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Efficient Preparation of Aminoacylated Dinucleoside Phosphates with N-FMOC Amino Acid Fluorides[1]

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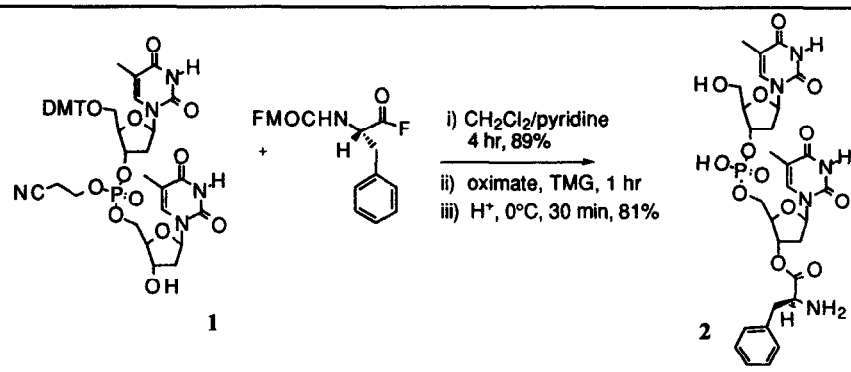
Abstract: A procedure for the efficient aminoacylation of oligonucleotides has been developed. Internucleoside phosphates are protected with the β–cyanoethyl group prior to aminoacylation with N-FMOC amino acid fluorides. Deprotection with oximate removes all protecting groups without disturbing the aminoacyl linkage. © 1997 Elsevier Science Ltd.

The synthesis of aminoacylated nucleosides, nucleotides, and oligonucleotides is of considerable interest as they are important intermediates in a number of biological systems.[2] We recently reported that FMOC protected ribo- and deoxyribonucleosides can be efficiently aminoacylated with N-FMOC amino acid fluorides.[3] We now describe an extension of this methodology which converts suitably protected dinucleoside phosphates to the corresponding 3'-aminoacylated derivative. The procedure provides easy and efficient access to aminoacylated oligonucleotides.

Methods for the preparation of 2'(3')-O-aminoacyl nucleotides have utilized amino acid anhydrides,[4] cyanomethyl esters,[5] or *in situ* activation by a number of coupling agents.[6] These synthetic procedures proceed in low yield or are limited in the nature of the N-protecting group on the amino acid.

Our initial attempts to aminoacylate a dinucleoside phosphate with N-FMOC amino acid fluorides was based on procedures in which a dinucleoside phosphate, protected on the bases with FMOC and on the phosphates with 2-chlorophenyl, was aminoacylated by BPOC- or HOBt-activated amino acids.[6f,h] When aminoacylation reactions of similarly protected dinucleoside phosphates were attempted with acid fluorides we observed loss of the 2-chlorophenyl protecting groups with resultant complex reaction mixtures. This is presumably the result of fluoride ion catalyzed deprotection of the aryl phosphate protecting group.[7]

A phosphate protecting group which is stable to fluoride ion is necessary for smooth aminoacylation. In addition, deprotection of the phosphate protecting group must proceed under conditions where the newly formed amino acid ester is stable. Our results show that the β-cyanoethyl group is a suitable protecting group.



Scheme 1

The β–cyanoethyl group has been used as a phosphate protecting group. However, it is typically removed with aqueous NH_4OH which readily hydrolyzes the aminoacyl linkage. Chládek has reported the use of the β–cyanoethyl group to protect guanosine-O^6.[8] In this case the β–cyanoethyl group was removed by treatment with anhydrous oximate, conditions where the aminoacyl group is stable.[6f,9]

Previous reports indicated that TBAF in THF removed β–cyanoethyl protecting groups,[10] thus apparently precluding the use of this protecting group during acylation reactions with acid fluorides. We have found that under anhydrous conditions the β–cyanoethyl group was stable to TBAF. Thus, treatment of dinucleoside phosphate **1**[11] with TBAF in dry THF for one hour resulted in no loss of the β–cyanoethyl group. However, the β–cyanoethyl group was easily removed by treatment with N^1,N^1,N^3,N^3-tetramethylguanidinium 2-nitrobenzaldehyde oximate. Thus the β–cyanoethyl group seemed a good choice for phosphate protection.

In the experiment of interest, shown in Scheme **1**, the β–cyanoethyl group proved stable during reaction of an amino acid fluoride with protected dinucleoside phosphate **1**. The β–cyanoethyl group was removed by oximate concurrently with removal of the FMOC protecting group to provide **2**. TLC analysis suggests that the aminoacylation and the deprotection reactions go in quantitative yield with isolated yields of 89% and 81% respectively.

In conjunction with our previous results on aminoacylation of ribo- and deoxyribonucleosides this work demonstrates that any ribo- or deoxyribooligonucleotide protected on the bases with FMOC and on the phosphates with β–cyanoethyl can be easily aminoacylated.

Experimental. To a solution of dried **1** (0.100 g, 0.111 mmol) in anh methylene chloride (2 mL) and pyridine (0.2 mL) was added N-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl fluoride (0.0864 g, 0.222 mmol). The resulting mixture was stirred at rt under argon for 4 hr.[3] The reaction mixture was diluted with $CHCl_3$ (50 mL) and extracted with cold water (30 mL). The aqueous layer was extracted with $CHCl_3$ (20 mL) and the organic layers were combined, dried over Na_2SO_4, filtered, and the solvent was evaporated taking care to completely remove all pyridine. TLC of the crude in $CHCl_3$/MeOH 9:1 showed quantitative conversion into product. The crude material was purified on a silica gel column eluting with CH_2Cl_2/acetone 1:1 to give a white solid (124 mg, 89%).[12] The solid (0.090 g, 0.071 mmol) was dissolved in anhydrous THF (1 mL). A freshly prepared solution of N^1,N^1,N^3,N^3-tetramethylguanidine (0.031 mL, 0.24 mmol) and O-nitrobenzaldoxime (0.0476 g, 0.28 mmol) in dry acetonitrile (1.5 mL) was added via a syringe. The resulting mixture was stirred under argon at room temperature for 1hr.[6f,6h,8] TLC in $CHCl_3$/MeOH 12:1 showed a quantitative conversion to a baseline DMT-containing material. The product was purified and the DMT-group deprotected as described[6f] to give the desired product **2** (40.1 mg, 81%).[13] TLC of the AcOH solution of the product in 1-butanol/H_2O/AcOH 8:1:1 on cellulose plate revealed only a single spot. NMR analysis showed that traces of solvents used during the deprotection were associated with the product. Quantitation by UV (ε= 18,500)[14] gave the purity of **3** as 94%.

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References and Notes

1. Abbreviations used: FMOC, [(9-fluorenylmethyl)oxy]carbonyl; T, thymidine; DMT, dimethoxytrityl; TBAF, tetrabutyl ammonium fluoride; BPOC, [[2-(4-biphenylyl)isopropyl]oxy]carbonyl; HOBt, 1-hydroxybenzotriazole.

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12. ^{1}H-NMR ($CDCl_3$, 250 MHz) d 9.22-9.29 (m, 2H), 7.75 (d, 2H, *J*= 7.5 Hz), 7.13-7.56 (m, 22H), 6.83 (m, 4H), 6.42 (m, 1H), 6.10 (m, 1H), 5.57 (dd, 1H, *J* = 16.0, 7.9 Hz), 5.16-5.28 (m, 2H), 4.66 (m, 1H), 4.12-4.46 (m, 8H), 3.77-3.86 (m, 7H), 3.36-3.55 (m, 2H), 3.09 (m, 2H), 2.33-2.75 (m, 6H), 1.89 (s, 3H), 1.41 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) d 171.5, 163.5, 158.9, 155.7, 150.5, 150.4, 150.2, 150.2, 144.0, 144.0, 143.8, 143.7, 141.3, 135.5, 135.5, 135.3, 135.0, 130.1, 130.1, 129.2, 128.8, 128.1, 128.1, 127.7, 127.4, 127.4, 127.3, 127.3, 127.1, 125.0, 120.0, 116.3, 116.1, 113.4, 111.8, 111.8, 111.6, 87.3, 85.5, 85.3, 84.5, 84.5, 84.4, 84.3, 82.1, 82.0, 79.9, 79.7, 79.7, 77.2, 74.6, 67.5, 67.4, 67.4, 67.1, 63.3, 63.2, 62.5, 62.5, 62.4, 62.4, 55.3, 55.3, 54.9, 47.1, 38.9, 38.2, 36.5, 19.7, 19.6, 19.5, 12.4, 11.7; HRMS (FAB, NBA/NaI) calcd for $[C_{68}H_{67}O_{17}N_6P + Na]^+$ 1293.4198, found 1293.4235.

13. ^{1}H-NMR (D_2O, 250 MHz) d 7.45 (m, 2H), 7.10-7.26 (m, 5H), 6.01 (t, 1H, *J*= 6.9Hz), 5.87 (t, 1H, J= 7.1Hz), 5.25 (br s, 1H), 4.31 (t, 1H, *J*= 7.2 Hz), 3.88-3.98 (m, 4H), 3.60 (m, 2H), 3.05-3.18 (m, 2H), 2.10-2.37 (m, 4H), 1.67(br s, 6H); ^{13}C-NMR (D_2O, 100 MHz, DSS external standard) d 171.6, 168.9, 168.7, 154.2, 154.1, 139.9, 139.6, 136.5, 132.0, 131.9, 130.8, 114.5, 114.2, 88.4, 88.4, 87.8, 87.3, 85.3, 85.2, 79.5, 77.9, 77.8, 67.7, 63.7, 56.5, 41.5, 40.2, 38.6, 14.3, 14.2; HRMS (FAB, glycerol/NaI) calcd for $[C_{29}H_{36}O_{11}N_5P + Na]^+$ 716.1945, found 716.1963.

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