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Published in:
Nature Chemistry

DOI:
10.1038/NCHEM.1402

nature chemistry

SUPPLEMENTARY INFORMATION

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Selective Transformations of Complex Molecules are Enabled by Aptameric Protective Groups

Content:

General

^{1}H-NMR-, ^{13}C-NMR, heteronuclear single-quantum correlation (HSQC) spectra and attached proton test (ATP) were recorded on a Varian Unity Inova (500 MHz and 600 MHz) and Oxford AS400 (400 MHz) NMR spectrometer at 25 °C. High res olution 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) equipp ed 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®), paromomycin sulfate salt (98%), *N,N*-dimethylformamide (DMF, 99%), *N*-hydroxysuccinimide (NHS, 98%), trifluoroacetic anhydride (99%), dichloromethane (DCM, 99.5%), tetrahydrofurane (THF, 99.9%), pyridine (99%), 4-pentynoic acid (95%), acetic acid (99%), isobutyric acid (99%), 4-methoxyophenyl isocyanate (99%), phenyl isocyanate (98%), 4-(dimethylamino)-phenyl isocyanate (97%) and toluene (99.8%) were purchased from *Sigma Aldrich* and used as received. 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 aptamers (82 – 91% purity) were purchased from *BioSpring* (Frankfurt am Main, Germany) and *riboxx* GmbH (Radebeul, Germany). Acetic acid *N*-hydroxysuccinimide esters **3a** was synthesized according to a literature procedure[1]. For the regioselective transformation Milli-Q water was treated with diethylpyrocarbonate (DEPC) and sterilized using an autoclave (121 °C, 20 min).

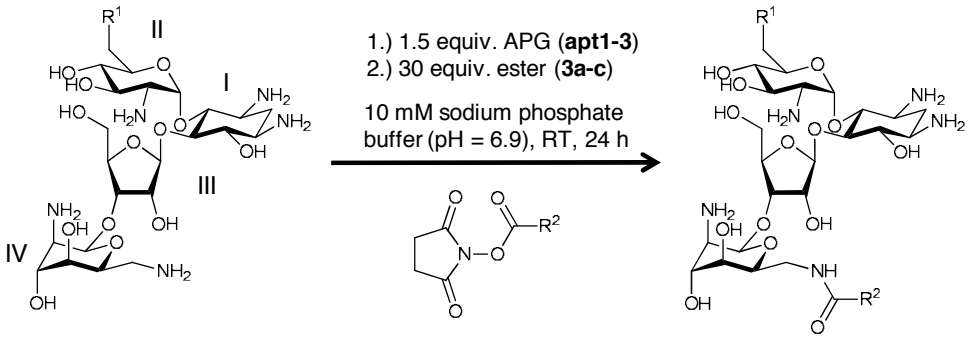
General procedure

1 General procedure for synthesis of activated ester 3b and 3c[2]

To trifluoroacetic acid anhydride (56.4 ml, 0.47 mol) was added *N*-hydroxysuccinimide (20 g, 0.196 mol) at 0 ℃. The reaction was allowed to war m up to r.t. over 24 hours. Then toluene (50 ml) was added and the volatile was removed in vacuo. The residue was taken up in dichloromethane (15 mL) and the remaining solvent was removed in vacuo. The resulting product, *N*-trifluoroacetoxysuccinimde, was used without any further purification for the next step. To a solution of carboxylic acid in 15 ml THF (15 mL) was added *N*-trifluoroacetoxysuccinimide (5 g, 23.7 mmol) and pyridine (2.1 ml, 26.4 mmol) at 0 ℃. The reaction mixture was allowed to warm up to r.t. over 24 h, before being quenched with water (20 mL). The aqueous phase was extracted twice with 1:1 mixture of diethyl ether and hexan (75 mL). The combinated organic phases were washed with saturated aqueous NH_4Cl solution (100 mL), saurated aqueous $NaHCO_3$ solution (100 mL) and brine (100 mL), dried over $MgSO_4$, filtered and concetrated to yield activated ester **3b** and **3c**.

2 General procedures for regioselective transformation of aminoglycosides

a) Amide formation of neomycin B



1 neomycin B $R^1 = NH_2$

3a R^2 = Me
3b R^2 = *i*-Pr
3c R^2 = 3-butynyl

4a $R^1 = NH_2$ R^2 = Me
5 $R^1 = NH_2$ R^2 = *i*-Pr
6 $R^1 = NH_2$ R^2 = 3-butynyl

Figure S1 Regioselective transformation of amino group in C6 position at ring IV.

A 6.1 mM RNA aptamer solution (sequences: 5'-GGA CUG GGC GAG AAG UUU AGU CC-3' (**apt1**), 5'-CUG CAG UCC GAA AAG GGC CAG-3' (**apt2**) or 5'-UGU AGG GCG AAA AGU UUU ACA-3' (**apt3**)) (816 μL, 4.98 μmol) in 10 mM sodium phosphate buffer (pH 6.8) was heated to 85 ℃ for 10 min and was afterwards kept at room te mperature for 15 min. Then a 4.8 mM solution of the aminoglycoside antibiotic (684 μL, 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.

Afterwards 30 equiv. NHS ester (98.5 μmol) dissolved in sodium phosphate buffer (1.5 mL, pH 7.4) (for activated ester **3a**) or in DMF (108 μl) (for activated esters **3b** and **3c**) were added and the reaction mixture was allowed to react for 24 hours at room temperature. After addition of a 7 wt. % ethylamine water solution (126 μL) and further incubation for 30 min at room temperature, the crude mixture was heated to 99 ℃ for 10 min. To the hot solution a 53 mM aqueous solution of didodecyldimethylammonium bromide (3 mL) was added to precipitate the RNA. After incubation for 15 min at room temperature and centrifugation for 30 min at 6 ℃ (16.1 u/s) the supernatant was freeze-dried and dissolved in water (400 μL). Each fraction (30 μL) was purified by HPLC using a Waters Spherisorb ODS-2C_{18} analytic column (water/acetone 1:0.9 containing 12.1 mM HFBA) at a flow rate of 1 ml/min at 40℃ to afford the antibiotic derivatives **4a**, **5** and **6**. After evaporation of acetone and freeze-drying the product was dissolved in D_2O (150 μL) for NMR-studies.

b) Urea bond formation of neomycin B and paromomycin

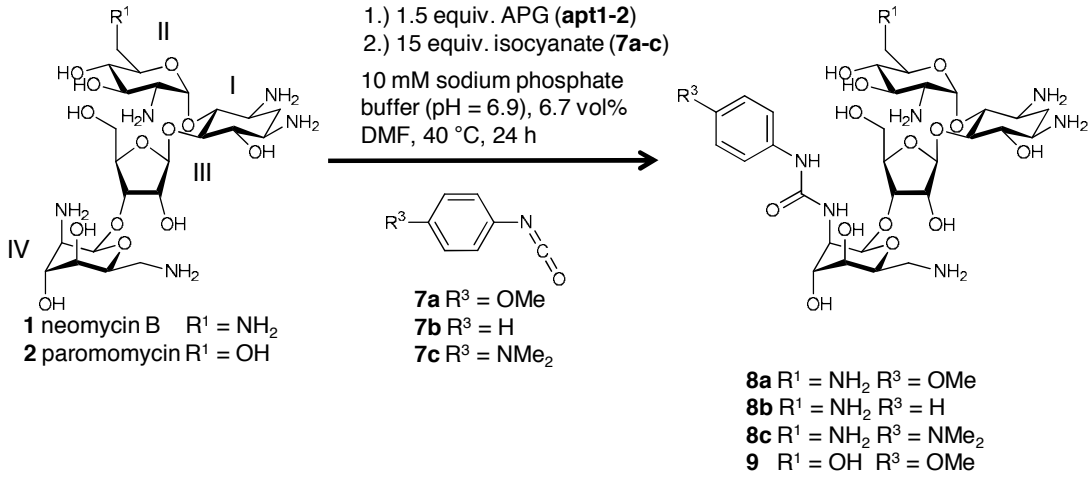


Figure S2 Regioselective transformation of amino group in C2 position at ring IV.

A 6.1 mM RNA aptamer solution (sequences: 5'-GGA CUG GGC GAG AAG UUU AGU CC-3' (**apt1**) or 5'-CUG CAG UCC GAA AAG GGC CAG-3' (**apt2**)) (816 μL, 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. Then a 4.8 mM solution of the aminoglycoside antibiotic (684 μL, 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. Afterwards 15 equiv. phenyl isocyanate **7a-c** (49.2 μmol) dissolved in DMF (108 μL) were added and the reaction mixture was allowed to react for 24 hours at 40 ℃. After addition of a 7 wt. % ethy lamine water solution (180 μL) and further incubation for 30 min at room temperature, a 2 M sodium hydroxide solution (240 μl) was added and the crude mixture was heated to 90 ℃ for 30 mi n. After cooling to room temperature each fraction (50 μL) 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℃ to afford the

antibiotic derivatives **8a-c** and **9**. After evaporation of acetone and freeze-drying the product was dissolved in D_2O (150 μL) for NMR-studies.

Analytical Data

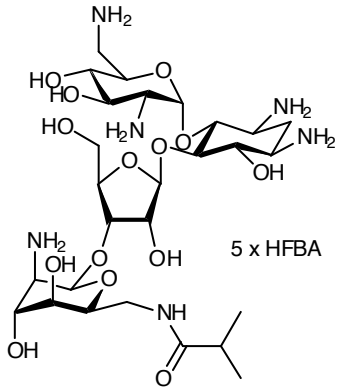
2,5-dioxopyrrolidin-1-yl isobutyrate 3b. The title compound was prepared according to the general procedure (1) described above. Product **3b** was obtained as a white solid reaching a yield of 72 % (2.33g, 12.6 mmol). ^{1}H-NMR ($CDCl_3$, 400MHz): δ[ppm] = 2.79 (q, J = 7.0 Hz, 1H, **C**H-$(CH_3)_2$); 2.715 (s, 4H, CH_2-CO); 1.22 (d, J = 7.6 Hz, 6H, CH(**C**$H_3)_2$). ^{13}C-NMR ($CDCl_3$, 400 MHz): δ[ppm] = 172.059 (1C, **C**O-CH); 169.378 (2C, **C**O-CH_2); 31.568 (1C, CH$(CH_3)_2$); 25.502 (1C, CO-**C**H_2); 18.631(1C, CH(**C**$H_3)_2$).

2,5-dioxopyrrolidin-1-yl pent-4-ynoate 3c. The title compound was prepared according to the general procedure (1) described above. Product **3c** was obtained as a white solid reaching a yield of 49 % (1.69, 8.7 mmol). ^{1}H-NMR ($CDCl_3$, 500MHz): δ[ppm] = 2.86 (t, J = 7.5 Hz, 2H, CH_2-C**H**$_2$-C≡CH); 2.82 (s, 4H, CH_2); 2.59 (t, J = 7.5 Hz, 2H, C**H**$_2$-CH_2-C≡CH); 2.04 (s, 1H, C≡C**H**). ^{13}C-NMR ($CDCl_3$, 400 MHz): δ[ppm] = 169.05 (2C, **C**O); 167.12 (1C, **C**O); 80.98 (1C, **C**≡CH); 70.13 (1C, C≡**C**H); 30.37 (1C, **C**H_2-CH_2-C≡CH); 25.66 (1C, CH_2), 14.16 (1C, CH_2-**C**H_2-C≡CH).

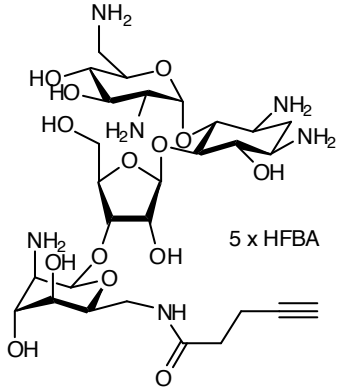
6´´´-*N*-acetyl neomycin B x 5 HFBA (4a). The title compound was prepared according to the general procedure (2.a) described above using NHS ester **3a**. Derivative **4a** was obtained as a white solid. For the measurement of regioselectivity and the characterization of the compound ^{1}H-NMR, HSQC as well as APT spectra were recorded and electrospray ionization (ESI)-MS was employed. The yield was determined by HPLC: R_t = 7.6 min, 76% conversion, 45% yield (apt1). TLC (Chloroform/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 (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´´, 5-H, 3-H´), 3.76 (dd, 1H, J = 12.5 Hz, J = 5.5 Hz, 5-H´´), 3.72- 3.68 (m, 2H, 4-H´´´, 6-H), 3.60 (dd, J = 14 Hz, J = 7.5 Hz, 1H, 6a-H´´´), 3.56 (m, 2H, 3-H, 2-H´´´), 3.53-3.41 (m, 4H, 6a-H´, 2-H´, 6b-H´´´, 4-H´), 3.38 (m, 1H, 1-H), 3.32 (dd, J = 14 Hz, J = 6 Hz, 1H, 6b-H´), 2.51 (dt, J = 12.5 Hz; J = 3.8 Hz, 1H, 2-H_e), 2.04 (s, 3H, CH_3), 1.89 (dd, J = 12.7 Hz, 1H, 2-H_a). APT (D_2O, 500 MHz) δ (p.p.m.) 174.49

(Carbonyl-C), 110.00 (C-1´´), 95.49 (C-1´), 95.51 (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-5´), 67.88 (C-3´), 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*-2-methylpropanoyl neomycin B x 5 HFBA (5). The title compound was prepared according to the general procedure (2.a) described above using NHS ester **3b**. Derivative **5** was obtained as a white solid. For the measurement of regioselectivity and the characterization of the compound ^{1}H-NMR and HSQC spectra were recorded and ESI-MS was employed. The yield was determined by HPLC: R_t = 9.65 min, 71% conversion, 31% yield (apt1). TLC (Chloroform/MeOH/17% NH_4OH 2:1:1 v/v/v) R_f = 0.50. ^{1}H-NMR (D_2O, 500 MHz) δ (p.p.m.) 5.98 (s, 1H, 1-H´), 5.38 (s, 1H, 1-H´´), 5.15 (s, 1H, 1-H´´´), 4.39 (d, J = 6.0 Hz, 1H, 3-H´´), 4.37 (m, 1H, 2-H´´), 4.21 (m, 1H, 3-H´´´), 4.17 (m, 1H, 4-H´´), 4.06-4.01 (m, 2H, 5-H´´´, 4-H), 3.96 (t, J = 10.3 Hz, 1H, 5-H´), 3.92 – 3.88 (m, 3H, 5-H´´, 5-H, 3-H´), 3.70 (dd, 1H, J = 14 Hz, J = 5.8 Hz, 5-H´´), 3.65- 3.61 (m, 2H, 4-H´´´, 6-H), 3.54-3.50 (m, 3H, 6a-H´´´, 3-H, 2-H´´´), 3.46 (m, 1H, 4-H´), 3.44-3.38 (m, 3H, 6a-H´, 2-H´, 6b-H´´´), 3.56-3.33 (m, 1H, 1-H), 3.28 (dd, J = 13.5 Hz, J = 6 Hz, 1H, 6b-H´), 2.47 (m, 2H, C**H**$(CH_3)_2$, 2-H_e), 1.86 (dd, J = 12.2 Hz, 1H, 2-H_a), 1.09 (s, J = 6.5 Hz, 6H, CH_3). ^{13}C-signals based on HSQC (D_2O, 500 MHz) δ (p.p.m.) 110.31 (C-1´´), 95.48 (C-1´), 95.18 (C-1´´´), 84.72 (C-5), 81.54 (C-4´´), 75.23 (C-4), 74.90 (C-3´´), 73.49 (C-2´´), 72.47 (C-5´´´), 72.39 (C-6), 70.42 (C-4´), 69.23 (C-5´), 67.87 (C-3´), 67.48 (C-3´´´), 66.08 (C-4´´´), 60.04 (C-5´´), 53.33 (C-2´), 50.87 (C-2´´´), 49.59 (C-1), 48.22 (C-3), 39.94 (C-6´), 39.03 (C-6´´´), 34.93 (**C**H$(CH_3)_2$), 27.95 (CH(**C**$H_3)_2$), 27.89 (C-2). MS (EI+) (*m/z*): found 685.36163 $[M+H]^+$, 707.34369 $[M+Na]^+$; calculated 685.36143 $[M+H]^+$, 707.34337 $[M+Na]^+$.

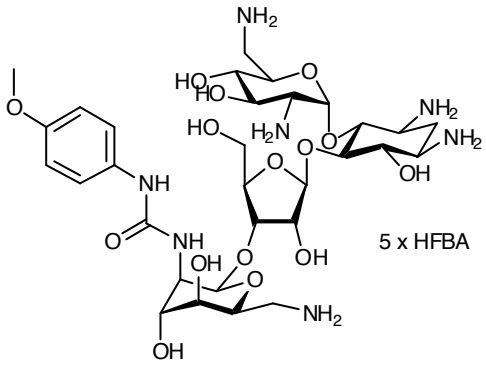


6´´´-*N*-4-pentinoyl neomycin B x 5 HFBA (6). The title compound was prepared according to the general procedure (2.a) described above using NHS ester **3c**. Derivative **6** was obtained as a white solid. For the measurement of regioselectivity and the characterization of the compound ^{1}H-NMR and HSQC spectra were recorded and ESI-MS was employed. The yield was determined by HPLC: R_t = 9.8 min, 83% conversion, 45% yield (apt1). TLC (Chloroform/MeOH/17% NH_4OH 2:1:1 v/v/v) R_f = 0.60. ^{1}H-NMR (D_2O, 500 MHz) δ (p.p.m.) 6.06 (d, J = 4 Hz, 1H, 1-H´), 5.43 (s, 1H, 1-H´´), 5.19 (s, 1H, 1-H´´´), 4.47 (d, J = 5.75 Hz, 1H, 3-H´´), 4.41 (m, 1H, 2-H´´), 4.26 (m, 1H, 3-H´´´), 4.22 (m, 1H, 4-H´´), 4.11 - 4.06 (m, 2H, 5-H´´´, 4-H),

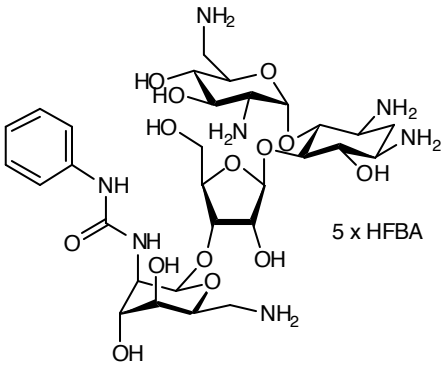


4.01 (t, J = 10 Hz, 1H, 5-H′), 3.97 – 3.94 (m, 3H, 5-H′′, 5-H, 3-H′), 3.77 (dd, J = 13 Hz, J = 5 Hz, 1H, 5-H′′), 3.75- 3.68 (m, 2H, 4-H′′′, 6-H), 3.61 (dd, J = 14 Hz, J = 7.5 Hz, 1H, 6a-H′′′), 3.56-3.53 (m, 2H, 3-H, 2-H′′′), 3.52 – 3.45 (m, 4H, 4-H′, 6a-H′, 2-H′, 6b-H′′′), 3.36 (m, 1H, 1-H), 3.33 (dd, J = 14 Hz, J = 6 Hz, 1H, 6b-H′), 2.56 – 2.43 (m, 6H, $(CH_2)_2$, 2-H_e, C≡C-H), 1.90 (dd, J = 12.3 Hz, 1H, 2-H_a). ^{13}C-signals based on HSQC (D_2O, 500 MHz) δ (p.p.m.) 110.11 (C-1′′), 95.54 (C-1′), 95.33 (C-1′′′), 84.71 (C-5), 81.56 (C-4′′), 75.17 (C-3′′), 75.12 (C-4), 73.52 (C-2′′), 72.72 (C-5′′′), 72.35 (C-6), 70.50 (C-4′), 83.47 (**C**≡CH), 70.35 (C≡**C**H), 69.24 (C-5′), 67.92 (C-3′), 67.51 (C-3′′′), 66.05 (C-4′′′), 60.05 (C-5′′), 53.33 (C-2′), 50.88 (C-2′′′), 49.52 (C-1), 48.20 (C-3), 39.90 (C-6′), 39.34 (C-6′′′), 34.23 (CO-**C**H_2-CH_2), 27.80 (C-2), 14.54 (CO-CH_2-**C**H_2). MS (EI+) (*m/z*): found 695.34564 $[M+H]^+$, 717.32770 $[M+Na]^+$; calculated 695.34578 $[M+H]^+$, 717.32772 $[M+Na]^+$.

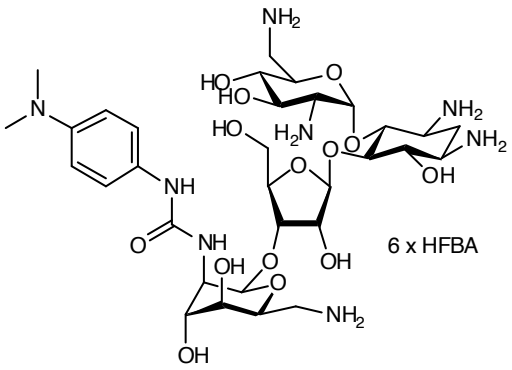
2′′′-*N*-[(4-methoxyphenyl)amino]carbonyl neomycin B x 5 HFBA (8a). The title compound was prepared according to the general procedure (2.b) described above using isocyanate **7a**. Derivative **8a** was obtained as a white solid. For the measurement of regioselectivity and the characterization of the compound ^{1}H-NMR, HSQC as well as APT spectra were recorded and ESI-MS was employed. The yield was determined by HPLC: R_t = 16.4 min, 44% conversion, 37% yield (apt1). TLC (Chloroform/MeOH/17% NH_4OH 2:1:1 v/v/v) R_f = 0.45. ^{1}H-NMR (D_2O, 500 MHz) δ (p.p.m.) 7.23 (d, J = 9 Hz, 2H, Ar-H3, Ar-H5), 7.00 (d, J = 9 Hz, 2H, Ar-H2, Ar-H6), 6.05 (d, J = 3.5 Hz, 1H, 1-H′), 5.42 (d, J = 1.5 Hz, 1H, 1-H′′), 5.11 (s, 1H, 1-H′′′), 4.43 (t, J = 5.25 Hz, 1H, 3-H′′), 4.33 (m, 1H, 2-H′′), 4.26-4.19 (m, 2H, 5-H′′′, 4-H′′), 4.12-4.07 (m, 2H, 4-H, 2-H′′′), 4.04 (m, 1H, 3-H′′′), 4.00 (t, J = 14.5 Hz, 1H, 5-H′), 3.95 – 3.88 (m, 3H, 5a-H′′, 5-H, 3-H′), 3.83 (s, 3H, OCH_3), 3.76-3.67 (m, 3H, 5b-H′′, 6-H, 4-H′′′), 3.55 (dt, J = 3 Hz, J = 9.5 Hz, 1H, 3-H), 3.61-3.41 (m, 3H, 2-H′, 4-H′, 6a-H′), 3.38-3.28 (m, 4H, 1-H, 6a-H′′′, 6b-H′′′, 6b-H′), 2.50 (dt, J = 11.5 Hz; J = 3 Hz, 1H, 2-H_e), 1.90 (dd, J = 12.7 Hz, 1H, 2-H_a). APT (D_2O, 500 MHz) δ (p.p.m.) 158.47 (Carbonyl-C), 156.10 (Ar-C4), 130.32 (Ar-C1), 125.11 (2C, Ar-C2,C6), 114.93 (2C, Ar-C3,C5), 110.12 (C-1′′), 98.72 (C-1′′′), 95.64 (C-1′), 84.75 (C-5), 82.05 (C-4′′), 75.82 (C-3′′), 74.95 (C-4), 74.31 (C-2′′), 72.79 (C-6), 70.61 (C-4′), 70.18 (C-5′′′), 67.78 (C-3′′′), 69.41 (C-3′), 68.07 (C-4′′′), 68.09 (C-5′), 60.00 (C-5′′), 55.58 (CH_3), 53.36 (C-2′), 51.13 (C-2′′′), 49.75 (C-1), 48.42 (C-3), 40.61 (C-6′′′), 39.95 (C-6′), 27.93 (C-2). MS (EI+) (*m/z*): found 764.36742 $[M+H]^+$, 786.34508 $[M+Na]^+$; calculated 764.36724 $[M+H]^+$, 786.34918 $[M+Na]^+$.



2´´´-*N*-(phenylamino)carbonyl neomycin B x 5 HFBA (8b). The title compound was prepared according to the general procedure (2.b) described above using isocyanate **7b**. Derivative **8b** was obtained as a white solid. For the measurement of regioselectivity and the characterization of the compound ^{1}H-NMR and HSQC spectra were recorded and ESI-MS was employed. The yield was determined by HPLC: R_t = 18.2 min, 52% conversion, 51% yield (apt1). TLC (Chloroform/MeOH/17% NH_4OH 2:1:1 v/v/v) R_f = 0.48. ^{1}H-NMR (D_2O, 500 MHz) ^{1}H-NMR (D_2O, 500 MHz) ^{1}H-NMR (D_2O, 500 MHz) δ (p.p.m.) 7.38 (t, J = 7.75 Hz, 2H, Ar-H3, Ar-H5), 7.30 (d, J = 8.0 Hz, 2H, Ar-H2, Ar-H6), 7.18 (t, J = 7.25 Hz, 1H, Ar-H4), 6.03 (d, J = 3.5 Hz, 1H, 1-H´), 5.40 (s, 1H, 1-H´´), 5.16 (s, 1H, 1-H´´´), 4.43 (t, J = 5.25 Hz, 1H, 3-H´´), 4.34 (m, 1H, 2-H´´), 4.24 (t, J = 4.5 Hz, 1H, 5-H´´´), 4.18 (m, 1H, 4-H´´), 4.11-4.03 (m, 3H, 4-H, 2-H´´´, 3-H´´´), 3.99 (t, J = 10.00 Hz, 1H, 5-H´), 3.95 – 3.85 (m, 3H, 5a-H´´, 5-H, 3-H´), 3.72-3.67 (m, 3H, 5b-H´´, 6-H, 4-H´´´), 3.54 (dt, J = 10Hz, J = 3 Hz, 1H, 3-H), 3.49-3.39 (m, 3H, 2-H´, 4-H´, 6a-H´), 3.36-3.31 (m, 3H, 1-H, 6a-H´´´, 6b-H´´´), 3.27 (dd, J = 14 Hz, J = 7 Hz, 1H, 6b-H´), 2.49 (dt, J = 12 Hz; J = 4 Hz, 1H, 2-H_e), 1.90 (dd, J = 12.5 Hz, 1H, 2-H_a). ^{13}C-signals based on HSQC spectrum (D_2O, 500 MHz) δ (p.p.m.) 129.50 (2C, Ar-C3,C5), 124.65 (Ar-C4), 121.87 (2C, Ar-C2,C6), 110.12 (C-1´´), 98.58 (C-1´´´), 95.343 (C-1´), 84.90 (C-5), 81.76 (C-4´´), 75.55 (C-3´´), 74.34 (C-4), 74.07 (C-2´´), 72.39 (C-6), 70.78 (C-4´), 70.42 (C-5´´´), 69.97 (C-3´´´), 69.47 (C-3´), 68.17 (C-4´´´), 68.10 (C-5´), 60.23 (C-5´´), 53.53 (C-2´), 50.92 (C-2´´´), 49.79 (C-1), 48.48 (C-3), 40.66 (C-6´´´), 40.18 (C-6´), 27.90 (C-2). MS (EI+) (*m/z*): found 734.35551 $[M+H]^+$, 756.33745 $[M+Na]^+$; calculated 734.35668 $[M+H]^+$, 756.33862 $[M+Na]^+$.

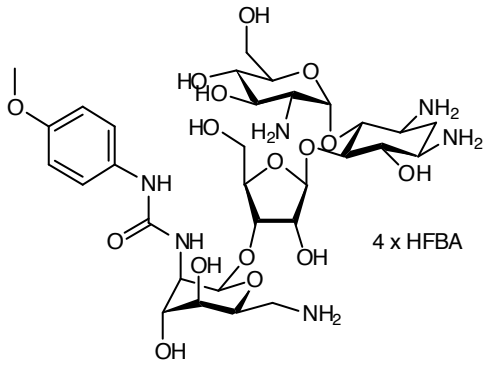


2´´´-*N*-[4(*N,N*-di-methylamino)phenyl]carbonyl neomycin B x 6 HFBA (8c). The title compound was prepared according to the general procedure (2.b) described above using isocyanate **7c**. Derivative **8c** was obtained as a white solid. For the measurement of regioselectivity and the characterization of the compound ^{1}H-NMR and HSQC spectra were recorded and ESI-MS was employed. The yield was determined by HPLC: R_t = 16.9 min, 55% conversion, 48% yield (apt1); 55% conv., 48% yield. TLC (Chloroform/MeOH/17% NH_4OH 2:1:1 v/v/v) R_f = 0.53. ^{1}H-NMR (D_2O, 500 MHz) δ (p.p.m.) 7.50 (m, 4H, Ar-H2, Ar-H3, Ar-H5, Ar-H6), 6.01 (d, J = 3.5 Hz, 1H, 1-H´), 5.39 (d, J = 2 Hz, 1H, 1-H´´), 5.12 (d, J = 1.5 Hz, 1H, 1-H´´´), 4.44 (t, J = 5.0 Hz, 1H, 3-H´´), 4.36 (m, 1H, 2-H´´), 4.25 (t, J = 4.25 Hz, 1H, 5-H´´´), 4.14 (m, 1H, 4-H´´), 4.11-4.08 (m, 3H, 4-H, 2-H´´´, 3-H´´´), 3.99 (m,



1H, 5-H´), 3.92 – 3.87 (m, 3H, 5a-H´´, 5-H, 3-H´), 3.77 (s,br, 1H, 4-H´´´), 3.73-3.66 (m, 2H, 5b-H´´, 6-H), 3.53 (td, J = 11.5 Hz, J = 3.5 Hz, 3-H´), 3.48-3.42 (m, 3H, 2-H´, 6a-H´, 4-H´), 3.41-3.31 (m, 3H, 6a-H´´´, 6b-H´´´, 1-H) , 3.27 (dd, J = 13.5 Hz, J = 6.5 Hz, 1H, 6b-H´), 3.24 (s, 6H, $N(CH_3)_2$), 2.48 (dt, J = 12.5 Hz; J = 2.5 Hz, 1H, 2-H_e), 1.90 (dd, J = 12.5 Hz, 1H, 2-H_a). ^{13}C-signals based on HSQC spectrum (D_2O, 500 MHz) δ (p.p.m.) 121.08, 121.19 (4C, Ar-C2,Ar-C3, Ar-C5,Ar-C6), 110.12 (C-1´´), 98.25 (C-1´´´), 95.41 (C-1´), 84.70 (C-5), 81.25 (C-4´´), 75.37 (C-3´´), 75.06 (C-4), 73.96 (C-2´´), 72.26 (C-6), 70.43 (C-4´), 70.18 (C-5´´´), 69.70 (C-3´´´), 69.24 (C-3´), 68.02 (C-4´´´^{I}), 67.83 (C-5´), 59.90 (C-5´´), 53.29 (C-2´), 50.58 (C-2´´´), 49.58 (C-1), 48.21 (C-3), 46.16 (2C, $N(CH_3)_2$), 40.41 (C-6´´´^{I}), 39.93 (C-6´), 27.72 (C-2). MS (EI+) (*m/z*): found 777.39821 $[M+H]^+$, 799.37998 $[M+Na]^+$; calculated 777.39887 $[M+H]^+$, 799.38082 $[M+Na]^+$.

2´´´-*N*-[(4-methoxyphenyl)amino]carbonyl paromomycin x 4 HFBA (9). The title compound



was prepared according to the general procedure (2.b) described above using isocyanate **7a**. Derivative **9** was obtained as a white solid. For the measurement of regioselectivity and the characterization of the compound ^{1}H-NMR and HSQC spectra were recorded and ESI-MS was employed. The yield was determined by HPLC: R_t = 8.1 min, 54% conversion, 49% yield (apt1). TLC (Chloroform/MeOH/17% NH_4OH 2:1:1 v/v/v) R_f = 0.65. ^{1}H-NMR (D_2O, 600 MHz) δ (p.p.m.) 7.24 (d, J = 9 Hz, 2H, Ar-H2), 7.01 (d, J = 9 Hz, 2H, Ar-H3), 5.81 (d, J = 3.5 Hz, 1H, 1-H´), 5.37 (d, J = 1.5 Hz, 1H, 1-H´´), 5.12 (s, 1H, 1-H´´´), 4.46 (t, J = 5.5 Hz, 1H, 3-H´´), 4.33 (m, 1H, 2-H´´), 4.23 (t, J = 5.5 Hz 1H, 5-H´´´), 4.18 (m, 1H, 4-H´´), 4.09-4.01 (m, 3H, 4-H, 2-H´´´, 3-H´´´), 3.93-3.85 (m, 4H, 5a-H´´, 6a-H´, 3-H´, 5-H), 3.83 (s, 3H, OCH_3), 3.80-3.68 (m, 4H, 5-H´, 5b-H´´, 6b-H´, 4-H´´´), 3.71 (t, J = 9.5 Hz, 1H, 6-H), 3.56 (t, J = 9.75 Hz, 1H, 3-H), 3.51 (t, J = 7.75 Hz, 1H, 4-H´), 3.44 (dd, J = 11.0 Hz, J = 4.0 Hz, m, 1H, 2-H´), 3.38-3.30 (m, 3H, 6a-H´´´, 6b-H´´´, 1-H), 2.50 (dt, J = 13.0 Hz; J = 4.0 Hz, 1H, 2-H_e), 1.86 (dd, J = 12.0 Hz, 1H, 2-H_a). ^{13}C-signals based on HSQC (D_2O, 500 MHz) δ (p.p.m.) 125.25 (Ar-C2, C6), 114.88 (Ar-C3, C5), 110.12 (C-1´´), 98.74 (C-1´´´), 96.24 (C-1´), 84.48 (C-5), 81.85 (C-4´´), 77.44 (C-4), 75.70 (C-3´´), 74.15 (C-2´´), 74.10 (C-5´), 72.43 (C-6), 70.46 (C-5´´´), 69.99(C-3´´´), 69.40 (C-4´), 69.01(C-3´), 68.26 (C-4´´´), 60.47, 60.33 (2C, C-5´´, C-6´), 55.79 (CH_3), 53.92 (C-2´), 51.13 (C-2´´´), 49.83 (C-1), 49.05 (C-3), 40.78 (C-6´´´), 28.20 (C-2). MS (EI+) (*m/z*): found 765.35071 $[M+H]^+$, 787.33215 $[M+Na]^+$; calculated 765.35126 $[M+H]^+$, 787.33320 $[M+Na]^+$.

NMR-spectra of neomycin B and antibiotic derivatives 8a and 9

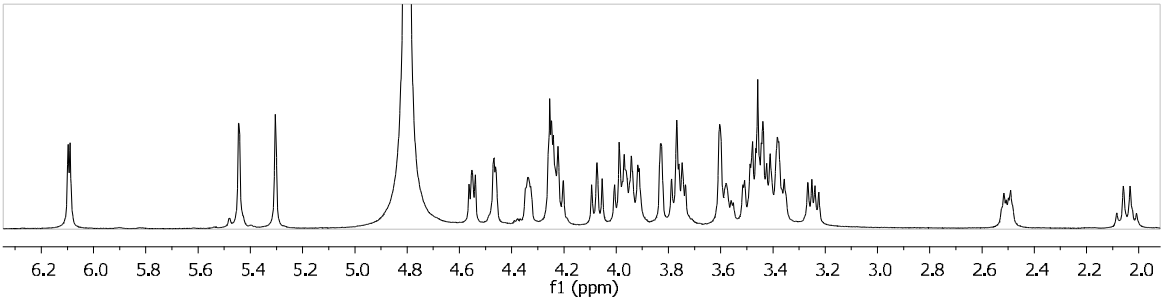


Figure S3a ^{1}H-NMR spectrum (500 MHz, D_2O) of neomycin B x 6 HFBA **1**.

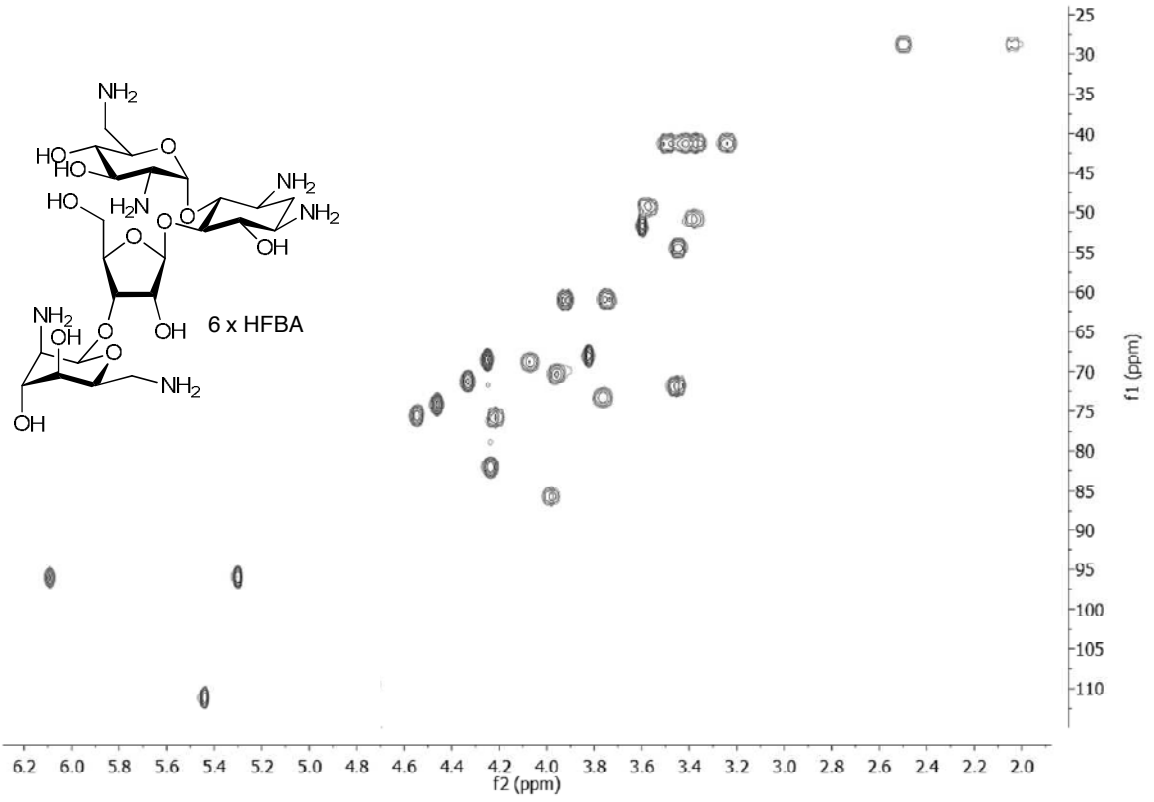


Figure S3b HSQC spectrum (500 MHz, D_2O) of neomycin B x 6 HFBA **1**.

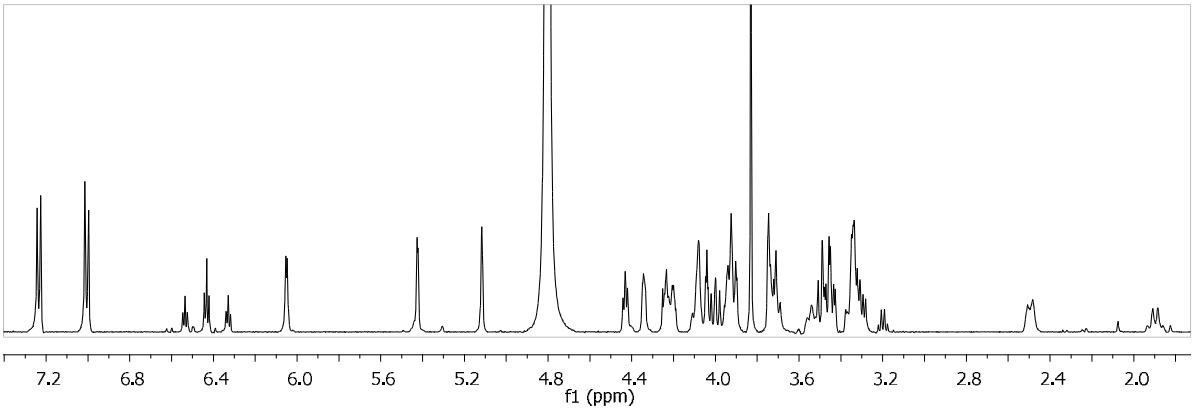


Figure S4 ^{1}H-NMR (500 MHz, D_2O) spectrum of 2´´´-*N*-[(4-methoxyphenyl)amino]carbonyl neomycin B x 5 HFBA **8a**.

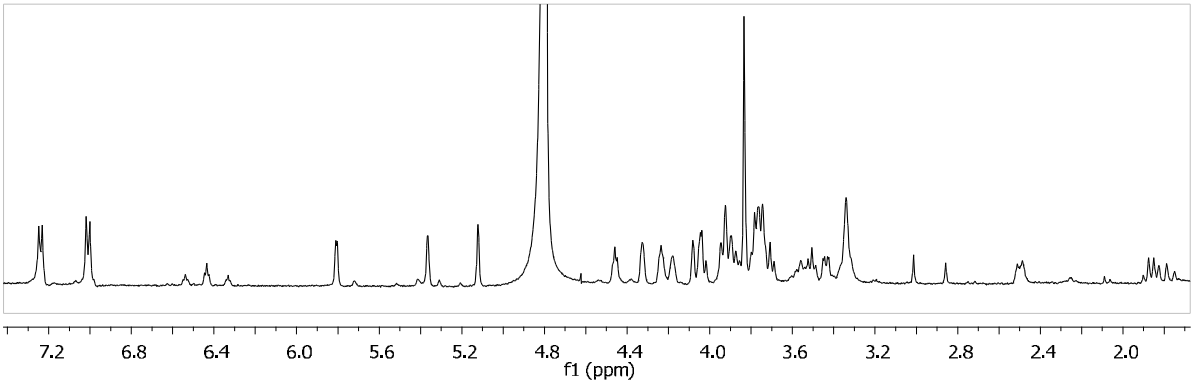


Figure S5a ^{1}H-NMR (600 MHz, D_2O) spectrum of 2´´´-*N* -[(4-methoxyphenyl)amino]carbonyl paromomycin x 4 HFBA **9**.

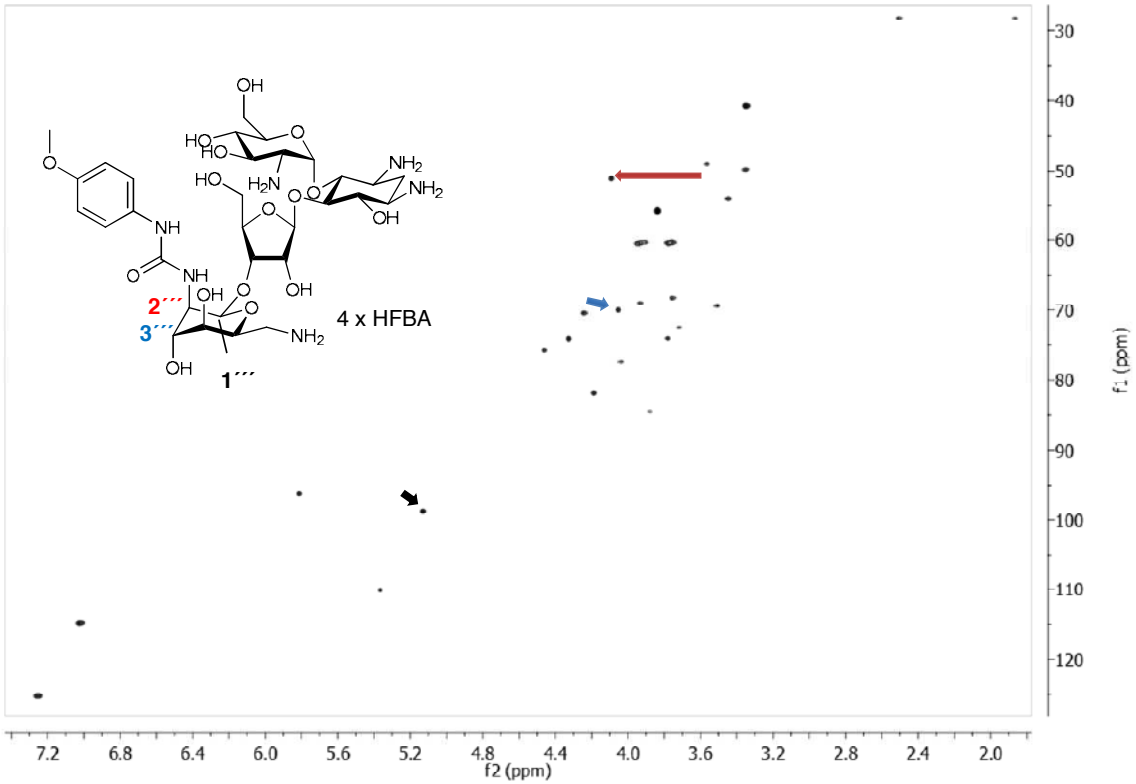


Figure S5b HSQC (600 MHz, D_2O) spectrum of 2´´´-*N* -[(4-methoxyphenyl)amino]carbonyl paromomycin x 4 HFBA **9**. Arrows indicate shift of specific signals due to the regioselective transformation of the amino group in C2 position of ring IV: *J*(C2´´´-H) coupling (red), *J*(C3´´´-H) coupling (blue), *J*(C1´´´-H) coupling (black).

Comparison of HSQC spectra and APT of monoacetylated neomycin B obtained in absence and presence of APG apt1.

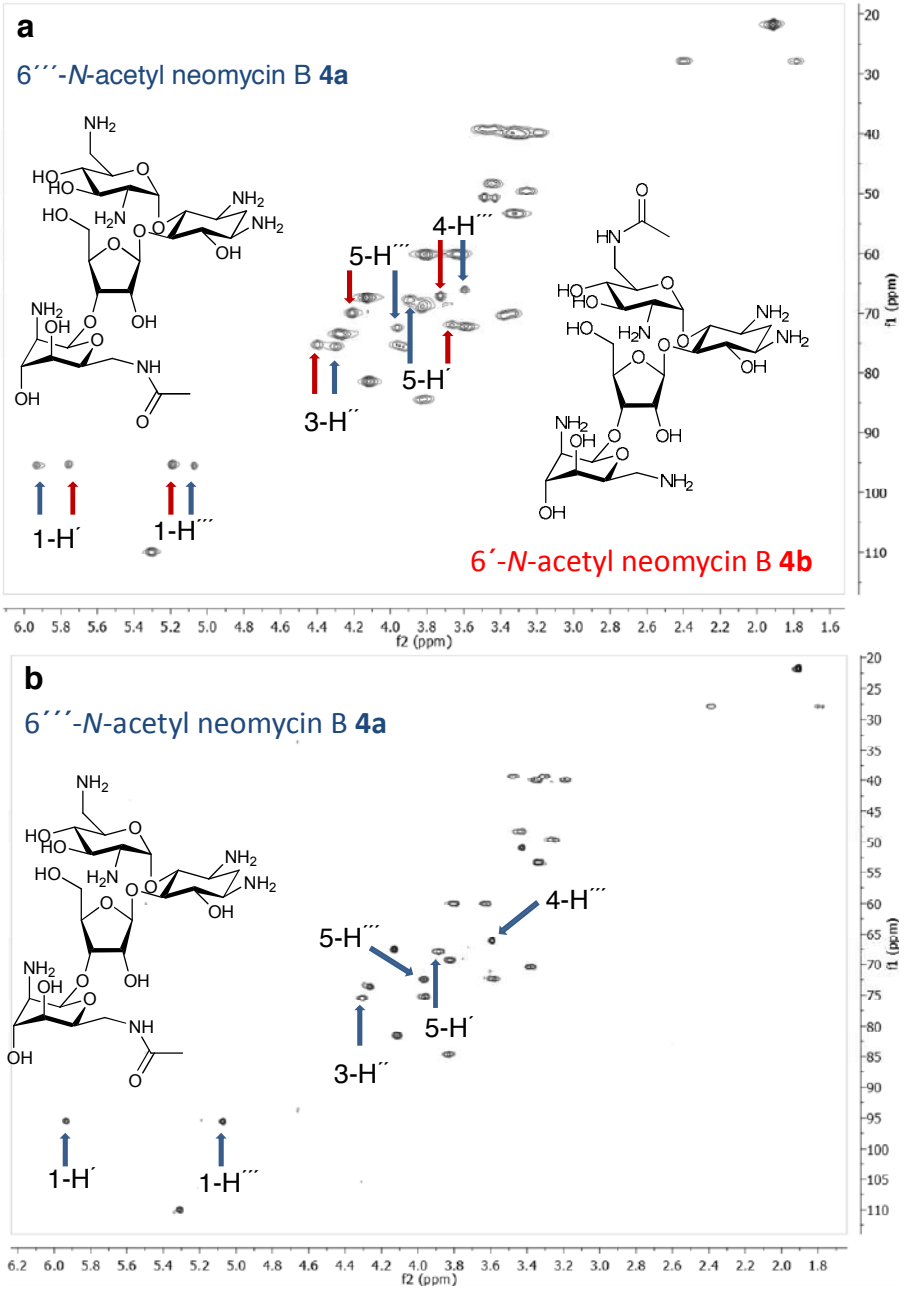


Figure S6 HSQC-NMR spectra (500 MHz, D_2O) of the isomeric mixture of monoacetylated neomycin B obtained without APG (a) and of 6´´´-*N*-acetyl neomycin B x 5 HFBA **4a** produced in the presence of APG **apt1** (b). Duplication of signals proves the formation of mainly two monoacetylated neomycin B isomers, i.e. the 6´´´-*N*-acetyl neomycin B **4a** and 6´-*N*-acetyl neomycin B **4b**, when no APG is employed. In contrast, utilization of **apt1** as APG results in only one detectable regioisomer **4a**.

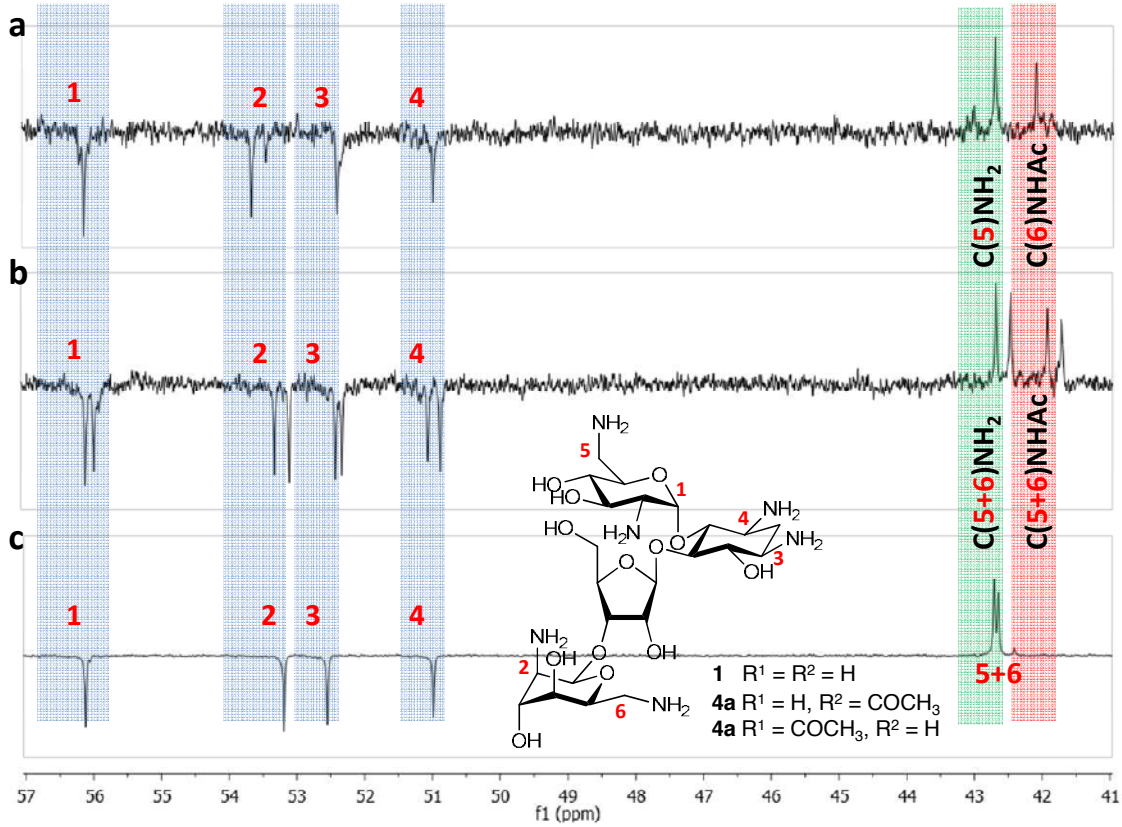


Figure S7 Attached Proton Tests (APT) of monoacetylated neomycin B after transformation in presence of RNA (a) and in absence of APG **apt1** (b) using NHS ester **3a**. APT of neomycin B **1** (c). Figure 7a proves the regioselective transformation of the amine group in C6 position of ring IV of neomycin B **1** to **4a**, while the spectrum b) shows the presence of two monoacetylated neomycin B derivatives, 6´´´-*N*-acetyl neomycin B **4a** and 6´-*N*-acetyl neomycin B **4b**.

HPLC- and HRMS- data

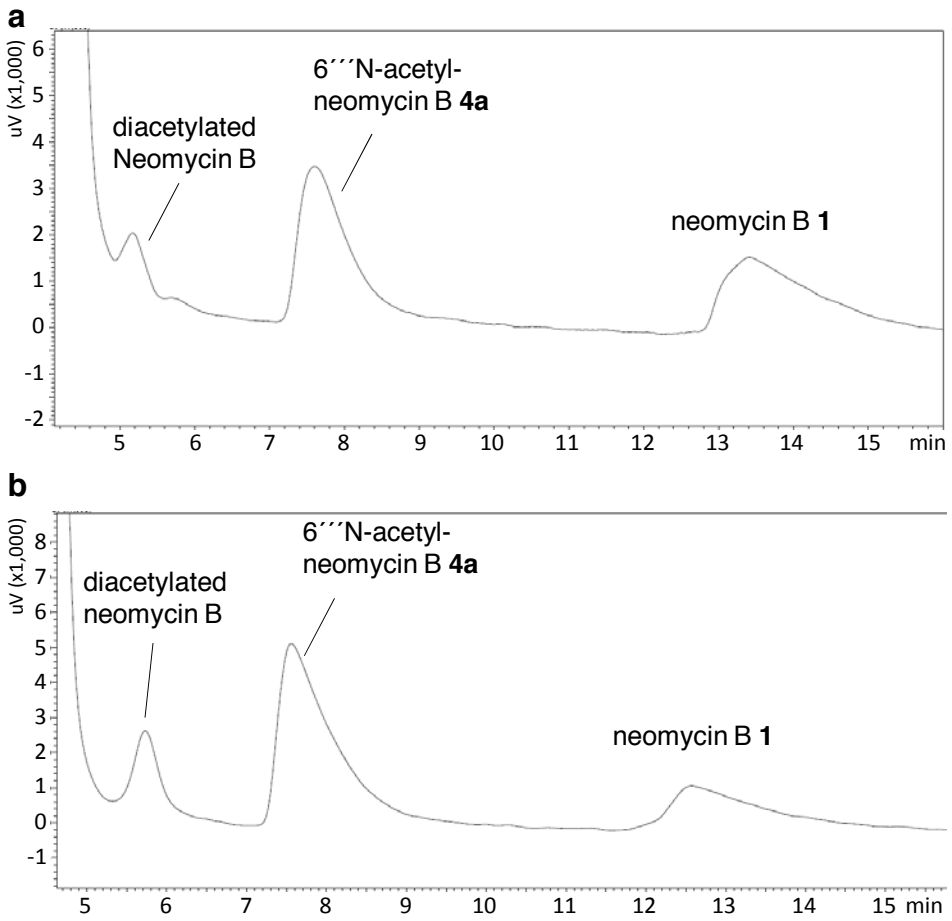


Figure S8 HPLC-Chromatogram of reaction mixture after transformation of neomycin B **1** using 30 equiv. of acetic acid NHS ester **3a** during protection with APGs **apt1** (a) and **apt2** (b).

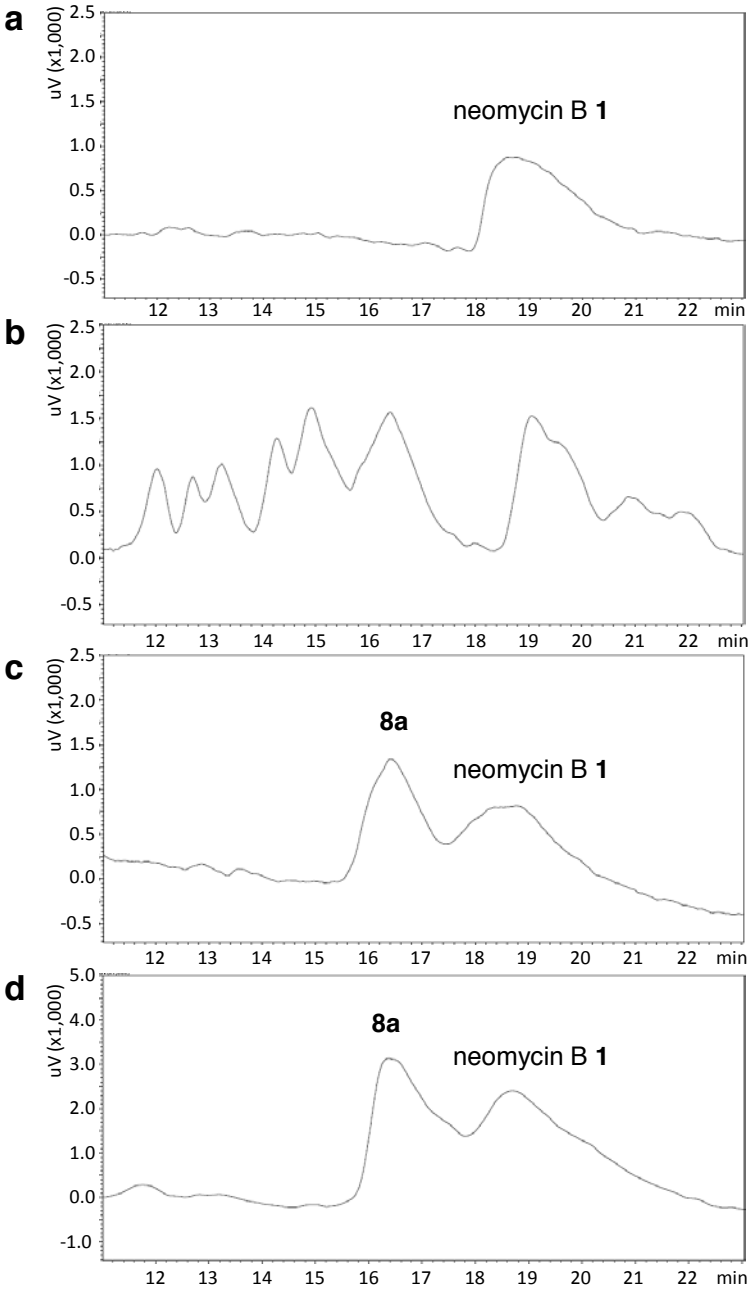


Figure S9 HPLC-Chromatogram of neomycin B (a) and its transformation applying 15 equiv. of 4-methoxyphenylisocyanate **7a** without APG (b) and in presence of APGs **apt1** (c) and **apt2** (d).

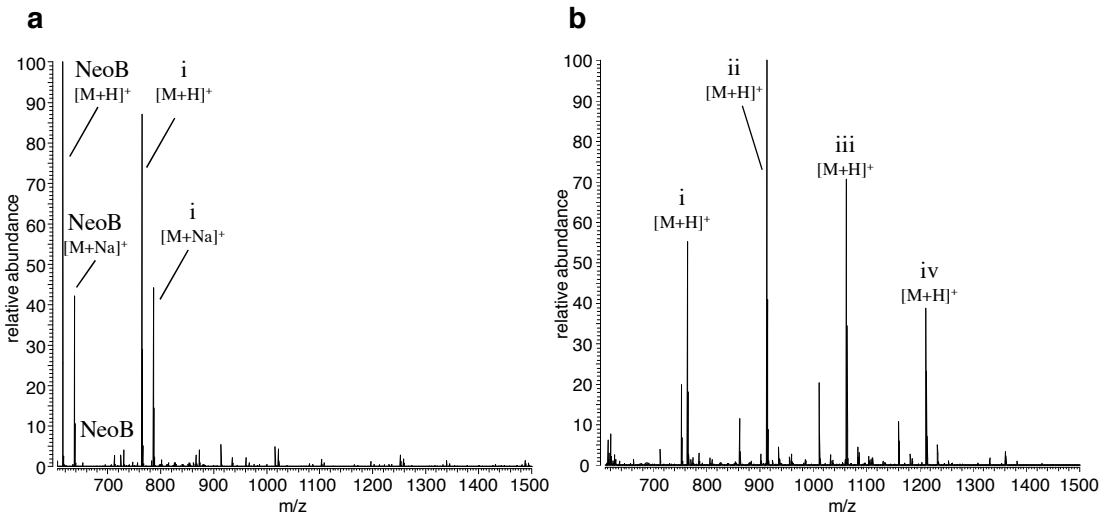


Figure S10 Electrospray Ionisation (ESI) mass spectra of reaction mixture of neomycin B **1** with 15 equiv. of isocyanate **7a** in the presence (a) and absence (b) of APG **apt1**. NeoB = neomycin B **1**, i-iv = number of reacted amino groups within neomycin B.

Conventional synthesis of neomycin B[3,4] modified at ring IV.

Figure S11 Synthesis of building block I

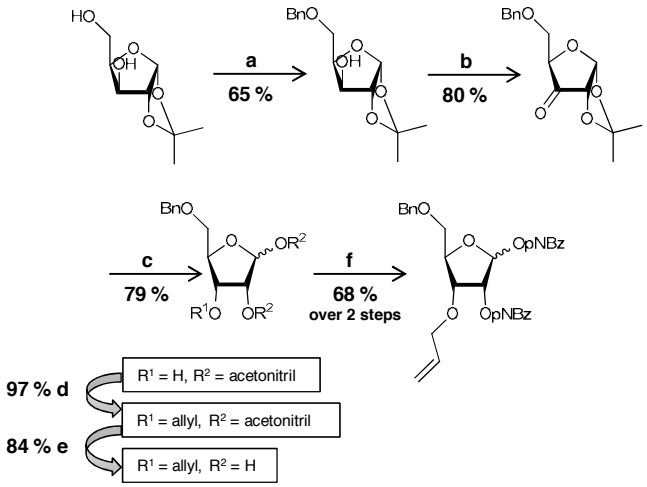


Figure S12 Synthesis of building block II

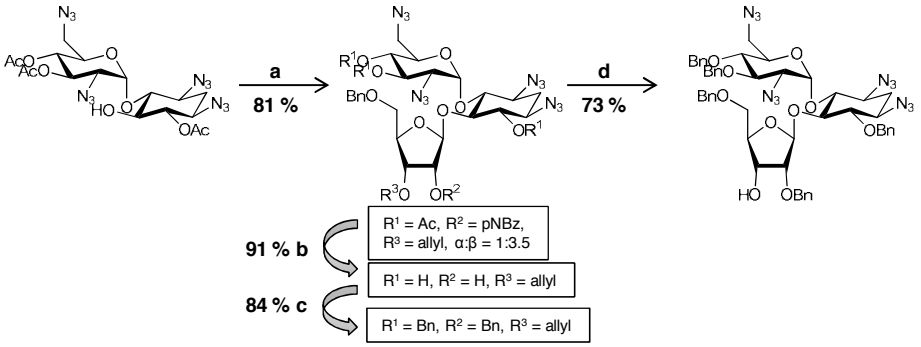


Figure S13 Connection of building block I and II

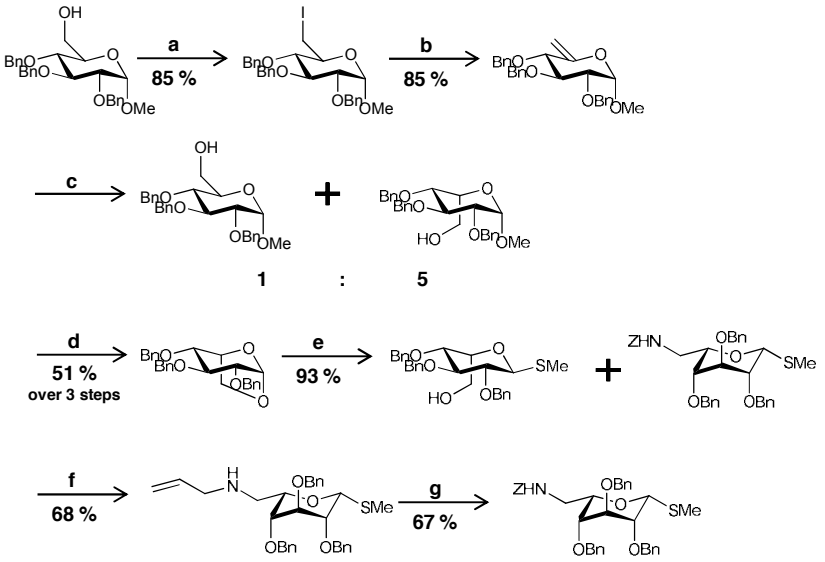


Figure S14 Synthesis of building block III

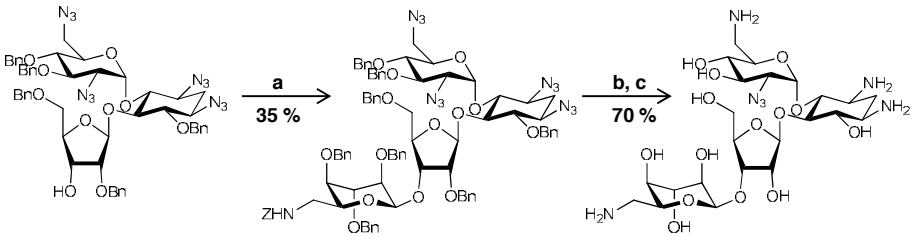


Figure S15 Connection of building blocks I+II and III

Antimicrobial tests

Materials

Following materials were purchased to carry out antimicrobial tests (company, cat. no.):

Mueller Hinton II Broth powder (BD - cat. no. 212322)
Agar (Roth - cat. no. 5210.2)
6 mm paper disks (BBL - cat. no. 231039)

Kirby-Bauer Test[5,6,7]

Antibiotic disk preparation. Paper disks (6 mm diameter, BBL Microbiology Systems) were wetted through with 20, 25, 30 and 35 µL of a solution containing the antibiotic sample at a concentration of 0.5 nmol/µL. The wet disks were dried in a desiccator overnight, and used the next day.

Culture preparation. A colony picked from a freshly made plate of the bacteria strain *E.coli* ATCC 25922 was used to inoculate 20 mL of Mueller-Hinton broth and the obtained culture was grown overnight at 37 ℃ and 250 RPM.

Kirby-Bauer test procedure. A refreshed culture was obtained by adding 1 mL of overnight culture to 99 mL of fresh Mueller-Hinton broth. The culture was grown at 37 ℃ and 250 RPM until it reached an OD_{600} of 0.132 (0.5 McFarland) and a series of Mueller-Hinton-Agar plates preheated at 37 ℃ were inoculated spreading 200 µL of that culture with sterile cotton. The plates were then dried for 30 minutes. Then on each plate 3 or 4 antibiotic paper disks were placed. The plates were incubated overnight at 37 °C and subsequently the diameter of the inhibition growth zone was measured.

MIC Test[5,6,7]

A 96-well, round-bottom plate was used to setup a culture made by 100 µL of *E.coli* ATCC 25922 culture in Mueller-Hinton with an OD_{600} of 0.132 (0.5 McFarland) and an established amount of antibiotic per well.
The 96-well plate was incubated overnight at 37 ℃ and 350 RPM. The OD_{600} of all wells were measured using an *E.coli* ATCC 25922 culture with an OD_{600} of 0.132 as reference and the MIC value was determined by taking the lowest concentration where no bacterial growth was observed.

References

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