Size-Specific Ligands for RNA Hairpin Loops

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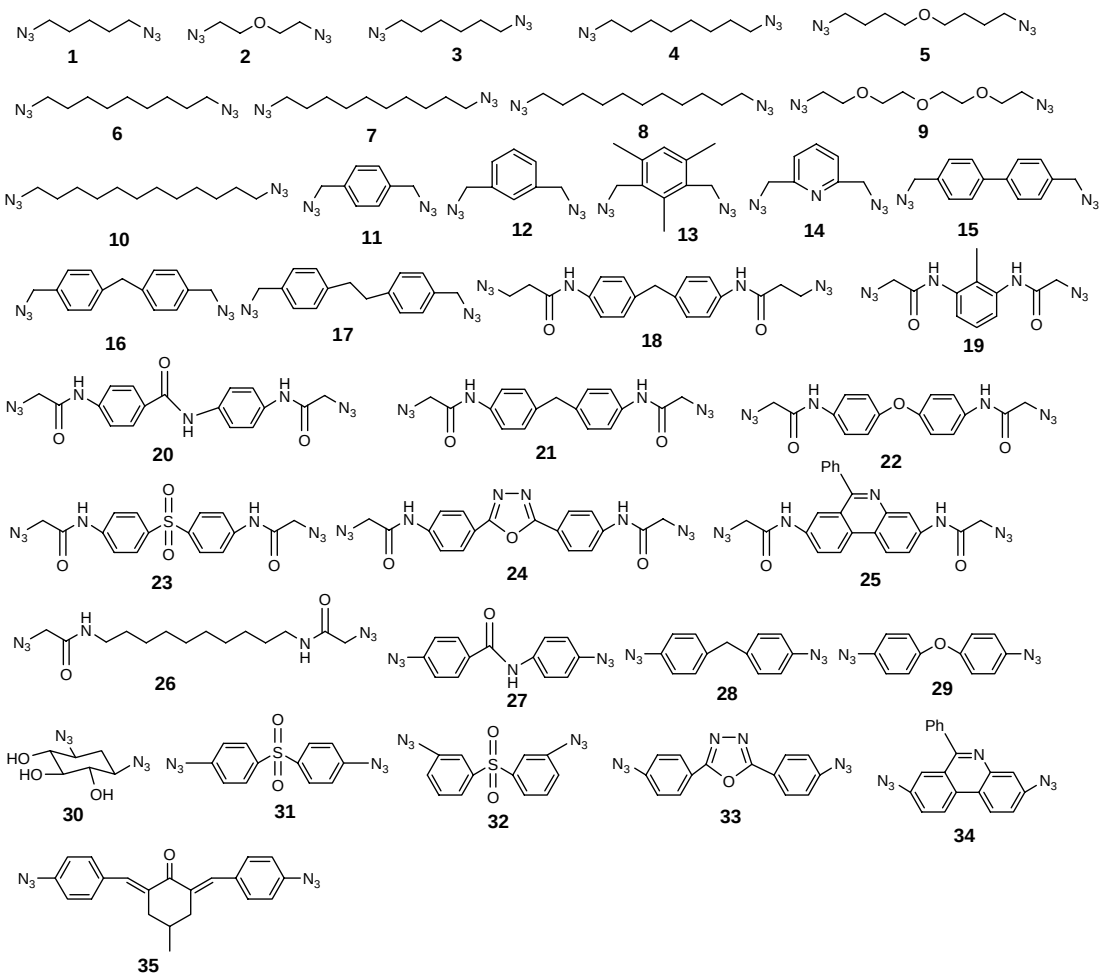
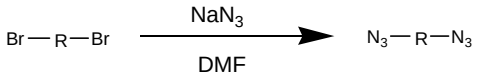
Supporting Information -- Methods

^{1}H NMR and ^{13}C NMR spectra were recorded on Varian unity 400 and on Varian unity 500 spectrometers in $CDCl_3$, CD_3OD, DMSO-d_6 or D_2O. The data is reported as follows : chemical shifts in ppm (δ), multiplicities are indicated as s-singlet; d-doublet; t-triplet; q-quarted; m-multiplet; br-broad. Coupling constants *J* are reported in Hz. Infrared spectra were recorded on Perkin Elmer spectrum BX spectrophotometer, and the peaks reported in cm^{-1}. Mass spectra data are reported in m/e (intensity to 100%). Analytical thin-layer chromatography was performed on Merck silica gel plated with F254 indicator. The plates were visualized by CAM stain. Melting points were determined on a Thomas-Hoover Capillary Melting point Apparatus and are uncorrected. Azide **30**[1] and starting material 2-deoxy-1,3-diazido-5,6-O-isopropylidene-streptamine was made following the previously published procedure.[2] Diazide **35** and p-toluenesulfonic acid resin were purchased from Aldrich. Azidomethyl polystyrene resin was made according to the literature protocol.[3] All other reagents were also purchased from Aldrich.

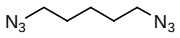
35 diazides



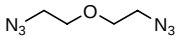
General scheme for the synthesis of the diazides 1-17



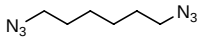
NaN_3 (1.0 g, 15.0 mmol) was added to a solution of the dibromo compound (10.0 mmol) in DMF (15.0 mL). The mixture was stirred at 60°C for 10h, at which point water (100.0 mL) was added and the product was extracted with ether (3 x 10 mL). The organic layer was washed three times with water (3 x 10 mL), the solvent was evaporated, and the compound was purified by chromatography on silica gel (hexane as eluent solvent) to give the pure products.



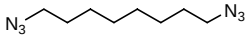
Yield 96%, liquid.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:
3.28 (t, J = 6.7, 4H), 1.68 (m, 4H), 1.40 (m, 2H).
^{13}C NMR (125 MHz, $CDCl_3$) δ ppm:
51.5 (x2), 28.9, 26.5 (x2).
IR (CH_2Cl_2): 2104, 1510 cm^{-1}.



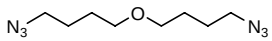
Yield 97%, liquid.
^{1}H NMR (500 MHz, $CDCl_3$) δ ppm:
3.70 (t, J = 6.7, 4H), 3.40 (t, J = 7.0, 4H).
^{13}C NMR (125 MHz, $CDCl_3$) δ ppm:
70.2 (x2), 50.9 (x2).
IR (CH_2Cl_2): 2110, 1510 cm^{-1}.



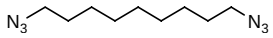
Yield 95%, liquid.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:
3.28 (t, J = 6.7, 4H), 1.68 (m, 4H), 1.48 (m, 4H).
^{13}C NMR (125 MHz, $CDCl_3$) δ ppm:
51.4 (x2), 28.6 (x2), 24.1 (x2).
IR (CH_2Cl_2): 2104, 1510 cm^{-1}.



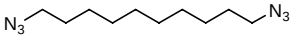
Yield 96%, liquid.
^{1}H NMR (500 MHz, $CDCl_3$) δ ppm:
3.30 (t, J = 6.7, 4H), 1.60 (m, 4H), 1.30-1.48 (m, 8H).
^{13}C NMR (125 MHz, $CDCl_3$) δ ppm:
51.6 (x2), 29.1 (x2), 28.9 (x2), 26.8 (x2).
IR (CH_2Cl_2): 2104, 1510 cm^{-1}.



Yield 97%, liquid.
^{1}H NMR (500 MHz, $CDCl_3$) δ ppm:
3.45 (t, J = 6.7, 4H), 3.30 (t, J = 7.0, 4H), 1.6-1.72 (m, 8H).
^{13}C NMR (125 MHz, $CDCl_3$) δ ppm:
70.3 (x2), 51.5 (x2), 27.0 (x2), 26.0 (x2).
IR (CH_2Cl_2): 2104, 1500 cm^{-1}.

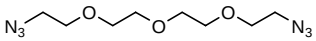


Yield 96%, liquid.
^{1}H NMR (500 MHz, $CDCl_3$) δ ppm:
3.25 (t, J = 6.7, 4H), 1.58-1.65 (m, 4H), 1.28-1.40 (m, 10H).
^{13}C NMR (125 MHz, $CDCl_3$) δ ppm:
51.6 (x2), 29.4 (x2), 29.2, 29.0 (x2), 26.8 (x2).
IR (CH_2Cl_2): 2104, 1500 cm^{-1}.

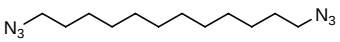


Yield 97%, liquid.
^{1}H NMR (500 MHz, $CDCl_3$) δ ppm:
3.24 (t, J = 6.7, 4H), 1.58-1.65 (m, 4H), 1.24-1.40 (m, 12H).
^{13}C NMR (125 MHz, $CDCl_3$) δ ppm:
51.6 (x2), 29.5 (x2), 29.3 (x2), 29.0 (x2), 26.9 (x2).
IR (CH_2Cl_2): 2104, 1508 cm^{-1}.

Yield 97%, liquid.
^{1}H NMR (500 MHz, $CDCl_3$) δ ppm:
3.24 (t, J = 6.7, 4H), 1.58-1.63 (m, 4H), 1.23-1.40 (m, 14H).
^{13}C NMR (125 MHz, $CDCl_3$) δ ppm:
51.6 (x2), 29.6 (x2), 29.5 (x2), 29.3, 29.0 (x2), 26.9 (x2).
IR (CH_2Cl_2): 2110, 1500 cm^{-1}.

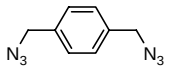


Yield 98%, liquid.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:
3.68 (t, J = 6.7, 12H), 3.40 (t, J = 6.7, 4H).
^{13}C NMR (100 MHz, $CDCl_3$) δ ppm:
70.9 (x2), 70.2 (x4), 50.8 (x2).
HRMS (ESI): m/e calcd for $C_8H_{16}N_6O_3$ ($M + H^+$) 245.1362, found 245.1348.
IR (CH_2Cl_2): 2104, 1500 cm^{-1}.

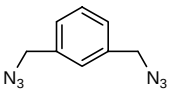


Yield 96%, liquid.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:
3.25 (t, J = 6.7, 4H), 1.58-1.63 (m, 4H), 1.23-1.40 (m, 16H).
^{13}C NMR (100 MHz, $CDCl_3$) δ ppm:
51.6 (x2), 29.7 (x2), 29.6 (x2), 29.3 (x2), 29.0 (x2), 26.9 (x2).

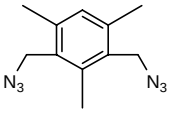
IR (CH_2Cl_2): 2104, 1500 cm^{-1}.



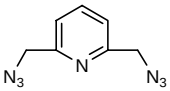
Yield 97%, oil.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:
7.38 (s, 4H), 4.58 (s, 4H).
^{13}C NMR (100 MHz, $CDCl_3$) δ ppm:
135.7 (x2), 128.9 (x4), 54.6 (x2).
HRMS (EI): m/e calcd for $C_8H_8N_6$ (M) 188.0810, found 188.0811.
IR (CH_2Cl_2): 2097, 1259 cm^{-1}.



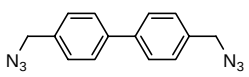
Yield 96%, liquid.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:
7.38-7.43 (m, 1H), 7.28-7.32 (m, 3H), 4.38 (s, 4H).
^{13}C NMR (100 MHz, $CDCl_3$) δ ppm:
136.3 (x2), 129.6 (x2), 128.2, 128.0, 54.7 (x2).
HRMS (EI): m/e calcd for $C_8H_8N_6$ (M) 188.0810, found 188.0809.
IR (CH_2Cl_2): 2100, 1259 cm^{-1}.



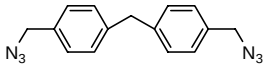
Yield 96%, white crystal.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:
6.98 (s, 1H), 4.41 (s, 4H), 2.42 (s, 3H), 2.37 (s, 6H).
^{13}C NMR (100 MHz, $CDCl_3$) δ ppm:
138.1 (x2), 137.8, 130.9 (x2), 130.3, 48.8 (x2), 20.3 (x2), 15.9.
HRMS (EI): m/e calcd for $C_{11}H_{14}N_6$ (M) 230.1279, found 230.1274.
IR (CH_2Cl_2): 2098, 1260 cm^{-1}.
m. p. = 38 ºC.



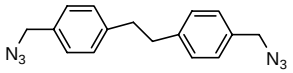
Yield 96%, oil.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:
7.58 (t, 1H), 7.28-7.32 (m, 2H), 4.48 (s, 4H).
^{13}C NMR (100 MHz, $CDCl_3$) δ ppm:
156.0, 138.3 (x2), 121.3 (x2), 55.5 (x2).
HRMS (EI): m/e calcd for $C_7H_7N_7$ (M.W) 189.0760, found 189.0759.
IR (CH_2Cl_2): 2101, 1265 cm^{-1}.



Yield 97%, liquid.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:
7.40-7.51 (m, 6H), 7.20-7.28 (m,2H), 4.18 (d,2H), 4.06 (d,2H).
^{13}C NMR (100 MHz, $CDCl_3$) δ ppm:
139.8 (x2), 133.8 (x2), 130.4 (x2), 129.6 (x2), 128.7(x2), 128.5 (x2), 52.8 (x2).
HRMS (EI): m/e calcd for $C_{14}H_{12}N_6$ (M) 264.2830, found 264.2829.
IR (CH_2Cl_2): 2100, 1265 cm^{-1}.

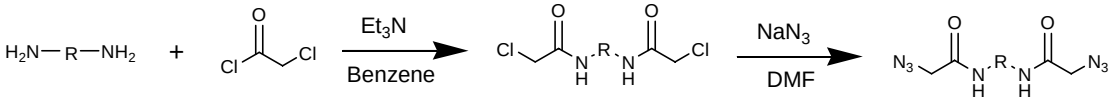


Yield 97%, liquid.
^{1}H NMR (500 MHz, $CDCl_3$) δ ppm:
7.28-7.30 (d, 4H), 7.20-7.23 (d, 4H), 4.32 (s, 4H), 4.00 (s, 2H).
^{13}C NMR (125 MHz, $CDCl_3$) δ ppm:
141.2 (x2), 133.5 (x2), 129.6 (x2), 128.7 (x2), 54.7 (x2), 41.4.
HRMS (EI): m/e calcd for $C_{15}H_{14}N_6$ (M) 278.1280, found 278.1282.
IR (CH_2Cl_2): 2101, 1260 cm^{-1}.



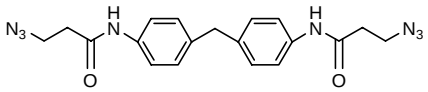
Yield 97%, white crystal.
^{1}H NMR (500 MHz, $CDCl_3$) δ ppm:
7.24-7.28 (m, 4H), 7.16-7.19 (d, 4H), 4.30 (s, 4H), 2.96 (s, 4H).
^{13}C NMR (125 MHz, $CDCl_3$) δ ppm:
141.9 (x2), 133.1 (x2), 129.2 (x2), 128.6 (x2), 54.7 (x2), 37.6 (x2).
HRMS (EI): m/e calcd for $C_{16}H_{16}N_6$ (M) 292.1436, found 292.1430.
IR (CH_2Cl_2): 2101, 1258 cm^{-1}.
m. p. = 54ºC.

General scheme for the synthesis of the diazides 18-26



The appropriate diamine (10.0 mmol) was dissolved in benzene (15.0 mL) and Et_3N (2.0 mL) was added. The flask was cooled to 0°C and chloroacetyl chloride (2.80 g, 25 mmol) was added over 20 min. The mixture was then warmed to room temperature and stirred for 5 h, at which point a white precipitate was collected. The precipitate was dissolved in DMF, water was added, and the white precipitate was collected again and dried to give the pure products (yield 97%).

The white solid (5.0 mmol) was dissolved in DMF (10.0 mL), NaN_3 (1.0 g, 15.0 mmol) was added, and the mixture was stirred at 60°C for 10h. After the reaction was finished, water (100.0 mL) was added to the reaction mixture, and the product was filtered and washed several times with water. Recrystalization from ethanol provided the pure products.



Yield 92%, white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm:

9.99 (s, 2H), 7.47 (d, 4H), 7.15 (d, 4H), 3.80 (s, 2H), 3.58 (t, J = 10.0, 4H), 2.58 (t, J = 10.0, 4H).

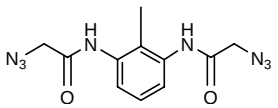
^{13}C NMR (100 MHz, DMSO-d_6) δ ppm:

169.1 (x2), 137.7 (x2), 136.9 (x2), 129.5 (x2), 119.9 (x2), 47.5 (x2), 40.7, 36.2 (x2).

HRMS (ESI): m/e calcd for $C_{19}H_{20}N_8O_2$ ($M + H^+$) 393.1787, found 393.1793.

IR (KBr): 3500, 2101, 1845 cm^{-1}.

m. p. = 178-180 ºC.



Yield 94%, white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm:

9.63 (s, 2H), 7.18-7.25 (m, 3H), 4.10 (s, 4H), 2.02 (s, 3H).

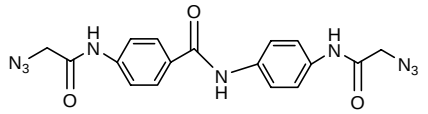
^{13}C NMR (125 MHz, DMSO-d_6) δ ppm:

167.1 (x2), 136.8, 128.3, 126.1, 123.8, 51.6 (x2), 13.5.

HRMS (ESI): m/e calcd for $C_{11}H_{12}N_8O_2$ ($M + H^+$) 289.1161, found 289.1159.

IR (KBr): 3545, 2101, 1800 cm^{-1}.

m. p. = 201-203 ºC.



Yield 94%, pale yellow solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm:

10.43 (s, 1H), 10.18 (s, 2H), 7.98-8.00 (m, 2H), 7.71-7.78 (m, 4H), 7.53-7.60 (m, 2H), 4.15 (s, 2H), 4.02 (s, 2H).

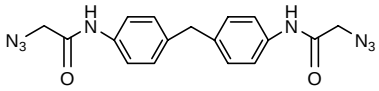
^{13}C NMR (125 MHz, DMSO-d_6) δ ppm:

167.5, 166.7, 165.3, 141.9, 135.7, 134.8, 130.3, 129.3, 121.5, 120.2, 119.1, 51.9, 51.8.

HRMS (ESI): m/e calcd for $C_{17}H_{15}N_9O_3$ ($M + H^+$) 394.1376, found 394.1384.

IR (KBr): 3500, 2101, 1820 cm^{-1}.

m. p. = 240-243 ºC.



Yield 90%, pale yellow solid.

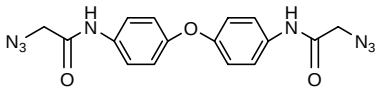
^{1}H NMR (500 MHz, DMSO-d_6) δ ppm:

10.10 (s, 2H), 7.48-7.50 (m, 4H), 7.16-7.18 (m, 4H), 4.00 (s, 4H), 3.82 (s, 2H).

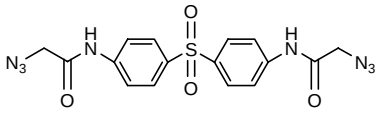
^{13}C NMR (125 MHz, DMSO-d_6) δ ppm:

166.7 (x2), 137.4 (x2), 137.0 (x2), 129.6 (x2), 120.1 (x2), 51.8 (x2), 40.5.

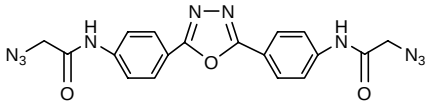
HRMS (ESI): m/e calcd for $C_{17}H_{16}N_8O_2$ ($M + H^+$) 365.1474, found 365.1484.
IR (KBr): 3510, 2101, 1800 cm^{-1}.
m. p. = 167-169 ºC.



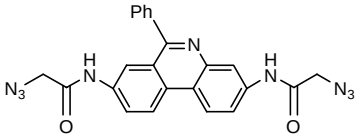
Yield 90%, white solid.
^{1}H NMR (500 MHz, DMSO-d_6) δ ppm:
10.16 (s, 2H), 7.48-7.50 (m, 4H), 6.98-7.00 (m, 4H), 4.00 (s, 4H).
^{13}C NMR (125 MHz, DMSO-d_6) δ ppm:
166.7 (x2), 153.4 (x2), 134.6 (x2), 121.7 (x2), 119.5 (x2), 51.8 (x2).
HRMS (ESI): m/e calcd for $C_{16}H_{14}N_8O_3$ ($M + H^+$) 367.1267, found 367.1263.
IR (KBr): 3500, 2101, 1810 cm^{-1}.
m. p. = 182-184 ºC.



Yield 92%, white solid.
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm:
10.58 (s, 2H), 7.92-7.98 (m, 4H), 7.76-7.79 (m, 4H), 4.03 (s, 4H).
^{13}C NMR (100 MHz, DMSO-d_6) δ ppm:
167.8 (x2), 143.5 (x2), 136.2 (x2), 129.2 (x2), 120.0 (x2), 51.9 (x2).
HRMS (ESI): m/e calcd for $C_{16}H_{14}N_8O_4S$ ($M + H^+$) 415.0937, found 415.0955.
IR (KBr): 3510, 2101, 1800 cm^{-1}.
m. p. = 186-188 ºC.



Yield 89%, pale yellow solid.
^{1}H NMR (500 MHz, DMSO-d_6) δ ppm:
10.60 (s, 2H), 8.06-8.10 (m, 4H), 7.79-7.81 (m, 4H), 4.10 (s, 4H).
^{13}C NMR (125 MHz, DMSO-d_6) δ ppm:
167.6 (x2), 164.1 (x2), 142.3 (x2), 128.2 (x2), 120.1 (x2), 118.9 (x2), 51.9 (x2).
HRMS (ESI): m/e calcd for $C_{18}H_{14}N_{10}O_3$ ($M + H^+$) 419.1329, found 419.1328.
IR (KBr): 3515, 2101, 1810 cm^{-1}.
m. p. = 247-249 ºC.



Yield 86%, pale yellow solid.
^{1}H NMR (500 MHz, DMSO-d_6) δ ppm:

10.50 (s, 2H), 8.68-8.82 (m, 2H), 8.35-8.40 (m, 2H), 8.20 (s, 1H), 7.85(s, 1H), 7.58-7.70 (m, 6H), 4.18 (s, 2H), 4.08 (s, 2H).

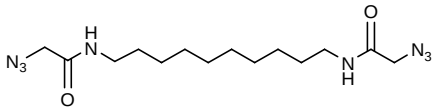
^{13}C NMR (125 MHz, DMSO-d_6) δ ppm:

167.4, 161.3, 143.9, 140.0, 139.2, 138.0, 130.2, 129.6, 129.4, 129.0, 125.1, 124.1, 123.7, 120.4, 119.9, 117.2, 52.0, 51.9.

HRMS (ESI): m/e calcd for $C_{23}H_{17}N_9O_2$ ($M + H^+$) 452.1583, found 452.1586.

IR (KBr): 3510, 2101, 1800 cm^{-1}.

m. p. = 210-212 ºC.



Yield 89%, white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ ppm:

6.30 (s, 2H), 4.00 (s, 4H), 3.23 (m, 4H), 1.55-1.57 (m, 4H), 1.20-1.36 (m, 12H).

^{13}C NMR (125 MHz, $CDCl_6$) δ ppm:

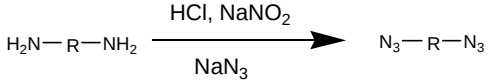
166.6 (x2), 52.9 (x2), 39.6 (x2), 29.6 (x2), 29.5 (x2), 29.3 (x2), 26.9 (x2).

HRMS (ESI): m/e calcd for $C_{14}H_{26}N_8O_2$ ($M + H^+$) 339.2257, found 339.2249.

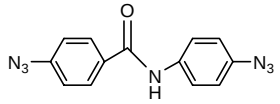
IR (KBr): 3300, 2101 cm^{-1}.

m. p. = 90-91 ºC.

General scheme for the synthesis of the diazides 27-34



A solution of $NaNO_2$ (4.0 g, 58.0 mmol) in water was added dropwise to a solution of the diamine (58.0 mmol) in 2 N HCl (120 mL) at 0-5ºC with vigorous stirring. The mixture was kept below 5ºC for 30 min, the diazonium salt solution was neutralized with $CaCO_3$, and then a solution of NaN_3 (4.5 g, 69.6 mmol) in water (10 mL) was added dropwise while the temperature was kept below 5ºC. The solid precipitate was filtered and washed twice with water (10 mL). Recrystallization from ethanol provided the pure products.



Yield 90%, red solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm:

10.35 (s, 1H), 8.00 (d, 2H), 7.80 (d, 2H), 7.28 (d, 2H), 7.10 (d, 2H).

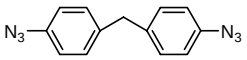
^{13}C NMR (125 MHz, DMSO-d_6) δ ppm:

165.0, 143.4, 137.0, 134.9, 131.8, 130.3, 122.5, 120.0, 119.6.

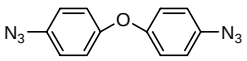
HRMS (ESI): m/e calcd for $C_{13}H_9N_7O$ ($M + H^+$) 280.0936, found 280.0947.

IR (CH_2Cl_2): 3510, 2101, 1800 cm^{-1}.

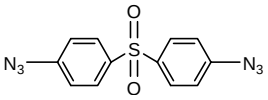
m. p. = 170-171 ºC.



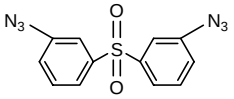
Yield 86%, yellow oil.
^{1}H NMR (500 MHz, CD_3Cl) δ ppm:
7.18 (d, 4H), 6.98 (d, 4H), 3.97 (s, 2H).
^{13}C NMR (125 MHz, CD_3Cl) δ ppm:
138.2, 137.8, 130.4, 119.3, 40.7.
HRMS (EI): m/e calcd for $C_{13}H_{10}N_6$ (M) 250.2562, found 250.2561.
IR (CH_2Cl_2): 2104, 1501 cm^{-1}.



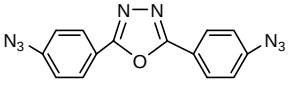
Yield 88%, pale yellow solid.
^{1}H NMR (500 MHz, CD_3Cl) δ ppm:
7.00 (s, 8H).
^{13}C NMR (125 MHz, CD_3Cl) δ ppm:
154.5 (x2), 135.3 (x2), 120.5 (x2), 120.3 (x2).
HRMS (EI): m/e calcd for $C_{12}H_8N_6O$ (M) 252.0760, found 252.0767.
IR (CH_2Cl_2): 2104, 1500 cm^{-1}.
m. p. = 67-68 ºC.



Yield 85%, yellow solid.
^{1}H NMR (400 MHz, CD_3Cl) δ ppm:
7.90 (d, 4H), 7.16 (d, 4H).
^{13}C NMR (100 MHz, CD_3Cl) δ ppm:
129.7 (x4), 119.9 (x4).
HRMS (EI): m/e calcd for $C_{12}H_8N_6O_2S$ (M) 300.0429, found 300.0427.
IR (CH_2Cl_2): 2104, 1510 cm^{-1}.
m. p. = 154-155 ºC.



Yield 85%, pale yellow solid.
^{1}H NMR (500 MHz, DMSO-d_6) δ ppm:
7.94 (d, 4H), 7.30 (d, 4H).
^{13}C NMR (125 MHz, DMSO-d_6) δ ppm:
145.7 (x2), 137.7 (x2), 130.0 (x2), 121.0 (x2).
HRMS (EI): m/e calcd for $C_{12}H_8N_6O_2S$ (M) 300.0429, found 300.0425.
IR (CH_2Cl_2): 2104, 1510 cm^{-1}.
m. p. = 107-108 ºC.



Yield 85%, pale yellow solid.
^{1}H NMR (500 MHz, $CDCl_3$) δ ppm:

8.12 (d, 4H), 7.18 (d, 4H).

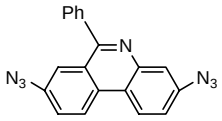
^{13}C NMR (125 MHz, $CDCl_3$) δ ppm:

164.1 (x2), 143.8 (x4), 128.7 (x4), 120.5 (x2), 119.9 (x2).

HRMS (ESI): m/e calcd for $C_{14}H_8N_8O$ ($M + H^+$) 305.0899, found 305.0898.

IR (CH_2Cl_2): 2104, 1510 cm^{-1}.

m. p. = 185-187 ºC.



Yield 83%, yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ ppm:

8.60 (d, 1H), 8.51 (d,1H), 7.92 (s, 1H), 7.70 (m, 3H), 7.56-7.60 (m,4H), 7.36 (d, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ ppm:

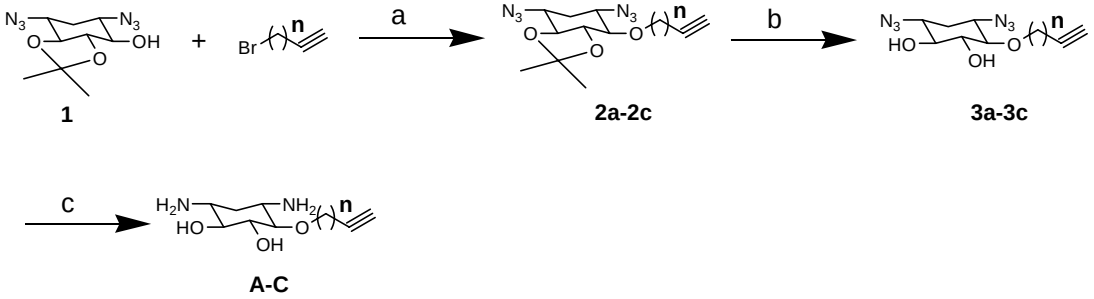
161.8, 141.0, 139.1, 130.7, 129.7, 129.4, 128.9, 126.0, 124.3, 123.7, 122.9, 120.8, 119.8, 119.0, 118.0.

HRMS (ESI): m/e calcd for $C_{19}H_{11}N_7$ ($M + H^+$) 338.1154, found 338.1149.

IR (CH_2Cl_2): 2110, 1510 cm^{-1}.

m. p. = 165-166 ºC.

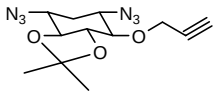
General scheme for the synthesis of the deoxystreptamine-alkynes



Reagents and Conditions: (a) NaH, DMF (b) CH_3COOH, 1,4-dioxane/H_2O. (c) PMe_3, NaOH, CH_3OH.

Synthetic procedures and compound characterization

To a solution of starting material **1**[2] (0.50 g, 1.97 mmol), NaH (0.40 g, 9.85 mmol, 60 % dispersion in mineral oil) and a catalytic amount of TBAI in anhydrous DMF were added. The bromo alkyne reagent (0.98 mmol) was then added dropwise. The mixture was stirred at room temperature for 2 h, at which point methanol (1 mL) was added to stop the reaction. The reaction mixture was poured into a solution of ice water and ether. The organic layer was washed with brine, and dried over Na_2SO_4. After removal of the solvent, purification by silica gel column chromatography (ethyl acetate in hexanes, 5-10% gradient) gave the pure products, R_f 0.30 (25 % ethyl acetate in hexanes).



2a

Yield: 97%, white foam.

^{1}H NMR (500 MHz, CD_3OD) δ ppm:

4.37-4.43 (m, 2H), 3.72-3.78 (m, 2H), 3.43-3.58 (m, 3H), 2.85 (s, 1H), 2.21 (ddd, J = 4.5, 4.5, 13.0, 1H), 1.42 (s, 3H), 1.41 (s, 3H), 1.38 (q, J = 12.4, 1H).

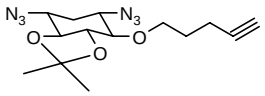
^{13}C NMR (125 MHz, CD_3OD) δ ppm:

112.2, 80.0, 79.9, 79.8, 79.1, 74.8, 60.7, 57.6, 57.5, 33.6, 25.8, 25.7.

HRMS (ESI): m/e calcd for $C_{12}H_{16}N_6O_3$ ($M + H^+$) 293.1362, found 293.1358.

IR (CH_2Cl_2) = 2103, 1220 cm^{-1}.

R_f = 0.5 (30% ethyl acetate in hexanes).



2b

Yield: 98%, colorless oil.

^{1}H NMR (500 MHz, CD_3OD) δ ppm:

3.91-3.96 (m, 1H), 3.68-3.74 (m, 2H), 3.50-3.57 (m, 1H), 3.42-3.49 (m, 3H), 2.28-2.32 (m, 2H), 2.21 (ddd, J = 4.5, 4.5, 13.0, 1H), 2.15 (s, 1H), 1.75-1.81 (m, 2H), 1.42 (s, 3H), 1.41 (s, 3H), 1.32 (q, J = 12.4, 1H).

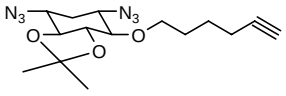
^{13}C NMR (125 MHz, CD_3OD) δ ppm:

111.9, 83.2, 81.9, 80.0, 79.8, 69.5, 68.3, 61.4, 57.5, 33.6, 29.0, 25.9, 25.7, 14.5.

HRMS (ESI): m/e calcd for $C_{14}H_{20}N_6O_3$ ($M + H^+$) 321.1311, found 321.1314.

IR (CH_2Cl_2) = 2102, 1227 cm^{-1}.

R_f = 0.5 (30% ethyl acetate in hexanes).



2c

Yield: 98%, colorless oil.

^{1}H NMR (500 MHz, CD_3OD) δ ppm:

3.84-3.90 (m, 1H), 3.68-3.74 (m, 1H), 3.60-3.63 (m, 1H), 3.51-3.57 (m, 1H), 3.42-3.49 (m, 3H), 2.17-2.21 (m, 4H), 1.68-1.73 (m, 2H), 1.59-1.64 (m, 2H), 1.42 (d, 6H), 1.34 (q, J = 12.4, 1H).

^{13}C NMR (125 MHz, CD_3OD) δ ppm:

111.9, 83.6, 81.8, 80.1, 79.8, 70.4, 68.3, 61.3, 57.5, 33.6, 28.8, 25.9, 25.7, 25.0, 17.5.

HRMS (ESI): m/e calcd for $C_{15}H_{22}N_6O_3$ ($M + H^+$) 335.1822, found 335.1821.

IR (CH_2Cl_2) = 2102, 1227 cm^{-1}.

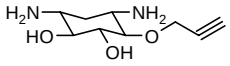
R_f = 0.5 (30% ethyl acetate in hexanes).

General Procedure for acetal removal:

To a solution of the above compounds (0.5 mmol) in 2:1 dioxane-water (10 mL) was added glacial acetic acid (8 mL). The mixture was stirred at 50-55 ºC for 10 h, at which point the reaction mixture was concentrated to dryness to provide a residue that was of sufficient purity to use directly in the next step. R_f 0.24 (10 % MeOH in CH_2Cl_2).

General procedure for the azide reduction:

The deprotected compound (0.5 mmol) from the previous step was dissolved in MeOH (5.0 mL) and 0.1 M aqueous NaOH (0.5 mL). PMe_3 (1 M in THF, 5 equiv.) was then added. The reaction mixture was stirred at 45-50 ºC for 5 hrs; when complete, the reaction mixture was cooled to room temperature and loaded on a short column (5 cm in height) of packed silica gel and Celite. The column was eluted with a series of solutions as the follows: MeOH, and MeOH/conc.NH_4OH (from 0-10% of conc. NH_4OH). The fractions containing the desired product were analyzed by TLC and collected. Removal of solvents gave the final product. R_f 0.35 (25 % NH_4OH in MeOH).



A

Yield: 90%, white foam.

^{1}H NMR (500 MHz, CD_3 OD) δ ppm:

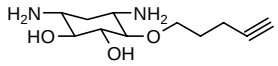
4.54-4.58 (m, 1H), 4.45-4.48 (m, 1H), 3.28 (t, 1H), 3.05-3.09 (m, 2H), 2.90 (s, 1H), 2.60-2.68 (m, 2H), 2.00 (ddd, J = 4.4, 4.4, 13.0, 1H), 1.25 (q, J = 12.4, 1H).

^{13}C NMR (125 MHz, CD_3OD) δ ppm:

85.6, 80.4, 77.7, 77.0, 74.7, 59.5, 51.2, 50.2, 35.6.

HRMS (ESI): m/e calcd for $C_9H_{16}N_2O_3$ ($M + H^+$) 201.1239, found 201.1230.

IR (net): 3410 (br), 1100 cm^{-1}.



B

Yield: 95%, white foam.

^{1}H NMR (400 MHz, CD_3 OD) δ ppm:

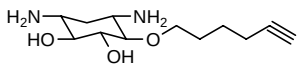
3.95-4.00 (m, 1H), 3.78-3.80 (m, 1H), 3.41-3.43 (m, 1H), 3.25-3.31 (m, 1H), 3.20-3.25 (m, 2H), 3.02 (t, 1H), 2.29-2.35 (m, 2H), 2.20 (s, 1H), 2.10 (ddd, J = 4.4, 4.4, 13.0, 1H), 1.78-1.82 (m, 2H), 1.22 (q, J = 12.4, 1H).

^{13}C NMR (100 MHz, CD_3OD) δ ppm:

84.7, 76.6, 75.9, 71.6, 68.3, 60.8, 60.4, 32.3, 29.3, 14.7.

HRMS (ESI): m/e calcd for $C_{11}H_{20}N_2O_3$ ($M + H^+$) 229.1552, found 229.1557.

IR (net): 3400 (br), 1100 cm^{-1}.



C

Yield: 95%, white foam.

^{1}H NMR (500 MHz, CD_3 OD) δ ppm:

3.95-4.01 (m, 1H), 3.60-3.64 (m, 1H), 3.25 (t, 1H), 3.02 (t, 1H), 2.86 (t, 1H), 2.58-2.70 (m, 2H), 2.18-2.22 (m, 3H), 1.98 (ddd, J = 4.4, 4.4, 13.0, 1H), 1.70-1.78 (m, 2H), 1.58-1.63 (m, 2H), 1.20 (q, J = 12.4, 1H).

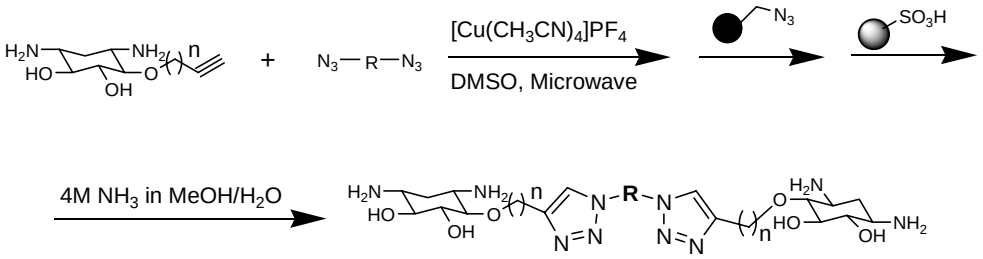
^{13}C NMR (125 MHz, CD_3OD) δ ppm:

86.6, 83.7, 78.2, 76.9, 72.2, 68.4, 51.3, 50.6, 35.9, 29.3, 25.2, 17.6.

HRMS (ESI): m/e calcd for $C_{12}H_{22}N_2O_3$ ($M + H^+$) 243.1709, found 243.1712.

IR (net): 3400 (br), 1100 cm^{-1}.

General scheme for the synthesis and catch-release purification of the deoxystreptamine dimer library

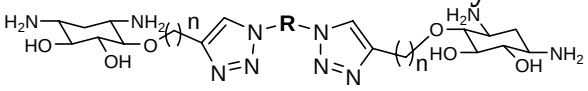


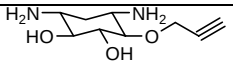
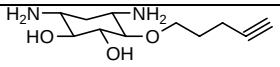
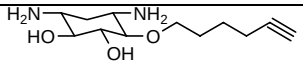
In a vial, $Cu(MeCN)_4PF_6$ (0.16 mg, 0.00044 mmol, 2.0 mol% with respect to alkyne) was added to a solution of azide (0.01 mmol) and 2-deoxystreptamine-alkyne (0.022 mmol) in DMSO (0.5 mL). The mixture was heated with domestic microwave (vials uncapped and microwave at full power) for 40 seconds. The azidomethyl polystyrene resin (0.1 g) was added, and the reaction mixture was heated again for 30 seconds (vials uncapped and microwave at full power). Reaction monitoring by TLC (MeOH/conc.NH_4OH 2:1) indicated that the 2-deoxystreptamine-alkyne had been completely consumed. The reaction mixture was collected by filtration and the resin was washed with DMSO (3 x 2 mL), and the filtrates were combined together. p-Toluenesulfonic acid resin (110 mg) was added. Agitation for 1 h at room temperature was followed by filtration and alternating washing of the resin with MeOH (5 x 5 mL), H_2O (5 x 5 mL), MeOH (5 x 5 mL), CH_2Cl_2 (5 x 5 mL).

The product was released via the following steps. The resin was first treated with 10 % triethylamine in methanol (10 mL). After the resin had been shaken for 30 min, it was filtered and the resin was washed twice with MeOH (2x 5 mL). Then the resin was then shaken with a 4 M ammonia methanol/water (2:1) solution (2.0 mL) for 1 h. The solution was filtered, and the resin was washed with MeOH/H_2O (2:1) (3 x 2 mL for 10 min). The combined filtrates were evaporated to dryness gave the dimer products.

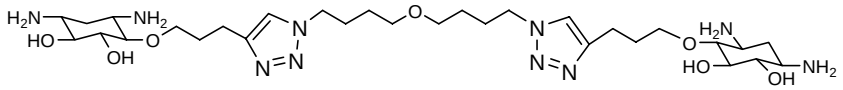
The library was analyzed by TLC and LC-MS (CH_3CN/ H_2O; UV detection at 200-400 nm). LC-MS was conducted on all 105 members of the library using a reversed-phase C18 column. The mass spectrometer was a Finnigan LCQ decaXP equipped with a Surveyor autosampler, a Surveyor PDA detector (200 – 400 nm) and a mass selective detector. The purity of each compound in the library and the mass observed for each compound in the library is listed in the Table below.

Table: Characterization of library members



	 A			 B			 C		
	Products			Products			Products		
Azide	M.W	M + 1	LC/MS Purity	M.W	M + 1	LC/MS Purity	M.W	M + 1	LC/MS Purity
1	554.6	556.3	92	610.8	611.4	91	638.8	639.4	87
2	556.6	558.2	89	612.7	613.4	88	640.8	641.4	96
3	568.7	570.2	91	624.8	625.4	94	652.5	653.5	97
4	596.7	598.3	96	652.8	653.5	81	680.9	681.5	94
5	612.7	614.5	93	668.8	669.5	93	696.6	697.5	97
6	610.7	612.5	90	666.9	667.5	95	694.9	695.5	91
7	624.8	626.4	97	680.9	681.5	80	708.9	709.5	86
8	638.8	640.5	94	694.9	695.5	81	723.0	723.5	80
9	644.7	646.5	95	700.8	701.5	87	728.9	729.5	98
10	652.8	654.5	86	708.9	709.5	80	737.0	737.5	83
11	588.7	590.4	97	644.8	645.4	91	672.8	673.4	97
12	588.7	590.3	97	644.8	645.4	87	672.8	673.4	97
13	630.7	632.5	90	686.9	687.4	93	714.9	715.5	98
14	589.7	591.2	94	645.8	646.4	97	673.8	674.4	98
15	664.8	666.5	95	720.9	721.4	93	748.9	749.4	89
16	678.8	680.5	93	734.9	735.4	90	762.9	763.5	90
17	692.8	694.2	90	748.9	749.5	92	777.0	777.5	84
18	792.9	794.5	95	849.0	849.5	97	877.0	877.5	91
19	688.7	690.5	97	744.9	745.4	94	772.6	773.5	87
20	793.8	795.5	89	849.9	850.5	82	878.0	878.5	96
21	764.8	766.5	86	820.9	821.5	93	849.0	849.5	93
22	766.8	768.3	88	822.9	823.4	89	851.0	851.5	91
23	814.9	816.5	95	871.0	871.4	96	899.0	899.5	89
24	818.8	820.5	87	875.0	875.4	90	903.0	903.5	94
25	851.9	853.5	90	908.0	909.0	80	936.1	936.5	99
26	738.9	740.4	92	795.0	796.0	97	823.0	823.5	81
27	679.7	681.4	86	735.8	736.5	96	763.9	764.5	87
28	650.7	652.5	87	706.8	707.6	90	734.9	735.5	84
29	652.7	654.5	95	708.8	709.5	91	736.9	737.4	84
30	614.6	616.4	93	670.8	671.5	95	698.8	699.4	92
31	700.8	702.3	91	756.9	757.5	95	784.9	785.4	87
32	700.8	702.3	90	756.9	757.5	98	784.9	785.4	94
33	704.7	706.5	83	760.9	762.1	87	788.9	789.5	84
34	737.8	739.5	89	793.9	795.0	86	822.0	822.5	85
35	770.9	772.5	85	827.0	828.0	87	855.0	855.5	80

Characterization of selected compounds



White solid.

^{1}H NMR (500 MHz, D_2O) δ ppm:

7.61 (s, 2H), 4.20 (t, J = 7.0, 4H), 3.68-3.72 (m, 2H), 3.48-3.64 (m, 2H), 3.24 (t, J = 7.0, 4H), 3.18 (t, J = 6.8, 2H), 2.98 (t, J = 7.0, 2H), 2.82 (t, J = 7.0, 2H), 2.56-2.62 (m, 8H), 1.78-1.83 (m, 6H), 1.70-1.75 (m, 6H), 1.30-1.38 (m, 4H), 1.12 (q, J = 12.4, 2H).

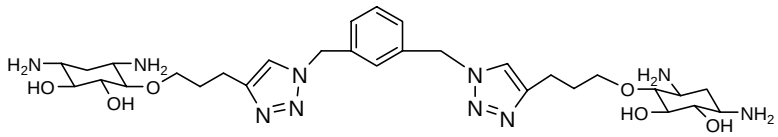
^{13}C NMR (125 MHz, D_2O) δ ppm:

147.7 (x2), 123.2 (x2), 86.0 (x2), 77.2 (x2), 75.5 (x2), 72.0 (x2), 69.5 (x2), 50.5 (x2), 50.0 (x2), 49.7 (x2), 35.0 (x2), 29.0 (x2), 26.3 (x2), 25.6 (x2), 21.1 (x2).

HRMS (ESI): m/e calcd for $C_{30}H_{56}N_{10}O_7$ ($M + H^+$) 669.4412, found 669.4422.

IR (KBr): 3500 (br), 1099 cm^{-1}.

Rf: 0.3 (MeOH/ NH_4OH 4:1)



White solid.

^{1}H NMR (500 MHz, D_2O) δ ppm:

7.59 (s, 2H), 7.20 (t, 1H), 7.10 (d, 2H), 6.90 (s, 1H), 5.30 (s, 4H), 3.68-3.72 (m, 2H), 3.48-3.54 (m, 2H), 3.18 (t, J = 7.0, 4H), 3.00 (t, J = 6.8, 2H), 2.81 (t, J = 7.0, 2H), 2.56- 2.60 (m, 8H), 1.78-1.83 (m, 6H), 1.70-1.75 (m, 6H), 1.12 (q, J = 12.4, 2H).

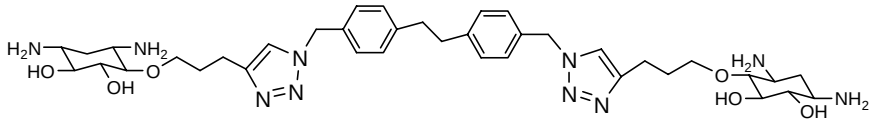
^{13}C NMR (125 MHz, D_2O) δ ppm:

148.1 (x2), 136.1 (x2), 129.8 (x2), 128.1, 127.0 (x2), 123.3, 85.9 (x2), 77.1 (x2), 75.5 (x2), 71.9 (x2), 53.4 (x2), 50.4 (x2), 49.7 (x2), 48.9 (x2), 34.8 (x2), 29.0 (x2), 12.3 (x2).

HRMS (ESI): m/e calcd for $C_{30}H_{48}N_{10}O_6$ ($M + H^+$) 645.3837, found 645.3834.

IR (KBr): 3500 (br), 1099 cm^{-1}.

Rf: 0.3 (MeOH/ NH_4OH 4:1)



White solid.

^{1}H NMR (500 MHz, D_2O) δ ppm:

7.18 (s, 2H), 6.80 (d, 4H), 6.48 (d, 4H), 5.00 (s, 4H), 3.60-3.68 (m, 2H), 3.38-3.43 (m, 2H), 3.18 (t, J = 7.0, 4H), 3.00 (t, J = 6.8, 2H), 2.80 (t, J = 7.0, 2H), 2.50- 2.60 (m, 4H), 2.30 (t, 4H), 2.06-2.12 (m, 4H), 1.78-1.83 (m, 2H), 1.48-1.50 (m, 4H), 1.02 (q, J = 12.4, 2H).

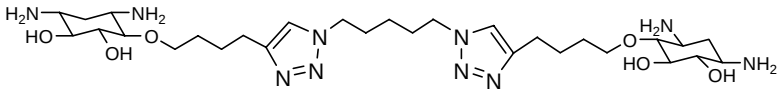
^{13}C NMR (125 MHz, D_2O) δ ppm:

147.9 (x2), 141.7 (x2), 132.9 (x2), 128.8 (x2), 128.3 (x2), 122.4 (x2), 85.9 (x2), 77.0 (x2), 75.6 (x2), 71.9 (x2), 53.3 (x2), 50.5 (x2), 49.7 (x2), 48.9 (x2), 36.0 (x2), 34.8 (x2), 29.1 (x2), 12.3 (x2).

HRMS (ESI): m/e calcd for $C_{38}H_{56}N_{10}O_6$ ($M + H^+$) 749.4463, found 749.4468.

IR (KBr): 3500 (br), 1100 cm^{-1}.

Rf: 0.3 (MeOH/ NH_4OH 4:1)



White solid.

^{1}H NMR (400 MHz, D_2O) δ ppm:

7.50 (s, 2H), 4.18 (t, J = 7.0, 4H), 3.68-3.78 (m, 2H), 3.48-3.56 (m, 2H), 3.18 (t, J = 7.0, 4H), 3.06 (t, J = 6.8, 2H), 2.86 (t, J = 7.0, 2H), 2.60-2.70 (m, 4H), 2.50 (t, J = 7.0, 2H), 1.80-1.90 (m, 2H), 1.60-1.70 (m, 4H), 1.47-1.51 (m, 4H), 1.38-1.43 (m, 4H), 1.12 (q, J = 12.4, 2H), 0.80-0.88 (m, 2H).

^{13}C NMR (100 MHz, D_2O) δ ppm:

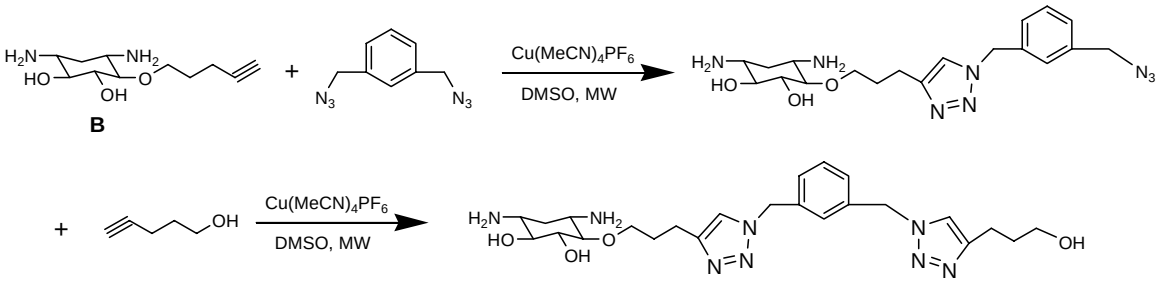
148.1 (x2), 123.1 (x2), 85.4 (x2), 76.3 (x2), 75.5 (x2), 72.9 (x2), 50.5 (x2), 49.8 (x2), 49.7 (x2), 33.9 (x2), 28.7 (x2), 28.4 (x2), 25.2 (x2), 24.2, 21.1 (x2).

HRMS (ESI): m/e calcd for $C_{29}H_{54}N_{10}O_6$ ($M + H^+$) 639.4306, found 639.4304.

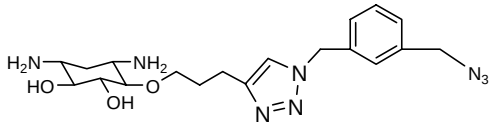
IR (KBr): 3500 (br), 1099 cm^{-1}.

Rf: 0.3 (MeOH/ NH_4OH 4:1)

Synthesis of mono deoxystreptamine derivatives



In a small vial, 2-deoxystreptamine-alkyne **B** (0.23 g, 1 mmol) and 1,3-Bis-azidomethyl-benzene (0.41 g, 2.2 mmol) were mixed in DMSO (1 mL); $Cu(MeCN)_4PF_6$ (7.4 mg, 0.02 mmol, 2.0 mol % with respect to alkyne) was added as a solid. The reaction was heated with domestic microwave (vials uncapped, microwave at full power) for 40 seconds, and the reaction mixture was then loaded on a short column of (5 cm in height) packed silica gel. The column was eluted with a series of solutions as the follows: MeOH, and MeOH/conc.NH_4OH (from 0-10% of conc. NH_4OH). The fractions containing desired product were analyzed by TLC and collected. Removal of solvents gave the final product 0.25 g (61 %). R_f 0.60 (25 % NH_4OH in MeOH).



white foam

^{1}H NMR (500 MHz, CD_3OD) δ ppm:

7.79 (s, 1H), 7.40 (t, 1H), 7.28-7.37 (m, 3H), 5.58 (s, 2H), 4.38 (s, 2H), 3.94-3.98 (m, 1H), 3.60-3.65 (m, 1H), 3.23 (t, J = 7.0, 1H), 3.03 (t, J = 6.8, 1H), 2.85 (t, J = 7.0, 1H), 2.80 (t, 2H), 2.58-2.70 (m, 2H), 1.92-1.99 (m, 3H), 1.20 (q, J = 12.4, 1H).

^{13}C NMR (125 MHz, CD_3OD) δ ppm:

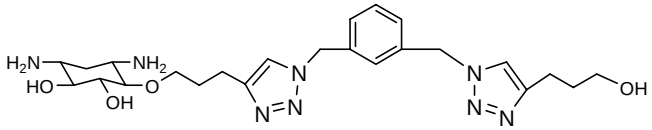
148.0, 136.9, 136.4, 129.3, 128.2, 127.7, 122.2, 86.6, 78.2, 76.7, 71.3, 53.9, 53.3, 51.3, 50.6, 36.0, 29.9, 21.6.

HRMS (ESI): m/e calcd for $C_{19}H_{28}N_8O_3$ ($M + H^+$) 417.2363, found 417.2344.

IR (KBr): 3500 (br), 1099 cm^{-1}.

Rf: 0.60 (MeOH/ NH_4OH 4:1)

The above product (0.20 g, 0.48 mmol) was mixed with 4-pentyn-1-ol (0.06 g, 0.72 mmol) in DMSO (1 mL). $Cu(MeCN)_4PF_6$ (5.3 mg, 0.014 mmol, 2.0 mol % with respect to alkyne) was added as a solid. The reaction was heated with domestic microwave (vials uncapped, microwave at full power) for 40 seconds, and the reaction mixture was loaded on a short column (5 cm in height) packed with silica gel. The column was eluted with a series of solutions as the follows: MeOH, and MeOH/conc.NH_4OH (from 0-10% of conc. NH_4OH). The fractions containing the desired product were collected. Removal of solvents gave the final product 0.21 g (91 %). R_f 0.40 (25 % NH_4OH in MeOH).



White foam.

^{1}H NMR (500 MHz, CD_3OD) δ ppm:

7.79 (s, 1H), 7.76 (s, 1H), 7.40 (t, 1H), 7.28 (d, 2H), 7.02 (s, 1H), 5.58 (d, 4H), 3.95-4.00 (m, 1H), 3.60-3.65 (m, 1H), 3.56-3.58 (t, J = 7.0, 2H), 3.23 (t, J = 7.0, 1H), 3.07 (t, J = 6.8, 1H), 2.93 (t, J = 7.0, 1H), 2.75-2.82 (m, 4H), 2.62-2.70 (m, 2H), 1.98-2.02 (m, 1H), 1.92-1.97 (m, 2H), 1.81-1.88 (m, 2H), 1.22 (q, J = 12.4, 1H).

^{13}C NMR (125 MHz, CD_3OD) δ ppm:

148.0, 136.7, 129.4, 127.9, 127.8, 127.3, 122.3, 122.2, 86.0, 77.6, 76.6, 71.2, 60.7, 53.2, 51.2, 50.5, 35.1, 32.0, 29.8, 21.5, 21.4.

HRMS (ESI): m/e calcd for $C_{24}H_{36}N_8O_4$ ($M + H^+$) 501.2938, found 501.2959.

IR (KBr): 3500 (br), 1099 cm^{-1}.

Rf: 0.40 (MeOH/ NH_4OH 4:1)

Reference:

(1) Greenberg, W. A.; Priestley, E. S.; Sears, P. S.; Alper, P. B.; Rosenbohm, C.; Hendrix, M.; Hung, S. C.; Wong, C. H. J. Am. Chem. Soc. 1999, 121(28), 6527-6541.
(2) Liu, X.; Thomas, J. R.; Hergenrother, P. J. J. Am. Chem. 2004, 126, 9196-9197.
(3) Lober, S. ; Rodriguez-Loaiza, P.; Gmeiner, P. Org. Lett. 2003, 123(46), 1753-1755.

Protocols for RNA binding and footprinting experiments

Materials

All reagents were obtained from Fisher unless otherwise stated. All solutions were made with Milli-Q purified water. Aminoglycosides were purchase from Sigma, and all RNA was purchased from Dharmacon Research. The *E. coli* tRNA mixture was purchased from Fluka.

Fluorescence Binding Assay

The ligand solutions were prepared as serial dilutions in TM_1 buffer (10 mM Tris, 1 mM $MgCl_2$, pH 7.5) at a concentration four times greater than the desired final concentration, to allow for the subsequent dilution during the addition of the RNA solution. 25 μL of the appropriate ligand solution was then added to a well of a black 96 well plate (Nunc 237105). Refolding of the RNA was performed using a thermocycler as follows. The RNA, stored in 10 mM Tris, 0.5 mM EDTA, pH 7.5, was first denatured by heating to 95 ºC for 2 min; the temperature was then dropped 0.1 ºC/sec until the temperature reached 25 ºC. After refolding, the RNA was diluted to working concentration of 37.5 nM through addition of the appropriate amount of TM_1 buffer (<4 μL added into 1900 μL of buffer). The tube was mixed by inversion, and then 75 μL of the RNA solution was added to each well containing ligand. This subsequent dilution brought the final RNA concentration to 28 nM. The fluorescence was measured on a Criterion Analyst AD (Molecular Devices) with an excitation filter of 485 ± 15 nm, an emission filter of 530 ± 15 nm, and a 505 nm dichroic cutoff mirror. The binding was allowed to proceed to equilibrium, which was monitored in 15 min intervals. Equilibrium was determined when three identical curve were obtained. Nearly all curves were fit to a single site model using TableCurve 2D v5.01 (equation 8108):

$$y = \frac{a * x}{Kd + x}$$

where a is the asymptotic limit. Those compounds that displayed a sigmodial shape were fit to a logistic dose-response curve (equation 8013):

$$y = \frac{a}{1 + \left(\frac{x}{Kd}\right)^c}$$

where a is the limit that the curve approaches. All binding assay were performed in quadruplicate. In all cases the error bars are the standard deviation from the mean.

The tRNA competition experiment was performed as outlined by Luedtke, N. W. *et al. Biochemistry* **2003**, 42, 11391. In brief, a 100-fold bases excess relative to the fluorescently labeled RNA was refolded in 1900 μL of 10 mM Tris pH 7.5, 2 mM $MgCl_2$, 100 mM NaCl at 95 ºC for 2 min and allowed to cool to ambient temperature. After the tRNA mixture was cooled the fluorescence binding assay was carried as described above

with the exception that the fluorescently labeled RNA was added to the refolded tRNA mixture.

Footprint Assay

The RNA was 5' radiolabeled with γ-ATP (Amersham Biosciences) using T4 polynucleotide kinase (Invitrogen) according to manufacturer protocols. The protocol for footprinting is largely derived from McPike M. P. *et al*, *Methods in Enzymology* **2001**, *340*, 431-449. The proper RNase concentration and reaction time to yield 'single-hit' kinetics (<20% enzymatic digestion) were determined first by varying RNase concentration in a series of 1:10 dilution in TM_1 with a constant digestion time of 1 min. When necessary the most optimal RNase concentration was held constant and time was varied until 'single-hit' kinetics were obtained.

The ligand solutions were prepared as serial dilutions in TM_5 (10 mM Tris, 5 mM $MgCl_2$, pH 7.5) at a concentration 2.5 times greater than the desired final concentration, to allow for the subsequent dilution during the addition of the RNA solution. 4 μL of the appropriate ligand solution were added a well of a 96 well V-bottom plate (Nunc 249662). Refolding of the RNA was preformed using a thermocycler. The RNA, stored in 10 mM Tris, 0.5 mM EDTA, pH 7.5 was first denatured by heating to 95 ºC for 2 min after which the temperature was dropped 0.1 ºC/sec until the temperature reached 25 ºC. 5 μL of the refolded RNA was added to each well containing ligand. Binding was allowed to proceed for 30 min. After incubation, 1 μL of RNase is added to each well and the digestion is stopped by the addition of Stop Buffer (8 M Urea, 50 mM EDTA, 2X TBE, bromphenol blue 0.05% (w/v), and xylene cyanol 0.05% (w/v)). The concentrations of RNase and digestion time are those that yield 'single-hit' kinetics. 18 μL of each sample were then run on a 20% Acrylamide (29:1), 8M Urea gel at 45 V for 5.25 hours. Gels were analyzed by Molecular Dynamics Storm 430 phosphoimager (Amersham Biosciences). All densitometeric analysis of were performed using ImageQuant 5.2 (Molecular Dynamics). In order to correct for loading errors, all the parent bands were normalized and each band considered for analysis was normalized to their respective parent band.

UV Melting Temperature Experiments

All RNA oligos used in the UV melting temperature experiments were generated by *in vitro* T7 transcription (see below). All experiments were conducted with 1 cm pathlength quartz cuvette. The absorbance versus temperature profiles were measured at 260 nm with a five second averaging time. Initially all experiments started at 20ºC where the temperature was held for 5 min prior to the start of the temperature ramp. The temperature ramp rate was 0.5ºC/min with 1.5 min equilibration time in between each temperature step. The buffer solution used contained 10 mM sodium cacodylate, 10 mM Mops pH 7.5, 0.1 mM EDTA and NaCl to bring the final concentration of Na^+ to 100 mM. To determine the T_m values, plots of absorbance vs. temperature were produced by Origin by Microcal. The first derivative of each plot was obtained and the local maximum of the first derivative data was recorded after performance of one smoothing operation.

T7 RNAP Expression and Purification

pT7-911 was the kind gift of Prof. Scott K. Silvermann. A glycerol stock of pT7-911 in XLI-Blue (Stratagene) was used to generate 10 mL overnight culture of LB/ampicillin (100 μg/mL). The overnight culture was incubated at 37 ºC with 225 rpm for 12-16 hours after which time the overnight culture was used to seed a 1L LB/ampicillin (100 μg/mL) induction culture. The induction culture was allowed to incubate at 37 ºC with 225 rpm until the OD_{600} reached 0.4-0.6; upon which IPTG was added to the induction culture to give a final concentration of 250 μM. The induction culture continued to incubate at 37 ºC with 225 rpm for a period of 4 hours. The cells where then harvested by centrifugation at 6000x*g* for 30 min. The supernatant discarded and the pellet was resuspended in 10 mL of cold Binding Buffer (10 mM Tris pH 7.5, 100 mM NaCl, 5 mM β-mercaptoethanol, 5% glycerol, 5 mM imidazole). Cells were lysed by subjection to a French Press at 10,000 psi twice. The soluble fraction was separated from the soluble/membrane fraction via centrifugation at 40,000x*g* for 30 min. The supernatant collected into a 15 mL Falcon tube containing 1.5 mL Ni-NTA resin slurry (Qiagen). The supernatant was allowed to batch load onto the Ni^{2+} column for 1 hour at 4 ºC. After the batch loading process, the supernatant- Ni-NTA argarose-resin was loaded onto an Econo-Pac Disposalbe Chromatography Column (Bio-rad). The column was washed with 10 mL of cold Binding Buffer, 10 mL cold Wash Buffer (identical to Binding Buffer except for 10 mM imidazole), and the His-tagged T7-RNAP was eluted with 10 mL of cold Elution Buffer (identical to Binding Buffer except 250 mM imidazole). All elution fractions were analyzed for the presence of protein using the Bradford dye reagent. All samples containing protein were combined and concentrated to ~3 mg/mL using the centricon centrifugal concentration device, 10,000 molecular weight cutoff (Millipore).

T7 RNAP Transcription Runoff

All DNA used in the transcription process was purchased from Integrated DNA Technologies. The T7 template DNA oligo (5-acg cac gct gta ata cga ctc act ata-3') was annealed to either the RNA **I** (tetraloop) template (5'mgmg cgc aca cgc gcc tat agt gag tcg tat tac agc gtg cgt-3'), RNA **II** (hexaloop) template (5'-mgmgc gct aca ctg cgc cta tag tga gtc gta tta cag cgt gcg t-3'), RNA **III** (heptaloop) template (5'- mgmgc gct act act gcg cct ata gtg agt cgt att aca gcg tgc gt -3'), or RNA **IV** (octaloop) template (5'- mgmgc gct act gac tgc gcc tat agt gag tcg tat tac agc gtg cgt-3'). All DNA templates used were PAGE purified, 20% Acrylamide (29:1), 8M Urea gel, on a 20% Denaturing gel prior to use, the templates were validated by MALDI-MS.

All T7 transcription assays were performed on a 10 nmol scale. The T7 template and its corresponding RNA hairpin loop template were annealed by adding 10 nmols of each template to a 1.7 mL centrifuge tube containing 20 mM Tris pH 8.0, 75 mM NaCl, 0.5 mM EDTA, in a final volume 1 mL. The 1.7 mL tube was incubated at 95 ºC for 3 min followed by incubation on ice for 5 min. This 1.0 mL template mixture was added to a 40 mL centrifugation tube (Nalge Nunc: 3146-0050) containing a 9 mL solution of Tris, NTPs, etc, such that the final concentration in 10 mL is as follows: 38 mM Tris pH 8.0, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, 1 mM GTP, 1 mM CTP, 1 mM UTP, 2 mM Spermine, 500 μL T7 RNAP. The transcription reaction was then incubated at 37 ºC for

6 hours after which 100 μL of 500 mM EDTA, 3 mL of 4 M NaCl, 30 mL cold 100% EtOH were added to the transcription reaction. The contents were then incubated at -80 ºC overnight. The following morning the tube was spun at 40,000x*g* for 30 min. The supernatant was discarded and the pellet was washed with cold ethanol (70%). After 1 hour incubation at –80 ºC the crude product was spun again at 40,000x*g* for 30 min. After decanting, the pellet was dried via lyophilization. After the pellet is dried to completeness, it was resuspended in a minimal volume (typically 300 μL) of 10 mM Tris, 0.5 mM EDTA, pH 7.5 and PAGE purified (20% Acrylamide (29:1), 8M Urea gel, 2 mm thickness). The molecular weights of all products were verified by MALDI-MS.