Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.
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Synthesis and evaluation of sensitive coumarin-based fluorogenic substrates for discovery of α-*N*-acetyl galactosaminidases through droplet-based screening

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List of abbreviations

ACN Acetonitrile
α-GalNAcases α-N-acetyl-galactosaminidase
3-CU 3-Carboxyumbelliferone
δ Chemical shift
DMF N,N-Dimethylformamide
DCM Dichloromethane
E. coli *Escherichia coli*
GalNAc α-*N*-acetyl-galactosamine
IPTG Isopropyl β-*D*-thiogalactoside
J Coupling constant
JB Jericho Blue. 8-Fluoro-3-carboxyumbelliferone
LB Lysogeny broth, also known as Luria broth
LRMS Low resolution mass spectroscopy
MU 4-Methylumbelliferone
PBS Phosphate buffered Saline
PB Pacific Blue. 6,8-difluoro-3-carboxyumbelliferone
TFMU 4-Trifluoromethylumbelliferone
THF Tetrahydrofuran

Synthesis and characterization of compounds

General Information

All chemicals were of analytical grade and purchased from the Sigma-Aldrich company, unless otherwise stated. All solvents were BOC standard grade and dried before use. Dichloromethane and pyridine were distilled from calcium hydride. Methanol was distilled from magnesium. THF was distilled from sodium. DMF was dried and stored over 4Å molecular sieves. Analytical thin-layer chromatography (TLC) was performed on aluminum-backed sheets of Silica Gel $60F_{254}$ (E. Merck) of thickness 0.2 mm. The plates were visualised using UV light (254 nm) and/or by exposure to 10% ammonium molybdate (2 M in H_2SO_4) followed by charring. Flash column chromatography was carried out using Silicycle (230-400 mesh). Proton and carbon NMR spectra were recorded on Bruker Advance 400inv, 400 dir and 300 Fourier Transform spectrometers fitted with a 5 mm BBI-Z probe. All spectra were recorded using an internal deuterium lock and are referenced internally using the residual solvent peak. Carbon and proton chemical shifts are quoted in parts per million (ppm) downfield of tetramethylsilane, fluorine chemical shifts are quoted downfield of trifluoroacetic acid. Coupling constants (*J*) are given in Hertz (Hz). Carbon NMR spectra were measured with broadband proton decoupling and were recorded with DEPT. Mass spectra were measured on a Waters/Micromass LCT instrument in methanol using electrospray ionisation (ESI) and recorded using the Time-Of-Flight (TOF) method.

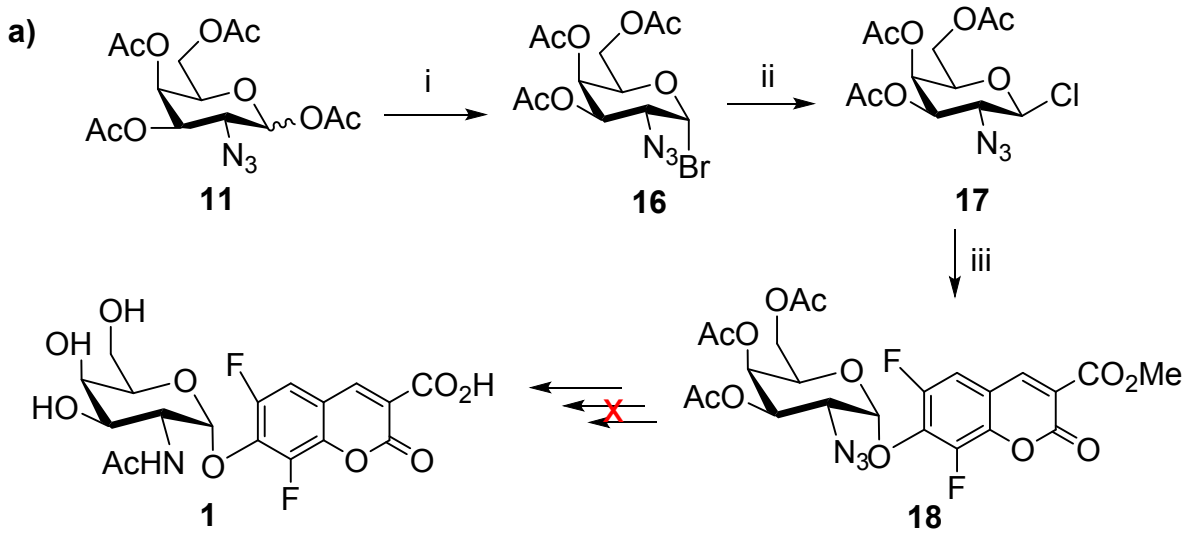
General Procedures

Glycosylation

A mixture of the silyl-protected glycosyl fluoride **14** (0.45 mmol, 1.5 equiv), the tetrabutylammonium salt of the coumarin (0.30 mmol) and 4 Å molecular sieves (100 mg) in anhydrous dichloromethane (10 mL) was stirred for 15 minutes at room temperature under N_2, and then cooled on an ice bath. To the mixture was added $BF_3{\cdot}Et_2O$ (0.17 mL, 1.38 mmol, 4.6 equiv) as one portion, the reaction mixture was stirred for 24 h at 0°C, diluted with dichloromethane (20 mL), filtered through Celite and washed with dichloromethane (20 mL). The combined dichloromethane filtrate was washed with saturated $NaHCO_3$ (30 mL) and brine (30 mL), dried over $MgSO_4$, filtered and concentrated. The resulting residue was purified by flash column chromatography (petroleum ether-ethyl acetate) to afford the product as a solid.

Synthesis and characterization of compounds

Attempted synthesis of GalNAc-α-Pacific Blue 1



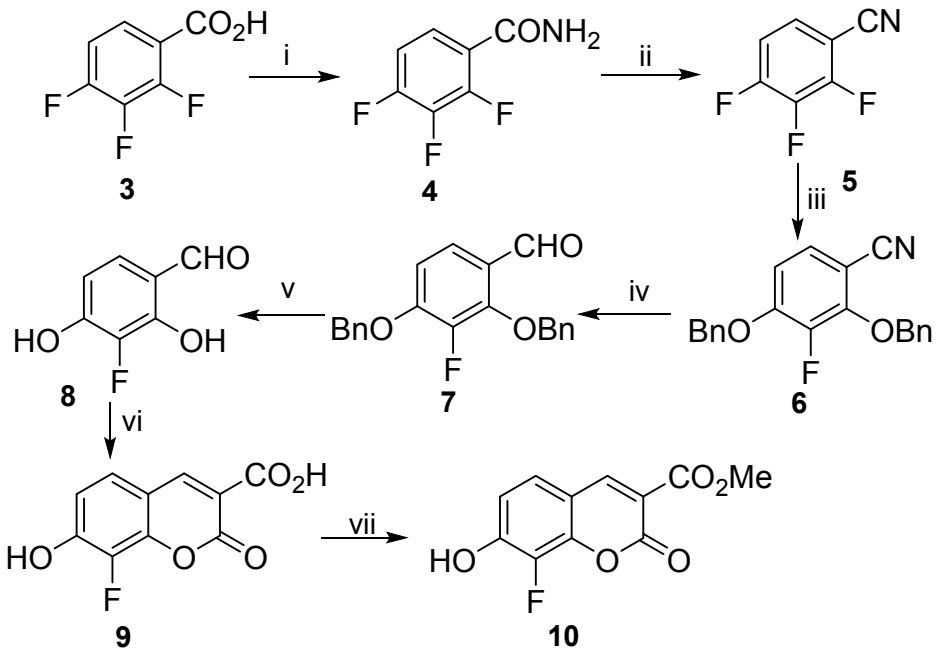
Scheme S1. Reagents and conditions: i. DCM, EtOAc, Ac_2O, $TiBr_4$, rt, 94%. ii. ACN, TEAC, rt. iii. ACN, Ag_2CO_3, $AgClO_4$, 3Å Molecular sieves, PB methyl ester, Lutidine, rt, 36%

While efficient syntheses of the Pacific Blue coumarin have been published[1,2] coupling of this in an alpha configuration to GalNAc has not. Such syntheses of alpha-configured GalNAc derivatives can be challenging.

Our initial approach (Scheme S1) involved synthesis of the per-O-acetylated 2-azido-2-deoxy beta glucosyl chloride from the alpha bromide[3–5] using tetraethylammonium chloride.[6,7] Displacement of the chloride by the methyl ester of the Pacific Blue coumarin[8-9] using a silver catalyst in acetonitrile yielded a mixture of the alpha and beta anomers in yields of 36 and 21% respectively.[6] While far from ideal, this approach provided material with which to assess optimal routes for deprotection and amide formation. However, all attempts to reduce the azide and acetylate it to give the acetamide resulted in rapid decomposition of the glycoside. We therefore inverted the order and first deprotected the sugar acetates under Zemplen conditions.[10–12] This was successful and the 2-azido glycoside was isolated. However, this unprotected glycoside decomposed very quickly. The planned glycoside therefore had no future as a screening substrate.

3-(Carboxyl methyl ester)-6,8-difluoro-umbelliferyl 2-Azido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl fluoride (**18**): To a solution of 2-azido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-D-galactopyranose (**11**) (537 mg, 1.44 mmol) in anhydrous DCM (8 mL) were added EtOAc (0.8 mL) and $TiBr_4$ (1.3 g, 3.54 mmol, 2.5 equiv), and the reaction mixture was stirred overnight at room temperature. Another portion of $TiBr_4$ (0.2 g) was added, and the reaction mixture was stirred for 2 days. The reaction mixture was diluted with DCM (75 mL), washed with cold $NaHCO_3$ (2 x 50 mL) and cold brine (50 mL), dried over $MgSO_4$, filtered and evaporated, dried under vacuum to afford **2-azido-2-deoxy-3,4,6-tri-*O*-**

acetyl-α-D-galactopyranosyl bromide (16) as a colorless syrup (532 mg, 94%). To the solution of bromide (532 mg) in dry acetonitrile (27 mL) was added tetraethylammonium chloride in dry acetonitrile (27 mL) in one portion, the reaction mixture was stirred for 20 minutes at room temperature under N_2. The reaction mixture was diluted with toluene (400 mL), washed with cold water (2 x 200 mL) and cold brine (200 mL), dried over $MgSO_4$, filtered and evaporated, dried under vacuum to afford **2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl chloride (17)** as a colorless syrup (470 mg, 100%). A suspension of PB methyl ester (160 mg, 0.63 mmol), lutidine (0.3 mL), Ag_2CO_3 (400 mg), $AgClO_4$ (60 mg) and 3Å molecular sieves in dry acetonitrile (15 mL) was stirred for 0.5 h at room temperature under N_2. To this suspension was added dropwise a solution of crude chloride (265 mg, 0.76 mmol, 1.2 equiv) in dry acetonitrile (15 mL), and the reaction mixture was stirred for 6 days at room temperature. The reaction mixture was diluted with acetone, filtered through Celite and washed with acetone, concentrated to give a brown residue. The brown residue was redissolved in EtOAc (150 mL), washed with 1 M HCl (50 mL) and brine (50 mL), filtered and concentrated. The resulting residue was purified by flash column chromatography (2:1 & 3:2 petroleum ether-ethyl acetate) to afford the product as a white solid (127 mg, 36% based on PB methyl ester) and its β-anomer (75 mg, 21%). ^{1}H NMR (d_6-Acetone, 400 MHz) α-anomer: δ 8.69 (s, 1 H, Ar-H), 7.68 (dd, 1 H, $J_{H,F}$ 2.2, $J_{H,F}$ 9.4 Hz, Ar-H), 5.42 (d, 1 H, $J_{1,2}$ 4.4 Hz, H-1), 5.18 (d, 1 H, H-4), 4.88 (dd, 1 H, $J_{3,4}$ 3.2 Hz, $J_{2,3}$ 10.8 Hz, H-3), 4.08 (m, 1 H, H-5), 4.00 (t, 1 H, H-2), 3.97 (dd, 1 H, $J_{5,6a}$ 3.6 Hz, H-6a), 3.88 (s, 3 H, OCH_3), 3.87 (dd, 1 H, $J_{5,6b}$ 7.6 Hz, $J_{6a,6b}$ 11.2 Hz, H-6b), 2.09 (s, 3 H, CH_3CO), 1.96 (s, 3 H, CH_3CO), 1.93 (s, 3 H, CH_3CO). ^{13}C NMR (d_6-Acetone, 100 MHz) α-anomer: δ 170.0, 169.9, 169.2, 162.9, 154.3, 151.5 (dd, $J_{C,F}$ 3.0 Hz & 245.0 Hz), 147.3 (d, $J_{C,F}$ 3.0 Hz), 142.6 (dd, $J_{C,F}$ 5.0 Hz & 252.0 Hz), 141.1 (dd, $J_{C,F}$ 2.0 Hz & 10.0 Hz), 133.8 (dd, $J_{C,F}$ 13.0 Hz & 18.0 Hz), 119.5, 116.1 (dd, $J_{C,F}$ 1.0 Hz & 10.0 Hz), 110.5 (dd, $J_{C,F}$ 4.0 Hz & 21.0 Hz), 83.7, 69.2, 67.8, 67.7, 62.2, 58.8, 52.3, 19.8 (3s). ESI-MS α-anomer: Calcd for $[C_{23}H_{21}F_2N_3O_{12} + Na]^+$: 592.1; Found m/z: 592.0. ^{1}H NMR (d_6-Acetone, 400 MHz) β-anomer: δ 8.65 (s, 1 H, Ar-H), 7.68 (dd, 1 H, $J_{H,F}$ 1.8, $J_{H,F}$ 10.2 Hz, Ar-H), 5.46 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.39 (d, 1 H, H-4), 5.12 (dd, 1 H, $J_{3,4}$ 3.2 Hz, $J_{2,3}$ 10.8 Hz, H-3), 4.31 (m, 1 H, H-5), 4.18-4.10 (m, 3 H, H-2 & H-6), 3.88 (s, 3 H, OCH_3), 2.09 (s, 3 H, CH_3CO), 2.04 (s, 3 H, CH_3CO), 1.92 (s, 3 H, CH_3CO). ^{13}C NMR (d_6-Acetone, 100 MHz) β-anomer: δ 170.1, 169.7, 169.3, 162.9, 154.3, 151.4 (d, $J_{C,F}$ 245.0 Hz), 147.4, 142.7 (d, $J_{C,F}$ 251.0 Hz), 141.3 (d, $J_{C,F}$ 7.0 Hz), 136.9 (dd, $J_{C,F}$ 10.0 Hz & 15.0 Hz), 118.9, 114.8 (d, $J_{C,F}$ 10.0 Hz), 110.9 (dd, $J_{C,F}$ 3.0 Hz & 21.0 Hz), 102.6, 71.7, 71.1, 66.5, 61.5, 61.0, [i]52.3, 19.8 (3s). ESI-MS β-anomer: Calcd for $[C_{23}H_{21}F_2N_3O_{12} + Na]^+$: 592.1; Found m/z: 592.0.



Scheme S2. Reagents and conditions: i. DCM, oxalyl chloride, DMF, rt; $CHCl_3$, NH_4OH, 0°C. ii. $POCl_3$, 80°C. iii. DMF, BnOH, K_2CO_3, 105°C. iv. DCM, DIBAL in cyclohexane (1 M), -78°C. v. MeOH, THF, H_2, Pd/C, rt. vi. H_2O, Meldrum's acid, NH_4OAc, rt. vii. MeOH, H_2SO_4, refluxed, 90%

For the discussion regarding the synthesis of Jericho Blue (Scheme S2), please refer to the manuscript.

2,3,4-Trifluorobenzamide (**4**): To a suspension of compound **3** (5 g, 28.6 mmol) in anhydrous DCM (40 mL), stirred at room temperature, was added oxalyl chloride (3 mL, 34.9 mmol, 1.2 equiv) as one portion, and then two drops of dry DMF were added (note: a lot of gas is generated). The reaction mixture was stirred for 23 h at room temperature while venting to the air to release the evolved gases. The solvents were evaporated under reduced pressure, co-evaporated with $CHCl_3$ three times to afford a black oil. To the black oil in $CHCl_3$ (22 mL), cooled with ice-bath, was quickly added ammonium hydroxide (31 mL) while stirring vigorously. After addition, the reaction mixture was stirred under the same conditions for another hour. The white precipitate was filtered and washed with water and dried under vacuum. The filtrate was separated into two layers and the aqueous phase then extracted with DCM (50 mL x 3). The combined organic phase was dried over $MgSO_4$, filtered and evaporated to afford another portion of product. The total amount of white solid was 4.39 g (88%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.99 (dddd, 1 H, Ar-H), 7.11 (dddd, 1 H, J 2.0, 5.6, 8.8, 16.0 Hz, Ar-H), 6.53 (s, 1 H, NH), 6.33 (s, 1 H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.5, 153.6 (ddd, J 3.6, 10.0, 255.6 Hz), 150.4 (ddd, 3.7, 11.1, 250.2 Hz), 139.9 (ddd, J 15.6, 16.8, 251.5 Hz), 126.2 (ddd, 1.3, 3.9, 6.4 Hz), 118.1 (m), 113.0 (dd, J 3.6, 17.4 Hz). ^{19}F-NMR ($CDCl_3$, 282 MHz): δ -127.5 (dd, J 11.6, 20.3 Hz), -134.6 (dd), -159.7 (t). EI-HRMS: Calcd for $C_7H_4F_3NO$: 175.02450; Found m/z: 175.02403.

2,3,4-Trifluorobenzonitrile (**5**): A mixture of compound **4** (4.39 g, 25.1 mmol) and phosphoryl chloride (20 mL) was stirred for 3 h at 80°C under N_2, diluted with diethyl ether (60 mL). The reaction mixture was slowly poured into a beaker containing ice water while cooling with an ice bath and stirred at 0°C. Saturated $NaHCO_3$ was added dropwise then the mixture extracted with diethyl ether (150 mL x 3). The combined organic phase was washed with saturated $NaHCO_3$ (200 mL) and brine (200 mL), dried over

$MgSO_4$, filtered and concentrated to afford a colorless oil (3.668, 93%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.45 (m, 1 H, Ar-H), 7.15 (dddd, 1 H, J 2.0, 6.8, 9.2, 16.0 Hz, Ar-H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 154.7 (ddd, J 3.0, 10.0, 260.0 Hz), 153.1 (ddd, 4.0, 12.0, 262.0 Hz), 140.4 (ddd, J 13.0, 16.0, 255.0 Hz), 128.1 (dd, 5.0, 9.0 Hz), 114.1 (dd, J 4.0, 19.0 Hz), 112.3, 99.5 (dd, J 4.0, 14.0 Hz). ^{19}F-NMR ($CDCl_3$, 282 MHz): δ -123.1 (dd, J 11.6, 20.3 Hz), -126.7 (t), -156.8 (t). EI-HRMS: Calcd for $C_7H_2F_3N$: 157.01393; Found m/z: 157.01386.

2,4-Bis(benzyloxy)-3-fluorobenzonitrile (**6**): To a solution of compound **5** (3.587 g, 22.8 mmol) in dry DMF (3.5 mL) were added benzyl alcohol (12 mL) and anhydrous K_2CO_3 (19 g), and the suspension was stirred for 18 h at 105°C under N_2. The reaction mixture was cooled down to room temperature, and diluted with EtOAc (20 mL), filtered and the solids washed with EtOAc (300 mL). The combined EtOAc solution was washed with water (150 mL x 2) and brine (150 mL), dried over $MgSO_4$, filtered and concentrated to afford an off-white solid. This was crystallized from ethanol to give a white solid (5.96 g). The mother liquor was concentrated and purified by flash column chromatography (1:1 petroleum ether-DCM) to afford another portion of product (0.7 g). The total amount of white solid was 6.66 g (88%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.52-7.33 (m, 10 H, Ar-H), 7.23 (dd, 1 H, J 2.0, 8.8 Hz, Ar-H), 6.74 (dd, 1 H, J 7.2 Hz, Ar-H), 5.34 (s, 2 H, CH_2), 5.18 (s, 2 H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 152.3 (d, J 9.0 Hz), 149.4 (d, J 10.0 Hz), 145.6 (d, J 248.0 Hz), 135.8, 135.4, 128.9, 128.8, 128.7, 128.66, 128.5, 127.5, 116.0 (J 4.0 Hz), 109.7, 100.2 (J 3.0 Hz), 76.3 (d, J 7.0 Hz), 71.7. ^{19}F-NMR ($CDCl_3$, 282 MHz): δ -148.9. EI-HRMS: Calcd for $C_{21}H_{16}FNO_2$: 333.11651; Found m/z: 333.11678.

2,4-Bis(benzyloxy)-3-fluorobenzaldehyde (7): To a solution of compound **6** (6.6 g, 19.8 mmol) in anhydrous DCM (25 mL), stirred at -78°C under N_2, was added, dropwise diisobutylaluminum hydride (25 mL, 1 M in cyclohexane). The reaction mixture was stirred for 4 h under the same conditions, stirred for 0.5 h at room temperature, and then cooled to 0°C. To the mixture was added dropwise HCl (0.5 M, 80 mL) with stirring to quench the reaction. After addition, the mixture was stirred overnight at room temperature and extracted with EtOAc (100 mL x 4). The organic phase was washed with water (200 mL x 2) and brine (200 mL), dried over $MgSO_4$, filtered and concentrated, then dried under vacuum to afford a slightly yellow solid (6.64 g, 100%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.18 (s, 1 H, CHO), 7.61 (dd, 1 H, J 2.0, 8.8 Hz, Ar-H), 7.51-7.36 (m, 10 H, Ar-H), 6.86 (dd, 1 H, J 7.2 Hz, Ar-H), 5.33 (s, 2 H, CH_2), 5.25 (s, 2 H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 188.2 (d, J 2.7 Hz), 153.4 (d, J 9.1 Hz), 150.1 (d, J 8.3 Hz), 145.4 (d, J 246.6 Hz), 136.0, 135.6, 128.9, 128.8, 128.7, 128.6, 128.5, 127.6, 123.7 (J 3.9 Hz), 123.6, 109.3, 76.9 (d, J 7.2 Hz), 71.5. ^{19}F-NMR ($CDCl_3$, 282 MHz): δ -150.5. EI-HRMS: Calcd for $C_{21}H_{17}FO_3$: 336.11617; Found m/z: 336.11641.

2,4-Dihydroxy-3-fluorobenzaldehyde (**8**): A mixture of compound **7** (6.57 g, 19.5 mmol), Pd/C (10%, 1.05 g) and methanol-THF (7:3, 63 mL) was evacuated and refilled with H_2 three times, and stirred for 5 h at room temperature under H_2 atmosphere. The reaction mixture was filtered through a short pad of Celite and washed with methanol and EtOAc. After evaporation of solvent, the resulting residue was purified by flash column chromatography (dry loaded, 80:20:1, petroleum ether-EtOAc-AcOH) to afford the product as a white solid (2.5 g, 82%). ^{1}H NMR (d_6-Acetone, 400 MHz): δ 11.4 (brs, 1 H, OH), 9.86 (s, 1 H, CHO), 7.47 (d, 1 H, J 8.8 Hz, Ar-H), 6.71 (t, 1 H, $J_{H,F}$ 7.6 Hz, Ar-H), 3.26 (brs, 1 H, OH). ^{13}C NMR (d_6-Acetone, 75 MHz): δ 195.6 (d, J 2.5 Hz), 152.7 (d, J 9.7 Hz), 151.3 (d, J 9.6 Hz), 139.3 (d, J 239.0 Hz), 130.0 (d, J 3.5 Hz), 115.9 (d, J 1.4 Hz), 109.4. ^{19}F-NMR ($CDCl_3$, 282 MHz): δ -165.0. EI-HRMS: Calcd for $C_7H_5FNO_3$: 156.02227; Found m/z: 156.02215.

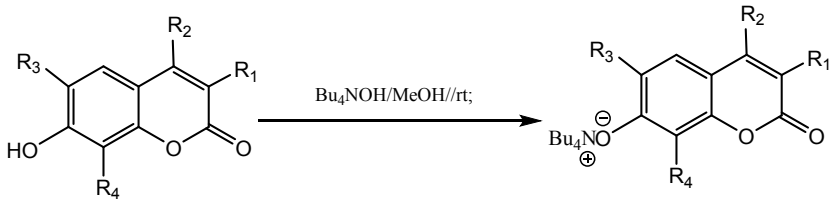
8-Fluoro-7-hydroxy-2-oxo-2H-chromene-3-carboxylic acid (Jericho Blue, **9**): To a suspension of compound **8** (2.2 g, 14.1 mmol) in water (65 mL) were added Meldrum's acid (2.22 g, 15.4 mmol) and ammonium acetate (330 mg, 4.3 mmol), the suspension was stirred for 3 h at room temperature, 10% more Meldrum's acid and ammonium acetate were added again and the mixture stirred for another 1.5 h under the same conditions. The reaction mixture was cooled down with an ice bath, HCl (2 M, 40 mL) was added and the mixture stirred for 15 minutes at 0°C, and then stored in the fridge for 1 h. The solid was filtered and washed with cold water, and then dried under vacuum to afford the final product as a slightly yellow solid (3.077 g, 97%). ^{1}H NMR (d_6-DMSO, 400 MHz): δ 11.5 (brs, 1 H, OH), 8.69 (s, 1 H, Ar-H), 7.55 (d, 1 H, J 8.2 Hz), 6.98 (t, 1 H, $J_{H,F}$ 8.0 Hz, Ar-H). ^{13}C NMR (d_6-DMSO, 75 MHz): δ 164.5, 156.7, 151.3 (d, J 8.7 Hz), 149.9 (d, J 1.6 Hz), 144.8 (d, J 8.5 Hz), 137.9 (d, J 243.1 Hz), 126.6 (d, J 3.5 Hz), 114.8, 114.0, 111.9. ^{19}F-NMR (d_6-DMSO, 282 MHz): δ -160.1. ESI-HRMS: Calcd for $[C_{10}H_5FNO_5 - H]^-$: 223.0043; Found m/z: 223.0047.

Methyl 8-Fluoro-7-hydroxy-2-oxo-2H-chromene-3-carboxylate (**10**): To a suspension of compound **9** (0.96 g, 4.3 mmol) in dry methanol (50 mL) were added concentrated H_2SO_4 (2 drops), and the suspension was refluxed overnight under N_2 (suspension-clear-suspension) [13,14]. The reaction mixture was cooled down to room temperature, and then to 0°C. The yellowish precipitate was filtered and washed with cold methanol to afford 14 (0.918 g, 90%). ^{1}H NMR (d_6-DMSO + D_2O, 400 MHz): δ 8.72 (s, 1 H, Ar-H), 7.57 (d, 1 H, J 8.8 Hz), 6.99 (t, 1 H, $J_{H,F}$ 8.0 Hz, Ar-H). ^{13}C NMR (d_6-DMSO, 75 MHz): δ 163.8, 155.8, 151.5 (d, J 8.7 Hz), 150.3 (d, J 2.0 Hz), 145.0 (d, J 8.3 Hz), 137.9 (d, J 243.2 Hz), 126.7 (d, J 3.5 Hz), 114.9, 113.3, 111.8, 52.9. ^{19}F-NMR (d_6-DMSO, 282 MHz): δ -160.1. ESI-MS: Calcd for $[C_{11}H_6FNO_5 - H]^-$: 237.2; Found m/z: 237.1.

Tetrabutylammonium coumarin salt preparation (**19a-19i)**

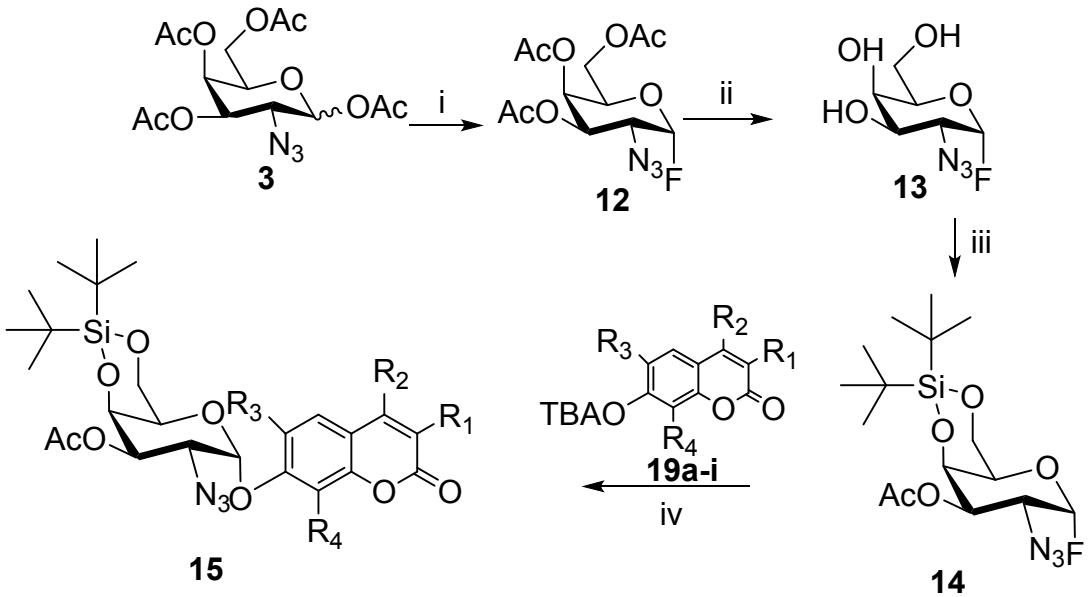
As is noted below, in order to render the coumarins soluble under the conditions used for glycoside formation they had to be used as their tetrabutylammonium salts. Conversion to this salt form was accomplished by treatment of **10** with tetrabutylammonium hydroxide in methanol as solvent (Scheme S3).[15] Use of any other alcohol resulted in partial transesterification of the methyl ester moiety. The crude salt obtained after evaporation of methanol was dissolved in a minimum amount of dichloromethane and precipitated out using diethyl ether to afford a yellow solid, which was dried under vacuum and used in the glycosylation reactions without further purification (**19a**-**19i**).

To a suspension of the coumarin (1 mmol) in MeOH (7.5 mL) was added dropwise 0.1 M tetrabutylammonium hydroxide in MeOH (10 mL, 1 equiv) with stirring, and the reaction mixture was stirred for 15 minutes at room temperature. After evaporation, the product was precipitated out with DCM and diethyl ether to afford the salts as a yellow solid. The solid was dried under vacuum and used directly without further purification.



19a: R_1=H, R_2=Me, R_3=H, R_4=H, 90%
19b: R_1=H, R_2=Me, R_3=F, R_4=F, 87%
19c: R_1=CO_2Me, R_2=H, R_3=H, R_4=F, 100%
19d: R_1=H, R_2=Me, R_3=Cl, R_4=H, 91%
19e: R_1=CO_2Me, R_2=H, R_3=Cl, R_4=H, 94%
19f: R_1=CO_2Me, R_2=H, R_3=H, R_4=Cl, 89%
19g: R_1=CO_2Me, R_2=H, R_3=H, R_4=H, 69%
19h: R_1=H, R_2=CF_3, R_3=H, R_4=H, 89%
19i: R_1=H, R_2=CF_3, R_3=F, R_4=F, 88%

Scheme S3. Reagents and conditions: 1 M Bu_4NOH in MeOH, MeOH, rt.



15a: R_1=H, R_2=Me, R_3=H, R_4=H
15b: R_1=H, R_2=Me, R_3=F, R_4=F
15c: R_1=CO_2Me, R_2=H, R_3=H, R_4=F
15d: R_1=H, R_2=Me, R_3=Cl, R_4=H
15e: R_1=CO_2Me, R_2=H, R_3=Cl, R_4=H
15f: R_1=CO_2Me, R_2=H, R_3=H, R_4=Cl
15g: R_1=CO_2Me, R_2=H, R_3=H, R_4=H
15h: R_1=H, R_2=CF_3, R_3=H, R_4=H
15i: R_1=H, R_2=CF_3, R_3=F, R_4=F

Scheme S4. Reagents and conditions: i). DCM, HF/Pyridine, 0°C, 93%. ii). MeOH, NH_3, 0°C, 95%. iii). Pyridine, di-*tert*-butylsilylbistriflate, 0°C-rt; Ac_2O, rt, 81%. iv). DCM, Coumarin.TBA salt (15a-i), 4Å Molecular sieves, $BF_3{\cdot}Et_2O$, 0°C.

As discussed in the paper, we initially used the method pioneered by Kiso in which a di-tert-butylsilylene protecting group installed across the 4 and 6 positions strongly directs α-glycosylation in Mitsunobu reactions.[16–18] However, we were not able to achieve the described yields. This is likely as a consequence of the low nucleophilicity of the low pKa coumarin derivatives as well as the insolubility of the coumarin derivatives in the appropriate solvents. However, once converted to their tetrabutylammonium salts (See scheme S3), these compounds dissolve well in dichloromethane. We finally shifted our strategy to use of a glycosyl fluoride donor and coupling under catalysis from $BF_3{\cdot}Et_2O$. The final scheme is shown in Scheme S4 and was used to carry out the couplings of the range of coumarin aglycones shown.

2-Azido-2-deoxy-D-galactose was generated from D-galactosamine hydrochloride by neutralisation using potassium carbonate in water then reaction with triflic azide.[19–23] After acetylation in pyridine and acetic anhydride, the protected azidogalactose **3** was treated with HF/pyridine in dichloromethane to convert it to the protected α-fluoride. Deacetylation was accomplished by dissolving this compound in dry methanol and bubbling in ammonia gas for 5 minutes at 0°C, then stirring overnight under the same conditions.[24] The 2-azido-2-deoxy-D-galactosyl fluoride product (**13**) was silylated at the 4,6-positions by treatment with di-tert-butyl bis(trifluoromethanesulfonate) in pyridine and then acetylated *in-situ* to give the protected azidogalactosyl fluoride **14** as the key intermediate. Glycoside formation was achieved by coupling the galactosyl fluoride with the tetrabutylammonium salt of the coumarin in

dichloromethane in the presence of boron trifluoride diethyl etherate. The resultant protected umbelliferyl α-azido-galactosides were purified by flash silica chromatography.

2-Azido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl fluoride (**12**): A solution of 2-azido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-D-galactopyranose (**11**) (2.24 g, 6 mmol) in anhydrous DCM (15 mL) was transferred to a 100 mL plastic bottle, cooled down to 0°C, and then HF/Pyridine (15 mL, 70% HF in pyridine) was added. The plastic bottle was capped and stirred overnight at 0°C. The reaction mixture was slowly poured (be very careful) into a plastic beaker with ice, saturated $NaHCO_3$ and solid $NaHCO_3$ cooled with an ice bath. The crude product was extracted with ethyl acetate (2 x 100 mL), washed with saturated $NaHCO_3$ (2 x 100 mL) and brine (100 mL), dried over $MgSO_4$, filtered and concentrated. The resulting residue was purified by flash column chromatography (5:1 petroleum ether-ethyl acetate) to afford the product as a colorless syrup (1.85 g, 93%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.64 (dd, 1 H, $J_{1,2}$ 2.4 Hz, $J_{H,F}$ 52.4 Hz, H-1), 5.49 (d, 1 H, H-4), 5.32 (dd, 1 H, $J_{3,4}$ 3.2 Hz, H-3), 4.38 (m, 1 H, H-5), 4.13 (dd, 1 H, $J_{5,6a}$ 6.0 Hz, $J_{6a,6b}$ 11.2 Hz, H-6a), 4.07 (dd, 1 H, $J_{5,6b}$ 4.4 Hz, H-6b), 3.78 (dddd, 1 H, $J_{2,3}$ 10.8 Hz, $J_{H,F}$ 24.8 Hz, H-2), 2.14 (s, 3 H, CH_3CO), 2.05 (s, 3 H, CH_3CO), 2.04 (s, 3 H, CH_3CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.4, 169.9, 169.7, 106.0 (d, $J_{C,F}$ 227.0 Hz), 69.3 (d, $J_{C,F}$ 3.0 Hz), 68.2, 66.9, 61.3, 57.5 (d, $J_{C,F}$ 24.0 Hz), 20.72, 20.66, 20.6. ^{19}F-NMR ($CDCl_3$, 282 MHz): δ -148.4. ESI-HRMS: Calcd for $[C_{12}H_{16}FN_3O_7 + Na]^+$: 356.0870; Found m/z: 356.0872.

2-Azido-2-deoxy-α-D-galactopyranosyl fluoride (**13**): To a solution of **12** (566 mg, 1.7 mmol) in anhydrous methanol (20 mL) was bubbled gaseous ammonia for 5 minutes at 0°C, and the reaction mixture then stirred overnight at 0°C. The solvent was evaporated at reduced pressure, and the resulting residue was purified by flash column chromatography (9:1 DCM-MeOH) to afford the product as a colorless syrup (333 mg, 95%). ^{1}H NMR (D_2O, 400 MHz): δ 5.69 (dd, 1 H, $J_{1,2}$ 2.4 Hz, $J_{H,F}$ 53.6 Hz, H-1), 3.98 (m, 3 H, H-3, 4 & 5). 3.77 (dd, 1 H, $J_{5,6a}$ 6.4 Hz, $J_{6a,6b}$ 11.2 Hz, H-6a), 3.73 (dd, 1 H, $J_{5,6b}$ 5.2 Hz, H-6b), 3.65 (dddd, $J_{2,3}$ 10.4 Hz, $J_{H,F}$ 26.8 Hz, H-2). ^{13}C NMR (CD_3OD, 100 MHz): δ 107.0 (d, $J_{C,F}$ 222.0 Hz), 73.8 (d, $J_{C,F}$ 10.0 Hz), 69.0, 68.1, 61.2, 60.2 (d, $J_{C,F}$ 24.0 Hz). ^{19}F-NMR (D_2O, 282 MHz): δ -148.8. ESI-HRMS: Calcd for $[C_6H_{10}N_3FO_4 + Na]^+$: 230.0553; Found m/z: 230.0558.

2-Azido-2-deoxy-3-*O*-acetyl-4,6-*O*-di-*tert*-butylsilylene-α-D-galactopyranosyl fluoride (**14**): To a solution of **13** (1.016 g, 4.91 mmol) in dry pyridine (70 mL), stirred at 0°C under N_2, was added di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (2.03 ml, 2.74 g, 6.23 mmol, 1.3 equiv) as one portion. The reaction mixture was stirred for 1 h under the same conditions and then stirred overnight at rt. To the reaction mixture was added acetic anhydride (3.2 mL) and the mixture was stirred for 1.5 h at room temperature. After evaporation of solvent, the resulting residue was redissolved in EtOAc (200 mL), washed with cold 1 M HCl (100 mL), cold $NaHCO_3$ (100 mL) and cold brine (100 mL), dried over $MgSO_4$, filtered and concentrated. The resulting residue was purified by flash column chromatography (15:1 petroleum ether-EtOAc) to afford **18** as a white solid (1.55 g, 81%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.73 (dd, 1 H, $J_{1,2}$ 2.8 Hz, $J_{H,F}$ 52.8 Hz, H-1), 5.10 (dd, 1H. $J_{3,4}$ 2.8 Hz, $J_{2,3}$ 11.2 Hz, H-3), 4.78 (d, 1 H, H-4), 4.28 (dd, 1 H, $J_{5,6a}$ 2.6 Hz, $J_{6a,6b}$ 13.2 Hz, H-6a), 4.21 (dd, 1 H, $J_{5,6b}$ 1.6 Hz, H-6b), 3,97 (m, 1 H, H-5), 3.95 (dddd, 1 H, $J_{H,F}$ 26.0 Hz, H-2), 2.18 (s, 3 H, CH_3CO), 1.03 (2 s, 18 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.4, 106.8 (d, $J_{C,F}$ 226.0 Hz), 71.2, 69.7, 69.5 (d, $J_{C,F}$ 2.0 Hz), 66.5, 57.1 (d, $J_{C,F}$ 23.0 Hz), 27.6, 27.2, 23.3, 20.9, 20.8. ^{19}F-NMR ($CDCl_3$, 282 MHz): δ -147.9. ESI-HRMS: Calcd for $[C_{16}H_{28}FN_3O_5Si + H]^+$: 390.1861; Found m/z: 390.1864.

4-Methylumbelliferyl 2-azido-2-deoxy-3-*O*-acetyl-4,6-*O*-di-*tert*-butylsilylene-α-D-galactopyranoside (**15a**): See general procedure B. The resulting residue was purified by flash column chromatography (4:1

petroleum ether-EtOAc) to afford **15a** as a white solid (71%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.53 (d, 1 H, J 8.8 Hz, Ar-H), 7.12 (d, 1 H, J 2.4 Hz, Ar-H), 7.03 (dd, 1 H, Ar-H), 6.18 (d, 1 H, J 0.8 Hz, Ar-H), 5.71 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 5.33 (dd, 1H. $J_{3,4}$ 2.8 Hz, $J_{2,3}$ 10.8 Hz, H-3), 4.79 (d, 1 H, H-4), 4.21 (dd, 1 H, $J_{5,6a}$ 2.0 Hz, $J_{6a,6b}$ 12.8 Hz, H-6a), 4.09 (dd, 1 H, $J_{5,6b}$ 1.2 Hz, H-6b), 4.06 (dd, 1 H, H-2), 3,84 (m, 1 H, H-5), 2.40 (d, 3 H, J 0.8 Hz), 2.21 (s, 3 H, CH_3CO), 1.07 (s, 9 H), 1.02 (s, 9 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.6, 161.0, 159.0, 155.1, 152.3, 125.9, 115.4, 113.7, 113.2, 104.4, 97.4, 71.2, 70.0, 68.5, 66.7, 56.7, 27.7, 27.3, 23.4, 21.0, 20.9, 18.8. ESI-HRMS: Calcd for $[C_{26}H_{35}FN_3O_8Si +H]^+$: 546.2272; Found m/z: 546.2275.

6,8-Difluoro-4-methylumbelliferyl 2-azido-2-deoxy-3-*O*-acetyl-4,6-*O*-di-*tert*-butylsilylene-α-D-galactopyranoside (**15b**): See general procedure B. The resulting residue was purified by flash column chromatography (6:1 petroleum ether-EtOAc) to afford **15b** as a white solid (74%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.17 (dd, 1 H, $J_{H,F}$ 2.0 Hz &10.0 Hz, Ar-H), 6.34 (s, 1 H, Ar-H), 5.68 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.34 (dd, 1H. $J_{3,4}$ 2.8 Hz, $J_{2,3}$ 10.8 Hz, H-3), 4.85 (d, 1 H, H-4), 4.30 (m, 2 H, H-5 & H-6a), 4.09 (m, 2 H, H-2 & H-6b), 2.40 (d, 3 H, J 1.2 Hz), 2.22 (s, 3 H, CH_3CO), 1.05 (s, 9 H), 1.02 (s, 9 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.5, 158.7, 152.0 (dd, $J_{C,F}$ 3.0 Hz & 245.0 Hz), 151.2, 144.2 (dd, $J_{C,F}$ 5.0 Hz & 253.0 Hz), 139.7 (dd, $J_{C,F}$ 4.0 Hz & 10.0 Hz), 135.3 (dd, $J_{C,F}$ 12.0 Hz & 16.0 Hz), 117.0 (d, $J_{C,F}$ 9.0 Hz), 116.0, 106.1 (dd, $J_{C,F}$ 4.0 Hz & 22.0 Hz), 102.1, 71.6, 70.4, 69.2, 67.1, 57.0, 27.7, 27.3, 23.4, 21.0, 20.8, 18.9. ^{19}F-NMR ($CDCl_3$, 282 MHz): δ -131.7 (d, J 3.1 Hz), -145.2 (d). ESI-HRMS: Calcd for $[C_{26}H_{33}F_2N_3O_8Si +Na]^+$: 604.1903; Found m/z: 604.1906.

3-(Carboxyl methyl ester)-8-fluoro-umbelliferyl 2-azido-2-deoxy-3-*O*-acetyl-4,6-*O*-di-*tert*-butylsilylene-α-D-galactopyranoside (**15c**): See general procedure B. The resulting residue was purified by flash column chromatography (4:1 & 3:1 petroleum ether-EtOAc) to afford **15c** as a white solid (70%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.50 (s, 1 H, Ar-H), 7.36 (dd, 1 H, J 0.8 Hz and 7.6 Hz, Ar-H), 7.21 (dd, 1 H, J 8.8 Hz, Ar-H), 5.74 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.30 (dd, 1H. $J_{3,4}$ 2.8 Hz, $J_{2,3}$ 10.8 Hz, H-3), 4.84 (d, 1 H, H-4), 4.23 (dd, 1 H, $J_{5,6a}$ 1.6 Hz, $J_{6a,6b}$ 12.8 Hz, H-6a), 4.12 (dd, 1 H, H-2), 4.06 (d, 1 H, H-6b), 3.96 (m, 1 H, H-5), 3.91 (s, 3 H, OCH_3), 2.12 (s, 3 H, CH_3CO), 1.03 (s, 9 H), 1.00 (s, 9 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.4, 163.5, 155.2, 149.0 (d, $J_{C,F}$ 8.0 Hz), 148.7 (d, $J_{C,F}$ 1.0 Hz), 144.6 (d, $J_{C,F}$ 7.0 Hz), 140.2 (d, $J_{C,F}$ 261.0 Hz), 125.0 (d, $J_{C,F}$ 4.0 Hz), 116.4, 114.5, 113.9, 98.9, 71.3, 69.8, 68.9, 66.6, 56.8, 53.0, 27.6, 27.3, 23.3, 20.9, 20.8. ^{19}F-NMR ($CDCl_3$, 282 MHz): δ -151.5 (s). ESI-HRMS: Calcd for $[C_{27}H_{34}FN_3O_{10}Si +Na]^+$: 630.1895; Found m/z: 630.1896.

6-Chloro-4-methyl-umbelliferyl 2-Azido-2-deoxy-3-*O*-acetyl-4,6-*O*-di-*tert*-butylsilylene-α-D-galactopyranoside (**15d**): See general procedure B. The resulting residue was purified by flash column chromatography (9:1 & 6:1 petroleum ether-EtOAc) to afford **15d** as a white solid (62%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.59 (s, 1 H, Ar-H), 7.21 (s, 1 H, Ar-H), 6.21 (d, 1 H, J 0.8 Hz, Ar-H), 5.74 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.33 (dd, 1H. $J_{3,4}$ 2.8 Hz, $J_{2,3}$ 10.8 Hz, H-3), 4.85 (d, 1 H, H-4), 4.20 (dd, 1 H, $J_{5,6a}$ 2.0 Hz, $J_{6a,6b}$ 12.8 Hz, H-6a), 4.15 (dd, 1 H, H-2), 4.06 (dd, 1 H, $J_{5,6b}$ 1.2 Hz, H-6b), 3.84 (m, 1 H, H-5), 2.38 (d, 3 H, J 0.8 Hz, Ar-CH_3), 2.20 (s, 3 H, CH_3CO), 1.06 (s, 9 H), 1.01 (s, 9 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.5, 160.4, 154.0, 153.2, 151.4, 125.8, 120.0, 115.8, 114.0, 104.5, 97.7, 71.5, 69.8, 68.8, 66.6, 56.8, 53.0, 27.6, 27.3, 23.3, 21.0, 20.9, 18.7. ESI-HRMS: Calcd for $[C_{26}H_{34}ClN_3O_8Si +Na]^+$: 602.1701; Found m/z: 602.1706.

3-(Carboxyl methyl ester)-6-chloro-umbelliferyl 2-azido-2-deoxy-3-*O*-acetyl-4,6-*O*-di-*tert*-butylsilylene-α-D-galactopyranoside (**15e**): See general procedure B. The resulting residue was purified by flash column chromatography (4:1 petroleum ether-EtOAc) to afford **15e** as a white solid (45%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.48 (s, 1 H, Ar-H), 7.65 (s, 1 H, Ar-H), 7.26 (s, 1 H, Ar-H), 5.77 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1),

5.33 (dd, 1H. $J_{3,4}$ 2.8 Hz, $J_{2,3}$ 10.8 Hz, H-3), 4.87 (d, 1 H, H-4), 4.22 (dd, 1 H, $J_{5,6a}$ 2.4 Hz, $J_{6a,6b}$ 12.8 Hz, H-6a), 4.19 (dd, 1 H, H-2), 4.08 (dd, 1 H, $J_{5,6b}$ 1.5 Hz, H-6b), 3.90 (s, 3 H, OCH_3), 3.82 (m, 1 H, H-5), 2.22 (s, 3 H, CH_3CO), 1.08 (s, 9 H), 1.03 (s, 9 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.5, 163.6, 156.4, 156.2, 155.4, 148.0, 130.2, 121.0, 116.4, 113.4, 103.8, 97.8, 71.4, 69.7, 69.0, 66.5, 56.7, 53.1, 27.6, 27.3, 23.3, 21.0, 20.9. ESI-HRMS: Calcd for $[C_{27}H_{34}ClN_3O_{10}Si + Na]^+$: 646.1600; Found m/z: 646.1605 .

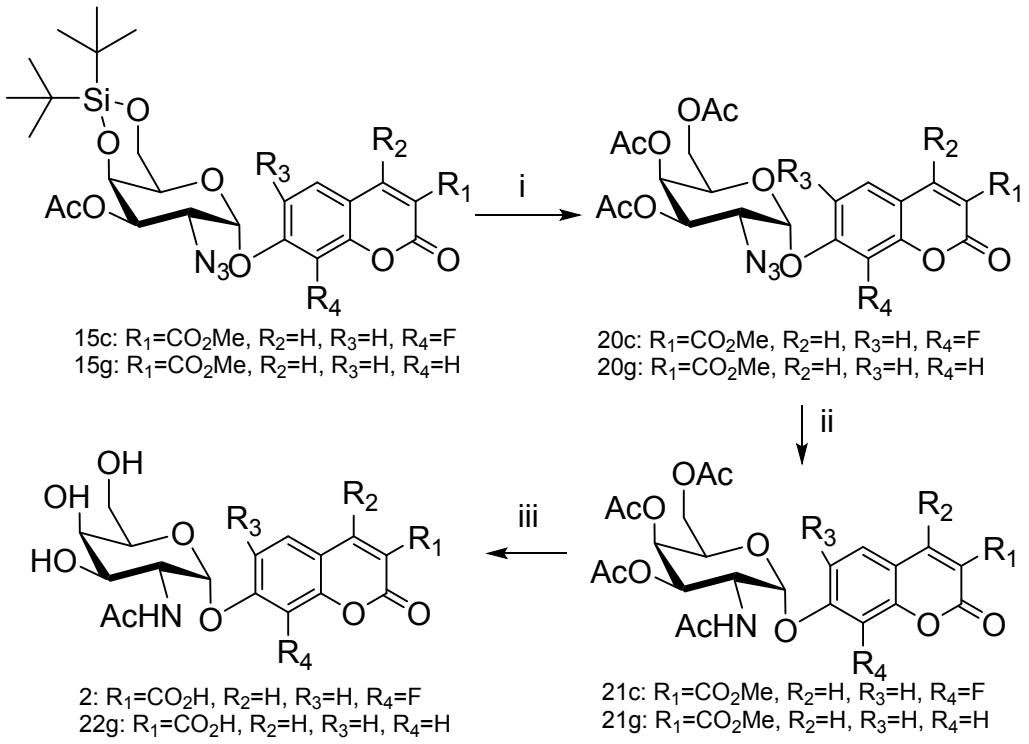
3-(Carboxyl methyl ester)-6-chloro-umbelliferyl 2-azido-2-deoxy-3-*O*-acetyl-4,6-*O*-di-*tert*-butylsilylene-α-D-galactopyranoside (**15f**): See general procedure B. The resulting residue was purified by flash column chromatography (4:1 & 3:1 petroleum ether-EtOAc) to afford **15f** as a white solid (65%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.51 (s, 1 H, Ar-H), 7.51 (d, 1 H, J 8.8 Hz, Ar-H), 7.23 (d, 1 H, Ar-H), 5.84 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.36 (dd, 1H. $J_{3,4}$ 2.8 Hz, $J_{2,3}$ 10.8 Hz, H-3), 4.87 (d, 1 H, H-4), 4.22 (dd, 1 H, $J_{5,6a}$ 2.0 Hz, $J_{6a,6b}$ 13.2 Hz, H-6a), 4.17 (dd, 1 H, H-2), 4.05 (dd, 1 H, $J_{5,6b}$ 0.8 Hz, H-6b), 3.93 (s, 3 H, OCH_3), 3.88 (m, 1 H, H-5), 2.20 (s, 3 H, CH_3CO), 1.06 (s, 9 H), 1.02 (s, 9 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.4, 163.6, 156.9, 155.6, 152.6, 148.8, 128.6, 116.1, 114.0, 112.1, 111.6, 97.7, 71.4, 69.8, 69.0, 66.6, 56.8, 53.1, 27.7, 27.3, 23.3, 21.0, 20.8. ESI-HRMS: Calcd for $[C_{27}H_{34}ClN_3O_{10}Si + Na]^+$: 646.1600; Found m/z: 646.1602.

3-(Carboxyl methyl ester)-umbelliferyl 2-azido-2-deoxy-3-*O*-acetyl-4,6-*O*-di-*tert*-butylsilylene-α-D-galactopyranoside (**15g**): See general procedure B. The resulting residue was purified by flash column chromatography (3:1 petroleum ether-EtOAc) to afford **15g** as a white solid (71%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.54 (s, 1 H, Ar-H), 7.56 (d, 1 H, J 8.8 Hz, Ar-H), 7.13 (d, 1 H, J 2.4 Hz, Ar-H), 7.06 (dd, 1 H, Ar-H), 5.73 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.31 (dd, 1H. $J_{3,4}$ 2.8 Hz, $J_{2,3}$ 10.8 Hz, H-3), 4.79 (d, 1 H, H-4), 4.21 (dd, 1 H, $J_{5,6a}$ 2.0 Hz, $J_{6a,6b}$ 12.8 Hz, H-6a), 4.11~4.07 (m, 2 H, H-2 & H-6b), 3.94 (s, 3 H, OCH_3), 3.82 (m, 1 H, H-5), 2.20 (s, 3 H, CH_3CO), 1.07 (s, 9 H), 1.02 (s, 9 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.6, 164.0, 161.4, 157.2, 156.8, 149.2, 131.2, 115.3, 114.8, 113.3, 103.8, 97.5, 71.2, 70.0, 68.7, 66.7, 56.3, 53.0, 27.6, 27.3, 23.4, 21.0, 20.9. ESI-HRMS: Calcd for $[C_{27}H_{35}N_3O_{10}Si + Na]^+$: 612.1989; Found m/z: 612.1992.

4-Trifluoromethyl-umbelliferyl 2-azido-2-deoxy-3-*O*-acetyl-4,6-*O*-di-*tert*-butylsilylene-α-D-galactopyranoside (**15h**): See general procedure B. The resulting residue was purified by flash column chromatography (15:1 petroleum ether-EtOAc) to afford **15h** as a white solid (69%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.68 (dd, 1 H, Ar-H), 7.18 (d, 1 H, J 2.4 Hz, Ar-H), 7.10 (dd, 1 H, J 8.8 Hz, Ar-H), 6.68 (s, 1 H, Ar-H), 5.74 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.33 (dd, 1H. $J_{3,4}$ 2.8 Hz, $J_{2,3}$ 11.2 Hz, H-3), 4.80 (d, 1 H, H-4), 4.22 (dd, 1 H, $J_{5,6a}$ 2.0 Hz, $J_{6a,6b}$ 12.8 Hz, H-6a), 4.09 (m, 2 H, H-2 & H-6b), 3,83 (m, 1 H, H-5), 2.21 (s, 3 H, CH_3CO), 1.08 (s, 9 H), 1.03 (s, 9 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.6, 159.9, 159.0, 156.1, 141.5 (q, $J_{C,F}$ 33.0 Hz), 126.8 (d, $J_{C,F}$ 2.0 Hz), 121.6 (q, $J_{C,F}$ 274.0 Hz), 114.7, 113.7 (q, $J_{C,F}$ 6.0 Hz), 108.9, 104.8, 97.5, 71.2, 70.0, 68.7, 66.7, 56.6, 27.7, 27.3, 23.4, 21.0, 20.9. ^{19}F-NMR ($CDCl_3$, 282 MHz): δ -65.2. ESI-HRMS: Calcd for $[C_{26}H_{32}F_3N_3O_8Si + Na]^+$: 622.1808; Found m/z: 622.1812.

6,8-Difluoro-4-trifluoromethylumbelliferyl 2-azido-2-deoxy-3-*O*-acetyl-4,6-*O*-di-*tert*-butylsilylene-α-D-galactopyranoside (**15i**): See general procedure B. The resulting residue was purified by flash column chromatography (15:1 petroleum ether-EtOAc) to afford **15i** as a white solid (45%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.32 (d, 1 H, $J_{H,F}$ 10.4 Hz, Ar-H), 6.85 (s, 1 H, Ar-H), 5.73 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 5.32 (dd, 1H. $J_{3,4}$ 2.8 Hz, $J_{2,3}$ 11.2 Hz, H-3), 4.85 (d, 1 H, H-4), 4.30 (d, 1 H, $J_{6a,6b}$ 13.2 Hz, H-6a), 4.25 (brs, 1 H, H-5), 4.13 (dd, 1 H, H-2), 4.09 (d, 1 H, H-6b), 2.21 (s, 3 H, CH_3CO), 1.03 (s, 9 H), 1.01 (s, 9 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.5, 156.8, 152.4 (dd, $J_{C,F}$ 4.0 Hz & 246.0 Hz), 144.4 (dd, $J_{C,F}$ 5.0 Hz & 254.0 Hz), 140.4 (m, 2 C), 136.7 (dd, $J_{C,F}$ 12.0 Hz & 16.0 Hz), 121.1 (q, $J_{C,F}$ 274.0 Hz), 116.9 (q, $J_{C,F}$ 6.0 Hz), 110.2 (d, $J_{C,F}$ 11.0 Hz), 107.2 (d, $J_{C,F}$ 23.0 Hz), 102.3, 70.9, 70.1, 69.4, 66.6, 57.0, 27.7, 27.3, 23.4, 21.0, 20.8. ^{19}F-NMR ($CDCl_3$, 282 MHz): δ -

65.5, 129.4 (d, J 2.5 Hz), -143.7 (d). ESI-HRMS: Calcd for $[C_{26}H_{30}F_5N_3O_8Si + Na]^+$: 658.1620; Found m/z: 658.1623.



Scheme S5. Reagents and conditions: i). THF, AcOH, TBAF, rt; Pyridine, Ac_2O, rt, **20c**: 65%; **20g**: 66%. ii). THF, H_2O, Ph_3P, silica gel, 50°C; Pyridine, Ac_2O, rt; **21c**: 72%; **21g**: 67%. iii). MeOH, Na, rt; THF, H_2O, LiOH, 0°C; **2**: 69%; **22g**: 71%.

Having demonstrated the utility of our synthetic approach for α-GalNAc glycosylation of coumarin derivatives, we carried two of the glycosides through the deprotection steps to assess the suitability of this approach and to provide fully deprotected substrate for testing within droplets (Scheme S5).

Treatment of the glycosides **15c** and **15g** with tetrabutylammonium fluoride removed the silyl protecting group,[16,25] after which the free hydroxyls were directly acetylated using acetic anhydride in pyridine to yield the per-O-acetylated azido galactosides (**20c** and **20g**). Reduction of the azide with triphenyl phosphine and direct acetylation gave the 2-acetamido sugars (**21c** and **21g**).[26–28] Final deprotection was achieved by use of sodium methoxide in dry methanol to remove the O-acetates, then hydrolysis with 1 M LiOH in water at 0°C to remove the methyl ester.[29] When hydrolysis was complete the solvent was evaporated under reduced pressure, then the residue was re-dissolved in water, Amberlite resin was added and stirred for 0.5 h. After filtration, the filtrate was loaded on a Sep-Pak, eluted with water and water/acetonitrile then concentrated by evaporating under reduced pressure. The solid obtained was redissolved in water and lyophilized to afford **2** and **22g** as solid products.

3-(Carboxyl methyl ester)-8-fluoroumbelliferyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranoside (**20c**): To a solution of **15c** (230 mg, 0.379 mmol) in anhydrous THF (6 mL) were added AcOH (114 μL) and TBAF (1 M in THF, 1.17 mL), and the reaction mixture was stirred overnight at room temperature under N_2. After evaporation, the resulting residue was purified by flash column chromatography (2:3 DCM-EtOAc) to afford the intermediate diol. To the diol were added pyridine (3

mL) and Ac_2O (2 mL), and the reaction mixture was stirred overnight at room temperature. Pyridine was removed by evaporation. To the resulting residue was added water (10 mL) and the mixture was stirred for 0.5 h at room temperature to quench the reaction, and then extracted with EtOAc (20 mL x 3). The organic phase was washed with 1 M HCl (30 mL), saturated $NaHCO_3$ (2 x 30 mL) and brine (30 mL), dried over $MgSO_4$, filtered and concentrated. The resulting residue was purified by flash column chromatography (3:2 & 1:1 petroleum ether-EtOAc) to afford the product **20c** as a slightly yellow foam (65%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.51 (s, 1 H, Ar-H), 7.37 (d, 1 H, Ar-H), 7.19 (dd, 1 H, J 6.4 Hz & 8.8 Hz, Ar-H), 5.73 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.52 (m, 2H, H-3 & H-4), 4.38 (m, 1 H, H-5), 4.09 (dd, 1 H, $J_{5,6a}$ 6.4 Hz, $J_{6a,6b}$ 11.2 Hz, H-6a), 4.04 (dd, 1 H, $J_{5,6b}$ 6.8 Hz, H-6b), 3.96 (dd, 1 H, $J_{2,3}$ 11.4 Hz, H-2), 3.91 (s, 3 H, OCH_3), 2.15 (s, 3 H, CH_3CO), 2.07 (s, 3 H, CH_3CO), 1.93 (s, 3 H, CH_3CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.3, 170.0, 169.8, 163.5, 155.2, 148.7 (d, $J_{C,F}$ 8.0 Hz), 148.6 (d, $J_{C,F}$ 2.0 Hz), 144.6 (d, $J_{C,F}$ 9.0 Hz), 140.3 (d, $J_{C,F}$ 253.0 Hz), 124.9 (d, $J_{C,F}$ 4.0 Hz), 116.7, 114.9, 114.3, 98.6, 68.6, 68.3, 67.2, 61.4, 57.3, 53.1, 20.71, 20.68, 20.66. ^{19}F-NMR ($CDCl_3$, 282 MHz): δ -150.7 (s). ESI-HRMS: Calcd for $[C_{23}H_{22}FN_3O_{12}+Na]^+$: 574.1085; Found m/z: 574.1085.

3-(Carboxyl methyl ester)-umbelliferyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranoside (**20g**): See the preparation of compound **20c**. The resulting residue was purified by flash column chromatography (3:2 petroleum ether-EtOAc) to afford the product **20g** as a syrup (66%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.52 (s, 1 H, Ar-H), 7.55 (d, 1 H, J 8.4 Hz, Ar-H), 7.10 (d, 1 H, J 2.0 Hz, Ar-H), 7.04 (dd, 1 H, Ar-H), 5.72 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 5.52 (m, 2H, H-3 & H-4), 4.24 (m, 1 H, H-5), 4.09 (dd, 1 H, $J_{5,6a}$ 5.6 Hz, $J_{6a,6b}$ 11.6 Hz, H-6a), 4.05 (dd, 1 H, $J_{5,6b}$ 7.2 Hz, H-6b), 3.91 (s, 3 H, OCH_3), 3.90 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-2), 2.16 (s, 3 H, CH_3CO), 2.07 (s, 3 H, CH_3CO), 1.92 (s, 3 H, CH_3CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.3, 170.0, 169.9, 163.9, 161.1, 157.1, 156.7, 149.1, 131.2, 115.5, 115.1, 113.5, 103.9, 97.2, 68.3, 68.2, 67.1, 57.2, 52.9, 20.73, 20.69, 20.6. ESI-HRMS: Calcd for $[C_{23}H_{23}N_3O_{12}+Na]^+$: 556.1179; Found m/z: 556.1183.

3-(Carboxyl methyl ester)-8-fluoroumbelliferyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranoside (**21c**): A mixture of **20c** (149 mg, 0.27 mmol), THF (8 mL), H_2O (2 mL), silica gel (50 mg) and Ph_3P (99 mg, 0.38 mmol, 1.4 equiv) was stirred overnight at 50°C. After completion of the reaction, the reaction mixture was diluted with THF (10 mL), filtered through Celite and washed with DCM (20 mL). The solvents were evaporated and co-evaporated with toluene under reduced pressure, and the resulting residue was dried for 2 h under vacuum. To the residue were added pyridine (3 mL) and Ac_2O (1 mL), and the mixture stirred overnight at room temperature. Pyridine was removed by evaporation. To the resulting residue was added water (10 mL) and the mixture stirred for 0.5 h at room temperature to quench the reaction, extracted with EtOAc (20 mL x 3). The organic phase was washed with 1 M HCl (30 mL), saturated $NaHCO_3$ (2 x 30 mL) and brine (30 mL), dried over $MgSO_4$, filtered and concentrated. The crude material was purified by flash column chromatography (6:1 DCM-acetone) to afford **21c** as a white foam (72%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.52 (s, 1 H, Ar-H), 7.37 (dd, 1 H, J 1.2 Hz & 8.8 Hz, Ar-H), 7.21 (dd, 1 H, Ar-H), 6.25 (d, J 8.8 Hz, NHAc), 5.73 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.50 (d, 1H, H-4), 5.40 (dd, 1 H, $J_{2,3}$ 11.6 Hz, $J_{3,4}$ 3.2 Hz, H-3), 4.78 (ddd, 1 H, H-2), 4.34 (m, 1 H, H-5), 4.13 (dd, 1 H, $J_{5,6a}$ 6.0 Hz, $J_{6a,6b}$ 10.8 Hz, H-6a), 4.06 (dd, 1 H, $J_{5,6b}$ 7.2 Hz, H-6b), 3.92 (s, 3 H, OCH_3), 2.18 (s, 3 H, CH_3CO), 2.07 (s, 3 H, CH_3CO), 2.03 (s, 3 H, CH_3CO), 1.95 (s, 3 H, CH_3CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.0, 170.9, 170.4, 170.3, 163.4, 155.2, 149.2 (d, $J_{C,F}$ 8.0 Hz), 148.7, 144.4 (d, $J_{C,F}$ 9.0 Hz), 140.1 (d, $J_{C,F}$ 251.0 Hz), 125.0 (d, $J_{C,F}$ 5.0 Hz), 116.5, 114.6, 114.1, 98.6, 68.6, 68.3, 67.2, 61.4, 57.3, 53.1, 20.71, 20.68, 20.66. ^{19}F-NMR ($CDCl_3$, 282 MHz): δ -153.0 (s). ESI-HRMS: Calcd for $[C_{25}H_{26}FNO_{13}+Na]^+$: 590.1286; Found m/z: 590.1289.

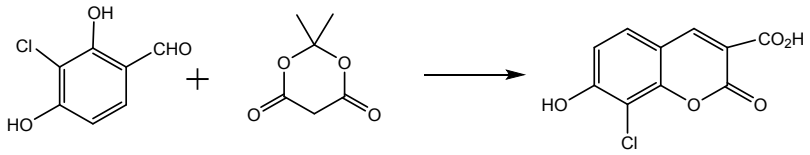
3-(Carboxyl methyl ester)-umbelliferyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranoside (**21g**): See the preparation of compound **21c**. The resulting residue was purified by flash column chromatography (1:4 petroleum ether-EtOAc) to afford **21g** as a white foam (67%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.52 (s, 1 H, Ar-H), 7.55 (d, 1 H, J 8.4 Hz, Ar-H), 7.10 (s, 1 H, Ar-H), 7.05 (d, 1 H, Ar-H), 6.11 (d, J 9.2 Hz, NHAc), 5.74 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 5.44 (brs, 1H, H-4), 5.38 (dd, 1 H, $J_{3,4}$ 2.8 Hz, $J_{2,3}$ 11.2 Hz, H-3), 4.77 (ddd, 1 H, H-2), 4.19 (m, 1 H, H-5), 4.10 (dd, 1 H, $J_{5,6a}$ 6.0 Hz, $J_{6a,6b}$ 11.2 Hz, H-6a), 4.03 (dd, 1 H, $J_{5,6b}$ 7.2 Hz, H-6b), 3.91 (s, 3 H, OCH_3), 2.17 (s, 3 H, CH_3CO), 2.03 (s, 3 H, CH_3CO), 1.98 (s, 3 H, CH_3CO), 1.91 (s, 3 H, CH_3CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.1, 170.6, 170.4, 170.3, 163.8, 161.4, 157.0, 156.8, 149.0, 131.2, 115.4, 114.2, 113.4, 104.4, 96.8, 68.2, 67.8, 67.0, 61.6, 52.9, 47.9, 23.3, 20.9, 20.8, 20.7. ESI-HRMS: Calcd for $[C_{25}H_{27}NO_{13}+H]^+$: 550.1561; Found m/z: 550.1558.

3-Carboxy-8-fluoroumbelliferyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**2**): To a solution of **21c** (110 mg, 0.194 mmol) in anhydrous MeOH (10 mL) was added a catalytic amount of sodium, and the reaction mixture stirred for 3 h at room temperature under N_2. After completion, the reaction mixture was neutralized with Amberlite, filtered and washed with MeOH and concentrated. The methyl ester intermediate was purified by flash column chromatography (9:1 DCM-MeOH) to afford a white solid (76 mg). A solution of the white solid in THF (22 mL) and water (13 mL) was treated with 1 M LiOH (1 M, 0.65 mmol, 3.8 equiv) at 0°C overnight. After evaporation, the resulting solid was redissolved in water (5 mL) and neutralized with amberlite (IR-120 hydrogen form), filtered and concentrated. The crude product was redissolved in water, loaded on C18 Sep-Pak, eluted with water and water-acetonitrile. The acetonitrile was evaporated, and the aqueous solution was lyophilized to afford the desired product **2** (69%). ^{1}H NMR (d_6-DMSO + D_2O, 400 MHz): δ 8.73 (s, 1 H, Ar-H), 7.67 (dd, 1 H, J 1.6 Hz & 7.6 Hz, Ar-H), 7.32 (dd, 1 H, Ar-H), 5.71 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.39 (dd, 1 H, $J_{2,3}$ 11.2 Hz, H-2), 3.88 (dd, 1 H, $J_{3,4}$ 3.2 Hz, H-3), 3.84 (d, 1 H, H-4), 3.74 (m, 1 H, H-5), 3.52 (dd, 1 H, $J_{5,6a}$ 5.6 Hz, $J_{6a,6b}$ 10.8 Hz, H-6a), 3.39 (dd, 1 H, $J_{5,6b}$ 6.4 Hz, H-6b), 1.85 (s, 3 H, CH_3CO). ^{13}C NMR (d_6-DMSO + D_2O, 100 MHz): δ 170.9, 164.3, 156.2, 149.5 (d, $J_{C,F}$ 10.2 Hz), 149.2, 144.1 (d, $J_{C,F}$ 11.5 Hz), 139.7 (d, $J_{C,F}$ 329.5 Hz), 126.2 (d, $J_{C,F}$ 5.3 Hz), 116.4, 114.9, 114.4, 98.5, 73.7, 68.2, 67.3, 60.7, 50.0, 23.1. ^{19}F-NMR (D_2O, 282 MHz): δ -155.5 (s). ESI-HRMS: Calcd for $[C_{18}H_{18}FNO_{10}+Na]^+$: 450.0812; Found m/z: 450.0809.

3-Carboxyumbelliferyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**22g**): See the preparation of compound **2**. The product was obtained as a white solid (71%). ^{1}H NMR (D_2O, 400 MHz): δ 8.33 (s, 1 H, Ar-H), 7.48 (d, 1 H, J 8.8 Hz, Ar-H), 7.03 (dd, 1 H, Ar-H), 6.94 d, 1 H, J 2.0 Hz, Ar-H), 5.67 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 4.40 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-2), 4.16 (dd, 1 H, $J_{3,4}$ 3.2 Hz, H-3), 4.08 (d, 1 H, H-4), 3.99 (m, 1 H, H-5), 3.78 (dd, 1 H, $J_{5,6a}$ 8.0 Hz, $J_{6a,6b}$ 12.0 Hz, H-6a), 3.72 (dd, 1 H, $J_{5,6b}$ 4.4 Hz, H-6b), 2.08 (s, 3 H, CH_3CO). ^{13}C NMR (d_6-DMSO + D_2O, 100 MHz): δ 175.0, 167.4, 161.7, 161.2, 155.8, 149.3, 131.6, 115.28, 115.26, 113.3, 103.5, 96.4, 72.6, 68.5, 67.7, 61.3, 49.7, 22.2. ESI-HRMS: Calcd for $[C_{18}H_{19}NO_{10}+Na]^+$: 432.0907; Found m/z: 432.0900.

Synthesis of fluorophores for Table 1:

Umbelliferone, methylumbelliferone and 4-(trifluoromethyl)-7-hydroxycoumarin (TFMU) were obtained from commercial vendors. All the rest of the fluorophores have been reported and were synthesized based on known procedures with the exception of Jericho Blue and 3-carboxy-8-chloroumbelliferone whose synthesis is reported here. We have reported the synthesis of 6-chloro-4-methylumbelliferone (6-ClMU) before[33]. The synthesis of 6-fluoro-4-methylumbelliferone (6-FMU), 6,8-difluoro-4-methylumbelliferone (6,8-F_2MU, also known as Marina Blue), 6,8-difluoro-4-(trifluoromethyl)-umbelliferone (6,8-F_2TFMU), 3-carboxyumbelliferone (3-CU) and 3-carboxy-6,8-diflouro-umbelliferone (known as Pacific Blue) is reported by Sun *et al*[1] and the synthesis of the 3-carboxy-6-chloro-umbelliferone (6-Cl-3-CU) is reported by Zhao et al[34]. In all cases, the synthesis is a condensation reaction of the appropriately substituted resorcinol. The methyl ester protected version of the carboxy containing fluorophores were prepared as reported for compound 10 above. A typical procedure is as follows:



Scheme S6. Synthesis of 3-carboxy-8-chloroumbelliferone

A mixture of 3-chloro-2,4-dihydroxylbenzaldehyde (1.02 g, 5.93 mmol), Meldrum's acid (0.934 g, 6.49 mmol, 1.1 equiv) and NH_4OAc (0.139 g, 1.8 mmol) in water (28 mL) was stirred for 3 hours at room temperature. More Meldrum's acid (187 mg) and NH_4OAc (28 mg) were added, and then the mixture was stirred overnight at the same condition. Then the reaction mixture was cooled with ice bath and to the mixture was added 2 M HCl (40 mL), and the mixture was stirred for 15 minutes at 0°C and left in a fridge overnight. The resulting solid was filtered and washed with cold water, dried under vacuum to afford 3-carboxy-8-chloroumbelliferone (or 3-carboxy-8-chloro-7-hydroxycoumarin) as a slightly yellow solid (1.382 g, 97%). ^{1}H NMR (d_6-DMSO + D_2O, 400 MHz): δ 8.67 (s, 1 H, Ar-H), 7.70 (d, 1 H, J 8.7 Hz), 7.01 (d, 1 H, Ar-H). ^{13}C NMR (d_6-DMSO + D_2O, 100 MHz): δ 163.9, 159.1, 156.4, 152.4, 149.4, 129.6, 113.3, 113.1, 111.4, 106.1. ESI-MS: Calcd for $[C_{10}H_5ClO_5 - H]^-$: 239.0; Found m/z: 239.0.

Testing the substrates for droplet-based screen

Testing of substrates in water/oil droplets

Water-in-oil droplets were generated using a PDMS droplet generator chip [Darwin Microfluidics] in conjunction with a pressure pump system (OB1 MK3+, Elveflow) using the fluorinated oil HFE-7500 (3M) along with 1% (w/w) EA surfactant [Raindance Technologies]. Droplets (60 μm diameter) were formed at a pressure of 200 mbar (oil) and 150 mbar (aqueous phase). For testing substrate leakage, the aqueous phase consisted of 0.5 X LB medium, 0.5 X PBS (pH 7.0) and 50 μM of the appropriate fluorophore. Droplets without fluorophore were also prepared in the same manner. Upon production, droplets were collected in 1.5 mL microcentrifuge tubes. The droplets containing fluorophores were then mixed 1:1 with droplets prepared without fluorophore. After mixing, an aliquot of the droplets was flowed into a PMMA Fluidic 157 chip [Microfluidic ChipShop] for visualization. The droplets were then imaged using a Zeiss Axio Observer 7 microscope with a UV LED for excitation (EX: 385/30 nm, EM 425/29 nm) and consistent exposure settings across samples. The remaining mixed droplets were left overnight at room temperature before being imaged again under identical conditions. The resulting images of the droplets are shown in Figure S1 and show that the only those fluorophores with a free carboxylic acid group remain inside the droplets (3-CU and JB) while the rest diffuse out and result and almost homogenously fluorescent droplets after overnight incubation.

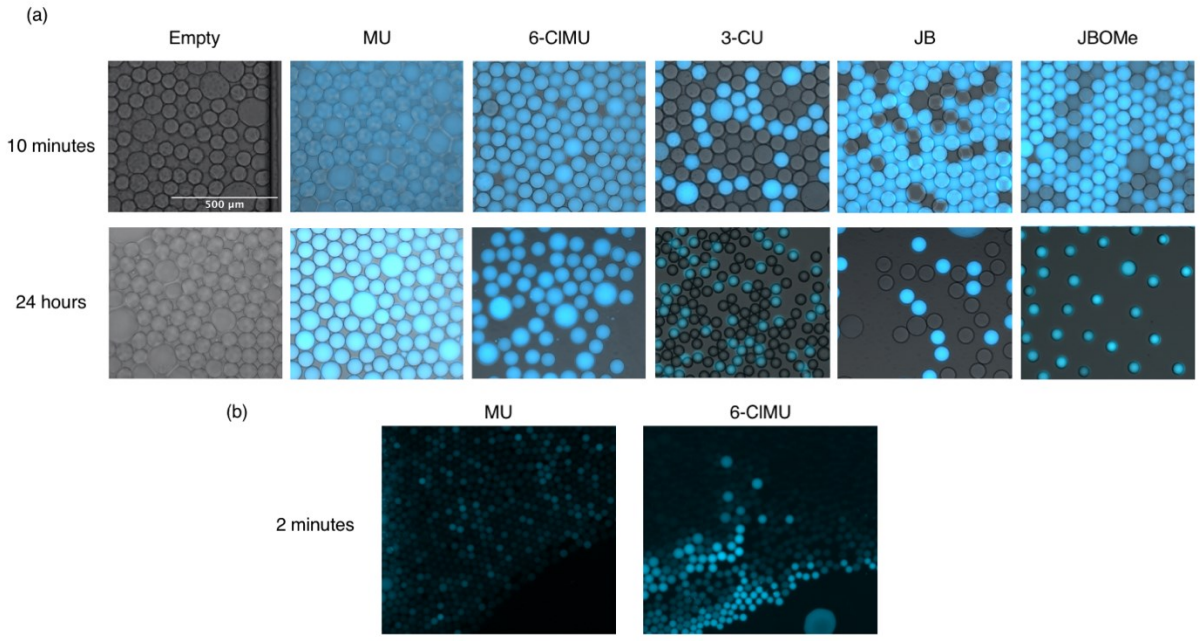


Figure S1. Leakage of different fluorophores between water-in-perfluorinated oil droplets. Aqueous droplets of 0.5X PBS and 0.5X LB +/- 50 μM fluorophore were generated in an oil phase HFE-7500 + 1% EA using a PDMS chip. These fluorophore containing droplets were then mixed with 1:1 with empty droplets (produced under identical conditions but without fluorophore) and an aliquot imaged via fluorescence microscopy (ex: 385/30 nm, em: 425/29 nm). The remaining mixed droplets were then left for 24 hours at room temperature before imaging again. (b) Droplets containing MU and 6-ClMU had intermixed completely before the initial 10-minute time point. To identify leakage, droplets were mixed as before and then imaged immediately over the course of two minutes. Images shown are representative images from two independent trials. Note that the brightness of the images has been adjusted to account for less efficient excitation of the non-carboxylated coumarin derivatives and for consistency.

For the mock screen, the aqueous phase contained 0.5 x PBS buffer, 0.5 x LB medium, 100 µg/ml kanamycin, 100 µM IPTG, 0.25 mg/ml BSA with 50 µM **2** and 6,600,000 cfu/ml of *Escherichia coli.* Under the conditions described the setup produces approximately 8,800,000 droplets per 1 ml water phase, allowing the encapsulation of, on average, 0.75 bacteria per droplet. For this experiment, the *E. coli* sample contained a mixture of *E. coli* cells strain BL21(DE3) expressing the gene for the α-*N*-acetyl-galactosaminidase EmGH109[30] encoded on a pET28a plasmid along with *E coli* cells of the same strain containing the empty pET28a plasmid, in a ratio of 1:1000. After generation, the droplets were collected in a 2 ml plastic vial and incubated overnight at 37°C. These incubated droplets were then transferred to a glass microscope slide and imaged using the same Zeiss Microscope (EX: 385/30 nm, EM 425/29 nm). The results are shown in Figure 1 in the main paper and discussed there.

The mock assay using mineral oil was also performed in a relatively similar manner. However, in this case, a blood group cleaving exo-α-GalNAcase GH36 from *Collinsella sp.* in pUC19[31] was used as our model hit, while cells containing empty pUC19 were used as our background control. In brief, colonies were picked and grown overnight in starter cultures of LB + 100 µg/mL carbenicillin with shaking at 37°C. They were then diluted 1:1000 into LB + carbenicillin and grown at 37°C until OD_{600} reached ~0.6. The concentration of cells was then set 125,000 cells/mL in varying ratios of GH36: empty pUC19 (from 1% positive – 50% positive) in an aqueous phase containing 25% Percoll, 0.25 mg/mL BSA, 50 µM IPTG, 100 µg/mL carbenicillin, 0.5 X PBS (pH 7.0), 0.5 X LB, and 50 µM **2.** Mineral oil with 4% ABIL EM90 (w/w) and 1% Brij L4 (w/w) was prepared for use as oil phase. The aqueous and oil phases were then flowed at 2 mL/h and 5 mL/h respectively into a PDMS chip made according to previous specifications[32] using syringe pumps [Harvard Apparatus]. Droplets produced had a diameter of 200 µm and therefore 230,000 droplets were produced from each mL of aqueous phase. Thus, on average ~0.5 cells were encapsulated in each droplet. The droplets were then collected on glass weigh boats and incubated at 37°C for 48 hours before imaging on a UV lamp. The results are shown in figure S2 and show that, similar to the experiment with fluorinated oil, the fluorescence signal of Jericho Blue is maintained inside the droplets even after long incubation time.

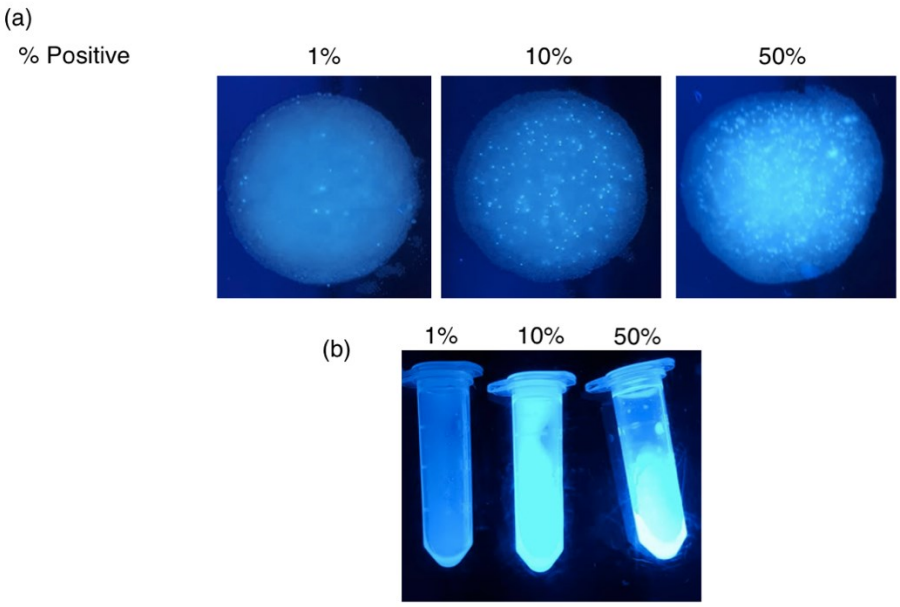


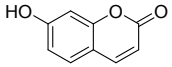
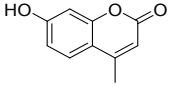
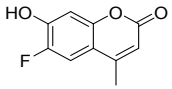
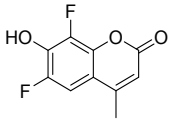
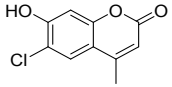
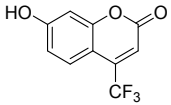
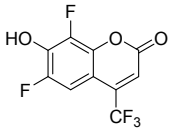
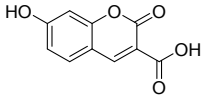
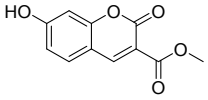
Figure S2. Mock screening for α-GalNAcases in water-in-mineral oil emulsions using Jericho Blue α-D-GalNAc. *E. coli* expressing a GH36 α-GalNAcase were mixed with *E. coli* carrying the equivalent empty pUC19 plasmid at varying ratios. Portions of these mixtures were then packaged into (a) water-in-mineral oil droplets or (b) left in a microcentrifuge tube at 37°C for 48 hours before visualization on a UV lamp table

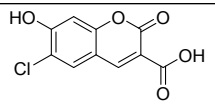
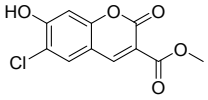
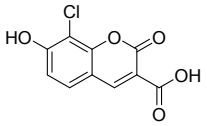
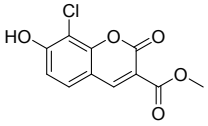
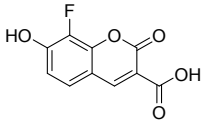
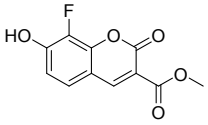
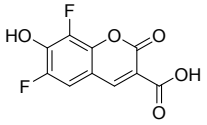
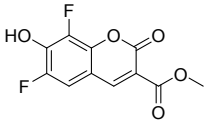
Determining the spectroscopic and chemical properties of coumarin derivatives

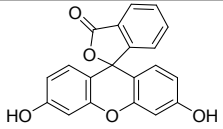
All the measurements were done using a Synergy H1 plate reader [Biotek] at room temperature and in clear flat-bottom 96-well plates [Corning]. To determine the excitation and emission wavelengths, a 100 µM solution of each compound was prepared in phosphate buffer saline (PBS) buffered at pH = 7.4, containing 5% DMSO. To determine the pKa values, 50 µM solutions of each compound was prepared in a range of buffers with various pH values (citrate-phosphate buffer, glycylglycine buffer, and glycine-NaOH buffer), the absorbance of each solution was measured at the λ_{max} of the anionic form of each compound, and the pK_a of each compound was determined by fitting the data to a pH titration curve using GraFit 7.0 (Erithacus software limited; www.erithacus.com/grafit). The graphs for all the compounds can be found below. It should be said that, we believe the major source of contradicting pK_a values found in literature is likely to be using fluorescent readings instead of absorbance. The linear range for fluorescent reads is substantially tighter compared to absorbance and this often means that using fluorescence leads to erroneous pK_a values, unless the measurements are done in very low concentrations of the fluorophores, where the fluorescence signal change in response to fluorophore concentration is in the linear range for all pH values.

The relative molar extinction coefficient and fluorescence brightness values were measured by making standard calibration curves. This was done by preparing a series of dilute solutions of each compound in PBS buffer, pH 7.4 as well as 100 mM glycine-NaOH buffer pH 10, followed by measuring the fluorescence and absorbance of the solutions at the respective maximum wavelengths for each compound. The slope of the line in each case determines the molar fluorescence brightness and molar extinction coefficient respectively. Under the conditions tested, these solutions exhibit a linear response range of up to 50 µM for absorbance and usually less than 5 µM for fluorescence. Note that the reported fluorescence intensities here depend on both the inherent fluorescence as well as the pK_a of 7-hydroxy (where applicable) for each compound. Since the absolute fluorescence brightness values depend on the instrument parameters (such as slit width, gain, etc.), all the data is reported relative to fluorescein, as a common standard with known characteristics and a bright fluorescence.

Table S1: Extended spectroscopic and chemical properties of coumarin derivatives

#	Compound	Acronym	λ_{max} [a] (nm)	λ_{ex} [a] (nm)	λ_{em} [a] (nm)	pK_a [b]	Rel. molar. ex. coef. @pH = 7.4 [c]	Rel. molar fluor. brightness @pH = 7.4 [c]	Rel. molar ex. coef. @pH = 10 [c]	Rel. molar fluor. brightness @pH = 10 [c]
1		**Umbelliferone**	362	365 (360-365)	455 (450-455)	7.56 ± 0.06	0.19	0.082	0.27	0.11
2		**MU**	360	365 (360-365)	450 (450-455)	7.67 ± 0.04	0.17	0.077	0.27	0.13
3		**6-FMU**	360	360 (360-365)	450 (445-450)	6.28 ± 0.02	0.33	0.12	0.34	0.12
4		**6,8-F_2MU**	360	360 (360-365)	450 (450-455)	4.90 ± 0.02	0.33	0.10	0.30	0.10
5		**6-ClMU**	365	365 (365-370)	450 (445-450)	6.14 ± 0.02	0.29	0.14	0.29	0.14
6		**TFMU**	385	390 (390-395)	505 (500-505)	7.14 ± 0.03	0.19	0.049	0.23	0.057
7		**6,8-F_2TFMU**	385	390 (390-395)	505 (500-505)	4.30 ± 0.02	0.22	0.036	0.20	0.033
8		**3-CU**	390	390 (390-395)	450 (450-455)	7.31 ± 0.03	0.30	0.26	0.40	0.32
9		**3-CUOMe**	401	400 (400-405)	450 (445-450)	6.50 ± 0.02	0.53	0.68	0.54	0.70

10		**6-Cl-3-CU**	390	395 (390-395)	450 (450-455)	5.71 ± 0.03	0.37	0.35	0.37	0.34
11		**6-Cl-3-CUOMe**	405	410 (405-410)	450 (445-450)	4.84 ± 0.02	0.52	0.58	0.52	0.57
12		**8-Cl-3-CU**	390	390 (390-395)	450 (450-455)	5.75 ± 0.02	0.38	0.32	0.39	0.31
13		**8-Cl-3-CUOMe**	404	410 (405-410)	450 (450-455)	4.87 ± 0.02	0.50	0.49	0.50	0.47
14		**JB**	390	390 (390-395)	450 (450-455)	6.01 ± 0.02	0.36	0.26	0.36	0.25
15		**JBOMe**	405	405 (405-410)	450 (450-455)	5.19 ± 0.02	0.53	0.45	0.51	0.43
16		**PB**	390	390 (390-395)	450 (450-455)	4.66 ± 0.05	0.35	0.31	0.35	0.29
17		**PBOMe**	405	405 (405-410)	450 (450-455)	3.73 ± 0.03	0.52	0.54	0.50	0.51

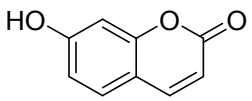
18		**Fluorescein**	491	484 (480-492)	519	6.20 ± 0.03	1	1	1	1

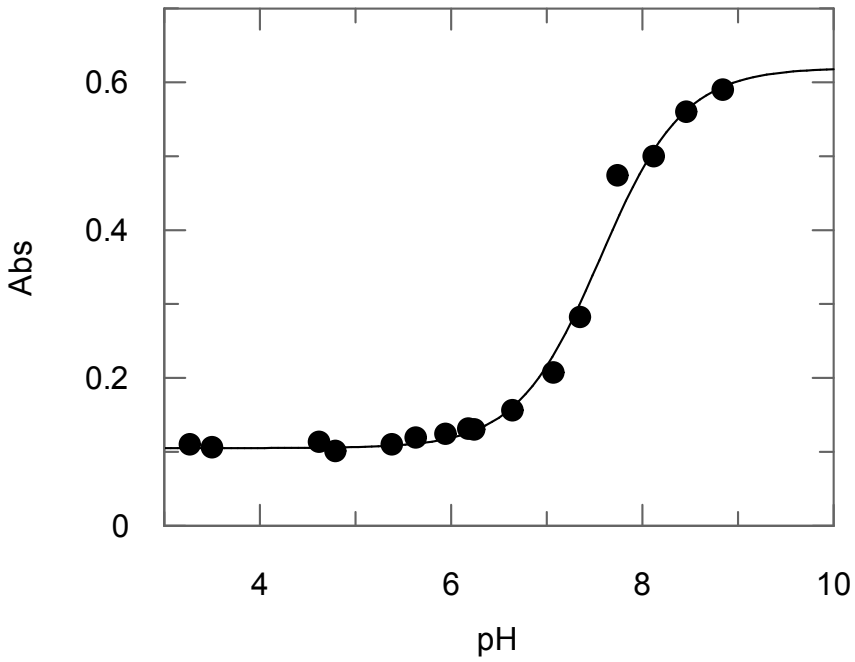
a) Measured for 100 µM solutions of each compounds in PBS buffer containing 5% DMSO. For excitation and emission wavelengths the values reported in parentheses are the range where the fluorescence signal is almost the same, as measured with the plate reader.

b) pKa of the (first) ionizable phenol; calculated based on measured absorbance of 50 µM solutions of the compounds in buffers with various pH values, each containing 5% DMSO.

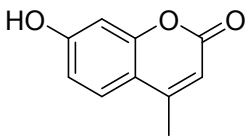
c) The slope of the graph of absorbance/fluorescence vs concentration of compound. Measured at maximum wavelengths of each compound in either PBS buffer or 100 mM glycine buffer. Values are reported relative to Fluorescein. An estimated 10% error should be considered for these values due to different purity grades of compounds from different sources.

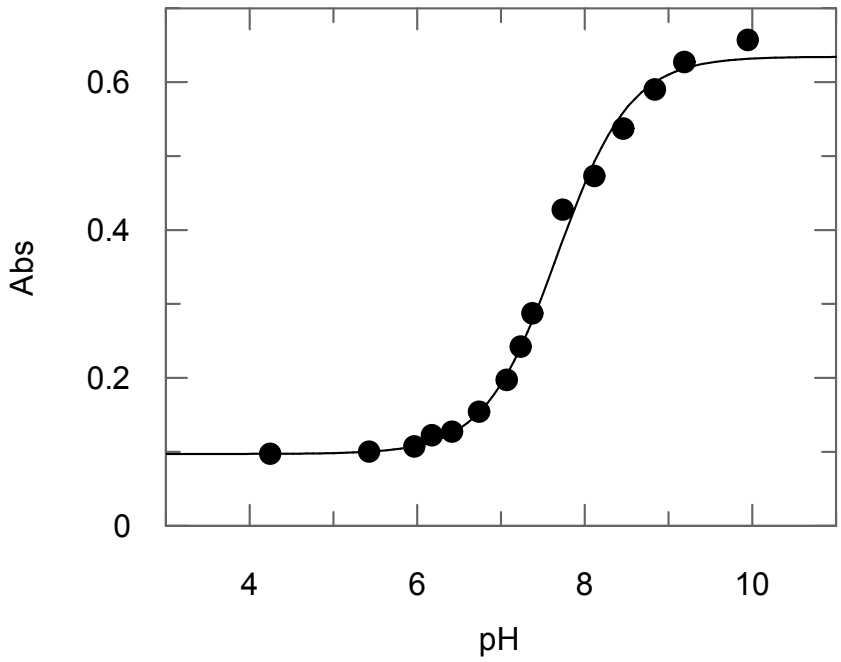
Plots of pKa determination



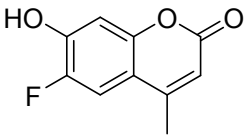


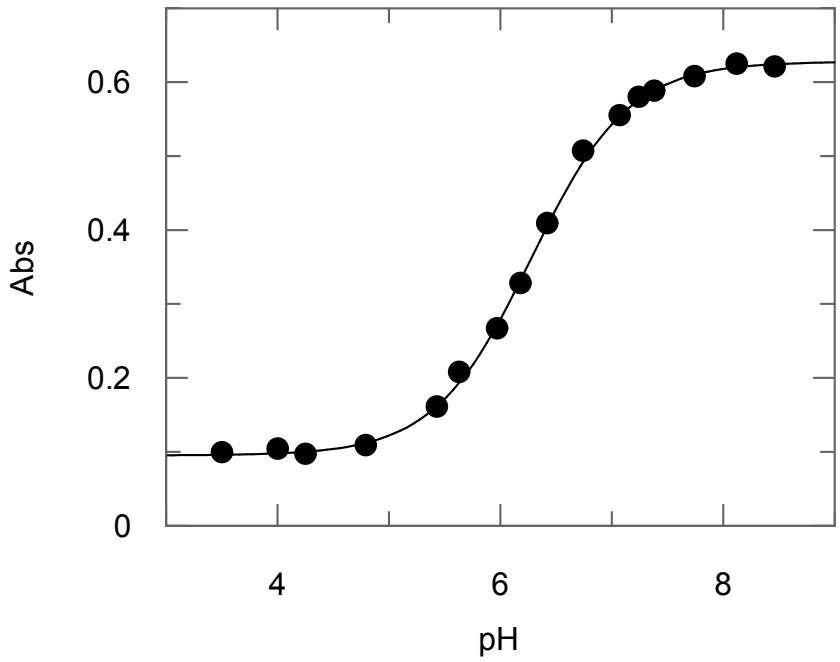
Parameter	Value	Std. Error
pKa	7.5603	0.0580
Limit1	0.1050	0.0066
Limit2	0.6195	0.0167



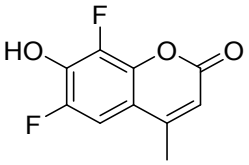


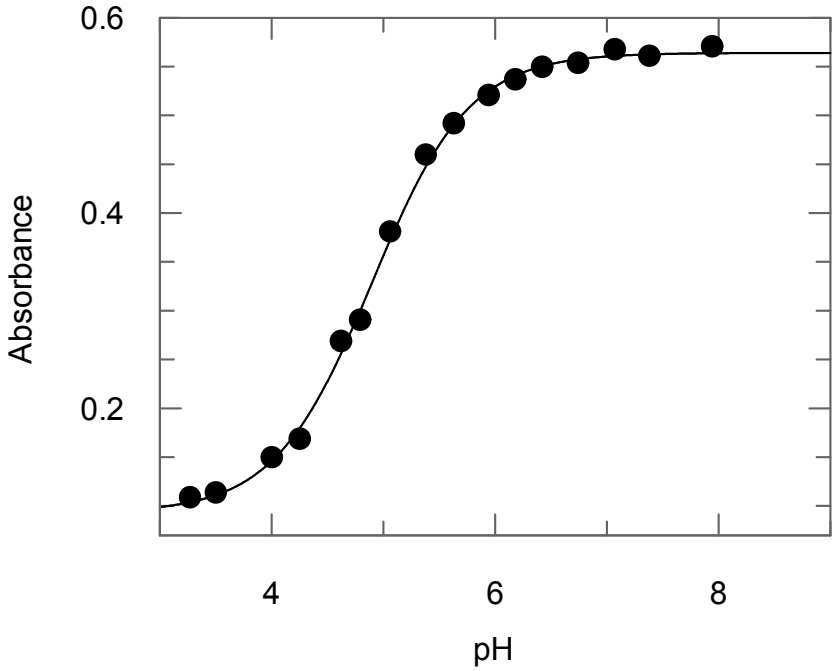
Parameter	Value	Std. Error
pKa	7.6729	0.0421
Limit1	0.0971	0.0078
Limit2	0.6344	0.0100



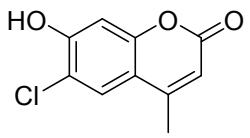


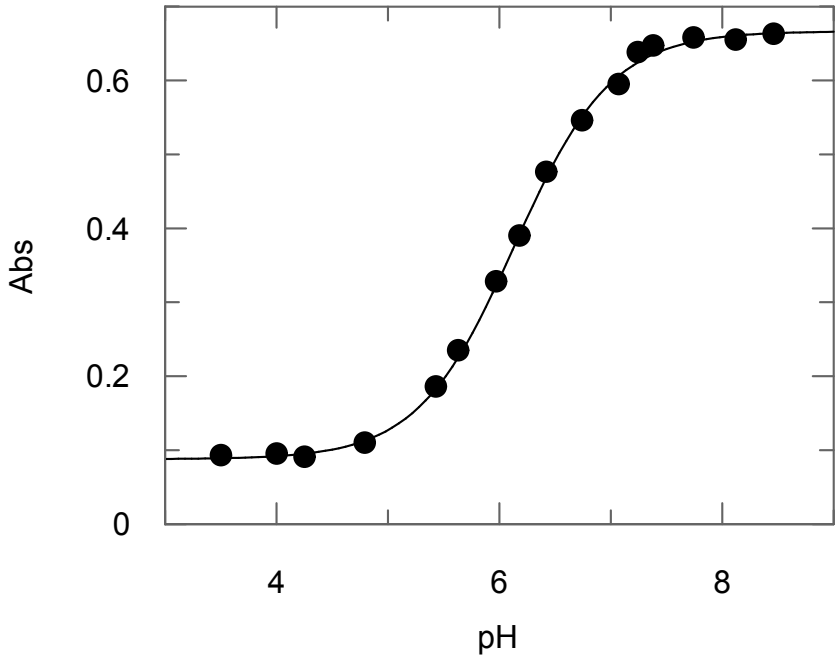
Parameter	Value	Std. Error
pKa	6.2773	0.0152
Limit1	0.0951	0.0032
Limit2	0.6277	0.0032



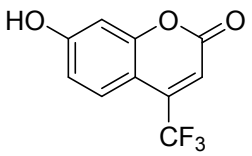


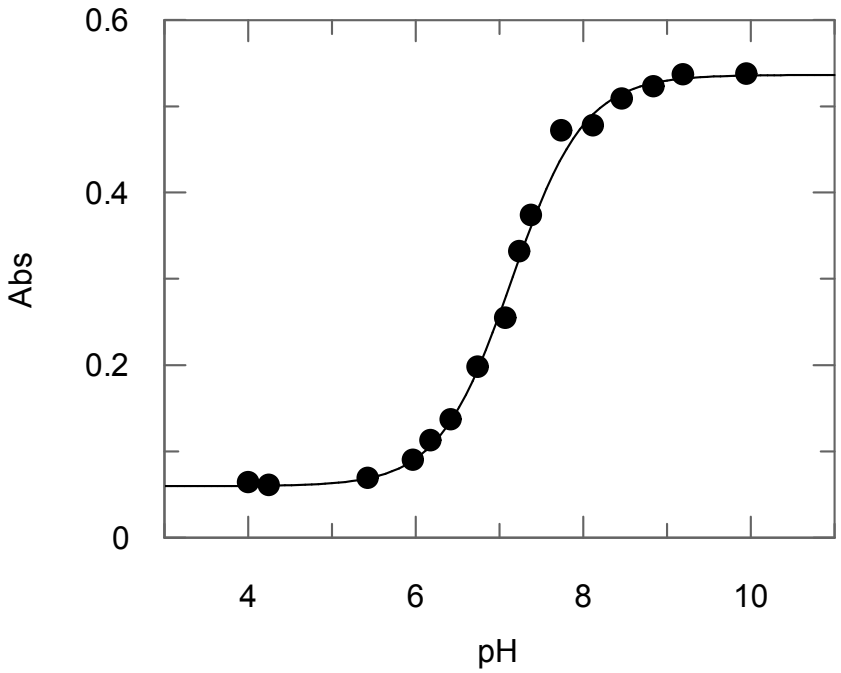
Parameter	Value	Std. Error
pKa	4.8984	0.0210
Limit1	0.0930	0.0050
Limit2	0.5640	0.0030



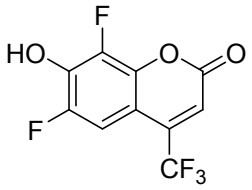


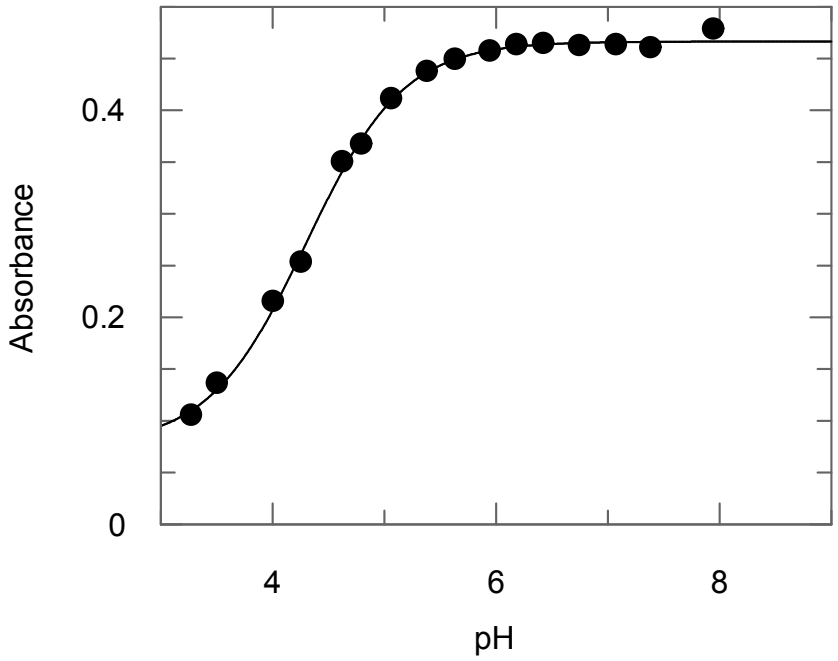
Parameter	Value	Std. Error
pKa	6.1381	0.0156
Limit1	0.0877	0.0037
Limit2	0.6666	0.0034



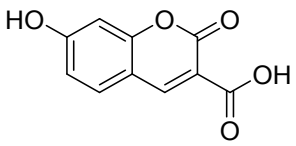


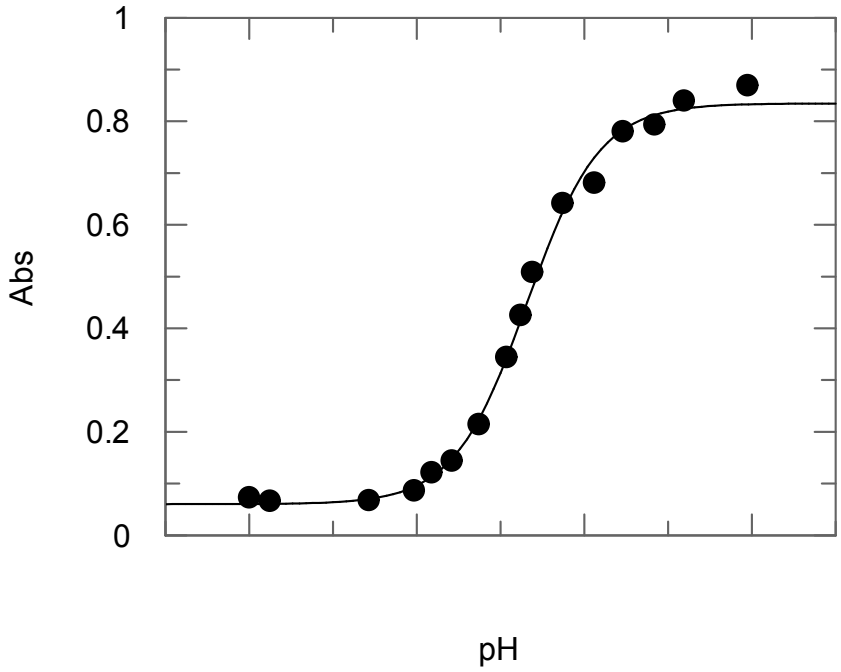
Parameter	Value	Std. Error
pKa	7.1443	0.0311
Limit1	0.0596	0.0060
Limit2	0.5364	0.0060



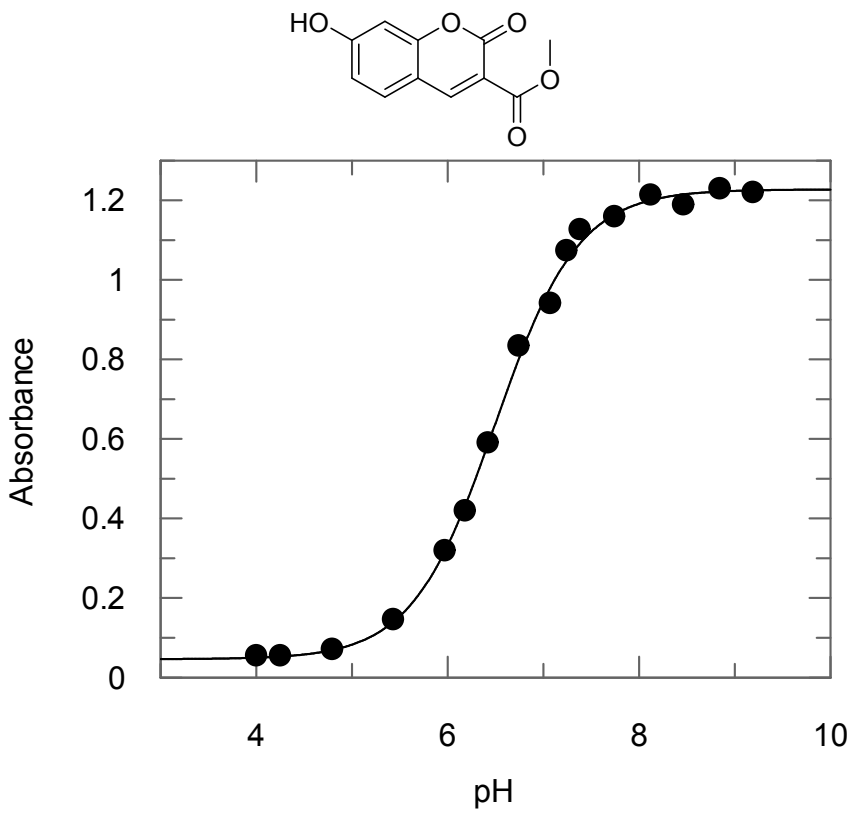


Parameter	Value	Std. Error
pKa	4.2998	0.0246
Limit1	0.0764	0.0062
Limit2	0.4664	0.0021

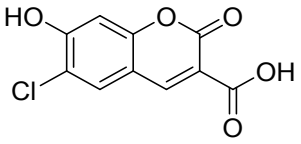


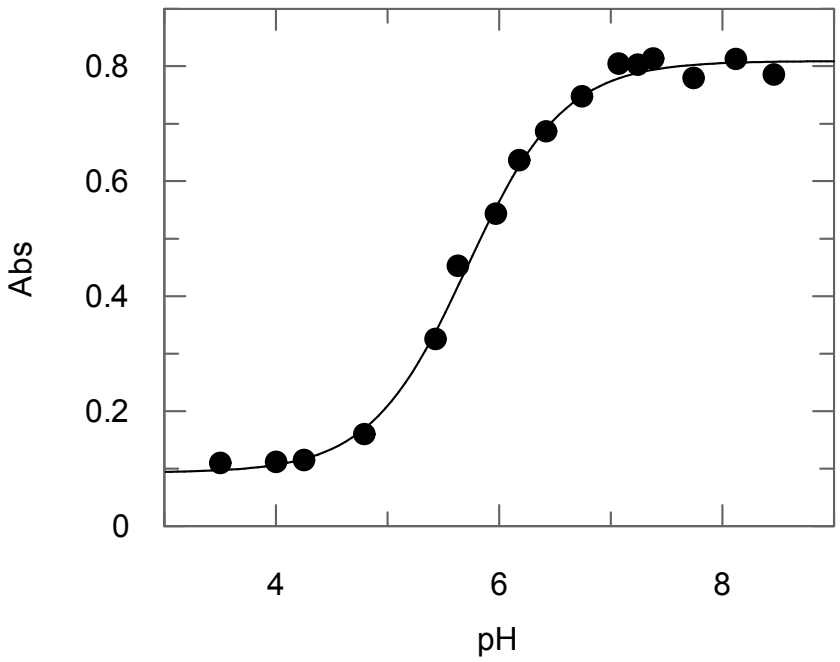


Parameter	Value	Std. Error
pKa	7.3136	0.0332
Limit1	0.0601	0.0098
Limit2	0.8345	0.0110



Parameter	Value	Std. Error
pKa	6.4967	0.0217
Limit1	0.0462	0.0110
Limit2	1.2273	0.0091





Parameter	Value	Std. Error
pKa	5.7140	0.0310
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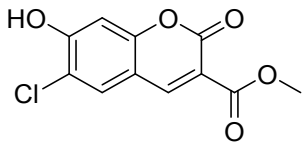
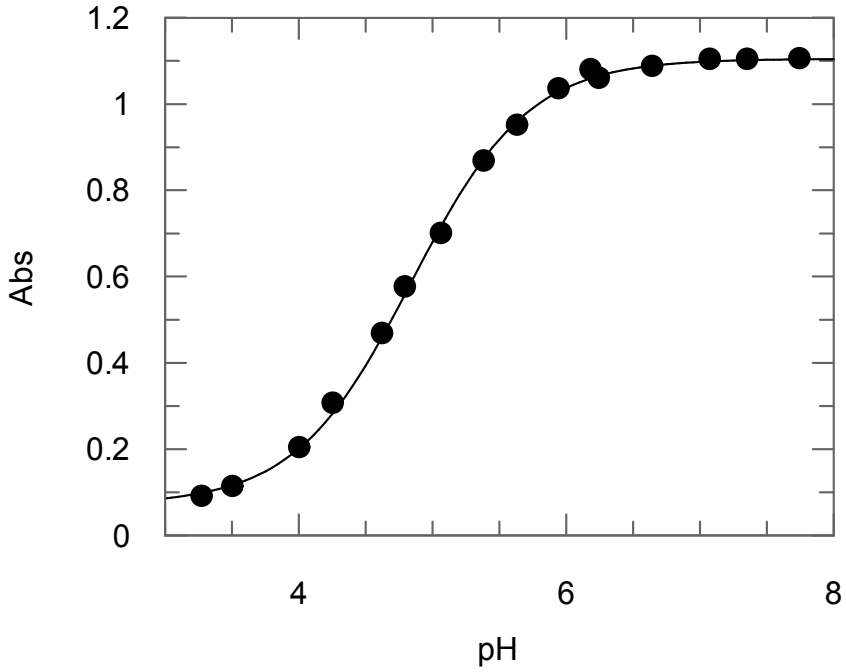
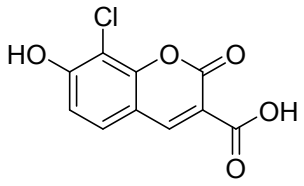
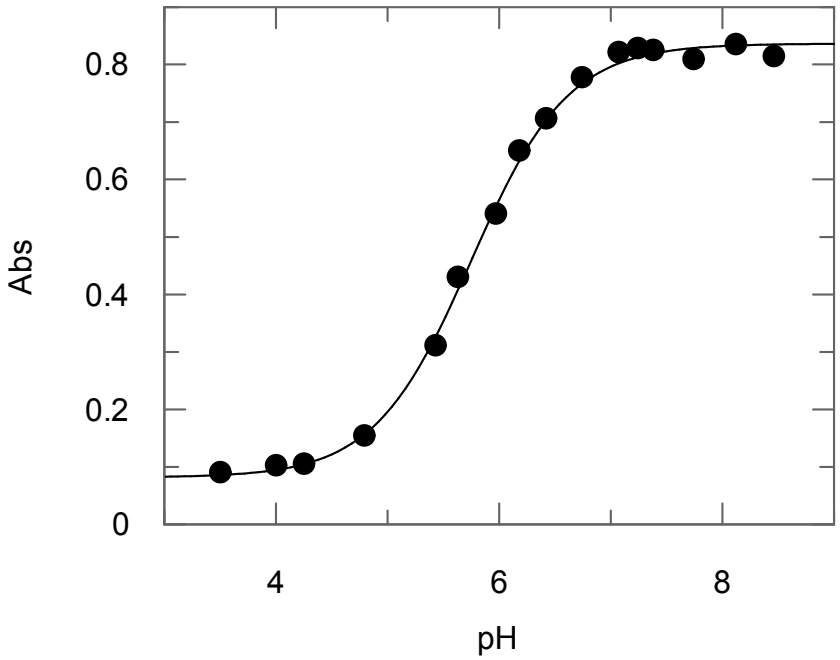
Limit2	0.8090	0.0072



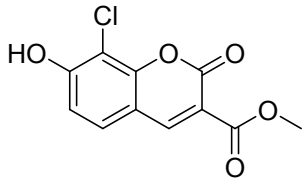


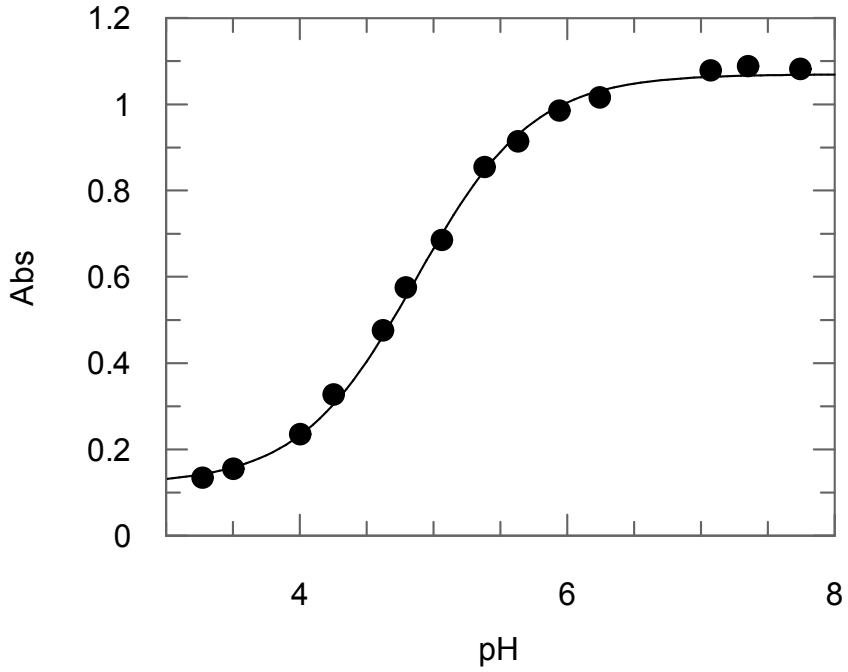
Parameter	Value	Std. Error
pKa	4.8440	0.0153
Limit1	0.0711	0.0081
Limit2	1.1048	0.0047



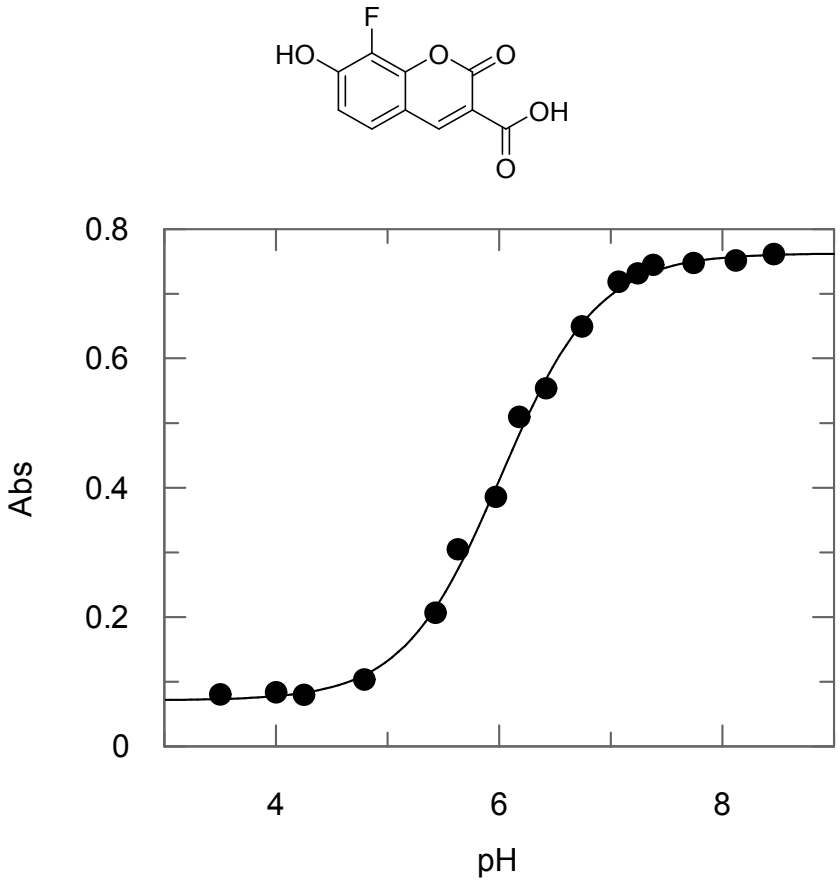


Parameter	Value	Std. Error
pKa	5.7503	0.0241
Limit1	0.0814	0.0081
Limit2	0.8363	0.0060

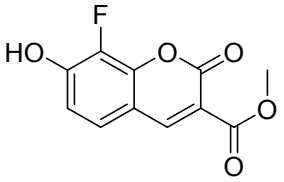


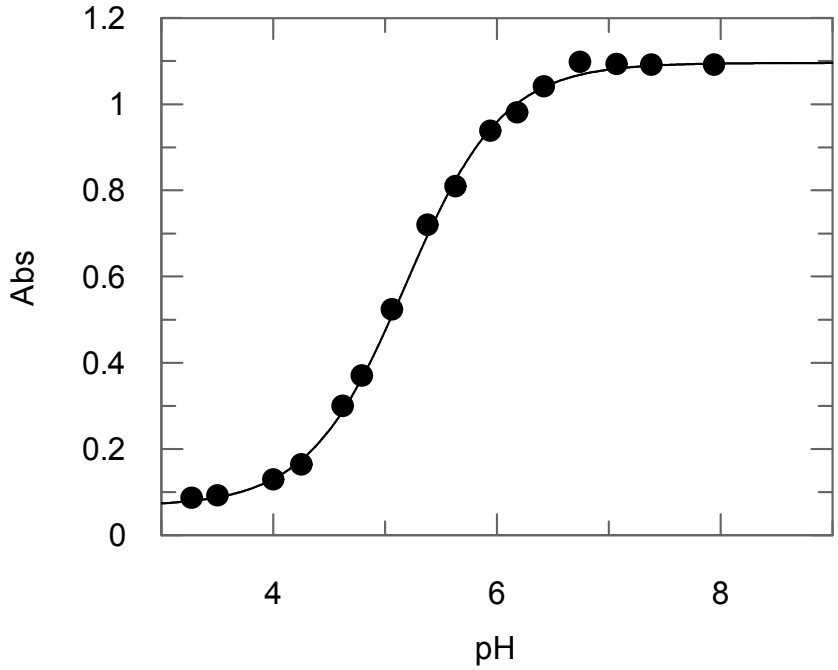


Parameter	Value	Std. Error
pKa	4.8689	0.0227
Limit1	0.1188	0.0108
Limit2	1.0696	0.0074

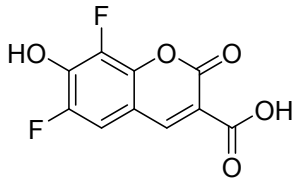


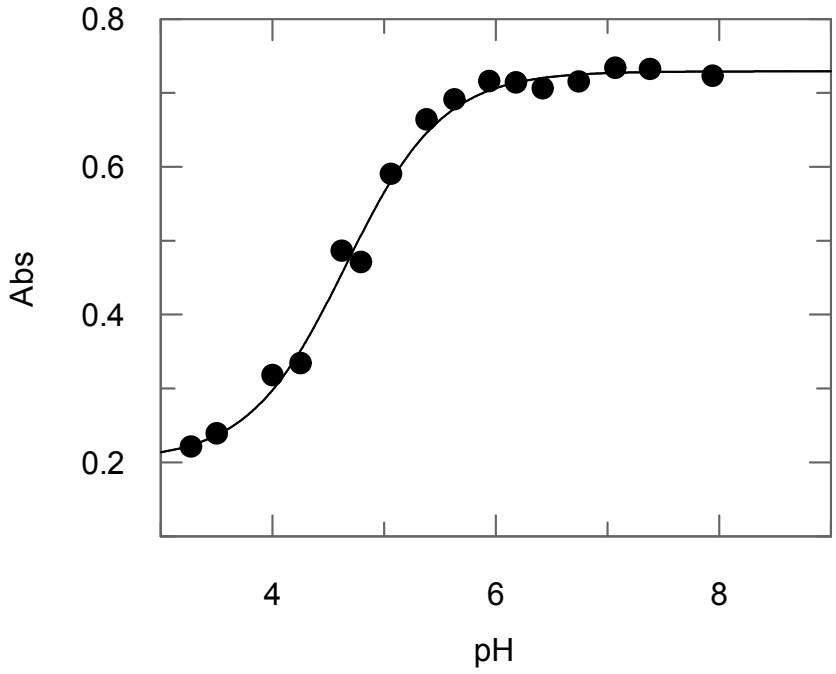
Parameter	Value	Std. Error
pKa	6.0136	0.0246
Limit1	0.0710	0.0072
Limit2	0.7624	0.0062



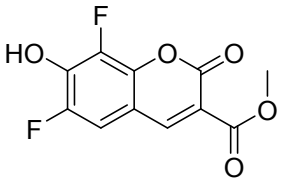


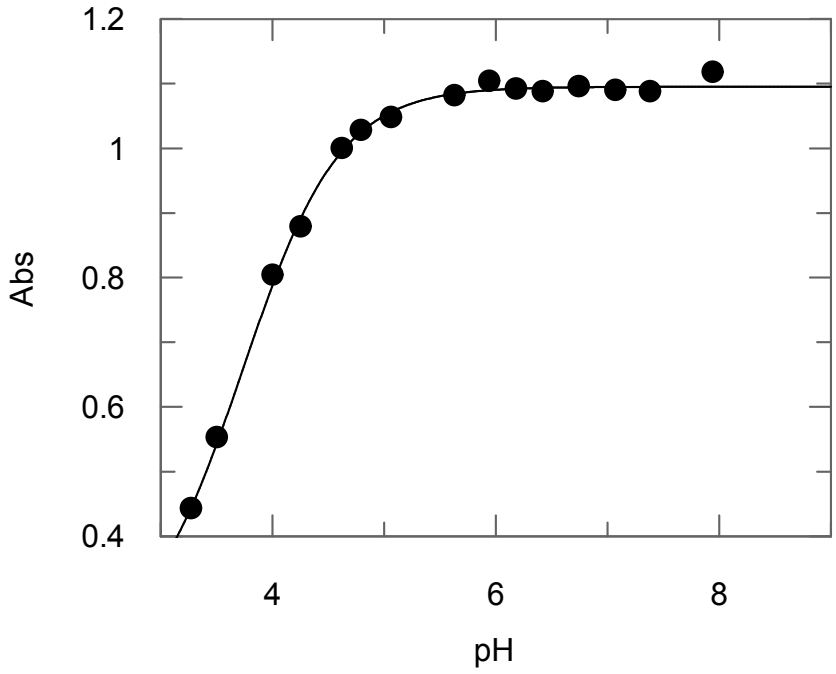
Parameter	Value	Std. Error
pKa	5.1855	0.0179
Limit1	0.0668	0.0082
Limit2	1.0959	0.0062



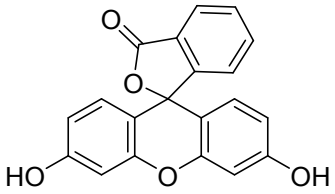


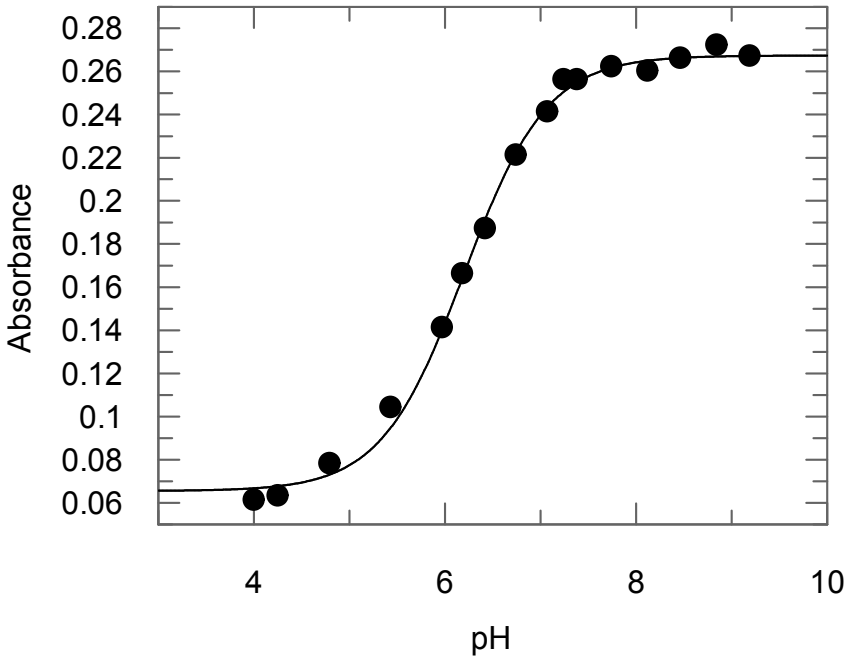
Parameter	Value	Std. Error
pKa	4.6556	0.0459
Limit1	0.2022	0.0136
Limit2	0.7292	0.0066





Parameter	Value	Std. Error
pKa	3.7344	0.0329
Limit1	0.2192	0.0258
Limit2	1.0953	0.0036





Parameter	Value	Std. Error
pKa	6.2024	0.0284
Limit1	0.0654	0.0026
Limit2	0.2674	0.0018

References

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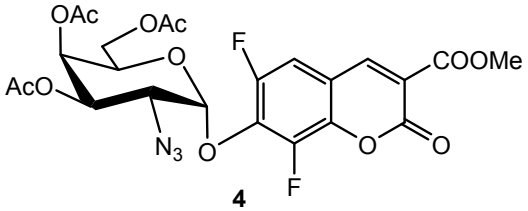
NMR spectra

NMR spectra



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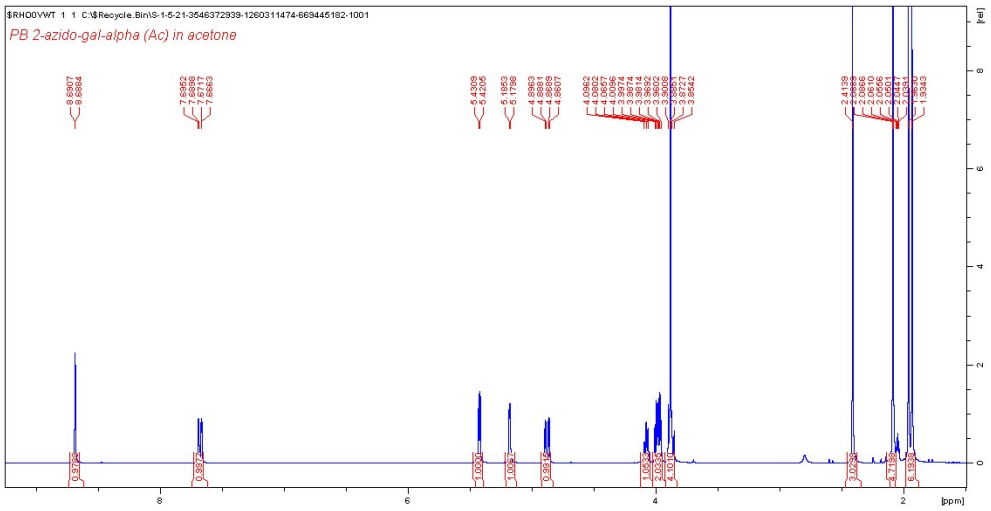

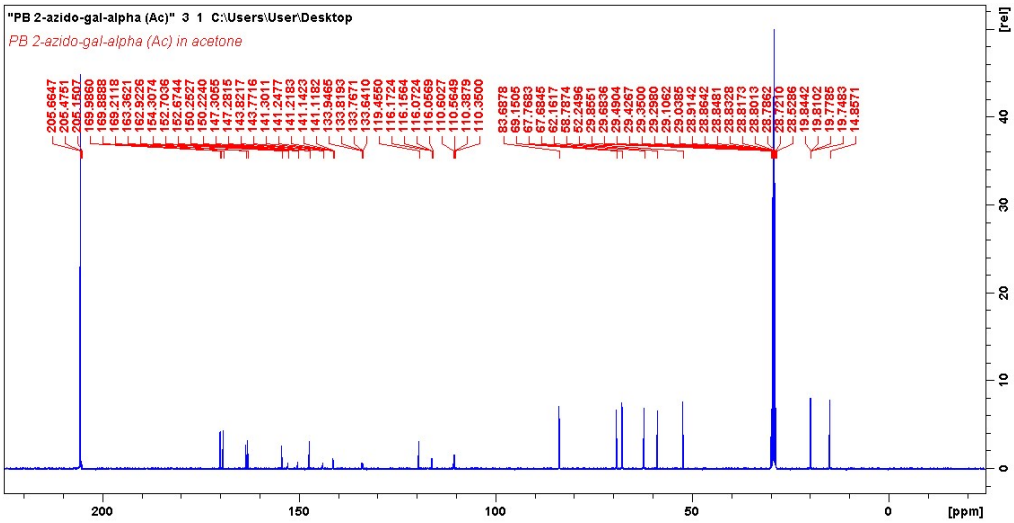

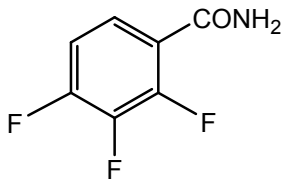
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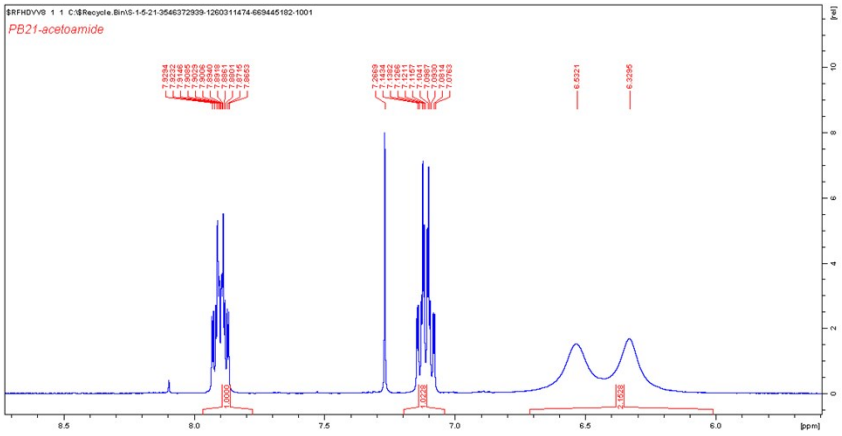
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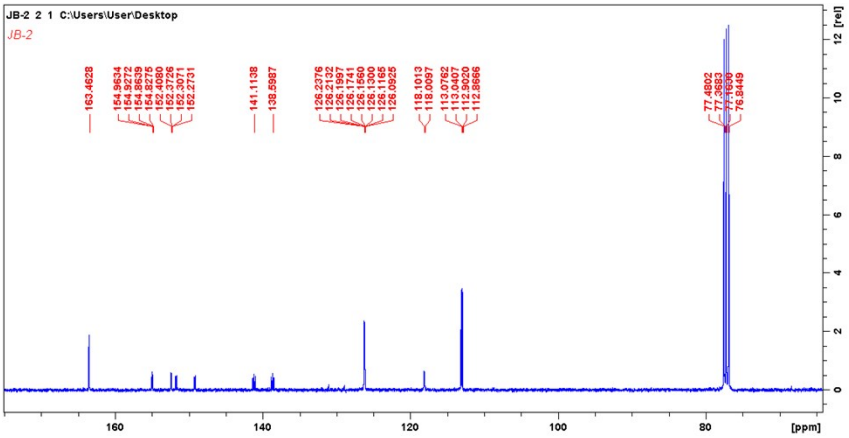
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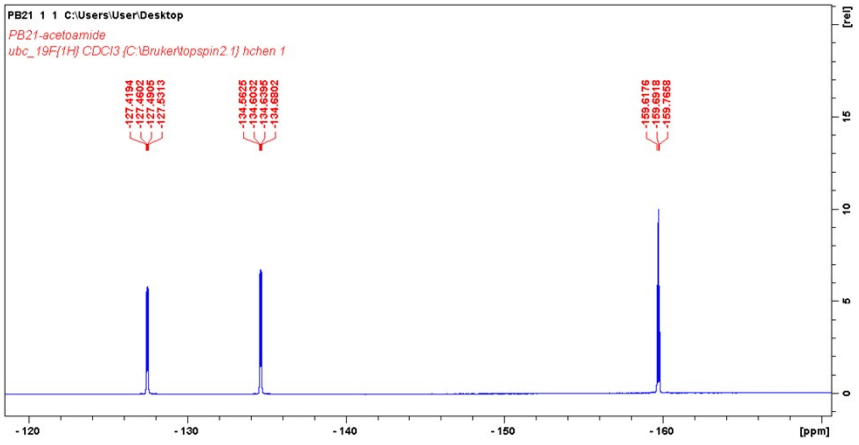
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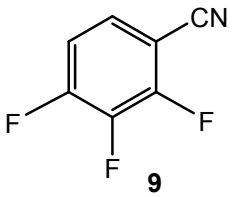
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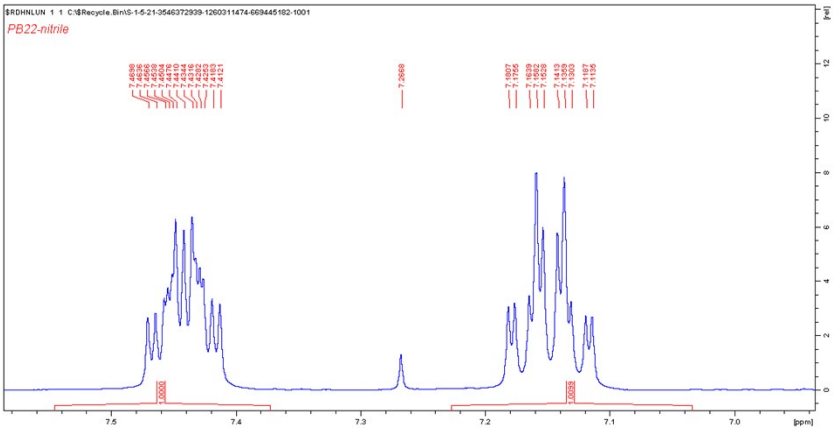
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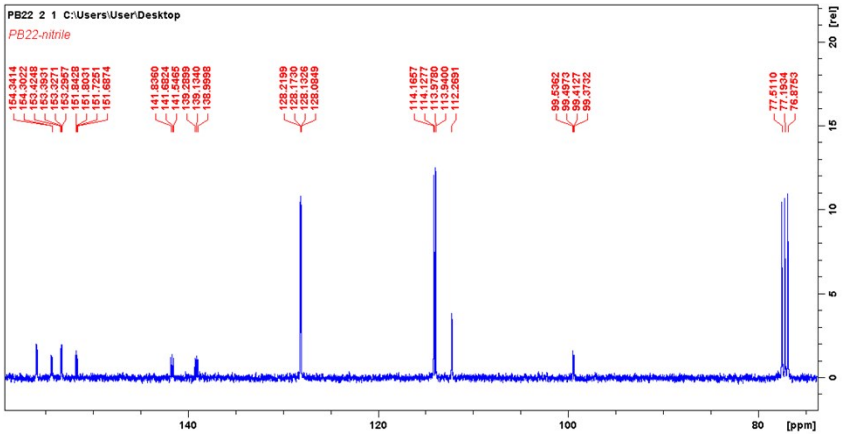
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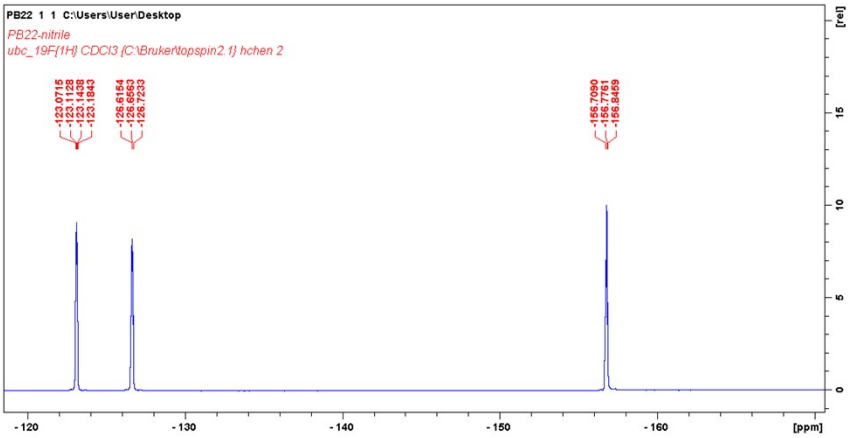
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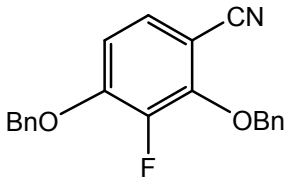
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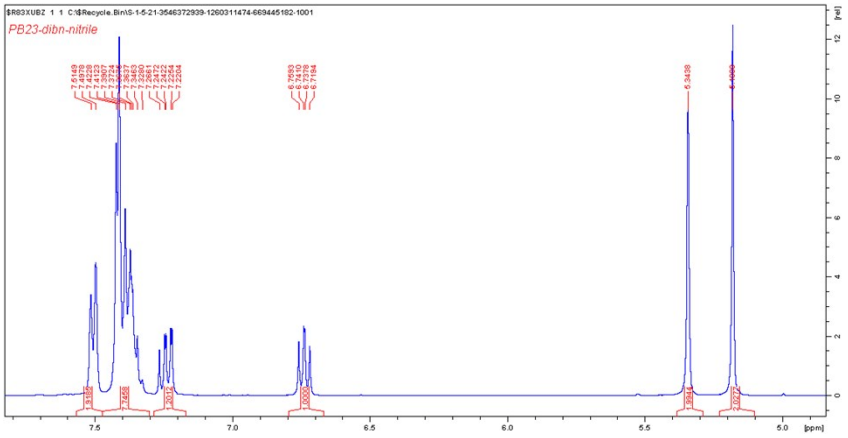
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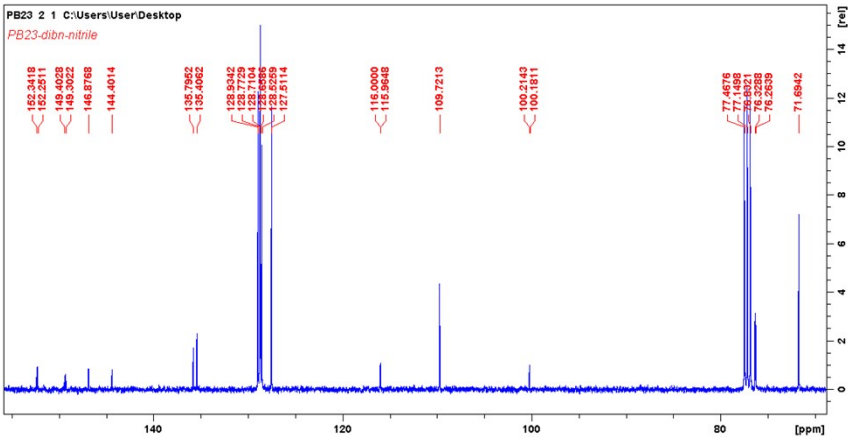
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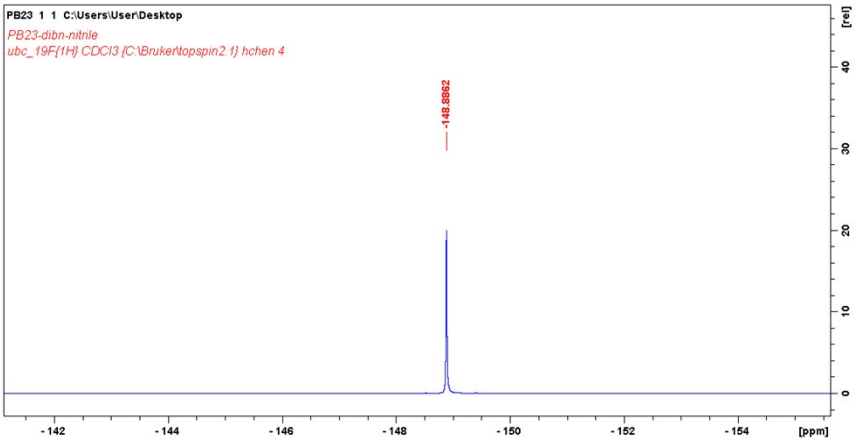
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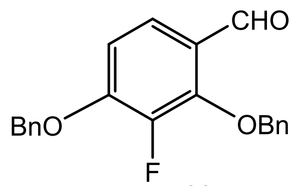
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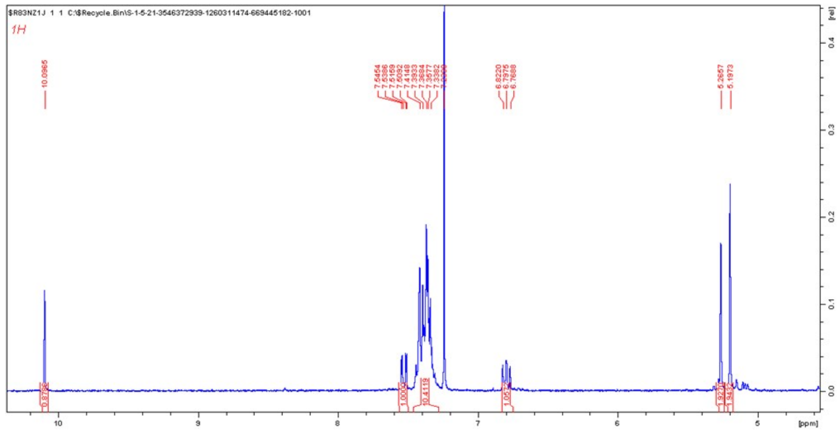
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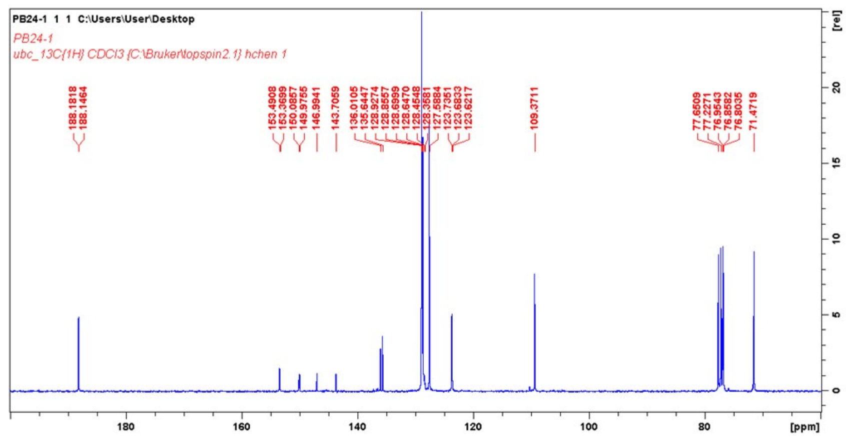
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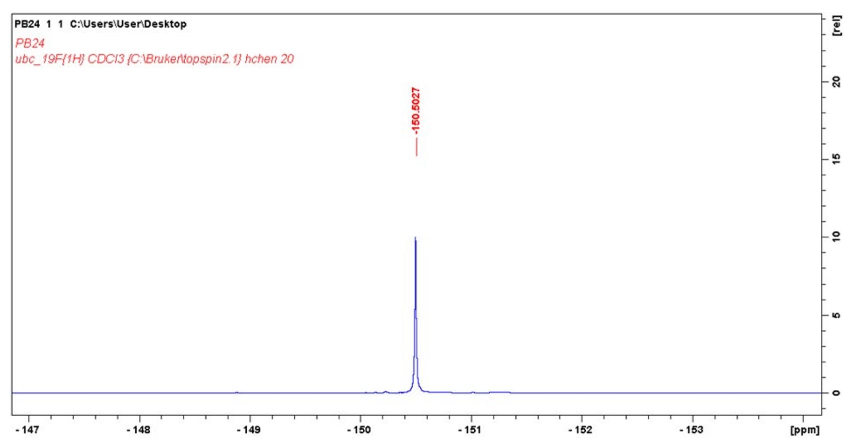
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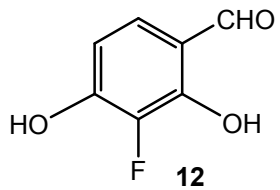
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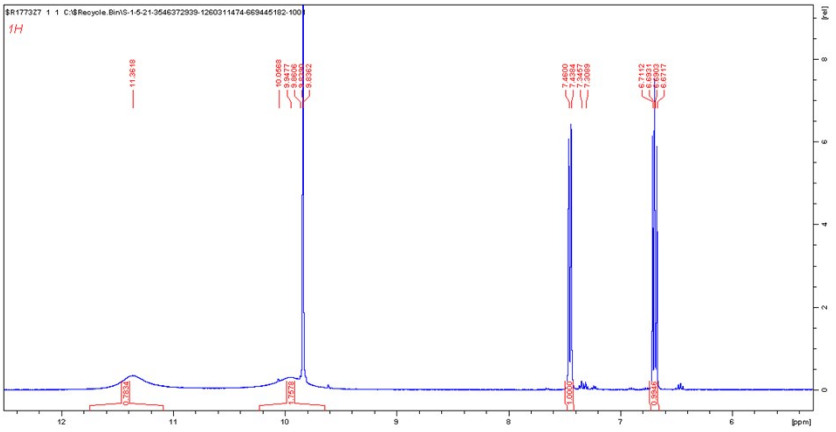
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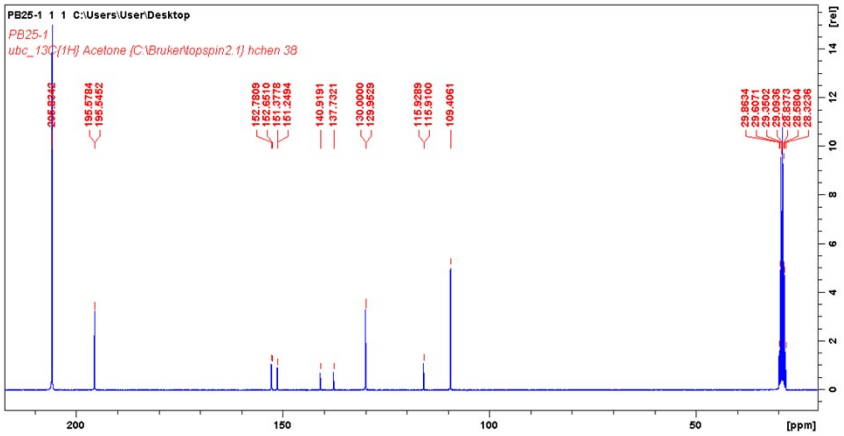
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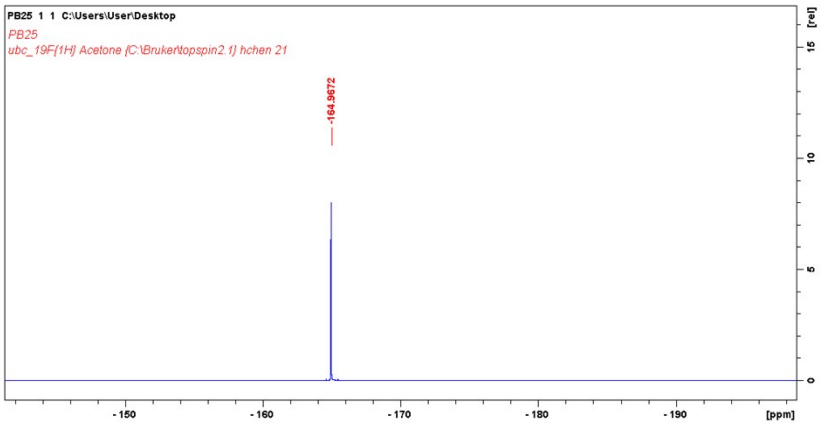
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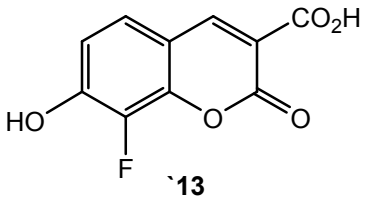
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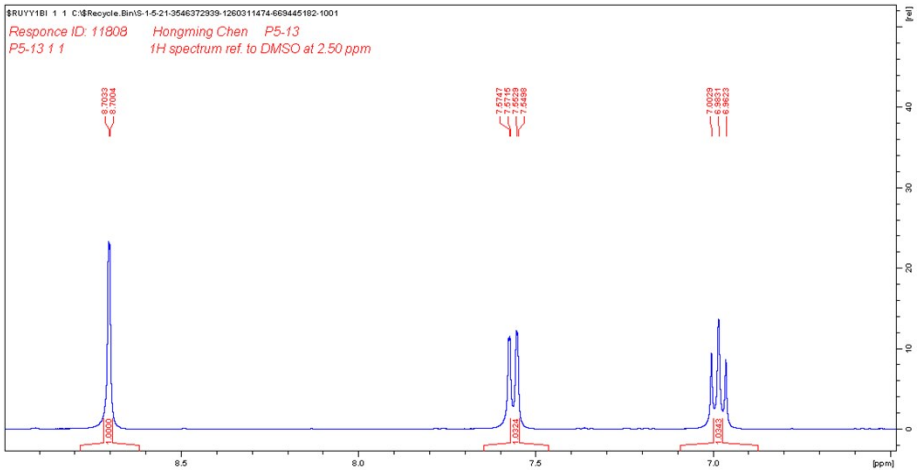
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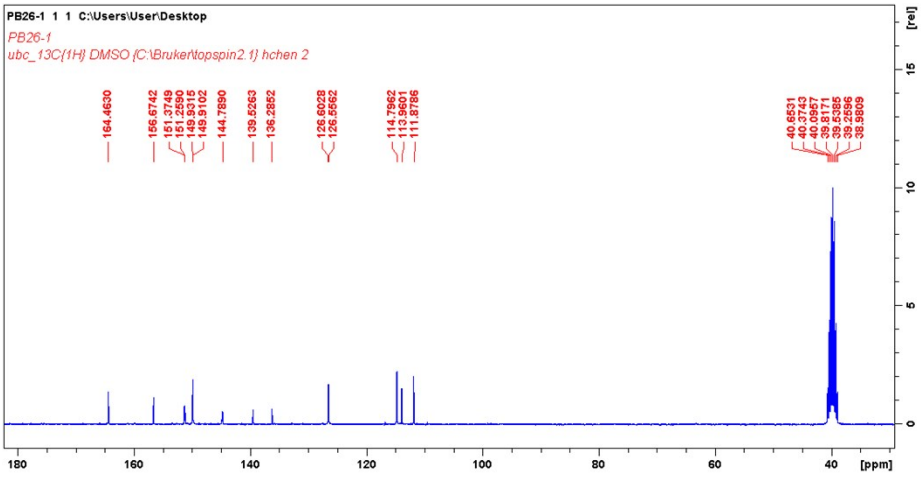
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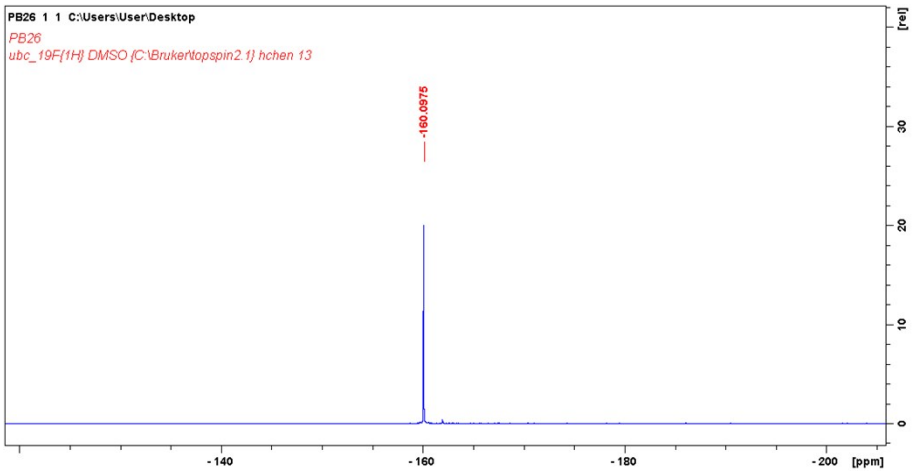
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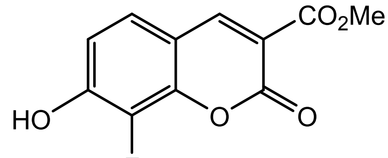
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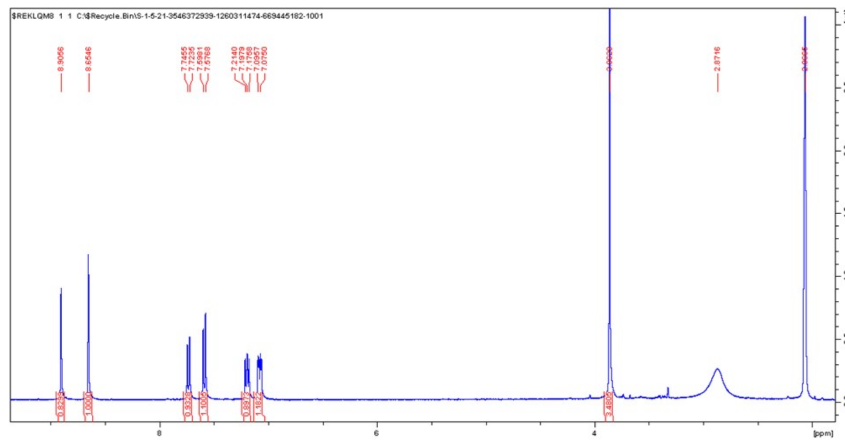

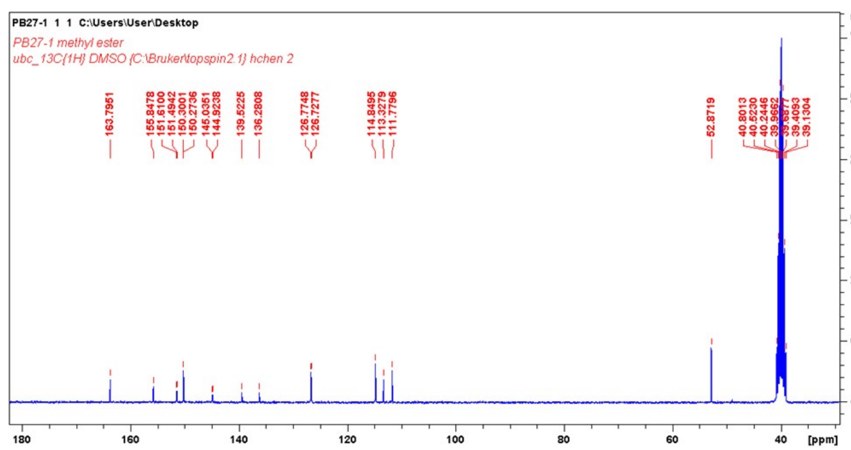
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PB27-1 methyl ester
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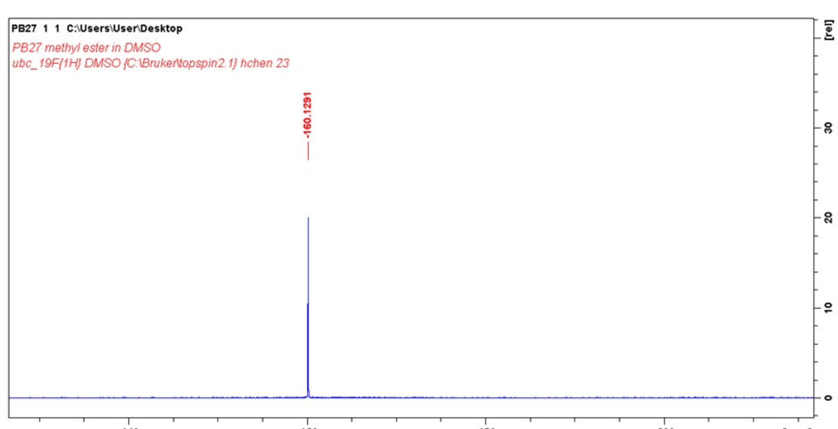
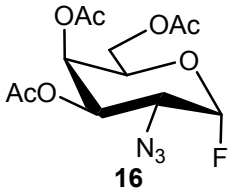
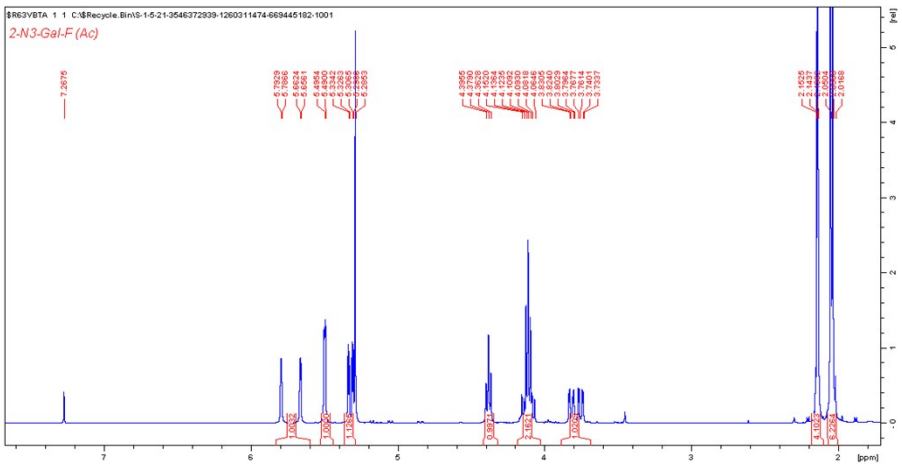
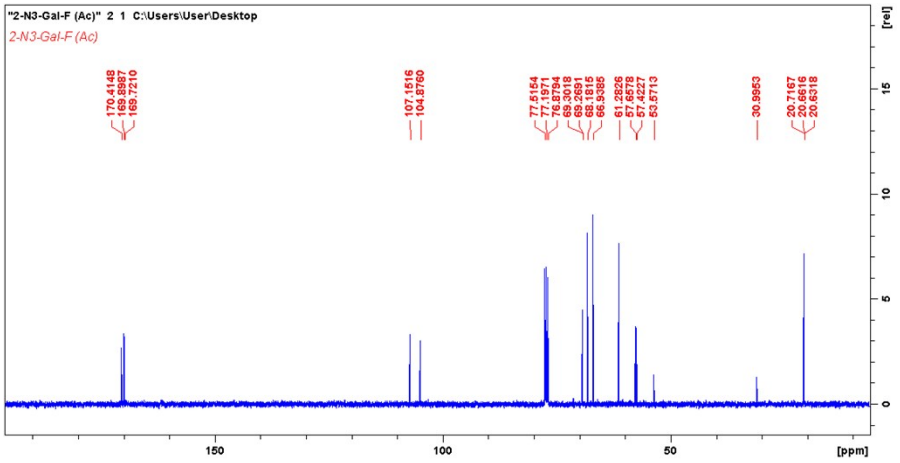
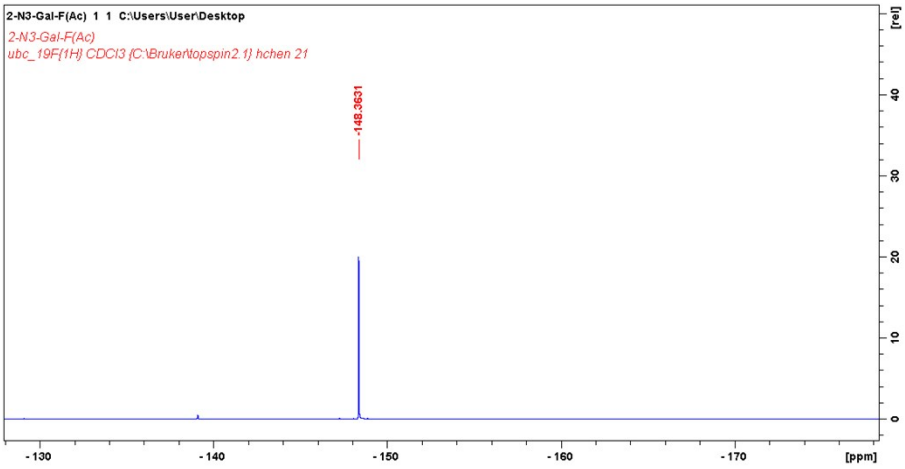

PB27 1 1 C:\Users\User\Desktop
PB27 methyl ester in DMSO
ubc_19F{1H} DMSO {C:\Bruker\topspin2.1} hchen 23
-160.1291

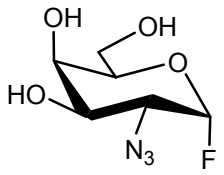

OAc
OAc
O
AcO
N3
F

16

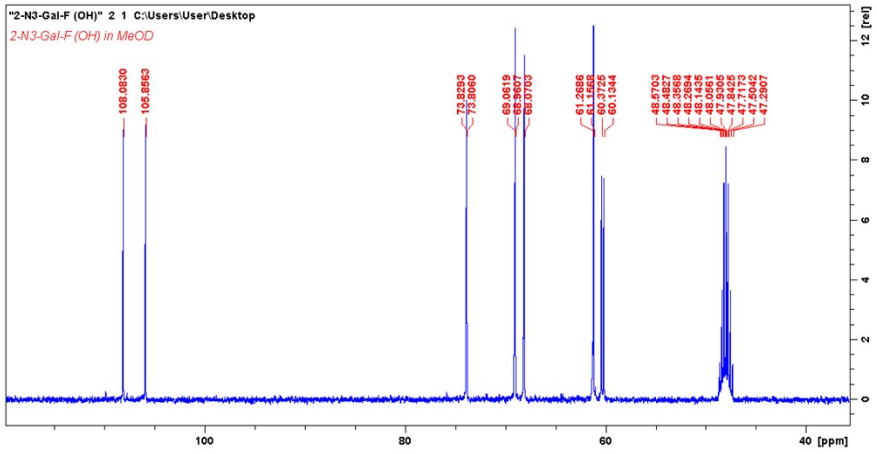

2-N3-Gal-F (Ac)
[rel]
[ppm]

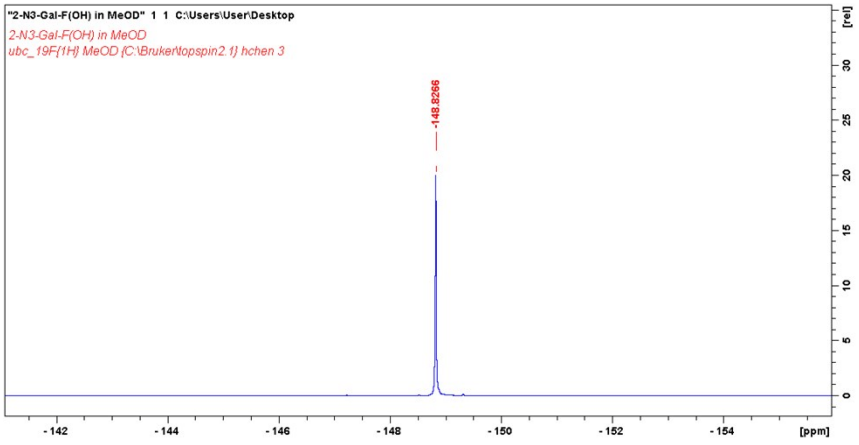

"2-N3-Gal-F (Ac)" 2 1 C:\Users\User\Desktop
2-N3-Gal-F (Ac)
170.4148
169.8987
169.7210
107.1516
104.9760
77.5154
77.1977
76.8794
69.3018
69.2691
68.1815
66.9085
61.2826
57.4229
57.4227
53.5713
30.9953
20.7167
20.6616
20.6318
[rel]
[ppm]

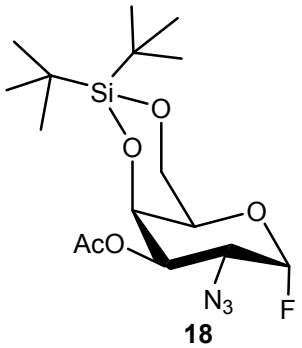

2-N3-Gal-F(Ac) 1 1 C:\Users\User\Desktop
2-N3-Gal-F(Ac)
ubc_19F{1H} CDCl3 (C:\Bruker\topspin2.1) hchen 21
-148.3631
[rel]
[ppm]


OH
OH
O
HO
N_3
F

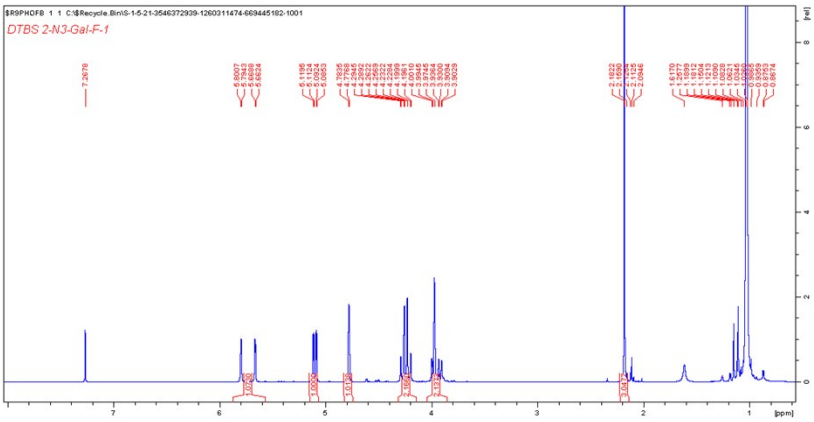
17

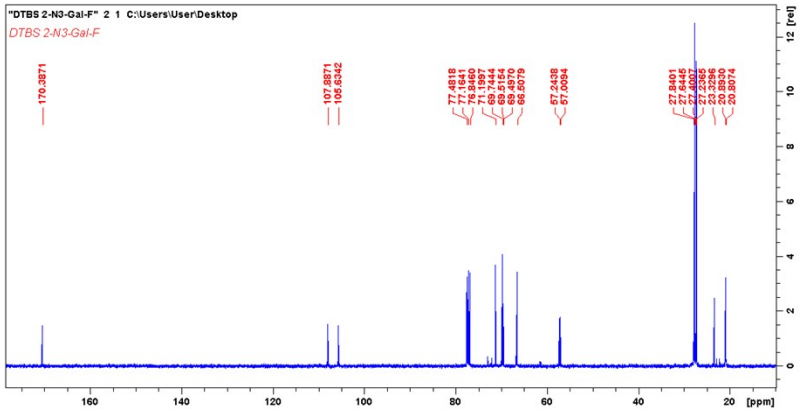

"2-N3-Gal-F (OH)" 2 1 C:\Users\User\Desktop
2-N3-Gal-F (OH) in MeOD
108.0830
106.8563
73.8293
73.8060
69.0619
68.9807
68.0703
61.2686
61.1668
60.3726
60.1344
48.5703
48.4827
48.3568
48.2564
48.1435
48.0561
47.9305
47.7415
47.7143
47.5042
47.2907
12 [rel]
100
80
60
40 [ppm]

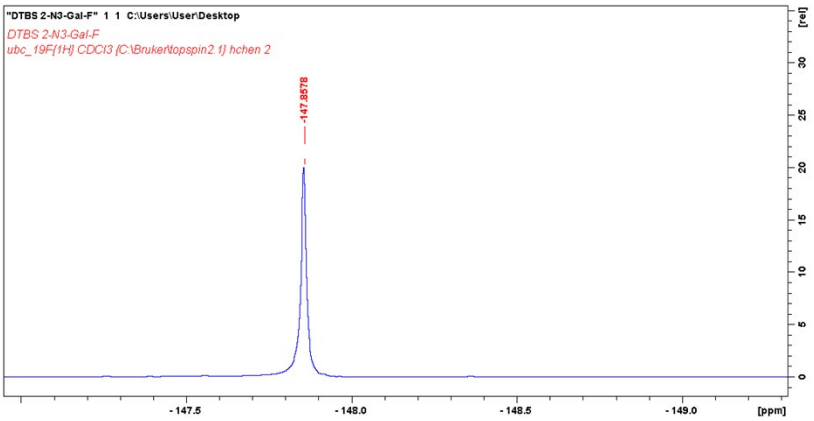

"2-N3-Gal-F(OH) in MeOD" 1 1 C:\Users\User\Desktop
2-N3-Gal-F(OH) in MeOD
ubc_19F{1H} MeOD {C:\Bruker\topspin2.1} hchen 3
-148.8266
[rel]
-142
-144
-146
-148
-150
-152
-154
[ppm]

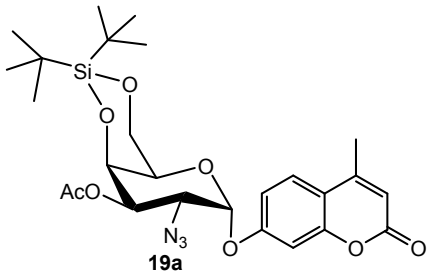

Si
O
O
O
AcO
N3
F

18

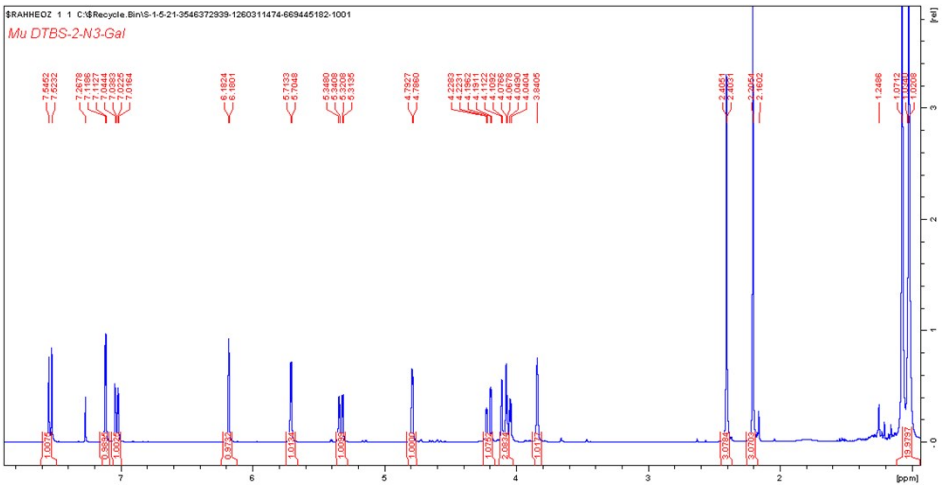

DTBS 2-N3-Gal-F-1
[ppm]
[rel]

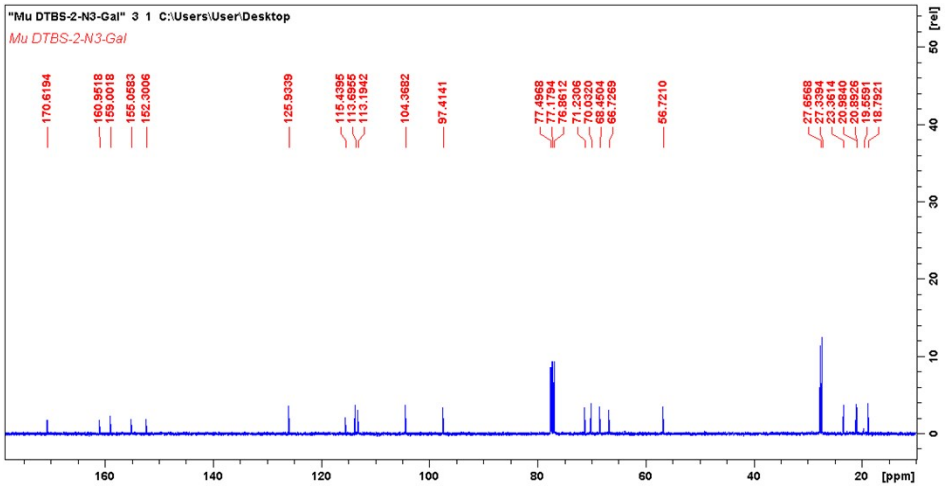

"DTBS 2-N3-Gal-F" 2 1 C:\Users\User\Desktop
DTBS 2-N3-Gal-F
170.3871
107.8871
105.6342
77.4818
77.1641
76.8460
74.1997
69.7444
69.5154
68.4570
68.3979
67.2458
67.0004
27.8401
27.6445
27.4490
27.2365
23.3286
20.8530
20.8014
160
140
120
100
80
60
40
20
[ppm]
12
[rel]

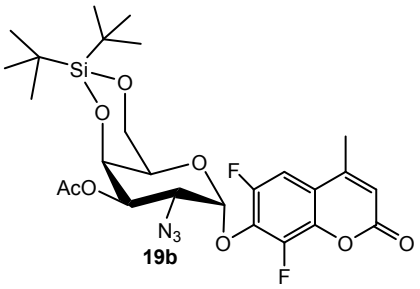

"DTBS 2-N3-Gal-F" 1 1 C:\Users\User\Desktop
DTBS 2-N3-Gal-F
ubc_19F{1H} CDCl3 {C:\Bruker\topspin2.1} hchen 2
-147.8578
-147.5
-148.0
-148.5
-149.0
[ppm]
[rel]

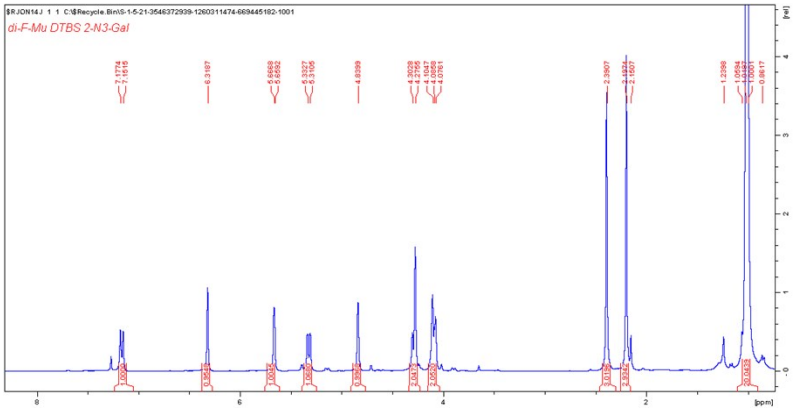

Si
O
O
O
AcO
N3
O
O
O

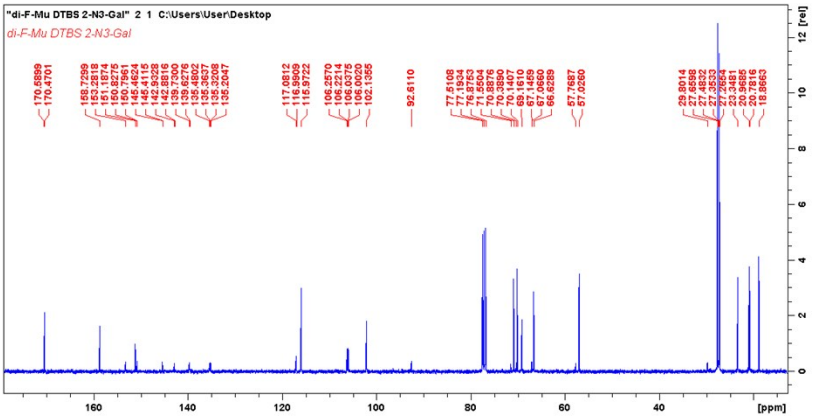
19a

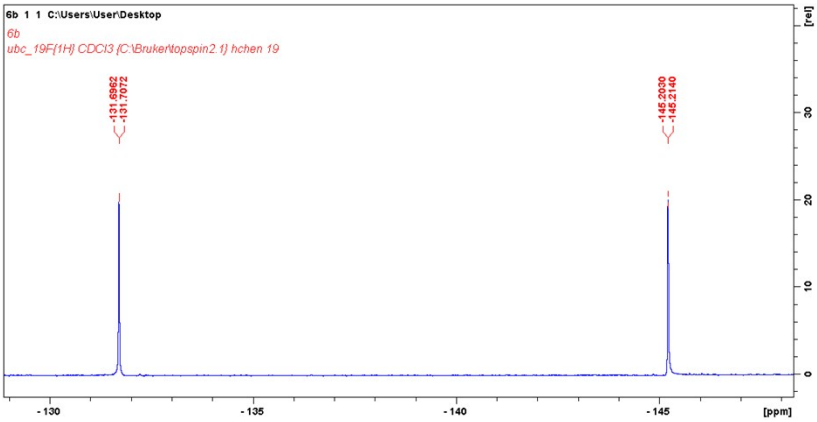

$RAHHEOZ 1 1 C:\$Recycle.Bin\S-1-5-21-3546372939-1260311474-669445182-1001
Mu DTBS-2-N3-Gal
[ppm]
[rel]

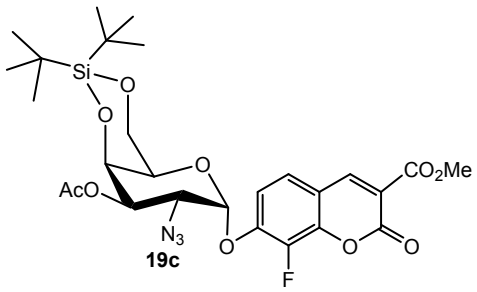

"Mu DTBS-2-N3-Gal" 3 1 C:\Users\User\Desktop
Mu DTBS-2-N3-Gal
170.6194
160.9518
159.0018
155.0583
152.3006
125.9339
115.4395
113.6955
113.1942
104.3682
97.4141
77.4968
77.1794
76.8612
71.2306
70.0320
68.4504
66.7269
56.7210
27.6568
27.3394
27.1294
23.3614
20.9840
20.8826
19.5591
18.7921
[ppm]
[rel]


Si
O
O
O
AcO
N3
O
F
O
O
F
19b

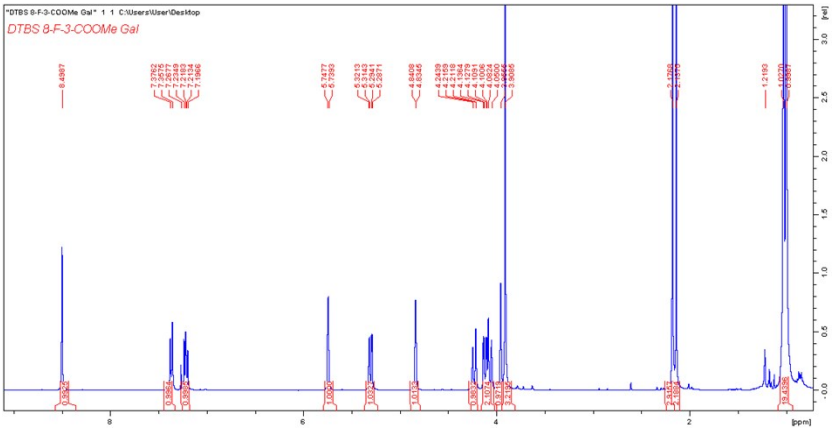

di-F-Mu DTBS 2-N3-Gal
[ppm]
[rel]

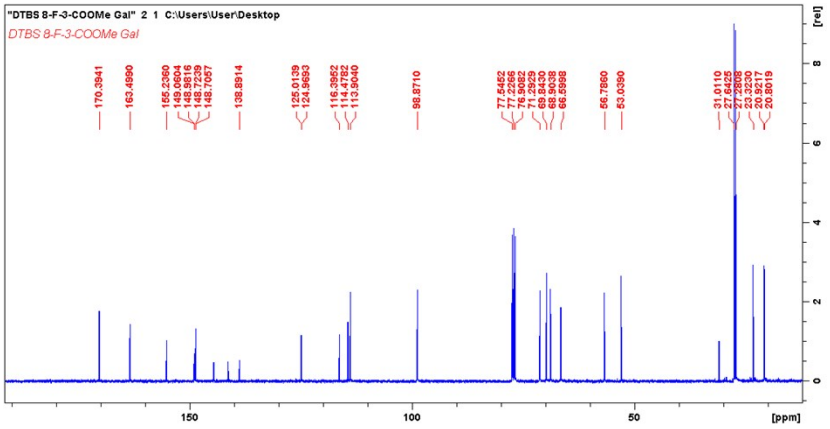

"di-F-Mu DTBS 2-N3-Gal" 2 1 C:\Users\User\Desktop
di-F-Mu DTBS 2-N3-Gal
[ppm]
[rel]

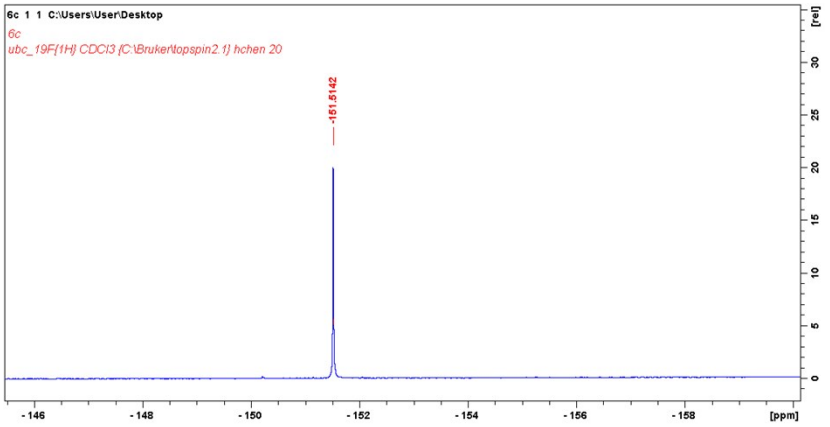

6b 1 1 C:\Users\User\Desktop
6b
ubc_19F{1H} CDCl3 {C:\Bruker\topspin2.1} hchen 19
-131.6962
-131.7072
-145.2030
-145.2140
[ppm]
[rel]

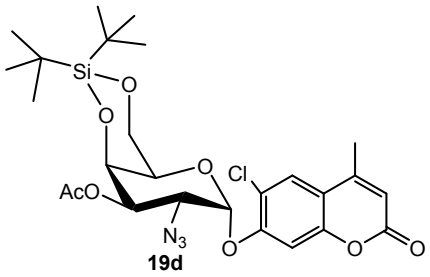

Si
O
O
O
AcO
N3
O
O
O
O
F
CO2Me

19c

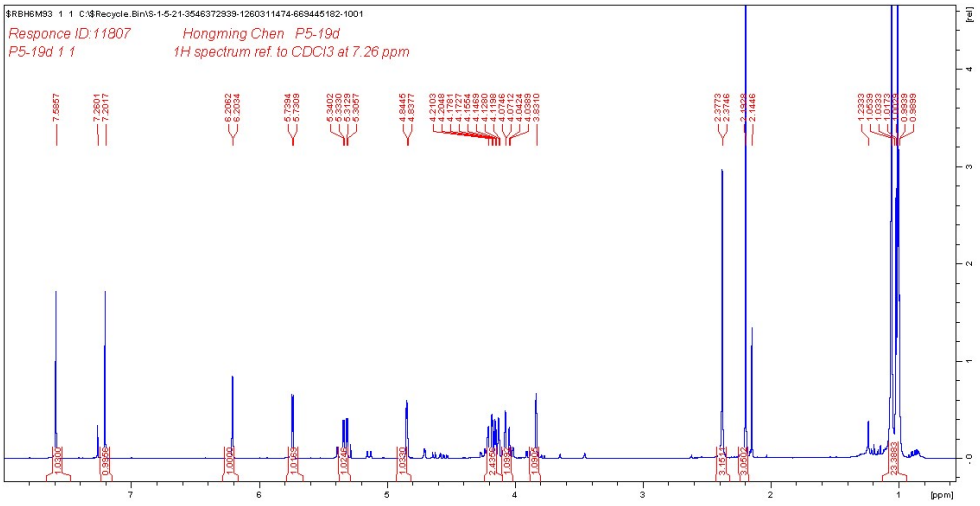

"DTBS 8-F-3-COOMe Gal" 1 1 C:\Users\User\Desktop
DTBS 8-F-3-COOMe Gal
[ppm]
[rel]

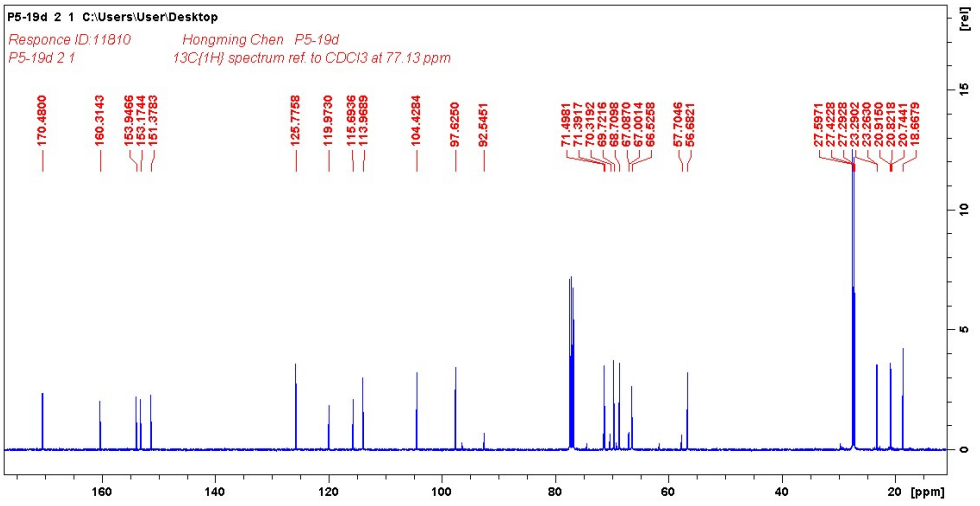

"DTBS 8-F-3-COOMe Gal" 2 1 C:\Users\User\Desktop
DTBS 8-F-3-COOMe Gal
170.3941
163.4690
155.2360
149.0604
148.8516
148.7238
148.7057
138.8914
125.0139
124.9693
116.3952
114.4782
110.3040
98.8710
77.5462
77.2266
76.9062
71.2929
69.8430
68.8938
68.8598
66.7860
53.0390
31.0110
27.6425
27.2808
23.3210
20.8517
20.8019
[ppm]
[rel]

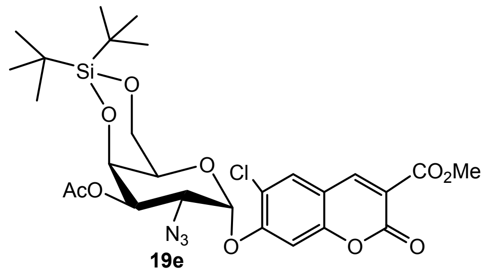

6c 1 1 C:\Users\User\Desktop
6c
ubc_19F{1H} CDCl3 (C:\Bruker\topspin2.1) hchen 20
-151.5142
[ppm]
[rel]


Si
O
O
O
AcO
N3
O
Cl
O
O

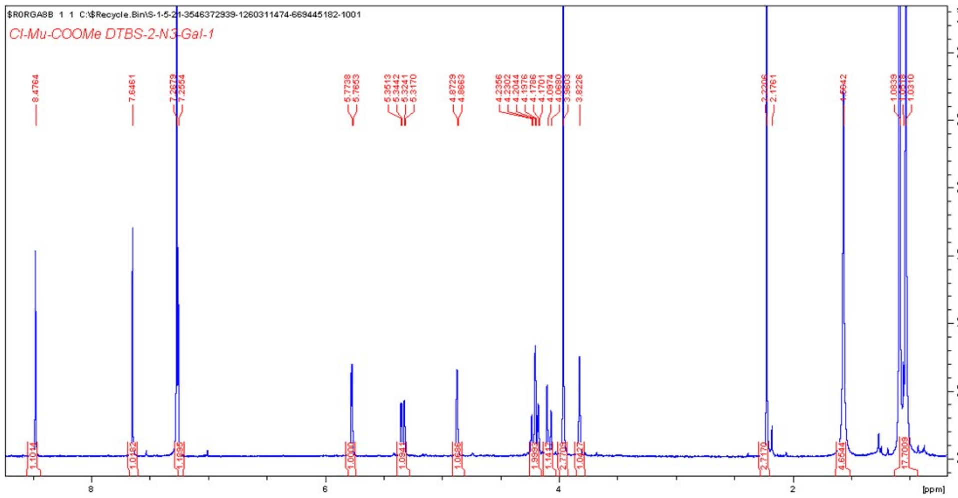
19d

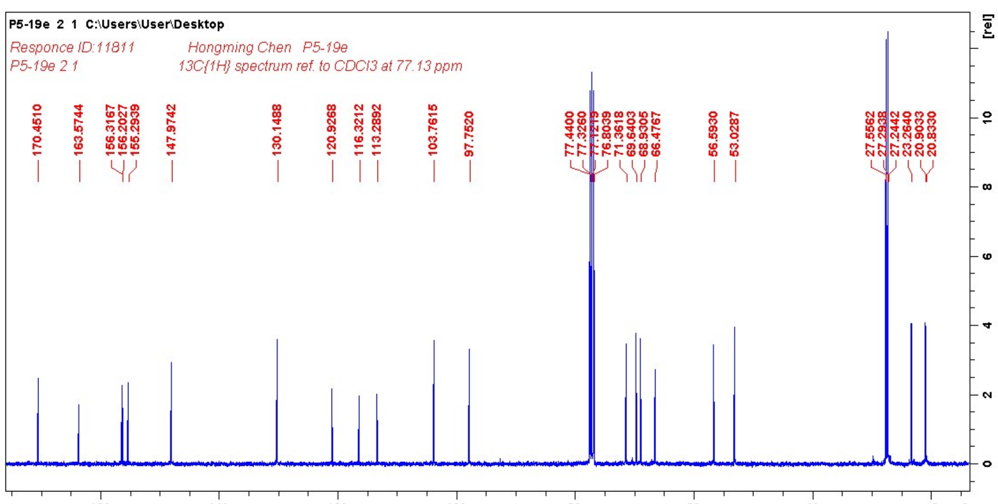

$RBH6M93 1 1 C:\$Recycle.Bin\S-1-5-21-3546372939-1260311474-669445182-1001
Responce ID:11807 Hongming Chen P5-19d
P5-19d 1 1 1H spectrum ref. to CDCl3 at 7.26 ppm
[rel]
[ppm]

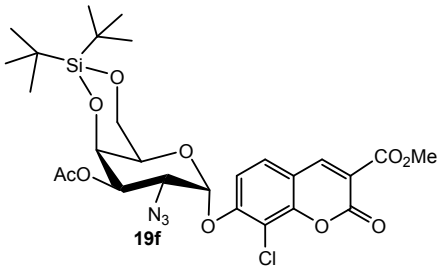

P5-19d 2 1 C:\Users\User\Desktop
Responce ID:11810 Hongming Chen P5-19d
P5-19d 2 1 13C{1H} spectrum ref. to CDCl3 at 77.13 ppm
170.4800
160.3143
153.9466
153.1744
151.3783
125.7758
119.9730
115.6936
113.9689
104.4284
97.6250
92.5451
71.4981
71.3917
70.3192
69.7216
69.7098
69.0870
67.0014
65.5258
57.7046
56.8821
27.5971
27.4228
27.2928
23.2902
23.2630
20.9510
20.8218
20.7441
18.6679
[rel]
[ppm]


Si
O
O
O
AcO
N3
Cl
CO2Me
O
O
O

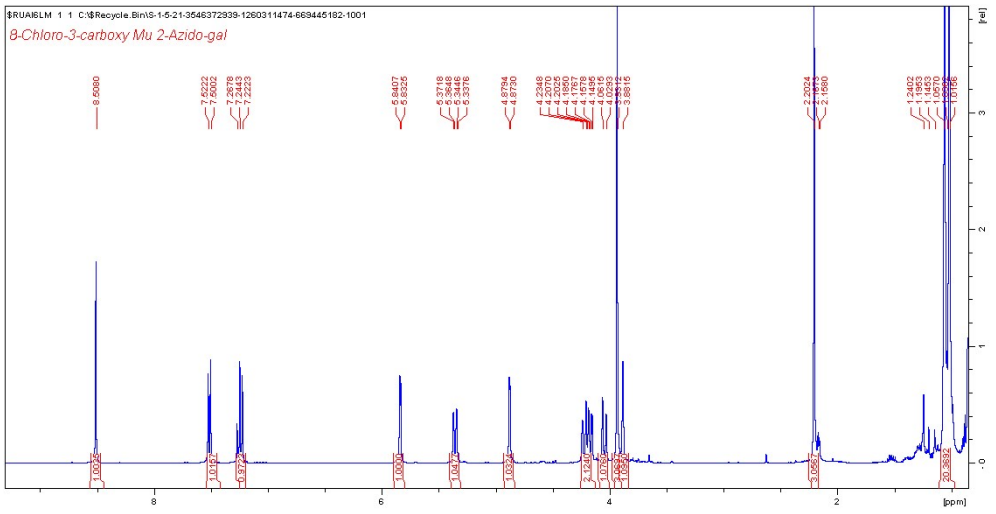
19e

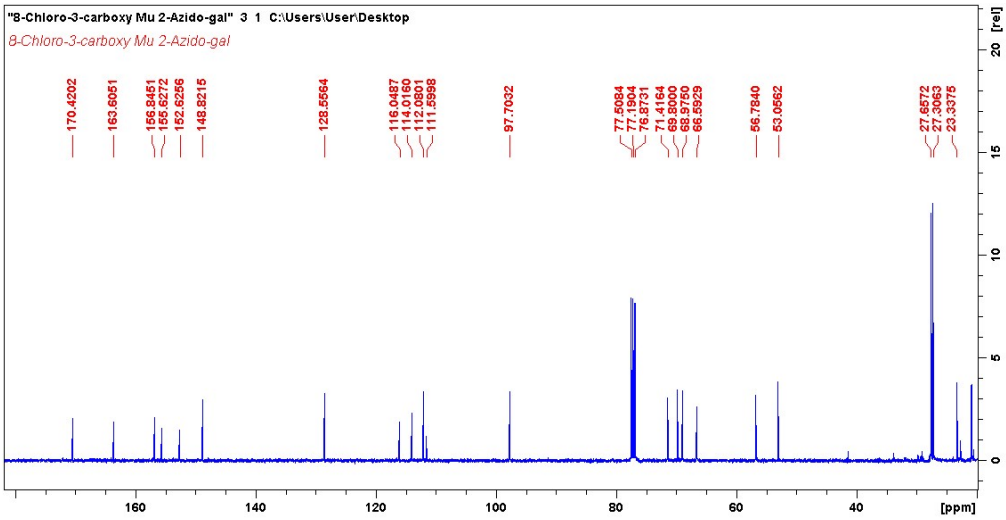

Cl-Mu-COOMe DTBS-2-N3-Gal-1
[ppm]
[rel]

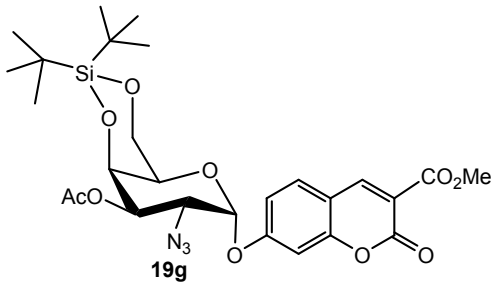

P5-19e 2 1 C:\Users\User\Desktop
Responce ID:11811
Hongming Chen P5-19e
P5-19e 2 1
13C{1H} spectrum ref. to CDCl3 at 77.13 ppm
170.4510
163.5744
156.3167
156.2027
155.2939
147.9742
130.1488
120.9268
116.3212
113.2892
103.7615
97.7520
77.4400
77.3260
77.1219
76.8039
71.3618
69.6403
68.9305
66.4767
56.5930
53.0287
27.5562
27.2938
27.2442
23.2640
20.9033
20.8330
[ppm]
[rel]


Si
O
O
O
AcO
N3
O
O
O
CO2Me
Cl

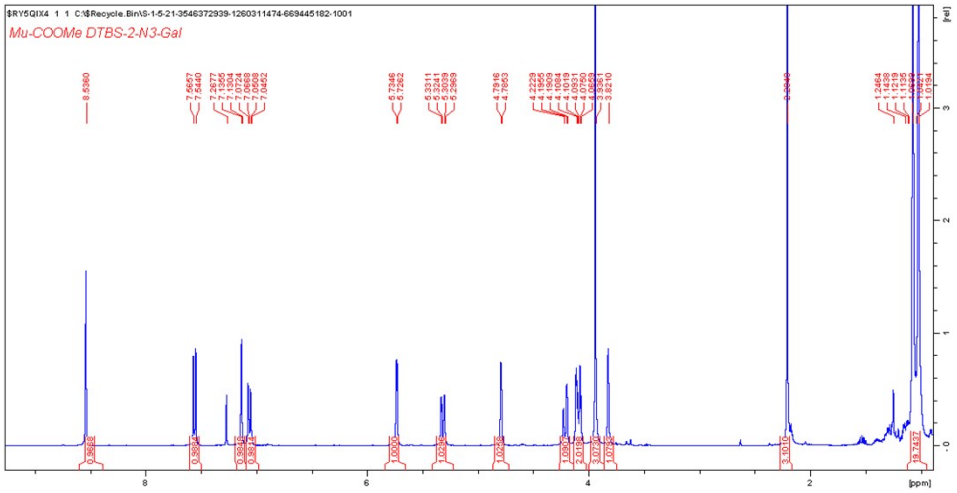
19f

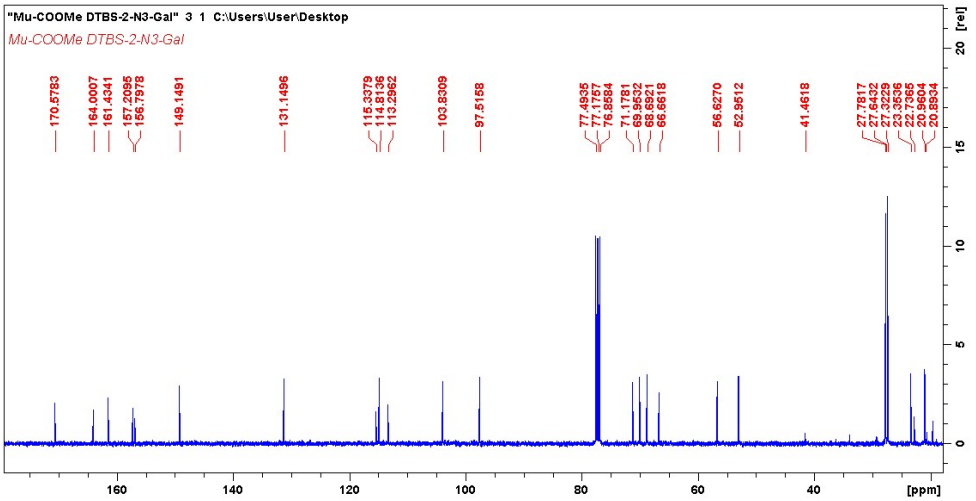

$RUAI6LM 1 1 C:\$Recycle.Bin\S-1-5-21-3546372939-1260311474-669445182-1001
8-Chloro-3-carboxy Mu 2-Azido-gal
[rel]
[ppm]

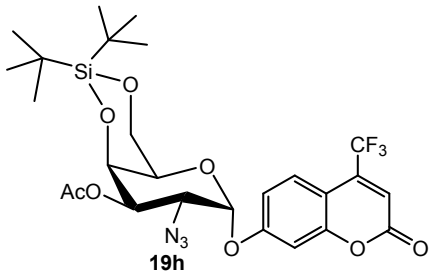

"8-Chloro-3-carboxy Mu 2-Azido-gal" 3 1 C:\Users\User\Desktop
8-Chloro-3-carboxy Mu 2-Azido-gal
170.4202
163.6051
156.8451
155.6272
152.6256
148.8215
128.5564
116.0487
114.0160
112.0891
111.5998
97.7032
77.5084
77.1904
76.8731
71.4164
69.8000
68.9750
66.5929
56.7840
53.0562
27.6572
27.3063
23.3375
[rel]
[ppm]


Si
O
O
O
AcO
N3
O
O
O
CO2Me

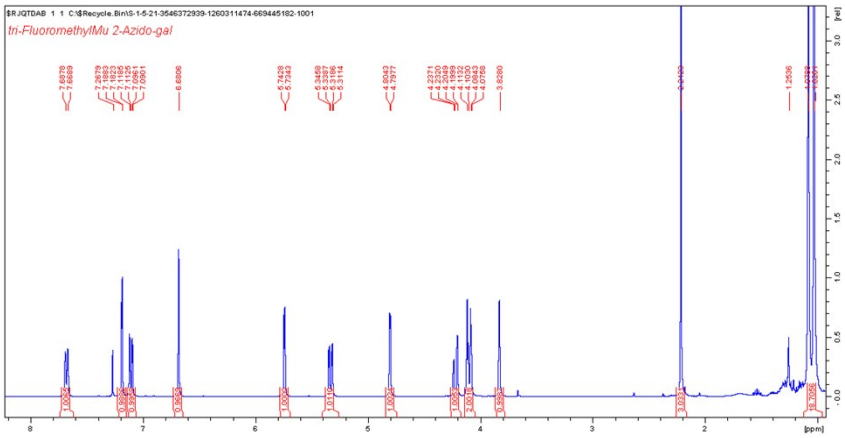
19g

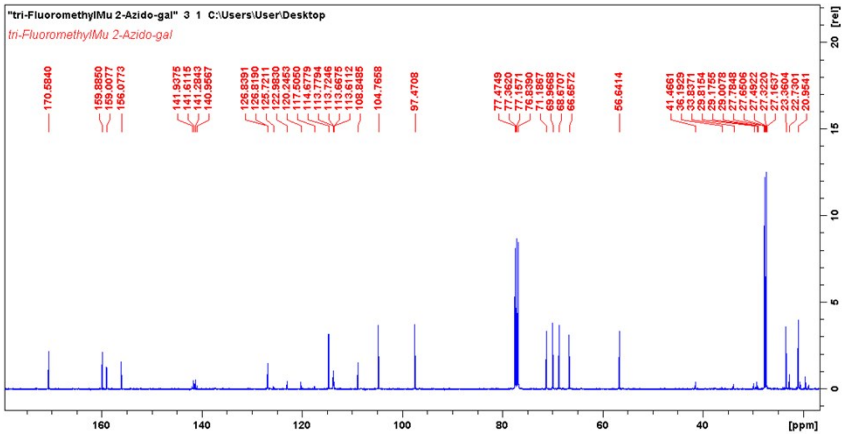

Mu-COOMe DTBS-2-N3-Gal
[ppm]
[rel]

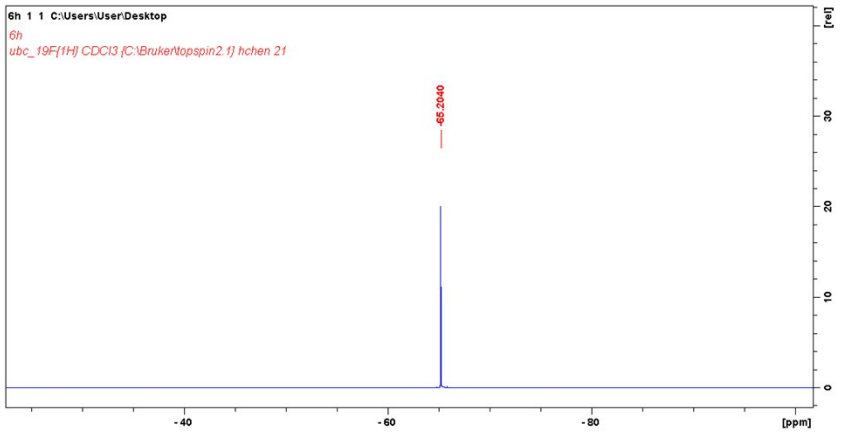

"Mu-COOMe DTBS-2-N3-Gal" 3 1 C:\Users\User\Desktop
Mu-COOMe DTBS-2-N3-Gal
170.5783
164.0007
161.4341
157.2095
156.7978
149.1491
131.1496
115.3379
114.8136
113.2962
103.8309
97.5158
77.4935
77.1757
76.8584
71.1781
69.9532
68.6921
66.6618
56.6270
52.9512
41.4618
27.7917
27.6432
27.3229
23.3536
22.7365
20.9604
20.8934
[ppm]
[rel]

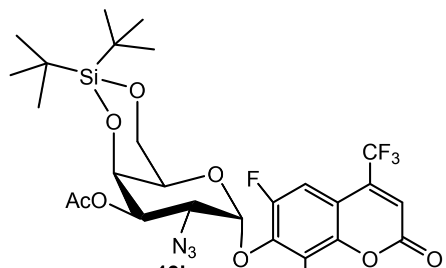


19h

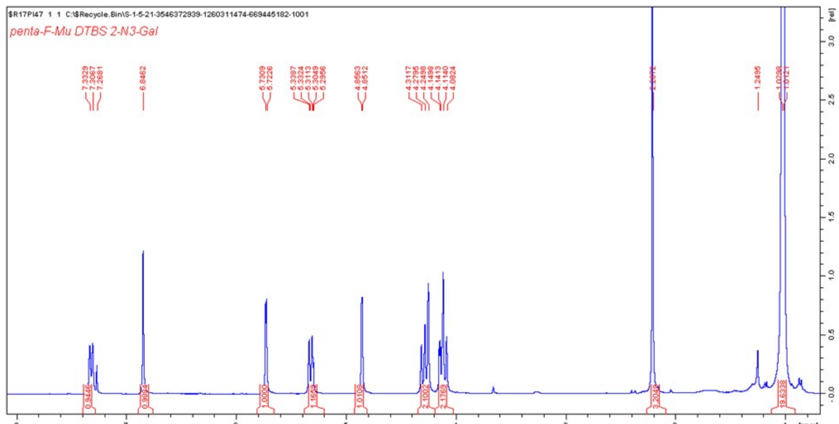

tri-FluoromethylMu 2-Azido-gal
[ppm]
[rel]

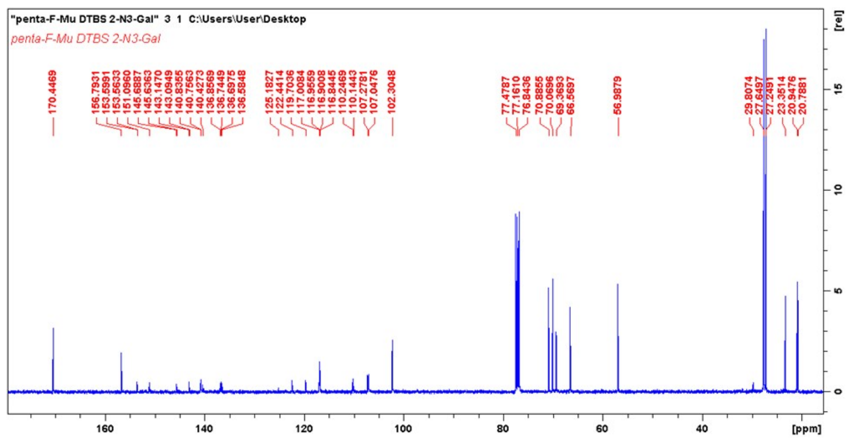

"tri-FluoromethylMu 2-Azido-gal" 3 1 C:\Users\User\Desktop
tri-FluoromethylMu 2-Azido-gal
[ppm]
[rel]


6h 1 1 C:\Users\User\Desktop
6h
ubc_19F{1H} CDCl3 {C:\Bruker\topspin2.1} hchen 21
-65.2040
[ppm]
[rel]


Si
O
O
O
AcO
N3
O
CF3
F
O
O
O
F

19i


penta-F-Mu DTBS 2-N3-Gal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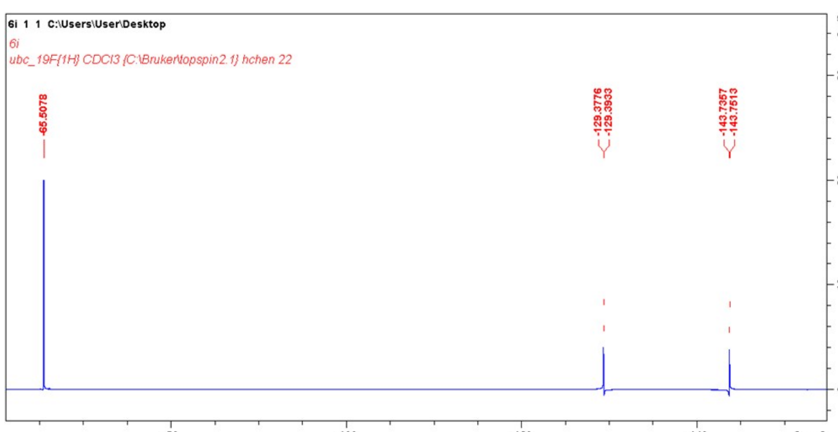
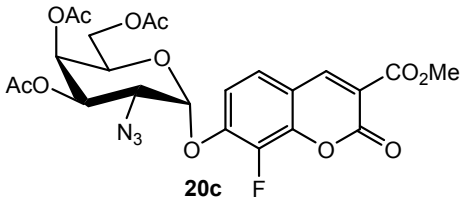
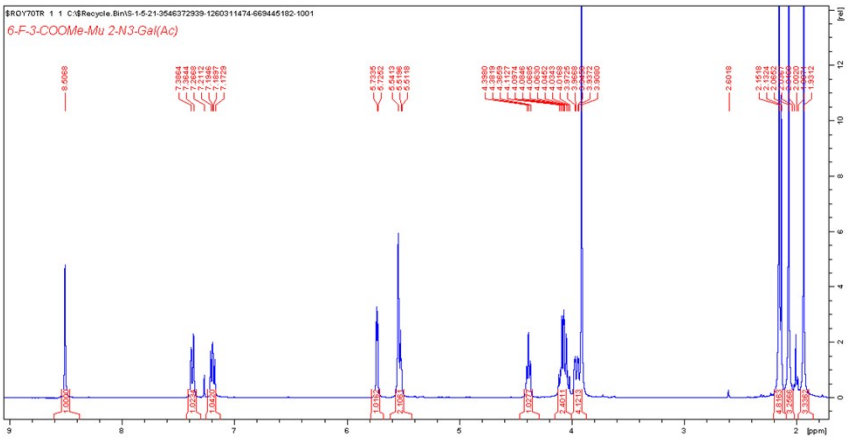
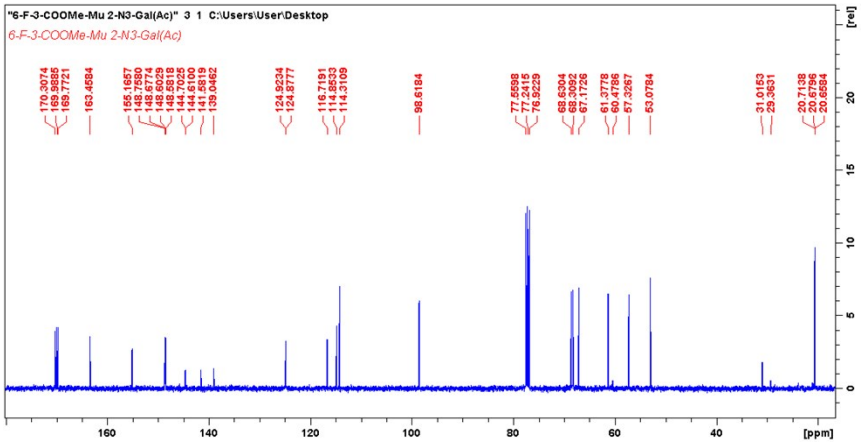
penta-F-Mu DTBS 2-N3-Gal

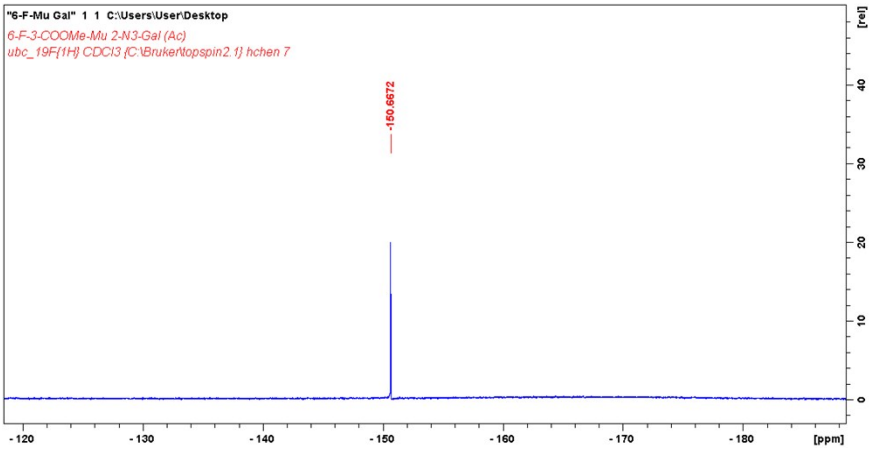

6i 1 1 C:\Users\User\Desktop
6i
ubc_19F{1H} CDCl3 {C:\Bruker\topspin2.1} hchen 22

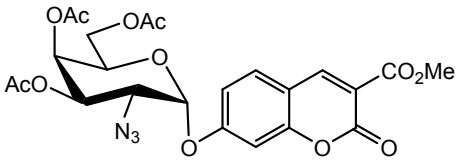

OAc
OAc
O
AcO
N3
O
O
O
CO2Me
F

20c

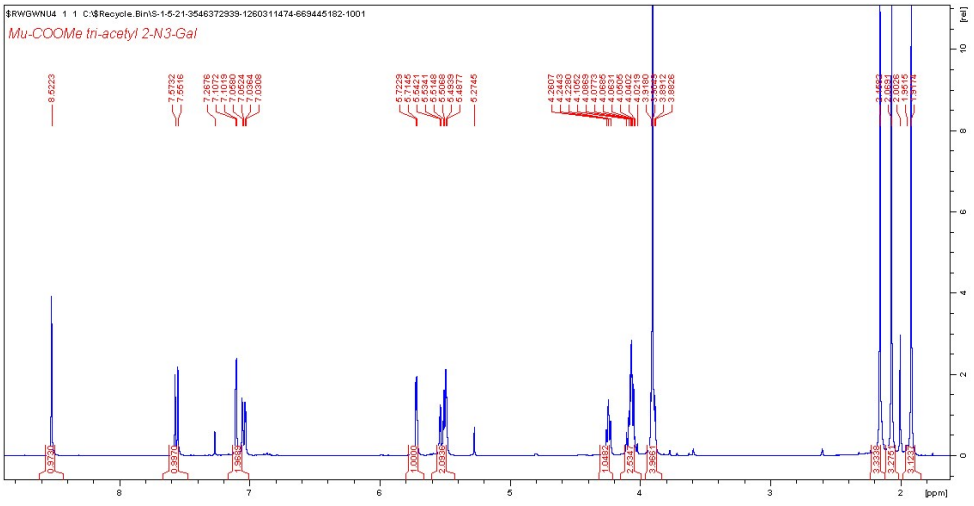

6-F-3-COOMe-Mu 2-N3-Gal(Ac)
[ppm]

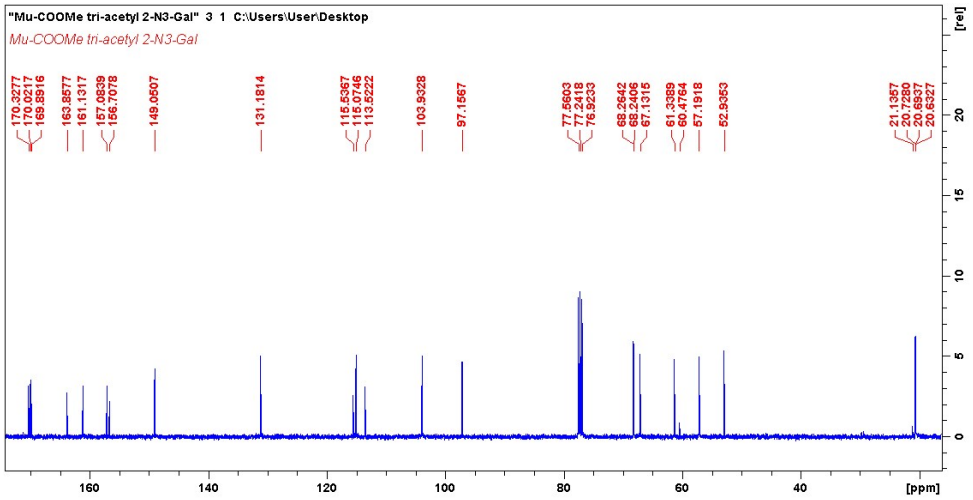

"6-F-3-COOMe-Mu 2-N3-Gal(Ac)" 3 1 C:\Users\User\Desktop
6-F-3-COOMe-Mu 2-N3-Gal(Ac)
[ppm]

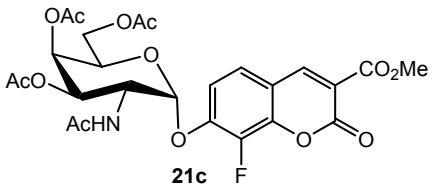

"6-F-Mu Gal" 1 1 C:\Users\User\Desktop
6-F-3-COOMe-Mu 2-N3-Gal (Ac)
ubc_19F{1H} CDCl3 {C:\Bruker\topspin2.1} hchen 7
-150.6672
[ppm]


OAc
OAc
O
AcO
N3
O
O
O
CO2Me

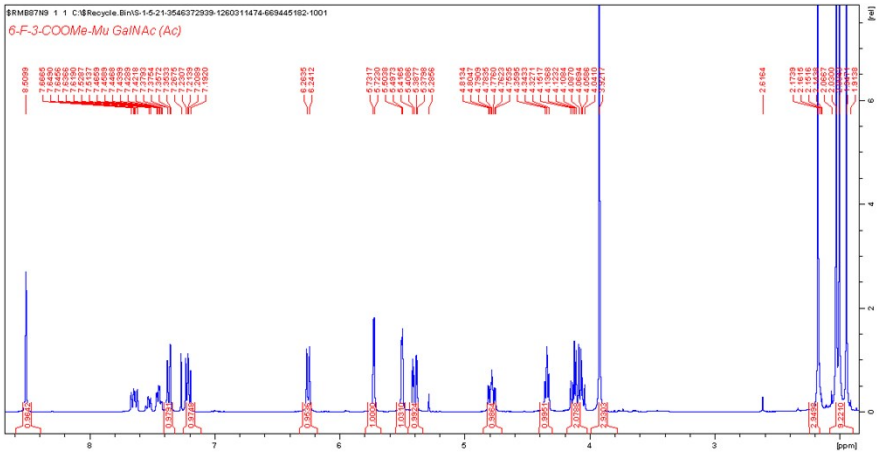
20g

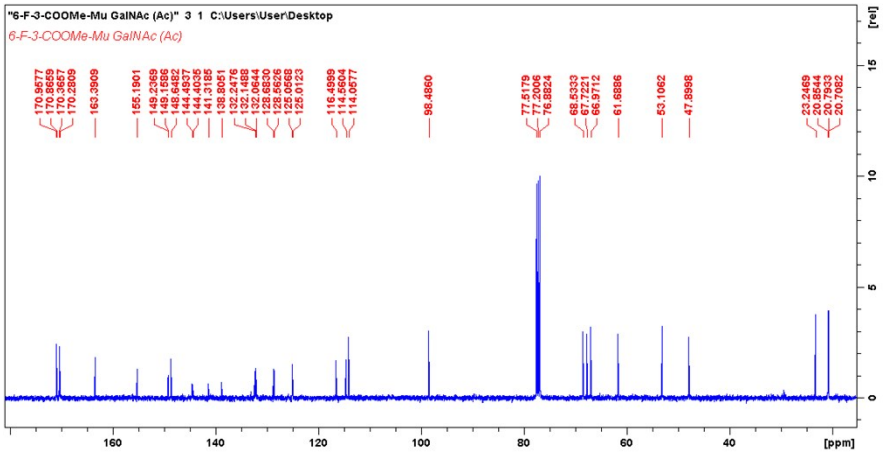

$RWGWNU4 1 1 C:\$Recycle.Bin\S-1-5-21-3546372939-1260311474-669445182-1001
Mu-COOMe tri-acetyl 2-N3-Gal
[rel]
[ppm]

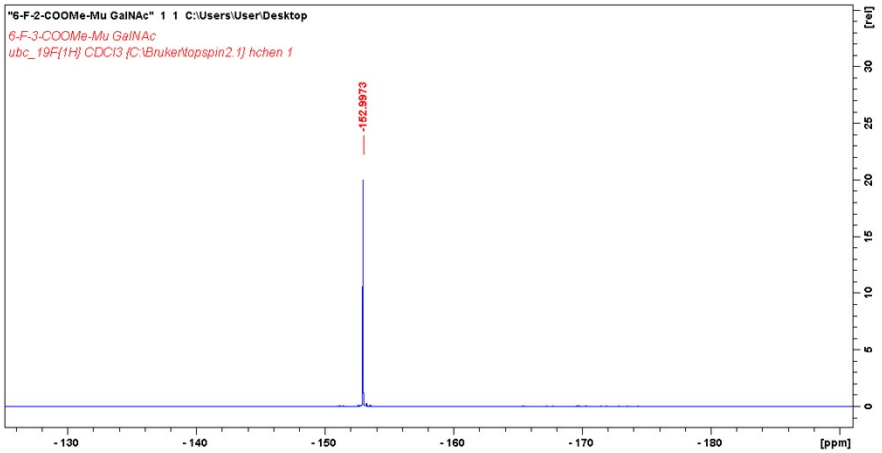

"Mu-COOMe tri-acetyl 2-N3-Gal" 3 1 C:\Users\User\Desktop
Mu-COOMe tri-acetyl 2-N3-Gal
170.3277
170.0217
169.8916
163.8577
161.1317
157.0839
156.7078
149.0507
131.1814
115.5367
115.3746
113.6222
103.9328
97.1567
77.5603
77.2418
76.9233
68.2642
68.2406
67.1315
61.3389
60.4764
57.1918
52.9353
21.1357
20.7280
20.6937
20.6327
[rel]
[ppm]

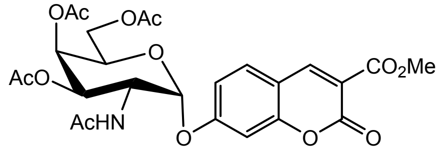

OAc
OAc
O
AcO
AcHN
O
O
O
CO2Me
F

21c

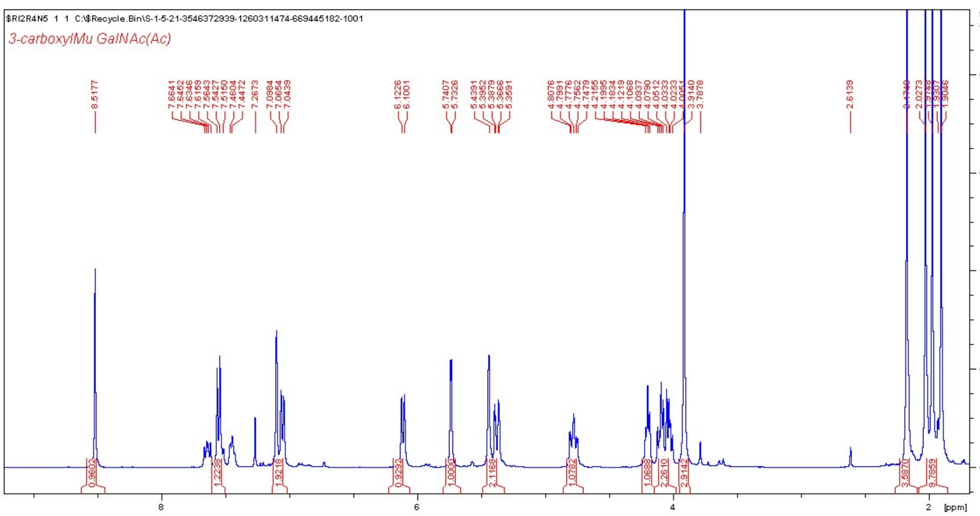

6-F-3-COOMe-Mu GalNAc (Ac)
[ppm]
[rel]

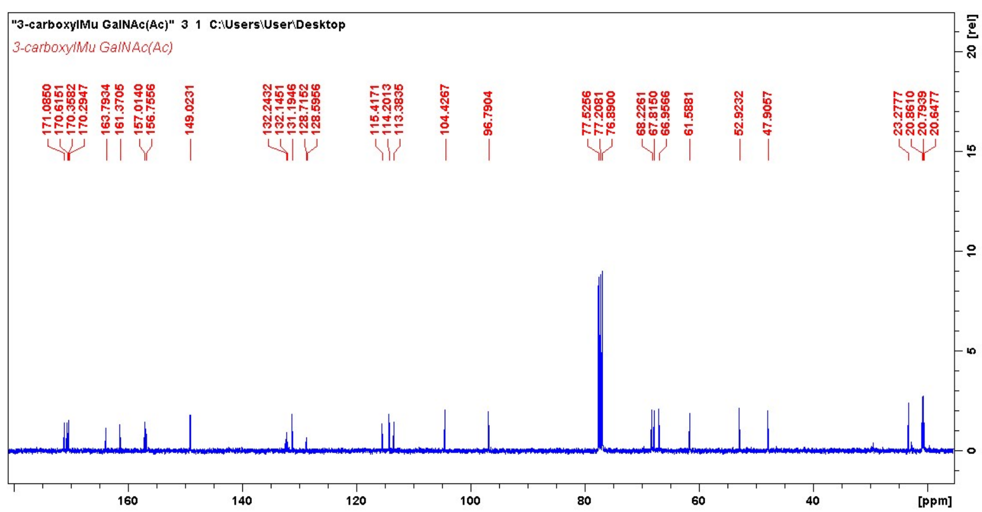

"6-F-3-COOMe-Mu GalNAc (Ac)" 3 1 C:\Users\User\Desktop
6-F-3-COOMe-Mu GalNAc (Ac)
170.9577
170.8859
170.3557
170.2809
163.3909
155.1901
149.2369
149.1686
148.8482
144.4557
144.4035
141.3185
138.8051
132.2476
132.1488
131.0644
128.6830
128.5626
125.0568
125.0123
116.4899
114.6604
114.4577
98.4860
77.5178
77.2056
76.8824
68.5333
67.7221
66.9712
61.6886
53.1062
47.8998
23.2469
20.8544
20.7933
20.7082
[ppm]
[rel]

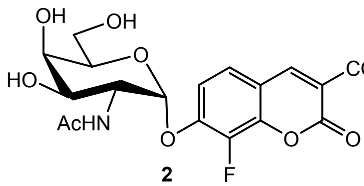

"6-F-2-COOMe-Mu GalNAc" 1 1 C:\Users\User\Desktop
6-F-3-COOMe-Mu GalNAc
ubc_19F{1H} CDCl3 {C:\Bruker\topspin2.1} hchen 1
-152.9973
[ppm]
[rel]


OAc
OAc
O
AcO
AcHN
O
CO2Me
O
O

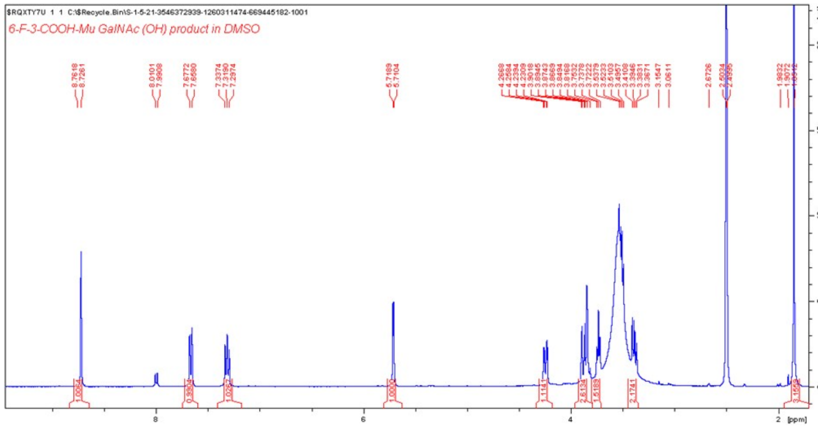
21g

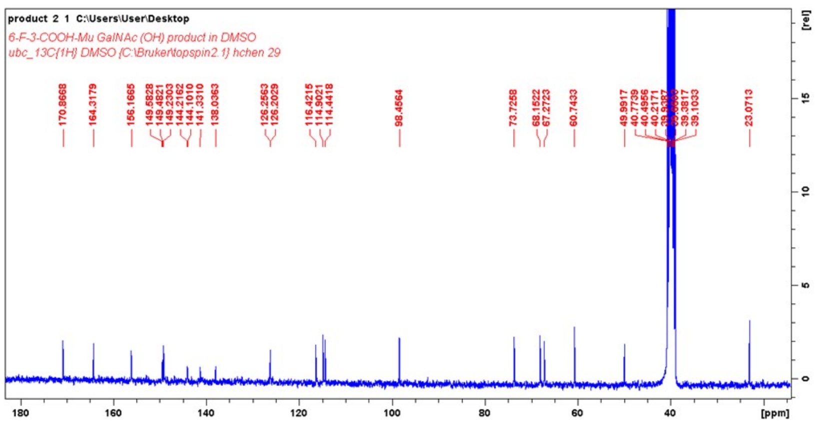

3-carboxylMu GalNAc(Ac)
[ppm]
[rel]

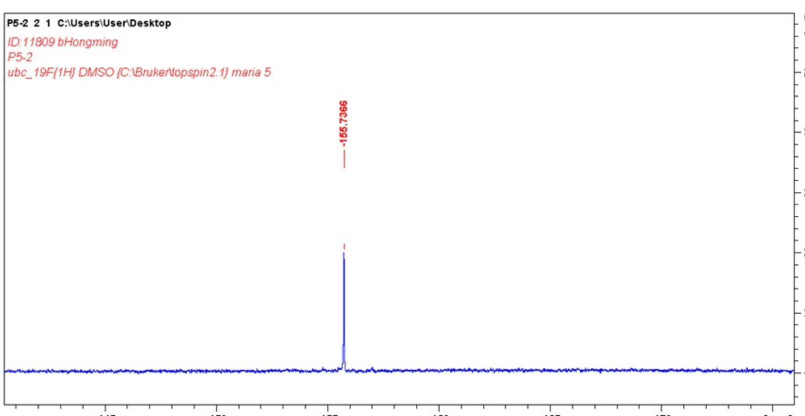

"3-carboxylMu GalNAc(Ac)" 3 1 C:\Users\User\Desktop
3-carboxylMu GalNAc(Ac)
171.0850
170.6151
170.3582
170.2947
163.7934
161.3705
157.0140
156.7556
149.0231
132.2432
132.1451
131.1946
128.7152
128.5956
115.4171
114.2013
113.3835
104.4267
96.7904
77.5256
77.2081
76.8900
68.2261
67.8150
66.9566
61.5881
52.9232
47.9057
23.2777
20.8610
20.7939
20.6477
[ppm]
[rel]

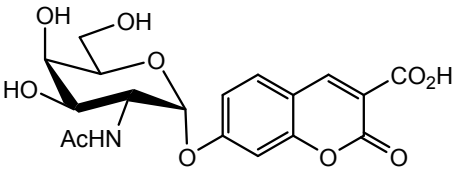


2

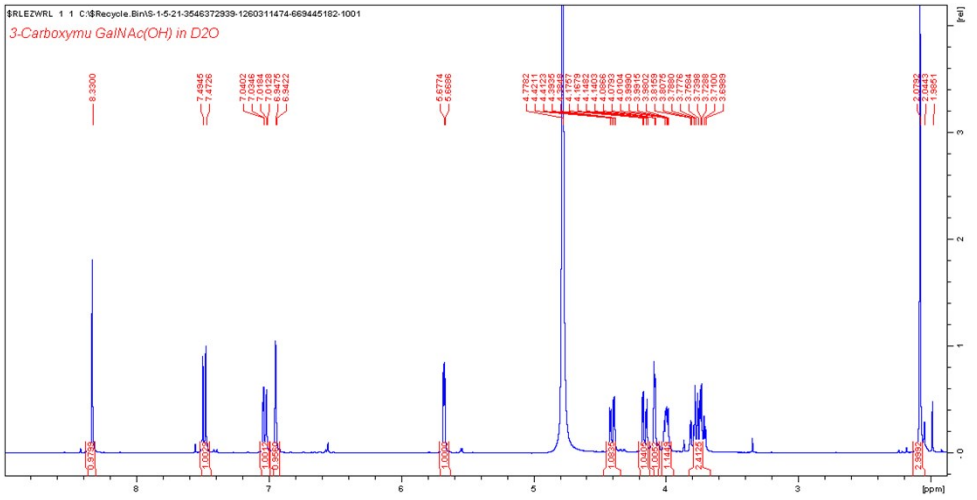

6-F-3-COOH-Mu GalNAc (OH) product in DMSO

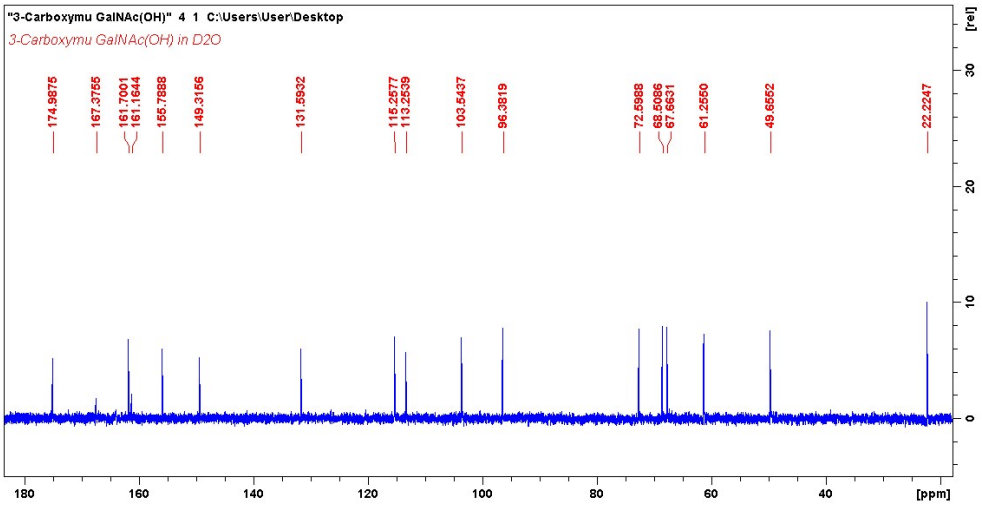

product 2 1 C:\Users\User\Desktop
6-F-3-COOH-Mu GalNAc (OH) product in DMSO
ubc_13C{1H} DMSO {C:\Bruker\topspin2.1} hchen 29


P5-2 2 1 C:\Users\User\Desktop
ID:11809 bHongming
P5-2
ubc_19F{1H} DMSO {C:\Bruker\topspin2.1} maria 5
-155.7366


OH
OH
O
HO
AcHN
O
O
O
CO2H

22g


$RLEZWRL 1 1 C:\$Recycle.Bin\S-1-5-21-3546372939-1260311474-669445182-1001
3-Carboxymu GalNAc(OH) in D2O
[rel]
[ppm]


"3-Carboxymu GalNAc(OH)" 4 1 C:\Users\User\Desktop
3-Carboxymu GalNAc(OH) in D2O
174.9875
167.3755
161.7001
161.1644
155.7888
149.3156
131.5932
115.2577
113.2559
103.5437
96.3819
72.5988
69.5086
67.6631
61.2560
49.6552
22.2247
[rel]
[ppm]