of 40% peracetic acid/acetic acid (25 mL) was added over 30 min. Upon completion of the addition, the solution was warmed to 0 °C over 30 min and then cooled to -10 °C, whereupon 25 mL of saturated aqueous $NaHSO_3$ was added dropwise over 30 min. Upon warming to room temperature, equal volumes of water and diethyl ether were added. The organic layer was separated, dried over $MgSO_4$, and evaporated to provide a yellow liquid, which was dissolved in boiling CCl_4 and allowed to cool to room temperature to produce **3** as a white solid (6.0 g, 91%): mp 124-126 °C; ^1H NMR (300 MHz, $CDCl_3$, ppm) 6.68 (s, 2 H), 5.38 (br s, 2 H), 3.86 (s, 3 H); ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm) 149.47, 138.52, 116.89, 111.76, 61.26. Anal. Calcd for $C_7H_7O_3Br$ (219.04): C, 38.39; H, 3.22; Br, 36.48. Found: C, 38.34; H, 3.27; Br, 36.66.

2-Methoxybenzene-1,3-diol (4). To a solution of **3** (5 g, 23 mmol) in ethyl acetate (30 mL) was added 5% Pd/C (500 mg), and this suspension was hydrogenated on a Paar shaker to produce **4** quantitatively (3.2 g) as a white solid after filtration of the suspension and evaporation of the filtrate: mp 85-87 °C (lit.[1] mp 84.5-85 °C); ^1H NMR (300 MHz, $CDCl_3$, ppm) 6.88 (t, 1 H, J = 8.3 Hz), 6.51 (d, 2 H, J = 8.2 Hz), 5.31 (br s, 2 H), 3.88 (s, 3 H); ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm) 148.97, 134.63, 124.80, 108.19, 61.17. Anal. Calcd for $C_7H_8O_3$ (140.14): C, 60.00; H, 5.75. Found: C, 59.76; H, 5.72.

3,5-Dibromo-2-methoxyphenol (6). The procedure described above was followed with the exception that 20 mL (32 mmol) of 1.6 M *n*-butyllithium and 3.2 g (32 mmol) of trimethyl borate were used. The product was obtained as a yellow oil, which was distilled (bp 120 °C (0.24 Torr)) to give 7.4 g (87%) of **6** as a colorless oil which solidified upon standing: mp 67-68 °C; ^1H NMR (300 MHz, $CDCl_3$, ppm) 7.22 (d, 1 H, J = 2.3 Hz), 7.08 (d, 1 H, J = 2.3 Hz), 5.75 (br s, 1 H), 3.90 (s, 3 H); ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm) 150.49, 143.91, 126.94, 118.43, 117.61, 116.33, 61.20. Anal. Calcd for $C_7H_6Br_2O_2$ (281.93): C, 29.82; H, 2.14; Br, 56.68. Found: C, 29.89; H, 2.18; Br, 56.93.

Acknowledgment. The assistance of the analytical departments located at Bound Brook, NJ, and Pearl River, NY, is gratefully acknowledged.

Registry No. 1, 607-99-8; 3, 133932-61-3; 4, 29267-67-2; 6, 79893-39-3.

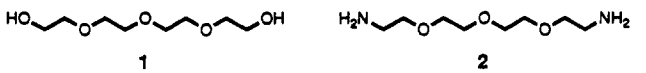
The Synthesis of Heterobifunctional Linkers for the Conjugation of Ligands to Molecular Probes

Carolyn R. Bertozzi[1] and Mark D. Bednarski*[,2]

Department of Chemistry, University of California, Berkeley, California 94720

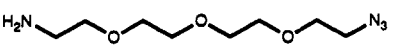
Received December 21, 1990

The availability of bifunctional water-soluble compounds with flexible dimensions is important for the conjugation of small molecules to proteins or molecular probes.[3,4] These bifunctional molecules can be used in antibody production, drug delivery, protein immobilization, and for the study of enzymes and receptors.[5-8] Polyethylene glycol (PEG) derivatives are ideal for these purposes because they are inexpensive, water soluble, and available in a variety of lengths. However, currently available symmetrical PEG derivatives such as diol **1** and diamine **2** are difficult to functionalize selectively.[9]



1 **2**

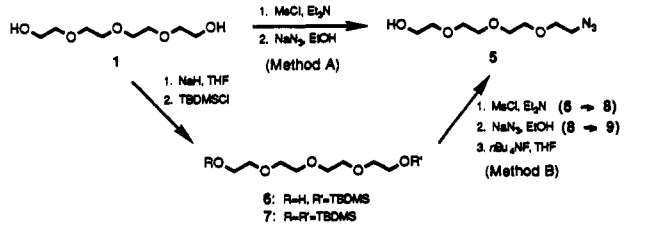
In this paper we describe the synthesis of a heterobifunctional PEG derivative **3**, which contains a free amine



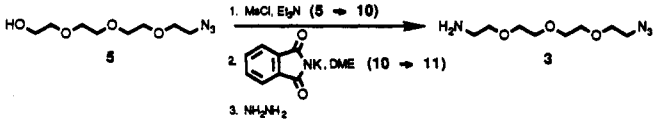
3

that can be conjugated to biological molecules directly by an amide linkage (or via the corresponding isothiocyanate) and an azide that can be reduced to an amine for conjugation to other molecules. The azide reduction can be accomplished by mild, biocompatible reagents such as 1,3-propanedithiol.[10,11] The byproducts of the reduction can be easily removed from the reaction by dialysis or lyophilization and in many cases their presence does not interfere with biological assays. Compound **3** can also be reacted with small organic soluble molecules for the synthesis of heterobifunctional compounds. We have used

Scheme I



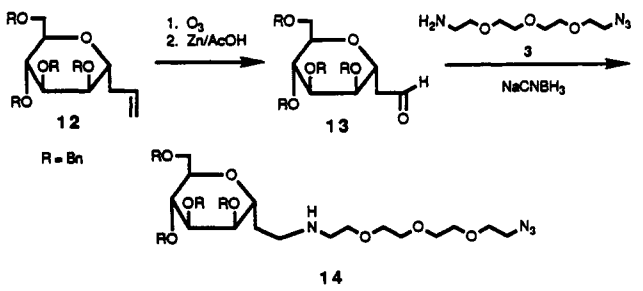
Scheme II



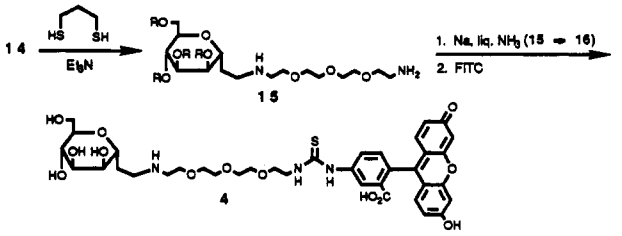
(1) Office of Naval Research predoctoral fellow; AT&T Bell Laboratories GRPW awardee.
(2) American Cancer Society Junior Faculty Awardee 1990-1993, Grant No. JFRA-261.
(3) Lee, R. T.; Wong, T.-C.; Lee, R.; Yue, L.; Lee, Y. C. *Biochemistry* **1989**, *28*, 1856.
(4) Fernandez-Santana, V.; Marino-Albernas, J. R.; Verez-Bencomo, V.; Perez-Martinez, C. S. *J. Carbohydr. Chem.* **1989**, *8*, 531.
(5) Antibody production: (a) Magnusson, G.; Ahlfors, S.; Dahmen, J.; Jansson, K.; Nilsson, U.; Noori, G.; Stenvall, K.; Tjornebo, A. *J. Org. Chem.* **1990**, *55*, 3932. (b) Peeters, J. M.; Hazendonk, T. G.; Beuvery, E. C.; Tesser, G. I. *J. Immunol. Methods* **1989**, *120*, 133. (c) Lemieux, R. U.; Bundle, D. R.; Baker, D. A. *J. Am. Chem. Soc.* **1975**, *97*, 4076.
(6) Drug delivery: (a) Park, B. K.; Kitteringham, N. R. *Drug Metab. Rev.* **1990**, *22*, 87. (b) Laguzza, B. C.; Nichols, C. L.; Briggs, S. L.; Cullinan, G. J.; Johnson, D. A.; Starling, J. J.; Baker, A. L.; Bumol, T. F.; Corvalan, J. R. F. *J. Med. Chem.* **1989**, *32*, 548. (c) Neville, D. M., Jr.; Srinivasachar, K.; Stone, R.; Scharff, J. *J. Biol. Chem.* **1989**, *264*, 14653.
(7) Protein immobilization: (a) Bhatia, S. K.; Shriver-Lake, L. C.; Prior, K. J.; Georger, J. H.; Calvert, J. M.; Bredehorst, R.; Ligler, F. S. *Anal. Biochem.* **1989**, *178*, 408. (b) Janda, K. D.; Ashley, J. A.; Jones, T. M.; McLeod, D. A.; Schloeder, D. M.; Weinhouse, M. I. *J. Am. Chem. Soc.* **1990**, *112*, 8886.
(8) Enzyme and receptor studies: (a) Kozikowski, A. P.; Tuckmantel, W. *Tetrahedron Lett.* **1989**, *30*, 4613. (b) Wang, G. T.; Matayoshi, E.; Huffaker, H. J.; Krafft, G. A. *Tetrahedron Lett.* **1990**, *31*, 6493. (c) Bertozzi, C. R.; Bednarski, M. D. *J. Am. Chem. Soc.* Submitted for publication.
(9) Jacobson, A. R.; Makris, A. N.; Sayre, L. M. *J. Org. Chem.* **1987**, *52*, 2592.
(10) Azide reduction with 1,3-propanedithiol can be accomplished in aqueous pyridine or DMF as well as in absolute methanol or ethanol: Bayley, H.; Sandring, D. N.; Knowles, J. R. *Tetrahedron Lett.* **1978**, 3633.
(11) Alternative methods of azide reduction that are compatible with most biological samples include the following. (a) Dithiothreitol (DTT) in physiological buffers: Staros, J. V.; Bayley, H.; Standring, D. N.; Knowles, J. R. *Biochem. Biophys. Res. Commun.* **1978**, *80*, 568. Cartwright, I. L.; Hutchinson, D. W.; Armstrong, V. W. *Nucleic Acids Res.* **1976**, *3*, 2331. (b) H_2S in aqueous pyridine: Adachi, T.; Yamada, Y.; Inoue, I. *Synthesis* **1977**, *45*. (c) Na_2S/triethylamine: Belinka, B. A., Jr.; Hassner, A. *J. Org. Chem.* **1979**, *44*, 4712. (d) Ph_3P/NH_4OH in pyridine: Mungall, W. S.; Greene, G. L.; Heavner, G. A.; Letsinger, R. L. *J. Org. Chem.* **1975**, *40*, 1659.

0022-3263/91/1956-4326$02.50/0

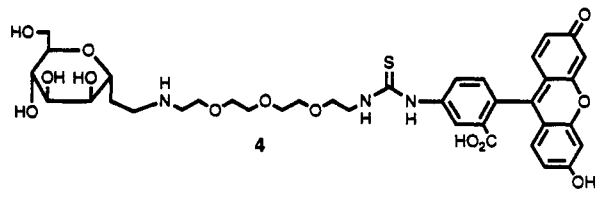
Scheme III



Scheme IV



compound 3 in the synthesis of a mannose–fluorescein conjugate, compound 4, for the study of cell-surface mannose-specific lectins.[8c]



Compound 3 was prepared from commercially available tetraethylene glycol 1 by two methods (Scheme I). The first method involves mesylation (MsCl, Et_3N) of 1 followed by reaction with sodium azide in ethanol to directly give azido alcohol 5 and the corresponding diazide, which are easily separated by silica gel chromatography. Alternatively, compound 1 can be monosilylated by using sodium hydride and *tert*-butyldimethylsilyl chloride (TBDMSCl) in THF to give a 4:1 mixture of mono- to disilylated products 6 and 7, which are also separable by chromatography.[12] Mesylation of compound 6 followed by treatment with sodium azide in ethanol and deprotection with tetra-*n*-butylammonium fluoride (*n*-Bu_4NF) gives compound 5. The monosilylated alcohol 6 can serve as a precursor to linkers with functional groups other than amines and azides.[13]

Compound 5 was mesylated and subjected to a Gabriel amine synthesis to give azido amine 3 (Scheme II). We have synthesized approximately 7 g of compound 3 from 1 using these procedures.

The synthesis of the mannose–fluorescein conjugate 4 begins with the construction of the *C*-glycoside of mannose 12 as previously described.[14] Compound 12 was ozonized and reduced with zinc/acetic acid to give aldehyde 13 (Scheme III).[15,16] Reductive amination of compound 13 with linker 3 gave mannose-linker conjugate 14. Reduction of the azide using 1,3-propanedithiol followed by debenzylation using sodium in liquid ammonia (Na, liquid NH_3) and reaction with fluorescein isothiocyanate (FITC) gave conjugate 4 (Scheme IV). We have used compound 4 to study mannose-specific lectins on bacterial cell surfaces.[8c]

In summary, we have synthesized a convenient azido amine linker 3 and demonstrated its use in the synthesis of a carbohydrate–fluorescein conjugate for studying carbohydrate-binding proteins. The linker is easily prepared from tetraethylene glycol, and the synthesis can be applied to other PEG derivatives of various lengths. We feel that compound 3 and its derivatives will find use in the conjugation of biomolecules to proteins, drugs, or other probes.

Experimental Section

General Procedures. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Dichloromethane (CH_2Cl_2) and triethylamine (Et_3N) were distilled from calcium hydride, tetrahydrofuran (THF) and dimethoxyethane (DME) were distilled from potassium/benzophenone ketyl, methanol was distilled from magnesium/iodine, and methanesulfonyl chloride was distilled from P_2O_5. The internal standard for ^1H and ^{13}C spectra determined in D_2O was 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt. Mass spectral data are tabulated as *m/e* (intensity expressed as percent of the base peak). Elemental analyses for all compounds characterized by high-resolution mass spectrometry were not available due to the viscosity of the compounds.

1-[(*tert*-Butyldimethylsilyl)oxy]-11-hydroxy-3,6,9-trioxaundecane (6). A solution of 200 g (1.04 mol) of tetraethylene glycol in 400 mL of dry THF was cooled to 0 °C under a nitrogen atmosphere, after which sodium hydride (80% dispersion in mineral oil, 9.4 g, 0.39 mol) was added. A solution of *tert*-butyldimethylsilyl chloride (60.8 g, 0.39 mol) in 250 mL of dry THF was then added via syringe over a 2-h period, and the mixture was stirred at room temperature for 30 min before removal of the THF in vacuo. The remaining residue was dissolved in cyclohexane and washed twice with water (50 mL). The cyclohexane layer was dried and concentrated in vacuo to afford 195 g of a slightly yellow oil, which was shown by ^1H NMR to be a 4:1 mixture of mono- and disilylated tetraethylene glycols, respectively. A portion of the crude mixture was loaded onto a 1-L bed of silica gel and eluted with a gradient of 1:0 to 1:1 cyclohexane/ethyl acetate. Removal of solvents in vacuo gave 25.0 g of monosilylated glycol 6. An analytical sample was purified by distillation using a Kugelrohr apparatus: bp 198–200 °C (0.10 Torr); IR (thin film) 3463, 2931, 2857, 1644, 1473, 1362, 1255, 1107, 940, 834, 778 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.04 (s, 6 H), 0.87 (s, 9 H), 2.64 (t, 1 H, J = 6.06), 3.53 (t, 2 H, J = 5.51), 3.58 (m, 2 H), 3.64 (m, 8 H), 3.70 (m, 2 H), 3.74 (t, 2 H, J = 5.26); ^{13}C NMR ($CDCl_3$) δ -5.32, 18.33, 25.89, 61.72, 62.68, 70.34, 70.61, 70.67, 72.48, 72.64; mass spectrum (GC–MS) 251.10 (M-57 (C_4H_9), 10) 89.05 (100). Anal. Calcd for $C_{14}H_{32}O_5Si$: C, 54.51; H, 10.46. Found: C, 53.49; H, 10.66.

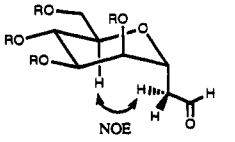
1-[(*tert*-Butyldimethylsilyl)oxy]-11-[(methanesulfonyl)oxy]-3,6,9-trioxaundecane (8). A solution of 20.5 g (66.7 mmol) of 6 in 400 mL of dry Et_3N was cooled to 0 °C under a nitrogen

(12) The use of sodium hydride produces the monosodium salt which seems to precipitate from the reaction mixture. Upon addition of TBDMSCl, monosilylation occurs by reaction of the sodium salt preferentially over the free alcohol.

(13) Bertozzi, C. R.; Bednarski, M. D. Unpublished results.

(14) Compound 12 was synthesized from methyl (2,3,4,6-tetra-*O*-benzyl)-α-D-mannopyranoside according to the procedure of Hosomi, A.; Sakata, Y.; Sakurai, H. *Carbohydr. Res.* **1987**, *171*, 223.

(15) The stereochemistry of compound 12 was confirmed by 2D NOE studies performed on aldehyde 13. A NOE was observed between the axial proton on C-4 of the pyranose ring and a methylene proton adjacent to the aldehyde on the carbon-linked side chain:



13

(16) Compound 13 has been synthesized previously using a similar method: Panek, J. S.; Sparks, M. A. *J. Org. Chem.* **1989**, *54*, 2034.

atmosphere. Methanesulfonyl chloride (15.3 g, 0.133 mol) was added via syringe over a 30-min period, and the solution was warmed to room temperature. After stirring for 3 h, the solution was concentrated in vacuo. The resulting mixture was dissolved in CH_2Cl_2 and washed twice with water (50 mL). The organic layer was dried and concentrated in vacuo to afford a brown residue, which was diluted with cyclohexane and filtered. The filtrate was concentrated in vacuo to afford a brown oil, which was used without further purification: ^1H NMR (400 MHz, $CDCl_3$) δ 0.04 (s, 6 H), 0.86 (s, 9 H), 3.05 (s, 3 H), 3.52 (t, 2 H, J = 5.5), 3.60–3.65 (m, 8 H), 3.72–3.75 (m, 4 H), 4.34–4.36 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ -5.32, 18.30, 25.87, 37.67, 62.66, 68.97, 69.23, 70.49, 70.61, 70.68, 72.64.

1-Azido-11-[(*tert*-butyldimethylsilyl)oxy]-3,6,9-trioxaundecane (9). A solution of 25.8 g (66.7 mmol) of crude mesylate 8 and 8.67 g (0.133 mol) of sodium azide in 400 mL of 95% ethanol was heated at reflux for 8 h. After cooling to room temperature, the ethanol was removed in vacuo, and the remaining mixture was diluted with 300 mL of cyclohexane. The solution was washed twice with water (50 mL), dried, and concentrated in vacuo to give the crude product, which was purified by silica gel chromatography eluting with a gradient of 20:1 to 15:1 cyclohexane/ethyl acetate to afford 17.0 g (73% based on 6) of a colorless oil: mp 210 °C dec; IR (thin film) 2861, 2104, 1472, 1464, 1361, 1350, 1291, 1252, 1111, 938, 834, 777, 661 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.04 (s, 6 H), 0.87 (s, 9 H), 3.37 (t, 2 H, J = 5.2), 3.54 (t, 2 H, J = 5.5), 3.64–3.67 (m, 10 H), 3.74 (t, 2 H, J = 5.3); ^{13}C NMR ($CDCl_3$) δ -5.31, 18.33, 25.89, 50.64, 62.67, 69.99, 70.63, 70.70, 72.62; mass spectrum (GC–MS) 305.2 (M – 28 (N_2), 43), 73.1 (100), 248.2 (88). Anal. Calcd for $C_{14}H_{31}O_4N_3Si$: C, 51.04; H, 8.26; N, 12.75. Found: C, 51.56; H, 9.64; N, 12.16.

1-Azido-11-hydroxy-3,6,9-trioxaundecane (5). Method A. A solution of 50.0 g (0.260 mol) of tetraethylene glycol, 50 mL of dry Et_3N, and 200 mL of dry ether was cooled to 0 °C under a nitrogen atmosphere. Methanesulfonyl chloride (15.0 g, 0.130 mol) was added over a 3-h period, after which the solution was allowed to warm slowly to room temperature. The reaction contents were concentrated in vacuo, and 300 mL of 95% ethanol and 18.0 (0.280 mol) of sodium azide were added. The mixture was heated at reflux for 24 h, cooled to room temperature, and concentrated in vacuo. The remaining mixture was diluted with 400 mL of ether, washed twice with brine (100 mL), and dried. Concentration in vacuo afforded the crude product, which was purified by silica gel chromatography eluting with a gradient of 1:1 to 3:1 ethyl acetate/hexanes to afford approximately 25 g (44%) of a viscous oil.

Method B. To a solution of 15.0 g (43.0 mmol) of 9 in 250 mL of THF was added 51.0 mL of a 1.0 M solution of tetra-*n*-butylammonium fluoride in THF (51.0 mmol). After 2 h the reaction was concentrated in vacuo to afford a brown viscous oil. The crude product was purified by silica gel chromatography, eluting with a gradient of 1:1 to 3:1 ethyl acetate/hexanes to afford 75.5 g (80%) of a viscous oil. An analytical sample can be obtained from the crude material from either method A or B by distillation using a Kugelrohr apparatus: bp 120–124 °C (0.10 Torr); IR (thin film) 3444, 2872, 2105, 1647, 1453, 1348, 1301, 1124, 936, 887, 851 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.54 (br t, 1 H, J = 5.9), 3.37 (t, 2 H, J = 5.2), 3.58–3.60 (m, 2 H), 3.64–3.67 (m, 10 H), 3.69–3.72 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 50.60, 61.66, 69.98, 70.27, 70.52, 70.59, 70.63, 72.43; mass spectrum (GC–MS) 191.2 (M – 28 (N_2), 3.7), 89.1 (100). Anal. Calcd for $C_8H_{17}O_4N_3$: C, 43.83; H, 7.82; N, 19.17. Found: C, 43.56; H, 8.17; N, 19.11.

1-Azido-11-[(methanesulfonyl)oxy]-3,6,9-trioxaundecane (10). A solution of 11.7 g (53.5 mmol) of compound 5 and 12 mL of dry Et_3N in 350 mL of dry CH_2Cl_2 was cooled to 0 °C under a nitrogen atmosphere. Methanesulfonyl chloride (7.35 g, 64.5 mmol) was added dropwise via syringe over a 20-min period, and the solution was warmed to room temperature and stirred for 1.5 h. The mixture was then washed twice with saturated aqueous $NaHCO_3$ (100 mL) and three times with water (50 mL). The organic layer was dried and concentrated in vacuo to afford a brown oil, which was used in the next step without further purification: ^1H NMR (400 MHz, $CDCl_3$) δ 3.04 (s, 3 H), 3.36 (t, 2 H, J = 5.2), 3.62–3.65 (m, 10 H), 3.73–3.75 (m, 2 H), 4.33–4.36 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 37.57, 50.57, 68.90, 69.23, 69.94, 70.50, 70.55, 70.58.

1-Azido-11-phthalimido-3,6,9-trioxaundecane (11). A solution of 13.2 (44.0 mmol) of mesylate 10 and 10.5 g (57.0 mmol) of potassium phthalimide in 400 mL of dry DME was heated at reflux for 30 min under a nitrogen atmosphere. After cooling to room temperature and concentration in vacuo, the resulting residue was diluted with benzene and the solids were filtered. Concentration of the filtrate in vacuo followed by purification of the crude product by silica gel chromatography (4:1 cyclohexane/ethyl acetate) afforded 11.4 g (75%) of phthalimide 11: mp 200 °C dec; IR (thin film) 2871, 2105, 1774, 1710, 1616, 1467, 1429, 1394, 1352, 1305, 1189, 1119, 1026, 920, 874, 794, 720 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.33 (t, 2 H, J = 5.2), 3.55–3.63 (m, 10 H), 3.71 (t, 2 H, J = 5.8), 3.86 (t, 2 H, J = 5.9), 7.67–7.69 (m, 2 H), 7.80–7.82 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 37.18, 50.57, 67.82, 69.90, 70.02, 70.53, 70.58, 123.12, 132.04, 133.84, 168.15; mass spectrum (EI) 320 (M – 28 (N_2), 2.95), 174 (100). Anal. Calcd for $C_{16}H_{20}O_5N_4$: C, 55.17; H, 5.79; N, 16.08. Found: C, 55.55; H, 5.73; N, 15.99.

1-Amino-11-azido-3,6,9-trioxaundecane (3). A solution of 11.3 g (32.5 mmol) of phthalimide 11 and 3.7 mL of 55% hydrazine hydrate in 150 mL of absolute methanol was heated at reflux for 2 h, during which time a white precipitate formed. The mixture was cooled to room temperature and concentrated in vacuo, after which the crude residue was diluted with 150 mL of water and 25 mL of concentrated HCl and heated at reflux for 1 h. The resulting suspension was cooled to 0 °C and the solids were filtered. The aqueous filtrate was neutralized with 1 M NaOH and then concentrated in vacuo. The residue was diluted with CH_2Cl_2, washed with 4 M NaOH, and dried over Na_2SO_4. Concentration in vacuo afforded 6.8 g (96%) of a colorless oil. Attempts to obtain an analytical sample by distillation using a Kugelrohr apparatus were accompanied by partial decomposition: bp 198–202 °C (0.10 Torr); IR (thin film) 3377, 2865, 2106, 1594, 1456, 1346, 1301, 1121, 1034, 992, 938, 854 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.31 (br s, 2 H), 2.78 (t, 2 H, J = 5.2), 3.31 (t, 2 H, J = 5.3), 3.43 (t, 2 H, J = 5.2), 3.54–3.61 (m, 10 H); ^{13}C NMR ($CDCl_3$) δ 41.63, 50.48, 69.86, 70.10, 70.45, 70.47, 70.52, 73.33; high-resolution mass spectrum (FAB$^+$) calcd for $C_8H_{19}N_4O_3$ $(MH)^+$ 219.1457, found 219.1453.

2-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)acetaldehyde (13). A solution of 19.0 g (34.4 mmol) of compound 12[14] in 500 mL of dry CH_2Cl_2 was cooled to -78 °C under a nitrogen atmosphere. Ozone was bubbled through the solution until saturated, after which the solution was purged with nitrogen to remove the excess ozone. Zinc dust (22.5 g, 340 mmol) and 8.25 mL of glacial acetic acid were then added, and the reaction was warmed to room temperature and stirred for 1 h. The suspension was filtered, and the filtrate was concentrated in vacuo. The crude product was purified by silica gel chromatography eluting with a gradient of 15:1 to 5:1 cyclohexane/ethyl acetate to afford 11.6 g (61%) of a thick colorless syrup: IR (thin film) 3435, 3087, 3063, 3029, 2923, 2867, 2730, 1954, 1877, 1810, 1726, 1605, 1585, 1496, 1454, 1365, 1207, 1102, 1028, 738, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.59 (ddd, 1 H, J = 2.5, 8.0, 16.3), 2.68 (ddd, 1 H, J = 2.1, 5.1, 16.3), 3.60 (dd, 1 H, J = 2.2, 7.8), 3.71 (dd, 1 H, J = 5.6, 10.2), 3.78 (m, 2 H), 3.83 (dd, 1 H, J = 6.7, 10.2), 3.98 (m, 1 H), 4.42–4.55 (m, 9 H), 7.19–7.35 (m, 20 H), 9.71 (t, 1 H, J = 2.3); ^{13}C NMR ($CDCl_3$) δ 45.55, 66.17, 68.18, 71.33, 72.45, 72.51, 73.23, 74.01, 74.22, 74.46, 75.71, 127.60, 127.69, 127.81, 127.85, 127.87, 128.08, 128.34, 128.39, 128.42, 137.66, 137.86, 137.94, 138.21, 200.55; high-resolution mass spectrum (FAB$^+$) calcd for $C_{36}H_{38}O_6Na$ $(M + Na)^+$ 589.2566, found 589.2573.

1-Azido-11-[[2-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)ethyl]amino]-3,6,9-trioxaundecane (14). A solution of amine linker 3 (3.00 g, 13.7 mmol) in 50 mL of absolute methanol was adjusted to pH 7 with methanolic HCl. This solution was then added to a solution of 5.00 g (8.80 mmol) of aldehyde 13 in 200 mL of absolute methanol and stirred for 10 min. Sodium cyanoborohydride (0.38 g, 6.00 mmol) was added, and the solution was stirred at room temperature for 24 h. The solution was adjusted to pH 10 with 4 M NaOH and then concentrated in vacuo. The resulting residue was diluted with 200 mL of water and extracted with one 150-mL portion and then two 50-mL portions of CH_2Cl_2. The organic extracts were dried and concentrated in vacuo to give the crude product which was purified by silica gel chromatography, eluting with ethyl acetate

(1% Et_3N) to afford 2.74 g (41%) of a colorless syrup: IR (thin film) 3330, 3088, 3062, 3029, 2867, 2102, 1955, 1884, 1810, 1454, 1362, 1302, 1103, 737, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.58–1.85 (m, 3 H), 2.60–2.75 (m, 4 H), 3.35 (t, 2 H, J = 5.2), 3.54 (t, 2 H, J = 5.3), 3.58–3.61 (m, 3 H), 3.63–3.66 (m, 8 H), 3.70 (dd, 1 H, J = 2.9, 9.8), 3.75–3.81 (m, 3 H), 3.84 (dd, 1 H, J = 6.6, 13.4), 4.07 (d app t, 1 H, J = 4.2, 8.9), 4.50–4.64 (m, 7 H), 4.71 (d, 1 H, J = 11.4), 7.18–7.20 (m, 2 H), 7.25–7.37 (m, 18 H); ^{13}C NMR ($CDCl_3$) δ 30.00, 46.61, 49.20, 50.56, 69.16, 69.93, 70.25, 70.53, 70.60, 71.41, 71.60, 72.01, 73.18, 73.35, 73.72, 74.87, 75.99, 127.37, 127.50, 127.53, 127.59, 127.74, 127.79, 127.87, 128.19, 128.22, 128.25, 128.26, 138.12, 138.20, 138.24, 138.33; high-resolution mass spectrum (FAB$^+$) calcd for $C_{44}H_{57}O_8N_4$ $(MH)^+$ 769.4176, found 769.4169.

1-Amino-11-[[2-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)ethyl]amino]-3,6,9-trioxaundecane (15). To a solution of 5.99 g (7.79 mmol) of compound 14 in 50 mL of absolute methanol were added 5.41 mL (38.9 mmol) of dry Et_3N and 4.22 g (38.9 mmol) of 1,3-propanedithiol under a nitrogen atmosphere. The solution was stirred at room temperature for 48 h and then concentrated in vacuo. The crude residue was purified by silica gel chromatography eluting with 40:1 chloroform/methanol (0.5% Et_3N) to give 5.15 g (89%) of a colorless oil: IR (thin film) 3316, 3062, 3029, 2916, 2867, 1954, 1880, 1812, 1455, 1363, 1101, 738, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.58–1.68 (m, 1 H), 1.73–1.84 (m, 1 H), 2.05 (br s, 3 H), 2.60–2.73 (m, 4 H), 2.82 (t, 2 H, J = 5.3), 3.48 (t, 2 H, J = 5.3), 3.52 (t, 2 H, J = 5.2), 3.55–3.57 (m, 3 H), 3.59–3.63 (m, 6 H), 3.67 (dd, 1 H, J = 2.6, 9.7), 3.74–3.84 (m, 4 H), 4.05 (d app t, 1 H, J = 4.2, 8.9), 4.48–4.62 (m, 7 H), 4.69 (d, 1 H, J = 11.3), 7.16–7.19 (m, 2 H), 7.24–7.35 (m, 18 H); ^{13}C NMR ($CDCl_3$) δ 29.94, 41.61, 46.65, 49.24, 69.19, 70.25, 70.44, 70.49, 71.53, 71.60, 72.13, 73.03, 73.25, 73.43, 73.71, 74.95, 76.12, 77.23, 127.45, 127.57, 127.60, 127.64, 127.67, 127.80, 127.84, 127.94, 128.26, 128.29, 128.31, 128.33, 138.19, 138.25, 138.29, 138.37; high-resolution mass spectrum (FAB$^+$) calcd for $C_{44}H_{59}O_8N_2$ $(MH)^+$ 743.4271, found 743.4270.

1-Amino-11-[[2-(α-D-mannopyranosyl)ethyl]amino]-3,6,9-trioxaundecane (16). To a solution of 3.20 g (4.31 mmol) of compound 15 in 30 mL of dry DME at −42 °C were added 75 mL of liquid ammonia under an atmosphere of ammonia. Sodium metal was added to the solution until a dark blue color persisted. The solution was stirred for 30 min, and the excess sodium was decomposed with a saturated solution of NH_4Cl in methanol. The ammonia was allowed to evaporate at room temperature, and the solution was concentrated in vacuo. The crude residue was dissolved in water/methanol and applied to a 100-mL column of Bio-Rad AG50W-X4 H^+ resin. The column was eluted first with water/methanol and then with 1 M NH_4OH (10% methanol). The fractions containing the product (detected with ninhydrin) were combined and concentrated in vacuo to afford 1.55 g (94%) of a slightly brown oil, which was used in the next step without further purification: IR (Nujol) 3698–2537 (br), 1586, 1154, 1026, 722 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 1.65–1.77 (m, 1 H), 1.97–2.10 (m, 1 H), 2.68–2.89 (m, 6 H), 3.52–3.78 (m, 16 H), 3.80–3.89 (m, 2 H), 3.95–4.01 (m, 1 H); ^{13}C NMR (D_2O) δ 26.55, 39.33, 45.07, 47.23, 60.91, 67.09, 68.71, 69.09, 69.13, 69.36, 70.45, 71.14, 73.54, 76.30; high-resolution mass spectrum (FAB$^+$) calcd for $C_{16}H_{35}O_8N_2$ $(MH)^+$ 383.2393, found 383.2398.

Mannose–Fluorescein Conjugate (4). Fluorescein isothiocyanate (FITC) (13 mg, 0.03 mmol) was added to a solution of compound 16 (13 mg, 0.03 mmol) in 20 mL of 100 mM $NaHCO_3$ buffer (pH 9). The solution was stirred for 6 h and neutralized with 1 M HCl. The crude reaction mixture was applied to a 10 mL column of Bio-Rad AG50W-X4 H^+ resin, and the column was washed with water and methanol to remove the unreacted FITC. The column was then rinsed with 1.5 M NH_4OH, and the fractions containing the product (detected by an orange color) were concentrated in vacuo to afford a fluffy orange solid. The product was characterized by ^1H NMR and mass spectrometry. Compound 4 also inhibited the mannose-specific adhesion of *E. coli* to yeast cells:[8c] ^1H NMR (400 MHz, D_2O) δ 1.55–1.80 (br m, 1 H), 1.85–2.15 (br m, 1 H), 2.80–3.10 (br m, 2 H), 3.20–3.85 (br m, 23 H), 6.20–6.53 (br m, 5 H), 6.70–7.60 (br m, 6 H); mass spectrum (FAB$^+$) 770 (M^+ − H).

Acknowledgment. This research was supported by National Institute of Health award R29 GM43037-02 and the Procter & Gamble University Exploratory Research Program. C.B. thanks the Office of Naval Research (ONR) and AT&T Bell Laboratories for graduate fellowships. M.B. thanks the American Cancer Society for a Junior Faculty Award.

Supplementary Material Available: ^1H and ^{13}C NMR spectra of all new compounds (23 pages). Ordering information is given on any current masthead page.

Selective Hydrogenations Promoted by Copper Catalysts. 1. Chemoselectivity, Regioselectivity, and Stereoselectivity in the Hydrogenation of 3-Substituted Steroids

Nicoletta Ravasio*

Centro CNR di Studio sulle Metodologie Innovative di Sintesi Organiche, Dipartimento di Chimica, Università di Bari, via Amendola 173, I-70126 Bari, Italy

Michele Rossi

Dipartimento di Chimica Inorganica e Metallorganica e Centro CNR, Università di Milano, via Venezian 21, I-20133 Milano, Italy

Received March 28, 1990

Catalytic hydrogenation of steroids has been widely investigated.[1-6] Among many catalytic systems, those based on Pd are of general applications for the specific reduction of olefinic double bonds owing to satisfactory performance in activity and chemoselectivity.

In many cases, however, poor stereoselectivity is observed as, for example, in the hydrogenation of 4-en-3-one steroids, where mixtures of 5α and 5β ketones are obtained. Useful modifications of the catalytic system have been proposed, as the use of acids or bases,[1] to increase the yield of 5β derivatives. Best results were obtained by means of substituted pyridines as solvents.[4]

On the contrary, homogeneous catalysts, based on noble-metal complexes, are of great importance for high chemoselectivity and, particularly, because they allow the preparation of pure 5α derivatives.[7-12] Limitations in their use are low activity and separation problems.

Although copper catalysts are widely used in industrial chemical processes for the hydrogenation of different compounds (e.g., CO to methanol;[13] fat esters and oxo

(1) Augustine, R. L. In *Organic Reactions in Steroid Chemistry*; Fried, J., Edwards, J. A., Eds.; Van Nostrand Reinhold: New York, 1972; Vol. 1, Chapter 3.
(2) Augustine, R. L. *Advances in Catalysis*; Academic Press: New York, 1976; Vol. 25, p 56.
(3) Nishimura, S.; Ishige, M.; Shiota, M. *Chem. Lett.* **1977**, 963.
(4) Tsuji, N.; Suzuki, J.; Shiota, M.; Takahashi, I.; Nishimura, S. *J. Org. Chem.* **1980**, *45*, 2729.
(5) Ishige, M.; Shiota, M. *Can. J. Chem.* **1980**, *58*, 1061.
(6) Nishimura, S.; Momma, Y.; Kawamura, H.; Shiota, M. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 780.
(7) Voelter, W.; Djerassi, C. *Chem. Ber.* **1968**, *101*, 58.
(8) Nishimura, S.; Tsuneda, K. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 852.
(9) Nishimura, S.; Ichino, T.; Akimoto, A.; Tsuneda, K. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 279.
(10) Nishimura, S.; Yumoto, O.; Tsuneda, K.; Mori, H. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2603.
(11) Suggs, J. W.; Cox, S. D.; Crabtree, R. H.; Quirk, J. M. *Tetrahedron Lett.* **1981**, *22*, 303.
(12) Kollar, L.; Toros, S.; Heil, B.; Tuba, Z. *J. Mol. Catal.* **1988**, *47*, 33.
(13) Klier, K. *Advances in Catalysis*; Academic Press: New York, 1982; Vol. 31, p 243.

0022-3263/91/1956-4329$02.50/0