Oligosaccharides

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Photodegradation of Target Oligosaccharides by Light-Activated Small Molecules**

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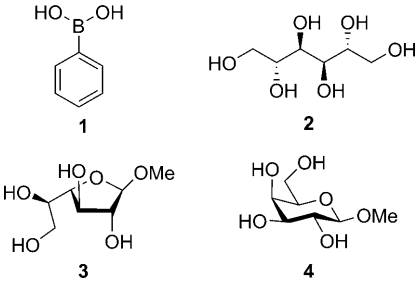
Carbohydrates in living cells play important roles in many biological events, including bacterial cell wall recognition, viral and bacterial infection, cell signaling, tumor cell metastasis and fertilization.[1] The development of innovative methods for selectively controlling specific functions of certain oligosaccharides has attracted much attention in the fields of chemistry, biology, and medicine. One major approach in the functional analysis of oligosaccharides is genetic knockout experiments of oligosaccharide-processing enzymes.[2] For example, in a 1994 study of the gene *Mgat-1*, which encodes *N*-acetylglucosaminyl transferase I (GlcNAc-TI), the absence of the gene in knockout mice was found to be embryonically lethal because of the loss of complex and hybrid N-glycan biosynthesis.[3,4] However, it is extremely difficult to knockout multiple genes simultaneously to remove a specific oligosaccharide. Because oligosaccharides share sugar components and sometimes exhibit similar functions, knocking out a gene that codes for an enzyme involved in many early synthetic processes may affect downstream pathways. Therefore, the possibility of developing a chemical agent that can selectively and directly degrade target oligosaccharides under mild conditions has attracted much attention. In general, however, it is difficult to achieve selective degradation of a target oligosaccharide, even with a chemical approach, because of the complexity of the oligosaccharide structures compared to those of DNA and proteins. Herein we report an innovative method for the degradation of target oligosaccharides induced by light-activated small molecules under mild conditions and without additives.

In our previous studies, certain anthraquinone (AQ) derivatives were found to be capable of degrading not only DNA[5] but also carbohydrates including β- and γ-cyclodextrins (CDs),[6] which show a high affinity with AQ derivatives, upon irradiation with long wavelength UV light and without further additives. In addition, it was found that the designed and synthesized AQ/lectin (peanut agglutinin; PNA) hybrid selectively degraded the target tumor-associated disaccharide Gal(β-1,3)GalNAc.[6]

On the basis of our preliminary and fundamental findings, we expected that a hybrid molecule consisting of an artificial receptor, which selectively binds to a target oligosaccharide, and a photodegrading AQ derivative could be used for selective photodegradation of target oligosaccharides. To investigate our hypothesis, we focused on the structure of D-galactofuranoside, which is a major sugar component of D-galactan in mycobacterial cell walls, as the target oligosaccharide. It has been reported previously that D-galactan is a central supporting structure of the cell wall of *Mycobacterium tuberculosis*,[7] and essential for cell growth and survival in the host. In addition, the main constituents (D-galactofuranose residues) are not found in mammalian metabolism. Therefore, D-galactans have been the subject of much attention as drug targets for new antituberculosis drugs without deleterious side effects.[8,9] Arylboronic acid was chosen as a recognition moiety. It is widely known that phenylboronic acid can bind to 1,2- or 1,3-diols through reversible boronate formation under physiological conditions.[10,11] Thus, the use of boronic acids is considered to be a promising approach for carbohydrate recognition.[12] Norrild and co-workers reported that D-glucose binds to boronic acids in water in its weakly populated furanose form but not in its pyranose form;[13] therefore, we envisaged that arylboronic acids would bind preferentially to an acyclic 1,2-diol on the exocyclic side chain of D-galactofuranoside, compared to 1,2- or 1,3-diols on the pyranosides, which are the majority of the sugar components in mammalian cells.

To confirm the difference in the affinity of phenylboronic acid (**1**) for methyl β-D-galactofuranoside (**3**) and methyl β-D-galactopyranoside (**4**; Scheme 1), we carried out a quantita-



Scheme 1. Chemical structures of phenylboronic acid (**1**) and several diols.

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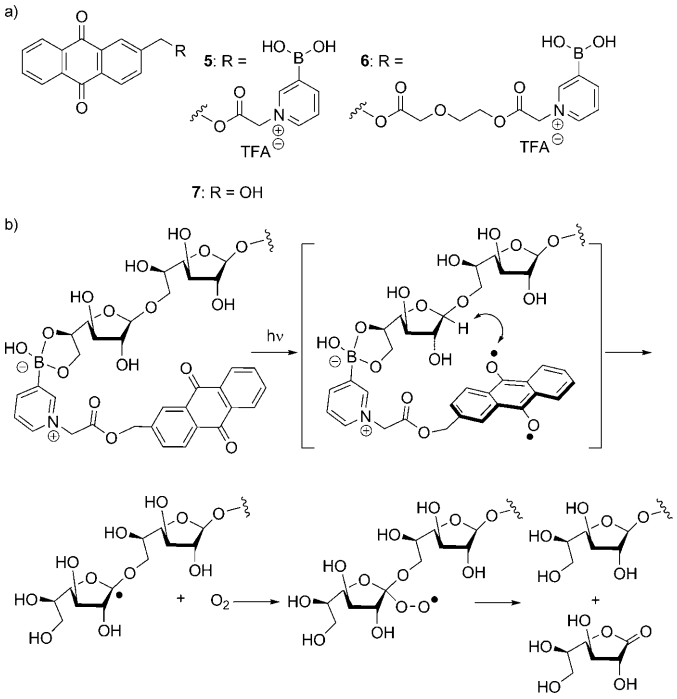
tive three-component alizarin red S (ARS) assay[14] with fluorescence measurements in neutral (buffered pH 7.4) water. As a result, the K_a value of **3** with respect to **1** (K_a = 158) was found to be 30-fold greater than that of **4** with **1** (K_a = 5), and, interestingly, also greater than that of D-mannitol (**2**) with **1** (K_a = 126).

On the basis of these favorable preliminary findings, we attempted to design a small molecule in which the AQ derivative was attached to an arylboronic acid that had a high affinity for D-galactofuranoside, thereby creating a hybrid consisting of both degradation and recognition sites for a target oligosaccharide. Our designed hybrid molecules **5** and **6** are shown in Scheme 2a. We chose a pyridinium boronic acid



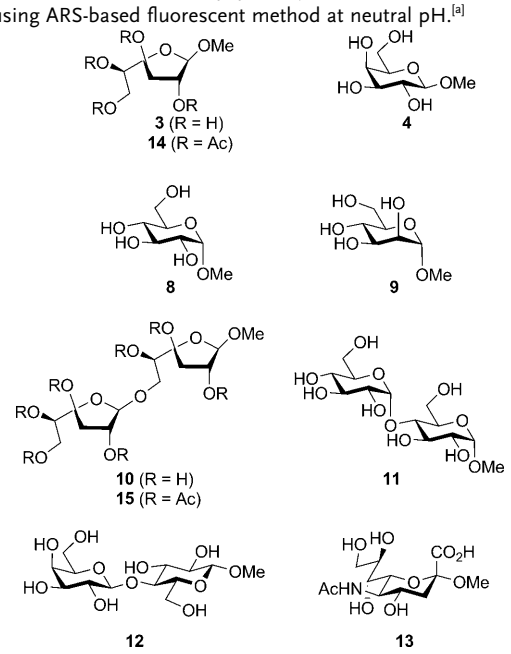
Scheme 2. One of the expected photodegradation pathways of D-galactofuranoside by a designed AQ/boronic acid hybrid (only oxidative cleavage of glycosidic bond is shown). a) Chemical structures of AQ/boronic acid hybrids **5** and **6**. b) One of the expected photodegradation pathways of D-galactofuranoside by a radical species produced by photoexcitation of the AQ moiety of hybrid **5** and O_2.

as a recognition moiety because a positive charge on the pyridine ring was expected to increase both the Lewis acidity of the boronic acid for tighter binding to the target oligosaccharide, and the water solubility of the hybrid molecule. In considering the length of the spacer moiety, we took into account the distance between the D-galactofuranoside ring and the AQ moiety after complexation of the hybrid with the 1,2-diol on the target oligosaccharide. On the basis of our previous work on the photodegradation of oligosaccharides[6] using light-activated AQ derivatives, and work reported by other groups on the oxidative scission of nucleotides[15] and oligosaccharides[16] by radical species, it seems certain that the abstraction of the anomeric and other protons from the glycoside is the one of the major driving forces. Thus, we expected that the photodegradation ability of the hybrid molecules would be influenced by the length of the spacer (Scheme 2b).

After chemical synthesis of our designed AQ/boronic acid hybrids **5** and **6** (see Scheme S1 in the Supporting Information), we carried out ARS assays of **5** with several glycosides, including methyl β-D-galactofuranoside (**3**), methyl β-D-galactopyranoside (**4**), methyl α-D-glucopyranoside (**8**), methyl α-D-mannopyranoside (**9**), methyl 6-*O*-(β-D-galactofuranosyl)β-D-galactofuranoside (**10**), methyl α-D-maltoside (**11**) methyl β-D-lactoside (**12**), and *N*-acetylneuraminic acid α-methylglycoside (Neu5Acα2Me) (**13**). One of the target glycosides, **10**, was synthesized as shown in Scheme S2 on the Supporting Information.

The results of the ARS binding assay are summarized in Table 1. It was found that **5** effectively bound to D-galactofuranosides **3** and **10** (entries 1 and 5); the K_a value obtained for **5** with **3** was slightly greater than that obtained for **1** with **3**. In sharp contrast the other glycosides, including Neu5Acα2Me (**13**), which possesses an extended glycerol side chain, showed low affinities with **5** (entries 2–4 and 6–8). These results clearly indicate that our designed molecule **5** binds

Table 1: Association constant (K_a) for hybrid molecule **5** with different diols using ARS-based fluorescent method at neutral pH.[a]



Entry	Diol	K_a [M^{-1}]
1	methyl β-D-galactofuranoside (**3**)	170
2	methyl β-D-galactopyranoside (**4**)	7
3	methyl β-D-glucopyranoside (**8**)	6
4	methyl α-D-mannopyranoside (**9**)	8
5	methyl 6-*O*-(β-D-galactofuranosyl)β-D-galactofuranoside (**10**)	174
6	methyl α-D-maltoside (**11**)	1
7	methyl β-D-lactoside (**12**)	1
8	*N*-acetylneuraminic acid α-methylglycoside (**13**)	5

[a] The K_a values were determined in 10% MeCN/0.1 M sodium phosphate buffer (pH 7.4) and are the average of at least two reproducible measurements.

favorably to acyclic 1,2-diols on the target glycosides rather than to 1,2- or 1,3-diols on other pyranosides and a glycerol side chain on sialoside.[17]

Next, we examined the photoinduced oligosaccharide-degrading activities of **5** and **6** (1.0 mM) against glycosides **3**, **4**, **8**, **9**, **10**, **11**, **12**, and **13** (1.0 mM each) in a 10% MeCN/0.1M phosphate buffer (pH 7.4) at 25°C for 10 minutes using a long-wavelength UV light (365 nm, 100 W) that was placed 10 cm from the sample. The progress of the photodegradation reaction was monitored by HPLC/UV (210 nm or 254 nm) analysis of the resulting photodegradation products after their acetylation (Ac_2O, pyridine; for **3**, **4**, and **8**–**12**) or *tert*-butyldiphenylsilylation (TBDPS) of a primary alcohol (TBDPSCl/imidazole in *N*,*N*-dimethylformamide; for **13**) and a subsequent short-pass silica gel column purification. The percentage degradation was calculated based on the peak area corresponding to each peracetylated glycoside or 9-*O*-TBDPS-Neu5Acα2Me, and the results are summarized in Figure 1. When 2-hydroxymethyl AQ (**7**) was used as a control, less than 10% degradation of the glycosides took place owing to its low affinity for glycosides. However, when **5** or **6** was exposed to glycoside **10** under photoirradiation, significant degradation took place, and the degradation activities of **5** and **6** were found to be almost the same. Glycoside **3** also underwent degradation by **5** and **6** under photoirradiation, although the efficiency was lower than that for **10**. These results clearly indicate that the selectivity of the hybrids, and thus their degradation ability, is highly dependent upon their affinity with the target glycosides. Moreover, it should be noted that the degradation of disaccharide **10** by **5** and **6** was much more effective than for the monosaccharide **3**. In addition, **5**, which possesses a short spacer, showed higher activity in the degradation of **3** than **6**, which suggests that the number of protons on the glycoside that can react with the photoactivated AQ has a significant influence upon the degradation ability of the hybrid molecule.

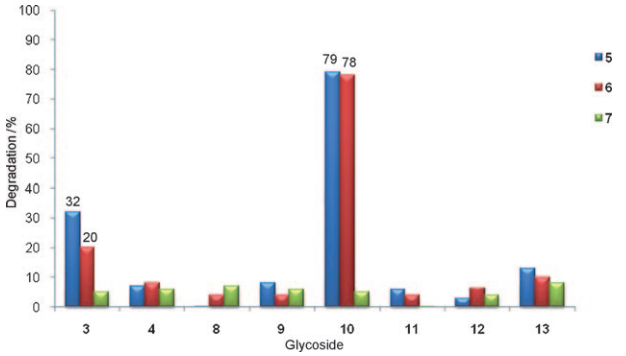


Figure 1. Photodegradation of glycosides by AQ/boronic acid hybrids. Each glycoside (1.0 mM) was incubated with AQ/boronic acid hybrid **5**, **6**, or 2-hydroxymethyl AQ (**7**) (1.0 mM) in 10% MeCN/0.1 M phosphate buffer (100 μL, pH 7.4) at 25°C for 10 min under irradiation with a UV lamp (365 nm, 100 W) placed 10 cm from the sample, and analyzed by HPLC methods (Mightysil RP-18 GP 5 mm, 4.6×150 mm; 40°C; detection by UV (210 nm or 254 nm) after total acetylation or silylation of a primary alcohol of the photodegradation products.

These degradation phenomena were confirmed by ESI/TOF MS analysis after the photoreaction and subsequent acetylation of the resulting products. The MS peak corresponding to the acetylated disaccharide **15** disappeared only after incubation of **10** with **5** under photoirradiation. In addition, the MS peak corresponding to the acetylated monosaccharide **14**, which resulted from the cleavage of **10** and subsequent acetylation, was detected as one of the major peaks (see Figure S1 in the Supporting Information). HPLC analysis also indicated the presence of **14**, and the chemical yield was calculated, based on the peak area, to be 13%.

Next, we conducted electron paramagnetic resonance (EPR) spin trapping experiments using 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) to confirm the generation of reactive oxygen species from the reaction of photoexcited AQ derivatives with O_2. It was found that photoirradiation of **5** in the presence of DMPO gave a product having an EPR spectrum that is characteristic of the DMPO hydroxyl radical spin adduct DMPO/·OH (Figure 2b), which results from the reaction of DMPO with ·OH or the decay of the DMPO superoxide anion spin adduct DMPO–·OOH (Figure 2c).[18,19] In addition, it was confirmed that no peaks corresponding to DMPO/·OOH or DMPO/·OH were detected either by treatment of DMPO with **5** without photoirradiation or by photoirradiation of DMPO in the absence of **5** (Figure 2a). These results indicate that oligosaccharide degradation (including oxidative cleavage of the glycosidic bond) arises

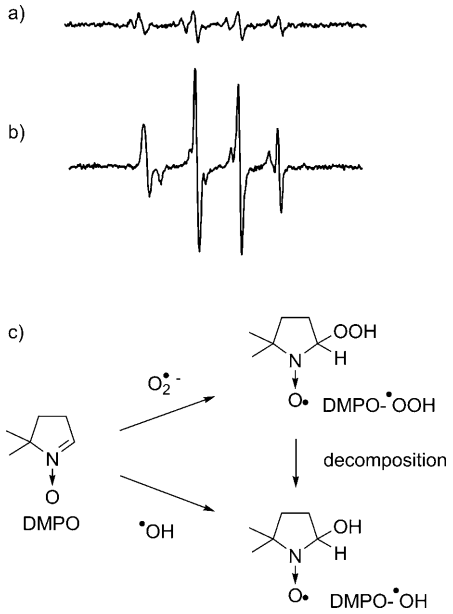


Figure 2. EPR spectrum obtained during photoirradiation of AQ/boronic acid hybrids in the presence of DMPO. AQ/boronic acid hybrid **5** (500 μM) and DMPO (500 mM) were incubated in 10%MeCN/0.1 M phosphate buffer (pH 7.4) containing 1.0 mM DETAPAC under irradiation with a UV lamp (365 nm, 100 W) placed 40 cm from a flat cell. a) Before irradiation. b) After 1 min irradiation. c) Possible pathways for the formation of DMPO/·OH. DETAPAC = diethylenetriaminepentaacetic acid.

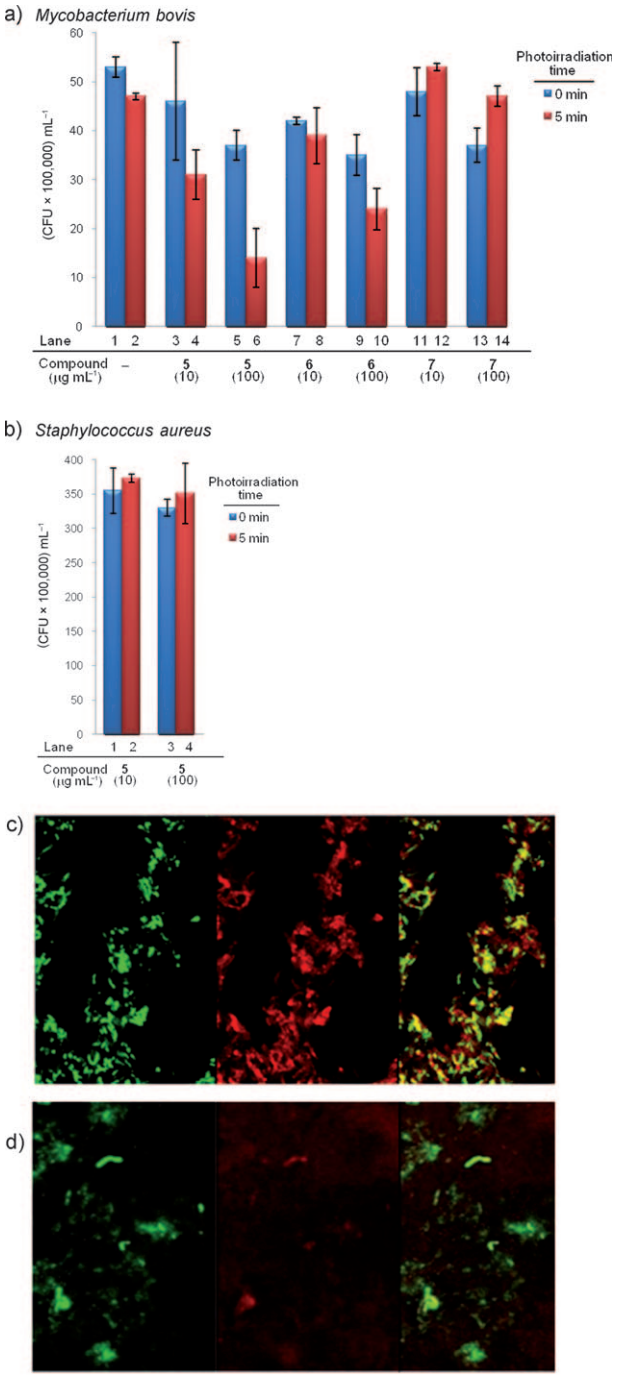


Figure 3. Bactericidal assay of AQ/boronic acid hybrids against BCG and *S. aureus*. a) Each compound (**5**–**7**, 10 and 100 μg mL^{-1}) was incubated with BCG under photoirradiation (368 nm, 30 W) for 0 and 5 min using a UV lamp placed 20 cm from the samples, and the bactericidal activity of each compound was determined by CFU after 21 days of incubation at 37 °C. b) Hybrid **5** (10 and 100 μg mL^{-1}) was incubated with *S. aureus* under photoirradiation (368 nm, 30 W) for 0 and 5 min using a UV lamp placed 20 cm from the samples. The bactericidal activity of **5** was determined by CFU after an 18 h incubation at 37 °C. Visualization of cell wall and DNA of BCG with different fluorescent dyes by confocal laser scanning microscopy after treatment with **5** without photoirradiation (c) or with photoirradiation (d). DNA is shown in green (left) and the cell wall is shown in red (middle); a composite image is also displayed (right).

from a radical species produced by the reaction of photoexcited AQ derivatives with O_2.

Finally, we examined the ability of our AQ/boronic acid hybrids to inhibit growth in *Mycobacterium bovis* BCG (ATCC 35734), which possesses the target oligosaccharides on the cell wall, in the presence and absence of photoirradiation (368 nm, 30 W, 5 min). Bacterial growth was determined based on the number of colony-forming units (CFU) after 21 days of incubation at 37 °C. The results are summarized in Figure 3 a. When photoirradiation was carried out in the absence of the AQ/boronic acid hybrid, the CFU numbers were almost the same as those for the control (Figure 3 a , lanes 1 and 2). When the cells were exposed to **5**, **6**, and **7** without photoirradiation, the CFU numbers decreased slightly in a dose-dependent manner as a result of the cytotoxicity of the compounds (Figure 3 a , lanes 3, 5, 7, 9, 11, and 13). However, when the bacterial cells were exposed to photoirradiation in the presence of **5** or **6**, the CFU numbers decreased significantly in line with the concentration of each compound (Figure 3 a , lanes 4, 6, 8, and 10), and in the presence of **7** the CFU numbers did not decrease in a concentration-dependent manner, even with photoirradiation (Figure 3 a , lanes 12 and 14).

In the case of *Staphylococcus aureus* (FDA209P), which does not possess the target oligosaccharides in its cell wall,[20] it was confirmed that **5**, at a concentration of both 10 and 100 μg mL^{-1}, did not show bactericidal activity even under photoirradiation (Figure 3 b, lanes 2 and 4). These results indicate that the AQ/boronic acid hybrids exhibited selective bactericidal activities against BCG.

To reveal whether **5** degraded the mycobacterial cell wall component with photoirradiation, the cell wall and DNA of BCG were detected by applying a combination of two fluorescent dyes (SynaptoRed for the cell wall and SYBR green for DNA) to samples incubated in the presence of **5** with or without photoirradiation, and subsequently analyzed using confocal laser scanning microscopy. The resulting images are shown in Figure 3 c and d. A comparison of these figures clearly shows that **5** caused effective degradation of the cell wall but not of the DNA under photoirradiation. Although we have not yet obtained direct evidence of photodegradation of the target oligosaccharides on the mycobacterial cell wall, these results suggest that selective photodegradation of the target oligosaccharides on the bacterial cell wall by **5** took place and induced significant whole-cell destruction.

In conclusion, we have developed a new and innovative method for selective degradation of a target oligosaccharide by photoirradiation using AQ/boronic acid hybrids under neutral conditions. The results presented herein will contribute to the molecular design of novel artificial oligosaccharide photodegradation agents. The development of oligosaccharide-photodegrading agents having high specificity and diversity is now under investigation in our laboratories. We hope this method will provide a means of controlling the specific functions of certain oligosaccharides.

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[1] *Comprehensive Glycoscience* (Eds: J. P. Kamerling, G.-J. Boons, Y. C. Lee, A. Suzuki, N. Taniguchi, A. G. J. Voragen), Elsevier, Oxford, **2007**.
[2] J. B. Lowe, J. D. Marth, *Annu. Rev. Biochem.* **2003**, *72*, 643 – 691.
[3] E. Ioffe, P. Stanley, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 728 – 732.
[4] M. Metzler, A. Gertz, M. Sarkar, H. Schachter, J. W. Schrader, J. D. Marth, *EMBO J.* **1994**, *13*, 2056 – 2065.
[5] K. Toshima, Y. Maeda, H. Ouchi, A. Asai, S. Matsumura, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2163 – 2165.
[6] M. Ishii, S. Matsumura, K. Toshima, *Angew. Chem.* **2007**, *119*, 8548 – 8551; *Angew. Chem. Int. Ed.* **2007**, *46*, 8396 – 8399.
[7] M. Daffe, P. J. Brennan, M. McNeil, *J. Biol. Chem.* **1990**, *265*, 6734 – 6743.
[8] K. Mikušová, T. Yagi, R. Stern, M. R. McNeil, G. S. Besra, D. C. Crick, P. J Brennan, *J. Biol. Chem.* **2000**, *275*, 33 890 – 33 897.
[9] M. S. Scherman, K. A. Winans, R. J. Stern, V. Jones, C. R. Bertozzi, M. R. McNeil, *Antimicrob. Agents Chemother.* **2003**, *47*, 378 – 382.
[10] J. P. Lorand, J. O. Edwards, *J. Org. Chem.* **1959**, *24*, 769 – 774.
[11] G. Springsteen, B. Wang, *Tetrahedron* **2002**, *58*, 5291 – 5300.
[12] T. D. James in *Boronic acids in Organic Synthesis and Chemical Biology* (Ed.: D. G. Hall), Wiley-VCH, Weinheim, **2005**, pp. 441 – 479.
[13] J. C. Norrild, H. Eggert, *J. Am. Chem. Soc.* **1995**, *117*, 1479 – 1484.
[14] G. Springsteen, B. Wang, *Chem. Commun.* **2001**, 1608 – 1609.
[15] W. K. Pogozelski, T. D. Tullius, *Chem. Rev.* **1998**, *98*, 1089 – 1107.
[16] B. C. Gilbert, D. M. King, B. Thomas, *Carbohydr. Res.* **1984**, *125*, 217 – 235.
[17] K. Djanashvili, L. Frullano, J. A. Peters, *Chem. Eur. J.* **2005**, *11*, 4010 – 4018.
[18] E. Wertz, B. Bolton in *Electron Spin Resonance*, McGraw-Hill, New York, **1972**.
[19] H. M. Swartz, J. R. Bolton, D. C. Borg in *Biological Application of Electron Spin Resonance*, Wiley-Interscience, New York, **1972**.
[20] W. W. Navarre, O. Schneewind, *Microbiol. Mol. Biol. Rev.* **1999**, *63*, 174 – 229.