A Convenient Way to *N*-Allyloxycarbonyl Protected Adenosine and Cytidine Derivatives

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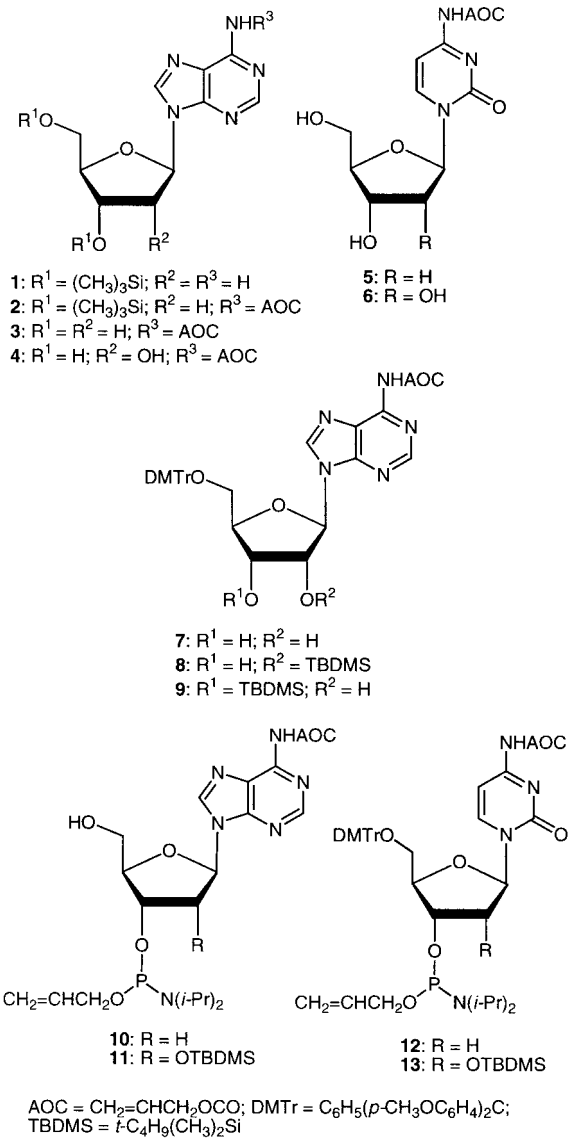
Abstract: A convenient method for the introduction of an allyloxycarbonyl protector to adenyl and cytosyl bases using commercially available allyloxycarbonyl chloride as the protecting reagent has been developed.

In oligonucleotide synthesis, the development of good protecting groups for amino functions of nucleoside bases is an important subject. The protector should be easily introduced and removed under mild conditions, because the internucleotide bond is sensitive to strong acids or bases. As a protector fulfilling such requirements, we developed the allyloxycarbonyl (AOC) group removable by a Pd(0)-catalyzed reaction under almost neutral conditions.[1-3] The AOC protection is useful for the synthesis of DNA and RNA oligomers, particularly, base-labile derivatives with artificial modifications. Actually, its utility has been demonstrated in the preparation of oligoDNAs containing alkaline-labile (5*R*)-5,6-dihydro-5-hydroxythymidine, known as a mutagenic and/or cytotoxic modification of RNA.[4] Further, AOC protection was effectively employed in the synthesis of oligoDNAs with modified backbones such as phosphonodiesters,[5] phosphotriesters,[5] and phosphorothiodiesters,[6] 2'-deoxy-3-isoadenosine-incorporated oligoDNAs,[7] cytidine-5'-monophosphono-*N*-acetylneuraminic acid (CMP-Neu5Ac),[8] branched-type RNAs,[9] and N^4-acetylcytidine-incorporated oligoRNAs.[10] However, the existing strategy has a drawback in the preparation of *N*-AOC adenosine and cytosine compounds: the protection needs rather expensive reagents and time-consuming multi-operations. For instance, the allyloxycarbonylation of these two bases requires allyl 1-benzotriazolyl carbonate (AOCOBT)[2,3,9] or 1-(allyloxycarbonyl)tetrazole (AOCTet)[3,9] as reagents, which are not commercially available but have to be prepared from allyloxycarbonyl chloride (AOCCl) using expensive 1-(hydroxy)benzotriazole or 1*H*-tetrazole. Further, in the preparation of the *N*-AOC-adenosines, moisture-sensitive *tert*-butyllithium[2] or *tert*-butylmagnesium chloride[9] is employed for the activation of the amino function of the nucleobase. These reagents require special care, like working under an argon atmosphere. This paper describes a simple access to the *N*-AOC-protected adenosine and cytidine derivatives using commercially available, less expensive AOCCl in the presence of *N*-methylimidazole.



Introduction of the AOC group to the N^6-position of 2'-deoxyadenosine was achieved in one pot via the following process. Initially, 2'-deoxyadenosine was treated with an excess of hexamethyldisilazane and a catalytic amount of $(NH_4)_2SO_4$[11] in refluxing dioxane to give the bis-silylated compound **1**. Subsequently, **1** was allyloxycarbonylated with AOCCl and *N*-methylimidazole, giving **2**, and finally the transient silyl protector was removed by triethylamine in methanol to afford **3** in 70 % isolated yield.[12] In a similar manner, N^6-AOC-adenosine **4**,[13] N^4-AOC-2'-deoxycytidine **5**,[14] and N^4-AOC-cytidine **6**[15] were prepared from the parent nucleosides in 89–99% overall yields. This method could not be applied to the amino group of the guanyl base.[16] According to literature methods or their modifications, the AOC-protected compounds, **3–6**, can be converted to the phosphoramidites, **10**,[3] **11**,[17-20] **12**,[3] and **13**,[10,17] requisite as monomer units for oligonucleotide synthesis. For example, **11** was obtained from **4** via (1) tritylation of the 5'-hydroxyl group by $C_6H_5(p\text{-}CH_3OC_6H_4)_2CCl$ in a pyridine–DMF mixture, giving **7**[18] (77% yield), (2) silylation with $t\text{-}C_4H_9(CH_3)_2SiCl$ and imidazole in DMF, producing **8**[19] (33% yield) together with the 3'-*O*-silylated derivative **9** (45% yield), and (3) the condensation of **8** and $(CH_2{=}CHCH_2O)P[N(i\text{-}C_3H_7)_2]_2$ in the presence of diisopropylammonium tetrazolide (81% yield).[20]

In conclusion, the present allyloxycarbonylation is superior in the respect of operational simplicity and cost-performance to previous methods.

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(12) The experimental procedure for the preparation of N^6-(allyloxycarbonyl)-2'-deoxyadenosine (**3**): A mixture of pre-dried 2'-deoxyadenosine (1.50 g, 5.97 mmol), hexamethyldisilazane (20 mL), and a catalytic amount of $(NH_4)_2SO_4$ in dioxane (20 mL) was heated under reflux for 2.5 h. The reaction mixture was concentrated and coevaporated twice with dry toluene, giving an oily residue, which was dissolved in dichloromethane (50 mL). To this solution were added *N*-methylimidazole (1.40 mL, 1.44 g, 17.8 mmol) and AOCCl (1.90 mL, 2.16 g, 17.8 mmol) and the resulting mixture was stirred at room temperature for 36 h. Concentration of the reaction mixture gave a viscous oil, which was dissolved in methanol (50 mL) containing triethylamine (13 mL). The resulting solution was stirred at room temperature for 12 h and then was evaporated to afford an oil. This crude material was subjected to silica gel column chromatography with a 1:25 mixture of methanol and dichloromethane as eluent to give **3** (1.40g, 70% yield) as a colorless foam; R_f 0.3 (a 1:9 methanol–dichloromethane mixture); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.30–2.36 (m, 1H), 2.72–2.79 (m, 1H), 3.49–3.64 (m, 2H), 3.86–3.89 (m, 1H), 4.42–4.44 (m, 1H), 4.64–4.65 (m, 2H), 4.98–5.00 (m, 1H), 5.22–5.43 (m, 3H), 5.92–6.00 (m, 1H), 6.42–6.45 (m, 1H), 8.62 (s, 1H), 8.68 (s, 1H), 10.60 (s, 1H).

(13) **4**: Mp 164–166 °C (crystallization from the reaction mixture); 89 % yield; R_f 0.3 (a 1:9 methanol–dichloromethane mixture); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 3.55–3.71 (m, 2H), 3.95–3.97 (m, 1H), 4.17 (m, 1H), 4.61–4.66 (m, 3H), 5.14–5.44 (m, 4H), 5.53 (s, 1H), 5.90–6.04 (m, 2H), 8.63 (s, 1H), 8.68 (s, 1H), 10.66 (s, 1H).

(14) **5**: A foam; 95% yield; R_f 0.4 (a 1:9 methanol–dichloromethane mixture); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.89–2.04 (m, 1H), 2.22–2.28 (m, 1H), 3.52–3.58 (m, 2H), 3.83–3.84 (m, 1H), 4.21 (m, 1H), 4.60–4.62 (m, 2H), 5.09–5.38 (m, 4H), 5.86–5.99 (m, 1H), 6.06–6.11 (m, 1H), 7.00–7.03 (d, 1H, *J* = 7.4 Hz), 8.30–8.32 (d, 1H, *J* = 7.4 Hz), 10.78 (s, 1H).

(15) **6**: Mp 103–107 °C (recrystallization from a methanol–ethyl acetate mixture); 99 % yield; R_f 0.3 (a 1:9 methanol–dichloromethane mixture); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.57–3.97 (m, 5H), 4.62–4.64 (m, 2H), 5.03 (d, 1H, *J* = 5.4 Hz), 5.15 (t, 1H, *J* = 4.9 Hz), 5.22–5.38 (m, 2H), 4.47 (d, 1H, *J* = 4.4 Hz), 5.77 (d, 1H, *J* = 2.4 Hz), 5.91–5.98 (m, 1H), 7.00 (d, 1H, *J* = 7.3 Hz), 8.40 (d, 1H, *J* = 7.3 Hz), 10.80 (s, 1H).

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(18) **7**: A yellow foam; 77% yield from **4**; R_f 0.76 (a 1:9 methanol–dichloromethane mixture); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 3.22–3.24 (m, 2H), 3.71 (s, 6H); 4.06–4.11 (m, 1H), 4.30–4.34 (m, 1H), 4.64–4.66 (m, 2H), 4.73–4.78 (m, 1H), 5.25–5.44 (m, 3H), 5.59–5.61 (m, 1H), 5.90–6.04 (m, 2H), 6.79–6.84 and 7.18–7.36 (2 m's, 13H), 8.56 (s, 2H), 10.65 (s, 1H).

(19) **8**: A colorless foam; 33 % yield from **7**; R_f 0.64 (a 3:2 ethyl acetate–hexane mixture); ^{1}H-NMR (270 MHz, DMSO-d_6) δ –0.15 (s, 3H), –0.05 (s, 3H), 0.73 (s, 9H), 3.27–3.68 (m, 2H), 3.71 (s, 6H), 4.11–4.28 (m, 2H), 4.63–4.65 (m, 2H), 4.86 (t, *J* = 4.78 Hz, 1H), 5.47–5.92 (m, 3H), 5.86–6.04 (m, 2H), 6.80–6.84 and 7.19–7.47 (2 m's, 13H), 8.54 (s, 1H), 8.62 (s, 1H), 10.65 (s, 1H). **9**: A colorless foam; 45 % yield from **7**; R_f 0.4 (a 3:2 ethyl acetate–hexane mixture); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 0.04 (s, 3H), 0.08 (s, 3H), 0.84 (s, 9H), 3.11–3.16 (m, 1H), 3.33–3.39 (m, 1H), 3.71 (s, 6H), 4.00–4.06 (m, 1H), 4.48–4.51 (m, 1H), 4.64–4.66 (m, 2H), 4.86–4.90 (m, 1H), 5.18–5.43 (m, 3H), 5.89–6.05 (m, 2H), 6.82–6.85 and 7.19–7.39 (2 m's, 13H), 8.54 (s, 1H), 8.57 (s, 1H), 10.67 (s, 1H).

(20) **11**: A colorless foam; 81% yield from **8**; R_f 0.42 (a 3:7 ethyl acetate–hexane mixture); ^{1}H-NMR (400 MHz, $CDCl_3$) δ –0.21 (s, 3H), –0.02 (s, 3H), 0.75 and 0.77 (2 s's, 9H), 1.15–1.28 (m, 12 H), 3.44–3.62 (m, 2H), 3.78 (s, 3H), 3.79 (s, 3H), 3.96–4.26 (m, 2H), 4.41-4–49 (m, 3H), 4.76–4.78 (m, 2H), 4.97–5.44 (m, 6H), 5.89–6.09 (m, 3H), 6.77–6.85 and 7.20–7.73 (2 m's, 13 H), 8.16–8.19 (m, 2H), 8.64 and 8.66 (2 s's, 1H); ^{31}P-NMR (400 MHz, $CDCl_3$, H_3PO_4 standard) δ 148.8, 151.0.