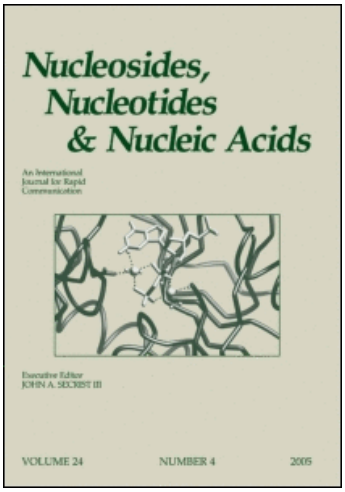

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Phosphitylation of Primary Carboxamides. Synthesis of Peptide-Oligonucleotide Conjugates with Acylphosphoramidate Linkages

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PHOSPHITYLATION OF PRIMARY CARBOXAMIDES. SYNTHESIS OF PEPTIDE-OLIGONUCLEOTIDE CONJUGATES WITH ACYLPHOSPHORAMIDATE LINKAGES.

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Abstract. N-acylphosphoramidates can be obtained from the reaction of phosphitylated primary carboxamides and an alcohol in the presence of an acid catalyst such as tetrazole and subsequent oxidation. The reaction is useful for the preparation of peptide-oligonucleotide conjugates.

The phosphitylation of hydroxyl groups and, in particular, the preparation of nucleoside-phosphoramidite derivatives, is usually accomplished by reaction with an electrophilic P^{III} reagent such as an alkoxybis(dialkylamino)phosphine[1] or a chloro(alkoxy)dialkylaminophosphine[2], of which the latter is known to be more reactive. We have seen that, in a similar way, primary carboxamides are nucleophilic enough to react with chloro(alkoxy)dialkylaminophosphines and a base and, much more slowly, with alkoxybis(dialkylamino)phosphines in the presence of an acid catalyst.

The preparation of an N-acylphosphoramidate taking advantage of this unprecedented reaction was first carried out using phenylacetamide as the compound bearing the carbamoyl ($CO-NH_2$) group. Thus, the reaction between phenylacetamide and chloro(2-cyanoethoxy)diisopropylaminophosphine in the presence of ethyldiisopropylamine afforded the N-acylphosphorodiamidite derivative $Ph-CH_2-CO-NH-P(OCNE)NiPr_2$ (**1**) which, in the presence of tetrazole, reacted with 5'-dimethoxytritylthymidine to give the N-acylphosphoramidite DMT-T-O-P(OCNE)-NH-CO-CH_2-Ph (**2**). Acylphosphoramidite **2** was oxidized with tBuOOH to an N-acylphosphoramidate diester derivative, DMT-T-P(O)(OCNE)-NH-CO-CH_2-Ph, **3**. The synthesis scheme is shown in Figure 1. The same N-acylphosphoramidate **3** was obtained by reaction between phenylacetamide and the standard thymidine-phosphoramidite synthon ($DMT-T-O-P(OCNE)NiPr_2$) in the presence of tetrazole, and subsequent oxidation. Treatment of **3** with base removed the cyanoethyl

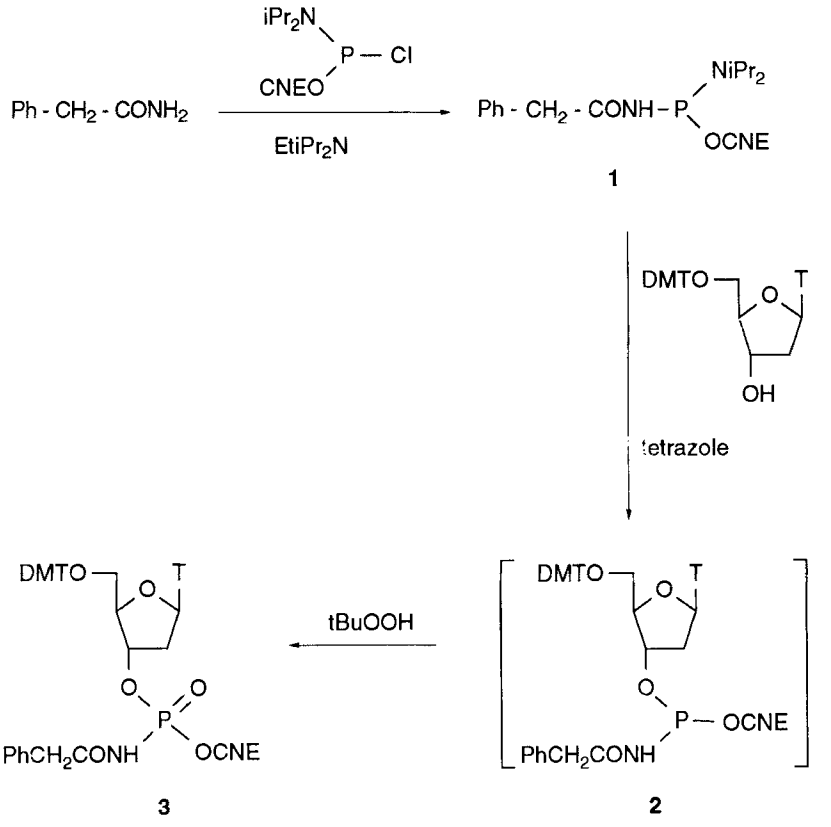
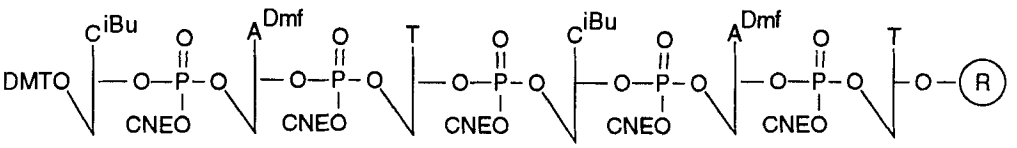


FIGURE 1. Synthesis of the N-acylphosphoramidate derivative DMT-T-O-P(O)(OCNE)-NH-CO-CH_2-Ph (**3**).

group to give the corresponding phosphoramidate monoester which was shown to be stable in a wide range of basic conditions[3].

This reaction scheme provides a new synthetic route to peptide-nucleotide conjugates with an acylphosphoramidate linkage and was successfully used for the preparation of Ac-Ser-Gly-Asp-NH-p$^{5'}$T (**4**) and Ac-Ser-Gly-Asp-NH-p$^{5'}$CATCAT (**5**, Figure 2). For both syntheses, the carbamoyl group of the protected peptide Ac-Ser(Ac)-Gly-Asp(OFm)-NH_2[4] (Fm=9-fluorenylmethyl) was phosphitylated by reaction with chloro(2-cyanoethoxy)-diisopropylaminophosphine and ethyldiisopropylamine. The resulting protected peptide-phosphorodiamidite, Ac-Ser(Ac)-Gly-Asp(OFm)-NH-P(OCNE)$NiPr_2$ (**6**), was anchored onto either thymidinyl-succinyl-polystyrene or the oligonucleotide-resin $C^{iBu}A^{Dmf}TC^{iBu}A^{Dmf}$T-succinyl-polystyrene[5] **7** in the presence of tetrazole (iBu=isobutyryl, Dmf=dimethylaminomethylene[6]).



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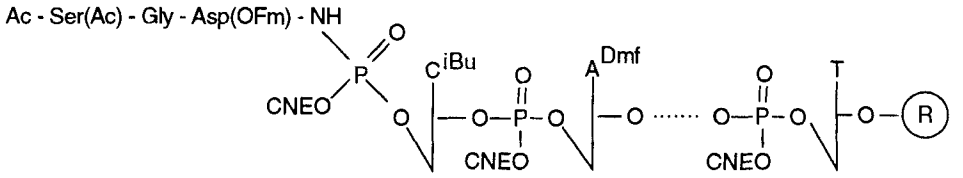
i) 3% TCA / DCM
ii) Ac-Ser(Ac)-Gly-Asp(OFm)-NH-P(OCNE)NiPr$_2$ (**6**), tetrazole
iii) aqueous I_2



conc. aq. NH_3, 55°C

Ac - Ser - Gly - Asp - NH - p $^{5'}$CATCAT.

5

FIGURE 2. Synthesis of the peptide-oligonucleotide conjugate Ac-Ser-Gly-Asp-NHp$^{5'}$CATCAT (**5**).

After oxidation with aqueous iodine, the target molecules were deprotected and detached from the resin by a treatment with concentrated aqueous ammonia at 55°C (6 h) and purified by reversed phase liquid chromatography. The products were obtained in a 22-26% range yield and were characterised by amino acid analysis after acid hydrolysis (**4**: Ser 0.76, Asp 0.96, Gly 1.00; **5**: Ser 0.70, Asp 0.94, Gly 1.00), nucleoside composition after digestion with snake venom phosphodiesterase and alkaline phosphatase in the case of **5** (dC 0.9, T 1.0, dA 1.1) and electrospray mass spectrometry (negative mode, **4**: m/z 643 $[M-2H+Na]^-$, 621 $[M-H]^-$, 310 $[M-2H]^{2-}$; **5**: m/z 724 $[M-5H+2Na]^{3-}$, 717 $[M-4H+Na]^{3-}$, 709 $[M-3H]^{3-}$, 537 $[M-5H+Na]^{4-}$, 532 $[M-4H]^{4-}$).

In summary, the synthetic method which we have reported for the synthesis of N-acylphosphoramidate derivatives makes use of mild reaction conditions which are convenient for the preparation of base-stable peptide-oligonucleotide conjugates not described before.

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3. N-acylphosphoramidate **3** is stable to: 0.05 M K_2CO_3 in MeOH/dioxane 1:1, 24 h, r.t.; 0.25 M LiOH in MeOH/dioxane/H_2O 1.5:1.5:1, 24 h, r.t.; conc. aq. NH_3/dioxane 1:1, 15 h, 55ºC.
4. Protected peptide Ac-Ser(Ac)-Gly-Asp(OFm)-NH_2 was obtained by acetylation of the side chain hydroxyl group of the serine residue of peptide Ac-Ser-Gly-Asp(OFm)-NH_2. For the preparation of Ac-Ser-Gly-Asp(OFm)-NH_2, see Robles, J.; Pedroso, E.; Grandas, A. *Int. J. Peptide Protein Res.*, **1994**, *43*, 359-362.
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