CHEMBIOCHEM

Supporting Information

DOI: 10.1002/cbic.200800642

Supporting Information

for

A Fluorescent Probe for the 70 S-Ribosomal GTPase-Associated Center

Sebastian Schoof, Sascha Baumann, Bernhard Ellinger, and Hans-Dieter Arndt*

HPLC methods

Varian ProStar, Diode Array Detector, flow = 1 mL/min, ternary gradient (A/B/C in v/v/v): A = bidest. H_2O, B = CH_3CN, C = 2% TFA in bidest. H_2O.

Method I:

A/B/C = 90:10:0 for 1 min, then to 0:100:0 in 16 min, then 0:100:0 for 2 min.

Column: 125 x 4 mm Nucleodur 5 µm C4 (Macherey & Nagel).

Method II:

A/B/C = 85:10:5 for 1 min, then to 0:95:5 in 10 min, then 0:95:5 for 3 min.

Column: 125 x 4 mm Nucleodur 3 µm C18 Gravity (Macherey & Nagel).

1. Preparation of truncated Thiostrepton derivatives

To a stirred solution of thiostrepton **1** (1.0 g, 0.601 mmol) in $CHCl_3$ (50 mL) was added $HNEt_2$ (5 mL) dropwise at 0°C. After 5 min the reaction was continued at RT for 3 h. The volatiles were coevaporated with toluene and the residue was purified by silica gel column chromatography (2-5 % MeOH in $CHCl_3$) to provide truncated thiostrepton **4** and minor amounts of **5**.

(**4**): 70% yield, colorless powder, m.p. > 240 °C decomp., R_f = 0.39 ($CHCl_3$/MeOH 9:1). HPLC: R_t =13.4 min (method I). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD 4:1) d = 9.70 (s, 1H), 8.63 (d, *J*=8.8, 1H), 8.12 (s, 1H), 8.10 (s, 1H), 8.00 (s, 1H), 7.86 (s, 1H), 7.40 (s, 1H), 7.14 (s, 1H), 6.97 (d, *J*=7.9, 1H), 6.92 (d, *J*=7.6, 1H), 6.73 (d, *J*=10.1, 1H),

6.46 (d, *J*=1.7, 1H), 6.25 – 6.15 (m, 2H), 6.06 (q, *J*=7.1, 1H), 5.67 (d, *J*=2.1, 1H), 5.63 – 5.57 (m, 2H), 5.48 (d, *J*=1.6, 1H), 5.18 (s, 1H), 5.16 – 5.12 (m, 2H), 4.81 (dd, *J*=8.9, 12.9, 1H), 4.60 – 4.52 (m, 1H), 4.28 – 4.23 (m, 3H), 3.93 – 3.86 (m, 1H), 3.68 – 3.60 (m, 2H), 3.51 – 3.43 (m, 2H), 3.36 – 3.25 (m, 1H), 3.00 (dd, *J*=11.4, 13.0, 1H), 2.80 (d, *J*=4.3, 1H), 2.78 – 2.71 (m, 1H), 2.13 (t, *J*=12.9, 1H), 1.79 – 1.71 (m, 1H), 1.55 (d, *J*=6.6, 3H), 1.45 (d, *J*=7.1, 3H), 1.42 – 1.37 (m, 1H), 1.26 (d, *J*=6.6, 3H), 1.21 (d, *J*= 6.6, 3H), 1.13 (d, *J*=6.4, 5H), 1.03 – 0.97 (m, 8H), 0.95 – 0.86 (m, 1H), 0.83 (d, *J*=6.9, 3H), 0.72 (t, *J*=7.3, 4H), 0.65 (d, *J*=6.2, 3H). HRMS calcd. for [$C_{69}H_{83}N_{18}O_{17}S_5$+$H^+$] 1595.478, found (ESI) 1595.482.

(**5**): 15 % yield, colorless powder, m.p. > 240 °C decomp., R_f = 0.28 ($CHCl_3$/MeOH 9:1). HPLC: R_t = 14.0 min (method I). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD 4:1) d 9.70 (s, 1H), 8.63 (d, *J* = 8.8, 1H), 8.12 (s, 1H), 8.10 (s, 1H), 8.00 (s, 1H), 7.86 (s, 1H), 7.40 (s, 1H), 7.14 (s, 1H), 6.97 (d, *J* = 7.9, 1H), 6.92 (d, *J* = 7.6, 1H), 6.73 (d, *J* = 10.1, 1H), 6.46 (d, *J* = 1.7, 1H), 6.25 – 6.15 (m, 2H), 6.06 (q, *J* = 7.1, 1 H), 5.67 (d, *J* = 2.1, 1H), 5.63 – 5.57 (m, 2H), 5.48 (d, *J* = 1.6, 1H), 5.18 (s, 1H), 5.16 – 5.12 (m, 2H), 4.81 (dd, *J* = 8.9, 12.9, 1H), 4.60 – 4.52 (m, 1H), 4.28 – 4.23 (m, 3H), 3.93 – 3.86 (m, 1H), 3.68 – 3.60 (m, 2H), 3.51 – 3.43 (m, 2H), 3.36 – 3.25 (m, 1H), 3.00 (dd, *J* = 11.4, 13.0, 1H), 2.80 (d, *J* = 4.3, 1H), 2.78 – 2.71 (m, 1H), 2.13 (t, *J* = 12.9, 1H), 1.79 – 1.71 (m, 1H), 1.55 (d, *J* = 6.6, 3H), 1.45 (d, *J* = 7.1, 3H), 1.42 – 1.37 (m, 1H), 1.26 (d, *J* = 6.6, 3H), 1.21 (d, *J* = 6.6, 3H), 1.13 (d, *J* = 6.4, 5H), 1.03 – 0.97 (m, 8H), 0.95 – 0.86 (m, 1H), 0.83 (d, *J* = 6.9, 3H), 0.72 (t, *J* = 7.3, 4H), 0.65 (d, *J* = 6.2, 3H). HRMS calcd. for [$C_{66}H_{80}N_{17}O_{16}S_5$+$H^+$] 1526.457, found (ESI) 1526.461.

Truncation **4** ? **5:**

To a stirred solution of **4** (100 mg, 0.060 mmol) in $CHCl_3$ (4.5 mL) was added $HNEt_2$ (0.5 mL) dropwise at 0°C. After 5 min the reaction was continued at RT. After 29 h additional $HNEt_2$ (0.5 mL) was added and the reaction mixture was stirred for 27 h. The volatiles were coevaporated with toluene and the residue was purified by silica gel column chromatography (2-5 % MeOH in $CHCl_3$) to give 46 mg of **5** (48%) as a colorless solid.

2. General procedure for the preparation of sulfa-Michael adducts 7-11

Truncated thiostrepton **4** (50 mg, 0.031 mmol) was dissolved in TFE (2 mL) and NaP_i buffer was added (1 mL, 50 mM, pH 9). The respective thiol was added (0.037 mmol, 1.2 eq) followed by 22 µL Et_3N (0.156 mmol, 5 eq). The reaction mixture was stirred

for 16 h under Ar atmosphere. The volatiles were removed and purification of the crude product by preparative HPLC (C4 column, CH_3CN/H_2O) gave the desired sulfa-Michael addition product after lyophilization of the corresponding fractions.

(**7**): 56 % yield, colorless powder. m.p. > 240 °C decomp., R_f = 0.21 ($CHCl_3$/MeOH 17:3). HPLC: R_t = 11.0 min (method I). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 4:1) d = 8.13 (s, 1H), 8.09 (d, *J*=3.9, 1H), 8.02 (s, 1H), 7.87 (s, 1H), 7.41 (s, 1H), 7.16 (s, 1H), 7.00 – 6.92 (m, 1H), 6.74 (d, *J*=10.1, 1H), 6.27 – 6.15 (m, 2H), 6.07 (q, *J*=7.0, 1H), 5.69 (s, 1H), 5.64 – 5.56 (m, 2H), 5.22 – 5.12 (m, 3H), 4.87 – 4.79 (m, 1H), 4.78 – 4.72 (m, 1H), 4.61 – 4.53 (m, 1H), 4.37 (d, *J*=9.7, 1H), 4.31 (d, *J*=9.1, 1H), 4.29 – 4.22 (m, 2H), 3.82 – 3.72 (m, 1H), 3.67 – 3.62 (m, 2H), 3.57 – 3.44 (m, 3H), 3.31 – 3.20 (m, 3H), 3.09 – 2.86 (m, 2H), 2.82 (d, *J*=4.1, 1H), 2.13 (d, *J*=13.4, 1H), 1.83 – 1.72 (m, 1H), 1.57 (d, *J*=6.5, 3H), 1.46 (d, *J*=7.0, 4H), 1.28 (t, *J*=6.5, 3H), 1.23 (d, *J*=6.6, 4H), 1.14 (d, *J*=6.4, 4H), 1.05 – 0.98 (m, 7H), 0.85 (d, *J*=6.9, 3H), 0.73 (t, *J*= 7.3, 3H), 0.66 (d, *J*=6.1, 3H). HRMS calcd. for [$C_{75}H_{94}N_{18}O_{22}S_6$+$H^+$] 1791.519, found (ESI) 1791.520.

(**8a**): 6 % yield, colorless powder. m.p. > 240 °C decomp., R_f = 0.35 ($CHCl_3$/MeOH 9:1). HPLC: R_t = 17.5 min (method I). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 4:1) d = 8.12 (s, 1H), 8.08 (s, 1H), 8.02 (s, 1H), 7.41 (s, 1H), 7.15 (s, 1H), 6.98 – 6.92 (m, 2H), 6.73 (d, *J*=10.1, 1H), 6.27 – 6.15 (m, 2H), 6.06 (q, *J*=7.0, 1H), 5.68 (d, *J*=2.0, 1H), 5.60 (s, 1H), 5.58 (s, 1H), 5.19 (s, 1H), 5.17 – 5.13 (m, 2H), 4.82 (dd, *J*=8.5, 12.0, 1H), 4.61 – 4.55 (m, 1H), 4.29 – 4.20 (m, 2H), 3.94 – 3.87 (m, 1H), 3.80 – 3.69 (m, 16H), 3.68 – 3.59 (m, 17H), 3.46 (s, 2H), 2.87 (d, *J*=6.9, 2H), 2.85 – 2.79 (m, 2H), 2.51 – 2.44 (t, *J*=7.4, 2H), 2.15 (dd, *J*=10.2, 22.6, 1H), 1.91 – 1.83 (m, 2H), 1.74 – 1.67 (m, 1H), 1.56 (d, *J*=6.7, 3H), 1.48 – 1.41 (m, 6H), 1.23 (d, *J*=6.7, 6H), 1.10 (s, 8H), 1.03 – 0.97 (m, 8H), 0.85 (d, *J*=6.9, 3H), 0.75 – 0.68 (m, 8H), 0.64 (d, *J*=6.3, 3H). HRMS calcd. for [$C_{77}H_{100}N_{18}O_{17}S_6$+$H^+$] 1741.591, found (ESI) 1741.592.

(**8b**): 6 % yield, colorless powder. m.p. > 240 °C decomp., R_f = 0.38 ($CHCl_3$/MeOH 9:1). HPLC: R_t = 17.7 min (method I). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 4:1) d = 8.12 (s, 1H), 8.08 (s, 1H), 8.02 (s, 1H), 7.41 (s, 1H), 7.15 (s, 1H), 6.94 (d, *J*=7.5, 1H), 6.73 (d, *J*=10.1, 1H), 6.27 – 6.16 (m, 2H), 6.06 (q, *J*=7.1, 1H), 5.68 (d, *J*=2.3, 1H), 5.64 – 5.56 (m, 2H), 5.18 (d, *J*=1.7, 1H), 5.17 – 5.13 (m, 2H), 4.82 (dd, *J*=9.0, 12.9, 1H), 4.61 – 4.53 (m, 2H), 4.31 – 4.19 (m, 2H), 3.94 – 3.88 (m, 1H), 3.68 – 3.61 (m, 2H), 3.52 – 3.44 (m, 2H), 3.31 – 3.22 (m, 1H), 3.05 – 2.97 (m, 1H), 2.87 (d, *J*=6.5,

2H), 2.81 (d, *J*=4.1, 1H), 2.79 – 2.71 (m, 1H), 2.52 – 2.40 (t, *J*=7.4, 2H), 2.19 – 2.09 (m, 1H), 1.81 – 1.73 (m, 1H), 1.56 (d, *J*=6.5, 3H), 1.49 – 1.39 (m, 6H), 1.27 (d, *J*=6.6, 3H), 1.23 (d, *J*=6.6, 3H), 1.16 – 1.05 (m, 13H), 1.04 – 0.98 (m, 6H), 0.85 (d, *J*=6.9, 3H), 0.76 – 0.67 (m, 7H), 0.65 (d, *J*=6.2, 3H). HRMS calcd. for [$C_{77}H_{100}N_{18}O_{17}S_6$+$H^+$] 1741.591, found (ESI) 1741.592.

(**9**): 32 % yield, colorless powder, m.p. > 240 °C decomp., R_f = 0.21 ($CHCl_3$/MeOH 9:1). HPLC: R_t = 12.5 min (method I). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD 4:1) d = 8.08 (s, 1H), 8.04 (s, 1H), 7.98 (s, 1H), 7.61 (d, *J*=5.9, 1H), 7.38 (s, 1H), 7.11 (s, 1H), 6.92 (d, *J*=7.8, 1H), 6.69 (d, *J*=10.0, 1H), 6.23 – 6.11 (m, 2H), 6.03 (q, *J*=7.5, 1H), 5.64 (s, 1H), 5.59 – 5.53 (m, 2H), 5.15 (s, 1H), 5.14 – 5.08 (m, 2H), 4.79 (dd, *J*=9.1, 12.3, 1H), 4.63 – 4.57 (m, 1H), 4.56 – 4.50 (m, 2H), 4.24 – 4.18 (m, 2H), 3.91 – 3.83 (m, 1H), 3.69 (s, 1H), 3.65 – 3.58 (m, 2H), 3.55 (d, *J*=5.0, 3H), 3.47 – 3.39 (m, 2H), 3.27 – 3.18 (m, 1H), 3.02 – 2.94 (m, 1H), 2.93 – 2.74 (m, 4H), 2.17 – 2.06 (m, 1H), 1.84 (s, 3H), 1.76 – 1.70 (m, 1H), 1.52 (d, *J*=6.5, 3H), 1.41 (d, *J*=7.1, 4H), 1.26 – 1.20 (m, 3H), 1.18 (d, *J*=6.6, 3H), 1.10 (d, *J*=6.3, 4H), 1.04 (s, 1H), 0.99 – 0.94 (m, 6H), 0.81 (d, *J*=7.0, 3H), 0.68 (t, *J*=7.3, 3H), 0.60 (d, *J*=6.4, 3H). HRMS calcd. for [$C_{75}H_{93}N_{19}O_{20}S_6$+$H^+$] 1772.524, found (ESI) 1772.525.

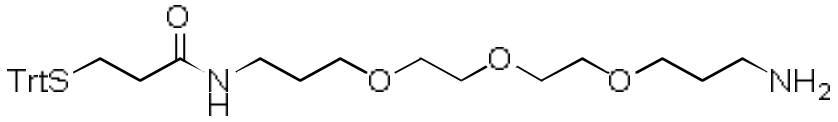
(**10**): 42 % yield, colorless powder, m.p. > 240 °C decomp., R_f = 0.10 ($CHCl_3$/MeOH 17:3). HPLC: R_t = 7.4 min (method I). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD 4:1) d = 8.12 (s, 1H), 8.08 (s, 1H), 8.01 (s, 1H), 7.87 (s, 1H), 7.41 (s, 1H), 7.15 (s, 1H), 7.00 – 6.91 (m, 2H), 6.73 (d, *J*=10.2, 1H), 6.28 – 6.15 (m, 2H), 6.06 (q, *J*=7.1, 1H), 5.68 (d, *J*=1.8, 1H), 5.64 – 5.55 (m, 2H), 5.18 (s, 1H), 5.17 – 5.13 (m, 2H), 4.82 (dd, *J*=9.0, 13.0, 1H), 4.70 – 4.61 (m, 1H), 4.61 – 4.52 (m, 2H), 4.29 – 4.22 (m, 2H), 3.92 – 3.86 (m, 1H), 3.68 – 3.61 (m, 2H), 3.52 – 3.44 (m, 2H), 2.93 – 2.78 (m, 3H), 2.16 – 2.09 (m, 1H), 1.92 – 1.85 (m, 3H), 1.81 – 1.72 (m, 1H), 1.56 (d, *J*=6.6, 3H), 1.45 (d, *J*=7.1, 3H), 1.31 – 1.25 (m, 3H), 1.23 (d, 2H), 1.14 (d, *J*=6.4, 3H), 1.03 – 0.97 (m, 6H), 0.85 (d, *J*=6.9, 3H), 0.72 (t, *J*=7.3, 3H), 0.65 (d, *J*=6.1, 3H). HRMS calcd. for [$C_{74}H_{91}N_{19}O_{20}S_6$+$H^+$] 1758.509, found (ESI) 1758.509.

(**11**): 26 % yield, colorless powder, m.p. > 240 °C decomp., R_f = 0.16 ($CHCl_3$/MeOH 17:3). HPLC: R_t = 8.6 min (method II). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD 4:1) d = 8.65 (d, *J*=8.7, 1H), 8.13 (s, 1H), 8.08 (d, *J*=1.5, 1H), 8.02 (s, 1H), 7.63 (d, *J*=5.3, 1H), 7.45 – 7.39 (m, 2H), 7.16 (s, 1H), 6.99 – 6.92 (m, 2H), 6.74 (d, *J*=10.2, 1H), 6.28 – 6.16 (m, 2H), 6.07 (q, *J*=7.1, 1H), 5.69 (s, 1H), 5.65 – 5.57 (m, 2H), 5.19 (s, 1H), 5.18

– 5.13 (m, 2H), 4.83 (dd, *J*=8.9, 13.0, 1H), 4.71 – 4.65 (m, 1H), 4.61 – 4.52 (m, 1H), 4.30 – 4.22 (m, 2H), 3.67 – 3.61 (m, 3H), 3.51 – 3.43 (m, 2H), 3.33 – 3.23 (m, 1H), 3.06 – 2.98 (m, 1H), 2.98 – 2.91 (m, 2H), 2.88 – 2.79 (m, 4H), 2.78 – 2.70 (m, 4H), 2.42 (d, *J*=5.5, 6H), 2.21 – 2.08 (m, 1H), 1.82 – 1.73 (m, 1H), 1.57 (d, *J*=6.6, 3H), 1.46 (d, *J*=7.1, 3H), 1.31 – 1.26 (m, 3H), 1.23 (d, *J*=6.6, 6H), 1.14 (d, *J*=6.4, 6H), 1.04 – 1.01 (m, 5H), 0.85 (d, *J*=6.9, 3H), 0.73 (t, *J*=7.3, 3H), 0.65 (d, *J*=6.2, 3H). HRMS calcd. for [$C_{73}H_{93}N_{19}O_{17}S_6$+$H^+$] 1700.539, found (ESI) 1700.541.

3. Preparation of Trityl-protected PEG-fluoresceine tag 13

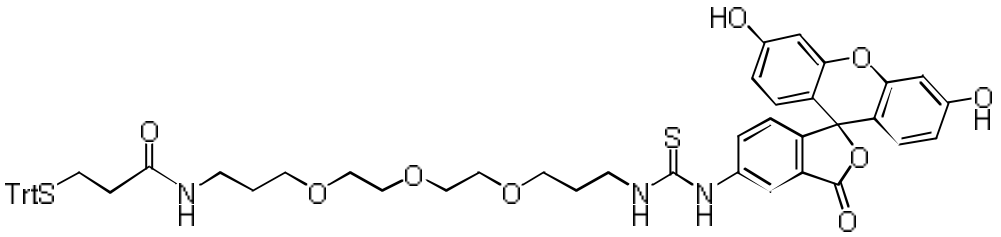
N-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-*b*-(tritylthio)propionamide (12)



To a solution of Trt-mercaptopropionic acid (697 mg, 2.0 mmol) and HBTU (1.52 g, 2.0 eq) in DMF (20 mL) was added $EtN(iPr)_2$ (349 µL, 1.0 eq) at 0°C. After 10 min the reaction mixture was added dropwise to 4,7,10-trioxa-1,13-tridecanediamine (4.38 mL, 10 eq) in CH_2Cl_2 (20 mL) and stirred over night. After removal of the volatiles the residue was redissolved in EtOAc (100 mL) and washed with sat. $NaHCO_3$ solution (60 mL). The aqueous layer was extracted with EtOAc (3 x 30 mL), the combined organic layers were washed with brine (60 mL), dried with Na_2SO_4, filtered and concentrated. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH 95:5) to afford amine **12** as colorless oil (601 mg, 54 %).

R_f: 0.28 (CH_2Cl_2/MeOH 10:1). ^{1}H NMR (400 MHz, $CDCl_3$) d = 7.42 – 7.36 (m, 6H), 7.31 – 7.27 (m, 5H), 7.24 – 7.18 (m, 3H), 7.05 (s, 2H), 6.06 (t, *J*=6.4, 1H), 3.73 (t, *J*= 5.2, 2H), 3.67 – 3.48 (m, 9H), 3.41 (t, *J*=5.4, 2H), 3.28 – 3.10 (m, 4H), 2.44 (t, *J*=7.1, 2H), 2.11 (t, *J*=7.1, 2H), 1.96 – 1.82 (m, 2H), 1.75 – 1.57 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) d = 172.82, 144.84, 129.76, 128.15, 126.96, 71.21, 70.49, 69.71, 69.56, 69.35, 68.10, 67.10, 41.75, 36.80, 35.33, 29.69, 27.73, 25.90. HRMS calcd. for [$C_{32}H_{42}N_2O_4S$+H^+] 551.2938, found (ESI) 551.2932.

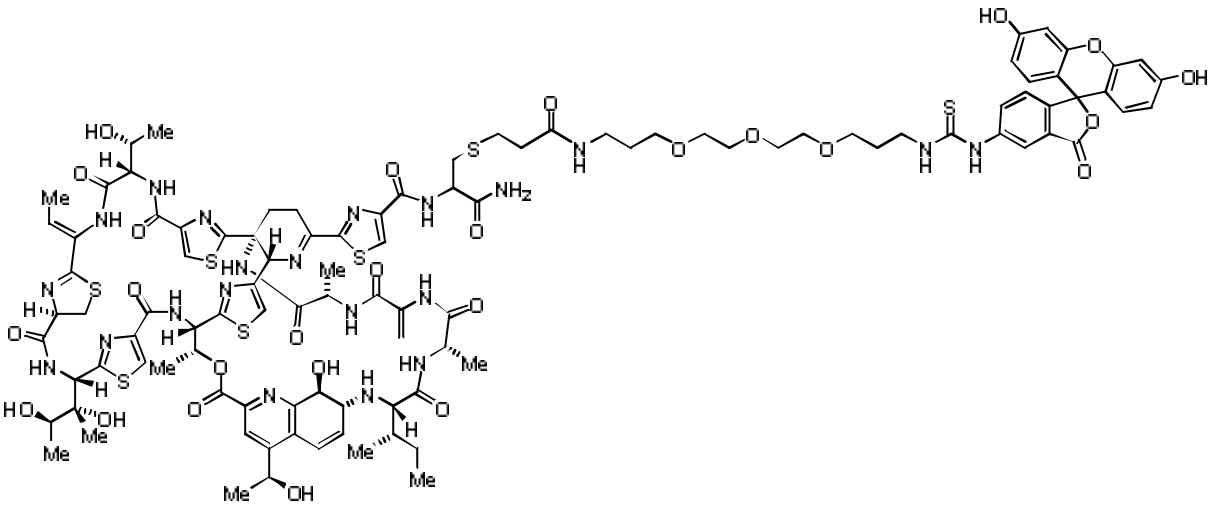
***N*-(1-(5'-fluoresceinylamino)-1-thiono-6,9,12-trioxa-2-azapentadecan-15-yl)-*b*-(tritylthio)propinonamide (13)**



To a solution of amine **12** (280 mg) in acetone/$CHCl_3$ (60 mL, 2:1) was added FITC (218 mg, 1.1 eq) and $EtN(iPr)_2$ (89 µL, 1 eq). After stirring for 16 h, the mixture was concentrated and subjected to silica gel chromatography (EtOAc / 5? 10 % MeOH) to give trityl-protected thiol **13** (214 mg, 45 %) as an orange solid.

R_f = 0.51 (EtOAc/MeOH 10:1). M. p. > 230 °C decomp., ^{1}H NMR (400 MHz, $CDCl_3$/ CD_3OD 4:1) d = 7.86 (d, *J*=1.9, 1H), 7.71 (dd, *J*=2.1, 4.4, 1H), 7.21 – 7.14 (m, 6H), 7.06 (t, *J*=7.4, 6H), 6.99 (t, *J*=7.0, 3H), 6.91 (d, *J*=8.3, 1H), 6.52 (d, *J*=2.4, 2H), 6.49 (d, *J*=8.6, 2H), 6.35 (dd, *J*=2.3, 8.6, 2H), 3.55 (s, 1H), 3.45 – 3.37 (m, 8H), 3.36 – 3.32 (m, 2H), 3.29 (t, *J*=6.1, 2H), 3.06 – 2.98 (m, 2H), 2.23 (t, *J*=7.3, 2H), 1.94 (t, *J*=7.2, 2H), 1.75 – 1.68 (m, 2H), 1.55 – 1.48 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$/ CD_3OD 4:1) d = 173.50, 145.87, 130.54, 130.25, 128.79, 127.64, 103.57, 71.33, 71.01, 70.95, 69.84, 67.66, 37.79, 35.94, 30.05, 29.67, 28.90, 14.55. HRMS calcd. for [$C_{53}H_{53}N_3O_9S_2$+H^+] 940.3296, found (ESI) 940.3302.

4. Preparation of PEG-fluoresceine-tagged probe 6



S-Trt-protected PEG-fluorescein-conjugate **13** (32 mg, 0.034 mmol, 1.1 eq) was dissolved in $CHCl_3$ (3 mL) containing TES (5 µL, 0.034 mmol, 1.1 eq). TFA (240 µL) was added and the mixture was stirred for 20 min. The volatiles were coevaporated with toluene and the residue was redissolved in TFE (6 mL) and NaP_i buffer was added (2 mL, 50 mM, pH 9). Truncated thiostrepton **4** (50 mg, 0.031 mmol) was added followed by Et_3N (44 µL, 0.313 mmol, 10 eq). The reaction mixture was stirred under Ar atmosphere for 16 h. The mixture was concentrated and purified by prep. HPLC (C4, CH_3CN/H_2O) to give probe **6** (23 mg, 32 %) as a yellow powder after lyophilization of the corresponding fractions.

(**6**): M.p. > 240 °C decomp., R_f = 0.36 ($CHCl_3$/MeOH 17:3). HPLC: R_t = 7.6 min (method I). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 4:1) d = 8.13 (s, 1H), 8.08 (d, *J*=1.3, 1H), 8.02 (s, 1H), 7.72 (s, 1H), 7.41 (s, 1H), 7.15 (s, 1H), 7.00 (d, *J*=8.5, 1H), 6.95 (d, *J*=9.1, 2H), 6.74 (d, *J*=9.8, 1H), 6.48 – 6.41 (m, 4H), 6.26 – 6.17 (m, 2H), 6.07 (q, *J*= 6.7, 1H), 5.68 (s, 1H), 5.62 – 5.58 (m, 2H), 5.20 – 5.13 (m, 3H), 4.82 (dd, *J*=9.0, 12.8, 1H), 4.66 – 4.61 (m, 1H), 4.58 – 4.54 (m, 1H), 4.28 – 4.23 (m, 2H), 3.69 – 3.57 (m, 5H), 3.46 (br s, 10H), 3.41 (s, 3H), 3.36 – 3.30 (m, 2H), 3.10 – 3.03 (m, 3H), 3.00 (d, *J*=11.7, 1H), 2.82 (d, *J*=5.1, 3H), 2.72 – 2.66 (m, 2H), 2.34 – 2.26 (m, 2H), 1.82 – 1.73 (m, 3H), 1.56 (d, *J*=6.8, 5H), 1.46 (d, *J*=7.2, 3H), 1.29 – 1.25 (m, 3H), 1.23 (d, *J*= 6.6, 3H), 1.14 (d, *J*=6.5, 3H), 1.04 – 0.98 (m, 6H), 0.96 (d, *J*=6.6, 2H), 0.85 (d, *J*=6.9, 3H), 0.73 (t, *J*=7.4, 3H), 0.65 (d, *J*=6.8, 3H). HRMS calcd. for [$C_{103}H_{121}N_{21}O_{26}S_7$+$H^+$] 2292.691, found (ESI) 2292.688.

5. Biochemistry

a) L11-protein: The L11 protein from *Thermus thermophilus* was expressed and purified as N-terminally His_6-tagged fusion-protein following procedures described elsewhere.[1]

b) RNA: A 58 nt fragment of *Escherichia coli* 23 S rRNA (1051-1109) was synthesized by in vitro transcription and purified as described elsewhere.[1]

c) Thiopeptides: Thiostrepton (**1**) was purchased from Calbiochem. Analytical samples of Nosiheptide (**2**) and Micrococcin (**3**) were gifts from Prof. Dr. Heinz G. Floss, Prof. T. Mahmud and Dr. K. Shin-Ya. Thiopeptide concentration was determined by UV using the following extinction coefficients:

thiostrepton (**1**): e_{280} = 0.027 cm^{-1} μM^{-1};
nosiheptide (**2**): e_{280} = 0.039 cm^{-1} μM^{-1};
micrococcin (**3**): e_{280} = 0.034 cm^{-1} μM^{-1};
probe (**6**): e_{492} = 0.078 cm^{-1} μM^{-1}.

All thiopeptide stock solutions were prepared in trifluoroethanol (TFE).

d) Electro-mobility-shift assay: For protein/RNA affinity determination radioactive EMSA were performed. The RNA was 5`-^{32}P-labeled using T4 polynucleotide kinase (Fermentas) following the suppliers protocol. ^{32}P-?-ATP was purchased from Amersham Biosciences (GE Healthcare). For binding reactions 1 nM labeled RNA and varying concentrations of protein were incubated in binding buffer (5 mM MOPS, 5 mM $MgCl_2$, 50 mM KCl, pH 8.0) at room temperature.

The EMSA were performed in 10% (w/v) acrylamide gels (37.5:1) using pre-cooled TBE + 5 mM $MgCl_2$ (1×) pH 8.0 as gel- and running buffer. After 30-60 min. equilibration the gels were run for approx. 120 min. at 30 mA at 4°C. Gels were dried, analyzed by autoradiography (FLA 5000 Phosphoimager, Kodak/Raytest) and evaluated using the AIDA software (Kodak/Raytest). Intensities were normalized (% Binding = shift / (shift + unshift)) and plotted against the protein concentration (Figure S1). Hill fitting of the data of three independent experiments yielded a K_{D1} of 17.7 nM ± 2.5 nM, respectively.

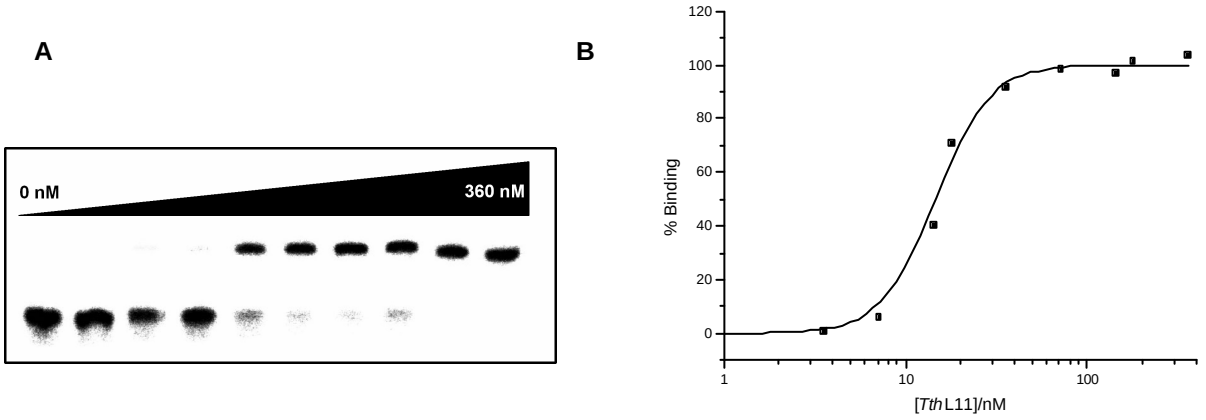


Figure S1. A) Autoradiograph of a representative EMSA of the titration of 1 nM ^{32}P-labeled RNA 58mer with 0; 3.6; 7.2; 14.4; 18; 36; 72; 144; 180; 360 nM *Tth*L11-protein. B) Repre-sentative plot of complex formation in dependence on the protein concentration. The data were fitted using the Hill equation.

e) Fluorescence polarisation experiments: Fluorescence polarization experiments were performed using a Tecan Safire II plate reader in 384 well plates (PerkinElmer Optiplate-384 F) at an excitation wavelength of 470 nm, emission wavelength of 520 nm, emission bandwidth of 5 nm, and 50 scans per well at 22°C.

Probe Affinity Determination

A typical binding reaction sample contained 5 nM of **6** in 5 mM MOPS; 5 mM $MgCl_2$; 50 mM KCl at pH 7.5; 5% (v/v) TFE as well as varying concentrations of protein L11 and/or the 23S rRNA-fragment (total volume: 50 µL). All samples were equilibrated for 12-24 hours before evaluation.

Equilibrium titrations of **6** with protein or RNA alone did not induce any change of the polarization signal, indicating that no binding was occurring (data not shown). Titration of 5 nM of **6** and 0.6 µM protein with increasing amounts of RNA induced a significant increase of the polarization signal, indicating a binding event. Anisotropy data were plotted against the RNA concentration and fitted by least-squares approximation (Origin V 7.5) with the equation below, which can be shown to analytically describe the binding of a ligand in two consecutively coupled equlibriums. The fitting process of five independent experiments gave a probe/complex K_{D2} of 0.14 nM ± 0.08 nM.

$$A = (A_{\max} - A_0)\left(\frac{c_{RNA} + T^* + \frac{k_1 k_2}{l} + k_2}{2T^*} - \sqrt{\left(\frac{c_{RNA} + T^* + \frac{k_1 k_2}{l} + k_2}{2T^*}\right)^2 - \frac{c_{RNA}}{T^*}}\right) + A_0$$

A: Anisotropy, A_{max}/A_o max/min

l: concentration of protein (L11, typically 0.6 µM)

c_{RNA}: concentration of rRNA fragment

k_1: K_{D1} of RNA/L11 complex formation

T^*: concentration of **6** (TS^*, 5 nM)

k_2: K_{D2} of TS^* + RNA/L11 complex formation

Displacement Titrations

For displacement titrations 5 nM of **6**, 5 nM RNA and 0.6 µM L11 protein in 5 mM MOPS; 5 mM $MgCl_2$; 50 mM KCl pH 7.5; 5% (v/v) TFE, were prepared with varying concentrations of the analytes (total volume: 50 µL). All tested compounds induced a significant decrease of the polarization signal, indicating a displacement of the fluo-

rescent probe from the macromolecular complex. After plotting against the thiopeptide concentration, the data were fitted to the Hill equation to yield IC_{50} values. Five independent experiments were averaged and converted into apparent K_D using the equation given below.

Table S1. *IC_{50}* values and apparent K_D from displacement titrations.

Compound	IC_{50}/µM	K_{app}/nM
Thiostrepton **1**	0.0071 ± 0.0017	0.20 ± 0.05
Nosiheptide **2**	0.0040 ± 0.0009	0.11 ± 0.02
Micrococcin **3**	0.062 ± 0.032	1.73 ± 0.90
4	0.0067 ± 0.0005	0.19 ± 0.01
5	0.0113 ± 0.0016	0.32 ± 0.05
Probe **6**	-	**0.14** ± **0.08**
7	0.0081 ± 0.002	0.22 ± 0.06
8a	0.128 ± 0.042	3.59 ± 1.20
8b	0.193 ± 0.072	5.41 ± 2.03
8a+b	0.115 ± 0.060	3.23 ± 1.70
9	0.0114 ± 0.003	0.32 ± 0.08
10	0.0112 ± 0.0014	0.32 ± 0.04
11	0.016 ± 0.004	0.44 ± 0.11

$$K_{app} \quad \frac{IC_{50}}{TS^*} \times k_2 \quad =$$

K_{app}: apparent K_D
T^*: concentration of **6** (TS^*, 5 nM)
k_2: K_{D2} of TS^* + RNA/L11 complex formation (0.14 nM)

6. Antibacterial Growth Inhibition Testing

All growth inhibition testing was conducted in 96 well plates equipped with diffusion-equilibrating covers (“System Duetz”, EnzyScreen bv, Leiden, NL) in an orbital shaker at 190 rpm at 37°C. Five independent colonies of *Staphylococcus aureus* (ATCC No. 25923 or No. 43300) were used as starters for a liquid preculture (20 mL). The bacteria were incubated until logarithmic growth phase was reached (OD_{600} 0,3 – 1), then diluted to 1-5×10^5 colony forming units per mL and used in the antimicrobial testing. Total volume per well was 100µL Müller-Hinton broth (Carl Roth, Germany) with 2% NaCl (Sigma Aldrich, Germany). 8 blank wells without bacteria, 4 wells with bacteria, and 4 wells with two known antibiotics (Sigma Aldrich, Germany) at their minimal inhibitory concentration (MIC) were used as controls (ampicillin: 0.26 mg/L; tetracycline: 1 mg/L). All compounds were prepared in twofold dilution series in trifluoroethanol (TFE), each done in duplicate. All concentrations given are final concentrations in the test volume; final concentration of TFE was 1% v/v. Up to 5% v/v TFE were found tolerable in independent control experiments. Bacterial growth was followed by OD determination during 24h. The reported MIC values correspond to the concentration where no growth was observed.

7. References

[1] S. Baumann, S. Schoof, S. D. Harkal, H.-D. Arndt, *J. Am. Chem. Soc.* **2008**, *130*, 5664–5666.