Pergamon

Tetrahedron Letters 40 (1999) 8049–8053

TETRAHEDRON LETTERS

Disaccharide synthesis on a soluble hyperbranched polymer

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Received 20 July 1999; revised 2 September 1999; accepted 7 September 1999

Abstract

A hyperbranched star polymer was employed as a soluble support for disaccharide synthesis as a practical alternative to a dendrimer support. The large number of terminal groups present on the support permit very high loading levels to be possible. Polymer bound intermediates could be directly analyzed by MALDI-TOF mass spectrometry due to the presence of a photolabile linker that is cleaved by the MALDI-TOF laser. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: carbohydrates; oligosaccharides; hyperbranched polymer; solution-phase synthesis.

Although the use of cross-linked insoluble polymers as supports for chemical synthesis has been an effective separation strategy for many classes of compounds, solid-phase synthesis is hampered by the heterogeneous reaction conditions that are required.[1] Factors such as the solvent-dependent swelling capacity of the support and the differential accessibility of the polymer-bound substrates lead to nonlinear kinetic behavior.[2] Therefore, adapting solution-phase chemistry to the solid-support can be complicated and time-consuming. To circumvent these difficulties, Janda and coworkers have exploited non-crosslinked, linear polymers such as polyethylene glycol (PEG)[3] as soluble supports that permit reaction homogeneity wherein reagent separation is achieved using SEC or precipitation. However, only very low loadings are possible because there are only one or two attachment points per polymer molecule.[4] Kim and co-workers at Merck[5] developed a first generation PAMAM dendrimer as a soluble support that was capable of high loading levels due to the large number of highly accessible, peripheral attachment points present on the dendrimer surface. Purification of soluble polymer-bound intermediates was accomplished by SEC and standard spectroscopic techniques could be used to follow the reaction progress on the support. However, the multi-step syntheses and associated high cost of dendrimers severely impedes the application of these materials to preparative scale processes.[6] Hyperbranched polymers provide a practical alternative to dendrimer supports because they maintain a highly branched structure that displays a large number of terminal groups on the surface of an approximately globular morphology similar to dendrimers; however, they are prepared in a single-step.[7]

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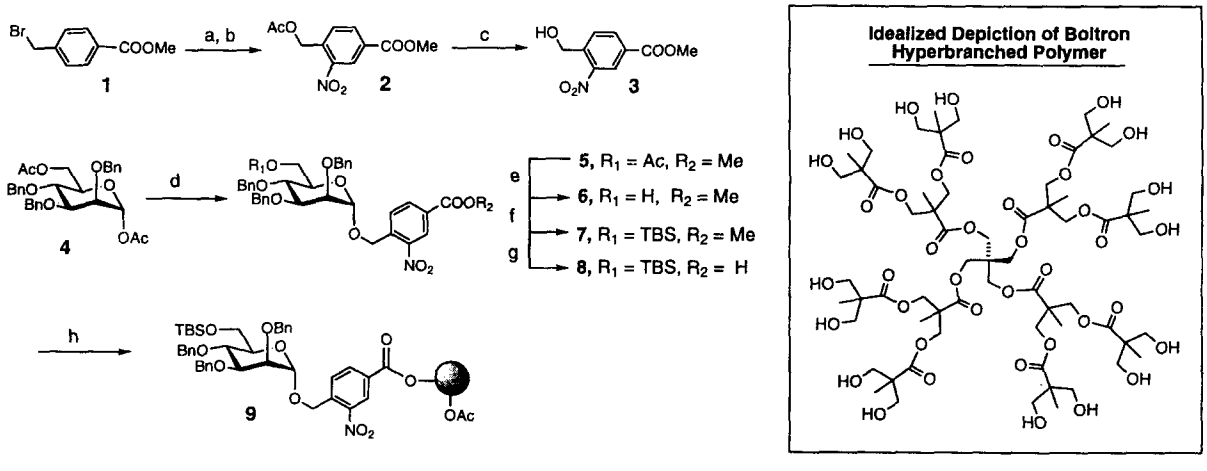
PII: S0040-4039(99)01709-8

In this paper, we report the use of a hyperbranched polyester as a soluble support for disaccharide synthesis. The polyester, initially reported by Hult,[8] is constructed in one-step from pentaerythritol as the 'star center' using dimethylolpropionic acid as the repeating AB_2 monomer (Scheme 1). The hyperbranched polyester offers all the advantages demonstrated by dendrimeric supports, but at extremely low cost ($5/kg): (1) The polymer-bound intermediates exhibit high solubility in most aprotic solvents (THF, ether, CH_2Cl_2, acetone) but very low solubility in methanol from which it can be quantitatively precipitated. SEC purification is also possible. (2) Although these materials are imperfectly branched, the majority of the terminal groups can be expected to reside near the surface of the polymer thus providing for high substrate accessibility even at high loading levels.[9] (3) The support undergoes rapid hydrolytic degradation to water-soluble materials thereby permitting product purification by extraction. (4) Direct mass spectral analysis of the polymer-bound disaccharides can be achieved by photolytic release of the disaccharide from the support with the MALDI-TOF laser.



Scheme 1. Synthesis of polymer–carbohydrate conjugate. Key: (a) HNO_3–H_2SO_4, 92%. (b) HOAc, NaOAc, 46%. (c) NH_3–CH_3OH, 76%. (d) **3**, BF_3–OC_2H_5, 78%. (e) K_2CO_3, CH_3OH, 75%. (f) TBDMSCl, $(C_2H_5)_3N$, CH_2Cl_2, 91%. (g) KOH, H_2O, 96%. (h) i. **8**, EDCI, DMAP, polymer. ii CH_3COCl, pyr

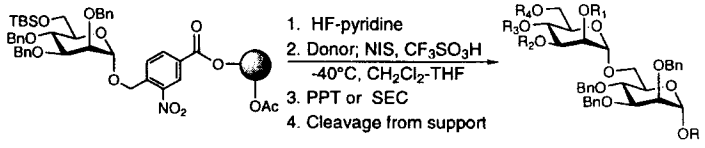
The nominal molecular weights of the polymers used in this work were 7250 (Boltron H-40) and 14 600 (Boltron H-50) daltons each theoretically containing 8.8 mmol of terminal hydroxyl attachment points per gram. Attachment to the support was accomplished using a photolabile *o*-nitrobenzyl alcohol linkage to permit cleavage by irradiation at 350 nm. Accordingly, linker **3** was prepared in three steps from methyl 4-bromomethylbenzoate and then attached to the first mannosyl acceptor by glycosylation with 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-mannose (**4**)[10] using BF_3–$O(C_2H_5)_2$ affording mannoside **5** in a 6:1 α/β anomeric ratio (Scheme 1). Exchange of the 6-acetoxy function for a *t*-butyldimethylsilyloxy group followed by basic hydrolysis of the methyl ester provided acceptor–linker conjugate **8**. The polymer was then loaded to ca. 40% capacity by treatment with **8** and EDCI followed by capping of the remaining hydroxy groups with acetyl chloride.[11] The polymer-supported substrate was then treated with HF–pyridine to liberate the 6-hydroxyl group of the acceptor and purified using an aqueous extractive work-up. The actual loading was determined by ^1H NMR to be 0.68–0.85 mmol/g[12] due to the significant increase in molecular weight upon attachment of the carbohydrate-linker conjugate.

Glycosylations were carried out by exposing the polymer-supported acceptor to an excess (5 equiv.) of a thioglycoside donor (**10**–**11**)[13] in the presence of *N*-iodosuccinimide (NIS) and catalytic trifluoromethanesulfonic acid (TfOH) in CH_2Cl_2–THF at –40°C (Scheme 2, Table 1).[14] Separation of the polymer-bound disaccharide from excess reagents and glycosyl donor could be accomplished by filtration

through Sephadex LH-20 size-exclusion gel using chloroform as the mobile phase. Although SEC purification was usually effective, we found that precipitation with methanol was more expedient. The ability to precipitate the polymer substrate with methanol was general for all the protected saccharides investigated. Subsequent irradiation at 350 nm for 24–36 h afforded cleavage from the support giving disaccharides **12–13**[15] in moderate overall yield following chromatographic isolation.[16] For comparison, glycosylation of **6** with **10–11** under identical reaction conditions followed by irradiation at 350 nm provided disaccharides **12–13** with identical (α/β) selectivities albeit in higher yield. Increasing the mass of the support to 14 600 daltons (Boltron H50) afforded no significant improvement in yield or selectivity.

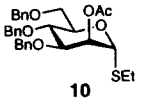
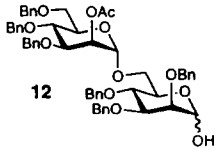
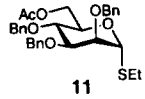
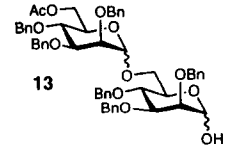
Since exposure of the support to aqueous base effects rapid degradation to dimethylolpropionic acid, we investigated hydrolytic cleavage of disaccharides **16** and **17**[17] from the support using NaOH in THF–H_2O at 60°C (Table 2). Due to the water-solubility of dimethylolpropionic acid, the disaccharides could be readily isolated from the polymer-derived by-products by acidification of the solution and extraction into ethyl acetate. In general, this process was more practical than photocleavage and provided improved yields of the disaccharides. This cleavage method also generated a protected anomeric center which simplified isolation.

In contrast to dendrimers which are typically monodisperse in structure, hyperbranched polymers exist as a polydisperse mixture. Monodispersity provides a capability to monitor progress on the support using techniques such as mass spectrometry for which monomolecularity is essential. Although the hyperbranched polyester support is polydisperse (pdi=1.4), direct mass spectral analysis of the disaccharide on the support is similarly possible because the MALDI-TOF laser (337 nm) cleaves the disaccharide from the support during analysis. For example, freshly precipitated polymer-bound disaccharide **17** exhibits a signal at *m/z*=1020 (M+Na) and 1036 (M+K) when analyzed by MALDI-TOF MS using α-cyano-4-hydroxycinnamic acid as the matrix (Fig. 1). The lack of any significant peaks at



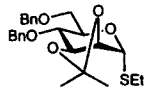
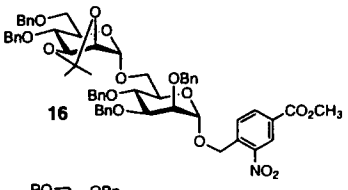
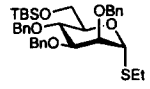
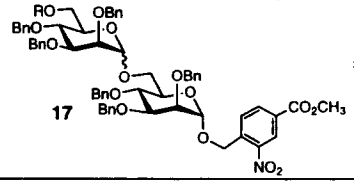
Scheme 2.

Table 1
Disaccharide synthesis (photocleavage from support)

Donor	Product	Pur.[a]	HB Poly.	Yield (α/β)
 **10**	 **12**	SEC	H-40	49 % (1:0)
		PPT	H-40	32 % (1.0)
		PPT	H-50	36 % (1:0)
		--	--	76% (1:0)[b]
 **11**	 **13**	SEC	H-40	78 % (1:1)
		PPT	H-40	42 % (1:1)
		PPT	H-50	27 % (1:1)
		--	--	75 % (1:1)[b]

(a) SEC = Size exclusion chromatography , LH-20 Sephadex; PPT = precipitation, CH_3OH. (b) Prepared from **6** as a control.

Table 2
Cleavage by hydrolytic degradation of polymer[a]

Donor	Product[b]	Yield (α/β)
 14	 16	53 % (5:1)
 15	 17	59 % (1:1)[c]

(a) Polymer (H-50)-disaccharide intermediates purified by precipitation into CH_3OH. (b) Crude products after hydrolytic cleavage (NaOH, THF-H_2O, 60°C) were esterified with CH_2N_2 in ether. (c) Isolated as a mixture of two compounds: R = TBDMS (**17a**, 26 %) and R = H (**17b**, 33 %).

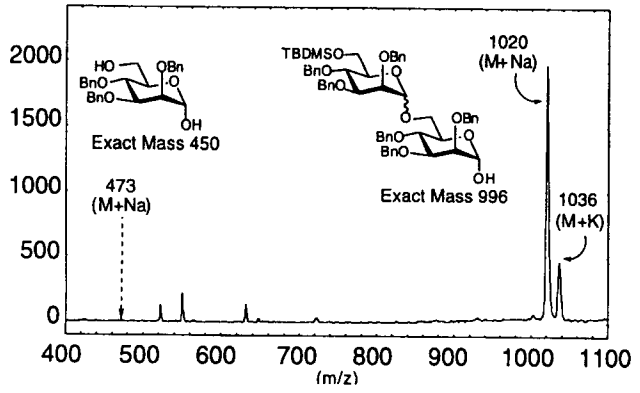


Figure 1. MALDI-TOF spectrum of polymer-supported **17**

m/z=473 indicated that the glycosylation had proceeded to completion; the moderate 59% yield is likely due to mechanical loss in the precipitation and potential error in estimating the initial loading level.

The high loading capacity and solubility of hyperbranched polymers offer a practical alternative to dendrimers for developing soluble supports with extremely high loading levels. As an illustration, glycosylation of 235 mg of the polymer-supported acceptor **9** with donor **14** afforded 109 mg (53%; theor.: 208 mg) of disaccharide **16**. In principle, this fact and the low cost of hyperbranched polymers should permit the use of these supports for preparative scale applications. Work is currently in progess to increase the loading level beyond 40% capacity and to progess to longer oligosaccharides. A limitation of this support resides in the ester linkages which are susceptible to cleavage with nucleophilic reagents; therefore, more robust hyperbranched systems will be developed in due course.

Acknowledgements

This work was supported by The Ohio State University through a University Seed Grant. The authors wish to thank Perstorp Polyols for the generous gift of the Boltron polymers.

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15. Selected data for **12–13**: **12**: The stereochemistry of the anomeric linkages was confirmed as α,α by the gated ^{13}C NMR spectrum (Barresi, F.; Hindsgaul, O. *Can. J. Chem.* **1994**, *72*, 1447): ^{13}C NMR (125 MHz, $CDCl_3$) 98.76 (J_{C-H}=169.1 Hz); 94.43 (J_{C-H}=169.4 Hz). Anal. calcd for $C_{56}H_{60}O_{12}$; C, 72.71; H, 6.54. Found: C, 72.50; H, 6.54. **13**: (1:1 α/β): ^{13}C NMR (100 MHz, $CDCl_3$) 100.7, 99.35, 97.15 (4 carbons). Anal. calcd for $C_{56}H_{60}O_{12}$; C, 72.71; H, 6.54. Found: C, 72.54; H, 6.57.
16. Yields were estimated (±5%) using the weight of initial polymer–substrate conjugate and the loading as determined by ^1H NMR.
17. Selected data for **16–17**: **16**: (5:1 α/β) The stereochemistry of the anomeric linkages of the major isomer was confirmed as α,α by the gated ^{13}C NMR spectrum: ^{13}C NMR (125 MHz, $CDCl_3$) 97.90 (J_{C-H}=168.5 Hz); 98.18 (J_{C-H}=168.4 Hz); MALDI-TOF MS for $C_{59}H_{63}NO_{15}Na$ (M+Na) calcd 1049; obsd 1049. Anal. calcd for $C_{59}H_{63}NO_{15}$; C, 69.06; N, 1.37; H, 6.19. Found: C, 69.21; N, 1.47; H, 6.28. **17b**: (R=H, 1:1 α:β): ^{13}C NMR (125 MHz, $CDCl_3$) 98.35, 98.90, 102.42, 101.8 ppm; MALDI-TOF MS for $C_{63}H_{65}NO_{15}Na$ (M+Na) calcd 1098; obsd 1098. Anal. calcd for $C_{63}H_{65}NO_{15}$; C, 70.31; N, 1.30; H, 6.09. Found: C, 70.20; N, 1.35; H, 6.13.