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Synthesis of Sulfated β-1,6-Linked Oligosaccharide Mimetics: A Novel Potent Inhibitor of HIV Replication

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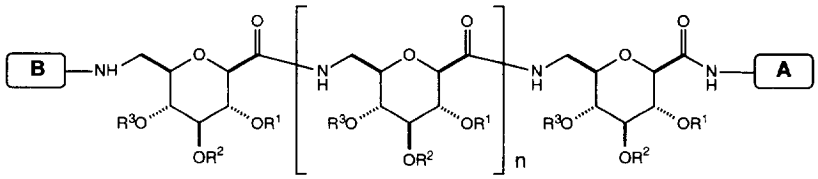
Abstract: A novel sulfated β(1→6)-linked oligosaccharide mimetics has been synthesized and found to be a potent inhibitor of HIV replication, with an IC_{50} of 1 μM. Copyright © 1996 Elsevier Science Ltd

Despite their potent inhibitory effect on HIV replication,[1-4] sulfated polysaccharides such as dextran sulfate and heparin have been abandoned as chemotherapeutic agents because of their anticoagulant activity, poor absorption and instability.[5,6] More recently, considerable effort has been devoted to developing a class of sulfated oligosaccharides with a hydrophobic aglycon as potential inhibitors of HIV infection that would exhibit less anticoagulant activity and toxicity.[7,8] Unfortunately, these compounds are natural oligosaccharides derivatives and are therefore susceptible to glycosidase digestion.[8] Thus, development of a new type of oligosaccharide mimetics that is resistant to glycosidases has been actively pursued. A group from Hoffman-La Roche has recently reported the synthesis of amido-linked oligosaccharides composed of nor-muramic acid[9] and (2→6)-linked 2-amino-2-deoxy-D-glucuronic acid derivatives.[10,11]

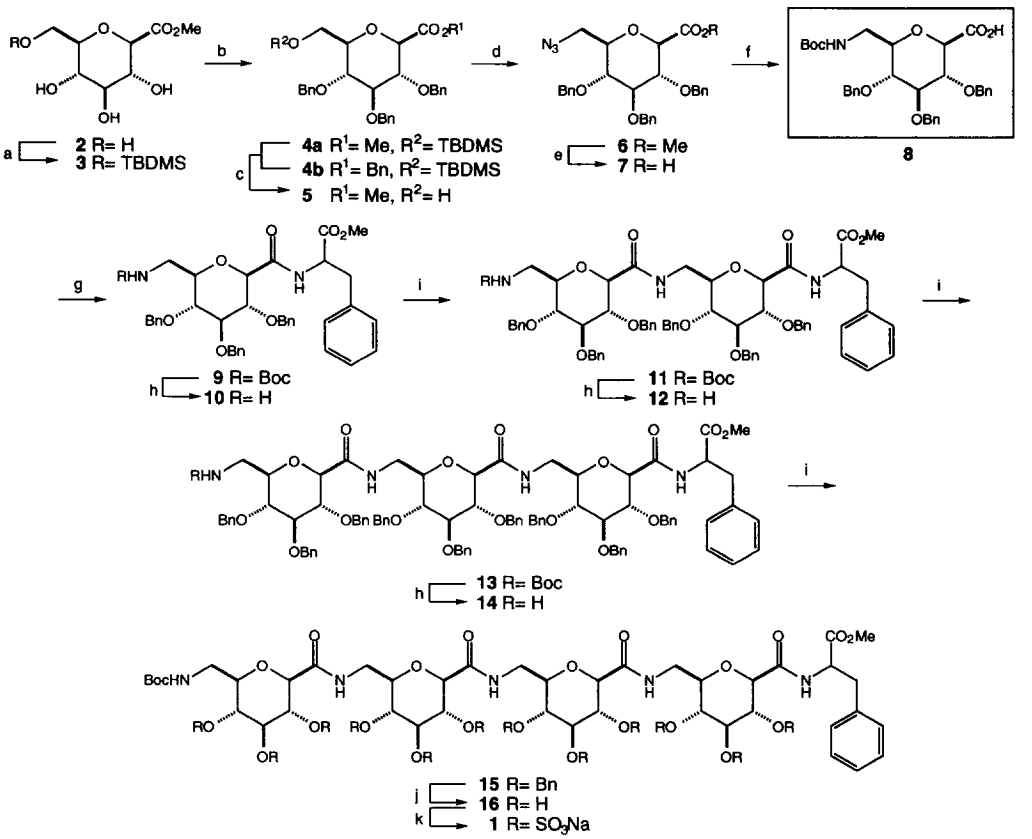


A,B= amino-containing compound; R^1, R^2, R^3 = H or SO_3Na

Fig. 1. General structure of β(1→6)-linked oligosaccharide analogue.

In the course of our study on the development of carbohydrate mimetics, we have synthesized a β(1→2)-linked carbopeptoid and have shown that its sulfated derivative is a potent inhibitor of HIV replication.[12] Herein, we report the synthesis of an even more potent inhibitor of HIV replication: a sulfated β(1→6)-linked oligosaccharide mimetic (shown in Fig. 1) which is interlinked via amido linkages between the *C*-1 β-carboxylate and the *C*-6 amino groups. The reducing end (*C*-terminus) is blocked with an amino compound **A**: in this case, **A** is phenylalanine, and the nonreducing end (*N*-terminus) is protected with **B**, in this case, a Boc group. Both the *C*- and *N*-termini can be modified or conjugated with a variety of functional moieties such as fluorescent groups.

Scheme 1 summarizes the synthesis of the tetrameric analogue, starting with a known methyl 2,6-anhydro-D-*glycero*-D-gulo-hepturonate (**2**).[13,14] For the introduction of the 6-amino group, **2** was selectively silylated at the 6-OH with TBDMSCl, giving **3**, and the remaining 2,3,4-hydroxyl groups of **3** were benzylated under the conditions



Scheme 1. Synthesis of a sulfated tetrameric β(1→6)-linked oligosaccharide analogue (1). Reagents and conditions: a) 1.2eq TBDMSCl, 2.5eq imidazole, DMF, 2h, 0°C, 83%; b) 6eq Ag_2O, 9eq BnBr, DMF, 20h, r.t., 75%; c) (i) AcOH/THF/H_2O (3:1:1), 15h, r.t., (ii) NaOMe/MeOH, 1h, r.t., 78% overall; d) (i) TsCl, pyridine, 12h, 0°C to r.t., (ii) NaN_3, DMF, 12h, 60°C, 81% overall; e) 0.13N LiOH, MeOH/THF/H_2O (3:3:1), 3h, r.t., 82%; f) (i) H_2, Pd-C/$BaSO_4$ (Lindlar catalyst), MeOH, 3h, r.t., (ii) 1.5eq Boc_2O, 2eq LiOH, MeOH/H_2O (3:1), 12h, r.t., 54% overall; g) 1.2eq phenylalanine methyl ester, 1.5eq DEPC, 3eq Et_3N, DMF, 16h, 0°C to r.t., 92%; h) 2*N* HCl/EtOAc, 3h, 0°C to r.t., i) 1.2eq **8**, 1.5eq DEPC, 3eq Et_3N, DMF, 16h, 0°C to r.t.; j) H_2, 10% Pd-C, MeOH, 16h, r.t., 86%; k) 10eq SO_3•NMe_3, DMF, 5 days, 50°C, 65%.

described by Nicolaou et al.[14] However, the benzylation of **3** with Ag_2O/BnBr, in our hands, gave a 1:1 mixture of a methyl ester (**4a**) and the corresponding benzyl ester (**4b**) in 75% yield.[15] Presumably, the basic condition caused an ester-exchange reaction between the CO_2Me and BnOH generated by the hydrolysis of BnBr.[16]

The silyl group of the mixture of **4a** and **4b** was removed under the acidic conditions,[14] and the crude product was treated with NaOMe in MeOH (in order to convert the benzyl ester to the corresponding methyl ester) to give **5** in 78% overall yield. Tosylation of the primary OH followed by the treatment with NaN_3 gave **6** in 81% overall yield. After the methyl ester of **6**[17] was hydrolyzed (LiOH/MeOH-H_2O),[18] giving **7**, the azido group of **7** was reduced with H_2/Lindlar catalyst,[19] and subsequently the amino group was protected with Boc group (Boc_2O)[20] to furnish the monomeric building block **8**.[17]

The monomeric component **8** was first coupled with phenylalanine (diethylphosphoryl cyanide (DEPC) and Et_3N)[21] as the *C*-terminal modification to give **9**[17] in 92% yield. The Boc group of **9** was removed with 2*N* HCl/EtOAc to give **10**, which was used for the next coupling reaction without further purification. The coupling of **10** and the monomeric component **8** was carried out again with DEPC and Et_3N to give **11**[17] in 90% yield. The same reaction steps were carried out: 1) removal of the Boc group from the *N*-terminus and 2) coupling of the monomeric component **8**, to the dimer **11** and then the trimer **12** easily produced the respective trimer (**13**:[17] 85% yield) and the tetramer (**15**:[17] 81% yield). The benzyl groups of **15** were removed by hydrogenolysis to give the tetramer **16**[17] in 86% yield after chromatographic purification on Sephadex G-25. Sulfation of **16** was conducted with SO_3•NMe_3 in anhydrous DMF for 4 days at 50 °C, and the final compound was purified by Sephadex G-25 chromatography with water (fractions that contain sulfated oligosaccharide analogue were detected with Azure A reagent)[22] to give the sulfated tetrameric β(1→6)-linked oligosaccharide analogue **1**[17] in 65% yield. Elemental analysis and mass spectroscopic data suggested that the average number of the sulfate groups per Glc unit was two.

The anti-HIV activity of tetramer **1** was assessed by measuring the protection of MT2 cells from HIV infection.[23] While neither the trimer analogue of **1** nor the non-sulfated tetramer **16** had any measurable inhibitory activity, the sulfated tetramer **1** showed a strong inhibitory potency, with an IC_{50} of 1 μM, which is almost equivalent to that of the natural oligosaccharide derivative with more than five sulfated Glc residues. The inhibition mechanism of the sulfated oligosaccharide is not clear yet, but is suggested to be dependent the degree of the sulfation.[24] The potency of the anti-HIV activity of the sulfated tetramer (**1**) compares favorably with other inhibitors of HIV replication such as AZT (zidovudine) which has a reported IC_{50} of 0.024 to 2.5 μM.[25]

In summary, we have demonstrated an efficient strategy for constructing a new type of carbohydrate mimetics via the β(1→6)-amido linkage and have shown that its sulfated product is a very potent inhibitor of HIV replication. We are now evaluating the relationship between structure and anti-HIV activity for a number of amido-linked oligosaccharide analogues with other positional linkages, and also assaying their stability and anticoagulant activity.

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15. The structures of **4a** and **4b** were assigned based on the ^{1}H NMR spectra of the *O*-desilylated derivatives from the mixture. **5** (Me ester from **4a**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.62 (s, 3H, CO_2CH_3), 4.56 (d, 1H, 0.5 × Bn), 4.69 (d, 1H, 0.5 × Bn), 4.75 (d, 1H, 0.5 × Bn), 4.84 (d, 1H, 0.5 × Bn), 4.88 (s, 2H, 1.0 × Bn). Bn ester derivative (from **4b**): ^{1}H NMR δ 4.36 (d, 1H, 0.5 × Bn), 4.58 (d, 1H, 0.5 × Bn), 4.60 (d, 1H, 0.5 × Bn), 4.72 (d, 1H, 0.5 × Bn), 4.76 (s, 1H, 1.0 × Bn), 4.99 (s, 1H, 1.0 × Bn). Additionally, upon treatment with NaOMe in MeOH, a mixture of the *O*-desilylated products gave a single product of **5**.

16. The *C*-1 CO_2Me-containing sugar derivatives, including glucose, galactaose, and glucosamine derivatives were very sensitive to basic conditions. Attempts to benzylate **2** in a standard way (addition of NaH first and then BnBr in DMF) or in the presence of I^- (Ag_2O/KI/BnBr in DMF) resulted in the formation of eliminated products as a major by-product. However, employment of mild conditions (Ag_2O/BnBr without I^-) or a reversed addition of the reagents (addition of NaH to a premixed suspension of the substrate, BnBr and Bu_4NI in THF) gave the products in moderate to good yields. See, Ichikawa, Y.; Manaka, A.; Kuzuhara, H. *Carbohydr. Res.* **1985**, *138*, 55-61.

17. Selected ^{1}H and ^{13}C NMR data, and the numbering is based on the regular carbohydrate numbering (the anomeric carbon is the *C*-1). **8**: ^{1}H NMR (300 MHz, CD_3OD) δ 1.45 (s, 9H, BocNH), 3.47-3.29 (m, 3H, H-2,5,6a), 3.54 (q, 1H, *J* 3.8,13.9 Hz, H-6b), 3.71 (m, 2H, H-1,4); ^{13}C NMR (75 MHz, CD_3OD) δ 166.5 (NHBoc), 172.5 (*C*-1 COOH). **9**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 (s, 9H, BocNH), 3.10 (q, 1H, *J* 6.2,14.0 Hz, β-proton of Phe), 3.15 (q, 1H, *J* 5.8,14.0 Hz, β-proton of Phe), 3.33-3.41 (m, 2H, H-2,5), 3.80 (d, 1H, J 9.0 Hz, H-1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.4 (tBu of Boc), 37.4, 52.3 ($CO_2\underline{C}H_3$), 52.6, 75.0, 75.5, 77.9, 78.5, 79.5, 80.4, 85.7, 155.7, 168.3, 171.6. **11**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (s, 9H, BocNH), 3.02 (m, 2H, β-protons of Phe); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.2 (tBu of Boc), 37.6, 52.3, 53.0, 74.8, 75.0, 75.4, 77.2, 78.2, 78.7, 79.4, 79.7, 80.3, 85.5, 85.7, 156.0, 168.2, 168.8, 171.8. **13**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (s, 9H, BocNH), 3.07 (m, 2H, β-protons of Phe); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.4 (tBu of Boc), 37.6, 52.3, 53.1, 74.8, 74.9, 75.3, 75.5, 77.3, 77.8, 78.1, 78.2, 78.4, 78.6, 79.3, 79.7, 80.1, 85.5, 85.6, 85.7, 156.0, 168.2, 168.8, 169.0, 171.6. **15**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (s, 9H, BocNH), 3.07 (m, 2H, β-protons of Phe); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.4 (tBu of Boc), 37.8, 52.2, 53.3, 74.7, 74.8, 75.0, 75.16, 75.21, 75.4, 75.6, 76.6, 76.8, 77.0, 77.2, 77.5, 78.0, 78.1, 78.3. 78.6, 78.9, 79.2, 79.4, 79.8, 80.1. 80.6, 85.4, 85.7, 85.8, 86.1, 156.1, 168.2, 168.9, 169.16, 169.22, 171.8. **16**: ^{1}H NMR (300 MHz, D_2O) δ 1.29 (s, 9H, BocNH), 2.95 (m, 2H, β-protons of Phe). **1**: ^{1}H NMR (300 MHz, D_2O) δ 1.29 (s, 9H, BocNH), 3.04 (m, 2H, β-protons of Phe), 3.59 (s, 3H, CO_2CH_3), 7.22-7.16 (m, 5H, Ph of Phe).

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