Supporting Information for

Synthesis and Biochemical Evaluation of *O*-acetyl-ADP-ribose and *N*-acetyl Analogs

*Lindsay R. Comstock and John M. Denu**

Department of Biomolecular Chemistry, University of Wisconsin-Madison

1300 University Ave., MSC 551, Madison, WI 53706

Corresponding Author: John M. Denu, Fax: +1 (608) 262-5253;

Tel: +1 (608)265-1859; E-mail: jmdenu@wisc.edu

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General Procedures:

Reactions were typically carried out with anhydrous solvents under an inert atmosphere of argon; those containing water were performed under normal atmospheric conditions. All reagents and anhydrous solvents were obtained from commercial suppliers and used as received. The silica gel used in column flash chromatography was Merck no. 9385, 60 Å, 230–400 mesh. Reversed-phase flash silica was prepared and used as previously described.[1] Analytical TLC was conducted on EM Science silica gel plates with detection by phosphomolybdic acid and/or UV light. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian MercuryPlus 300 or Bruker AC300 spectrometer using TMS or solvent as the internal reference. ^{13}C-NMR spectra for **2** and **3** were recorded on a Varian Unity 500. Chemical shifts are reported in ppm, in δ units. Mass spectra were obtained from the University of Wisconsin-Madison, Department of Chemistry or Biotechnology Center mass spectrometry facility.

Analytical HPLC was performed using a Shimadzu series 2010C HPLC with either a PolyHydroxyethyl A (HILIC) column (300 Å, 5 µm, 4.5 x 200 mm, PolyLC, Inc.) or C18 column (90 Å, 10 µm, 4.6 x 250 mm, Vydac) and detected at both 214 and 260 nm. All mobile phases were filtered through a Millipore 0.20-µm nylon filter prior to use. Compound separation for HILIC utilized a gradient system comprising of ACN (solvent A) and 10 mM NH_4OAc (solvent B) using a flow rate of 0.5 mL/min. The gradient was run isocratically with 20% B for 9 min followed by a linear gradient of 20-60% B over a 30-min period. Under these conditions, diadenosine 5'-pyrophosphate (AppA) and the desired acetylated ADPr co-eluted (23.5 min). Compound separation for C18 utilized a gradient system comprising of H_2O with 0.05% TFA (solvent A) and ACN with 0.02% TFA (solvent B) using a flow rate of 0.5 mL/min. The gradient was run isocratically with 0% B for 2 min followed by a linear gradient of 0-8% B over a 20-min period. The gradient was then increased to 100% over the next 5 min. Under these conditions, diadenosine 5'-pyrophosphate (AppA) eluted at 20 min., where the desired acetylated ADPr eluted around 15 min.

Preparative HPLC was performed using a Beckman Biosys 510 HPLC with either a HILIC column (300 Å, 5 µm, 9.4 x 250 mm, PolyLC, Inc.) or C18 column (90 Å, 10 µm, 10 x 250 mm, Vydac) and detected at 260 nm. For HILIC, compound separation utilized a gradient system comprising ACN (solvent A) and 10 mM NH_4OAc (solvent B) using a flow rate of 4 mL/min. The gradient was run isocratically in 20% B for 5 min followed by a linear gradient of 20-40% B over a 40-min period. For C18, compound separation utilized a gradient system comprising of H_2O with 0.05% TFA (solvent A) and ACN with 0.02% TFA (solvent B) using a flow rate of 4 mL/min. The gradient was run isocratically in 0% B for 2 min followed by a linear gradient of 0-8% B over a 20-min period. The gradient was then increased to 100% over the next 20 min.

General Phosphatase Assay:

The following method was modified from a previously described procedure.[2] The methodology employed calf intestine phosphatase (CIP, New England Biolabs) to hydrolyze the phosphate ester from the ribose sugar and the total amount of free phosphate in solution was determined using a colorimetric assay. Identification of fractions containing inorganic phosphate and/or the phosphorylated sugar followed the following steps: 1. Reaction contained 50 mM Tris (pH 7.8), 10 mM $MgCl_2$, 5U CIP, and 25 µL phosphate sample (200 µL final volume).* 2. Each tube was incubated at 37°C for 5 min to liberate free phosphate. 3. The reactions were quenched with 600 µL of the molybdate-ascorbic acid quench solution and followed with incubation at 42°C for 20 min. 4. A_{820} was obtained to identify fractions containing the phosphorylated ribose sugar. Additional no enzyme controls to distinguish fractions containing free inorganic phosphate from the desired sugar were performed.

*Fractions containing MeOH resulted in a negligible effect on CIP activity.

Characterization of Synthetic Intermediates:

3,5-Di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose (**5**)[3]: 5-*O*-Benzyl-3-oxo-1,2-*O*-isopropylidene-α-D-xylofuranose was prepared from **4** as previously described in 65% yield.[4] To 5-*O*-benzyl-3-oxo-1,2-*O*-isopropylidene-α-D-xylofuranose (1.461 g, 5.252 mmol) in 22.5 mL dry MeOH was added $NaBH_4$ (0.0993 g, 2.626 mmol). After stirring for 1 h, the reaction was quenched with H_2O and the solvent was evaporated *in vacuo*. The resulting residue was re-suspended in EtOAc and washed ($NaHCO_3$). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. To the crude ribofuranose in 29 mL dry DMF at 0°C was added NaH (5.777 mmol), followed by BnBr (2.70 g, 15.76 mmol). After stirring for 30 min., the reaction was quenched with 400 µL AcOH and the solvent was removed. The residue was re-suspended in EtOAc, subjected to an aqueous workup (H_2O, $NaHCO_3$, EtOAc, brine), dried over Na_2SO_4, and evaporated *in vacuo*. Flash chromatography on silica (3:1 Hexanes/EtOAc) afforded **5** (1.55 g, 80%). ^{1}H NMR ($CDCl_3$) δ 7.35-7.25 (m, 10H), 5.76 (d, J= 3.7 Hz, 1H), 4.73 (d, J= 11.8 Hz, 1H), 4.59-4.47 (m, 4H), 4.21-4.16 (m, 1H), 3.86 (dd, J= 9.1, 4.5 Hz, 1H), 3.76 (dd, J= 11.3, 1.9 Hz, 1H), 3.56 (dd, J= 11.3, 3.7 Hz, 1H), 1.59 (s, 3H), 1.36 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 137.9, 137.6, 128.2, 128.1, 127.7, 127.5, 127.4, 112.6, 104.0, 77.9, 77.1, 73.2, 71.9, 68.0, 26.7, 26.4. HRMS-EI: calcd for $C_{22}H_{26}O_5$ (M + Na^+), 393.1678, obsd 393.1677.

1,3,5-Tri-*O*-benzyl-α-D-ribofuranose (**6**)[5]: To 3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose (**5**) (0.656 g, 1.77 mmol) in 21 mL 1:1 dioxane/H_2O was added 1.5 mL Dowex-50WX2-100 (H^+-form) (as a 1:1 slurry in H_2O). After the suspension was stirred at 80°C for 22 h, the reaction was cooled to ambient temperature and the resin was filtered; the solvent was evaporated and co-stripped with EtOH (to remove trace H_2O). After drying *in vacuo*, the resulting material was washed with 1:1 Et_2O/Petroleum Ether (with vigorous shaking). This process was repeated (typically 2-3 times) until all contaminants were removed in the organic layer to result in product (0.486 g), which a portion

was taken directly forward. The 3,5-di-*O*-benzyl-D-ribofuranose (0.322 g, 0.974 mmol) in 39 mL MeOH (HPLC grade) was added Bu_2SnO (0.339 g, 1.364 mmol). The reaction was heated at reflux for 2 h. Upon cooling to ambient temperature, the solvent was removed and the clear oil was dried under vacuum. To the resulting dibutylstannylene in 1.33 mL anhydrous DMF was added K_2CO_3 (0.431 g, 3.117 mmol). Benzyl bromide (0.450 g, 2.630 mmol) was added drop wise to the rapidly stirring suspension and stirred for an additional 27 h. The mixture was filtered through Celite and washed with several portions of $CHCl_3$. The resulting organic was washed with water, dried over Na_2SO_4, and evaporated *in vacuo*. Flash chromatography on silica (3:1 Hexanes/EtOAc) afforded **6** (0.305 g, 74 %). ^{1}H NMR ($CDCl_3$) δ 7.38-7.22 (m, 15H), 5.08 (d, J= 4.6 Hz, 1H), 4.87 (d, J= 12.2. Hz, 1H), 4.71 (d, J= 12.0 Hz, 1H), 4.59 (ABq, J= 12.0 Hz, 2H), 4.49 (ABq, J= 12.1 Hz, 2H), 4.23 (dd, J= 7.4, 4.1 Hz, 1H), 4.15 (ddd, J= 11.5, 7.0, 4.5 Hz, 1H), 3.82 (dd, J= 7.1, 3.1 Hz, 1H), 3.46 (dd, J= 10.6, 4.1 Hz, 1H), 3.39 (dd, J= 10.6, 4.1 Hz, 1H), 3.03 (d, J= 11.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 138.2, 138.1, 128.6, 128.5, 127.93, 127.90, 127.8, 127.7, 100.8, 82.3, 76.8, 73.7, 73.0, 72.2, 70.3, 69.2. HRMS-ESI: calcd for $C_{26}H_{28}O_5$ (M + Na^+), 443.1834, obsd 443.1828.

1,3,5-Tri-*O*-benzyl-2-*O*-acetyl-α-D-ribofuranose (Intermediate to **7**): To 1,3,5-tri-*O*-benzyl-α-D-ribofuranose (**6**) (0.208 g, 0.494 mmol) in 2.5 mL anhydrous pyridine was added acetic anhydride (0.856 g, 8.390 mmol). After stirring for 6 h, the reaction was poured into ice water and extracted with $CHCl_3$ (three times). The organic layers were combined, dried over Na_2SO_4, and evaporated *in vacuo*. Flash chromatography on silica (3:1 Hexanes/EtOAc) afforded product (0.221 g, 97 %). ^{1}H NMR ($CDCl_3$) δ 7.35-7.25 (m, 15H), 5.26 (d, J= 4.6 Hz, 1H), 4.94 (dd, J= 7.0, 4.6 Hz, 1H), 4.86 (d, J= 12.5 Hz, 1H), 4.66 (ABq, J= 12.5 Hz, 2H), 4.54-4.42 (m, 3H), 4.24 (dt=q, J= 8.0, 3.9 Hz, 1H), 4.05 (dd, J= 6.8, 4.8 Hz, 1H), 3.49 (dd, J= 10.6, 3.2 Hz, 1H), 3.36 (dd, J= 10.7, 4.1 Hz, 1H), 2.16 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.7, 138.1, 138.1, 138.0, 128.6, 128.5, 128.4, 128.2, 128.0, 127.91, 127.88, 127.7, 99.8, 81.4, 75.6, 73.6, 73.2, 72.1, 69.5, 21.0. HRMS-EI:

calcd for $C_{28}H_{30}O_6$ ($M + Na^+$), 486.2018, obsd 486.2025.

1,3-Di-*O*-benzyl-2-*O*-acetyl-α-D-ribofuranose (**7**): To 1,3,5-tri-*O*-benzyl-2-*O*-acetyl-α-D-ribofuranose (0.159 g, 0.3431 mmol) in 768 µL 1:1 MeOH/AcOH containing 0.08% pyridine (v/v) was added 15.3 mg 10% Pd/C. The reaction was stirred under 50 psi H_2 for 1 d. The Pd/C was filtered off through Celite and washed with MeOH. The solvent was evaporated *in vacuo*. Flash chromatography on silica (2:1 Hexanes/EtOAc) afforded **7** (0.063 g, 49 %); this reaction typically gave yields of 40-50%. Approximately 5-15% of unreacted starting material could be recovered. ^{1}H NMR ($CDCl_3$) δ 7.34-7.25 (m, 10H), 5.23 (d, J= 4.4 Hz, 1H), 4.91 (dd, J= 6.7, 4.4 Hz, 1H), 4.83 (d, J= 12.4 Hz, 1H), 4.66 (ABq, J= 12.5 Hz, 2H), 4.49 (d, J= 12.1 Hz, 1H), 4.17-4.13 (m, 1H), 4.03 (dd, J= 6.7, 5.5 Hz, 1H), 3.69 (ddd, J= 12.1, 4.2, 3.1 Hz, 1H), 3.42 (ddd, J= 12.1, 7.8, 3.5 Hz, 1H), 2.15 (s, 3H), 1.89 (dd, J= 7.7, 4.3 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 170.7, 138.0, 137.7, 128.6, 128.5, 128.2, 127.9, 127.8, 99.8, 82.3, 75.1, 73.4, 72.2, 69.6, 62.0, 21.0. HRMS-ESI: calcd for $C_{21}H_{24}O_6$ ($M + Na^+$), 395.1471, obsd 395.1467.

1,3-Di-*O*-benzyl-2-*O*-acetyl-α-D-ribofuranose 5-hydrogen phosphate (TEA salt) (Intermediate to **8**): To 1,3-di-*O*-benzyl-2-*O*-acetyl-α-D-ribofuranose (**7**) (0.0645 g, 0.173 mmol) in 470 µL dry THF at 0°C was added TEA (0.1577 g, 1.559 mmol) and followed by $POCl_3$ (0.0398 g, 0.260 mmol) drop wise. After stirring at 0°C for 2 h, a few ice chips were added and stirred for an additional hour. The solvent was evaporated and dried under vacuum. The desired product was purified away from free inorganic phosphate using reversed-phase flash chromatography and a step-wise gradient of MeOH (25-100% in degassed H_2O).[1] After identifying the desired fractions by the phosphatase assay (described above in general procedures), the fractions were combined to yield **11** as TEA salt (0.0810 g, 85%). ^{1}H NMR ($CDCl_3$) δ 7.39-7.22 (m, 10H), 5.22 (d, J= 4.4 Hz, 1H), 4.90 (dd, J= 6.5, 4.3 Hz, 1H), 4.81 (d, J= 12.7 Hz, 1H), 4.63 (s, 2H), 4.58 (d, J= 12.7

Hz, 1H), 4.29-4.27 (m, 1H), 4.20 (dd, J= 6.5, 4.4 Hz, 1H), 4.00 (m, 2H), 3.01 (bq, 6H), 2.09 (s, 3H), 1.26 (bt, 9H); ^{13}C NMR ($CDCl_3$) δ 170.5, 138.5, 138.1, 128.33, 128.30, 128.1, 127.7, 127.6, 127.5, 99.7, 81.6, 75.9, 73.0, 72.4, 69.3, 64.7, 45.6, 20.9, 8.6; ^{31}P NMR ($CDCl_3$) δ 2.2. HRMS-ESI: calcd for $C_{21}H_{24}O_9P$ $(M - H)^-$, 451.1163, obsd 451.1256.

2-*O*-Acetyl-D-ribofuranose 5-hydrogen phosphate (TEA salt) (**8**) : To 1,3-di-*O*-benzyl-2-*O*-acetyl-α-D-ribofuranose 5-hydrogen phosphate (**7**) (as the TEA salt) (0.0608 g, 0.1098 mmol) in 4.2 mL EtOH was added 10% Pd/C (0.0417 g). The reaction was stirred under 50 psi H_2 for 2 d. The Pd/C was filtered off through Celite and washed with MeOH. The solvent was evaporated to give **8** (0.036 g, 88 %). Multiple signals were observed by NMR for the phosphorus and all ribose protons and carbons. This observation is attributable to a combination of the mixture of α and β anomers and the previously observed transesterification to the 3-position.[6] ^{1}H NMR (CD_3OD) δ 5.43-5.09 (m, 1H), 4.96-4.90 (m, 1H), 4.39-4.17 (m, 1H), 4.14-4.05 (m, 1H), 4.03-3.95 (m, 2H), 3.20 (bq, 6H), 2.12-2.09 (m, 3H), 1.32 (bt, 9H); ^{13}C NMR (CD_3OD) δ 172.1, 171.9, 103.6, 101.0, 97.5, 96.8, 83.1, 83.0, 80.7, 80.6, 79.0, 75.6, 75.4, 74.5, 73.7, 71.7, 67.6, 67.53, 67.47, 58.3, 47.4, 21.2, 20.85, 20.77, 18.4, 9.1; ^{31}P NMR (CD_3OD) δ 2.2, 1.9, 1.84, 1.78. HRMS-ESI: calcd for $C_7H_{12}O_9P$ $(M - H)^-$, 271.0224, obsd 271.0249.

3-Azido-3-deoxy-6-*O*-benzyl-1,2-*O*-isopropylidene-α-D-allofuranose (**10**): 3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**9**)[7] (1.218 g, 4.272 mmol) was dissolved in 17 mL 70% aqueous AcOH and stirred overnight. The solvent was evaporated *in vacuo* and co-stripped with EtOH three times to remove all traces of acid; the resulting oil was dried under vacuum. To the crude furanose in 23 mL dry toluene was added Bu_2SnO (1.276 g, 5.126 mmol); the reaction was then refluxed overnight with azeotropic removal of water. The Dean-Stark trap was then removed and replaced with a standard reflux condenser. BnBr (0.71 mL, 5.981 mmol) and TBAB (0.689 g, 2.136 mmol) were

added and stirred at 110°C for an additional 6 h. Upon cooling to ambient temperature, the solvent was evaporated and the residue was dried *in vacuo*. Flash chromatography on silica (20 → 50% EtOAc in Hexanes) provided **10** (0.935 g, 65%). ^{1}H NMR ($CDCl_3$) δ 7.34 (m, 5H), 5.76 (d, J= 3.6 Hz, 1H), 4.69 (dd, J= 4.5, 4.0 Hz, 1H), 4.56 (s, 2H), 4.13 (dd, J= 9.1, 4.3 Hz, 1H), 4.08-4.03 (m, 1H), 3.66-3.54 (m, 3H), 2.82 (d, J= 3.3 Hz, 1H), 1.56 (s, 3H), 1.35 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 137.7, 128.5, 127.94, 127.92, 113.2, 104.1, 80.8, 77.9, 73.5, 70.7, 70.0, 60.5, 26.6, 26.5. HRMS-ESI: calcd for $C_{16}H_{21}N_3O_5$ ($M + Na^+$), 358.1379, obsd 358.1396.

2-Azido-2-deoxy-5-*O*-benzyl-1,3-*O*-bis-(*tert*-butyldimethylsilyl)-D- ribofuranose (**12**): To 3-azido-3-deoxy-6-*O*-benzyl-1,2-*O*-isopropylidene-α-D-allofuranose (**10**) (0.558 g, 1.665 mmol) in 7.5 mL 1:1 dioxane/H_2O was added 650 µL Dowex-50WX2-100 (H^+-form) (as a 1:1 slurry in H_2O). After stirring at 80°C for 20 h, the reaction was cooled; the resin was filtered off and washed with 1.1 mL dioxane. $NaIO_4$ (0.374 g, 1.749 mmol) in 2.8 mL H_2O was added slowly to the stirring solution and stirred for one hour. Additional $NaIO_4$ (0.119 g, 0.556 mmol) in 860 µL H_2O was added and stirred for an additional hour. Finally, $NaHCO_3$ (0.168 g, 1.999 mmol) was added in small portions and the mixture was stirred overnight. The resulting suspension was filtered through Celite and washed with EtOAc. The combined filtrates were concentrated, brought up in EtOAc, and washed with H_2O; the organic was dried over Na_2SO_4 and evaporated *in vacuo*. The material was purified on silica (3:1 $CHCl_3$/EtOAc) to afford **11** (0.267 g, 1.007 mmol) and taken directly forward. The diol was brought up in 5.2 mL dry DMF and imidazole (0.343 g, 5.04 mmol) and TBSCl (0.455 g, 3.02 mmol) were added. After stirring overnight, the reaction was washed (NH_4Cl twice, EtOAc, brine), dried over Na_2SO_4 and evaporated *in vacuo*. Flash chromatography on silica (0 → 5% EtOAc in Hexanes) afforded **12** as a mixture of α and β anomers (1:6 ratio) (0.492 g, 60%). Signals for the two anomers could be discerned by ^{1}H NMR ($CDCl_3$): **α** - δ 7.34-7.28 (m, 5H), 5.52 (d, J= 4.3 Hz, 1H), 4.53 (ABq, J= 12.1 Hz,

2H), 4.29 (dd, J= 7.0, 3.3 Hz, 1H), 4.19 (dd, J= 6.4, 3.5 Hz, 1H), 3.57-3.51 (m, 2H), 3.04 (dd, J= 6.8, 4.3 Hz, 1H), 0.94 (s, 9H), 0.89 (s, 9H), 0.17 (s, 3H), 0.16 (s, 3H), 0.12 (s, 3H), 0.01 (s, 3H); **β** - δ 7.34-7.27 (m, 5H), 5.20 (d, J= 1.4 Hz, 1H), 4.57 (s, 2H), 4.45 (dd, J= 6.3, 5.0 Hz, 1H), 4.06 (td, J= 6.2, 3.5 Hz, 1H), 3.62 (dd, J= 10.5, 3.5 Hz, 1H), 3.55-3.49 (m, 2H), 0.90 (s, 9H), 0.87 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H), 0.09 (s, 3H), 0.08 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 138.3, 138.1, 128.6, 128.5, 127.9, 127.7, 100.5, 98.9, 85.0, 82.5, 73.8, 73.7, 73.5, 71.2, 69.5, 68.9, 62.4, 26.0, 25.9, 25.8, 18.2, 18.1, 18.0, -4.1, -4.3, -4.5, -4.7, -4.88, -4.91, -5.1. HRMS-EI: calcd for $C_{24}H_{43}N_3O_4Si_2$ (M + Na^+), 516.2690, obsd 516.2696.

2-*N*-Acetyl-2-deoxy-5-*O*-benzyl-1,3-*O*-bis-(*tert*-butyldimethylsilyl)-D-ribofuranose (Intermediate to **13**): To 2-azido-2-deoxy-5-*O*-benzyl-1,3-*O*-bis-(*tert*-butyldimethylsilyl)-D-ribofuranose (**12**) (0.492 g, 0.998 mmol) in 14.6 mL 4:2:1:1 pyridine/7N methanolic NH_3/MeOH/H_2O was added PPh_3 (0.707 g, 2.69 mmol). After stirring overnight, the solvent was evaporated off and co-stripped with EtOH (2x) to remove trace water. After drying under vacuum, the resulting solid was dissolved in 4.3 mL dry CH_2Cl_2 and cooled to 0°C. Pyridine was added (0.237 g, 2.993 mmol), followed by acetic anhydride (0.122 g, 1.197 mmol) drop wise. The reaction was allowed to warm to rt and stirred for 2 h; the reaction was washed (H_2O, CH_2Cl_2), dried over Na_2SO_4 and evaporated *in vacuo*. Flash chromatography on silica (3:1 Hexanes/ EtOAc) afforded product as a mixture of α and β-anomers (1:6 ratio) (0.427 g, 84%). Signals for the two anomers could be discerned by ^{1}H NMR ($CDCl_3$): **α** - δ 7.34-7.27 (m, 5H), 5.95 (bd, 1H), 5.38 (d, J= 4.3Hz, 1H), 4.52 (m, 2H), 4.37-4.34 (m, 1H), 4.16-4.14 (m, 1H), 4.14-4.12 (m, 1H), 3.54-3.50 (m, 2H), 1.99 (s, 3H), 0.90 (s, 18H), 0.12 (s, 3H), 0.10 (s, 3H), -0.02 (s, 3H), -0.04 (s, 3H); **β** - δ 7.34-7.27 (m, 5H), 5.99 (bd, 1H), 5.32 (s, 1H), 4.58 (m, 2H), 4.27 (t, J= 5.6 Hz, 1H), 4.02 (t, J= 5.6 Hz, 1H), 4.00-3.98 (m, 1H), 3.65-3.56 (m, 2H), 2.00 (s, 3H), 0.88 (s, 18H), 0.13 (s, 3H), 0.09 (s, 3H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 170.3, 169.2, 138.2, 138.0, 128.5, 128.4, 128.0, 127.9, 127.7, 101.8, 96.8, 85.3, 83.6, 73.6, 73.5, 72.4, 72.3, 71.5, 69.9, 59.6, 54.3, 25.8,

23.4, 23.3, 18.1, 18.0, -4.1, -4.4, -4.6, -4.9, -5.0, -5.1,-5.3. HRMS-EI: calcd for $C_{26}H_{47}NO_5Si_2$ (M + Na^+), 532.2891, obsd 532.2885.

2-*N*-Acetyl-2-deoxy-1,3-*O*-bis-(*tert*-butyldimethylsilyl)-D-ribofuranose (**13**) : To 2-*N*-acetyl-2-deoxy-5-*O*-benzyl-1,3-*O*-bis-(*tert*-butyldimethylsilyl)-D-ribofuranose (0.248 g, 0.486 mmol) in 20.3 mL EtOH was added 10% Pd/C (0.160 g). The reaction was stirred under 50 psi H_2 for 2 d. The Pd/C was filtered off through Celite and washed with EtOH. The solvent was evaporated and dried *in vacuo*. The two anomers of **13** were separable by column chromatography on silica using a gradient from 2:1 Hexanes/EtOAc → 2:1 EtOAc/Hexanes: (β-anomer: 0.143 g, α-anomer: 0.030 g, 85 % overall). **α**: ^{1}H NMR ($CDCl_3$) δ 5.97 (bd, J= 8.8 Hz, 1H), 5.39 (d, J= 4.3 Hz, 1H), 4.27 (ddd, J= 8.8, 7.3, 4.3 Hz, 1H), 4.14 (dd, J= 7.5, 2.8 Hz, 1H), 4.08 (dt, J= 4, 2.8 Hz, 1H), 3.78 (ddd, J= 11.9, 5.2, 3.3 Hz, 1H), 3.63 (ddd, J= 11.9, 7.6, 4.2 Hz, 1H), 2.00 (s, 3H), 1.77 (dd, J= 7.7, 5.2 Hz, 1H), 0.91 (s, 9H), 0.90 (s, 9H), 0.12 (s, 3H), 0.10 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 169.5, 96.9, 86.7, 70.8, 62.8, 54.7, 25.8, 23.3, 18.1, 18.0, -4.2, -4.5, -4.9, -5.3. **β**: ^{1}H NMR ($CDCl_3$) δ 6.07 (bd, J= 4 Hz, 1H), 5.37 (s, 1H), 4.74 (t, J= 6.2 Hz, 1H), 4.00 (dt, J= 6, 2.5 Hz, 1H), 3.91 (dd, J= 6, 4.8 Hz, 1H), 3.80 (dt, J= 12, 2.1 Hz, 1H), 3.58 (dd, J= 12.2, 10, 2.6 Hz, 1H), 2.59 (dd, J= 10, 2.6 Hz, 1H), 2.03 (s, 3H), 0.92 (s, 9H), 0.91 (s, 9H), 0.20 (s, 3H), 0.17 (s, 3H), 0.12 (s, 3H), 0.10 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.7, 101.3, 85.8, 69.5, 61.8, 60.3, 25.81, 25.76, 23.3, 18.1, 18.0, -4.7, -4.8, -4.9, -5.0. HRMS-ESI: calcd for $C_{19}H_{41}NO_5Si_2$ (M + Na^+), 442.2421, obsd 442.2409.

2-*N*-Acetyl-2-deoxy-1,3-*O*-bis-(*tert*-butyldimethylsilyl)-β-D-ribofuranose 5-hydrogen phosphate (TEA salt) (**14**) : To 2-*N*-acetyl-2-deoxy-1,3-*O*-bis-(*tert*-butyldimethylsilyl)-β-D-ribofuranose (**13**) (0.096 g, 0.229 mmol) in 620 µL dry THF at 0°C was added TEA (0.208 g, 2.06 mmol), followed by $POCl_3$ (0.053 g, 0.343 mmol) drop wise. After stirring cold for 2 h, a few ice chips were added and stirred for an additional hour. The solvent was evaporated off and the desired

product was purified from free inorganic phosphate using reversed-phase flash chromatography utilizing a step-wise gradient of MeOH (25-100% in degassed H_2O).[1] After identifying the desired fractions by the phosphatase assay (described above in general procedures), the fractions were combined to yield **14** as the TEA salt (0.128 g, 93%). ^{1}H NMR ($CDCl_3$) δ 6.07 (bd, 1H), 5.16 (d, J= 1.9 Hz, 1H), 4.38-4.33 (m, 1H), 4.03-3.96 (m, 2H), 3.92-3.86 (m, 2H), 3.01 (bq, 6H), 1.94 (s, 3H), 1.26 (bt, 9H), 0.84 (s, 9H), 0.82 (s, 9H), 0.09 (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H), 0.01 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.0, 101.8, 84.0, 72.5, 66.6, 59.2, 45.5, 25.9, 25.8, 23.3, 18.1, 18.0, 8.7, -3.8, -4.3, -4.6, -5.1; ^{31}P NMR ($CDCl_3$) δ 2.1. HRMS-ESI: calcd for $C_{19}H_{42}NO_8PSi_2$ $(M - H)^-$, 498.2114, obsd 498.2106.

5-*O*-Benzyl-3-*O*-trifluoromethanesulfonyl-1,2-*O*-isopropylidene-α-D-xylofuranose (Intermediate to **15**): To 5-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose[3] (2.00 g, 7.14 mmol) in 150 mL dry CH_2Cl_2 at -10°C was added pyridine (2.26 mL, 27.85 mmol). Trifluoromethanesulfonic anhydride (1.44 mL, 8.57 mmol) was added drop wise and the reaction was slowly warmed to 0°C over an hour. Saturated $NaHCO_3$ was added to quench the reaction and followed by aqueous workup ($NaHCO_3$, CH_2Cl_2). The organic layer was dried over Na_2SO_4 and evaporated. Flash chromatography on silica (4:1 Hexanes/ EtOAc) gave the desired product (2.58 g, 87%). ^{1}H NMR ($CDCl_3$) δ 7.36-7.30 (m, 5H), 5.99 (d, J= 3.7 Hz, 1H), 5.28 (d, J= 2.6 Hz, 1H), 4.73 (d, J= 3.9 Hz, 1H), 4.55 (ABq, J= 11.6 Hz, 2H), 4.54-4.49 (m, 1H), 3.78 (dd, J= 9.6, 5.8 Hz, 1H), 3.67 (dd, J= 9.6, 7.6 Hz, 1H), 1.49 (s, 3H), 1.32 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 137.5, 128.7, 128.21, 128.17, 116.5, 113.2, 104.8, 88.4, 83.2, 77.5, 74.1, 66.2, 26.7, 26.5. HRMS-EI: calcd for $C_{13}H_{19}F_3O_8S$ $(M + Na^+)$, 435.0701, obsd 435.0701.

3-Azido-3-deoxy-5-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose (**15**)[8]: To LiF (0.570 g, 21.97 mmol) in 15.7 mL dry DMF at 100°C was added $TMSN_3$ (2.531 g, 21.97 mmol). After stirring for 1 h, 5-*O*-benzyl-3-*O*-trifluoromethanesulfonyl-1,2-

O-isopropylidene-α-D-xylofuranose (2.58 g, 6.28 mmol) in 15.7 mL dry DMF was added and the reaction was stirred for an additional 5 h. Upon cooling to ambient temperature, the reaction was washed ($NaHCO_3$ twice, $CHCl_3$), dried over Na_2SO_4 and dried *in vacuo*. Flash chromatography on silica (3:1 Hexanes/ Et_2O) provided **15** (0.843 g, 44%). ^{1}H NMR ($CDCl_3$) δ 7.33-7.27 (m, 5H), 5.79 (d, J= 3.8 Hz, 1H), 4.67 (dd, J= 4.3, 3.9 Hz, 1H), 4.58 (ABq, J= 12.1 Hz, 2H), 4.20-4.15 (m, 1H), 3.78 (dd, J= 11.3, 2.3 Hz, 1H), 3.61 (dd, J= 11.3, 3.7 Hz, 1H), 3.56 (dd, J= 9.5, 4.7 Hz, 1H), 1.55 (s, 3H), 1.34 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 137.3, 128.4, 127.8, 127.7, 127.6, 113.0, 104.2, 79.9, 77.3, 73.7, 67.8, 60.5, 26.41, 26.39. HRMS-EI: calcd for $C_{15}H_{19}N_3O_4$ ($M + Na^+$), 328.1273, obsd 328.1285.

3-Azido-3-deoxy-5-*O*-benzyl-1,2-*O*-bis-(*tert*-butyldimethylsilyl)-β-D-ribofuranose (**16**): To 3-azido-3-deoxy-5-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose (0.843 g, 2.763 mmol) in 12.5 mL 1:1 dioxane/H_2O was added 1 mL Dowex-Dowex-50WX2-100 (H^+-form) (as a 1:1 slurry in H_2O). After the suspension was stirred at 80°C for 22 h, the reaction was cooled and the resin was filtered off; the solvent was evaporated and co-stripped with EtOH (to remove trace H_2O). Upon drying completely *in vacuo*, the crude material was brought up in 25 mL dry DMF and imidazole (0.940 g, 13.81 mmol) and TBSCl (1.250 g, 8.288 mmol) were added. After stirring overnight, the reaction was washed (NH_4Cl twice, EtOAc, brine), dried over Na_2SO_4 and evaporated to dryness. Flash chromatography on silica (0→ 5% EtOAc in Hexanes) afforded **16** (0.877 g, 64%) predominantly as the β-anomer (α-hydrogen signals at C1 were < 1%). ^{1}H NMR ($CDCl_3$) 7.35-7.27 (m, 5H), 5.12 (s, 1H), 4.59 (s, 2H), 4.32-4.26 (m, 1H), 4.05 (d, J= 4.2 Hz, 1H), 3.67-3.56 (m, 3H), 0.92 (s, 9H), 0.85 (s, 9H), 0.15 (s, 3H), 0.12 (s, 3H), 0.09 (s, 3H), 0.07 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 138.3, 128.5, 127.9, 127.8, 102.8, 79.4, 78.7, 73.7, 72.1, 62.3, 25.9, 25.8, 18.3, 18.0, -4.1, -4.70, -4.73, -5.07. HRMS-EI: calcd for $C_{24}H_{43}N_3O_4Si_2$ ($M + Na^+$), 516.2690, obsd 516.2676.

3-*N*-Acetyl-3-deoxy-5-*O*-benzyl-1,2-*O*-bis-(*tert*-butyldimethylsilyl)-β-D-ribofuranose (Intermediate to **17**): To 3-azido-3-deoxy-5-*O*-benzyl-1,2-*O*-bis-(*tert*-butyldimethylsilyl)-β-D-ribofuranose (**16**) (0.688 g, 1.395 mmol) in 20.6 mL 4:2:1:1 pyridine/7N methanolic NH_3/MeOH/H_2O was added PPh_3 (0.988 g, 3.77 mmol). After stirring overnight, the solvent was evaporated off and co-stripped with EtOH (2x) to remove trace water. After drying under vacuum, the resulting material was dissolved in 5.5 mL dry CH_2Cl_2 and cooled to 0°C. Pyridine was added (0.331 g, 4.184 mmol), followed by acetic anhydride (0.171 g, 1.674 mmol) drop wise. The reaction was allowed to warm to rt and stirred for 2 h; the reaction was washed (H_2O, CH_2Cl_2), dried over Na_2SO_4 and evaporated *in vacuo*. Flash chromatography on silica (2:1 Hexanes/ EtOAc) yielded product (0.669 g, 94%). ^{1}H NMR ($CDCl_3$) δ 7.36-7.21 (m, 5H), 5.80 (bd, J= 8.9 Hz, 1H), 5.14 (s, 1H), 4.56 (s, 2H), 4.47 (td, J= 8.9, 4.5 Hz, 1H), 4.01 (td, J= 8.1, 3.0 Hz, 1H), 3.93 (d, J= 4.5 Hz, 1H), 3.71 (dd, J= 10.3, 2.9 Hz, 1H), 3.58 (dd, J= 10.3, 8.1 Hz, 1H), 1.96 (s, 3H), 0.91 (s, 9H), 0.86 (s, 9H), 0.09 (s, 9H), 0.06 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 169.6, 138.4, 128.4, 127.9, 127.6, 102.8, 81.8, 77.8, 73.6, 73.5, 52.0, 25.8, 25.7, 23.4, 18.2, 17.9, -4.1, -4.5, -4.9, -5.2. HRMS-ESI: calcd for $C_{26}H_{47}NO_5Si_2$ (M + Na^+), 532.2891, obsd 532.2902.

3-*N*-Acetyl-3-deoxy-1,2-*O*-bis-(*tert*-butyldimethylsilyl)-β-D-ribofuranose (**17**): To 3-*N*-acetyl-3-deoxy-5-*O*-benzyl-1,2-*O*-bis-(*tert*-butyldimethylsilyl)-β-D-ribofuranose (0.278 g, 0.545 mmol) in 22.7 mL EtOH was added 10% Pd/C (0.185 g). The reaction was stirred under 50 psi H_2 for 2 d. The Pd/C was filtered off through Celite and washed with EtOH. The solvent was evaporated and dried *in vacuo*. Flash chromatography on silica (1:1 Hexanes/ EtOAc) afforded **17** (0.203 g, 89%). ^{1}H NMR ($CDCl_3$) δ 6.08 (bd, J= 8.7 Hz, 1H), 5.13 (s, 1H), 4.47-4.40 (m, 1H), 3.99 (d, J= 4.8 Hz, 1H), 3.93-3.87 (m, 1H), 3.72-3.67 (m, 2H), 3.40 (dd, J= 8.9, 5.1 Hz, 1H), 2.02 (s, 3H), 0.95 (s, 9H), 0.89 (s, 9H), 0.14 (s, 6H), 0.11 (s, 3H), 0.10 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.8, 102.2, 84.3, 78.6, 64.6, 53.1, 25.8, 25.7, 23.3, 18.2, 17.9, -4.1, -4.4, -4.9, -5.2. HRMS-ESI: calcd for $C_{19}H_{41}NO_5Si_2$

(M + Na^+), 442.2421, obsd 442.2437.

3-*N*-Acetyl-3-deoxy-1,2-*O*-bis-(*tert*-butyldimethylsilyl)-β-D-ribofuranose 5-hydrogen phosphate (TEA salt) (**18**) : To 3-*N*-acetyl-3-deoxy-1,2-*O*-bis-(*tert*-butyldimethylsilyl)-β-D-ribofuranose (**17**) (0.100 g, 0.239 mmol) in 650 µL dry THF at 0°C was added TEA (0.217 g, 2.15 mmol), followed by $POCl_3$ (0.055 g, 0.358 mmol) drop wise. After stirring at 0°C for 2 h, a few ice chips were added and stirred for an additional hour. The solvent was evaporated off and the desired product was purified from free inorganic phosphate using reversed-phase flash chromatography and a step-wise gradient of MeOH (25-100% in degassed H_2O).[1] After identifying the desired fractions by the phosphatase assay (described above in general procedures), the fractions were combined to give **18** as the TEA salt (0.129 g, 90%). ^{1}H NMR ($CDCl_3$) δ 6.73 (bd, 1H), 5.01 (s, 1H), 4.21-4.11 (m, 2H), 4.07 (d, J= 3.8 Hz, 1H), 4.02-3.89 (m, 2H), 3.01 (bq, 6H), 1.95 (s, 3H), 1.25 (bt, 9H), 0.83 (s, 9H), 0.82 (s, 9H), 0.03 (s, 6H), 0.00 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 170.5, 103.3, 80.0, 68.32, 68.26, 53.8, 45.5, 25.9, 25.8, 23.4, 18.2, 18.0, 8.6, -4.0, -4.77, -4.82, -5.1; ^{31}P NMR ($CDCl_3$) δ 2.3. HRMS-ESI: calcd for $C_{19}H_{42}NO_8PSi_2$ $(M - H)^-$, 498.2114, obsd 498.2107.

2-*N*-Acetyl-2-deoxy-D-ribofuranose 5-hydrogen phosphate (TEA salt) (**19**) : To 2-*N*-acetyl-2-deoxy-1,3-*O*-bis-(*tert*-butyldimethylsilyl)-β-D-ribofuranose 5-hydrogen phosphate (TEA salt) (**14**) (0.0200 g, 0.033 mmol) in 300 µL ACN was added 165 µL Dowex-50WX2-100 (H^+-form) (as a 1:1 slurry in H_2O). The reaction was stirred for 2 d. The reaction was passed over Dowex-50WX8 (TEA-form) and the solvent was evaporated. Upon drying under vacuum, the desired product was passed over a reversed-phase silica plug and washed with H_2O (5 mL). The solvent was evaporated to yield **19** as the TEA salt (0.0120 g, 97%). A combination of *N*-Acetyl rotomers and α and β anomers (3:5 ratio) was observed by NMR.[9] ^{1}H NMR [distinct signals for the anomeric position (C-1) and acetyl could be discerned] (D_2O) δ 5.46 (m, 0.62 H), 5.26 (m, 0.38 H), 4.38-4.29 (m,

1H), 4.27- 4.24 (m, 1H), 4.20-4.08 (m, 1H), 4.0-3.92 (m, 2H), 3.17 (q, J= 7.3 Hz, 6H), 2.06, 2.04 (s, s, 3 H), 1.25 (t, J= 7.3 Hz, 9H); ^{13}C NMR [multiple signals were observed for the combination of rotomers/anomers of the ribofuranose carbons and the acetyl group only] (D_2O) δ 174.8, 174.5, 174.2, 100.1, 95.8, 95.0, 84.3, 84.1, 82.9, 82.7, 70.1, 69.7, 65.8, 65.7, 65.2, 65.1, 57.9, 54.2, 46.8, 22.0, 21.9, 8.4; ^{31}P NMR ($CDCl_3$) δ 1.0. HRMS-ESI: calcd for $C_7H_{14}NO_8P$ (M - H)$^-$, 270.0384, obsd 270.0415.

3-*N*-Acetyl-3-deoxy-D-ribofuranose 5-hydrogen phosphate (TEA salt) (**20**) : To 3-*N*-acetyl-3-deoxy-1,2-*O*-bis-(*tert*-butyldimethylsilyl)-β-D-ribofuranose 5-hydrogen phosphate (TEA salt) (**18**) (0.0229 g, 0.0381 mmol) in 340 µL ACN was added 190 µL Dowex-50WX2-100 (H^+-form) (as a 1:1 slurry in H_2O). The reaction was stirred for 2 d. The reaction was passed over Dowex-50WX8 (TEA-form) and the solvent was evaporated. Upon drying under vacuum, the desired product was passed over a reversed-phase silica plug and washed with H_2O (5 mL). The solvent was evaporated to afford **20** as the TEA salt (0.0133 g, 94%). A mixture of α and β anomers (1:3 ratio) were observed by NMR. ^{1}H NMR [distinct signals for the anomeric position (C-1) could be discerned] (D_2O) δ 5.44 (d, J= 3.6 Hz, 0.25H), 5.27 (s, 0.75H), 4.40-4.29 (m, 1H), 4.24-4.19 (m, 1H), 4.16-4.09 (m, 1H), 4.07-3.95 (m, 1H), 3.92-3.84 (m, 1H), 3.18 (q, J= 7.4 Hz, 6H), 2.03 (s, 3H), 1.25 (t, J= 7.4 Hz, 9H); ^{13}C NMR [signals for both anomers could be discerned for the ribofuranose carbons and the acetyl carbonyl only] (D_2O) δ 174.7, 174.5, 101.9, 96.9, 79.7, 79.5, 74.4, 74.3, 69.9, 66.5, 51.8, 51.3, 46.8, 22.0, 8.4; ^{31}P NMR ($CDCl_3$) δ 1.0. HRMS-ESI: calcd for $C_7H_{14}NO_8P$ (M - H)$^-$, 270.0384, obsd 270.0417.

O-Acetyl-ADP-ribose (**1**): The tri-*n*-octylammonium salt of AMP (prepared via titration of an equal molar amount of AMP and tri-n-octylamine in MeOH)[10] (0.0228 g, 0.0325 mmol) was co-evaporated from 100 µL DMF (to remove trace water) and brought up in a final volume of 165 µL DMF. Diphenyl phospho-

chloridate (0.0131 g, 0.0488 mmol) was added, followed immediately by tributylamine (0.0120 g, 0.0650 mmol). After stirring for 3 h, the solvent was evaporated *in vacuo*. The resulting residue was chilled to 0°C and 1 mL ether was added with shaking to precipitate the desired product. After 30 min, the ether was removed by decantation and the remaining precipitate was co-evaporated from DMF (100 µL). Upon drying under vacuum, 65 µL DMF was added to the activated AMP and 2-*O*-acetyl-D-ribofuranose 5-hydrogen phosphate (TEA salt) (**8**) (0.0243 g, 0.0650 mmol) in 165 µL DMF was added, followed immediately by 510 µL pyridine. After stirring for 1 d, the solvent was evaporated off and dried under vacuum. The crude material was dissolved in 40 mL 10 mM NH_4OAc (pH 4.8) and loaded to a column of DEAE-cellulose (Whatman DE52) (2.5 x 50 cm); after washing with 50 mL of buffer, a linear gradient was formed between 10 and 500 mM NH_4OAc (250 mL each) and fractions were collected (10 mL). An additional 50 mL 500 mM NH_4OAc was passed over the column. The absorbance was measured at 260 nm and fractions containing the desired product were combined and lyophilized. **1** eluted in the first major band of material (the second band of material contained diadenosine 5'-pyrophosphate [AppA]). The resulting material contained contaminants which were removed by rechromatography with preparative HILIC, followed by preparative C18 (flow rate of 1 mL/min was utilized). The peaks corresponding to the two isomers were collected and lyophilized after each column to give the desired product[6] (2-*O*AADPr: 0.660 mg; 3-*O*AADPr: 0.130 mg; 4.1% overall): Analytical HPLC (HILIC) retention time = 21.4 min; analytical HPLC (C18) retention time = 16.0, 17.8 min. HRMS-ESI: calcd for $C_{17}H_{24}N_5O_{15}P_2^-$ $(M - H)^-$, 600.0750, obsd 600.0728.

2'-*N*-Acetyl-ADP-ribose (**2**): A procedure very similar to that described for **1** was carried out containing the tri-*n*-octylammonium salt of AMP (0.0254 g, 0.00363 mmol) with **19** (0.0270 g, 0.0725 mmol). The crude material was dissolved in 40 mL 10 mM NH_4HCO_3 (pH 8) and loaded to a column of DEAE-cellulose

(Whatman DE52) (2.5 x 50 cm); after washing with 50 mL 10 mM NH_4HCO_3, a linear gradient was formed between 10 and 500 mM NH_4HCO_3 (250 mL each) and fractions were collected (10 mL). An additional 50 mL 500 mM NH_4HCO_3 was passed over the column. The absorbance was measured at 260 nm and fractions containing the desired product were combined and lyophilized. **2** eluted in the first band of material (the second band of material contained diadenosine 5'-pyrophosphate [AppA]). The desired product contained contaminants (including AppA) which were removed by rechromatography with preparative HILIC, followed by preparative C18. The peak corresponding to the desired product was collected and lyophilized after each column to give the desired product (**2**) as a white flocculent powder (0.0046 g, 21.0%): Analytical HPLC (HILIC) retention time = 23.1 min; analytical HPLC (C18) retention time = 15.4 min. A combination of *N*-Acetyl rotomers and α and β anomers (1:1 ratio) were observed by NMR. ^{1}H NMR [distinct signals for the anomeric position (C-1') and acetyl could be discerned] (D_2O) δ 8.62 (s, 1H), 8.41 (s, 1H), 6.14 (d, J= 5.3 Hz, 1H), 5.45-5.41 (m, 0.5H), 5.25-5.22 (m, 0.5H), 4.74-4.70 (m, 1H), 4.54-4.50 (m, 1H), 4.40-4.37 (m, 1H), 4.34-4.30 (m, 1H), 4.28-4.20 (m, 3H), 4.20-4.15 (m, 1H), 4.12-4.08 (m, 1H), 4.05-4.02 (m, 1H), 2.04, 2.03, 2.00 (s, s, s, 3H); ^{13}C NMR [multiple signals were observed for each carbon due to the combination of rotomers/anomers] (D_2O) δ 177.2, 177.0, 176.8, 165.7, 165.4, 165.2, 152.5, 150.8, 147.4, 144.8, 121.0, 120.8, 120.1, 117.8, 115.4, 104.4, 102.7, 99.3, 98.2, 90.5, 86.8, 86.7, 86.44, 86.38, 84.94, 84.87, 84.8, 84.6, 82.03, 81.99, 81.9, 81.8, 77.3, 76.7, 72.8, 72.4, 72.3, 72.0, 70.1, 69.0, 68.8, 68.4, 67.7, 65.1, 60.3, 56.5, 54.4, 54.0, 24.5, 24.44, 24.42, 24.40; ^{31}P NMR (D_2O) δ -10.4 (m). HRMS-ESI: calcd for $C_{17}H_{25}N_6O_{14}P_2^-$ $(M - H)^-$, 599.0909, obsd 599.0875.

3'-*N*-Acetyl-ADP-ribose (**3**): A procedure very similar to that described for **1** was carried out containing the tri-*n*-octylammonium salt of AMP (0.0295 g, 0.0421 mmol) with **20** (0.0313 g, 0.00841 mmol). Purification of the desired product was carried out utilizing the same gradients as performed for **2**. The peak

corresponding to the desired product after preparative HPLC was collected and lyophilized to give the desired product (**3**) as a white flocculent powder (0.0054 g, 21.5%): Analytical HPLC (HILIC) retention time = 23.9 min; analytical HPLC (C18) retention time = 15.0 min. A mixture of α and β anomers (1:3 ratio) were observed by NMR. ^{1}H NMR [distinct signals for the anomeric position (C-1') could be discerned] (D_2O) δ 8.62 (s, 1H), 8.40 (s, 1H), 6.15 (d, J= 5.4 Hz, 1H), 5.41 (d, J= 3.6 Hz, 0.25H), 5.25 (s, 0.75H), 4.74 (t, J= 5.2 Hz, 1H), 4.53 (t, J= 4.4 Hz, 1H), 4.41-4.37 (m, 1H), 4.34-4.30 (m, 1H), 4.27-4.19 (m, 3H), 4.17-4.13 (m, 1H), 4.12-4.06 (m, 1H), 4.03-3.92 (m, 1H), 2.00 (s, 3H); ^{13}C NMR [distinct signals for the α and β anomers could be discerned for multiple carbons] (D_2O) δ 177.1, 165.8, 165.5, 152.9, 151.1, 147.9, 144.9, 122.5, 120.2, 117.8, 115.5, 104.4, 99.4, 90.6, 86.8, 82.1, 81.9, 77.3, 76.8, 72.9, 72.4, 70.2, 68.8, 67.7, 54.5, 54.0, 24.6, 24.5; ^{31}P NMR (D_2O) δ -10.4 (m). HRMS-ESI: calcd for $C_{17}H_{25}N_6O_{14}P_2^-$ $(M - H)^-$, 599.0909, obsd 599.0886.

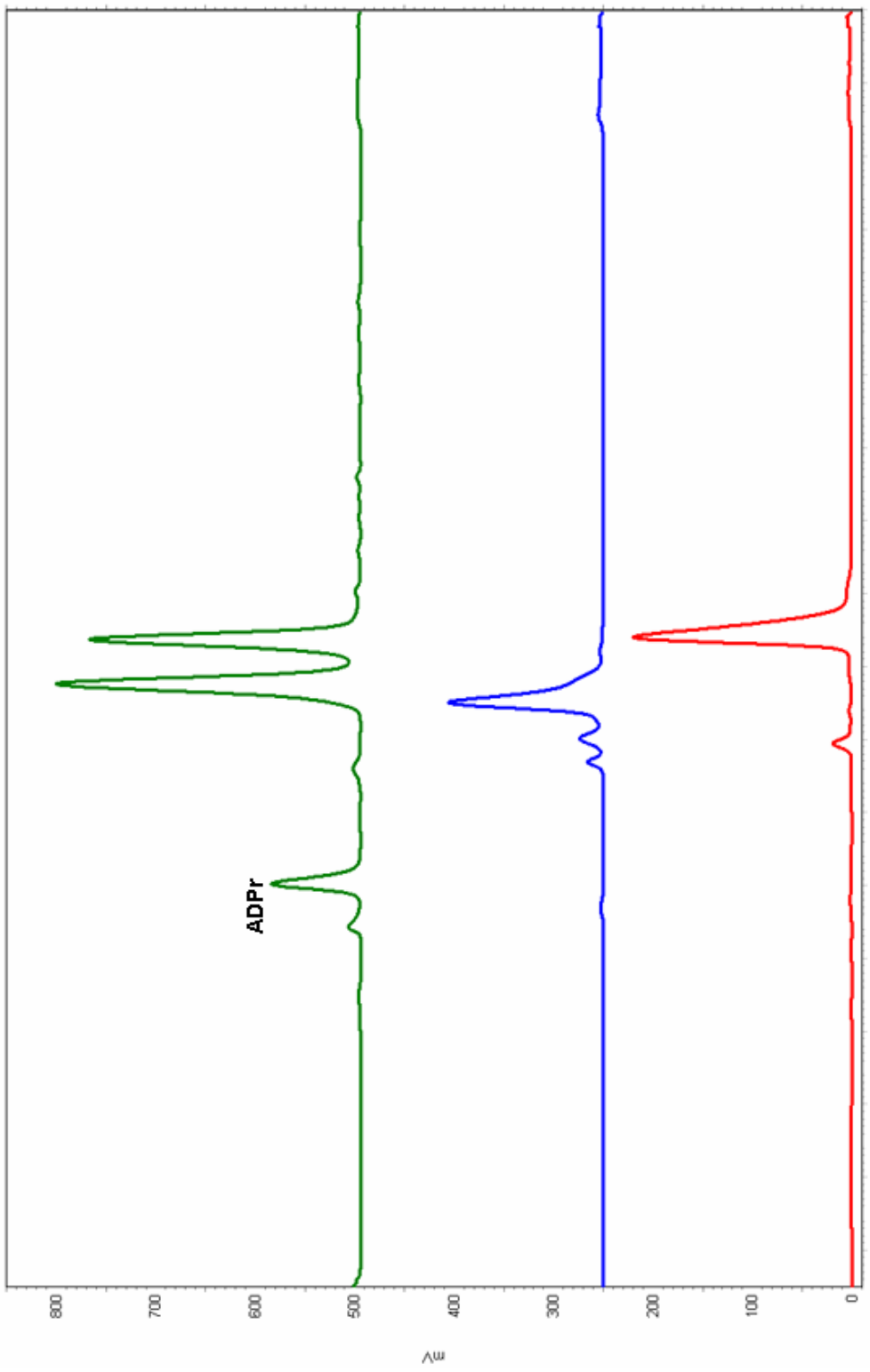
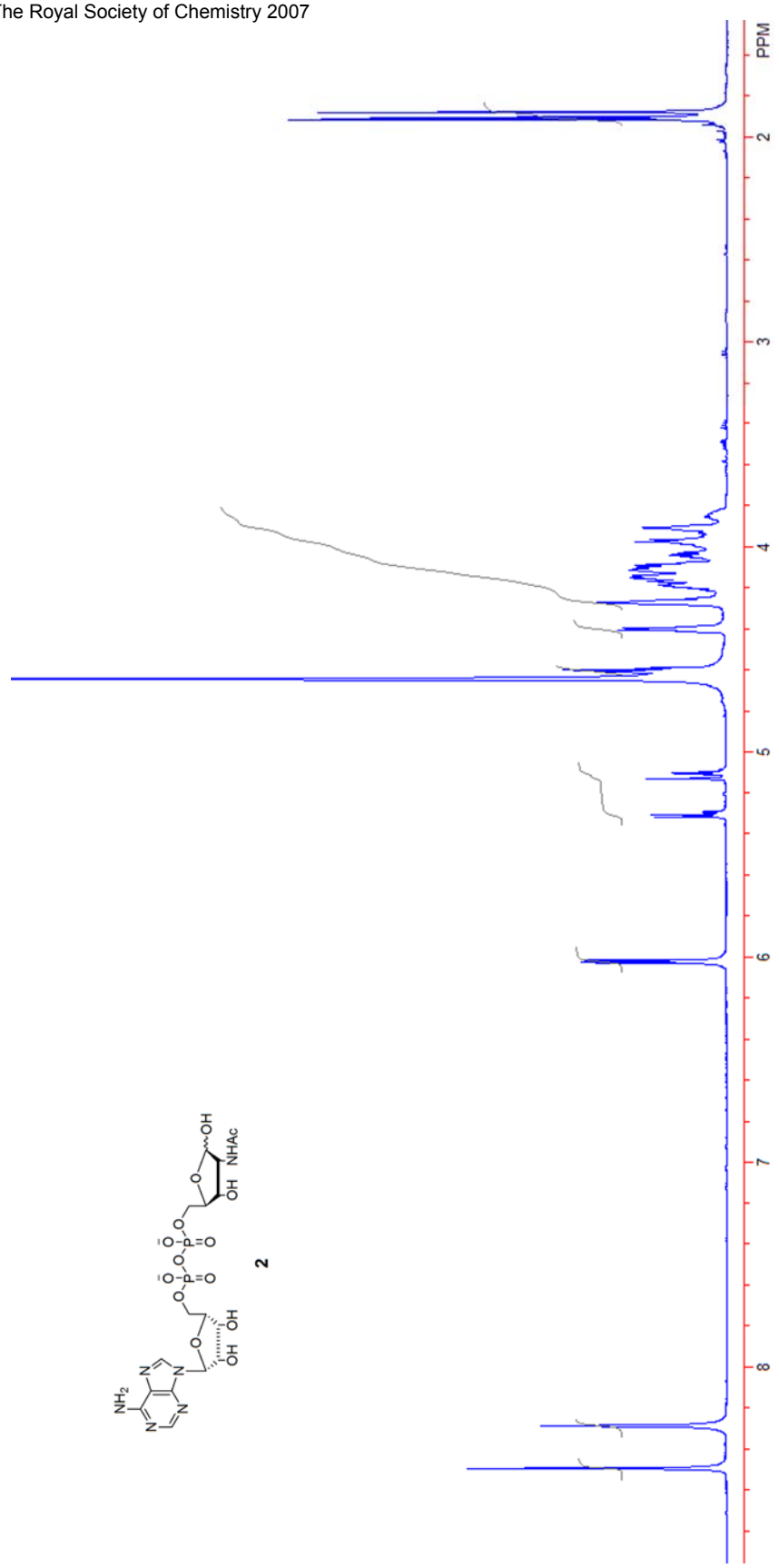
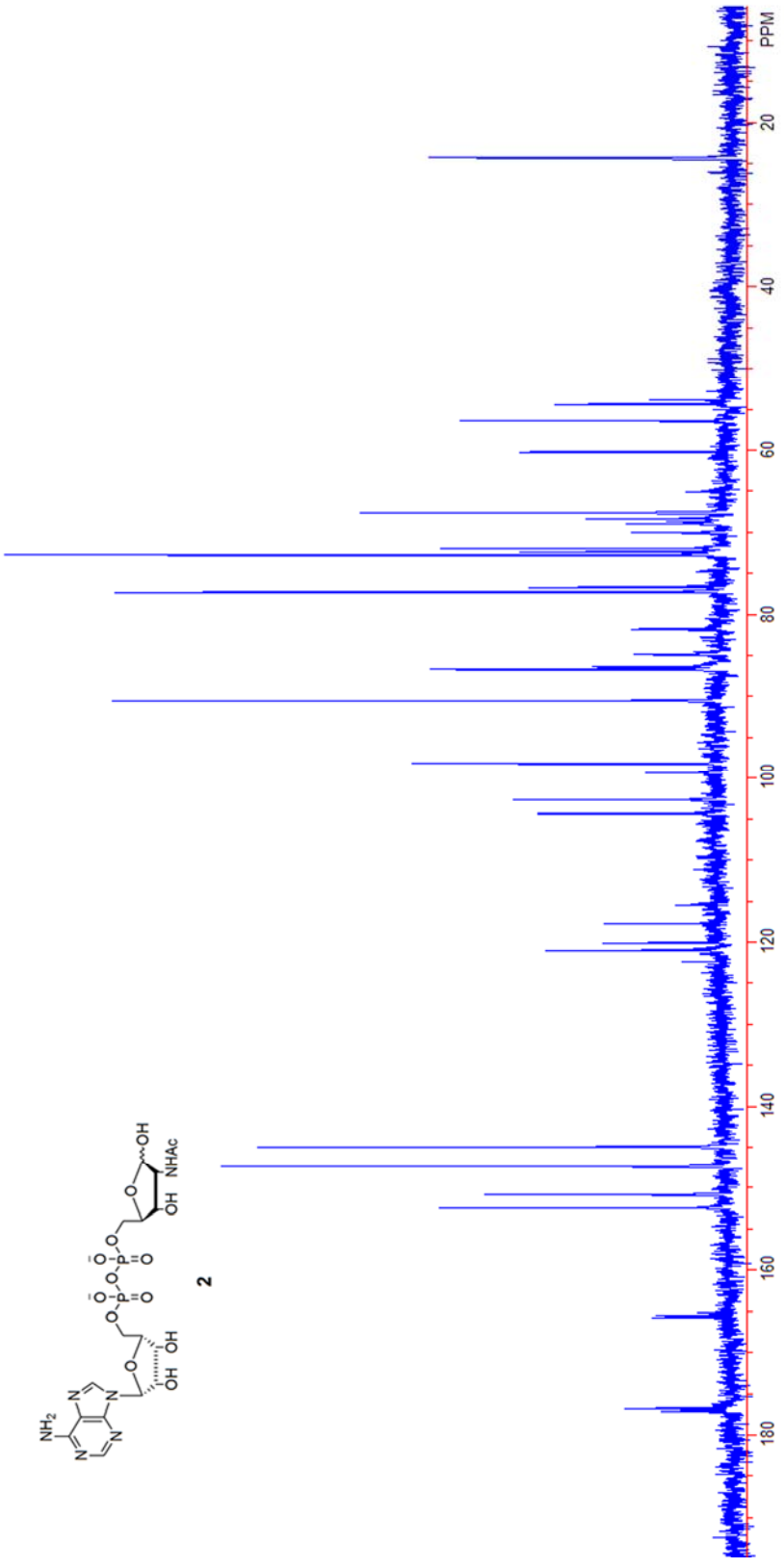
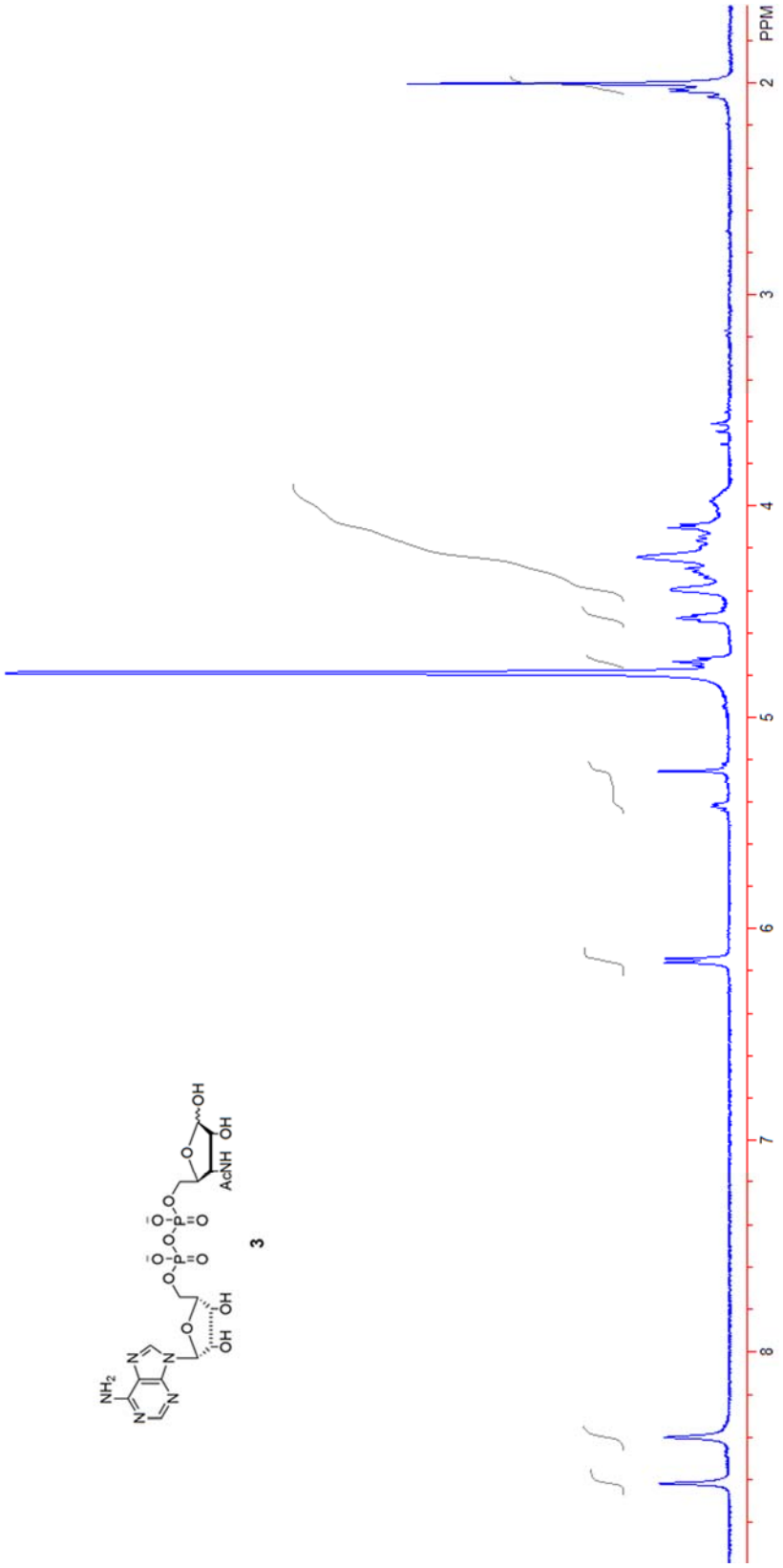
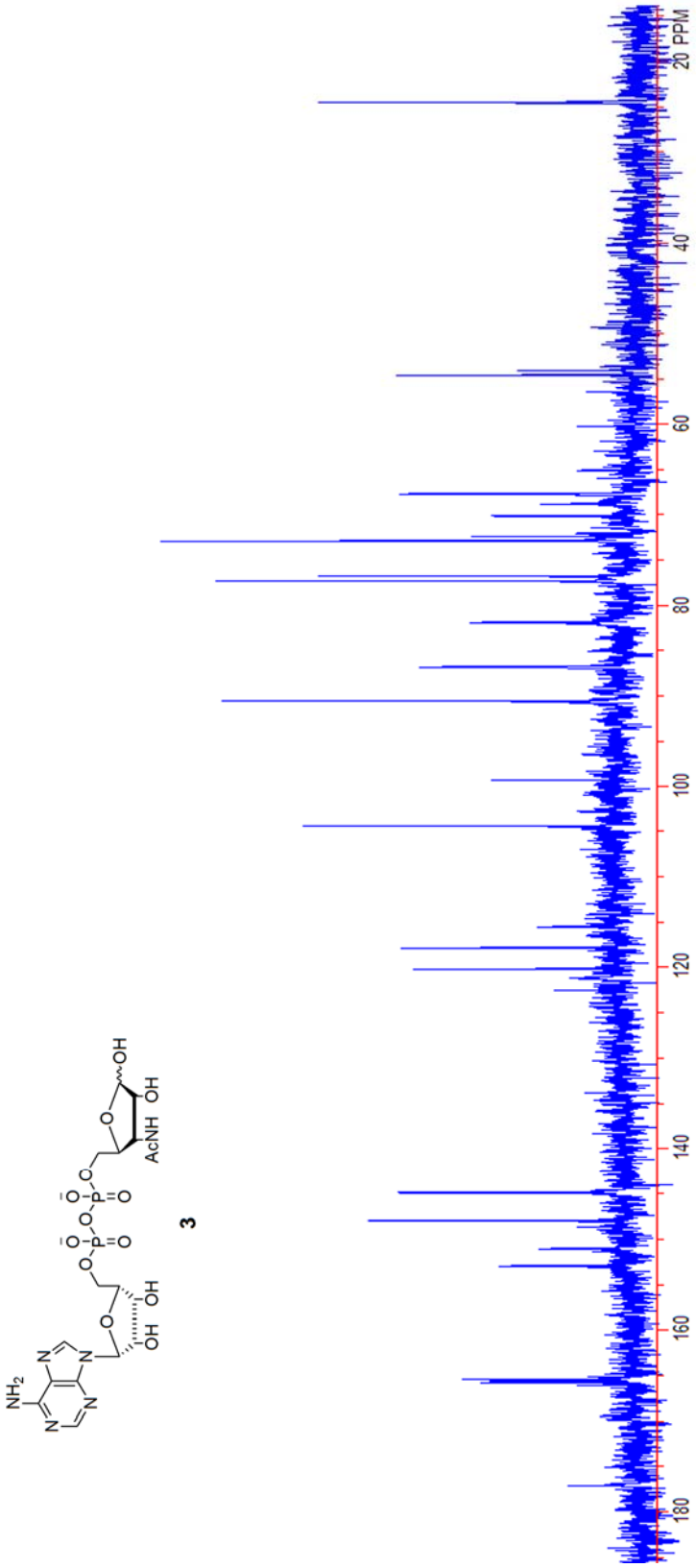


Figure S1. HPLC of 1 compared to *O*AADPr. Purified 2-OAADPr (red) and 3-OAADPr (blue) from the condensation of **21** and **8** are compared versus enzymatically generated OAADPr (green) on C18. HPLC method follows that described in general procedures.


PPM
2
3
4
5
6
7
8
NHAc
OH
OH
OH
OH
NH2
2


PPM
20
40
60
80
100
120
140
160
180
NHAc
OH
NH2
2


PPM
2
3
4
5
6
7
8
AcNH
OH
NH2
3


20 PPM
40
60
80
100
120
140
160
180
AcNH
OH
NH2
3

HPLC Stability Studies:

To validate the installation of an *N*-acetyl as a non-hydrolyzable substitution, the stabilities of both 2'- and 3'-*N*AADPr were tested under conditions previously described by our laboratory.[11] Reactions were carried out with both analogs (final concentration of 500 μM) in 50 mM Tris (pH 7.5 @ 37°C) containing 1 mM DTT. Samples were incubated at 37°C over 3 days and four time points were collected (0, 24, 48, and 72 h). Each sample was quenched with H_2O containing 0.05% TFA and analyzed by HPLC (analytical C18) using the method described in general procedures. Each trace was integrated (Shimadzu EZStart version 7.2.1 SP1) to determine peak area and analog percentage is shown in the following table:

	0 h	24 h	48 h	72 h
2'-*N*AADPr	98.7%	98.7%	98.2%	98.3%
3'-*N*AADPr	95.7%	98.2%	98.0%	98.0%

* Both 2'- and 3'-*N*AADPr contained trace impurities at 12.9 and 12.5 min respectively and contributed to the observed degradation products visible at 23.3 min in each study.

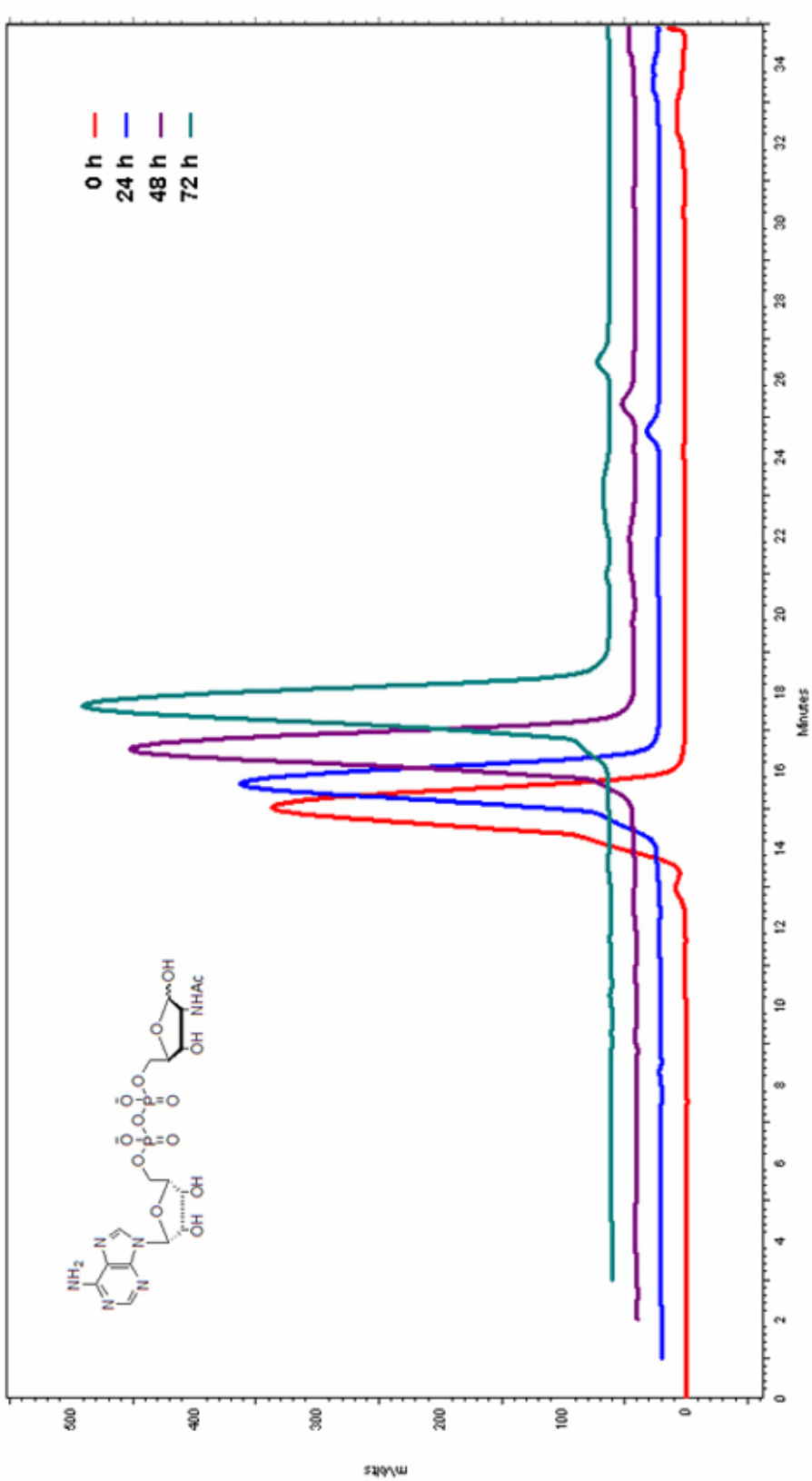


Figure S2. Time course stability analysis of 2-*N*AADPr. 2-NAADPr (500 μM final concentration) was incubated at 37°C and time points were collected (0h, red; 24h, blue; 48 h, purple; 72h, teal). HPLC (C18) method follows that described in general procedures.

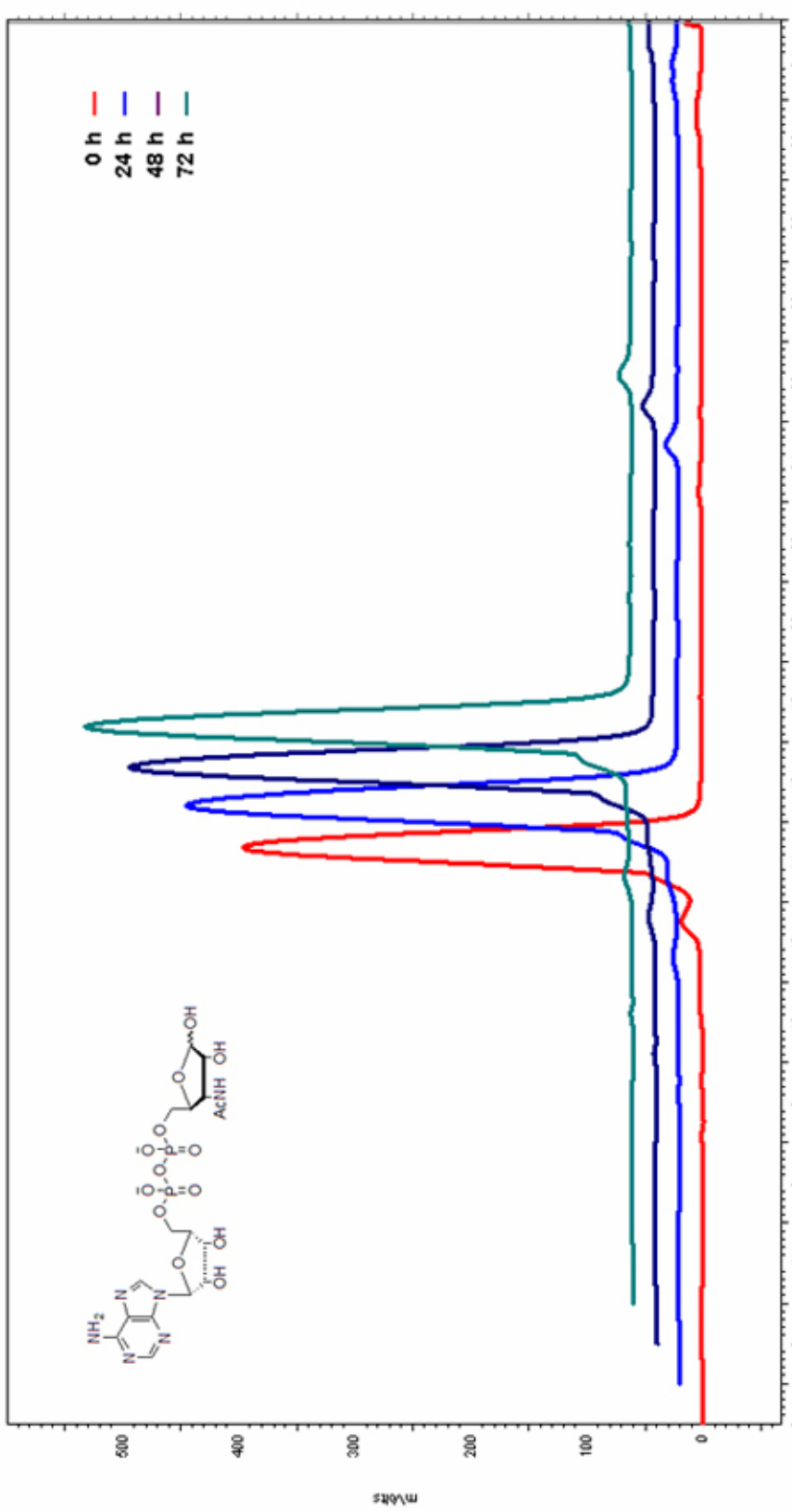


Figure S3. Time course stability analysis of 3-NAADPr. 3-NAADPr (500 µM final concentration) was incubated at 37°C and time points were collected (0h, red; 24h, blue; 48 h, purple; 72h, teal). HPLC (C18) method follows that described in general procedures.

MacroH2A Binding Studies

MacroH2A1.1 overexpression and purification (plasmid kindly provided by A. Ludurner, European Molecular Biology Laboratory) was carried out using standard protocols.[12] Protein quantification was performed with the Bradford assay using BSA as the standard.[12]

Binding assays were performed using isothermal titration calorimetry (ITC) on a VP-ITC instrument (MicroCal) at 25°C.[13] Proteins were dialyzed into ITC buffer (50 mM KH_2PO_4, pH 6.5, 1 mM DTT) overnight prior to use. ADPr, *O*AADPr, 2'-*N*AADPr, and 3'-*N*AADPr were suspended in ITC buffer and quantitated by absorbance at 260 nm (15,400 OD $M^{-1}cm^{-1}$). mH2A1.1 *macro* domain protein concentration was 38-53 μM and ligand concentrations ranged from 380-440 μM. The resulting raw binding heats were converted, after background heat correction, to binding enthalpies (ΔH) and dissociation constants (K_d) using the Origin software (Originlab, USA).

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7. **9** was prepared using procedures similar to **15**. Spectral data was in agreement with that described in: (a) Z. G. Gao, H. T. Duong, T.Sonina, S. K. Kim, P. V. Pompaey, S. Van Calenbergh, L. Mamedova, H. O. Kim, M. J.

Kim, A. Y. Kim, B. T. Liang, L. S. Jeong, K. A. Jacobson, *J. Med. Chem.,* 2006, **49**, 2689; (b) H. H. Baer, Y. Gan, *Carbohydr. Res.,* 1991, **210**, 233.

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9. VT NMR experiments were performed (^{1}H, 500 MHz) and the mutliplicity of the anomeric hydrogens were evaluated as a function of an increase in temperature. Compared to the complex multiplets observed at 25°C, the peak shape for the two observed anomeric hydrogen resonances were broadened significantly with an increase in temperature (at both 40 and 60°C). No $\Delta\delta$ between the two peaks were observed. From this data, it can be concluded that a combination of *N*-Acetyl rotomers and α and β anomers is observed by NMR (^{1}H and ^{13}C). *Personal communication*, Hans J. Reich (University of Wisconsin-Madison).
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