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Synthesis, structural, and biological studies on a pseudodisaccharide containing a bicyclic, bridged carba-sugar

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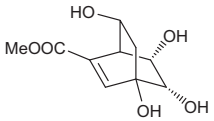
ABSTRACT

Two carba-sugar containing pseudodisaccharide diastereomers have been synthesized from a racemic bicyclooctene derivative. The determination of the absolute configurations was carried out by means of CD measurements, CD calculations and X-ray diffraction. The compounds showed moderate competitive inhibitory effects on chondroitin AC-I lyase.

1. Introduction

Recently[1] we have published the synthesis of a bridged analog of the cytotoxic pericosine antibiotic in racemic form (Fig. 1).



rac-**1**

Figure 1. Pericosine analog *rac*-**1**.

Due to the apparent similarity of *rac*-**1** to uronic acid, *rac*-**1** can be regarded as a carba-uronic acid analog. Since a structural resemblance to the parent sugar generally induces interesting consequences in the biological activity, it was obvious for us to find an application of this compound as a precursor for the synthesis of a pseudodisaccharide derivative. The synthesis of carba-sugars is a developing area of carbohydrate chemistry,[2] but until now, bridged carba-analogs have not been reported.

Glycosaminoglycan-degrading enzymes, including chondroitin AC lyases, have found widespread application for the analysis of various polysaccharides.[3,4] Chondroitin AC lyases catalyze the cleavage of the glycosidic bond on the non-reducing end of the uronic acid part of chondroitin sulfates. The mechanism of the cleavage is based on a β-elimination of the 4-O-substituent of the uronic acid (Scheme 1).[4]

In order to obtain an inhibitor of chondroitin AC lyase, Rye and Withers synthesized uronic acid containing thio-linked disaccharide, which proved to be a weak competitive inhibitor of the enzyme with K_i = 45 mM.[5]

In order to study the role of a bridged carba-uronic acid as a D-glucuronic acid substitute in a potential lyase enzyme inhibitor, we decided to synthesize a pseudodisaccharide using the synthetic intermediate *rac*-**2** as a starting compound.

2. Results and discussion

The conjugate addition of the thiolate of **3** onto the activated double bond of *rac*-**2** led to the formation of a mixture of two diastereomers **4a** and **4b** (Scheme 2). The latter compounds could be obtained in pure form after column chromatography.

In the diastereomers, the absolute stereochemistry of the aglycon could not be deduced by NMR spectroscopy from the known absolute configuration of the sugar moiety because of the free rotation about the sulfur–carbon bonds of the thioglycoside. Thus, ECD spectroscopy was applied to determine the absolute configuration. Diastereomers **4a** and **4b** gave almost mirror image ECD spectra, which confirmed that the sugar moiety has only a minor effect on the ECD properties. Compound **4a** gave a negative n→π* ECD transition as a long plateau in the range of 275–350 nm, while **4b** had a positive n→π* CE (Fig. 2).

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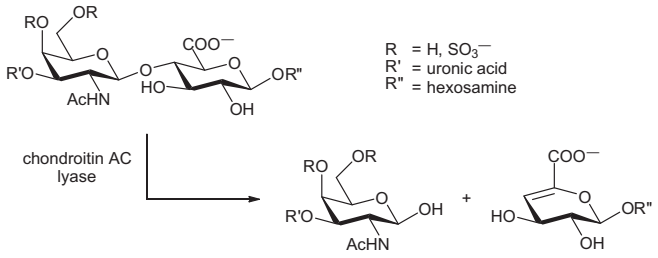
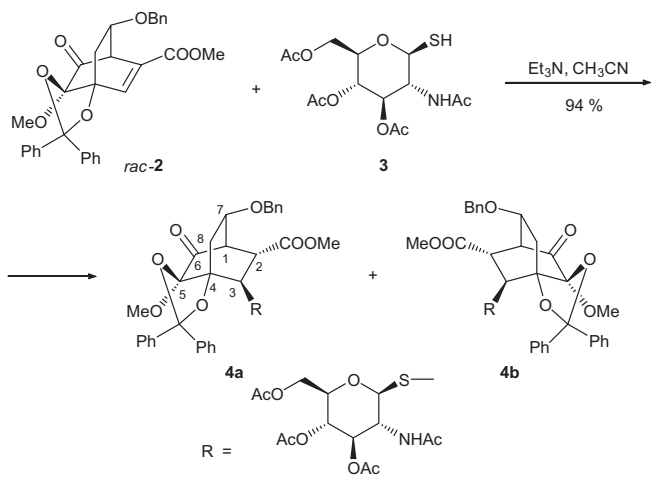


Scheme 1. Proposed elimination mechanism of chondroitin AC lyase.[4]



Scheme 2. Preparation of diastereomers **4a** and **4b** from *rac*-**2**.

However, the n→π* transition could not be used to determine the configuration unambiguously. The application of the octant rule is not reliable, since the orientation of the groups in the sectors, especially in the front ones, cannot be estimated for certainty. Thus the ketone carbonyl of **4a** was reduced to a hydroxyl group and its benzyl group was removed in order to simplify the molecule for a TDDFT ECD calculation (**4a**→**4d**). As a result, the negative ECD band above 270 nm disappeared, while the other regions of the spectrum did not show any significant changes (Fig. 2). In **4d**, it is the diphenylmethylidene acetal chromophore and the C-4 and C-5 stereogenic centers of the 1,3-dioxolane ring that determine the CEs of the ECD spectrum. In order to determine the absolute configuration of **4d**, a TDDFT ECD calculation was carried out on the theoretical model structure **4e**, in which the thioglycoside moiety was replaced by a SMe group (Scheme 3). This simplification could be made, since the diastereomers **4a** and **4b** gave nearly mirror image ECD spectra regardless of the presence of the same thioglycoside moiety. The ECD calculation was performed in order to correlate the experimental ECD curves with the absolute configuration at C-4 and C-5 of **4d**. This allowed us to assign the absolute configuration of **4a** and **4b**.

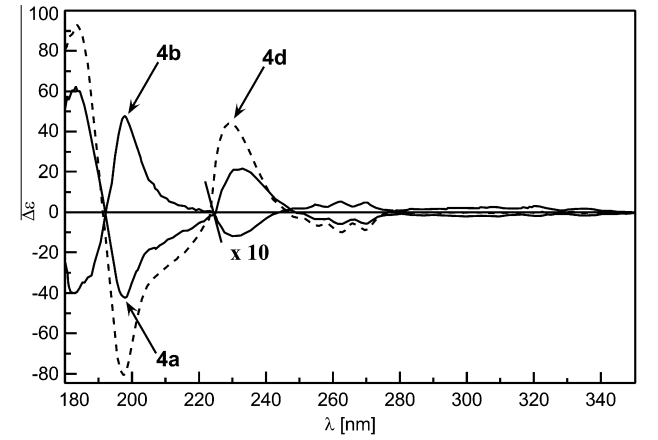
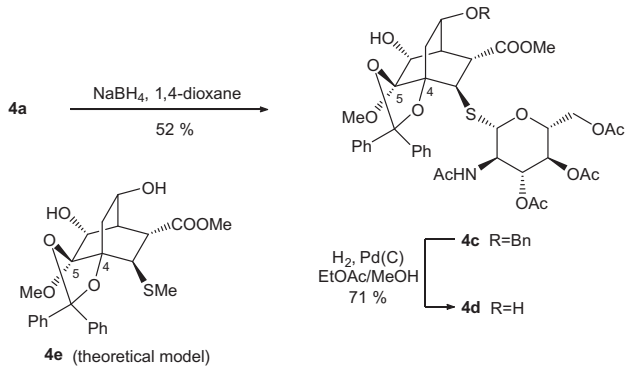


Figure 2. Experimental ECD spectra of **4a**, **4b**, and **4d** in acetonitrile. Δε Values are multiplied by 10 above 225 nm.



Scheme 3. Preparation of **4d** and the structure of its theoretical model **4e**.

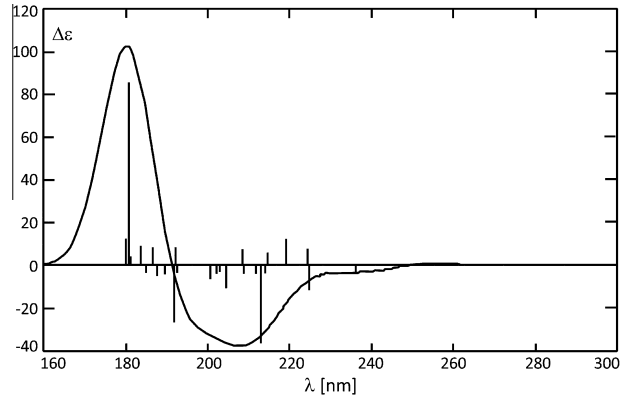


Figure 3. ECD spectra of the theoretical model structure **4e** calculated at the B3LYP/TZVP//HF/6-31G* level of time-dependent density functional theory (Δε in 1000 $cm^2\ mol^{-1}$).

The general shape of the calculated CD curve of **4e** (Fig. 3) nicely reproduces the experimental CD spectrum of compound **4d** in Figure 2. Thus, a very weak negative band calculated at 274 nm (not resolved in Fig. 3) corresponds to the negative part of the experimental CD curve around 260 nm. A slightly stronger positive CD band calculated at 256 nm could be correlated with the observed positive Cotton effect at 229 nm. Moreover, the strong negative and the strong positive Cotton effects observed at 199 and 184 nm can be assigned to those calculated at 208 and 180 nm.

In this way, the absolute configurations (1*R*,2*R*,3*R*,4*R*,5*R*,7*R*) and (1*S*,2*S*,3*S*,4*S*,5*S*,7*S*) have been assigned to the bicyclooctane skeleton of **4a** and **4b**, respectively. These results were supported by X-ray diffraction measurements on **4b** (Fig. 4). Single crystals were grown from a methanol–butanol–water mixture. The asymmetric unit contains four independent molecules of **4b** in the P1 space group resulting in a large unit cell. Solvent areas and one acetyl group remained disordered, resulting in CheckCIF errors, but this does not influence the overall correctness of the absolute structure determination of **4b**.

After Zemplén deacetylation of **4a**, the carbonyl group was reduced resulting in a **6a** quasi-axial alcohol with complete stereose-

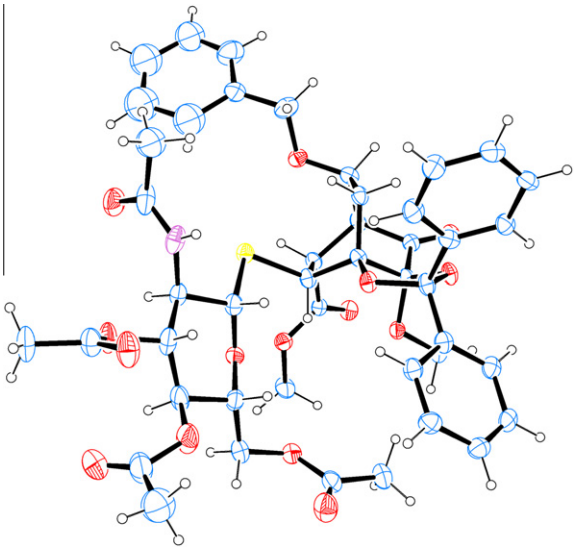
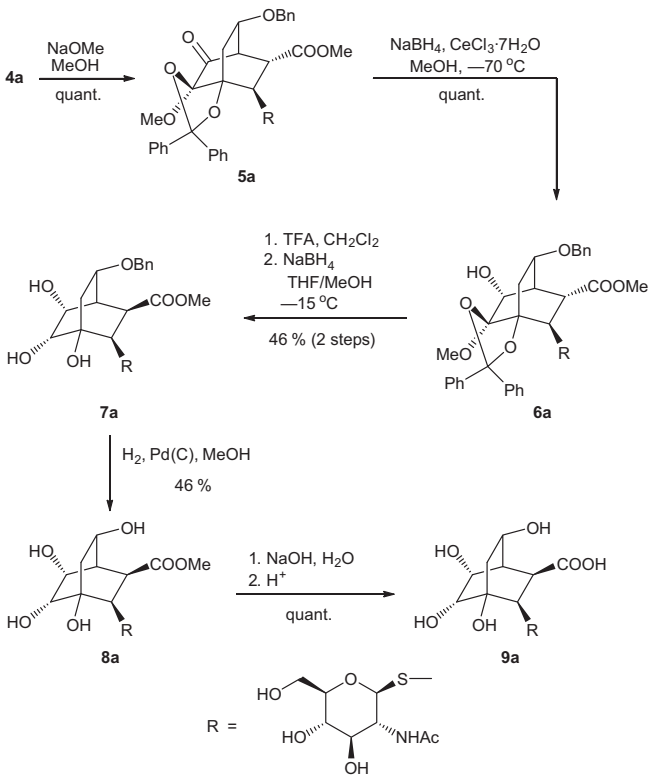
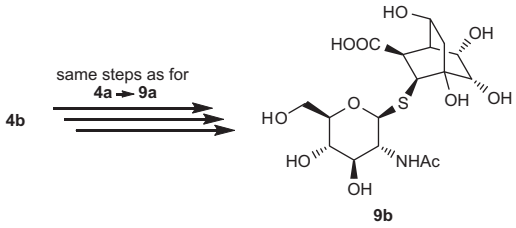


Figure 4. ORTEP view of **4b**.

lectivity. The configuration of the newly formed stereogenic center at C6 was supported by the NOEs of the H6 with H7 and the 6-OH with 5-OMe. In the subsequent steps the diphenylmethylene acetal protecting group was removed by acid hydrolysis and the deliberated carbonyl group was stereoselectively reduced to give **7a** with a delicate stereoheptade generated step by step from the achiral aromatic methyl gallate.[1] It should be noted that during the sodium borohydride reduction, an inversion of configuration of the stereogenic center bearing the carboxylic residue in **7a** as detected. The benzyl ether and methyl ester protecting functions of **7a** were removed by catalytic hydrogenation and careful hydrolysis (Scheme 4). All of these (**4a**→**9a**) chemical transformations were also conducted on diastereomer **4b,** resulting in **9b** (Scheme 5).



Scheme 4. Functionalization of the diastereomer **4a**.



Scheme 5. Functionalization of the diastereomer **4b**.

Both compounds **9a** and **9b** showed competitive inhibition of chondroitin AC-I lyase with K_i values of 2.4×10^{-6} and 1.7×10^{-6} M, respectively (Fig. 5). A K_m value of 1.9×10^{-6} M was determined for the enzyme without an inhibitor. The K_m/K_i values for both compounds were close to 1, indicating comparable enzyme-binding affinities for the natural substrate and inhibitors. Compound **9b** was a slightly better inhibitor than compound **9a**.

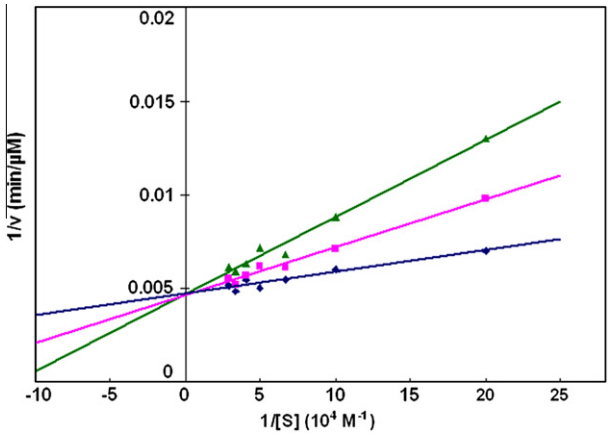


Figure 5. Lineweaver–Burk plot showing the inhibition by **9a** and **9b**. Experimental procedures are detailed in supporting information. Kinetics analysis displayed a competitive inhibition for both compounds **9a** (red square) and **9b** (green triangle) by having the same *y*-intercept as uninhibited enzyme (blue diamond).

3. Conclusion

It is very interesting that both enantiomeric moieties of the **9a** and **9b** pseudodisaccharide diastereomers, bridged carba-uronic acid analogs, could mimic D-glucuronic acid almost equally as well in binding to the enzyme. The low substrate specificity of chondroitin lyase AB is remarkable. These results were as expected based on previous literature that suggests thioglycosides typically bind to glycosidases with similar affinities as their oxygen-substituted counterparts.[6]

4. Experimental

4.1. General

Solutions were concentrated in vacuo at 40 °C. Organic phases were dried over Na_2SO_4. Dry MeOH was distilled over Mg. TLC was performed on Merck Kieselgel F_{254} plates, spots were made visible using UV light (254 nm) and/or spraying with acidic (H_2SO_4) ammonium molybdate solution followed by heating. Column chromatography was carried out using Merck Kieselgel

60 silica (0.063–0.200 mm). NMR spectra were recorded on a Bruker Avance DRX 500 or Bruker Avance 400 spectrometer, at the given frequency and in the given solvent. Chemical shifts are given in ppm and coupling constants in hertz. NMR assignments were based on 2D COSY and 2D HSQC experiments. Mass spectra were obtained on a Bruker microTOF-Q (ESI-QqTOF) spectrometer. CD spectra were recorded with a J-810 spectropolarimeter. Melting points were measured on a Büchi Melting Point B-540 device and are uncorrected. X-ray data were collected on an Oxford Diffraction SuperNova diffractometer equipped with Atlas CCD detector (Cu Kα radiation, $\lambda = 1.54184$ Å). The structure was solved using the SIR-92 software[7] and refined on F^2 using SHELX-97 program,[8] publication material was prepared with the WINGX-97 suite.[9] Crystallographic data (excluding structure factors) for the structure **4b** in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC 834844. 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).

4.2. (1*R*,2*R*,3*R*,4*R*,5*R*,7*R*)-7-Benzyloxy-4,5-dihydroxy-5-methoxy-2-methoxycarbonyl-6-oxo-4,5-*O*-diphenylmethylidenebicyclo-[2.2.2]octan-3-yl 2′-acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-1′-thio-β-D-glucopyranoside 4a and (1*S*,2*S*,3*S*,4*S*,5*S*,7*S*)-7-benzyloxy-4,5-dihydroxy-5-methoxy-2-methoxycarbonyl-6-oxo-4,5-*O*-diphenylmethylidenebicyclo[2.2.2]octan-3-yl 2′-acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-1′-thio-β-D-glucopyranoside 4b

A solution of **2** (1.47 g, 2.87 mmol), 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranose[10,11] (1.25 g, 3.44 mmol), and Et_3N (0.93 ml, 6.67 mmol) in MeCN (65 ml) was stirred at room temperature for 4 h. The reaction mixture was concentrated and the residue was subjected to column chromatography (hexane/CH_2Cl_2/acetone 6:4:1→5:4:1→4:4:1) to furnish the two diastereomers: 1.00 g (40%) **4a** and 1.07 g (43%) **4b**. 0.28 g (11%) of the product was eluted as a mixture of **4a** and **4b** which was separated using preparative TLC (CH_2Cl_2/EtOAc 7:3) to furnish a further 0.17 g (7%) of **4a** and 0.10 g (4%) of **4b**. Single crystals of **4b** were grown by very slow evaporation of methanol–butanol–water mixture. *Compound **4a***: mp: 160–163 °C. HRMS: *m*/*z* calcd for $C_{45}H_{49}NO_{15}SNa$ $[M+Na]^+$ 898.2721, found 898.2750 (ESI-TOF). $[\alpha]_D^{23} = -36.0$ (*c* 0.10, CH_2Cl_2). CD (MeCN, λ [nm] (Δε), $c = 2.58 \times 10^{-4}$): 332sh (−0.14), 318sh (−0.15), 305 (−0.18), 268sh (−0.54), 261 (−0.57), 254sh (−0.35), 232 (2.15), 216sh (−8.89), 206sh (−17.50), 197 (−42.03), 183 (62.57). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.59–7.22 (m, 15H, Ar), 5.69 (d, 1H, $J_{2'}$ = 9.1, NH), 5.41 (t, 1H, $J_{2'}\sim J_{4'}\sim$10, H-3′), 5.21 (t, 1H, $J_{3'}\sim J_{5'}\sim$10, H-4′), 5.19 (d, 1H, $J_{2'}$ = 10.3, H-1′), 4.45 (d, 1H, J_{gem} = 11.8, OCH_2Ph), 4.42 (t, 1H, $J_2\sim{}^4J_{8b}\sim$3, H-3), 4.39 (dd, 1H, $J_{6'a}$ = 12.2, $J_{5'}$ = 5.4, H-6′b), 4.35 (d, 1H, J_{gem} = 11.8, OCH_2Ph), 4.26 (dd, 1H, $J_{6'b}$ = 12.2, $J_{5'}$ = 2.4, H-6′a), 4.19 (dt, 1H, J_{NH} = 9.1, $J_{1'}\sim J_{3'}\sim$10, H-2′), 3.90 (ddd, 1H, $J_{4'}$ = 10.1, $J_{6'a}$ = 5.4, $J_{6'b}$ = 2.4, H-5′), 3.72 (dd, 1H, J_{8b} = 8.7, J_1 = 4.3, H-7), 3.71 (s, 3H, COOMe), 3.52 (t, 1H, $J_1\sim J_3\sim$3, H-2), 3.23 (t, 1H, $J_2\sim J_7\sim$3.5, H-1), 2.86 (s, 3H, 5-OMe), 2.12 (d, 1H, J_{8b} = 15.7, H-8a), 2.09 (s, 3H, Ac), 2.07 (s, 3H, Ac), 1.94 (s, 3H, Ac), 1.80 (s, 3H, Ac), 1.73 (ddd, 1H, J_{8a} = 15.7, J_7 = 8.7, $^4J_3$ = 2.5, H-8b). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 197.4 (C-6), 172.5, 170.9, 170.7, 170.0, 169.4 (4×CH_3CO + *C*OOMe), 143.4–125.0 (Ar), 113.5 (*C*Ph_2), 102.0 (C-5), 87.1 (C-4), 84.4 (C-1′), 76.0 (C-5′), 73.6 (C-3′), 70.8 (C-7), 70.2 (*C*H_2Ph), 68.7 (C-4′), 62.6 (C-6′), 54.2 (C-2′), 52.4 (COO*C*H_3), 50.4 (C-1), 50.1 (5-OMe), 48.9 (C-2), 44.6 (C-3), 31.7 (C-8), 23.1, 20.7, 20.6, 20.5 (4×*C*H_3CO).

*Compound **4b***: mp: 125–128 °C. HRMS: *m*/*z* calcd for $C_{45}H_{49}NO_{15}SNa$ $[M+Na]^+$ 898.2721, found 898.2753 (ESI-TOF). $[\alpha]_D^{23} = +8.3$ (*c* 0.10, CH_2Cl_2). CD (MeCN, λ [nm] (Δε), $c = 2.07 \times 10^{-4}$): 332sh (0.21), 319 (0.28), 305sh (0.26), 268sh (0.49), 260 (0.54), 254 (0.38), 230 (−1.18), 205sh (14.74), 197 (45.42), 182 (−42.34). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8 7.60–7.24 (m, 15H, Ar), 5.82 (d, 1H, $J_{2'}$ = 9.3, NH), 5.48 (t, 1H, $J_{2'}\sim J_{4'}\sim$10, H-3′), 5.38 (d, 1H, $J_{2'}$ = 10.3, H-1′), 5.20 (t, 1H, $J_{3'}\sim J_{5'}\sim$10, H-4′), 4.57 (t, 1H, $J_2\sim{}^4J_{8b}\sim$3, H-3), 4.43 (d, 1H, J_{gem} = 11.8, OCH_2Ph), 4.35 (dd, 1H, $J_{6'a}$ = 12.2, $J_{5'}$ = 5.7, H-6′b), 4.35 (d, 1H, J_{gem} = 11.8, OCH_2Ph), 4.24 (dd, 1H, $J_{6'b}$ = 12.2, $J_{5'}$ = 2.3, H-6′a), 4.14 (dt, 1H, J_{NH} = 9.3, $J_{1'}\sim J_{3'}\sim$10, H-2′), 3.88 (ddd, 1H, $J_{4'}$ = 10.1, $J_{6'b}$ = 5.7, $J_{6'a}$ = 2.3, H-5′), 3.71 (dd, 1H, J_{8b} = 8.7, J_1 = 4.9, H-7), 3.71 (s, 3H, COOMe), 3.47 (t, 1H, $J_1\sim J_3\sim$3, H-2), 3.24 (t, 1H, $J_2\sim J_7\sim$3.5, H-1), 2.87 (s, 3H, 5-OMe), 2.30 (d, 1H, J_{8b} = 15.5, H-8a), 2.09 (s, 3H, Ac), 2.08 (s, 3H, Ac), 1.90 (s, 3H, Ac), 1.89 (s, 3H, Ac), 1.69 (ddd, 1H, J_{8a} = 15.5, J_7 = 8.7, $^4J_3$ = 2.5, H-8b). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 197.6 (C-6), 172.5, 170.9, 170.7, 170.0, 169.5 (4×CH_3CO + *C*OOMe), 143.4–125.1 (Ar), 113.8 (*C*Ph_2), 103.3 (C-5), 88.3 (C-4), 84.1 (C-1′), 76.1 (C-5′), 73.5 (C-3′), 70.5 (C-7), 70.0 (*C*H_2Ph), 68.7 (C-4′), 62.5 (C-6′), 54.2 (C-2′), 52.4 (COO*C*H_3), 50.3 (C-1), 50.2 (5-OMe), 45.8 (C-2), 43.2 (C-3), 31.3 (C-8), 23.2, 20.74, 20.67, 20.5 (4×*C*H_3CO).

4.3. (1*R*,2*R*,3*R*,4*R*,5*S*,6*R*,7*R*)-7-Benzyloxy-4,5,6-trihydroxy-5-methoxy-2-methoxy-carbonyl-4,5-*O*-diphenylmethylidenebicyclo[2.2.2]octan-3-yl 2′-acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-1′-thio-β-D-glucopyranoside 4c

To a solution of **4a** (399 mg, 0.46 mmol) in 1,4-dioxane (10 ml) at 15 °C, $NaBH_4$ (24 mg, 0.63 mmol) was added. The mixture was stirred for 3 h at 15 °C and then concentrated. The residue was partitioned between EtOAc and an aq citric acid solution (0.3%). The organic phase was extracted with saturated $NaHCO_3$ solution, dried and concentrated. The crude was purified by column chromatography (hexane/EtOAc 4:6) to obtain 206 mg (52%) of **4c** as a white solid. Mp: 120–123 °C. HRMS: *m*/*z* calcd for $C_{45}H_{51}NO_{15}SNa$ $[M+Na]^+$ 900.2877, found 900.2844 (ESI-TOF). $[\alpha]_D^{23} = -26.9$ (*c* 0.13, CH_2Cl_2). CD (MeCN, λ [nm] (Δε), $c = 1.59 \times 10^{-4}$): 268sh (−0.84), 261 (−0.96), 254sh (−0.64), 248sh (−0.25), 228 (4.45), 214sh (−21.55), 207 (−31.00), 197 (−80.46), 183 (93.08). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.55–7.19 (m, 15H, Ar); 5.83 (d, 1H, $J_{2'}$ = 8.3, NH); 5.64 (dd, 1H, $J_{2'}$ = 10.3, $J_{4'}$ = 9.3, H-3′); 5.26 (d, 1H, $J_{2'}$ = 10.4, H-1′); 5.17 (dd, 1H, $J_{5'}$ = 10.1, $J_{3'}$ = 9.3, H-4′); 4.54 (d, 1H, J_6 = 10.6, OH); 4.44 (d, 1H, J_{gem} = 12.0, CH_2Ph); 4.40 (dd, 1H, $J_{6'a}$ = 12.2, $J_{5'}$ = 5.5, H-6′b); 4.30 (d, 1H, J_{gem} = 12.0, CH_2Ph); 4.29 (t, 1H, $J_2\sim{}^4J_{8b}\sim$3, H-3); 4.21 (dd, 1H, $J_{6'b}$ = 12.2, $J_{5'}$ = 2.4, H-6′a); 3.99 (dt, 1H, J_{NH} = 8.3, $J_{1'}\sim J_{3'}\sim$10, H-2′); 3.88 (ddd, 1H, $J_{4'}$ = 10.1, $J_{6'b}$ = 5.5, $J_{6'a}$ = 2.4, H-5′); 3.85 (d, 1H, J_{OH} = 10.6, H-6); 3.72 (dd, 1H, J_{8b} = 8.3, J_1 = 4.4, H-7); 3.78 (s, 3H, COOMe); 3.44 (t, 1H, $J_1\sim J_3\sim$3, H-2); 2.86 (s, 3H, 5-OMe); 2.58 (t, 1H, $J_2\sim J_7\sim$3.5, H-1); 2.07 (s, 3H, Ac); 2.03 (s, 3H, Ac); 1.97 (s, 3H, Ac); 1.90 (d, 1H, J_{8b} = 15.2, H-8a); 1.80 (s, 3H, Ac); 1.61 (ddd, 1H, J_{8a} = 15.2, J_7 = 8.5, $^4J_3$ = 2.7, H-8b). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.6, 170.7, 170.5, 170.4, 169.6 (4xCH_3CO + *C*OOMe); 144.6-125.1 (Ar); 112.2 (*C*Ph_2); 106.2 (C-5); 86.2 (C-4); 83.1 (C-1′); 76.0 (C-5′); 74.0 (C-6); 72.9 (C-3′); 72.0 (C-7); 69.6 (*C*H_2Ph); 69.2 (C-4′); 62.9 (C-6′); 55.1 (C-2′); 52.8 (COO*C*H_3); 52.6 (5-OMe); 48.6 (C-2); 44.1 (C-3); 41.4 (C-1); 30.4 (C-8); 23.3 (*C*H_3CO); 20.7 (3C, *C*H_3CO).

4.4. (1*R*,2*R*,3*R*,4*R*,5*S*,6*R*,7*R*)-4,5,6,7-Tetrahydroxy-5-methoxy-2-methoxycarbonyl-4,5-*O*-diphenylmethylidenebicyclo[2.2.2]-octan-3-yl 2′-acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-1′-thio-β-D-glucopyranoside 4d

To a solution of **4c** (49 mg, 62 μmol) in EtOAc (2 ml) and MeOH (10 ml), Pd(C) (43 mg, 10 % Pd) was added. The mixture was stirred

under an H_2 atmosphere overnight. The Pd(C) was removed by filtration through Celite. The filtrate was concentrated and the crude product was subjected to column chromatography (EtOAc) to obtain 35 mg (71 %) of **4d** as a white solid. Mp: 130–135 °C. HRMS: *m*/*z* calcd for $C_{38}H_{45}NO_{15}SNa$ $[M+Na]^+$ 810.2408, found 810.2428 (ESI-TOF). $[\alpha]_D^{23} = -18.1$ (*c* 0.24, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.58–7.17 (m, 10H, Ar), 5.85 (d, 1H, $J_{2'}$ = 8.8, NH), 5.41 (dd, 1H, $J_{2'}$ = 10.3, $J_{4'}$ = 9.3, H-3′), 5.17 (d, 1H, $J_{2'}$ = 10.5, H-1′), 5.15 (dd, 1H, $J_{5'}$ = 10.0, $J_{3'}$ = 9.3, H-4′), 4.51 (d, 1H, J_6 = 10.7, OH), 4.43 (dd, 1H, $J_{6'a}$ = 12.2, $J_{5'}$ = 5.6, H-6′b), 4.38 (t, 1H, $J_2{\sim}{}^4J_{8b}{\sim}3$, H-3), 4.20–4.09 (m, 2H, H-2′+H-6′a), 4.01 (dd, 1H, J_{8b} = 8.6, J_1 = 4.4, H-7), 3.85 (d, 1H, $J_{6\text{-OH}}$ = 10.6, H-6), 3.81 (s, 3H, COOMe), 3.79 (ddd, 1H, $J_{4'}$ = 10.0, $J_{6'b}$ = 5.6, $J_{6'a}$ = 2.6, H-5′), 3.44 (t, 1H, $J_1{\sim}J_3{\sim}3$, H-2), 2.85 (s, 3H, 5-OMe), 2.48 (t, 1H, $J_2{\sim}J_7{\sim}3.5$, H-1), 2.07 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.95 (s, 3H, Ac), 1.81 (ddd, 1H, J_{8a} = 15.8, J_7 = 8.6, $^4J_3$ = 2.5, H-8b), 1.55 (d, 1H, J_{8b} = 15.8, H-8a). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.1, 170.9, 170.6, 170.4, 169.4 (4xCH_3CO + *C*OOMe), 144.5-125.1 (Ar), 112.5 (*C*Ph_2), 106.0 (C-5), 85.6 (C-4), 83.1 (C-1′), 76.0 (C-5′), 74.0 (C-6), 73.3 (C-3′), 68.8 (C-4′), 65.8 (C-7), 62.5 (C-6′), 54.1 (C-2′), 53.0 ($COOCH_3$), 52.5 (5-OMe), 48.0 (C-2), 44.2 (C-3), 43.6 (C-1), 34.3 (C-8), 23.2, 20.7, 20.65, 20.6 (4C, *C*H_3CO).

4.5. (1*R*,2*R*,3*R*,4*R*,5*R*,7*R*)-7-Benzyloxy-4,5-dihydroxy-5-methoxy-2-methoxycarbonyl-6-oxo-4,5-*O*-diphenylmethylidenebicyclo-[2.2.2]octan-3-yl 2′-acetamido-2′-deoxy-1′-thio-β-D-glucopyranoside 5a and (1*S*,2*S*,3*S*,4*S*,5*S*,7*S*)-7-benzyloxy-4,5-dihydroxy-5-methoxy-2-methoxycarbonyl-6-oxo-4,5-*O*-diphenylmethylidenebicyclo[2.2.2]octan-3-yl 2′-acetamido-2′-deoxy-1′-thio-β-D-glucopyranoside 5b

To a solution of **4a**/**4b** (710 mg, 0.81 mmol) in dry MeOH (30 ml), NaOMe solution (1.0 ml, *c* = 0.15 M in MeOH) was added and the mixture was stirred at room temperature for 3 h. The reaction mixture was neutralized using a Serdolit-Red cation-exchanger. The resin was filtered off, washed with MeOH and the filtrate was concentrated. The residue was coevaporated with CH_2Cl_2/hexane to furnish **5a**/**5b** (608/613 mg, quant.) as an off-white powder, which was used in the next step without purification. Analytically pure product can be obtained by column chromatography (CH_2Cl_2/MeOH 94:6). *Compound* ***5a***: mp: 143–146 °C. HRMS: *m*/*z* calcd for $C_{39}H_{43}NO_{12}SNa$ $[M+Na]^+$ 772.2404, found 772.2433 (ESI-TOF). $[\alpha]_D^{23} = -52.6$ (*c* 0.31, CH_2Cl_2). CD (MeCN, λ [nm] ($\Delta\varepsilon$), $c = 2.39 \times 10^{-4}$): 332sh (−0.18), 318 (−0.23), 305sh (−0.21), 268sh (−0.39), 261 (−0.42), 255sh (−0.23), 231 (1.97), 214sh (−6.63), 197 (−33.56), 187 (33.94). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.60–7.14 (m, 16H, Ar+NH), 5.78 (br s, 1H, 3′-OH), 5.32 (br s, 1H, 4′-OH), 5.14 (d, 1H, $J_{2'}$ = 9.8, H-1′), 4.41 (t, 1H, $J_2{\sim}{}^4J_{8b}{\sim}3$, H-3), 4.37 (d, 1H, J_{gem} = 12.0, OCH_2Ph), 4.31 (d, 1H, J_{gem} = 12.0, OCH_2Ph), 4.10–3.82 (m, 4H, H-2′+H-3′+H-6′), 4.01 (t, 1H, $J_{3'}{\sim}J_{5'}{\sim}9.5$, H-4′), 3.87 (br s, 1H, 5′-OH), 3.68 (dd, 1H, J_{8b} = 8.9, J_1 = 4.5, H-7), 3.66–3.60 (m, 1H, H-5′), 3.63 (s, 3H, COOMe), 3.56 (t, 1H, $J_1{\sim}J_3{\sim}3$, H-2), 3.22 (t, 1H, $J_2{\sim}J_7{\sim}4$, H-1), 2.85 (s, 3H, 5-OMe), 2.12 (d, 1H, J_{8b} = 15.3, H-8a), 1.96 (s, 3H, Ac), 1.72 (ddd, 1H, J_{8a} = 15.3, J_7 = 8.9, $^4J_3$ = 2.5, H-8b). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 197.7 (C-6), 172.9, 172.2 (CH_3CO + *C*OOMe), 143.4–125.1 (Ar), 113.6 (*C*Ph_2), 102.0 (C-5), 87.3 (C-4), 84.8 (C-1′), 79.9 (C-5′), 75.8 (C-3′), 70.6 (C-7), 70.3 (C-4′), 70.1 (*C*H_2Ph), 61.8 (C-6′), 55.9 (C-2′), 52.7 ($COOCH_3$), 50.4 (C-1), 50.2 (5-OMe), 49.0 (C-2), 44.8 (C-3), 31.8 (C-8), 23.4 (*C*H_3CO).

Compound ***5b***: mp: 150–153 °C. HRMS: *m*/*z* calcd for $C_{39}H_{43}NO_{12}SNa$ $[M+Na]^+$ 772.2404, found 772.2429 (ESI-TOF). $[\alpha]_D^{23} = +15.8$ (*c* 0.31, CH_2Cl_2). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.60-7.12 (m, 16H, Ar+NH), 5.29 (d, 1H, $J_{2'}$ = 10.2, H-1′), 4.54 (t, 1H, $J_2{\sim}{}^4J_{8b}{\sim}3$, H-3), 4.33 (d, 1H, J_{gem} = 12.0, OCH_2Ph), 4.27 (d, 1H, J_{gem} = 12.0, OCH_2Ph), 4.04–3.96 (m, 3H, H-3′+H-6′), 3.93 (dt, 1H, J_{NH} = 9.2, $J_{1'}{\sim}J_{3'}{\sim}10$, H-2′), 3.83 (t, 1H, $J_{3'}{\sim}J_{5'}{\sim}9$, H-4′), 3.64 (dd, 1H, J_{8b}=8.8, J_1 = 4.4, H-7), 3.61-3.58 (m, 1H, H-5′), 3.62 (s, 3H, COOMe), 3.41 (t, 1H, $J_1{\sim}J_3{\sim}3$, H-2), 3.19 (t, 1H, $J_2{\sim}J_7{\sim}4$, H-1), 2.83 (s, 3H, 5-OMe), 2.30 (d, 1H, J_{8b} = 15.2, H-8a), 2.01 (s, 3H, Ac), 1.70–1.63 (m, 1H, H-8b). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 197.6 (C-6), 172.7, 172.2 (CH_3CO + *C*OOMe), 143.4–125.1 (Ar), 113.7 (*C*Ph_2), 102.2 (C-5), 88.4 (C-4), 84.9 (C-1′), 80.1 (C-5′), 76.0 (C-3′), 70.7 (C-4′), 70.1 (C-7), 69.8 (*C*H_2Ph), 62.1 (C-6′), 56.1 (C-2′), 52.6 ($COOCH_3$), 50.2 (C-1), 50.15 (5-OMe), 46.2 (C-2), 43.8 (C-3), 31.3 (C-8), 23.5 (*C*H_3CO).

4.6. (1*R*,2*R*,3*R*,4*R*,5*S*,6*R*,7*R*)-7-Benzyloxy-4,5,6-trihydroxy-5-methoxy-2-methoxycarbonyl-4,5-*O*-diphenylmethylidenebicyclo[2.2.2]octan-3-yl 2′-acetamido-2′-deoxy-1′-thio-β-D-glucopyranoside 6a and (1*S*,2*S*,3*S*,4*S*,5*R*,6*S*,7*S*)-7-benzyloxy-4,5,6-trihydroxy-5-methoxy-2-methoxycarbonyl-4,5-*O*-diphenylmethylidenebicyclo[2.2.2]octan-3-yl 2′-acetamido-2′-deoxy-1′-thio-β-D-glucopyranoside 6b

To a solution of $CeCl_3{\cdot}7H_2O$ (1.47 g, 3.95 mmol) in MeOH (30 ml) at −30 °C was added $NaBH_4$ (121 mg, 3.20 mmol). The mixture was stirred for 40 min at −30 °C. After cooling the mixture to −70 °C, a solution of **5a**/**5b** (608 mg, 0.81 mmol) in MeOH (9 ml) was added dropwise over 20 min. The reaction mixture was stirred at −70 °C for 2 h, then allowed to warm up to room temperature and evaporated. The residue was separated between CH_2Cl_2 (100 ml) and a 0.25% aq citric acid solution (40 ml). The organic phase was extracted with a saturated $NaHCO_3$ solution. The combined aq extracts were shaken with CH_2Cl_2, then the combined organic extracts were dried, filtered, and evaporated. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 93:7) to furnish **6a**/**6b** (612 mg, quant./523 mg, 86%) as a white solid. *Compound* ***6a***: mp: 144–147 °C. HRMS: *m*/*z* calcd for $C_{39}H_{45}NO_{12}SNa$ $[M+Na]^+$ 774.2560, found 774.2571 (ESI-TOF). $[\alpha]_D^{23} = -37.2$ (*c* 0.31, CH_2Cl_2). CD (MeCN, λ [nm] ($\Delta\varepsilon$), $c = 2.47 \times 10^{-4}$): 268sh (−0.71), 261 (−0.78), 254sh (−0.45), 229 (4.04), 207sh (−3.75), 197 (−56.10), 186 (52.60). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.60–7.09 (m, 16H, Ar+NH), 5.06 (d, 1H, $J_{2'}$ = 9.1, H-1′), 4.48 (d, 1H, J_6 = 10.8, 6-OH), 4.37 (d, 1H, J_{gem} = 12.1, OCH_2Ph), 4.28 (br s, 1H, H-3), 4.26 (d, 1H, J_{gem} = 12.1, OCH_2Ph), 4.05 (br s, 2H, H-6′), 4.01–3.90 (m, 2H, H-2′+H-3′), 3.90–3.82 (m, 1H, H-4′), 3.82 (d, 1H, $J_{6\text{-OH}}$ = 10.8, H-6), 3.70 (s, 3H, COOMe), 3.68 (dd, 1H, J_{8b} = 8.7, J_1 = 4.3, H-7), 3.59–3.51 (m, 1H, H-5′), 3.47 (t, 1H, $J_1{\sim}J_3{\sim}3$, H-2), 2.81 (s, 3H, 5-OMe), 2.54 (t, 1H, $J_2{\sim}J_7{\sim}4$, H-1), 1.94 (s, 3H, Ac), 1.79 (d, 1H, J_{8b} = 15.1, H-8a), 1.65–1.56 (m, 1H, H-8b). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 176.5, 172.2 (CH_3CO + *C*OOMe), 144.5–125.2 (Ar), 112.2 (*C*Ph_2), 106.0 (C-5), 85.9 (C-4), 84.7 (C-1′), 79.9 (C-5′), 75.7 (C-3′), 74.0 (C-6), 71.8 (C-7), 70.4 (C-4′), 69.5 (*C*H_2Ph), 61.9 (C-6′), 55.9 (C-2′), 53.1 ($COOCH_3$), 52.5 (5-OMe), 48.9 (C-2), 45.4 (C-3), 41.2 (C-1), 30.7 (C-8), 23.4 (*C*H_3CO).

Compound ***6b***: mp: 155–157 °C. HRMS: *m*/*z* calcd for $C_{39}H_{45}NO_{12}SNa$ $[M+Na]^+$ 774.2560, found 774.2578 (ESI-TOF). $[\alpha]_D^{23} = +5.6$ (*c* 0.30, CH_2Cl_2). CD (MeCN, λ [nm] ($\Delta\varepsilon$), $c = 2.42 \times 10^{-4}$): 268sh (0.64), 261 (0.77), 254sh (0.50), 230 (−3.00), 210sh (17.30), 197 (70.20), 186 (−44.92). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.57–7.11 (m, 15H, Ar), 6.87 (d, 1H, $J_{2'}$ = 6.5, NH), 5.17 (d, 1H, $J_{2'}$ = 9.7, H-1′), 4.54 (d, 1H, J_6 = 10.8, 6-OH), 4.35 (t, 1H, $J_2{\sim}{}^4J_{8b}{\sim}2$, H-3), 4.32 (d, 1H, J_{gem} = 12.1, OCH_2Ph), 4.21 (d, 1H, J_{gem} = 12.1, OCH_2Ph), 3.99 (br s, 2H, H-6′), 3.90–3.79 (m, 4H, H-6+H-2′+H-3′+H-4′), 3.70 (s, 3H, COOMe), 3.65 (dd, 1H, J_{8b} = 8.6, J_1 = 4.4, H-7), 3.59–3.53 (m, 1H, H-5′), 3.24 (t, 1H, $J_1{\sim}J_3{\sim}3$, H-2), 2.82 (s, 3H, 5-OMe), 2.53 (t, 1H, $J_2{\sim}J_7{\sim}4$, H-1), 1.88 (s, 3H, Ac), 2.09 (d, 1H, J_{8b} = 14.7, H-8a), 1.62–1.54 (m, 1H,

H-8b). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 176.7, 172.3 (CH_3CO+-*C*OOMe), 144.5–125.2 (Ar), 112.6 (*C*Ph_2), 106.3 (C-5), 87.5 (C-4), 85.2 (C-1′), 79.8 (C-5′), 76.7 (C-6; from 2D-HSQC), 73.8 (C-3′), 71.4 (C-7), 70.7 (C-4′), 69.2 (*C*H_2Ph), 62.1 (C-6′), 56.1 (C-2′), 52.9 (COO*C*H_3), 52.5 (5-OMe), 45.4 (C-2), 44.9 (C-3), 41.2 (C-1), 29.9 (C-8), 23.3 (*C*H_3CO).

4.7. (1*R*,2*S*,3*R*,4*R*,5*R*,6*R*,7*R*)-7-Benzyloxy-4,5,6-trihydroxy-2-methoxycarbonyl-bicyclo[2.2.2]octan-3-yl 2′-acetamido-2′-deoxy-1′-thio-β-D-glucopyranoside 7a and (1*S*,2*R*,3*S*,4*S*,5*S*,6*S*,7*S*)-7-benzyloxy-4,5,6-trihydroxy-2-methoxycarbonyl-bicyclo[2.2.2]octan-3-yl 2′-acetamido-2′-deoxy-1′-thio-β-D-glucopyranoside 7b

To a solution of **6a**/**6b** (390 mg, 0.52 mmol) in CH_2Cl_2 (20 ml), TFA (0.2 ml) was added and stirred at room temperature for 1 h. The reaction mixture was diluted with dry toluene (20 ml) and evaporated, then a further 10 ml of dry toluene was evaporated from the residue. The crude product was dissolved in THF/MeOH (9:1, 20 ml), cooled to −15 °C, then $NaBH_4$ (85 mg, 2.25 mmol) was added. After stirring the reaction mixture at −15 °C for 30 minutes, it was diluted with MeOH (15 ml) and neutralized using Serdolit-Red cation-exchanger at room temperature. The resin was filtered off and washed with MeOH. To the filtrate aq. AcOH (96%, 0.3 ml) was added and evaporated to a volume of approximately 10 ml. The residue was then coevaporated with MeOH (20 ml) to a volume of approximately 5 ml. Traces of AcOH were removed by coevaporating the residue with 2 × 20 ml dry toluene. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 83:17) to furnish **7a**/**7b** (133 mg, 46%/162 mg, 56%) as a white powder if coevaporated with CH_2Cl_2/hexane. *Compound* ***7a***: mp: 180–190 °C (dec). HRMS: *m*/*z* calcd for $C_{25}H_{35}NO_{11}SNa$ $[M+Na]^+$ 580.1829, found 580.1810 (ESI-TOF). $[\alpha]_D^{23} = -24.5$ (*c* 0.20, MeOH). CD (MeCN, λ [nm] ($\Delta\varepsilon$), $c = 3.10 \times 10^{-4}$): 236 (−0.18), 213 (2.43). ^{1}H NMR (500 MHz, CD_3OD): δ = 7.36–7.24 (m, 5H, Ar), 4.66 (d, 1H, $J_{2'}$ = 10.5, H-1′), 4.49 (br s, 2H, OCH_2Ph), 3.93 (dd, 1H, J_2 = 7.9, $^4J_{8b}$ = 2.3, H-3), 3.90 (dd, 1H, J_5 = 8.6, J_1 = 3.7, H-6), 3.89 (dd, 1H, $J_{6'b}$ = 12.1, $J_{5'}$=2.2, H-6′a), 3.79 (t, 1H, $J_{1'}$∼$J_{3'}$∼9, H-2′), 3.75–3.70 (m, 1H, H-7), 3.69 (s, 3H, COOMe), 3.66 (d, 1H, J_6 = 8.6, H-5), 3.63 (dd, 1H, $J_{6'a}$ = 12.1, $J_{5'}$ = 5.9, H-6′b), 3.43 (t, 1H, $J_{2'}$∼$J_{4'}$∼9, H-3′), 3.32 (t, 1H, $J_{3'}$∼$J_{5'}$∼9, H-4′), 3.39–3.33 (m, 1H, H-5′), 2.99 (dd, 1H, J_3 = 7.9, J_1 = 2.1, H-2), 2.69 (m, 1H, H-1), 1.95 (s, 3H, Ac), 1.79 (dd, 1H, J_{8b} = 14.1, J_7 = 4.9, H-8a), 1.74 (ddd, 1H, J_{8a} = 14.1, J_7 = 9.2, $^4J_3$ = 2.3, H-8b). ^{13}C NMR (125 MHz, CD_3OD): δ = 176.6, 174.0 (CH_3CO + *C*OOMe), 139.8, 129.5, 128.9, 128.8 (Ar), 87.6 (C-1′), 82.3 (C-5′), 77.3 (C-3′), 72.7 (C-4), 72.3 (C-7), 71.9 (C-4′), 71.5 (C-5), 71.4 (*C*H_2Ph), 66.7 (C-6), 63.0 (C-6′), 56.4 (C-2′), 52.6 (COO*C*H_3), 46.4 (C-3), 46.2 (C-2), 42.9 (C-1), 37.0 (C-8), 23.1 (CH_3*C*O).

Compound ***7b***: mp: 180–190 °C (dec). HRMS: *m*/*z* calcd for $C_{25}H_{35}NO_{11}SNa$ $[M+Na]^+$ 580.1829, found 580.1838 (ESI-TOF). $[\alpha]_D^{23} = +26.1$ (*c* 0.20, MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ = 7.37–7.24 (m, 5H, Ar), 4.84 (d, 1H, $J_{2'}$ = 10.5, H-1′), 4.51 (br s, 2H, OCH_2Ph), 4.11 (dd, 1H, J_2 = 7.1, $^4J_{8b}$ = 2.5, H-3), 3.87 (dd, 1H, J_5 = 8.6, J_1 = 3.7, H-6), 3.79 (dd, 1H, $J_{6'b}$ = 12.1, $J_{5'}$ = 2.5, H-6′a), 3.73 (ddd, 1H, J_{8b} = 9.6, J_{8a} = 4.2, J_1 = 2.7, H-7), 3.71–3.65 (m, 2H, H-2′+H-6′b), 3.67 (s, 3H, COOMe), 3.61 (d, 1H, J_6 = 8.6, H-5), 3.44 (t, 1H, $J_{2'}$∼$J_{4'}$∼9, H-3′), 3.37 (t, 1H, $J_{3'}$∼$J_{5'}$∼9, H-4′), 3.25 (ddd, 1H, $J_{4'}$ = 9.5, $J_{6'b}$ = 5.5, $J_{6'a}$ = 2.5, H-5′), 3.14 (dd, 1H, J_3 = 7.1, J_1 = 2.5, H-2), 2.66 (m, 1H, H-1), 1.98 (dd, 1H, J_{8b}=14.0, J_7 = 4.2, H-8a), 1.97 (s, 3H, Ac), 1.70 (ddd, 1H, J_{8a} = 14.0, J_7=9.6, $^4J_3$ =2.5, H-8b). ^{13}C NMR (125 MHz, CD_3OD): δ = 177.0, 173.9 (CH_3CO + *C*OOMe), 139.8, 129.5, 128.9, 128.8 (Ar), 86.1 (C-1′), 81.6 (C-5′), 77.7 (C-3′), 73.5 (C-4), 72.6 (C-7), 71.9 (2C, C-5+C-4′), 71.4 (*C*H_2Ph), 67.0 (C-6), 63.1 (C-6′), 57.0 (C-2′), 52.6 (COO*C*H_3), 43.8 (C-3), 45.8 (C-2), 42.9 (C-1), 36.2 (C-8), 23.2 (CH_3*C*O).

4.8. (1*R*,2*S*,3*R*,4*R*,5*R*,6*R*,7*R*)-4,5,6,7-Tetrahydroxy-2-methoxycarbonyl-bicyclo[2.2.2]octan-3-yl 2′-acetamido-2′-deoxy-1′-thio-β-D-glucopyranoside 8a and (1*S*,2*R*,3*S*,4*S*,5*S*,6*S*,7*S*)-4,5,6,7-tetrahydroxy-2-methoxycarbonyl-bicyclo[2.2.2]octan-3-yl 2′-acetamido-2′-deoxy-1′-thio-β-D-glucopyranoside 8b

To a solution of **7a**/**7b** (128 mg, 0.23 mmol) in MeOH (15 ml) Pd(C) (94 mg, 10 % Pd) was added. After flushing the flask with argon the suspension was hydrogenized using a H_2-filled balloon for 24 h. The mixture was filtered through Celite to remove Pd(C) and then concentrated. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 6:4) to furnish 45 mg (42%) of **8a**/**8b** (45 mg, 42%/55 mg, 54%) as a colorless film, which was then lyophilized to obtain a hygroscopic white substance. *Compound* ***8a***: HRMS: *m*/*z* calcd for $C_{18}H_{29}NO_{11}SNa$ $[M+Na]^+$ 490.1359, found 490.1356 (ESI-TOF). $[\alpha]_D^{23} = -41.8$ (*c* 0.12, MeOH).^{1}H NMR (500 MHz, CD_3OD): δ = 4.69 (d, 1H, $J_{2'}$ = 10.2, H-1′), 3.87 (dd, 1H, J_2=8.3, $^4J_{8b}$ = 2.5, H-3), 3.95–3.88 (m, 3H, H-6+H-7+H-6′a), 3.76 (t, 1H, $J_{1'}$∼$J_{3'}$∼10, H-2′), 3.69 (s, 3H, COOMe), 3.65 (d, 1H, J_6 = 8.4, H-5), 3.64 (dd, 1H, $J_{6'a}$ = 12.1, $J_{5'}$ = 6.1, H-6′b), 3.45 (t, $J_{2'}$∼$J_{4'}$∼9, 1H, H-3′), 3.40–3.32 (m, 2H, H-4′+H-5′), 2.98 (dd, 1H, J_3 = 8.3, J_1 = 1.9, H-2), 2.47–2.45 (m, 1H, H-1), 1.96 (s, 3H, Ac), 1.75 (ddd, 1H, J_{8a} = 14.1, J_7 = 9.7, $^4J_3$ = 2.5, H-8b), 1.63 (dd, 1H, J_{8b} = 14.1, J_7 = 5.1, H-8a). ^{13}C NMR (125 MHz, CD_3OD): δ = 176.7, 173.9 (CH_3*C*O + -*C*OOMe), 87.8 (C-1′), 82.3 (C-5′), 77.4 (C-3′), 72.7 (C-4), 72.0 (C-4′), 71.3 (C-5), 67.0 (C-6), 64.7 (C-7), 63.1 (C-6′), 56.6 (C-2′), 52.6 (COO*C*H_3), 47.0 (C-3), 46.5 (C-1), 45.6 (C-2), 39.0 (C-8), 23.1 (CH_3*C*O).

Compound ***8b***: HRMS: *m*/*z* calcd for $C_{18}H_{29}NO_{11}SNa$ $[M+Na]^+$ 490.1359, found 490.1347 (ESI-TOF). $[\alpha]_D^{23} = +28.2$ (*c* 0.12, MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ = 4.86 (d, 1H, $J_{2'}$ = 10.5, H-1′), 4.11 (dd, 1H, J_2 = 7.4, $^4J_{8b}$ = 2.4, H-3), 3.96 (ddd, 1H, J_{8b} = 9.7, J_{8a} = 4.6, J_1=2.6, H-7), 3.90 (dd, 1H, J_5 = 8.7, J_1 = 3.7, H-6), 3.77 (dd, 1H, $J_{6'a}$ = 12.1, $J_{5'}$ = 2.5, H-6′a), 3.71–3.65 (m, 2H, H-2′+H-6′b), 3.67 (s, 3H, COOMe), 3.60 (d, 1H, J_6 = 8.7, H-5), 3.43 (t, 1H, $J_{2'}$∼$J_{4'}$∼9, H-3′), 3.38 (m, 1H, $J_{3'}$∼$J_{5'}$∼9, H-4′), 3.24-3.19 (m, 2H, H-2+H-5′), 2.45–2.43 (m, 1H, H-1), 1.97 (s, 3H, Ac), 1.86 (dd, 1H, J_{8b} = 13.7, J_7 = 4.6, H-8a), 1.71 (ddd, 1H, J_{8a} = 13.7, J_7 = 9.7, $^4J_3$ = 2.4, H-8b). ^{13}C NMR (125 MHz, CD_3OD): δ = 177.3, 173.9 (CH_3CO + *C*OOMe), 86.0 (C-1′), 81.5 (C-5′), 77.9 (C-3′), 73.6 (C-4), 71.7 (C-4′), 71.8 (C-5), 66.9 (C-6), 65.1 (C-7), 62.9 (C-6′), 57.0 (C-2′), 52.5 (COO*C*H_3), 44.1 (C-3), 46.4 (C-1), 45.4 (C-2), 38.1 (C-8), 23.2 (CH_3*C*O).

4.9. (1*R*,2*S*,3*R*,4*R*,5*R*,6*R*,7*R*)-4,5,6,7-Tetrahydroxy-2-carboxy-bicyclo[2.2.2]octan-3-yl 2′-acetamido-2′-deoxy-1′-thio-β-D-glucopyranoside 9a and (1*S*,2*R*,3*S*,4*S*,5*S*,6*S*,7*S*)-4,5,6,7-tetrahydroxy-2-carboxybicyclo[2.2.2]octan-3-yl 2′-acetamido-2′-deoxy-1′-thio-β-D-glucopyranoside 9b

To a solution of **8a**/**8b** (5 mg, 11 μmol) in H_2O (200 μl), aq NaOH solution (210 μl, 0.1 M) was added. After stirring for 1.5 h, the reaction mixture was neutralized using a Serdolit-Red cation-exchanger. The resin was filtered off, washed with H_2O, and the filtrate was lyophilized to obtain **9a**/**9b** (5 mg/5 mg, quant.) as a hygroscopic substance. *Compound* ***9a***: HRMS: *m*/*z* calcd for $C_{17}H_{27}NO_{11}SNa$ $[M+Na]^+$ 476.1203, found 476.1192 (ESI-TOF). $[\alpha]_D^{23} = -51.8$ (*c* 0.18, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 4.65 (d, 1H, $J_{2'}$ = 10.5, H-1′), 3.94–3.85 (m, 3H, H-6+H-7+H-6′a), 3.83–3.74 (m, 2H, H-3+H-2′), 3.68–3.60 (m, 2H, H-4′+H-6′b), 3.42–3.32 (m, 3H, H-5+H-3′+H-5′), 3.04 (br d, 1H, J_3∼9, H-2), 2.17 (m, 1H, H-1), 1.99 (s, 3H,

Ac), 1.71 (ddd, 1H, J_{8a} = 13.9, J_7 = 9.2, $^4J_3$ = 2.2, H-8b), 1.60 (dd, 1H, J_{8b} = 13.9, J_7 = 5.0, H-8a). ^{13}C NMR (100 MHz, CD_3OD): δ = 182.8, 174.0 (CH_3CO + COOH), 87.1 (C-1′), 82.3 (C-5′), 78.0 (C-3′), 73.0 (C-4), 72.5 (C-4′), 72.0 (C-5), 68.1 (C-6), 66.2 (C-7), 63.0 (C-6′), 56.5 (C-2′), 50.2 (C-2), 49.1 (C-3; from 2D-HSQC), 44.7 (C-1), 38.3 (C-8), 23.1 (CH_3CO).

Compound ***9b***: HRMS: *m*/*z* calcd for $C_{17}H_{27}NO_{11}SNa$ $[M+Na]^+$ 476.1203, found 476.1199 (ESI-TOF). $[\alpha]_D^{23} = +35.4$ (*c* 0.18, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 4.79 (d, 1H, $J_{2'}$ = 10.4, H-1′), 3.94–3.79 (m, 4H, H-3+H-6+H-7+H-6′a), 3.71–3.59 (m, 3H, H-5+H-2′+H-6′b), 3.50 (t, 1H, $J_{2'}\sim J_{4'}\sim$9, H-3′), 3.33–3.25 (m, 2H, H-4′+H-5′; partially covered by CHD_2OD), 3.00 (br d, 1H, $J_3\sim$9, H-2), 2.15 (br s, 1H, H-1), 1.98 (s, 3H, Ac), 1.77–1.67 (m, 2H, H-8). ^{13}C NMR (100 MHz, CD_3OD): δ = 182.7, 173.9 (CH_3CO+COOH), 84.8 (C-1′), 82.1 (C-5′), 77.7 (C-3′), 73.7 (C-4), 72.7 (C-4′), 72.0 (C-5), 68.2 (C-6), 66.3 (C-7), 63.0 (C-6′), 57.1 (C-2′), 49.1 (C-2; from 2D-HSQC), 46.1 (C-3), 44.6 (C-1), 37.9 (C-8), 23.2 (CH_3CO).

4.10. Enzyme inhibitory tests

Chondroitin AC-I lyase from *Flavobacterium heparinum*[12] was prepared as a recombinant enzyme from *Escherichia coli* in Professor Cygler's laboratory at the Biotechnology Research Institute in Montreal as previously described.[13] Assays were performed in quartz cuvettes (1 cm path length) using a total solution volume of 500 μl. Mixtures containing the desired amount of chondroitin sulfate A substrate and inhibitor **9a** or **9b** (6 μM) in 100 mM sodium phosphate buffer, pH 6.0 were pre-incubated at 25 °C for 10 min. Chondroitin AC lyase was then added to a final concentration of 0.01 μM. The reaction solution was quickly mixed and the absorbance at 232 nm was monitored at 37 °C over a 5 min period of time. Initial reaction rates were calculated using an extinction coefficient of 3800 M^{-1} cm^{-1} for the enzymatic disaccharide products obtained from chondroitin sulfate A.

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References

1. Fejes, Zs.; Mándi, A.; Komáromi, I.; Bényei, A.; Naesens, L.; Fenyvesi, F.; Szilágyi, L.; Herczegh, P. *Tetrahedron* **2009**, *65*, 8171.
2. Arjona, O.; Gómez, A. M.; López, J. C.; Plumet, J. *Chem. Rev.* **2007**, *107*, 1919.
3. Lunin, V. V.; Li, Y.; Linhardt, R. J.; Miyazono, H.; Kyogashima, M.; Kaneko, T.; Bell, A. W.; Cygler, M. *J. Mol. Biol.* **2004**, *337*, 367.
4. Yip, V. L. Y.; Withers, S. G. *Org. Biomol. Chem.* **2004**, *2*, 2707.
5. Rye, C. S.; Withers, S. G. *Carbohydr. Res.* **2004**, *339*, 699.
6. Driguez, H. *Top. Curr. Chem.* **1997**, *187*, 85.
7. Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A. *J. Appl. Cryst.* **1993**, *26*, 343.
8. Sheldrick, G. M. *Acta Cryst. A* **2008**, *A64*, 112.
9. Farrugia, L. J. *J. Appl. Cryst.* **1999**, *32*, 837.
10. Horton, D.; Wolfrom, M. L. *J. Org. Chem.* **1962**, *27*, 1794.
11. Wang, L.-X.; Lee, Y. C. *J. Chem. Soc., Perkin Trans. 1* **1996**, 581.
12. Gu, K.; Linhardt, R. J.; Laliberte, M.; Zimmermann, J. *Biochem. J.* **1995**, *312*, 569.
13. Huang, W.; Boju, L.; Tkalec, L.; Su, H.; Yang, H. O.; Gunay, N. S.; Linhardt, R. J.; Kim, Y. S.; Matte, A.; Cygler, M. *Biochemistry* **2001**, *40*, 2359.