Enzymatic Synthesis of L-Fucose and Analogs

Chi-Huey Wong,* Ramón Alajarín, Francisco Morís-Varas, Olga Blanco, and Eduardo García-Junceda

Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California 92037

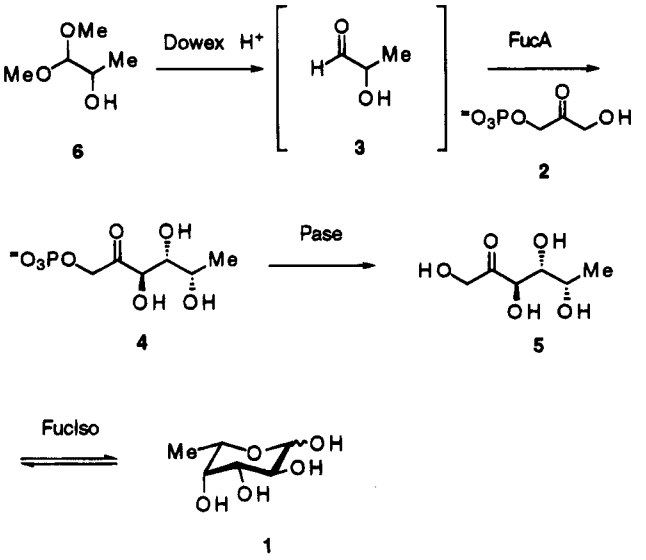
Received May 4, 1995

L-fucose **1** is a naturally occurring sugar widely found in nature. It is found in many bacterial and plant glycosides and polysaccharides,[1] sometimes sulfated,[2] in oligosaccharides of human milk,[3] and in many glycolipids[4] and glycoproteins[5] including several families of blood-group antigens.[5a] It is also found in cell-surface oligosaccharides, such as the tetrasaccharide sialyl Lewis x (Le^x) on neutrophils, as part of selectin ligands involved in cell adhesion[6] and cancer metastasis processes.[7]

Given the importance of sialyl Le^x and derivatives[8,9] as potencial therapeutic agents for the treatment of inflammatory diseases,[9c] the development of an efficient synthesis of **1** has been a subject of interest in glycotechnology.[8,10,11]

A few methods were reported for the synthesis of **1**, and they all gave low yields *via* multichemical transformations: It was prepared from L-arabinose[12] (1%, nine steps), from D-galactose[13] (15%, 4.88 mmol, 4 steps), from D-glucose[14] (19.3%, 1.69 mmol, five steps), and from D-mannose[15] (54.8%, six steps) or methyl α-D-mannopyranoside[16] (24%, 0.26 mmol, eight steps). The main natural source for **1** is Fucoidan extracted from kelp.[17] To our knowledge, there is no method available for the enzymatic preparation of **1**.

Scheme 1



We here report an enzymatic synthesis of **1** (Scheme 1) from dihydroxyacetone phosphate **2** (DHAP) and DL-lactaldehyde **3** catalyzed by L-fuculose-1-phosphate aldolase, followed by reaction with acid phosphatase and L-fucose isomerase. Two methods were carried out: the stepwise synthesis and the sequential synthesis. Both procedures have also been succesfully used to synthetize six L-fucose analogs modified at position 5.

The methods are strategically the same as those previously described for the synthesis of L-glucose,[18] except that the aldolase of choice is L-fuculose-1-phosphate aldolase instead of L-rhamnulose-1-phosphate aldolase because of the inverted stereochemistry in C-4. Both the stepwise and the sequential approaches to the synthesis of **1** involve L-fuculose-1-phosphate **4** and L-fuculose **5** as intermediates. The synthesis of **4** has been previously carried out from **2** and **3**[19a,c,d] *via* recombinant L-fuculose aldolase-catalyzed aldolic condensation, using the L-enantiomer of **3**[19a,b,d] or *via* L-fucose isomerase-catalyzed isomerization of **1** coupled with L-rhamnulose kinase[19a,c] reaction. Compound **5** has been also prepared from **1** using the cell-free extract of an *Escherichia coli* mutant strain in the presence of borate buffer,[20] *via* bacterial oxidation of L-fucitol by *Aerobacter suboxidans*[21] and from **4** *via* enzyme-catalyzed phosphate hydrolysis.[19a,d]

In the stepwise method **2** and **3** were enzymatically condensed using L-fuculose-1-phosphate aldolase to yield

(1) (a) Lindberg, B.; Svensson, S. *MTP Int. Rev. Sci. Ser. One, Carbohydr.* **1973**, *7*, 319. (b) Jann, K.; Jann, B. In *Surface Carbohydrates of the Prokaryotic Cell*; Sutherland, I. W., Ed.; Academic Press: New York, 1977; pp 247–287. (c) Aspinall, G. O.; Stephen, A. M. *MTP Int. Rev. Sci., Ser. One, Carbohydr.* **1973**, *7*, 285.

(2) (a) Percival, E. *Methods Carbohydr. Chem.* **1962**, *1*, 195. (b) Larsen, B.; Haug, A.; Painter, T. J. *Acta Chem. Scand.* **1966**, *20*, 219. (c) Chandrasekaran, E. V.; Jain, R. K.; Larsen, R. D.; Wlasichuk, K.; Matta, K. L. *Biochemistry* **1995**, *34*, 2925.

(3) Kobata, A. In *The Glycoconjugates*; Horowitz, M. I., Pigman, W., Eds.; Academic Press: New York, 1977; Vol. 1, pp 423–440.

(4) (a) Hakomori, S.-I. *Prog. Biochem. Pharmacol.* **1975**, *10*, 167. (b) Mckibbin, J. M. *J. Lipid Res.* **1978**, *19*, 131.

(5) (a) Lloyd, K. O. *MTP Int. Rev. Sci., Ser. Two, Carbohydr.* **1976**, *7*, 251. (b) Kornfeld, R.; Kornfeld, S. *Annu. Rev. Biochem.* **1976**, *45*, 217.

(6) (a) Phillips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.-I.; Paulson, J. C. *Science* **1990**, *250*, 1130. (b) Waltz, G.; Arufto, A.; Kalanus, W.; Bevilacqua, M.; Seed, B. *Science* **1990**, *250*, 1132. (c) Lowe, J. B.; Stoolman, L. M.; Nair, R. P.; Larsen, R. D.; Berhend, T. L.; Marks, R. M. *Cell* **1990**, *63*, 475.

(7) (a) Paulson, J. C. In *The Receptors*; Conn, M., Ed.; Academic Press: New York, 1985; Vol. 2, pp 131–219. (b) Paulson, J. C. In *Adhesion: its role in inflammatory disease*; Harlan, J., Liu, D., Eds.; W. H. Freeman: New York, 1992; Chapter 2, p 19. (c) Springer, T. A.; Lasky, L. A. *Nature* **1991**, *349*, 196. (d) Lasky, L. A. *Science* **1992**, *258*, 964. (e) Rice, G. E.; Bevilacqua, M. P. *Science* **1989**, *246*, 1303. (f) Bevilacqua, M P.; Pober, J. S. Hendrick, D. L.; Cotran, R. S.; Gimbrone, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 9238.

(8) Ichikawa, Y.; Lin, Y.-C.; Dumas, D. P.; Shen, G.-J.; García-Junceda, E.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, L. E.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 9283.

(9) (a) DeFrees, S. A.; Gaeta, F. C. A.; Lin, Y.-C.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 7549. (b) DeFrees, S. A.; Kosh, W.; Way, W.; Paulson, J. C.; Sabesan, S.; Halcomb, R. L.; Huang, D.-H.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 66. (c) Mulligan, M. S.; Paulson, J. C.; DeFrees, S.; Zheng, Z.-L.; Lowe, J. B.; Ward, P. A. *Nature* **1993**, *364*, 149.

(10) From Aldrich Co.: $11.50/g.

(11) Flowers, H. M. *Adv. Carbohydr. Chem. Biochem.* **1981**, *39*, 279.

(12) Tanimura, A. *Eisei Shikenjo Hokoku* **1959**, *77*, 123; *Chem. Abstr.* **1961**, *55*, 12306.

(13) Dejter-Juszynski, M.; Flowers, H. M. *Carbohydr. Res.* **1973**, *28*, 144.

(14) Chiba, T.; Tejima, S. *Chem. Pharm. Bull.* **1979**, *27*, 2838.

(15) Gesson, J.-P.; Jaquesy, J.-C.; Mondon, M.; Petit, P. *Tetrahedron Lett.* **1992**, *33*, 3637.

(16) Defaye, J.; Andrée, G.; Wong, C.-C. *Carbohydr. Res.* **1981**, *95*, 131.

(17) Schweiger, R. G. (To Kelco Co.), U.S. Pat. 3,240,775, March 15, 1966, Appl. July 30, 1962; *Chem. Abstr.* **1966**, *65*, 2342f.

(18) Alajarín, R.; García-Junceda, E.; Wong, C.-H. *J. Org. Chem.* **1995**, *60*, 4294.

(19) (a) Fessner, W.-D.; Schneider, A.; Eyrisch, O.; Sinerius, G.; Badía, J. *Tetrahedron: Asymmetry* **1993**, *4*, 1183. (b) Fessner, W.-D.; Sinerius, G.; Schneider, A.; Dreyer, M.; Schulz, G. E.; Badía, J.; Aguilar, J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 555. (c) Fessner, W.-D.; Badía, J.; Eyrisch, O.; Schneider, A.; Sinerius, G. *Tetrahedron Lett.* **1992**, *33*, 5231. (d) Liu, K. K.-C.; Kajimoto, T.; Chen, L.; Zhong, Z.; Ichikawa, Y.; Wong, C.-H. *J. Org. Chem.* **1991**, *56*, 6280.

(20) Green, M.; Cohen, S. S. *J. Biol. Chem.* **1956**, *219*, 557.

(21) Williams, D. T.; Jones, J. K. N. *Can. J. Chem.* **1967**, *45*, 741.

0022-3263/95/1960-7360$09.00/0

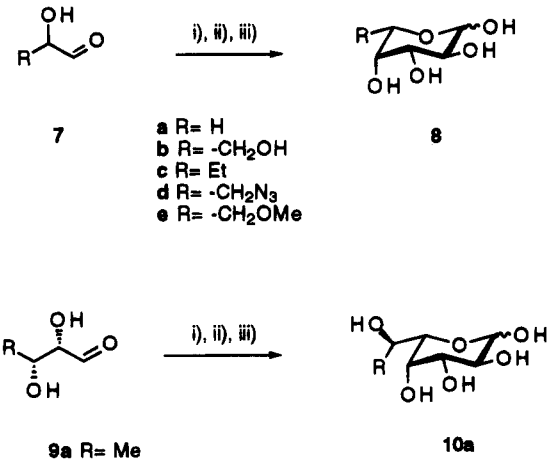
L-fuculose-1-phosphate **4** which was isolated as barium salt. Compound **3** was prepared from DL-lactaldehyde dimethyl acetal **6** as previously reported[22] and used directly without further purification. Crude **4** was converted to sodium salt by treatment with Dowex 50W-X8 (H^+ form) followed by addition of NaOH. Subsequent phosphate ester hydrolysis with acid phosphatase yielded **5**, the physical data of which were in accordance with the literature values.[19d] Compound **5** was isomerized with L-fucose isomerase to give a 90:10 mixture[23] of **1** and **5** which after separation by Dowex 50W-X8 (Ba^{2+} form) chromatography with water/ethanol (1:1) yielded pure **1** (75.2 mg, 0.459 mmol, 38.6% from DHAP).

In the sequential method the aldol products were used directly without isolation in the subsequent phosphatase and isomerase reactions to give the same equilibrium ratio of 90:10 for **1** and **5**. Further isolation by silica gel and Dowex chromatographies gave **1** in a higher yield (152 mg, 0.927 mmol, 55.2%). The NMR spectra and optical rotation of **1** obtained by both methods were in complete agreement with those from an authentic sample.

As described, both methods were carried out on a small scale (0.4−1 mmol) and a large excess of D,L-**3** (six equiv) was used in order to ensure a good kinetic selection of L-**3**.[19c,24a] We also used large amounts of isomerase (1500 units/mmol)[24b] and a long reaction time (12 h) to ensure that equilibrium was reached. The sequential process was then scaled up (543 mg, 3.3 mmol, 33.1%), and both the reaction time and the amount of enzymes were substantially reduced.[25] Furthermore, continuous extraction and crystallization were used as an alternative to replace chromatographic purification of **1** in order to reduce the cost and to make the process more appropriate for larger scale reactions.[26]

We have also explored the substrate specifity of the isomerase, and some fucose analogs have been prepared using this combined enzymatic process (Scheme 2). By this way, D-arabinose (**8a**) and 6-methyl-L-fucose (**8c**) were prepared from **7a** (commercially available) and **7c**,[27] respectively, using the stepwise method, and L-galactose (**8b**), 6-azido-L-fucose (**8d**), 6-methoxy-L-fucose (**8e**), and 7-deoxy-D-glycero-L-galactoheptose (**10a**) were prepared from **7b**,[28a] **7d**,[28b] **7e**,[22,27] and **9a**,[28a] respectively, using the sequential method. When **9b**[28a] was used as substrate, the product **10b** was, however, not a substrate for the isomerase (Scheme 3). Similarly, **11a** and **11b** are substrates for the aldolase, but the products are not substrates for the isomerase. In addition, **11b** gave a mixture of **12b** and **12c**.[29] Compound **14** (prepared from compound **13** and used directly without characterization for aldolase reaction) was not a substrate for the aldolase.

Scheme 2



i) Fuculose aldolase/DHAP. ii) Acid phosphatase. iii) Fucose isomerase.

In summary, the sequential method was shown to be a better process and could be scaled up for the synthesis of **1** and some analogs. Work is in progress to prepare new fucose derivatives for the synthesis of sialyl Lewis x mimetics.

Experimental Section

Materials and Methods. Acid phosphatase (EC 3.1.3.2) was purchased from Sigma. All chemicals and solvents were purchased from Aldrich. Dowex 50W-X8 (200−400 mesh, H^+ form) was thoroughly washed with purified water prior to use. Aldol condensation was monitored enzymatically by DHAP consumption.[30] The phosphatase-catalyzed hydrolysis was monitored by TLC (silica gel 60 from Merck). Isomerization was monitored by ^{13}C-NMR (100 MHz) analysis of the methyl group of **1** (δ = 15.9) and **5** (δ_{major} = 15.0, δ_{minor} = 14.0) in the cyclized form. Nuclear magnetic resonance (^1H: 400 MHz; ^{13}C: 100 MHz) spectra were obtained using HDO (in D_2O; δ = 4.8) and CH_3CN (in D_2O; δ = 1.30) as internal references. Flash chromatography was carried out with silica gel 60 (230−400 mesh).

Enzyme Preparation. The *E. coli* cells containing recombinant L-fucose isomerase prepared in this laboratory and deposited with ATCC (ATCC no. 87024)[31] were grown aerobically to late logarithmic phase (OD_{600} 0.9) at 37 °C in four 3 L flasks

(22) Durrwachter, J. R.; Drueckhammer, D. G.; Nozaki, K.; Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1986**, *108*, 7812.

(23) This is the equilibrium ratio for **1** and **5** (ref 20).

(24) (a) When D,L-aldehyde was supplied in equimolar ratio with DHAP a 1:1 mixture of 5-*epi*-ketose 1-phosphates was obtained. When racemic aldehyde was supplied in excess, the diastereomerically pure L-ketose 1-phosphate was obtained. (b) To our knowledge, the only reported synthetic procedure for the isomerization is the conversion from **1** to **4** *via* **5** using fucose isomerase and rhamnulokinase (ref 19a,c). Only 30 units of isomerase per millimole of **1** was used in the procedure, but in this case the process was favored by the presence of kinase.

(25) In a single experiment, starting from **6** (50 mmol) and **2** (10.0 mmol), and using a similar procedure to the sequential method (see Experimental Section) in the presence of less amounts of enzymes (aldolase: 30 units/mmol; phosphatase: 40 units/mmol; isomerase: 328 units/mmol). The reactions were complete in shorter reaction times (3 h for aldolase, 18 h for phosphatase and 9 h for isomerase reaction).

(26) The bacterial source is relatively inexpensive: The price for one vial of aldolase or isomerase-containing recombinant *E. coli* cells is $61 from ATCC (no. 86984 for aldolase and no. 87024 for isomerase, see ref 32 and ref 31, respectively).

(27) Korobi, Y.; Myles, D. C.; Whitesides, G. M. *J. Org. Chem.* **1992**, *57*, 5899.

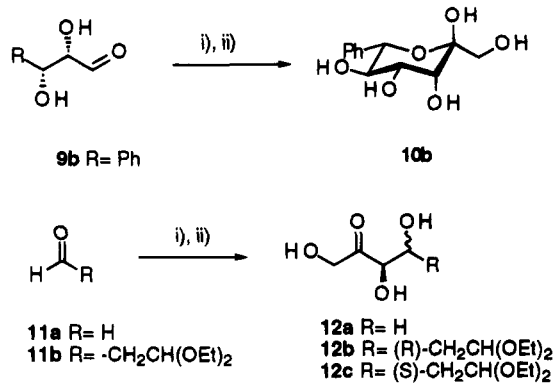
(28) (a) Henderson, I.; Sharpless, K. B.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 558. (b) Durrwachter, J. R.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 4175.

(29) Although the reaction of aldehyde **11b** and DHAP catalyzed by purified fuculose aldolase has been previously reported to be selective in the C−C bond formation (ref 19b), we have found that the same reaction catalyzed by a crude extract of fuculose aldolase prepared by this laboratory (ref 32b) was not selective. We first checked whether compound **12c** was formed *via* contaminated rhamnulose aldolase or fructose aldolase (in this case **12c** should be the enantiomeric structure) present in the fuculose aldolase crude extract. Compound **11b** was, however, not a substrate for purified rhuA or commercially available RAMA in similar conditions to those used in the fucA-catalyzed reaction. Since the stereochemistry at C-3 is completely controlled by the aldolase and the stereochemistry at C-4 also depends of the substrate (ref 19b), we assigned a (3*R*,4*R*) stereochemistry for **12b** and a (3*R*,4*S*) stereochemistry for **12c** based on the coupling constant $J_{H3,H4}$ (**12b**: J_{trans} = 4.7 Hz; **12c**: J_{cis} = 2.5 Hz).

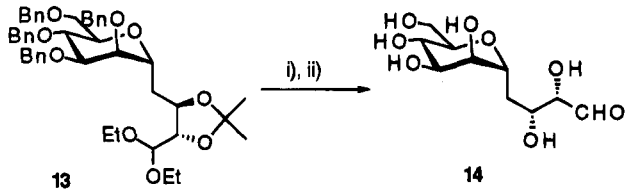
(30) Bergmeyer, H. U. *Methods of Enzymatic Analysis*, 3rd ed.; Verlag Chemie: Deerfield, FL, 1984; Vol. 2, pp 146−7.

(31) García-Junceda, E.; Shen, G.-J.; Alajarín, R.; Wong, C.-H. *Bioorg. Med. Chem.* (in press).

Scheme 3



i) Fuculose aldolase/DHAP. ii) Acid phosphatase.



i) H_2/Pd-C. ii) HCl

containing LB medium (1 L each) supplemented with ampicillin (250 mg/mL), followed by induction with IPTG (250 mM). The culture was kept overnight (OD_{600} 2.1) at 30 °C. Cells were harvested by centrifugation (10,000*g*; 30 g of wet cells were obtained), resuspended in Tris-HCl buffer (50 mM, pH 7.5, 80 mL), and disrupted by repeatedly passing through a French Press (~1600 lb/in.^{2}) five times. Cellular debris was removed by centrifugation (16000*g*), and the clear supernatant (total activity 66000 units) was used for isomerization reaction. For the preparation of aldolase, the *E. coli* cells prepared in this laboratory and deposited with ATCC (ATCC no. 86984)[32] were grown as previously decribed, and the crude extract (total activity 1720 units) obtained was directly used for aldol reaction. The aldolase[32b] and isomerase[33] were assayed according to the procedures described previously. One unit of enzyme represents one micromole of product formed per minute.

Stepwise Synthesis of 1. L-Fuculose-1-phosphate Barium Salt 4. Compound **6**[22] (1.51 g; 12.6 mmol) was dissolved in purified water (13 mL) and treated with Dowex AG50W-X8 resin (H^+ form, 400 mesh) until pH 2.8. The mixture was heated at 50 °C for 8 h, and the resin was filtered off and washed with water (2 × 5 mL). A solution of **2**[34] was added (5.76 mL; 2.10 mmol) and the pH was adjusted to 6.8 with 6 N NaOH (0.6 mL). L-Fuculose aldolase crude extract[32b] (63 units; 6.5 mL) was added, and the mixture was stirred at room temperature until 91% of **2**[30] was consumed. After adjusting the pH to 7.0 with 2 N NaOH, $BaCl_2 \cdot 2H_2O$ (1.03 g, 2 equiv) was added and the mixture was stirred for 1 h at room temperature. The precipitate was centrifuged (30 min × 4500 rpm), the supernatant was decanted and treated with acetone (2 vol), and the mixture was kept at 4 °C overnight. The precipitate was collected by centrifugation (30 min × 4500 rpm), washed with Et_2O/EtOH (1:1) (10 mL), and dried under high vacuum. A white solid of crude **4** (0.64 g, 80%) was obtained. This salt was used without further purification[35] for the next step.

L-Fuculose (5). Crude **4** (0.50 g) was powdered, dispersed in H_2O (9 mL), and treated with Dowex 50W-X8 resin (H^+ form, 400 mesh) until pH 2.8 for 30 min. The resin was filtered off and washed with water (2 × 2 mL), and the filtrate was adjusted to pH 4.7 with 6 N HCl. Acid phosphatase (250 units) was added, the mixture was heated at 37 °C for 21 h, the pH was adjusted to 7.0 with saturated $Ba(OH)_2$ solution, and methanol (2 vol) was added. The precipitate was filtered off through Celite and the solvent removed under reduced pressure. The residue was chromatographed on silica gel with $CHCl_3$/MeOH (3:1). Compound **5** (123 mg, 46% from DHAP) was obtained as a slightly yellowish oil: $[\alpha]_D = 0°$ (*c* 1, water), [lit.[19d] $[\alpha]_D = 0°$ (*c* 1.9, water)]. NMR (^1H and ^{13}C) data were in accordance with literature values.[19d] A sample was taken and converted to the 2-nitrophenylhydrazone derivative: mp 158–160° C (lit.[20] 162–163 °C).

L-Fucose (1). Compound **5** (89.6 mg; 0.546 mmol) obtained as described above was dissolved in Tris-HCl buffer (50 mM, pH 7.5; 2 mL) containing mercaptoethanol (2 mM) and $MnCl_2$ (2 mM). An aliquot of the L-fucose isomerase crude extract (1 mL, 820 units) was added and the mixture was stirred overnight at room temperature. Methanol (2 vol) was added, and the mixture was filtered through Celite. The solvent was distilled under reduced pressure and the residue chromatographed on Dowex 50W-X8 resin (Barium form, 400 mesh, 2.5 × 9.5 cm)[36] with water/ethanol (1:1). Compound **1** (75.2 mg, 84%) was obtained as a yellowish oil: $[\alpha]_D = -73.5°$ (*c* 1.1, water), [lit.[16] $[\alpha]_D = -77°$ (*c* 1, water), lit.[15] $[\alpha]_D = -80°$ (*c* 0.35, water), lit.[14] $[\alpha]_D = -76.1°$ (*c* 0.65, water), lit.[13] $[\alpha]_D = -75°$ (*c* 0.8, water)]. NMR (^1H and ^{13}C) data were identical to those from an authentic sample.

Sequential Synthesis of 1. Compound **6** (1.21 g; 10.1 mmol) was dissolved in water (3 mL), and Dowex 50W-X8 (H^+ form, 200–400 mesh) was added until pH 2.8. The mixture was heated at 50 °C for 8 h, and then the resin was filtered off and washed with water (2 × 0.5 mL). A solution of **2**[34] (4.62 mL, 1.68 mmol) was added, and the mixture was adjusted to pH 6.8 with 6 N NaOH (0.4 mL). L-Fuculose aldolase[32b] (5.2 mL, 50 units) was added, the mixture was stirred gently at room temperature for 17 h (DHAP analysis[30] indicates 95% conversion), and the pH was adjusted to 4.7 with 6 N HCl (0.4 mL). Acid phosphatase (0.45 mL, 225 units) was added and the mixture heated at 37 °C for 19 h. The mixture was cooled to room temperature, and the pH was adjusted to 7.5 with 6 N NaOH (0.6 mL). $MnCl_2$ (30 μL, 0.7 M) and L-fucose isomerase[31] (3 mL, 2460 units) were added. The mixture was stirred at room temperature for 24 h and adjusted to pH 7.0 with 2 N HCl. Methanol (2 vol) was then added, and the precipitate was filtered off through Celite. The solvent was removed under reduced pressure and the residue chromatographed on silica gel with $CHCl_3$/MeOH (3:1). The fractions containing **1** were pooled and then concentrated, and the residue was chromatographed on Dowex 50W-X8 (barium form, 200–400 mesh)[36] with water/ethanol (1:1) to yield **1** as a white-yellowish solid (152 mg, 0.927 mmol, 55.2%). NMR (^1H and ^{13}C) data were identical to those of an authentic sample: mp 134–136 °C, [lit.[16] 137–138 °C, lit.[15] 152–153 °C, lit.[14] 139–140 °C, lit.[13] 137–139 °C]; $[\alpha]_D = -74.1°$ (*c* = 1.1, water), [lit.[16] $[\alpha]_D = -77°$ (*c* = 1, water), lit.[15] $[\alpha]_D = -80°$ (*c* = 0.35, water), lit.[14] $[\alpha]_D = -76.1°$ (*c* 0.65, water), lit.[13] $[\alpha]_D = -75°$ (*c* 0.8, water)].

Scaled Up Sequential Synthesis of 1. Compound **6** (6.0 g, 50 mmol) was hydrolyzed in water (50 mL) with Dowex 50W-X8 resin (H^+ form, 200–400 mesh, 6.0 g, pH 2.8) for 4 h at 60 °C. After the resin was filtered off and washed with water, a solution of **2**[34] (18.60 mL, 10.0 mmol) was added, the mixture adjusted to pH 6.8 with 6 N NaOH (1.8 mL), and L-fuculose aldolase[32b] (24 mL, 305 units) added. The mixture was readjusted to pH 6.8 with 6 N NaOH (0.2 mL), and the solution was degassed with argon and protected from light. After 3 h at room temperature (DHAP analysis[30] indicates 95% conversion), the mixture was adjusted to pH 4.7 with 6 N HCl (1.8 mL), acid phosphatase (1.33 mL, 400 units) was added, and the mixture was heated at 37 °C for 18 h. The mixture was then cooled to room temperature and adjusted to pH 7.5 with 6 N NaOH (2.6 mL). $MnCl_2$ (1 mL, 0.11 M) and L-fucose isomerase[31] (4 mL, 3280 units) were added. The mixture was stirred at room temperature for 9 h and adjusted to pH 7.0 with 6 N HCl. Methanol (2

(32) (a) Chou, W.-C.; Chen, L.; Fang, J.-M.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 6191. (b) García-Junceda, E.; Shen, G.-J.; Sugai, G.-J.; Wong, C.-H. *Bioorg. Med. Chem.* **1995**, *3*, 945.

(33) Dische, Z.; Borenfreund, E. *J. Biol. Chem.* **1951**, *192*, 583.

(34) Jung, S.-H.; Jeong, J.-H.; Miller, P.; Wong, C.-H. *J. Org. Chem.* **1994**, *59*, 7182.

(35) Straub, A.; Effenberger, F.; Fischer, P. *J. Org. Chem.* **1990**, *55*, 3926.

(36) Liu, K. K.-C.; Pederson, R. L.; Wong, C.-H. *J. Chem. Soc. Perkin. Trans. 1* **1991**, 2669.

vol) was then added, and the precipitate was filtered off through Celite and washed with MeOH (4 × 25 mL). MeOH was removed under reduced pressure (bath temp < 25 °C), water (100 mL) was added on the aqueous residue, and the mixture was continuously extracted with AcOEt for 48 h. The organic extract was discarded and the aqueous solution evaporated under reduced pressure (bath temp < 25 °C). The residue was treated with methanol (50 mL), and the white solid formed was filtered off, washed with more methanol (2 × 15 mL), and discarded. Charcoal was added to the filtrate, and the mixture was heated at 30 °C for 15 min. After filtration to remove charcoal, methanol was removed, and toluene was added time to time to remove traces of water (bath temp < 25 °C). After removing the solvents, the residue was dissolved in hot absolute ethanol (12.5 mL), seeded with **1**, and stored at 4 °C for 2 days. Compound **1** (543 mg, 3.3 mmol, 33.1%) was obtained as a white solid whose physical and spectroscopic data were in accordance with those from an authentic sample: mp 139-140 °C; $[\alpha]_D$ = −76.6° (*c* = 0.9, water).

D-Arabinose (8a) (80 mg, 0.54 mmol, 30%). NMR (^{1}H and ^{13}C) data were identical to those of an authentic sample. $[\alpha]_D$ = −19° (*c* = 0.5, MeOH, 30 mim), $[[\alpha]_D$ = −104.3° (*c* = 3, water, 20 h) from Aldrich Co.].

L-Galactose (8b) (60 mg, 0.32 mmol, 58%). NMR (^{1}H and ^{13}C) data were identical to those of an authentic sample. $[\alpha]_D$ = −70.2° (*c* = 1, water), $[[\alpha]_D$ = −77° (*c* = 1, water) from Aldrich Co.].

6-Methyl-L-fucose (8c) (89 mg, 0.50 mmol, 25%). $[\alpha]_D$ = −47.6 (*c* = 0.5, MeOH, 30 min), [lit.[37] $[\alpha]_D$ = −26.2° (*c* = 1, pyridine), lit.[15] $[\alpha]_D$ = −33° (*c* = 1.2, water)]. ^{1}H-NMR (400 MHz, D_2O) δ: 5.03 (H1α, d, $J_{H1\alpha,H2\alpha}$ = 3.7 Hz), 4.39 (H1β, d, $J_{H1\beta,H2\beta}$ = 8 Hz), 3.73 (H5α, m), 3.69 (H4α, H4β, d, *J* = 3 Hz), 3.66 (H3α, d, $J_{H2\alpha,H3\alpha}$ = 3.5 Hz), 3.62 (H2α, d, $J_{H2\alpha,H3\alpha}$ = 3.5 Hz), 3.45 (H3β, dd, $J_{H2\beta,H3\beta}$ = 10 Hz, $J_{H3\beta,H4\beta}$ = 3.5 Hz), 3.35 (H5β, t, $J_{H5\beta,CH2}$ = 3 Hz), 3.29 (H2β, dd, $J_{H1\beta,H2\beta}$ = 8 Hz, $J_{H2\beta,H3\beta}$ = 10 Hz), 1.4−1.51 (CH_2, α and β), 0.77 (CH_3, α and β). ^{13}C-NMR (100 MHz, D_2O) δ: 96.42 (C1β), 92.23 (C1α), 76.43, 73.15, 72.04, 69.72 (β anomer), 71.81, 70.22, 69.51, 68.45 (α anomer), 23.01 (CH_2, α and β), 9.41 (CH_3, α and β). HRMS Calcd for $C_7H_{14}O_5$ + H^+ 179.0919, found 179.0914.

6-Azido-L-fucose (8d) (31 mg, 0.15 mmol, 20%). $[\alpha]_D$ = −3.25 (*c* = 0.4, MeOH, 10 min), [lit.[37], $[\alpha]_D$ = −56.4 (*c* = 1, H_2O)]. ^{1}H-NMR (400 MHz, D_2O) δ: 5.10 (H1α, d, $J_{H1\alpha,H2\alpha}$ = 3.7 Hz), 4.42 (H1β, d, $J_{H1\beta,H2\beta}$ = 8 Hz), 4.10 (H5α, ddd, $J_{H4\alpha,H5\alpha}$ = 1 Hz, $J_{CH2,H5\alpha}$ = 4.6 Hz, $J_{CH2,H5\alpha}$ = 8.7 Hz), 3.78 (H4α, dd, $J_{H4\alpha,H5\alpha}$ = 1 Hz, $J_{H4\alpha,H3\alpha}$ = 3 Hz), 3.72 (H4β, dd, $J_{H4\beta,H5\beta}$ = 1 Hz, $J_{H4\beta,H3\beta}$ = 3.5 Hz), 3.68 (H3α, dd, $J_{H4\alpha,H3\alpha}$ = 3 Hz, $J_{H2\alpha,H3\alpha}$ = 10.5 Hz), 3.64 (H5β, ddd, $J_{H5\beta,H4\beta}$ = 1 Hz, $J_{CH2,H5\beta}$ = 4.4 Hz, $J_{CH2,H5\beta}$ = 8.4 Hz), 3.62 (H2α, dd, $J_{H2\alpha,H1\alpha}$ = 3.7 Hz, $J_{H2\alpha,H3\alpha}$ = 10.5 Hz), 3.48 (H3β, dd, $J_{H2\beta,H3\beta}$ = 10 Hz, $J_{H3\beta,H4\beta}$ = 3.5 Hz), 3.31 (H2β, dd, $J_{H1\beta,H2\beta}$ = 8 Hz, $J_{H2\beta,H3\beta}$ = 10 Hz). ^{13}C-NMR (100 MHz, D_2O) δ: 96.45 (C1β), 92.36 (C1α), 73.43, 72.67, 71.71, 69.11 (β anomer), 69.65, 69.03, 68.90, 68.21 (α anomer), 50.91 (C6α), 50.74 (C6β). HRMS Calcd for $C_6H_{11}N_3O_5$ + H^+ = 206.06986, observed 206.0693.

6-Methoxy-L-fucose (8e) (63 mg, 0.3 mmol, 25%). $[\alpha]_D$ = −14.4 (*c* = 1, MeOH, 4 h), [lit.[37], $[\alpha]_{25}$ = −80.6 (*c* = 1, pyridine, eq)]. ^{1}H-NMR (400 MHz, D_2O) δ: 5.10 (H1α, d, $J_{H1\alpha,H2\alpha}$ = 3.6 Hz), 4.42 (H1β, d, $J_{H1\beta,H2\beta}$ = 8 Hz), 4.08 (H5α, t, $J_{CH2,H5\alpha}$ = 6 Hz), 3.81 (H4α, d, $J_{H4\alpha,H3\alpha}$ = 3 Hz), 3.75 (H4β, d, $J_{H4\beta,H3\beta}$ = 3.5 Hz), 3.6−3.7 (H2α, H3α, H5β, overlapped signals), 3.58 (CH_3O, α and β), 3.47−3.52 (H3β and CH_2 α and β, overlapped signals), 3.32 (H2β, dd, $J_{H1\beta,H2\beta}$ = 8 Hz, $J_{H2\beta,H3\beta}$ = 10 Hz). ^{13}C-NMR (100 MHz, D_2O) δ: 96.31 (C1β), 92.19 (C1α), 73.17, 72.61, 71.82, 71.61, 69.46, 68.93, 68.44, 68.19, 59.29 (CH_3), 58.35 (C6β), 58.20 (C6α). HRMS Calcd for $C_7H_{14}O_6$ + Na^+ 217.0688, observed 217.0647.

7-Deoxy-D-glycero-L-galactoheptose (10a) (18 mg, 0.09 mmol, 28%). ^{1}H-NMR (major β piranose form, D_2O) δ: 4.55 (dd, 1H, *J* = 7.6 and 4.5 Hz); 4.02 (m, 1H); 3.82 (t, 1H, *J* = 3.2 Hz); 3.62 (dd, 1H, *J* = 10.0 and 3.0 Hz); 3.49 (t, 1H, *J* = 7.9 Hz); 3.37 (d, *J* = 7.3 Hz, 1H); 1.22 (d, 3H, *J* = 6.4 Hz). ^{13}C-NMR (major β pyranose form, D_2O) δ 95.5, 78.3, 72.1, 70.9, 67.9, 66.0, 16.7. HRMS Calcd for $C_7H_{14}O_6$ + Na^+ 217.0688, found 217.0691.

6-Phenyl-D-galacto-2-hexulose (10b) (60 mg, 32%).^{1}H-NMR (major β pyranose form) (D_2O) δ: 7.41−7.31 (5H, aromatic); 5.28 (s, 1H); 4.08 (t, *J* = 3.7 Hz, 1H); 3.95 (d, *J* = 3.5 Hz, 2H); 3.69 (d, *J* = 11.8 Hz, 1H); 3.49.(d, *J* = 11.8 Hz, 1H). ^{13}C-NMR (D_2O) δ: 128.3−127.0, 9.5, 73.8, 73.4, 69.0, 65.2, 63.6. HRMS Calcd for $C_{12}H_{16}O_6$ + H^+ 257.1025, found 257.1016.

D-Erythrulose (12a) (130 mg, 1.08 mmol, 21.7%). $[\alpha]_D$ = −16.7 (*c* = 0.52, water) [L-erythrulose: lit.[38] $[\alpha]_D$ = + 13° (*c* = 2.44, water); lit.[39] $[\alpha]_D$= + 13.3° (*c* = 2.25, water)].

Compounds 12b and 12c were obtained as a mixture (1:1) after phosphatase reaction (64.9 mg, 16.4%). ^{1}H-NMR (D_2O) δ: 4.57 (dd, 2H, $J_{H1,H1'}$ = 19.4 Hz, H_1 and H_1' in **12b**); 4.55 (dd, 2H, $J_{H1,H1'}$ = 19.6 Hz, H_1 and H_1' in **12c**); 4.32 (d, 1H, $J_{H3,H4}$ = 4.7 Hz, H_3 in **12b**; 4.31 (d, 1H, $J_{H3,H4}$ = 2.5 Hz, H_3 in **12c**); 4.15 (ddd, $J_{H4,H5}$ = 9.2 Hz, $J_{H4,H5'}$ = 4.2 Hz, $J_{H4,H3}$ = 2.5 Hz, 1H, H_4 in **12c**); 3.99 (ddd, $J_{H4,H5}$ = 9.6 Hz, $J_{H4,H5'}$ = 3.4 Hz, $J_{H4,H3}$ = 4.7 Hz, 1H, H_4 in **12b**); 3.77 (m, 4H, CH_2Me); 3.62 (m, 4H, CH_2Me); 1.86 (m, 4H, $2H_5$); 1.21 (m, 12H, Me) ppm (H_6 overlaps with HDO signal). ^{13}C-NMR (D_2O) δ: 213.2 and 212.8 (CO); 101.3 and 101.1 (C_6); 78.2 and 78.1 (C_1); 69.2, 68.8, 67.0, 66.5, 63.8, 63.7, 63.3, 63.2; 37.1 and 36.3 (C_5); 14.7 (Me). HRMS Calcd for $C_8H_{14}O_5$ + H^+ 191.0919, found 191.0914.

Compound 13. ^{1}H-NMR ($CDCl_3$) δ: 7.43−7.17 (m, 20H), 4.82 (d, *J* = 11.2 Hz, 1H), 4.72 (d, *J* = 11.2 Hz, 1H), 4.63 (d, *J* = 12.8 Hz, 1H), 4.58 (d, *J* = 7.0 Hz, 1H), 4.54 (d, *J* = 12.0 Hz, 1H), 4.51 (m, 1H), 4.48 (d, *J* = 12.0 Hz, 1H), 4.04 (ddd, *J* = 9.8, 8.1 and 1.7 Hz, 1H), 3.37 (m, 1H), 3.79−3.59 (m, 8H), 3.51 (dd, *J* = 9.4 and 7.1 Hz, 1H), 2.09 (ddd, *J* = 14.2, 9.3 and 1.7 Hz, 1H), 1.59 (ddd, *J* = 14.2, 10.0 and 4.7 Hz, 1H), 1.34 (s, 3H), 1.32 (s, 3H), 1.20 (t, *J* = 7.0 Hz, 3H), 1.16 (t, *J* = 7.0 Hz, 3H). ^{13}C-NMR ($CDCl_3$) δSPCLN 138.6 128.3−127.4, 109.2, 103.1, 80.6, 78.7, 75.5, 75.2, 74.5, 74.4, 73.4, 73.3, 71.6, 71.3, 69.7, 64.2, 62.1, 33.6, 27.4, 26.8, 15.4, 15.2. HRMS calcd for $C_{45}H_{56}O_9$ M + Cs^+ 873.2979, found 873.2946.

Acknowledgment. We thank the N.I.H. for financial support (NIH-GM44154), Ministerio de Educación y Ciencia (Spain) for postdoctoral fellowships to R.A. and F.M.-V., Consellería de Educación (Xunta de Galicia, Spain) which partially supported a postdoctoral fellowship to O.B., and C.S.I.C. (Spain) for a postdoctoral fellowship to E.G.-J.

JO950846Z

(37) May, A. J.; Sartorelli, A. C. *J. Med. Chem.* **1979**, *22*, 971.

(38) De Wilde, H.; De Clerq, P. *Tetrahedron Lett.* **1987**, *28*, 4757.

(39) Collins, P. M. *Carbohydrates*; Chapman and Hall Chemistry Source Books: New York, 1987.