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Glyco-optimization of aminoglycosides: new aminoglycosides as novel anti-infective agents ☆

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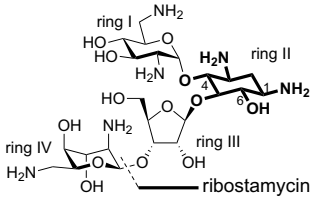
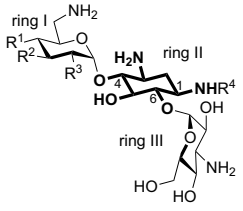
Abstract—Glyco-optimization (OPopS^{TM}) of aminoglycosides has been performed by replacing the existing sugar moiety with a variety of sugar derivatives. Glycosylation of the 6-position of nebramine provided a library of novel 4,6-linked aminoglycosides (AMGs). Among them, compounds **8b**,**g**,**i**,**l**, and **8u** with 2″-amino, 2″,3″-diamino, 2″,4″-diamino, 3″,4″-diamino, 3″-amino groups, respectively, showed significant antimicrobial activity against Gram-(+) and -(−) bacteria. Several were particularly potent against *Pseudomonus aeruginosa* with MICs in the 1–2 μg/mL range.

1. Introduction

Aminoglycosides (AMGs) are a clinically important class of antibiotics with broad-spectrum activity (Fig. 1). They are particularly useful for virulent Gram-negative organisms,[1] for which few therapeutic options are available. AMGs are composed of aminosugars and an aminocyclitol core (2-deoxystreptamine: 2-DOS). They inhibit protein synthesis as well as interfere with the fidelity of RNA translation by binding to the 16S subunit of bacterial ribosomal RNA.[2] However, application of AMGs is limited due to their intrinsic toxicities (nephrotoxicity and ototoxicity) and AMG-resistant pathogens have recently emerged.[3] Therefore, development of safer and more potent AMG-based antibiotics is badly needed. Because the sugar components of AMGs play key roles in the antibacterial activity and toxicity, we have applied a 'glyco-optimization' approach by replacing the existing sugar moieties with a variety of sugar structures in order to evaluate their potency and toxicity.



neomycin B



amikacin: R^1 = OH, R^2 = OH, R^3 = OH, R^4 = AHB

tobramycin: R^1 = OH, R^2 = H, R^3 = NH_2, R^4 = H

Figure 1. Representative structures of 4,5- and 4,6-linked subclasses of 2-deoxystreptamine (2-DOS) antibiotics, respectively. The 2-DOS moiety is highlighted in bold. AHB ≡ (*S*)-4-amino-2-hydroxybutyryl.

☆ Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2004.05.004

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Chemical modification of the AMG class of molecules has been well documented;[4] however, systematic replacement of the sugars substituents has not been well explored until recently due to the inherit problem that carbohydrate-based medicinal chemistry is laborious. Complementary work by Chang and co-workers at Utah has shown that the 5-linked disaccharide (ring III and IV) in neomycin B and recently the 6-linked monosaccharide in kanamycin (ring III) could be replaced by novel amino sugars while retaining a significant degree of antimicrobial activity.[5] Our strategy was to use a tobramycin-derived pseudo-disaccharide because of tobramycin's clinical importance and then modify the 6-linked sugar using our glyco-optimization technology to gain insight into the sugar moiety's contribution to antimicrobial activity.

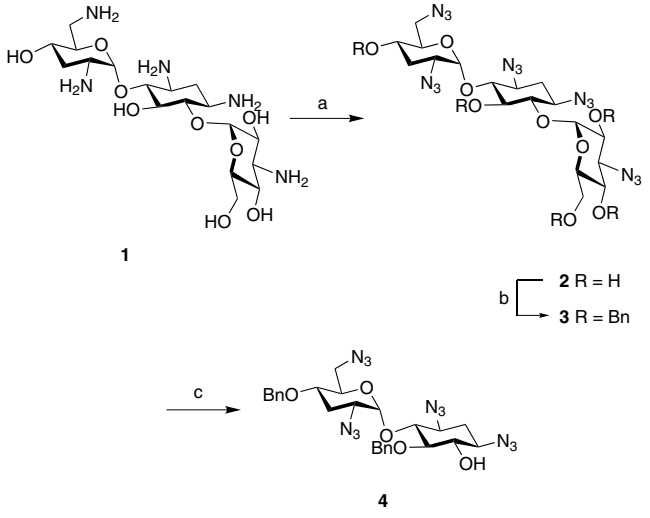
2. Preparation of a nebramine aglycone

The suitably protected nebramine aglycone **4** was obtained by the degradation of tobramycin (**1**) as outlined in Scheme 1. Treatment of **1** with trifluoromethanesulfonyl azide[6] gave per-azido tobramycin **2**, quantitatively, which was benzylated to afford a fully protected tobramycin **3**. Regioselective, acidic hydrolysis of **3** gave the desired protected nebramine **4** with the free 6-OH group in good yield.

3. Glyco-optimization (OPopS^{TM})

3.1. Pseudo-trisaccharide preparation

We first performed glycosylation of the nebramine derivative **4** with a series of monosaccharides with various functionalities **6a**–**t**. Glycosylation of acceptor **4** with a series of *p*-tolylthioglycoside derivatives generated a library of novel pseudo-trisaccharides. These thioglycoside donors **6a**–**t**, with high to low reactivity values, were activated by a combination of NIS–TfOH affording pseudo-trisaccharides **7a**–**y** in good to excellent yield (42–92%) followed by deprotection to afford **8a**–**y** (Scheme 2 and Table 1, respectively).[7] For glycosylation with donor **6r**, we employed NIS–AgOTf as the activating reagent due to the presence of the *N*,*N*-dimethyl group. The α-isomers were favored as expected for thioglycosides with nonparticipating groups (i.e., H, OBn, and N_3 in the present study) at the 2-position, while **6n**, possessing the β-directing 2-*O*-Bz, gave the β-isomer as expected.



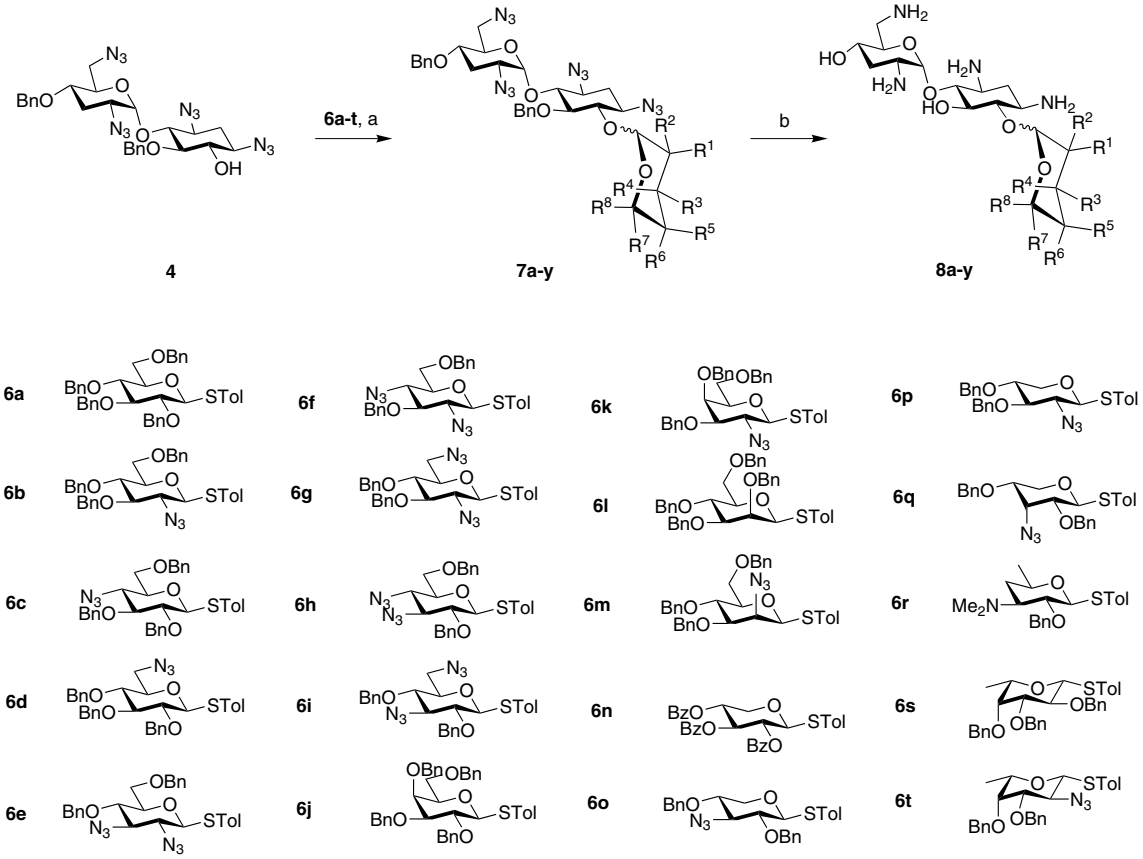
Scheme 1. Preparation of 4′,5-*O*-dibenzyl-per-azidonebramine **4**. Reagents and conditions: (a) N_3Tf, $CuSO_4$ (cat.), TEA, DCM–MeOH–H_2O; (b) NaH, BnBr, DMF, 0 °C to rt; (c) H_2SO_4, MeOH, reflux.

3.2. Pseudo-tetrasaccharide preparation via OPopS^{TM}

OPopS^{TM} is Optimer's proprietary chemistry technology for glyco-optimization and the creation of oligosaccharide diversity. In the process, a reaction is started with a highly reactive thioglycoside sugar donor with no free OH group (donor A) and a less reactive thioglycoside acceptor with one free OH group (donor B). The reactivity of donor A is normally 10 times greater than that of donor B, which serves as an acceptor during the first reaction. The in situ formed disaccharide A–B, conveniently monitored by TLC, is subsequently activated with an additional equivalent of activator in the presence of the final acceptor, in this case nebramine derivative **4**, to produce a diverse series of pseudo-tetrasaccharides in good yields (Scheme 3). Because of the difference in the reactivity of thioglycoside derivatives and the ability to monitor each step of the process by TLC, the sequence of the final products has been shown to be highly predictable.[8] Thus, using this powerful glyco-optimization strategy we have prepared a first set of diverse 4,6-linked pseudo-tetrasaccharide AMG derivatives from a selection of aminosugars in an attempt to identify a unique AMG antibiotic.

3.3. Deprotection

Global deprotection of the fully protected pseudo-tri- and pseudo-tetrasaccharides was carried out in three steps: (1) *O*-debenzoylation with NaOMe, (2) Staudinger reduction for the transformation of azido group to amino group, and (3) *O*-debenzylation by hydrogenolysis over 20% $Pd(OH)_2$/carbon without any major problems. The final AMG derivatives (**8a**–**y** and **10a**–**n**) were purified using preparative-LCMS on C-18 and a modified mobile phase.[9] The compounds were obtained as hygroscopic pentafluoropropionic acid (PFPA) salts. The salts were converted to free-base on Dowex® 50WX4-400 and eluted with aqueous ammonia hydroxide and freeze-dried to obtain a white powder. NMRs were obtained to assess the anomeric ratios. The spectra of free-bases could not be interpreted due to broad signals and multiple protonation states. Addition of a drop of 0.1 M DCl in D_2O produced sharp signals and the anomeric ratios were reported in Table 1. In general, the deprotection and purification process yield-

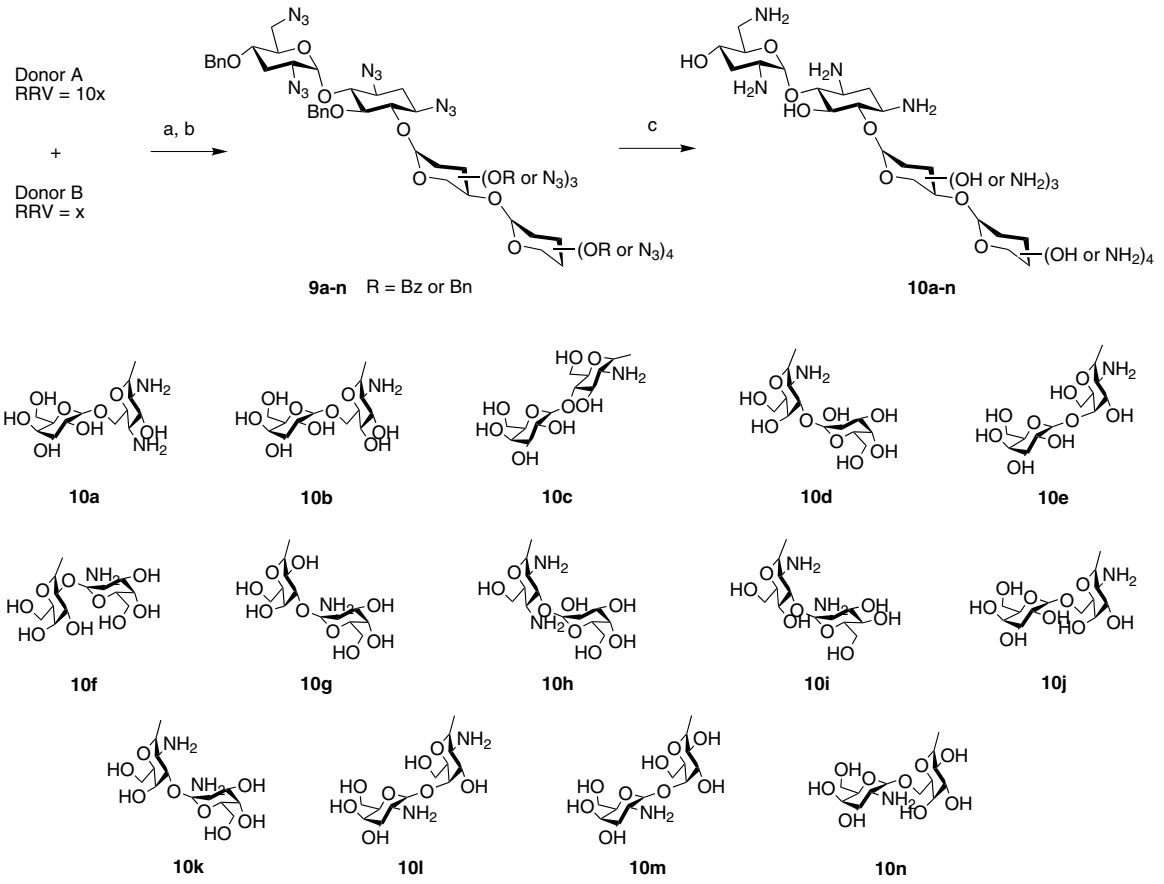


Scheme 2. Synthesis of novel 4,6-linked 2-DOS derivatives **8a**–**y** through glycosylation of **4** with a series of tolylthioglycosides **6a**–**t**. Reagents and conditions: (a) NIS, TfOH (cat.); (b) (i) NaOMe, MeOH; (ii) $P(CH_3)_3$, THF, H_2O; (iii) 20% $Pd(OH)_2$/C, AcOH–H_2O.

Table 1. Fully de-protected trisaccharides **8a**–**y**

No.	α/β	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8
8a	3	H	OH	OH	H	H	OH	CH_2OH	H
8b	α	H	NH_2	OH	H	H	OH	CH_2OH	H
8c	β	H	NH_2	OH	H	H	OH	CH_2OH	H
8d	α	H	OH	OH	H	H	NH_2	CH_2OH	H
8e	β	H	OH	OH	H	H	NH_2	CH_2OH	H
8f	α	H	OH	OH	H	H	OH	CH_2NH_2	H
8g	α	H	NH_2	NH_2	H	H	OH	CH_2OH	H
8h	β	H	NH_2	NH_2	H	H	OH	CH_2OH	H
8i	α	H	NH_2	OH	H	H	NH_2	CH_2OH	H
8j	β	H	NH_2	OH	H	H	NH_2	CH_2OH	H
8k	α	H	NH_2	OH	H	H	OH	CH_2NH_2	H
8l	α	H	OH	NH_2	H	H	NH_2	CH_2OH	H
8m	β	H	OH	NH_2	H	H	NH_2	CH_2OH	H
8n	α	H	OH	NH_2	H	H	OH	CH_2NH_2	H
8o	1.5	H	OH	OH	H	OH	H	CH_2OH	H
8p	1.1	H	NH_2	OH	H	OH	H	CH_2OH	H
8q	α	OH	H	OH	H	H	OH	CH_2OH	H
8r	α	NH_2	H	OH	H	H	OH	CH_2OH	H
8s	β	H	OH	OH	H	H	OH	H	H
8t	α	H	NH_2	OH	H	H	OH	H	H
8u	4	H	OH	NH_2	H	H	OH	H	H
8v	5	H	OH	H	NH_2	H	OH	H	H
8w	α	H	OH	NMe_2	H	H	H	Me	H
8x	α	OH	H	H	OH	H	OH	H	Me
8y	α	NH_2	H	H	OH	H	OH	H	Me

ed a 25–50% recovery of final product. Representative characterization data for compound **8b** is provided.[10] Pseudo-tetrasaccharides were purified in a similar fashion.



Scheme 3. Synthesis of novel 4,6-linked 2-DOS derivatives **10a**–**n** through glycosylation of **4** with a series of disaccharides prepared in situ. Reagents and conditions: (a) NIS, TfOH (cat.); (b) **4**, NIS, TfOH, or **4**, NIS, AgOTf; (c) (i) NaOMe, MeOH; (ii) $P(CH_3)_3$, THF, H_2O; (iii) 20% $Pd(OH)_2/C$, AcOH–H_2O.

4. Biological evaluation

Minimum inhibitory concentrations (MICs) in μg/mL values were determined after 16–20 h incubation of compounds **8a**–**y** and **10a**–**n** in comparison with tobramycin against reference strains *Staphylococcus aureus* ATCC 29213; *Enterococcus faecalis* ATCC 29212; *Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853, 35151, and PAO-1 and *Staphylococcus aureus* MRSA 33591.

None of the β-linked glycosides evaluated showed significant antimicrobial activity (Table 2). In sugars lacking amines, their derivatives, such as, glucose **8a** and galactose **8o**, showed moderate activity against *P. aeruginosa*, 16 and 32 μg/mL, respectively. The α-linked sugars with a 2-amino group (2-amino-2-deoxy-glucose **8b** and 2-amino-2-deoxy-xylose **8u**) showed potent activity with a MIC value of 2 μg/mL against *P. aeruginosa*. Interestingly, the corresponding β-anomer **8c** was devoid of activity. Among glucose derivatives containing various regioisomers of diamino groups (**8g**,**i**,**k**,**l**,**n**) **8g**,**i**, and **8l** (*P. aeruginosa*, 2 μg/mL) showed potent activity. Their β-anomers showed week activity. The epimer of the 2-amino position (**8r** vs **8b**) and the 3-amino epimer (**8v** vs **8u**) were also found to be inactive.

The crystal structure of tobramycin complexed with 16S-RNA has revealed hydrogen bonds between the O6 and N7 of G_{1405} and 2″-OH and 3″-NH_2 of tobramycin, respectively.[11] The MIC data suggest that maintaining this interaction is critical for activity and that reversing the 2″-OH and 3″-NH_2 to 2″-NH_2 and 3″-OH is sufficient to maintain the interaction. Thus, the ubiquitous 1,2-hydroxy amine motif in the *trans* or (*gluco*) orientation was found to be a versatile ligand capable of recognizing the Hoogsteen face of guanosine (vide infra), similar observations been previously noted.[12] It has been noted that a lack of a strong correlation exists between strength of RNA binding and antibiotic activity.[13] Therefore, we used a transcription–translation assay on select compounds to clarify that these new compounds were targeting ribosomal RNA.[13a] Compounds with significant antimicrobial activity were indeed confirmed to inhibit in vitro translation. Similar work on a series of 6-linked kanamycins in press by Chang and co-workers[5b] noted that substitution of the 6″-position resulted in a surprising decrease in activity, a similar observation was made for compound **8n**. This decrease in activity may be due to a subtle balance between RNA target-selectivity and binding affinity.

Antibacterial activities of the pseudo-tetrasaccharide aminoglycoside derivatives were not impressive. Struc-

Table 2. In vitro antimicrobial activities of pseudo-trisaccharides (**8a**–**y**)

No.	MIC μg/mL						
	Sa	*Ef*	*Ec*	*Pa*	*Pa**	*Pa***	*Sa**
1	0.5	8–32	0.25–1	0.25–1	0.5	0.5	>64
8a	32	>64	32	16	16	8	>64
8b	4	32	8	2	1	1	>64
8c	>64	>64	>64	>64	64	64	>64
8d	8	>64	16	8	16	8	>64
8e	>64	>64	>64	>64	>64	>64	>64
8f	32	>64	64	>64	—	—	>64
8g	1	32	—	2	1	1	>64
8h	64	>64	>64	64	—	—	>64
8i	1	32	2	2	1	1	>64
8j	8	>64	32	32	16	16	>64
8k	8	>64	32	16	16	8	>64
8l	1	64	—	2	1	2	>64
8m	32	>64	—	>64	>64	>64	>64
8n	2	>64	8	16	8	4	>64
8o	64	>64	32	32	16	16	>64
8p	64	>64	—	>64	>64	64	>64
8q	>64	>64	>64	>64	>64	>64	>64
8r	64	>64	>64	64	>64	64	>64
8s	>64	>64	>64	>64	>64	>64	>64
8t	16	>64	8	16	—	—	>64
8u	2	>64	2	2	—	—	>64
8v	32	>64	>64	>64	—	—	>64
8w	32	>64	16	>64	32	64	>64
8x	>64	>64	>64	>64	>64	>64	>64
8y	>64	>64	>64	>64	—	—	>64

Sa, *Staphylococcus aureus* ATCC 29213; *Ef*, *Enterococcus faecalis* ATCC 29212; *Ec*, *Escherichia coli* ATCC 25922; *Pa*, *Pseudomonas aeruginosa* ATCC 27853; *Pa**, *Pseudomonas aeruginosa* ATCC 35151; *Pa***, *Pseudomonas aeruginosa* PAO-1; *Sa**, *Staphylococcus aureus* MRSA 33591.

tural studies of the 4,5-linked AMG paromomycin bound to 16S RNA show that III and IV ring can be accommodated by the major groove of the RNA.[14] On the other hand structural studies of the 4,6-linked subclasses (e.g., tobramycin and gentamicin),[11] clinically relevant AMGs, indicate ring III is positioned against the wall of the major grove. Our studies indicate that the target 16S ribosomal site would not allow bulky modification through the 6-OH group of nebramine. Thus, the lack of activity of our four-ringed structures is entirely consistent with the available structural data.

5. Conclusion

We have performed OPopS™ on an aminoglycoside and successfully generated a series of aminoglycoside derivatives with new sugar moieties linked through the 6-position of nebramine. The 1,2-hydroxy amine motif on ring III in the *trans* or (*gluco*) orientation was found to be important for the recognition of the Hoogsteen face of guanosine (vida infra) and 2-amino-2-deoxy-glucose was identified as a ubiquitous aminosugar able to replace the 3-amino-3-deoxy-glucose of tobramycin. Aminoglycoside derivatives with such a novel sugar motif may have improved toxicity and activity against resistant pathogens. Our results demonstrated that glyco-optimization could be a powerful tool for identifying a new lead compound from drugs with sugar components that play a key role in activity or resistance profiles. Evaluation of the in vivo efficacy and toxicity is ongoing.

Acknowledgements

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References and notes

1. Dax, S. L. *Antibacterial Chemotherapeutic Agents*; Blackie Academic and Professional: London, 1997; pp 208–240.
2. Davies, J.; Davies, B. D. *J. Biol. Chem.* **1968**, *243*, 3312.
3. (a) Mingeot-Leclercq, M.-P.; Tulkins, P. M. *Antimicrob. Agents Chemother.* **1999**, *43*, 1003; (b) Mingeot-Leclercq, M.-P.; Glupczynski, Y.; Tulkins, P. M. *Antimicrob. Agents Chemother.* **1999**, *43*, 727.
4. Kotra, L. P.; Mobashery, S. *Curr. Org. Chem.* **2001**, *5*, 193.
5. (a) Chang, C.-W. T.; Hui, Y.; Elchert, B.; Wang, J.; Li, J.; Rai, R. *Org. Lett.* **2002**, *4*, 4603; (b) Li, J.; Wang, J.; Czyryca, P. G.; Chang, H.; Orsak, T. W.; Evanson, R.; Chang, C.-W. T. *Org. Lett.* **2004**, *6*, 1381.
6. Alper, P. B.; Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6029.
7. General procedure for the glycosylation between acceptor **4** and donors **6a**–**t**: To a flask containing flame-dried molecular sieves was added acceptor **4** (1.0 equiv), corresponding donors **6a**–**t** (1.5 equiv), and NIS (1.6 equiv) under nitrogen. The flask was cooled to −78 °C and anhydrous CH_2Cl_2 was added. The mixture was stirred at −78 °C for 20 min. Then freshly prepared 1.0 M TfOH in

diethyl ether (0.15–0.3 equiv) was added and the reaction allowed to warm to −20 °C over 1 h. The reaction was quenched with solid $Na_2S_2O_3$, $NaHCO_3$, and a few drops of H_2O, stirred until colorless, then diluted with CH_2Cl_2, filtered, washed with $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated. The product was purified by flash chromatography on silica gel (gradient elution 2–15% EtOAc–Hex).

8. Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C. H. *J. Am. Chem. Soc.* **1999**, *121*, 734.
9. Inchauspe, G.; Samain, D. *J. Chromatogr.* **1984**, *303*, 277.
10. Deprotection of **7b** to give compound **8b**: To a solution of trisaccharide **7b** (162 mg, 0.155 mmol) in THF was added a 1 M solution of trimethylphosphine in THF (11.5 mL) in five portions and water (100 μL). The solution was stirred overnight and an additional portion of water (2–3 mL) was added. The solution was stirred 0.5 h and evaporated to dryness. The residue was dried under high-vacuum, dissolved in AcOH–H_2O (1:1, 10 mL) and subjected to hydrogenolysis in the presence of 20% (wt) $Pd(OH)_2$ on carbon (100 mg) under hydrogen (1 atm). The solution was stirred overnight, filtered, and concentrated. The product was purified by preparative-LCMS (YMC HPLC column (75 × 30 mm ID, S-5 μm, 12 nm) using a gradient of 10–30% MeCN with 0.1% PFPA (v/v) in water with 0.1% PFPA (v/v) at a flow rate of 20 mL/min). The fractions containing product **8b** were collected and concentrated as PFPA salts. The product was transformed into its free base on Dowex® 50WX4-400 and eluted with 0–6% aqueous ammonia, concentrated and lyophilized to give **8b** as white solid (27 mg, 37.5% yield). NMR data of **8b**: ^{1}H NMR (D_2O with a drop of DCl): δ 1.02–1.44 (m, 2H, CHH, CHH), 1.67–1.89 (m, 1H, CHH), 2.22–2.44 (m, 1H, CHH), 2.75–3.82 (m, 16H), 5.39 (d, $J = 3.9$ Hz, 1H, anomeric proton), 5.54 (d, $J = 3.3$ Hz, 1H, anomeric proton). ^{13}C NMR (D_2O with a drop of DCl): δ 29.4, 30.3, 39.9, 47.9, 48.3, 49.5, 54.1, 60.3, 69.5, 69.4, 70.0, 70.1, 73.2, 73.9, 78.5, 82.8, 94.3, 96.9; Mass spectrum (ESI), m/z 468.5 $(M+1)^+$.
11. (a) Vicens, Q.; Westhof, E. *J. Mol. Biol.* **2003**, *326*, 1175; (b) Vicens, Q.; Westhof, E. *Chem. Biol.* **2002**, *9*, 747.
12. (a) Hendrix, M.; Alper, P. B.; Priestley, E. S.; Wong, C.-H. *Angew. Chem., Int. Ed.* **1997**, *36*, 95; (b) Wong, C.-H.; Hendrix, M.; Manning, D. D.; Rosenbohm, C.; Greenberg, W. A. *J. Am. Chem. Soc.* **1998**, *120*, 8319.
13. (a) Greenberg, W. A.; Priestley, E. S.; Sears, P. S.; Alper, P. B.; Rosenbohn, C.; Hendrix, M.; Hung, S.-C.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 6527; (b) Wong, C.-H.; Hendrix, M.; Priestley, E. S.; Greenberg, W. A. *Chem. Biol.* **1998**, *5*, 397.
14. (a) Fourmy, D.; Recht, M. I.; Blanchard, S. C.; Puglisi, J. D. *Science* **1996**, *274*, 1367; (b) Vicens, Q.; Westhof, E. *Structure* **2001**, *9*, 647.