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Short and Efficient Synthesis of Alkyne-Modified Amino Glycoside Building Blocks

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In the light of recent progress in RNA biology, the need for molecules that bind to RNA and thus may be suited to manipulating RNA-mediated processes is steadily increasing. We present a very short and efficient synthetic route to alkyne-modified neamine and 2-deoxystreptamine derivatives on a half-gram scale. These derivatives are suitable for constructing a library of potential divalent RNA binders by copper-catalysed 1,3-dipolar cycloaddition to diazides ("click chemistry"). The conjugate dimers thus formed inhibited Dicer-mediated micro-RNA maturation with IC_{50} values between 0.6 and 15 μM.

Introduction

During the last decade, the scientific view of RNA underwent a dramatic change. Although the discovery of self-splicing catalytic RNA[1] and the existence of riboswitch-mediated regulation of gene expression[2] had already been widely accepted several years ago, these observations seemed to be restricted to a few species and thus were mainly of academic interest. Since the discovery of RNA interference,[3] and more recently the discovery of microRNAs (miRNAs) in mammals,[4] a whole plethora of RNA-related phenomena has (re)gained increased scientific attention. Today RNA is not only a state-of-the-art tool and a potential drug candidate, for example, siRNAs, antisense molecules, aptamers[5] and ribozymes,[6] RNA itself is a potential drug target, like ribosomal RNA, mRNA,[7] viral RNA,[8–10] miRNAs[11–13] and riboswitches.[14] To study the function of potential RNA drug targets by creating loss-of-function phenotypes, the use of antisense molecules or siRNAs that either block or degrade the target RNA is the most straightforward approach. However, in spite of the tremendous effort put into the development of therapeutic oligonucleotides in recent years, there are only a few products on the market to date and no systemically acting oligonucleotide has gained FDA approval so far.[15] Moreover, very recent results have added new complexity to the use of RNA as a drug because it has been shown that a number of the siRNAs that are in clinical trials against age-related macular degeneration act by a completely unspecific activation of the extracellular toll-like receptor TLR3.[16]

Thus, the manipulation of RNA-mediated processes by small molecules may offer alternative approaches towards pharmacological applications. In contrast to nucleic acids and nucleic acid mimics, amino glycosides that target prokaryotic ribosomal RNA have been widely used in the treatment of human disease for more than half a century.[17] In spite of their significant toxicity, amino glycosides play a leading role in the treatment of enterococcal, mycobacterial and severe Gram-negative bacterial infections and are the most commonly used antibiotics worldwide.[18]

Since the discovery of streptomycin by Schatz and Waksman,[17] a reasonable effort has been made to expand the class of naturally occurring amino glycosides by synthetic derivatives and analogues. Owing to their complex structures, only a few attempts to synthesize de novo amino sugars or amino cyclitols have been reported.[19–21] More recent strategies involve the chemical modification of existing amino glycoside building blocks.[22] Examples include conjugation with intercalating residues or the dimerization of whole amino glycosides[23] or substructures like 2-deoxystreptamine (2-DOS)[24,25] and neamine.[26–28] However, owing to the laborious chemo- and regioselective protection and deprotection procedures involved in this synthetic strategy, most libraries based on the combinatorial conjugation of amino glycoside building blocks reported so far barely exceed 20 members. Such libraries should be far too small for the identification of selective RNA binders. Moreover, because RNA structures are very dynamic and difficult to predict, a rational design of selective small-molecule RNA binders is not in sight. Hence, the synthesis of libraries of small molecules targeting RNA is gaining ever increasing attention. In the course of our efforts to identify RNA binders that inhibit miRNA maturation,[29,30] we decided to

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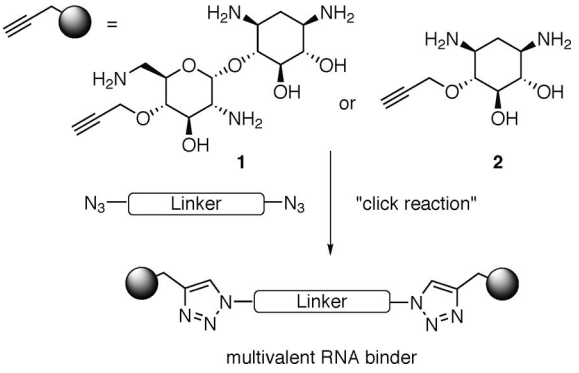
synthesize a library of neamine and 2-deoxystreptamine (2-DOS) dimers by the copper-catalyzed 1,3-dipolar cycloaddition of alkynes to bifunctional azides (Scheme 1).



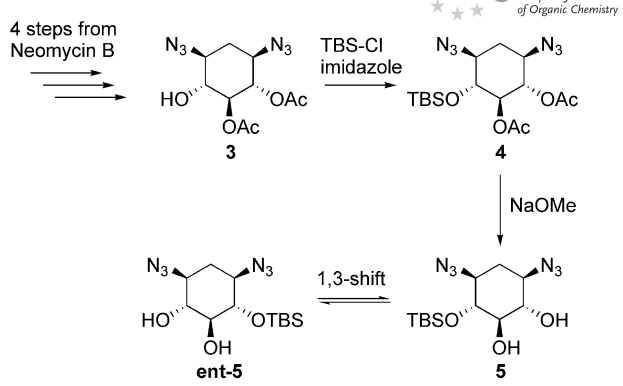
Scheme 1. Alkyne-modified neamine and 2-deoxystreptamine for dimerization by "click chemistry".

Results and Discussion

An elegant method to overcome the synthetic limitations of extensive protecting group chemistry is the use of chemoselective conjugation procedures like the Sharpless–Meldal variant of the Huisgen reaction,[31,32] which makes the post-conjugation steps obsolete. Recently, a library of 105 2-DOS conjugates constructed by this so-called "click chemistry" was reported by Hergenrother and co-workers.[25] Interestingly, several members of this library showed a highly size-selective binding affinity towards artificial terminal RNA hairpin loops, underscoring the benefits of a powerful synthetic strategy towards amino glycoside mimics. Inspired by these results, we wanted to expand this approach to an analogous conjugation of the more complex neamine building block. This endeavour was mainly driven by the idea that the larger interaction surface of the neamine scaffold relative to that of 2-DOS might lead to even more potent and more selective RNA-binding conjugates.

Although the neamine and the 2-DOS moiety can be readily obtained by acidolysis of neomycin B on a multigram scale,[33,34] derivatization essentially includes regio- and enantio-controlled arrangement of protecting groups. As an example, the esterase-mediated racemic resolution of peracetylated 2-DOS diazide is a common route to the enantiopure protected 2-DOS building block.[35] However, this procedure includes the use of expensive enzymes and laborious protection and deprotection procedures. Moreover, in our hands, the optically pure and partially deprotected compound **5** underwent racemization through the migration of the silyl group under basic deprotection conditions (Scheme 2).[36]

As an alternative to the racemic resolution of acetylated 2-DOS we decided to make use of the 2,6-dideoxydiaminoglucose residue of neamine as a protecting group.[37] Neomycin B sulfate was subjected to acid hydrolysis to yield

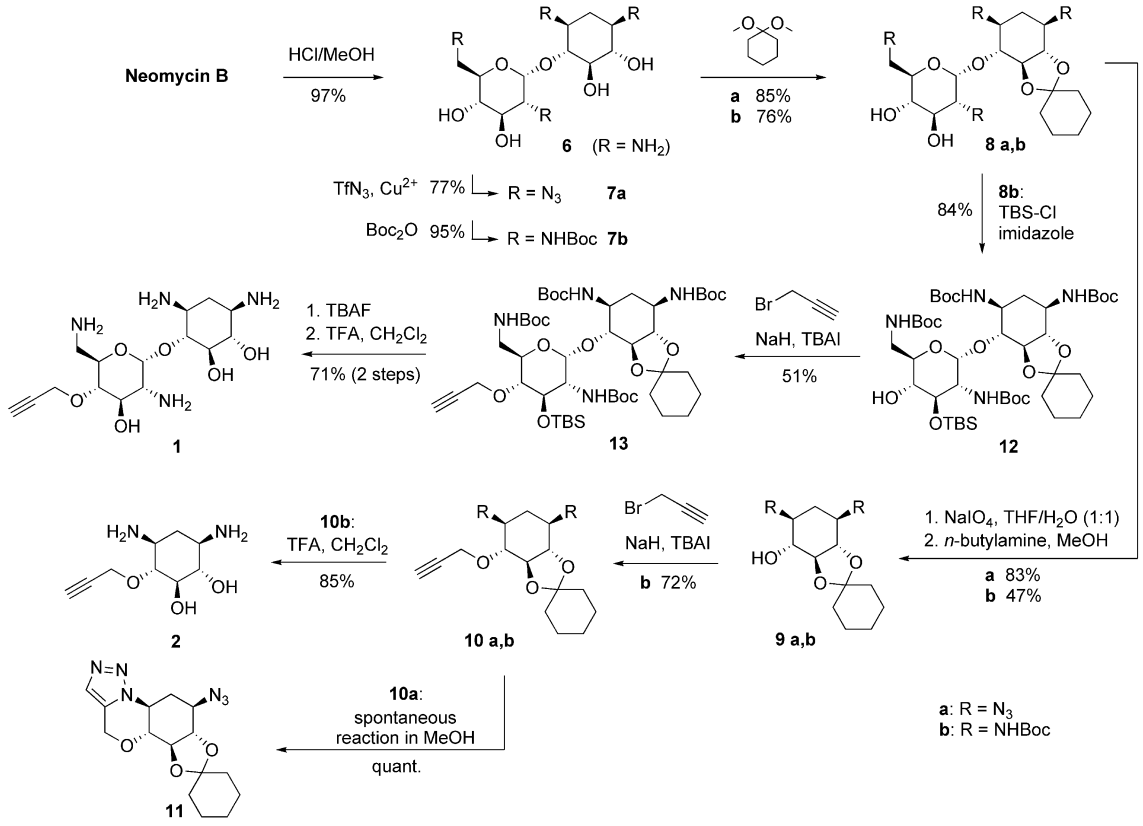


Scheme 2. Silyl shift as observed for some of the 2-DOS derivatives.

neamine **6**, which was subsequently converted into either the corresponding azide **7a** or the Boc-protected amine **7b**, as described previously (Scheme 3).[37] The protected neamine was treated with an excess of cyclohexanone dimethyl ketal to give the 3′,4′:5,6-diketal. The diketal was then allowed to equilibrate to the more stable 5,6-monoketal **8a** or **8b** under acidic conditions. For **8a**, this detour resulted in a doubling of the reaction yield compared with the literature.[38]

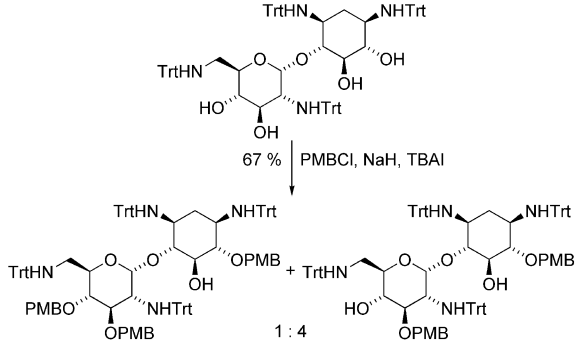
In the case of the Boc-protected derivative **8b**, however, the yields were considerably lower. Notably, the unconverted **7b** was nearly quantitatively recovered upon LC purification. To form the alkyne-modified 2-DOS, in the next step, the vicinal diol was cleaved by using sodium periodate and 2-DOS was liberated from the intermediate amino aldehyde under alkaline conditions to form the protected 2-DOS building blocks **9a** and **9b**. With these compounds in hand, we introduced the alkyne moiety by using sodium hydride and propargyl bromide. To our surprise, we were unable to isolate the alkyne **10a** after column chromatography. A more detailed investigation revealed that **10a** completely underwent an intermolecular cycloaddition reaction within 12 h at room temperature to form the triazole **11** (see the Supporting Information for more details). This cycloaddition occurred in methanolic solution, even when the reaction vessel was protected from light and after several rounds of extraction with EDTA to ensure complete removal of copper and other divalent cations. A similar reaction at elevated temperature has been described previously.[39] However, this phenomenon was restricted to the propargyl-substituted 2-DOS derivative. Longer residues (pentynyl or hexynyl residues) did not react in this way (data not shown). Finally, deprotection of the Boc-protected derivative **10b** afforded the desired optically active building block **2** in good yield.

To synthesize an alkyne-linked enamine, we first envisioned a synthesis analogous to the method published by Riguet et al.[40] In this approach, the amino groups of neamine were protected with trityl groups, which are removable under very mild conditions. In fact, owing to their intrinsic lability we had problems with spontaneous cleavage of the trityl protecting groups during liquid chromatography, even when neutral or alkaline aluminium oxide was



Scheme 3. Synthesis of neamine **1** and the 2-DOS derivative **2**.

used as the stationary phase. The main difficulties with this methodology, however, arose with the partial protection of the hydroxy functions as PMB ethers, which resulted in a mixture of the two- and the desired three-fold protected neamine. Instead of the reported equimolar product formation, in the best case we obtained a molar ratio of about 4:1, which makes this approach very ineffective (Scheme 4).

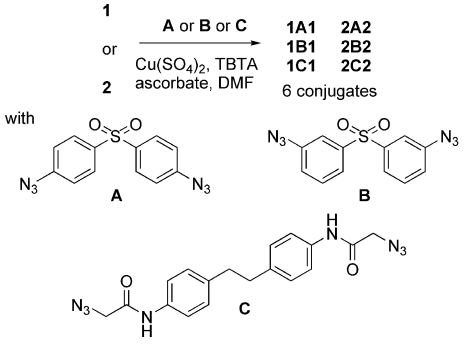


Scheme 4.

Owing to these difficulties, we decided to rely on the chemistry established for the synthesis of the 2-DOS derivative **2**. We treated the intermediate ketal **8b** with 1.2 equiv. of TBDMS chloride, which gave the regioisomer **12** in 84% isolated yield.[41] When **12** was treated with sodium hydride and 10 equiv. of propargyl bromide, a disubstituted product was formed, which was not subjected to detailed structural characterization (data not shown). Only minimal amounts of this side-product were observed when 2 equiv. of propargyl bromide were added. This, however, led to only moderate reaction yields of the desired product **13**. After cleavage of the silyl ether and acidic treatment of the intermediate **14**, the neamine **1** was isolated in good yield.

To test whether the newly developed neamine building block **1** and the 2-DOS derivative **2** are amenable to the copper-catalyzed 1,3-cycloaddition to diazides, conjugation was attempted with three different linkers (Scheme 5). In contrast to the method described by Hergenrother and coworkers,[25] we obtained the best conjugation yields in a mixture of DMF and water using a mixture of copper(II) sulfate and sodium ascorbate in the presence of the tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) ligand.[42]

The conjugates were purified in parallel and the procedure included liquid chromatography on silica gel followed by chromatography on RP-18, mainly to remove coeluted silica gel. After purification we noticed a massive loss of substance with the isolated yields varying between 15 and 35%. In contrast to the 2-DOS derivative **2**, which was completely converted into the divalent conjugates in all cases according to TLC, the conjugation yields for the neamine building block **1** varied and in some cases the mono-conjugation products were also observed. Possibly, the unprotected amino groups of **1** are able to complex copper ions, thus poisoning the catalyst. However, because we need only milligram amounts of each substance for biological studies, the product loss was favoured over the de-



Scheme 5. Synthesis of the neamine and 2-DOS conjugates.

protection of each compound after the coupling step. When the coupling step was performed on a larger scale, however, the isolated yields were approximately 75% (see the Exp. Sect.).

Biological Studies

With the idea in mind that a small molecule binding to a hairpin-shaped precursor miRNA (pre-miRNA) may lead to inhibition of the Dicer-mediated cleavage and thus the formation of a mature miRNA, we tested the six conjugates in a fluorescence-based assay developed in our group.[29] In a preliminary study, we showed that the promiscuous RNA binder kanamycin A is able to inhibit the maturation of the let-7 RNA with an IC_{50} between 50 and 100 μM.[30] In contrast, all six 2-DOS and neamine conjugates showed much better inhibition of let-7 than kanamycin A. In fact, the IC_{50} values for the 2-DOS and the neamine conjugates were in the ranges of 10–20 and 0.5–1.5 μM, respectively (Table 1).

Table 1. Inhibition of miRNA maturation by the click conjugates.

Conjugate	Inhibition of miRNA maturation: IC_{50} [μM]
1A1	1.31 ± 0.02
1B1	0.63 ± 0.04
1C1	1.10 ± 0.06
2A2	15.1 ± 0.37
2B2	11.1 ± 0.11
2C2	14.7 ± 3.09

Conclusion

In this study we have developed a short, straightforward and cost-effective synthesis of alkyne-conjugated neamine and 2-DOS. These compounds were easily synthesized in half-gram amounts. These compounds were subjected to copper-catalyzed 1,3-dipolar cycloaddition with aromatic and aliphatic dazides to yield conjugate dimers. Thus, we have developed a method for the synthesis of a small library of 2-DOS and neamine dimers. The binding affinity of these dimers to RNA was then tested and the inhibition values for Dicer-mediated let-7 maturation were in most cases one-to-two orders of magnitude better than that of kanamycin A. We will report on the biological investigation of such molecules, including the issue of selectivity between different miRNAs, in due course.

Experimental Section

General: All reagents were purchased in the highest quality available and were used without further purification. Thin-layer chromatography was carried out on aluminium plates from Merck (Kieselgel 60 F_{254}). Flash chromatography was performed on silica gel 60 from Merck and octadecyl-modified silica gel (end-capped) from Machery & Nagel. ^1H and ^{13}C NMR spectra were recorded with a Bruker DPX 300 spectrometer. Chemical shifts are reported in parts per million relative to a residual solvent signal: ^1H ($CDCl_3$) = 7.26 ppm, ^{13}C ($CDCl_3$) = 77.0 ppm; ^1H (CD_3OD) = 3.31 ppm, ^{13}C (CD_3OD) = 49.05 ppm; ^1H (D_2O) = 4.79 ppm. High-resolution mass spectra (HRMS) were obtained by electrospray ionization (ESI) with a Hewlett–Packard GCMS 5995-A spectrometer. Optical rotations were measured with a Perkin–Elmer Polarimeter 241 in a 10 cm cell.

Neamine (**6**)[34] and compounds **7a**,[34] **7b**,[37] **8b**,[37] and **12**[41] were synthesized as described previously. The conjugates **2A2** and **2B2** have been described before.[25]

1,3,2′,6′-Tetraazido-5,6-*O*-cyclohexylideneneamine (8a): 1,1-Dimethoxycyclohexane (2.98 g, 3.15 mL, 20.7 mmol) and *p*-toluenesulfonic acid monohydrate (cat. amount) were added to a solution of 1,3,2′,6′-tetraazidoneamine (**7a**; 1.47 g, 3.45 mmol) in dry dimethylformamide (2.5 mL). The reaction was heated at 50 °C and 25 mbar for 5 h in a rotary evaporator and then quenched by the addition of triethylamine (1 mL). After evaporation the yellow oil was dissolved in chloroform (30 mL) and washed consecutively with water (10 mL) and sat. $NaHCO_3$ (10 mL). The organic layer was dried with magnesium sulfate and the solvent was removed in vacuo. Finally, the 1,3,2′,6′-tetraazido-3′,4′,5,6-di-*O*-cyclohexylideneneamine [R_f = 0.79 (50% ethyl acetate in cyclohexane)] was fully converted into the desired 1,3,2′,6′-tetraazido-5,6-*O*-cyclohexylideneneamine by the following procedure: dry dimethylformamide (25 mL), *p*-toluenesulfonic acid monohydrate (cat. amount) and dry methanol (0.7 mL) were added to 1,3,2′,6′-tetraazido-3′,4′,5,6-di-*O*-cyclohexylideneneamine. This solution was heated for 8 h in a rotary evaporator (50 °C, 25 mbar) and quenched with triethylamine (1 mL). After evaporation, the crude product was purified by chromatography on silica gel (25→33% ethyl acetate in cyclohexane) to yield **8a** (1.49 g, 2.94 mmol, 85%) as a colourless oil. R_f = 0.37 (50% ethyl acetate in cyclohexane). $[a]_D^{22}$ = +93.7 (*c* = 1.0, chloroform). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.36–1.55 (m, 3 H, 2_{eq}-CH_2, CH_2), 1.57–1.70 (m, 8 H, CH_2), 2.32 (td, *J* = 5.0, 13.3 Hz, 1 H, 2_{ax}-CH_2), 3.25 (dd, *J* = 3.6, 10.5 Hz, 1 H, 2′-CH_2), 3.42 (m, 1 H, 6-CH), 3.47–3.61 (m, 5 H, 3-, 4-, 5′-CH, 6′-CH_2), 3.62–3.69 (m, 1 H, 1-CH), 3.79–3.85 (m, 1 H, 5-CH), 3.90–4.02 (m, 2 H, 3′-, 4′-CH), 5.48 (d, *J* = 3.6 Hz, 1 H, 1′-CH) ppm. ^{13}C NMR (75 MHz, CD_3CN): δ = 2 × 23.6 and 24.7 (CH_2), 33.6 (2-CH_2), 35.9 and 36.1 (CH_2), 51.0 (6′-CH_2), 57.0 (1-CH), 60.7 (3-CH), 62.4 (2′-CH), 70.9, 71.0 and 71.2 (3′-, 4′-, 5′-CH), 76.9 (5-CH), 79.1 and 79.2 (4-, 6-CH), 96.1 (1′-CH), 113.5 (C_q) ppm. HRMS: calcd. for $C_{18}H_{30}N_{13}O_6$ $[M + NH_4]^+$ 524.2442; found 524.2454.

1,3-Diazido-5,6-*O*-cyclohexylidene-2-deoxystreptamine (9a): A solution of 1,3,2′,6′-tetraazido-5,6-*O*-cyclohexylideneneamine (2.67 g, 5.28 mmol) in methanol (190 mL) was cooled to 0 °C. After addition of $NaIO_4$ (8.47 g, 39.6 mmol), the reaction mixture was stirred for 15 h at room temperature. The formation of the desired

dialdehyde was monitored by TLC [R_f = 0.74 (50% ethyl acetate in cyclohexane)]. The precipitate was filtered through Celite and the solution was concentrated in vacuo. The residue was dissolved in ethyl acetate (260 mL) and washed with water (260 mL) and sat. $NaHCO_3$ (290 mL). The organic layer was dried with Na_2SO_4 and the solvents were evaporated. The solid was then dissolved in methanol (260 mL) and *n*-butylamine (1.15 g, 1.56 mL, 15.8 mmol) was added dropwise. After 2 h stirring at room temperature *n*-butylamine (0.74 g, 1 mL, 10.1 mmol) was added again and the mixture was stirred for an additional 24 h. The crude product was purified by chromatography on silica gel (0→5% ethyl acetate in cyclohexane) to yield **9a** (1.30 g, 4.40 mmol, 83%) as yellow crystals. R_f = 0.43 (30% ethyl acetate in cyclohexane). $[\alpha]_D^{22}$ = +13.3 (*c* = 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.38–1.47 (m, 3 H, 2_{eq}-CH_2, CH_2), 1.61–1.69 (m, 8 H, CH_2), 2.33 (td, *J* = 4.9, 13.6 Hz, 1 H, 2_{ax}-CH_2), 3.05 (br., 1 H, OH), 3.36–3.47 (m, 3 H, 4-, 5-, 6-CH), 3.61–3.80 (m, 2 H, 1-, 3-CH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.6, 23.7 and 24.9 (CH_2), 33.7 (2-CH_2), 36.2 and 36.2 (CH_2), 57.4 (1-CH), 62.4 (3-CH), 74.2 (4-CH), 79.1 and 79.2 (5-, 6-CH), 113.6 (C_q) ppm. HRMS: calcd. for $C_{12}H_{18}N_6O_3$ $[M + H]^+$ 295.1513; found 295.1518.

1,3-Bis-*N*-(*tert*-butyloxycarbonyl)-5,6-*O*-cyclohexylidene-2-deoxystreptamine (9b): $NaIO_4$ (18.8 g, 88.5 mmol) was added to an ice-cold solution of **8b** (9.44 g, 11.8 mmol) in tetrahydrofuran/water (1:1, 200 mL). The solution was stirred overnight at room temperature. The white precipitate was filtered through Celite and the filtrate was concentrated to dryness in vacuo. The white residue was dissolved in ethyl acetate (100 mL) and the resulting solution was washed with water (200 mL) and brine (200 mL). The organic layer was dried (Na_2SO_4) and the solvent was removed under reduced pressure. The residue was dissolved in methanol (275 mL) and *n*-butylamine (2.59 g, 35.4 mmol) was added slowly. After stirring overnight at room temperature the solvent was removed under reduced pressure. The residue was purified by flash chromatography (30→50% ethyl acetate in cyclohexane) to yield **9b** (2.49 g, 5.54 mmol, 47%) as a white solid. R_f = 0.18 (40% ethyl acetate in cyclohexane). $[\alpha]_D^{22}$ = –23.0 (*c* = 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.37 (m, 21 H, CH_2, CH_3, 2_{eq}-CH_2), 1.52–1.64 (m, 8 H, CH_2), 1.92 (br., 1 H, OH), 2.40–2.58 (m, 1 H, 2_{ax}-CH_2), 3.31–3.37, 4.41–3.48, 3.51–3.57 and 3.63–3.72 (m, 5 H, 1-, 3-, 4-, 5-, 6-CH), 4.74 (d, *J* = 5.9 Hz, 2 H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.6, 23.6 and 2 × 24.9 (CH_2), 6 × 28.3 (CH_3), 36.1 and 36.3 (2-CH_2, CH_2), 49.2 (1-CH), 53.4 (3-CH), 74.1 (4-CH), 77.9 (5-CH), 79.8 and 80.4 [$C_q(CH_3)_3$], 80.7 (6-CH), 112.6 (C_q), 155.3 and 156.5 [C_q(O)] ppm. HRMS: calcd. for $C_{22}H_{39}N_2O_7$ $[M + H]^+$ 443.2752; found 443.2754; calcd. for $C_{22}H_{42}N_3O_7$ $[M + NH_4]^+$ 460.3017; found 460.3021; calcd. for $C_{22}H_{38}N_2NaO_7$ $[M + Na]^+$ 465.2571; found 465.2578.

1,3-Bis-*N*-(*tert*-butyloxycarbonyl)-5,6-*O*-cyclohexylidene-4-*O*-propargyl-2-deoxystreptamine (10b): Streptamine derivative **9b** (1.43 g, 3.23 mmol) was dissolved in dry tetrahydrofuran (45 mL) and cooled to 0 °C and then NaH (0.26 g, 6.47 mmol, 60% in mineral oil) was added. After stirring for 1 h at 0 °C a catalytic amount of tetrabutylammonium iodide (TBAI) and a solution of propargyl bromide (0.96 g, 6.47 mmol, 80% in toluene) in dry tetrahydrofuran (5 mL) were added. The ice-bath was removed and the reaction was stirred for 3 d at room temperature. The addition of TBAI was repeated every 24 h. Water (5 mL) was used to quench the reaction, which was then poured into a mixture of water (30 mL) and diethyl ether (60 mL). The aqueous layer was extracted with diethyl ether (2 × 50 mL) and the organic layer was washed with sat. $NaHCO_3$ (30 mL). All the aqueous layers were re-extracted with diethyl ether (2 × 50 mL) and the combined organic layers were dried with Na_2SO_4 and the solvents evaporated. Purification by chromatography on silica gel (0→2% methanol in chloroform) provided **10b** as a colourless solid (1.11 g, 2.31 mmol, 72%). R_f = 0.34 (30% ethyl acetate in cyclohexane). ^{1}H NMR (300 MHz, CD_3OD): δ = 1.44 (m, 21 H, CH_2, CH_3, 2_{eq}-CH_2), 1.65 (m, 8 H, CH_2), 2.40–2.58 (td, *J* = 4.6, 13.2 Hz, 1 H, 2_{ax}-CH_2), 2.81 (t, *J* = 2.4 Hz, 1 H, ≡CH), 3.38–3.71 (m, 5 H, 1-, 3-, 4-, 5-, 6-CH), 4.37 (dq, *J* = 2.4, 15.6 Hz, 2 H, O-CH_2) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 2 × 24.9 and 26.2 (CH_2), 3 × 28.8 and 3 × 28.8 (CH_3), 2 × 37.3 and 37.5 (2-CH_2, CH_2), 50.1 and 52.8 (1-, 3-CH), 59.1 (O-CH_2), 2 × 75.7 [$C_q(CH_3)_3$], 79.8, 80.3, 80.6, 81.0 and 82.3 (4-, 5-, 6-CH and *C*≡*C*H), 112.3 (C_q), 157.7 and 158.0 [C_q(O)] ppm. HRMS: calcd. for $C_{25}H_{41}N_2O_7$ $[M + H]^+$ 481.2908; found 481.2914.

Pentacyclic Internal Cycloaddition Product 11: 1,3-Diazido-5,6-*O*-cyclohexylidene-2-deoxystreptamine (0.20 g, 0.68 mmol) and a small portion of tetrabutylammonium iodide were dissolved in dry toluene (1 mL) in a dry Schlenk flask. Then NaH (0.14 g, 3.40 mmol, 60% in mineral oil) and after 15 min propargyl bromide (0.11 g, 0.08 mL, 0.75 mmol, 80% in toluene) were added over a period of 5 min. After 24 h the reaction was quenched by the addition of water (1 mL). Subsequently the solution was poured into a mixture of diethyl ether (10 mL) and water (10 mL) and extracted three times with diethyl ether (3 × 5 mL). The collected organic phases were washed with brine (5 mL) and dried with magnesium sulfate. After removal of the solvent, the residue was purified by chromatography on silica gel (0→5% ethyl acetate in cyclohexane) to give **11** (0.22 g, 0.67 mmol, 99%) as a colourless solid. R_f = 0.44 (30% ethyl acetate in cyclohexane) and 0.02 after 16 h in CD_3OD. ^{1}H NMR (300 MHz, CD_3OD): δ = 1.40–1.50 (m, 2 H, CH_2), 1.60–1.95 (m, 9 H, CH_2, 2_{eq}-CH_2), 3.19 (td, *J* = 4.6, 13.1 Hz, 1 H, 2_{ax}-CH_2), 3.69 (dd, *J* = 8.8, 10.1 Hz, 1 H, 6-CH), 3.82 (t, *J* = 9.2 Hz, 1 H, 5-CH), 3.94 (t, *J* = 9.3 Hz, 1 H, 4-CH), 4.03–4.12 (m, 1 H, 5-CH), 4.22–4.34 (m, 1 H, 1-CH), 4.98 (td, *J* = 0.9, 15.5 Hz, 1 H, O-CH*H*), 5.21 (d, *J* = 15.5 Hz, 1 H, O-CH*H*), 7.54 (s 1 H, C_q=*C*H) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 2 × 24.7 and 26.0 (CH_2), 31.6 (2-CH_2), 37.1 and 37.2 (CH_2), 58.2 and 58.9 (1-, 3-CH), 63.4 (O-CH_2), 77.5 (4-CH), 78.7 (5-CH), 81.6 (6-CH), 114.3 (C_q), 129.5 and 132.7 (C_q=*C*H) ppm. HRMS (ESI): calcd. for $C_{15}H_{21}N_6O_3$ $[M + H]^+$ 333.1670; found 333.1669; calcd. for $C_{15}H_{20}N_6NaO_3$ $[M + Na]^+$ 355.1489; found 355.1489.

4-*O*-Propargyl-2-deoxystreptamine (2): The fully protected alkyne **10b** (0.72 g, 1.52 mmol) was dissolved in dichloromethane (8 mL) and trifluoroacetic acid (4 mL) was added. After 30 min the mixture was concentrated in vacuo and purified by chromatography on silica gel [0→10% NH_4OH (28–30%) in methanol] followed by a purification with reversed-phase chromatography to remove eluted silica gel. Yield: 0.55 g, 1.29 mmol, 85%, colourless solid. R_f = 0.38 [10% NH_4OH (28–30%) in methanol]. $[\alpha]_D^{22}$ = +31.5 (*c* = 1.0, water). ^{1}H NMR (300 MHz, CD_3OD): δ = 1.84 (q, *J* = 12.3 Hz, 1 H, 2_{eq}-CH_2), 2.43 (m, 1 H, 2_{ax}-CH_2), 2.99 (t, *J* = 2.3 Hz, 1 H, ≡CH), 3.16–3.28 (m, 2 H, 1-, 3-CH), 3.39–3.57 (m, 3 H, 4-, 5-, 6-CH), 4.60 (m, 2 H, O-CH_2) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 30.0 (2-CH_2), 50.7 and 51.6 (1-, 3-CH), 60.6 (O-CH_2), 74.4 (5-CH), 77.1 (≡CH), 77.7 and 80.1 (4-, 6-CH), 80.7 (C_q≡) ppm. HRMS: calcd. for $C_9H_{17}N_2O_3$ $[M + H]^+$ 201.1239; found 201.1234.

1,3,2′,6′-Tetrakis-*N*-*tert*-(butyloxycarbonyl)-5,6-*O*-cyclohexylidene-3′-*O*-(*tert*-butyldimethylsilyl)-4′-*O*-propargylneamine (13): NaH (0.09 g, 2.18 mmol, 60% suspension in mineral oil) was added to an ice-cold solution of **12** (1.00 g, 1.09 mmol) in anhydrous tetrahydrofuran (15 mL) under argon. After stirring for 1 h at room temperature, a small amount of tetrabutylammonium iodide and, within 5 min, propargyl bromide (0.32 g, 2.18 mmol) were added.

After stirring the solution for 2 d at room temperature, the reaction was quenched by the addition of methanol (1 mL). The solution was poured into a mixture of water (30 mL) and diethyl ether (30 mL). The aqueous layer was separated and extracted with diethyl ether (2 × 30 mL). The organic layer was back-extracted with brine (30 mL), dried (Na_2SO_4) and concentrated to dryness under reduced pressure. The yellow residue was purified by flash chromatography (20→40% ethyl acetate in cyclohexane) to yield **13** (0.53 g, 0.56 mmol, 51%) as a white foam. R_f = 0.50 (33% ethyl acetate in cyclohexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.03 [s, 6 H, $Si(CH_3)_2$], 0.81 [s, 9 H, $SiC_q(CH_3)_3$], 1.38–1.59 (m, 47 H, CH_2, CH_3, 2_{eq}-CH_2), 2.18 (m, 1 H, C≡CH), 2.48–2.52 (m, 1 H, 2_{ax}-CH_2), 3.44–4.19 (m, 13 H, 1-, 3-, 4-, 5-, 6-, 2′-, 3′-, 4′-, 5′-CH, 6′-CH_2, O-CH_2), 4.65–4.98 (m, 5 H, NH, 1′-CH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = –5.1 and –4.1 [$Si(CH_3)_2$], 18.2 [SiC_q-$(CH_3)_3$], 23.7, 24.9 and 26.8 (CH_2), 3 × 25.8 [$SiC_q(CH_3)_3$], 28.1–28.4 (12 × CH_3), 2 × 36.0 and 36.2 (2-CH_2, CH_2), 38.5 (6′-CH_2), 46.7 (O-CH_2), 49.0, 53.9 and 55.0 (1-, 3-, 2′-CH), 71.0, 71.4, 71.5, 72.1 and 72.9 (4-, 5-, 3′-, 4′-, 5′-CH), 78.4, 79.0, 80.0 and 80.8 ($C_q$$CH_3$), 79.8 (C≡*C*H), 80.3 (6-CH), 81.4 (*C*≡CH), 99.2 (1′-CH), 112.4 (C_q), 2 × 154.9, 155.1 and 155.4 [C_q(O)] ppm. HRMS: calcd. for $C_{47}H_{83}N_4O_{14}Si$ [M + H]$^+$ 955.5670; found 955.5670; calcd. for $C_{47}H_{86}N_5O_{14}Si$ [M + NH_4]$^+$ 972.5935; found 972.5932; calcd. for $C_{47}H_{82}N_4NaO_{14}Si$ [M + Na]$^+$ 977.5489; found 977.5484.

4′-*O*-Propargylneamine (1): Tetrabutylammonium fluoride trihydrate (TBAF; 0.97 g, 3.08 mmol) was added to a solution of **13** (0.67 g, 0.7 mmol) in dry tetrahydrofuran (15 mL) and the solution was stirred for 20 h at room temperature. The ice-cold solution was quenched with water (10 mL), extracted with DCM (3 × 40 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the resulting residue was purified by flash chromatography (33→50% ethyl acetate in cyclohexane) to yield the desilylated intermediate (0.47 g, 0.56 mmol, 81%) as a white solid. R_f = 0.29 (50% ethyl acetate in cyclohexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.38–1.57 (m, 47 H, CH_2, CH_3, 2_{eq}-CH_2), 2.18 (t, *J* = 2.21 Hz, 1 H, C≡CH), 2.38–2.44 (m, 1 H, 2_{ax}-CH_2), 3.22–4.35 (m, 13 H, 1-, 3-, 4-, 5-, 6-, 2′-, 3′-, 4′-, 5′-CH, 6′-CH_2, O-CH_2), 4.84–5.17 (m, 5 H, NH, 1′-CH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.5, 23.6 and 24.8 (CH_2), 28.1–28.3 (12 × CH_3), 2 × 35.9 and 36.2 (2-CH_2, CH_2), 38.7 (6′-CH_2), 47.3 (O-CH_2), 48.9, 50.9 and 54.6 (1-, 3-, 2′-CH), 71.0, 71.8, 71.9, 72.2 and 72.3 (4-, 5-, 3′-, 4′-, 5′-CH), 79.4, 79.6, 79.8 and 79.9 ($C_q$$CH_3$), 80.2 (C≡*C*H), 80.4 (6-CH), 81.4 (*C*≡CH), 98.5 (1′-CH), 112.4 (C_q), 155.0, 155.1, 156.5 and 156.8 [C_q(O)] ppm. HRMS: calcd. for $C_{41}H_{69}N_4O_{14}$ [M + H]$^+$ 841.4805; found 841.4805; calcd. for $C_{41}H_{72}N_5O_{14}$ [M + NH_4]$^+$ 858.5070; found 858.5076; calcd. for $C_{41}H_{68}N_4NaO_{14}$ [M + Na]$^+$ 863.4624; found 863.4629.

This white solid (0.22 g, 0.26 mmol) was stirred in DCM (1.5 mL), water (0.7 mL) and trifluoroacetic acid (1.0 mL) for 2 h at room temperature. The solution was concentrated to dryness and the residue was purified by flash chromatography [0→20% NH_4OH (28–30%) in methanol]. To separate any silica gel eluted, the residue obtained was purified again by flash chromatography on a reversed phase to yield **1** (0.19 g, 0.23 mmol, 88%) as a white solid. R_f = 0.17 [10% NH_4OH (28–30%) in methanol]. ^{1}H NMR (300 MHz, D_2O): δ = 1.82–1.95 (q, *J* = 12.5 Hz, 1 H, 2_{eq}-CH_2), 2.46–2.50 (td, *J* = 3.8, 12.4 Hz, 1 H, 2_{ax}-CH_2), 3.00 (t, *J* = 2.5 Hz, 1 H, C≡CH), 3.17–3.66 and 3.87–4.10 (m, 13 H, 1-, 3-, 4-, 5-, 6-, 2′-, 3′-, 4′-, 5′-CH, 6′-CH_2, O-CH_2), 5.94 (d, *J* = 3.7 Hz, 1 H, 1′-CH) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 29.2 (2-CH_2), 37.0 (6′-CH_2), 47.4 (O-CH_2), 48.4, 48.8 and 53.6 (1-, 3-, 2′-CH), 68.8, 69.5, 71.0, 72.6, 75.0, 75.2 and 78.9 (4-, 5-, 6-, 3′-, 4′-, 5′-CH, C≡*C*H), 76.6 (*C*≡CH), 97.0 (1′-CH) ppm. HRMS: calcd. for $C_{15}H_{29}N_4O_6$ [M + H]$^+$ 361.2082; found 361.2080; calcd. for $C_{15}H_{30}N_4O_6$ [M + 2H]$^{2+}$ 181.1077; found 181.1076.

General Procedure for the Synthesis of the Conjugates: TBTA (15 mol-%, 0.09 M solution in DMF) was added to a solution of the alkyne-substituted 2-deoxystreptamine (**2**) or neamine (**1**) (33 μmol, 0.05 M in DMF) and the corresponding diazide (15 μmol). After degassing, a freshly prepared sodium ascorbate solution (30 mol-%, 1 M in water) was added followed by 15 mol-% of a Cu^{II} sulfate solution (0.35 M in water) and the mixture was shaken for 10 d at 50 °C. Then the solution was evaporated to a minimum and the residue was purified by column chromatography on silica gel using a gradient from 0 to 15% (for 2-DOS derivatives) or 0 to 30% (for neamine derivatives) aqueous ammonium hydroxide solution (28–30%) in methanol. To separate any silica gel eluted, the residue obtained was dissolved in water (2 mL) and purified again through a reversed-phase column. The isolated substances were pure by NMR and HPLC–MS or UPLC–MS.

When the reactions were carried out on a larger scale (165 μM of alkyne in a 0.15 M DMF stock solution), the isolated yields were all around 75%, respectively.

Conjugate 1A1: ^{1}H NMR (300 MHz, D_2O): δ = 1.86 (q, *J* = 12.6 Hz, 2 H, 2_{eq}-CH_2), 2.41 (dt, *J* = 12.4, 4.1 Hz, 2 H, 2_{ax}-CH_2), 3.24–3.68 (m, 16 H, 1-, 3-, 4-, 5-, 6-, 4′-CH, 6′-CH_2), 3.91–4.09 (m, 6 H, 2′-, 3′-, 5′-CH), 4.54 (s, 4 H, O-CH_2), 5.91 (d, *J* = 3.8 Hz, 2 H, 1′-CH), 7.98–8.01 and 8.13–8.16 (m, 8 H, CH_{ar}), 8.71 (s, 2 H, C_q=CH) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 28.1 (2-CH_2), 41.9 (O-CH_2), 47.7 (6′-CH_2), 48.3 and 49.6 (1-, 3-CH), 53.4, 68.0, 68.9, 70.9, 72.4, 75.1 and 77.3 (4-, 5-, 6-, 2′-, 3′-, 4′-, 5′-CH), 95.7 (1′-CH_2), 2 × 121.7 (CH_{ar}), 125.0 (C_q=*C*H), 2 × 129.6 (CH_{ar}), 138.7, 139.7 and 140.0 (2 × $C_{q\text{-}ar}$, C_q=CH) ppm. HRMS: calcd. for $C_{42}H_{65}N_{14}O_{14}S$ [M + H]$^+$ 1021.4520; found 1021.4542.

Conjugate 1B1: ^{1}H NMR (300 MHz, D_2O): δ = 1.86 (q, *J* = 12.6 Hz, 2 H, 2_{eq}-CH_2), 2.46 (dt, *J* = 12.4, 4.1 Hz, 2 H, 2_{ax}-CH_2), 3.25–3.68 (m, 16 H, 1-, 3-, 4-, 5-, 6-, 4′-CH, 6′-CH_2), 3.93–4.10 (m, 6 H, 2′-, 3′-, 5′-CH), 4.55 (s, 4 H, O-CH_2), 5.93 (br., 2 H, 1′-CH), 7.78–7.83, 8.06–8.12 and 8.42 (m, 8 H, CH_{ar}), 8.71 (s, 2 H, C_q=CH) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 28.1 (2-CH_2), 42.0 (O-CH_2), 47.8 (6′-CH_2), 48.4 and 49.6 (1-, 3-CH), 53.4, 68.0, 68.1, 70.9, 72.4, 75.1 and 77.3 (4-, 5-, 6-, 2′-, 3′-, 4′-, 5′-CH), 95.8 (1′-CH_2), 119.9 (CH_{ar}), 125.0 (C_q=*C*H), 126.6, 128.5 and 131.8 (CH_{ar}), 136.9, 138.6 and 140.9 (2 × $C_{q\text{-}ar}$, C_q=CH) ppm. HRMS: calcd. for $C_{42}H_{65}N_{14}O_{14}S$ [M + H]$^+$ 1021.4520; found 1021.4528.

Conjugate 1C1: ^{1}H NMR (300 MHz, D_2O): δ = 1.90 (q, *J* = 12.6 Hz, 2 H, 2_{eq}-CH_2), 2.47 (dt, *J* = 12.4, 4.1 Hz, 2 H, 2_{ax}-CH_2), 2.84 (br., 4 H, CH_2), 3.26–3.70 (m, 16 H, 1-, 3-, 4-, 5-, 6-, 4′-CH, 6′-CH_2), 3.94–4.09 (m, 6 H, 2′-, 3′-, 5′-CH), 4.54 (s, 4 H, O-CH_2), 5.94 (d, *J* = 3.8 Hz, 2 H, 1′-CH), 7.13–7.16 and 7.28–7.32 (m, 8 H, CH_{ar}), 8.24 (s, 2 H, C_q=CH) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 28.1 (2-CH_2), 36.1 (CH_2), 42.0 (O-CH_2), 47.7 (6′-CH_2), 48.4 and 49.6 (1-, 3-CH), 52.4 [*C*H_2C_q(O)], 53.4, 68.0, 68.9, 71.0, 72.4, 75.1 and 77.2 (4-, 5-, 6-, 2′-, 3′-, 4′-, 5′-CH), 95.7 (1′-CH_2), 2 × 118.2 (CH_{ar}), 127.8 (C_q=*C*H), 2 × 129.2 (CH_{ar}), 134.0, 137.7 and 139.5 (2 × $C_{q\text{-}ar}$, C_q=CH), 165.8 [C_q(O)] ppm. HRMS: calcd. for $C_{48}H_{75}N_{16}O_{14}$ [M + H]$^+$ 1099.5643; found 1099.5676.

Conjugate 2A2: ^{1}H NMR (300 MHz, D_2O): δ = 1.81 (q, *J* = 12.5 Hz, 2 H, 2_{eq}-CH_2), 2.43 (dt, *J* = 4.2, 12.5 Hz, 2 H, 2_{ax}-CH_2), 3.24–3.43 (m, 4 H, 1-, 3-CH), 3.51–3.66 (m, 6 H, 4-, 5-, 6-CH), 4.95 (d, *J* = 12.3 Hz, 2 H, O-C*H*H), 5.14 (d, *J* = 12.3 Hz, 2 H, O-C*H*H), 7.91 (d, *J* = 8.8 Hz, 4 H, CH_{ar}), 8.06 (d, *J* = 8.8 Hz, 4 H, CH_{ar}), 8.53 (s, 2 H, C_q=CH) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 28.1 (2-CH_2), 49.0 and 49.8 (1-, 3-CH), 64.6 (O-CH_2), 72.4, 75.4

and 79.8 (4-, 5-, 6-CH), 2×121.4 (CH_{ar}), 123.3 (C_q=*C*H), 2×129.6 (CH_{ar}), 139.5, 140.1 and 144.8 (2×$C_{q\text{-}ar}$, C_q=CH) ppm. HRMS: calcd. for $C_{30}H_{41}N_{10}O_8S$ $[M + H]^+$ 701.2824; found 701.2820.

Conjugate 2B2: ^{1}H NMR (300 MHz, D_2O): δ = 1.82 (q, J = 12.5 Hz, 2 H, 2_{eq}-CH_2), 2.43 (dt, J = 4.1, 12.2 Hz, 2 H, 2_{ax}-CH_2), 3.25–3.43 (m, 4 H, 1-, 3-CH), 3.52–3.69 (m, 6 H, 4-, 5-, 6-CH), 4.92–4.97 (m, 2 H, O-C*H*H), 5.12–5.16 (m, 2 H, O-C*H*H), 7.65–7.79 and 7.91–8.06 (m, 6 H, CH_{ar}), 8.31 (br., 2 H, CH_{ar}), 8.51 (s, 2 H, C_q=CH) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 28.1 (2-CH_2), 49.0 and 49.8 (1-, 3-CH), 64.6 (O-CH_2), 72.4, 75.4 and 79.8 (4-, 5-, 6-CH), 119.6 (CH_{ar}), 123.1 (C_q=*C*H), 126.3, 128.2 and 131.7 (CH_{ar}), 2×137.0 and 140.9 (2×$C_{q\text{-}ar}$, C_q=CH) ppm. HRMS: calcd. for $C_{30}H_{41}N_{10}O_8S$ $[M + H]^+$ 701.2824; found 701.2823.

Conjugate 2C2: ^{1}H NMR (300 MHz, D_2O): δ = 1.79 (q, J = 12.5 Hz, 2 H, 2_{eq}-CH_2), 2.41 (dt, J = 4.3, 12.5 Hz, 2 H, 2_{ax}-CH_2), 2.78 (br., 4 H, CH_2), 3.22–3.34 (m, 4 H, 1-, 3-CH), 3.50–3.64 (m, 6 H, 4-, 5-, 6-CH), 4.88–4.92 (m, 2 H, O-C*H*H), 5.07–5.11 (m, 2 H, O-C*H*H), 5.36 [br., 4 H, CH_2C(O)], 7.05–7.17 and 7.23–7.32 (m, 8 H, CH_{ar}), 8.06 (s, 2 H, C_q=CH) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 28.1 (2-CH_2), 36.1 (CH_2), 49.0 and 49.8 (1-, 3-CH), 52.4 [*C*H_2C_q(O)], 64.6 (O-CH_2), 72.4, 75.4 and 79.7 (4-, 5-, 6-CH), 2×121.4 (CH_{ar}), 126.6 (C_q=*C*H), 2×129.1 (CH_{ar}), 134.0, 139.4 and 143.8 (2×$C_{q\text{-}ar}$, C_q=CH), 165.9 [C_q(O)] ppm. HRMS: calcd. for $C_{36}H_{51}N_{12}O_8$ $[M + H]^+$ 779.3947; found 779.3964.

Supporting Information (see also the footnote on the first page of this article): Conversion of **10a** into **11** (NMR data), NMR spectra for all 2-DOS and neamine conjugates, analytical UPLC data, inhibition of let-7 miRNA: IC_{50} curves for all conjugates.

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