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FULL PAPER

Diverse Synthesis of Natural Trehalosamines and Synthetic 1,1′-Disaccharide Aminoglycosides

Yen-Chu Lu,[a] Soumik Mondal,[a] Ching-Chi Wang,[a] Chun-Hung Lin,[b] Kwok-Kong Tony Mong*[a]

Dedication to Prof Chi-Huey Wong on occasion of his 70th Birthday

Abstract: We report a general strategy for diverse synthesis of ten disaccharide aminoglycosides including natural 2-trehalosamine (**1**), 3-trehalosamine (**2**), 4-trehalosamine (**3**), neotrehalosyl 3,3′-diamine (**8**), and synthetic aminoglycosides (**4**–**7**, **9**, and **10**). The aminoglycoside compounds feature different anomeric configurations and numbers of the amino groups. The key step for the synthesis is the glycosylation coupling of a stereo-directing donor with a configuration stable TMS glycoside acceptor. Either of the donor or acceptor can be substituted with an azido group. The aminoglycosides prepared in present study are characterized with the 1D and 2D NMR spectroscopy.

Introduction

Trehalosamines are 1,1′-disaccharide aminoglycosides isolated from *Streptomyces* and *Bacillus* species.[1] These aminoglycoside compounds feature a 1,1′-glycosidic bond that joins two monosaccharide units at the anomeric centers. Trehalosamines and the related analogues are of interest to scientists due to the unique biological properties. For example, 2-trehalosamine was active against *Mycobacterium tuberculosis* in previous studies.[1a] Neotrehalosyl 3,3′-diamine has been shown to inhibit the growth of *Staphylococcus aureus*.[1e] Pentapeptide derivatives of 6-trehalosamine inhibit the fibrillogenesis of amyloid β(1-42) peptide.[2] Synthetic disaccharide aminoglycosides exhibit binding property toward 16S RNA.[3]

Over the past, different synthetic routes were developed for synthesies of trehalosamines and related analogues. Majority of the routes rely on regioselective functionalization of available trehalose. These approaches require extensive optimization of reaction conditions, but their application scope is narrow.[4] Another limitation is that only disaccharide aminoglycosides with 1*α*→1′*α* anomeric configuration can be accessed. In nature, 1,1′-disaccharide may exist in other anomeric configurations. For example, neotrehalosyl 3,3'-diamine possesses a 1*α*→1'*β* glycosidic bond.[5] From synthetic perspective, non-symmetric 1,1′-disaccharides with different anomeric configurations are better prepared by coupling of two different glycosyl building blocks using a 1,1′-glycosylation method.

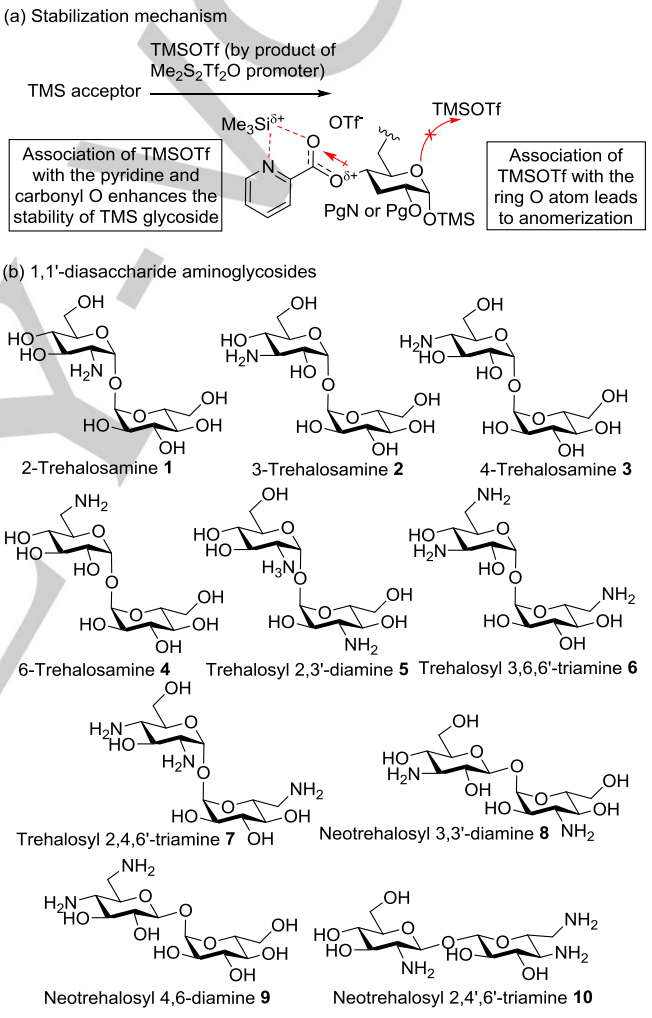

Figure 1. (a) Stabilization of TMS glycoside by Pico protecting group. (b) Structure of 1,1′-disaccharide aminoglycosides **1**-**10**.

In the literature, there are only few reports concerning the preparation of 1,1′-disaccharide aminoglycosides by the glycosylation approach.[6-8] Paulsen reported the synthesis of 2-trehalosamine from coupling of a glucosyl hemiacetal with a 2-azido-2-deoxy-D-*gluco*-pyranosyl chloride, but the stereoselectivity of the reaction was moderate.[6] Koto employed 2-(2,4-dintroanilino) substituted-2-deoxy-glucosyl bromide for preparation of 2-trehalosamine, and four different anomers were

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produced in the reaction conditions.[8] These studies highlight the challenge arising from 1,1′-glycosylation. In 2013, Anjum et al. prepared the neotrehalosyl 3,3′-diamine by self-coupling of 3-azido-3-deoxy-D-*gluco*-pyranose trichloroacetimidate with excellent selectivity.[7] This method is restricted to a particular glycosyl trichloroacetimidate and the application scope is narrow . Therefore, a versatile strategy for synthesis of 1,1′-disaccharide aminoglycosides remains desirable.

Recently, we have developed a glycosylation method for coupling of configuration stable trimethylsilyl (TMS) glycoside acceptors and stereo-directing thioglycoside donors with excellent stereocontrol.[9] Stability of the TMS glycoside stems from a picolinoyl (Pico) group at C4 position, which coordinates with TMSOTf (a by-product of the glycosylation promoter) that impedes *α*→*β*-anomerization of the glycoside (Figure 1a). We reason that this method should constitute a technical platform for construction of trehalosamines and related aminoglycosides. Herein, we report for the first time a general method for synthesis of naturally occurring 2-trehalosamine **1**,[1a] 3-trehalosamine **2**,[1b] 4-trehalosamine **3**,[1d] neotrehalosyl 3,3′-diamine **8**;[1e] and related non-symmetrical disaccharide aminoglycoside analogues **4**–**7**, **9**, and **10** (Figure 1b).

Results and Discussion

2.1 General consideration and Building Block Preparation

Retrosynthetic analysis of disaccharide aminoglycosides **1**–**10** is given in Figure 2. Targets **1**–**10** are the deprotection products of 1,1′-disaccharides **11**–**20** (Figure 2a). Disconnection of the glycosidic bonds at **11**–**20** revealed thioglycosyl donors **21**–**26** (Figure 2b) and Pico protected TMS glycoside acceptors **27α**–**31α** and **29β** (Figure 2c).

The construction of 1*α*→1'*α*-linked disaccharides **11**–**17** required *α*-directing 4,6-*O*-benzylidene thioglycoside donors **21**–**24** and TMS *α*-acceptors **27α–29α**.[10] The *α*-directing capacity of the donor is attributed to the conformation restrain property of the benzylidene group.[11] For the construction of 1*β*→1'*α*-linked disaccharides **18**–**19**, *β*-directing donors **25** and **26** with TMS *α*-glycoside acceptors **30α** and **31α** were employed; whereas the *β*-directing ability of the donor is derived from the neighbouring group participation (NPG) effect. The building blocks for 1*β*→1'*β*-linked disaccharide **20** were TMS *β*-glycoside acceptor **29β** and *β*-directing donor **26**.

Among the building blocks in Figure 3b and 3c, thioglycosyl donors **22**–**24**, TMS *α*-glycoside acceptors **27α**, **29α**, and **31α** were known compounds and their preparation followed the literature procedures.[9],[10] The syntheses of thioglycosides **21**, **25**, **26**, TMS *α*-glycosides **28α**, **30α**, and TMS *β*-glycoside **29β** are outlined in Schemes 1a–1d.

3-Azido-containing building blocks **21**, **25**, and **30α** were derived from diacetonide glucofuranose **32**, which was converted to 3-azido-3-deoxy-D-*gluco*-furanose **33** through an oxidation–reduction–substitution protocol (Scheme 1a).[12] Subsequent deprotection of the acetonide groups of **33** followed

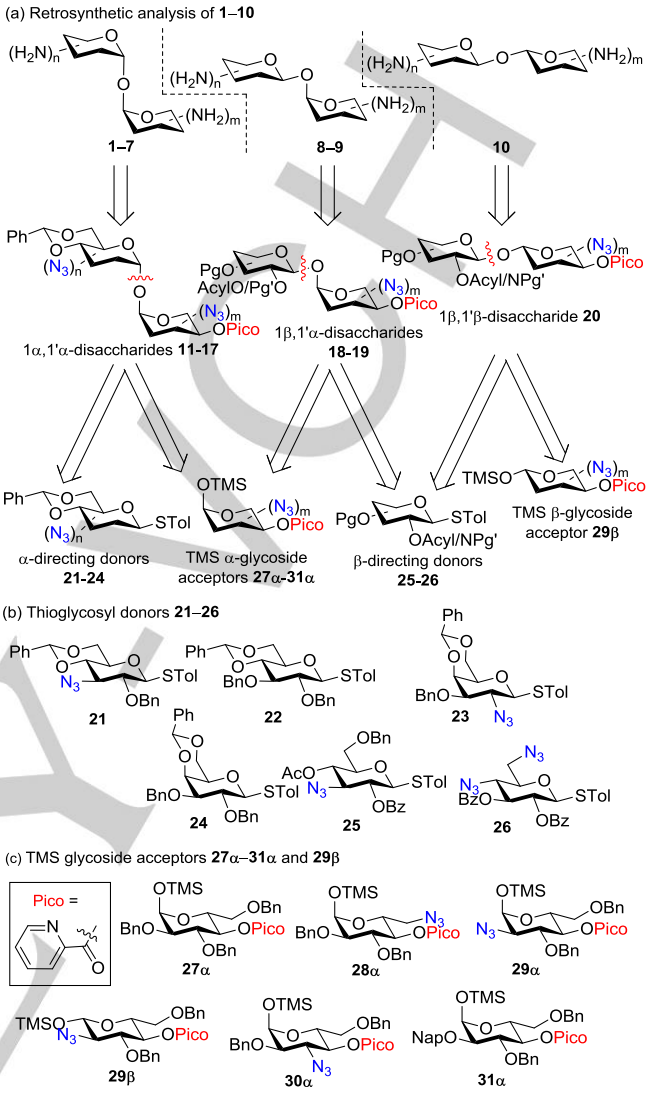


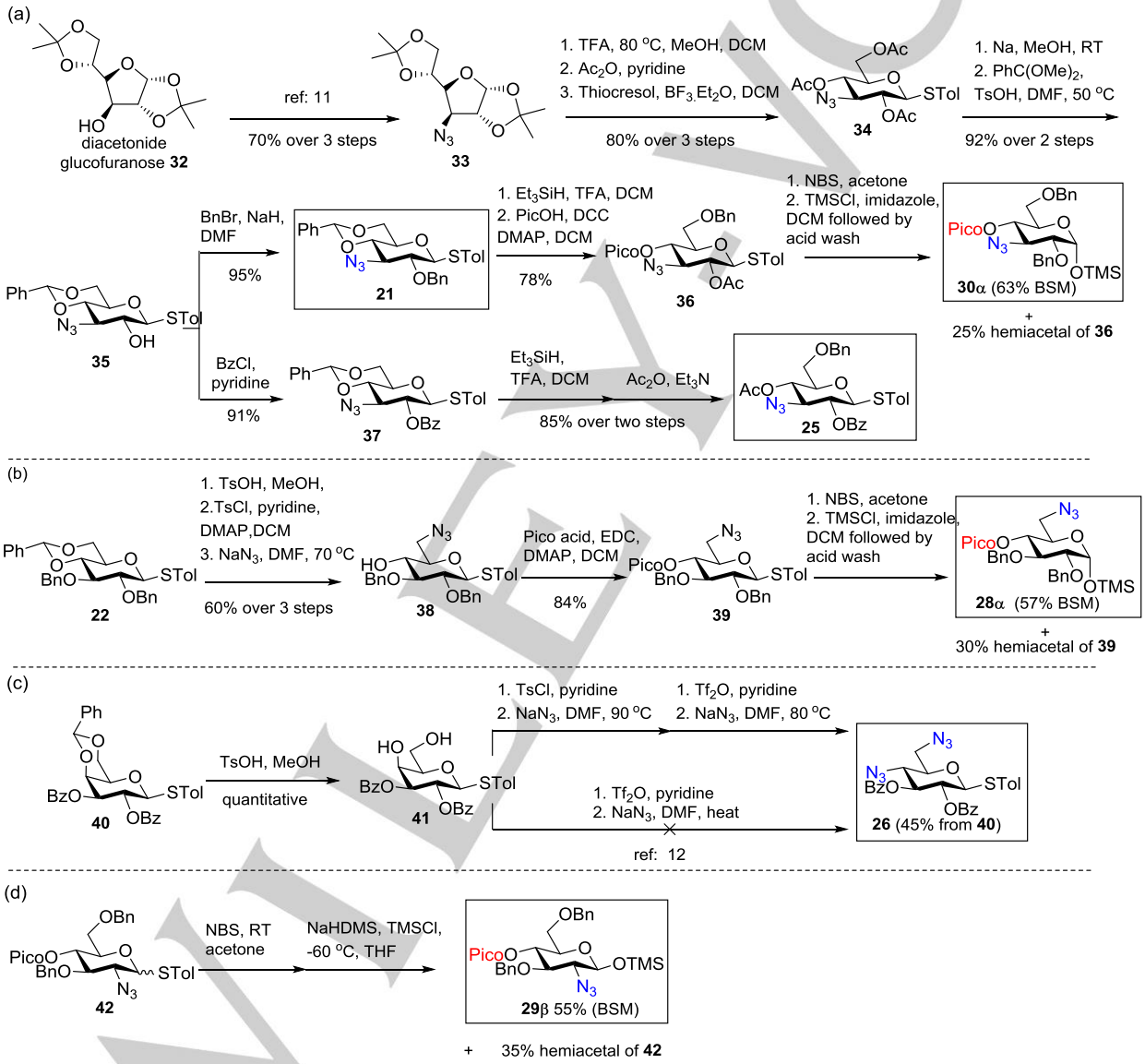
Figure 2. (a) Retrosynthetic analysis of aminoglycoside targets **1**–**10**. (b) Thioglycosyl donors. (c) TMS glycoside acceptors.

by acetylation and thioglycosidation produced per-*O*-acetyl 3-azido-3-deoxy-thio-D-*gluco*-pyranoside **34**. Deacetylation of **34** and protection of 4,6-diol with a benzylidene acetal afforded intermediate **35** with a C2 hydroxyl, which by benzylation was converted to 3-azido-3-deoxy-thio-D-*gluco*-pyranosyl donor **21**. **21** could also serve as an starting substrate for preparation of TMS 3-azido-3-deoxy-*α*-D-*gluco*-pyranoside **30α**. Thus, reductive cleavage of the benzylidene group of **21** using triethylsilane (Et_3SiH) and trifluoroacetic acid (TFA) produced a thioglycoside intermediate with a C4 hydroxyl, which was protected with a Pico group to afford fully protected **36** (Scheme 1a). Hydrolysis of the thio-acetal group in **36** with *N*-bromosuccinimide (NBS) produced a hemiacetal, which by treatment with trimethylchlorosilane (TMSCl) and imidazole was converted to TMS 3-azido-3-deoxy-D-*gluco*-pyranoside **30** as a ~1.5:1 *α*:*β* mixture. The *β*-anomer of **30** was selectively hydrolysed by washing the mixture (in DCM)

with diluted $HCl_{(aq)}$ (~0.5 N). As such, desired *α*-anomer **30α** was isolated in 63% recovered yield along with ~25% of the hemiacetal. In addition to **21**, advanced intermediate **35** could **be** used for preparation of *β*-directing 3-azido-3-deoxy-thio-D-*gluco*- via a three-step sequence, namely benzoylation, reductive ring cleavage, and acetylation, in 77% overall yield.

TMS 6-azido-6-deoxy-*α*-D-*gluco*-pyranoside **28α** was derived from known glucopyranoside **22** (Scheme 1b).[10] Acid hydrolysis of the benzylidene acetal at **22** gave a 4,6-diol intermediate, the C6 hydroxyl of **22** was converted to a tosyl group for substitution with sodium azide (NaN_3). As such, 6-azido-6-deoxy-thio-*β*-D-*gluco*-pyranoside **38** was afforded in 60% yield (from **22**). Then, a Pico group was introduced at C4 hydroxyl of **38** to furnish fully protected 3-azido-3-deoxy-thio-D-*gluco*-pyranoside **39**, which was converted to TMS 6-azido-6-deoxy-*α*-D-*gluco*-pyranoside acceptor **28α** through hydrolysis of the thioacetal followed by silylation.

Preparation of *β*-directing 4,6-diazido-4,6-dideoxy-thio-D-*gluco*-pyranoside donor **26** commenced with 2-azido thiogalactoside **40** (Scheme 1c).[13] Benzoylation of **40** followed by deprotection of the benzylidene group gave 4,6-diol **41**. Initial



Scheme 1. Preparation of α-directing thioglycoside donors **21**, **25**, **26** and TMS glycoside acceptors **28α**, **29β**, and **30α**.

effort for simultaneous conversion of the two hydroxyls of **41** to azido groups through a literature protocol, i.e. triflation and NaN_3 substitution, did not meet with success.[13] Alternatively, a stepwise approach was devised; whereas the 6-hydroxyl of **41**

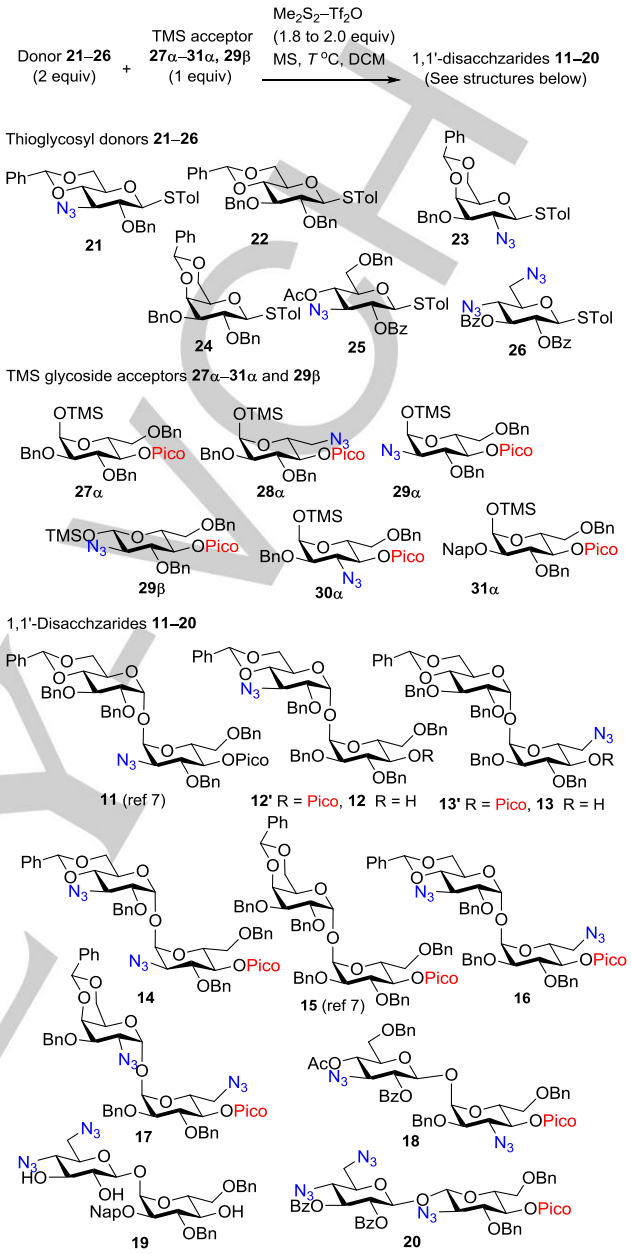
was selectively converted to a tosyl group, then followed by NaN_3 substitution. The, the 4-hydroxyl group of the 6-azido substituted product was then subjected to triflation and substitution with NaN_3 to produce donor **26** in 45% overall yield (from **40**).

For preparation of TMS 2-azido-2-deoxy-thio-D-*gluco*-*β*-glucoside **29β**, known 2-azido-2-deoxy-thio-D-*gluco*-pyanoside **42** was first hydrolysed to a hemiacetal (Scheme 1d). In contrast to the preparation of TMS *α*-glycosides **28α** and **30α**, a stronger base, i.e. sodium bis(trimethylsilyl)amide (NaHDMS) (2.0 equiv) was applied instead of imidazole. Under the kinetic controlled conditions, the hemiacetal was presumably deprotonated to give an alkoxide, which reacted with TMSCl (1.05 equiv) to give desired TMS *β*-glycoside **29β** in 55% yield along with 35% of unreacted hemiacetal of **42**. We reason that the use of the alkoxide nucleophile may bias the formation of the *β*-anomer, and the exact mechanism needs further investigation. Of noted, different stoichiometric amounts of NaHMDS and TMSCl were employed to improve the conversion, but in vain.

2.2 Synthesis of Protected 1,1'-Disaccharides 11–20

With the thioglycosyl donors and Pico protected TMS glycoside acceptors in hands, we initiated the synthesis of protected disaccharides **11**–**20** (Scheme 2, Table 1). In general, 2.0 equiv. of a thioglycosyl donor and 1.0 equiv. of a TMS glycoside acceptor in DCM (50 mM) were treated with 1.8 to 2.0 equiv. of freshly prepared dimethyldisulfide–triflic anhydride (Me_2S_2–Tf_2O) complex at ≤ 0 ºC reaction temperature.[14] The exact temperature was optimized for each donor/acceptor pair. For example, the construction of 1*α*→1'*α*-linked disaccharides **11**–**17** was performed at 0 ºC (Entries 1–7), but at the same temperature, the *β*-selectivity of a participating thio-D-*galacto*-pyranoside donor was eroded.[9] Therefore for the construction of disaccharides **18** and **19**, a lower –20 ºC temperature was applied (Entries 8 and 9). For the construction of 1*β*→1'*β*-linked disaccharide **20**, –40 ºC temperature was needed to avoid the *β*→*α* anomerization of TMS *β*-glycoside **29β**, which is more acid labile than the *α*-counterpart, i.e. **28α** (Entry 10). The glycosylation products given in entries 2, 3 and 9 were contaminated with some impurities. Therefore, the acyl protecting groups of the glycosylation products were removed before the NMR characterization.

In general, the yields of glycosylation spanned from acceptable ~42% to good 75%. Excellent stereoselectivity was observed and no other anomers could be isolated from the reaction mixture. On some occasions, small amount of the TMS acceptor remained. The yield of glycosylation was generally higher for D-*galacto* donors (**23**, **24**) than for D-*gluco* donors (**21**, **22**) (Entries 5 and 7). Such a reactivity-yield correlation pattern is consistent with different stereoelectronic properties of the sugar scaffolds. The anomeric configurations of the disaccharide products **11**–**20** were unequivocally confirmed by the $^3J_{H1H2}$ and/or $^1J_{C1H1}$ coupling constants



Scheme 2. Synthesis of 1,1′-disaccharides **11–20**.

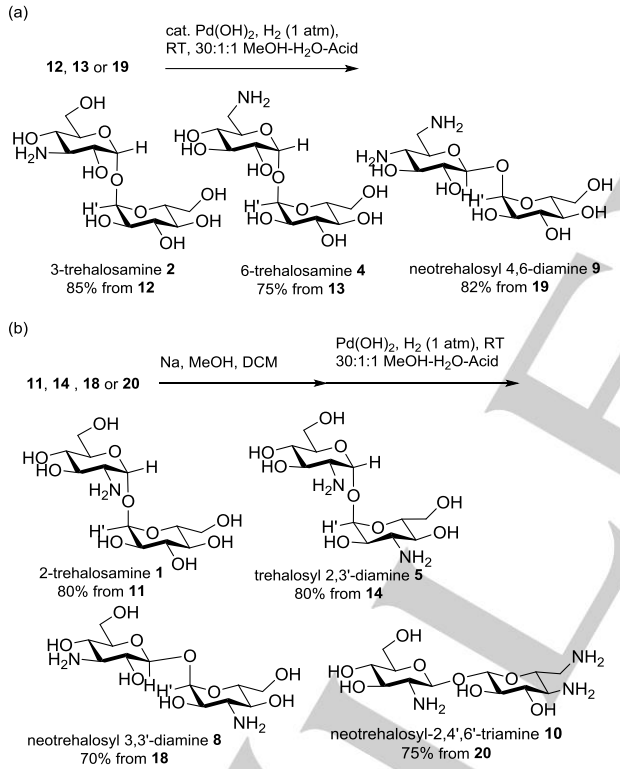
Table 1. Construction of protected 1,1-disaccharide aminoglycosides **11**-**20**

Entry	Donor	Acceptor	*T* °C	Product (%)
1	**22**	**29α**	0	**11** (60)[a]
2	**21**	**27α**	0	**12** (42)[b]
3	**22**	**28α**	0	**13** (52)[b]
4	**21**	**29α**	0	**14** (62)
5	**24**	**27α**	0	**15** (75)[a]
6	**21**	**28α**	0	**16** (63)
7	**23**	**28α**	0	**17** (65)
8	**25**	**30α**	-20	**18** (45)
9	**26**	**31α**	-20	**19** (43)[b]
10	**26**	**29β**	-40	**20** (45)

[a] The same glycosylation reactions were performed in previous studies, but in a smaller scale (ref 7). [b] The crude glycosylation product was hydrolysed to facilitate NMR characterization and the yield was based on the glycosylation and hydrolysis reactions.

2.3 Synthesis of Trehalosamines and 1,1'-Disaccharide Aminoglycosides 1–10

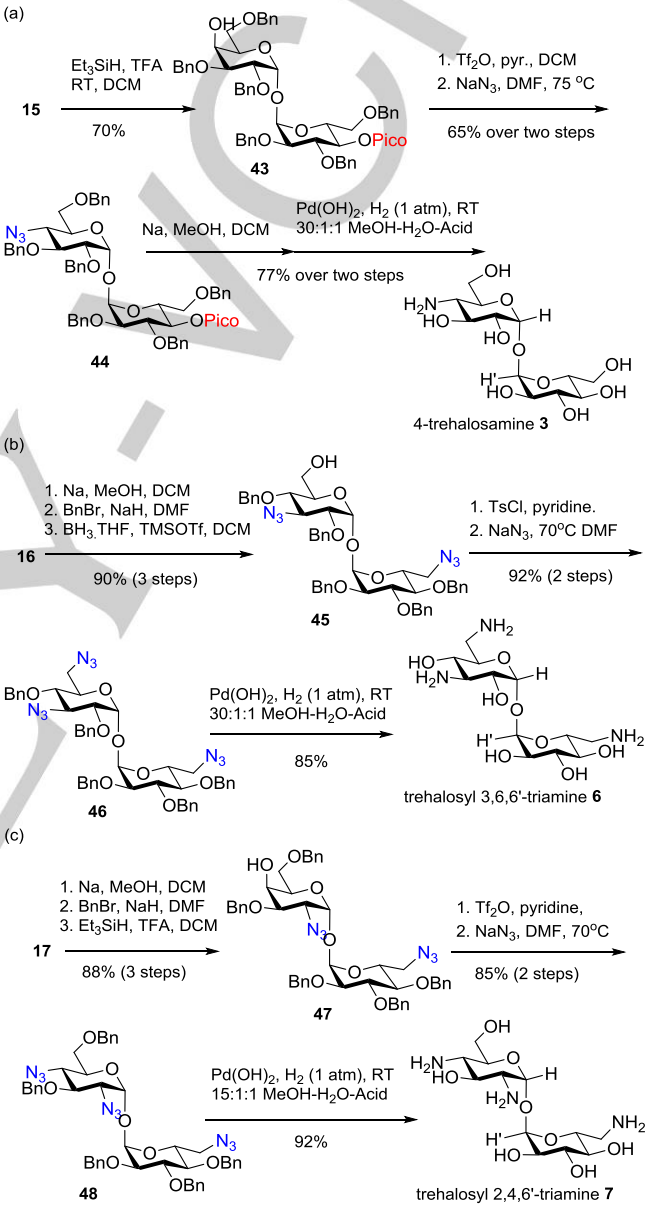
Having prepared disaccharides **11**–**20**, the stage was set to conclude the synthesis of targets **1**–**10** (Schemes 3 and 4). Thus, 3-trehalosamine **2**, 6-trehalosamine **4** and neotrehalosyl 4,6-diamine **9** could be obtained from disaccharides **12**, **13**, and **19** through a one-pot hydrogenolysis and azide-reduction protocol (Scheme 3a). Among various palladium catalysts, Pearlman catalyst ($Pd(OH)_2$) was found to be more effective than Pd/C.[15] The reaction was better conducted in a MeOH and H_2O cosolvent mixture under H_2 (1 atm); whereas, a trace amount of 1 N HCl (or neat acetic acid) was added to accelerate the reactions. Upon 24 h reactions, desired trehalosamines **2**, **4**, and neotrehalosyl 4,6-diamine **9** were obtained in high 75%–85% yields.



Scheme 3. (a) Synthesis of disaccharide aminoglycosides **2**, **4**, and **9**. (b) Synthesis of disaccharide aminoglycosides **1**, **5**, **8**, and **10**.

The synthesis of 2-trehalosamine **1**, trehalosyl 2,3′-diamine **5**, neotrehalosyl 3,3′-diamine **8**, and neotrehalosyl 2,4′,6′-triamine **10** employed disaccharides **11**, **14**, **18**, and **20**, respectively (Scheme 3b). The disaccharide substrate was first hydrolysed in basic conditions to deprotect the acyl groups. Subsequent one-pot hydrogenolysis and azide-reduction afforded desired aminoglycoside targets **1**, **5**, **8**, and **10** in 70%–80% overall yields (two steps).

For the synthesis of 4-trehalosamine **3**, trehalosyl 3,6,6'-triamine **6**, and trehalosyl 2,4,6'-triamine **7**, the amino group at C4 position of **3**, at C6 position of **6**, and at C4 position of **7** were introduced after the glycosylation (Scheme 4).



Scheme 4. (a) Synthesis of 4-trehalosamine **3**. (b) Synthesis of trehalosyl 3,6,6'-triamine **6**. (c) Synthesis of trehalosyl 2,4,6'-triamine **7**.

In previous synthesis, 4-trehalosamine **3** was prepared by selective functionalization of a trehalose substrate and the amino group was introduced as an azide through a tedious double inversion protocol.[4f] By using the 1,1′-glycosylation method, a more direct synthetic route was posible (Scheme 4a). Thus, Gal-

α-(1→1′)-α-Glc disaccharide **15** was employed for the procurement of **3** (Structure of **15** in Scheme 2). The benzylidene group of **15** was cleaved with Et_3SiH and TFA in DCM to afford disaccharide **43** in ca 70% yield. As the 4-hydroxyl of **43** has an axial orientation, it could be directly subjected to triflation and the crude triflation product was substituted with NaN_3 to give 4-azido-4-deoxy-trehalose **44**. As such, the double inversion protocol was eliminated. Subsequent hydrolysis of the Pico group of **44** and one-pot hydrogenolysis and azide-reduction furnished 4-trehalosamine **3** in 77% yield.

Trehalosyl 3,6,6′-triamine **6** could be derived from disaccharide **16**, which contained two azido groups at C3 and C6′ positions, respectively (Structure of **16** in Scheme 2) (Scheme 4b). The third azido group was introduced at C6 position of **16** after the glycosylation. Initially, the benzylidene group of **16** was cleaved in reduction conditions to give a free 6-hydroxyl for azido group introduction. However, the presence of the Pico group rendered the cleavage of the benzylidene ring difficult, which probably is attributed to the interference from the basic property of the Pico group. Thus, the Pico group of **16** was first replaced with a benzyl ether group. Then, the benzylation product was subjected to reductive ring opening with BH_3.THF and TMSOTf. As such, desired disaccharide **45** was obtained in 90% overall yield (three steps). Next, the 6-hydroxyl of **45** was converted to a tosyl group, which underwent substitution with NaN_3 to give protected 3,6,6′-triazido substituted trehalose **46**. One-pot hydrogenolysis and azide-reduction of **46** furnished trehalosyl 3,6,6′-triamine **6** in a high 85% yield.

The synthesis of trehalosyl 2,4,6′-triamine **7** started with disaccharide scaffold **17** (Structure of **17** in Scheme 2) (Scheme 4c). Inspired by the synthesis of **6** above, the Pico group of **17** was first replaced with the Bn protecting group. The benzylation product was treated with Et_3SH and TFA to cleave the benzylidene ring to furnish intermediate **47**, which has a 4-hydroxyl group in axial orientation. Conversion of the hydroxyl group to triflate followed by NaN_3 substitution afforded protected 2,4,6′-triazido substituted trehalose **48**. One-pot hyrogenolysis and azide reduction of **48** completed the synthesis of trehalosyl 2,4,6′-triamine **7**.

Trehalosamines **1**–**4**, neotrehalosyl diamine **8**, and disaccharide aminoglycosides **5**–**7**, **9**, and **10** obtained in above syntheses were extensively characterized with 1D and 2D NMR spectroscopy. The anomeric proton, carbon, and the position of the amino substitution are clearly indicated in HSQC and/or COSY spectra. Table 2 summarises the chemical shifts (*δ*, ppm) of the anomeric protons (H-1 and H-1′) (Columns 3 and 5) and the corresponding coupling constants ($^3J_{H1H2}$ and $^3J_{H1'H2'}$) (Columns 4 and 6) of compounds **1**-**10**.

Table 2. ^{1}H chemical shifts (*δ*) and H1-H2 coupling constants ($^3J_{H1-H2}$) at the anomeric centres of **1**–**10**

Entry	1,1′-Disaccharide aminoglycoside	*δ* of H1 (ppm)	$^3J_{H1H2}$ (Hz)	*δ* of H1′ (ppm)	$^3J_{H1'H2'}$ (Hz)
1	**1**	5.39	4.0	5.21	3.5
2	**2**	5.16	4.0	5.13	3.5
3	**3**	5.13	4.0	5.04	4.0
4	**4**	5.12	3.5	5.16	4.0
5	**5**	5.43	4.0	5.25	3.5
6	**6**	5.24	4.0	5.21	4.0
7	**7**	5.47	3.5	5.25	3.5
8	**8**	4.80	8.4	5.33	3.6
9	**9**	4.57	8.0	5.14	4.0
10	**10**	4.89	10.2	5.09	10.2

Conclusions

In summary, we have applied 1,1′-glycosylation strategy for preparation of a small library of natural trehalosamines (**1**–**3**), neotrehalosyl 3,3′-diamine (**8**), and synthetic 1,1′-disaccharide aminoglycosides (**4**–**7**, **9**, **10**). These disaccharide aminoglycosides feature different anomeric configurations and distribution patterns of amino groups. Their structures are characterized extensively with NMR spectroscopic methods. Exploration of the bactericidal properties of these compounds is in progress and the results will be reported in due course.

Experimental Section

Procedures for preparation of thioglycoside donors **21**, **25**, **26** and TMS glycoside acceptors **28α**, **29β**, and **30α** (Scheme 1), post-glycosylation modification (Scheme 4) and one-pot hyrodrogenolysis and azide reduction (Scheme 3) are given in SI.

General glycosylation procedure: To a mixture of thioglycosyl donor (**21**–**26**, 2.0 equiv.), TMS α-glycoside (**27α**–**31α** and **29β**, 1.0 equiv.) or TMS β-glycoside (**29β**) and freshly activated 4Å MS, DCM (50 to 100 mM w.r.t acceptor) was added and stirred at RT under N_2 for 15 min. Then the temperature was decreased to 0 ºC (or –20 ºC for coupling of **23**/**28α** and **26**/**31α** or –40 ºC for coupling of **26**/**29β**) and the solution was stirred for another 15 min before the addition of promoter. In another pear-shaped flask, a solution of dimethyldisulfide (Me_2S_2, 1.4-2.0 equiv.) in DCM (1 M) was treated with triflic anhydride (Tf_2O, same equiv. as Me_2S_2) under N_2 at 0 °C (see SI for specific amounts of Me_2S_2 and Tf_2O). After standing for 20 min at 0 °C, the Me_2S_2-Tf_2O promoter was transferred slowly to the donor and acceptor mixture. The resulting mixture was stirred at 0 °C or at optimized temperature until the glycosylation was complete. Then the reaction was quenched by Et_3N (same equiv. as promoter) followed by filtration to remove MS. The DCM filtrate was directly concentrated for flash chromatography to obtain the 1,1′-disaccharides **11**, **12′**, **13′**, **14**, **15**, **16**, **17**, **18**, **19′** and **20**. 1,1′-Disaccharides **12′**, **13′**, and **19′** were taken to hydrolysis to give **12**′, **13**′, and **19** (in SI).

For 2-trehalosamine **1**, R_f 0.18 (*i*PrOH:NH_4OH, 2:1); $[\alpha]_D^{20}$ +170.0 (*c* 0.5, H_2O) (Lit.: $[\alpha]_D^{20}$ +176.0, *c* 2.0 in H_2O);[1a] ^{1}H NMR (500 MHz, D_2O): δ 5.39 (d, *J* = 4.0 Hz, 1H, H-1), 5.21 (d, *J* = 3.5 Hz, 1H, H-1′), 3.94 (dd, *J* = 9.0, 10.5 Hz, 1H, H-4), 3.82 (dd, *J* = 11.5, 2.0 Hz, 1H, H-5′), 382 – 3.58 (m, 5H including H-6 × 2, H-6′ × 2, H-3′), 3.63 (dd, *J* = 10.5, 4.0 Hz, 1H, H-2′) – 3.59 (dt, *J* = 5.5, 2.0 Hz, 1H, H-5), 3.48 (t, *J* = 10.0 Hz, 1H, H-3), 3.398 (dd, *J* = 11.0, 4.0 Hz, 1H, H-2), 3.397 (dd, *J* = 10.0, 9.5 Hz, 1H, H-4′). ^{13}C NMR (125 MHz, D_2O): δ 93.3 (C-1′), 90.6 (C-1), 72.7 (C-5′), 72.5 (C-5), 72.0 (C-3′), 70.5 (C-2′), 69.42 (C-4′), 69.41 (C-4), 69.2 (C-3), 60.5, 60.0, 53.4 (C-2). HRMS (ESI): calcd for $C_{12}H_{23}NNaO_{10}^+$, 364.1214; found: 364.1205 [M + Na]$^+$.:

For 3-trehalosamine **2**, R_f 0.22 (*i*PrOH/NH_4OH, 2/1); $[\alpha]_D^{20}$ +168.0 (*c* 0.2, CH_3OH), (Lit.: $[\alpha]_D^{20}$ +161.0º, *c* =13.7 in H_2O);[1b] ^{1}H NMR (500 MHz, D_2O): *δ* 5.16 (d, *J* = 4.0 Hz, 1H, H-1), 5.13 (d, *J* = 3.5 Hz, 1H, H-1′), 3.83 (dd, *J* = 3.5, 10.5 Hz, 1H, H-2), 3.81 – 3.60 (m, 7H), 3.63 (t, *J* = 10.0 Hz, 1H, H-4), 3.58 (dd, *J* = 10.0, 3.5 Hz, 1H, H-2'), 3.50 (t, *J* = 10.5 Hz, 1H, H-3), 3.39 (t,

J = 9.5 Hz, 1H, H-3'). ^{13}C NMR (125 MHz, D_2O): *δ* 93.3 (C-1', $^1J_{H'C'}$ = 170.8 Hz), 92.2 (C-1, $^1J_{HC'}$ = 171.9 Hz), 72.4, 72.3, 71.9, 70.9 (C-2'), 69.6 (C-3'), 67.6 (C-2), 65.8 (C-4), 60.48, 59.80, 54.8 (C-3). HRMS (ESI): calcd for $C_{12}H_{23}NNaO_{10}^+$, 364.1214; found: 364.1218 $[M + Na]^+$.

For 4-trehalosamine **3**, R_f 0.18 (*i*PrOH/NH_4OH, 2/1); $[\alpha]_D^{20}$ +173.0º (*c* 0.3, CH_3OH), (Lit.: $[\alpha]_D^{20}$ +179.0º *c* 0.5 in H_2O);[1d] ^{1}H NMR (500 MHz, CD_3OD): *δ* 5.13 (d, *J* = 4.0 Hz, 1H, H-1), 5.04 (d, *J* = 4.0 Hz, 1H'), 4.08 (dt, *J* = 10.5, 4.0 Hz, 1H, H-5), 3.97 (t, *J* = 10 Hz, 1H, H-3), 3.81-3.70 (m, 6H), 3.67 (dd, *J* = 10.0, 4.0 Hz, 1H, H-2), 3.59 (dd, *J* = 10.0, 4.0 Hz, 1H, H-2'), 3.38 (dd, *J* = 9.0 Hz, 1H, H-3'), 3.41 (t, *J* = 10.3 Hz, 2H, H-4'), 3.23 (d, *J* = 10.5 Hz, 1H, H-4). ^{13}C NMR (101 MHz, CD_3OD): *δ* 93.5 (C-1'), 93.4 (C-1), 72.4, 72.2, 70.9 (C-2), 70.8 (C-2'), 69.5 (C-4'), 68.5 (C-3), 68.4 (C-5), 60.4, 60.3, 52.5 (C-4). HRMS (ESI): calcd for $C_{12}H_{23}NNaO_{10}^+$ requires 364.1214; found: *m/z* 364.1220 $[M + Na]^+$.

For 6-trehalosamine **4**, R_f 0.16 (*i*PrOH/NH_4OH, 2/1); $[\alpha]_D^{20}$ +142.0 (*c* 0.35, CH_3OH); ^{1}H NMR (500 MHz, D_2O): *δ* 5.16 (d, *J* = 4.0 Hz, 1H, H-1'), 5.12 (d, *J* = 3.5 Hz, 1H, H-1), 3.92 (td, *J* = 3.0, 9.5 Hz, 1H, H-5), 3.81 – 3.75 (m, 4H), 3.69 (dd, *J* = 12.0, 4.0 Hz, 1H, H-6b'), 3.61 (dd, *J* = 3.5, 10.0 Hz, 1H, H-2'), 3.58 (dd, *J* = 4.0, 10.0 Hz, 1H, H-2), 3.40 – 3.35 (two signals (dd and t) overlapped, 2H, H-6a and H-4'), 3.30 (t, *J* = 9.5 Hz, 1H, H-4), 3.08 (dd, *J* = 9.0, 13,5 Hz, 1H, H-6b). ^{13}C NMR (125 MHz, D_2O): *δ* 93.3 (C-1), 93.2 (C-1'), 72.3, 72.0, 71.8, 71.2 (C-4), 70.8 (C-2), 70.5 (C-2'), 69.3 (C-4'), 67.9 (C-5), 60.2 (C-6'), 40.3 (C-6). HRMS (ESI): calcd for $C_{12}H_{23}NNaO_{10}^+$, 364.1214; found: 364.1212 $[M + Na]^+$

For trehalosyl 2,3′-diamine **5**, R_f 0.23 (*i*PrOH/NH_4OH, 2/1); $[\alpha]_D^{20}$ +175.0 (*c* 0.8, CH_3OH); ^{1}H NMR (500 MHz, D_2O): δ 5.43 (d, *J* = 4.0 Hz, 1H, H-1), 5.25 (d, *J* = 3.5 Hz, 1H, H-1′), 3.96 (dd, *J* = 9.0, 10.5 Hz, 1H, H-3), 3.91 (dd, *J* = 4.0, 11.0 Hz, 1H, H-2′), 3.86 – 3.78 (m, 3H), 3.73 (dd, *J* = 5.0, 12.5 Hz, 2H), 3.69 – 3.65 (m, 2H), 3.54 (d, *J* = 10.5 Hz, 1H, H-3′), 3.50 (t, *J* = 9.5 Hz, 1H, H-4), 3.43 (dd, *J* = 3.5, 11.0 Hz, 1H, H-2). ^{13}C NMR (125 MHz, D_2O): δ 92.4 (C-1′), 91.0 (C-1), 72.74, 72.70, 69.5 (C-3), 69.3 (C-4), 67.2, 65.7, 60.2, 60.0, 54.5 (C-3′), 53.5 (C-2). HRMS (ESI): calcd for $C_{12}H_{24}NNaO_9^+$, 363.1374; found: 363.1366 $[M + Na]^+$.

For trehalosyl 3,6,6′-triamine **6**, R_f 0.18 (*i*PrOH:NH_4OH = 2:1); $[\alpha]_D^{20}$ +165.0 (*c* 0.7, CH_3OH); ^{1}H NMR (500 MHz, D_2O): *δ* 5.24 (d, *J* = 4.0 Hz, 1H, H-1), 5.21 (d, *J* = 4.0 Hz, 1H, H-1′), 4.04 (td, *J* = 2.5, 9.0 Hz, 1H, H-5), 3.98 (ddd, *J* = 3.0, 8.5, 10.5 Hz, 1H, H-5′), 3.93 (dd, *J* = 3.5, 10.5 Hz, 1H, H-2), 3.80 (t, *J* = 9.5 Hz, 1H, H-3′), 3.66 (dd, *J* = 4.0, 10.0 Hz, 1H, H-2′), 3.59 (t, *J* = 10.0 Hz, 1H, H-4), 3.55 (t, *J* = 10.5 Hz, 1H, H-3), 3.46 – 3.31 (m, 3H), 3.12 (ddd, *J* = 4.0, 8.0, 12.0 Hz, 2H). ^{13}C NMR (125 MHz, D_2O): δ 94.5 (C-1′), 93.3 (C-1), 72.1 (C-3′), 71.2 (C-4′), 70.7 (C-2′), 68.4 (C-5′), 68.36 (C-5), 67.8 (C-4), 67.4 (C-2), 54.5 (C-3), 40.5 (C-6 or C-6′), 40.2 (C-6 or C-6′). HRMS (ESI): calcd for $C_{12}H_{25}N_3NaO_8^+$, 362.1534; found: 362.1526 $[M + Na]^+$.

For trehalosyl 2,4,6′-triamine **7**, R_f 0.18 (*i*PrOH/NH_4OH, 2/1); $[\alpha]_D^{20}$ +128.0 (*c* 0.75, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 5.47 (d, *J* = 3.5 Hz, 1H, H-1), 5.25 (d, *J* = 3.5 Hz, 1H, H-1'), 4.22 (t, *J* = 10.0 Hz, 1H, H-3), 4.18 (dt, *J* = 4.0, 10.5 Hz, 1H, H-5), 3.86 – 3.75 (m, 4H), 3.68 (dd, *J* = 4.0, 10.5 Hz, 1H, H-2'), 3.55 (dd, *J* = 3.5, 10.5 Hz, 1H, H-2), 3.43 – 3.33 (m, 3H, including H-4, H-4', and H-6a'), 3.16 (dd, *J* = 7.5, 13.5 Hz, 1H, H-6b'). ^{13}C NMR (125 MHz, D_2O): δ 93.5 ($^1J_{CH}$ = 173 Hz, C-1'), 90.9 ($^1J_{CH}$ = 175 Hz, C-1), 71.3, 70.7, 69.9 (C-2'), 68.6, 68.5, 65.5 (C-3), 59.8 (C-6), 53.3 (C-2), 52.1 (C-4), 40.0 (C-6'). HRMS (ESI): calcd for $C_{12}H_{26}N_3O_8^+$, 340.1709; found: 340.1714 $[M + H]^+$.

For neotrehalosyl 3,3′-diamine **8**, R_f 0.2 (*i*PrOH/NH_4OH, 2/1); $[\alpha]_D^{20}$ +173.0 (*c* 0.3, CH_3OH); ^{1}H NMR (600 MHz, CD_3OD): *δ* 5.33 (d, *J* = 3.6 Hz, 1H, H-1′), 4.80 (d, *J* = 8.4 Hz, 1H, covered by the signal of D_2O but the ^3J values and position could be identified from the correlation signals in the COSY and HSQC spectra), 4.01 (bs, *J* = 6.6 Hz, 1H), 3.91 – 3.89 (m, 3H including H-2′), 3.82 – 3.76 (m, 2H), 3.74 – 3.69 (m, 2H), 3.64 (dd, *J* = 10.8, 8.4 Hz, 1H, H-2), 3.61 – 3.60 (bm, 1H), 3.64 (t, *J* =10.8 Hz, H-3′), 3.28 (t, *J* = 10.2 Hz, 1H, H-3), 1.94 (s, 6H, C*H*$_3CO_2^- \times 2$). ^{13}C NMR (151 MHz, D_2O): δ 181.0 ($CH_3CO_2^- \times 2$), (102.73 (C-1), 99.1 (C-1′), 76.9, 72. 6, 69.6, 68.0, 65.7, 59.9, 59.8 (C-3), 57.5 (C-3′), 55.0, 23.1 (C$H_3CO_2^- \times 2$). HRMS (ESI): calcd for $C_{12}H_{25}N_2O_9^+$, 341.1560; found: 341.1564 $[M + H]^+$.

For neotrehalosyl-4,6-diamine **9**, R_f 0.2 (*i*PrOH/NH_4OH, 2/1); $[\alpha]_D^{20}$ +15.4 (*c* 0.13, CH_3OH); ^{1}H NMR (500 MHz, D_2O): δ 5.14 (d, *J* = 4.0 Hz, 1H, H-1′), 4.57 (d, *J* = 8.0 Hz, 1H, H-1), 3.77 – 3.74 (m, 1H, H-5′), 3.72 – 3.67 (m, 2H, H-6′), 3.64 (dt, *J* = 10.0, 2.5 Hz, 1H, H-3′), 3.52 (dd, *J* = 3.5, 10.0 Hz, 1H, H-2′), 3.49 – 3.41 (m, 2H including H-3 and H-5), 3.39 (t, J = 8.0 Hz, 1H, H-2), 3.37 (t, *J* = 8.0 Hz, 1H, H-4′), 2.85 (h, *J* = 12.5, 9.0, 3.5 Hz, 1H, H-6a), 2.68 – 2.60 (m, 1H, H-6b), 2.57 (t, *J* = 10.0 Hz, 1H, H-4). ^{13}C NMR (125 MHz, D_2O): *δ* 102.7 (C-1, $^1J_{CH}$ = 156 Hz), 100.4 (C-1′, $^1J_{CH}$ = 169.1 Hz), 73.5, 73.4, 72.7, 72.6, 71.3, 71.1, 69.1, 60.2 (C-4), 57.2 (C-6). HRMS (ESI): calcd for $C_{12}H_{25}N_2O_9^+$, 341.1555; found: 341.1589 $[M + H]^+$.

For neotrehalosyl 2,4',6'-diamine **10**, R_f 0.25 (*i*PrOH/NH_4OH, 2:1); $[\alpha]_D^{20}$ 16.7 (*c* 0.6, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 5.09 (d, *J* = 8.5 Hz, 1H, H-1′), 4.89 (d, *J* = 8.5 Hz, 1H, H-1), 4.01 (td, *J* = 2.5, 9.5 Hz, 1H, H-5), 3.87 (dd, *J* = 2.0, 12.5 Hz, 1H, H-6′), 3.71 (dd, *J* = 5.5, 12.5 Hz, 1H, H-6′), 3.67 (q, *J* = 9.5 Hz, 2H, including H-3 and H-3′), 3.54 – 3.42 (m, 4H, including H-2, H-4′, H-5′and H-6), 3.28 – 3.17 (m, 2H, including H-4 and H-6), 3.12 (dd, *J* = 9.0, 11.0 Hz, 1H, H-2′).^{13}C NMR (125 MHz, D_2O): *δ* 98.3 (C-1, $^1J_{CH}$ = 166.1 Hz), 95.3 (C-1′, $^1J_{CH}$ = 167.0 Hz), 76.4 (C-2), 72.2, 71.7, 71.5, 69.4, 69.0, 60.2 (C-6′), 55.0 (C-2′), 52.9 (C-4), 39.8(C-6). HRMS (ESI): calcd for $C_{12}H_{26}N_3O_8^+$, 341.1714; found: 340.1708 $[M + H]^+$.

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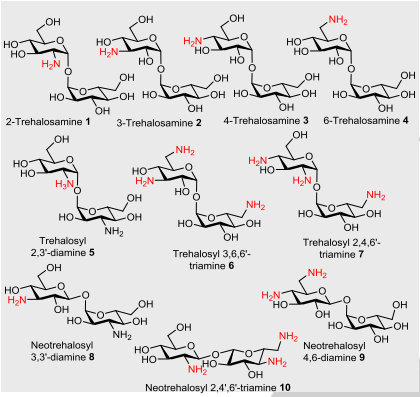

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