ChemPubSoc Europe

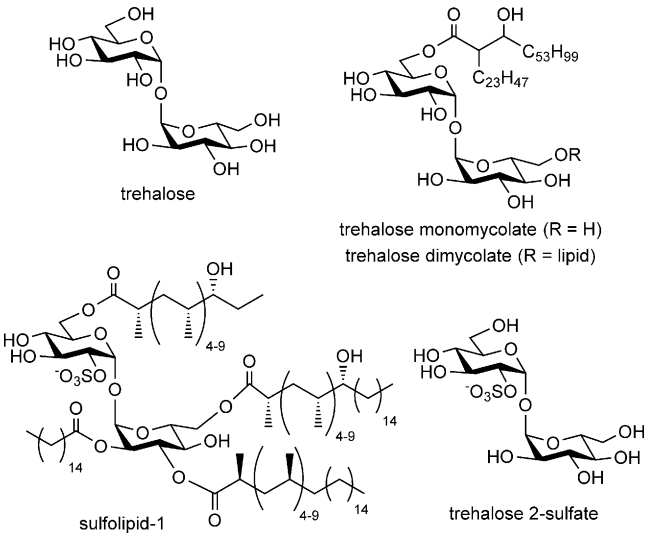
DOI: 10.1002/cbic.201402288

Chemoenzymatic Synthesis of Trehalose Analogues: Rapid Access to Chemical Probes for Investigating Mycobacteria

Bailey L. Urbanek,[a] Douglas C. Wing,[a] Krystal S. Haislop,[b] Chelsey J. Hamel,[a] Rainer Kalscheuer,[c] Peter J. Woodruff,[b] and Benjamin M. Swarts*[a]

Trehalose analogues are emerging as valuable tools for investigating *Mycobacterium tuberculosis*, but progress in this area is slow due to the difficulty in synthesizing these compounds. Here, we report a chemoenzymatic synthesis of trehalose analogues that employs the heat-stable enzyme trehalose synthase (TreT) from the hyperthermophile *Thermoproteus tenax*. By using TreT, various trehalose analogues were prepared quickly (1 h) in high yield (up to >99% by HPLC) in a single step from readily available glucose analogues. To demonstrate the utility of this method in mycobacteria research, we performed a simple "one-pot metabolic labeling" experiment that accomplished probe synthesis, metabolic labeling, and imaging of *M. smegmatis* in a single day with only TreT and commercially available materials.

Trehalose is a C_2-symmetric disaccharide consisting of two glucose molecules linked by a 1,1-α,α-glycosidic bond. Although trehalose is not present in mammals, it is widespread elsewhere in nature, where it primarily functions as an energy source and as a protectant against desiccation, osmotic stress, and changes in temperature.[1] Trehalose metabolism is required for virulence in a number of pathogenic organisms, most notably *Mycobacterium tuberculosis* (*Mtb*), which is the causative agent of human tuberculosis (TB).[2] *Mtb* is characterized by its complex cell envelope, which contains a variety of trehalose glycolipids that are involved in cell-wall biosynthesis and that contribute to pathogenesis (Scheme 1).[3] The essentiality of trehalose metabolism in *Mtb*—coupled with its absence in humans—makes it an attractive target for drug and diagnostic development, a notion that is underscored by the recent identification of numerous antimycobacterial compounds that inhibit trehalose glycolipid transport.[4]



Scheme 1. Chemical structures of trehalose and some naturally occurring trehalose derivatives found in mycobacteria.

Trehalose analogues are valuable tools for investigating trehalose metabolism in mycobacteria. Recently, trehalose analogues modified with detectable tags, including fluorescein and azido groups, have been used as probes for imaging live mycobacteria.[5] This capability enables experiments on cell-wall structure and dynamics, substrate promiscuity of trehalose-processing machinery, and antibiotic modes of action. These types of studies could potentially be extended to animal models of *Mtb* infection, due to the absence of trehalose in mammals. Trehalose analogues have also been evaluated as antimycobacterial compounds due to their potential ability to disrupt essential metabolic pathways,[6] as exemplified by 6-azido-6-deoxy-α,α′-trehalose (6-TreAz), which inhibits growth of *M. aurum* by acting on cell-wall biosynthesis.[7] In addition, synthetic analogues of several mycobacterial glycolipids, including trehalose monomycolate (TMM), trehalose dimycolate (TDM), and sulfolipid-1 (SL-1), have proven useful for investigating and modulating immune response to mycobacterial infection.[8] For instance, trehalose-6,6′-dibehenate, an analogue of TDM, mimics the immune activation properties of TDM and has been shown to be an effective adjuvant for vaccine development.[9]

Despite their value, the development and application of trehalose analogues in TB research remains limited, in part due to the difficulties associated with their chemical synthesis. Specifically, the C_2 symmetry and 1,1-α,α-glycosidic bond of trehalose

[a] *B. L. Urbanek,[+] D. C. Wing,[+] C. J. Hamel, Prof. B. M. Swarts*
Department of Chemistry, Central Michigan University
Mount Pleasant, MI 48859 (USA)
E-mail: ben.swarts@cmich.edu

[b] *K. S. Haislop, Prof. P. J. Woodruff*
Department of Chemistry, University of Southern Maine
Portland, ME 04104 (USA)

[c] *Dr. R. Kalscheuer*
Institute for Medical Microbiology and Hospital Hygiene
Heinrich-Heine-University Duesseldorf
40225 Duesseldorf (Germany)

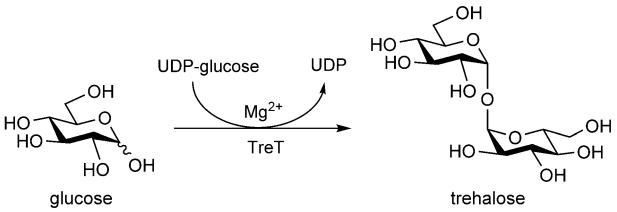
[+] *These authors contributed equally to this work.*

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cbic.201402288.

pose significant synthetic challenges. To address this issue, a number of chemical methods have been developed for trehalose desymmetrization/regioselective hydroxy group manipulation[10] and the formation of 1,1-α,α-glycosidic linkages.[11] Collectively, these methods are versatile and important for preparing various types of trehalose analogues, particularly those with complex structures bearing multiple sites of modification, such as SL-1 analogues.[12] However, chemical syntheses of trehalose analogues are usually lengthy and low-yielding, which motivated us to develop a complementary approach. Here, we describe a chemoenzymatic method for the rapid and efficient synthesis of trehalose analogues that is both accessible to non-chemists and well-suited to generating trehalose-based probes to investigate mycobacteria.

We first sought to identify a trehalose biosynthesis pathway that could be adapted for the chemoenzymatic synthesis of trehalose analogues. Several natural pathways exist that could, in principle, be exploited for synthetic applications.[1] Two enzymes are particularly fitting for this purpose because they employ simple and readily available glucose (Glc) analogues as substrates: trehalose phosphorylase (TreP), which catalyzes the cleavage of trehalose-6-phosphate to release Glc and β-D-Glc-1-phosphate; and trehalose synthase (TreT), which catalyzes the formation of trehalose directly from Glc and uridine diphosphate glucose (UDP-Glc). TreP was recently explored for its ability to produce trehalose analogues for biopreservation applications by running the enzyme in the reverse "synthetic" direction, but substrate specificity tests were limited to natural sugars, and no yields or product characterization data were reported.[13] Further, the donor in these reactions, β-D-Glc-1-phosphate, is prohibitively expensive and difficult to synthesize. Studies focused on the characterization of TreT from *Thermotoga maritima* and *Pyrococcus horikoshii* showed that these enzymes could process a few monosaccharide acceptors other than Glc—mannose, galactose, and fructose were tested—albeit with low conversion and long reaction times.[14] In addition, a multistep chemoenzymatic approach to synthesizing 2-deoxy-2-fluoro-α,α′-trehalose (2-fluoroTre) has been reported, but it required the use of three enzymes and a Glc-6-phosphate analogue intermediate, thus limiting synthetic efficiency and versatility.[5a] Overall, the reported results are encouraging, but the use of enzymes for the practical and efficient synthesis of trehalose analogues has remained underdeveloped.

We decided to pursue trehalose analogue synthesis using TreT from the hyperthermophile *Thermoproteus tenax* (Scheme 2).[15] In addition to its simple acceptor and donor substrates and excellent thermostability, TreT from *T. tenax* cannot degrade trehalose,[15] which distinguishes it from TreT enzymes in other organisms. We hypothesized that these characteristics would make TreT from *T. tenax* ideal for synthetic applications. To initiate the study, we expressed and purified TreT from *E. coli* as reported[15] and screened it for reactivity by using a panel of monofunctionalized Glc analogues. The Glc analogues that were tested contained fluoro-, deoxy-, azido-, and stereochemical modifications occurring at all positions of the sugar ring, which afforded a systematic evaluation of TreT substrate promiscuity. Reactions were performed in 50 mM HEPES buffer (pH 7.4) containing 10 mM Glc analogue, 40 mM UDP-Glc, 20 mM $MgCl_2$, and 9.8 μM TreT (reaction volume 50 μL). The reactions were incubated at 70 °C with gentle shaking for 1 h, quenched by addition of cold acetone, and analyzed by HPLC and high-resolution ESI mass spectrometry (Figures S1–S18 in the Supporting Information).



Scheme 2. Synthesis of trehalose from Glc and UDP-Glc by TreT from *T. tenax*.

The Glc analogues that we evaluated were remarkably well tolerated by TreT (Table 1). In most cases, the corresponding trehalose analogue products were generated in excellent yield after only 1 h, which highlighted the efficiency, rapidity, and generality of the method. Fluoro-, deoxy-, azido-, and stereochemical modifications of the Glc 2-, 3-, and 6-positions were generally accepted, except for azido substitution at the 2-position and inversion of the 3-OH group. Glc analogues bearing 4-position alterations were poor substrates (n.d.–26% yield), indicating a strict specificity at this position. Nonetheless, 4-position-modified products were observed in low yield; this, suggests that mutation of the active site of TreT might allow for improved substrate tolerance. Finally, 5-thio-D-glucose, the sole 5-position-modified Glc analogue that was tested, was converted to the novel compound 5-thio-trehalose in quantitative yield. The 5-thio-D-glucose reaction was performed in 10 mM dithiothreitol (DTT), indicating that TreT retains activity in the presence of DTT, which can facilitate access to other thio-modified trehalose analogues.

Selected reactions were run on a semi-preparative scale (5–10 mg) to evaluate the scalability of the method and to confirm product structure by NMR spectroscopy. Semi-preparative reactions were performed as described above (reaction volume: 1.5–2.0 mL), and the products were readily purified by silica gel chromatography. Consistent with the small-scale results, 2-fluoroTre, 2-deoxyTre, 3-fluoroTre, 6-TreAz, and 5-thio-Tre were obtained in isolated yields of 92–97%, which demonstrated that scale-up of the TreT reaction is feasible. ^{1}H and ^{13}C NMR analysis established the product structures, including the assignment of 1,1-α,α-stereochemistry for newly formed glycosidic bonds (Supporting Information). No regio- or stereoisomers of the desired trehalose analogue products were detected.

As discussed above, previously reported efforts to assess the substrate specificity of trehalose-synthesizing enzymes have primarily been limited to natural sugars, for example, Glc epimers, for the purpose of developing new compounds for the preservation of biomaterials.[13] In the present work, we considerably expanded this focus by emphasizing the incorporation of unnatural functionalities that are useful for glycobiology re-

Table 1. Synthesis of trehalose analogues from Glc analogues and UDP-Glc by TreT from *T. tenax*.

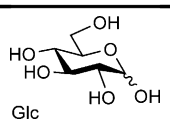
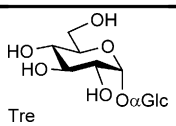
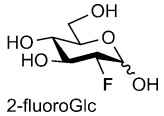
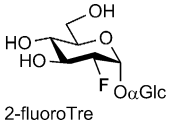
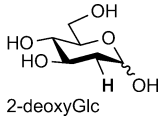
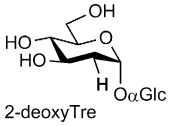
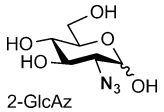
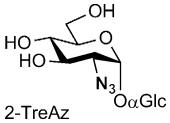
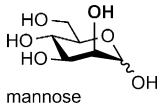
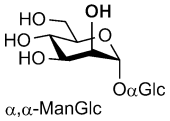
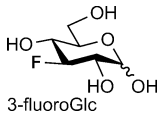
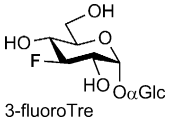
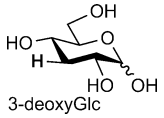
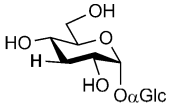
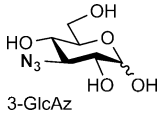
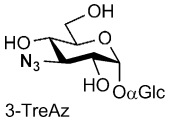
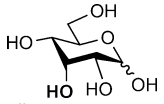
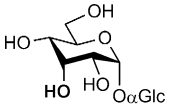
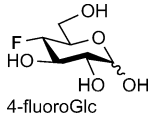
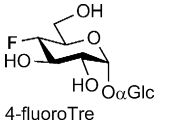
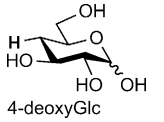
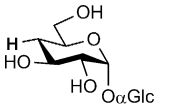
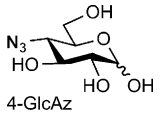
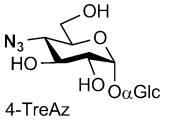
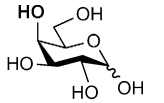
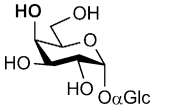
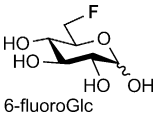
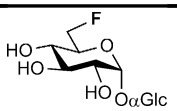
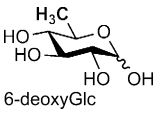
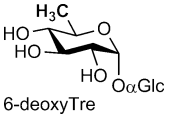
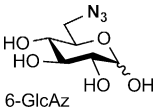
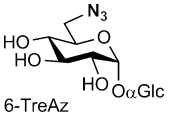
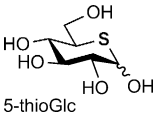
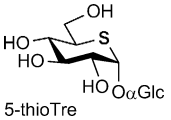
	Substrate	Product	Conversion [%][a]
1	 Glc	 Tre	>99
2	 2-fluoroGlc	 2-fluoroTre	>99
3	 2-deoxyGlc	 2-deoxyTre	>99
4	 2-GlcAz	 2-TreAz	n.d.
5	 mannose	 α,α-ManGlc	87
6	 3-fluoroGlc	 3-fluoroTre	>99
7	 3-deoxyGlc	 3-deoxyTre	75
8	 3-GlcAz	 3-TreAz	95
9	 allose	 α,α-AllGlc	n.d.
10	 4-fluoroGlc	 4-fluoroTre	7
11	 4-deoxyGlc	 4-deoxyTre	26
12	 4-GlcAz	 4-TreAz	n.d.
13	 galactose	 α,α-GalGlc	12

Table 1. (Continued)

	Substrate	Product	Conversion [%][a]
14	 6-fluoroGlc	 6-fluoroTre	98
15	 6-deoxyGlc	 6-deoxyTre	87
16	 6-GlcAz	 6-TreAz	>99
17	 5-thioGlc	 5-thioTre	>99

[a] Conversions determined by HPLC. n.d.: not detected.

search.[16] For instance, deoxy-, fluoro-, and thio-modified sugar analogues have been used as structure–activity probes, inhibitors of glycan-processing enzymes, and hydrolytically stable structural mimics. In addition, detectable sugar analogues—ranging from sugars bearing reactive chemical tags to radioactive sugars—are broadly used for biological research and clinical diagnostics. For example, azido sugars are commonly used in conjunction with bioorthogonal chemistry to metabolically label and image glycoconjugates in living systems,[17] and ^{18}F-labeled sugars are used in the clinic for positron emission tomography (PET) imaging of cancer.[18] We expect that the TreT reaction could facilitate the extension of these techniques to TB research by expediting access to unnatural trehalose analogues.

To evaluate the applicability of the TreT method to rapidly generating trehalose-based probes for imaging mycobacteria, we performed a one-day experiment that encompassed probe synthesis, metabolic labeling, and imaging of *M. smegmatis*, which is an avirulent model organism that is frequently used in TB research (Figure 1 A). First, TreT was used to convert commercially available 6-azido-6-deoxy-D-glucose (6-GlcAz) to 6-TreAz (Table 1, entry 16), an established chemical reporter for metabolic labeling of mycobacterial glycolipids.[5b] Next, we capitalized on the biocompatibility of enzymatic reactions by directly diluting the aqueous TreT reaction mixture into live *M. smegmatis* cell suspension, which we termed "one-pot metabolic labeling." After incubation for 4 h, 6-TreAz-labeled mycobacteria were washed, fixed, and reacted with an azide-reactive fluorophore, alkyne-488, via Cu-catalyzed azide–alkyne cycloaddition (CuAAC).[19] As shown in Figure 1 B, fluorescence microscopy revealed strong labeling of wild-type *M. smegmatis* that was treated with a reaction mixture containing 6-TreAz (+TreT). No fluorescence was observed when bacteria were treated with a reaction mixture lacking TreT (−TreT), which consisted only of unreacted substrates. The same experiments were

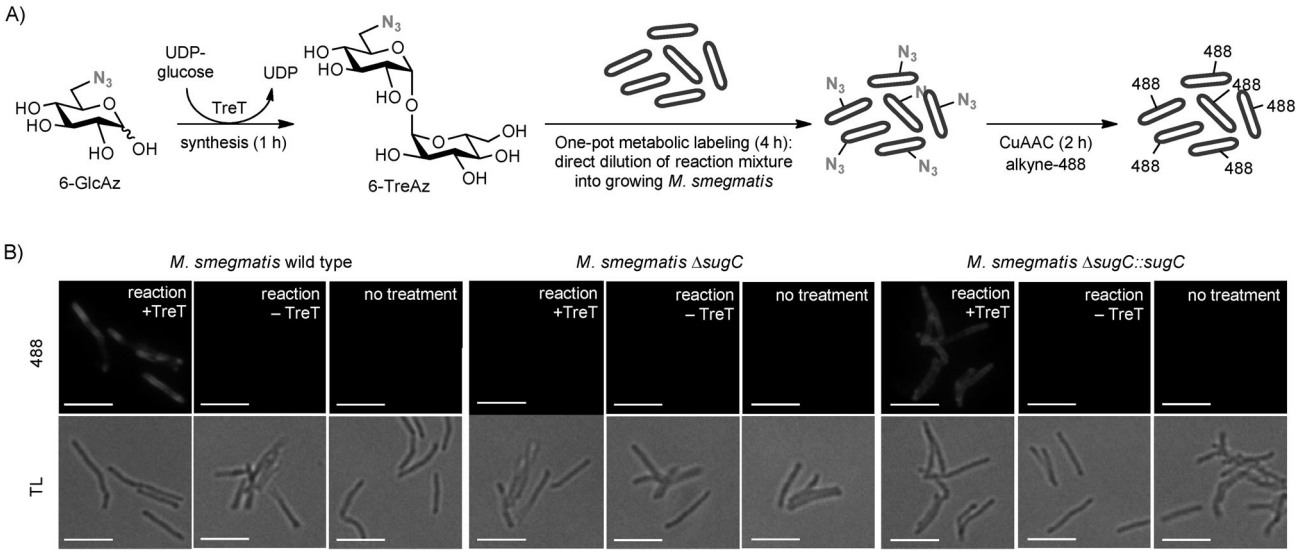


Figure 1. One-pot metabolic labeling of *M. smegmatis* enabled by TreT. A) Experimental workflow for single-day probe synthesis, metabolic labeling, and imaging of *M. smegmatis*. B) Fluorescence microscopy analysis of 6-TreAz-treated (or untreated) *M. smegmatis* wild-type, Δ*sugC* mutant, and Δ*sugC*::*sugC* complement. TL, transmitted light. Scale bars: 5 μm.

performed in *M. smegmatis* Δ*sugC*,[20] a mutant missing the trehalose transporter required for 6-TreAz uptake and labeling,[5b] as well as its complement, *M. smegmatis* Δ*sugC*::*sugC*. As predicted, fluorescence was abolished in the Δ*sugC* mutant and restored in the complement, confirming that 6-TreAz labeling proceeded via the expected pathway. Importantly, direct treatment of *M. smegmatis* with the TreT reaction mixture had no effect on bacterial growth or appearance. This operationally simple experiment provides a model for rapidly preparing and administering trehalose analogues to investigate mycobacteria. In contrast, traditional methods for synthesizing and purifying these compounds can require weeks of work by a trained chemist in a well-equipped synthesis laboratory.

In conclusion, the thermostable trehalose synthase TreT from *T. tenax* can convert a broad variety of Glc analogues into trehalose analogues in high yield in 1 h. The types of sugar modification that were chosen for this study are useful for numerous research applications and will open new avenues for investigating mycobacterial pathogenesis. To demonstrate this point, we exploited the speed and biocompatibility of the TreT reaction to quickly generate and use an azide-modified trehalose analogue to image mycobacteria. We anticipate that this approach could be adapted to the development of in vivo diagnostic tools, such as [^{18}F]-modified trehalose analogues, which were originally proposed as potential PET probes for TB by Davis, Barry, and co-workers.[5a] For instance, TreT might be used to rapidly convert existing pipelines of 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG), which is regularly used for PET imaging of cancer, to [^{18}F]-2-fluoroTre (Table 1, entry 2) for PET imaging of TB disease progression and response to chemotherapy. More broadly, it is expected that the TreT method will provide easy and efficient access to novel trehalose analogues for a diverse range of biological studies. At present, we are continuing to develop this method by investigating enzyme kinetics, expanding substrate scope, and exploring approaches for enzyme reuse.

Acknowledgements

This work was supported by CMU start-up funds to B.M.S. and the Research Corporation for Science Advancement (20185 to P.J.W.). R.K. acknowledges support from the Juergen Manchot Foundation. We thank Dr. Robin Hood for assistance with NMR and MS instrumentation. We are grateful to Michael Boyce for critical reading of the manuscript.

Keywords: chemoenzymatic synthesis · click chemistry · glycolipids · mycobacteria · trehalose

[1] A. D. Elbein, Y. T. Pan, I. Pastuszak, D. Carroll, *Glycobiology* **2003**, *13*, 17R – 27R.
[2] H. Tournu, A. Fiori, P. Van Dijck, *PLoS Pathog.* **2013**, *9*, e1003447.
[3] a) P. J. Brennan, H. Nikaido, *Annu. Rev. Biochem.* **1995**, *64*, 29 – 63; b) H. Marrakchi, M.-A. Lanéelle, M. Daffé, *Chem. Biol.* **2014**, *21*, 67 – 85.
[4] a) A. E. Grzegorzewicz, H. Pham, V. A. K. B. Gundi, M. S. Scherman, E. J. North, T. Hess, V. Jones, V. Gruppo, S. E. M. Born, J. Korduláková, S. S. Chavadi, C. Morisseau, A. J. Lenaerts, R. E. Lee, M. R. McNeil, M. Jackson, *Nat. Chem. Biol.* **2012**, *8*, 334 – 341; b) V. La Rosa, G. Poce, J. O. Canseco, S. Buroni, M. R. Pasca, M. Biava, R. M. Raju, G. C. Porretta, S. Alfonso, C. Battilocchio, B. Javid, F. Sorrentino, T. R. Ioerger, J. C. Sacchettini, F. Manetti, M. Botta, A. De Logu, E. J. Rubin, E. De Rossi, *Antimicrob. Agents Chemother.* **2012**, *56*, 324 – 331; c) S. A. Stanley, S. S. Grant, T. Kawate, N. Iwase, M. Shimizu, C. Wivagg, M. Silvis, E. Kazyanskaya, J. Aquadro, A. Golas, M. Fitzgerald, H. Dai, L. Zhang, D. T. Hung, *ACS Chem. Biol.* **2012**, *7*, 1377 – 1384; d) S. P. S. Rao, S. B. Lakshminarayana, R. R. Kondreddi, M. Herve, L. R. Camacho, P. Bifani, S. K. Kalapala, J. Jiricek, N. L. Ma, B. H. Tan, S. H. Ng, M. Nanjundappa, S. Ravindran, P. G. Seah, P. Thayalan, S. H. Lim, B. H. Lee, A. Goh, W. S. Barnes, Z. Chen, K. Gagaring, A. K. Chatterjee, K. Pethe, K. Kuhen, J. Walker, G. Feng, S. Babu, L. Zhang, F. Blasco, D. Beer, M. Weaver, V. Dartois, R. Glynne, T. Dick, P. W. Smith, T. T. Diagana, U. H. Manjunatha, *Sci. Transl. Med.* **2013**, *5*, 214ra168.

[5] a) K. M. Backus, H. I. Boshoff, C. S. Barry, O. Boutureira, M. K. Patel, F. D'Hooge, S. S. Lee, L. E. Via, K. Tahlan, C. E. Barry, B. G. Davis, *Nat. Chem. Biol.* **2011**, *7*, 228 – 235; b) B. M. Swarts, C. M. Holsclaw, J. C. Jewett, M. Alber, D. M. Fox, M. S. Siegrist, J. A. Leary, R. Kalscheuer, C. R. Bertozzi, *J. Am. Chem. Soc.* **2012**, *134*, 16123 – 16126.
[6] a) J. D. Rose, J. A. Maddry, R. N. Comber, W. J. Suling, L. N. Wilson, R. C. Reynolds, *Carbohydr. Res.* **2002**, *337*, 105 – 120; b) J. Wang, B. Elchert, Y. Hui, J. Y. Takemoto, M. Bensaci, J. Wennergren, H. Chang, R. Rai, C. W. Chang, *Bioorg. Med. Chem.* **2004**, *12*, 6397 – 6413; c) S. Gobec, I. Plantan, J. Mravljak, U. Švajger, R. A. Wilson, G. S. Besra, S. L. Soares, R. Appelberg, D. Kikelj, *Eur. J. Med. Chem.* **2007**, *42*, 54 – 63.
[7] J. T. Belisle, V. D. Vissa, T. Sievert, K. Takayama, P. J. Brennan, G. S. Besra, *Science* **1997**, *276*, 1420 – 1422.
[8] a) S. A. Gilmore, M. W. Schelle, C. M. Holsclaw, C. D. Leigh, M. Jain, J. S. Cox, J. A. Leary, C. R. Bertozzi, *ACS Chem. Biol.* **2012**, *7*, 863 – 870; b) B. L. Stocker, A. A. Khan, S. H. Chee, F. Kamena, M. S. M. Timmer, *ChemBioChem* **2014**, *15*, 382 – 388.
[9] a) H. Schoenen, B. Bodendorfer, K. Hitchens, S. Manzanero, K. Werninghaus, F. Nimmerjahn, E. M. Agger, S. Stenger, P. Andersen, J. Ruland, G. D. Brown, C. Wells, R. Lang, *J. Immunol.* **2010**, *184*, 2756 – 2760; b) E. M. Agger, I. Rosenkrands, J. Hansen, K. Brahimi, B. S. Vandahl, C. Aagaard, K. Werninghaus, C. Kirschning, R. Lang, D. Christensen, M. Theisen, F. Follmann, P. Andersen, *PLoS ONE* **2008**, *3*, e3116.
[10] V. A. Sarpe, S. S. Kulkarni, *Trends Carbohydr. Res.* **2013**, *5*, 8 – 33.
[11] M. A. Chaube, S. S. Kulkarni, *Trends Carbohydr. Res.* **2013**, *4*, 1 – 19.
[12] a) C. D. Leigh, C. R. Bertozzi, *J. Org. Chem.* **2008**, *73*, 1008 – 1017; b) A. Lemétais, Y. Bourdreux, P. Lesot, J. Farjon, J.-M. Beau, *J. Org. Chem.* **2013**, *78*, 7648 – 7657; c) B. Gau, A. Lemetais, M. Lepore, L. F. Garcia-Alles, Y. Bourdreux, L. Mori, M. Gilleron, G. De Libero, G. Puzo, J. M. Beau, J. Prandi, *ChemBioChem* **2013**, *14*, 2413 – 2417.
[13] J. Van der Borght, C. Chen, L. Hoflack, L. Van Renterghem, T. Desmet, W. Soetaert, *Appl. Environ. Microbiol.* **2011**, *77*, 6939 – 6944.
[14] a) H.-M. Kim, Y.-K. Chang, S.-I. Ryu, S.-G. Moon, S.-B. Lee, *J. Mol. Catal. B* **2007**, *49*, 98 – 103; b) S.-I. Ryu, J.-E. Kim, N. T. Huong, E.-J. Woo, S.-K. Moon, S.-B. Lee, *Enzyme Microb. Technol.* **2010**, *47*, 249 – 256.
[15] T. Kouril, M. Zaparty, J. Marrero, H. Brinkmann, B. Siebers, *Arch. Microbiol.* **2008**, *190*, 355 – 369.
[16] L. L. Kiessling, R. A. Splain, *Annu. Rev. Biochem.* **2010**, *79*, 619 – 653.
[17] E. M. Sletten, C. R. Bertozzi, *Angew. Chem. Int. Ed.* **2009**, *48*, 6974 – 6998; *Angew. Chem.* **2009**, *121*, 7108 – 7133.
[18] P. Som, H. L. Atkins, D. Bandoypadhyay, J. S. Fowler, R. R. MacGregor, K. Matsui, Z. H. Oster, D. F. Sacker, C. Y. Shiue, H. Turner, C.-N. Wan, A. P. Wolf, S. V. Zabinski, *J. Nucl. Med.* **1980**, *21*, 670 – 675.
[19] a) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2002**, *41*, 2596 – 2599; *Angew. Chem.* **2002**, *114*, 2708 – 2711; b) C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057 – 3064.
[20] R. Kalscheuer, B. Weinrick, U. Veeraraghavan, G. S. Besra, W. R. Jacobs, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 21761 – 21766.

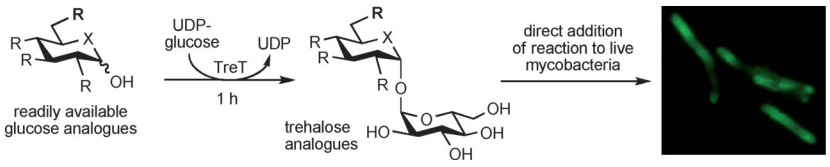
Received: June 5, 2014
Published online on ■■ ■■, 0000

COMMUNICATIONS

*B. L. Urbanek, D. C. Wing, K. S. Haislop, C. J. Hamel, R. Kalscheuer, P. J. Woodruff, B. M. Swarts**

■■ – ■■

Chemoenzymatic Synthesis of Trehalose Analogues: Rapid Access to Chemical Probes for Investigating Mycobacteria



Trehalose tools for TB: A one-step chemoenzymatic method for the rapid and efficient synthesis of trehalose analogues was developed. This method enabled facile preparation and administration of a trehalose-based probe for detecting mycobacteria, which might enable the development of new diagnostic tools for tuberculosis (TB) research.