Nucleotides; XXIII[1] Synthesis of Protected 2′-Deoxyribonucleoside-3′-phosphotriesters containing the *p*-Nitrophenylethyl Phosphate Blocking Group

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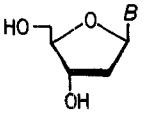
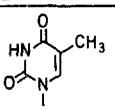
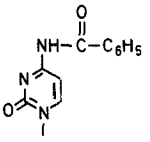
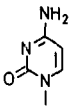
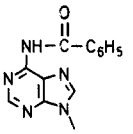
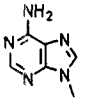
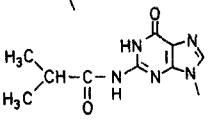
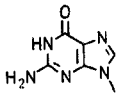
Recent developments in oligonucleotide synthesis[2-6] have revealed that the phosphotriester approach[7,8,9] offers advantages over the older, classical phosphodiester method[10]. The choice of a versatile phosphate blocking group is a crucial problem in this strategy and has led to the introduction of numerous functions including the 2-cyanoethyl[11-14], trihaloethyl[15,16,17], phenyl[18], and substituted phenyl groups[19,20]. The striking properties of the *p*-nitrophenylethyl (NPE) group[21-24] for phosphate and aglycon protection facilitate the synthetic as well as the deprotection steps.

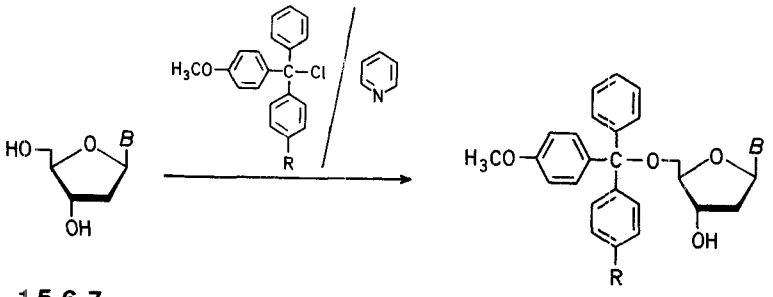
As a continuation of our efforts to provide new versatile building blocks for oligonucleotide syntheses we now describe the preparation of protected phosphotriesters of all the four common 2′-deoxyribonucleosides, thymidine (**1**), 2′-deoxycytidine (**2**), 2′-deoxyadenosine (**3**), and 2′-deoxyguanosine (**4**), containing the 2,5-dichlorophenyl and 2-(4-nitrophenyl)-ethyl group on the phosphate function. The amino groups of the starting 2′-deoxynucleosides **2–4** were first protected by benzoylation (**2** and **3**) and isobutyrylation (**4**), respectively.



No.	B		No.	B	
1		[T]	5		[C^{Bz}]
2		[C]	6		[A^{Bz}]
3		[A]	7		[$G^{i\text{-}Bu}$]
4		[G]			

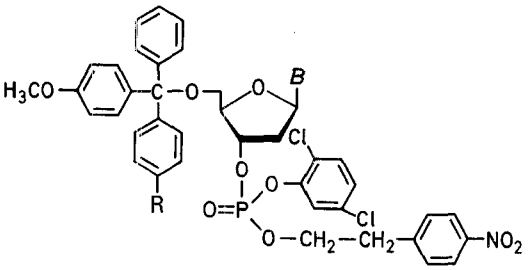
Introduction of the *p*-methoxytrityl or *p,p′*-dimethoxytrityl group by known methods on to compounds **1, 5–7** gives the products **8–15**, some of which have not been fully characterized in the literature (see experimental part and Table 1). In the next step, the 5′-protected compounds **8–15** are phosphorylated with 2,5-dichlorophenyl phosphorodichloridate in the presence of 1,2,4-triazole (2 equivalents) in absolute pyridine to form first the corresponding phosphordiester-triazolides, which on addition of 2-(4-nitrophenyl)-ethanol react within 6 h to give the fully protected phosphotriesters **16–23**. Isolation and purification is achieved by silica gel column chromatography and in the cases of **16–19** by recrystallization from ethanol/*n*-hexane to give colourless crystals in good yields. Characterization and structural proof is based on microanalysis, U.V. and ^{1}H-N.M.R. spectra (Table 2).

U.V.-Absorption spectra: Cary Spectrometer Model 118; ^{1}H-N.M.R.-spetra: Bruker HFX-90 and Jeol JNM-MH-100 instruments, in $CDCl_3$ with TMS as internal standard; T.L.C. on thin layer plates silica gel F 1500 LS 254 (Schleicher and Schüll); column chromatography on silica gel 60, particle size 0.063–0.2 mm of (Merck/Darmstadt); – the substances were dried in a vacuum dessicator over phosphorus pentoxide; melting points are not corrected.



1,5,6,7 8-15

No.	8,16	9,17	10,18	11,19	12,20	13,21	14,22	15,23
B	T	C^{Bz}	A^{Bz}	$G^{i\text{-}Bu}$	T	C^{Bz}	A^{Bz}	$G^{i\text{-}Bu}$
R	H	H	H	H	OCH_3	OCH_3	OCH_3	OCH_3



16-23

N^6-Benzoyl-2′-deoxycytidine (5)[15,25]:
Method A: 2′-Deoxycytidine hydrochloride monohydrate (5.8 g, 0.02 mol) is dissolved in water (20 ml) and then stirred with Amberlite A-26 ($OH^{\ominus}$-form; 8 g) for 30 min at room temperature until the solution is neutral. After filtration and washing with water (500 ml), the solution is evaporated to dryness. The residue is recrystallized from methanol to give colourless crystals of **2**; yield: 4.5 g (99%).

Compound **2** (4.5 g, 19.8 mmol) is coevaporated with absolute pyridine (4 × 10 ml) and then dissolved in this solvent (50 ml). Benzoyl chloride (10 ml) is added and, after stirring for 3 h at room temperature, the solution is poured slowly on to ice (600 ml). The mixture is then extracted with ethyl acetate (4 × 150 ml). Separation of the organic layer, drying with sodium sulfate, and evaporation gives *tetrabenzoyl-2′-deoxycytidine*[26]; yield: 12.1 g (95%).

This material (1.92 g, 3 mmol) is dissolved in a mixture of tetrahydrofuran (80 ml), methanol (80 ml), and water (20 ml), chilled with ice to 0 °C and then treated with 1 normal sodium hydroxide solution (15 ml) for about 15 min until no starting material is detected by T.L.C. The mixture is neutralized by addition of DOWEX 50WX4 (pyridinium form), filtered, washed with methanol (300 ml), and then evaporated to dryness. The residue is treated with ether (200 ml), the precipitate collected, and dried to give colourless crystals; yield: 0.79 g (80%); m.p. 194 °C (Lit.[25], m.p. 194 °C).

Method B: Compound **2** (9.1 g) is dissolved in absolute methanol (500 ml). To the boiling solution is added five times at intervals of 2 h benzoic anhydride (10 g, 0.083 mol) each. After reflux for 10 h in total, the precipitate is filtered off. The filtrate is evaporated to dryness, the residue is treated with water (100 ml) and ether, (100 ml), and then filtered again. The precipitate is recrystallized from water to give the product; yield: 11.4 g (86%); m.p. 193–194 °C (Lit.[15], m.p. 190–192 °C).

N^2,3′,5′-Triisobutyryl-2′-deoxyguanosine[30]:
2′-Deoxyguanosine (**4**; 8.02 g, 30 mmol) is coevaporated with absolute pyridine (2 × 50 ml), then the residue is suspended in 20 ml of the same solvent (20 ml) + chloroform (100 ml). Isobutyryl chloride (16.0 g, 150 mmol) in dry chloroform (60 ml) is added dropwise with stirring at 0 °C within 30 min. The mixture is stirred at room temperature for 2 h and then poured on to ice (1000 ml). Extraction with chloroform (3 × 600 ml) drying over with sodium sulfate, evaporation wth toluene (50 ml), and co-evaporation with *n*-hexane (3 × 25 ml) gives a colourless amorphous foam; yield: 11.5 g (90%).

$C_{22}H_{31}N_5O_7$	calc.	C 55.34	H 6.54	N 14.67
(477.5)	found	54.89	6.45	14.71

N^2-Isobutyryl-2′-deoxyguanosine (7)[27,28]:
N^2,3′,5′-Triisobutyryl-2′-deoxyguanosine[30] (0.95 g, 2 mmol) is dissolved in ethanol (40 ml) and, after cooling to 0 °C, 1 normal sodium hydroxide solution (15 ml) is added. The solution is stirred for 15 min until no starting material is detected by T.L.C. and is then neutralized by addition of DOWEX 50WX4 (pyridinium form). The resin is filtered off, washed with ethanol (500 ml), and the filtrate evaporated to dryness. The residue gives on recrystallization from water colourless crystals; yield: 0.52 g (77%); m.p. 185–187 °C.

$C_{14}H_{19}N_5O_5$	calc.	C 49.84	H 5.68	N 20.76
(337.3)	found	49.71	5.83	20.89

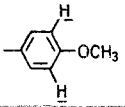
N^4-Benzoyl-5′-*O*-monomethoxytrityl-2′-deoxycytidine (9)[26]:
Compound **5**[26] (5.6 g, 18 mmol) is coevaporated with absolute pyridine (3 × 30 ml) and then dissolved in the same solvent (100 ml). Monomethoxytrityl chloride (6.8 g, 22 mmol) is added and the mixture is stirred for 20 h at room temperature. After addition of methanol (10 ml) and stirring for another 30 min, the mixture is evaporated to dryness. The residue is dissolved in chloroform (200 ml), washed with water (150 ml), the organic layer dried with sodium sulfate and then concentrated to a small volume which is placed on a silica gel column (35 × 3 cm) for chromatographic separation eluting first with chloroform (300 ml) and then with chloroform/methanol (99/1), which elutes the product **9**. Evaporation of the main fraction and reprecipitation from a small amount chloroform in *n*-hexane (500 ml) with stirring gives **9** as a colourless amorphous powder; yield: 9.5 g (88%)

$C_{36}H_{33}N_3O_6$	calc.	C 71.63	H 5.51	N 6.96
(603.6)	found	71.08	5.82	6.78

N^2-Isobutyryl-5′-*O*-monomethoxytrityl-2′-deoxyguanosine (11)[27,29]:
Compound **7** (7.42 g, 22 mmol) is coevaporated with absolute pyridine (2 × 25 ml) and then dissolved in the same solvent (80 ml). Monomethoxytrityl chloride (12.3 g, 40 mmol) is added and stirred for 6 h at room temperature. After addition of methanol (30 ml), the mixture is evaporated to dryness, the residue is extracted with chloroform (400 ml) and then washed with water (250 ml). The organic layer is separated, dried with sodium sulfate and concentrated to a small volume, which is put on a silica gel column for chromatography. Separation and elution is achieved first by chloroform/methanol (99/1; 500 ml), then (98/2; 500 ml), and finally (90/10), which elutes the product **11**. The main fraction is evaporated to dryness and reprecipitated by dissolving in chloroform (20 ml) and slow dropwise addition to a stirred mixture of *n*-hexane/ether (1/1, 400 ml) to give a colourless amorphous powder; yield: 11.4 g (85%).

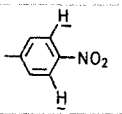
$C_{34}H_{35}N_5O_5$	calc.	C 66.98	H 5.78	N 11.48
(609.7)	found	67.28	5.80	11.22

Table 1. U.V. and ^{1}H-N.M.R. Data of 5′-Mono- and 5′-Dimethoxytrityl-2′-deoxynucleosides **8–15**

Product	U.V. (CH_3OH) λ_{max} [nm] (log ε)	^{1}H-N.M.R. ($CDCl_3$)[a] δ [ppm]		
		H_3CO	H-1′	
8	231 (4.15)[b]; 266 (4.00)	3.78 (s)	6.43 (pt, J = 7.3 Hz)[c]	6.84 (d, J = 8.85 Hz)
9	231 (4.41); 258 (4.36); 304 (4.04)	3.79 (s)	6.30 (pt, J = 5.8 Hz)[c]	6.86 (d, J = 9.20 Hz)
10	233 (4.42); 281 (4.30)	3.76 (s)	6.47 (pt, J = 6.4 Hz)[c]	6.79 (d, J = 8.85 Hz)
11	235 (4.26); 254 (4.24); 259 (4.24); 275 (4.11); 281 (4.11)	3.68 (s)	6.16 (pt, J = 6.4 Hz)[c]	6.70 (d, J = 8.90 Hz)
12	234 (4.36)[b]; 267 (4.06)	3.76 (s)	6.43 (pt, J = 6.1 Hz)[c]	6.84 (d, J = 8.90 Hz)
13	235 (4.53); 258 (4.40); 304 (4.05)	3.78 (s)	6.30 (pt, J = 5.5 Hz)[c]	6.85 (d, J = 7.7 Hz)
14	233 (4.50); 278 (4.32)	3.75 (s)	6.47 (pt, J = 6.4 Hz)[c]	6.78 (d, J = 8.80 Hz)
15	236 (4.36); 252 (4.26); 260 (4.24); 273 (4.12); 280 (4.11)	3.68 (s)	6.17 (pt, J = 5.8 Hz)[c]	6.70 (d, J = 8.55 Hz)

[a] Only selected values given.
[b] Shoulder.
[c] pt = pseudotriplet.

Table 2. U.V. and ^{1}H-N.M.R. Data of 2′-Deoxynucleoside-3′ 2,5-Dichlorophenyl 2-(4-Nitrophenyl)-ethyl Phosphates **16–23**

Product	U.V. (CH_3OH) λ_{max} [nm] (log ε)	^{1}H-N.M.R. ($CDCl_3$)[a] δ [ppm]				
		—O—CH_2—CH_2—	OCH_3	—O—CH_2—CH_2—	H-1′	
16	262 (4.29)	3.04 (t)	3.71 (s)	4.43 (t)	6.44 (pt)[b]	8.07 (d)
17	260 (4.54); 305 (4.12)	3.12 (t)	3.80 (s)	4.46 (t)	6.38 (pt)[b]	8.14 (d)
18	229 (4.54); 277 (4.44)	3.10 (t)	3.77 (s)	4.48 (t)	6.50 (pt)[b]	8.10 (d)
19	228 (4.48)[c]; 253 (4.42)[c]; 260 (4.46); 273 (4.39)[c]; 280 (4.35)[c]	3.11 (t)	3.78 (s)	4.45 (t)	6.17 (pt)[b]	8.11 (d)
20	229 (4.46)[c]; 266 (4.31)	3.10 (m)	3.78 (s)	4.44 (m)	6.47 (q)	8.11 (d)
21	229 (4.60)[c]; 238 (4.55)[c]; 261 (4.54); 300 (4.12)[c]	3.10 (t)	3.78 (s)	4.45 (t)	6.31 (q)	8.10 (d)
22	225 (4.64); 229 (4.64); 277 (4.50)	3.16 (m)[d]	3.76 (s)	4.42 (m)[d]	6.48 (q)	8.10 (d)
23	225 (4.58); 230 (4.58); 237 (4.47)[c]; 255 (4.41)[c]; 261 (4.42); 273 (4.38); 280 (4.35)[c]	3.14 (m)[d]	3.76 (s)	4.44 (m)[d]	6.10 (pt)[b]	8.11 (d)

[a] Only selected values given.
[b] pt = pseudotriplet.
[c] Shoulder.
[d] Overlapping with other resonances.

N^4-Benzoyl-5′-*O*-dimethoxytrityl-2′-deoxycytidine (13)[26]:
N^4-Benzoyl-2′-deoxycytidine (**5**; 4.96 g, 15 mmol) is dissolved in absolute pyridine (60 ml), 4,4′-dimethoxytrityl chloride (6.78 g, 20 mmol) is added and then the mixture is stirred for 1 h at room temperature. The reaction is stopped by addition of methanol (30 ml) and then extracted with chloroform (3 × 200 ml) and 0.1 molar phosphate buffer (pH 7; 3 × 200 ml). The organic layer is dried with sodium sulfate and evaporated to dryness followed by several coevaporations with benzene. The residue is purified by short column chromatography (∅ 10 cm) on silica gel (100 g) successively with dichloromethane (1.5 l), dichloromethane/methanol (99/1, 2 l), and dichloromethane/methanol (95/5, 2 l). Evaporation of the last fraction and reprecipitation of the residue from a small amount of chloroform into *n*-hexane/diethyl ether (9/1) gives **13** as a colourless amorphous solid; yield: 8.17 g (86%).

$C_{37}H_{35}N_3O_7 \cdot 0.5\,H_2O$	calc.	C 69.14	H 5.64	N 6.53
(642.7)	found	69.28	5.60	6.59

N^2-Isobutyryl-5′-*O*-dimethoxytrityl-2′-deoxyguanosine (15):
Compound **7** (5.06 g, 15 mmol) is evaporated with absolute pyridine (2 × 25 ml) and then dissolved in the same solvent (80 ml). Dimethoxytrityl chloride (6.8 g, 20 mmol) is added and the mixture is stirred at room temperature for 2 h. Subsequently methanol (50 ml), chloroform (300 ml), and 0.5 molar phosphate buffer pH 7 (300 ml) are added and, after shaking, the organic layer is separated. Extraction is repeated with chloroform and phosphate buffer. The organic layers are combined, dried with sodium sulfate, evaporated in vacuum, and coevaporated with benzene (3 × 100 ml) to form a solid foam. Purification is achieved by column chromatography (20 × 4 cm) on silica gel with successive elution by chloroform/triethylamine (99/1; 2 l) and chloroform/triethylamine/methanol (98/1/1; 3 l). The main fraction is collected, evaporated, and the residue reprecipitated from a small amount of chloroform into *n*-hexane (1.5 l) with stirring to give **15** as a colourless amorphous solid; yield: 8.1 g (85%).

$C_{35}H_{37}N_5O_7$	calc.	C 65.71	H 5.83	N 10.95
(636.7)	found	65.66	5.82	10.84

2′-Deoxynucleoside-3′ 2,5-Dichlorophenyl 2-(4-Nitrophenyl)-ethyl Phosphates 16–23; General Procedure:
2,5-Dichlorophenyl phosphorodichloridate (4.2 g, 15 mmol) is added to a solution of freshly sublimed 1,2,4-triazole (2.28 g, 33 mmol) in absolute pyridine (8 ml) with ice-cooling and stirred for 45 min. Then a solution of the protected 2′-deoxynucleoside **8–15** (10 mmol) in absolute pyridine (15 ml) is added dropwise within 60

min with stirring. After 15–30 min all starting material has disappeared (T.L.C. monitoring), and 2-(4-nitrophenyl)-ethanol (3.2 g, 19 mmol) is added. The mixture is stirred for 6 h at room temperature, then concentrated to one third of its volume, diluted with chloroform (300 ml), washed with 0.5 molar phosphate buffer pH 7 (200 ml), and water (200 ml). The organic layer is separated, dried with sodium sulfate and then evaporated to dryness followed by coevaporation with benzene (2 × 100 ml) to remove traces of pyridine. The residue is dissolved in a small amount of chloroform and put on two silica gel columns (40 × 2.5 cm) for chromatography. The colums are eluted first with chloroform (400 ml) followed by mixtures of chloroform/methanol or dichloromethane/methanol/triethylamine. The main fraction of the elution is collected, evaporated, and the residue reprecipitated from a small amount of chloroform into a stirred mixture of *n*-hexane/ether (1/1, 400 ml) or (9/1, 1000 ml).

5'-O-Monomethoxytritylthymidine-3' 2,5-Dichlorophenyl 2-(4-Nitrophenyl)-ethyl Phosphate **(16)**[21]:
Chromatography in chloroform/methanol (98/2) gives a colourless amorphous powder; yield: 7.73 g (87%); which is recrystallized from ethanol/*n*-hexane (1/1, 700 ml) to afford colourless crystals; yield: 7.42 g (84%); m.p. 105–108°C.

$C_{44}H_{40}Cl_2N_3O_{11}P$	calc.	C 59.47	H 4.54	N 4.73
(888.7)	found	59.51	4.58	4.90

N^4-*Benzoyl-5'-O-monomethoxytrityl-2'-deoxycytidine-3' 2,5-Dichlorophenyl 2-(4-Nitrophenyl)-ethyl Phosphate* **(17)**:
Chromatography in chloroform/methanol (99/1) gives a colourless amorphous solid; yield: 8.05 g (82%); which can be recrystallized from ethanol/*n*-hexane to form crystals; m.p. 123–126°C.

$C_{50}H_{43}Cl_2N_4O_{11}P$	calc.	C 61.42	H 4.43	N 5.73
(977.8)	found	61.43	4.41	5.44

N^6-*Benzoyl-5'-O-monomethoxytrityl-2'-deoxyadenosine-3' 2,4-Dichlorophenyl 2-(4-Nitrophenyl)-ethyl Phosphate* **(18)**:
Chromatography is performed with chloroform/methanol (99/1) and gives, on recrystallization of the main fraction from ethanol/*n*-hexane (2/1), colourless crystals; yield: 8.27 g (83%); m.p. 92–95°C.

$C_{51}H_{43}Cl_2N_6O_{10}P$	calc.	C 61.14	H 4.32	N 8.38
(1001.8)	found	60.84	4.09	8.24

N^2-*Isobutyryl-5'-O-monomethoxytrityl-2'-deoxyguanosine-3' 2,5-Dichlorophenyl 2-(4-Nitrophenyl)-ethyl Phosphate* **(19)**:
The chromatographic separation is performed first with chloroform/methanol (99/1, 400 ml) followed by a mixture (98/2) of the same solvents to elute the main fraction. Recrystallization from ethanol/*n*-hexane (2/1) gives colourless crystals; yield: 7.68 g (80%); m.p. 105–108°C.

$C_{48}H_{45}Cl_2N_6O_{11}P$	calc.	C 58.60	H 4.61	N 8.54
(983.8)	found	58.68	4.61	8.59

5'-O-Dimethoxytritylthymidine-3' 2,5-Dichlorophenyl 2-(4-Nitrophenyl)-ethyl Phosphate **(20)**:
Column chromatography is performed first with dichloromethane/triethylamine (99.5/0.5, 3 l) to remove excess of *p*-nitrophenylethanol. The product is then eluted by dichloromethane/methanol/triethylamine (98.5/1/0.5), the main fraction is evaporated, and the residue reprecipitated from a small amount of chloroform into a stirred mixture of *n*-hexane/ether (9/1, 1 l) to give a colourless powder; yield: 7.34 g (80%).

$C_{45}H_{42}Cl_2N_3O_{12}P$	calc.	C 58.83	H 4.61	N 4.75
(918.7)	found	59.06	4.70	4.55

N^4-*Benzoyl-5'-O-dimethoxytrityl-2'-deoxycytidine-3' 2,5-Dichlorophenyl 2-(4-Nitrophenyl)-ethyl Phosphate* **(21)**:
Silica gel column chromatography is achieved analogously to the preceeding procedure and work-up gives a colourless amorphous powder; yield: 8.8 g (88%).

$C_{51}H_{45}Cl_2N_4O_{12}P$	calc.	C 60.78	H 4.50	N 5.56
(1007.8)	found	60.76	4.53	5.67

N^6-*Benzoyl-5'-O-dimethoxytrityl-2'-deoxyadenosine-3' 2,5-Dichlorophenyl 2-(4-Nitrophenyl)-ethyl Phosphate* **(22)**:
Column chromatography and work-up of the mixture is performed first with dichloromethane/triethylamine (99.5/0.4) and then with dichloromethane/methanol/triethylamine (97.5/2/0.5) to give, on reprecipitation of the main fraction, a colourless solid; yield: 8.98 g (87%).

$C_{52}H_{45}Cl_2N_6O_{11}P$	calc.	C 60.53	H 4.39	N 8.14
(1031.9)	found	60.41	4.56	8.05

N^2-*Isobutyryl-5'-O-dimethoxytrityl-2'-deoxyguanosine-3' 2,5-Dichlorophenyl 2-(4-Nitrophenyl)-ethyl Phosphate* **(23)**:
Column chromatography on elution first with dichloromethane/triethylamine (99.5/0.5) and then with mixtures of dichloromethane/methanol/triethylamine (97.5/2/0.5 and 96.5/3/0.5) affords, after reprecipitation of the main fraction, a colourless amorphous powder; yield: 7.3 g (72%).

$C_{49}H_{47}Cl_2N_6O_{12}P$	calc.	C 58.05	H 4.67	N 8.29
(1013.8)	found	57.83	4.52	8.28

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[1] Nucleotides XXII: F. Himmelsbach, B.S. Schulz, T. Trichtinger, R. Charubala, W. Pfleiderer, *Tetrahedron* **40**, 59 (1984).
[2] H. Kössel, H. Seliger, *Fortschr. Chem. Org. Naturstoffe* **32**, 297 (1975).
[3] V. Amarnath, A.D. Broom, *Chem. Rev.* **77**, 183 (1977).
[4] M. Ikehara, E. Ohtsuka, A.F. Markham, *Adv. Carbohydr. Chem. Biochem.* **36**, 135 (1979).
[5] E. Ohtsuka, M. Ikehara, D. Söll, *Nucleic Acids Res.* **10**, 6553 (1982).
[6] S.A. Narang, *Tetrahedron* **39**, 3 (1983).
[7] C.B. Reese, *Phosphorus Sulfur* **1**, 245 (1976).
[8] C.B. Reese, *Tetrahedron* **34**, 3143 (1978).
[9] J.H. van Boom, *Heterocycles* **7**, 1197 (1977).
[10] P.T. Gilham, H.G. Khorana, *J. Am. Chem. Soc.* **80**, 6212 (1958).
[11] G.M. Tener, *J. Am. Chem. Soc.* **83**, 159 (1961).
[12] R.L. Letsinger, K.K. Ogilvie, *J. Am. Chem. Soc.* **89**, 4801 (1967).
[13] R.L. Letsinger, K.K. Ogilvie, *J. Am. Chem. Soc.* **91**, 3350 (1968).
[14] R.L. Letsinger, K.K. Ogilvie, P.S. Miller, *J. Am. Chem. Soc.* **91**, 3360 (1969).
[15] F. Eckstein, I. Rizk, *Chem. Ber.* **102**, 2362 (1967).
[16] K. Itakura, N. Katagiri, C.P. Bahl, R.H. Wightman, S. Narang, *J. Am. Chem. Soc.* **97**, 7327 (1975).
[17] J.H. van Boom, P.M.J. Burgers, R. Crea, G. van der Marel, G. Wille, *Nucleic Acids Res.* **4**, 747 (1977).
[18] C.B. Reese, K. Saffhill, *J. Chem. Soc. Chem. Commun.* **1968**, 767.
[19] J.H. van Boom, P.M.J. Burgers, P.H. van Deursen, R. Arentzen, C.B. Reese, *Tetrahedron Lett.* **1974**, 3785.
[20] K. Itakura, N. Katagiri, S.A. Narang, *Can. J. Chem.* **52**, 3689 (1974).
[21] E. Uhlmann, W. Pfleiderer, *Helv. Chim. Acta* **64**, 1688 (1981).
[22] G. Silber, D. Flockerzi, R.S. Varma, R. Charubala, E. Uhlmann, W. Pfleiderer, *Helv. Chim. Acta* **64**, 1704 (1981).
[23] D. Flockerzi, E. Uhlmann, W. Pfleiderer, *Helv. Chim. Acta* **66**, 2018 (1983).
[24] F. Himmelsbach, B.S. Schulz, T. Trichtinger, R. Charubala, W. Pfleiderer, *Tetrahedron* **40**, 59 (1984).
[25] D.H. Rammler, H.G. Khorana, *J. Am. Chem. Soc.* **84**, 3112 (1962).
[26] H. Schaller, G. Weimann, B. Lerch, H.G. Khorana, *J. Am. Chem. Soc.* **85**, 3821 (1963).
[27] H. Büchi, H.G. Khorana, *J. Mol. Biol.* **72**, 251 (1972).
[28] J. Stawinski, T. Hozumi, S.A. Narang, C.P. Bahl, R. Wu, *Nucleic Acids Res.* **4**, 353 (1977).
[29] P. Bühlmayer, G. Graf, F. Waldmeier, C. Tamm, *Helv. Chim. Acta* **63**, 2469 (1980).
[30] B.L. Gaffney, L.A. Marky, R.A. Jones, *Tetrahedron* **40**, 3 (1984)