Supporting Information

Materials and Methods

Commercial reagents were used without further purification. High-resolution mass spectrometry was performed at the Advanced Instrumentation for Molecular Structure (AIMS) facility at the University of Toronto. HPLC analysis was performed using a C18 reversed phase semi-preparative (250mm x 10 mm) and analytical (250mm x 4.6 mm) columns. Ribonucleoside reactions were eluted with 10% acetonitrile (HPLC grade) and 0.1% trifluoroacetic acid (TFA) in deionized water, and ribonucleotide reactions with 8% acetonitrile (HPLC grade) and 0.1% TFA in deionized water. The flow rate was 3.0 mL/min for semi-preparative column, and 1.0 mL/min for analytical column at room temperature. Eluting species were detected at 263 nm.

Synthesis

Bis(tetraethylammonium salt) ethyl phosphate

Ethyl dichlorophosphate was added to a ten-fold excess of water in an ice-cooled round-bottom flask over ten minutes. The reaction mixture was stirred for one hour, and hydrochloric acid generated as by-product was removed by rotary evaporation. The resulting ethyl phosphoric acid was neutralized with two equivalents of tetraethylammonium hydroxide (35% w/w solution) and freeze-dried to yield a white paste. (80% yield)

\[ \text{1H NMR (300 MHz, D}_2\text{O): } \delta 3.74 (2H, m, OCH}_2\text{CH}_3), 2.98 (16H, q, +N(CH}_2\text{CH}_3)_4), 1.20 – 1.10 (27H, m, +N(CH}_2\text{CH}_3)_4, OCH}_2\text{CH}_3) \]

\[ \text{31P NMR (121 MHz, D}_2\text{O): } \delta 1.68 \]

\[ \text{α-N-t-BOC-phenylalanyl ethyl phosphate (BOCPheEP)} \]

1. DCC (1.3 eq.)

\[ \text{2. CH}_2\text{Cl}_2 \]

\[ \text{α-N-t-Boc-phenylalanine (2 eq) was first activated with dicyclohexylcarbodiimide (DCC, 2 eq) in dry dichloromethane for three minutes. Bis(tetraethylammonium) ethyl phosphate (1.0 eq), pre-dissolved in dry dichloromethane, was added, and stirred for 1 hour at room temperature. Dicyclohexylurea (DCU) precipitated as white solid and the solution was filtered. The products were extracted with water. The solution was freeze-dried to yield a colorless, glassy solid (80 – 85% yield), which was used without further purification.} \]
\( ^1H \text{NMR (300 MHz, D}_2\text{O):} \delta \text{ 7.2-7.3 (5H, m, Ar), 4.5 (m, 1H, CHCO), 4.0 (2H, quintet, ArCH}_2\text{), 3.3 (10H, m, POCH}_2\text{CH}_3\text{), }^3\text{N(CH}_2\text{CH}_3\text{)}_4\text{), 1.3 (9H, s, C(CH}_3\text{)}_3\text{), 1.2 (15H, m, POCH}_2\text{CH}_3\text{, }^3\text{N(CH}_2\text{CH}_3\text{)}_4\text{)}\)

\( ^3P \text{NMR (121 MHz, D}_2\text{O):} \delta \text{ -6.14} \)

\text{MS-ESI (-): calculated m/z 372.1230, found m/z 372.1217}

Deprotection of \( \alpha\)-\( N\)-t-BOC-phenylalanyl ethyl phosphate (PheEP)

\( \alpha\)-\( N\)-t-BOC-phenylalanyl ethyl phosphate was dissolved in a minimum amount of trifluoroacetic acid (TFA) in a flask that had been flushed with dry nitrogen. The reaction was stirred until the bubbling from evolved carbon dioxide ceased. Excess TFA was removed under vacuum and the resulting oil was precipitated with dry cold acetone. Isolation by vacuum filtration produced PheEP as solid white powder (30% yield).

\( ^1H \text{NMR (300 MHz, D}_2\text{O):} \delta \text{ 7.3 (5H, m, Ar), 4.4 (1H, CHCO), 3.8 (2H, quintet, POCH}_2\text{CH}_3\text{), 3.2 (2H, m, ArCH}_2\text{), 2.1 (2H, s, NH}_2\text{), 1.1 (3H, t, POCH}_2\text{CH}_3\text{)}\)

\( ^3P \text{NMR (121 MHz, D}_2\text{O):} \delta \text{ -6.48} \)

\text{MS-ESI (-): calculated m/z 272.0693, found m/z 272.0699}

\text{HPLC Analysis}

![HPLC Chromatogram](image)

HPLC chromatogram for the lanthanum-catalyzed reaction of adenosine with PheEP to give two Phe-monoesters of adenosine. (HRMS: calculated m/z = 415.1724, found m/z = 415.1711, 415.1720) Yield of ester products is approximately 60%.

\text{Reaction conditions: [adenosine] = [PheEP] = [La(OTf)}_3\text{] = 10 mM in pH 6 MES buffer (100 mM) at 25°C.}
HPLC chromatogram for the reaction of adenosine with PheEP in the absence of lanthanum. \([\text{adenosine}] = [\text{PheEP}] = 10 \text{ mM} \) in pH 6 MES buffer (100 mM) at 25°C.

HPLC chromatogram for the lanthanum-catalyzed reaction of cytidine with PheEP to give two Phe-monoesters of cytidine. (HRMS: calculated m/z = 391.1612, found m/z = 391.1599, 391.1605) Yield of ester products is approximately 70%.

**Reaction conditions:** \([\text{cytidine}] = [\text{PheEP}] = [\text{La(OTf)}_3] = 10 \text{ mM} \) in pH 6 MES buffer (100 mM) at 25°C.
HPLC chromatogram for the reaction of cytidine with PheEP in the absence of lanthanum. [cytidine] = [PheEP] = 10 mM in pH 6 MES buffer (100 mM) at 25°C.

HPLC chromatogram for the reaction of uridine with PheEP in the absence of lanthanum. [uridine] = [PheEP] = 10 mM in pH 6 MES buffer (100 mM) at 25°C.
HPLC chromatogram for the lanthanum-catalyzed reaction of 2'-deoxycytidine with PheEP. [2'-deoxycytidine] = [PheEP] = [La(OTf)$_3$] = 10 mM in pH 6 MES buffer (100 mM) at 25°C.

HPLC chromatogram for the lanthanum-catalyzed reaction of 5'-AMP with PheEP to give Phe-monoesters of 5'-AMP. (HRMS: calculated m/z = 493.1242, found m/z = 493.1197, 493.1263)

**Reaction conditions:** [5'-AMP] = [PheEP] = [La(OTf)$_3$] = 10 mM in pH 6 MES buffer (100 mM) at 25°C.
HPLC for the lanthanum-catalyzed reaction of 5’-CMP with PheEP to give Phe-monoesters of 5’-CMP. (HRMS: calculated m/z = 469.1129, found m/z = 469.1143, 469.1156)

**Reaction conditions:** \([5’-\text{CMP}] = [\text{PheEP}] = [\text{La(OTf)}_3] = 10 \text{ mM} \) in pH 6 MES buffer (100 mM) at 25°C.
Spectral data for phenylalanyl monoester of uridine

HRMS: calculated m/z = 392.1452, found m/z = 392.1454, 392.1444

\( ^1H \text{ NMR (400 MHz, CD}_3\text{OD):} \) \( \delta \) 8.03 (1H, d, C7-H), 7.92 (1H, d, C7'-H), 7.31 – 7.42 (5H, m, aromatic), 6.06 (1H, d, C1-H), 5.89 (1H, d, C1'-H), 5.74 (1H, d, C6'-H), 5.73 (1H, d, C6-H), 5.40 (2H, dd, C11-H), 5.31 (2H, dd, C11'-H), 4.42 – 4.48 (2H, m, C2-H, C2'-H, C3-H, C3'-H), 3.87 – 3.89 (1H, m, C4-H, C4'-H), 3.64 – 3.78 (2H, m, C5-H, C5'-H)
$^{13}$C NMR (100 MHz, CD$_3$OD): $\delta$ 165.9 (C10, C10'), 142.8 (C6), 142.2 (C6'), 129.0 – 130.6 (aromatic), 103.5 (C7'), 102.7 (C7), 90.8 (C1), 89.0 (C1'), 86.4 (C2), 84.2 (C2'), 76.8 (C3'), 75.8 (C3), 73.9 (C4), 71.4 (C4'), 62.6 (C5'), 62.3 (C5), 55.3 (C11), 55.2 (C11'), 37.6 (C12, C12')
ESI-MS (TOF +) spectrum of PheEP polymerization in 0.25 M, pH 8 HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer

Exact Mass: 294.14

Exact Mass: 312.15

Exact Mass: 459.22

Exact Mass: 606.28