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The first chemical synthesis of F-4-GlcAβ(1→3)GlcNAc-UDP with the potential of novel substrate and enzyme inhibitor for hyaluronic acid synthases (HASs)

Guohua Wei [a,b], Vipin Kumar [a], Jun Xue [a], Robert D. Locke [a], Khushi L. Matta [a,*]

[a] Cancer Biology, Roswell Park Cancer Institute, Buffalo, NY 14263, USA
[b] State key laboratory of Environmental Chemistry and Ecotoxicology, Research center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

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ABSTRACT

The first chemical synthesis of F-4-GlcAβ(1→3)GlcNAc-UDP is described here. This compound can serve as a novel substrate to study the catalytic mechanism of hyaluronic acid synthases (HASs) and has potential to be donor for these enzymes that extend HA chain at the reducing end. Moreover, it may also behave as inhibitor for the enzymes that act on non-reducing end in the assembly of HA chain.

1. Introduction

Hyaluronan (HA), a non-sulfated linear glycosaminoglycan (GAG) polymer, composed of repeating disaccharide units of glucuronic acid and *N*-acetylglucosamine linked together via alternating β-1,4 and β-1,3 glycosidic bonds. It is one of the chief components of the extracellular matrix and contributes significantly in multiple biological and physiological functions such as wound healing, inflammation, angiogenesis, and tumor growth.[1] Hyaluronan synthases (HASs) are the enzyme which catalyze the polymerization of HA using UDP-sugar precursors. These are the first glycosyltransferases, unlike the majority of other known glycosyltransferases, demonstrated to be capable of transferring two distinct sugars (GlcUA and GlcNAc) alternatively only to one end of growing HA polymer chain.[1a,e,2] This unique bifunctional nature of HASs in the assembly of HA molecule is recently reviewed by Weigel et al.[3] Added to the inimitability of these enzymes, there are two mutually exclusive possibilities for the direction of HA biosynthesis. Bacterial HASs add sugars at the non-reducing ends of the acceptor to produce the HA polymer, whereas, vertebrate enzymes transfer the sugars to the reducing end (Fig. 1). For the biosynthesis at the reducing end, the UDP released during each transfer step comes from an HA-UDP intermediate formed by addition to the previous sugar.[1e,2,3] The intermediate, (HA)-UDP, acts at the C-3 position of UDP-GlcNAc releasing the UDP of HA, and keeping the UDP of GlcNAc intact. This divergence calls for an explanation. Hitherto, no three-dimensional structure for any HASs is available, therefore, our understanding of the detailed mechanisms whereby hyaluronan influences cell behavior is still very incomplete.[3]

Interfacing chemistry between biology[4] has been the real guide and theme for our laboratories over the last three decades. A chemical synthetic approach provides a series of well-defined oligosaccharide structures that can lead to the identification of the previously unrecognized glycosyltransferases activities and, therefore, to the discovery of new biosynthetic pathways in the assembly of glycans. The modifications to the basic acceptor structures by the introduction of *O*-Me or fluoro groups can result in highly specific acceptors or inhibitors for the individual glycosyltransferases. In this context, a series of novel acceptors have been synthesized to study sulfotransferases, sialyltransferases, and fucosyltransferases.[5] We now extend our theme of interfacing chemistry between biology of HA to focus our attention on development of tools to understand the metabolism of this molecule. We describe herein the first chemical synthesis of F-4-GlcAβ(1→3)GlcNAc-UDP (**1**) which may have the potential to be a specific donor for the HASs enzyme that extend the HA chain at the reducing end whereas this modified analog may function as inhibitor for the enzyme that act at non-reducing end in the assembly of HA molecule.

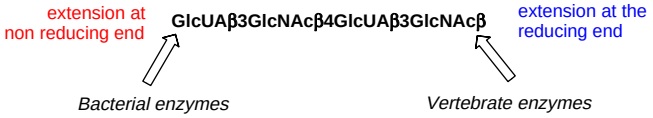

Figure 1. Extension of HA polymer.

* Corresponding author. Tel.: +1 716 845 2397; fax: +1 716 845 8768.
E-mail address: khushi.matta@roswellpark.org (K.L. Matta).

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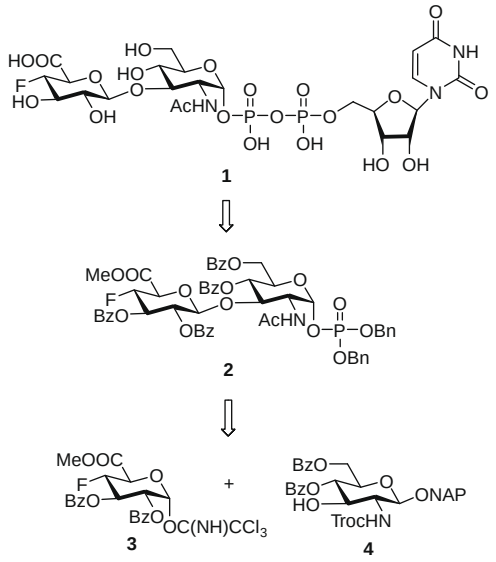


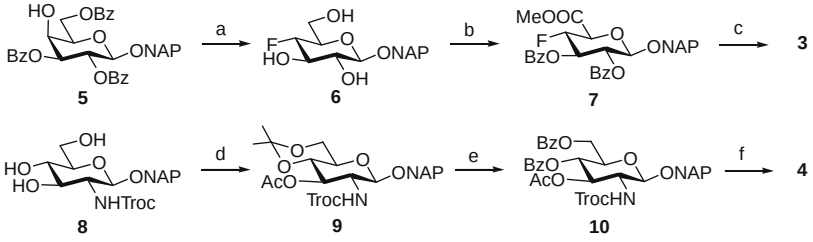
Figure 2. Retrosynthetic analysis of target compound F-4-GlcAβ(1→3)GlcNAc-UDP 1.

2. Results and discussion

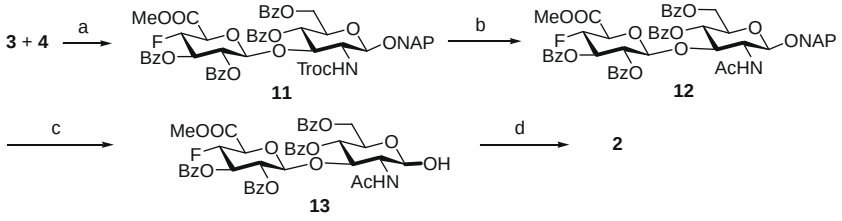
As shown in retrosynthetic analysis (Fig. 2), the key intermediate in our approach to the title compound **1** is the protected F-4-GlcAβ(1→3)GlcNAc-1-phosphate derivative (**2**) that could be derived from monosaccharide building blocks **3** and **4**. For the preparation of **3** and **4**, NAP as an anomeric protecting group attracts our attention as this group, like *p*-methoxybenzyl, can be mildly removed with DDQ oxidation (reported from our laboratory). It may be added here that many starting monosaccharides having NAP group did not require chromatography. Moreover, the compounds being solid were easy to handle.[6] The preference of benzoyl group over acetyl group for protection of the 2-OH position of GlcA in donor **3**, circumvented the possible formation of side product due to transacetylation during O-glycosylation.[7]

The synthesis of **3** commenced with compound **5**, which was treated with diethylaminosulfur trifluoride (DAST)[8] followed by deprotection of benzoyl groups to afford **6** in 85.4% yield (over over two steps). Selective oxidation of 6-OH of **6** to a carboxylic acid by 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and $Ca(ClO)_2$[9] followed by methyl esterification and benzoylation gave **7** with 82% overall yield in three steps. Chemoselective removal of NAP with DDQ[10] and subsequent treatment with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) afforded imidate **3** in 78% yield.[11] Next, synthesis of monosaccharide acceptor **4** was initiated using already known compound **8**.[6] Installation of isopropylidene group and subsequent acetylation provided **9** in good yield. Treatment of **9** with 80% HOAc to remove the isopropylidene followed by benzoylation gave **10** in high yield. Finally, selective removal of 3-OAc group of **10** with *p*-TsOH in CH_2Cl_2–MeOH (9:1) proceeded smoothly[12] to afford **4** in 89% yield (Scheme 1).

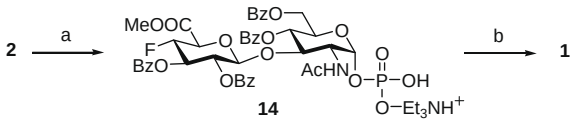
With the required building blocks in hand, the multistep synthesis of the phosphate **2** was initiated. First, coupling of the F-4-GlcA donor **3** and the suitable protected acceptor **4** was carried out with $BF_3{\cdot}Et_2O$ in the presence of 4 Å molecular sieves in toluene to give the desired disaccharide **11** with complete β1→3 stereoselectivity in 89% yield.[7a,8,13] Substitution of dichloromethane as a solvent proved to be detrimental for above-mentioned glycosylation reaction. Also, TMSOTf was found ineffective for the same glycosylation as a promoter in comparison with $BF_3{\cdot}Et_2O$. Not many expedient methods are available for the synthesis of GlcAβ1→3 linkage[8,13b–e] and suffer from certain drawbacks. For example, a synthesis reported by Kong and co-workers yielded both α- and β1→3 linked products in equimolar ratio.[13b] Withers and co-workers followed an alternative route, to obtain F-4-GlcAβ1→3 linked disaccharide. First, the synthesis of F-4-Glcβ1→3 linked disaccharide was carried out followed by oxidation of F-4-Glc moiety to achieve final product. However, the overall yield of this indirect conversion was only moderate.[8] Similar roundabout synthetic sequence was also accounted by Yeung et al.[13c] Fraser-Reid and co-workers utilized GlcA imidate donor to glycosylate 3-OH of suitably protected *n*-pentenyl acceptor but resulted in 3:1 mixture of the desired β1→3 linked disaccharide and the corresponding orthoester.[13d] Recently, Rele et al. reported a stereospecific



Scheme 1. Preparation of the key building blocks **3** and **4**. Reagents and conditions: (a) (i) DAST, CH_2Cl_2, −30 °C, 4 h, 89%; (ii) NaOMe, MeOH, rt, 4 h, 96%; (b) (i) TEMPO, $Ca(ClO)_2$, KBr, Bu_4NBr, CH_2Cl_2, satd aq $NaHCO_3$, 0 °C, 1 h; (ii) MeI, $KHCO_3$, DMF, rt, 10 h; (iii) BzCl, py, rt, 4 h, 82% for three steps; (c) (i) DDQ, CH_2Cl_2–MeOH (4:1), rt, 12 h; (ii) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 2 h, 78% for two steps; (d) (i) 2,2-dimethoxylpropane, *p*-TsOH, DMF, 0 °C, 4 h; (ii) Ac_2O, py, rt, 4 h, 88% for two steps; (e) (i) 80% HOAc, 70 °C, 4 h; (ii) BzCl, py, rt, 4 h, 92% for two steps; (f) *p*-TsOH, CH_2Cl_2–MeOH (9:1), rt, 24 h, 89%.



Scheme 2. Preparation of disaccharide monophosphate derivative **2**. Reagents and conditions: (a) $BF_3{\cdot}Et_2O$, toluene, 4 Å MS, 0 °C, 2 h, 89%; (b) Zn, Ac_2O, AcOH, THF, rt, 3 h, 86%; (c) DDQ, CH_2Cl_2–MeOH (4:1), rt, 12 h, 89%; (d) LHMDS, $[(BnO)_2P]_2O$, THF, −70 to 0 °C, 3 h, 83%.



Scheme 3. Synthesis of F-4-GlcAβ(1→3)GlcNAc-UDP **1**. Reagents and conditions: (a) H_2, Pd–C, EtOAc–MeOH (1:1), Et_3N, rt, 6 h, 91%; (b) (i) UMP-morpholidate, 1*H*-tetrazole, DMF-py (3:1), rt, 2 d; (ii) 3 M NaOH, MeOH, rt, 10 h; (iii) RP column HPLC; gel-filtration column HPLC, 42% from **14**.

synthesis of β1→3 linked disaccharide by the glycosylation of *n*-pentenyl glycoside acceptor with GlcA donor without obtaining the corresponding orthoester as the side product.[13e] In conclusion, the efficacy of glycosylation with a donor can depend on the nature of the sugar alcohol and the protecting groups present there in. Thus, it is also perceptible from above discussion that the glycosylation reaction described by us is extremely efficient and superior to most of the previously described syntheses for β1→3 linkage. Our glycosylation reaction provides a route to obtain F-4-GlcAβ1→3 linked disaccharide with complete β1→3 stereoselectivity and high yield.

To proceed further in our synthetic sequence, NHTroc group of **11** was transformed to NHAc using Zn–Ac_2O to furnish **12** in 86% yield. Selective removal of NAP with DDQ in CH_2Cl_2–MeOH (4:1) and subsequent phosphorylation with tetrabenzyl pyrophospgate[14] gave the benzyl-protected anomeric phosphate **2** as the desired α anomer in high yield. Appearance of significant signals in the ^{1}H NMR spectrum of **2** at $\delta = 5.71$ (dd, $J_{1,2} = 3.4$ Hz, $J_{1,P} = 6.4$ Hz, 1H, H-1of GlcNAc), and the signal in the ^{13}P NMR of **2** at $\delta = -1.91$ (s, 1P) confirmed that the newly formed linkage between the disaccharide and phosphoric acid moiety was α (Scheme 2).

Deprotection of the benzyl groups in compound **2** by hydrogenation over Pd–C provided **14** in very high yield. Coupling of **14** with UMP-morpholidate in the presence of 1*H*-tetrazole in DMF–pyridine (3:1)[14] followed by deprotection of the benzoyl groups and the methyl ester using 3 M NaOH afforded the target compound **1**[15] in 42% yield over two steps after isolation and purification by reverse-phase column HPLC and gel-filtration column HPLC (Scheme 3).

3. Conclusions

In conclusion, we successfully accomplished the first chemical synthesis of F-4-GlcAβ(1→3)GlcNAc-UDP. During the course of our synthesis, we have described a very efficient glycosylation reaction which provides a facile access to the desired F-4-GlcAβ1→3 linked disaccharide with complete stereoselectivity and high yield. We believe that this compound will serve as a novel substrate to study the catalytic mechanism of HASs. The strategy as described here could be extended to develop a novel assay for these enzymes using synthetic acceptors/donors.

Acknowledgments

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7. (a) Brown, R. T.; Carter, N. E.; Mayalrap, S. P.; Scheinmann, F. *Tetrahedron* **2000**, *56*, 7591; (b) In a separate study in our laboratory, the reaction of commonly used methyl 2,3,4-tri-*O*-acetyl-1-*O*-trichloroacetimidoyl-α/β-D-glucopyranosyluronate with NAP 2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside did not proceed to give the desired β1→3 disaccharide in a satisfactory yield but afforded the transacetylated product viz., NAP 3-acetyl-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside. Hence, it is evident that the glycosylation with GlcA donor can depend upon the nature of the acceptor also.
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15. *The selected physical data of key compounds is listed*: Compound **3**: $[\alpha]_D^{25}$ +8.9 (*c* 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.86 (s, 3H, COOMe), 4.50 (dd, $J_{5,4} = 9.6$ Hz, $J_{5,F} = 4.8$ Hz, 1H, H-5), 5.01 (ddd, $J_{4,3} = 10.0$ Hz, $J_{4,F} = 49.6$ Hz, 1H, H-4), 5.19 (dd, $J_{2,1} = 4.0$ Hz, $J_{2,3} = 9.6$ Hz, 1H, H-2), 6.11 (ddd, $J_{3,2} = J_{3,4} = 10.0$ Hz, $J_{3,F} = 14.6$ Hz, 1H, H-3), 6.62 (d, $J_{1,2} = 3.6$ Hz, 1H, H-1), 7.35–8.01 (m, 10H, Ph), 8.87 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 52.5, 69.4, 71.6 (d, *J* = 18.0 Hz), 72.3 (d, *J* = 17.7 Hz), 86.3 (d, *J* = 17.7 Hz, C-4), 90.1, 92.3, 159.7, 164.7, 164.9, 167.5. MALDITOF-MS: calcd for $C_{23}H_{19}Cl_3FNO_8$: 561.02; found: 584.34 $[M+Na]^+$. Compound **4**: $[\alpha]_D^{25}$ +52.5 (*c* 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 3.21 (s, 1H), 3.85 (t, *J* = 10.6 Hz, 1H), 4.22 (m, 1H), 4.35 (m, 2H), 4.45 (dd, *J* = 4.6, 11.5 Hz, 1H), 4.58 (d, *J* = 12.0 Hz, 1H), 4.65 (d, *J* = 12.0 Hz, 1H), 4.72 (m, 2H), 4.88 (d, *J* = 8.6 Hz, 1H), 5.15 (d, *J* = 9.0 Hz, 1H), 5.63 (t, *J* = 9.6 Hz, 1H), 6.90–8.0 (m, 17H). MALDITOF-MS: calcd for $C_{34}H_{30}Cl_3NO_9$: 701.10; found: 724.45 $[M+Na]^+$. Compound **11**: $[\alpha]_D^{25}$ +20.6 (*c* 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 3.39 (s, 3H), 3.95–3.98 (m, 2H), 4.35–4.41 (m, 2H), 4.51–4.57 (m, 2H), 4.60 (d, *J* = 7.8 Hz, 1H, H-1′), 4.62–4.68 (m, 2H), 4.78–4.80 (m, 1H), 4.83 (d, *J* = 8.0 Hz, 1H, H-1), 4.95 (d, *J* = 12.0 Hz, 1H), 5.04–5.10 (m, 2H), 5.34–5.36 (m, 2H), 5.58 (ddd, *J* = 10.0, 14.6 Hz, 1H), 6.97–8.04 (m, 27H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 52.0, 58.1, 62.9, 69.8, 70.7, 71.4, 71.5 (d, *J* = 8.4 Hz), 71.7, 72.0, 73.4 (d, *J* = 19.4 Hz), 77.6, 87.9 (d, $J_{4',F} = 185.4$ Hz, C-4′), 94.8, 97.9, 100.9, 153.2, 163.9, 164.4, 164.7, 165.3, 165.6. ^{19}F NMR (323.6 MHz, $CDCl_3$) δ (ppm) = −122.13 (dd, $J_{F,4'} = 51.4$ Hz, $J_{F,3'} = 16.4$ Hz). MALDITOF-MS: calcd for $C_{55}H_{47}Cl_3FNO_{16}$: 1101.19; found: 1124.51 $[M+Na]^+$. Compound **2**: $[\alpha]_D^{25}$ +32.3 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 1.83 (s, 3H), 3.41 (s, 3H), 3.89–3.92 (m, 1H), 4.15–4.25 (m, 3H), 4.39–4.49 (m, 2H), 4.68–4.73 (m, 2H), 4.98–5.11 (m, 6H), 5.71 (dd, $J_{1,2} = 3.4$ Hz, $J_{1,P} = 6.4$ Hz, 1H, H-1), 5.78 (d, *J* = 9.2 Hz, 1H, NHAc), 6.12 (ddd, $J_{3',2'} = J_{3',4'} = 10.0$ Hz, $J_{3',F} = 15.5$ Hz, 1H, H-3′), 6.90–8.04 (m, 30H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 23.2, 53.2, 56.1 ($J_{C\text{-}2,P} = 6.9$ Hz, C-2), 62.1, 69.3 ($J_{CH2Ph,P} = 4.9$ Hz, $POCH_2Ph$), 69.5 ($J_{CH2Ph,P} = 4.9$ Hz, $POCH_2Ph$), 69.8, 71.7, 72.5 (CH, d, *J* = 8.8 Hz), 72.8, 73.8 (d, *J* = 19.8 Hz), 77.6, 86.9 (d, $J_{4',F} = 186.2$ Hz, C-4′), 95.4 ($J_{C\text{-}1,P} = 6.1$ Hz, C-1), 100.9, 163.9, 164.6, 164.7, 165.5, 168.6, 170.1. ^{19}F NMR (323.6 MHz, $CDCl_3$) δ (ppm) = −121.32 (dd, $J_{F,4'} = 52.2$ Hz, $J_{F,3'} = 16.8$ Hz). ^{31}P NMR (202 MHz, $CDCl_3$) δ (ppm) = −1.91 (s, 1P). MALDITOF-MS: calcd for $C_{57}H_{53}FNO_{18}P$: 1089.30; found: 1112.61 $[M+Na]^+$. Compound **1**: $[\alpha]_D^{25}$ +12.5 (*c* 0.6, H_2O); ^{1}H NMR (400 MHz, D_2O) δ (ppm) = 1.93 (s, 3H), 3.51 (m, 1H), 3.62 (ddd, $J_{3',2'} = J_{3',4'} = 9.6$ Hz, $J_{3',F} = 15.6$ Hz, 1H, H-3′), 3.68–3.92 (m, 5H), 3.96 (dd, $J_{5',4'} = 9.2$ Hz, $J_{5',F} = 2.6$ Hz, 1H, H-5′), 4.01 (ddd, $J_{2,1} = 3.6$ Hz, $J_{2,3} = 10.0$ Hz, $J_{2,P} = 3.1$ Hz, 1H, H-2), 4.08 (m, 1H), 4.15 (m, 1H), 4.16–4.18 (m, 1H), 4.23–4.26 (m, 2H), 4.35 (ddd, $J_{4',3'} = J_{4',5'} = 9.4$ Hz, $J_{4',F} = 51.6$ Hz, 1H, H-4′), 4.51 (d, $J_{1',2'} = 7.9$ Hz, 1H, H-1′), 5.43 (dd, $J_{1,2} = 3.2$ Hz, $J_{1,P} = 8.1$ Hz, 1H, H-1), 5.82 (d, 1H), 5.85 (d, 1H), 7.85 (d, 1H). ^{13}C NMR (100 MHz, D_2O) δ (ppm) = 23.4, 57.2 ($J_{C\text{-}2,P} = 8.8$ Hz, C-2), 62.8, 66.2 (d, *J* = 3.8 Hz), 70.7, 70.9, 71.7, 74.5 (d, *J* = 8.8 Hz), 74.7, 75.8 (d, *J* = 19.8 Hz), 77.6, 81.2, 85.0 (d, *J* = 9.8 Hz), 89.8, 93.9 (d, $J_{4',F} = 185.2$ Hz, C-4′), 96.8 ($J_{C\text{-}1,P} = 6.0$ Hz, C-1), 102.3, 103.8, 141.5, 153.4, 168.1, 174.5, 175.1. ^{19}F NMR (323.6 MHz, D_2O) δ (ppm) = −122.02 (dd, $J_{F,4'} = 50.2$ Hz, $J_{F,3'} = 16.2$ Hz). ^{31}P NMR (202 MHz, D_2O): δ: −11.2 (d, *J* = 23.8 Hz), −13.2 (d, *J* = 23.6 Hz). MALDITOF-MS: calcd for $C_{23}H_{34}FN_3O_{22}P_2$: 785.11; found: 783.95 $[M-H]^-$.