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Photolabile Protecting Groups for Nucleosides: Synthesis and Photodeprotection Rates

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Abstract: *o*-Nitrobenzyloxycarbonyl and a number of related groups have been tested for the photolabile protection of nucleoside 5'-hydroxyls. The rates of photodeprotection were found to vary by approximately 17-fold in a series of 5'-O-protected thymidine derivatives irradiated at 365 nm under identical conditions. The homologous 2-(*o*-nitrophenyl)ethoxycarbonyl group and its derivatives were found to be removed approximately 2-fold faster than the corresponding *o*-nitrobenzyloxycarbonyl group, possibly due to an increased rate of α-hydrogen abstraction by the photo-excited nitro group. Photolysis rates were affected by substitutions on both the phenyl ring and α-carbon, with the strongest rate enhancements caused by the presence of a methyl or second *o*-nitrophenyl group in the α-position. Among the ring-substituted derivatives studied, *o*-nitro and *o*-iodo had the strongest enhancement effects on photodeprotection, while an *o*-fluoro group reduced the rate of photodeprotection. In general, substitutions at other positions on the phenyl ring had less effect on photolysis rates. © 1997 Elsevier Science Ltd.

The use of photolabile protecting groups in nucleic acid[1-8], carbohydrate[9], and peptide[10-12] chemistry has been well established. More recently, photolabile protection of the 5'-hydroxyl of 2'-deoxyribonucleoside 3'-phosphoramidites has been employed in the solid-phase synthesis of DNA probe arrays.[13-16] Useful photolabile protecting groups must be stable to mild chemical treatments, but photolytically cleaved in high yield by irradiation at wavelengths which do not damage the protected molecule.

The *o*-nitrobenzyl group or groups containing this photosensitive moiety have been used for photolabile protection of hydroxyl, carboxyl, amino, thiol, and carbonyl functions.[17] The removal of photolabile groups from a protected hydroxyl oxygen by irradiation at wavelengths >320 nm involves abstraction of a hydrogen from the α-methylene carbon by the excited nitro group followed by rearrangement to *o*-nitrosobenzaldehyde and the deprotected alcohol. In the case of the 2-nitrobenzyloxycarbonyl group, carbon dioxide is also released (Fig. 1). The quantum yield of photodeprotection may be strongly influenced by substitution on the phenyl ring or methylene carbon of the *o*-nitrobenzyl group. Reichmanis *et al.*[18] reported a 5-fold increase in quantum yield for photocleavage of the *o*-nitrobenzyl esters of trimethylacetic acid when the parent ester is substituted with an α-methyl group.

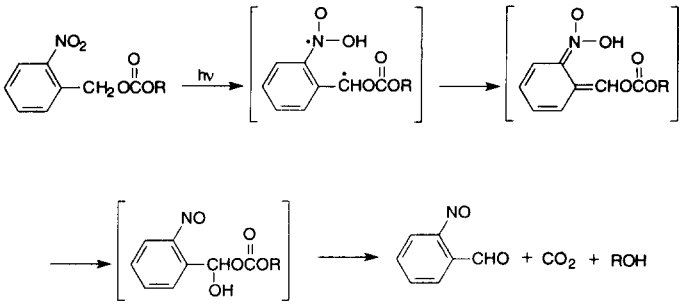


Fig. 1. Photolysis of 2-nitrobenzyl carbonate esters.

The reported photochemical reactions of *o*-nitro groups with ß-carbons[19,20] suggested that the homologous *o*-nitrophenylethyl group might also be useful as a photocleavable protecting function. The photocyclization of 2-(*o*-nitrophenyl)ethanol to N-hydroxyoxindole[20] was postulated to involve the initial formation of 2-(*o*-hydroxylaminophenyl) acetic acid which could cyclize with cleavage of the Cß-O bond (Fig. 2). Analogous photoreactions involving 2-(*o*-nitrophenyl)ethyl ethers or carbonate esters would release alcohols. The 2-(*o*-nitrophenyl)ethyl carbonate esters were, in fact, found to be photocleavable. This report describes the synthesis of deoxynucleosides protected at the 5'-O position with the *o*-nitrobenzyloxycarbonyl group, the 2-(*o*-nitrophenyl)ethyloxycarbonyl group and a number of their substituted derivatives, and comparison of their photodeprotection rates.

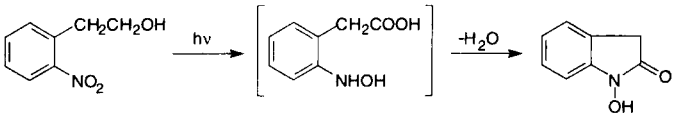


Fig. 2. Photocyclization of 2-(*o*-nitrophenyl)ethanol.[20]

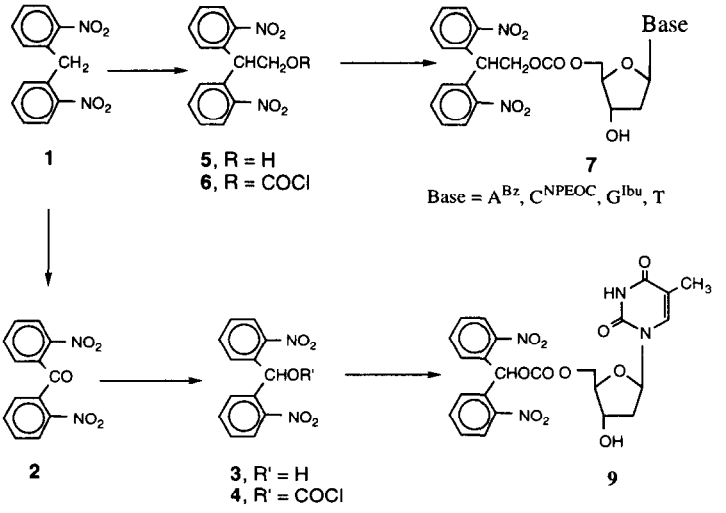
RESULTS AND DISCUSSION

Synthesis

The synthesis of photolabile 5'-O-(*o*-nitrophenyl)alkoxycarbonyl nucleosides is illustrated in Schemes 1 and 2. Di-(*o*-nitrophenyl)methane (**1**) was prepared following the literature procedure.[21] Chromic anhydride oxidation of **1** gave 2,2'-dinitrobenzophenone (**2**), which was then converted to di(*o*-nitrophenyl)methanol (**3**) by reduction with sodium borohydride (Scheme 1). Alternatively, compound (**1**) was converted to 2,2'-di-(*o*-nitrophenyl)ethanol (**5**) by treatment with paraformaldehyde and benzyltrimethylammonium hydroxide (triton B)

in dimethylsulfoxide. The structure of compounds **3** and **5** were confirmed on the basis of ^1H NMR and elemental analysis. Treatment of compounds **3** and **5** with phosgene yielded the corresponding chloroformate

Scheme 1



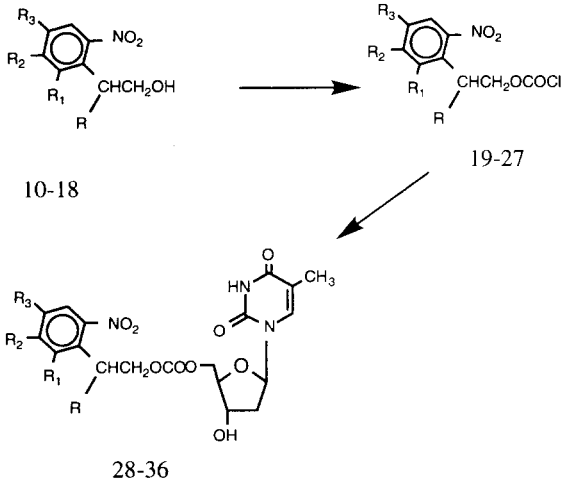
derivatives **4** and **6**, respectively. Compound **4** was used for next step without characterization The disappearance of the OH proton and a downfield shift of 0.64 ppm of the $CHC\underline{H}_2$ proton peak in the NMR spectrum of compound **6** compared to **5** clearly indicate the formation of an ester linkage. Reaction of compound **6** with amine-protected 2'-deoxyribonucleosides gave the corresponding 5'-O-[2,2'-di-(2-nitrophenyl)ethoxycarbonyl]-2'-deoxynucleosides (**7a-d**) in 54-70% yield. We found that addition of **6** to a solution of 2'-deoxynucleoside in pyridine at 0-5 °C exclusively yielded 5'-O-monosubstituted derivatives; however, a mixture of 5'-O-monosubstituted and 3',5'-O-disubstituted derivatives was found when the reaction was carried out at room temperature.

Similarly, the reaction of 2-(*o*-nitrophenyl)ethanol and its ring-substituted derivatives with phosgene under basic condition yielded the corresponding chloroformate derivatives (**19-27**, Scheme 2). Treatment of thymidine with compounds **19-27** resulted in the 5'-O-protected thymidines (**28-36**), which were characterized by UV, NMR and elemental analysis.

Photodeprotection Rates

Photolytic decay rates were measured under identical conditions (365 nm, 18 mW/cm^2, 100 mM in methanol-water, 1:1, v/v) for a number of thymidine derivatives in which the 5'-hydroxyl was protected with either an *o*-nitrobenzyloxycarbonyl (NBOC) or 2-(*o*-nitrophenyl)ethoxycarbonyl (NPEOC) group. The

photolytic half-life ($t_{1/2}$) for each compound is given in Table 1. In general, the effects of NBOC substitutions on the relative photolysis rates were consistent with those previously observed for *o*-nitrophenyl derivatives.[10,11] The dimethoxy substituted derivative, 5'-O-(6-nitroveratryloxycarbonyl)thymidine (**37**) had a slightly increased photolysis rate (~15%) relative to the unsubstituted parent compound (**38**), while the quantum yield was lowered by approximately 25-fold due to increased absorbance at 365 nm. The presence of a second *o*-nitrophenyl group on the α-carbon in 5'-O-di-[(*o*-nitrophenyl)methoxycarbonyl]thymidine (**9**) resulted in an



Compound #	R	R_1	R_2	R_3
10, 19, 28	H	NO_2	H	H
11, 20, 29	H	I	H	H
12, 21, 30	H	Br	H	H
13, 22, 31	H	Cl	H	H
14, 23, 32	H	Cl	H	Cl
15, 24, 33	H	H	CH_3O	H
16, 25, 34	H	H	H	H
17, 26, 35	H	F	H	H
18, 27, 36	CH_3	H	H	H

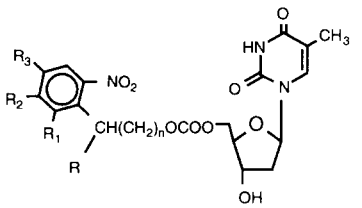
Scheme 2

8.6-fold increase in photolysis rate compared to compound **38**. The large rate enhancement in the latter case is presumably due to the effect of increased ease of α-CH abstraction[22] because of an aromatic substitution as well as the presence of a second photoreactive *o*-nitro group.

The NPEOC derivatives had photolysis rates approximately 1.8-fold greater than the corresponding NBOC derivatives; i.e., compounds **7a** and **34** were photolyzed ~1.8 times faster than **9** and **38**, respectively. As in the case of the *o*-nitrobenzyl group, substitutions on the ring or α-carbon of the 2-(*o*-nitrophenyl)ethyl group can significantly affect the photolysis rate of the NPEOC derivatives. The highly electronegative *o*-fluoro substituent in **35** reduced the rate by 40%, while *o*-substitution with the larger halogen atoms in **29-31** increased the rate in the order: Cl ≤ Br < I. Quantum yields for the *o*-halo derivatives also increased in the same order, although the effect was less pronounced. Substitution of methoxy- or chloro- groups in the *meta*- and/or *para*-positions had less effect on the photolysis rate. The effect of *o*-substitution on quantum yield may be due to either inductive or steric effects. Bulky substituents such as the iodo atom may cause increased steric interaction between the nitro and alkyl groups, while the *o*-fluoro substituent would be expected to have little steric effect. Interestingly, introduction of a second *o*-nitro group (**28**), which can also participate in

photoreactions with the side chain[18], increased the photolysis rate only slightly compared to the *o*-iodo derivative. The greatest increase in the photolysis rate of NPEOC derivatives, however, was achieved by substitutions on the α-carbon. The presence of a second α-(*o*-nitrophenyl) group in **7a** resulted in a rate increase of approximately 8.4-fold relative to the parent compound **34**, similar to the rate difference observed in compounds **9** and **38**. The presence of the α-methyl group in compound **36** resulted in a 9.4-fold increase relative to **34**.

Table 1: Photolysis data for 5'-O- protected thymidines (**7a**, **9**, **28-38**)*



No.	n	R	R_1	R_2	R_3	$\varepsilon_{365\ nm}$	$t_{1/2}$, min	Φ
36	1	CH_3	H	H	H	294	0.66	0.35
7a	1	*o*-nitrophenyl	H	H	H	457	0.74	0.20
9	0	*o*-nitrophenyl	H	H	H	424	1.33	0.12
28	1	H	NO_2	H	H	370	2.26	0.080
29	1	H	I	H	H	285	2.38	0.10
30	1	H	Br	H	H	224	4.00	0.076
31	1	H	Cl	H	H	216	4.48	0.070
32	1	H	Cl	H	Cl	199	4.72	0.072
33	1	H	H	CH_3O	H	1690	5.08	0.0079
34	1	H	H	H	H	257	6.20	0.042
35	1	H	F	H	H	206	8.83	0.037
37	0	H	H	CH_3O	CH_3O	5100	9.96	0.0013
38	0	H	H	H	H	179	11.42	0.033

*Photolysis conditions are described in materials and methods section.

In addition to the rapid photolysis of 5'-O-[2,2'-di-(2-nitrophenyl)ethoxycarbonyl]-T (**7a**), we found that it is cleanly converted to the deprotected thymidine in high yield. In order to determine the photolysis yield, **7a** was labeled by reaction of its 3'-OH with [^{3}H]acetic anhydride, and the resulting compound was photolysed for 5 min in methanol-water. HPLC analysis before and after photolysis showed >95% conversion of 3'-O-

[^{3}H]acetyl-5'-O-[2,2'-di-(2-nitrophenyl)ethoxycarbonyl]-T to 3'-O-[^{3}H]acetyl-T with no formation of new radioactive intermediates or side products (Fig. 3 a, b).

The rates of photodeprotection of NPEOC vs. NBOC derivatives and the effects of α-substitution are consistent with the relative rates of hydrogen abstraction from alkyl carbon by a photo-activated *o*-nitro group: primary < secondary < tertiary.[23,24] While the possibility of direct attack of the excited nitro group on ß-carbon cannot presently be ruled out in the case of NPEOC derivatives, this mechanism appears less likely in view of the relatively low yields reported for photocyclization of *o*-nitro groups with ß-carbon.[19,20] The observed effects of NPEOC phenyl ring substituents on photolysis rates are also more consistent with a mechanism involving α-hydrogen abstraction, as neither their inductive nor steric effects are as likely to influence reactions

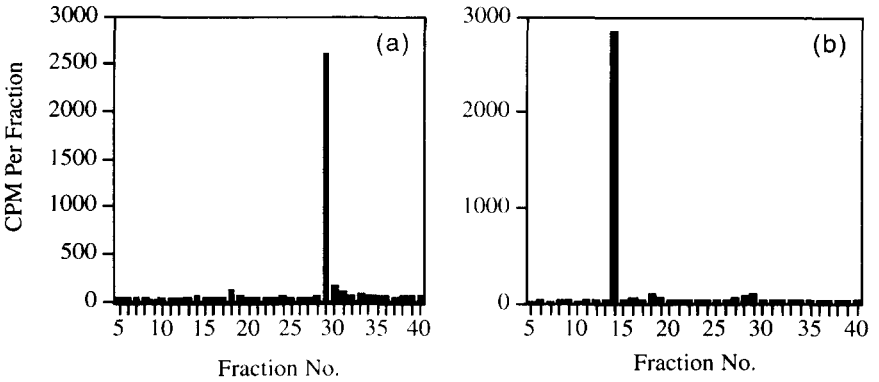


Fig. 3. (a) HPLC analysis of 3'-O-[^{3}H]acetyl-5'-O-[2,2'-di-(2-nitrophenyl)ethoxycarbonyl]-T solution before irradiation at 365 nm; (b) analysis after 5 min irradiation to give 3'-O-[^{3}H]acetyl-T.

at the ß-carbon. A possible mechanism for the photocleavage of NPEOC derivatives is indicated in Fig. 4, where an *aci*-nitro intermediate analogous to that for NBOC derivatives (Fig. 1) could undergo rearrangement to photolabile *o*-nitrostyrene with ß-elimination of the protected group. A photolabile intermediate was observed

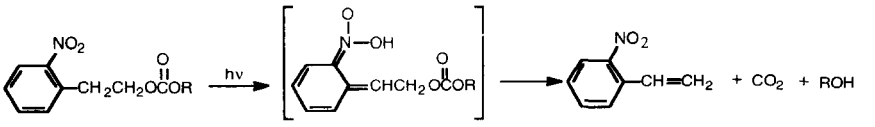


Fig. 4. Possible mechanism of NPEOC-OR photocleavage.

by HPLC during the photolysis of NPEOC compounds. However, further studies will be required to elucidate the mechanism of this reaction.

CONCLUSIONS

The results shown in Table 1 demonstrate that the rate of photoremoval of the *o*-nitrobenzyloxycarbonyl group can be enhanced by more than an order of magnitude by elongation of the side-chain with a single methylene moiety and addition of a methyl or *o*-nitrophenyl substituent to the α-carbon. The enhancement effects of these two modifications appeared to be essentially independent of one another, i.e., α-substitution of either the NBOC or NPEOC groups with an *o*-nitrophenyl moiety resulted in a similar enhancement factor. The photolysis rates of NPEOC derivatives could also be increased by up to ~3-fold by appropriate substitution of the phenyl ring. The photolabile protective groups examined in this study are stable to chemical reagents encountered in the nucleoside 3'-phosphoramidites, and solid-phase oligonucleotide synthesis. 3'-Phosphoramidites of all four 5'-O-[2,2'-di-(2-nitrophenyl)ethoxycarbonyl]-dNs (**7a-d**) have been prepared and used for the photochemical synthesis of oligonucleotides on glass substrates in high yield.[25]

MATERIALS AND METHODS

General

All solvents, chemicals, and reagents were analytical grade and were used without further purification unless otherwise indicated. Phosgene was purchased as a 20% solution in toluene from Fluka Chemical Co. The melting points were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Baker analyzed silica gel (60-200 mesh) was used for flash column chromatography. Thin layer chromatography (TLC) was performed using glass plates coated with 250 micron layers of silica gel GF precoated glass plates (Analtech, Inc.). Spots on the TLC plates were detected by visualization under short-wave ultraviolet (UV) light or by heating the chromatogram at 100 °C after spraying with 5% sulfuric acid in methanol. ^{1}H-NMR spectra were recorded on a Gemini 200 or 250 MHz spectrometer. Chemical shifts are reported in ppm (δ) down field from the internal tetramethylsilane standard (TMS=0 ppm). The signals are expressed as s (singlet), d (doublet), t (triplet), m (multiplet), or bs (broad singlet). The presence of exchangeable protons was confirmed by treatment with deuterium oxide followed by reintegration of the NMR spectrum.

Photolysis

5'-O-protected thymidine derivatives (100 μM) were dissolved in methanol-water, 1:1, v:v. An aliquot (25 μL) of the sample was analyzed by HPLC using a NOVA-PAK C_{18} column (Waters) eluted with a linear

gradient of acetonitrile in 0.1 M triethylammonium acetate, pH 7, and monitoring absorbance of the eluent at 260 nm. The remaining solution was transferred to a 2 mm-pathlength stoppered quartz cuvette and irradiated with a 200 watt mercury arc lamp using a 365 nm bandpass and 345 nm cutoff filters to produce a light intensity of approximately 18 milliwatts/cm^2 in wavelength region centered at 365 nm. Aliquots of the irradiated solution were taken at four to five time intervals and analyzed for the loss of starting material by HPLC. The half-life ($t_{1/2}$) was calculated from the log plot of peak area at time t (A_t) divided by peak area at time zero (A_0) versus the irradiation time. The experiments were repeated two to four times for each compound and the mean half-life ($t_{1/2}$) is reported in Table 1. For determination of quantum yields, the light intensity was measured by chemical actinometry using the potassium ferrioxalate method[26] and by spectral radiometry in the 340-400 nm region. Molar extinction coefficients were determined for each compound in methanol-water, 1:1, v:v. The quantum yields were calculated from the following equation[27]:

$$\Phi = \frac{(0.693)(6.02 \times 10^{20})}{(2.303) t_{1/2} \sum \varepsilon_\lambda Z_\lambda}$$

where Z_λ is the light intensity (photons cm^{-2} sec^{-1}) and ε_λ is the molar extinction coefficient, respectively, at the wavelength interval centered at λ. The products of $\varepsilon_\lambda Z_\lambda$ at 1 nm intervals were summed over the region 340-400 nm (less than 1% of the total light energy was distributed in the 340-350 nm or 390-400 nm regions).

The photolysis of 3'-O-[^{3}H]acetyl-5'-O-[2,2'-di-(2-nitrophenyl)ethoxycarbonyl]-T was carried out in methanol-water, 1:2 v/v. A 30 µL sample of non-irradiated solution (~50 nmol/mL) was analyzed by HPLC. Fractions (1.0 mL) were collected, mixed with scintillation fluid and analyzed for ^{3}H on a Beckman LS 6000IC scintillation counter: a radioactive peak (~2850 cpm) corresponding to 3'-O-acetyl-5'-O-[2,2'-di-(2-nitrophenyl)ethoxycarbonyl] -T eluted in fraction 29 (Fig. 3a). An aliquot (100 µL) of the solution was placed in a 2 mm-pathlength cuvette as above and irradiated for 5.0 min. A 30 µL aliquot of the photolysis solution was analyzed by HPLC as described before. A radioactive peak (~2860 cpm) eluted in fraction 14 (Fig. 3b), corresponding to the position of authentic thymidine 3'-acetate (Sigma).

Compounds

Di-(2-nitrophenyl)methanol (3). Sodium borohydride (227 mg) was added to a solution of 2,2'-dinitrobenzophenone[21] (544 mg, 2.0 mmol) in a mixture of dioxane (12 mL) and methanol (20 mL). TLC (methylene chloride: petroleum ether 2:1, v:v) of the reaction mixture showed that all of the starting material (R_f = 0.52) was converted to a more polar compound (R_f = 0.4) after 30 min. The solvent was removed *in vacuo* and the residue was stirred with water (20 mL) for 1h. The separated solid was removed via filtration, washed with cold water and dried to yield 495 mg (90%) of pure compound **3**, mp 122-123 °C (lit.[28] mp 126 °C). UV (CH_3CN) λ_{max} [nm] (log ε): 258 (3.86); ^{1}H-NMR (200 MHz, $CDCl_3$): δ 8.06 (d, J=1.3 Hz, 2H, Ar-H), 7.5

(m, 6H, Ar-H), 6.93 (d, J=5.0 Hz, 1H, >CH), 3.66 (d, J=4.23 Hz, 1H, OH). Anal. calcd. for $C_{13}H_{10}N_2O_5$ (274.24): C, 56.94; H, 3.68; N, 10.21. Found: C, 57.08; H, 3.74; N, 10.08.

Di-(2-nitrophenyl)methyl chloroformate (4). Di-(2-nitrophenyl)methanol (**3**, 137 mg, 0.50 mmol) was dissolved in methylene chloride (7 mL) and mixed with a 20% solution of phosgene in toluene (7 mL). DBU (1,8-diazabicyclo[5.4.0]undec-7-ene; 100 mL) was added and the mixture was stirred at room temperature. After ~42 h an additional aliquot (50 mL) of DBU was added. Approximately 8 h after the second addition of DBU, TLC (methylene chloride) indicated >90% conversion to the corresponding chloroformate. The reaction solution was passed through a column (1 x 5 cm) of silica gel and the column was eluted with methylene chloride. The solvent was evaporated and the residue was redissolved in dry methylene chloride (~0.25 M) and stored at 4 °C.

2,2'-Di-(2-nitrophenyl)ethyl alcohol (5). Di-(2-nitrophenyl)methane[21] (3.5 g, 13.6 mmol) was dissolved in dimethylsulfoxide (3 mL). Paraformaldehyde (310 mg) and benzyltrimethylammonium hydroxide (Triton B, 0.18 mL, 40% solution in methanol) were added and the mixture was stirred at 90 °C for 16 h. Solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography. Elution of the column with 30% methylene chloride in petroleum ether gave unreacted starting material (1.0 g, 28%). Continued elution of the column with methylene chloride yielded 2.25 g (57%) of **5**. An analytical sample was prepared by crystallization from methanol (mp 150-151 °C). UV (CH_3CN) λ_{max} [nm] (log ε): 209 (sh, 4.30), 255 (sh, 3.91); ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.93 (dd, J=8.02 Hz, 2H, Ar-H), 7.62-7.37 (m, 6H, Ar-H), 5.50 (t, J=6.22 Hz, 1H, -$CHCH_2$), 4.35 (t, J=5.21 Hz, 2H, -$CHCH_2$-), 2.14 (t, J=3.2 Hz, 1H, CH_2OH). Anal. calcd. for $C_{14}H_{12}N_2O_5$ (288.23): C, 58.33; H, 4.20; N, 9.72. Found: C, 58.31; H, 4.04; N, 9.62.

2,2'-Di-(2-nitrophenyl)ethyl chloroformate (6). Compound **5** (288 mg, 1.0 mmol) was dissolved in anhydrous tetrahydrofuran (5 mL) under argon atmosphere. The solution was cooled in an ice bath and a 20% solution of phosgene in toluene (3 mL) was added dropwise. After complete addition, the cooling bath was removed and the mixture was stirred for 3 h at room temperature. The TLC (methylene chloride) of the reaction mixture showed that all of the starting material had been consumed. The solvents were removed *in vacuo* to yield crude compound **6** (320 mg, 92%). The crude material was used for next step without further purification. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.95 (d, J=8.06 Hz, 2H, Ar-H), 7.56 (t, J=7.50 Hz, 2H, Ar-H), 7.44 (d, J=7.85 Hz, 2H, Ar-H), 7.35 (d, J=7.77 Hz, 2H, Ar-H), 5.75 (t, J=5.87 Hz, 1H, -$CHCH_2$-), 4.99 (d, J=6.23 Hz, 2H, -$CHCH_2$).

5'-O-[2,2'-Di-(2-nitrophenyl)ethoxycarbonyl]thymidine (7a). Thymidine (1.5 g, 6.2 mmol) was dried under vacuum for 6 h and dissolved in anhydrous pyridine (20 mL) under nitrogen atmosphere. The mixture was cooled in an ice bath and a solution of compound **6** (2.3 g, 6.56 mmol) in anhydrous methylene chloride (20 mL) was added dropwise. The reaction mixture was stirred at room temperature for 3 h and the solvent was removed *in vacuo*. The residue was dissolved in methylene chloride (100 mL), and washed with 0.1N hydrochloric acid (2x50 mL) followed by water (2x50 mL). All of the aqueous layers were combined and re-extracted with methylene chloride (2x50 mL). The organic portion was dried (Na_2SO_4), filtered and the

solvent was removed *in vacuo*. The crude product was purified on a silica gel column. Elution of the column (0-3% methanol in methylene chloride) gave 380 mg (7%) of 3', 5'-O-bis-(2-[2,2'-dinitrodiphenyl]ethoxycarbonyl)thymidine and 2.4 g (70%) of compound **7a**. UV (CH_3CN) λ_{max} [nm] (log ε): 206 (sh, 4.53), 260 (4.28), 345 (sh, 2.93); ^{1}H-NMR (200 MHz, $CDCl_3$): δ 9.5 (s, 1H, NH), 7.96 (d, J=7.25 Hz, 2H, Ar-H), 7.52 (t, J=7.4 Hz, 2H, Ar-H), 7.43 (m, 3H, Ar-H and H-6), 7.28 (d, J=3.21 Hz, 2H, Ar-H), 6.28 (t, J=6.8 Hz, 1H, H-1'), 5.77 (t, J=6.85 Hz, 1H, -$CHCH_2$-), 5.29 (s, 1H, 3'-OH), 4.87 (m, 2H, -$CHCH_2$-), 4.37 (m, 2H, H-5'), 4.11 (d, J= 2.0 Hz, 1H, H-3'), 3.55 (t, J= 1.0 Hz, 1H, H-4'), 2.3 and 2.16 (m, 2H, H-2'), 1.59 (s, 3H, CH_3). Anal. calcd. for $C_{25}H_{24}N_4O_{11}$. $1H_2O$ (574.43): C, 52.27; H, 4.57; N, 9.76. Found: C, 52.04; H, 4.44; N, 9.57.

5'-O-[2,2'-Di-(2-nitrophenyl)ethoxycarbonyl]-N^6-benzoyl-2'-deoxyadenosine (7b). Compound **7b** was prepared in 54% yield following the procedure described for **7a**, starting with N^6-benzoyldeoxyadenosine (500 mg, 1.4 mmol) and compound **6** (400 mg). UV (CH_3CN) λ_{max} [nm] (log ε): 230 (sh, 4.38), 260 (sh, 4.30), 275 (4.37), 337 (sh, 3.23); ^{1}H-NMR (200 MHz, $CDCl_3$): δ 9.25 (bs, 1H, NH), 8.73 (s, 1H, H-8), 8.16 (s, 1H, H-2), 8.05 (d, J=6.68 Hz, 2H, Ar-H), 7.97 (d, J=6.23 Hz, 2H, Ar-H), 7.55 (m, 5H, Ar-H), 7.45 (m, 4H, Ar-H), 6.48 (t, J=6.23 Hz, 1H, H-1'), 5.78 (t, J=6.59 Hz, 1H, $CHCH_2$), 5.29 (s, 1H, OH), 4.85 (d, J=6.31 Hz, 2H, $CHCH_2$), 4.72 (m, 1H, H-5'), 4.39 (t, J=3.75 Hz, 1H, H-3'), 4.23 (t, J=3.87 Hz, 1H, H-4'), 2.82 and 2.56 (m, 2H, H-2'). Anal. calcd. for $C_{32}H_{27}N_7O_{10}.H_2O$ (687.57): C, 55.9; H, 4.26; N, 14.26. Found: C, 56.23; H, 4.14; N, 14.26.

5'-O-[2,2'-Di-(2-nitrophenyl)ethoxycarbonyl]-N^2-isobutyryl-2'-deoxyguanosine (7c). Compound **7c** was prepared in 58% yield from the reaction of N^2-isobutyryl-2'-deoxyguanosine (1.0 g, 3.0 mmol) with compound **6** (4.4 mmol) in methylene chloride/pyridine as described for **7a**. UV (CH_3CN) λ_{max} [nm] (log ε): 253 (4.35), 258 (4.35), 277 (sh, 4.21), 344 (sh, 2.90); ^{1}H-NMR (200 MHz, $CDCl_3$): δ 10.01 (s, 1H, NH), 7.92 (d, J=7.77 Hz, 2H, Ar-H), 7.83 (s, 1H, H-8), 7.55 (m, 2H, Ar-H), 7.43 (t, J=7.11 Hz, 4H, Ar-H), 6.21 (t, J=6.48 Hz, 1H, H-1'), 5.71 (t, J=6.68 Hz, 1H, $CHCH_2$), 5.29 (s, 1H, OH), 4.82 (d, J=6.59, Hz, 2H, $CHCH_2$), 4.72 (m, 1H, H-3'), 4.34 (m, 2H, H-5'), 4.19 (m, 1H, H-4'), 2.84 (m, 1H, -CH), 2.66 and 2.45 (m, 2H, H-2'), 1.25 and 1.21 (2xs, 2x3H, 2xCH_3). Anal. calcd. for $C_{29}H_{29}N_7O_{11}$ (651.54): C, 53.46; H, 4.45; N, 15.05. Found: C 53.13; H, 4.50; N, 15.05.

5'-O-[2,2'-Di-(2-nitrophenyl)ethoxycarbonyl]-N^4-[2-(*p*-nitrophenyl)ethoxycarbonyl]-2'-deoxycytidine (7d). Compound **7d** was prepared in 69% yield starting with N^4-[2-(*p*-nitrophenyl) ethoxycarbonyl]-2'-deoxycytidine following the procedure described for **7a**. UV (CH_3CN) λ_{max} [nm] (log ε): 208 (sh, 4.70), 239 (4.37), 268 (4.29), 350 (sh, 2.98); ^{1}H-NMR (200 MHz, $CDCl_3$): δ 8.18 (d, J=7.98 Hz, 2H, Ar-H), 7.97 (d, J=8.06 Hz, 2H, Ar-H), 7.93 (d, J=6.22 Hz, 1H, H-5), 7.58 (d, J=7.47 Hz, 2H, Ar-H), 7.44-7.35 (m, 6H, Ar-H), 7.08 (d, J=7.04 Hz, 1H, H-6), 6.22 (t, J=6.05 Hz, 1H, H-1'), 5.78 (t, J=6.6 Hz, 1H, $CHCH_2$), 5.29 (d, J=0.94 Hz, 1H, 3'-HO), 4.86 (m, 3H, $CHCH_2$ and H-3'), 4.44-4.34 (m, 3H, OCH_2 and H-4'), 4.19 (t, J=2 Hz, 2H, H-5'), 3.11 (t, J=6.41 Hz, 2H, CH_2-Ar), 2.65 (m, 1H, H-2'), 2.079 (m, 1H, H-2'). Anal. calcd. for $C_{33}H_{30}N_6O_{14}$. $0.5H_2O$ (743.56): C, 53.30; H, 4.21; N, 11.31. Found: C, 53.22; H, 4.28; N, 10.99.

3'-O-[^{3}H]acetyl-5'-O-[2,2'-di-(2-nitrophenyl)ethoxycarbonyl]thymidine. [^{3}H]acetic anhydride (Amersham; 500 mCi/mmol) was diluted to a specific activity of 5 mCi/mmol with unlabeled acetic anhydride. Compound **7a** (1.1 mg, 2.0 mmol) was dissolved in 29 mL of anhydrous pyridine and mixed with 21 mL of the diluted [^{3}H]acetic anhydride. After ~48 h at room temperature, the solution was mixed with an equal volume of water, neutralized with saturated $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was evaporated and the labeled compound was redissolved in methylene chloride and purified by silica gel chromatography (1-3% methanol in methylene chloride). HPLC (see above) of the purified compound showed that 95% of the radioactivity co-eluted with a sample of 3'-acetyl-5'-O-[2,2'-di-(2-nitrophenyl)ethoxycarbonyl]T prepared by similar reaction of **7a** in pyridine with unlabeled acetic anhydride.

5'-O-[Di-(2-nitrophenyl)methoxycarbonyl]thymidine (9). Compound **9** was prepared in 28% yield starting with thymidine and **4** (~0.5 mmol) following the procedure described for **7a**. The crude product was purified on a preparative TLC plate (1000 micron, coated with fluorescent silica gel). The plate was developed in 10% methanol in methylene chloride. Silica gel containing the major band was removed from the plate and eluted with ethyl acetate. Evaporation of the solvent gave compound **9** as a foam. UV (CH_3CN) λ_{max} [nm] (log ε): 202 (4.45), 216 (sh, 4.29), 260 (4.23), 340 (sh, 2.90); ^{1}H-NMR (200 MHz, $CDCl_3$): δ 9.33 (bs, 1H, NH), 8.13 (dt, J= 1.2 and 8.0 Hz, 2H, Ar-H), 7.89 (s, 1H, H-6), 7.6 (m, 4H, Ar-H), 7.3 (m, 2H, Ar-H), 6.35 (t, J=8.0 Hz, 1H, H-1'), 5.3 (s, 1H, OH), 4.54 (m, 2H, H-5'), 4.38 (m, 1H, H-4'), 4.12 (m, 1H, H-3'), 2.38 and 2.19 (m, 2H, H-2'), 1.59 (s, 3H, CH_3). Anal. calcd. for $C_{24}H_{22}N_4O_{11}.1H_2O$ (560.34): C, 51.44; H, 4.32; N, 10.00. Found: C, 51.88; H, 4.44; N, 9.61.

2-(2,4-Dichloro-6-nitrophenyl)ethanol (14). A mixture of 2,4-dichloro-6-nitrotoluene (1.03 g, 5 mmol) and paraformaldehyde (0.15 g, 5 mmol) in dimethylsulfoxide (2.5 mL) was treated with a solution of potassium *tert*-butoxide (90 mg, 0.8 mmol) in *tert*-butanol (1 mL) for 5 min at room temperature and then at 80 °C for 30 min. The mixture was brought to room temperature, neutralized with acid and diluted with water (30 mL). The solution was extracted with ethyl acetate and the organic phase was dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by silica gel column chromatography (12x2 cm). Elution of the column with 10-30% ethyl acetate in petroleum ether gave 0.77 g (65% yield) of compound **14**. UV (CH_3OH) λ_{max} [nm] (log ε): 205 (4.32), 218 (sh, 4.20), 254 (sh, 3.40), 292 (3.08); ^{1}H-NMR (250 MHz, $CDCl_3$): δ 7.73 (d, J=2.0 Hz, 1H, Ar-H), 7.65 (d, J=2.0 Hz, 1H, Ar-H), 3.92 (m, α-CH_2), 3.26 (t, ß-CH_2). Anal. calcd. for $C_8H_7Cl_2NO_3$ (236.1): C, 40.71; H, 2.99; N 5.93. Found: C 40.36; H, 2.96; N, 5.85.

2-(2,6-Dinitrophenyl)ethyl chloroformate (19). A solution of 2-(2,6-dinitrophenyl)ethanol[29] (**10**, 4.08 g, 19.23 mmol) and triethylamine (2.7 mL, 19.28 mmol) in anhydrous tetrahydrofuran (30 mL) was added to a cold solution (0 °C) of diphosgene (3.81 g, 19.28 mmol) in anhydrous tetrahydrofuran (10 mL) over a period of 20 min with stirring. After 30 min the cooling bath was removed and stirring was continued at room temperature for 2 h. The mixture was filtered over celite and washed with tetrahydrofuran. The solvent was removed *in vacuo* to give compound **19** (5.13 g, 97% yield) as a brownish solid, mp 84-85 °C. UV (CH_3CN) λ_{max} [nm] (log ε): 233 (4.02), 292 (sh, 3.11), 331 (sh, 2.82); ^{1}H-NMR (250 MHz, CDCl3): δ 8.10 (d,

J=8.2 Hz, 2H, Ar-H-3, Ar-H-5), 7.67 (t, J=8.1 1H, Ar-H-4), 4.67 (t, 2H, α-CH_2), 3.50 (t, 2H, ß-CH_2). Anal. calcd. for $C_9H_7ClN_2O_6$ (274.6): C, 39.36; H, 2.57; N, 10.20. Found: C, 39.40; H, 2.60; N, 10.20.

2-(2-Iodo-6-nitrophenyl)ethyl chloroformate (20). A solution of 20% phosgene in toluene (15 mL) was slowly added to a solution of 2-(2-iodo-6-nitrophenyl)ethanol (**11**, 879 mg, 3 mmol) in tetrahydrofuran (6.0 mL) with cooling under nitrogen atmosphere. TLC (methylene chloride) after 30 min stirring at room temperature showed complete conversion of the starting material into the product. The solvent was removed *in vacuo* and the residue was used for the next step without further purification.

2-(2-Bromo-6-nitrophenyl)ethyl chloroformate (21). Compound **21** was prepared in 99% yield following the procedure described for compound **19**, starting with 2-(2-bromo-6-nitrophenyl)ethanol[30] (**12**, 0.5 g, 2.03 mmol). UV (CH_3OH) λ_{max} [nm] (log ε): 205 (sh, 4.14), 211 (4.16), 254 (3.52), 297 (sh, 3.05). ^{1}H-NMR (250 MHz, $CDCl_3$): δ 7.85 (d, 2H, Ar-H), 7.33 (t, 1H, Ar-H), 4.62 (t, 2H, α-CH_2), 3.47 (t, 2H, ß-CH_2). Anal. calcd. for $C_9H_7BrClNO_4$ (308.5): C, 35.04; H, 2.29; N 4.54. Found: C, 35.50; H, 2.58; N, 4.50.

2-(2-Chloro-6-nitrophenyl)ethyl chloroformate (22). Phosgene gas was bubbled to a solution of 2-(2-chloro-6-nitrophenyl)ethanol[31] (**13**, 31 g, 0.15 mol) in anhydrous tetrahydrofuran (190 mL) at room temperature with stirring for 2.5 h. Excess reagent and solvent were removed *in vacuo* to give compound **22** (39.4 g, 97% yield) as a yellow syrup. UV (CH_3CN) λ_{max} [nm] (log ε): 211 (4.14), 253 (3.53), 300 (sh, 3.02). ^{1}H-NMR (250 MHz, $CDCl_3$): δ 7.81 (dd, 1H, Ar-H), 7.69 (dd, 1H, Ar-H), 7.41 (t, 1H, Ar-H), 4.63 (t, 2H, α-CH_2), 3.46 (t, 2H, ß-CH_2). Anal. calcd. for $C_9H_7Cl_2NO_4$ (264.1): C, 40.94; H, 2.67; N, 5.30. Found: C, 41.05; H, 2.73; N, 5.00.

2-(2,4-Dichloro-6-nitrophenyl)ethyl chloroformate (23). Compound **23** was prepared in 94% yield following the procedure described for compound **19**, starting with 2-(2,4-dichloro-6-nitrophenyl)ethanol (**14**, 0.5 g, 2.12 mmol). UV (CH_3OH) λ_{max} [nm] (log ε): 204 (4.35), 217 (4.25) 252 (sh, 3.47), 295 (3.10). ^{1}H-NMR (250 MHz, $CDCl_3$): δ 7.82 (d, J=1.8 Hz, 1H, Ar-H), 7.70 (d, J=1.8 Hz, 1H, Ar-H), 4.59 (t, 2H, α-CH_2), 3.42 (t, 2H, ß-CH_2). Anal. calcd. for $C_9H_6Cl_3NO_4$ (298.5): C, 36.21; H, 2.03; N, 4.69. Found: C, 36.37; H, 2.30; N 4.60.

2-(5-Methoxy-2-nitrophenyl)ethyl chloroformate (24). Compound **24** was prepared in 96% yield following the procedure described for compound **22**, starting with 2-(5-methoxy-2-nitrophenyl)ethanol[29] (**15**, 3.0 g, 15 mmol). UV (CH_3CN) λ_{max} [nm] (log ε): 222 (sh, 3.87), 230 (3.90), 303 (3.87). ^{1}H-NMR (250 MHz, $CDCl_3$): δ 8.12 (d, 1H, Ar-H), 6.88 (dd, 1H, Ar-H), 6.79 (d, J=2.8 Hz, 1H, Ar-H), 4.63 (t, 2H, α-CH_2), 3.89 (s, 3H, OCH_3), 3.35 (t, 2H, ß-CH_2). Anal. calcd. for $C_{10}H_{10}ClNO_5$ (259.6): C, 46.26; H, 3.88; N, 5.39: Found: C, 46.42; H, 4.00; N, 5.50.

2-(2-Nitrophenyl)ethyl chloroformate (25). A solution of 2-(2-nitrophenyl)ethanol[32] (**16**, 5.2 g, 31 mmol) in tetrahydrofuran (20 mL) was treated with phosgene. The mixture was stirred at room temperature for

1.5 h. The excess solvent and reagent were removed *in vacuo* to yield compound **25** (6.69 g, 94%) as a yellow oil. UV (CH_3CN) λ_{max} [nm] (log ε): 202 (4.12), 218 (sh, 3.74), 256 (3.70), 209 (sh, 3.16), 346 (sh, 2.59); ^{1}H-NMR (250 MHz, $CDCl_3$): δ 7.99 (dd, 1H, Ar-H-3), 7.48 (m, 3H, Ar-H), 4.62 (t, α-CH_2), 3.3 (t, ß-CH_2). Anal. calcd. for $C_9H_8ClNO_4$ (229.6): C, 47.08; H, 3.51; N 6.10. Found: C, 47.30; H, 3.70; N, 6.00.

2-(2-Fluoro-6-nitrophenyl)ethyl chloroformate (26). Compound **26** was prepared in 93% yield as a brownish syrup following the procedure described for **19**, starting with 2-(2-fluoro-6-nitrophenyl)ethanol[28], (**17**, 1.85 g, 10 mmol). UV (CH_3OH) λ_{max} [nm] (log ε): 204 (4.04), 251 (3.67), 293 (sh, 3.23); ^{1}H-NMR (250 MHz, $CDCl_3$): δ 7.83 (d, J=7.7 Hz, 1H, Ar-H), 7.44 (m, 2H, Ar-H), 4.63 (t, 2H, α-CH_2), 3.37 (dt, J=1.6, 6.4 Hz, 2H, ß-CH_2). Anal. calcd. for $C_9H_7FClNO_4$ (247.6): C, 43.66; H, 2.85; N, 5.66. Found: C, 43.97; H, 3.02; N, 5.59.

2-(2-Nitrophenyl)propyl chloroformate (27). Compound **27** was prepared in 96% yield following the procedure described for compound **19**, starting with 2-(2-nitrophenyl)propanol[33] (**18**, 0.5 g, 2.76 mmol). UV (CH_3OH) λ_{max} [nm] (log ε): 205 (sh, 4.07), 2.18 (sh, 3.75), 251 (3.59). ^{1}H-NMR (250 MHz, $CDCl_3$): δ 7.84 (dd, J=1.2, 8.0 Hz, 1H, Ar-H), 7.62 (m, 1H, Ar-H), 7.44 (m, 2H, Ar-H), 4.50 (d, J=6.3 Hz, 2H, α-CH_2), 3.80 (sextet, 2H, ß-CH_2), 1.42 (d, J=7.0 Hz, CH_3). Anal. calcd. for $C_{10}H_{10}ClNO_4$ (243.7): C, 49.3; H, 4.14; N, 5.75. Found: C, 49.71; H, 4.32; N, 5.70.

5'-O-[2-(2,6-Dinitrophenyl)ethoxycarbonyl]thymidine (28). Compound **28** was prepared in 75% yield following the procedure described for compound **34**, starting with thymidine and compound **19**, at -50 °C. The residue was purified on a silica gel column (15x3.5 cm). Elution of the column with 5-50% acetone in methylene chloride gave 1.15 g (64% yield) of compound **28**. UV (CH_3OH) λ_{max} [nm] (log ε): 206 (4.28), 244 (sh, 4.07), 256 (4.08), 335 (sh, 2.72). ^{1}H-NMR (250 MHz, DMSO-*d6*): δ 11.32 (s, 1H, NH), 8.27 (d, 2H, Ar-H), 7.78 (t, 1H, Ar-H), 7.40 (s, 1H, H-6), 6.18 (t, 1H, H-1'), 5.44 (d, 1H, 3'-OH), 4.37 (t, 2H, α-CH_2), 4.23 (m, 3H, H-3', H-5'), 3.91 (m, 1H, H-4'), 3.29 (t, 2H, ß-CH_2), 2.15 (m, 2H, H-2'), 1.71 (s, 3H, CH_3). Anal. calcd. for $C_{19}H_{20}N_4O_{11}$ (480.4): C, 47.51; H, 4.20; N, 11.66. Found: C, 47.40; H, 4.15; N, 11.57.

5'-O-[2-(2-Iodo-6-nitrophenyl)ethoxycarbonyl]thymidine (29). Thymidine (484.4 mg, 2 mmol) was dissolved in anhydrous pyridine (5 mL) under nitrogen atmosphere. The mixture was cooled to 0 °C, and a solution of compound **20** (crude, 889 mg, ~2.5 mmol) in methylene chloride (10 mL) was added dropwise. After 30 min stirring at room temperature the solvent was removed and the residue was purified by silica gel column chromatography. Elution of the column with 0-1.5% methanol in methylene chloride gave 769 mg (68% yield) of compound **29**. UV (CH_3CN) λ_{max} [nm] (log ε): 226 (4.17), 261 (4.06), 322 (2.96); ^{1}H-NMR (200 MHz, DMSO-*d6*): δ 8.25 (d, J=7.20 Hz, 1H, Ar-H), 7.92 (d, J=8.0 Hz, 2H, Ar-H), 7.43 (s, 1H, H-6), 7.28 (t, J=8.0 Hz, 1H, Ar-H), 6.20 (t, J=6.6 Hz, 1H, H-1'), 5.74 (s, 1H, 3'-OH), 5.43 (d, J=4.4 Hz, 1H, H-4'), 4.29 (m, 4H, H-5 & α-CH_2), 3.94 (m, 1H, H-3'), 3.91 (d, J=4.0 Hz, 2H, ß-CH_2), 2.16 (m, 2H,

H-2'), 1.74 (s, 1H, CH_3). Anal. calcd. for $C_{19}H_{20}IN_3O_9$ (561.29): C, 40.66; H, 3.59; N, 7.49. Found: C, 40.49; H, 3.70; N, 7.29.

5'-O-[2-(2-Bromo-6-nitrophenyl)ethoxycarbonyl]thymidine (30). Compound **30** was prepared following the procedure described for compound **34**, starting with thymidine and compound **21** (1.72 g, 5.7 mmol). The reaction was carried out at -50 to -20 °C and the product was purified on a silica gel column. Elution of the column with 1-5% methanol in methylene chloride gave 1.58 g (73% yield) of compound **30**. UV (CH_3OH) λ_{max} [nm] (log ε): 210 (4.37), 263 (4.06). ^{1}H-NMR (250 MHz, $CDCl_3$): δ 8.35 (s, 1H, NH), 7.85 (d, 1H, Ar-H), 7.76 (d, 1H, Ar-H), 7.38 (s, 1H, H-6), 7.30 (t, 1H, Ar-H), 6.37 (t, 1H, H-1'), 4.45 (m, 5H, H-3', H-5' & α-CH_2), 4.15 (m, 1H, H-4'), 3.47 (t, 2H, ß-CH_2), 2.41 (m, 2H, 3'-OH, H-2'), 2.25 (m, 1H, H-2'), 1.86 (s, 3H, CH_3). Anal. calcd. for $C_{19}H_{20}BrN_3O_9$ (514.3): C, 44.37; H, 3.92; N, 8.17. Found: C, 44.31; H, 3.96; N, 8.11.

5'-O-[2-(2-Chloro-6-nitrophenyl)ethoxycarbonyl]thymidine (31). Compound **31** was prepared following the procedure described for compound **34** starting with thymidine and compound **22** (1.3 g, 5 mmol). The reaction was carried out at -50 to -30 °C and the product was purified on a silica gel column. Elution of the column with 2-9% methanol in methylene chloride gave 1.55 g (80% yield) of compound **31**. UV (CH_3OH) λ_{max} [nm] (log ε): 210 (4.36), 263 (4.05). ^{1}H-NMR (250 MHz, $CDCl_3$): δ 8.11 (s, 1H, NH), 7.74 (d, J=8.2 Hz, 1H, Ar-H), 7.67 (d, J=8.0 Hz, 1H, Ar-H), 7.39 (t, 1H, Ar-H), 7.38 (s, 1H, H-6), 6.36 (t, 1H, H-1'), 4.44 (m, 5H, H-3', H-5' & α-CH_2), 4.15 (q, 1H, H-4'), 3.45 (t, 2H, ß-CH_2), 2.38 (m, 1H, H-2'), 2.26 (m, 1H, H-2'), 1.86 (s, 3H, CH_3). Anal. calcd. for $C_{19}H_{20}ClN_3O_9$ (469.8): C, 48.57; H, 4.29; N, 8.94. Found: C, 48.53; H, 4.34; N, 8.91.

5'-O-[2-(2,4-Dichloro-6-nitrophenyl)ethoxycarbonyl]thymidine (32). Compound **32** was prepared following the procedure described for compound **34**, starting with thymidine and compound **23** (12.8 g, 4.3 mmol). The reaction was carried out at -50 to -15 °C and the product was purified on a silica gel column. Elution of the column with 1-3% methanol in methylene chloride gave 1.36 g (81% yield) of compound **32**. UV (CH_3OH) λ_{max} [nm] (log ε): 203 (4.51), 214 (sh, 4.38). ^{1}H-NMR (250 MHz, $CDCl_3$): δ 8.91 (s, 1H, NH), 7.75 (d, J=2.1 Hz, 1H, Ar-H), 7.68 (d, J=2.1 Hz, 1H, Ar-H), 7.37 (s, 1H, H-6), 6.37 (t, 1H, H-1'), 4.63 (m, 5H, H-3', H-5' & α-CH_2), 4.16 (m, 1H, H-4'), 3.40 (t, 2H, ß-CH_2), 2.86 (d, 1H, 3'-OH), 2.42 (m, 1H, H-2'), 2.24 (m, 1H, H-2'), 1.86 (s, 3H, CH_3). Anal. calcd. for $C_{19}H_{19}Cl_2N_3O_9$ (504.3): C, 45.25; H, 3.80; N, 8.33. Found: C, 45.02; H, 3.89; N, 8.04.

5'-O-[2-(5-Methoxy-2-nitrophenyl)ethoxycarbonyl]thymidine (33). Compound **33** was prepared following the procedure described for compound **34**, starting with thymidine and compound **24** (1.65 g, 6.3 mmol). The reaction was carried out at -30 to -15 °C and the product was purified on a silica gel column. Elution of the column with 6% methanol in methylene chloride gave 1.35 (71% yield) of compound **33**. UV (CH_3OH) λ_{max} [nm] (log ε): 202 (4.30), 234 (sh, 3.94), 270 (4.09), 302 (sh, 3.84). ^{1}H-NMR (250 MHz, $CDCl_3$): δ 8.79 (s, 1H, NH), 8.06 (1H, Ar-H), 7.34 (s, 1H, H-6), 6.85 (dd, 1H, Ar-H), 6.77 (d, J=2.7, 1H, Ar-H), 6.32 (t, 1H, H-1'), 4.43 (m, 5H, H-3', H-5' & α-CH_2), 4.12 (q, 1H, H-4'), 3.85 (s, 3H, OCH_3),

3.33 (m, 2H, ß-CH_2), 2.75 (d, 1H, 3'-OH), 2.38 (m, 1H, H-2'), 2.18 (m, 1H, H-2'), 1.83 (s, 3H, CH_3). Anal. calcd. for $C_{20}H_{23}N_3O_{10}$ (465.4): C, 51.61; H, 4.98; N, 9.03. Found: C, 51.31; H, 5.09; N, 8.63.

5'-O-[2-(2-Nitrophenyl)ethoxycarbonyl]thymidine (34). Thymidine (1 g, 4.13 mmol) was evaporated with dry pyridine (2x10 mL) and then dissolved in dry pyridine (10 mL). The solution was cooled to -30 °C and a solution of **25** (1.45 g, 6.31 mmol) was added dropwise. Stirring was continued for 5 h at -30 to -15 °C. The mixture was diluted with methylene chloride (150 mL) and water (150 mL) was added. The organic phase was separated and the aqueous phase was re-extracted with methylene chloride (2 x 150 mL). The organic extracts were combined and dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified on a silica gel column (15x3.5 cm). Elution of the column with 5-50% acetone in methylene chloride gave 1.15 g (64% yield) of compound **34**. UV (CH_3OH) λ_{max} [nm] (log ε): 205 (4.13), 262 (4.12), 334 (sh, 2.64). ^{1}H-NMR (250 MHz, $CDCl_3$): δ 8.92 (s bs, 1H, NH), 7.96 (dd, 1H, Ar-H), 7.55 (t, 1H, Ar-H), 7.42 (m, 3H, H-6, & 2 Ar-H), 6.35 (t, 1H, H-1'), 4.44 (m, 5H, H-3', H-5' & α-CH_2), 4.15 (q, H-4'), 3.34 (m, 2H, ß-CH_2), 2.89 (d, 1H, 3'-OH), 2.41 (m, 1H, H-2'), 2.22 (m, 1H, H-2'), 1.85 (s, 3H, CH_3). Anal. calcd. for $C_{19}H_{21}N_3O_9$ (435.4): C, 52.41; H, 4.86; N, 9.65. Found: C, 52.07; H, 5.15; N, 9.65.

5'-O-[2-(2-Fluoro-6-nitrophenyl)ethoxycarbonyl]thymidine (35). Compound **35** was prepared following the procedure described for compound **34**, starting with thymidine and compound **26** (1.4 g, 4.5 mmol). The reaction was carried out at -50 °C. The residue was purified on a silica gel column. Elution of the column with 1-4% methanol in methylene chloride gave 3',5'-O-bis(2-[2-fluoro-6-nitrophenyl]ethoxycarbonyl)-thymidine followed by 1.31 g (70% yield) of compound **35**. UV (CH_3OH) λ_{max} [nm] (log ε): 205 (4.29), 261 (4.10). ^{1}H-NMR (250 MHz, DMSO-d_6): δ 7.89 (d, J=7.6 Hz, 1H, Ar-H), 7.64 (m, 2H, Ar-H),7.74 (s, 1H, H-6), 6.20 (t, J=6.6 Hz, 1H, H-1'),5.47 (d, J=3.8 Hz, 1H, 3'-OH), 4.38 (t, J=6.6 Hz, 2H, α-CH_2), 4.27 (m, 3H, H-4', & H-5'), 3.93 (m, 1H, H-3'), 3.25 (t, J=6.2 Hz, 2H, ß-CH_2), 2.16 (m, 2H, H-2'), 1.74 (3H, CH_3). Anal. calcd. for $C_{19}H_{20}FN_3O_9$ (453.4): C, 50.34; H, 4.45; N, 9.27. Found: C, 50.22; H, 4.49; N, 9.18.

5'-O-[2-(2-Nitrophenyl)propoxycarbonyl]thymidine (36). Compound **36** was prepared following the procedure described for compound **34**, starting with thymidine and compound **27** (1.31 g, 5.4 mmol). The reaction was carried out at -50 to -20 °C and the product was purified on a silica gel column. Elution of the column with 1-6% methanol in methylene chloride gave 1.31 g (71% yield) of compound **36** as a colorless solid. UV (CH_3OH) λ_{max} [nm] (log ε): 206 (4.33), 262 (4.09). ^{1}H-NMR (250 MHz, $CDCl_3$): δ 8.66 (s, 1H, NH, diastereomer), 8.64 (s, 1H, NH, diastereomer), 7.77 (m, 1H, Ar-H), 7.59 (t, 1H, Ar-H), 7.43 (m, 2H, Ar-H), 7.33 (s, 1H, H-6, diastereomer), 7.30 (s, 1H, H-6, diastereomer), 6.34 (t, 1H, H-1', diastereomer), 6.32 (t, 1H, H-1', diastereomer), 4.29 (m, 5H, H-3', H-5' & α-CH_2), 3.80 (m, 2H, ß-CH_2), 2.62 (d, J=7.0 Hz, 1H, 3'-OH, diastereomer), 2.60 (d, J=4.4 Hz, 1H, 3'-OH, diastereomer), 2.39 (m, 1H, H-2'), 2.18 (m, 1H, H-2'), 1.86 (s, 3H, CH_3, diastereomer), 1.75 (s, 3H, CH_3, diastereomer), 1.38 (d, J=7.0 Hz, 3H, CH_3, diastereomer), 1.37 (d, J=7.0 Hz, 3H, CH_3, diastereomer). Anal. calcd. for $C_{20}H_{23}N_3O_9$ (449.4): C, 53.45; H, 5.16; N, 9.35. Found: C, 53.14; H, 5.21; N, 9.16 .

5'-O-(6-Nitroveratryloxycarbonyl)thymidine (37). 6-Nitroveratryloxycarbonyl chloride (413 mg, 1.5 mmol) was added to a stirred solution of thymidine (242 mg, 1.0 mmol) in dry pyridine (10 mL) at 0-5 °C. After stirring at room temperature for 14h, pyridine was stripped off and the residue was dissolved in methylene chloride, extracted with 0.01 N HCl saturated with NaCl, dried (Na_2SO_4), filtered and solvent was evaporated. The crude compound was purified by silica gel chromatography to yield 196 mg (41%) of pale yellow crystals, mp 193-194 °C (uncorr.). Fab-MS (m/e): Calculated ($C_{20}H_{23}N_3O_{11}$) 481.4; Found: 482.20 [M+H]. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.23 (bs, 1H, NH), 7.64 (d, J=1.2 Hz, 1H, Ar-H), 7.30 (s, 1H, Ar-H), 7.12 (s, 1H, H-6), 6.15 (t, J=6.4 Hz, 1H, H-1'), 5.42 (m, 3H, 3'-OH, H-3' and H-4'), 4.30 (m, 2H, H-5'), 3.83, 3.82 (2s, 2x3H, CH_3O), 3.28 (s, 2H, CH_2-), 2.10 (m, 1H, H-2'), 2.08 (m, 1H, H-2'), 1.6 (s, 3H, CH_3).

5'-O-(2-Nitrobenzyloxycarbonyl)thymidine (38). 2-Nitrobenzyloxycarbonyl chloride (842 mg in 2 mL CH_2Cl_2, obtained from the reaction of 2-nitrobenzyl alcohol and 20% phosgene solution in toluene) was added to a stirred solution of thymidine (363 mg, 1.5 mmol) in anhydrous pyridine (3 mL) under argon. After 18 h the solvent from the reaction mixture was removed *in vacuo* . The residue was dissolved in CH_2Cl_2 (50 mL) and washed with 0.1 N HCl (2x25 mL), saturated solution of sodium hydrogen carbonate (2x25 mL) followed by water (25 mL). The organic phase was dried (Na_2SO_4), filtered and solvent was removed *in vacuo*. The residue on silica gel column purification (0-3% methanol in dichloromethane) yielded 395 mg (63%) of pure compound. Fab-MS (m/e): Calculated ($C_{18}H_{19}N_3O_9$) 421.53; Found: 422.18 [M+H]. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.3 (bs, 1H, NH), 8.14 (d, J=7.2 Hz, 1H, Ar-H), 7.79 (t, J=7.6 Hz, 1H, Ar-H), 7.66 (t, J=7.6 Hz, 2H, Ar-H), 7.43 (s, 1H, H-6), 6.12 (t, J=6.8 Hz, 1H, H-1'), 5.51 (s, 1H, 3'-OH), 5.45 (d, J=4.4 Hz, 1H, H-3'), 4.34 and 4.26 (2xm, 3H, H-4' and H-5'), 2.50 (s, 2H, CH_2-), 2.15 (m, 1H, H-2'), 2.11 (m, 1H, H-2'), 1.10 (s, 3H, CH_3).

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