Supporting Information

A new and convenient approach for the preparation of β-cyanoethyl protected trinucleotide phosphoramidites

Matthäus Janczyk, a,b Bettina Appel, a Danilo Springstubbe, a Hans-Joachim Fritz c and Sabine Müller

Ernst-Moritz-Arndt-Universität Greifswald
Institut für Biochemie
Felix-Hausdorff-Str. 4
17487 Greifswald, Germany
Fax: 49 3834 864471
Tel: 49 3834 8622843

E-mail: smueller@uni-greifswald.de
Index

Contents of Supporting Information page #
General information ........................................................................................................ 3
1. N-acylation.................................................................................................................. 3
2. 5'-Dimethoxytritylation .............................................................................................. 4
3. 3'-Silylation ................................................................................................................ 4
4. Removal of the 5'-DMT group .................................................................................... 4
5. Nucleoside Phosphoramidite .................................................................................... 5
6. Coupling reaction I ..................................................................................................... 5
7. Removal of the 5'-DMT group .................................................................................... 5
8. Coupling reaction II .................................................................................................. 6
9. Deprotection of the 3'-OH-group .............................................................................. 6
10. Trinucleotide phosphoramidite ................................................................................. 6
Analytical data of 5'-O-DMT 3'-OH-trinucleotides .................................................... 7
Table S1. MALDI data and yields of dinucleotide compounds .................................... 12
Table S2. MALDI data and yields of trinucleotide compounds .................................... 13
Table S3. MALDI data of hexamers ............................................................................. 14
Figure S1. HPLC diagrams ........................................................................................ 15
Literature ....................................................................................................................... 16
Appendix: $^{31}$P NMR of trinucleotide compounds ...................................................... 17
**General information**

Dry methylene chloride, dry methanol and dry tetrahydrofuran were obtained from Fluka. Pyridine was dried overnight over KOH, heated to reflux for 4 h, distilled off and stored over molecular sieve. Diiisopropyl ethyl amine was stored over calcium hydride and distilled before use. All other reagents, chemicals, buffers and solvents were obtained as the highest commercially available grade. Solvents used for column chromatographie were freshly distilled. Other chemicals were used without further purification. Reactions were carried out at room temperature unless stated otherwise. Silica gel for column chromatography (0.063-0.2 mm) was obtained from Sigma-Aldrich. All products were visualized on TLC plates (Macherey-Nagel Fertigfolien Alugram Sil G/UV254) at 254 nm ultraviolet light. Reversed-phase HPLC were performed on an ÄKTA Purifier (GE Healthcare); Column EC 250/4 Nucleodur 100-5 C18 ec (Macherey-Nagel), flow rate 1 ml/min, Buffer A 5% acetonitrile in deionized water, Buffer B 70% acetonitrile in deionized water. NMR spectra were recorded with TMS as the internal standard on Bruker Avance 300 MHz. Mass spectra were recorded on a Bruker Microflex MALDI-TOF, VG Autospec (FAB), and ESI Bruker Esquire LC (Ion Trap), respectively. UV measurements were obtained from an Ultrospec 2100 pro (Amersham Biosciences). The hexamers were synthesized by the phosphoramidite method on a DNA-synthesizer (Gene Assembler Special, Pharmacia) at 0.2 µmol scale as described elsewhere.\(^1\) Standard PAC-phosphoramidites as well as CPG supports were obtained from ChemGenes or Link Technologies. BMT (emp Biotech) was used as activator.\(^2\)

1. **N-acylation**\(^3\)

10 mmol of either dA, dG or dC were coevaporated twice with 50 ml dry pyridine in a 500 ml round-bottom flask and finally suspended in 100 ml dry pyridine. After cooling down in an ice-bath 5 ml trimethylchlorosilane (approximately 5 equivalents) were added slowly. After 30 minutes 5 equivalents of the acylation reagent were added (8.5 ml isobutyric anhydride for dG and dA, 6 ml benzoyl chloride for dC). The solution was stirred for 2 h. The reaction was stopped by adding 20 ml cold water to the solution followed by 20 ml of concentrated ammonia. The reaction solution was
kept in an ice-bath for 30 minutes. After concentration in vacuo the resulting oil was dissolved in 50 ml water. The aqueous layer was washed with small amount of ether. In the case of dG crystallization from water begins within minutes. N-acylated dA and dC were purified from anorganic salts by silica chromatography (CH₂Cl₂:MeOH 80:20). Rf-Wert (CH₂Cl₂:MeOH 8:2): 0.5.

2. 5'-Dimethoxytritylation³

10 mmol of N-acylated desoxyribonucleoside (or thymidine) were coevaporated twice with 50 ml dry pyridine and dissolved in 100 ml dry pyridine. Dimethylaminopyridine (DMAP) (61 mg, 0.05 mmol) and dry triethylamine (TEA) (2 ml, 15 mmol) were added. After adding dimethoxytritylchloride (4.7 g, 15 mmol) the solution was stirred for 4 h. The reaction was stopped by adding 200 ml water. The product was extracted with ether. The ether was removed in vacuo and the crude product was purified via silica gel chromatography (CH₂Cl₂:MeOH = 99:1→95:5). Rf (CH₂Cl₂:MeOH 9:1): 0.5.

3. 3'-Silylation⁴

5 mmol of 5'-dimethoxytritylated desoxynucleoside were dissolved in 20 ml anhydrous tetrahydrofuran and 3 ml anhydrous pyridine. AgNO₃ (2.2 g, 1.3 equivalents) and TBDMSCl (2 g, 1.3 equivalents) were added and the mixture was stirred overnight. After adding saturated NaHCO₃-solution the crude compound was isolated with dichloromethane. After washing with sat. aq. NaHCO₃ the organic layer was dried with sodiumsulfate. After removing the solvents in vacuo the silylated nucleoside was used for further synthesis without any purification. Rf (CH₂Cl₂, MeOH 95:5): 0.8.

4. Removal of the 5'-DMT group⁵

5 mmol of 3'-silylated-5'-tritylated desoxyribonucleoside were dissolved in 100 ml of dichlormethane containing 3% trichloracetic acid. The mixture was stirred for 10 minutes and poured into saturated NaHCO₃ in a separating funnel. After washing the organic phase with sat. aq. NaHCO₃ the organic layer was dried with sodiumsulfate. The solvent was removed in vacuo, the product was purified via silica gel chromatography. Rf-Wert (CH₂Cl₂:MeOH 9:1): 0.5.
5. Nucleoside Phosphoramidite

1 mmol of the 5'-DMT protected desoxynucleoside was coevaporated twice with 5.5 ml of a mixture of dry dichloromethane/dry pyridine (20:1) and dissolved in 10 ml dry dichloromethane. 1 ml freshly distilled anhydrous diisopropylethylamine was added to the mixture. 1.2 equivalents of 2-cyanoethyl-N,N-diisopropylchlorophosphoramidit were slowly added while stirring. After five minutes 1 ml anhydrous methanol, five minutes later 4 ml triethylamine and finally 10 ml ethylacetate were added. The mixture was washed twice with sat. aq. NaHCO₃ and twice with brine. The organic layer was dried with sodiumsulfate and the solvent was removed in vacuo. The crude product could be used for coupling reactions without further purification. Rf-Wert (CH₂Cl₂ : ethyl acetate: triethylamine 45:45:10): 0.9 (Desoxyguanosin: 0.4).

6. Coupling reaction I

1 mmol nucleoside phosphoramidite and 0.8 mmol 5'-deprotected nucleoside were dissolved in 10 ml anhydrous acetonitrile. Tetrazole (10 mmol) was added. After 30 minutes a 0.1 M solution of iodine in lutidine/tetrahydrofuran/water 1:2:1 was added to the mixture until no decolourization of the mixture was detectable. The solvents were removed in vacuo, the remaining brown foam was dissolved in 50 ml dichlormethane. The organic layer was washed with 50 ml 6% sodiumbicarbonate, then with 50 ml sat. aq. NaHCO₃ and with 50 ml water. After the organic layer was dried with Na₂SO₄ the solvent was removed in vacuo. The dinucleotide was purified by silica gel chromatography (CH₂Cl₂:MeOH = 9:1, Rf = 0.6). (See table S1 for yields and MALDI results.)

7. Removal of the 5'-DMT group

The dinucleotide was dissolved in 10 ml dichlormethane containing 3 % of trichloracetic acid. The mixture was washed with saturated NaHCO₃-solution. The organic layer was dried with Na₂SO₄ and the solvent was removed in vacuo. The product was purified by silica gel chromatography (CH₂Cl₂: MeOH = 9:1; Rf = 0.4). (See table S1 for yields and MALDI results.)
8. Coupling reaction II\(^5\)

1.2 mmol of the desoxyribonucleoside phosphoramidite were dissolved in 10 ml anhydrous acetonitrile. 1 mmol of the 5' unprotected dinucleotide was added to the mixture. After two minutes tetrazole (10 mmol) was added, the mixture was stirred for another 1 h. A 0.1 M solution of iodine in lutidine/tetrahydrofuran/water 1:2:1 was given to the mixture until no decolourization of the iodine solution was detectable. The solvents were removed in vacuo, and the remaining brown foam was dissolved in 50 ml dichlormethane. The organic layer was washed with 50 ml 6% sodiumbicarbonate solution, 50 ml saturated NaHCO\(_3\)-solution and 50 ml water. The organic layer was dried with Na\(_2\)SO\(_4\) and the solvents were removed in vacuo. The trinucleotide was purified by silica gel chromatography (CH\(_2\)Cl\(_2\):MeOH 95:5; R\(_f\) = 0.7). (See table S2 for yields and MALDI results.)

9. Deprotection of the 3'-OH-group\(^5\)

0.5 mmol of the 3'-TBDMS protected trinucleotide was dissolved in 10 ml of anhydrous dimethylformamide. 0.25 ml of TEA/3xHF was added. The mixture was stirred over night until no starting material was detectable on TLC. The mixture was diluted with 50 ml saturated NaHCO\(_3\), the product was extracted with 30 ml dichlormethane. The organic layer was dried with Na\(_2\)SO\(_4\), the solvents were removed in vacuo and the 3'-unprotected trinucleotide was purified by RP-HPLC (for conditions see General Information, page S3). The yield was virtually quantitative in all cases. The purity of all compounds was controlled and confirmed by thin layer chromatography and in some cases additionally by HPLC. The identity of all compounds was confirmed by MALDI mass spectrometry. (see experimental data in next chapter and figure S1a and S1b).

10. Trinucleotide phosphoramidite\(^3\)

0.5 mmol of the 3'-unprotected trinucleotide were dissolved in 5 ml anhydrous dichlormethane. 0.55 mmol of 2-cyanoethyl-\(N,N,N',N'\)-tetraisoproplyphosphoramidite reagent and 0.6 mmol BMT were added to this mixture which was stirred for two hours. After completed reaction the solvent was removed under reduced pressure. After coevaporation with 2 ml of anhydrous dichlormethane the phosphoramidite was
dried over night at oil pump vacuum. An aqueous workup did not take place. The trinucleotide was used for solid phase synthesis. Rf-Wert (CH₂Cl₂:MeOH 9:1): 0.7. See below for analytical data.

**Analytical data of 5'-O-DMT 3'-OH-trinucleotides**

**DMTAAAOH:**

Rf- value (CH₂Cl₂: methanol = 9:1) = 0.6.

³¹P-NMR (DMSO-d⁶, 300 MHz): δ = -2.78, -2.74 (2 x phosphortriester diastereomer), -2.58 (br, 2 x phosphortriester diastereomer).

MALDI⁺-MS: calculated: 1496.42 g/mol, found: 1519.15 g/mol (Na-peak).

UV: λ_max = 258 nm.

**DMTACCOH:**

Rf- value (CH₂Cl₂: methanol = 9:1) = 0.6.

³¹P-NMR (DMSO-d⁶, 300 MHz): δ = -2.572 (br, 2 x phosphortriester diastereomer), -2.467, -2.435 (2 x phosphortriester diastereomer).

MALDI⁺-MS: calculated: 1516.4 g/mol, found: 1539.753 g/mol (Na-peak).

UV: λ_max = 260 nm.

**DMTATCOH:**

Rf- value (CH₂Cl₂: methanol = 9:1) = 0.6

³¹P-NMR (DMSO-d⁶, 300 MHz): δ = -2.62, -2.60, -2.55, -2.42 (4 x phosphortriester diastereomer).

MALDI⁺-MS: calculated: 1427.3 g/mol, found: 1450.805 g/mol (Na-peak).

UV: λ_max = 259 nm.

**DMTATGOH:**

Rf- value (CH₂Cl₂: methanol = 9:1) = 0.6.

³¹P-NMR (DMSO-d⁶, 300 MHz): δ = -2.64 (br, 2 x phosphortriester diastereomer), 2.56 (br, 2 x phosphortriester diastereomer).
MALDI+−MS: calculated: 1432.47 g/mol, found: 1456.2 g/mol (Na-peak).
UV: \( \lambda_{\text{max}} = 261 \) nm.

**DMTCACOH:**

Rf value (CH\(_2\)Cl\(_2\): methanol = 9:1) = 0.6.

\(^{31}\)P-NMR (DMSO-d\(_6\), 300 MHz): \( \delta = -2.83, -2.73, -2.42, -2.40 \) (4 x phosphortriester diastereomer).
MALDI+−MS: calculated: 1516.4 g/mol, found: 1540.04 g/mol (Na-peak).
UV: \( \lambda_{\text{max}} = 259 \) nm.

**DMTCAGOH:**

Rf value (CH\(_2\)Cl\(_2\): methanol = 9:1) = 0.6.

\(^{31}\)P-NMR (DMSO-d\(_6\), 300 MHz): \( \delta = -2.85, -2.79 \) (2 x phosphostriester diastereomer), -2.53 (br, 2 x phosphortriester diastereomer).
MALDI+−MS: calculated: 1522.4 g/mol, found: 1545.6 g/mol (Na-peak).
UV: \( \lambda_{\text{max}} = 258 \) nm.

**DMTCATOH:**

Rf value (CH\(_2\)Cl\(_2\): methanol = 9:1) = 0.6.

\(^{31}\)P-NMR (DMSO-d\(_6\), 300 MHz): \( \delta = -2.83, -2.74 \) (2 x phosphortriester diastereomer), -2.46 (2 x phosphortriester diastereomer).
MALDI+−MS: calculated: 1426.45 g/mol, found: 1450.84 g/mol (Na-peak).
UV: \( \lambda_{\text{max}} = 260 \) nm.

**DMTCCAOH:**

Rf value (CH\(_2\)Cl\(_2\): methanol = 9:1) = 0.6.

\(^{31}\)P-NMR (DMSO-d\(_6\), 300 MHz): \( \delta = -2.68, -2.60 \) (2 x br, 4 x phosphortriester diastereomer).
MALDI+−MS: calculated: 1516.40 g/mol, found: 1539.51 g/mol (Na-peak).
UV: \( \lambda_{\text{max}} = 259 \) nm.

**DMTCTGOH:**

S8
Rf-value (CH$_2$Cl$_2$: methanol = 9:1) = 0.6.

$^{31}$P-NMR (DMSO-d$_6$, 300 MHz): $\delta =$ -2.71, -2.62 (2 x phosphortriester diastereomer), -2.54 (br, 2 x phosphortriester diastereomer).

MALDI*-MS: calculated: 1442.44 g/mol, found: 1466.22 g/mol (Na-peak).

UV: $\lambda_{\text{max}} = 259$ nm.

**DMTGATOH:**

Rf-value (CH$_2$Cl$_2$: methanol = 9:1) = 0.6.

$^{31}$P-NMR (DMSO-d$_6$, 300 MHz): $\delta =$ -3.14, -2.85 (2 x br, 4 x phosphortriester diastereomer).

MALDI*-MS: calculated: 1433.31 g/mol, found: 1456.41 g/mol (Na-peak).

UV: $\lambda_{\text{max}} = 260$ nm.

**DMTGCAOH:**

Rf-value (CH$_2$Cl$_2$: methanol = 9:1) = 0.6.

$^{31}$P-NMR (DMSO-d$_6$, 300 MHz): $\delta =$ -3.69, -3.65, 2.70, 2.60 (4 x phosphortriester diastereomer).

MALDI*-MS: calculated: 1521.49 g/mol, found: 1545.68 g/mol (Na-peak).

UV: $\lambda_{\text{max}} = 259$ nm.

**DMTGCGOH:**

Rf-value (CH$_2$Cl$_2$: methanol = 9:1) = 0.6.

$^{31}$P-NMR (DMSO-d$_6$, 300 MHz): $\delta =$ -2.84, -2.74, -2.56, -2.45 (4 x phosphortriester diastereomer).

MALDI*-MS: calculated: 1537.49 g/mol, found: 1561.76 g/mol (Na-peak).

UV: $\lambda_{\text{max}} = 261$ nm.

**DMTGCTOH:**

Rf-value (CH$_2$Cl$_2$: methanol = 9:1) = 0.6.

$^{31}$P-NMR (DMSO-d$_6$, 300 MHz): $\delta =$ -2.67--2.45 (4 x phosphortriester diastereomer).

MALDI*-MS: calculated: 1443.30 g/mol, found: 1466.07 g/mol (Na-peak).

UV $\lambda_{\text{max}} = 259$ nm.
DMTGGCOH:

Rf value (CH₂Cl₂: methanol = 9:1) = 0.6.

$^{31}$P-NMR (DMSO-d₆, 300 MHz): $\delta = -2.69, 2.65, -2.47, -2.39$ (4 x phosphortriester diastereomer).

MALDI$^+$-MS: calculated: 1538.41 g/mol, found: 1561.50 g/mol (Na-peak).

UV: $\lambda_{\text{max}} = 258$ nm.

DMTGGTOH:

Rf value (CH₂Cl₂: methanol = 9:1) = 0.6.

$^{31}$P-NMR (DMSO-d₆, 300 MHz): $\delta = -2.70$ (br, 2 x phosphortriester diastereomer), -2.51, -2.49 (2 x phosphortriester diastereomer).

MALDI$^+$-MS: calculated: 1449.31 g/mol, found: 1472.7 g/mol (Na-peak).

UV: $\lambda_{\text{max}} = 258$ nm.

DMTGTAOH:

Rf value (CH₂Cl₂: methanol = 9:1) = 0.6.

$^{31}$P-NMR (DMSO-d₆, 300 MHz): $\delta = -2.70$ (2 x phosphortriester diastereomer), -2.58 (br, 2 x phosphortriester diastereomer).

MALDI$^+$-MS: calculated: 1433.31 g/mol, found: 1456.38 g/mol (Na-peak).

UV $\lambda_{\text{max}} = 259$ nm.

DMTGTOOH:

Rf value (CH₂Cl₂: methanol = 9:1) = 0.5.

$^{31}$P-NMR (DMSO-d₆, 300 MHz): $\delta = -2.70$ (br, 2x phosphortriester diastereomer), -2.51, -2.49 (2 x phosphortriester diastereomer).

MALDI$^+$-MS: calculated: 1354.41 g/mol, found: 1377.12 g/mol (Na-peak).

UV: $\lambda_{\text{max}} = 261$ nm.

DMTTGGOH:

Rf value (CH₂Cl₂: methanol = 9:1) = 0.6

$^{31}$P-NMR (DMSO-d₆, 300 MHz): $\delta = -2.80, -2.66, -2.65, -2.50$ (4 x phosphortriester diastereomer).
MALDI⁺-MS: calculated: 1449.31 g/mol, found: 1472.47 g/mol (Na-peak).
UV: $\lambda_{\text{max}} = 258$ nm.

**DMTTTCOH:**

R$_f$- value (CH$_2$Cl$_2$: methanol = 9:1) = 0.5

$^{31}$P-NMR (DMSO-d$_6$, 300 MHz): $\delta = -2.70, -2.52, -2.46, -2.43$ (4 x phosphortriester diastereomer).

MALDI⁺-MS: calculated: 1347.40 g/mol, found: 1371.32 g/mol (Na-peak).
UV: $\lambda_{\text{max}} = 260$ nm.

**DMTTTTOH:**

R$_f$-value (CH$_2$Cl$_2$: methanol = 9:1) = 0.5.

$^{31}$P-NMR (DMSO-d$_6$, 300 MHz): $\delta = 2.71, -2.53, -2.48, -2.45$ (4 x phosphortriester diastereomer).

MALDI⁺-MS: calculated: 1258.37 g/mol, found: 1281.89 g/mol (Na-peak).
UV: $\lambda_{\text{max}} = 259$ nm.
Table S1. MALDI data and yields of dinucleotide compounds. Found masses are given as Na-peak (m/z).

<table>
<thead>
<tr>
<th>Dinucleotide</th>
<th>5'-O-DMT 3'-O-TBDMS</th>
<th>5'-OH 3'-O-TBDMS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>found mass</td>
<td>calcd. mass</td>
</tr>
<tr>
<td>AA</td>
<td>1196</td>
<td>1173</td>
</tr>
<tr>
<td>AC</td>
<td>1206</td>
<td>1183</td>
</tr>
<tr>
<td>AG</td>
<td>1212</td>
<td>1189</td>
</tr>
<tr>
<td>AT</td>
<td>1117</td>
<td>1094</td>
</tr>
<tr>
<td>CA</td>
<td>1206</td>
<td>1183</td>
</tr>
<tr>
<td>CC</td>
<td>1216</td>
<td>1193</td>
</tr>
<tr>
<td>CG</td>
<td>1222</td>
<td>1199</td>
</tr>
<tr>
<td>CT</td>
<td>1127</td>
<td>1104</td>
</tr>
<tr>
<td>GC</td>
<td>1222</td>
<td>1199</td>
</tr>
<tr>
<td>GG</td>
<td>1228</td>
<td>1205</td>
</tr>
<tr>
<td>GT</td>
<td>1133</td>
<td>1110</td>
</tr>
<tr>
<td>TA</td>
<td>1117</td>
<td>1094</td>
</tr>
<tr>
<td>TC</td>
<td>1127</td>
<td>1104</td>
</tr>
<tr>
<td>TG</td>
<td>1133</td>
<td>1110</td>
</tr>
<tr>
<td>TT</td>
<td>1038</td>
<td>1015</td>
</tr>
</tbody>
</table>
Table S2. MALDI data and yields of trinucleotide compounds. Found masses are given as Na-peak (m/z).

<table>
<thead>
<tr>
<th>Trinucleotide</th>
<th>5'-O-DMT 3'-O-TBDMS</th>
<th>5'-O-DMT 3'-OH*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>found mass</td>
<td>calcd. mass</td>
</tr>
<tr>
<td>AAA</td>
<td>1633</td>
<td>1610</td>
</tr>
<tr>
<td>ACC</td>
<td>1653</td>
<td>1630</td>
</tr>
<tr>
<td>ATC</td>
<td>1563</td>
<td>1540</td>
</tr>
<tr>
<td>ATG</td>
<td>1569</td>
<td>1546</td>
</tr>
<tr>
<td>CAC</td>
<td>1654</td>
<td>1629</td>
</tr>
<tr>
<td>CAG</td>
<td>1658</td>
<td>1635</td>
</tr>
<tr>
<td>CAT</td>
<td>1563</td>
<td>1540</td>
</tr>
<tr>
<td>CCA</td>
<td>1652</td>
<td>1629</td>
</tr>
<tr>
<td>CTG</td>
<td>1579</td>
<td>1556</td>
</tr>
<tr>
<td>GAT</td>
<td>1569</td>
<td>1546</td>
</tr>
<tr>
<td>GCA</td>
<td>1658</td>
<td>1635</td>
</tr>
<tr>
<td>GCG</td>
<td>1674</td>
<td>1651</td>
</tr>
<tr>
<td>GCT</td>
<td>1580</td>
<td>1557</td>
</tr>
<tr>
<td>GGC</td>
<td>1674</td>
<td>1651</td>
</tr>
<tr>
<td>GGT</td>
<td>1586</td>
<td>1563</td>
</tr>
<tr>
<td>GTA</td>
<td>1569</td>
<td>1546</td>
</tr>
<tr>
<td>GTT</td>
<td>1491</td>
<td>1468</td>
</tr>
<tr>
<td>TGG</td>
<td>1586</td>
<td>1563</td>
</tr>
<tr>
<td>TTC</td>
<td>1484</td>
<td>1461</td>
</tr>
<tr>
<td>TTT</td>
<td>1395</td>
<td>1372</td>
</tr>
</tbody>
</table>

* removal of the 3'-O-TBDMS group was virtually quantitative in all cases
Table S3. MALDI data of hexamers. Found masses are given as Na-peak (m/z). Coupled phosphoramidite trinucleotide building blocks are given in bold letters.

<table>
<thead>
<tr>
<th>6mer 5’ → 3’</th>
<th>calcd. mass</th>
<th>found mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAAATTA</td>
<td>1798</td>
<td>1798</td>
</tr>
<tr>
<td>ACC TTC</td>
<td>1726</td>
<td>1726</td>
</tr>
<tr>
<td>ATCTTA</td>
<td>1765</td>
<td>1765</td>
</tr>
<tr>
<td>ATGTTC</td>
<td>1781</td>
<td>1781</td>
</tr>
<tr>
<td>CACTTA</td>
<td>1750</td>
<td>1750</td>
</tr>
<tr>
<td>CAG TTC</td>
<td>1766</td>
<td>1766</td>
</tr>
<tr>
<td>CATTTA</td>
<td>1765</td>
<td>1765</td>
</tr>
<tr>
<td>CCATTC</td>
<td>1726</td>
<td>1726</td>
</tr>
<tr>
<td>CTGTTC</td>
<td>1781</td>
<td>1779</td>
</tr>
<tr>
<td>GATTTA</td>
<td>1805</td>
<td>1805</td>
</tr>
<tr>
<td>GCATTC</td>
<td>1766</td>
<td>1766</td>
</tr>
<tr>
<td>GC GTTC</td>
<td>1782</td>
<td>1782</td>
</tr>
<tr>
<td>GCTTTC</td>
<td>1757</td>
<td>1757</td>
</tr>
<tr>
<td>GGC TTC</td>
<td>1806</td>
<td>1806</td>
</tr>
<tr>
<td>GGT TTA</td>
<td>1821</td>
<td>1821</td>
</tr>
<tr>
<td>GTATTA</td>
<td>1805</td>
<td>1805</td>
</tr>
<tr>
<td>GTTTTC</td>
<td>1772</td>
<td>1772</td>
</tr>
<tr>
<td>TG GTTA</td>
<td>1821</td>
<td>1821</td>
</tr>
<tr>
<td>TTCTTA</td>
<td>1756</td>
<td>1756</td>
</tr>
<tr>
<td>TTTTTA</td>
<td>1771</td>
<td>1771</td>
</tr>
</tbody>
</table>
Figure S1

Figure S1: HPLC diagram of pure compounds a) DMT-GCG-OH (88% buffer B) and b) DMT-TGG-OH (87% buffer B); gradient: 0% B for 5 min, 0 – 100% B in 23.5 min, 100% B for 30 min.
Literature


Appendix

$^{31}$P NMR spectra of data of 5'-O-DMT 3'-OH-trinucleotides

DMTAAAOH
DMTATCOH
DMTCACOH
DMTCATOH
DMTCCAOH
DMTGCGOH
DTGCGOH
DMTGTTOH
DMTTGGOH
DMTTTCOH