Hyperbranched Polyester Supported Disaccharide Synthesis: The Effect of Loading Level on Glycosylation Efficiency

Eric Assen B. Kantchev, Jon R. Parquette**

The Ohio State University, Department of Chemistry, Columbus, OH 43210, USA
E-mail: parquett@chemistry.ohio-state.edu

Received 26 April 2005

Abstract: The efficiency of glycosylation of glycosyl acceptors supported on a hyperbranched polyester (Boltorn H-50) by an activated trichloroacetamidate donor was investigated as a function of the polymer loading. Loading of the hyperbranched polyester with an *o*-nitrobenzyl photolabile linker at 25% loading capacity (corresponding to 1.36 mmol/g) and using five equivalents of the glycosyl donor was optimal for quantitative transformation (>98%) at both the mono- and disaccharide stage. The disaccharide product obtained under these optimized conditions was isolated in 16% yield over five steps.

Key words: carbohydrates, glycosylations, polymers, dendrimers, solid-phase

Cross-linked polystyrene resins, first developed in the context of solid-phase peptide synthesis,[1] have gained increased popularity for synthesis of other classes of organic compounds.[2] The facile removal of excess reagents and soluble by-products from the polymer-attached product(s) by simple filtration constitutes the main advantage of the resin supports, enabling high-throughput and automated synthetic protocols. Limitations of these resins include solvent-dependent swelling behavior, nonlinear reaction kinetic profiles that hamper the adaptation of synthetic protocols and analytical techniques to heterogeneous conditions. Linear soluble polymers such as poly(ethyleneglycol) (PEG) have been employed as alternatives to the resin supports to circumvent some of these limitations.[3] Soluble supports allow reactions to be conducted in solution, which provides a kinetic advantage and facilitates the characterization of polymer-bound intermediates by solution-phase spectroscopy. Precipitation, size-exclusion chromatography (SEC) or membrane filtration separates the supported intermediates from soluble reagents. However, the intrinsically low levels attainable with many linear soluble polymers, such as in PEG, limit this strategy to small-scale applications. To address this limitation, dendrimers,[4] hyperbranched polymers[5] and dendronized solid supports have been investigated as high-loading supports for organic synthesis.[6] Dendrimers and hyperbranched polymers possess an $(x - 1)n + 1$ (n = degree of polymerization) number of terminal groups for an AB_x-type repeat unit.[7] Furthermore, the putatively globular structure of most dendrimers and hyperbranched polymers suggests enhanced reagent accessibility of the terminal groups. Accordingly, branched materials provide a potential for increased loading levels compared with supports based on strictly linear systems. The high synthetic investment associated with preparation of dendrimers renders their utilization as supports prohibitively expensive. To provide a low-cost alternative to dendrimers, we reported the first example of the use of a soluble, hyperbranched polyester [Boltorn H40 and H50 (**1**),[8] Figure 1] to support the synthesis of oligosaccharides.[9] In addition, cluster glycoside synthesis based on Boltorn H20 and H30 polymer cores was recently reported.[10] The focus of this work is to determine the optimal/maximal loading level of this hyperbranched support for the synthesis of oligosaccharides via the trichloroacetamidate glycosyl coupling protocol.

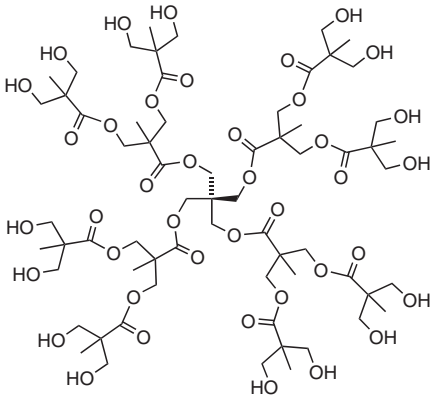
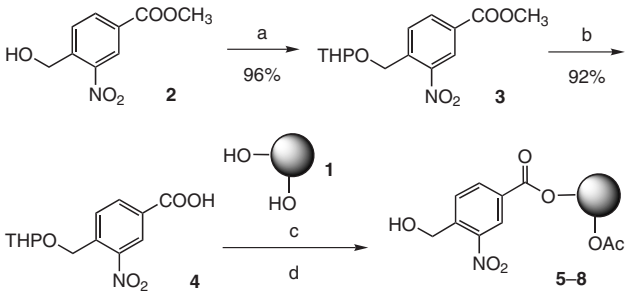


Figure 1 Idealized depiction of Boltorn hyperbranched polymers.

The efficiency of the trichloroacetamidate glycosylation protocol[11] was explored as a function of the loading of the hyperbranched support and the stoichiometry of glycosyl donor. Accordingly, four polymer samples (**5**–**8**) were functionalized with a photolabile linker at loading levels ranging from 25% to 100% (Scheme 1). The photolabile linkage moiety, **4**, was prepared by THP-protection of methyl-4-hydroxymethyl-3-nitrobenzoate[12] (**2**) and hydrolysis with KOH. The THP-protected carboxylic acid **4** was then loaded (EDCI, cat. DMAP) onto the Boltorn H-50 polymer (**1**) in 25%, 50%, 75% and 100% of its theoretical loading capacity (8.8 mmol/g), followed by peracetylation of the remaining hydroxyl groups with acetyl chloride. The THP protecting group was removed with cat. HCl in methanol–dichloromethane. Polymers **5**–**7** (25–75% loading) were purified by precipitation into

SYNLETT 2005, No. 10, pp 1567–1570
Advanced online publication: 07.06.2005
DOI: 10.1055/s-2005-869863; Art ID: S04405ST

methanol; however, the fully loaded polymer, **8**, exhibited solubility in methanol and, therefore, was precipitated into CH_2Cl_2. The linker-polymer conjugates loaded at 25% (**5**) and 50% (**6**) showed good agreement between calculated and observed loading values (Table 1), suggesting complete incorporation of the linker. However, the polymers loaded at 75% (**7**) and 100% (**8**) afforded loading levels considerably less than the theoretical value. The lower efficiency of linker incorporation at higher loading levels may be a consequence of differing steric accessibilities that exist among the terminal hydroxyl groups.



Scheme 1 Reagents and conditions: (a) DHP, cat. TsOH, CH_2Cl_2; (b) KOH, H_2O–THF, then dil. H_2SO_4; (c) EDCI, cat. DMAP, THF–pyridine, then excess CH_3COCl; (d) cat. HCl, MeOH–CH_2Cl_2.

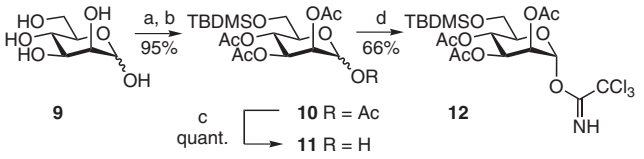
Table 1 Loaded Linker-Polymer Conjugates

Compound	Capacity (%)	Yield (%)[a]	Loading (mmol/g)[b]
5	25	68	1.36 (1.32)
6	50	38	1.55 (1.49)
7	75	49	2.20 (2.91)
8	100	25	2.34 (3.41)

[a] Isolated yields of loaded polymer obtained by precipitation into MeOH (**5**–**7**) or CH_2Cl_2 (**8**).
[b] Determined by ^{1}H NMR using DMF as an internal standard. The theoretical loading was calculated at the loading capacity indicated based on 8.8 mmol/g OH groups in the Boltorn H50 polymer assuming 100% coupling and acetyl capping efficiency.

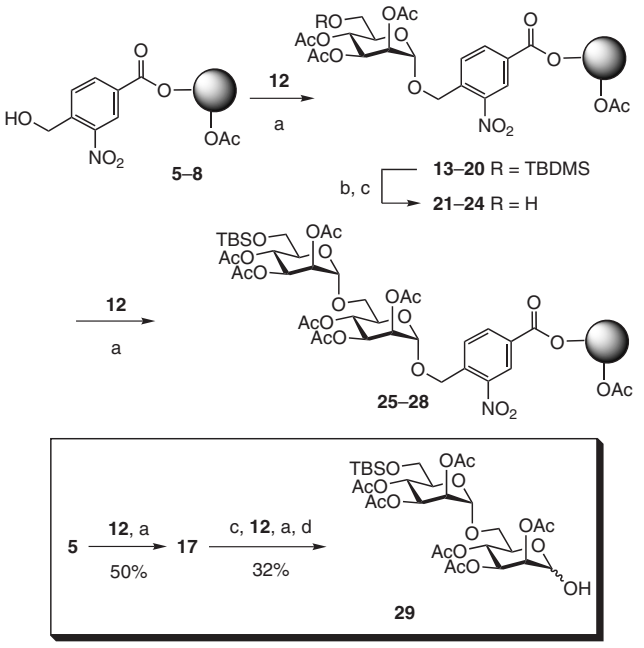
The polymer-supported acceptors **5**–**8** were each glycosylated with trichloroacetamidate **12** to determine the effect of loading level on coupling efficiency. Accordingly, donor **12** was prepared by selective silylation of D-mannose at C-6 followed by acetylation of the remaining hydroxyl groups (Scheme 2). Subsequent cleavage of the anomeric acetyl group with hydrazine monoacetate and reaction with trichloroacetonitrile provided trichloroacetamidate **12** in 66% yield.

Exposure of polymers **5**–**8** to trichloroacetamidate **12** in the presence of $BF_3{\cdot}OEt_2$ in THF at room temperature resulted in complete consumption of **12** within 15 minutes (Scheme 3). Following precipitation of the polymer into methanol, the glycosylation efficiencies were determined by ^{1}H NMR on the support using the normalized ratio of the *o*-nitrobenzyl linker protons at δ = 7.6–8.7 ppm to the



Scheme 2 *Reagents and conditions*: (a) TBDMSCl, cat. DMAP, pyridine; b) $(CH_3COO)_2O$; (c) $NH_2NH_2{\cdot}AcOH$, DMF–THF (1:2); d) CCl_3CN, cat. NaH, CH_2Cl_2.

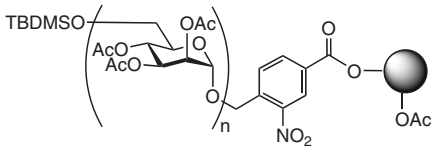
6-TBDMS methyl protons at δ = 0.0–0.2 ppm in $CDCl_3$ (Table 2). The monoglycosylated products **17**–**20** were then capped with acetic anhydride in pyridine to block all unreacted hydroxyl groups. Treatment with HF·pyridine in THF cleaved the 6-TBDMS group affording acceptors **21**–**24** after partitioning between water and ethyl acetate. Subsequent glycosylation of **21**–**24** with donor **12** afforded polymer-bound disaccharides **25**–**28** after precipitation into methanol.



Scheme 3 *Reagents and conditions*: (a) cat. $BF_3{\cdot}OEt_2$, THF; (b) $(CH_3COO)_2O$ (capping); (c) HF·pyridine, THF; (d) hν (350 nm), THF.

Table 2 presents the efficiency for each glycosylation step as a function of the loading capacity of the support and the stoichiometry of the glycosyl donor. Several points are noteworthy: (1) At a 25% loading level (n = 1), whereas the maximal efficiency occurs using five equivalents of donor, the greatest increase in efficiency takes place going from one (43%) to two (81%) equivalents of donor (entries 1 and 2); (2) Increasing the loading capacity from 25% to 100% afforded a corresponding linear decrease in efficiency from 98% to 33% (entries 5–8); (3) The second glycosylation step (n = 2) experienced the most significant decrease in efficiency upon increasing from 25% to 50%, but exhibited similar efficiencies at 75% and 100% loading levels (entries 11 and 12). The decrease in

Table 2 Glycosylation Efficiency as a Function of Loading Level[a]



glycosylation product (**13**–**20**, **25**–**28**)

Entry	Acceptor	n	Loading (mmol/g)	**12** (Equiv)	Efficiency[b]	Product
1	**5**	1	25% (1.36)	1	0.43	**13**
2	**5**	1	25% (1.36)	2	0.81	**14**
3	**5**	1	25% (1.36)	3	0.88	**15**
4	**5**	1	25% (1.36)	4	0.92	**16**
5	**5**	1	25% (1.36)	5	0.98	**17**
6	**6**	1	50% (1.55)	5	0.68	**18**
7	**7**	1	75% (2.20)	5	0.48	**19**
8	**8**	1	100% (2.34)	5	0.33	**20**
9	**21**	2	25% (n.d.)	5	1.00[c]	**25**
10	**22**	2	50% (n.d.)	5	0.40[c]	**26**
11	**23**	2	75% (n.d.)	5	0.25[c]	**27**
12	**24**	2	100% (n.d.)	5	0.24[c]	**28**

[a] Purified by precipitation into MeOH, centrifugation, and drying in vacuum.
[b] Determined by ^{1}H NMR in $CDCl_3$.
[c] Yield from **5**–**8** over two glycosylation steps (Scheme 3).

glycosylation efficiency at very high loading levels is, in part, a consequence of the imperfect branching of hyperbranched polymers. A recent detailed study on the molecular mass analysis of lower generation Boltorn polyesters using SEC in polar solvents has shown that Boltorn H-40 has a higher M_W (6700 vs. 5100) and wider PDI (2.6 vs. 1.8) compared to the values provided by the manufacturer.[13] Accordingly, in contrast to a perfect dendritic structure, the glycosyl acceptors would not be uniformly presented at the periphery of the polymer. At higher loading levels, the less accessible internal sites react less efficiently with glycosyl donors. Similarly, the yield of chemical reactions conducted on dendrimeric supports depends on the dendrimer generation. For example, Bradley and coworkers found that complete Fmoc-deprotection of the terminal aminogroups of polyurea dendrimer-derivatized, high loading polystyrene beads is slower at higher dendrimer generation.[14] Lee-Ruff et al. observed that photochemical coupling of cyclobutanones to polyhydroxy dendritic PEG supports proceeds with lower efficiency at higher generation or increased branching.[15]

Although quantitative conversion to **17** was observed by NMR at the 25% loading level, only 50% yield of the monosaccharide product **17** was recovered on preparative scale (Scheme 3)[16] due to the partial solubility of the acetate protected, polymer-bound monosaccharide in methanol.[17] However, deprotection and glycosylation of **17** with trichloroacetamidate donor **12**, followed by irradiation at 350 nm for 48 hours in THF afforded dissacharide **29** as a mixture of equilibrating anomers in 32% yield (3 steps).[18] The major anomer was assigned to be α,α based on the $^1J_{C-H}$ coupling constants of the anomeric carbons.[19]

In conclusion, we determined the efficiency of glycosylation of Boltorn H-50 supported glycosyl acceptors as a function of both the polymer loading and equivalents of activated donor. The combination of 25% loading level (1.36 mmol/g) and 5 equivalents glycosyl donor was optimal for quantitative transformation (>98%) at both the mono- and disaccharide stage.

Acknowledgment

This work was supported by The Ohio State University through a University Seed Grant and graduate assistantship to E. A. K. The generous gift of large samples of Boltorn polymers from the manufacturer, Perstorp polyols, is greatly appreciated.

References

(1) *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*; Chan, W. C.; White, P. D., Eds.; Oxford University Press: Oxford UK, **2000**.

(2) (a) Dörwald, F. Z. *Organic Synthesis on Solid Phase*, 2nd ed.; Wiley-VCH: New York, **2002**. (b) Seneci, P. *Solid Phase Synthesis and Combinatorial Technologies*; Wiley Interscience: New York, **2002**.

(3) Reviews: (a) Bergbreiter, D. E. *Curr. Opin. Drug Discovery Dev.* **2001**, *4*, 736. (b) Osburn, P. L.; Bergbreiter, D. E. *Prog. Polym. Sci.* **2001**, *26*, 2015. (c) Toy, P. H.; Janda, K. D. *Acc. Chem. Res.* **2000**, *33*, 546. (d) Gravert, D. J.; Janda, K. D. *Chem. Rev.* **1997**, *97*, 489.

(4) General introduction to dendrimers: Newkome, G. R.; Moorefield, C. N.; Vögtle, F. *Dendrimers and Dendrons: Concepts, Syntheses, Applications*; Wiley-VCH: New York, **2001**.

(5) (a) *Star and Hyperbranched Polymers*; Mishra, M. K.; Kobayashi, S., Eds.; Plastic Engineering Series 53, Marcel Dekker: New York, **1999**. (b) Frechet, J. M.; Hawker, C. J. *React. Funct. Polym.* **1995**, *26*, 127.

(6) Reviews: (a) Gebbink, R. J. M.; Klein, K.; Cornelis, A.; Van Klink, G. P. M.; Van Koten, G. *Rev. Mol. Biotechnol.* **2002**, *90*, 183. (b) Haag, R. *Chem.–Eur. J.* **2001**, *7*, 327.

(7) Kim, Y. H.; Webster, O. *Hyperbranched Polymers,* In *Star and Hyperbranched Polymers*, 53; Mishra, M. K.; Kobayashi, S., Eds.; Plastic Engineering Series 53, Marcel Dekker: New York, **1999**, 201.

(8) (a) Manufacturer specifications for Boltorn H-40: 64 terminal OH groups, nominal M_W 7,316 and PDI 1.8; Boltorn H-50: 128 terminal OH groups, nominal M_W 14,600 and PDI 2.0. (b) Preparation of Boltorn polyesters: Malmstroem, E.; Johansson, M.; Hult, A. *Macromolecules* **1995**, *28*, 1698.

(9) Kantchev, A. B.; Parquette, J. R. *Tetrahedron Lett.* **1999**, *40*, 8049.

(10) Arce, E.; Nieto, P. M.; Diaz, V.; Castro, R. G.; Bernad, A.; Rojo, J. *Bioconjugate Chem.* **2003**, *14*, 817.

(11) Reviews: (a) Knerr, L.; Schmidt, R. R. In *Solid Support Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries*; Seeberger, P. H., Ed.; John Wiley and Sons: New York, **2001**, 67–98. (b) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.

(12) Zehavi, U.; Sadeh, S.; Herchman, M. *Carbohydr. Res.* **1983**, *124*, 23.

(13) Zagar, E.; Zigon, M. *J. Chromatogr. A* **2004**, *1034*, 77.

(14) Lebreton, S.; Newcombe, N.; Bradley, M. *Tetrahedron Lett.* **2002**, *43*, 2479.

(15) Zhong, J.-H.; Fishman, A.; Lee-Ruff, E. *Org. Lett.* **2002**, *4*, 4415.

(16) **Representative Experimental Procedures.**
Compound 17. The glycosyl acceptor **5** (25% loading capacity, 1.36 mmol/g; 0.33 g, 0.45 mmol, 100 mol%) and the glycosyl donor **12** (1.27 g, 2.25 mmol, 500 mol%) were dissolved in dry THF (4.5 mL). After a homogeneous solution had formed, $BF_3{\cdot}OEt_2$ (90 μL, 102 mg, 0.72 mmol, 160 mol%) was added and the solution was stirred for 30 min. The solution was concentrated in vacuo and then precipitated with MeOH (50 mL). After centrifugation, decantation of the supernatant, rinsing of the polymer mass with MeOH (3 × 10 mL) and drying under high vacuum, compound **17** (0.275 g, 0.23 mmol, 50%) was obtained. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.08 (s, 6 H), 0.91 (s, 9 H), 4.95–5.42 (m, 5 H), 7.95 (br s, 1 H), 8.32 (br s, 1 H), 8.67 (br s, 1H).
Compound 25. To a solution of **17** (0.275 g, 0.225 mmol, 100 mol%) in THF, HF·pyridine (0.5 mL, 0.5 g, 5.0 mmol, 222 mol%) was added and the solution stirred for 12 h. The mixture was diluted with EtOAc (15 mL) and washed with H_2O (5 mL), 10% H_2SO_4 (5 mL) and sat. $NaHCO_3$ (5 mL). After drying ($MgSO_4$) and evaporation of the solvent, compound **21** was obtained as yellow foam and used directly for the next step. The monosaccharide donor **21** was glycosylated using the glycosyl donor **12** (0.64 g, 1.13 mmol, 500 mol%), $BF_3{\cdot}OEt_2$ (45 μL, 51 mg, 0.36 mmol, 160 mol%), and dry THF (2.5 mL) as described for **17** above. The polymer-immobilized disaccharide **25** was isolated after precipitation into MeOH (25 mL). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.09 (s, 3 H), 0.10 (s, 3 H), 1.28 (s, 9 H), 4.80–5.45 (m, 10 H), 7.95 (br s, 1 H), 8.32 (br s, 1 H), 8.67 (br s, 1 H).
Disaccharide 29. Compound **25** (as obtained from the previous step) was dissolved in dry THF (2.5 mL) in a quartz vessel, purged with Ar for 10 min, then irradiated with UV light (350 nm) for 48 h. The solution was evaporated and the crude mixture applied directly onto a silica gel column. After chromatography (hexanes–EtOAc, 2:1), compound **29** (50 mg, 0.072 mmol, 32% from **21**) was isolated as colorless oil. Major anomer (α,α): ^{1}H NMR (400 MHz, $CDCl_3$): δ = –0.04 (s, 3 H), –0.02 (s, 3 H), 0.84 (s, 9 H), 1.91 (s, 3 H), 1.92 (s, 3 H), 1.96 (s, 3 H), 1.98 (s, 3 H), 2.05 (s, 3 H), 2.08 (s, 3 H), 3.48 (m, 1 H), 3.62–3,82 (m, 5 H), 4.16 (m, 1 H), 4.75 (d, J = 1.6 Hz, 1 H), 5.13–5.28 (m, 5 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = –5.01, –4.95, 18.8, 21.1, 21.1, 21.2, 21.2, 21.2, 21.2, 26.3, 62.9, 66.9, 67.2, 67.7, 69.3, 69.6, 69.7, 70.1, 70.4, 71.9, 92.5 ($^1J_{C–H}$ = 172.6 Hz), 97.7 ($^1J_{C–H}$ = 170.1 Hz), 170.1, 170.2, 170.3, 170.5, 170.6, 170.7. Anal. Calcd for $C_{30}H_{17}O_{17}Si$: C, 50.84; H, 6.83. Found: C, 50.72; H, 6.64.

(17) In contrast, benzyl-protected oligosaccharides loaded on acetate-capped Boltorn polyesters at comparable loading levels were completely insoluble in MeOH (ref. 9). Such behavior is in agreement with the notion that the terminal groups largely determine the solubility properties of dendrimers and hyperbranched polymers.

(18) (a) In addition to losses during precipitation into MeOH, cleavage of the *o*-nitrobenzyl linker with UV light generally suffers from low efficiency. See: Holmes, C. P. *J. Org. Chem.* **1997**, *62*, 2370. (b) The disaccharide yield is comparable with yields obtained previously utilizing the UV light cleavage protocol (ref. 9).

(19) Barresi, F.; Hindsgaul, O. *Can. J. Chem.* **1994**, *72*, 1447.