Supplementary Material

Comparison of affinity ranking by target-directed dynamic combinatorial chemistry and surface plasmon resonance

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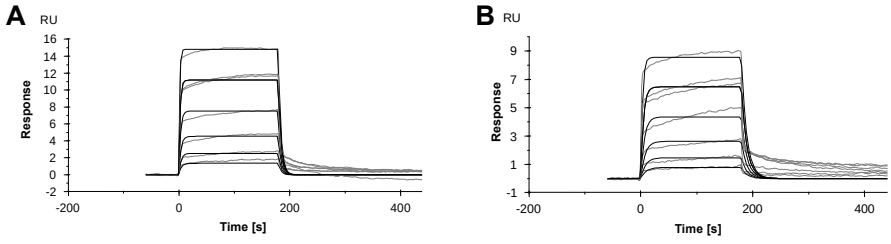
Surface Plasmon Resonance Experiments

According to an established procedure,[S1] SPR experiments were conducted using a Biacore T200 system (GE Healthcare). In brief, dilution series with two-fold increasing concentrations were delivered over a streptavidin (SA) chip with immobilized $FimH_{FL-B}$. The reference cell was capped with biotin-poly(ethylenglycol)amine. Starting from stock solutions of compounds (50 mM in DMSO), dilution series were prepared in buffer (HBS-EP; GE Healthcare). Compounds were injected for 180 s at a flow rate of 30 µL/min, followed by an 800 s dissociation phase. The sensorgrams were referenced and blank subtracted and fitted according to a 1:1 binding model. Complete data are given in Table S1 and the sensorgrams are depicted in Figure S1.

Table S1. Results from SPR measurements.

	k_{on} [1/Ms]	Compound	K_D [nM]	$t_{1/2}$ [s]
3a	$6.18 \cdot 10^5$	0.22	359	3.13
3c	$2.93 \cdot 10^5$	0.11	358	6.60
3d	$4.31 \cdot 10^5$	0.12	267	6.03
3e	$3.92 \cdot 10^5$	0.19	492	3.59
3g	$4.41 \cdot 10^5$	0.20	461	3.41
3h	$4.41 \cdot 10^5$	0.28	642	2.45
3j	$4.61 \cdot 10^5$	0.21	462	3.26
3k	$4.23 \cdot 10^5$	0.18	427	3.84
3l	$9.52 \cdot 10^5$	0.05	508	14.3
3m	$5.86 \cdot 10^5$	0.28	484	2.44
3n	$5.62 \cdot 10^5$	0.22	390	3.16
3o	$4.87 \cdot 10^5$	0.21	440	3.24
3p	$5.35 \cdot 10^5$	0.20	377	3.44
3q	$1.56 \cdot 10^5$	0.04	286	15.6
3r	$4.49 \cdot 10^5$	0.15	337	4.57
3s	$4.96 \cdot 10^5$	0.27	536	2.60
3t	$3.09 \cdot 10^5$	0.12	376	5.96



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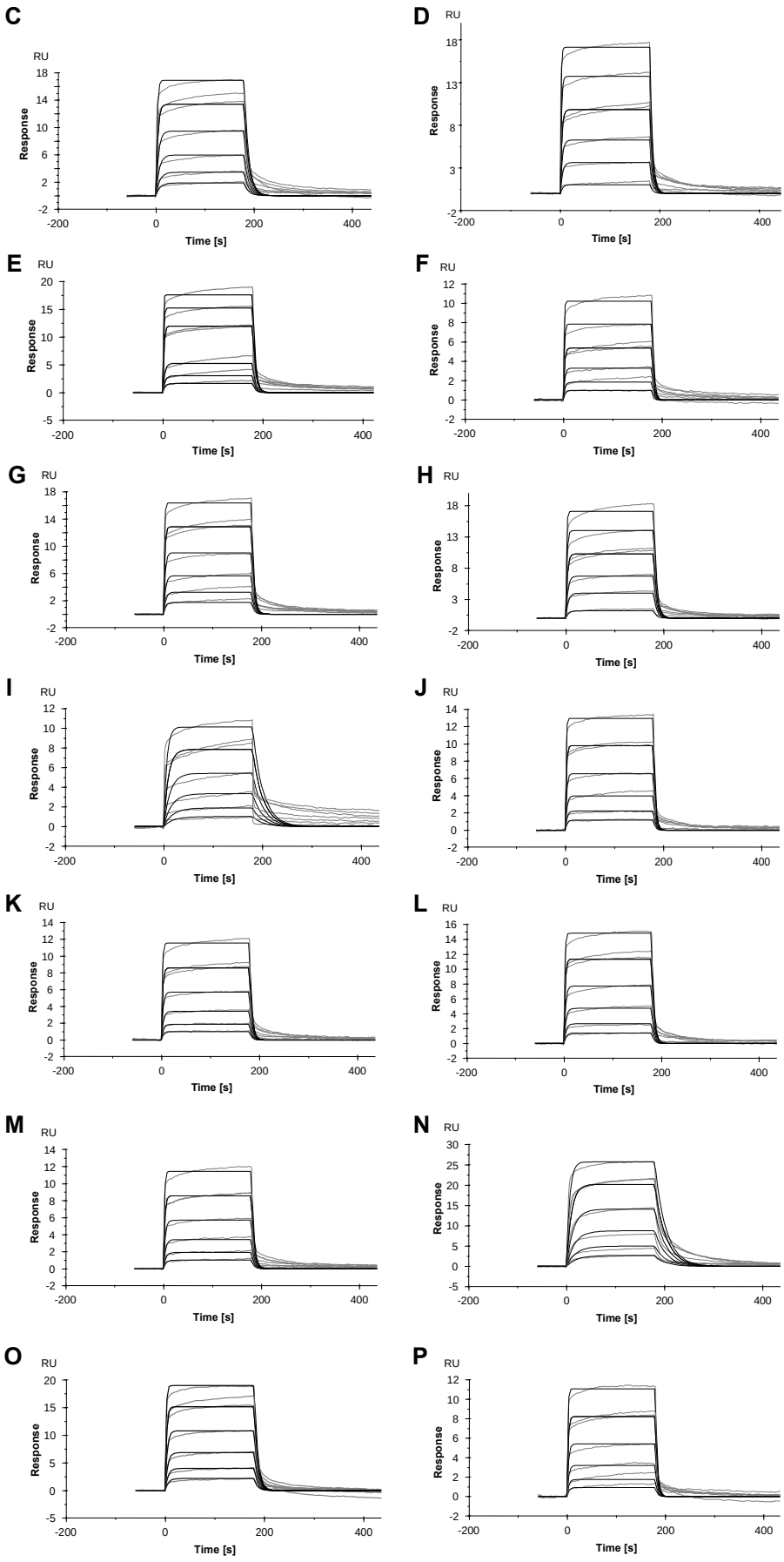

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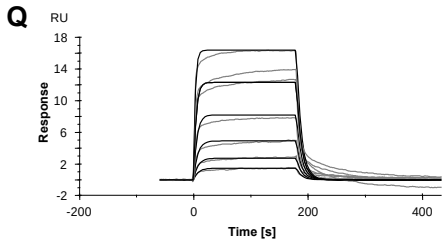

Figure S1. Multi-cycle kinetics of $FimH_{FL-B}$ with two-fold increasing concentrations of A) **3a** (24-750nM); B) **3c** (24-750 nM); C) **3d** (24-750 nM); D) **3e** (24-1500 nM, without 47 nM); E) **3g** (39-2500 nM); F) **3h** (47-1500 nM); G) **3j** (39-1250 nM); H) **3k** (24-1500 nM, without 47 nM); I) **3l** (40-1250 nM); J) **3m** (31-1000 nM); K) **3n** (24-750 nM); L) **3o** (31-1000 nM); M) **3p** (24-750 nM); N) **3q** (24-750 nM); O) **3r** (31-1000 nM); P) **3s** (31-1000 nM); Q) **3t** (24-750 nM).

Fluorescence Polarization Assay

Experiments were conducted as previously described,[S2-3] using a non-biotinylated version of the full-lenth FimH protein ($FimH_{FL}$) and a fluorescently labeled FimH antagonist (**11**, Figure S2),[S2] whose K_D = 137 nM for $FimH_{FL}$ has been previously determined in a direct binding assay.[S3] Stock solutions of the compounds at 50 mM in DMSO were prepared. Starting from 600 µM, 1:2 dilution series were prepared in assay buffer (20 mM HEPES buffer, 150 mM NaCl, 50 µg/mL BSA, pH 7.4). Measurements were done at constant concentrations of $FimH_{FL}$ (300 nM) and fluorescently labeled antagonist (10 nM). The mixtures were incubated for 1 h in 96-well-plates (Corning, flat bottom, non-binding surface). Fluorescence polariza-tion was measured with a Synergy™ H1 Multi-Mode microplate reader (BioTek Intruments). Equilibrium dissociation constants were determined using Prism (GraphPad Software) and the Wang equation.[S4] The results are depicted in Table S2.

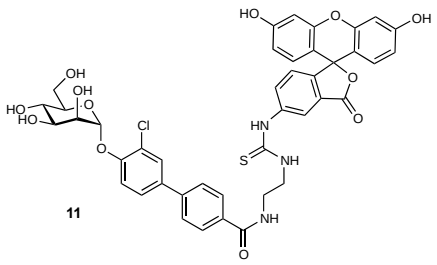

Figure S2. Fluorescently labeled FimH antagonist **11** used in the fluorescence polarization assay.[S2,S3]

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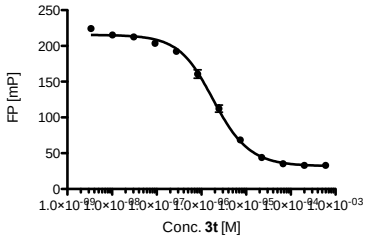
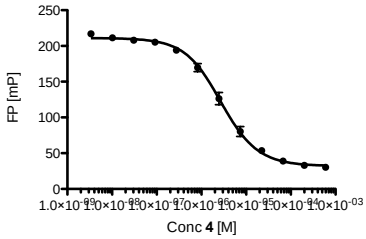
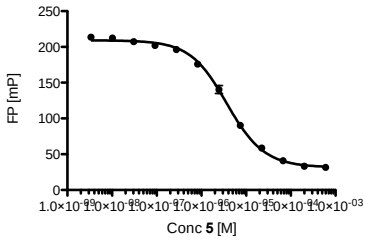
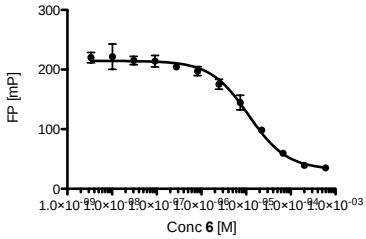
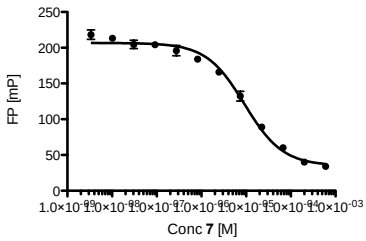
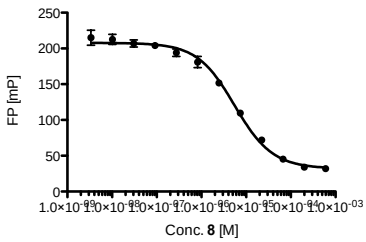
Table S2. Affinities obtained by fluorescent polarization assay.

Compound	Curve	K_D [nM]
3t		515.0
5		783.6
6		1097
7		3498
8		2828
9		1673

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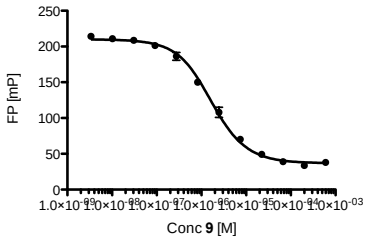
10		447.5

HPLC Traces of DCC Experiments

Purity of Target Compounds

HRMS of Target Compounds

The LC/HRMS analysis were carried out using an Agilent 1100 LC equipped with a photodiode array detector and a Micromass QTOF I equipped with a 4 GHz digital-time converter. The results are summarized in Table S9.

Table S7. Results of HRMS analysis of acylhydrazones and bioisosteres.

Compound	Formula for $[M+Na]^+$	HRMS [m/z]	
		calcd	**found**
3a	$C_{19}H_{20}FN_3NaO_7$	444.1183	444.1181
3c	$C_{18}H_{20}FN_3NaO_7S$	464.0904	464.0905
3d	$C_{19}H_{22}FN_3NaO_7S$	478.1060	478.1061
3e	$C_{19}H_{19}ClFN_3NaO_7$	478.0793	478.0799
3g	$C_{19}H_{22}FN_3NaO_7$	446.1339	446.1341
3h	$C_{19}H_{21}FN_2NaO_8$	447.1182	447.1182
3j	$C_{21}H_{23}FN_2NaO_8$	473.1336	473.1336
3k	$C_{22}H_{22}FN_3NaO_7$	482.1339	482.1340
3l	$C_{23}H_{24}FN_3NaO_7$	496.1496	496.1496
3m	$C_{21}H_{23}FN_2NaO_7$	457.1387	457.1387
3n	$C_{19}H_{21}FN_2NaO_7S$	463.0951	463.0954
3o	$C_{20}H_{20}ClFN_2NaO_7$	477.0841	477.0841
3p	$C_{19}H_{20}ClFN_2NaO_7S$	497.0561	497.0561
3q	$C_{18}H_{18}ClFN_2NaO_7S$	483.0405	483.0406
3r	$C_{24}H_{23}FN_2NaO_7$	493.1387	493.1388
3s	$C_{21}H_{20}F_4N_2NaO_7$	511.1104	511.1107
3t	$C_{22}H_{20}ClFN_2NaO_7S$	533.0561	533.0562
5	$C_{20}H_{23}FN_2NaO_7$	445.1387	445.1386
6	$C_{20}H_{23}FN_2NaO_7$	445.1387	445.1392
7	$C_{20}H_{23}FN_2NaO_7$	445.1387	445.1385
8	$C_{20}H_{23}FN_2NaO_6S$	461.1159	461.1161
9	$C_{20}H_{23}FN_2NaO_6S$	461.1159	461.1160
10	$C_{21}H_{22}FNNaO_8$	458.1227	458.1227

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HPLC of Target Compounds

System: Agilent 1100/1200 with UV detector (190-410 nm) and Agilent 380 ELSD detector. Column: Waters Atlantis T3, 3 µm, 2.1 × 100 mm (Waters Corporation). A: H_2O + 0.01% TFA; B: MeCN + 0.01% TFA. Detection: Light scattering (Nebulizer control 70%, drift tube temperature 50 °C, gas pressure 50 psi, gain 500). Gradient: 5% B → 95% B (20 min); flow rate: 0.5 mL/min. The results of the purity analysis are summarized in Table S8.

Table S8. HPLC analysis of target compounds.

Compound	Retention time [min]	Purity (%)
3a	5.990	>99.50
3c	7.316	>99.50
3d	7.247	>99.50
3e	7.484	>99.50
3f[1]	7.627	>99.50
3g	7.625	>99.50
3h	7.667	>99.50
3j	7.922	>99.50
3k	7.937	>99.50
3l	8.142	>99.50
3m	8.335	>99.50
3n	8.289	>99.50
3o	8.718	>99.50
3p	8.871	>99.50
3q	8.885	>99.50
3r	9.214	>99.50
3s	9.335	>99.50
3t	9.858	>99.50
3u[1]	10.087	>99.50
5	7.677	>99.50
6	8.178	>99.50
7	8.380	>99.50
8	8.849	>99.50
9	7.960	>99.50
10	7.962	>99.50

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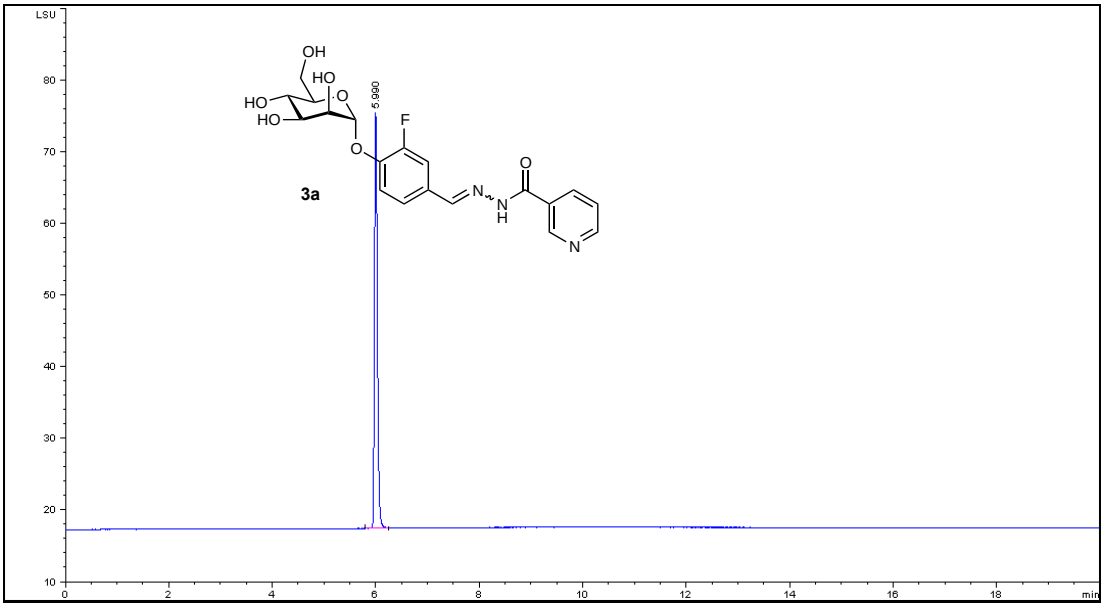
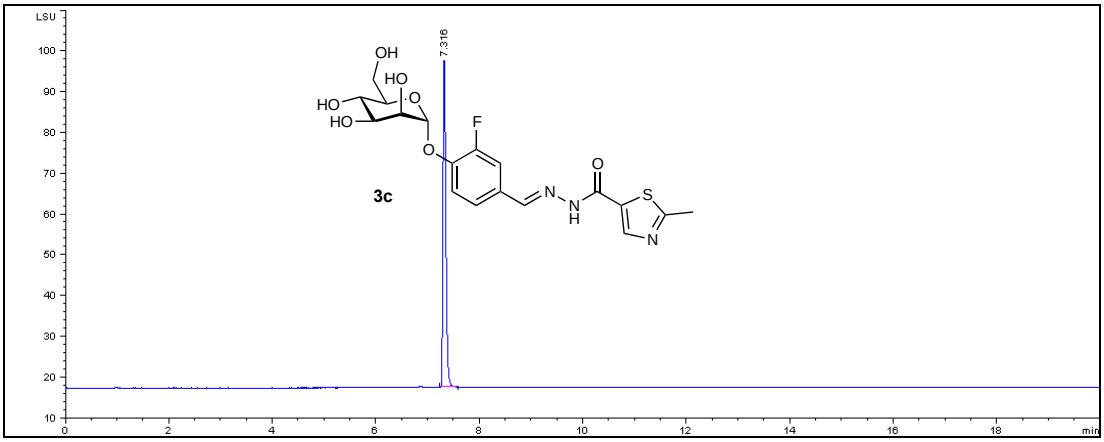
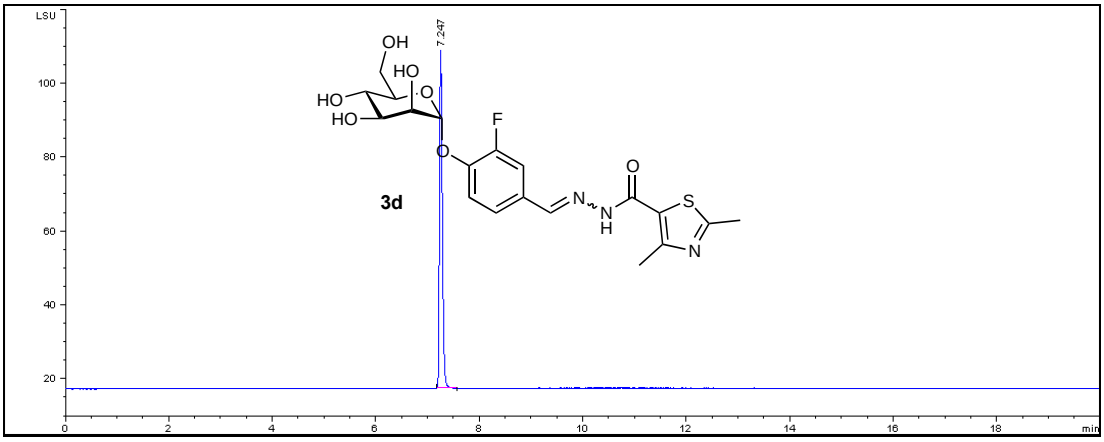
HPLC Traces of Target Compounds







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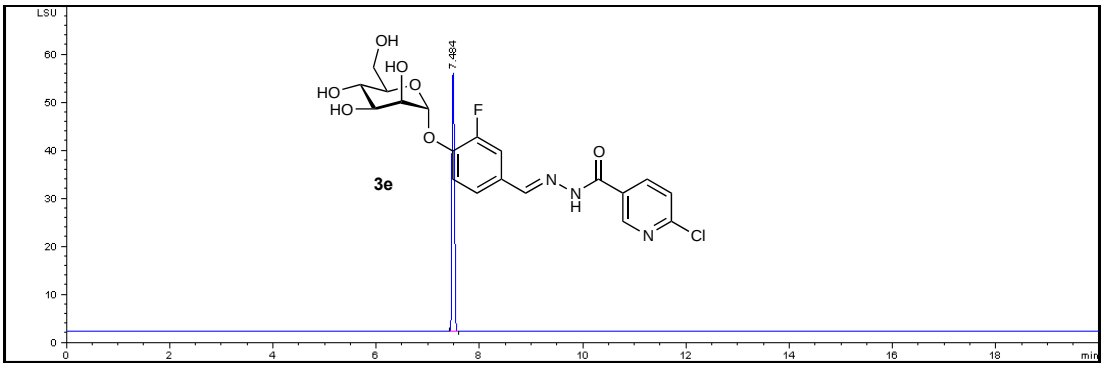

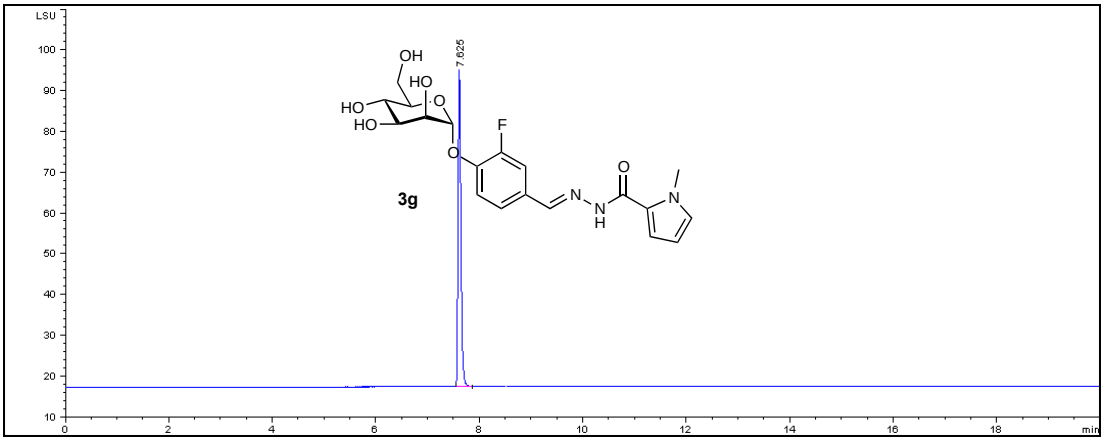

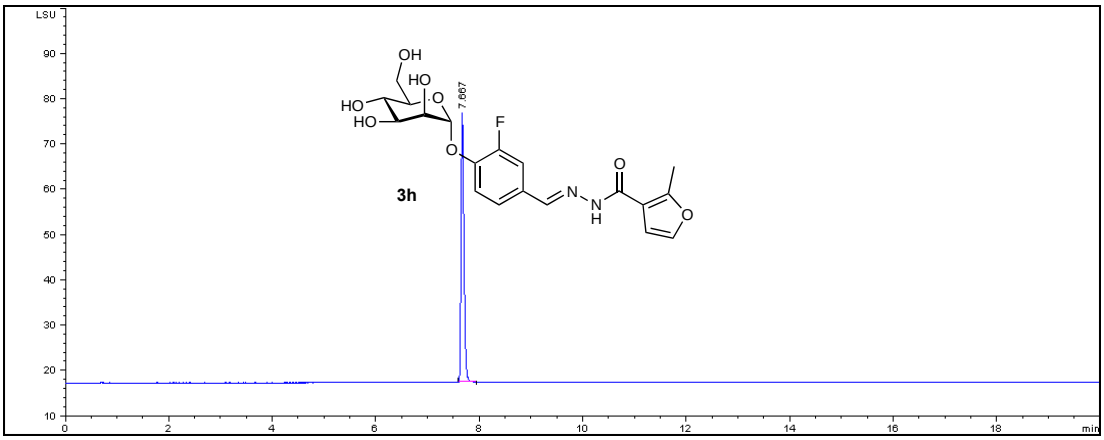

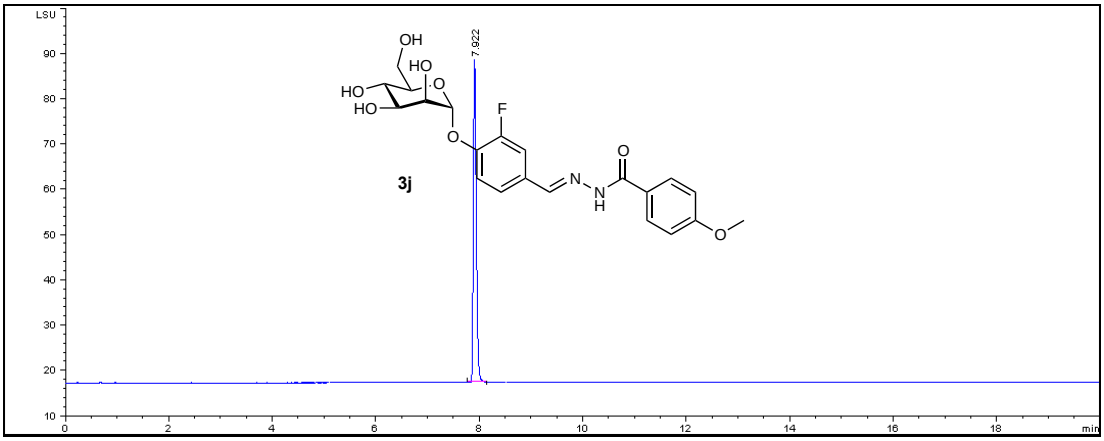

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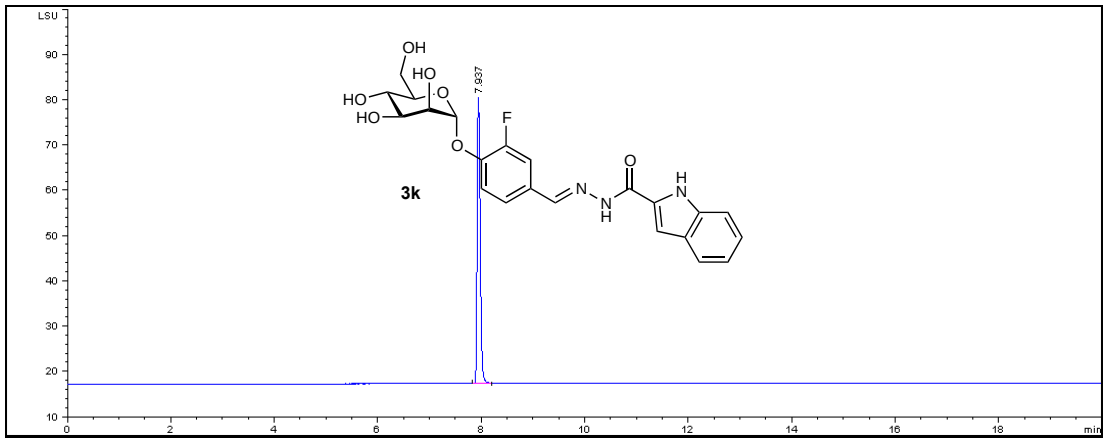

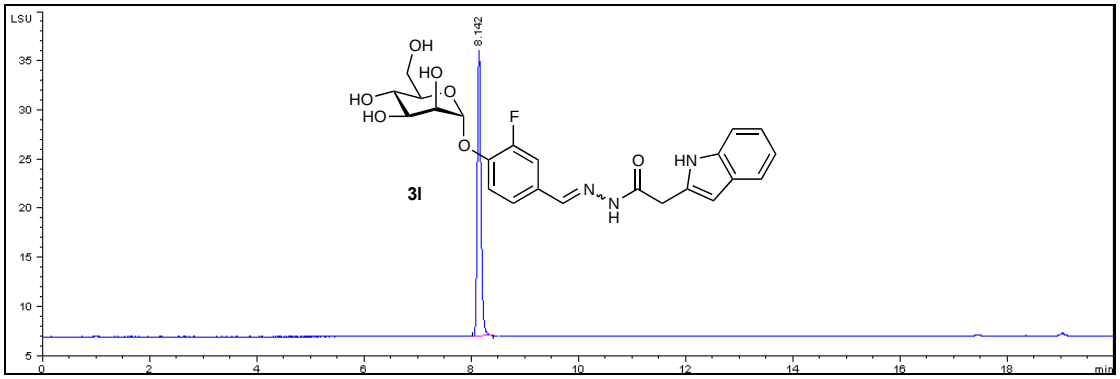

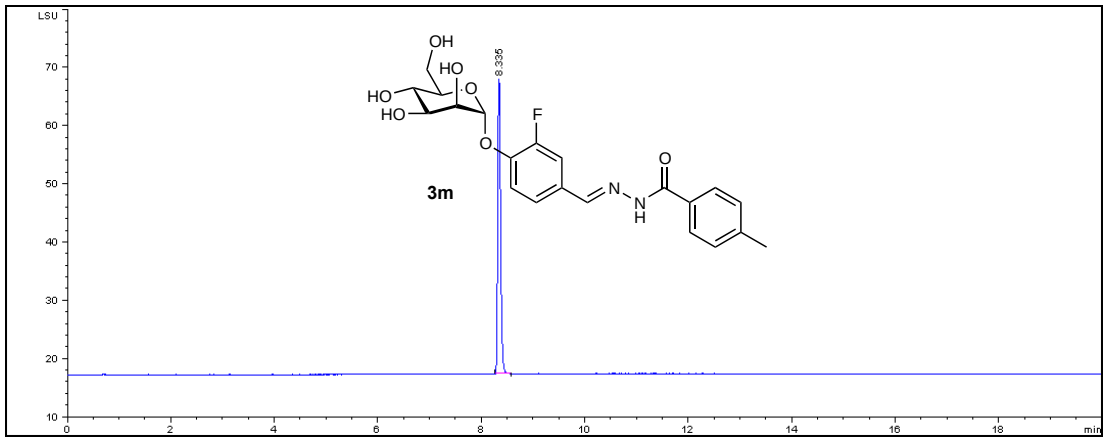

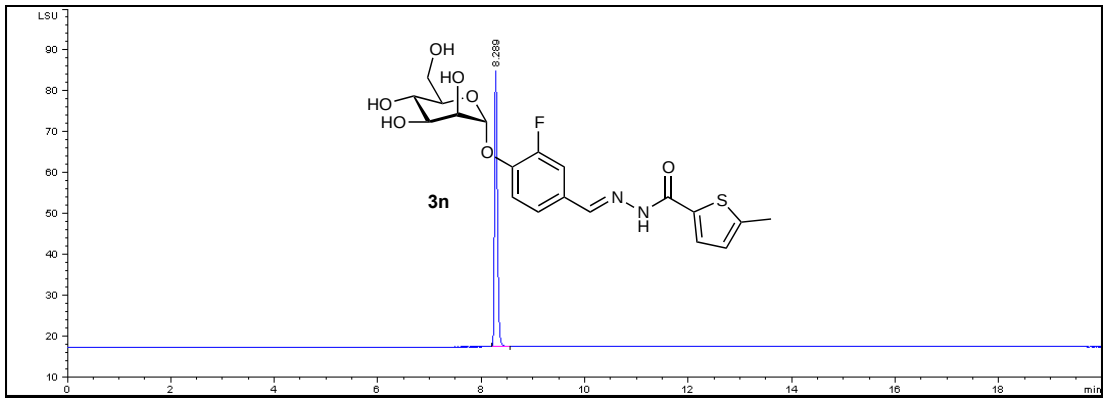

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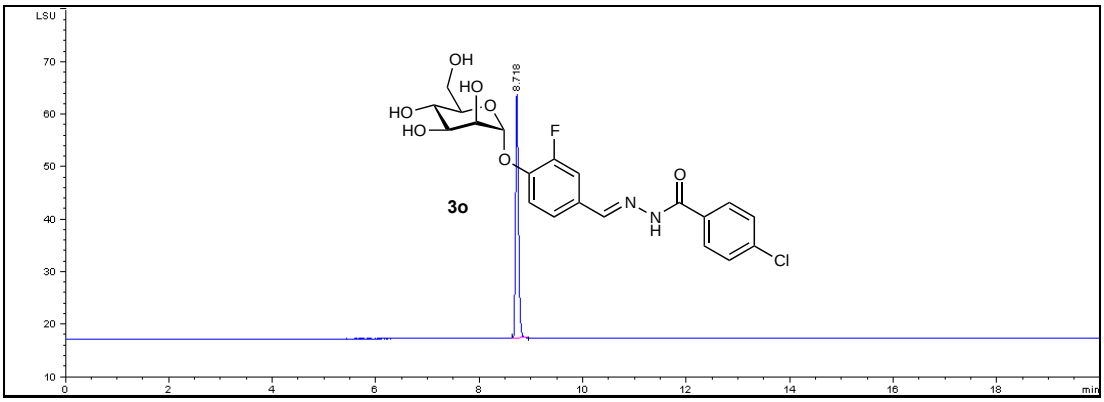

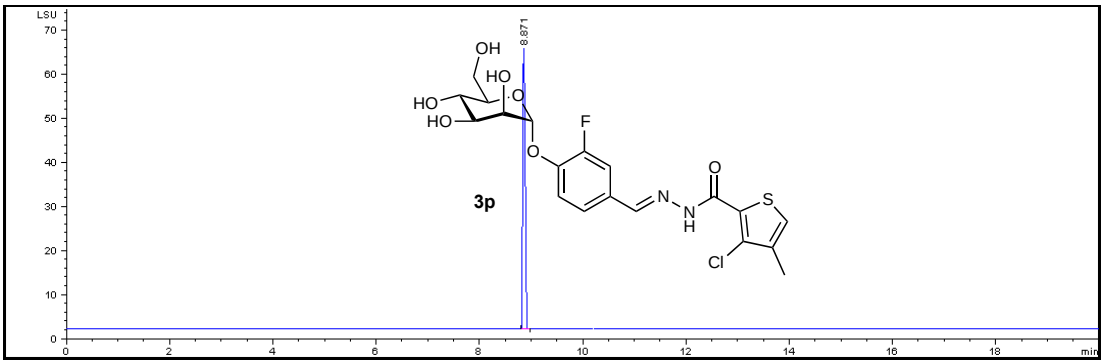

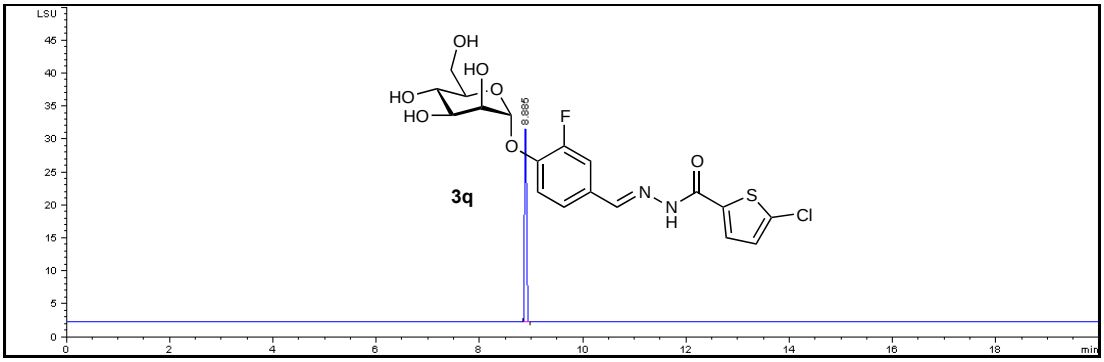

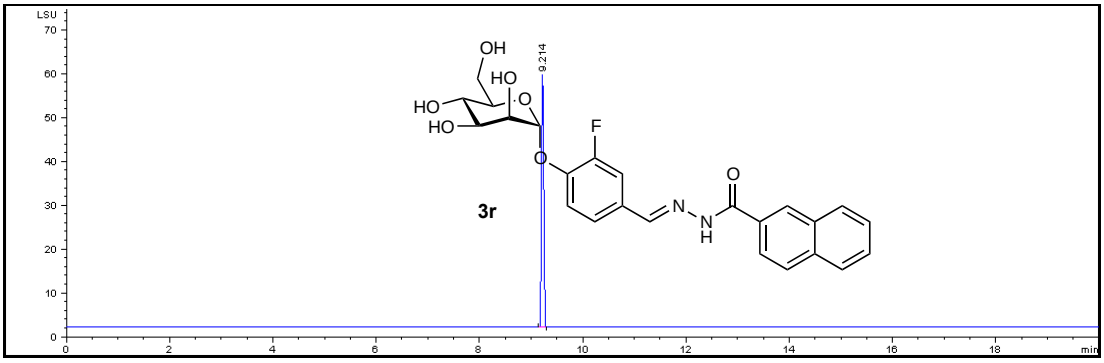

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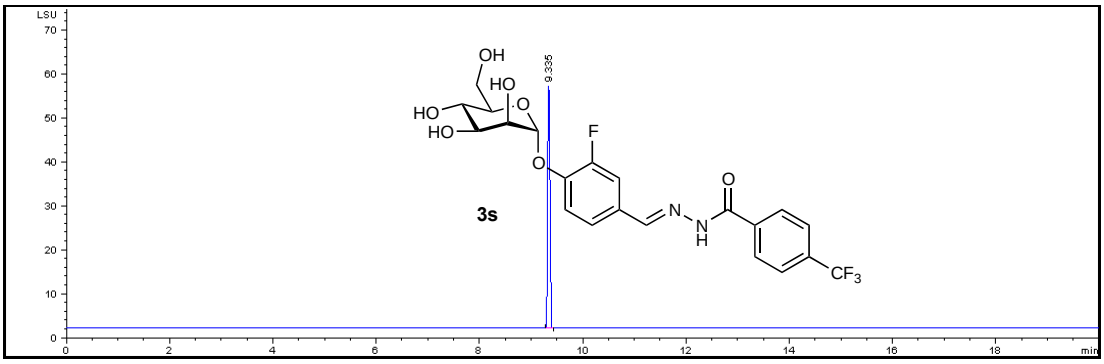

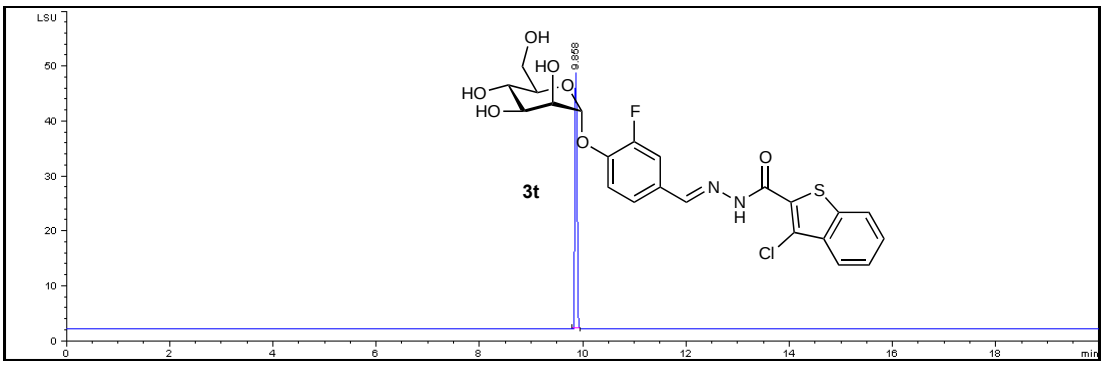

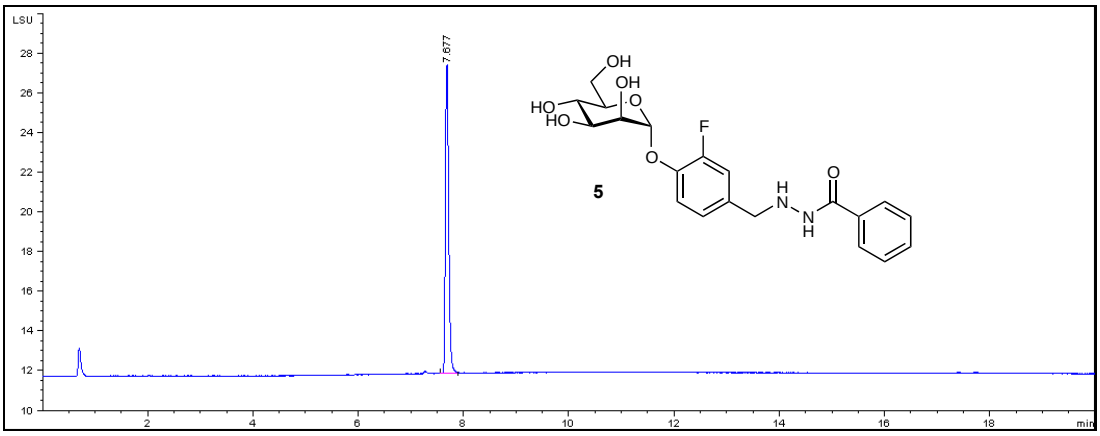

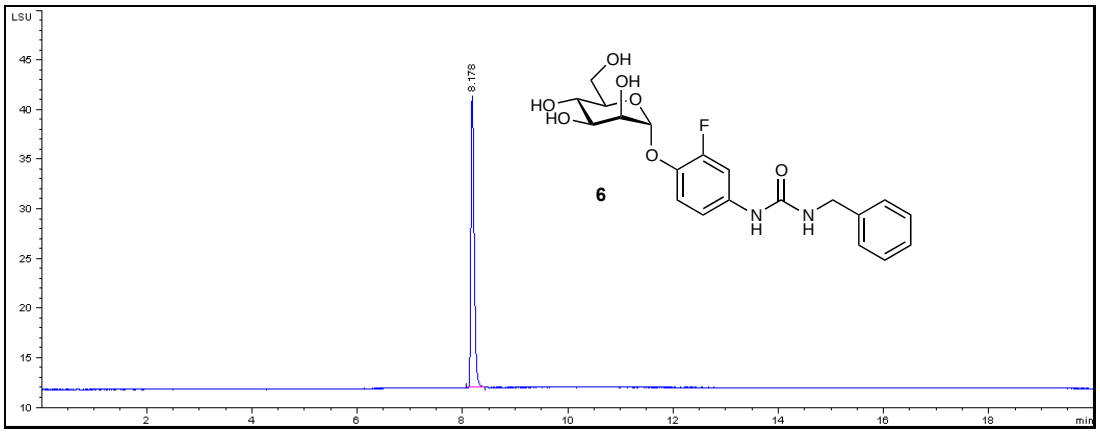

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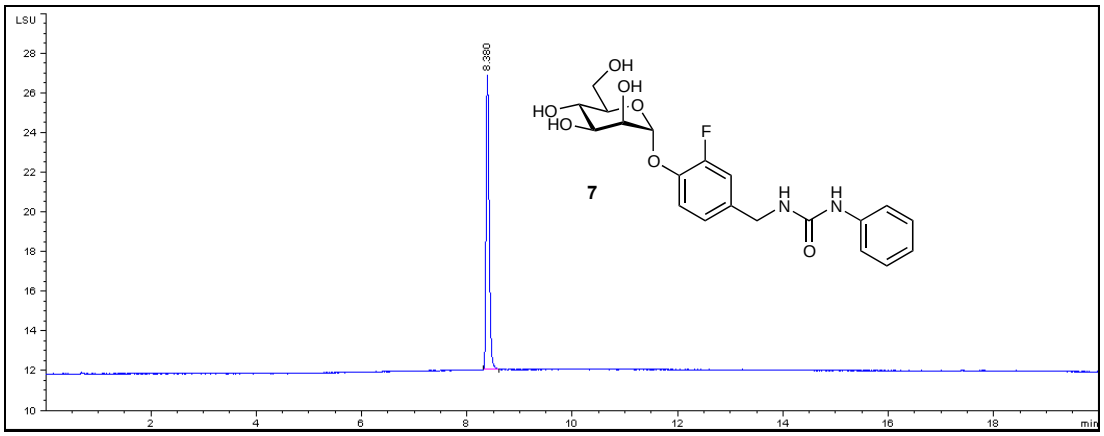

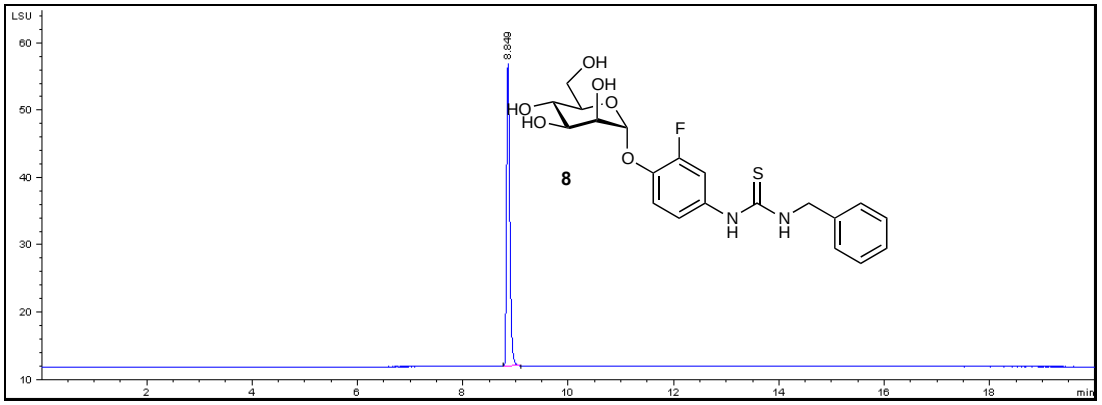

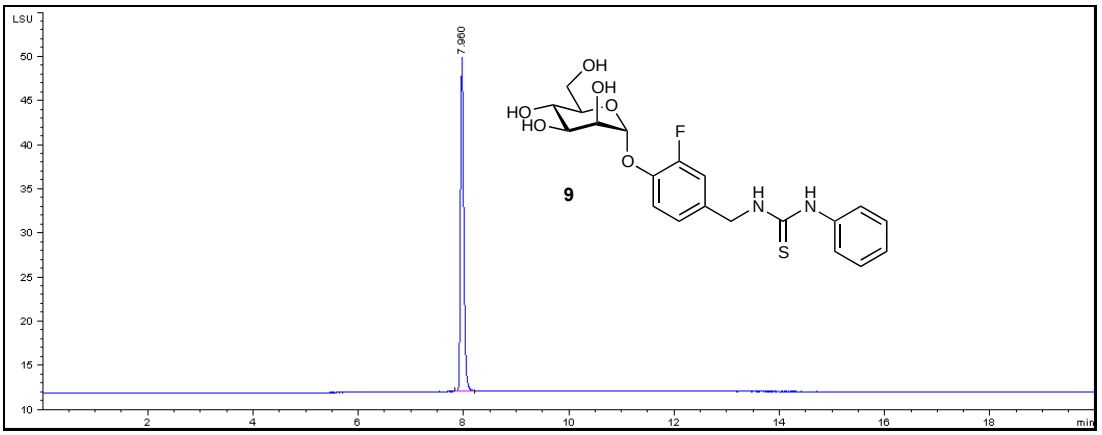

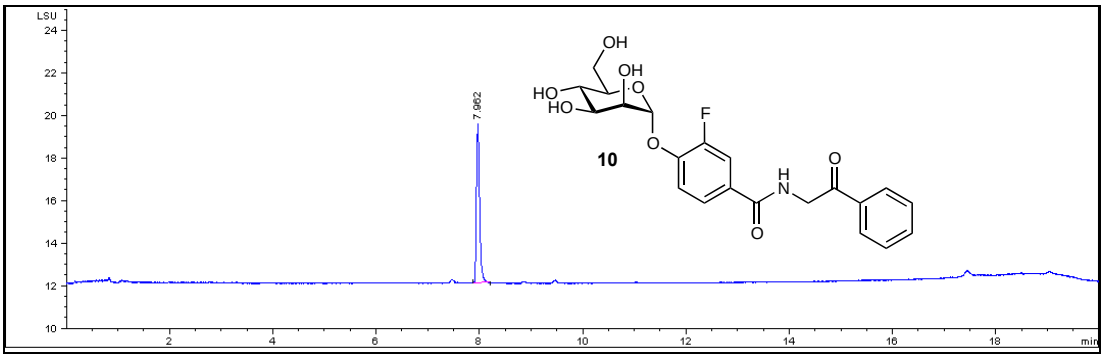

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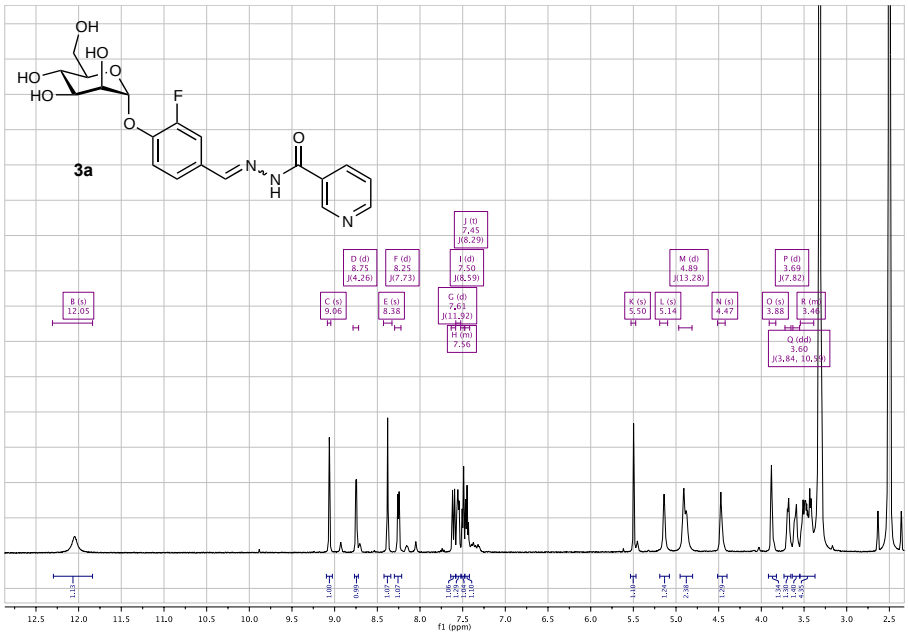
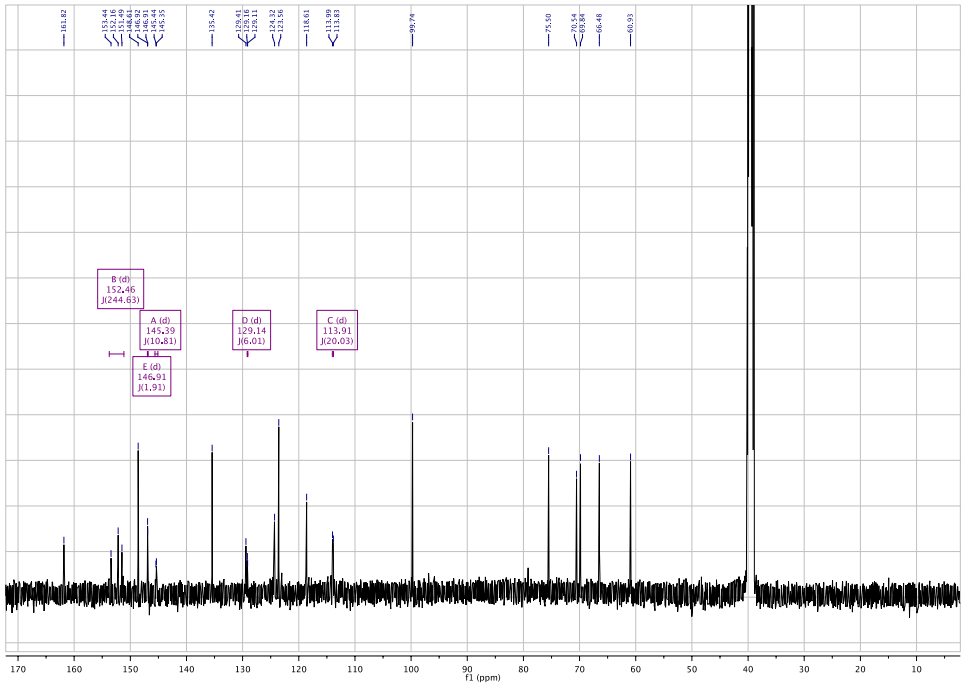
^{1}H & ^{13}C NMR Spectra of Target Compounds





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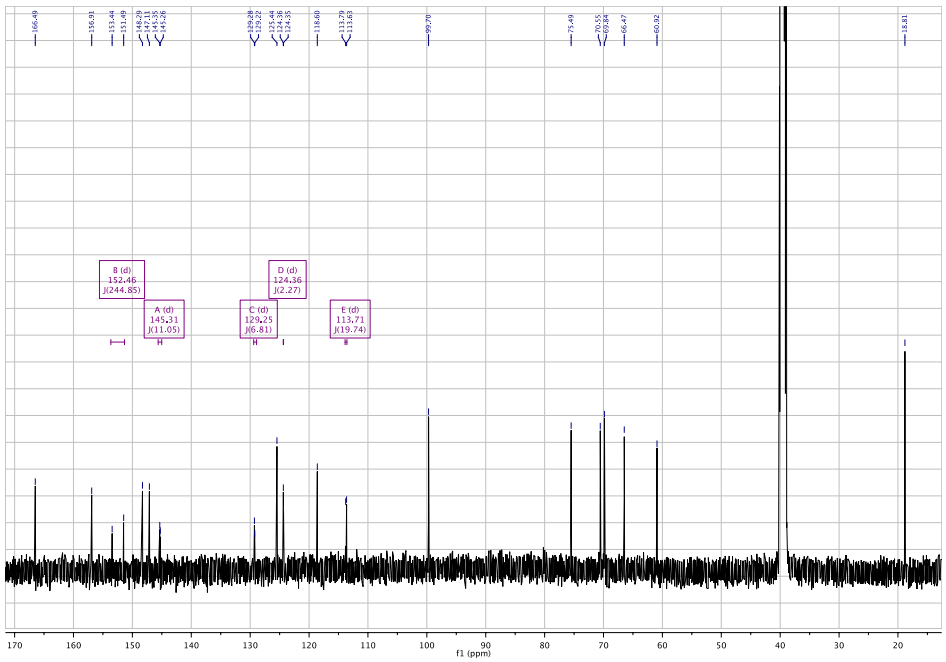

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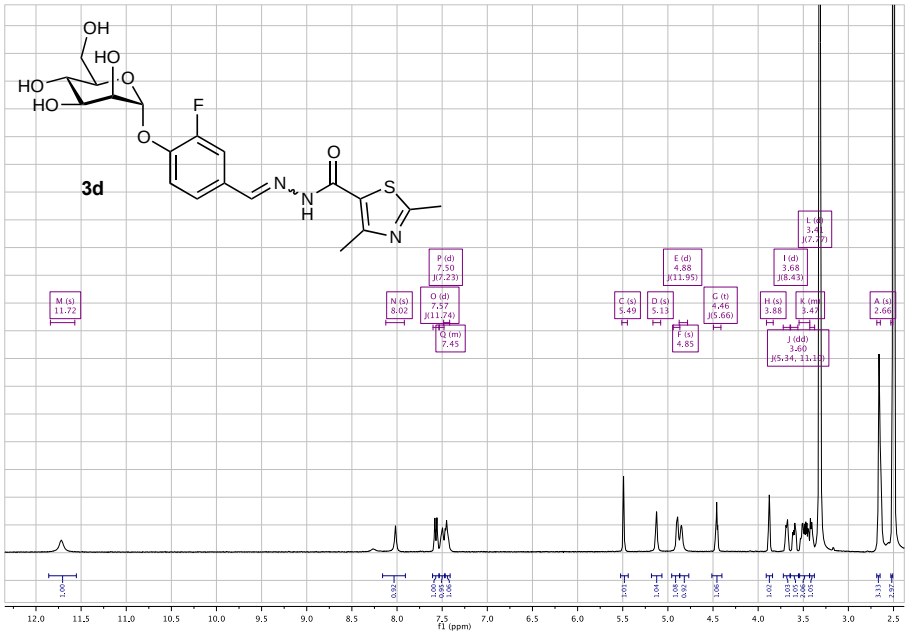

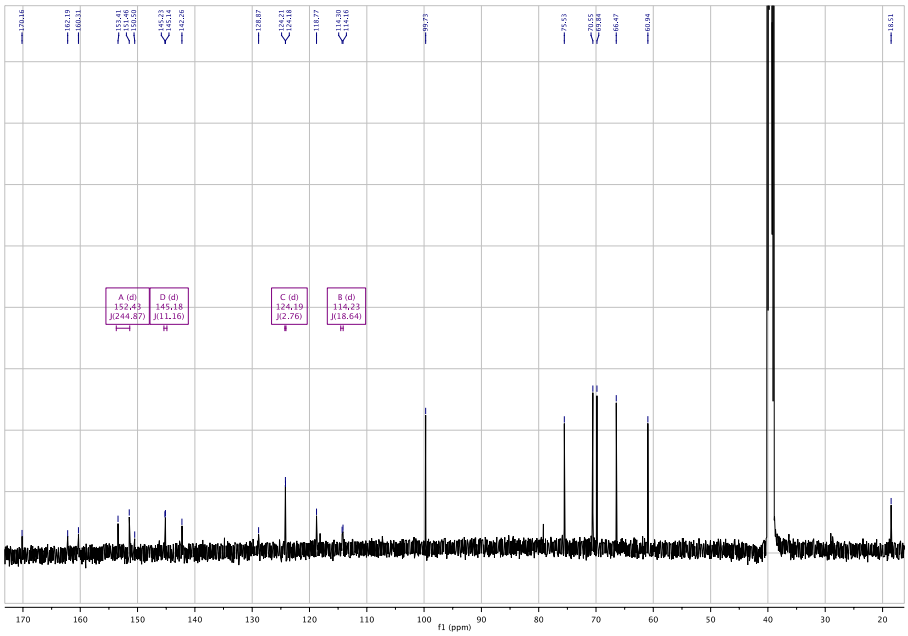

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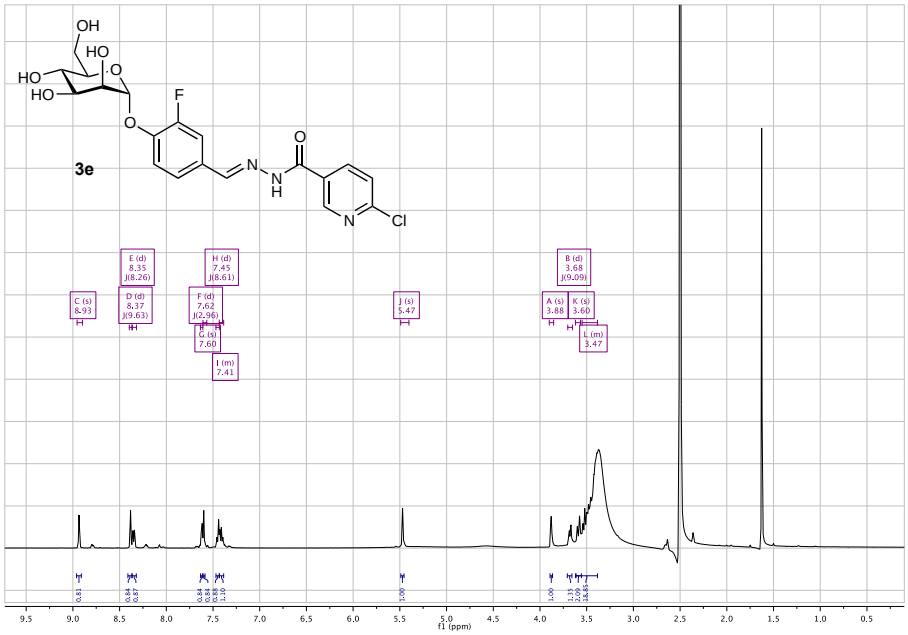

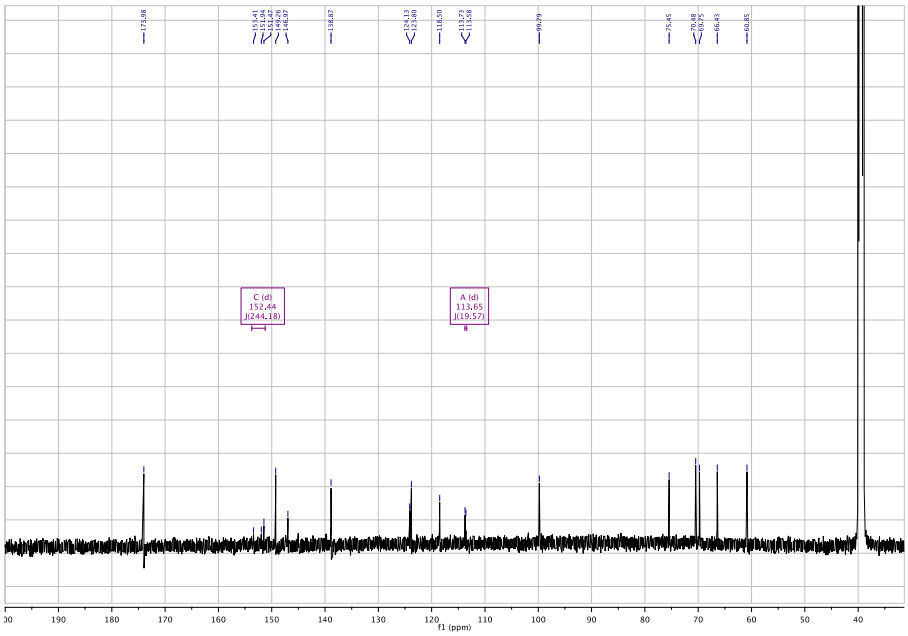

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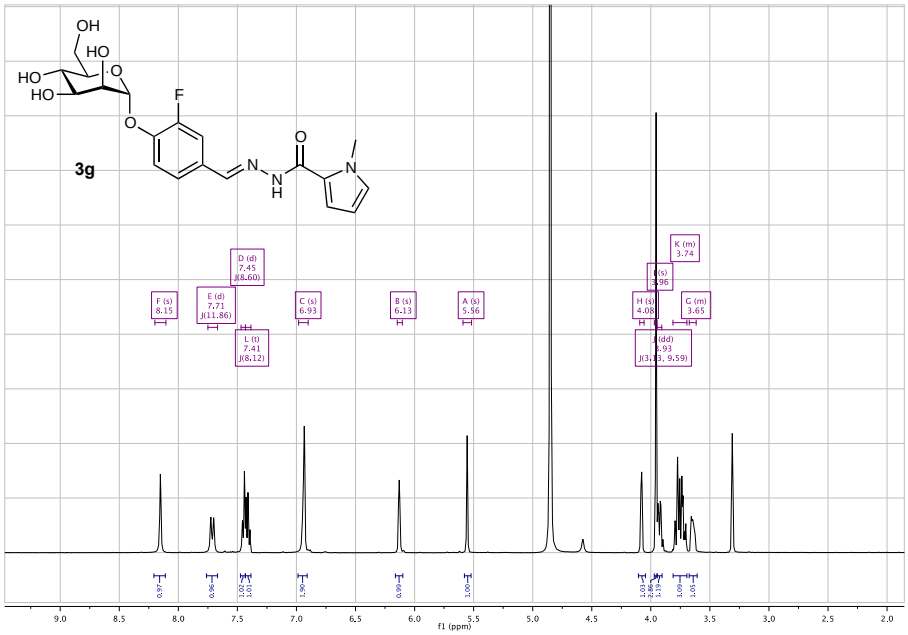

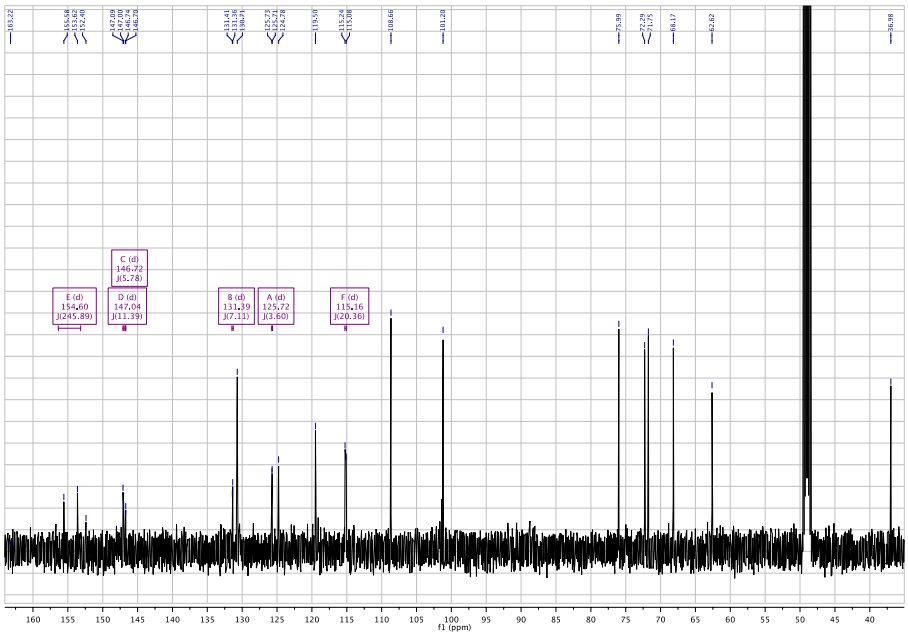

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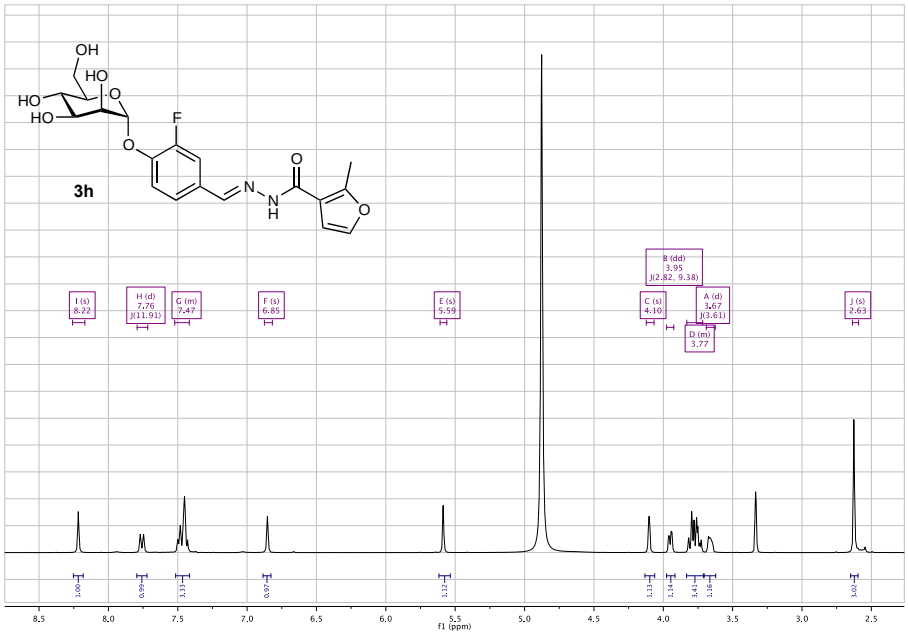

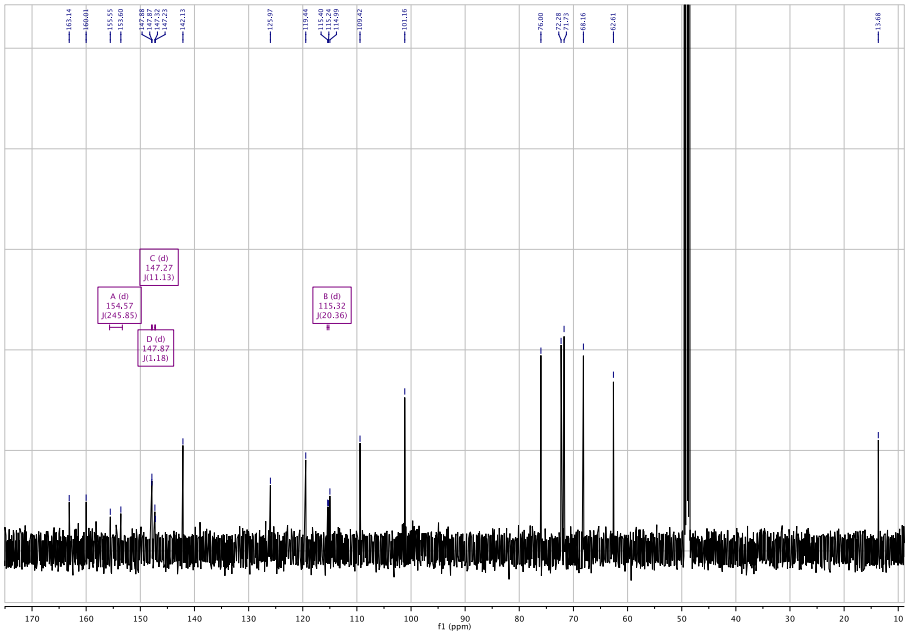

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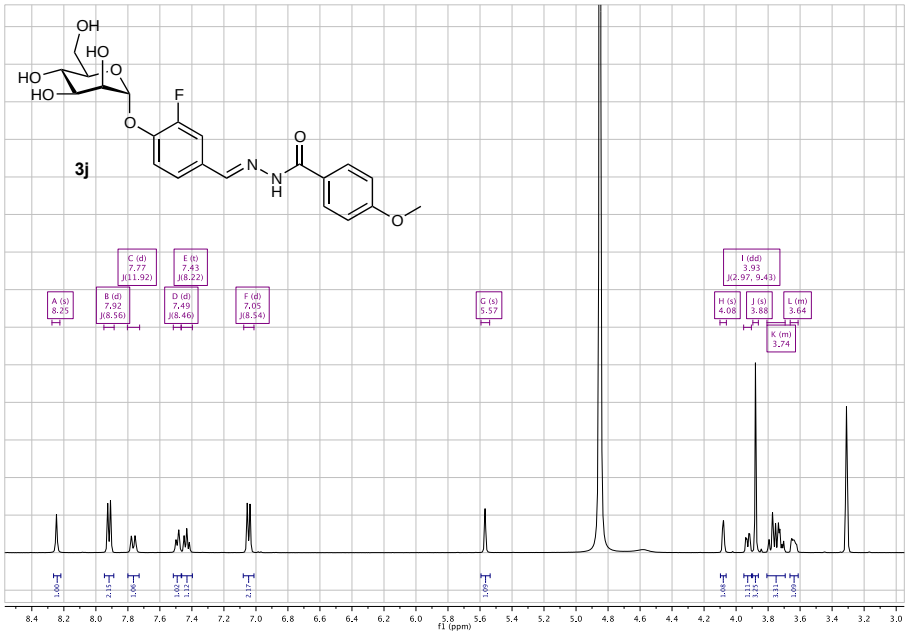

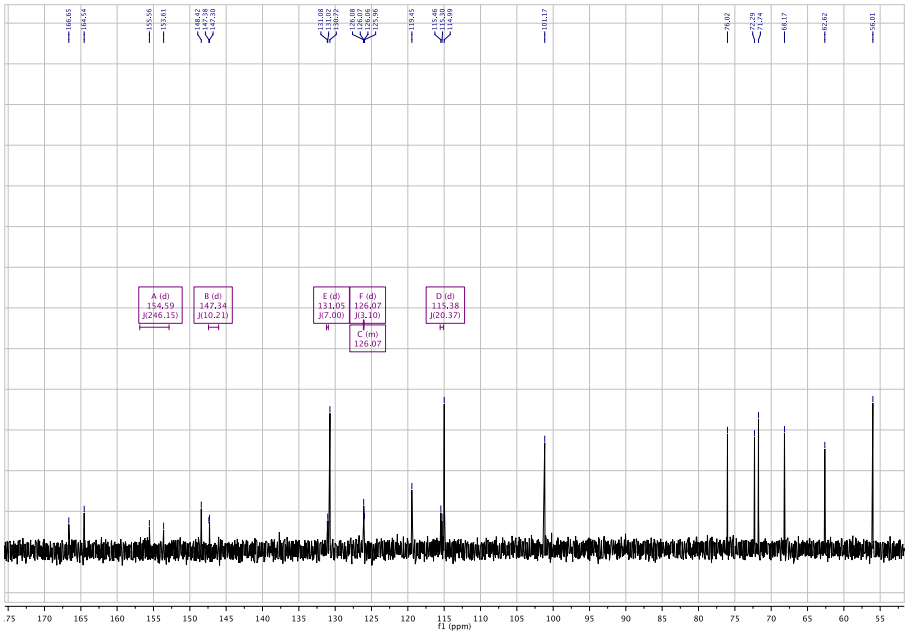

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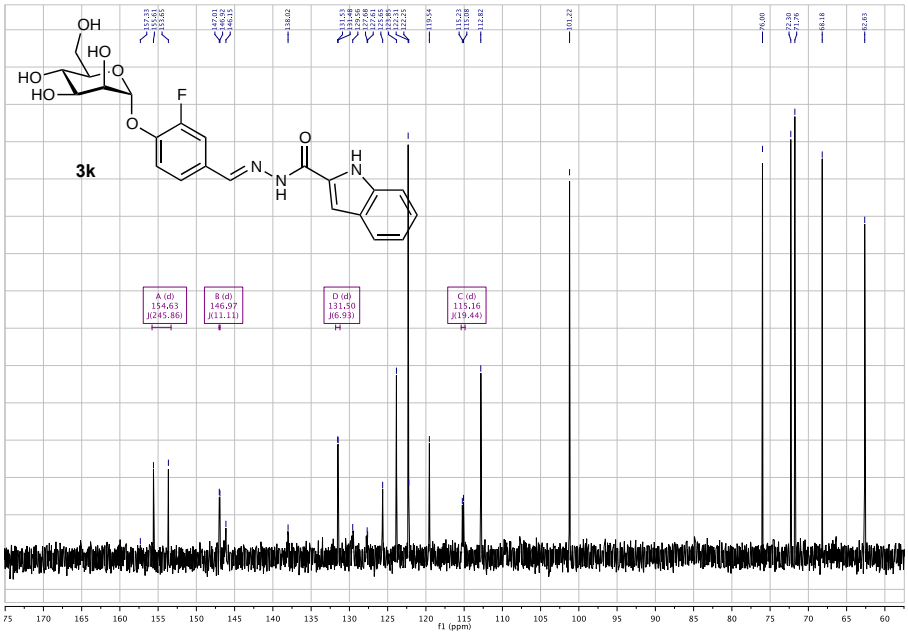

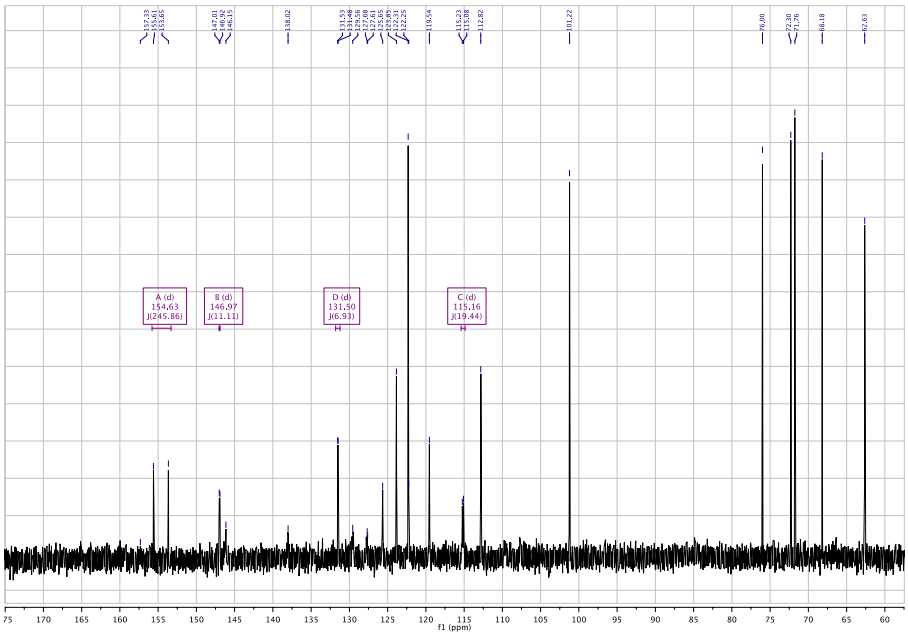

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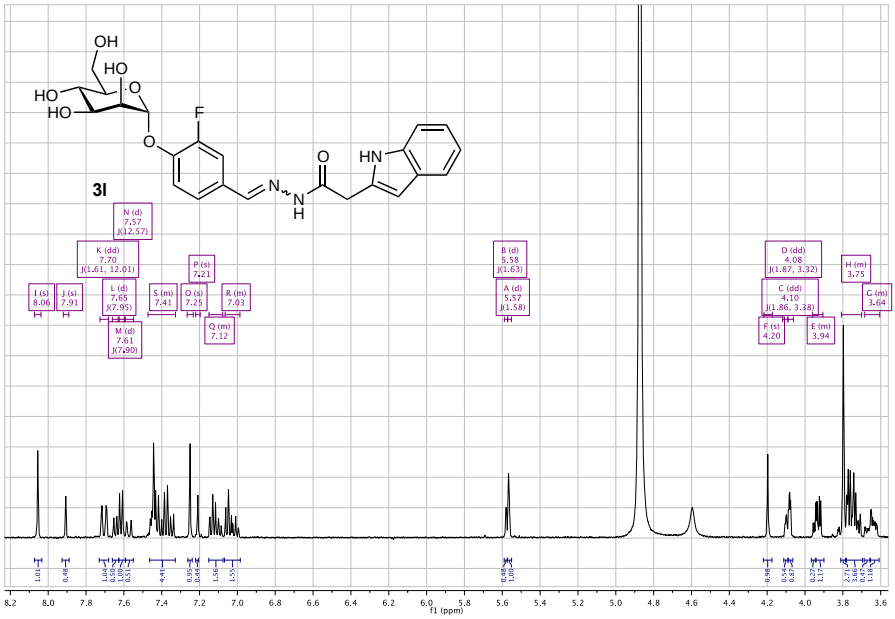

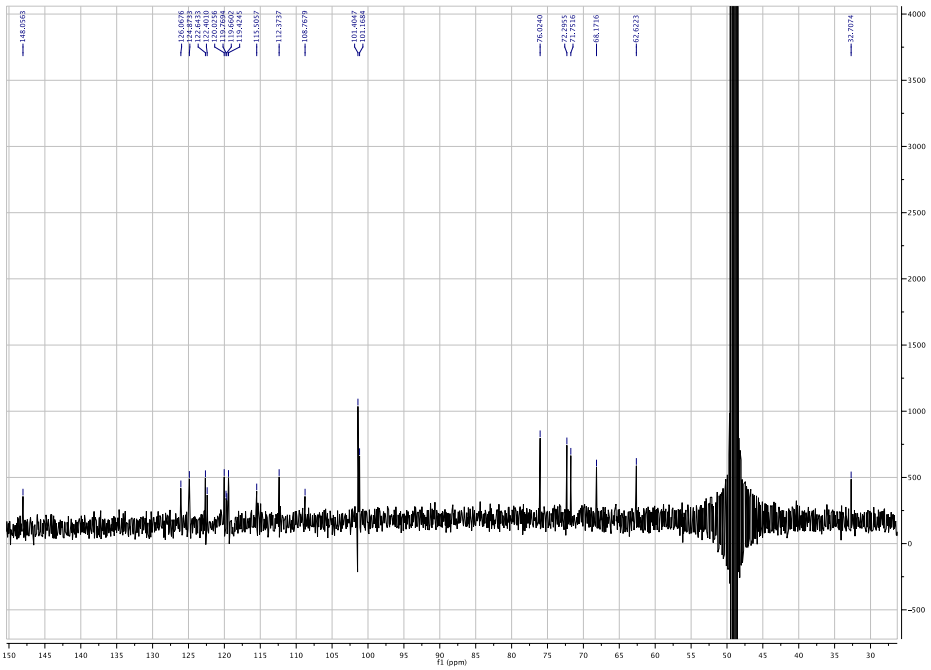

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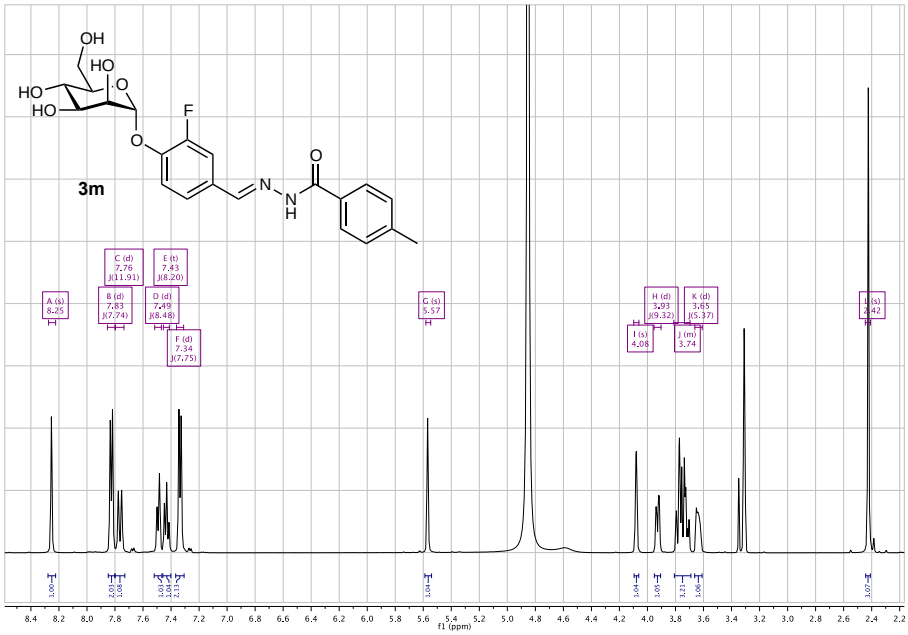

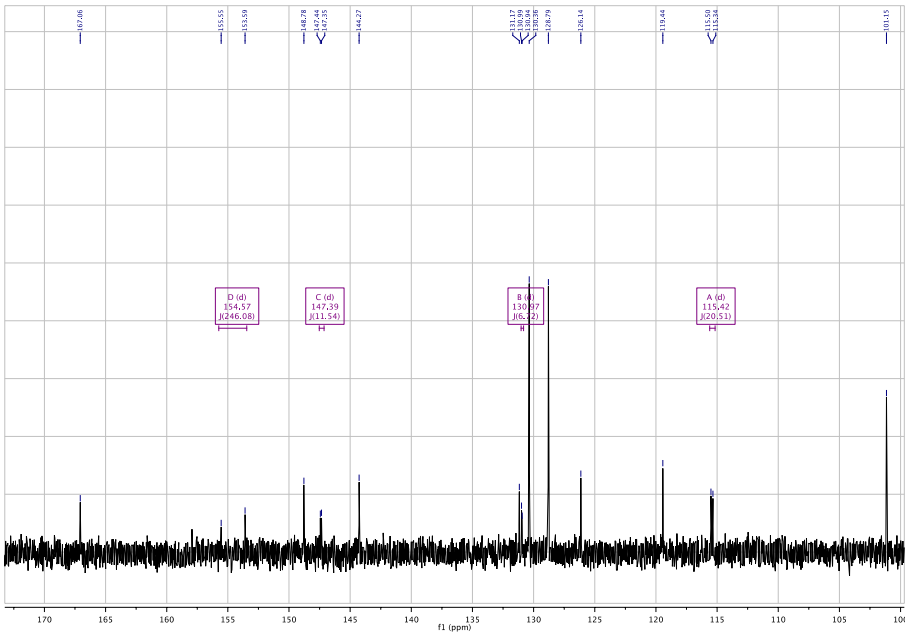

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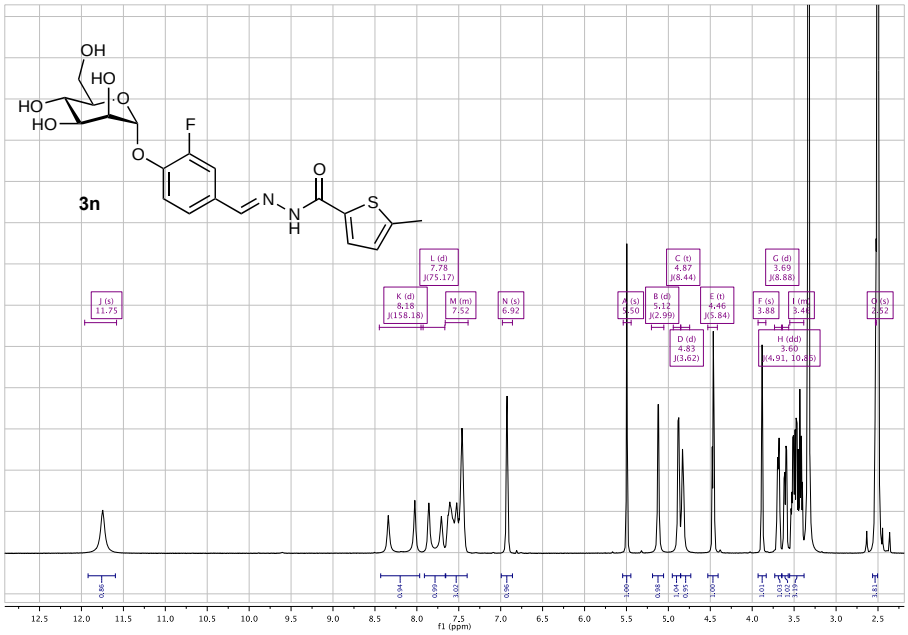

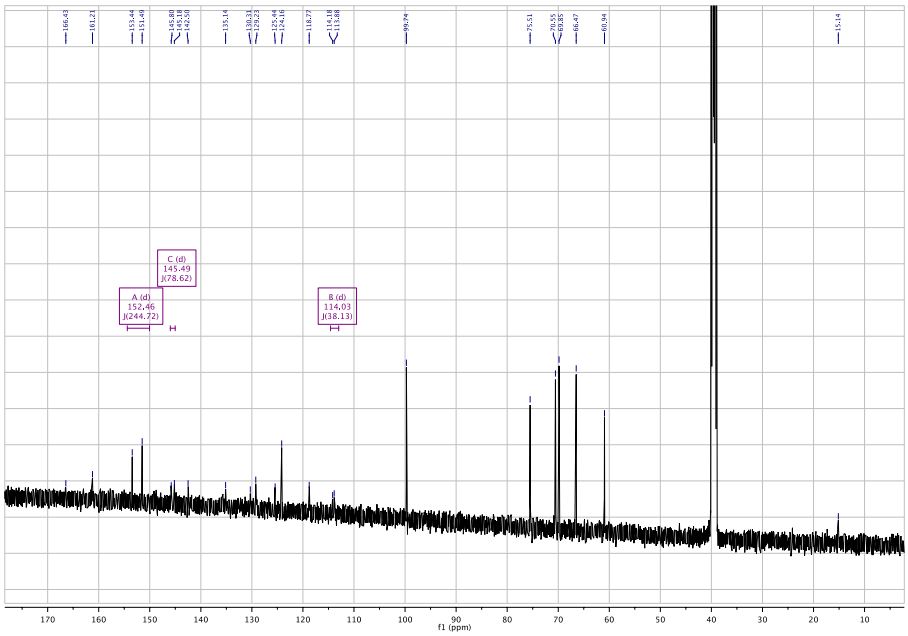

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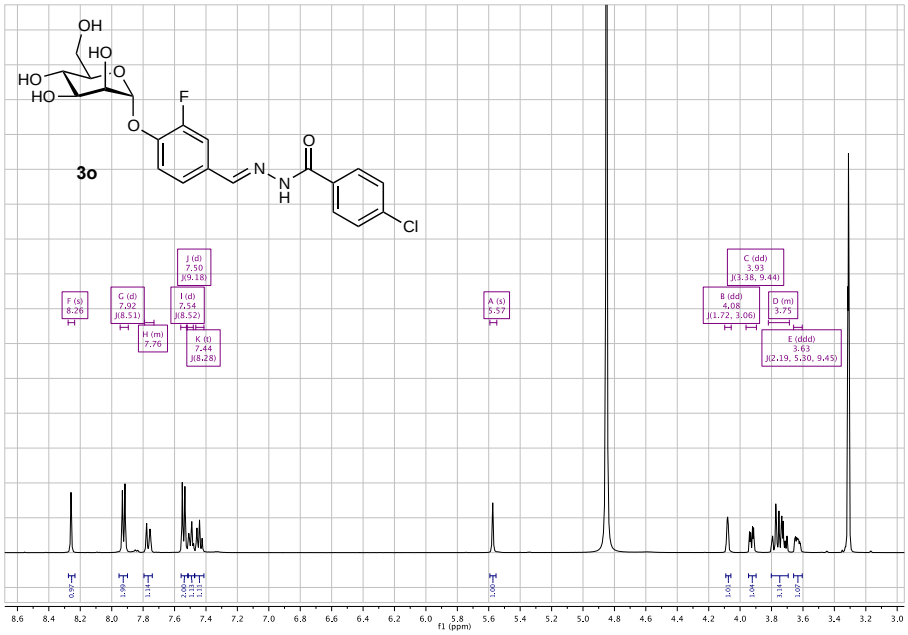

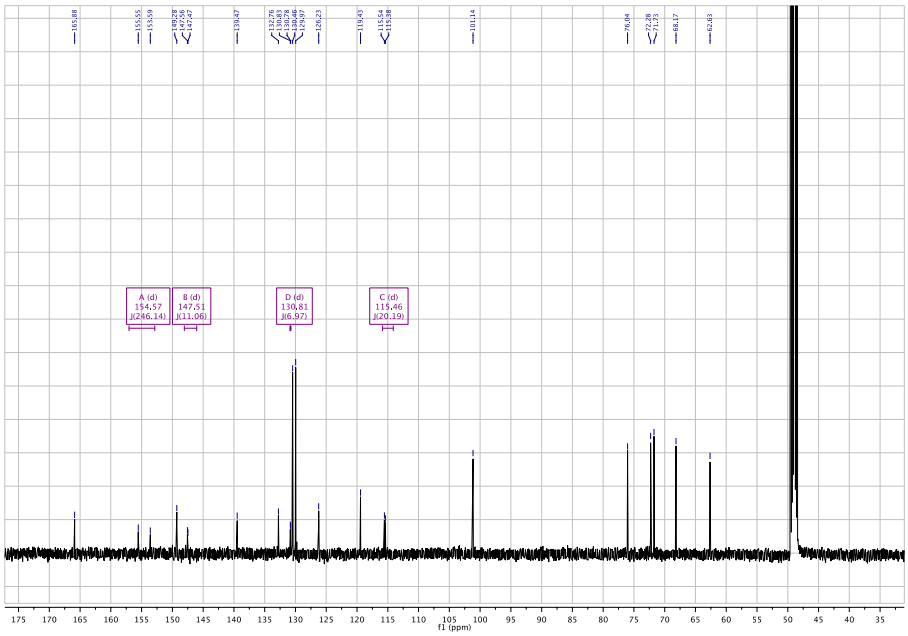

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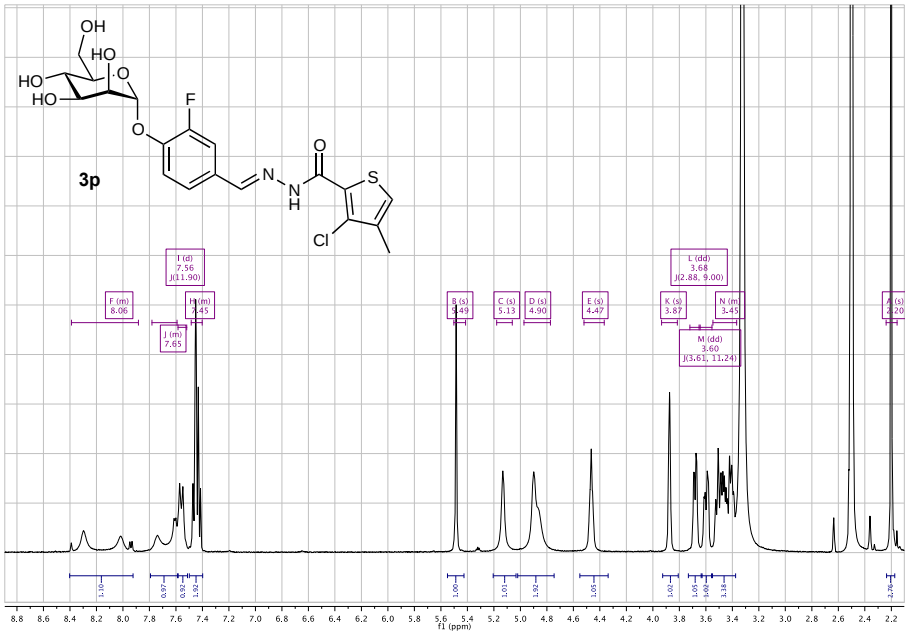

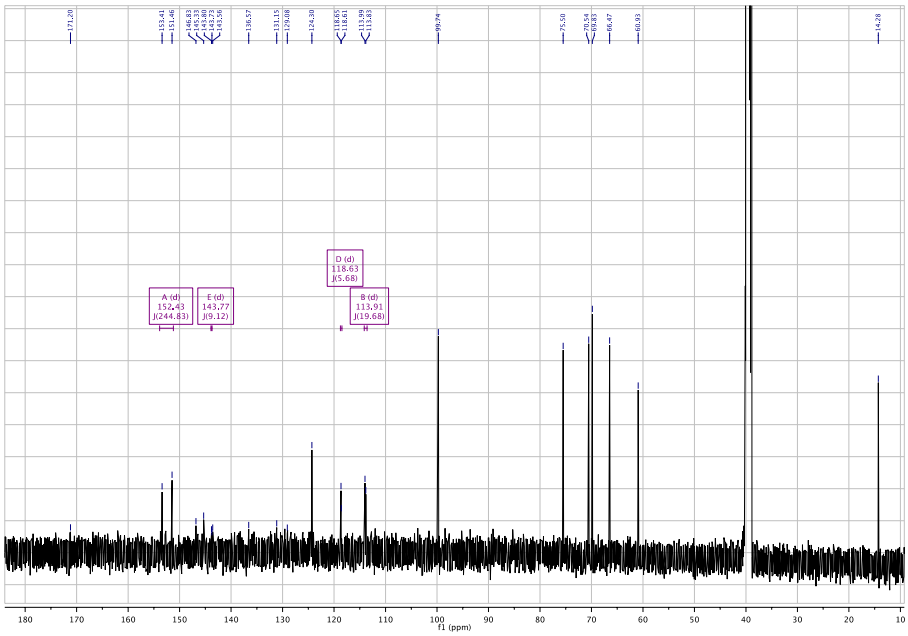

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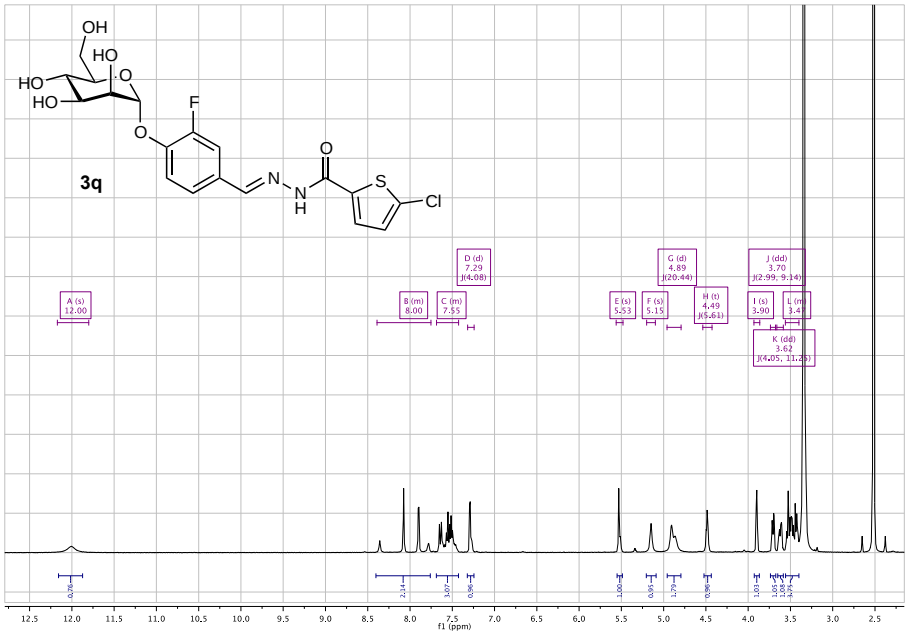

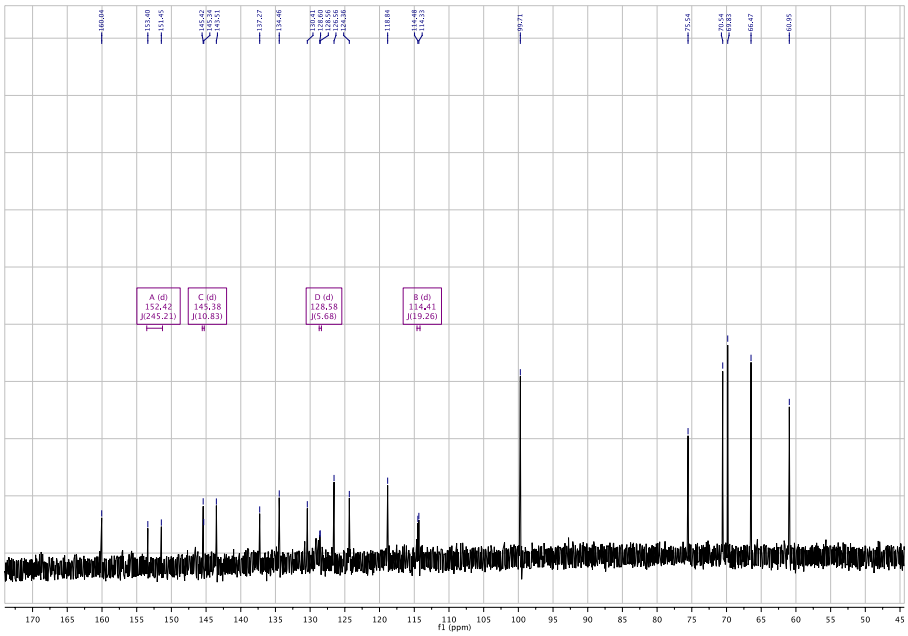

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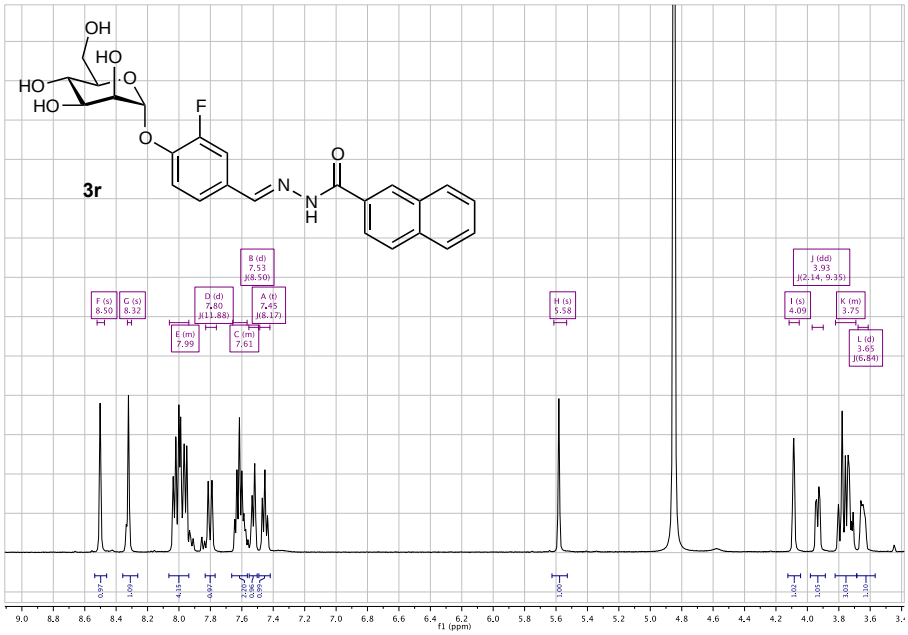

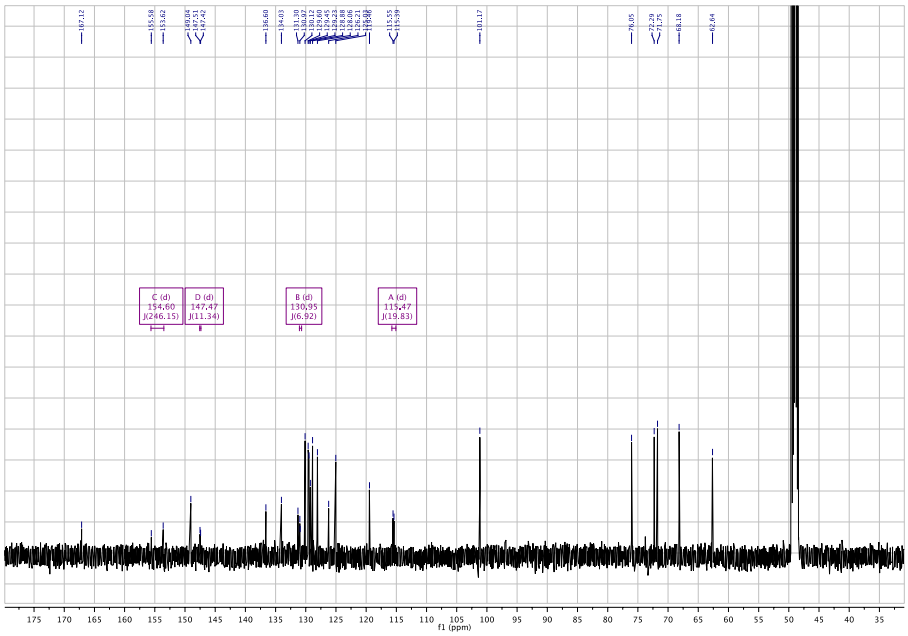

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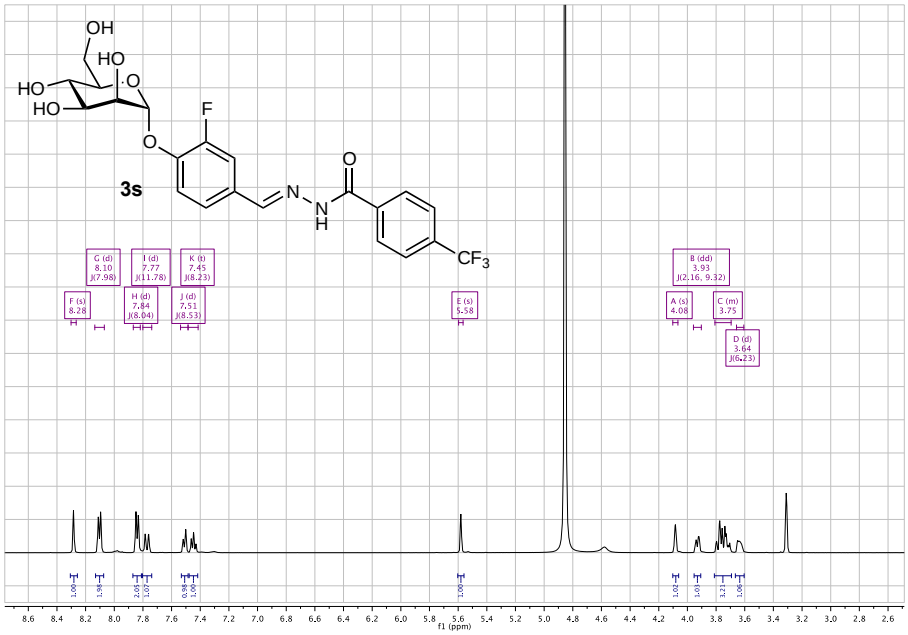

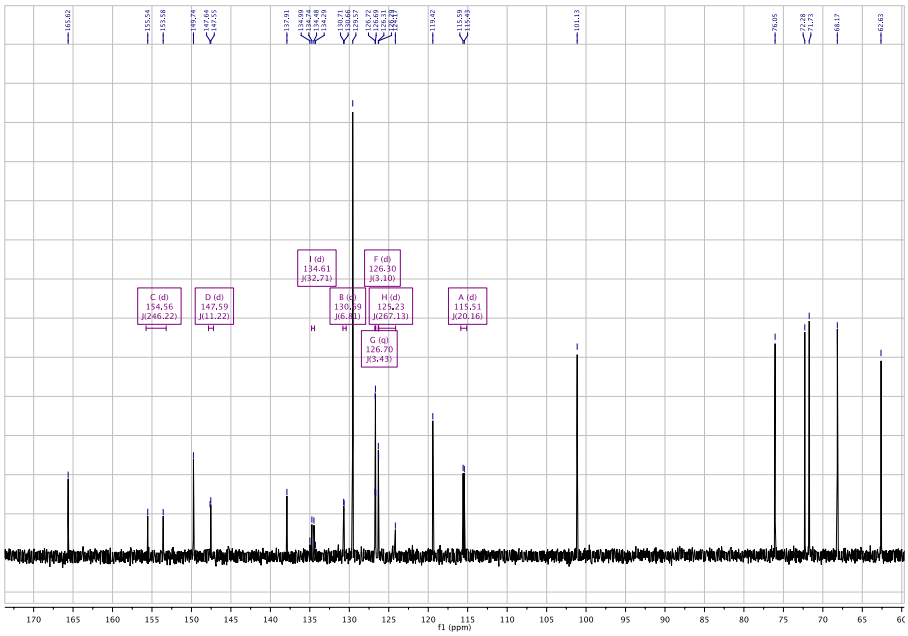

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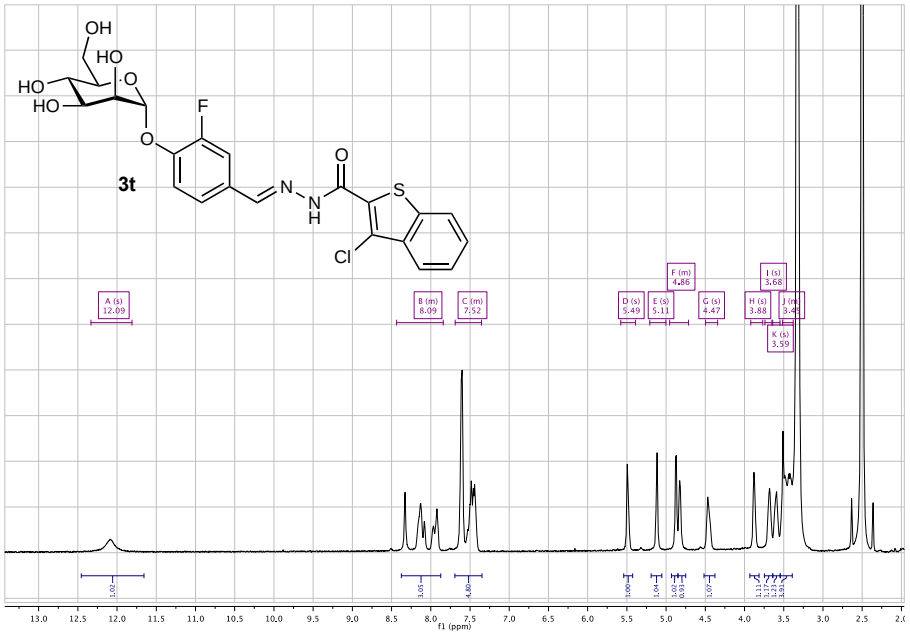

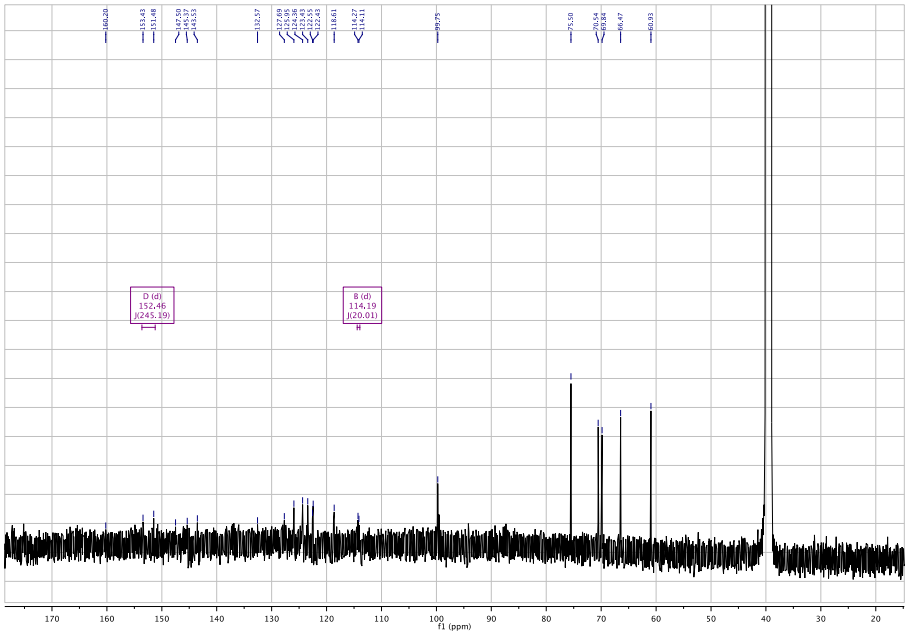

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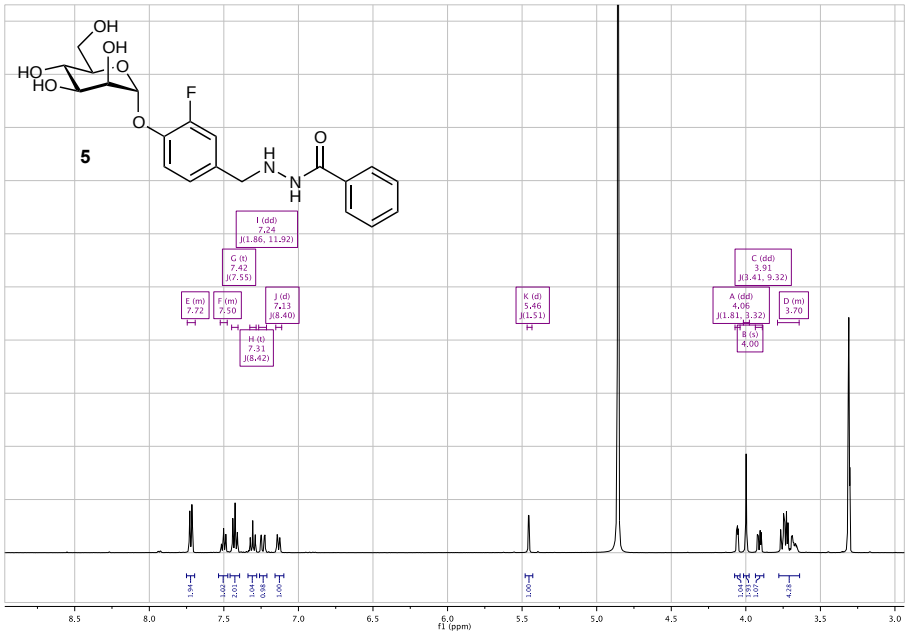

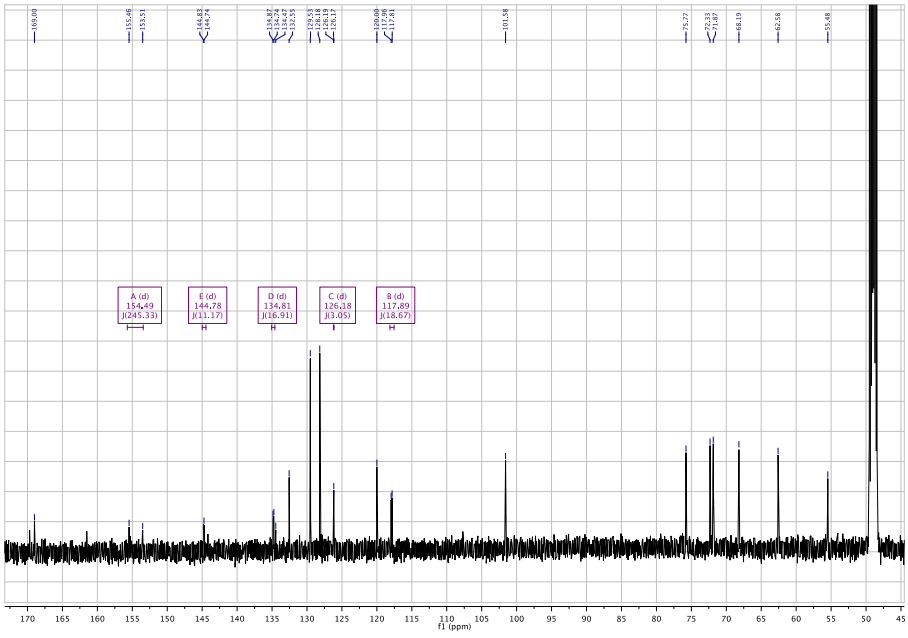

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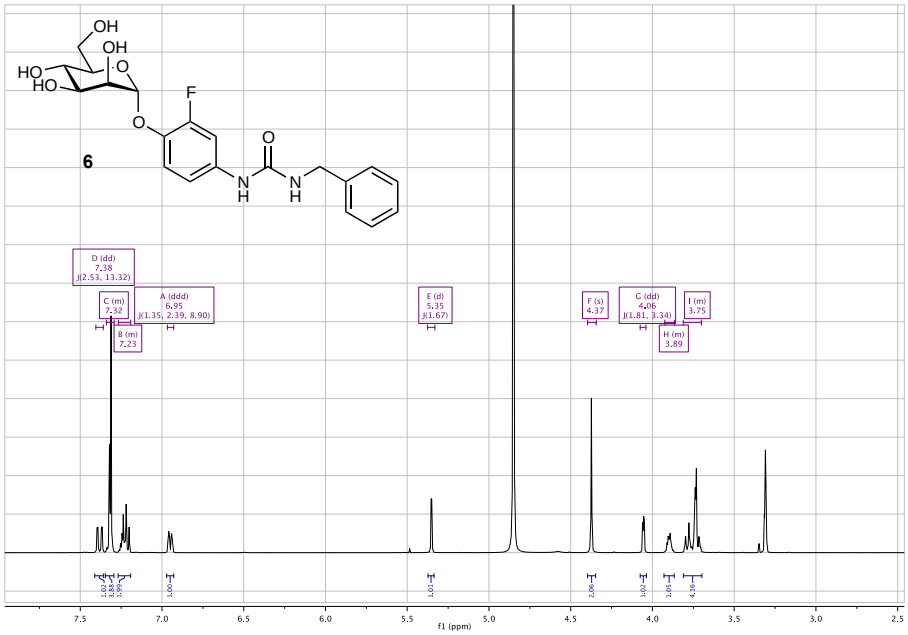

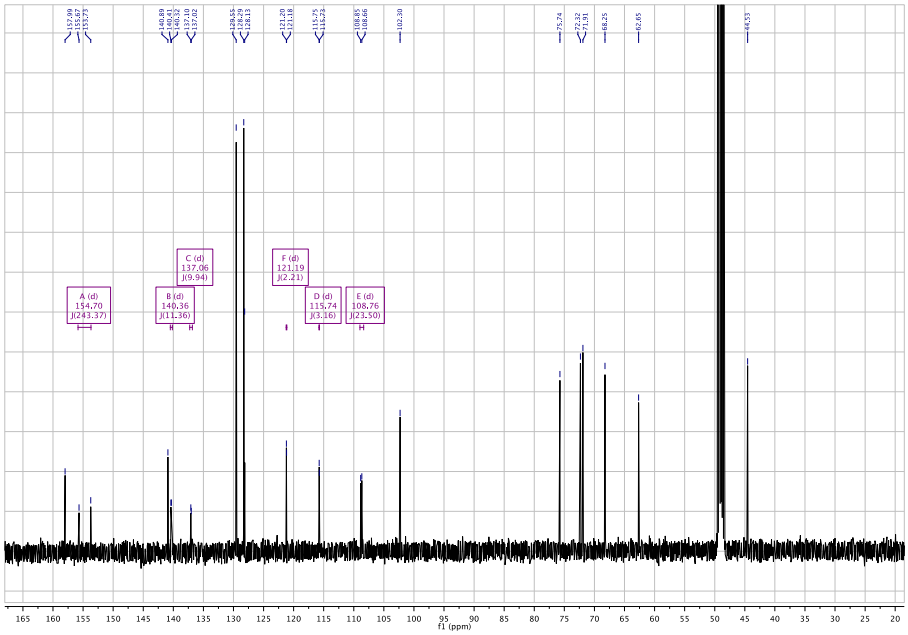

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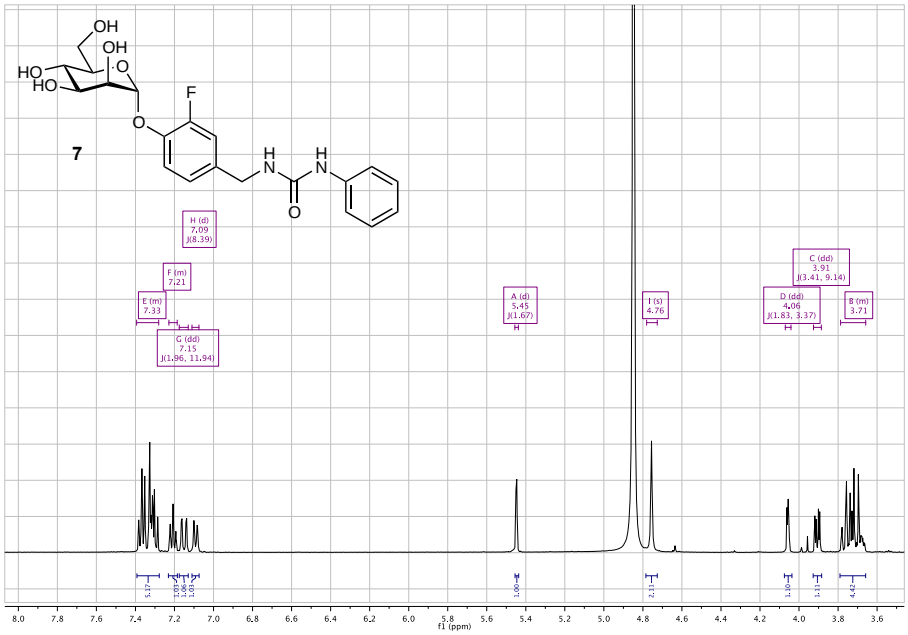

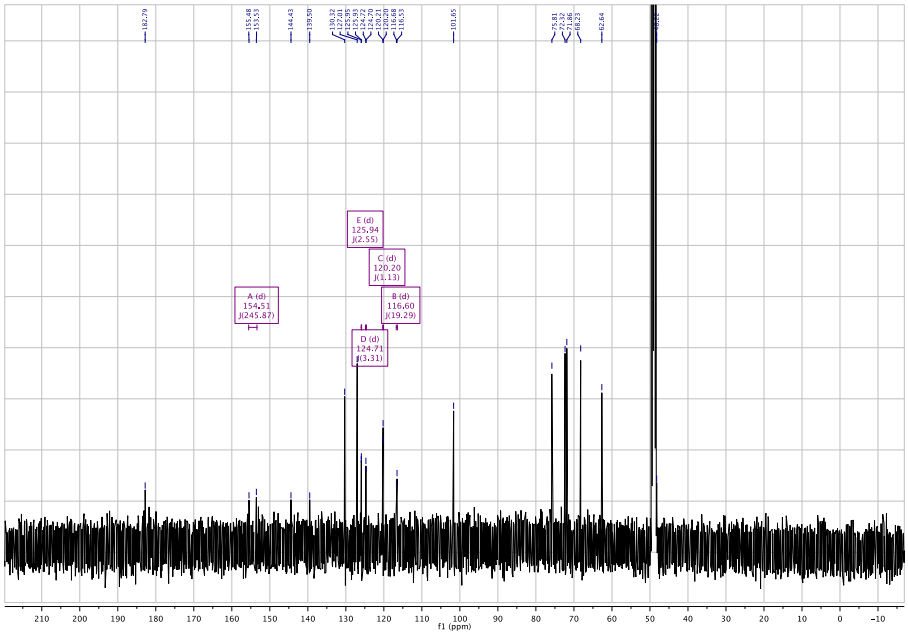

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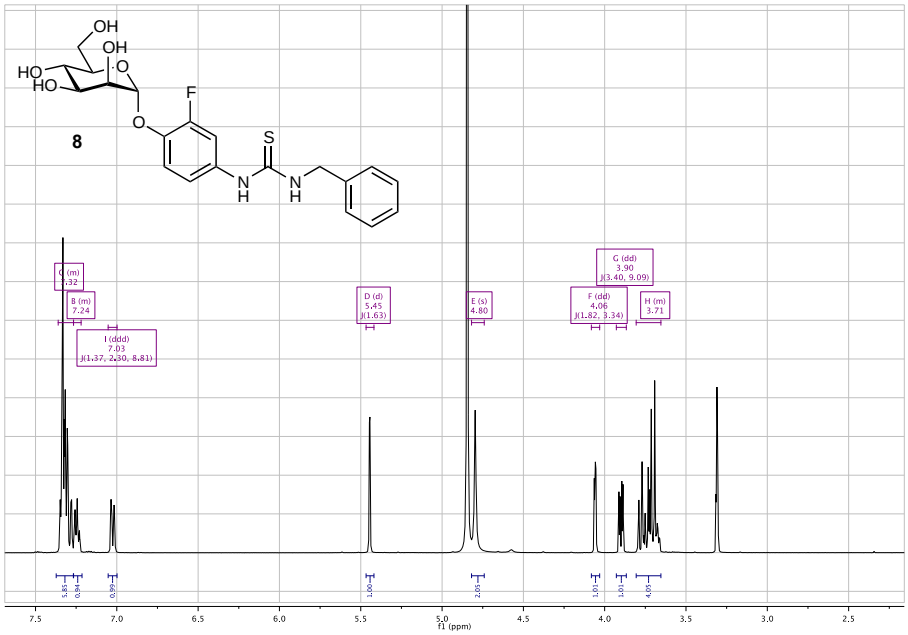

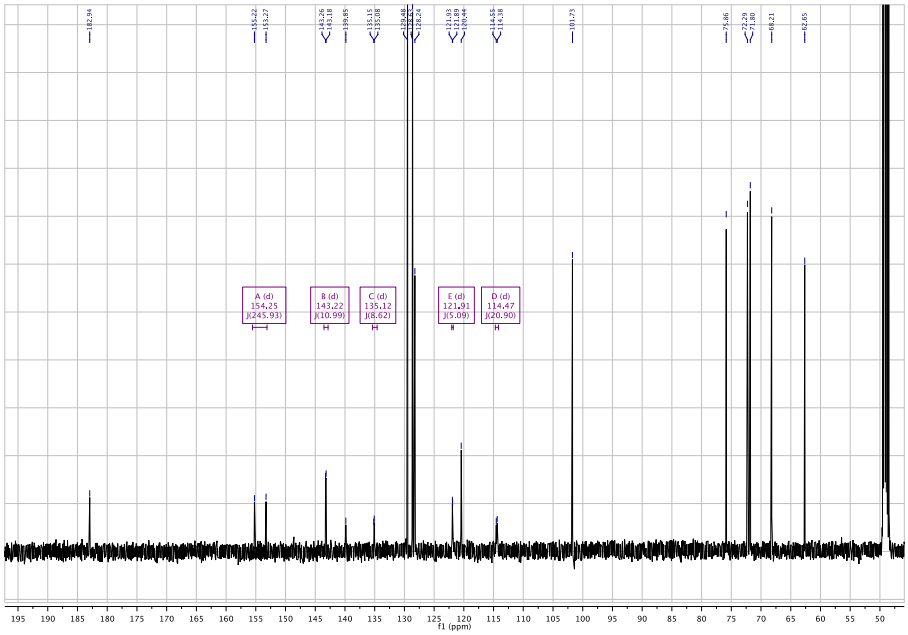

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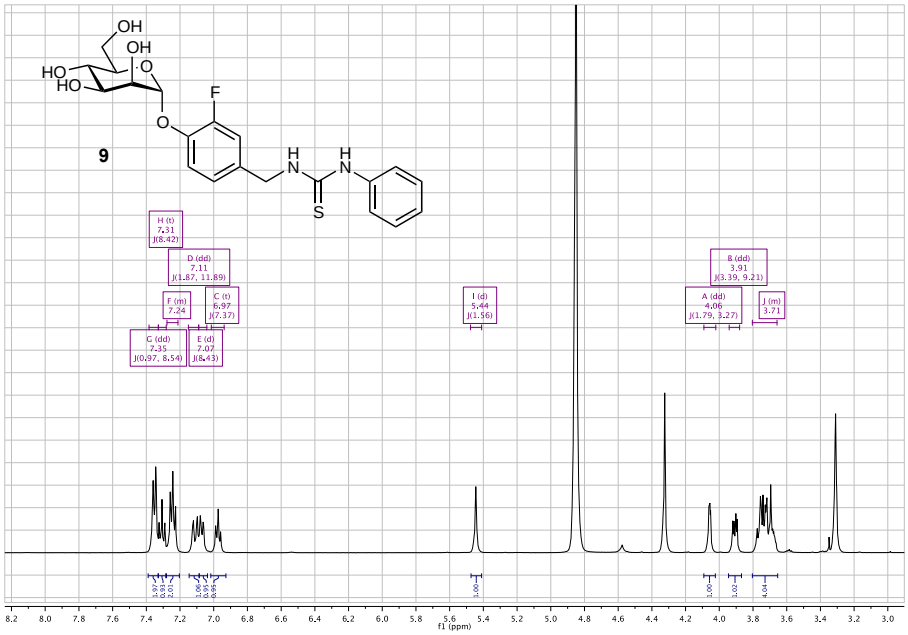

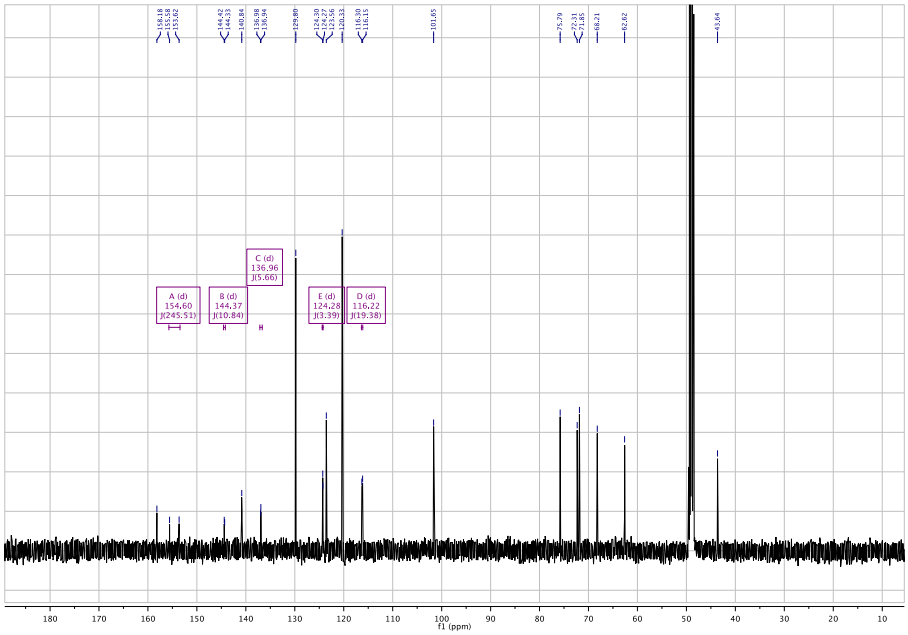

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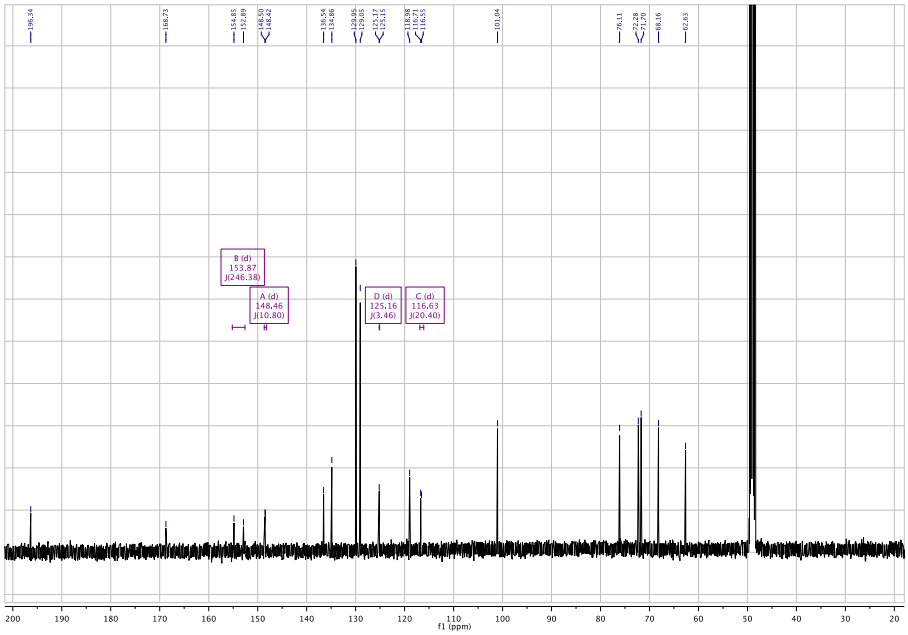

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References

1. Frei, P.; Pang, L.; Silbermann, M.; Eriş, D.; Mühlethaler, T.; Schwardt, O.; Ernst, B., Target-directed Dynamic Combinatorial Chemistry: A Study on Potentials and Pitfalls as Exemplified on a Bacterial Target. *Chem. Eur. J.* **2017**, *23*, 11570-11577.
2. Kleeb, S.; Pang, L.; Mayer, K.; Eris, D.; Sigl, A.; Preston, R. C.; Zihlmann, P.; Sharpe, T.; Jakob, R. P.; Abgottspon, D.; Hutter, A. S.; Scharenberg, M.; Jiang, X.; Navarra, G.; Rabbani, S.; Smiesko, M.; Lüdin, N.; Bezençon, J.; Schwardt, O.; Maier, T.; Ernst, B., FimH Antagonists: Bioisosteres To Improve the in Vitro and in Vivo PK/PD Profile. *J. Med. Chem.* **2015**, *58*, 2221-2239.
3. Mayer, K.; Eris, D.; Schwardt, O.; Sager, C. P.; Rabbani, S.; Kleeb, S.; Ernst, B., Urinary Tract Infection — Which Conformation of the Bacterial Lectin FimH Is Therapeutically Relevant? *J. Med. Chem.* **2017**, *60*, 5646-5662.
4. Wang, Z.-X., An exact mathematical expression for describing competitive binding of two different ligands to a protein molecule. *FEBS Lett.* **1995**, *360*, 111-114.

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