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A New Method for Conjugation of Carbohydrates to Proteins Using an Aminooxy-Thiol Heterobifunctional Linker

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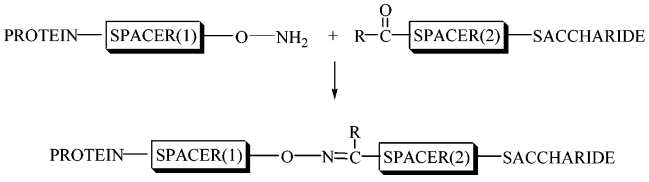

A new, efficient, and mild protocol is presented for the coupling of saccharides to proteins. First, a heterobifunctional aminooxy-thiol linker is coupled to the bromoacylated protein to introduce aminooxy groups through thioether linkages. Condensation of the aminooxylated protein and aldehydo/keto-derivatized carbohydrates affords covalent saccharide−protein constructs. Uncoupled saccharide can be recovered in its original form. The scope of our protocol is exemplified by the coupling of neutral mono- and tetrasaccharides and a negatively charged ribitol-phosphate construct to BSA.

Interest in conjugates of carbohydrates, ranging from mono- to polysaccharides, and proteins dates back to the 1920s when the synthesis and the first serological data for this group of compounds were reported.[1] Protein conjugates of capsular polysaccharides of a range of human pathogens are among the most successful carbohydrate-based drugs, and include licensed human vaccines against *Haemophilus influenzae* type b, *Streptococcus pneumoniae*, and *Neisseria meningitides* group C. Recent progress in synthesis of carbohydrates prompted an interest in vaccines based on synthetic oligosaccharides covalently attached to proteins.[2] A conjugate of a synthetic fragment of the capsular polysaccharide (CPS) of *H. influenzae* type b with tetanus toxoid elicits anti-CPS antibodies in humans at similar titers to those elicited by the polysaccharide-based vaccine.[3,4]

The expensive nature of oligosaccharide synthesis requires an efficient bioconjugation method.[5] Thus, conjugation has to be performed in an aqueous solution, near physiological pH, at ambient temperature, in a short time to avoid denaturation of the protein.[5−9] Ideally, the cyclic structure of the reducing-end moiety should be preserved, and the linker should be attached at this terminus of the saccharide chain. The activating moieties on both the saccharide and the protein should be stable to hydrolytic decomposition. Preferentially, the coupling reaction should proceed with an equimolar ratio of the saccharide and the reactive moieties on the protein. If an excess of the saccharide is necessary, the recovery of the uncoupled saccharide hapten in reusable form is also desirable. Most bioconjugation methods fail to meet these requirements. Recently proposed protocols include the use of homobifunctional linkers such bis-succinimide esters of dicarboxylic acids,[10] bis-*p*-nitrophenyl esters of adipic acid,[11] the Diels−Alder cycloaddition reaction,[12] and the Huisgen cycloaddition between an azide and an acetylenic bond.[13] Other linkers, such as diethyl and dimethyl squarates,[14,15] stand out in recent popularity. Occasional reports on the immune response to the spacer moiety present further challenges in glycoconjugate vaccine synthesis, and it has been found recently that conjugates prepared by the squarate method exhibited decreased immunogenicity.[16] Here we describe a highly efficient method for covalent attachment of mono- and oligosaccharides to proteins which also allows the recovery of uncoupled material in its original, reactive form.

Our approach is based on the well-documented oxime formation between an *O*-alkyl hydroxylamine and aldehydo or keto groups. While chemoselective coupling through oxime formation has precedent in preparing carbohydrate−peptide[17−20] and carbohydrate−lipid conjugates[21] as well as peptide−oligonucleotide[22] and bifunctional oligonucleotide constructs,[23] to our knowledge oxime formation has not been used to prepare saccharide−protein conjugates. According to our protocol, the protein is equipped with a spacer that carries an aminooxy group, whereas the carbohydrate part features an

(1) Avery, O. T.; Goebel, W. F. *J. Exp. Med.* **1929**, *50*, 521−533.
(2) Pozsgay, V. *Adv. Carbohydr. Chem. Biochem.* **2000**, *56*, 153−199.
(3) Fernández-Santana, V.; Cardoso, F.; Rodriguez, A.; Carmenate, T.; Peña, L.; Valdés, Y.; Hardy, E.; Mawas, F.; Heynngnezz, L.; Rodríguez, M. C.; Figueroa, I.; Chang, J.; Toledo, M. E.; Musacchio, A.; Hernández, I.; Izquierdo, M.; Cosme, K.; Roy, R.; Verez-Bencomo, V. *Infect. Immun.* **2004**, *72*, 7115−7123.
(4) Peeters, C. C. A. M.; Evenberg, D.; Hoogerhout, P.; Käyhty, H.; Saarinen, L.; van Boeckel, C. A. A.; van der Marel, G. A.; van Boom, J. H.; Poolman, J. T. *Infect. Immun.* **1992**, *60*, 1826−1833.
(5) Langenhan, J. M.; Thorson, J. S. *Curr. Org. Synth.* **2005**, *2*, 59−81.
(6) Pozsgay, V. *Glycoconjugate J.* **1993**, *10*, 133−141.
(7) Stowell, C. P.; Lee, Y. C. *Adv. Carbohydr. Chem. Biochem.* **1981**, *37*, 225−281.
(8) Davis, B. G. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3215−3237.
(9) Roy, R. *Drug Discovery Today: Technol.* **2004**, *1*, 327−336.
(10) Lahmann, M.; Bulow, L.; Teodorovic, P.; Gyback, H.; Oscarson, S. *Glycoconjugate J.* **2004**, *21*, 251−256.
(11) Wu, X. Y.; Ling, C. C.; Bundle, D. R. *Org. Lett.* **2004**, *6*, 4407−4410.
(12) Pozsgay, V.; Vieira, N. E.; Yergey, A. *Org. Lett.* **2002**, *4*, 3191−3194.
(13) Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 3192−3193.
(14) Tietze, L. F.; Schröter, C.; Gabius, S.; Brinck, U.; Goerlach-Graw, A.; Gabius, H.-J. *Bioconjugate Chem.* **1991**, *2*, 148−153.
(15) Tietze, L. F.; Arlt, M.; Beller, M.; Glüsenkamp, K.-H.; Jähde, E.; Rajewsky, M. F. *Chem. Ber.* **1991**, *124*, 1215−1221.
(16) Mawas, F.; Niggemann, J.; Jones, C.; Corbel, M. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Infect. Immun.* **2002**, *70*, 5107−5114.
(17) Cervigni, S. E.; Dumy, P.; Mutter, M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1230−1232.
(18) Peri, F.; Dumy, P.; Mutter, M. *Tetrahedron* **1998**, *54*, 12269−12278.
(19) Brask, J.; Jensen, K. J. *J. Pept. Sci.* **2000**, *6*, 290−299.
(20) Marcaurelle, L. A.; Shin, Y. S.; Goon, S.; Bertozzi, C. R. *Org. Lett.* **2001**, *3*, 3691−3694.
(21) Perouzel, E.; Jorgensen, M. R.; Keller, M.; Miller, A. D. *Bioconjugate Chem.* **2003**, *14*, 884−898.
(22) Singh, Y.; Defrancq, E.; Dumy, P. *J. Org. Chem.* **2004**, *69*, 8544−8546.
(23) Edupuganti, O. P.; Singh, Y.; Defrancq, E.; Dumy, P. *Chem.−Eur. J.* **2004**, *10*, 5988−5995.

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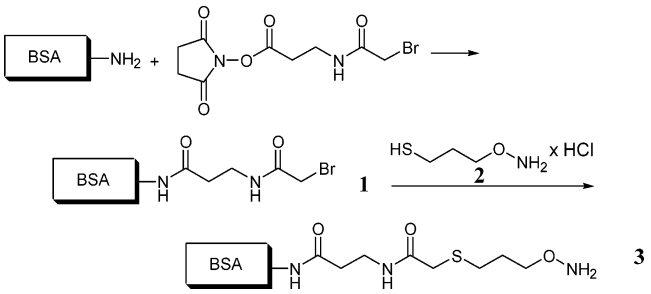
SCHEME 1. Synthesis of Aminooxylated Bovine Serum Albumin



aldehydo or a keto group in its aglycon. Condensation between the carbonyl and the aminooxy groups leads to a stable oxime linkage between the two counterparts.

Aminooxy groups have been created in the protein bovine serum albumin (BSA) in a two-step protocol as illustrated in Scheme 1. First, the protein was treated with the commercially available bromoacetylating reagent succinimidyl 3-(bromoacetamido)propionate that introduced thiol-reactive bromoacetamido moieties in BSA (→ **1**). Under our usual conditions, an average of 30−35 bromoacetyl moieties were incorporated per BSA molecule, as determined by MALDI-TOF MS using sinapinic acid as the matrix, with a yield of 80−90% based on Lowry assay[24] of the BSA recovered by Sephadex G-50 chromatography. The half-life of the activated ester moiety in the bromoacylating reagent in PBS (pH = 7.4) at room temperature is about 5 h as found by NMR spectroscopy. Under these conditions, the bromoacetyl moiety remains stable, as determined by NMR and mass spectrometric measurements. This finding is in some contrast with the observation that chloride ion may replace the bromine in the bromoacetamido group, thus converting it into a less reactive species.[25] In subsequent experiments, the bromoacetylated protein was coupled with the heterobifunctional linker **2**, which has terminal aminooxy and thiol groups, respectively, to form the aminooxylated BSA (**3**) through stable thioether linkages. Linker **2**, a colorless crystalline material, was synthesized by a modification of a published procedure[26] on a 10-g scale. Compound **2** showed no signs of decomposition after more than six months, when stored at ambient temperature. Because replacement of bromine in **1** by the linker **2** results in small increase in the molecular weight only (27 Da/residue), the average number of aminooxy groups per BSA was calculated on the assumption that the difference between the molecular weights of BSA and the aminooxylated BSA **3** is due entirely to the incorporation of the linkers as shown in Scheme 1. However, we have no evidence that every single bromoacetyl moiety in **1** is converted to thioethers. We cannot exclude other processes such as inter- or intramolecular substitution of the bromine with the protein's amino groups or hydrolysis of the bromoacetyl moiety. However, this uncertainty has no effect on the ultimate success of the protocol, as further described below.

The feasibility of our approach was tested with four L-rhamnose derivatives **11** and **13**−**15**, having either aldehydo (**11** and **13**) or keto groups (**14** and **15**) in their aglyconic moieties (Figure 1). These reagents were selected (i) to map the reactivity of the amine (**8**) and hydrazide derivatives (**12**) with the activated oxocarboxy-

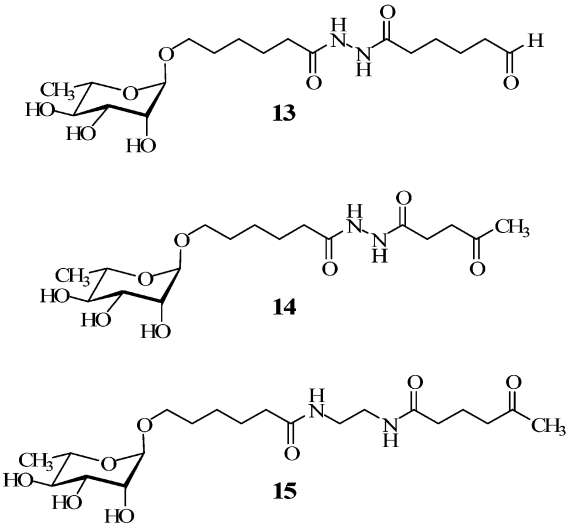


FIGURE 1. L-Rhamnose derivatives **13**−**15**.

lic acids designed to introduce reactive carbonyl groups (see below) and (ii) to compare the reactivities of the aldehydo versus the keto derivatives for oxime formation. Compound **11** was obtained from acetobromo-L-rhamnose (**4**) and 5-methoxycarbonylpentanol[27] (**5**) (Scheme 2). Intermediate **6** was deacetylated (→ **7**) and then treated with 1,2-diaminoethane to afford **8**. N-Acylation of **8** with the heterobifunctional spacer[28] **9** (→ **10**) followed by acid-catalyzed hydrolysis of the intermediate acetal afforded the aldehyde **11**. Compound **12** was prepared similarly, except that the ester **7** was converted to hydrazide **12** by treatment with hydrazine, from which acylation with **9** followed by hydrolysis afforded **13**. Compound **14** was obtained from hydrazide **12** by treatment with levulinic anhydride, and compound **15** was prepared by reaction of the amine **8** with the anhydride of 5-ketohexanoic acid, followed by Bio-Gel P-2 chromatography using 0.02 M pyridine−acetic acid as the eluent, in a yield of 80−90%. The monosaccharide aldehydes (**11**, **13**) and ketones (**14**, **15**) were coupled to aminooxylated BSA **3** at room temperature at pH 5.5 and 7.0, respectively, for 24 h, affording the conjugates as shown in Table 1. In a time-course experiment, we found that the reaction is >90% completed within 6 h. The average level of incorporation was calculated from the increase of the molecular weight of the aminooxylated protein as determined by MALDI-TOF MS, with sinapinic acid as the matrix. Using a 2.5 to 2.7-fold molar excess of the aldehydo haptens **11** or **13**, we substituted two-thirds of the available aminooxy groups in **3** at pH 5.5 (expts 1 and 2). Similar results were obtained with the keto haptens **14** and **15** (expts 3 and 4). A change of the pH from 5.5 to 7.0 had no effect on the average incorporation level (expts 4 and 5). The ability to run the conjugation reactions at neutral pH allows maximum preservation of the antigenicity of the protein. Interestingly, when less than an equimolar

(24) Lowry, J. O.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. *J. Biol. Chem.* **1951**, *193*, 265−275.
(25) Inman, J. K.; Highet, P. F.; Kolodny, N.; Robey, F. A. *Bioconjugate Chem.* **1991**, *2*, 458−463.
(26) Bauer, L.; Suresh, K. S.; Ghosh, B. K. *J. Org. Chem.* **1965**, *30*, 949−951.
(27) Sabesan, S.; Paulson, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 2068−2080.
(28) Pozsgay, V. *J. Org. Chem.* **1998**, *63*, 5983−5999.

SCHEME 2. Synthesis of the Rhamnose Aldehyde 11

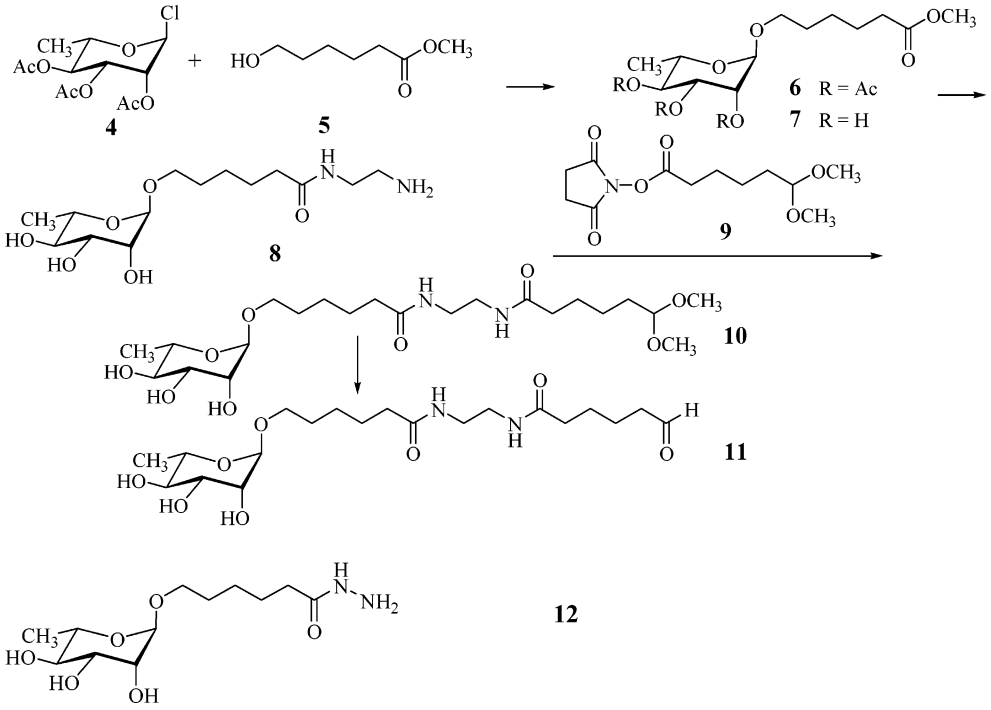
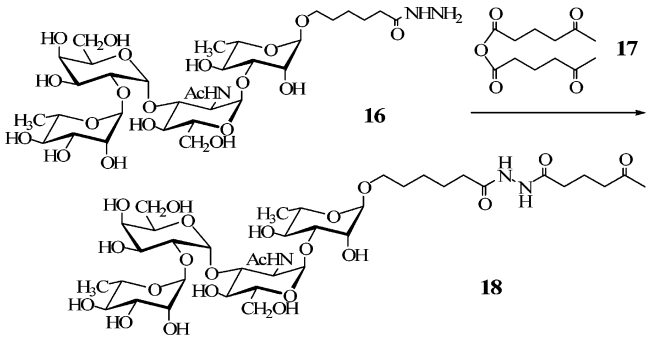


TABLE 1. Conjugation of Haptens 11, 13, 14, 15, and 19 to Aminooxylated BSA 3

expt	amount of BSA (μmol)	ratio of aminooxy groups and BSA[a] (mol/mol)	amount of aminooxy group[a] (μmol)	hapten	amount of hapten (μmol)	pH	molecular mass of conjugate[a] (kDa)	number of bound haptens[a]
1	0.067	34	2.3	**11**	5.8	5.5	83.8	22
2	0.067	34	2.3	**13**	6.2	5.5	83.0	21
3	0.067	34	2.3	**14**	6.2	5.5	82.8	22
4	0.067	34	2.3	**15**	6.2	5.5	83.5	21
5	0.067	34	2.3	**15**	6.2	7.0	83.2	21
6	0.067	34	2.3	**15**	2.0	5.5	83.7	21
7	0.067	34	2.3	**19**	3.7	7.0	81.2	17

[a] Average value.

SCHEME 3. Synthesis of Keto-Tetrasaccharide 18



amount of the keto hapten **15** was used (expt 6), the incorporation level showed no decrease relative to the experiments using an excess of the haptens (expts 1−5). Thus, an excess of the hapten is not necessary for efficient conjugation to occur. The procedure is also suitable for the attachment of higher-membered oligosaccharides to proteins. For example, when a PBS solution of aminooxylated BSA **3** containing an average of 34 aminooxy groups was treated with the ketohexanoyl derivative **18** obtained by treatment of the tetrasaccharide[28] **16** with the anhydride of 5-ketohexanoic acid (**17**) (Scheme 3), MALDI-TOF MS indicated the incorporation of an average of 17 tetrasaccharide chains per BSA (Table 2). The average number of attached ligands was the same when the tetrasaccharide **18** was used in a 10% molar excess only (expt 2), whereas the incorporation level deceased to 11 when 0.55 molar equivalents of the tetrasaccharide were used relative to the aminooxy groups on the protein (expt 3). Just as was the case with the monosaccharide conjugations, uncoupled tetrasaccharide **18** could be recovered in its original keto form and may be recycled, using either gel filtration or ultrafiltration through a 10 kDa cutoff Amicon membrane. Our protocol is also well-suited for the conjugation of charged haptens to proteins. For example, when the D-ribitol-phosphate derivative **20** obtained from the aminoethyl phosphoester[29] **19** by treatment with the anhydride 5-ketohexanoic acid (**17**) (Scheme 4) was allowed to react with aminooxylated BSA **3**, the average number of haptens per BSA was found to be 17, as determined by MALDI-TOF mass spectrometry.

In summary, a new simple and high-yielding protocol is presented for covalent coupling of oligosaccharides to proteins under mild conditions, using a commercially available linker and an easily accessible aminooxy-thiol. The method is likely to be applicable to the prepara-

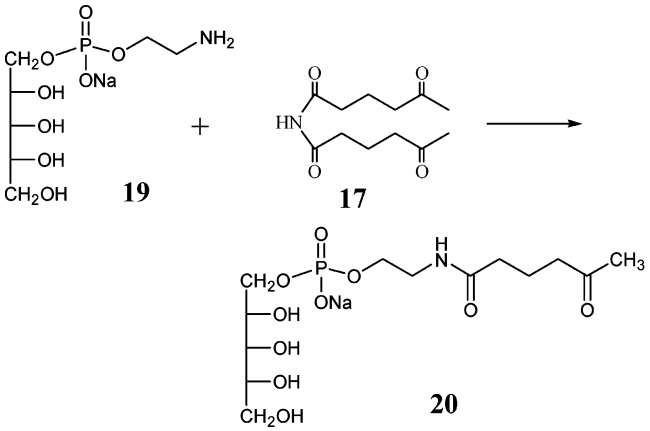
(29) Fekete, A.; Hoogerhout, P.; Kubler-Kielb, J.; Pozsgay, V. National Institutes of Health, Bethesda, MD. Unpublished results, 2005.

TABLE 2. Conjugation of Tetrasaccharide Keto Derivative 18 to Aminooxylated BSA 3

expt	amount of BSA (μmol)	molar ratio of aminooxy groups and BSA[a]	amount of aminooxy groups[a] (μmol)	amount of compd **18** (μmol)	pH	molecular mass of conjugate[a] (kDa)	number of bound haptens[a]
1	0.067	34	2.3	3.4	6.8	89.9	17
2	0.067	34	2.3	2.2	6.8	89.2	17
3	0.067	34	2.3	1.1	6.8	84.4	11

[a] Average value.

SCHEME 4. Synthesis of Keto-Ribitol-Phosphate 20



tion of polysaccharide−protein glycoconjugate vaccines. Another potential use is the construction of carbohydrate-modified solid surfaces that may serve as tools for glycomics such as biosensors and immunoadsorbents for the explorations of interactions of carbohydrates with other biological molecules.

Experimental Section

Preparation of Aminooxylated Bovine Serum Albumin 3. A solution of BSA (90 mg, 1.4 μmol containing 81 μmol of lysine) in 1.5 mL of PBS containing 0.1% glycerol and 1 mM EDTA (buffer A) at pH 7.4 was treated with a solution of succinimidyl 3-(bromoacetamido)propionate (20 mg, 65 μmol) in DMSO (100 μL) at 22 °C at pH 7.4 using an autotitrator. After 2 h, the mixture was applied to a Sephadex G-50 column (1 × 50 cm) in buffer A. The void volume fractions were pooled and analyzed by MALDI-TOF MS. Average MW: 73.1 kDa. Average yield of **1**: 85% as determined by the Lowry assay.[24] Bromoacylated BSA (**1**) (90 mg, 43 μmol of bromoacetyl groups) was treated with a solution of *O*-3-(thiopropyl)hydroxylamine hydrochloride (**2**) (15 mg, 105 μmol) in 1 M K_2HPO_4 (100 μL) for 2 h at 22 °C at pH 7.4 followed by gel filtration as described for **2**. The void volume fractions were pooled and analyzed by MALDI-TOF MS. Average MW: 74.0 kDa. Average yield of **4**: 80% (Lowry).[24]

(Methoxycarbonyl)pentyl 2,3,4-tri-*O*-Acetyl-α-L-rhamnopyranoside (6). To a stirred mixture of acetochloro-L-rhamnose (15 g, 49 mmol), DTBMP (7.0 g, 34 mmol), **5** (10.0 g, 77 mmol), and powdered 4 A molecular sieves (5 g) in CH_2Cl_2 (50 mL) was added AgOTf (15.0 g, 58 mmol) at 0 °C. After 10 min, the mixture was treated with Bu_4NBr (excess). After 5 min, the mixture was shaken with aqueous $NaHCO_3$ and was filtered through Celite. Extractive workup followed by column chromatography on silica gel (4:1 hexanes−EtOAc) to afford **6** (6.0 g) as a syrup: NMR ($CDCl_3$) ^{1}H δ 5.27 (dd, J = 6.0 Hz, J = 9.7 Hz), 5.20 (dd, J = 2.0 Hz, J = 3.3 Hz), 5.04 (t, J = 9.7 Hz), 4.69 (d, J = 2.0 Hz), 3.84 (dq, J = 6.3 Hz), 3.66 (s), 3.40 (m), 2.32 (t, J = 7.4 Hz), 2.13 (s), 2.03 (s), 1.97 (s), 1.62 (m), 1.38 (m), 1.20 (d, J = 6.3 Hz); HRMS *m/z* Calcd for $C_{19}H_{30}O_{10}Na$: 441.1737. Found: 441.1760. Anal. Calcd for $C_{19}H_{30}O_{10}$: C, 54.54; H, 7.23. Found: C, 54.52; H, 7.19.

(Methoxycarbonyl)pentyl α-L-Rhamnopyranoside (7). To a solution of **6** (5.5 g) in MeOH was added NaOMe (cat) at 22 °C. After 3 h, the solution was treated with Dowex 50WX8−100 (H^+) followed by filtration, concentration, and chromatography on silica gel (4:1 → 1:1 hexanes−EtOAc) to afford **7** (3.6) as a crystalline solid: NMR (D_2O) ^{1}H δ 4.79 (d, J = 1.6 Hz), 3.92 (dd, J = 1.6 Hz, J = 3.0 Hz), 3.73 (dd, J = 3.0 Hz, J = 9.3 Hz), 3.69 (s), 3.71−3.66 (m), 3.54 (dq), 3.44 (t, J = 9.3 Hz), 2.41 (t, J = 7.4 Hz), 1.67−1.59 (m), 1.44−1.34 (m), 1.29 (d, J = 6.3 Hz); HRMS *m/z* Calcd for $C_{13}H_{24}O_7Na$: 315.1420. Found: 315.1406. Anal. Calcd for $C_{13}H_{24}O_7$: C, 53.41; H, 8.28. Found: C, 53.60; H, 8.20.

[(2-Aminoethyl)aminocarbonyl]pentyl α-L-Rhamnopyranoside (8). A solution of **7** (1.5 g) in 1,2-diaminoethane (10 mL) was kept at 80 °C for 12 h. The solution was concentrated at reduced pressure at 80 °C. Water was added to and evaporated from the residue (3 × 20 mL). Silica gel column chromatographic purification of the residue (MeOH) afforded **8** (1.7 g) as an amorphous solid: NMR (D_2O) ^{1}H δ 4.78 (d, J = 1.5 Hz), 3.91 (dd), 3.74−3.64 (m), 3.52 (dq), 3.43 (dd, J = 4.0 Hz, J = 9.7 Hz), 3.39 (t, J = 5.9 Hz), 3.24 (t, J = 6.0 Hz), 3.18 (t, J = 6.2 Hz), 2.97 (t, J = 6.0 Hz), 2.29−2.24 (m), 1.62 (m), 1.36 (m), 1.28 (d, J = 6.3 Hz); HRMS *m/z* Calcd for $C_{14}H_{29}N_2O_6$: 321.2026. Found: 321.2020. Anal. Calcd for $C_{14}H_{28}N_2O_6 \times 3H_2O$: C, 44.91; H, 9.15. Found: C, 45.15; H, 8.79.

Compound 10. To a solution of compound **8** (350 mg, 1.05 mmol) in MeOH was added **9** (590 mg, 2.16 mmol) at 22 °C. After 5 h, the solution was concentrated under reduced pressure. Extractive workup (CHC_3/H_2O) followed by freeze-drying of the aqueous layer afforded a solid. Chromatographic purification (1:1 MeOH−EtOAc) afforded compound **10** (360 mg): NMR (D_2O) ^{1}H δ 4. 2 (br s), 3.91 (br), 3.72 (dd, J = 3.35 Hz, J = 9.1 Hz), 3.69−3.63 (m), 3.51 (dq), 3.43 (t, J = 9.1 Hz), 3.30 (s), 2.23 (m), 1.59 (m), 1.35 (m), 1.28 (d, J = 6.3 Hz); HRMS *m/z* Calcd for $C_{20}H_{36}N_2O_8Na$: 455.2369; found: 455.2378. Anal. Calcd for $C_{22}H_{42}N_2O_9$: C, 55.21; H, 8.85. Found: C, 55.35; H, 8.96.

Compound 11. A solution of **10** (440 mg) in 2:1 H_2O−AcOH was kept at 22 °C for 6 h and then was concentrated to afford **11** (420 mg) as an amorphous solid: NMR (D_2O) ^{1}H δ 9.67 (m), 5.02 (t, J = 5.7 Hz), 4.78 (br s), 3.91 (dd), 3.73 (dd, J = 3.3 Hz, J = 9.7 Hz), 3.52 (dq), 3.43 (t, J = 9.7 Hz), 2.24 (m), 1.60 (m), 1.36 (m), 1.28 (d, J = 6.3 Hz); HRMS *m/z* Calcd for $C_{20}H_{36}N_2O_8Na$: 455.2369. Found: 455.2366. Anal. Calcd for $C_{20}H_{36}N_2O_9 \times 2H_2O$: C, 51.27; H, 8.60. Found: C, 51.98; H, 8.39.

General Protocol for the Conjugation of Aminooxylated BSA with Aldehydo and Keto Rhamnoses 11, 13−15. To a solution of aminooxylated BSA (5 mg, 0.067 μmol, containing approximately 2.3 μmol of aminooxy groups) in 1.0 mL of buffer A was added 6.2 μmol of either **13**, **14**, or **15** or 5.8 μmol of **11**. The pH of this solution was adjusted to 5.5 with 0.2 M HCl to a final volume of 1.3 mL. After 12 h at 37 °C, the reaction mixture was applied to a Sephadex G-50 column eluted with buffer A. The void volume fractions were pooled and analyzed by MALDI-TOF MS. Average yield of the reaction: 85% (Lowry).[24] The molecular masses of the conjugates so obtained are listed in Table 1.

Acknowledgment. We acknowledge Andrew Lees' suggestions on the use of oxime chemistry for bioconjugation.

Supporting Information Available: Full experimental details and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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