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Preliminary Communications

Mixed SAMs and MALDI–ToF MS: Preparation of *N*-glycosylamine derivative and thioctic acid methyl ester bearing 1,2-dithiolane groups and detection of enzymatic reaction on Au

Hani Mutlak A. Hassan *, Beatrice A. Maltman

Manchester Interdisciplinary Biocentre (MIB), University of Manchester, 131 Princess Street, Manchester M1 7DN, UK

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ABSTRACT

Herein, we report an enzymatic galactosylation reaction of β-glucopyranosylamide **4** and thioctic acid methyl ester **5** bearing 1,2-dithiolane groups to form a new system of mixed self-assembled monolayers (SAMs) on gold. Characterization of the enzymatic activity was conveniently achieved by mass spectrometry.

1. Introduction

In 1983, Nuzzo and Allara discovered the formation of self-assembled monolayers (SAMs) of alkanethiolates on gold and are pioneers of this field [1]. Since then, SAMs have been employed in various arenas of chemical science; interfacial chemical reactions, detection of ligand–protein interactions, drug discovery, to mention a few [2]. Matrix-assisted laser desorption/ionization and time-of-flight mass spectrometry (MALDI–ToF MS) has previously been utilized to confirm molecules adsorbed to a gold surface. While, unfortunately, the majority of applications have used SAMs with alkanethiolate structures for biological studies [3], we were attracted in using the 1,2-dithiolane group for anchoring β-glucopyranosylamide **4** along with thioctic acid methyl ester **5** on gold, to form mixed SAMs *via* the 1,2-dithiolane rings, and examining whether the desired galactosylation enzymatic reaction of these mixed SAMs (see Fig. 1) could be verified by mass spectrometry. The 1,2-dithiolane group tends to form stable SAMs than their thiol counterpart [4], presumably because of the cyclic disulfide structure, and thus could offer favorable substrate conformation for binding to biomolecules. Herein, we show the use of mixed SAMs and MALDI–ToF MS analysis allowed a rapid and simple characterization of a galactosylation reaction of 1,2-dithiolane-based carbohydrate **4** on a gold surface.

2. Experimental

*Synthesis of 1-amino-1-deoxy-β-D-N-acetylglucoside (**2**).* To a microwave reaction tube was added *N*-acetyl-D-glucosamine **1** (250 mg, 1.1 mmol), ammonium carbonate (1.25 g, 13 mmol), and anhydrous $(CH_3)_2SO$ (900 μL). The tube was sealed and irradiated under microwave conditions (10 W, 250 psi, 40 °C) for 1.5 h. After lyophilization, β-glycosylamine **2** was obtained as a white solid in 91% yield. The crude product **2** was used in the next step without purification. Analytical data were consistent with the literature [6]. *Synthesis of 2,3,4,6-tetrahydroxyl-1-N-thioctoyl-β-D-glucopyranosylamide (**4**).* To an oven dried round-bottomed flask was added thioctic acid **3** (1,2-dithiolane-3-pentanoic acid) (247 mg, 1.2 mmol), HBTU (1.36 g, 3.6 mmol), HOBt (162 mg, 1.2 mmol), and anhydrous $(CH_3)_2NCHO$ (9 mL). The reaction mixture was stirred for 15 min and transferred to a round-bottomed flask containing β-glycosylamine **2** (0.220 g, 1 mmol) and stirring continued at rt overnight, under N_2 atmosphere. The solvent was removed under reduced pressure and the crude product was purified *via* silica gel column chromatography (5 → 10% CH_3OH in $CHCl_3$) to give **4** in an oil form. The product was then azeotropically dried with toluene to afford a yellow solid (270 mg, 66%). Analytical data were consistent with the literature [8]. *Synthesis of methyl 5-(1,2-dithiolan-3-yl)pentanoate (**5**).* To a stirred solution of thioctic acid **3** (206 mg, 1 mmol) in anhydrous CH_3OH (100 mL) was added catalytic amount of conc. H_2SO_4. The reaction mixture was stirred at rt overnight. After workup, **5** was obtained as a yellow oil (218 mg, 99%) and used for SAMs studies without purification. Analytical data were consistent with the literature [5]. *Preparation procedure of self-assembled monolayers.* A gold substrate was

* Corresponding author.
E-mail address: hani.mutlak.a.hassan@gmail.com (H.M.A. Hassan).

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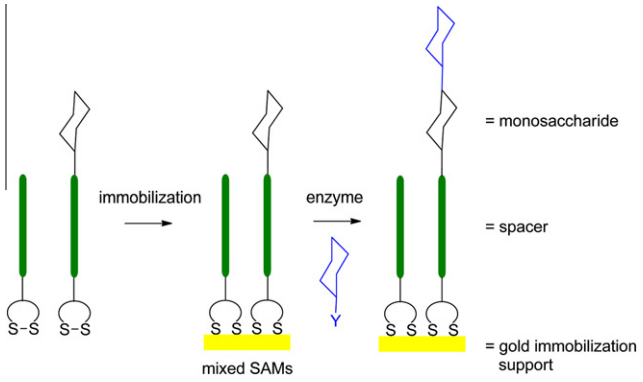
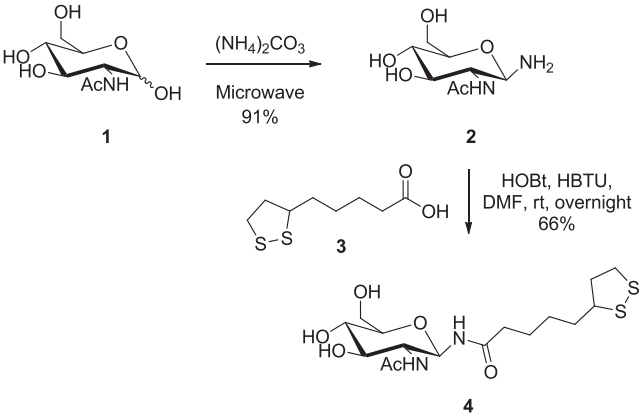
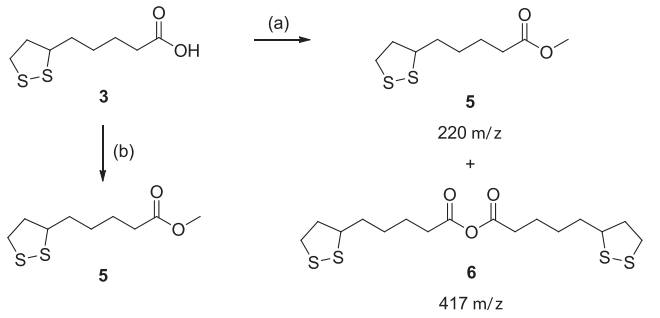


Fig. 1. Schematic representation of immobilization and galactosylation process on Au.



Scheme 1. Synthesis of β-glucopyranosylamide **4** bearing 1,2-dithiolane group for immobilization on Au.



Scheme 2. Reagents and conditions: (a) CH_3OH, conc. H_2SO_4, 0.08 M, rt, overnight; (b) CH_3OH, conc. H_2SO_4, 0.01 M, rt, overnight. Formation of ester **5** and anhydride **6** observed by TLC and MS and its exclusive formation under different esterification conditions.

immersed into a solution of **4** and **5** (1:25 ratio) in ethanol (1 mL; 1 mM final concentration) and left overnight at rt. The substrate was then washed thoroughly with ethanol and dried with N_2. *Enzymatic galactosylation procedure.* The gold substrate was immersed into a solution of β-1,4-galactosyltransferase (10 μL, 0.06 units), 1% UDP-Gal (50 μL), MOPS buffer (100 μL, pH 7.5, 500 mM), $MnCl_2$ (50 μL, 200 mM), and water (790 μL). After the solution was shaken gently at 30 °C overnight, the substrate was washed thoroughly with ethanol and water then dried with N_2 and analyzed by MALDI–ToF MS.

3. Results and discussion

The study commenced by first preparing β-glycosylamine **2** which was obtained in good yield (91%) by microwave irradiation of *N*-acetylglucosamine **1** with ammonium carbonate (modification of the Kochetkov amination) [6]. Glycosylamines are an important motif in carbohydrate science, particularly in glycopeptides and glycoproteins [7]. Coupling of **2** with the commercially available thioctic acid **3** using HOBt and HBTU as peptide coupling

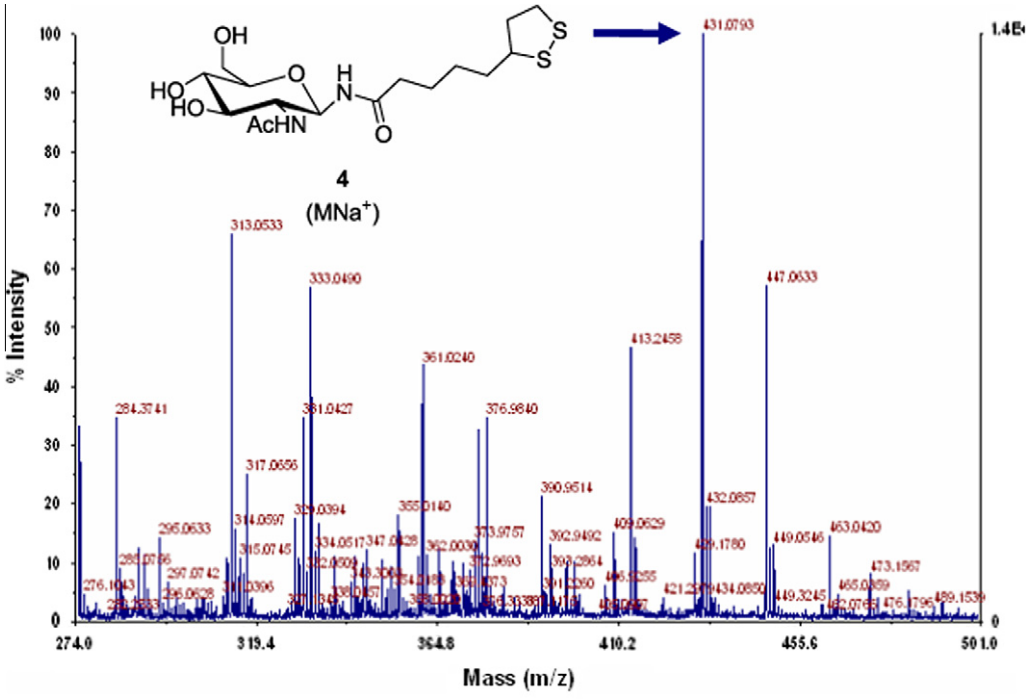


Fig. 2. MALDI–ToF MS spectrum displaying desired molecular mass of **4** plus sodium and potassium adducts (at 431 and 447 *m*/*z*, respectively) after immobilization on Au.

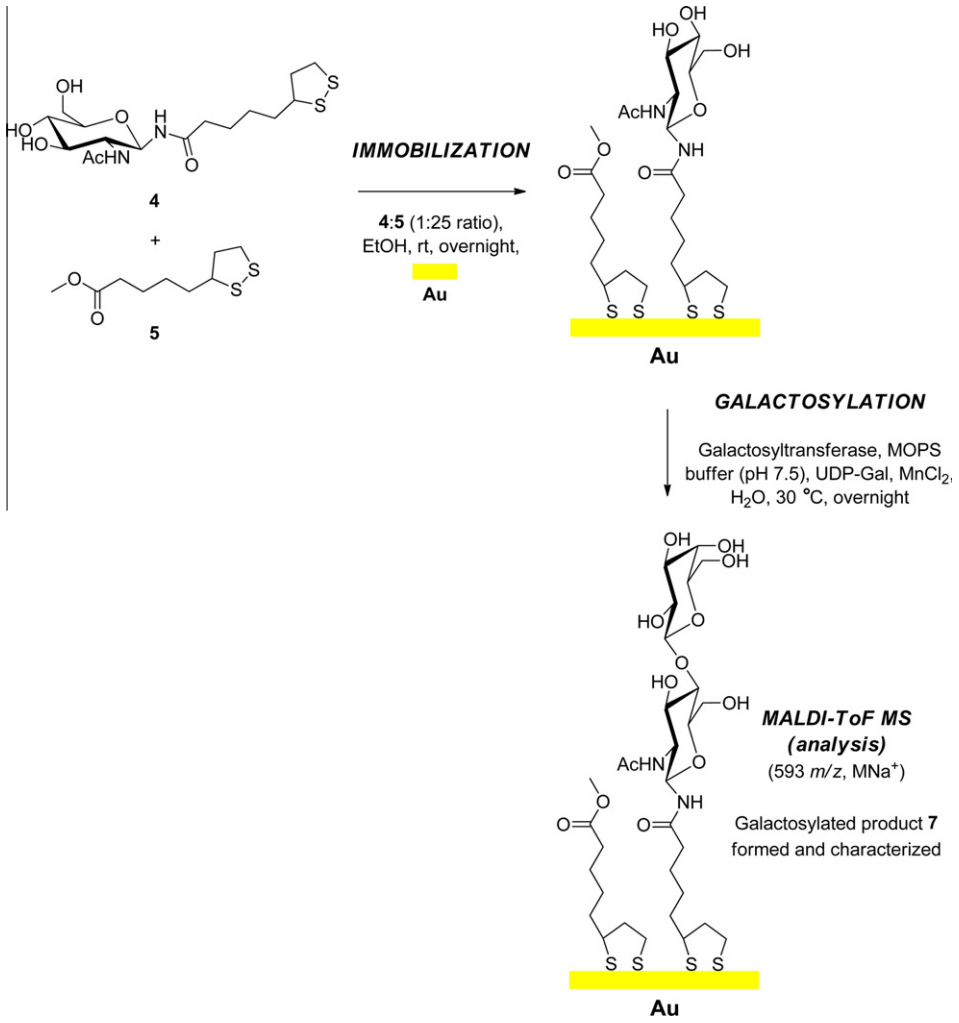


Fig. 3. Illustration of immobilization of **4** and **5** on Au and galactosylation reaction to give disaccharide **7**.

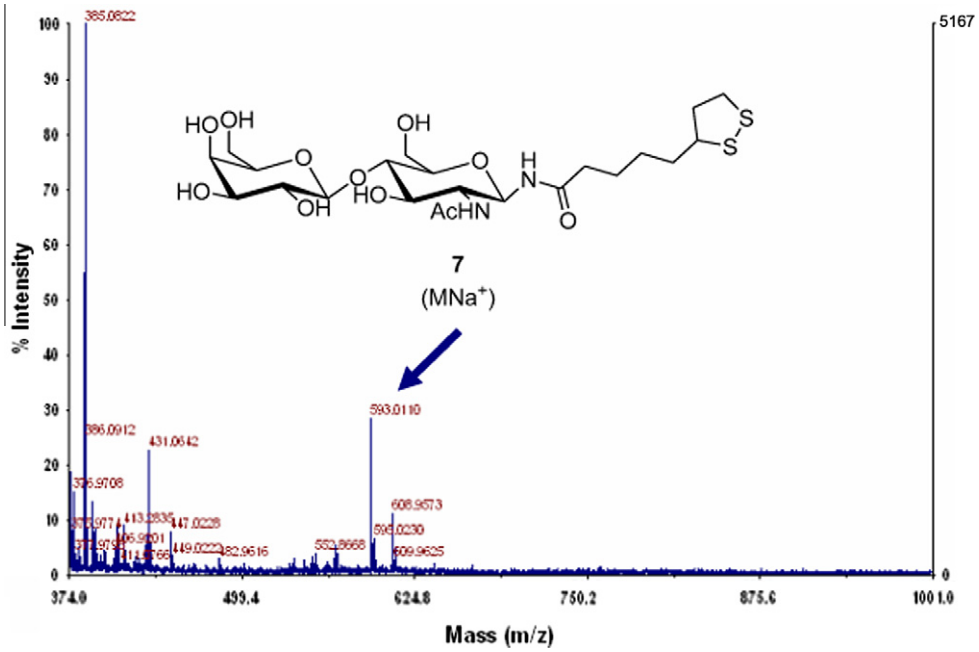


Fig. 4. MALDI–ToF MS confirmed successful galactosylation product (593 *m*/*z*, MNa^+).

reagents delivered the desired β-glucopyranosylamide **4** [8] in 66% isolated yield in a short two-step synthetic pathway (Scheme 1). This straightforward route benefits in obtaining only the β-anomer directly from **1**. Because the employment of mixed monolayers on gold aids recognition of biological events [2], we decided to synthesize thioctic acid methyl ester **5** as it was envisioned that this compound (as apposed to acid **3**) would not pose unwanted interference with the enzymatic reaction once co-immobilized on gold. The first attempted generation of ester **5** was performed by subjecting thioctic acid **3** to esterification conditions (CH_3OH, cat. conc. H_2SO_4, rt, overnight, 0.08 M). Analysis by TLC and MS indicated the formation of the desired product **5** along with anhydride **6** (two molecules of thioctic acid **3** underwent a condensation reaction).

We then exclusively generated **5** under higher dilution conditions (CH_3OH, cat. conc. H_2SO_4, rt, overnight, 0.01 M) in 99% yield. The modified reaction conditions prevented self-condensation to arise and gave **5** as the sole product (Scheme 2).

Having synthesized β-glucopyranosylamide **4** and ester **5**, we performed a preliminary immobilization experiment to test whether compound **4** could effectively be chemisorbed on the gold surface. By incubating a solution of **4** and **5** (1:25 ratio) at rt overnight with a gold chip and examining potential immobilization on the gold surface using MALDI–ToF MS, we established that a successful attachment of **4** on gold has taken place with a characteristic molecular mass signal at 431 m/z (MNa^+) observed together with the potassium adduct (447 m/z) (Fig. 2).

The stage was set to carry out our desired enzymatic galactosylation reaction (Fig. 3). The carbohydrate-terminated substrate **4** was first immobilized on gold and galactosylation was conducted by immersing the gold substrate into a solution containing β-1,4-galactosyltransferase, UDP-Gal, MOPS buffer (pH 7.5), $MnCl_2$, and water, leaving the system incubated at 30 °C overnight. Pleasingly, analysis by MALDI–ToF MS revealed a characteristic mass peak at 593 m/z (MNa^+), therefore giving robust evidence that the enzymatic modification to give the desired disaccharide product **7** has successfully occurred (Fig. 4). Although the starting material **4** was not fully converted to **7**, the combination of **4** and **5** to form mixed SAMs seems to have conveniently worked well, despite the short alkyl chain (spacer) in compound **4** between the carbohydrate ligand and the gold surface.

4. Conclusion

In this communication, we have shown that the use of mixed SAMs and MALDI–ToF MS provided a highly efficient detection of an enzymatic activity, employing β-glucopyranosylamide **4** together with thioctic acid methyl ester **5** (both bearing 1,2-dithiolane groups) to form a new system of mixed monolayers on gold. We wish to extend the use of the thioctic acid structure for other endeavors such as discovering inhibitors of enzymes using SAMs and MALDI–ToF MS technology.

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References

[1] R.G. Nuzzo, D.L. Allara, J. Am. Chem. Soc. 105 (1983) 4481–4483.
[2] J.C. Love, L.A. Estroff, J.K. Kriebel, R.G. Nuzzo, G.M. Whitesides, Chem. Rev. 105 (2005) 1103–1169.
[3] (a) Z.A. Gurard-Levin, M. Mrksich, Annu. Rev. Anal. Chem. 1 (2008) 767–800;
(b) M. Mrksich, ACS Nano 2 (2008) 7–18;
(c) J. Su, M. Mrksich, Angew. Chem. Int. Ed. Engl. 41 (2002) 4715–4718;
(d) B.T. Houseman, M. Mrksich, Angew. Chem. Int. Ed. Engl. 38 (1999) 782–785;
(e) A. Sanchez-Ruiz, S. Serna, N. Ruiz, M. Martin-Lomas, N.-C. Reichardt, Angew. Chem. Int. Ed. 50 (2011) 1801–1804;
(f) W.-S. Yeo, D.-H. Min, R.W. Hsieh, G.L. Greene, M. Mrksich, Angew. Chem. Int. Ed. 44 (2005) 5480–5483;
(g) Y.-K. Kim, S.-R. Ryoo, S.-J. Kwack, D.-H. Min, Angew. Chem. Int. Ed. 48 (2009) 3507–3511.
[4] J. Sharma, R. Chhabra, C.S. Andersen, K.V. Gothelf, H. Yan, Y. Liu, J. Am. Chem. Soc. 103 (2008) 7820–7821.
[5] B. Adger, M.T. Bes, G. Grogan, R. McCague, S. Pedragosa-Moreau, S.M. Roberts, R. Villa, P.W.H. Wan, A.J. Willetts, Bioorg. Med. Chem. 5 (1997) 253–261.
[6] For a microwave-assisted Kochetkov amination, see: (a) M.A. Brun, M.D. Disney, P.H. Seeberger, ChemBioChem 7 (2006) 421–424;
(b) M. Bejugam, S.L. Flitch, Org. Lett. 6 (2004) 4001–4004.
[7] K.C. Nicolaou, S.A. Snyder, A.Z. Nalbandian, D.A. Longbottom, J. Am. Chem. Soc. 126 (2004) 6234–6235.
[8] This compound was recently prepared by a different approach, see: M. Kadalbajoo, J. Park, A. Opdahl, H. Suda, C.A. Kitchens, J.C. Garno, J.D. Batteas, M.J. Tarlov, P. DeShong, Langmuir 23 (2007) 700–707.