Convenient and Regioselective Synthesis of Nucleoside Phosphoramidate Monoesters

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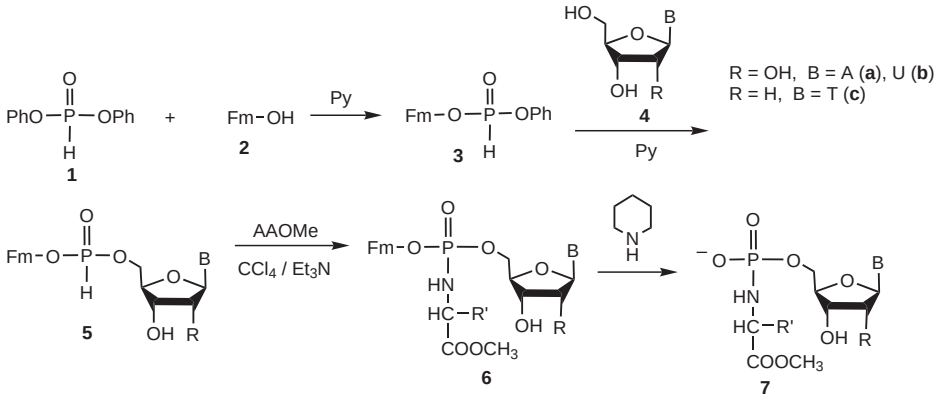
Abstract: We have developed a convenient and efficient method for the synthesis of nucleoside phosphoramidate monoesters. This step-wise synthesis, consisting of an ester exchange, reaction of the 5′-OH of nucleosides with fluorenylmethyl aryl phosphite, and an Atherton–Todd reaction, gave fluorenylmethyl nucleoside 5′-phosphoramidates; final removal of the fluorenylmethyl group yielded the desired target products in good yields.

Key words: phosphoramidate, ester-exchange, diphenyl phosphite, nucleoside, Atherton–Todd reaction

In general, the biological activity of antiviral and anticancer nucleosides is dependent on the host cell kinase activity producing their active triphosphorylated forms. However, the phosphorylating yields of these nucleosides are very low because they are unnatural substrates for the corresponding kinases. The development of nucleoside prodrugs capable of undergoing intracellular activation to the corresponding nucleotides has become an area of intense interest.[1] Nucleoside amino acid phosphoramidates show promise as potential pronucleotides.[1a,b] Moreover, nucleoside phosphoramidate monoesters are potent antiviral and/or anticancer agents with enhanced activity and reduced cytotoxicity.[2] These nucleoside phosphoramidate monoesters are thought to exert their biological functions through a P–N bond cleavage by phosphoramidases to yield the corresponding nucleoside monophosphates.[2d,3] These studies have provided valuable insight to the design of phosphoramidate-based pronucleotides.[1a,b] Chlorophosphate[3a,4] and DCC-mediated[5] coupling methods are usually applied to the synthesis of this kind of phosphoramidates, however, most of these methods required the 2′,3′-OH and NH_2 to be protected prior to phosphorylation of 5′-OH. Wagner et al.[3b] used direct phosphorylation of nucleosides – treatment of 5′-OH phosphorus oxychloride in triethyl phosphate, hydrolysis to nucleoside monophosphate,[5] and finally coupling of the nucleoside monophosphate with the amino acid methyl esters in *tert*-butanol and water using DCC, afforded the corresponding phosphoramidates. The method usually needs a long reaction time, the overall yields are low, and purification of the nucleoside monophosphate over an ion-exchange column (BioRad AG1-X8) is troublesome, so it is necessary to develop a new general and efficient approach to nucleoside phosphoramidate monoesters.

The Atherton–Todd reaction is considered to be an effective approach for the construction of the P–N bond from H-phosphonates; the synthetic route is summarized in Scheme 1.



Scheme 1 Synthetic route of nucleoside phosphoramidate monoesters

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The ester-exchange reaction with fluorenylmethanol (Fm-OH) and 1.2 equivalents of diphenyl phosphite (^{31}P NMR, 1.40 ppm) in anhydrous pyridine at –5 °C under a nitrogen atmosphere led to **3** with a minor amount of difluorenylmethyl phosphite as side product (ca. 6%, ^{31}P NMR determination, ^{31}P NMR, δ = 9.00 ppm); the reaction was completed within ten minutes. The corresponding nucleoside (1.4 equiv) in pyridine was added dropwise to the resulting solution at –5 °C, the solution was warmed to 40 °C, and the reaction was complete within 1 h. ^{31}P NMR spectroscopy showed that the major product fluorenylmethyl nucleoside 5′-*H*-phosphonate (^{31}P NMR, δ 9.60–10.00ppm) along with side products difluorenylmethyl phosphite and dinucleoside phosphite (^{31}P NMR, δ 10.50 ppm) were present in the solution. Pyridine was removed from the solution under reduced pressure; subsequent Atherton–Todd reaction of the residue **5** (the crude product fluorenylmethyl nucleoside 5′-*H*-phosphonate) with 1.2 equivalents of amino acid methyl ester in THF in the presence of CCl_4 and Et_3N provided fluorenylmethyl nucleoside 5′-phosphoramidate (**6**) in 73–82% isolated overall yields. Treatment of **6** with piperidine in CH_2Cl_2 led to the target products **7**. A total isolated yield of 65–75% was achieved (**1**→**7**, Table 1).

Table 1 ^{31}P NMR and Total Yields of the Synthesized Compounds

Compound	NuOH	R′	^{31}P NMR (δ/ppm)	Yield (%)
7aa	A	CH_3	7.29	67
7ab	A	$CH(CH_3)_2$	6.83	73
7ac	A	CH_2Ph	6.82	73
7ad	A	CH_2COOCH_3	6.65	66
7ae	A	CH_2OH	6.77	65
7ba	U	CH_3	6.58	70
7bb[6]	U	$CH(CH_3)_2$	7.01	73
7bc	U	CH_2Ph	6.85	73
7bd	U	CH_2COOCH_3	7.02	71
7be	U	CH_2OH	6.80	66
7ca	T	CH_3	6.83	68
7cb	T	$CH(CH_3)_2$	6.86	74
7cc	T	CH_2Ph	6.57	75
7cd	T	CH_2COOCH_3	7.00	70
6bb[6]	U	$CH(CH_3)_2$	8.70, 8.50	82

Regioselective reaction of fluorenylmethyl aryl phosphite with the 5′-OH of nucleosides was confirmed by ^{1}H NMR spectroscopy. The 5′-CH_2 of the free nucleosides should appear as doublets in the ^{1}H NMR spectrum, which is a result of the coupling with 4′-CH, however, the ^{1}H NMR spectra of nucleoside phosphoramidates (**6** and **7**) showed multiple peaks due the coupling of the H in P-5′-OCH_2 with P and H in 4′-CH. The regioselective phosphorylation of 5′-OH is attributed to the high reactivity of 5′-OH compared with 2′,3′-OH, and NH_2.

Compared with the previous approaches to nucleoside phosphoramidates, this method has its advantages. The reactions are operationally simple and fast, the total yields are high, and the desired target products are easily purified.

In conclusion, we have developed a new method for the synthesis of nucleoside phosporamidate monoesters, regioselective ester-exchange, reaction of the 5′-OH of nucleosides with fluorenylmethyl aryl phosphite, and Atherton–Todd reaction gave fluorenylmethyl nucleoside 5′-phosporamidates; removal of the fluorenylmethyl yielded the desired target products in good yields.

Fluorenylmethyl phenyl *H*-phosphonate (3): Fm-OH (196 mg, 1 mmol) in anhydrous pyridine (3 mL) was added dropwise to diphenyl phosphite (281 mg, 1.2 mmol) in anhydrous pyridine (3 mL), and the solution was stirred at –5 °C. The reaction was complete within 10 min. ^{31}P NMR (pyridine): δ = 5.30 ppm.

Fluorenylmethyl nucleoside 5′-*H*-phosphonates (5): Nucleoside (1.4 mmol) in anhydrous pyridine (3 mL) was added to a solution of the above. The resulting solution was stirred for about 1 h at 40 °C, and concentrated under reduced pressure to provide the crude product fluorenylmethyl nucleoside 5′-*H*-phosphonate **5** (mixture of diastereoisomers, ratio 1:1), ^{31}P NMR (pyridine): δ = 9.8 ppm (two peaks, $^1J_{P\text{-}H}$ = 692 Hz).

Fluorenylmethyl nucleoside 5′-phosphoramidates (6): Amino acid methyl ester hydrogen chloride (1.2 mmol), Et_3N (2 mmol), and CCl_4 (0.5 mL) were mixed together in anhydrous THF (5 mL); the crude product fluorenylmethyl nucleoside 5′-*H*-phosphonate (**5**) in anhydrous THF (2 mL) was added dropwise to the solution at –5 °C, and the solution was stirred for 30 min at r.t. The Atherton–Todd reaction produced fluorenylmethyl nucleoside 5′-phosphoramidate **6** in almost quantitative yield (^{31}P NMR). The solvent was removed by rotary evaporation, the residue was dissolved in CH_2Cl_2 (5 mL), and then the solution was washed with 0.1 M HCl (3 × 3 mL). Purification by silica gel column chromatography (CH_2Cl_2–MeOH, 10:1) gave the fluorenylmethyl nucleoside 5′-phosphoramidate **6** (mixture of diastereoisomers, ratio 1:1). ^{31}P NMR: δ = 8.60 (2 peaks). Overall yield from **1** to **6**: 73–82%.

Nucleoside 5′-phosphoramidate monoesters (7): Piperidine (1 mL) was added to **6** in CH_2Cl_2 (5 mL) and the solution was stirred for 5 min. The solvent and piperidine were removed on a rotary evaporator. Purification by silica gel column chromatography (PrOH–H_2O–NH_3×H_2O, 7:1:2) gave the desired target product nucleoside phosphoramidates **7** in 65–75% (total isolated yield from **1** to **7**). These compounds were identified by ^{31}P, ^{1}H, and ^{13}C NMR spectroscopy and ESI-MS.

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(6) Spectroscopic data of selected compounds: Fm-U-P-LeuOCH$_3$ (**6bb**). Overall yield from **1** to **6**: 82%. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.77–0.82 (d, 6 H, CH_3, ^3J = 6.00 Hz), 1.26 (m, 2 H, β-CH_2), 1.40 (m, 1 H, γ-CH), 3.49–3.62 (m, 4 H, OCH_3, α-CH), 3.85 (m, 5′-CH_2), 3.99 (Fm-CH), 4.02 (m, 1 H, 4′-CH), 4.14 (t, 1 H, 3′-CH), 4.25 (t, 1 H, 2′-CH), 5.21 (2 H, Fm-CH_2), 5.81 (1 H, 1′-CH), 5.62 (d, 1 H, 3-CH), 7.74 (d, 1 H, 2-CH), 7.20–7.55 (8 H, Fm-Ar-). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.67 (CH_3), 22.73 (CH_3), 24.54 (γ-CH), 43.84 (β-CH_2), 48.09 (Fm-CH), 52.34 (OCH_3), 52.86 (α-CH), 64.80 (5′-CH_2), 68.46 (2′-CH), 69.84 (3′-CH), 72.40 (Fm-CH_2), 83.10 (4′-CH), 89.81 (1′-CH), 102.70 (3-CH), 120.12, 124.98, 127.26, 127.97, 140.34, 141.42 (Fm-Ar), 143.17 (2-CH), 151.21 (6-C), 163.98 (3-C), 174.68 (CO). ^{31}P NMR (121 MHz, $CDCl_3$): δ = 8.70, 8.50. ESI-MS (Positive): *m*/*z* = 630 $[M + H]^+$. **U-P-LeuOCH$_3$ (7bb)**. Total yield from **1** to **7**: 73%. ^{1}H NMR (300 MHz, D_2O): δ = 0.73–0.75 (d, 6 H, CH_3, ^3J = 6.00 Hz), 1.38 (m, 2 H, β-CH_2), 1.54 (m, 1 H, γ-CH), 3.60–3.62 (4 H, m, OCH_3, α-CH), 3.83–3.94 (m, 5′-CH_2), 4.12 (m, 1 H, 4′-CH), 4.18 (t, 1 H, 3′-CH), 4.23 (t, 1 H, 2′-CH), 5.84 (1 H, 1′-CH), 5.83 (d, 1 H, 3-CH), 7.85 (d, 1 H, 2-CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.45 (CH_3), 21.98 (CH_3), 24.26 (γ-CH), 43.09 (d, β-CH_2, $^3J_{P\text{-}C}$ = 7.16 Hz), 52.61 (OCH_3), 53.48 (α-CH), 63.60 (5′-CH_2), 69.91 (2′-CH), 73.87 (3′-CH), 83.53 (d, 4′-CH, $^3J_{P\text{-}C}$ = 8.61 Hz), 88.65 (1′-CH), 102.69 (3-CH), 141.86 (2-CH), 151.81 (6-C), 166.19 (3-C), 178.27 (CO). ^{31}P NMR (121 MHz, D_2O): δ = 7.01. ESI-MS (Negative): *m*/*z* = 450 $[M - H]^-$.