

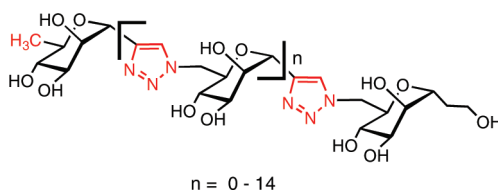
## Modular Approach to Triazole-Linked 1,6- $\alpha$ -D-Oligomannosides to the Discovery of Inhibitors of *Mycobacterium tuberculosis* Cell Wall Synthetase

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Aiming at developing inhibitors of mannosyltransferases, the enzymes that participate in the biosynthesis of the cell envelope of *Mycobacterium tuberculosis*, the synthesis of a range of designed triazole-linked 1,6-oligomannosides up to a hexadecamer has been accomplished by a modular approach centered on the Cu(I)-catalyzed azide–alkyne cycloaddition as key process. The efficiency and fidelity of the cycloaddition are substantiated by high yields (76–96%) and exclusive formation of the expected 1,4-disubstituted triazole ring in all oligomer assembling reactions. Key features of oligomers thus prepared are the anomeric carbon–carbon bond of all mannoside residues and the 6-deoxymannoside capping residue. Suitable bioassays with dimer, tetramer, hexamer, octamer, decamer, and hexadecamer showed variable inhibitor activity against mycobacterial  $\alpha$ -(1,6)-mannosyltransferases, the highest activity ( $IC_{50} = 0.14$ – $0.22$  mM) being registered with the hexamannoside and octamannoside.

### Introduction

Among infectious diseases that were thought to be defeated in the last century, tuberculosis (TB) has reappeared overbearingly<sup>1</sup> and is presently a leading cause of mortality in many parts of the world, including Western countries. Tuberculosis is especially diffused in sub-Saharan Africa, where it is epidemic because of an increased susceptibility conferred by HIV infection.<sup>2</sup> The convergence of TB and HIV poses difficult problems because antiviral and antitubercular drugs given together may present high levels of

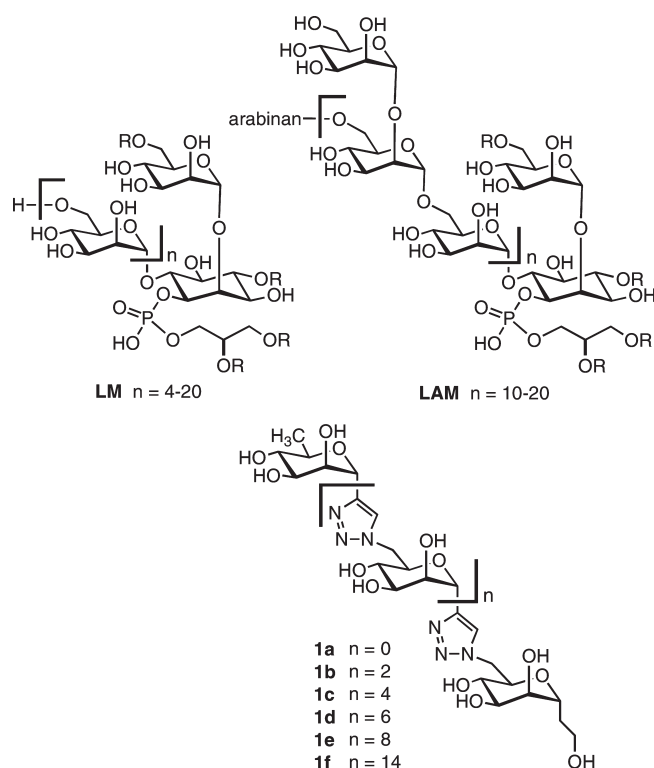
incompatibility. Various drugs are currently available with different modes of action to treat TB.<sup>3</sup> Nevertheless, drug resistance continues to emerge, and therefore, there is an increasing demand for rapid development of new antitubercular drugs<sup>4</sup> targeting essential functions of its etiological agent, *Mycobacterium tuberculosis* (Mtb). As this bacterium invades and colonizes macrophage cells, its ability to survive within this inhospitable environment has been attributed to its robust cell wall.<sup>5</sup> Therefore, an ideal TB drug target is the biosynthesis of the mycobacterial cell envelope. Indeed, two

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**FIGURE 1.** Structures of lipomannans LMs and lipoarabinomannans (LAMs) of Mtb cell walls (R stands for various fatty acid acyl groups) and designed triazole-tethered oligomannosides **1** featuring a capping 6-deoxymannose fragment.

of the available antibiotics used to treat TB, ethambutol and isoniazid, act by preventing the formation of a complete cell wall.<sup>6</sup> This is composed of various glycopospholipids such as mycolyl-arabinogalactan (mAG)<sup>7</sup> and phosphatidylinositol mannosides (PIMs).<sup>8</sup> The latter, in addition to being important in their own right, may also be hyperglycosylated to form other wall components such lipomannans (LMs) and lipoarabinomannans (LAMs).<sup>9</sup> These glycolipids all contain a common  $\alpha$ -1,6-linked mannoside core as shown, for example, in LMs and LAMs (Figure 1). Synthetic approaches to oligomannan fragments of the Mtb cell wall were described in

various instances.<sup>10</sup> Moreover, in a series of papers, Lowary and co-workers have described the preparation and biochemical evaluation of fluoro, methoxy, amino, and deoxy analogues<sup>11</sup> of octyl 1,6- $\alpha$ -D-dimannoside<sup>12</sup> ( $\alpha$ -D-Manp-(1,6)- $\alpha$ -D-Manp-O(CH<sub>2</sub>)<sub>7</sub>CH<sub>3</sub>) through selective substitution of the C6 hydroxyl group. Compounds have been also prepared in which two of the hydroxyl groups on the nonreducing residue of the above disaccharide were replaced.<sup>13</sup> These compounds were all considered potential substrates and inhibitors for  $\alpha$ -1,6-mannosyltransferase ( $\alpha$ -(1,6)-ManT) activity required for the biosynthesis of LM and LAM mannan cores. Along the same vein, Watt and Williams prepared a series of hydrophobic octyl 6-deoxy  $\alpha$ -1,6-linked oligomannosides up to a tetramer via iterative glycosylation.<sup>14</sup> The rationale behind these synthetic efforts was that deoxygenation of the 6-position of the oligomannosyl chain should prevent these compounds acting as substrates for the  $\alpha$ -(1,6)-ManTs. Oligomannosides prepared by Watt and Williams featured the natural *O*-glycosidic anomeric linkage and, therefore, were prone to undergo a facile cleavage by hydrolases. Moreover, the chain lengths of these compounds might be too short for an efficient mimicry of the oligomannoside chains of LMs and LAMs, which are in fact constituted of 10–20 mannose units. Hence, we have developed a new approach to more stable and higher oligomers **1**, all preserved from hydrolytic degradation by virtue of anomeric carbon–carbon bonds and triazole rings as interglycosidic linkers (Figure 1). In fact, we envisaged the Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC), first chronicled by Sharpless and Meldal groups,<sup>15</sup> as a suitable tool for the assembly of a number of mannose residues via linear and/or convergent strategies. The usefulness of this valuable reaction for oligomannoside assembly was demonstrated in recent work from our laboratory.<sup>16</sup> Moreover, we thought that in the oligomers thus obtained the triazole ring could act not only as a robust linker impervious to chemical and enzymatic degradation but could also participate in hydrogen-bonding and dipole interactions, thereby favoring molecular recognition processes.<sup>17</sup>

## Results and Discussion

We set out to prepare triazole-linked oligomannosides **1** (TOM) by a modular approach involving CuAAC reactions as shown in Scheme 1. While this scheme exemplifies only the synthesis of a high oligomer featuring 16 mannose

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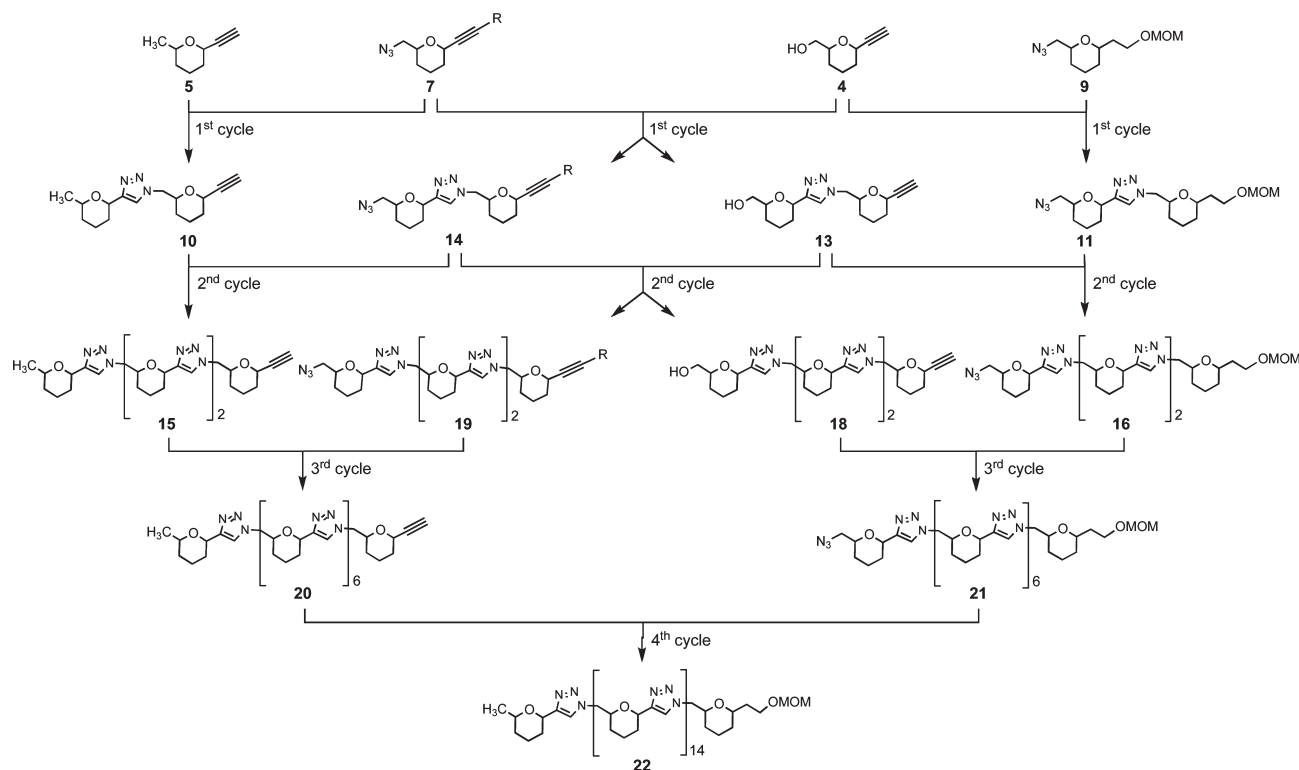
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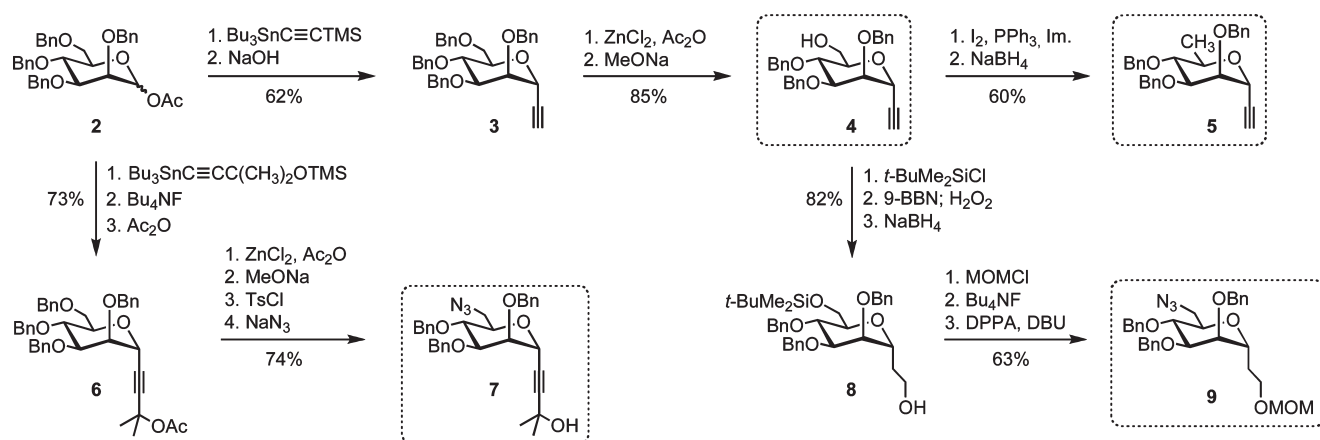
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SCHEME 1. Convergent Synthesis of Triazole-Linked Hexadecamannoside (R = Protective Group)



SCHEME 2



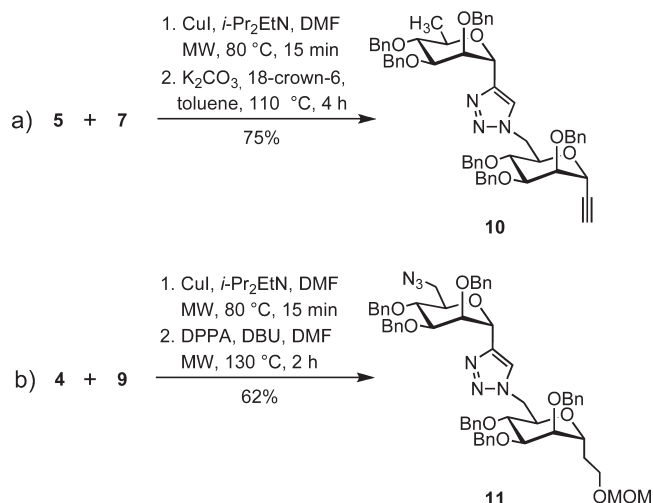
residues, it can be realized that the lower oligomers can be equally prepared from suitable precursors showed therein. Details on each reaction sequence constituted of the CuAAC and functional group transformation as well as the synthesis of a range of oligomers are presented below.

**Preparation of Starting Monosaccharidic Building Blocks.** Although previously reported in a succinct manner,<sup>16</sup> the synthetic routes to these building blocks are presented briefly in Scheme 2. Compounds **5** and **9** were finalized to act as a capping residue and a platform, respectively, in the prepared oligosaccharides, while **4** and **7** were the first pair of elongating units. The methyl group in **5** constituted a key structural motif to prevent the enzyme-promoted oligomerization, while the protected hydroxyethyl group at the anomeric carbon of **9** formed a stable terminal C-glycoside unit. Upon removal of

the methoxymethyl (MOM) group by acid hydrolysis, the free hydroxyethyl group could serve as a point of the attachment of fatty acids via esterification. The 3-hydroxy-3-methylbutynyl (HMB) group in **7** acted as a masked ethynyl group. In fact, HMB is a formal adduct of acetone to the ethynyl group from which the former can be easily removed (deacetonation) simply by basic treatment. This protection/deprotection strategy avoided homopolymer formation via intermolecular Cu(I)-catalyzed cycloaddition.<sup>18</sup> The use of HMB represented two optimized conditions, i.e., stability under the CuAAC conditions and ease of deacetonation.

(18) It is well known that the CuAAC takes place with terminal alkynes. Exceptionally, iodoalkynes do react as well. See: Hein, J. E.; Tripp, J. C.; Krasnova, L.; Sharpless, B. K. B.; Fokin, V. V. *Angew. Chem., Int. Ed.* **2009**, *48*, 8018–8021.

SCHEME 3

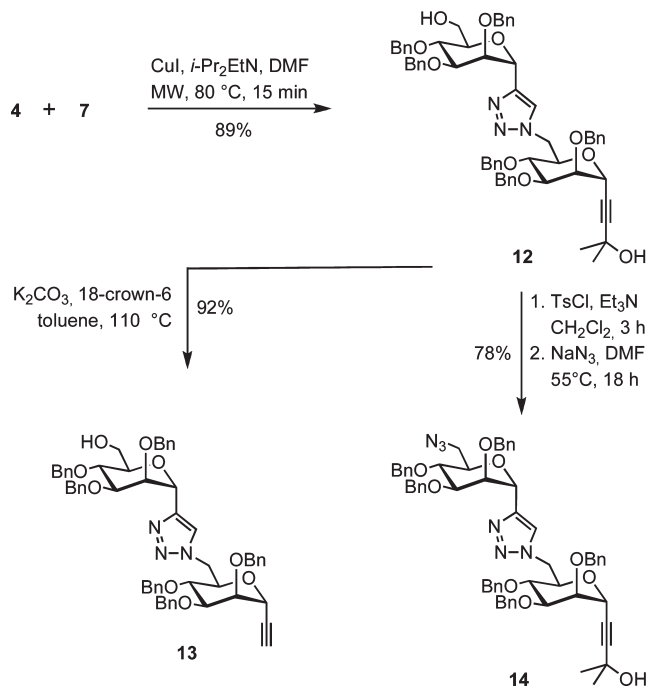


On the other hand, the ethynyl protection as cobalt hexacarbonyl complex or trimethylsilyl derivative<sup>16b</sup> appeared unsatisfactory as these groups were partially removed under the CuAAC conditions.

As shown in Scheme 2, the preparation of all starting building blocks relied on the use of tetra-*O*-benzylmannopyranosyl acetate **2** as the common precursor. Succinctly, the 3-hydroxy-3-methylbutynyl group was introduced by glycosylation of **2** with 1-tributylstannyl-3-methyl-3-trimethylsilyloxy-1-butyne followed by sequential desilylation–acetylation of the hydroxy group. Compound **6** thus obtained was selectively debenzylated, then deacetylated, and finally transformed into the target azide **7** by selective activation of the primary hydroxy group as tosylate and reaction with sodium azide.<sup>16b</sup> Coupling of **2** with 1-tributylstannyl-2-trimethylsilyl-ethyne, followed by desilylation, yielded the ethynyl glycoside **3** that was selectively debenzylated to afford **4**.<sup>16a</sup> This compound was transformed into the target 6-deoxy derivative **5** by free hydroxy group iodination and hydride reduction.<sup>16b</sup> The preparation of the azide **9** involved first the protection of the free hydroxy group of **4** as silyl derivative and then hydroboration–oxidation of the alkyne function to give the hydroxyethyl *C*-mannoside **8**. The latter was converted into the methoxymethyl (MOM) derivative that in turn was transformed into the target azide **9** by desilylation and treatment with diphenyl phosphoryl azide (DPPA) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).<sup>16a</sup>

**Synthesis of Triazole-Tethered 1,6- $\alpha$ -D-Oligomannoside Building Blocks and Assembly of the Hexadecamer.** To demonstrate the feasibility of the synthetic strategy outlined in Scheme 1, the synthesis of the hexadecasaccharide **1f** was first performed. This involved execution of four consecutive cycles each one being constituted of CuAAC and azidation or/and deacetonation reactions. All cycloadditions were performed under the conditions optimized in our recent work,<sup>16b</sup> that is, by microwave heating at  $80^\circ\text{C}$  (external vessel wall temperature) for 15 min of equimolar azide and alkyne mixture in DMF in the presence of catalytic CuI (0.2 equiv) and *N,N*-diisopropylethylamine (2 equiv) as a ligand. The deacetonation of the 3-hydroxy-3-methylbutynyl group to give the free ethynyl moiety was efficiently carried out under mild basic conditions ( $\text{K}_2\text{CO}_3$ , crown ether) in refluxing toluene. Finally,

SCHEME 4



the transformation of the primary hydroxy group into azide was carried out by either direct azidation with DPPA or by one-pot tosylation and substitution with sodium azide.

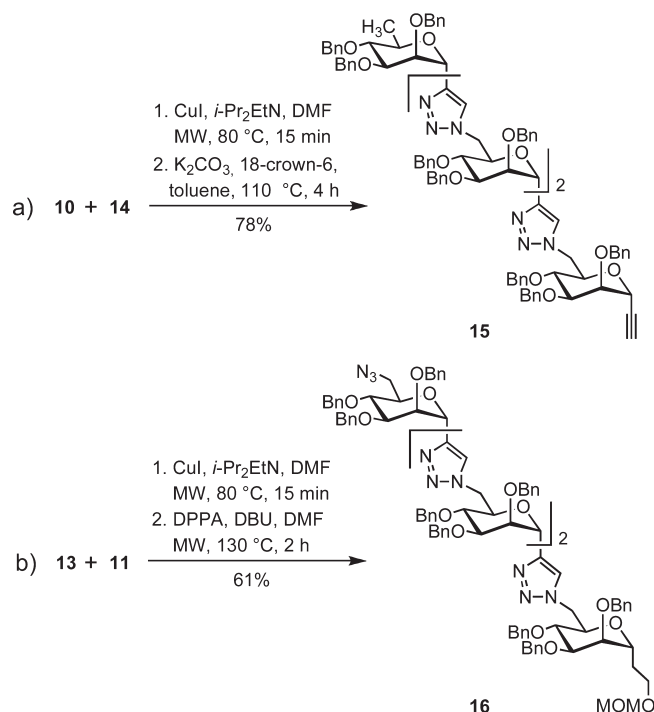
**First Cycle.** This involved (a) coupling of alkyne **5** with azide **7** followed by deacetonation to give the dimannoside **10** (Scheme 3, eq a); (b) coupling of alkyne **4** with azide **9** followed by azidation to give **11** (Scheme 3, eq b); and (c) coupling of alkyne **4** with azide **7** to give **12** and transformation of the latter into **13** and **14** by deacetonation and azidation, respectively (Scheme 4).

**Second Cycle.** This involved (a) coupling of alkyne **10** with azide **14** followed by deacetonation to give the tetramannoside **15** (Scheme 5, eq a); (b) coupling of alkyne **13** with azide **11** followed by azidation to give **16** (Scheme 5, eq b); (c) coupling of alkyne **13** with azide **14** to give **17** and transformation of the latter into **19** and **18** by azidation and deacetonation, respectively (Scheme 6).

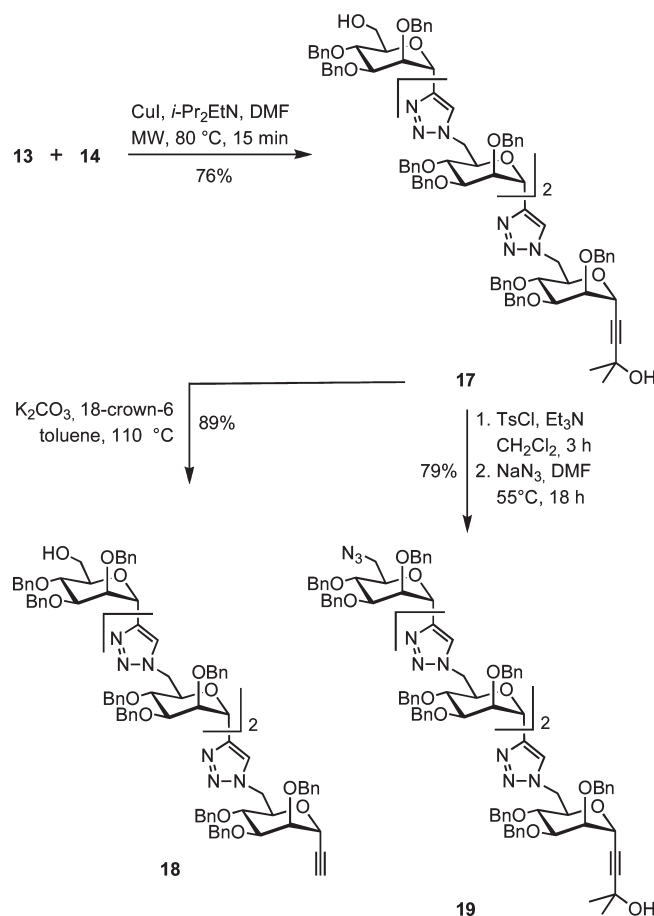
**Third Cycle.** This involved (a) coupling of alkyne **15** with azide **19** followed by deacetonation to give the octamannoside **20** (Scheme 7, eq a); (b) coupling of alkyne **18** with azide **16** followed by azidation to give **21** (Scheme 7, eq b).

**Fourth Cycle.** This involved only the final Cu(I)-catalyzed coupling of octamannosides **20** and **21** to give the protected hexadecamannoside **22** (Scheme 8). MALDI-TOF mass spectrometry of **22** revealed  $m/z = 7761.40$  for the corresponding hydrogen adduct, as compared to the calculated  $m/z = 7761.15$ . This compound was subjected to the protecting group removal (demethoxymethylation by  $\text{CF}_3\text{CO}_2\text{H}$  and debenzylation by  $\text{BCl}_3$ ) and acetylation by  $\text{Ac}_2\text{O}$  to give the peracetylated derivative **23**, which was purified and characterized by NMR and MS analyses. Finally the removal of all acetyl protecting groups from **23** under mild basic conditions using  $\text{NH}_3$  in methanol and water at room temperature afforded the target TOM **1f** (calcd  $m/z = 3413.10$   $[\text{M} + \text{Na}]^+$ , found  $m/z = 3412.99$ ) in 9.4% overall yield from the monosaccharidic building blocks.

SCHEME 5

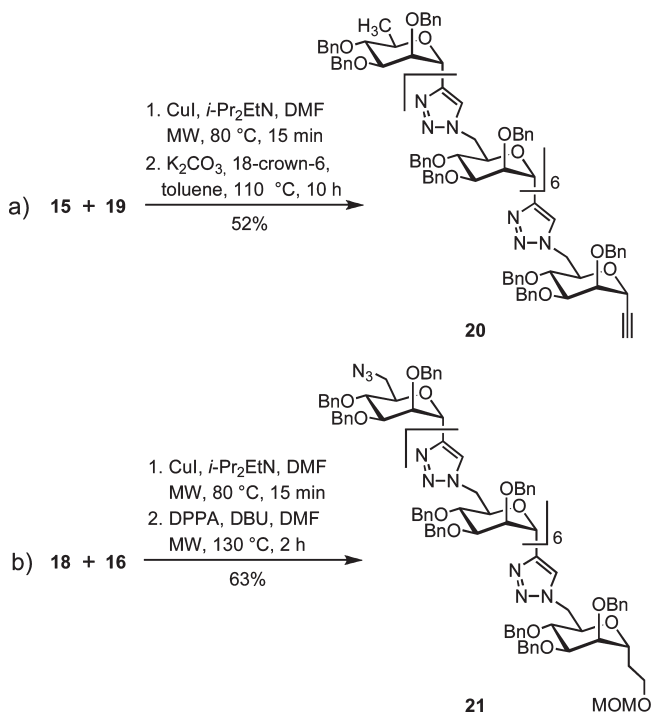


SCHEME 6

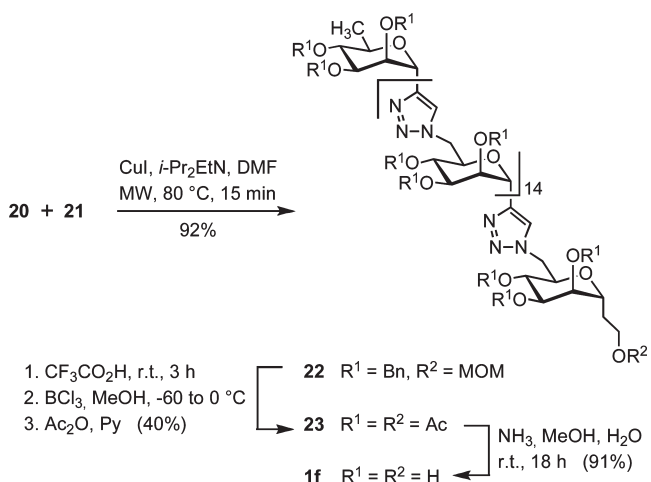


Before closing this section, a few aspects regarding Schemes 3–8 are worth considering. First, it can be noticed

SCHEME 7



SCHEME 8



that all intermolecular CuAAC reactions took place with great efficiency regardless of the complexity of the substrates to give exclusively a single cycloadduct. The structure of the 1,4-disubstituted 1,2,3-triazole moieties of the oligosaccharides was confirmed by  $^{13}\text{C}$  NMR spectroscopy.<sup>19</sup> It can be also noticed that the two functional group transformations, i.e., deacetonation and azidation, were simple and efficient processes that were performed without any damage of the substrates.

**Synthesis of a Set of TOMs (1).** From an inspection of Scheme 1 it can be easily realized that the Cu(I)-catalyzed

(19) The 1,4-disubstitution pattern of the triazole ring in all prepared oligomannosides was supported by the large and positive  $\Delta(\delta_{\text{C4}} - \delta_{\text{C5}})$  value (17–21 ppm) in their  $^{13}\text{C}$  NMR spectra as observed in other compounds obtained by click CuAAC in our laboratory. See ref 16a and: (a) Dondoni, A.; Marra, A. *J. Org. Chem.* **2006**, *71*, 7546–7557. (b) Dondoni, A.; Giovannini, P. P.; Massi, A. *Org. Lett.* **2004**, *6*, 2929–2932. (c) Marra, A.; Vecchi, A.; Chiappe, C.; Melai, B.; Dondoni, A. *J. Org. Chem.* **2008**, *73*, 2458–2461.



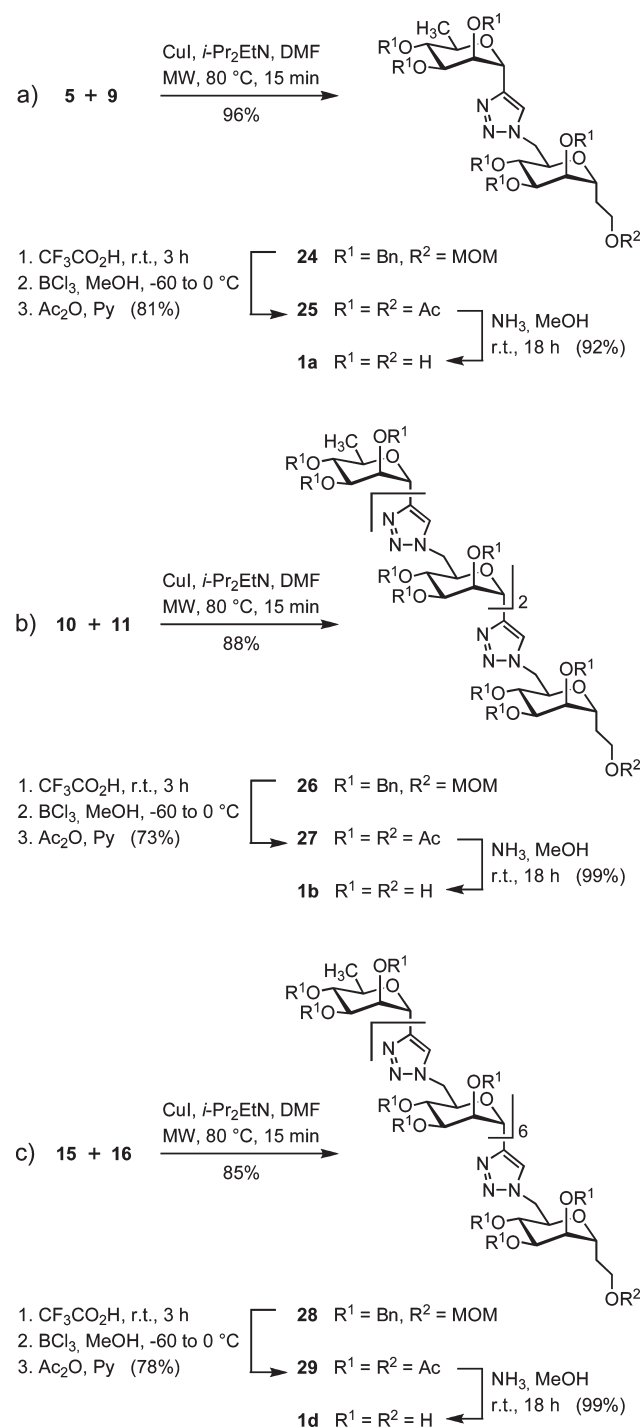
cycloadditions of the alkynes on the left side with the azides on the right side can give rise to the formation of *all* oligomers of type **1** starting from the dimannoside up to the hexadecamannoside. Even higher oligomers should be accessible by the extension of Scheme 1. Nevertheless, considering that the aim of the present work consisted of the discovery of Mtb cell envelope synthetase inhibitors, we decided to prepare only a set of compounds that could give some insight on the effect of the chain length on their biological activity. Suitable reaction partners required for the CuAAC-based synthesis of the target oligomers were synthesized as shown in Schemes 2, 3, and 5. Thus, the CuAAC of alkyne **5** with azide **9** afforded the fully protected dimannoside **24** (Scheme 9, eq a). The removal of MOM and benzyl protective groups and acetylation transformed this compound into the peracetylated derivative **25**, which was purified and characterized by NMR and MS. Finally, the removal of all acetyl groups from **25** by  $\text{NH}_3$  in methanol afforded the target TOM **1a** (71.5% yield from **5**), suitable for the planned biological tests. The same procedure was followed for the preparation of the tetramannoside **1b** starting from the cycloaddition of alkyne **10** with azide **11** (Scheme 9, eq b) and octamannoside **1d** starting from the cycloaddition of alkyne **15** with azide **16** (Scheme 9, eq c). The oligomannosides **1b** and **1d** were obtained in 43.5% and 32.8% overall yield, respectively, from the monosaccharidic building blocks.

#### In vitro $\alpha$ -(1,6)-Mannosyltransferases Inhibition by TOMs.

The inhibition of the mycobacterial  $\alpha$ -(1,6)-mannosyltransferases by triazole-linked oligomannosides TOM-2 (**1a**), TOM-4 (**1b**), TOM-8 (**1d**), and TOM-16 (**1f**), as well as hexamannoside TOM-6 (**1c**) and decamannoside TOM-10 (**1e**), these being synthesized as reported,<sup>16b</sup> was evaluated in vitro using a well-established assay<sup>11–13</sup> based on membrane extracts from *Mycobacterium smegmatis*. These membrane extracts contain polyprenolphosphomannose (PPM)-dependent  $\alpha$ -(1,6)-mannosyltransferases that are involved in the synthesis of the  $\alpha$ -1,6-linked mannoside core present in the mycobacterial cell wall LAMs.<sup>20</sup> It has been demonstrated that an octyl dimannoside [ $\alpha$ -Manp(1,6)- $\alpha$ -Manp-O(CH<sub>2</sub>)<sub>7</sub>-CH<sub>3</sub>] is the minimum acceptor substrate<sup>12</sup> for the above  $\alpha$ -(1,6)-mannosyltransferases, whereas their donor substrate, i.e., phosphodecaprenyl  $\beta$ -D-mannopyranoside, is formed from guanosine diphosphate (GDP)–mannose.<sup>21</sup>

Each triazole-linked oligomannoside **1a–f** (at 1.0 mM) was incubated at 37 °C for 30 min with [<sup>14</sup>C]<sub>1</sub>-labeled phosphodecaprenyl  $\beta$ -D-mannopyranoside (generated in situ from GDP-[<sup>14</sup>C]mannose), *M. smegmatis* membrane fractions, and  $\alpha$ -Manp(1,6)- $\alpha$ -Manp-O(CH<sub>2</sub>)<sub>7</sub>CH<sub>3</sub> disaccharidic acceptor (at 0.2 mM). After these experiments, we were pleased to observe that all the oligomannosides led to a significant inhibition of [<sup>14</sup>C]Manp incorporation from decaprenyl P-[<sup>14</sup>C]mannose onto the disaccharidic acceptor (101 cpm, no enzyme control; 13,627 cpm, no inhibitor; 8,174 cpm, TOM-2 (**1a**); 7,498 cpm, TOM-4 (**1b**); 401 cpm, TOM-6 (**1c**); 656 cpm, TOM-8 (**1d**); 6,680 cpm, TOM-10 (**1e**); 4,739 cpm, TOM-16 (**1f**). It has to be noted, however, that the hexamannoside TOM-6 (**1c**) and the octamannoside TOM-8 (**1d**),

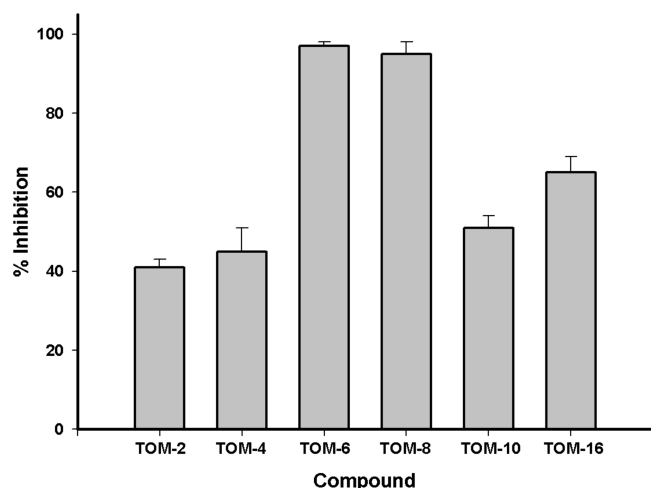
SCHEME 9



consistently over several independent experiments (Figure 2), showed the highest activity (ca. 95% inhibition). Subsequent experiments where TOM concentrations were varied provided inhibitory IC<sub>50</sub> values for TOM-2 (1.24 mM), TOM-4 (1.15 mM), TOM-6 (0.14 mM), TOM-8 (0.22 mM), TOM-10 (0.95 mM), and TOM-16 (0.84 mM). These values demonstrated that TOM-6 and TOM-8 were stronger  $\alpha$ -(1,6)-ManTs inhibitors than the octyl 6'-amino-O-disaccharides prepared by Tam and Lowary (IC<sub>50</sub> = 0.8–1.2 mM).<sup>11c</sup> Very recent attempts to prepare other mycobacterial mannosyltransferase inhibitors were met with failure.<sup>22</sup> In particular, a

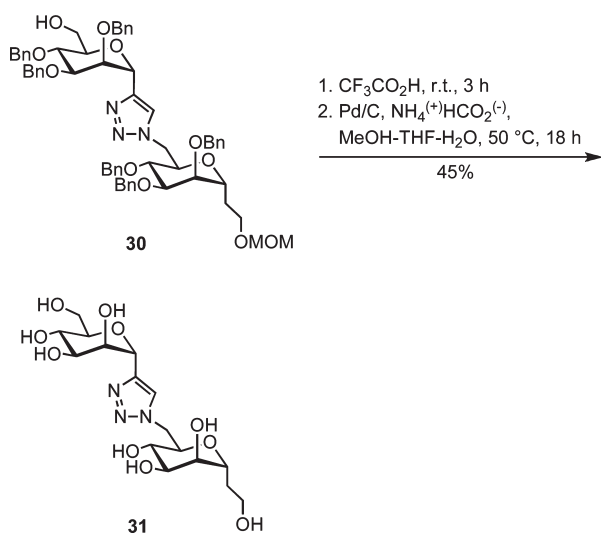
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**FIGURE 2.** In vitro inhibition of mycobacterial  $\alpha$ -(1,6)-mannosyltransferase enzyme activity by TOM-2–TOM-16 (**1a–f**). The assay utilizes *M. smegmatis* membranes,  $\alpha$ -Manp(1,6)- $\alpha$ -Manp-O(CH<sub>2</sub>)<sub>7</sub>-CH<sub>3</sub> disaccharidic acceptor (0.2 mM), 0.25  $\mu$ Ci of [<sup>14</sup>C]GDP-Man, and **1a–f** (1.0 mM). The results are expressed as percent inhibition of the incorporation of [<sup>14</sup>C]mannose into the acceptor and are representative of three independent experiments with each assay performed in triplicate.

#### SCHEME 10



series of 15 monosaccharidic alkyl and cycloalkyl  $\alpha$ -D-mannopyranoside derivatives, including 6-deoxygenated analogues, were prepared and evaluated as inhibitors. However, all these compounds were totally inactive toward the  $\alpha$ -(1,6)-ManTs.<sup>22</sup>

Following these results, it was important to establish whether the presence of a deoxygenated capping residue was a key structural feature for the mycobacterial  $\alpha$ -(1,6)-mannosyltransferase inhibition. To this end, the triazole-linked 1,6-dimannoside **31** was prepared from the known<sup>16a</sup> protected derivative **30** (Scheme 10) and tested by the standard assay described for TOM-2. Quite surprisingly, this compound turned out to be not only a poor mannosyl

acceptor up to 4 mM but also a weak inhibitor of the  $\alpha$ -(1,6)-ManTs ( $IC_{50}$  = 0.96 mM). Thus, while the deoxygenated 6-position at the reducing end ensures the total inertness of TOMs toward mannosyltransferases, this structural feature appears to be not crucial for inhibition.

#### Conclusion

In summary, a modular strategy has been established for the preparation of a set of C-oligomannosides featuring the triazole ring as the interglycosidic linker. In fact, the key reaction for the assembly of these oligomers consists of the very efficient click azide–alkyne cycloaddition (CuAAC). The efficiency and fidelity of this reaction were maintained regardless of the structural complexity of building blocks to be assembled. The biological experiments indicated that TOM-6 (**1c**) and TOM-8 (**1d**) are endowed with the optimal chain length for the interaction with PPM-dependent  $\alpha$ -(1,6)-mannosyltransferases. Although the core- $\alpha$ -(1,6)-LM is around 20 Man units in length, a TOM with a minor number of Man units binds efficiently to the  $\alpha$ -(1,6)-mannosyltransferase, suggesting that the binding groove of the TOM-6 oligosaccharide fits optimally into the protein. Very likely the 1,4-disubstituted triazole ring spacers contribute substantially to the overall length of these non-natural oligomannosides. In addition, it is worth noting that the presence of a non-natural C-glycosidic linkage and triazole linker in oligomannosides **1a–f** does not perturb their molecular recognition properties toward these mycobacterial  $\alpha$ -(1,6)-mannosyltransferases.

#### Experimental Section

**General Experimental Section.** All moisture-sensitive reactions were performed under a nitrogen atmosphere using oven-dried glassware. Anhydrous solvents were dried over standard drying agents<sup>23</sup> and freshly distilled prior to use. Reactions were monitored by TLC on silica gel 60 F<sub>254</sub> with detection by charring with sulfuric acid. Flash column chromatography<sup>24</sup> was performed on silica gel 60 (40–63  $\mu$ m). Optical rotations were measured at 20  $\pm$  2 °C in the stated solvent; [ $\alpha$ ]<sub>D</sub> values are given in deg·mL·g<sup>-1</sup>·dm<sup>-1</sup>. <sup>1</sup>H NMR (300 and 400 MHz) and <sup>13</sup>C NMR spectra (75 MHz) were recorded from CDCl<sub>3</sub> solutions at room temperature unless otherwise specified. Peak assignments were aided by <sup>1</sup>H–<sup>1</sup>H COSY and gradient-HMQC experiments. In the <sup>1</sup>H NMR spectra reported below, the *n* and *m* values quoted in geminal or vicinal proton–proton coupling constants *J*<sub>*n,m*</sub> refer to the number of the corresponding sugar protons. The closed vessel MW experiments were performed using a single-mode cavity Biotage Initiator microwave reactor; the temperature was measured externally on the outside vessel wall by an IR sensor; the reaction time was counted when the reaction mixture reached the preset temperature. Monosaccharides **4**,<sup>16a</sup> **5**,<sup>16b</sup> **6**,<sup>16b</sup> **7**,<sup>16b</sup> **8**,<sup>16a</sup> and **9**<sup>16a</sup> and disaccharides **10**,<sup>16b</sup> **11**,<sup>16a</sup> and **30**<sup>16a</sup> were prepared as described in our previous papers.

**MS Analysis.** For accurate mass measurements, the compounds were analyzed in positive-ion mode by electrospray hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer (Q-TOF) fitted with a Z-spray electrospray ion source. The capillary source voltage and the cone voltage were set at 3500 and 35 V, respectively; the source temperature was

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kept at 80 °C; nitrogen was used as a drying gas at a flow rate of ca. 50 L/h. The time-of-flight analyzer was externally calibrated with NaI from  $m/z$  300 to 2000 to yield an accuracy near to 5 ppm. When necessary an internal lock mass was used to further increase the mass accuracy. Accurate mass data were collected by directly infusing samples (10 pmol/ $\mu$ L in 1:1 CH<sub>3</sub>CN–H<sub>2</sub>O containing 10 mM ammonium formate) into the system at a flow rate of 5  $\mu$ L/min. Compounds **1b**, **1d**, **1f**, **20–23**, **28**, and **29** were analyzed by MALDI TOF mass spectrometry using a pulsed nitrogen laser ( $\lambda$  = 337 nm). Each compound was dissolved in H<sub>2</sub>O (**1b**, **1d**, **1f**) or 1:1 CHCl<sub>3</sub>–acetonitrile (**20–23**, **28**, **29**), and prior to the acquisition of spectra, 1  $\mu$ L of this solution was diluted with 1  $\mu$ L of saturated  $\alpha$ -cyano-4-hydroxycinnamic acid matrix solution (10 mg/mL in 1:1 EtOH–H<sub>2</sub>O, containing 0.1% of CF<sub>3</sub>CO<sub>2</sub>H). Ca. 1  $\mu$ L of the resulting mixture was placed onto the mass spectrometer's sample target and dried at room temperature. Once the liquid was completely evaporated, the sample was loaded into the mass spectrometer and analyzed. The instrument was operated in positive ion reflectron mode with the source voltage set to 12 kV. The pulse voltage was optimized at 1999 V, and the detector and reflectron voltages were set to 5200 and 2350 V, respectively. Measurements were performed in the mass range  $m/z$  800–5000 with a suppression mass gate set to  $m/z$  500 to prevent detector saturation from matrix cluster peaks and an extraction delay of 600 ns. The instrument was externally calibrated using a polyethylene glycol mix as standard. A mass accuracy near to the nominal (50 ppm) was achieved for each standard. The protonated monoisotopic mass of ACTH peptide ( $m/z$  2465.199) was used as internal lock mass to further improve the peptide mass accuracy near to 10–20 ppm. The monoisotopic masses were calculated according to the reported<sup>25</sup> atomic weights of the elements.

**3,7-Anhydro-4,5,6-tri-*O*-benzyl-1,2,8-trideoxy-1-*C*-[2-(2-hydroxypropyl)-8-[4-(2',3',4'-tri-*O*-benzyl- $\alpha$ -D-mannopyranosyl)-1*H*-1,2,3-triazol-1-yl]-D-glycero-D-talo-oct-1-ynitol (12).** A mixture of alkyne **4** (420 mg, 0.92 mmol), azide **7** (497 mg, 0.92 mmol), *N,N*-diisopropylethylamine (320  $\mu$ L, 1.84 mmol), CuI (34 mg, 0.18 mmol), and DMF (4 mL) in a vial sealed with a Teflon septum and aluminum crimp was subjected to microwave irradiation for 15 min at 80 °C and then cooled to room temperature, diluted with AcOEt (150 mL), washed with an aqueous solution of EDTA disodium salt (0.05 M, 3  $\times$  30 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated. The residue was eluted from a column of silica gel with 3:2 cyclohexane–AcOEt to give **12** (818 mg, 89%) as an amorphous solid:  $[\alpha]_D = -13.6$  (c 0.9, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz)  $\delta$  7.72 (s, 1H, H-5 tr), 7.46–7.26 (m, 30H, Ar), 5.23 (d, 1H,  $J_{1',2'} = 1.8$  Hz, H-1'), 4.96 and 4.71 (2 d, 2H,  $J = 11.0$  Hz, PhCH<sub>2</sub>), 4.89 and 4.59 (2 d, 2H,  $J = 11.0$  Hz, PhCH<sub>2</sub>), 4.82 and 4.77 (2 d, 2H,  $J = 12.5$  Hz, PhCH<sub>2</sub>), 4.77 (dd, 1H,  $J_{2',3'} = 3.0$  Hz, H-2'), 4.74 (d, 1H,  $J_{3,4} = 2.0$  Hz, H-3), 4.72 and 4.61 (2 d, 2H,  $J = 11.5$  Hz, PhCH<sub>2</sub>), 4.68 (dd, 1H,  $J_{7,8a} = 2.2$ ,  $J_{8a,8b} = 14.3$  Hz, H-8a), 4.66 (s, 2H, PhCH<sub>2</sub>), 4.62 and 4.59 (2 d, 2H,  $J = 12.0$  Hz, PhCH<sub>2</sub>), 4.52 (dd, 1H,  $J_{7,8b} = 7.0$  Hz, H-8b), 4.00–3.90 (m, 4H, H-3', H-5, H-6, H-7), 3.80 (dd, 1H,  $J_{5',6'a} = 3.0$ ,  $J_{6'a,6'b} = 12.0$  Hz, H-6'a), 3.72 (dd, 1H,  $J_{4,5} = 3.0$  Hz, H-4), 3.68 (dd, 1H,  $J_{5',6'b} = 6.2$  Hz, H-6'b), 3.62 (dd, 1H,  $J_{3',4'} = J_{4',5'} = 9.0$  Hz, H-4'), 3.43 (ddd, 1H, H-5'), 2.85 (s, 1H, OH), 2.20 (bs, 1H, OH), 1.31 (s, 6H, 2 CH<sub>3</sub>); <sup>13</sup>C NMR  $\delta$  144.5 (C-4 tr), 138.4 (C), 138.0 (C), 137.9 (C), 137.70 (C), 137.67 (C), 128.5 (CH), 128.4 (CH), 128.3 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 127.4 (CH), 123.7 (C-5 tr), 95.1 (C), 80.4 (CH), 79.4 (CH), 76.4 (CH), 75.3 (CH<sub>2</sub>), 75.1 (CH<sub>2</sub>), 75.0 (CH<sub>2</sub>), 74.8 (CH), 74.7 (CH), 74.3 (CH), 73.2 (CH), 72.8 (CH<sub>2</sub>), 72.1 (CH<sub>2</sub>), 71.9 (CH<sub>2</sub>), 71.5 (CH), 66.4 (CH), 64.4 (C), 62.9 (CH<sub>2</sub>), 50.8 (CH<sub>2</sub>), 31.2 (CH<sub>3</sub>), 30.9 (CH<sub>3</sub>); HRMS (ESI/Q-TOF)  $m/z$  calcd for C<sub>61</sub>H<sub>66</sub>N<sub>3</sub>O<sub>10</sub> (M + H)<sup>+</sup> 1000.4748, found 1000.4714.

**3,7-Anhydro-4,5,6-tri-*O*-benzyl-1,2,8-trideoxy-8-[4-(2',3',4'-tri-*O*-benzyl- $\alpha$ -D-mannopyranosyl)-1*H*-1,2,3-triazol-1-yl]-D-glycero-D-talo-oct-1-ynitol (13).** A mixture of **12** (355 mg, 0.36 mmol), anhydrous K<sub>2</sub>CO<sub>3</sub> (20 mg, 0.15 mmol), 18-crown-6 ether (19 mg, 0.07 mmol), and toluene (3 mL) was stirred at 110 °C for 4 h and then concentrated. The residue was eluted from a column of silica gel with 2:1 cyclohexane–AcOEt to give **13** (308 mg, 92%) as an amorphous solid:  $[\alpha]_D = +28.9$  (c 1.1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz) selected data  $\delta$  7.68 (s, 1H, H-5 tr), 7.43–7.21 (m, 30H, Ar), 5.25 (d, 1H,  $J_{1',2'} = 2.0$  Hz, H-1'), 4.54 (dd, 1H,  $J_{7,8a} = 2.4$ ,  $J_{8a,8b} = 14.3$  Hz, H-8a), 4.07 (ddd, 1H,  $J_{6,7} = 9.7$ ,  $J_{7,8b} = 5.2$  Hz, H-7), 4.04 (dd, 1H,  $J_{4,5} = 3.0$ ,  $J_{5,6} = 9.3$  Hz, H-5), 4.00 (dd, 1H,  $J_{3',4'} = 9.4$ ,  $J_{4',5'} = 9.0$  Hz, H-4'), 3.91 (dd, 1H,  $J_{2',3'} = 2.9$  Hz, H-3'), 3.51 (dd, 1H, H-5), 3.48 (ddd, 1H,  $J_{5',6'a} = 3.2$ ,  $J_{5',6'b} = 5.2$  Hz, H-5'), 2.49 (d, 1H,  $J_{1,3} = 2.3$ , H-1), 2.07 (bs, 1H, OH); <sup>13</sup>C NMR  $\delta$  144.6 (C-4 tr), 138.4 (C), 138.0 (C), 137.8 (C), 137.5 (C), 128.4 (CH), 128.3 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 127.5 (CH), 123.9 (C-5 tr), 80.2 (CH), 80.0 (CH), 78.2 (CH), 77.6 (C), 76.1 (CH), 75.3 (CH<sub>2</sub>), 75.2 (CH), 75.0 (CH<sub>2</sub>), 74.8 (CH), 74.2 (CH), 74.1 (CH), 73.1 (CH<sub>2</sub>), 72.7 (CH<sub>2</sub>), 72.1 (CH<sub>2</sub>), 72.0 (CH<sub>2</sub>), 71.8 (CH<sub>2</sub>), 71.4 (CH), 66.1 (CH), 62.7 (CH<sub>2</sub>), 50.6 (CH<sub>2</sub>); HRMS (ESI/Q-TOF)  $m/z$  calcd for C<sub>58</sub>H<sub>60</sub>N<sub>3</sub>O<sub>9</sub> (M + H)<sup>+</sup> 942.4330, found 942.4366.

**3,7-Anhydro-8-[4-(6'-azido-2',3',4'-tri-*O*-benzyl-6'-deoxy- $\alpha$ -D-mannopyranosyl)-1*H*-1,2,3-triazol-1-yl]-4,5,6-tri-*O*-benzyl-1,2,8-trideoxy-1-*C*-[2-(2-hydroxypropyl)-D-glycero-D-talo-oct-1-ynitol (14).** A mixture of alcohol **12** (440 mg, 0.44 mmol), freshly distilled Et<sub>3</sub>N (0.19 mL, 1.32 mmol), and *p*-toluenesulfonyl chloride (126 mg, 0.66 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (4 mL) was stirred at room temperature for 3 h and then diluted with CH<sub>2</sub>Cl<sub>2</sub> (40 mL), washed with 1 M phosphate buffer at pH 7 (2  $\times$  15 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated. The residue was filtered through a short column of silica gel with 2:1 cyclohexane–AcOEt to give the 8'-*O*-tosyl derivative as a white solid (545 mg). A mixture of this compound, sodium azide (143 mg, 2.20 mmol), and anhydrous DMF (3 mL) was stirred at 55 °C for 19 h and then cooled to room temperature, diluted with AcOEt (40 mL), washed with H<sub>2</sub>O (2  $\times$  15 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated. The residue was eluted from a column of silica gel with 3:1 cyclohexane–AcOEt to give **14** (352 mg, 78%) as a syrup:  $[\alpha]_D = +32.1$  (c 0.8, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz) selected data  $\delta$  7.81 (s, 1H, H-5 tr), 7.44–7.20 (m, 30H, Ar), 5.24 (d, 1H,  $J_{1',2'} = 1.7$  Hz, H-1'), 4.96 and 4.72 (2 d, 2H,  $J = 10.7$  Hz, PhCH<sub>2</sub>), 4.89 and 4.57 (2 d, 2H,  $J = 11.0$  Hz, PhCH<sub>2</sub>), 4.81 (dd, 1H,  $J_{2',3'} = 3.0$  Hz, H-2'), 4.77 (d, 1H,  $J_{3,4} = 2.0$  Hz, H-3), 3.72 (dd, 1H,  $J_{4,5} = 3.5$  Hz, H-4), 3.53 (ddd, 1H,  $J_{4',5'} = 9.5$ ,  $J_{5',6'a} = 7.5$ ,  $J_{5',6'b} = 2.3$  Hz, H-5'), 3.38 (dd, 1H,  $J_{6'a,6'b} = 13.0$  Hz, H-6'a), 3.32 (dd, 1H, H-6'b), 2.40 (s, 1H, OH), 1.32 and 1.31 (2 s, 6H, 2 CH<sub>3</sub>); <sup>13</sup>C NMR  $\delta$  144.3 (C-4 tr), 138.2 (C), 137.9 (C), 137.7 (C), 137.6 (C), 137.5 (C), 128.3 (CH), 128.2 (CH), 127.9 (CH), 127.7 (CH), 127.6 (CH), 127.5 (CH), 127.4 (CH), 124.3 (C-5 tr), 94.9 (C), 80.3 (CH), 79.3 (CH), 76.4 (CH), 75.2 (CH), 75.2 (CH<sub>2</sub>), 74.9 (CH<sub>2</sub>), 74.39 (CH), 74.35 (CH), 73.9 (CH), 73.1 (CH), 72.5 (CH<sub>2</sub>), 71.9 (CH<sub>2</sub>), 71.69 (CH<sub>2</sub>), 71.65 (CH<sub>2</sub>), 71.1 (CH), 66.2 (CH), 64.4 (C), 51.5 (CH<sub>2</sub>), 50.6 (CH<sub>2</sub>), 31.1 (CH<sub>3</sub>), 30.8 (CH<sub>3</sub>); HRMS (ESI/Q-TOF)  $m/z$  calcd for C<sub>61</sub>H<sub>65</sub>N<sub>6</sub>O<sub>9</sub> (M + H)<sup>+</sup> 1025.4813, found 1025.4814.

**Tetramannoside Alkyne (15).** A mixture of alkyne **10** (110 mg, 0.12 mmol), azide **14** (122 mg, 0.12 mmol), *N,N*-diisopropylethylamine (42  $\mu$ L, 0.24 mmol), CuI (5 mg, 24  $\mu$ mol), and DMF (0.6 mL) in a vial sealed with a Teflon septum and aluminum crimp was subjected to microwave irradiation for 15 min at 80 °C and then cooled to room temperature, diluted with AcOEt (50 mL), washed with 0.05 M aqueous solution of EDTA (3  $\times$  10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated. A mixture of this residue, anhydrous K<sub>2</sub>CO<sub>3</sub> (7 mg, 50  $\mu$ mol), 18-crown-6 ether (6 mg, 24  $\mu$ mol), and toluene (1 mL) was stirred at 110 °C for 4 h and then concentrated. The residue was eluted from a column of



silica gel with 2:1 cyclohexane–AcOEt to give **15** (176 mg, 78%), identical to the product described by us in a previous paper.<sup>16b</sup>

**Tetramannoside azide (16).** A mixture of alkyne **13** (68 mg, 65  $\mu$ mol), azide **11** (62 mg, 65  $\mu$ mol), *N,N*-diisopropylethylamine (23  $\mu$ L, 0.13 mmol), CuI (2.5 mg, 13  $\mu$ mol), and DMF (1 mL) in a vial sealed with a Teflon septum and aluminum crimp was subjected to microwave irradiation for 15 min at 80 °C and then cooled to room temperature, diluted with AcOEt (50 mL), washed with 0.05 M aqueous solution of EDTA (2  $\times$  15 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated. A mixture of this residue, diphenyl phosphoryl azide (43  $\mu$ L, 0.20 mmol), 1,8-diazabicyclo[5.4.0.]undec-7-ene (19  $\mu$ L, 0.13 mmol), sodium azide (22 mg, 0.33), and anhydrous DMF (1 mL) in a vial sealed with a Teflon septum and aluminum crimp was subjected to microwave irradiation for 2 h at 130 °C and then cooled to room temperature, diluted with AcOEt (50 mL), washed with saturated aqueous Na<sub>2</sub>CO<sub>3</sub> (20 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated. The residue was eluted from a column of silica gel with 3:1 cyclohexane–AcOEt to give **16** (81 mg, 61%), identical to the product described by us in a previous paper.<sup>16a</sup>

**Protected Tetramannoside Alkyne (17).** The coupling of azide **14** (178 mg, 0.17 mmol) with alkyne **13** (165 mg, 0.14 mmol) was performed as described for the synthesis of **12** to give, after column chromatography on silica gel (from 2:1 to 1:1 cyclohexane–AcOEt), **17** (259 mg, 76%) as an amorphous solid:  $[\alpha]_D = +6.8$  (*c* 0.8, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz) selected data  $\delta$  7.79 (s, 1H, H-5 tr), 7.39–7.12 (m, 62H, Ar, 2 H-5 tr), 5.36, 5.25, and 5.23 (3 d, 3H,  $J_{1',2'} = 1.5$  Hz,  $J_{1'',2''} = 1.5$  Hz,  $J_{1''',2'''} = 1.5$  Hz, H-1', H-1'', H-1'''), 4.96 (d, 1H,  $J_{3,4} = 1.5$  Hz, H-3), 3.03 (s, 1H, OH), 2.40 (dd, 1H,  $J_{6''a,OH} = 5.0$ ,  $J_{6''b,OH} = 7.2$  Hz, OH), 1.24 and 1.22 (2 s, 6H, 2 CH<sub>3</sub>); <sup>13</sup>C NMR  $\delta$  144.5 (C-4 tr), 143.4 (C-4 tr), 138.5 (C), 138.3 (C), 138.1 (C), 138.0 (C), 137.9 (C), 137.6 (C), 128.7–127.5 (CH), 124.3 (C-5 tr), 124.0 (C-5 tr), 95.0 (C), 80.0 (CH), 79.5 (CH), 76.6 (CH), 75.3 (CH), 75.1 (CH<sub>2</sub>), 74.9 (CH<sub>2</sub>), 74.6 (CH), 73.1 (CH), 72.6 (CH<sub>2</sub>), 72.4 (CH<sub>2</sub>), 72.3 (CH), 71.8 (CH<sub>2</sub>), 71.6 (CH<sub>2</sub>), 71.4 (CH<sub>2</sub>), 71.1 (CH), 66.1 (CH), 64.2 (C), 62.7 (CH<sub>2</sub>), 51.1 (CH<sub>2</sub>), 50.5 (CH<sub>2</sub>), 31.1 (CH<sub>3</sub>), 30.9 (CH<sub>3</sub>); HRMS (ESI/Q-TOF) *m/z* calcd for (C<sub>119</sub>H<sub>125</sub>N<sub>9</sub>O<sub>18</sub>)/2 (M + 2H)<sup>2+</sup> 983.9571, found 983.9586.

**Tetramannoside Alkyne (18).** The deprotection of tetramannoside **17** (75 mg, 0.04 mmol) was performed as described for the synthesis of **13** to give, after column chromatography on silica gel (2:1 cyclohexane–AcOEt), **18** (65 mg, 89%) as an amorphous solid:  $[\alpha]_D = +2.8$  (*c* 1.1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz) selected data  $\delta$  7.79 (s, 1H, H-5 tr), 7.47 (s, 1H, H-5 tr), 7.42–7.17 (m, 61H, Ar, 1 H-5 tr), 5.35, 5.26, and 5.23 (3 d, 3H,  $J_{1',2'} = 1.7$  Hz,  $J_{1'',2''} = 1.7$  Hz,  $J_{1''',2'''} = 1.7$  Hz, H-1', H-1'', H-1'''), 2.33 (bs, 1H, OH), 2.32 (d, 1H,  $J_{1,3} = 2.2$  Hz, H-1); <sup>13</sup>C NMR selected data  $\delta$  144.6 (C-4 tr), 143.8 (C-4 tr), 143.6 (C-4 tr), 138.5–137.5 (C), 128.9–127.6 (CH), 124.4 (C-5 tr), 124.2 (C-5 tr), 124.0 (C-5 tr), 67.8 (CH), 62.8 (CH<sub>2</sub>), 51.1 (CH<sub>2</sub>), 50.9 (CH<sub>2</sub>), 50.5 (CH<sub>2</sub>); HRMS (ESI/Q-TOF) *m/z* calcd for (C<sub>116</sub>H<sub>119</sub>N<sub>9</sub>O<sub>17</sub>)/2 (M + 2H)<sup>2+</sup> 954.9362, found 954.9412.

**Tetramannoside Azide (19).** The alcohol **17** (150 mg, 0.08 mmol) was azidated as described for the preparation of **14** to give, after column chromatography on silica gel (2:1 cyclohexane–AcOEt), **19** (118 mg, 79%) as a syrup:  $[\alpha]_D = +4.3$  (*c* 1.1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz) selected data  $\delta$  7.91 (1s, 1H, H-5 tr), 7.41–7.06 (m, 62H, Ar, 2 H-5 tr), 5.40, 5.27, and 5.26 (3 d, 3H,  $J_{1',2'} = 1.5$  Hz,  $J_{1'',2''} = 1.5$  Hz,  $J_{1''',2'''} = 1.5$  Hz, H-1', H-1'', H-1'''), 4.99 (d, 1H,  $J_{3,4} = 2.0$  Hz, H-3), 2.80 (s, 1H, OH), 1.23 and 1.19 (2 s, 6H, 2 CH<sub>3</sub>); <sup>13</sup>C NMR:  $\delta$  144.5 (C-4 tr), 143.5 (C-4 tr), 138.5 (C), 138.4 (C), 138.3 (C), 138.1 (C), 138.0 (C), 137.6 (C), 128.7–127.5 (CH), 124.7 (C-5 tr), 124.4 (C-5 tr), 124.1 (C-5 tr), 95.0 (C), 80.3 (CH), 80.2 (CH), 80.0 (CH), 79.6 (CH), 77.2 (CH), 76.7 (CH), 75.5 (CH), 75.2 (CH), 75.0 (CH<sub>2</sub>), 74.7 (CH), 74.5 (CH), 73.9 (CH), 73.2 (CH), 72.6 (CH), 72.5 (CH<sub>2</sub>), 72.3 (CH), 71.8 (CH<sub>2</sub>), 71.6 (CH<sub>2</sub>), 71.4 (CH<sub>2</sub>), 71.1 (CH), 70.6 (CH<sub>2</sub>), 70.3 (CH<sub>2</sub>), 70.0 (CH<sub>2</sub>), 66.1

(CH), 64.2 (C), 61.8 (CH<sub>2</sub>), 51.8 (CH<sub>2</sub>), 51.3 (CH<sub>2</sub>), 51.0 (CH<sub>2</sub>), 50.5 (CH<sub>2</sub>), 31.2 (CH<sub>3</sub>), 30.9 (CH<sub>3</sub>); HRMS (ESI/Q-TOF) *m/z* calcd for (C<sub>119</sub>H<sub>124</sub>N<sub>12</sub>O<sub>17</sub>)/2 (M + 2H)<sup>2+</sup> 996.4604, found 996.4620.

**Octamannoside Alkyne (20).** The alkyne **15** (97 mg, 0.05 mmol) was allowed to react with azide **19** (102 mg, 0.05 mmol) as described for the preparation of **15** to give, after trituration with CH<sub>3</sub>OH, **20** (101 mg, 52%) as an amorphous solid:  $[\alpha]_D = -172.3$  (*c* 0.7, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz) selected data  $\delta$  7.84 (s, 1H, H-5 tr), 6.02 (bs, 2H, 2 anom H), 5.96, 5.88, 5.80, 5.47, and 5.41 (5 bs, 5H, 5 anom H), 1.40 (d, 3H,  $J = 6.0$  Hz, CH<sub>3</sub>); <sup>13</sup>C NMR selected data  $\delta$  145.0 (C-4 tr), 143.5 (C-4 tr), 143.3 (C-4 tr), 143.0 (C-4 tr), 139.6–137.5 (C), 128.5–126.7 (CH), 124.9–123.9 (C-5 tr), 18.5 (CH<sub>3</sub>); MALDI-TOF MS *m/z* calcd for C<sub>232</sub>H<sub>234</sub>N<sub>21</sub>O<sub>32</sub> (M + H)<sup>+</sup> 3828.53, found 3828.41.

**Octamannoside Azide (21).** The alkyne **18** (55 mg, 0.03 mmol) was allowed to react with azide **16** (58 mg, 0.03 mmol) as described for the preparation of **16** to give, after trituration with CH<sub>3</sub>OH, **21** (71 mg, 63%) as an amorphous solid:  $[\alpha]_D = -138.6$  (*c* 1.1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz) selected data  $\delta$  7.92 (s, 1H, H-5 tr), 5.98 (bs, 2H, 2 anom. H), 5.94, 5.86, 5.71, 5.50, and 5.44 (5 bs, 5H, 5 anom. H), 3.10 (s, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR selected data  $\delta$  144.5–143.1 (C-4 tr), 139.5–137.6 (C), 128.3–126.7 (CH), 125.5–123.9 (C-5 tr); MALDI-TOF MS *m/z* calcd for C<sub>234</sub>H<sub>241</sub>N<sub>24</sub>O<sub>34</sub> (M + H)<sup>+</sup> 3933.63, found 3933.40.

**Hexadecamannoside (22).** The alkyne **20** (50.6 mg, 13  $\mu$ mol) was allowed to react with azide **21** (52 mg, 13  $\mu$ mol) as described for the preparation of **12** to give, after trituration with CH<sub>3</sub>CN, **22** (93 mg, 92%) as an amorphous solid:  $[\alpha]_D = -3.2$  (*c* 0.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz) selected data  $\delta$  7.88 (s, 1H, H-5 tr), 3.17 (s, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR selected data  $\delta$  143.4 (C-4 tr), 139.8–137.5 (C), 128.2–126.7 (CH), 124.8 (C-5 tr); MALDI-TOF MS *m/z* calcd for C<sub>466</sub>H<sub>474</sub>N<sub>45</sub>O<sub>66</sub> (M + H)<sup>+</sup> 7761.15, found 7761.40.

**Hexadecamannoside (23).** To a solution of **22** (75 mg, 9.7  $\mu$ mol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added trifluoroacetic acid (200  $\mu$ L). The solution was kept at room temperature for 3 h and then concentrated. To a stirred, cooled (–60 °C) solution of the residue in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (2 mL) was added a 1 M solution of BCl<sub>3</sub> in CH<sub>2</sub>Cl<sub>2</sub> (930  $\mu$ L, 0.93 mmol). The solution was stirred at –60 °C for 1 h and, after an additional 1 h stirring at 0 °C, diluted with MeOH (0.5 mL), stirred at 0 °C for 30 min, diluted with Et<sub>3</sub>N (0.5 mL), concentrated, and dried under high vacuum to give a white solid. A suspension of the residue in pyridine (2 mL) and acetic anhydride (1 mL) was stirred at room temperature for 24 h, diluted with MeOH (1 mL), and concentrated. The residue was eluted from a column of silica gel with AcOEt to give **23** (18 mg, 40%) as an amorphous solid:  $[\alpha]_D = +32.9$  (*c* 0.4, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz) selected data  $\delta$  7.99 (s, 1H, H-5 tr), 7.73–7.62 (14 s, 14H, 14 H-5 tr); MALDI-TOF MS: *m/z* calcd for C<sub>226</sub>H<sub>279</sub>N<sub>45</sub>NaO<sub>114</sub> (M + Na)<sup>+</sup> 5472.93, found 5472.88.

**Hexadecamannoside (1f).** To a solution of **23** (18 mg, 3.8  $\mu$ mol) in MeOH (2 mL) was added a 2 M solution of ammonia in MeOH (2 mL). The solution was kept at room temperature overnight and then concentrated to give **1f** (10 mg, 91%) as an amorphous solid:  $[\alpha]_D = +4.7$  (*c* 0.3, DMSO); <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O) selected data:  $\delta$  7.80–7.36 (15 s, 15H, 15 H-5 tr); MALDI-TOF MS *m/z* calcd for C<sub>128</sub>H<sub>181</sub>N<sub>45</sub>NaO<sub>65</sub> (M + Na)<sup>+</sup> 3413.10, found 3412.99.

**3,7-Anhydro-4,5,6-tri-O-benzyl-2,8-dideoxy-1-methoxymethyl-8-[4-(2',3',4'-tri-O-benzyl-6'-deoxy- $\alpha$ -D-mannopyranosyl)-1H-1,2,3-triazol-1-yl]-D-glycero-D-talo-octitol (24).** The coupling of azide **9** (12 mg, 0.023 mmol) with alkyne **5** (10 mg, 0.023 mmol) was performed as described for the synthesis of **12** to give, after column chromatography on silica gel (3:1 cyclohexane–AcOEt), **24** (21 mg, 96%) as an amorphous solid:  $[\alpha]_D = +21.7$  (*c* 1.1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz) selected data  $\delta$  7.65 (s, 1H, H-5 tr), 7.43–7.22 (m, 30H, Ar), 5.18 (d, 1H,  $J_{1',2'} = 2.0$  Hz, H-1'),

4.92 and 4.60 (2 d, 2H,  $J = 10.8$  Hz, PhCH<sub>2</sub>), 4.74 (dd, 1H,  $J_{7,8a} = 6.5$ ,  $J_{8a,8b} = 14.3$  Hz, H-8a), 4.47 (dd, 1H,  $J_{7,8b} = 3.0$  Hz, H-8b), 4.18 (ddd, 2H,  $J = 4.3$ , 5.0, 9.0 Hz, 2 H-1), 3.80 (dd, 1H,  $J_{3,4} = 3.0$ ,  $J_{4,5} = 7.5$  Hz, H-4), 3.62 (dd, 1H, H-3), 3.54 (dd, 1H,  $J_{5,6} = 6.8$  Hz, H-5), 3.28 (s, 3H, CH<sub>3</sub>), 1.80–1.70 (m, 2H, 2 H-2), 1.33 (d, 3H,  $J = 6.0$  Hz, 3 H-6'); <sup>13</sup>C NMR  $\delta$  145.2 (C-4 tr), 138.7 (C), 138.6 (C), 138.0 (C), 137.8 (C), 128.5 (CH), 128.4 (CH), 128.2 (CH), 128.1 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 127.5 (CH), 127.4 (CH), 123.8 (C-5 tr), 96.4 (CH<sub>2</sub>), 80.5 (CH), 80.2 (CH), 77.2 (CH), 75.9 (CH), 75.1 (CH<sub>2</sub>), 74.9 (CH), 74.0 (CH<sub>2</sub>), 72.6 (CH), 72.5 (CH<sub>2</sub>), 72.4 (CH<sub>2</sub>), 71.8 (CH<sub>2</sub>), 71.0 (CH), 70.4 (CH), 70.2 (CH), 63.6 (CH<sub>2</sub>), 55.2 (CH), 50.5 (CH<sub>2</sub>), 29.8 (CH<sub>2</sub>), 26.9 (CH<sub>2</sub>), 18.2 (CH<sub>3</sub>); HRMS (ESI/Q-TOF)  $m/z$  calcd for C<sub>60</sub>H<sub>67</sub>N<sub>3</sub>NaO<sub>10</sub> (M + Na)<sup>+</sup> 1012.4724, found 1012.4786.

**1,4,5,6-Tetra-*O*-acetyl-3,7-anhydro-2,8-dideoxy-8-[4-(2',3',4'-tri-*O*-acetyl-6'-deoxy- $\alpha$ -D-mannopyranosyl)-1*H*-1,2,3-triazol-1-yl]-D-glycero-D-talo-octitol (25).** The deprotection of **24** (18 mg, 18  $\mu$ mol) was performed as described for the synthesis of **23** to give, after column chromatography on silica gel (AcOEt), **25** (10 mg, 81%) as an amorphous solid:  $[\alpha]_D = +118.7$  (c 0.6, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz) selected data  $\delta$  7.83 (s, 1H, H-5 tr); <sup>13</sup>C NMR selected data  $\delta$  170.1 (C=O), 141.8 (C-4 Tr), 124.3 (C-5 Tr), 50.5 (CH<sub>2</sub>), 29.8 (CH<sub>2</sub>), 27.9 (CH<sub>2</sub>), 17.6 (CH<sub>3</sub>); HRMS (ESI/Q-TOF)  $m/z$  calcd for C<sub>30</sub>H<sub>42</sub>N<sub>3</sub>O<sub>16</sub> (M + H)<sup>+</sup> 700.2565, found 700.2619.

**3,7-Anhydro-2,8-dideoxy-8-[4-(6'-deoxy- $\alpha$ -D-mannopyranosyl)-1*H*-1,2,3-triazol-1-yl]-D-glycero-D-talo-octitol (1a).** The deprotection of **25** (8 mg, 11  $\mu$ mol) was performed as described for the synthesis of **1f** to give, after a column chromatography on Sephadex LH20 (1:1 CH<sub>2</sub>Cl<sub>2</sub>–MeOH), **1a** as an amorphous solid (4.2 mg, 92%): <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O) selected data  $\delta$  7.96 (s, 1H, H-5 tr), 5.10 (bs, 1H, H-1'); HRMS (ESI/Q-TOF)  $m/z$  calcd for C<sub>16</sub>H<sub>28</sub>N<sub>3</sub>O<sub>9</sub> (M + H)<sup>+</sup> 406.1826, found 406.1815.

**Tetramannoside (26).** The coupling of azide **11** (15 mg, 0.015 mmol) with alkyne **10** (13.8 mg, 0.015 mmol) was performed as described for the synthesis of **12** to give, after column chromatography on silica gel (2:1 cyclohexane–AcOEt), **26** (26 mg, 88%) as an amorphous solid:  $[\alpha]_D = -34.5$  (c 0.7, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz) selected data  $\delta$  7.80, 7.69, and 7.43 (3 s, 3H, 3 H-5 tr), 7.42–7.14 (m, 60H, Ar) 5.29, 5.23, and 5.20 (3 d, 3H,  $J_{1',2'} = 1.8$  Hz,  $J_{1'',2''} = 1.8$  Hz,  $J_{1''',2'''} = 1.8$  Hz, H-1', H-1'', H-1'''), 3.22 (s, 3H, CH<sub>3</sub>), 1.34 (d, 3H,  $J = 6.0$  Hz, CH<sub>3</sub>); <sup>13</sup>C NMR selected data  $\delta$  145.2 (C-4 tr), 143.9 (C-4 tr), 143.8 (C-4 tr), 138.7–137.9 (C), 128.5–127.3 (CH), 124.4 (C-5 tr), 124.3 (C-5 tr), 123.9 (C-5 tr), 96.4 (CH<sub>2</sub>), 63.8 (CH<sub>2</sub>), 55.2 (CH), 50.8 (CH<sub>2</sub>), 50.7 (CH<sub>2</sub>), 29.7 (CH<sub>2</sub>), 18.4 (CH<sub>3</sub>).

**Tetramannoside (27).** The deprotection of **26** (20 mg, 10  $\mu$ mol) was performed as described for the synthesis of **23** to give, after column chromatography on silica gel (AcOEt), **27** (10 mg, 73%) as an amorphous solid:  $[\alpha]_D = +3.9$  (c 0.7, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz) selected data  $\delta$  8.15, 7.77, and 7.68 (3 s, 3H, 3 H-5 tr); <sup>13</sup>C NMR selected data  $\delta$  170.5–170.0 (C=O), 29.7 (CH<sub>2</sub>), 20.8–20.7 (CH<sub>3</sub>), 17.6 (CH<sub>3</sub>); HRMS (ESI/Q-TOF)  $m/z$  calcd for C<sub>58</sub>H<sub>76</sub>N<sub>9</sub>O<sub>30</sub> (M + H)<sup>+</sup> 1378.4698, found 1378.4749.

**Tetramannoside (1b).** The deprotection of **27** (9 mg, 6.5  $\mu$ mol) was performed as described for the synthesis of **1f** to give **1b** as an amorphous solid (5 mg, quantitative): HRMS (MALDI-TOF)  $m/z$  calcd for C<sub>32</sub>H<sub>49</sub>N<sub>9</sub>NaO<sub>17</sub> (M + Na)<sup>+</sup> 854.3144, found 854.3214.

**Octamannoside (28).** The coupling of azide **16** (21 mg, 10.6  $\mu$ mol) with alkyne **15** (20 mg, 10.6  $\mu$ mol) was performed as described for the synthesis of **12** to give, after trituration with CH<sub>3</sub>OH, **28** (35 mg, 85%) as an amorphous solid:  $[\alpha]_D = -150.4$  (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz) selected data  $\delta$  7.88, 6.50, 6.38, 6.36, and 6.24 (5 s, 5H, 5 H-5 tr), 6.00 (bs, 2H, 2 anom H), 5.94, 5.87, 5.71, 5.46, and 5.41 (5 bs, 5H, 5 anom. H), 3.09 (s, 3H, CH<sub>3</sub>), 1.39 (d, 3H,  $J = 6.0$  Hz, CH<sub>3</sub>); <sup>13</sup>C NMR selected data  $\delta$  145.0–143.1 (C-4 tr), 139.5–137.5 (C), 128.4–126.7 (CH),

125.0–124.0 (C-5 tr), 96.0 (CH<sub>2</sub>), 64.2 (CH<sub>2</sub>), 54.8 (CH), 18.5 (CH<sub>3</sub>); MALDI-TOF MS  $m/z$  calcd for C<sub>234</sub>H<sub>242</sub>N<sub>21</sub>O<sub>34</sub> (M + H)<sup>+</sup> 3892.62, found 3892.57.

**Octamannoside (29).** The deprotection of **28** (34 mg, 8.7  $\mu$ mol) was performed as described for the synthesis of **23** to give, after column chromatography on silica gel (AcOEt), **29** (19 mg, 78%) as an amorphous solid:  $[\alpha]_D = -52.1$  (c 0.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz) selected data  $\delta$  7.98 (s, 1H, H-5 tr), 7.72 (s, 2H, 2 H-5 tr), 7.70 (s, 2H, 2 H-5 tr), 7.68 and 7.63 (2 s, 2H, 2 H-5 tr), 1.20 (d, 3H,  $J = 6.0$  Hz, CH<sub>3</sub>); MALDI-TOF MS  $m/z$  calcd for C<sub>114</sub>H<sub>143</sub>N<sub>21</sub>NaO<sub>58</sub> (M + Na)<sup>+</sup> 2758.49, found 2758.48.

**Octamannoside (1d).** The deprotection of **29** (9 mg, 3.4  $\mu$ mol) was performed as described for the synthesis of **1f** to give **1d** (6 mg, quantitative) as an amorphous solid: HRMS (MALDI-TOF)  $m/z$  calcd for C<sub>64</sub>H<sub>94</sub>N<sub>21</sub>O<sub>33</sub> (M + H)<sup>+</sup> 1684.6323, found 1684.5648.

**3,7-Anhydro-2,8-dideoxy-8-[4-( $\alpha$ -D-mannopyranosyl)-1*H*-1,2,3-triazol-1-yl]-D-glycero-D-talo-octitol (31).** To a solution of **30** (22 mg, 0.02 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added trifluoroacetic acid (400  $\mu$ L). The solution was kept at room temperature for 3 h and then concentrated. To a solution of the residue in 4:2:1 CH<sub>3</sub>OH–THF–H<sub>2</sub>O (4 mL) were added ammonium formate (110 mg, 1.75 mmol) and 10% palladium on carbon (212 mg, 0.20 mmol). The mixture was stirred at 50 °C for 18 h in a sealed vial and then cooled to room temperature, filtered through a pad of Celite, and concentrated. The residue was eluted from a column of Sephadex LH20 (1  $\times$  20 cm, d  $\times$  h) with 3:1 H<sub>2</sub>O–CH<sub>3</sub>OH to give **31** (4 mg, 45%) as an amorphous solid:  $[\alpha]_D = -6.1$  (c 0.4, H<sub>2</sub>O); <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) selected data  $\delta$  7.97 (s, 1H, H-5 tr), 5.10 (d, 1H,  $J_{1',2'} = 2.2$  Hz, H-1'), 4.46 (dd, 1H,  $J_{7,8b} = 9.0$ ,  $J_{8a,8b} = 14.2$  Hz, H-8b), 4.41 (dd, 1H,  $J_{2',3'} = 3.2$  Hz, H-2'); <sup>13</sup>C NMR (D<sub>2</sub>O)  $\delta$  146.5 (C-4 tr), 128.3 (C-5 tr), 78.2 (CH), 77.7 (CH), 75.7 (CH), 75.2 (CH), 74.1 (CH), 73.7 (CH), 73.4 (CH), 72.7 (CH), 71.4 (CH), 69.9 (CH), 63.7 (CH<sub>2</sub>), 60.3 (CH<sub>2</sub>), 54.2 (CH<sub>2</sub>), 32.3 (CH<sub>2</sub>); HRMS (ESI/Q-TOF)  $m/z$  calcd for C<sub>16</sub>H<sub>28</sub>N<sub>3</sub>O<sub>10</sub> (M + H)<sup>+</sup> 422.1775, found 422.1767.

**Evaluation of Inhibitor Activity of 1a–f in the PPM1/ $\alpha$ (1,6)-Mannosyltransferase Assay.** The  $\alpha$ -Manp(1,6)- $\alpha$ -Manp-O(CH<sub>2</sub>)<sub>7</sub>-CH<sub>3</sub> disaccharide acceptor<sup>12</sup> (0.8  $\mu$ L, stored as a 20 mM ethanol stock) was dried under a stream of argon in a microcentrifuge tube (1.5 mL), placed in a vacuum desiccator for 15 min to remove any residual solvent, and then resuspended in 8  $\mu$ L of a 1% aqueous solution of Igepal. Assays were performed by incubating each TOM compound (**1a–f**) at a final concentration of 1.0 mM with 2.4  $\mu$ M GDP-[U-<sup>14</sup>C]mannose (321 mCi/mmol, 0.25  $\mu$ Ci), 62.5  $\mu$ M ATP, 10  $\mu$ M MgCl<sub>2</sub>, 62.5  $\mu$ M DTT, 12.5  $\mu$ M NaF, 0.25 mM decaprenolphosphate (in 1% CHAPS) and *M. smegmatis* membrane fractions corresponding to 500  $\mu$ g. The final volume of the assays was adjusted to 80  $\mu$ L with 50 mM MOPS (pH 7.9), with a 0.2 mM final concentration of  $\alpha$ -Manp(1,6)- $\alpha$ -Manp-O(CH<sub>2</sub>)<sub>7</sub>-CH<sub>3</sub> acceptor. The reaction mixtures were then incubated at 37 °C for 30 min. A 1:1 CHCl<sub>3</sub>/CH<sub>3</sub>OH (533  $\mu$ L) solution was added to the incubation tubes, and the entire contents were centrifuged at 1800g. The supernatant was recovered and dried under a stream of argon, resuspended in 1:1 EtOH/H<sub>2</sub>O (1 mL), and loaded onto a pre-equilibrated (1:1 EtOH/H<sub>2</sub>O) 1 mL Whatmann strong anion-exchange (SAX) cartridge, which was washed with 3 mL of EtOH. The eluate was dried, and the resulting products were partitioned between the two phases arising from a mixture of *n*-butanol (3 mL) and H<sub>2</sub>O (3 mL). The resulting organic phase was recovered following centrifugation at 3500g, the aqueous phase was again extracted twice with 3 mL of water-saturated *n*-butanol, and the pooled extracts were back-washed twice with water-saturated *n*-butanol (3 mL). The water-saturated *n*-butanol fraction was dried and resuspended in 200  $\mu$ L of *n*-butanol. The total counts per minute of radiolabeled material extractable into the *n*-butanol phase was measured by scintillation counting using 10% of the labeled material and

10 mL of EcoScintA. The incorporation of [ $^{14}\text{C}$ ]Man was determined by subtracting counts present in control assays (incubation of the reaction components in the absence of  $\alpha\text{-Manp}(1,6)\text{-}\alpha\text{-Manp-O}(\text{CH}_2)_7\text{CH}_3$ ).

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**Supporting Information Available:** Copies of the NMR spectra of the new products. This material is available free of charge via the Internet at <http://pubs.acs.org>.