Synthesis and Properties of Nonpolar DNA (Arylalkyl)phosphonates

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Dedicated to Prof. Dr. *Wolfgang Pfleiderer* on the occasion of his 75th birthday

The eight (arylalkyl)-modified phosphoramidites (=(arylalkyl)phosphonamidites) **1**–**8** (*Fig. 2*) were synthesized (*Schemes 1–3*) and incorporated at different positions into 2′-deoxyoligonucleotides. The [*P*(*R*)]- and [*P*(*S*)]-diastereoisomers of the hexanucleotides **32**–**39** (*Table 1*) and of the dodecanucleotides **41**–**45** (*Table 2*) obtained were separated by means of reversed-phase HPLC. UV, CD, and fluorescence spectroscopy were used to investigate the thermal stability (T_m) and the structural changes of their DNA duplexes with 5′-d(CGCGCG)-3′ and 5′-d(ATGATTGACCTG)-3′, respectively. The T_m values significantly depend on the place of modification (*Table 2*). A dangling-end effect is observed when the [3-(anthracen-9-yl)propyl]-modified **8** is attached at the 5′-terminus (see duplex with **45c**). In the case of the incorporation of aromatic moieties tethered *via* a methylene linker to the P-atom (benzyl- and (naphthalen-1-ylmethyl)-modified **1** and **6**, resp.), the duplexes with the [*P*(*R*)]-oligonucleotides are more stable than those with the [*P*(*S*)]-isomers, whereas in the case of longer alkyl chains at the P-atom (see **2**–**5**), the T_m values show the reverse tendency. The observed T_m differences are assigned to changes in base stacking (*Figs. 6* and *7*).

1. Introduction. – Backbone-modified oligonucleotides have been well-studied as antisense oligonucleotides [1]. The most common strategy is to replace or block the negatively charged O-atom to give phosphorothioates, phosphorotriesters, or methylphosphonates [2]. To yield appropriate intracellular concentrations of these antisense oligonucleotides, the lipophilic cell membrane must be easily passed by the oligonucleotides. The maximum intracellular oligonucleotide concentration is *ca.* 10% of the extracellular concentration [3]. Therefore, cationic lipids like lipofectin can be used as uptake enhancers to increase intracellular oligonucleotide concentrations [4].

Recently synthesized nonpolar benzyl-modified oligonucleotides have shown better *in vitro* and *in vivo* inhibitory activity against hepatitis-C virus gene expression than phosphorothioates or methylphosphonates [5][6]. Therefore, it might be possible that the more lipophilic modifications show the same or even better inhibitory activity and could be taken up by the cells more effectively. For this reason, we decided to synthesize new modifications with enhanced lipophilicity.

The substitution of one of the nonbinding O-atoms at the P-atom by an arylalkyl group creates [*P*(*R*)]- and [*P*(*S*)]-configured diastereoisomers (*Fig. 1*). In the case of methylphosphonates [7] as well as for benzylphosphonates [8], different properties for the diastereoisomers were observed. Benzyl-modified oligonucleotides show higher thermal stability (T_m) and enhanced stability against nucleophilic attack as compared to their methyl analogues. In the case of methylphosphonates, the [*P*(*R*)]-isomer

exhibits higher T_m values than the [*P*(*S*)]-isomer, whereas benzylphosphonates show the opposite feature [8].

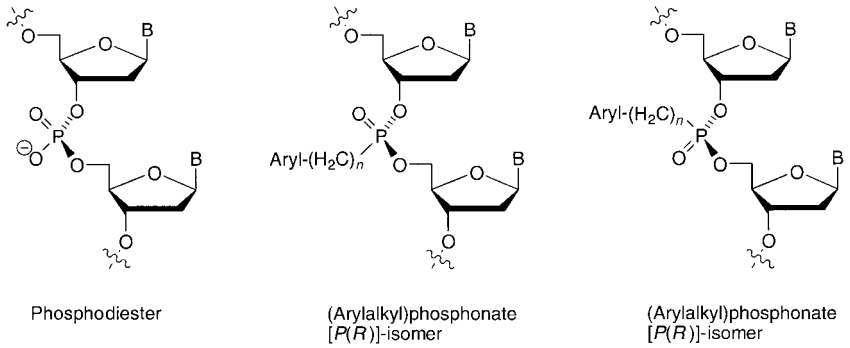
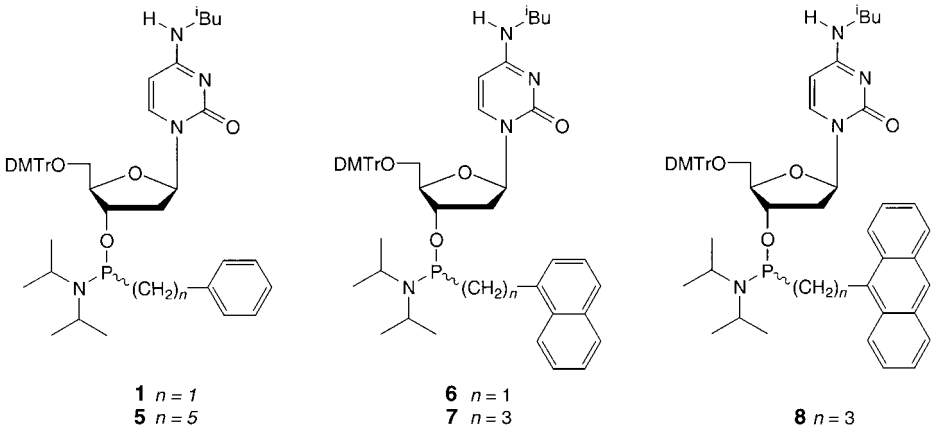


Fig. 1. *[P(S)]- and [P(R)]-Configuration of arylalkyl-backbone-modified oligonucleotides*

Here we describe the synthesis of (arylalkyl)-modified building blocks **1**–**8** (*Fig. 2*) which allow the incorporation into oligodeoxyribonucleotides *via* solid-phase synthesis at every strand position. Further, we investigated the properties of the modified, diastereoisomerically pure oligonucleotides in view of their lipophilicity, thermal stability, and structural changes.

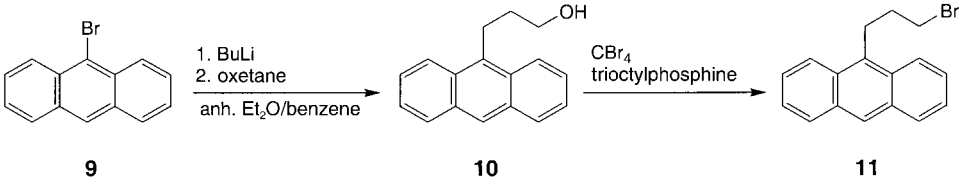


DMTr = 4,4′-dimethoxytrityl, ^{i}Bu = isobutyryl

Fig. 2. *Monomer building blocks* **1**–**8** *for the incorporation into DNA oligonucleotides*

Results and Discussion. – 1. *Phosphonamidite Syntheses.* To incorporate the arylalkyl backbone modifications into 2′-deoxyoligonucleotides, we used the phosphoramidite solid-phase synthesis. Therefore, we synthesized the eight different phosphonamidites (= modified phosphoramidites) **1**–**8** (*Fig. 2*) using modified phosphitylating agents (*Scheme 2*). The benzyl-modified phosphoramidite **1** was prepared as described in [8][9].

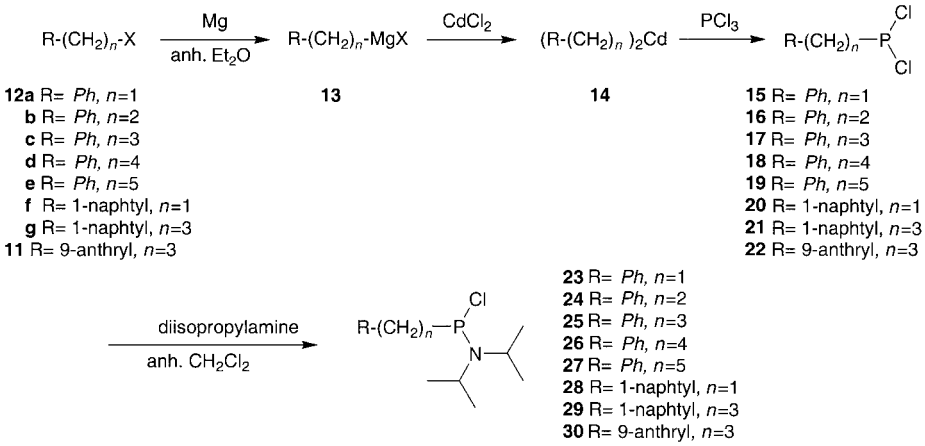
Scheme 1. *Synthesis of 9-(3-Bromopropyl)anthracene* (**11**)



Starting compounds were the corresponding arylalkyl bromides or chlorides, most of which were commercially available. The 9-(3-bromopropyl)anthracene (**11**) was synthesized according to *Searles* [10] (*Scheme 1*) from 9-bromoanthracene (**9**), which was treated with BuLi at $-78°$ and then alkylated *in situ* with oxetane to give **10** in 32% yield. Treatment of alcohol **10** with CBr_4 and trioctylphosphine gave **11** in 86% yield. The phenylbutyl bromide (**12d**), 5-phenylpentyl bromide (**12e**), and 1-(3-bromopropyl)naphthalene (**12g**) were prepared as described in [10][11].

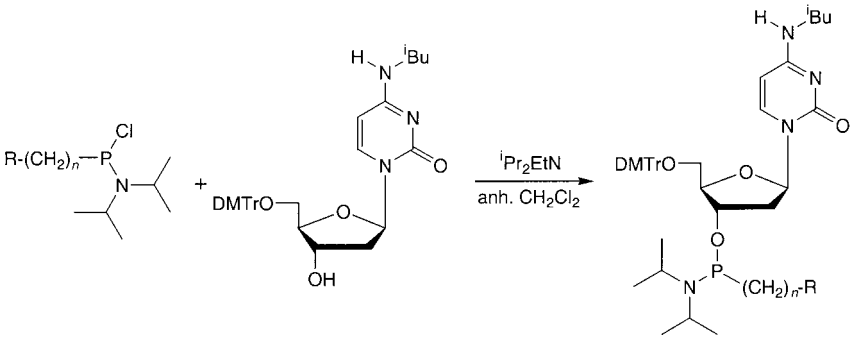
The arylalkylhalides **11** and **12a–g** were converted with Mg to *Grignard* reagents, and subsequent transmetallation with cadmium chloride reduced the reactivity of the metallorganic compounds (*Scheme 2*). The (arylalkyl)phosphonous dichlorides were obtained by the reaction of the organocadmium compounds **14** with phosphorous trichloride at $-78°$ under Ar. The phosphonous dichlorides **15–20** were purified by distillation and obtained in 54–64% yield, except for the highly viscous oily **19** (39% yield). Unfortunately, workup of **21** and **22** by distillation, chromatography, or crystallization was not possible. Therefore, yields were calculated by NMR spectroscopy. The modified phosphitylating agents **23–30** were obtained by reaction of the (arylalkyl)phosphonous dichlorides **15–22** with 2 equiv. diisopropylamine at $-35°$. The *N,N*-diisopropyl-*P*-(phenylalkyl)phosphonamidous chlorides **23–27** were purified by distillation and obtained in 38–84% yield. In the case of the compounds **28–30**, workup was not possible. Therefore, these modified phosphitylating agents were used for further reactions without purification.

Scheme 2. *Synthesis of* N,N-*Diisopropyl*-P-*(arylalkyl)phosphonamidous Chlorides* **23–30**



Treatment of the modified phosphitylating agents **23**–**30** with 5′-*O*-(4,4′-dimethoxytrityl)- and N^4-isobutyryl-protected 2′-deoxycytidine in the presence of $^iPr_2EtN$ at room temperature gave the corresponding modified phosporamidite building blocks **1**–**8** (*Scheme 3*). The (phenylalkyl)phosphonamidites **1**–**5** were isolated in excellent yields (73–89%) as white foams. The (naphthylalkyl)phosphonamidites **6** and **7** as well as the (anthrylpropyl)phosphonamidite **8** decomposed very easily during the workup; thus, they were purified immediately by flash chromatography on short columns, and the eluting mixture hexane/AcOEt was treated with 1% Et_3N. Under these optimized conditions, **6**–**8** were obtained in acceptable yields (31–58%).

Scheme 3. *Synthesis of Phosphonamidites* **1**–**8**



23 R= *Ph*, *n*=1
24 R= *Ph*, *n*=2
25 R= *Ph*, *n*=3
26 R= *Ph*, *n*=4
27 R= *Ph*, *n*=5
28 R= 1-naphtyl, *n*=1
29 R= 1-naphtyl, *n*=3
30 R= 9-anthryl, *n*=3

1 R= *Ph*, *n*=1
2 R= *Ph*, *n*=2
3 R= *Ph*, *n*=3
4 R= *Ph*, *n*=4
5 R= *Ph*, *n*=5
6 R= 1-naphtyl, *n*=1
7 R= 1-naphtyl, *n*=3
8 R= 9-anthryl, *n*=3

DMTr = 4,4′-dimethoxytrityl, ^{i}Bu = isobutyryl

2. *Oligodeoxynucleotides.* 2.1. *Synthesis and Separation of Diastereoisomers.* The oligodeoxynucleotides **31**–**45** were synthesized on an *Expedite* (*PerSeptive Biosystems*) synthesizer by phosphoramidite chemistry. The coupling times for modified amidites were enhanced to 300 s; therewith, the coupling yields were always higher than 96%, except for the (anthrylpropyl)phosphonamidite **8** (90%). The $(MeO)_2$Tr-on synthesized dodecanucleotides and hexanucleotides were deprotected (amino groups) and cleaved from the controlled-pore-glass (CPG) support with ethylenediamine (EDA) mix (= ethane-1,2-diamine/EtOH/MeCN/H_2O 50 : 24 : 24 : 2) for 3 h at room temperature. The crude oligodeoxynucleotides were purified by reversed-phase HPLC (*Poros*®-*R3* column, see *Exper. Part*) with an eluting gradient of 0–30% MeCN in 0.1M $(Et_3NH)OAc$ (pH 7.0) within 10 min (flow rate 5 ml/min). Afterwards, the 5-*O*-$(MeO)_2$ group was deprotected with 80% AcOH/H_2O within 30 min and the product precipitated with EtOH at $-20°$. Finally, purification and separation of the diastereoisomers was achieved by reversed-phase HPLC (*RP-18e*, see *Exper. Part*), the gradient of eluents (0.1M $(Et_3NH)OAc$ (pH 7.0)/MeCN) varying from 0–20% to 0–40% MeCN in 20–50 min (flow rate 1 ml/min) depending on the kind and place of the

modification. Details for separation of the single-stranded diastereoisomers are shown in *Tables 1* and *2*. *Fig. 3* shows a representative HPLC profile, that of the (anthrylpropyl)-modified dodecanucleotide **45a**. The inset represents the purity control by HPLC of the diastereoisomerically pure [*P*(*S*)]-configured oligonucleotide. In all cases, the separation of the diastereoisomers was better than 98%. The fast-eluting isomer is, in analogy to methyl-modified oligonucleotides [12][13], [*P*(*R*)]-con-

Table 1. *Thermal Stability* (T_m) *of Diastereoisomerically Pure Monomodified DNA Duplexes: Oligonucleotide 5′-d(GC***X***GCG)-3′* (**X** = modified nucleotide derived from **1**–**8** (*Fig. 2*, *Scheme 3*)) *Paired with 5′-d(CGCGGC)-3′*[a])

X[b])	Oligonucleotide	[*P*(*S*)]-Isomer		[*P*(*R*)]-Isomer		Reversed-phase HPLC separation[c])
		T_m [°]	ΔT_m [°]	T_m [°]	ΔT_m [°]	
C	**31**	44.3	–	44.3	–	–
1	**32**	41.0	−3.3	45.5	+1.2	0–30% *B* in 30 min
2	**33**	39.8	−4.5	37.8	−6.5	0–30% *B* in 30 min
3	**34**	39.3	−5.0	37.1	−7.2	0–30% *B* in 30 min
4	**35**	38.9	−5.4	36.6	−7.7	0–30% *B* in 30 min
5	**36**	38.9	−5.4	36.2	−8.1	0–30% *B* in 40 min
6	**37**	40.8	−3.5	46.0	+1.7	0–25% *B* in 40 min
7	**38**	36.8	−7.5	34.4	−9.9	0–25% *B* in 50 min
8	**39**	31.7	−12.6	30.0	−14.3	0–27% *B* in 50 min

[a]) Buffer: 10 mM phosphate, 140 mM NaCl, pH 7.0, oligomer concentration 16 μM. [b]) **X** = C or derived from phosphonamidites **1**–**8**. [c]) Eluents: *A* 0.1M (Et_3NH)OAc (pH 7.0); *B* MeCN.

Table 2. *Thermal Stability* (T_m) *of Diastereoisomerically Pure Monomodified DNA Duplexes: Oligonucleotide 5′-d(***X***AGGT***X***AAT***X***AT)-3′* (**X** = modified nucleotide derived from **1**, **3**, or **6**–**8** (*Fig. 2*, *Scheme 3*); oligonucleotides **a**, 1 **X** at central position; oligonucleotides **b**, 1 **X** at the third-last position to the 3′-terminus; oligonucleotides **c**, 1 **X** at the 5′-terminus) *Paired with 5′-d(ATGATTGACCTG)-3′*[a])

X[b])	Oligonucleotide	[*P*(*S*)]-Isomer		[*P*(*R*)]-Isomer		Reversed-phase HPLC separation[c])
		T_m [°]	ΔT_m [°]	T_m [°]	ΔT_m [°]	
C	**40**	46.4	–	46.4	–	–
1	**41a**	43.8	−2.6	46.5	+0.1	5–22% *B* in 40 min
	41b	44.6	−1.8	46.2	−0.2	5–18% *B* in 40 min
	41c	44.8	−1.6	44.6	−1.8	5–22% *B* in 40 min
6	**42a**	43.7	−2.7	46.9	+0.5	5–25% *B* in 40 min
	42b	45.7	−0.7	46.5	+0.1	5–17% *B* in 50 min
	42c	45.8	−0.6	45.7	−0.7	5–20% *B* in 40 min
3	**43a**	44.3	−2.1	43.4	−3.0	5–30% *B* in 40 min
	43b	45.6	−0.8	43.6	−2.8	5–17% *B* in 40 min
	43c	45.6	−0.8	43.6	−2.8	0–30% *B* in 30 min
7	**44a**	41.5	−4.9	39.5	−6.9	5–25% *B* in 40 min
	44b	43.2	−3.2	41.4	−5.0	7–20% *B* in 40 min
	44c	47.2	+0.8	45.2	−1.2	5–25% *B* in 40 min
8	**45a**	40.6	−5.6	39.5	−6.9	10–25% *B* in 45 min
	45b	44.1	−2.3	43.4	−3.0	10–18% *B* in 45 min
	45c	51.2	+4.8	47.3	+0.9	10–25% *B* in 45 min

[a]) [b]) [c]) See *Table 1*.

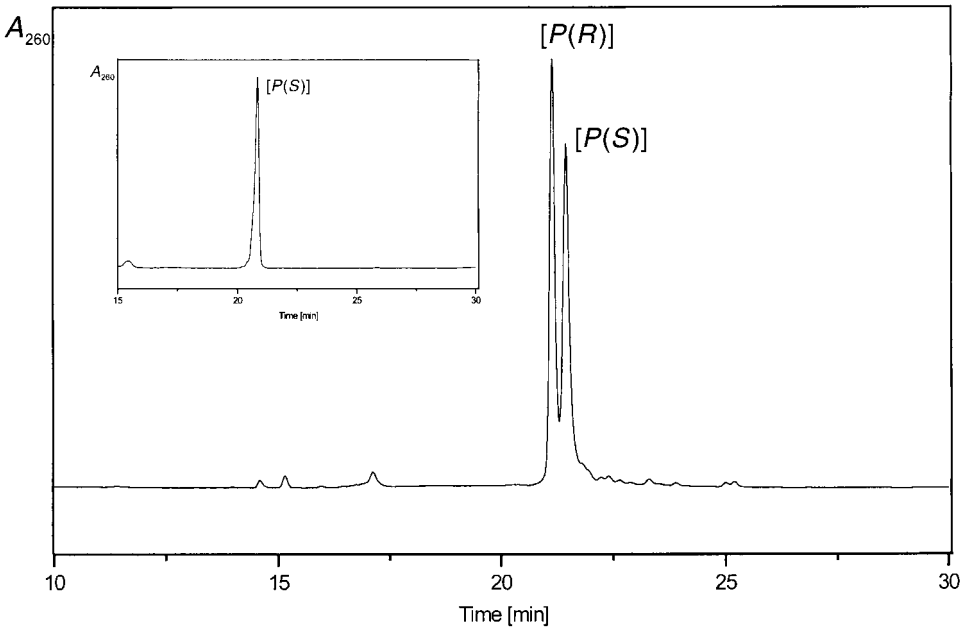


Fig. 3. *Reversed-phase HPLC profile of diastereoisomer separation of single-stranded 5′-d(CAGGT***8***AAT-CAT)-3′* (**45a**). For HPLC conditions, see text and *Table 1* and *2*, respectively. Insert: *HPLC purity control.*

figured, and the slow-eluting isomer is [*P*(*S*)]-configured. The pure oligonucleotides were subsequently desalted (*Sephadex-G25*) and characterized by MALDI-TOF mass spectra. The detected masses were in good agreement with the calculated values.

2.2. *Lipophilicity of Modified Oligonucleotides.* The lipophilic character of differently modified oligonucleotides was investigated with the [*P*(*S*)]-configured hexanucleotides 5′-d(GC**X**GCG)-3′ (**X** = modified nucleotide derived from **1**–**8**, see *Fig. 2*) by using reversed-phase HPLC (*RP-18e*, see *Exper. Part*). An MeCN gradient of 0–30% in 0.1M (Et_3NH)OAc buffer (pH 7.0) within 40 min was used to determine the elution times.

In addition, the influence on lipophilicity of the modified nonpolar (arylalkyl)-phosphonates in **32**–**39** as compared to the corresponding polar phosphorothioates in **46** (= **31** with a $-PO_3S^-$– link at C(3′) of **X**) and to the unmodified phosphodiester in **31** (**X** = C) was examined with the single-strand oligonucleotides (*Fig. 4*). As expected, the lipophilicity increased from a retention time of 19.92 min for benzyl-modified **32** to 28.61 min for (anthrylpropyl)-modified oligonucleotide **39** compared to the parent hexanucleotide **31** (15.57 min) and the phosphorothioate-containing **46** (17.37 min). The lipophilicity also increased with extension of the alkyl-chain length as reflected by higher retention times in constant steps of 1.90 ± 0.13 min per methylene group from benzyl-modified oligonucleotide **32** to 5-(phenylpentyl)-modified oligonucleotide **36**. Interestingly, extension of the aromatic ring system from the (phenylpropyl) to the (naphthylpropyl) modification (**34** *vs.* **38**) showed a difference in retention time of 3.31 min, whereas, from the (naphthylpropyl) to the (anthrylpropyl) modification (**38**

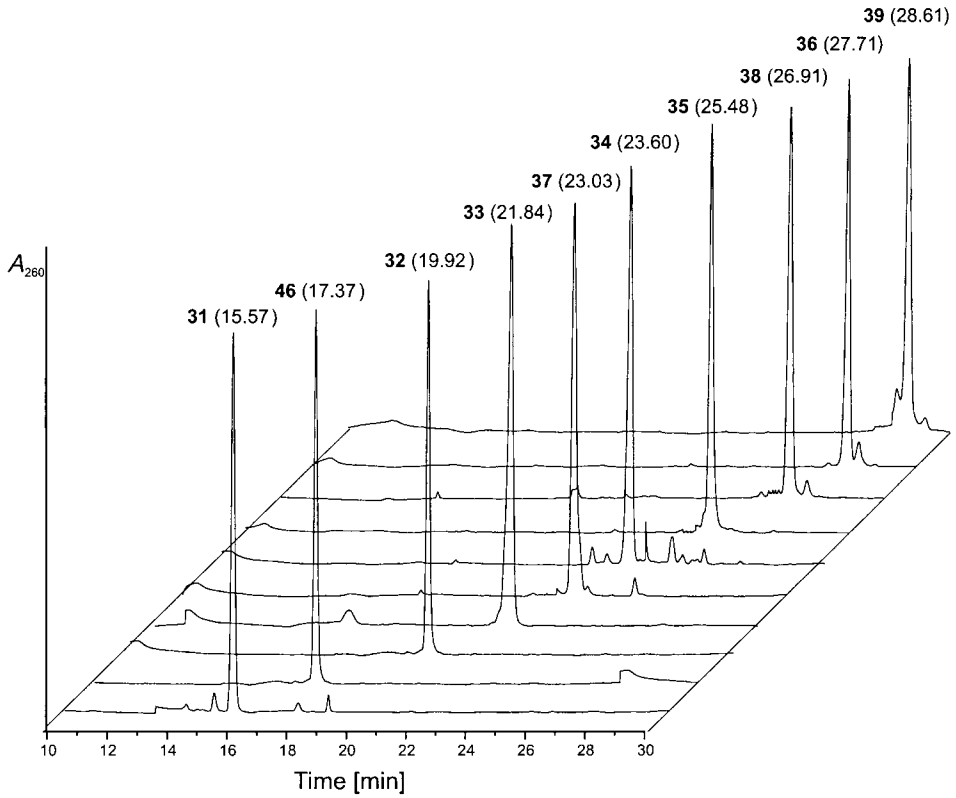


Fig. 4. *Reversed-phase HPLC profiles of the pure single-strand hexamers* **32**–**39** ([*P*(*S*)]-isomer), *the unmodified oligonucleotide* **31**, *and the thioate-modified oligonucleotide* **46**. Gradient: 0–30% *B*/*A* in 40 min at 1 ml/min was used. Buffer *A*, 0.1M (Et_3NH)OAc (pH 7.0); *B* MeCN. Retention times t_R in min.

vs. **39**), an increase of only 1.70 min was observed. On the other hand, the difference in retention time between the benzyl and the (phenylpropyl) modification (**32** *vs.* **34**) (3.68 min) was nearly identical to that between the (naphthylmethyl) and the (naphthylpropyl) modifications (**37** *vs.* **38**) (3.88 min). Summarizing, these data demonstrate the possibility to adjust the lipophilicity of oligonucleotides with different (arylalkyl) backbone modifications.

2.3. *Thermal Denaturation Study of Duplexes.* UV Melting profiles of DNA duplexes were recorded in a phosphate buffer (NaH_2PO_4, Na_2HPO_4; pH 7.0) containing 140 mM NaCl at an oligonucleotide concentration of 9 μM for each strand at wavelengths of 274 nm for CG hexanucleotides and 260 nm (concentration of 4 μM for each strand) for random dodecanucleotides. Each melting curve was determined twice within a temperature range of 10–75° and a heating rate of 0.5°/min.

First the modifications at the central position of a CG hexanucleotide, *i.e.*, of (5′-d(GC**X**GCG)-3′ (**X** = modified nucleotide derived from **1**–**8**, see *Fig. 2*) were tested. The diastereoisomerically pure [*P*(*R*)]- and [*P*(*S*)]-isomers of oligonucleotides **32**–**39**

were hybridized with the complementary target oligonucleotide 5′-d(CGCGCG)-3′. The melting temperatures obtained are shown in *Table 1*.

The benzyl-modified [*P*(*R*)]-**32** and (naphthylmethyl)-modified [*P*(*R*)]-**37** gave duplexes with T_m values that are 1.2° and 1.7° higher than those of the parent duplex **31**·[5′-d(CGCGCG)-3′], whereas the diastereoisomers [*P*(*S*)]-**32** and [*P*(*S*)]-**37** gave duplexes of decreased stability ($\Delta T_m = -3.3$ and −3.5°, resp.). With the change of the alkyl-chain length in the modification from benzyl to (phenylethyl), (phenylpropyl), (phenylbutyl), and (phenylpentyl) (see **32**–**36**, resp.), the T_m value of the duplexes decreased significantly ($\Delta T_m = -4.1$ to −8.1°) for both diastereoisomers compared to their unmodified analogue **31**·[5′-d(CGCGCG)-3′] [14]. In these cases, we observed that the [*P*(*S*)]-isomers are more stable than the [*P*(*R*)]-isomers. An explanation of these results are possible interactions like groove binding or other π-π contacts of the benzene ring ([*P*(*S*)]-isomer) to the DNA duplex. The duplexes with (naphthylpropyl)-modified **38** as well as with (anthrylpropyl)-modified **39** (incorporation at the central position of the hexanucleotide) exhibited a considerable reduction of the thermal stability for both the [*P*(*S*)]- and [*P*(*R*)]-isomers. Possible explanations for these findings are: *i*) the modified backbones are less solvated by H_2O molecules and *ii*) lower stacking effects are present due to a possible duplex deformation.

To investigate the thermal stability based on the location of the modification, we incorporated the building blocks **1**, **3**, and **6**–**8** at three different positions in a randomized dodecanucleotide (*Table 2*). Modifications were at the central position (oligonucleotides **a**), at the third-to-last position to the 3′-terminus (oligonucleotides **b**), or at the 5′-terminus (oligonucleotides **c**), *i.e.*, 5′-d(**X**AGGT**X**AAT**X**AT)-3′, each with one **X** = modified nucleotide derived from **1**, **3**, and **6**–**8** (see *Fig. 2*). *Table 2* summarizes the T_m values for the duplexes obtained with the [*P*(*R*)]- and [*P*(*S*)]-isomers of **41**–**45** containing the modifications derived from **1**, **3**, and **6**–**8** at different positions (oligonucleotides **a**–**c**). The results discussed above for the hexanucleotides **32**–**39** (*Table 1*) were confirmed by the T_m values of the duplexes obtained with the centrally modified [*P*(*R*)]- and [*P*(*S*)]-configured dodecanucleotides **41a**, **42a**, **43a**, **44a**, and **45a**. In general, the stabilization or destabilization of the duplexes obtained with **41a**–**45a** ([*P*(*R*)]- and [*P*(*S*)]-configured) were significantly lower than those of the corresponding hexanucleotides **32**, **34**, and **37**–**39**.

In the case of the third-to-last-3′-terminally and 5′-terminally benzyl- and (naphthylmethyl)-modified [*P*(*S*)]-isomers of dodecanucleotides **41b**,**c** and **42b**,**c**, a slight increase of duplex stabilization was observed compared to the centrally modified oligonucleotides **41a** and **42a**, respectively, whereas the [*P*(*R*)]-isomers of **41b**,**c** and **42b**,**c** showed the reverse tendency. The duplexes obtained with the 5′-terminally modified **41c** and **42c** exhibited nearly identical T_m values for both diastereoisomers.

These findings are in contrast to the results we obtained from the duplexes of (phenylpropyl)-modified **43**, (naphthylpropyl)-modified **44**, and (anthrylpropyl)-modified **45**. Generally, in these cases, duplexes obtained with [*P*(*S*)]-isomers showed higher T_m values than those with [*P*(*R*)]-isomers, and duplexes obtained with 5′-terminally modified oligonucleotides (**c** series) were more stable than those with third-last-3′-terminally ones (**b** series), and the latter exhibited higher T_m values than duplexes obtained with the corresponding centrally modified oligomers (**a** series). An explanation for these observations is the ‘dangling-end’ effect. Originally, dangling end

described a duplex stabilization by a single nucleotide residue at the 5′-terminus [15]. Further studies showed that nonpolar DNA-base mimics like naphthalene, phenanthrene, and pyrene stack considerably more strongly than their natural counterparts [16][17]. In our case, the 5′-terminally anthracene-modified [*P*(*S*)]-**45c** raised the T_m of the duplex by $+4.8°$. The effect of naphthalene-modified [*P*(*S*)]-**44c** was lower with ΔT_m only $+0.8°$. To investigate further the effects of size and lipophilicity of the backbone modifications, we measured CD and fluorescence spectra.

2.4. *Fluorescence Spectra*. Different fluorescence properties were obtained upon interaction of polycyclic aromatic chromophores with oligonucleotides. These properties are to some extent dependent on the nature of the nucleotides with which the chromophore interacts [18], and also in the manner in which the chromophore is tethered (*e.g.*, intercalation, groove binding) [19]. The oligonucleotide-tethered anthracene has been shown to give fluorescence-emission enhancement upon duplex formation [20] when the (anthracen-2-ylmethyl) moiety tethered to a uridine 2′-OH group was incorporated into the oligomer. In the case of oligonucleotides containing an (anthracen-9-ylmethyl) modification at the $NH_2-C(2)$ group of 2′-deoxyguanosine, a quenching of fluorescence was observed [21].

We examined the fluorescence properties of the dodecanucleotides **45a** and **45c** in the single-strand state and as double-strand DNA duplex with 5′-d(ATGATTGACCTG)-3′ (*Fig. 5*). The fluorescence measurements were carried out at 20° in

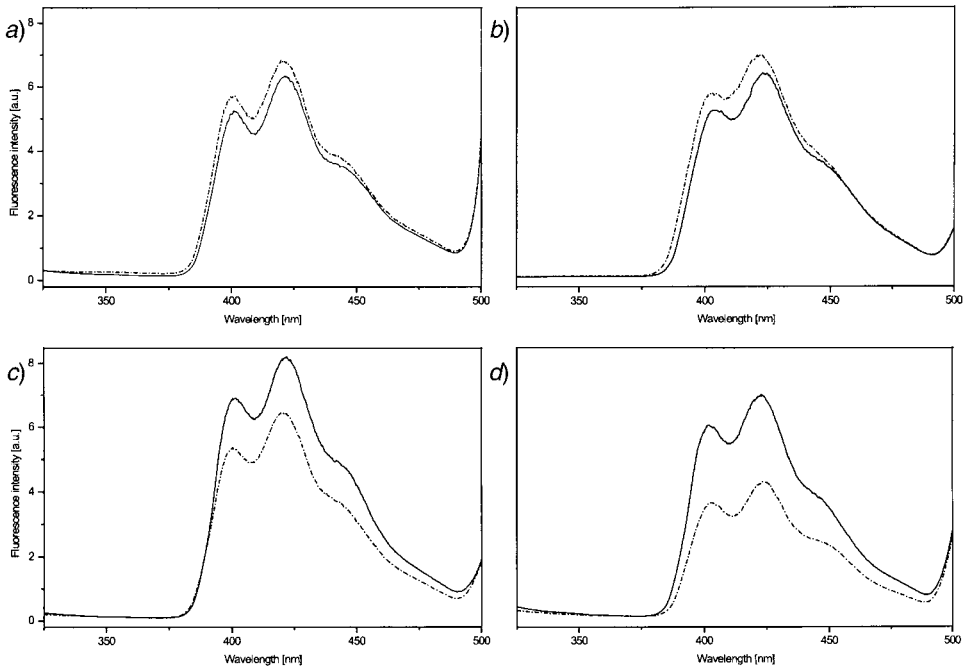


Fig. 5. *Fluorescence emission spectra* (λ_{ex} 255 nm) *of the (anthracen-9-ylpropyl)-modified dodecanucleotides* **45a** *and* **45c** *as single-strand* (—) *and as double-strand duplex* (–·–) *with 5′-d(ATGATTGACCTG)-3′* a) [P(R)]-**45a**, b) [P(S)]-**45a**, c) [P(R)]-**45c**, *and* d) [P(S)]-**45c** (see *Table 2*)

the phosphate buffer used to determine the T_m values at a single-strand concentration of 0.2 μM. The fluorescence-emission maxima of the (anthracen-9-ylpropyl)-modified single-strand oligomers [*P*(*S*)]-**45a**,**c** exhibited a red shift of 3 nm upon conversion to the duplex structure (*Fig. 5*,*b* and *d*), whereas the [*P*(*R*)]-analogues showed no red shift (*Fig. 5*,*a* and *c*). In the case of [*P*(*R*)]- and [*P*(*S*)]-**45c**, a decrease in fluorescence yield was observed (*Fig. 5*,*c* and *d*). The reduction of 21 and 37%, respectively, is far less than typically observed with intercalative binding, but it indicates the postulated dangling-end effect [18][22]. It is noteworthy that there is a direct correlation between higher T_m values (*Table 2*) and the obtained stronger fluorescence quenching.

2.5. *Circular-Dichroism Spectra*. CD Spectra were recorded at 350–210 nm with oligonucleotide concentrations of 4 μM of each strand in sodium phosphate buffer (pH 7.0) containing NaCl (140 mM). The temperature of the measurement was 10° to ensure that only duplex DNA was present. *Fig. 6* shows the CD spectra of 5′-d(ATGATTGACCTG)-3′ duplexes with the 5′-terminally modified [*P*(*S*)]- and [*P*(*R*)]-**43c**–**45c** compared with that of the corresponding duplex obtained with the unmodified analogue **40**. All 5′-terminally modified dodecanucleotides exhibited a typical curve for a B-type helix with a maximum at *ca.* 285 nm and a strong minimum at *ca.* 250 nm. In the cases of (phenylpropyl)-modified [*P*(*S*)]- and [*P*(*R*)]-**43c**, (naphthylmethyl)-modified [*P*(*S*)]- and [*P*(*R*)]-**44c**, and the (anthrylpropyl)-modified [*P*(*R*)]-**45c**, a significant shift to longer wavelengths at the positive ellipticity of *ca.* 6 nm was observed compared to the parent duplex obtained with **40** (281 nm). In contrast to this, the CD spectra of the duplex obtained with (anthrylpropyl)-modified [*P*(*S*)]-**45c** showed identical spectra to those of the duplex obtained with **40**. The

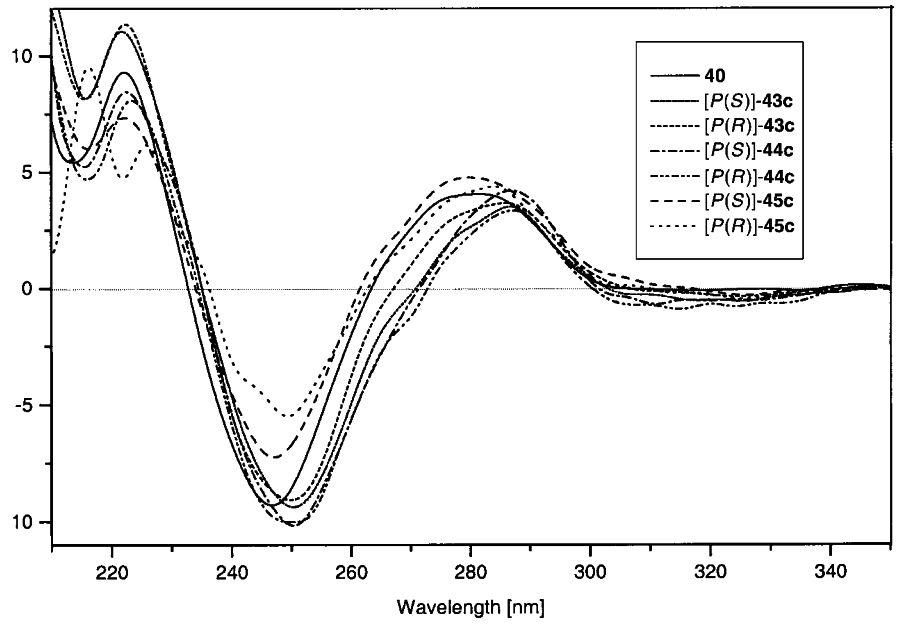


Fig. 6. *CD Spectra of duplexes obtained with 5′-terminally modified dodecadeoxynucleotides* **43c**–**45c**

intensity at *ca*. 285 nm corresponds to the number of paired and unpaired bases as well as to the extent of base-stacking interactions [23]. At this maximum, intensity differences were significant and correlated with the T_m values determined for these duplexes (*Table 2*).

Fig. 7 shows the CD spectra of the duplexes obtained with (naphthylmethyl)-modified dodecanucleotides **42a**–**c**. As described above (*Fig. 6*), for all spectra, a shift between 5 and 7 nm to longer wavelengths was observed. A striking observation was the significant ellipticity decrease of the positive band depending on the place of modification. The duplexes obtained with 5′-terminally modified [*P*(*S*)]- and [*P*(*R*)]-**42c** showed nearly identical ellipticities, whereas those with centrally modified diastereoisomers exhibited differences in the spectra. Thus, all the curves indicated that (arylalkyl)-modifications have significant influences on stacking effects in DNA duplexes, which is documented in a higher ellipticity at *ca*. 285 nm for more stable duplexes and a decreased ellipticity for duplexes with lower T_m values.

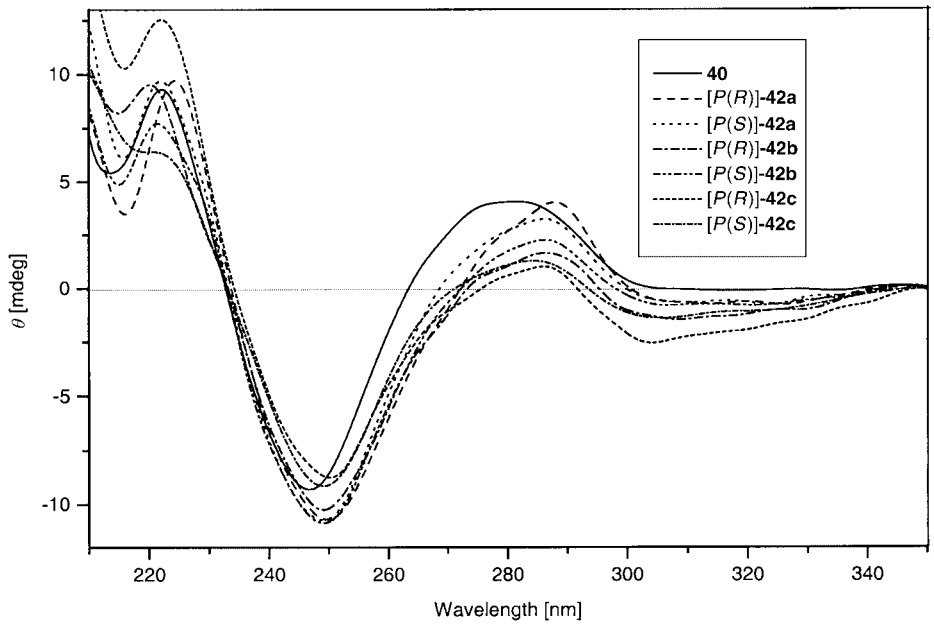


Fig. 7. *CD Spectra of duplexes obtained with (naphthylmethyl)-modified dodecadeoxynucleotides* **42a**–**c**

We thank Dr. *Thomas J. Lehmann* for supplying us with benzyl-modified phosporamidites. *S. A*. would also like to thank the *Fonds der Chemischen Industrie* and the *BMBF* for a Ph. D. fellowship and financial support.

Experimental Part

General. The 4-(bromobutyl)benzene (**12d**), (5-bromopentyl)benzene (**12e**), and 1-(3-bromopropyl)naphthalene (**12g**) were prepared according to published procedures [10][11]. The other (bromoalkyl)- and (chloroalkyl)arenes were obtained from *Aldrich*. Et_2O was distilled from sodium metal/benzophenone ketyl (=sodium oxidodiphenylmethyl), and the other anh. solvents, *e.g.*, CH_2Cl_2 and pyridine, were obtained from *Fluka* and used without further purification. Humidity-sensitive reactions were conducted in dried glassware

under a positive pressure of dry Ar. Dry MeCN ($H_2O < 30$ ppm) for the phosphitylation reaction was purchased from *PerSeptive Biosystems*. Oligonucleotide syntheses: *Expedite-8909* synthesizer from *PerSeptive Biosystems*. Flash column chromatography (FC): silica gel *60* (40–63 μm) from *Merck*. TLC: silica gel *60* F_{254} plates from *Merck*. HPLC: *Poros® Oligo™ R3* from *Applied Biosystems*, *RP-18e* column (5 μm) *Purospher®STAR* from *Merck*; desalting with a *Sephadex-G25* column from *Pharmacia*. UV/Melting profiles: *Varian Cary-1* UV/VIS spectrophotometer, *Cary* temp. controller, 10-mm cuvette. Fluorescence Spectra: *F-4500* fluorescence spectrophotometer from *Hitachi*. CD Spectra: *Jasco 710* spectropolarimeter. NMR: *Bruker AM-250* (^{1}H) and *Bruker AMX-400* (^{1}H,^{31}P) spectrometers; δ in ppm, J in Hz; for convenience, primed locants are given to the monosaccharide moiety and unprimed ones to the nucleobase moiety. MS: *PerSeptive-Biosystems* MALDI-TOF spectrometer *Voyager DE*; ESI = electrospray ionization.

9-(3-Hydroxypropyl)anthracene (**10**). To a soln. of 9-bromoanthracene (**9**; 8.0 g, 31 mmol) in anh. Et_2O (50 ml) under Ar at $-78°$ (acetone/liq. N_2), 1.5M BuLi in hexane (18.7 ml, 30 mmol) was added. Then the mixture was warmed within 1 h to 0°. Oxetane (2.36 ml, 35 mmol) was added, and the mixture was refluxed. After 2 h, benzene (70 ml) was added and refluxing continued for 4 h. After cooling to r.t., the mixture was hydrolyzed with sat. NH_4Cl soln. (100 ml), the aq. layer extracted with Et_2O, and the combined org. phase dried ($MgSO_4$) and evaporated. The residue was purified by FC (hexane/AcOEt 4:1): **10** (2.0 g, 32%). Yellow solid. TLC (hexane/AcOEt 2:1): R_f 0.46. ^{1}H-NMR (250 MHz, (D_6)DMSO): 8.45–8.34 (*m*, 3 arom. H); 8.05 (*m*, 2 arom. H); 7.58–7.47 (*m*, 4 arom. H); 4.71 (*t*, $J = 5.1$, OH); 3.68–3.59 (*m*, $CH_2CH_2CH_2O$); 1.87 (*m*, $CH_2CH_2CH_2O$).

9-(3-Bromopropyl)anthracene (**11**). A soln. of **10** (6.21 g, 26.3 mmol) and tetrabromomethane (20.63 g, 62.3 mmol) in anh. Et_2O (100 ml) was treated with trioctylphosphine (27.4 ml, 61.5 mmol) in Et_2O (40 ml) at 0°. After stirring for 20 h at r.t., the Et_2O was evaporated and the residue purified by FC (hexane/AcOEt 6:1): **11** (6.75 g, 86%). Yellow solid. TLC (hexane/AcOEt 4:1): R_f 0.55. ^{1}H-NMR (250 MHz, $CDCl_3$): 8.22 (*m*, 3 arom. H); 7.91 (*m*, 2 arom. H); 7.48–7.35 (*m*, 4 arom. H); 3.71 (*t*, $J = 6.8$, $ArCH_2$); 3.52 (*t*, $J = 6.4$, CH_2Br); 2.32 (*m*, CH_2CH_2Br).

(2-Phenylethyl)phosphonous Dichloride (**16**). The reaction and workups described herein were all performed under Ar. The (2-bromoethyl)benzene (**12b**; 46.4 g, 0.37 mol) and Mg turnings (8.98 g, 0.37 mol) in anh. Et_2O (350 ml) were refluxed for 1.5 h. The soln. was cooled in an ice bath and stirred vigorously whilst powdered anh. $CdCl_2$ (33.65 g, 0.185 mol) was added rapidly. After stirring for 2 h at 0°, the salt was removed by filtration under positive pressure of Ar. The filtrate was added during 30 min to a vigorously stirred soln. of PCl_3 (152.3 g, 1.11 mol) in anh. Et_2O (100 ml). After the addition was complete, the mixture was stirred at r.t. for 2.5 h. The white precipitate was filtered off under positive pressure of Ar. The filter cake was washed with Et_2O and the combined Et_2O soln. evaporated. Distillation gave **16** (47.2 g, 62%). Colorless liquid. B.p. 102°/10^{-2} mbar. ^{1}H-NMR (250 MHz, $CDCl_3$): 7.35–7.22 (*m*, 5 arom. H); 3.01 (*m*, $PhCH_2$); 2.64 (*m*, CH_2P). ^{31}P-NMR (250 MHz, $CDCl_3$): 193.12.

(3-Phenylpropyl)phosphonous Dichloride (**17**). As described above for **16**, with (3-bromopropyl)benzene (**12c**; 40 g, 263 mmol) Mg turnings (6.45 g, 265 mmol), anh. Et_2O (200 ml), $CdCl_2$ (24.0 g, 131 mmol), PCl_3 (56.9 ml, 650 mmol), and anh. Et_2O (70 ml). Distillation gave **17** (32.75 g, 54%). Colorless liquid. B.p. 120°/10^{-2} mbar. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.29–7.10 (*m*, 5 arom. H); 3.45 (*t*, $J = 6.5$, $PhCH_2$); 2.71 (*t*, $J = 7.5$, CH_2P); 2.02 (*m*, $CH_2CH_2CH_2P$). ^{31}P-NMR (400 MHz, $CDCl_3$): 195.23.

(4-Phenylbutyl)phosphonous Dichloride (**18**). As described above for **16**, with (4-bromobutyl)benzene (**12d**; 19.5 g, 91 mmol), Mg turnings (2.26 g, 93 mmol), anh. Et_2O (150 ml) (3 h under reflux), $CdCl_2$ (8.23 g, 44.9 mmol), PCl_3 (31 ml, 354 mmol), and anh. Et_2O (40 ml). Distillation gave **18** (13.6 g, 64%). Colorless liquid. B.p. 110°/10^{-3} mbar. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.35 (*m*, 2 arom. H); 7.24 (*m*, 3 arom. H); 2.72 (*t*, $J = 7.1$, $PhCH_2$); 2.37 (*m*, CH_2P); 1.84 (*m*, $CH_2CH_2CH_2P$). ^{31}P-NMR (400 MHz, $CDCl_3$): 195.86.

(5-Phenylpentyl)phosphonous Dichloride (**19**). As described above for **16**, with (5-bromopentyl)benzene (**12e**; 20.0 g, 88 mmol), Mg turnings (2.19 g, 90 mmol), anh. Et_2O (150 ml) (4 h under reflux), $CdCl_2$ (8.05 g, 44 mmol), PCl_3 (30.8 ml, 353 mmol), and anh. Et_2O (50 ml). Distillation gave **19** (8.9 g, 39%). Colorless liquid. B.p. 129°/10^{-2} mbar. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.31–7.15 (*m*, 5 arom. H); 3.23 (*t*, $J = 7.7$, $PhCH_2$); 2.48 (*t*, $J = 7.8$, CH_2P); 1.89–1.61 (*m*, $CH_2CH_2CH_2CH_2P$); 1.50 (*m*, $CH_2CH_2CH_2P$). ^{31}P-NMR (400 MHz, $CDCl_3$): 195.70.

(Naphthalen-1-ylmethyl)phosphonous Dichloride (**20**). As described above for **16**, with 1-(chloromethyl)-naphthalene (**12f**; 32.8 g, 186 mmol), Mg turnings (4.51 g, 186 mmol), anh. Et_2O (200 ml), $CdCl_2$ (17.0 g, 93 mmol), PCl_3 (64.9 ml, 742 mmol), and anh. Et_2O (100 ml). Distillation gave **20** (26.0 g, 57%). Colorless oil. B.p. 132°/10^{-5} mbar. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.08 (*m*, 1 arom. H); 7.91 (*m*, 1 arom. H); 7.69 (*m*, 1 arom. H); 7.63–7.44 (*m*, 4 arom. H); 3.50 (2*s*, CH_2P). ^{31}P-NMR (400 MHz, $CDCl_3$): 180.51.

[3-(Naphthalen-1-yl)propyl]phosphonous Dichloride (**21**). As described above for **16**, with 1-(3-bromopropyl)naphthalene (**12g**; 8.0 g, 32 mmol), Mg turnings (0.78 g, 32 mmol), anh. Et_2O (50 ml) (12 h under reflux), $CdCl_2$ (2.93 g, 16 mmol), PCl_3 (17.4 ml, 199 mmol), and anh. Et_2O (30 ml). Distillation of the yellow and highly viscous crude **21** was not possible. Yield determined by ^{1}H- and ^{31}P-NMR: 3.0 g (34%). ^{1}H-NMR (400 MHz, $CDCl_3$): 8.05 (*m*, 1 arom. H); 7.89 (*m*, 1 arom. H); 7.73 (*m*, 1 arom. H); 7.57–7.35 (*m*, 4 arom. H); 3.07 (*m*, ArCH_2); 2.20 (*m*, CH_2P); 1.78 (*m*, CH_2CH_2P). ^{31}P-NMR (400 MHz, $CDCl_3$): 195.46.

[3-(Anthracen-9-yl)propyl]phosphonous Dichloride (**22**). As described above for **16**, with 9-(3-bromopropyl)anthracene (**11**; 7.0 g, 22.4 mmol), Mg turnings (0.58 g, 24 mmol), anh. Et_2O (50 ml) (12 h under reflux), without $CdCl_2$, PCl_3 (16.9 ml, 192 mmol), and anh. Et_2O (30 ml). Distillation of the yellow and highly viscous crude **22** was not possible. Yield determined by ^{1}H- and ^{31}P-NMR: 4.06 g (54%). ^{1}H-NMR (400 MHz, $CDCl_3$): 8.33–8.21 (*m*, 3 arom. H); 8.01 (*m*, 2 arom. H); 7.61–7.43 (*m*, 4 arom. H); 3.64 (*m*, ArCH_2); 2.28 (*m*, CH_2P); 1.93 (*m*, 2 CH_2CH_2P). ^{31}P-NMR (400 MHz, $CDCl_3$): 194.78.

N,N-*Diisopropyl*-P-*(2-phenylethyl)phosphonamidous Chloride* (**24**). The reactions and workups described herein were all performed under Ar. To a vigorously stirred soln. of **16** (23.6 g, 114 mmol) in anh. CH_2Cl_2 (100 ml), ^{i}Pr$_2$NH (29.45 ml, 209 mmol) was added at $-35°$ within 30 min. The mixture was stirred for 2 h at r.t., and the precipitate was filtered off under positive pressure of Ar. The filter cake was washed with CH_2Cl_2 and the combined CH_2Cl_2 soln. evaporated. The crude product was purified by distillation: **24** (20.3 g, 66%). Colorless liquid. B.p. 124°/10^{-3} mbar. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.24–7.10 (*m*, 5 arom. H); 3.62 (*m*, Me$_2$C*H*); 2.71 (*m*, PhCH_2); 2.33–2.25 (*m*, CH_2P); 1.23–1.09 (*m*, *Me*$_2$CH). ^{31}P-NMR (400 MHz, $CDCl_3$): 139.37.

N,N-*Diisopropyl*-P-*(3-phenylpropyl)phosphonamidous Chloride* (**25**). As described above for **24**, with **17** (32.75 g, 148 mmol), ^{i}Pr$_2$NH (40.5 ml, 287 mmol), and anh. CH_2Cl_2 (150 ml). Distillation gave **25** (35.6 g, 84%). Colorless oil. B.p. 145°/10^{-3} mbar. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.27–7.09 (*m*, 5 arom. H); 3.40 (*m*, PhCH_2); 2.66 (*m*, Me$_2$C*H*); 2.00 (*m*, CH_2P); 1.81–1.64 (*m*, CH_2CH_2P); 1.19–1.04 (*m*, *Me*$_2$CH). ^{31}P-NMR (400 MHz, $CDCl_3$): 140.68.

N,N-*Diisopropyl*-P-*(4-phenylbutyl)phosphonamidous Chloride* (**26**). As described above for **24**, with **18** (6.74 g, 29 mmol), ^{i}Pr$_2$NH (7.0 ml, 50 mmol), and anh. CH_2Cl_2 (30 ml). Distillation gave **26** (6.62 g, 77%). Colorless oil. B.p. 140°/10^{-5} mbar. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.31 (*m*, 2 arom. H); 7.20 (*m*, 3 arom. H); 3.43 (*m*, Me$_2$C*H*); 2.78 (*t*, *J* = 7.5, PhCH_2); 2.15 (*m*, CH_2P); 1.73 (*m*, 4 CH_2CH_2CH_2P); 1.30–1.15 (*m*, *Me*$_2$CH). ^{31}P-NMR (400 MHz, $CDCl_3$): 141.90.

N,N-*Diisopropyl*-P-*(5-phenylpentyl)phosphonamidous Chloride* (**27**). As described above for **24**, with **19** (8.9 g, 36 mmol), ^{i}Pr$_2$NH (10.0 ml, 71 mmol), and anh. CH_2Cl_2 (40 ml). Distillation gave **27** (4.2 g, 38%). Colorless highly viscous oil. B.p. 155°/10^{-5} mbar. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.23–7.10 (*m*, 5 arom. H); 3.41 (*m*, PhCH_2); 2.55 (*m*, Me$_2$C*H*); 2.01 (*m*, CH_2P); 1.66 (*m*, CH_2CH$_2$CH_2CH$_2$P); 1.41 (*m*, 2 CH_2CH$_2$CH$_2$P); 1.30–1.08 (*m*, *Me*$_2$CH). ^{31}P-NMR (400 MHz, $CDCl_3$): 139.13.

N,N-*Diisopropyl*-P-*(naphthalen-1-ylmethyl)phosphonamidous Chloride* (**28**). As described above for **24**, with **20** (3.4 g, 14 mmol), ^{i}Pr$_2$NH (3.7 ml, 26 mmol), and anh. CH_2Cl_2 (15 ml). After evaporation of the solvent, a white solid was obtained. Yield of **28** determined by ^{1}H- and ^{31}P-NMR: 2.54 g (59%). ^{1}H-NMR (400 MHz, $CDCl_3$): 8.02 (*m*, 1 arom. H); 7.75 (*m*, 1 arom. H); 7.63 (*m*, 1 arom. H); 7.49–7.30 (*m*, 4 arom. H); 3.84 (*m*, CH_2P); 3.29 (*m*, Me$_2$C*H*); 1.05 (*m*, *Me*$_2$CH). ^{31}P-NMR (400 MHz, $CDCl_3$): 141.23.

N,N-*Diisopropyl*-P-*[3-(naphthalen-1-yl)propyl]phosphonamidous Chloride* (**29**). As described above for **24**, with **21** (3.0 g, 11 mmol), ^{i}Pr$_2$NH (3.0 ml, 21 mmol), and anh. CH_2Cl_2 (10 ml). After evaporation of the solvent, a white highly viscous oil was obtained. Yield of **29** determined by ^{1}H- and ^{31}P-NMR: 2.61 g (70%). ^{1}H-NMR (400 MHz, $CDCl_3$): 8.00 (*m*, 1 arom. H); 7.80 (*m*, 1 arom. H); 7.66 (*m*, 1 arom. H); 7.46–7.26 (*m*, 4 arom. H); 3.34 (*m*, Me$_2$C*H*); 3.04 (*m*, ArCH_2); 1.75 (*m*, CH_2P); 1.48 (*m*, CH_2CH$_2$P); 1.31 (*m*, *Me*$_2$CH). ^{31}P-NMR (400 MHz, $CDCl_3$): 140.10.

N,N-*Diisopropyl*-P-*[3-(anthracen-9-yl)propyl]phosphonamidous Chloride* (**30**). As described above for **24**, with **22** (2.28 g, 7.1 mmol), ^{i}Pr$_2$NH (1.9 ml, 13.4 mmol), and anh. CH_2Cl_2 (10 ml). After evaporation of the solvent, a yellow solid was obtained. Yield of **30** determined by ^{1}H- and ^{31}P-NMR (2.03 g, 74%). ^{1}H-NMR (400 MHz, $CDCl_3$): 8.31–8.20 (*m*, 3 arom. H); 8.03 (*m*, 2 arom. H); 7.56–7.41 (*m*, 4 arom. H); 3.58 (*m*, ArCH_2); 3.42 (*m*, Me$_2$C*H*); 2.25 (*m*, CH_2P); 1.93 (*m*, CH_2CH$_2$P); 1.49 (*m*, *Me*$_2$CH). ^{31}P-NMR (400 MHz, $CDCl_3$): 140.28.

2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)*-N^4*-isobutyrylcytidine 3′-[*N,N-*Diisopropyl*-P-*(2-phenylethyl)phosphonamidite]* (**2**). To a soln. of 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N*^{4}-isobutyrylcytidine (0.70 g, 1.16 mmol) and ^{i}Pr$_2$EtN (0.90 ml, 5.0 mmol) in anh. CH_2Cl_2 (10 ml), **24** (0.38 g, 1.4 mmol) was added at r.t. under Ar. After stirring for 1 h, the mixture was cooled to 0° and diluted with CH_2Cl_2 (10 ml). The reaction was quenched by adding sat. aq. $NaHCO_3$ soln. (5 ml). The aq. layer was then extracted with CH_2Cl_2 (2 × 20 ml), the combined

org. layer dried ($MgSO_4$) and evaporated, and the crude oil purified by FC (AcOEt/hexane/Et_3N 69:30:1): **2** (714 mg, 73%). White foam. TLC (AcOEt/hexane/Et_3N 69:30:1): R_f 0.33, 0.23. ^{1}H-NMR (400 MHz, $CDCl_3$): 9.34 (*s*, NH); 8.16, 8.04 (2*d*, *J* = 7.3, H–C(6)); 7.33–7.12 (*m*, 14 arom. H); 7.05 (2*d*, *J* = 7.1, H–C(5)); 6.74 (*m*, 4 arom. H); 6.19, 6.14 (2*t*, *J* = 6.3, H–C(1′)); 4.45, 4.39 (2*m*, H–C(3′)); 4.11, 4.06 (2*m*, H–C(4′)); 3.70 (2*s*, 2 MeO); 3.45 (*m*, H–C(5′)); 3.36 (*m*, Me_2CH); 2.63 (*m*, Me_2CHCO); 2.58 (*m*, 2 $PhCH_2$); 2.48 (*m*, H_α–C(2′)); 2.13 (*m*, H_β–C(2′)); 1.83 (*m*, CH_2P); 1.12–0.99 (*m*, Me). ^{31}P-NMR (400 MHz, $CDCl_3$): 128.35, 127.47. ESI-MS: 835.7 ($[M+H]^+$).

2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)*-N^4-*isobutyrylcytidine 3′-[*N,N-*Diisopropyl*-P-*(3-phenylpropyl)phosphonamidite]* (**3**). As described above for **2**, with 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N*^{4}-isobutyrylcytidine (1.00 g, 1.67 mmol), ^{i}Pr_2EtN (1.00 ml, 5.6 mmol), anh. CH_2Cl_2 (15 ml), and **25** (0.60 g, 2.1 mmol). The crude oil was purified by FC (AcOEt/hexane/Et_3N 69:30:1): **3** (1.23 g, 87%). White foam. TLC (AcOEt/hexane/Et_3N 69:30:1): R_f 0.34, 0.25. ^{1}H-NMR (400 MHz, $CDCl_3$): 9.26 (*s*, NH); 8.15, 8.06 (2*d*, *J* = 7.2, H–C(6)); 7.35–7.09 (*m*, 14 arom. H); 7.01 (2*d*, *J* = 7.3, H–C(5)); 6.76 (*m*, 4 arom. H); 6.14 (*m*, H–C(1′)); 4.47, 4.40 (2*m*, H–C(3′)); 4.08 (*m*, H–C(4′)); 3.72 (2*s*, 2 MeO); 3.39–3.28 (*m*, H–C(5′), $PhCH_2$); 3.20 (*m*, Me_2CH); 2.63 (*m*, Me_2CH-CO); 2.52 (*m*, H_α–C(2′)); 2.09 (*m*, H_β–C(2′)); 1.91–1.72 (*m*, CH_2CH_2P); 1.19–1.02 (*m*, Me). ^{31}P-NMR (400 MHz, $CDCl_3$): 128.20, 127.90. ESI-MS: 849.7 ($[M+H]^+$).

2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)*-N^4-*isobutyrylcytidine 3′-[*N,N-*Diisopropyl*-P-*(4-phenylbutyl)phosphonamidite]* (**4**). As described above for **2**, with 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N*^{4}-isobutyrylcytidine (500 mg, 0.83 mmol), ^{i}Pr_2EtN (0.57 ml, 3.16 mmol), anh. CH_2Cl_2 (10 ml), and **26** (0.42 g, 1.4 mmol). The crude oil was purified by FC (AcOEt/hexane/Et_3N 69:30:1): **4** (0.64 g, 89%). White foam. TLC (AcOEt/hexane/Et_3N 69:30:1): R_f 0.36, 0.26. ^{1}H-NMR (400 MHz, $CDCl_3$): 9.37 (*s*, NH); 8.22, 8.11 (2*d*, *J* = 7.5, H–C(6)); 7.41–7.10 (*m*, 14 arom. H); 7.04 (*m*, H–C(5)); 6.83 (*m*, 4 arom. H); 6.29, 6.21 (2*t*, *J* = 5.9, H–C(1′)); 4.52, 4.46 (2*m*, H–C(3′)); 4.19, 4.11 (2*m*, H–C(4′)); 3.78 (2*s*, 2 MeO); 3.49–3.37 (*m*, H–C(5′), Me_2CH); 2.71–2.54 (*m*, Me_2CHCO, H_α–C(2′), $PhCH_2$); 2.20–2.10 (*m*, H_β–C(2′), CH_2P); 1.89–1.71 (*m*, $CH_2CH_2CH_2P$); 1.32–1.15 (*m*, Me). ^{31}P-NMR (400 MHz, $CDCl_3$): 129.10, 128.80. ESI-MS: 863.8 ($[M+H]^+$).

2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)*-N^4-*isobutyrylcytidine 3′-[*N,N-*Diisopropyl*-P-*(5-phenylpentyl)phosphonamidite]* (**5**). As described above for **2**, with 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N*^{4}-isobutyrylcytidine (600 mg, 1.0 mmol), ^{i}Pr_2EtN (0.72 ml, 4.0 mmol), anh. CH_2Cl_2 (10 ml), and **27** (0.53 g, 1.7 mmol). The crude oil was purified by FC (AcOEt/hexane/Et_3N 69:30:1): **5** (0.76 g, 86%). White foam. TLC (AcOEt/hexane/Et_3N 69:30:1): R_f 0.34, 0.22. ^{1}H-NMR (400 MHz, $CDCl_3$): 9.21 (*s*, NH); 8.18, 8.07 (2*d*, *J* = 7.5, H–C(6)); 7.20–7.02 (*m*, 14 arom. H, H–C(5)); 6.76 (*m*, 4 arom. H); 6.23, 6.14 (2*t*, *J* = 5.8, H–C(1′)); 4.48, 4.36 (2*m*, H–C(3′)); 4.16, 4.04 (2*m*, H–C(4′)); 3.72 (2*s*, 2 MeO); 3.42–3.31 (*m*, H–C(5′), Me_2CH); 3.25 (*m*, $PhCH_2$); 2.65 (*m*, Me_2CH-CO); 2.60–2.45 (*m*, H_α–C(2′), CH_2P); 2.15 (*m*, H_β–C(2′)); 1.81–1.62 (*m*, $CH_2CH_2CH_2CH_2P$); 1.27–1.15 (*m*, Me). ^{31}P-NMR (400 MHz, $CDCl_3$): 128.80, 128.46. ESI-MS: 878.8 ($[M+H]^+$).

2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)*-N^4-*isobutyrylcytidine 3′-[*N,N-*Diisopropyl*-P-*(naphthalen-1-ylmethyl)phosphonamidite]* (**6**). As described above for **2**, with 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N*^{4}-isobutyrylcytidine (300 mg, 0.5 mmol). ^{i}Pr_2EtN (0.36 ml, 2.0 mmol), anh. CH_2Cl_2 (7 ml), and **28** (0.31 g, 1.0 mmol) (reaction time: 1.5 h). The crude oil was purified by FC (AcOEt/hexane/Et_3N 69:30:1): **6** (251 mg, 58%). White foam. TLC (AcOEt/hexane/Et_3N 69:30:1): R_f 0.34, 0.27. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.89 (*s*, NH); 8.13, 8.07 (2*d*, *J* = 7.1, H–C(6)); 7.85 (*m*, 1 arom. H); 7.65 (*m*, 1 arom. H); 7.51 (*m*, 1 arom. H); 7.34–7.12 (*m*, 12 arom. H); 7.05 (*m*, H–C(5)); 6.83 (*m*, 4 arom. H); 6.26 (*m*, H–C(1′)); 4.53 (*m*, H–C(3′)); 4.26 (*m*, H–C(4′)); 3.79 (2*s*, 2 MeO); 3.62 (*m*, CH_2P); 3.51–3.32 (*m*, H–C(5′), Me_2CH); 2.71 (*m*, Me_2CHCO); 2.54 (*m*, H_α–C(2′)); 2.19 (*m*, H_β–C(2′)); 1.38–1.10 (*m*, Me). ^{31}P-NMR (400 MHz, $CDCl_3$): 126.74, 126.55. ESI-MS: 899.6 ($[M+H]^+$).

2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)*-N^4-*isobutyrylcytidine 3′-[*N,N-*Diisopropyl*-P-*[3-(naphthalen-1-yl)propyl]phosphonamidite]* (**7**). As described above for **2**, with 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N*^{4}-isobutyrylcytidine (400 mg, 0.67 mmol), ^{i}Pr_2EtN (0.43 ml, 2.4 mmol), anh. CH_2Cl_2 (10 ml), and **21** (0.67 g, 2.0 mmol) (reaction time: 2 h). The crude oil was purified by FC (AcOEt/hexane/Et_3N 49:50:1): **7** (187 mg, 31%). White foam. TLC (AcOEt/hexane/Et_3N 49:50:1): R_f 0.29, 0.22. ^{1}H-NMR (400 MHz, $CDCl_3$): 9.24 (*s*, NH); 8.12, 8.06 (2*d*, *J* = 7.4, H–C(6)); 7.98 (*m*, 1 arom. H); 7.85 (*m*, 1 arom. H); 7.72 (*m*, 1 arom. H); 7.45–7.21 (*m*, 12 arom. H); 7.05 (*m*, H–C(5)); 6.85 (*m*, 4 arom. H); 6.22 (*m*, H–C(1′)); 4.38 (*m*, H–C(3′)); 4.26 (*m*, H–C(4′)); 3.78 (2*s*, 2 MeO); 3.41 (*m*, Me_2CH); 3.23 (*m*, $ArCH_2$, H–C(5′)); 2.65 (*m*, Me_2CHCO); 2.48 (*m*, H_α–C(2′)); 2.15–2.02 (*m*, H_β–C(2′), CH_2P); 1.75 (*m*, CH_2CH_2P); 1.46–1.15 (*m*, Me). ^{31}P-NMR (400 MHz, $CDCl_3$): 127.95, 127.65. ESI-MS: 899.6 ($[M+H]^+$).

2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)*-N^4-*isobutyrylcytidine 3′-[*N,N-*Diisopropyl*-P-*[3-(anthracen-9-yl)propyl]phosphonamidite]* (**8**). As described above for **2**, with 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N*^{4}-isobutyr-

ylcytidine (310 mg, 0.52 mmol), $^{i}Pr_2EtN$ (0.30 ml, 1.7 mmol), anh. CH_2Cl_2 (10 ml), and **22** (0.58 g, 1.5 mmol) (reaction time: 2 h). The crude oil was purified by FC (AcOEt/hexane/Et_3N 49 : 50 : 1): **8** (270 mg, 55%). Yellow foam. TLC (AcOEt/hexane/Et_3N 49 : 50 : 1): R_f 0.42, 0.34. ^{1}H-NMR (400 MHz, $CDCl_3$): 9.10 (*s*, NH); 8.31 – 8.10 (*m*, 3 arom. H, H–C(6)); 8.02 (*m*, 2 arom. H); 7.53 – 7.16 (*m*, 13 arom. H, H–C(5)); 6.86 (*m*, 4 arom. H); 6.28 (*m*, H–C(1′)); 4.52 (*m*, H–C(3′)); 4.17 (*m*, H–C(4′)); 3.82 (2*s*, 2 MeO); 3.62 (*m*, CH_2P); 3.57 – 3.31 (*m*, H–C(5′), Me_2CH, $ArCH_2$); 2.70 – 2.58 (*m*, Me_2CHCO, H_α–C(2′)); 2.21 (*m*, H_β–C(2′)); 1.89 (*m*, CH_2P); 1.68 (*m*, CH_2CH_2P); 1.35 – 1.07 (*m*, Me). ^{31}P-NMR (400 MHz, $CDCl_3$): 128.38, 127.92. ESI-MS: 949.7 ($[M+H]^+$).

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