

Supporting Information

for

Artificial bioconjugates with naturally occurring linkages: the use of phosphodiester

Takao Shoji¹, Hiroki Fukutomi¹, Yohei Okada² and Kazuhiro Chiba^{*,1}

Address: ¹Department of Applied Biological Science, Tokyo University of Agriculture and Technology,

3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan and ²Department of Chemical Engineering, Tokyo

University of Agriculture and Technology, 2-24-16 Naka-cho, Koganei, Tokyo 184-8588, Japan

Email: Kazuhiro Chiba - chiba@cc.tuat.ac.jp

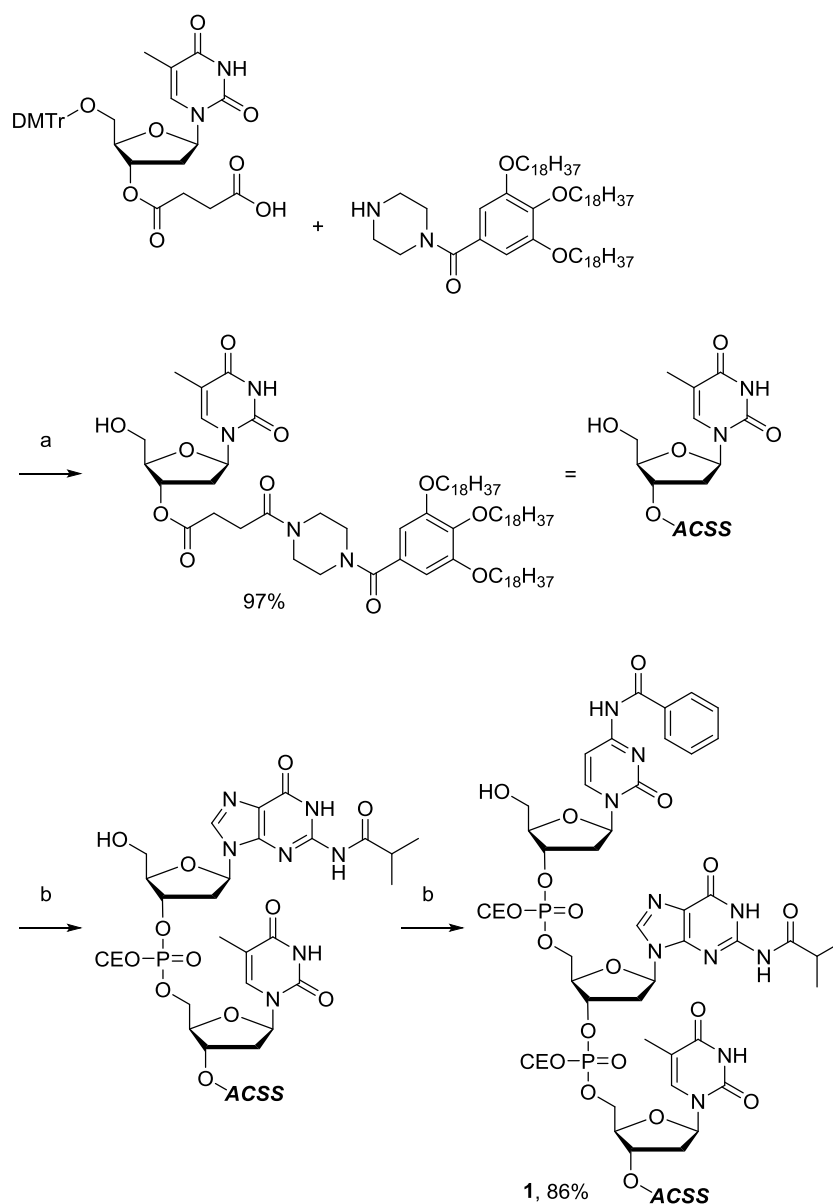
* Corresponding author

**Additional schemes and figures, general remarks, synthesis and characterization data,
including copies of ¹H and ¹³C NMR**

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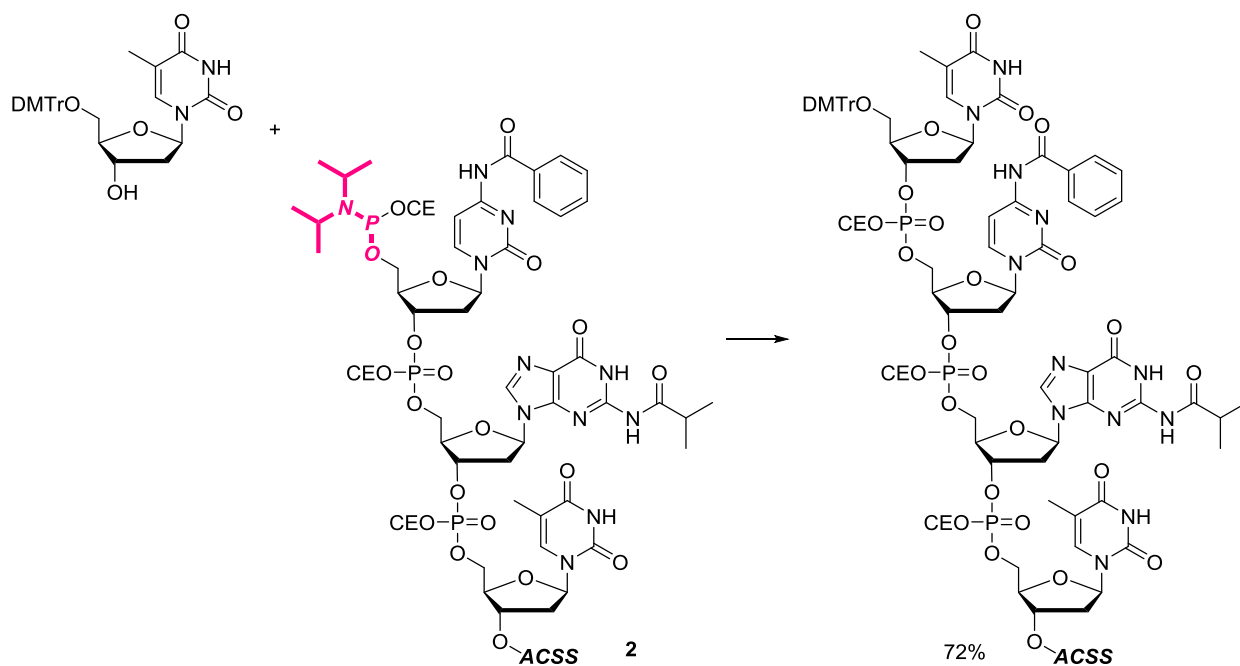
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1. Additional schemes and figures



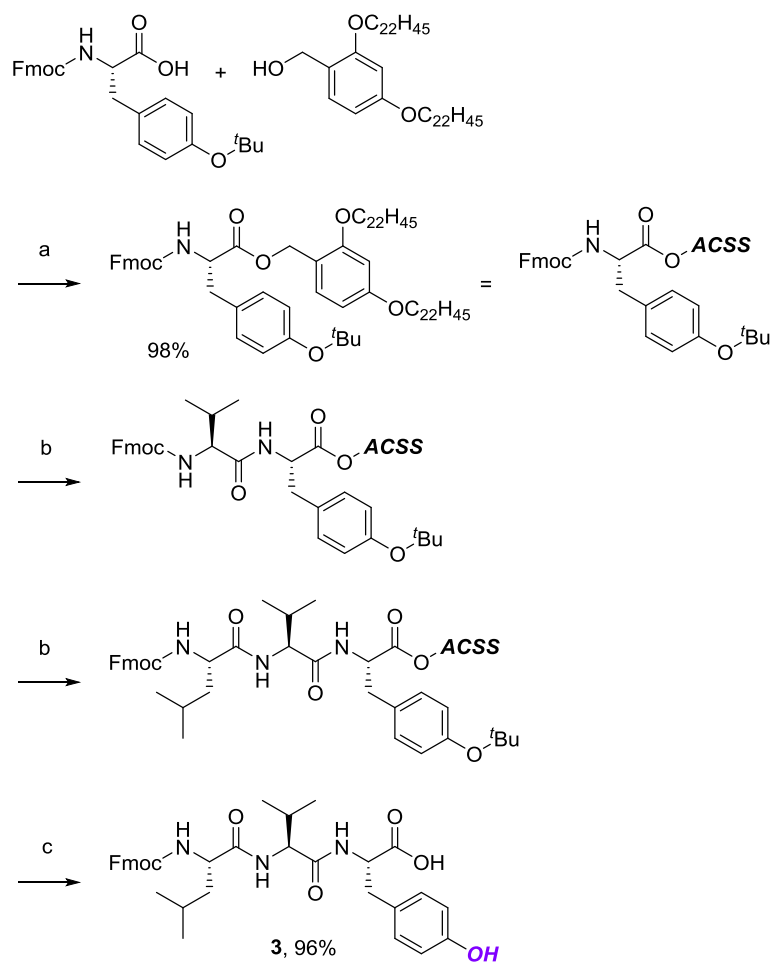
Reagents and conditions: (a) (i) DIPCI (4.0 eq), HOBT (4.0 eq), DIPEA (4.0 eq), CH₂Cl₂, rt, 1h; (ii) 3% DCA/CH₂Cl₂, rt, 5 min, 97% over 2 steps. (b) (i) DMT-Phosphoramidite (2.0 eq), BMT (2.0 eq), CH₂Cl₂/CH₃CN (10:1, v/v), rt, 10 min; (ii) 1% Ac₂O, 1% Pyridine, 1% NMI/CH₂Cl₂, rt, 10 min; (iii) 0.67% BPO/DMP/CH₂Cl₂, rt, 10 min; (iv) 3% DCA/CH₂Cl₂, rt, 5 min, 86% over 6 steps.

Scheme S1: Preparation of the supported trinucleotide **1**.



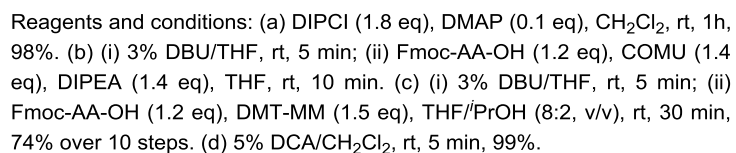
Reagents and conditions: (i) BMT (2.0 eq), CH₂Cl₂/CH₃CN (10:1, v/v), rt, 10 min; (ii) 1% Ac₂O, 1% Pyridine, 1% NMI/CH₂Cl₂, rt, 10 min; (iii) 0.67% BPO/DMP/CH₂Cl₂, rt, 10 min, 72% over 3 steps.

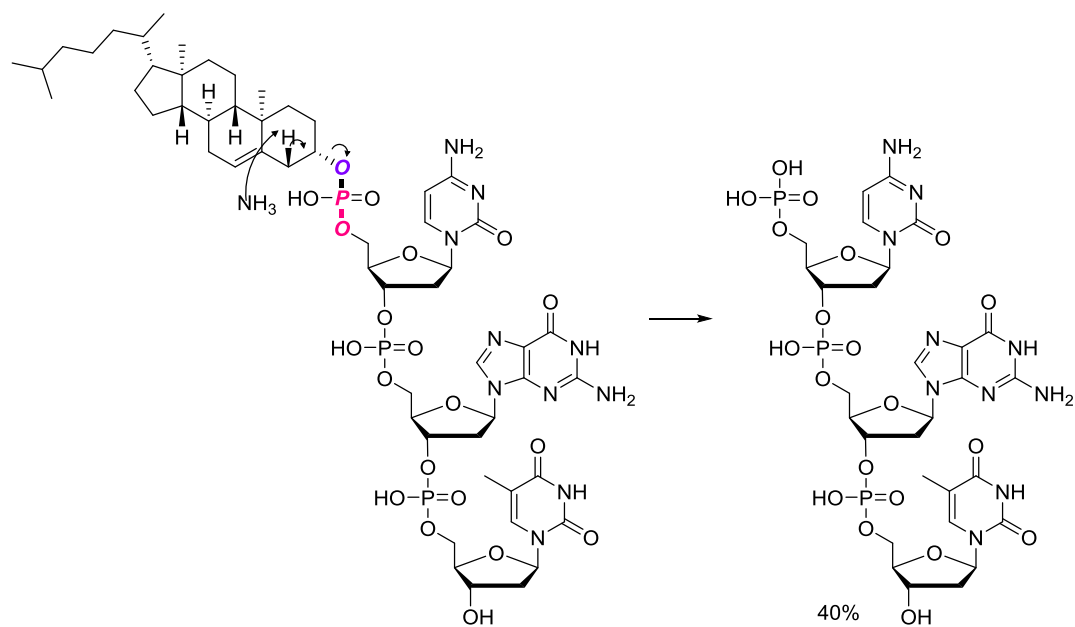
Scheme S2: Conjugation between 5'-activated supported trinucleotide **2** and unactivated DMT-dT.



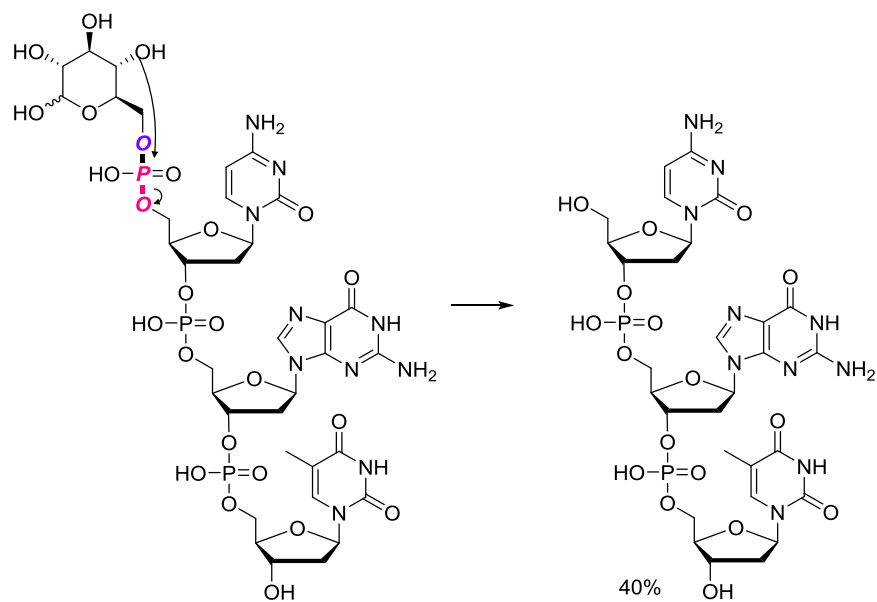
Reagents and conditions: (a) DIPCI (1.8 eq), DMAP (0.1 eq), CH_2Cl_2 , rt, 1h, 98%. (b) (i) 3% DBU/THF, rt, 5 min; (ii) Fmoc-AA-OH (1.2 eq), COMU (1.4 eq), DIPEA (1.4 eq), THF, rt, 10 min. (c) 10% TFA/ CH_2Cl_2 , rt, 5 min, 96% over 5 steps.

Scheme S3: Preparation of the tripeptide **3**.

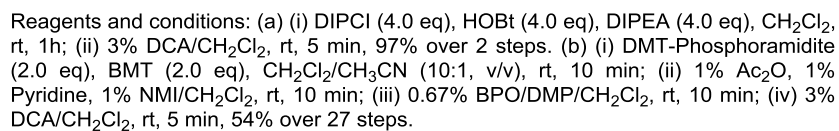




Scheme S5: Side reaction accompanied with the deprotection of the lipid-conjugate.



Scheme S6: Side reaction accompanied with the deprotection of the sugar-conjugate.



S8

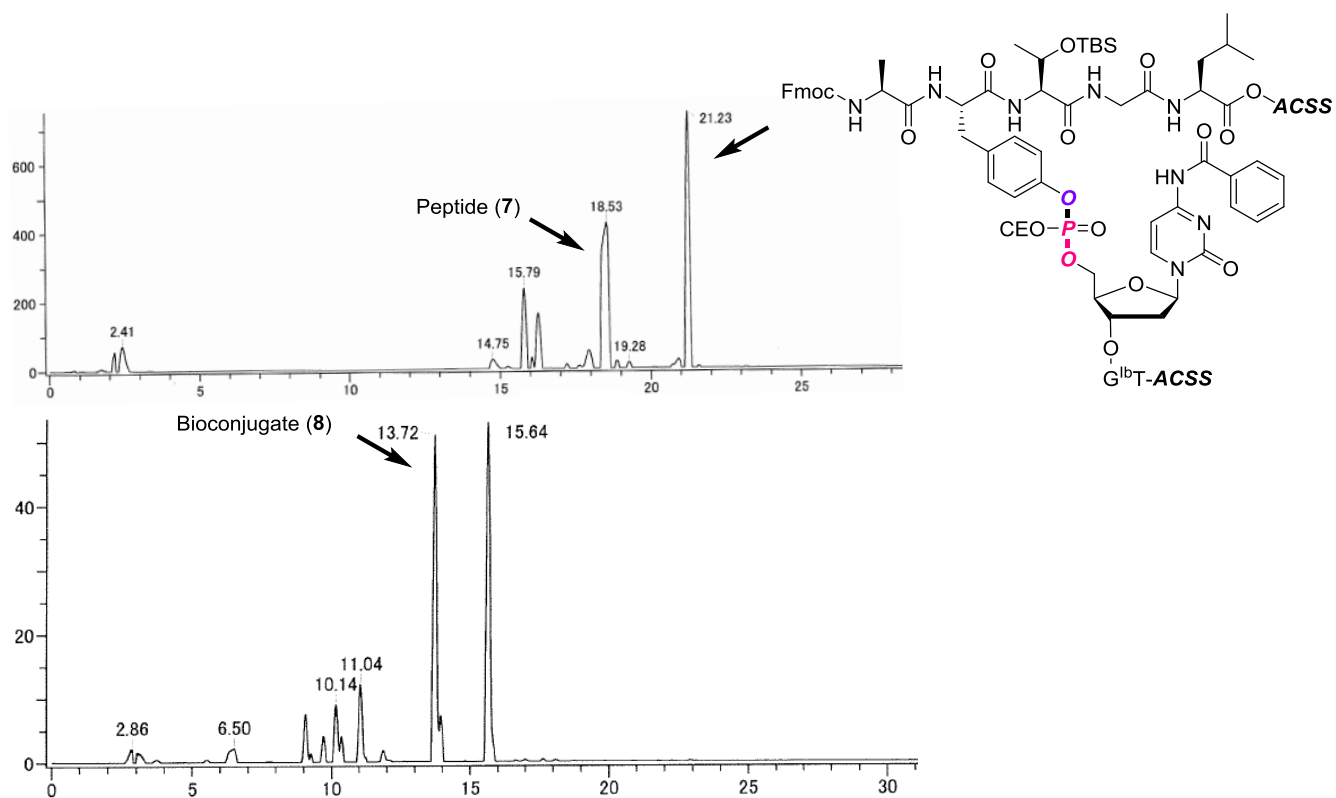


Figure S1: Crude HPLC spectra of protected and deprotected pentapeptide **7**-conjugate.

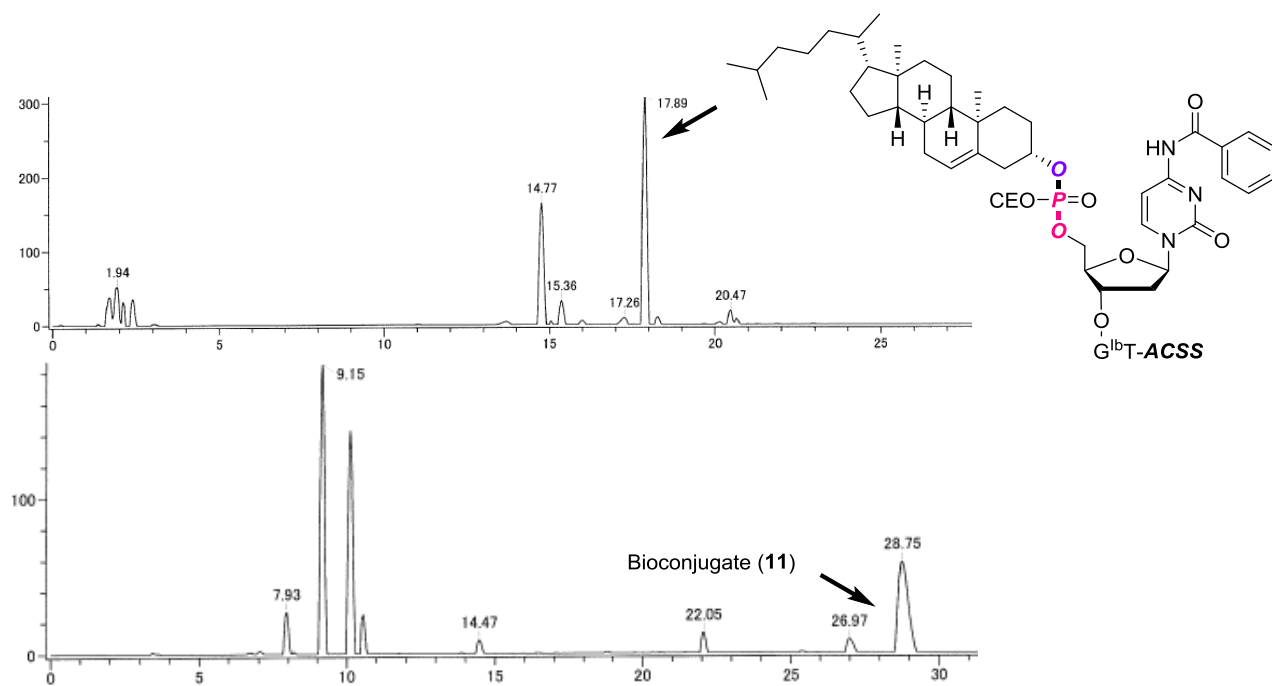


Figure S2: Crude HPLC spectra of protected and deprotected lipid 9-conjugate.

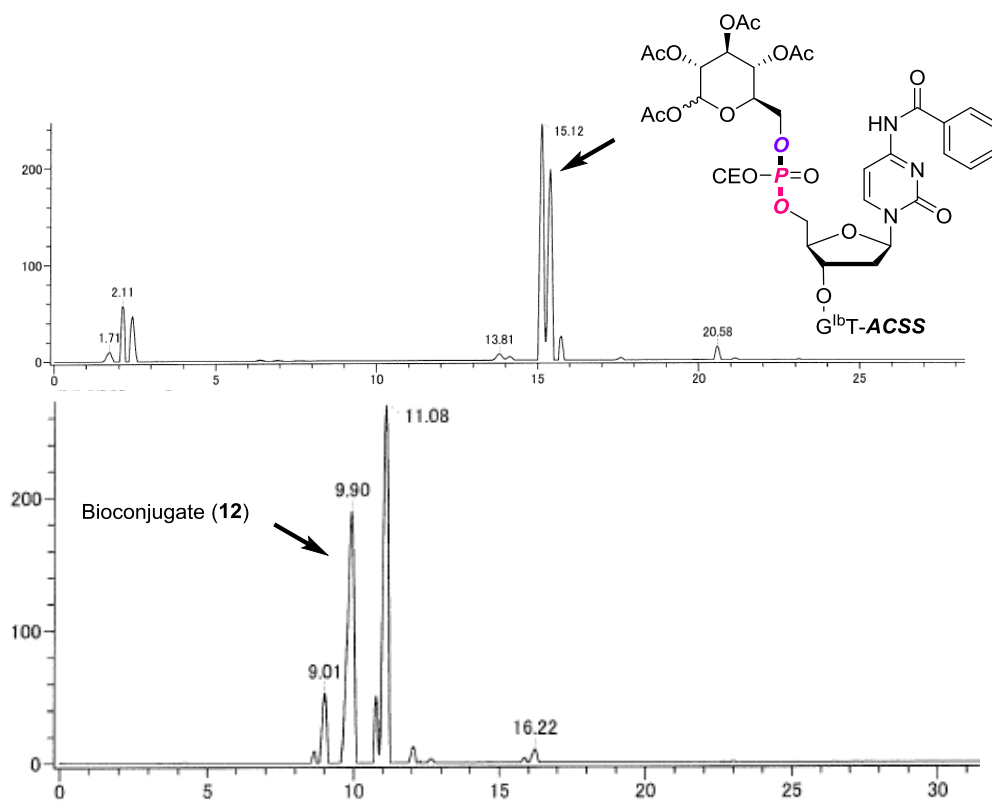
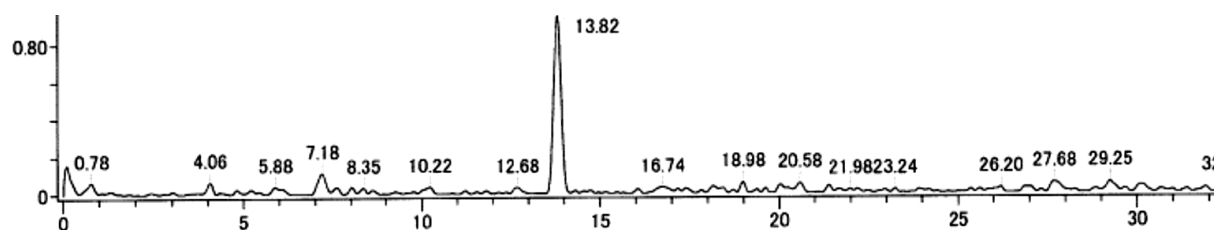
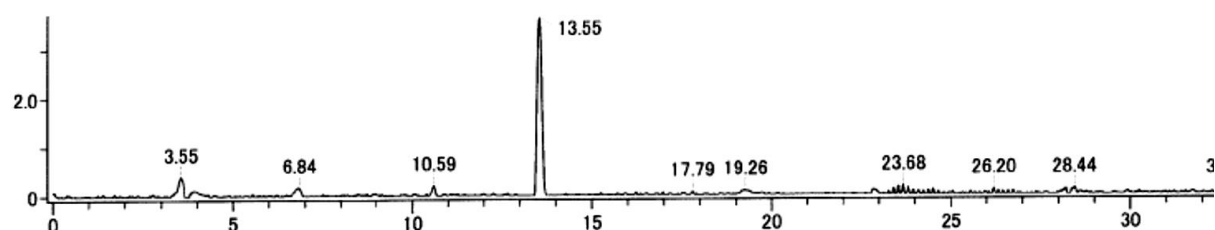


Figure S3: Crude HPLC spectra of protected and deprotected sugar **10**-conjugate.

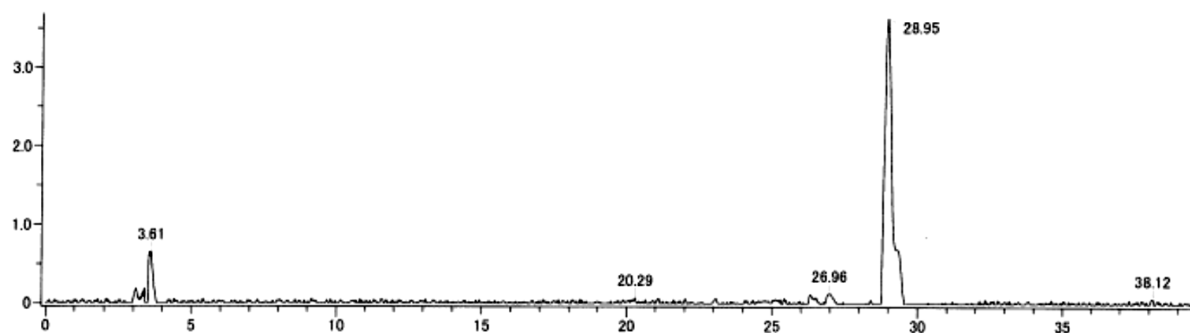
Bioconjugate 5



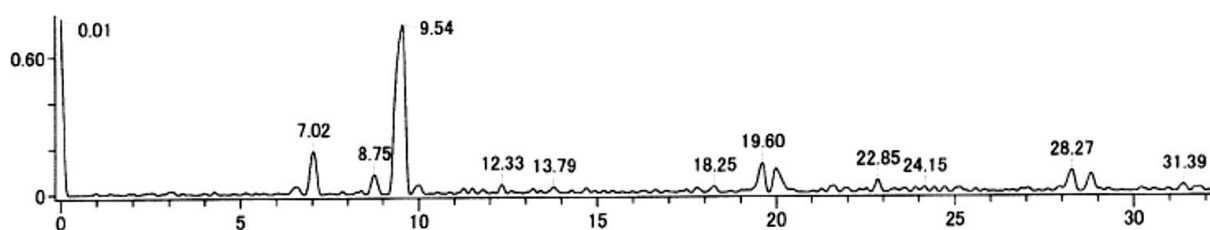
Bioconjugate 8



Bioconjugate 11



Bioconjugate 12



Bioconjugate 16

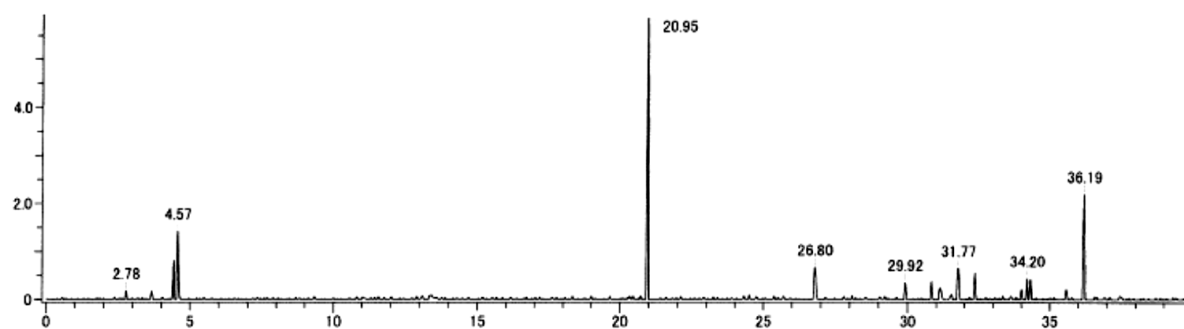


Figure S4: HPLC spectra of deprotected bioconjugates 5, 8, 11, 12, and 16 after purification.

2. General remarks

All reagents and solvents were purchased from commercial sources and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) carried out on silica gel plates, with detection by UV absorption (254 nm) and by heating the plates after dipping them in a solution of 12 molybdo(VI) phosphoric acid n-hydrate in 95% ethanol. Silica gel (particle size 40–50 μm) was used for column chromatography. ^1H NMR spectra were collected on 600 or 400 MHz NMR spectrometers using the deuterated solvent as an internal deuterium reference. Chemical shift data are given in δ units calibrated with residual protic solvent. The multiplicity of a signal is indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; m, multiplet. ^{13}C NMR spectra were collected on 150 or 100 MHz spectrometers with proton decoupling using the deuterated solvent as an internal carbon reference. Chemical shift data are given in δ units calibrated with residual solvent. ^{31}P NMR quantitative spectra were collected on 242.95 or 161.83 MHz spectrometers inverse gated proton decoupling. High-resolution mass spectra (HRMS) were collected on electrospray ionization (ESI)- or matrix assisted laser desorption ionization (MALDI)-time-of-flight (TOF) spectrometers.

Abbreviations: BMT, 5'-(benzylmercapto)-1*H*-tetrazole; MS, molecular sieve; dT, 2'-deoxythymidine; dC^(Bz), *N*⁴-benzoyl-2'-deoxycytidine; dG^(Ib), *N*²-isobutyryl-2'-deoxyguanosine, dA^(Bz), *N*⁶-benzoyl-2'-deoxyadenosine; NMI, *N*-methylimidazole; BPO, butanone peroxide; DMP, dimethylphthalate; DCA, dichloro acetic acid; THF, tetrahydrofuran; Fmoc, 9-fluorenylmethoxycarbonyl; DIPCI, diisopropylcarbodiimide; DMAP, 4-dimethylaminopyridine; COMU, *N*-[(1-cyano-2-ethoxy-2-oxoethylideneaminoxy)dimethylamino(morpholino)]carbenium

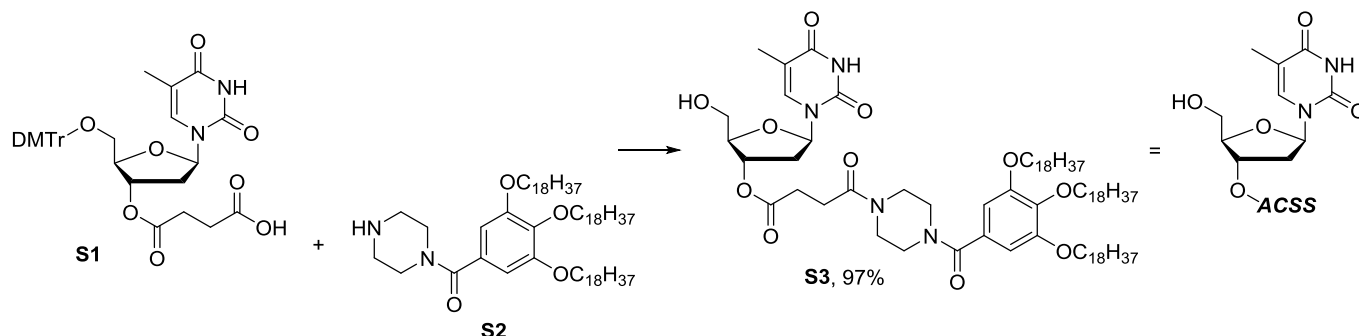
hexafluorophosphate; DIPEA, diisopropylethylamine; DMT-MM,

4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride; DBU,

1,8-diazabicyclo[5.4.0]undec-7-ene; TBDMS, *tert*-butyldimethylsilyl; TFA, trifluoroacetic acid.

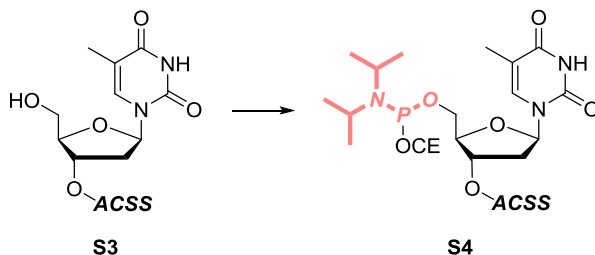
3. Synthesis and characterization data

Supported dT (S3)



The titled compound was synthesized according to the reported procedure.¹ ¹H NMR (CDCl₃, 400 MHz) δ 8.066 (0.7H, br), 7.469 (1H, s), 6.576 (2H, s), 6.203 (1H, dd, J = 8.25, 6.19 Hz), 5.928 (0.5H, s), 5.36 (1H, ddd, J = 6.19, 2.75, 2.75 Hz), 4.112 (1H, dd, J = 4.67, 2.75 Hz), 4.011-3.920 (6H, m), 3.921-3.859 (2H, m), 3.825-3.307 (7H, m), 3.196-3.059 (1.5H, m), 2.676 (4H, m), 2.478-2.369 (2H, m), 1.916 (3H, s), 1.821-1.652 (6H, m), 1.519-1.378 (6H, m), 1.365-1.160 (84H, m), 0.866 (9H, t, J = 6.87 Hz).

5'-Phosphitylation of the Supported dT



To a solution of the supported dT **S3** (132 mg, 0.10 mmol) in CH₂Cl₂ (4 mL) stirred at r.t. was added BMT (61.8 mg, 0.32 mmol), 3 Å MS, and 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite (101 μ L, 0.30 mmol). The resulting reaction mixture was stirred at rt for 20 min, diluted with MeCN, and collected by vacuum filtration. The residue was repeatedly washed with MeCN and dried in vacuo to give the 5'-phosphitylated product **S4** in 93% (141 mg, 0.093 mmol) as a white solid. ¹H NMR (600 MHz, CDCl₃) δ 8.003 (1H, br), 7.598 (1H, d, J = 48.54 Hz), 6.576 (2H, s), 6.334 (1H, ddd, J = 33.89, 9.16, 5.04 Hz), 5.337

(1H, dd, $J = 27.02, 5.95$ Hz), 4.201 (1H, d, $J = 12.36$ Hz), 4.018-3.839 (8H, m), 3.821-3.745 (2H, m), 3.730-3.376 (8H, m), 2.726-2.586 (6H, m), 2.422 (1H, dddd, $J = 16.03, 13.74, 5.50, 4.58$), 2.158 (1H, dddd, $J = 8.93, 7.79, 5.95, 3.21$ Hz), 1.960 (3H, d, $J = 27.02$ Hz), 1.812-1.686 (6H, m), 1.576 (2H, s), 1.501-1.383 (6H, m), 1.370-0.955 (96H, m), 0.865 (9H, t, $J = 6.87$ Hz); ^{13}C NMR (150 MHz, CDCl_3) δ 172.777, 170.775, 169.774, 163.605, 153.336, 150.447, 139.692, 135.593, 129.853, 117.429, 111.375, 111.079, 105.835, 85.069, 84.640, 84.382, 75.774, 75.515, 73.608, 69.404, 64.198, 63.540, 63.387, 58.772, 58.544, 58.467, 58.258, 43.374, 43.260, 43.145, 37.882, 37.558, 32.009, 30.398, 29.806, 29.606, 29.444, 29.234, 26.183, 24.839, 14.198, 12.663, 12.530; ^{31}P NMR (242.95 MHz, CDCl_3) δ 150.191, 149.217, 14.835, 9.691, 8.311; IR ν_{max} (cm^{-1} , KBr) 3509.81, 3182.93, 3071.08, 2912.95, 2851.24, 2360.44, 2338.59, 1696.09, 1640.16, 1577.49, 1470.46, 1426.10, 1380.78, 1324.86, 1273.75, 1217.83, 1200.47, 1155.15, 1110.80, 1048.12, 1031.73, 974.84, 885.17, 716.43, 558.29; HRMS $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{88}\text{H}_{155}\text{N}_6\text{O}_{12}\text{PNa}$ 1542.1333, found 1542.1309.

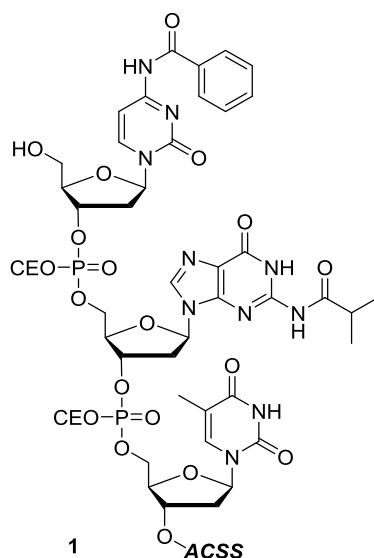
General procedure for elongation of oligonucleotides

Carried out according to the reported procedure.¹ To a solution of supported oligonucleotide (1.0 mmol) in dichloromethane (88 mL) stirred at rt was added amidite monomer and 0.25 M BMT/MeCN (8.8 mL). The resulting reaction mixture was stirred at rt for 20 min, followed by the addition of 1% Ac_2O , 1% pyridine, and 1% NMI. The resulting reaction mixture was stirred at rt for 10 min, followed by the addition of 0.67% BPO/DMP/ CH_2Cl_2 . The resulting reaction mixture was stirred at rt for 10 min, diluted with MeOH (100 mL), and collected by vacuum filtration. The residue was repeatedly washed with MeOH and dried in vacuo to give the elongated product as a white solid.

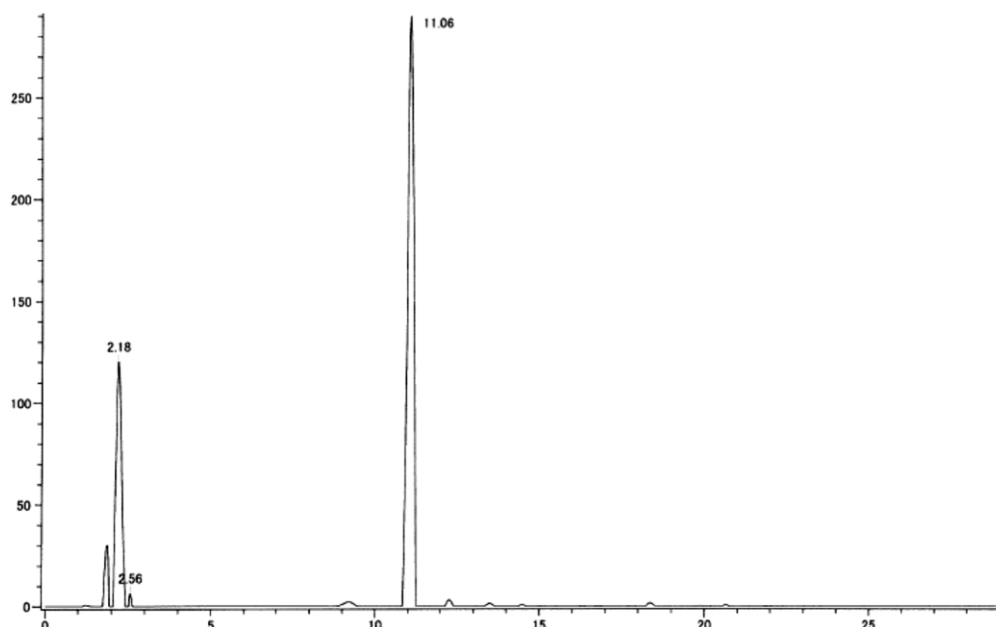
General procedure for deprotection of DMTr

To a solution of supported oligonucleotide (1.0 mmol) in CH_2Cl_2 (20 mL) stirred at rt was added 5% DCA/ CH_2Cl_2 (20 mL). The resulting reaction mixture was stirred at rt for 1 min, diluted with MeOH (100 mL), neutralized with TEA and collected by vacuum filtration. The residue was repeatedly washed with MeOH and dried in vacuo to give the deprotected product as a white solid.

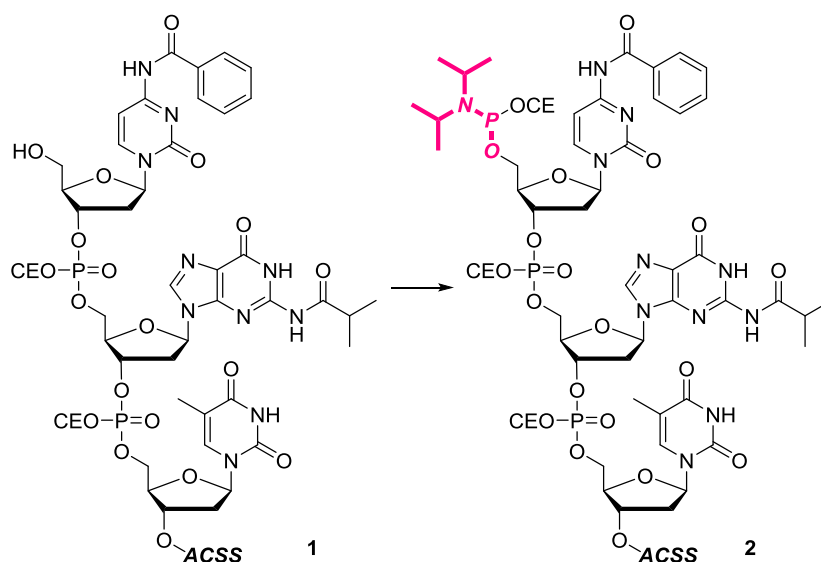
Supported trinucleotide 1, Scheme S1



White solid. ^{31}P NMR (242.95 MHz, CDCl_3) δ -1.624 - -2.680; HRMS $[\text{M} + \text{Na}]$ calculated for $\text{C}_{115}\text{H}_{178}\text{N}_{14}\text{O}_{25}\text{P}_2\text{Na}$ 2240.2455, found. 2240.2443. Purity was checked by HPLC analysis conditions using a C8 column with H_2O and $\text{THF}:\text{MeCN}=8:2$ (70 to 100%, 20 min) as an eluent at flow rate of 0.20 mL/min at 50 °C.



5'-Phosphitylation of the supported trinucleotide 1, see Table 1

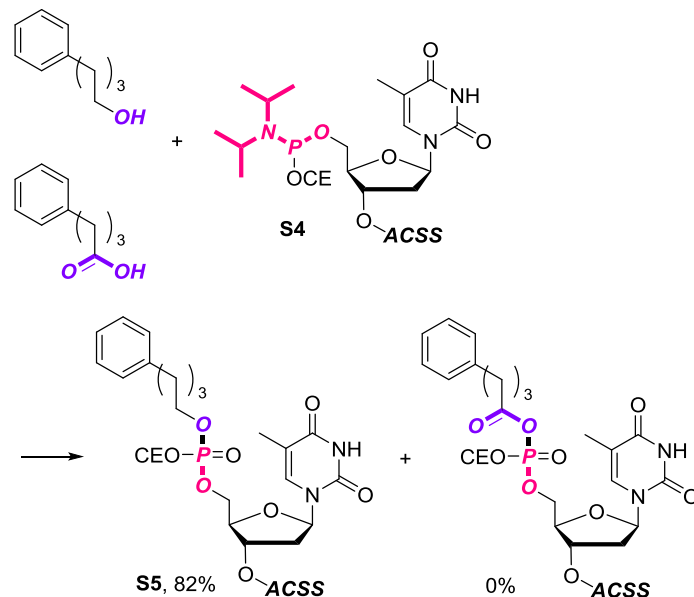


To a solution of the supported trinucleotide **1** (110 mg, 0.050 mmol) in CH_2Cl_2 (2 mL) stirred at rt was added BMT (29.0 mg, 0.15 mmol), 3 Å MS, and 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite (51.0 μL , 0.15 mmol). The resulting reaction mixture was stirred at r.t. for 30 min, diluted with MeCN, and collected by vacuum filtration. The residue was repeatedly washed with MeCN and dried in vacuo to give the 5'-phosphitylated product in 91% (110 mg, 0.045 mmol) as a white solid. ^{31}P NMR (242.95 MHz,

CDCl₃) δ 150.1624, 150.083, 149.362, 149.298, 14.781, 9.502, 9.015, -1.624 - -3.329; HRMS [M + Na]⁺

calculated for C₁₂₄H₁₉₅N₁₆O₂₆P₃Na 2240.3534, found 2440.3541.

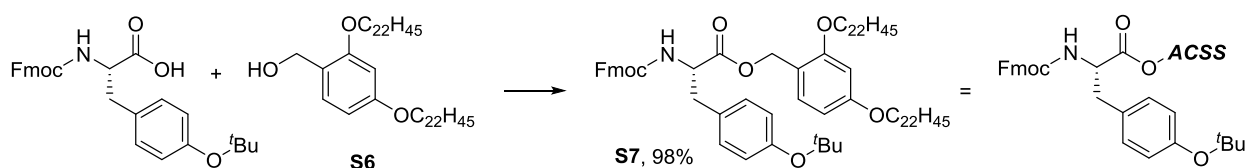
Competition experiment between alcohol and carboxylic acid, see Scheme 1



To a solution of the supported dT **S4** (152.0 mg, 0.1 mmol) in CH₂Cl₂/MeCN (10:1, v/v, 4 mL) stirred at rt was added BMT (60 mg, 0.3 mmol), 3 Å MS, 4-phenyl-1-butanol (31 mg, 0.2 mmol), and 4-phenylbutanoic acid (33 mg, 0.2 mmol). The resulting reaction mixture was stirred at rt for 30 min, followed by the addition of 0.67% BPO/DMP/CH₂Cl₂. The resulting reaction mixture was stirred at rt for 30 min, diluted with MeOH (20 mL), and collected by vacuum filtration. The residue was repeatedly washed with MeOH and dried in vacuo to give the desired product in 82% (129.9 mg, 0.082 mmol) as a white solid. ¹H NMR (600 MHz, CDCl₃) δ 8.461 (0.2H, br), 7.582-7.376 (1H, m), 7.269-7.241 (2H, m, overlapped on CDCl₃), 7.178-7.127 (2H, m), 6.575 (2H, s), 6.377-6.194 (1H, m), 5.372-5.270 (1H, m), 4.421-4.182 (4H, m), 4.135-4.099 (1H, m), 4.019-3.865 (6H, m), 3.823-3.338 (5H, m), 2.801-2.705 (1.5H, m), 2.700-2.548 (5.5H, m), 2.476-2.361 (1H, m), 2.241-2.058 (1H, m), 1.920 (3H, t, J = 10.65 Hz), 1.833-1.745 (4H, m), 1.734-1.630 (6H, m), 1.475-1.413 (6H, m), 1.379-1.241 (87H, m), 1.192 (2H, d, J = 6.19 Hz), 0.864 (9H, t, J = 7.22 Hz); ¹³C

NMR (150 MHz, CDCl₃) δ 172.653, 170.777, 169.676, 163.213, 153.360, 150.258, 142.455, 139.927, 138.998, 135.053, 129.835, 128.495, 126.072, 122.108, 119.571, 116.335, 111.844, 105.917, 84.604, 82.641, 74.340, 73.622, 69.447, 68.844, 676.551, 62.141, 37.094, 35.265, 32.000, 30.401, 29.941, 29.798, 29.444, 29.242, 29.166, 29.108, 27.979, 27.203, 26.179, 22.760, 19.840, 14.239, 12.506; ³¹P NMR (242.95 MHz, CDCl₃) δ -0.785, -1.083; IR ν_{max} (cm⁻¹, KBr) 3414.35, 3211.86, 3071.08, 2912.95, 2851.24, 2349.84, 2248.59, 1701.87, 1640.16, 1583.27, 1465.63, 1431.89, 1386.57, 1329.68, 1267.97, 1245.79, 1222.65, 1160.94, 1121.40, 997.98, 766.57, 716.43, 699.07, 671.11, 552.51; HRMS [M + Na] calculated for C₉₂H₁₅₄N₅O₁₄PNa 1607.1122, found 1607.1117.

Supported Tyr(*t*-Bu) (S7)



To a solution of Fmoc-Tyr(*t*-Bu)-OH (1.65 g, 3.6 mmol) and compound **S6** (1.5 g, 2.0 mmol) in CH₂Cl₂ (50 mL) stirred at rt was added DIPC1 (1.6 mL, 3.6 mmol) and DMAP (180 μ L, 0.4 mmol). The resulting reaction mixture was stirred at rt for 2 h, diluted with MeCN (100 mL), and collected by vacuum filtration. The residue was repeatedly washed with MeCN and dried in vacuo to give the titled compound **S7** in 98% yield (2.35 g, 1.96 mmol) as white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.755 (2H, d, *J* = 7.56 Hz), 7.559 (2H, d, *J* = 7.56 Hz), 7.390 (2H, t, *J* = 7.56 Hz), 7.296 (2H, t, *J* = 7.56 Hz), 7.161 (1H, d, *J* = 8.25 Hz), 6.898 (2H, d, *J* = 8.25 Hz), 6.816 (2H, d, *J* = 8.25 Hz), 6.436 (1H, s), 6.410 (1H, d, *J* = 8.25 Hz), 5.274 (1H, d *J* = 8.25 Hz), 5.152 (2H, dd, *J* = 23.37, 11.68 Hz), 4.652 (1H, dd, *J* = 13.75, 5.50 Hz), 4.394 (1H, dd, *J* = 10.65, 7.56 Hz), 4.311 (1H, dd, *J* = 10.65, 7.56 Hz), 4.188 (1H, t, *J* = 7.56 Hz), 3.933 (4H, dd, *J* = 13.75, 6.87 Hz),

3.061 (2H, ddd, $J = 34.02, 14.43, 5.50$ Hz), 1.833-1.693 (4H, m), 1.465-1.378 (4H, m), 1.379-1.192 (81H, m), 0.873 (6H, t, $J = 7.56$ Hz); ^{13}C NMR (150 MHz, CDCl_3) δ 171.543, 161.116, 158.703, 155.562, 154.452, 144.025, 143.872, 141.382, 131.817, 130.544, 129.998, 127.767, 127.144, 125.239, 125.201, 124.138, 120.030, 116.449, 115.827, 104.567, 99.713, 78.371, 68.260, 68.202, 66.996, 63.070, 54.845, 47.262, 32.029, 29.807, 29.760, 29.721, 29.530, 29.463, 29.377, 28.926, 26.159, 22.799, 14.229, 2.002; IR ν_{max} (cm^{-1} , KBr) 3330.46, 2911.99, 2861.84, 2638.14, 2465.54, 2403.83, 2319.95, 2281.38, 2253.41, 2019.10, 1895.68, 1733.69, 1617.02, 1594.84, 1510.95, 1460.81, 1382.71, 1337.39, 1293.04, 1259.29, 1181.19, 1136.83, 1036.55, 918.91, 896.74, 830.21, 780.07, 757.89, 740.53, 718.35, 640.25, 612.29, 573.72, 545.76, 500.44, 461.87, 416.55; HRMS $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{79}\text{H}_{123}\text{NO}_7\text{Na}$ 1220.9192, found 1220.9187.

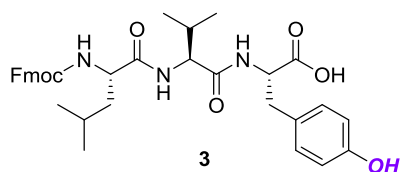
General procedure for coupling of peptide

To a solution of supported peptide (1.0 mmol) and Fmoc-AA-OH in THF (20 mL) stirred at rt was added COMU (1.4 mmol) and DIPEA (1.4 mmol). The resulting reaction mixture was stirred at rt for 10 min, diluted with MeCN (60 mL), and collected by vacuum filtration. The residue was repeatedly washed with MeCN and dried in vacuo to give the coupled product as a white solid.

General procedure for deprotection of Fmoc

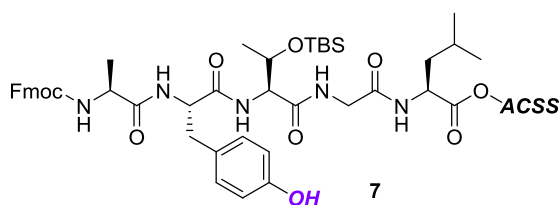
To a solution of supported peptide (1.0 mmol) in THF (19.4 mL) stirred at rt was added DBU (600 μL). The resulting reaction mixture was stirred at rt for 5 min, diluted with MeCN (60 mL), neutralized 1 N HCl (4 mL) and collected by vacuum filtration. The residue was repeatedly washed with MeCN and dried in vacuo to give the deprotected product as a white solid.

Tripeptide 3, Scheme S3



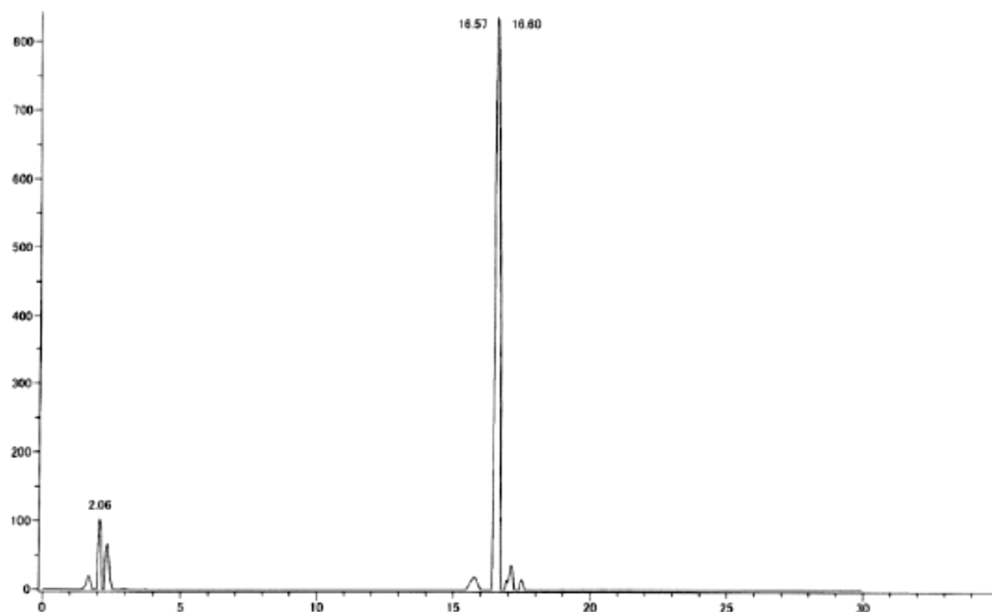
95% (from **S7** over 5 steps) as white solid. ^1H NMR (600 MHz, CD_3OD) δ 8.24 (0.5H, m), 7.82 (1H, m), 7.77 (2H, m), 7.63 (2H, m), 7.36 (2H, m), 7.28 (2H, m), 6.99 (2H, m), 6.65 (2H, m), 4.55 (1H, m), 4.34 (2H, m), 4.18-4.17 (3H, m), 3.04-3.02 (1H, m), 2.87-2.85 (1H, m), 1.99 (1H, m), 1.61 (1H, m), 1.51-1.45 (2H, m), 0.92-0.86 (12H, m); ^{13}C NMR (150 MHz, CD_3OD) δ 173.96, 173.10, 155.95, 144.06, 143.76, 141.26, 129.96, 127.42, 126.85, 124.87, 119.56, 114.86, 66.34, 58.45, 53.92, 53.64, 40.55, 36.25, 31.01, 24.54, 22.19, 20.50, 18.35, 17.29 ;IR ν_{max} (cm^{-1} , KBr) 3290.93, 3068.19, 2962.13, 2872.45, 1717.30, 1672.95, 1639.20, 1538.92, 1521.56, 1455.03, 1399.10, 1365.35, 1332.57, 1293.04, 1259.29, 1226.5, 1170.58, 1108.87, 1030.77, 986.41, 930.49, 830.21, 734.75, 684.61, 595.90, 528.40; HRMS $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{35}\text{H}_{41}\text{N}_3\text{O}_7\text{Na}$ 638.2837, found 638.2829.

Pentapeptide 7 in Figure S2

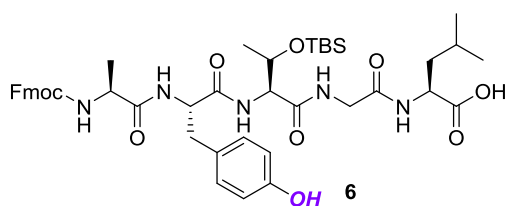


74% (from **S6** over 10 steps) as white solid. ^1H NMR (600 MHz, CDCl_3) δ 7.759 (2H, d, $J = 7.33$ Hz), 7.578 (2H, d, $J = 7.33$ Hz), 7.296 (2H, t, $J = 7.33$ Hz), 7.310 (2H, t, $J = 7.33$ Hz), 7.190 (1H, d, $J = 8.70$ Hz), 7.031 (2.5H, d, $J = 8.24$ Hz), 6.703 (2.5H, d, $J = 8.24$ Hz), 6.628 (1H, d, $J = 5.50$ Hz), 6.419-6.399 (2H, m), 6.208

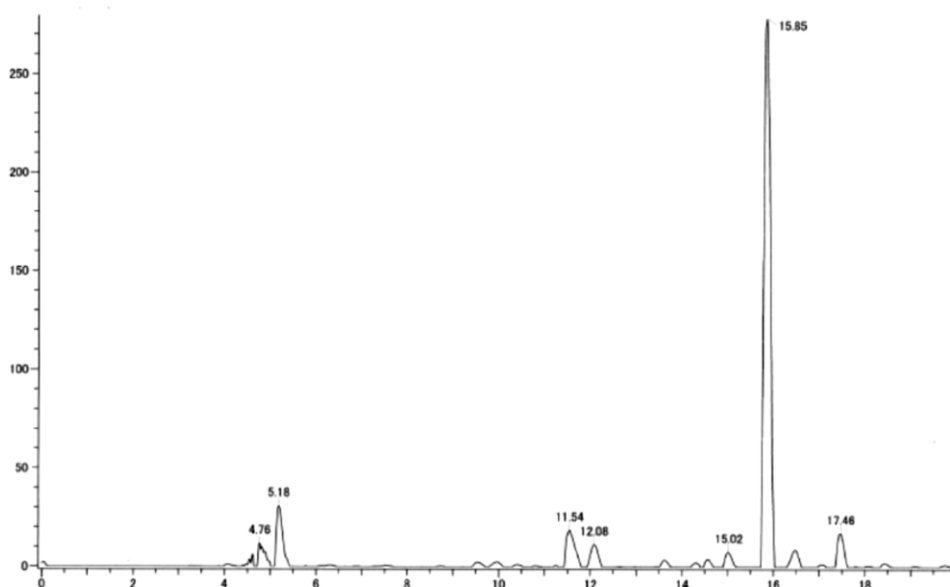
(1H, d, $J = 6.41$ Hz), 5.765 (1H, br), 5.288 (1H, br), 5.149 (2H, dd, $J = 41.67, 11.91$ Hz), 4.568-4.485 (2H, m), 4.436-4.315 (3H, m), 4.203 (3H, t, $J = 6.41$ Hz), 3.996 (1H, dd, $J = 16.03, 6.41$ Hz), 3.919 (4H, t, $J = 6.87$ Hz), 3.751-3.718 (5H, m), 3.534-3.459 (1H, m), 3.073 (1H, dd, $J = 13.28, 5.95$ Hz), 2.877-2.819 (1H, m), 1.841 (4H, t, $J = 6.41$ Hz), 1.753 (4H, ddd, $J = 15.11, 7.33, 6.41$ Hz), 1.655-1.498 (13H, m), 1.464-1.394 (4H, m), 1.365-1.179 (71H, m), 1.006 (2H, d, $J = 5.95$ Hz), 0.924-0.849 (12H, m), 0.814 (8H, s), 0.029 (3H, s), -0.044 (3H, s); ^{13}C NMR (150 MHz, CDCl_3) δ 171.281, 169.402, 160.974, 158.523, 141.437, 134.868, 130.329, 127.870, 127.212, 127.183, 125.085, 123.455, 120.108, 116.809, 104.519, 99.723, 68.212, 68.059, 63.235, 58.715, 51.297, 47.188, 43.412, 41.095, 39.295, 38.473, 34.164, 32.009, 29.787, 29.740, 29.692, 29.511, 29.463, 29.444, 29.358, 29.234, 26.145, 26.097, 25.773, 25.697, 24.906, 22.903, 22.770, 21.912, 17.907, 14.198, 11.471, 10.108, 6.142, -4.499, -5.119; IR ν_{max} (cm^{-1} , KBr) 3295.75, 3070.08, 2918.73, 2851.24, 2462.65, 2360.44, 2338.27, 2248.59, 1696.09, 1640.16, 1617.02, 1589.06, 1515.78, 1465.63, 1448.28, 1375.00, 1324.86, 1256.40, 1184.08, 1155.15, 1121.40, 1087.66, 1043.30, 980.63, 941.09, 834.06, 778.14, 738.60, 716.43, 665.32, 541.90; HRMS $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{96}\text{H}_{155}\text{N}_5\text{O}_{12}\text{SiNa}$ 1622.1367, found 1622.1362. Purity was checked by HPLC analysis using a C8 column with H_2O and $\text{THF}:\text{MeCN}=8:2$ (70 to 100%, 20 min) as an eluent at flow rate of 0.20 mL/min at 50 °C.



Pentapeptide 6 in Figure S2



99% (from **7** over 1 step) as white solid. HRMS $[M + Na]^+$ calculated for $C_{45}H_{61}N_5O_{10}SiNa$ 882.4080, found 882.4107. Purity was checked by HPLC analysis using a C18 column with 0.1% TFA/ H_2O and 0.1% TFA/MeCN (20 to 100%, 20 min) as an eluent at flow rate of 1.0 mL/min at 40 °C.



Bioconjugations and deprotection, see Scheme 4 and Table 2

A) Bioconjugations of tripeptide **3 and pentapeptide **6** to 5'-activated supported trinucleotide **2****

To a solution of 5'-activated supported trinucleotide **2** (0.025 mmol) in CH₂Cl₂ (10:1, v/v, 2 mL) stirred at rt was add 5 M peptide **3** or **6**/DMF (100 µL) and 0.25 M BMT/MeCN (200 µL). The resulting reaction mixture was stirred at rt for 30 min, followed by the addition of 0.67% BPO/DMP/CH₂Cl₂. The resulting reaction mixture was stirred at rt for 30 min, diluted with MeOH (10 mL), and collected by vacuum filtration. The residue was repeatedly washed with MeOH and dried in vacuo to give the desired bioconjugate as a white solid.

B) Bioconjugation of supported pentapeptide (7**) to 5'-activated supported trinucleotide **2****

To a solution of 5'-activated supported trinucleotide **2** (0.025 mmol) in CH₂Cl₂ (10:1, v/v, 6.4 mL) stirred at rt was add supported pentapeptide **7** and 0.75 M BMT/MeCN (200 µL). The resulting reaction mixture was stirred at rt for 30 min, followed by the addition of 0.67% BPO/DMP/CH₂Cl₂. The resulting reaction mixture was stirred at rt for 30 min, diluted with MeOH (30 mL), and collected by vacuum filtration. The residue was repeatedly washed with MeOH and dried in vacuo to give the desired bioconjugate as a white solid.

C) Bioconjugation of lipid **9 and suger **10** to 5'-activated supported trinucleotide **2****

To a solution of 5'-activated supported trinucleotide **2** (0.025 mmol) in CH₂Cl₂ (10:1, v/v, 2 mL) stirred at rt was add lipid **9** or suger **10** and 0.25 M BMT/MeCN (200 µL). The resulting reaction mixture was stirred at

r. for 30 min, followed by the addition of 0.67% BPO/DMP/CH₂Cl₂. The resulting reaction mixture was stirred at rt for 30 min, diluted with MeOH (10 mL), and collected by vacuum filtration. The residue was repeatedly washed with MeOH and dried in vacuo to give the desired bioconjugates as white solids.

D) Deprotection and cleavage of ACSS in tripeptide **3** conjugate

Protected tripeptide conjugates (5 μmol) was added to 28% NH₃ aq/EtOH (3:1, v/v). The resulting reaction mixture was stirred at 70 °C for 3 h, followed by evaporation. The residue was diluted with 50 mM AcOH/NH₃ buffer and purified by C18 column by using 20% MeCN/50 mM AcOH/NH₃ buffer as eluting solvent. The crude mixture was dried by lyophilization. The resulting crude mixture was purified by HPLC to give the desired bioconjugate **5**.

E) Deprotection and cleavage of ACSS in lipid **9** or sugar **10** conjugates

Protected lipid or sugar conjugates (5 μmol) was added to 28% NH₃ aq/EtOH (3:1, v/v). The resulting reaction mixture was stirred at 70 °C for 3 h, followed by evaporation. The residue was diluted with 50% MeCN/50 mM AcOH/NH₃ buffer or 50 mM AcOH/NH₃ buffer and filtered by membrane filter (0.45 μm). The filtrate solution was dried by lyophilization. The resulting crude mixture was purified by HPLC to give the desired bioconjugates **11** or **12**.

F) Deprotection and cleavage of ACSS in pentapeptide **7** conjugate

Protected pentapeptide conjugates (5 μmol) was added to 5% DCA/CH₂Cl₂ (1 mL). The resulting mixture was stirred at rt for 5 min, diluted with MeCN (5 mL), neutralized with TEA and collected by vacuum

filtration. The residue was added to CH₂Cl₂ (1 mL) and filtered by membrane filter (0.45 μm). The filtrate solution was evaporated and dried in vacuo. The crude mixture was added to 28% NH₃ aq/EtOH (3:1, v/v). The resulting reaction mixture was stirred at 70 °C for 3 h, followed by evaporation. The residue was diluted with 50 mM AcOH/NH₃ buffer and filtered by membrane filter (0.45 μm). The filtrate solution was dried by lyophilization. The resulting crude mixture was TEA 3HF (1 mL) and DMF (1 mL). The resulting mixture was stirred at rt for 24 h, diluted with 50 mM AcOH/NH₃ buffer (1 mL) and purified by C18 column by using 20% MeCN/50 mM AcOH/NH₃ buffer as eluting solvent. The crude mixture was dried by lyophilization. The resulting crude mixture was purified by HPLC to give the desired bioconjugate **8**.

Protected tripeptide conjugate

Crude product as white solid; ³¹P NMR (242.95 MHz, CDCl₃) δ 12.426, 11.722, 9.664, 9.069, -1.570- -3.194, -5.387, -7.959; HRMS [M + Na]⁺ calculated for C₁₅₃H₂₂₁N₁₈O₃₄P₃Na 2971.5256, found 2971.5280.

Deprotected tripeptide conjugate 5

White solid; HRMS [M - H]⁻ calculated for C₄₉H₆₇N₁₃O₂₄P₃ 1314.3640, found.1314.3623.

HPLC spectra are Figure 3 in the Manuscript and Figure S4.

Protected pentapeptide conjugate

Crude product as white solid. ³¹P NMR (242.95 MHz, CDCl₃) δ 9.637, 9.096, -1.651- -2.680; HRMS [M + Na]⁺ calculated for C₂₁₄H₃₃₅N₂₀O₃₉P₃SiNa 3953.3720, found 3953.3739.

Deprotected pentapeptide conjugate 8

White solid. HRMS $[M - H]^-$ calculated for $C_{53}H_{73}N_{15}O_{27}P_3$ 1444.4019, found 1444.4007.

HPLC spectra are Figure S1 and S4.

Protected lipid conjugate

Crude product as white solid. ^{31}P NMR (242.95 MHz, $CDCl_3$) δ 9.967-9.806, 9.402-9.321, -1.104- -1.993;

HRMS $[M + Na]^+$ calculated for $C_{145}H_{226}N_{15}O_{28}P_3Na$ 2741.5827, found 2741.5825.

Deprotected lipid conjugate 11

White solid. HRMS $[M - H]^-$ calculated for $C_{56}H_{82}N_{10}O_{20}P_3$ 1307.4925, found 1307.4902.

HPLC spectra are Figure S2 and S4.

Protected sugar conjugate

Crude product as white solid. ^{31}P NMR (242.95 MHz, $CDCl_3$) δ 9.536, 9.051, -1.078- -2.829; HRMS $[M +$

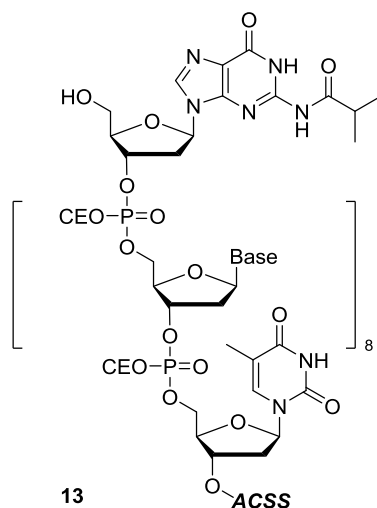
$Na]^+$ calculated for $C_{132}H_{200}N_{15}O_{37}P_3Na$ 2704.3368, found 2704.3371.

Deprotected sugar conjugate 12

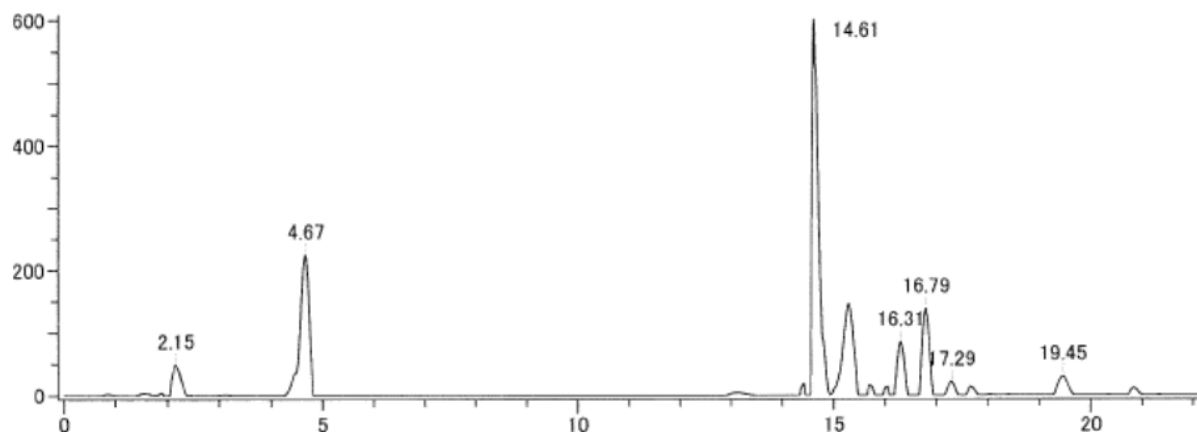
White solid. HRMS $[M - H]^-$ calculated for $C_{35}H_{48}N_{10}O_{25}P_3$ 1101.2010, found 1101.1994.

HPLC spectra are Figure S3 and S4.

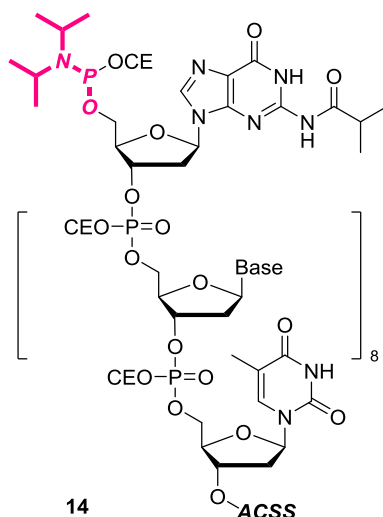
Supposed decanucleotide 13, Scheme S7



Carried out according to the general elongation of oligonucleotide and deprotection of DMTr. 54% yield (0.163 mmol) from **S3** (0.3 mmol) as white solid. ^{31}P NMR (242.95 MHz, CDCl_3) δ -2.020; HRMS $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{243}\text{H}_{310}\text{N}_{49}\text{O}_{71}\text{P}_9\text{Na}$ 5351.9684 found 5351.9656. Purity was checked by HPLC analysis using a C8 column with H_2O and $\text{THF}:\text{MeCN}=8:2$ (70 to 100%, 20 min) as an eluent at flow rate of 0.20 mL/min at 50 °C.



5'-Phosphitylation of the Supported decanucleotide 14, see Scheme 3



Carried out according to the procedure of 5'-phosphitylation of supported trinucleotide. 88% yield as white solid. ^{31}P NMR (242.95 MHz, CDCl_3) δ 149.839, 149.000, 14.402, -2.003- -2.139.

Bioconjugations and deprotection, see Scheme 4

All protocol was carried out according to the procedure of pentapeptide conjugate (8).

Protected pentapeptide conjugate 15

Crude product as white solid. ^{31}P NMR (242.95 MHz, CDCl_3) δ 10.048, 9.159, -2.128; HRMS $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{342}\text{H}_{467}\text{N}_{55}\text{O}_{85}\text{P}_{10}\text{SiNa}$ 7065.0949, found 7065.0951.

Deprotected pentapeptide conjugate 16

White solid. HRMS $[\text{M} - \text{H}]^-$ calculated for $\text{C}_{121}\text{H}_{158}\text{N}_{43}\text{O}_{67}\text{P}_{10}$ 3594.7660, found 3594.7685.

HPLC spectra are Figure 4 in the Manuscript and S4.

(1) Kim, S.; Matsumoto, M.; Chiba, K. *Chem. Eur. J.* **2013**, *19*, 8615 – 8620.

