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Synthesis of Oligonucleotides Containing 3'-Alkyl Amines Using *N*-Isobutyryl Protected Deoxyadenosine Phosphoramidite.

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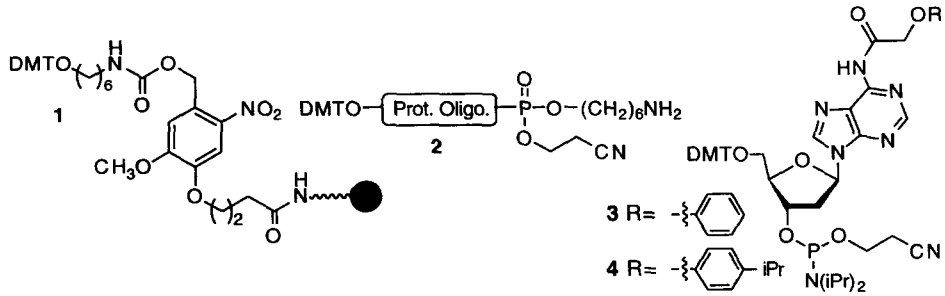
Abstract: Oligonucleotides were synthesized using the *N*-isobutyryldeoxyadenosine protected β-cyanoethyl phosphoramidite, **9**. The oligonucleotides that were synthesized using **9** can be deprotected using concentrated NH_4OH at 55°C in 2 h. No depurination was detected via anion exchange HPLC. The *N*-isobutyryl protected deoxyadenosine did not undergo transamidation with nucleobase and phosphate protected oligonucleotides containing primary alkyl amines, and will be useful in the synthesis of bioconjugates using protected biopolymers.

We previously reported on solid phase oligonucleotide synthesis supports that enable one to site specifically incorporate alkaline labile nucleotides into oligonucleotides.[1] These solid phase supports (e.g. **1**) are also useful for preparing protected oligonucleotides containing a single functional group at their 3'-termini (e.g. **2**), making them potentially useful for the synthesis of oligonucleotide conjugates.[2] We and others observed that benzamide protecting groups are incompatible with the photochemical conditions typically employed to cleave protecting groups and linkers to solid phase supports.[2b,3] We subsequently found that phenoxyacetyl (PAC) protected deoxyadenosine (**3**, PAC-dA), and other commercially available "fast deprotecting" phosphoramidites were compatible with photolabile synthesis supports.[4,5] However, while developing methodology for the synthesis of oligonucleotide bioconjugates using **2**, we observed that significant amounts of the phenoxyacetyl and isopropylphenoxyacetyl groups introduced via **3** and **4** (PAC-dG), respectively, were transferred to the 3'-terminal alkyl amine when concentrated solutions of **2** were left at room temperature for several hours.[6] Transamidation of the isopropylphenoxyacetyl protecting group from PAC-dG did not pose a serious limitation to bioconjugation studies involving **2**, because isobutyryl protected deoxyguanosine was compatible with photochemical cleavage of oligonucleotides from **1**.[3] However, a different nitrogen protecting group for deoxyadenosine was required. We wish to report that *N*-isobutyryldeoxyadenosine phosphoramidite **9** is compatible with photocleavable solid phase synthesis support **1**, and does not undergo transamidation in solutions



of **2**.[7] Furthermore, **9** is readily deprotected in 2 hours, and can be used in general as a fast deprotecting phosphoramidite for automated DNA synthesis.

Electrospray mass spectrometry (ESMS) was invaluable for deducing that the exocyclic amine protecting groups of PAC-dA (**3**) and PAC-dG (**4**) underwent transamidation in **2**. ESMS analysis of **5** (prepared on **1**), following photolytic cleavage and ammonolysis, revealed the presence of biopolymers containing either a phenoxyacetyl, or an isopropylphenoxyacetyl group (Figure 1). ESMS analysis of oligonucleotides prepared on commercially available succinato supports using fast deprotecting PAC phosphoramidites did not indicate the presence of any residual protecting groups of the type observed in **5** (data not shown). Further evidence that a phenoxyacetyl group was transferred to the terminal 3'-alkyl amine was based upon the later retention time of **5** compared to **6**, as measured by anion exchange HPLC, as well as its resistance to cleavage by concentrated NH_4OH (55°C, 12 h).[8]

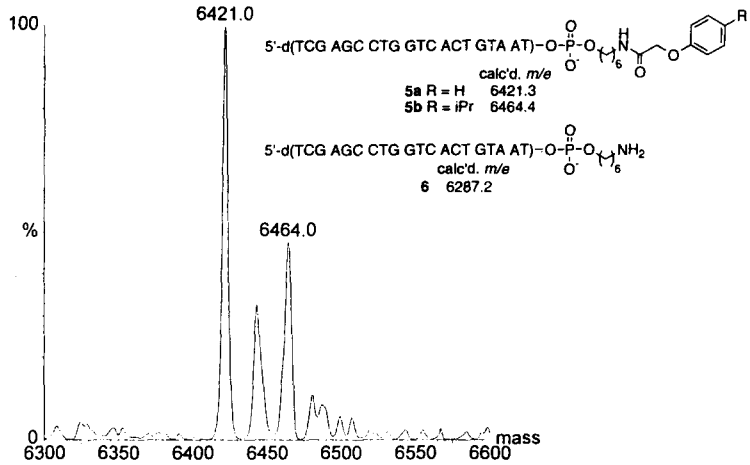
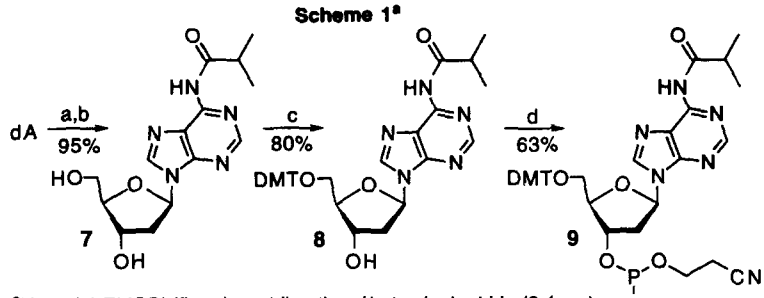


Figure 1. Electrospray mass spectral analysis of **5**. The unassigned peak (*m/e* = 6443.0) corresponds to **5a** + Na. Photolytic cleavage and deprotection were carried out as previously described.[2,4a]

In selecting a suitable amine protecting group for deoxyadenosine, we considered the lability of the group to concentrated NH_4OH, as well as the effect of the group on the acid lability of the glycosidic bond. The isobutyryl group was an attractive candidate, because it was known to be compatible with the photochemical cleavage of oligonucleotides from solid phase supports.[2,3] Furthermore, when used for protecting deoxycytidine and deoxyguanosine, it did not undergo transamidation with a primary amine. To our knowledge, the isobutyryl group has not been used for protecting deoxyadenosine during oligonucleotide synthesis.[5,7] The requisite β-cyanoethyl phosphoramidite (**9**) was synthesized in a straightforward manner from deoxyadenosine (Scheme 1). The exocyclic amine of deoxyadenosine was protected using a transient protection.[9] The free nucleoside (**7**) was then carried on to **9** via standard procedures.[10]

The stability of the glycosidic bond in *N*-isobutyryl protected deoxyadenosine to the acidic deprotection step during DNA synthesis was established via the preparation of 5'-d(T_{14}**9**T_4, **10**) on **1**. Standard

Scheme 1[a]



[a]Key: (a) TMSCl (5 eq.), pyridine then *i*-butyrylanhydride (2.4 eq.) (b) NH_4OH, 0°C (c) DMTCl (1.5 eq.), pyridine, 25°C (d) 2-Cyano-ethyl diisopropylchlorophosphoramidite (1.3 eq.), diisopropylethyl amine (4 eq.), CH_2Cl_2, 0°C

oligonucleotide synthesis cycles were used for the synthesis of oligonucleotides. Anion exchange HPLC analysis of **10** did not show any 5'-d$(T)_{14}$, indicating that the glycosidic bond in *N*-isobutyryl protected deoxyadenosine survived 15 cycles of dedimethoxytritylation.[11] The integrity of **10** was also confirmed by ESMS, in which no isobutyryl adducts were observed (data not shown). The lability of the *N*-isobutyryl group in deoxyadenosine to concentrated NH_4OH was investigated using **7** as substrate. We determined by reverse phase HPLC that the isobutyryl protecting group was completely cleaved in concentrated NH_4OH within 3 h at 55 °C. ESMS of **11** (Figure 2a) which was prepared on succinato resin using **9** and commercially available fast deprotecting deoxycytidine and deoxyguanosine phosphoramidites, confirmed that *N*-isobutyryl protected deoxyadenosine can be employed as a relatively fast (2 h, 55°C) deprotecting phosphoramidite.[5]

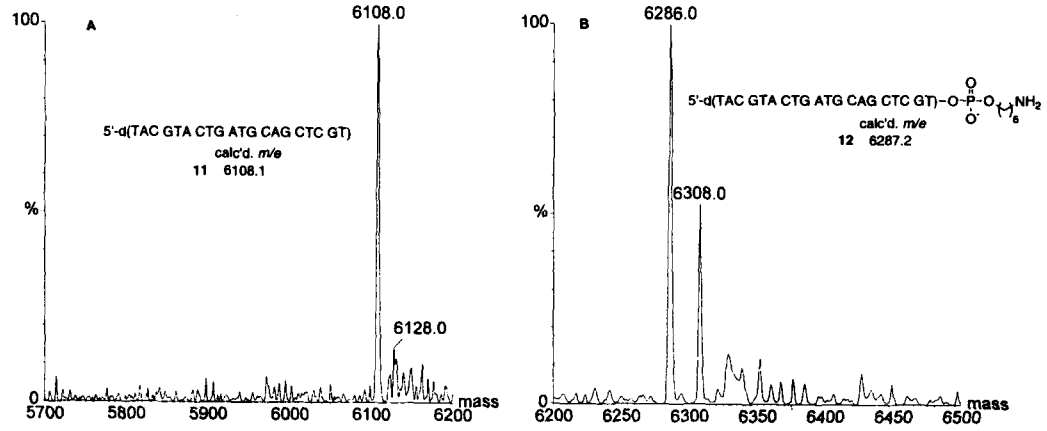


Figure 2. Electrospray mass spectral analysis of a) **11** b) **12**. Resin bound **11** was treated with concentrated NH_4OH at 55 °C for 2 h. Oligonucleotide **12** was cleaved photolytically and deprotected as previously described.[2,4a]

The proclivity of the isobutyryl group to undergo transamidation when present in **2** was initially examined using a **7** as a model compound. No degradation was observed by thin layer chromatography when the free

nucleoside (**7**) was stirred in the presence of *n*-butylamine (0.13 M) at 25 °C for 2 h. The utility of **9** for the synthesis of oligonucleotides containing alkyl amines was demonstrated via its employment (along with *N*-isobutyryldeoxycytidine and *N*-isobutyryldeoxyguanosine protected phosphoramidites) in the preparation of **12** on **1**. In contrast to **5**, following ammonolysis, only the molecular ion and alkali metal adducts of **12** were observed by ESMS (Figure 2b), demonstrating that transamidation did not occur.

In conclusion, we report a protecting group for the exocyclic amine of deoxyadenosine, which is compatible with the photochemical cleavage of protected oligonucleotides containing 3'-alkyl amines from solid phase supports. Phosphoramidite **9** is readily prepared, and should be of general use as a fast deprotecting phosphoramidite for the synthesis of oligonucleotides.

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References and Footnotes

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6. McMinn, D. L.; Greenberg, M. M. (manuscript in preparation).
7. While this letter was in preparation, **9** has become offered as a phosphoramidite compatible with **1** (*The Glen Report* **1996**, *9*, 2.). The synthesis of **9**, its compatibility with primary alkyl amines, and its general utility in oligonucleotide synthesis have not been reported.
8. Anion exchange HPLC of oligonucleotides were carried out on a Vydac #3040L54 weak anion exchange column (4.6 × 250 mm); Solvent A: $(NH_4)_2HPO_4$ (0.1 M, pH 6.7), 20% CH_3CN; Solvent B: $(NH_4)_2HPO_4$ (0.3 M, pH 6.7), 20% CH_3CN; 0-100% B, 30 min, linearly; 1.0 mL/min. Ret. time: **5a**, 21.6 min; **5b**, 23.4 min; **6**, 18.9 min.
9. Gao, X.; Gaffney, B. L.; Hadden, S.; Jones, R. A. *J. Org. Chem.* **1986**, *51*, 755.
10. **9:** ^{1}H NMR ($CDCl_3$) δ 8.63 (s, 1H), 8.36 (s, 1H), 8.11 (s, 1H), 7.43 - 7.23 (m, 9H), 6.80 (d, 4 H, J = 8.7 Hz), 6.46 (t, 1 H, J = 5.0 Hz), 4.60 (m, 1H), 4.13 (m, 1H), 3.79 (s, 1H), 3.42 (m, 2H), 3.2 (m, 1H), 2.95 - 2.52 (m, 2H), 2.12 (m, 1H), 1.32 (d, 6 H, J = 6.9 Hz); ^{31}P NMR ($CDCl_3$) δ 149.56, 149.44; IR (thin film) 2933, 1723, 1608, 1583, 1509, 1462, 1250, 1177, 1094, 910, 729 cm^{-1}.
11. Anion exchange HPLC was carried out as described in reference 8. Ret. time: **10**, 15.6 min; 5'-d$(T)_{14}$, 6.6 min.

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