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Synthesis of diuridine 3′,5′-boranophosphate: *H*-phosphonate approach

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Abstract

Diuridine 3′,5′-boranophosphate, the RNA analogue of boranophosphate nucleic acids, was synthesized by a new approach via the *H*-phosphonate. Two diastereomers of diuridine 3′,5′-boranophosphate were separated by reverse phase HPLC.

Oligonucleotides in which one of the non-bridging phosphate oxygen atoms is replaced by a borane group (BH_3) represent a novel class of modified nucleic acids.[1–5] The borane group (BH_3) in oligonucleoside boranophosphates is isoelectronic with the oxygen (O) in natural oligonucleotides and the sulfur (S) in oligonucleoside phosphorothioates,[6] and is isosteric with the methyl group (CH_3) in oligonucleoside methylphosphonates.[7] The oligonucleoside boranophosphates are nuclease-resistant[8,9] and carry the same negative charge as natural DNA and RNA. They are of special interest as potential antisense, antigene, and ribozyme agents to control gene expression.[8]

Oligodeoxyribonucleoside (DNA) boranophosphates have been chemically prepared using the phosphoramidite approach[1] and more recently using the *H*-phosphonate approach.[10–13] For oligoribonucleoside (RNA) boranophosphates, only the synthesis of diuridine boranophosphate using the phosphoramidite approach was reported.[14] This phosphoramidite method however has certain disadvantages. First, the borane–dimethylsulfide complex, used in the boronation step, is a strong reducing agent which may cause base modification.[12,13] Second, the 5′-dimethoxytrityl (DMT) protected phosphoramidite is not a suitable precursor because the boranophosphate group is incompatible with the DMT cations released during deprotection of the oligonucleotide.[12,15] To overcome these problems, the *H*-phosphonate approach has been applied to synthesize boranophosphate DNA analogues: dithymidine 3′,5′-boranophosphate[10,12] and oligothymidine boranophosphates.[11–13] Using a similar approach, we report here the successful synthesis of a boranophosphate RNA analogue, diuridine 3′,5′-boranophosphate, via silylation of an *H*-phosphonate precursor followed by boronation.

In initial studies, 5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)uridin-3′-yl *H*-phosphonate **1a** was condensed with 2′,3′-*O*-dibenzoyluridine **2a** to produce one diastereomer of the *H*-

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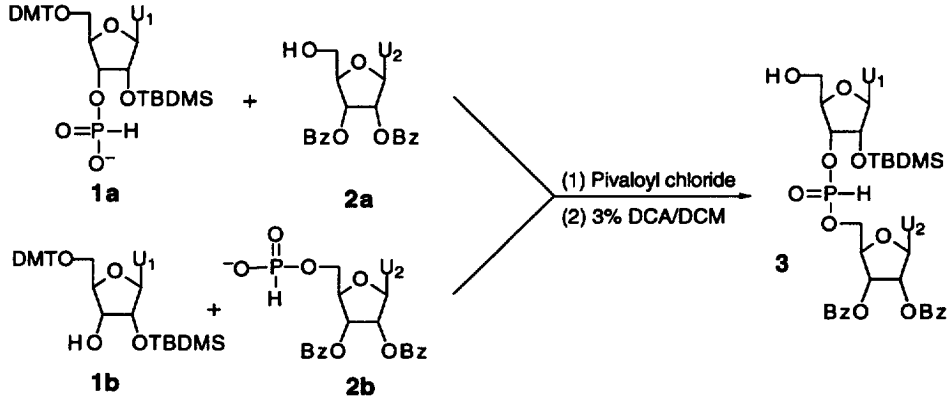
phosphonate diester in excess (ca. 81% of the major isomer at δ 10.52 ppm and 19% of the minor isomer at δ 9.21 ppm by ^{31}P NMR[16] in $CDCl_3$). The subsequent DMT-removal and silica gel chromatographic purification (0–15% methanol in dichloromethane as solvent) resulted in only one isomer of 2′-*O*-(*tert*-butyldimethylsilyl)-uridyl-(3′→5′)-2′,3′-*O*-dibenzoyluridine 3′-*H*-phosphonate **3** (^{31}P NMR, DMSO-d_6, δ 11.05 ppm). This result is interesting from the view point of stereoselectivity of condensation but may not be preferable when comparable amounts of both diastereomers are desired.[17] Ahmer et al. showed that the content of the minor *H*-phosphonate isomer could be increased from 15–20% to 40% by condensing a ribonucleoside 5′-*H*-phosphonate with a ribonucleoside having a free 3′-hydroxy group.[17] Thus, we decided to synthesize diribonucleoside 3′,5′-boranophosphate **6** using a three-step approach, which involves a condensation of a protected uridine 5′-*H*-phosphonate (**2b**) with uridine having a free 3′-hydroxy group (**1b**), followed by a two-step boronation reaction.



To a solution of 2′,3′-*O*-dibenzoyluridine-5′-yl-*H*-phosphonate[18] **2b** (92.6 mg, 0.15 mmol) and 5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-uridine **1b** (132 mg, 0.2 mmol) in anhydrous pyridine (4.0 mL) was added pivaloyl chloride (100 μL, 0.75 mmol). The condensation was completed within 30 min. The ^{31}P NMR ($CDCl_3$) spectrum of the reaction mixture showed two singlets at δ 10.51 ppm (60%) and δ 9.13 ppm (40%). Subsequent treatment with 3% dichloroacetic acid in dichloromethane (3% DCA/DCM) removed the 5′-DMT protecting group. The resulting 2′-*O*-(*tert*-butyldimethylsilyl)-uridyl-(3′→5′)-2′,3′-dibenzoyluridine 3′-*H*-phosphonate **3** was obtained after chromatography on a silica gel column as a mixture of two isomers.[19]

The silylation of the *H*-phosphonate diester **3** with *N,O*-bis(trimethylsilyl)acetamide (BSA) in THF followed by in situ treatment with a borane-*N,N*-diisopropylethylamine complex (DIPEA·BH_3) resulted in the formation of the borane-phosphite **4** (boranophosphate triester), which was confirmed as a broad peak (δ 102–108 ppm) in the ^{31}P NMR ($CDCl_3$) spectrum.[1,13] The diastereomeric ratio of the two isomers of compound **4** could not be determined from the ^{31}P NMR spectra because of peak broadening.[20] We and others have shown that boronation of phosphite triester to boranophosphate appears to proceed with retention of configuration at the phosphorus atom.[21,22] Without purification, **4** was hydrolyzed by ethanol:concentrated ammonia (1:3, v/v) to give 2′-*O*-(*tert*-butyldimethylsilyl)-uridyl-(3′→5′)-uridine boranophosphate **5**. Compound **5** (a diastereomeric mixture) was purified by ion-exchange chromatography on QA-52 cellulose with a linear gradient of 0.005 M to 0.2 M NH_4HCO_3 (pH 9.6).[23]

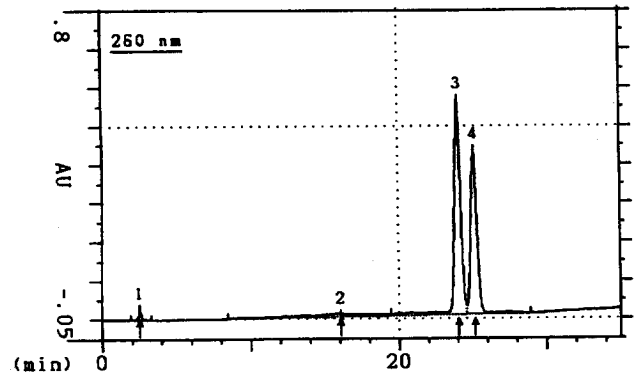
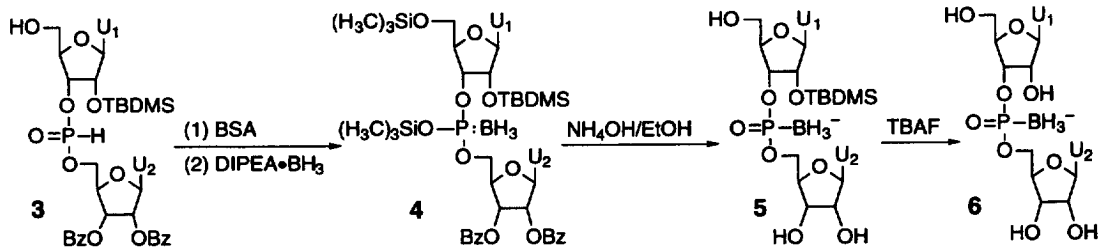


Figure 1. Separation of diastereomers of diuridine 3′,5′-boranophosphate **6** by HPLC. The elution was carried out on a Waters Delta Pak C18 column (15 μ, 100 Å, 3.9×300 mm) with a linear gradient of 0–30% methanol in 50 mM triethylammonium acetate (pH 6.8) at a flow rate of 1.5 mL/min. The retention times for two isomers of **6** (peaks 3 and 4) were 24.03 min (55%) and 25.13 min (39%)



Treatment of compound **5** with tetrabutylammonium fluoride (TBAF) removed the 2′-*O*-*tert*-butyldimethylsilyl group and yielded diuridine 3′,5′-boranophosphate **6**.[24] Compound **6** was purified and the two diastereomers were separated by reverse phase HPLC (Fig. 1).

In summary, we successfully synthesized diuridine 3′,5′-boranophosphate through silylation of its *H*-phosphonate precursor in solution phase followed by reaction with an amine·borane complex. The procedure decribed here for the synthesis of diuridine 3′,5′-boranophosphate may be adaptable to solid phase synthesis of diribonucleoside 3′,5′-boranophosphates and oligoribonucleoside boranophosphates.

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19. Compound **3**: yield: 76%. MS (FAB$^+$): $[M+Na+MeOH]^+$ 911.2 (calcd [M] 856.9 for $C_{38}H_{45}N_4O_{15}PSi$). ^{31}P NMR ($CDCl_3$): δ 10.78 (62%) and 9.81 (38%). ^{31}P NMR (DMSO-d_6): δ 11.08 and 11.04. ^{1}H NMR (DMSO-d_6): δ 8.27 (m, 2H, H6), 7.90, 7.79 (2d, 4H, H–Ar), 7.61 (m, 2H, H–Ar), 7.44, 7.38 (2t, 4H, H–Ar), 6.18, 6.11 (2m, 2H, H1′), 5.89 (d, 1H, $H_{U2}5$), 5.83, 5.78 (2t, 1H, $H_{U2}3'$), 5.72 (m, 2H, $H_{U2}2'$, $H_{U1}5$), 4.90, 4.77 (2m, 1H, $H_{U1}3'$), 4.60 (m, 1H, $H_{U2}4'$), 4.41 (m, 3H, $H_{U2}5'$, $H_{U1}2'$), 4.14 (unresolved m, 1H, $H_{U1}4'$), 3.59 (m, 2H, $H_{U1}5'$), 0.76 (s, 9H, *t*-BuSi), –0.05 (s, 6H, MeSi).
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23. Compound **5**: overall yield (**3** to **5**): 40%. MS (FAB$^+$): $[M+H]^+$ 663.0 (calcd [M] 661.5 for $C_{24}H_{39}N_4O_{13}PSi$). ^{31}P NMR (D_2O): δ 92–96 (br). ^{1}H NMR (D_2O): δ 7.81, 7.73 (d, m, 2H, H6), 5.80, 5.75 (2m, 4H, H5, H1′), 0.68, 0.67 (2s, 9H, *t*-BuSi), –0.06, –0.07 (2s, 6H, MeSi), –0.2 to +0.6 (br, 3H, BH_3).
24. Compound **6**: yield: 74%. MS (FAB$^-$): $[M]^-$ 547.1 (calcd 547.2 for $C_{18}H_{25}BN_4O_{13}P$). ^{31}P NMR (D_2O): δ 93–97 (br). ^{1}H NMR (D_2O): δ 7.84, 7.76 (2d, 2H, H6), 5.78–5.66 (m, 4H, H5, H1′), –0.25 to +0.65 (br, 3H, BH_3).