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Probing the limitations of the fluorous content for tag-mediated microarray formation†

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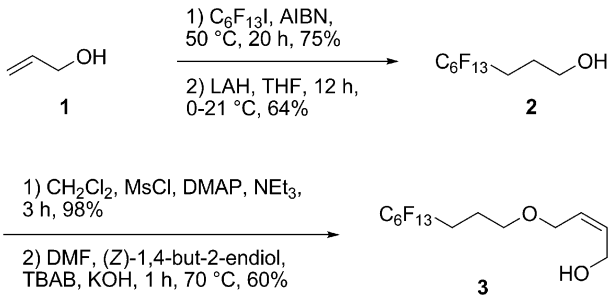
The synthesis of a di-perfluorohexyl tag is reported for use in a fluorous-based carbohydrate microarray. A comparative microarray study with this di-perfluorohexyl tag and a mono-perfluorooctyl and mono-perfluorohexyl tag found the increased fluorous content conducive to better spot morphology and easier washing protocols without precluding reuse of the fluorous slide.

Perfluorinated compounds (PFCs) have been extensively used in the past decade in commercial, industrial, and research studies, but now PFCs are becoming a major environmental concern.[1–7] The most environmentally persistent PFCs are perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). Recent studies have found that the growing amounts of PFOS and PFOA found in water correlate with the bioaccumulation of PFCs in human and animals globally.[1–7] However, unlike the longer PFCs, C6 and shorter perfluorous chains are not environmentally persistent.[8] Growing safety and health issues with these 8-carbon-length fluorocarbons threaten their continued bulk manufacturing and therefore also the inexpensive use of this fluorous chain length in a variety of applications in which environmental escape of the PFCs is not a concern. However, such octylfluorous tags have already proven useful not only for efficient separations using fluorous solid phase extraction (FSPE)[9] but also as a convenient fluorous handle for microarray formation.[10] Fluorous microarrays rely on non-covalent fluorous–fluorous interactions between the fluorous tail linked to the molecule used for screening and the fluorous coated glass slide.[11,12] In addition to their use for carbohydrates, such octylfluorous-based microarrays have also been successful for screening other small molecule–protein interactions.[13,14] More recently, biotin was adhered to a fluorous-coated glass slide and it was demonstrated that C_8F_{17}-tagged molecules were better for fluorous biotin–avidin microarrays than the C_6F_{13}-tagged molecules in terms of spot intensity, size and spot morphology.[15] Given that a shorter fluorous tag was likely not an option, we set out to discover a fluorous tag that could form strong enough non-covalent interactions for robust fluorous microarray studies without reliance on the C_8F_{17} motif.



Scheme 1 Synthetic route to the mono-C_6F_{13}-allyl fluorous tag.

First a route to the desired mono-C_6F_{13}-tag was designed based on the synthesis of the known mono-C_8F_{17}-allyl tag for direct comparison.[16] We started the synthetic route with perfluorohexyl iodide as an inexpensive precursor to obtain our desired product **3** (Scheme 1). Radical addition of perfluorohexyl iodide to allyl alcohol followed by reduction of the iodide provided perfluorohexyl alcohol **2**. Alcohol **2** was then mesylated for displacement by (*Z*)-1,4-but-2-endiol to provide the desired allyl tag **3** in 28% overall yield from allyl alcohol.

As one hexylfluorous tag was insufficient for good spot formation on a fluorous slide, we next designed a new tag containing two C_6F_{13}-moieties for direct comparison with the related mono-C_8F_{17}- and mono-C_6F_{13}-tagged carbohydrates described above. Such a di-C_6F_{13}-tag would still need to allow the attached sugar to orient away from the slide surface in such a way that protein-binding could take place. As many sugars come in the form of glycolipids, these structures were seen as inspiration for the design of compound **7** with two fluorous tails attached to a group that served as a UV-active and removable linker (Scheme 2). Using ceric ammonium nitrate[17] the fluorous linker could be readily removed from the di-C_6F_{13}-tagged peracetylated glucose. The desired di-C_6F_{13}-tag synthesis then started with the known diester **4** made by addition of two allyl groups to diethylmalonate followed by radical addition of perfluorohexyl iodide to the resulting alkenes.[18] The presence of the diesters precluded use of lithium aluminium hydride to remove the iodides; therefore, microwave-assisted conditions using zinc in acidic medium were developed for iodide removal to produce, after base-mediated saponification, diacid **5**. The diacid was immediately subjected to heat-mediated

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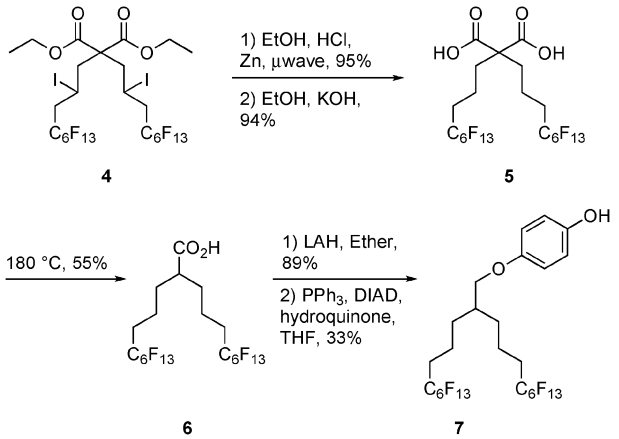

Scheme 2 Synthetic route to the di-C_6F_{13} fluorous tag.

decarboxylation to produce monoacid **6**.[19,20] This monoacid was reduced to the alcohol for a Mitsunobu reaction analogous to those previously carried out with other fluorous alcohols[21] to produce the desired di-fluorous tag **7** in a 14% overall yield from diester **4**. The di-fluorous tag was found to be soluble to at least 1 M concentrations in the common solvents dichloromethane and toluene at room temperature.

With the necessary fluorous tags **3** and **7** in hand, the next step was their glycosylation with peracetylated trichloroacetimidate-activated[22] mannose, glucose, and rhamnose glycosyl donors. After Zemplen deacylation and reduction of the alkene, nine compounds (Fig. 1) were obtained to perform microarray experiments with fluorescein isothiocyanate-labeled concanavalin A (FITC-ConA). ConA is known to bind to terminally α-linked D-mannose, whereas β-D-glucose and α-L-rhamnose are not ligands for this plant lectin.[23] Previously reported FSPE protocols for the octylfluorous tagged monosaccharides were used for the purification of our new fluorous-tagged monosaccharides.[21] Interestingly, the same solvents could be used for eluting the compounds with a single as for a double fluorous hexyl moiety. The aromatic ring also does not override the fluorous content in the FSPE protocol.

In order to compare the performance of the three fluorous-tags on the fluorous-coated glass slide, the carbohydrate microarray study was set up as shown in Fig. 1. To make a 250 μM concentration of the carbohydrates, the fluorous-tagged monosaccharides were dissolved in methanol/DMSO/water (2 : 6 : 2).[24] These monosaccharides were then spotted multiply in groups of nine onto a commercially available fluoroalkylsilane-derivatized glass slide using a standard microarray spotting robot.[24,25] The slides were then incubated with a 200 μM solution of FITC-ConA in phosphate-buffered saline (PBS) for one hour. After incubation, the slide was washed with a 1× PBS containing 1% BSA solution twice and then washed once with distilled water.[26] Next, the slide was scanned using a General Scanning ProScan Array HT at 488 nm to visualize the carbohydrate–ConA binding (Fig. 1). Finally, the data collected from the scan were processed through ImaGene® 8.0 software to obtain the intensities of each fluorous-tagged monosaccharide. (For details of spotting and scanning see ESI.†)

Multiple spotting and scanning experiments revealed several key experimental details. We found that spotting on a new

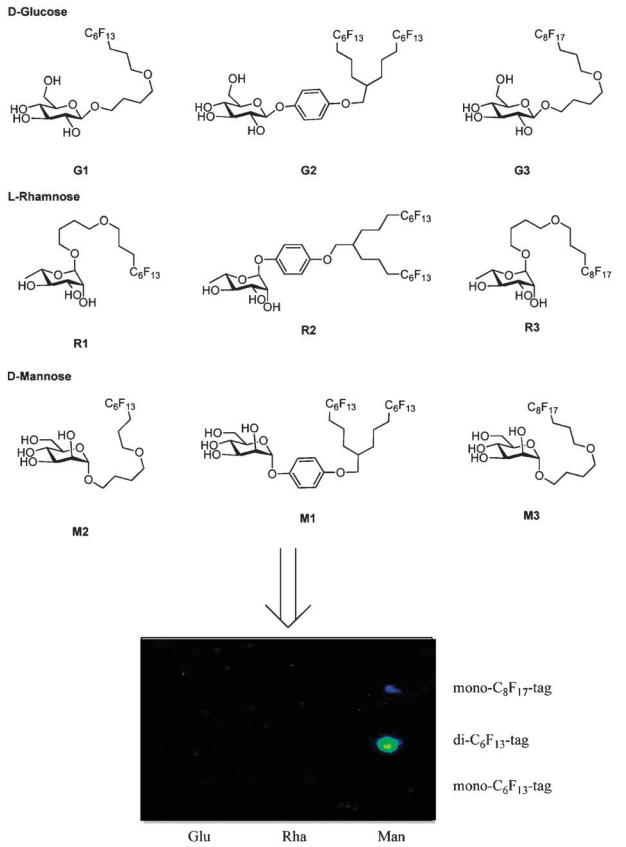

Fig. 1 The nine fluorous linked saccharides that were spotted at 250 μM concentration on the microarray slide and then screened for binding to ConA-FITC. The slide was visualized using a fluorescent scanner at 488 nm.

fluorous glass slide resulted in uneven spots; washing the slides with a 1 : 1 dichloromethane : methanol solution before printing solved this problem. The morphology of the spots was found to also be affected by several physical factors like temperature, humidity and drying time. We printed the slides under three different humidity conditions (60%, 65% and 70% humidity) while maintaining the temperature at 22 °C and observed that the slides printed at 70% humidity showed the best spots with no donut effect.

We speculate that the higher water content in air helps the hydrophilic carbohydrates to better orient on the glass slide to create the non-covalent fluorous–fluorous interactions with the fluorous molecules on the slide. Drying of the slide after printing also had an effect on the spot morphology. Less donut effect was observed when the slides were dried for a longer period of time. After testing for various drying times, we concluded that the slides should be kept in the humidity chamber for 18 hours and then outside of it for 2 hours before incubating.

After optimal spotting and drying conditions were found, comparisons among the three different fluorous tags were made. Fluorescence scans after various washing protocols show that the di-C_6F_{13}-tag-containing sugars were robust and could withstand more than two washings with 1× PBS containing 1% BSA. In many cases, the mono-C_6F_{13}-tag containing sugars were being washed away when washed more than

once with the PBS solution containing BSA, whereas the intensity of the di-C_6F_{13}-tag containing sugars was the same even after washing multiple times. There was only a slight decrease in the intensity of the $-C_8F_{17}$ tag containing sugars with multiple washings with PBS as shown by the scans. Fig. 1 shows all nine of the fluorous-linked monosaccharides spotted on the same fluorous slide. As shown, only two of these fluorous-linked monosaccharides are seen bound to the ConA-FITC. The relatively weak non-covalent fluorous interaction of the mono-C_6F_{13}-tag with the slide due to less fluorous content precluded its visualization. Note also that the β-D-glucose and α-L-rhamnose compounds did not bind at all, as expected. From the slide we can see also that the di-C_6F_{13}-tag has a larger spot size and is brighter than the mono-C_8F_{17}-tag. Despite the apparent greater adhesion of the sugars attached to the di-C_6F_{13}-tag to the slides, the fluorous slides could still be washed and reused at least five times without significantly increasing background noise.

By software analysis of spot intensities, we can conclude that the spot intensity of the sugars attached to the di-C_6F_{13}-tag is two to four times more intense than that of the mono-C_8F_{17}-tag and its binding ability is superior to both the mono-C_8F_{17}- and -C_6F_{13}-tag. The synthesis of the di-C_6F_{13}-tag is relatively simple and has high yielding steps which could be carried out on a larger scale. Clearly, the standard mono-C_8F_{17} tag can be effectively and efficiently replaced by the di-C_6F_{13}-tag. Given its comparable behaviour on fluorous silica gel, this new tag could also possibly replace the octylfluorous tag in the purification of compounds using FSPE in both manual and automated syntheses.

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