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## A PRACTICAL SYNTHESIS OF GLYCINAMIDE RIBONUCLEOTIDE<sup>1</sup>

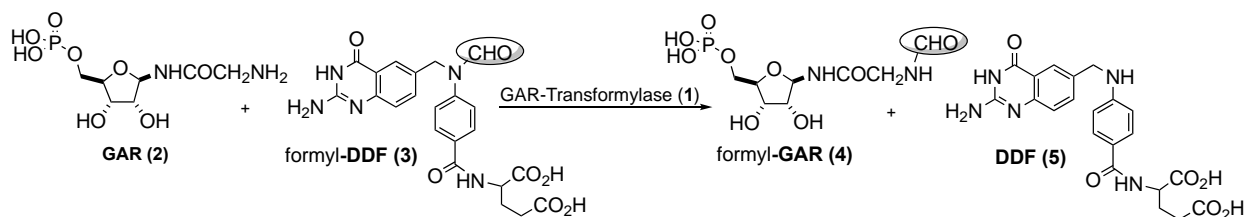
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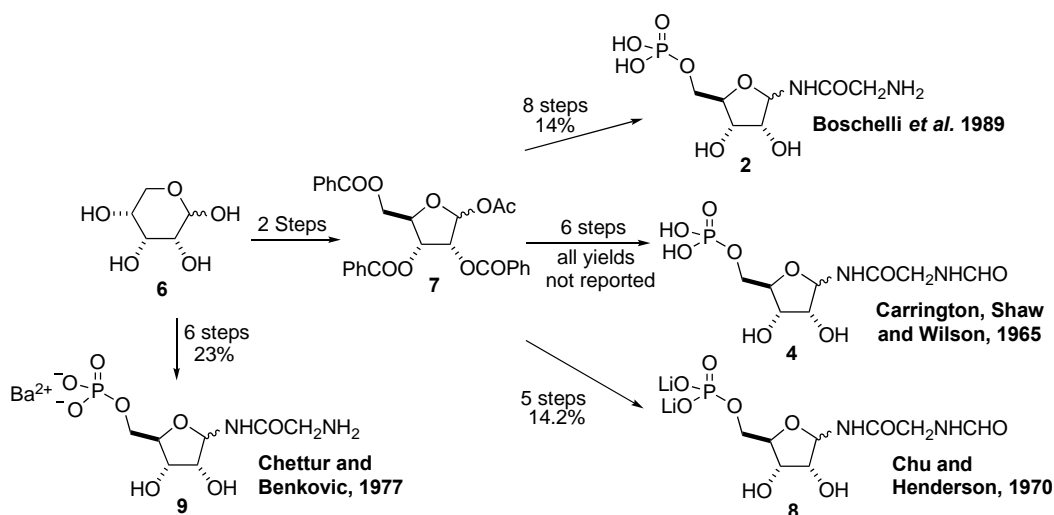
**Abstract** – A practical nine-step synthesis of an alpha-/beta-anomeric mixture of glycinamide ribonucleotide (GAR) has been developed. The synthesis was accomplished in nine steps from D-ribose and occurred in 9.5% overall yield. The route as devised provided material on the multi-milligram scale.

### INTRODUCTION

Glycinamide ribonucleotide transformylase (GART) is an enzyme that catalyzes the formation of *N*-formylglycinamide ribonucleotide (fGAR) from glycinamide ribonucleotide (GAR) in the third step of the *de novo* purine biosynthesis pathway.<sup>2-4</sup> As the *N*-formylation of GAR is crucial to the formation of the nucleic acid building blocks of DNA, the inhibition of GART in humans is a potential target for the development of anti-cancer agents,<sup>5,6</sup> whereas its inhibition in bacteria is a potential route for antibiotics design. In humans GART is part of a multi-enzyme complex, whereas in *E. coli*, it exists as an isolated protein.<sup>7</sup> Thus, it is easier to develop an assay to monitor the function of *E. coli* GART, as it catalyzes the transfer of a formyl group from formyldeazafolic acid (fDDF) to GAR (Scheme 1).<sup>8-10</sup> As part of an effort to better understand the biochemical function of GART, we desired synthetic access to GAR and fDDF, as neither are commercially available. While access to viable quantities to fDDF was achieved following the route published by Davoll and Johnson,<sup>11,12</sup> we ran into difficulties getting adequate quantities of GAR following published routes.<sup>13-16</sup> Benkovic has published a short 6-step synthesis of GAR from the *D-ribo*-pyranosylamine, however, we found the preparation/purification of this starting material to be difficult.<sup>17</sup> Part of these difficulties were a result of the lack of full experimental details and characterization data in the published route. Herein we disclose a novel composite route a 1:1 mixture of  $\alpha$ -/ $\beta$ -anomers of GAR. In addition, we disclose the full experimental details for GAR and the intermediates that we isolated in route. While only the  $\beta$ -isomer of GAR is a substrate for GART, Caperrelli *et al.* and others have shown that the presence of the  $\alpha$ -isomer in the 1:1 mixture of anomers has no deleterious effects on the enzyme activity.<sup>4,8,18</sup>

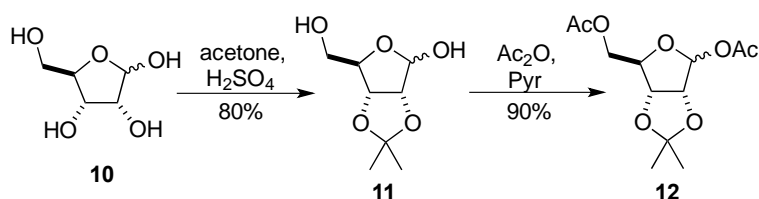


Our new route to GAR is a hybrid of the previous routes and builds mostly on the routes reported by Boschelli *et al.*<sup>15</sup> and Chu and Henderson<sup>16</sup> (Scheme 2). This revised route simplified the protecting group strategy and allowed for the easy purification of the final product, as well as the intermediates formed in route.

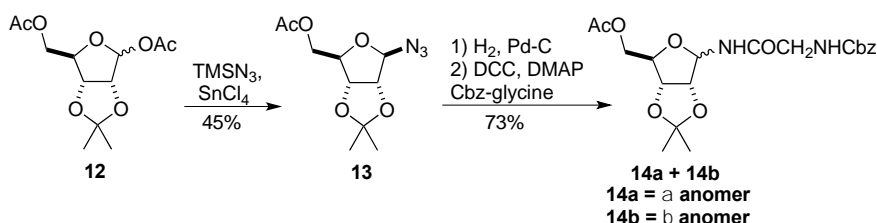


## RESULTS AND DISCUSSION

Our route to GAR started with D-ribose **10**, which was suitably protected as the C-2/3-acetonide **11** (Scheme 3) using acetone in presence of catalytic H<sub>2</sub>SO<sub>4</sub>. Both the anomeric and C-5 hydroxyl groups then protected as acetates using acetic anhydride in the presence of pyridine to yield bis-acetate **12**.<sup>19</sup>

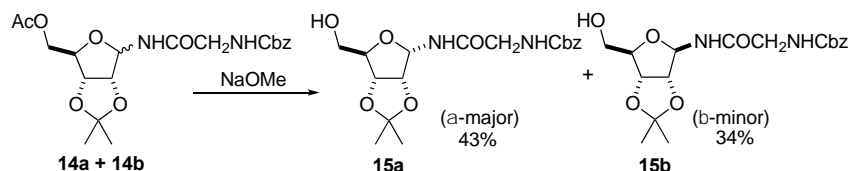


The anomeric acetate in **12** was converted to azide using  $\text{TMSN}_3$  and  $\text{SnCl}_4$  resulting in  $\beta$ -azide **13** (4).<sup>19</sup> The azide **13** was reduced with hydrogen over Pd-C to form an equilibrating mixture of anomeric amines, which was used without further purification. The crude mixture of amines was coupled with Cbz-glycine by DCC/DMAP-mediated amide coupling to give a 1:1 mixture of glycosidic amides **14**.<sup>15</sup> this stage, the mixture of anomers could not be readily separated.



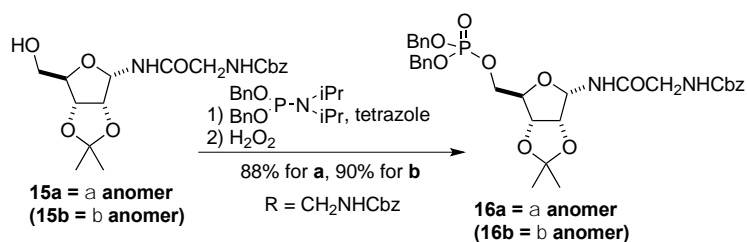
Scheme 4. Synthesis of glycinamide derivative of D-ribose

The C-5 acetate in **14** was removed with NaOMe to give the ribose derivative with a free hydroxyl group at the C-5 position (Scheme 5). Fortuitously, using column chromatography the mixture of anomers **15a** and **15b** could be separated. The  $\alpha$ -anomer **15a** being the major product was isolated in a 43% yield and  $\beta$ -anomer **15b** in a 34% yield. It is important to note that both anomers, as well as the mixture, can be taken forward to satisfactory GAR for the enzymatic assay. However, the separation of the two anomers led to easier analysis of spectral data.



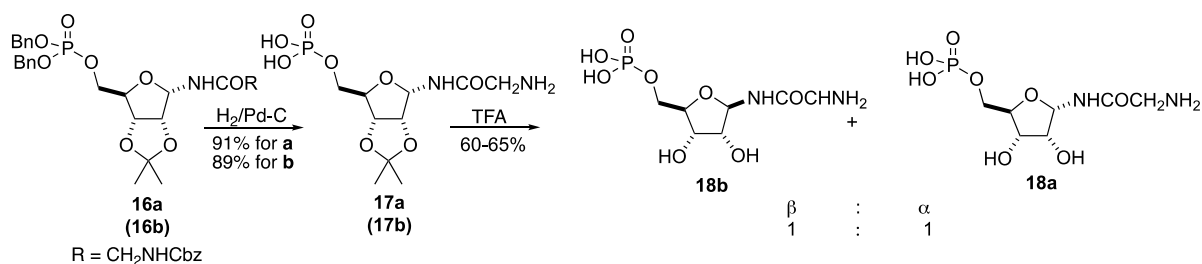
Scheme 5. Acetate deprotection and separation of anomers

Separately, the two anomers **15a** and **15b** were converted into the 5-dibenzoyloxyphosphates **16a** and **16b** (88 and 90% yield, respectively) by a two-step phosphorylation sequence (Scheme 6). Specifically, the primary alcohols were treated with dibenzyl *N,N*-diisopropylphosphoramidite and tetrazole. Once TLC analysis indicated consumption of starting material, excess  $\text{H}_2\text{O}_2$  was added to oxidize the *in situ* formed phosphite.<sup>20,21</sup>



Scheme 6. Installation of phosphate

Reductive debenzoylation of phosphate **16a** with 1 atm. of hydrogen gas over Pd-C produced the acetonide protected  $\alpha$ -GAR **17a**, as a single anomer (Scheme 7). Removal of the acetonide on **17** with aqueous TFA gave the desired product, GAR as a 1:1 mixture of anomers, **18a** and **18b**.<sup>20</sup> Despite careful monitoring of the reaction time and pH, we were not able to find conditions that removed the acetonide without anomerization. In fact, when the  $\beta$ -anomer **16b** was subjected to the same 2-step reaction sequence, an identical mixture of anomers of GAR (**18a/b**) was isolated in similar yields. The 5-hydroxy-ribose **15b** when subjected to the same reaction steps gave similar yields and also resulted in a 1:1  $\alpha/\beta$ -mixture of anomeric GAR.

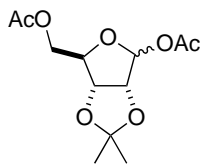


Scheme 7. Deprotection steps to anomeric mixture of GAR

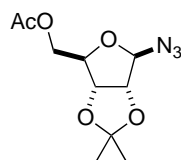
In conclusion, a practical route is described to a 1:1  $\alpha/\beta$ -mixture of GAR that routinely provided material in multi-milligram quantities. The synthetic material produced from the outlined route served as expected as the formyl-group accepting component in the formylation reaction with fDDF that is catalyzed by GART.

## EXPERIMENTAL

**General Methods and Materials:** Commercial reagents were used without further purification. Dry methylene dichloride (DCM) was obtained from an in-house dry solvent system which employs nitrogen gas pressure to pass solvent through activated alumina columns. Air and moisture sensitive reactions were carried out under nitrogen atmosphere with help of septa and syringes. Silica coated glass backed thin layer chromatography plates were developed in solvents (% by volume) and stained with *p*-anisaldehyde or potassium permanganate. For compound purification flash column chromatography was performed using 60-200 mesh silica gel. GAR (**18**) and acetonide of GAR (**17**) can be purified if needed by eluting through C-18 silica gel with water. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on 400 MHz and 500 MHz spectrometers. Chemical shift for internal standard CDCl<sub>3</sub> was set to  $\delta$  7.26 for <sup>1</sup>H NMR and  $\delta$  77.36 for <sup>13</sup>C NMR. For IR, samples were analyzed neat on a Bruker Alpha-P FT-IR spectrometer. High-resolution mass spectrometry data were obtained from the mass spectrometry center at Barnett Institute at Northeastern. Optical rotations were measured on a Jasco P-2000 digital polarimeter, concentration and solvent of choice are mentioned in parentheses.

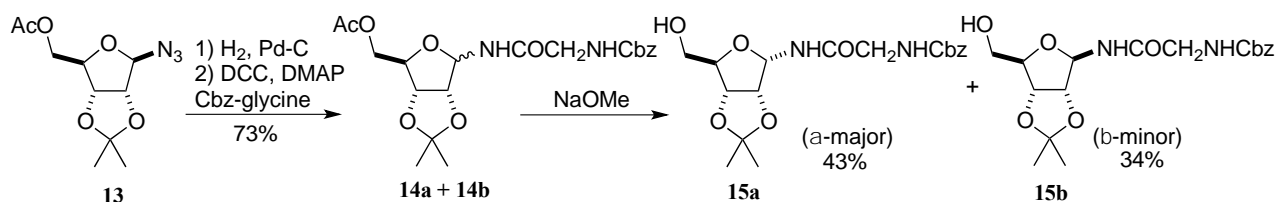


**2,3-*O*-Isopropylidene-D-ribofuranose 1,5-diacetate (12).** To a solution of D-ribose (5 g, 33.3 mmol) (**10**) in acetone (50 mL) was slowly added conc. H<sub>2</sub>SO<sub>4</sub> (0.25 mL) at room temperature and the reaction mixture was stirred for 2.5 h. To the reaction mixture was added solid NaHCO<sub>3</sub> (10 g) and the solution was filtered. The residue was washed with acetone (2 x 10 mL). The filtrate and washings were combined and concentrated under reduced pressure. The product was purified by silica gel chromatography eluting with 35% EtOAc/Hexanes to obtain 2,3-*O*-isopropylidene-ribose as clear colorless viscous liquid (5.56 g, 29.3 mmol, 88%). *R<sub>f</sub>* (40% EtOAc/Hexanes) = 0.25. This compound was dissolved in dry DCM (72 mL) and the solution was cooled to 0 °C and pyridine (10.4 mL, 10.21 g, 129 mmol) was added dropwise with stirring. After 10 min, anhydrous acetic anhydride (11 mL, 11.95 g, 117 mmol) was added dropwise followed by DMAP (355 mg, 2.9 mmol) at 0 °C. The resulting mixture was stirred for 1 h while allowing to gradually warm to room temperature. The reaction mixture was diluted with DCM (120 mL) and poured over ice cold water (50 mL) in a separatory funnel and washed with ice cold dilute HCl (1 N, 50 mL, 2-3 times, until all pyridine is removed). The organic layer was further washed with sat. aq. NaHCO<sub>3</sub> followed by brine and dried over Na<sub>2</sub>SO<sub>4</sub>. The organic layer was filtered off. The filtrate was concentrated under reduced pressure. The product was purified by silica gel chromatography eluting with 12% EtOAc/Hexanes to yield clear colorless viscous liquid (6.72 g, 24.3 mmol, 83%). *R<sub>f</sub>* (20% EtOAc/Hexanes) = 0.45; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.21(s, 1H), 4.71 (s, 2H), 4.46 (m, 1H), 4.12 (m, 2H), 2.09 (s, 3H), 2.06 (s, 3H), 1.49 (s, 3H), 1.33 (s, 1H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 170.8, 169.6, 113.6, 102.5, 85.7, 85.4, 81.9, 64.4, 26.8, 25.4, 21.5, 21.1. IR (neat): ν 2988.7, 2941.7, 1738.8, 1435.7, 1371.9, 1209.0 cm<sup>-1</sup>. HRMS calculated for [C<sub>12</sub>H<sub>18</sub>O<sub>7</sub>Na<sup>+</sup>] 297.0945, found 297.0945. [α]<sub>D</sub><sup>22</sup> -60 (*c* 1.98, CH<sub>2</sub>Cl<sub>2</sub>).



**2,3-*O*-Isopropylidene-5-*O*-acetyl-β-D-ribofuranosyl azide (β) (13).** The diacetate (**12**) (5 g, 18.1 mmol) was dissolved in dry DCM (50 mL), TMSN<sub>3</sub> (2.62 mL, 2.29 g, 19.9 mmol) was added to it at room temperature with stirring. Neat SnCl<sub>4</sub> (18 mL, 1 M in DCM) was diluted with dry DCM (20 mL) and added to the reaction mixture at room temperature and stirred for 5 h. The reaction mixture was diluted with Et<sub>2</sub>O and washed with water, sat. aq. NaHCO<sub>3</sub> and brine. The Et<sub>2</sub>O extract was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The product was purified by silica gel chromatography eluting with 9% EtOAc/Hexanes to give a clear colorless liquid (2.25 g, 8.8 mmol, 48.2%). *R<sub>f</sub>* (30%

EtOAc/Hexanes) = 0.65;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.49 (s, 1H), 4.63 (d,  $J = 6$  Hz, 1H), 4.40 - 4.45 (m, 2H), 4.10 - 4.19 (m, 2H), 2.05 (s, 3H), 1.44 (s, 3H), 1.27 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  170.9, 113.7, 97.2, 85.8, 85.4, 82.3, 64.1, 26.8, 25.2, 21.1. IR (neat):  $\nu$  2988.6, 2943.8, 2109.8, 1742.6, 1457.2, 1373.6, 1210.1  $\text{cm}^{-1}$ . HRMS Calculated for  $[\text{C}_{10}\text{H}_{15}\text{N}_3\text{O}_5\text{Na}^+]$  280.0904, found 280.0885.  $[\alpha]_{\text{D}}^{24} -286$  ( $c$  0.43,  $\text{CH}_2\text{Cl}_2$ )



**2,3-*O*-Isopropylidene-5-hydroxy-1-*N*-(benzyloxycarbonyl)glycyl)-*D*-ribofuranosylamine (15a and 15b).** The 2,3-*O*-isopropylidene-5-*O*-acetyl- $\beta$ -*D*-ribofuranosyl azide (**13**) (1 g, 3.9 mmol) was dissolved in MeOH (16 mL). Catalytic Pd-C was added and stirred under  $\text{H}_2$  for 6 - 8 h at atmospheric pressure. Upon completion the reaction mixture was filtered through celite, the filtrate was concentrated under reduced pressure, dried at pump and subjected to the following step without further purification. The product was a thick colorless liquid. In a reaction flask Cbz-glycine (1.22 g, 5.8 mmol) was dissolved in dry DCM (20 mL). The solution was cooled to 0  $^\circ\text{C}$  and DCC (1.2 g, 5.8 mmol) was added followed by DMAP (71.2 mg, 0.6 mmol) with stirring. The reaction was stirred at 0  $^\circ\text{C}$  for 30 min. The amine from the previous step was dissolved in dry DCM (18 mL) and added to the reaction mixture and stirred overnight. The reaction mixture was cooled to 0  $^\circ\text{C}$  and filtered through celite and washed with cold DCM. The filtrate and washings were collected and concentrated under reduced pressure. The 5-*O*-acetyl derivative (**14**) was purified by silica gel chromatography eluting with 60% EtOAc/Hexanes to obtain the coupling product as a mixture of  $\alpha$  and  $\beta$  anomers as a sticky, syrupy, clear liquid (1.2 g, 2.8 mmol, 73.0%).  $R_f$  (70% EtOAc/Hexanes) = 0.4. HRMS Calculated for  $[\text{C}_{20}\text{H}_{26}\text{N}_2\text{O}_8\text{Na}^+]$  445.1581, found 445.1556.

#### Deprotection of 5-*O*-acetyl derivative and separation of anomers:

The 5-*O*-acetyl derivative (**14a** and **14b**) (650 mg, 1.5 mmol) was dissolved in MeOH (12 mL) and cooled to 0  $^\circ\text{C}$ . NaOMe (3.4 mL, 1.7 mmol, 0.5 N in MeOH) was then added dropwise with stirring at 0  $^\circ\text{C}$ . The reaction was stirred for 30 min. The reaction was quenched by adding ice-cold 1 N HCl (10 mL) and the mixture was extracted with  $\text{Et}_2\text{O}$  (40 mL, twice). The combined  $\text{Et}_2\text{O}$  layer was washed with sat. aq.  $\text{NaHCO}_3$ , brine and dried over  $\text{Na}_2\text{SO}_4$ . The  $\text{Et}_2\text{O}$  extract was then concentrated under reduced pressure. The product, a mixture of  $\alpha$  and  $\beta$  anomers, was purified by silica gel chromatography eluting with 75% EtOAc/Hexanes to provide the less polar  $\beta$  anomer as a white solid (200 mg, 0.53 mmol, 34.1%). The  $\alpha$  anomer has a smaller  $R_f$  value, elutes at 90% EtOAc/Hexanes and is a low-melting solid

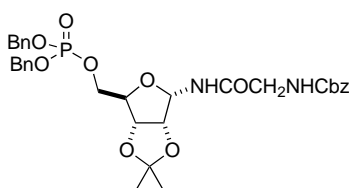
(253 mg, 0.67, 43%).

Data for alpha anomer (**15a**):

$R_f$  (EtOAc) = 0.20,  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.32 (m, 4H), 7.19 (m, 1H), 5.91 (m, 1H), 5.8 (dd,  $J$  = 8.4, 4 Hz, 1H), 5.10 (s, 2H), 4.72 (d,  $J$  = 5.6 Hz, 1H), 4.66 (m, 1H), 4.1 (s, 1H), 3.82 - 4.02 (m, 3H), 3.52 - 3.74 (m, 2H), 2.73 (s, 1H), 1.46 (s, 3H), 1.32 (s, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  170.5, 156.9, 136.4, 128.8, 128.5, 128.4, 113.3, 83.1, 82.1, 80.7, 79.6, 67.4, 63.6, 44.8, 26.3, 24.9. IR (neat):  $\nu$  3323.7, 2931.9, 2359.8, 2339.9, 1681.8, 1505.6, 1455.0, 1415.9, 1381.3, 1270.0, 1208.8  $\text{cm}^{-1}$ . HRMS Calculated for  $[\text{C}_{18}\text{H}_{24}\text{N}_2\text{O}_7\text{Na}^+]$  403.1476, found 403.1475.  $[\alpha]_{\text{D}}^{24}$   $-7.6$  ( $c$  0.55,  $\text{CH}_2\text{Cl}_2$ ).

Data for beta anomer (**15b**):

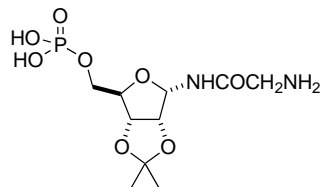
$R_f$  (EtOAc) = 0.22;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.74 (d,  $J$  = 7.6 Hz, 1H), 7.36 (bs, 4H), 5.75 (d,  $J$  = 8.8 Hz, 1H), 5.67 (m, 1H), 5.10 (s, 2H), 4.77 (d,  $J$  = 5.6 Hz, 1H), 4.55 (d,  $J$  = 4.4 Hz, 1H), 4.25 (s, 1H), 3.70 - 3.90 (m, 2H), 3.55 - 3.70 (m, 2H), 2.88 (bs, 1H), 1.51 (s, 3H), 1.31 (s, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  169.3, 157.0, 136.5, 129.0, 128.8, 128.7, 113.2, 87.1, 86.6, 86.4, 82.1, 67.6, 63.8, 45.1, 27.0, 25.4. IR (neat):  $\nu$  3323.4, 2359.4, 2339.9, 2319.6, 1691.0, 1652.0, 1519.9, 1454.9, 1378.2, 1334.6, 1270.1, 1206.8  $\text{cm}^{-1}$ . HRMS Calculated for  $[\text{C}_{18}\text{H}_{24}\text{N}_2\text{O}_7\text{Na}^+]$  403.1476, found 403.1471.  $[\alpha]_{\text{D}}^{24}$   $-37$  ( $c$  0.14,  $\text{CH}_2\text{Cl}_2$ ).



### 2,3-*O*-Isopropylidene-1-*N*-(benzyloxycarbonylglycyl)-*D*-ribofuranosylamine-5-dibenzylphosphate

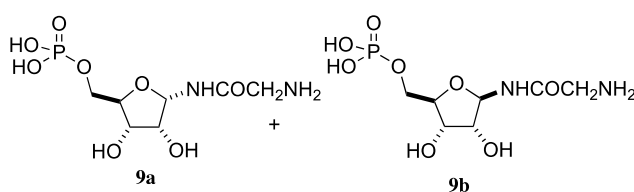
( $\alpha$ ) (**16a**). To a solution of 2,3-*O*-isopropylidene-1-*N*-(benzyloxycarbonylglycyl)-*D*-ribofuranosylamine (**15a**) (430 mg, 1.1 mmol) in dry DCM (5.6 mL) was added a solution of dibenzyl *N,N*-diisopropylphosphoramidite (0.76 mL, 780.9 mg, 2.3 mmol) in dry DCM (3.75 mL) with stirring. Tetrazole (5 mL, 2.3 mmol, 0.45 M in MeCN) was then added and stirred for 1 h. The reaction mixture was cooled to 0 °C,  $\text{H}_2\text{O}_2$  (4 mL, 35% in  $\text{H}_2\text{O}$ ) was added and stirred at 0 °C for 45 min, TLC was checked. Upon complete conversion of the intermediates to product, the reaction was quenched by dropwise addition of sat. aq.  $\text{Na}_2\text{SO}_3$  with stirring for 5-10 min. The reaction mixture was then extracted with EtOAc (10 mL, twice). The EtOAc extract was washed with sat. aq.  $\text{NaHCO}_3$  and brine and dried over  $\text{Na}_2\text{SO}_4$ . The product was purified by silica gel chromatography eluting with 70% EtOAc/Hexanes to obtain the product as a viscous translucent liquid (637 mg, 1 mmol, 88%)  $R_f$  (70% EtOAc/Hexanes) = 0.4;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.25 - 7.37 (m, 14H), 7.06 - 7.12 (m, 1H), 5.98 (m, 1H), 5.73 (dd,  $J$  = 8.8, 4.4 Hz, 1H), 4.98 - 5.12 (m, 6H), 4.57 (d,  $J$  = 6 Hz, 1H), 4.39 (m, 1H), 4.07 (bs, 1H), 3.90 - 3.98 (m, 2H), 3.78 - 3.90 (m, 3H), 1.42 (s, 3H), 1.25 (s, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  169.5, 156.7, 136.3, 135.6 (m, 2Cs), 128.82, 128.77, 128.6, 128.3, 128.2, 128.2, 113.2, 81.7, 81.0, 80.1 (d,  $J$  = 8 Hz), 79.1, 69.74 (d,

$J = 3$  Hz), 69.70 (d,  $J = 3$  Hz), 68.4 (d,  $J = 6$  Hz), 67.1, 44.7, 26.1, 24.7. IR (neat):  $\nu$  3307.6, 2926.0, 2853.8, 2360.3, 2337.0, 1691.9, 1514.2, 1454.8, 1376.8, 1267.5, 1210.0, 1157.8, 1079.0  $\text{cm}^{-1}$ . HRMS Calculated for  $[\text{C}_{32}\text{H}_{37}\text{N}_2\text{O}_{10}\text{PNa}^+]$  663.2078, Found 663.2073.  $[\alpha]_{\text{D}}^{24} -14$  ( $c$  0.135,  $\text{CH}_2\text{Cl}_2$ ).



### 2,3-*O*-Isopropylidene-*D*-ribofuranosylamine-5-phosphate ( $\alpha$ ) (17a).

2,3-*O*-Isopropylidene-1-*N*-(benzyloxycarbonyl)glycyl)-*D*-ribofuranosylamine-5-dibenzylphosphate ( $\alpha$ ) (16a) (180 mg, 0.28 mmol) was dissolved in 4.5 mL EtOH and reduced with Pd-C under hydrogen overnight. Water (2 mL) was added with stirring and the Pd-C was filtered off. The filtrate was concentrated under reduced pressure, water was removed azeotropically with toluene to yield (83 mg, 0.25 mmol, 91%) debenzylated product as a colorless syrupy liquid.  $R_f$  (70% EtOAc/Hexanes) = 0;  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  5.94 (d,  $J = 4.4$  Hz, 1H), 5.03 (d,  $J = 6.4$  Hz, 1H), 4.91 (dd,  $J = 5.6, 4.4$  Hz, 1H), 4.29 (d,  $J = 2.8$  Hz, 1H), 3.90 - 3.92 (m, 4H), 1.55 (s, 3H), 1.41 (s, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ )  $\delta$  168.0, 113.5, 81.9, 81.8, 81.3 (d,  $J = 8.3$  Hz), 79.0, 65.7 (d,  $J = 5.3$  Hz), 40.8, 25.1, 23.5. IR (neat):  $\nu$  3326.7, 2930.2, 1689.6, 1651.9, 1519.8, 1206.2, 1156.2, 1088.7, 1036.6  $\text{cm}^{-1}$ . HRMS Calculated for  $[\text{C}_{10}\text{H}_{19}\text{N}_2\text{O}_8\text{PNa}^+]$  349.0771, found 349.0774.  $[\alpha]_{\text{D}}^{24} -38$  ( $c$  0.17,  $\text{H}_2\text{O}$ ).



**GAR: mixture of alpha and beta (1:1) (18a and 18b).** The acetone of glycinamide ribonucleotide 17a (75 mg, 0.23 mmol) was dissolved in 2.5 mL water. TFA (0.35 mL) was added. The reaction mixture was stirred for 5 h at room temperature. The water and TFA was evaporated off under reduced pressure to afford (39 mg, 0.14 mmol, 60%) glycinamide ribonucleotide as a colorless syrupy liquid.  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  5.65 (d,  $J = 4$  Hz), 5.36 (d,  $J = 4.2$  Hz), 4.13 - 4.18 (m), 3.96 - 4.04 (m), 3.75 - 3.92 (m), 3.75 (s), 3.72 (s).  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ )  $\delta$  168.18, 168.16, 83.6, 82.8 (d,  $J = 9$  Hz), 81.0 (d,  $J = 8$  Hz), 80.5, 74.0, 70.8, 70.7, 70.2, 65.0 (d,  $J = 5$  Hz), 64.8 (d,  $J = 5$  Hz), 40.8, 40.7. IR (neat):  $\nu$  3215.7, 2929.6, 1674.0, 1536.2, 1434.4, 1129.8, 1027.8  $\text{cm}^{-1}$ . HRMS Calculated for  $[\text{C}_7\text{H}_{15}\text{N}_2\text{O}_8\text{PH}^+]$  287.0639, found 287.0645.  $[\alpha]_{\text{D}}^{24} -21$  ( $c$  0.10,  $\text{H}_2\text{O}$ ).

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