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Synthesis and Separation of Diastereomers of Uridine 2′,3′-Cyclic Boranophosphate

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Abstract—The first boron-containing 2′,3′-cyclic phosphate-modified analogue, uridine 2′,3′-cyclic boranophosphate (2′,3′-cyclic-UMPB), was synthesized. 5′-*O*-Protected uridine was cyclophosphorylated by diphenyl *H*-phosphonate to yield uridine 2′,3′-cyclic *H*-phosphonate, which upon silylation followed by boronation and subsequent acid treatment gave 2′,3′-cyclic-UMPB in high yield. The two diastereomers of 2′,3′-cyclic-UMPB were separated by HPLC. An alternative method for synthesis of uridine 2′,3′-cyclic phosphorothioate (2′,3′-cyclic-UMPS) via *H*-phosphonate was also described.

2′,3′-Cyclic phosphate esters of nucleosides are intermediates in the ribonuclease-catalyzed hydrolysis of ribonucleic acid (RNA), and are themselves substrates for ribonucleases.[1] Nucleoside 2′,3′-cyclic phosphorothioate analogues have been exploited to study the mechanism of ribonuclease catalysis.[2] Comparison of the configuration of reactants and products where the phosphate reaction center has been replaced by a phosphorothioate gives important information on the stereochemical course of ribonuclease-catalyzed reactions.[3–5] For example, pancreatic ribonuclease (RNase A) exhibits a preference for the Rp (or *endo*) isomers of uridine 2′,3′-cyclic phosphorothioate (2′,3′-cyclic-UMPS) and cytidine 2′,3′-cyclic phosphorothioate (2′,3′-cyclic-CMPS).[3] These phosphorothioate analogues have been used to investigate the stereoselectivity of ribozyme cleavage reactions.[6] Likewise, nucleoside 2′,3′-cyclic boranophosphate, in which a non-bridging oxygen of the cyclic phosphate is replaced by an isoelectronic borane group (BH_3),[7,8] should provide another useful tool for investigating the mechanisms of the ribonuclease-catalyzed reactions.

The first synthesis of a 2′,3′-cyclic phosphate-modified analogue, 2′,3′-cyclic-UMPS, was described by Eckstein in 1968.[9] The Rp (or *endo*) isomer was isolated by fractional crystallization of the triethylammonium salt[3] and used as reference to determine the absolute configurations of other chiral phosphorothioate analogues.[2] This method involved thiophosphorylation of 5′-*O*-acetyl[4,9] or 5′-*O*-DMT[10] protected nucleoside by triimidazolyl-1-phosphine-sulfide followed by water and ammonia treatment (9–44% yields). Another approach utilized 2-chloro-4*H*-1,3,2-benzodioxa-phosphorin-4-one to phosphorylate the 5′-*O*-acetyl ribonucleoside.[11] Sulfurization of the phosphorylated product followed by ammonia treatment gave 2′,3′-cyclic-NMPS in 32–45% yields. In another method, cyclization of nucleoside 3′(2′)-phosphorothioate derivatives in the presence of *N,N′*-dicyclohexyl-carbodiimide (DCC) in pyridine or *N*-cyclohexyl-*N′*-(3-trimethyl-ammonium-1-propyl)-carbodiimide led to the formation of 2′,3′-cyclic-NMPS in 86–96% yields.[12]

Here, we propose a new approach for synthesis of 2′,3′-cyclic-UMPS, which involves sulfurization of the intermediate uridine 2′,3′-cyclic *H*-phosphonate **4** (Scheme 1). Intermediate **4** was obtained by condensation (cyclization) of a mixture of uridine 3′- and 2′-*H*-phosphonates[13] (**2** and **3**) by pivaloyl chloride. The condensation (cyclization) step was completed within 15 min, as suggested by ^{31}P NMR ($CDCl_3$) spectra of the reaction mixture in which two signals at δ 7.2 and 6.6 (for compounds **2** and **3**) were shifted to δ 21.0 and 19.0. Without purification, the reaction mixture containing intermediate **4** was oxidized with elemental sulfur in carbon disulfide to yield 5′-*O*-DMT-uridine 2′,3′-cyclic phosphorothioate **5**. The formation of intermediate **5** was confirmed by the appearance of the two single peaks at δ 80.0 and 78.7 in ^{31}P NMR ($CDCl_3$) spectra of the reaction mixture. The

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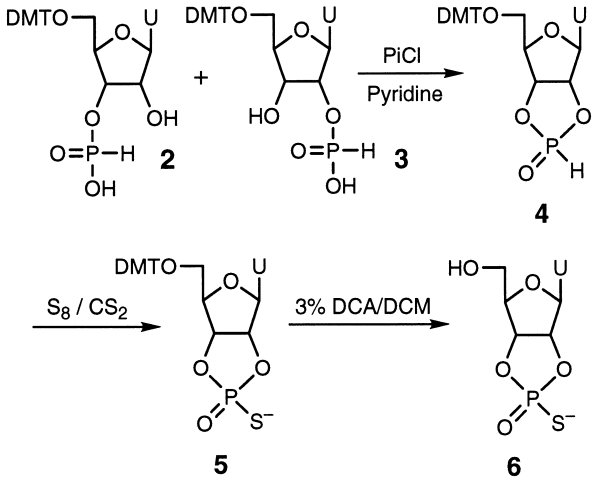
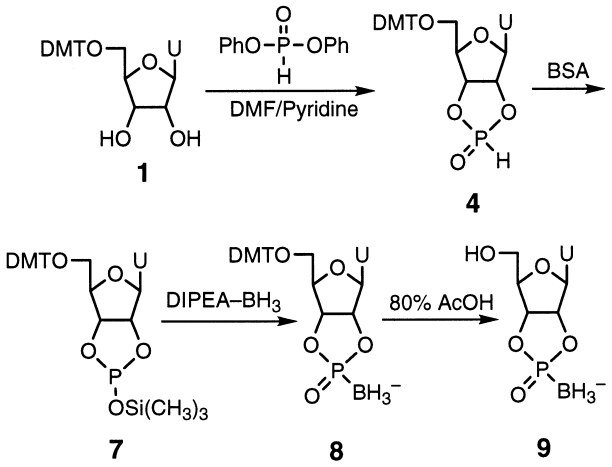


Scheme 1.



Scheme 2.

final deprotection of the 5′-*O*-DMT group with 3% dichloroacetic acid in dichloromethane gave the desired 2′,3′-cyclic-UMPS **6** (40% overall, **2**/**3**→**6**).[14] The Sp (*exo*)- and Rp (*endo*)-diastereomers of 2′,3′-cyclic-UMPS **6** were separated by HPLC (Fig. 1A).[15]

Synthesis of the uridine 2′,3′-cyclic boranophosphate analogue **9** (Scheme 2) was not as straightforward as that of the phosphorothioate analogue 2′,3′-cyclic-UMPS **6**. The boronation requires the intermediate formation of a cyclic phosphite triester **7** by silylation of *H*-phosphonate **4** using *N,O*-bis(trimethylsilyl)acetamide (BSA).[16–18] However, the silylation of intermediate **4** (prepared by cyclization of compound **2** and **3**, Scheme 1) did not give the phosphite triester intermediate **7**, presumably because intermediate **7** could react with the excess pivaloyl chloride and water formed in the condensation step (Scheme 1, **2**/**3**→**4**). Attempts to purify intermediate **4** using an extraction work up (ethyl acetate or dichloromethane with water) failed because of its instability and reactivity. Therefore, we developed an alternative way to prepare intermediate **4** as shown in Scheme 2.

We found that the reaction of diphenyl *H*-phosphonate with 5′-*O*-DMT-uridine **1** for 20 min gave the desired intermediate **4** (^{31}P NMR ($CDCl_3$) δ 27.8 and 23.9). Without purification, silylation of intermediate **4** with BSA (5 min) yielded the phosphite triester **7**, as indicated by two signals at δ 140.1 and 136.6 in ^{31}P NMR ($CDCl_3$) spectra of the reaction mixture. Subsequent boronation of the phosphite triester **7** and simultaneous removal of the trimethylsilyl group were achieved by addition of borane-*N,N*-diisopropylethyl-amine complex.[16–18] After 4 h, two broad peaks centered at δ 127.6 and 123.9 appeared in ^{31}P NMR ($CDCl_3$) spectra of the reaction mixture, confirming the formation of 5′-*O*-DMT-uridine 2′,3′-cyclic boranophosphate **8**. After

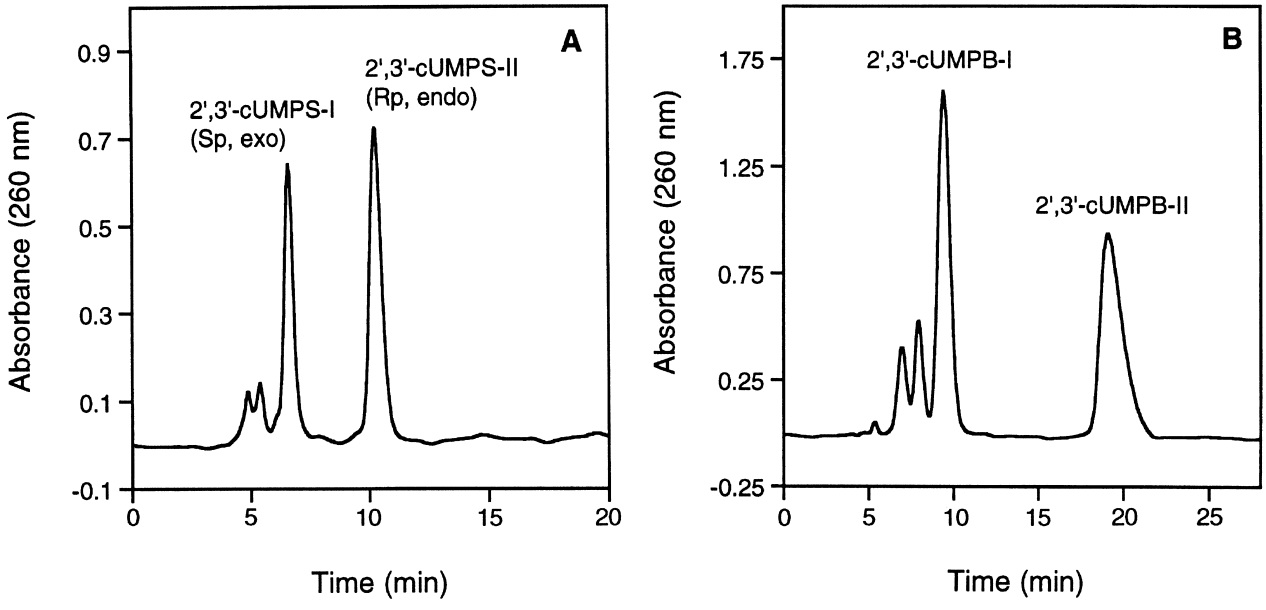


Figure 1. Isocratic separation of the two diastereomers of (A) 2′,3′-cyclic-UMPS **6** and (B) 2′,3′-cyclic-UMPB **9** by HPLC. Elution was carried out on a Waters Delta Pak C18 column (15 μ, 300 Å, 7.8×300 mm) with 6% (for **6**) or 5% (for **9**) methanol in 100 mM triethylammonium acetate (TEAA, pH 6.8) at a flow rate of 3.0 mL/min.

deprotection of the 5′-*O*-DMT group by acid treatment and ion-exchange chromatography purification, the desired 2′,3′-cyclic UMPB **9** was obtained in good yield (70% overall, **1**→**9**).[19] The two diastereomers of 2′,3′-cyclic UMPB **9**, arbitrarily named as isomer I and II, were separated by HPLC (Fig. 1B).[20]

During the preparation of this manuscript, a new efficient method utilizing the same intermediate 2′,3′-cyclic *H*-phosphonate **4** to synthesize 2′,3′-cyclic-NMPS **6** has been reported.[21] The reaction of 5′-*O*-DMT protected nucleosides with diphenyl *H*-phosphonate in pyridine led to the formation of intermediate **4**, which upon sulfurization and the subsequent removal of 5′-DMT, gave 2′,3′-cyclic-NMPS in 78–93% yields.[21]

In summary, the first 2′,3′-cyclic boranophosphate analogue, 2′,3′-cyclic-UMPB, has been synthesized by an *H*-phosphonate approach, which involves the silylation of a 2′,3′-cyclic *H*-phosphonate intermediate followed by boronation.[16–18] The availability of the two diastereomers of 2′,3′-cyclic-UMPB should be useful for determining the absolute configurations of other boranophosphate analogues.[22–28] The potential of cyclic boranophosphates as substrates or inhibitors for ribonucleases should also provide valuable information about the mechanisms of such ribonuclease-catalyzed reactions.

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13. 5′-*O*-DMT-uridine 3′-**2** and 2′-*H*-phosphonates **3** (72%, ^{31}P NMR ($CDCl_3$) δ 8.5 (s), 7.2 (s); MS (FAB$^-$) 610.08 (calcd 610.56 for $C_{30}H_{31}N_2O_{10}P$)) were synthesized by phosphorylation of 5′-*O*-DMT-uridine **1** by 4-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one and subsequent treatment with triethylammonium bicarbonate (TEAB) (Marugg, J. E.; Tromp, M.; Kuyl-Yeheskiely, E.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1986**, *27*, 2661).
14. 2′,3′-Cyclic-UMPS **6** (40%): ^{31}P NMR (D_2O) δ 77.6 (s), 76.3 (s); ^{1}H NMR (D_2O) δ 7.56, 7.55 (2d, J = 8.0 Hz, 1H, H-6), 5.83, 5.75 (2d, J = 3.2 Hz, 1H, H-1′), 5.70, 5.69 (2d, J = 8.0 Hz, 1H, H-5), 5.08 (m, 1H, H-2′), 4.88–4.78 (m, 1H, H-3′), 4.26, 4.17 (2m, 1H, H-4′), 3.76–3.63 (m, 2H, H-5′); MS (FAB$^-$) 320.98 (calcd 321.20 for $C_9H_{10}N_2O_7PS$).
15. 2′,3′-Cyclic-UMPS **6**, isomer I (Sp, *exo*): rt = 6.58 min (33%); ^{31}P NMR (D_2O) δ 77.6 (s); ^{1}H NMR (D_2O) δ 7.54 (d, J = 8.4 Hz, 1H, H-6), 5.74 (m, 1H, H-1′), 5.67 (d, J = 7.6 Hz, 1H, H-5), 5.05 (m, 1H, H-2′), 4.80 (m, 1H, H-3′), 4.15 (m, 1H, H-4′), 3.73–3.60 (m, 2H, H-5′); MS (FAB$^-$) 321.01 [M]$^-$ (calcd 321.20 for $C_9H_{10}N_2O_7PS$). 2′,3′-Cyclic-UMPS **6**, isomer II (Rp, *endo*): rt = 10.24 min (47%); ^{31}P NMR (D_2O) δ 76.1 (s); ^{1}H NMR (D_2O) δ 7.52 (d, J = 8.0 Hz, 1H, H-6), 5.79 (m, 1H, H-1′), 5.64 (d, J = 8.0 Hz, 1H, H-5), 5.01 (m, 1H, H-2′), 4.78 (m, 1H, H-3′), 4.20 (m, 1H, H-4′), 3.70–3.58 (m, 2H, H-5′); MS (FAB$^-$) 321.00 [M]$^-$ (calcd 321.20 for $C_9H_{10}N_2O_7PS$).
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19. 2′,3′-Cyclic-UMPB **9** (70%): ^{31}P NMR (D_2O) δ 120.7 (q, J = 125.85 Hz), 116.10 (q, J = 124.88 Hz); ^{1}H NMR (D_2O) δ 7.48 (d, J = 8.0 Hz, 1H, H-6), 5.74 (2d, J = 3.2 Hz, 1H, H-1′), 5.64 (m, 1H, H-5), 4.98–4.92 (m, 1H, H-2′), 4.79 (q, J = 5.6 Hz, 1H, H-3′), 4.17, 4.01 (2q, J = 4.0 Hz, 1H, H-4′), 3.68–3.56 (m, 2H, H-5′); MS (FAB$^-$) 303.07 (calcd 303.00 for $C_9H_{13}BN_2O_7P$).
20. 2′,3′-Cyclic-UMPB **9**, isomer I: rt = 9.40 min (39%); ^{31}P NMR (D_2O) δ 120.8 (q, J = 145.71 Hz); ^{1}H NMR (D_2O) δ 7.38 (d, J = 7.6 Hz, 1H, H-6), 5.75 (m, 1H, H-1′), 5.60 (d, J = 7.2 Hz, 1H, H-5), 5.03 (m, 1H, H-2′), 4.79 (m, 1H, H-3′), 4.19 (q, J = 4.0 Hz, 1H, H-4′), 3.74–3.62 (m, 2H, H-5′); MS (FAB$^-$) 303.0 [M]$^-$ (calcd 303.00 for $C_9H_{13}BN_2O_7P$). 2′,3′-Cyclic-UMPB **9**, isomer II: rt = 19.11 min (43%); ^{31}P NMR (D_2O) δ 116.1 (q, J = 130.17 Hz); ^{1}H NMR (D_2O) δ 7.41 (d, J = 7.6 Hz, 1H, H-6), 5.68 (d, J = 2.8 Hz, 1H, H-1′), 5.62 (d, J = 7.6 Hz, 1H, H-5), 5.05 (m, J = 3.2 Hz, 1H, H-2′), 4.87 (q, J = 6.0 Hz, 1H, H-3′), 4.02 (q, J = 3.2 Hz, 1H, H-4′), 3.75–3.63 (m, 2H, H-5′); MS (FAB$^-$) 303.0 [M]$^-$ (calcd 303.00 for $C_9H_{13}BN_2O_7P$).
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