Homogenous Enzymatic Synthesis Using a Thermo-Responsive Water-Soluble Polymer Support

Xuefei Huang,[a] Krista L. Witte,[a] David E. Bergbreiter,[b,*] Chi-Huey Wong[a,*]

[a] Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037, USA
Fax: (+1) 858–784–2409, e-mail: wong@scripps.edu
[b] Department of Chemistry, Texas A & M University, College Station, TX 77843, USA
Fax: (+1) 979–845–4719, e-mail: bergbreiter@tamu.edu

Received April 30, 2001; Accepted July 3, 2001

Abstract: Several enzymes immobilized on thermo-responsive polyacrylamide polymers are nearly as active as their soluble forms, and can be recovered for reuse after gentle heating and precipitation. Carbohydrates attached to these polymers have been used for enzymatic glycosylation, and the products have been isolated by thermal precipitation followed by release from the polymer, thus greatly simplifying product purification in water.

Keywords: enzyme immobilization; glycosylations; polymers; thermo-responsive

Introduction

Insoluble polymer resins have been commonly utilized as supports for organic synthesis since the reaction intermediates and products can be easily isolated and purified.[1] Despite these advantages, however, the heterogeneity of a solid phase can lead to potential complications, e.g., low reactivity, non-linear kinetics, stereochemical complexity and analytical difficulty.[2] In addition, a number of reactions in solution are not applicable to solid phase. Soluble polymers can potentially combine the advantages of both solid-phase and solution-phase chemistry by providing a homogeneous stage for synthesis with facile isolation of products during work-up through precipitation and filtration.[3] There are many forms of soluble polymers, including the commonly used poly(ethylene glycol) (PEG) and polyacrylamide.[4] The copolymers of *N*-*i*-propylacrylamide (NIPAm) and functionalized monomers have been shown to be thermo-responsive and exhibit inverse temperature-dependent solubility in water.[5,6] These polymers are soluble in cold water, but become insoluble and precipitate out of the solution once the temperature is higher than their lower critical solution temperatures (LCST), presumably due to the enhancement of hydrophobic interactions between the side chains at higher temperatures.[6,7] The LCST of these polymers can thus be readily tailored by modifying the side chain, as well as the ratio of NIPAm to the functionalized monomer. These thermo-responsive polymers have been utilized in the design of smart chemical catalysts, the catalytic activities of which can be switched on and off depending on the solution temperature.[8,9] They have also been used for immobilization of proteins[10] but no synthetic application has been exploited. The potential of NIPAm polymers and copolymers as well as other functionally similar peptides in purification of biological macromolecules has been described.[11] Herein, we present our studies of enzymes attached to such polymers as recyclable biocatalysts and enzymatic carbohydrate synthesis with glycosyl acceptors immobilized on the polymer through a cleavable linker. Due to the high solubility of oligosaccharides in water and the high cost of enzymes, it is desirable to carry out enzymatic synthesis in such systems, which would allow the recovery of enzymes and products by gentle heating after the reaction.

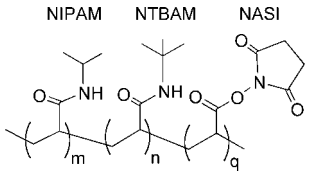
Results and Discussion

Four different thermo-responsive polymers, which have LCST's between 20 and 30 °C (Figure 1), were investigated for use as supports for enzymes.[5,9] Near or sub-ambient LCST values of these selected polymers are essential for maintaining high enzymatic activities after recycling of the enzymes. Enzymes are immobilized through amide linkages by reaction with the *N*-acrylylsuccinamide (NASI) groups. In order to find the optimum conditions for enzyme immobiliza-

  1615-4150/01/34306–7-675–681 $ 17.50-.50/0

tion and recovery, different temperatures for coupling and precipitation were evaluated utilizing subtilisin BPN' as the test enzyme and succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide as the substrate. While subtilisin immobilized on polymer A (NIPAm:NASI = 10:1) had very low activities (data not shown), it showed the highest activity (about 70% of that of the soluble enzyme) when immobilized on polymer D (NIPAm:NTBAm:NASI = 20:5:2) with a coupling temperature of 5 °C and precipitation temperature of 21 °C (Figure 2). Precipitation of the polymer under higher temperatures led to decreased enzymatic activity. Polymer B (NIPAm:NASI = 20:1) also showed good results with a coupling temperature of 14 °C and precipitation temperature of 30 °C. Polymers B and D and their respective optimized coupling and precipitation temperatures were thus used for the studies of other enzymes. The effect of precipitation pH on the enzyme activities was also studied by measuring the activity of subtilisin immobilized on polymer D. The highest activity of immobilized subtilisin was obtained when it was precipitated at pH 4.5. To investigate the general utility of these polymers, other enzymes, including bovine liver β-galactosidase, bovine milk galactosyl transferase, papain, and glucose 6-phosphate dehydrogenase V from baker's yeast, were also immobilized on polymers B and D under the same conditions, and it was found that these enzymes were efficiently immobilized and retained most of their activities (data not shown).

The activity of the immobilized subtilisin was analyzed after precipitation and re-solubilization. Subtilisin retained about 50% of its activity after three rounds of precipitation and re-solubilization. The decrease of enzymatic activities was not due to the release of enzymes from the polymer support, as the supernatant after filtration did not show any enzymatic activity. Interestingly, the catalytic activity of the immobilized enzyme increased slowly over time as the polymer was re-solubilized at low temperature,



Polymer	m	n	q	LCST
A	10	0	1	30 °C
B	20	0	1	28 °C
C	20	1	1	22 °C
D	20	5	2	20 °C

Figure 1. Structure and LCST of the polymers studied.

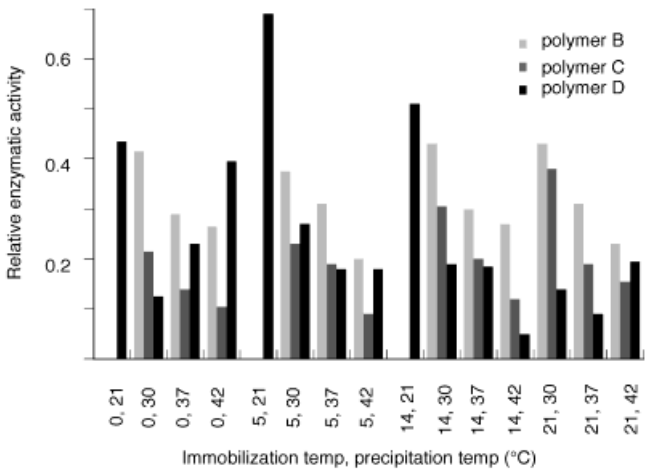


Figure 2. Effects of immobilization temperature and precipitation temperature on immobilized subtilisin activity. The immobilization yield was estimated based on the activity of enzyme used in loading and the activity of unloaded enzyme.

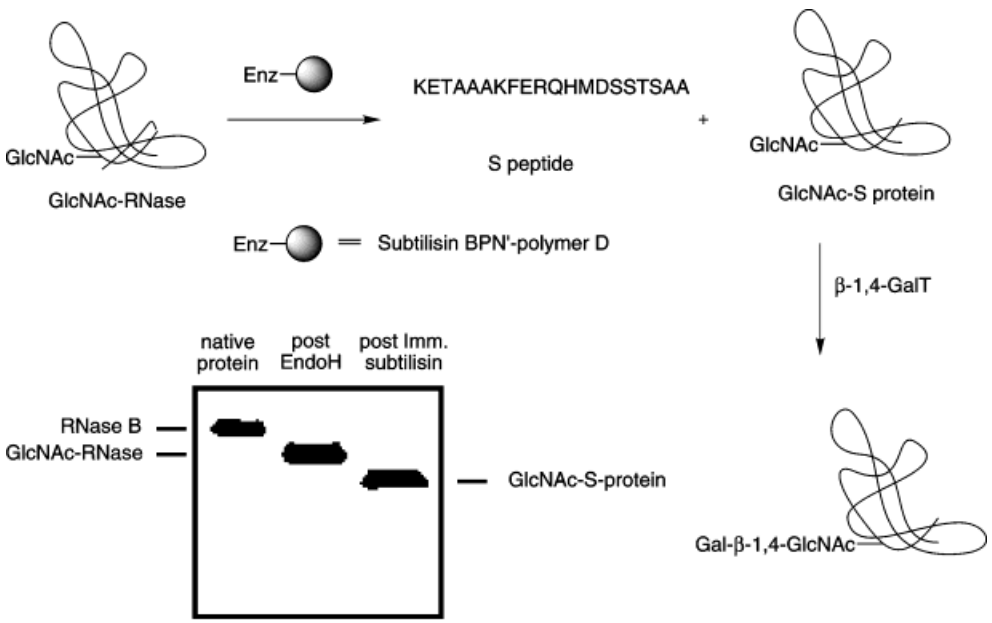


Figure 3. Limited proteolysis of GlcNAc-RNase by immobilized subtilisin BPN'.

probably because the enzyme in the re-solubilized polymer is slowly restored to its fully active conformation.

With the immobilized subtilisin in hand, we carried out the proteolysis of a modified form of the glycoprotein ribonuclease (RNase) B. The glycan of RNase B was removed by the glycosidase Endo H to give a monoglycosylated protein, which was proteolytically cleaved to give two fragments, peptide S and GlcNAc-protein S (Figure 3). Due to the easy removal of the immobilized protease after limited proteolysis, the possible complication of undesired degradation caused by protease contamination was avoided. A precipitation is all that is required to isolate GlcNAc-protein S. If subtilisin not bound to a polymer were used, however, the resulting GlcNAc-protein S had to be isolated after proteolysis by column chromatography.[12] After removal of the subtilisin-polymer conjugate, the GlcNAc-protein S was glycosylated to generate a re-modeled glycoprotein bearing a well-defined sugar moiety.[12]

These thermo-responsive polymers are also useful supports in oligosaccharide synthesis. To show this, a glycosyl acceptor was attached to the polymer through linker **1**, **2**, or **3** (Figure 4), and enzymatic glycosylation of the immobilized glycosyl acceptor was carried out. With linker **1**, the immobilized GlcNAc was converted to LAcNAc in low yield (~ 30%) by β-1,4-galactosyl transferase while in the case of linker **2**, the polymer was insoluble in water. Therefore, studies were focused on the more hydrophilic linker **3**. The fourteen-atom chain between the polymer and the acceptor (linker **3**) was selected so that the immobilized acceptors have the dynamics and mobility like their low molecular weight analogues, which would allow us to monitor the progress of the reaction by NMR spectroscopy.[5,8]

Linker **3** was prepared by reacting bromoacetic acid with *N*-Boc-aminoethylethylene glycol (**4**) to give carboxylic acid **5** in 40% yield. Coupling of acid **5** with 4-aminomethylphenol,[13] followed by glycosylation with 2-acetamido-2-deoxy-α-D-glucopyranosyl chloride 3,4,6-triacetate[14] under phase-transfer conditions and acid deprotection gave amine **6** in 78% yield for the three steps. Amine **6** was immobilized on polymer A to provide the *N*-acetylglucosamine (GlcNAc) bearing polymer **7**. Galactosylation of the immobi-

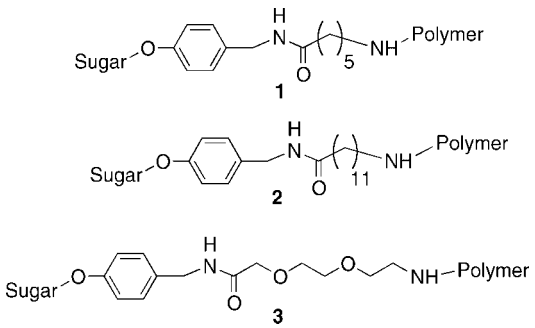
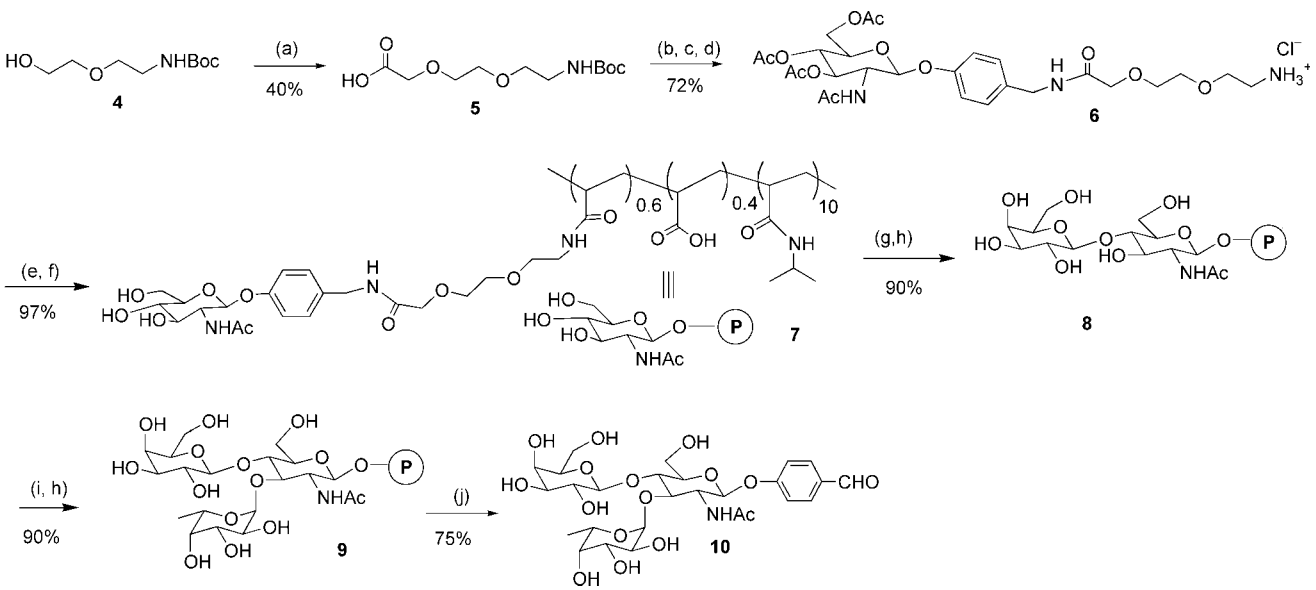


Figure 4. Linkers **1** – **3**.



Scheme 1. (a) NaH, DMF, bromoacetic acid, rt, 3 h; (b) 4-aminomethylphenol, BOP, *N*,*N*-diisopropylethylamine (DIPEA), THF, rt, 3 h; (c) 2-acetamido-2-deoxy-α-D-glucopyranosyl chloride 3,4,6-triacetate, Cs_2CO_3, tetrabutylammonium hydrogen sulfate, CH_2Cl_2:H_2O (3:1), pH 9.5, rt, 12 h; (d) 4 M HCl in dioxane, CH_2Cl_2, rt, 0.5 h, 100%; (e) polymer A, DIPEA, BOP, THF:CH_3CN (1:1), rt, 12 h; (f) MeOH, NaOH, rt, 5 min; (g) 1.2 equiv. UDP-gal, 0.25% Triton X-100, 100 mM $MnCl_2$, β-1,4-GalT from bovine milk (12.5 mU/mL), alkaline phosphatase (0.5 U/mL) in HEPES pH 7.4 buffer, rt, 1 day; (h) EDTA, 55 °C, centrifuge; (i) 1.2 equiv. GDP-fuc, 0.25% Triton X-100, 100 mM $MnCl_2$, FucT V (30 mU/mL), alkaline phosphatase (0.5 U/mL) in MES pH 6.0 buffer, rt, 1 day; (j) CAN, CH_3CN:H_2O (3:1), rt, 1 h.

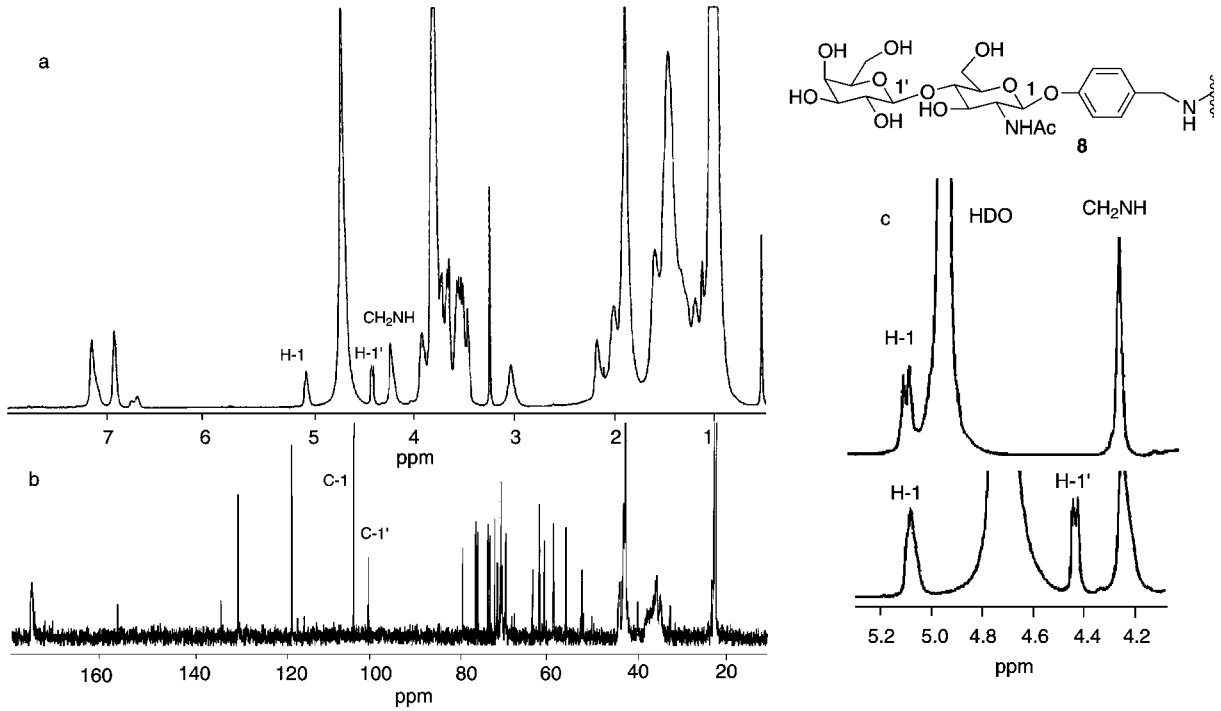
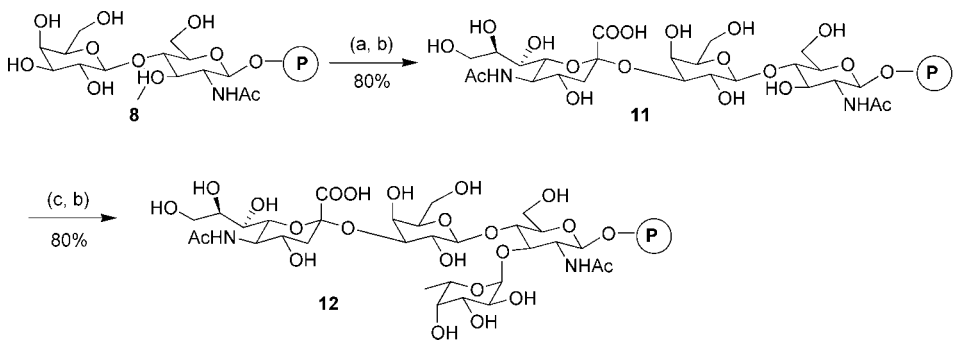


Figure 5. (**a**) ^{1}H NMR of polymer **8** in D_2O (600 MHz); (**b**) ^{13}C NMR of polymer **8** in D_2O (150 MHz); (**c**) Comparison between expanded ^{1}H NMR of polymer **7** (*upper trace*) and polymer **8** (*lower trace*). The coupling constant of H-1' of polymer **8** (7.4 Hz) is indicative of the β linkage between galactose and GlcNAc.

lized GlcNAc **7** was attempted with 100 mU bovine milk β-1,4-galactosyltransferase with 1 equivalent of donor UDP-galactose in *N*-(2-hydroxyethyl)-piperazine-*N*'-(2-ethanesulfonic acid) (HEPES) buffer at pH 7.4 (Scheme 1). Greater than 95% of the polymer was recovered after reaction by heating the solution to 55 °C followed by centrifugation. About 70% of the GlcNAc was galactosylated as determined by ^{1}H NMR without cleaving the sugars off the polymer. Polymer **7** was fully galactosylated to give the *N*-acetyllactosamine (LacNAc) bearing polymer **8** with one more round of galactosylation.(Figure 5) The LacNAc polymer **8** was further fucosylated using bovine α-1,3-fucosyl transferase and GDP-fucose as donor, and the Le^{x} bearing polymer **9** was isolated by heating the solution to 55 °C. Fucosylation was estimated to be complete based on the ^{1}H NMR spectrum of the polymer-bound product. The improvement on the extent of glycosylation is presumably due to the more hydrophilic nature of LacNAc polymer **8** compared to the GlcNAc polymer **7**. The Le^{x} trisaccharide was oxidatively released from the polymer **9** using ceric ammonium nitrate (CAN) to yield Le^{x} glycoside **10** bearing an aldehyde functionality, which can be potentially utilized for conjugation with proteins or amine-containing supports through reductive amination.[15]

The LacNAc bearing polymer **8** was also sialylated using α-2,3-sialyltransferase (Scheme 2). Due to the



Scheme 2. (a) 1.2 equiv. CMP-sialic acid, 0.25% Triton X-100, 100 mM $MnCl_2$, α-2,3-sialT (100 mU/mL), alkaline phosphatase (0.5 U/mL) in HEPES pH 7.4 buffer, rt, 1 day; (b) i. pyridine, Ac_2O; ii. centrifuge; iii. 0.1 N NaOH; (c) 1.2 equiv. GDP-fuc, 0.25% Triton X-100, 100 mM $MnCl_2$, FucT V (30 mU/mL), alkaline phosphatase (0.5 U/mL) in MES pH 6.0 buffer, rt, 1 day.

highly hydrophilic nature of the sialyl-LacNAc, the LCST of the polymer becomes higher than 90 °C so that the polymer cannot be recovered by raising the temperature of the solution. In order to solve this problem, the crude mixture after sialylation was subject to peracetylation using pyridine and acetic anhydride. Although soluble at 0 °C, the polymer was insoluble in water at room temperature after peracetylation and separated from the reaction mixture. After deacetylation, pure polymer **11** bearing sialyl-LacNAc was obtained. The extent of sialylation was higher than 90% after only one round of sialylation. Polymer **11** was subjected to fucosylation with fucosyl transferase (Scheme 2). After peracetylation, centrifugation, and deacetylation, polymer **12** containing sialyl Lewis X (SLe^x) tetrasaccharide was obtained with the extent of fucosylation higher than 90%. Polymers **11** and **12** may be useful for studying their multivalent interaction with selectins[16] as well as with influenza hemagglutinin and neuraminidase.[17]

Conclusions

In summary, we have demonstrated that a number of enzymes can be immobilized onto thermo-responsive polyacrylamide polymers and recovered for reuse after gentle heating. The immobilized enzymes showed comparable activities as their soluble forms. Oligosaccharides have also been synthesized on the polymer through enzymatic glycosylation and the progress of glycosylation has been followed by NMR. The trisaccharide Le^x was synthesized in 60% yield for the three steps from polymer **7** with no chromatographic purification of intermediates. Compared to other solution-phase[18] and solid-phase[19] oligosaccharide syntheses, this new method of oligosaccharide synthesis greatly simplifies product purification and eliminates the need of size exclusion chromatography to separate the desired product from the reaction mixture.

Experimental Section

Materials

All enzymes were purchased from Sigma and Calbiochem. All polymers were synthesized as described previously and have typical M_v values of ca. 8.5×10^5.[5]

General Procedures for Immobilization of Enzyme

Stock solutions were made for the polymer (50 mg/mL in DMF) and the enzyme to be immobilized (10 mg/mL in 0.1 M borate, 0.2 M NaCl, pH 8.5). All solutions were chilled to 0 °C. Aliquots of enzyme (100 μL) and polymer (100 μL) were added to 800 μL buffer (0.1 M borate, 0.2 M NaCl) in Eppendorf tubes at 0 °C. The reaction mixture was mixed well and kept at the coupling temperature for ten to twelve hours, after which the solution was adjusted to pH 4.5 with 10% acetic acid. The solution was gently warmed to the precipitation temperature for 20 min and the precipitate was isolated by centrifugation and vigorously washed with aliquots of warmed acetate buffer (100 mM pH 4.5). The immobilized enzyme was then re-dissolved in an appropriate buffer at 4 °C for future use.

Assay of Subtilisin BPN'

Assays were performed at 4 °C with prechilled solution. To a solution of succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (4 mM) in 100 mM of tris buffer (120 μL, pH 8.0) in a UV cuvette, an aliquot of the immobilized enzyme solution (20 μL) was added. The change of absorbance at 410 nm was monitored for the next 30 sec and the rate of hydrolysis was determined.

Limited Proteolysis of GlcNAc-RNase with Immobilized Subtilisin BPN'

An aliquot of immobilized subtilisin (100 μL) in 100 mM of MES buffer (pH 6.0) was added to a solution of GlcNAc-RNase (900 μL, 3.3 mg/mL) in 100 mM of HEPES buffer (pH 8.0) at 4 °C. After 4 h at 4 °C, the solution was adjusted to pH 4.5 with 10% acetic acid and warmed to 21 °C. The immobilized subtilisin was removed by centrifugation to give the pure GlcNAc-protein S as shown by the 20% SDS-tricine gel.

Procedures for Immobilization of Glycosyl Acceptor 6

To a solution of amine **6** (100 μmol) in THF:acetonitrile (1:1, 10 mL) was added a polymer, DIPEA, and BOP. The reaction mixture was kept at room temperature for 12 h, after which the solvent was evaporated on a rotary evaporator. Dichloromethane (10 mL) was added to generate a solution. Upon addition of water (10 mL), a white precipitate immediately formed. The solution was decanted and the precipitate was washed with dichloromethane (10 mL) and water (10 mL). The precipitate was re-dissolved in methanol (5 mL) and a solution of 0.2 N NaOH (500 μL) was added. After 30 min at room temperature, the solution was adjusted to pH 2 and the solvent was evaporated by rotary evaporator. The resulting solid was re-dissolved in water at 0 °C. Gentle heating to 37 °C precipitated the polymer-bound **6**. The polymer precipitate was isolated by centrifugation, re-dissolved in water, and analyzed by solution-state NMR spectroscopy.

General Procedures for Glycosylation

The immobilized glycosyl acceptor (1 equiv.) and sugar nucleotide (1.2 equiv.) were dissolved in an appropriate buffer (25 mM) containing 0.25% Triton X-100 and $MnCl_2$ (20 mM). Glycosyltransferase (25 mU/mL) and alkaline phosphatase (0.5 U/mL) were added to the reaction. The mixture was gently stirred at room temperature for 1 d and a white precipitate was formed. The precipitate was filtered off and EDTA

(8 mg) was added. The solution was heated to 55 °C, centrifuged and the supernatant was decanted. The precipitate obtained was washed with hot water (55 °C) and re-dissolved in cold water. In the cases where the polymer LCST became greater than 90 °C, the solvent was evaporated after glycosylation. Pyridine (1 mL) and acetic anhydride (1 mL) were added. After stirring at room temperature for 3 h, all solvents were evaporated and the mixture was washed with 0.1 N hydrochloric acid (10 mL × 3). The solid was collected by centrifugation and re-dissolved in methanol (5 mL). A solution of 0.01 N NaOH (0.5 mL) was added and the mixture was stirred at room temperature for 1 h. The solution was adjusted to pH 4 with 0.1 N hydrochloric acid and the solvent was evaporated.

Buffers, Enzymes and Sugar Nucleotides used for each Glycosylation

Galactosylaton: 100 mM HEPES buffer, pH 7.4, β-1,4-galactosyltransferase, UDP-galactose.

Fucosylation: 100 mM 4-morpholineethanesulfonic acid (MES) buffer, pH 6.0, α-1,3-fucosyltransferase V, GDP-fucose.

Sialylation: 100 mM HEPES buffer, pH 7.4, α-2,3-sialyltransferase, CMP-sialic acid.

Le^X Trisaccharide 10

To a solution of polymer **9** (10 mg) in CH_3CN/H_2O (3:1, 1 mL) was added CAN (20 mg). The mixture was stirred at room temperature for 1 hour and the solvent was evaporated. Le^X trisaccharide **10** was purified by C-18 reverse phase silica gel chromatography (eluent 20% MeOH in H_2O).

Polymer 9: ^{1}H NMR (500 MHz, CD_3OD): δ = 7.27 (d, *J* = 7.5 Hz, 1H), 6.97 (d, *J* = 7.5 Hz, 1H), 5.05 – 5.18 (m, 2H), 4.49 (d, *J* = 7.8 Hz, 1H), 4.38 (s, 2H); ^{13}C NMR (500 MHz, CD_3OD): δ = 175.95, 158.32, 134.16, 128.20, 114.89, 103.90, 100.57, 100.43.

Le^x 10: ^{1}H NMR (600 MHz, D_2O): δ = 9.78 (s, 1H), 7.91 (d, *J* = 8.4 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 5.30 (d, *J* = 8.2 Hz, 1H), 5.10 (d, *J* = 4.0 Hz, 1H), 4.42 (d, *J* = 7.8 Hz, 1H), 3.40 – 4.32 (m, 16H), 1.95 (s, 3H), 1.15 (d, *J* = 6.6 Hz, 1H); HRMS (MALDI-FTMS $C_{27}H_{39}NO_{16}Na$): calcd.: 656.2161, found: 656.2176.

Polymer 11: ^{1}H NMR (600 MHz, CD_3OD): δ = 7.23 (d, *J* = 7.2 Hz, 1H), 6.97 (d, *J* = 7.2 Hz, 1H), 5.04 (d, *J* = 8.4 Hz, 1H), 4.47 (d, *J* = 7.2 Hz, 1H), 4.37 (s, 2H), 2.70 (dd, *J* = 4.8, 12.2 Hz, 1H).

Polymer 12: ^{1}H NMR (500 MHz, CD_3OD): δ = 7.23 (d, *J* = 7.2 Hz, 1H), 6.97 (d, *J* = 7.2 Hz, 1H), 5.05 – 5.15 (m, 2H), 4.52 (d, *J* = 7.2 Hz, 1H), 4.38 (s, 2H), 2.75 (dd, *J* = 4.8, 12.2 Hz, 1H).

Acknowledgements

This research is supported by the National Institutes of Health (GM 44154 to C. H. W.) and by the National Science Foundation (CHE-9707710) and the Robert A. Welch Foundation to (D. E. B.). We thank Dr. Brenda Case for her initial synthesis of samples of possible polymer supports.

References

[1] A. R. Vaino, K. D. Janda, *J. Comb. Chem.* **2000**, *2*, 579–596; J. S. Früchtel, G. Jung, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 17–42; P. H. H. Hermkens, H. C. J. Otenhejm, D. Rees, *Tetrahedron* **1996**, *52*, 4527–4554; L. A. Thompson, J. A. Ellman, *Chem. Rev.* **1996**, *96*, 555–600; S. J. Shuttleworth, S. M. Allin, P. K. Sharma, *Synthesis* **1997**, 1217–1239.

[2] R. Liang, L. Yan, J. Loebach, M. Ge, Y. Uozumi, K. Sekanina, N. Horan, J. Gildersleeve, C. Thompson, S. Andri, K. Biswas, W. C. Still, D. Kahne, *Science* **1996**, *274*, 1520–1522; P. Hodge, *Chem. Soc. Rev.* **1997**, *26*, 417–424; W. Li, B. Yan, *J. Org. Chem.* **1998**, *63*, 4092–4097; J. C. Briggs, P. Hodge, Z.-P. Zhang, *Tetrahedron.* **1997**, *53*, 3943–3956.

[3] P. H. Toy, K. D. Janda, *Acc. Chem. Res.* **2000**, *33*, 546–554; D. J. Gravert, K. D. Janda, *Chem. Rev.* **1997**, *97*, 489–509; V. N. R. Pillai, M. Mutter, *Acc. Chem. Res.* **1981**, *14*, 122–130.

[4] D. E. Bergbreiter, *Med. Res. Rev.* **1999**, *19*, 439–450; S.-I. Nishimura, K. Yamada, *J. Am. Chem. Soc.* **1997**, *119*, 10555–10556.

[5] D. E. Bergbreiter, B. L. Case, Y.-S. Liu, J. W. Caraway, *Macromol.* **1998**, *31*, 6053–6062.

[6] H. G. Schild, *Prog. Polym. Sci.* **1992**, *17*, 163–249 and reference cited therein.

[7] D. E. Bergbreiter, L. Zhang, V. M. Mariagnanam, *J. Am. Chem. Soc.* **1993**, *115*, 9295–9296.

[8] D. E. Bergbreiter, *Catalysis Today* **1998**, *42*, 389–397.

[9] D. E. Bergbreiter, J. W. Caraway, *J. Am. Chem. Soc.* **1996**, *118*, 6092–6093.

[10] J. P. Chen, A. S. Hoffman, *Biomaterials* **1990**, *11*, 631–634; M. Matsukata, T. Aoki, K. Sanui, N. Ogata, A. Kikuchi, Y. Sakurai, T. Okano, *Bioconjugate Chem.* **1996**, *7*, 96–101; K. Hoshino, M. Taniguchi, H. Kawaberi, Y. Takeda, S. Morohashi, T. Sasakura, *J. Ferment. Bioeng.* **1997**, *83*, 246–252.

[11] I. Y. Galaev, B. Mattiasson, *Trends Biotechnol.* **1999**, *17*, 335–40. D. Meyer, A. Chilkoti, *Nat. Biotechnol.* **1999**, 17, 1112–1115.

[12] K. Witte, P. Sears, R. Martin, C.-H. Wong, *J. Am. Chem. Soc.* **1997**, *119*, 2114–2118.

[13] A. G. Johnston, D. A. Leigh, A. Murphy, J. P. Smart, M. D. Deegan, *J. Am. Chem. Soc.* **1996**, *118*, 10662–10663.

[14] D. Horton, in *Organic Synthesis. Coll. Vol. V*, (Ed.: H. E. Baumgarten), John Wiley & Sons, New York, **1973**, pp. 1–5.

[15] J. Danishefsky, J. R. Allen, *Angew. Chem. Int. Ed.* **2000**, *39*, 836–863.

[16] J. K. Welply, S. Z. Abbas, P. Scudder, J. L. Keene, K. Broschat, S. Casnocha, C. Gorka, C. Steininger, S. C. Howard, J. J. Schmuke, *Glycobiology* **1994**, *4*, 259–265; G. Weitz-Schmidt, D. Stokmaier, G. Scheel, N. E. Nifant'ev, A. B. Tuzikov, N. V. Bovin, *Anal. Biochem.* **1996**, *238*, 184–190; O. Renkonen, S. Toppila, L. Penttila, H. Salminen, J. Helin, H. Maaheimo, C. E. Costello, J. P. Turunen, R. Renkonen, *Glycobiology* **1997**, *7*, 453–461; S. Toppila, J. Lauronen, P. Mattila, J. P. Turunen, L. Penttila, T. Paavonen, O. Renkonen, R. Renkonen, *Eur. J. Immunol.* **1997**, *27*, 1360–1365; G. Weitz-Schmidt, D. Stokmaier, G. Scheel, N. Nifant'ev, A. B.

Tuzikov, N. V. Bovin, *Anal. Biochem.* **1996**, *238*, 184–190; R. Stahn, H. Schafer, F. Kernchen, J. Schreiber, *Glycobiology* **1998**, *8*, 311–319.
[17] X.-L. Sun, Y. Kanie, C.-T. Guo, O. Kanie, Y. Suzuki, C.-H. Wong, *Eur. J. Org. Chem.* **2000**, 2643–2653; M. Mammen, S.-K. Choi, G. M. Whitesides, *Angew. Chem. Int. Ed.* **1998**, *37*, 2754–2794; T. Furuike, S. Aiba, T. Suzuki, T. Takabashi, Y. Suzuki, K. Yamada, S. Nishimura, *J. Chem. Soc., Perkin Trans. 1* **2000**, 2000–2005.
[18] P. Sears, C.-H. Wong, *Science* **2001**, 291, 2344–2350.
[19] P. H. Seeberger, W.-C. Haase, *Chem. Rev.* **2001**, 200, 4349–4393.