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A NEW PROTECTED ACYL PROTECTING GROUP FOR EXOCYCLIC AMINO FUNCTIONS OF NUCLEOBASES

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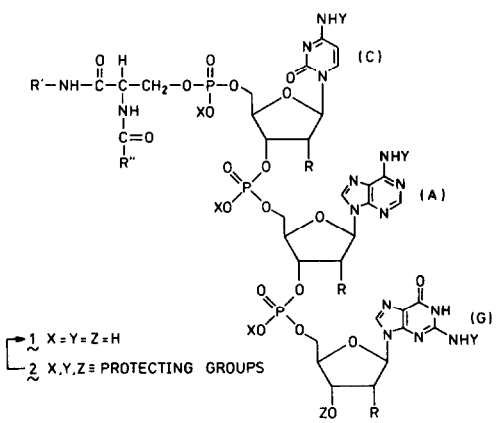
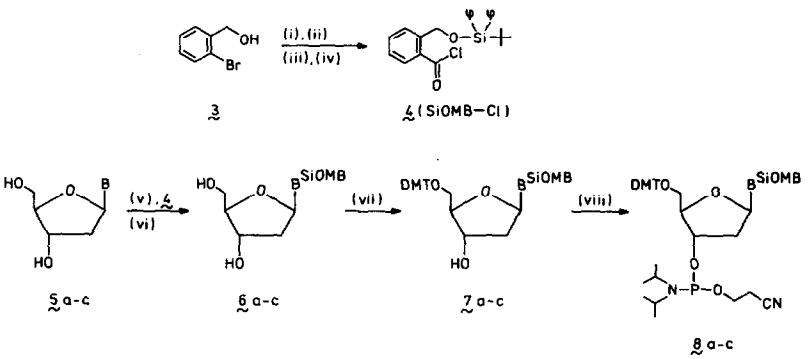
ABSTRACT: 2-(*Tert*-butyldiphenylsilyloxymethyl)benzoyl chloride (SiOMB-Cl) reacts with the per-O-trimethylsilylated d-nucleosides C,G and A to give, after removal of the $Si(Me)_3$ groups and 5'-O-protection with 4,4'-dimethoxytrityl chloride (DMT-Cl), the corresponding N-SiOMB-5'-O-DMT derivates, the SiOMB groups of which can easily be removed by fluoride ion. The SiOMB protected nucleosides proved to be suitable building units for the preparation of DNA fragments in solution and on a solid support.

Nucleoproteins are naturally occurring biopolymers[1] in which the 5'-terminal hydroxyl group of nucleic acids (DNA or RNA) is covalently linked through a phosphodiester bond with the hydroxyl groups of the L-amino acids serine, threonine and (or) tyrosine in proteins. The results so far obtained[2] in preparing nucleopeptide fragments [*i.e.*, 1(R=H)] containing the rather base-labile nucleotide-(P-O)-serine bond clearly indicated that a general and reliable route toward this type of biopolymers was *inter alia* severely hampered[3] by the lack of a suitable blocking group (*i.e.*, Y in **2**) for the exocyclic amino functions of the nucleobases adenine, cytosine and guanine.



As part of a programme to develop protecting groups, which can be removed selectively under neutral conditions from the protected precursor **2** of nucleopeptide **1**, we report that the exocyclic amino protecting group 2-(*tert*-butyldiphenylsilyloxymethyl)benzoyl (SiOMB) can be deblocked rapidly with fluoride ion. The design of the protected acyl protecting group is based on the following considerations[4]. It is well-known that esters of 2-(hydroxymethyl)benzoic acid are very prone[5] to base-catalyzed cyclization. Protection of the benzylic hydroxyl group with the rather acid and base resistant *tert*-butyldiphenylsilyl function[6] may engender the SiOMB group compatible with conditions normally applied in the synthesis of nucleic acids.

The preparation of the SiOMB-chloride **4** is outlined in Scheme 1. Thus, transformation of 2-bromo-benzylalcohol **3** to give SiOMB-Cl (**4**) could be realized in four steps with an overall yield of 66%. Protection (see Scheme 1) of the exocyclic amino function of persilylated **5a** (1 mmol) with **4** (1.2 mmol) in dry pyridine afforded the N^4-SiOMB cytidine derivative **6a** which was treated, without *prior* purification, with DMT-Cl (step vii) to give N^4-SiOMB-5'-O-DMT **7a** in a good yield. Unfortunately, the reaction of **4** with persilylated d-adenosine and d-guanosine derivatives **5b** and **5c**, respectively, was accompanied by the formation of unwanted byproducts (for more details see Table 1). Nonetheless, the resulting crude products **6b,c** thus obtained could be further processed to afford **7b** and **7c** in acceptable yields (Table 1).



Scheme 1. *Reagents*: (i) *tert*-butyldiphenylsilyl chloride. (ii) Mg, Et_2O (iii) CO_2-gas. (iv) Oxalyl chloride, toluene. (v) $(Me_3Si)_2NH/Me_3SiCl$ (Ref. 7). (vi) $MeOH/H_2O$.(vii) 4,4'-Dimethoxytrityl chloride. (viii) 2-cyanoethyl-N,N-diisopropyl-chlorophosphoramidite (Ref. 8).

Table 1. Yields and other relevant data on compounds 7 and 8.

Starting compounds	N-SiOMB-5'-O-DMT Derivatives	Yield[a] (%)	Phosphor-amidites	Yield (%)	^{31}P-NMR[d] data
5a(B=C)	7a	82	8a	85	149.9/149.2
5b(B=A)	7b	75[b]	8b	90	149.1
5c(B=G)	7c	75[c]	8c	82	149.5

[a] Based on 5. [b] See under 9 in ref. and notes. [c] See under 10 in ref. and notes.
[d] In p.p.m. relative to 85% H_3PO_4.

At this stage, we were anxious to examine the susceptibility of the SiOMB group toward fluoride ion. Contrary to expectation[6] we observed that treatment of 7a-c with $(n\text{-}Bu)_4NF$ (2 eq.), in the absence (dry dioxane) or presence (pyridine/H_2O, 1:1) of water, resulted in complete removal of the SiOMB group within 5 and 45 min. at 20°C, respectively. In comparison, removal of the 5'-O-SiOMB from compound 9, obtained by the reaction of 4 with 2',3'-O-isopropylidene-uridine, with 'dry' $(n\text{-}Bu)_4NF$ was, as expected[6], complete after 5 h at 20°C. The relatively fast and quantitive fluoride ion-assisted deblocking of the SiOMB group from 7a-c may be tentatively explained by the transition-state stabilization as depicted in Fig. 1, in which *inter alia* the strong hydrogen bond between the fluoride ion and the amino proton plays a pivotal role. The above assumption may explain the extremely slow removal (more than 5 h) of one SiOMB group from the N^6-di-SiOMB d-adenosine derivative 10[9] by fluoride ion (dry conditions).

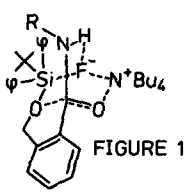
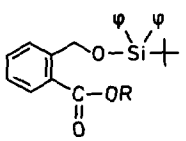


FIGURE 1

7a-c



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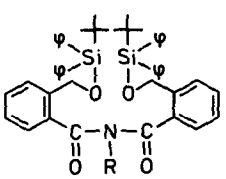

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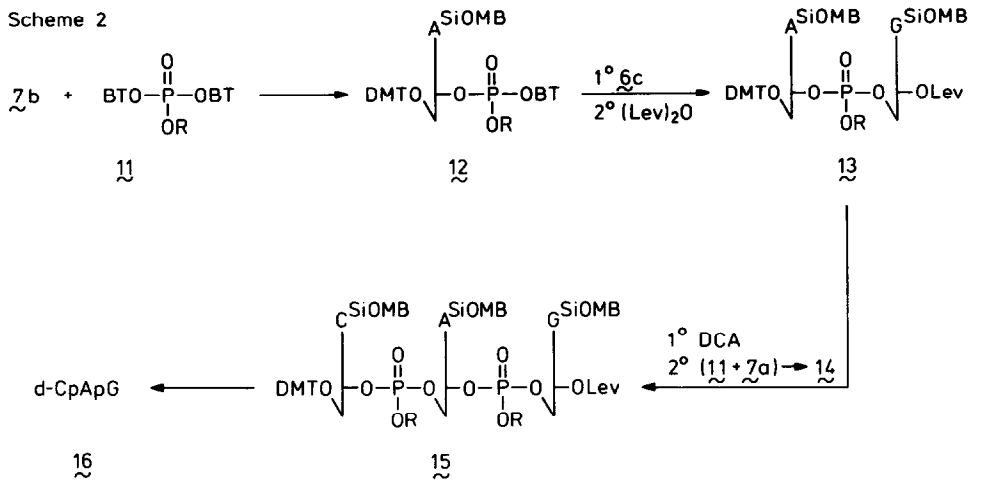
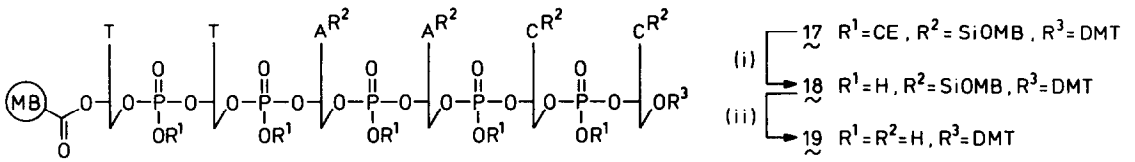
The use of the SiOMB group in the synthesis of DNA is illustrated (Scheme 2) by the assemblage of the trimer 16 *via* the 1-hydroxybenzotriazole-promoted phosphotriester approach[11]. Thus, phosphorylation of 7b with reagent 11 (BT = benzotriazolyl) gave intermediate 12. *In situ* coupling of 12 with 6c, in dry pyridine, and subsequent acylation with

laevulinic acid anhydride afforded dimer **13** in 80% yield. Acidolysis of the 5'-O-DMT group with dichloroacetic acid (DCA) followed by condensation with the 3'-phosphorylated intermediate **14**, prepared *in situ* by phosphorylation of **7a** with **11**, furnished, after purification by silica gel column chromatography, trimer **15** in 90% yield. Simultaneous

Scheme 2



deblocking of the SiOMB and 2-chlorophenyl (R) groups from **15** with $(n\text{-Bu})_4NF$ (wet conditions)[12], followed by hydrazinolysis[13] and acidic hydrolysis (HOAC/H_2O) afforded homogeneous **16** in a yield of 90% (based on **15**). Trimer **16** thus obtained was in every aspect, FPLC-analysis, ^1H- and ^{31}P-NMR spectroscopy, identical with the same trimer prepared by a similar approach[11] starting from the normally used N-acyl protected d-nucleosides.The application of the N-SiOMB protected phosphoramidite d-nucleosides **8a-c**, which were obtained in good yield (Table 1) by phosphitylation (step viii in Scheme 1) of compounds **7a-c**, toward the assemblage of d-CCAATT on a solid support (monobeads, Pharmacia) using an automated Gene Assembler (Pharmacia), is outlined in Scheme 3. The immobilized fully-protected hexamer **17** was assembled[6] by stepwise elongation of immobilized (3'-O-succinyl linkage) thymidine with 1H-tetrazole-mediated coupling of the appropriate phosphoramidite derivatives (*i.e.*, **7a-b** and the corresponding 3'-phosphoramidite of thymidine), followed by oxidation (I_2, collidine, H_2O, acetonitrile) of the resulting phosphite-triester, and subsequent acidolysis of the DMT-group. The efficacy of each elongation step was, as gauged spectrophotometrically by the released DMT-cation, higher than 97%. Apart from the successful conclusion of the solid-phase synthesis of hexamer **17**, it is also of interest to note that the removal of the cyanoethyl (R^1) and SiOMB (R^2) groups proceeded without hydrolysis of the succinyl linkage.



Scheme 3. *Reagents*: (i) Et_3N/pyridine/H_2O (3:1:1, v/v), 1 h, 20°C. (ii) $(n\text{-Bu})_4NF$(2 eq.)/pyridine/H_2O (1:1, v/v), 2 h, 20°C (iii) 2% DCA in $ClCH_2CH_2Cl$, 4 min. (iv) 25% aq. NH_3/EtOH (3:1, v/v), 1 h, 20°C.

Thus, removal of the CE (step i) and SiOMB (step ii) from **17** afforded the still immobilized[14] hexamers **18** and **19**, respectively. Further deblocking of **19** (steps iii and iv) gave, after purification (Sephadex G-50), homogeneous d-CCAATT which was identical, on the basis of FPLC-analysis and ^1H-NMR data, with the same fragment prepared *via* a similar solid-phase approach[6] using N-acyl-5'-O-DMT-d-nucleoside- phosphoroamidites. In conclusion, the mild fluoride ion-assisted removal of the SiOMB group from exocyclic amino functions of nucleobases will be of great value for the synthesis[15] of nucleopeptides (*e.g.*, **1**) containing the base-labile nucleotide-(P-O)-serine linkage. Further, the recently

developed[16] solid-phase synthesis of RNA using N-phenoxyacetyl-5'-O-DMT-2'-O-*tert*-butyldimethylsilyl protected ribonucleosides may be improved substantially by applying the corresponding N-SiOMB protected ribonucleosides. At present, we are exploring whether the SiOMB-amino-protecting group-strategy can also be applied toward the preparation of peptides and oligosaccharides.

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3. In order to prevent the occurrence of the unwanted β-elimination in the deblocking of **2** to give **1** the commonly used N-acyl (*i.e.* benzoyl or anisoyl protecting groups Y in **2**) were replaced in earlier studies (see ref. 2b-c) by the 2-nitrophenylsulfenyl (in the case of d-adenosine or d-cytosine) or the di-n-butylaminomethylene group (in the case of d-guanosine).
4. The use of protected acyl protecting groups [*i.e.*, 2-(dibromomethyl) and 2-(isopropylthiomethoxymethyl)benzoyl] was reported for the first time by C.B. Reese *et al.* [see: J. Chem. Soc. Chem. Commun. 987 (1979) and Nucleosides, Nucleotides, **4**, 117 (1985)] and, later on, by M.J. Gait *et al.* [see: Nucleosides, Nucleotides, **6**, 341 (1987)] for the temporary protection of the 5'-hydroxy function of ribonucleosides.
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9. Reaction of **5b** (B=A) with **4**, followed by tritylation, gave apart from **7b** the N^6-di-SiOMB product **10**. The latter product could easily be converted into **7b** by short treatment with NaOMe/MeOH.
10. In the reaction of **5c** (B=G) with **4** we observed (TLC-analysis) the formation of the N^2-SiOMB product **6c** and an unidentified byproduct which, however, could be converted into **6c** by short treatment with DCA in CH_2Cl_2.
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14. This was derived from the observation that FPLC-analysis of the separate eluates, collected after executing steps i and ii followed by acidolysis, did not reveal the presence of d-CCAATT.
15. We reported (ref.2) earlier that the nucleotide-(P-O)-serine bond in **1** survived prolonged fluoride ion (wet conditions) treament.
16. T. Wu, K.K. Ogilvie and R.T. Pon, Nucl. Acids Res., **17**, 3501 (1989).

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