Facile Purification of large-scale steric selected bicyclic carbohydrates by SepaBean™

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Chromatography Application Note ANSS-003

Carbohydrates $C_n(H_2O)_n$ are the most abundant class of organic compounds found in living organisms and a major source of energy. They are formed in plants by photosynthesis, a chemical reaction powered by sunlight using CO_2 and H_2O as starting materials. Carbohydrates are vital for plants and animals, either as an energy source or building blocks.

The photosynthesis stores energy in the chemical bonds of carbohydrate (glucose), When glucose is broken down by cellular respiration or other processes, the energy stored in the chemical bonds are converted into the primary fuel molecule for cells, ATP.

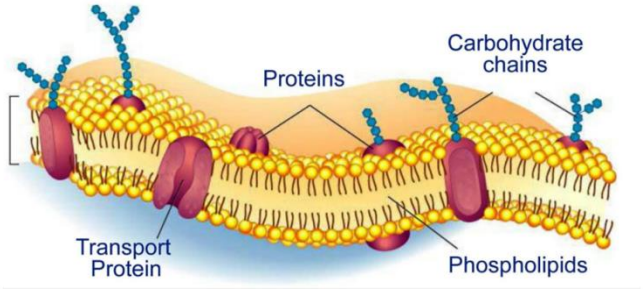


Figure 1. Proteins are embedded in the cell membranes. Carbohydrate chains which are attached to some proteins help cells recognizing and communicating with each other.

Carbohydrates are also an essential building block for life: 1. Cellulose, chitin, and peptidoglycans are tough fibrous structural carbohydrates with cross-linked hydrogen or peptide bonds; 2. Ribose, a 5-carbon cyclic carbohydrate is the backbone of RNA; 3. A group of carbohydrates and their derivatives are identity markers on the outer surface of many cells, which are essential in multicellular organisms recognizing and communicating with each other. (**Figure 1**).[1]

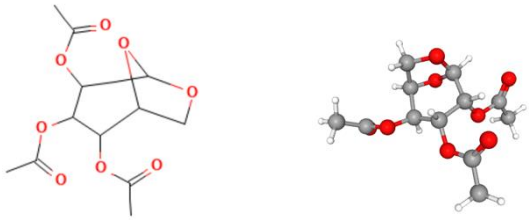


Figure 2. The 3D chemical structure and crystal structure of an important 1,6-anhydrohexopyranoses: levoglucosan, triacetate.

Because of the important functions of carbohydrates, many efforts in the chemistry community have been dedicated to their synthesis, and the synthesis of their derivatives. 1,6-anhydrohexopyranoses have proven to be a very important basic building block of various carbohydrate derivatives. For example, they can be synthetically converted to rifamycin S, indanomycin, thromboxane B2, (+)-biotin, tetrodotoxin, quinone, and macrolide antibiotics. One of the 1,6-anhydrohexopyranoses with three acetate, levoglucosan triacetate (3,4-diacetyloxy-6,8-dioxabicyclo[3.2.1]octan-2-yl) acetate) has been first crystalized in 1974[2], by *Leung et al*, to study their steric and strain factors contribute to the selective opening of the anhydro ring, as shown in **Figure 2**. Its [3.2.1]bicyclic framework imposes high stereo- and regioselectivities, and the 6-member pyranose ring is locked in the $_1C^4$ conformation creates stereocenters.[3]

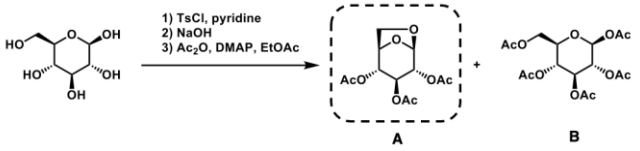
As shown below, *Zottola et al* have developed a very

[1] https://rwu.pressbooks.pub/bio103/chapter/carbohydrates

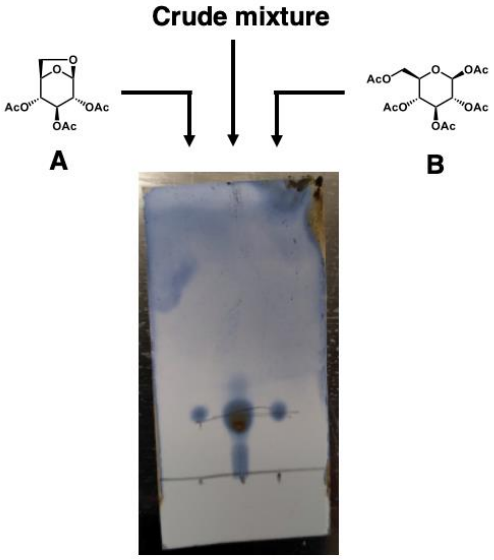
[2] F Leung; R H Marchessault *Can. J. Chem* V52 N13 (**1974**) 2516.

[3] Mark A Zottola; Ricardo Alonso; Gregory D Vite; Bert Fraser-Reid *J. Org. Chem.* V54 N26 (**1989**) 6123.

simple gram-scale synthesis for levoglucosan triacetate **A**.



Following Zottola's procedure, the synthesis of **A** on the multi-gram scale, only yield less than 50% yield of **A**, therefore no crystallization occurs. The by-product **B** co-eluted on normal phase silica TLC (solvent, 60% hexane: 40% ethyl acetate).



Therefore, we chose to use SepaBean™ automatic flash chromatography system with SepaFlash™ reverse phase C18 column (UltraPure irregular C18, 40-63 µm, 60 Å, carbon content 17%, end-capped, surface area 500 ㎡/g). As shown in the graph below, 1.5 g of the crude mixture was loaded on an 80g C18 column, about 1.8% sample loading, with 75% water and 25% acetonitrile as the solvent mixture, target compound **A** was eluted within the first 12 mins, followed by the by-product **B**.

More than 85% purity of **A** was obtained, which crystallized out readily from methyl tert-butyl ether, yielding a final product >99% pure. Proton NMR was used for the determination of sample purity.

This application note is a good example that automatic flash chromatography SepaBean™ is a facile and simple purification method. In this example

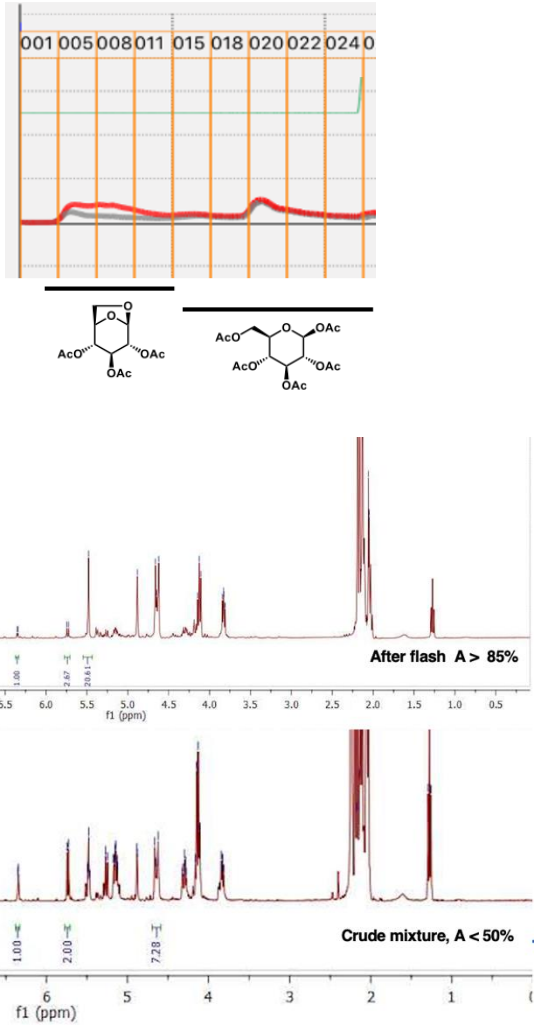


*Figure 4. Proton NMR of crude mixture with less than 50% compound **A** before flash chromatography; compound **A** with more than 85% purity after the flash purification.*

here, two carbohydrates cannot separate on regular phase silica TLC, however, flash chromatography with our workhorse reverse phase C18 column can readily separate those two compounds. No complicated method development is necessary. Within 20 min, the product is isolated with a molar purity of 85%. On an 80g SepaFlash™ C18, recommended sample loading is between 105 mg ~ 2.1 g. Research scientists can quickly load a relatively large quantity of samples on a big column. It is important to note that the C18 column is reusable 40~60 times under proper storage. Flash chromatography is a powerful tool for the purification of carbohydrates and many other synthetic compounds.

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