A Modular System for the Preparation of Diazirine-Labeled Mannose Derivatives Using Thiourea Bridging

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Abstract: The protein FimH is a bacterial lectin, which is utilized by Escherichia coli to adhere to the glycocalyx of potential host cells. FimH has specificity for α-mannosyl residues, as revealed by biological as well as X-ray studies. To further investigate the molecular details of carbohydrate binding to FimH, photolabile mannose derivatives are important tools to covalently mark carbohydrate binding sites on FimH. Here, a modular approach for the synthesis of photolabile diazirine-labeled mannosides and mannosyl clusters is reported. The convergent synthesis utilizes thiourea bridging of the amino-functionalized diazirine 4 with NCS-functionalized carbohydrates.

Key words: carbohydrates, glyoclusters, photolabeling, diazirines, thiourea

Escherichia coli bacteria possess protein appendages, called fimbriae or pili, to adhere to the glycocalyx of their potential host cells. The so-called type 1 fimbriae are mannose-specific and can interact with terminal α-mannosyl residues of the glycocalyx.1 Type 1 fimbriae are known to be critical factors for the ability of E. coli to colonize the human bladder and persist in the urinary tract.2 The lectin domain of type 1 fimbriae is formed by a protein named FimH.3 X-ray studies4 have revealed a carbohydrate recognition domain (CRD) at the tip of the protein which can accommodate one α-mannosyl residue together with one water molecule. Additional carbohydrate binding sites on FimH have not been identified, whereas testing results with multivalent cluster mannosides5 as well as theoretical studies6 suggest, that there could be more than just one carbohydrate binding site on FimH. Here, a modular approach for the synthesis of photolabile diazirine-labeled mannoses and mannosyl clusters is reported. The convergent synthesis utilizes thiourea bridging of the amino-functionalized diazirine 4 with NCS-functionalized carbohydrates.

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Key words: carbohydrates, glyoclusters, photolabeling, diazirines, thiourea

Escherichia coli bacteria possess protein appendages, called fimbriae or pili, to adhere to the glycocalyx of their potential host cells. The so-called type 1 fimbriae are mannose-specific and can interact with terminal α-mannosyl residues of the glycocalyx.1 Type 1 fimbriae are known to be critical factors for the ability of E. coli to colonize the human bladder and persist in the urinary tract.2 The lectin domain of type 1 fimbriae is formed by a protein named FimH.3 X-ray studies4 have revealed a carbohydrate recognition domain (CRD) at the tip of the protein which can accommodate one α-mannosyl residue together with one water molecule. Additional carbohydrate binding sites on FimH have not been identified, whereas testing results with multivalent cluster mannosides5 as well as theoretical studies6 suggest, that there could be more than just one mannose-interacting domain on FimH.

To further investigate carbohydrate binding to the bacterial adhesin FimH, we decided to use the well-known photolabeling methodology.7 Therefore, mannose derivatives were targeted, which carry a photolabile diazirine group. Ideally, incubation of such functionalized sugar ligands with specific lectins, such as FimH, leads to covalent attachment of the sugar moiety to the CRD of the lectin upon irradiation.

As it is unknown at which position and distance of the sugar ring a diazirine group should ideally be attached, a synthetic pathway allowing a maximum of structural flexibility is advantageous. Such a modular approach for the synthesis of diazirine-labeled mannose derivatives also offers an option to improve receptor binding. Binding of α-mannosides such as methyl α-D-mannoside (MeMan) to FimH is weak with IC50 values in the millimolar range. By incorporation of aryl aglycon moieties, as found in p-nitrophenyl-α-D-mannoside (pNPMa), carbohydrate binding to FimH is improved by approximately 100 times.8 Consequently, we envisaged a synthesis of photolabile mannose derivatives which is compatible with fine-tuning of binding potencies such as the appropriate incorporation of phenyl rings.

Thus, our work on the synthesis of diazirine-labeled carbohydrates was based on a convergent approach involving an amino-functionalized diazirine and an isothiocyanato-functionalized ligand, which can be combined by the thiourea bridging strategy9 (Equation 1).

Equation 1 Convergent synthetic strategy for the preparation of diazirine-functionalized sugar derivatives by thiourea bridging.

Synthesis of the amino-functionalized diazirine 4 started with (±)-1-aminopropan-2-ol (1) (Scheme 1). After the amino group was reacted with Boc anhydride,10 the protected product was instantaneously oxidized in a Swern reaction11 leading to the ketone 2 in an overall yield of 93%. A keto group can be turned into a diazirine ring using hydroxylamine-O-sulfonic acid in liquid ammonia, followed by oxidative treatment of the intermediate diaziridine with iodine.12 This procedure delivered the Boc-protected diazirine 3 in 70% yield, which could be easily deprotected with hydrochloric acid to give the amino hydrochloride 4. The diazirine 4 is a photoactive group to be introduced into isothiocyanato-functionalized molecules via thiourea bridging.

To explore the feasibility of thiourea bridging with the amino-functionalized diazirine 4, two isothiocyanato-functionalized sugars were used, the mannosyl isothiocyanate513 and the NCS-functionalized phenyl mannoside8,14 respectively, which were both prepared according to literature-known procedures (Scheme 2). The hydrochloride 4 was converted into the free amine by adding an excess of diisopropylethyl amine (DIPEA) and was then stirred with the carbohydrate components at room temper-
atute for 2 h. This delivered the protected diazirine-labeled mannose derivatives 6 and 9, respectively (Scheme 2), which could be de-O-acetylated according to Zemplén15 at 0 °C to give the unprotected photolabile ligands 7 and 10 in quantitative yields.

Interestingly, when the deprotection reaction was carried out at room temperature, 7 was obtained as an anomeric mixture with an α/β ratio of 4:1. The anomeric configuration was assigned on the basis of the values of $J_{C-1,H-1}$ as measured in the $^{13}$C gated-decoupling NMR experiment. When deprotection was carried out at 0 °C, no anomerization occurred.

After thiourea bridging employing the amine 4 had been successful, the scope of this reaction pathway was further evaluated. Two mannoses, one with an alkyl, the other one with an aryl aglycon, were selected and functionalized at the 6-position following known procedures.14,17 Thus, the respective mannose was first regioselectively 6-O-tosylated, acetylated and then subjected to nucleophilic displacement to give the 6-azido-6-deoxy derivatives, which were finally converted into the 6-deoxy-6-isothiocyanato mannoses 11 and 14 (Scheme 3) via a Staudinger-type reaction with carbon disulfide and triethyl phosphate.18 Indeed, thiourea bridging with the amino-functionalized diazirine 4 under the above mentioned standard conditions again proceeded in very good yields to give the photolabile mannoses 13 and 16 after deacetylation.

In extension of our work, it became our goal to apply this approach for the synthesis of multivalent carbohydrate ligands by thiourea bridging. Recently, we have reported about the graded reactivity of differently isothiocyanato-functionalized acetyl-protected mannose derivatives in thiourea bridging.19 The difference in reactivity was most pronounced when mannolyl isothiocyanates were compared to 6-deoxy-6-isothiocyanato-mannosides. Therefore we planned to synthesize the di-NCS-functionalized mannose derivative 20 (Scheme 4) to eventually address.

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**Scheme 1** Synthesis of the amino-functionalized diazirine 4. 
**Reagents and conditions:** (i) Boc$_2$O, MeOH, r.t., 1 h; (ii) oxalyl chloride, DMSO, anhyd CH$_2$Cl$_2$, −55 °C, Et$_3$N, 15 min, 93% over two steps; (iii) NH$_2$, H$_2$NOSO$_3$H, MeOH–CH$_2$Cl$_2$, −50 → −20 °C → r.t., 16 h; (iv) 10% I$_2$/MeOH, Et$_3$N, 0 °C, 70% over two steps; (v) HCl, Et$_2$O, r.t., 1 h, 99%.

**Scheme 2** Synthesis of the photolabile mannose derivatives 7 and 10. 
**Reagents and conditions:** (i) 4, DIPEA, r.t., 2 h, CH$_2$Cl$_2$, 83% for 6; 62% for 9; (ii) 1 M NaOMe/MeOH (0.1 equiv/OH), 0.5 h, r.t., 0 °C, 97% for 7, quant for 10.

**Scheme 3** Synthesis of the photoactive mannose derivatives 13 and 16. 
**Reagents and conditions:** (i) 4, DIPEA, r.t., 2 h, CH$_2$Cl$_2$, 62% for 12; quant for 15; (ii) 1 M NaOMe/MeOH (0.1 equiv/OH), 0.5 h, r.t., 98% for 13; 91% for 16.

**Scheme 4** Synthesis of di-NCS-functionalized sugar 20 and its regioselective reaction to 21. 
**Reagents and conditions:** (i) CS$_2$, P(OEt)$_3$, toluene, 8 h, 80 °C, 76%; (ii) 33% HBr–AcOH, Ac$_2$O, 1.5 h, r.t., 68%; (iii) KSCN (10 equiv), 190 °C, 7 min, 74%; (iv) 4, DIPEA, r.t., CH$_2$Cl$_2$, 88%.
both NCS functions with a branched oligoamine for clustering and the diazirine-functionalized amine 4 for photoactivation in subsequent reactions.

Synthesis of 20 started with the 6-azido-6-deoxy mannose derivative 17,20 which was converted into the corresponding isothiocyanate 18 using carbon disulfide and triethyl phosphate (Scheme 4). Then, the anomeric acetyl group was substituted in HBr–AcOH to deliver the functionalized α-mannosyl bromide 19 in good yield with the 6-isothiocyanato group surviving the rather harsh reaction conditions. Melting of 19 with an excess of potassium thiocyanate gave the 1,6-diisothiocyanato mannose derivative 20 in over 70% yield. In the 13C NMR spectrum of 20 the peaks corresponding to both NCS-carbon atoms can be easily distinguished from one another, 1-NCS resonating at 144.7 and 6-NCS at 135.1 ppm.21 When 20 was stirred with the diazirine-labeled amine 4 the NMR signal at 144.7 ppm disappeared indicating that only the anomeric isothiocyanato function reacted as expected19 to deliver the thiourea derivative 21 in excellent yield. Finally, 21 was treated with tris(2-aminoethyl)amine (22) leading to the trivalent mannosyl cluster 23, which was deprotected according to Zemplén15 giving rise to 24 (Scheme 5). The somewhat low yield in the coupling step is due to difficulties in separating the product from side products.

This contribution has two essential take home messages: (i) the amino-functionalized diazirine 4 is suited for the introduction into NCS-functionalized carbohydrates, e.g. by thiourea bridging within a wide scope; and (ii) this chemistry can be combined with carbohydrate clustering by utilizing the graded reactivity of the different NCS groups in disothiocyanato-functionalized carbohydrates such as 20 to give rise to photolabeled glyoclusters such as 24.

Photolabeling experiments with the herein reported diazirines will eventually be carried out in analogy to the literature, which has reported successful protein labeling with diazirine-functionalized sugars.22 Our first photolabeling studies show, that a thiourea function is compatible with photoactivation of diazirines.

In conclusion, the modular synthetic approach presented in this paper offers a general possibility for the introduction of a diazirine function into various types of biologically interesting molecules by thiourea bridging. The method is orthogonal to ligand clustering based on the graded reactivity of different NCS groups and thus also allows to investigate multivalency effects using photolabeled conjugates.

Optical rotations were measured with a Perkin-Elmer model 241 polarimeter (20 °C, 589 nm, length of cuvette: 1 dm). Reactions were monitored by TLC on silica gel GF254 (Merck) with detection under UV and by charring with 10% H3SO4 in EtOH and subsequent heating. Flash column chromatography was performed on silica gel 60 (40–63 μm, Merck). NMR spectra were recorded on Bruker AMX 400 or Bruker DRX 500 instruments. Chemical shifts are relative to TMS or the solvent peaks of CDCl3 (7.24 ppm 1H, 77.0 ppm 13C) or acetone-d6 (2.04 ppm 1H, 29.3 ppm 13C). Where necessary, assignments were based on COSY and HSQC experiments. IR spectra were taken with a Perkin Elmer FT-IR Paragon 1000 (KBr). MALDI-TOF mass spectra were measured with a Bruker Biflex III with 19 kV acceleration voltage. DHB (c = 10 μg/μL in 40% MeCN–H2O, 0.1% TFA) was used as the matrix. Ionization was effected with a nitrogen laser at 337 nm. ESI mass spectra were measured with an Applied Biosystems Mariner ESI-TOF 5280.

Scheme 5 Synthesis of the photoactive mannosyl cluster 24.
Reagents and conditions: (i) CH2Cl2, 2 h, 0 °C, 30%; (ii) 1 M NaOMe/MeOH (0.1 equiv/OH), 1 h, 0 °C, 69%.

2-(Oxopropyl)carbaminic Acid tert-Butyl Ester (2)
Di-tert-butyl dicarbonate (6.0 g, 28 mmol) was dissolved in MeOH (20 mL) and dichloromethane-2-ol (1.8 mL, 1.72 g, 23 mmol) was added slowly. The mixture was stirred for 1 h at r.t. Then, the solvent was removed in vacuo and the residue taken up in CH2Cl2, washed with conc. NaHCO3 solution and dried (Na2SO4). The crude product was directly used in the following Swern oxidation. Oxalyl chloride (2.2 mL, 3.2 g, 25 mmol) was dissolved in anhyd CH2Cl2 (25 mL) at –55 °C and a solution of DMSO (3.6 mL, 3.9 g, 51 mmol) in anhyd CH2Cl2 (6 mL) was slowly added. After 5 min, a solution of the crude Boc-protected alcohol obtained from 1 in CH2Cl2 (10 mL) was added dropwise to the reaction mixture which was then stirred for 15 min at 0 °C. After the addition of Et3N (8 mL), the solution was warmed to r.t., washed with conc. NaHCO3 solution, dried (Na2SO4) and filtered. Flash column chromatography (cyclohexane–EtOAc, 3:1) delivered the product as a yellowish solid; yield: 3.7 g (93%); mp 48–49 °C.

1H NMR (300 MHz, CDCl3): δ = 5.26 (br s, 1 H, NH), 4.04 (d, JCH2NH = 4.8 Hz, 2 H, CH2), 2.19 (s, 3 H, CH3), 1.45 (s, 9 H, t-C6H13). 13C NMR (75.47 MHz, CDCl3): δ = 203.4 (C=O), 155.5 [OC(O)NH], 79.8 [C(CH3)], 50.9 (CH2), 28.3 [C(CH3)], 27.1 (CH3).

MS (EI): m/z = 173.1 [M]+ (calcd m/z = 173.11) for C6H13NO3.
MS (CI): m/z = 174.1 [M + H]+ (calcd m/z = 174.12) for C4H7NO3, Anal Calc'd for C4H7NO3: C, 55.47; H, 8.73; N, 8.09. Found: C, 55.39; H, 8.79; N, 8.11

(2-Aziripryl)carboxaminic Acid tert-Butyl Ester (3)

Ketone 2 (3.6 g, 21 mmol) was dissolved in 2:1 mixture of anhyd MeOH and CH2Cl2 (30 mL) and cooled to –50 °C. Ammonia (100 mL) was condensed into the solution and the mixture was stirred for 4 h at –20 °C. Then, a solution of hydroxylamine-O-sulfonic acid (2.85 g, 25 mmol) in MeOH (10 mL) was added at –50 °C. After 1 h, the mixture was allowed to warm to r.t. and was stirred overnight. Solids were removed by filtration and the filtrate was concentrated and taken up in Et2O (20 mL). After the addition of Et3N (10 mL), I2 (10% in MeOH) was added until the solution remained yellow. Once more, the solution was concentrated and taken up in Et2O and H2O. The aqueous phase was extracted with Et2O (2×) and the combined organic phases were washed with Na2S2O3 solution and H2O. The aqueous phase was extracted with Et2O (2×) and the combined organic phases were washed with Na2S2O3 solution and H2O. After drying (Na2SO4) and filtration, column chromatography (cyclohexane–EtOAc, 8:1) delivered the product as a colorless solid; yield: 2.6 g (70%).

IR (KBr): 1686 cm⁻¹ (C=O).

1H NMR (300 MHz, CDCl3): δ = 4.47 (br s, 1 H, NH), 3.13 (d, JCHHN = 6.4 Hz, 2 H, CH2), 1.44 (s, 9 H, C(CH3)3). 13C NMR (75.47 MHz, CDCl3): δ = 155.6 [OC(O)NH], 43.9 [CH2(CN)], 28.3 [C(CH3)], 28.6 [C(NCN)], 17.8 (CH3). MS (Cl): m/z = 186.1 [M + H]+ (calcd m/z = 186.1). In the presence of MeOH (2×) and the combined organic phases were washed with Na2S2O3 solution and H2O. After drying (Na2SO4) and filtration, column chromatography (cyclohexane–EtOAc, 8:1) delivered the product as a colorless solid; yield: 2.6 g (70%).

1-Amino-2-azipropyl Hydrochloride (4)
The diazirine 3 (150 mg, 0.8 mmol) was dissolved in EtO (1 mL), conc. HCl (0.5 mL) was added and the mixture was stirred for 30 min at r.t. Then the solution was concentrated and lyophilized; yield: 94 mg (99%).

1H NMR (300 MHz, D2O): δ = 2.94 (s, 2 H, CH2), 1.13 (s, 3 H, CH3).

Diazeirine-Labeled Mannosyl Derivatives 6, 9, 12, 15 by Thiourea Bridging; General Procedure

To a solution of the isothiocyanato-functionalized mannose derivative (1.1 equiv) in anhyd CH2Cl2 (10 mL), was added dropwise a solution of the isothiocyanato-functionalized mannose derivative (1 equiv) in DIPEA (50 μL) and anhyd CH2Cl2 (1 mL). The course of the reaction was monitored by TLC (cyclohexane–EtOAc). After completion of the reaction (2 h), the solvent was removed in vacuo and the crude product was purified by flash column chromatography with the eluents described.

N-(2-Aziripryl)-N'-(2,3,4,6-tetra-O-acetyl-a-d-mannopyranosylthiourido) (6)

Yield: 83%; fine colorless amorphous solid; Rf 0.27 (cyclohexane–EtOAc, 1:1); [α]D20 +58.9 (c = 0.95, CHCl3).

1H NMR (300 MHz, CDCl3): δ = 7.26 (br s, 1 H, man-NH), 6.71 (br t, 1 H, NHCH3), 5.32–5.16 (m, J1,2 = 1.5 Hz, J3,4 = 9.3 Hz, 4 H, H-1 to H-4), 4.29 (dd, J4,5 = 5.7 Hz, 1 H, H-5), 3.61 (dd, J4,5 = 2.6 Hz, 1 JCH = 12.5 Hz, 1 H, H-6'), 4.01 (dd, J5,6 = 9.3 Hz, 5 H, H-5, 3.65 (dd, J5,6 = 15.0 Hz, JCHHN = 5.7 Hz, 2 H, NHCH3)) 2.12, 2.06, 2.02, 1.98 [4 s, 4 x 3 H, 4 x C(O)CH3].

13C NMR (75.47 MHz, CDCl3): δ = 140.7 [C(O)CH3], 118.9 [man-CH2CH2arom], 125.4 [CH2NH], 124.6 [C-6], 114.9 [C-5], 109.5 [C-4], 104.5 [C-3], 101.3 [C-2], 66.7 [C-1], 55.8 [C-2 to C-5], 55.2 [O2NCarom(Cx,x = 0.5), 3,4 = 15.0 Hz, J3,4 = 9.3 Hz, 2 H, H-5, 3.65 (dd, J5,6 = 15.0 Hz, JCHHN = 5.7 Hz, 2 H, NHCH3)) 2.12, 2.06, 2.02, 1.98 [4 s, 4 x 3 H, 4 x C(O)CH3].

1H NMR (300 MHz, CDCl3): δ = 7.26 (br s, 1 H, NH), 6.71 (br t, 1 H, NHCH3), 5.32–5.16 (m, J1,2 = 1.5 Hz, J3,4 = 9.3 Hz, 4 H, H-1 to H-4), 4.29 (dd, J4,5 = 5.7 Hz, 1 H, H-5), 4.11 (dd, J4,5 = 2.6 Hz, 1 JCH = 12.5 Hz, 1 H, H-6'), 4.01 (dd, J5,6 = 9.3 Hz, 5 H, H-5, 3.65 (dd, J5,6 = 15.0 Hz, JCHHN = 5.7 Hz, 2 H, NHCH3)) 2.12, 2.06, 2.02, 1.98 [4 s, 4 x 3 H, 4 x C(O)CH3].

13C NMR (75.47 MHz, CDCl3): δ = 140.7 [C(O)CH3], 118.9 [man-CH2CH2arom], 125.4 [CH2NH], 124.6 [C-6], 114.9 [C-5], 109.5 [C-4], 104.5 [C-3], 101.3 [C-2], 66.7 [C-1], 55.8 [C-2 to C-5], 55.2 [O2NCarom(Cx,x = 0.5), 3,4 = 15.0 Hz, J3,4 = 9.3 Hz, 2 H, H-5, 3.65 (dd, J5,6 = 15.0 Hz, JCHHN = 5.7 Hz, 2 H, NHCH3)) 2.12, 2.06, 2.02, 1.98 [4 s, 4 x 3 H, 4 x C(O)CH3].

Synthesis 2006, No. 6, 952–958 © Thieme Stuttgart · New York
4-Nitrophenyl-6-deoxy-6′-(2′-azipropylthioiroueo)-α-D-mannopyranoside (16)

Yield: 91%; colorless amorphous solid; Rf 0.72 (EtOAc–MeOH, 1:1); [α]D20 +84.5 (c = 1.01, MeOH).

1H NMR (500 MHz, CD3OD): δ = 8.22 (d, J = 9.3 Hz, 2 H, H-3), 7.25 (d, J = 9.3 Hz, 2 H, H-5), 5.65 (dd, J1,2 = 1.9 Hz, 1 H, H-1), 4.05 (dd, J = 1.9, 3.3 Hz, 1 H, H-2), 3.90 (dd, J2,3 = 3.3 Hz, J1,2 = 9.0 Hz, 1 H, H-3), 3.86–3.57 (m, 4 H, H-4, H-5, H-6, H-6′), 3.55–3.28 (m, 2 H, CH, H-2), 0.99 (s, 3 H, CH3).

13C NMR (125.75 MHz, CD3OD): δ = 185.1 (C=O), 162.5 (mann-OCm, 2), 143.9 (O(Nc)m, 126.8 (O(Nc)m(C2,6)), 117.85 (mann-OCm(C6)), 99.9 (C-1), 74.1, 71.7, 71.4, 69.1 (C-2 to C-5), 48.0 (CH2), 46.2 (C-6), 50.1 (C[CH(N)]), 62.3 (CH2) Hz, 3.79 (dd, J = 3.7, 6, 6′ Hz, 1 H, H-6), 2.21, 2.11, 2.03 (s, 3 H, 3 × CH2). Such signals are often observed in thioamides and their derivatives, corroborating the proposed structure.

ESI-MS: m/z = 440.1054 [M + Na]+ (calcd m/z = 440.1054) for C20H39N4O5S + Na.

2,3,4-Tetra-O-acetyl-6-deoxy-6-isothiocyanato-D-mannopyranose (18)

To a solution of the azide (17) in dry THF (10 ml), was added Et3N (3.0 ml) and stirred with Ac2O (1.1 ml) for 0.5 h at r.t. or 0 °C. After complete removal of the acetyl groups, the mixture was re-crystallized from EtOAc–MeOH (3:1) to give 18 as colorless crystals.

1H NMR (300 MHz, CDCl3): δ = 6.12 (d, J1,2 = 2.0 Hz, 1 H, H-1), 5.80 (d, J1,2 = 1.2 Hz, 1 H, H-1′), 5.48 (dd, J = 1.2, 3.1 Hz, 1 H, H-2′), 3.58–3.53 (m, 2 H, H-2′, H-3′), 2.57–2.52 (m, 1 H, H-3′), 5.14 (dd, J2,3 = 1.3 Hz, J1,2 = 9.9 Hz, 1 H, H-3′), 4.00 (dd, J3,4 = 9.3 Hz, Jk,6′ = 4.5 Hz, Jg,6′ = 3.5 Hz, 1 H, H-5′), 3.74 (dd, J = 3.4, 4.4, 9.3 Hz, 1 H, H-6′), 3.71 (dd, J6,6′ = 15.1 Hz, 1 H, H-6′), 3.58 (dd, J = 4.5, 15.1 Hz, 1 H, H-6′), 2.20, 2.18, 2.09, 2.03 (4 × 3 H, CH3O), 2.23, 2.12, 2.04, 2.02 (4 × 3 H, CH3O) Hz, 13C NMR: δ = 170.0, 169.9, 169.6, 168.0 (C=O), 90.2 (C-1), 70.9, 68.3, 68.1, 66.4 (C-2 to C-5), 45.8 (C-6), 20.8, 20.8, 20.6, 20.6 [4 CH3O] Hz. The crude product was purified by HPLC.

Methyl 6-2′(Azipropylthioiroueo)-6-deoxy-6′-D-mannopyranoside (13)

Yield: 98%; colorless amorphous solid; Rf 0.60 (EtOAc–MeOH, 1:1); [α]D20 +131.3 (c = 0.60, MeOH).

1H NMR (500 MHz, CD3OD): δ = 20.48 (br s, 1 H, H-1), 3.95–3.83 (br m, 1 H, H-1), 3.83 (dd, J = 1.6 Hz, 1 H, H-2), 3.70 (dd, J1,2 = 3.3 Hz, J2,3 = 9.5 Hz, 1 H, H-3′), 3.68–3.53 (m, Jk,6′ = 9.5 Hz, 5 H, H-4, H-5, H-6′), 3.40 (s, 3 H, OCH3), 1.09 (s, 3 H, CH3).

13C NMR (125.75 MHz, CD3OD): δ = 142.4 (C=O), 137.7 (C-1, 2′), 72.7, 71.9, 68.3 (C-2 to C-5), 62.6 (C-6), 45.7 (CH3O), 26.9 (CH3O), 18.4 (CH3O) Hz.

ESI-MS: m/z = 343.1046 [M + Na]+ (calcd m/z = 343.1047) for C13H17N3O5S + Na.

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CH₂Cl₂ was evaporated in vacuo. The remaining mixture was heated to 190 °C for 8 min. The cooled melt was dissolved in a 1:1 mixture of H₂O and CH₂Cl₂ (60 mL) and the aqueous phase was extracted with CH₂Cl₂ (2 ×). After drying (Na₂SO₄) and filtration, column chromatography (cyclohexane–EtOAc, 3:1) afforded the product as an amorphous colorless solid; yield: 1.06 g (74%); [α]D²⁰ +116.6 (c = 0.9, CHCl₃).

IR (KBr): 2118 (NCS), 1543 cm⁻¹ [C(NN)].

1H NMR (300 MHz, CDCl₃): δ = 5.6 (dd, J₂,₃ = 2.1 Hz, 1 H, H-1), 5.4–5.3 (m, 3 H, 3 H-2, H-3, H-4), 4.1 (ddd, J₃,₄ = 8.3 Hz, J₃,₅ = 4.6 Hz, J₄,₅ = 3.3 Hz, 1 H, H-5), 3.8 (dd, J₅,₆ = 15.1 Hz, 1 H, H-6), 3.6 (dd, J₂ = 4.6, 15.1 Hz, 1 H, H-6), 2.2, 2.1, 2.0 [3 s, 3 × 3 H, 3 × C(O)CH₃].

13C NMR (125.75 MHz, CDCl₃): δ = 47.6 (CH₂NH), 45.8 (C-6), 25.5 [C(NN)], 20.8, 20.7, 20.7 [3 C(O)CH₃], 18.4 (CH₃).

Anal. Calcd for C₃₉H₇₀N₁₉O₁₂S₆: C, 43.29; H, 4.15; N, 7.21. Found: C, 43.69; H, 4.25; N, 7.22

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