Supporting Information

Discovery and Optimization of the First Highly Effective and Orally Available Galectin-3 Inhibitors for Treatment of Fibrotic Disease

Fredrik R. Zetterberg,*[,a] Alison MacKinnon,*[,b] Thomas Brimert,[c,d] Lise Gravelle,[e] Richard E. Johnsson,[c] Barbro Kahl-Knutson,[d] Hakon Leffler,[f] Ulf J. Nilsson,[a,g] Anders Pedersen,[e] Kristoffer Peterson,[a,g] James A. Roper,[h] Hans Schambye,[e] Robert J. Slack,[h] Susan Tantawi, [e]

[a]Galecto Biotech AB, Sahlgrenska Science Park, Medicinaregatan 8 A, SE-413 46 Gothenburg, Sweden

[b]Galecto Biotech ApS, Nine Edinburgh Bioquarter, 9 Little France Rd, Edinburgh EH16 4UX, UK

[c]Red Glead Discovery AB, Medicon Village, SE-223 63, Lund, Sweden

[d]Biochemistry and Structural Biology, Center for Molecular Protein Science, Department of Chemistry, Lund University, Box 124, SE-221 00, Lund, Sweden

[e]Galecto Biotech ApS, Cobis Science Park, Ole Maaloes Vej 3, DK-2200, Copenhagen, Denmark

[f]Department of Laboratory Medicine, Lund University, Box 124, SE-221 00, Lund, Sweden

[g]Centre for Analysis and Synthesis, Department of Chemistry, Lund University, Box 124, SE-221 00, Lund, Sweden

[h]Galecto Biotech ApS, Stevenage Bioscience Catalyst, Stevenage, Hertfordshire, SG1 2FX UK

Corresponding Authors

*Fredrik R. Zetterberg; e-mail: FZ@galecto.com; phone: +46 763240495

*Alison MacKinnon; e-mail: am@galecto.com; phone: +44 7711832576

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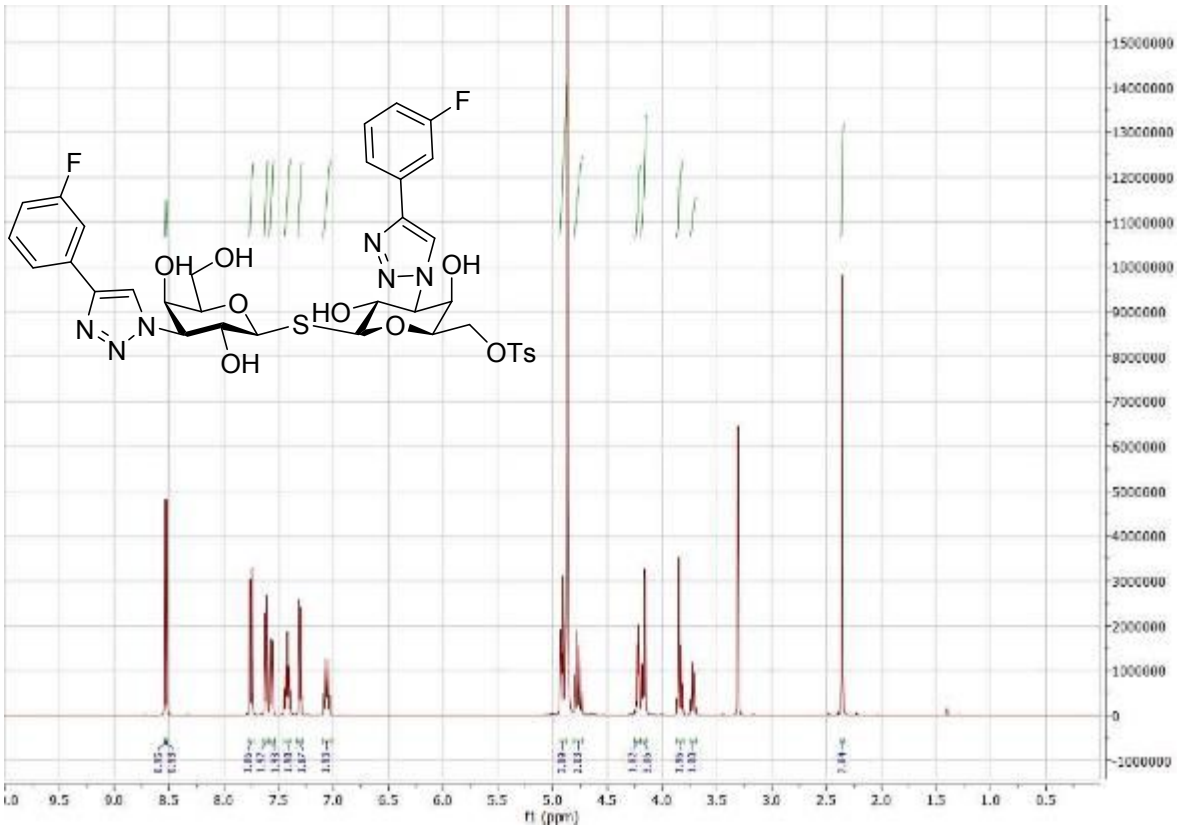
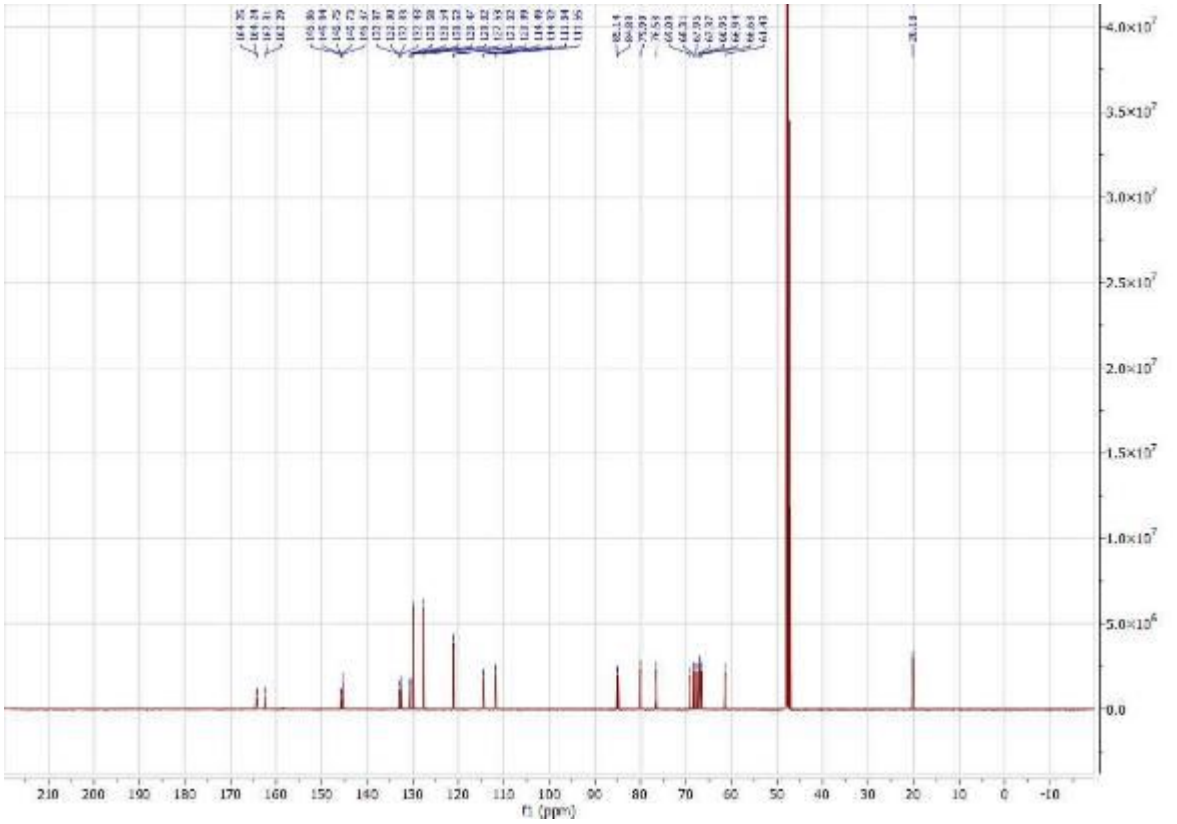
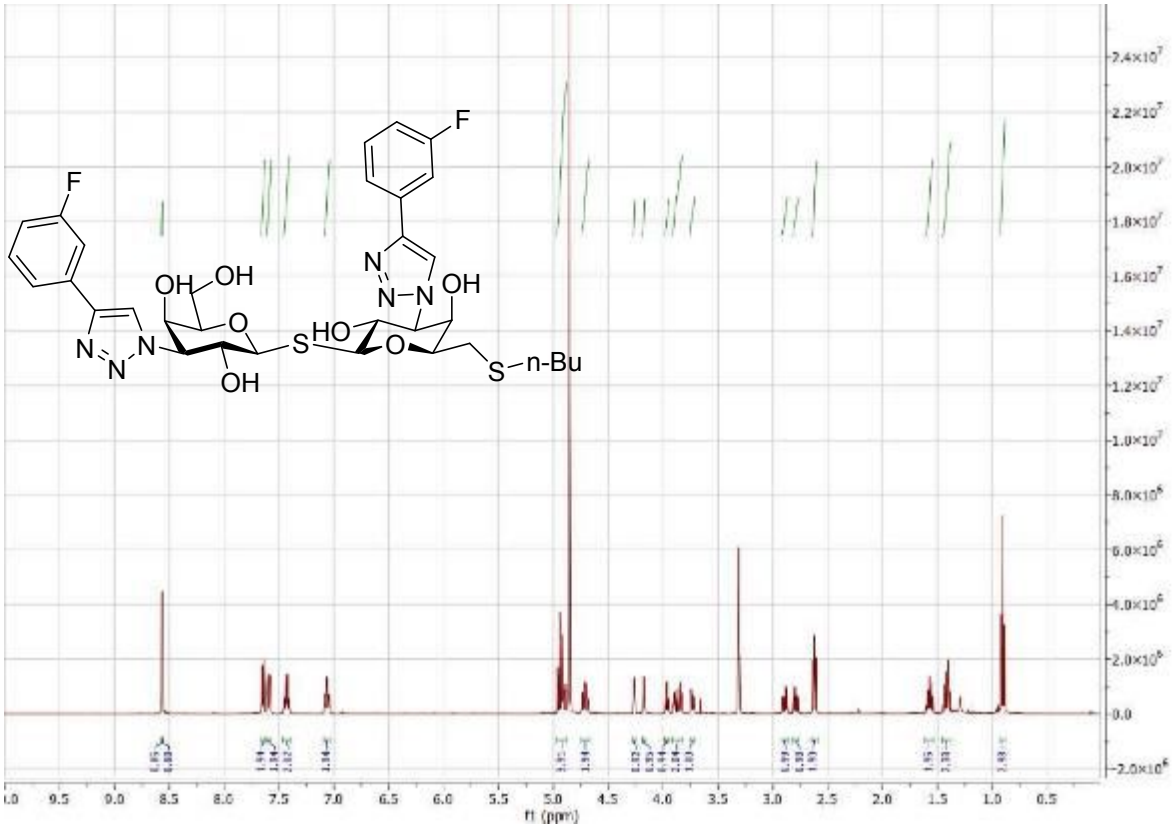
NMR spectra of synthesized compounds

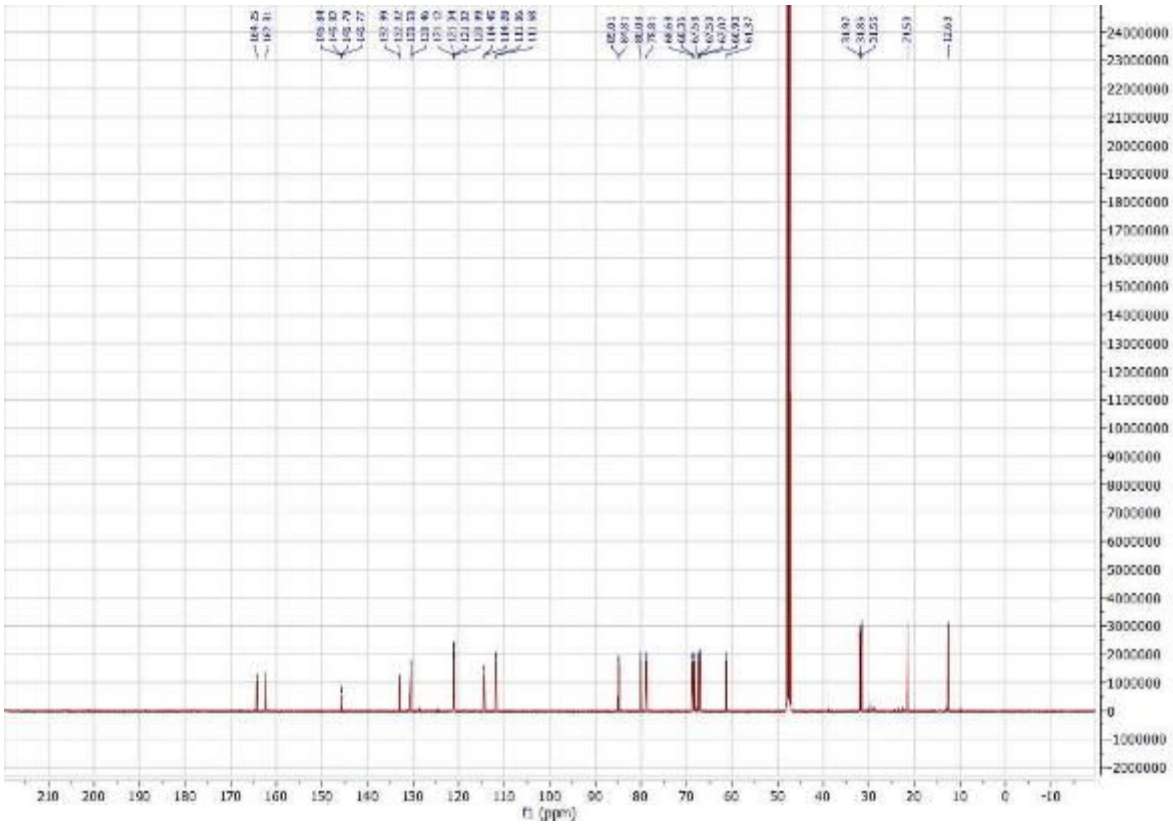
3,3'-Dideoxy-3,3'-bis-[4-(3-fluorophenyl)-1*H*-1,2,3-triazol-1-yl]-6-(4-methylphenyl)sulfonyl-1,1'-sulfanediyl-di-β-D-galactopyranoside 2



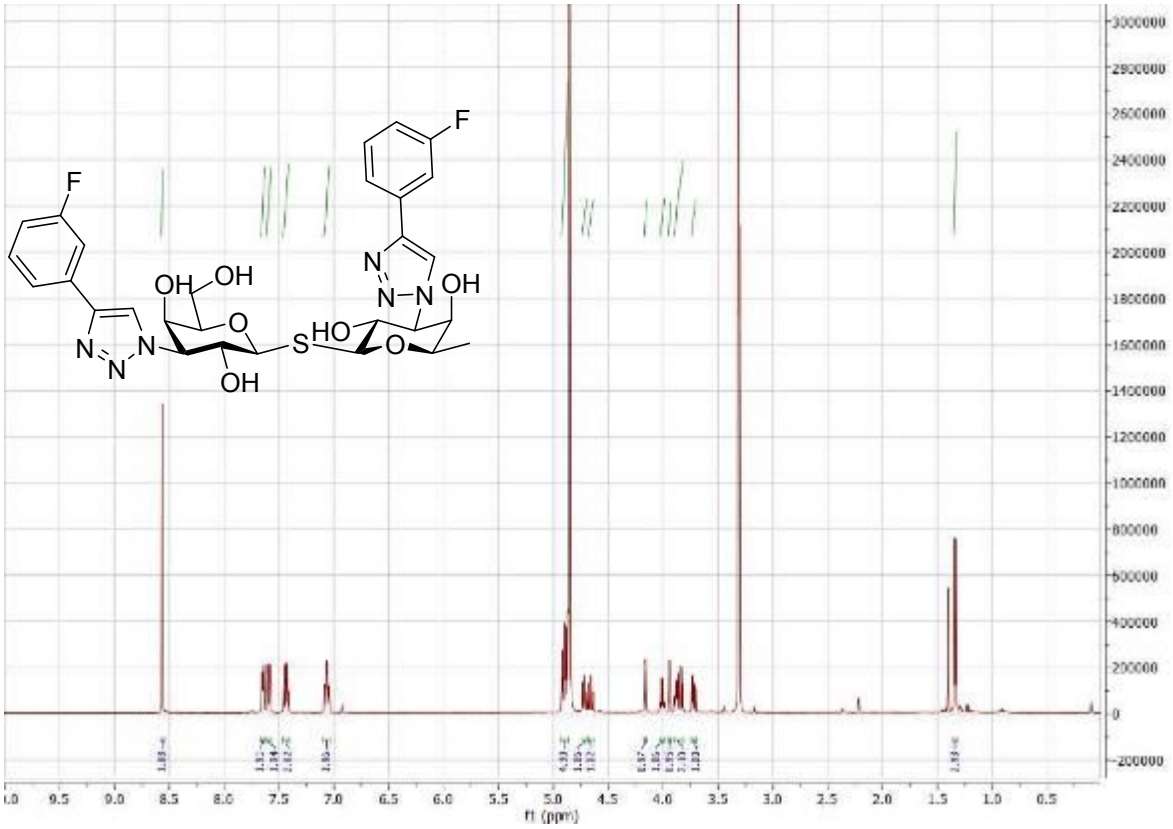


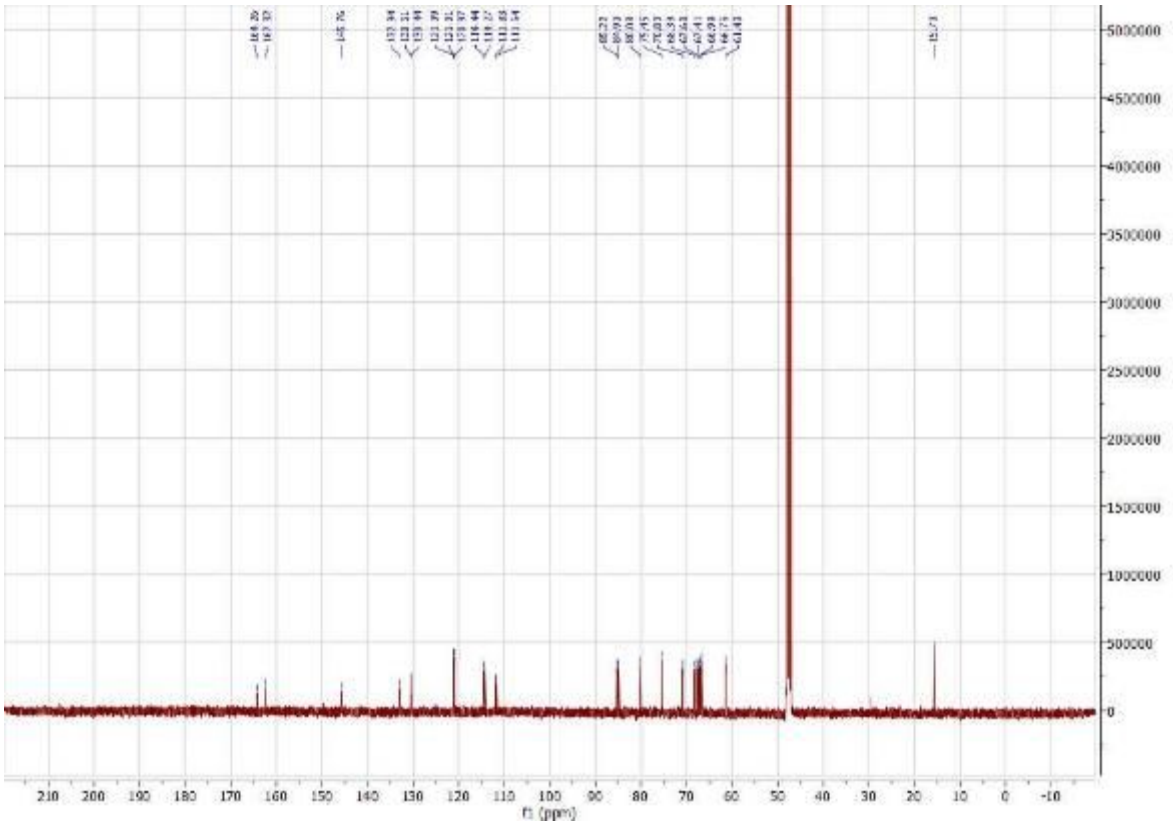
6-*S*-Butyl-3,3',6-trideoxy-3,3'-bis-[4-(3-fluorophenyl)-1*H*-1,2,3-triazol-1-yl]-1,1'-sulfanediyl-di-β-D-galactopyranoside 3



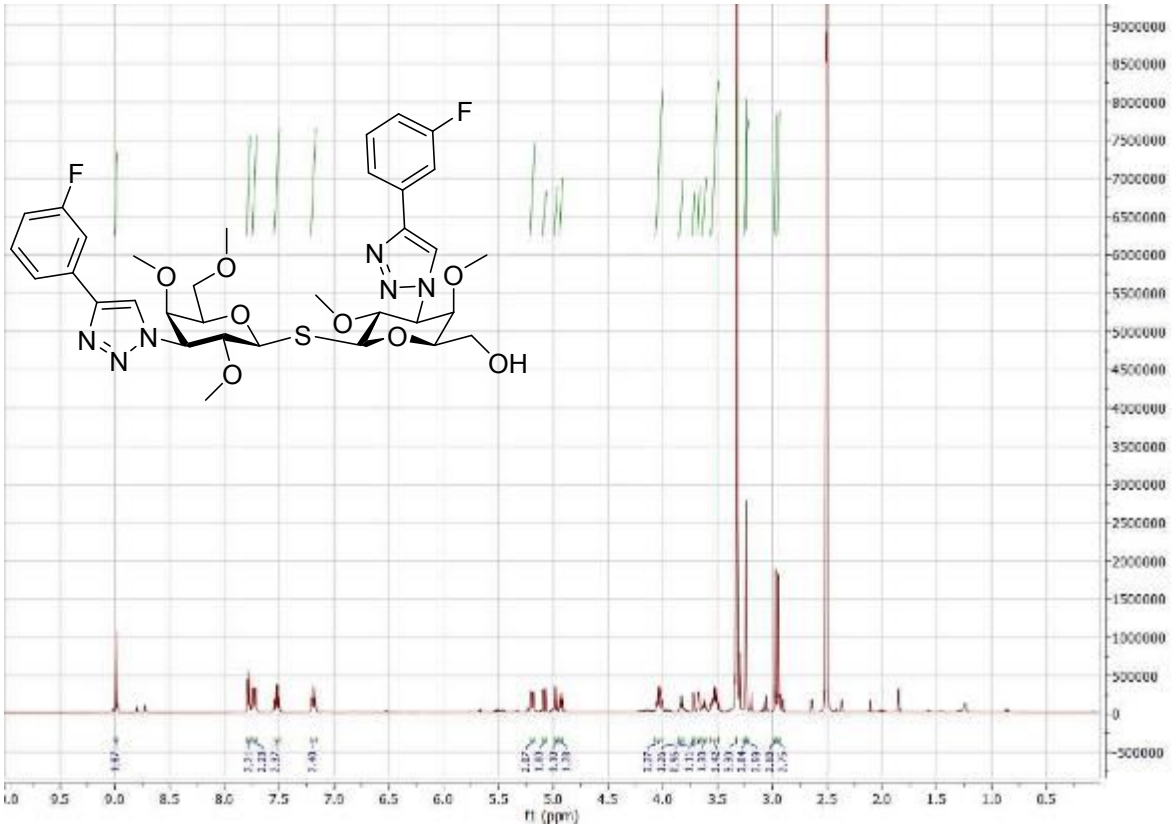


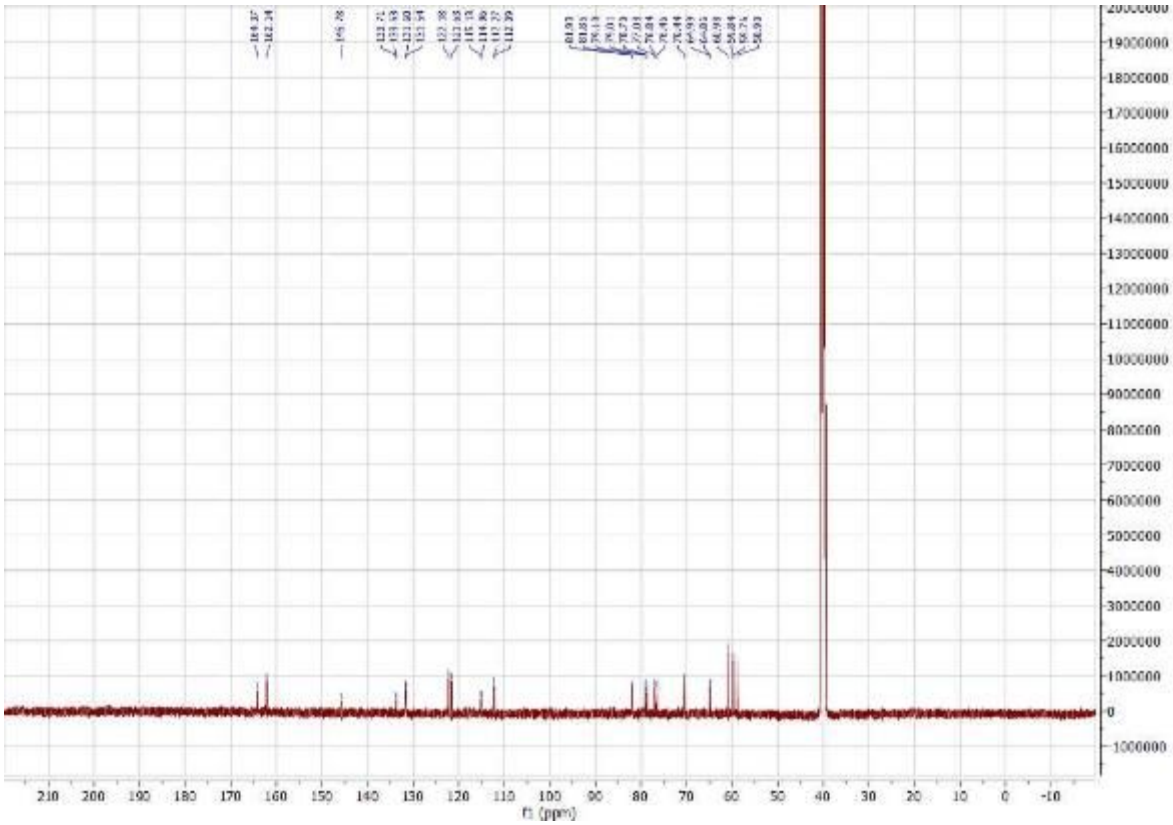
3,3',6-Trideoxy-3,3'-bis-[4-(3-fluorophenyl)-1*H*-1,2,3-triazol-1-yl]-1,1'-sulfanediyl-di-β-D-galactopyranoside 4





3,3’-Dideoxy-3,3’-bis-[4-(3-fluorophenyl)-1*H*-1,2,3-triazol-1-yl]-2,2’,4,4’,6-penta-*O*-methyl-1,1’-sulfanediyl-di-β-D-galactopyranoside 5a





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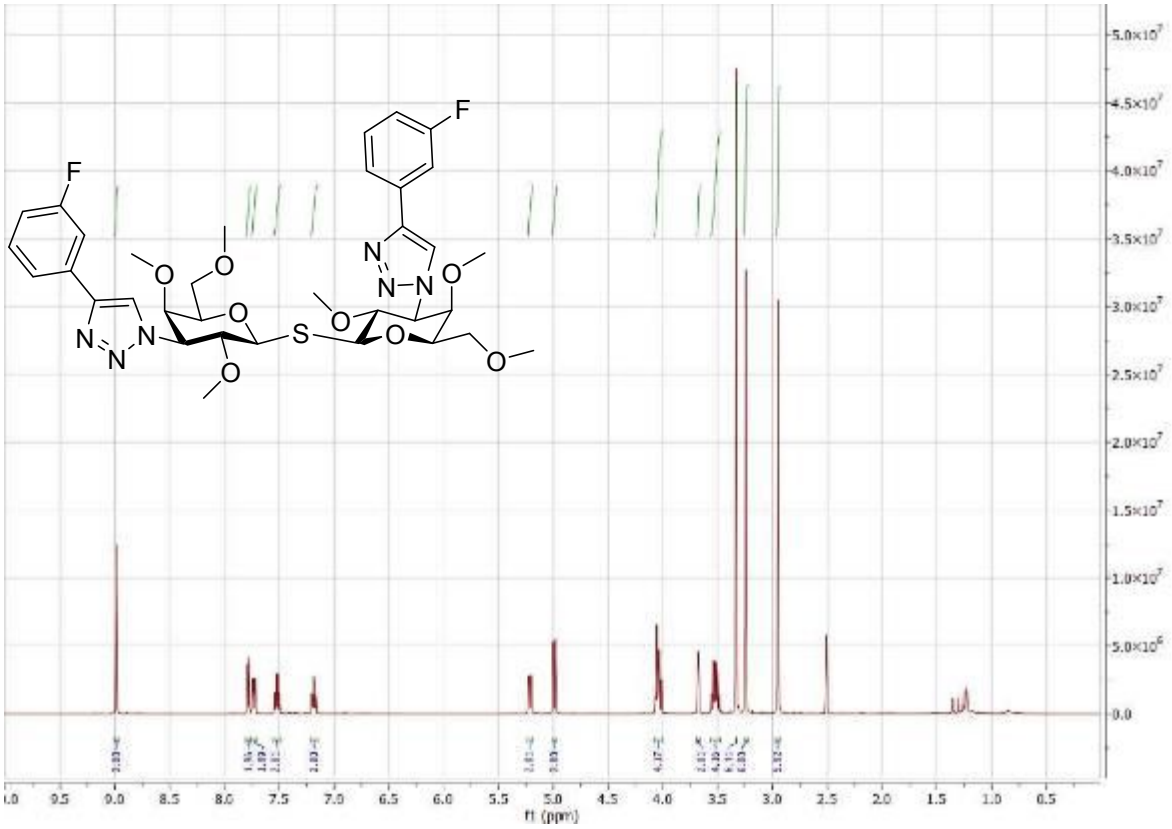
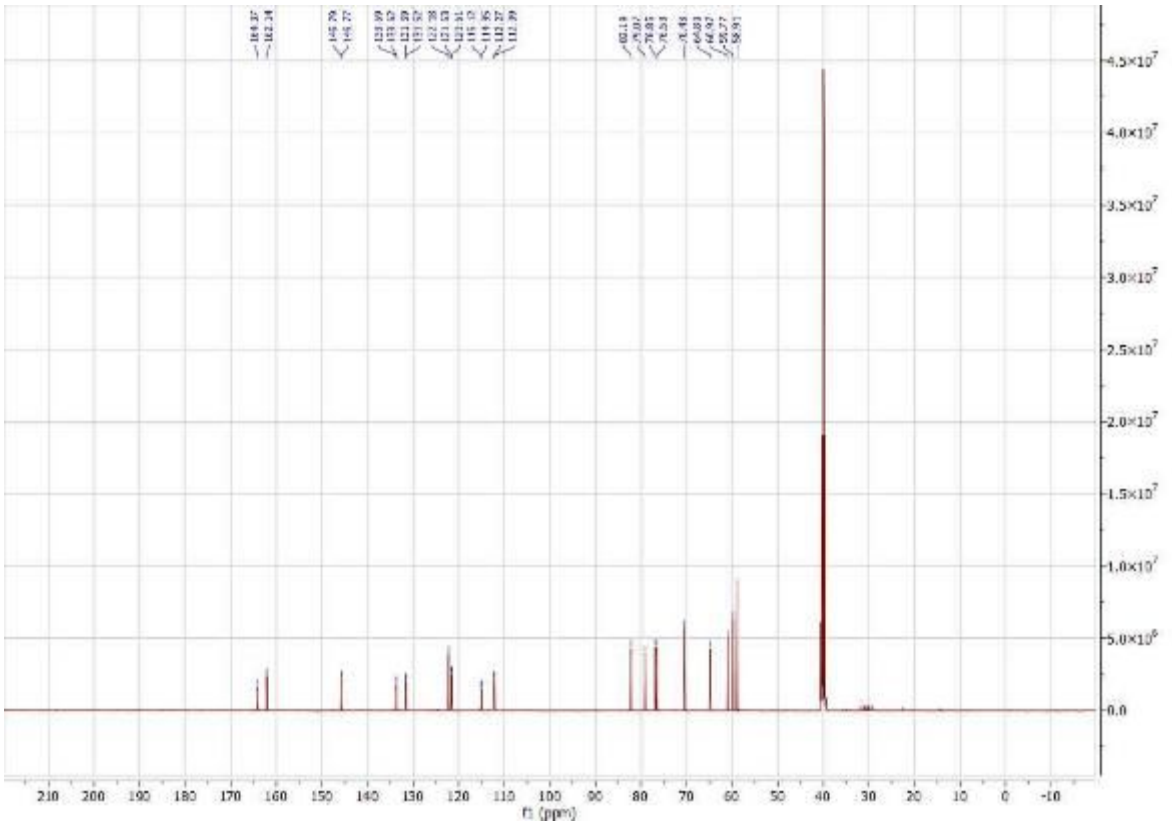
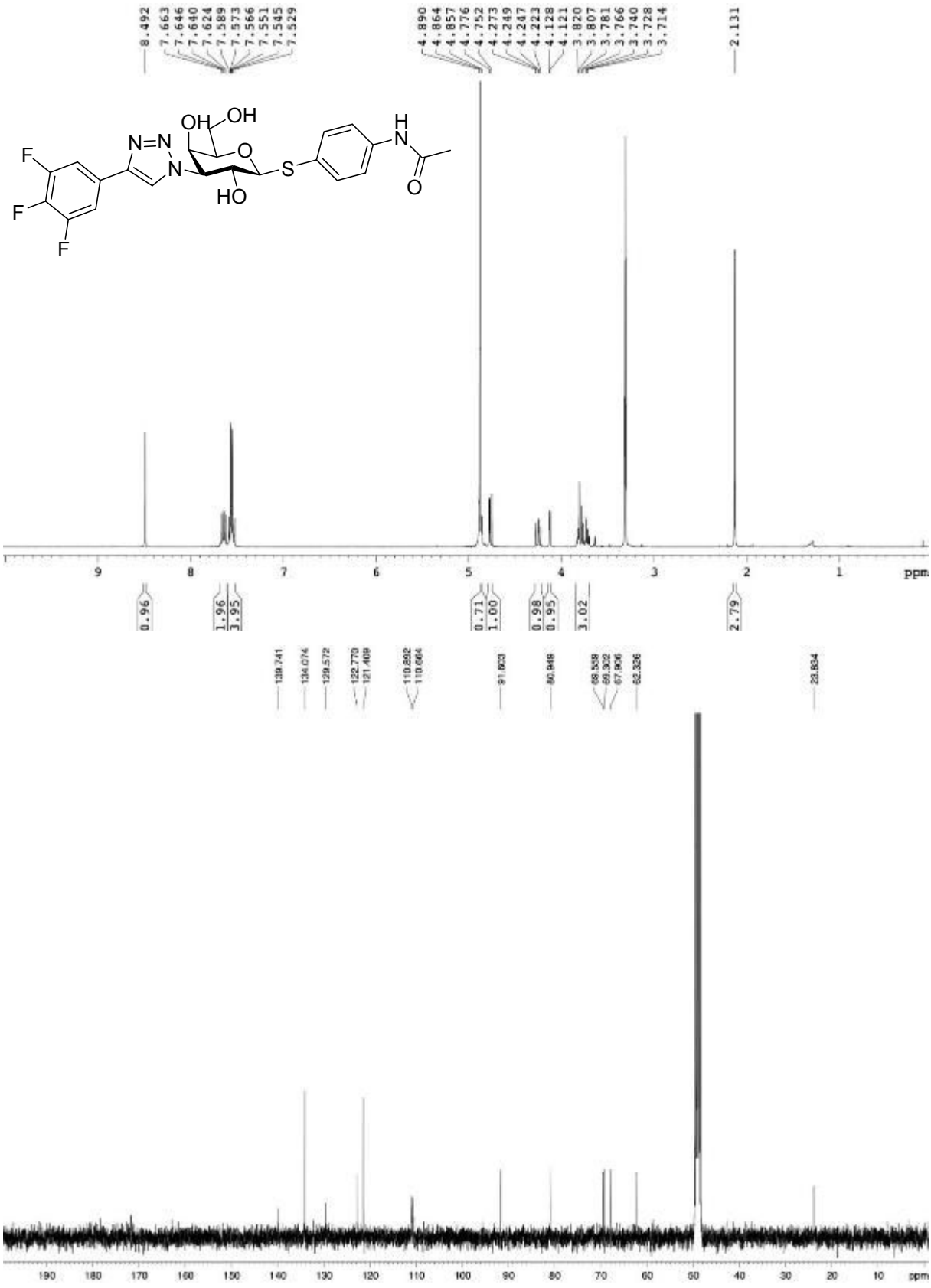
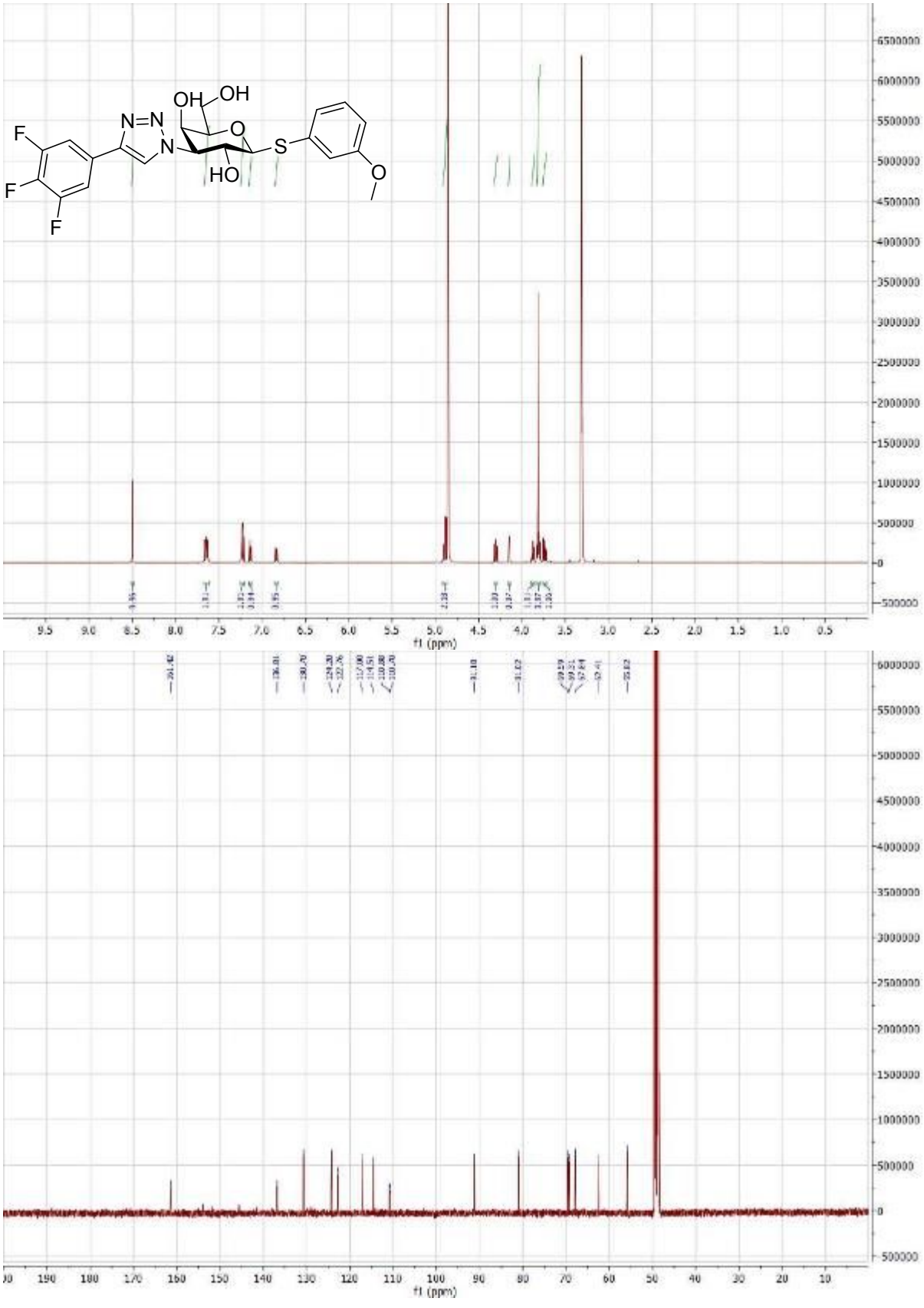




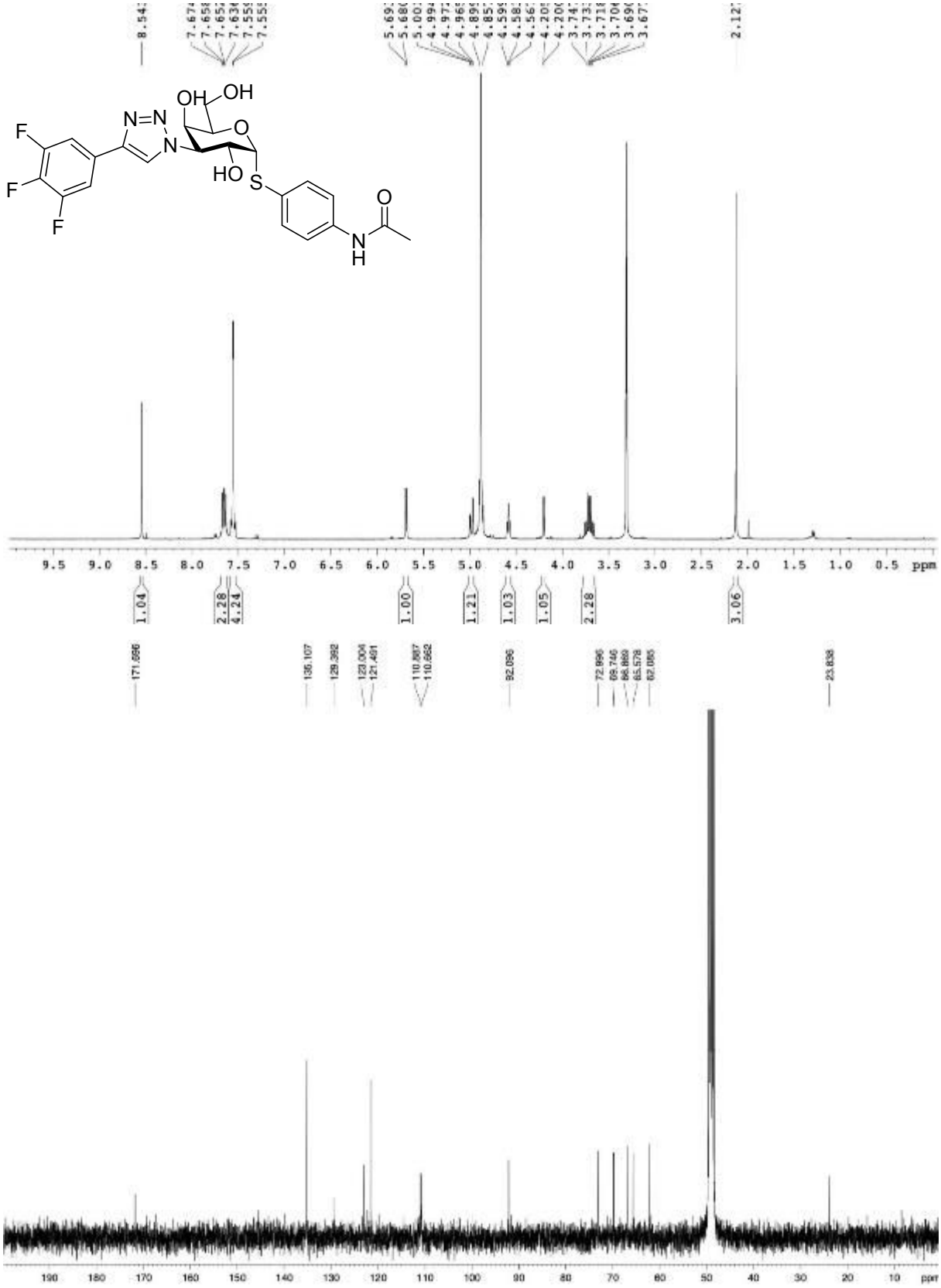
4-Acetamidophenyl 3-deoxy-3-[4-(3,4,5-trifluorophenyl)-1*H*-1,2,3-triazol-1-yl]-1-thio-β-D-galactopyranoside 8a



3-Methoxyphenyl 3-deoxy-3-[4-(3,4,5-trifluorophenyl)-1*H*-1,2,3-triazol-1-yl]-1-thio-β-D-galactopyranoside 8b



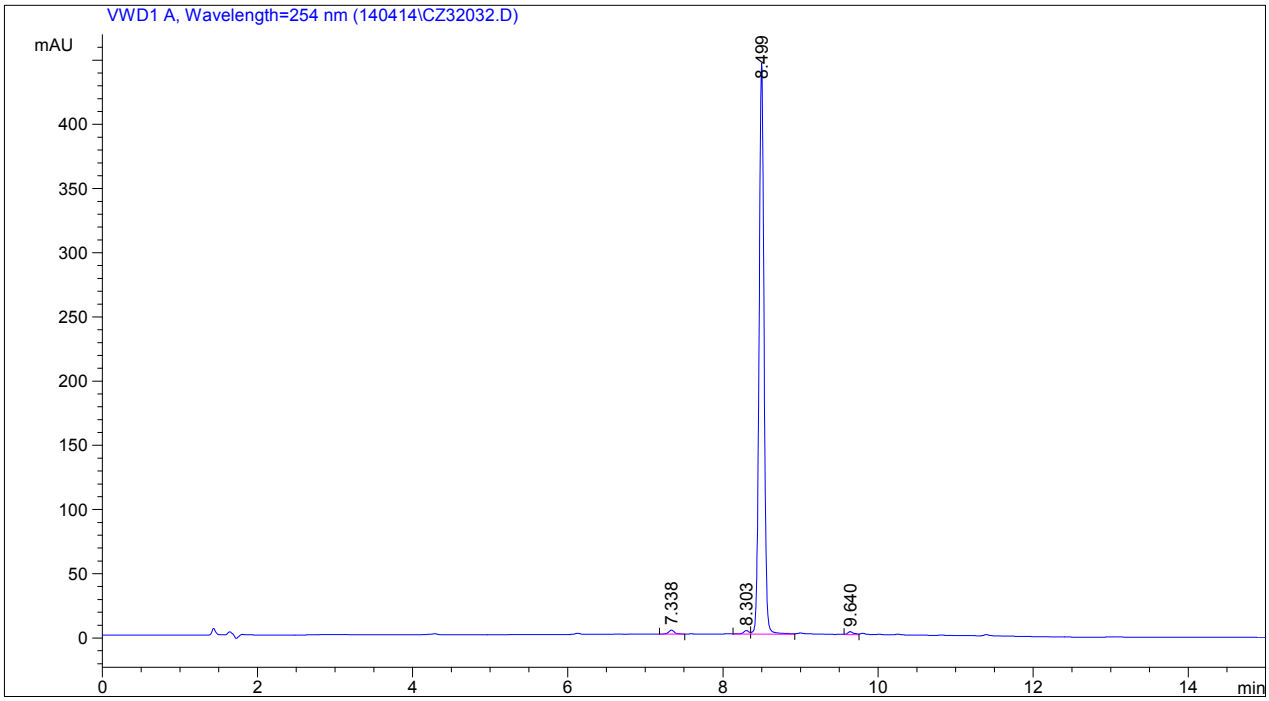
4-Acetamidophenyl 3-deoxy-3-[4-(3,4,5-trifluorophenyl)-1*H*-1,2,3-triazol-1-yl]-1-thio-α-D-galactopyranoside 11a



5-Bromopyridin-3-yl 3-deoxy-3-[4-(3,4,5-trifluorophenyl)-1*H*-1,2,3-triazol-1-yl]-1-thio-α-D-galactopyranoside 11d

Purity chromatograms

6-*S*-Butyl-3,3',6-trideoxy-3,3'-bis-[4-(3-fluorophenyl)-1*H*-1,2,3-triazol-1-yl]-1,1'-sulfanediyl-di-β-D-galactopyranoside 3



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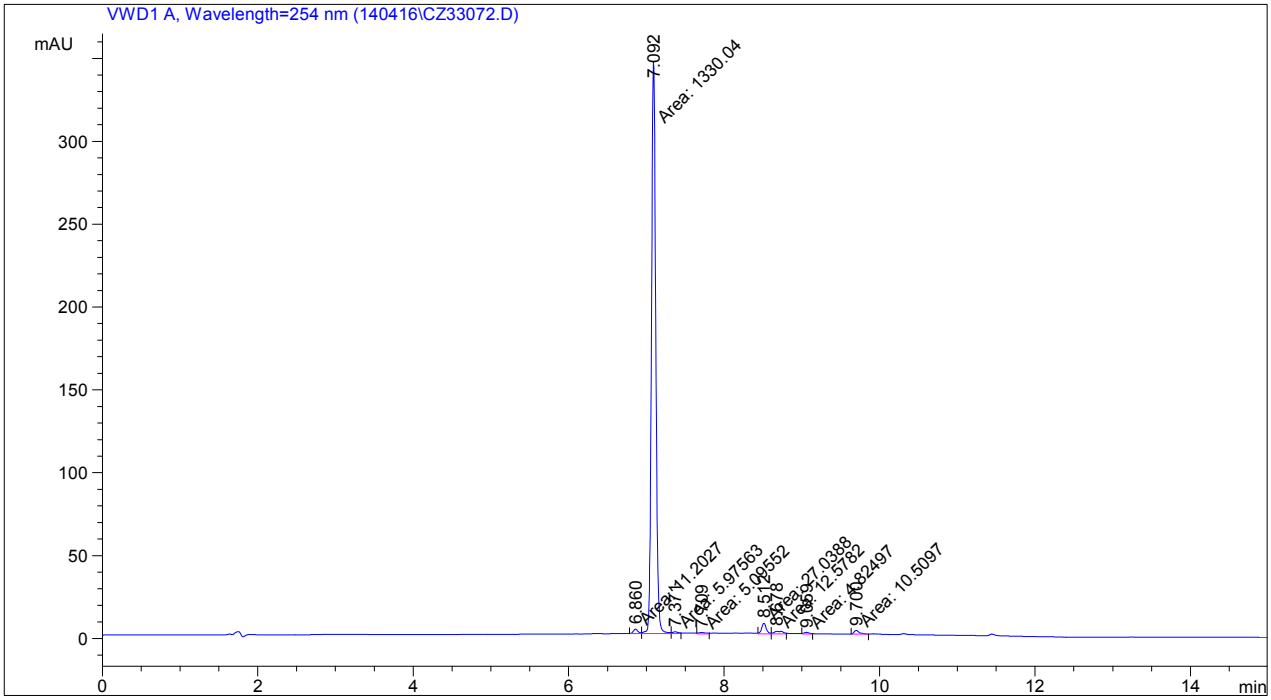
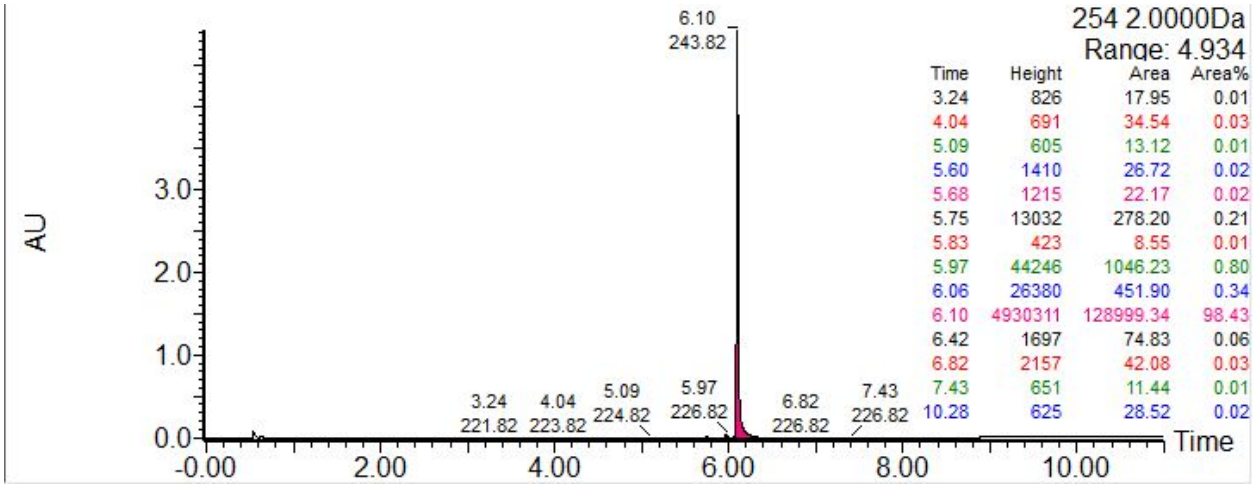
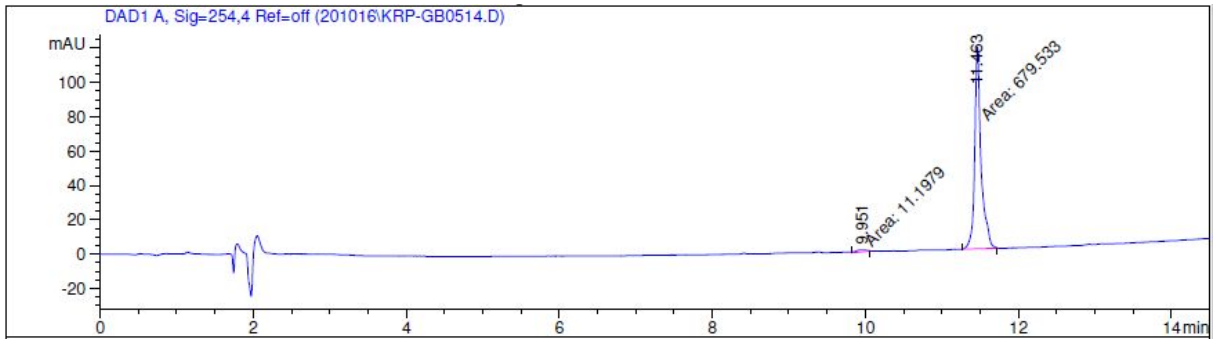


3,3'-Dideoxy-3,3'-bis-[4-(3-fluorophenyl)-1*H*-1,2,3-triazol-1-yl]-2,2',4,4',6-penta-*O*-methyl-1,1'-sulfanediyl-di-β-D-galactopyranoside 5a



3,3'-Dideoxy-3,3'-bis-[4-(3-fluorophenyl)-1*H*-1,2,3-triazol-1-yl]-2,2',4,4',6,6'-hexa-*O*-methyl-1,1'-sulfanediyl-di-β-D-galactopyranoside 5b



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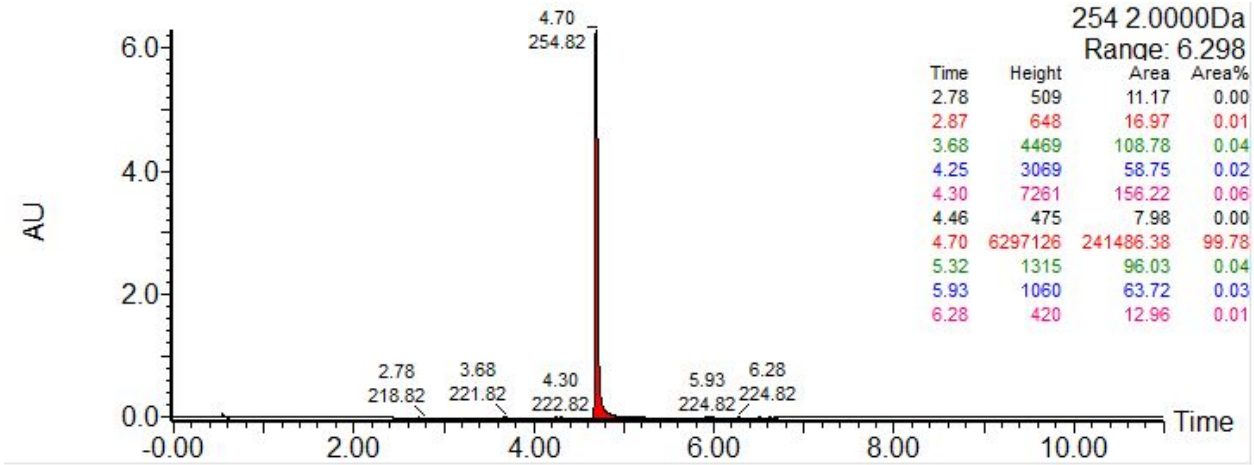
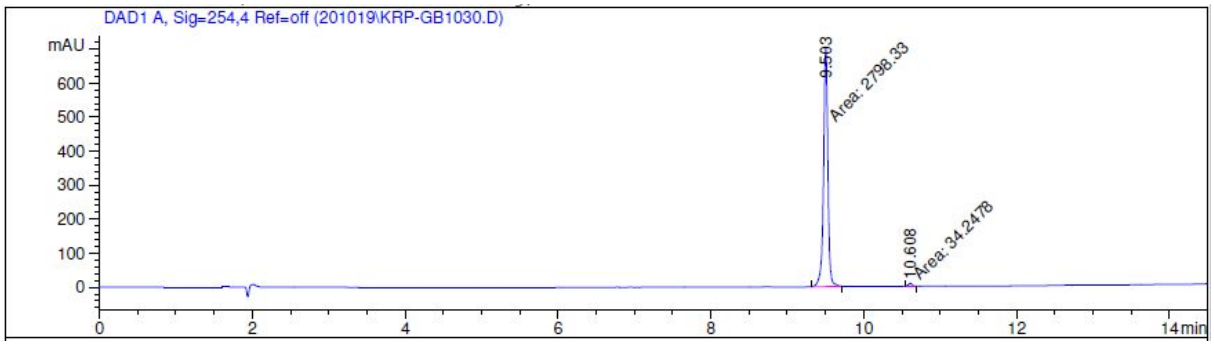
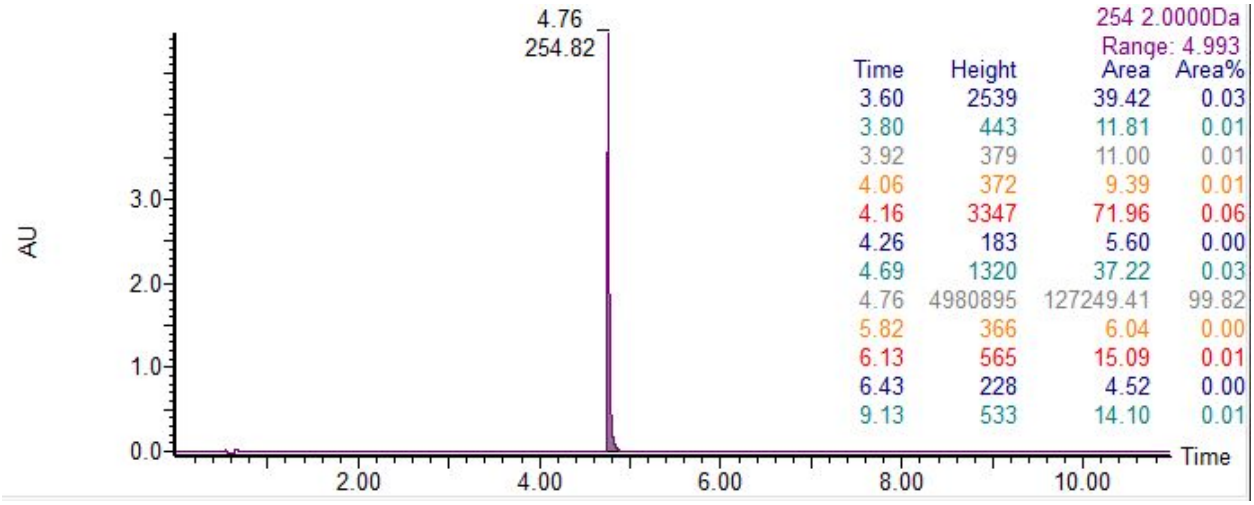


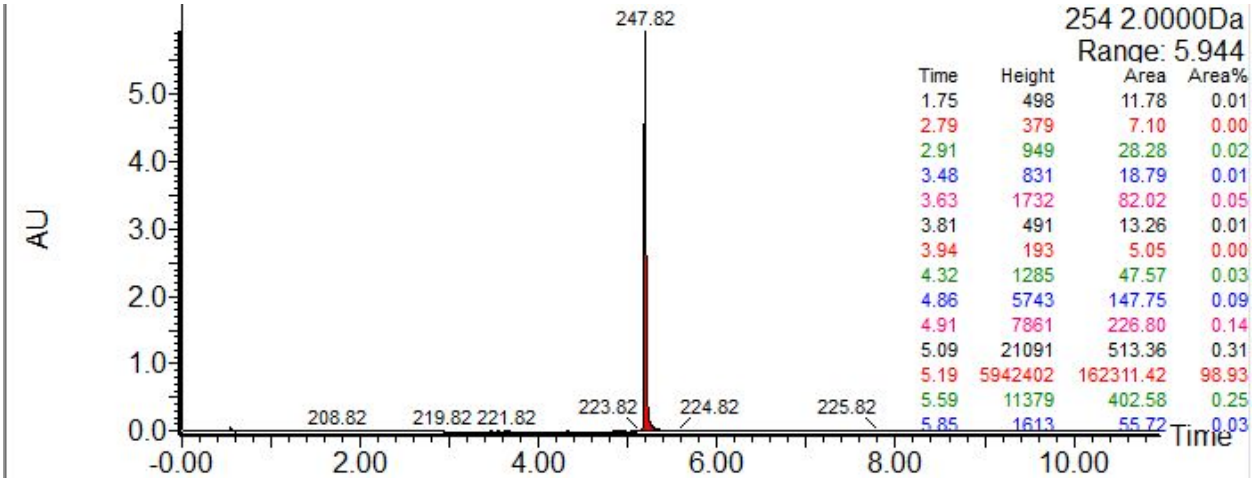
3-Methoxyphenyl 3-deoxy-3-[4-(3,4,5-trifluorophenyl)-1*H*-1,2,3-triazol-1-yl]-1-thio-β-D-galactopyranoside 8b



4-Acetamidophenyl 3-deoxy-3-[4-(3,4,5-trifluorophenyl)-1*H*-1,2,3-triazol-1-yl]-1-thio-α-D-galactopyranoside 11a



5-Bromopyridin-3-yl 3-deoxy-3-[4-(3,4,5-trifluorophenyl)-1*H*-1,2,3-triazol-1-yl]-1-thio-α-D-galactopyranoside 11d



Surface Plasmon Resonance (SPR) Analysis. To detect the affinity of **11d** to human and mouse full-length galectin-1 and -3, SPR analysis was performed with BIAcore™ (Cytiva, USA) in the presence of 5% DMSO at 25°C. Target protein was immobilized onto a CM3 sensorchip via amine coupling and different concentrations of **11d** were injected at a flow rate of 30 µl/min. SPR response was recorded as a function of time at 25°C between proteins, and k_{on} and k_{off} were determined using global fitting parameters with a 1:1 binding model and K_d calculated from steady-state fitting**; 11d** binding to galectin was stable at 25°C for the duration of the experiment (Table S1).

Table S1. SPR binding affinity and kinetics for **11d** against human and mouse galectin-3 and -1.

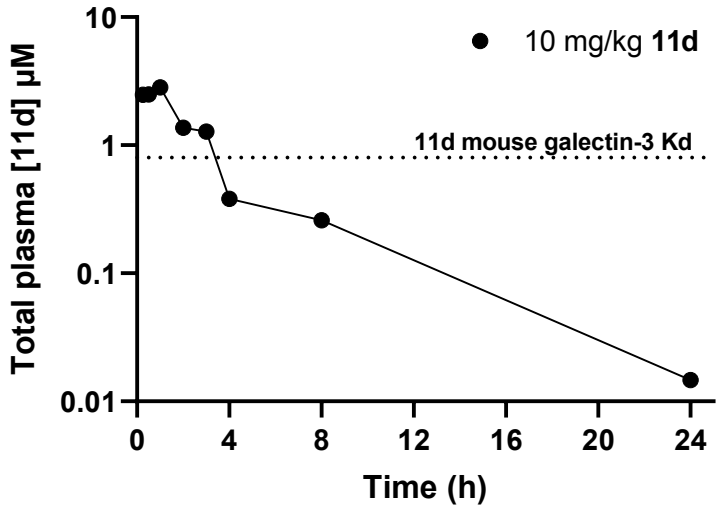
	Human Galectin-3	**Mouse Galectin-3**	**Human Galectin-1**	**Mouse Galectin-1**
K_d **(µM)**	0.037	2.2	3.7	5.1
k_{on} **(M.s^{-1})**	4.77 x 10^6	7.26 x 10^4	7.86 x 10^5	3.68 x 10^4
k_{off} **(s^{-1})**	0.21	0.21	3.63	0.25

Cell cytotoxicity A549 cells (Sigma Aldrich, Gillingham, UK) were seeded in 96-well plates (5000 cells per well in Ham's F12 Kaighn's medium [Thermofisher Scientific] supplemented with 10% FCS maintained in a humidified incubator at 37°C, 5% CO_2) and treated with increasing concentrations of galectin-3 inhibitor from 123 nM to 90 μM for 72 h. Cell viability was determined using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay (PromoCell, Germany) as per the manufacturer's instructions. Absorbance values were read on a plate reader (CLARIOstar Plus, BMG Labtech) at 570 nm with a reference wavelength of 630 nm; background corrected results (calculated as OD570-OD630) were expressed as a percentage of DMSO-treated cells. Non-linear regression (using [Inhibitor] vs response – variable slope [four parameter] equation) was applied to determine IC_{50} values (Graphpad Prism, San Diego, CA, USA). Individual experiments were performed in duplicate and data shown are the mean ± standard deviation (SD) from at least 2 separate occasions.

Table S2. Cell cytotoxicity of **11d** in A549 cells measured by MTT assay.

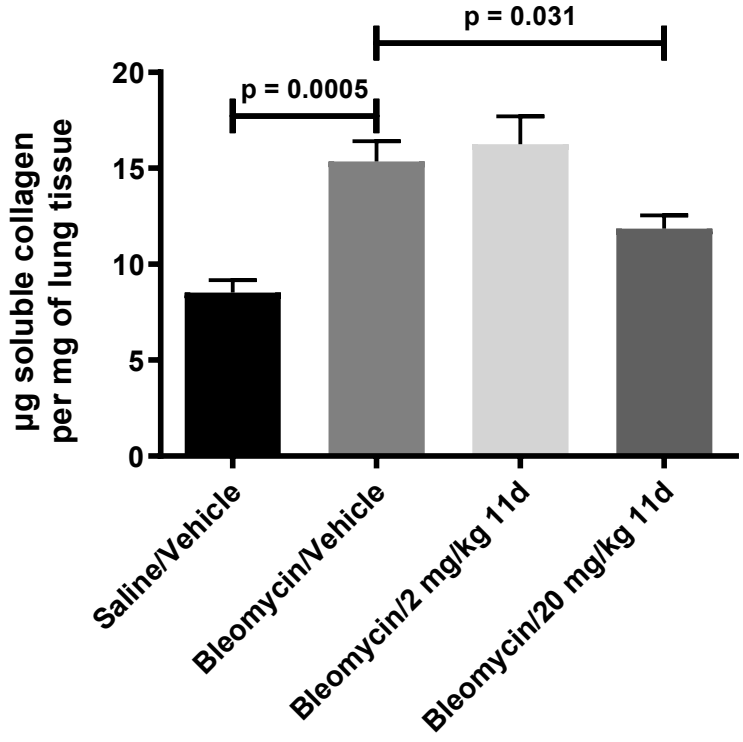
Compound	***IC*$_{50}$ (μM)**	**Maximum % Reduction in Viability**
11d	>90	43.4 ± 5.80

Figure S1. Pharmacokinetic profile of **11d** in female C57BL/6 mice following oral administration with total blood concentrations determined by LC-MS/MS.



Bleomycin-induced lung fibrosis. Male C57BL/6 mice were dosed with 33 µg bleomycin sulfate (Apollo Scientific) in 50 µl saline via oropharyngeal route under brief isofluorane anaesthesia at Day 0. The galectin-3 inhibitor **11d** was prepared at a concentration of 0.2 and 2 mg/mL in 10% solutol:90% PEG300 and administered orally at a dose volume of 10 mL/kg b.i.d on Days 12 to 26 post-bleomycin instillation. Following termination, collagen content in the left lung lobe was determined by Sircol™ (Biocolor) assay as per the manufacturer's instructions.

Figure S2. Effect of compound **11d** dosing on lung collagen in a murine bleomycin-induced lung fibrosis model. Mice received 33 μg bleomycin in saline at Day 0 with compound **11d** dosed orally twice daily from Days 12 to 26 post-bleomycin instillation (n=5–8 per group).



Specificity screen of 11b and d towards 87 different targets

The specificity of compound **11b** and **d** were tested against a panel of 87 different targets (enzymes, receptors and ion channels, supplementary material, S20, part of a safety screen provided by Eurofins (PanLabs).

11b

Cat #	Assay Name	Species	Conc.	% Inh.	IC_{50}*	K_i	n_H
214510	Calcium Channel L-Type, Benzothiazepine	rat	10 µM	50			
214600	Calcium Channel L-Type, Dihydropyridine	rat	10 µM	59			
215000	Calcium Channel L-Type, Phenylalkylamine	rat	10 µM	55			
271700	Serotonin (5-Hydroxytryptamine) 5-HT_{2B}	hum	10 µM	50			
220320	Transporter, Dopamine (DAT)	hum	10 µM	82			

Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*	K_i	n_H	R
Compound: GB1107-03, PT #: 1189509										
107710	ATPase, Na^+/K^+, Heart, Pig	368169	pig	2	10 µM	6				
104010	Cholinesterase, Acetyl, ACES	368252	hum	2	10 µM	0				
116020	Cyclooxygenase COX-1	368115	hum	2	10 µM	10				
118010	Cyclooxygenase COX-2	368116	hum	2	10 µM	-12				
140010	Monoamine Oxidase MAO-A	368071	hum	2	10 µM	5				
140120	Monoamine Oxidase MAO-B	368072	hum	2	10 µM	11				
107300	Peptidase, Angiotensin Converting Enzyme	368065	rabbit	2	10 µM	-3				
112510	Peptidase, CTSG (Cathepsin G)	368255	hum	2	10 µM	5				
152000	Phosphodiesterase PDE3	368188	hum	2	10 µM	17				
154000	Phosphodiesterase PDE4	368189	hum	2	10 µM	5				
178010	Protein Serine/Threonine Kinase, PKC, Non-Selective	368276	rat	2	10 µM	-12				
174990	Protein Tyrosine Kinase, Insulin Receptor	368270	hum	2	10 µM	0				
176020	Protein Tyrosine Kinase, LCK	368182	hum	2	10 µM	11				
200510	Adenosine A_1	368090	hum	2	10 µM	31				
200610	Adenosine A_{2A}	368090	hum	2	10 µM	5				
203100	Adrenergic α_{1A}	368060	rat	2	10 µM	16				
203200	Adrenergic α_{1B}	368061	rat	2	10 µM	1				
203400	Adrenergic α_{1D}	368352	hum	2	10 µM	-9				
203630	Adrenergic α_{2A}	368096	hum	2	10 µM	10				
203710	Adrenergic α_{2B}	368097	hum	2	10 µM	3				
204010	Adrenergic β_1	368154	hum	2	10 µM	0				
204110	Adrenergic β_2	368113	hum	2	10 µM	11				
206000	Androgen (Testosterone)	368155	hum	2	10 µM	42				
210030	Angiotensin AT_1	368341	hum	2	10 µM	13				
212620	Bradykinin B_2	368214	hum	2	10 µM	8				
214510	Calcium Channel L-Type, Benzothiazepine	368062	rat	2	10 µM	50				
214600	Calcium Channel L-Type, Dihydropyridine	368098	rat	2	10 µM	59				
215000	Calcium Channel L-Type, Phenylalkylamine	368416	rat	2	10 µM	55				
216000	Calcium Channel N-Type	368143	rat	2	10 µM	-1				
217030	Cannabinoid CB_1	368050	hum	2	10 µM	21				
217100	Cannabinoid CB_2	368058	hum	2	10 µM	16				
217510	Chemokine CCR1	368342	hum	2	10 µM	-7				
244500	Chemokine CXCR2 (IL-$8R_B$)	368344	hum	2	10 µM	-3				

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*	K_i	n_H	R
218030	Cholecystokinin CCK_1 (CCK_A)	368142	hum	2	10 µM	27				
218130	Cholecystokinin CCK_2 (CCK_B)	368142	hum	2	10 µM	-1				
219500	Dopamine D_1	368048	hum	2	10 µM	15				
219600	Dopamine D_{2L}	368040	hum	2	10 µM	10				
219700	Dopamine D_{2S}	368047	hum	2	10 µM	3				
224010	Endothelin ET_A	368216	hum	2	10 µM	-9				
226010	Estrogen ERα	368250	hum	2	10 µM	-4				
226810	$GABA_A$, Chloride Channel, TBOB	368158	rat	2	10 µM	49				
226600	$GABA_A$, Flunitrazepam, Central	368105	rat	2	10 µM	1				
226630	$GABA_A$, Ro-15-1788, Hippocampus	368343	rat	2	10 µM	-4				
228610	$GABA_{B1A}$	368212	hum	2	10 µM	12				
232030	Glucocorticoid	368094	hum	2	10 µM	24				
232600	Glutamate, AMPA	368064	rat	2	10 µM	-6				
232700	Glutamate, Kainate	368167	rat	2	10 µM	18				
237000	Glutamate, Metabotropic, $mGlu_5$	368357	hum	2	10 µM	5				
232810	Glutamate, NMDA, Agonism	368208	rat	2	10 µM	12				
232910	Glutamate, NMDA, Glycine	368209	rat	2	10 µM	-2				
233000	Glutamate, NMDA, Phencyclidine	368108	rat	2	10 µM	-16				
234000	Glutamate, NMDA, Polyamine	368210	rat	2	10 µM	13				
239000	Glycine, Strychnine-Sensitive	368211	rat	2	10 µM	5				
239610	Histamine H_1	368089	hum	2	10 µM	11				
239710	Histamine H_2	368051	hum	2	10 µM	9				
250460	Leukotriene, Cysteinyl $CysLT_1$	368354	hum	2	10 µM	7				
251100	Melanocortin MC_1	368279	hum	2	10 µM	3				
251350	Melanocortin MC_4	368279	hum	2	10 µM	2				
252610	Muscarinic M_1	368308	hum	2	10 µM	17				
252710	Muscarinic M_2	368093	hum	2	10 µM	5				
252810	Muscarinic M_3	368093	hum	2	10 µM	4				
252910	Muscarinic M_4	368307	hum	2	10 µM	9				
257010	Neuropeptide Y Y_1	368218	hum	2	10 µM	4				
258590	Nicotinic Acetylcholine	368106	hum	2	10 µM	12				
258700	Nicotinic Acetylcholine α1, Bungarotoxin	368107	hum	2	10 µM	0				
260130	Opiate δ_1 (OP1, DOP)	368059	hum	2	10 µM	-2				
260210	Opiate κ(OP2, KOP)	368059	hum	2	10 µM	6				
260410	Opiate µ(OP3, MOP)	368101	hum	2	10 µM	-3				
265010	Platelet Activating Factor (PAF)	368095	hum	2	10 µM	0				

Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*	K_i	n_H	R
265600	Potassium Channel [K_{ATP}]	368166	ham	2	10 µM	0				
265900	Potassium Channel hERG	368110	hum	2	10 µM	36				
267500	PPARγ	368148	hum	2	10 µM	4				
299005	Progesterone PR-B	368039	hum	2	10 µM	11				
271110	Serotonin (5-Hydroxytryptamine) 5-HT_{1A}	368213	hum	2	10 µM	4				
271230	Serotonin (5-Hydroxytryptamine) 5-HT_{1B}	368042	hum	2	10 µM	2				
271650	Serotonin (5-Hydroxytryptamine) 5-HT_{2A}	368161	hum	2	10 µM	7				
271700	Serotonin (5-Hydroxytryptamine) 5-HT_{2B}	368100	hum	2	10 µM	50				
271800	Serotonin (5-Hydroxytryptamine) 5-HT_{2C}	368163	hum	2	10 µM	17				
271910	Serotonin (5-Hydroxytryptamine) 5-HT_3	368213	hum	2	10 µM	-8				
279510	Sodium Channel, Site 2	368104	rat	2	10 µM	44				
255520	Tachykinin NK_1	368217	hum	2	10 µM	41				
202000	Transporter, Adenosine	368207	gp	2	10 µM	49				
220320	Transporter, Dopamine (DAT)	368045	hum	2	10 µM	82				
226400	Transporter, GABA	368151	rat	2	10 µM	13				
204410	Transporter, Norepinephrine (NET)	368044	hum	2	10 µM	45				
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	368063	hum	2	10 µM	2				
287530	Vasopressin V_{1A}	368345	hum	2	10 µM	-3				

11d

Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*	K_I	n_H	R
Compound: GB1211-02, PT #: 1192815										
107710	ATPase, Na^+/K^+, Heart, Pig	375396	pig	2	10 µM	-3				
104010	Cholinesterase, Acetyl, ACES	375394	hum	2	10 µM	-4				
116020	Cyclooxygenase COX-1	375331	hum	2	10 µM	13				
118010	Cyclooxygenase COX-2	375332	hum	2	10 µM	-3				
140010	Monoamine Oxidase MAO-A	375398	hum	2	10 µM	5				
140120	Monoamine Oxidase MAO-B	375399	hum	2	10 µM	9				
107300	Peptidase, Angiotensin Converting Enzyme	375395	rabbit	2	10 µM	-1				
112510	Peptidase, CTSG (Cathepsin G)	375397	hum	2	10 µM	-4				
152000	Phosphodiesterase PDE3	375469	hum	2	10 µM	2				
154000	Phosphodiesterase PDE4	375470	hum	2	10 µM	-21				
178010	Protein Serine/Threonine Kinase, PKC, Non-Selective	375538	rat	2	10 µM	1				
174990	Protein Tyrosine Kinase, Insulin Receptor	375536	hum	2	10 µM	-15				
176020	Protein Tyrosine Kinase, LCK	375537	hum	2	10 µM	-5				
200510	Adenosine A_1	375278	hum	2	10 µM	4				
200610	Adenosine A_{2A}	375279	hum	2	10 µM	6				
203100	Adrenergic α_{1A}	375262	rat	2	10 µM	3				
203200	Adrenergic α_{1B}	375263	rat	2	10 µM	11				
203400	Adrenergic α_{1D}	375314	hum	2	10 µM	7				
203630	Adrenergic α_{2A}	375280	hum	2	10 µM	-3				
203710	Adrenergic α_{2B}	375353	hum	2	10 µM	1				
204010	Adrenergic β_1	375272	hum	2	10 µM	3				
204110	Adrenergic β_2	375281	hum	2	10 µM	5				
206000	Androgen (Testosterone)	375333	hum	2	10 µM	1				
210030	Angiotensin AT_1	375518	hum	2	10 µM	7				
212620	Bradykinin B_2	375316	hum	2	10 µM	-5				
214510	Calcium Channel L-Type, Benzothiazepine	375307	rat	2	10 µM	5				
214600	Calcium Channel L-Type, Dihydropyridine	375261	rat	2	10 µM	-13				
215000	Calcium Channel L-Type, Phenylalkylamine	375277	rat	2	10 µM	5				
216000	Calcium Channel N-Type	375348	rat	2	10 µM	-3				
217030	Cannabinoid CB_1	375228	hum	2	10 µM	2				
217100	Cannabinoid CB_2	375359	hum	2	10 µM	21				
217510	Chemokine CCR1	375425	hum	2	10 µM	-12				
244500	Chemokine CXCR2 (IL-8R_B)	375352	hum	2	10 µM	11				

Note: Items meeting criteria for significance (≥50% stimulation or inhibition) are highlighted.
* Batch: Represents compounds tested concurrently in the same assay(s).
gp=Guinea pig; ham=Hamster; hum=Human

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*	K_i	n_H	R
218030	Cholecystokinin CCK_1 (CCK_A)	375319	hum	2	10 µM	-5				
218130	Cholecystokinin CCK_2 (CCK_B)	375319	hum	2	10 µM	-1				
219500	Dopamine D_1	375264	hum	2	10 µM	-4				
219600	Dopamine D_{2L}	375367	hum	2	10 µM	15				
219700	Dopamine D_{2S}	375282	hum	2	10 µM	-8				
224010	Endothelin ET_A	375317	hum	2	10 µM	13				
226010	Estrogen ERα	375320	hum	2	10 µM	2				
226810	$GABA_A$, Chloride Channel, TBOB	375349	rat	2	10 µM	19				
226600	$GABA_A$, Flunitrazepam, Central	375266	rat	2	10 µM	-2				
226630	$GABA_A$, Ro-15-1788, Hippocampus	375305	rat	2	10 µM	-9				
228610	$GABA_{B1A}$	375240	hum	2	10 µM	1				
232030	Glucocorticoid	375549	hum	2	10 µM	-2				
232600	Glutamate, AMPA	375231	rat	2	10 µM	1				
232700	Glutamate, Kainate	375342	rat	2	10 µM	-13				
237000	Glutamate, Metabotropic, $mGlu_5$	375431	hum	2	10 µM	10				
232810	Glutamate, NMDA, Agonism	375309	rat	2	10 µM	-1				
232910	Glutamate, NMDA, Glycine	375310	rat	2	10 µM	-13				
233000	Glutamate, NMDA, Phencyclidine	375283	rat	2	10 µM	4				
234000	Glutamate, NMDA, Polyamine	375355	rat	2	10 µM	-4				
239000	Glycine, Strychnine-Sensitive	375343	rat	2	10 µM	-4				
239610	Histamine H_1	375267	hum	2	10 µM	-16				
239710	Histamine H_2	375404	hum	2	10 µM	-1				
250460	Leukotriene, Cysteinyl $CysLT_1$	375312	hum	2	10 µM	6				
251100	Melanocortin MC_1	375335	hum	2	10 µM	1				
251350	Melanocortin MC_4	375335	hum	2	10 µM	-3				
252610	Muscarinic M_1	375244	hum	2	10 µM	-4				
252710	Muscarinic M_2	375243	hum	2	10 µM	8				
252810	Muscarinic M_3	375243	hum	2	10 µM	6				
252910	Muscarinic M_4	375244	hum	2	10 µM	1				
257010	Neuropeptide Y Y_1	375324	hum	2	10 µM	8				
258590	Nicotinic Acetylcholine	375269	hum	2	10 µM	11				
258700	Nicotinic Acetylcholine α1, Bungarotoxin	375270	hum	2	10 µM	5				
260130	Opiate δ_1 (OP1, DOP)	375247	hum	2	10 µM	0				
260210	Opiate κ(OP2, KOP)	375247	hum	2	10 µM	2				
260410	Opiate µ(OP3, MOP)	375247	hum	2	10 µM	1				
265010	Platelet Activating Factor (PAF)	375334	hum	2	10 µM	21				

Note: Items meeting criteria for significance (≥50% stimulation or inhibition) are highlighted.
* Batch: Represents compounds tested concurrently in the same assay(s).
gp=Guinea pig; ham=Hamster; hum=Human

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*	K_i	n_H	R
265600	Potassium Channel [K_{ATP}]	375522	ham	2	10 µM	25				
265900	Potassium Channel hERG	375253	hum	2	10 µM	20				
267500	PPARγ	375300	hum	2	10 µM	0				
299005	Progesterone PR-B	375344	hum	2	10 µM	0				
271110	Serotonin (5-Hydroxytryptamine) 5-HT_{1A}	375328	hum	2	10 µM	7				
271230	Serotonin (5-Hydroxytryptamine) 5-HT_{1B}	375330	hum	2	10 µM	-7				
271650	Serotonin (5-Hydroxytryptamine) 5-HT_{2A}	375510	hum	2	10 µM	7				
271700	Serotonin (5-Hydroxytryptamine) 5-HT_{2B}	375252	hum	2	10 µM	12				
271800	Serotonin (5-Hydroxytryptamine) 5-HT_{2C}	375274	hum	2	10 µM	8				
271910	Serotonin (5-Hydroxytryptamine) 5-HT_3	375329	hum	2	10 µM	0				
279510	Sodium Channel, Site 2	375275	rat	2	10 µM	23				
255520	Tachykinin NK_1	375340	hum	2	10 µM	-3				
202000	Transporter, Adenosine	375519	gp	2	10 µM	15				
220320	Transporter, Dopamine (DAT)	375285	hum	2	10 µM	18				
226400	Transporter, GABA	375341	rat	2	10 µM	9				
204410	Transporter, Norepinephrine (NET)	375260	hum	2	10 µM	12				
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	375311	hum	2	10 µM	8				
287530	Vasopressin V_{1A}	375322	hum	2	10 µM	5				

In vitro assessment of effects of 11b and 11d on human potassium channel using human embryonic kidney (HEK) 293 cells transfected with a human ether-a-go-go-related gene (hERG)

In vitro assessment were performed at IPS Therapeutique (Sherbrooke, Canada)

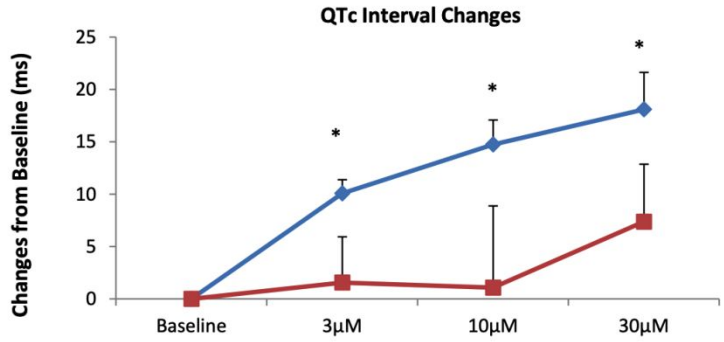
The transfected HEK293 cells were submitted to five (5) minutes of exposure to each concentration (0.1, 1 and 10µM) in presence of closed circuit perfusion (2mL/min). Five (5)minutes for washout periods in presence of a flow-through perfusion (2mL/min) in addition to a closed circuit perfusion (2mL/min). The positive control (100 nM E-4031) was added to naivel cells obtained from the same cell line and same passage for a period of 5 minutes in presence of a closed circuit perfusion (2mL/min). Cells were under continuous stimulation of the pulses protocol throughout the experiments and cell currents were recorded after 5 minutes of exposure to each condition. Whole-cell currents elicited during a voltage pulse were recorded in baseline conditions and following the application of the selected concentrations of test article. Currents were also recorded following a washout period. The cells were depolarized for one second from the holding potential (-80 mV) to a maximum value of +40 mV, starting at -40mV and progressing in 10 mV increments. The membrane potential was then repolarized to -55 mV for one second, and finally returned to -80 mV. Whole-cell tail current amplitude was measured at a holding potential of -55mV, following activation of the current from-40to +40 mV. Current amplitude was measured at the maximum (peak) of this tail current. Current density was obtained by dividing current amplitude by cell capacitance measured prior to capacitive transient minimisation. All data points presented have been corrected for solvent effects and time-dependent current run-down.

Compound	Statistically significant inhibition starting at (μM)	Maximal inhibition (%)	IC50 (μM)
11b	0.1	61	2.9
11d	1	36	not applicable

***In vivo* assessment of QTc effect of 11b and 11d in anestesized guinea pig**

The evaluation of QTc in anestesized guinea pigs were performed at **IPS Therapeutique (Sherbrooke, Canada).** The guinea pigs were anaesthetized with a mixture of 1.5 to 2.0% isoflurane USP (Abbott Laboratories, Montreal Canada) in 95% O_2 and 5% CO_2. The left jugular vein was cannulated to administer the drug or vehicle. The guinea pigs received three consecutive intravenous injections (i.v.) of 11b and 11d in a cumulative-dose scheme to obtain 3, 10 or 30 μM target plasma concentrations. Each dose was administered ~10 minutes following completion of the assessment from the previous dose. Calculations were based on a body weight of 325-335g and a blood volume of 64 mL/kg. Since the test articles were administered intravenously, 100% bioavailability was assumed. The control-vehicle group received intravenous injections of the same volume of solvent (DMSO/saline). ECG leads were placed on the animal in a lead 1-2-3 configuration. A continuous recording of the ECG was made, starting prior to the first injection, and lasting at least 10 minutes past the last injection (n=3). The PR, RR, QRS and QT intervals were measured in millisecond using cursor readings in the Clampfit 10.2.0.14 module of the pClamp 10.2.0.14 software (Axon Instrument Inc., Foster City, California, USA, [now Molecular Devices Inc.]). Three PR, RR, QRS and QT intervals were measured for each condition and then averaged in Microsoft Excel 2007. All test article data points presented have been corrected for the changes attributable to vehicle exposure and experimental time.

Effect of 11b(blue line) and 11d(red line) on QTc intervals of guinea pigs.



Co-crystallization of galectin-3 C-terminal domain with compound 11d

Galectin-3C (C-terminal domain, J. Seetharaman, A. Kanigsberg, R. Slaaby, H. Leffler, S. H. Barondes, J. M. Rini, *J. Biol. Chem.* **1998**, *273*, 13047-13052.
) solutions (15 µL, 20 mg/mL in 10 mM sodium phosphate buffer pH 7.5, 100 mM NaCl, and 10 mM ß-mercaptoethanol) were mixed with **11d** (0.5 µL, 50 mM in 100% DMSO). The sample was centrifuged at 13500 rpm for 5 min. A seed bead solution (Hampton Research) was used made from crushed apo galectin-3C crystals in a 40-fold diluted seed solution (diluted reservoir solution; see below) was used to seed the crystallisation drops. Drops were setup in MRC 3-well plates and stored at 16 °C with 200 nL galectin-3C: **11d** complex, 30 nL apo galectin-3C seed solution, and 200 nL reservoir solution (30% PEG 4000, 0.1 M Tris/HCl pH 7.5, 0.1 M $MgCl_2$, 7.9 mM β- mercaptoethanol) and then equilibrated against 1 ml of reservoir solution containing 30% PEG 4000, 0.1 M Tris/HCl pH 7.5, 0.1M $MgCl_2$, 0.4M NaSCN, 7.9 mM β-mercaptoethanol. Large crystals formed (approx. 0.1-0.2 mm) were briefly soaked in a cryoprotectant solution (25.5% PEG4000. 15 % glycerol, 85 mM Tris/HCl pH 7.5, 85 mM $MgCl_2$, 250 mM NaSCN, 8 mM β-mercaptoethanol, and 1 mM **11d**). After soaking, the crystal was flash-cooled in liquid N_2.

Data collection and structure solution of galectin-3C in complex with 11d

Data sets were collected at 100K at station PXIII, Swiss Light Source, Villigen, Switzerland (λ = 1.0000 Å) equipped with a Pilatus 2M-F detector. 900 images were collected using an exposure time of 0.1 s and an oscillation of 0.2° per image. The data were processed using XDS and Aimless to 1.05 Å. (W. Kabsch, *Acta Crystallogr. Sect. D Biol. Crystallogr.* **2010**, *66*, 125-132.) A previously-delivered complex determined to 1.2 Å resolution (pdb id 5OAX) (K. Peterson, R.

Kumar, O. Stenström, P. Verma, P. R. Verma, M. Håkansson, B. Kahl-Knutsson, F. Zetterberg, H. Leffler, M. Akke, D. T. Logan, U. J. Nilsson; Systematic Tuning of Fluoro-galectin-3 Interactions Provides Thiodigalactoside Derivatives with Single-Digit nM Affinity and High Selectivity *J. Med. Chem.,* 2018, **61**, 1164-1175, DOI: 10.1021/acs.jmedchem.7b01626) with the ligand removed was used as a starting model for both structures. CCP4-style restraints for **11d** were generated using the JLigand program.(A. A. Lebedev, P. Young, M. N. Isupov, O. V. Moroz, A. A. Vagin, G. N. Murshudov, *Acta Crystallogr. Sect. D Biol. Crystallogr.* **2012**, *68*, 431-440.) After initial rigid body and atomic refinement of the protein coordinates in Refmac5 (Murshudov, G.N., Skubak, P., Lebedev, A.A., Pannu, N.S., Steiner, R.A., Nicholls, R.A., Winn, M.D. Long, F. and Vagin, A.A. (2011) REFMAC5 for the refinement of macromolecular crystal structures, *Acta Crystallogr. D Biol. Crystallogr.* **67**, 355-367.), the coordinates of the compounds were fitted to the electron density using Coot(P. Emsley, B. Lohkamp, W. G. Scott, K. Cowtan, *Acta Crystallogr. Sect. D Biol. Crystallogr.* **2010**, *66*, 486-501.). The structures were refined until convergence using Refmac5. Individual anisotropic B-factors for each atom were refined. Hydrogen atoms were added in the riding positions. Water molecules were added to positive difference density peaks more than 5 standard deviations above the mean and present in the 2m|Fo|-d|Fc| map at the 1 σ level. Molecular images were generated using PyMOL (Schrodinger LLC). Model validation and analysis were performed using MolProbity. (V. B. Chen, W. B. Arendall, J. J. Headd, D. A. Keedy, R. M. Immormino, G. J. Kapral, L. W. Murray, J. S. Richardson, D. C. Richardson, *Acta Crystallogr. Sect. D Biol. Crystallogr.* **2010**, *66*, 12-21.)

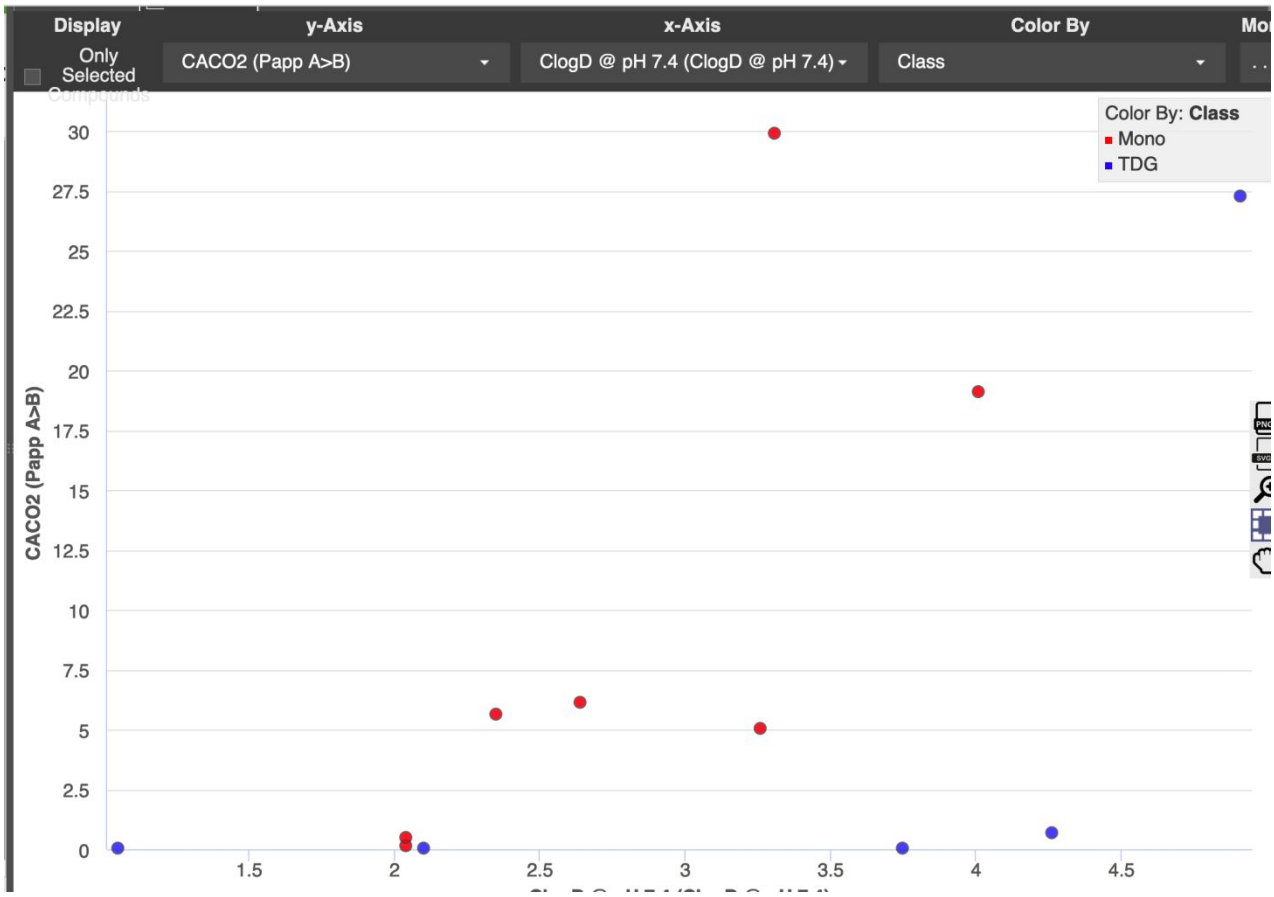
Coordinates have been deposited in the Protein Data Bank with accession numbers 7ZQX.

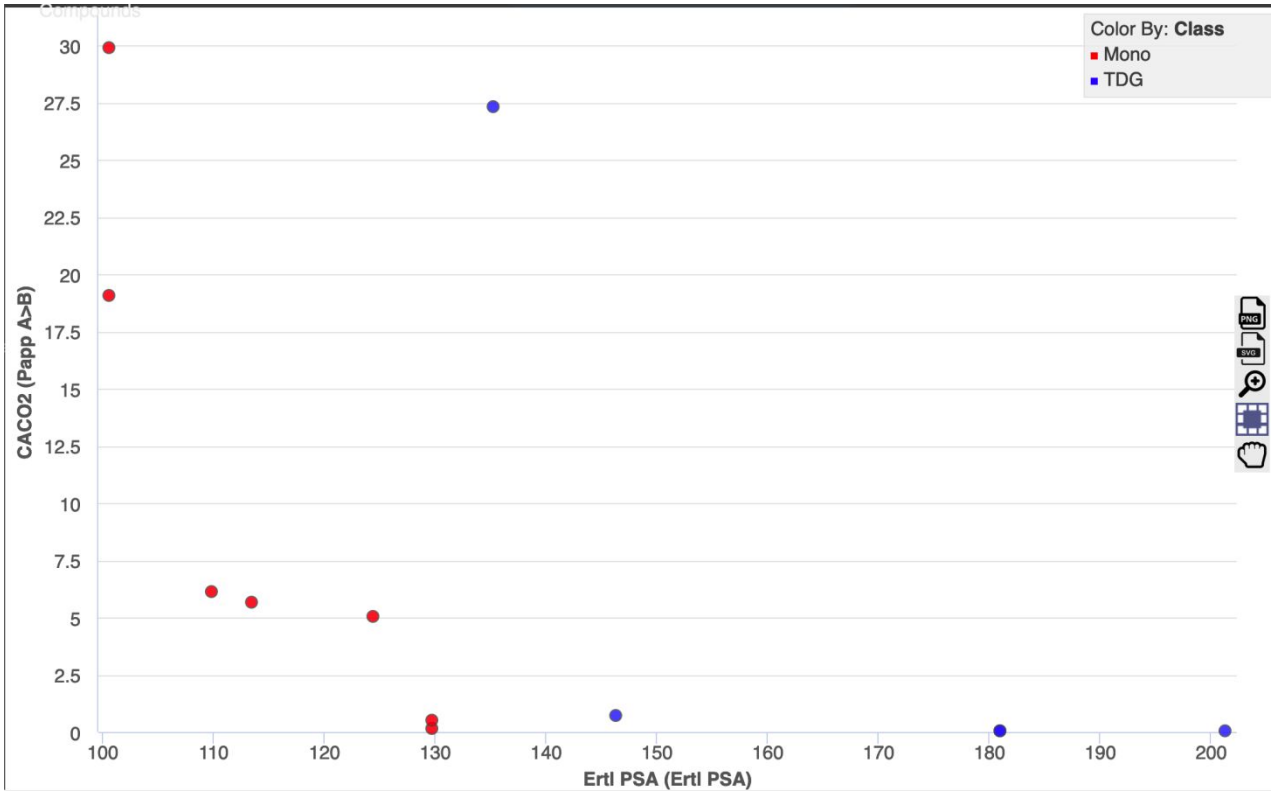
Data processing and refinement statistics.. Values in parentheses are for the highest resolution shell, unless noted otherwise.

Resolution (Å)	29.06 - 1.05 (1.07 - 1.05)
Wavelength (Å)	1.0000
Space group	P212121
Unit cell (Å, °)	a = 36.40, b = 58.09, c = 62.58

Completeness (%)	100.0 (99.9)
Redundancy	6.1 (5.5)
No. of observations / unique reflections	383 590 / 62 710
$\langle I/\sigma(I) \rangle$	21.1 (3.0)
CC(1/2) (%)	100.0 (87.4)
R_{merge} (I) (%)	3.7 (54.5)
R_{model} (F) (%)	10.9 (17.6)
R_{free} (F) (%)	12.9 (18.3)
No. of non-hydrogen atoms	1656
No. of water molecules	263
rms deviations from ideal geometry: Bond lengths (Å)	0,021
Bond angles (deg)	2.4
Mean B-factor (protein, $Å^2$)	11.1
Mean B-factor (**11d**, $Å^2$)	12.9
Mean B-factor (solvent and ions, $Å^2$)	25.4
Ramachandran plot quality	
Favoured regions (%)	97.0
Allowed regions (%)	3.0
Outliers (%)	0

Plots of passage A>B over CACO-2 over ClogD(pH 7.4) and ertlPSA for compounds included in the manuscript. Monogalctosides denoted Mono(red) and thidodigalactosides denoted TDG(blue).




Compounds
Color By: Class
Mono
TDG
CACO2 (Papp A>B)
30
27.5
25
22.5
20
17.5
15
12.5
10
7.5
5
2.5
0
100
110
120
130
140
150
160
170
180
190
200
Ertl PSA (Ertl PSA)