Electronic Supplementary Material (ESI) for Dalton Transactions.

Supplementary Information

The first indoleamine-2,3-dioxygenase-1 (IDO1) inhibitors containing carborane

Christopher J.D. Austin, Jan Kahlert, Fatiah Issa, Joseph A. Ioppolo, John H. Reed, Jennifer A. Ong, Jason R. Smith, Joanne F. Jamie, David Hibbs, and Louis M. Rendina*.

Table of Contents

IDO1 Inhibition Assays

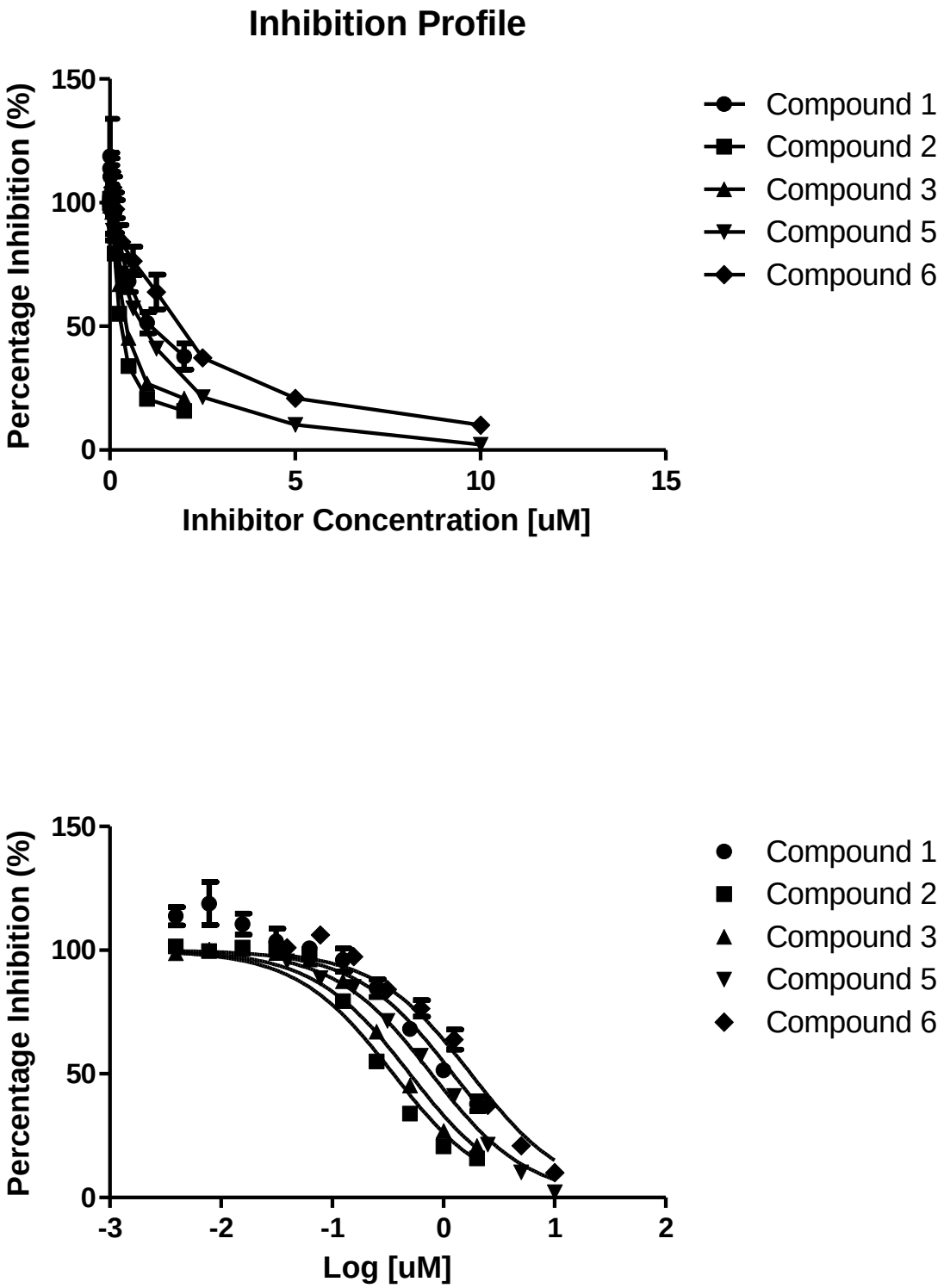


Figure S1: Inhibition profiles for tested IDO1 inhibitors

***In Silico* Docking Studies**

Benzyl: 5-oxo ligation poses

Inspection of the 5-oxo poses shows 46 (90%) of the structures have resulted in Arg231 moving across closer to the active site. Of the remaining 5 structures, 4 show Arg231 in the same position as their starting structure, with only one structure resulting in movement of Arg231 away from the active site.

The average distance between the protonatable amino group and the carboxylate carbon of the haem propanoate arm is 3.2 Å, compared to 3.5 Å for the starting structure. In all cases the hydroxyl group has moved behind the benzyl group, to be in proximity of the Arg231 residue. This pose position is in contrast to the starting structure, where the hydroxyl group rests between the haem propanoate group and the carbonyl group of Gly262. This suggests that Arg231 is moving into the active site entrance (in the crystal structure (2D0T)[1] Arg231 is held in place by two CHES buffer molecules) which in turn moves the benzyl group. As a result, the hydroxyl group shifts to accommodate the movement of the benzyl group.

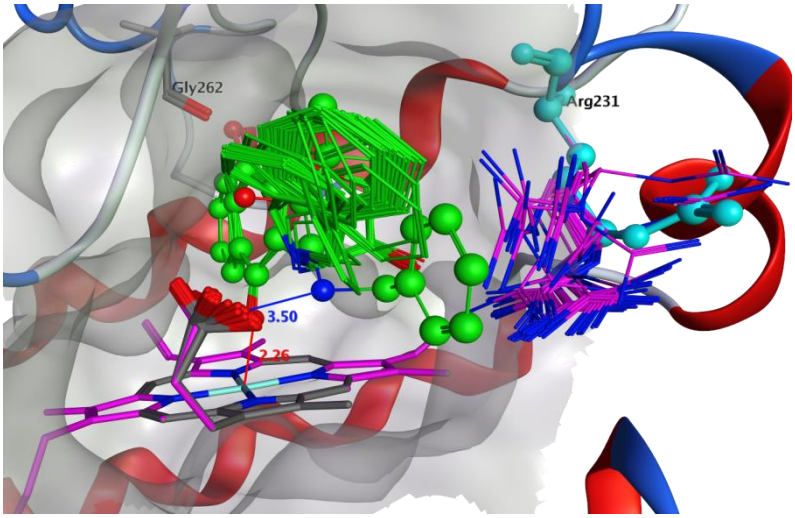


Figure S2: *In silico* 'What-if' docking simulation results for **2** with the 5-oxo group as the haem ligand. **2** drawn with carbons (green); ball and stick atoms represent the starting **2** pose. Carbon atoms (pink) show Arg231 position (light blue ball and stick – starting position) and starting position of haem group. Dark-blue atoms are nitrogen, red atoms are oxygen.

Benzyl: 10-oxo ligation poses

Inspection of the 10-oxo ligation poses (23 total) gives an average distance of 4.2 Å between the **2** amino group and haem carboxylate moiety, unchanged from the starting position. For Arg231 positioning, most structures (14 poses – 61%) remain in a similar position to the starting pose. In 6 structures (26%), Arg231 moves towards the active site. In the remaining 3 structures (13%), Arg231 orientates in a more open position, away from **2** and the active site.

Similarly, the majority of **2** benzyl groups remain in a similar pose to the starting position. In two instances (9%) the benzyl moiety moves towards the Arg231 residue, however proximity to Arg231 remains in only one of these. When compared to 5-oxo ligation poses, the amino moiety in the 10-oxo group has an increased average distance to the haem propanoate and the benzene ring has, on average, an increased distance to Arg231.

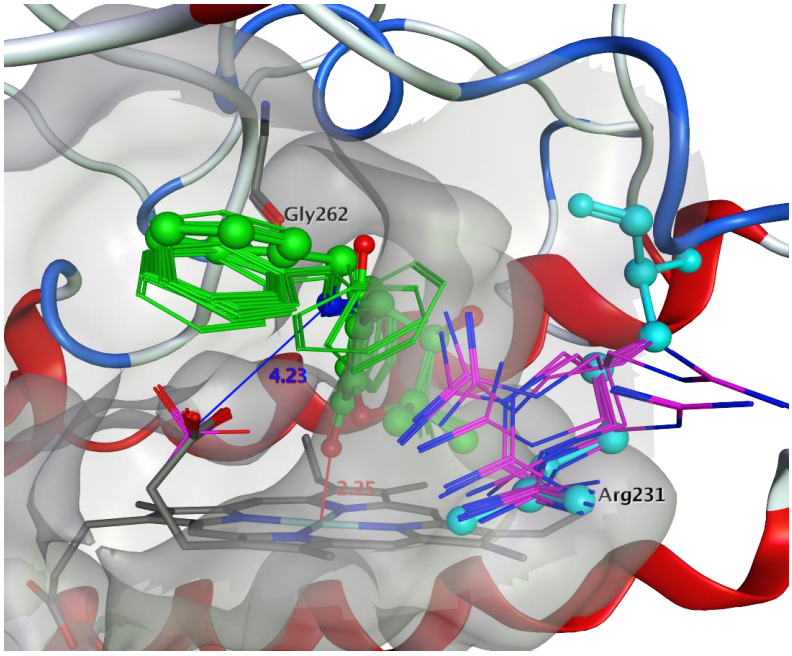


Figure S3: *In silico* 'What-if'docking simulation results for **2** with the 10-oxo group as the haem ligand. **2** drawn with green carbons; ball and stick atoms represent the starting **2** pose. Pink carbons show Arg231 position (light blue ball and stick atoms – starting position) and starting position of haem group. Dark-blue atoms are nitrogen, red atoms are oxygen.

These results suggest that 5-oxo ligation is the more preferred pose of **2** in the current crystal structure due to increased interaction with both the haem propanoate arm and Arg231. The benzyl group extends into the active site entrance void (in a pose consistent with observed SAR data[2]) and may interact with Arg231 *via* a cation-pi interaction.

4-Substituted Adamantyl-Pyranonaphtoquinones

Compared to the benzyl-substituted compounds, the 4-substituted adamantyl-pyranonaphthoquinone structures gave rise to fewer poses within the IDO1 active site (6 for ligation through the 5-oxo atoms and 16 for ligation through 10-oxo atoms). This is most likely due to the bulk of the ligand's adamantane cage, which is less able to freely rotate within the IDO1 active site entrance void, when compared to the benzyl ring of **2**, producing fewer simulated docking poses. However, modelling simulations indicate that bulky substituents (of a similar volume to a *closo*-carborane cage) at the 4-position of the pyranonapthoquinone framework are accommodated by the IDO1 active site entrance void, in both 5-oxo and 10-oxo ligation poses.

Adamantyl: 5-oxo ligation poses

Inspection of the 5-oxo haem-bound ligand poses demonstrates little variation in adamantyl-position from the initial docking pose (Figure S4). The average distance between the protonatable amino moiety of the ligand and the haem propanoate arm is 4.5 Å, the same as the initial docking position. The ligand hydroxyl group maintains a distance to the IDO1 backbone Gly262 carbonyl group of 2.8 Å and Arg231 remains in an open position (*i.e.* does not move to interact with the 5-oxo adamantyl-ligand as observed in the 5-oxo benzyl-ligand analogue (Figure S2)).

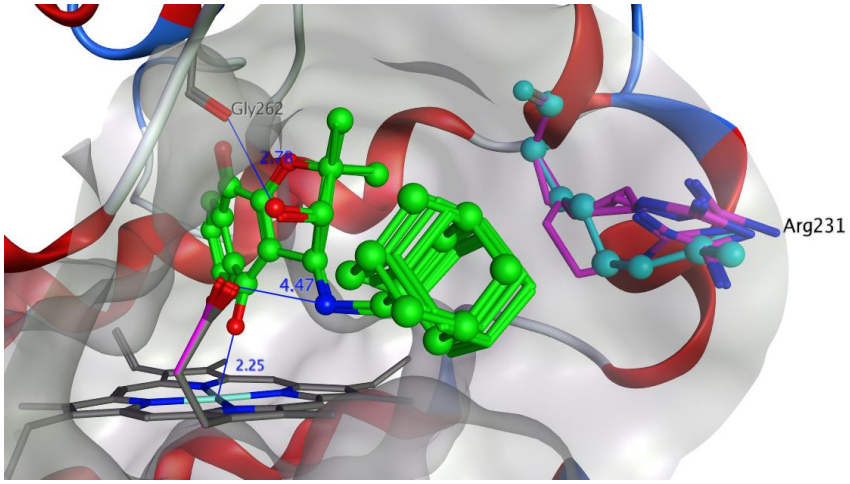


Figure S4: *In silico* 'What-if' docking simulation results for 4-substituted adamantyl-pyranonaphtoquinone with the 5-oxo group as the haem ligand. Adamantyl- pyranonaphtoquinone drawn with green carbons; ball and stick atoms represent the starting pose. Pink carbons show Arg231 position (light blue ball and stick atoms – starting position) and starting position of haem group. Dark-blue atoms are nitrogen, red atoms are oxygen.

Adamantyl: 10-oxo ligation poses

In contrast, 10 of 16 (62.5%) 10-oxo haem-bound ligation poses show movement in Arg231 towards the (now re-orientated) hydroxyl group of the 4-substituted adamantyl-pyranonaphthoquinone, indicating an increased interaction between the ligand and Arg231. However, little variation in the position of the adamantyl moiety, or the protonatable amino group was observed. All (16) ligand poses maintain ~3.5 Å between the ligand's amino-group and the haem propanoate arm.

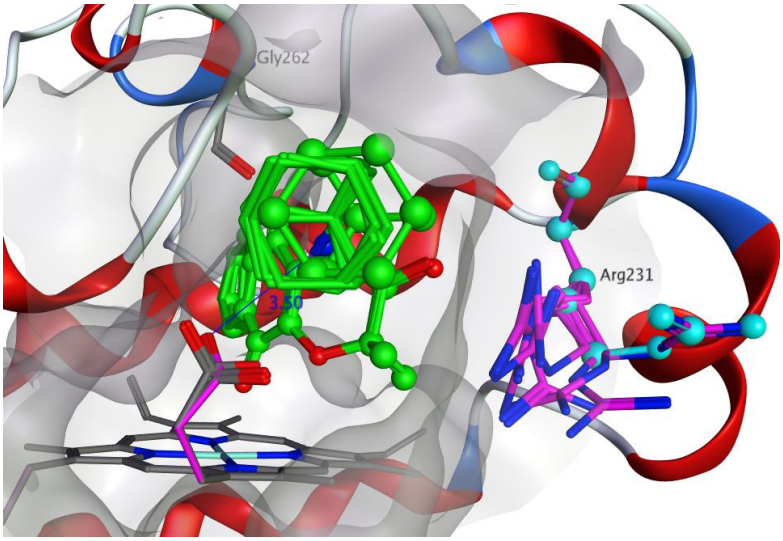
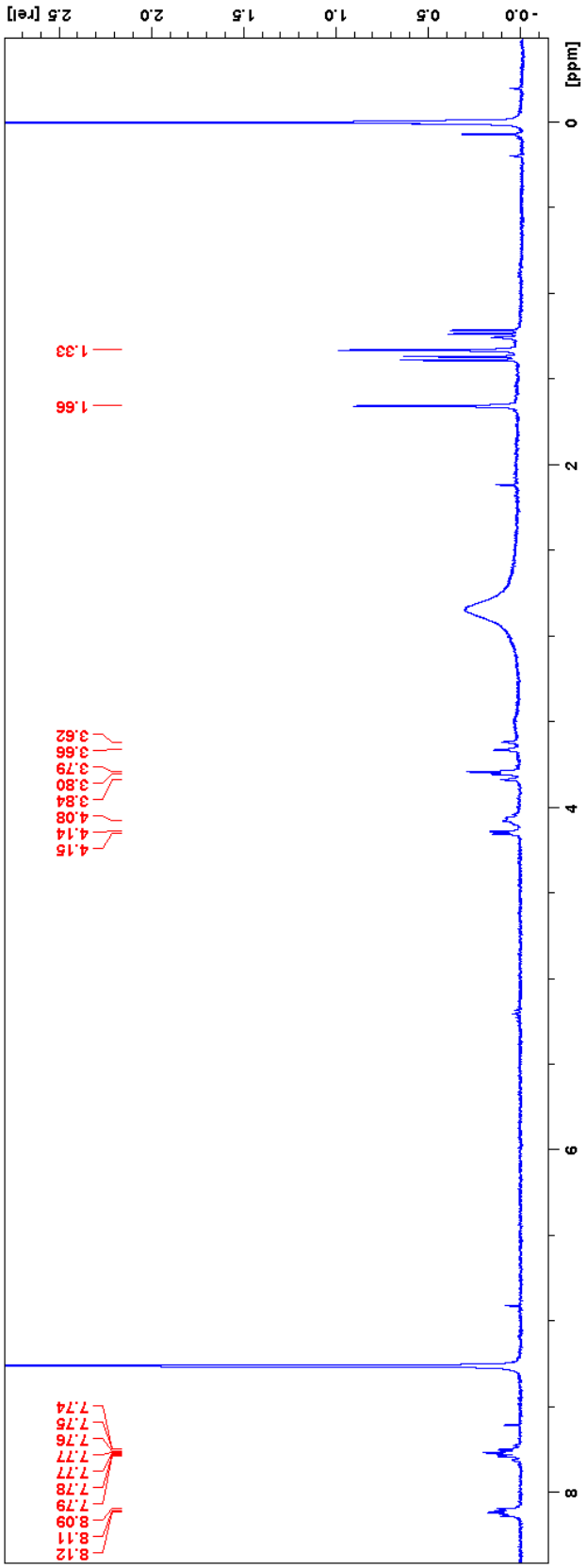


Figure S5: *In silico* 'What-if' docking simulation results for 4-substituted adamantyl-pyranonaphtoquinone with the 10-oxo group as the haem ligand. Adamantyl- pyranonaphtoquinone drawn with green carbons; ball and stick atoms represent the starting pose. Pink carbons show Arg231 position (light blue ball and stick atoms – starting position) and starting position of haem group. Dark-blue atoms are nitrogen, red atoms are oxygen.

Interestingly, our modelling suggests that the 10-oxo ligation position is favoured in the adamantyl-substituted ligand docking (16 poses vs. 6 poses for the 5-oxo adamantyl- ligand); which is in contrast with the benzyl-substituted **2** simulations, in which 5-oxo heme ligation was the preferred docking orientation. Additionally, for both 5-oxo and 10-oxo adamantyl docking, the cation-pi interaction between ligand and protein (specifically to Arg231) observed in docked benzyl-substituted pyranonapthoquinoes is lost. The small reduction in IDO1 inhibitory activity observed in our carborane derivatives **5** and **6** (when compared to the benzyl equivalents **2** and **3**, respectively) may be due to the loss of this important ligand-protein interaction.

NMR Spectra

Compound 5: ^{1}H NMR



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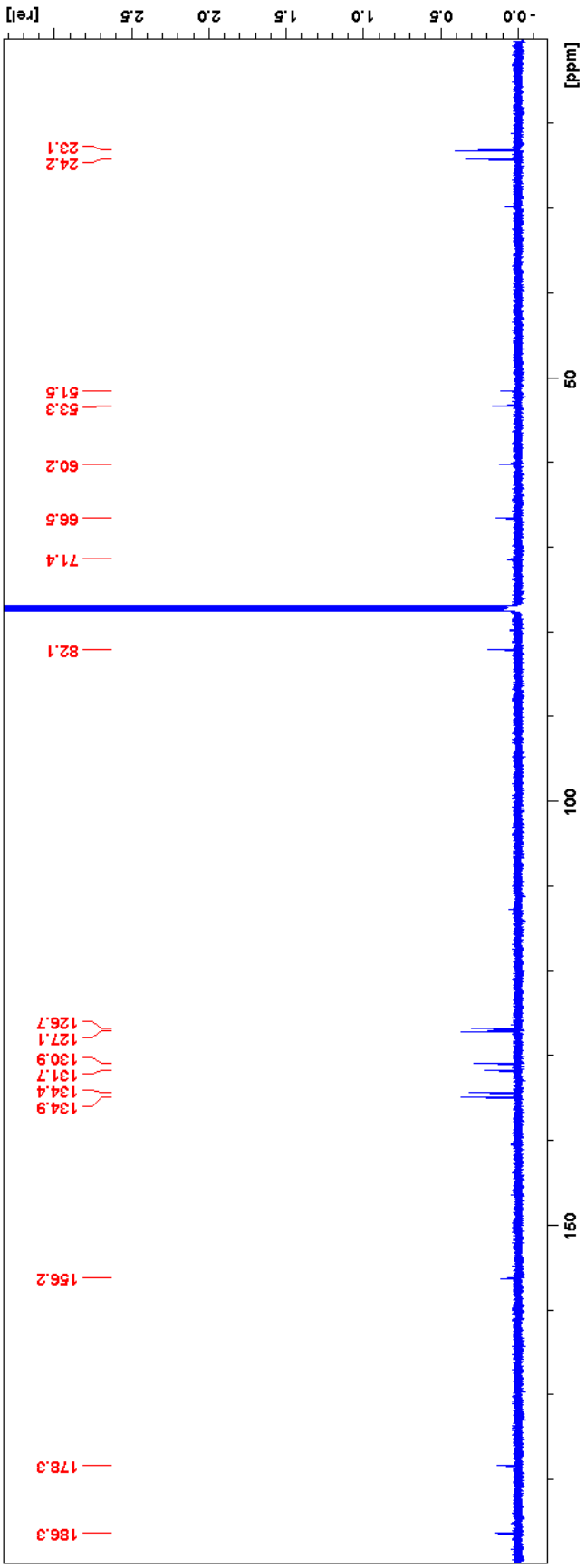
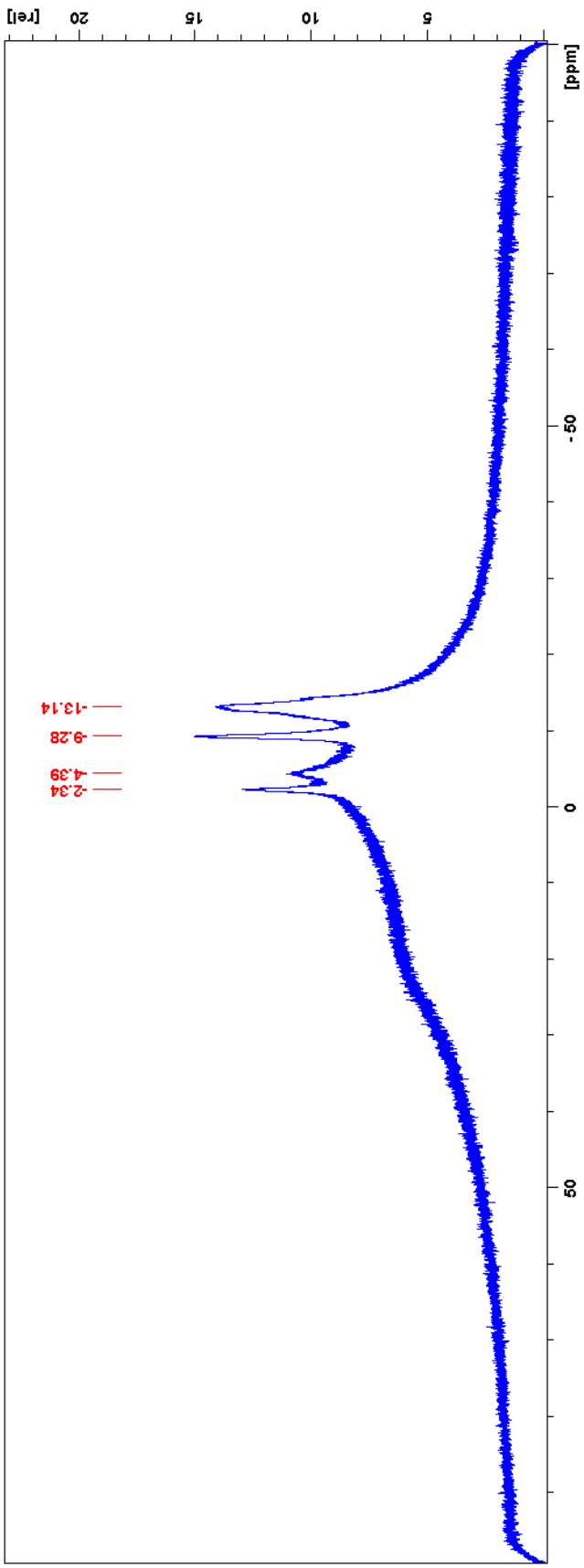


Compound 5: ^{11}B NMR



Compound 5: HR-ESI-MS

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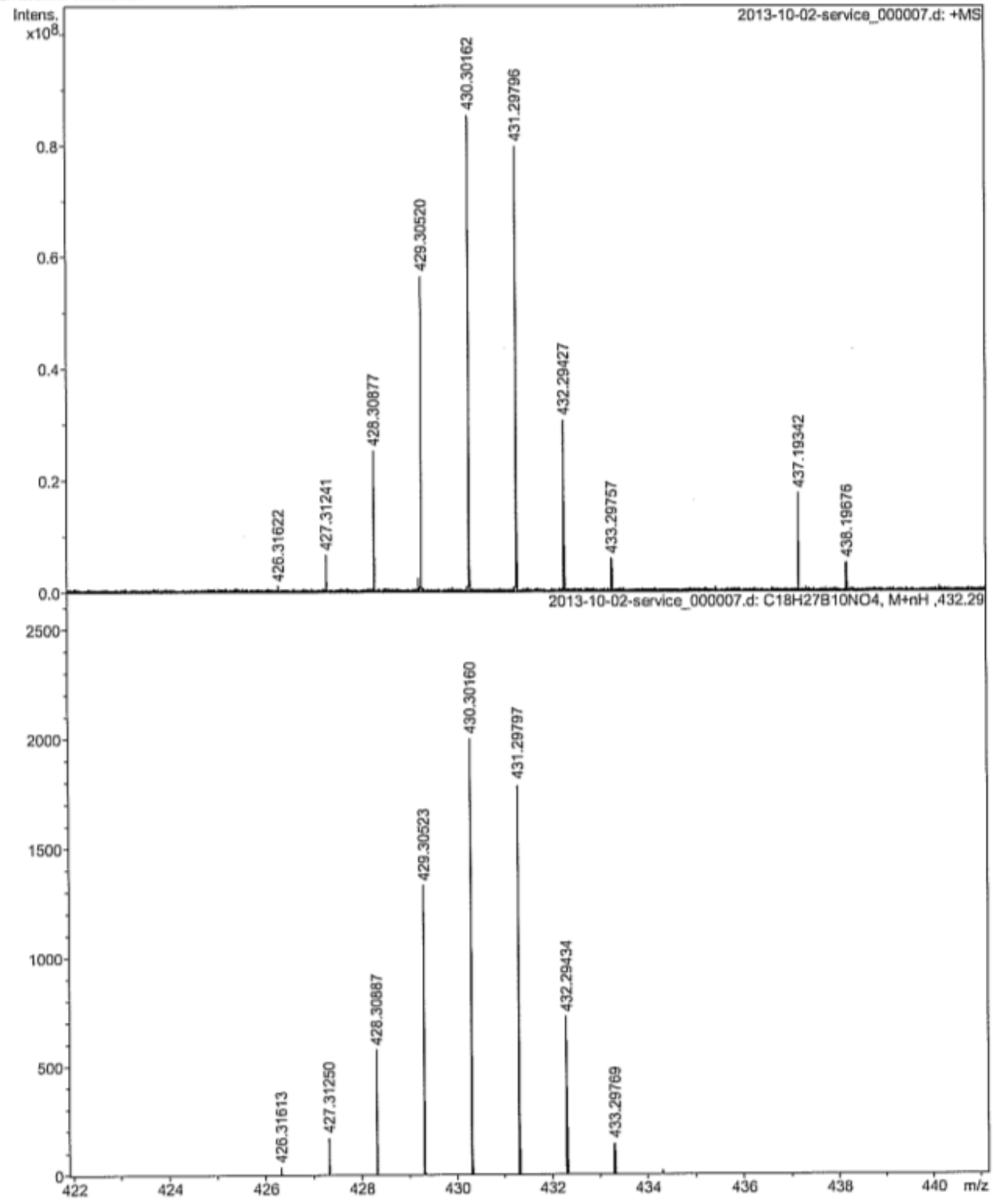
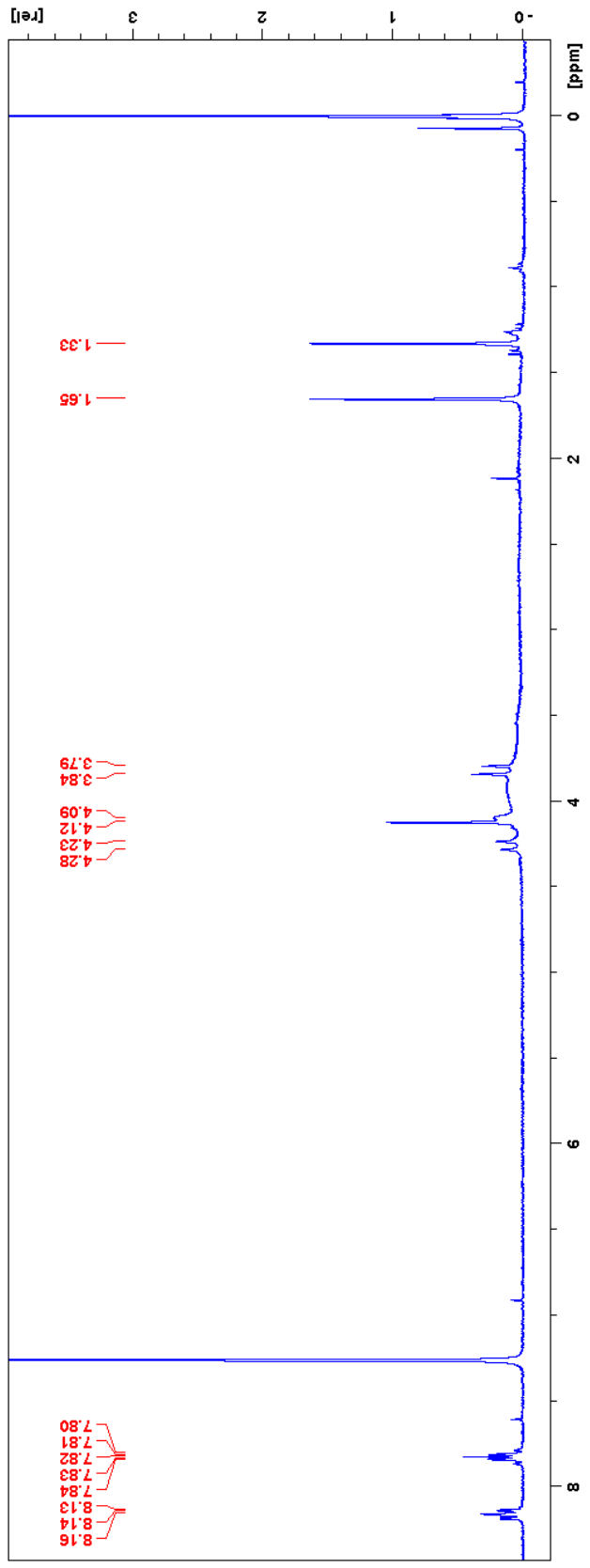
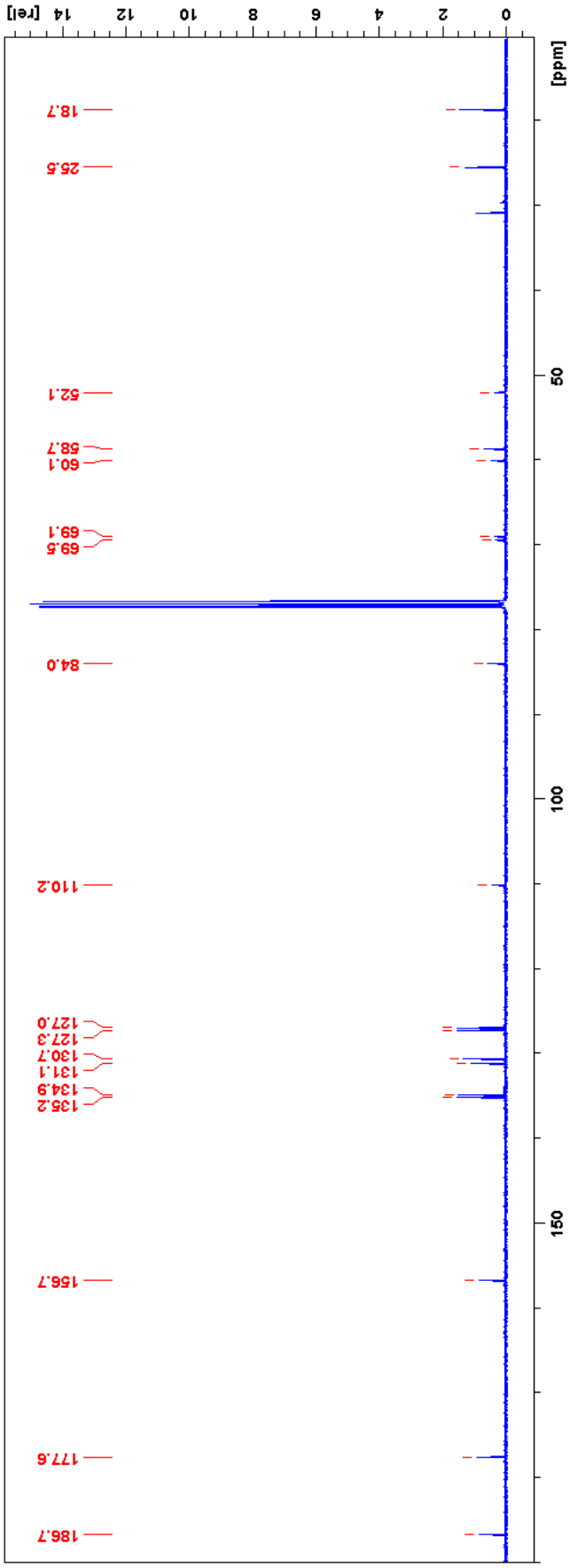


NMR Spectra

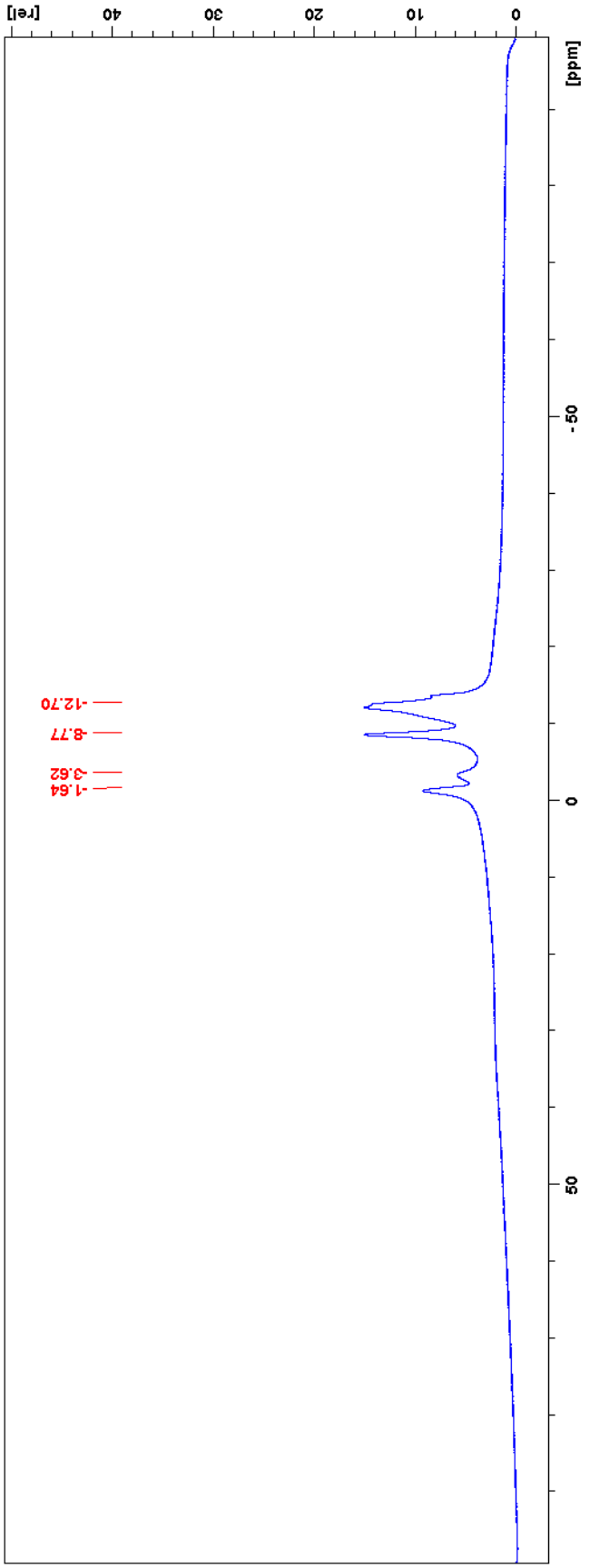
Compound 6: ^{1}H NMR



Compound 6: ^{13}C NMR



Compound 6: ^{11}B NMR



Compound 6: HR-ESI-MS

Generic Display Report

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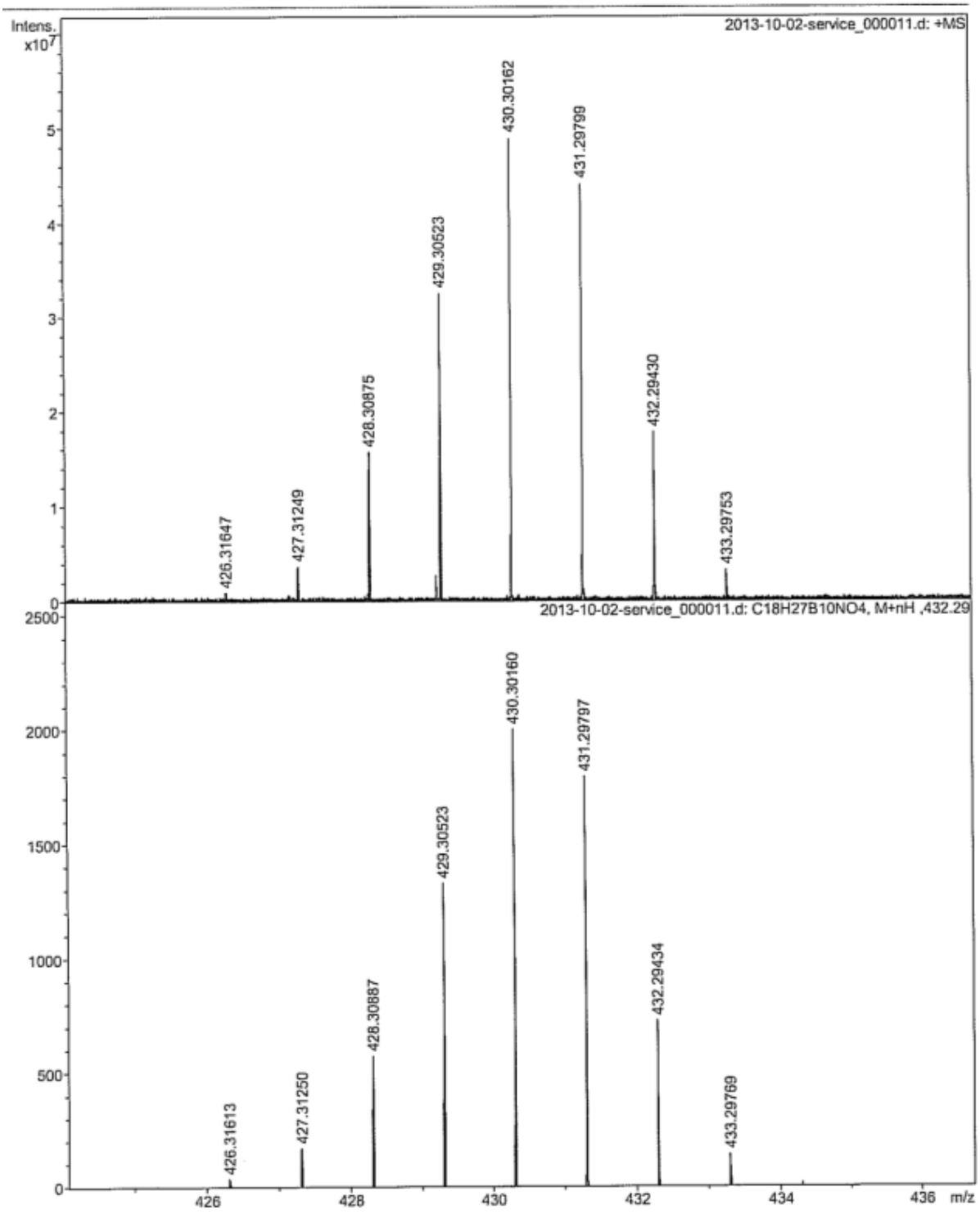

References

1. H. Sugimoto, S. Oda, T. Otsuki, T. Hino, T. Yoshida and Y. Shiro, *Proc. Natl. Acad. Sci. U.S.A.*, 2006, **103**, 2611-2616.
2. S. Kumar, W. P. Malachowski, J. B. DuHadaway, J. M. LaLonde, P. J. Carroll, D. Jaller, R. Metz, G. C. Prendergast and A. J. Muller, *J. Med. Chem.*, 2008, **51**, 1706-1718.