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Synthesis of Novel Iminosugar-Based Trehalase Inhibitors by Cross-Metathesis Reactions

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The synthesis of a novel class of trehalase inhibitors composed of iminopyranose or iminofuranose residues linked at the pseudoanomeric carbon through an alkyl chain is described. A set of six novel compounds was prepared by the same reaction sequence involving the Grubbs Ru–carbene-catalyzed cross-metathesis (CM) of different *N*-Cbz-protected allyl *C*-iminoglycosides as the key step in homo- or heterodimerization reactions. The target products, obtained with the CM reaction, were fully hydrogenated by catalytic hydrogenolysis, and preliminary biological screening of the products as inhibitors of commercially available porcine trehalase was performed.

Introduction

Trehalase (EC 3.2.1.28) is an extremely specific inverting glycosidase that hydrolyzes trehalose [α-D-glucopyranosyl-α-D-glucopyranoside (**1**) Figure 1][1] to two glucose units. Trehalose assumes considerable relevance in nature,[2] and its hydrolysis is a process essential to vital functions of several organisms, in particular fungi and insects, while being almost absent in mammalian metabolism. Thus, trehalose processing enzymes such as trehalases are attractive targets for the search of inhibitors, as valuable tools for studying the molecular physiology of trehalase function and sugar metabolism in insects, and as potential novel insecticides.[3] Some natural pseudodisaccharides, such as validoxylamine A (**2**),[4] trehazolin (**3**),[5] casuarine-6-*O*-α-D-glucoside (**4**),[6,7] and its analogues (i.e., **5** and **6**)[8] have been shown to be potent inhibitors of trehalase. In addition, it is well known that iminosugars[9,10] are potent glycosidases inhibitors due to the presence of the endocyclic nitrogen, which is able to mimic the transition state of the enzymatic reaction. Hence, a few iminosugar-based compounds (such as **4**–**8**, Figure 1) have been also proposed as trehalase inhibitors.[5,11,12]

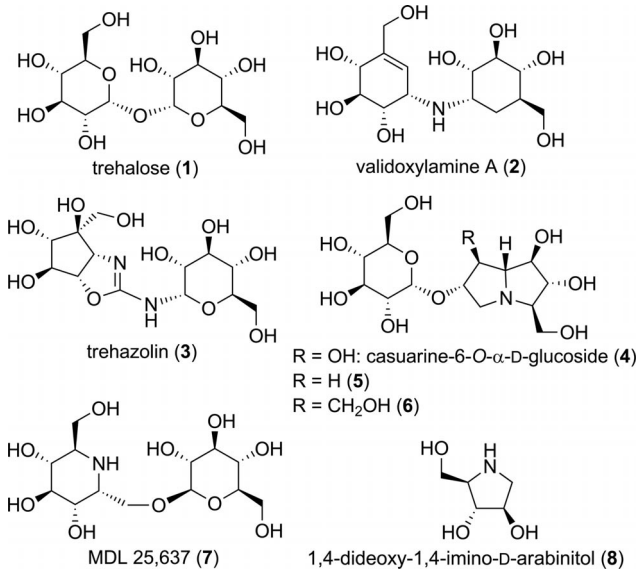

Figure 1. Chemical structure of trehalose (**1**), validoxylamine A (**2**), trehazolin (**3**), casuarine-6-*O*-α-D-glucoside (**4**), its analogues (i.e., **5**, **6**), and other iminosugar-based trehalase inhibitors (i.e., **7** and **8**).

In the search for new inhibitors that might be specific towards insect trehalase,[8,12] we herein propose the synthesis of iminosugar-based trehalase inhibitors, possessing a pseudodisaccharidic structure, obtained by cross-metathesis (CM) reactions between suitably functionalized piperidine or pyrrolidine iminosugars.

Results and Discussion

We report therein the synthesis and preliminary biological assays of novel trehalase inhibitors (compounds **10**,

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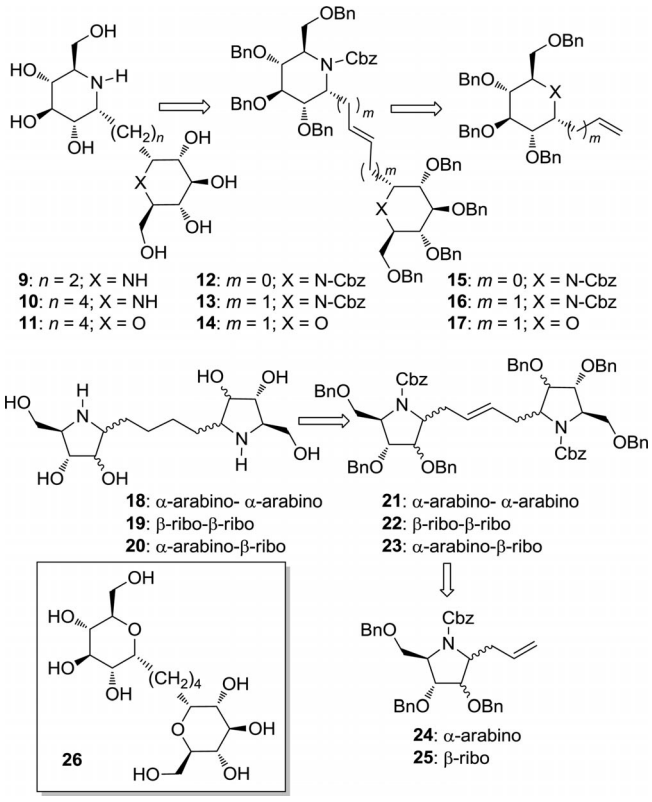
11, **18**–**20**; Scheme 1) containing one or two iminosugar moieties, namely, the nojirimycin core or the 1,4-dideoxy-1,4-imino-D-arabinitol or the 1,4-dideoxy-1,4-imino-D-ribitol scaffolds. To mimic the natural disaccharidic substrates, the two units were linked by a short and flexible alkyl bridge; the Grubbs Ru–carbene-catalyzed CM reaction[13] was used to connect the two moieties suitably functionalized with a double bond, as outlined in the retrosynthetic scheme (Scheme 1).

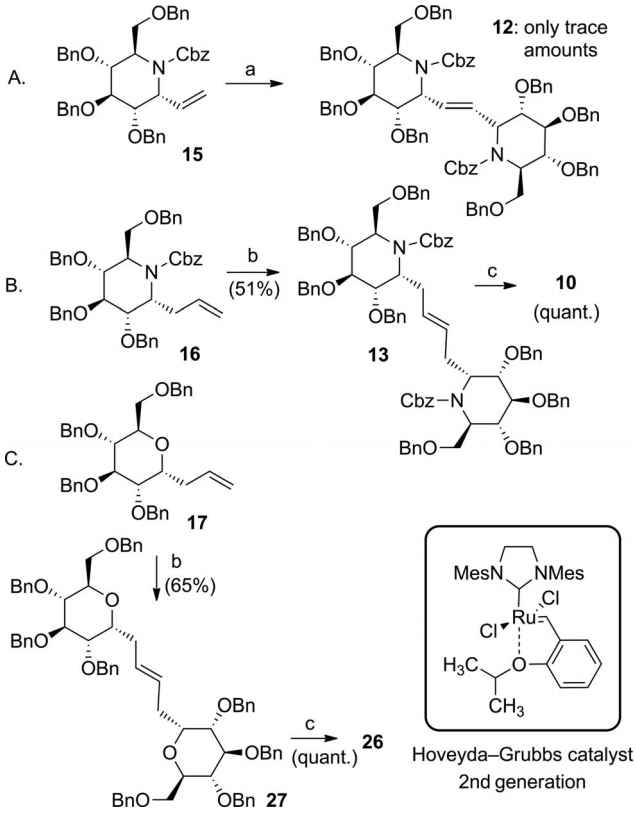


Scheme 1. Retrosynthetic scheme for the synthesis of trehalase inhibitors.

As the starting point, we decided to synthesize nojirimycin dimers **9** and **10**, linked through a two and four carbon atom spacer, respectively, having in our hands fully protected α-*C*-vinyl nojirimycin **15**[14] and protected α-*C*-allyl nojirimycin **16** (Scheme 1).[15] Each monomer was homodimerized by CM using the 2nd generation Grubbs catalyst in dry dichloromethane as solvent.

It should be pointed out that the endocyclic nitrogen atom has to be protected as a carbamate, as recent reports[16] showed that the CM is not compatible with the endocyclic *N*-Bn group (found as protecting group in our iminosugar synthesis), very likely because of the coordinating ability of the latter with the Ru–carbene catalyst. Thus, the *N*-carbobenzyloxynojirimycin was used in the present study.

Initially, protected α-vinyl nojirimycin **15** was treated with 20% in weight Grubbs 1,3-dimesityl-4,5-dihydroimidazol-2-ylideneruthenium carbene (Scheme 2, inset) in dichloromethane at room temperature, but the reaction did not afford the expected product. Hence, different conditions were tried (DCM at r.t., DCM at 40 °C, DCE at 80 °C, DCE plus ultrasonication at 50 °C), and desired product **12** was obtained only in trace amounts (Scheme 2A). The extremely scarce reactivity observed for vinyl nojirimycin derivative **15** towards CM is supported also by similar examples reported on vinyl-*C*-glycosides.[17]



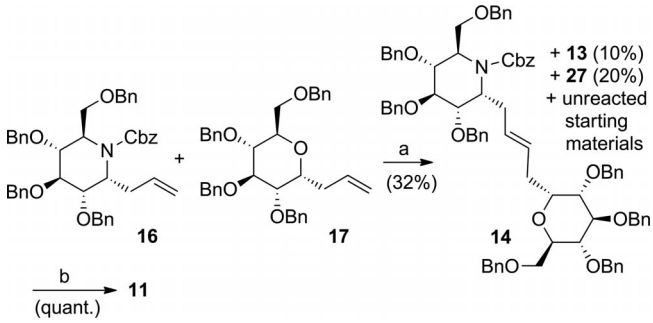
Scheme 2. Homodimerization of pyranose structures by CM. Reagents and conditions: (a) Hoveyda–Grubbs catalyst 2nd generation, DCE, 80 °C, overnight; (b) Hoveyda–Grubbs catalyst 2nd generation, DCM, r.t., overnight; (c) $Pd(OH)_2/C$, H_2, EtOAc/EtOH = 1:1.

We proceeded further with the homodimerization of α-*C*-allyl nojirimycin **16** (Scheme 2B), and in this case the CM reaction afforded compound **13** (see general procedure) in 51% yield as the (*E*) alkene (>95%). The yield, despite being quite low, is comparable to those obtained in different CM reactions reported on the same substrate.[18] Dimer product **13** was fully deprotected by Pd-catalyzed hydrogenation.

To assess the relevance of the nitrogen atom in the inhibition assays with iminosugar-based trehalase inhibitors, an additional homodimerization by CM was performed on α-*C*-allyl glucoside **17** (Scheme 2C), which afforded dimer **27** in 65% yield; that compound in turn gave compound **26** after catalytic hydrogenolysis.

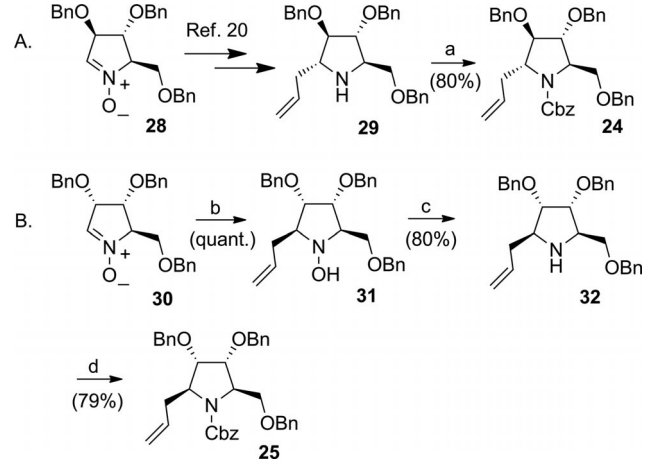
To gain better insight on the role of nitrogen, a heterodimer containing one nojirimycin unit and one glucose unit was also synthesized (Scheme 3) by CM. Due to the inertness of vinyl derivatives, we decided to concentrate our attention only on *C*-allyl monomers (α-*C*-allyl nojirimycin **16** and α-*C*-allyl glucoside **17**). The CM reaction was performed by using a slight excess amount of α-*C*-allyl gluco-

side **17**, which gave desired compound **14** in 32% yield. The remaining material was composed of glucose and nojirimycin homodimers **27** and **13** together with unreacted starting alkenes **16** and **17**. Final catalytic hydrogenation afforded compound **11**.



Scheme 3. Heterodimerization of pyranose structures by CM. Reagents and conditions: (a) Hoveyda–Grubbs catalyst 2nd generation, DCM, r.t., overnight; (b) $Pd(OH)_2/C$, H_2, EtOAc/EtOH = 1:1.

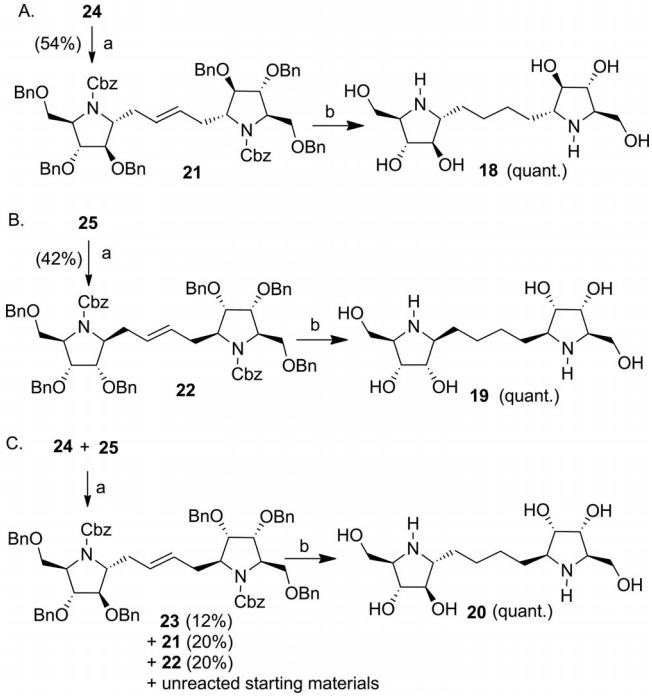
Due to the reported activity against trehalases of some iminofuranoses, such as compound **8**[11] (Figure 1), we envisaged the possibility to expand our set of potential inhibitors also to pseudodisaccharides obtained by CM of *C*-allyl-iminofuranoses. Two different allyl-iminofuranoses were synthesized and used for the CM reaction (Scheme 4), possessing a 1,4-dideoxy-1,4-imino-D-arabinitol (**24**) or 1,4-dideoxy-1,4-imino-D-ribitol (**25**) core, respectively.



Scheme 4. Synthesis of the iminofuranose scaffolds. Reagents and conditions: (a) CbzCl, H_2O/dioxane, $NaHCO_3$, r.t., 16 h; (b) allylmagnesium bromide, THF, 0 °C, 3 h; (c) Zn, AcOH/H_2O, r.t., 40 min; (d) CbzCl, H_2O/dioxane, $NaHCO_3$, r.t., 20 h.

The synthesis of α-C-allyl iminoarabinofuranosyl derivative **24** (Scheme 4A) proceeded by Grignard addition to nitrone **28**,[19] as recently described.[20] Final carbamoylation of intermediate **29** by Cbz-chloride in water/dioxane[21] afforded **24** (80%).[20] On the contrary, the synthesis of β-C-allyl iminoribofuranosyl derivative **25** is not reported in the literature and is presented here from nitrone **30**,[22] as illustrated in Scheme 4B. The Grignard reaction of nitrone **30** occurred with total stereoselectively and only the *anti* isomer was formed, according to the expected stereochemical outcome; reduction of hydroxylamine **31** to the corresponding amine **32** followed by carbamoylation afforded β-C-allyl iminoribofuranosyl derivative **25** suitable to be employed in the CM reaction.

The two scaffolds were then used for homodimerization and heterodimerization reactions by CM (Scheme 5). The CM reactions were performed by following the general procedure, and afforded dimers **21**, **22**, and **23** in 54, 42, and 12% yield, respectively. As expected, the heterodimerization proceeded with a similar reaction outcome to that observed for six-membered ring derivatives (homodimerization and starting material byproducts). Finally, hydrogenolysis afforded deprotected compounds **18**, **19**, and **20** (see the general procedure).



Scheme 5. Dimerization of furanose structures by CM. Reagents and conditions: (a) Hoveyda–Grubbs catalyst 2nd generation, DCM, r.t., overnight; (b) $Pd(OH)_2/C$, H_2, EtOAc/EtOH = 1:1.

All the deprotected compounds (i.e., **10**, **11**, **18**–**20**, **26**) were tested for their inhibitory activity against porcine trehalase. To examine the potential of each member of the library as trehalase inhibitor, preliminary screening assays at a fixed concentration (1 mM) of potential inhibitors was carried out, and dose–response curves were established for the most active compounds to determine the K_i values. Experiments were performed at a fixed substrate concentration, close to the K_m value (2.5 mM) in the presence of increasing concentrations of the inhibitor. The inhibitory activity is shown as a percentage at the fixed concentration in Figure 2.

At a concentration of 1 mM, compounds **11**, **18**, **19**, and **20** showed 25, 14, 28, and 43% inhibition, respectively, with respect to the control in the absence of an inhibitor, whereas

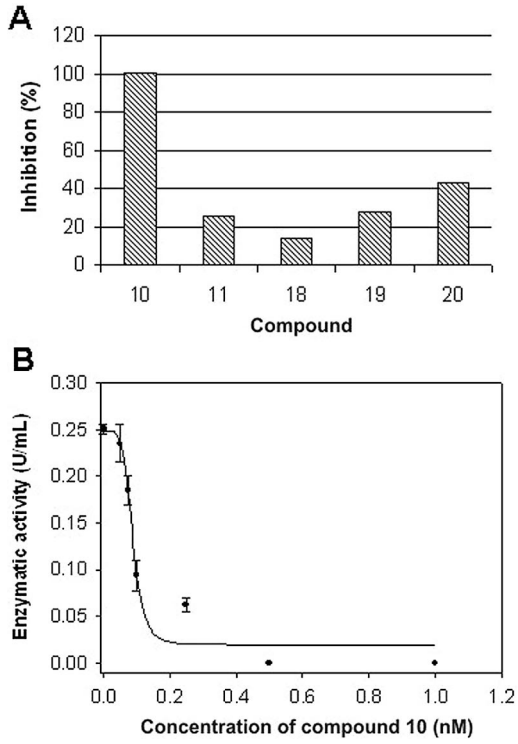


Figure 2. (A) Inhibition data of the synthesized compounds against porcine trehalase observed at 1 mM inhibitor concentration. (B) Dose–response curve of compound **10** (concentrations ranging from 50 μM to 1 mM) on porcine trehalase.

compound **10**, which is the nojirimycin dimer, was the most active derivative of the series, showing 100% inhibition at a concentration of 1 mM (Figure 2A). For this compound, a dose–response curve was performed (Figure 2B), affording IC_{50} = 88 μM and K_i = 44 μM. On the other hand, reference glucose dimer **26** did not show any inhibition at a concentration of 1 mM.

Conclusions

A series of iminosugars was synthesized by cross-metathesis reactions, and the series was preliminarily assayed for their activity against porcine trehalase. The collected data showed that very small inhibition was observed with iminoarabinofuranosyl dimers, whereas a significant inhibition could be detected with nojirimycin dimer **10**. It is worth noting that nojirimycin–glucose heterodimer **11** showed inhibitory activity in between that of nojirimycin homodimer **10** and glucose homodimer **26**, which was fully inactive. These results highlight that the nitrogen atom is relevant for inhibition, as usually reported for iminosugars, and that probably in the present case an additive effect occurs. Work is underway in our laboratories to expand the scope of this reaction and to investigate the role of the length of the alkyl chain connecting the two iminosugar moieties in more detail.

Experimental Section

General Methods: All solvents were dried with molecular sieves for at least 24 h prior to use. When dry conditions were required, the reaction was performed under an Ar atmosphere. Thin-layer chromatography (TLC) was performed on silica gel $60F_{254}$ coated glass plates (Merck) with UV detection when possible, charring with a conc. H_2SO_4/EtOH/H_2O (10:45:45) solution, or with a solution of $(NH_4)_6Mo_7O_{24}$ (21 g), $Ce(SO_4)_2$ (1 g), conc. H_2SO_4 (31 mL) in water (500 mL) and then heating to 110 °C for 5 min. Flash column chromatography was performed on silica gel 230–400 mesh (Merck). Routine ^1H and ^{13}C NMR spectra were recorded at 400 MHz (^1H) and at 100.57 MHz (^{13}C) with a Varian Mercury instrument or with a Varian Gemini 200 MHz instrument, 50.29 MHz (^{13}C) when specified. Chemical shifts are reported in parts per million downfield from TMS as an internal standard. Mass spectra were recorded with a System Applied Biosystems MDS SCIEX instrument (Q TRAP, LC–MS–MS, turbon ion spray) or with a System Applied Biosystem MDS SCIEX instrument (Q STAR elite nanospray). ESI full MS were recorded with a Thermo LTQ instrument by direct inlet; relative percentages are shown in brackets. Elemental analyses (C, H, N) were performed with a Perkin–Elmer series II 2400 analyzer or with a Perkin–Elmer CHNS/O 2400 analyze, and all synthesized compounds showed a purity of more than 95%.

General Procedure for the Cross-Metathesis Reaction: To a 0.1 M solution of the appropriate allyl monomers in anhydrous CH_2Cl_2 was added the Grubbs catalyst (5% in weight). The mixture was stirred overnight at room temperature and concentrated. The residue was purified directly on a silica gel column using a suitable eluent. All the products obtained by CM were the (*E*) isomers (>95% as determined by NMR).

General Procedure for the Hydrogenolysis Reaction: A 0.02 M solution of the appropriate dimer dissolved in EtOAc/EtOH (1:1) was treated with $Pd(OH)_2$/C (100% in weight). The reaction was stirred for 5 d under a H_2 atmosphere. Palladium was then removed by filtration through a Celite pad followed by washing with EtOH and water. Evaporation of the solvents afforded the corresponding deprotected compounds in quantitative yields.

Homodimer 10: ^1H NMR (D_2O): δ = 4.09–2.90 (m, 14 H, 1-H, 2-H, 3-H, 4-H, 5-H, 6-H), 1.90–0.91 (m, 8 H, CH_2) ppm. ^{13}C NMR (D_2O): δ = 74.5, 71.6, 70.5, 58.0, 56.2 (C-1, C-2, C-3, C-4, C-5), 59.8 (C-6), 28.3, 26.8 (CH_2) ppm. MS (TOF): *m*/*z* = 381.22 [M + H]$^+$; found 381.50. $C_{16}H_{32}N_2O_8$ (380.44): calcd. C 50.51, H 8.48, N 7.36; found C 50.54, H 8.49, N 7.34.

Heterodimer 11: ^1H NMR (D_2O): δ = 3.99–2.78 (m, 14 H, 1,1′-H, 2,2′-H, 3,3′-H, 4,4′-H, 5,5′-H, 6,6′-H), 1.12 (m, 4 H, CH_2), 0.99 (br. s, 4 H, CH_2) ppm. ^{13}C NMR (D_2O): δ = 78.2, 75.9, 75.0, 74.5, 73.9, 73.0,71.6, 70.5, 58.1, 57.6 (C-1,1′, C-2,2′, C-3,3′, C-4,4′, C-5,5′), 63.7, 59.8 (C-6,6′), 28.1, 28.0, 26.9, 25.9 (CH_2) ppm. MS (TOF): *m*/*z* = 382.21 [M + H]$^+$; found 382.50. $C_{16}H_{31}NO_9$ (381.42): calcd. C 50.38, H 8.19, N 3.67; found C 50.34, H 8.20, N 3.65.

Protected Homodimer 13: Flash column chromatography (petroleum ether/EtOAc, 85:15). ^1H NMR ($CDCl_3$): δ = 7.46–7.02 (m, 50 H, ArH), 5.48 (br. s, 2 H, CH=CH), 5.06 (s, 4 H, CH_2 Cbz), 4.66–4.32 (m, 16 H, OCH_2Ph), 4.30 (m, 2 H, 1-H), 4.15 (m, 2 H, 3-H), 3.91 (m, 2 H, 4-H), 3.81 (d, *J* = 7.7 Hz, 2 H, 6a-H), 3.67 (m, 4 H, 2-H, 6b-H), 3.56 (m, 2 H, 5-H), 2.48 (m, 2 H, CH_2-CH=CH), 2.39 (m, 2 H, CH_2-CH=CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 156.1 (C=O, Cbz), 138.6–136.8 (C Ar), 129.7–127.0 (CH=CH, CH Ar), 84.5, 82.1, 81.7, 80.7, 77.2, 76.5, 54.8, 53.4 (C-1,1′, C-2,2′, C-3,3′, C-4,4′, C-5,5′), 73.4–72.8 (OCH_2Ph), 72.2, 72.0 (C-6,6′), 67.4, 66.4

(CH_2 Cbz), 31.0, 30.0 (CH_2-CH=CH) ppm. MS (TOF): *m*/*z* = 1367.66 $[M + H]^+$; found 1367.00. $C_{88}H_{90}N_2O_{12}$ (1367.69): calcd. C 77.28, H 6.63, N 2.05; found C 77.34, H 6.62, N 2.05.

Protected Heterodimer 14: Flash column chromatography (toluene/EtOAc, 98:2). ^1H NMR ($CDCl_3$): δ = 7.33–7.08 (m, 45 H, ArH), 5.48 (m, 1 H, CH=CH), 5.35 (m, 1 H, CH=CH), 5.02 (s, 2 H, CH_2 Cbz), 4.84 (d, *J* = 10.9 Hz, 1 H, OCH_2Ph), 4.75–4.70 (m, 2 H, OCH_2Ph), 4.60–4.19 (m, 13 H, OCH_2Ph), 4.11 (m, 1 H, 1-H), 3.94 (m, 1 H, 1′-H), 3.87–3.43 (m, 12 H, 2-H, 3-H, 4-H, 5-H, 6a-H, 6b-H), 2.50 (m, 1 H, CH_2-CH=CH), 2.37 (m, 1 H, CH_2-CH=CH), 2.23 (m, 2 H, CH_2-CH=CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 156.2 (C=O Cbz), 139.0–136.7 (C Ar), 130.3–127.5 (CH=CH, CH Ar), 82.7, 82.6, 81.7, 80.8, 80.3, 78.3, 74.3, 71.3, 54.8, 53.3 (C-1,1′, C-2,2′, C-3,3′, C-4,4′, C-5,5′), 75.6, 75.2, 73.7, 73.1, 73.0, 72.8, 72.3, 72.0 (OCH_2Ph), 69.1, 67.5 (C-6,6′), 60.7 (CH_2 Cbz), 30.0, 28.8 (CH_2-CH=CH) ppm. MS (TOF): *m*/*z* = 1234.60 $[M + H]^+$; found 1234.80. $C_{80}H_{83}NO_{11}$ (1234.54): calcd. C 77.83, H 6.78, N 1.13; found C 77.79, H 6.77, N 1.14.

Homodimer 18: ^1H NMR (D_2O): δ = 4.19–3.05 (m, 12 H, 1-H, 2-H, 3-H, 4-H, 5-H), 1.88–1.58 (m, 4 H, CH-CH_2-CH_2), 1.48–1.27 (m, 4 H, CH_2-CH_2-CH_2-CH_2) ppm. ^{13}C NMR (D_2O): δ = 79.1 73.7 (C-2, C-3), 64.9, 64.0 (C-1, C-4), 60.6 (C-5), 27.4 (CH-CH_2-CH_2), 26.5 (CH_2-CH_2-CH_2-CH_2) ppm. $C_{14}H_{28}N_2O_6$ (320.39): calcd. C 52.48, H 8.81, N 8.74; found C 52.41, H 8.80, N 8.75.

Homodimer 19: ^1H NMR (D_2O): δ = 4.30–3.17 (m, 12 H, 1-H, 2-H, 3-H, 4-H, 5-H), 1.80–1.49 (m, 4 H, CH-CH_2-CH_2), 1.47–1.26 (m, 4 H, CH_2-CH_2-CH_2-CH_2) ppm. ^{13}C NMR (D_2O): δ = 75.8, 73.0 (C-2, C-3), 65.2, 62.9 (C-1, C-4), 59.9 (C-5), 32.2 (CH-CH_2-CH_2), 27.8 (CH_2-CH_2-CH_2-CH_2) ppm. $C_{14}H_{28}N_2O_6$ (320.39): calcd. C 52.48, H 8.81, N 8.74; found C 52.43, H 8.80, N 8.73.

Heterodimer 20: ^1H NMR (D_2O): δ = 4.17–3.19 (m, 12 H, 1-H, 2-H, 3-H, 4-H, 5-H), 1.82–1.50 (m, 4 H, CH-CH_2-CH_2), 1.48–1.27 (m, 4 H, CH_2-CH_2-CH_2-CH_2) ppm. ^{13}C NMR (D_2O): δ = 80.7, 76.9, 75.8, 73.0 (C-2, C-2′, C-3, C-3′), 64.9, 64.9 63.9, 63.9 (C-1, C-1′, C-4, C-4′), 60.6, 59.9 (C-5, C-5′), 32.2, 32.2 (CH-CH_2-CH_2), 27.7, 27.7 (CH_2-CH_2-CH_2-CH_2) ppm. $C_{14}H_{28}N_2O_6$ (320.39): calcd. C 52.48, H 8.81, N 8.74; found C 52.52, H 8.82, N 8.74.

Protected Homodimer 21: Flash column chromatography (petroleum ether/EtOAc, 87.5:12.5). ^1H NMR ($CDCl_3$): δ = 7.48–6.97 (m, 40 H, ArH), 5.39–5.25 (m, 2 H, CH=CH), 5.23–4.98 (m, 4 H, CH_2 Cbz), 4.69–4.21 (m, 12 H, OCH_2Ph), 4.21–3.40 (m, 12 H, 1-H, 2-H, 3-H, 4-H, 5-H), 1.26 (t, *J* = 7.1 Hz, 4 H, CH_2-CH=CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 154.4 (C=O, Cbz), 138.7–136.7 (C Ar), 129.9 (CH=CH), 128.7–127.6 (CH Ar), 83.6, 82.7, 64.5, 63.0 (C-1, C-2, C-3, C-4), 73.2, 71.2 (OCH_2Ph), 68.9, 68.0 (C-5,5′), 67.1 (CH_2 Cbz), 29.9 (CH_2-CH=CH) ppm. MS (TOF): *m*/*z* = 1127.54 $[M + H]^+$; found 1127.20. $C_{72}H_{74}N_2O_{10}$ (1127.38): calcd. C 76.71, H 6.62, N 2.48; found C 76.75, H 6.61, N 2.48.

Protected Homodimer 22: Flash column chromatography (petroleum ether/EtOAc, 80:20). ^1H NMR ($CDCl_3$): δ = 7.36–7.11 (m, 40 H, ArH), 5.51–5.25 (m, 2 H, CH=CH), 5.17–5.03 (m, 4 H, CH_2 Cbz), 4.58–4.22 (m, 12 H, OCH_2Ph), 4.21–3.41 (m, 12 H, 1-H, 2-H, 3-H, 4-H, 5-H), 1.25 (t, *J* = 7.1 Hz, 4 H, CH_2-CH=CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 155.9 (C=O, Cbz), 138.5–136.9 (C Ar), 128.7–127.7 (CH=CH, CH Ar), 77.6, 76.6, 61.9, 61.3 (C-1, C-2, C-3, C-4), 73.3, 71.7 (OCH_2Ph), 68.3 (C-5), 67.1 (CH_2 Cbz), 30.0 (CH_2-CH=CH) ppm. MS (TOF): *m*/*z* = 1127.54 $[M + H]^+$; found 1127.30. $C_{72}H_{74}N_2O_{10}$ (1127.38): calcd. C 76.71, H 6.62, N 2.48; found C 76.77, H 6.63, N 2.48.

Protected Heterodimer 23: Flash column chromatography (petroleum ether/EtOAc, 85:15). ^1H NMR ($CDCl_3$): δ = 7.56–7.00 (m, 40 H, ArH), 5.54–5.24 (m, 2 H, CH=CH), 5.23–4.94 (m, 4 H, CH_2 Cbz), 4.69–4.21 (m, 12 H, OCH_2Ph), 4.20–3.40 (m, 12 H, 1-H, 2-H, 3-H, 4-H, 5-H), 1.29–1.24 (m, 4 H, CH_2-CH=CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 156.3 (C=O, Cbz), 138.5–136.9 (C Ar), 128.7–127.8 (CH=CH, CH Ar), 82.9, 76.7, 62.6, 61.3 (C-1, C-2, C-3, C-4), 73.3, 71.4 (OCH_2Ph), 68.9 (C-5), 67.1 (CH_2 Cbz), 29.9 (CH_2-CH=CH) ppm. $C_{72}H_{74}N_2O_{10}$ (1127.38): calcd. C 76.71, H 6.62, N 2.48; found C 76.67, H 6.63, N 2.48.

Pyrrolidine 25: Allyl ammine **32** (575 mg, 1.3 mmol) was dissolved in water (5 mL) and $NaHCO_3$ (218 mg, 2.6 mmol) was added. The suspension was stirred until complete dissolution of the salt, then dioxane (6 mL) was added. The resulting mixture was cooled to 0 °C with an ice bath and CbzCl (186 μL, 1.3 mmol) was added dropwise. After 10 min, the ice bath was removed, and the reaction mixture was stirred for 20 h at room temperature. After addition of EtOAc (27 mL) and water (8 mL), the aqueous layer was separated and washed with EtOAc (3 × 10 mL); the combined organic extracts were washed with 1 M HCl (2 × 10 mL) and brine and dried with Na_2SO_4. The solvent was evaporated, and the residue was purified by flash column chromatography (petroleum ether/EtOAc, 2:1) to afford pure **25** (590 mg, 1.02 mmol, 79%) as a colorless oil. ^1H NMR ($CDCl_3$): δ = 7.51–7.10 (m, 20 H, ArH), 5.73–5.29 (m, 1 H, CH_2CH=CH_2), 5.18–5.11 (m, 2 H, CH_2 Cbz), 5.05–4.82 (m, 2 H, CH_2CH=CH_2), 4.53–4.40 (m, 6 H, OCH_2Ph), 4.20–4.01 (m, 3 H, 1-H, 2-H, 4-H) 3.79 (t, *J* = 4.2 Hz, 1 H, 3-H), 3.72–3.46 (m, 2 H, 5-H), 2.50–2.32 (m, 2 H, CH_2CH=CH_2) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 151.1 (C=O Cbz), 137.9–136.3 (C Ar), 133.8 (CH_2CH=CH_2), 128.1–127.0 (C Ar), 117.2 (CH_2CH=CH_2), 78.4 (C-3), 77.4 (C-2), 72.9, 71.3, 71.0 (OCH_2Ph), 67.8 (C-5), 66.6 (CH_2 Cbz), 61.5–60.43 (C-1, C-4), 36.4–35.9 (CH_2CH=CH_2) ppm. MS (ESI): *m*/*z* (%) = 600.28 (79) $[M + Na]^+$. $C_{37}H_{39}NO_5$ (577.72): calcd. C 76.92, H 6.80, N 2.42; found C 76.85, H 6.81, N 2.42.

Homodimer 26: ^1H NMR (D_2O): δ = 3.90–3.75 (m, 2 H, 1-H), 3.66 (dd, *J* = 12.2, 1.6 Hz, 2 H, 6a-H), 3.59–3.41 (m, 6 H, 2-H, 5-H, 6b-H), 3.41–3.30 (m, 2 H, 3-H), 3.16 (t, *J* = 9.2 Hz, 2 H, 4-H), 1.63–1.50 (m, 2 H, CH_2), 1.49–1.37 (m, 2 H, CH_2), 1.36–1.26 (m, 2 H, CH_2), 1.25–1.17 (m, 2 H, CH_2) ppm. ^{13}C NMR (D_2O): δ = 78.4, 76.0, 75.0, 74.0, 73.1 (C-1, C-2, C-3, C-4, C-5), 63.9 (C-6), 26.9, 26.1 (CH_2) ppm. MS (TOF): *m*/*z* = 383.19 $[M + H]^+$; found 383.17. $C_{16}H_{30}O_{10}$ (382.41): calcd. C 50.52, H 7.91; found C 50.47, H 7.92.

Protected Homodimer 27: Flash column chromatography (petroleum ether/EtOAc, 85:15). ^1H NMR ($CDCl_3$): δ = 7.43–7.18 (m, 40 H, ArH), 5.55 (br. d, *J* = 21.9 Hz, 2 H, CH=CH), 4.94 (d, *J* = 11.0 Hz, 2 H, OCH_2Ph), 4.85–4.78 (m, 4 H, OCH_2Ph), 4.70–4.61 (m, 6 H, OCH_2Ph), 4.52–4.41 (m, 4 H, OCH_2Ph), 4.10 (m, 2 H, 1-H), 3.88–3.51 (m, 12 H, 2-H, 3-H, 4-H, 5-H, 6a-H, 6b-H), 2.47 (m,4 H, CH_2-CH=CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 139.0, 138.5, 138.4, 138.2 (C Ar), 128.7–127.8 (CH=CH, CH Ar), 82.6, 80.3, 78.2, 74.2, 71.3 (C-1, C-2, C-3, C-4, C-5), 75.7, 75.3, 73.7, 73.2 (OCH_2Ph), 69.0 (C-6), 29.9, 28.8 (CH_2-CH=CH) ppm. MS (TOF): *m*/*z* = 1101.55 $[M + H]^+$; found 1101.60. $C_{72}H_{76}O_{10}$ (1101.39): calcd. C 78.52, H 6.96; found C 78.59, H 6.94.

Pyrrolidine 31: To a cooled (0 °C) solution of nitrone **30** (960 mg, 2.3 mmol) in anhydrous THF (30 mL) was dropwise added allylmagnesium bromide (1.0 M in diethyl ether, 6.9 mL, 6.9 mmol). After stirring for 3 h at 0 °C, the reaction was quenched with saturated aqueous NH_4Cl (20 mL). The reaction mixture was diluted with diethyl ether (25 mL), the organic layer was separated, and the aqueous layer was extracted with diethyl ether (2 × 20 mL). The combined organic extracts were washed with brine (20 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure to

afford crude **31** (1.05 g, quantitative), which was employed in the following step without further purification. A sample of crude product was purified by flash column chromatography (petroleum ether/EtOAc, 3:1) to give pure **31** as a white solid. M.p. 63–65 °C. ^{1}H NMR ($CDCl_3$): δ = 7.32–7.29 (m, 15 H, ArH), 5.94–5.83 (m, 1 H, CH_2CH=CH_2), 5.08–5.00 (m, 2 H, CH_2CH=CH_2), 4.58–4.51 (m, 5 H, OCH_2Ph), 4.43 (d, J = 11.7 Hz, 1 H, OCH_2Ph), 3.72 (t, J = 5.4 Hz, 1 H, 3-H), 3.56 (dd, J = 5.3, 2.3 Hz, 2 H, 5-H), 3.47 (dd, J = 7.3, 5.8 Hz, 1 H, 2-H), 3.39–3.30 (m, 2 H, 1-H, 4-H), 2.46–2.32 (m, 2 H, CH_2CH=CH_2) ppm. ^{13}C NMR ($CDCl_3$): δ = 138.2, 138.1, 138.0 (C Ar), 135.5 (CH=CH_2), 128.4–127.7 (C Ar), 116.7 (CH=CH_2), 77.3 (C-2), 75.4 (C-3), 73.3 (C-4), 71.9, 71.7, 71.4 (OCH_2Ph), 70.1 (C-1), 69.7 (C-5), 36.0 (CH_2CH=CH_2) ppm. MS (ESI): m/z (%) = 482.42 (100) $[M + Na]^+$. $C_{29}H_{33}NO_4$ (459.58): calcd. C 75.79, H 7.24, N 3.05; found C 75.69, H 7.21, N 3.07.

Pyrrolidine 32: A solution of hydroxylamine **31** (923 mg, 2.0 mmol) in acetic acid (10 mL) and water (10 mL) was treated with Zn powder (2.6 g, 40.0 mmol). The resulting mixture was stirred at room temperature for 40 min, diluted with water (50 mL), and treated with Na_2CO_3 (9.3 g) until bubbling of CO_2 stopped. The reaction mixture was extracted with DCM (3 × 50 mL), and the combined organic extracts were washed with 3 M NaOH (50 mL) and brine, dried (Na_2SO_4), and evaporated under reduced pressure to give pure ammine **32** (704 mg, 1.6 mmol, 80%) as a colorless oil, which did not need further purification. An analytically pure sample was obtained after purification by flash column chromatography ($CHCl_3$/Et_2O, 2:1). ^{1}H NMR ($CDCl_3$): δ = 7.36–7.24 (m, 15 H, ArH), 5.78 (ddt, J = 17.1, 10.1, 7.0 Hz, 1 H, CH_2CH=CH_2), 5.08–5.01 (m, 2 H, CH_2CH=CH_2), 4.60–4.45 (m, 6 H, OCH_2Ph), 3.74 (t, J = 5.3 Hz, 1 H, 3-H), 3.51–3.40 (m, 4 H, 2-H, 4-H, 5-H), 3.35–3.30 (m, 1 H, 1-H), 2.41–2.35 (m, 1 H, CH_2CH=CH_2), 2.08 (td, J = 14.2, 6.9 Hz, 1 H, CH_2CH=CH_2) ppm. ^{13}C NMR ($CDCl_3$): δ = 138.3–138.2 (C Ar), 135.2 (CH=CH_2), 128.3–127.6 (C Ar), 117.1 (CH_2CH=CH_2), 81.3 (C-2), 78.2 (C-3), 73.2, 71.8, 71.6 (OCH_2Ph), 71.1 (C-5), 61.6 (C-4), 60.6 (C-1), 38.4 (CH_2CH=CH_2) ppm. MS (ESI): m/z (%) = 444.42 (100) $[M + H]^+$. $C_{29}H_{33}NO_3$ (443.58): calcd. C 78.52, H 7.50, N 3.16; found C 78.59, H 7.48, N 3.15.

Enzyme Assay: Trehalase activity was measured through a coupled assay with glucose-6-phosphate dehydrogenase and hexokinase according to Wegener et al.[23] All enzyme assays were performed in triplicates at 30 °C by using sample volumes varying from 5 to 20 μL in 1 mL test and using a Cary3 UV/Vis Spectrophotometer. Enzyme activities were analyzed by Cary Win UV application software for Windows XP. The specific activity (U mg^{-1}) was expressed as μmol min^{-1} mg protein^{-1}. Values were expressed as mean ±S.E. of replicated.

Supporting Information (see footnote on the first page of this article): Details of the enzyme assays and selected copies of the NMR spectra.

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