Utilizing Fluorine-labelled 2-Aminobenzamidoximes to Distinguish Aldoses via ^{19}F-NMR

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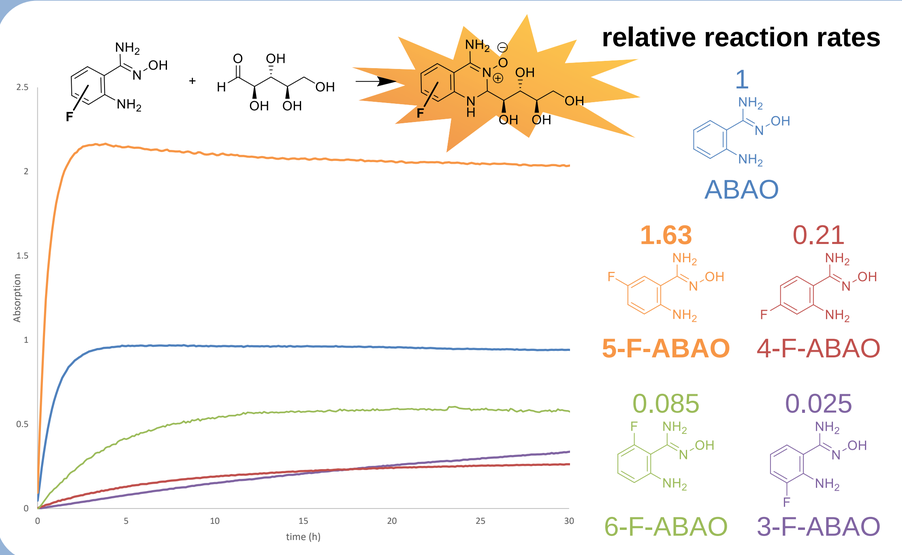
ABAOs and sugars

Aldoses are a group of sugars distinguished only by their many stereocenters, leading to different properties and reactivities. Here we demonstrate a simple approach to differentiate such sugars in a mixture.

The aldehyde-selective reagent 2-aminobenzamidoxime (ABAO) was introduced by Kitov et al.[1] for the quantification of aldehydes with a range of interesting applications.[2] We recently employed the reaction of ABAO with aldoses for the determination of their open-chain-contents based on a kinetic assay.[3] Now we set out to differentiate the formed products of different sugars in ^{19}F-NMR by introducing a fluorine label on ABAO. ^{19}F-NMR exhibits high sensitivity, a wide span of shifts and produces one signal per F-ABAO-aldose adduct, which are excellent traits for the purpose we envisioned.

Optimization of F-label position



relative reaction rates

1 — ABAO

1.63 — 5-F-ABAO

0.21 — 4-F-ABAO

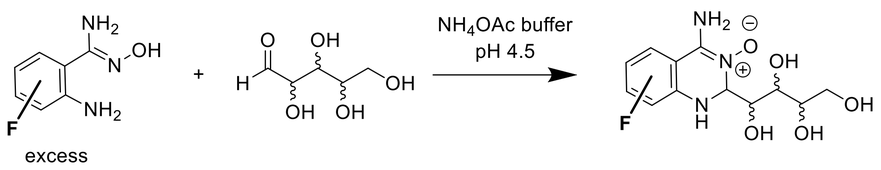
0.085 — 6-F-ABAO

0.025 — 3-F-ABAO

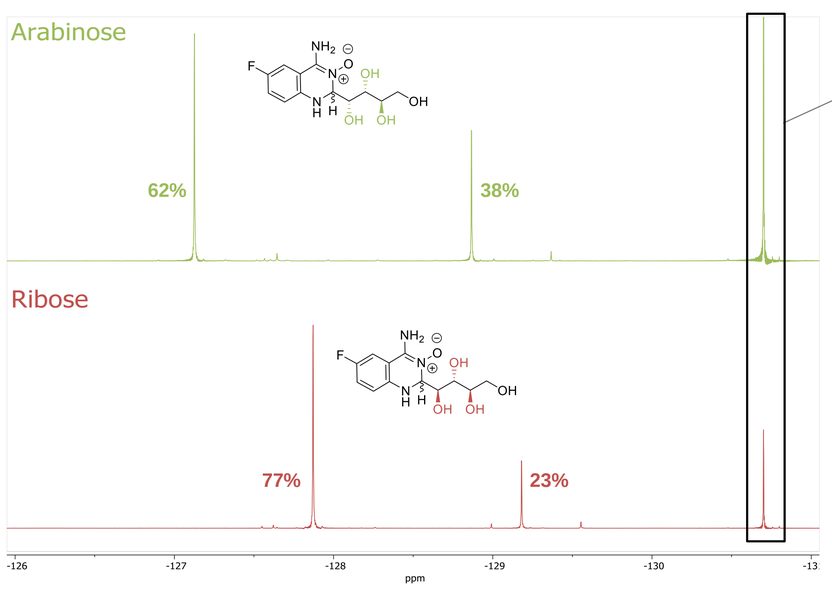
Aiming for the best suited F-ABAO, we first set out to find the ideal position for the fluorine label of the four possible ones. One important factor to consider is the reaction speed of the four candidates. Reaction rates of ABAO derivatives were compared by tracking the rate of adduct formed with ribose by measuring the absorption of the strongly absorbing product at 405 nm. This revealed significant differences not only in reaction speed, but also in the final absorption maximum, as shown in the graph on the left.

5-F-ABAO is the fastest reacting of the four derivatives, even outperforming unlabeled ABAO. Comparing ^{19}F-NMR spectra of F-ABAO adducts with different sugars also revealed 5-F-ABAO to be the best suited candidate in respect to the separation of peaks, making it the clear favorite for this application.

The idea at a glance



^{19}F-NMR



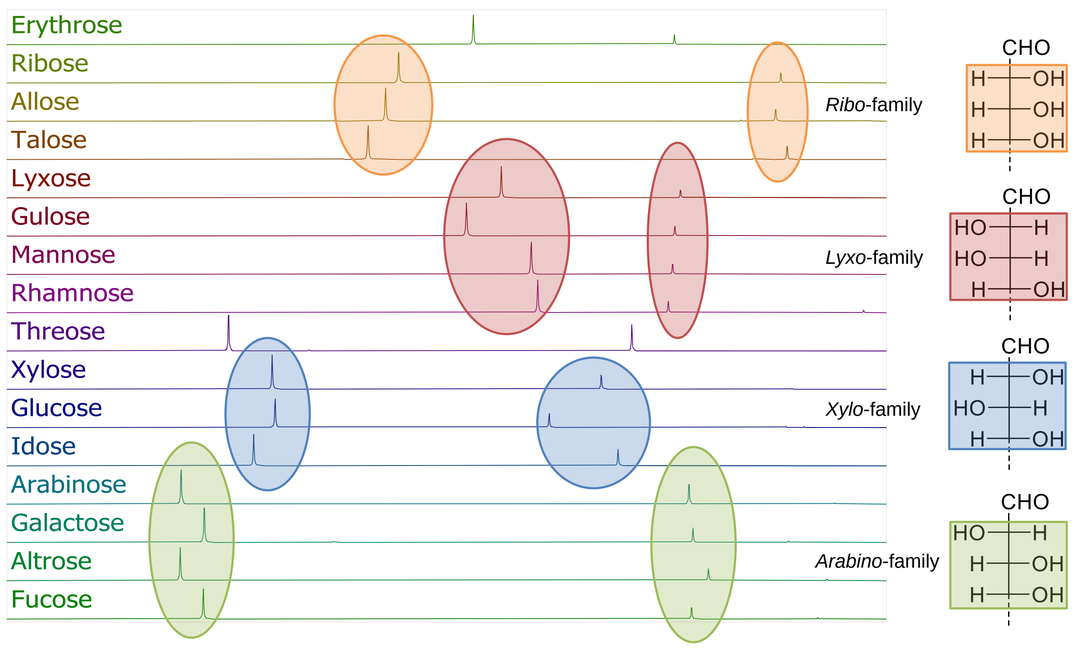
An excess of F-ABAO is reacted with an aldose in NH_4OAc buffer. ^{19}F-NMR spectra are recorded either directly in the deuterated reaction solvent (D_2O buffered with NH_4OAc) or in DMSO-d6 after lyophilization.
The ^{19}F-NMR spectra on the right are the adducts of ribose and arabinose with 5-F-ABAO, measured in DMSO.
Noteworthy, during the reaction a new stereocenter on the former aldehyde position is formed, resulting in two diastereomers visible in ^{19}F-NMR. As shown with arabinose and ribose, the observed ratios of these diastereomers vary from sugar to sugar.

5-F-ABAO is added in excess to ensure a fast reaction and complete conversion. Additionally, the signal of the remaining unreacted 5-F-ABAO can be used as an internal standard in ^{19}F-NMR.

Conclusion:
We found that 5-F-ABAO is able to distinguish the first three stereocenters of sugars (shown below), which is especially impressive because these stereocenters have a distance of 8 atoms between them and the F-label.

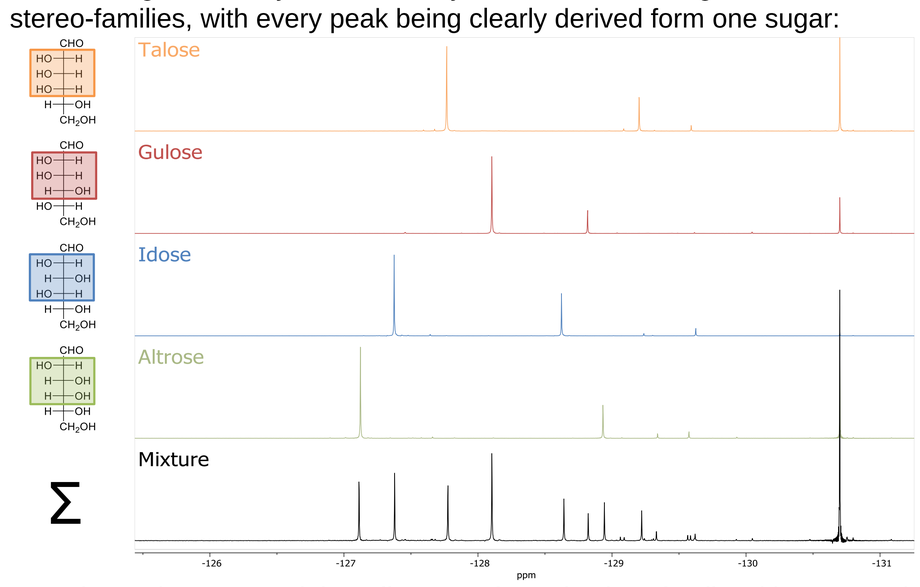
Sugars cluster into stereo-families

All four F-ABAO derivatives were reacted with all standard aldotetroses, -pentoses, -hexoses and the 6-deoxy-sugars fucose and rhamnose.
^{19}F-NMR spectra were recorded either with DMSO-d6 or buffered D_2O as solvent.
The best performing solvent-derivative combination for differentiating the various sugar-adduct shifts was utilizing 5-F-ABAO and recording in DMSO-d6 (shown below). Sugars within the same stereo- family are grouped together within a small range of chemical shifts, enabling the classification of an unknown sugar into a specific stereo-family.

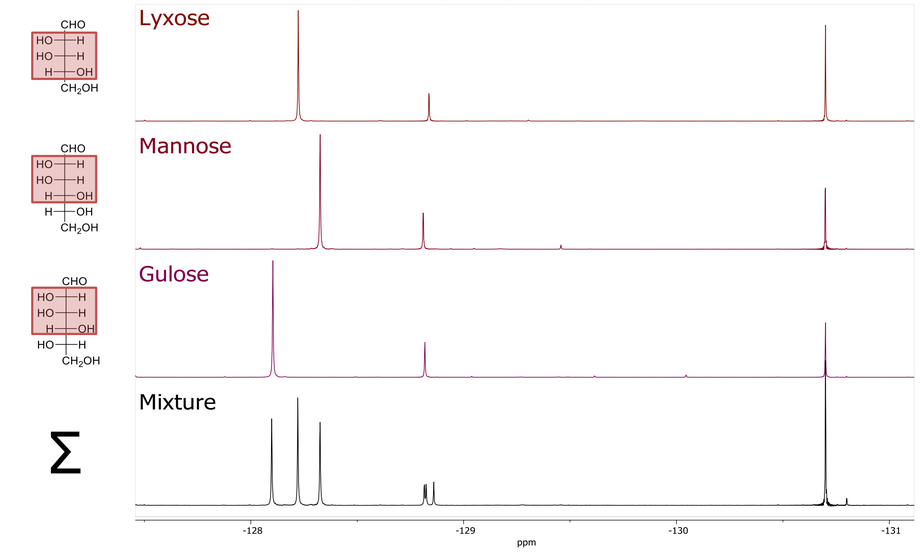


Challenging mixture analysis

Showcasing the ability of our assay to differentiate sugars from different stereo-families, with every peak being clearly derived form one sugar:



Even in a mixture containing all sugars from the *lyxo*-family with two peaks overlapping, at least one diagnostic peak per sugar remains.



Acknowledgements

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1. Kitov, P. I.; Vinals, D. F.; Ng, S.; Tjhung, K. F.; Derda, R., *J Am Chem Soc* **2014,** *136* (23), 8149-8152.
2. Ressmann, A. K.; Schwendenwein, D.; Leonhartsberger, S.; Mihovilovic, M. D.; Bornscheuer, U. T.; Winkler, M.; Rudroff, F., *Advanced Synthesis & Catalysis* **2019,** *361* (11), 2538-2543.
3. Kalaus, H.; Reichetseder, A.; Scheibelreiter, V.; Rudroff, F.; Stanetty, C.; Mihovilovic, M. D., *European Journal of Organic Chemistry* **2021,** *2021* (18), 2589-2593.