OBC
www.rsc.org/obc

"Click chemistry" *en route* to pseudo-starch

Laurence Marmuse,[a] Sergey A. Nepogodiev*[a] and Robert A. Field*[a,b]

[a] *Centre for Carbohydrate Chemistry, School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich, UK NR4 7TJ. E-mail: s.nepogodiev@uea.ac.uk*

[b] *Department of Biological Chemistry, John Innes Centre, Norwich, UK NR4 7UH. E-mail: r.a.field@uea.ac.uk*

Received 29th March 2005, Accepted 3rd May 2005
First published as an Advance Article on the web 11th May 2005

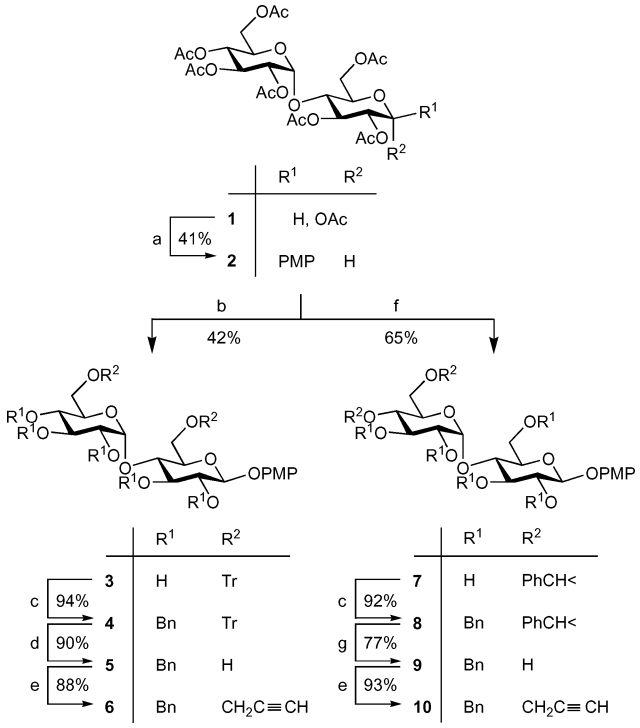
Rapid assembly of starch fragment analogues was achieved using "click chemistry". Specifically, two hexadecasaccharide mimics containing two parallel maltoheptaosyl chains linked *via* [1,2,3]-triazoles to a maltose core were synthesized using Cu(I)-catalyzed [3 + 2] dipolar cycloaddition of azido saccharides and 6,6′- and 4′,6′-dipropargylated *p*-methoxyphenyl maltoside.

Detailed knowledge of structural features is essential for understanding the biosynthesis of starch components and their assembly into the starch granule. Through this understanding, rational alteration of the pathway of starch biosynthesis using genetic manipulation promises to deliver a new generation of novel raw materials.[1] Starch is composed of two polysaccharides: an essentially linear α-(1→4)-D-glucan amylose and the highly branched macromolecule amylopectin, consisting of relatively short (1→4)-D-glucan chains attached through α-(1→6)-linkages to an α-(1→4)-D-glucan backbone.[2] It is believed that local organization of these branched chains of amylopectin is responsible for the ordered semi-crystalline structure of the starch granule in which crystalline regions alternate with amorphous zones. The short branches of amylopectin form double helices that are stacked in the crystalline regions, whereas amorphous zones are occupied by amylopectin fragments incorporating (1→6)-branch points. To gain an insight into the role of such branch points in the propagation of double helices, computer modelling[3,4] and NMR spectroscopic studies[4] have been undertaken on amylopectin fragments. However, obtaining experimental information about these fragments is hampered by the fact that they represent only a small fragment of the total polysaccharide material. Therefore synthetic, well-defined fragments of amylopectin incorporating α-(1→6)-branch points would be useful tools for physicochemical and biochemical studies.

Synthesis of oligosaccharides related to starch have been reported in the literature,[5] but poor stereoselectivity in the 1,2-*cis*-glucosylation reaction is known[6] to be a serious obstacle in the assembly of the large branched fragments. To enforce interactions of two parallel oligosaccharide chains, they need to be attached to a template, as in the case of a cellulose II mimic developed by Vasella and co-workers.[7] Application of the template concept to the construction of amylopectin fragment analogues requires development of a simple and efficient strategy for conjugation of long-chain maltooligosaccharides to a template. One of the reactions that can satisfy these requirements is Huisgen's 1,3-dipolar cycloaddition[8] of azides and terminal acetylenes, yielding triazoles. The potential of this reaction has been recently enhanced by the discovery[9] that Cu(I) catalyzes formation of a single regioisomer of substituted 1,2,3-triazoles, making this reaction one of the most powerful "click chemistry"[10] transformations. In carbohydrate chemistry this methodology has been successfully applied for the synthesis of multivalent saccharides[11] and cyclodextrin analogues.[12]

Continuing our efforts on generating synthetic amylopectin fragments[6] we describe herein an approach to the construction of amylopectin analogues composed of two linear maltoheptaose chains attached to a maltose template through heterocyclic bridges. Two types of molecules, with attachment points at the 4′,6′ and 6,6′ positions of a maltose template, were selected as targets. The strategy for the introduction of a matching pair of reactive groups, suitable for 1,3-dipolar cycloaddition, requires the simple and efficient introduction of azide and alkyne groups into suitable building blocks. The branching template chosen was a dipropargylated maltose derivative, whereas linear chains containing an azido group in the reducing terminal anomeric positions comprised the cycloaddition partner.

Isomeric di-*O*-propargyl derivatives **6** and **10** were synthesized starting from readily available[13] maltose peracetate **1** (Scheme 1). Glycosidation of **1** with *p*-methoxyphenol in the presence of $BF_3{\cdot}OEt_2$ gave an α,β mixture of aryl glycosides from which pure β-anomer **2** was isolated by crystallisation in 41% yield. Deacetylation of **2** followed by selective protection of primary OH groups *via* alkylation with TrCl in pyridine afforded 6,6′-di-*O*-trityl derivative **3** in 42% overall yield. For the synthesis of 4′,6′ di-*O*-propargyl maltoside **10**, glycoside **2** was deacetylated and selectively benzylidenated to produce acetal **7** in 65% overall yield. After benzylation of the remaining hydroxy groups in **3** and **7**, acid-labile temporary triphenylmethyl and benzylidene groups in **4** and **8** were removed to give diols **5** and **9** in 90% and 77% yield, respectively. Reactions of dialkoxides prepared *in situ* from diols **5** and **9** with propargyl bromide led to target

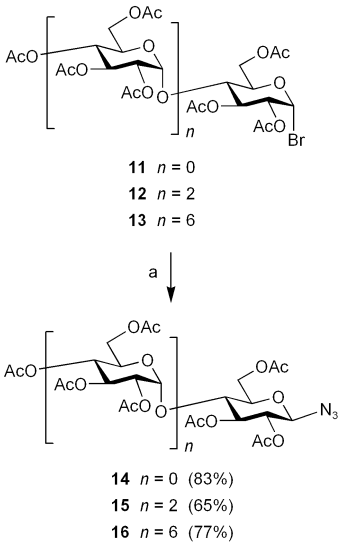


Scheme 1 Reagents and conditions: (a) *p*-methoxyphenol, CH_2Cl_2, $BF_3{\cdot}OEt_2$; (b) 1. MeOH, NaOMe 2. TrCl, pyridine; (c) BnBr, NaH; (d) TsOH, MeOH–CH_2Cl_2; (e) $CH{\equiv}CCH_2Br$, NaH, THF–DMPU; (f) 1. NaOMe, MeOH, 2. $PhCH(OMe)_2$, TsOH, DMF; (g) 90% AcOH.

DOI: 10.1039/b504293c

di-*O*-propargyl maltosides **6** and **10** in 88% and 93% yield, respectively.

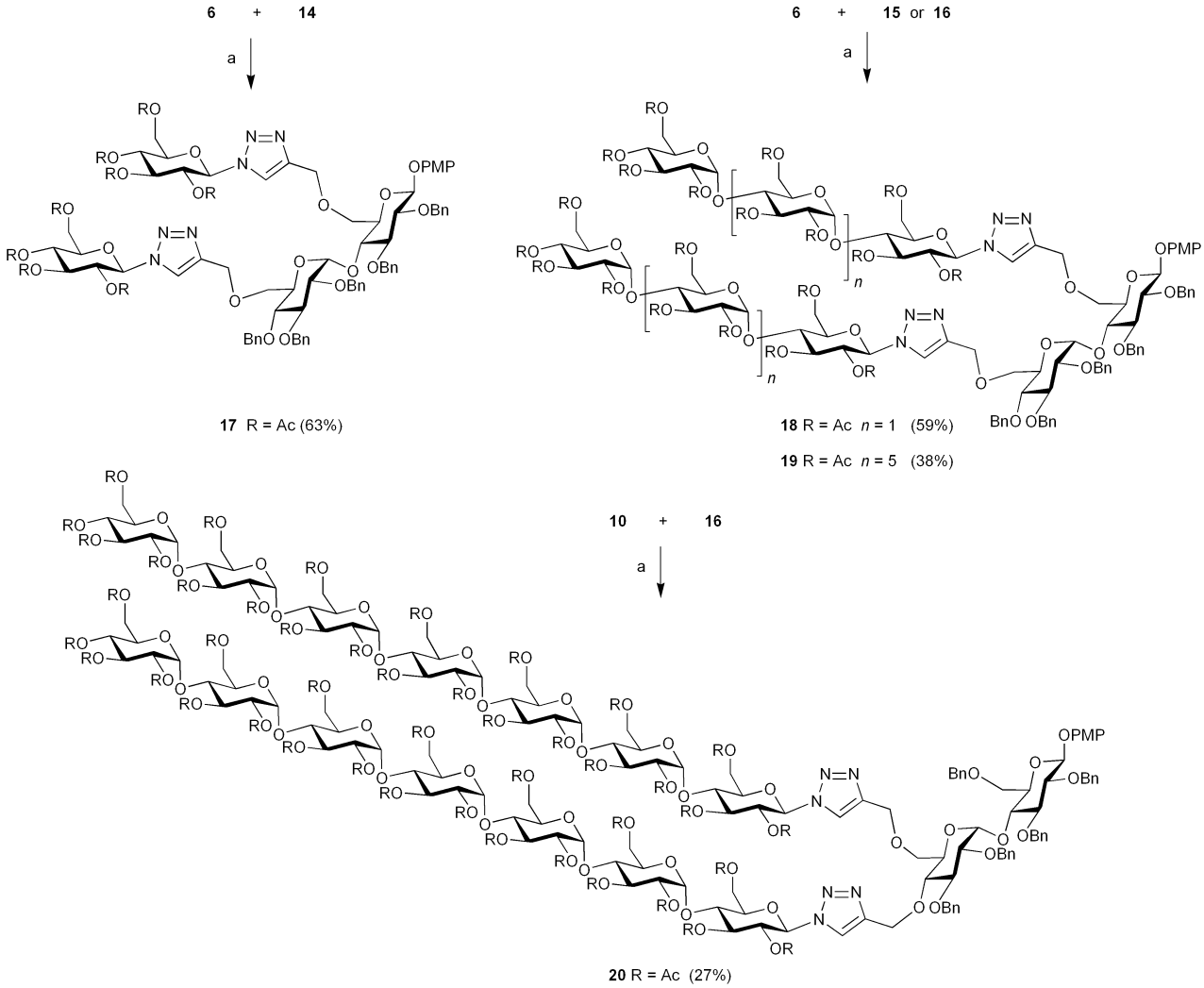
A series of peracetylated β-glycosyl azides was synthesized by reaction[14] of Me_3SiN_3 with glucosyl bromide **11**, maltotriosyl bromide **12**,[15] and maltoheptaosyl bromide **13**[15] in the presence of Bu_4NF. Thus glycosyl azides **14**,[14] **15**, and **16** were prepared in 65–83% yield (Scheme 2).



Scheme 2 Reagents and conditions: Me_3SiN_3, TBAF, THF.

The results of 1,3-dipolar cycloaddition of dipropargylated maltosides and azidoglucosides are shown in Scheme 3. All reactions were carried out using $(Ph_3P)_3$·CuBr as a catalyst in the presence of DIPEA as a base as described previously,[11a] except that, instead of microwave irradiation, a longer reaction time (12 h) at room temperature was applied. The yields of cycloaddition reactions varied between 65 and 27%, decreasing when increasing length of the azidooligosaccharide chain.

The structure of cycloaddition products was confirmed by NMR spectroscopy and mass spectrometry. Both ^1H and ^{13}C NMR spectra of **17**–**20** revealed very close but distinguishable resonances (δ_H *ca.* 5.7–5.9 and δ_C *ca.* 85–86) corresponding to the anomeric center of the glucopyranose residues attached to N-1 of the triazole unit. From the rest of the considerably overlapping resonances of acetylated glucoparanose residues, only clusters corresponding to signals of α-anomeric (δ_C *ca.* 95–96) and C-6 (δ_C *ca.* 61.5–62) carbon atoms were reliably assignable. Characteristic signals of anomeric carbon atoms ($\delta_{C\text{-}1}$ *ca.* 102 and $\delta_{C\text{-}1'}$ *ca.* 97), as well as resonances corresponding to *p*-methoxyphenyl group (δ_{OMe} *ca.* 55.5 and aromatics δ *ca.* 115 and δ_C *ca.* 118) were observed in the ^{13}C NMR spectra of compounds **17**–**20**. The [1,2,3]-triazole unit was evident from ^1H NMR spectra by the presence of two separate resonances (δ_H *ca.* 7.7). Therefore, NMR data clearly indicated formation of a single isomer in each case, which for the copper(I)-catalyzed cycloaddition reaction is known to be the 1,4-substituted [1,2,3]-triazole.[9b] Regioselectivity of cycloaddition in the synthesis of 6,6′-di-substituted derivatives **17**–**19** also followed from the observation of only one pair of



Scheme 3 Synthesis of templated bis-glucopyranoside (**17**), bis-maltotrioside (**18**), and bis-maltoheptaosides (**19** and **20**). Reagents and conditions: (a) $(Ph_3P)_3CuBr$, DIPEA, toluene, 12 h, room temperature.

doublets of aromatic protons (δ_H *ca.* 6.9 and δ_H *ca.* 7.0) belonging to the anomeric *p*-methoxyphenyl group in the ^{1}H NMR spectra. We noted previously[6] that the chemical shifts of these signals are highly sensitive to the stereochemistry of a substituent at the 6 position of a *p*-methoxyphenyl β-maltoside unit. All triazole-bridged products were analyzed by MALDI-TOF MS, giving the expected sodium adducts of molecular ions: 1744.7 (**17**), 2897.1 (**18**), 5202.7 (**19**), and 5207.6 (**20**).

In summary, we have described the first application of "click chemistry" based on cycloaddition of substituted azide and alkynes to the synthesis of well-defined branched oligosaccharide mimics. Starting from dipropargylated maltoside and azido maltooligosaccharides this modular approach allowed the construction of a number of [1,2,3]-triazole-based analogues of amylopectin fragments in one simple coupling step. These analogues include two isomeric hexadecasaccharide analogues which have potential for templating formation of double helixes between two parallel maltoheptaosyl chains attached to a core maltose unit. Studies to investigate such assembly processes are ongoing.

Acknowledgements

We thank the EPSRC and the Weston Foundation for financial support. The EPSRC Mass Spectrometry Service Centre, Swansea are acknowledged for invaluable support.

References

1 (*a*) A. M. Smith, *Curr. Opin. Plant Biol.*, 1999, **2**, 223–229; (*b*) C. J. Slattery, I. H. Kavakli and T. W. Okita, *Trends Plant Sci.*, 2000, **5**, 291–298; (*c*) A. G. Heyer, J. R. Lloyd and J. Kossmann, *Curr. Opin. Biotechnol.*, 1999, **10**, 169–174.

2 (*a*) D. J. Gallant, B. Bouchet and P. M. Baldwin, *Carbohydr. Polym.*, 1997, **32**, 177–191; (*b*) R. F. Tester, J. Karkalas, in *Biopolymers: Polysaccharides II, vol. 6*, Wiley-VCH, Weinheim, 2002, pp. 383–438; (*c*) A. Buleon, P. Colonna, V. Planchot and S. Ball, *Int. J. Biol. Macromol.*, 1998, **23**, 85–112; (*d*) A. Imberty, A. Buleon, V. Tran and S. Pérez, *Starch/Staerke*, 1991, **43**, 375–384.

3 (*a*) A. Imberty and S. Pérez, *Int. J. Biol. Macromol.*, 1989, **11**, 177–185; (*b*) A. Buleon and V. Tran, *Int. J. Biol. Macromol.*, 1990, **12**, 345–352; (*c*) A. Neszmelyi and J. Hollo, *Starch/Staerke*, 1989, **41**, 1–3.

4 F. Corzana, M. S. Motawia, C. Hervé du Penhoat, F. van den Berg, A. Blennow, S. Pérez and S. B. Engelsen, *J. Am. Chem. Soc.*, 2004, **126**, 13144–13155.

5 (*a*) M. S. Motawia, C. E. Olsen, K. Enevoldsen, J. Marcussen and B. L. Møller, *Carbohydr. Res.*, 1995, **277**, 109–123; (*b*) I. Damager, C. E. Olsen, B. L. Møller and M. S. Motawia, *Carbohydr. Res.*, 1999, **320**, 19–30; (*c*) M. S. Motawia, K. Larsen, C. E. Olsen and B. L. Møller, *Synthesis*, 2000, **11**, 1547–1556; (*d*) I. Damager, C. E. Olsen, B. L. Møller and M. S. Motawia, *Synthesis*, 2002, 418–426; (*e*) I. Damager, C. E. Olsen, A. Blennow, K. Denyer, B. L. Møller and M. S. Motawia, *Carbohydr. Res.*, 2003, **338**, 189–197.

6 L. Marmuse, S. A. Nepogodiev and R. A. Field, *Tetrahedron: Asymmetry*, 2005, **16**, 477–485.

7 B. Bernet, J. W. Xu and A. Vasella, *Helv. Chim. Acta*, 2000, **83**, 2072–2114.

8 R. Huisgen, in *1,3-Dipolar Cycloaddition Chemistry, vol. 1*, ed. A. Padwa, Wiley, 1984, pp. 1–176.

9 (*a*) V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596–2599; (*b*) C. W. Tørnoe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064; (*c*) V. O. Rodionov, V. V. Fokin and M. G. Finn, *Angew. Chem., Int. Ed.*, 2005, **44**, 2210–2215.

10 C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004–2021.

11 (*a*) F. Perez-Balderas, M. Ortega-Munoz, J. Morales-Sanfrutos, F. Hernández-Mateo, F. G. Calvo-Flores, J. A. Calvo-Asin, J. Isac-García and F. Santoyo-González, *Org. Lett.*, 2003, **5**, 1951–1954; (*b*) S. Chittaboina, F. Xie and Q. Wang, *Tetrahedron Lett.*, 2005, **46**, 2331–2336.

12 (*a*) K. D. Bodine, Y. Gin and M. S. Gin, *J. Am. Chem. Soc.*, 2004, **126**, 1638–1639; (*b*) B. Hoffmann, B. Bernet and A. Vasella, *Helv. Chim. Acta*, 2002, **85**, 265–287.

13 K. P. R. Kartha, M. Aloui and R. A. Field, *Tetrahedron Lett.*, 1996, **37**, 8807–8810.

14 E. D. Soli, A. S. Manoso, M. C. Patterson, P. DeShong, D. A. Favor, R. Hirschmann and A. B. Smith, III, *J. Org. Chem.*, 1999, **64**, 3171–3177.

15 E. Farkas, L. Jánossy, J. Harangi, L. Kandra and A. Lipták, *Carbohydr. Res.*, 1997, **303**, 407–415.