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Highly Efficient Synthesis Of 2'-*O*-Amino Nucleosides And Their Incorporation In Hammerhead Ribozymes

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Abstract: 2'-*O*-Amino nucleosides were prepared from arabino nucleosides by triflate displacement with N-hydroxy phthalimide. The corresponding phosphoramidites suitable for automated oligonucleotide synthesis, were also prepared. Incorporation of 2'-*O*-amino uridine into position U7 or 2'-*O*-amino adenosine into position A9 of a hammerhead ribozyme resulted in a slight improvement of catalytic rates. © 1998 Elsevier Science Ltd. All rights reserved.

Structural modifications of oligonucleotides are becoming increasingly important as their possible clinical applications emerge.[1-5] As a continuation of our studies towards structure-activity relationships in hammerhead ribozymes,[6-9] we are interested in the introduction of the hydroxylamino functionality into ribozyme sequences. One could expect certain effects on ribozyme cleavage activity if a hydroxylamino group is placed in close proximity to the cleavage site due to the nucleophillic nature of the hydroxylamine residue. Alternatively, an *O*-amino group at the 2'-position of a nucleoside monomeric unit could provide enhanced overall ribozyme stability towards cellular nucleases. Furthermore, the presence of an *O*-amino group in an oligonucleotide provides a unique opportunity for introduction of post-synthetic modifications via oxime formation.

This communication describes the first, to the best of our knowledge, syntheses of 2'-*O*-amino uridine and adenosine and their corresponding phosphoramidites.

Two-step conversion of an alcohol to the aminooxy alcohol through the corresponding *O*-phthalimido-derivative using N-hydroxyphthalimide as a nucleophile was first reported by Mitsunobu.[10-12] This procedure was successfully applied to sugars,[13-15] modified disaccharides and deoxynucleosides to modify the 5'- or 3'-OH group.[16-20] However, we were unable to produce 2'-*O*-phthalimido nucleosides in yields of greater than 10%, using various conditions for Mitsunobu inversion of the corresponding 3',5'-*O*-protected arabino-precursors. As a possible alternative to the Mitsunobu reaction we decided to attempt the displacement of suitably protected 2'-arabino-triflates with N-hydroxy phthalimide.

Arabinonucleosides **1**(B= Ade or Ura) were protected with the Markiewicz group and then treated with triflurormethanesulfonic anhydride (ara-U) or trifluoromethanesulfonic chloride (ara-A) to give arabino derivatives **3** or **5.** Inversion of configuration by substitution with N-hydroxy phthalimide in the presence of DBU provided ribonucleosides **6** or **7** in nearly quantitative yield. One-pot two-step deprotection (Et_3N•HF, 3 h, rt., followed by addition of 40% aq solution of methylamine, 1.5 h, rt.) afforded 2'-*O*-amino nucleosides **9**[21] in 80% yield, which proves the compatibility of phthaloyl protecting group with oligonucleotide synthesis and deprotection. The exocyclic amino group of adenosine derivative **7** was selectively *t*-butylbenzoylated to provide fully protected compound **8**. Careful Markiewicz group deprotection of derivatives **6** or **8** with Et_3N•HF afforded hydroximides **10** (B_1= Ura or N_6-*t*-BuBz-Ade). We have found that Markiewicz group deprotection in compounds **6** and **7** with Et_3N•HF/dichloromethane (225 min.[22], rt.) is accompanied by partial opening of a phthalimido cycle. This side reaction brings the yield of desired 2'-*O*-*N*-phthaloylnucleosides down to 50-55%. We also showed that the resulting 2'-*O*-*N*-nucleoside phthalamides can be quantitatively converted to

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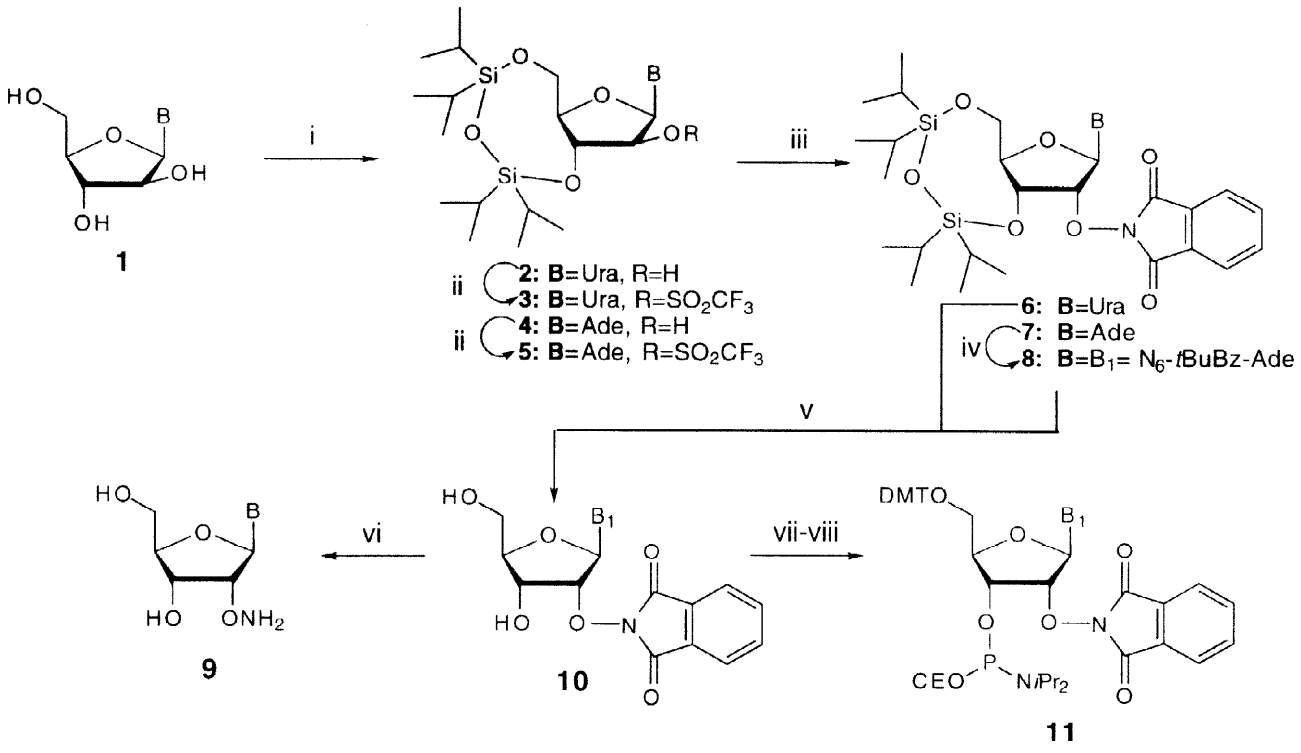


FIGURE 1.Synthesis Of 2'-*O*-Amino Nucleosides And Their Phosphoramidites. Reagents and conditions: i) TIPS-Cl/Pyr; ii) CF_3SO_2Cl, DMAP/CH_2Cl_2, 0°C, 2h; iii) PhthNOH, DBU/MeCN, 30 min., rt.; iv) *t*-BuBzCl/Pyr; v) Et_3N•HF; vi) $MeNH_2/H_2O$; vii) DMT-Cl/Pyr; viii) 2-cyanoethyl N,N-diisopropyl chlorophosphoramidite, DIPEA/CH_2Cl_2.

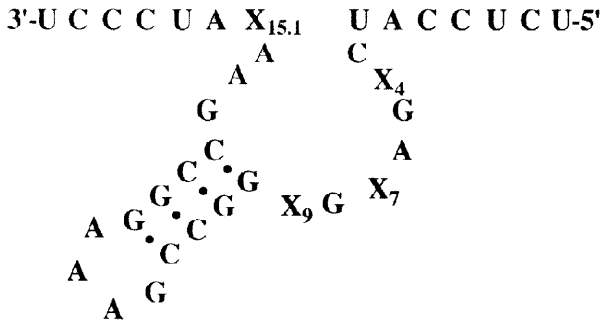
2'-*O*-amino nucleosides by treatment with 40% aq methylamine (1h, rt.). However, we didn't detect the formation of this side-product during deprotection of the N_6-blocked adenosine derivative **8**. The inversion of configuration at C-2 from arabino in nucleosides **1** to ribo in nucleosides **9** is evident from increase of the value of $J_{1',2'}$ from 4.8 Hz for **1** to 6.4-6.8 Hz in ribo derivatives **9**[21,23]. Application of the standard procedures of dimethoxytritylation and phosphitylation to these compounds resulted in formation of the corresponding phosphoramidites **11** (B_1= Ura or N_6-*t*-BuBz-Ade)[24].

A typical experimental procedure is as follows:

To an ice-cooled solution of 3',5'-*O*-(tetraisopropyldisiloxane-1,3-di-yl)-1-β-D-arabinofuranosyl-uracil (4 g, 8.2 mmol) in dichloromethane was added trifluoromethane sulfonic anhydride (1.66 mL, 9.86 mmol) and the reaction mixture was stirred at -5 °C for 30 min. The solution was then diluted with dichloromethane and washed with cold 1% aq acetic acid, then with saturated aq sodium bicarbonate and brine. The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The resulting derivative **3** (Fig.1) was dissolved in anhydrous acetonitrile (70 mL) and N-hydroxyphthalimide (1.74 g, 10.66 mmol) was added. A solution of DBU (1.6 mL, 10.66 mmol) in acetonitrile (5 mL) was added dropwise to the reaction mixture under vigorous stirring. After 30 min., the dark orange reaction mixture was diluted with dichloromethane (250 mL) and extracted with saturated aq sodium bicarbonate solution (3x250 mL). The resulting colorless organic layer was washed with brine and evaporated to give 3.6 g (70%) of compound **6** (Fig. 1) which was used without further purification.

Fully protected monomer blocks were incorporated into various positions of ribozyme model sequences using standard protocols for solid-phase RNA synthesis[25]. The presence of 2'-*O*-amino nucleosides in the ribozymes was proven by base-compositional analysis[7].

Ribozyme sequence and sites of 2'-*O*-amino nucleoside incorporation are shown in Fig 2. The ribozyme cleavage reaction of a 17-mer RNA substrate containing the recognition sequence 5'- AGG GAU UAA UGG



Rz 1: X_4=2'-ONH$_2$-U, X_7=U, X_9=$X_{15.1}$=A

Rz 2: X_4=X_7=2'-ONH$_2$-U, X_9=$X_{15.1}$=A

Rz 3: X_4=X_7=U, X_9=A, $X_{15.1}$=2'-ONH$_2$-A

Rz 4: X_4= U, X_7=2'-ONH$_2$-U, X_9=$X_{15.1}$=A

Rz 5: X_4=X_7=U, X_9=2'-ONH$_2$-A, $X_{15.1}$=A

FIGURE 2 Hammerhead Ribozymes Sites Modified With 2'-*O*-Amino Nucleosides

AGA -3' was studied under single-turnover conditions (10 mM $MgCl_2$, pH 6.5, 37 °C)[8] and the results are represented in Fig.3. Modifications of U4 (Rz 1), U4 and U7 (Rz 2) or A15.1 (Rz 3) resulted in a dramatic loss of activity (100 fold). However, ribozymes with a single replacement of U7 with 2'-*O*-NH_2-U (Rz 4) or A9 with 2'-*O*-NH_2-A (Rz 5) demonstrated slightly enhanced activity (Fig.3).

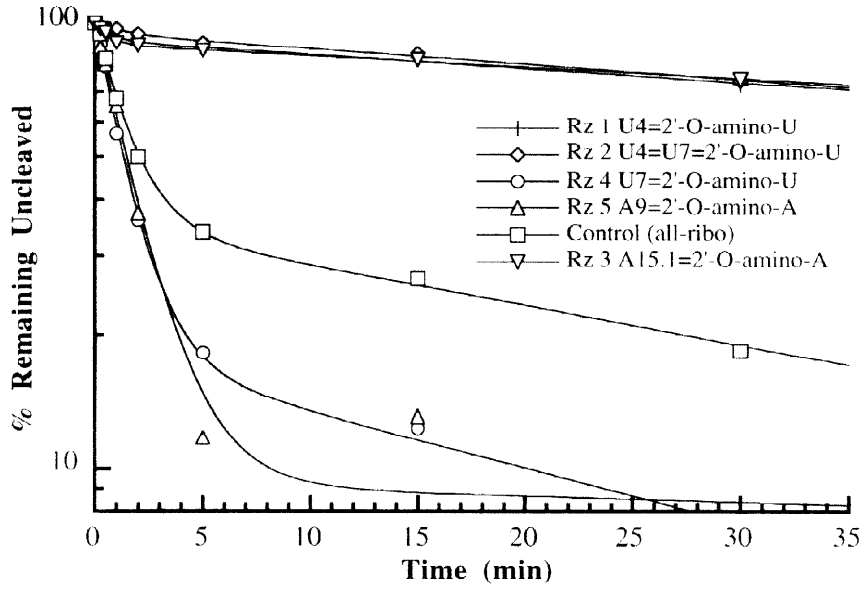


FIGURE 3 Cleavage Rates Of The Ribozymes, Containing 2'-*O*-Amino Nucleosides

In conclusion, we have identified a straightforward route to 2'-*O*-amino-nucleosides and their phosphoramidites suitable for automated solid-phase synthesis. Incorporation of 2'-*O*-amino nucleosides into a hammerhead ribozyme had significant effects on activity. The investigation of post-synthetic modifications of these ribozymes via oxime formation is in progress and will be reported in due course.

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21. 400 Mhz ^{1}H-NMR (DMSO-d_6), δ p.p.m., **9** (B=Ura) : 11.15 (bs, 1H, N3-H); 7.93 (d, 1H, 6-H, $J_{6,5}$ 8.0); 6, 25 (s, 2H, 2'-ONH_2); 5.88 (d, 1H, 1'-H, $J_{1',2'}$ 6.4); 5.65 (d, 1H, 5-H, $J_{5,6}$ 8.0); 5.13 (m, 2H, 5'-OH, 3'-OH); 4.13 (m, 1H, 3'-H); 4.08 (m, 1H, 2'-H); 3.84 (dd, 1H, 4'-H $J_{4',5'}$3.0, $J_{3',4'}$2.8); 3.54 (m, 2H, 5'-H_2). FAB-MS: 260.1 (M+H) Calculated for $C_9H_{13}N_3O_6$: 259.22
9 (B=Ade): 8.34 (s, 1H, 2-H); 8.12 (s, 1H, 8-H); 7.32 (s, 2H, 6-NH_2), 6.27 (s, 2H, 2'-ONH_2); 5.99 (d, 1H, 1'-H, $J_{1',2'}$ 6.8); 5,42 (dd 1H, 5'-OH); 5.15 (d, 1H, 3'-OH, $J_{3',OH}$4.4); 4.64 (dd, 1H, 2'-OH, $J_{2',3'}$4.8); 4.34 (m, 1H, 3'-H, $J_{3',4'}$4.4); 3.96 (dd, 1H, 4'-H, $J_{4',5'}$3.6); 3.65 (m, 1H, 5'-H_a, $J_{5'a,OH}$4.8, $J_{5'a,5'b}$12.0); 3.53 (m, 1H, 5'-H_b, $J_{5'b,OH}$7.6). FAB-MS: 283.12 (M+H) Calculated for $C_{10}H_{14}N_6O_4$ 282.26.
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