Supporting Information

Aptameric Protective Groups Tolerate Many Different Reagents and Reactions for Regioselective Modifications of Neomycin B

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General

^{1}H-NMR-, heteronuclear single-quantum coherence (HSQC) spectra and attached proton test (ATP) were recorded on a Varian Unity Inova (500 MHz and 600 MHz) NMR spectrometer at 25 °C. High resolution mass spectrometry (HRMS) was carried out on a LTQ ORBITRAP XL instrument (Thermo Scientific) employing electron impact ionization in positive ion mode (EI+). Chromatographic separations were carried out on a *Shimadzu* VP series high performance liquid chromatography (HPLC) modular system (DGU-14A3 Online Vacuum-Degasser, two LC-20 AT pumps, SIL-20A auto sampler, CTP-20 A column oven, RID-10 refractive detector, FRC-10 A fraction collector and Shimadzu LCsolution software). HPLC purification was performed with a Waters Spherisorb ODS-2 C_{18} analytical column (250 x 4.6 mm, spherical particles of 5 µm and 80 Å pore size) using isocratic elution at 40 °C. A pH-meter (Hanna Instruments pH 209) equipped with a glass combination electrode was used for pH adjustments of the reaction buffers.

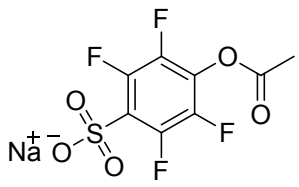
Materials

All chemicals and reagents were purchased from commercial suppliers and used without further purification, unless otherwise noted. Neomycin B trisulfate x hydrate (VETRANAL®), *N*,*N*-dimethylformamide (DMF, 99 %), dichloromethane (DCM, 99.5 %), tetrahydrofurane (THF, 99.9 %), pyridine (99 %), acetic acid (99 %), propyl isocyanate (99 %), isopropyl isocyanate (98 %), *tert*-butyl isocyanate (97 %), 2-iminothiolane hydrochloride (98 %), toluene (99.8 %), sulfuryl chloride (97 %), sodium azide (95 %), acetonitril (99.8 %), imodazole (99 %), methanolic 3 N HCl solution, sulfuric acid (30 % SO_3) and di-cyclohexylcarbodiimde (99 %), Isatoic anhydride (96 %), 3,4-epoxy-1-butene (98 %) were purchased from Sigma-Aldrich and used as received. 2,3,5,6-tetrafluoro phenol (98 %) was purchased from *Acros Organics*. For HPLC purification heptafluorobutyric acid (HFBA) (*Fluka*, puriss. p.a., for ion chromatography) and acetone (*Sigma-Aldrich*, HPLC grade) were used. Ultrapure water (specific resistance > 18.4 MΩ cm) was obtained by Milli-Q water purification system (*Sartorius*®). RNA aptamer (82 – 91 % purity) were purchased from *BioSpring* (Frankfurt am Main, Germany) and *riboxx* GmbH (Radebeul, Germany). For the regioselective transformation Milli-Q water was treated with diethylpyrocarbonate (DEPC) and sterilized using an autoclave (121 °C, 20 min).

General procedures for reagents 7 and 11

(Both compounds were synthesized according to published procedures[1],[2])

Sodium 4-(acetoxy)-2,3,5,6-tetrafluorobenzenesulfonate 7.[1]

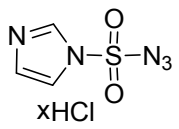


10.1 g of 2,3,5,6-tetrafluorophenol (61.4 mmol) were taken up in 22 mL fuming sulphuric acid (30 % SO_3) and stirred at ambient temperature for 18 h before pouring the mixture into 200 mL iced brine. The product was precipitated by adding 6 g of NaCl and stirred until no further precipitate was formed. This mixture was filtered through a sintered glass disc and the collected solids were taken up in 330 mL boiling acetonitril, filtered while hot, and allowed to cool slowly to ambient temperature. The colourless crystalline product was collected by filtration and dried in vacuum yielding 5.42 g (20.2 mmol, 33 % yield) of 4-sulfo-tetrafluoro phenol sodium salt. 270 mg of this sodium salt (1.0 mmol) and 53.8 µL of acetic acid (0.94 mmol) were dissolved in 30 mL acetone. After 230 mg 1,3-dicyclohexylcarbodiimide (1.1 mmol) were added the mixture was stirred at room temperature for 20 h. The resulting precipitate was removed by filtration and the filtrate was concentrated under reduced pressure. The crude mixture was purified by column chromatography using silica gel as stationary phase and a 4:1 acetone/chloroform mixture as mobile phase.

163 mg (0.56 mmol, 56 % yield), white solid. R_f (acetone/chloroform 4:1) = 0.55.

^{1}H-NMR (D_2O, 400MHz): δ (p.p.m.) = 2.46 (s, 3H, CH_3-CO). ^{13}C-NMR (D_2O, 50.43 MHz): δ (p.p.m.) = 170.07 (1C, CO); 147.01 (dq), 144.09 (ddd) (2C, 3-C-Ar, 5-C-Ar); 145.03 (dq), 142.10 (ddd) (2C, 2-C-Ar, 6-C-Ar), 131.14 (1C, C-SO_2); 127.61 (1C, C-CO-CH_3) ; 22.82 (1C, CH_3-CO).

Synthesis of diazo-transfer reagent Imidazole-1-sulfonyl Azide Hydrochloride 11.[2]



Sulfuryl chloride (1.6 mL, 20 mmol) was added dropwise to an ice-cooled suspension of sodium azide (1.3 g, 20 mmol) in acetonitril (20 mL) and the mixture was stirred overnight at room temperature. Imidazole (2.6 g, 38 mmol) was added portion-wise to the ice-cooled mixture and the resulting slurry was stirred for additional 3 h at room temperature. The mixture was diluted with ethyl acetate (40 mL), washed with water (2 x 40 mL) and then with saturated aqueous sodium hydrogen carbonate (2 x 40 mL), dried over $MgSO_4$ and filtered. The filtrate was cooled in an ice-batch and a 3 M HCl methanolic solution (10 mL) was added drop wise to precipitate the product. Finally, the filter cake was washed with EtOAc (3 x 10 mL) to obtain **11**. Yield: 1.9 g (9.1 mmol, 45 % yield). ^{1}H-NMR (D_2O, 400MHz) δ (p.p.m.) 9.53 (s, 1H, H-2), 8.07 (s, 1H, H-5), 7.67 (s, 1H, H-4). ^{13}C-NMR (D_2O, 400MHz) δ (p.p.m.) 137.6, 122.6, 120.18. MS (EI+) (*m/z)*: found 174.0078 $[M\text{-}Cl]^+$, calc. 174.0080 $[M\text{-}Cl]^+$.

General procedures for antibiotic modifications

a) Site-selective acylation using STP ester 7, 2-iminothiolane hydrochloride 9 and isatoic anhydride 17.

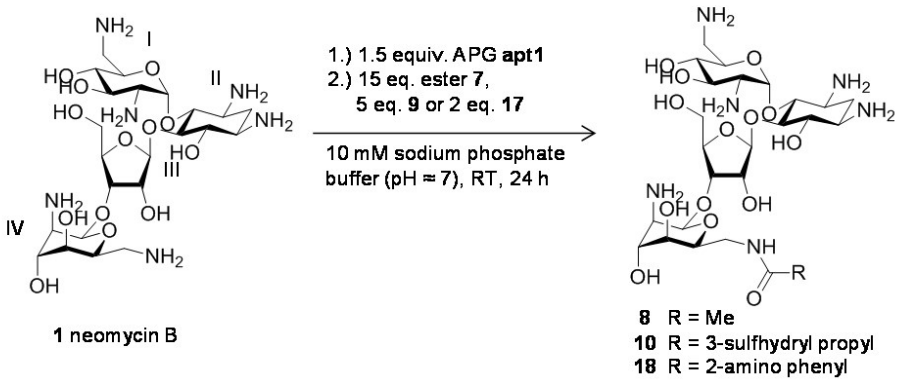


Figure S1 Regioselective acylation of amino group in C6 position of antibiotic's ring IV.

900 µL of a 5.54 mM RNA aptamer solution (4.98 µmol) in 10 mM sodium phosphate buffer (pH 6.8) was heated to 85 °C for 10 min and was afterwards kept at room temperature for 15 min. 684 µL of a 4.8 mM solution of neomycin B sulphate (3.28 µmol) in 10 mM sodium phosphate buffer (pH 7.4) was added and the mixture was allowed to stand for 30 min at room temperature. Then, 15 equiv. STP-ester **7** (49.2 µmol) 500 µL 10 mM sodium phosphate buffer (pH 7.4), or 5 equiv. 2-iminothiolane hydrochloride **9** (16.4 µmol) dissolved in 42 µL 10 mM sodium phosphate buffer (pH 7.4), or 2 equiv. isoatoic anhydride **17** (6.56 µmol) dissolved in 108 µL DMF were added and the reaction mixture was allowed to react for 24 hours at room temperature. The pH of the final reaction mixture was approx. 8. After addition of 180 µL of a 7 wt. % ethylamine water solution and further incubation for 30 min at room temperature, 486 µl of a 2 M sodium hydroxide solution were added and the crude mixture was heated to 90 °C for 30 min. After cooling to room temperature each 50 µL fraction was purified by HPLC using a *Waters* Spherisorb ODS-2C_{18} analytic column (water/acetone 1:0.81 containing 16.9 mM HFBA) at a flow rate of 1 ml/min at 40°C to afford the antibiotic derivatives **8**, **10** and **18**. After evaporation of acetone and freeze-drying the product was taken up in 500 µL water for yield determination by HPCL using same conditions as described above. The remaining solution (approx. 450 µL) was freeze-dried and the product was taken up in 150 µL of D_2O for NMR-studies.

b) Site-selective azide introduction using diazo-transfer reagent 11.

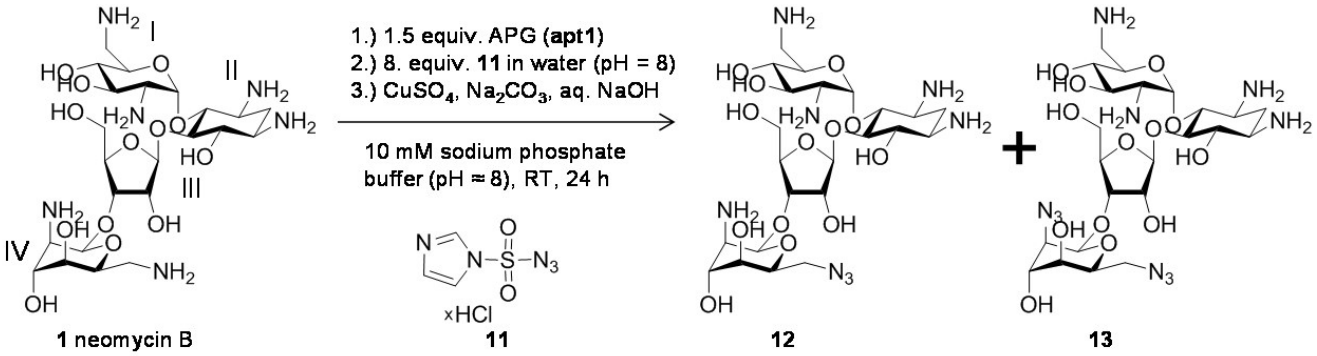


Figure S2 Regioselective azide introduction in C2 and C6 position of antibiotic's ring IV.

900 µL of a 5.54 mM RNA aptamer solution (4.98 µmol) in 10 mM sodium phosphate buffer (pH 6.8) was heated to 85 °C for 10 min and was afterwards kept at room temperature for 15 min. 684 µL of a 4.8 mM solution of neomycin B sulphate (3.28 µmol) in 10 mM sodium phosphate buffer (pH 7.4) was added and the mixture was allowed to stand for 30 min at room temperature. 540 µL of 4.8 mM aqueous solution of diazo-transfer reagent **11** (10 mg/mL), which was adjusted to approx. pH 8 with 25 µL of 2 M NaOH solution, was added into the solution of the antibiotic **1** - **apt1** complex. After 59 µL of an aqueous solution of sodium carbonate (10 mg/mL) and 50 µL of an aqueous solution of copper sulfate (2 mg/mL, 0.19 mol%) were added the mixture was reacted for 24 hours at room temperature. The pH of the final reaction mixture was approx. 8. After addition of 180 µL of a 7 wt. % ethylamine water solution and further incubation for 30 min at room temperature, 375 µl of a 2 M NaOH solution were added and the crude mixture was heated to 90 °C for 30 min. After cooling to room temperature each 50 µL fraction was purified by HPLC using a *Waters* Spherisorb ODS-2C_{18} analytic column (water/acetone 1.0 : 0.81 containing 16.9 mM HFBA) at a flow rate of 1 ml/min at 40 °C to afford the antibiotic derivatives **12** and **13**. After evaporation of acetone and freeze-drying the product was taken up in 500 µL water for yield determination by HPCL using same conditions as described above. The remaining solution (approx. 450 µL) was freeze-dried and the product was taken up in 150 µL of D2O for NMR-studies.

c) Site-selective urethane transformation using aliphatic isocyanate 14.

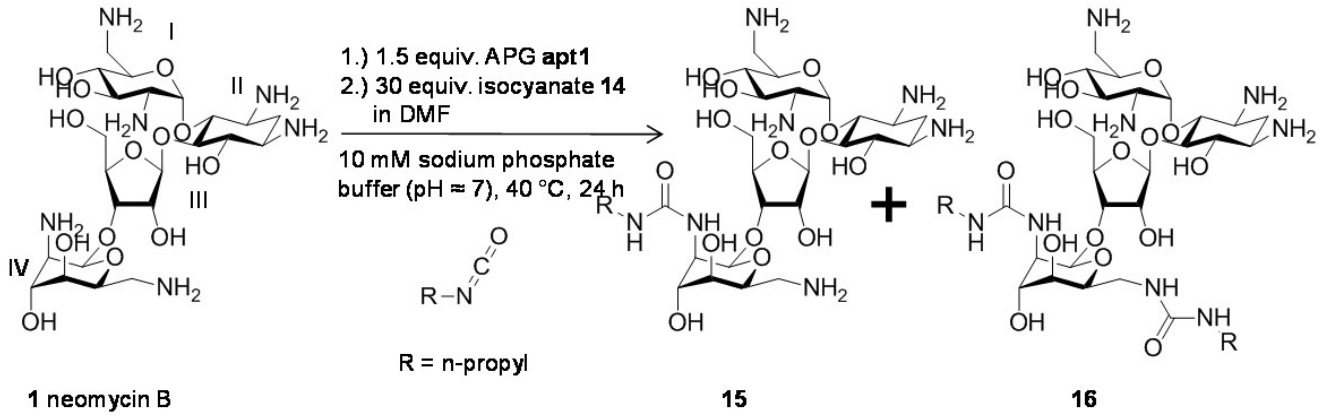


Figure S3 Urea bond formation in C2 and C6 positions of antibiotic's ring IV.

900 µL of a 5.54 mM RNA aptamer solution (4.98 µmol) in 10 mM sodium phosphate buffer (pH 6.8) was heated to 85 °C for 10 min and was afterwards kept at room temperature for 15 min. 684 µL of a 4.8 mM solution of the aminoglycoside antibiotic (3.28 µmol) in 10 mM sodium phosphate buffer (pH 7.4) was added and the mixture was allowed to stand for 30 min at room temperature. 30 equiv. (49.2 µmol) of aliphatic isocyanate **14** dissolved in 108 µL DMF were added and the reaction mixture was allowed to react for 24 hours at 40 °C. The pH of the final reaction mixture was approx. 7. After addition of 180 µL of a 7 wt. % ethylamine water solution and further incubation for 30 min at room temperature, 280 µl of a 2 M sodium hydroxide solution was added and the crude mixture was heated to 90 °C for 30 min. After cooling to room temperature each 50 µL fraction was purified by HPLC using a Waters Spherisorb ODS-2C_{18} analytic column (water/acetone 1.0 : 0.81 containing 16.9 mM HFBA) at a flow rate of 1 ml/min at 40 °C to afford the antibiotic derivative **15** and **16**. After evaporation of acetone and freeze-drying the product was taken up in 500 µL water for yield determination by HPCL using same conditions as described above. The remaining solution (approx. 450 µL) was freeze-dried and the product was taken up in 150 µL of D_2O for NMR-studies.

d) Site-selective alkylation using 3,4-epoxy-1-butene 19.

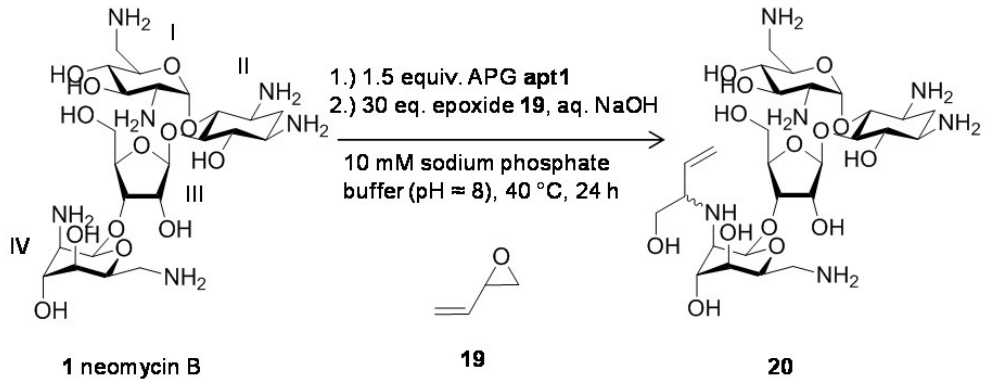
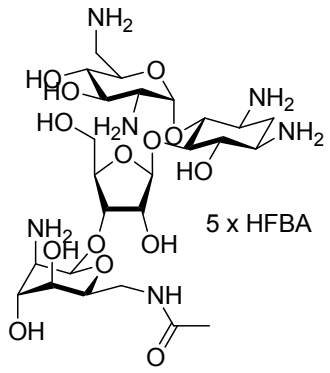


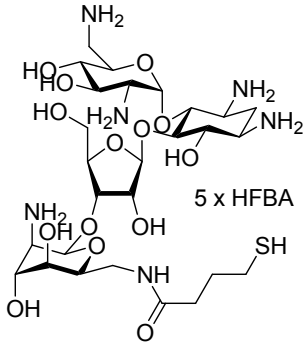
Figure S4 Regioselective alkylation of amino group in C2 position of antibiotic's ring IV.

900 µL of a 5.54 mM RNA aptamer solution (4.98 µmol) in 10 mM sodium phosphate buffer (pH 6.8) was heated to 85 °C for 10 min and was afterwards kept at room temperature for 15 min. 684 µL of a 4.8 mM solution of neomycin B sulphate (3.28 µmol) in 10 mM sodium phosphate buffer (pH 7.4) was added and the mixture was allowed to stand for 30 min at room temperature. Then, 30 equiv. of 3,4-epoxy-1-butene **19** (49.2 µmol) in 108 µL DMF were added and a pH value of approx. 8 was adjusted by adding of 90 µL aq. 0.25 M NaOH solution. The reaction mixture was allowed to react for 24 hours at 40 °C. After addition of 180 µL of a 7 wt. % ethylamine water solution and further incubation for 30 min at room temperature, 486 µl of a 2 M sodium hydroxide solution were added and the crude mixture was heated to 90 °C for 30 min. After cooling to room temperature each 50 µL fraction was purified by HPLC using a *Waters* Spherisorb ODS-2C_{18} analytic column (water/acetone 1:0.81 containing 16.9 mM HFBA) at a flow rate of 1 ml/min at 40 °C to afford the antibiotic derivative **20**. After evaporation of acetone and freeze-drying the product was taken up in 500 µL water for yield determination by HPCL using same conditions as described above. The remaining solution (approx. 450 µL) was freeze-dried and the product was taken up in 150 µL of D_2O for NMR-studies.

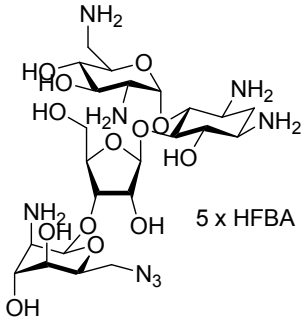
Analytical Data



6´´´-*N*-acetyl neomycin B x 5 HFBA (8). The title compound was prepared according to the general procedure described above. Derivative **8** was obtained as a white solid. For the measurement of regioselectivity and the characterization of the compound ^{1}H-NMR, HSQC as well as APT spectra were recorded and electrospray ionization (ESI)-MS was employed. The yield was determined by HPLC: R_t = 9.7 min, 30 % yield. TLC ($CHCl_3$/MeOH/17% NH_4OH 2:1:1 v/v/v) R_f = 0.52. ^{1}H-NMR (D_2O, 500 MHz) δ (p.p.m.) 6.06 p.p.m. (d, J = 4 Hz, 1H, 1-H´), 5.44 (d, J = 2 Hz, 1H, 1-H´´), 5.20 (d, J = 1.5 Hz, 1H, 1-H´´´), 4.44 (t, J = 5.75 Hz, 1H, 3-H´´), 4.39 (dd, J = 5 Hz, J = 2 Hz, 1H, 2-H´´), 4.26 (t, J = 3 Hz, 1H, 3-H´´´), 4.24 (m, 1H, 4-H´´), 4.09 (t, J = 6.75 Hz, 1H, 5-H´´´), 4.07 (m, 1H, 4-H), 4.01 (t, J = 10 Hz, 1H, 5-H´), 3.98 – 3.92 (m, 3H, 5-H_a´´, 5-H, 3-H´), 3.76 (dd, 1H, J = 12.5 Hz, J = 5.5 Hz, 5-H_b´´), 3.72- 3.68 (m, 2H, 4-H´´´, 6-H), 3.60 (dd, J = 14 Hz, J = 7.5 Hz, 1H, 6-H_a´´´), 3.56 (m, 2H, 3-H, 2-H´´´), 3.53-3.41 (m, 4H, 6-H_a´, 2-H´, 6-H_b´´´, 4-H´), 3.38 (m, 1H, 1-H), 3.32 (dd, J = 14 Hz, J = 6 Hz, 1H, 6-H_b´), 2.51 (dt, J = 12.5 Hz; J = 3.8 Hz, 1H, 2-H_{eq}), 2.04 (s, 3H, CH_3), 1.89 (dd, J = 12.7 Hz, 1H, 2-H_{ax}). APT (D_2O, 125.7 MHz) δ (p.p.m.) 174.49 (Carbonyl-C), 110.00 (C-1´´), 95.51 (C-1´´´), 95.49 (C-1´), 84.62 (C-5), 81.66 (C-4´´), 75.39 (C-3´´), 75.29 (C-4), 73.58 (C-2´´), 72.45 (C-5´´´), 72.42 (C-6), 70.35 (C-4´), 69.22 (C-3´), 67.88 (C-5´), 67.56 (C-3´´´), 66.10 (C-4´´´), 60.00 (C-5´´), 53.15 (C-2´), 50.90 (C-2´´´), 49.65 (C-1), 48.16 (C-3), 39.85 (C-6´), 39.33 (C-6´´´), 27.88 (C-2), 21.74 (CH_3). MS (EI+) (*m/z)*: found 657.33008 $[M+H]^+$, 679.31226 $[M+Na]^+$; calculated 657.33013 $[M+H]^+$, 679.31207 $[M+Na]^+$.

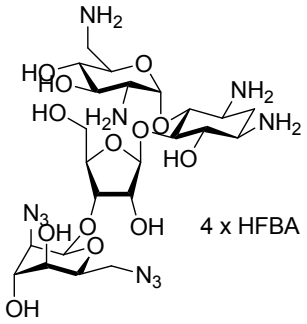


6´´´-*N*-γ-sulfhydryl-butanonyl neomycin B x 5 HFBA (10). The title compound was prepared according to the general procedure described above. Derivative **10** was obtained as a white solid. For the measurement of regioselectivity and the characterization of the compound ^{1}H-NMR, HSQC as well as APT spectra were recorded and electrospray ionization (ESI)-MS was employed. The yield was determined by HPLC: R_t = 16.3 min, 30 % yield. ^{1}H-NMR (D_2O, 500 MHz) δ (p.p.m.) 6.03 (s, 1H, 1-H´), 5.42 (s, 1H, 1-H´´), 5.18 (s, 1H, 1-H´´´), 4.44-4.35 (m, 2H, 3-H´´, 2-H´´), 4.26-4.12 (m, 2H, 3-H´´´, 4-H´´), 4.11-4.04 (m, 2H, 4-H, 5-H´´´), 4.00 (t, J = 10.3 Hz, 1H, 3-H´), 3.96-3.89 (m, 3H, 5-H, 5-H´, 5-H_a´´), 3.77-3.67 (m, 3H, 5-H_b´´, 6-H, 4-H´´´), 3.58-3.43 (m, 7H, 3-H, 2-H´´´, 6-H_a´´´, 6-H_b´´´, 6-H_a´, 4-H´, 2-H´), 3.37 (dt, J = 10.5 Hz, J = 4.5 Hz, 1H, 1-H), 3.28 (dd, J = 14 Hz, J = 6 Hz, 1H, 6-H_b´), 2.75 (q, J = 7 Hz, 2H, γ-CH_2), 2.50 (dt, J = 12.5 Hz, J = 3.5 Hz, 1H, 2-H_{eq}), 2.38 (t, J = 7.3 Hz, 2H, α-CH_2), 2.02-1.87 (m, 3H, β-CH_2, 2-H_{ax}). ^{13}C-signals based on HSQC (D_2O, 500 MHz) δ (p.p.m.) 110.3 (C-1´´), 95.9 (C-1´´´), 95.6 (C-1´), 84.9 (C-5), 81.8 (C-4´´), 76.1 (C-3´´), 75.4 (C-4), 74.1 (C-2´´), 72.8 (C-5´´´), 72.5 (C-6), 70.8 (C-4´), 69.5 (C-3´), 68.2 (C-5´), 67.7 (C-3´´´), 66.4 (C-4´´´), 60.5 (C-5´´), 53.6 (C-2´), 51.1 (C-2´´´), 49.9 (C-1), 48.5 (C-3), 40.2 (C-6´), 39.5 (C-6´´´), 37.0 (γ-CH_2), 34.2 (α-CH_2), 28.0 (C-2), 24.6 (β-CH_2). MS (EI+) (*m/z)*: found 717.32732 $[M+H]^+$, 739.30863 $[M+Na]^+$; calculated 717.33350 $[M+H]^+$, 739.31544 $[M+Na]^+$.

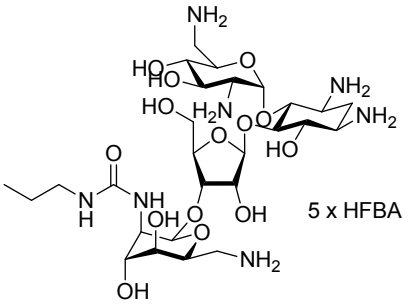


6´´´-*azido* neomycin B x 5 HFBA (12). The title compound was prepared according to the general procedure described above. Derivative **12** was obtained as a white solid. For the measurement of regioselectivity and the characterization of the compound ^{1}H-NMR, HSQC as well as APT spectra were recorded and electrospray ionization (ESI)-MS was employed. The yield was determined by HPLC: R_t = 13.0 min, 47 % yield. ^{1}H-NMR (500 MHz, D_2O, 25 °C, TMS) δ (p.p.m.) 6.00 (d, J = 3.5 Hz, 1H, 1-H´), 5.43 (s, 1H, 1-H´´), 5.26 (s, 1H, 1-H´´´), 4.49 (dd, J = 7 Hz, J = 5 Hz, 1H , 3-H´´), 4.44 (d, J = 4.5 Hz, 1H, 2-H´´), 4.26-4.20 (m, 3H, 3-H´´´, 4-

H´´, 5-H´´´), 4.06 (t, J = 9.5 Hz, 1H, 4-H), 4.01-3.92 (m, 4H, 3-H´, 5-H_a´´, 5-H, 5-H´), 3.78-3.66 (m, 4H, 5-H_b´´, 6-H_a´´´, 4-H´´´, 6-H), 3.52-3.36 (m, 2H, 2-H´´´, 3-H), 3.51-3.43 (m, 4H, 4-H´, 6-H_a´, 6-H_b´´´, 2-H´), 3.37 (dt, J = 11.8 Hz, J = 4.5 Hz, 1H, 1-H), 3.30 (dd, J = 14 Hz, J = 6.5 Hz, 1H, 6-H_b´), 2.50 (dt, J = 12.5 Hz, J = 4.3 Hz, 1H , 2-H_{eq}), 1.90 (dd, J = 12.5 Hz, 1H, 2-H_{ax}). ^{13}C-signals based on HSQC (500 MHz, D_2O, 25 °C, TMS) δ (p.p.m.) 110.2 (C-1´´), 95.6 (C-1´), 95.2 (C-1´´´), 84.6 (C-5), 81.4 (C-4´´), 75.3 (C-4), 75.1 (C-3´´), 73.7 (C-5´´´), 73.4 (C-2´´), 72.5 (C-6), 70.5 (C-4´), 69.3 (C-3´), 67.9 (C-5´), 67.7 (C-3´´´), 66.5 (C-4´´´), 60.2 (C-5´´), 53.4 (C-2´), 50.9 (C-6´´´), 50.8 (C-2´´´), 49.6 (C-1), 48.2 (C-3), 39.9 (C-6´), 27.9 (C-2). MS (EI+) (*m/z)*: found 641.30845 $[M+H]^+$, 663.28839 $[M+Na]^+$; calculated 641.31006 $[M+H]^+$, 663.29200 $[M+Na]^+$.

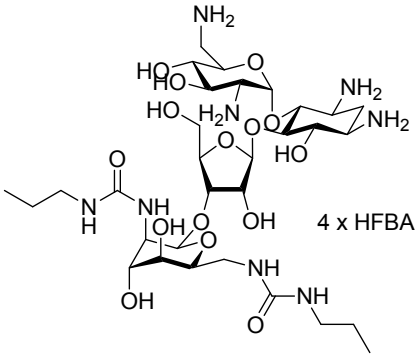


2´´´, 6´´´-diazido neomycin B x 5 HFBA (13). The title compound was prepared according to the general procedure described above. Derivative **13** was obtained as a white solid. For the measurement of regioselectivity and the characterization of the compound ^{1}H-NMR, HSQC as well as APT spectra were recorded and electrospray ionization (ESI)-MS was employed. The yield was determined by HPLC: R_t = 8.9 min, 17 % yield. ^{1}H-NMR (500 MHz, D_2O, 25 °C, TMS) δ (p.p.m.) 6.04 (d, J = 3 Hz, 1H, 1-H´), 5.47 (s, 1H, 1-H´´), 5.17 (d, J = 1 Hz, 1H , 1-H´´´), 4.49 (dd, J = 5.5 Hz, J = 4 Hz, 1H, 3-H´´), 4.45 (dd, J = 4 Hz, J = 2 Hz, 1H, 2-H´´), 4.28 (dt, J = 5.3 Hz, J = 2 Hz, 1H, 4-H´´), 4.16-4.13 (m, 2H, 3-H´´´, 5-H´´´), 4.08 (t, J = 8.3 Hz, 1H, 4-H), 4.03-3.94 (m, 4H, 5-H´, 3-H´, 5-H_a´´, 5-H), 3.90 (m, 1H, 2-H´´´), 3.78 (dd, J = 10.5 Hz, J = 4.5 Hz, 1H, 5-H_b´´), 3.72-3.67 (m, 2H, 6-H_a´´´, 6-H), 3.62 (m, 1H, 4-H´´´), 3.56 (dt, J = 9.3 Hz, J = 3.5 Hz, 1H, 3-H), 3.53-3.44 (m, 4H, 6-H_a´, 6-H_b´´´, 2-H´, 4-H´), 3.39 (dt, J = 9.5 Hz, J = 3.5 Hz, 1H, 1-H), 3.32 (dd, J = 11.5 Hz, J = 5 Hz, 1H, 6-H_b´), 2.52 (dt, J = 10.5 Hz, J = 3.5 Hz, 1H, 2-H_{eq}), 1.92 (dd, J = 10.5 Hz, 1H, 2-H_{ax}). ^{13}C-signals based on HSQC (500 MHz, D_2O, 25 °C, TMS) δ (p.p.m.) 110.2 (C-1´´), 98.1 (C-1´´´), 95.7 (C-1´), 84.6 (C-5), 81.5 (C-4´´), 75.3 (C-4), 74.7 (C-3´´), 74.3 (C-5´´´), 73.2 (C-2´´), 72.4 (C-6), 70.3 (C-4´), 69.2 (C-3´´´), 69.0 (C-3´), 67.8 (C-5´), 67.2 (C-4´´´), 60.3 (C-5´´), 59.6 (C-2´´´), 53.2 (C-2´), 50.8 (C-6´´´), 49.8 (C-1), 48.2 (C-3), 39.8 (C-6´), 28.1 (C-2). MS (EI+) (*m/z)*: found 667.29664 $[M+H]^+$, 689.27895 $[M+Na]^+$; calculated 667.30056 $[M+H]^+$, 689.28250 $[M+Na]^+$.

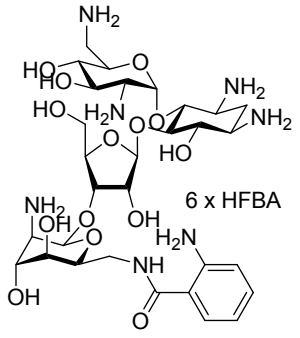


2´´´-*N*-(propylamino)carbonyl neomycin B x 5 HFBA (15). The title compound was prepared according to the general procedure described above. Derivative **15** was obtained as a white solid. For the measurement of regioselectivity and the characterization of the compound ^{1}H-NMR, HSQC as well as APT spectra were recorded and electrospray ionization (ESI)-MS was employed. The yield was determined by HPLC: R_t = 11.6 min, 23 % yield. ^{1}H-NMR (500 MHz, D_2O, 25 °C, TMS) δ (p.p.m.) 6.03 (d, J =4 Hz, 1H, 1-H´), 5.40 (s, 1H, 1-H´´), 5.07 (s, 1H, 1-H´´´), 4.40 (t, J = 5.5 Hz, 1H, 3-H´´), 4.32 (dd, J = 4 Hz, J = 2 Hz, 1H, 2-H´´), 4.22 (t, J = 4.3 Hz, 1H, 5-H´´´), 4.15 (dt, J = 6.8 Hz, J = 3 Hz, 1H, 4-H´´), 4.08 (t, J = 9.8 Hz, 1H, 4-H), 4.01-3.97 (m, 3H, 5-H´, 3-H´´´, 2-H´´´), 3.95-3.88 (m, 3H, 3-H´, 5-H, 5-H_a´´), 3.74-3.67 (m, 3H, 4-H´´´, 5-H_b´´, 6-H), 3.54 (dt, J = 11 Hz, J = 3.5 Hz, 1H, 3-H), 3.49-3.41 (m, 3H, 4-H´, 6-H_a´, 2-H´), 3.39-3.22 (m, 3H, 6-H_a´´´, 6-H_b´´´, 1-H), 3.29 (dd, J = 13.5 Hz, J = 6.5 Hz, 1H, 6-H_b´), 3.04 (q, J = 6.6 Hz, 2H, α-CH_2), 2.49 (dt, J = 12.5 Hz, J = 3.8 Hz, 1H, 2-H_{eq}), 1.89 (dd, J = 12.5 Hz, 1H, 2-H_{ax}), 1.45 (q, J = 7.2 Hz, 2H, β-CH_2), 0.85 (t, J = 7.3 Hz, 3H, CH_3). ^{13}C-signals based on HSQC (500 MHz, D_2O, 25 °C, TMS) δ (p.p.m.) 110.0 (C-1´´), 98.8 (C-1´´´), 95.4 (C-1´), 84.8 (C-5), 81.7 (C-4´´), 75.8 (C-3´´), 75.1 (C-4), 74.1 (C-2´´), 72.5 (C-6), 70.5 (C-4´), 70.3 (C-5´´´), 70.0 (C-3´´´), 69.4 (C-3´), 68.1 (C-4´´´), 68.0 (C-5´), 60.1 (C-5´´), 53.4 (C-2´), 50.9 (C-2´´´), 49.6 (C-1), 48.3 (C-3), 41.6 (α-CH_2), 40.5 (C-6´´´), 39.9 (C-6´), 27.9 (C-2), 22.4 (β-CH_2), 10.3 (CH_3); signal of quaternary carbon (CO) is missing due to the fact that it is not detectable by

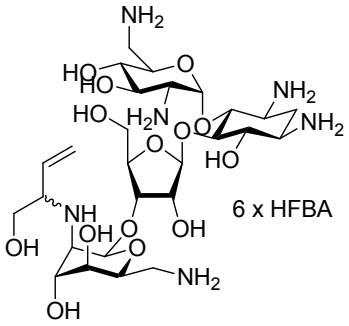
HSQC spectroscopy. MS (EI+) (*m/z)*: found 700.36911 $[M+H]^+$, 722.34964 $[M+Na]^+$; calculated 700.37233 $[M+H]^+$, 722.35427 $[M+Na]^+$.



2´´´, 6´´´-bis-*N*-(propylamino)carbonyl neomycin B x 5 HFBA (16). The title compound was prepared according to the general procedure described above. Derivative **16** was obtained as a white solid. For the measurement of regioselectivity and the characterization of the compound ^{1}H-NMR, HSQC as well as APT spectra were recorded and electrospray ionization (ESI)-MS was employed. The yield was determined by HPLC: R_t = 9.6 min, 31 % yield. ^{1}H-NMR (500 MHz, D_2O, 25 °C, TMS) δ (p.p.m.) 6.05 (d, J = 4 Hz, 1H, 1-H´), 5.39 (s, 1H, 1-H´´), 4.98 (s, 1H, 1-H´´´), 4.37-4.32 (m, 2H, 3-H´´, 2-H´´), 4.16 (dt, J = 5.5 Hz, J = 2.5 Hz, 1H, 4-H´´), 4.09 (t, J = 9.8 Hz, 1H, 4-H), 4.02-3.98 (m, 3H, 3-H´´´, 5-H´, 5-H´´´), 3.96-3.89 (m, 4H, 2-H´´´, 5-H, 3-H´, 5-H_a´´), 3.73-3.68 (m, 2H, 5-H_b´´, 6-H), 3.65 (m, 1H, 4-H´´´), 3.56 (dt, J = 11.3 Hz, 3.5 Hz, 1H, 3-H), 3.50 (t, J = 9.5 Hz, 1H, 4-H´), 3.48-3.35 (m, 5H, 6-H_a´, 2-H´, 6-H_a´´´, 6-H_b´´´, 1-H), 3.31 (dd, J = 14 Hz, J = 6.5 Hz, 1H, 6-H_b´), 3.06 (2xq, J = 6.5 Hz, 4H, α-CH_2), 2.50 (dt, J = 12 Hz, J = 3.8 Hz, 1H, 2-H_{eq}), 1.90 (dd, J = 12.5 Hz, 1H, 2-H_{ax}), 1.47 (2xq, J = 7 Hz, 4H, β-CH_2), 0.87 (2xt, J = 7.5 Hz, 6H, CH_3).^{13}C-signals based on HSQC (500 MHz, D_2O, 25 °C, TMS) δ (p.p.m.) 110.2 (C-1´´), 99.3 (C-1´´´), 95.8 (C-1´), 85.0 (C-5), 82.2 (C-4´´), 76.1 (C-3´´), 75.4 (C-4), 74.4 (C-2´´), 73.6 (C-5´´´), 72.7 (C-6), 70.7 (C-4´), 70.3 (C-3´´´), 69.5 (C-3´), 68.2 (C-5´), 67.1 (C-4´´´), 60.5 (C-5´´), 53.7 (C-2´), 51.4 (C-2´´´), 50.0 (C-1), 48.6 (C-3), 41.9 (2C, α-CH_2), 40.2 (C-6´), 40.0 (C-6´´´), 28.0 (C-2), 22.6 (2C, β-CH_2), 10.6 (2C, CH_3); signals of quaternary carbons (2x CO) are missing due to the fact that they are not detectable by HSQC spectroscopy. MS (EI+) (*m/z)*: found 785.42184 $[M+H]^+$, 807.40231 $[M+Na]^+$; calculated 807.40703 $[M+H]^+$, 679.31207 $[M+Na]^+$.



6´´´-*N*-anthranoyl neomycin B x 5 HFBA (18). The title compound was prepared according to the general procedure described above. Derivative **18** was obtained as a white solid. For the measurement of regioselectivity and the characterization of the compound ^{1}H-NMR, HSQC as well as APT spectra were recorded and electrospray ionization (ESI)-MS was employed. The yield was determined by HPLC: R_t = 10.2 min, 52 % yield. TLC ($CHCl_3$/MeOH/17% NH_4OH 2:1:1 v/v/v) R_f = 0.54. ^{1}H-NMR (500 MHz, D_2O, 25 °C, TMS) δ (p.p.m.) 7.66 (dd, J = 7.7 Hz, J = 2.5 Hz, 1H, Ar-H2), 7.55 (t, J = 8.3 Hz, 1H, Ar-H5), 7.23 (m, 2H, Ar-H3, Ar-H4), 5.99 (d, J = 4 Hz, 1H, 1-H´), 5.42 (d, J = 2.5 Hz, 1H, 1-H´´), 5.24 (d, J = 1.0 Hz, 1H, 1-H´´´), 4.38 (t, J = 5.5 Hz, 1H, 3-H´´), 4.34 (dd, J = 5 Hz, J = 2.5 Hz, 1H, 2-H´´), 4.28 (t, J = 3 Hz, 1H, 3-H´´´), 4.25-4.21 (m, 2H, 4-H´´, 5-H´´´), 4.06 (t, J = 9.8 Hz, 1H, 4-H), 3.99 (t, J = 10 Hz, 1H, 5-H´), 3.95 – 3.89 (m, 2H, 3-H´, 5-H), 3.84- 3.77 (m, 3H, 4-H´´´,5-H_a´´, 6-H_a´´´), 3.70-3.60 (m, 3H, 5-H_b´´, 6-H, 6-H_b´´´), 3.58-3.54 (m, 2H, 3-H, 2-H´´´), 3.49-3.44 (m, 2H, 6-H_a´, 4-H´), 3.39-3.34 (m, 1H, 1-H, 2-H´), 3.29 (dd, J = 13.5 Hz, J = 6.5 Hz, 1H, 6-H_b´), 2.50 (dt, J = 12.5 Hz; J = 3.8 Hz, 1H, 2-H_{eq}),1.90 (dd, J = 12.5 Hz, 1H, 2-H_{ax}). ^{13}C-signals based on HSQC (500 MHz, D_2O, 25 °C, TMS) δ (p.p.m.) 132.8 (Ar-C5), 128.5 (Ar-C2), 119.6, 119.4 (2C, Ar-C3, Ar-C4), 109.9 (C-1´´), 95.9 (C-1´´´), 95.4 (C-1´), 84.7 (C-5), 81.8 (C-4´´), 76.1 (C-3´´), 75.2 (C-4), 73.9 (C-2´´), 72.5 (C-5´´´), 72.4 (C-6), 70.6 (C-4´), 69.2 (C-3´), 67.9 (C-5´), 67.6 (C-3´´´), 66.2 (C-4´´´), 60.4 (C-5´´), 53.4 (C-2´), 51.0 (C-2´´´), 49.6 (C-1), 48.3 (C-3), 40.1 (C-6´), 39.6 (C-6´´´), 27.9 (C-2), signals of quaternary carbons are missing due to the fact that they are not detectable by HSQC spectroscopy. MS (EI+) (*m/z)*: found 734.35566 $[M+H]^+$, 756.33733 $[M+Na]^+$; calculated 734.35668 $[M+H]^+$, 756.33862 $[M+Na]^+$.



2´´´-*N*-[rac-1-(hydroxymethyl)prop-2-en-1yl] neomycin B x 6 HFBA (20). The title compound was prepared according to the general procedure described above. Derivative **20** was obtained as a white solid. For the measurement of regioselectivity and the characterization of the compound ^{1}H-NMR, HSQC as well as APT spectra were recorded and electrospray ionization (ESI)-MS was employed. The yield was determined by HPLC: R_t = 13.1 min, 38 % yield. TLC ($CHCl_3$/MeOH/17% NH_4OH 2:1:1 v/v/v) R_f = 0.45. ^{1}H-NMR (600 MHz, D_2O, 25 °C, TMS) δ (p.p.m.) 6.05 (s, 1H, 1-H´), 5.64 (m, 1H, ($CH=CH_2$), 5.47-5.31 (m, 4H, 1-H´´, 1-H´´´, $CH=CH_2$), 4.51 (m, 1H, 3-H´´), 4.43-4.33 (m, 2H, 2- H´´, 5-H´´´), 4.30-4.25 (m, 2H, 4-H´´, 3-H´´´), 4.08 (t, J = 9.0 Hz, 1H, 4-H), 4.02 (t, J = 10.2 Hz, 1H, 5-H´), 3.98-3.90 (m, 3H, 3-H´, 5-H, 5-H_a´´), 3.86 (m, 1H, 4-H´´´), 3.80-3.70 (m, 4H, 5-H_b´´, 6-H, HO-CH_2-CHR-$CH=CH_2$), 3.68-3.61 (m, 2H, 2-H´´´, HO-CH_2-CHR-$CH=CH_2$), 3.58-3.31 (m, 7H, 1-H, 2-H´, 3-H, 4-H´, 6-H_a´, 6-H_a´´´, 6-H_b´´´),3.31 (dd, J = 13.8 Hz, J = 6.6 Hz, 1H, 6-H_b´), 2.50 (dt, J = 12.6 Hz; J = 3.6 Hz, 1H, 2-H_{eq}), 1.90 (dd, J = 12.0 Hz, 1H, 2-H_{ax}). ^{13}C-signals based on HSQC (D_2O, 125.7 MHz) δ (p.p.m.) 124.9 (**C**H=CH_2), 117.7 ($CH=CH_2$), 109.7 (C-1´´), 95.1 (C-1´´´), 94.9 (C-1´), 84.3 (C-5), 81.2 (C-4´´), 75.1 (C-3´´, C-4), 73.3 (C-2´´), 72.1 (C-6), 70.2 (C-4´), 69.7 (C-5´´´), 69.0 (HO-CH_2-CHR-$CH=CH_2$), 68.9 (C-3´), 67.6 (C-5´), 67.1 (C-3´´´), 66.9 (C-4´´´), 62.1 (HO-CH_2-CHR-$CH=CH_2$), 59.9 (C-5´´), 53.0 (C-2´), 50.4 (C-2´´´), 49.3 (C-1), 47.9 (C-3), 39.8, 39.6 (C-6´, C-6´´´), 27.7 (C-2). MS (EI+) (*m/z)*: found 685.36152 $[M+H]^+$, 707.34113 $[M+Na]^+$; calculated 685.36143 $[M+H]^+$, 707.34337 $[M+Na]^+$.

^{1}H-NMR and HSQC spectra of neomycin B and derivatives 8, 10, 12, 13, 15, 16, 18 and 20.

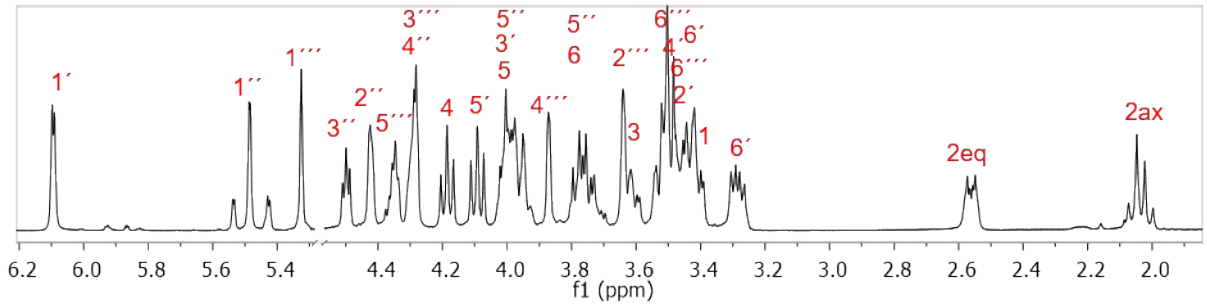


Figure S5 ^{1}H-NMR spectrum (500 MHz, D_2O) of neomycin B x 6 HFBA **1**.

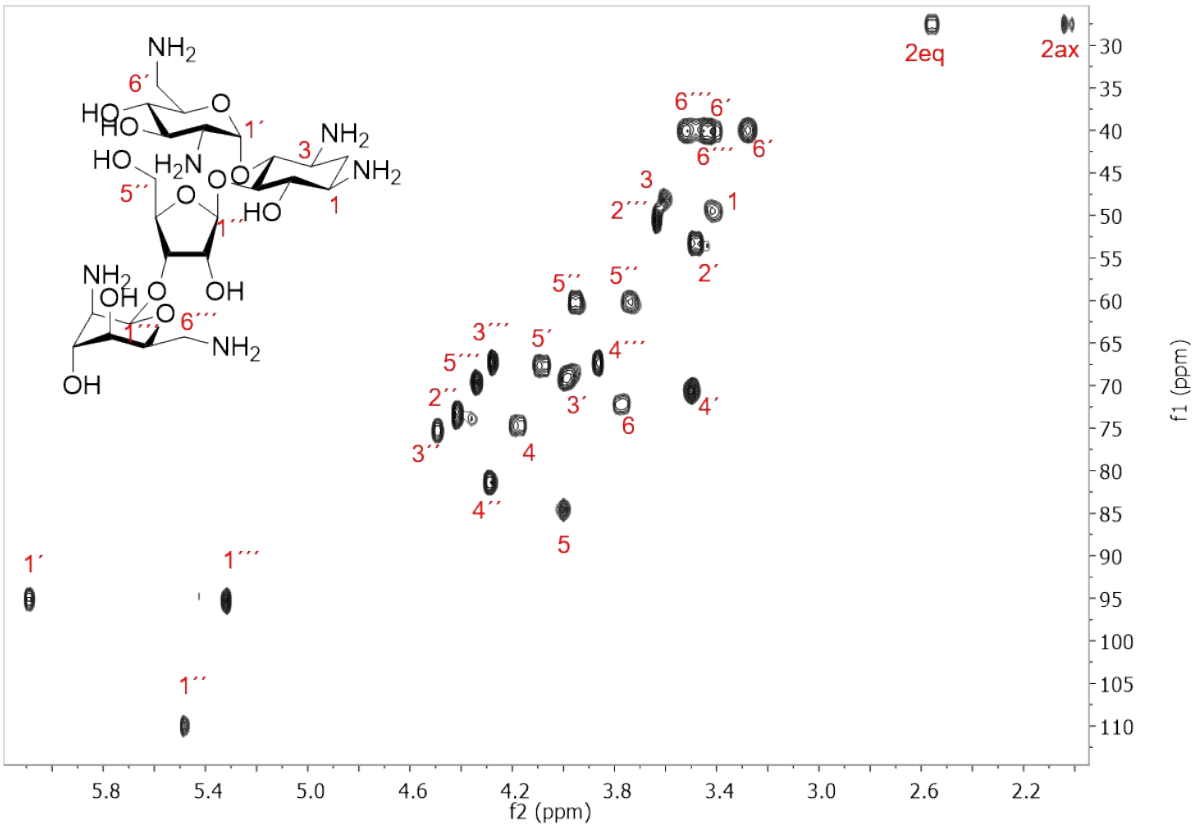


Figure S6 HSQC spectrum (500 MHz, D_2O) of neomycin B x 6 HFBA **1**

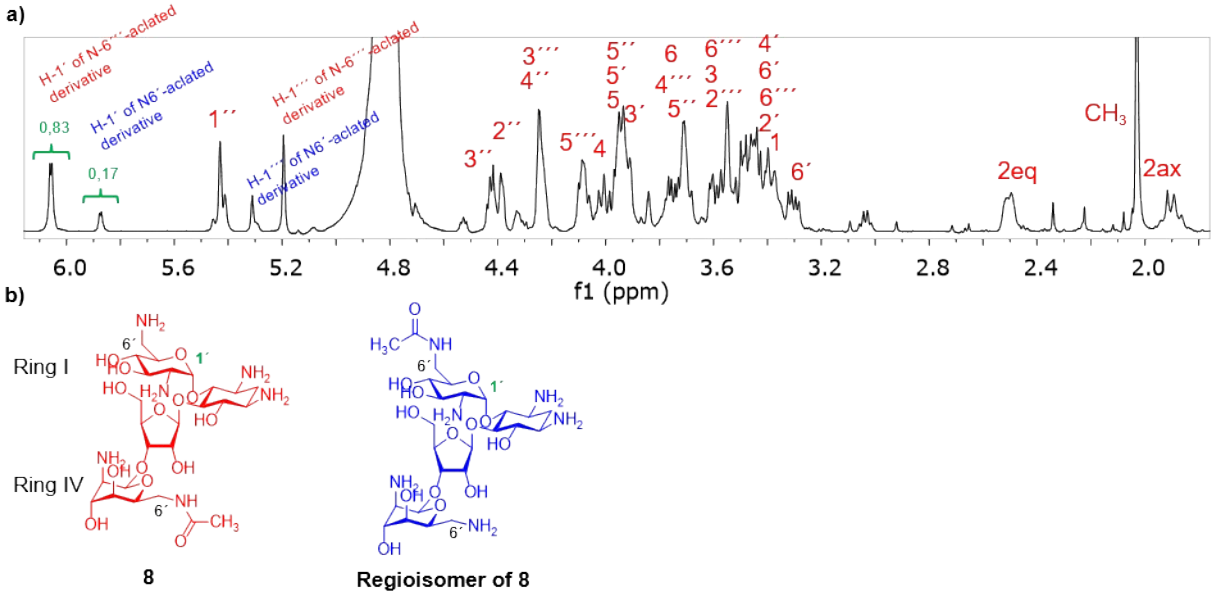


Figure S7 ^{1}H-NMR (500 MHz, D_2O) spectrum of 6´´´-*N*-acetyl neomycin B x 5 HFBA **8** (a) and structures of derivative **8** (red) and regioisomer 6´-*N*-acetyl neomycin B (blue) acetylated at ring IV (b). Integrals corresponding to the 1-H´ signal of both isomers are shown in green (a).

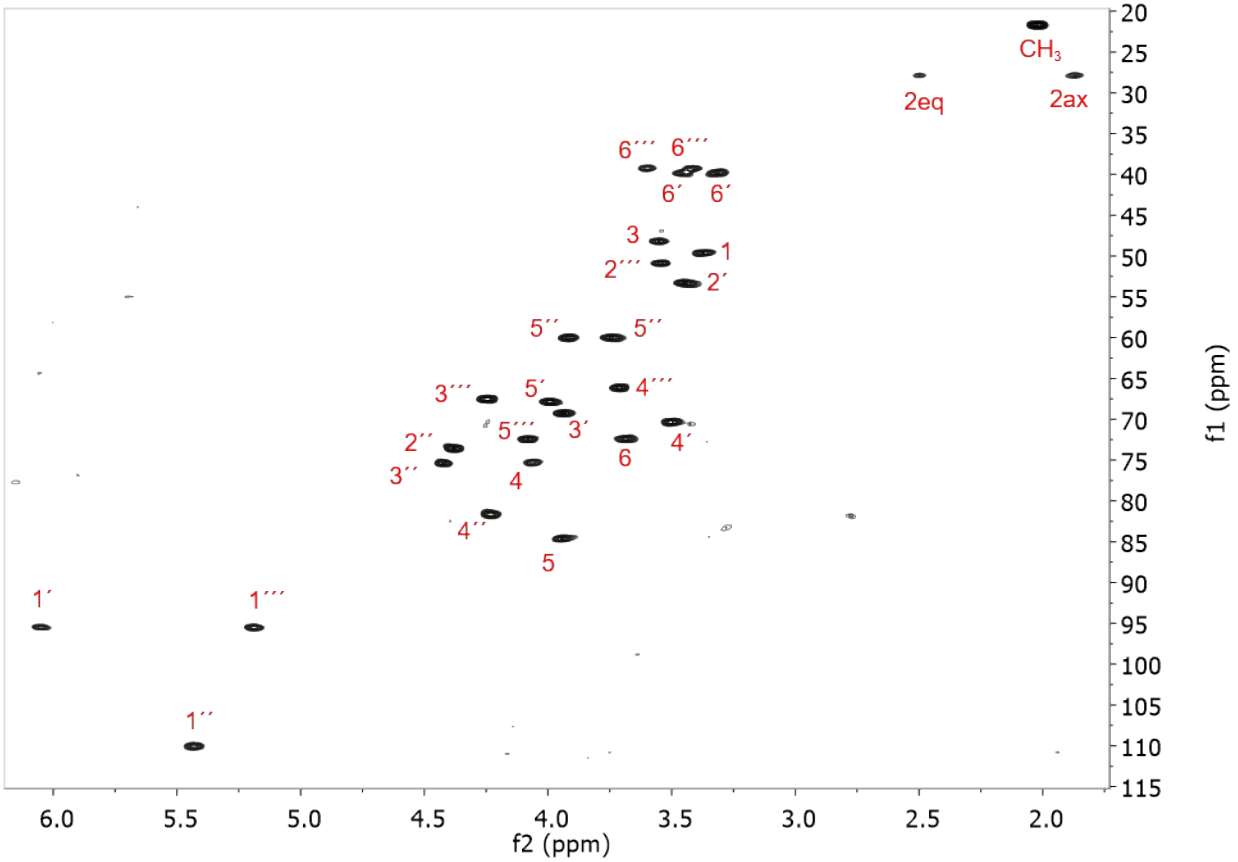


Figure S8 HSQC (500 MHz, D_2O) spectrum of 6´´´-*N*-acetyl neomycin B x 5 HFBA **8** including assignment of corresponding *J*(C-H) coupling signals.

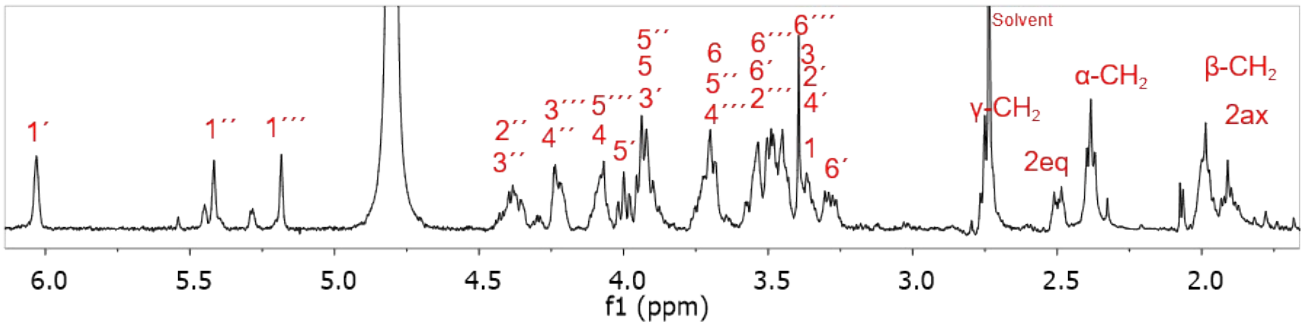


Figure S9 ^{1}H-NMR (500 MHz, D_2O) spectrum of 6´´´-*N*-γ-sulfhydryl-butanoyl neomycin B x 5 HFBA **10.**

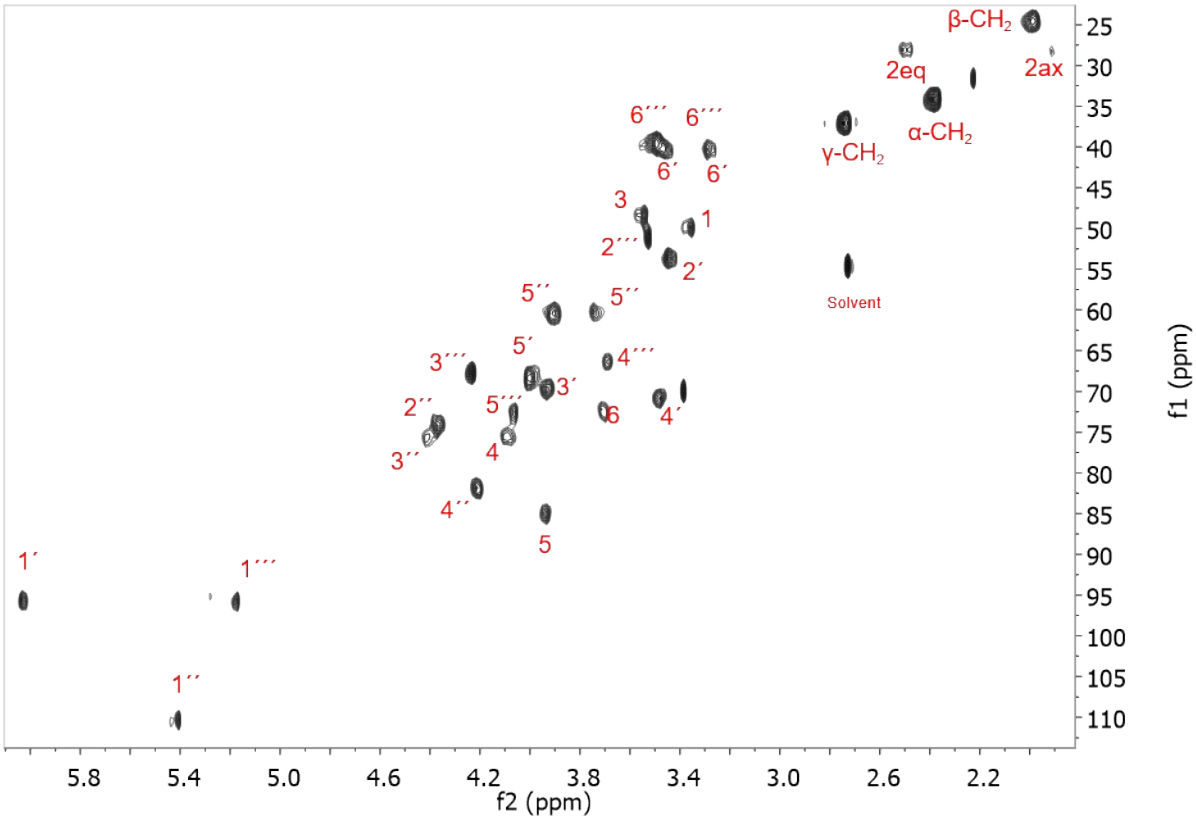


Figure S10 HSQC (500 MHz, D_2O) spectrum of 6´´´-*N*-γ-sulfhydryl-butanoyl neomycin B x 5 HFBA **10** including assignment of corresponding *J*(C-H) coupling signals.

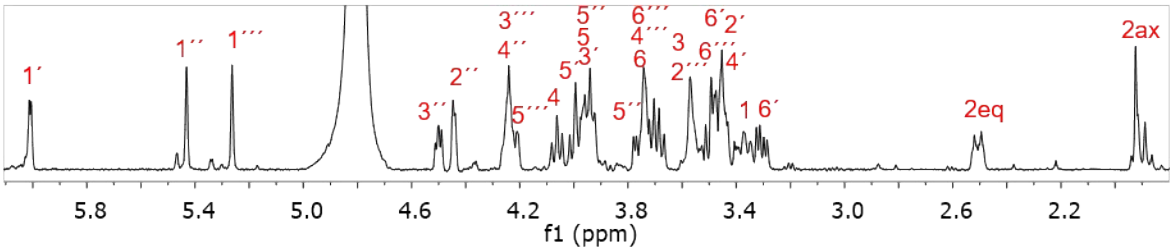


Figure S11 ^{1}H-NMR (500 MHz, D_2O) spectrum of 6´´´-azido neomycin B x 5 HFBA **12**.

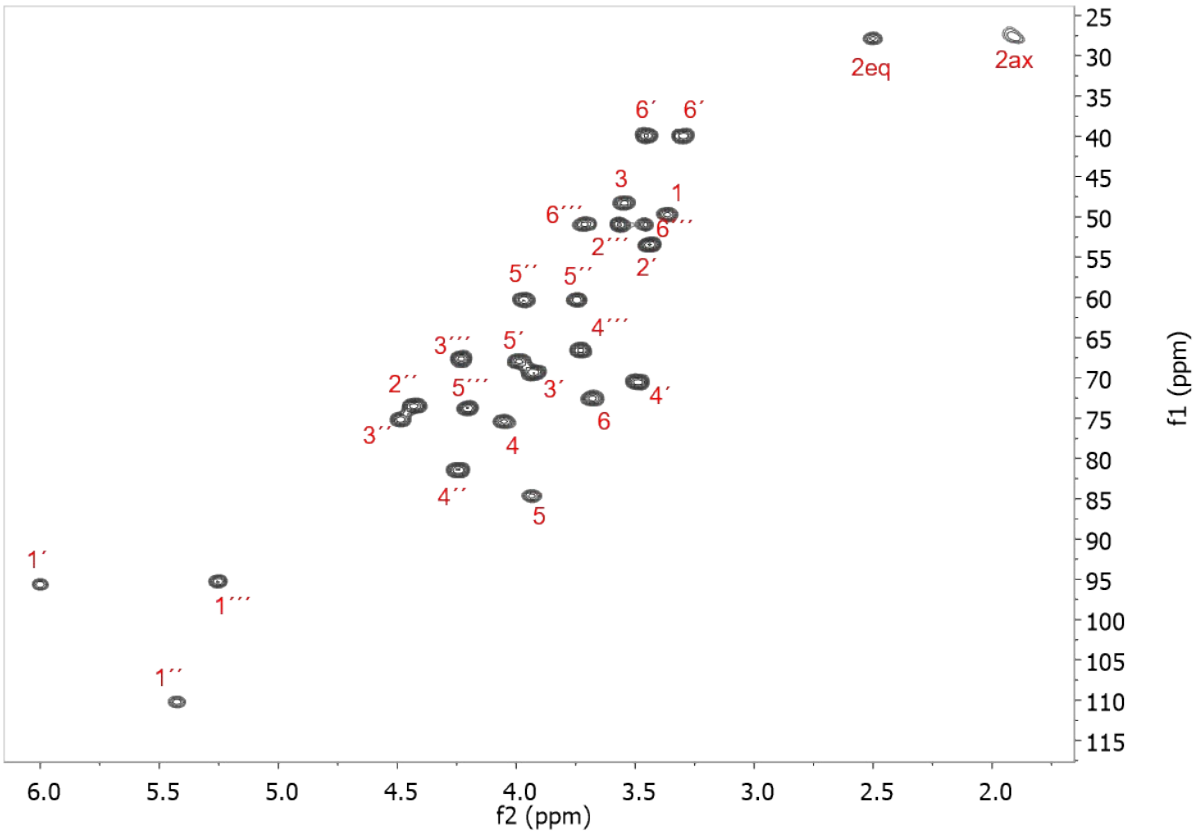


Figure S12 HSQC (500 MHz, D_2O) spectrum of 6´´´-azido neomycin B x 5 HFBA **12** including assignment of corresponding *J*(C-H) coupling signals.

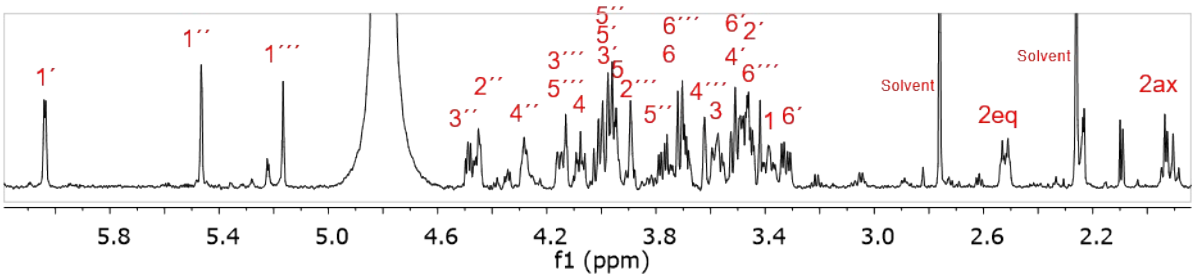


Figure S13 HSQC (500 MHz, D_2O) spectrum of 2´´´, 6´´´-diazido neomycin B x 5 HFBA **13**.

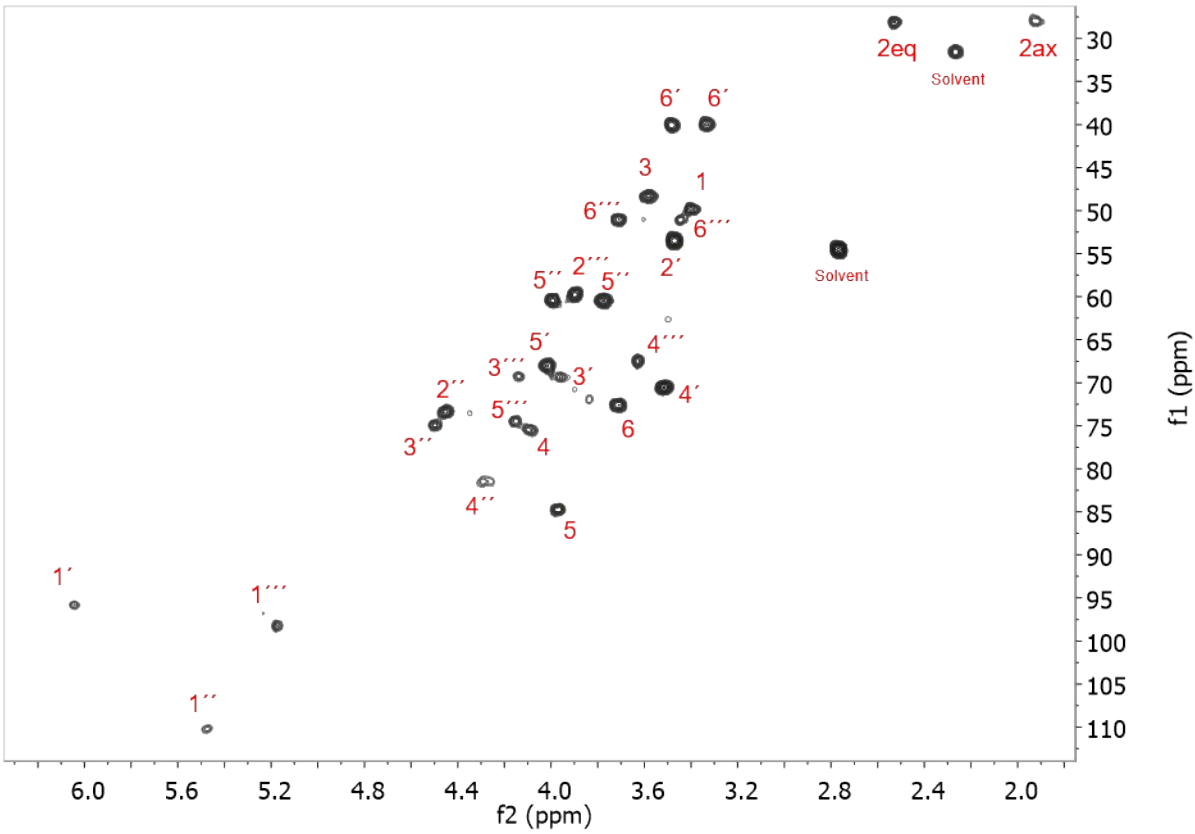


Figure S14 ^{1}H-NMR (500 MHz, D_2O) spectrum of 2´´´, 6´´´-diazido neomycin B x 5 HFBA **13** including assignment of corresponding *J*(C-H) coupling signals.

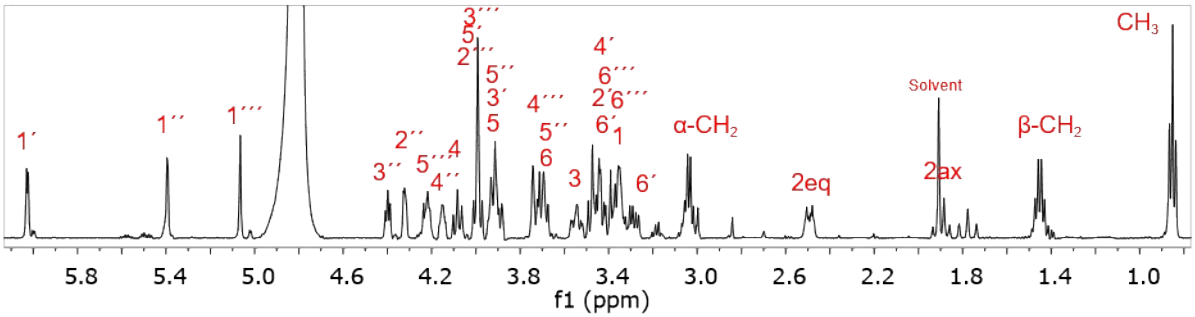


Figure S15 ^{1}H-NMR (500 MHz, D_2O) spectrum of 2´´´-*N*-(propylamino)carbonyl neomycin B x 5 HFBA **15**.

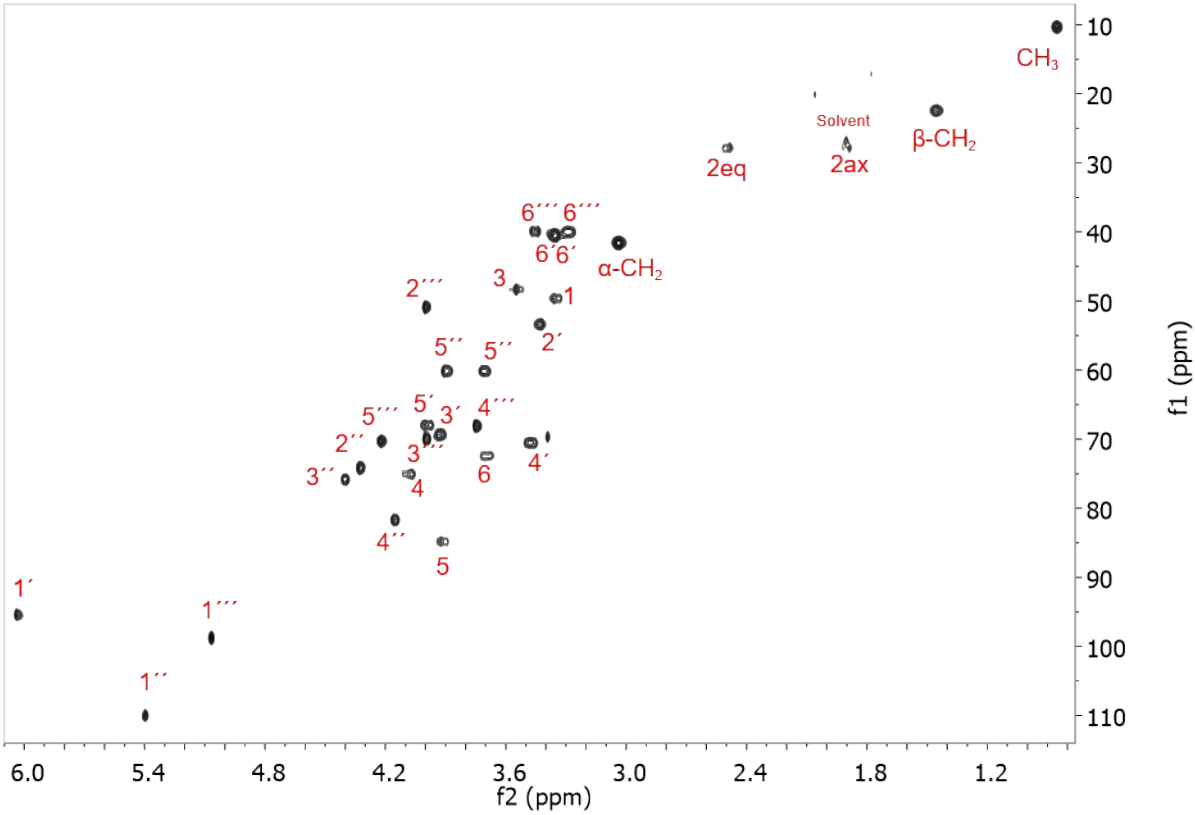


Figure S16 HSQC (500 MHz, D_2O) spectrum of 2´´´-*N*-(propylamino)carbonyl neomycin B x 5 HFBA **15** including assignment of corresponding *J*(C-H) coupling signals.

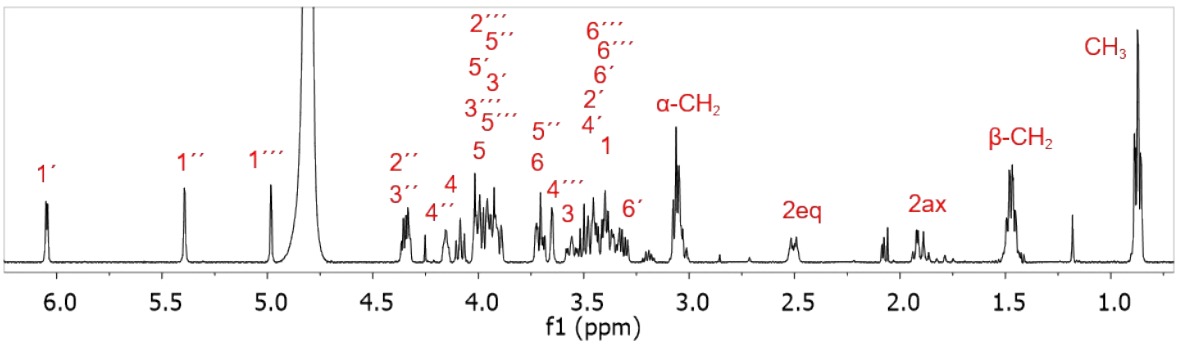


Figure S17 ^{1}H-NMR (500 MHz, D_2O) spectrum of 2´´´, 6´´´ bis-*N*-(propylamino)carbonyl neomycin B x 5 HFBA **16**.

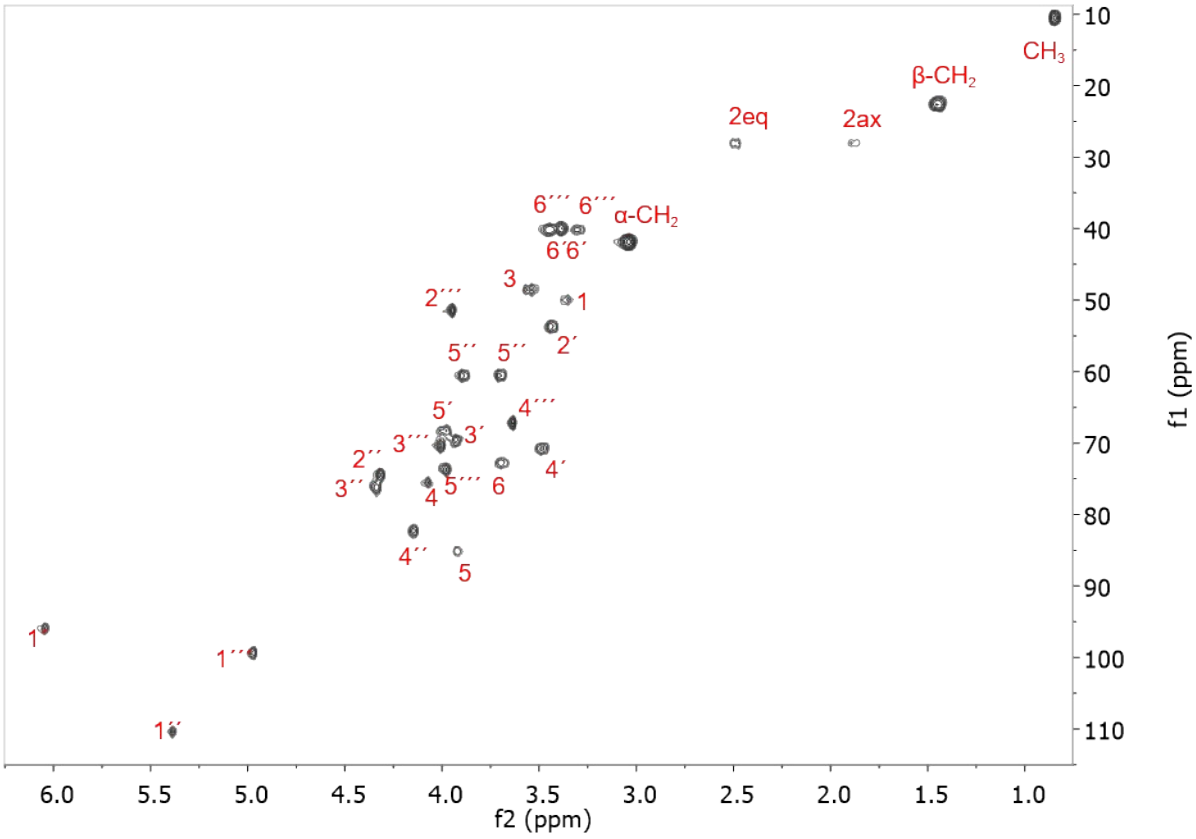


Figure S18 HSQC (500 MHz, D_2O) spectrum of 2´´´, 6´´´ bis-*N*-(propylamino)carbonyl neomycin B x 5 HFBA **16** including assignment of corresponding *J*(C-H) coupling signals.

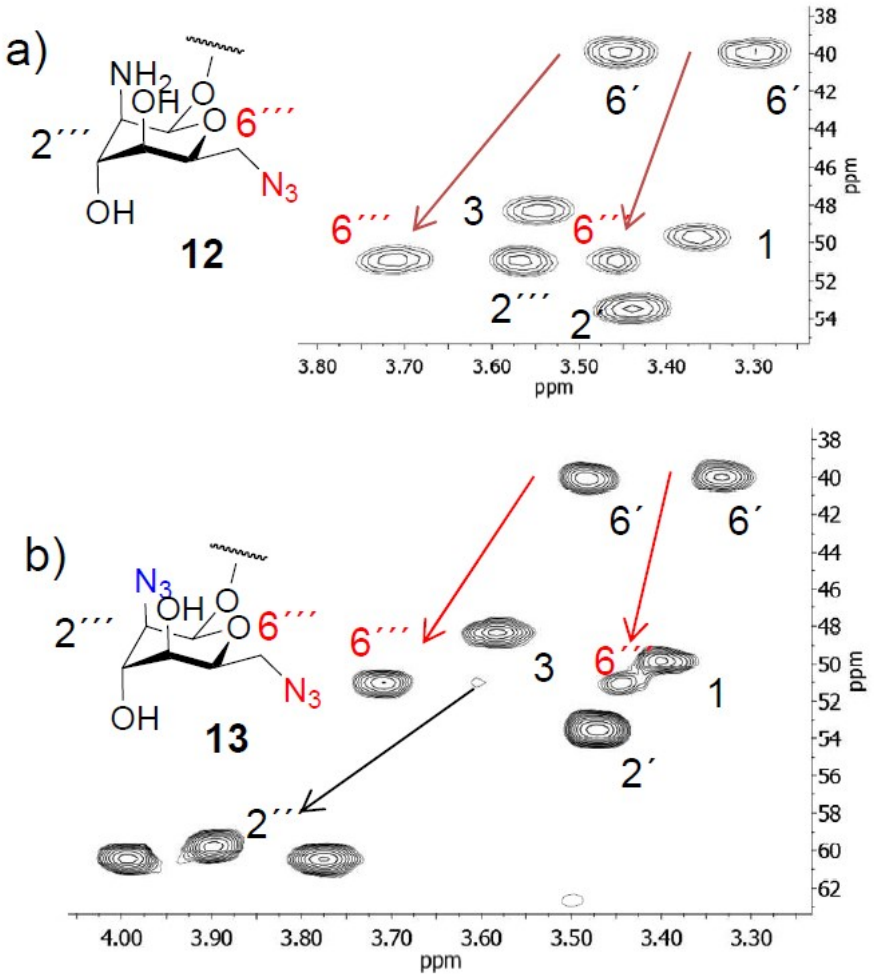


Figure S19. Sections of HSQC-spectra (500 MHz) of *mono*-azido derivative **12** (a) and *di*-azido derivative **13** (b). Chemical shifts of the corresponding *J*(C-H) coupling indicate the selective transformation of the amino group in C6 (red arrow) and C2 position (black arrow) at neomycin's B ring IV into an azido group.

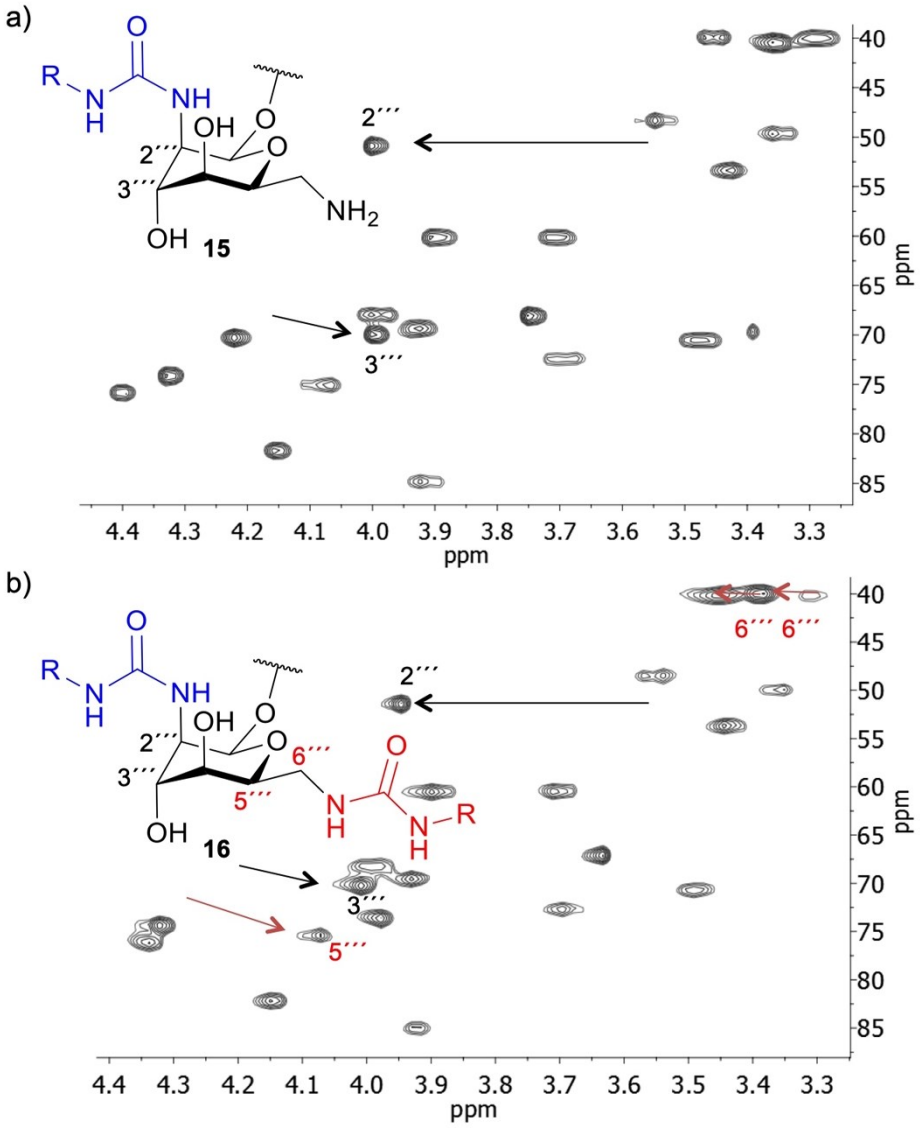


Figure S20. Sections of HSQC-spectra (500 MHz) of *mono*-urea- **15** (a) and *di*-urea derivative **16** (b) (R = propyl). Chemical shifts of the corresponding *J*(C-H) coupling indicate the introduction of urea group at the C2 (black arrows) and C6 position at neomycin's B ring IV.

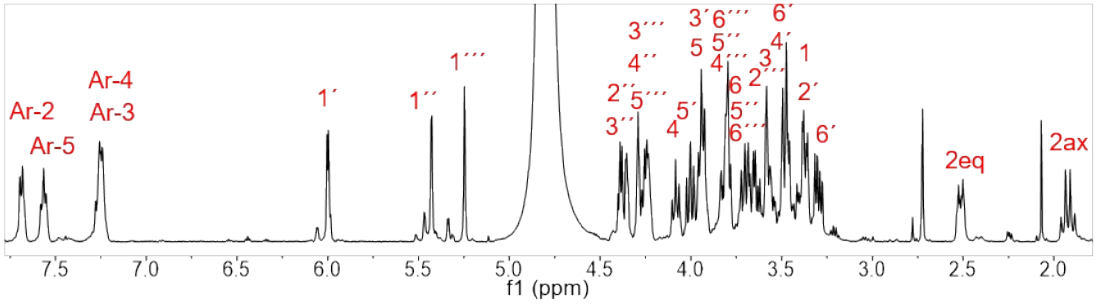


Figure S21 ^{1}H-NMR (500 MHz, D_2O) spectrum 2´´´-*N*-anthranoyl neomycin B x 5 HFBA **18**.

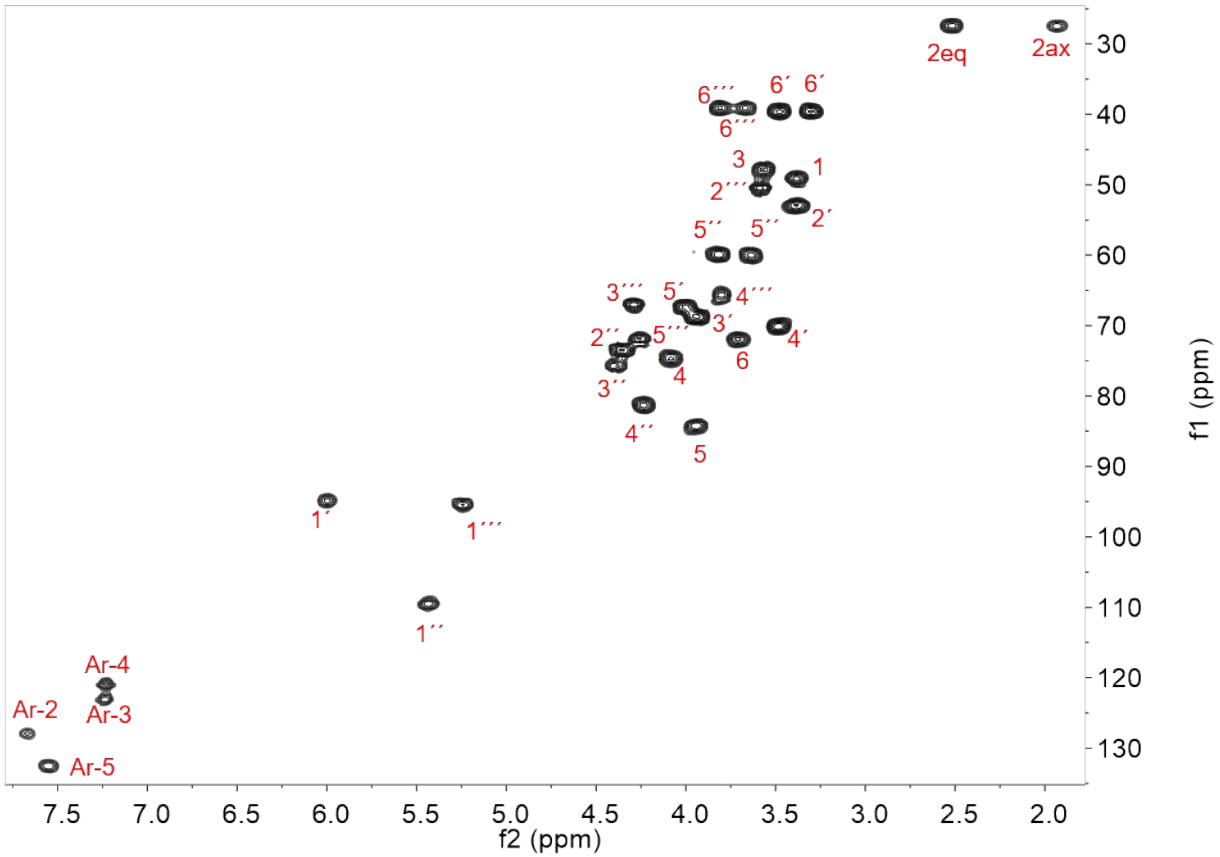


Figure S22 HSQC (500 MHz, D_2O) spectrum of 2´´´-*N*-anthranoyl neomycin B x 5 HFBA **18** including assignment of corresponding *J*(C-H) coupling signals.

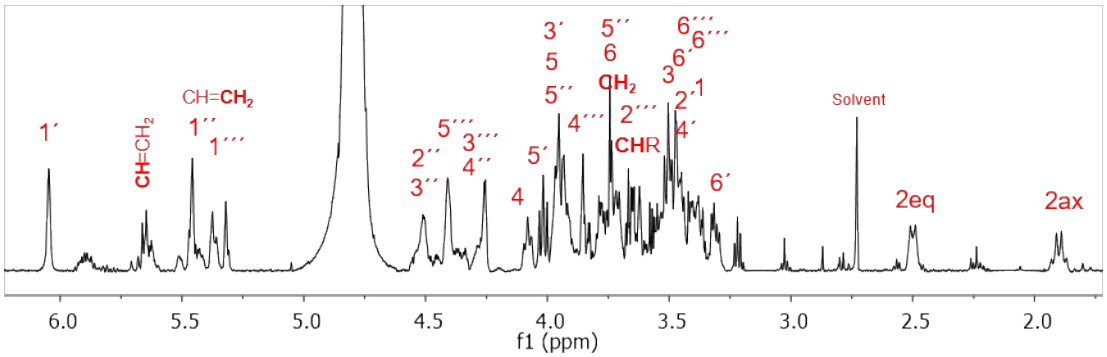


Figure S23 ^{1}H-NMR (600 MHz, D_2O) spectrum of 6´´´-*N*-[rac-1-(hydroxymethyl)prop-2-en-1-yl] neomycin B x 5 HFBA **20.**

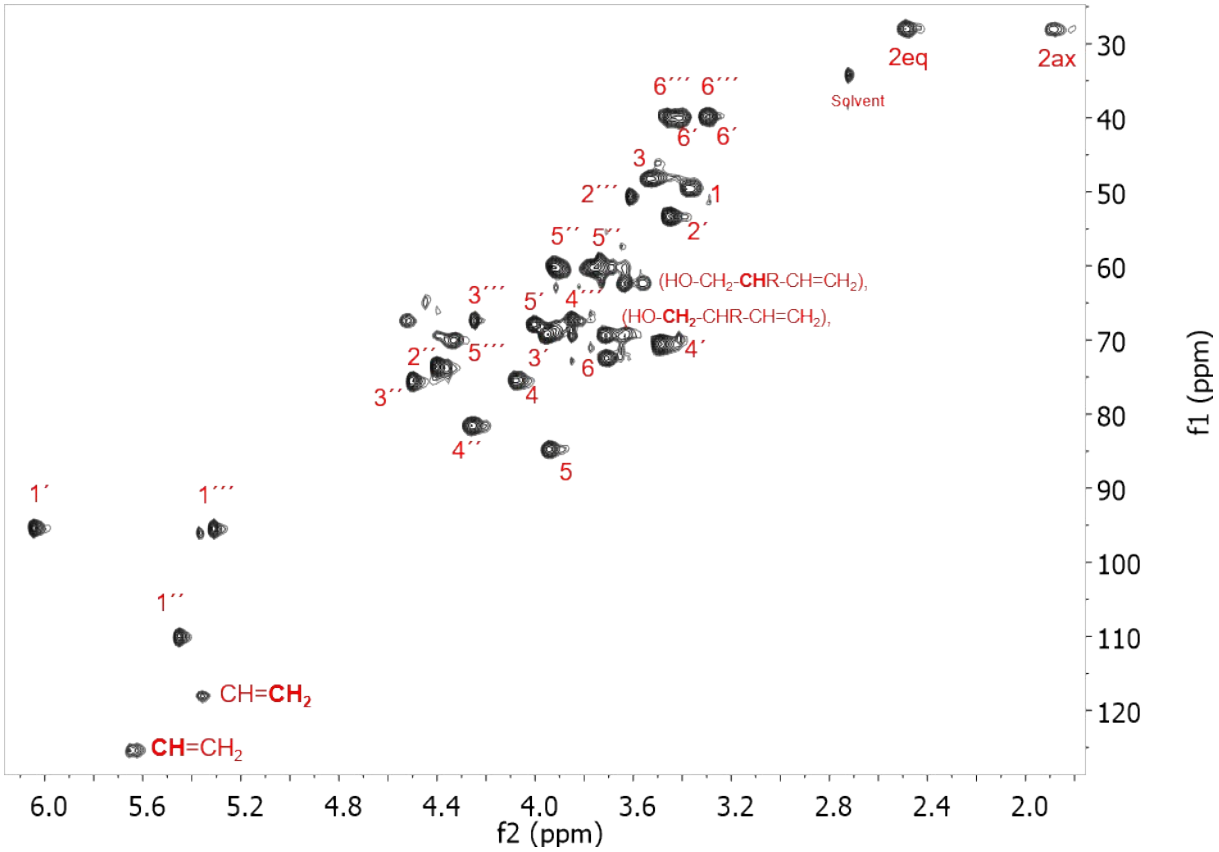


Figure S24 HSQC (500 MHz, D_2O) spectrum of 6´´´-*N*-[rac-1-(hydroxymethyl)prop-2-en-1-yl] neomycin B x 5 HFBA **20** including assignment of corresponding *J*(C-H) coupling signals.

High resolution mass spectra

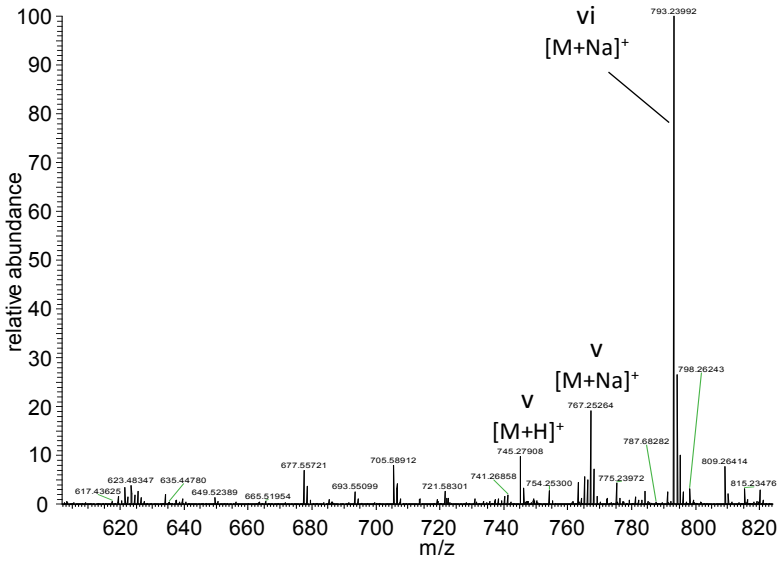


Figure S25 ESI-MS spectrum after transformation of neomycin B **1** with 8 equiv. diazo-transfer reagent **11** in absence of APG (v-vi = number of introduced azide groups).

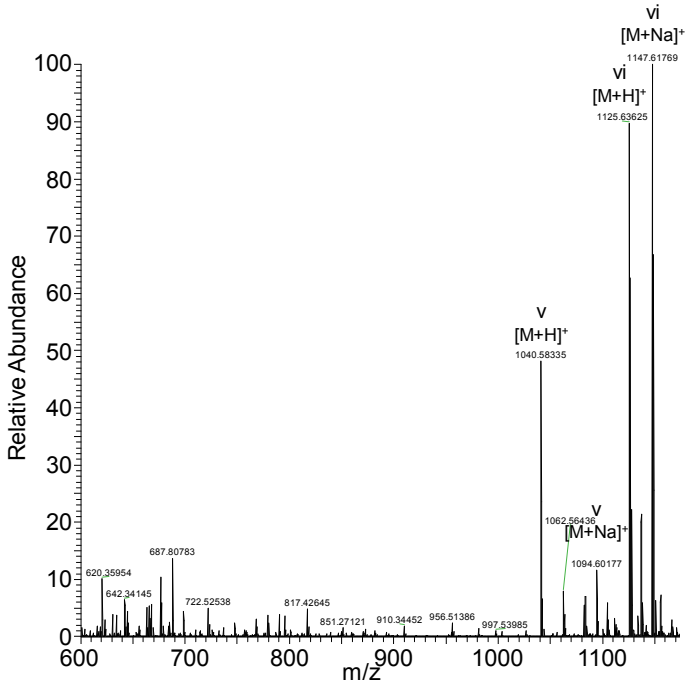


Figure S26 ESI-MS spectrum after transformation of neomycin B **1** with 30 equiv. propyl isocyanate **14** in absence of APG (v-vi = number of introduced urea groups).

High performance liquid chromatography (HPLC)

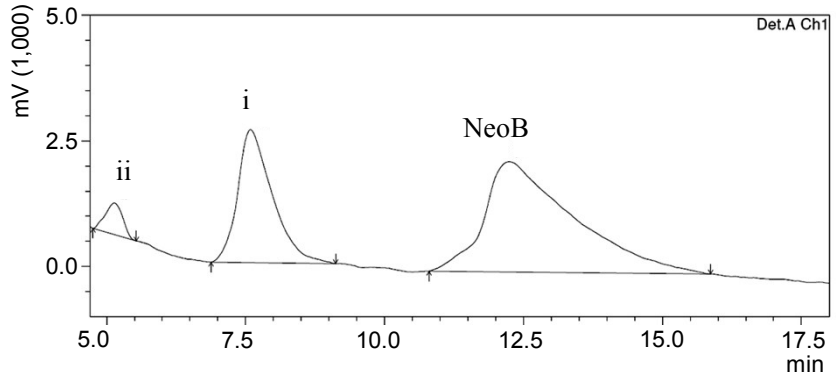


Figure S27 HPLC chromatogram after transformation of neomycin B **1** (NeoB) with 15 equiv. STP-ester **7** in presence of APG (i-ii = number of reacted amino groups).

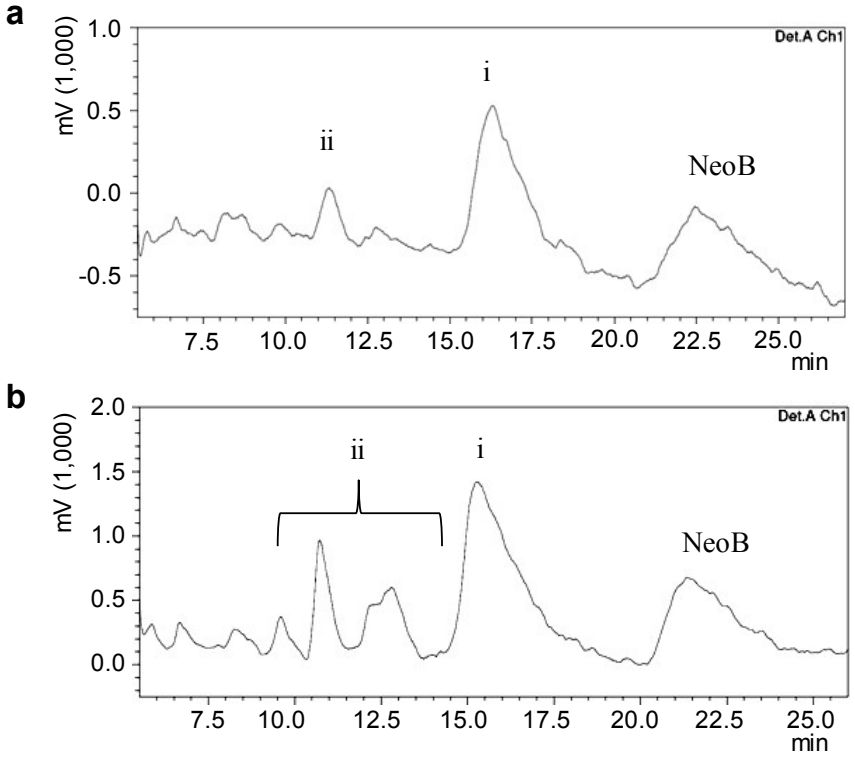


Figure 28 HPLC chromatogram after transformation of **1** (NeoB) using 5 equivalents (a) and 30 equivalents (b) of *Traut's* reagents **9** in presence of APG (i-ii = number of reacted amino groups).

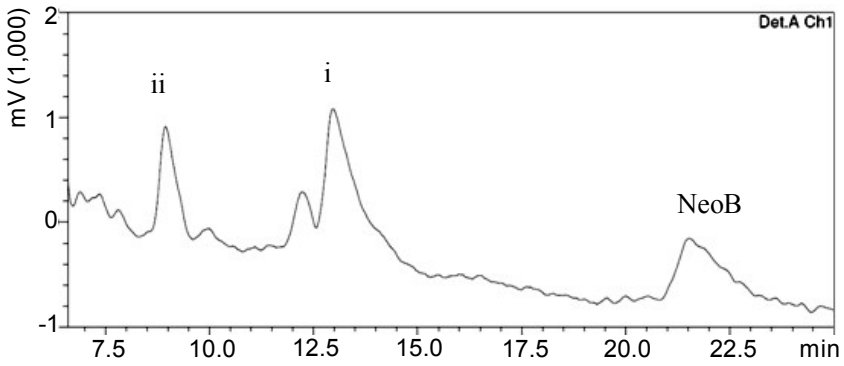


Figure S29 HPLC chromatogram after transformation of neomycin B **1** (NeoB) using 8 equiv. of diazo-transfer reagent **11** in presence of APG (i-ii = number of reacted amino groups).

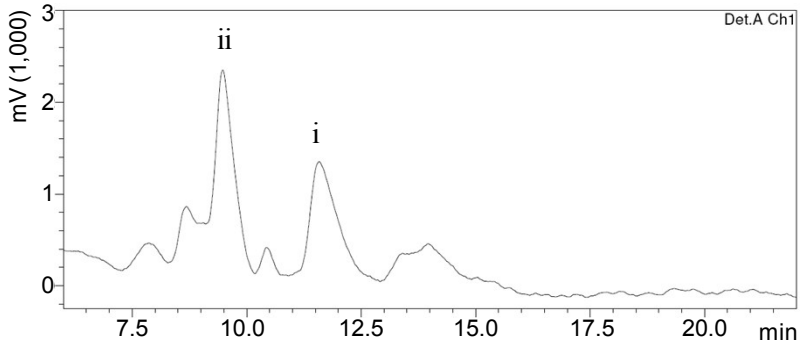


Figure S30 HPLC chromatogram after transformation of neomycin B **1** (NeoB) with 30 equiv. propyl isocyanate **14** in presence of APG (i-ii = number of reacted amino groups).

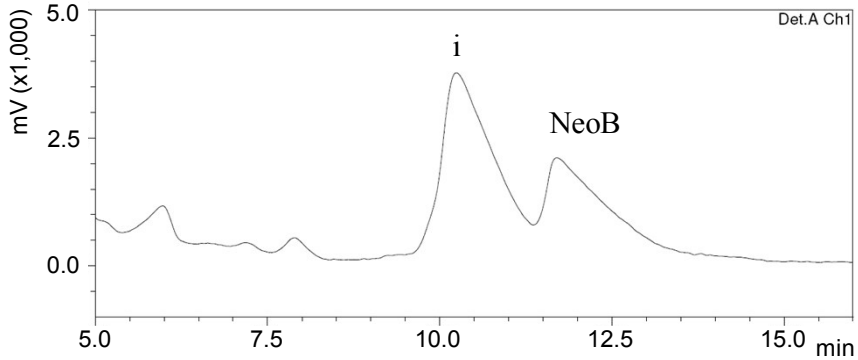


Figure S31 HPLC chromatogram after transformation of neomycin B **1** (NeoB) with 2 equiv. isatoic anhydride **17** in presence of APG (i = number of introduced reacted amino groups).

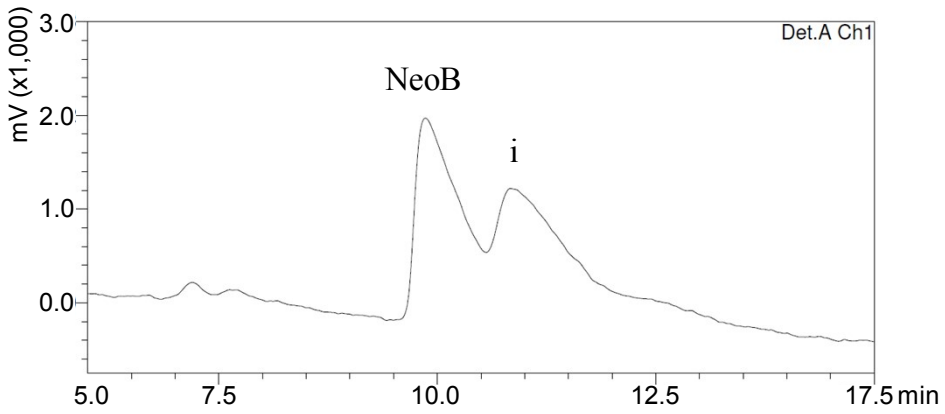


Figure S32 HPLC chromatogram after transformation of neomycin B **1** (NeoB) with 30 equiv. 3,4-epoxy-1-butene **19** in presence of APG (i = number of reacted amino groups).

Control experiments using 1.5 eq. of acetyl STP ester 7 and *Traut's* reagent 9 in absence of RNA aptamer

To demonstrate that the presented transformations at ring IV are regioselective due to the protection of ring I, II and III by the RNA aptamer, we performed the acylation and the thiol group introduction using 1.5 eq. acetyl STP-ester **7** and 1.5 eq. *Traut's* reagent **9**, respectively in absence of RNA aptamer. As shown in Figure S29, the transformation of neomycin B in absence of RNA aptamer using 1.5 eq. STP ester **7** results mainly in *mono*- and *di*-acetyl neomycin B derivatives. To demonstrate that mono-acetyl neomycin B derivatives are not separable by HPLC, the fraction containing mono-acetyl neomycin B derivatives was collected and analyzed by NMR spectroscopy. As proven by ^{1}H-NMR spectroscopy (Fig. S36a), the mono-acylation of neomycin B in absence of RNA aptamer results in approx. a 1:1 mixture of two regioisomers exhibiting the amide bond in at the C6 position of ring II and ring IV, respectively. This result is in accordance with the reference reaction performed in previous studies using an activated succinimide ester in absence of RNA aptamers.[3] In contrast, the derivatization of neomycin B in presence of an APG using STP ester **7** results mainly in one regioisomer, i.e. 6´´´-*N*-acetyl neomycin B (**8**) (Figure S36b).

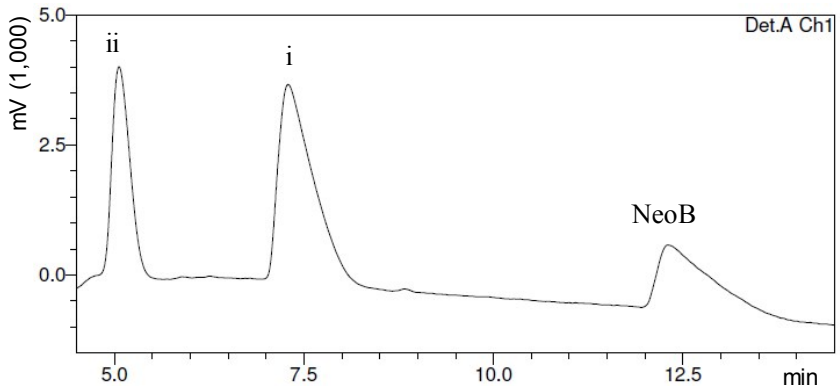


Figure S33 HPLC chromatogram after transformation of neomycin B **1** (NeoB) using 1.5 equiv. STP-ester **7** in absence of RNA aptamer (i-ii = number of acetylated amino groups).

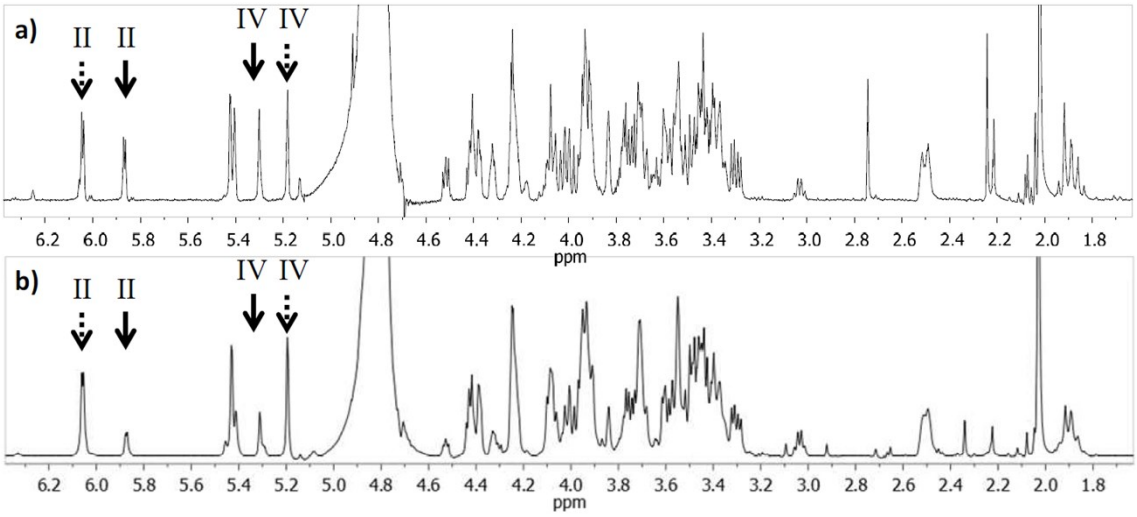


Figure S34 ^{1}H-NMR (500 MHz, D_2O) spectra of mono-acetyl neomycin B derivates formed using 1.5 equiv. STP ester **7** in absence of RNA aptamer (a) and employing 15 equiv. **7** in presence of the APG (b) Arrows indicate signals of the anomeric protons of ring II and IV of 6´´´-*N*-acetyl neomycin B (dashed arrows) and 6´-*N*-acetyl neomycin B (non-dashed arrows).

In contrast to the STP ester **7**, the modification of neomycin B using 1.5 eq. *Traut*'s reagent **9** in absence of RNA aptamer results in a mixture of neomycin B derivatives exhibiting only a single sulfhydryl group (see HPLC elugram, Fig. S35). However, as shown by NMR spectroscopy (Fig. S34), also this reaction is not selective in absence of RNA aptamer and results in an undefined mixture of mono-functionalized neomycin B derivatives, which are not separable by HPLC. The NMR in Figure S38 shows five signals of similar integrals in the range for anomeric protons of neomycin B (5.0-6.5 p.p.m.). This is clearly an indication for a mixture of different regioisomers considering that neomycin B and its derivative are carrying only three anomeric protons, which result in three signals in the same range.

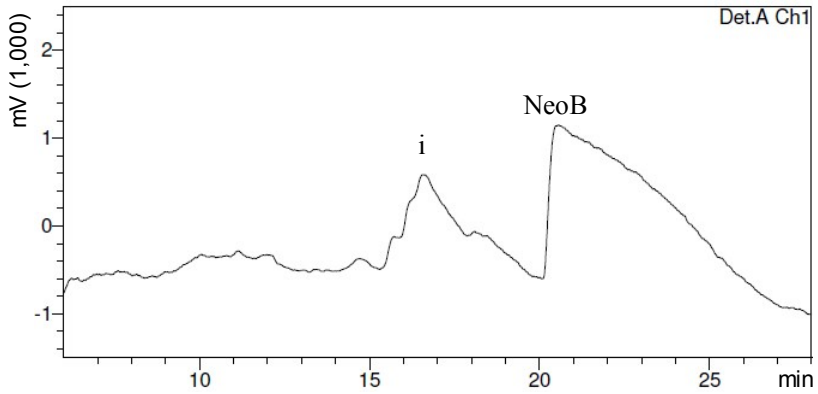


Figure S35 HPLC chromatogram after transformation of **1** (NeoB) using 1.5 eq. *Traut's* reagents **9** in absence of RNA aptamer (i = number of acylated amino groups).

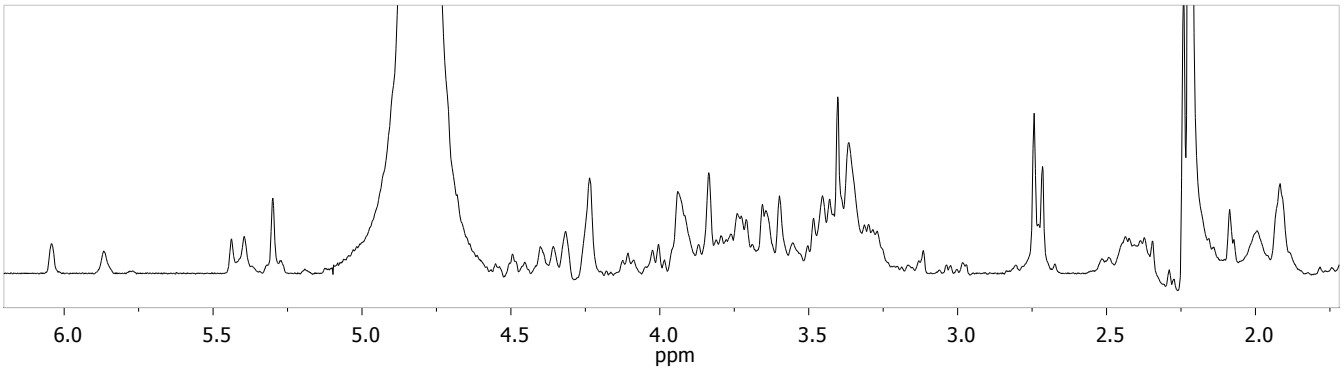


Figure S36 ^{1}H-NMR (500 MHz, D_2O) spectrum of mono-*N*-γ-sulfhydryl-butanoyl neomycin B derivatives formed using 1.5 eq. *Traut's* reagent **9** in absence of RNA aptamer.

Ionic Exchange Chromatography for Detection of RNA Degradation.

Anion Exchange Chromatography was performed in 80 °C with DNAPac PA200 analytical column (4x250 mm) using following buffers: A 25 mM Tris-HCl pH 7.5 and B 1 M $NaClO_4$ in buffer A. Linear gradient of buffer B in buffer A was employed (100 % B in 22.5 min, flow 1.2 mL/min). The run was monitored with wavelength of 260 nm with a PDA detector.

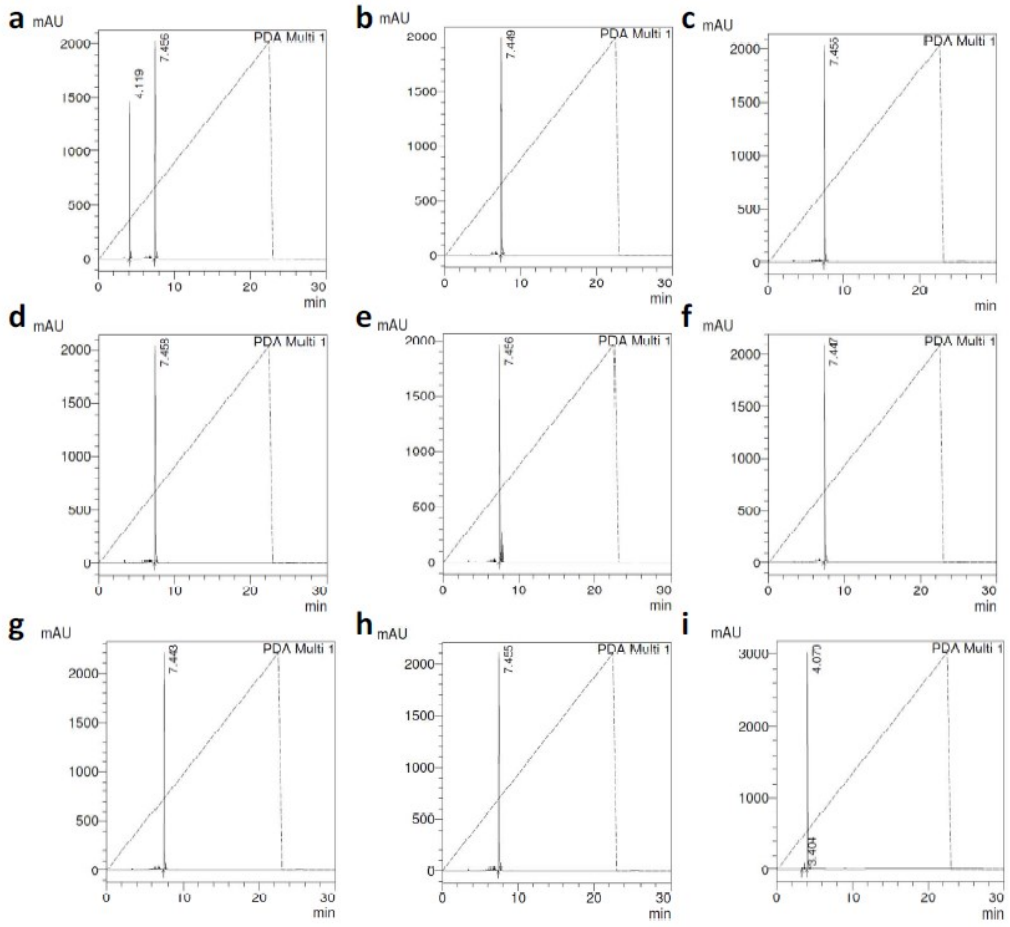


Figure S37 Anion Exchange Chromatography of reaction mixture after transformation of neomycin B in presence of 1.5 equiv. APG with STP ester **7** (a), *Traut's* reagent **9** (b), imidazole-1-sulfonyl azide hydrochloride **11** (c), n-propyl isocyanate **14** (d), isatoic anhydride **17** (e), 3,4-epoxy-1-butene **19** (f). Anion Echange Chromatography of RNA aptamer (g), neomycin B-APG complex (h) and STP ester **7** (i).

As shown in Figure S37, no degradation of RNA was detected after a reaction time of 24 hours. The additional peak in chromatogram of Figure S37a corresponds to the STP ester **7** (compare Figure S37i). Furthermore, to evaluate if the RNA aptamer reacted with the reagents we applied MALDI-TOF mass spectrometry. No case of RNA modifications of the APG were detected (data not shown).

References

(1) Gee, K. R.; Archer, E. A.; Kang, H. C. 4-Sulfotetrafluoropßhenyl (STP) Esters: New Water-Soluble Amine-Reactive Reagents for Labeling Biomolecules. *Tetrahedron Letters* **1999**, *40*, 1471-1474.

(2) Goddard-Borger, E. D.; Stick, R. V. An Efficient, Inexpensive, and Shelf-Stable Diazotransfer Reagent: Imidazole-1-sulfonyl Azide Hydrochloride. *Organic Letters* **2007**, *9*, 3797-3800.

(3) Bastian, A.A.; Marcozzi, A.; Herrmann, A. Selective transformations of complex molecules are enabled by aptameric protective groups. *Nat. Chem.* **2012**, *4*, 789-793.