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Total Synthesis and Structural Revision of TMG-chitotriomycin, a Specific Inhibitor of Insect and Fungal β-*N*-Acetylglucosaminidases

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TMG-chitotriomycin (**1**) was recently disclosed by Kanzaki and co-workers from the culture filtrate of *Streptomyces anulatus* NBRC13369, which exhibited potent and selective inhibition against the β-*N*-acetylglucosaminidase (GlcNAcase) of insects and fungi but no activity against the enzyme of humans and plants.[1] This unique inhibitory spectrum could lead to new insights for understanding the molecular mechanisms of the important chitin-degrading systems occurring in a wide variety of organisms and the development of new antifungal and insecticidal agents.[2] The proposed structure of **1** (Figure 1), determined by spectral and constitutive sugar analyses of the authentic sample and its alditol derivatives, was also intriguing in that this tetrasaccharide possesses a unique *N*,*N*,*N*-trimethyl-D-glucosamine (TMG) residue α-(1→4)-linked at the nonreducing end of a chitotriose;[3] moreover, the presence of the trimethylammonium could astonishingly result in epimerization at the remote C2 of the reducing-end GlcNAc unit.[1]

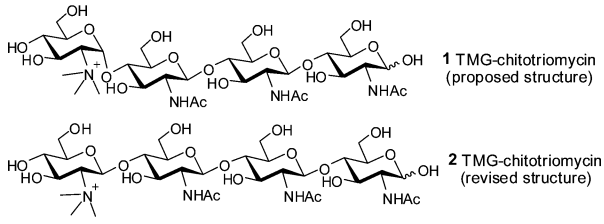
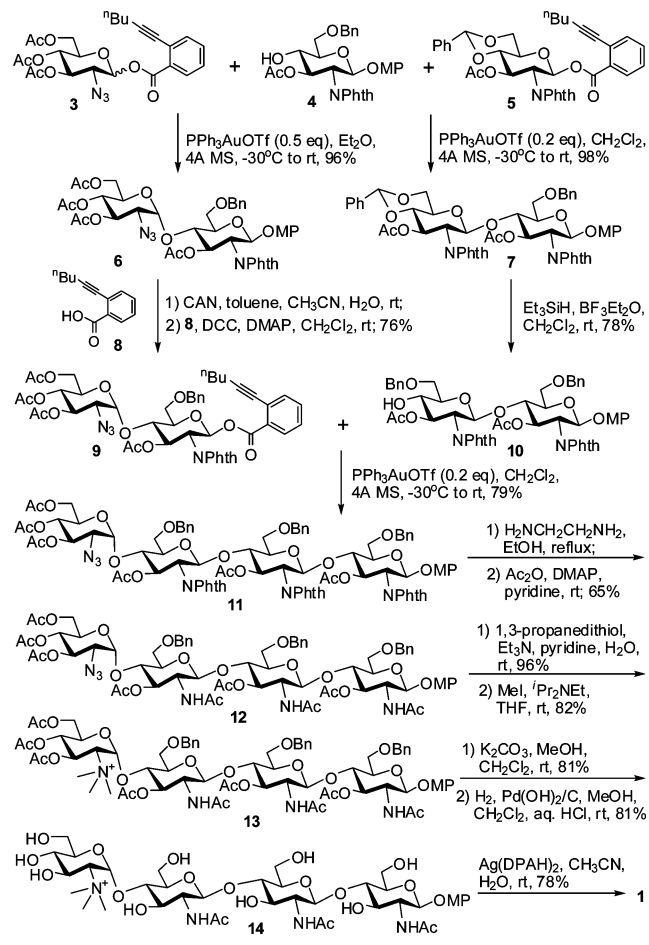


Figure 1

In this work, we developed a convergent [2 + 2] approach to synthesize the unique tetrasaccharide **1** (Scheme 1),[4] where the sterically demanding (1→4)-glycosidic linkages were assembled successfully by our newly developed glycosylation protocol with glycosyl *ortho*-hexynylbenzoates as donors and Au(I) as the catalyst.[5] The 2-azido and 2-*N*-phthalimido (Phth) groups in the monosaccharide units were employed to secure the formation of the α- and β-glycosidic linkages, respectively, and to distinguish the final elaboration of the trimethylammonium and acetamide functions. The *p*-methoxyphenyl (MP) group was used to protect the anomeric hydroxyl groups.

Thus, the required three monosaccharide building blocks **3**−**5** were readily prepared from glucosamine.[6] The coupling of the 2-azidoglucopyranosyl *o*-hexynylbenzoate **3** with glucosamine-4−OH derivative **4** in Et_2O (at −30 °C to rt) demanded an excess amount of the donor **3** (1.8 equiv) and a larger loading of the catalyst $Ph_3PAuOTf$ (0.5 equiv) for complete consumption of the acceptor **4**, providing the desired α-linked disaccharide **6** in 96% yield with no β-anomer being detected. The coupling of the 2-*N*-Phth-glucopyranosyl *o*-hexynylbenzoate **5** (1.3 equiv) with acceptor **4**

Scheme 1



in CH_2Cl_2 (−30 °C to rt) proceeded smoothly in the presence of 0.2 equiv of $Ph_3PAuOTf$, affording the β-linked disaccharide **7** exclusively (98%). Disaccharide **6** was subjected to selective removal of the anomeric *O*-MP protection with ceric ammonium nitrate (CAN) and subsequent ester formation (with *o*-hexynylbenzoic acid **8**) to afford the disaccharide *o*-hexynylbenzoate **9** (76% for two steps). Disaccharide **7** was converted into a new acceptor **10** via selective opening of the 4,6-*O*-benzylidene acetal (Et_3SiH, BF_3OEt_2, CH_2Cl_2, rt, 78%).[7] Coupling of disaccharides **9** and **10** under the catalysis of $Ph_3PAuOTf$ (0.2 equiv) in CH_2Cl_2 at −30 °C to rt provided the desired β-linked tetrasaccharide **11** in a satisfactory 79% yield.

To convert the fully masked tetrasaccharide **11** into the target TMG-chitotriomycin (**1**), model studies were carried out on disaccharide **6** to find the optimal sequence and conditions for those

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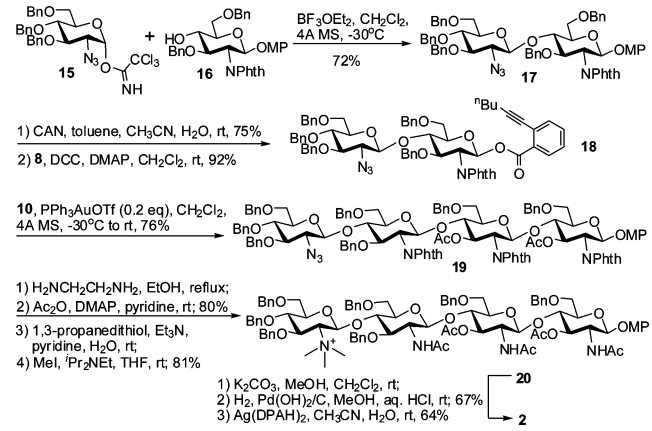
transformations (data not shown). It was noted that the trimethylammonium residue could not survive under the conditions required for removal of the *N*-Phth groups. Thus, the three *N*-Phth groups in **11** were first removed ($NH_2CH_2CH_2NH_2$, EtOH, reflux)[8] and the resulting $-NH_2$ acetylated to give **12** (65%). The azido group in **12** was reduced into an amino group with 1,3-propanedithiol (NEt_3, pyridine, H_2O, rt)[9] in an excellent yield of 96%; other conditions, such as reduction with PPh_3 or Zn/HOAc, led to much lower yields (~50%) because of migration of the *O*-Ac group onto the nascent amino group. The resulting amino group was then converted readily into the trimethylammonium residue with excess amounts of MeI in the presence of $^iPr_2NEt$ in THF at rt, leading to **13** (82%). The *O*-Ac groups in **13** were then removed with K_2CO_3 in a MeOH/CH_2Cl_2 mixed solvent at rt (81%), and the remaining *O*-Bn groups were cleaved via hydrogenolysis over $Pd(OH_2)_2$/C in the same solvent in the presence of a tiny amount of HCl(aq) at rt (81%), affording the *p*-methoxyphenyl β-glycoside of TMG-chitotriomycin (**14**). The final removal of the anomeric MP group proved to be difficult, as treatment of **14** with CAN under a variety of conditions led to complex mixtures. Fortunately, a mild oxidizing agent, bis(hydrogen dipicolinate)silver(II),[10] effected the cleavage of the anomeric MP group, furnishing the target tetrasaccharide **1** (78%).

The synthetic compound **1** was apparently not the natural TMG-chitotriomycin, as determined by a comparison of their ^{1}H NMR spectra. The biggest discrepancy arose from the chemical shift of the anomeric proton of the TMG residue: that of the natural product appeared at 5.38 ppm ($J \approx 3.0$ Hz) and that of synthetic **1** at 6.24 ppm ($J = 2.7$ Hz). After carefully examining the present synthesis and the structural evidence provided by Kanzaki and co-workers,[1] we suspected that the TMG might be β-linked to the chitotriose in the natural product instead of α-linked as in the previously assignment. The small $H_{1,2}$ coupling constant might be attributed to the conformational deviation of the TMG residue from the $^4C_1$ commonly adopted by glucosamine residues. This is strongly supported by the assignment of the β-TMG residue in the alditol derivatives of TMG-chitotriomycin to be in a twist−boat conformation.[6]

With a modification of the synthetic approach leading to tetrasaccharide **1**, the synthesis of the revised structure **2** was straightforward (Scheme 2). Thus, the β-linked disaccharide **17** was assembled stereoselectively via coupling of 2-azidoglucopyranosyl α-trichloroacetimidate **15** with glucosamine-4−OH derivative **16** in the presence of BF_3OEt_2 in CH_2Cl_2 at −30 °C (72%).[4b,6] The *p*-methoxybenzyl disaccharide **17** was then converted into the corresponding *o*-hexynylbenzoate **18** (69% for two steps) in a manner similar to that for the **6** → **9** conversion. The Au(I)-catalyzed glycosidic coupling of the disaccharides **18** and **10** worked equally as well as that of **9** and **10** to give **11**, providing the whole β-linked tetrasaccharide **19** in 76% yield. Steps similar to those for **11** → **1** were then employed for the conversion of **19** into the newly proposed TMG-chitotriomycin (**2**) (seven steps, 28%).

The ^{1}H NMR spectrum of the synthetic tetrasaccharide **2** was virtually identical to that of the authentic TMG-chitotriomycin.[1,6] Also, the ESI-MS spectrum of **2**, as described for the authentic sample, developed an intensified [$M^+ + 1$] peak (at *m*/*z* 832) after CD_3OD treatment, indicating the deuterium exchange at H2 of the reducing-end GlcNAc; however, the same was not observed for synthetic **1**. These results indicated that the β-orientation of the TMG unit is also critical in causing the epimerization at the reducing-end C2; however, further research is required to clarify this unusual property of TMG-chitotriomycin.

Scheme 2



In summary, the tetrasaccharides **1** and **2** with the unique TMG unit α- and β-(1→4)-linked to a chitotriose, respectively, have been efficiently synthesized in a convergent [2 + 2] manner in which the construction of the sterically demanding (1→4)-glycosidic linkages was achieved by the newly developed Au(I)-catalyzed glycosylation protocol with stable glycosyl *o*-hexynylbenzoates as donors. The present total synthesis has led to the unambiguous revision of the structure of TMG-chitotriomycin from **1** to **2** and shall provide ready access to this intriguing natural metabolite and its derivatives for further exploration of the selective inhibition of GlcNAcases, which are important and widely occurring in the chitin-degrading systems in nature.

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Supporting Information Available: Experimental details, characterization data, and ^{1}H NMR spectra for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Usuki, H.; Nitoda, T.; Ichikawa, M.; Yamaji, N.; Iwashita, T.; Komura, H.; Kanzaki, H. *J. Am. Chem. Soc.* **2008**, *130*, 4146.
(2) (a) Merzendorfer, H.; Zimoch, L. *J. Exp. Biol.* **2003**, *206*, 4393. (b) Horsch, M.; Mayer, C.; Sennhauser, U.; Rast, D. M. *Pharmacol. Ther.* **1997**, *76*, 187.
(3) The synthetic TMG has been reported. See: (a) Reckendorf, W. M.; Sandner, S. *Tetrahedron Lett.* **1988**, *29*, 2047. (b) Falkowski, L.; Beszczynski, M.; Jarzebski, A.; Stefanska, B. *Pol. J. Chem.* **1983**, *57*, 1353.
(4) For some relevant examples of the synthesis of chitooligosaccharide derivatives, see: (a) Nicolaou, K. C.; Bockovich, N. J.; Carcanague, D. R.; Hummel, C. W.; Even, L. F. *J. Am. Chem. Soc.* **1992**, *114*, 8701. (b) Wang, L. X.; Li, C.; Wang, Q. W.; Hui, Y. Z. *Tetrahedron Lett.* **1993**, *34*, 7763. (c) Ikeshita, S.; Sakamoto, A.; Nakahara, Y.; Nakahara, Y.; Ogawa, T. *Tetrahedron Lett.* **1994**, *35*, 3123. (d) Tailler, D.; Jacquinet, J. C.; Beau, J. M. *J. Chem. Soc. Chem. Commun.* **1994**, 1827.
(5) Li, Y.; Yang, Y.; Yu, B. *Tetrahedron Lett.* **2008**, *49*, 3604.
(6) See the Supporting Information for details.
(7) (a) Deninno, M. P.; Etienne, J. B.; Duplantier, K. C. *Tetrahedron Lett.* **1995**, *36*, 669. (b) Debenham, S. D.; Toone, E. J. *Tetrahedron: Asymmetry* **2000**, *11*, 385.
(8) (a) Kanie, O.; Crawley, S. C.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **1993**, *243*, 139. (b) Hansson, J.; Garegg, P. J.; Oscarson, S. *J. Org. Chem.* **2001**, *66*, 6234.
(9) (a) Stauch, T.; Greilich, U.; Schmidt, R. R. *Liebigs Ann.* **1995**, 2101. (b) Galan, M. C.; Venot, A. P.; Glushka, J.; Imberty, A.; Boons, G. J. *J. Am. Chem. Soc.* **2002**, *124*, 5964.
(10) (a) Kloc, K.; Mlochowski, J.; Syper, L. *Chem. Lett.* **1980**, 725. (b) Noshita, T.; Sugiyama, T.; Kitazumi, Y.; Oritani, T. *Tetrahedron Lett.* **1994**, *35*, 8259.

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