DOI: 10.1002/ejoc.200700528

Investigation on Condensing Agents for Phosphinate Ester Formation with Nucleoside 5′-Hydroxyl Functions[‡]

Anna Winqvist[a] and Roger Strömberg*[a]

Keywords: Oligonucleotides / Nucleotides / Phosphinates / Phosphonates / Methylenephosphonates / Condensation reactions

Condensation of a uridine 3′-deoxy-3′-*C*-methylenephosphinate with thymidine and guanosine derivatives to form methylenephosphinate esters was investigated. A number of different condensing agents were compared, and these include pivaloyl chloride, triisopropylbenzenesulfonyl chloride (TPS-Cl), phosphonium and uronium derivatives, numerous chlorophosphates and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (OXP). The phosphonium derivatives gave slow condensations or oxidative side reactions (hydroxybenzotriazole derivatives) during preactivation of the methylenephosphinate. Pivaloyl chloride gave long coupling times, and competing 5′-*O*-pivaloylation was detected. TPS-Cl gave rapid condensation but also rapid oxidation of the product. Most chlorophosphates gave competing 5′-*O*-phosphorylation of the nucleoside component, as well as base phosphorylation. However, 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinane (DMOCP) gave a rather efficient formation of dinucleoside methylenephosphinates at a decent rate. However, O^6-protection of guanines could become necessary with this reagent, since upon extended reaction time traces of O^6-phosphorylation were detected even with a low concentration (60 mM) of DMOCP (2 equiv. to phosphinate). Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (OXP) can, unlike DMOCP, be used in nearly equimolar amounts to phosphinate. Under such conditions OXP gives virtually quantitative condensation at a rate comparable to that of 2 equiv. of DMOCP and with no side reactions detected. We could also not detect any decomposition of OXP-preactivated phosphinate. Nucleophilic catalysts, more powerful than pyridine (*N*-methylimidazole, iodide and 4-methoxypyridine), accelerated the reactions with OXP, but preactivation in the absence of the 5′-OH component led to decomposition of the activated phosphinate.

Introduction

A large number of modified nucleic acid fragments have been developed in the past. This number increased drastically due to the development of antisense therapy.[1–3] The interest in modified RNA has increased further by the potential of using siRNA for therapeutics.[4,5] Recently, modified RNA has also become interesting for the development of the potential use of siRNA in disease treatment. In addition, modified di- and oligonucleotides have been used as potential enzyme inhibitors and in investigations of enzymatic mechanisms for a number of decades, and the use has increased with the number of analogues available.[6] An interesting modification that was introduced in dinucleotides already in 1970[7] is internucleoside 3′-deoxy-3′-*C*-methylenephosphonate linkages. Since then little has been reported on this type of modification. Mazur et al.[8] reported a trinucleotide containing 3′-deoxy-3′-*C*-methylenephosphonates, and recently Collingwood et al.[9] reported incorporation of these linkages into oligodeoxyribonucleotides.

The early work by Moffatt and later by Mazur occurred before the development of the H-phosphonate approach[10–15] to oligonucleotide synthesis. After the introduction of this methodology, it seemed likely that an analogous approach via alkylhydrogenphosphinates should be possible for incorporation of methylenephosphonate linkages. An early attempt at the basic concept demonstrated efficient coupling of methylphosphinate with a nucleoside to give the corresponding phosphinate ester, which was further converted into the phosphonate and thiophosphonate analogues.[16] More recently, thymidine 3′-deoxy-3′-*C*-methylenephosphinate building blocks, together with pivaloyl chloride as condensing agent, were used for introduction of methylenephosphonate linkages into oligodeoxyribonucleotides.[7] The ISIS Pharmaceuticals group has since also reported on the synthesis of nucleoside 3′-deoxy-3′-*C*-methylenephosphinate building blocks[17] and use of four different coupling agents[18] for internucleoside linkage formation, although these coupling agents were only compared with a deoxyribonucleoside 3′-deoxy-3′-*C*-methylene-

[‡] Nucleoside 3′-Deoxy-3′-*C*-Methylenephosphinates, 3. Part 1: A. Winqvist, R. Strömberg, *Eur. J. Org. Chem*. **2002**, 1509–1515. Part 2: A. Winqvist, R. Strömberg, *Eur. J. Org. Chem*. **2002**, 3140–3144.

[a] Department of Biosciences and Nutrition, Novum, Karolinska Institutet,
17177 Stockholm, Sweden
E-mail: Roger.Stromberg@biosci.ki.se

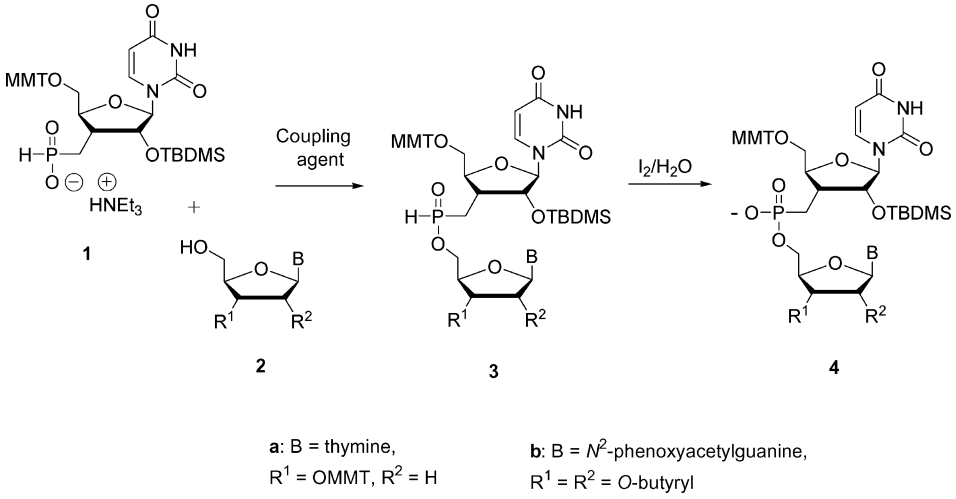
phosphinate which is sterically considerably less hindered than the corresponding protected ribonucleoside 3′-deoxy-3′-*C*-methylenephosphinate.

We have recently reported on the development of synthetic methods for key steps towards ribonucleoside 3′-deoxy-3′-*C*-methylenephosphinates, i.e., 3′-carbon extension at the nucleoside level,[19] synthesis of methylenephosphinate building blocks[20] and oxidation of methylenephosphinate linkages.[21] In this paper we report on an evaluation of condensing agents for the formation of the methylenephosphinate internucleoside linkage.

Results

In the condensation of a nucleoside 3′-deoxy-3′-*C*-methylenephosphinate building block with the 5′-hydroxyl function of a nucleoside (or elongating oligonucleotide) there are a number of aspects to consider. The formation of the internucleoside linkage should be sufficiently fast for practical usage. Although a relatively long condensation time can be acceptable for the formation of dinucleotides and in occasional syntheses of short oligonucleotide analogues, it would be generally preferred if an oligonucleotide analogue of, e.g., 20–24 units could be synthesized from one day to another. For a high coupling yield, reagents that can compete with the activated phosphinate for the nucleoside 5′-hydroxyl function should be avoided. In addition, side reactions at the lactam functions of the nucleobases are best kept at a minimum.

To investigate the above aspects, the phosphinate building block **1** was condensed with two different 5′-hydroxyl components, thymidine derivative **2a** and guanosine derivative **2b**, to give the 3′-deoxy-3′-*C*-methylenephosphinate esters **3a** and **3b**, respectively (Scheme 1). A number of condensing agents were evaluated (Figure 1) using ^{31}P NMR spectroscopy to monitor the course of the reactions. The products **3a** and **3b** were isolated and characterised, and as these were isomeric mixtures they were also further converted into the achiral compounds **4a** and **4b**, respectively, by oxidation with iodine in pyridine/water.[19] The first condensing agent investigated was pivaloyl chloride, which is the most commonly used in H-phosphonate-based oligonu-



Scheme 1.

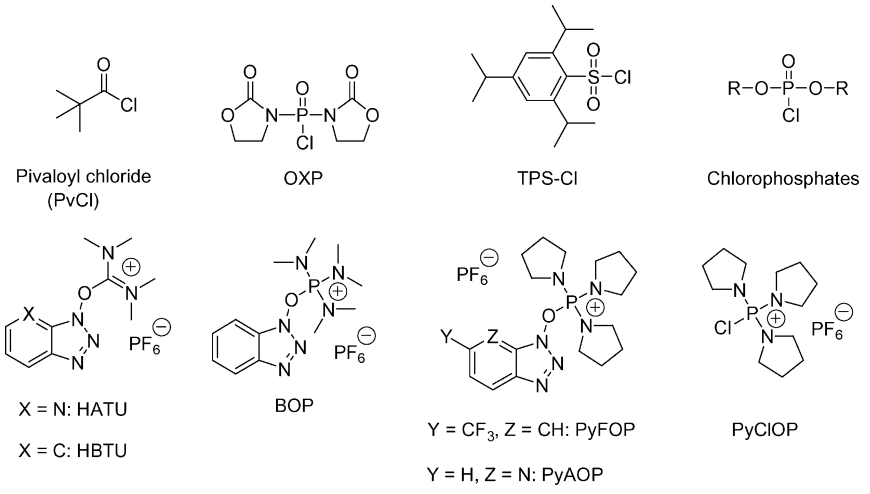


Figure 1. Condensing agents used for the coupling of **1** and **2** (for R see text or Table 1).

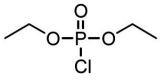
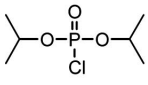
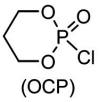
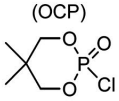
cleotide synthesis.[22,23] Coupling of **1** (30 mM) with **2a** (28.5 mM) in pyridine/acetonitrile (1:3, v/v) in the presence of 60 mM pivaloyl chloride gave **3a**. The rate of condensation was relatively low, especially when compared to the corresponding reaction with H-phosphonates. After 11 min, about 50% of **3a** was formed, whereas the corresponding reaction for H-phosphonates is generally complete at the time needed to record the first spectrum (less than 2–4 min).[24] The reaction did not give more than approximately 95% product, and TLC analysis revealed that minor amounts of 5′-*O*-pivaloylated **2a** were formed.

The reagent 2,4,6-triisopropylbenzenesulfonyl chloride (TPS-Cl) has also been reported to give efficient condensation with H-phosphonates, although oxidation of both H-phosphonate monoesters[25] and diesters[26] occurred. Condensation of **1** and **2a** with 60 mM TPS-Cl in pyridine/acetonitrile (1:3, v/v) gave complete and rapid conversion into **3a** (^{31}P NMR: δ = 37.0, 37.7 ppm) in less than the time required to record the first spectrum. However, a product (^{31}P NMR: δ = 42.3, 42.6 ppm) from a subsequent reaction was detected, amounting to 30% after about 3 h. The chemical shift alone is not definite evidence, but this together with the rapid conversion to the phosphonate anion **4a** upon addition of water makes it a reasonable suggestion that the compound formed is the corresponding chlorophosphonate. A similar reaction has previously been reported with H-phosphonate diesters.[24]

Chlorophosphates can be efficient condensing agents and were introduced early in H-phosphonate couplings[8,27] and have later been used at low temperature in H-phosphonate-based larger scale oligonucleotide synthesis in solution.[28,29] A chlorophosphate was also used in condensation of methylphosphinate with a nucleoside.[14] In addition, 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinane (DMOCP) has been used for methylenephosphinate linkage formation with deoxyribonucleotide 3′-deoxy-3′-*C*-methylenephosphinate building blocks.[16]

Reactions were carried out with **1**, **2a** and 60 mM or 120 mM of chlorophosphate in pyridine/acetonitrile (1:3, v/v). Several tested diaryl chlorophosphates (diphenyl, di-*o*-tolyl, or di-*o*-chlorophenyl chlorophosphate) proved to be too reactive, and although coupling was fast, competing 5′-*O*-phosphorylation of **2a** (6–40%) occurred. In addition, substantial amounts of phosphorylation at the lactam functionalities (the O^4-position of uracil or thymidine) were observed also with the less reactive diethyl chlorophosphate. To verify that this kind of side products forms under these specific conditions, we carried out additional experiments where 2′,3′,5′-tri-*O*-butyryluridine was treated with 120 mM diethyl chlorophosphate in pyridine/acetonitrile (1:3, v/v). Mass spectral analysis of this reaction mixture after 24 h revealed the presence of both a phosphorylated species (m/z = 591 [M – H$^+$]) and the product [the 6-(*N*-pyridinium)-2-aminopurine derivative[30]] of its subsequent reaction with pyridine (m/z = 516). More hindered dialkyl chlorophosphates gave higher selectivity. Diisopropyl chlorophosphate, 2-chloro-2-oxo-1,3,2-dioxaphosphorinane (OCP) and 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinane (DMOCP) seemed promising, at least at 60 mM concentration (Table 1). At this lower concentration no competing side reactions were detected with diisopropyl chlorophosphate, OCP and DMOCP. DMOCP proved less reactive and did not give side products, even at 120 mM, in the reaction of **1** and **2a**. However, subsequent side reactions were observed at the O^6-position of guanosine, when the DMOCP coupling was carried out with **2b**. The reaction of **1**, **2b** and 120 mM DMOCP gave 15% O^6-phosphorylation after leaving the condensation mixture for about 3 h. Even though the phosphodiester formed from the reagent, in the use of 60 mM DMOCP, seemed to trap all excess chlorophosphate, we observed that this trapping is somewhat slower than the coupling. This in turn leaves some chlorophosphate to react with the base. In fact, trace amounts (≤1%) of O^6-phosphorylation were detected even before

Table 1. Reaction of **1** with **2a** in the presence of dialkyl chlorophosphates.[a]

Entry	Dialkyl chlorophosphate	Conc.	Time for formation of **3a**[b]		5′-*O*-phosphorylation of **2a**[d]		O^4-phosphorylation of **3a**		
		[mM]	75 % product	90 % product	after coupling	δ of adduct	after 10 min	after 40 min	δ of adduct
1-1		60	n.e.[c]	< 4 min	2 %	–0.6 ppm	n.o.[e]	n.o.[e]	–8.1 ppm
		120	n.e.[c]	< 4 min	11 %		10 %	27 %	
1-2		60	n.e.[c]	< 4 min	n.o.[e]	–2.5 ppm	n.o.[e]	n.o.[e]	–10.1 ppm
		120	n.e.[c]	< 4 min	1 %		10 %	21 %	
1-3	 (OCP)	60	n.e.[c]	< 4 min	n.o.[e]	–6.8 ppm	n.o.[e]	n.o.[e]	–15.7 ppm
		120	n.e.[c]	< 4 min	2 %		3 %	13 %	
1-4	 (DMOCP)	60	8 min	11 min	n.o.[e]	–	n.o.[e]	n.o.[e]	–
		120	5 min	7 min	n.o.[e]		n.o.[e]	n.o.[e]	

[a] Reaction carried out in acetonitrile/pyridine (3:1, v/v). [b] Signals of two isomeric products observed at δ = 37 and 37.7 ppm. [c] n.e. = not estimated, since no data could be collected by NMR spectroscopy before a reaction time of 4 min. [d] The amount of competing phosphorylation of **2a** was estimated from the NMR spectra after the coupling reaction was complete. [e] n.o. = not observed.

completion of the condensation reaction, implying that it is difficult to completely avoid this side reaction. The amount of the side product increased slightly upon standing (still within ≤ 1%) until all chlorophosphate had been converted into pyrophosphate (by reaction with the formed phosphate anion) after which no further significant increase was detected upon standing overnight. As this condensing agent otherwise seemed promising, further analysis of the product of this side reaction was carried out. First 2′,3′,5′-tri-*O*-butyryl-N^2-phenoxyacetylguanosine was treated with 120 mM DMOCP in pyridine/acetonitrile (1:3, v/v), and MS analysis after 48 h revealed the presence of both a phosphorylated adduct (*m*/*z* = 776 [M – H$^+$]) and the product from subsequent reaction with pyridine (*m*/*z* = 689). In addition MS analysis of the reaction mixture of **1**, **2b** and DMOCP was carried out which revealed the presence of a DMOCP adduct of the U-G dimer (*m*/*z* = 1380.5 [M – H$^+$]).

Sekine et al.[31] introduced a number of uronium and phosphonium reagents as condensing agents for *H*-phosphonates. A drawback in the use of some of these reagents was that the preactivation of H-phosphonates, in the absence of alcohol, resulted in oxidative decomposition. For the present study a few reagents, i.e., HBTU, HATU, BOP, PyFOP, PyAOP and PyClOP were selected (Figure 1). The condensations of **1** with **2a** were carried out by the use of 60 mM of the various uronium and phosphonium reagents in pyridine/acetonitrile (1:3, v/v). The two uronium reagents, HBTU and HATU, gave relatively slow reactions. With HATU a higher rate was obtained than with HBTU, giving 90% conversion into the product in about 18 min. Major differences in reactivity were also observed among the phosphonium reagents (Table 2). The use of PyFOP or PyAOP as condensing agents gave fast coupling reactions. However, PyAOP gave detectable amounts of oxidation products during the condensation time. PyFOP gave fast couplings (both at concentrations of 60 mM or 120 mM of the condensing reagent) in which reactions were nearly complete upon recording of the first spectrum (≥ 90% conversion after about 4 min). No side reactions were observed during these condensations, i.e., with alcohol present from the beginning. However, when phosphinate **1** was treated with 60 mM PyFOP in pyridine/acetonitrile (1:3, v/v), in the absence of an alcohol component, the activated phosphinate underwent further conversions relatively fast (within 5 min) into an intermediate that decomposed to give the corresponding *C*-phosphonate. Addition of an alcohol to this intermediate, before it decomposed, did not result in the formation of a phosphinate ester. Hence, the oxidative side reaction can be a drawback in the use of condensing reagents such as PyFOP or PyAOP when these reagents are used in a synthesis that involves premixing of the condensing agent with the phosphinate before addition of the alcohol.

To avoid the use of hydroxybenzotriazole derivatives, the coupling of phosphinate **1** with alcohol **2a** using 60 mM PyClOP in pyridine/acetonitrile (1:3, v/v) was performed. This gave about 50% conversion into products in 1.5 h. To accelerate the reaction, catalysis by *N*-methylimidazole was examined. Coupling of phosphinate **1** with alcohol **2a** using 60 mM PyClOP and 0.5 M *N*-methylimidazole in pyridine/acetonitrile (1:3, v/v) gave ca 90% conversion into products in about 30 min. Thus, the reaction rate was significantly increased, but still considerably lower than with the least reactive chlorophosphates.

Table 2. Condensations of **1** with **2a** (to **3a**) as aided by carbonium or phosphonium salts.[a]

Entry	Coupling agent	Conc. [mM]	Time for the formation of 75% product	90% product
2–1	HBTU	60	3.3 h	4.6 h
2–2	HATU	60	10 min	18 min
2–3	BOP	60	1.1 h	3.0 h
2–4	PyFOP	60	n.e.[c]	<4 min
2–5	PyFOP	120	n.e.[c]	<4 min
2–6	PyClOP	60	50% in 1.5 h	–
2–7	PyClOP/*N*-methylimidazole[b]	60	–	0.5 h

[a] Reaction carried out in acetonitrile/pyridine (3:1, v/v). [b] The concentration of *N*-methylimidazole was 0.5 M. [c] n.e. = not estimated, since no data could be collected by NMR spectroscopy before a reaction time of 4 min.

The reagent bis(2-oxo-3-oxazolidinyl)phosphinic chloride (OXP) has been used as a condensing reagent in formation of carboxylic acid derivatives[32,33] and has also been reported for *H*-phosphonate couplings[25] as well as for the condensation of methylphosphinate[14] and thymidine 3′-deoxy-3′-*C*-methylenephosphinate,[16] although in the last case the efficiency reported was discouraging. Coupling of phosphinate **1** with **2a** was carried out at various concentrations of OXP (36–120 mM) in neat pyridine or pyridine/acetonitrile (1:3, v/v), as shown in Table 3. The rate was somewhat affected by the solvent composition, being slightly faster in a pyridine/acetonitrile mixture (1:3, v/v) than in neat pyridine. No side reactions were observed in the coupling to **2a**. However, when phosphinate **1** was condensed with **2b** by the use of 60 mM OXP in pyridine or a mixture of pyridine/acetonitrile, traces of a side product were observed after 1 h (presumably from reaction with the lactam function of the guanine base). MS analysis after prolonged treatment revealed the presence of the pyridinium adduct (*m*/*z* = 1293.5). A positive finding was then that the reaction rates were independent of the concentration of OXP, unlike the reaction with DMOCP, which suggests a fast activation step that is followed by a rate-limiting substitution step. When the coupling between **1** and **2a** was carried out by the use of 36 mM (1.2 equiv.) OXP in pyridine/acetonitrile (1:3, v/v), conversion into the product occurred at a rate similar to that of the reaction with 60 mM OXP (about 90% in 11 min). In the coupling of **1** to the guanosine derivative **2b**, we also did not observe any side reactions when the reaction was carried out with 36 mM OXP (1.2 equiv. to **1**). The reaction went to completion, and no O^6-phosphorylation was detected even after leaving the mixture overnight. Interestingly, the internucleoside linkage formation occurred at a higher rate with **2b** than with **2a**; at a concentration of 36 mM OXP the coupling was more than 90% complete when the first spectrum was recorded (4 min).

Table 3. Formation of **3a** in the condensations of **1** with **2a**, aided by OXP.

Entry	Coupling agent (solvent system)	Conc. [mM]	Time for the formation of 75% product	90% product
3–1	OXP (pyridine)	60	11 min	17 min
3–2	OXP (pyridine)	120	11 min	17 min
3–3	OXP (pyridine/CH_3CN)	60	6 min	11 min
3–4	OXP (pyridine/CH_3CN)	36	6 min	11 min
3–5	OXP/*N*-methylimidazole[b] (pyridine/CH_3CN)	36	n.e.[a]	<4 min
3–6	OXP/2,6-lutidine[b] (pyridine/CH_3CN)	36	6 min	12 min
3–7	OXP/4-methoxypyridine[b] (pyridine/CH_3CN)	36	4 min	7 min
3–8	OXP/sodium iodide[b] (pyridine/CH_3CN)	36	n.e.[a]	<4 min

[a] n.e. = not estimated since no data could be collected by NMR before 4 min. reaction time. [b] The concentrations of *N*-methylimidazole, 2,6-lutidine, 4-methoxypyridine and sodium iodide were all 0.5 M.

Treatment of phosphinate **1** with OXP in the absence of the 5′-hydroxyl component, using similar conditions, gave an activated 3′-deoxy-3′-*C*-methylenephosphinate derivative that was stable (several signals at δ = 33–34 ppm). Subsequent addition of the 5′-hydroxyl component **2a** to the preactivated phosphinate building block gave **3a**, at a rate comparable to that obtained when **2a** was present before the addition of OXP. Attempts to further increase the reaction rate in the condensation between **1** and **2a** were carried out using nucleophilic assistance and/or base catalysis. In the first instance *N*-methylimidazole was utilized for this purpose. Indeed, reaction of **1** with **2a** using 36 mM OXP and 0.5 M *N*-methylimidazole in pyridine/acetonitrile (1:3, v/v) gave a significant increase in the reaction rate. Complete conversion into the product occurred within the time required to record the first spectrum. However, when the treatment of **1** with OXP in the presence of 0.5 M *N*-methylimidazole was performed in the absence of the 5′-hydroxyl component, the activated phosphinate building block was not stable. Side reactions took place forming unidentified products that failed to give **3a** upon addition of **2a**. Instead, we considered the use of a less basic nucleophile (4-methoxypyridine), a poorly nucleophilic base (2,6-lutidine) and a nonbasic nucleophile (iodide) (Table 3). Treatment of **1** and **2a** with 36 mM OXP and 2,6-lutidine (0.5 M) in pyridine/acetonitrile (1:3, v/v) gave a rate similar to that of the reaction with only pyridine present, suggesting no strong base catalysis. On the other hand, similar reactions with added 4-methoxypyridine or iodide were faster. The reaction in the presence of 4-methoxypyridine gave 90% product in 7 min, and the reaction with added sodium iodide was complete when recording the first spectrum (less than 4 min). This clearly suggests that nucleophilic catalysis is operating. Although this can occur by displacing the chloride on OXP, as reported for several chlorophosphates,[34] this would not affect the rate since the reaction is independent on the OXP concentration. Presumably then, nucleophilic catalysis takes place on the intermediate(s) formed from the reaction of OXP with **1**. When **1** was activated with OXP in the presence of both iodide and 4-methoxypyridine, but in the absence of alcohol, the same problem as with *N*-methylimidazole appeared, i.e., consumption of the initially formed intermediate. Again, this led to incomplete coupling upon addition of **2a**. With iodide the consumption of the initial intermediate was rapid and essentially over within 10 min, but with 4-methoxypyridine the first active intermediate had a longer life time (ca 50% remaining after about 20 min).

Discussion

Condensation of methylenephosphinate **1** with nucleoside **2a** by the aid of the investigated phosphonium derivatives was either slow or (with hydroxybenzotriazole derivatives) gave oxidative side reactions during condensation or preactivation of **1**. Some of these reagents, in particular PyFOP, could be useful in solution synthesis but seem less suitable for situations where preactivation of phosphinate with the condensing reagent typically is preferred, e.g. in solid-phase oligo(nucleoside methylenephosphonate) synthesis via phosphinate intermediates. Acyl chlorides as condensing reagents give side reactions at the lactam function of guanosine; however, the resulting side product is reported to be cleaved by ammonolysis (as commonly used in the final deprotection of oligonucleotides) to regenerate the original structure of the nucleobase.[24] Nevertheless, pivaloyl chloride is less than ideal since competing 5′-*O*-acylation leads to non-quantitative coupling. TPS-Cl gives fast condensation but causes relatively fast oxidation of the product. TPS-Cl could in principle still be used as a coupling agent, but adds little advantage compared to several other investigated alternatives. For oligonucleotide synthesis, further studies would be also needed to see whether the oxidized intermediates would cause further problems that could not be readily circumvented.

Most chlorophosphates gave competing 5′-*O*-phosphorylation of the nucleoside component **2a**, in addition to the known problem of O^4- and O^6-phosphorylation of the uracil/thymine and guanine bases.[35–38] However, a couple of the least reactive chlorophosphates (diisopropyl chlorophosphate, OCP and DMOCP) can be of interest for the formation of internucleoside methylenephosphinate linkages. There are some limitations, though. Competing 5′-*O*-phosphorylation of **2a** or **2b** could be avoided, but O^6-phosphorylation of the guanine base was still observed in all cases even at 60 mM concentrations. However, with 60 mM DMOCP this side reaction is relatively minor which sug-

gests that this reagent could be suitable in the synthesis where low levels of O^6-phosphorylation are acceptable. In the oligo(nucleoside methylenephosphinate) synthesis, where repeated coupling takes place, it should be used with caution since concomitant O^6-phosphorylation may become too prominent if longer sequences are to be made. It has been reported that in the presence of various nucleophiles (pyridine or 1,2,4-triazole derivatives) further substitution of the O^6-phosphorylated species occur.[35,36] Studies on the cleavage of these groups under ammonolytic conditions, which are commonly used for the deprotection of oligonucleotides, showed that both the regenerated original structure of the nucleobase and the corresponding amino derivative could be formed. The latter reaction which leads to a modification of the base is most pronounced with guanosine (converted into 2,6-diaminopurine), and the different tested deblocking conditions gave substantial amounts of base modification.[36] To avoid this problem, O^6-protection of guanosine[39,40] would be recommended. If O^6-protection is employed together with O^4-protection of uridines (or thymidines), diisopropyl chlorophosphate or OCP could perhaps be advantageous, since condensations are more rapid with these reagents than with DMOCP.

The use of OXP for the formation of oligo(nucleoside methylenephosphinate)s seems to be the most general solution, at least if complete avoidance of side reactions is desired. Due to that the rate is independent on the concentration of OXP, this reagent can be used at near equimolar amounts to the phosphinate building block and still give a decent coupling time. Under such conditions we could not observe any side reactions, neither during condensation nor in the reaction mixture afterwards. The OXP-promoted condensation can be catalysed by nucleophiles such as *N*-methylimidazole, iodide and 4-methoxypyridine. However, the use of these nucleophilic catalysts is less suitable if preactivation of the phosphinate is done, since the initially formed active intermediate decomposes relatively fast, which leads to incomplete condensation. On the other hand, in the use of 36 mM OXP in pyridine/acetonitrile (1:3, v/v) without any additional nucleophilic catalyst, gave no detectable side reactions and a reasonable time of condensation also upon preactivation of the phosphinate.

Conclusions

In the present study a large number of condensing reagents were evaluated for coupling of the phosphinate building block **1** to 5′-hydroxyl components **2a** or **2b**, to achieve the 3′-deoxy-3′-*C*-methylenephosphinate esters **3a** or **3b**. Several of the condensing reagents that were examined were most effective in the synthesis of internucleoside methylenephosphinate linkages. Condensations using PyFOP were fast and efficient, but only if premixing of coupling agent and phosphinate in the absence of hydroxyl component can be avoided. Couplings using diisopropyl chlorophosphate, OCP and especially DMOCP proceeded at acceptable reaction times and could find all use in solution syntheses. Some caution is needed though, since the lactam functionalities of guanosine and uridine/thymidine (in the case of diisopropyl chlorophosphate and OCP) do not stay completely intact, and further O^4 (of U or T) and O^6 (of G) protection may be necessary if one wishes to avoid this completely.

The use of OXP for the formation of internucleoside methylenephosphinate linkages seems to be the most general solution. Although several of the other investigated condensing agents can be considered, OXP in nearly equimolar amounts to the phosphinate building block seems to be the safest choice with respect to minimisation of side reactions while still retaining a reasonable condensation time. With OXP as coupling reagent no side reactions could be detected at near equimolar amounts of the reagents. The phosphinate **1** was also stable after activation by OXP prior to coupling which could make the reagent useful not only for solution synthesis where the alcohol component is present from the start, but also when phosphinate is preactivated in the absence of alcohol. It certainly seems worth to try to optimise coupling time and other conditions with OXP in the synthesis of oligonucleoside (methylenephosphinate)s.

Experimental Section

General Remarks and Methods: NMR spectra were recorded with a Bruker Avance DRX-400 instrument (400.13 MHz in ^{1}H, 162.00 MHz in ^{31}P, and 100.62 MHz in ^{13}C). ^{13}C and ^{1}H chemical shifts are given in ppm, downfield from external TMS. ^{31}P chemical shifts are given downfield from external H_3PO_4 (2%, v/v) in D_2O. Most assignments of ^{1}H and ^{13}C NMR signals were made by standard ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC experiments. Silica gel column chromatography was generally carried out using Matrex silica, 60 Å (35–70 μm, Amicron). TLC analysis was carried out on precoated plates Silica Gel 60 F_{254} (Merck), with detection by UV light and/or by charring with 8% sulfuric acid in methanol. Solutions were concentrated under reduced pressure at temperatures not exceeding 40 °C. Pivaloyl chloride was purchased from Fluka and distilled before use. PyAOP and PyFOP[41] as well as OCP and DMOCP[42] were synthesized essentially according to the published methods, the two latter ones were additionally recrystallised from dichloromethane/toluene or dichloromethane/hexane. OXP was purchased from Aldrich. 2-*N*-Phenoxyacetylguanosine was prepared essentially according to a published method[43] using the acid anhydride for acylation, and the crude product was further purified by crystallization from methanol. Other reagents and solvents were of ordinary commercial grade unless otherwise stated. Diisopropyl chlorophosphate was purchased from Toronto Research Chemicals Inc. Diethyl chlorophosphate was purchased from Aldrich. BOP and HBTU were purchased from Nova Biochem, and HATU was purchased from Perseptive Biosystems. Acetonitrile (p.a.) was dried with molecular sieves (3 Å). Pyridine (p.a.) was dried with molecular sieves (4 Å). The phosphinate building block **1** was prepared as described previously.[18]

3′-*O*-(4-Methoxytrityl)thymidine (2a):[44] Thymidine (5 g, 20.6 mmol) was dried by evaporation of added pyridine (25 mL) and was dissolved in dry pyridine (50 mL). The solution was cooled to 0 °C (ice bath), and pivaloyl chloride (2.8 mL, 22.7 mmol,

1.1 equiv.) was added. The reaction mixture was left in a slowly melting ice bath for 1 h and then stirred at room temperature for an additional 3 h. 4-Methoxytrityl chloride (12.7 g, 41.3 mmol, 2 equiv.) was added, and the reaction mixture was stirred at 20 °C for 85 h. The reaction mixture was diluted with toluene (200 mL) and washed with saturated aqueous $NaHCO_3$ (2× 200 mL). The combined water layers were washed with CH_2Cl_2 (100 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was further dried by evaporation of added toluene (100 mL) and was then dissolved in THF/ethanol (1:1, v/v; 150 mL). 2 M aqueous sodium hydroxide (75 mL, 150 mmol, 7.3 equiv.) was added, and the reaction mixture was stirred at 60–70 °C for 3 h. The mixture was cooled to 20 °C, and ammonium chloride (8.5 g, 159 mmol, 7.7 equiv.) was added. The mixture was concentrated to remove the organic solvents. The residual aqueous mixture was washed with CH_2Cl_2 (2× 150 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography (30–80% ethyl acetate in toluene) to give **2a** (9.2 g, 87%). ^{1}H NMR ($CDCl_3$, 20 °C): δ = 1.61–1.66 (m, 1 H, 2′$_a$-C*H*), 1.78 (s, 3 H, CH_3), 1.82–1.86 (m, 1 H, 2′$_b$-C*H*), 2.39 (dd, J = 4.0, 6.5 Hz, 1 H, 5′-O*H*), 3,19–3.25 (m, 1 H, 5′-H$_a$) 3.53–3.59 (m, 1 H, 5′-H$_b$), 3.72 (s, 3 H, OCH_3) 3.87–3.91 (m, 1 H, 4′-H), 4.29 (d, J = 6.3 Hz, 1 H, 3′-H), 6.06 (dd, $J_{1',2'}$ = 5.8, 8.8 Hz, 1 H, 1′-H), 6.77 (d, J = 8.9 Hz, 2 H, MMT) 7.07–7.28 (m, 9 H, 6-H, MMT), 7.38 (d, J = 7.5 Hz, 4 H, MMT) 8.53 (s, 1 H, NH) ppm.

2′,3′-Di-*O*-butyryl-2-*N*-(phenoxyacetyl)guanosine (2b): 5′-*O*-(4-Methoxytrityl)-2-*N*-phenoxyacetylguanosine[45] (1 g, 1.45 mmol) was dried by evaporation of added pyridine (2× 5 mL) and dissolved in dry pyridine (2 mL). Butyric anhydride (0.52 mL, 3.2 mmol, 2.2 equiv.) was added, and the reaction mixture was stirred at 20 °C for 20 h. The mixture was concentrated and the residue dissolved in CH_2Cl_2 (60 mL). The solution was washed with saturated aqueous $NaHCO_3$ (30 mL). The water layer was washed with CH_2Cl_2 (20 mL). The combined organic layers were dried (Na_2SO_4), diluted with toluene (20 mL) and concentrated. The residue was further dried by evaporation of added toluene (10 mL) and dissolved in CH_2Cl_2 (5 mL). 1-Butanol (2.7 mL, 29.0 mmol, 20 equiv.) was added followed by *p*-toluenesulfonic acid monohydrate (0.83 g, 4.35 mmol, 3 equiv.), and the reaction mixture was stirred at 20 °C for 30 min. The mixture was diluted with CH_2Cl_2 (50 mL) and washed with saturated aqueous $NaHCO_3$ (50 mL). The water layer was washed with CH_2Cl_2 (20 mL). The combined organic layers were dried (Na_2SO_4), diluted with toluene (20 mL) and concentrated. The residue was purified by recrystallisation (CH_2Cl_2/diethyl ether) to give **2b** (0.65 g, 80%). ^{1}H NMR ($CDCl_3$, 20 °C): δ = 0.89 [t, J = 7.4 Hz, 3 H, C(O)CH$_2$CH$_2$CH_3], 1.00 [t, J = 7.4 Hz, 3 H, C(O)CH$_2$CH$_2$CH_3], 1.58 [sext, J = 7.4 Hz, 2 H, C(O)CH$_2$CH_2CH$_3$], 1.70 [sext, J = 7.4 Hz, 2 H, C(O)CH$_2$-CH_2CH$_3$], 2.26 [t, J = 7.4 Hz, 2 H, C(O)CH_2CH$_2$CH$_3$], 2.39 [t, J = 7.4 Hz, 2 H, C(O)CH_2CH$_2$CH$_3$], 3.86–4.02 (m, 2 H, 5′-H$_a$ and 5′-H$_b$), 4.33 (s, 1 H, 4′-H), 4.71 (s, 2 H, CH_2OPh), 4.95 (d, J = 8.7 Hz, 1 H, OH), 5.71 (d, J = 5.3 Hz, 1 H, 3′-H), 5.85–5.89 (m, 1 H, 2′-H), 5.95 (d, $J_{1',2'}$ = 7.3 Hz, 1 H, 1′-H), 7.05 (d, J = 8.3 Hz, 2 H, Ph), 7.10 (t, J = 7.4 Hz, 1 H, Ph), 7.37 (t, J = 7.9 Hz, 2 H, Ph), 7.77 (s, 1 H, 8-H), 9.30 (s, 1 H, NH), 11.8 (s, 1 H, NH) ppm. ^{13}C NMR ($CDCl_3$, 20 °C): δ = 13.7 [C(O)CH$_2$CH$_2$CH_3], 13.8 [C(O)CH$_2$CH$_2$CH_3], 18.3 [C(O)CH$_2$CH_2CH$_3$], 18.5 [C(O)-CH$_2$CH_2CH$_3$], 35.6 [C(O)CH_2CH$_2$CH$_3$], 36.0 [C(O)CH_2CH$_2$CH$_3$], 62.6 (C-5′), 66.6 (CH_2OPh), 71.9 (C-3′), 72.7 (C-2′), 85.8 (C-4′), 88.1 (C-1′), 115.0, 123.4, 125.2, 130.1, 139.1 (C-8), 146.7, 146.8, 155.0, 156.4, 169.7, 171.9, 172.6 ppm. $C_{26}H_{31}N_5O_9$ (557.56): calcd. C 56.01, H 5.60, N 12.56; found C 55.94, H 5.63, N 12.60.

3′-*O*-(4-Methoxytrityl)thymidine [2′-*O*-(*tert*-Butyldimethylsilyl)-3′-deoxy-5′-*O*-(4-methoxytrityl)uridine 3′-*C*-methylenephosphinate] (3a): To a solution of **2a** (35.2 mg, 0.068 mmol) and phosphinate **1** (57 mg, 0.072 mmol, 1.05 equiv.) in dry pyridine/acetonitrile (6:10, v/v; 1.6 mL) was added a solution of PyFOP (84.7 mg, 0.144 mmol, 2.10 equiv.) in dry acetonitrile (0.8 mL). The reaction mixture was stirred at 20 °C for 30 min. The mixture was diluted with CH_2Cl_2 (10 mL) and washed with saturated aqueous $NaHCO_3$ (10 mL). The water layer was washed with CH_2Cl_2 (5 mL). The combined organic layers were dried (Na_2SO_4), toluene (5 mL) was added, and the solution was concentrated. The residue was further dried by evaporation of added toluene (5 mL) and purified by silica gel column chromatography (30–100% ethyl acetate in CH_2Cl_2) to give **3a** as an isomeric mixture (in an isomeric of ratio of 53:47 as determined by relative integrals of ^{1}H NMR signals, 80.7 mg, 99%). The isomeric mixture was used for the next step. ^{1}H NMR (CD_3CN, 20 °C): δ = 0.09 (s, 3 H, SiCH$_3$), 0.20 (s, 3 H, SiCH$_3$), 0.85 [s, 9 H, SiC(CH$_3$)$_3$], 1.38–1.54 (2× m, 1 H, CH_2P), 1.64–1.89 (2× m, 2 H, 2′-H$_{(T)}$), 1.96–2.03 (2× m, 1 H, CH_2P), 2.47–2.67 (2× m, 1 H, 3′-H$_{(U)}$), 3.30 and 3.33 (2× dd, ^2J = 11.7, ^3J = 2.9, 3.2 Hz, 1 H, 5′-H$_{b(U)}$), 3.49 and 3.54 (2× dd, ^2J = 10.6, 12.9, ^3J = 2.5 Hz, 1 H, 5′-H$_{a(U)}$), 3.61–3.72 (2× m, 2 H, 5′-H$_{a(T)}$ and 5′-H$_{b(T)}$), 3.73 and 3.75 (2× s, 6 H, 2× OCH$_3$), 3.84–3.88 and 3.92–3.95 (2× m, 1 H, 4′-H$_{(T)}$), 3.97–4.07 (2× m, 1 H, 4′-H$_{(U)}$), 4.16–4.20 and 4.25–4.28 (2× m, 1 H, 3′-H$_{(T)}$), 4.43 and 4.46 (2× d, J = 4.2, 4.4 Hz, 1 H, 2′-H$_{(U)}$), 5.17 and 5.18 (2× d, J = 8.1 Hz, 1 H, 5-H$_{(U)}$), 5.61 and 5.62 (2× s, 1 H, 1′-H$_{(U)}$), 6.09–6.17 (2× m, 1 H, 1′-H$_{(T)}$), 6.83–6.90, 7.12–7.36 and 7.40–7.50 (3× m 29 H, 6-H$_{(T)}$, 2× MMT), 6.94 and 7.02 (2× dd, $^1J_{P,H}$ = 546, 548 Hz, $^3J_{P,H}$ = 2 Hz, 1 H, PH), 7.90 and 7.93 (2× d, J = 8.4 Hz, 1 H, 6-H$_{(U)}$), 8.97 (br. s, 1 H, NH), 9.15 and 9.22 (2× br. s, 1 H, NH) ppm. ^{31}P NMR (CD_3CN, 20 °C): δ = 37.8, 37.9 ppm. $C_{66}H_{73}N_4O_{13}PSi$ (1189.38): calcd. C 66.65, H 6.19, N 4.71; found C 66.47, H 6.24, N 4.76.

2′,3′-Di-*O*-butyryl-2-*N*-(phenoxyacetyl)guanosine [2′-*O*-(*tert*-Butyldimethylsilyl)-3′-deoxy-5′-*O*-(4-methoxytrityl)uridine 3′-*C*-methylenephosphinate] (3b): To a solution of **2b** (20 mg, 0.036 mmol) and phosphinate **1** (30 mg, 0.038 mmol, 1.05 equiv.) in dry pyridine/acetonitrile (6:10, v/v; 0.8 mL) was added a solution of PyFOP (44 mg, 0.075 mmol, 2.1 equiv.) in dry acetonitrile (0.4 mL). The reaction mixture was stirred at 20 °C for 30 min. The mixture was diluted with CH_2Cl_2 (10 mL) and washed with saturated aqueous $NaHCO_3$ (10 mL). The water layer was washed with CH_2Cl_2 (5 mL). The combined organic layers were dried (Na_2SO_4), toluene (5 mL) was added, and the solution was concentrated. The residue was further dried by evaporation of added toluene (5 mL) and purified by silica gel column chromatography (2–5% methanol in CH_2Cl_2) to give **3b** as an isomeric mixture (in an isomeric ratio of 52:48 as determined by relative integrals of ^{1}H NMR signals, 41 mg, 93%). The isomeric mixture was used for the next step. ^{1}H NMR (CD_3CN, 20 °C): δ = 0.07 and 0.13 (2× s, 3 H, SiCH$_3$), 0.20 and 0.22 (2× s, 3 H, SiCH$_3$), 0.86–1.01 [15 H, 2× C(O)CH$_2$CH$_2$CH_3 and SiC(CH$_3$)$_3$], 1.49–1.71 [5 H, CH_2P and 2× C(O)CH_2CH$_2$CH$_3$], 2.14 (m, 1 H, CH_2P), 2.24–2.43 [4 H, 2× C(O)CH$_2$CH_2CH$_3$], 2.63–2.75 (2× m, 1 H, 3′-H$_{(U)}$), 3.30–3.40 (m, 1 H, 5′-H$_{b(U)}$), 3.49–3.58 (m, 1 H, 5′-H$_{a(U)}$), 3.74 and 3.77 (2× s, 3 H, CH$_3$O), 4.01–4.07 (2× m, 1 H, 4′-H$_{(U)}$), 4.35–4.56 (4 H, 2′-H$_{(U)}$, 5′-H$_{a(G)}$, 5′-H$_{b(G)}$ and 4′-H$_{(G)}$), 4.69–4.78 (2 H, CH_2OPh), 5.16 and 5.18 (2× d, J = 6.6 Hz, 1 H, 5-H$_{(U)}$), 5.62–5.84 (3 H, 2′-H$_{(G)}$, 3′-H$_{(G)}$ and 1′-H$_{(U)}$), 5.93 and 5.97 (2× d, J = 5.4 and 6.6 Hz) (1 H, 1′-H$_{(G)}$), 7.12 and 7.14 (2× d, $^1J_{P,H}$ = 554 and 556 Hz for the respective isomer, 1 H, PH), 6.84–7.46 (19 H, MMT and Ph), 7.76 and 7.81 (2× s, 1 H, 8-H), 7.94 and 7.97 (2× d, J = 6.6 Hz, 1 H, 6-H$_{(U)}$), 9.25 (br. s, 1 H, NH), 10.7–11.2 (2× br. s, 1 H, NH), 11.8 (br. s, 1

H, NH) ppm. ^{31}P NMR (CD_3CN, 20 °C): δ = 39.7 and 40.3 ppm. $C_{62}H_{74}N_7O_{16}PSi$ (1232.36): calcd. C 60.43, H 6.05, N 7.96; found C 60.25, H 6.03, N 8.01.

Triethylammonium 3′-*O*-(4-Methoxytrityl)thymidine [2′-*O*-(*tert*-Butyldimethylsilyl)-3′-deoxy-5′-*O*-(4-methoxytrityl)uridine 3′-*C*-methylenephosphonate] (4a): Compound **3a** (50 mg, 0.042 mmol) was dissolved in pyridine (1.25 mL), and a solution of iodine (50 mg, 197 mmol, 4.7 equiv.) in pyridine/water (96:4, v/v; 1.25 mL) was added. The reaction mixture was stirred at 20 °C for 24 h. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with a combined solution of aqueous sodium thiosulfate (10%, w/w; 10 mL) and 2 M aqueous TEAB (10 mL). The water layer was washed with CH_2Cl_2 (10 mL). The combined organic layers were washed with 2 M aqueous TEAB (10 mL). The resulting aqueous layer was washed with CH_2Cl_2 (2× 5 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was further dried by evaporation of added toluene (5 mL) and purified by silica gel column chromatography (5–20% methanol in CH_2Cl_2 containing 0.1% triethylamine) to give **4a** (48 mg, 95%). ^{1}H NMR (CD_3OD, 20 °C): δ = 0.19 (s, 3 H, $SiCH_3$), 0.21 (s, 3 H, $SiCH_3$), 0.92 [s, 9 H, $SiC(CH_3)_3$], 1.25–1.42 [10 H, CH_2P and $N(CH_2CH_3)_3$], 1.74–1.82 (5 H, 2′-$H_{a(T)}$, 2′-$H_{b(T)}$ and $CH_{3(T)}$), 1.87–2.00 (m, 1 H, CH_2P), 2.57–2.66 (m, 1 H, 3′-$H_{(U)}$), 3.14 [q, J = 7.3 Hz, 6 H, $N(CH_2CH_3)_3$], 3.40–3.53 (3 H, 5′-$H_{b(T)}$, 5′-$H_{a(U)}$ and 5′-$H_{b(U)}$), 3.71 (s, 6 H, 2× CH_3O), 3.79 (2 H, 4′-$H_{(T)}$ and 5′-$H_{a(T)}$), 4.11–4.17 (m, 1 H, 4′-$H_{(U)}$), 4.27–4.32 (m, 1 H, 3′-$H_{(T)}$), 4.61–4.65 (m, 1 H, 2′-$H_{(U)}$), 5.13 (d, J = 8.0 Hz, 1 H, 5-$H_{(U)}$), 5.76 (d, $J_{1′,2′}$ = 2.2 Hz, 1 H, 1′-$H_{(U)}$), 6.26 (dd, $J_{1′,2′a}$ = 6.1, $J_{1′,2′b}$ = 8.1 Hz, 1 H, 1′-$H_{(T)}$), 6.82–7.45 (28 H, 2× MMT), 7.59 (s, 1 H, 6-$H_{(T)}$), 8.05 (d, J = 8.0 Hz, 1 H, 6-$H_{(U)}$) ppm. ^{13}C NMR (CD_3OD, 20 °C): δ = –4.4 ($SiCH_3$), –4.3 ($SiCH_3$), 9.2 [$N(CH_2CH_3)_3$], 12.7 ($CH_{3(T)}$), 19.0 [$SiC(CH_3)_3$], 23.6 (d, $^1J_{C,P}$ = 138 Hz, CH_2P), 26.5 [$SiC(CH_3)_3$], 39.6 (C-3′$_{(U)}$), 39.9 (C-2′$_{(T)}$), 47.7 [$N(CH_2CH_3)_3$], 55.8 (2× OCH_3), 64.5 (C-5′$_{(U)}$), 65.1 (C-5′$_{(T)}$), 76.6 (C-3′$_{(T)}$), 78.5 (C-2′$_{(U)}$), 85.1 (d, $^3J_{4′,P}$ = 13.3 Hz, C-4′$_{(U)}$), 86.4 and 86.5 (C-4′$_{(T)}$ and C-1′$_{(T)}$), 88.5, 88.8, 91.5 (C-1′$_{(U)}$), 101.8 (C-5$_{(U)}$), 111.9, 114.3, 114.4, 128.2, 128.3, 129.1, 129.7, 129.8, 131.8 131.9, 135.9, 136.9 (C-6$_{(T)}$), 138.1, 142.4 (C-6$_{(U)}$), 145.5, 146.1, 146.2, 152.0, 152.3, 160.3, 160.4, 166.2, 166.3 ppm. ^{31}P NMR (CD_3OD, 20 °C): δ = 22.7 ppm. MS (ES+/TOF): calcd. for $[C_{72}H_{89}N_5O_{14}PSi]^+$ 1306.5913; found 1306.5912.

Triethylammonium 2′,3′-Di-*O*-butyryl-2-*N*-(phenoxyacetyl)guanosine [2′-*O*-(*tert*-Butyldimethylsilyl)-3′-deoxy-5′-*O*-(4-methoxytrityl)uridine 3′-*C*-methylenephosphonate] (4b): Compound **3b** (39 mg, 0.032 mmol) was dissolved in pyridine (1 mL), and a solution of iodine (38 mg, 150 mmol, 4.7 equiv.) in pyridine/water (96:4, v/v; 1 mL) was added. The reaction mixture was stirred at 20 °C for 24 h. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with a combined solution of aqueous sodium thiosulfate (10%, w/w; 10 mL) and 2 M aqueous TEAB (10 mL). The water layer was washed with CH_2Cl_2 (10 mL). The combined organic layers were washed with 2 M aqueous TEAB (10 mL). The resulting aqueous layer was washed with CH_2Cl_2 (2× 5 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was further dried by evaporation of added toluene (5 mL) and purified by silica gel column chromatography (5–20% methanol in CH_2Cl_2 containing 0.1% triethylamine) to give **4b** (38 mg, 89%). ^{1}H NMR ([D_6]DMSO/D_2O, 4:1, v/v; 60 °C): δ = 0.09 (s, 6 H, 2× $SiCH_3$), 0.78 [t, J = 7.3 Hz, 3 H, $C(O)CH_2CH_2CH_3$], 0.82 [s, 9 H, $SiC(CH_3)_3$], 0.93 [t, J = 7.4 Hz, 3 H, $C(O)CH_2CH_2CH_3$], 1.18 [t, J = 7.3 Hz, 6 H, 2× $N(CH_2CH_3)_3$], 1.30–1.37 (m, 1 H, CH_2P), 1.45 [h, J = 7.2 Hz, 2 H, $C(O)CH_2CH_2CH_3$], 1.59 [h, J = 7.3 Hz, 2 H, $C(O)CH_2CH_2CH_3$], 1.81–1.91 (m, 1 H, CH_2P), 2.21 [dt, J = 7.2 and 3.5 Hz 2 H, $C(O)CH_2CH_2CH_3$], 2.36 [dt, J = 7.2 and 1.6 Hz, 2 H, $C(O)CH_2CH_2CH_3$], 2.47–2.56 (m, 1 H, 3′-$H_{(U)}$), 3.02 [q, J = 7.4 Hz, 1 H, 2× $N(CH_2CH_3)_3$], 3.31 (br. d, J = 10 Hz, 1 H, 5′-$H_{a(U)}$), 3.44 (dd, ^2J = 11, $J_{4′,5′}$ = 3.7 Hz, 1 H, 5′-$H_{b(U)}$), 3.69 (s, 3 H, OCH_3), 3.82–3.88 (m, 1 H, 5′-$H_{a(G)}$), 4.12–4.27 (3× m, 1 H, 4′-$H_{(U)}$, 1 H, 5′-$H_{b(G)}$, 1 H, 4′-$H_{(G)}$), 4.53 (dd, $J_{2′,3′}$ = 5.0 Hz, 1 H, 2′-$H_{(U)}$), 4.78 (d, ^2J = 16.2 Hz, 1 H, CH_2OPh), 4.89 (d, ^2J = 16.2 Hz, 1 H, CH_2OPh), 5.10 (d, J = 8.9 Hz, 1 H, 5-$H_{(U)}$), 5.59 (dd, ^3J = 4.2 and 2.3 Hz, 1 H, 3′-$H_{(G)}$), 5.68 (d, $J_{1′,2′}$ = 2.8 Hz, 1 H, 1′-$H_{(U)}$), 6.01–6.05 (2 H, 1′-$H_{(G)}$ and 2′-$H_{(G)}$), 6.82–7.38 (19 H, MMT and Ph), 7.73 (d, ^2J = 8.1 Hz, 1 H, 6-$H_{(U)}$), 8.12 (s, 1 H, 8-$H_{(G)}$) ppm. ^{13}C NMR ([D_6]DMSO/D_2O, 4:1, v/v; 60 °C): δ = –5.18 ($SiCH_3$), –5.08 ($SiCH_3$), 8.60 [$N(CH_2CH_3)_3$], 13.0 [$C(O)CH_2CH_2CH_3$], 13.3 [$C(O)CH_2CH_2CH_3$], 17.56 and 17.58 [$SiC(CH_3)_3$ and $C(O)CH_2CH_2CH_3$], 17.8 [$C(O)CH_2CH_2CH_3$], 22.4 (d, $^1J_{C,P}$ = 135 Hz, CH_2P), 25.5 [$SiC(CH_3)_3$], 34.9 [$C(O)CH_2CH_2CH_3$], 35.2 [$C(O)CH_2CH_2CH_3$], 38.4 (C-3′$_{(U)}$), 46.2 [$N(CH_2CH_3)_3$], 55.0 (OCH_3), 62.5 (C-5′$_{(U)}$), 63.7 (C-5′$_{(G)}$), 66.1 (CH_2OPh), 71.3 (C-3′$_{(G)}$), 71.8 (C-2′$_{(G)}$), 76.9 (d, $^3J_{C,P}$ = 6.8 Hz, C-2′$_{(U)}$), 82.6 (d, $^3J_{C,P}$ = 7.3 Hz, C-4′$_{(G)}$), 83.3 (d, $^3J_{C,P}$ = 11.3 Hz, C-4′$_{(U)}$), 86.5 and 86.8 [$C(Ar)_3$ and C-1′$_{(G)}$], 89.6 (C-1′$_{(U)}$), 100.9 (C-5$_{(U)}$), 113.2, 114.5, 121.2, 121.3, 126.9, 127.00, 127.8, 127.9, 128.1, 129.5, 130.1, 134.7, 140.0 (C-6$_{(U)}$ and C-8$_{(G)}$), 143, 8, 143.9, 147.2, 148.5, 150.3, 157.6, 158.3, 163.2, 171.6, 171.7, 172.1 ppm. ^{31}P NMR ([D_6]DMSO/D_2O, 4:1, v/v; 60 °C): δ = 20.51 ppm. MS (ES+/TOF): calcd. for $[C_{68}H_{90}N_8O_{17}PSi]^+$ 1349.5931; found 1349.5934.

2′,3′,4′-Tri-*O*-butyryluridine (5): Uridine (100 mg, 0.41 mmol) was dried by evaporation of added pyridine (2× 2 mL) and dissolved in dry pyridine (4 mL). Butyric anhydride (0.23 mL, 1.43 mmol, 3.5 equiv.) was added, and the reaction mixture was stirred at 20 °C for 25 h. The reaction mixture was concentrated. The residue was diluted with CH_2Cl_2 (20 mL) and washed with saturated aqueous $NaHCO_3$ (10 mL). The water layer was washed with CH_2Cl_2 (2× 10 mL). The combined organic layers were dried (Na_2SO_4), diluted with toluene (5 mL) and concentrated. The residue was purified by silica gel column chromatography (stepwise gradient of 1–3% methanol in CH_2Cl_2). The product was obtained as an oil and lyophilised from 1,4-dioxane to give **5** as an oil (184 mg, 99%). ^{1}H NMR ([D_6]DMSO, 20 °C): δ = 0.82–0.92 [9 H, $C(O)CH_2CH_2CH_3$], 1.50–1.56 [6 H, $C(O)CH_2CH_2CH_3$], 2.28–2.35 [6 H, $C(O)CH_2CCH_3$], 4.21–4.34 (3 H, 4′-H, 5′-H_a and 5′-H_b), 5.35 (t, J = 5.2 Hz, 1 H, 2′-H), 5.46 (t, J = 5.5 Hz, 1 H, 3′-H), 5.72 (d, J = 8.0 Hz, 1 H, 5-H), 5.88 (dd, J = 5.2 and 1.9 Hz, 1 H, 1′-H), 7.71 (d, J = 8.0 Hz, 1 H, 6-H), 11.5 (s, 1 H, NH) ppm. ^{13}C NMR ($CDCl_3$, 20 °C): δ = 13.9 [$C(O)CH_2CH_2CH_3$], 14.0 [$C(O)CH_2CH_2CH_3$], 14.1 [$C(O)CH_2CH_2CH_3$], 18.6 [$C(O)CH_2CH_2CH_3$], 18.68 [$C(O)CH_2CH_2CH_3$], 18.71 [$C(O)CH_2CH_2CH_3$], 36.0 [$C(O)CH_2CH_2CH_3$], 36.1 [$C(O)CH_2CH_2CH_3$], 36.4 [$C(O)CH_2CH_2CH_3$], 63.4 (C-5′), 70.5 and 73.0 (C-2′ and C-3′), 80.6 (C-4′), 87.6 (C-1′), 103.5 (C-5), 139.3 (C-6), 150.2, 162.6, 172.4, 172.9 ppm. $C_{21}H_{30}N_2O_9$ (454.48): calcd. C 55.50, H 6.65, N 6.16; found C 55.30, H 6.70, N 6.20.

2′,3′,4′-Tri-*O*-butyryl-2-*N*-(phenoxyacetyl)guanosin (6): 2-*N*-(Phenoxyacetyl)guanosine (100 mg, 0.24 mmol) was dried by evaporation of added pyridine (2× 2 mL) and dissolved in dry pyridine (4 mL). Butyric anhydride (0.16 mL, 0.96 mmol, 3.5 equiv.) was added, and the reaction mixture was stirred at 20 °C for 25 h. The reaction mixture was concentrated. The residue was diluted with CH_2Cl_2 (20 mL) and washed with saturated aqueous $NaHCO_3$ (10 mL). The water layer was washed with CH_2Cl_2 (2× 10 mL). The combined organic layers were dried (Na_2SO_4). Toluene (5 mL) was added, and the organic layer was concentrated. The residue was purified by silica gel column chromatography (stepwise gradient of

1–3% methanol in CH_2Cl_2). The product was obtained as an oil and lyophilised from 1,4-dioxane to give crystalline **6** (122 mg, 81%). ^{1}H NMR ([D$_6$]DMSO, 20 °C): δ = 0.79–0.94 [9 H, C(O)-$CH_2CH_2CH_3$], 1.47–1.59 [6 H, C(O)$CH_2CH_2CH_3$], 2.22–2.44 [6 H, C(O)$CH_2CH_2CH_3$], 4.29–4.43 (3 H, 4′-H, 5′-H$_a$ and 5′-H$_b$), 4.88 (s, 2 H, CH_2OPh), 5.55 (dd, J = 5.9 and 3.8 Hz, 1 H, 3′-H), 5.87 (t, J = 6.0 Hz, 1 H, 2′-H), 6.09 (d, J = 6.1 Hz, 1 H, 1′-H) 6.97–7.01 and 7.28–7.56 (5 H, Ph), 8.27 (s, 1 H, 8-H), 11.69 (br. s, 1 H, NH), 11.85 (br. s, 1 H, NH) ppm. ^{13}C NMR ($CDCl_3$, 20 °C): δ = 13.57 [2× C(O)$CH_2CH_2CH_3$] 13.64 [C(O)$CH_2CH_2CH_3$], 18.2 [2× C(O)$CH_2CH_2CH_3$], 18.3 [C(O)$CH_2CH_2CH_3$], 35.6 [C(O)-$CH_2CH_2CH_3$], 35.7 [C(O)$CH_2CH_2CH_3$], 35.8 [C(O)$CH_2CH_2CH_3$], 62.6 (C-5′), 66.8 (CH_2OPh), 70.1 (C-3′), 72.9 (C-2′), 79.8 (C-4′), 86.8 (C-1′), 114.9, 122.7, 122.9, 129.9, 138.1 (C-8), 146.4, 147.3, 155.1, 156.4, 169.7, 172.0, 172.3, 173.1 ppm. $C_{30}H_{37}N_5O_{10}$ (627.65): calcd. C 57.41, H 5.94, N 11.16; found C 57.24, H 6.01, N 11.14.

General Procedure for Analysis of the Coupling of Phosphinate 1 to 2a

Method A (for Acyl Chlorides as Coupling Reagents): A solution of phosphinate **1** (8.6 mg, 10.8 μmol) in dry pyridine (50 μL) was transferred to an NMR tube. The solution was concentrated and the residue was dried at 50 mbar overnight. The NMR tube with compound **1** was equipped with a co-axial inner tube containing H_3PO_4 (2%, v/v) in D_2O, and a solution of 57 mM **2a** in dry acetonitrile/pyridine (3:1, v/v; 180 μL, 10.3 μmol, 0.95 equiv.) was added. A 120 or 240 mM solution of the coupling agent in dry acetonitrile/pyridine (3:1, v/v; 180 μL, 21.6 or 43.2 mmol, 2 or 4 equiv.) was added, and the reaction was monitored by ^{31}P NMR spectroscopy. The signals of the two isomeric products were observed at δ = 37.7 and 37.0 ppm for all reactions carried out in acetonitrile/pyridine (3:1, v/v) with a variation of 0.1–0.2 ppm. The formation of product was measured using the integral from the H_3PO_4 signal as reference. The integral of the product was plotted against time, and this was used to obtain times for 75 and 90% completion of reactions. When the reaction was complete an additional ^{1}H-coupled spectrum was recorded.

Method B (for OXP as Coupling Reagent): The reaction and analysis of the coupling using 36 mM OXP in actonitrile/pyridine (3:1, v/v) was carried out essentially according to Method A, except that a reagent solution of 72 mM OXP (13.0 mmol, 1.2 equiv.) in 180 μL of dry acetonitrile/pyridine (3:1, v/v) or pyridine was used. The reaction and analysis of the coupling using 120 or 60 mM OXP in neat pyridine or acetonitrile/pyridine (3:1, v/v) was carried out essentially according to Method A, except that the solvent proportions of the stock solutions were different. Either neat pyridine was used as solvent or in the case where the reactions were carried out in a mixture of acetonitrile/pyridine (3:1, v/v), the reaction mixture was prepared by adding first a solution of 57 mM **2a** in dry acetonitrile (180 μL, 10.3 μmol, 0.95 equiv.) to the NMR tube with compound **1** (8.6 mg, 10.8 μmol) and then a solution of 240 or 120 mM OXP in dry acetonitrile/pyridine (1:1, v/v; 180 μL, 21.6 or 43.2 mmol, 2 or 4 equiv.). The signals of the two isomeric products were observed at δ = 37.7 and 37.0 ppm for all reactions carried out in acetonitrile/pyridine (3:1, v/v) and at δ = 37.0 and 36.1 ppm in pyridine, with a variation of 0.1–0.2 ppm.

Method C (for Chlorophosphates, Uronium or Phosphonium Salts as Coupling Reagents): The reaction and analysis of the coupling was carried out essentially according to Method A, except that the solvent proportions of the stock solutions were different. The reaction mixtures were prepared by adding first a solution of 57 mM **2a** in dry acetonitrile/pyridine (1:1, v/v;180 μL, 10.3 μmol, 0.95 equiv.) to the NMR tube with compound **1** (8.6 mg, 10.8 μmol) and then a solution of 120 or 240 mM coupling reagent in dry acetonitrile (180 μL, 21.6 or 43.2 mmol, 2 or 4 equiv.).

In case of using a chlorophosphate as coupling agent, the 5′-*O*-phosphorylated side products were identified by addition of an internal standard (ca. 0.5 equiv.). The internal standard was prepared in advance prior to use by mixing 120 mM chlorophosphate in dry acetonitrile (45 μL, 5.4 mmol) and 53 mM **2a** in dry acetonitrile/pyridine (1:1, v/v; 90 μL, 4.8 mmol). Side products resulting from O^4-phosphorylation were detected by ^{31}P NMR spectroscopy and in several cases also by mass pectrometry (see above). Signals that corresponded to compounds to hydrolysis, such as remaining chlorophosphate, were also identified by their absence in the spectra taken after the addition of water (10–20 μL) to the reaction mixture.

Procedure for the Coupling of Phosphinate 1 to 2b and Analysis of the Formation of Side Products by Subsequent Reaction of the Guanosine Moiety by the Coupling Reagents DMOCP and OXP: The coupling of compound **1** to **2b** using 120 (or 60) mM DMOCP or 120 (or 60) mM OXP were carried out according to Methods B and C, respectively. After complete coupling, the formation of side products was monitored by ^{31}P NMR spectroscopy. The nature of side products resulting from O^6-phosphorylation or reaction of OXP at the O^6-position of guanosine was, apart from being detected by ^{31}P NMR spectroscopy, confirmed by mass spectrometry (ESI-TOF) after a reaction time of 6 h, which gave the correct mass for the corresponding phosphorylated **3b** in the reaction mixture containing DMOCP (ES+/TOF: m/z = 1380.5 [$C_{67}H_{84}N_7O_{19}P_2Si$]$^+$) and the pyridinium adduct (which is formed in a subsequent step after the reaction at the O^6-position) in the reaction mixture containing OXP (ES+/TOF: m/z = 1293.5 [$C_{67}H_{78}N_8O_{15}PSi$]$^+$).

Acknowledgments

We thank the Swedish Research Council and the Swedish Foundation for Strategic Research for financial support.

[1] A. De Mesmaeker, R. Häner, P. Martin, H. E. Moser, *Acc. Chem. Res.* **1995**, *28*, 366–374.
[2] S. M. Freier, K.-H. Altmann, *Nucleic Acids Res.* **1997**, *25*, 4429–4443.
[3] E. Uhlmann, A. Peyman, *Chem. Rev.* **1990**, *90*, 543–584.
[4] D. M. Dykxhoorn, J. Lieberman, *Cell* **2006**, *126*, 231–235.
[5] T. Tuschl, *ChemBioChem* **2001**, *2*, 239–245.
[6] M. Warashina, Y. Takagi, W. J. Stec, K. Taira, *Curr. Opin. Biotechnol.* **2000**, *11*, 354–362, and references cited therein.
[7] H. P. Albrecht, G. H. Jones, J. G. Moffatt, *J. Am. Chem. Soc.* **1970**, *92*, 5511.
[8] A. Mazur, B. E. Tropp, R. Engel, *Tetrahedron* **1984**, *40*, 3949–3956.
[9] S. P. Collingwood, M. E. Douglas, F. Natt, U. Pieles, *Phosphorus Sulfur Silicon Relat. Elem.* **1999**, *144–146*, 645–648.
[10] P. J. Garegg, T. Regberg, J. Stawinski, R. Strömberg, *Chem. Scr.* **1985**, *25*, 280–282.
[11] P. J. Garegg, T. Regberg, J. Stawinski, R. Strömberg, *Chem. Scr.* **1986**, *26*, 59–62.
[12] B. C. Froehler, M. D. Matteucci, *Tetrahedron Lett.* **1986**, *27*, 469–472.
[13] P. J. Garegg, I. Lindh, T. Regberg, J. Stawinski, R. Strömberg, C. Henrichson, *Tetrahedron Lett.* **1986**, *27*, 4051–4054.
[14] P. J. Garegg, I. Lindh, T. Regberg, J. Stawinski, R. Strömberg, C. Henrichson, *Tetrahedron Lett.* **1986**, *27*, 4055–4058.
[15] B. C. Froehler, P. G. Ng, M. D. Matteucci, *Nucleic Acids Res.* **1986**, *14*, 5399–5407.

[16] J. Stawinski, R. Strömberg, T. Szabo, *Coll. Czech. Chem. Commun.* **1990**, *55*, 145–148.
[17] H. An, T. Wang, M. A. Maier, M. Manoharan, B. S. Ross, P. D. Cook, *J. Org. Chem.* **2001**, *66*, 2789–2801.
[18] P. D. Cook, H. An, T. Wang, M. Manoharan, *PCT Int. Appl.* **2001**, WO01/02415.
[19] A. Winqvist, R. Strömberg, *Eur. J. Org. Chem.* **2001**, 4305–4311.
[20] A. Winqvist, R. Strömberg, *Eur. J. Org. Chem.* **2002**, 1509–1515.
[21] A. Winqvist, R. Strömberg, *Eur. J. Org. Chem.* **2002**, 3140–3144.
[22] J. Stawinski, R. Strömberg, *Trends Org. Chem.* **1993**, *4*, 31–67.
[23] R. Strömberg, J. Stawinski, *Current Protocols in Nucleic Acids Chemistry*, John Wiley and Sons, New York, **2000**, chapters 3.4.1–3.4.11.
[24] P. J. Garegg, T. Regberg, J. Stawinski, R. Strömberg, *Nucleosides Nucleotides* **1987**, *6*, 655–662.
[25] P. J. Garegg, J. Stawinski, R. Strömberg, *J. Chem. Soc. Perkin Trans. 2* **1987**, 1209–1214.
[26] T. Regberg, J. Stawinski, R. Strömberg, *Nucleosides Nucleotides* **1988**, *7*, 23–35.
[27] R. Strömberg, J. Stawinski, *Nucleic Acids Symp. Ser.* **1987**, *18*, 185–188.
[28] C. B. Reese, Q. Song, *Nucleic Acids Res.* **1999**, *27*, 963–971.
[29] C. B. Reese, Q. Song, *J. Chem. Soc. Perkin Trans. 1* **1999**, 1477–1486.
[30] R. W. Adamiak, E. Biala, B. Skalski, *Nucleic Acids Res.* **1985**, *13*, 2989–3003.
[31] T. Wada, Y. Sato, F. Honda, S.-I. Kawahara, M. Sekine, *J. Am. Chem. Soc.* **1997**, *119*, 12710–12721.
[32] J. Diago-Meseguer, A. L. Palomo-Coll, J. R. Fernandez-Lizarbe, A. Zugaza-Bilbao, *Synthesis* **1980**, 547–551.
[33] J. Cabre-Castellvi, A. Palomo-Coll, A. L. Palomo-Coll, *Synthesis* **1981**, 616–620.
[34] R. Strömberg, J. Stawinski, *Nucleosides Nucleotides* **1987**, *6*, 815–820.
[35] C. B. Reese, A. Ubasawa, *Nucleic Acids Symp. Ser.* **1980**, 5–21.
[36] C. B. Reese, K. H. Richards, *Tetrahedron Lett.* **1985**, *26*, 2245–2248.
[37] R. W. Adamiak, E. Biala, Z. Gdaniec, S. Mielewczyk, B. Skalski, *Chem. Scr.* **1986**, *26*, 3–6.
[38] R. W. Adamiak, E. Biala, Z. Gdaniec, S. Mielewczyk, B. Skalski, *Chem. Scr.* **1986**, *26*, 7–11.
[39] C. B. Reese, P. A. Skone, *J. Chem. Soc. Perkin Trans. 1* **1984**, 1263–1271.
[40] T. Trichtinger, R. Charubala, W. Pfleiderer, *Tetrahedron Lett.* **1983**, *24*, 711–714.
[41] T. Høeg-Jensen, C. E. Olsen, A. Holm, *J. Org. Chem.* **1994**, *59*, 1257–1263.
[42] C. Patois, L. Ricard, P. Savignac, *J. Chem. Soc. Perkin Trans. 1* **1990**, 1577–1581.
[43] C. Chaix, D. Molko, R. Teoule, *Tetrahedron Lett.* **1989**, *30*, 71–74.
[44] K. K. Ogilvie, R. L. Letsinger, *J. Org. Chem.* **1967**, *32*, 2365–2366.
[45] T. Wu, K. K. Ogilvie, R. T. Pon, *Tetrahedron Lett.* **1988**, *29*, 4249–4252.

Received: June 8, 2007
Published Online: February 11, 2008

