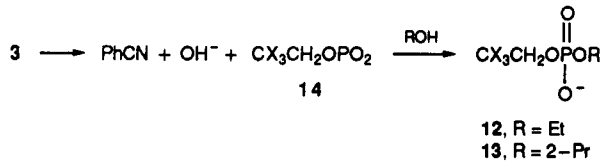
Table I. Summary of Results from Heating 3a in Different Solvents[a]

expt	E/Z ratio of 3a	solvent	temp, °C	time, h	products (yield, %)
1	90/10	water	100	14	s.m. recovered (100)
2	90/10	MeOH	64	12	s.m. recovered (100)
3	90/10	EtOH	78	12	**12a** (100) + PhCN (98)
4	90/10	2-PrOH	82	6	**13a** (100) + PhCN (N.D)
5	90/10	EtOH 2-PrOH 7:3	80	10	**12a** (65) + **13a** (35) + PhCN (95)
6	90/10	MeCN + 5 equiv of 2-PrOH	80	19	**13a** (100) + PhCN (100)
7	90/10	PhMe	111	72	s.m. (50) + **4a** (50) + PhCN (N.D.)
8	90/10	THF	67	24	s.m. (50) + **4a** (50) + PhCN (N.D.)
9	90/10	MeCN	80	14	**4a** (100) + PhCN (94)
10	90/10	EtCN	97	22	s.m. (27) + **4a** (73) + PhCN (N.D.)
11	20/80	2-PrOH	82	27	unreacted isomerized s.m. *E*:*Z* = 64:36
12	20/80	MeCN	80	5	isomerized s.m. (*E*:*Z* = 62:38)
13	20/80	MeCN	80	12	(*E*)-**3a** (50) + (*Z*)-**3a** (10) + **4a** (40)
14	20/80	MeCN	80	22	**4a** (100) + PhCN (99)

[a] Reaction mixtures were monitored by ^{31}P NMR spectroscopy. The durations of reactions given for 100% yields are accurate within 30 min. Chemical shifts of products: **4a** δ −11.3 (t); **12a** δ 0.45 (quint); **13a** δ −0.33 (q). Benzonitrile was determined by high performance liquid chromatography on a RP-18 column using methanol:water (60:40). s.m. = starting material. N.D. = not determined.

Scheme V[a]



[a] **a**, X = F; **b**, X = Cl.

Table II. Summary of Results from Heating 3b in Different Solvents[a]

expt	E/Z of 3b	solvent	temp, °C	time, h	products (yield, %)
1	75/25	2-PrOH	82	10	**13b** (100) + PhCN (95)
2	13/87	2-PrOH	82	44	(*E*)-**3b** (23) + **13b** (77) + PhCN (73)
3	75/25	MeCN	80	16	**4b** (100) + PhCN (97)
4	13/87	MeCN	80	15	(*E*)-**3b** (22) + (*Z*)-**3b** (29) **4b** (49) + PhCN (N.D.)
5	90/10	PhMe	111	72	s.m. (86) + **4b** (14) + PhCN

[a] Reaction mixtures were monitored by ^{31}P NMR spectroscopy. The durations of reactions given for 100% yields are accurate within 30 min. Chemical shifts of products: **4b** δ −12 (t); **13b** δ −1.9 (q). Benzonitrile was determined by high performance liquid chromatography on a RP-18 column using methanol:water (60:40). s.m. = starting material. N.D. = not determined.

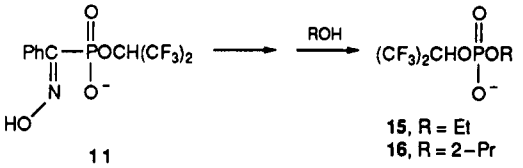
Table III. Summary of Results from Heating 11 (*E*/*Z* = 93:7) in Different Solvents[a]

expt	solvent	temp, °C	time, h	products (yield, %)
1	EtOH	78	21	s.m. (35) + PhCN (66) + **15** (65)
2	2-PrOH	82	6	**16** (100) + PhCN (92)
3	MeCN	80	21	s.m. (42) + **17** (58) + PhCN (N.D.)

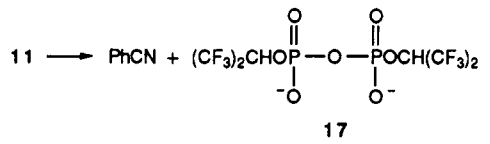
[a] Reaction mixtures were monitored by ^{31}P NMR spectroscopy. The durations of reactions given for 100% yields are accurate within 30 min. Chemical shifts of products: **17** δ −11.2 (d); **15** δ −1.0 (m); **16** δ −1.9 (q). Benzonitrile was determined by high performance liquid chromatography on a RP-18 column using methanol:water (60:40). s.m. = starting material. N.D. = not determined.

solvents, the results in Table I illustrate the dependence of rate on the polarity of the medium. For example, from the data listed in Table I it appears that **3a** undergoes complete fragmentation in acetonitrile several hundreds times faster (taking into account the difference in boiling points between the two solvents) than in toluene. It appears that in polar solvents the reaction rate is enhanced. This rate enhancement is likely to be the result of increased stabilization of the transition state leading to fragmentation and of better solvation of the leaving hydroxy group

Scheme VI



Scheme VII



by the more polar solvents. In aprotic solvents, in the absence of reactive compounds, dimerization of the metaphosphate is observed, as seen in the fragmentation of other types of metaphosphate precursors in such conditions.[8,12,13]

(ii) Stereospecificity. Examination of Table I reveals that the predominantly (*Z*)-**3a** does not undergo fragmentation in 2-propanol in 27 h. On the other hand, heating (*Z*)-**3a** in acetonitrile causes first a slow isomerization to the (*E*)-isomer, which is then followed by fragmentation. Similarly it can be seen in Table II that predominantly (*E*)-**3b** undergoes complete fragmentation in refluxing 2-propanol in 10 h, while the predominantly (*Z*)-**3b** gives in the same solvent in 44 h 77% fragmentation and 23% isomerization to the (*E*)-isomer. A similar trend is seen in refluxing acetonitrile. Thus, it appears that the fragmentation is a specific characteristic of the (*E*)-isomers.

It was noted earlier in our laboratory that the acid-catalyzed fragmentation of methyl hydrogen (α-hydroxyiminobenzyl)phosphonate is specific for the (*E*)-isomer.[3] This was interpreted in terms of stereoelectronic assistance by the nonbonding electron pairs of the P–O oxygens in the C–P bond-breaking process.[3] It appears that the present reaction too can be rationalized in similar trends. Additional analogy can be found between the present fragmentation and the Beckmann fragmentation of oximes.[14] Such fragmentation, which occurs when the

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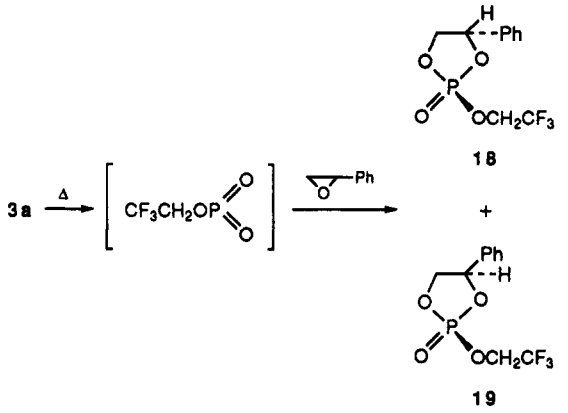
(13) Ramirez, F.; Marecek, J. F.; Yemul, S. S. *J. Am. Chem. Soc.* **1982**, *104*, 1345.

(14) Deslongchamps, P. *Stereoelectronic effects in organic chemistry*; Pergamon Press: Oxford, 1983; pp 298–9, and references cited therein.

Scheme VIII



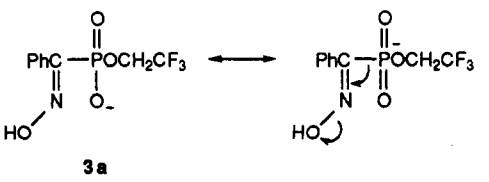
potential migrating group, oriented *anti* to the N–OH group, can form a relatively stable carbonium ion,[15] also yields the corresponding nitrile.

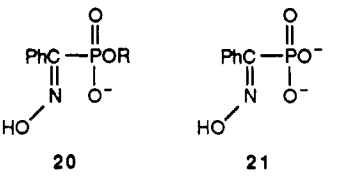
Trapping of Metaphosphate by Styrene Oxide. Metaphosphates are highly electrophilic species in solution.[16,17] In addition to their capacity to phosphorylate amines[10] and hydroxy compounds,[1-5,9,13,16] they have also been shown to perform electrophilic substitutions on activated aromatic rings, such as anilines[18] and *N*-methylpyrrole.[19] On the other hand, it has been established that some low-coordination phosphorus compounds react with epoxides with ring opening followed by subsequent formation of cyclic or polymeric products.[20] Recently, successful trapping of metaphosphate by epoxide was reported.[21] When we carried out the fragmentation of **3a** in the presence of 5 equiv of styrene oxide by reflux in dry acetonitrile, a solid product was formed. Examination of this product by ^{31}P NMR revealed only two signals in the range 17.6–17.8 ppm,[22] consistent with the formation of structures **18** and **19** in the reaction (Scheme VIII). The product (a mixture of two diastereoisomers which were not separated) was isolated and the structures were also confirmed by ^{1}H NMR and mass spectrometry.

Conclusion

In order to rationalize the thermal behavior of compounds of type **3** described here, we consider the contrast in behavior between the α-hydroxyimino phosphonic cids and monoesters. While simple monoester anions **20** were stable for 30 h in refluxing acetonitrile, dianions **21** exhibit a tendency to undergo fragmentation.[1]

Scheme IX





This indicates that one negative charge on the phosphonate group is insufficient, but two charges are adequate to provide electrons for the C–P bond fission and the departure of the oxime hydroxy group. It seems therefore reasonable to assume that the polyhaloalkyl and similar groups (e.g. *p*-chlorophenyl[23]) exert their influence through their electron-withdrawing effect. We assume that the contribution of hexavalent phosphorus resonance forms (Scheme IX) would result in an increase in the electron density on the phosphorus, which in turn would assist in the C–P bond breaking (as indicated) and the departure of the hydroxide leaving group.

The results presented indicate that α-hydroxyimino phosphonate esters with electron-withdrawing polyhaloalkoxy groups have the potential to phosphorylate hydroxy groups under neutral conditions and to yield 2,2,2-trihaloethyl or 1,1,1,3,3,3-hexafluoro-2-propyl phosphates. Since such groups are known to be useful phosphate protecting groups,[6] it appears that phosphonates of type **3** and **11** have the potential of becoming useful reagents.

Experimental Section

General. For the instruments used and for the preparation of compounds **3a** and **3b** see ref 5.

1,1,1,3,3,3-Hexafluoro-2-propyl Methyl Benzoylphosphonate (9). To a solution of methyl benzoylphosphonochloridate (8,[8] 21.8 g, 0.1 mol) in dry dichloromethane (70 mL) was added dropwise with stirring at 0 °C under nitrogen a solution of pyridine (9 mL, 0.11 mol) and 1,1,1,3,3,3-hexafluoro-2-propanol (16.8 g, 0.1 mol) in dry dichloromethane (70 mL). After the reaction mixture had been stirred for 2 h at ambient temperature, the solvent was removed at reduced pressure and the residue was taken up in anhydrous ester. Pyridinium chloride was removed by filtration and evaporation of the ether yielded 31 g (0.088 mol, 88%) of crude **9** as an oil: ^{31}P NMR (CH_2Cl_2) δ −1.3 (quint); IR (neat) CH 3055, C=O 1650, C=C 1592, P=O 1260 cm^{-1}. This product was used immediately without further purification for the synthesis of compound **10**.

Lithium 1,1,1,3,3,3-Hexafluoro-2-propyl Benzoylphosphonate (10). A solution of **9** (17.5 g, 0.05 mol) in dry acetonitrile (100 mL) was added to a solution of lithium bromide (4.8 g, 0.055 mol) in dry acetonitrile (30 mL). After the reaction mixture was stirred overnight at room temperature, the precipitated salt was filtered off, washed with dry acetonitrile, and dried in air: yield 95%; IR (KBr) 3050, 1650, 1594, 1260, 1090 cm^{-1}; ^{31}P NMR (D_2O) δ −2.0 (d, J = 11 Hz); ^{1}H NMR (D_2O) δ 8.17 (2H), 7.7 (1H, t), 7.58 (2H, t), 5.41 (1H, m). Anal. Calcd for $C_{10}H_6F_6O_4PLi$: C, 35.1, H, 1.75. Found: C, 35.4, H, 2.03.

(15) Although carbonium ions and metaphosphates[16] are very different from the standpoint of electronic configuration, there are certain features common to both types of these reactive intermediates. They both are frequently formed in dissociative-type reactions, they both are electrophilic, planar, and they both are intermediates leading to racemizations.

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(22) Signals in the range of 17–18 ppm are characteristic of 5-membered cyclic phosphates: Gallagher, M. J. In *Phosphorus-31 NMR Spectroscopy in Stereochemical Analysis*; Verkade, J. G., Quin, L. D.; Eds.; VCH Publishers: Deerfield Beach, FL, 1987; pp 308–310.

(23) Breuer, E.; Mahajna, M. Unpublished results.

Lithium 1,1,1,3,3,3-hexafluoro-2-propyl (α-Hydroxyiminobenzyl)phosphonate (11). Hydroxylamine free base was prepared by neutralizing hydroxylamine hydrochloride (8.34 g, 0.12 mol) in methanol (50 mL) with sodium methoxide freshly prepared by dissolving sodium (2.8 g, 0.12 mol) in methanol. After the precipitated sodium chloride was filtered off, the solvent was removed under reduced pressure and the residue was dissolved in dry dichloromethane (50 mL) was added to a suspension of **10** (34 g, 0.1 mol) in dry dichloromethane (150 mL). After the reaction mixture was stirred at room temperature for 3 h, the solvent was removed at reduced pressure and the residue salt was washed several times with dry acetonitrile and dried in air; yield 85%; IR (KBr) 3050, 1640, 1594, 1260, 1090 cm^{-1}; ^{31}P NMR (D_2O) δ 4.24 (d, J = 11 Hz) (93%, (*E*)-11), −0.5 (d, J = 11 Hz) (7%, (*Z*)-11); ^1H NMR (D_2O) δ 7.51–7.35 (5H, m), 5.3 (1H, m). No good analysis could be obtained for this compound. The low values obtained for C, H, and N, together with the good ^{31}P and ^1H NMR spectra, indicate the probable presence of an inorganic impurity, which could not be removed.

Thermal Fragmentation of 3 and 11. A suspension of **3** (100 mg) in dry solvent (10 mL) was heated to reflux. The progress of the reaction was monitored by ^{31}P NMR spectroscopy. After removal of the solvent the residue was also analyzed by IR and HPLC for benzonitrile.

Trapping of Metaphosphate by Styrene Oxide. A suspension of **3a** (360 mg, 1.25 mmol) and styrene oxide (0.7 mL, 6 mmol) in dry acetonitrile (15 mL) was refluxed for 15 h. The reaction mixture was cooled in an ice bath. The white precipitate was filtered, washed several times with dry acetone and dry acetonitrile, and dried to yield 280 mg (80%) of **18** and **19** as a mixture of diastereoisomers: MS m/z 282 (M^+); ^{31}P NMR ($CDCl_3$) δ 17.6, 17.8 (1:1); ^1H NMR ($CDCl_3$) δ 7.54–7.43 (5H, m), 5.5 (1H, m), 4.55 (2H, m), 4.15 (2H, m).

Diisopropylsilyl-Linked Oligonucleotide Analogs: Solid-Phase Synthesis and Physicochemical Properties

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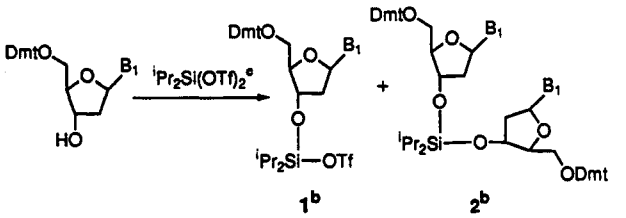
A novel synthetic method has been developed for efficient preparation of silyl-linked oligodeoxyribonucleotide analogs. The method allows, for the first time, automated solid-phase synthesis of long oligomers uniformly linked with the silyl internucleoside bridge. Synthesis of a thymidylate decanucleotide analog (14) illustrates this advance. The preparation of chimeric oligodeoxyribonucleotides containing single or multiple diisopropylsilyl backbone structures along with natural phosphodiester links is also described. These mixed backbone DNA strands were soluble and chemically stable in buffered aqueous solutions, as required for physicochemical study. These oligomers demonstrated excellent stability toward cleavage by 3′-exonuclease and good binding affinity with complementary oligonucleotides.

Introduction

Analogs of antisense oligodeoxyribonucleotides are of interest as potential antiviral, antibacterial, and anticancer agents.[1] To overcome the enzyme lability and drug delivery limitations of natural phosphate-linked antisense oligomers, various oligonucleotide analogs have been prepared.[2] Practical utility requires these analogs to have good cell penetration properties, resistance to degradation by nucleases, sequence specific hybridization to target nucleic acids, and an accessible chemical synthesis. The dialkylsilyl internucleoside linkage, first described in 1985,[3a] is attractive due to its neutral, achiral, and lipophilic properties and apparently simple synthesis. The development of this backbone, however, has been hampered for two reasons. The reported synthesis gives low yields of even relatively short oligomers, and the silyl ether products are insoluble in aqueous systems.

We have developed a method for high yielding solution synthesis of short oligomers and automated solid-phase synthesis of long oligomers of any sequence containing the diisopropylsilyl internucleoside linkage. This method involves preparation of intermediate 3′-*O*-diisopropylsilyl triflate **1** free from 3′,3′ dimers **2** which result from self-condensation. The formation of this self-condensation product in the initial silylation step[3b] has been a major impediment to successful solid-phase synthesis of oligonucleotide analogs uniformly linked by the dialkylsilyl internucleoside bridge. To permit evaluation of the physicochemical and biological properties of the dialkylsilyl internucleoside linkage, we have also developed a method for synthesis of mixed dialkylsilyl–phosphodiester backbone DNA strands, which are soluble and chemically stable in buffered aqueous systems and suitable for physicochemical study.

Scheme I



[a]Dmt = dimethoxytrityl, iPr = isopropyl, OTf = trifluoromethanesulfonyl. [b]**a**, B_1 = T; **b**, B_1 = A^{Bz}; **c**, B_1 = C^{Bz}; **d**, B_1 = G^{iB}; Bz = benzoyl, iB = isobutyryl. [c]Method 1: imidazole; ratio 1:2 = 1:1. Method 2: 2,6-di-*tert*-butyl-4-methylpyridine (Dtbp); ratio 1:2 > 20:1.

Results and Discussion

Our initial synthesis of the diisopropylsilyl-linked dinucleotide analog **3a** involved silylation of 5′-*O*-(dimethoxytrityl)thymidine with bis(trifluoromethanesulfonyl)diisopropylsilane and imidazole (Scheme I). This reaction gave significant amounts of the undesired 3′,3′ symmetrically-linked dinucleoside **2** as evidenced by TLC. Introduction of thymidine (Scheme II) followed by chromatographic isolation provided only 20% of the desired 3′,5′-linked dinucleoside **3a** along with 3′,3′-dimers **2a** and **4a** in 60% and 10% yields, respectively. Variations of reaction conditions including temperature, addition rate, stoichiometry, and concentration were applied for optimization of the ratio 1:2 with little success. Thus, it was necessary to develop a new silylation protocol that minimized formation of the self-condensation product **2**.

Since the symmetric dimer **2** results from a relatively hindered transition state involving attack of a secondary

† Department of Medicinal Chemistry.
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• Abstract published in *Advance ACS Abstracts*, November 15, 1993.

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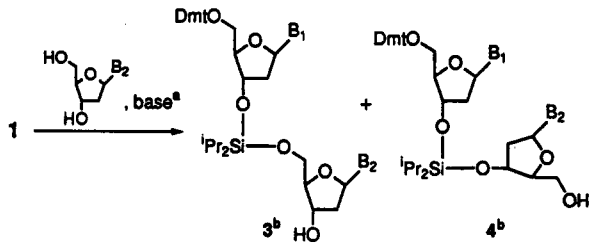
Scheme II



[a]Method 1: imidazole; ratio 3:4 = 2:1, yield of 3 20%. Method 2: Dtbp; ratio 3:4 > 20:1, yield of 3 70%. [b]**a**, $B_1 = B_2 = T$; **b**, $B_1 = T$, $B_2 = A^{Bz}$; **c**, $B_1 = T$, $B_2 = C^{Bz}$; **d**, $B_1 = T$, $B_2 = G^{iB}$; **e**, $B_1 = A^{Bz}$, $B_2 = T$; **f**, $B_1 = C^{Bz}$, $B_2 = T$; **g**, $B_1 = G^{iB}$, $B_2 = T$.

hydroxyl group on the 3′-silyl intermediate **1**, we reasoned that use of a hindered base as proton scavenger might slow the rate of formation of the 3′,3′ dimer **2** by increasing the activation energy for this undesired reaction path. The same effect should also enhance formation of the 3′,5′ coupling product, when using unprotected nucleosides in the coupling step. Indeed, when imidazole was replaced with the hindered base 2,6-di-*tert*-butyl-4-methylpyridine, almost none of the self-condensation product **2a** (<5%) was formed. Coupling by introduction of unprotected thymidine resulted in the formation of the dinucleoside **3a** in high yield. Thin layer chromatographs of this reaction show nearly quantitative formation of the dimers **3**. The product **4a** corresponding to reaction at the unprotected secondary hydroxyl was not observed. The advantage of not using 3′-OH protection can be exploited by continuing the reaction to trinucleoside **7** in a single pot. Thus the oligonucleoside **3a** was silylated without isolation and coupled with another molecule of unprotected thymidine to give, after chromatographic purification, the 3′,5′-linked trinucleoside **7** (Scheme III) in 55% yield. Higher yields (76%) of the trimer **7** were obtained in a stepwise (2 + 1) reaction that involved isolation and purification of the dimer unit. Isolation of diisopropylsilyl-linked nucleosides by silica gel chromatography is accompanied by some loss, presumably due to hydrolytic cleavage of silyl–ether linkages. Small-scale purification can be performed by reverse-phase HPLC. Unequivocal proof of the 3′,5′ nature of the silyl internucleoside link was obtained by ^1H–^{29}Si long range heteronuclear multiple quantum correlation NMR spectroscopy.[4]

The hindered base procedure was similarly useful for silylation of N^6-benzoyl-2′-deoxy-5′-*O*-(dimethoxytrityl)-adenosine, N^4-benzoyl-2′-deoxy-5′-*O*-(dimethoxytrityl)-cytidine, and N^2-isobutyryl-2′-deoxy-5′-*O*-(dimethoxytrityl)-guanosine. In each case, the 3′-silylated species **1** was the sole product. Coupling to unprotected thymidine gave the 3′,5′-linked heterodinucleosides A-Si-T **3e**, C-Si-T **3f**, and G-Si-T **3g**, respectively, in good yields (60–75%). Similar syntheses of T-Si-A **3b**, T-Si-C **3c**, and T-Si-G **3d** were also accomplished. Our next aim was to chain-extend the silyl-linked oligonucleosides. A tetrathymidylate oligomer **12** was synthesized by 3′-O-silylation of 5′,3′-linked trimer **7** followed by coupling with thymidine. The silylation reaction of trimer proceeded smoothly to give a single 3′-O-silylated species. No trace of the self-condensation product was observed. Stoichiometric reaction with thymidine gave a 50–60% condensation, leading to a 30% isolated yield of the tetrathymidylate analog after preparative reverse-phase HPLC.

(4) Hewitt, J. M.; Lenhart, W. C.; Moore, R. N.; Saha, A. K.; Weis, A. L. *Nucleosides Nucleotides*, **1992**, *11*(9), 1661–1666.

Syntheses of penta- and hexathymidine analogs were planned as [3 + 2] and [3 + 3] sequences, the first component being the 5′-protected trinucleoside **7** and the second component the detritylated di- or trinucleosides **5** or **8** (Scheme II). The latter compounds were prepared by reaction of dinucleoside **3a** and trinucleoside **7**, respectively, with 3% trichloroacetic acid in methylene chloride followed by flash chromatography on silica gel. Yields in these detritylation reactions were high (70–80%), indicating good acid stability of the diisopropylsilyl link. The 3′-O-silylation of the 5′-*O*-dimethoxytrityl trimer **7** proceeded well, as described for the tetramer above. However, coupling efficiency to the deprotected di- and trinucleosides **5** and **8**, respectively, was low and only minor amounts (20–30% by TLC) of the desired oligomers were formed. Evidence for formation of these oligomers was obtained from good intensity molecular and fragmentation ions in their FAB mass spectra as well as NMR of partially purified products.

Solution syntheses of higher chain length (>5–6 nucleobases) diisopropylsilyl-linked oligodeoxynucleotide analogs, though feasible, tend to be tedious and complex. This problem was solved by developing a scheme for solid-phase automated synthesis (Scheme III), which includes high purity synthesis of the monomeric synthon, its stabilization for room temperature handling and storage, and rapid coupling reactions. The 3′-*O*-diisopropylsilyl triflate intermediate **1a**, prepared at −40 °C, underwent some detritylation when warmed to room temperature. Stability against detritylation was achieved by adding an excess of imidazole (2 equiv) to the reaction mixture prior to warming to room temperature. The mixture was diluted with acetonitrile to 0.1 M to reduce the viscosity of the medium. The reagent solution remained colorless for several weeks and was stored under anhydrous conditions at −20 °C until use in a DNA synthesizer. Purity analysis for this synthon was according to high resolution NMR whereby no trace of the 3′,3′-dimer was observed.

Strands of varying lengths up to a 10-mer of thymidines containing the all-silicon backbone were synthesized reproducibly by solid-phase methodology (Scheme IV). The overall coupling efficiency based on the DMT assay was 96.3%. A synthesis was performed whereby the end-capping procedure after every coupling was omitted. This alteration to the synthesis cycle produced a mixture having shorter (9, 8, 7, etc.) lengths of the intended 10-mer strand. This mixture produced a good sizing ladder and retention time marker on reverse-phase HPLC. After synthesis the CPG solid support was cleaved with 6:3:1 NH_4OH (30% aqueous)/2-propanol/ CH_3CN with good recoveries as determined from the 260-nm absorbance reading (16–20 optical density units from 1-μm scale). The crude product from each synthesis was analyzed by reverse-phase HPLC in 0.01 M TEAA (pH 7.5)/acetonitrile gradient. A major peak was observed for each synthesis. The retention time increased as the chain length of the synthesized product increased: 5′-TSiTSiT-3′ 12.72 min; 5′ TSiTSiTSiTSiT-3′ 16.06 min; 5′ TSiTSiTSiTSiTSiTSiTSiTSiT-3′ 21.01 min. The trimer was identical in all respects to the same compound prepared by solution-phase methodology. The penta- and the decanucleosides were also characterized by proton NMR and FAB–mass spectroscopy.

The silyl-linked decathymidylate molecule **14** synthesized by solid phase was soluble in polar organic solvents but insoluble in aqueous systems. For determination of the physicochemical properties of the diisopropylsilyl