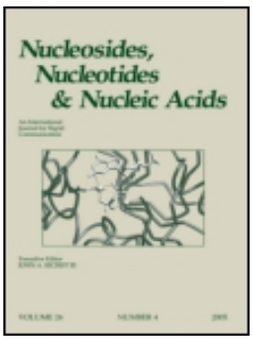

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6-(Levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl and 2-(Levulinyloxymethyl)-5-methoxy-4-nitrobenzoyl Groups as Novel Base-Labile Groups for 5′-Hydroxy Protection in Solid-Phase Oligonucleotide Synthesis

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ABSTRACT

The 6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl (LMM*o*NBz) and 2-(levulinyloxymethyl)-5-methoxy-4-nitrobenzoyl (LMM*p*NBz) groups were developed as novel base-labile groups for 5′-hydroxy protection in solid-phase oligonucleotide synthesis. A comparative study of the utility of LMM*o*NBz, LMM*p*NBz, and 2-(levulinyloxymethyl)-5-nitrobenzoyl (LMNBz) groups is described.

Key Words: Base-labile protecting group; 5′-Hydroxy protection; Oligonucleotide synthesis.

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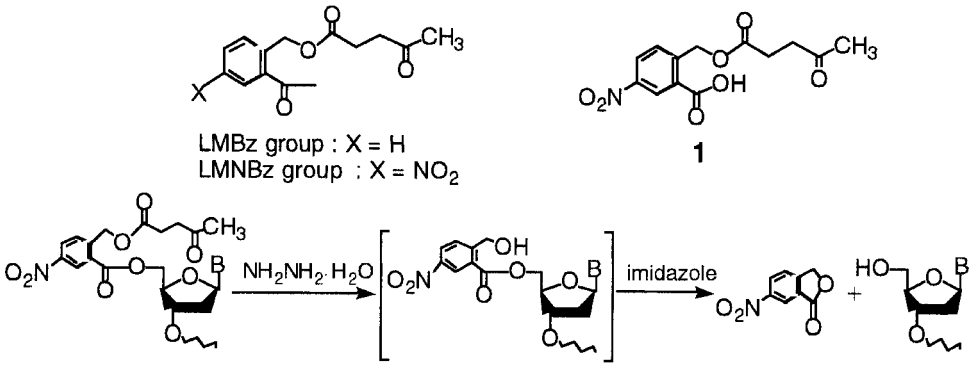
INTRODUCTION

Although reliable methods of solid-phase oligodeoxyribonucleotide (DNA) synthesis using the 4,4′-dimethoxytrityl (DMTr) group to protect the 5′-hydroxy group of nucleotide units have been established, the DMTr group is not ideal for acidic deprotection at successive cycles, giving rise to depurination, particularly N^6-benzoyl-2′-deoxyadenosine residues.[1] When the DMTr group is used to protect the 5′-hydroxy group in oligoribonucleotide (RNA) synthesis in combination with the acid-labile tetrahydropyran-2-yl (Thp) group to protect the 2′-hydroxy group, it has been shown that repeated acidic treatment leads to significant loss of the Thp group and subsequent phosphoryl migration.[2] Clearly there is a need for an alternative 5′-hydroxy protecting group for certain applications in oligonucleotide synthesis. Consequently, various protecting groups for the 5′-hydroxy group of 2′-deoxyribonucleoside and ribonucleoside 3′-phosphoramidites have been reported,[3] as exemplified by the levulinyl group[4] and the modified 2-hydroxymethylbenzoyl group,[5] both of which are removable under basic conditions. The Lev group is easily removed under mild conditions by treatment with 0.5 M hydrazine monohydrate in 1:4 acetic acid - pyridine at room temperature, although the yield of 5′-*O*-levulinylation is unsatisfactory due to low regioselectivity (30–46% for 2′-*O*-Thf-U, -C^{Bz}, and -A^{Bz})[4d]. Based on the chemistry of these protecting groups, we previously reported the utility of the 2-(levulinyloxymethyl)-5-nitrobenzoyl (LMNBz) group to compare it to the 2-(levulinyloxymethyl)benzoyl (LMBz) group, which has higher regioselectivity, yet allows easy removal of the Lev group.[6] The protecting reagent, 2-(levulinyloxymethyl)-5-nitrobenzoic acid (**1**), was easily prepared from phthalide. The LMNBz group was introduced at the 5′-position of 2′-deoxyribonucleosides (**2**) and 2′-*O*-Thp-ribonucleosides (**3**) by treatment with **1** (1.1 equiv) in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) (2.2 equiv) in pyridine at room temperature, with yields over 63%, regardless of the nucleoside structure (Sch. 2). Moreover, the LMNBz group was removed by consecutive treatments with 0.5 M hydrazine monohydrate in 1:4 acetic acid/pyridine, and 0.5 M imidazole in acetonitrile. Using the LMNBz protecting group, the synthesis of both DNA- and RNA-type oligomers was efficiently performed



Scheme 1.

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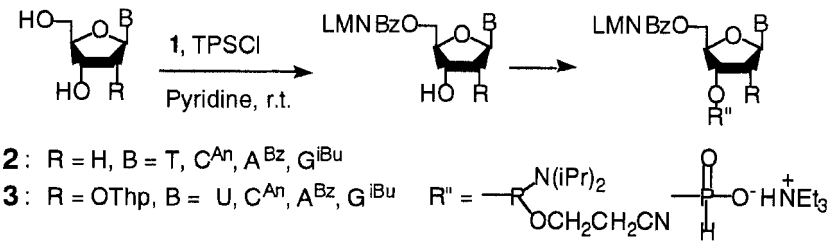

Scheme 2.

on controlled pore glass (CPG) supports using the phosphoramidite[6] and *H*-phosphonate methods.[7] Introducing the nitro group to the *m*-position of the LMBz group enhances susceptibility of the LMNBz group to the processes of both hydrazinolysis and deprotonation with imidazole (Sch. 1).

Furthermore, we developed *o*- and *p*-nitrobenzoyl derivatives, 6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl (LMM*o*NBz) and 2-(levulinyloxymethyl)-5-methoxy-4-nitrobenzoyl (LMM*p*NBz), as novel base-labile groups to protect the 5′-hydroxy group in solid-phase oligonucleotide synthesis,[a] and performed a comparative study of the LMM*o*NBz, LMM*p*NBz, and LMNBz groups. The results of this study will be described in full herein.

RESULTS AND DISCUSSION

Synthesis of 5′-*O*-LMM*o*NBz- and -LMM*p*NBz-2′-deoxyribonucleosides [9 (*o*) and 9 (*p*)]. The protecting reagents, 6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoic acid (LMM*o*NBzOH) [**4** (*o*-NO_2)] and 2-(levulinyloxymethyl)-5-methoxy-4-nitrobenzoic acid (LMM*p*NBzOH) [**4** (*p*-NO_2)], were easily prepared from *m*-anisic acid (**5**). Treatment of **5** with formaldehyde in the presence of conc. hydrochloric acid resulted in the formation of 5-methoxyphthalide (**6**) exclusively. After alkaline hydrolysis of **6**, the resulting mixture was adjusted at ca. pH 2 with conc. hydrochloric acid. 2-(Hydroxymethyl)-5-methoxybenzoic acid (**7**) was obtained as a white crystalline solid in 54% yield from **5**. Treatment of **7** with levulinic anhydride in the presence of 1-methylimidazole led to the formation of 2-(levulinyloxymethyl)-5-methoxybenzoic acid (**8**) in 64% yield. Compound **8** was then treated with a mixture of conc. nitric acid and sulfuric acid to afford LMM*o*NBzOH [**4** (*o*-NO_2) (34% yield) and LMM*p*NBzOH [**4** (*p*-NO_2)] (21% yield) (Sch. 3).

Introducing LMM*o*NBz and LMM*p*NBz to the 5′-position of 2′-deoxyribonucleosides (**2**) was accomplished by treatment with **4** (*o*- and *p*-NO_2) (1.1 equiv), respectively, in the presence of TPSCl (2.2 equiv) in pyridine at room temperature.

[a]A portion of the present work was presented on the occasion of the XVth International Round Table "Nucleosides, Nucleotides and Nucleic Acids", Leuven, Belgium, September 10–14, 2002.[8]

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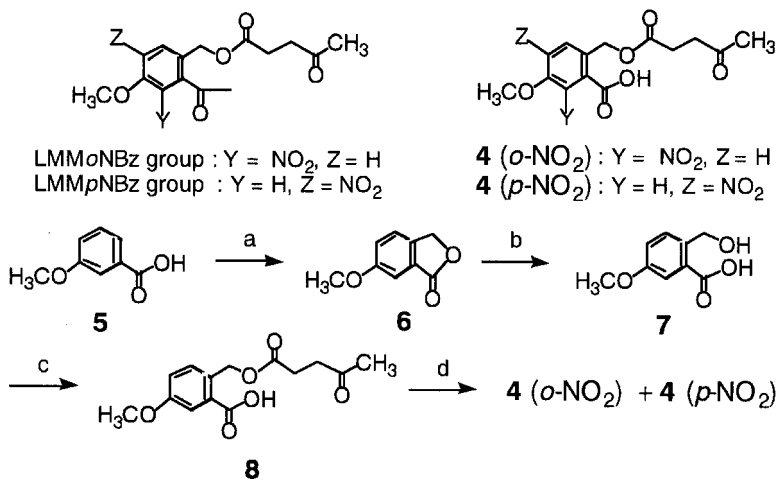
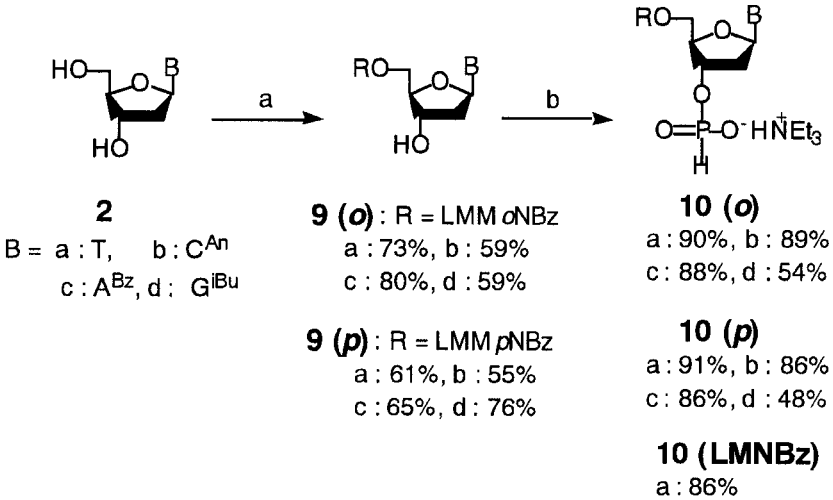


Scheme 3. Conditions: (a) HCHO, conc. HCl/1,4-dioxane, 50–55°C, 3 days, (b) 1) KOH, 85% MeOH-H_2O, 55°C, 30 min, 2) conc. HCl, (c) levulinic anhydride, 1-methylimidazole, 1,4-dioxane, r.t., 1 h, (d) HNO_3, H_2SO_4 in an ice-salt bath, 20 min.

The corresponding 5′-*O*-benzoyl derivatives **9** (*o* and *p*) were obtained in 55–80% yields (Sch. 4).

Synthesis of Oligodeoxyribonucleosides

We used manual synthesis[9] to perform a basic study of solid-phase oligonucleotide synthesis using the *H*-phosphonate method,[10] as shown in Sch. 5. 5′-*O*-LMNBz-,



Scheme 4. Conditions: (a) 4 (*o*- or *p*-NO_2), TPSCl, pyridine, r.t., 2–3 h, (b) 1) tris-(1,2,3-triazolyl)phosphine, *N*-methylmorpholine, CH_2Cl_2, 0°C, 30 min, 2) 1 M TEAB aq.

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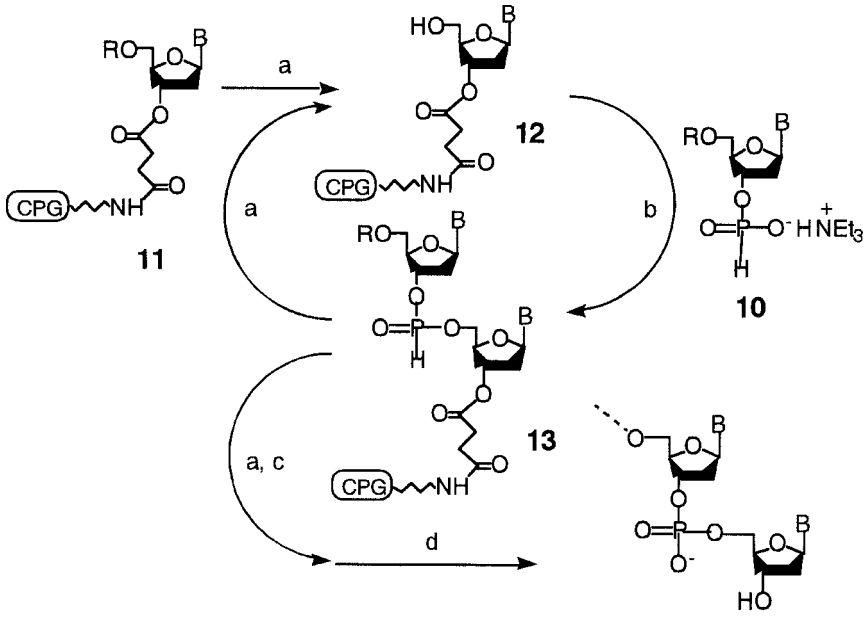

Scheme 5. Conditions (a) 1) 0.5 M $NH_2NH_2H_2O$, 2:3:5 CH_3CO_2H-pyridine-CH_3CN, 15 min, 2) 0.5 M imidazole, CH_3CN, 5 min, (b) 0.05 M **10**, 0.25 M pivaloyl chloride, 1:1 pyridine-CH_3CN, r.t., 10 min, (c) 3% I_2, H_2O-19% pyridine-76% THF, r.t., 10 min, (d) conc. NH_4OH, r.t., 3 h and 55°C. 6 h.

-LMM*o*NBz-, and -LMMN*p*Bz-2′-deoxyribonucleoside 3′-*H*-phosphonates [**10** (*o*, *p*, and LMNBz)] were prepared by treating the respective 5′-*O*-LMNBz-, -LMM*o*NBz-, and -LMM*p*NBz-nucleosides [**9** (*o*, *p*, and LMNBz)] with tris(1,2,4-triazolyl)phosphine (3–5 equiv) and subsequent hydrolysis with aqueous triethylammonium bicarbonate (Sch. 4).[10] Prior to oligonucleotide synthesis, CPG support was functionalized with the 5′-*O*-LMNBz-, -LMM*o*NBz-, and -LMMN*p*Bz-2′-deoxyribonucleoside derivatives [**9** (*o*, *p*, and LMNBz)] in the usual manner.[11]

At first, we performed a comparative study of the LMM*o*NBz, LMM*p*NBz, and LMNBz groups to synthesize the oligonucleotides, as exemplified by the synthesis of dimer (TpT) using 5′-*O*-DMTr-thymidine 3′-*H*-phosphonate [**10a** (DMTr)][8] on CPGs [**11a** (*o*, *p*, and LMNBz)]. Following deprotection of the LMM*o*NBz, LMM*p*NBz, and LMNBz groups of the thymidine linked to CPG support [**11a** (*o*, *p*, and LMNBz)] [Sch. 5, condition (a)], the *H*-phosphonate monomer [**10a** (DMTr)] was coupled to the supported thymidine (**12**) in the presence of pivaloyl chloride as the condensing agent. After assembling the *H*-phosphonate dimer, the DMTr group was removed, and the *H*-phosphonate dimer was oxidized with I_2/H_2O to yield the desired phosphodiester-linked dimer. The TpT was then cleaved from the support, and was analyzed with reversed-phase HPLC [Fig. 1, HPLC peak area ratio of T ($t_R = 3.2$ min) and TpT ($t_R = 13.1$ min): T/TpT = 5/95 (I), 11/89 (II), and 22/78 (III)]. Superiority of the LMM*o*NBz protection was clearly evident in the reversed-phase HPLC chromatograms. The superior nature of LMM*o*NBz protec-

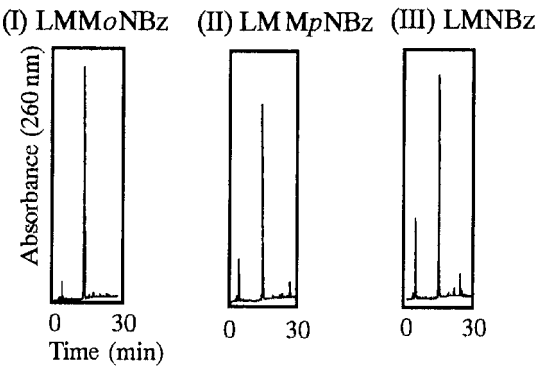

Figure 1. Reversed-phase HPLC profiles of crude products of TpT preparations using the 5′-*O*-DMTr-thymidine 3′-*H*-phosphonate **10a** (**DMTr**) derivative on CPGs [**11a** (*o*, *p*, and **LMNz**)], respectively.

tion over LMNBz protection prompted us to conduct further comparative studies, as exemplified by our synthesis of tetramer (TpTpTpT), using 5′-*O*-LMM*o*NBz, -LMM*p*NBz-, and -LMNBz-thymidine 3′-*H*-phosphonates [**10a** (*o*, *p*, and LMNBz)] on the commercially available 5′-*O*-DMTr-thymidine which was linked to CPG support [**11a** (DMTr)]. After deprotection of the DMTr group of the 5′-*O*-DMTr-thymidine linked to CPG support [**11a** (DMTr)], the *H*-phosphonate monomers [**10a** (*o*, *p*, and LMNBz)] were respectively coupled to the supported thymidine (**12a**). Following deprotection of the LMM*o*NBz, LMM*p*NBz, and LMNBz groups of the growing oligomer linked to CPG support, the *H*-phosphonate monomers [**10a** (*o*, *p*, and LMNBz)] were coupled to the growing oligomer (Sch. 5). After assembling *H*-phosphonate tetramer, the LMM*o*NBz, LMM*p*NBz and LMNBz groups were removed, and the *H*-phosphonate tetramer was oxidized with I_2/H_2O. The TpTpTpT was then cleaved from the support, and was analyzed with reversed-phase

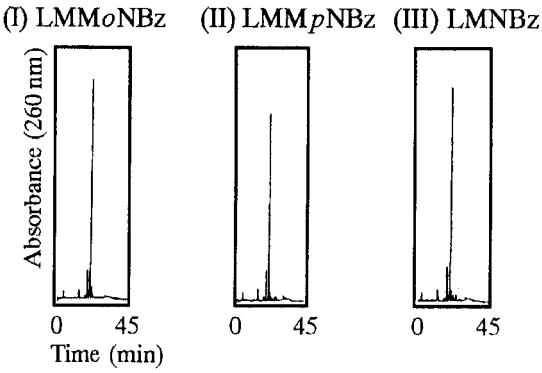

Figure 2. Reversed-phase HPLC profiles of crude products of TpTpTpT preparations using thymidine 3′-*H*-phosphonate derivatives [**10a** (*o*, *p*, and **LMNz**)] on CPG **11a** (**DMTr**), respectively.

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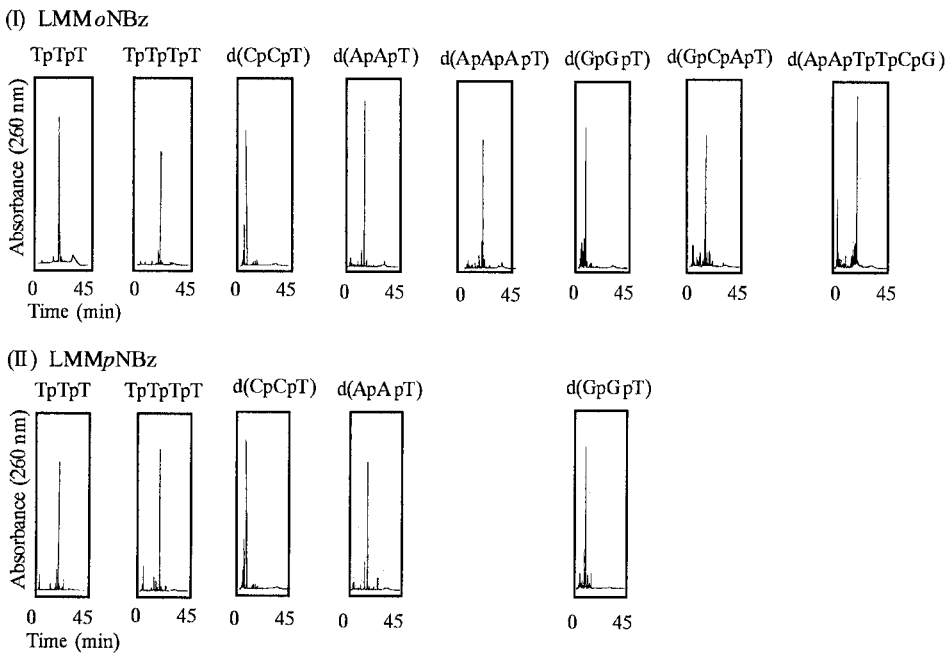

Figure 3. Reversed-phase HPLC profiles of crude products of oligodeoxyribonucleotide preparations using 3′-*H*-phosphonate monomers [**10** (*o* and *p*)] on CPGs [**11** (*o* and *p*)], respectively. Conditions of reversed-phase HPLC: column μBONDASPHERE 5μ C18 (3.9 mm ID × 150 mm L); elution buffer 7.25–50% acetonitrile/0.1 M TEAA (pH 7); flow rate 1 mL/min.

HPLC [Fig. 2, HPLC peak area ratio of TpT ($t_R = 13.1$ min), TpTpT ($t_R = 18.4$ min), and TpTpTpT ($t_R = 20.1$ min): TpT/TpTpT/TpTpTpT = 8/11/81 (I), 12/13/75 (II), and 10/14//76 (III)]. Oligomer synthesis using the LMMN*o*Bz group formed an oligomer with slightly better efficiency than oligomer synthesis reactions using the LMM*p*NBz and LMNBz groups.

Furthermore, using 5′-*O*-LMM*o*NBz- and -LMM*p*NBz-2′-deoxyribonucleoside 3′-*H*-phosphonates [**10a–d** (*o* and *p*)], oligodeoxyribonucleotides [TpTpT, TpTpTpT, d(CpCpT), d(ApApT), d(ApApApT), d(GpGpT), d(GpCpApT), and d(ApApTpTpCpG)] were synthesized on CPGs [**11a** (*o* and *p*)], respectively, as shown in Sch. 5. The HPLC tracings of each of the resulting mixtures are shown in Fig. 3. The enzymatic degradation of these oligodeoxyribonucleotides gave satisfactory data to prove their structures (Fig. 4).[3d,4c,4d]

Introducing the nitro group to the *o*-position of the benzoyl group is likely to enhance susceptibility of the 2-hydroxymethylbenzoyl derivative, which was produced by hydrazinolysis of the 2-(levulinyloxymethyl)benzoyl derivative, to the process of intramolecular cyclization by treatment with imidazole (Sch. 1). Thus, we confirmed the superiority of the LMM*o*NBz group over the LMMN*p*Bz and LMNBz, as shown in Fig. 1–3. The LMMN*o*Bz protecting goup is basically useful for 5′-hydroxy protection in solid-phase oligonucleotide synthesis, and is expected to be free from the incompatibility due to the use of the DMTr protecting group.

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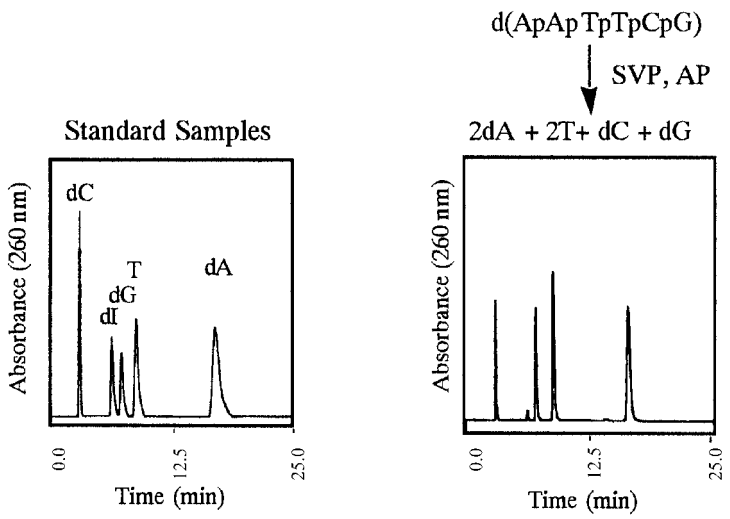

Figure 4. Reversed-phase HPLC analysis of products obtained by digestion of the completely unmasked hexamer with snake venom phosphodiesterase (SVP) and alkaline phosphatase (AP), and of standard samples. 2′-Deoxyinosine was produced from 2′-deoxyadenosine due to contamination with adenosine deaminase. Conditions of reversed-phase HPLC: column μBONDASPHERE 5μ C18 (3.9 mm ID × 150 mm L); elution buffer 3% acetonitrile/0.06 M TEAA (pH 7); flow rate 1 mL/min.

EXPERIMENTAL

Column chromatography was performed on silica gel (Wakogel C-300, purchased from Wako Pure Chemicals, Co. Ltd.) by the use of methanol/chloroform, and triethylamine/methanol/chloroform. TLC was conducted on Merck silica gel F_{254} by developing with 1:9 methanol/chloroform. High performance liquid chromatography (HPLC) was conducted on μBONDASPHRE 5μ C18 (3.9 mm × 150 mm L) for purification of oligodeoxyribonucleotiedes and analyses of the digestion of oligodeoxyribonucleotides with snake venom phosphodiesterase and alkaline phoaphatase. Melting points were determined by a Yanaco Micro-melting-point apparatus, and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian GEMINI-300 apparatus with $CDCl_3$ or DMSO-d_6 as an internal standard. ^{31}P-NMR spectra were recorded on a Brucker DRX 400 apparatus with 85% H_3PO_4 as an external standard. Mass spectra were recorded on a VG AutoSpecE apparatus. Elemental analyses were achieved with a Perkin-Elmer 240-002 apparatus.

2-(Hydroxymethyl)-5-methoxybenzoic acid (7). To a solution of *m*-anisic acid (**5**) (3.80 g, 25 mmol) in 1,4-dioxane (16 mL) was added a 37% formaldehyde solution (12 mL) and conc. hydrochloric acid (20 mL). The mixture was stirred for 3 days at 50°C. The resulting mixture was combined with chloroform (200 mL) and was washed with water (100 mL × 2). The organic layer was evaporated to give 5-methoxyphthalide (**6**) (3.28 g). Without further purification, **6** was treated with potassium hydroxide in 85:15 methanol/water (20 mL) for 30 min at 50°C. After evaporation,

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the residue was dissolved in water (100 mL) and was washed with diethyl ether (10 mL × 3). The aqueous solution was acidified (ca. pH 2) with conc. hydrochloric acid. The resulting precipitates were gathered by filtration, and were washed with chilled water (20 mL) to give a white powder of **7** (2.49 g, 54% yield from *m*-anisic acid): m.p. 120–121°C; ^{1}H-NMR ($CDCl_3$) δ 3.18 (s, 3H, OCH_3), 4.67 (s, 2H, PhCH_2), 7.01 (dd, 1H, $J = 2.8$ Hz and 8.4 Hz, H-4), 7.30 (d, 1H, $J = 2.8$ Hz, H-6), and 7.58 (d, 1H, $J = 8.4$ Hz, H-3). *Anal.* calcd. for $C_9H_{10}O_4$; C, 59.34; H, 5.53. Found: C, 59.18; H, 5.63.

2-(Levulinyloxymethyl)-5-methoxybenzoic acid (8). To a solution of levulinic acid (3.1 mL, 30 mmol) in 1,4-dioxane (60 mL) was added *N*,*N*′-dicyclohexylcarbodiimide (DCC) (3.1 g, 15 mmol). The mixture was stirred for 3 h at room temperature. After removing the precipitate by filtration, **7** (1.82 g, 10 mmol) was added to the filtrate, and the mixture was stirred for 1 h at room temperature in the presence of 1-methylimidazole (1.2 mL, 15 mmol). After evaporation, the residue was dissolved in a saturated aqueous sodium carbonate solution (50 mL), and the solution was washed with diethyl ether (10 mL × 3). The aqueous layer was acidified (ca. pH 2) with conc. hydrochloric acid. The resultant precipitate was gathered by filtration, and was washed with chilled water (20 mL) to give a white powder of **8** (1.69 g, 64%): m.p. 134.5–136°C; ^{1}H-NMR ($CDCl_3$) δ 2.19 (s, 3H, COCH_3), 2.65 (t, 2H, $J = 6.5$ Hz, OCOCH$_2$CH_2COCH$_3$), 2.77 (t, 2H, $J = 6.5$ Hz, OCOCH_2CH$_2$COCH$_3$), 3.86 (s, 3H, OCH_3), 5.47 (s, 2H, PhCH_2), 7.11 (dd, 1H, $J = 2.8$ Hz and 8.6 Hz, Ph-*H*), 7.42 (d, 1H, $J = 8.6$ Hz, Ph-*H*), and 7.60 (d, 1H, $J = 2.8$ Hz, Ph-*H*). *Anal.* calcd. for $C_9H_{10}O_4$; C, 59.99; H, 5.75. Found: C, 60.10; H, 5.78.

6-(Levulinyloxymethyl)-3-methoxy-2-nitrobenzoic acid (LMM*o*NBzOH) [4 (*o*-NO_2)] and 2-(levulinyloxymethyl)-5-methoxy-4-nitrobenzoic acid (LMM*p*NBzOH) [4 (*p*-NO_2)]. A solution of **8** (0.84 g, 3 mmol) in 98% sulfuric acid (3.2 mL)/61% nitric acid (4.2 mL) was stirred for 20 min with cooling in an ice-salt bath. The resulting mixture was added to a 5% aqueous sodium hydrogen carbonate solution (50 mL) and was washed with diethyl ether (50 mL × 3). The aqueous layer was acidified (pH 4) with 1 M hydrochloric acid and was extracted with chloroform (50 mL × 3). The organic layer was dried over anhydrous magnesium sulfate and then was evaporated to give crude LMM*p*NBzOH [**4** (*p*-NO_2)], which was purified by crystallization from diethyl ether as a white powder (0.201 g, 21%). The aqueous layer was acidified (pH 3) with 1 M hydrochloric acid and was extracted with chloroform (50 mL × 3). The organic layer was dried over anhydrous magnesium sulfate and then was evaporated to give crude LMM*o*NBzOH [**4** (*o*-NO_2)], which was purified by crystallization from diethyl ether as a white powder (0.336 g, 34%).

LMM*o*NBzOH [**4** (*o*-NO_2)]: m.p. 136–137°C; ^{1}H-NMR ($CDCl_3$) δ 2.19 (s, 3H, COCH_3), 2.57 (t, 2H, $J = 6.0$ Hz, OCOCH$_2$CH_2COCH$_3$), 2.76 (t, 2H, $J = 6.0$ Hz, OCOCH_2CH$_2$COCH$_3$), 3.93 (s, 3H, OCH_3), 5.26 (s, 2H, PhCH_2), 7.13 (d, 1H, $J =$ 8.7 Hz, Ph-*H*), and 7.51 (d, 1H, $J = 8.7$ Hz, Ph-*H*). *Anal.* calcd. for $C_{14}H_{15}NO_8$; C, 51.70; H, 4.65; N, 4.31. Found: C, 51.68; H, 4.74; N, 4.31.

LMM*p*NBzOH [**4** (*p*-NO_2)]: m.p. 174°C; ^{1}H-NMR ($CDCl_3$) δ 2.19 (s, 3H, COCH_3), 2.64 (t, 2H, $J = 6.3$ Hz, OCOCH$_2$CH_2COCH$_3$), 2.78 (t, 2H, $J = 6.3$ Hz, OCOCH_2CH$_2$COCH$_3$), 3.99 (s, 3H, OCH_3), 5.43 (s, 2H, PhCH_2), 7.72 (s, 1H,

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Ph-*H*), and 7.91 (s, 1H, Ph-*H*). *Anal.* calcd. for $C_{14}H_{15}NO_8$; C, 51.70; H, 4.65; N, 4.31. Found: C, 51.75; H, 4.75; N, 4.11.

5′-*O*-[6-(Levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl]thymidine [9a (*o*)]. After azeotropic evaporation from pyridine (2 mL × 3), thymidine (**2a**) (0.242 g, 1 mmol) and LMM*o*NBzOH [**4** (*o*-NO_2)] (0.357 g, 1.1 mmol) were dissolved in pyridine (4 mL). 2,4,6-Triisopropylbenzenesulfonyl chloride (TPSCl) (0.666 g, 2.2 mmol) was added to the solution, which was then stirred at room temperature for 2 h. The resulting mixture was quenched with water (1 mL) with stirring, and was extracted with ethyl acetate (50 mL × 2). The extracts were combined and washed with a saturated aqueous sodium hydrogen carbonate solution (50 mL × 2) and then with water (50 mL). After drying over anhydrous magnesium sulfate, the organic layer was evaporated to dryness, and the residue was subjected to chromatographic separation on a column of silica gel by the use of a chloroform/methanol system to give **9a** (*o*) (0.399 g, 73%) as a colorless foam: ^{1}H-NMR ($CDCl_3$) δ 1.72 (s, 3H, CH_3 of thymidine), 2.18 (s, 3H, COCH_3), 2.15–2.22 (m, 1H, H-2′), 2.36–2.47 (m, 1H, H-2″), 2.54 (t, 2H, $J = 6.2$ Hz, OCOCH_2CH_2COCH_3), 2.75 (t, 2H, $J = 6.2$ Hz, OCOCH_2CH_2COCH_3), 3.09 (d, 1H, $J = 4.1$ Hz, 3′-OH), 3.95 (s, 3H, OCH_3), 4.13–4.15 (m, 1H, H-4′), 4.43–4.50 (m, 2H, H-3′, 5′), 4.56–4.61 (m, 1H, H-5″), 5.19, 5.26 (2d, 2H, $J = 12.8$ Hz, PhCH_2), 6.27 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.6$ Hz, H-1′), 7.19 (d, 1H, $J = 8.5$ Hz, Ph-*H*), 7.21 (s, 1H, H-6), 7.59 (d, 1H, $J = 8.5$ Hz, Ph-*H*), and 8.34 (br s, 1H, N^3-*H*). *Anal.* calcd. for $C_{24}H_{27}N_3O_{12}\cdot H_2O$; C, 50.79; H, 5.15; N, 7.40. Found: C, 50.60; H, 5.12; N, 7.21.

***N*^{4}-Anisoyl-5′-*O*-[6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl]-2′-deoxycytidine [9b (*o*)].** Compound **9b** (*o*) was obtained in 59% yield (0.393 g) as a colorless foam by treating *N*^{4}-anisoyl-2′-deoxycytidine (**2b**) (0.361 g, 1 mmol) with LMM*o*NBzOH [**4** (*o*-NO_2)] (0.357 g, 1.1 mmol) in the presence of TPSCl (0.666 g, 2.2 mmol) in pyridine (4 mL) for 3 h and a subsequent work-up as described above. ^{1}H-NMR ($CDCl_3$) δ 2.17 (s, 3H, COCH_3), 2.16–2.25 (m, 1H, H-2′), 2.54 (t, 2H, $J = 6.2$ Hz, OCOCH_2CH_2COCH_3), 2.65–2.67 (m, 1H, H-2″), 2.75 (t, 2H, $J = 6.2$ Hz, OCOCH_2CH_2COCH_3), 3.88 (s, 3H, OCH_3 of An group), 3.95 (s, 3H, OCH_3 of LMMoNBz group), 4.2–4.28 (m, 1H, H-4′), 4.39–4.43 (m, 1H, H-3′), 4.53–4.67 (m, 2H, H-5′, and 5″), 5.21 (s, 2H, PhCH_2), 6.25 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.0$ Hz, H-1′), 6.98 (d, 2H, $J = 8.8$ Hz, Ph-$H \times 2$ of An group), 7.21 (d, 1H, $J = 8.8$ Hz, Ph-*H* of LMM*o*NBz group), 7.22–7.43 (m, 1H, H-5), 7.58 (d, 1H, $J = 8.8$ Hz, Ph-*H* of LMM*o*NBz group), 7.85 (d, 2H, $J = 8.8$ Hz, Ph-$H \times 2$ of An group), 8.03 (d, 1H, $J = 6.6$ Hz, H-6), and 8.80 (br s, 1H, N^4-*H*). *Anal.* calcd. for $C_{31}H_{32}N_4O_{13}\cdot 0.5\ H_2O$; C, 54.95; H, 4.91; N, 8.27. Found: C, 54.66; H, 4.92; N, 8.16.

***N*^{6}-Benzoyl-5′-*O*-[6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl]-2′-deoxyadenosine [9c (*o*)].** Compound **9c** (*o*) was obtained in 80% yield (01.010 g) as a colorless foam by treating *N*^{6}-benzoyl-2′-deoxyadenosine (**2c**) (0.675 g, 1.90 mmol) with LMM*o*NBzOH [**4** (*o*-NO_2)] (0.680 g, 2.09 mmol) in the presence of TPSCl (1.266 g, 4.18 mmol) in pyridine (4 mL) for 2 h and a subsequent work-up as described above. ^{1}H-NMR ($CDCl_3$) δ 2.17 (s, 3H, COCH_3), 2.53–2.63 (m, 3H, H-2′

and $OCOCH_2CH_2COCH_3$), 2.70–2.75 (m, 2H, $OCOCH_2CH_2COCH_3$), 3.00–3.05 (m, 1H, H-2″), 3.92 (s, 3H, OCH_3), 4.28–4.31 (m, 1H, H-4′), 4.54–4.58 (m, 2H, H-5′ and 5″), 4.75–4.78 (m, 1H, H-3′), 5.19, 5.26 (2d, 2H, $J = 12.7$ Hz, $PhCH_2$), 6.46 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.6$ Hz, H-1′), 7.16 (d, 1H, $J = 8.9$ Hz, Ph-*H* of LMM*o*NBz group), 7.50–7.60 (m, 4H, Ph-*H* of LMM*o*NBz group and Ph-$H \times 3$ of Bz group), 8.03 (d, 2H, $J = 8.5$ Hz, Ph-$H \times 2$ of Bz group), 8.16 (s, 1H, H-8), 8.72 (s, 1H, H-2), and 9.10 (br s, 1H, N^6-*H*). *Anal*. calcd. for $C_{31}H_{30}N_6O_{11} \cdot 1.5\ H_2O$; C, 53.99; H, 4.82; N, 12.19. Found: C, 54.05; H, 4.66; N, 12.22.

N^2-Isobutyryl-5′-*O*-[6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl]-2′-deoxyguanosine [9d (*o*)]. Compound **9d** (*o*) was obtained in 59% yield (0.382 g) as a colorless foam by treating N^2-isobutyryl-2′-deoxyguanosine (**2d**) (0.337 g, 1 mmol) with LMM*o*NBzOH [**4** (*o*-NO_2)] (0.357 g, 1.1 mmol) in the presence of TPSCl (0.666 g, 2.2 mmol) in pyridine (4 mL) for 2 h and a subsequent work-up as described above. ^{1}H-NMR ($CDCl_3$) δ 1.25(d, 6H, $J = 6.8$ Hz, $CH(CH_3)_2$), 2.16 (s, 3H, $COCH_3$), 2.48–2.53 (m, 3H, H-2′ and $OCOCH_2CH_2COCH_3$), 2.70–2.85 (m, 4H, $OCOCH_2CH_2COCH_3$, $CH(CH_3)_2$, and H-2″), 3.92 (s, 3H, OCH_3), 4.26–4.29 (m, 1H, H-4′), 4.48–4.63 (m, 2H, H-5′ and 5″), 4.81–4.84 (m, 1H, H-3′), 5.05, 5.07 (2d, 2H, $J = 12.9$ Hz, $PhCH_2$), 6.21 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.5$ Hz, H-1′), 7.16 (d, 1H, $J = 8.8$ Hz, Ph-*H* of LMM*o*NBz group), 7.53 (d, 1H, $J = 8.8$ Hz, Ph-*H* of LMM*o*NBz group), 7.81 (s, 1H, H-8), 9.72 (br s, 1H, N^1-*H*), and 12.20 (br s, 1H, N^2-*H*). *Anal*. calcd. for $C_{28}H_{32}N_6O_{12} \cdot H_2O$; C, 50.76; H, 5.17; N, 12.68. Found: C, 51.01; H, 5.17; N, 12.60.

5′-*O*-[2-(Levulinyloxymethyl)-5-methoxy-4-nitrobenzoyl]thymidine [9a (*p*)]. Compound **9a** (*p*) was obtained in 61% yield (0.308 g) as a colorless foam by treating thymidine (**2a**) (0.242 g, 1 mmol) with LMM*p*NBzOH [**4** (*p*-NO_2)] (0.357 g, 1.1 mmol) in the presence of TPSCl (0.666 g, 2.2 mmol) in pyridine (4 mL) for 2 h and a subsequent work-up as described above. ^{1}H-NMR ($CDCl_3$) δ 1.83 (s, 3H, CH_3 of thymidine), 2.21 (s, 3H, $COCH_3$), 2.26–2.35 (m, 1H, H-2′), 2.40–2.49 (m, 1H, H-2″), 2.62 (t, 2H, $J = 6.0$ Hz, $OCOCH_2CH_2COCH_3$), 2.78 (t, 2H, $J = 6.0$ Hz, $OCOCH_2CH_2COCH_3$), 3.09 (d, 1H, $J = 4.1$ Hz, 3′-OH), 4.01 (s, 3H, OCH_3), 4.19–4.23 (m, 1H, H-4′), 4.50–4.69 (m, 3H, H-3′, 5′, and 5″), 5.33, 5.41 (2d, 2H, $J = 13.5$ Hz, $PhCH_2$), 6.27 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.7$ Hz, H-1′), 7.13 (s, 1H, H-6), 7.66 (s, 1H, Ph-*H*), 7.95 (s,1H, Ph-*H*), and 8.67 (s, 1H, N^3-*H*). *Anal*. calcd. for $C_{24}H_{27}N_3O_{12} \cdot H_2O$; C, 50.79; H, 5.15; N, 7.40. Found: C, 50.52; H, 5.09; N, 7.25.

N^4-Anisoyl-5′-*O*-[2-(levulinyloxymethyl)-5-methoxy-4-nitrobenzoyl]-2′-deoxycytidine [9b (*p*)]. Compound **9b** (*p*) was obtained in 55% yield (0.367 g) as a colorless foam by treating N^4-anisoyl-2′-deoxycytidine (**2b**) (0.361 g, 1 mmol) with LMM*p*NBzOH [**4** (*p*-NO_2)] (0.357 g, 1.1 mmol) in the presence of TPSCl (0.666 g, 2.2 mmol) in pyridine (4 mL) for 2 h and a subsequent work-up as described above. ^{1}H-NMR ($CDCl_3$) δ 2.20 (s, 3H, $COCH_3$), 2.24–2.33 (m, 1H, H-2′), 2.62 (t, 2H, $J = 6.3$ Hz, $OCOCH_2CH_2COCH_3$), 2.74–2.81 (m, 3H, H-2″ and $OCOCH_2CH_2COCH_3$), 3.88 (s, 3H, OCH_3 of An group), 4.01 (s, 3H, OCH_3 of LMM*p*NBz group), 4.36–4.40 (m, 1H, H-4′), 4.48–4.51 (m, 1H, H-3′), 4.67–4.69 (m, 2H, H-5′ and 5″), 5.35, 5.41 (2d, 2H, $J = 13.5$ Hz, $PhCH_2$), 6.25 (t, 1H, $J_{1',2'} = J_{1',2''} =$

6.1 Hz, H-1′), 6.98 (d, 2H, $J = 8.8$ Hz, Ph-$H \times 2$ of An group), 7.40–7.49 (m, 1H, H-5), 7.64 (s, 1H, Ph-*H* of LMM*p*NBz group), 7.85 (d, 2H, $J = 8.8$ Hz, Ph-$H \times 2$ of An group), 7.94 (s,1H, Ph-*H* of LMM*p*NBz group), 7.95–7.99 (m, 1H, H-6), and 8.70 (br s, 1H, N^4-*H*). *Anal*. calcd. for $C_{31}H_{32}N_4O_{13}\cdot H_2O$; C, 54.23; H, 4.99; N, 8.16. Found: C, 54.41; H, 4.95; N, 8.08.

N^6-Benzoyl-5′-*O*-[2-(levulinyloxymethyl)-5-methoxy-4-nitrobenzoyl]-2′-deoxyadenosine [9c (*p*)]. Compound **9c** (*p*) was obtained in 65% yield (0.431 g) as a colorless foam by treating N^6-benzoyl-2′-deoxyadenosine (**2c**) (0.355 g, 1 mmol) with LMM*p*NBzOH [**4** (*p*-NO_2)] (0.357 g, 1.1 mmol) in the presence of TPSCl (0.666 g, 2.2 mmol) in pyridine (4 mL) for 2 h and a subsequent work-up as described above. ^{1}H-NMR ($CDCl_3$) δ 2.22 (s, 3H, COCH_3), 2.59–2.84 (m, 5H, H-2′, OCOCH$_2$CH_2COCH$_3$, and OCOCH_2CH$_2$COCH$_3$), 3.14–3.16 (m, 1H, H-2″), 3.96 (s, 3H, OCH_3), 4.35–4.37 (m, 1H, H-4′), 4.68–4.71 (m, 2H, H-5′ and 5″), 4.90–4.93 (m, 1H, H-3′), 5.23, 5.44 (2d, 2H, $J = 13.3$ Hz, PhCH_2), 6.45 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.2$ Hz, H-1′), 7.49 (s, 1H, Ph-*H* of LMM*p*NBz group), 7.51–7.62 (m, 3H, Ph-$H \times 3$ of Bz group), 7.90 (s, 1H, Ph-*H* of LMM*p*NBz group), 8.04 (d, 2H, $J = 7.4$ Hz, Ph-$H \times 2$ of Bz group), 8.14 (s, 1H, H-8), 8.70 (s, 1H,H-2), and 8.96 (br s, 1H, N^6-*H*). *Anal*. calcd. for $C_{31}H_{30}N_6O_{11}\cdot 1.5\ H_2O$; C, 53.99; H, 4.82; N, 12.24. Found: C, 54.23; H, 4.79; N, 12.07.

N^2-Isobutyryl-5′-*O*-[2-(levulinyloxymethyl)-5-methoxy-4-nitrobenzoyl]-2′-deoxyguanosine [9d (*p*)]. Compound **9d** (*p*) was obtained in 76% yield (0.491 g) as a colorless foam by treating N^2-isobutyryl-2′-deoxyguanosine (**2d**) (0.337 g, 1 mmol) with LMM*p*NBzOH [**4** (*p*-NO_2)] (0.357 g, 1.1 mmol) in the presence of TPSCl (0.666 g, 2.2 mmol) in pyridine (4 mL) for 2 h and a subsequent work-up as described above. m.p. 106–108°C (from methanol); ^{1}H-NMR ($CDCl_3$) δ 1.17, 1.21 (2d, 6H, $J = 6.4$ Hz, CH(CH_3)$_2$), 2.18 (s, 3H, COCH_3), 2.53–2.63 (m, 3H, H-2′ and OCOCH$_2$CH_2COCH$_3$), 2.76–2.85 (m, 4H, OCOCH_2CH$_2$COCH$_3$, CH(CH$_3$)$_2$, and H-2″), 3.91 (s, 3H, OCH_3), 4.28–4.33 (m, 1H, H-4′), 4.52–4.58 (m, 1H, H-5′), 4.72–4.77 (m, 1H, H-5''), 5.01–5.04 (m, 1H, H-3′), 5.29 (s, 2H, PhCH_2), 6.19 (t, 1H, $J_{1',2'} = J_{1',2''} = 5.4$ Hz, H-1′), 7.59 (s, 1H , Ph-*H* of LMM*p*NBz group), 7.84 (s, 1H, Ph-*H* of LMM*p*NBz group), 7.88 (s, 1H, H-8), 10.18 (br s, 1H, N^1-*H*), and 12.26 (br s, 1H, N^2-*H*). *Anal*. calcd. for $C_{28}H_{32}N_6O_{12}\cdot 1.5\ H_2O$; C, 50.07; H, 5.25; N, 12.51. Found: C, 50.33; H, 5.41; N, 12.59.

5′-*O*-[6-(Levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl]thymidine 3′-*H*-phosphonate triethylammonium salt [10a (*o*)]. To a solution of PCl_3 (0.22 mL, 2.5 mmol) and *N*-methylmorpholine (2.75 mL, 25 mmol) in dichloromethane (25 mL) was added 1,2,4-triazole (0.573 g, 8.3 mmol) at room temperature to generate tris(1,2,4-triazolyl)phosphine. After 30 min the reaction mixture was cooled to 0°C, and **9a** (*o*) {0.274 g, 0.5 mmol, dried by azeotropic evaporation from pyridine (2 mL × 3) and acetonitrile (2 mL)} in dichloromethane (7 mL) were added dropwise over 10 min. After 20 min the reaction mixture was quenched by treatment with 1 M aqueous triethylammonium bicarbonate (TEAB, pH 8.5) (20 mL) with stirring, and was extracted with chloroform (20 mL × 3). The organic layer was dried over

anhydrous magnesium sulfate and then was evaporated. The residue was subjected to chromatographic separation on a column of silica gel by the use of a chloroform/methanol/triethylamine system to give **10a** (*o*) (0.313 g, 90% yield) as a colorless foam: ^{1}H-NMR ($CDCl_3$) δ 1.34 (t, 9H, $J = 7.3$ Hz, $CH_2CH_3 \times 3$), 1.73 (s, 3H, CH_3 of thymidine), 2.16 (s, 3H, $COCH_3$), 2.17–2.22 (m, 1H, H-2′), 2.50–2.56 (m, 3H, H-2″ and $OCOCH_2CH_2COCH_3$), 2.72 (t, 2H, $J = 6.4$ Hz, $OCOCH_2CH_2COCH_3$), 3.05 (q, 6H, $J = 7.3$ Hz, $CH_2CH_3 \times 3$), 3.93 (s, 3H, OCH_3), 4.34–4.37 (m, 1H, H-4′), 4.56–4.64 (m, 2H, H-5′ and 5''), 4.76–4.79 (m, 1H, H-3′), 5.18, 5.24 (2d, 2H, $J = 13.1$ Hz, $PhCH_2$), 6.29 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.8$ Hz, H-1′), 6.93 (d, 1H, $J_{P,H} = 621.3$ Hz, P-*H*), 7.17 (d, 1H, $J = 8.8$ Hz, Ph-*H*), 7.25 (s, 1H, H-6), 7.58 (d, 1H, $J = 8.8$ Hz, Ph-*H*), and 8.50 (br s, 1H, N^3-*H*). ^{31}P-NMR ($CDCl_3$) δ 4.64.

***N*^{4}-Anisoyl-5′-*O*-[6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl]-2′-deoxycytidine 3′-*H*-phosphonate triethylammonium salt [10b (*o*)].** Compound **10b** (*o*) was obtained in 89% yield (0.371 g) as a colorless foam by treating **9b** (*o*) (0.344 g, 0.5 mmol) with tris(1,2,4-triazolyl)phosphine (2.5 mmol) and a subsequent work-up as described above. ^{1}H-NMR ($CDCl_3$) δ 1.35 (t, 9H, $J = 7.3$ Hz, $CH_2CH_3 \times 3$), 2.16 (s, 3H, $COCH_3$), 2.16–2.20 (m, 1H, H-2′), 2.56 (t, 2H, $J = 6.1$ Hz, $OCOCH_2CH_2COCH_3$), 2.74 (t, 2H, $J = 6.1$ Hz, $OCOCH_2CH_2COCH_3$), 279–2.87 (m, 1H, H-2″), 3.09 (q, 6H, $J = 7.3$ Hz, CH_2CH_3 x 3), 3.88 (s, 3H, OCH_3 of An group), 3.95 (s, 3H, OCH_3 of LMM*o*NBz group), 4.45–4.46 (m, 1H, H-4′), 4.55–4.71 (m, 2H, H-5′ and 5″), 4.75–4.80 (m, 1H, H-3′), 5.18, 5.27 (2d, 2H, $J = 12.9$ Hz, Hz, $PhCH_2$), 6.25 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.2$ Hz, H-1′), 6.92 (d, 1H, $J_{P,H} = 622.2$ Hz, P-*H*), 6.98 (d, 2H, $J = 8.8$ Hz, Ph-$H \times 2$ of An group), 7.18 (d, 1H, $J = 8.8$ Hz, Ph-*H* of LMM*o*NBz group), 7.31–7.39 (m, 1H, H-5), 7.58 (d, 1H, $J = 8.8$ Hz, Ph-*H* of LMM*o*NBz group), 7.85 (d, 2H, $J = 8.8$ Hz, Ph-$H \times 2$ of An group), 8.03–8.9 (m, 1H, H-6), and 8.60 (br s, 1H, N^4-*H*). ^{31}P-NMR ($CDCl_3$) δ 4.63.

***N*^{6}-Benzoyl-5′-*O*-[6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl]-2′-deoxyadenosine 3′-*H*-phosphonate triethylammonium salt [10c (*o*)].** Compound **10c** (*o*) was obtained in 88% yield (0.365 g) as a colorless foam by treating **9c** (*o*) (0.331 g, 0.5 mmol) with tris(1,2,4-triazolyl)phosphine (2.5 mmol) and a subsequent work-up as described above. ^{1}H-NMR ($CDCl_3$) δ 1.36 (t, 9H, $J = 7.3$ Hz, $CH_2CH_3 \times 3$), 2.12 (s, 3H, $COCH_3$), 2.48 (t, 2H, $J = 6.5$ Hz, $OCOCH_2CH_2COCH_3$), 2.67 (t, 2H, $J = 6.5$ Hz, $OCOCH_2CH_2COCH_3$), 2.78–2.79 (m, 1H, H-2′), 2.94–2.97 (m, 1H, H-2″), 3.10 (q, 6H, $J = 7.3$ Hz, $CH_2CH_3 \times 3$), 3.91 (s, 3H, OCH_3), 4.50–4.54 (m, 1H, H-4′), 4.57-4.71 (m, 2H, H-5′ and 5″), 5.02–5.05 (m, 1H, H-3′), 5.10, 5.15 (2d, 2H, $J = 13.0$ Hz, $PhCH_2$), 6.51 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.6$ Hz, H-1′), 6.99 (d, 1H, $J_{P,H} = 621.9$ Hz, P-*H*), 7.15 (d, 1H, $J = 8.9$ Hz, Ph-*H* of LMM*o*NBz group), 7.50–7.60 (m, 4H, Ph-*H* of LMM*o*NBz group and Ph-$H \times 3$ of Bz group), 8.02 (d, 2H, $J = 7.5$ Hz, Ph-$H \times 2$ of Bz group), 8.20 (s, 1H, H-8), 8.73 (s, 1H, H-2), and 9.03 (br s, 1H, N^6-*H*). ^{31}P-NMR ($CDCl_3$) δ 4.38.

***N*^{2}-Isobutyryl-5′-*O*-[6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl]-2′-deoxyguanosine 3′-*H*-phosphonate triethylammonium salt [10d (*o*)].** Compound **10d** (*o*) was obtained in 54% yield (0.218 g) as a colorless foam by treating **9d** (*o*) (0.322 g, 0.5 mmol) with tris(1,2,4-triazolyl)phosphine (1.5 mmol) and a subsequent work-up

as described above. ^{1}H-NMR ($CDCl_3$) δ 1.20–1.25 (m, 6H, CH(CH_3)$_2$, 1.34 (t, 9H, $J = 7.3$ Hz, $CH_2CH_3 \times 3$), 2.16 (s, 3H, $COCH_3$), 2.50–2.53 (m, 3H, H-2′ and $OCOCH_2CH_2COCH_3$), 2.69–2.74 (m, 4H, $OCOCH_2CH_2COCH_3$, $CH(CH_3)_2$, and H-2″), 3.08 (q, 6H, $J = 7.3$ Hz, $CH_2CH_3 \times 3$), 3.94 (s, 3H, OCH_3), 4.25–4.27 (m, 1H, H-4′), 4.40–4.43 (m, 1H, H-5′), 4.61–4.64(m, 1H, H-5''), 4.70 (s, 2H, $PhCH_2$), 6.04–6.10 (m, 2H, H-1′ and 3′), 7.00 (d, 1H, $J_{P,H} = 626.4$ Hz, P-*H*), 7.16 (d, 1H, $J = 8.8$ Hz, Ph-*H* of LMM*o*NBz group), 7.51 (d, 1H, $J = 8.8$ Hz, Ph-*H* of LMM*o*NBz group), 7.64 (s, 1H, H-8), 10.99 (br s, 1H, N^1-*H*), and 11.65 (br s, 1H, N^2-*H*). ^{31}P-NMR ($CDCl_3$) δ 4.23.

5′-*O*-[2-(Levulinyloxymethyl)-5-methoxy-4-nitrobenzoyl]thymidine 3′-*H*-phosphonate triethylammonium salt [10a (*p*)]. Compound **10a** (*p*) was obtained in 91% yield (0.319 g) as a colorless foam by treating **9a** (*p*) (0.274 g, 0.5 mmol) with tris(1,2,4-triazolyl)phosphine (2.5 mmol) and a subsequent work-up as described above. ^{1}H-NMR ($CDCl_3$) δ 1.36 (t, 9H, $J = 7.3$ Hz, $CH_2CH_3 \times 3$), 1.84 (s, 3H, CH_3 of thymidine), 2.20 (s, 3H, $COCH_3$), 2.28–2.32 (m, 1H, H-2′), 2.58–2.62 (m, 1H, H-2″), 2.66 (t, 2H, $J = 6.4$ Hz, $OCOCH_2CH_2COCH_3$), 2.80 (t, 2H, $J = 6.4$ Hz, $OCOCH_2CH_2COCH_3$), 3.09 (q, 6H, $J = 7.3$ Hz, $CH_2CH_3 \times 3$), 4.03 (s, 3H, OCH_3), 4.43–4.46 (m, 1H, H-4′), 4.63–4.68 (m, 2H, H-5′ and 5″), 4.89–4.92 (m, 1H, H-3′), 5.42, 5.47 (2d, 2H, $J = 14.4$ Hz, $PhCH_2$), 6.30 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.7$ Hz, H-1′), 6.96 (d, 1H, $J_{P,H} =$ 620.6 Hz, P-*H*), 7.18 (s, 1H, H-6), 7.73 (s, 1H, Ph-*H*), 7.96 (s,1H, Ph-*H*), and 8.63 (br s, 1H, N^3-*H*). ^{31}P-NMR ($CDCl_3$) δ 4.07.

***N*^{4}-Anisoyl-5′-*O*-[2-(levulinyloxymethyl)-5-methoxy-4-nitrobenzoyl]-2′-deoxycytidine 3′-*H*-phosphonate triethylammonium salt [10b (*p*)].** Compound **10b** (*p*) was obtained in 86% yield (0.360 g) as a colorless foam by treating **9b** (*p*) (0.344 g, 0.5 mmol) with tris(1,2,4-triazolyl)phosphine (2.5 mmol) and a subsequent work-up as described above. ^{1}H-NMR ($CDCl_3$) δ 1.34 (t, 9H, $J = 7.3$ Hz, $CH_2CH_3 \times 3$), 2.17 (s, 3H, $COCH_3$), 2.24–2.27 (m, 1H, H-2′), 2.64 (t, 2H, $J = 6.4$ Hz, $OCOCH_2CH_2COCH_3$), 2.78 (t, 2H, $J = 6.4$ Hz, $OCOCH_2CH_2COCH_3$), 3.02–3.08 (m, 1H, H-2″), 3.05 (q, 6H, $J = 7.3$ Hz, $CH_2CH_3 \times 3$), 3.88 (s, 3H, OCH_3 of An group), 4.00 (s, 3H, OCH_3 of LMM*p*NBz group), 4.60–4.65 (m, 1H, H-4′), 4.67–4.69 (m, 2H, H-5′ and 5″), 4.69–4.70 (m, 1H, H-3′), 5.39, 5.46 (2d, 2H, $J =$ 14.2 Hz, $PhCH_2$), 6.24 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.2$ Hz, H-1′), 6.93 (d, 1H, $J_{P,H} =$ 620.4 Hz, P-*H*), 6.98 (d, 2H, $J = 8.8$ Hz, Ph-$H \times 2$ of An group), 7.40–7.49 (m, 1H, H-5), 7.67 (s, 1H, Ph-*H* of LMM*p*NBz group), 7.85 (d, 2H, $J = 8.8$ Hz, Ph-$H \times 2$ of An group), 7.93 (s,1H, Ph-*H* of LMM*p*NBz group), and 7.94–7.98 (m, 1H, H-6). ^{31}P-NMR ($CDCl_3$) δ 4.05.

***N*^{6}-Benzoyl-5′-*O*-[2-(levulinyloxymethyl)-5-methoxy-4-nitrobenzoyl]-2′-deoxyadenosine 3′-*H*-phosphonate triethylammonium salt [10c (*p*)].** Compound **10c** (*p*) was obtained in 86% yield (0.357 g) as a colorless foam by treating **9c** (*p*) (0.331 g, 0.5 mmol) with tris(1,2,4-triazolyl)phosphine (2.5 mmol) and a subsequent work-up as described above. ^{1}H-NMR ($CDCl_3$) δ 1.31 (t, 9H, $J = 7.3$ Hz, $CH_2CH_3 \times 3$), 2.16 (s, 3H, $COCH_3$), 2.62 (t, 2H, $J = 6.3$ Hz, $OCOCH_2CH_2COCH_3$), 2.75 (t, 2H, $J = 6.3$ Hz, $OCOCH_2CH_2COCH_3$), 2.78–2.83 (m, 1H, H-2′), 3.00 (q, 6H, $J = 7.3$ Hz, Hz, $CH_2CH_3 \times 3$), 3.10–3.13 (m, 1H, H-2″), 3.97 (s, 3H, OCH_3), 4.58–4.61 (m, 1H,

H-4′), 4.64–4.78 (m, 2H, H-5′ and 5″), 5.17–5.19 (m, 1H, H-3′), 5.38 (s, 2H, PhCH_2), 6.50 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.6$ Hz, H-1′), 6.99 (d, 1H, $J_{P,H} = 618.7$ Hz, P-*H*), 7.27–7.65 (m, 3H, Ph-*H* × 3 of Bz group), 7.64 (s, 1H, Ph-*H* of LMM*p*NBz group), 7.91 (s, 1H, Ph-*H* of LMM*p*NBz group), 8.04 (d, 2H, $J = 7.2$ Hz, Ph-*H* × 2 of Bz group), 8.14 (s, 1H, H-8), 8.73 (s, 1H,H-2), and 8.98 (br s, 1H, N^6-*H*). ^{31}P-NMR ($CDCl_3$) δ 4.36.

N^2-Isobutyryl-5′-*O*-[2-(levulinyloxymethyl)-5-methoxy-4-nitrobenzoyl]-2′-deoxyguanosine 3′-*H*-phosphonate triethylammonium salt [10d (*p*)]. Compound **10d** (*p*) was obtained in 48% yield (0.196 g) as a colorless foam by treating **9d** (*p*) (0.322 g, 0.5 mmol) with tris(1,2,4-triazolyl)phosphine (1.5 mmol) and a subsequent work-up as described above. ^{1}H-NMR ($CDCl_3$) δ 1.24 (d, 6H, $J = 6.8$ Hz, $CH(CH_3)_2$), 1.36 (t, 9H, $J = 7.3$ Hz, CH_2CH_3 × 3), 2.19 (s, 3H, $COCH_3$), 2.59–2.62 (m, 3H, H-2′ and $OCOCH_2CH_2COCH_3$), 2.72–2.79 (m, 3H, $OCOCH_2CH_2COCH_3$ and H-2″), 3.05–3.11 (m, 7H, $CH(CH_3)_2$ and CH_2CH_3 × 3), 3.97 (s, 3H, OCH_3), 4.42–4.44 (m, 1H, H-4′), 4.48–4.53 (m, 1H, H-5′), 4.82–4.86 (m, 1H,H-5″), 5.34, 5.40 (2d, 2H, $J = 14.4$ Hz, PhCH_2), 5.55–5.60 (m, 1H, H-3′), 6.14 (dd, 1H, $J_{1',2'} = 7.4$ Hz and $J_{1',2''} = 4.2$ Hz, H-1′), 6.97 (d, 1H, $J_{P,H} = 623.2$ Hz, P-*H*), 7.60 (s, 1H , Ph-*H* of LMM*p*NBz group), 7.69 (s, 1H, Ph-*H* of LMM*p*NBz group), 7.90 (s, 1H, H-8), 10.39 (br s, 1H, N^1-*H*), and 12.40 (br s, 1H, N^2-*H*). ^{31}P-NMR ($CDCl_3$) δ 3.60.

5′-*O*-[2-(Levulinyloxymethyl)-5-nitrobenzoyl]thymidine 3′-*H*-phosphonate triethylammonium salt [10a (LMNBz)]. Compound **10a** (LMNBz) was obtained in 86% yield (0.30 g) as a colorless foam by treating **9a** (LMNBz) (0.26 g, 0.5 mmol) with tris(1,2,4-triazolyl)phosphine (2.5 mmol) and a subsequent work-up as described above. ^{1}H-NMR ($CDCl_3$) δ 1.28 (t, 9H, $J = 7.3$ Hz, CH_2CH_3 × 3), 1.71 (s, 3H, CH_3 of thymidine), 2.16 (s, 3H, $COCH_3$), 2.22–2.29 (m, 1H, H-2′), 2.53–2.56 (m, 1H, H-2″), 2.65 (t, 2H, $J = 6.2$ Hz, $OCOCH_2CH_2COCH_3$), 2.78 (t, 2H, $J = 6.2$ Hz, $OCOCH_2CH_2COCH_3$), 3.01 (q, 6H, $J = 7.2$ Hz, CH_2CH_3 × 3), 4.37–4.40 (m, 1H, H-4′), 4.57–4.66 (m, 2H, H-5′ and 5″), 4.89–4.93 (m, 1H, H-3′), 5.55, 5.60 (2d, 2H, $J = 16.3$ Hz, PhCH_2), 6.28 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.7$ Hz, H-1′), 6.91 (d, 1H, $J_{P,H} = 621$ Hz, P-*H*), 7.16 (s, 1H, H-6), 7.76 (d, 1H, $J = 8.6$ Hz, Ph-*H*), 8.34 (dd, 1H, $J = 8.8$ Hz and $J = 2.3$ Hz, Ph-*H*), and 8.74 (d, 1H, $J = 2.3$ Hz, Ph-*H*). ^{31}P-NMR ($CDCl_3$) δ 4.09.

Functionalization of the CPG Support with a Nucleoside 3′-(Carboxy)propionate Derivative

1) A solution of **9a** (*o*) (0.164 g, 0.3 mmol) in pyridine (2 mL) was treated with succinic anhydride (0.060 g, 0.6 mmol) and 4-dimethylpyridine (DMAP) (0.018 g, 0.45 mmol) at room temperature for 1 day with stirring. The resulting mixture was quenched by treatment with chilled water (1 mL) at room temperature for 30 min with stirring and evaporated to dryness. The residue was subjected to chromatographic separation on a column of silica gel by the use of a chloroform/methanol system to give 3′-*O*-[3-(carboxy)propionyl]-5′-*O*-[6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl]thymidine (0.192 g, 99% yield) as a colorless foam: ^{1}H-NMR ($CDCl_3$) δ 1.71 (s, 3H, CH_3 of thymidine), 2.17 (s, 3H, $COCH_3$), 2.12–2.22 (m, 1H, H-2′),

2.44–2.50 (m, 1H, H-2″), 2.54 (t, 2H, $J = 6.5$ Hz, $OCOCH_2CH_2COCH_3$), 2.66–2.76 (m, 6H, $OCOCH_2CH_2COCH_3$, $OCOCH_2CH_2COOH$, and $OCOCH_2CH_2COOH$), 3.95 (s, 3H, OCH_3), 4.28–4.29 (m, 1H, H-4′), 4.50–4.63 (m, 2H, H-5′ and 5″), 5.22 (s, 2H, $PhCH_2$), 5.18–5.27 (m, 1H, H-3′), 6.25 (dd, 1H, $J_{1',2'} = 8.7$ Hz, $J_{1',2''} = 5.4$ Hz, Hz, H-1′), 7.19 (d, 1H, $J = 8.9$ Hz, Ph-*H*), 7.30 (s, 1H, H-6), 7.59 (d, 1H, $J = 8.9$ Hz, Ph-*H*), and 9.86 (s, 1H, N^3-*H*).

After azeotropic evaporation from pyridine (1 mL × 3), 3′-*O*-[3-(carboxy)propionyl]-5′-*O*-[6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl]thymidine (0.162 g, 0.25 mmol) and 1-hydroxybenzotriazole (0.051 g, 0.375 mmol) were dissolved in dried 1,4-dioxane (3 mL), DCC (0.077 g, 0.375 mmol) was added to the solution, and the mixture was stirred for 3 h at room temperature. The resulting precipitates were removed by filtration, and the filtrate was poured onto the CPG support (Long Chain Amino-Alkyl Controlled-Pore Glass, 500 A, 80–120 mesh; purchased from Funakoshi) (0.5 g), which was in advance subjected to azeotropic evaporation from pyridine (2 mL × 3). The mixture was shaken for 12 h at room temperature in the presence of 1-methyimidazole (0.1 mL, 1.25 mmol). The CPG loaded with 6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl]thymidine derivative was filtered off and washed with acetonitrile (5 mL) and methanol (5 mL). Unreacted amino groups of the CPG support were capped by treatment with 1:1:8 acetic anhydride/2,6-lutidine/THF and 1 : 9 1-methylimidzole/THF for 30 min at room temperature, followed by successive washing of CPG [**11a** (*o*)] with acetonitrile (5 mL) and methanol (5 mL). After deprotection of the LMM*o*NBz group by consecutive traetmant with 0.5 M hydazine monohydrate in 4:1 pyridine/acetic acid at room temperature for 15 min and with 0.5 M imidazole in acetonitrile at room temperature for 5 min and removing of thymidine (**2a**) from the CPG support by treatment with conc. aqueous ammonia at room temperature for 3 h using 9.8 mg of CPG [**11a** (*o*)], thymidine content in CPG [**11a** (*o*)] was determined to be 55.79 μmol / g by UV spectroscopy {thymidine, λ_{max} 267 nm in H_2O ($\varepsilon = 9650$)}.

2) 3′-*O*-[3-(Carboxy)propionyl]-5′-*O*-[2-(levulinyloxymethyl)-5-methoxy-4-nitrobenzoyl]thymidine was obtained in 92% yield (0.180 g) as a colorless foam by treating **9a** (*p*) (0.164 g, 0.3 mmol) with succinic anhydride (0.060 g, 0.6 mmol) in the presence of DMAP (0.018 g, 0.45 mmol) in pyridine (2 mL) for 1 day and a subsequent work-up as described above. ^{1}H-NMR ($CDCl_3$) δ 1.85 (s, 3H, CH_3 of thymidine), 2.20 (s, 3H, $COCH_3$), 2.27–2.37 (m, 1H, H-2′), 2.51–2.58 (m, 1H, H-2″), 2.63–2.70 (m, 6H, $OCOCH_2CH_2COCH_3$, $OCOCH_2CH_2COOH$, and $OCOCH_2CH_2COOH$), 2.80 (t, 2H, $J = 6.3$ Hz, $OCOCH_2CH_2COCH_3$), 4.01 (s, 3H, OCH_3), 4.36–4.41 (m, 1H, H-4′), 4.52–4.66 (m, 2H, H-5′, and 5″), 5.35–5.37 (m, 1H, H-3′), 5.41 (s, 2H, $PhCH_2$), 6.23 (dd, 1H, $J_{1',2'} = 8.3$ Hz, $J_{1',2''} = 7.8$ Hz, H-1′), 7.19 (s, 1H, H-6), 7.71 (s, 1H, Ph-*H*), 7.93 (s,1H, Ph-*H*), and 9.82 (s, 1H, N^3-*H*).

3′-*O*-[3-(Carboxy)propionyl]-5′-*O*-[2-(levulinyloxymethyl)-5-methoxy-4-nitrobenzoyl]thymidine (0.162 g, 0.25 mmol) was introduced to the CPG support as described for the synthesis of CPG [**11a** (*p*)]. After deprotection of the LMMpNBz group and removing of thymidine (**2a**) from the CPG support using 10.3 mg of CPG [**11a** (*p*)], thymidine content of CPG [**11a** (*p*)] was determined to be 55.79 μmol/g by UV spectroscopy.

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3) 3′-*O*-[3-(Carboxy)propionyl]-N^2-isobutyryl-5′-*O*-[6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl]-2′-deoxyguanosine was obtained in 86% yield (0.259 g) as a colorless foam by treating **9d** (*o*) (0.193 g, 0.3 mmol) with succinic anhydride (0.060 g, 0.6 mmol) in the presence of DMAP (0.018 g, 0.15 mmol) in pyridine (3 mL) for 1 day and a subsequent work-up as described above. ^{1}H-NMR ($CDCl_3$) δ 1.25 (d, 6H, $J = 6.9$ Hz, $CH(CH_3)_2$), 2.16 (s, 3H, $COCH_3$), 2.48–2.91 (m, 7H, H-2′, 2″, $OCOCH_2CH_2COCH_3$, $OCOCH_2CH_2COOH$, and $CH(CH_3)_2$), 3.89 (s, 3H, OCH_3), 4.37–4.40 (m, 1H, H-4′), 4.56–4.60 (m, 2H, H-5′ and 5″), 5.09 (s, 2H, $PhCH_2$), 5.40–5.44 (m, 1H, H-3′), 6.13 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.8$ Hz, H-1′), 7.17 (d, 1H, $J = 8.9$ Hz, Ph-*H* of LMM*o*NBz group), 7.54 (d, 1H, $J = 8.8$ Hz, Ph-*H* of LMM*o*NBz group), 7.84 (s, 1H, H-8), 9.84 (br s, 1H, N^1-*H*), and 12.17 (br s, 1H, N^2-*H*).

3′-*O*-[3-(Carboxy)propionyl]-N^2-isobutyryl-5′-*O*-[6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoyl]-2′-deoxyguanosine was introduced to the CPG support as described for the synthesis of CPG [**11d** (*o*)]. After deprotection of the LMMoNBz group, removing of N^2-isobutyryl-2′-deoxyguanosine (**2d**) and deprotection of the iBu group by treatment conc. aqueous ammonia at 55°C for 6 h using 10.3 mg of CPG [**11d** (*o*)], 2′-deoxyguanosine content of CPG [**11d** (*o*)] was determined to be 21.11 μmol/g by UV spectroscopy {2′-deoxyguanosine, λ_{max} 254 nm in H_2O ($\varepsilon = 13000$)}.

Synthesis of Oligothymidylic Acids

After chain elongation with a simple syringe-based reaction system[10] using a column of CPG (**11a**) (Sch. 5), the resulting oligothymidylic acid was detached from the CPG support by treatment with conc. aqueous ammonia (4 mL) for 3 h at room temperature. After evaporation of the aqueous ammonia, the residual deprotected oligothymidylic acid was analyzed by reverse-phase HPLC (see Fig. 1 and 2).

Synthesis of Oligodeoxyribonucleotides

After chain elongation with a simple syringe-based reaction system[10] using a column of CPG (**11**) (Sch. 5), the resulting oligomer was detached from the CPG support by treatment with conc. aqueous ammonia (4 mL) for 3 h at room temperature. The resulting solution was heated in a sealed vial at 55°C for 6 h. After evaporation of the aqueous ammonia, the residue was dissolved in water (10 mL) and was washed with ethyl acetate (5mL × 3). The aqueous layer was evaporated, and the residual deprotected oligomer was purified by reverse-phase HPLC (see Fig. 3). The main peak was collected [yield of TpTpT, 16.4 A_{260} units from CPG **11a** (*o*) (1 μmol) and 8.7 A_{260} units from CPG **11a** (*p*) (1 μmol); yield of TpTpTpT, 14.0 A_{260} units from CPG **11a** (*o*) (1 μmol) and 12.7 A_{260} units from CPG **11a** (*p*) (1 μmol); yield of d(CpCpT), 9.8 A_{260} units from CPG **11a** (*o*) (1 μmol) and 9.8 A_{260} units from CPG **11a** (*p*) (1 μmol); yield of d(ApApT), 15.4 A_{260} units from CPG **11a** (*o*) (1 μmol) and 7.4 A_{260} units from CPG **11a** (*p*) (1 μmol); yield of d(ApApApT), 15.4 A_{260} units from CPG **11a** (*o*) (1 μmol); yield of d(GpGpT), 9.9 A_{260} units from CPG **11a** (*o*) (1 μmol) and 8.9 A_{260} units from CPG **11a** (*p*) (1 μmol);

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yield of d(GpCpApT), 7.6 A_{260} units from CPG **11a** (*o*) (1 μmol); yield of d(ApApTpTpCpG), 11.0 A_{260} units from CPG **11d** (*o*) (1 μmol)].

Enzymatic Digestion of Oligodeoxyribonucleotides

The fully deprotected oligonucleotides (1.0 A_{260} unit) were each dissolved in 30 mM Tris/HCl (pH 8.0) (70 μL) containing 6 mM $MgCl_2$, and snake venom phosphodiesterase (2.8 mg/0.7 mL, SIGMA) (3 μL) and alkaline phosphatase (1500 u/83 μL, Roche) (1 μL) were added. The resulting mixture was incubated at 37°C for 24 h and was heated at 90°C for 2 min.[3d,4c,4d] Digestion products were analyzed by reversed-phase HPLC with 2% acetonitrile/0.04 M TEAA (pH 7.0) as an eluent (see Fig. 4). 2′-Deoxyinosine was produced from 2′-deoxyadenosine due to contamination with adenosine deaminase.[4d]

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