Tetrahedron Letters, Vol. 33, No. 17, pp. 2357-2360, 1992
Printed in Great Britain

0040-4039/92 $5.00 + .00
Pergamon Press Ltd

Synthesis of Alkylphosphon(othio)ate Analogues of DNA

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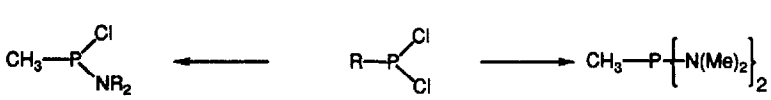
Abstract: Easily accessible bis(diisopropylamino)alkylphosphines (alkyl is methyl, *n*-butyl or *n*-octyl) are convenient reagents for the synthesis of DNA fragments having an alkylphosphonate or alkylphosphonothioate linkage at a predetermined site in solution and on solid support.

Oligodeoxynucleotides in which one or more of the natural internucleosidic (3'-5') phosphodiester bonds have been replaced by non-charged methylphosphonate linkages are valuable tools in biophysical and biological (*e.g.* protein-DNA interaction, antisense) studies[1]. The assembly of oligodeoxynucleotide methylphosphonates has been accomplished using reagents **2a**[2], **2b**[3] or **3**[4] which, in turn, are accessible starting with commercially available methyldichlorophosphine[5] (**1**, R = Me). However, the introduction of other alkylphosphonate bonds by a similar route is less convenient due to the poor accessibility of the requisite alkyldichlorophosphines (*i.e.* **1**; R = *n*-butyl or *n*-octyl).

In order to study[6] the effect of increasing chain length on the affinity of d-oligonucleotide alkylphosphon(othio)ates for complementary d-oligonucleotides, we report here that the conversion of bis(diisopropylamino)chlorophosphine (**4**)[7] into the bis(diisopropylamino)alkylphosphines **6a-c** opened the way to a solid-phase synthesis of oligodeoxynucleotides containing alkylphosphonate or alkylphosphonothioate linkages at preselected positions (*e.g.* hexamers **19a-c** and **20**, respectively).



2a : R = Me
b : R = iPr

1 : R = Me

3

Earlier studies from our laboratory revealed that crystalline and readily accessible bis(diisopropylamino)chlorophosphine (**4**)[7] could not only be applied to the formation of internucleosidic 1*H*-phosphonate linkages[8], but also to the preparation of the interesting phosphitylating reagents **5a**[9] and **5b**[10]. Apart from this, it was to be expected[11] that reagent **4** would also be a convenient starting compound for the synthesis of the bis(diisopropylamino)alkylphosphines **6a-c**. Indeed, replacement of the chlorine atom in **4** by an alkyl group using *n*-butyl lithium or methyl(*n*-octyl)magnesium halides, following a reported[11] procedure, gave the *three reagents* **6a-c** *as homogeneous liquids (see Table 1).*

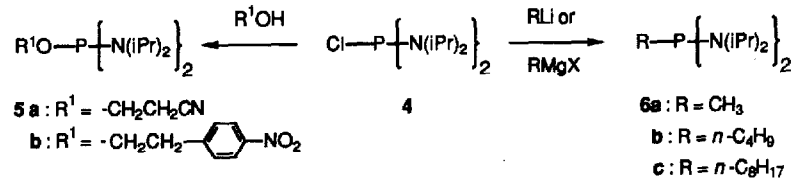


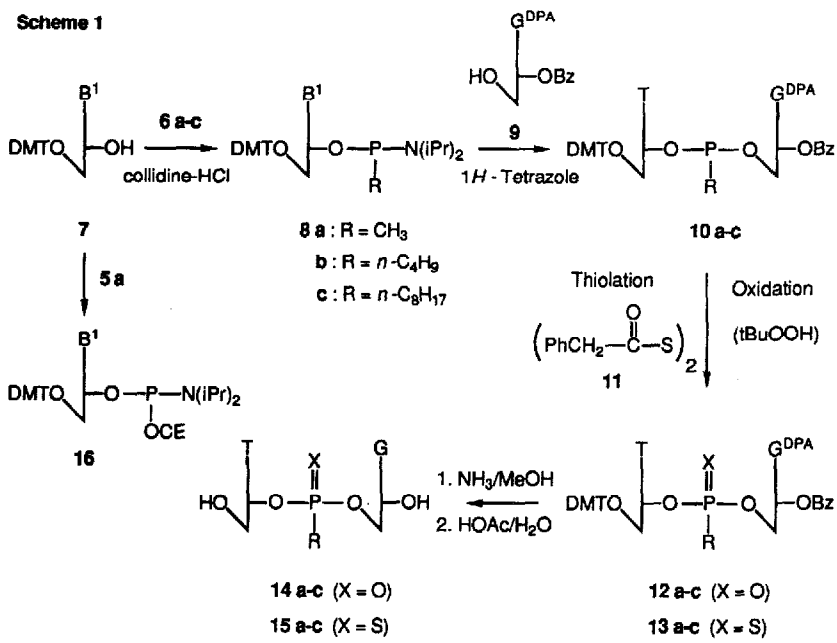
Table 1. Relevant data of bis(diisopropylamino)alkylphosphines **6**.

RLi or RMgX[a]	Product	Yield[b] (%)	B.p. (°C/kPa)	^{31}P NMR (ppm)	Mass $[M]^+$
MeMgBr	**6a**	68	77- 78/0.78	39.7	246
n-BuLi	**6b**	62	119-122/1.42	47.7	288
n-OctMgCl	**6c**	69	152-154/0.99	47.8	345

[a] Commercially available (M solution in ether or hexane). [b] Based on **4**.

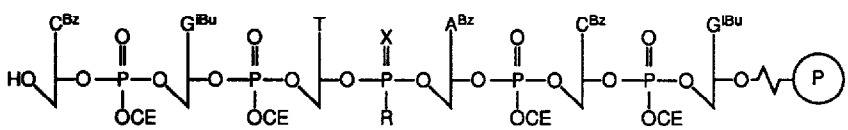
In order to evaluate the properties of the new reagents **6a-c**, the synthesis of the alkylphosphonate dimers **14a-c** in solution was first explored (Scheme 1). For example, in the first stage, a solution of 5'-*O*-dimethoxytritylthymidine (**7**, B^1 = T, 1.0 mmol) in dry dichloromethane (5 mL) was reacted with a two-fold excess of the methyl derivative **6a** in the presence of 2,4,6-collidine-HCl (0.1 mmol)[6]. After 24 h at 20°C, work-up and purification (flash column chromatography) gave homogeneous **8a** (B^1 = T) in 85% yield[12]. In a similar fashion, the respective thymidine *n*-butyl and *n*-octyl phosphonamidites **8b** and **8c** were obtained in nearly the same yield[12].

Scheme 1



In the second stage, the individual alkylphosphonamidites **8a-c** (B^1 = T; 0.55 mmol each) in dry dichloromethane (5 mL) were condensed with 2-*N*-diphenylacetyl-3'-*O*-benzoyl-2'-deoxyguanosine[13] (**9**; 0.50 mmol) in the presence of 1*H*-tetrazole (1.38 mmol). ^{31}P NMR analysis of the reaction mixture indicated in each case rapid formation of the dinucleoside phosphonites **10a-c**[14]. *In situ* oxidation of **10a-c** with *t*-butylhydroperoxide for 5 min at 20°C resulted, after work-up and purification by silica gel column chromatography, in the isolation of the fully protected modified dimers **12a-c**[15]. Removal of the diphenylacetyl and benzoyl groups from **12a-c** by ammonolysis (NH_3/MeOH) for 48 h at 50°C and subsequent acidic hydrolysis of the DMT group afforded, after purification (Sephadex LH-20), the modified TG dimers **14a-c**[16], the identity and homogeneity of which was unambiguously ascertained by ^{31}P- and ^{1}H-NMR spectroscopy. Apart from the successful preparation of the TG dimers **14a-c**, it was also demonstrated that the intermediate phosphonites **10a-c** could be converted smoothly into the corresponding alkylphosphonothioates **15a-c**. Thus, thiolation of **10a-c** (0.50 mmol) in dichloromethane (5 mL) with phenylacetyl disulfide (**11**)[10,17] (1.0 mmol) for 5 min at 20°C gave, after isolation and purification, the fully protected TG dimers **13a-c**[18]. Deblocking of the latter compounds, under the same conditions as mentioned for the conversion of **12a-c** into **14a-c**, yielded the homogeneous dimers **15a-c**[16].

The ease of preparing intermediate dimers **10a-c** and their high propensity toward oxidation and thiolation, urged us to apply the alkylphosphonamidites **8a-c** in a solid-phase synthesis of the modified hexamers **19a-c** and **20**, using an automated Gene Assembler and controlled pore glass as the solid support.



17 a-c X = O ⟶ **19 a-c** d-[CpGpTp(O,R)ApCpG]

18 X = S, R = CH_3 ⟶ **20** d-[CpGpTp(S,CH_3)ApCpG]

To this end, the immobilized fully protected hexamers **17a-c** were assembled by stepwise elongation of immobilized (3'-*O*-succinyl linkage) 2-*N*-isobutyryl-2'-deoxyguanosine via 1*H*-tetrazole-mediated coupling of the appropriate phosphoramidites **16** (B = T, C^{Bz}, A^{Bz} or G^{iBu}), prepared by phosphitylation of **7** with **5a**[19], and **8a-c** (B^1 = T) followed by oxidation (I_2/H_2O) and subsequent acidolysis of the DMT group. The efficiency of each elongation step was, as gauged spectrophotometrically by the released DMT cation, higher than 97%. Cleavage of the succinyl linkage and removal of all protective groups by ammonolysis (NH_3/MeOH, 50°C, 24 h) gave, after purification (Sephadex G-50), the target hexamers **19a-c**, the identity and homogeneity of which was firmly established by ^{31}P NMR spectroscopy and FPLC analysis. In the same way, the immobilized methylphosphonothioate-containing hexamer **18** was prepared by thiolation of the intermediate methylphosphonite linkage between the thymidine and 6-*N*-benzoyl-2'-deoxyadenosine unit of the growing chain. Ammonolysis of **18** resulted in the isolation of **20**, the methylphosphonothioate linkage of which was *inter alia* confirmed by ^{31}P NMR and FPLC analysis[20].

In conclusion, the new and readily available bis(diisopropylamino)alkylphosphines **6a-c** promise to be convenient reagents for the future preparation of alkylphosphon(othio)ate analogues of nucleic acids and other biopolymers.

References and notes

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12. ^{31}P NMR (CH_2Cl_2) data of compounds **8a-c** (B^1 = T): **8a**; 120.91, 120.55. **8b**; 127.62, 127.38. **8c**; 127.59, 127.41. R_f values (ethyl acetate/triethylamine, 95/5, v/v) of compounds **8a-c**: **8a**; 0.51. **8b**; 0.53. **8c**; 0.58. In a similar way, compounds **8a-c** (B^1 = C^{Bz}, A^{Bz} or G^{iBu}) were obtained.
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14. ^{31}P NMR (CH_2Cl_2) data of compounds **10a-c**: **10a**; 184.86, 184.16. **10b**; 189.12, 188.76. **10c**; 189.30, 188.48.
15. Yields of compounds **12a-c**: **12a**; 87%. **12b**; 86%. **12c**; 89%. ^{31}P NMR (CH_2Cl_2) data of compounds **12a-c**: **12a**; 33.85, 32.40. **12b**; 35.66, 34.36. **12c**; 36.63, 34.39. R_f values (dichloromethane/methanol, 92/8, v/v) of compounds **12a-c**: **12a**; 0.53, 0.51. **12b**; 0.56. **12c**; 0.60. Mass spectrometric $[M+Na]^+$ data of compounds **12a-c**: **12a**; 1193.4. **12b**; 1234.6. **12c**; 1290.8.
16. All deprotected compounds were characterized by ^{31}P NMR spectroscopy as well as mass spectrometry.
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18. Yields of compounds **13a-c**: **13a**; 84%. **13b**; 82%. **13c**; 85%. ^{31}P NMR (CH_2Cl_2) data of compounds **13a-c**: **13a**; 99.73, 98.64. **13b**; 104.35, 103.26. **13c**; 104.32, 103.32. R_f values (dichloromethane/methanol, 92/8, v/v) of compounds **13a-c**: **13a**; 0.56, 0.53. **13b**; 0.58. **13c**; 0.63. Mass spectrometric $[M+Na]^+$ data of compounds **13a-c**: **13a**; 1209.2. **13b**; 1253.9. **13c**; 1307.4.
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(Received in UK 6 February 1992)