Regioselective Esterification of Various D-Glucopyranosides: Synthesis of a Fully Protected Disaccharide Unit of Hyaluronic Acid

Xin-An Lu, Chien-Hung Chou, Cheng-Chung Wang, Shang-Cheng Hung*

Institute of Chemistry, Academia Sinica, Taipei 115, Taiwan
Fax +886(2)27831237; E-mail: schung@chem.sinica.edu.tw.

Received 28 May 2003

Dedicated to Professor Ray Lemieux, in honor of his many contributions to organic chemistry.

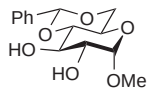
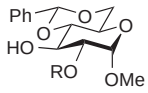
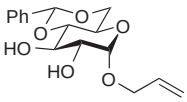
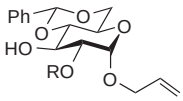
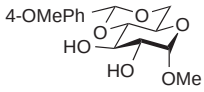
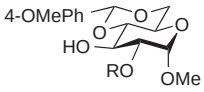
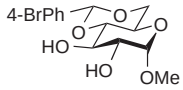
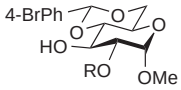
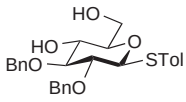
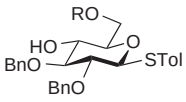
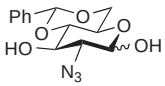
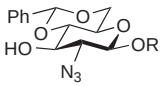
Abstract: A highly regioselective esterification of various D-glucopyranosides with triethylamine and acid anhydrides in excellent yields is described here. Its application toward the synthesis of a fully protected disaccharide unit of hyaluronic acid is also highlighted.

Key words: carbohydrates, esterifications, hyaluronic acid

Regioselective protection of individual hydroxy groups is of crucial importance in carbohydrate chemistry.[1] For the preparation of selectively protected glycosyl acceptors or donors toward the synthesis of oligosaccharides or glycoconjugates, acetyl (Ac) and benzoyl (Bz) are generally used as electron-withdrawing protecting groups to block single hydroxyls. Some strategies have been reported for regioselective introduction of acyl groups via direct treatment with benzoyloxybenzotriazole[2] (BzOBT, commercially non-available), or selective activation of hydroxy groups through stannylene acetals[3] as well as enzymes.[4] These methodologies mostly have their advantages and disadvantages, which may give low selectivity and yields, involving tedious purification of regioisomers. To tackle this problem, we have employed a very simple combination of triethylamine with acid anhydrides as mild esterification reagents to study the regioselectivity of various D-glucopyranosides.

Table 1 illustrates the results of regioselective acetylation and benzoylation on a variety of D-glucopyranosyl diols.[5] Initially, benzoylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **1** with 1.4 equivalents of benzoic anhydride and 9 equivalents of triethylamine in dichloromethane at room temperature led to the corresponding 2-benzoate **2**[6,2] in excellent yield (92%, entry 1) as a sole product. Similar phenomenon was observed when Ac_2O was used as an acetylating agent, affording the desired 2-acetate **3**[7] in 80% yield (entry 2). It should be noted that a random esterification occurs if pyridine is used in place of triethylamine. In entries 3-8, the α-allyl glucopyranoside **4**, *p*-methoxybenzylidene acetal **7**, and *p*-bromobenzylidene acetal **10** were selected to examine the compatibility of substituted groups at the anomeric, O4, and O6 positions, and the corresponding 2-esters **5**,[8] **6**,[9] **8**, **9**, **11**, and **12** were obtained in good yields, respectively. Owing to the inductive effect of the two oxygen atoms at the anomeric center, the C2-oxide formed in triethylamine solution reacts predominantly with various anhydrides to furnish the esters in high selectivity. As expected from the steric considerations, a clear-cut preference was observed for 6-O-protection during acylation of the 4,6-diol **13**, to give the corresponding 6-benzoate **14** (entry 9) and 6-acetate **15** (entry 10) in 83% and 79% yields, respectively.

Table 1 Regioselective Esterification of Various D-Glucopyranosides with Triethylamine and Acid Anhydrides at Room Temperature

Entry	Glucopyranoside	Product	Yield (%)
			
1	**1**	**2**: R = Bz	93
2		**3**: R = Ac	80
			
3	**4**	**5**: R = Bz	78
4		**6**: R = Ac	64
			
5	**7**	**8**: R = Bz	86
6		**9**: R = Ac	69[a]
			
7	**10**	**11**: R = Bz	88
8		**12**: R = Ac	65[a]
			
9	**13**	**14**: R = Bz	83
10		**15**: R = Ac	79
			
11	**16**	**17**: R = Bz	93
12		**18**: R = Ac	92

[a] Compounds **7** and **10** were recovered in 13% and 18% yields, respectively.

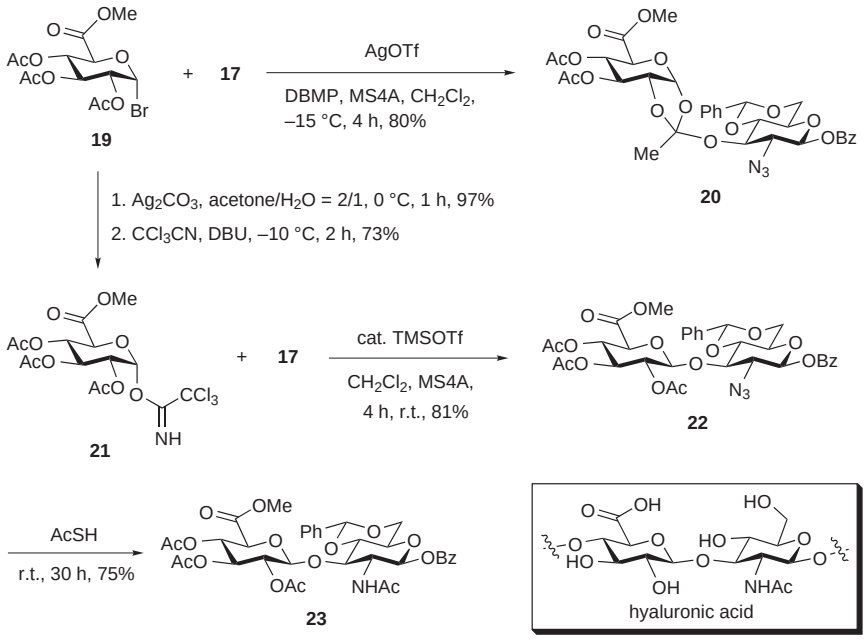
Synlett 2003, No. 9, Print: 11 07 2003.
Art Id.1437-2096,E;2003,0,09,1364,1366,ftx,en;S03703ST.pdf.

Since D-glucosamine is a typical component of numerous biomolecules, for example, glycosaminoglycans, blood group antigens, *N*-glycoproteins and GPI anchors, it was thought worthwhile to examine the regioselective discrimination of the hydroxy groups at C1 and C3. In entry 11, 2-azido-2-deoxy-4,6-*O*-benzylidene-D-glucopyranose **16**, generated from D-glucosamine hydrochloride in two straightforward steps,[10] was subjected to benzoylation under these conditions. It was heartening to see the formation of the corresponding β-anomeric benzoate **17** as a sole isomer (93%) in a highly regio- and stereoselective manner. Its absolute configuration was unambiguously determined through the X-ray single crystal analysis.[11] Similarly, acetylation of **16** (entry 12) furnished the β-anomeric acetate **18** in 92% yield. The high regio- and stereoselectivity in this case is perhaps induced by a close interplay of various factors. The higher reactivity of the anomeric hydroxy group stems out from its higher acidity in mild basic conditions, to gererate higher proportion of anomeric alkoxide that reacts preferentially with bulky acylating agents resulting in observed regioselctivity. Along with this, the kinetic streoelectronic effect[12] and 1,3-diaxial repulsion orient the oxide toward the equatorial position, giving the β-isomer, exclusively.

Hyaluronic acid (HA), an ubiquitous glycosaminoglycan found in almost all tissues, possesses unique viscoelastic and rheological properties.[13] It plays significant roles in a diverse set of biological processes including cell adhesion, hemopoiesi and angiogenesis.[12] HA is a negatively charged linear polysaccharide consisting of β-1,4-linked repeating disaccharide units of β-1,3-linked D-glucuronic acid and *N*-acetyl-D-glucosamine. The literature has documented some strategies to prepare HA-related molecules. Enzymatic synthesis from UDP-2-acetamido-2-deoxy-α-D-glucopyranose and UDP-α-D-glucuronic acid catalyzed by HA synthase could lead to a polymer (n = 1,500).[14] Chemical methods having either a D-glucosamine[15] or a D-glucuronic acid[15d,16] residue at the reducing end have been investigated.

From the basic structure of the disaccharide repeating-unit in HA, a free hydroxy group at the C3 position of D-glucosamine residue is required for further glycosylation. With the key synthon **17** in hands, our approach to the synthesis of HA-disaccharide is outlined in Scheme 1. It starts from the glycosyl bromide **19**, which can be conveniently prepared from commercially available D-glucurolactone in three steps.[17] Silver trifluoromethanesulfonate-activated coupling of the donor **19** with the alcohol **17** in the presence of 2,6-di-*t*-butyl-4-methylpyridine (DBMP) yielded the single orthoester **20** (80%) without isolation of any desired product. On the other hand, hydrolysis of compound **19** with silver carbonate in acetone and water (97%) followed by treatment with trichloroacetnitrile employing 1,8-diazabicyclo[4.3.0]undecane (DBU) as a base provided the corresponding trichloroacetimidate **21** (73%), which was subjected to couple with the glycosyl acceptor **17** to give the desired disaccharide **22**[18] in 81% yield. The β-configuration of the newly formed glycosidic bond is determined according to the *trans*-diaxial coupling constant ($J_{1,2}$ = 8.0 Hz) of anomeric proton in the D-glucuronate unit. Reaction of compound **22** with thioacetic acid afforded the expected *N*-acetyl derivative **23**[18] (75%), a fully protected HA-disaccharide unit.

In conclusion, we have successfully developed a highly regioselective acetylation and benzoylation of the D-glucopyranosyl 2,3-diols at O2, D-glucopyranosyl 4,6-diols at O6, and D-glucosamine-derived 1,3-diol at O1, using a very simple and mild reagent combination. The preparation of a fully protected HA-disaccharide via assembly of



Scheme 1

the key building block **17** with the trichloroacetimidate **21** followed by transformation of N_3 into NAc group is also carried out efficiently. Applications of the disaccharides **22** toward the synthesis of HA-related oligosaccharides are currently under investigation.

Acknowledgment

We thank Mr. Yuh-Sheng Wen for X-ray single crystal structural analysis and the National Science Council of Taiwan R. O. C. (NSC 91-2113-M-001-004 and NSC 91-2323-B-001-006) for financial support.

References

(1) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 3rd ed.; John Wiley and Sons, Inc.: New York, **1999**.

(2) Kim, S.; Chang, H.; Kim, W. J. *J. Org. Chem.* **1985**, *50*, 1751.

(3) Grindley, T. B. *Adv. Carbohydr. Chem. Biochem.* **1998**, *53*, 17.

(4) Schelhaas, M.; Waldmann, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2056.

(5) Following is the general procedure for the regioselective esterification of various D-glucopyranosyl diols. To a solution of the diol in CH_2Cl_2 (10 mL/g) was added acid anhydride (1.4 equiv) at r.t. under nitrogen. After stirring for 10 min, Et_3N (9 equiv) was added to the mixture, and the whole solution was kept stirring overnight. MeOH (5 equiv) was added to quench the reaction, and the resulting solution was evaporated under reduced pressure. The syrup was dissolved in EtOAc, and the mixture was sequentially washed by water, aq sat. $NaHCO_3$(twice), and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by either recrystallization in ethanol or flash column chromatography on silica gel to give the desired product.

(6) Szeja, W. *Carbohydr. Res.* **1983**, *115*, 240.

(7) (a) Dasgupta, F.; Hay, G. W.; Szarek, W. A.; Shilling, W. L. *Carbohydr. Res.* **1983**, *114*, 153. (b) Eby, R.; Webster, K. T.; Schuerch, C. *Carbohydr. Res.* **1984**, *129*, 111. (c) Horton, D.; Priebe, W.; Varela, O. *Carbohydr. Res.* **1985**, *144*, 317. (d) Hanessian, S.; Kagotani, M. *Carbohydr. Res.* **1990**, *202*, 67.

(8) Li, Z.-J.; Huang, H.-Q.; Cai, M.-S. *Carbohydr. Res.* **1994**, *265*, 227.

(9) Zhang, S.-Q.; Li, Z.-J.; Wang, A.-B.; Cai, M.-S.; Feng, R. *Carbohydr. Res.* **1998**, *308*, 281.

(10) Hung, S.-C.; Thopate, S. R.; Wang, C.-C. *Carbohydr. Res.* **2001**, *330*, 177.

(11) Colorless crystals from chloroform/hexane, $C_{20}H_{19}N_3O_6$, fw = 397.14, crystal dimensions: $0.43 \times 0.31 \times 0.19$ mm^3, crystal system: orthorhombic, space group: P212121, unit-cell dimensions: a = 8.7073(7), b = 11.0684(21), c = 19.784(6) Å, V = 1906.7(7) Å^3, Z = 4, ρ_{calcd} = 1.384 gcm^{-3}, wavelength = 0.7107 Å, F(000) = 831.85, mu = 0.10 mm^{-1}, 2θ(max) = 50.0. The deposition number at the Cambridge Crystallographic Data Centre is CCDC 162431.

(12) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.

(13) *The Chemistry, Biology and Medical Applications of Hyaluronan and its Derivatives*; Laurent, T. C., Ed.; Portland Press: London, **1998**.

(14) De Luca, C.; Lansing, M.; Martini, I.; Crescenzi, F.; Shen, G.-J.; O'Regan, M.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 5869.

(15) (a) Slaghek, T. M.; Nakahara, Y.; Ogawa, T. *Tetrahedron Lett.* **1992**, *33*, 4971. (b) Slaghek, T. M.; Nakahara, Y.; Ogawa, T.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1994**, *255*, 61. (c) Halkes, K. M.; Slaghek, T. M.; Hyppönen, T. K.; Kruiskamp, P. H.; Ogawa, T.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1998**, *309*, 161. (d) Yeung, B. K. S.; Hill, D. C.; Janicka, M.; Petillo, P. A. *Org. Lett.* **2000**, *2*, 1279.

(16) (a) Slaghek, T. M.; Hyppönen, T. K.; Ogawa, T.; Kamerling, J. P.; Vliegenthart, J. F. G. *Tetrahedron Lett.* **1993**, *34*, 7939. (b) Slaghek, T. M.; Hyppönen, T. K.; Ogawa, T.; Kamerling, J. P.; Vliegenthart, J. F. G. *Tetrahedron: Asymmetry* **1994**, *5*, 2291. (c) Carter, M. B.; Petillo, P. A.; Anderson, L.; Lerner, L. E. *Carbohydr. Res.* **1994**, *258*, 299. (d) Coutant, C.; Jacquinet, J.-C. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1573. (e) Blatter, G.; Jacquinet, J.-C. *Carbohydr. Res.* **1996**, *288*, 109.

(17) Fischer, B.; Nudelman, A.; Ruse, M.; Herzig, J.; Gottlieb, H. E.; Keinan, E. *J. Org. Chem.* **1984**, *49*, 4988.

(18) The selected physical data of key compounds is listed. Compound **22**: $[\alpha]_D^{25}$ –103.1 (*c* 1.0, $CHCl_3$). Mp 209–210 °C. IR ($CHCl_3$): ν = 2956 (w), 2115 (s), 1751 (s), 1635 (m), 1374 (m), 1219 (s), 912 (w)cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09 (d, *J* = 7.2 Hz, 2 H, ArH), 7.63 (t, *J* = 7.2 Hz, 1 H, ArH), 7.49 (t, *J* = 7.6 Hz, 2 H, ArH), 7.44–7.37 (m, 5 H, ArH), 5.81 (d, *J* = 8.3 Hz, 1 H, H-1), 5.53 (s, 1 H, benzylidene), 5.25–5.14 (m, 2 H, H-3′, H-4′), 5.05 (t, *J* = 8.3 Hz, 1 H, H-2′), 4.84 (d, *J* = 8.0 Hz, 1 H, H-1′), 4.35 (dd, *J* = 10.3, 4.8 Hz, 1 H, H-5), 3.84–3.73 (m, 5 H, H-2, H-3, H-4, H-6, H-5′), 3.64–3.60 (m, 1 H, H-6′), 3.61 (s, 3 H, OMe), 2.08 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 1.99 (s, 3 H, OAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.08 (C), 169.34 (C), 169.30 (C), 166.77 (C), 164.37 (C), 136.76 (C), 134.09 (CH), 130.08 (CH), 129.17 (CH), 128.65 (CH), 128.31 (CH), 125.90 (CH), 101.54 (CH), 100.84 (CH), 93.68 (CH), 80.01 (CH), 79.39 (CH), 72.42 (CH), 72.08 (CH), 71.55 (CH), 69.25 (CH), 68.21 (CH_2), 67.06 (CH), 65.02 (CH), 52.69 (CH_3), 20.55 (CH_3), 20.42 (CH_3). HRMS (FAB, MH$^+$) calcd for $C_{33}H_{36}N_3O_{15}$: 714.2146. Found: 714.2111. Anal. Calcd for $C_{33}H_{35}N_3O_{15}$: C, 55.54; H, 4.94; N, 5.89. Found: C, 55.41; H, 4.64; N, 5.55. Compound **23**: $[\alpha]_D^{25}$ –66.2 (*c* 0.9, $CHCl_3$). Mp 188–189 °C. IR ($CHCl_3$): ν = 3417 (w), 2955 (m), 1756 (s), 1751 (s), 1735 (s), 1654 (m), 1249 (s), 1084 (s), 753 (m)cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 (d, *J* = 7.5 Hz, 2 H, ArH), 7.60 (t, *J* = 7.5 Hz 1 H, ArH), 7.48–7.44 (m, 4 H, ArH), 7.41–7.37 (m, 3 H, ArH), 6.29 (d, *J* = 7.9 Hz, 1 H, H-1), 5.87 (d, *J* = 7.9 Hz, 1 H, NH), 5.52 (s, 1 H, benzylidene), 5.23–5.15 (m, 2 H, H-3′, H-4′), 5.00 (t, *J* = 7.7 Hz, 1 H, H-2′), 4.90 (d, *J* = 7.7 Hz, 1 H, H-1′), 4.45 (t, *J* = 8.7 Hz, 1 H, H-3), 4.36 (dd, *J* = 8.9, 3.3 Hz, 1 H, H-6), 3.95–3.89 (m, 2 H, H-2, H-4), 3.82–3.72 (m, 3 H, H-5, H-5′, H-6), 3.62 (s, 3 H, OMe), 1.99 (s, 3 H, OAc), 1.98 (s, 3 H, OAc), 1.96 (s, 3 H, OAc), 1.94 (s, 3 H, OAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.42 (C), 170.05 (C), 169.43 (C), 169.39 (C), 167.08 (C), 164.81 (C), 136.97 (C), 133.82 (CH), 130.09 (CH), 129.12 (CH), 128.65 (CH), 128.60 (CH), 128.29 (CH), 126.07 (CH), 101.51 (CH), 99.67 (CH), 92.38 (CH), 79.76 (CH), 77.32 (CH), 72.09 (CH), 71.56 (CH), 69.24 (CH), 68.57 (CH_2), 66.63 (CH), 55.43 (CH), 52.68 (CH_3), 23.29 (CH_3), 20.53 (CH_3), 20.43 (CH_3). HRMS (FAB, MH$^+$) calcd for $C_{35}H_{40}NO_{16}$: 730.2347. Found: 730.2360. Anal. Calcd for $C_{35}H_{39}NO_{16}$: C, 57.61; H, 5.39; N, 1.92. Found: C, 57.58; H, 5.33; N, 1.85.