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Multicomponent Domino Synthesis and Antibacterial Activity of Neomycin–Sugar Conjugates

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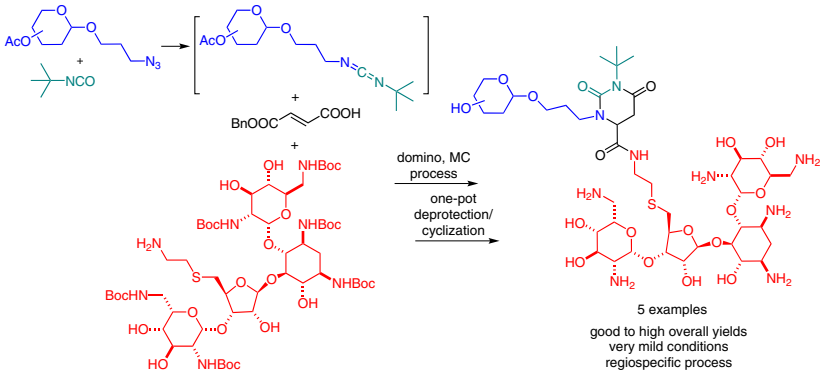
This manuscript is dedicated to the memory of Vincenzo Volonterio



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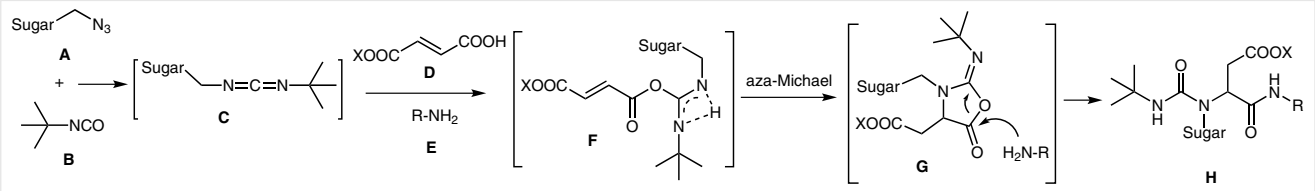
Abstract An efficient multicomponent domino process that works under mild conditions was used for the synthesis of systematically modified neomycin–sugar conjugates. The final aminoglycoside derivatives were tested against methicillin-resistant *Staphylococcus aureus*, *Klebsiella*, and *E. coli* strains, and show activity comparable to the parent antibiotics. Such a strategy can impact multicomponent combinatorial syntheses of diverse biologically active conjugates.

Key words multicomponent reaction, domino reaction, aminoglycosides, carbodiimides, antibiotics

The discovery of streptomycin led off the aminoglycoside antibiotic era with the subsequent introduction of several derivatives (e.g., neomycin, kanamycin) capable of curing previously untreatable life-threatening infections.[1–5] However, aminoglycoside side effects and the emergence of bacterial resistance have slowed down their use, raising interest in finding new derivatives that overcome their reduced efficacy and undesirable effects.[6,7] In this context, the aminoglycoside scaffold remains an excellent starting point for new antibiotics. Indeed, the design, synthesis, and antibacterial activity of modified aminoglycosides or aminoglycoside conjugates continue to be reported and investigated.[8–15] However, the synthetic procedures exploited for the synthesis of aminoglycoside derivatives require multistep pathways, which are not ideal for the production of diverse libraries.

To aid in this process, domino multicomponent reactions (MCRs),[16–21] providing access to molecular diversity by conducting successive reactions in a reaction vessel starting from three or more reactants, can markedly increase the probability of finding novel active and selective aminoglycoside antibiotics. Likely because of their multifunctional nature, the MC synthesis of aminoglycoside derivatives has received little attention and, to the best of our knowledge, only one example has been reported dealing with the functionalization of a fully protected neamine core, by the well-known Ugi MC reaction.[22] Herein, we report the application of an innovative MC process recently developed in our laboratories[23–25] for the synthesis of a collection of systematically modified neomycin–sugar conjugates. The new derivatives have been tested against pathogenic and drug-resistant Gram-positive and Gram-negative bacterial strains. Due to the flexibility and the mild conditions required for the process, we anticipate that such a strategy could be used in the future for the synthesis of libraries of new, differently substituted aminoglycoside conjugates and derivatives.

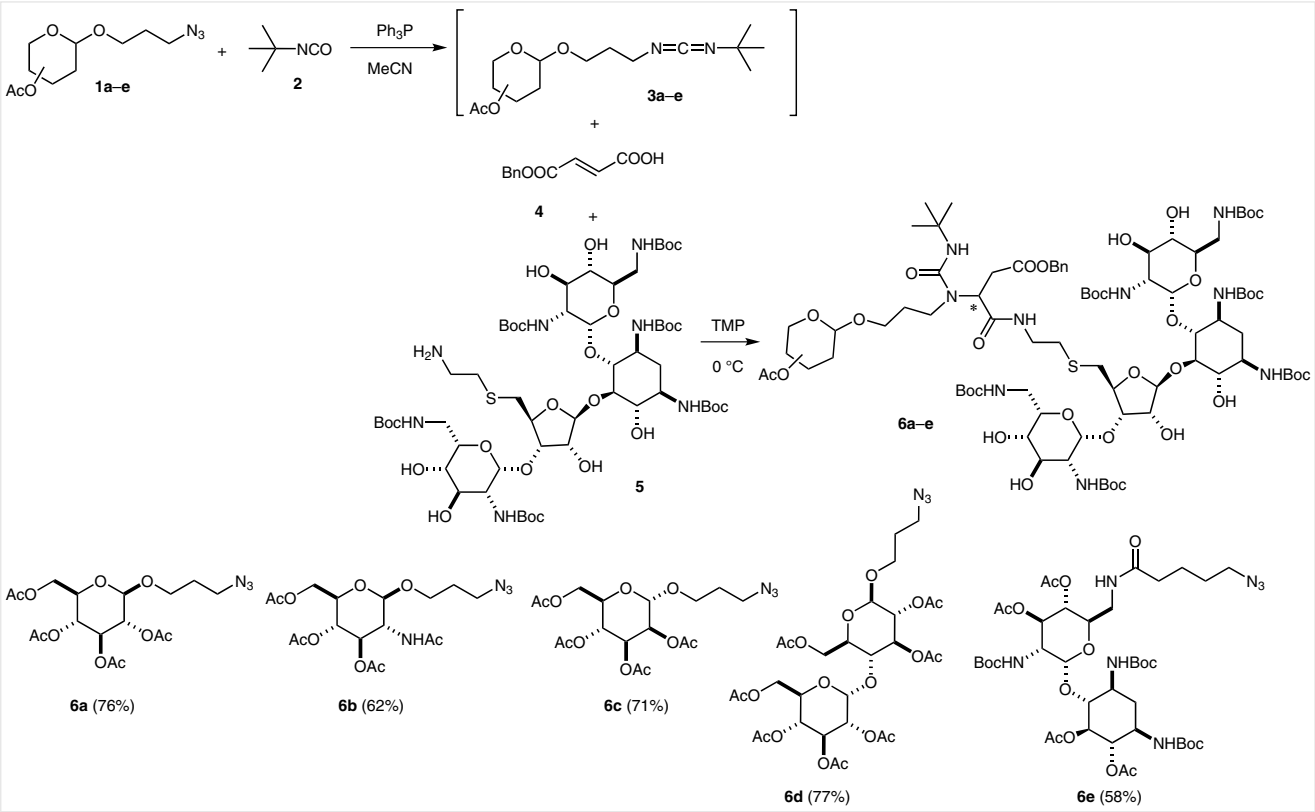
Recently, we have introduced a regiospecific MC domino process, which is efficiently executed under very mild conditions and utilizes easily accessible components such as glycosyl azides, iso(thio)cyanates, fumaric acid monoesters, and amines (Scheme 1).[26–31]



Scheme 1 Mechanism of the MC domino process

Thus, glycosyl azides **A** react with *tert*-butyl isocyanate (**B**) in the presence of Ph_3P (Staudinger reaction followed by aza-Wittig reaction) giving rise to the formation of carbodiimides **C**. The reaction can be easily followed by TLC and the resulting carbodiimide used in situ. Once carbodiimide **C** is formed, the temperature is lowered to 0 °C and 2,4,6-trimethylpyridine (TMP), amine **E**, then fumaric acid monoester **D** are added in this sequence. Acid **D** reacts with carbodiimide **C** giving rise to the formation of *O*-acylisourea **F** which undergoes an intramolecular aza-Michael reaction producing the intermediate **G.** This step is highly regioselective since the attack arises from the less sterically hindered primary sugar amine moiety rather than the bulky *tert*-butylamino moiety. Thus, the last step of the domino process is the nucleophilic attack of amine **E**, which becomes involved in the reaction once intermediate **G** is already formed, at the carbonyl of intermediate **G** leading to the formation of final compound **H**.

The efficiency and versatility of this process was likely to be compatible with the aminoglycoside skeleton and prompted us to explore the possibility of functionalizing aminoglycosides, in particular neomycin, with carbohydrates. A priori, properly functionalized neomycin derivatives can participate either as the azide, iso(thio)cyanate, or amine component, leading to the formation of three different conjugates. We envisioned the easiest way to initially study application of the process to aminoglycoside chemistry would be use of a neomycin amine derivative as the nucleophilic component. Indeed, due to the high solubility of neomycin derivative **5** in the reaction medium (MeCN at 0 °C, Scheme 2), the difficult protection of all the neomycin hydroxyl groups, which would have been otherwise neces-

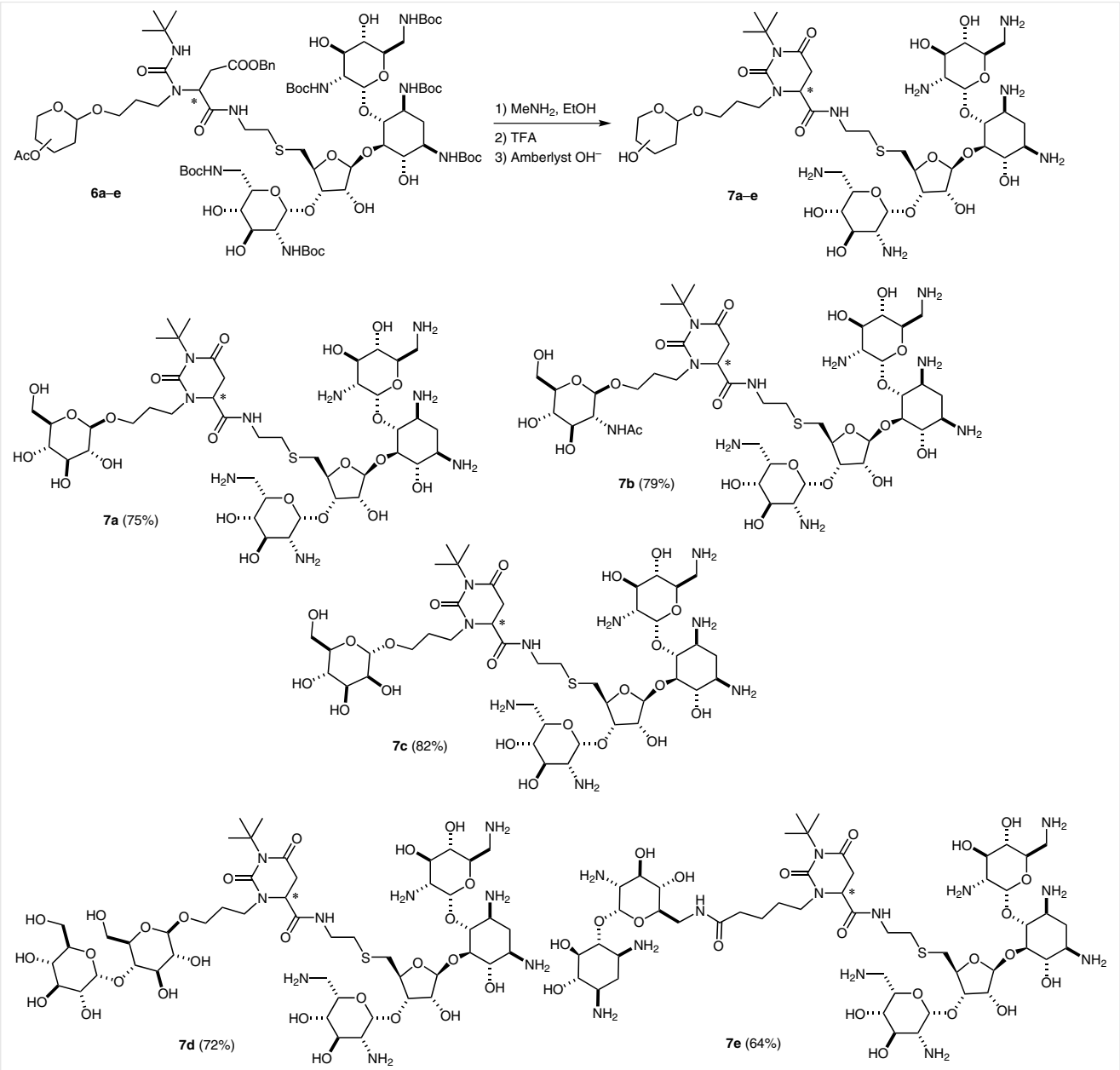


Scheme 2 MC domino synthesis of neomycin conjugates **6a–e**

sary due to the possible interference of these groups in the Staudinger step, can be avoided. Accordingly, we reacted (3-azidopropyl)-*O*-acetyl-β-glucoside **1a** with *tert*-butyl isocyanate (**2**) in the presence of Ph_3P in MeCN at room temperature. Once the formation of the corresponding carbodiimide **3a** was accomplished (ca. 12 h, as monitored by TLC), the reaction temperature was lowered to 0 °C, and TMP and a solution of neomycin derivative **5** in MeCN were added, followed by fumaric acid monobenzyl ester (**4**). The neomycin–glucose conjugate **6a** was obtained as the only regioisomer of an equimolecular mixture of two diastereoisomers in satisfactory yield (Scheme 2).[32]

The complete regioselectivity was likely achieved because the less sterically hindered primary *N*-substituent on the carbodiimide intermediate is much more reactive than the tertiary *tert*-butyl *N*′-substituent in the intramolecular aza-Michael step (see Scheme 1).[26–31]

Encouraged by this result, we went on to use several appropriately protected glycosyl azides such as *N*-acetylglucosamine **1b**, mannose **1c**, and disaccharide maltose **1d**. In all cases, the MC process worked well giving rise to the re-



Scheme 3 Synthesis and structures of the final neomycin–sugar conjugates **7a–e**

giospecific formation of the corresponding neomycin conjugates **6b–d**, respectively, all in good yields (Scheme 2). Finally, to link together two aminoglycosides, a strategy that has resulted in the discovery of more potent antibiotics in the past,[8–15] we carried out the reaction starting with neamine derivative **1e**. In this case, protection of the neamine hydroxyl groups was required to avoid complications in the Staudinger/aza-Wittig reaction, forming the corresponding carbodiimide **3e**. This reaction also proceeded smoothly, producing neomycin–neamine conjugate **6e** in a satisfactory yield.

The conjugates **6a–e** were subjected to deprotection of the hydroxyl and amino groups. Surprisingly, when glucose–neomycin conjugate **6a** was deacetylated with ethanolic methylamine, the *tert*-butylurea moiety reacted readily with the benzyl ester yielding dihydrouracil heterocycle derivative **7a** (Scheme 3).[33] Any attempts to selectively deprotect the hydroxyl groups without cyclization, such as treatment with catalytic NaOMe or NaOH, failed, giving rise to the formation of the corresponding dihydrouracil derivative in lower yields. This unexpected cyclization likely reflects a favorable conformation of precursor **6a**, and perhaps the stability of the resulting six-membered ring. Indeed, such cyclization was observed with all the conjugates, giving rise to the formation of the corresponding dihydrouracil–neomycin derivatives in very good yields. Finally, upon treatment of the cyclized intermediates with trifluoroacetic acid (TFA) in CH_2Cl_2, followed by neutralization of the resulting TFA salts with basic Amberlyst resin, the fully deprotected conjugates **7a–e** were also obtained in excellent yields.

Neomycin–sugar derivatives **7a–e** were tested against strains of resistant Gram-positive and Gram-negative bacteria, to determine their antibacterial potency. Neomycin B was used as reference and tetracycline, tobramycin, vancomycin, and ciprofloxacin served as positive controls for different bacterial strains. Minimum inhibitory concentrations (MIC) in μg/mL were determined using microdilution assay.

The results obtained with Gram-negative bacterial strains are summarized in Table 1. Disappointingly, neomycin conjugates **7a–e** were not active against drug-sensitive or drug-resistant *Pseudomonas aeruginosa* strains PA01, PA103, and PA ATCC 27853 (Table 1, entries 8–10), nor against most strains of *Acinetobacter baumannii* (AB), a Gram-negative opportunistic pathogen capable of causing serious infections in immune-compromised patients (Table 1, entries 1–3). However, in the latter family, conjugates **7a**,**b**,**d** were slightly active against AB ATCC 17978, though less active than neomycin B and tetracycline (Table 1, entry 2). On the other hand, the conjugates showed good activity against *E. coli* ATCC 25922 and K12 (Table 1, entries 4 and 5), with derivatives **7a**,**b**,**d**,**e** as potent as the positive control ciprofloxacin in the case of strain K12. Finally, compounds **7a**,**b**,**d** were very active against *Klebsiella pneumoniae* ATCC 700603 (Table 1, entry 7), even more potent than the control tetracycline, with the maltose–neomycin derivative **7d** in particular having an MIC value of 6.25 μg/mL.

With respect to Gram-positive bacteria, the conjugates **7a–e** were tested against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (SE). In the case of MRSA, two different strains were used, and conjugates **7a**,**b**,**d**,**e** appeared effective only against ATCC 33591, though less effective than the parent neomycin B and vancomycin control (Table 2, entries 1 and 2). Noteworthy results were obtained against SE ATCC 12228,

Table 1 Minimum Inhibitory Concentration (MIC) against Gram-Negative Strains

		MIC (μg/mL)								
Entry	Gram-negative bacteria	Neo B[e]	**7a**	**7b**	**7c**	**7d**	**7e**	Cipr[e]	Tetr[e]	Tobr[e]
1	AB ATCC 5075[a]	>50	>50	>50	>50	>50	>50		6.25	
2	AB ATCC 17978[a]	0.78	25	12.5	50	12.5	50		6.25	
3	AB ATCC 19606[a]	25	>50	>50	>50	>50	>50		6.25	
4	EC ATCC 25922[b]	1.56	25	12.5	25	12.5	50	6.25		
5	EC K12[b]	3.12	12.5–6.25	12.5–6.25	25	6.25	12.5	12.5–6.25		
6	KP GNR1100[c]	6.25	50	50	>50	50	>50		6.25	
7	KP ATCC 700603[c]	6.25	12.5	12.5	50	6.25	25		25	
8	PA01[d]	12.5–6.25	>50	>50	>50	>50	>50			0.39
9	PA103[d]	1.5	>50	>50	>50	>50	>50			0.39
10	PA ATCC 27853[d]	50–25	>50	>50	>50	50	>50			0.39

[a] AB = *Acinetobacter baumannii*.
[b] EC = *Escherichia coli*.
[c] KP = *Klebsiella pneumoniae*.
[d] PA = *Pseudomonas aeruginosa*.
[e] Neo B = neomycin B, used as reference; Cipr = ciprofloxacin, Tetr = tetracycline, Tobr = tobramycin, used as positive controls.

Table 2 Minimum Inhibitory Concentration (MIC) against Gram-Positive Strains

		MIC (μg/mL)						
Entry	Gram-positive bacteria	Neo B[c]	**7a**	**7b**	**7c**	**7d**	**7e**	Vancomycin[c]
1	MRSA TCH1516[a]	>50	>50	>50	>50	>50	>50	3.12
2	MRSA ATCC 33591[a]	3.12	12.5	12.5	>50	6.25	12.5	3.12
3	SE ATCC 12228[b]	0.39	1.56	0.78	3.12	0.78	1.56	6.25–3.12
4	SE ATCC 1457[b]	≥50	50	≥50	≥50	25	25–12.5	6.25

[a] MRSA = methicillin-resistant *Staphylococcus aureus*.
[b] SE = *Staphylococcus epidermidis*.
[c] Neo B = neomycin B, used as reference; vancomycin used as positive control.

with all the conjugates being more active than vancomycin, and derivatives **7b**,**d** only slightly less potent than neomycin B (Table 2, entry 3).

To summarize, by applying a new MC domino process to aminoglycoside components, we have efficiently synthesized a collection of systematically modified neomycin–sugar conjugates, starting from easily accessible reactants under mild conditions and in good yield. The deprotection reaction of the conjugates triggered an unexpected cyclization reaction leading to an aminoglycoside scaffold which is tethered to the carbohydrate moiety through a dihydrouracil linker. The resulting conjugates were tested for their antibacterial activity against resistant Gram-positive and Gram-negative bacterial strains. Although the conjugates exhibited limited improvement in potency, the chemistry described here could be exploited for the simple preparation of new collections or libraries of differently substituted aminoglycoside conjugates, thus facilitating the discovery of new biologically active compounds for diverse applications.[34–36]

Commercially available reagent-grade solvents were employed, without purification. TLC was run on silica gel 60 F254 (Merck). Flash chromatography was performed with silica gel 60 (60–200 μm, Merck). ^{1}H NMR spectra were recorded on 400-MHz spectrometers. Chemical shifts are expressed in ppm (δ), using TMS as internal standard for ^{1}H and ^{13}C nuclei (δ^H and δ^C = 0.00). ESI-MS was performed with an Esquire 3000 Plus ion-trap mass spectrometer equipped with an ESI source. Elemental analyses of the basic neomycin conjugates were obtained on FlashEA 1112 NC analyzers. Glycosyl azides **1** were prepared as described in the literature.[7] Neomycin derivative **5** was obtained as reported.[9] Mueller Hinton broth used for sensitivity testing was obtained from Hardy Diagnostics (Santa Maria, CA, USA). Polystyrene 96-well microplates for minimum inhibitory concentration (MIC) testing were purchased from Corning Inc. (Corning, NY, USA). Bacterial strains for sensitivity testing, including nine strains from the American Type Culture Collection (ATCC; Manassas, VA, USA): hospital-associated MRSA strain 33591, rendered resistant to rifampicin by serial passage; USA300 MRSA strain TCH1516; SE strains 12228 and 1457; *Klebsiella pneumoniae* strain 700603; *Pseudomonas aeruginosa* strains 27853, PA01, and PA103; *E. coli* strains 25922 and K12; *Acinetobacter baumannii* strains 5075, 19606, and 17978; *K. pneumoniae* strain GNR1100 (respiratory isolate), a clinical isolate obtained from a tertiary academic hospital in the New York metropolitan area. MIC values for aminoglycosides were determined using broth microdilution in accordance with the Clinical Laboratory Standards Institute guidelines (Performance Standards for Antimicrobial Susceptibility Testing, 19th Informational Supplement M100-S19, Clinical and Laboratory Standards Institute,Wayne, PA, USA, 2008). For the determination of the values, a VersaMax plate reader (Molecular Devices, Mountain View, CA, USA), set at 600 nm wavelength, was used.

Neomycin Conjugates 6a–e; General Procedure for the Multicomponent Synthesis

To a stirred solution of glycosyl azide **1** (1 equiv) in MeCN (0.1 M), *tert*-butyl isocyanate (**2**, 1.05 equiv) followed by Ph_3P (1.05 equiv) were added at r.t. The solution was stirred until complete formation of the corresponding carbodiimide **3** was achieved (TLC monitoring). The temperature was lowered to 0 °C and TMP (1 equiv), a solution of neomycin derivative **5** (1 equiv) in a minimum amount of MeCN, then a solution of fumaric acid monobenzyl ester (**4**, 1 equiv) in a minimum amount of MeCN were added. The temperature was slowly left to reach r.t. and the reaction, when finished (TLC monitoring, ca. 3 h), was quenched with aqueous 1 N HCl. The mixture was extracted with EtOAc, the organic phases were collected and dried over Na_2SO_4, the solvent was removed under reduced pressure, and the crude was purified by flash chromatography to provide compounds **6a–e** as yellow oils.

Conjugate 6a

Mixture of two diastereoisomers; yield: 154 mg (76%).

R_f = 0.32 (CH_2Cl_2–MeOH, 90:10).

^{1}H NMR (400 MHz, CD_3OD): δ = 7.37–7.35 (m, 5 H, aromatics), 5.62 (br s, 1 H), 5.32 (br s, 1 H), 5.26–5.25 (m, 2 H), 5.15–5.14 (m, 2 H, -OCH*H*Ph and CH Asp), 5.10 (d, *J* = 12.8 Hz, 1 H, -OC*H*HPh), 5.02–5.00 (m, 2 H), 4.94–4.90 (m, 2 H), 4.67–4.65 (m, 2 H, -CHCH_2OAc), 4.27–4.26 (m, 2 H), 4.15–4.11 (m, 3 H), 3.91–3.78 (m, 4 H), 3.58–3.22 (m, 18 H), 2.89–2.88 (m, 2 H, -CH_2CH_2S-), 2.74–2.72 (m, 4 H, -CH_2COOBn and -SCH_2CH-), 2.05–2.00 (m, 13 H, H-2 neomycin and 4 × $COCH_3$), 1.77–1.76 (m, 2 H, -$CH_2CH_2CH_2$-), 1.46–1.43 (m, 55 H, H-2 neomycin and 6 × $COOC(CH_3)_3$), 1.35 (s, 9 H, -NHC$(CH_3)_3$).

^{13}C NMR (100.6 MHz, CD_3OD): δ = 170.1, 170.0, 169.8, 169.7, 169.4, 168.8, 168.4, 156.1, 155.7, 155.4, 155.3, 155.0, 134.6, 126.8, 126.7, 126.6, 126.5, 126.4, 126.3, 120.2, 99.3, 99.2, 99.0, 79.9, 78.0, 77.8,

77.6, 72.8, 71.7, 71.5, 70.6, 70.2, 70.1, 68.8, 67.2, 67.0, 66.2, 65.8, 64.9, 64.8, 60.3, 54.9, 54.2, 50.9, 49.5, 49.4, 41.2, 39.9, 37.3, 33.0, 32.9, 29.9, 27.7, 27.6, 27.0, 26.3, 26.2, 26.0, 18.1, 17.9.

ESI-MS: *m*/*z* (%) = 1990.4 $[M + Na]^+$ (100), 1968.4 $[M + H]^+$ (12).

Anal. Calcd for $C_{88}H_{143}N_9O_{38}S$: C, 53.73; H, 7.33; N, 6.41. Found: C, 53.76; H, 7.31; N, 6.43.

Conjugate 6b

Mixture of two diastereoisomers; yield: 163 mg (62%).

R_f = 0.12 (CH_2Cl_2–MeOH, 90:10).

^{1}H NMR (400 MHz, CD_3OD): δ = 7.40–7.38 (m, 5 H, aromatics), 5.36 (br s, 1 H), 5.26–5.18 (m, 5 H), 5.14 (d, *J* = 12.4 Hz, 1 H, -OC*H*HPh), 5.02–5.01 (m, 1 H), 4.98 (s, 1 H), 4.90–4.88 (m, 1 H), 4.67–4.65 (m, 3 H), 4.30–4.28 (m, 3 H), 4.15–4.11 (m, 3 H), 3.96–3.94 (2 H), 3.83–3.81 (m, 4 H), 3.58–3.22 (m, 14 H), 2.94–2.92 (m, 2 H, -CH_2CH_2S-), 2.80–2.76 (m, 4 H, -CH_2COOBn and -SCH_2CH-), 2.05–2.00 (m, 13 H, H-2 neomycin, 4 × $COCH_3$), 1.81–1.79 (m, 2 H, -$CH_2CH_2CH_2$-), 1.50–1.48 (m, 55 H, H-2 neomycin and 6 × COOC(CH3)3), 1.46 (s, 9 H, -NHC(CH_3)$_3$).

^{13}C NMR (100.6 MHz, CD_3OD): δ = 170.3, 169.6, 169.5, 169.1, 168.5, 156.2, 155.7, 155.4, 155.0, 126.8, 126.6, 126.5, 108.3, 99.6, 99.5, 98.0, 96.6, 78.0, 77.7, 76.7, 72.9, 71.8, 71.6, 71.4, 70.7, 70.3, 70.2, 68.9, 67.7, 67.5, 66.5, 65.7, 65.6, 64.9, 60.6, 55.1, 52.7, 49.4, 41.5, 40.0, 39.0, 37.5, 37.0, 35.5, 33.1, 32.9, 32.7, 29.9, 28.5, 27.7, 27.6, 27.3, 27.1, 27.0, 26.4, 26.2, 26.1, 20.3, 20.2, 20.1, 18.0, 17.9.

ESI-MS: *m*/*z* (%) = 1989.1 $[M + Na]^+$ (100).

Anal. Calcd for $C_{88}H_{144}N_{10}O_{37}S$: C, 53.76; H, 7.38; N, 7.12. Found: C, 53.78; H, 7.33; N, 7.13.

Conjugate 6c

Mixture of two diastereoisomers; yield: 172 mg (71%).

R_f = 0.35 (CH_2Cl_2–MeOH, 90:10).

^{1}H NMR (400 MHz, CD_3OD): δ = 7.37–7.35 (m, 5 H, aromatics), 5.27–5.24 (m, 3 H), 5.17–5.16 (m, 3 H), 5.12–5.11 (m, 2 H), 4.94 (m, 1 H), 4.83–4.81 (m, 2 H), 4.68–4.66 (m, 2 H, -CHCH_2OAc), 4.24–4.21 (m, 2 H), 4.11–4.10 (m, 2 H), 4.02–4.00 (m, 1 H), 3.92–3.91 (m, 1 H), 3.78–3.77 (m, 1 H), 3.72–3.71 (m, 1 H), 3.49–3.26 (m, 19 H), 3.21–3.11 (m, 1 H), 2.90–2.89 (m, 2 H, -CH_2CH_2S-), 2.82–2.81 (m, 2 H, -CH_2COOBn), 2.75–2.73 (m, 2 H, -SCH_2CH-), 2.13–2.03 (m, 13 H, H-2 neomycin and 4 × $COCH_3$), 2.03–2.01 (m, 2 H, -$CH_2CH_2CH_2$-), 1.46–1.44 (m, 55 H, H-2 neomycin and 6 × $COOC(CH_3)_3$), 1.35 (s, 9 H, -NHC(CH_3)$_3$).

^{13}C NMR (100.6 MHz, CD_3OD): δ = 170.2, 169.8, 169.7, 168.9, 168.8, 168.7, 156.1, 155.8, 155.4, 155.1, 134.6, 126.9, 126.7, 126.6, 96.2, 80.0, 78.0, 77.7, 76.6, 72.9, 71.9, 70.7, 70.3, 68.9, 68.1, 67.2, 65.1, 65.0, 64.8, 64.2, 61.0, 58.8, 55.4, 54.2, 49.3, 33.2, 33.0, 27.3, 27.2, 27.1, 27.0, 26.4, 26.3, 26.2, 26.1, 18.1, 18.0, 17.9.

ESI-MS: *m*/*z* (%) = 1990.4 $[M + Na]^+$ (21), 1968.4 $[M + H]^+$ (100).

Anal. Calcd for $C_{88}H_{143}N_9O_{38}S$: C, 53.73; H, 7.33; N, 6.41. Found: C, 53.75; H, 7.36; N, 6.44.

Conjugate 6d

Mixture of two diastereoisomers; yield: 156 mg (77%).

R_f = 0.43 (CH_2Cl_2–MeOH, 90:10).

^{1}H NMR (400 MHz, CD_3OD): δ = 7.37–7.35 (m, 5 H, aromatics), 5.38–5.36 (m, 3 H), 5.32–5.29 (m, 2 H), 5.28 (dd, *J* = 9.2, 3.2 Hz, 1 H), 5.16–5.14 (m, 2 H), 5.11 (m, 1 H), 5.04 (d, *J* = 12.8 Hz, 1 H, -OC*H*HPh), 5.02 (d, *J* = 12.8 Hz, 1 H, -OCH*H*Ph), 4.95–4.94 (m, 2 H), 4.87–4.86 (m, 3 H), 4.79–4.77 (m, 1 H), 4.67–4.65 (m, 2 H, -CHCH2OAc), 4.55 (m, 1 H, -CHC*H*HOAc), 4.52 (m, 1 H, -CHCH*H*OAc), 4.25–4.23 (m, 3 H), 4.13–4.11 (m, 3 H), 3.92–3.90 (m, 3 H), 3.78–3.76 (m, 4 H), 3.51–3.20 (m, 22 H), 2.89–2.88 (m, 2 H, -CH_2CH_2S-), 2.74–2.72 (m, 4 H, -CH_2COOBn and -SCH_2CH-), 2.05–2.00 (m, 13 H, H-2 neomycin and 4 × $COCH_3$), 1.77–1.76 (m, 2 H, -$CH_2CH_2CH_2$-), 1.46–1.43 (m, 55 H, H-2 neomycin and 6 × $COOC(CH_3)_3$), 1.35 (s, 9 H, -NHC(CH_3)$_3$).

^{13}C NMR (100.6 MHz, CD_3OD): δ = 169.5, 169.2, 169.0, 168.8, 168.4, 156.2, 155.4, 155.0, 134.6, 126.8, 126.6, 126.5, 126.4, 94.4, 80.0, 78.0, 77.6, 73.9, 72.9, 72.2, 71.8, 70.9, 70.2, 69.0, 68.1, 67.2, 67.1, 66.2, 65.7, 64.9, 61.5, 60.4, 55.6, 50.9, 49.4, 33.0, 32.7, 29.9, 27.9, 27.6, 27.1, 27.0, 26.4, 26.2, 26.1, 18.4, 18.2, 18.1, 18.0, 17.9, 17.8.

ESI-MS: *m*/*z* (%) = 2277.7 $[M + Na]^+$ (100).

Anal. Calcd for $C_{100}H_{159}N_9O_{46}S$: C, 53.25; H, 7.11; N, 5.59. Found: C, 53.26; H, 7.14; N, 5.61.

Conjugate 6e

Mixture of two diastereoisomers; yield: 97 mg (58%).

R_f = 0.23 (CH_2Cl_2–MeOH, 80:20).

^{1}H NMR (400 MHz, CD_3OD): δ = 7.37–7.35 (m, 5 H, aromatics), 5.32 (br s, 1 H), 5.28–5.27 (m, 2 H), 5.18–5.11 (m, 3 H), 5.10–5.08 (m, 2 H, -OC*H*HPh and CH Asp), 4.95 (s, 1 H), 4.90–4.84 (m, 2 H), 4.23–4.20 (m, 2 H), 3.92–3.89 (m, 2 H), 3.73–3.71 (m, 5 H), 3.49–3.11 (m, 24 H), 2.89–2.88 (m, 2 H, -CH_2CH_2S-), 2.74–2.72 (m, 4 H, -CH_2COOBn and -SCH_2CH-), 2.25 (m, 2 H, -NHCOCH_2-), 1.98–1.93 (m, 14 H, H-2 neomycin and neamine, 4 × $COCH_3$), 1.55–1.51 (m, 4 H, -$CH_2CH_2CH_2CH_2$-), 1.46–1.43 (m, 83 H, H-2 neomycin and neamine, 9 × $COOC(CH_3)_3$), 1.30 (s, 9 H, -NHC(CH_3)$_3$).

^{13}C NMR (100.6 MHz, CD_3OD): δ = 173.6, 173.2, 170.2, 169.8, 169.2, 168.9, 168.4, 168.0, 156.1, 155.4, 155.0, 154.7, 134.6, 130.9, 130.8, 130.4, 96.7, 78.3, 78.0, 77.8, 77.7, 76.2, 74.0, 73.0, 70.7, 70.2, 67.4, 66.8, 65.2, 65.0, 64.9, 55.6, 51.5, 49.8, 49.2, 37.4, 36.8, 33.8, 33.5, 33.1, 32.6, 28.2, 27.9, 27.2, 26.4, 26.2, 26.1, 26.0, 25.9, 21.4, 21.3, 18.3, 18.2, 18.0, 17.9.

ESI-MS: *m*/*z* (%) = 2474.0 $[M + Na]^+$ (100).

Anal. Calcd for $C_{111}H_{183}N_{13}O_{45}S$: C, 54.38; H, 7.52; N, 7.43. Found: C, 54.40; H, 7.56; N, 7.45.

Neomycin–Dihydrouracil Conjugates 7a–e; General Procedure

To a stirred solution of a neomycin conjugate **6** (1 equiv) in EtOH (0.1 M), a ca. 8.03 M solution of $MeNH_2$ in EtOH (1:1 v/v) was added at r.t. After the reaction was complete (TLC monitoring, ca. 2 h), the organic solvents were evaporated. The crude was treated with TFA in CH_2Cl_2 (50% v/v) for 2 h. The solvents were evaporated and co-evaporated with toluene twice. The obtained crude was dissolved in water and washed twice with CH_2Cl_2. The aqueous solution was lyophilized to obtain the clean neomycin–dihydrouracil–sugar conjugates **7**·6 TFA as fluffy white solids. The resulting salts were dissolved in water and activated basic Amberlyst resin was added until basic pH was reached. The mixture was filtered and the water lyophilized to obtain conjugates **7** as fluffy white solids.

Conjugate 7a·TFA

Mixture of two diastereoisomers; yield: 67 mg (75%).

^{1}H NMR (400 MHz, D_2O): δ = 6.00 (d, *J* = 4.0 Hz, 1 H, H-1′ neomycin), 5.35 (s, 1 H, H-1″ neomycin), 5.25 (s, 1 H, H-3′ neomycin), 4.36 (m, 2 H), 4.26 (m, 2 H), 4.18 (m, 3 H), 4.05 (t, *J* = 9.6 Hz, 1 H), 3.97 (t, *J* = 9.2 Hz, 1 H), 3.86 (m, 5 H), 3.78 (s, 1 H), 3.68–3.28 (m, 15 H), 3.19 (m, 3

H), 3.05 (m, 1 H), 2.87 (m, 2 H), 2.65 (m, 4 H), 2.43 (m, 1 H, 2-desoxystreptamine C*H*H), 1.88 (m, 1 H, 2-desoxystreptamine CH*H*), 1.81 (m, 2 H, -OCH$_2$C*H*$_2$CH$_2$N-), 1.50 (s, 9 H, C(CH$_3$)$_3$).

^{13}C NMR (100.6 MHz, D$_2$O): δ = 175.9, 170.8, 162.6 (q, *J* = 36.2 Hz, CF$_3$COOH), 158.8, 116.3 (q, *J* = 290.7 Hz, *C*F$_3$COOH), 110.4, 102.3, 95.6, 95.0, 85.5, 80.2, 78.7, 76.0, 75.9, 75.8, 75.1, 73.7, 73.3, 72.5, 70.8, 70.2, 69.8, 68.1, 67.8, 67.6, 67.4, 60.9, 58.3, 56.4, 56.1, 53.6, 51.0, 49.7, 48.5, 40.6, 40.3, 38.4, 38.0, 34.5, 30.5, 27.9, 27.3, 27.2.

ESI-MS: *m*/*z* (%) = 1112.4 [M + Na]$^+$ (21), 1090.4 [M + H]$^+$ (100).

Anal. Calcd for C$_{43}$H$_{79}$N$_9$O$_{21}$S: C, 47.37; H, 7.30; N, 11.56. Found: C, 47.39; H, 7.31; N, 11.58.

Conjugate 7b·TFA

Mixture of two diastereoisomers; yield: 54 mg (79%).

^{1}H NMR (400 MHz, D$_2$O): δ = 6.01 (d, *J* = 4.0 Hz, 1 H, H-1′ neomycin), 5.38 (s, 1 H, H-1″ neomycin), 5.28 (s, 1 H, H-3′ neomycin), 4.38 (m, 2 H), 4.28 (m, 2 H), 4.20 (m, 2 H), 4.05 (t, *J* = 10.0 Hz, 1 H), 3.99 (t, *J* = 8.8 Hz, 1 H), 3.88 (m, 3 H), 3.81 (s, 1 H), 3.67 (m, 3 H), 3.58–3.29 (m, 17 H), 3.26 (dd, *J* = 13.6, 6.8 Hz, 1 H), 3.06 (m, 1 H), 2.85 (m, 2 H), 2.77–2.73 (m, 4 H), 2.67 (s, 3 H, -NHCOC*H*$_3$), 2.47 (m, 1 H, 2-desoxystreptamine C*H*H), 1.89 (m, 1 H, 2-desoxystreptamine CH*H*), 1.74 (m, 2 H, -OCH$_2$C*H*$_2$CH$_2$N-), 1.51 (s, 9 H, C(CH$_3$)$_3$).

^{13}C NMR (100.6 MHz, D$_2$O): δ = 176.0, 175.9, 174.9, 174.6, 170.9, 162.7 (q, *J* = 36.2 Hz, CF$_3$COOH), 159.0, 158.7, 116.5 (q, *J* = 291.7 Hz, *C*F$_3$COOH), 112.1, 110.5, 102.1, 101.5, 95.8, 95.1, 85.6, 80.4, 78.9, 78.8, 76.1, 76.0, 75.3, 74.2, 74.1, 74.0, 73.9, 72.6, 70.9, 70.8, 70.4, 70.2, 70.1, 69.8, 68.2, 67.9, 67.7, 67.6, 61.0, 60.9, 60.8, 59.3, 57.2, 56.4, 56.1, 55.8, 55.7, 53.9, 53.8, 51.1, 49.8, 48.7, 40.7, 40.4, 38.8, 38.7, 38.6, 38.5, 38.3, 34.7, 34.6, 34.5, 34.3, 30.8, 30.7, 29.9, 28.8, 28.1, 28.0, 27.3, 26.1, 22.4, 22.3.

ESI-MS: *m*/*z* (%) = 1131.3 [M + H]$^+$ (25), 566.1 [M + H]$^{2+}$ (100).

Anal. Calcd for C$_{45}$H$_{82}$N$_{10}$O$_{21}$S: C, 47.78; H, 7.31; N, 12.38. Found: C, 47.81; H, 7.30; N, 12.40.

Conjugate 7c·TFA

Mixture of two diastereoisomers; yield: 64 mg (82%).

^{1}H NMR (400 MHz, D$_2$O): δ = 6.02 (d, *J* = 3.6 Hz, 1 H, H-1′ neomycin), 5.38 (s, 1 H, H-1″ neomycin), 5.28 (s, 1 H, H-3′ neomycin), 4.29 (m, 2 H), 4.22 (m, 2 H), 4.11 (m, 2 H), 4.08 (t, *J* = 8.8 Hz, 1 H), 4.01 (t, *J* = 9.6 Hz, 1 H), 3.88 (m, 5 H), 3.71 (s, 1 H), 3.68–3.28 (m, 16 H), 3.14 (m, 1 H), 3.05 (m, 1 H), 2.87 (m, 2 H), 2.65 (m, 4 H), 2.48 (m, 1 H, 2-desoxystreptamine C*H*H), 1.92 (m, 1 H, 2-desoxystreptamine CH*H*), 1.82 (m, 2 H, -OCH$_2$C*H*$_2$CH$_2$N-), 1.52 (s, 9 H, C(CH$_3$)$_3$).

^{13}C NMR (100.6 MHz, D$_2$O): δ = 175.8, 175.7, 174.9, 170.7, 164.9, 162.2 (q, *J* = 37.2 Hz, CF$_3$COOH), 158.6, 158.5, 116.1 (q, *J* = 290.7 Hz, *C*F$_3$COOH), 110.4, 100.0, 99.9, 95.7, 94.9, 85.5, 80.2, 78.8, 75.1, 73.8, 72.8, 72.7, 72.5, 70.8, 70.3, 69.7, 68.1, 67.6, 67.5, 66.9, 65.4, 65.2, 61.0, 58.3, 56.2, 56.0, 53.7, 51.1, 49.7, 48.6, 40.6, 40.4, 38.7, 38.6, 38.3, 37.0, 34.6, 34.5, 34.3, 34.2, 31.6, 30.7, 30.6, 28.0, 27.9, 27.8, 27.1, 26.3, 21.2.

ESI-MS: *m*/*z* (%) = 1112.4 [M + Na]$^+$ (52), 1090.4 [M + H]$^+$ (100).

Anal. Calcd for C$_{43}$H$_{79}$N$_9$O$_{21}$S: C, 47.37; H, 7.30; N, 11.56. Found: C, 47.38; H, 7.30; N, 11.55.

Conjugate 7d·TFA

Mixture of two diastereoisomers; yield: 62 mg (72%).

^{1}H NMR (400 MHz, D$_2$O): δ = 6.13 (d, *J* = 4.0 Hz, 1 H, H-1′ neomycin), 5.49 (s, 1 H, H-1″ neomycin), 5.44 (s, 1 H, H-3′ neomycin), 5.38 (s, 1 H, H-1′ maltose), 4.48 (m, 3 H), 4.39 (m, 2 H), 4.31 (m, 2 H), 4.18 (t, *J* = 9.6 Hz, 1 H), 4.11 (t, *J* = 8.0 Hz, 1 H), 4.07 (m, 4 H), 3.98 (m, 2 H), 3.68–3.28 (m, 22 H), 3.17 (m, 1 H), 2.96 (m, 2 H), 2.80 (m, 6 H), 2.58 (m, 1 H, 2-desoxystreptamine C*H*H), 2.01 (m, 1 H, 2-desoxystreptamine CH*H*), 1.94 (m, 2 H, -OCH$_2$C*H*$_2$CH$_2$N-), 1.63 (s, 9 H, C(CH$_3$)$_3$).

^{13}C NMR (100.6 MHz, D$_2$O): δ = 176.0, 170.8, 162.8 (q, *J* = 35.2 Hz, CF$_3$COOH), 158.8, 116.2 (q, *J* = 291.7 Hz, *C*F$_3$COOH), 110.5, 102.1, 99.6, 95.6, 95.0, 85.5, 78.7, 78.6, 77.0, 76.9, 76.3, 76.2, 75.1, 74.6, 73.7, 73.1, 72.9, 72.8, 72.7, 72.6, 72.5, 71.7, 70.7, 70.2, 69.6, 69.5, 69.4, 68.0, 67.8, 67.7, 67.6, 67.4, 60.8, 60.7, 60.5, 58.2, 53.6, 50.9, 49.6, 48.9, 48.4, 40.5, 40.4, 40.2, 38.5, 38.4, 38.3, 38.2, 34.4, 34.1, 34.0, 30.4, 28.1, 27.9, 27.3, 27.1.

ESI-MS: *m*/*z* (%) = 1252.5 [M + H]$^+$ (100).

Anal. Calcd for C$_{49}$H$_{89}$N$_9$O$_{26}$S: C, 46.99; H, 7.16; N, 10.07. Found: C, 47.01; H, 7.18; N, 10.09.

Conjugate 7e

Mixture of two diastereoisomers; yield: 34 mg (64%).

^{1}H NMR (400 MHz, D$_2$O): δ = 5.66 (s, 1 H, H-1′ neomycin), 5.65 (s, 1 H, H-1′ neamine), 5.43 (s, 1 H, H-1″ neomycin), 5.34 (s, 1 H, H-3′ neomycin), 4.41 (m, 1 H), 4.36–4.32 (m, 2 H), 4.17 (m, 2 H), 4.05 (t, *J* = 9.6 Hz, 1 H), 4.01 (t, *J* = 8.0 Hz, 1 H), 3.88–3.83 (m, 7 H), 3.57–3.10 (m, 14 H), 3.07 (m, 4 H), 2.76–2.71 (m, 6 H), 2.49 (m, 3 H), 2.26 (m, 5 H), 2.91 (m, 2 H, 2-desoxystreptamine C*HH*), 1.63 (m, 2 H), 1.48 (m, 2 H), 1.29 (s, 9 H, C(CH$_3$)$_3$).

^{13}C NMR (100.6 MHz, CD$_3$OD): δ = 174.2, 173.9, 172.6, 168.6, 157.8, 156.2, 108.7, 95.8, 95.7, 78.6, 74.1, 71.4, 71.3, 70.8, 70.7, 70.5, 70.2, 67.6, 67.5, 66.4, 53.1, 50.2, 48.7, 48.5, 48.0, 47.6, 39.3, 38.9, 38.5, 37.4, 33.6, 33.4, 28.1, 27.9, 27.2, 27.1, 26.3, 26.2, 21.3.

ESI-MS: *m*/*z* (%) = 1274.2 [M + H]$^+$ (100).

Anal. Calcd for C$_{51}$H$_{95}$N$_{13}$O$_{22}$S: C, 48.06; H, 7.51; N, 14.29. Found: C, 48.08; H, 7.55; N, 14.32.

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0035-1562727.

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