4, 28.4 (C-2, C-3, POM); 29.5 (*C*H$_3$, Lev); 27.6 (*C*H$_2$α, Lev); 12.4(*C*H$_3$, thymine).

Preparation of dimer ***20***.

a) From donor ***17***. - To a cooled (0°C) mixture of **17** (202 mg, 0.4 mmol), **12** (125 mg, 0.3 mmol) and powdered molecular sieves (5Å) in 1,2-dichloroethane (7 mL) was added a freshly prepared solution of NIS (90 mg, 0.4 mmol) and TfOH (5.3 µL, 60 µmol) in 1:1 1,2-dichloroethane-diethylether (4 mL). After 1 min, the reaction mixture was filtered, diluted with dichloromethane, washed with aq. $Na_2S_2O_3$ (1M, 2x 10 mL) and aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated. The residue was chromatographed on Sephadex LH-20 with 1:1 dichloromethane-methanol to give dimer **20** (223 mg, 85%). ^{1}H NMR data ($CDCl_3$): δ 7.92-7.43 (m, 12 H, *H*-arom.); 6.34-6.21 (m, 2 H, 2x H-1'); 5.35 (m, 1 H, H-3', *T1*); 4.84 (AB, 2 H, OC*H*$_2$O); 4.54-4.19 (m, 5 H, H-4' *T1*, H-3',4',5' *T2*); 4.03 (s, 2 H, C*H*$_2$, MAc); 3.89-3.74 (m, 2 H, H-5', *T1*); 3.41 (s, 3 H, C*H*$_3$, MAc); 2.84-2.37 (m, 8 H, 2x H-2', 2x C*H*$_2$, Lev); 1.96, 1.91 (2x s, 6 H, 2x C*H*$_3$, thymine). ^{13}C NMR data ($CDCl_3$): δ 206.5 (Cγ, Lev); 172.2 (*C*=O, Lev); 169.7 (*C*=O, MAc); 168.7 (2x *C*=O, Bz); 162.4 (2x C-2); 149.1 (2x C-4); 135.2-129.0 (*C*-arom.); 111.0, 110.9 (2x C-5); 94.7 (OCH$_2$O); 85.3, 84.6, 83.2, 82.7 (2x C-1', 2x C-4'); 77.0 (C-3', *T2*); 74.7 (C-3, *T1*); 69.3 (*C*H$_2$, MAc); 67.9 (C-5', *T1*); 63.4 (C-5', *T2*); 59.2 (OCH$_3$, MAc); 37.7, 37.6, 37.1 (2x C-2', *C*H$_2$β Lev); 29.6 (*C*H$_3$, Lev); 27.6 (*C*H$_2$α, Lev); 12.5, 12.4 (2x *C*H$_3$, thymine).

Anal. Calcd. for $C_{43}H_{46}N_4O_{16}$: C 59.0, H 5.3; found: C 59.0, H 5.5%.

b) From donor ***19***. - Compound **19** (162 mg, 0.3 mmol) was condensed with **12** (104 mg, 0.25 mmol) in the presence of NIS-*cat.*TfOH as described above. Work-up (after 10 min) and purification afforded **20** (96 mg, 44%).

5'-O-Levulinoyl-3'-O-methylthiomethyl-thymidine (**22**). - Compound **3** (0.60 g, 1 mmol) was treated with 90% acetic acid and processed, as described for the synthesis of **17**, to give **21** (0.27 g, 0.9 mmol). Subsequent acylation

with levulinic anhydride furnished **22** (0.29 g, 81%). ^{1}H NMR data ($CDCl_3$): δ 10.1 (s, 1 H, H-3); 7.36 (s, 1 H, H-6); 6.28 (dd, 1 H, H-1′, $J_{1,2a}$~$J_{1,2b}$ 6.7 Hz); 4.66 (AB, 2 H, OCH_2S); 4.47-4.21 (m, 4 H, H-3′, H-4′, H-5′); 2.86-2.34 (m, 6 H, H-2′, 2x CH_2 Lev); 2.21, 2.15 (2x s, 6 H, SCH_3, CH_3 Lev); 1.94 (CH_3, thymine). ^{13}C NMR data ($CDCl_3$): δ 206.3 (Cγ, Lev); 172.2 (*C*=O, Lev); 163.9 (C-2); 150.3 (C-4); 135.0 (C-6); 111.0 (C-5); 84.8, 81.8 (C-1′, C-4′); 75.6 (C-3′); 73.7 (OCH$_2$S); 63.6 (C-5′); 37.6, 37.2 (C-2′, CH$_2$β Lev); 29.5 (*C*H$_3$, Lev); 27.6 (*C*H$_2$α, Lev); 13.6 (*C*H$_3$S); 12.4 (*C*H$_3$, thymine).

Preparation of dimer ***23***. - Coupling of donor **22** (120 mg, 0.3 mmol) with acceptor **6** (79 mg, 0.25 mmol) was performed in 1,2-dichloroethane (5 mL) at 0°C in the presence of NIS (68 mg, 0.3 mmol) and TfOH (4 µL, 45 µmol). After 1 min, the reaction mixture was filtered and processed as described for the preparation of **20**, to afford **23** (140 mg, 84%). ^{1}H NMR data ($CDCl_3$): δ 10.11 (s, 2 H, 2x H-3); 7.44, 7.37 (2x s, 2 H, 2x H-6); 6.35 (dd, 1 H, H-1′, $J_{1,2a}$~$J_{1,2b}$ 7.2 Hz); 6.26 (dd, 1 H, H-1′, $J_{1,2a}$~$J_{1,2b}$ 6.8 Hz); 5.36 (m, 1 H, H-3, *T1*); 4.84 (AB, 2 H, OCH_2O); 4.52-4.20 (m, 5 H, H-4′ *T1*, H-3′,4′,5′ *T2*); 4.10 (s, 2 H, CH_2, MAc); 3.98-3.71 (m, 2 H, H-5′, *T1*); 3.46 (s, 3 H, OCH_3, MAc); 2.86-2.15 (m, 8 H, 2x H-2′, 2x CH_2 Lev); 2.18 (s, 3 H, CH_3, Lev); 1.92, 1.91 (2x CH_3, thymine). ^{13}C NMR data ($CDCl_3$): δ 206.4 (Cγ, Lev); 172.2 (*C*=O, Lev); 169.7 (*C*=O, MAc); 163.9, 163.8 (2x C-2); 150.5, 150.3 (2x C-4); 135.1 (2x C-6); 111.1, 111.0 (2x C-5); 94.6 (OCH$_2$O); 84.9, 84.4, 83.0, 82.4 (2x C-1′, 2x C-4′); 77.0 (C-3′, *T2*); 74.8 (C-3′, *T1*); 69.3 (CH$_2$, MAc); 67.9 (C-5′, *T1*); 63.4 (C-5′, *T2*); 59.2 (CH$_3$, MAc); 37.5, 37.1 (2x C-1′, CH$_2$β Lev); 29.5 (*C*H$_3$, Lev); 27.6 (*C*H$_2$α, Lev); 12.5, 12.3 (2x *C*H$_3$, thymine).

Anal. Calcd. for $C_{29}H_{38}N_4O_{14}$: C 52.3, H 5.8; found: C 52.0, H 5.6%.

Deblocking of ***20*** *to give dimer* ***24***. - Compound **23** (175 mg, 0.2 mmol) was dissolved in dioxane (5 mL) wherupon aq.NH_4OH (25%, 10 mL) was added. The resulting mixture was left for 2 h and concentrated. The oily residue was washed with dichloromethane (2x 20 mL) and purified by chromatography on Sephadex S100 (HiLoad, HR) with 0.1 M triethylammonium bicarbonate to give **24** (90 mg, 92%). ^{1}H NMR data (D_2O): δ 7.77, 7.64 (2x s, 2 H, 2x H-6); 6.3-6.2 (m, 2 H, 2x H-1′); 4.85 (AB, 2 H, OCH_2O); 1.88, 1.87 (2x s, 6 H, 2x CH_3, thymine). ^{13}C NMR data (D_2O): δ 166.4 (2x C-2); 152.4 (2x C-2); 137.9 (2x C-6); 111.6, 111.5 (2x C-5); 96.0 (OCH$_2$O); 86.9, 86.2 (2x bs, 2x C-1′, 2x C-4′); 78.8 (C-3′, *T2*); 72.2 (C-3′, *T1*); 69.0 (C-5′, *T1*); 62.8 (C-5′, *T2*); 40.8, 38.9 (2x C-2′); 12.7, 12.5 (2x *C*H$_3$, thymine).

Anal. Calcd. for $C_{21}H_{28}N_4O_{10}$: C 50.8, H 5.7; found: C 50.5, H 6.0%.

Removal of the levulinoyl group of ***20*** *to give* ***25***. - To a solution of **20** (218 mg, 0.25 mmol) in 4:1 pyridine-ethylacetate (3 mL) was added a freshly pepared mixture of 1M hydrazine in 3:2 pyridine-acetic acid (3 mL). After 1 min, the reaction mixture was diluted with dichloromethane, extracted with water (2x 20 mL) and aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated. Silica gel chromatography (eluens: 95:5 dichloromethane-acetone) then afforded **25** (150 mg, 78%). ^{13}C NMR data ($CDCl_3$): δ 170.1 (*C*=O, MAc); 168.9, 168.8 (2x *C*=O, Bz); 162.7, 162.6 (2x C-2); 149.2 (2x C-4); 136.2-129.1 (*C*-arom.); 111.2, 110.9 (2x C-5); 94.1 (OCH$_2$O); 86.0, 85.2, 84.9, 83.4 (2x C-1′, 2x C-4′); 76.2 (C-3′, *T2*); 74.8 (C-3′, *T1*); 69.5 (CH$_2$, MAc); 67.7 (C-5′, *T1*); 62.0 (C-5′, *T2*); 59.4 (CH$_3$, MAc); 37.7, 37.4 (2x C-2′); 12.7, 12.5 (2x *C*H$_3$, thymine).

Synthesis of trimer ***26***. - To a cooled (0°C) and stirred mixture of donor **17** (120 mg, 0.24 mmol), acceptor **25** (150 mg, 0.195 mmol) and powdered molecular sieves (5Å) in 1,2-dichloroethane (5 mL) was added a solution of NIS (54 mg, 0.24 mmol) and TfOH (3.2 µL, 36 µmol) in 1:1 dichloroethane-diethyl ether (2.4 mL). After 1 min, the reaction mixture was filtered. The filtrate was diluted with dichloromethane, washed successively with aq. $Na_2S_2O_3$ (1M, 2x 10 mL) and aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated. Purification was effected

by chromatography on Sephadex LH-20 (eluens 1:1 dichloromethane-methanol) to give **26** (199 mg, 83%). ^{13}C NMR data ($CDCl_3$): δ 206.4 (Cγ, Lev); 172.3 (*C*=O, Lev); 169.7 (*C*=O, MAc); 168.8, 168.7 (*C*=O, Bz); 162.5, 162.4 (3x C-2); 149.1 (3x C-4); 135.5-129.0 (*C*-arom.); 111.0 (double intensity), 110.9 (3x C-5); 95.3, 94.5 (2x OCH_2O); 85.5 (double intensity), 84.8, 83.4, 83.2, 82.7 (3x C-1′, 3x C-4′); 77.8, 77.4 (2x C-3′, *T2*, *T3*); 74.6 (C-3′, *T1*); 69.4 (CH_2, MAc); 68.3, 67.9 (2x C-5′, *T1*, *T2*); 63.6 (C-5′, *T3*); 59.2 (CH_3, MAc); 37.9, 37.6, 37.4, 37.1 (3x C-2′, $CH_2\beta$ Lev); 29.6 (CH_3, Lev); 27.7 ($CH_2\alpha$, Lev); 12.6, 12.5 (double intensity) (CH_3, thymine).

Deblocking of 26 to give 27. - Aq. NH_4OH (25%, 10 mL) was added to a solution of **26** (123 mg, 0.1 mmol) in dioxane (4 mL). After 2 h, the mixture was concentrated and the residue was purified by chromatography on Sephadex S100 (HiLoad, HR) with 0.1 M triethylammonium bicarbonate. The appropriate fractions were collected and lyophillized to furnish **27** (68 mg, 91%). ^1H NMR data ($CDCl_3$): δ 6.4-6.2 (m, 3 H, 3x H-1′); 4.87, 4.85 (2x AB, 4 H, OCH_2O). ^{13}C NMR data ($CDCl_3$): δ 166.4 (3x C-2); 152.3 (3x C-4); 138.0 (3x C-6); 111.9 (double intensity), 111.8 (3x C-5); 96.1 (bs, 2x OCH_2O); 86.8, 86.6, 86.3, 85.1 (3x C-1′, 3x C-′); 79.1 (2x C-3′, *T2*, *T3*); 72.1 (C-3′, *T1*); 69.1 (bs, 2x C-5′, *T1,T2*); 62.7 (C-5′, *T3*); 40.5, 38.8, 38.6 (3x C-2′); 12.7 (bs), 12.5 (3x CH_3, thymine).

Anal. Calcd. for $C_{32}H_{42}N_6O_{15}$: C 51.2, H 5.6; found: C 50.8, H 5.9%.

Removal of the MAc group of 13 to give 28. - Compound **13** (0.32 g, 0.3 mmol) was dissolved in 1:1 dichloromethane-methanol (5 mL) whereupon potassium-*tert.*-butoxide (5 mg) was added. After 15 min, the reaction mixture was taken up into dichloromethane, washed with water (20 mL) and aq. NaCl (1.5M, 10 mL), and dried ($MgSO_4$). Evaporation of the solvent yielded a residue which was chromatographed on silica gel with 95:5 dichloromethane-acetone to give **28** (0.29 g, 96%). ^{13}C NMR data ($CDCl_3$): δ 168.9 (*C*=O, Bz); 162.6 (C-2); 149.2 (C-4); 158.6-113.2 (*C*-arom.); 111.3, 110.6 (2x C-5); 94.8 (OCH_2O); 86.9 (C_{quat}, DMTr); 85.1, 85.0 (double intensity), 84.4 (2x C-1′, 2x C-4′); 78.3 (C-3′, *T2*); 71.2 (C-3′, *T1*); 68.0 (C-5′, *T1*); 63.4 (C-5′, *T2*); 55.1 (OCH_3, DMTr); 40.2, 38.4 (2x C-2′); 12.5, 11.7 (2x CH_3, thymine).

Preparation of amidite 30. - To a stirred solution of **28** (0.29 g, 0.29 mmol) and DIPEA (0.1 mL, 0.6 mmol) in 1,2-dichloroethane (5 mL) was added 2-cyanoethoxy(N,N-diisopropylamino)chlorophosphine (**29**) (0.11 g, 0.45 mmol). After 10 min, the reaction mixture was extracted with aq. NaCl (1.5M, 2x 10 mL) and aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated. Chromatography of the residue on silica gel (eluens: 98:2 ethylacetate-triethylamine) gave **30** (0.99 g, 82%). ^{31}P NMR data ($CDCl_3$): δ 149.4 and 149.3.

Preparation of tetramer 32. - 1-*H*-Tetrazole (71 mg, 1 mmol) was added to a stirred solution of **25** (155 mg, 0.2 mmol) and **30** (99 mg, 0.24 mmol) in acetonitrile (3 mL). After stirring for 5 min, *tert.*BuOOH ((90%, 54 μL, 0.6 mmol) was introduced while stirring was continued for another 10 min. Concentration gave crude **31**, which was redissolved in 90% acetic acid and heated at 50°C for 30 min. The reaction mixture was diluted with dichloromethane, extracted with water (2x 15 mL) and aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated. Purification was effected by silica gel chromatography (eluens: 95:5 dichloromethane-acetone). The appropriate fractions were concentrated and redissolved in dioxane (10 mL). Aq. ammoniumhydroxide (25%, 20 mL) was introduced slowly and the mixture was left for 3 h. The reaction mixture was concentrated and the residue was chromatographed on Sephadex S100 (HiLoad, HR) with 0.1 M triethylammonium bicarbonate to give **32** (190 mg, 82%). ^{13}C NMR data (D_2O): δ 167.7, 167.5 (4x C-2); 152.7, 152.6 (4x C-4); 137.9, 137.7 (4x C-6); 112.0, 111.9, 111.7 (4x C-5); 95.5 (bs, 2x OCH_2O); 85.8, 85.6, 85.2 (4x C-1′, 2x C-4′, *T1,T4*); 84.4, 83.9 (2x C-4′, *T2,T3*, $^3J_{CP}$ 7.33 and 8.79 Hz); 78.3 (2x C-3′, *T2,T4*); 75.6 (C-3′, *T3*, $^2J_{CP}$ 5.86 Hz); 70.9 (C-3′, *T1*); 68.0, 67.8 (2x C-5′,

T1,T3); 65.5 (C-5', *T2*); 61.6 (C-5', *T4*); 39.1, 38.1, 37.5 (4x C-2'); 12.4 (4x *C*H $_3$, thymine).

5'-O-methylthiomethyl-3'-O-methoxyacetyl-N ^{3}-benzoyl-thymidine (**33**). - To a cooled (0°C) mixture of **12** (0.84 g, 2 mmol) and dimethylsulphide (1.46 mL, 20 mmol) in acetonitrile (15 mL) was added within 30 min benzoyl peroxide (2 g, 8 mmol). After 2 h, the reaction mixture was concentrated. The remaining oil was dissolved in dichloromethane, extracted with water (2x 20 mL) and aq. $NaHCO_3$ (0.9M, 20 mL), dried ($MgSO_4$) and concentrated. The residue was applied on a column of silica gel, which was eluted with 7:3 dichloromethane-hexane. The appropriate fractions were concentrated to give **33** (0.69 g, 72%). ^{1}H NMR data ($CDCl_3$): δ 7.95-7.44 (m, 6 H, *H*-arom.); 6.36 (dd, 1 H, H-1', $J_{1,2a}$ 5.7 Hz, $J_{1,2b}$ 8.5 Hz); 5.38 (m, 1 H, H-3'); 4.73 (AB, 2 H, OC*H*$_2$S); 4.23 (m, 1 H, H-4'); 4.04 (s, 2 H, C*H*$_2$, MAc); 3.84 (m, 2 H, H-5'); 3.43 (s, 3 H, OC*H*$_3$, MAc); 2.50-2.22 (m, 2 H, H-2'); 2.19 (s, 3 H, SC*H*$_3$); 1.96 (s, 3 H, C*H*$_3$, thymine). ^{13}C NMR data ($CDCl_3$): δ 169.6 (*C*=O, Bz), 162.4 (C-2); 149.1 (C-4); 135.0-128.9 (*C*-arom.); 111.0 (C-5); 84.7, 83.4 (C-1', C-4'); 75.8 (O*C*H$_2$S); 75.3 (C-3'); 69.3 (*C*H$_2$, MAc); 67.9 (C-5'); 59.2 (O*C*H$_3$, MAc); 37.5 (C-2'); 14.3 (S*C*H$_3$); 12.5 (*C*H$_3$, thymine).

Preparation of dimers 35 and 38. - A solution of NIS (68 mg, 0.3 mmol) and TfOH (4 µL, 45 µmol) in 1:1 1,2-dichloroethane-diethyl ether (3 mL) was added to a cooled (0°C) mixture of **33** (143 mg, 0.3 mmol) and **34** (135 mg, 0.25 mmol) in 1,2-dichloroethane (5 mL). After 1 min, the reaction mixture was filtered. The filtrate was diluted with dichloromethane, extracted successively with aq. $Na_2S_2O_3$ (1M, 2x 20 mL) and aq. $NaHCO_3$ (0.9M, 20 mL), dried ($MgSO_4$) and concentrated. The remaining oil was chromatographed on Sephadex LH-20 with 1:1 dichloromethane-methanol to give **35** (220 mg, 91%, α:β = 8:1). ^{1}H NMR data ($CDCl_3$): α-isomer, δ 6.33 (dd, 1 H, H-1', *T*, $J_{1,2a}$ 5.7 Hz, $J_{1,2b}$ 8.5 Hz); 5.15 (d, 1 H, H-1, $J_{1,2}$ 3.6 Hz). ^{13}C NMR data ($CDCl_3$): α-isomer, δ 169.5 (*C*=O, Mac), 168.6 (*C*=O, Bz); 162.4 (C-2, *T*); 149.2 (C-4, *T*); 138.4-127.4 (*C*-arom.); 111.1 (C-5, *T*); 94.0 (C-1, J_{CH} 171 Hz); 92.0 (O*C*H$_2$O); 84.7, 83.2 (C-1', C-4', *T*); 81.8, 79.1, 77.5, 71.1 (C-2,3,4,5); 75.4 (C-3', *T*); 75.5, 75.0, 74.9, 73.3 (benzyl-*C*H$_2$); 69.3 (*C*H$_2$, MAc); 68.5, 68.3 (C-5' *T*, C-6); 59.2 (O*C*H$_3$, MAc); 37.7 (C-2', *T*); 12.6 (*C*H$_3$, thymine).
Similarly, NIS-TfOH mediated condensation of **33** (143 mg, 0.3 mmol) with **37** (82 mg, 0.25 mmol) furnished, after purification on silica gel (eluens: 97:3 dichloromethane-acetone), **38** (159 mg, 84%). ^{13}C NMR data ($CDCl_3$): δ 169.2 (*C*=O MAc, *C*=O serine); 168.7 (*C*=O, Bz); 162.4 (C-2, *T*); 155.7 (*C*=O, CBz); 149.1 (C-4, *T*); 135.9-127.9 (*C*-arom.); 111.0 (C-5, *T*); 95.7 (O*C*H$_2$O); 84.6, 83.2 (C-1', C-4', *T*); 74.9 (C-3', *T*); 69.3 (*C*H$_2$, MAc); 68.4 (C-5', *T*); 67.7, 67.3, 66.9 (*C*H$_2$ serine, benzyl-*C*H$_2$); 59.2 (O*C*H$_3$, MAc); 54.2 (*C*H, serine); 37.4 (C-2', *T*); 12.5 (*C*H$_3$, thymine).

Deblocking of dimers 35 and 38. - Compound **35** (194 mg, 0.2 mmol) was dissolved in 7:3 dioxane-water (8 mL) and hydrogenated in the presence of palladium on charcoal (10% Pd, 100 mg) for 24 h. The catalyst was removed by filtration and the filtrate was concentrated. The residue was dissolved in ammoniumhydroxide (25%, 15 mL) and left for 2 h. The mixture was concentrated and the residue was chromatographed on Sephadex S100 (HiLoad, HR) with 0.1 M triethylammonium bicarbonate to give **36** (60 mg, 70%). ^{13}C NMR data ($CDCl_3$): δ 166.9 (C-2, *T*); 152.0 (C-4, *T*); 138.0 (C-6, *T*); 111.7 (C-5, *T*); 96.1 (C-1); 92.9 (O*C*H$_2$O); 85.7, 85.6 (C-1', C-4', *T*); 73.5, 72.9, 71.6, 70.0 (C-2,3,4,5); 71.4 (C-3', *T*); 68.6 (C-5', *T*); 61.0 (C-6); 39.1 (C-2', *T*); 12.2 (*C*H $_3$, thymine).

Anal. Calcd. for $C_{17}H_{26}N_2O_{11}$: C 47.0, H 6.0; found: C 46.8, H 6.2%.

Similarly, processing of **38** (150 mg, 0.2 mmol), as described above, afforded **39** (60 mg, 83%). ^{1}H NMR data ($CDCl_3$): δ 175.2 (*C*=O, serine); 167.2 (C-2, *T*); 152.4 (C-4, *T*); 138.0 (C-6, *T*); 111.9 (C-5, *T*); 96.0 (O*C*H$_2$O); 85.8, 85.5 (C-1', C-4', *T*); 71.4 (C-3', *T*); 68.2 (C-5', *T*); 67.6 (*C*H $_2$, serine); 62.8 (*C*H, serine); 38.9 (C-2', *T*); 12.3 (*C*H$_3$, thymine).

Anal. Calcd. for $C_{14}H_{21}N_3O_8$: C 46.8, H 5.9; found: C 46.8, H 6.0%.

Synthesis of pyrophosphate analogue 42. - A solution of NIS (90 mg, 0.4 mmol) and dibenzyl phosphate **40** (110 mg, 0.4 mmol) in tetrahydrofuran (4 mL) was added to a mixture of **33** (143 mg, 0.3 mmol) and powdered molecular sieves (5Å) in 1,2-dichloroethane (5 mL). After stirring for 20 min, the reaction mixture was filtered. The filtrate was taken up in dichloromethane, washed with aq. $Na_2S_2O_3$ (1M, 2x 10 mL) and aq. $NaHCO_3$ (0.9M, 10 mL), dried ($MgSO_4$) and concentrated to give **41**. ^{1}H NMR data ($CDCl_3$): δ 7.91-7.35 (m, 16 H, *H*-arom.); 6.33 (dd, 1 H, H-1′, $J_{1,2a}$ 5.7 Hz, $J_{1,2b}$ 9.0 Hz); 5.32 (m, 1 H, H-3′); 5.21 (AB, 2 H, OCH_2O, J_{HP} 12.2 Hz); 5.09, 5.05 (2x AB, 4 H, benzyl-CH_2); 4.16 (m, 1 H, H-4′); 4.01 (s, 2 H, CH_2, MAc); 3.95 (m, 2 H, H-5′); 3.41 (s, 3 H, OCH_3, MAc); 2.38- 2.17 (m, 2 H, H-2′); 1.91 (s, 3 H, CH_3, thymine). ^{13}C NMR data ($CDCl_3$): δ 169.6 (*C*=O, MAc); 168.7 (*C*=O, Bz); 162.4 (C-2), 149.1 (C-4); 135.3- 127.8 (*C*-arom.); 111.3 (C-5); 92.1 (d, OCH_2O, J_{CP} 3.4 Hz); 84.5, 82.9 (C-1′, C-4′); 75.3 (C-3′); 69.4, 69.3, 69.2 (C-5′, CH_2 MAc, benzyl-CH_2); 59.2 (OCH_3, MAc); 37.2 (C-2′); 12.5 (CH_3, thymine).

Deblocking of **41** was effected in a similar way as described for the preparation of **36** and **38** to give **42** as the triethylammonium salt. Compound **42** thus obtained was applied on a column of Dowex (Na^+-form) and eluted with water. The UV-positive fraction (254 nm) was lyophillized to give **42** as the corresponding Na^+-salt (60 mg, 61%). ^{31}P NMR data (D_2O): δ -1.03. ^{1}H NMR data (D_2O): δ 7.62 (s, 1 H, H-6); 6.28 (dd, 1 H, H-1′, $J_{1,2a}$~$J_{1,2b}$ 6.8 Hz); 5.09 (AB, 2 H, OCH_2O, J_{HP} 10.4 Hz); 4.53 (m, 1 H, H-3′); 4.13 (m, 1 H, H-4′); 3.71 (m, 1 H, H-5′); 2.36 (m, 2 H, H-2′); 1.88 (s, 3 H, CH_3, thymine). ^{13}C NMR data (D_2O): δ 152.2 (C-4); 138.0 (C-6); 112.0 (C-5); 91.6 (d, OCH_2O, J_{CP} 4.4 Hz); 85.6, 85.5 (C-1′, C-4′); 71.4 (C-3′); 68.9 (C-5′); 38.9 (C-2′); 12.2 (CH_3, thymine).

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