

ORIGINAL PAPER

Key side products due to reactivity of dimethylmaleoyl moiety as amine protective group

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Dimethylmaleoyl (DMM) moiety has become an important amine protective group in sugar chemistry. We disclose herein that DMM-containing D-glucosamine analogues, because of their electrophilic nature, are prone to reactions with strong nucleophiles, such as hydrazine, resulting in a set of undesired side products that are difficult to detect, yet proved to be problematic for organic synthesis.

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Introduction

The dimethylmaleoyl (DMM) moiety has been widely used as an amine protective group in carbohydrate chemistry since it favours the formation of β -anomeric glycoside bond (El Ashry & Aly, 2007; Heseck et al., 2004a, 2004b; Aly et al., 2001, 1998). The DMM group was used for protection of D-glucosamine in our syntheses of bacterial cell wall fragments (Fuda et al., 2005; Heseck et al., 2004a, 2004b). There are several advantages of using DMM in sugar chemistry, including the ease of functionality introduction into building units, the facility of multiple DMM groups' simultaneous removal at the end of the synthesis and good solubility of the DMM derivatives in common organic solvents required for the glycosylation reaction and for purification.

Three glycosyl donors and three glycosyl acceptors were prepared based on DMM D-glucosamine derivatives which were in turn used in the syntheses of cell wall fragments. Four of them are shown in Fig. 1, leading to the synthesis of tetrasaccharide VI. These efforts included a 37-step synthesis of a 2000 Da fragment VII prepared through the intermediary steps leading to the formation of the key DMM-containing intermediate VI (Heseck et al., 2004a). The cell wall fragment VII was useful in the elucidation of the bac-

terial peptidoglycan, the major constituent of the cell wall, solution structure (Meroueh et al., 2006), and for studies in structural biology of proteins binding to peptidoglycan (Cho et al., 2007; Perez-Dorado et al., 2007). A need for relatively large quantities of this hard-to-access sample for currently ongoing projects exists, which prompted the efforts disclosed herein.

As is true for any multistep synthesis, including that of VII, steps giving clean transformations, without the need for purification, are highly desirable. If a product can be purified by crystallisation instead of column chromatography, this would prove advantageous. Needless to say, crystallisation of products is not guaranteed, especially for sugar derivatives. Furthermore, scale-up is an issue, since smaller scale reactions might not be amenable to scale-up in some instances, necessitating additional methodology development. In such cases when scale-up can be attempted, compromises among sample purity, yield, timescale, and the ease of execution come into play.

One of the key glycosyl donors for the syntheses of D-glucosamine-containing molecules, such as VII, is compound III. The synthetic route to compound III is given in Fig. 2. During the scale-up synthesis of glycosyl donor III (tens of gram scale), the yields of the reactions often fluctuated. Although most intermediates had to be purified by column chromatogra-

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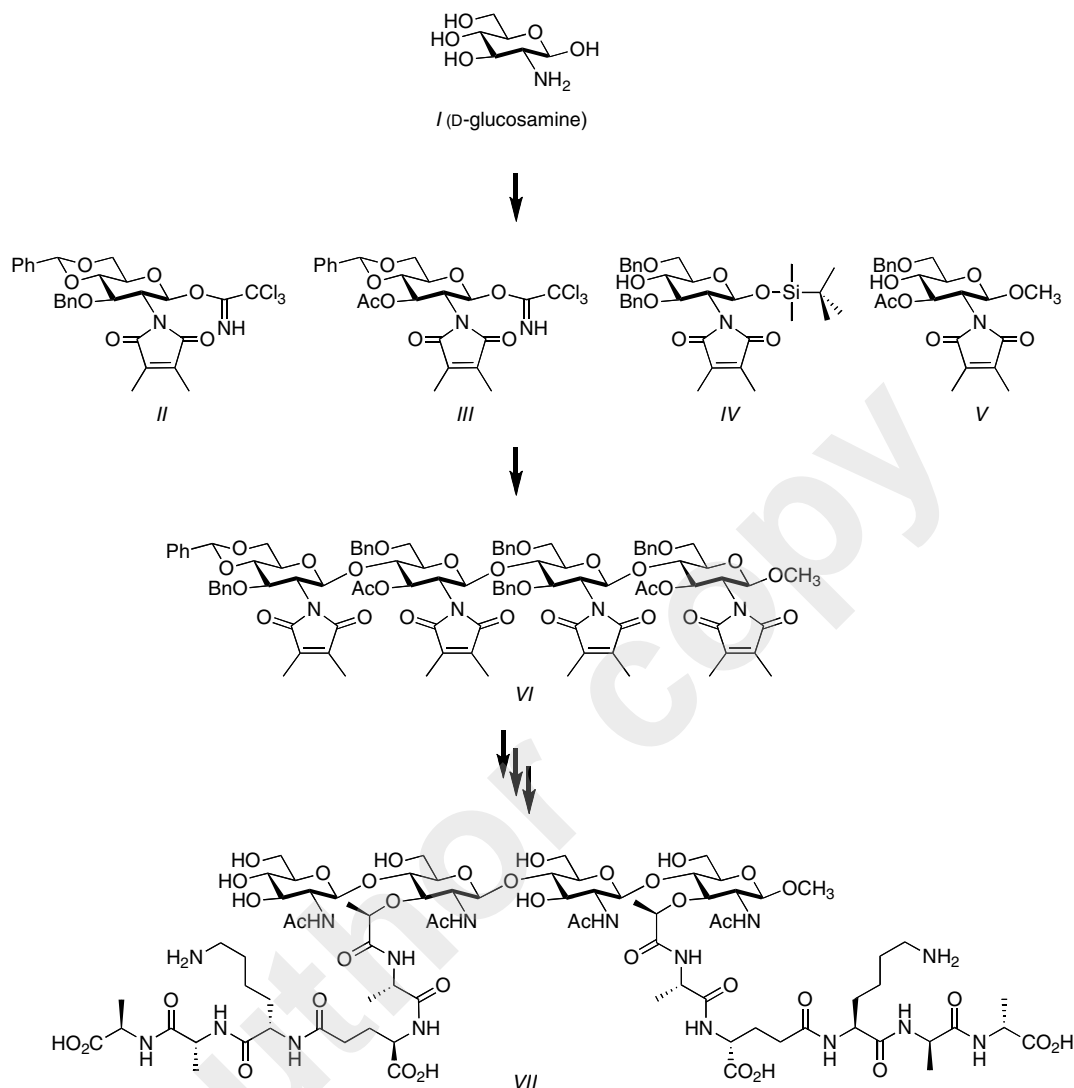


Fig. 1. Synthesis of a bacterial cell wall fragment (*VII*).

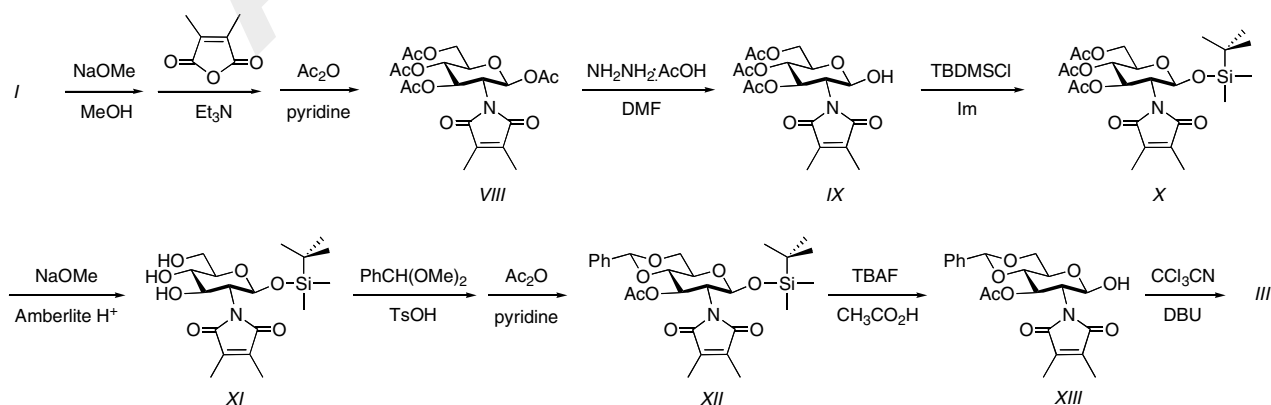


Fig. 2. Synthesis of glycosyl donor *III* starting from D-glucosamine.

phy, the samples behaved differently in large scale in cases when the products could be triturated in smaller scale syntheses. It was concluded that these problems were caused by certain impurities that were difficult

to detect. As described below, the culprit impurities in our case give simple spectra which could not be detected or were easily dismissed as insignificant in the more complex spectra of these highly functionalised

aminosugar analogues. Existence of these undetected impurities can, however, be problematic as they can e.g. react with the reagents in various synthetic steps differently; even compromise the calculation of the key reagents quantity for each step. The large-scale preparation allowed to isolate three distinct hard-to-detect impurities which contributed to poorer sample properties. Structures of these impurities were elucidated by X-ray crystallography.

Experimental

^1H and ^{13}C NMR spectra were recorded on a Varian INOVA 500-MHz spectrometer. Mass spectra were recorded on JEOL JMS-AX505HA and Finnigan-MAT 8430 for high-resolution magnetic sector mass spectrometers. Thin-layer chromatography (TLC) was performed using the Whatman precoated K6F silica gel 60 Å (0.25 mm thickness plates). TLC plates were visualised by immersion in a *p*-anisaldehyde or cerium(IV) sulphate solution and warmed on a hot plate. Flash chromatography was carried out with silica gel 60, 230–400 mesh (0.040–0.063 mm particle size) purchased from EM Science. All organic reagents were purchased from either Sigma Aldrich Chemical Company or Acros Organics. All reactions were performed under a nitrogen atmosphere, unless noted otherwise.

Conversion of D-glucosamine to compound *III*, shown in Fig. 2, was carried out according to the procedure developed in our laboratory (Hesek et al., 2004a, 2004b).

Compounds *XIV*, *XV*, and *XVI* were isolated as follows: A mixture of byproducts was collected and combined during the synthesis of compound *XI* starting from D-glucosamine. The resulting mixture was subjected to several runs of preparative TLC using hexane/ethyl acetate ($\varphi_{\text{r}} = 3 : 1$ to $1 : 1$) as eluent yielding compounds *XIV*, *XV*, and *XVI*. ^1H NMR and ^{13}C NMR spectra of compounds *XIV*, *XV*, and *XVI* are given in Fig. 3.

Crystals of suitable size for single-crystal X-ray diffraction analysis were obtained by diffusion of diethyl ether into a CH_2Cl_2 solution at room temperature overnight. Crystals were examined using Infineum V8512 oil and placed on a MiTeGen mount, and they were transferred to the 120 K (or 200 K for compound *XV*) N_2 stream of a Bruker SMART Apex CCD diffractometer. Unit cell parameters were determined from reflections with $I > 10 \sigma(I)$ from three orthogonal sets of $30 \times 0.5^\circ \omega$ scans. Data collection strategy was calculated using COSMO, included in the Apex2 suite of programs (Bruker, 2008), to maximise the coverage of reciprocal space in a minimum amount of time. Average 4-fold redundancy of measurements was sought. Data were corrected for the Lorentz and polarisation effects, as well as for absorption. On cooling to 100 K, crystals of compound *XV* were shattered.

Thus, data collection was performed at 200 K.

Structure solution and refinement were performed using the programs of the SHELXTL software package (Sheldrick, 2008). Crystallographic data (excluding structure factors) for compounds *XIV*, *XV*, and *XVI* were deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers 696326, 696327 and 696328. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Results and discussion

Identification of putative impurities in each step of the synthesis was attempted. As the large-scale synthesis was performed and the products were isolated, remainders of the samples for the first four steps of the synthesis (Fig. 2), up to compound *XI*, were collected. Combining all impurities over several steps is not a general approach, but it was necessary in this case as the amount of impurities was very small. ^1H NMR spectrum of the combined impurities prior to purification showed multiple singlet peaks at around $\delta = 2$, along with signals from compound *XI* (Fig. 3A). Each component was isolated and carefully analysed. The large-scale preparation allowed to isolate three distinct impurities together with the reagent, 2,3-dimethylmaleic anhydride, from the mixtures.

These impurities were somewhat difficult to detect in the reaction mixtures since they often ran along with the desired products during chromatography. Impurities of unknown structures were designated as compounds *XIV*, *XV*, and *XVI*. NMR spectra for these compounds are given in Figs. 3B and 3C.

All showed singlet resonances were observed at around $\delta = 2$ for ^1H NMR and $\delta = 9$ for ^{13}C NMR. Together with the additional signals at $\delta = 137.7$ and $\delta = 167.2$ in the ^{13}C NMR spectra, these are typical resonances originating from the DMM group. For compound *XIV*, ^1H NMR shows an extra broad singlet at $\delta = 3.88$, integrated to two protons, with no significant change in ^{13}C NMR. This could be a DMM derivative with exchangeable protons. For compound *XVI*, the ^1H NMR spectrum showed an extra signal at $\delta = 2.35$ with integration of 6 and ^{13}C NMR resonances at $\delta = 24.9$ and $\delta = 169.2$, which can be ascribed to two symmetric acetyl groups. Mass spectrometry gave m/z values of 141, 249, 225 for samples *XIV*, *XV*, and *XVI*, respectively. The NMR and MS results led to the assignment of the possible structures for *XIV*, *XV*, and *XVI*, as show in Fig. 3D. Although the formation of five-membered *XIVa* and *XVIa* are favoured entropically, it was not possible to differentiate them from six-membered *XIVb* and *XVIb* by NMR and MS data. These compounds were crystallised and the structures for the unknown *XIV*, *XV*, and *XVI* were ultimately

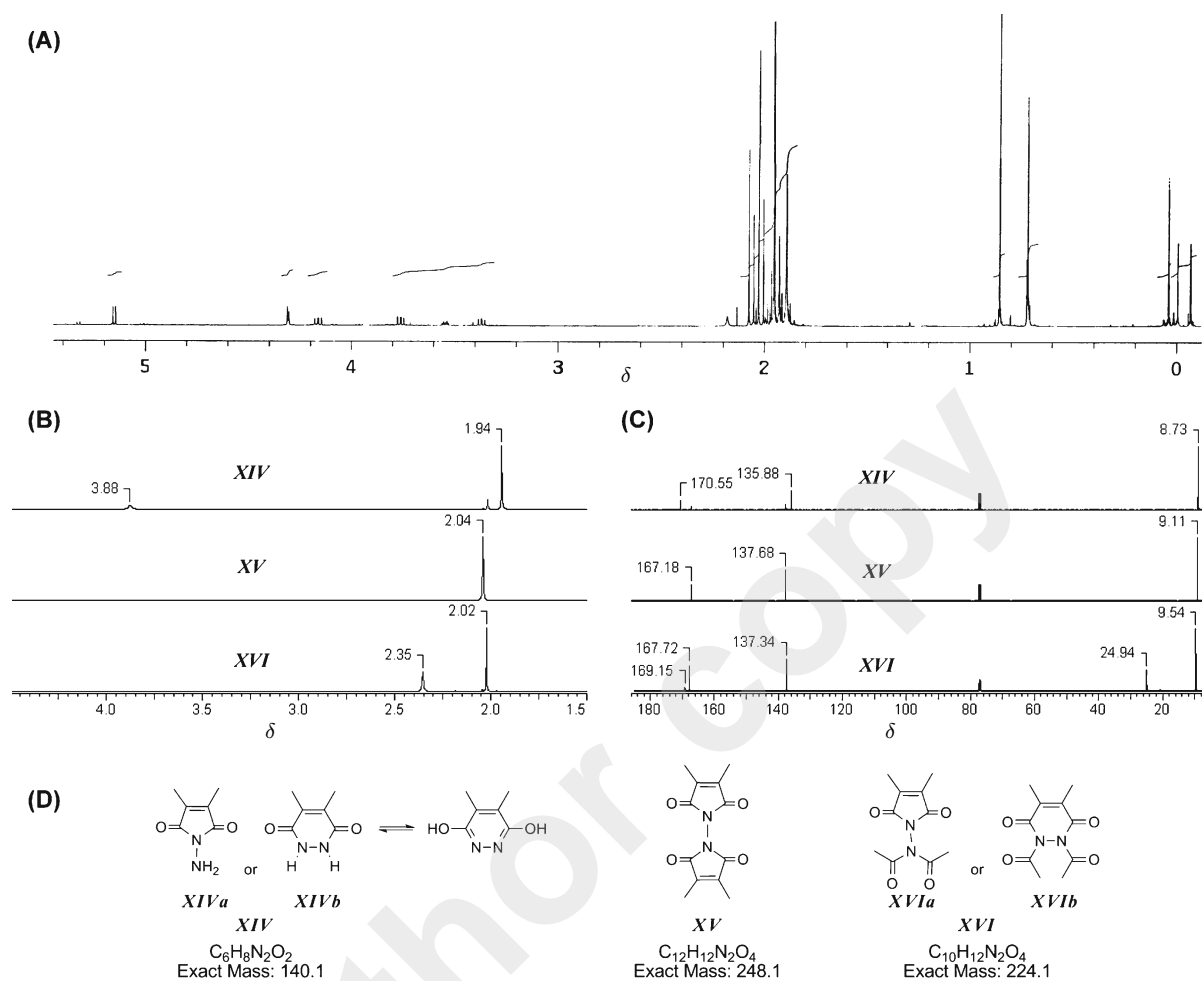


Fig. 3. ^1H NMR spectrum of combined impurities prior to purification (A); ^1H NMR (B) and ^{13}C NMR (C) spectra of the three isolated side products (XIV, XV, and XVI) in CDCl_3 ; possible structures of compounds XIV, XV, and XVI, consistent with the NMR and MS data (D).

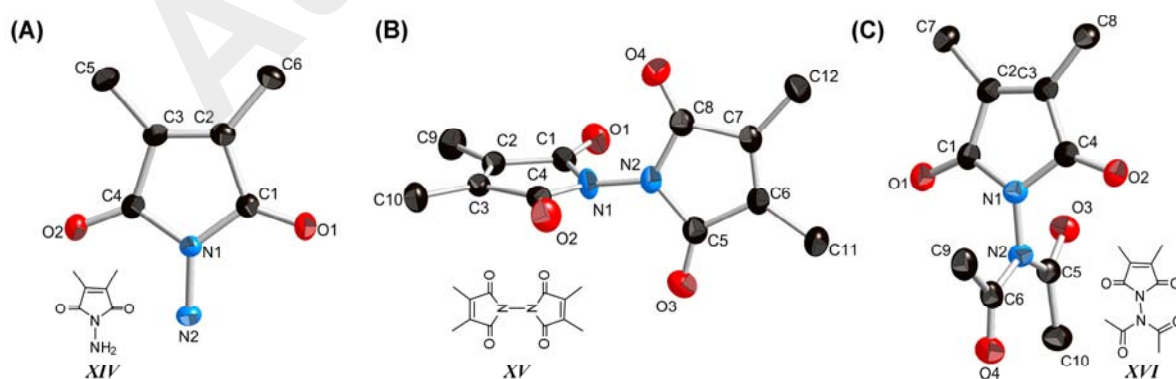


Fig. 4. ORTEP diagrams of compounds XIV, XV, and XVI (panels A, B, and C, respectively), at the 50 % probability level. Hydrogen atoms were omitted for clarity.

assigned unequivocally by the determination of their X-ray structures (Fig. 4).

X-ray structures revealed that five- instead of six-membered rings are present in these compounds (XIV, XV, and XVI). The DMM rings are planar, except that of compound XVI, where the imide nitrogen (N1)

is out of plane, by 0.12 Å. The individual acetamido groups in compound XVI are nearly planar, however, two planes, defined by an individual acetyl group, are rotated by 7° with respect to each other. The two carbonyl groups are not symmetrically disposed as the dipoles of the individual carbonyls of the acetyl

Table 1. Crystal data and structure refinement for compounds *XIV*, *XV*, and *XVI*

	<i>XIV</i>	<i>XV</i>	<i>XVI</i>
Empirical formula	C ₆ H ₈ N ₂ O ₂	C ₁₂ H ₁₂ N ₂ O ₄	C ₁₀ H ₁₂ N ₂ O ₄
Formula weight	140.14	248.24	224.22
Temperature/K	120(2)	200(2)	120(2)
Crystal system	Monoclinic	Triclinic	Monoclinic
Space group	P 2 ₁ /c	P $\bar{1}$	P 2 ₁ /n
<i>a</i> /Å	13.4460(3)	7.6699(3)	11.6526(2)
<i>b</i> /Å	13.0028(3)	8.6159(4)	7.2897(2)
<i>c</i> /Å	7.7782(2)	10.4086(7)	13.3030(3)
α /°	90	98.365(3)	90
β /°	90.298(1)	100.013(2)	104.598(1)
γ /°	90	115.850(2)	90
Unit-cell volume/Å ³	1359.89(6)	590.27(5)	1093.53(4)
Formula units per unit cell, <i>Z</i>	8	2	4
Absorption coefficient/mm ⁻¹	0.882	0.107	0.903
Density/(g cm ⁻³)	1.369	1.397	1.362
Crystal size/mm ³	0.26 × 0.21 × 0.17	0.14 × 0.10 × 0.06	0.38 × 0.38 × 0.06
θ range/°	3.29–69.41	2.05–27.52	4.51–69.59
Data/restraints/parameters	2511/0/198	2685/0/168	2002/0/149
<i>R</i> ₁ [<i>I</i> > 2 σ (<i>I</i>)], <i>wR</i> ₂ (all data)	0.0333, 0.0887	0.0388, 0.1063	0.0359, 0.0973
Largest difference peak and hole/(e Å ⁻³)	0.233 and -0.198	0.223 and -0.198	0.218 and -0.184

groups point in opposing directions. The two DMM rings in *XV* are rotated by 80° with respect to each other. Compound *XIV* crystallised as two independent molecules identical in conformation but forming a linear hydrogen-bonded chain parallel to the crystallographic *c*-axis.

A search in literature revealed that compounds *XIV* and *XV* have already been known (Hedaya et al., 1966; Horning & Amstutz, 1955). Compound *XV* (bis-(dimethylmaleic)-hydrazide) was prepared from two equivalents of dimethylmaleic anhydride and hydrazine hydrate in acetic acid (Hedaya et al., 1966). Compound *XIV* (*N*-aminodimethylmaleimide) was prepared by the reaction of dimethylmaleic anhydride and hydrazine hydrate in the ratio of 1 : 1 in refluxing ethanol (Horning & Amstutz, 1955). Diacetyl derivative of *XIV* (compound *XVI*) is a new compound (Table 1). The formation of a six-membered ring structure, such as in compound *XIVb* (dimethylmaleic hydrazide), requires the use of hydrazine dihydrochloride or hydrazine sulphate in refluxing water (Horning & Amstutz, 1955; Steck et al., 1954).

Since three DMM-based impurities were available, their TLC values were compared to those of four glucosamine derivatives in the hexane/ethyl acetate ($\varphi_r = 1 : 1$) system. As mentioned earlier, these impurities were hardly visible under a UV lamp (typical TLC detection), except for 2,3-dimethylmaleic anhydride. Compounds *XIV* and *XVI* were visualised as yellow spots by *p*-anisaldehyde staining, and as white spots by cerium sulphate staining, while compound *XV* was not detectable in similar conditions. Glucosamine derivatives (*VIII–XI*) were visualised as dark purple spots with anisaldehyde staining and as black spots by cerium sulphate staining. These three DMM compounds show similar *R_f* values as glucosamine deriva-

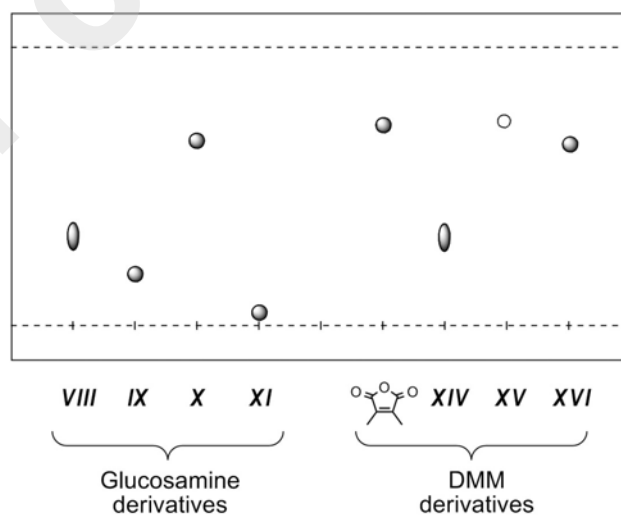


Fig. 5. TLC comparison of glucosamine derivatives (*VIII–XI*) and isolated DMM-based impurities (*XIV–XVI*) in hexane/ethyl acetate ($\varphi_r = 1 : 1$).

tives. For example, compound *XIV* runs together with compounds *VIII*, or *IX*, and compound *XVI* has similar *R_f* as compound *X* (Fig. 5). This precise analysis helped to check the reactions described in Fig. 2 and to improve the synthesis.

All three impurities can result either from the reaction of dimethylmaleic anhydride and hydrazine acetate traces or from the reaction of DMM with the glucosamine derivatives. The fact that hydrazine is a very reactive supernucleophile is widely known. The use of hydrazine acetate is intended to moderate the reactivity of hydrazine. Some impurities, such as compound *XIV*, can also serve as nucleophiles in any step of the synthesis. But as it is evident from the above text, ex-

cessive reactivity of hydrazine acetate is still a problem indicating that the step has to be performed with utmost caution. Furthermore, compound *VIII* prepared in larger scale had to be carefully purified, to get rid of these hard to detect impurities to make the synthesis of Fig. 2 the most efficient. These observations were critical in the improvement of the presented synthesis on a multigram-scale.

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