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Solid Phase Synthesis of a Model Nucleopeptide with a Phosphodiester Bond between the 5' End of a Trinucleotide and a Serine Residue

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Key words: nucleopeptides; solid phase; phosphite triester; serine-nucleoside phosphate diester bond.

Abstract: *The model nucleopeptide Boc-Ser(pTCT)-NHcHex has been assembled on a polystyrene solid support using the phosphite triester approach. Fmoc and cyanoethyl groups have been used to protect the exocyclic amine of cytosine and phosphate groups respectively. Final deprotection has been carried out by treatment with methanolic potassium carbonate.*

The replication of the DNA of some viruses, among which the best known are the *Bacillus* phage ϕ29 and the adenoviruses, is known to be initiated by the covalent attachment of a protein to the 5' end[1, 2]. In these nucleoproteins, as well as in other RNA-protein complexes, a covalent phosphodiester bond is established between the terminal 5'-OH and a residue of serine[3, 4], threonine[5] or tyrosine[6-8].

In the last few years, syntheses of nucleopeptides with phosphodiester bonds to these three amino acids have been carried out by different methodologies[9-15]. The main problem associated with the preparation of such compounds remains the combination of protecting groups to be used in the synthesis of hybrid structures in which a nucleoside is linked to a residue of serine (or, to a lesser extent, threonine) *via* a phosphate linker[16]. These phosphate linkages are rather labile to basic treatments, where they may easily undergo β-elimination to yield a dehydroalanine derivative and an oligonucleotide 5'-phosphate[10].

In this paper we report an alternative approach to the synthesis of nucleopeptides with a phosphodiester bond between the 5' end of an oligonucleotide and a residue of serine. We have obtained the model nucleopeptide Boc-Ser(pTCT)-NHcHex[17] by the phosphite triester methodology[18] on a polystyrene solid matrix[19]. All phosphate groups have been protected with the standard cyanoethyl group, and the exocyclic amine of cytosine with the Fmoc group[20-22]. The synthetic scheme is outlined in figure 1.

The trinucleotide **1** has been assembled manually on an insoluble polystyrene support following standard synthetic procedures with modifications rendering the phosphite triester methodology compatible with the use of polystyrene[19, 23]. Coupling of the O-cyanoethoxy-N,N-diisopropylaminophosphoramidite of Boc-Ser-NHcHex[24] **2** and subsequent oxidation have been carried out under identical conditions to all other synthetic cycles. Cleavage of the nucleopeptide-resin bond and elimination of the base-labile protecting groups (Fmoc, cyanoethyl) have been accomplished by treatment of the fully protected nucleopeptide **3** with 0.05 M potassium carbonate in methanol/dioxane 1/1. This reagent has been used to cleave ester functions[25] and base-labile protecting groups in the synthesis of phosphate-methylated DNA fragments[26]. Although it has been reported that removal of Fmoc groups requires long reaction times[26] (up to 36 h), a five hour treatment of **3** at room temperature (15 equivalents of carbonate/reacting functional group) has allowed us to obtain crude **4** in 95% yield[27].

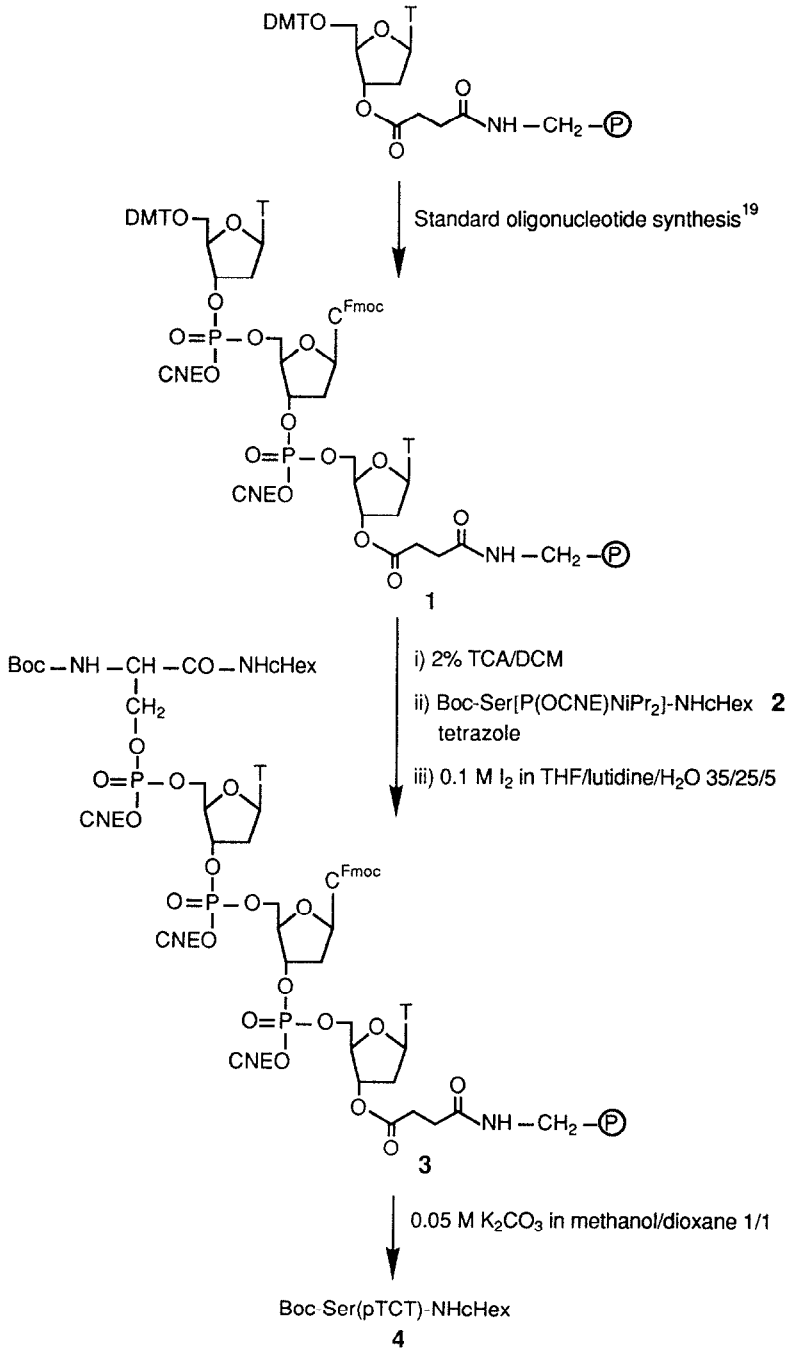

Figure 1. Synthesis of the model nucleopeptide Boc-Ser(pTCT)-NHcHex.

The model nucleopeptide Boc-Ser(pTCT)-NHcHex has been purified by ion-exchange chromatography on a DEAE-Sephadex column by elution with a gradient of triethylammonium bicarbonate buffer (from 0.1 M to 0.5 M) (70% cleavage and purification yield). The sodium salt of **4** has finally been obtained after chromatography using DOWEX 50 X4 (Na^+ salt). Purified **4** shows a single peak on analytical HPLC[27] and has been characterized by ^1H- and ^{31}P-NMR[28].

In conclusion, we have shown that this approach is a good alternative for the preparation of nucleopeptides with a base-labile phosphodiester bond, which can be easily obtained in good yields. The protecting groups chosen can be removed in a single deprotection step, under mild conditions that leave the phosphate bond between the aminoacid and the nucleoside unaltered. Furthermore, the use of a solid support for the protection of the 3' end obviously prevents many intermediate purification steps in comparison with a synthesis carried out in solution. Our work now is directed towards the preparation of nucleopeptides of larger size.

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17. Abbreviations used are: Boc: *t*-butoxycarbonyl; cHex: cyclohexyl; DMT: 4,4'-dimethoxytrityl; CNE: cyanoethyl; Fmoc: 9-fluorenylmethoxycarbonyl; P : copoly(styrene-1%-divinylbenzene). ^{31}P-NMR spectra have been recorded using 85% H_3PO_4 as an external reference. R_f values for phosphoramidites refer to separations on silica gel plates using the solvent mixture ethyl acetate/dichloromethane/pyridine 50/50/2.
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22. 5'-O-DMT-N^4-Fmoc-cytidine was synthesised following the procedure described in reference 21 for the synthesis of the monomethoxytrityl derivative. Phosphitylation was carried out by reaction with 1.2 equivalents of O-cyanoethoxy-bis(diisopropylamino)phosphine and 0.5 equivalents of tetrazole in dichloromethane. After usual work-up and precipitation from hexanes, **2** was obtained in 85% yield (^{31}P-NMR: δ 147.9; Rf: 0.6).
23. Solid phase synthesis was carried out on 100 mg of DMT-T-succinyl-polystyrene with a substitution degree of 0.12 μmol/mg following the procedure described in reference 19 with the exception of the neutralisation step with triethylamine after incorporation of the base-labile C^{Fmoc} derivative. 10 equivalents of nucleoside phosphoramidites, 20 equivalents of **2** and 40 equivalents of tetrazole were used at the corresponding coupling steps.
24. Boc-Ser-NHcHex was synthesised from commercial Boc-serine by activation with 1 equivalent of both N-methylmorpholine and *iso*butylchloroformate in dichloromethane at -15°C (10 min) and subsequent reaction with 5 equivalents of cyclohexylamine (1 hr, room temperature). After extractions with 1M $KHSO_4$ and brine, the product was purified by silica gel column chromatography and obtained in a 60% yield after crytallisation from ethyl acetate/hexanes (m. p : 108-110°C); ^{1}H-RMN ($CDCl_3$, 200 MHz): δ 6.80 (1H, s, NHcHex), 5.65 (1H, s, Boc-NH), 4.10 (2H, m, $C^{\beta}H_2$), 3.8-3.6 (2H, m, $C^{\alpha}H$, CH cHex), 3.2 (1H, s, OH), 1.9-1.1 (10H, m, CH_2 cHex), 1,45 (9H, s, Boc). **2** was obtained by phosphitylation of Boc-Ser-NHcHex under the same reaction conditions described above (ref. 22) in a 80% yield (^{31}P-NMR: δ 148.8 and 149.8; Rf: 0.70).
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27. After filtration and washings, the reaction was quenched by addition of 1% acetic acid. Crude **4** was obtained after evaporation of solvents at low pressure and lyophilisation. HPLC analysis (Spherisorb ODS, linear gradient from 0.01% aqueous triethylammonium acetate to acetonitrile/water 1/1 in 20 min, 1 mL/min, detection wavelength: 260 nm) showed the target nucleopeptide **4** with a retention time of 12 min and a minor peak at 8.5 min which corresponds to the trinucleotide TCT (less than 5% with relation to **4**).
28. ^{31}P-NMR (D_2O, pD=6.5): broad band centered at -3.6 ppm. ^{1}H-NMR (200 MHz, D_2O, pD=6.5): The most significant signals are: δ 7.88 (1H, d, H-6 dC); 7.73 (2H, s, 2xH-5 dT); 6.38-6.28 (3H, m, 3xH-1', dC, dT); 6.07 (1H, d, H-5 dC); 2.53-2.48 and 2.38-2.28 (6H, m, 6xH-2', dC, dT); 1.92 (9H, s, Boc); 1.78-1.18 (10H, m, CH_2 cHex); 1.42 (6H, s, 2xCH_3 dT).

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