F-LABELLED 2-AMINOBENZAMIDOXIMES AS ALDEHYDE-SELECTIVE PROBES FOR ^{19}F-NMR BASED QUALITATIVE ANALYSIS OF ALDOSES

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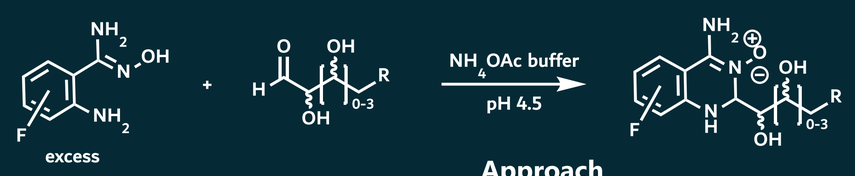
The idea at a glance



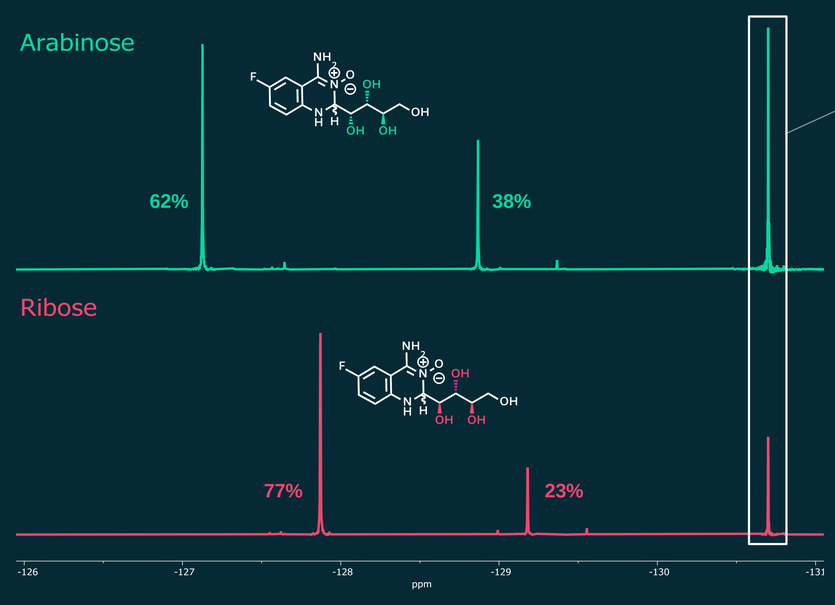
Approach

Utilizing sensitive ^{19}F-NMR, we sought out to apply F-labeled ABAOs (2-aminobenzamidoximes) as probes for the qualitative analysis of aldose mixtures. We systematically examined all four possible F-ABAO derivatives with a broad range of aldoses to find the best suited F-ABAO regarding peak separation and reaction kinetics. As exemplified with arabinose and ribose on the right, reaction with 5-F-ABAO leads to distinctive peaks in ^{19}F-NMR even though there are only subtle differences in their stereochemistry.

Result

We found that 5-F-ABAO can distinguish aldoses with subtle differences, with differing stereocenters 8 atoms apart from the F-label. Additionally, 5-F-ABAO is the fastest reacting F-ABAO derivative.

^{19}F-NMR



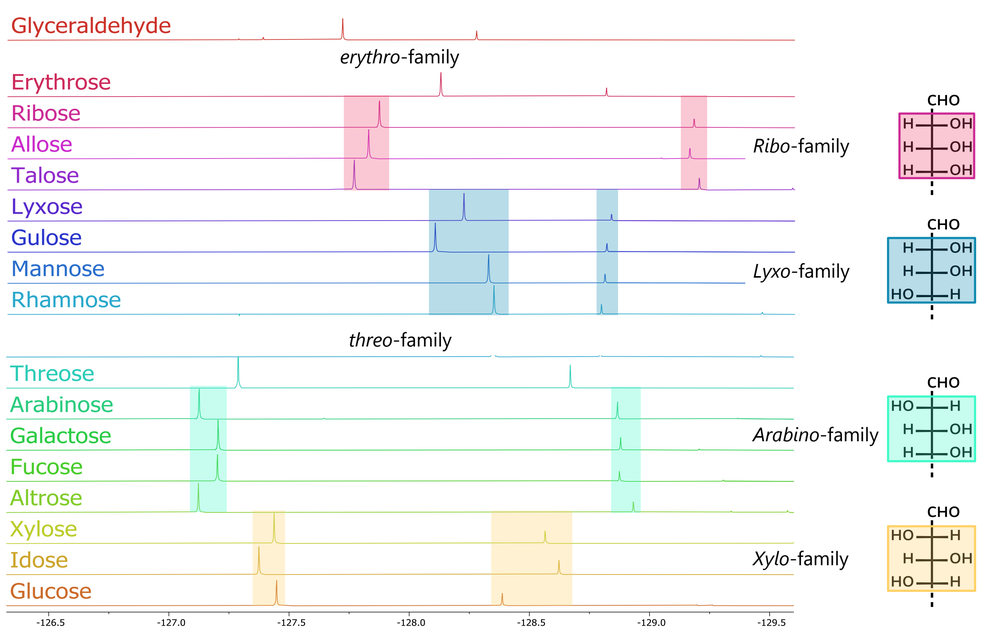
5-F-ABAO is added in excess to ensure a fast reaction and to serve as an internal standard to calibrate the chemical shift.

Motivation

Aldoses are important chiral natural compounds distinguished by their many stereocenters, leading to different properties and reactivities. 2-aminobenzamidoxime (ABAO) is an aldehyde-selective reagent introduced by Kitov *et al.*[1] We recently employed the reaction of ABAO with aldoses to determine their open-chain-contents *via* a kinetic assay.[2] Here we demonstrate a simple qualitative approach to differentiate aldoses in a mixture. Introducing a fluorine label enables the use of sensitive ^{19}F-NMR.

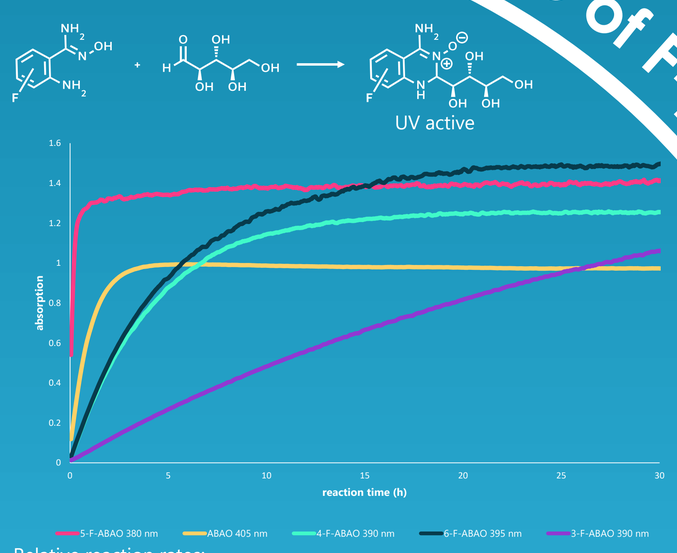
Sugars cluster into *stereo*-families

The fluorine label was tested on all four possible aromatic positions, shown here are the results of the best candidate: the spectra of **5-F-ABAO** reacted with a broad range of aldoses. Subtle differences in stereochemistry lead to different chemical shifts in ^{19}F-NMR, making the sugars distinguishable. The peaks of the sugars are grouped into their "stereofamilies".

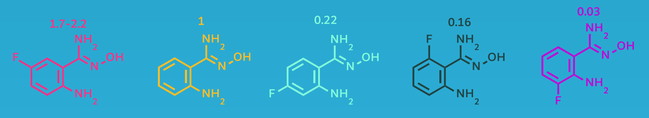


All F-ABAO derivatives were reacted with glyceraldehyde, all standard aldotetroses, -pentoses, -hexoses and the 6-deoxy-sugars fucose and rhamnose in aqueous buffer. Water was removed *via* lyophilization and ^{19}F-NMR spectra were recorded in DMSO-d6.

Kinetic influence of F-position

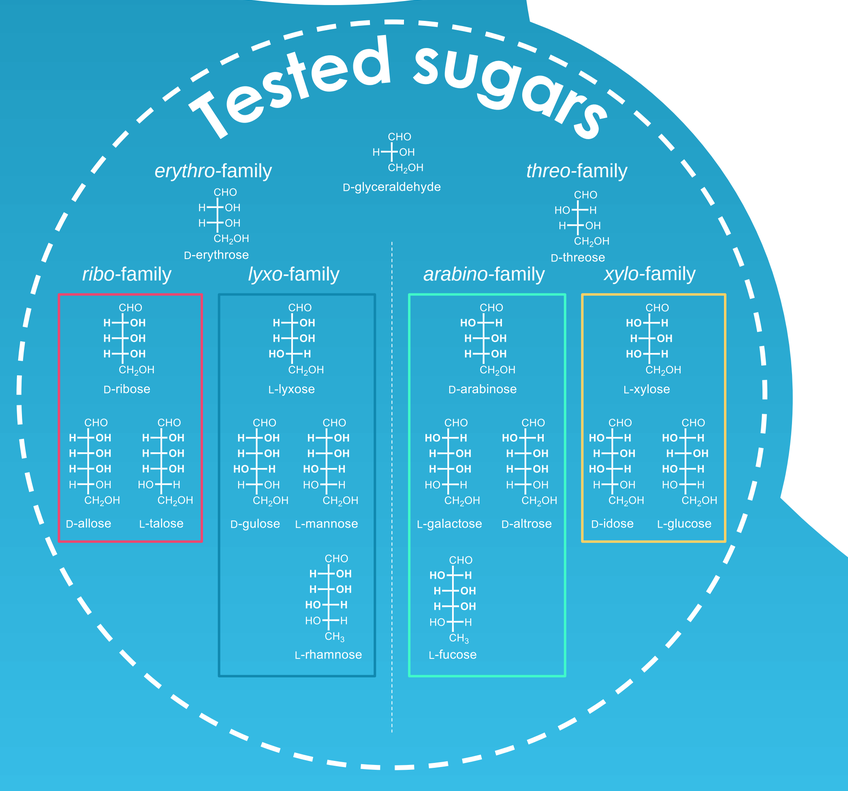


Relative reaction rates:



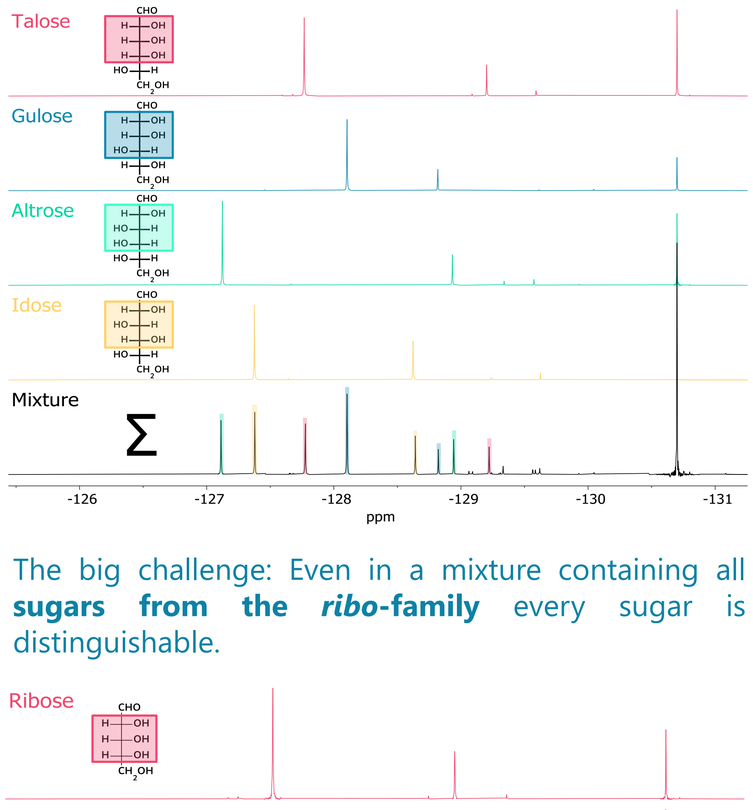
An important factor to consider is the reaction speed of the four candidates. Reaction rates of ABAO derivatives were compared by tracking the rate of adduct formed with ribose by measuring the absorption of the strongly absorbing product at various wavelengths. This revealed significant differences in reaction speed as shown on the graph above. **5-F-ABAO** is the fastest reacting of the four derivatives, even outperforming unlabeled ABAO. As **5-F-ABAO** is also the best suited candidate in respect to the separation of ^{19}F-NMR peaks, it is the clear favorite for this application.

Tested sugars



Mixture analysis

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



The big challenge: Even in a mixture containing all **sugars from the *ribo*-family** every sugar is distinguishable.

1. Kitov, P. I.; Vinals, D. F.; Ng, S.; Tjhung, K. F.; Derda, R., *J Am Chem Soc* **2014,** *136*, 8149-8152.
2. Kalaus, H.; Reichetseder, A.; Scheibelreiter, V.; Rudroff, F.; Stanetty, C.; Mihovilovic, M. D., *Eur. J. Org. Chem.* **2021,** *2021*, 2589-2593.

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