Directing the biological activities of heparan sulfate oligosaccharides using a chemoenzymatic approach*

(*Supplementary Information*)

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Running title: Chemoenzymatic synthesis of heparan sulfate

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*Chemical synthesis of hexasaccharide **1**.*

1. **Synthesis of 10 by the one-pot procedure:** After the donor **11** (50 mg, 49.4 μmol) and activated molecular sieve MS-4 Å (500 mg) were stirred for 30 minutes at room temperature in dichloromethane (DCM) (3 mL), the solution was cooled to −78 °C, followed by addition of AgOTf (38 mg, 148 μmol) in Et_2O (1 mL). The mixture was stirred for 5 minutes at −78 °C and then *p*-TolSCl (7.8 μL, 49.4 μmol) was added into the solution. The mixture was vigorously stirred for 10 minutes, followed by addition of a solution of acceptor **12** (42.1 mg, 46.9 μmol) in CH_2Cl_2 (1 mL). The reaction mixture was stirred for 2 hours from −78 to −20 °C and then the mixture was cooled down to −78 °C, followed by sequential additions of AgOTf (12.7 mg, 49.4 μmol) in Et_2O (1 mL), acceptor **13** (32.4 mg, 39.5 μmol) in CH_2Cl_2 (1 mL). The mixture was stirred for 5 minutes at −78 °C and then *p*-TolSCl (7.4 μL, 39.5 μmol) was added into the solution. The reaction mixture was stirred for 3 hours from −78 to −20 °C and then was quenched with Et_3N (40 μL), concentrated under vacuum to dryness. The resulting residue was diluted with CH_2Cl_2 (30 mL), followed by filtration. The organic phase was washed with saturated aqueous $NaHCO_3$, H_2O and then dried over Na_2SO_4, filtered and concentrated. Silica gel column chromatography (2:1 Hexanes–EtOAc) afforded **10** as white solid (69.7 mg, 71%). $[\alpha]^{25}_D$ +56.6 (c = 0.8, CH_2Cl_2); ^{1}H-NMR (600 MHz, $CDCl_3$): δ -0.04 (s, 3H, CH_3Si), -0.01 (s, 3H, CH_3Si), 0.88 (s, 9H, $(CH_3)_3CSi$), 1.08 (t, 3H, J = 7.2 Hz, OCH2), 1.98 (s, 3H, $COCH_3$), 1.99 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.10 (s, 3H, COCH3), 2.11 (s, 3H, COCH3), 2.18 (s, 3H, $COCH_3$), 2.31-2.33 (m, 2H), 2.42-2.55 (m, 6H), 2.60-2.62 (m, 2H), 2.66-2.69 (m, 2H), 3.18-3.21 (m, 1H), 3.23-3.27 (m, 2H), 3.43-3.46 (m, 1H), 3.49-3.55 (m, 2H), 3.60-3.68 (m, 5H), 3.71-4.10 (m, 19H), 4.15-4.20 (m, 3H), 4.25-

4.26 (m, 1H), 4.30-4.33 (m, 1H), 4.51-4.53 (m, 1H), 4.56-4.57 (m, 2H), 4.59-4.76 (m, 8H), 4.80-4.86 (m, 2H), 5.03-5.05 (m, 1H), 5.11-5.13 (m, 1H), 5.22 (t, 1H, J = 7.8 Hz), 5.29 (t, 1H, J = 8.4 Hz), 5.35 (t, 1H, J = 8.4 Hz), 5.37 (d, 1H, J = 4.2 Hz), 5.44 (d, 1H, J = 4.2 Hz), 5.49 (d, 1H, J = 3.6 Hz), 7.07-7.46 (m, 36H), 7.53-7.58 (m, 3H), 8.01-8.06 (m, 6H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ -5.0, -3.8, 15.0, 17.9, 20.7, 20.8, 25.8 (×2), 27.5, 27.6, 27.7, 29.7, 29.8 (×2), 36.6, 37.6, 37.7, 37.8, 61.9, 62.5, 62.6, 63.0, 63.6 (×2), 65.2, 69.5, 69.6, 70.9, 71.4, 72.4, 72.6, 72.7, 73.9, 74.1, 74.2, 74.3, 74.4, 74.6, 74.9, 75.0 (×2), 75.1 (×2), 75.6, 77.4, 77.5, 77.7, 80.2, 82.8, 82.9 (×2), 97.6, 97.7, 98.0, 100.3, 100.6, 100.7, 127.1, 127.4 (×2), 127.5 (×2), 127.6 (×4), 127.7, 128.1, 128.2 (×2), 128.3, 128.4, 128.6, 128.9, 129.0, 129.6, 129.7 (×2), 133.2, 133.5 (×2), 137.2, 137.3, 137.4, 137.8, 138.3, 138.4, 164.9 (×2), 165.0, 170.5 (×2), 170.6, 171.7, 171.8, 172.1, 206.2, 206.3 (×2). HRMS: $[M+Na]^+$ $C_{128}H_{149}N_9NaO_{40}Si$ calcd 2502.9569, obsd 2502.9532. gHMQC (without ^{1}H decoupling): $^1J_{C1,H1}$ = 174.9, 173.9, 173.9, 161.9, 161.9, 159.6 Hz.

Ethyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate (14). Compound **10** (150 mg, 0.06 mmol) was dissolved in pyridine (2.4 mL) and acetic acid (1.6 mL). The mixture was cooled down to 0 °C, followed by addition hydrazine monohydrate (45 μl, 0.9 mmol). The mixture was stirred at 0 °C for 2h and then was quenched by acetone (0.28 mL). The mixture was stirred at

room temperature for another 1h and the acetone was evaporated under vaccum. The residue was diluted with EtOAc (50 mL) and washed with saturated $NaHCO_3$, 10% HCl and water. The organic phase was dried over Na_2SO_4, filtered and the solvents were removed in vacco. Silica gel column chromatography (1:1 Hexanes–EtOAc) afforded the triol compound as white solid (125 mg, 95%). To a solution of the triol (100 mg, 0.045 mmol) in DCM (2 mL) was consecutively added H_2O (2 mL), 1 M KBr (0.15 mL, 0.15 mmol), 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) (21.4 mg, 0.135 mmol), 0.5 M $NaHCO_3$ (2 mL) and Bu_4NBr (442 mg, 1.35 mmol) at room temperature. The mixture was cooled down to 0° C, followed by slow addition of NaOCl (0.95mL, 13.5 mmol). The pH value of the mixture was calibrated with 0.5 M NaOH to maintain at pH=10 and the resulting solution was warmed up slowly to room temperature (maintain at pH=10). After stirring for 3 h, the DCM was evaporated under vacuum and the residue was diluted with EtOAc (50 mL) and washed with 10% HCl, saturated $NaHCO_3$ and water. The organic phase was dried over Na_2SO_4, filtered and the solvents were removed in vacco. Without separation, the resulting residue was dissolved in DCM (5 mL), followed by addition of phenyldiazomethane in diethyl ether (30 μL, 0.09 mmol). The mixture was stirred at room temperature for 3 h and then was diluted with DCM (50 mL) and the organic phase was washed with saturated $NaHCO_3$ and then dried over Na_2SO_4, filtered and the solvents were removed in vacco. Silica gel column chromatography (2:1 Hexanes–EtOAc) afforded compound **14** as white solid (60 mg, 53% for two steps). $[\alpha]^{25}_{D}$ +57.6 (c = 1, CH_2Cl_2); ^{1}H-NMR (600 MHz, $CDCl_3$): δ -0.04 (s, 3H, CH_3Si), -0.02 (s, 3H, CH_3Si), 0.84 (s, 9H, $(CH_3)_3CSi$), 1.03 (t, 3H, J = 7.2 Hz, OCH_2), 1.99 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 3.10-3.12 (m, 1H), 3.17-3.20 (m,

1H), 3.27-3.29 (m, 1H), 3.42-3.57 (m, 4H), 3.61-3.77 (m, 7H), 3.82-3.99 (m, 6H), 4.04-4.17 (m, 3H), 4.19-4.23 (m, 2H), 4.27-4.43 (m, 5H), 4.48-4.69 (m, 12H), 4.74-4.86 (m, 4H), 4.90-4.95 (m, 2H), 5.05-5.09 (m, 2H), 5.23 (t, 1H, J = 8.4 Hz), 5.36 (t, 1H, J = 8.4 Hz), 5.40 (d, 1H, J = 4.2 Hz), 5.42 (d, 1H, J = 3.6 Hz), 5.44 (t, 1H, J = 8.4 Hz), 5.51 (d, 1H, J = 3.6 Hz), 7.03-7.56 (m, 54H), 8.00-8.01 (m, 6H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ -5.1, -3.8, 14.2, 14.9, 17.9, 18.0, 20.8, 20.9 (×2), 25.8, 60.4, 61.4, 62.3, 62.8 (×2), 63.8, 65.3, 65.4, 67.1, 67.3, 67.7, 69.1, 69.2, 70.8, 71.2, 73.4, 73.7, 74.1, 74.2, 74.3 (×3), 74.6 (×2), 74.9, 75.0, 75.2, 75.5, 75.6, 77.4, 77.6, 77.7, 77.9, 80.3, 82.3, 82.6, 82.7, 97.0, 97.1, 97.4, 100.7, 101.2, 101.3, 127.0, 127.1, 127.5 (×2), 127.6 , 127.7 (×3), 127.8, 128.2 (×2), 128.3 (×4), 128.4 (×2), 128.5 (×3), 128.6 (×2), 128.7, 128.8 (×2), 129.5, 129.7, 129.8, 129.9, 131.2, 133.3, 133.6, 134.4, 134.5, 134.7, 136.9, 137.0, 137.2, 137.8, 137.9, 164.6, 164.7, 164.9, 166.8, 167.1, 167.8, 170.5, 170.6, 170.8. HRMS: $[M+Na]^+$ $C_{134}H_{143}N_9NaO_{37}Si$ calcd 2520.9252, obsd 2520.9298.

Ethyl 2-amino-2-deoxy-α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyluronic acid-(1→4)-2-amino-2-deoxy-α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyluronic acid-(1→4)-2-amino-2-deoxy-α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyluronic acid (1). Compound **6** (55 mg, 0.022 mmol) was dissolved in pyridine (1.5 mL). The mixture was cooled down to 4 °C, followed by addition of HF (0.75 mL, 65-70% in pyridine). The mixture was stirred at room temperature overnight and then the solvent was evaporated under vaccum. The residue was diluted in DCM (50 mL) and washed with saturated $NaHCO_3$ and then dried over Na_2SO_4, filtered and the solvents were removed in vacco. Silica gel column chromatography (6:1 Hexanes–EtOAc) afforded the alcohol as

white solid (40.5 mg, 77%). The mixture of the alcohol (15 mg, 6.28 μmol), THF (0.8 mL) and aqueous LiOH (3mg in 0.2 mL of H_2O) was cooled to –5 °C, followed by addition of H_2O_2 (0.3 mL, 30%). The mixture was stirred at room temperature for 16 h and then MeOH (0.8 mL) and NaOH (30 mg in 0.2 mL of H_2O) was added. The mixture was stirred for another 24 h and then was acidified with 10% HCl, concentrated to dryness. The resulting residue was purified by quickly passing through a short silica gel column (4:1, CH_2Cl_2–MeOH). The mixture of the obtained solid and $Pd(OH)_2$ (50 mg) in MeOH/H_2O/HOAc (3 mL/1 mL/1 mL) was stirred under H_2 at room temperature overnight and then filtered. The filtrate was concentrated to dryness under vacuum and then was co-evaporated with H_2O (10 mL) three times to remove the HOAc. The aqueous phase was further washed with CH_2Cl_2 (5 mL × 3) and EtOAc (5 mL×3) and then the aqueous phase was dried under vacuum. The crude product was further purified by size exlusion chromatography (G-15) to afford compound **1** (acetate salt) as white solid (6.5 mg, 83% for two steps). $[\alpha]^{25}_D$ +47.6 (c = 0.5, H_2O); ^{1}H-NMR (600 MHz, D_2O): δ 1.07 (t, 3H, J = 7.2 Hz), 2.98 (br, 1H), 3.02-3.04 (m, 1H), 3.10-3.11 (m, 3H), 3.16-3.19 (m, 1H), 3.24-3.27 (m, 2H), 3.33-3.37 (m, 1H), 3.52-3.82 (m, 29H), 4.34-4.39 (m, 3H), 5.47 (d, 1H, J = 3.0 Hz), 5.48 (d, 1H, J = 3.0 Hz), 5.51 (d, 1H, J = 3.0 Hz); ^{13}C-NMR (150 MHz, D_2O): δ 14.4, 23.4, 54.9, 55.3, 59.6, 60.3, 66.4, 69.7, 70.9, 72.0, 72.2, 72.3, 73.1, 73.2, 73.8, 76.3, 76.4, 76.6, 77.0, 78.4, 98.8 (×2), 99.1, 102.0, 102.6 (×2), 162.1, 175.2, 175.3, 181.7; HRMS: $[M+H]^+$ $C_{38}H_{64}N_3O_{31}$ calcd 1058.3524, obsd 1058.3547. gHMQC (without ^{1}H decoupling): $^1J_{C1,H1}$ = 173.7, 170.8, 170.8, 161.1, 161.1, 161.1 Hz.

Supplementary Figure Legends

Supplementary Fig 1S. Synthetic scheme of hexasaccharide 1.

Supplementary Fig 2S. Structural characterization of heptasaccharide 3. *Panel A* shows the PAMN-HPLC chromatogram of heptasaccharide **3**. The impurities were indicated by arrows. *Panel B* shows the MS spectrum of HPLC-purified heptasaccharide **3**. *Panel C* shows the RPIP-HPLC chromatogram of disaccharide analysis of heparin lyase-digested *N*-[^{35}S]sulfo-labeled heptasaccharide **3**. *Panel D* shows the reaction involved in digestion of heptasaccharide **3** using heparin lyases. The ratio of two resultant ^{35}S-labeled disaccharides (GlcUA-[*N*-^{35}S]GlcNS/ΔUA2S-[*N*-^{35}S]GlcNS) was measured to be 1:1.8, which is very close to the theoretical value of 1:2 as described in *Panel D*. *Panel E* shows the HPLC chromatogram of the disaccharide analysis of nitrous acid degraded 2-*O*-[^{35}S]sulfo-labeled heptasaccharide **3**. The elution position of IdoUA2S-AnMan was determined by coeluting with the disaccharide standard (Xia, et al. 2002). *Panel F* shows the reaction involved in the degradation of heptasaccharide **3** with nitrous acid. The sites of ^{35}S-label are colored in red.

Supplementary Figure 3S. Structural characterization of heptasaccharide 4. *Panel A* shows the DEAE-HPLC profile of ^{35}S-labeled heptasaccharide **4**. *Panel B* shows the MS spectrum of HPLC-purified heptasaccharide **4**. *Panel C* shows the RPIP-HPLC chromatogram of the disaccharide analysis of heparin lyase-digested *N*-[^{35}S]sulfo heptasaccharide. *Panel D* shows the reaction involved in the degradation of heptasaccharide **4** with heparin lyases. The ratio of the resultant two ^{35}S-labeled disaccharides (GlcUA-[*N*-^{35}S]GlcNS6S/ΔUA2S-[*N*-^{35}S]GlcNS6S) was determined to be 1:1.8, which is very close to the theoretical value of 1:2 as described in *Panel D*.

Supplementary Figure 4S. HPLC chromatograms of heptasaccharide 4 with or without digestion of β-glucuronidase digestion. *Panel A* shows the DEAE-HPLC chromatogram of heptasaccharide **4**. *Panel B* shows the DEAE-HPLC chromatogram of heptasaccharide **4** digested with β-glucuronidase. The reaction involved in the digestion of heptasaccharide **4** with β-glucuronidase is shown on the right. As observed, the retention time of the ^{35}S-labeled peak was shifted from 32 min to 38 min, suggesting that heptasaccharide **4** is susceptible to the digestion of β-glucuronidase. Thus, we conclude that a GlcUA unit is present at the nonreducing end of heptasaccharide **4** based on the substrate specificity of β-glucuronidase (Liu, et al. 1999, Weïwer, et al. 2008).

Supplementary Figure 5S. Structural characterization of heptasaccharide 3'. *Panel A* shows the PAMN-HPLC chromatogram of ^{35}S-labeled heptasaccharide **3'**. *Panel B* shows the RPIP-HPLC chromatogram of nitrous acid-degraded heptasaccharide **3'**. Because only ^{35}S-labeled disaccharide with a structure of [2-*O*-^{35}S]GlcUA2S-AnMan was observed, we concluded that 2-*O*-sulfation of GlcUA residue was successful.

Supplementary Figure 6S. Structural characterization of heptasaccharide 4'. *Panel A* shows the DEAE-HPLC profile of ^{35}S-labeled heptasaccharide **4**'. *Panel B* shows the RPIP-HPLC chromatogram of the disaccharide analysis of heparin lyase-digested *N*-[^{35}S]- labeled heptasaccharide **4'**. The reaction involved in the degradation of heptasaccharide 4' with heparin lyases is shown on the right. The ^{35}S-label was specifically introduced to the *N*-position of the heptasaccharide **4**'. The digestion of heptasaccharide **4**' should yield two ^{35}S-labeled disaccharides, GlcUA-GlcNS6S and ΔUA2S-GlcNS6S, with a ratio of 1:2. Panel B showed the digestion of heptasaccharide **4**' with heparin lyases resulted in two disaccharides, GlcUA-GlcNS6S and ΔUA2S-

GlcNS6S, with a ratio of 1:1.2, somewhat different from 1:2. We did not observe any disaccharide with the structure of GlcUA-GlcNS or ΔUA2S-GlcNS, representing incomplete 6-*O*-sulfation. Therefore, we can conclude that heptasaccharide **4'** was fully 6-*O*-sulfated as anticipated. Taken together, our data suggest that three 6-*O*-sulfo groups were present in heptasaccharide **4**'.

Supplementary Figure 7S. HPLC chromatogram of heptasaccharide 2, octasaccharide 5 and nonasaccharide 6. Panel A shows the PAMN-HPLC profile of heptasaccharide 2. Panel B shows the PAMN-HPLC profile of octasaccharide 5. Panel C shows the PAMN-HPLC profile of nonasaccharide **6**. The chemical structures of heptasaccharide **2**, octasaccharide **5** and nonasaccharide **6** are shown on the right. The retention time of major ^{35}S-labeled peak was altered from 31 min (*Panel A*) to 24 min (*Panel B*) and to 29 min (*Panel C*). These data suggest that the anticipated extension from heptasaccharide to nonasaccharide was successful.

Supplementary Figure 8S. Structural characterization of nonasaccharide 7. *Panel A* shows the PAMN-HPLC chromatogram of ^{35}S-labeled nonasaccharide **7**. *Panel B* shows the chemical structure of nonasaccharide 7. *Panel C* shows the RPIP-HPLC profile of heparin-lyase digested *N*-[^{35}S]sulfated nonasaccharide **7**. *Panel D* shows the reaction involved in the digestion of nonasaccharide **7** using heparin lyases. In this experiment, the ^{35}S-label was specifically introduced to the *N*-position of the nonasaccharide **7**. The digestion of nonasaccharide **7** should yield two ^{35}S-labeled disaccharides, ΔUA-GlcNS and ΔUA2S-GlcNS, with a ratio of 1:2 (*Panel D*). Our data showed that the digestion of nonasaccharide **7** with heparin lyases resulted in two disaccharides, ΔUA-GlcNS and ΔUA2S-GlcNS, with a ratio of 1.7 (*Panel C*), which is

close to the theoretically calculated values of 1:2 (*Panel D*). Our results suggest that two 2-*O*-sulfo groups were present in nonasaccharide **7**.

Supplementary Figure 9S. RPIP-HPLC chromatogram of nitrous acid-degraded nonasaccharide **7**. A 2-*O*-[^{35}S]sulfated nonasaccharide **7** was prepared. RPIP-HPLC analysis of the nitrous acid-degraded nonasaccharide **7** yielded a single ^{35}S-labeled disaccharide with a structure of IdoUA2S-AnMan. The results proved that the iduronic acid residues carry 2-*O*-sulfo groups.

Supplementary Figure 10S. Structural characterization of nonasaccharide 9. *Panel A* shows the DEAE-HPLC profile of ^{35}S-labeled nonasaccharide **9**. *Panel B* shows the chemical structure of nonasaccharide **9**. *Panel C* shows the RPIP-HPLC of nitrous acid-degraded 3-*O*-[^{35}S]sulfated nonasaccharide **9**. Unlike the nitrous acid degradations used above, nonasaccharide **9** was first deacetylated by exposing to hydrazine followed by nitrous acid degradation at pH 4.5. The resultant oligosaccharide was further degraded by nitrous acid at pH 1.5, resulting the complete degradation to disaccharides. The reactions involved in a two-step nitrous acid degradation are shown in *Panel D*. Because we observed the disaccharide with a structure of GlcUA-[3-*O*-^{35}S]AnMan3S6S, the structure of nonasaccharide was proved to be the one anticipated.

Reference

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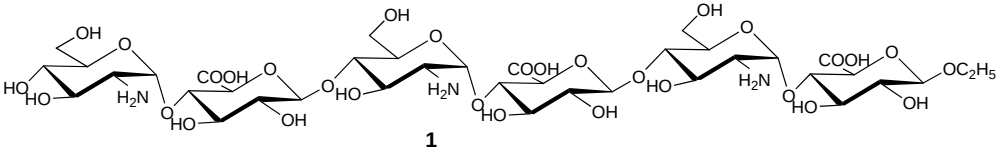
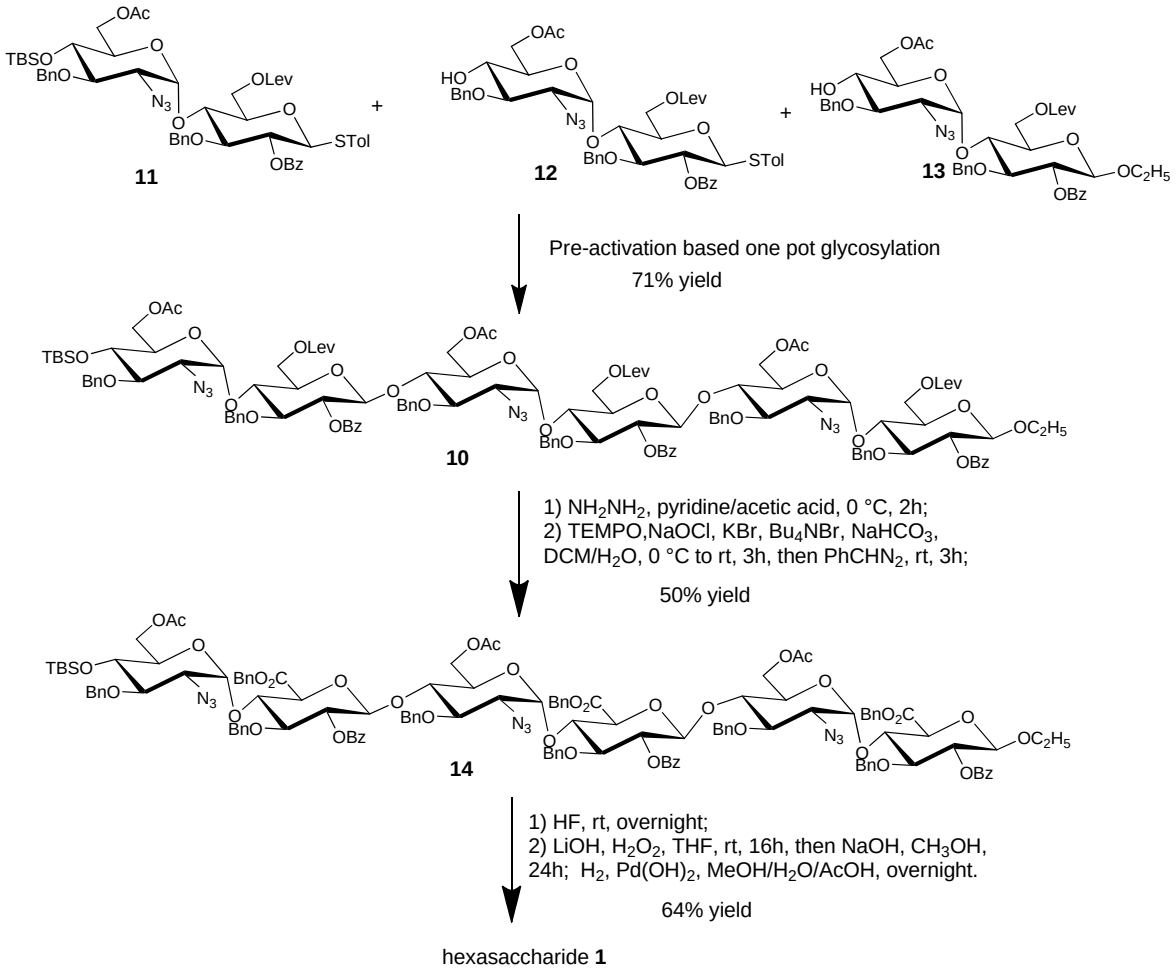
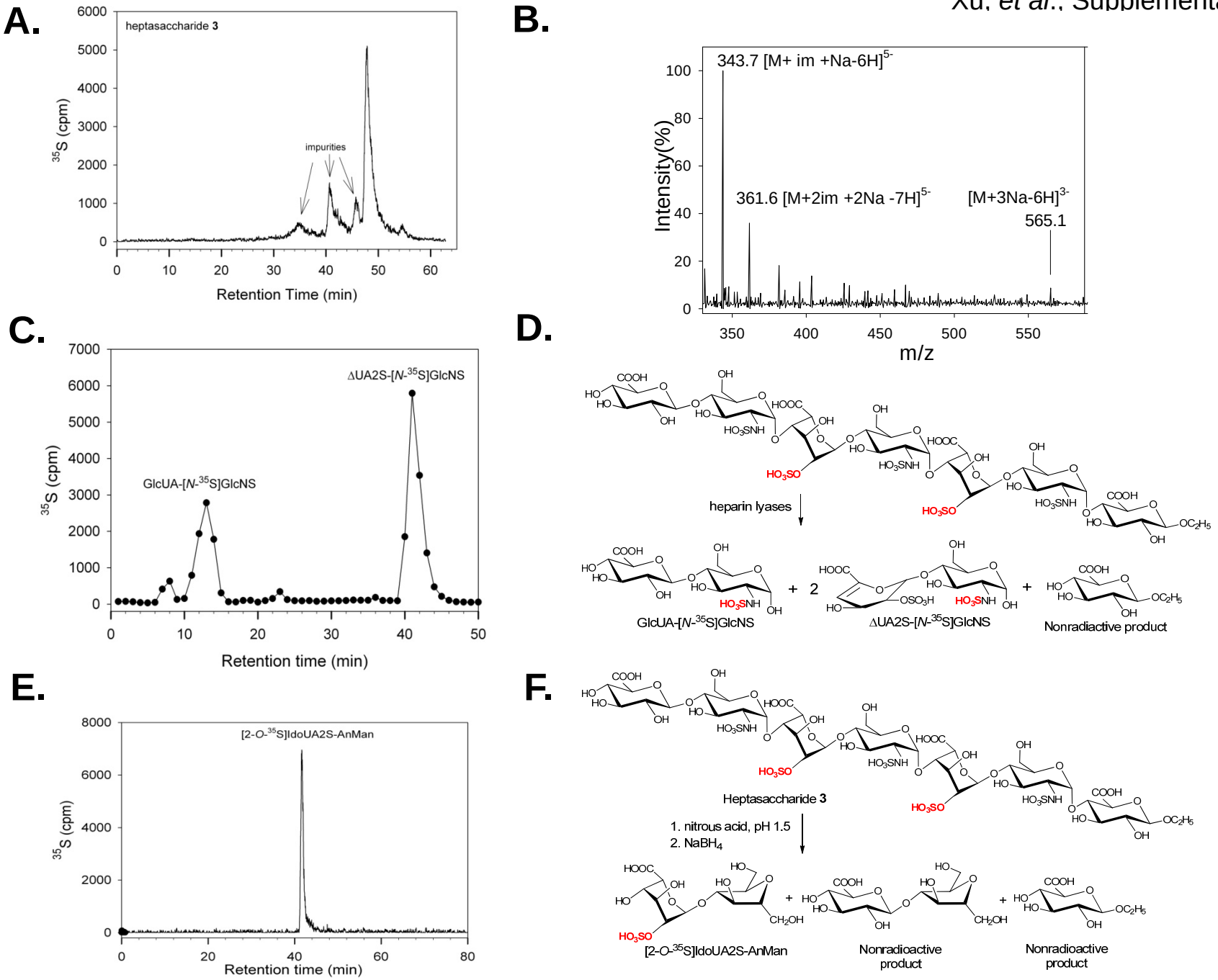


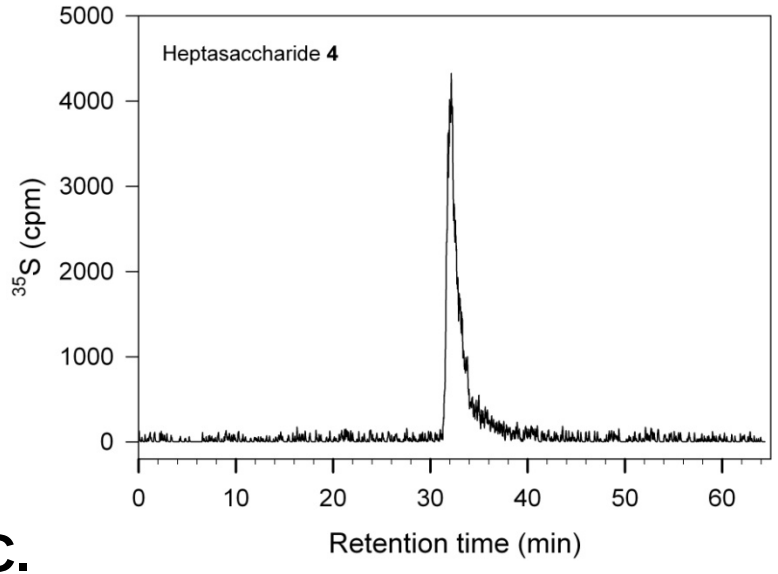
1

Scheme 1. Chemical Synthesis of Hexasaccharide **1**.

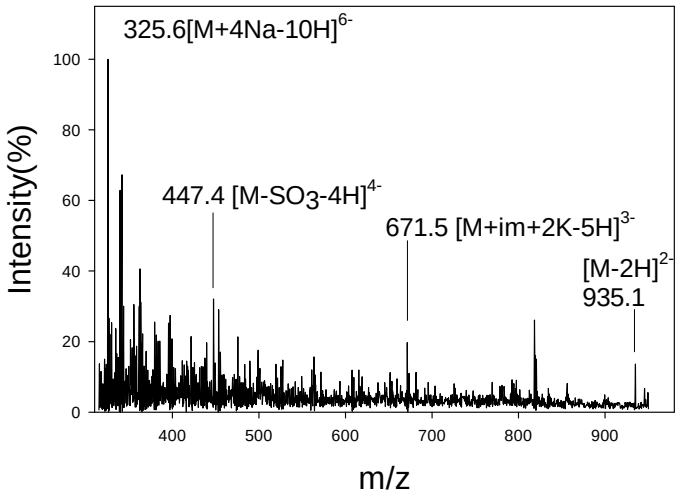



A.
heptasaccharide 3
impurities
^{35}S (cpm)
Retention Time (min)
B.
343.7 [M+ im +Na-6H]$^{5-}$
361.6 [M+2im +2Na -7H]$^{5-}$
[M+3Na-6H]$^{3-}$
565.1
Intensity(%)
m/z
C.
ΔUA2S-[N-^{35}S]GlcNS
GlcUA-[N-^{35}S]GlcNS
^{35}S (cpm)
Retention time (min)
D.
heparin lyases
GlcUA-[N-^{35}S]GlcNS
ΔUA2S-[N-^{35}S]GlcNS
Nonradiactive product
E.
[2-O-^{35}S]IdoUA2S-AnMan
^{35}S (cpm)
Retention time (min)
F.
Heptasaccharide 3
1. nitrous acid, pH 1.5
2. NaBH$_4$
[2-O-^{35}S]IdoUA2S-AnMan
Nonradioactive product
Nonradioactive product

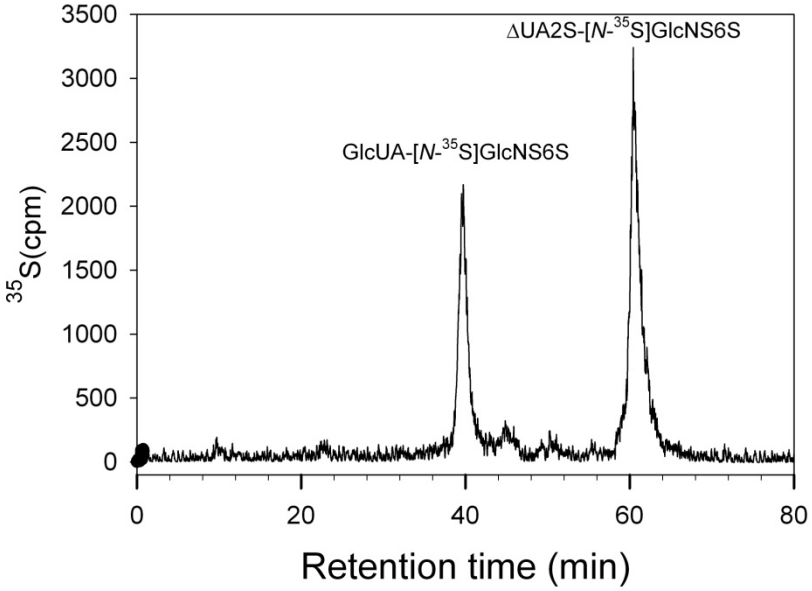
A.


Heptasaccharide 4
^{35}S (cpm)
Retention time (min)

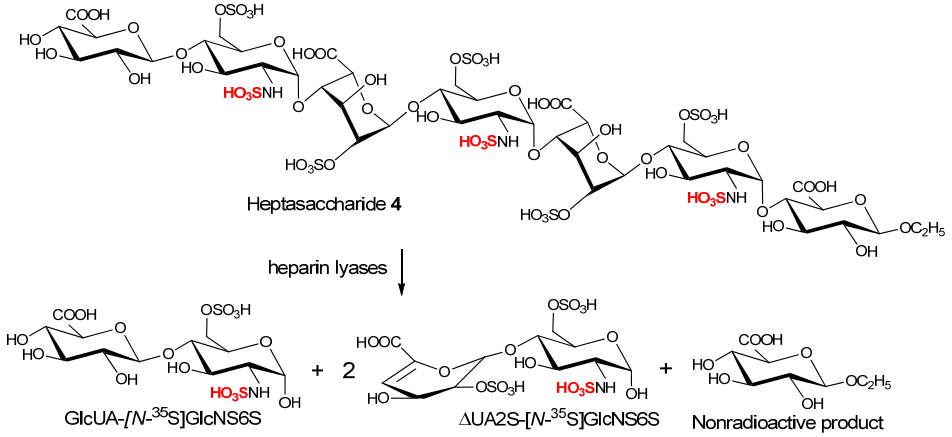
B.

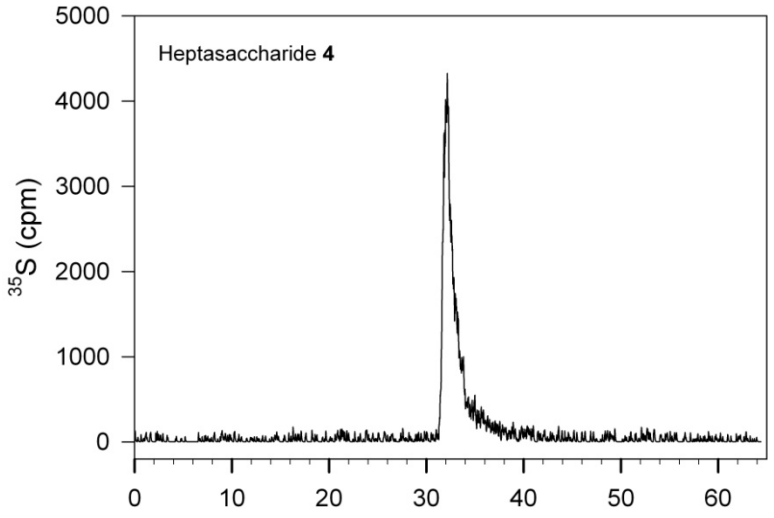

325.6[M+4Na-10H]$^{6-}$
447.4 [M-SO3-4H]$^{4-}$
671.5 [M+im+2K-5H]$^{3-}$
[M-2H]$^{2-}$
935.1
Intensity(%)
m/z

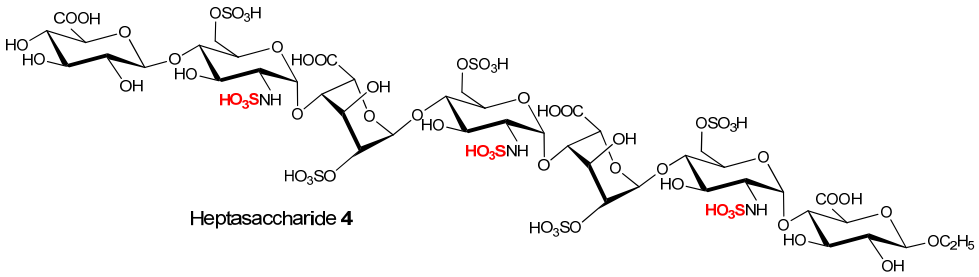
C.


ΔUA2S-[N-^{35}S]GlcNS6S
GlcUA-[N-^{35}S]GlcNS6S
^{35}S(cpm)
Retention time (min)

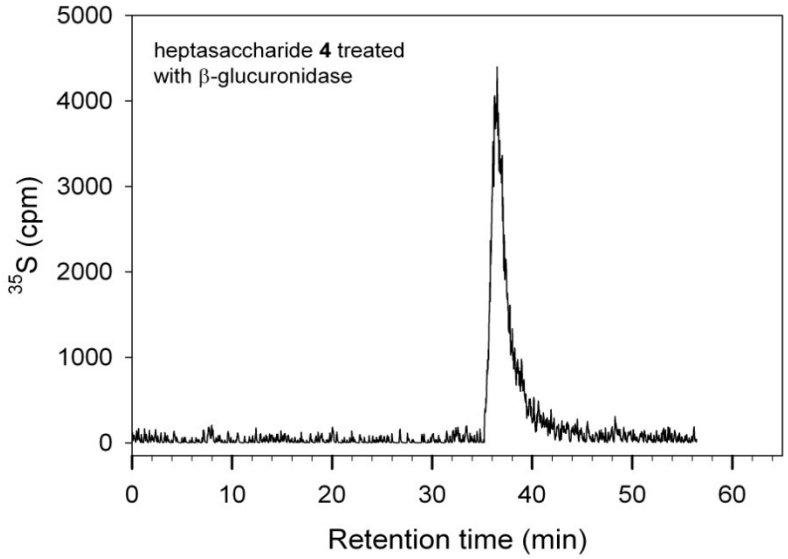
D.

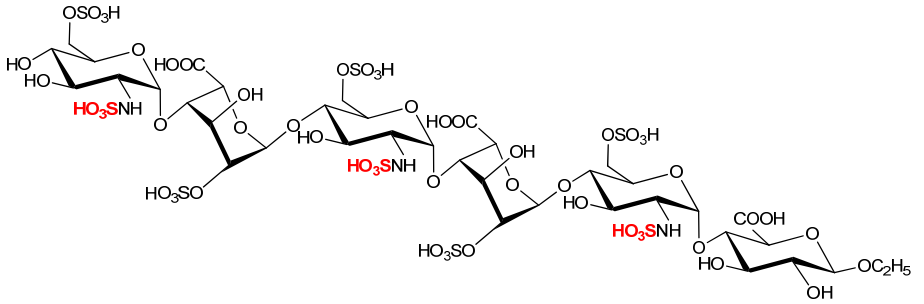

Heptasaccharide 4
heparin lyases
GlcUA-[N-^{35}S]GlcNS6S
ΔUA2S-[N-^{35}S]GlcNS6S
Nonradioactive product



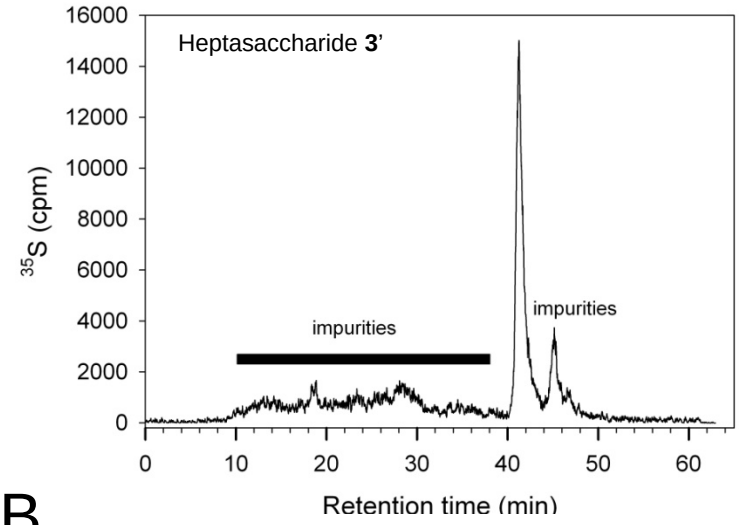


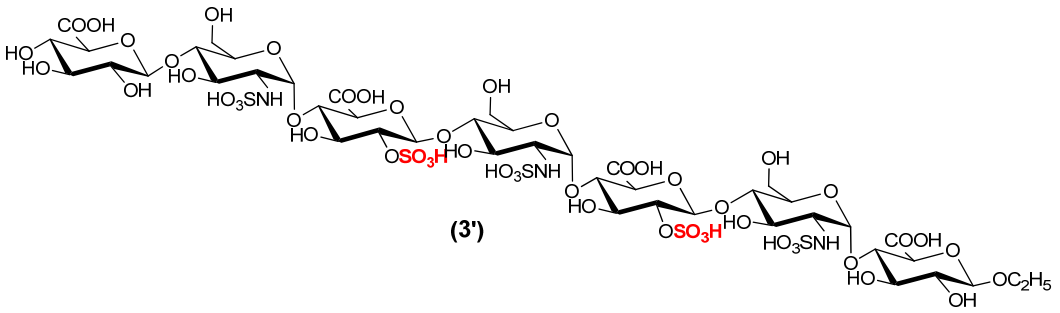
β-glucuronidase



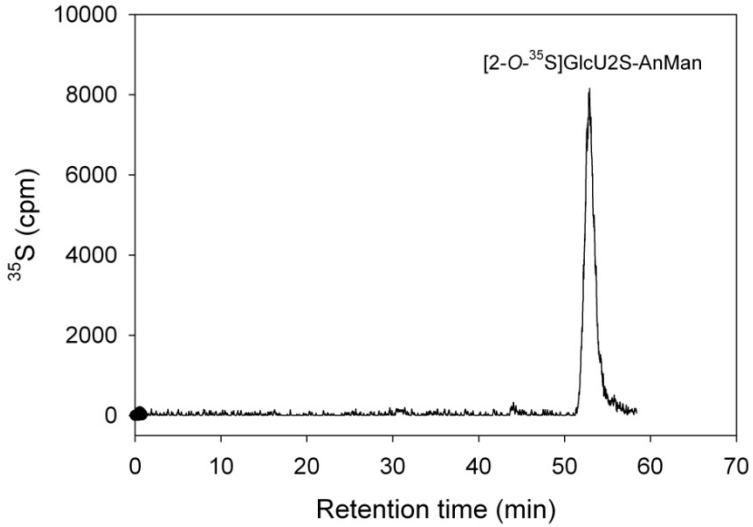


A.

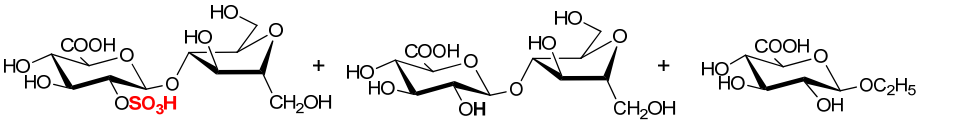




B.

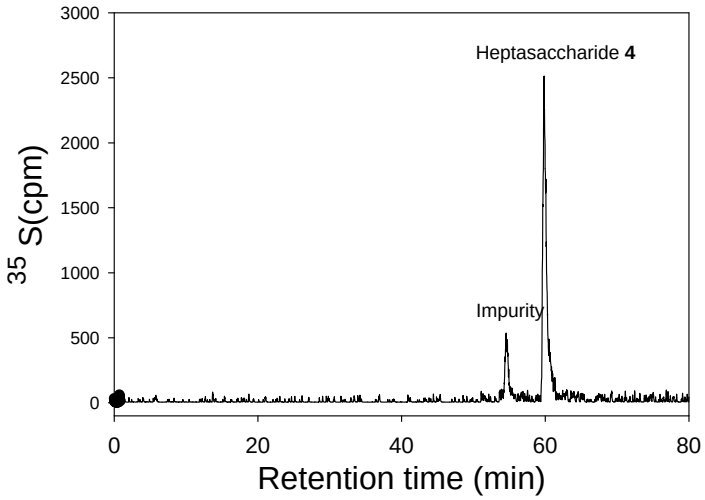


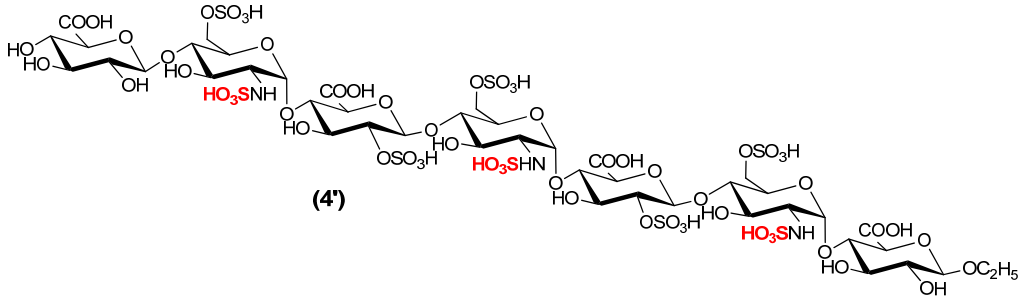
1. Nitrous acid, pH 1.5
2. $NaBH_4$



[2-*O*-^{35}S]GlcUA2S-AnMan Nonradioactive product Nonradioactive product

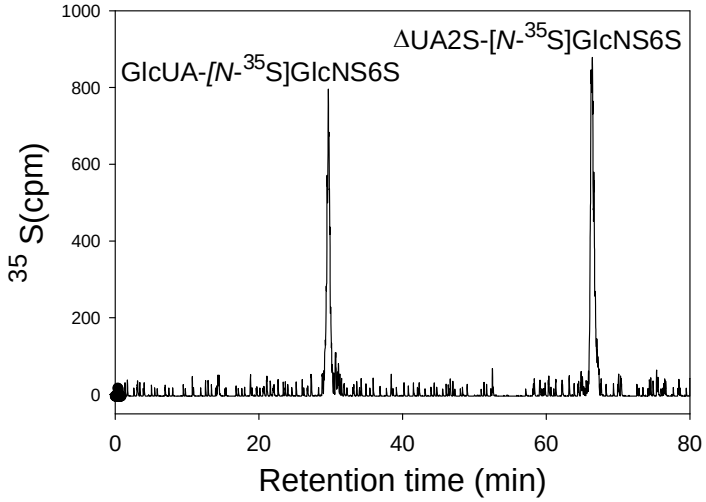
A.

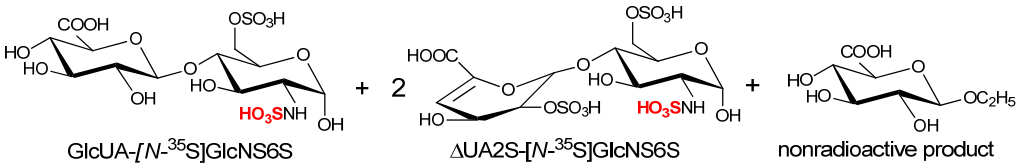




Heparin lyases

B.

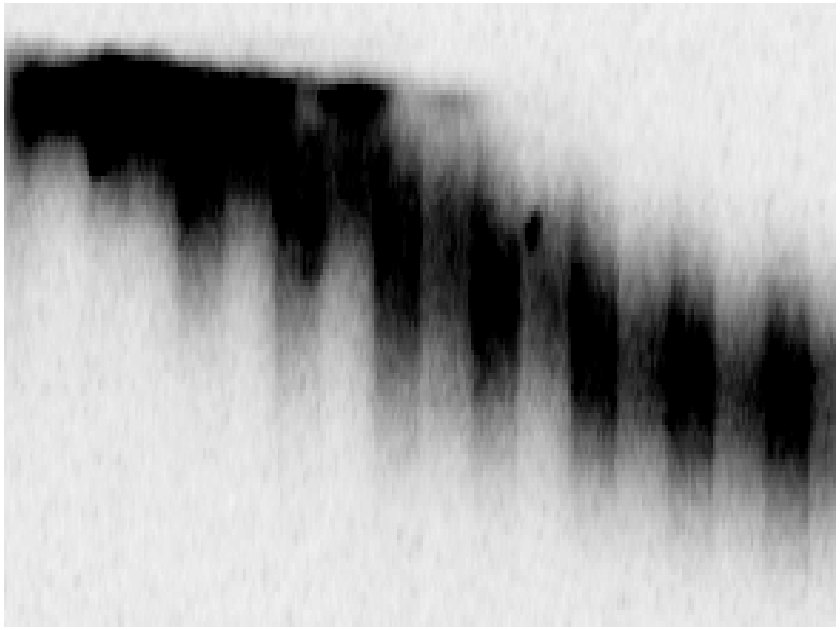




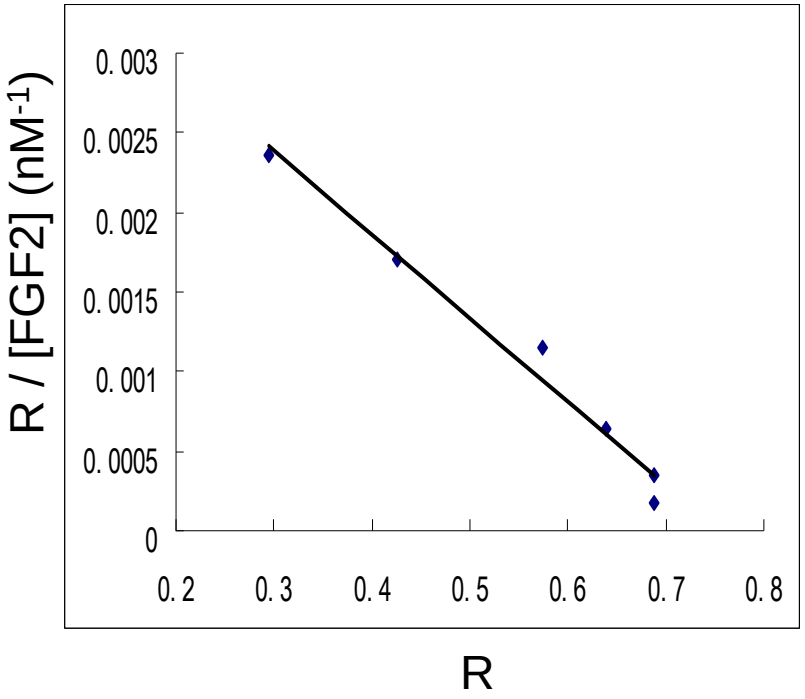
A

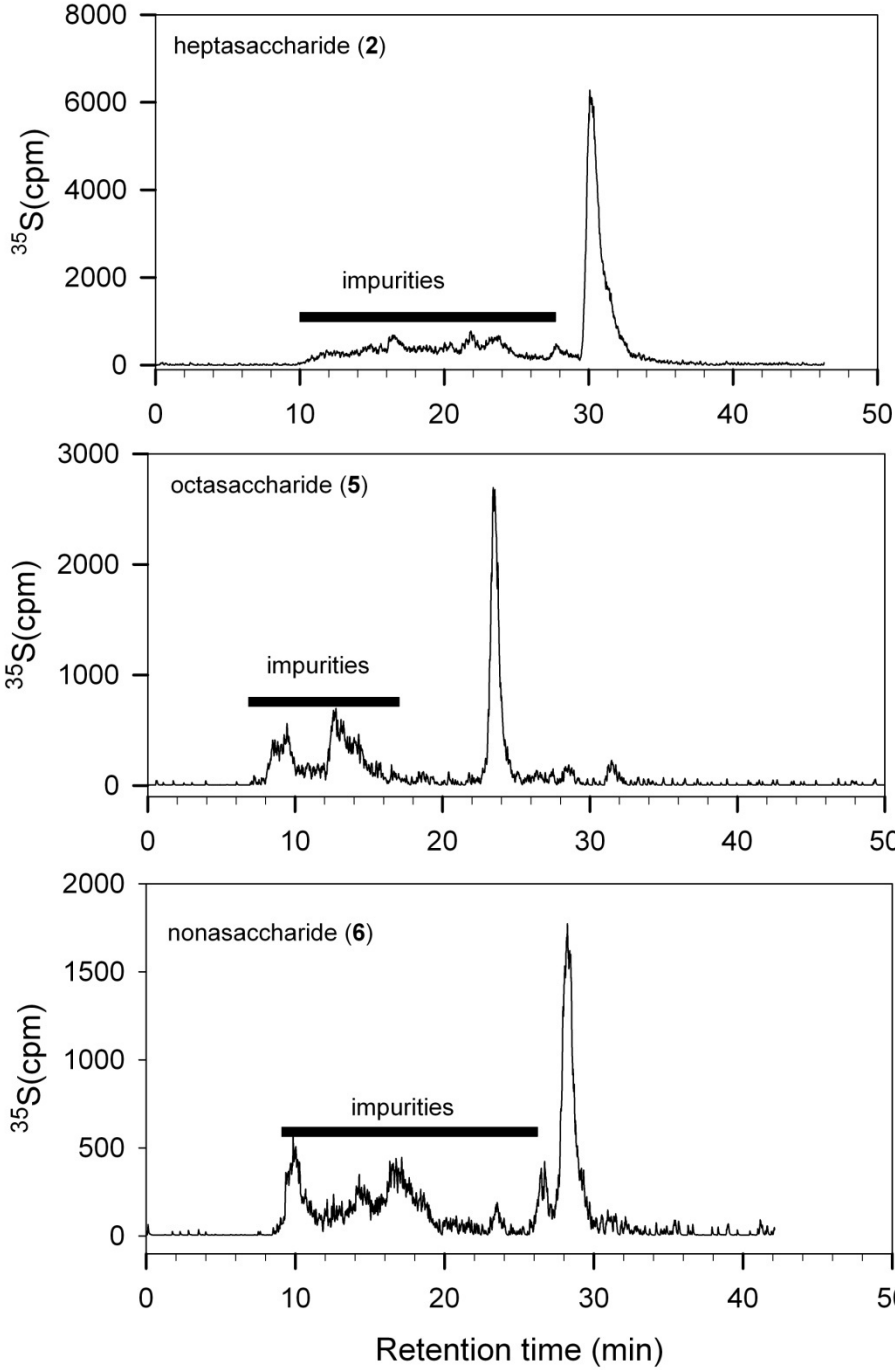
FGF2 (nM)

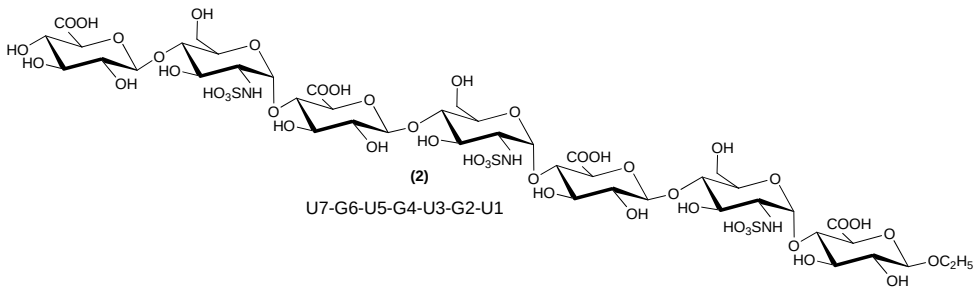
4000 2000 1000 500 250 125 64 32 0

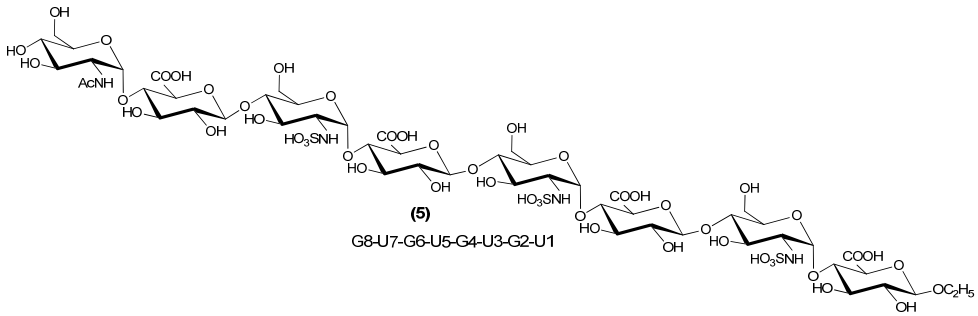


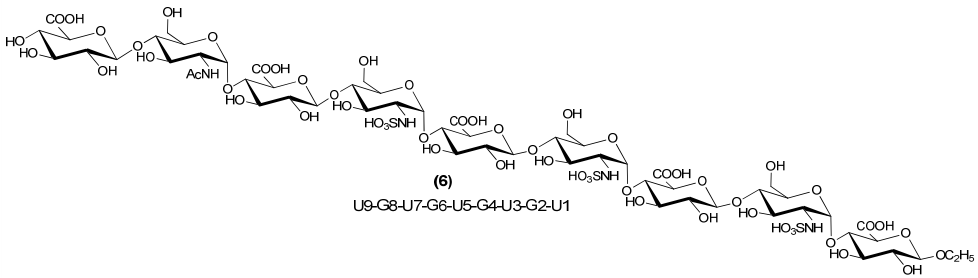
B



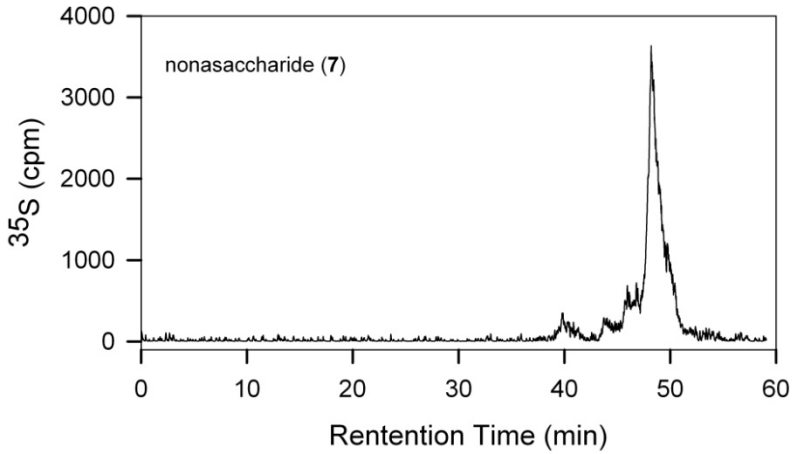

heptasaccharide (2)
impurities
octasaccharide (5)
impurities
nonasaccharide (6)
impurities
35S(cpm)
Retention time (min)


(2)
U7-G6-U5-G4-U3-G2-U1

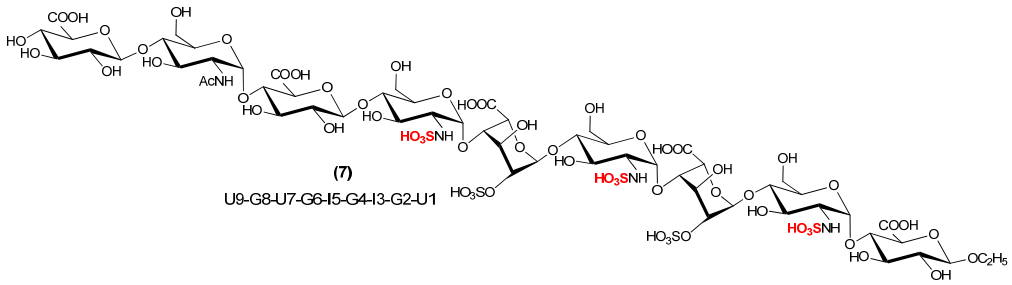

(5)
G8-U7-G6-U5-G4-U3-G2-U1


(6)
U9-G8-U7-G6-U5-G4-U3-G2-U1

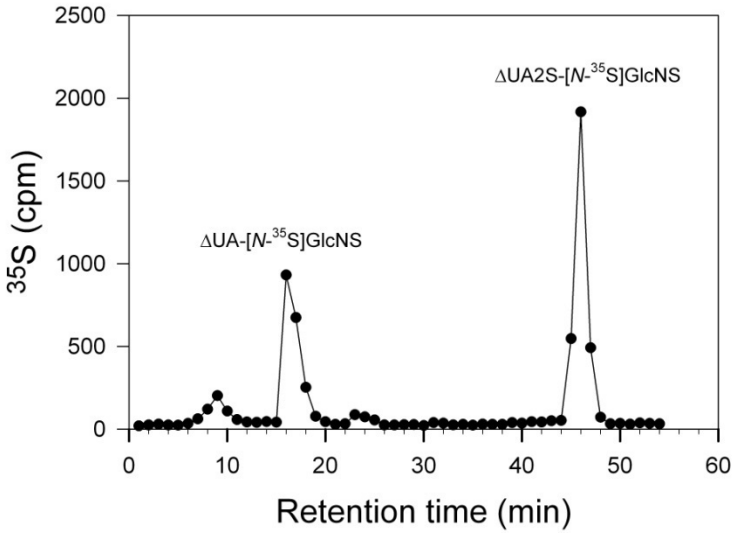
A.


nonasaccharide (7)
^{35}S (cpm)
4000
3000
2000
1000
0
0
10
20
30
40
50
60
Rentention Time (min)

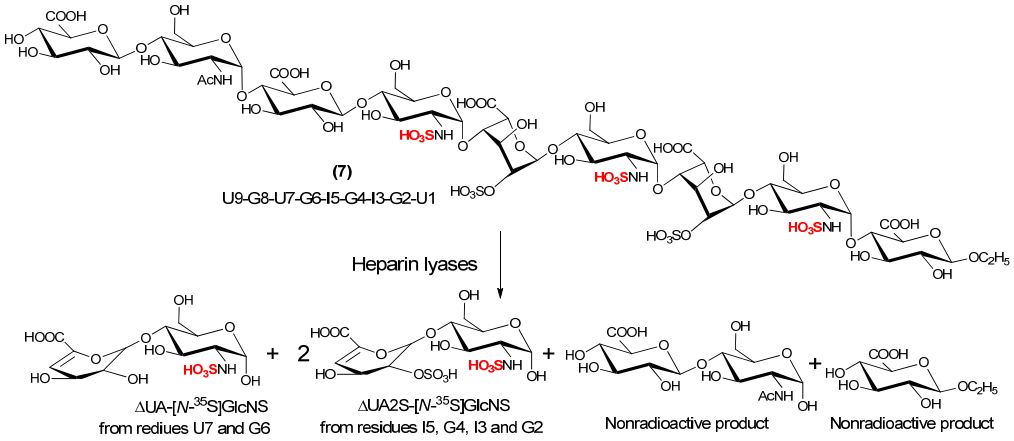
B.

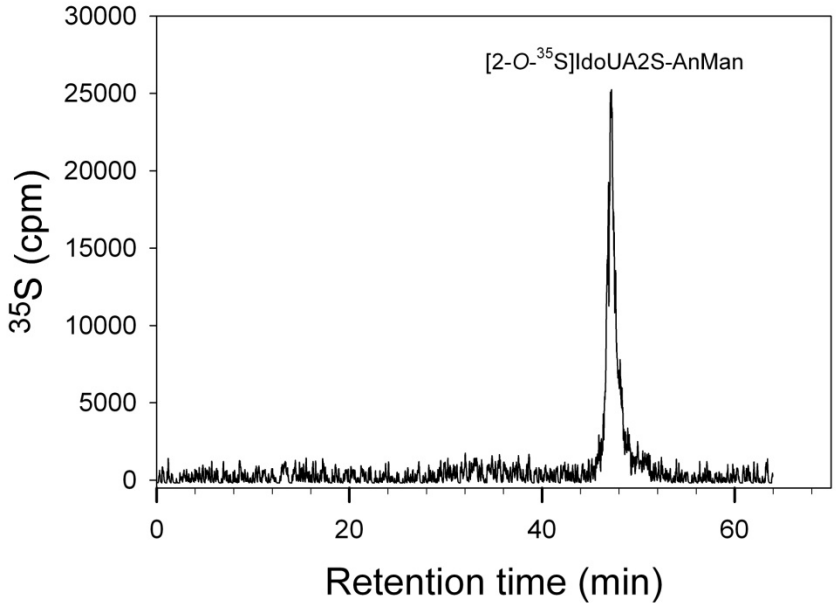

(7)
U9-G8-U7-G6-I5-G4-I3-G2-U1

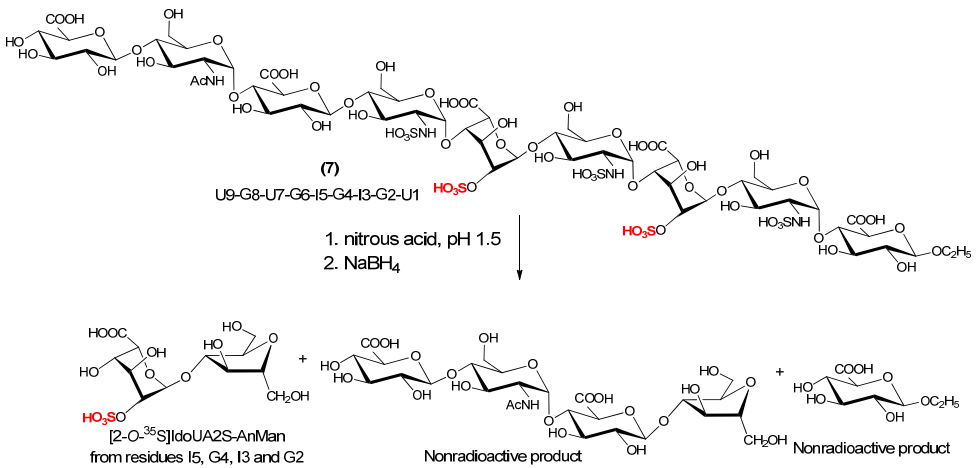
C.


ΔUA2S-[N-^{35}S]GlcNS
ΔUA-[N-^{35}S]GlcNS
^{35}S (cpm)
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
Retention time (min)

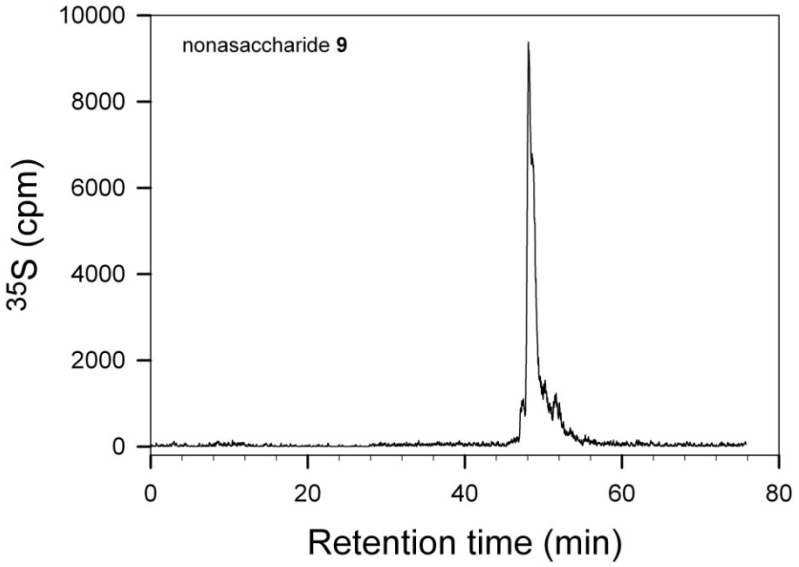
D.


(7)
U9-G8-U7-G6-I5-G4-I3-G2-U1
Heparin lyases
ΔUA-[N-^{35}S]GlcNS
from rediues U7 and G6
ΔUA2S-[N-^{35}S]GlcNS
from residues I5, G4, I3 and G2
Nonradioactive product
Nonradioactive product

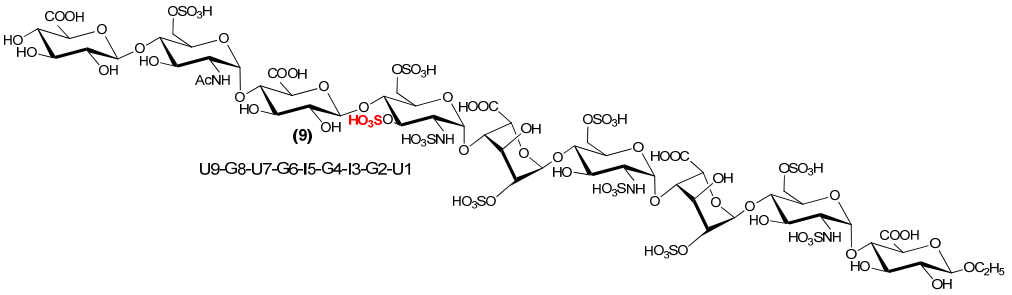

[2-O-35S]IdoUA2S-AnMan
35S (cpm)
30000
25000
20000
15000
10000
5000
0
0
20
40
60
Retention time (min)


(7)
U9-G8-U7-G6-I5-G4-I3-G2-U1
1. nitrous acid, pH 1.5
2. NaBH4
[2-O-35S]IdoUA2S-AnMan
from residues I5, G4, I3 and G2
Nonradioactive product
Nonradioactive product

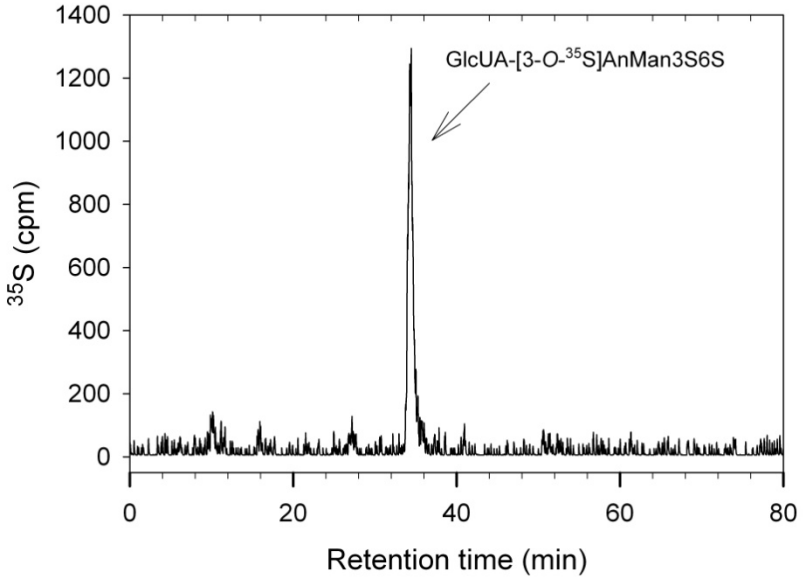
A.


nonasaccharide 9
^{35}S (cpm)
Retention time (min)
0
2000
4000
6000
8000
10000
0
20
40
60
80

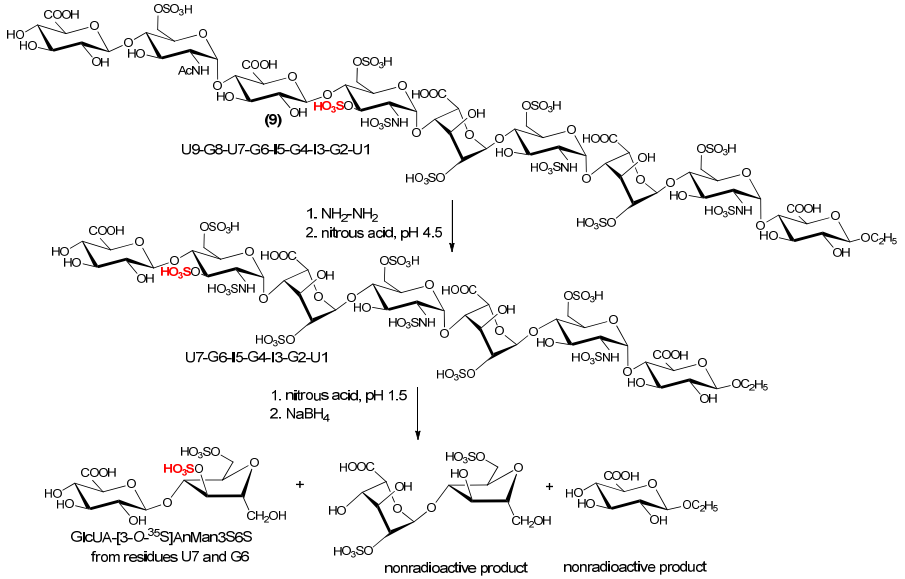
B.


(9)
U9-G8-U7-G6-I5-G4-I3-G2-U1

C.


GlcUA-[3-O-^{35}S]AnMan3S6S
^{35}S (cpm)
Retention time (min)
0
200
400
600
800
1000
1200
1400
0
20
40
60
80

D.


(9)
U9-G8-U7-G6-I5-G4-I3-G2-U1
1. NH_2-NH_2
2. nitrous acid, pH 4.5
U7-G6-I5-G4-I3-G2-U1
1. nitrous acid, pH 1.5
2. $NaBH_4$
GlcUA-[3-O-^{35}S]AnMan3S6S
from residues U7 and G6
nonradioactive product
nonradioactive product