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Design and synthesis of substrates for newborn screening of Maroteaux–Lamy and Morquio A syndromes

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ABSTRACT

In continued efforts to develop enzymatic assays for lysosomal storage diseases appropriate for newborn screening laboratories we have synthesized novel and specific enzyme substrates for Maroteaux–Lamy (MPS VI) and Morquio A (MPS IVA) diseases. The sulfated monosaccharide derivatives were found to be converted to product by the respective enzyme in blood from healthy patients but not by blood from patients with the relevant lysosomal storage disease. The latter result shows that the designed substrates are highly selective for the respective enzymes.

The mucopolysaccharidoses (MPSs) are a class of lysosomal storage diseases caused by the deficiency in an enzyme in glycosaminoglycan catabolism. These syndromes result in non-degraded or partially degraded glycosaminoglycans amassing in the lysosome resulting in irreversible multisystemic organ damage.[1–4] Recently, treatments have become available for some of the MPS syndromes; however optimal benefits from these treatments would require commencement of treatment prior to the onset of the irreversible symptoms. In continuance of our efforts on neonatal diagnosis of lysosomal storage diseases by tandem mass spectrometry,[5,6] we carried out the synthesis of two novel substrates that can be used to assay *N*-acetyl galactosamine 4-sulfatase, the enzyme deficient in Maroteaux–Lamy syndrome (MPS VI) and galactose 6-sulfatase, the enzyme deficient in Morquio A syndrome (MPS IVA).

The assays require substrates that are readily prepared, specific for the enzymes, and tagged for detection in the newborn screening laboratory. Natural substrates for the sulfatases are oligosaccharides containing a sulfate on the terminal sugar. While these oligosaccharides would be selective substrates for the enzymes, they are difficult to prepare on the scale needed for worldwide newborn screening (~10 g/year). In contrast, aryl sulfates are readily available but show low specificity between the sulfatase enzymes. We based our substrate design (Fig. 1) on the terminal sugar of the natural substrates, that is, *N*-acetylgalactosamine 4-sulfate and galactose 6-sulfate. The anomeric carbon of these sugars was coupled to an umberferryl moiety, which could be used for fluorescence assays in laboratories lacking tandem mass spectrometers. A carbon chain with a fragmentable *N*-*tert*-butyl carbamate was attached to direct the fragmentation of the parent ion in the mass spectrometer along a single reaction pathway, which increases the sensitivity of the

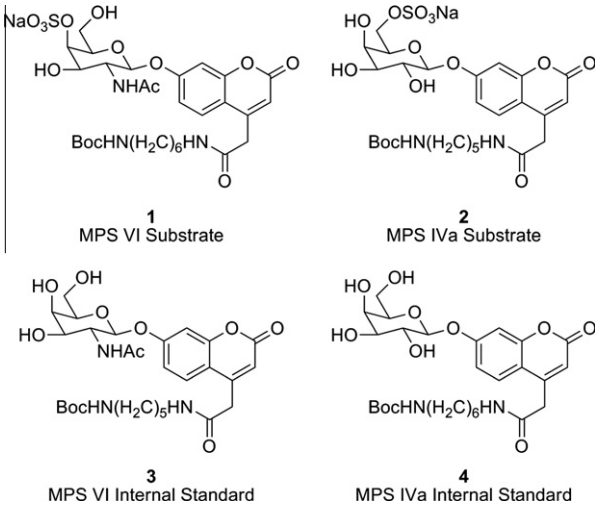

Figure 1. Maroteaux–Lamy substrate **1** and internal standard **3** and Morquio A substrate **2** and internal standard **4**.

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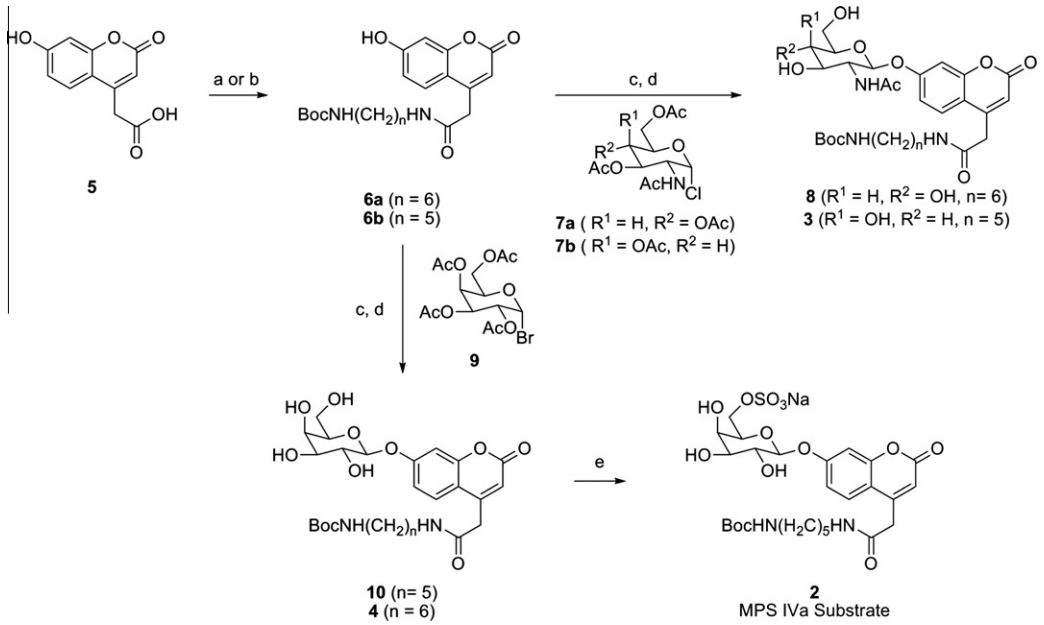
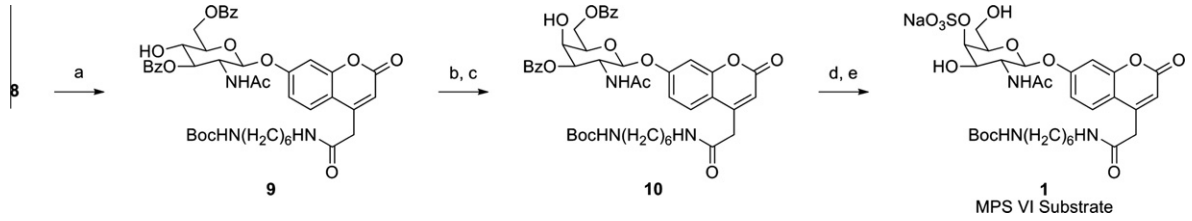


Scheme 1. Preparation of MPS IVA substrate **2** and internal standards **3** and **4**. Reagents and conditions: (a) *N*-Boc-1,6-hexanediamine, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, DMF, $CH2Cl_2$, overnight, 52%; (b) *N*-Boc-1,5-pentanediamine, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, DMF, CH_2Cl_2, overnight, 62%; (c) glycosyl chloride (**7a**, **7b**) or glycosyl bromide (**9**), tetrabutylammonium hydrogensulfate, aq NaOH (1 M), DCM, 30–60 min; (d) sodium methoxide, MeOH, $CHCl_3$, 4 h, **8** (from **6a** and **7a**): 53% over two steps; **3** (from **6b** and **7b**): 50% over two steps; **10** (from **6b** and **9**): 28% over two steps, **4** (from **6a** and **9**): 35% over two steps; (e) sulfur trioxide–pyridine complex, pyridine, 5 h; DOWEX 50WX8 (Na+ form), 45%.



Scheme 2. Preparation of MPS VI substrate **1**. Reagents and conditions: (a) benzoyl chloride (2.1 equiv), pyridine, 0 °C, 3 h, 58%; (b) triflic anhydride, pyridine, DCM, −20 °C, 3 h; (c) sodium nitrite, DMF, overnight, 51% over two steps; (d) sulfur trioxide–pyridine complex, pyridine, 3 h; DOWEX 50WX8 (Na+ form), 50%; (e) sodium methoxide, MeOH, 5 d, 80%.

tandem mass spectrometry assay. The linker chain lengths were chosen such that the mass of the products and internal standards do not coincide with the masses of any of the other neonatal assays developed earlier in our laboratory, thereby allowing the assays to be multiplexed in the mass spectrometry analysis.

The synthesis of both the compounds (Schemes 1 and 2) started with the amide coupling of 7-hydroxycoumarinyl-4-acetic acid with either mono-Boc protected 1,6-hexanediamine or 1,5-pentanediamine. The resulting substituted coumarins were glycosylated with two different glycosyl halide donors under phase transfer catalysis to afford the β-glycosides.[7] The acetate esters were then deprotected to afford glycosides **8**, **3**, **4** and **10**. Glycosides **3** and **4** are the desired internal standards for the enzymatic assays and were used without further elaboration. The MPS IVA substrate **2** was readily synthesized from glycoside **10** by selective sulfation of the primary 6-hydroxyl group over the secondary 2-, 3- and 4-hydroxyl groups as revealed by the downfield shift of H-6 in the sulfate relative to the non-sulfate.[8]

The MPS VI substrate (**1**) required further synthetic manipulations in order to install the sulfate at the more hindered 4-hydroxy of the sugar. Therefore, the less hindered 3- and 6-hydroxyls were selectively benzoylated to afford dibenzoate **9**. The glucosamine was then converted to a galactosamine by inversion of the 4-hydroxyl by formation of the triflate and displacement with sodium nitrite to afford **10**. Finally, sulfation of the free hydroxyl followed by cleavage of the benzoate protecting groups gave the desired MPS VI substrate (**1**).

Full synthetic details are provided as Supplementary data.

With the two substrates and two internal standards in hand, enzymatic activity was studied. The enzymatic activity was measured by incubating a solution of substrate and internal standard with a 2 mm diameter dried blood spot punch for 16 h. The amount of product was quantified by tandem mass spectrometer using the internal standards. For MPS VI, the range of activity measured with 10 dried blood spots from healthy individuals was 1.6–10.5 μmol h^{-1} (L blood)$^{-1}$ compared to 0.08 μmol h^{-1} (L blood)$^{-1}$ using a dried blood spot from an MPS VI patient. For MPV IVA, the values are 0.021–0.35 μmol h^{-1} (L blood)$^{-1}$ for 30 dried blood spots from healthy patients and 0.00039–0.00043 μmol h^{-1} (L blood)$^{-1}$ from six patients with MPS IVA. These results serve to illustrate that the substrates are highly specific for the respective enzymes. Full details of the assay including assay statistics and performance on a large number of dried blood spots will be reported elsewhere.

In conclusion, we have synthesized substrates for two lysosomal sulfatases, *N*-acetylgalactosamine 4-sulfatase and galactose 6-sulfatase, they are highly specific for their respective enzymes. The syntheses developed can be readily scaled to prepare the ~10 g/year of material needed to support worldwide newborn

screening. Further evaluation of these reagents is under investigation in newborn screening laboratories.

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Supplementary data

Supplementary data (experimental procedure for the synthesis and spectral data) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.080.

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