Supplemental Information: The dehydration of N-acetylglucosamine (GlcNAc) to enantiopure dihydroxyethyl acetamidofuran (Di-HAF)

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Materials and methods

S1. Reagents

All reagents were acquired from commercial sources and used without further purification. Specifically, N-acetyl-D-glucosamine was purchased from Carbosynth Ltd, Silica gel (ultra pure, 40-60 um, 60A) and molecular sieves (3 Å, powder <50 µM) from Acros Organics, Celite 535 from Carl Roth and phenylboronic acid from AmBeed. Thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F254 aluminum plates. Visualization of compounds by TLC was done by potassium permanganate stain.

S2. Apparatus

^{1}H-NMR and ^{13}C-NMR analyses were performed on a Bruker Avance NEO (400 MHz) at 25 °C. High-resolution mass spectrometry (HRMS) was performed on a Thermo Scientific LTQ Orbitrap XL (FTMS). Infrared spectra (IR) spectra were recorded on a Nicolet 380 FT-IR of Thermo Electron corporation. DSC was recorded on a TA instruments DSC Q20 and enantiomeric excess (*ee*) was determined by chiral HPLC on a waters Acquity UPC^{2}.

S3. Chiral LC method

Chiral SFC Method : 31875A UPC^2 5m AMY MA1

System : Waters Acquity UPC^2 system with UV detector and QDA detector
Column : Acquity UPC^2 Trefoil AMY (3.0 x 150 mm; 2.5 µm)

Mobile Phase A : CO_2
Mobile Phase B : 0.2% Ammonia in MeOH
Pump Flow : 2.5 mL/Min
UV detection : 240 nm
Injection Volume : 1.0 µL
Total Run Time : 5.0 Min
Column Temperature : 40 °C
ABPR : 2500 psi
Mass Detection : MS Scan ES positive and negative
Mass Range : 100 – 1250 Da
Pump Program : Gradient

Time (Min)	%A	%B	Curve
Initial	98.0	2.0	initial
4.0	75.0	25.0	6
4.1	98.0	2.0	6

Experimental procedures

S4. Synthesis of Di-HAF on 5 g scale

(R)-N-(5-(1,2-Dihydroxyethyl)furan-3-yl)acetamide (Di-HAF). Triflic acid (3.4 g, 2.0 mL, 1 eq, 23 mmol) was added to a suspension of N-acetylglucosamine (**GlcNAc**) (5.0 g, 1 eq, 23 mmol), phenylboronic acid (4.1 g, 1.5 eq, 34 mmol) and molecular sieves (3 Å , powdered, 25 g) in pyridine (125 mL). The flask was flushed with nitrogen and heated to reflux (116 °C). After 30 min the mixture was cooled down to room temperature. Pinacol (5.3 g, 2 eq, 45 mmol) and potassium carbonate (6.2 g, 2 eq, 45 mmol) were added and stirring was continued for 30 min. The mixture was poured into a stirred suspension of silica (15 g) in heptanes (1.2 L) and the suspension was filtered over a patch of silica (ca. 350 mL silica). The residue was washed with heptanes (1 L), ethyl acetate (1 L) and subsequently with dichloromethane-methanol (9 : 1, 2 L). The dichloromethane-methanol solution was concentrated in vacuo to afford a brown oil (4.06 g). The crude was crystallized from acetonitrile (20 mL) to afford Di-HAF as a light brown solid (3.05 g, 16 mmol, yield: 73%, 99.3% *ee,* see S3). ^{1}H-NMR (400 MHz, D_2O) δ 7.74 (s, 1H), 6.31 (s, 1H), 4.65 (t, J = 6.0 Hz, 1H), 3.80 – 3.68 (m, 2H), 2.02 (s, 3H). ^{13}C-NMR (100 MHz, D_2O) δ 171.93, 152.55, 132.64, 124.27, 102.97, 67.36, 63.27, 21.95. HRMS (ESI) calculated for $C_8H_{11}NO$ ($M+H^+$):186.07608; found ($M+H^+$): 186.07620 (see S10)

S5. Synthesis of Di-HAF on 50 g scale

(R)-N-(5-(1,2-Dihydroxyethyl)furan-3-yl)acetamide (Di-HAF). Triflic acid (33.9 g, 20.1 mL, 1 eq, 226 mmol) was added to a suspension of N-acetylglucosamine (**GlcNAc**) (50.0 g, 1 eq, 226 mmol), phenylboronic acid (41.3 g, 1.5 eq, 339 mmol) and molecular sieves (3 Å , powdered, 250 g) in pyridine (1.25 L). The flask was flushed with nitrogen and heated to reflux (116 °C). After 30 min the mixture was cooled down to room temperature. Pinacol (53.4 g, 2 eq, 452 mmol) and potassium carbonate (62.5 g, 2 eq, 452 mmol) were added and stirring was continued for 30 min. The mixture was filtered over celite (1 cm) and the residue was washed with pyridine (0.5 L). The filtrate was concentrated in vacuo (40 °C) to a volume of approximately 0.5 L and poured into a suspension of silica (150 g) in heptane (4 L). The suspension was filtered over a patch of silica (ca. 350 mL silica). The residue was washed with heptanes (1 L), ethyl acetate (4 L) and subsequently with dichloromethane-methanol (9:1, 8 L). The dichloromethane-methanol solution was concentrated *in vacuo* to afford a brown oil (44 g). The crude was crystallized from acetonitrile (150 mL) to afford **Di-HAF** as a light brown solid (30.5 g, 165 mmol, yield: 73%, 99% ee). ^{1}H-NMR (400 MHz, D_2O) δ 7.74 (s, 1H), 6.31 (s, 1H), 4.65 (t, J = 6.0 Hz, 1H), 3.80 – 3.68 (m, 2H), 2.02 (s, 3H).

S6. Dehydration of ManAc to afford Di-HAF

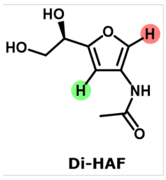
(R)-N-(5-(1,2-Dihydroxyethyl)furan-3-yl)acetamide (Di-HAF). Triflic acid (3.4 g, 2.0 mL, 1 eq, 23 mmol) was added to a suspension of N-acetyl mannosamine (**ManAc**) (5.0 g, 1 eq, 23 mmol), phenylboronic acid (4.1 g, 1.5 eq, 34 mmol) and molecular sieves (3 Å , powdered, 25 g) in pyridine (125 mL). The flask was flushed with nitrogen and heated to reflux (116 °C). After 30 min the mixture was cooled down to room temperature. Pinacol (5.3 g, 2 eq, 45 mmol) and potassium carbonate (6.2 g, 2 eq, 45 mmol) were added and stirring was continued for 30 min. The mixture was poured into a stirred suspension of silica (15 g) in heptane (1.2 L) and the suspension was filtered over a patch of silica (ca. 350 mL silica). The residue was washed with heptane (1 L), ethyl acetate (1 L) and subsequently with dichloromethane-methanol (9 : 1, 2 L). The dichloromethane-methanol solution was concentrated in vacuo to afford a brown oil (4.1 g). The crude was crystallized from acetonitrile (20 mL) to afford **Di-HAF** as a light brown solid (1.3 g, 6.8 mmol, yield: 33%). ^{1}H-NMR (400 MHz, D_2O) δ 7.74 (s, 1H), 6.31 (s, 1H), 4.65 (t, J = 6.0 Hz, 1H), 3.80 – 3.68 (m, 2H), 2.02 (s, 3H) (NMR data were identical to those obtained starting from GlucNAc).

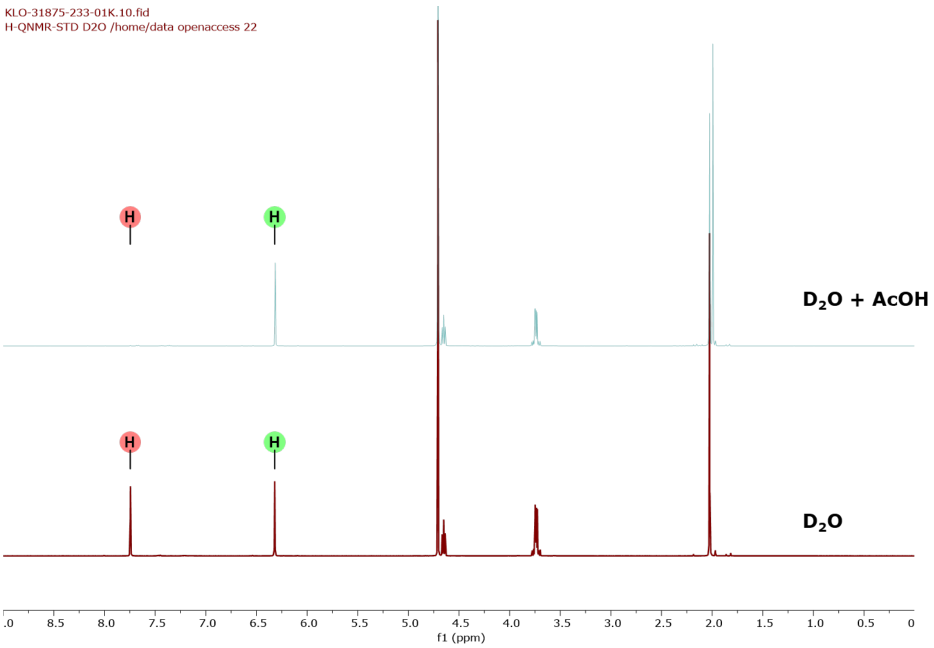
S7. Dehydration of Di-HAf to 3A5AF

N-(5-acetylfuran-3-yl)acetamide (3A5AF). A suspension of (R)-N-(5-(1,2-dihydroxyethyl)furan-3-yl)acetamide (**Di-HAF)** (500 mg, 2.7 mmol, 1 eq.) and pyridine hydrochloride (624 mg, 5.4 mmol, 2 eq.) in pyridine (10 mL) was heated to reflux temperature (116 °C) under a nitrogen atmosphere. The mixture was stirred for 3 hours. The volatiles were removed in vacuo (45 °C) and the crude was purified by automated column chromatography (silica; DCM/MeOH) to afford N-(5-acetylfuran-3-yl)acetamide (**3A5AF**) as a beige solid (290 mg, 1.73 mmol, yield: 64%). 1H-NMR (400 MHz, DMSO-d_6) δ 10.21 (s, 1H), 8.17 (s, J = 0.9 Hz, 1H), 7.18 (s, J = 0.8 Hz, 1H), 2.40 (s, 3H), 2.02 (s, 3H) (spectroscopic data corresponded to literature[13]).

S8. H/D-exchange NMR experiment

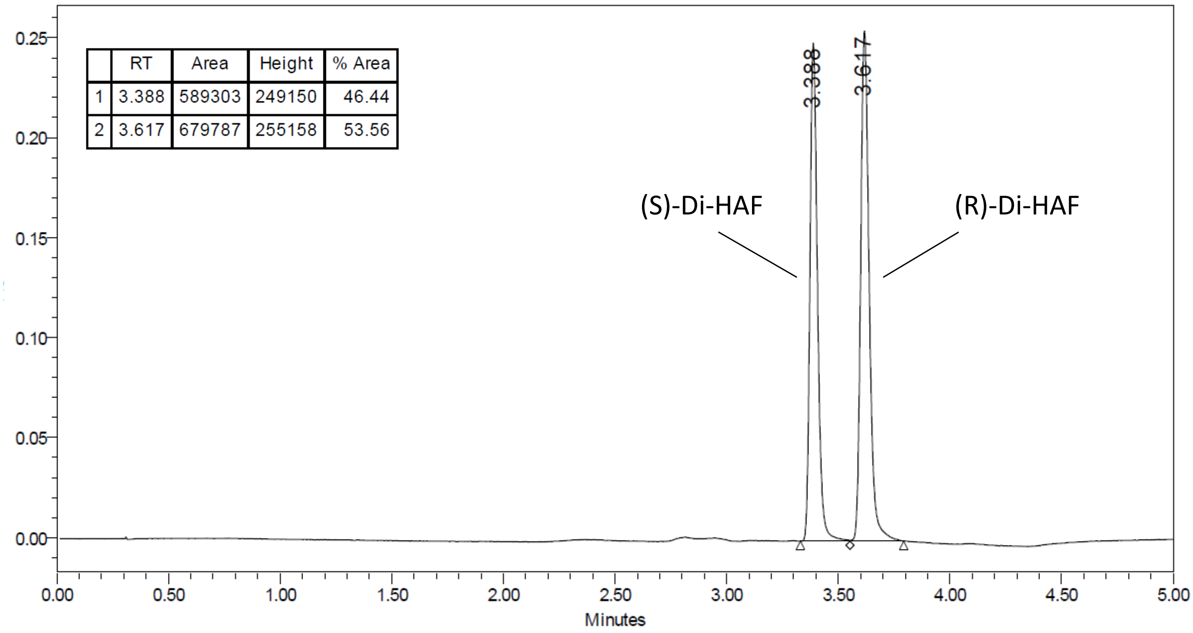
(R)-N-(5-(1,2-dihydroxyethyl)furan-3-yl-2-d)acetamide-*d₁* (Di-HAF-*d₁*). (R)-N-(5-(1,2-Dihydroxyethyl)furan-3-yl)acetamide (**Di-HAF**) (20 mg, 0.11 mmol, 1 eq.) was dissolved in deuterium oxide (0.6 mL) and acetic acid was added (6.5 mg, 0.11 mmol, 1.0 eq.). The mixture was kept at room temperature for 24 hours. ^{1}H-NMR showed complete disappearance of the signal at 7.7 ppm, corresponding to the C2 position of the furan ring. ^{1}H-NMR (400 MHz, D_2O) δ 6.31 (s, 1H), 4.65 (t, J = 6.4, 5.6, 0.6 Hz, 1H), 3.80 – 3.68 (m, 2H), 2.03 (s, 3H).





S9. Preparation of a mixture (R)-Di-HAF and (S)-Di-HAF for chiral HLPC method development

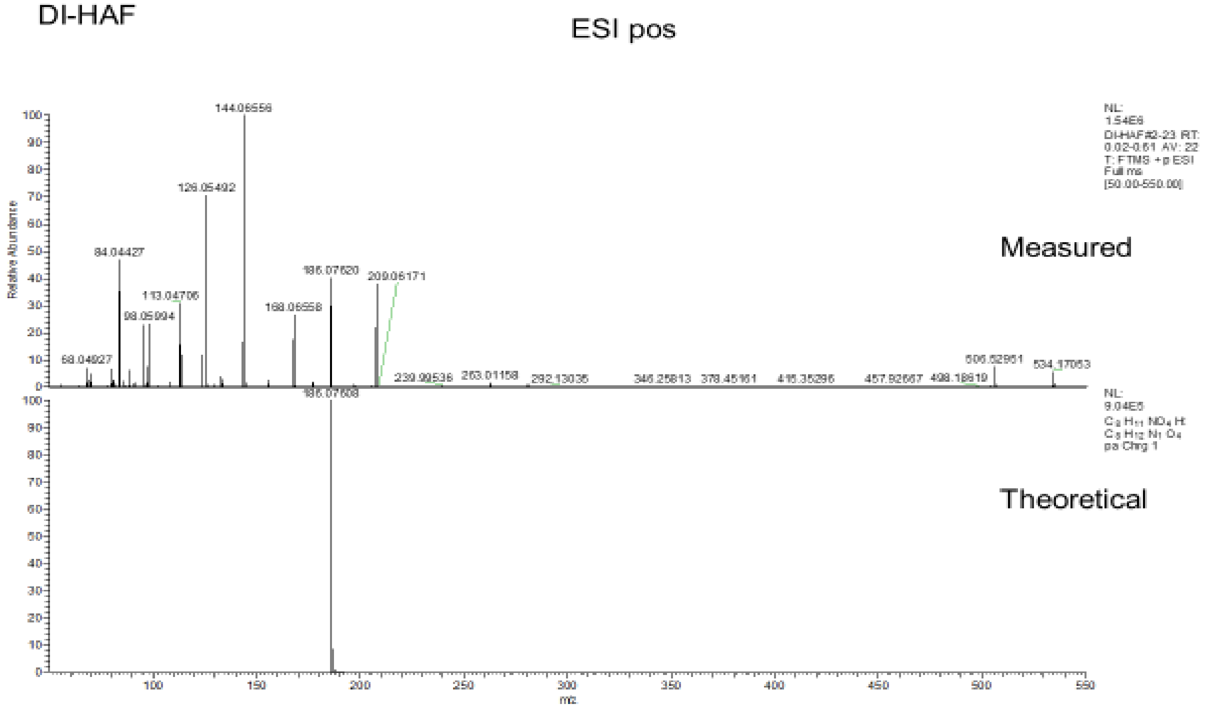
(R/S)-N-(5-(1,2-dihydroxyethyl)furan-3-yl-2-d)acetamide. Diisopropyl azodicarboxylate (**DIAD**) (682 mg, 656 µL, 2.5 eq, 3.38 mmol) was added to a stirred suspension of N-(5-(1,2-dihydroxyethyl)furan-3-yl)acetamide (**Di-HAF**) (250 mg, 1 eq, 1.35 mmol), triphenylphosphine (885 mg, 2.5 eq, 3.38 mmol) and TFA (462 mg, 312 µL, 3 eq, 4.05 mmol) in THF (15 mL). The temperature was kept below 30 °C. A solution was obtained which was stirred for 30 minutes. A mixture of methanol (10 mL) and K_2CO_3 (933 mg, 5 eq, 6.75 mmol) was added and stirring was continued for an additional hour. The reaction mixture was concentrated *in vacuo* (45 °C) and the crude product was purified by automated column chromatography to afford a mixture of enantiomers (46:54) as an orange oil (75 mg, yield: 30%).

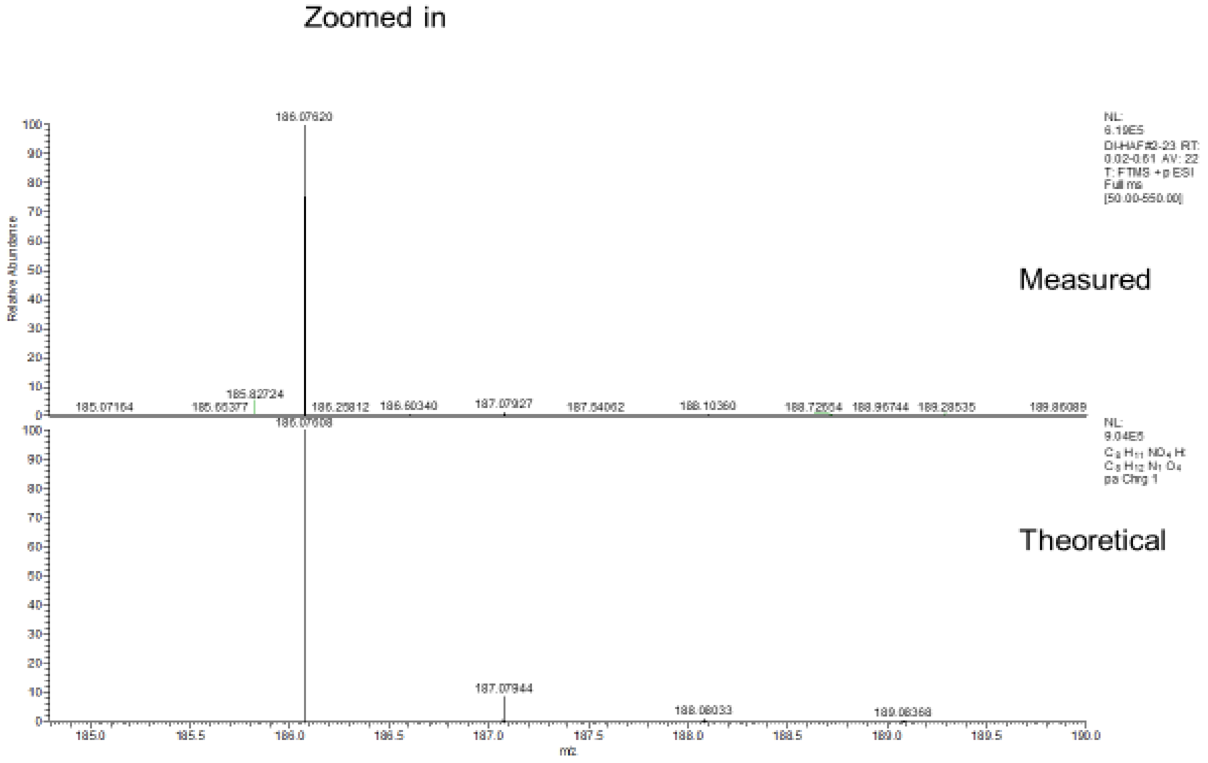


	RT	Area	Height	% Area
1	3.388	589303	249150	46.44
2	3.617	679787	255158	53.56

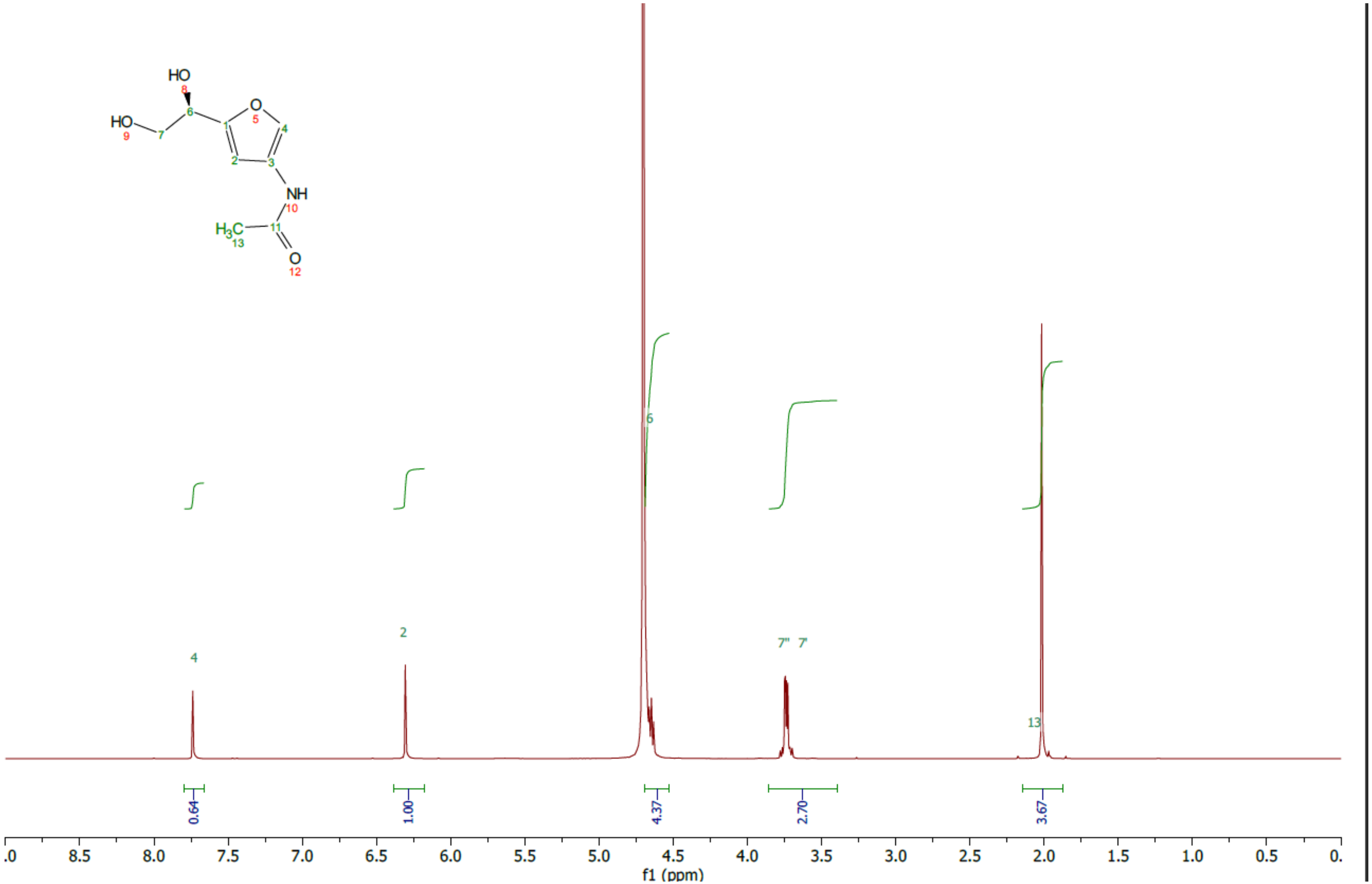
Characterization of Di-HAF

S10. HRMS of Di-HAF

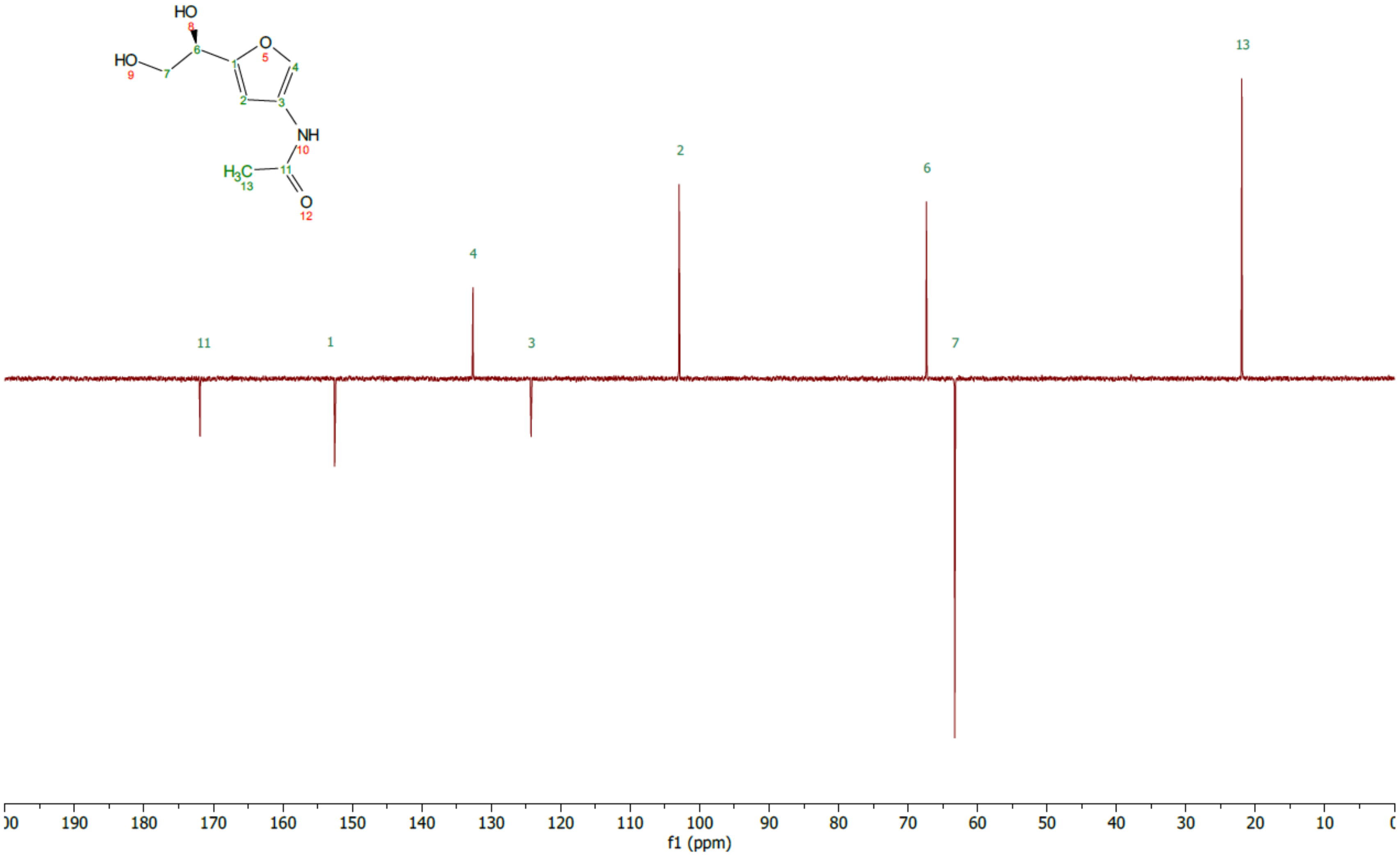




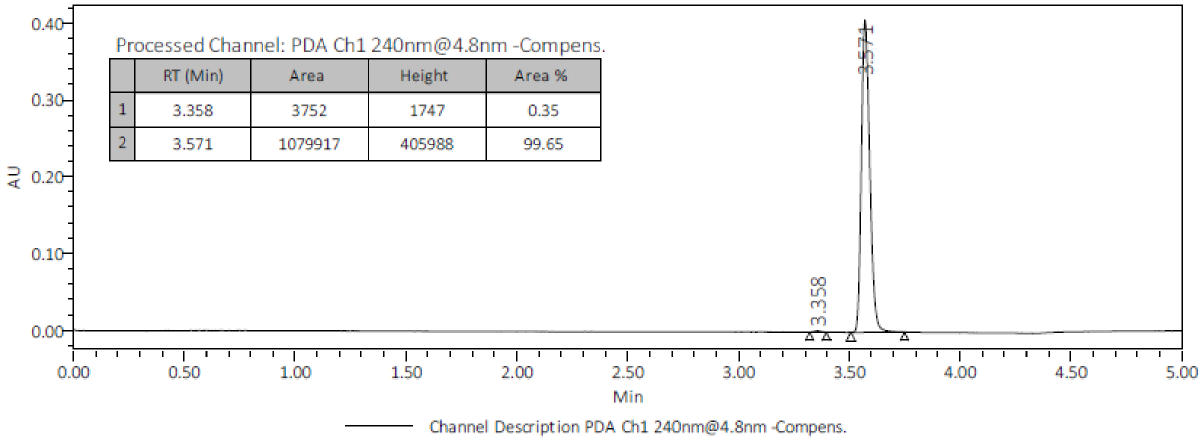
S11. ^{1}H-NMR spectrum of Di-HAF (D_2O)



S12. ^{13}C-NMR spectrum of Di-HAF (D_2O)

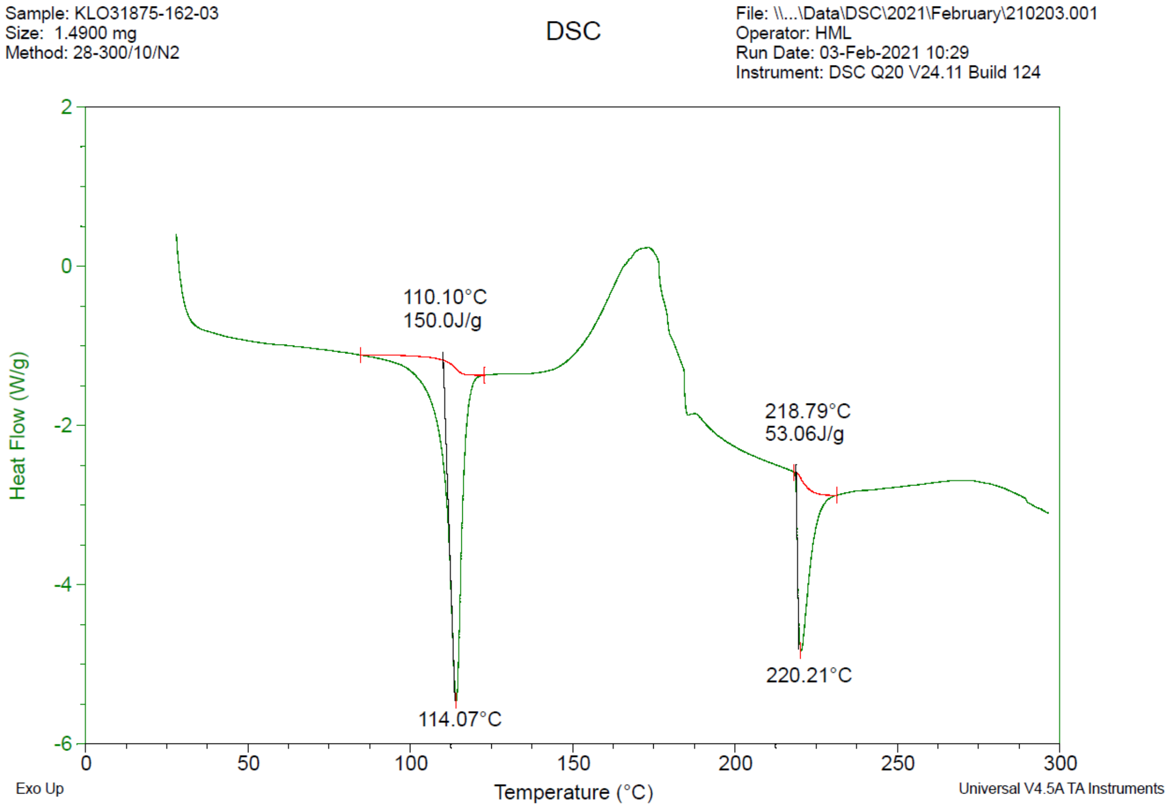


S13. Chiral HLPC analysis Di-HAF (5 g scale)


Processed Channel: PDA Ch1 240nm@4.8nm -Compens.

	RT (Min)	Area	Height	Area %
1	3.358	3752	1747	0.35
2	3.571	1079917	405988	99.65

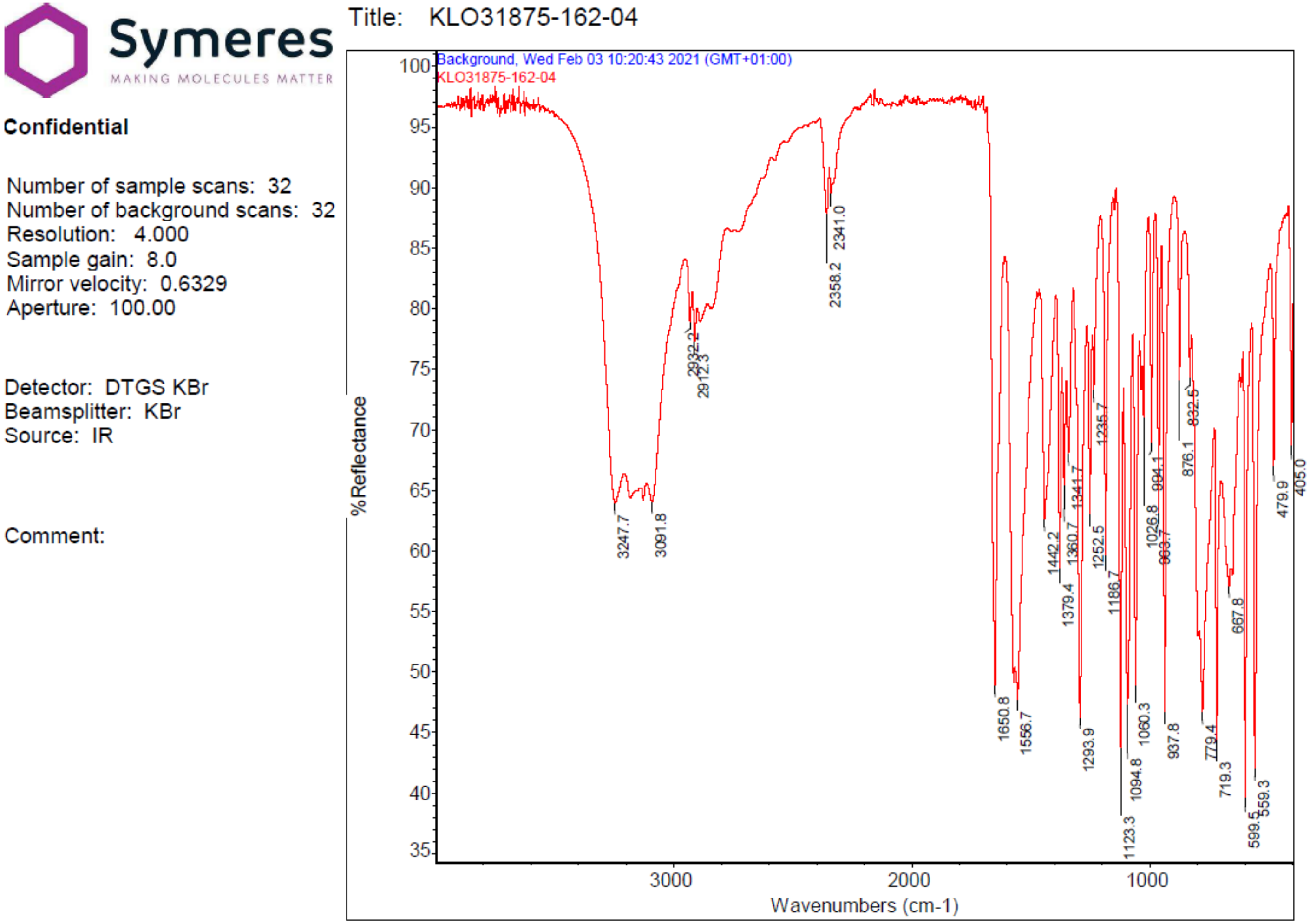
S14. DSC Di-HAF


Sample: KLO31875-162-03
Size: 1.4900 mg
Method: 28-300/10/N2

DSC

File: \\...\Data\DSC\2021\February\210203.001
Operator: HML
Run Date: 03-Feb-2021 10:29
Instrument: DSC Q20 V24.11 Build 124

S15. IR spectrum Di-HAF



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Number of sample scans: 32
Number of background scans: 32
Resolution: 4.000
Sample gain: 8.0
Mirror velocity: 0.6329
Aperture: 100.00

Detector: DTGS KBr
Beamsplitter: KBr
Source: IR

Comment:

Title: KLO31875-162-04

S16. ^{1}H-NMR spectrum of N-acetylglucosamine (pyridine-d_5)