BEILSTEIN JOURNAL OF ORGANIC CHEMISTRY

Synthesis of the pentasaccharide repeating unit of the *O*-antigen of *E. coli* O117:K98:H4

Pintu Kumar Mandal

Full Research Paper

Open Access

Address:
Medicinal and Process Chemistry Division, CSIR-Central Drug Research Institute, BS-10/1, Sector 10, Jankipuram extension, Sitapur Road, Lucknow, 226 031, India

Email:
Pintu Kumar Mandal - pintuchem06@gmail.com

Keywords:
Escherichia coli; glycosylation; lipopolysaccharide; *O*-antigen; pentasaccharide

Beilstein J. Org. Chem. **2014,** *10,* 2724–2728.
doi:10.3762/bjoc.10.287

Received: 19 August 2014
Accepted: 06 November 2014
Published: 20 November 2014

Associate Editor: S. Flitsch

Abstract

The pentasaccharide repeating unit of the *O*-antigen of *E. coli* O117:K98:H4 strain has been synthesized using a combination of sequential glycosylations and [3 + 2] block synthetic strategy from the suitably protected monosaccharide intermediates. Thioglycosides and glycosyl trichloroacetimidate derivatives have been used as glycosyl donors in the glycosylations.

Introduction

Escherichia coli becomes an important human pathogen in recent years owing to the emergence of new pathogenic strains [1]. Several diseases, such as meningitis and sepsis [2], diarrhoeal outbreaks [3] and urinary tract infections [4] are associated with pathogenic *Escherichia coli* (*E. coli*) strains. *E. coli* strains have been found to produce the Shiga toxin (Stx), heat-labile (LT) or heat-stable (ST) enterotoxins, cytotoxic necrotizing factors (CNF1 and CNF2) and hemolysins (α-Hly and E-Hly) [5,6] and are responsible for hemorrhagic colitis and haemolytic-uremic syndroms in humans [7]. The different strains of *E.coli* as well as bacteria belonging to different genera, e.g., *Shigella*, *Salmonella*, and *Klebsiella* show serological cross-reactions within the species [8]. The *E. coli* O117 strain emerged as a significant cause for septicaemia, bovine diarrhoea in new born children and human [9]. Together with other *E. coli* strains *E. coli* 0117 strains are responsible for pyelonephritis which is sexually transmitted by a woman that spread up to 60 to 80% of community acquired urinary tract or travelled through the bloodstream to the kidneys [10,11]. The *O*-specific polysaccharide of *E. coli* O117:K98:H4 is a linear pentasaccharide repeating unit consisting of D-galactosamine, D-glucose, D-galactose, and L-rhamnose (Figure 1) [12].

→ 3)-α-D-Gal*p*NAc-(1→ 4)-β-D-Gal*p*NAc-(1 →3)-α-L-Rha*p*-(1→ 4)-α-D-Glc*p*-(1→ 4)-β-D-Gal*p*-(1→

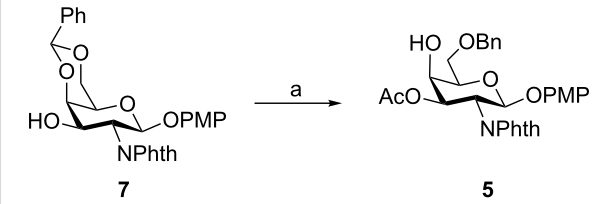
Figure 1: Structure of the pentasaccharide repeating unit of the *O*-specific polysaccharide of *E. coli* O117:K98:H4.

Vaccination is the recent thrust in the drug discovery program to prevent bacterial infections. Several bacterial *O*-antigens have been chosen for the development of glycoconjugate vaccine candidates against infectious diseases [13-16]. As a consequence, a significant quantity of oligosaccharides is required to evaluate their immunological properties for detailed understanding of the role of *O*-antigens in the pathogenicity of the *E. coli* strains. Development of chemical synthetic strategies would be useful to get large quantities of the oligosaccharides. As a part of the ongoing studies on the synthesis of bacterial cell wall oligosaccharides [17-19], a straightforward synthesis of the pentasaccharide repeating unit of the *O*-specific polysaccharide of *E. coli* O117:K98:H4 as its 3-aminopropyl glycoside is presented herein (Figure 2). The 3-aminopropyl group would be suitable for attachment of the pentasaccharide to any surface or carrier proteins.

Results and Discussion

The target pentasaccharide **1** has been synthesized as its 3-aminopropyl glycoside using a combination of sequential and [3 + 2] block glycosylation strategy. A trisaccharide acceptor **11** and a disaccharide trichloroacetimidate donor **14** were synthesized from the appropriately protected monosaccharide intermediates **2** [20], **3** [21], **4** [22], **5** and **6** [23] (Figure 2) derived from the commercially available aldoses. Trisaccharide acceptor **11** was then glycosylated with disaccharide trichloroacetimidate donor **14** to form pentasaccharide derivative **15**, which was finally deprotected to give target pentasaccharide **1** (see below Scheme 2). Some of the notable features of this synthetic strategy are (a) application of iodonium ion mediated general glycosylation conditions; (b) nitrosyl tetrafluoroborate ($NOBF_4$) mediated activation of glycosyl trichloroacetimidate donor; (c) the attachment of an aminopropyl linker at the anomeric center; (d) glycosylation and removal of the *p*-methoxybenzyl (PMB) group in one-pot.

Treatment of *p*-methoxyphenyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-galactopyranoside (**7**) [24] (prepared from D-galactosamine hydrochloride in six steps) with acetic anhydride in pyridine followed by regioselective reductive opening of the benzylidene acetal on treatment with sodium cyanoborohydride in the presence of HCl/Et_2O [25] furnished *p*-methoxyphenyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (**5**) in 77% yield over two steps (Scheme 1).



Scheme 1: Reagents and conditions: (a) (i) acetic anhydride, pyridine, room temperature, 2 h; (ii) $NaBH_3CN$, HCl/Et_2O, 5 °C, 2 h, 77% overall yield.

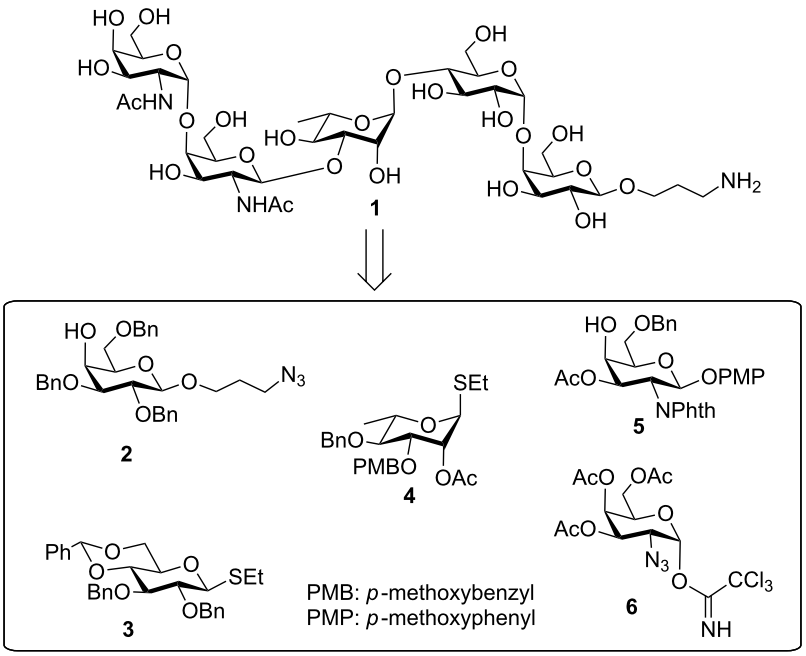
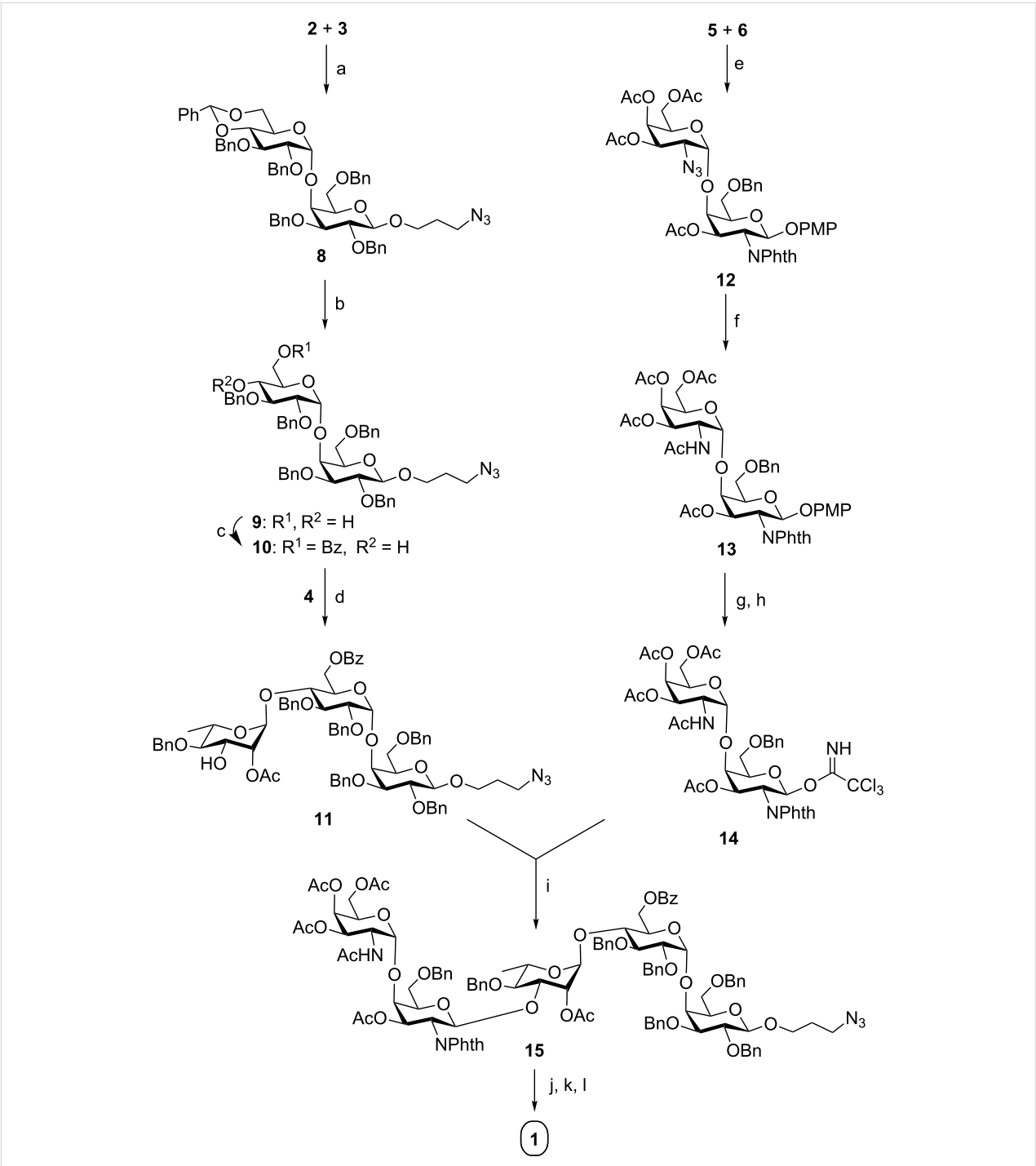


Figure 2: Structure of the synthesized pentasaccharide (**1**) and its precursor intermediates.

Trisaccharide acceptor **11** could be synthesized following the reaction pathway depicted in Scheme 2. Glycosylation of 3-azidopropyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**2**) with the thioglycoside donor **3** in the presence of *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) [26,27] gave disaccharide derivative **8** in 72% yield. NMR spectroscopy confirmed the formation of compound **8** [δ 5.04 (d, J = 3.6 Hz, 1H, H-1_B), 4.38 (d, J = 7.6 Hz, 1H,



Scheme 2: Reagents: (a) *N*-iodosuccinimide (NIS), TMSOTf, CH_2Cl_2, MS 4 Å, −30 °C, 1 h, 72%; (b) $HClO_4/SiO_2$, CH_3CN, rt, 20 min, 85%; (c) benzoyl cyanide, DCM/pyridine, rt, 2 h, 80%; (d) *N*-iodosuccinimide (NIS), TfOH, CH_2Cl_2, MS 4 Å, −30 °C, 1 h, then 0 °C, 1 h, 77%; (e) $NOBF_4$, Et_2O/CH_2Cl_2 (3:1), −15 °C, 1 h, 75%; (f) PPh_3, THF, 6 h, then Ac_2O, pyridine, rt, 1 h, 84%;(g) CAN, CH_3CN, H_2O, rt, 1.5 h; (h) CCl_3CN, DBU, CH_2Cl_2, −10 °C, 1 h; (i) $NOBF_4$, CH_2Cl_2, −15 °C, 1 h, 70%; (j) i) $NH_2NH_2{\cdot}H_2O$, CH_3CH_2OH, 80 °C, 8 h, ii) acetic anhydride, pyridine, rt, 1 h; (k) H_2, 20% $Pd(OH)_2/C$, CH_3OH, rt, 24 h; (l) 0.1 M CH_3ONa, CH_3OH, rt, 3 h, 58% overall yield.

H-1_A) in ^{1}H NMR and at δ 103.9 (C-1_A), 100.5 (C-1_B) in ^{13}C NMR spectra]. Following an earlier report [28], cleavage of the benzylidene acetal from compound **8** catalyzed by perchloric acid on silica ($HClO_4/SiO_2$) [28,29] afforded 3-azidopropyl (2,3-di-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**9**) in 85% yield. Selective 6-*O*-benzoylation of compound **9** was accomplished with benzoyl cyanide [30] to furnish disaccharide acceptor **10** in 80% yield. Compound **10** was reacted with 3-*O*-PMB protected L-rhamnosylthioglycoside donor **4** and NIS/TfOH [26,27] to yield the trisaccharide derivative by an iodonium ion catalyzed glycosylation. Participation of the 2-*O*-acetyl group in donor **4** ensured the α-selectivity of the glycosylation. Following an earlier report [19], raising the temperature of the reaction mixture after the glycosylation led to the removal of the PMB group in the same pot [31] to furnish trisaccharide acceptor **11** in 77% yield. The formation of compound **11** was supported by NMR spectral analysis [signals at δ 4.85 (d, J = 2.4 Hz, 1H, H-1_B), 4.84 (brs, 1H, H-1_C), 4.18 (d, J = 7.4 Hz, 1H, H-1_A) and at δ 104.1 (C-1_A), 98.7 (C-1_B), 97.6 (C-1_C) in the ^{1}H and ^{13}C NMR spectra respectively]. In another experiment, coupling of 2-azido-α-D-galactosyl trichloroacetimidate derivative **6** and compound **5** in the presence of $NOBF_4$ [32] in Et_2O/CH_2Cl_2 gave disaccharide derivative **12** in 75% yield. The formation of compound **12** was confirmed by NMR spectroscopic analysis [signals at δ 5.92 (d, J = 8.1 Hz, 1H, H-1_D), 5.06 (d, J = 2.7 Hz, 1H, H-1_E) in the ^{1}H NMR and δ 99.1 (C-1_E), 97.7 (C-1_D) in the ^{13}C NMR, respectively]. Reduction of the azido groups were carried out by treatment with triphenylphosphine [33], then the product was acetylated using acetic anhydride and pyridine to give disaccharide derivative **13** in 84% overall yield in two steps. The anomeric PMP group of compound **13** was oxidatively cleaved using ceric(IV) ammonium nitrate (CAN) [15] and the hemeacetal thus obtained was reacted with trichloroacetonitrile in the presence of DBU [34] to afford the desired disaccharide trichloroacetimidate derivative **14** in 77% yield. It was used directly without further purification [17] (Scheme 2). Finally, glycosylation of trisaccharide acceptor **11** with the trichloroacetimidate donor **14** in the presence of $NOBF_4$ [32] in CH_2Cl_2 furnished pentasaccharide derivative **15** in 70% yield. Formation of compound **15** was supported by NMR spectral analysis [signals at δ 5.10 (d, J = 3.3 Hz, 1H, H-1_E), 5.00 (d, J = 2.4 Hz, 1H, H-1_B), 4.92 (brs, 1H, H-1_C), 4.69 (d, J = 7.7 Hz, 1H, H-1_D),4.35 (d, J = 7.8 Hz, 1H, H-1_A) and at δ 104.0 (C-1_A), 101.2 (C-1_D), 98.9 (C-1_B), 98.3 (C-1_C), 97.4 (C-1_E) in the ^{1}H and ^{13}C NMR spectra, respectively]. The *N*-phthaloyl group was removed using hydrazine hydrate and the free amine thus formed was acetylated using acetic anhydride in pyridine [35]. Then the removal of the benzyl group was accomplished by hydrogenation using Pearlman's catalyst [36]. Finally the acetates were removed by Zemplén de-O-acetylation [37] using sodium methoxide to afford the target pentasaccharide **1** in 58% overall yield (Scheme 2).

Conclusion

In summary, a [3 + 2] block glycosylation strategy has been developed to synthesize a pentasaccharide 3-aminopropyl glycoside (**1**) corresponding to the *O*-antigen of *E. coli* O117:K98:H4 strain. The in situ removal of the PMB ether in one-pot following the glycosylation reaction reduced the overall number of steps.

Supporting Information

Supporting Information File 1

Experimental part.
[http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-10-287-S1.pdf]

Acknowledgements

The author gratefully acknowledges financial support by the Department of Science and Technology (DST), India under Fast Track Proposal Scheme for Young Scientists (CS-127/2012) and SAIF Division of CSIR-CDRI for providing the spectroscopic and analytical data. CDRI communication no. 8849.

References

1. Johnson, J. R.; Russo, T. A. *J. Lab.Clin. Med.* **2002,** *139,* 155–162. doi:10.1067/mlc.2002.121550
2. Robbins, J. B.; McCracken, G. H.; Gotschlich, E. C.; Ørskov, F.; Ørskov, I.; Hanson, L. A. *N. Engl. J. Med.* **1974,** *290,* 1216–1220. doi:10.1056/NEJM197405302902202
3. Johnson, J. R. *Clin. Microbiol. Rev.* **1991,** *4,* 80–128.
4. Besser, R. E.; Lett, S. M.; Weber, J. T.; Doyle, M. P.; Barrett, T. J.; Wells, J. G.; Griffin, P. M. *JAMA, J. Am. Med. Assoc.* **1993,** *269,* 2217–2220. doi:10.1001/jama.1993.03500170047032
5. Gay, C. C.; Besser, T. E. *Escherichia coli septicaemia in calves,* 1st ed.; CAB International: Wallingford, UK, 1994; p 75.
6. Salvadori, M. R.; Valadares, G. F.; da Silva Leite, D.; Blanco, J.; Yano, T. *Braz. J. Microbiol.* **2003,** *34,* 230–235. doi:10.1590/S1517-83822003000300009
7. Eklund, M. F.; Scheutz, F.; Siitonen, A. *J. Clin. Microbiol.* **2001,** *39,* 2829–2834. doi:10.1128/JCM.39.8.2829-2834.2001
8. Ørskov, F.; Ørskov, I. *Methods Microbiol.* **1984,** *14,* 43–112. doi:10.1016/S0580-9517(08)70447-1
9. Blanco, J.; González, E. A.; Blanco, M.; Garabal, J. I.; Alonso, M. P.; Fernández, S.; Villanueva, R.; Aguilera, A.; García, M. A.; Torres, J.; Rey, A.; Jansen, W. H.; Guinée, P. A. M. *J. Med. Microbiol.* **1991,** *35,* 162–167. doi:10.1099/00222615-35-3-162
10. Hebelka, M.; Lincoln, K.; Sandberg, T. *Scand. J. Infect. Dis.* **1993,** *25,* 141–143.
11. Chang, S. L.; Shortliffe, L. D. *Pediatr. Clin. North Am.* **2006,** *53,* 379–400. doi:10.1016/j.pcl.2006.02.011
12. Leslie, M. R.; Parolis, H.; Parolis, L. A. S. *Carbohydr. Res.* **1999,** *323,* 103–110. doi:10.1016/S0008-6215(99)00232-3

13. Verez-Bencomo, V.; Fernández-Santana, V.; Hardy, E.; Toledo, M. E.; Rodríguez, M. C.; Heynngnezz, L.; Rodriguez, A.; Baly, A.; Herrera, L.; Izquierdo, M.; Villar, A.; Valdés, Y.; Cosme, K.; Deler, M. L.; Montane, M.; Garcia, E.; Ramos, A.; Aguilar, A.; Medina, E.; Toraño, G.; Sosa, I.; Hernandez, I.; Martínez, R.; Muzachio, A.; Carmenates, A.; Costa, L.; Cardoso, F.; Campa, C.; Diaz, M.; Diaz, R. *Science* **2004,** *305,* 522–525. doi:10.1126/science.1095209
14. Roy, R. *Drug Discovery Today: Technol.* **2004,** *1,* 327–336. doi:10.1016/j.ddtec.2004.10.005
15. Werz, D. B.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2005,** *44,* 6315–6318. doi:10.1002/anie.200502615
16. Meeks, M. D.; Saksena, R.; Ma, X.; Wade, T. K.; Taylor, R. K.; Kovác, P.; Wade, W. F. *Infect. Immun.* **2004,** *72,* 4090–4101. doi:10.1128/IAI.72.7.4090-4101.2004
17. Mandal, P. K.; Misra, A. K. *Glycoconjugate J.* **2008,** *25,* 713–722. doi:10.1007/s10719-008-9120-1
18. Mandal, P. K.; Branson, T. R.; Hayes, E. D.; Ross, J. F.; Gavín, J. A.; Daranas, A. H.; Turnbull, W. B. *Angew. Chem., Int. Ed.* **2012,** *51,* 5143–5146. doi:10.1002/anie.201109068
19. Mandal, P. K.; Dhara, D.; Misra, A. K. *Beilstein J. Org. Chem.* **2014,** *10,* 293–299. doi:10.3762/bjoc.10.26
20. Joosten, J. A. F.; Loimaranta, V.; Appeldoorn, C. C. M.; Haataja, S.; El Maate, F. A.; Liskamp, R. M. J.; Finne, J.; Pieters, R. J. *J. Med. Chem.* **2004,** *47,* 6499–6508. doi:10.1021/jm049476+
21. Crich, D.; Bowers, A. A. *J. Org. Chem.* **2006,** *71,* 3452–3463. doi:10.1021/jo0526688
22. Mukherjee, C.; Misra, A. K. *Glycoconjugate J.* **2008,** *25,* 611–624. doi:10.1007/s10719-008-9107-y
23. Kalidasan, K.; Su, Y.; Wu, X.; Yao, S. Q.; Uttamchandani, M. *Chem. Commun.* **2013,** *49,* 7237–7239. doi:10.1039/c3cc42836b
24. Zhang, Z.; Magnusson, G. *Carbohydr. Res.* **1996,** *295,* 41–55. doi:10.1016/S0008-6215(96)90118-4
25. Garegg, P. J.; Hultberg, H.; Wallin, S. *Carbohydr. Res.* **1982,** *108,* 97–101. doi:10.1016/S0008-6215(00)81894-7
26. Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990,** *31,* 1331–1334. doi:10.1016/S0040-4039(00)88799-7
27. Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990,** *31,* 4313–4316. doi:10.1016/S0040-4039(00)97609-3
28. Agnihotri, G.; Misra, A. K. *Tetrahedron Lett.* **2006,** *47,* 3653–3658. doi:10.1016/j.tetlet.2006.03.133
29. Chakraborti, A. K.; Gulhane, R. *Chem. Commun.* **2003,** 1896–1897. doi:10.1039/b304178f
See for a preparation of $HClO_4$-SiO_2.
30. Abbas, S. A.; Haines, A. H. *Carbohydr. Res.* **1975,** *39,* 358–363. doi:10.1016/S0008-6215(00)86148-0
31. Bhattacharyya, S.; Magnusson, B. G.; Wellmar, U.; Nilsson, U. J. *J. Chem. Soc., Perkin Trans. 1* **2001,** 886–890. doi:10.1039/b009448j
32. Sau, A.; Santra, A.; Misra, A. K. *Synlett* **2012,** *23,* 2341–2348. doi:10.1055/s-0032-1317135
33. Zou, W.; Sandbhor, M.; Bhasin, M. *J. Org. Chem.* **2007,** *72,* 1226–1234. doi:10.1021/jo062057v
34. Schmidt, R. R.; Jung, K.-H. In *Preparative Carbohydrate Chemistry;* Hanessian, S., Ed.; Marcel Dekker: New York, NY, 1997; pp 283–312.
35. Kanie, O.; Crawley, S. C.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **1993,** *243,* 139–164. doi:10.1016/0008-6215(93)84087-M
36. Pearlman, W. M. *Tetrahedron Lett.* **1967,** *8,* 1663–1664. doi:10.1016/S0040-4039(00)70335-2
37. Zemplén, G. *Ber. Dtsch. Chem. Ges.* **1926,** *59,* 1254–1266. doi:10.1002/cber.19260590626

License and Terms