

Synthesis and antibacterial activity of novel neamine derivatives:  
preponderant role of the substituent position on the neamine core†Nicolas Gernigon,<sup>a</sup> Valérie Bordeau,<sup>b</sup> Fabienne Berrée,<sup>a</sup> Brice Felden\*<sup>b</sup> and Bertrand Carboni\*<sup>a</sup>

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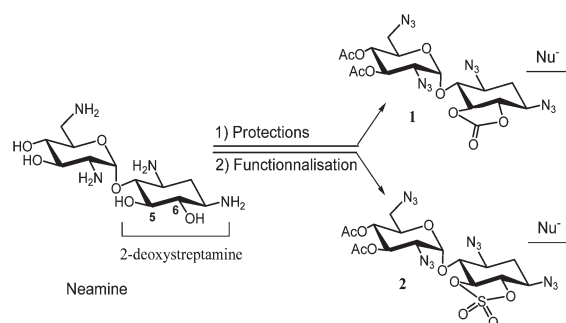
A series of neamine derivatives were prepared from the cyclic carbonate and sulfate of 1,3,2',6'-tetraazido-3',4',-di-*O*-acetylneamine. Ring opening reactions with diversely substituted amines result in the formation of the corresponding carbamates or sulfonic acids with good overall yields. The antibacterial activities of the synthesized products against *E. coli* (DH5 $\alpha$ ) and *S. aureus* (RN4220) were evaluated. With isolated single regioisomers, the preponderant effect of the 5-positions of the carbamate substituent on the neamine core was demonstrated.

## Introduction

Aminoglycosides have been used as antibacterials for decades, but it is only recently that they have been recognized for their antiviral properties and their importance in the treatment of genetic diseases.<sup>1</sup> Their use, however, has been slowed down by problems associated with bacterial resistance, nephrotoxicity or ototoxicity resulting from the non-specific binding of RNAs.<sup>2</sup> Aminoglycosides exert their biological functions by binding to the A-site and decoding the region of the prokaryotic ribosomal RNA small subunit.<sup>3</sup> They interact with unpaired adenine residues in the decoding loop, displacing the unpaired adenines and locking them into a “flipped-out” orientation similar to that which occurs during mRNA decoding.<sup>4</sup> Consequently, they interfere with the protein synthesis in bacteria, in turn inducing cell death.

The key structural feature of this family of antibiotics is streptomine, a 1,3-diaminocyclohexanetetraol unit or 2-deoxystreptomine in cases where the 2-hydroxy function is deoxygenated as in most aminoglycosides (Scheme 1).<sup>5</sup> Analysis of the structural elements common to the natural or semisynthetic aminoglycoside family highlights that a number of them (*e.g.* neomycin, ribostamycin, kanamycin B, arbekacin) contain a neamine core.<sup>2</sup> While showing itself to have a modest antibiotic activity, it constitutes however a minimal motif which is required for binding

to the A-site of the 16S ribosomal RNA.<sup>1</sup> Therefore, not surprisingly, this pseudodisaccharide was used as a starting scaffold to generate new potential antibiotics with the aim of circumventing microbial resistance while retaining activity and decreasing the associated toxicity.<sup>6</sup> However, even though major progresses have been made in the understanding of RNA–ligand recognition principles in recent years, the design of RNA-specific ligands remains a difficult task. RNA is a dynamic target and mutual interactions can greatly modify their conformations.<sup>7</sup> The most significant contributions related to association phenomena result from electrostatic interactions between the protonated amino groups and the negatively charged phosphates of the oligoribonucleotide backbone.<sup>8</sup> Keeping this in mind, we decided to maintain these key functional groups and have developed a synthetic strategy that allows the easy introduction of polar substituents on the 5- or 6-*O*-position of the neamine core. Two compounds **1** and **2** possessing, respectively, a cyclic carbonate or a sulfate moiety at the 5,6-positions were selected as key building blocks (Scheme 1).<sup>9</sup> These five-membered rings are likely to undergo a variety of ring opening reactions in the presence of various nucleophiles.<sup>10</sup>



Scheme 1 Synthetic routes to neamine derivatives.

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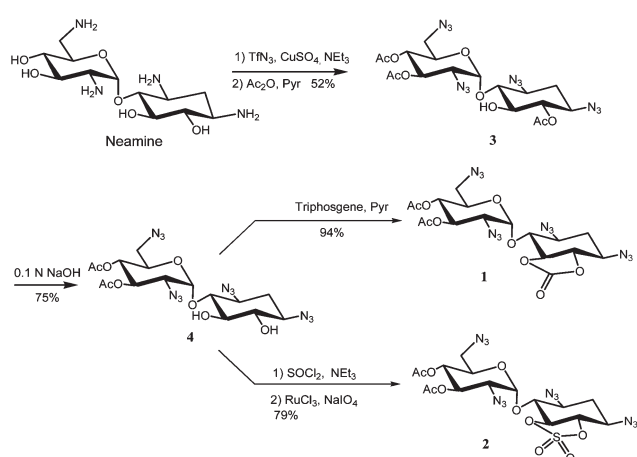
† Electronic supplementary information (ESI) available: Synthesis of **3** and **4**, biological assays for **8a** and **8a + 8b** and <sup>1</sup>H and <sup>13</sup>C NMR spectra of neamine derivatives **1**, **2** and **5–18**. See DOI: 10.1039/c2ob07065k

In this report, we describe the synthesis of these two novel neamine derivatives, **1** and **2**, their reactivity towards amines and a biological evaluation of the antibacterial activities of the resulting products on Gram positive and negative bacteria.

## Results and discussion

Neamine hydrochloride was prepared using a reported procedure through methanolysis of the commercially available neomycin sulfate.<sup>11</sup> It was then protected as its tetraazido derivative in a 82% yield using freshly prepared triflyl azide in the presence of copper sulfate.<sup>12,13</sup> The choice of this amino protecting group, first reported by Wong and co-workers,<sup>12</sup> offers advantages compared with carbamate or *N*-trityl groups.<sup>14</sup> These take into account a better stability, an easier chemoselective deprotection, and a greater solubility and the absence of rotamers that complicate the interpretation of the NMR spectra. The regioselective triacetylation at positions 6, 3' and 4' was undertaken with excess of acetic anhydride in a 1 : 1 CH<sub>2</sub>Cl<sub>2</sub>-pyridine mixture to give compound **3**.<sup>15</sup> Side products, mainly the tetraacetylated derivative, were easily separated by column chromatography and converted after treatment with MeONa in methanol to the corresponding tetrahydroxy derivative to be recycled.<sup>16</sup> Treatment of **3** with 1.3 equivalents of NaOH 0.1 N in THF regioselectively produced the free 5, 6 diol **4**.<sup>17</sup> This results in the disappearance of the peaks at 4.94 ppm (*H*-6) and at 169.2 and 20.1 ppm (COCH<sub>3</sub>), respectively in the <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra. Compound **4** can be converted to the corresponding carbonate **1** with triphosgene in CH<sub>2</sub>Cl<sub>2</sub> in the presence of pyridine in a 94% yield. In parallel, the sulfate **2** was synthesized by using thionyl chloride and subsequent oxidation with ruthenium trichloride in the presence of NaIO<sub>4</sub> (yield, 79%). **1** and **2** were characterised by spectroscopic data (<sup>1</sup>H NMR, <sup>13</sup>C NMR and mass spectra) in agreement with the proposed structures (Scheme 2).

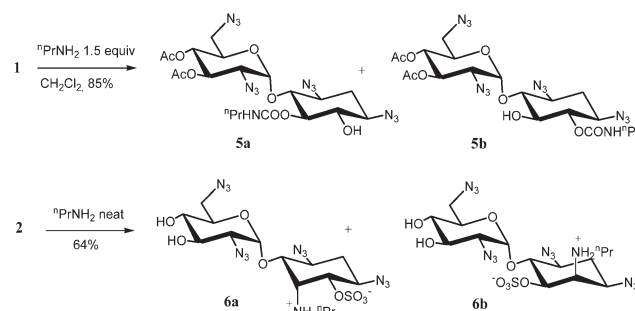
With these two building blocks in hand, we first studied their reactivity towards propylamine as a model experiment. The nucleophilic attack on the carbonate **1** was followed by ring opening to give the carbamates **5a** and **5b**.<sup>18</sup> A complete conversion was observed after 2 h at room temperature in methylene



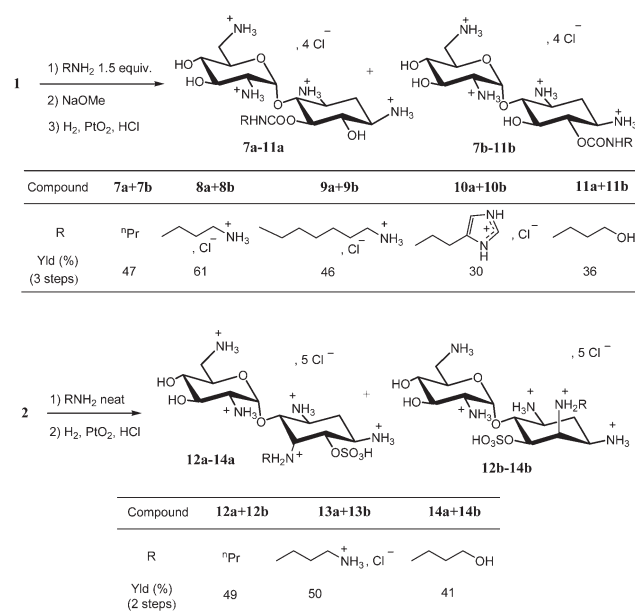
**Scheme 2** Synthesis of the carbonate- and sulfate-containing neamine derivatives **1** and **2**.

chloride. Examination of the <sup>1</sup>H NMR spectrum of the crude material revealed the presence of two regioisomers (1 : 1), as indicated by two signals of anomeric protons at 5.54 and 5.30 ppm. By contrast, the ring opening of the cyclic sulphate **2** did not occur under these conditions. Thus, after an optimization process, the reaction was carried out by using propylamine as solvent. Here too, a 1 : 1 mixture of products **6a** and **6b** (two H-1' at 5.38 and 5.37 ppm and two double doublets at 4.64 and 4.34 ppm for HC(OSO<sub>3</sub>H), H-5 or H-6) was obtained and a complete deacetylation occurring at the 3' and 4' positions was observed (Scheme 3).

We then initiated the synthesis of other neamine derivatives and hypothesized that introducing additional polar substituents on the neamine core would result in higher interactions within the 16S rRNA binding site.<sup>8</sup> The previous transformations were therefore applied similarly to other amines possessing supplementary amino, hydroxy or imidazole groups. Once the carbamates were prepared, they were treated with sodium methylate in methanol, followed by hydrogenation in the presence of platinum oxide and hydrochloric acid,<sup>19</sup> inducing the formation of fully deprotected neamine derivatives **7–11** (Scheme 4). In the case of the sulfonic acids, the azido groups were directly reduced



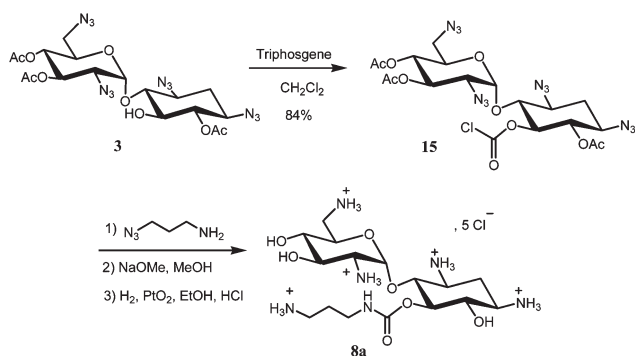
**Scheme 3** Ring opening of **1** and **2** with *n*-propylamine.



**Scheme 4** Synthesis of the neamine derivatives **7–14**.

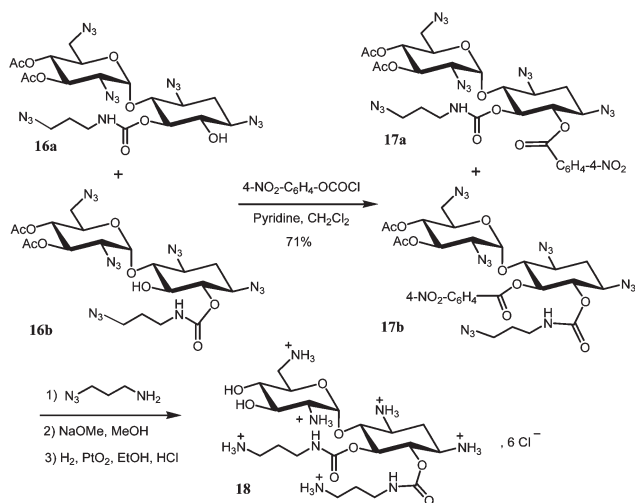
under identical conditions after the ring opening of the cyclic sulfate moiety to produce compounds **12–14**.

To compare the influence of the position of the carbamate substituent on the neamine core, we tried to separate regioisomers **a** and **b**. Many attempts were realized at different steps of the synthetic sequence. Finally, the separation of **8a** and **8b** was achieved just before the last hydrogenation step by HPLC on a C<sub>18</sub> reversed-phase column (Scheme 4). Furthermore, to unmistakably establish the structure of each isomer, **8a** was separately synthesized from the triacetate derivative **3**. After treatment with triphosgene to obtain the chloroformate **15** in 84% yield, the addition of 3-azidopropylamine, followed by deprotection of the hydroxyl groups and hydrogenation with H<sub>2</sub> in the presence of PtO<sub>2</sub> and hydrochloric acid, afforded compound **8a** in 61% overall yield (three steps, Scheme 5).



Scheme 5 Synthesis of neamine derivative **8a**.

At last, to increase the panel of the neamine derivatives and to increase the number of ammonium groups present on the aminoglycoside core, the bis-carbamate **18** was prepared from **16**, an intermediate in the preparation of **8** (1 : 1 mixture of two regioisomers). Addition of 4-nitrophenyl chloroformate gave the carbonates **17a + 17b** in 71% yield. A sequence similar to that described above afforded product **18** (53% yield, three steps, Scheme 6).



Scheme 6 Synthesis of the bis-carbamate **18**.

Table 1 The MICs of the aminoglycoside derivatives **7–14** and **18** compared to neamine ( $\mu\text{g mL}^{-1}$ )

Entry	Compound	<i>E. coli</i> (strain DH5 $\alpha$ )	<i>S. aureus</i> (strain RN4220)
a	Neamine	30 $\pm$ 10	22 $\pm$ 8
b	<b>7a + 7b</b>	>400	>400
c	<b>8a + 8b</b>	70 $\pm$ 15	35 $\pm$ 7
d	<b>8a</b>	50 $\pm$ 15	45 $\pm$ 5
e	<b>8b</b>	>100	>100
f	<b>9a + 9b</b>	250 $\pm$ 50	50 $\pm$ 5
g	<b>10a + 10b</b>	250 $\pm$ 50	250 $\pm$ 50
h	<b>11a + 11b</b>	150 $\pm$ 50	200 $\pm$ 50
i	<b>12a + 12b</b>	>400	>400
j	<b>13a + 13b</b>	50 $\pm$ 10	60 $\pm$ 10
k	<b>14a + 14b</b>	200 $\pm$ 50	300 $\pm$ 50
l	<b>18</b>	250 $\pm$ 50	300 $\pm$ 50

The comparative antibacterial activities of **7–14**, **18** and neamine were evaluated by determining the minimum inhibitory concentrations (MICs) against *E. coli* (DH5 $\alpha$ ) and *S. aureus* (RN4220) cells as described in the Experimental section (Table 1).

The antibacterial activities of eleven new neamine derivatives against gram positive and negative bacteria are presented in Table 1. The introduction of an OCONH-*n*Pr substituent instead of an hydroxyl group at position 5 or 6 causes a loss of activity (entries a and b). When the *n*-propyl extremity of the carbamate moiety was replaced by a 3-aminopropyl, the activity was restored close to that of neamine (entries a and c), whereas the MICs are higher in the cases of OCONH-(CH<sub>2</sub>)<sub>3</sub>-OH or OCONH-(CH<sub>2</sub>)<sub>2</sub>-imidazole (entries g and h). Furthermore, introducing two aminopropyl chains abolished the antibacterial activity against gram positive and negative bacteria (entry l). It is worthy to note the negative influence of an increase in the number of carbon atoms in the aminoalkyl chain observed for *E. coli*, while this modification has little effect for *S. aureus* (RN4220) (entries c and f). It suggests that despite strong sequence conservation, the decoding sites of each bacterium have a slightly different fold. Similar observations can also be drawn with the sulfonic acid derivatives (entries i, j and k). The most remarkable result of these antibacterial assays is the critical influence of the position of the carbamate substituent on the neamine core (entries d and e). The comparison of the two isolated regioisomers **8a** and **8b** demonstrated that compound **8a** is the most active (see the ESI<sup>†</sup> for details). It indicates that carbamate substitution at position 6 is detrimental to the antibacterial activity whereas substitutions at position 5 are tolerated and have only a minor negative functional influence compared to the neamine (Table 1).

## Conclusions

In this study, we developed efficient methods for synthesizing cyclic carbonates and sulfates of 1,3,2',6'-tetraazido-3',4',-di-*O*-acetyneamine which are key precursors for the preparation of novel neamine derivatives. The reported ring opening reactions are applicable to different nucleophiles, allowing various chemical modifications of other aminoglycosides used in clinics. Although the final products showed antibacterial activities

weaker than that of neamine, biological tests revealed the determinant influence of the position of the carbamate substituent on the antibacterial activity. This report is expected to serve as a basis to generate compounds of higher activities against human and animal bacterial pathogens.

## Experimental section

### General procedures

Tetrahydrofuran was distilled from sodium/benzophenone, methylene chloride from  $P_2O_5$  and methanol from sodium. Analytical thin layer chromatography was performed on Merck Silica Gel 60 F254 plates. Silica gel 60H (200–300 mesh) was used for column chromatography. The compounds were characterized by  $^1H$  and  $^{13}C$  NMR techniques including COSY, HMQC and HMBC experiments.  $^1H$  NMR spectra (300 MHz, 500 MHz) and  $^{13}C$  NMR (75 MHz, 125 MHz) were recorded on a Bruker AC 300 and AC 500 spectrometer with TMS as the internal reference. Chemical shifts are given in ppm and coupling constants  $J$  in Hz. High-resolution mass spectra (HRMS) were recorded on a Bruker MicrOTOF-Q II spectrometer (Centre Regional de Mesures Physiques de l'Ouest). Optical rotations were measured by a Rudolph Research Analytical Autopol III Automatic Polarimeter. The HPLC separation of the neamine derivative **8** was carried out on a  $C_{18}$  reversed-phase column (Interchim,  $10 \times 250$  mm) on an Agilent 1200 system with diode array UV detector (220 nm). Samples were eluted at a flow rate of  $3 \text{ mL min}^{-1}$ , using the gradient: 0.1% TFA in acetonitrile/0.1% TFA in water 40/60 v/v. 3-Azidopropylamine and 6-azidohexylamine were prepared according to reported procedures.<sup>20</sup>

#### 1. Synthesis of cyclic carbonate **1** and sulfate **2**. 5,6-[Carbonylbis(oxy)]-3',4'-di-O-acetyl-1,3,2',6'-tetraazidoneamine **1**

To a solution of **4** (2 g, 3.9 mmol) in anhydrous  $CH_2Cl_2$  (40 mL) and pyridine (3.15 mL, 39 mmol) at  $-78^\circ C$  under an Ar atmosphere was added a solution of triphosgene (1.7 g, 5.85 mmol) in anhydrous  $CH_2Cl_2$  (10 mL). The reaction was stirred overnight and allowed to warm to room temperature. The solution was diluted with  $CH_2Cl_2$  (50 mL) and neutralized with saturated  $NH_4Cl$  aqueous solution (10 mL). The organic phase was separated from the aqueous phase, washed with HCl 1N ( $2 \times 5$  mL), saturated  $NaHCO_3$  ( $1 \times 5$  mL), dried over  $MgSO_4$  and filtered. The filtrate was evaporated under reduced pressure and purified by column chromatography using 20% to 30% EtOAc in cyclohexane to give **1** (1.99 g, 3.7 mmol, 94%) ( $R_f$  0.7 EtOAc–cyclohexane 2 : 3).  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  5.49 (dd,  $J = 9.2, 9.2$  Hz, 1H, H-3'), 5.42 (d,  $J = 3.6$  Hz, 1H, H-1'), 5.05 (dd,  $J = 9.3, 9.3$  Hz, 1H, H-4'), 4.25 (ddd,  $J = 4.3, 4.3, 10.1$  Hz, 1H, H-5'), 4.20 (dd,  $J = 9.9, 9.9$  Hz, 1H, H-5), 4.10 (dd,  $J = 10.2, 10.2$  Hz, 1H, H-6), 4.02 (dd,  $J = 8.9, 8.9$  Hz, 1H, H-4), 3.99–3.94 (m, 1H, H-1), 3.73–3.68 (m, 1H, H-3), 3.46 (dd,  $J = 3.7, 10.7$  Hz, 1H, H-2'), 3.38 (d,  $J = 4.4$  Hz, 2H, H-6'), 2.58 (ddd,  $J = 5.2, 5.2, 14.0$  Hz, 1H, H-2<sub>eq</sub>), 2.12 (s, 3H,  $CH_3$ ), 2.08 (s, 3H,  $CH_3$ ), 1.64 (ddd,  $J = 12.5, 12.5, 12.5$  Hz, 1H, H-2<sub>ax</sub>);  $^{13}C$  NMR (125 MHz,  $CDCl_3$ )  $\delta$  170.0 ( $CH_3-C=O$ ), 169.7 ( $CH_3-C=O$ ), 152.5 ( $C=O$ ), 96.4 (C-1'), 80.8 (C-5), 80.6 (C-6), 75.7 (C-4), 69.9 (C-5'), 69.7 (C-4'), 69.1 (C-3'), 60.6 (C-2'), 59.7 (C-3), 56.1 (C-1), 50.9 (C-6'), 33.6 (C-2), 20.7

( $CH_3$ ), 20.6 ( $CH_3$ ); HRMS (electrospray) Calcd. for  $C_{17}H_{20}N_{12}O_9Na$  [ $M + Na$ ]<sup>+</sup>: 559, 1374, found: 559, 1371. [ $\alpha$ ] +148.1 ( $c$  0.45,  $CH_2Cl_2$ ).

#### 3',4'-Di-O-acetyl-5,6-[sulfonylbis(oxy)]-1,3,2',6'-tetraazidoneamine **2**

To a solution of **4** (2 g, 3.9 mmol) in anhydrous  $CH_2Cl_2$  (40 mL) with  $NEt_3$  (1.9 mL, 13.7 mmol) at  $0^\circ C$  was added  $SOCl_2$  (425  $\mu$ L, 5.9 mmol). The reaction was refluxed for 2 h. The solution was cooled to room temperature, neutralized by saturated  $NH_4Cl$  aqueous solution (10 mL). The layers were separated and the organic layer was concentrated under reduced pressure. The residue was taken up with EtOAc (50 mL), washed with  $H_2O$  ( $2 \times 5$  mL), brine ( $1 \times 5$  mL), dried over  $MgSO_4$  and concentrated to obtain the corresponding sulfite which was used without further purification. It was dissolved in a mixture of  $CCl_4$  (20 mL) and acetonitrile (20 mL) and treated with  $NaIO_4$  (1.26 g, 5.9 mmol),  $RuCl_3$  (40 mg, 5%mol) and water (20 mL). After stirring for 2 h, the reaction mixture was diluted with diethyl ether (50 mL) and the layers were separated. The organic layer was washed with  $H_2O$  ( $2 \times 10$  mL), saturated  $NaHCO_3$  ( $1 \times 10$  mL), dried over  $MgSO_4$ , and concentrated *in vacuo*. The residue was purified by column chromatography using 20% to 25% EtOAc in cyclohexane to obtain **2** (1.8 g, 3.1 mmol, 79%) ( $R_f$  0.7 EtOAc–cyclohexane 2 : 3).  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  5.46 (dd,  $J = 9.9, 9.9$  Hz, 1H, H-3'), 5.32 (d,  $J = 3.7$  Hz, 1H, H-1'), 5.05 (dd,  $J = 9.8, 9.8$  Hz, 1H, H-4'), 4.61 (dd,  $J = 10.2, 10.2$  Hz, 1H, H-5), 4.48 (dd,  $J = 10.1, 10.1$  Hz, 1H, H-6), 4.27 (ddd,  $J = 3.4, 4.9, 9.8$  Hz, 1H, H-5'), 4.09–3.95 (m, 2H, H-1, H-4), 3.71 (ddd,  $J = 5.2, 9.1, 14.2$  Hz, 1H, H-3), 3.47 (dd,  $J = 3.6, 10.6$  Hz, 1H, H-2'), 3.39–3.33 (m, 2H, H-6'), 2.59 (ddd,  $J = 5.1, 5.1, 14.1$  Hz, 1H, H-2<sub>eq</sub>), 2.11 (s, 3H,  $CH_3$ ), 2.08 (s, 3H,  $CH_3$ ), 1.67 (ddd,  $J = 12.1, 12.1, 12.1$  Hz, 1H, H-2<sub>ax</sub>);  $^{13}C$  NMR (75 MHz,  $CDCl_3$ )  $\delta$  170.1 ( $CH_3-C=O$ ), 169.7 ( $CH_3-C=O$ ), 96.9 (C-1'), 84.5 (C-5), 84.2 (C-6), 75.4 (C-4), 70.0 (C-5'), 69.6 (C-4'), 69.0 (C-3'), 60.6 (C-2'), 58.8 (C-3), 55.6 (C-1), 50.8 (C-6'), 32.9 (C-2), 20.7 ( $CH_3$ ), 20.6 ( $CH_3$ ); HRMS (electrospray) Calcd. for  $C_{16}H_{20}N_{12}O_{10}NaS$  [ $M + Na$ ]<sup>+</sup>: 595.1044, found: 595.1050. [ $\alpha$ ] +136.7 ( $c$  0.64,  $CH_2Cl_2$ ).

**2. Preparation of the neamine derivatives 7–11 via the cyclic carbonate **1**.** *General procedure A: addition of amines to carbonate **1**:* To a solution of **1** (200 mg, 0.37 mmol) in anhydrous  $CH_2Cl_2$  (5 mL) was added the amine (0.58 mmol). The reaction was stirred at room temperature for 2 h. Quantitative conversion of the starting material was observed by TLC. Solvent was eliminated under reduced pressure and the residue was taken up with EtOAc (10 mL). The organic layer was washed with HCl 1N ( $2 \times 1$  mL),  $H_2O$  ( $2 \times 1$  mL), brine (1 mL), dried over  $MgSO_4$  and concentrated *in vacuo*. The residue was purified through silica gel chromatography.

*General procedure B: cleavage of the acetates:* Sodium methoxide was first prepared by slow addition, under an Ar atmosphere, of sodium (56 mg, 2 mmol) to anhydrous methanol (5 mL). 0.05 mL of this solution (0.02 mmol) was slowly added to a solution of the acetate (0.2 mmol) in anhydrous methanol (5 mL). The reaction mixture was stirred overnight at room temperature. The solution was diluted with methanol (5 mL) before the addition of a cation-exchange resin (Amberlyst<sup>®</sup> 15,

20–50 mesh, H<sup>+</sup> form). The mixture was stirred for 1 h. The resin was filtered off and the filtrate was concentrated *in vacuo*.

**General procedure C: reduction of the azide functions:** Azide (0.2 mmol) was dissolved in anhydrous methanol (5 mL). PtO<sub>2</sub> (100% wt). HCl 12 N (370 μL) was added and the reaction was stirred overnight under an atmospheric pressure of dihydrogen at room temperature. The mixture was filtered through celite, solvents were eliminated under reduced pressure and the residue was lyophilised to give the final product which was used without further purification.

**2.1 Preparation of 7a + 7b.** 3',4'-Di-O-acetyl-5-[[propylamino]carbonyloxy]-1,3,2',6'-tetraazidoneamine **5a** and 3',4'-di-O-acetyl-6-[[propylamino]carbonyloxy]-1,3,2',6'-tetraazidoneamine **5b**

By following the general procedure A, a 1 : 1 mixture of regioisomers **5a** + **5b** was obtained (187 mg, 0.31 mmol, 85%) (chromatography conditions: 20% to 30% EtOAc in cyclohexane, *R<sub>f</sub>* 0.4 EtOAc–cyclohexane 2 : 3). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 5.54 (d, *J* = 3.3 Hz, 1H, H-1'), 5.48 (dd, *J* = 10.5, 10.5 Hz, 1H, H-3'), 5.45 (dd, *J* = 10.6, 10.6 Hz, 1H, H-3'), 5.30 (d, *J* = 3.2 Hz, 1H, H-1'), 5.21 (t, *J* = 5.6 Hz, 1H, NH), 5.05 (dd, *J* = 9.7, 9.7 Hz, 2H, H-4'), 4.97 (t, *J* = 5.6 Hz, 1H, NH), 4.86 (dd, *J* = 8.9, 8.9 Hz, 1H, CH–OC=O–NHR), 4.75 (dd, *J* = 9.8, 9.8 Hz, 1H, CH–OC=O–NHR), 4.52–4.44 (m, 1H, H-5'), 4.42–4.34 (m, 1H, H-5'), 4.11 (d, *J* = 6.2 Hz, 1H, OH), 3.98 (d, *J* = 3.3 Hz, 1H, OH), 3.69 (dd, *J* = 8.8 Hz, 1H), 3.62–3.10 (m, 19H), 2.42–2.30 (m, 2H, H-2<sub>eq</sub>), 2.10 (s, 6H, CH<sub>3</sub>–C=O), 2.06 (s, 6H, CH<sub>3</sub>–C=O), 1.70–1.45 (m, 4H, H-2<sub>ax</sub>, H-2''), 0.94 (t, *J* = 7.2 Hz, 6H, H-3''); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 170.2 (CH<sub>3</sub>–C=O), 169.9 (CH<sub>3</sub>–C=O), 169.7 (CH<sub>3</sub>–C=O), 169.7 (CH<sub>3</sub>–C=O), 156.8 (C=O), 156.1 (C=O), 98.5 (C-1'), 98.5 (C-1'), 82.5, 78.6, 78.1, 77.0, 76.3, 75.0, 70.8, 70.8, 70.0, 69.4, 69.3, 69.3, 61.4 (C–N<sub>3</sub>), 60.9 (C–N<sub>3</sub>), 60.3 (C–N<sub>3</sub>), 58.7 (C–N<sub>3</sub>), 58.3 (C–N<sub>3</sub>), 58.1 (C–N<sub>3</sub>), 50.8 (C-6'), 50.7 (C-6'), 43.2 (C-1''), 43.1 (C-1''), 31.8 (C-2), 31.6 (C-2), 23.0 (C-2''), 22.9 (C-2''), 20.7 (CH<sub>3</sub>–C=O), 20.7 (CH<sub>3</sub>–C=O), 20.6 (CH<sub>3</sub>–C=O), 20.6 (CH<sub>3</sub>–C=O), 11.1 (C-3''), 11.1 (C-3''); HRMS (electrospray) Calcd. for C<sub>20</sub>H<sub>29</sub>N<sub>13</sub>O<sub>9</sub>Na [M + Na]<sup>+</sup>: 618.2109, found: 618.2090.

5-[[propylamino]carbonyloxy]-1,3,2',6'-tetraazidoneamine and 6-[[propylamino]carbonyloxy]-1,3,2',6'-tetraazidoneamine

By following the general procedure B, a 1 : 1 mixture of regioisomers was obtained (71 mg, 0.14 mmol, 69%). (*R<sub>f</sub>* 0.2 EtOAc–cyclohexane 2 : 3). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 5.54 (d, *J* = 3.3 Hz, 1H, H-1'), 5.30 (d, *J* = 3.2 Hz, 1H, H-1'), 5.21 (t, *J* = 5.6 Hz, 1H, NH), 4.97 (t, *J* = 5.6 Hz, 1H, NH), 4.86 (dd, *J* = 8.9, 8.9 Hz, 1H, CH–OC=O–NHR), 4.75 (dd, *J* = 9.8, 9.8 Hz, 1H, CH–OC=O–NHR), 4.52–4.44 (m, 1H, H-5'), 4.42–4.34 (m, 1H, H-5'), 4.11 (d, *J* = 6.2 Hz, 1H, OH), 3.98 (d, *J* = 3.3 Hz, 1H, OH), 3.69 (dd, *J* = 8.8 Hz, 1H), 3.62–3.10 (m, 27H), 2.42–2.30 (m, 2H, H-2<sub>eq</sub>), 1.70–1.45 (m, 4H, H-2<sub>ax</sub>, H-2''), 0.94 (t, *J* = 7.2 Hz, 6H, H-3''); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 156.8 (C=O), 156.1 (C=O), 98.5 (C-1'), 98.5 (C-1'), 82.5, 78.6, 78.1, 77.0, 76.3, 75.0, 70.8, 70.8, 70.0, 69.4, 69.3, 69.3, 61.4 (C–N<sub>3</sub>), 60.9 (C–N<sub>3</sub>), 60.3 (C–N<sub>3</sub>), 58.7 (C–N<sub>3</sub>), 58.3 (C–N<sub>3</sub>), 58.1 (C–N<sub>3</sub>), 50.8 (C-6'), 50.7 (C-6'), 43.2 (C-1''), 43.1 (C-1''), 31.8 (C-2), 31.6 (C-2), 23.0 (C-2''), 22.9 (C-2''), 11.1 (C-3''), 11.1 (C-3''); HRMS (electrospray) Calcd. for C<sub>16</sub>H<sub>25</sub>N<sub>13</sub>O<sub>7</sub>Na [M + Na]<sup>+</sup>: 534.1898, found: 534.1901.

5-[[propylamino]carbonyloxy]-neamine tetrahydrochloride **7a** and 6-[[propylamino]carbonyloxy]-neamine tetrahydrochloride **7b**

By following the general procedure C, a 1 : 1 mixture of regioisomers **7a** and **7b** was obtained (90 mg, 0.16 mmol, 81%). <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O) δ 5.81 (d, *J* = 3.2 Hz, 1H, H-1'), 5.45 (d, *J* = 3.4 Hz, 1H, H-1'), 4.80 (dd, *J* = 9.0, 9.0 Hz, 1H), 4.20 (dd, *J* = 9.8, 9.8 Hz, 1H), 4.00–2.90 (m, 24H), 2.50–2.34 (m, 2H, H-2<sub>eq</sub>), 1.96–1.76 (m, 2H, H-2<sub>ax</sub>), 1.44–1.26 (quint., *J* = 7.1 Hz, 4H, H-2''), 0.75 (t, *J* = 7.3 Hz, 6H, H-3''); <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O) δ 157.3 (C=O), 157.1 (C=O), 95.7 (C-1'), 93.5 (C-1'), 77.2, 76.8, 74.0, 73.4, 73.4, 71.2, 70.6, 70.2, 69.7, 69.2, 68.0, 68.0, 53.4, 53.1, 49.4, 48.8, 48.2, 48.0, 42.5 (C-6'), 42.5 (C-6'), 40.1 (C-1''), 39.9 (C-1''), 28.2 (C-2), 27.9 (C-2), 22.1 (C-2''), 22.1 (C-2''), 10.5 (C-3''), 10.5 (C-3''); HRMS (electrospray) Calcd. for C<sub>16</sub>H<sub>33</sub>N<sub>5</sub>O<sub>7</sub>Na [M + Na]<sup>+</sup>: 430.2278, found: 430.2277.

**2.2 Preparation of 8a + 8b.** 5-[[3-azidopropylamino]carbonyloxy]-3',4'-di-O-acetyl-1,3,2',6'-tetraazidoneamine and 6-[[3-azidopropylamino]carbonyloxy]-3',4'-di-O-acetyl-1,3,2',6'-tetraazidoneamine

By following the general procedure A, a 1 : 1 mixture of regioisomers was obtained (200 mg, 0.31 mmol, 84%). (Chromatography conditions: 20% to 30% EtOAc in cyclohexane) (*R<sub>f</sub>* 0.4 EtOAc–cyclohexane 2 : 3). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 5.55 (t, *J* = 5.2 Hz, 1H, NH), 5.48 (d, *J* = 3.7 Hz, 1H, H-1'), 5.42 (dd, *J* = 9.6, 9.6 Hz, 2H, H-3'), 5.28 (d, *J* = 4.2 Hz, 1H, H-1'), 5.25 (t, *J* = 6.0 Hz, 1H, NH), 5.03 (dd, *J* = 9.7, 9.7 Hz, 2H, H-4'), 4.87 (dd, *J* = 9.2, 9.2 Hz, 1H, CH–OC=O–NHR), 4.76 (dd, *J* = 9.8, 9.8 Hz, 1H, CH–OC=O–NHR), 4.46 (ddd, *J* = 3.9, 6.7, 9.8 Hz, 1H, H-5'), 4.35 (ddd, *J* = 2.9, 4.7, 9.9 Hz, 1H, H-5'), 4.04 (d, *J* = 7.2 Hz, 1H, OH), 3.94 (d, *J* = 4.0 Hz, 1H, OH), 3.70–3.20 (m, 22H), 2.40–2.30 (m, 2H, H-2<sub>eq</sub>), 2.09 (s, 6H, CH<sub>3</sub>), 2.04 (s, 6H, CH<sub>3</sub>), 1.86–1.74 (m, 4H, H-2''), 1.62 (ddd, *J* = 12.6, 12.6, 12.6 Hz, 1H, H-2<sub>ax</sub>), 1.47 (ddd, *J* = 12.2, 12.2, 12.2 Hz, 1H, H-2<sub>ax</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 170.2 (CH<sub>3</sub>–C=O), 170.0 (CH<sub>3</sub>–C=O), 169.8 (CH<sub>3</sub>–C=O), 169.7 (CH<sub>3</sub>–C=O), 156.7 (C=O), 156.1 (C=O), 98.6 (C-1'), 98.5 (C-1'), 82.7, 78.6, 77.9, 76.7, 76.1, 74.8, 70.8, 69.9, 69.4, 69.3, 69.3, 69.2, 61.4 (C–N<sub>3</sub>), 60.8 (C–N<sub>3</sub>), 60.1 (C–N<sub>3</sub>), 58.6 (C–N<sub>3</sub>), 58.3 (C–N<sub>3</sub>), 58.1 (C–N<sub>3</sub>), 50.8 (C-6'), 50.6 (C-6'), 49.0 (C-3''), 49.0 (C-3''), 39.0 (C-1''), 38.8 (C-1''), 31.7 (C-2), 31.7 (C-2), 28.8 (C-2''), 28.7 (C-2''), 20.7 (CH<sub>3</sub>), 20.6 (CH<sub>3</sub>), 20.6 (CH<sub>3</sub>), 20.6 (CH<sub>3</sub>); HRMS (electrospray) Calcd. for C<sub>20</sub>H<sub>28</sub>N<sub>16</sub>O<sub>9</sub>Na [M + Na]<sup>+</sup>: 659.2123, found: 659.2134.

5-[[3-azidopropylamino]carbonyloxy]-1,3,2',6'-tetraazidoneamine and 6-[[3-azidopropylamino]carbonyloxy]-1,3,2',6'-tetraazidoneamine

By following the general procedure B, we obtained a mixture (1 : 1) of white solids (100 mg, 0.18 mmol, 91%). (*R<sub>f</sub>* 0.20 EtOAc–cyclohexane 2 : 3). <sup>1</sup>H NMR (300 MHz, Acetone-d<sub>6</sub>) δ 6.66 (s, br, 1H), 6.52 (s, br, 1H), 5.64 (d, *J* = 3.7 Hz, 1H, H-1'), 5.30 (d, *J* = 3.4 Hz, 1H, H-1'), 4.92 (dd, *J* = 9.1, 9.1 Hz, 1H), 4.81 (dd, *J* = 9.8, 9.8 Hz, 1H), 4.26–4.20 (m, 1H, H-5'), 4.20–4.14 (m, 1H, H-5'), 3.94 (dd, *J* = 8.9, 10.2 Hz, 1H), 3.91 (dd, *J* = 8.8, 10.4 Hz, 1H), 3.80–3.62 (m, 8H), 3.62–3.40 (m, 14H), 3.40–3.20 (m, 7H), 3.12 (dd, *J* = 3.4, 10.4 Hz, 1H, H-2'), 2.45–2.30 (m, 2H, H-2<sub>eq</sub>), 1.90–1.75 (m, 4H, H-2''), 1.68 (ddd, *J* = 12.5, 12.5, 12.5 Hz, 1H, H-2<sub>ax</sub>), 1.53 (ddd, *J* = 12.2, 12.2,

12.2 Hz, 1H, H-2<sub>ax</sub>); <sup>13</sup>C NMR (75 MHz, Acetone-d<sub>6</sub>) δ 156.5 (C=O), 156.1 (C=O), 98.6 (C-1'), 98.5 (C-1'), 80.2, 77.7, 77.5, 76.7, 75.5, 75.0, 72.1, 71.9, 71.6, 71.5, 71.4, 70.8, 63.4 (C-N<sub>3</sub>), 62.9 (C-N<sub>3</sub>), 60.5 (C-N<sub>3</sub>), 59.4 (C-N<sub>3</sub>), 59.3 (C-N<sub>3</sub>), 58.6 (C-N<sub>3</sub>), 51.4 (C-6'), 51.4 (C-6'), 48.7 (C-3''), 48.6 (C-3''), 38.3 (C-1''), 38.1 (C-1''), 31.7 (C-2), 31.6 (C-2), 29.5 (C-2''), 29.3 (C-2''); HRMS (electrospray) Calcd. for C<sub>16</sub>H<sub>24</sub>N<sub>16</sub>O<sub>7</sub>Na [M + Na]<sup>+</sup>: 575.1911, found: 575.1912.

5-[[[3-Azidopropylamino]carbonyl]oxy]-neamine pentahydrochloride **8a** and 6-[[[3-azidopropylamino]carbonyl]oxy]-neamine pentahydrochloride **8b**

By following the general procedure C, we obtained **8a** and **8b** (97 mg, 0.16 mmol, 80%) as a mixture (1 : 1) of white solids. <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O) δ 5.86 (d, *J* = 3.9 Hz, 1H, H-1'), 5.51 (d, *J* = 3.8 Hz, 1H, H-1'), 4.88 (dd, *J* = 9.2, 9.2 Hz, 1H), 4.80 (dd, *J* = 9.7, 9.7 Hz, 1H), 4.30 (dd, *J* = 10.1, 10.1 Hz, 1H), 4.10–3.10 (m, 23H), 3.00–2.92 (m, 4H), 2.50 (ddd, *J* = 4.3, 4.3, 12.9 Hz, 1H, H-2<sub>eq</sub>), 2.44 (ddd, *J* = 3.3, 3.3, 11.7 Hz, 1H, H-2<sub>eq</sub>), 1.95 (ddd, *J* = 12.6, 12.6, 12.6 Hz, 1H, H-2<sub>ax</sub>), 1.91 (ddd, *J* = 12.6, 12.6, 12.6 Hz, 1H, H-2<sub>ax</sub>), 1.86–1.76 (m, 4H, H-2''); <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O) δ 157.5 (C=O), 157.2 (C=O), 96.0 (C-1'), 93.6 (C-1'), 77.5, 77.0, 74.3, 73.5, 73.3, 71.4, 70.7, 70.3, 69.8, 69.3, 68.1, 68.1, 53.5, 53.1, 49.4, 48.8, 48.2, 48.0, 40.1 (C-6'), 40.0 (C-6'), 37.7, 37.7, 37.1, 37.1, 28.3, 27.9, 27.0, 27.0; HRMS (electrospray) Calcd. for C<sub>16</sub>H<sub>35</sub>N<sub>6</sub>O<sub>7</sub> [M + H]<sup>+</sup>: 423.2567, found: 423.2571.

2.3 Preparation of **9a** + **9b**. 5-[[[3-Azidoethylamino]carbonyl]oxy]-3',4'-di-O-acetyl-1,3,2',6'-tetraazidoneamine and 6-[[[3-azidoethylamino]carbonyl]oxy]-3',4'-di-O-acetyl-1,3,2',6'-tetraazidoneamine

By following the general procedure A, a (1 : 1) mixture of regioisomers was obtained (195 mg, 0.29 mmol, 78%). (Chromatography conditions: 20% to 30% EtOAc in cyclohexane) (*R*<sub>f</sub> 0.40 EtOAc–cyclohexane 2 : 3). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 5.52 (d, *J* = 3.4 Hz, 1H, H-1'), 5.45 (dd, *J* = 9.4, 9.4 Hz, 2H, H-3'), 5.28 (d, *J* = 3.7 Hz, 1H, H-1'), 5.14 (t, *J* = 5.8 Hz, 1H, NH), 5.05 (dd, *J* = 10.1, 10.1 Hz, 2H, H-4'), 4.96 (t, *J* = 5.8 Hz, 1H, NH), 4.86 (dd, *J* = 9.1, 9.1 Hz, 1H, CH–OC=O–NHR), 4.74 (dd, *J* = 9.8, 9.8 Hz, 1H, CH–OC=O–NHR), 4.48 (ddd, *J* = 2.8, 4.5, 10.1 Hz, 1H, H-5'), 4.38 (ddd, *J* = 2.7, 4.7, 10.0 Hz, 1H, OH), 3.74–3.62 (m, 1H), 3.62–3.15 (m, 21H), 2.45–2.30 (m, 2H, H-2<sub>eq</sub>), 2.10 (s, 3H, CH<sub>3</sub>), 2.10 (s, 3H, CH<sub>3</sub>), 2.07 (s, 3H, CH<sub>3</sub>), 2.06 (s, 3H, CH<sub>3</sub>), 1.70–1.30 (m, 18H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 170.2 (CH<sub>3</sub>–C=O), 170.0 (CH<sub>3</sub>–C=O), 169.7 (CH<sub>3</sub>–C=O), 169.7 (CH<sub>3</sub>–C=O), 156.8 (C=O), 156.1 (C=O), 98.5 (C-1'), 98.5 (C-1'), 82.7, 78.7, 78.0, 76.9, 76.3, 75.0, 70.8, 69.9, 69.4, 69.4, 69.3, 69.2, 61.4 (C-N<sub>3</sub>), 60.9 (C-N<sub>3</sub>), 60.3 (C-N<sub>3</sub>), 58.7 (C-N<sub>3</sub>), 58.3 (C-N<sub>3</sub>), 58.1 (C-N<sub>3</sub>), 51.3 (C-6''), 51.3 (C-6''), 50.8 (C-6'), 50.7 (C-6'), 41.4 (C-1''), 41.2 (C-1''), 31.8 (C-2''), 31.6 (C-2''), 29.6 (C-2), 29.5 (C-2), 28.7 (C-5''), 28.7 (C-5''), 26.3, 26.3, 26.2, 26.1, 20.7 (CH<sub>3</sub>), 20.7 (CH<sub>3</sub>), 20.6 (CH<sub>3</sub>), 20.6 (CH<sub>3</sub>); HRMS (electrospray) Calcd. for C<sub>23</sub>H<sub>33</sub>N<sub>16</sub>O<sub>9</sub>Na<sub>2</sub> [M + 2Na]<sup>+</sup>: 723.2412, found: 723.2438.

5-[[[3-Azidoethylamino]carbonyl]oxy]-1,3,2',6'-tetraazidoneamine and 6-[[[3-azidoethylamino]carbonyl]oxy]-1,3,2',6'-tetraazidoneamine

By following the general procedure B, a 1 : 1 mixture of regioisomers was obtained (85 mg, 0.15 mmol, 72%). (*R*<sub>f</sub> 0.20

EtOAc–cyclohexane 2 : 3). <sup>1</sup>H NMR (300 MHz, Acetone-d<sub>6</sub>) δ 6.57 (t, *J* = 5.4 Hz, 1H, NH), 6.38 (t, *J* = 5.5 Hz, 1H, NH), 5.64 (d, *J* = 3.6 Hz, 1H, H-1'), 5.31 (d, *J* = 3.6 Hz, 1H, H-1'), 4.88 (dd, *J* = 9.3, 9.3 Hz, 1H, CH–OC=O–NHR), 4.78 (dd, *J* = 9.2, 9.2 Hz, 1H, CH–OC=O–NHR), 4.26–4.12 (m, 2H, H-5'), 3.93 (dd, *J* = 10.3, 10.3 Hz, 1H), 3.90 (dd, *J* = 9.1, 9.1 Hz, 1H), 3.80–3.39 (m, 14H), 3.38–3.25 (m, 11H), 3.25–3.05 (m, 5H), 2.44–2.28 (m, 2H, H-2<sub>eq</sub>), 1.67 (ddd, *J* = 12.4, 12.4, 12.4 Hz, 1H, H-2<sub>ax</sub>), 1.65–1.30 (m, 17H); <sup>13</sup>C NMR (75 MHz, Acetone-d<sub>6</sub>) δ 156.6 (C=O), 156.0 (C=O), 98.5 (C-1'), 98.5 (C-1'), 80.2, 77.7, 77.6, 76.6, 75.6, 75.1, 72.0, 71.9, 71.5, 71.5, 71.3, 70.9, 63.4 (C-N<sub>3</sub>), 63.1 (C-N<sub>3</sub>), 60.6 (C-N<sub>3</sub>), 59.4 (C-N<sub>3</sub>), 59.3 (C-N<sub>3</sub>), 58.6 (C-N<sub>3</sub>), 51.4 (C-6''), 51.4 (C-6''), 51.1 (C-6'), 51.0 (C-6'), 40.9 (C-1''), 40.6 (C-1''), 31.6 (C-2''), 31.5 (C-2''), 29.6 (C-2), 29.5 (C-2), 28.6 (C-5''), 28.5 (C-5''), 26.2, 26.1, 26.0, 26.0; HRMS (electrospray) Calcd. for C<sub>19</sub>H<sub>30</sub>N<sub>16</sub>O<sub>7</sub>Na [M + Na]<sup>+</sup>: 617.2381, found: 617.2372.

5-[[[3-Azidoethylamino]carbonyl]oxy]-neamine pentahydrochloride **9a** and 6-[[[3-azidoethylamino]carbonyl]oxy]-neamine pentahydrochloride **9b**

By following the general procedure C, a 1 : 1 mixture of regioisomers **9a** and **9b** was obtained (105 mg, 0.16 mmol, 82%). <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O) δ 5.80 (d, *J* = 3.6 Hz, 1H, H-1'), 5.43 (d, *J* = 3.8 Hz, 1H, H-1'), 5.00–4.70 (m, 3H), 4.24 (dd, *J* = 9.9, 9.9 Hz, 1H), 4.05–3.75 (m, 5H), 3.70 (dd, *J* = 10.6, 10.6 Hz, 1H), 3.65–2.90 (m, 16H), 2.90–2.80 (m, 4H, H-1''), 2.50–2.30 (m, 2H, H-2<sub>eq</sub>), 2.00–1.75 (m, 2H, H-2<sub>ax</sub>), 1.60–1.30 (m, 8H, H-2'', H-5''), 1.30–1.10 (m, 8H, H-3'', H-4''); <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O) δ 157.3 (C=O), 157.1 (C=O), 95.8 (C-1'), 93.6 (C-1'), 77.3, 73.6, 73.5, 73.4, 71.3, 70.7, 70.3, 69.8, 69.3, 69.2, 68.1, 68.0, 53.5, 53.1, 49.4, 48.8, 48.2, 48.1, 40.6, 40.2, 40.2, 40.0, 39.5, 39.5, 28.5, 28.3, 28.0, 27.9, 26.6, 26.6, 25.4, 25.4, 25.3, 25.2; HRMS (electrospray) Calcd. for C<sub>19</sub>H<sub>41</sub>N<sub>6</sub>O<sub>7</sub> [M + H]<sup>+</sup>: 465.3037, found: 465.3040.

2.4 Preparation of **10a** + **10b**. 3',4'-Di-O-acetyl-5-[[[2-(1H-imidazol-3-yl)ethyl]amino]carbonyl]oxy]-1,3,2',6'-tetraazidoneamine and 3',4'-di-O-acetyl-6-[[[2-(1H-imidazol-3-yl)ethyl]amino]carbonyl]oxy]-1,3,2',6'-tetraazidoneamine

By following the general procedure A, a 1 : 1 mixture of regioisomers was obtained (175 mg, 0.27 mmol, 73%). (Chromatography conditions: 10% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) (*R*<sub>f</sub> 0.35 MeOH–CH<sub>2</sub>Cl<sub>2</sub> 1 : 10). <sup>1</sup>H NMR (300 MHz, Acetone-d<sub>6</sub>) δ 7.64 (s, 1H, H-6''), 7.63 (s, 1H, H-6''), 6.91 (s, 2H, H-4''), 6.71 (t, *J* = 5.4 Hz, 1H, NH), 6.49 (t, *J* = 5.5 Hz, 1H, NH), 5.91 (d, *J* = 3.4 Hz, 1H, H-1'), 5.47 (dd, *J* = 9.1, 9.1 Hz, 1H, H-3'), 5.47 (d, *J* = 3.9 Hz, 1H, H-1'), 5.41 (dd, *J* = 9.3, 10.7 Hz, 1H, H-3'), 5.04 (dd, *J* = 10.0, 10.0 Hz, 1H, H-4'), 5.03 (dd, *J* = 10.0, 10.0 Hz, 1H, H-4'), 4.97 (dd, *J* = 9.2, 9.2 Hz, 1H, CH–OC=O–NHR), 4.83 (dd, *J* = 9.8, 9.8 Hz, 1H, CH–OC=O–NHR), 4.51 (ddd, *J* = 3.4, 4.7, 9.6 Hz, 2H, H-5'), 3.90–3.55 (m, 9H), 3.55–3.25 (m, 11H), 2.90–2.70 (m, 4H, H-2''), 2.50–2.30 (m, 2H, H-2<sub>eq</sub>), 2.10 (s, 3H, CH<sub>3</sub>), 2.05 (s, 3H, CH<sub>3</sub>), 2.04 (s, 3H, CH<sub>3</sub>), 2.02 (s, 3H, CH<sub>3</sub>), 1.75 (ddd, *J* = 12.3, 12.3, 12.3 Hz, 1H, H-2<sub>ax</sub>), 1.58 (ddd, *J* = 12.1, 12.1, 12.1 Hz, 1H, H-2<sub>ax</sub>); <sup>13</sup>C NMR (75 MHz, Acetone-d<sub>6</sub>) δ 169.5 (CH<sub>3</sub>–C=O), 169.4 (CH<sub>3</sub>–C=O), 169.3 (CH<sub>3</sub>–C=O), 169.2 (CH<sub>3</sub>–C=O), 156.2 (C=O), 156.0 (C=O), 135.0 (C-6''), 135.0 (C-6''), 135.0 (C-3''), 135.0 (C-3''), 116.0 (C-4''), 113.2 (C-4''), 98.1 (C-1'), 98.1 (C-1'), 80.0, 78.2, 77.5, 77.1, 75.2, 74.8, 69.8, 69.8, 69.3, 69.3, 69.2, 69.2, 60.6 (C-N<sub>3</sub>),

60.6 (C-N<sub>3</sub>), 60.4 (C-N<sub>3</sub>), 59.0 (C-N<sub>3</sub>), 58.9 (C-N<sub>3</sub>), 58.4 (C-N<sub>3</sub>), 50.8 (C-6'), 50.7 (C-6'), 41.4 (C-1''), 41.3 (C-1''), 31.5 (C-2), 31.4 (C-2), 26.9 (C-2''), 26.9 (C-2''), 19.8 (CH<sub>3</sub>), 19.8 (CH<sub>3</sub>), 19.8 (CH<sub>3</sub>); HRMS (electrospray) Calcd. for C<sub>22</sub>H<sub>30</sub>N<sub>15</sub>O<sub>9</sub> [M + H]<sup>+</sup>: 648.2351, found: 648.2348.

5-[[[(2-(1H-imidazol-3-yl)ethyl)amino]carbonyl]oxy]-1,3,2',6'-tetraazidoneamine and 6-[[[(2-(1H-imidazol-3-yl)ethyl)amino]carbonyl]oxy]-1,3,2',6'-tetraazidoneamine

By following the general procedure B, a 1:1 mixture of regioisomers was obtained (67 mg, 0.12 mmol, 59%). (*R*<sub>f</sub> 0.2 MeOH-CH<sub>2</sub>Cl<sub>2</sub> 1:10). <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) δ 8.12 (s, 1H, H-6''), 8.10 (s, 1H, H-6''), 7.09 (s, 1H, H-4''), 7.08 (s, 1H, H-4''), 5.66 (d, *J* = 3.6 Hz, 1H, H-1'), 5.25 (d, *J* = 3.7 Hz, 1H, H-1'), 4.87 (dd, *J* = 9.4, 9.4 Hz, 1H, CH-OC=O-NHR), 4.65 (dd, *J* = 9.8, 9.8 Hz, 1H, CH-OC=O-NHR), 4.25–4.17 (m, 2H, H-5'), 3.88 (dd, *J* = 8.9, 10.5 Hz, 1H), 3.83 (dd, *J* = 8.9, 10.4 Hz, 1H), 3.69 (dd, *J* = 9.0, 9.0 Hz, 1H), 3.65–3.35 (m, 23H), 3.13 (dd, *J* = 3.8, 10.5 Hz, 1H, H-2'), 3.06 (dd, *J* = 3.8, 10.5 Hz, 1H, H-2'), 2.94–2.84 (quint., *J* = 7.0 Hz, 4H, H-2''), 2.36–2.28 (m, 2H, H-2<sub>eq</sub>), 1.55 (ddd, *J* = 12.4, 12.4, 12.4 Hz, 1H, H-2<sub>ax</sub>), 1.47 (ddd, *J* = 12.1, 12.1, 12.1 Hz, 1H, H-2<sub>ax</sub>); <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD) δ 156.8 (C=O), 156.7 (C=O), 134.1 (C-6''), 134.1 (C-6''), 133.2 (C-3''), 133.2 (C-3''), 116.5 (C-4''), 116.4 (C-4''), 98.6 (C-1'), 98.4 (C-1'), 79.5, 77.7, 77.1, 77.0, 75.1, 74.6, 72.0, 71.9, 71.3, 71.2, 70.7, 70.4, 62.8 (C-N<sub>3</sub>), 60.2 (C-N<sub>3</sub>), 59.2 (C-N<sub>3</sub>), 59.2 (C-N<sub>3</sub>), 58.4 (C-N<sub>3</sub>), 58.4 (C-N<sub>3</sub>), 51.2 (C-6'), 51.1 (C-6'), 40.2 (C-1''), 40.0 (C-1''), 31.6 (C-2), 31.5 (C-2), 25.9 (C-2''), 25.8 (C-2''); HRMS (electrospray) Calcd. for C<sub>18</sub>H<sub>26</sub>N<sub>15</sub>O<sub>7</sub> [M + H]<sup>+</sup>: 564.2140, found: 564.2148.

5-[[[(2-(1H-imidazol-3-yl)ethyl)amino]carbonyl]oxy]-neamine pentahydrochloride **10a** and 6-[[[(2-(1H-imidazol-3-yl)ethyl)amino]carbonyl]oxy]-neamine pentahydrochloride **10b**

By following the general procedure C, a 1:1 mixture of regioisomers was obtained (90 mg, 0.14 mmol, 70%). <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O) δ 8.49 (s, 2H, H-6''), 7.18 (s, 2H, H-4''), 5.84 (d, *J* = 3.4 Hz, 1H, H-1'), 5.42 (d, *J* = 3.6 Hz, 1H, H-1'), 4.80 (dd, *J* = 9.1, 9.1 Hz, 1H, CH-OC=O-NHR), 4.26 (dd, *J* = 9.6, 9.6 Hz, 1H, CH-OC=O-NHR), 4.05–3.75 (m, 6H), 3.71 (dd, *J* = 10.2, 10.2 Hz, 1H), 3.65–3.15 (m, 17H), 2.90–2.80 (m, 4H), 2.50–2.35 (m, 2H, H-2<sub>eq</sub>), 2.00–1.80 (m, 2H, H-2<sub>ax</sub>); <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O) δ 157.2 (C=O), 157.0 (C=O), 133.1 (C-6''), 133.1 (C-6''), 130.6 (C-3''), 130.5 (C-3''), 116.3 (C-4''), 116.2 (C-4''), 95.7 (C-1'), 93.4 (C-1'), 77.3, 76.7, 74.2, 73.4, 73.2, 71.2, 70.7, 70.3, 69.8, 69.2, 68.1, 68.0, 53.5, 53.1, 49.4, 48.8, 48.2, 48.0, 40.2, 40.0, 39.5, 39.4, 28.2, 27.9, 24.4, 24.4; HRMS (electrospray) Calcd. for C<sub>18</sub>H<sub>34</sub>N<sub>7</sub>O<sub>7</sub> [M + H]<sup>+</sup>: 460.2520, found: 460.2526, calcd. for C<sub>18</sub>H<sub>33</sub>N<sub>7</sub>O<sub>7</sub>Na [M + Na]<sup>+</sup>: 482.2339, found: 482.2332.

2.5 Preparation of **11a** + **11b**. 3',4'-Di-O-acetyl-5-[[[(3-hydroxypropylamino)carbonyl]oxy]-1,3,2',6'-tetraazidoneamine and 3',4'-di-O-acetyl-6-[[[(3-hydroxypropylamino)carbonyl]oxy]-1,3,2',6'-tetraazidoneamine

By following the general procedure A, a 1:1 mixture of regioisomers was obtained (147 mg, 0.24 mmol, 64%). (Chromatography conditions: 20% to 30% EtOAc in cyclohexane) (*R*<sub>f</sub> 0.35 EtOAc-cyclohexane 2:3). <sup>1</sup>H NMR (300 MHz, Acetone-d<sub>6</sub>) δ 5.87 (t, *J* = 5.7 Hz, 1H, NH), 5.61 (t, *J* = 5.8 Hz, 1H, NH), 5.55 (d, *J* = 3.5 Hz, 1H, H-1'), 5.46 (dd, *J* = 9.4, 9.4 Hz, 1H, H-3'),

5.41 (dd, *J* = 9.4, 9.4 Hz, 1H, H-3'), 5.29 (d, *J* = 3.3 Hz, 1H, H-1'), 5.03 (dd, *J* = 10.1, 10.1 Hz, 1H, H-4'), 5.02 (dd, *J* = 9.3, 9.3 Hz, 1H, H-4'), 4.87 (dd, *J* = 9.2, 9.2 Hz, 1H, CH-OC=O-NHR), 4.73 (dd, *J* = 9.8, 9.8 Hz, 1H, CH-OC=O-NHR), 4.52 (s, br, 1H), 4.47 (ddd, *J* = 2.9, 4.3, 10.1 Hz, 1H, H-5'), 4.38 (ddd, *J* = 2.9, 4.4, 9.9 Hz, 1H, H-5'), 4.27 (d, *J* = 3.3 Hz, 1H, OH), 3.75–3.60 (m, 5H), 3.60–3.45 (m, 7H), 3.45–3.20 (m, 12H), 2.35 (ddd, *J* = 3.4, 3.4, 13.2 Hz, 2H, H-2<sub>eq</sub>), 2.08 (s, 6H, CH<sub>3</sub>), 2.05 (s, 6H, CH<sub>3</sub>), 1.72 (quint., *J* = 5.6 Hz, 4H, H-2''), 1.62 (ddd, *J* = 12.6, 12.6, 12.6 Hz, 1H, H-2<sub>ax</sub>), 1.48 (ddd, *J* = 12.2, 12.2, 12.2 Hz, 1H, H-2<sub>ax</sub>); <sup>13</sup>C NMR (75 MHz, Acetone-d<sub>6</sub>) δ 169.5 (CH<sub>3</sub>-C=O), 169.4 (CH<sub>3</sub>-C=O), 169.2 (CH<sub>3</sub>-C=O), 169.2 (CH<sub>3</sub>-C=O), 156.7 (C=O), 156.3 (C=O), 98.2 (C-1'), 98.2 (C-1'), 80.4, 78.2, 77.6, 77.6, 76.8, 75.6, 74.9, 69.9, 69.8, 69.4, 69.3, 69.2, 60.7 (C-N<sub>3</sub>), 60.6 (C-N<sub>3</sub>), 59.7 (C-N<sub>3</sub>), 59.2 (C-N<sub>3</sub>), 59.1 (C-N<sub>3</sub>), 58.9 (C-N<sub>3</sub>), 58.8 (C-N<sub>3</sub>), 58.5 (C-N<sub>3</sub>), 50.7 (C-6'), 50.7 (C-6'), 38.3 (C-1''), 38.1 (C-1''), 32.7 (C-2''), 32.6 (C-2''), 31.4 (C-2), 31.3 (C-2), 19.8 (CH<sub>3</sub>), 19.8 (CH<sub>3</sub>), 19.8 (CH<sub>3</sub>).

5-[[[(3-Hydroxypropylamino)carbonyl]oxy]-1,3,2',6'-tetraazidoneamine and 6-[[[(3-hydroxypropylamino)carbonyl]oxy]-1,3,2',6'-tetraazidoneamine

By following the general procedure B, a 1:1 mixture of regioisomers was obtained (77 mg, 0.15 mmol, 73%). (*R*<sub>f</sub> 0.15 EtOAc-cyclohexane 2:3). <sup>1</sup>H NMR (500 MHz, Acetone-d<sub>6</sub>) δ 5.65 (d, *J* = 3.7 Hz, 1H, H-1'), 5.30 (d, *J* = 3.8 Hz, 1H, H-1'), 4.91 (dd, *J* = 9.4, 9.4 Hz, 1H, CH-OC=O-NHR), 4.79 (dd, *J* = 9.8, 9.8 Hz, 1H, CH-OC=O-NHR), 4.22 (ddd, *J* = 2.5, 5.2, 9.9 Hz, 1H, H-5'), 4.17 (ddd, *J* = 2.3, 5.2, 9.8 Hz, 1H, H-5'), 3.93 (dd, *J* = 8.7, 10.5 Hz, 1H), 3.89 (dd, *J* = 8.7, 10.5 Hz, 1H), 3.80–3.40 (m, 18H), 3.35–3.30 (m, 5H), 3.16 (dd, *J* = 3.7, 10.5 Hz, 1H, H-2'), 2.39 (ddd, *J* = 4.5, 4.5, 12.8 Hz, 1H, H-2<sub>eq</sub>), 2.35 (ddd, *J* = 4.1, 4.1, 12.7 Hz, 1H, H-2<sub>eq</sub>), 1.74 (m, 4H, H-2''), 1.67 (ddd, *J* = 12.4, 12.4, 12.4 Hz, 1H, H-2<sub>ax</sub>), 1.53 (ddd, *J* = 12.4, 12.4, 12.4 Hz, 1H, H-2<sub>ax</sub>); <sup>13</sup>C NMR (75 MHz, Acetone-d<sub>6</sub>) δ 156.8 (C=O), 156.4 (C=O), 98.6 (C-1'), 98.5 (C-1'), 80.1, 77.8, 77.5, 76.7, 75.5, 74.9, 72.1, 71.9, 71.5, 71.4, 71.3, 70.9, 63.4 (C-N<sub>3</sub>), 63.3 (C-N<sub>3</sub>), 60.5 (C-N<sub>3</sub>), 59.4 (C-N<sub>3</sub>), 59.3 (C-N<sub>3</sub>), 59.2 (C-N<sub>3</sub>), 59.1 (C-N<sub>3</sub>), 58.6 (C-N<sub>3</sub>), 51.4 (C-6'), 51.4 (C-6'), 38.2 (C-1''), 38.2 (C-1''), 32.6 (C-2''), 32.4 (C-2''), 31.7 (C-2), 31.6 (C-2); HRMS (electrospray) Calcd. for C<sub>16</sub>H<sub>25</sub>N<sub>13</sub>O<sub>8</sub>Na [M + Na]<sup>+</sup>: 550.1847, found: 550.1852.

5-[[[(3-Hydroxypropylamino)carbonyl]oxy]-neamine tetrahydrochloride **11a** and 6-[[[(3-hydroxypropylamino)carbonyl]oxy]-neamine tetrahydrochloride **11b**

By following the general procedure C, a 1:1 mixture of regioisomers **11a** and **11b** was obtained (89 mg, 0.16 mmol, 78%). <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O) δ 5.84 (d, *J* = 3.5 Hz, 1H, H-1'), 5.45 (d, *J* = 3.7 Hz, 1H, H-1'), 4.82 (dd, *J* = 9.1, 9.1 Hz, 1H, CH-OC=O-NHR), 4.28 (dd, *J* = 9.6, 9.6 Hz, 1H, CH-OC=O-NHR), 4.00–3.80 (m, 2H, H-5'), 3.97 (dd, *J* = 10.4, 10.4 Hz, 1H), 3.92 (dd, *J* = 10.1, 10.1 Hz, 1H), 3.83 (dd, *J* = 9.3, 9.3 Hz, 1H), 3.73 (dd, *J* = 9.8, 9.8 Hz, 1H), 3.65–3.00 (m, 22H), 2.50–2.35 (m, 2H, H-2<sub>eq</sub>), 2.00–1.75 (m, 2H, H-2<sub>ax</sub>), 1.63 (quint., *J* = 6.4 Hz, 4H, H-2''); <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O) δ 157.3 (C=O), 157.1 (C=O), 95.7 (C-1'), 93.5 (C-1'), 77.3, 76.7, 74.2, 73.5, 73.3, 71.3, 70.7, 70.3, 69.8, 69.2, 68.1, 68.1, 59.1, 59.1, 53.5, 53.1, 49.4, 48.8, 48.3, 48.1, 40.2 (C-6'), 40.0 (C-6'), 37.7 (C-1''), 37.6 (C-1''), 31.1 (C-2''), 31.1 (C-2''), 28.2

(C-2), 27.9 (C-2); HRMS (electrospray) Calcd. for  $C_{16}H_{33}N_5O_8Na [M + Na]^+$ : 446.2227, found: 446.2236.

**3. Preparation of the neamine derivatives 12–14 via the cyclic sulfate 2.** *General procedure D: ring opening of the sulfate 2:* **2** (200 mg, 0.35 mmol) was dissolved in pure amine (1 mL) and the mixture was stirred overnight at room temperature. The reaction was diluted with EtOAc (10 mL), washed with HCl 1N ( $3 \times 2$  mL), H<sub>2</sub>O ( $2 \times 1$  mL), brine (1 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure. The residue was purified through silica gel chromatography.

**3.1 Preparation of 12a + 12b.** *5-(N-Propylamino)-6-sulfoxy-1,3,2',6'-tetraazidoneamine 6a and 6-(N-propylamino)-5-sulfoxy-1,3,2',6'-tetraazidoneamine 6b*

By following the general procedure D, a 1 : 1 mixture of regioisomers was obtained (123 mg, 0.22 mmol, 64%). (Chromatography conditions: 40% to 50% EtOAc in cyclohexane) ( $R_f$  0.25 EtOAc–cyclohexane 2 : 3). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  5.71 (t,  $J = 6.0$  Hz, 1H, NH), 5.38 (t,  $J = 4.0$  Hz, 1H, H-1'), 5.37 (t,  $J = 4.0$  Hz, 1H, H-1'), 5.14 (t,  $J = 6.0$  Hz, 1H, NH), 4.80–4.70 (m, 5H), 4.64 (dd,  $J = 8.5, 8.5$  Hz, 1H), 4.34 (dd,  $J = 9.7, 9.7$  Hz, 1H), 4.28–4.15 (m, 1H, H-5'), 4.15–4.05 (m, 1H, H-5'), 3.99 (dd,  $J = 9.7, 9.7$  Hz, 2H), 3.85–3.70 (m, 1H), 3.70–3.10 (m, 20H), 2.50–2.30 (m, 2H, H-2<sub>eq</sub>), 1.70–1.50 (m, 6H, H-2'', H-2<sub>ax</sub>), 0.98 (t,  $J = 7.3$  Hz, 3H, H-3''), 0.97 (t,  $J = 7.3$  Hz, 3H, H-3''); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  98.8 (C-1'), 97.6 (C-1'), 86.3, 84.3, 80.7, 76.0, 75.0, 74.7, 72.1, 72.0, 71.7, 71.5, 71.4, 70.8, 60.5 (C–N<sub>3</sub>), 59.5 (C–N<sub>3</sub>), 58.9 (C–N<sub>3</sub>), 58.5 (C–N<sub>3</sub>), 51.4 (C–N<sub>3</sub>), 48.8 (C–N<sub>3</sub>), 48.5 (C–N<sub>3</sub>), 48.5 (C–N<sub>3</sub>), 41.3 (C-6'), 41.1 (C-6'), 36.4, 36.4, 31.5 (C-2), 31.5 (C-2), 11.1 (C-3''), 11.1 (C-3''); HRMS (electrospray) Calcd. for  $C_{15}H_{24}N_{13}O_8S [M - H]^-$ : 546.1592, found: 546.1580.

*5-(N-Propylamino)-6-sulfoxyneamine pentahydrochloride 12a and 6-(N-propylamino)-5-sulfoxyneamine pentahydrochloride 12b*

By following the general procedure C, a 1 : 1 mixture of regioisomers **12a** and **12b** was obtained (96 mg, 0.15 mmol, 77%). <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O)  $\delta$  5.88 (d,  $J = 3.7$  Hz, 1H, H-1'), 5.65 (d,  $J = 3.4$  Hz, 1H, H-1'), 4.85–4.60 (m, 2H, H-5'), 4.50 (dd,  $J = 9.7, 9.7$  Hz, 1H), 4.34 (dd,  $J = 9.8, 9.8$  Hz, 1H), 4.10–2.80 (m, 22H), 2.55–2.35 (m, 2H, H-2<sub>eq</sub>), 1.90 (ddd,  $J = 12.8, 12.8, 12.8$  Hz, 2H, H-2<sub>ax</sub>), 1.75 (quint.,  $J = 7.6$  Hz, 4H, H-2''), 0.98 (t,  $J = 7.3$  Hz, 3H, H-3''), 0.97 (t,  $J = 7.3$  Hz, 3H, H-3''); <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O)  $\delta$  95.8 (C-1'), 93.9 (C-1'), 83.4, 80.3, 76.5, 74.9, 72.9, 71.6, 70.6, 70.5, 69.4, 69.3, 68.0, 67.6, 49.7, 49.7, 49.0, 48.6, 48.2, 48.1, 40.8, 40.7, 40.1, 39.6, 28.0, 27.9, 27.0, 26.8, 11.1, 11.1; HRMS (electrospray) Calcd. for  $C_{15}H_{38}N_5O_8S [M + H]^+$ : 448.2441, found: 448.2428.

**3.2 Preparation of 13a + 13b.** *5-(N-(3-Azidopropylamino))-6-sulfoxy-1,3,2',6'-tetraazidoneamine and 6-(N-(3-azidopropylamino))-5-sulfoxy-1,3,2',6'-tetraazidoneamine*

By following the general procedure D, a 1 : 1 mixture of regioisomers was obtained (154 mg, 0.26 mmol, 76%). (Chromatography conditions: 40% to 50% EtOAc in cyclohexane) ( $R_f$  0.25 EtOAc–cyclohexane 2 : 3). <sup>1</sup>H NMR (300 MHz, Acetone-d<sub>6</sub>)  $\delta$  5.55 (d,  $J = 3.8$  Hz, 1H, H-1'), 5.53 (d,  $J = 3.8$  Hz, 1H, H-1'), 4.63 (dd,  $J = 9.0, 9.0$  Hz, 1H), 4.42 (dd,  $J = 9.7, 9.7$  Hz, 1H), 4.23 (ddd,  $J = 2.4, 5.3, 9.8$  Hz, 1H, H-5'), 4.15 (ddd,  $J = 2.5, 5.1, 9.8$  Hz, 1H, H-5'), 4.00–3.23 (m, 25H), 3.20 (dd,  $J = 3.8,$

10.5 Hz, 1H, H-2'), 2.50 (ddd,  $J = 4.4, 4.4, 13.0$  Hz, 1H, H-2<sub>eq</sub>), 2.41 (ddd,  $J = 4.1, 4.1, 13.3$  Hz, 1H, H-2<sub>eq</sub>), 1.80 (quint.,  $J = 6.4$  Hz, 4H, H-2''), 1.72 (ddd,  $J = 11.1, 11.1, 11.1$  Hz, 1H, H-2<sub>ax</sub>), 1.60 (ddd,  $J = 12.2, 12.2, 12.2$  Hz, 1H, H-2<sub>ax</sub>); <sup>13</sup>C NMR (75 MHz, Acetone-d<sub>6</sub>)  $\delta$  98.8 (C-1'), 97.6 (C-1'), 86.3, 84.3, 80.7, 76.0, 75.0, 74.7, 72.1, 72.0, 71.7, 71.5, 71.4, 70.8, 63.6, 63.2, 60.5 (C–N<sub>3</sub>), 59.5 (C–N<sub>3</sub>), 58.9 (C–N<sub>3</sub>), 58.5 (C–N<sub>3</sub>), 51.4 (C–N<sub>3</sub>), 48.8 (C–N<sub>3</sub>), 48.5 (C–N<sub>3</sub>), 48.5 (C–N<sub>3</sub>), 41.3 (C-6'), 41.1 (C-6'), 36.4, 36.4, 31.5 (C-2), 31.5 (C-2); HRMS (electrospray) Calcd. for  $C_{15}H_{23}N_{16}O_8S [M - H]^-$ : 587.1606, found: 587.1610.

*5-(N-(3-Azidopropylamino))-6-sulfoxyneamine hexahydrochloride 13a and 6-(N-(3-azidopropylamino))-5-sulfoxyneamine hexahydrochloride 13b*

By following the general procedure C, a 1 : 1 mixture of regioisomers **13a** and **13b** was obtained (88 mg, 0.13 mmol, 66%). <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O)  $\delta$  5.88 (d,  $J = 3.7$  Hz, 1H, H-1'), 5.65 (d,  $J = 3.4$  Hz, 1H, H-1'), 4.85–4.60 (m, 2H, H-5'), 4.50 (dd,  $J = 9.7, 9.7$  Hz, 1H), 4.34 (dd,  $J = 9.8, 9.8$  Hz, 1H), 4.10–2.80 (m, 26H), 2.55–2.35 (m, 2H, H-2<sub>eq</sub>), 1.90 (ddd,  $J = 12.8, 12.8, 12.8$  Hz, 2H, H-2<sub>ax</sub>), 1.75 (quint.,  $J = 7.6$  Hz, 4H, H-2''); <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O)  $\delta$  95.8 (C-1'), 93.9 (C-1'), 83.4, 80.3, 76.5, 74.9, 72.9, 71.6, 70.6, 70.5, 69.4, 69.3, 68.0, 67.6, 53.5, 52.6, 49.7, 49.7, 49.0, 48.6, 48.2, 48.1, 40.8, 40.7, 40.1, 39.6, 28.0, 27.9, 27.0, 26.8; HRMS (electrospray) Calcd. for  $C_{15}H_{33}N_6O_8S [M - H]^-$ : 457.2081, found 457.2084.

**3.3 Preparation of 14a + 14b.** *5-(N-(3-Hydroxypropylamino))-6-sulfoxy-1,3,2',6'-tetraazidoneamine and 6-(N-(3-hydroxypropylamino))-5-sulfoxy-1,3,2',6'-tetraazidoneamine*

By following the general procedure D, a 1 : 1 mixture of regioisomers was obtained (138 mg, 0.25 mmol, 71%). (Chromatography conditions: 40% to 50% EtOAc in cyclohexane) ( $R_f$  0.2 EtOAc–cyclohexane 2 : 3). <sup>1</sup>H NMR (300 MHz, Acetone-d<sub>6</sub>)  $\delta$  6.49 (t,  $J = 5.7$  Hz, 1H, NH), 6.22 (t,  $J = 5.9$  Hz, 1H, NH), 5.34 (d,  $J = 3.6$  Hz, 1H, H-1'), 5.33 (d,  $J = 3.5$  Hz, 1H, H-1'), 4.62 (dd,  $J = 8.9, 8.9$  Hz, 1H), 4.40 (dd,  $J = 9.7, 9.7$  Hz, 1H), 4.25 (ddd,  $J = 2.5, 5.3, 9.8$  Hz, 1H, H-5'), 4.15 (ddd,  $J = 2.5, 5.1, 9.8$  Hz, 1H, H-5'), 4.00–3.40 (m, 21H), 3.40–3.30 (m, 4H), 3.21 (dd,  $J = 3.8, 10.5$  Hz, 1H, H-2'), 2.49 (ddd,  $J = 4.3, 4.3, 13.0$  Hz, 1H, H-2<sub>eq</sub>), 2.40 (ddd,  $J = 4.2, 4.2, 13.0$  Hz, 1H, H-2<sub>eq</sub>), 1.86–1.74 (quint.,  $J = 3.6$  Hz, 4H, H-2''), 1.73 (ddd,  $J = 12.4, 12.4, 12.4$  Hz, 1H, H-2<sub>ax</sub>), 1.59 (ddd,  $J = 12.1, 12.1, 12.1$  Hz, 1H, H-2<sub>ax</sub>); <sup>13</sup>C NMR (75 MHz, Acetone-d<sub>6</sub>)  $\delta$  98.8 (C-1'), 97.7 (C-1'), 86.2, 84.3, 80.6, 76.1, 75.0, 74.7, 72.1, 72.0, 71.7, 71.5, 71.4, 70.8, 63.6, 63.1, 59.8 (C–N<sub>3</sub>), 59.4 (C–N<sub>3</sub>), 59.3 (C–N<sub>3</sub>), 59.3 (C–N<sub>3</sub>), 58.9 (C–N<sub>3</sub>), 58.5 (C–N<sub>3</sub>), 51.4 (C-6'), 51.4 (C-6'), 41.7 (C-2''), 41.4 (C-2''), 32.0, 31.9, 31.5 (C-2), 31.5 (C-2); HRMS (electrospray) Calcd. for  $C_{15}H_{24}N_{13}O_9S [M - H]^-$ : 562.1541, found: 562.1549.

*5-(N-(3-Hydroxypropylamino))-6-sulfoxyneamine pentahydrochloride 14a and 6-(N-(3-hydroxypropylamino))-5-sulfoxyneamine pentahydrochloride 14b*

By following the general procedure C, a 1 : 1 mixture of regioisomers **14a** and **14b** was obtained (75 mg, 0.12 mmol, 58%). <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O)  $\delta$  5.86 (d,  $J = 3.6$  Hz, 1H, H-1'), 5.66 (d,  $J = 3.4$  Hz, 1H, H-1'), 4.80–4.60 (m, 2H, H-5'), 4.47 (dd,  $J = 9.8$  Hz, 1H), 4.31 (dd,  $J = 9.8$  Hz, 1H), 4.10–3.75 (m, 7H), 3.70–3.45 (m, 8H), 3.45–3.25 (m, 6H), 3.25–3.15 (m, 5H), 2.45 (m, 2H, H-2<sub>eq</sub>), 1.91 (ddd,  $J = 12.6, 12.6, 12.6$  Hz,



2H, H-2<sub>ax</sub>), 1.76–1.64 (quint.,  $J = 6.4$  Hz, 4H, H-2''); <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O)  $\delta$  95.9 (C-1'), 93.9 (C-1'), 83.2, 80.1, 76.7, 74.8, 72.9, 71.4, 70.6, 70.5, 69.5, 69.3, 68.0, 67.7, 58.6, 58.6, 53.4, 52.6, 49.6, 48.6, 48.2, 48.1, 40.7, 40.6, 40.1, 39.6, 31.2, 31.1, 28.0, 27.9; HRMS (electrospray) Calcd. for C<sub>15</sub>H<sub>32</sub>N<sub>5</sub>O<sub>9</sub>S [M – H]<sup>–</sup>: 458.1921, found: 458.1918.

#### 4. Synthesis of **8a**. 5-[(Chlorocarbonyl)oxy]-1,3,2',6'-tetraazido-6,3',4'-tri-O-acetylneamine **15**

To a solution of **3** (2 g, 3.6 mmol) and pyridine (2.63 mL, 32.4 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (40 mL) under an Ar atmosphere was added a solution of triphosgene (3.2 g, 10.8 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (10 mL). The reaction mixture was stirred overnight. The solution was then diluted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and neutralized with saturated NH<sub>4</sub>Cl aqueous solution (10 mL). The organic phase was separated, washed with HCl 1N (2 × 5 mL), saturated NaHCO<sub>3</sub> (1 × 5 mL), dried over MgSO<sub>4</sub> and filtered. The filtrate was evaporated under reduced pressure to give **15** (1.9 g, 3.1 mmol, 84%) which was used without any other purification due to its poor stability ( $R_f$  0.75 EtOAc–cyclohexane 2 : 3). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  5.40 (dd,  $J = 9.4$ , 10.3 Hz, 1H, H-3'), 5.16 (d,  $J = 3.8$  Hz, 1H, H-1'), 5.08–4.96 (m, 3H, H-4', H-6, H-5), 4.40 (ddd,  $J = 3.0$ , 4.6, 10.1 Hz, 1H, H-5'), 3.76–3.44 (m, 4H), 3.42–3.24 (m, 2H), 2.46 (ddd,  $J = 4.5$ , 4.5, 13.4 Hz, 1H, H-2<sub>eq</sub>), 2.13 (s, 3H, CH<sub>3</sub>), 2.08 (s, 3H, CH<sub>3</sub>), 2.04 (s, 3H, CH<sub>3</sub>), 1.67 (ddd,  $J = 12.8$ , 12.8, 12.8 Hz, 1H, H-2<sub>ax</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  170.0 (CH<sub>3</sub>–C=O), 169.7 (CH<sub>3</sub>–C=O), 169.6 (CH<sub>3</sub>–C=O), 150.2 (C=O), 98.7 (C-1'), 80.7, 78.7, 73.1, 70.6, 69.7, 69.2, 61.0 (C–N<sub>3</sub>), 58.1 (C–N<sub>3</sub>), 57.4 (C–N<sub>3</sub>), 50.7 (C-6'), 31.5 (C-2), 20.6 (CH<sub>3</sub>), 20.6 (CH<sub>3</sub>), 20.4 (CH<sub>3</sub>).

#### 5-[(3-Azidopropylamino)carbonyl]oxy]-1,3,2',6'-tetraazido-6,3',4'-tri-O-acetylneamine

To a solution of **15** (200 mg, 0.33 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added 3-azidopropylamine (50 mg, 0.50 mmol). The reaction was stirred at room temperature for 2 h. Quantitative conversion of the starting material was observed by TLC. The solvent was eliminated under reduced pressure and the residue was taken up with EtOAc (10 mL). The organic layer was washed with HCl 1N (2 × 1 mL), H<sub>2</sub>O (2 × 1 mL), brine (1 × 1 mL), dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The residue was purified through silica gel chromatography to give a white solid (209 mg, 0.31 mmol, 93%). (Chromatography conditions: 20% to 30% of EtOAc in cyclohexane) ( $R_f$  0.50 EtOAc–cyclohexane 2 : 3). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  5.41 (dd,  $J = 9.2$ , 10.8 Hz, 1H, H-3'), 5.26 (t,  $J = 5.9$  Hz, 1H, NH), 5.22 (d,  $J = 3.8$  Hz, 1H, H-1'), 5.03 (dd,  $J = 9.7$ , 9.7 Hz, 1H, H-4'), 5.00 (dd,  $J = 9.4$ , 10.0 Hz, 1H, H-6), 4.90 (dd,  $J = 10.0$ , 10.0 Hz, 1H, H-5), 4.33 (ddd,  $J = 2.7$ , 4.8, 10.1 Hz, 1H, H-5'), 3.72–3.66 (m, 1H), 3.64 (dd,  $J = 9.8$ , 9.8 Hz, 1H), 3.60–3.52 (m, 1H), 3.40–3.20 (m, 7H), 2.42 (ddd,  $J = 4.5$ , 4.5, 13.3 Hz, 1H, H-2<sub>eq</sub>), 2.05 (s, 3H, CH<sub>3</sub>), 2.03 (s, 3H, CH<sub>3</sub>), 2.01 (s, 3H, CH<sub>3</sub>), 1.80–1.72 (m, 2H, H-2''), 1.61 (ddd,  $J = 12.5$ , 12.5, 12.5 Hz, 1H, H-2<sub>ax</sub>); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  170.1 (CH<sub>3</sub>–C=O), 169.9 (CH<sub>3</sub>–C=O), 169.7 (CH<sub>3</sub>–C=O), 154.7 (C=O), 98.4 (C-1'), 78.1, 74.6, 74.3, 69.6, 69.4, 69.3, 60.6 (C–N<sub>3</sub>), 58.4 (C–N<sub>3</sub>), 57.4 (C–N<sub>3</sub>), 50.7 (C-6'), 48.8 (C-3''), 38.7 (C-1''), 31.6 (C-2''), 28.8 (C-2), 20.6 (CH<sub>3</sub>), 20.6

(CH<sub>3</sub>), 20.5 (CH<sub>3</sub>); HRMS (electrospray) Calcd. for C<sub>22</sub>H<sub>30</sub>N<sub>16</sub>O<sub>10</sub>Na [M + Na]<sup>+</sup>: 701.2228, found: 701.2223.

#### 5-[(3-Azidopropylamino)carbonyl]oxy]-1,3,2',6'-tetraazido-neamine

By following the general procedure B, the corresponding O-deprotected neamine derivative was obtained (83 mg, 0.15 mmol, 75%). ( $R_f$  0.20 EtOAc–cyclohexane). <sup>1</sup>H NMR (300 MHz, Acetone-d<sub>6</sub>)  $\delta$  6.66 (s, br, 1H), 5.29 (d,  $J = 3.6$  Hz, 1H, H-1'), 4.92 (dd,  $J = 9.2$ , 9.2 Hz, 1H, H-5), 4.22 (ddd,  $J = 2.2$ , 4.9, 9.6 Hz, 1H, H-5'), 3.91 (dd,  $J = 8.7$ , 10.5 Hz, 1H), 3.80–3.60 (m, 3H), 3.60–3.40 (m, 8H), 3.40–3.20 (m, 3H), 3.14 (dd,  $J = 3.6$ , 10.4 Hz, 1H, H-2'), 2.35 (ddd,  $J = 3.7$ , 3.7, 12.8 Hz, 1H, H-2<sub>eq</sub>), 1.84 (quint.,  $J = 6.7$  Hz, 2H, H-2''), 1.52 (ddd,  $J = 12.2$ , 12.2, 12.2 Hz, 1H, H-2<sub>ax</sub>); <sup>13</sup>C NMR (75 MHz, Acetone-d<sub>6</sub>)  $\delta$  156.6 (C=O), 98.6 (C-1'), 77.7, 77.5, 75.5, 72.1, 71.5, 70.9, 63.0 (C–N<sub>3</sub>), 60.4 (C–N<sub>3</sub>), 59.3 (C–N<sub>3</sub>), 51.4 (C-6'), 48.6 (C-3''), 38.3 (C-1''), 31.7 (C-2), 29.4 (C-2''); HRMS (electrospray) Calcd. for C<sub>16</sub>H<sub>24</sub>N<sub>16</sub>O<sub>7</sub>Na [M + Na]<sup>+</sup>: 575.1911, found: 575.1912.

#### 5-[(3-Azidopropylamino)carbonyl]oxy]-neamine pentahydrochloride **8a**

By following the general procedure C, **8a** was obtained (97 mg, 0.16 mmol, 87%). <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O)  $\delta$  5.50 (d,  $J = 3.8$  Hz, 1H, H-1'), 4.88 (dd,  $J = 9.2$ , 9.2 Hz, 1H), 4.30 (dd,  $J = 10.1$ , 10.1 Hz, 1H), 3.96 (dd,  $J = 10.1$ , 10.1 Hz, 1H), 4.90–3.10 (m, 10H), 3.00–2.92 (m, 2H), 2.45 (ddd,  $J = 3.3$ , 3.3, 11.7 Hz, 1H, H-2<sub>eq</sub>), 1.91 (ddd,  $J = 12.6$ , 12.6, 12.6 Hz, 1H, H-2<sub>ax</sub>), 1.86–1.76 (m, 2H, H-2''); <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O)  $\delta$  157.4 (C=O), 93.6 (C-1'), 77.5, 73.2, 71.4, 70.3, 69.8, 68.1, 53.1, 49.4, 48.8, 40.0 (C-6'), 37.7, 37.0, 27.9, 27.0; HRMS (electrospray) Calcd. for C<sub>16</sub>H<sub>35</sub>N<sub>6</sub>O<sub>7</sub> [M + H]<sup>+</sup>: 423.2562, found: 423.2565.

#### 5. Synthesis of **8b**. 6-[(3-Azidopropylamino)carbonyl]oxy]-1,3,2',6'-tetraazidoneamine

**8b** was obtained from the 1 : 1 mixture previously prepared, by separation with reversed-phase HPLC (C<sub>18</sub> reversed-phase column, Interchim, 10 × 250 mm). <sup>1</sup>H NMR (300 MHz, Acetone-d<sub>6</sub>)  $\delta$  5.76 (t,  $J = 5.4$  Hz, 1H), 5.62 (d,  $J = 3.7$  Hz, 1H, H-1'), 4.81 (dd,  $J = 9.8$ , 9.8 Hz, 1H), 4.18 (ddd,  $J = 2.6$ , 5.2, 9.8 Hz, 1H, H-5'), 4.10 (br, 1H), 3.94 (dd,  $J = 8.9$ , 10.3 Hz, 2H), 3.71–3.45 (m, 10H), 3.40–3.32 (m, 3H), 2.45 (ddd,  $J = 4.4$ , 4.4, 12.9 Hz, 1H, H-2<sub>eq</sub>), 1.89 (quint.,  $J = 6.6$  Hz, 2H, H-2''), 1.70 (ddd,  $J = 12.4$ , 12.4, 12.4 Hz, 1H, H-2<sub>ax</sub>); <sup>13</sup>C NMR (75 MHz, Acetone-d<sub>6</sub>)  $\delta$  156.1 (C=O), 98.5 (C-1'), 80.1, 76.75, 75.1, 72.0, 71.6, 71.4, 63.5 (C–N<sub>3</sub>), 59.4 (C–N<sub>3</sub>), 58.6 (C–N<sub>3</sub>), 51.5 (C-6'), 48.6 (C-3''), 38.1 (C-1''), 31.7 (C-2), 29.3 (C-2''). [ $\alpha$ ] +13.75 ( $c$  0.8, H<sub>2</sub>O).

#### 6-[(3-Azidopropylamino)carbonyl]oxy]-neamine pentahydrochloride **8b**

By following the general procedure C, **8b** was obtained (115 mg, 0.19 mmol, 94%). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$  5.88 (d,  $J = 3.9$  Hz, 1H, H-1'), 4.82 (dd,  $J = 9.6$ , 9.6 Hz, 1H), 4.04 (dd,  $J = 9.5$ , 9.5 Hz, 1H), 3.99–3.93 (m, 2H), 3.88 (dd,  $J = 9.4$ , 9.4 Hz, 1H), 3.61–3.52 (m, 2H), 3.48–3.40 (m, 3H), 3.29–3.15 (m, 3H), 2.99 (ddd,  $J = 7.5$ , 7.5, 7.5 Hz, 2H), 2.52 (ddd,  $J = 4.3$ , 4.3, 12.8 Hz, 1H, H-2<sub>eq</sub>), 1.96 (ddd,  $J = 12.7$ , 12.7, 12.7 Hz, 1H, H-2<sub>ax</sub>), 1.84 (quint.,  $J = 6.8$  Hz, 2H, H-2''); <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O)  $\delta$  157.2 (C=O), 96.0 (C-1'), 77.0, 74.3, 73.5,

70.6, 69.3, 68.1, 53.5, 48.2, 48.0, 40.1 (C-6'), 37.7, 37.1, 28.2, 26.9.  $[\alpha] +57.1$  ( $c$  0.35, H<sub>2</sub>O).

**6. Synthesis of 18.** 5-[[[3-Azidopropylamino)carbonyl]oxy]-3',4'-di-O-acetyl-6-(4-nitrophenylcarbonate)-1,3,2',6'-tetraazidoneamine **17a** and 6-[[[3-azidopropylamino)carbonyl]oxy]-3',4'-di-O-acetyl-5-(4-nitrophenylcarbonate)-1,3,2',6'-tetraazidoneamine **17b**

To a solution of **16a** and **16b** (150 mg, 0.24 mmol), previously obtained in the preparation of **8a** and **8b**, in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added 4-nitrophenyl chloroformate (476 mg, 2.36 mmol). The reaction was stirred at room temperature overnight. Quantitative conversion of the starting material was observed by TLC. The solvent was eliminated under reduced pressure and the residue was taken up with EtOAc (20 mL). The organic layer was washed with NaOH 1N (5 × 1 mL), H<sub>2</sub>O (2 × 1 mL), HCl 1N (2 × 1 mL), H<sub>2</sub>O (2 × 1 mL), brine (1 × 1 mL) and dried over MgSO<sub>4</sub>. The residue was purified through silica gel chromatography using 30% of EtOAc in cyclohexane to afford a 1 : 1 mixture of **17a** and **17b** (135 mg, 0.17 mmol, 71%) ( $R_f$  0.50 EtOAc–cyclohexane 2 : 3). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.29 (d,  $J$  = 9.1 Hz, 4H, H-6''), 7.40 (d,  $J$  = 9.1 Hz, 2H, H-5''), 7.38 (d,  $J$  = 9.1 Hz, 2H, H-5''), 5.46 (dd,  $J$  = 9.3, 10.4 Hz, 1H, H-3'), 5.44 (dd,  $J$  = 9.2, 10.6 Hz, 1H, H-3'), 5.36 (t,  $J$  = 6.0 Hz, 1H, NH), 5.30–5.15 (m, 4H), 5.10–4.90 (m, 4H), 4.72 (dd,  $J$  = 10.0, 10.0 Hz, 1H), 4.50–4.40 (m, 2H, H-5'), 3.90–3.50 (m, 6H), 3.50–3.20 (m, 14H), 2.55–2.40 (m, 2H, H-2<sub>eq</sub>), 2.08 (s, 3H, CH<sub>3</sub>), 2.08 (s, 3H, CH<sub>3</sub>), 2.05 (s, 3H, CH<sub>3</sub>), 2.04 (s, 3H, CH<sub>3</sub>), 1.90–1.60 (m, 6H, H-2'', H-2<sub>ax</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  170.0 (CH<sub>3</sub>–C=O), 169.9 (CH<sub>3</sub>–C=O), 169.7 (CH<sub>3</sub>–C=O), 169.6 (CH<sub>3</sub>–C=O), 155.3, 155., 154.9, 154.7, 151.9, 151.8, 145.7, 145.6, 126.2, 126.2, 125.5, 125.4, 121.8, 121.8, 121.6, 121.6, 99.0 (C-1'), 98.4 (C-1'), 80.0, 79.5, 78.8, 78.1, 74.9, 74.2, 69.9, 69.7, 69.6, 69.6, 69.3, 69.3, 60.8 (C–N<sub>3</sub>), 60.8 (C–N<sub>3</sub>), 58.3 (C–N<sub>3</sub>), 58.2 (C–N<sub>3</sub>), 57.9 (C–N<sub>3</sub>), 57.2 (C–N<sub>3</sub>), 50.7 (C-6'), 50.7 (C-6'), 49.0 (C-3''), 48.8 (C-3''), 39.0 (C-1''), 38.7 (C-1''), 31.6 (C-2''), 31.4 (C-2''), 28.9 (C-2), 28.9 (C-2), 26.9 (CH<sub>3</sub>), 26.9 (CH<sub>3</sub>), 26.9 (CH<sub>3</sub>), 26.9 (CH<sub>3</sub>); HRMS (electrospray) Calcd. for C<sub>27</sub>H<sub>31</sub>N<sub>17</sub>O<sub>13</sub>Na [M + Na]<sup>+</sup>: 824.2185, found: 824.2169.

5,6-Di-[[[3-azidopropylamino)carbonyl]oxy]-3',4'-di-O-acetyl-1,3,2',6'-tetraazidoneamine

To a solution of **17a** + **17b** (135 mg, 0.17 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added 3-azidopropylamine (26 mg, 0.26 mmol). The reaction was stirred at room temperature for 2 h. Quantitative conversion of the starting material was observed by TLC. The solvent was eliminated under reduced pressure and the residue was taken up with EtOAc (10 mL). The organic layer was washed with NaOH 1N (5 × 1 mL), H<sub>2</sub>O (2 × 1 mL), HCl 1N (2 × 1 mL), H<sub>2</sub>O (2 × 1 mL), brine (1 × 1 mL) and dried over MgSO<sub>4</sub>. The residue was purified through silica gel chromatography using 25% of EtOAc in cyclohexane to afford the desired product (113 mg, 0.14 mmol, 87%). ( $R_f$  0.40 EtOAc–cyclohexane 2 : 3). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  5.43 (dd,  $J$  = 9.5, 10.4 Hz, 1H, H-3'), 5.33 (t,  $J$  = 5.7 Hz, 1H, NH), 5.24 (d,  $J$  = 3.7 Hz, 1H, H-1'), 5.23 (m, 1H, NH), 5.03 (dd,  $J$  = 10.0, 10.0 Hz, 2H, H-4', CH–OC=O–NHR), 4.81 (dd,  $J$  = 10.0, 10.0 Hz, 1H, CH–OC=O–NHR), 4.50–4.42 (m, 1H, H-5'), 3.75–3.50 (m, 3H), 3.45–3.15 (m, 11H), 2.41 (ddd,  $J$  = 4.3, 4.3,

13.2 Hz, 1H, H-2<sub>eq</sub>), 2.08 (s, 3H, CH<sub>3</sub>), 2.05 (s, 3H, CH<sub>3</sub>), 1.78 (quint.,  $J$  = 6.6 Hz, 4H, H-2''), 1.63 (ddd,  $J$  = 12.6, 12.6, 12.6 Hz, 1H, H-2<sub>ax</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  169.9 (CH<sub>3</sub>–C=O), 169.7 (CH<sub>3</sub>–C=O), 155.1 (C=O), 154.9 (C=O), 98.4 (C-1'), 78.1, 75.3, 74.7, 69.7, 69.4, 69.3, 60.7 (C–N<sub>3</sub>), 58.5 (C–N<sub>3</sub>), 57.9 (C–N<sub>3</sub>), 50.7 (C-6), 48.9 (C-3''), 48.8 (C-3''), 38.8 (C-1''), 38.5 (C-1''), 31.6 (C-2), 29.0 (C-2''), 28.9 (C-2''), 26.9 (CH<sub>3</sub>), 26.9 (CH<sub>3</sub>); HRMS (electrospray) Calcd. for C<sub>24</sub>H<sub>34</sub>N<sub>20</sub>O<sub>10</sub>Na [M + Na]<sup>+</sup>: 785.2665, found: 785.2659.

5,6-Di-[[[3-azidopropylamino)carbonyl]oxy]-1,3,2',6'-tetraazidoneamine

By following the general procedure B, the deacylated product was obtained (102 mg, 0.15 mmol, 75%). ( $R_f$  0.25 EtOAc–cyclohexane 2 : 3). <sup>1</sup>H NMR (300 MHz, Acetone-d<sub>6</sub>)  $\delta$  6.50 (d,  $J$  = 5.9 Hz, 2H, OH), 5.25 (d,  $J$  = 3.5 Hz, 1H, H-1'), 5.03 (dd,  $J$  = 9.6, 9.6 Hz, 1H, CH–OC=O–NHR), 4.88 (dd,  $J$  = 10.0, 10.0 Hz, 1H, CH–OC=O–NHR), 4.28 (s, 2H, NH), 4.26–4.16 (m, 1H, H-5'), 3.90 (dd,  $J$  = 8.9, 10.3 Hz, 1H), 3.83–3.65 (m, 3H), 3.65–3.50 (m, 2H), 3.50–3.35 (m, 5H), 3.35–3.20 (m, 4H), 3.13 (dd,  $J$  = 3.7, 10.5 Hz, 1H, H-2'), 2.45 (ddd,  $J$  = 4.1, 4.1, 12.8 Hz, 1H, H-2<sub>eq</sub>), 1.82 (quint.,  $J$  = 7.0 Hz, 4H, H-2''), 1.72 (ddd,  $J$  = 12.1, 12.1, 12.1 Hz, 1H, H-2<sub>ax</sub>); <sup>13</sup>C NMR (75 MHz, Acetone-d<sub>6</sub>)  $\delta$  155.3 (C=O), 155.2 (C=O), 98.6 (C-1'), 77.4, 74.7, 74.4, 72.1, 71.5, 70.7, 62.9 (C–N<sub>3</sub>), 59.2 (C–N<sub>3</sub>), 58.3 (C–N<sub>3</sub>), 51.3 (C-6), 48.7 (C-3''), 48.6 (C-3''), 38.2 (C-1''), 38.0 (C-1''), 31.5 (C-2), 29.1 (C-2''), 29.0 (C-2''); HRMS (electrospray) Calcd. for C<sub>20</sub>H<sub>30</sub>N<sub>20</sub>O<sub>8</sub>Na [M + Na]<sup>+</sup>: 701.2453, found: 701.2446.

5,6-Di-[[[3-azidopropylamino)carbonyl]oxy]-neamine hexahydrochloride **18**

By following the general procedure C, **18** was obtained (119 mg, 0.16 mmol, 81%). <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O)  $\delta$  5.45 (d,  $J$  = 3.8 Hz, 1H, H-1'), 5.06 (dd,  $J$  = 7.5, 7.5 Hz, 1H, CH–OC=O–NHR), 4.40 (dd,  $J$  = 8.8, 10.1 Hz, 1H, CH–OC=O–NHR), 3.98 (dd,  $J$  = 8.8, 10.3 Hz, 1H), 3.92–3.84 (m, 1H, H-5'), 3.72–3.58 (m, 2H), 3.45 (dd,  $J$  = 9.0, 9.0 Hz, 1H), 3.50–3.34 (m, 4H), 3.34–3.20 (m, 2H), 3.20–3.10 (m, 2H), 3.00–2.90 (m, 4H, H-1''), 2.50 (ddd,  $J$  = 4.1, 4.1, 12.6 Hz, 1H, H-2<sub>eq</sub>), 2.00 (ddd,  $J$  = 12.7, 12.7 Hz, 1H, H-2<sub>ax</sub>), 1.78 (quint.,  $J$  = 7.5 Hz, 4H, H-2''); <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O)  $\delta$  156.6 (C=O), 156.5 (C=O), 93.5 (C-1'), 75.7, 72.9, 72.8, 70.2, 69.9, 68.0, 53.1, 48.5, 47.7, 39.9 (C-6'), 37.9 (C-3''), 37.8 (C-3''), 37.0 (C-1''), 37.0 (C-1''), 27.8 (C-2), 26.9 (C-2''), 26.9 (C-2''); HRMS (electrospray) Calcd. for C<sub>20</sub>H<sub>43</sub>N<sub>8</sub>O<sub>8</sub> [M + H]<sup>+</sup>: 523.3204, found: 523.3201.

**7. Biological evaluation.** MICs (minimum inhibitory concentrations) were determined in a Mueller–Hinton (MH) broth according to the following procedure: *E. coli* (DH5 $\alpha$ ) and *S. aureus* strains (RN4220) cells were grown in 10 mL of a Mueller–Hinton broth overnight at 37 °C. Subsequently 100  $\mu$ L of this suspension was mixed with 9.9 mL of fresh Mueller–Hinton and incubated at 37 °C for 3–5 h. 5 mL of this inoculum was added to 45 mL of fresh Mueller–Hinton broth containing phenol red at 0.02 g L<sup>-1</sup> and 1% glucose. 90  $\mu$ L of the resulting mixture was introduced to a sterile 96-wells microplate. Stock solutions ranging from 50  $\mu$ M to 10 mM in HEPES (10 mM, pH = 7.4) were prepared for the chemicals described in Scheme 3, 4 and 5. The various concentrations of the

compounds were added from the freshly prepared stock solutions to the microplate. Negative (MH broth without bacteria) and positive (MH with bacteria without the chemical that was replaced by HEPES) controls were included and the microplate was then incubated overnight at 37 °C. The MICs were determined by visual examination of the microplates, by observing the change of colour of the growth media to a bright yellow resulting from the acid production of the growing bacteria in the course of the assay (test). No colour change reflected the absence of growth, because there was no acid production. For each chemical, MIC determinations were performed independently at least four times, in duplicate for each set of experiments, the MICs collected being reproducible.

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