Supporting Information

Ketone-DNA: A Versatile Post-synthetic DNA Decoration Platform

Subhakar Dey and Terry L. Sheppard*

Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208-3113

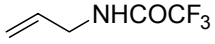
Experimental Protocols

General considerations. All reported melting points were determined using a Mel-Temp capillary melting point apparatus and are not corrected. ^{1}H-NMR spectra were recorded on a Mercury 400 (Varian) spectrometer at 400-MHz for ^{1}H (100-MHz for ^{13}C-NMR, 161.9-MHz for ^{31}P-NMR and 376.4-MHz for ^{19}F-NMR), unless noted otherwise. Chemical shifts were reported in parts per million (ppm) on the δ scale relative to residual proton signals: $CDCl_3$ (δ 7.26), CD_2Cl_2 (δ 5.32), DMSO-d_6 (δ 2.49), CD_3CN (δ 1.94) or TMS (δ 0.00). ^{13}C-NMR spectra were reported in ppm on the δ scale relative to: $CHCl_3$ (δ 77.0), CD_3CN (δ 1.39), CD_2Cl_2 (δ 54.0), or DMSO-d_6 (δ 39.5). ^{19}F-NMR and ^{31}P-NMR spectra were reported in ppm on the δ scale relative to $CFCl_3$ (δ 0.00) and H_3PO_4 (δ 0.00) respectively as external references. All NMR experiments were performed at RT. Proton assignments were based on COSY experiments. Splitting patterns are designated as follows: singlet (s), doublet (d), triplet (t), quintuplet (q), multiplet (m). High-resolution mass spectral (HRMS) data were obtained on a micromass ZAB-SE spectrometer and are reported in units of *m/z* for M^+ or the highest mass fragment derived from M. Abbreviations used: DMAP = 4-dimethylaminopyridine, DIPCDI = 1,3-diisopropylcarbodiimide, HOBt = 1-hydroxybenzotriazole, TFA = trifluoroacetic acid.

Solvents and Reagents. All moisture-sensitive reactions were performed in an inert, dry atmosphere of N_2. Reagent grade solvents were used for either chromatography or extraction. CH_2Cl_2, and pyridine were distilled over CaH_2 (0-1 mm grain size) under an inert atmosphere (N_2). DMTCl (Chemgenes) was crystallized from CH_2Cl_2-hexanes. *Bis*-(diisopropylamino)-2-cyanoethoxyphosphine was obtained from Chemgenes. Diisopropyl-ammonium tetrazolide was synthesized in our laboratory following a standard procedure.[1] DNA synthesis reagents were

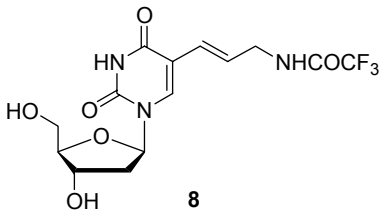
obtained from Glen Research. *N*-(aminooxyacetyl)-*N′*-(D-biotinoyl) hydrazine, trifluoroacetic acid salt (ARP) (**2**) was obtained from Molecular probes Inc. 7-Amino-4-methylcoumarin-3-acetyl (AMCA) hydrazide was obtained from Pierce. Amino acid derivatives, peptide coupling reagents and resin were obtained from Calbiochem-Novabiochem. All other chemicals were obtained from Aldrich chemical company. T4 polynucleotide kinase was obtained from US Biochemical and [γ-^{32}P] ATP (7000 Ci/mmol) was purchased from ICN.

Chromatography. Thin-layer chromatography (TLC) analysis was performed using silica gel 60 F-254 plates (0.25 mm thickness, EM Science). The plates were visualized first with UV illumination followed by charring with either **A**: 0.3% (w/v) ninhydrin solution in (97:3) EtOH-AcOH, **B**: 5% *p*-anisaldehyde in 95:5:1 EtOH-AcOH-H_2SO_4, or **C**: "Verghn's reagent"[2] (12.5 g ammonium molybdate and 0.5 g ceric sulfate dissolved in 250 mL of 10% aq H_2SO_4). Flash chromatography was performed using silica gel (230-400 mesh, Bodman Industries).



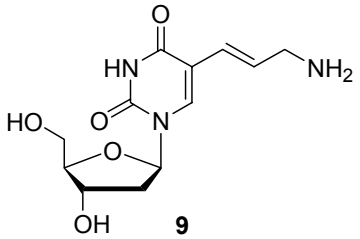
7

N-allyltrifluoroacetamide (7). Trifluoroacetic anhydride (37.2 mL, 263.2 mmol) was added drop-wise, via a syringe, over a period of 45 min to an ice-cold solution of allylamine (40 mL, 533 mmol) under N_2 atmosphere with stirring. The reaction mixture then was warmed gradually to RT and stirred for another 11 h. Product **7** (38.1 g, 95%) was isolated from the colorless viscous liquid by a short-path distillation under reduced pressure (0.1-0.5 mm Hg, 90 °C bath temperature). A dry ice-acetone bath was used to cool the collection flask during distillation. ^{19}F-NMR ($CDCl_3$) δ -76.54. ^{1}H NMR data were in accordance with literature values.[3] Density was determined by weighing 1 mL of the liquid, d = 1.234 g mL^{-1}.



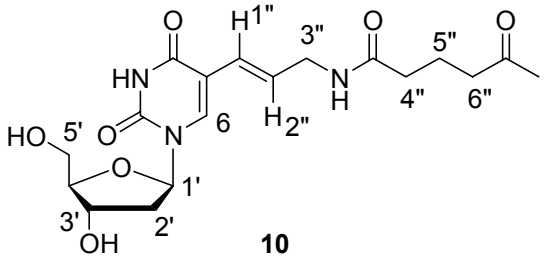
8

5-(3''-trifluoroacetamidoallyl)-2'-deoxyuridine (8). 5-iododeoxyuridine **6** (2.0 g, 5.6 mmol) was dissolved in 20 mL of warm (70 °C) DMF and then cooled to RT. To the cooled solution, NaOAc buffer (20 mL, 0.1 M, pH = 5.2) and *N*-allyltrifluoroactamide **7** (5.6 mL, 47.6 mmol) were added. The solution was purged with nitrogen and a solution of $Na_2[PdCl_4]$ (1.41 g, 4.82 mmol) in DMF (20 mL) was added while stirring vigorously. The reaction flask then was placed in a preheated (80 °C) oil bath. After 2 h, TLC analysis (9:1 CH_2Cl_2-MeOH; 5 cm plate, double development; R_f(starting material) = 0.44, green: char **B**; R_f (product) = 0.40, purple: char **B**) showed complete consumption of the starting material to product. The reaction mixture was concentrated by rotary evaporation to a thick-brown oil. The oil was purified by flash chromatography on silica gel (6 x 25 cm bed, sample was loaded onto the column with 1:3 MeOH-CH_2Cl_2. Column was packed and eluted with 1.5:8.5 MeOH-CH_2Cl_2, collecting 50 mL fractions. Fractions 23-33 contained TLC pure material.) to obtain 1.62 g (76%) of **8**. The reaction profile was same irrespective of the scale of the reaction. ^{1}H NMR data were in accordance with literature values.[3]



9

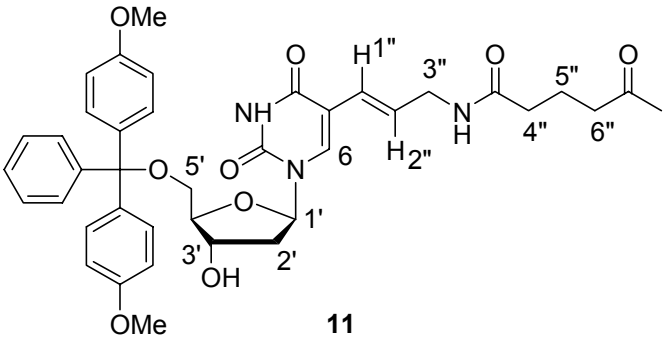
5-(3''-aminoallyl)-2'-deoxyuridine (9). Compound **8** (113 mg, 0.30 mmol) was treated with 50 mL of ammonium hydroxide (28% aq. solution) in a tightly capped flask for 8 h. TLC analysis showed complete consumption of the starting material to product. After rotary evaporation of the ammonium hydroxide, the product **9** was isolated as a white foam (114 mg), which was used in the next reaction without further purification. R_f = 0.60 (6:2:1 MeOH-EtOAc-NH_4OH, char **A**).



10

5-(3''-δ-ketohexanamidoallyl)-2'-deoxyuridine (10). Compound **9** (1.036 g, 3.66 mmol) was dissolved in DMF (10 mL) and DIPEA was added (956 µL, 5.49 mmol), followed by a solution of 5-oxohexanoic acid anhydride[4] (1.29 g, 5.68 mmol) in DMF (15 mL) and stirred at RT for 1 h. TLC analysis showed complete consumption of starting material to a single product. After evaporation of DMF, the oily residue was purified by flash chromatography on silica gel (4 x 27 cm bed, column was packed, loaded, and eluted with 1.5:8.5 MeOH-CH_2Cl_2, collecting 50 mL fractions. Fractions 21-32 contained TLC pure material) to obtain 1.316 g (91%) of **10**. R_f = 0.31, 1.5:8.5 MeOH-CH_2Cl_2, char **B**; mp 154-155 °C; ^{1}H-NMR (DMSO-d_6) δ 11.40 (s, 1H, NH), 8.00 (s, 1H, H-6), 7.98 (t, J = 5.6 Hz, 1H, NH), 6.38 (m, 1H, H-2″), 6.01-6.16 (2H, H-1′ + H-1″), 5.24 (s, 1H, -OH), 5.11 (s, 1H, -OH), 4.24 (broad s, 1H, H-3′), 3.77 (m, 1H, H-4′), 3.71 (t, J = 5.6 Hz, 2H, H-3″), 3.61 (dd, 1H, $J_{5'a,4'}$ = 2.8 Hz, $J_{5'a,5'b}$ = 11.6 Hz, H-5′a), 3.55 (dd, 1H, $J_{5'b,4'}$ = 2.8 Hz, $J_{5'a,5'b}$ = 11.6 Hz, H-5′b), 2.40 (t, J = 6.8 Hz, 2H, H-4″), 2.04-2.13 (7H, 2 x H-6″ + 2 x H-2′ + COC<u>H</u>$_3$), 1.65 (q, J = 7.6 Hz, 2H, H-5″); ^{13}C-NMR (DMSO-d_6) δ 207.9, 171.2, 161.9, 149.3,

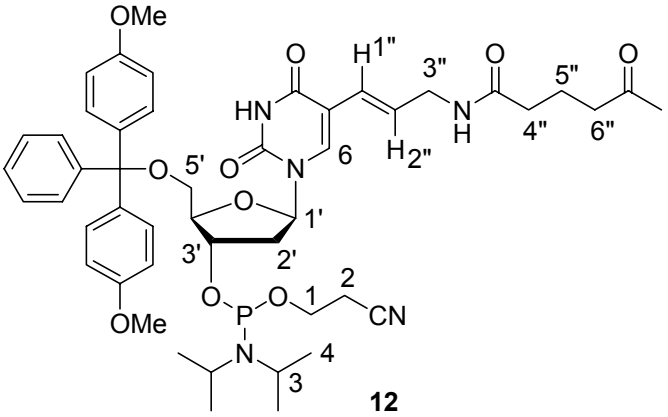
137.1, 126.6, 122.1, 110.2, 87.4, 84.2, 70.2, 61.1, 42.1, 40.9, 34.4, 29.8, 19.5; FAB (*m/z*): MNa^+ calculated for $C_{17}H_{23}N_3O_7Na$, 418.1590; found, 418.1598.



11

5'-O-(4-dimethoxytrityl)-5-(3"-δ-ketohexanamidoallyl)-2'-deoxyuridine (11). 4,4'-dimethoxytriphenylmethyl chloride (DMTCl, 469 mg, 1.38 mmol) was added as a solution in pyridine (5 mL), while stirring, to an ice cold solution of **10** (456 mg, 1.15 mmol) and DMAP (35 mg, 0.287 mmol) in 5 mL of dry pyridine. The ice-bath was removed after 5 min and the reaction was continued for another 11 h at RT, when TLC analysis showed formation of a single product with complete consumption of the starting material. MeOH (10 mL) was added to the reaction mixture and solvents were removed by rotary evaporation. The light-yellow oily residue was purified by flash chromatography on silica gel (4 x 16 cm bed, column was packed, loaded, and eluted with 1:9 MeOH-CH_2Cl_2 + 1% Et_3N (v/v), collecting 30 mL fractions. Fractions 6-20 contained TLC pure **11** (450 mg). Repurification of fraction-5 gave another 264 mg of pure product.). Net yield = 89%. R_f = 0.70, 1:9 MeOH-CH_2Cl_2 + 1% Et_3N (v/v), char **B**; mp 91-92 °C; ^{1}H-NMR (CD_2Cl_2) δ 9.21 (s, 1H, NH), 7.74 (s, 1H, H-6), 7.26-7.44 (9H, Ar-H), 6.85-6.87 (4H, Ar-H), 6.25-6.36 (2H, H-1′ + H-2″), 5.56-5.60 (apparent d, 1H, H-1″), 5.52 (t, 1H, J = 5.6 Hz, NH), 4.55 (m, 1H, H-3′), 4.07 (m, 1H, H-4′), 3.79 (s, 6H, 2 x O$\underline{CH_3}$), 3.59 (m, 2H, H-3″), 3.42 (dd, 1H, $J_{5'a,4'}$ = 3.2 Hz, $J_{5'a,5'b}$ = 10.4 Hz, H-5′a), 3.34 (dd, 1H, $J_{5'b,4'}$ = 3.2 Hz, $J_{5'a,5'b}$ = 10.4

Hz, H-5′b) 2.86 (m, 1H, -OH), 2.43 (t, 2H, *J* = 6.8 Hz, H-4″), 2.29-2.46 (m-under peak at 2.43, 2H, 2 x H-2′), 2.09 (s, 3H, $COC\underline{H}_3$), 1.96 (t, 2H, *J* = 7.6 Hz, H-6″), 1.76 (q, 2H, *J* = 7.6 Hz, H-5″); ^{13}C-NMR (CD_2Cl_2) δ 208.6, 172.1, 162.2, 159.0, 149.8, 144.8, 137.2, 135.9, 135.8, 130.4, 130.3, 129.4, 128.4, 128.0, 127.4, 123.1, 113.7, 112.0, 87.2, 86.7, 85.4, 72.5, 64.0, 55.8, 43.0, 42.2, 41.7, 35.6, 30.3, 20.1; FAB (*m/z*): MNa^+ calculated for $C_{39}H_{43}N_3O_9Na$, 720.2897; found, 720.2899.



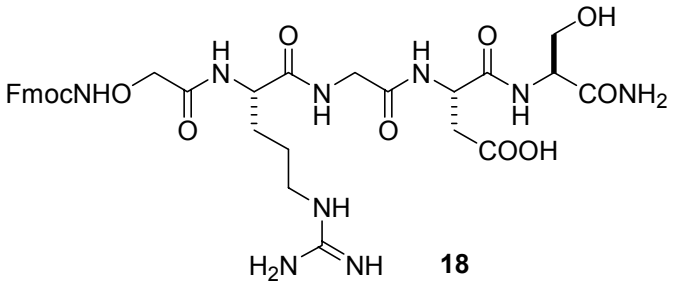
12

3'-O-(2-cyanoethyl N,N-diisopropylphosphoramidyl)-5'-O-(4-dimethoxytrityl)-5-(3''-δ-ketohexanamidoallyl)-2'- deoxyuridine (12), (mixture of diastereomers). To a stirred solution of **11** (450 mg, 0.644 mmol) and diisopropyl-ammonium tetrazolide (55 mg, 0.323 mmol) in 3.5 mL of dry CH_2Cl_2, *bis*-(diisopropylamino)-2-cyanoethoxyphosphine (243 µL, 0.708 mmol) was added. After 1h, TLC analysis showed the presence of starting material, and another 0.2 equivalents (42 µL, 0.128 mmol) of *bis*-(diisopropylamino)-2-cyanoethoxyphosphine added. After another hour TLC analysis showed complete consumption of the starting material. The reaction mixture was diluted with 100 mL of CH_2Cl_2 and the organic layer was washed with $NaHCO_3$ solution (2% aq. soln, 2 x 30 mL) followed by brine (30 mL), dried over Na_2SO_4 and evaporated to a white foam. Product was purified by flash chromatography on silica gel (4 x 16

cm bed, column was packed, loaded, and eluted with 1:4 acetone-EtOAc, collecting 75 mL fractions. Fractions 4-8 contained TLC pure material.) to give 492 mg (85%) of **12**. R_f = 0.49, 1:9 acetone-EtOAc, char **B**; ^{1}H-NMR (CD_3CN, 500 MHz) δ 9.53 (s, 1H, NH), 7.65, 7.62 (s, 1H, H-6), 7.23-7.47 (9H, Ar-H), 6.85-6.88 (4H, Ar-H), 6.28 (m, 1H, H-2″), 6.18-6.24 (2H, NH + H-1′), 5.72 (m, 1H, H-1″), 4.61 (m, 1H, H-3′), 4.12, 4.09 (m, 1H, H-4′), 3.75, 3.74 (s, 6H, 2 x $OC\underline{H}_3$), 3.52-3.66 (6H, 2 x H-1, 2 x H-3, 2 x H-3″), 3.33 (m 2H, 2 x H-5′), 2.63, 2.51 (t, *J* = 6.0 Hz, 2 x H-2), 2.40 (t, 2H, *J* = 7.0 Hz, 2 x H-4″), 2.05 (s, 3H, $COC\underline{H}_3$), 1.99 (m, 2H, 2 x H-6″), 1.70 (q, 2H, *J* = 7.5 Hz, H-5″), 1.02-1.16 (12H, 12 x H-4); ^{13}C-NMR (CD_3CN, 125 MHz) δ 209.3, 172.9, 163.1, 159.7, 150.6, 145.9, 136.7, 136.6, 136.5, 131.1, 129.0, 128.6, 128.5, 128.0, 123.5, 123.4, 179.6, 119.4, 114.2, 112.3, 112.2, 86.2, 85.9, 85.8, 74.22, 73.8, 73.7, 64.8, 64.2, 64.0, 59.5, 59.4, 56.0, 55.9, 44.0, 43.9, 43.1, 42.2, 40.4, 40.2, 35.7, 31.4, 30.1, 25.0, 24.9, 24.8, 21.1, 21.0, 20.9, 20.5, 19.8, 14.1; ^{31}P-NMR (CD_2Cl_2) δ 148.53, 148.36; FAB (*m/z*): MNa^+ calculated for $C_{48}H_{60}N_5O_{10}PNa$, 920.3975; found, 920.3979.

Synthesis of aminooxy conjugates.

Aminooxy RGD-peptide conjugate (18).[5]

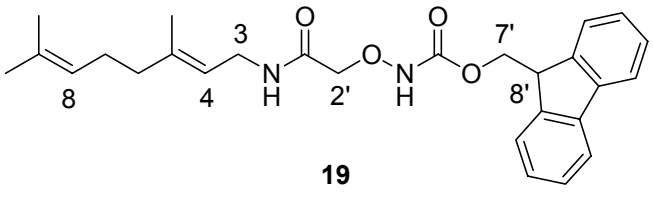


18

Aminooxy-RGD-peptide was assembled on Rink amide-MBHA resin (0.054 mmol scale) as described.[6] However, PyBOP was used as a coupling agent instead of HATU. For $FmocNHOCH_2COOH$[7] coupling, a combination of HOBt and DIPCDI was used as the activating

agent. The following amino acid derivatives were used for the synthesis: Fmoc-Ser(Trt)-OH, Fomc-Arg(Pbf)-OH, Fmoc-Asp-(O-Bu^t)-OH, Fmoc-Gly-OH and Fmoc-NHO-CH_2-COOH. Deprotection and cleavage of the peptide from the resin was affected by 95% TFA (4 mL) for 30 min followed by filtration (nylon filter) and evaporation of TFA under N_2 flow. Crude peptide then was precipitated by Et_2O (5 mL), dissolved in water (2 mL) and extracted twice with EtOAc (3 mL). The aqueous layer was filtered through a C-18 cartridge (2 x 5 mm, 40 µm particle size) and the cartridge was washed with acetonitrile (2 x 0.5 mL). After removal of acetonitrile under reduced pressure, the peptide was purified by reverse phase HPLC to give 13 mg of purified peptide after lyophilization (Vydac 218 TP1022, 19 x 250 mm column; flow = 10 mL/min, solvent A = 0.1% TFA in water; solvent B = 0.1% TFA in acetonitrile; gradient: 3% increment in B/min, starting from 15% B; detection wavelength = 220 and 254 nm, t_R = 13.5 min). FAB (*m/z*): MH^+ calculated for $C_{32}H_{42}N_9O_{11}$, 728.3004; found, 728.3000.

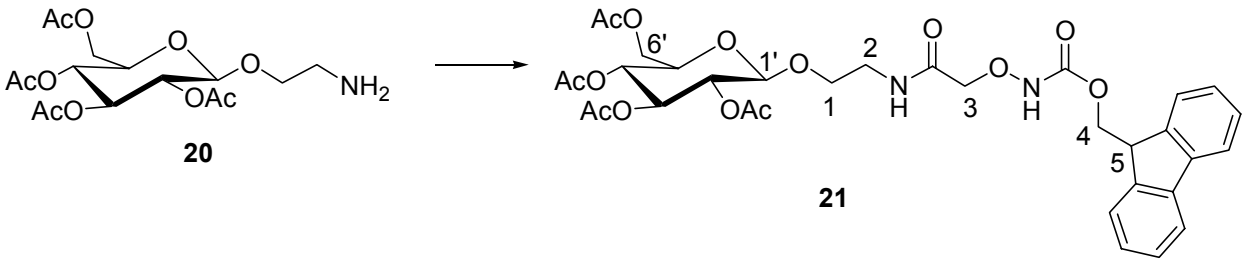
***Aminoxy geranylamine conjugate (19*)**. Fmoc-aminooxy acetic anhydride: To an ice cold solution of FmocNHOCH$_2$-COOH (1 g, 3.19 mmol) in 250 mL of dry THF, was added a solution of DCC (323 mg, 1.563 mmol) in THF (5 mL). The reaction was stirred for 19 h at RT. DCU formed in the reaction was removed by filtration. Removal of solvent produced a white foam, which was used immediately in the next reaction.



19

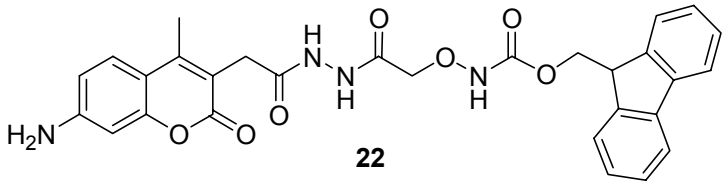
To a solution of Fmoc-aminooxy acetic anhydride (485 mg, 0.79 mmol) in 3 mL of THF, geranyl amine (200 µL, 1.08 mmol) was added. After 1 h, TLC analysis showed formation of one major product. The solvent was evaporated and the organic residue was dissolved in EtOAc (100 mL),

washed with HCl (1N aq., 25 mL), $NaHCO_3$ (2% aq. soln., 2 x 50 mL) and brine (3 x 50 mL). The EtOAc layer was dried over Na_2SO_4 and evaporated to an off-white solid. The product **19** was purified by flash chromatography on silica gel (3 x 17 cm bed, column was packed and eluted with 1:1 hexanes-EtOAc, sample loaded with $CHCl_3$, collecting 50 mL fractions and fractions 8-12 contained TLC pure material) to give 305 mg (71%) of pure **19**. R_f = 0.61, 1:4 hexanes-EtOAc, char **C**; mp 176-176 °C; ^{1}H-NMR ($CDCl_3$) δ 8.50 (s, 1H, NH), 7.25-7.77 (8H, Ar-H), 5.19 (t, 1H, *J* = 8 Hz, H-4), 5.05 (m, 1H, H-8), 4.47 (d, 2H, *J* = 6.8 Hz, 2 x H-7′), 4.34 (s, 2H, 2 x H-2′), 4.21 (t, 1H, *J* = 6.8 Hz, H-8′), 3.90 (t, 2H, *J* = 6.0 Hz, 2 x H-3), 3.49 (m, 1H, NH), 1.06-2.07 (13H, 3 x CH_3 + 2 x CH_2); ^{13}C-NMR ($CDCl_3$) δ 167.9, 158.0, 142.9, 141.1, 139.6, 131.4, 127.8, 127.0, 124.7, 123.7, 119.9, 119.2, 76.1, 68.0, 49.1, 46.8, 39.5, 37.1, 33.9, 26.5, 25.7, 25.0, 17.7, 16.4; FAB (*m/z*): MH^+ calculated for $C_{27}H_{33}N_2O_4$, 449.2440; found, 449.2439.



Aminooxy glucose conjugate (21). To a solution of FmocNHO-CH_2-COOH (150 mg, 0.48 mmol) and HOBt (65 mg, 0.48 mmol) in 4 mL of 1:1 CH_2Cl_2-DMF, DIPCDI (40 µL, 0.24 mmol) was added and mixed for 5 min. The solution then was added to the amine **20**[8] (187 mg, 0.48 mmol) and stirred for 2 h, when TLC analysis showed formation of one product. Solvents were removed under reduced pressure and the oily residue was purified by flash chromatography on silica gel (2.5 x 25 cm bed, column was packed, loaded and eluted with EtOAc, collecting 50 mL fractions. Fractions 3-4 contained TLC pure material) to give 182 mg (55%) of pure **21**. R_f = 0.48, EtOAc, char **C**; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.36 (s, 1H, NH), 7.76 (d, 2H, *J* = 7.0 Hz,

Ar-H), 7.57 (d, 2H, J = 8.0 Hz, Ar-H), 7.40 (t, 2H, J = 7.5 Hz, Ar-H), 7.31 (t, 2H, J = 7.5 Hz, Ar-H), 5.20 (t, 1H, J = 9.5 Hz), 5.06 (t, 1H, J = 9.5 Hz), 4.99 (dd, 1H, J_1 = 8.0 Hz, J_2 = 9.5 Hz), 4.51-5.42 (3H), 4.34 (s, 2H, H-3), 3.40-4.25 (8H), 2.06 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 170.8, 170.2, 169.2, 169.3, 168.6, 157.9, 143.2, 141.3, 127.9, 127.1, 124.9, 120.1, 120.0, 100.7, 75.9, 72.5, 71.8, 71.2, 68.3, 68.2, 67.8, 61.7, 46.8, 38.8, 20.7, 20.6, 20.5; FAB (*m/z*): MH^+ calculated for $C_{33}H_{39}N_2O_{14}$, 687.2401; found, 687.2399.



22

Aminooxy AMCA-hydrazide conjugate (22). FmocNHO-CH_2-COOH (2.55 mg, 8.08 mmol), HOBt (1.09 mg, 8.08 mmol) and DIPCDI (1.26 µL, 8.08 mmol) were dissolved in DMF-DCM (1:1, 50 µL) and mixed for 10 min. The solution was then added to AMCA-hydrazide (2 mg, 8.08 mmol in 100 µL of DMSO), mixed, and allowed to stand for 2 h at RT. TLC analysis showed formation of a major product. Product **22** was purified by flash chromatography on silica gel (0.5 x 13 cm bed, column was packed, loaded and eluted with EtOAc) to give 3.8 mg of **22** (95%). R_f = 0.44, EtOAc, char **C**, fluorescent at 365 nm; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 9.96 (s, 1H), 9.87 (s, 1H), 7.88 (d, 2H, J = 7.5 Hz), 7.68 (d, 2H, J = 7.5 Hz), 7.45 (d, 1H, J = 8.5 Hz), 7.40 (t, 2H, J = 7.5 Hz), 7.31 (t, 2H, J = 7.5 Hz), 6.56 (d, 1H, J = 8.5 Hz), 6.39 (s, 1H), 6.05 (s, 2H), 4.36 (d, 2H, J = 7.5 Hz), 4.27 (s, 2H), 4.23 (t, 1H, J = 7.0 Hz), 3.47 (s, 2H), 2.27 (s, 2H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 161.6, 154.2, 152.5, 150.36, 143.51, 142.6, 140.7, 139.42, 137.42, 128.9, 127.30, 126.31, 121.4, 120.1, 112.5, 111.3, 109.8, 109.3, 98.3, 73.4, 21.9, 14.9. FAB (*m/z*): MH^+ calculated for $C_{29}H_{26}N_4O_7$, 543.1880; found, 543.1880.

General method for Fmoc group removal from aminooxy conjugates. Fmoc-aminooxy derivative **18**, **19**, **21** or **22** was treated with 2% DBU solution in DMF (4 equivalents) for 8 min, followed by quenching of the reaction by acetic acid.

For **1**, (2.2 mg) DMF was removed under reduced pressure and 440 μL of water added to the reaction mixture. The aqueous layer was extracted with Et_2O (2 x 300 μL) and pH was adjusted by NH_4OH to 6.70.

For **21**, after quenching, NH_4OH (700 μL) was added to the reaction mixture (53 μmole scale) and allowed to stand at RT for 3 h to obtain **3**. After removal of ammonia and solvent by Speedvac concentrator, the residue was dissolved in 1 mL of 9:1 DMF-water and the pH was adjusted by AcOH (2μL) to 7.60.

For **4** and **5**, the pH was adjusted to 6-7 (in DMF) by dilute NH_4OH or AcOH.

DNA synthesis, deprotection, and purification. Standard unmodified DNA oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, Iowa). Modified oligonucleotides were synthesized in > 99% average coupling efficiency on a Pharmacia Gene Assembler Plus at the 1.3 μmole scale using phosphoramidites with labile base protecting groups for the natural nucleotides (Glen Research, "Ultramild": Ac for dC, *i*-Pr-PAC for dG, and PAC for dA). After the synthesis, the oligonucleotide was deprotected and cleaved from the sold support by treatment with 1.5 mL of 28% aqueous NH_3 at 37 °C for 16 h. Following deprotection, excess NH_3 was removed using a Speedvac concentrator, the solid support was filtered and washed with H_2O (2 x 500 μL) and the resulting aqueous solution (~1.5 mL) was filtered through a C-18 cartridge (2 x 0.5 cm bed, 40 μm particle size). Finally the C-18 cartridge was washed with H_2O-CH_3CN (1:3, 3 x 1 mL) and washings were united with the aqueous filtrate. After removal of

excess CH_3CN, purification was achieved by either PAGE (see under gel electrophoresis) or RP-HPLC.

Crude *ketone*-DNAs were analyzed (Figure S1) by RP-HPLC to assess the purity. (C18-XTerra, 4.6 x 50 mm column; flow = 1 mL/min, solvent A = 0.1 M triethylammonium acetate in water, pH = 7.2; solvent B = A + 25% acetonitrile, pH = 7.2; gradient: 1.66% increment in

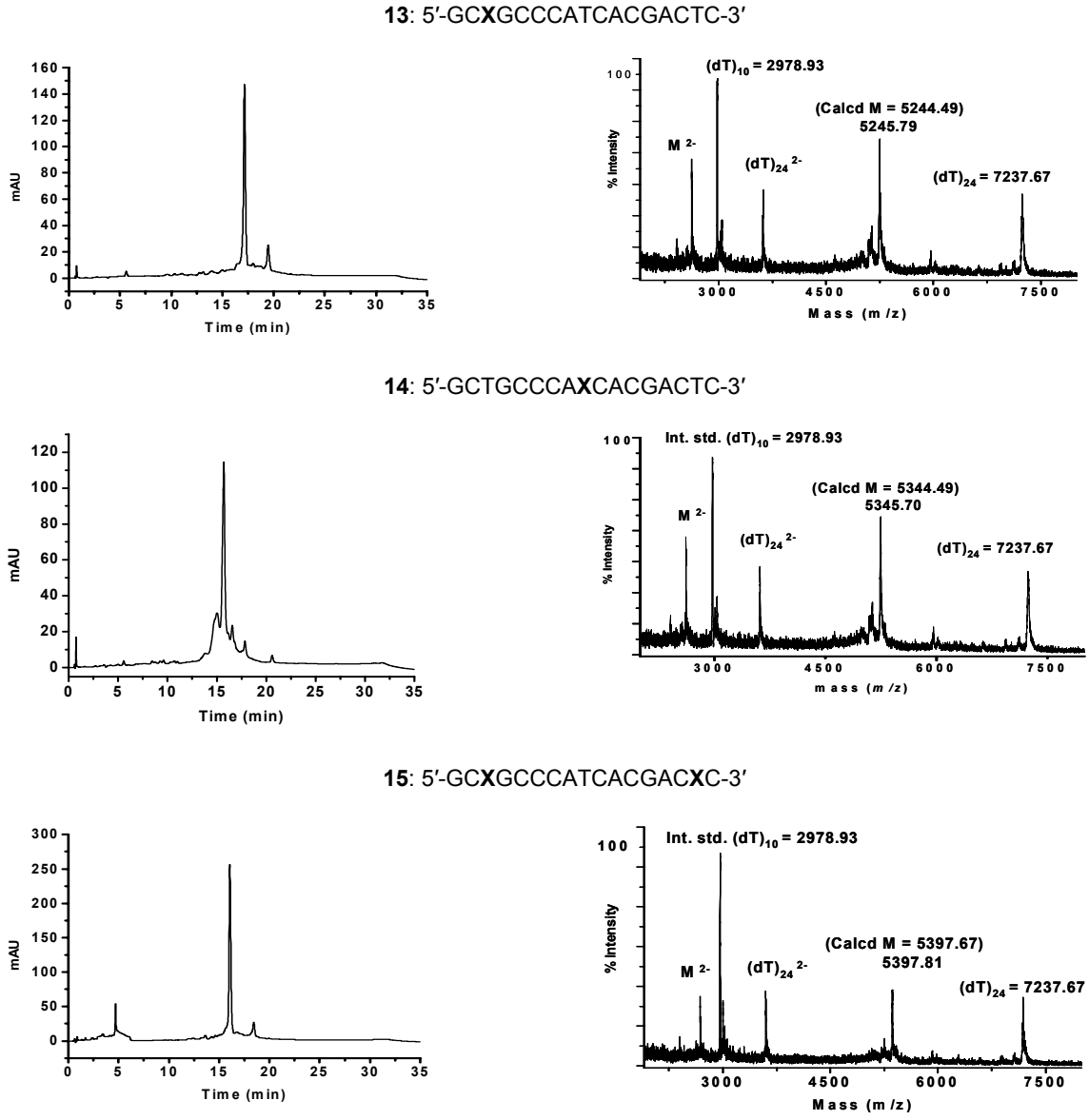


Figure S1. RP-HPLC profile (left panels) and MALDI-TOF mass (right panels) analysis of the crude *ketone*-DNAs **13**, **14**, and **15**.

B/min, starting from 15 % B; detection wavelength = 254 nm). *Ketone*-DNA: **13**, t_R = 17.1 min; **14**, t_R = 15.7 min; **15**, t_R = 16.1 min.

Ketone-DNAs were also purified by semi-preparative RP-HPLC and stored as lyophilized powders at -20 °C. (Vydac 218 TP1022, 19 x 250 mm column; flow = 10 mL/min, solvent A = 0.1 M triethylammonium acetate in water, pH = 7.2; solvent B = A + 25% acetonitrile, pH = 7.2; gradient: 1.4% increment in B/min, starting from 35 % B; detection wavelength = 254 nm). All purified oligonucleotide solutions were quantitated by measuring the UV absorption at 260 nm and using extinction coefficients calculated by the nearest-neighbors method.[9] The *ketone* nucleoside analog was assigned an extinction coefficient equivalent to a thymidine residue.

Oligonucleotide 5′-radiolabeling. Oligonucleotide (20 pmol) was incubated at 37 °C for 40 min with 24.5 units of polynucleotide kinase (USB) in a 20 μL reaction containing 10 mM Tris-acetate, 10 mM magnesium acetate, and 50 mM potassium acetate and 24 pmol (8.4 Ci/μL) [γ-^{32}P]ATP (7000 Ci/mmol). The reaction was purified by QIAquick nucleotide removal kit using protocol described by the supplier.[10] The labeled oligonucleotides were quantitated by liquid scintillation counting.

Aminooxy-conjugate ligation procedure. *Ketone*-DNAs (single strand concentration = 5 μM) were incubated with 100-fold excess of the modifiers in the appropriate buffer for the specified time at 37 °C. Authentic pH values of the reaction medium (at specified temperatures) or reagents were recorded with a micro-pH electrode (Orion, Beverly, MA). The final volume of all reactions was 175 μL. For reactions monitored by gel electrophoresis, 6400 kcpm of 5′-labeled DNAs were added to each reaction mixture–as tracer.

pH dependent ligation: The following buffers were used to perform the ligation reaction, as described above, between *ketone*-DNA **13** and aminooxy RGD-peptide **1**. In all cases 10× buffer was diluted by water followed by addition of **13** and **1**.

(i) Acetate buffer: 50 mM acetate. Ionic strength due to buffer alone = 36 mM. (100 mL of 10× buffer was constituted by titration a solution of acetic acid (6.005 g) in water by NaOH (1N). After 10 fold dilution at 37 °C, pH of buffer alone was 5.00). pH of the reaction medium = 4.44 at 37 °C.

(ii) Phosphate buffer: 10 mM PO_4^{3-}. (100 mL of 10× buffer was constituted by mixing 170 mg of Na_2HPO_4 and 1.056 g of NaH_2PO_4 in water. pH was adjusted to 6.40 at 22 °C by NaOH (1N). After 10 fold dilution at 37 °C, pH of buffer alone was 6.00). pH of the reaction medium at 37 °C = 6.24.

(iii) PBS buffer: 25 mM PO_4^{3-}. Ionic strength = 150 mM (100 mL of 10× buffer was constituted by mixing 1.08 g of NaH_2PO_4 and 2.2578 g of Na_2HPO_4 and 5.448 g of NaCl in water. pH was adjusted to 6.85 at 22 °C by NaOH (1N). After 10-fold dilution at 37 °C, pH of buffer alone was 6.75). pH of the reaction medium at 37 °C = 6.96.

13–RGD-peptide ligation reaction at different pHs (analyzed by Gel electrophoresis) shows that the reaction is very fast at lower pH as expected and within a reasonable time period at pH 7.

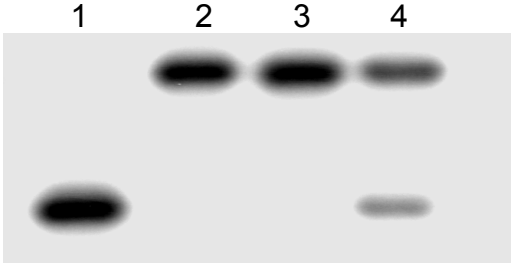


Figure 2S. Ligation reaction of **13** and **1** at different pHs at 37 °C after 6 h. Lane 1: **13** only; Lane 2, pH 4.44 (acetate buffer); lane 3, pH 6.24 (phosphate buffer); lane 4, pH 6.96 (PBS buffer).

RP-HPLC conditions for ligated products. Ligated products were resolved from the *ketone*-DNA **13** using a C-18 XTerra (4.6 x 50 mm) column (buffer A = 0.1 M triethylammonium acetate in water, pH = 7.2; buffer B = A + 50% acetonitrile, pH = 7.2. Flow = 1 ml/min, 254 nm detection) and a gradient profile as described below.

13-2 Conjugate: 0-40% B within 55 min; $t_{R\ (\mathbf{13\text{-}2})}$ = 33.0 min, $t_{R\ (\mathbf{13})}$ = 30.0 min.

13-3 Conjugate: 0-25% B within 55 min; $t_{R\ (\mathbf{13\text{-}3})}$ = 46.1 min, $t_{R\ (\mathbf{13})}$ = 45.1 min.

13-4 Conjugate: 0-35% B within 55 min; $t_{R\ (\mathbf{13\text{-}4})}$ = 36.5 min, $t_{R\ (\mathbf{13})}$ = 34.6 min.

13-5 Conjugate: 0-60% B within 45 min; $t_{R\ (\mathbf{13\text{-}5})}$ = 21.0 min, $t_{R\ (\mathbf{13})}$ = 18.6 min.

Gel electrophoresis. Ligated products were resolved from the starting *ketone*-DNAs using a denaturing (for single stranded labeling) or non-denaturing (for duplex labeling) 20% PAGE (29:1 acrylamide:bisacrylamide) gels. TBE (0.89 M Tris, 0.89 M borate, 10 mM Na_2EDTA, pH = 8.5) buffer was used for casting (including 8 M urea for denaturing gels) and running gels. An equal volume of the sample and "gel load buffer" (20% (w/v) sucrose, 0.05 M EDTA, 0.05% (w/v) SDS, 0.05% (w/v) bromophenol blue, 0.05% (w/v) xylene cyanol) were mixed and 10 µL of the solution was loaded on the gel. Gels dimensions were 0.75 x 280 mm and were run at a constant temperature of 35-40 °C (approximately at 20 mA, 1950 V). Gels were run until the bromophenol blue dye had migrated approximately 255 mm.

For routine gel purifications, oligonucleotides (150 µL x ~350 µM) were loaded and run on gel as described above (denaturing gel, dimension:1.5 x 400 mm, 2 x 1″ lanes). After electrophoresis (bromophenol blue dye migration ~375 mm), the oligonucleotide was imaged by UV shadowing, excised with a sterilized razor blade and the gel slices were soaked in "crush & soak" buffer (10 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, pH 7.5) at RT for 10 h. After filtration of the gel pieces through a 0.2 micron filter, DNAs were desalted using RP-HPLC

(C18-XTerra 4.6 x 50 mm column, flow 1 mL/min, 254 nm detection, 15 min washing with water followed by elution of DNA with 9:1 acetonitrile-water). Approximately 25-30% of the oligonucleotide was recovered in each case.

Radioactive bands in polyacrylamide gels were visualized using a Molecular Dynamics Storm Phosphorimager and quantitated using ImageQuant software.

UV-melting and CD experiments. Complexes were constituted by heating eqimolar solutions (2 μM single strand concentration) of the appropriate *ketone*-DNA and complementary DNA in phosphate-buffer (25 mM PO_4^{3-}, 0.1 mM EDTA disodium salt, pH 7.0, ionic strength adjusted with NaCl to 150 mM) to 95 °C for 5 min, followed by slow cooling to RT. All measurements were conducted in a 1-cm pathlength quartz cell equipped with a temperature probe. The absorbance at 260 nm was recorded as a function of temperature using a Cary 500 (Varian) double beam UV-VIS-NIR spectrophotometer equipped with a peltier thermostated sample holder with heating/cooling rates of 1 °C/min. Data were collected and evaluated using the "Thermal" module of the Cary WinUV software package. The melting temperature, T_m (defined as the temperature at which 50% of the complex is dissociated into its constituent components) was determined from the maximum of the first derivative of the melting curves. CD spectra were recorded on a JASCO J-715 spectropolarimeter at 22 °C and are baseline corrected (step resolution = 0.5 nm, speed = 100 nm/min, accumulation = 8, response = 1 s, bandwidth = 1 nm, sensitivity = 20 mdeg)

MALDI-TOF mass spectrometry.[11] MALDI-matrix was constituted by mixing 1:8 volume ratio of 2′, 4′, 6′-trihydroxy acetophenone (0.2 M in 1:1 CH_3CN, H_2O) and ammonium citrate (0.3 M aqueous) solution. By mixing equal volumes of matrix and DNA (internal standards, *ketone*-DNAs or *ketone*-DNA–aminooxy ligand conjugates; desalted, 2μM) MALDI samples

were constituted. For calibration purposes, 25% (molar ratio) of internal standards $(dT)_{10}$ and $(dT)_{24}$ were used. Spectra were acquired using a PerSeptive Biosystems (Framingham, MA) Voyager DE-Pro MALDI-TOF instrument operated in the linear negative ion, reflector mode. The laser energy was kept at 2800-3000, accelerating voltage at 25000 V, grid voltage at 90%, guide wire voltage at 0.025%, and the delay time at 125 ns. Each spectrum was an average of 128-256 laser shots.

13: calcd. M^- = 5244.49, found 5245.79;

14: calcd M^- = 5244.49, found, 5245.70;

15: calcd. M^- = 5397.67, found, 5397.81;

13-1 (RGD) Conjugate: calcd. M^- = 5731.96, found 5731.95;

13-2 (Biotin) Conjugate: calcd. M^- = 5557.87, found 5558.98;

13-3 (Glucose) Conjugate: calcd. M^- = 5522.75, found 5524.05;

13-4 (Geranyl) Conjugate: calcd. M^- = 5452.79, found 5452.65;

13-5 (AMCA) Conjugate: calcd. M^- = 5546.77, found 5547.35.

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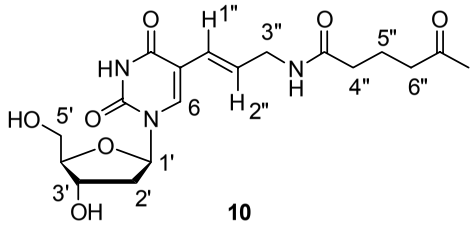

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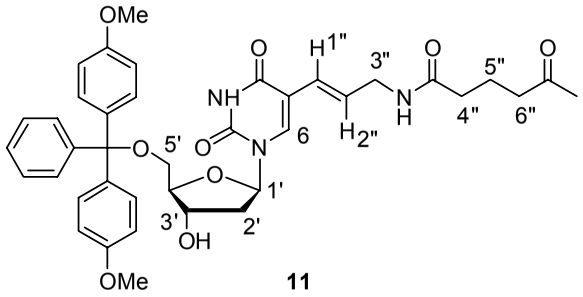
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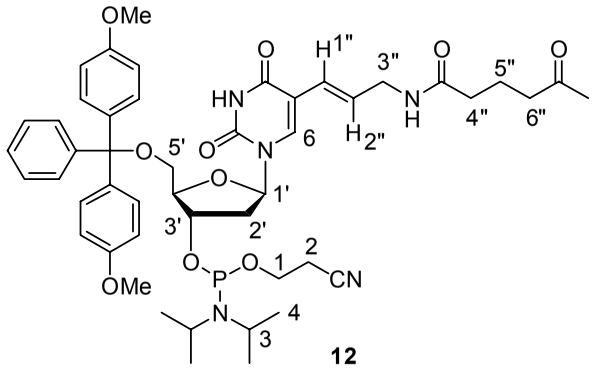
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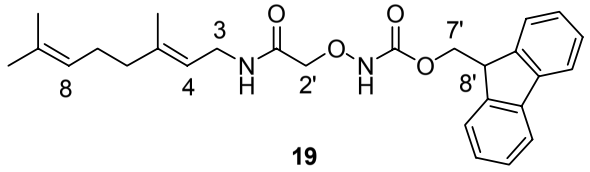

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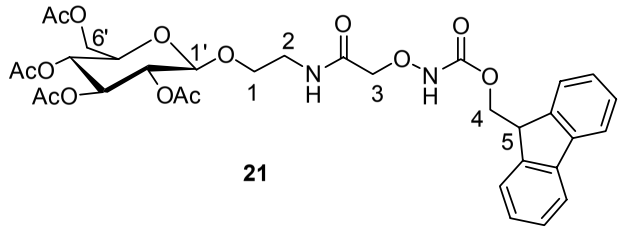
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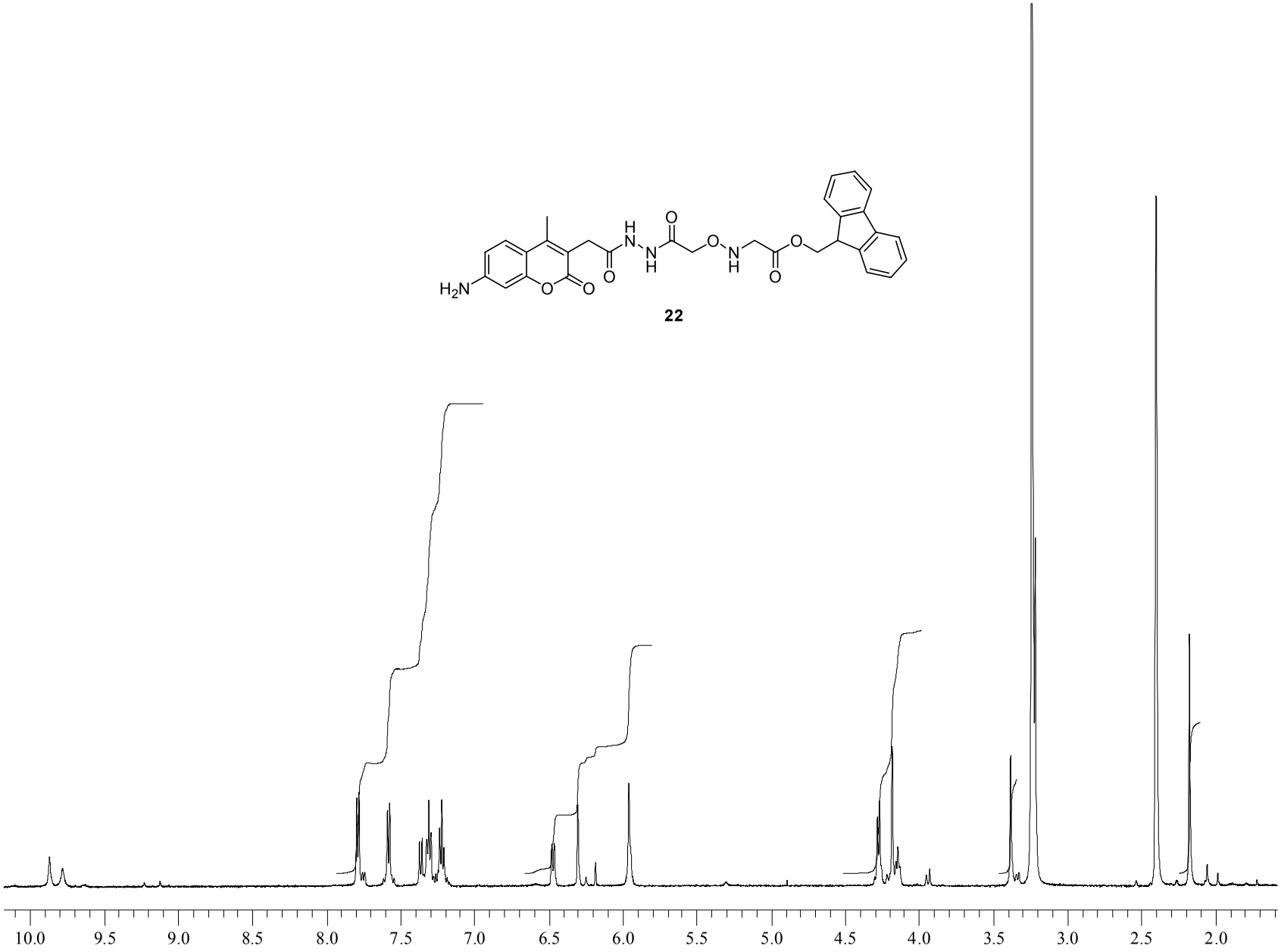
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