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# Structure-Guided Design of C3-Branched Swainsonine as Potent and Selective Human Golgi α-Mannosidase (GMII) Inhibitor

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**The human Golgi α-mannosidase, hGMII, removes two mannose residues from GlcNAc-Man$_5$GlcNAc$_2$ to produce GlcNAcMan$_3$GlcNAc$_2$, the precursor of all complex *N*-glycans including tumour-associated ones. The natural product GMII inhibitor, swainsonine, blocks processing of cancer-associated *N*-glycans, but also inhibits the four other human α-mannosidases, rendering it unsuitable for clinical use. Our previous structure-guided screening of iminosugar pyrrolidine and piperidine fragments identified two micromolar hGMII inhibitors occupying the enzyme active pockets in adjacent, partially overlapping sites. Here we demonstrate that fusing these fragments yields swainsonine-configured indolizidines featuring a C3-substituent that act as selective hGMII inhibitors. Our structure-guided GMII-selective inhibitor design complements a recent combinatorial approach that yielded similarly configured and substituted indolizidine GMII inhibitors, and holds promise for the potential future development of anti-cancer agents targeting Golgi *N*-glycan processing.**

Swainsonine (**1**, Fig. 1) is a natural product indolizidine alkaloid and an inhibitor of the human Golgi α-mannosidase, hGMII (MAN2A1).[1,2] hGMII, a retaining glycoside hydrolase from the CAZyme family GH38, catalyzes the hydrolysis of both the terminal α-1,3-linked and α-1,6-linked mannoses from GlcNAcMan$_5$GlcNAc$_2$ N-glycans, which is essential for further processing towards complex *N*-glycoproteins. Alteration of *N*-glycan branching through GMII modulation impacts the metastatic potential of cancer cells, and for this reason swainsonine is an interesting starting point for the development of antitumor agents. Swainsonine has been subject to Phase I and Phase II clinical trials, but its nonselective inhibition of other human GH38 α-mannosidases (MAN2A2, MAN2B1, MAN2B2, MAN2C1) hampers its further development.[3] Specifically, inhibition of lysosomal α-mannosidase (MAN2B1) causes side-effects similar to the lysosomal storage disease, α-mannosidosis. Thus, swainsonine derivatives that selectively inhibit GMII without blocking the action of the other human mannosidases, and particularly that of the lysosomal mannosidase, MAN2B1, may succeed where swainsonine has failed in becoming effective, clinical anticancer agents.

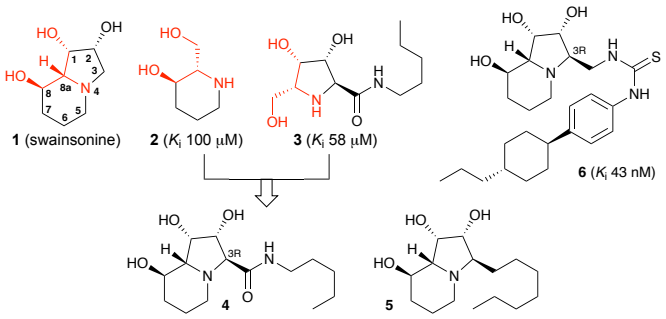


Fig. 1. Iminosugars subject to the here-presented studies: swainsonine (**1**), the piperidine (**2**) and pyrrolidine (**3**) fragments identified from our previous studies ($K_i$ values for hGMII taken from the literature[4]) based on which we designed C3-branched, swainsonine-configured title compounds **4** and **5**. Similarly configured indolizidine **6** was recently[7] reported as a potent and selective competitive hGMII inhibitor in a natural product-driven combinatorial chemistry approach.

hGMII, like the other human α-mannosidases, is a retaining exoglycosidase that cleaves substrate α-mannosides in a two-step, double displacement mechanism during which a transiently covalent enzyme-substrate adduct is formed. We recently developed a fluorescence polarization activity-based protein profiling (FP-ABPP) assay in which 358 glycomimetics were screened on their *Drosophila* GMII (dGMII) inhibitory potency.[4] This resulted in the discovery of several hits, including the micromolar inhibitors **2** and **3** (Fig. 1). These compounds capture different parts of swainsonine (**1**) with the swainsonine-configured 2-amino-1,3-diol moiety (shown in red – the 1-8a-8 stretch) represented in both pyrrolidine and piperidine fragments. The remaining three carbons in piperidine **2** feature as C5-C6-C7 in swainsonine, and the remaining two pyrrolidine carbons in **3** as C2 (as secondary alcohol, with the same stereochemistry) and C3. In contrast to swainsonine, however,

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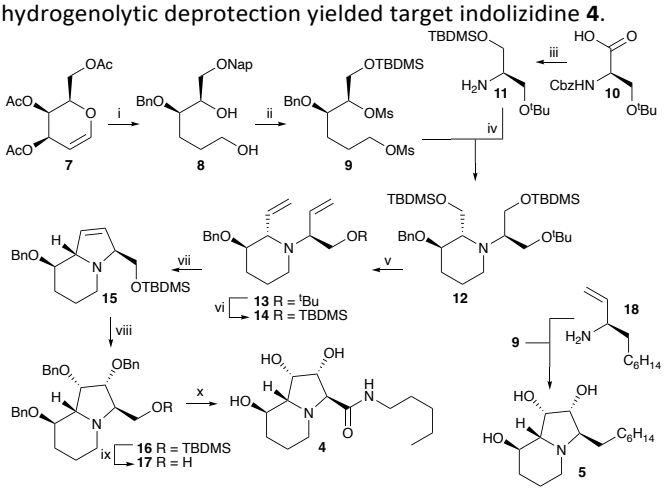
pyrrolidine **3** carries an exocyclic substituent at this C3 carbon. X-ray studies on dGMII (a close homologue of hGMII) complexed to either piperidine **2** or pyrrolidine **3** showed these fragments to occupy adjacent, partially overlapping positions within the enzyme active site. Moreover, the structure of dGMII complexed to **3** revealed the amide group of the appendage hydrogen bonded to the catalytic acid base residue. These data suggested that fusing the piperidine and pyrrolidine structural elements, while keeping the *trans*-configured (with respect to the 1,2-diol) C3 side chain functionality as in **3**, may lead to swainsonine analogues that inhibit GMII with improved selectivity. This idea was enforced by several studies[5-7] on C3-substituted swainsonine analogues, the most compelling of which is the recent report by Chen *et al*.[7] They identified in a natural product-inspired, computational chemistry guided combinatorial chemistry approach, a series of (3R)-substituted swainsonine derivatives including indolizidine **6** as a low nanomolar ($K_i$ 0.043 μM) hGMII inhibitor with 106-fold selectivity over the other human α-mannosidases. With this reasoning in mind, we decided to 'grow' our pyrrolidine (**2**) and piperidine (**3**) fragments into full-fledged, C3-substituted swainsonines. This required an efficient route of synthesis, which we established for the preparation of compound **4** as depicted in Scheme 1.

The route of synthesis starts with a Ferrier rearrangement and subsequent protective- and functional group manipulations to transform tri-*O*-acetyl-D-galactal **7** into orthogonally protected 1,5-diol **8**. Bismesylation then gives intermediate **9**, which is susbsequently transformed, with inversion of stereochemistry at the secondary alcohol carbon, into piperidine **12** by double $S_N2$ displacement with secondary amine **11** (itself prepared in three steps from partially protected D-serine **10**). Removal of both silyl protective groups was followed by Swern oxidation of both primary alcohols, and then a double Wittig event to give diene **13**. At this stage and in the optimal route we swapped the *tert*-butyl ether for a TBDMS ether (**13** to **14**), for reasons explained further down. The subsequent ring-closing metathesis (RCM, **14** to **15**) proved far from trivial, and all Grubbs-type ruthenium catalysts we tried, also under conditions (addition of acid) that protects the catalyst from the free tertiary amine, proved abortive. Usage of Schrock's molybdenum catalyst fortunately proved productive, yielding indolizine intermediate **15** in decent (50%) and reproducible yield along with recovery of reusable starting material. The next key step – dihydroxylation – as well proved more eventful than anticipated. Here we found that the *tert*-butyl analogue of **15** (thus the RCM product of **13**, which can be obtained with equal efficiently), upon treatment with $OsO_4$ predominantly returns the epimeric (with respect to swainsonine) cis-diol. Treatment of **15** with $OsO_4$ and TMEDA followed by benzylation gave the desired protected cis-diol **16** as the major product, together with a significant portion of the epimeric one (3:1, 64% overall yield in this key step). Addition of ethylene diamine following the dihydroxylation step was required to break up the intermediate osmate ester, and for this reason stoichiometric $OsO_4$ was needed to achieve an effective dihydroxylation. Removal of the silyl protective group followed by Jones oxidation of the resultant primary alcohol **17**, condensation of the carboxylic acid with heptylamine and final global hydrogenolytic deprotection yielded target indolizidine **4**.




Scheme 1. Reagents and conditions: (i) 1. $SnCl_4$, *i*PrOH, DCM, r.t., 16 h, 62%; 2. $H_2$, Pd/C, EtOH, r.t., 3 h, 90%; 3. NaOMe, MeOH, r.t., 2 h, 99%; 4. NapBr, Taylor's catalyst, KI, $K_2CO_3$, MeCN, 65 °C, 3 h, 89%; 4. BnBr, NaH, TBAI, DMF, r.t., 16 h, 94%; 5. 4:1 AcOH/1 M HCl, 60 °C, 16 h, quant.; 6. $NaBH_4$, EtOH, r.t., 3 h, 98%; (ii) 1. MsCl, pyr, 0 °C, 2 h, quant.; 2. DDQ, 3:1 DCM/$H_2O$, r. t., 3 h, quant; 3. TBSCl, imidazole, DCM, 0 °C, 2 h, 96%; (iii) 1. isobutyl chloroformate, N-methylmorpholine, $NaBH_4$, DCM, -15 °C, 2 h, quant; 2. TBSCl, imidazole, DCM, 0 °C to r.t., 2 h, 89%; 3. Pd/C, $H_2$, EtOH, r.t., 16 h, 98%; (iv) DIPEA, MeCN, 50 °C to 70 °C, 4 days, 77%; (v) 1. TBAF, THF, r.t., 16 h, 94%; 2. oxalyl chloride, DMSO, $Et_3N$, DCM, -78 °C to 0 °C, 5 h; 3. $MeP(Ph)_3Br$, NaHMDS, THF, -78 °C to 0 °C, 16 h, 36% (over 2 steps); (vi) 1. TFA, $H_2O$, 0 °C to r.t., 2 h, 77%; 2. TBSCl, imidazole, DCM, 0 °C, 2 h, 94%; (vii) Schrock-Hoveyda catalyst, benzene, r.t., 16 h, 50%; (viii) 1. $OsO_4$, TMEDA, DCM, -78 °C, then ethylenediamine, r.t., 16 h, 48% (together with 16% stereoisomeric cis-diol); 2. BnBr, NaH, DMF, r.t., 4 h, 70%; (ix) TBAF, THF, r.t., 2 h, 82%; (x) 1. $CrO_3$, $H_2SO_4$, $H_2O$, acetone, 60 °C, 30 min, 42%; 2. heptylamine, HATU, DIPEA, r.t., 16 h, 92%; 3. Pd/C, $H_2$, HCl, dioxane, r.t., 16 h, 89%.

With the aim to demonstrate the generality of the route of synthesis, we prepared similarly configured indolizidine **5**, now by reacting bismesylate **9** with allylamine **18** with the alkyl side-chain already installed. The ensuing route of the thus obtained piperidine (see the Supporting Information for details) towards indolizidine **5** proceeded through the same general sequence of events (liberation of the diol, double Swern oxidation followed by double Wittig olefination, then RCM and then dihydroxylation) with comparable efficiency as described for **12**. With branched swainsonines **4** and **5** in hand, we then determined their inhibition constants, together with that of swainsonine **1** as inhibitors of both hGMII and dGMII in a fluorogenic substrate assay with concentration-dependent inhibition of enzymatic 4-methylumbelliferyl-α-D-mannoside (4-MU-α-D-man) hydrolysis as the readout. Both enzymes are potently inhibited by swainsonine ($K_i$ hGMII 40 nM, $K_i$ dGMII 20 nM). As we reported previously, piperidine **2** ($K_i$ hGMII 100 μM, $K_i$ dGMII 58 μM) and pyrrolidine **3** ($K_i$ hGMII 41 μM, $K_i$ dGMII 11 μM) are about three-fold weaker inhibitors for both enzymes. 3(R)-Heptyl-swainsonine **5** ($K_i$ hGMII 308 μM, $K_i$ dGMII 167 μM) actually turns out to be a weaker inhibitor compared to the two fragments. Amide-modified swainsonine **4** in contrast ($K_i$ hGMII 15 μM, $K_i$ dGMII 6 μM) is somewhat (relative to pyrrolidine **3**) to considerably (relative to piperidine **2**) more potent as

inhibitor of both dGMII and hGMII. Besides the presence of a C3 sidechain, also the nature of this pharmacophore is therefore of influence on inhibition potency. This is supported by the work of Chen *et al*.[7] who performed extensive optimization studies on this part of the scaffold, arriving at the nanomolar hGMII inhibitor **6** (Fig. 1).

Table 1. A) Inhibition constants ($K_i$ values in μM) of swainsonine **1**, piperidine **2** , pyrrolidine **3** and branched indolizidines **4** and **5** as dGMII and hGMII inhibitors. B) $IC_{50}$ values for compounds **1**-**5** in lysates of human epithelial cells overexpressing MAN2A1/hGMII, MAN2A2, MAN2B1, MAN2B2 or MAN2C1.

| A | | | B | | | | |
|---|---|---|---|---|---|---|---|
| | **dGMII** | **hGMII** | **MAN2A1** | **MAN2A2** | **MAN2B1** | **MAN2B2** | **MAN2C1** |
| **1** | 0,08 ± 0.008 | 0,04 ± 0.009 | 2.4 ± 0.2 | 30 ± 3.0 | 1.6 ± 0.06 | 15 ± 0.6 | 24 ± 0.5 |
| **2** | 58 ± 3 | 100 ± 7 | >100 | >100 | >100 | >100 | >100 |
| **3** | 10.8 ± 0.8 | 41 ± 3 | 98 ± 16 | >100 | 58 ± 2.6 | 13 ± 0.25 | 0.12 ± 0.007 |
| **4** | 5.6 ± 0.6 | 15 ± 5 | 9.1 ± 2.0 | 66 ± 11 | 24 ± 1.3 | 1.5 ± 0.05 | >100 |
| **5** | 167 ± 13 | 308 ± 7 | 102 ± 12 | >100 | >100 | >100 | >100 |

The selectivity of compound **4**, being the most potent of the two branched swainsonine derivatives, was determined next in a fluorogenic substrate assay side by side with swainsonine **1**, piperidine **2** and pyrrolidine **3**. For this, extracts of cells in which either of the five human α-mannosidases (MAN2A1/hGMII, MAN2A2, MAN2B1, MAN2B2, MAN2C1) were brought to overexpression are first treated with these competitive inhibitors at varying concentrations, and subsequently with 4-methylumbelliferyl α-D-mannoside. Swainsonine **1** was revealed to be the most potent hGMII inhibitor also in these assays (table 1B, values for MAN2A1), with the inhibitory potency of compounds **2**-**5** towards overexpressed hGMII more or less following the trent observed for recombinant dGMII. We note that comparing trents and absolute values observed from the two assays should be done with caution: the origin (species) of the enzymes is different. As well, the second assay makes use of samples in which one mannosidase is brought to overexpression but in which the other four are also present at endogenous expression levels. Yet these assays do allow interpretation of some trents to evaluate whether our branched swainsonine derivatives hold merit as GMII-selective inhibitors. Which we believe is the case, to some extent. Swainsonine **1** is, besides MAN2A1 (hGMII), also the most potent inhibitor of MAN2A2 and MAN2B1 of the series, and together with pyrrolidine **3**, also the most potent MAN2B2 inhibitor. Rather strikingly, compound **3** proved to be the most potent MAN2C1 inhibitor, outperforming swainsonine **1** in this regard, with the other compounds inactive up until 100 micromolar. The reported broad spectrum activity of swainsonine **1** is therefore also apparent from these studies. Piperidine **2** proved inactive for all five enzymes and this holds true as well for alkylindolizidine **5**. Pyrrolidine **3** gives a mixed picture, and is besides being a remarkable MAN2C1 inhibitor also as noted on par with swainsonine for MAN2B2. It is 10-fold less active for hGMII (MAN2A1) in this assay compared to indolizidine **4**. This latter compound turns out to be the second-most active hGMII inhibitor after swainsonine in this assay, which matches the result from the kinetics assay on dGMII. It is also rather selective, although MAN2B2 and especially MAN2B1 are inhibited rather potently as well.

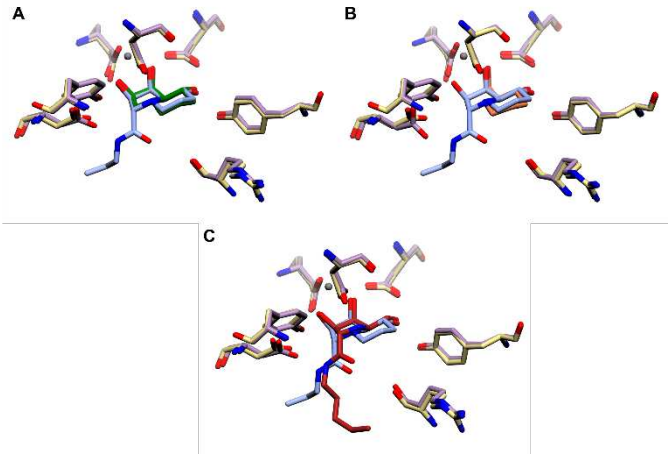



Fig. 2. Comparison of the binding pockets of dGMII in complex with inhibitors **1**-**4**. Images were created and visualised in CCP4mg (v. 2.10.11). The structure of **4** is displayed in blue cylinder form throughout. A) Binding pocket of dGMII:**4** with active site residues (yellow) SSM superimposed with the binding pocket of dGMII (lilac residues) in complex with **1** (green) (PDB: 3BLB). B) Binding pocket of dGMII:**4** with active site residues (yellow) SSM superimposed with the binding pocket of dGMII (lilac residues) in complex with inhibitor **2** (orange) (PDB: 6RRX). C) Binding pocket of dGMII:**4** with active site residues (yellow) SSM superimposed with the binding pocket of dGMII (lilac residues) in complex with inhibitor **3** (red) (PDB: 6RRN).

Aiming to further investigate the mode of action of these inhibitors we soaked dGMII with **4** and **5** to produce three complexed crystal structures resolved to 2.14 Å and 2.47 Å resolution respectively. Both **4** and **5** were only partially modelled with electron density missing for part of, or all of (in the case of **5**) the alkyl chain (see also the Supporting Information). As dictated by the stereochemistry at C3 it is likely that these alkyl chains point towards the "+1" sugar binding pocket but due to the likely flexibility of these chains, and their inability to be resolved in the electron density, we cannot be completely certain. Unsurprisingly, the structure of these complexes resembled typical GH38 family type folds, with DALI servers[8] identifying high structural similarity to other GH38 enzymes such as bovine lysosomal α-mannosidase (PDB: 1O7D, 27% sequence identity, rmsd of 2.0 Å across 250 Cα residues[9]) and *Streptococcus pyogenes* α-mannosidase (PDB: 2WYH, 14% sequence identity, rmsd of 3.3 Å across 905 Cα residues).[10] Both dGMII and ligand contribute in coordinating the active site zinc[4], and the octahedral zinc complex is formed by the 1- and 2-hydroxyls of the pyrrolidine ring together with His90, Asp92, Asp204 and His471 (Fig. S5). The overall binding motif of **4** and

**5** emulates that of swainsonine; coordinated by a series of hydrogen bonds in the active site (Fig. S6 and Fig. 2A). These hydroxyls on the pyrrolidine ring are also within hydrogen bonding distance of Asp92, Asp204 and Asp472. The hydroxyl attached to C1 of the piperidine fragment is hydrogen bonded to Asp472 and Tyr727. This hydrogen bonding network is also seen separately for each of the piperidine and pyrrolidine iminosugar fragments **2** and **3** (Fig. 2B and C).[4] The nitrogen of the ring in both **4** and **5** is coordinated by hydrogen bonds of approximately 2.7 Å and 3.2 Å respectively, from Asp204 (Fig. S6). Compound **4** displays a $K_i$, towards both dGMII and hGMII >20-fold higher than that of compound **5**. The additional hydrogen bond between the amide of **4** and acid/base residue Asp341, which is absent for compound **5** (Fig. S6 and Fig. 2), may explain this difference in inhibitory potency. This interaction also appears in other lyxo-configured pyrrolidine inhibitors such as **3** and other previously reported low μM inhibitors (Fig. 2).[4] By molecular docking, Chen and coworkers uncovered a hydrogen bonding network to exist between Tyr354 and a thiourea substituent in one of their inhibitors.[7] We do not observe this hydrogen bonding in our inhibitors with the corresponding residue (Tyr269 from dGMII) being positioned >4 Å away from any possible coordinating partner such as the amide nitrogen in **4** or nitrogen in the bicyclic ring of both **4** and **5**. Additionally, Chen and colleagues noticed the occurrence of sigma–π interactions and hydrophobic interactions between the 4-alkylcyclohexyl substituent in **6** with Tyr352/His358 and Gln150/Tyr316 in hGMII. They did not observe these interactions with hLM (MAN2B1), which may explain improved selectivity of this inhibitor for hGMII.

In conclusion, we here report a flexible and convergent strategy for the synthesis of C3-substituted swainsonines, which we demonstrate here in the synthesis of indolizidines **4** and **5**. Underscoring the literature report on the more elaborate indolizidine **6**,[7] compound **4** proves both more potent and more selective than the piperidine (**2**) and pyrrolidine (**3**) fragments as hGMII inhibitor. Structural studies corroborate the hypothesis behind our studies and indolizidine **4** occupies the partially overlapping sites where we previously[4] found compounds **2** and **3** to reside. The structural overlap with the swainsonine **1** bound structure is remarkable and additional interactions with the indolizidine **4** exocyclic amide bond with the enzyme active site likely contribute to the enhanced inhibitor potency (note that indolizidine **5** with a simple alkyl chain branch is less active). Chen and co-workers recently demonstrated that elaboration at this position yields nanomolar, selective hGMII inhibitors.[7] They arrived at the same 3(*R*)-substituted swainsonine scaffold following a strategy totally different from ours: combinatorial and natural product-inspired versus fragment based and structure-guided. Our approach supports the notion that hGMII, which has for several decades been regarded as a potential drug target, is druggable: compounds may be discovered that are selective for GMII over the four other human α-mannosidases.[11-13] Besides this, we feel our approach, selecting hits from our pyrrolidine/piperidine iminosugar library in an FP-ABPP assay, and then in a structure-guided fashion elaborate these hits to arrive at more potent and more selective inhibitors, should translate well to other disease-related glycosidases.

## Data availability

The data supporting this article have been included as part of the Supplementary Information.

## Conflicts of interest

There are no conflicts to declare.

#### Acknowledgements

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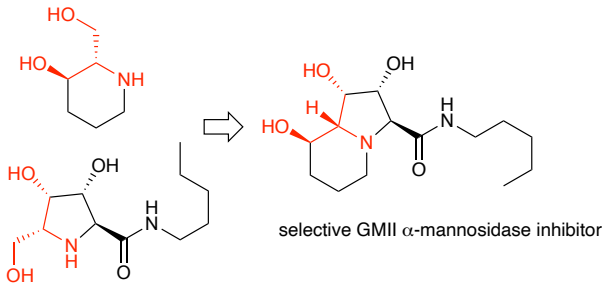
GRAPHICAL ABSTRACT




selective GMII α-mannosidase inhibitor

*Supporting Information*

# Structure-Guided Design of C3-Branched Swainsonine as Potent and Selective Human Golgi α-Mannosidase (GMII) Inhibitor

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**Protein expression and purification**

Drosophila Golgi α-mannosidase (dGMII) and human Golgi α-mannosidase (hGMII) were expressed in *Trichoplusia ni* cells (Invitrogen) and purified as previously reported[1], stored at -80 °C and thawed prior to use. Human GH38 α-mannosidases MAN2A1, MAN2A2, MAN2B1, MAN2B2 and MAN2C1 were overexpressed in HEK293T cells as previously reported.[1]

**Binding affinity assay**

Initial screening to assess binding affinity was carried out using a Nano-DSF melting temperature ($T_m$) assay. Purified dGMII and hGMII were diluted to a final concentration of 1 $mg.mL^{-1}$ in a buffer containing 20 mM HEPES, 20 mM NaCl and 1 mM DTT pH 7.4, and incubated for 2-3 hrs at room temperature with final concentrations of 1 mM of inhibitors **1**, **4** or **5**. A sample without any inhibitor was included as a reference for the $T_m$ of the apo dGMII/hGMII protein. Capillaries were then used to load the complexes into a Prometheus (NanoTemper) Nano-DSF machine to assess protein stability by monitoring the fluorescence of tryptophan and tyrosine residues. The temperature ramp was set from 20-95 ºC with a change of 1 ºC/min and an excitation power of 27%. $T_m$ values were calculated from the melting curves detailed in Figure S1 and S2 and Table S1. Two repeats were performed for each data set.

**$K_i$ determination**

The $K_i$ values of inhibitors for both dGMII and hGMII were determined by monitoring concentration-dependent decrease in accumulation of the fluorophore 4-methylumbelliferone when incubating 4-methylumbelliferyl-α-D-mannopyranoside with either of the two enzymes. Reactions were carried out in a buffer containing 50 mM MES pH 5.5, 1 mM $ZnSO_4$ and 0.1% (w/v) BSA in FluoroNunc™ 96-well plates (ThermoFisher). Inhibitor concentrations varied from 0-300 nM (swainsonine **1**), 0-200 µM (**4**) or 0-400 µM (**5**). Inhibitors were incubated, at room temperature, for approximately 30 minutes, with either dGMII or hGMII at final concentrations of 10 nM. 4-Methylumbelliferyl-α-D-mannopyranoside (Sigma Aldrich) was added to the reaction mixture to initiate the reaction at a fixed concentration of either 1 mM or 1.5 mM for dGMII or 750 µM or 1 mM for hGMII. Fluorescent intensity measurements were recorded using a BMG Labtech Clariostar plate reader in a continuous fashion with a top optic reader and a focal height of 5 mm. RFU were recorded at $\lambda_{ex}$ = 320-360 nm and $\lambda_{em}$ = 430-450 nm wavelengths with readings every 36 s for 120 cycles. The gain was set to 500 and reactions recorded at 25 ºC, and reactions were incubated at 500 rpm for 10 s before the first cycle was recorded. RFUs for each inhibitor concentration were plotted against time to work out the velocities. The inverse of the initial velocities ($1/V_o$) for each set of inhibitor concentrations were plotted in a set of Dixon plots at both 4-methylumbelliferyl-α-D-mannopyranoside concentrations for each protein. OriginPro (OriginLab®) was used to perform linear regression and extrapolation to obtain the intercept of the lines which correspond to the $K_i$ values for each inhibitor and protein complex. All fluorescence units were normalised to samples containing no inhibitors. Dixon plots are detailed in SI Figure 3.

**Selectivity determination**

HEK293T cells overexpressing human GH38 α-mannosidases MAN2A1, MAN2A2, MAN2B1, MAN2B2 and MAN2C1 were cultured in DMEM with 10% FCS (v/v), 0.1% penicillin/streptomycin (w/v) and 1% Glutamax (v/v) under 5% $CO_2$ at 37 °C in the presence of 100 µg/mL Zeocin to promote selection. Once confluent, cells were washed 3x with PBS, aliquoted and pellets were stored at -80 °C until use. Cell pellets were homogenised (Sonics VibraCell, 5s at 20% amplitude) in 150 mM McIlvaine buffer at pH 5.5 (MAN2A1, MAN2A2), pH 4.5 (MAN2B1, MAN2B2) or pH 6.7 (MAN2C1) containing either 2 mM $ZnCl_2$ (MAN2A1, MAN2A2, MAN2B1, MAN2B2) or 2 mM $CoCl_2$ (MAN2C1). For $IC_{50}$ measurements, 12.5 µL of homogenate (50 µg MAN2A1 or MAN2A2, 12.5 µg MAN2B1 or MAN2B2, MAN2C1 2.5 µg) was pre-incubated with 12.5 µL of compound (up to a concentration of 100 µM, final DMSO concentration 0.5%) at 37 °C for 30 min. Then, 100 µL 4-methylumbelliferyl-α-D-mannopyranoside (Glycosynth) substrate mix containing 0.1% (w/v) bovine serum albumin and 1 mM $ZnCl_2$ or $CoCl_2$ in 150 mM McIlvaine buffer at the corresponding pH (10 mM for MAN2A1, MAN2A2 and MAN2B1, 4mM for MAN2B2, 2 mM for MAN2C1) was then added, and further incubated for 30 min at 37 °C. After stopping the reaction with 200 µL excess 1 M NaOH-glycine (pH 10.3), liberated 4-MU fluorescence was measured with a fluorimeter LS55 (Perkin Elmer) at $\lambda_{Ex}$ 366 nm and $\lambda_{Em}$ 445 nm. Measurements were performed in 3 biological replicates with technical duplos. Results were processed and analysed using GraphPad Prism 9.0.

**X-ray crystallography and structural refinement**

Crystals were grown in screens optimised from previous conditions[1]. Crystals were set up in 48-well MAXI plates in a sitting drop vapour diffusion method. The best crystals grew in conditions containing 100 mM succinate pH 7.0 and 6-12% (w/v) PEG 3350 at a protein concentration of 10 $mg.mL^{-1}$ and seeded in a ratio of 200:100:400 nL of protein:seed:mother liquor. Crystals were soaked for 3-4 hr or overnight with final inhibitor concentrations of 10 mM. Crystals were then either cryoprotected with either 15 % (w/v) 2-methyl-2,4-pentanediol (MPD) or 25 % (w/v) ethylene glycol and fished and flash cooled in liquid nitrogen. Crystals were tested in-house for diffraction using a Rigaku XtaLAB Synergy-R diffractometer equipped with a microfocus MicroMax 007HF rotating anode generator with a specialised confocal MaxFlux optic. The best crystals were then sent to the Diamond Light Source synchrotron. Data was processed using the Xia2-DIALS pipeline. Structure generation and refinement was carried out using either CCP4i2 (V.8.0.011) or the CCP4 cloud interface. The atomic model that was used for molecular replacement was previously reported (PDB: 6RQZ)[1]. Iterative cycles of REFMAC, followed by manual buildings in COOT was used to generate the final structural outcome. Omit maps for inhibitors were calculated in the CCP4i2 interface. Refinement statistics are outlined in SI Table 3. Structures were deposited in the Protein Data Base (PDB) under accession codes 9FTR and 9FTQ for the dGMII:**4** and dGMII:**5** complexes, respectively.

**Supplementary Table 1**. $T_m$ for dGMII in complex with various ligands

| **Sample Name** | **Onset $T_m$ (°C)** | **$T_m$ (°C)** |
|---|---|---|
| Apo dGMII | 36.6 ± 3.89 | 50.9 ± 1.74 |

| | | |
|---|---|---|
| dGMII:**1** | 63.1 ± 5.70 | 74.1 ± 4.32 |
| dGMII:**4** | 49.5 ± 4.21 | 61.1 ± 2.02 |
| dGMII:**5** | 57.0 ± 1.20 | 65.7 ± 0.20 |
| Apo hGMII | 44.9 ± 0.04 | 54.5 ± 0.01 |
| hGMII:**1** | 47.0 ± 0.46 | 72.8 ± 0.22 |
| hGMII:**4** | 49.6 ± 0.31 | 61.7 ± 0.22 |
| hGMII:**5** | 48.4 ± 0.08 | 60.8 ± 0.16 |

**Supplementary Table 2**. $K_i$-values for compounds **1**, **4** and **5** as dGMII and hGMII inhibitors

| **Compound** | **dGMII** ($K_i$, μM) | **hGMII** ($K_i$, μM) |
|---|---|---|
| **1** | 0.0847 ± 0.008 | 0.0441 ± 0.009 |
| **4** | 5.63 ± 1.00 | 14.9 ± 5.04 |
| **5** | 167 ± 13.2 | 308 ± 6.57 |

**Supplementary Table 3**. X-ray data collection and refinement statistics for dGMII in complex with inhibitors

| | **dGMII:4** | **dGMII:5** |
|---|---|---|
| Beamline | Diamond i03 | Diamond i03 |
| Wavelength (Å) | 0.9762 | 0.9763 |
| Resolution (Å) | 57.68-2.14 (2.20-2.14) | 74.98-2.47 (2.57-2.47) |
| Space Group | $P2_12_12_1$ | $P2_12_12_1$ |
| Unit cell (Å) | a = 89.11; b = 91.94; c = 133.24 α = β = γ = 90° | a = 88.91; b = 90.91; c = 132.60 α = β = γ = 90° |
| Number of molecules in the asymmetric unit | 1 | 1 |
| Unique reflections | 61056 (4448) | 39207 (4329) |
| Completeness (%) | 100.0 (100.0) | 99.9 (100.0) |
| $R_{merge}$ (%) | 0.045 (0.352) | 0.037 (0.215) |
| $R_{p.i.m.}$ | 0.045 (0.352) | 0.037 (0.215) |
| Multiplicity | 1.9 (1.9) | 1.9 (1.9) |
| $<I/\sigma(I)$ | 9.6 (1.0) | 8.0 (0.9) |
| Overall B from Wilson plot ($Å_2$) | 32 | 41 |
| $CC_{1/2}$ | 0.997 (0.682) | 0.998 (0.94) |
| $R_{cryst}/R_{free}$ (%) | 17.9/25.9 | 20.8/29.8 |
| r.m.s.d. 1-2 bonds (Å) | 0.0144 | 0.0074 |
| r.m.s.d. 1-3 angles (°) | 2.5800 | 2.0720 |
| Avge main chain B ($Å^2$) | 37 | 52 |
| Avge side chain B ($Å^2$) | 38 | 52 |

| | | |
|---|---|---|
| Avge water B ($Å^2$) | 37 | 38 |
| Avge ligand B ($Å^2$) | 28 | 39 |
| Avge Zinc B ($Å^2$) | 27 | 43 |

**Figure S1**. Melting point curves for dGMII against a range of designed and known ligands. $T_m$ values calculated from a series of nanoDSF assays where the recorded 350:330nm fluorescence ratio (*y*) is plotted against temperature values in Celsius, °C, (*x*). Colours denote different ligands complexed, which is indicated in the legend. Curves and first derivatives are a representation of one repeat.

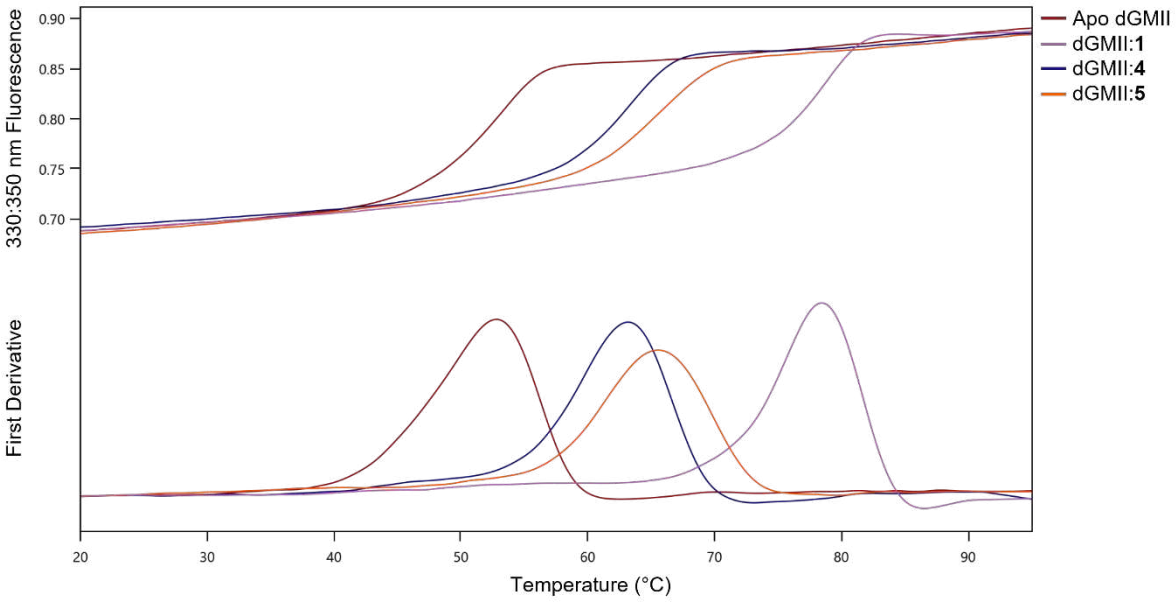



**Figure S2**. Melting point curves for hGMII against a range of designed and known ligands. $T_m$ values calculated from a series of nanoDSF assays where the recorded 350:330nm fluorescence ratio (*y*) is plotted against temperature values in Celsius, °C, (*x*). Colours denote different ligands complexed, which is indicated in the legend. Curves and first derivatives are a representation of one repeat.

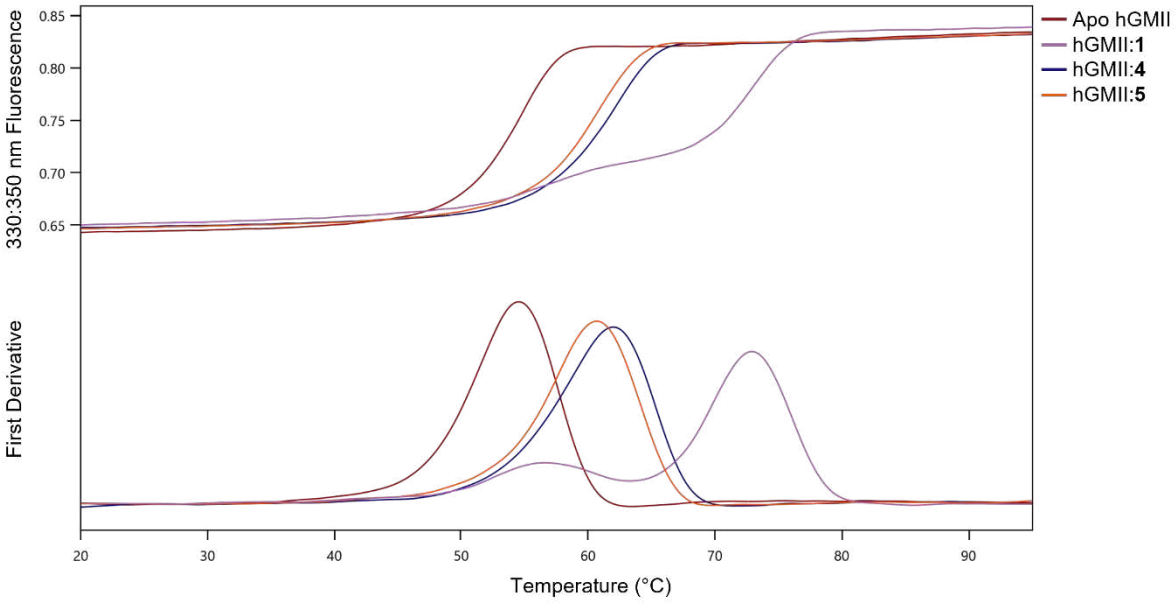



**Figure S3**. Dixon plots for calculating inhibitory constants for dGMII and hGMII with designed ligands and swainsonine. Dixon plots for dGMII (left panels) in complex with various ligands were calculated

from two standard concentrations of 4-Methylumbelliferyl *α*-D-mannopyranoside, 1.5 mM (green) and 1 mM (black), for hGMII (right panels) standard concentrations of 4-methylumbelliferyl *α*-D-mannopyranoside at 0.75 mM (blue) and 1 mM (black) were used. **A** dGMII in complex with **1** and calculated reciprocal initial velocity values (s. $\mu M^{-1}$) against inhibitor concentration (μM). **B** dGMII in complex with **4** and calculated reciprocal initial velocity values (s. $\mu M^{-1}$) against inhibitor concentration (μM). **C** dGMII in complex with **5** and calculated reciprocal initial velocity values (s. $\mu M^{-1}$) against inhibitor concentration (μM). **D** hGMII in complex with **1** and calculated reciprocal initial velocity values (s. $\mu M^{-1}$) against inhibitor concentration (μM). **E** hGMII in complex with **4** and calculated reciprocal initial velocity values (s. $\mu M^{-1}$) against inhibitor concentration (μM). **F** hGMII in complex with **5** and calculated reciprocal initial velocity values (s. $\mu M^{-1}$) against inhibitor concentration (μM).

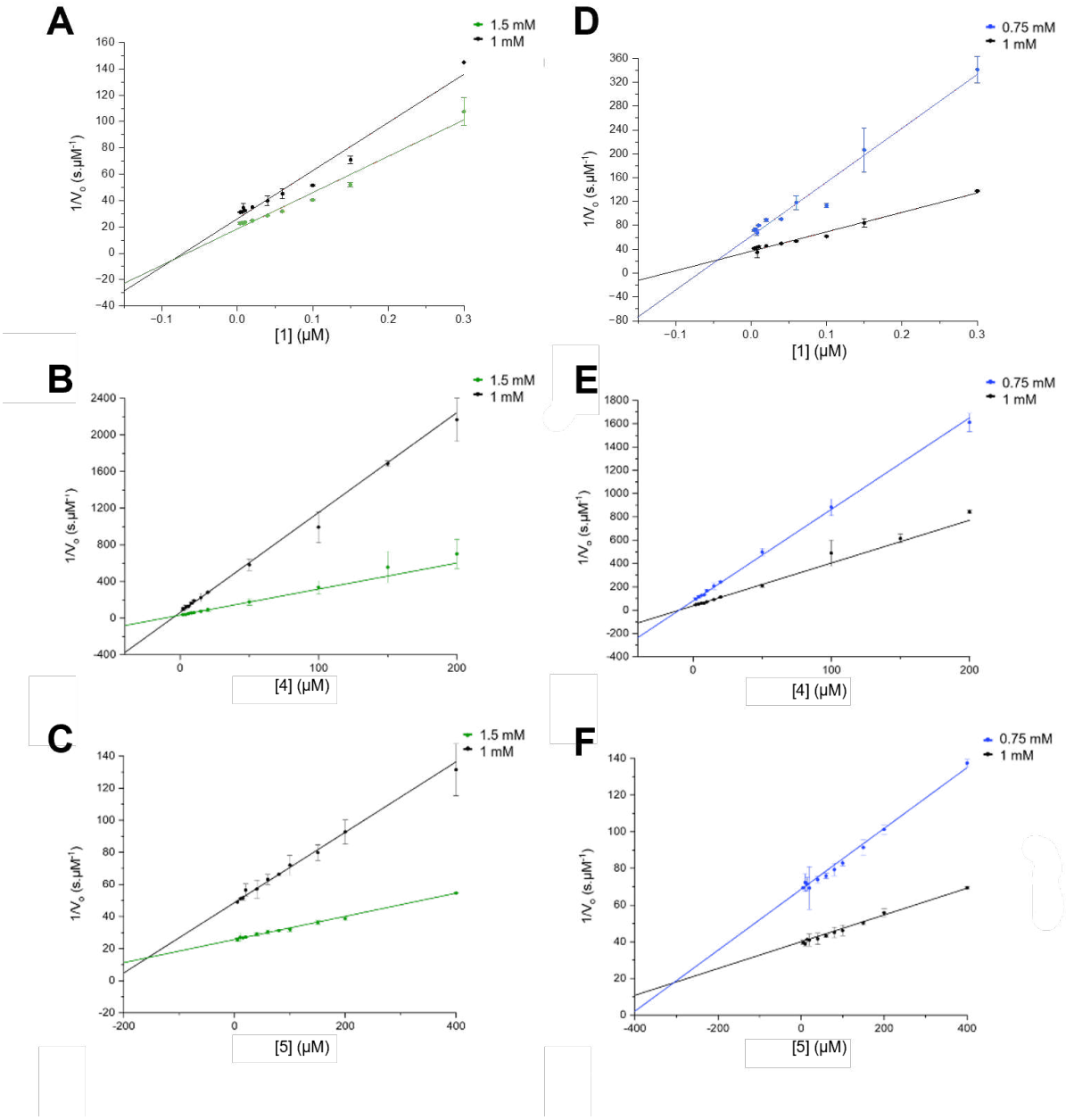



**Figure S4.** Ligand densities from the complexed crystal structures with designed inhibitors **4** and **5**. Images were created and visualised in CCP4mg (v. 2.10.11). Active site zincs are displayed in grey spheres throughout, and ligands are displayed in green cylinder form. All omit maps were calculated in

the CCP4i2 interface or obtained from the PDB and are displayed in grey chickenwire. $F_o$-$F_c$ maps are all contoured at 3 σ except for **5** (**B**) which is contoured at 2.5 σ. **A** Omit map for **4** in the dGMII active site. **B** Omit map for **5** in the dGMII active site.

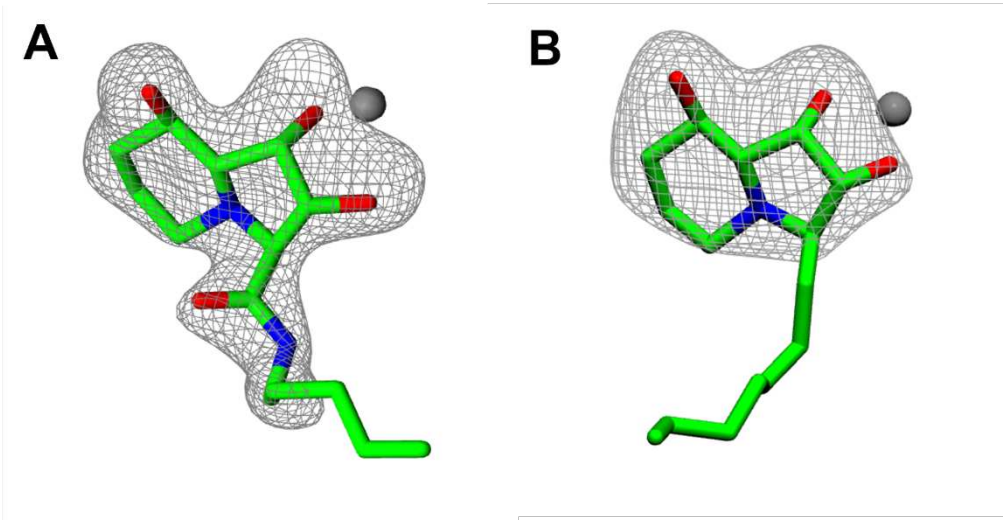



**Figure S5**. Octahedral coordination of the active site zinc in the active sites of complexed dGMII structures with designed inhibitors **4** and **5**. Images were created and visualised in CCP4mg (v. 2.10.11). Active site zincs are displayed in grey spheres throughout, and ligands are displayed in blue cylinder form. All bonds are indicated by blue dashed lines with all distances being displayed in angstroms (σ). **A** Binding pocket of dGMII:**4** with active site residues (yellow) and **4** forming a bonding network to the active site zinc. **B** Binding pocket of dGMII:**5** with active site residues (grey) and **5** forming a bonding network to the active site zinc.

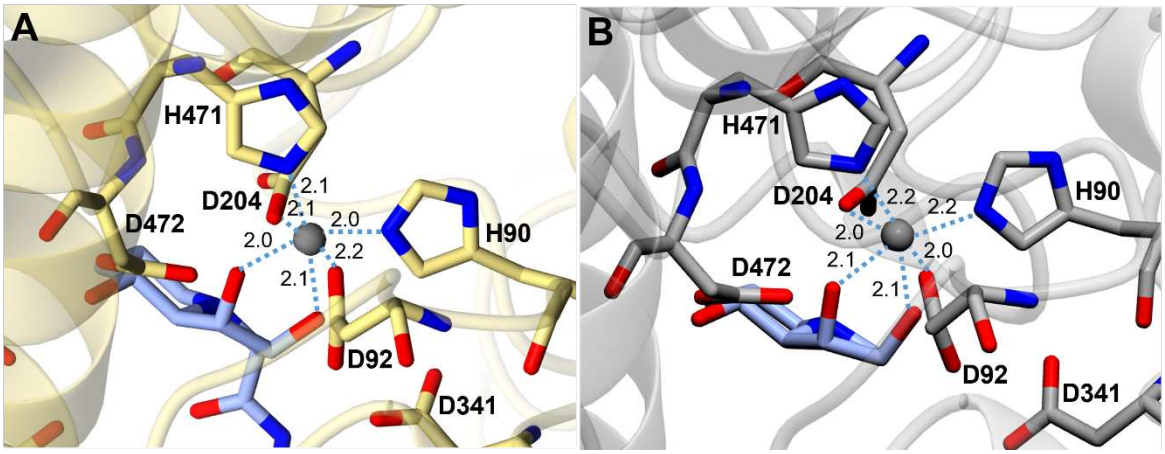
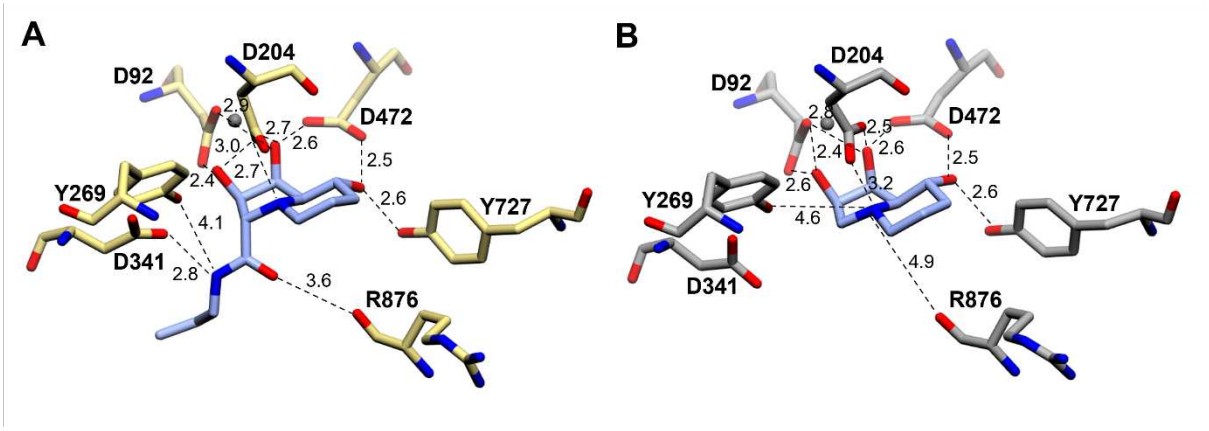


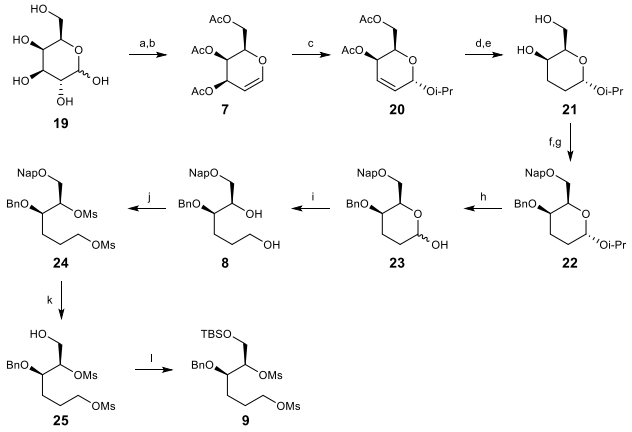
**Figure S6**. Binding pockets and secondary coordination spheres of dGMII in complex with **4** and **5**. Images were created and visualised in CCP4mg (v. 2.10.11). All ligands are displayed in blue cylinder form throughout. All bonds are indicated by black dashed lines with all distances being displayed in angstroms (σ). Water molecules are displayed in red spheres and zinc in grey sphere form. **A** Binding pocket of dGMII:**4** with active site residues (yellow) and **4** forming a series of hydrogen bonding networks. **B** Binding pocket of dGMII:**5** with active site residues (grey) and **5** forming a series of hydrogen bonding networks**.**



**Chemical synthesis**

**General information**: All moisture and oxygen sensitive reactions were carried out dry and under an argon atmosphere. Dry solvents were obtained by storage on flame dried molecular sieves 3 Å or 4 Å. All commercially available chemicals were used as received without further purification. All reactions were followed by thin layer chromatography using Merck Silica gel 60 $F_{254}$ aluminum sheets. Detection was done by UV (254 nm), staining with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25 g/L), $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10 g/L) in 10% sulfuric acid in water, a solution of $KMnO_4$ (20 g/L) and $K_2CO_3$ (10 g/L) in water, or a solution of ninhydrin (1 g/L) in 0.5% acetic acid in acetone, followed by heating at 150 °C. Flash column chromatography was performed with Screening Devices BV silica gel (particle size of 40-63 µm, pore diameter of 60 Å) with indicated eluents. $^1H$ and $^{13}C$ NMR spectra were recorded on a Bruker AV-400 (400 and 101 MHz respectively), a Bruker AV-500 (500 and 126 MHz respectively) and a Bruker DMX 850 (850 and 214 MHz respectively). Chemical shifts are given in ppm (δ) relative to tetramethyl silane (TMS) or the residual solvent peak as internal standard and coupling constants are given in Hz. Reversed phase HPLC purification was performed on a Thermo Finnigan Surveyor HPLC system with a Phenomenex Gemini $C_{18}$ column (4.6 mm x 50 mm, 5 µm particle size). High resolution mass spectrometry analysis was performed with a Thermo Finnigan LTQ Orbitrap mass spectrometer equipped with an electronspray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL/min, capillary temperature 250 °C) with resolution R = 60000 at *m/z* 400 (mass range *m/z* 150 - 2000) and dioctyl phthalate (*m/z* = 301.28428) as lock mass. The high-resolution mass spectrometer was calibrated before measurements with a calibration mixture (Thermo Finnigan).

**Synthesis of bismesylate 9**




**Scheme 1**. *Reagents and conditions*: a) $Ac_2O$, $HClO_4$, 0 °C, 1 h, then HBr in AcOH, r.t., 16 h; b) Zn, $NH_4Cl$, EtOAc, r.t., 16 h, 70% (over 2 steps); c) $SnCl_4$, *i*PrOH, DCM, r.t., 16 h, 62%; d) $H_2$, Pd/C, EtOH, r.t., 3 h, 90%; e) NaOMe, MeOH, r.t., 2 h, 99%; f) NapBr, 2-APB, KI, $K_2CO_3$, MeCN, 65 °C, 3 h, 89%; g) BnBr, NaH, TBAI, DMF, r.t., 16 h, 94%; h) 4:1 AcOH/1 M HCl, 60 °C, 16 h, quant.; i), $NaBH_4$, EtOH, r.t., 3 h, 98%; j) MsCl, pyr, 0 °C, 2 h, quant.; k) DDQ, 3:1 DCM/$H_2O$, r.t., 3 h, quant; l) TBSCl, imidazole, DCM, 0 °C, 2 h, 96%.

**Compound 7**: A suspension of galactose (60.0 g, 333 mmol) in acetic anhydride (240 mL) was cooled to 0 °C with an ice bath. A catalytic amount of perchloric acid (20 drops) was

slowed added, after which the ice bath was removed, and the suspension was stirred for 1 hour at room temperature. The reaction was cooled to 0 °C, a solution of HBr in acetic acid (33 wt.%, 480 mL, 2.67 mol, 8 eq.) was added dropwise and the reaction was stirred at room temperature overnight. The mixture was then poured into ice water (1 L) and extracted with DCM (3x). The organic layers were washed with water (1x) and concentrated *in vacuo*. The residue was redissolved in EtOAc (666 mL, 0.50 M) followed by the addition of zinc (130 g, 2.00 mol, 6 eq.) and $NH_4Cl$ (107 g, 2.00 mol, 6 eq.) and the reaction was stirred overnight. The reaction was then filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (30% → 50% $Et_2O$/pentane) gave compound **7** (63.8 g, 234 mmol) in 70% yield as a colourless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 6.46 (dd, *J* = 6.3, 1.7 Hz, 1H), 5.61 – 5.48 (m, 0H), 5.43 (dt, *J* = 4.5, 1.6 Hz, 1H), 4.73 (ddd, *J* = 6.3, 2.7, 1.5 Hz, 1H), 4.36 – 4.30 (m, 1H), 4.30 – 4.18 (m, 2H), 2.13 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.7, 170.5, 170.3, 145.6, 99.0, 72.9, 64.0, 63.9, 62.1, 21.0, 20.9, 20.8. HRMS: $[M+Na]^+$ calculated for $[C_{12}H_{16}O_7Na]^+$: 297.07882, found 295.07856.

**Compound 20**: To a solution of compound **7** (50.6 g, 186 mmol) in DCM (929 mL) at 0 °C was added isopropanol (28.4 mL, 372 mmol, 2 eq.). A solution of $SnCl_4$ (1 M in DCM, 18.6 mL, 18.6 mmol, 0.1 eq.) was added dropwise at 0 °C and the reaction was stirred at room temperature overnight. The reaction was neutralized with sat. aq. $NaHCO_3$, diluted with EtOAc and the mixture was washed with $H_2O$ (1x). The organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (20% → 40% $Et_2O$/pentane) gave compound **20** (33.5 g, 123 mmol) in 66% yield as a colourless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 6.10 (ddd, *J* = 10.0, 5.4, 0.9 Hz, 1H), 6.04 – 5.94 (m, 1H), 5.17 (d, *J* = 2.8 Hz, 1H), 5.01 (dd, *J* = 5.4, 2.3 Hz, 1H), 4.43 – 4.35 (m, 1H), 4.25 – 4.19 (m, 2H), 4.00 (hept, *J* = 6.1 Hz, 1H), 2.08 (s, 3H), 2.06 (s, 3H), 1.25 (d, *J* = 6.2 Hz, 3H), 1.18 (d, *J* = 6.2 Hz, 4H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.5, 131.3, 125.1, 92.5, 70.7, 66.8, 63.2, 63.1, 23.6, 22.1, 21.0, 20.9. HRMS: $[M+Na]^+$ calculated for $[C_{13}H_{20}O_6Na]^+$: 295.11521, found 295.11485.

**Compound 26**: Compound **20** (34.9 g, 128 mmol) was co-evaporated with toluene under argon (3x) and dissolved in ethanol (341 mL). The solution was purged for 45 min with argon after which, palladium on carbon (10% wt. loading, 4.11 g) was added and the mixture was purged with argon for another 15 minutes. The mixture was then purged with hydrogen gas for 15 min and the reaction was stirred at room temperature for 3 hours under hydrogen atmosphere. The reaction was filtered over celite and concentrated *in vacuo* and the crude product was purified by silica gel column chromatography (20% → 40% $Et_2O$/pentane) to afford compound **26** (31.8 g, 116 mmol) in 90% yield as a colourless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 4.99 (d, *J* = 3.1 Hz, 1H), 4.92 (s, 1H), 4.19 – 4.14 (m, 1H), 4.14 – 4.01 (m, 2H), 3.91 (hept, *J* = 6.2 Hz, 1H), 2.10 (s, 3H), 2.08 – 2.02 (m, 4H), 1.94 (tt, *J* = 13.6, 3.7 Hz, 1H), 1.81 (dq, *J* = 13.5, 3.2 Hz, 1H), 1.57 – 1.48 (m, 1H), 1.22 (d, *J* = 6.3 Hz, 3H), 1.15 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.9, 170.7, 94.9, 68.9, 67.1, 67.0, 63.8, 24.9, 23.4, 22.6, 21.8, 21.3, 20.9. HRMS: $[M+Na]^+$ calculated for $[C_{13}H_{22}O_6Na]^+$: 297.13086, found 297.13054.

**Compound 21**: To a solution of compound **26** (28.3 g, 103 mmol) in MeOH (275 mL, 0.375 M) was added NaOMe (4.37 M in MeOH, 7.09 mL, 0.3 eq.). After stirring for 2 hours at room temperature, the reaction was quenched with Amberlite $H^+$ (pH ≈ 5), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (2% → 4% MeOH/DCM) gave compound **21** (19.4 g, 102 mmol) in 99% yield as a colourless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 5.00 (d, *J* = 2.8 Hz, 1H), 3.99 – 3.77 (m, 5H), 3.07 (s, 1H), 2.61 (s, 1H), 2.14 – 1.91 (m, 2H), 1.75 – 1.64 (m, 1H), 1.55 – 1.44 (m, 1H), 1.19 (d, *J* = 6.3 Hz, 3H), 1.14 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 95.0, 69.4, 68.2, 67.1, 64.9, 25.5, 24.1, 23.5, 21.5. HRMS: $[M+Na]^+$ calculated for $[C_9H_{18}O_4Na]^+$: 213.10979, found 213.10973.

**Compound 27**: Compound **21** (18.0 g, 94.4 mmol) was co-evaporated with toluene under argon (3x) and dissolved in dry MeCN (315 mL). Potassium iodide (12.0 g, 104 mmol, 1.1 eq.), $K_2CO_3$ (14.3 g, 104 mmol, 1.1 eq.), 2-aminoethyl diphenylborinate (6.37 g, 28.3 mmol, 0.3 eq.) and 2-(bromomethyl)naphthalene (31.3 g, 142 mmol, 1.5 eq.) were added sequentially and the reaction was heated to 65 °C and stirred for 3 hours. The reaction mixture was concentrated and redissolved in EtOAc. The organic phase was washed with $H_2O$ (2x) and brine (1x), and the aqueous layers extracted with EtOAc (1x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (20% → 40% EtOAc/PE) gave **27** (27.8 g, 84.2 mmol) as a colourless oil in 89% yield. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.71 (m, 4H), 7.52 – 7.42 (m, 3H), 5.02 (d, *J* = 3.2 Hz, 1H), 4.80 – 4.66 (m, 2H), 4.02 – 3.98 (m, 1H), 3.98 – 3.92 (m, 1H), 3.90 (s, 1H), 3.76 – 3.68 (m, 2H), 3.23 (s, 1H), 2.13 (tt, *J* = 13.4, 4.1 Hz, 1H), 2.07 – 1.93 (m, 1H), 1.78 – 1.67 (m, 1H), 1.53 – 1.43 (m, 1H), 1.23 (d, *J* = 6.3 Hz, 3H), 1.16 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 135.2, 133.2, 133.0, 128.2, 127.8, 127.7, 126.5, 126.1, 125.9, 125.6, 95.0, 73.7, 71.7, 68.7, 68.0, 66.1, 25.4, 24.0, 23.4, 21.4. HRMS: $[M+Na]^+$ calculated for $[C_{20}H_{26}O_4Na]^+$: 353.17233, found 353.17197.

**Compound 22**: Compound **27** (53.7 g, 163 mmol) was dissolved in DMF (1.00 L) and cooled to 0 °C and NaH (13.0 g, 325 mmol, 2 eq.) was slowly added. The reaction was stirred for 15 min at 0 °C after which BnBr (38.7 mL, 325 mmol, 2 eq.) and TBAI (3.95 g, 16.3 mmol, 0.1 eq.) were added. The reaction was stirred overnight at room temperature and subsequently quenched with MeOH. Most of the solvent was removed *in vacuo* and $Et_2O$ and $H_2O$ were added. The two phases were separated, and the aqueous layers were extracted with $Et_2O$ (1x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography (10% → 30% $Et_2O$/pentane) to obtain compound **22** (64.4 g, 153 mmol) as a colourless oil in 94% yield. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.71 (m, 5H), 7.49 – 7.38 (m, 3H), 7.32 – 7.19 (m, 5H), 5.00 (d, *J* = 3.2 Hz, 1H), 4.72 (d, *J* = 12.1 Hz, 1H), 4.65 (d, *J* = 6.0 Hz, 1H), 4.62 (d, *J* = 6.0 Hz, 1H), 4.39 (d, *J* = 12.1 Hz, 1H), 4.11 (td, *J* = 6.3, 1.3 Hz, 1H), 3.97 (hept, *J* = 6.2 Hz, 1H), 3.74 – 3.60 (m, 2H), 3.60 – 3.54 (m, 1H), 2.15 – 2.00 (m, 1H), 1.97 – 1.83 (m, 2H), 1.50 (dt, *J* = 13.2, 3.0 Hz, 1H), 1.24 (d, *J* = 6.3 Hz, 3H), 1.14 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 138.7, 136.0, 133.3, 133.0, 128.3, 128.1, 127.9, 127.9, 127.7, 127.6, 126.4, 126.1, 125.8, 125.8, 94.5, 73.5,

71.1, 70.9, 70.4, 69.7, 67.9, 24.8, 23.5, 21.5, 20.9. HRMS: $[M+Na]^+$ calculated for $[C_{27}H_{32}O_4Na]^+$: 443.21928, found 443.21872.

**Compound 23**: A solution of compound **22** (11.8 g, 28.0 mmol) in a mixture of AcOH/1M HCl (280 mL, 4:1) was stirred overnight at 60 °C. After cooling the reaction to room temperature, it was slowly poured into sat. aq. $NaHCO_3$ (1.12 L) at 0°C. The aqueous phase was extracted with EtOAc (3x) and combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. After purification by silica gel column chromatography (30% → 60% EtOAc/PE) compound **23** (10.6 g, 28.0 mmol) was obtained in quantitative yield as a colourless oil as a mixture of isomers (3:2, α:β). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.66 (m, 5H), 7.49 – 7.36 (m, 4H), 7.33 – 7.17 (m, 5H), 5.37 (d, *J* = 2.5 Hz, 1H), 4.79 (d, *J* = 7.2 Hz, 0H), 4.76 – 4.70 (m, 1H), 4.65 – 4.59 (m, 2H), 4.40 – 4.33 (m, 1H), 4.31 – 4.23 (m, 1H), 3.79 – 3.75 (m, 0H), 3.75 – 3.63 (m, 1H), 3.59 – 3.53 (m, 1H), 3.50 (s, 1H), 3.46 (s, 0H), 3.20 (s, 0H), 2.82 (s, 1H), 2.18 – 1.99 (m, 1H), 1.99 – 1.82 (m, 1H), 1.82 – 1.67 (m, 1H), 1.66 – 1.44 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 138.5, 135.7, 133.4, 133.1, 128.4, 128.3, 128.3, 128.0, 128.0, 128.0, 127.8, 127.7, 126.8, 126.8, 126.2, 126.0, 126.0, 126.0, 96.3, 91.7, 77.2, 73.8, 73.7, 71.2, 71.0, 70.9, 70.8, 70.1, 70.0, 69.8, 27.9, 25.1, 24.3, 20.3. HRMS: $[M+Na]^+$ calculated for $[C_{24}H_{26}O_4Na]^+$: 401.17233, found 401.17193.

**Compound 8**: Compound **23** (10.3 g, 27.1 mmol, 1 eq.) was dissolved in EtOH (136 mL, 0.2 M) and $NaBH_4$ (1.03 g, 27.1 mmol, 1 eq.) was slowly added. After stirring for 3 hours at room temperature, the reaction was quenched with $H_2O$ at 0 °C. Additional $H_2O$ was added and the aqueous phase was extracted with DCM (3x). The combined organic layers were dried with $MgSO_4$, filtered and concentrated *in vacuo*. Silica gel column chromatography (2% → 10% MeOH/DCM) purification gave compound **8** (10.1 g, 26.5 mmol) as a colourless oil in 98% yield. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.71 (m, 4H), 7.56 – 7.40 (m, 3H), 7.35 – 7.20 (m, 5H), 4.70 (s, 2H), 4.66 – 4.46 (m, 2H), 3.89 (p, *J* = 10.2, 4.9 Hz, 1H), 3.69 – 3.49 (m, 6H), 2.59 (d, *J* = 5.4 Hz, 1H), 1.79 – 1.56 (m, 7H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 138.2, 135.5, 133.3, 133.1, 128.6, 128.4, 128.1, 128.0, 127.9, 127.8, 126.8, 126.3, 126.1, 126.0, 79.1, 73.7, 72.8, 71.7, 71.2, 62.9, 28.7, 26.7. HRMS: $[M+Na]^+$ calculated for $[C_{24}H_{28}O_4Na]^+$: 403.18798, found 403.18766.

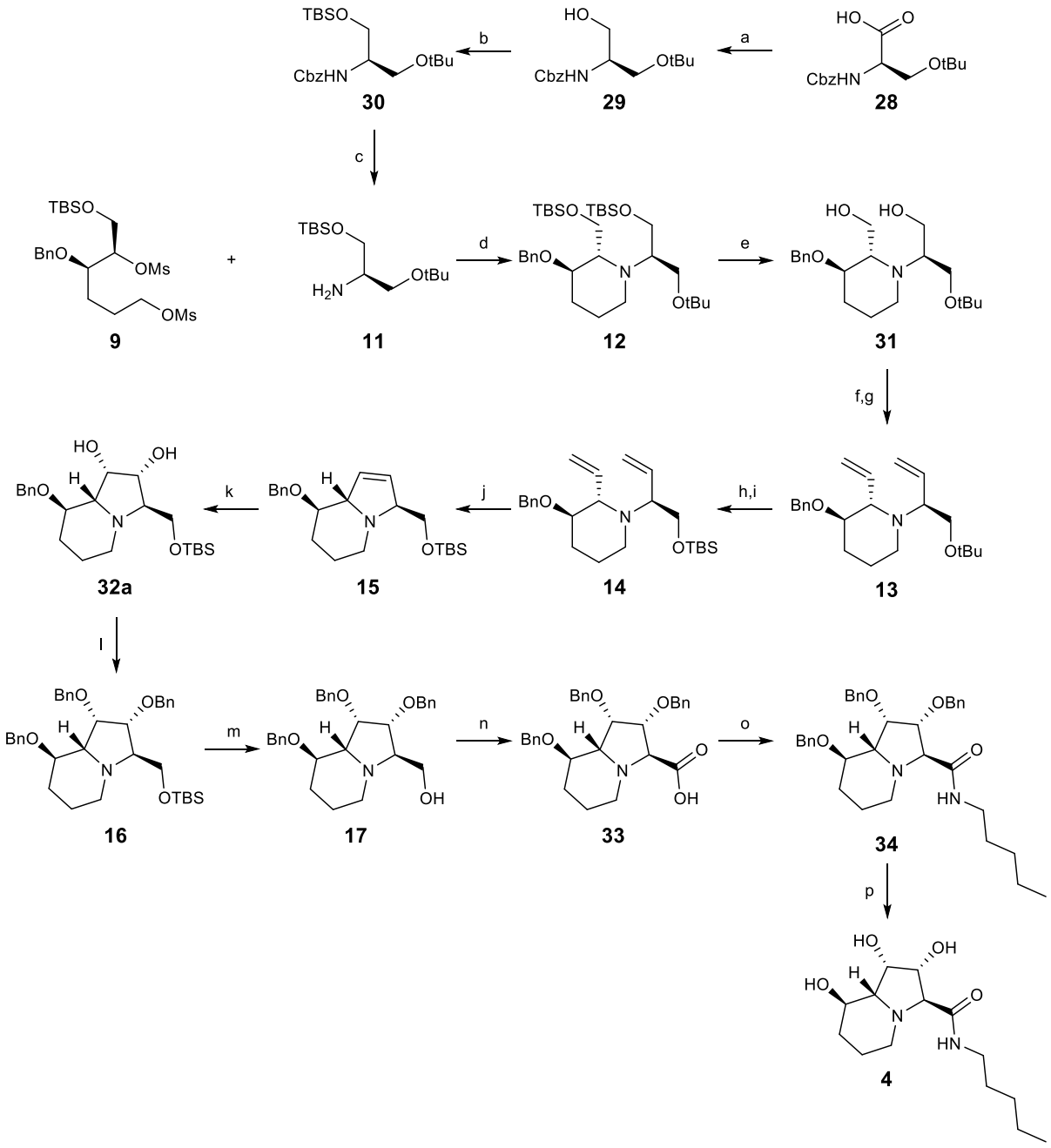
**Compound 24**: A solution of compound **8** (7.6 g, 20 mmol) in pyridine (80 mL) was cooled to 0 °C. Methanesulfonyl chloride (3.9 mL, 50 mmol, 2.5 eq.) was added slowly and the reaction was stirred for 2 hours at 0 °C. The reaction was quenched with $H_2O$ and additional $H_2O$ was added. The aqueous phase was extracted with EtOAc and the organic layer was washed with 1M HCL (1x), sat. aq. $NaHCO_3$ and brine. The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (20% → 50% EtOAc/PE) gave compound **24** (11 g, 20 mmol) in quantitative yield as a colourless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.89 – 7.67 (m, 4H), 7.57 – 7.37 (m, 3H), 7.37 – 7.11 (m, 5H), 4.93 – 4.85 (m, 1H), 4.77 – 4.65 (m, 2H), 4.65 – 4.52 (m, 2H), 4.21 – 4.09 (m, 2H), 3.86 – 3.74 (m, 2H), 3.74 – 3.65 (m, 1H), 3.03 (s, 3H), 2.93 (s, 3H), 1.90 – 1.65 (m, 3H), 1.63 – 1.47 (m, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 137.5, 134.8, 133.3, 133.2, 129.2, 128.7, 128.6, 128.4, 128.3, 128.3,

128.0, 127.9, 127.0, 126.5, 126.3, 125.8, 125.4, 81.7, 77.5, 73.8, 73.0, 69.6, 68.7, 38.6, 37.4, 25.8, 25.4. HRMS: $[M+Na]^+$ calculated for $[C_{26}H_{32}O_8S_2Na]^+$: 559.14308, found 559.14320.

**Compound 25**: Compound **24** (1.61 g, 3.00 mmol) was dissolved in a mixture of DCM/$H_2O$ (3:1, 40 mL) and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (2.04 g, 9.00 mmol, 3 eq.) was added. The reaction was stirred at room temperature for 3 hours and subsequently quenched with sat. aq. $Na_2S_2O_3$. Additional $H_2O$ was added, and the mixture was extracted with DCM (2x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (1% → 3% MeOH/DCM) furnished compound **25** (1.19 g, 3.00 mmol) as pale-yellow oil in quantitative yield. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.28 (m, 5H), 4.82 – 4.71 (m, 1H), 4.68 – 4.55 (m, 2H), 4.23 – 4.12 (m, 2H), 4.01 – 3.78 (m, 2H), 3.74 (q, *J* = 4.4 Hz, 1H), 3.07 (s, 3H), 2.99 (s, 3H), 1.92 – 1.72 (m, 3H), 1.68 – 1.55 (m, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 137.4, 128.8, 128.4, 128.3, 83.0, 77.2, 73.0, 69.6, 61.8, 38.5, 37.5, 25.9, 25.2. HRMS: $[M+Na]^+$ calculated for $[C_{15}H_{24}O_8S_2Na]^+$: 419.108048, found 419.08050.

**Compound 9**: A solution of compound **25** (1.8 g, 4.6 mmol) and imidazole (0.40 g, 5.9 mmol, 1.3 eq.) in DCM (11 mL) was cooled to 0 °C. *tert*-butyldimethylsilyl chloride (0.82 g, 5.5 mmol, 1.2 eq.) was added and the reaction was stirred for 2 hours at 0 °C. The reaction was quenched by the addition of MeOH, and the mixture was washed with $H_2O$. The aqueous layer was extracted with DCM (2x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. After silica gel column chromatography (20% → 40% EtOAc/PE) compound **9** (2.2 g, 4.4 mmol) was obtained in 96% yield as a colourless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.28 (m, 5H), 4.71 – 4.54 (m, 3H), 4.22 – 4.14 (m, 2H), 3.95 – 3.77 (m, 1H), 3.71 (p, *J* = 8.5, 4.0 Hz, 1H), 3.04 (s, 2H), 2.98 (s, 2H), 1.91 – 1.68 (m, 3H), 1.63 – 1.47 (m, 2H), 0.90 (s, 7H), 0.08 (d, *J* = 5.0 Hz, 5H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 137.6, 128.7, 128.4, 128.3, 83.8, 77.4, 73.1, 69.6, 62.0, 38.5, 37.5, 26.0, 25.9, 25.5, 18.4, -5.3. HRMS: $[M+Na]^+$ calculated for $[C_{21}H_{39}O_8S_2Si_1]^+$: 511.18501, found 511.18512.

**Synthesis of indolizidine 4**




**Scheme 2**. *Reagents and conditions*: a) isobutyl chloroformate, N-methylmorpholine, $NaBH_4$, DCM, -15 °C, 2 h, quant; b) TBSCl, imidazole, DCM, 0 °C to r.t., 2 h, 89%; c) Pd/C, $H_2$, EtOH, r.t., 16 h, 98%; d) DIPEA, MeCN, 50 °C to 70 °C, 4 days, 77%; e) TBAF, THF, r.t., 16 h, 94%; f) oxalyl chloride, DMSO, $Et_3N$, DCM, -78 °C to 0 °C, 5 h; g) $MeP(Ph)_3Br$, NaHMDS, THF, -78 °C to 0 °C, 16 h, 36% (over 2 steps); h) TFA, $H_2O$, 0 °C to r.t., 2 h, 89%; i) TBSCl, imidazole, DCM, 0 °C, 2 h, 96%; j) Schrock-Hoveyda catalyst, benzene, r.t., 16 h, 50%; k) $OsO_4$, TMEDA, DCM, -78 °C then ethylenediamine, r.t., 16 h, **32a**: 48% and **32b**: 16%; l) BnBr, NaH, DMF, r.t., 4 h, 70%; m) TBAF, THF, r.t., 2 h, 82%; n) $CrO_3$, $H_2SO_4$, $H_2O$, acetone, 60 °C to 0 °C, 30 min, 42%; o) amylamine, HATU, DIPEA, r.t., 16 h, 92%; p) Pd/C, $H_2$, HCl, dioxane, r.t., 16 h, 89%.

**Compound 29**: Z-D-ser(OtBu)-OH (1.48 g, 5.00 mmol) was dissolved in DME (10 mL) and cooled to -15 °C. N-methylmorpholine (0.55 mL, 5.00 mmol, 1 eq.) and isobutyl chloroformate (1.14 mL, 5.00 mmol, 1 eq.) were added dropwise and the mixture was stirred for 1 hour at -15 °C. The resulting suspension was filtered, and the filtrate was cooled to -15 °C. A solution of $NaBH_4$ (3 M in $H_2O$, 2.5 mL, 7.5 mmol, 1.5 eq.) was added followed by the addition of $H_2O$ (125 mL) and the mixture was stirred for 2 hours at room temperature. The reaction was extracted with DCM (3x) and the organic layers were washed with 1 M HCl (1x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (30% → 40% EtOAc/PE) gave compound **29** (1.25 g, 4.44 mmol) as a white solid in 89% yield. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.29 (m, 5H), 5.52 (d, *J* = 8.1 Hz, 1H), 5.11 (s, 2H), 3.89 – 3.83 (m, 1H), 3.83 – 3.76 (m, 1H), 3.76 – 3.67 (m, 1H), 3.59 (d, *J* = 3.4 Hz, 2H), 1.17 (s, 9H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 136.6, 128.7, 128.3, 77.5, 77.2, 76.8, 74.0, 67.0, 64.9, 63.8, 51.7, 27.4. HRMS: $[M+Na]^+$ calculated for $[C_{15}H_{23}NO_4Na]^+$: 304.15193, found 304.15163.

**Compound 30**: Compound **29** (12.3 g, 43.9 mmol) was co-evaporated with toluene under argon (3x) and dissolved in DCM (43.9 mL). Imidazole (3.59 g, 52.7 mmol, 1.2 eq.) was added and the solution was cooled to 0 °C. TBSCl (7.60 g, 50.5 mmol, 1.15 eq.) was added slowly and the reaction was stirred for 2 hours at room temperature. The mixture was poured into sat. aq. $NaHCO_3$ and extracted with DCM (3x). The organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. After silica gel column chromatography, compound **30** (15.6 g, 39.5 mmol) was obtained in 89% yield as a colorless liquid. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.29 (m, 5H), 5.14 (d, *J* = 7.7 Hz, 1H), 5.10 (s, 2H), 3.82 – 3.61 (m, 2H), 3.61 – 3.54 (m, 1H), 3.51 (dd, *J* = 8.9, 3.3 Hz, 1H), 3.33 (dd, *J* = 8.6, 6.0 Hz, 1H), 1.15 (s, 9H), 0.88 (s, 9H), 0.07 – 0.02 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 156.1, 136.7, 128.7, 128.4, 128.3, 77.5, 77.2, 76.8, 73.0, 66.8, 61.3, 59.3, 52.3, 27.6, 26.0, 18.4, -5.4. HRMS: $[M+H]^+$ calculated for $[C_{21}H_{38}NO_4Si]^+$: 396.25646, found 396.25611.

**Compound 11**: After co-evaporation with toluene (3x), compound **30** (0.79 g, 2.0 mmol) was dissolved in dry EtOH (5.3 mL) under an argon atmosphere. The solution was purged with argon for 15 minutes and palladium on carbon (10% wt. loading, 64 mg) was added followed by purging with argon for another 15 minutes. The mixture was then purged with hydrogen for 15 min and stirred under a hydrogen atmosphere overnight at room temperature. The reaction was filtered over celite and concentrated *in vacuo* and purified by silica gel column chromatography (2% → 10% MeOH/DCM) to obtain compound **11** (0.51 g, 1.95) in 98% yield as a colourless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 3.60 (dd, *J* = 9.8, 4.9 Hz, 1H), 3.50 (dd, *J* = 9.8, 5.9 Hz, 1H), 3.36 (dd, *J* = 8.7, 5.0 Hz, 1H), 3.22 (dd, *J* = 8.7, 6.4 Hz, 1H), 2.97 – 2.87 (m, 1H), 1.60 (s, 2H), 1.17 (s, 9H), 0.89 (s, 9H), 0.05 (s, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 72.8, 65.3, 63.7, 53.2, 27.7, 26.0, 18.4, -5.3. HRMS: $[M+H]^+$ calculated for $[C_{13}H_{32}NO_2Si]^+$: 262.21968, found 262.21952.

**Compound 12**: Compound **9** (2.87 g, 5.61 mmol) and compound **11** (3.80 g, 14.5 mmol, 2.6 eq.) were dissolved in MeCN (11 mL). DIPEA (5.85 mL, 33.6 mmol, 6 eq.) was added and the

mixture was stirred at 50 °C for 3 days. After full consumption of the starting material, the reaction was stirred overnight to 70 °C. The reaction was concentrated *in vacuo* and purified by silica gel column chromatography (2% → 10% $Et_2O$/pentane) to give compound **12** (2.56 g, 4.41 mmol) in 79% yield as a pale-yellow oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.22 (m, 5H), 4.67 – 4.44 (m, 2H), 3.99 – 3.85 (m, 2H), 3.80 – 3.66 (m, 2H), 3.50 – 3.45 (m, 1H), 3.45 – 3.38 (m, 1H), 3.36 – 3.30 (m, 1H), 3.20 – 3.12 (m, 1H), 2.87 – 2.79 (m, 1H), 2.67 (dt, *J* = 7.4, 3.8 Hz, 1H), 2.54 – 2.43 (m, 1H), 2.09 – 1.97 (m, 1H), 1.73 – 1.57 (m, 1H), 1.45 – 1.32 (m, 2H), 1.15 (s, 9H), 0.90 – 0.86 (m, 18H), 0.05 – 0.01 (m, 12H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 139.4, 128.4, 127.8, 127.4, 75.1, 72.4, 71.0, 66.1, 61.9, 60.9, 60.2, 59.6, 46.8, 29.2, 27.7, 26.1, 26.1, 23.8, 18.4, -5.4. HRMS: $[M+H]^+$ calculated for $[C_{32}H_{62}NO_4Si_2]^+$: 580.42119, found 580.41971.

**Compound 31**: To a solution of compound **12** (0.64 g, 1.1 mmol, 1 eq.) in THF (5.1 mL, 0.2 M) was added TBAF (1 M in THF, 3.3 mL, 3.3 mmol, 3 eq.) and the mixture was stirred at room temperature overnight. The reaction was diluted with DCM and washed with $H_2O$ (1x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (2% → 10% MeOH/DCM) gave compound **31** (0.31 g, 0.89 mmol) in 88% yield as a yellow solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.21 (m, 5H), 5.00 (s, 1H), 4.67 – 4.48 (m, 2H), 3.98 (dd, *J* = 11.9, 10.2 Hz, 1H), 3.44 – 3.37 (m, 1H), 3.34 – 3.11 (m, 7H), 2.86 (td, *J* = 12.6, 2.7 Hz, 1H), 2.71 (dd, *J* = 12.8, 5.2 Hz, 1H), 1.97 – 1.86 (m, 1H), 1.86 – 1.71 (m, 1H), 1.46 – 1.32 (m, 2H), 1.23 (s, 9H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 138.2, 128.5, 127.8, 127.7, 74.5, 73.8, 70.5, 67.3, 65.8, 60.8, 60.0, 59.1, 36.4, 27.4, 25.5, 21.1. HRMS: $[M+H]^+$ calculated for $[C_{20}H_{34}NO_4]^+$: 352.24824, found 352.24795.

**Compound 13**: A solution of oxalyl chloride (0.69 mL, 8.0 mmol) in DCM (8.0 mL) was cooled to -78 °C. Next, DMSO (0.71 mL, 10 mmol, 5 eq.) was dissolved in DCM (5.0 mL, 2 M) and added dropwise to the oxalyl chloride solution at -78 °C. After stirring for 40 minutes, a solution of compound **31** (0.70 g, 2.0 mmol, 1 eq.) in DCM (4.0 mL, 0.5 M) was added dropwise to the reaction mixture and the reaction was stirred for 2 hours at -78 °C. Triethylamine (3.4 mL, 24 mmol, 12 eq.) was added and the mixture was allowed to warm to 0 °C while stirring over 2 hours. The off-white suspension was diluted with DCM and washed $H_2O$ (1x) and the aqueous layer was extracted with DCM (1x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was used in the next step without purification. Methyltriphenylphosphonium bromide (1.4 g, 4.2 mmol, 2 eq.) was suspended in dry THF (12 mL, 0.33 M) and cooled to -78 °C. Sodium bis(trimethylsilyl)amide (1 M in THF, 4.2 mL, 4.2 mmol, 2.1 eq.) was added dropwise and the reaction was stirred for 2 hours while warming to 0 °C. The bright yellow suspension was cooled back to -78 °C and a solution of the aldehyde from the previous step in dry THF (2.7 mL, 0.75 M) was added dropwise. The reaction mixture was stirred overnight while warming to room temperature and quenched with sat. aq. $NH_4Cl$. The mixture was diluted with DCM and washed with $H_2O$ (1x). The aqueous layer was extracted with DCM (2x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. After purification by silica gel column chromatography (20% → 50% $Et_2O$/pentane) compound **13** was obtained as a pale-yellow oil. To remove trace impurities, the compound was redissolved in $Et_2O$ and extracted with 1 M HCl (2x). The combined

aqueous layers were basified with 3M NaOH and extracted with DCM (2x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Another purification by silica gel column chromatography (20% → 50% $Et_2O$/pentane) gave compound **13** (230 mg, 0.894 mmol) as a colourless and odourless oil in 92% yield. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.21 (m, 5H), 5.84 – 5.59 (m, 2H), 5.35 – 5.05 (m, 4H), 4.60 – 4.40 (m, 2H), 3.60 – 3.50 (m, 1H), 3.51 – 3.40 (m, 2H), 3.21 – 3.10 (m, 1H), 2.87 (t, *J* = 8.7 Hz, 1H), 2.78 (dt, *J* = 11.2, 1.3 Hz, 1H), 2.22 (td, *J* = 11.5, 2.6 Hz, 1H), 2.15 – 2.02 (m, 1H), 1.68 (dp, *J* = 13.4, 3.5 Hz, 1H), 1.50 – 1.34 (m, 1H), 1.32 – 1.18 (m, 1H), 1.15 (s, 9H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 139.1, 138.9, 134.1, 128.3, 127.9, 127.5, 119.5, 119.0, 78.6, 72.9, 71.7, 69.4, 64.6, 61.7, 46.4, 30.2, 27.7, 23.8. HRMS: $[M+H]^+$ calculated for $[C_{22}H_{34}NO_2]^+$: 344.25841, found 344.25827.

**Compound 35**: Compound **13** (0.34 g, 0.99 mmol) was dissolved in TFA (5.9 mL) and cooled to 0 °C. 10 drops of $H_2O$ were added, and the solution was stirred at room temperature for 2 hours. The reaction was diluted with DCM (100 mL) and the organic phase was washed with 1 M NaOH (2x). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (2% → 5% MeOH/DCM) gave compound **35** (0.25 g, 0.89 mmol) as a colourless oil in 89% yield. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.23 (m, 6H), 5.75 – 5.53 (m, 2H), 5.40 – 5.04 (m, 4H), 4.53 (q, *J* = 11.6 Hz, 2H), 3.79 – 3.66 (m, 1H), 3.51 (t, *J* = 10.5 Hz, 1H), 3.41 (dd, *J* = 10.3, 5.7 Hz, 1H), 3.21 – 3.10 (m, 1H), 2.97 (t, *J* = 8.7 Hz, 1H), 2.86 – 2.76 (m, 1H), 2.22 – 2.04 (m, 2H), 1.74 (dp, *J* = 12.5, 3.2 Hz, 1H), 1.51 – 1.34 (m, 1H), 1.34 – 1.20 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 138.8, 138.5, 132.0, 128.4, 127.9, 127.6, 120.2, 120.0, 78.7, 71.7, 69.1, 61.5, 60.1, 43.9, 30.3, 23.8. HRMS: $[M+H]^+$ calculated for $[C_{18}H_{26}NO_2]^+$: 288.19581, found 288.19571.

**Compound 14**: A solution of compound **35** (0.13 g, 0.46 mmol, 1 eq.) and imidazole (47 mg, 0.70 mmol) in DCM (1.1 mL) was cooled to 0 °C. TBSCl (84 mg, 0.56 mmol, 1.2 eq.) was added and the mixture was stirred at 0 °C for 2 hours. The reaction was quenched by addition of MeOH and washed with $H_2O$ (1x). The organic phase was dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (5% → 20% $Et_2O$/pentane) gave compound **14** (0.18 g, 0.44 mmol) in 96% yield as a colourless oil. $^1$H NMR (850 MHz, $CDCl_3$) δ 7.33 – 7.29 (m, 4H), 7.26 – 7.23 (m, 1H), 5.79 – 5.73 (m, 1H), 5.68 – 5.62 (m, 1H), 5.36 – 5.04 (m, 4H), 4.57 – 4.45 (m, 2H), 3.71 – 3.65 (m, 2H), 3.55 – 3.50 (m, 1H), 3.16 – 3.12 (m, 1H), 2.85 (t, *J* = 8.7 Hz, 1H), 2.80 (dt, *J* = 11.3, 2.9 Hz, 1H), 2.21 (td, *J* = 11.6, 2.5 Hz, 1H), 2.10 (dq, *J* = 11.6, 3.5 Hz, 1H), 1.68 (dq, *J* = 13.2, 3.4 Hz, 1H), 1.46 – 1.38 (m, 1H), 1.27 – 1.19 (m, 1H), 0.87 (s, 9H), 0.04 – 0.01 (m, 6H). $^{13}$C NMR (214 MHz, $CDCl_3$) δ 139.1, 139.1, 133.8, 128.3, 127.9, 127.5, 119.4, 119.3, 78.7, 71.6, 69.5, 65.7, 63.4, 46.3, 30.2, 26.1, 23.8, 18.5, -5.1, -5.1. HRMS: $[M+H]^+$ calculated for $[C_{24}H_{40}NO_2Si]^+$: 402.28228, found 402.28181.

**Compound 15**: This reaction was carried out under Schlenk conditions with flame-dried glassware. Compound **14** (583 mg, 1.45 mmol) was dissolved in dry benzene (14.5 mL) and added to a flask containing (341 mg, 0.436 mmol, 0.3 eq.) under argon atmosphere, and the dark red/brown solution was stirred overnight at room temperature. The reaction was

quenched by exposing to air for 1 hour and used as solution for silica gel column chromatography (5% → 20% $Et_2P$/pentane) to obtain starting material **14** (0.241 mg, 0.600 mmol) in 41% yield and the product compound **15** (270 mg, 0.722 mmol) as a pale-yellow oil in 50% yield. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.25 (m, 5H), 6.19 (dt, *J* = 6.1, 1.7 Hz, 1H), 5.85 (dt, *J* = 6.2, 1.6 Hz, 1H), 4.66 – 4.43 (m, 2H), 3.87 – 3.77 (m, 1H), 3.72 – 3.64 (m, 1H), 3.55 – 3.50 (m, 1H), 3.50 – 3.45 (m, 1H), 3.15 – 3.04 (m, 1H), 2.80 – 2.68 (m, 1H), 2.26 – 2.11 (m, 1H), 1.70 (s, 0H), 1.63 – 1.45 (m, 1H), 1.37 – 1.20 (m, 1H), 0.89 (s, 9H), 0.06 – 0.03 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 139.0, 132.9, 130.8, 128.5, 127.8, 127.7, 79.6, 71.0, 70.1, 68.1, 65.1, 45.9, 30.0, 26.0, 22.8, 18.4, -5.2, -5.3.

**Compound 32a and 32b**: Compound **15** (0.27 g, 0.72 mmol, 1 eq.) and N,N,N',N'-tetramethylethylenediamine (0.12 mL, 0.79 mmol) were dissolved in DCM (34 mL, 0.021 M) and the solution was cooled to -78 °C. A solution of $OsO_4$ (0.020 M in DCM, 39 mL, 0.76 mmol, 1.05 eq.) was slowly added and the solution was stirred at -78 °C for 1 hour. Ethylenediamine (0.96 mL, 14 mmol, 20 eq.) was added and the mixture was stirred overnight while warming to room temperature. The reaction was concentrated *in vacuo* and purified by silica gel column chromatography (2% → 10% MeOH/DCM) to give compound **32a** (0.14 g, 0.34 mmol) in 48% yield and **32b** (47 mg, 0.12 mmol) in 16% yield. Data for **32a**: $^1$H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.22 (m, 5H), 4.75 – 4.48 (m, 1H), 4.23 – 4.12 (m, 2H), 3.75 (ddd, *J* = 37.4, 10.8, 3.5 Hz, 2H), 3.61 – 3.50 (m, 1H), 3.00 (q, *J* = 3.5 Hz, 1H), 2.91 (d, *J* = 10.4 Hz, 1H), 2.80 (dd, *J* = 9.2, 3.4 Hz, 1H), 2.56 (td, *J* = 11.8, 2.9 Hz, 1H), 2.28 – 2.17 (m, 1H), 1.73 – 1.63 (m, 1H), 1.60 – 1.44 (m, 1H), 1.27 – 1.11 (m, 1H), 0.89 (s, 9H), 0.05 (d, *J* = 1.5 Hz, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 138.8, 128.7, 128.1, 128.0, 74.0, 73.4, 71.2, 70.7, 68.5, 68.2, 62.3, 45.6, 29.8, 26.0, 23.3, 18.2, -5.4. HRMS: $[M+Na]^+$ calculated for $[C_{22}H_{38}NO_4Si]^+$: 408.25646, found 408.25634. Data for **32b**: $^1$H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.23 (m, 5H), 4.74 – 4.44 (m, 2H), 4.30 – 4.20 (m, 1H), 4.07 – 3.97 (m, 1H), 3.75 (d, *J* = 3.2 Hz, 2H), 3.32 – 3.08 (m, 3H), 3.05 – 2.94 (m, 1H), 2.90 – 2.77 (m, 2H), 2.63 (td, *J* = 12.3, 2.9 Hz, 1H), 2.26 – 2.17 (m, 1H), 1.67 – 1.52 (m, 4H), 1.52 – 1.37 (m, 1H), 1.35 – 1.14 (m, 2H), 0.98 – 0.80 (m, 10H), 0.12 – 0.09 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 128.6, 127.9, 127.8, 76.0, 73.2, 71.9, 70.5, 70.4, 62.7, 60.9, 46.1, 30.2, 26.0, 22.2.

**Compound 16**: Compound **32a** (0.12 g, 0.29 mmol) was co-evaporated with toluene (3x) and dissolved in DMF (2.9 mL) under argon. NaH (24 mg, 0.60 mmol, 2.1 eq.) was added and the mixture was stirred for 15 minutes at room temperature followed by addition of benzyl bromide (0.15 mL, 0.63 mmol, 4.4 eq.). The reaction was stirred for 4 hours at room temperature and subsequently quenched with MeOH. The reaction was diluted with DCM and washed with $H_2O$ (1x). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (30% → 60% $Et_2O$/pentane) gave compound **16** (0.12 g, 0.20 mmol) as a colourless oil in 70% yield. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.18 (m, 15H), 4.86 – 4.69 (m, 2H), 4.67 – 4.37 (m, 4H), 4.24 – 4.18 (m, 1H), 4.03 (d, *J* = 5.2 Hz, 1H), 3.82 – 3.70 (m, 2H), 3.63 – 3.56 (m, 1H), 3.25 (dt, *J* = 5.8, 3.2 Hz, 1H), 2.98 – 2.91 (m, 1H), 2.76 (dd, *J* = 8.9, 3.1 Hz, 1H), 2.54 (td, *J* = 11.4, 3.4 Hz, 1H), 2.30 – 2.21 (m, 1H), 1.69 – 1.51 (m, 4H), 1.23 – 1.08 (m, 1H), 0.90 – 0.88 (m, 1H), 0.84 (s, 9H), 0.02 (s, 3H), -0.04 (s, 3H). $^{13}$C NMR

(101 MHz, $CDCl_3$) δ 139.2, 139.2, 138.8, 128.4, 128.3, 128.3, 127.9, 127.7, 127.6, 127.6, 127.5, 80.3, 76.9, 74.7, 73.8, 72.1, 70.5, 68.5, 65.3, 61.9, 45.8, 30.0, 26.0, 23.5, 18.2, -5.4, -5.5. HRMS: $[M+H]^+$ calculated for $[C_{36}H_{50}NO_4Si]^+$: 588.35036, found 588.35011.

**Compound 17**: To a solution of compound **16** (0.12 g, 0.20 mmol) in THF (1.0 mL) was added TBAF (1 M in THF, 0.60 mL, 0.60 mmol, 3 eq.). After stirring for 2 hours at room temperature, the reaction was diluted with DCM and the organic phase was washed with $H_2O$ (1x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (2% → 5% MeOH/DCM) to obtain compound **17** (78 mg, 0.16 mmol) as a colourless oil in 82% yield. $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.16 (m, 15H), 4.83 – 4.44 (m, 6H), 4.07 (t, *J* = 4.8 Hz, 1H), 4.01 (t, *J* = 4.7 Hz, 1H), 3.97 – 3.90 (m, 1H), 3.71 – 3.63 (m, 1H), 3.59 – 3.51 (m, 1H), 3.23 (q, *J* = 3.4 Hz, 1H), 3.09 (dd, *J* = 8.9, 4.3 Hz, 1H), 2.87 (dt, *J* = 12.4, 3.5 Hz, 1H), 2.72 – 2.60 (m, 1H), 2.22 – 2.12 (m, 1H), 1.67 – 1.55 (m, 2H), 1.38 – 1.22 (m, 2H). $^{13}C$ NMR (101 MHz, $CDCl_3$) δ 139.1, 138.9, 138.7, 128.5, 128.4, 128.0, 127.8, 127.7, 127.5, 127.5, 80.9, 79.1, 73.5, 73.3, 72.9, 71.1, 66.3, 65.8, 60.7, 45.8, 29.8, 20.9. HRMS: $[M+Na]^+$ calculated for $[C_{30}H_{36}N_1O_4]^+$: 474.26389, found 474.26354.

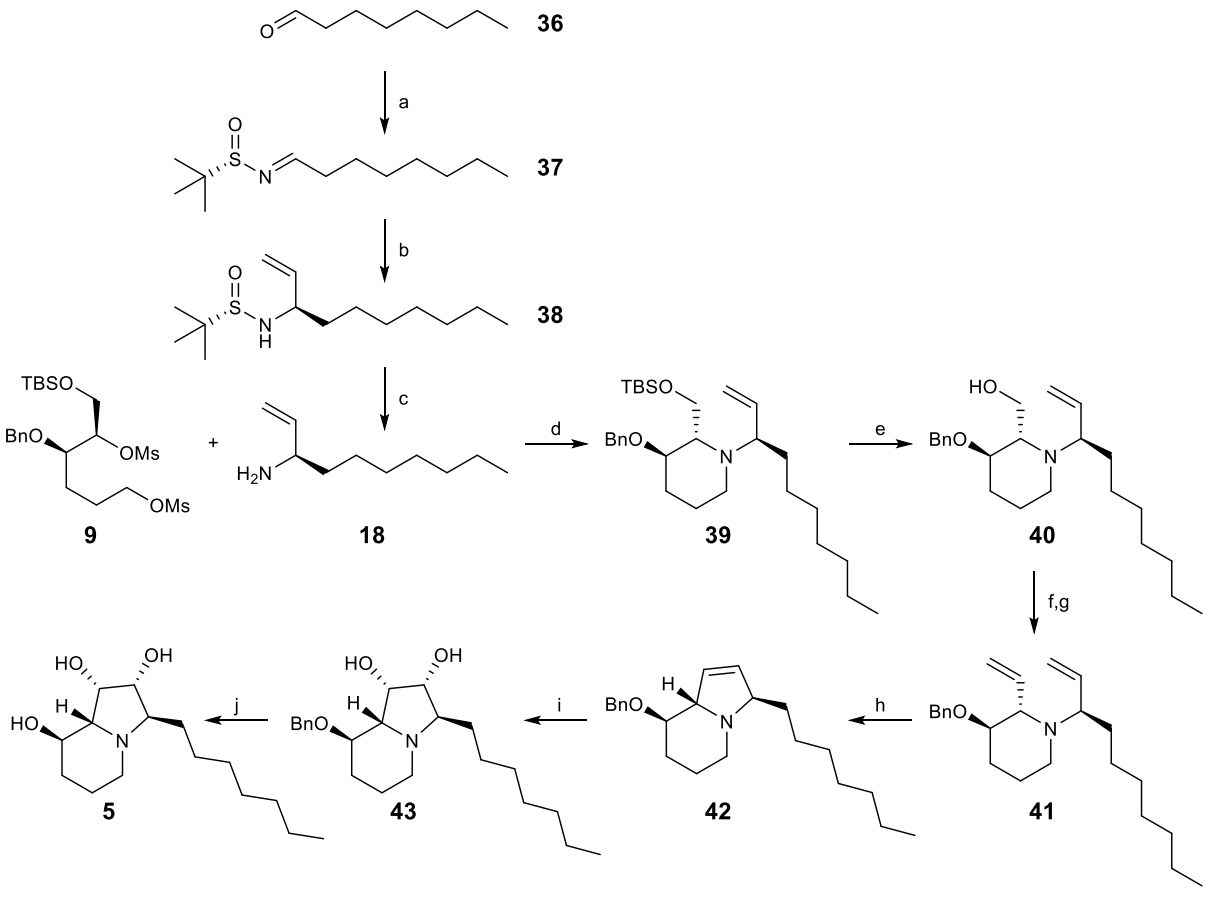
**Compound 33**: A stock solution of $HCrO_4^-$ (Jones reagent) was prepared by dissolving conc. $H_2SO_4$ (8.7 mL, 0.16 mol) in $H_2O$ (30 mL) followed by the addition of $CrO_3$ (2.1 g, 21 mmol). Compound **17** (77.8 mg, 0.164 mmol) was dissolved in mixture of acetone/$H_2O$ (3:2, 4.11 mL) and the pH was adjusted to pH ≈ 4 with 1 M $H_2SO_4$ aq. The solution was heated to 60 °C and the stock solution of Jones reagent (1.46 mL) was slowly added. The reaction was then stirred for 30 minutes at 60 °C and subsequently cooled to 0 °C, quenched with $Na_2S_2O_3$ sat. aq. and neutralized with $NaHCO_3$ sat. aq. The aqueous phase was extracted with DCM (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (3% → 8% MeOH/DCM) gave compound **33** (33.7 mg, 69.1 µmol) in 42% yield as a light brown solid. $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.13 (m, 15H), 4.90 – 4.64 (m, 4H), 4.57 – 4.39 (m, 4H), 4.16 (s, 1H), 3.89 (d, *J* = 10.3 Hz, 2H), 3.64 (s, 1H), 3.17 (s, 2H), 2.09 – 1.95 (m, 1H), 1.88 (s, 1H), 1.64 – 1.43 (m, 2H). $^{13}C$ NMR (101 MHz, $CDCl_3$) δ 170.7, 138.2, 138.0, 137.9, 128.5, 128.5, 128.5, 128.1, 128.1, 127.9, 127.8, 127.7, 81.6, 78.4, 74.1, 72.7, 72.4, 71.7, 71.0, 49.4, 26.8, 19.1. HRMS: $[M+Na]^+$ calculated for $[C_{30}H_{34}NO_5]^+$: 488.24315, found 488.24310.

**Compound 34**: Compound **33** (18 mg, 36 µmol) was dissolved in DMF (1.0 mL) and DIPEA (13 µL, 72 µmol, 2 eq.) and HATU (14 mg, 38 µmol, 1.05 eq.) were subsequently added. The mixture was stirred for 5 minutes at room temperature followed by the addition of amylamine (42 µL, 0.36 mmol, 10 eq.). The reaction was stirred overnight at room temperature and diluted with DCM. The organic phase was washed with $H_2O$ (1x) and the organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (10% → 30% EtOAc/PE) gave compound **34** (18 mg, 33 µmol) in 92% yield. $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.15 (m, 15H), 6.88 (t, *J* = 5.9 Hz, 1H), 4.88 – 4.59 (m, 4H), 4.56 – 4.46 (m, 2H), 4.11 (dd, *J* = 5.4, 2.5 Hz, 1H), 4.04 – 3.93 (m, 2H), 3.59 (d, *J* = 2.5 Hz, 1H), 3.32 – 3.10 (m, 3H), 2.77 – 2.60 (m, 2H), 2.17 – 2.05 (m, 1H), 1.77 (s, 1H), 1.62 –

1.38 (m, 4H), 1.38 – 1.19 (m, 6H), 0.88 (t, *J* = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.9, 139.2, 138.7, 138.6, 128.4, 128.4, 128.3, 128.2, 127.8, 127.6, 127.4, 82.6, 79.6, 73.7, 72.7, 72.2, 71.4, 70.2, 64.7, 47.1, 39.1, 30.6, 29.4, 29.1, 22.4, 20.6, 14.1. HRMS: $[M+Na]^+$ calculated for $[C_{35}H_{45}N_2O_4]^+$: 557.33738, found 557.33709.

**Compound 4**: Compound **33** (5.0 mg, 9.0 µmol) was co-evaporated with toluene under argon (3x) and dissolved in dioxane (0.50 mL, 18 mM). $H_2O$ (0.2 mL) was added followed by the addition of 1 M HCl aq. (0.2 mL). the solution was purged with argon for 15 minutes and a catalytic amount of palladium on carbon was added. The mixture was then purged with argon for another 15 minutes and subsequently purged with hydrogen for 15 minutes and stirred overnight under hydrogen atmosphere at room temperature. The reaction was then filtered over a Whatman filter and purified by HPLC to obtain compound **4** (2.3 mg, 8.0 µmol) in 89% yield. $^1$H NMR (850 MHz, $D_2O$ + 2 eq. NaOD) δ 4.41 (t, *J* = 4.9 Hz, 1H), 4.29 (dd, *J* = 5.2, 3.4 Hz, 1H), 3.77 – 3.71 (m, 1H), 3.48 (d, *J* = 4.4 Hz, 1H), 3.32 – 3.27 (m, 1H), 3.17 (dt, *J* = 13.7, 7.0 Hz, 1H), 2.77 (dd, *J* = 9.3, 3.4 Hz, 1H), 2.76 – 2.73 (m, 1H), 2.31 (td, *J* = 11.9, 2.8 Hz, 1H), 2.06 – 2.03 (m, 1H), 1.72 – 1.68 (m, 1H), 1.57 – 1.48 (m, 4H), 1.36 – 1.27 (m, 5H), 1.26 – 1.19 (m, 1H), 0.88 (t, *J* = 7.0 Hz, 3H). $^{13}$C NMR (214 MHz, $D_2O$) δ 173.0, 74.1, 70.6, 70.0, 69.4, 66.2, 45.6, 39.2, 31.8, 28.3, 28.0, 22.5, 21.6, 13.2. HRMS: $[M+Na]^+$ calculated for $[C_{14}H_{27}N_2O_4]^+$: 287.19653, found 287.19624.

**Synthesis of indolizidine 5**



**Scheme 3**. *Reagents and conditions*: a) (*S*)-*tert*-butylsulfinamide, pyridinium *p*-toluenesulfonate, $MgSO_4$, DCM, r.t., 16 h, 80%; b) vinylMgBr, toluene, r.t., 16 h, 77%; c) 4M HCl in dioxane, MeOH, 0 °C to r.t., 1 h, 98%; d) DIPEA, MeCN, 50 °C, 3 days, 55%; e) TBAF, THF, r.t., 16h, 98%; f) oxalyl chloride, DMSO, $Et_3N$, DCM, -78 °C to 0 °C, 5 h; g) $MeP(Ph)_3Br$, NaHMDS, THF, -78 °C to 0 °C, 16 h, 37% (over 2 steps); h) Schrock-Hoveyda catalyst, benzene, r.t., 16 h, 87%; i) $OsO_4$, TMEDA, DCM, -78 °C then ethylenediamine, r.t., 16 h, 39%; j) Pd/C, $H_2$, HCl, dioxane/$H_2O$, r.t., 16 h, 90%.

**Compound 37**: To a solution of (*S*)-*tert*-butylsulfinamide (0.61 g, 5.0 mmol) in DCM (10 mL) was added pyridinium *p*-toluenesulfonate (62 mg, 0.25 mmol, 0.05 eq.), $MgSO_4$ (3.0 g, 25 mmol, 5 eq.) and octanal (0.78 mL, 5.0 mmol, 1 eq.). The resulting mixture was stirred overnight at room temperature and subsequently filtered over Celite and concentrated *in vacuo*. Purification by silica gel column chromatography (10% → 20% $Et_2O$/pentane) gave compound **37** (0.92 g, 4.0 mmol) in 80% yield as a colourless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.06 (t, *J* = 4.8 Hz, 1H), 2.55 – 2.46 (m, 2H), 1.61 (p, *J* = 7.4 Hz, 3H), 1.38 – 1.23 (m, 8H), 1.19 (s, 9H), 0.91 – 0.84 (m, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.0, 56.6, 36.3, 31.8, 29.3, 29.2, 28.2, 25.7, 24.8, 22.7, 22.5, 14.2. HRMS: $[M+H]^+$ calculated for $[C_{36}H_{50}NO_4Si]^+$: 232.17296, found 232.17283.

**Compound 38**: Compound **37** (0.92 g, 4.0 mmol) was co-evaporated with toluene (3x) under an argon atmosphere. The residue was dissolved in dry toluene (400 mL) followed by the addition of vinylmagnesium bromide (1 M in THF, 8 mL, 8 mmol, 2 eq.). The resulting solution was stirred overnight at room temperature and subsequently quenched with $H_2O$ and concentrated *in vacuo*. The residue was redissolved in $Et_2O$ and washed with 1M HCL (1x), sat. aq. $NaHCO_3$ (1x) and brine (1x). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (20% → 50% EtOAc/PE) gave compound **38** (0.84 g, 3.1 mmol) in 77% yield as a colourless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 5.88 – 5.75 (m, 1H), 5.27 – 5.13 (m, 2H), 3.78 – 3.66 (m, 1H), 3.07 (d, *J* = 6.3 Hz, 1H), 1.69 – 1.57 (m, 1H), 1.54 – 1.40 (m, 1H), 1.37 – 1.23 (m, 10H), 1.21 (s, 9H), 0.91 – 0.84 (m, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 140.1, 116.5, 58.9, 55.9, 35.4, 31.9, 29.5, 29.3, 25.6, 22.8, 22.7, 14.2. HRMS: $[M+H]^+$ calculated for $[C_{14}H_{30}NOS]^+$: 260.20426, found 260.20412.

**Compound 18**: A solution of compound **38** (1.77 g, 6.47 mmol) in MeOH (64.7 mL) was cooled to 0 °C. HCl (4M in dioxane, 3.24 mL, 12.9 mmol, 2 eq.) was added and mixture was stirred for 1 hour while warming to room temperature. The reaction was concentrated *in vacuo* and the residue was redissolved in DCM and washed with 1 M HCl (1x). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (2% → 10% MeOH/DCM) gave the hydrochloride salt of compound **38** which was neutralized by dissolving in DCM and washing with 1 M NaOH (1 x) to obtain compound **41** (0.983 g, 6.33 mmol) in 98% yield as a colourless liquid. $^1$H NMR (400 MHz, $CDCl_3$) δ 5.84 – 5.71 (m, 1H), 5.17 – 4.95 (m, 2H), 3.32 – 3.22 (m, 1H), 1.45 – 1.37 (m, 1H), 1.35 – 1.19 (m, 11H), 0.91 – 0.82 (m, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 143.6, 113.5, 54.7, 37.7, 32.0, 29.7, 29.4, 26.2, 22.8, 14.2. HRMS: $[M+H]^+$ calculated for $[C_{10}H_{22}N]^+$: 156.17468, found 156.17463.

**Compound 39**: Compound **9** (1.7 g, 3.3 mmol) was dissolved in dry MeCN (6.5 mL) and compound **18** (0.983 g, 6.33 mmol, 2 eq.) and DIPEA (1.7 mL, 9.8 mmol, 3 eq.) were added. The resulting mixture was stirred for 3 days at 50 °C and subsequently concentrated *in vacuo*. Purification by silica gel column chromatography (5% → 40% EtOAc/PE) gave compound **39** (0.85 g, 1.8 mmol) in 55% yield as a colourless oil. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.21 (m, 5H), 5.89 – 5.74 (m, 1H), 5.17 – 5.05 (m, 2H), 4.64 – 4.43 (m, 2H), 3.91 – 3.78 (m, 2H), 3.49 – 3.38 (m, 2H), 2.74 – 2.64 (m, 2H), 2.38 – 2.27 (m, 1H), 2.08 – 1.97 (m, 1H), 1.79 – 1.51 (m, 2H), 1.45 – 1.10 (m, 14H), 0.93 – 0.82 (m, 10H), 0.04 – 0.02 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 140.7, 139.2, 128.4, 127.9, 127.5, 115.5, 74.9, 70.9, 64.4, 61.7, 60.8, 45.0, 32.1, 30.2, 29.5, 28.7, 27.5, 27.1, 26.1, 22.8, 18.5, 14.3, -5.2. HRMS: $[M+H]^+$ calculated for $[C_{29}H_{52}NO_2Si]^+$: 474.37618, found 474.37570.

**Compound 40**: To a solution of compound **39** (0.85 g, 1.8 mmol) in THF (3.6 mL) was added TBAF (1 M in THF, 2.7 mL, 2.7 mmol, 1.5 eq.) and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with DCM and washed with $H_2O$ (1x). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (0% → 6% MeOH/DCM) gave compound **40** (0.63 g, 1.8 mmol) in 98% yield as a colourless oil. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.24 (m, 5H), 5.85 – 5.72 (m, 1H), 5.22 – 5.07 (m, 2H), 4.66 – 4.48 (m, 2H), 3.93 – 3.82 (m, 1H), 3.75 – 3.67 (m, 1H), 3.52 – 3.40 (m, 2H), 2.89 – 2.80 (m, 1H), 2.72 (s, 1H), 2.40 (t, *J* = 10.3 Hz, 1H), 2.16 – 2.05 (m, 1H), 1.79 – 1.64 (m, 1H), 1.64 – 1.52 (m, 1H), 1.49 – 1.09 (m, 12H), 0.87 (d, *J* = 6.8 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 139.5, 138.7, 128.5, 127.9, 127.7, 116.5, 75.1, 71.3, 62.2, 60.3, 58.1, 44.1, 32.0, 30.0, 29.4, 29.2, 27.1, 27.1, 22.8, 21.8, 14.2. HRMS: $[M+H]^+$ calculated for $[C_{23}H_{38}NO_2]^+$: 360.28971, found 360.28931.

**Compound 41**: Oxalyl chloride (0.26 mL, 3.1 mmol) was dissolved in DCM (3.1 mL) and the solution was cooled to -78 °C. A solution of DMSO (0.28 mL, 3.9 mmol, 5 eq.) in DCM (2.0 mL, 2 M) was added dropwise to the oxalyl chloride solution at -78 °C and the mixture was stirred for 40 minutes at -78 °C. A solution of compound **40** (0.28 g, 0.76 mmol, 1 eq.) in DCM (1.6 mL, 0.5 M) was added dropwise to the reaction mixture and the reaction was stirred for 2 hours at -78 °C. Triethylamine (1.3 mL, 3.9 mmol, 12 eq.) was added and the mixture was allowed to warm to 0 °C while stirring over 2 hours. The off-white suspension was diluted with DCM and washed $H_2O$ (1x) and the aqueous layer was extracted with DCM (1x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was used in the next step without purification. To a suspension of methyltriphenylphosphonium bromide (0.56 g, 1.6 mmol, 2 eq.) in dry THF (4.7 mL, 0.33 M) at -78 °C was added sodium bis(trimethylsilyl)amide (1 M in THF, 1.6 mL, 1.6 mmol, 2.1 eq.) dropwise and the reaction was stirred for 2 hours while warming to 0 °C. The bright yellow suspension was cooled back to -78 °C and a solution of the aldehyde from the previous step in dry THF (1.0 mL, 0.75 M) was added dropwise. The reaction was stirred overnight while warming to room temperature and quenched with sat. aq. $NH_4Cl$. The mixture was diluted with DCM and washed with $H_2O$ (1x). The aqueous layer was extracted with DCM (2x) and the combined organic layers were dried

over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (5% → 20% $Et_2O$/pentane) compound **41** (0.24 g, 0.67 mmol) was obtained as a pale-yellow oil in 86% yield. To remove trace impurities, compound **41** (90 mg, 0.25 mmol) was purified by HPLC followed by another purification by silica gel column chromatography (5% → 20% $Et_2O$/pentane) to give compound **41** (33 mg, 93 µmol) as a colourless and odourless oil in 37% yield. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.20 (m, 5H), 5.86 – 5.65 (m, 2H), 5.37 – 5.25 (m, 2H), 5.14 – 5.04 (m, 2H), 4.59 – 4.45 (m, 2H), 3.39 – 3.29 (m, 1H), 3.26 – 3.15 (m, 1H), 3.01 (t, *J* = 8.5 Hz, 1H), 2.81 – 2.71 (m, 1H), 2.20 – 2.04 (m, 2H), 1.72 – 1.63 (m, 2H), 1.61 – 1.50 (m, 1H), 1.47 – 1.34 (m, 1H), 1.34 – 1.17 (m, 11H), 1.07 (s, 1H), 0.88 (d, *J* = 6.7 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 139.6, 139.1, 138.8, 128.3, 127.9, 127.5, 118.9, 115.8, 78.7, 71.6, 69.0, 61.6, 45.1, 32.0, 30.2, 30.0, 29.4, 27.0, 25.0, 23.7, 22.8, 14.3. HRMS: $[M+H]^+$ calculated for $[C_{24}H_{38}NO]^+$: 356.29479, found 356.29444.

**Compound 42**: This reaction was performed under Schlenk-conditions under argon with flame dried glassware. Compound **41** (33 mg, 93 µmol) was dissolved in dry benzene (1.0 mL) and added to a flask containing 2,6-Diisopropylphenylimidoneophylidene[racemic-BIPHEN]molybdenum (21 mg, 28 µmol). The mixture was stirred overnight at room temperature overnight and subsequently quenched by exposure to air. Purification by silica gel column chromatography (5% → 20% $Et_2O$/pentane) gave compound **42** (27 mg, 81 µmol) as a colourless oil in 87%. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.24 (m, 5H), 6.14 (d, *J* = 6.1 Hz, 1H), 5.87 – 5.80 (m, 1H), 4.71 – 4.52 (m, 2H), 3.31 (td, *J* = 9.9, 4.3 Hz, 1H), 3.24 – 3.12 (m, 1H), 3.00 – 2.88 (m, 2H), 2.25 – 2.11 (m, 2H), 1.80 – 1.48 (m, 5H), 1.46 – 1.05 (m, 15H), 0.88 (t, *J* = 6.8 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 139.1, 133.3, 129.8, 128.5, 127.7, 127.6, 125.7, 78.7, 74.3, 71.2, 68.4, 47.2, 32.9, 32.0, 31.0, 30.5, 30.1, 29.9, 29.4, 26.3, 25.4, 22.8, 14.3. HRMS: $[M+H]^+$ calculated for $[C_{22}H_{34}NO]^+$: 328.26349, found 328.26312.

**Compound 43**: A solution of compound **42** (18 mg, 55 µmol) and N,N,N',N'-tetramethylethylenediamine (9.1 µL, 61 µmol, 1.1 eq.) in DCM (2.5 mL) was cooled to -78 °C. A solution of $OsO_4$ (0.020 M in DCM, 3.0 mL, 58 µmol, 1.05 eq.) was slowly added and the solution was stirred at -78 °C for 1 hour. Ethylenediamine (37 µL, 0.55 mmol, 10 eq.) was added and the mixture was stirred overnight while warming to room temperature. The reaction was concentrated *in vacuo* and purified by silica gel column chromatography (2% → 5% MeOH/DCM) to give compound **43** (7.8 mg, 22 µmol) in 39% yield. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.38 – 7.32 (m, 4H), 7.31 – 7.27 (m, 1H), 4.73 – 4.52 (m, 1H), 4.26 (dd, *J* = 6.0, 4.1 Hz, 1H), 4.13 (t, *J* = 6.4 Hz, 1H), 3.60 (td, *J* = 10.5, 4.5 Hz, 1H), 3.05 – 3.00 (m, 1H), 2.28 – 2.21 (m, 1H), 2.12 – 2.05 (m, 1H), 1.88 (dd, *J* = 9.2, 4.0 Hz, 1H), 1.76 – 1.69 (m, 2H), 1.61 – 1.38 (m, 4H), 1.38 – 1.15 (m, 12H), 0.87 (t, *J* = 6.9 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 138.9, 128.6, 128.0, 127.9, 73.6, 71.9, 71.0, 70.5, 69.6, 69.4, 50.9, 32.0, 30.4, 30.2, 29.4, 27.3, 26.8, 23.7, 22.8, 14.3. HRMS: $[M+H]^+$ calculated for $[C_{22}H_{36}NO_3]^+$: 362.26897, found 362.26870.

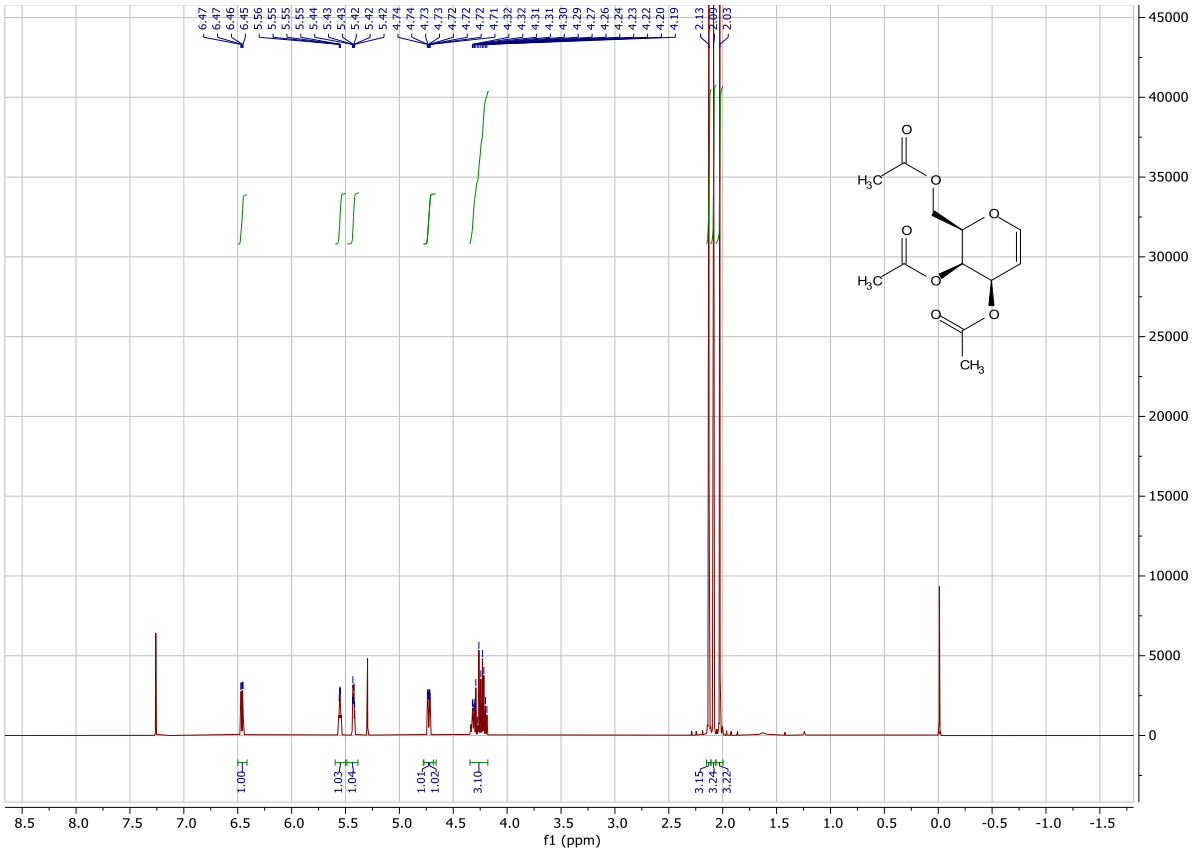
**Compound 5**: Compound **43** (7.2 mg, 20 µmol) was dissolved in dioxane/$H_2O$ (1: 1, 1.0 mL) and HCl (1 M, 40 µL, 40 µmol, 2 eq.) was added. The solution was purged with argon for 15 minutes followed by the addition of palladium on carbon (10% wt. loading, catalytic) and the

mixture was purged with argon for another 15 minutes. The reaction was purged with hydrogen for 15 minutes and stirred under hydrogen atmosphere overnight at room temperature. The mixture was filtered over a Whatman filter and concentrated *in vacuo*. Purification by silica gel column chromatography (2% → 10% $H_2O$/MeCN) gave compound **5** (4.9 mg, 18 µmol) in 90% yield. $^1$H NMR (500 MHz, $CDCl_3$) δ 4.47 (dd, *J* = 7.8, 5.5 Hz, 1H), 4.45 – 4.41 (m, 1H), 3.99 (td, *J* = 11.0, 4.5 Hz, 1H), 3.56 (dd, *J* = 11.9, 3.2 Hz, 1H), 3.30 – 3.26 (m, 1H), 2.88 (dd, *J* = 10.1, 3.0 Hz, 1H), 2.80 (td, *J* = 12.9, 3.3 Hz, 1H), 2.17 (dq, *J* = 11.8, 3.4 Hz, 1H), 1.99 (d, *J* = 15.1 Hz, 1H), 1.91 (dp, *J* = 16.2, 5.3 Hz, 1H), 1.85 – 1.71 (m, 1H), 1.71 – 1.63 (m, 1H), 1.58 (tt, *J* = 11.8, 5.4 Hz, 1H), 1.54 – 1.44 (m, 1H), 1.44 – 1.22 (m, 10H), 0.97 – 0.85 (m, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 73.2, 71.5, 69.3, 68.9, 64.7, 51.6, 49.5, 49.3, 49.2, 49.0, 48.8, 48.7, 48.5, 32.9, 32.9, 30.7, 30.2, 27.4, 26.3, 23.7, 22.2, 14.4. HRMS: $[M+H]^+$ calculated for $[C_{15}H_{30}NO_3]^+$: 272.22202, found 272.22180.

**Reference**


1. Z. Armstrong, C.-L. Kuo, D. Lahav, B. Liu, R. Johnson, T. J. M. Beenakker, C. de Boer, C.-S. Wong, E. R. van Rijssel, M. F. Debets, B. I. Florea, C. Hissink, R. G. Boot, P. P. Geurink, H. Ovaa, M. van der Stelt, G. M. van der Marel, J. D. C. Codée, J. M. F. G. Aerts, L. Wu, H. S. Overkleeft and G. J. Davies, *J. Am. Chem. Soc.*, 2020, **142**, 13021-13029.

## NMR Spectra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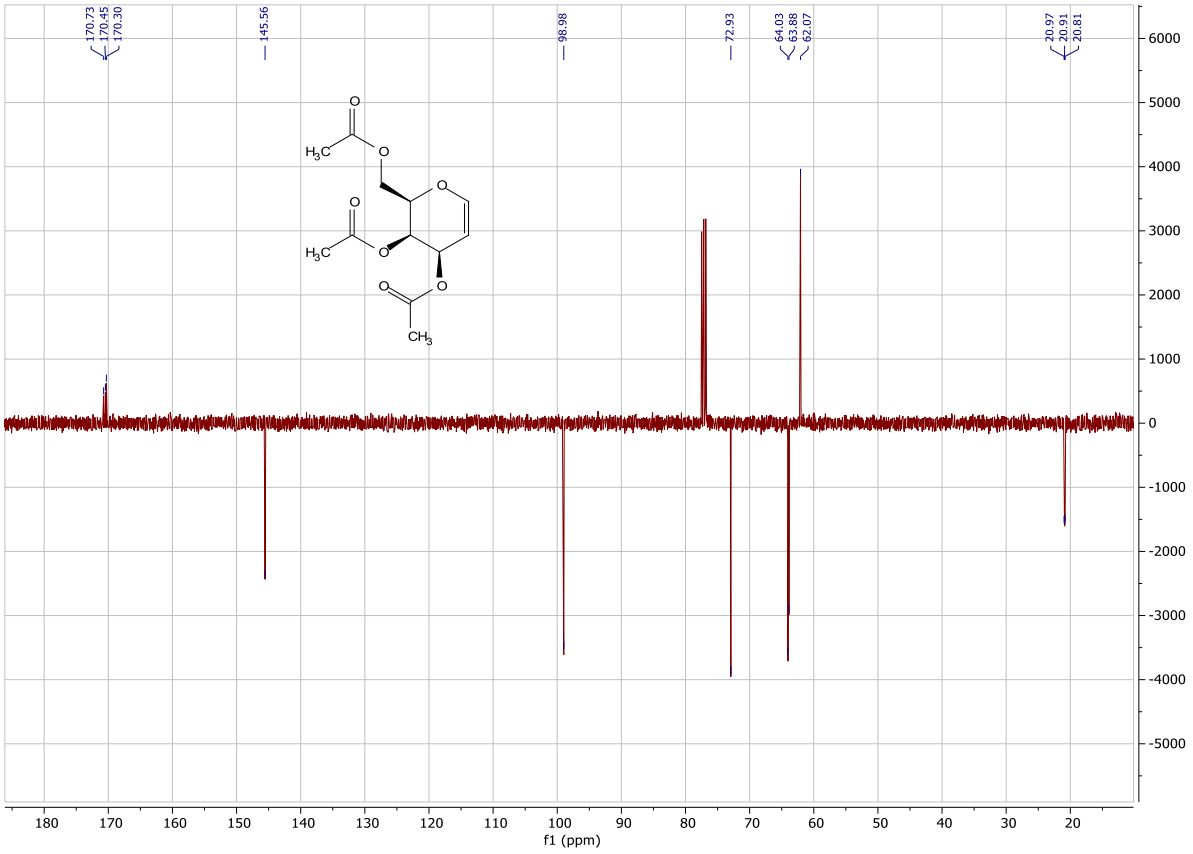
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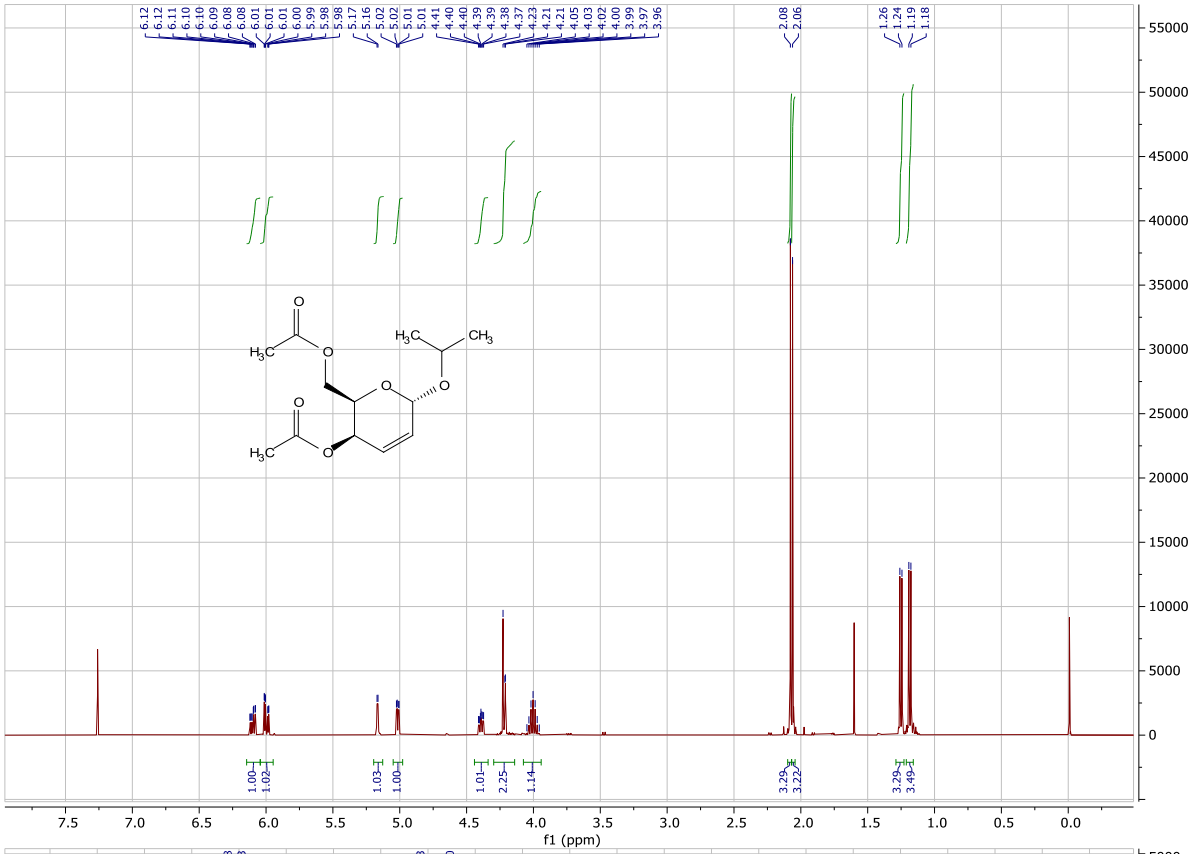
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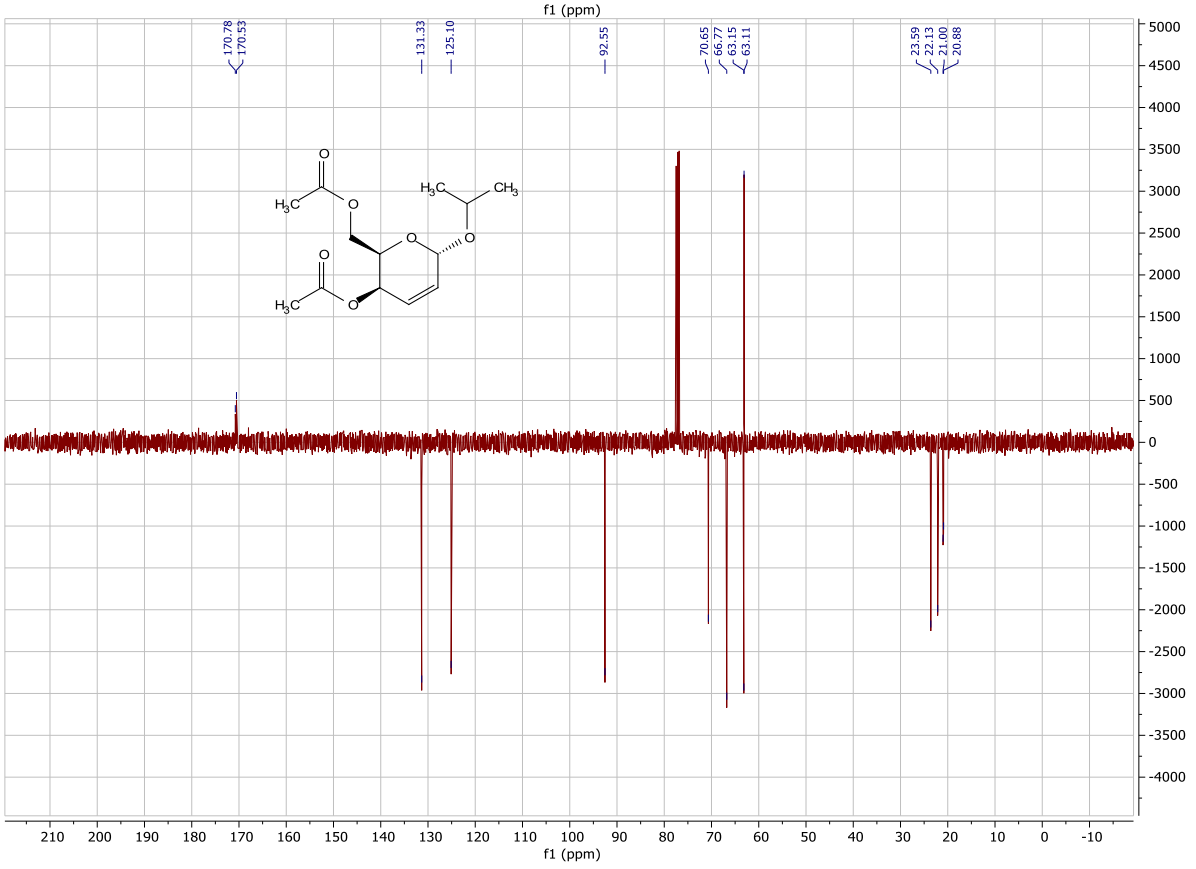
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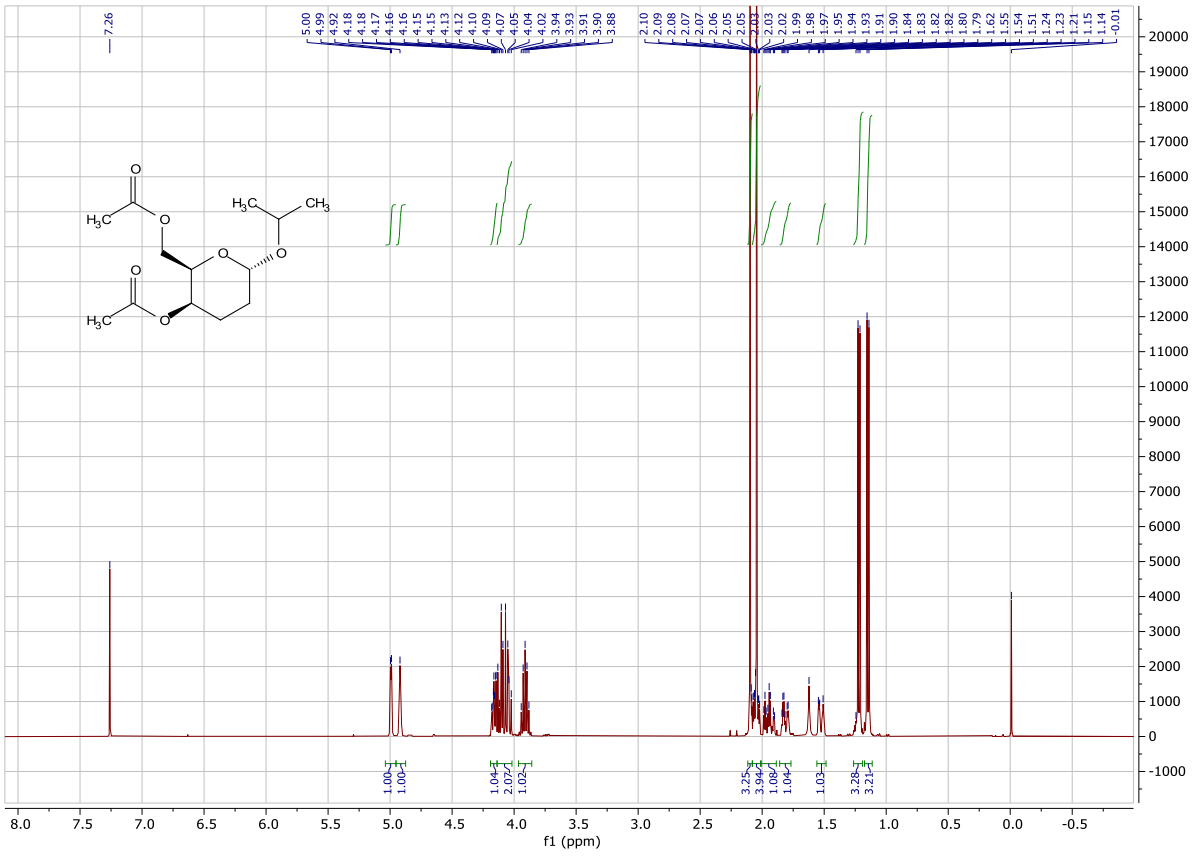
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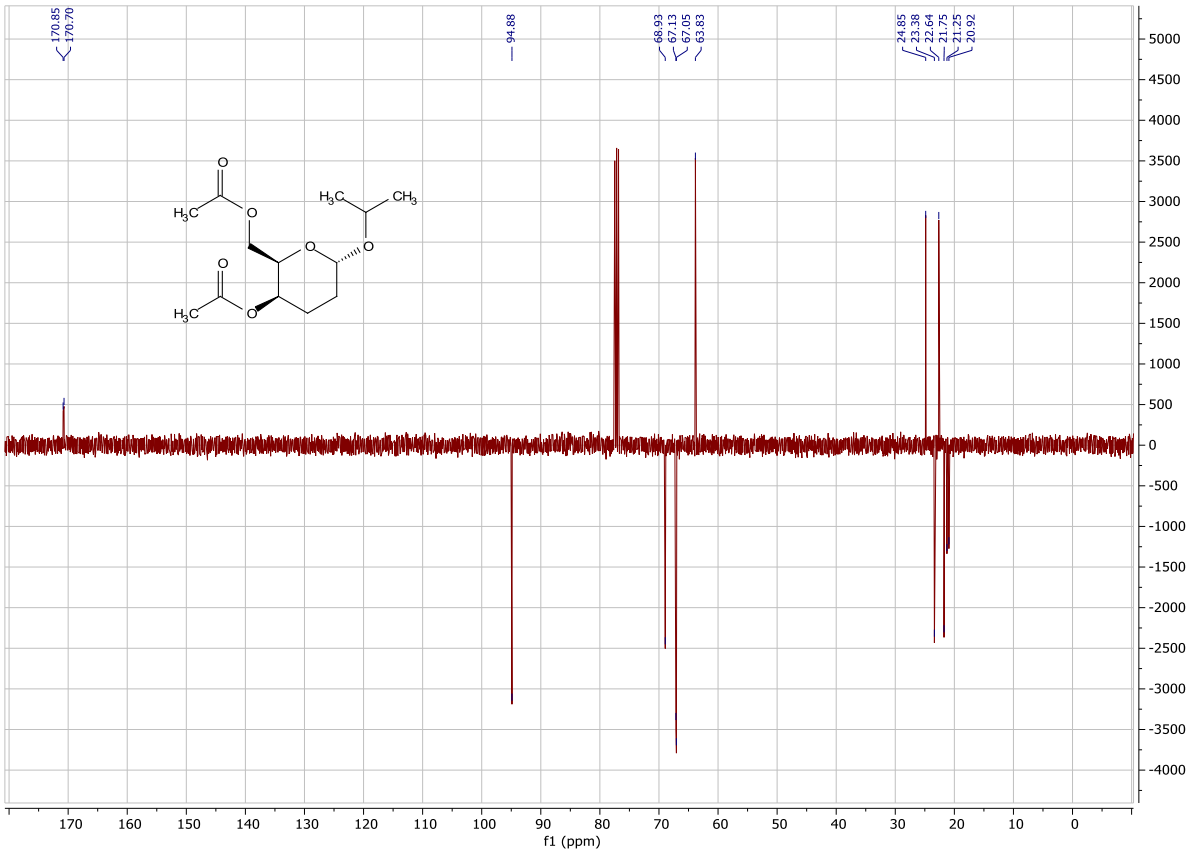
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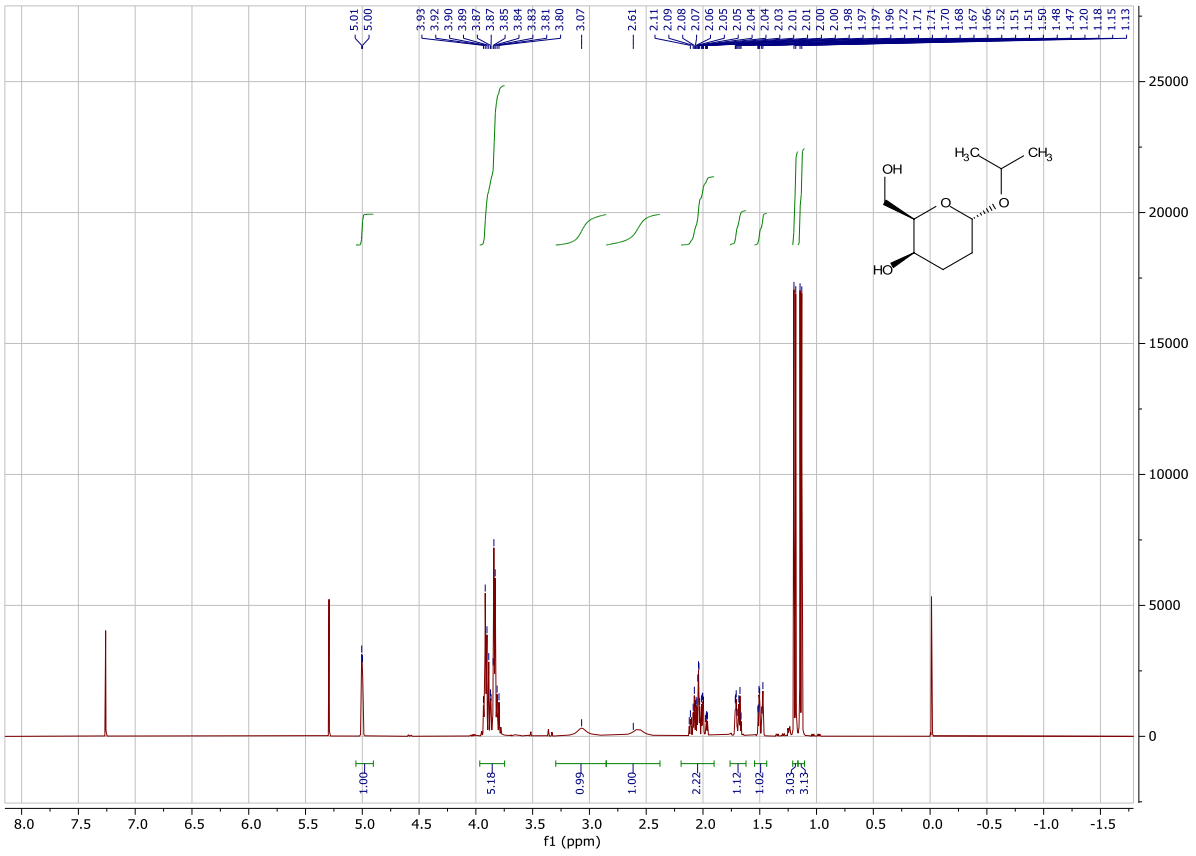
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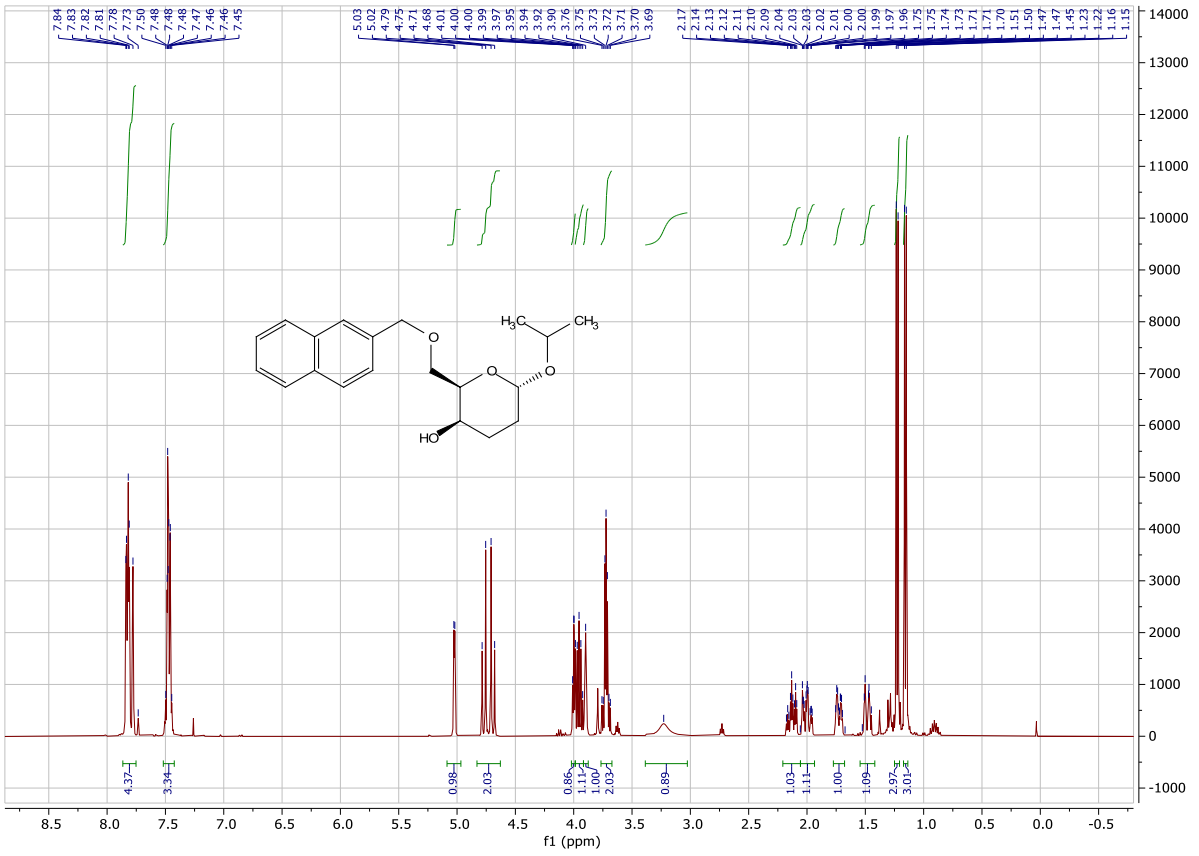
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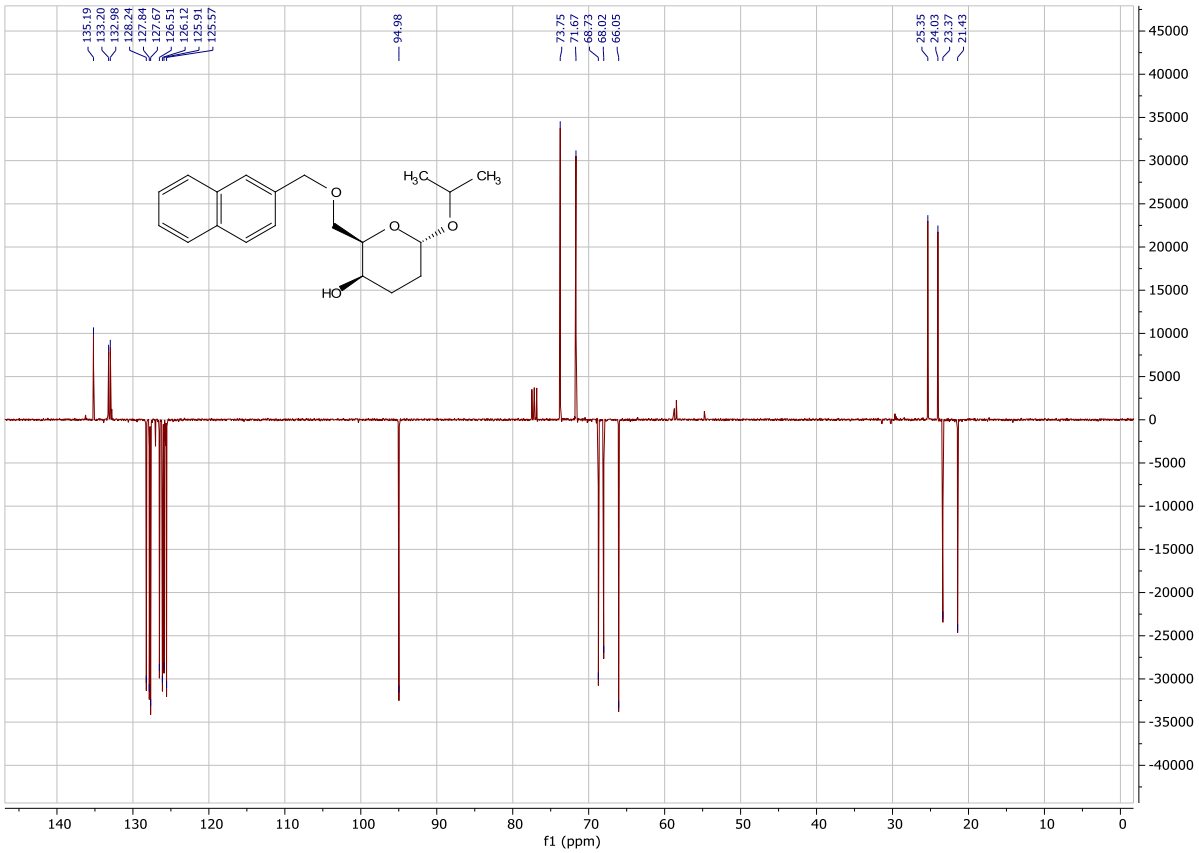
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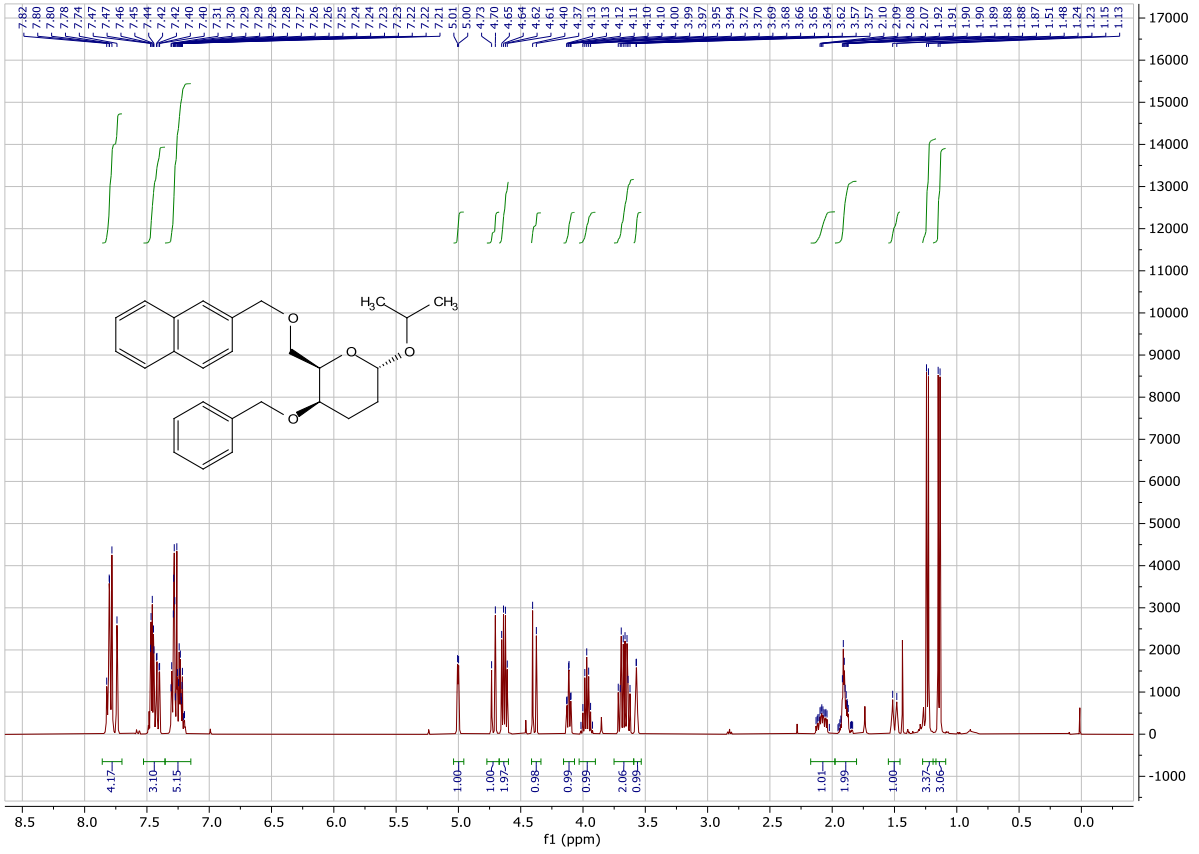
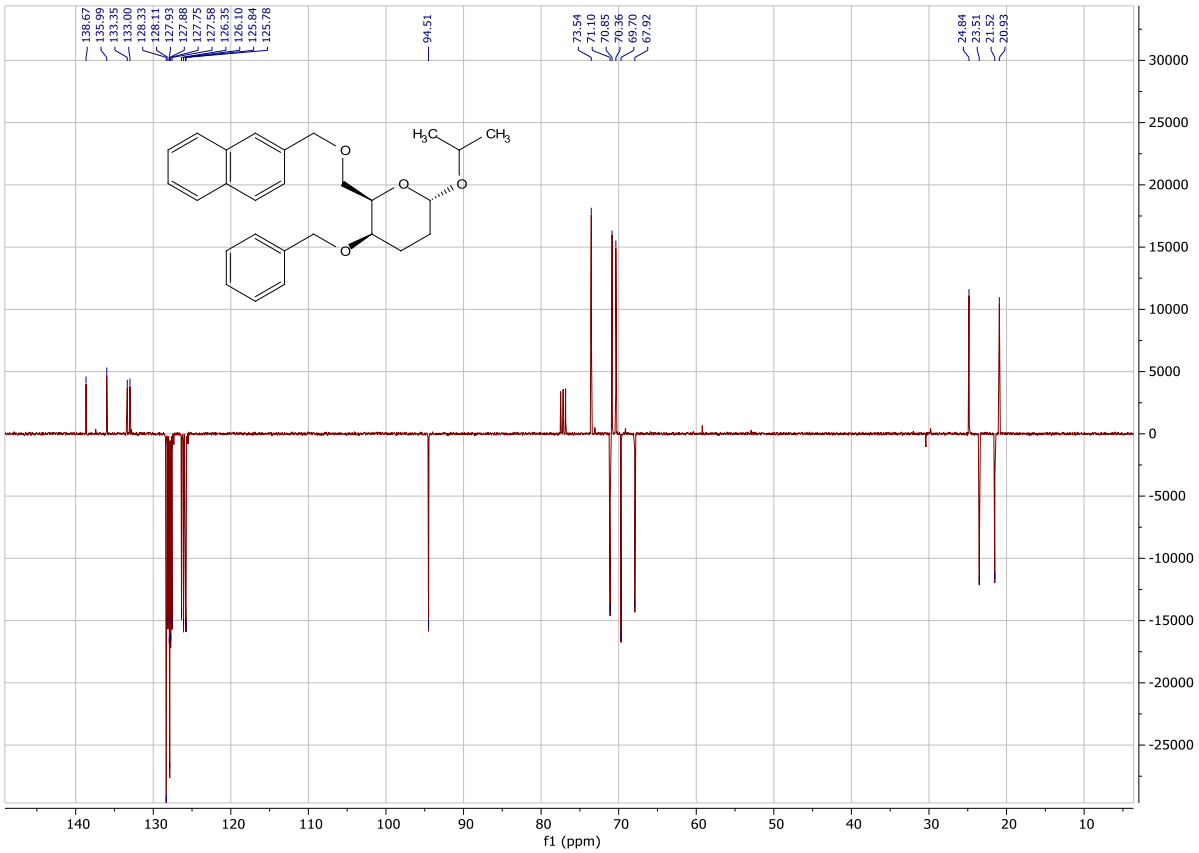
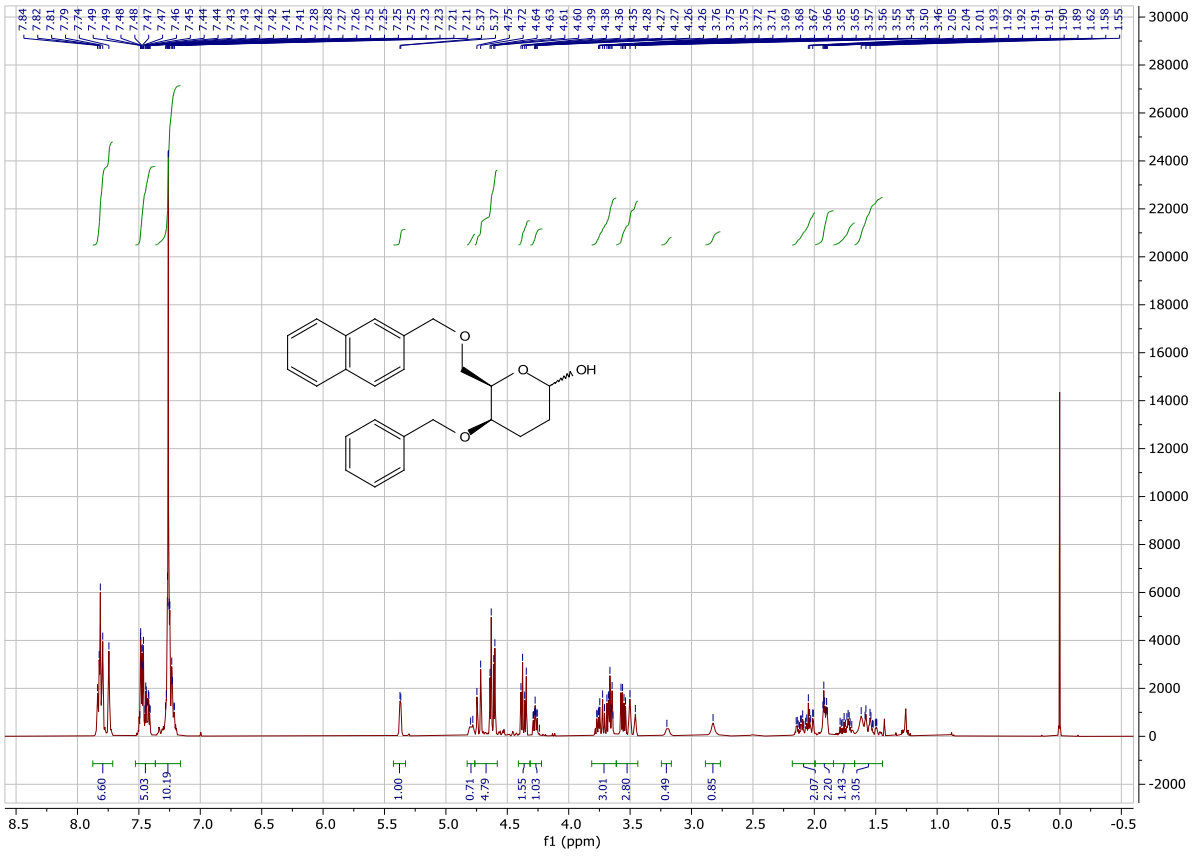
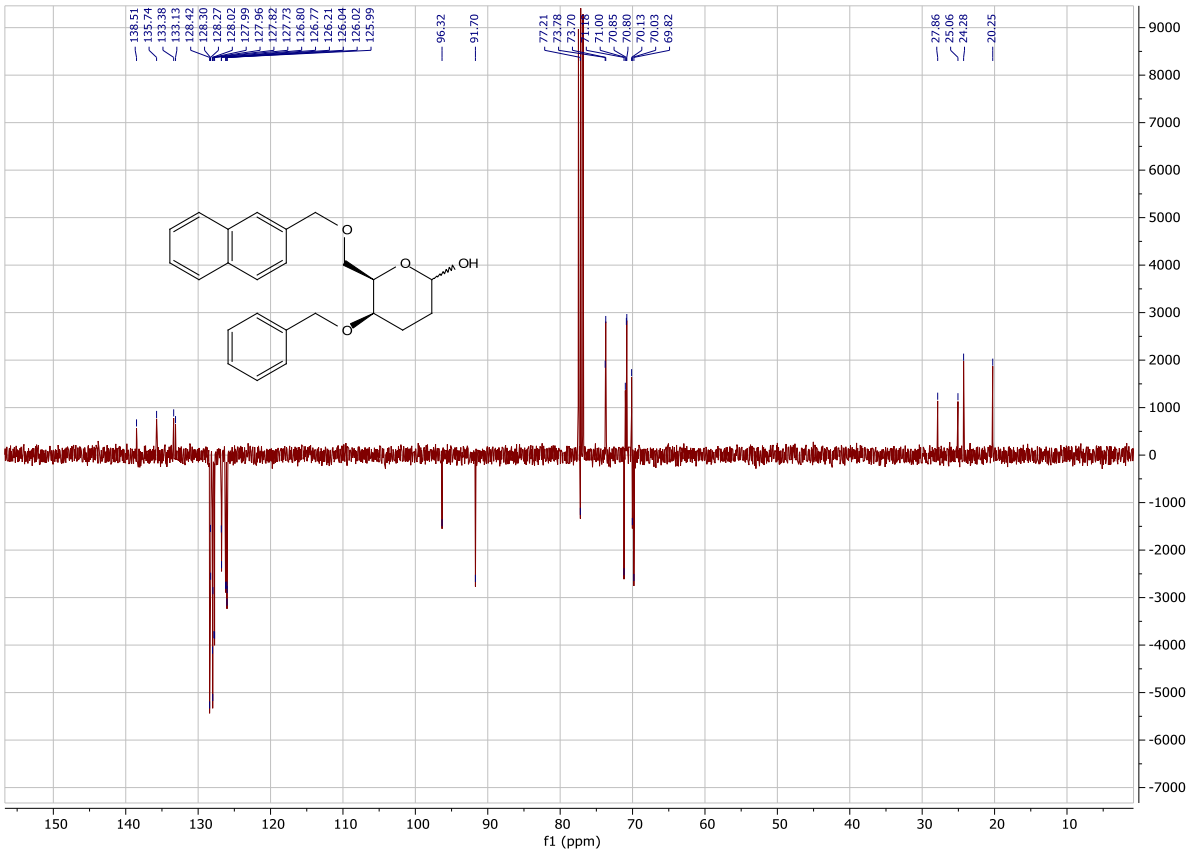
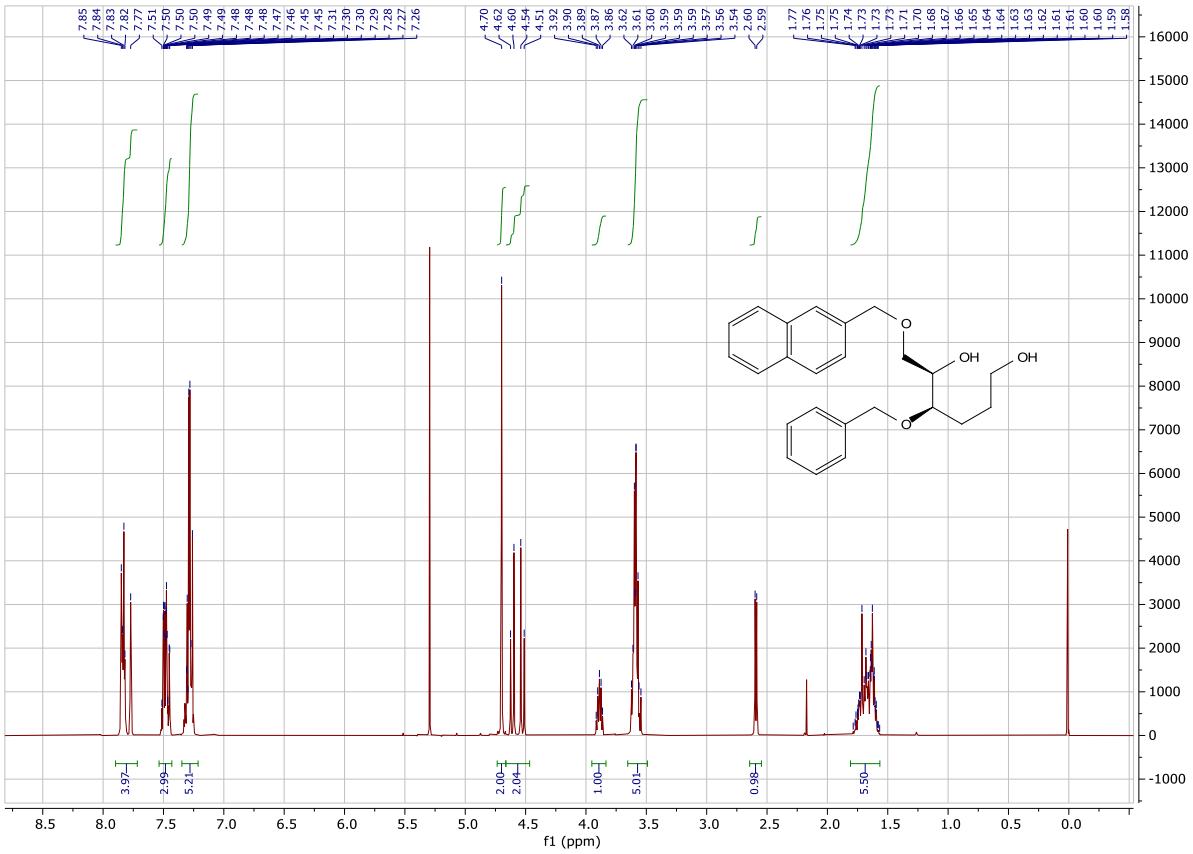
126.51
126.12
125.91
125.57
94.98
73.75
71.67
68.73
68.02
66.05
25.35
24.03
23.37
21.43
H3C
CH3
O
O
O
HO
f1 (ppm)

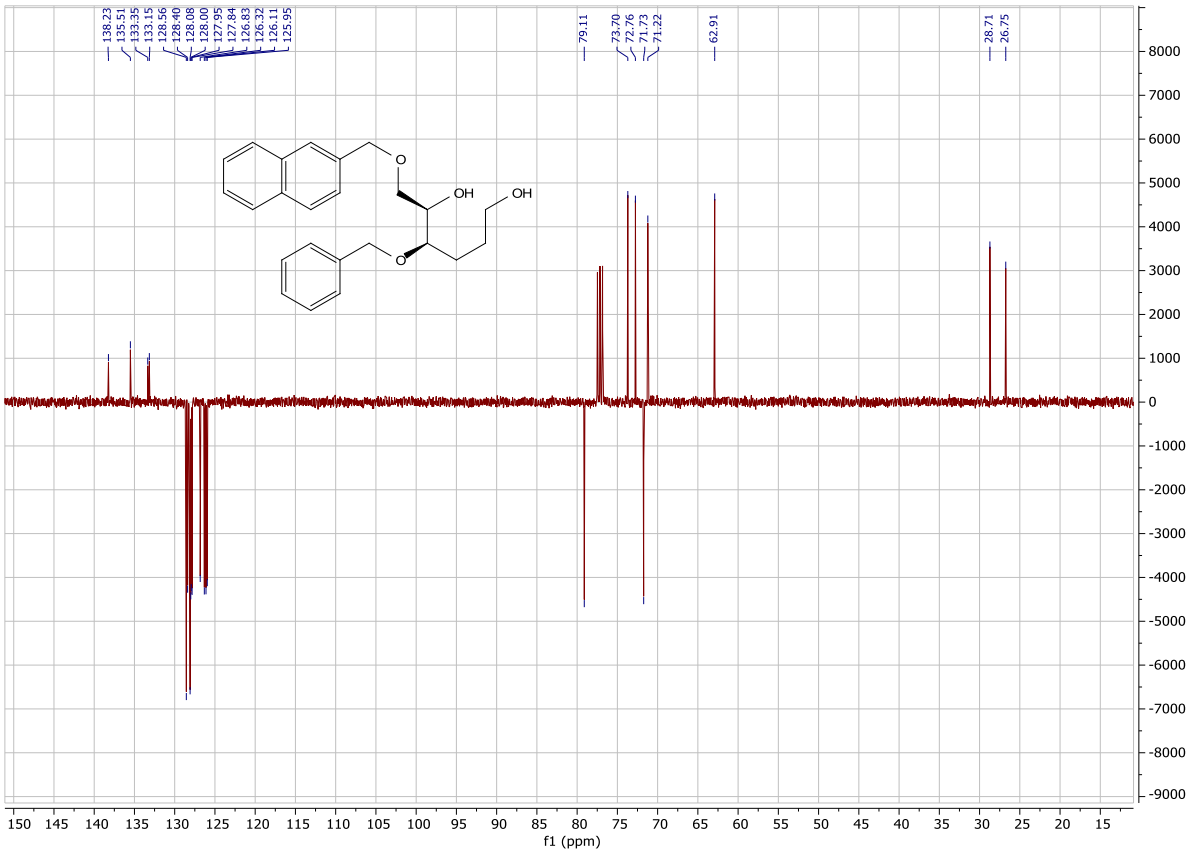

7.82 7.80 7.80 7.78 7.74 7.47 7.47 7.46 7.45 7.44 7.42 7.42 7.40 7.40 7.31 7.30 7.29 7.29 7.28 7.28 7.27 7.26 7.26 7.25 7.24 7.24 7.23 7.23 7.22 7.22 7.21 5.01 5.00 4.73 4.70 4.65 4.64 4.62 4.61 4.40 4.37 4.13 4.13 4.12 4.11 4.10 4.10 4.00 3.99 3.97 3.95 3.94 3.72 3.70 3.69 3.68 3.66 3.65 3.64 3.62 3.57 3.57 2.10 2.09 2.08 2.07 1.92 1.91 1.90 1.90 1.89 1.88 1.88 1.87 1.51 1.48 1.24 1.23 1.15 1.13
H3C CH3
O O O O O
4.17 3.10 5.15 1.00 1.00 1.97 0.98 0.99 0.99 2.06 0.99 1.01 1.99 1.00 3.37 3.06
f1 (ppm)

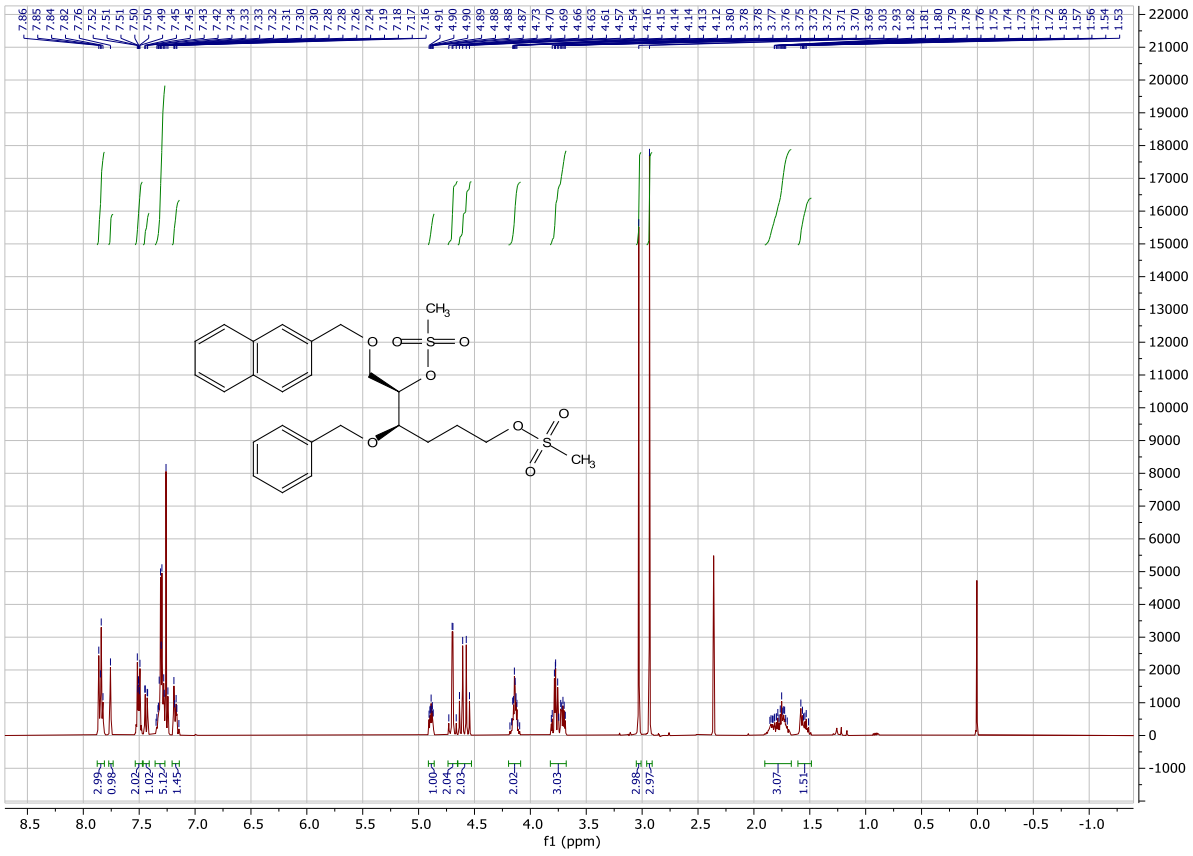

138.67 135.99 133.35 133.00 128.33 128.11 127.93 127.88 127.75 127.58 126.35 126.10 125.84 125.78 94.51 73.54 71.10 70.85 70.36 69.70 67.92 24.84 23.51 21.52 20.93
H3C CH3
O O O O O
f1 (ppm)

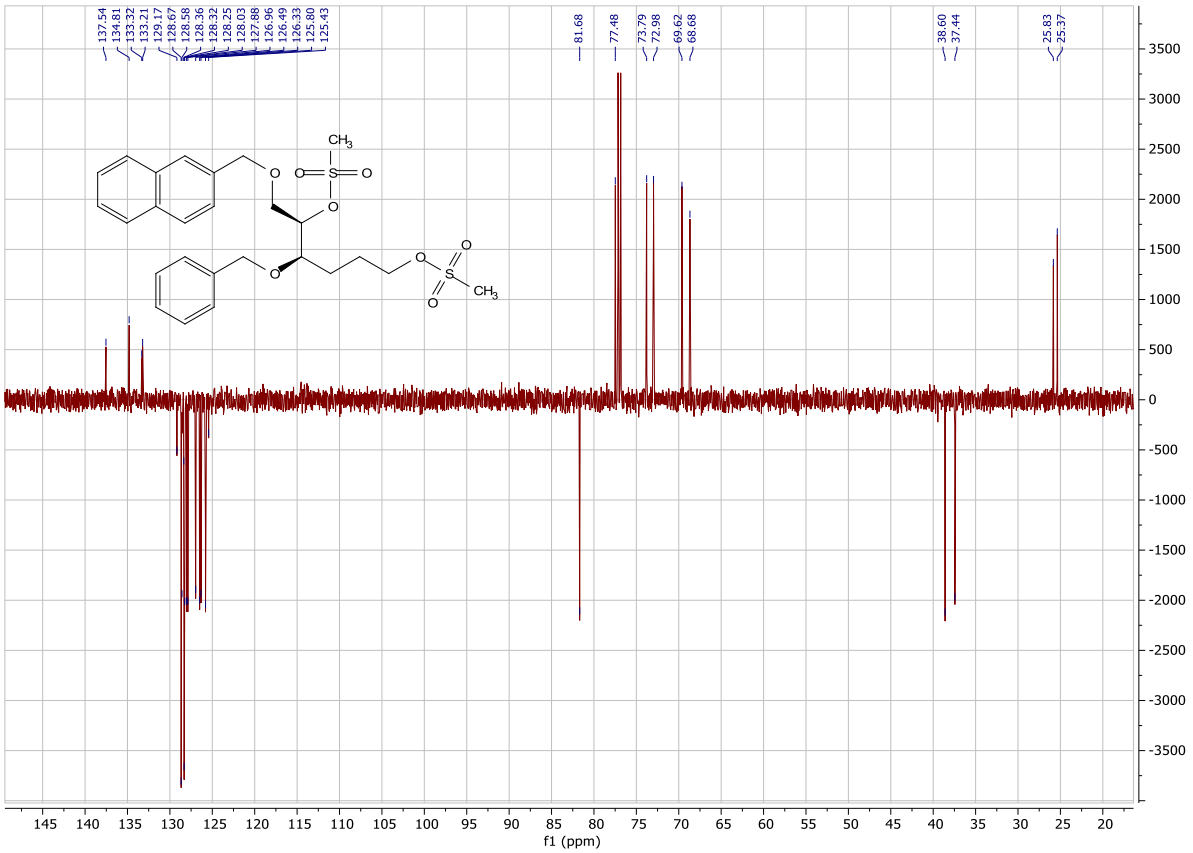

7.84 7.82 7.81 7.79 7.74 7.49 7.49 7.48 7.48 7.47 7.47 7.46 7.45 7.44 7.43 7.42 7.42 7.41 7.41 7.28 7.28 7.27 7.26 7.25 7.25 7.25 7.25 7.23 7.23 7.21 7.21 5.37 5.37 4.75 4.72 4.64 4.63 4.61 4.60 4.39 4.38 4.36 4.35 4.28 4.27 4.27 4.26 4.26 3.76 3.75 3.75 3.72 3.71 3.69 3.68 3.67 3.66 3.65 3.65 3.57 3.56 3.55 3.54 3.50 3.46 2.05 2.04 2.01 1.93 1.92 1.92 1.91 1.91 1.90 1.89 1.62 1.58 1.55
O
O
O
OH
6.60 5.03 10.19 1.00 0.71 4.79 1.55 1.03 3.01 2.80 0.49 0.85 2.07 2.20 1.43 3.05
f1 (ppm)

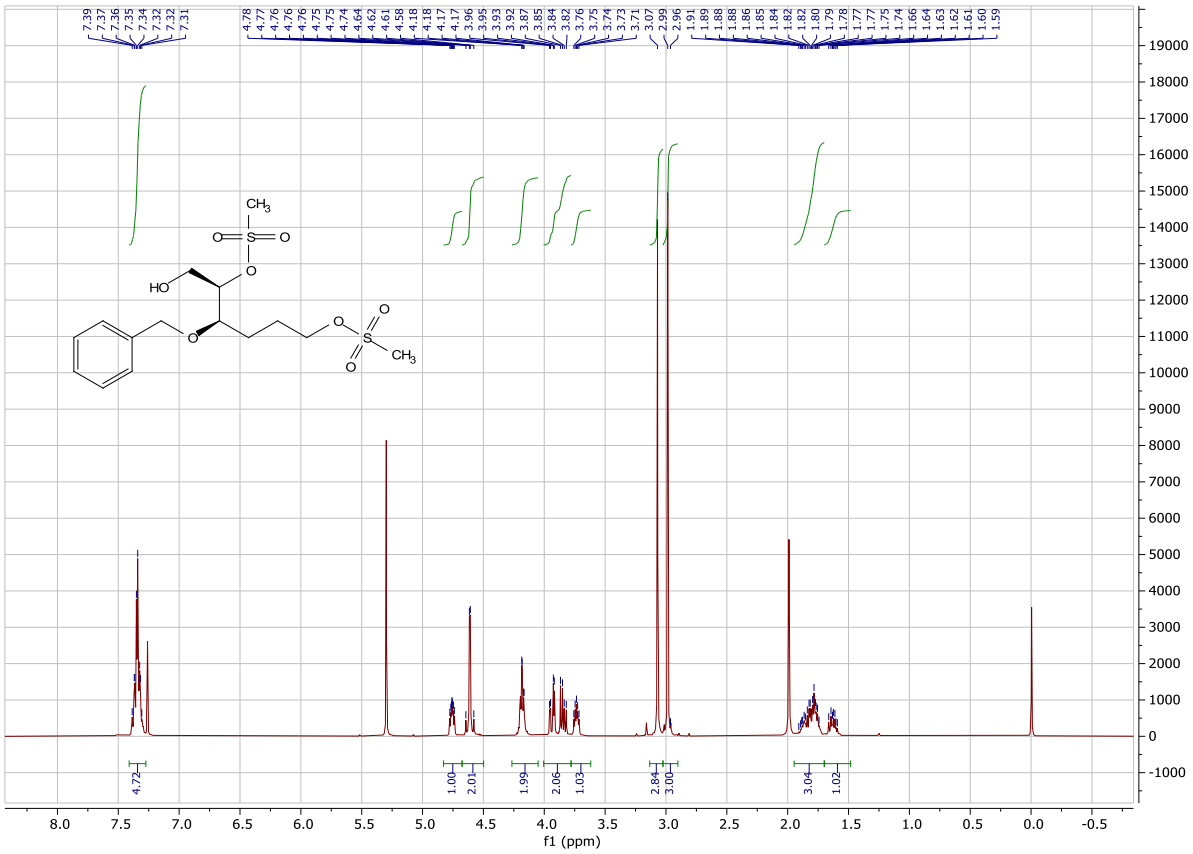

138.51 135.74 133.38 133.13 128.42 128.30 128.27 128.02 127.99 127.96 127.82 127.73 126.80 126.77 126.21 126.04 126.02 125.99
96.32 91.70
77.21 77.00 73.78 73.70 71.88 71.00 70.85 70.69 70.13 70.03 69.82
27.86 25.06 24.28 20.25
O
O
O
OH
f1 (ppm)

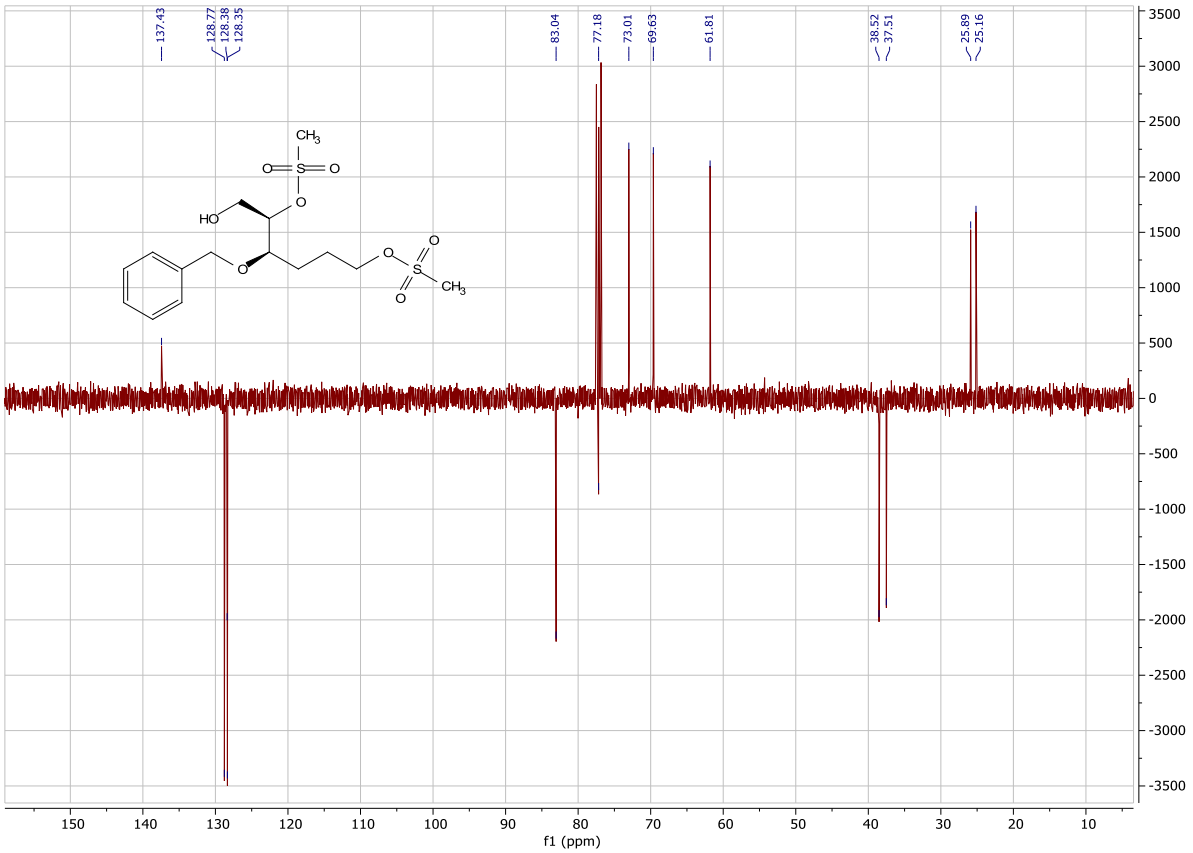

7.85 7.84 7.83 7.82 7.77 7.51 7.50 7.50 7.50 7.49 7.49 7.48 7.48 7.48 7.47 7.46 7.45 7.45 7.31 7.30 7.30 7.29 7.28 7.27 7.26
4.70 4.62 4.60 4.54 4.51 3.92 3.90 3.89 3.87 3.86 3.62 3.61 3.60 3.59 3.59 3.59 3.57 3.56 3.54 2.60 2.59
1.77 1.76 1.75 1.75 1.74 1.73 1.73 1.73 1.71 1.70 1.68 1.67 1.66 1.65 1.64 1.64 1.63 1.63 1.62 1.61 1.61 1.60 1.60 1.59 1.58
O
OH
OH
O
3.97
2.99
5.21
2.00
2.04
1.00
5.01
0.98
5.50
f1 (ppm)

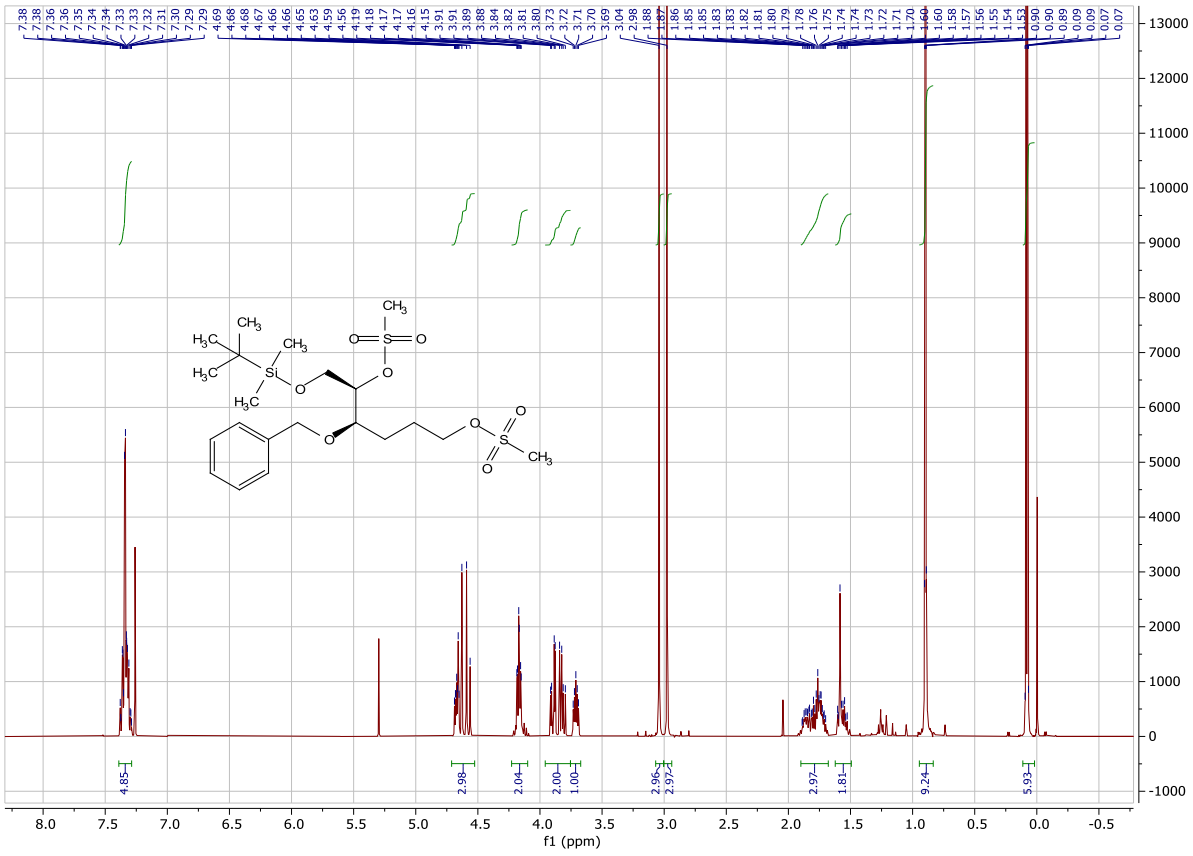

138.23 135.51 133.35 133.15 128.56 128.40 128.08 128.00 127.95 127.84 126.83 126.32 126.11 125.95
79.11 73.70 72.76 71.73 71.22 62.91 28.71 26.75
O
OH
OH
O
f1 (ppm)

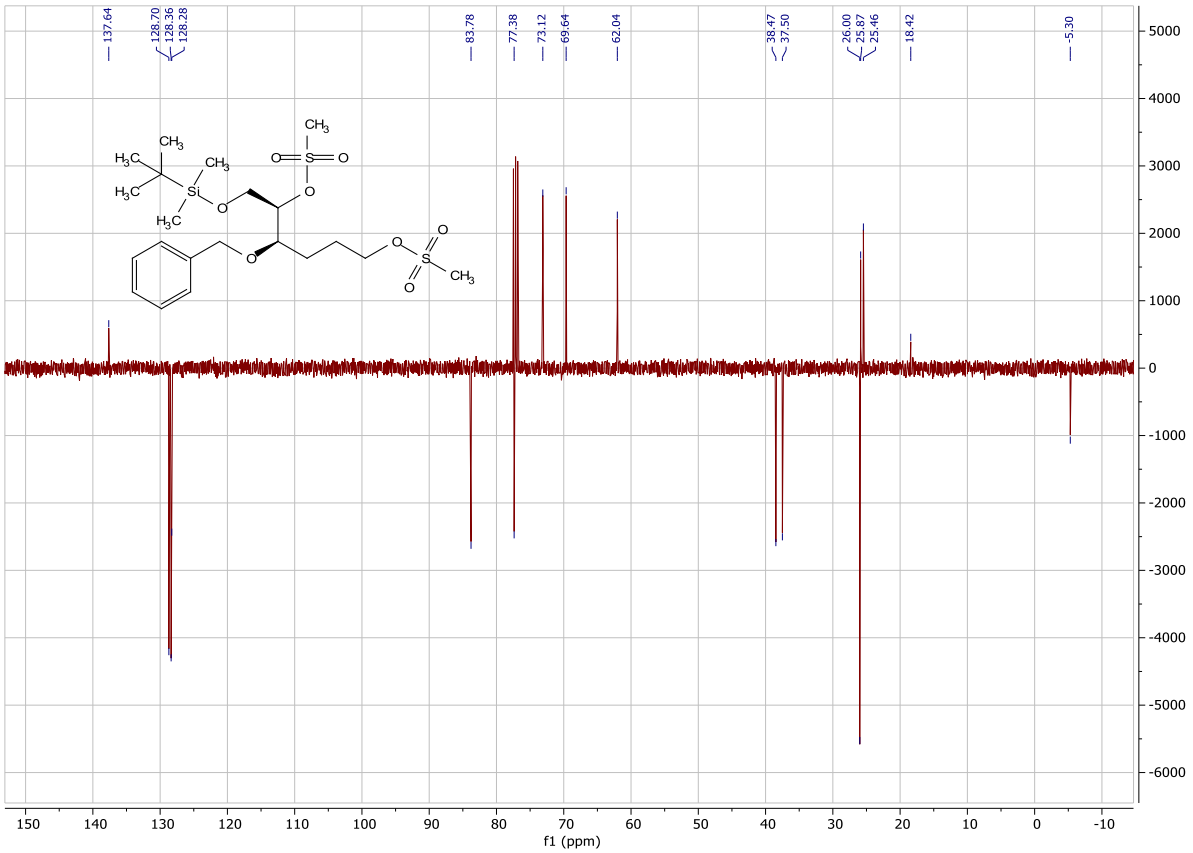

f1 (ppm)

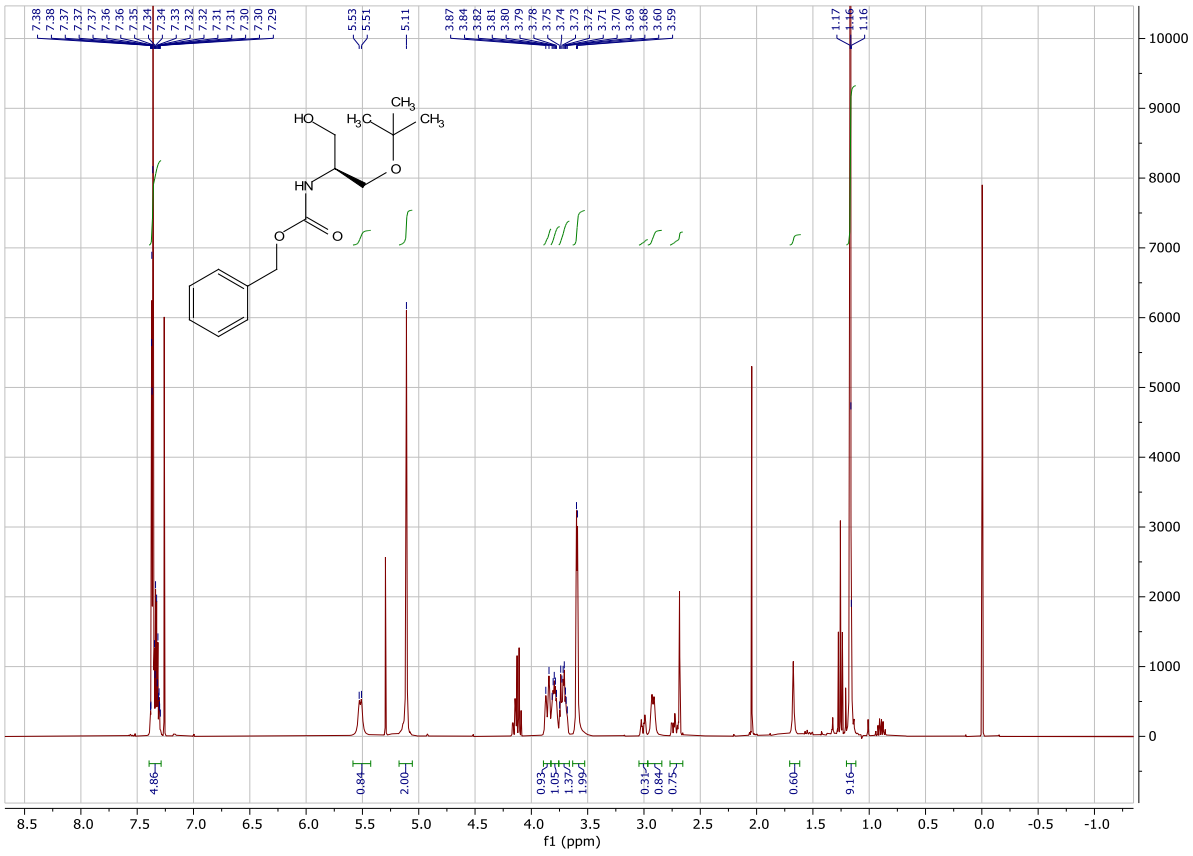

f1 (ppm)

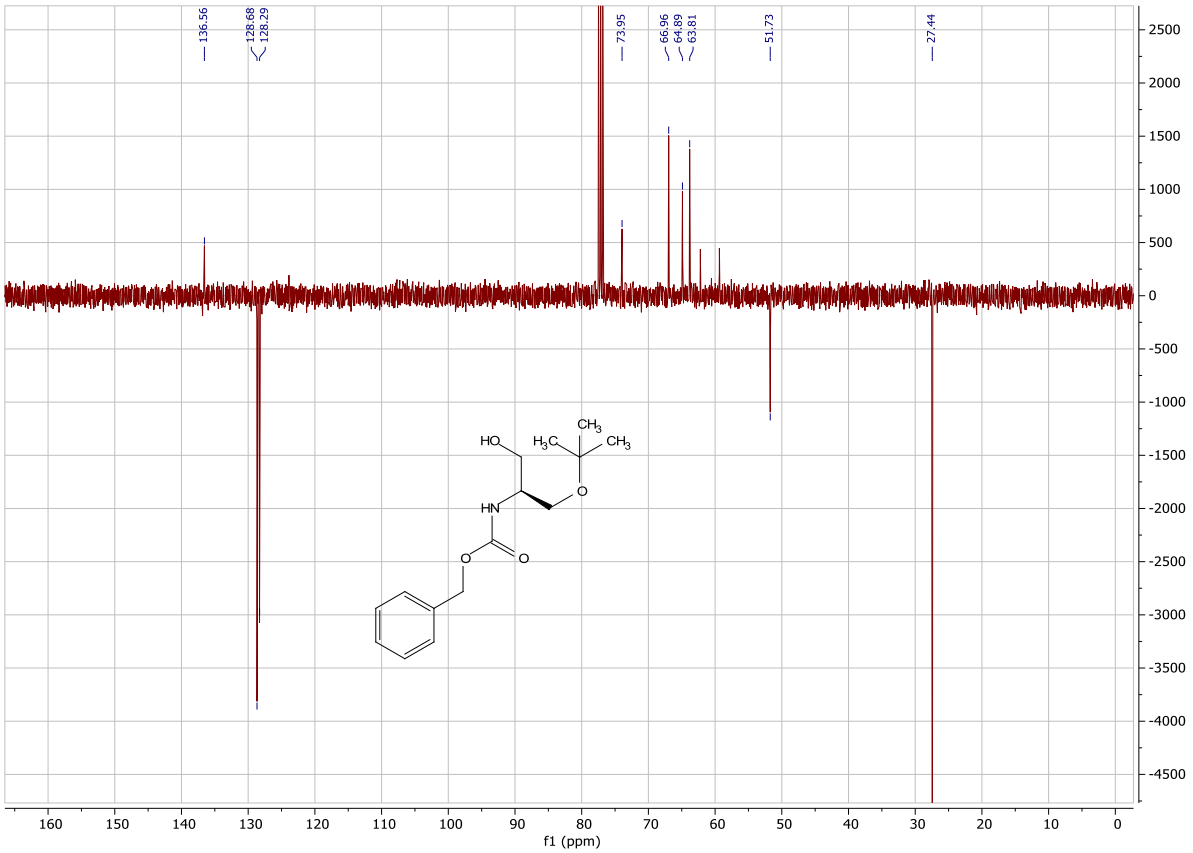

7.39 7.37 7.36 7.35 7.34 7.32 7.32 7.31
4.78 4.77 4.76 4.76 4.76 4.75 4.75 4.74 4.64 4.62 4.61 4.58 4.18 4.18 4.17 4.17 3.96 3.95 3.93 3.92 3.87 3.85 3.84 3.82 3.76 3.75 3.74 3.73 3.71 3.07 2.99 2.96 1.91 1.89 1.88 1.88 1.86 1.85 1.84 1.82 1.82 1.80 1.79 1.78 1.77 1.77 1.75 1.74 1.66 1.64 1.63 1.62 1.61 1.60 1.59
CH3
O=S=O
O
HO
O
O
O
O=S=O
CH3
4.72
1.00
2.01
1.99
2.06
1.03
2.84
3.00
3.04
1.02
f1 (ppm)

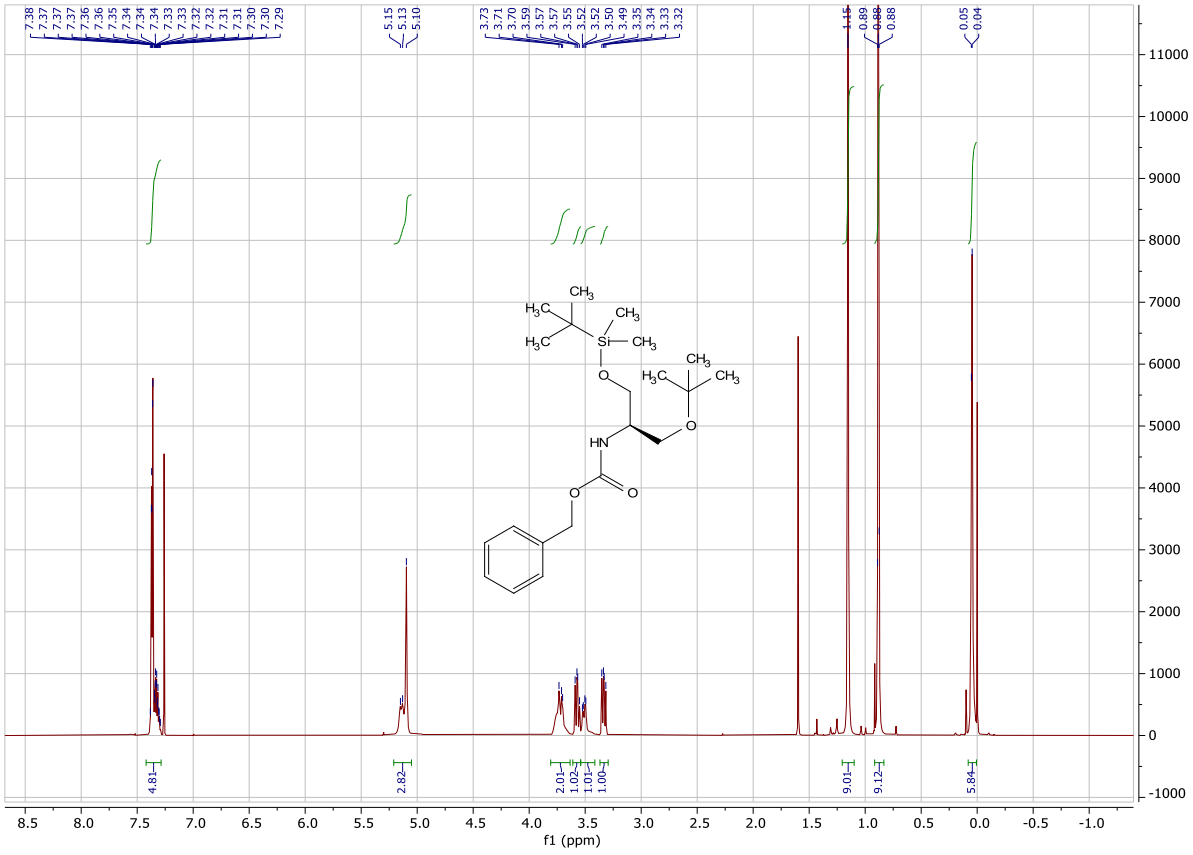

137.43
128.77
128.38
128.35
83.04
77.18
73.01
69.63
61.81
38.52
37.51
25.89
25.16
CH3
O=S=O
O
HO
O
O
O
O=S=O
CH3
f1 (ppm)

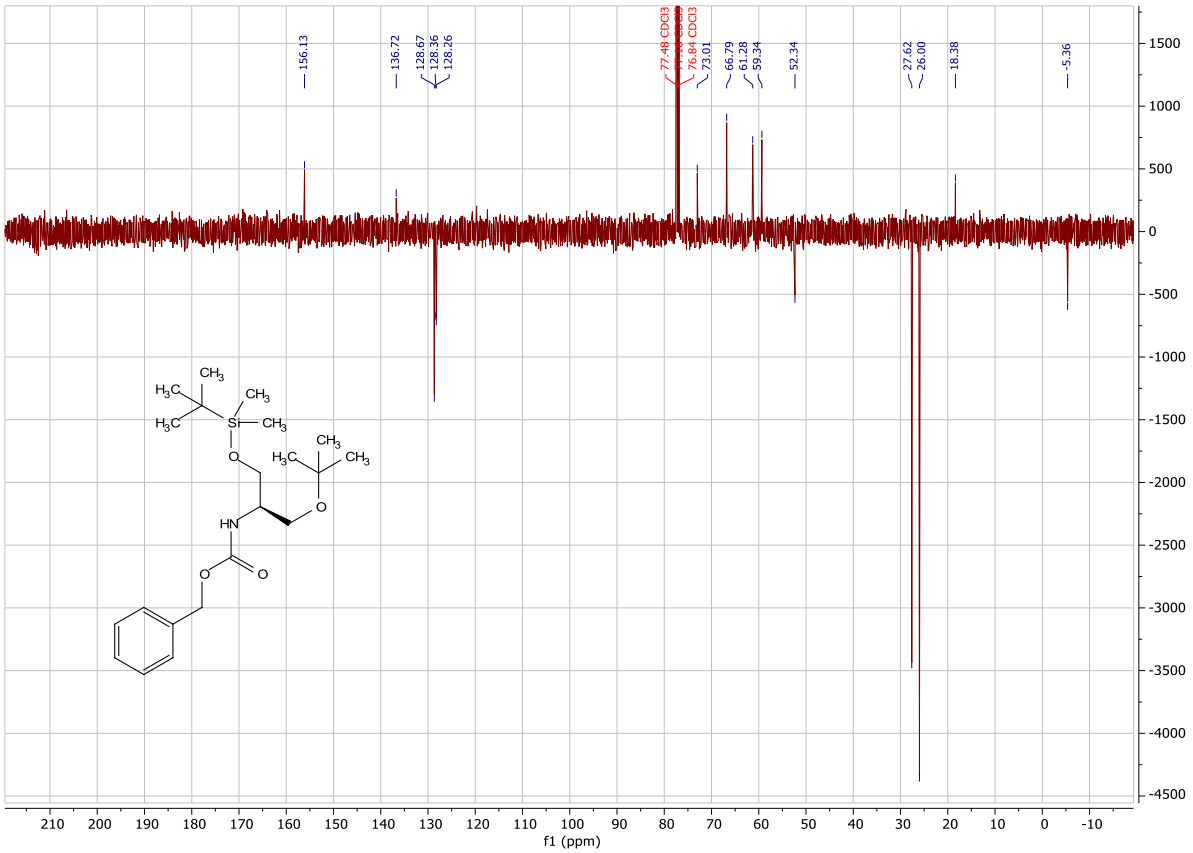

f1 (ppm)

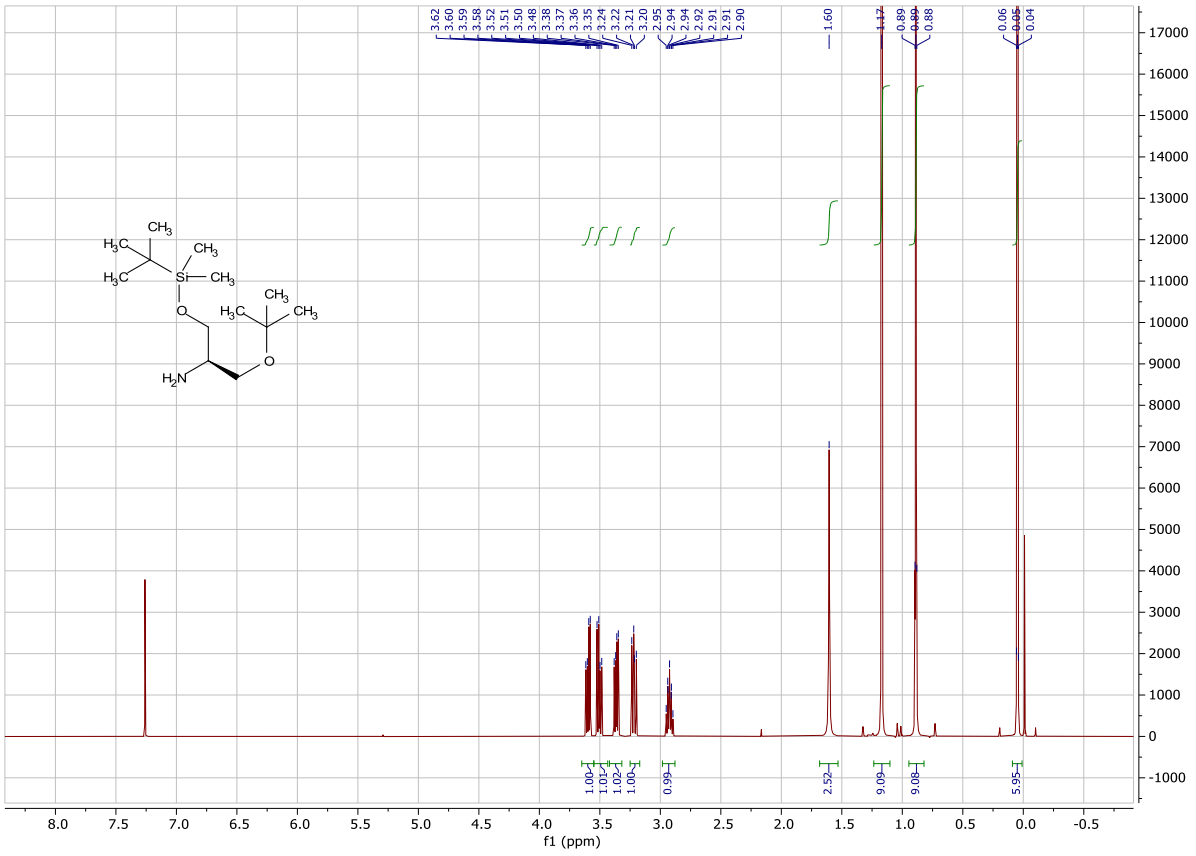

f1 (ppm)

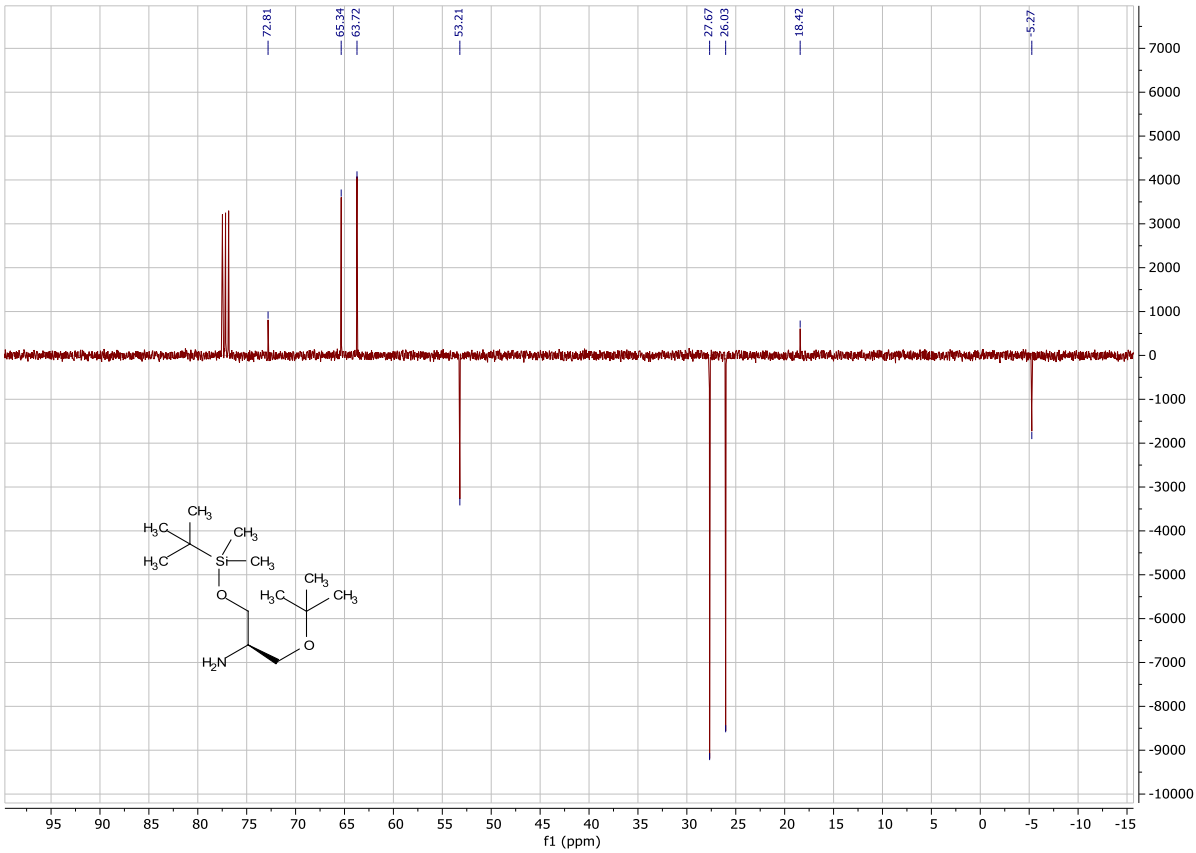

7.38 7.38 7.37 7.37 7.37 7.36 7.36 7.35 7.34 7.34 7.33 7.32 7.32 7.31 7.31 7.30 7.30 7.29
5.53 5.51
5.11
3.87 3.84 3.82 3.81 3.80 3.79 3.78 3.75 3.74 3.73 3.72 3.71 3.70 3.69 3.68 3.60 3.59
1.17 1.16 1.16
HO
H3C
CH3
CH3
O
HN
O
O
4.86
0.84
2.00
0.93
1.05
1.37
1.99
0.31
0.84
0.75
0.60
9.16
f1 (ppm)

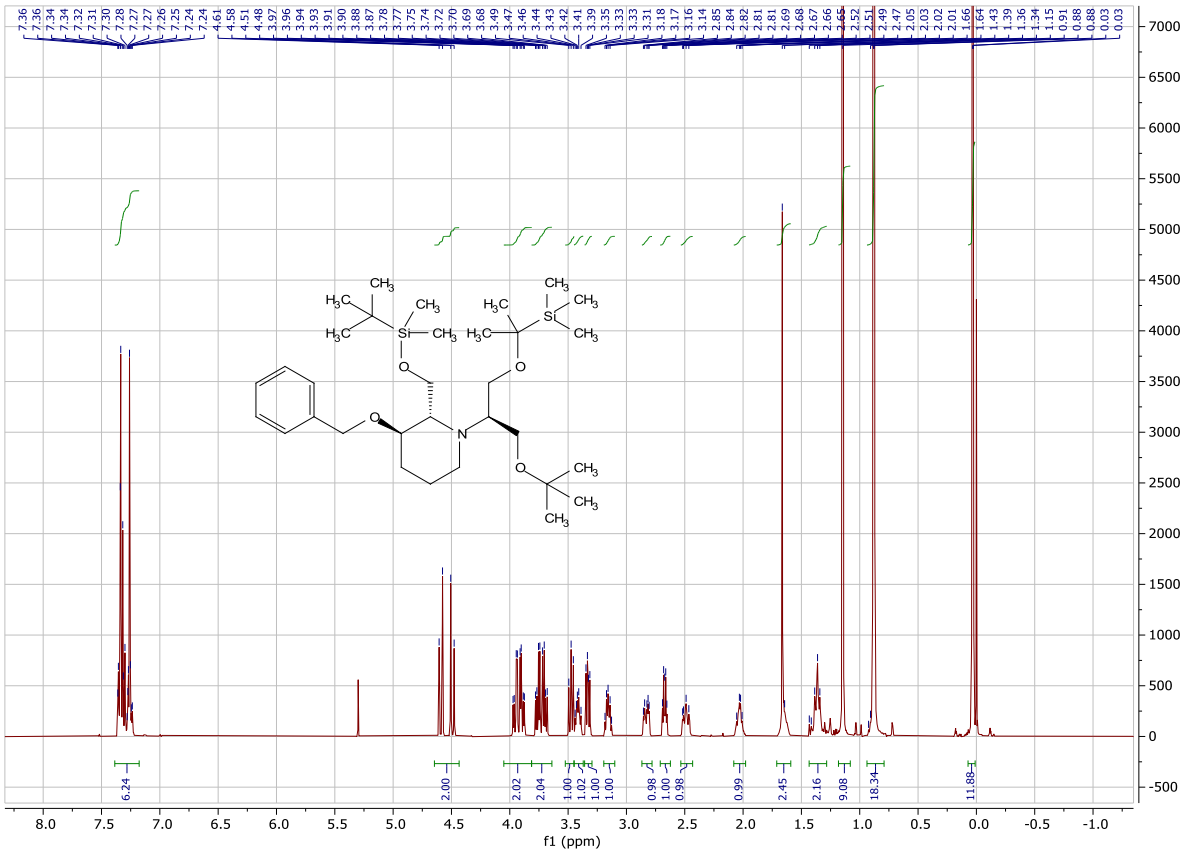

136.56
128.68
128.29
73.95
66.96
64.89
63.81
51.73
27.44
HO
H3C
CH3
CH3
O
HN
O
O
f1 (ppm)

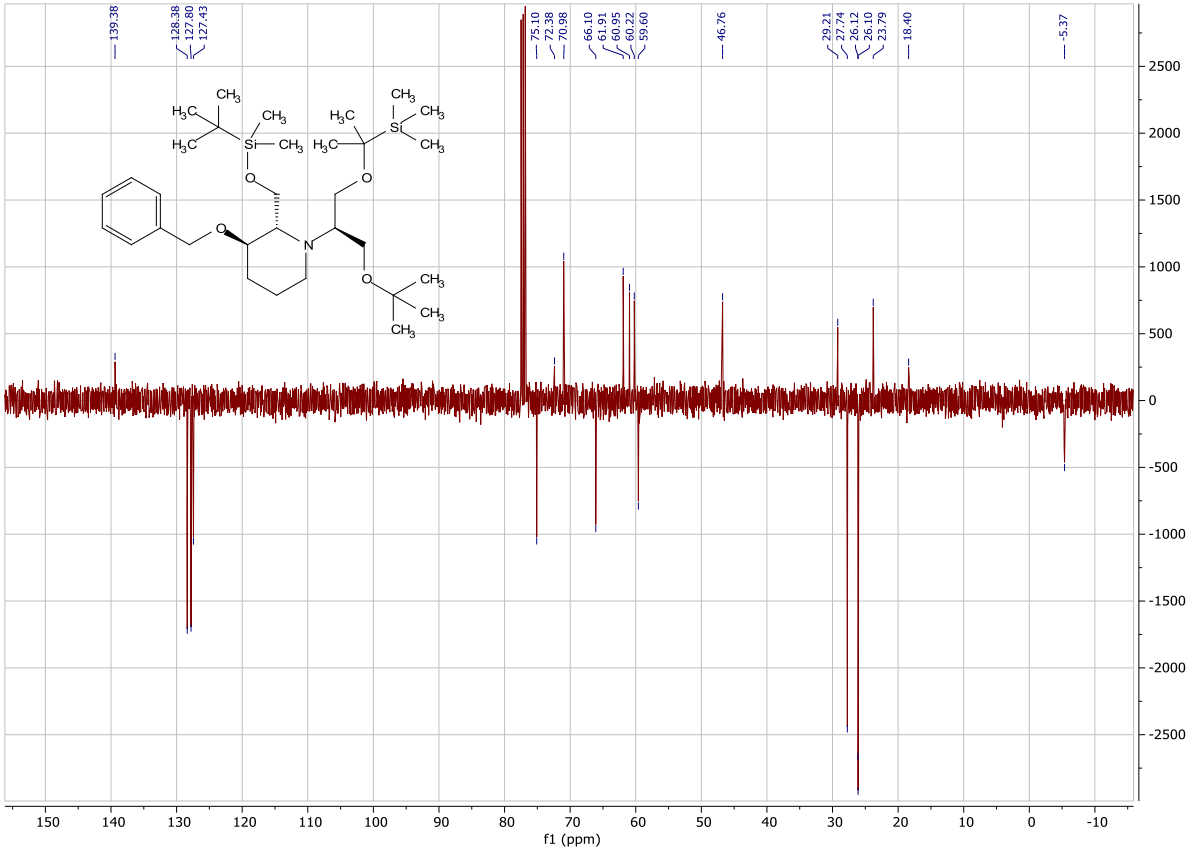

7.38 7.37 7.37 7.37 7.36 7.36 7.35 7.34 7.34 7.34 7.33 7.33 7.32 7.32 7.31 7.31 7.30 7.30 7.29
5.15 5.13 5.10
3.73 3.71 3.70 3.59 3.57 3.57 3.55 3.52 3.52 3.50 3.49 3.35 3.34 3.33 3.32
1.15 0.89 0.88 0.88
0.05 0.04
4.81 2.82 2.01 1.02 1.01 1.00 9.01 9.12 5.84
f1 (ppm)

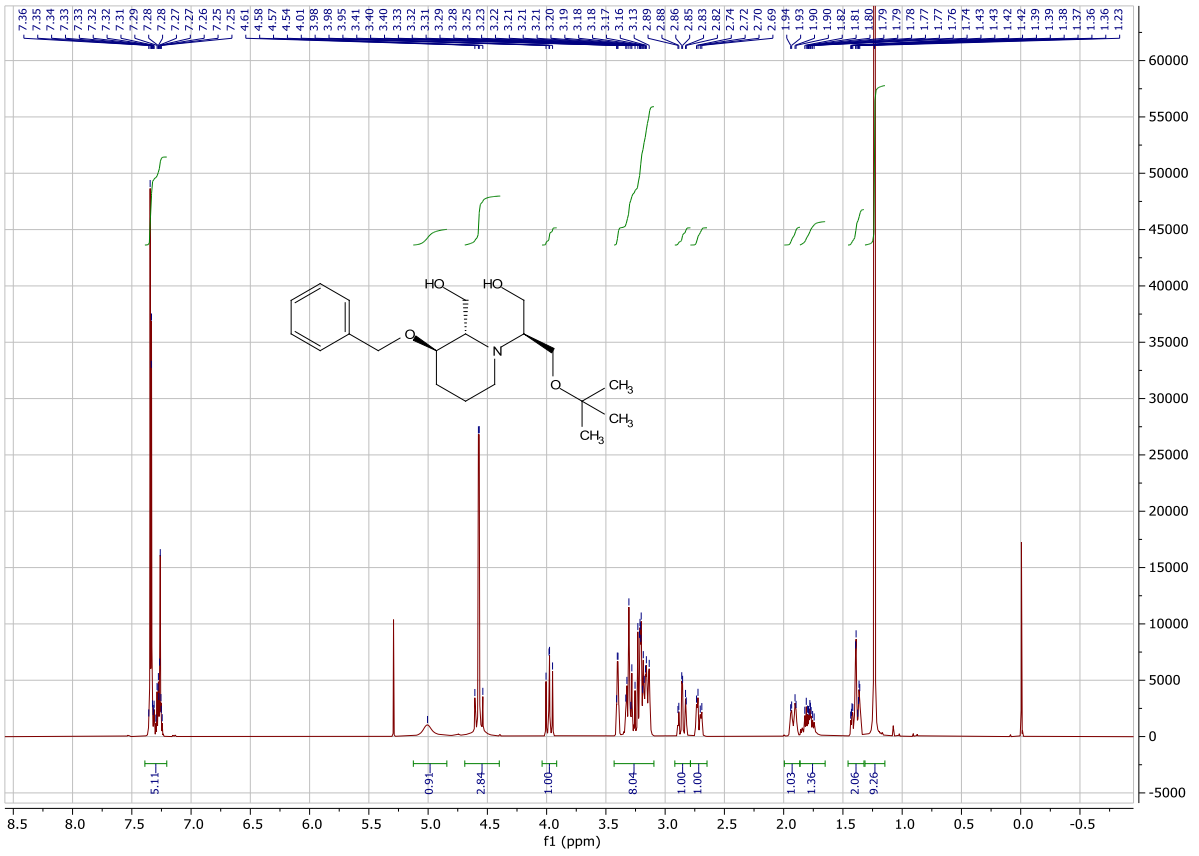

156.13 136.72 128.67 128.36 128.26
77.48 CDCl3 77.16 CDCl3 76.84 CDCl3 73.01 66.79 61.28 59.34 52.34 27.62 26.00 18.38 -5.36
f1 (ppm)

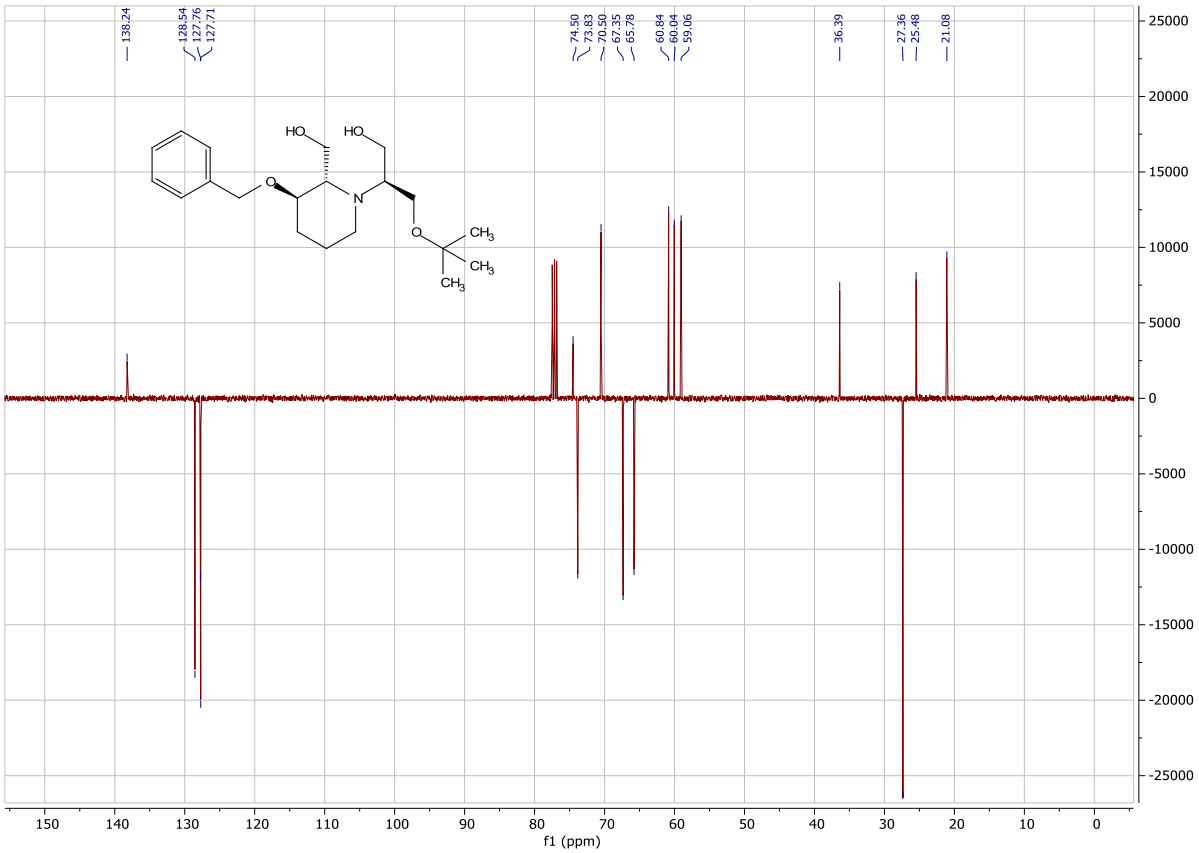

3.62
3.60
3.59
3.58
3.52
3.51
3.50
3.48
3.38
3.37
3.36
3.35
3.24
3.22
3.21
3.20
2.95
2.94
2.94
2.92
2.91
2.91
2.90
1.60
1.17
0.89
0.89
0.88
0.06
0.05
0.04
1.00
1.01
1.02
1.00
0.99
2.52
9.09
9.08
5.95
f1 (ppm)

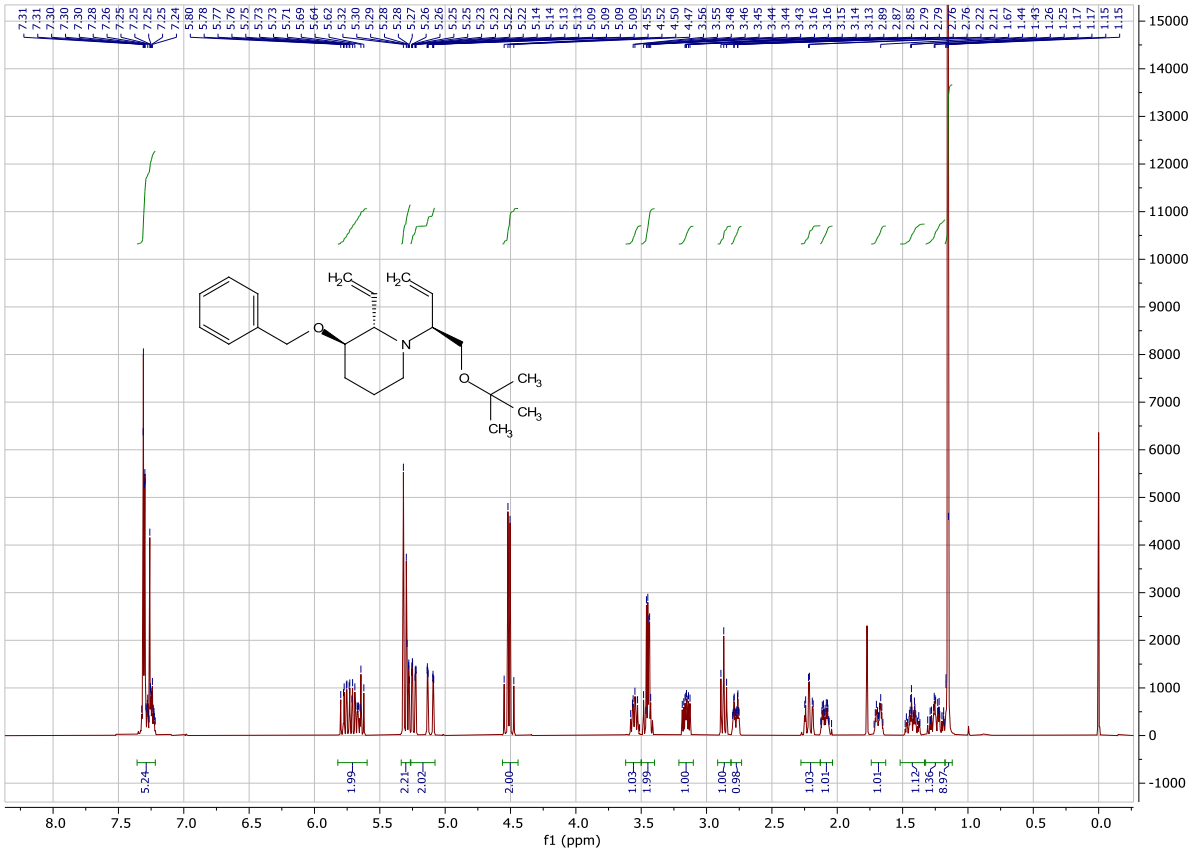

72.81
65.34
63.72
53.21
27.67
26.03
18.42
-5.27
f1 (ppm)


f1 (ppm)

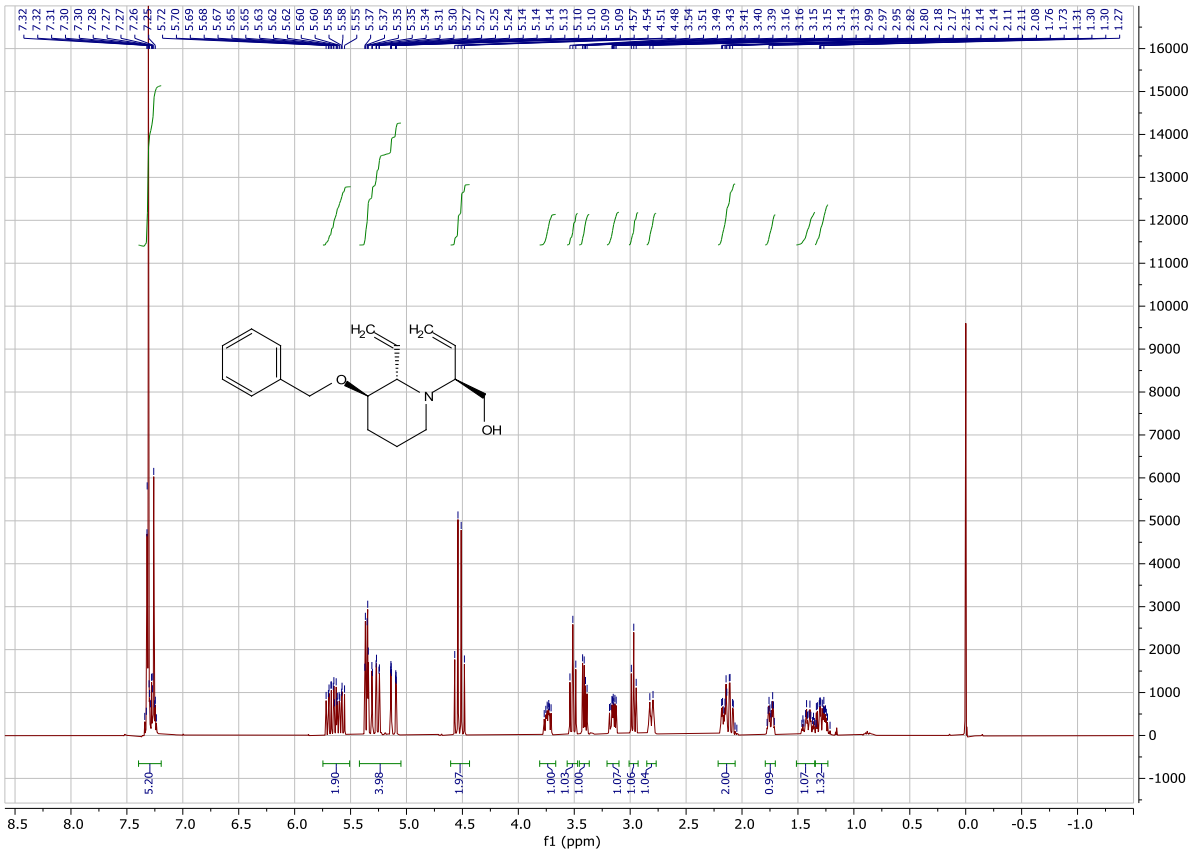

f1 (ppm)

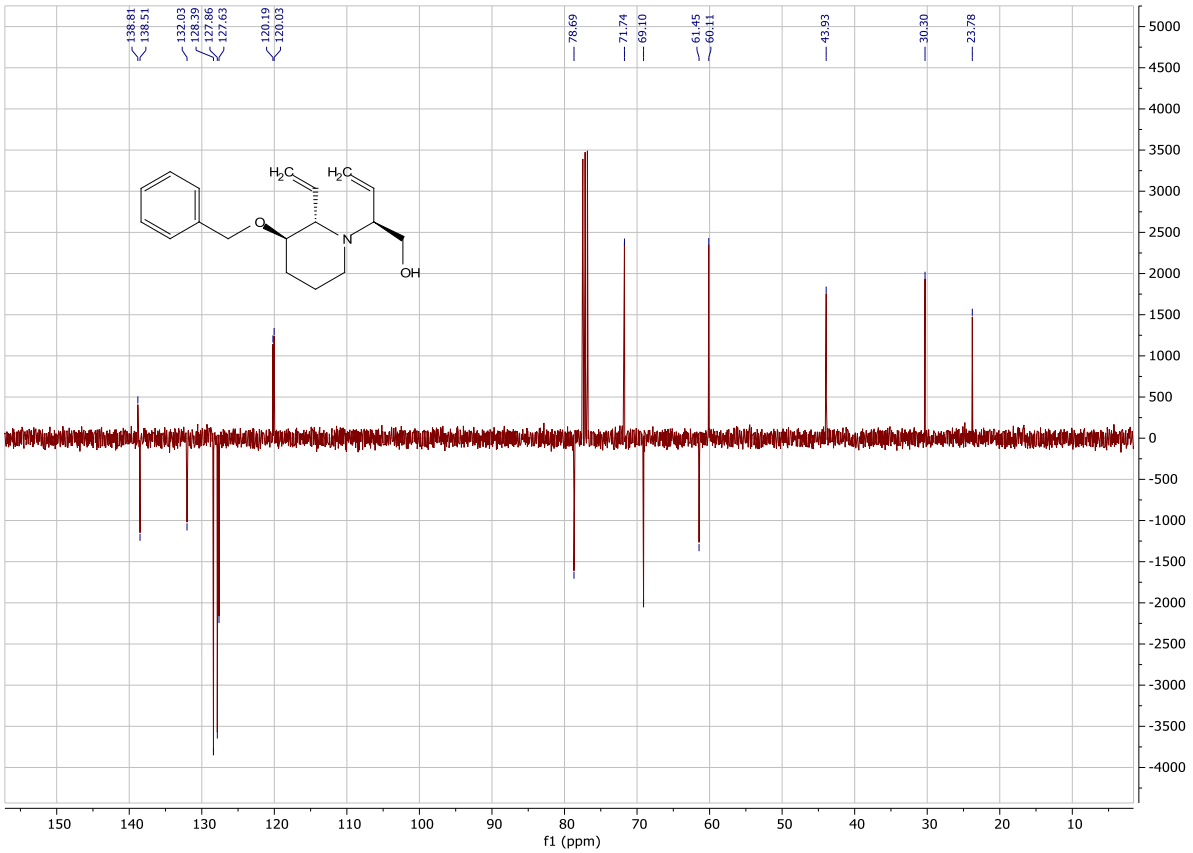

HO
HO
O
N
O
CH3
CH3
CH3
f1 (ppm)

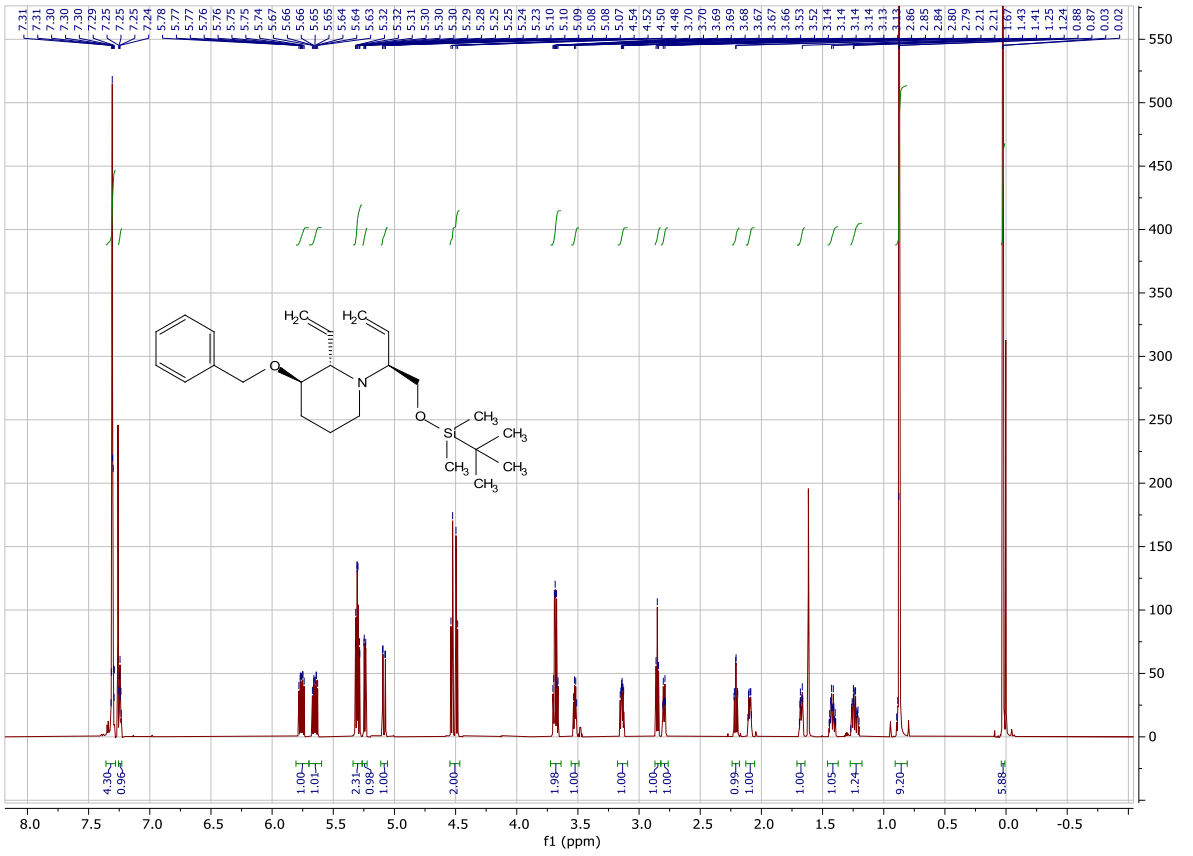

HO
HO
O
N
O
CH3
CH3
CH3
f1 (ppm)

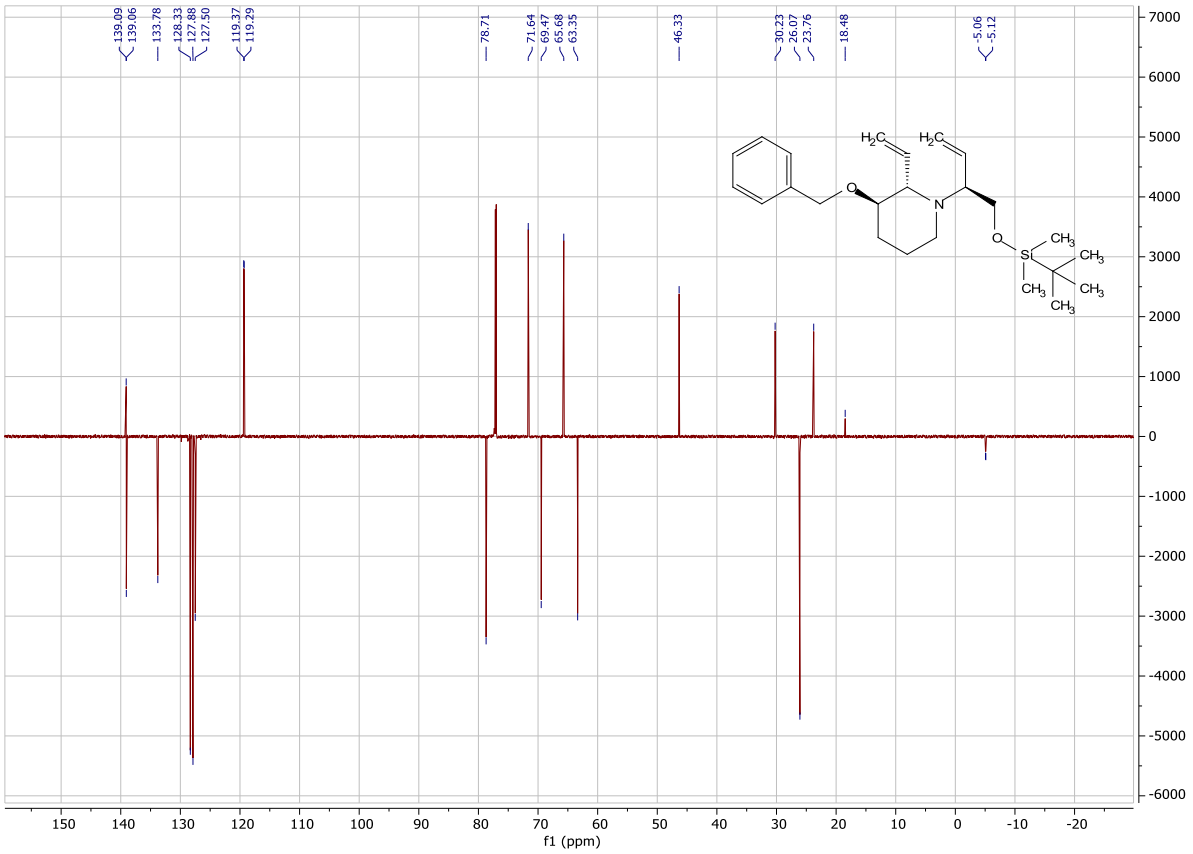

f1 (ppm)

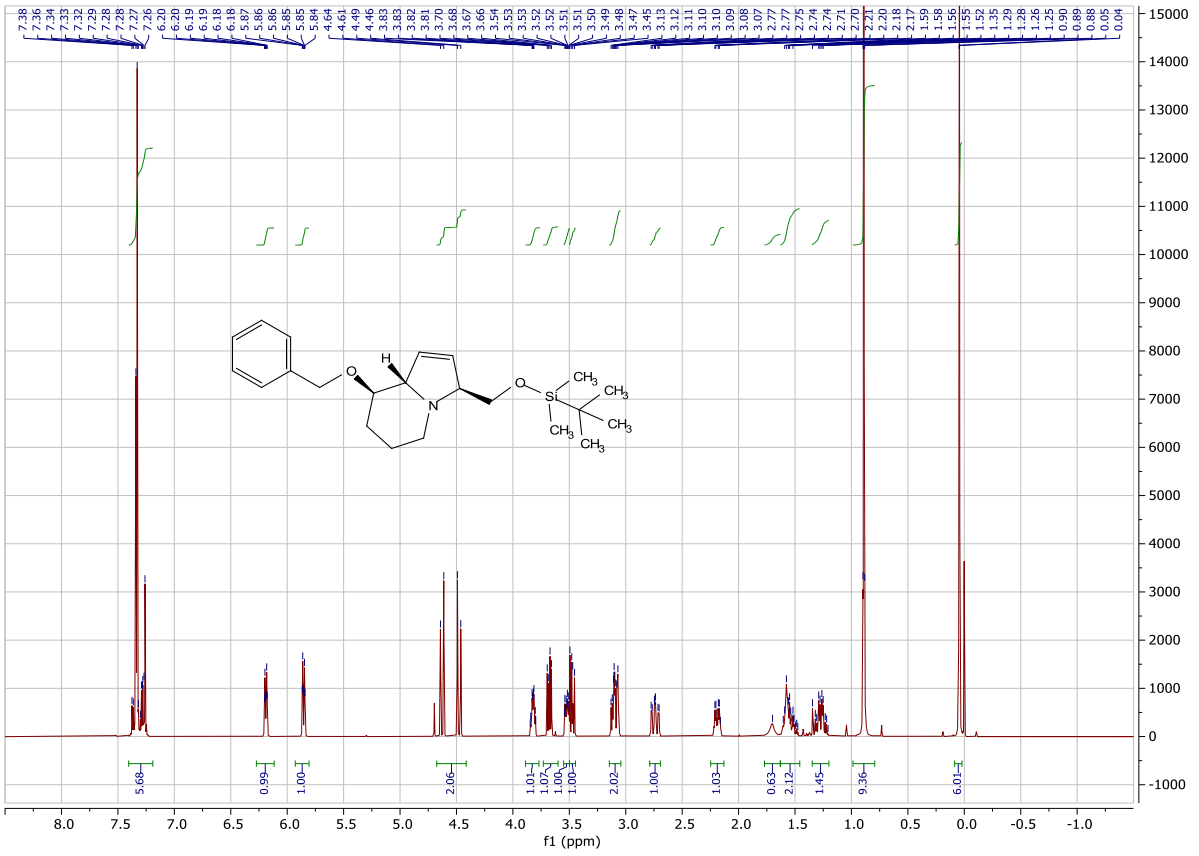

f1 (ppm)

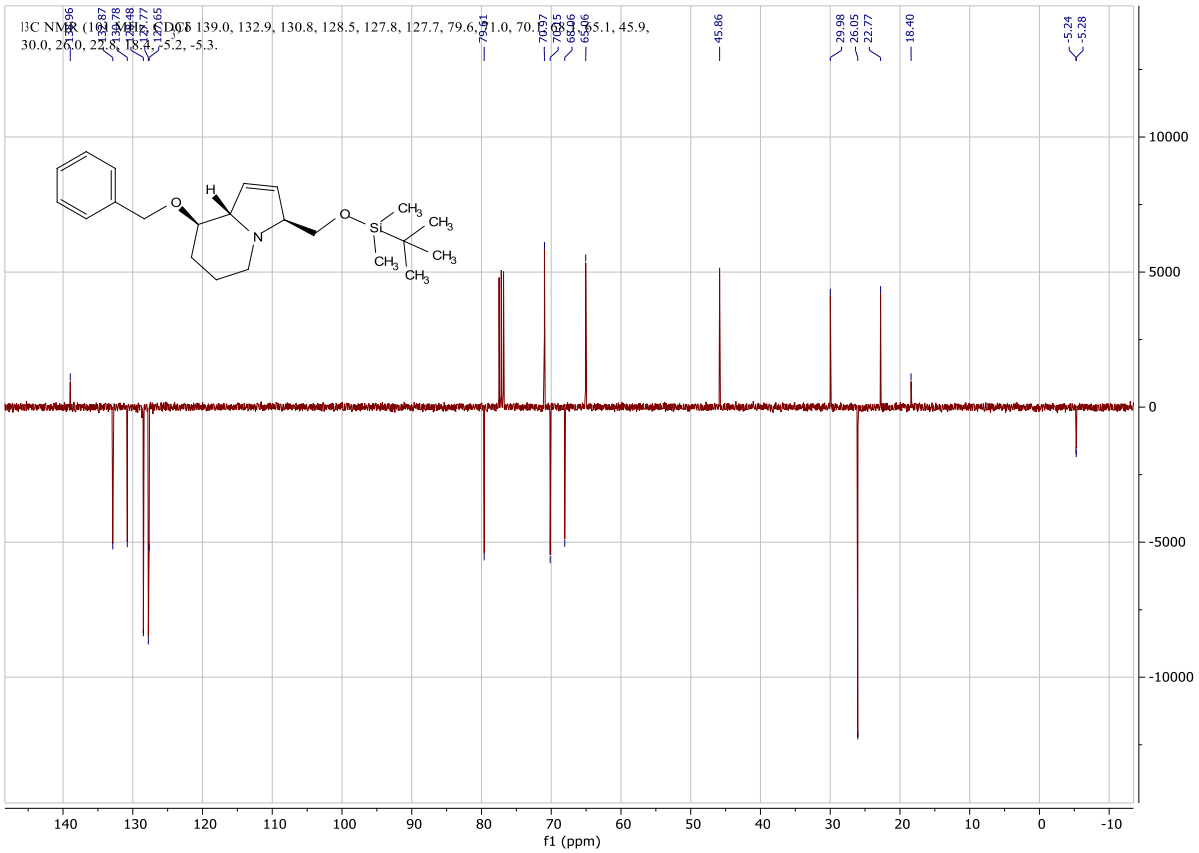

7.32 7.32 7.31 7.30 7.30 7.28 7.27 7.27 7.26 7.25 5.72 5.70 5.69 5.68 5.67 5.65 5.65 5.63 5.62 5.62 5.60 5.60 5.58 5.58 5.55 5.37 5.37 5.35 5.35 5.34 5.31 5.30 5.27 5.27 5.25 5.24 5.14 5.14 5.14 5.13 5.10 5.10 5.09 5.09 4.57 4.54 4.51 4.48 3.54 3.51 3.49 3.43 3.41 3.40 3.39 3.16 3.15 3.15 3.14 3.13 2.99 2.97 2.95 2.82 2.80 2.18 2.17 2.15 2.14 2.14 2.11 2.11 2.08 1.76 1.73 1.31 1.30 1.30 1.27
H2C
H2C
O
N
OH
5.20 1.90 3.98 1.97 1.00 1.03 1.00 1.07 1.06 1.04 2.00 0.99 1.07 1.32
f1 (ppm)

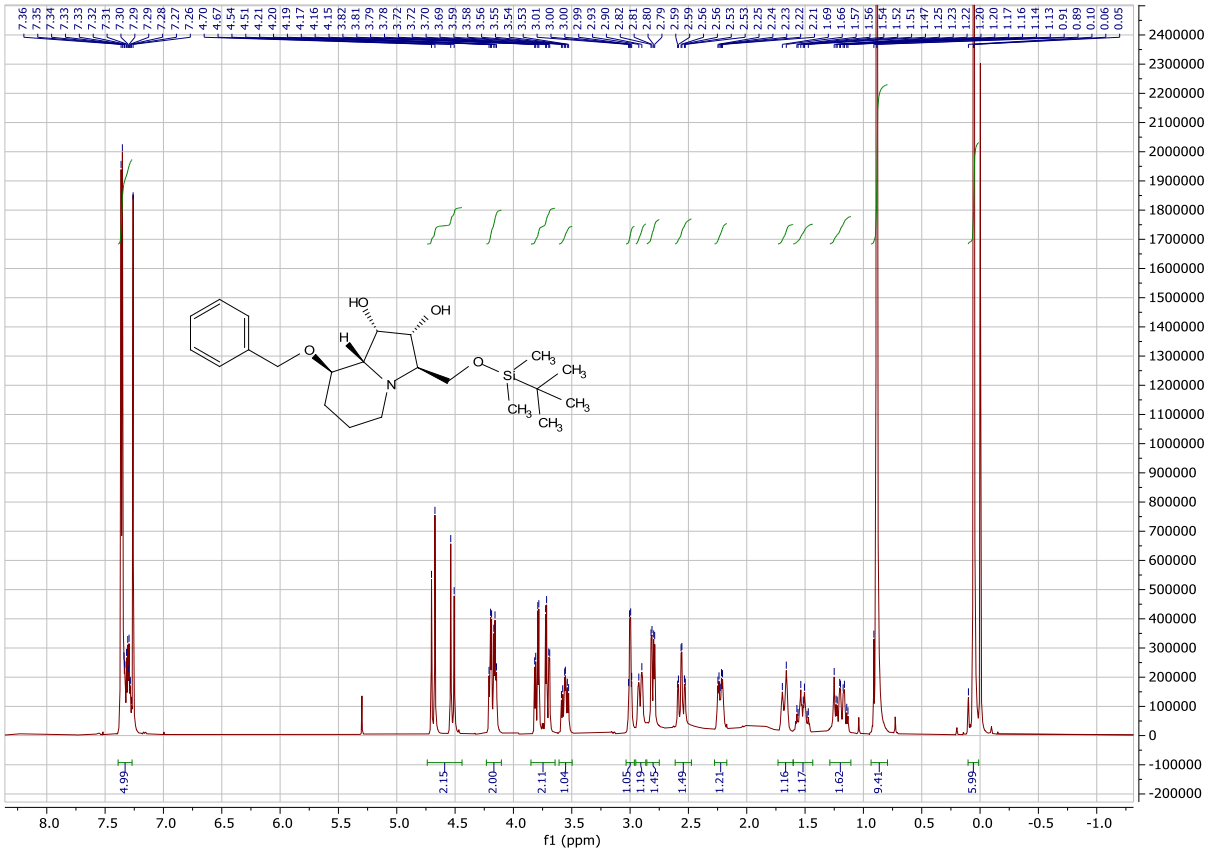

138.81 138.51 132.03 128.39 127.86 127.63 120.19 120.03 78.69 71.74 69.10 61.45 60.11 43.93 30.30 23.78
H2C
H2C
O
N
OH
f1 (ppm)

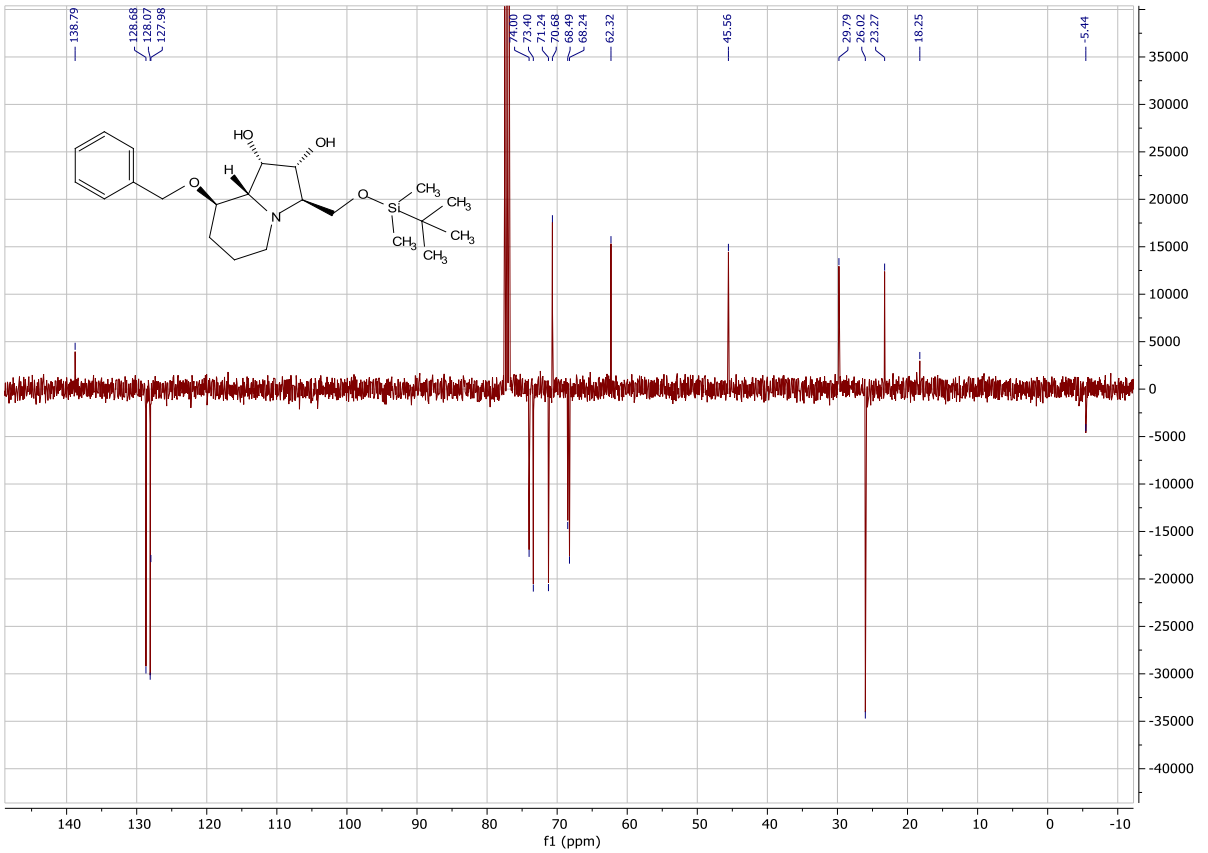

f1 (ppm)


f1 (ppm)


f1 (ppm)


13C NMR (101 MHz, CDCl3 139.0, 132.9, 130.8, 128.5, 127.8, 127.7, 79.6, 71.0, 70.7, 65.1, 45.9, 30.0, 26.0, 22.8, 18.4, -5.2, -5.3.
f1 (ppm)


7.36 7.35 7.34 7.33 7.33 7.32 7.31 7.30 7.29 7.29 7.29 7.28 7.27 7.26 4.70 4.67 4.54 4.51 4.21 4.20 4.19 4.17 4.16 4.15 3.82 3.81 3.79 3.78 3.72 3.72 3.70 3.69 3.59 3.58 3.56 3.55 3.54 3.53 3.01 3.00 3.00 2.99 2.93 2.90 2.82 2.81 2.80 2.79 2.59 2.59 2.56 2.56 2.53 2.53 2.25 2.24 2.23 2.22 2.21 1.69 1.66 1.57 1.56 1.54 1.52 1.51 1.47 1.25 1.23 1.22 1.20 1.20 1.17 1.16 1.14 1.13 0.91 0.89 0.10 0.06 0.05
HO
OH
H
O
N
O
Si
CH3
CH3
CH3
CH3
CH3
4.99 2.15 2.00 2.11 1.04 1.05 1.19 1.45 1.49 1.21 1.16 1.17 1.62 9.41 5.99
f1 (ppm)


138.79 128.68 128.07 127.98 74.00 73.40 71.24 70.68 68.49 68.24 62.32 45.56 29.79 26.02 23.27 18.25 -5.44
HO
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H
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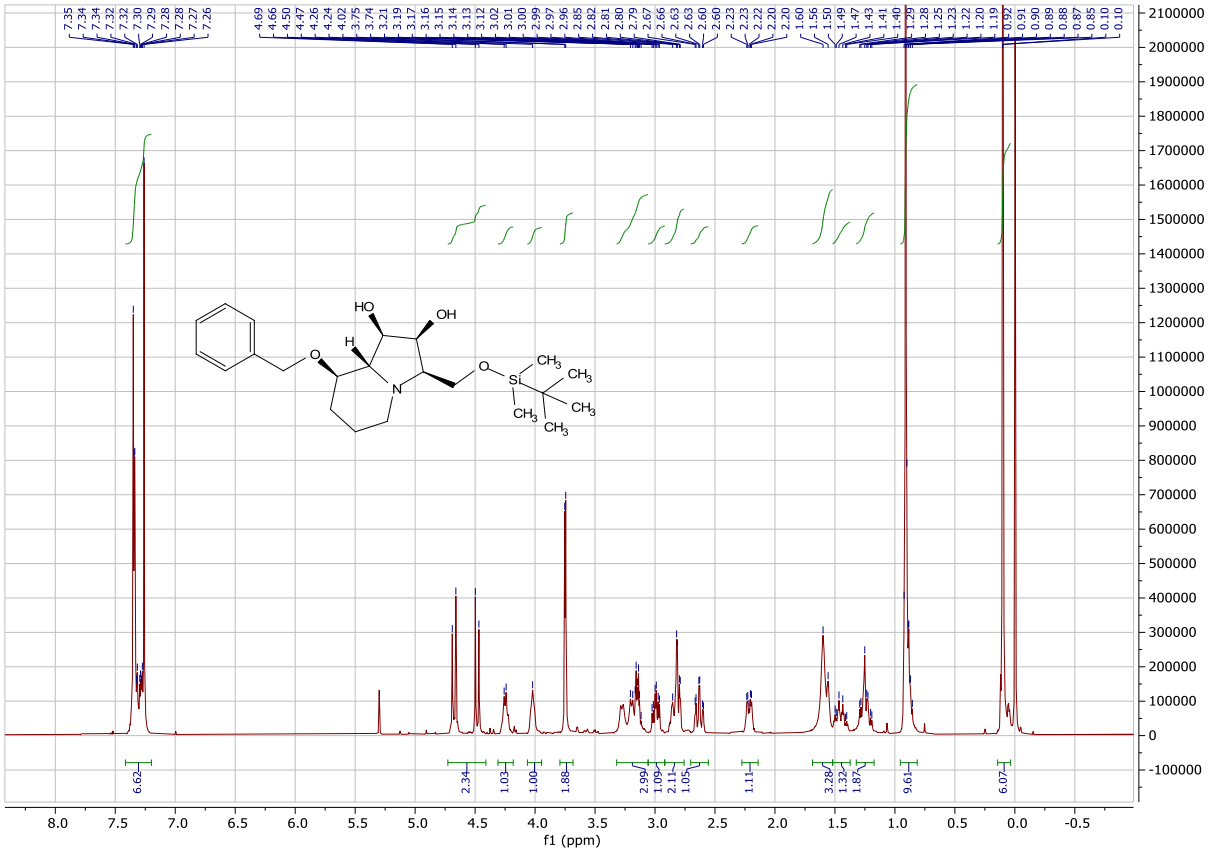
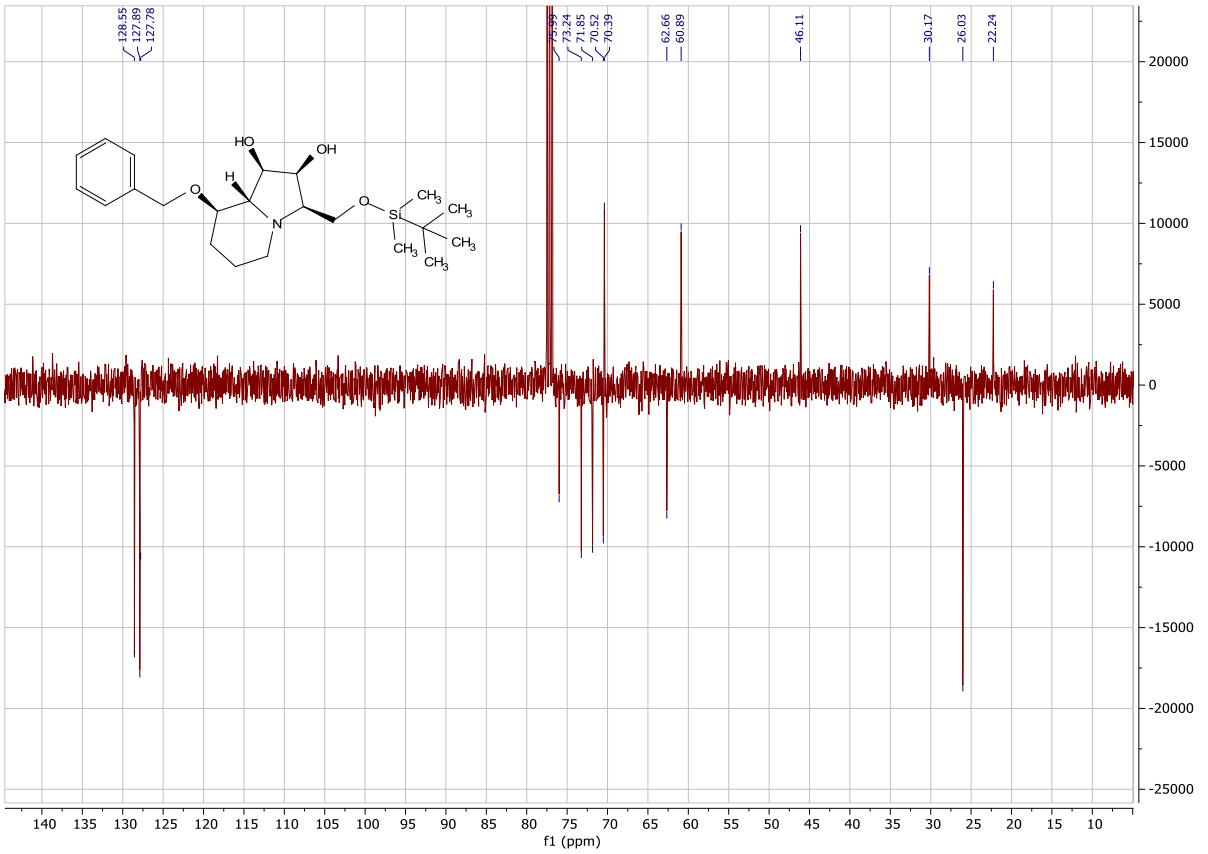
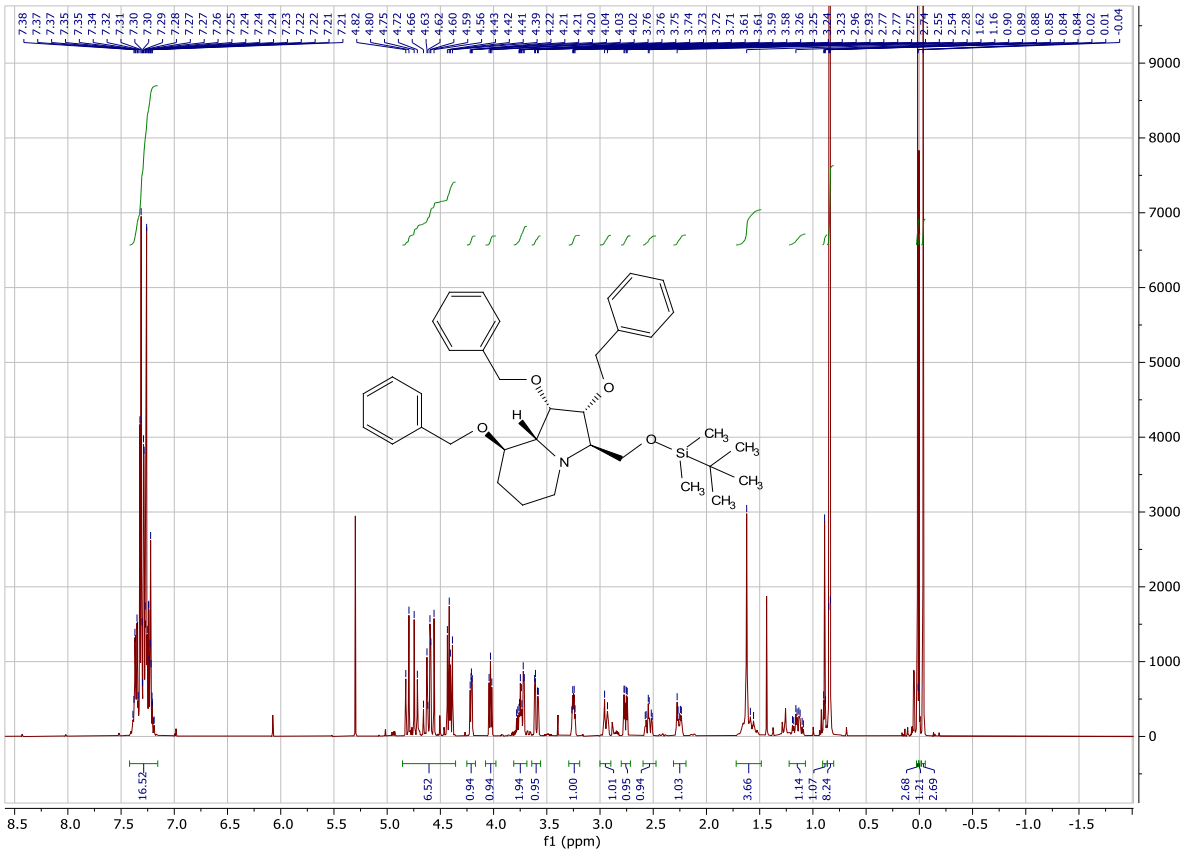
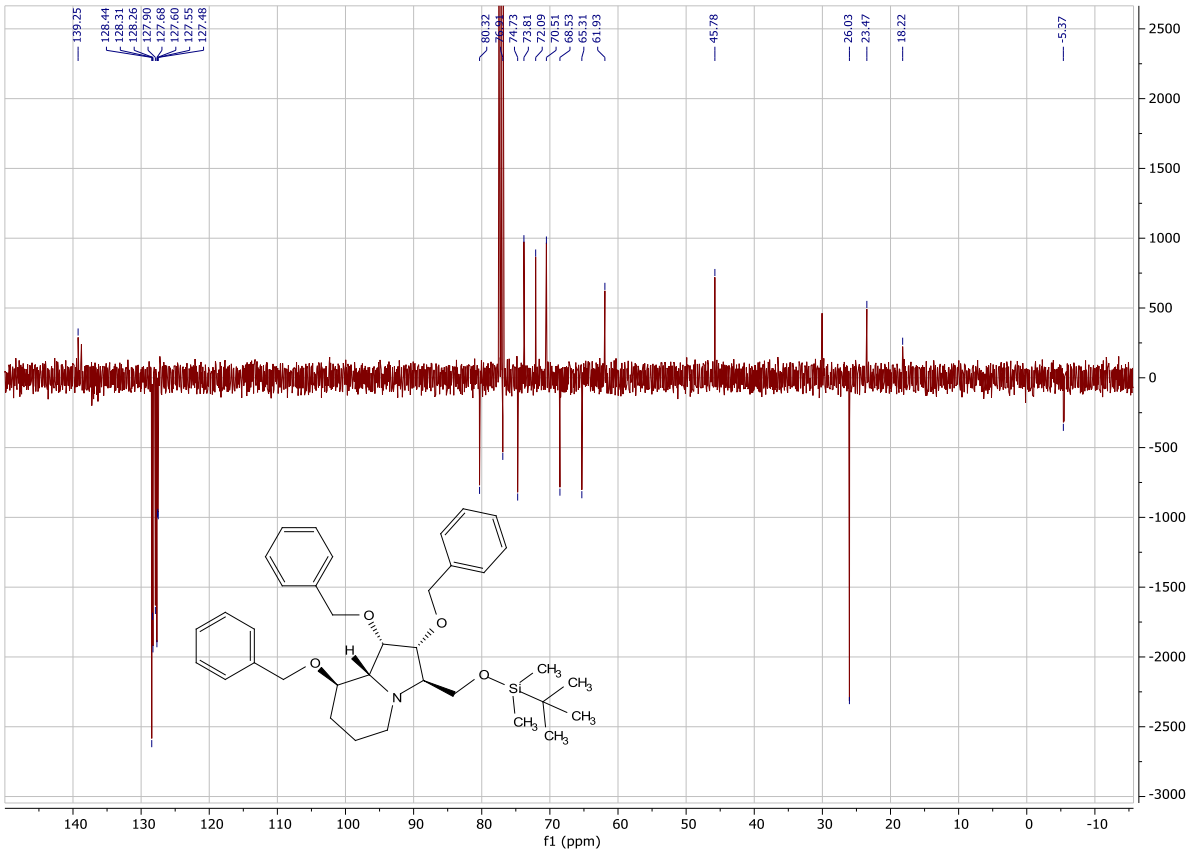
O
Si
CH3
CH3
CH3
CH3
CH3
f1 (ppm)


HO
OH
H
O
N
O
Si
CH3
CH3
CH3
CH3
CH3
f1 (ppm)


128.55
127.89
127.78
75.99
73.24
71.85
70.52
70.39
62.66
60.89
46.11
30.17
26.03
22.24
HO
OH
H
O
N
O
Si
CH3
CH3
CH3
CH3
CH3
f1 (ppm)


f1 (ppm)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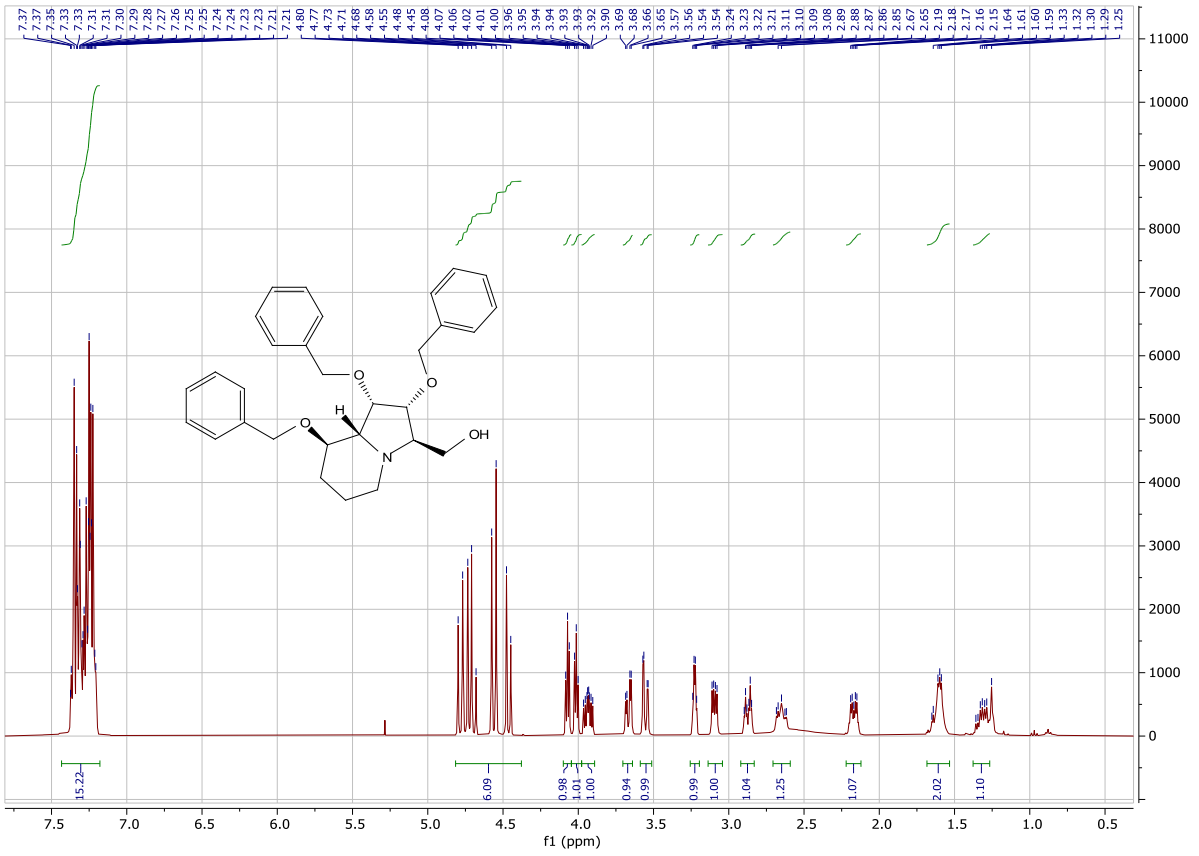
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11000 10000 9000 8000 7000 6000 5000 4000 3000 2000 1000 0
O O H O OH N
15.22 6.09 0.98 1.01 1.00 0.94 0.99 0.99 1.00 1.04 1.25 1.07 2.02 1.10
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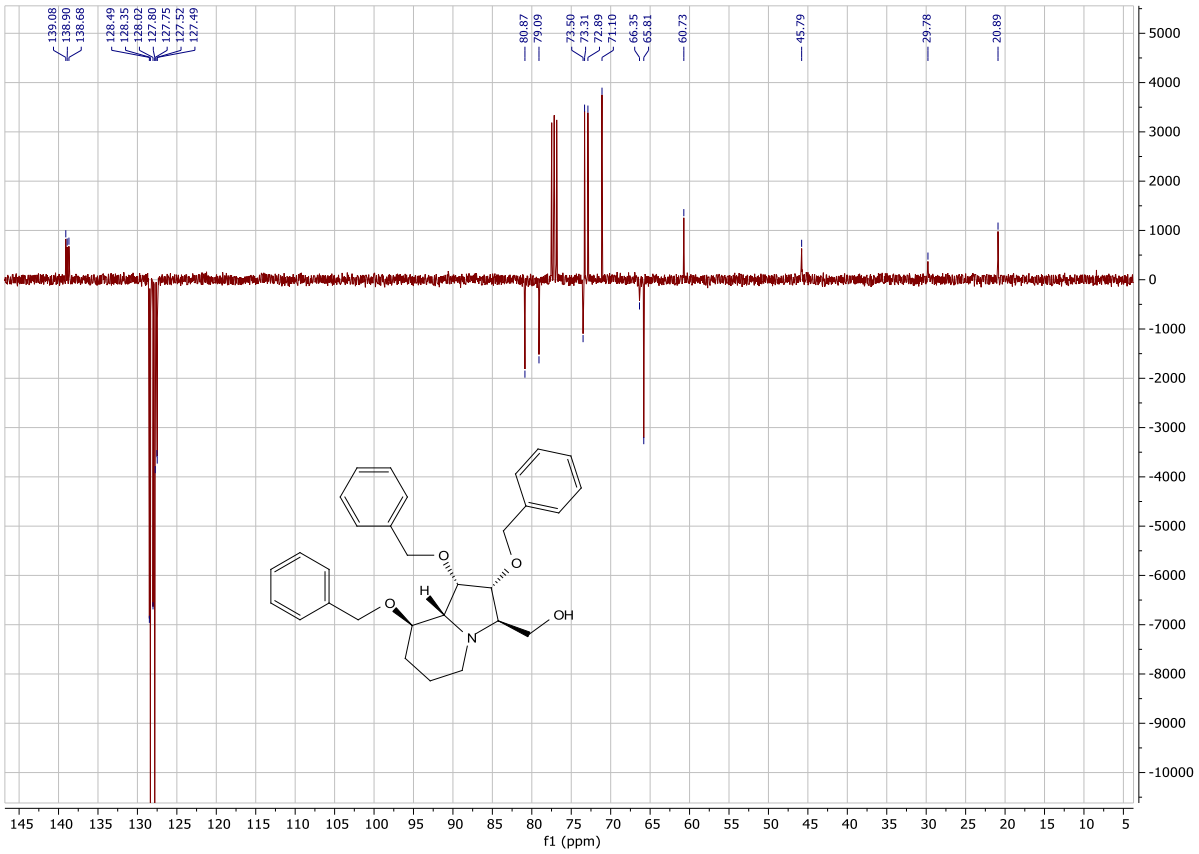
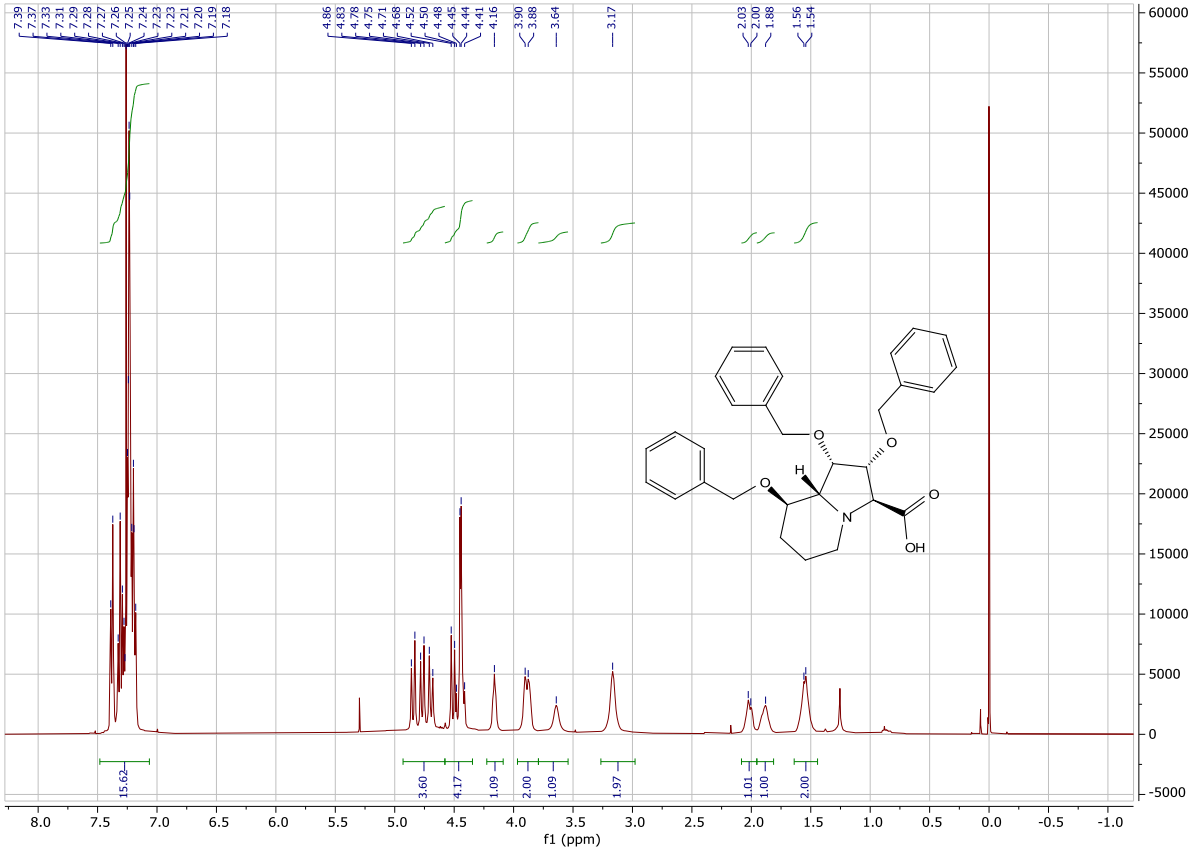
f1 (ppm)


139.08 138.90 138.68 128.49 128.35 128.02 127.80 127.75 127.52 127.49
80.87 79.09 73.50 73.31 72.89 71.10 66.35 65.81 60.73 45.79 29.78 20.89
5000 4000 3000 2000 1000 0 -1000 -2000 -3000 -4000 -5000 -6000 -7000 -8000 -9000 -10000
O O H O OH N
145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5
f1 (ppm)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7.31
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7.27
7.26
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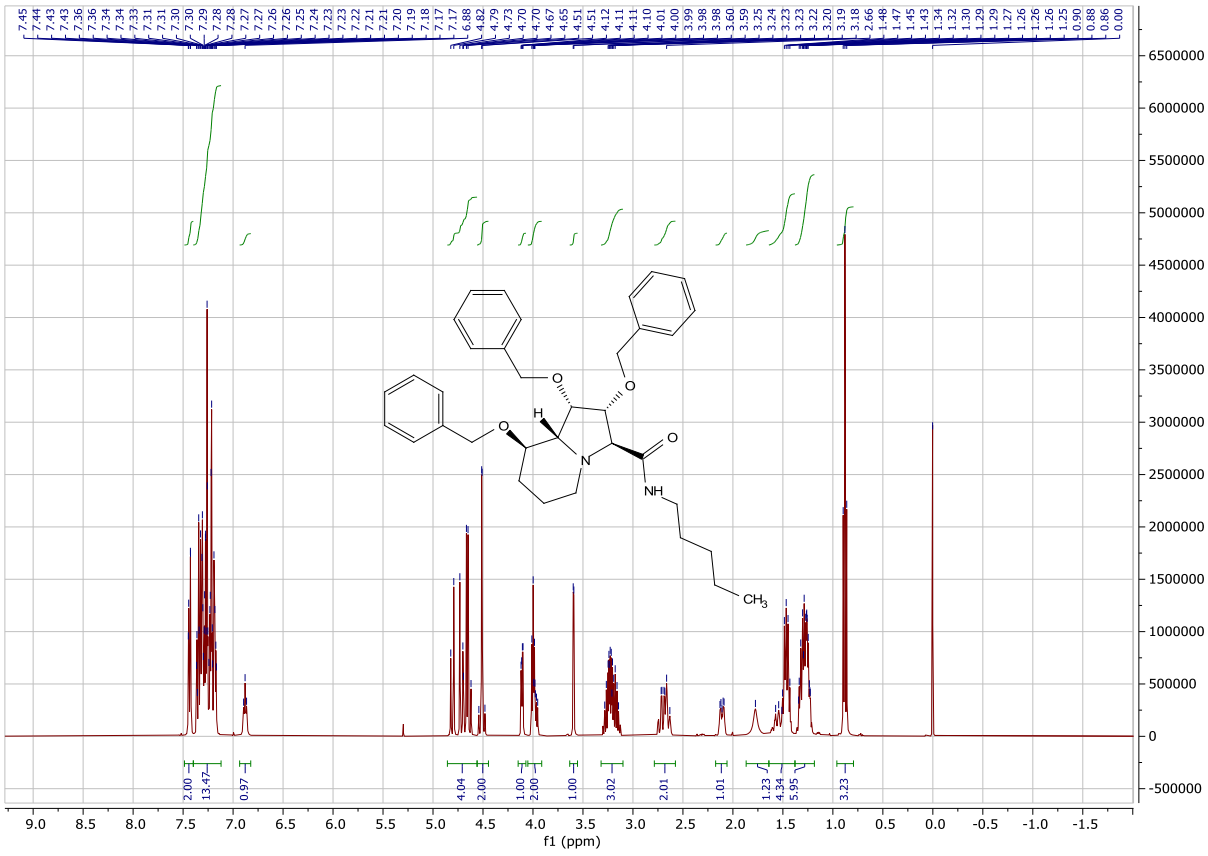
7.24
7.23
7.23
7.21
7.20
7.19
7.18
4.86
4.83
4.78
4.75
4.71
4.68
4.52
4.50
4.48
4.45
4.44
4.41
4.16
3.90
3.88
3.64
3.17
2.03
2.00
1.88
1.56
1.54
15.62
3.60
4.17
1.09
2.00
1.09
1.97
1.01
1.00
2.00
f1 (ppm)


70.73
49.37
26.83
19.15
f1 (ppm)


7.45 7.44 7.43 7.43 7.36 7.36 7.34 7.34 7.33 7.31 7.31 7.30 7.30 7.29 7.28 7.28 7.27 7.27 7.26 7.26 7.25 7.24 7.23 7.23 7.22 7.21 7.21 7.20 7.19 7.18 7.17 7.17 6.88 4.82 4.79 4.73 4.70 4.70 4.67 4.65 4.51 4.51 4.12 4.11 4.11 4.10 4.01 4.00 3.99 3.98 3.98 3.60 3.59 3.25 3.24 3.23 3.23 3.22 3.20 3.19 3.18 2.66 1.48 1.47 1.45 1.43 1.34 1.32 1.30 1.29 1.29 1.27 1.26 1.26 1.26 1.25 0.90 0.88 0.86 0.00
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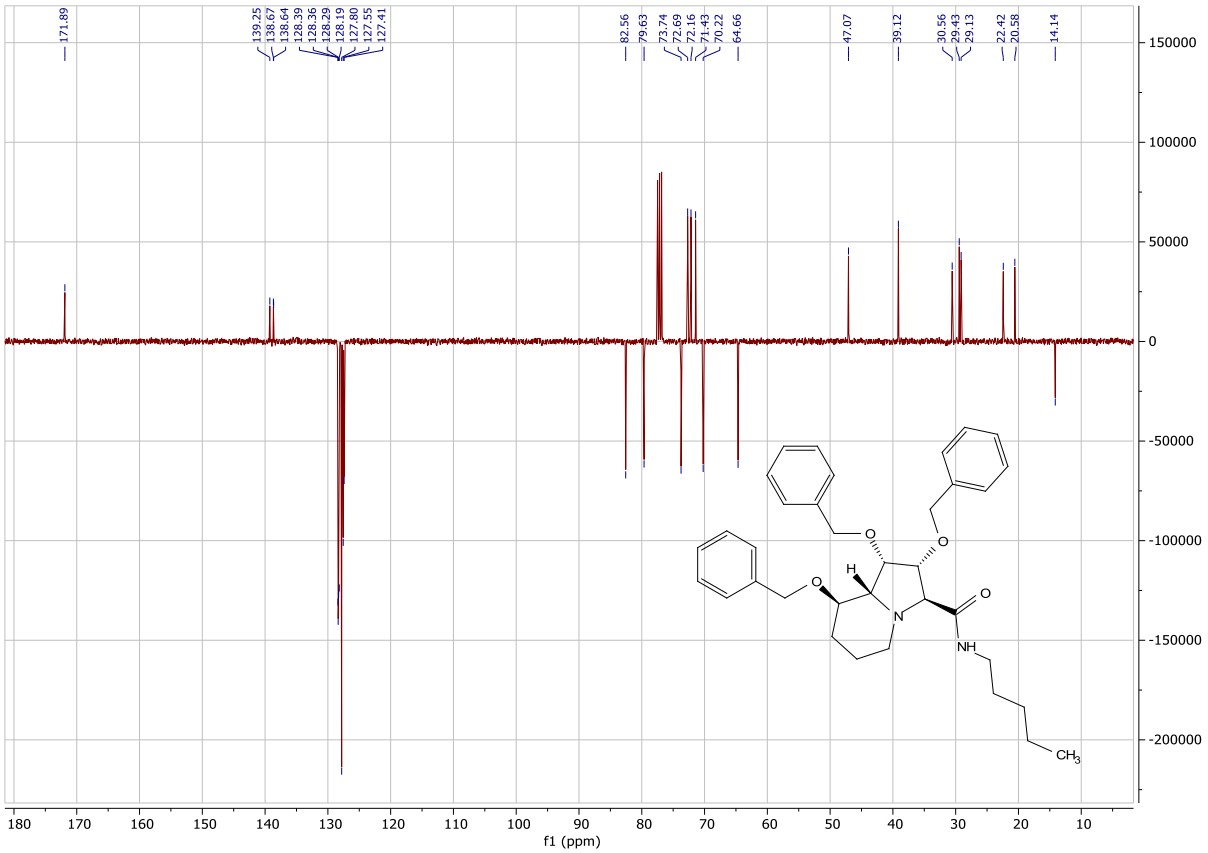
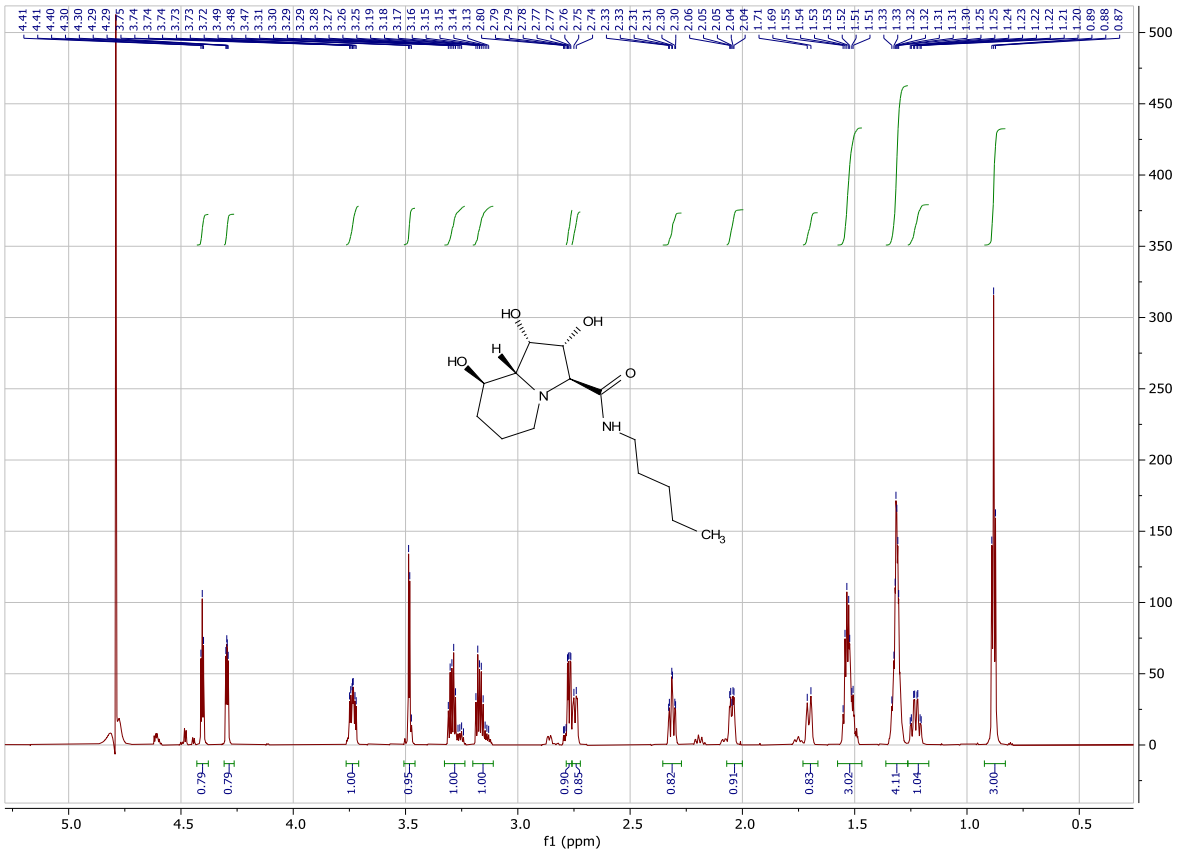
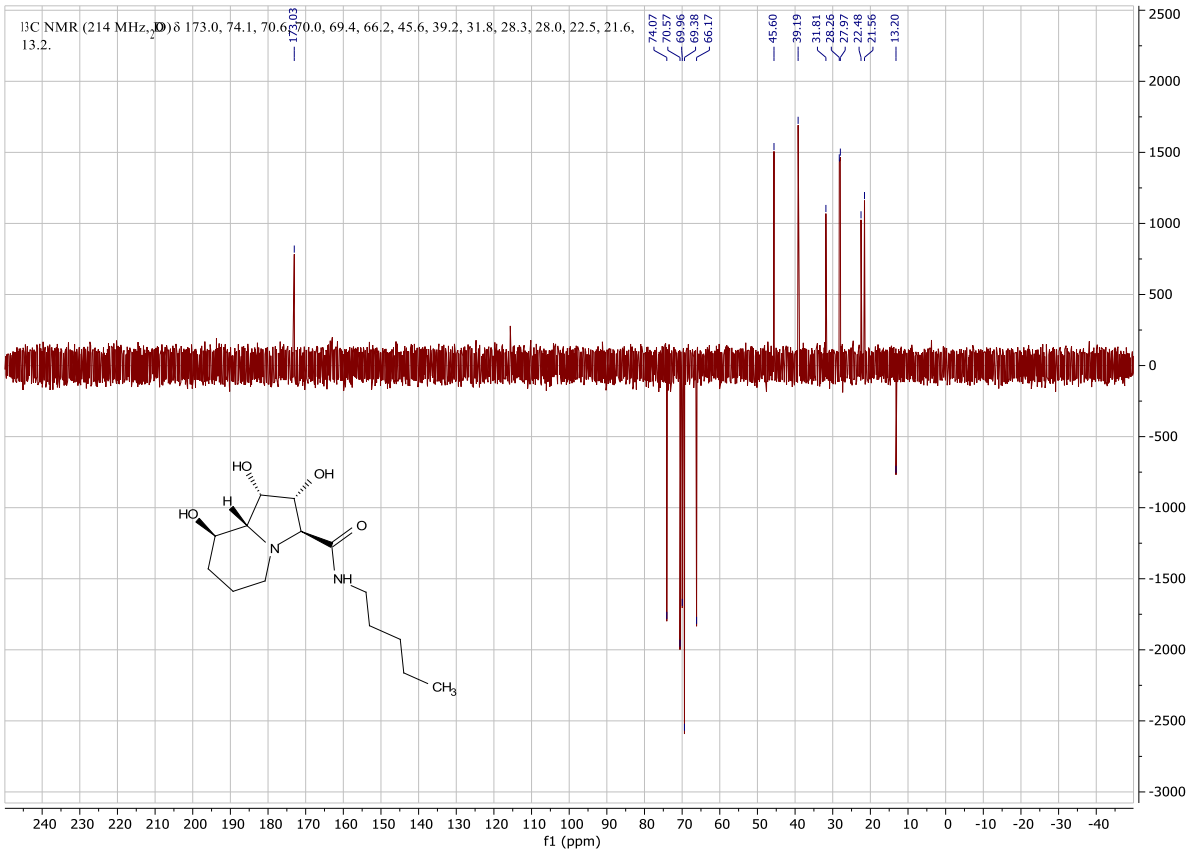
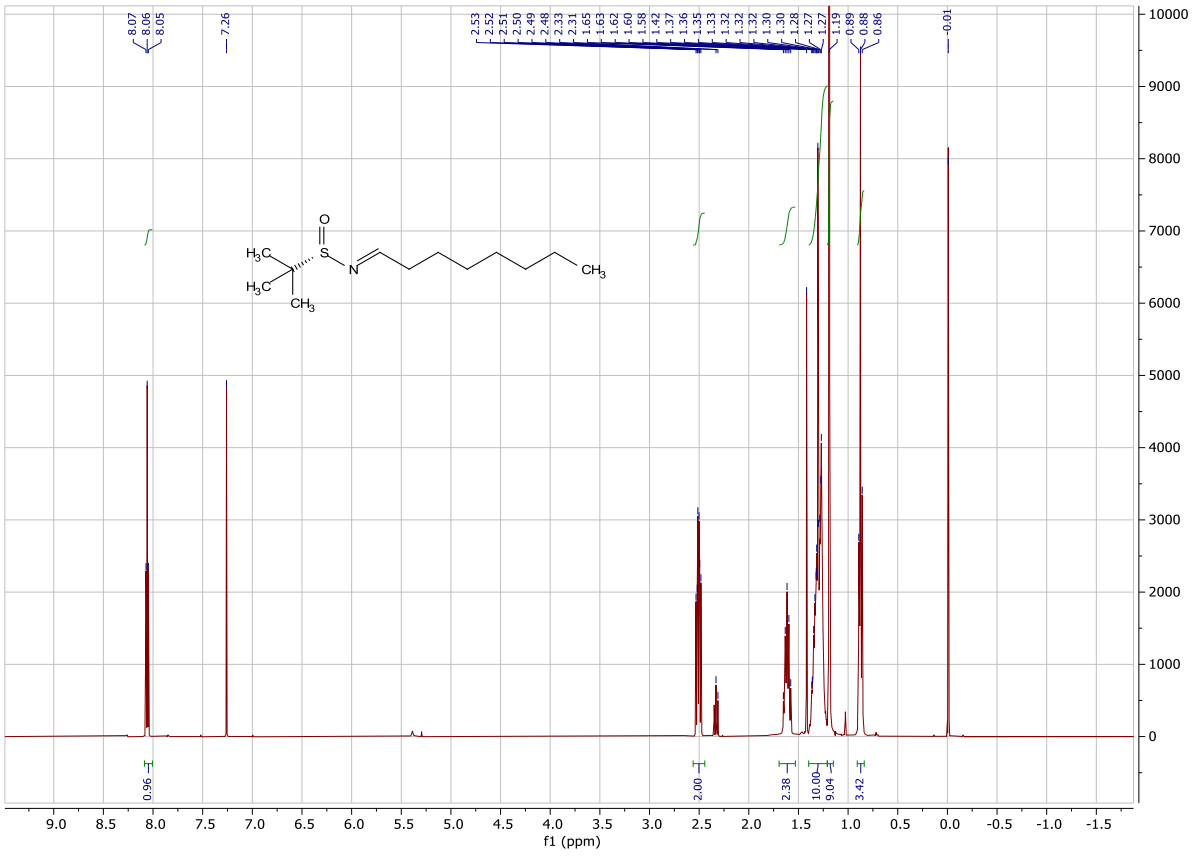
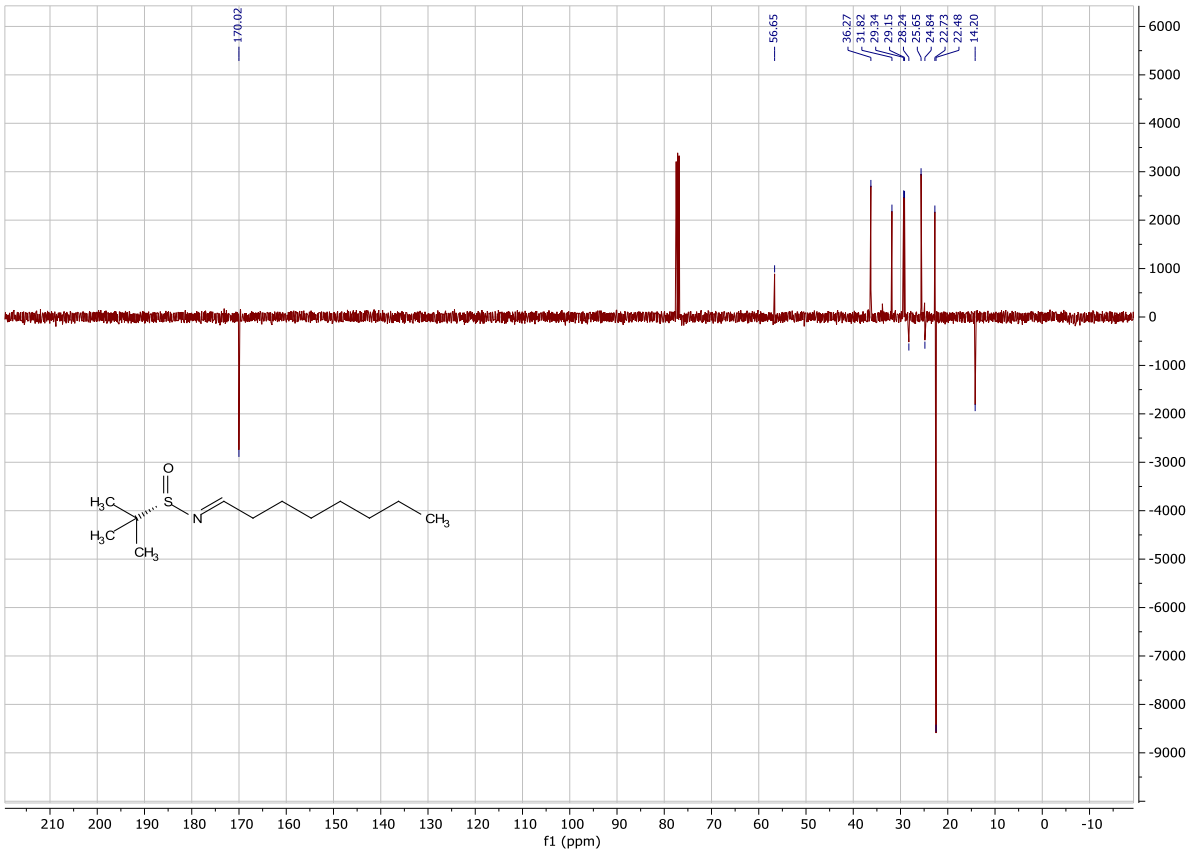
f1 (ppm)

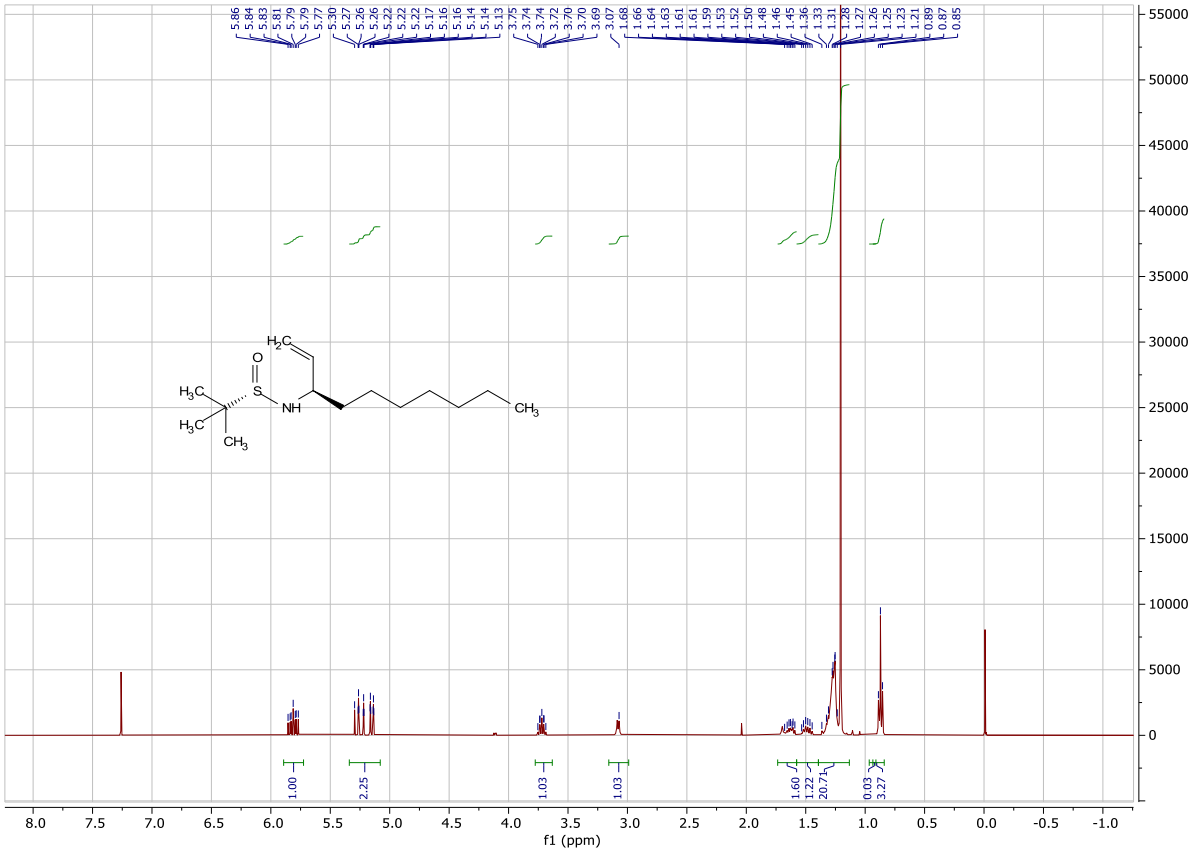

171.89 139.25 138.67 138.64 128.39 128.36 128.29 128.19 127.80 127.55 127.41 82.56 79.63 73.74 72.69 72.16 71.43 70.22 64.66 47.07 39.12 30.56 29.43 29.13 22.42 20.58 14.14
f1 (ppm)

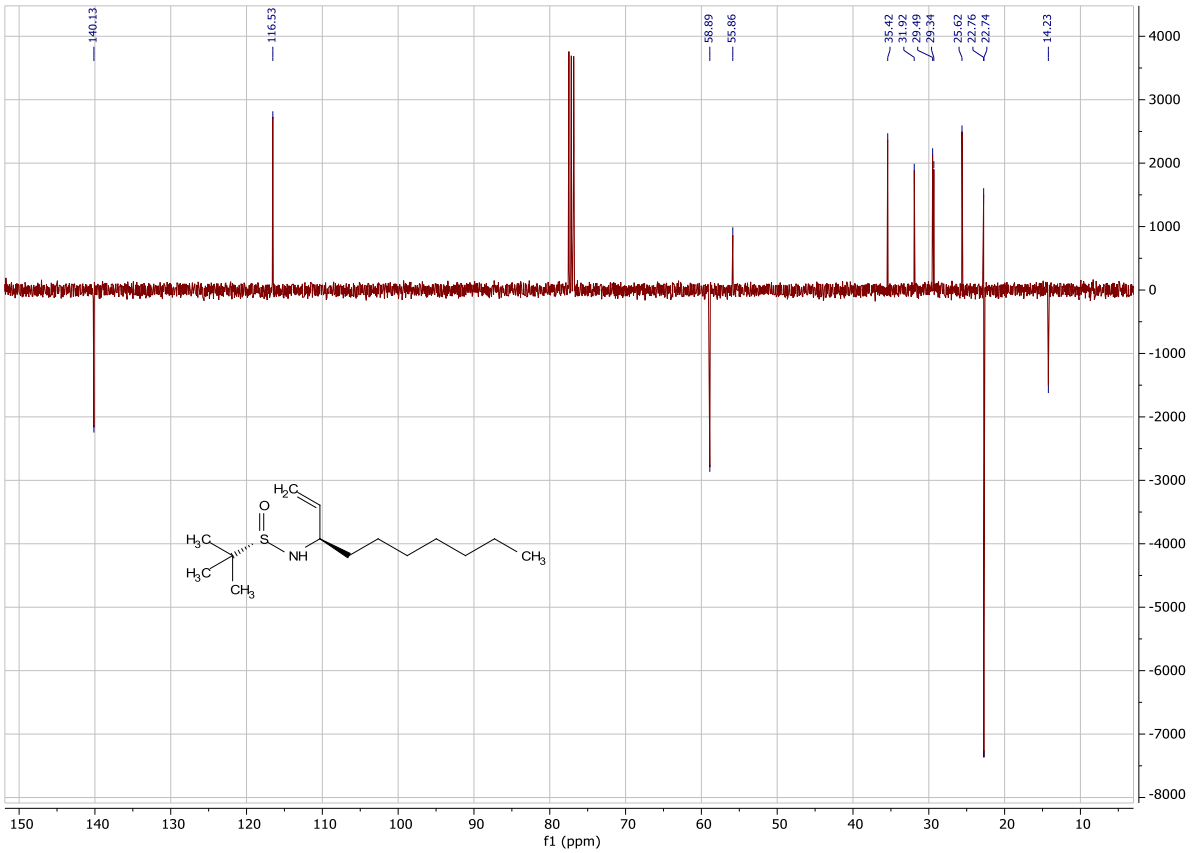

HO
OH
H
HO
N
O
NH
CH3
f1 (ppm)

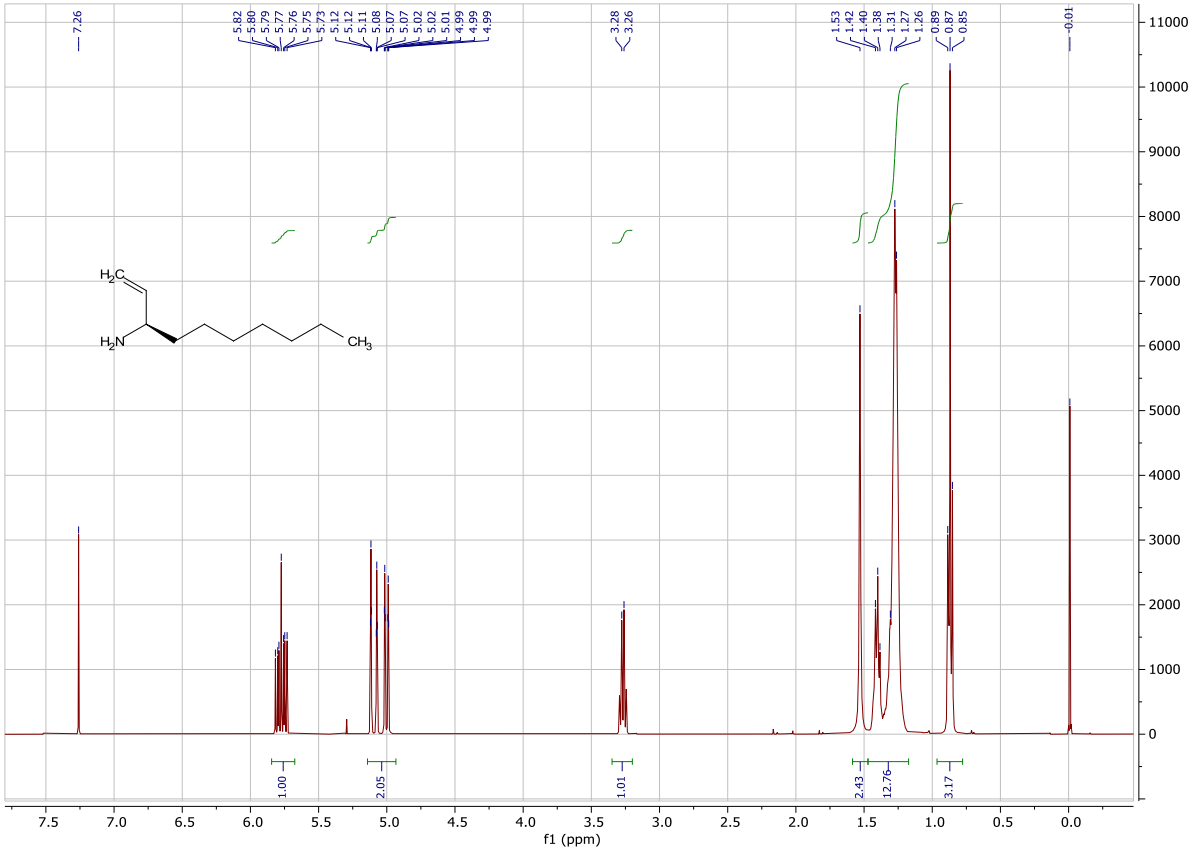

13C NMR (214 MHz, D2O) δ 173.0, 74.1, 70.6, 70.0, 69.4, 66.2, 45.6, 39.2, 31.8, 28.3, 28.0, 22.5, 21.6, 13.2.
HO
OH
H
HO
N
O
NH
CH3
f1 (ppm)

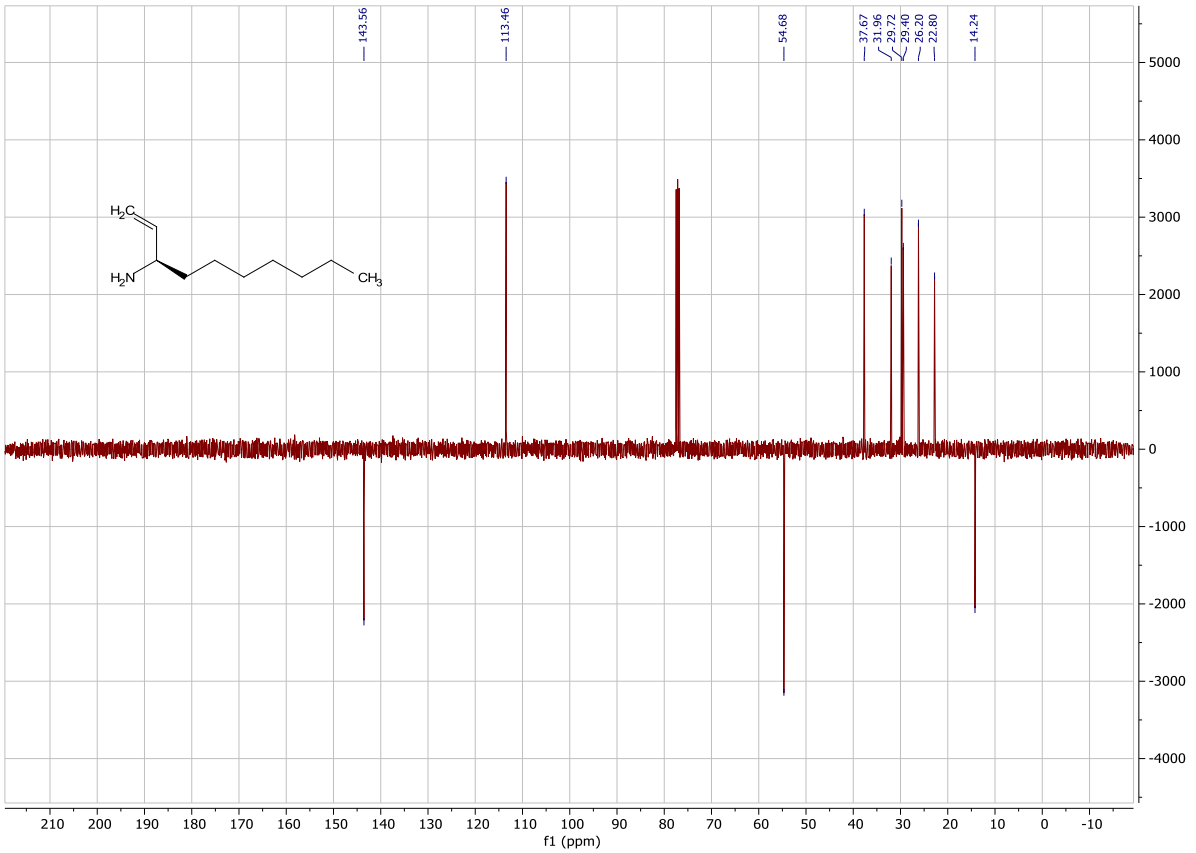

8.07
8.06
8.05
7.26
2.53
2.52
2.51
2.50
2.49
2.48
2.33
2.31
1.65
1.63
1.62
1.60
1.58
1.42
1.37
1.36
1.35
1.33
1.32
1.32
1.30
1.30
1.28
1.27
1.27
1.19
0.89
0.88
0.86
-0.01
0.96
2.00
2.38
10.00
9.04
3.42
f1 (ppm)

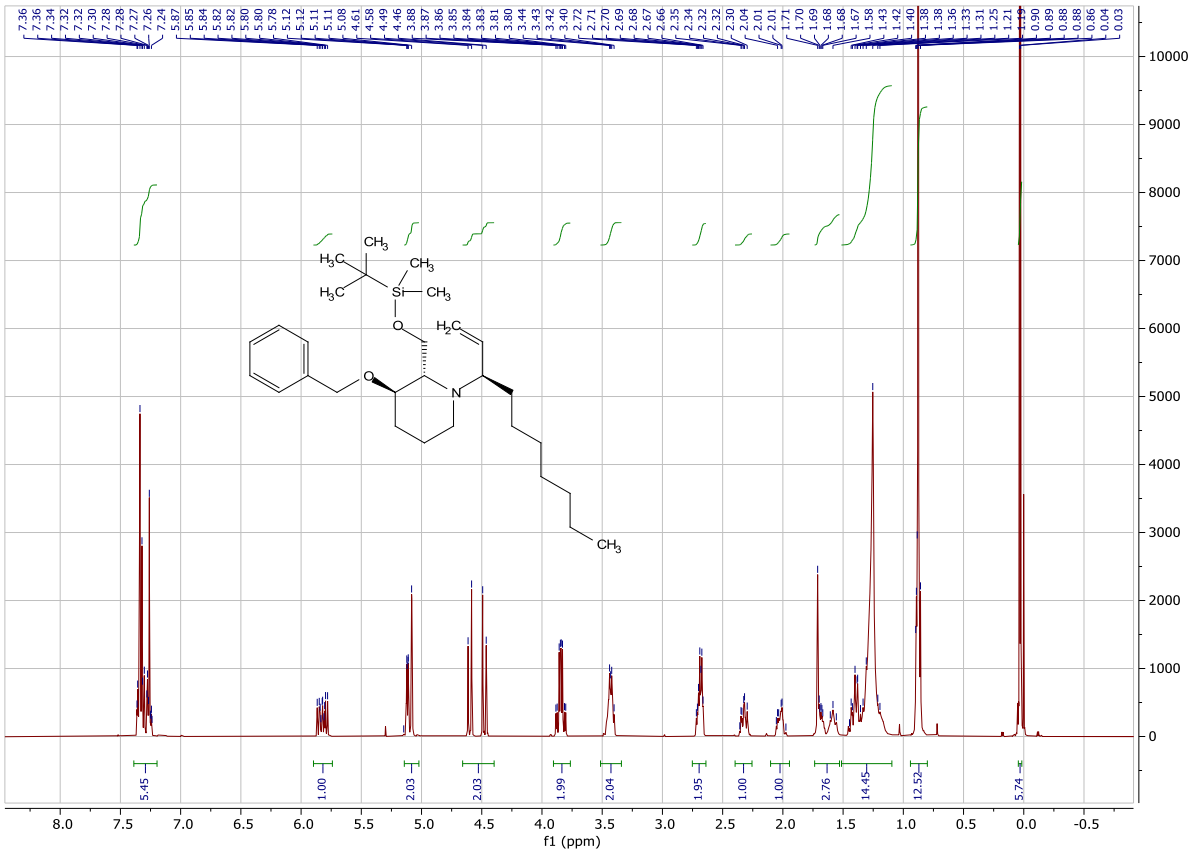

170.02
56.65
36.27
31.82
29.34
29.15
28.24
25.65
24.84
22.73
22.48
14.20
f1 (ppm)

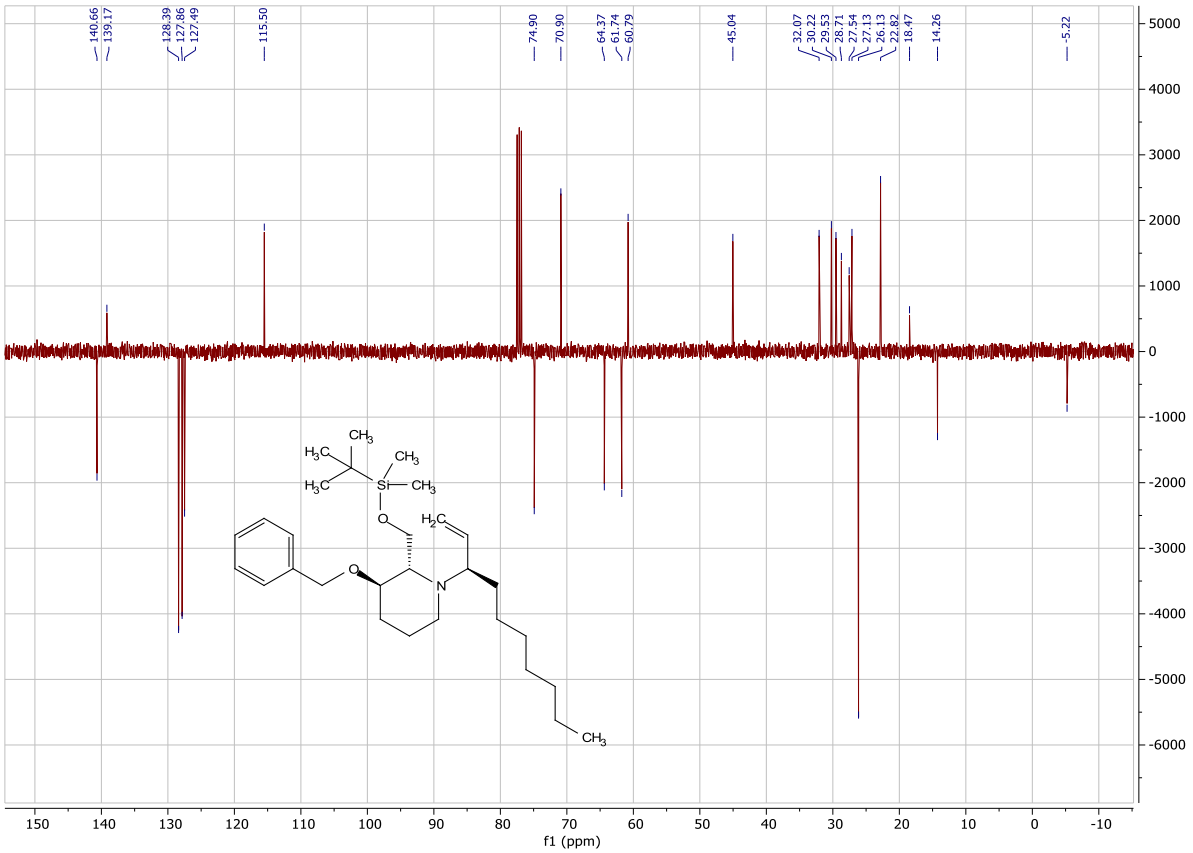

5.86 5.84 5.83 5.81 5.79 5.77 5.30 5.27 5.26 5.22 5.22 5.17 5.16 5.14 5.13 3.75 3.74 3.74 3.72 3.70 3.69 3.07 1.68 1.66 1.64 1.63 1.61 1.61 1.59 1.53 1.52 1.50 1.48 1.46 1.45 1.36 1.33 1.31 1.28 1.27 1.26 1.25 1.23 1.21 0.89 0.87 0.85
1.00 2.25 1.03 1.03 1.60 1.22 20.71 0.03 3.27
f1 (ppm)

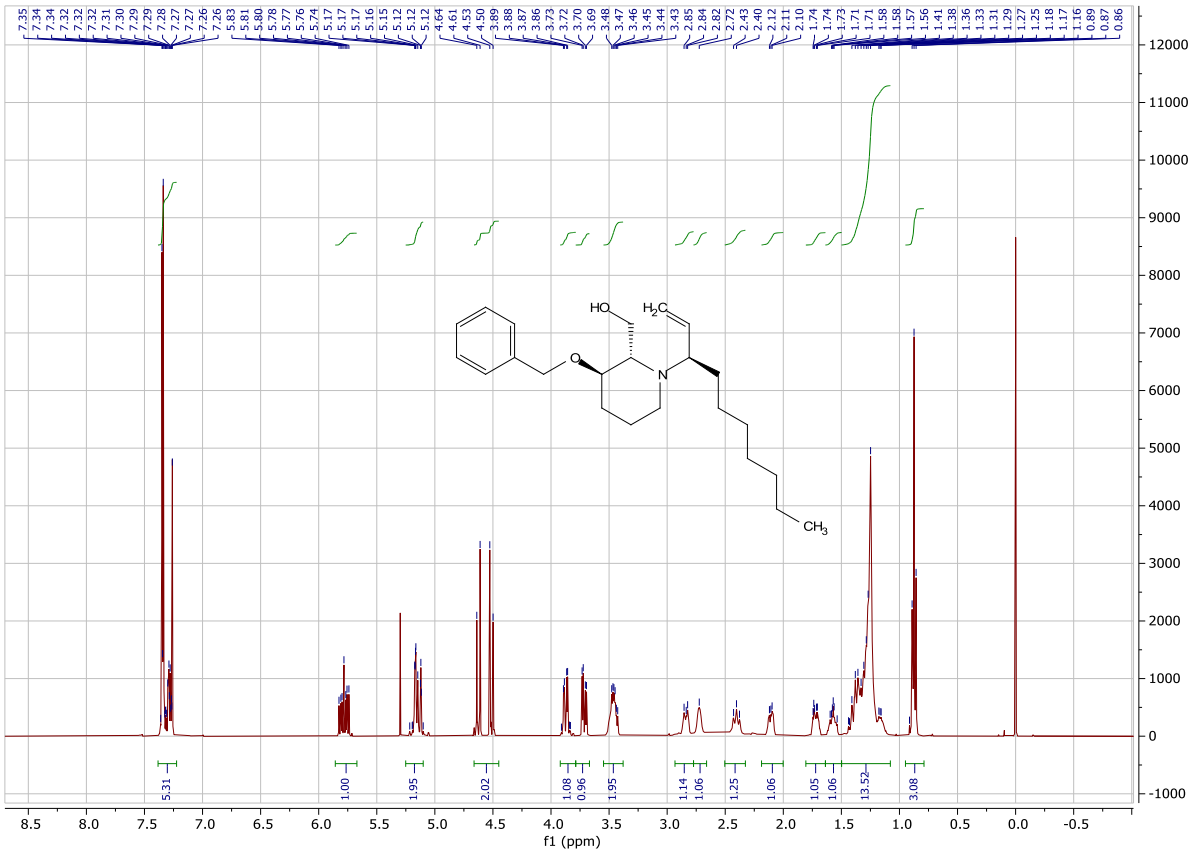

140.13 116.53 58.89 55.86 35.42 31.92 29.49 29.34 25.62 22.76 22.74 14.23
f1 (ppm)

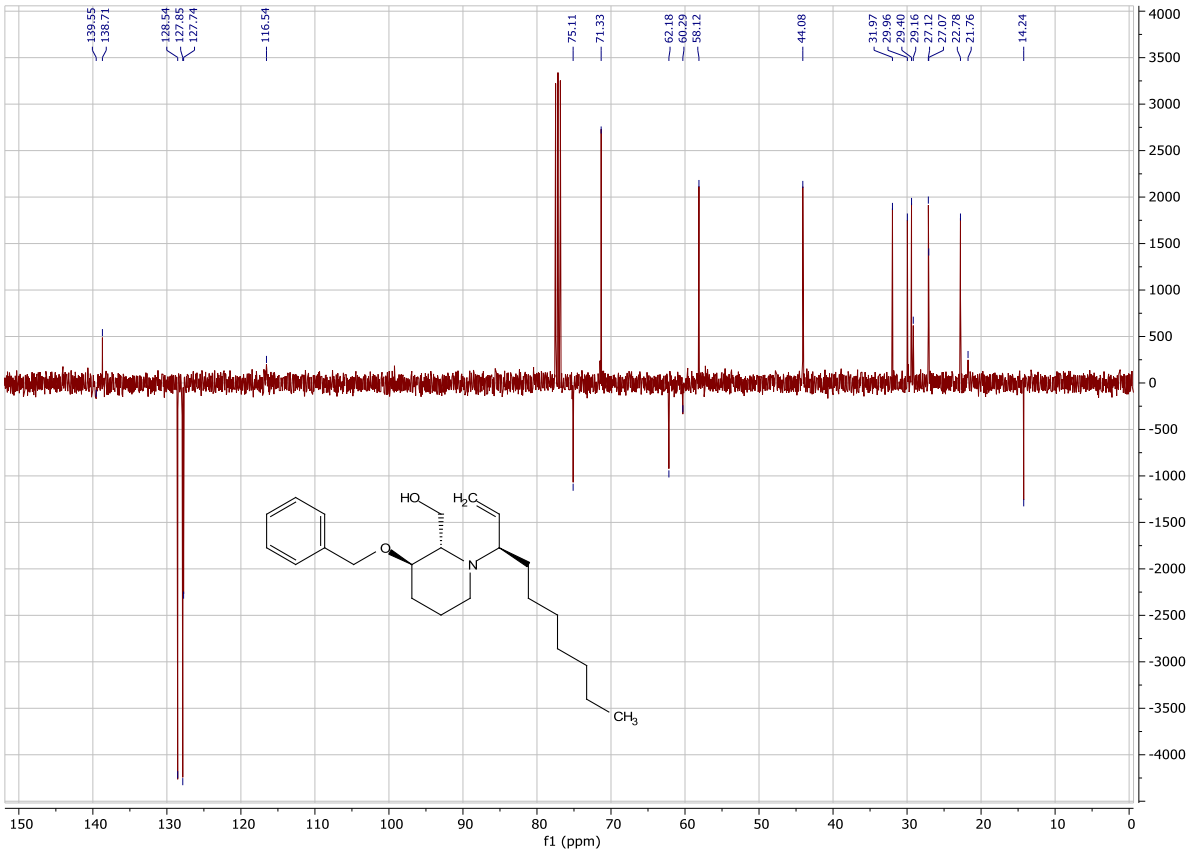

7.26
5.82
5.80
5.79
5.77
5.76
5.75
5.73
5.12
5.12
5.11
5.08
5.07
5.07
5.02
5.02
5.01
4.99
4.99
4.99
3.28
3.26
1.53
1.42
1.40
1.38
1.31
1.27
1.26
0.89
0.87
0.85
-0.01
H2C
H2N
CH3
1.00
2.05
1.01
2.43
12.76
3.17
f1 (ppm)

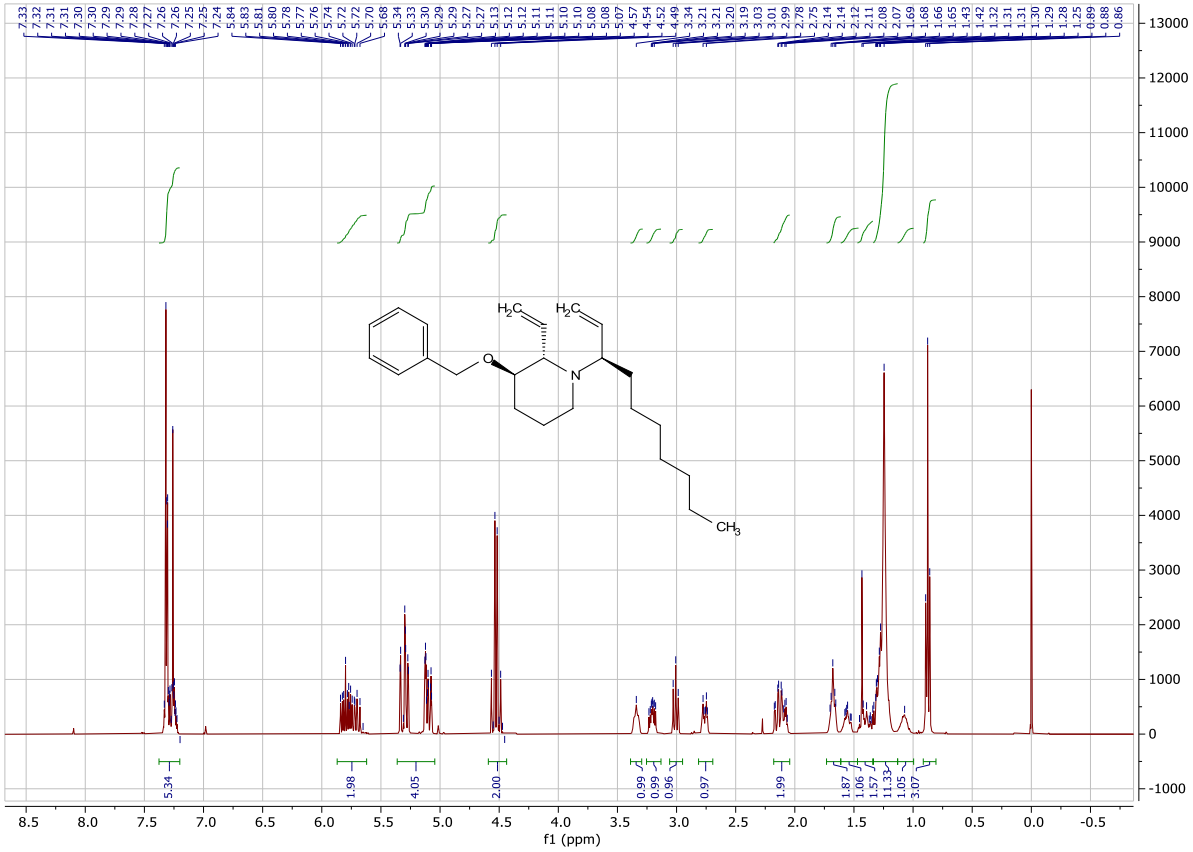

143.56
113.46
54.68
37.67
31.96
29.72
29.40
26.20
22.80
14.24
H2C
H2N
CH3
f1 (ppm)


7.36 7.36 7.34 7.32 7.30 7.28 7.28 7.27 7.26 7.24
5.87 5.85 5.84 5.82 5.82 5.80 5.80 5.78 5.12 5.12 5.11 5.11 5.08 4.61 4.58 4.49 4.46 3.88 3.87 3.86 3.85 3.84 3.83 3.81 3.80 3.44 3.43 3.42 3.40 2.72 2.71 2.70 2.69 2.68 2.67 2.66 2.35 2.34 2.32 2.32 2.30 2.04 2.01 2.01 1.71 1.70 1.69 1.68 1.68 1.67 1.58 1.43 1.42 1.40 1.38 1.38 1.36 1.33 1.31 1.25 1.21 0.90 0.89 0.88 0.88 0.86 0.04 0.03
5.45 1.00 2.03 2.03 1.99 2.04 1.95 1.00 1.00 2.76 14.45 12.52 5.74
f1 (ppm)


140.66 139.17 128.39 127.86 127.49 115.50 74.90 70.90 64.37 61.74 60.79 45.04 32.07 30.22 29.53 28.71 27.54 27.13 26.13 22.82 18.47 14.26 -5.22
f1 (ppm)


HO
H2C
O
N
CH3
f1 (ppm)


HO
H2C
O
N
CH3
f1 (ppm)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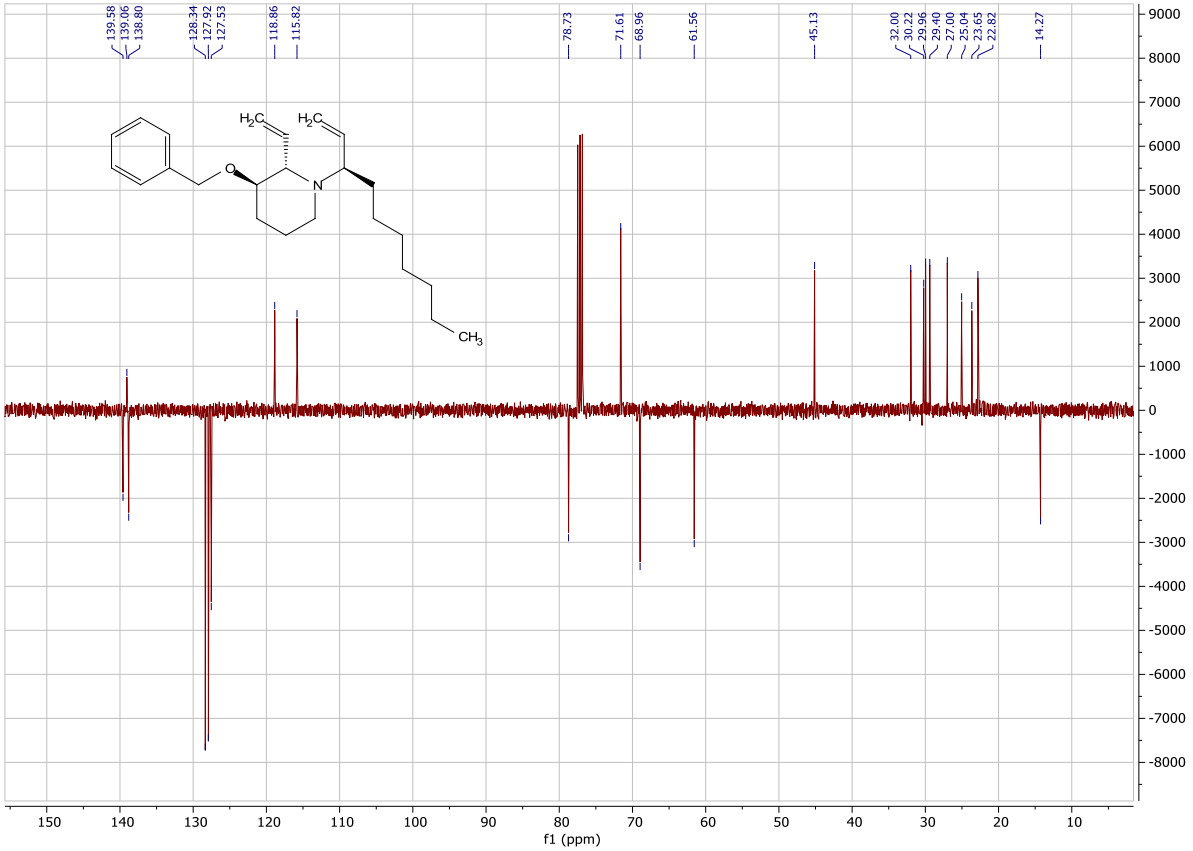
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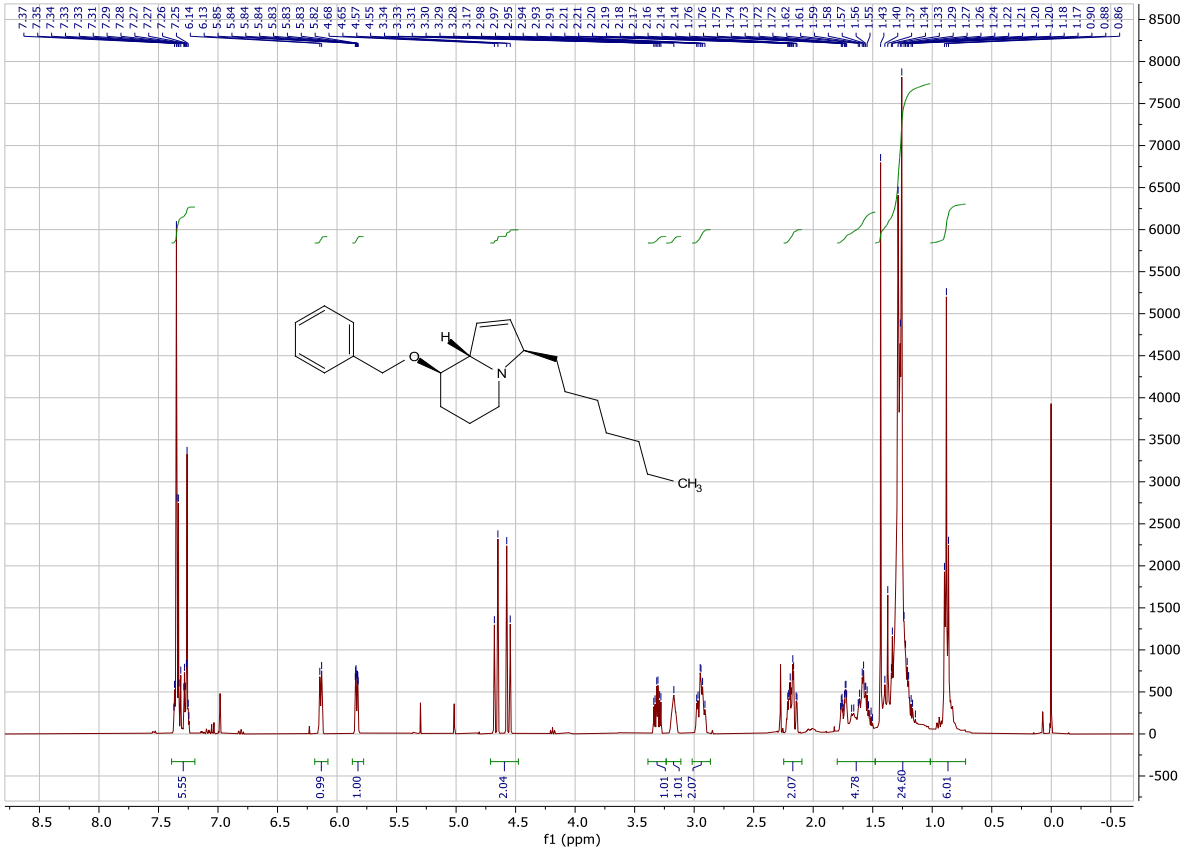
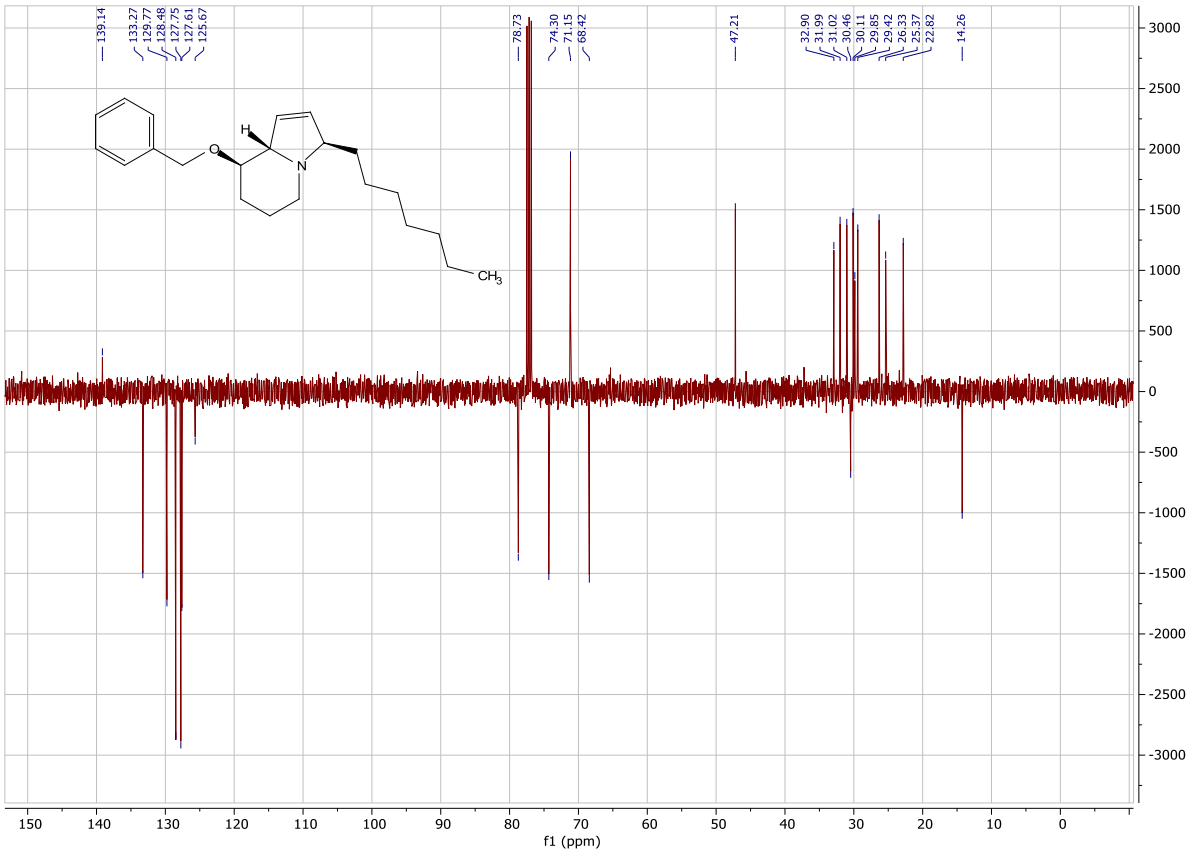
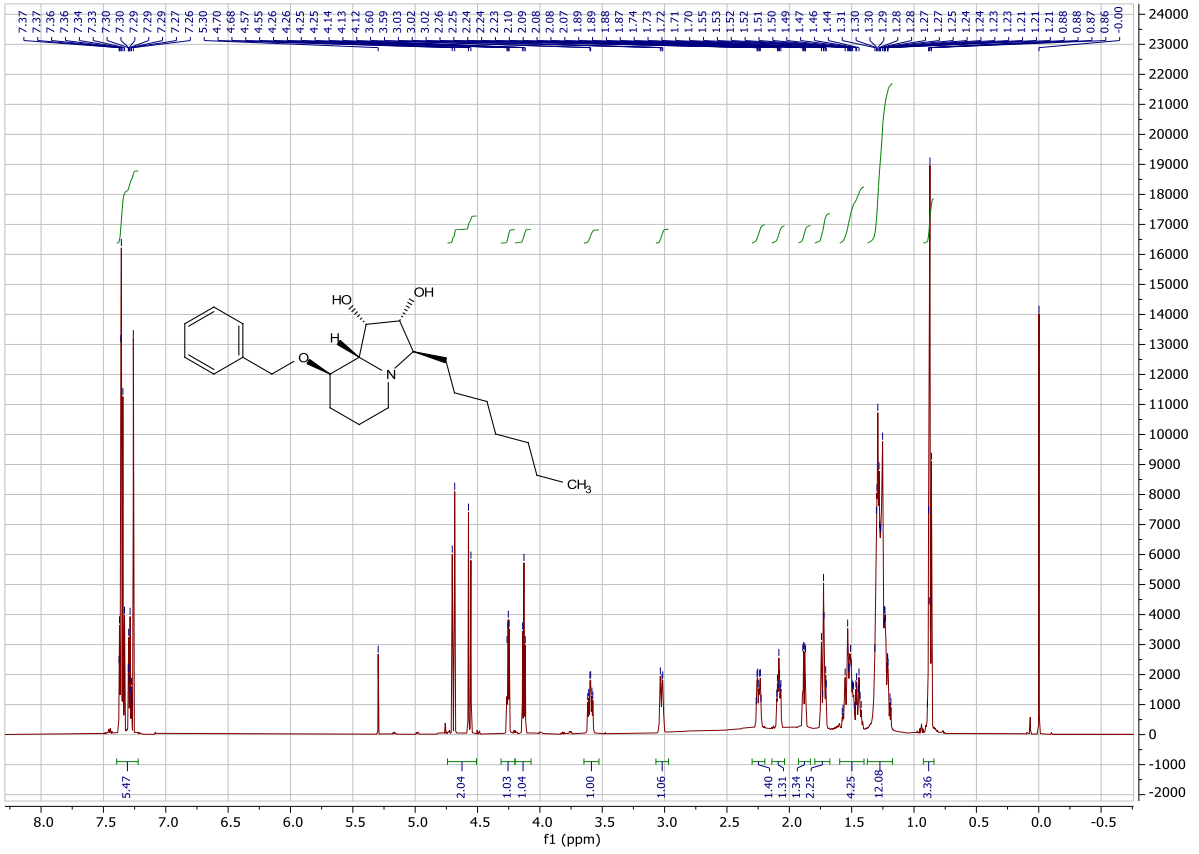

7.37 7.35 7.34 7.33 7.33 7.31 7.29 7.28 7.27 7.27 7.26 7.25 6.14 6.13 5.85 5.84 5.84 5.84 5.83 5.83 5.82 4.68 4.65 4.57 4.55 3.34 3.33 3.31 3.30 3.29 3.28 3.17 2.98 2.97 2.95 2.94 2.93 2.91 2.21 2.21 2.20 2.19 2.18 2.17 2.16 2.14 2.14 1.76 1.76 1.75 1.74 1.73 1.72 1.62 1.61 1.59 1.58 1.57 1.56 1.55 1.43 1.40 1.37 1.34 1.33 1.29 1.27 1.26 1.24 1.22 1.21 1.20 1.20 1.18 1.17 0.90 0.88 0.86
H
O
N
CH3
5.55 0.99 1.00 2.04 1.01 1.01 2.07 2.07 4.78 24.60 6.01
f1 (ppm)


139.14 133.27 129.77 128.48 127.75 127.61 125.67 78.73 74.30 71.15 68.42 47.21 32.90 31.99 31.02 30.46 30.11 29.85 29.42 26.33 25.37 22.82 14.26
H
O
N
CH3
f1 (ppm)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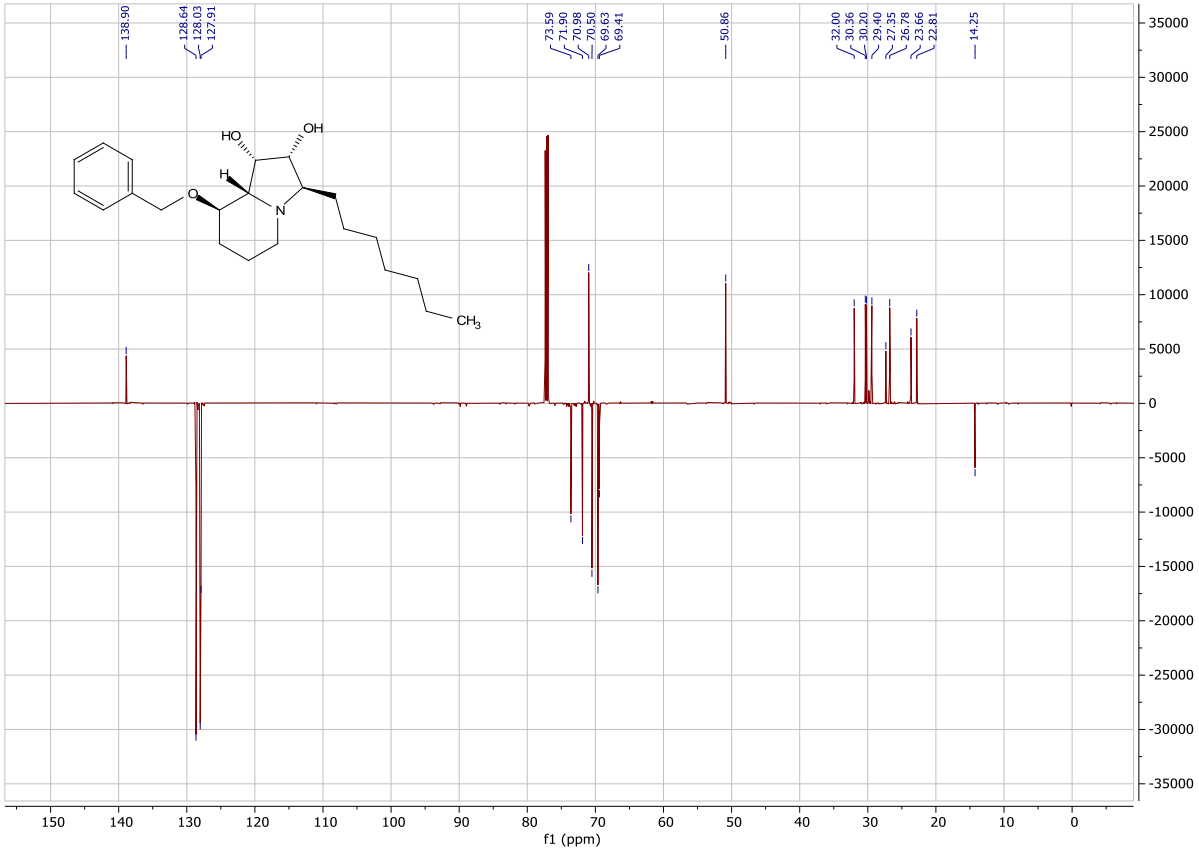
H
O
N
CH3
f1 (ppm)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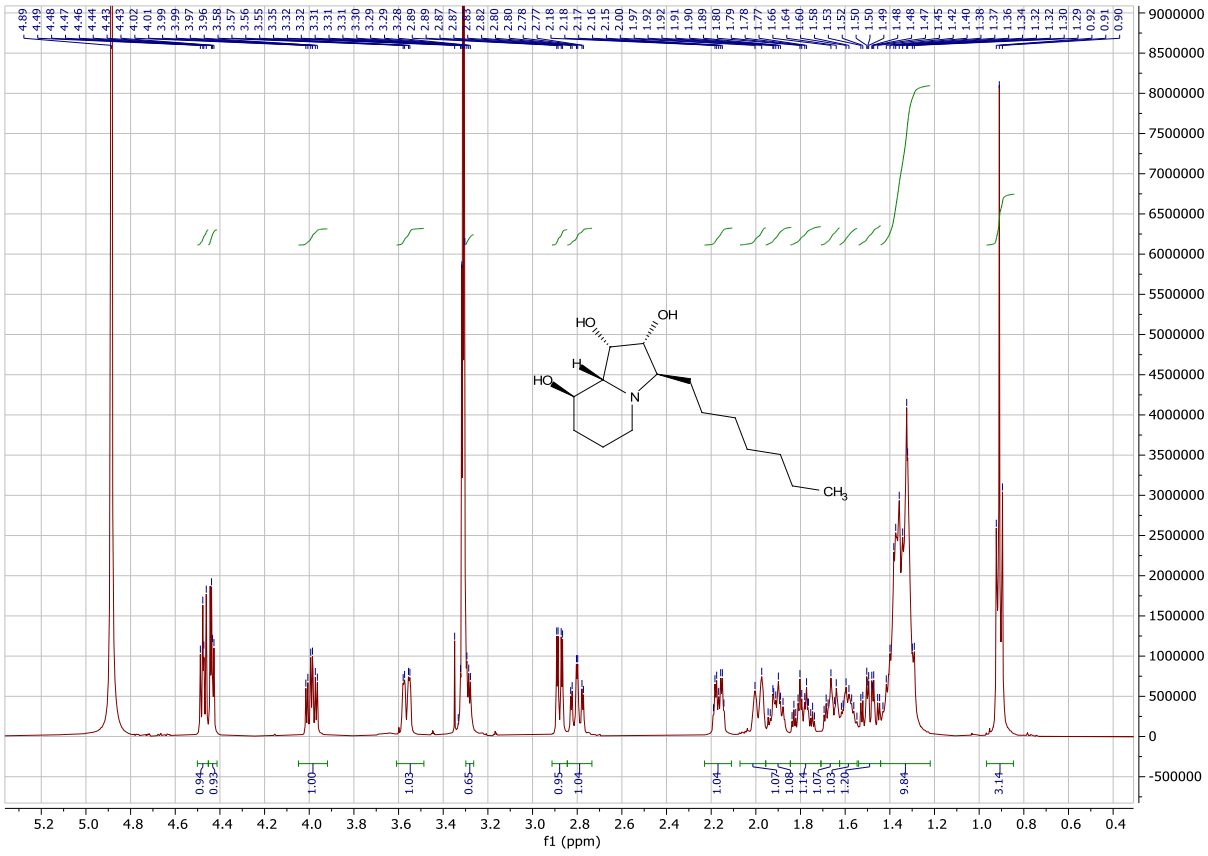
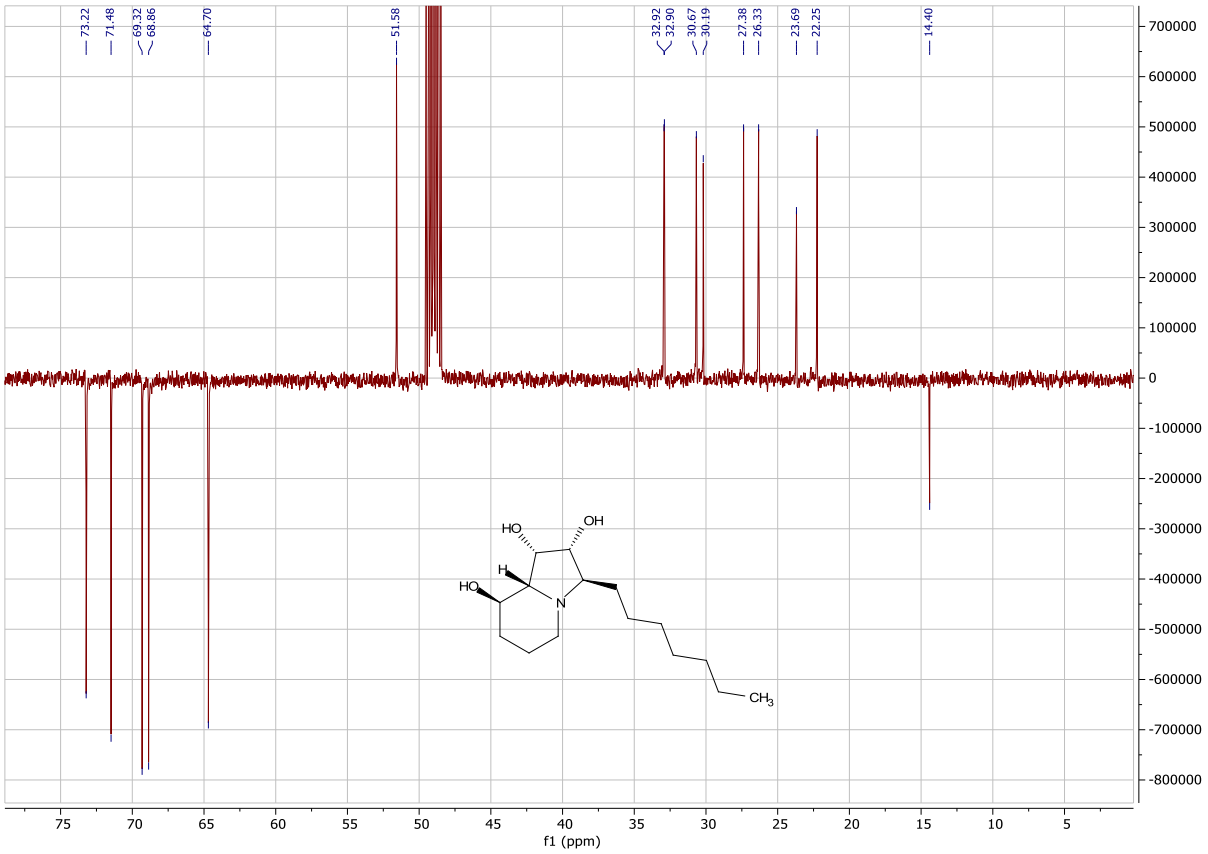
OH
H
O
N
CH3
f1 (ppm)


HO
OH
H
HO
N
CH3
f1 (ppm)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51.58
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30.67
30.19
27.38
26.33
23.69
22.25
14.40
HO
OH
H
HO
N
CH3
f1 (ppm)

## References


1. Z. Armstrong, C. L. Kuo, D. Lahav, B. Liu, R. Johnson, T. J. M. Beenakker, C. de Boer, C. Wong, E. R. van Rijssel, M. F. Debets, B. I. Florea, C. Hissink, R. G. Boot, P. P. Geurink. H. Ovaa, M. van der Stelt, G. M. van der Marel, J. D. C. Codée, J. M. F. G. Aerts, L. Wu, H. S. Overkleeft, G. J. Davies, *J. Am. Chem. Soc*. 2020, **142**, 13021-13029.