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Oxocarbenium ion cyclizations for the synthesis of disaccharide mimetics of 2-amino-2-deoxy-pyranosides: Application to the carbasugar of β-galactosamine-(1,4)-3-O-methyl-D-chiro-inositol

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ABSTRACT

The synthesis of the carbasugar of β-galactosamine-(1,4)-3-O-methyl-D-chiro-inositol (INS-2), a potential tool for studying glucose metabolism, is described. The synthetic strategy, entails an oxocarbenium ion cyclization on a *chiro*-inositol derived, thioacetal-enol ether to give a carbocyclic enol ether, which is elaborated to the 2-amino-2-deoxy carbasugar framework via a 2-oximo derivative.

1. Introduction

The 2-amino 2-deoxysugars *N*-acetylglucosamine (GluNAc) and *N*-acetylgalactosamine (GalNAc) are widely occurring on glycoproteins and polysaccharides that are involved in a variety of cellular processes including cell division, neuronal development, angiogenesis, blood coagulation, inflammation, tumor progression and microbial and viral infection [1–5]. Less common stereochemical variants of GluNAc and GalNAc comprise natural products with notable biological properties [6]. Accordingly, mimetics of 2-amino-2-deoxy-sugars are of interest as molecular probes of a variety of biological pathways and potential therapeutics [7–10]. We have been interested in C- [11–14] and carba [15–21] glycoside isosteres, wherein the ring or glycoside oxygen is replaced with a "CH_2" respectively (cf **1**–**3**, Fig. 1). As these analogues are ethers and not glycosides, unlike their parent glycosides, they are resistant to enzymatic and chemical hydrolysis, and for this reason are potentially useful for interrogating glycosyl processing enzymes and as stable therapeutics [22]. Their nuanced conformational properties relative to their O-glycoside parents, together with the "O" to "CH_2" have also led to their use as probes of carbohydrate protein binding [23,24].

Against this backdrop, and as a launchpad for a general approach to disaccharide type mimetics of 2-amino-2-deoxy-disaccharides, we undertook the synthesis C- and carba glycosides, **2** and **3** respectively, of the novel inositol glycan pseudo disaccharide, β-galactosamine-(1,4)-3-*O*-methyl-D-chiro-inositol **1** (INS-2) [25,26] This glycan has been shown to be an insulin-mimetic and insulin sensitizing [27,28]. It has been hypothesized that these properties are connected to activation of two related protein phosphatases, mitochondrial pyruvate dehydrogenase phosphatase (PDHP), and a Mg-dependent protein Ser/Thr phosphatase called PP2Ca, which respectively dephosphorylate the rate limiting enzymes of oxidative glucose disposal (i.e. PDH), and non-oxidative glucose disposal (glycogen synthase, GS). To identify a metabolically stable analog of INS-2 for potential therapeutic application as well as structure-activity relationships, we compared the activity of INS and C–INS–2 against PDHP and PP2Ca [25]. Like INS-2, C–INS–2 activated PDHP, but in contrast to INS-2, failed to activate PP2Ca. This result suggests that the glycoinositol binding site on the two phosphatases are different, and that INS-2 binds to distinctive sites to activate phosphatases for insulin signaling. *In silico* binding of INS-2 and C–INS–2 and an allosteric receptor site on PP2Ca suggested that the different activity of INS-2 and C–INS–2 may be due to their different conformational properties. However, another explanation is that the glycosidic oxygen in INS-2 (which is absent in C–INS–2), may be directly involved in binding. In this context, the binding and activity of the carba–INS–2 **3**, which is expected to have similar conformational properties to C–INS–2, but contains a pseudo-glycosidic oxygen, may elucidate the molecular basis of activity. Herein, we describe the synthesis of carba–INS–2.

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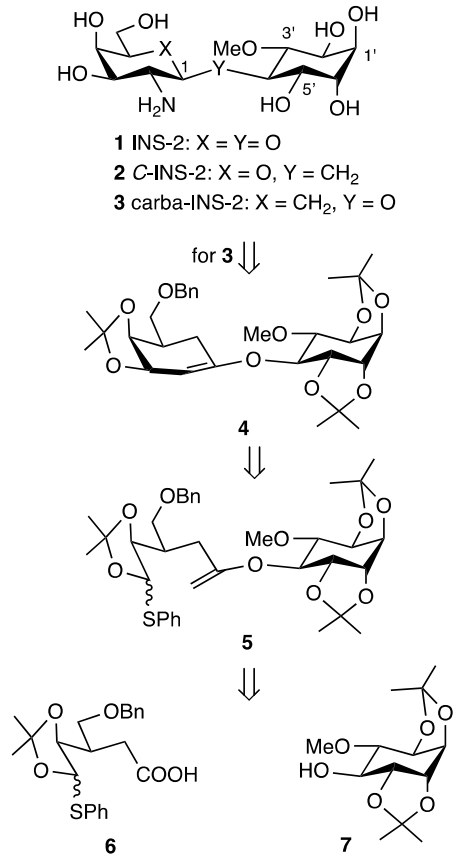
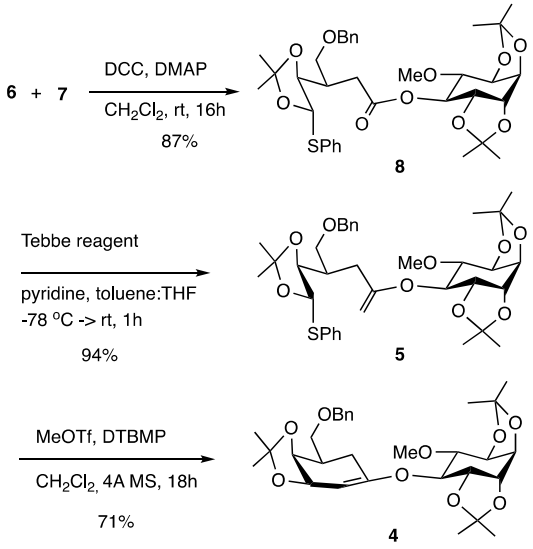


Fig. 1. Synthesis plan for carba–INS–2.

2. Results and discussion

The synthesis of **3** followed an oxocarbenium ion mediated, "glycosyl" ring forming strategy that we used for C–INS–2 and related carba-disaccharides [29–31]. Thus, **3** could be obtained from the elaboration of the cyclic enol ether **4** which would come from an oxocarbenium ion cyclization on the enol ether-thioacetal **5** (Fig. 1). The latter in turn can be assembled from acid **6** and alcohol **7** [29,32]. This approach to carba-glycosides is particularly relevant to disaccharide type frameworks in that it addresses both the synthesis of the unnatural "CH_2" mutation in the "glycosyl" segment and the challenging ether link to the "aglycone" residue. By comparison more conventional methods for carbasugars employ the coupling of preformed "glycone" and "aglycone" segments, but this strategy is somewhat limited to carba-monosaccharides with relatively simple "aglycone" or carba disaccharides with primary ether linkages [16,33].

DCC promoted esterification of acid **6,** which we had previously prepared from D-lyxose, and alcohol **7** provided ester **8** (Scheme 1) [29, 32]. Next, Tebbe olefination on **8** afforded enol ether **5** [34,35]. Because of the sensitivity of this material to acid, chromatography over silica gel was performed with triethylamine in the mobile phase. It should be noted that because of the presence of approximately 15% of the C3 epimer in **6** (which was not separable from **6**), the subsequent ester and enol ether derivatives were obtained as inseparable mixtures of **8** and **5** and minor amounts of their respective C3 epimers. The key oxocarbenium cyclization was executed by exposure of **5** to methyl triflate in dichloromethane in the presence of 2,6-ditert-butyl-4-methylpyridine (DTBMP) and molecular sieves. The enol ether **4** was produced in 71% yield, together with a minor amount of the C3 epimer, which was separated by chromatographically. Interestingly, consistent with our earlier results for related oxocarbenium cyclizations, none of the regioisomeric cyclic enol ether was observed. This selectivity may be a result of torsional effects on the deprotonation step leading to **4** [36].



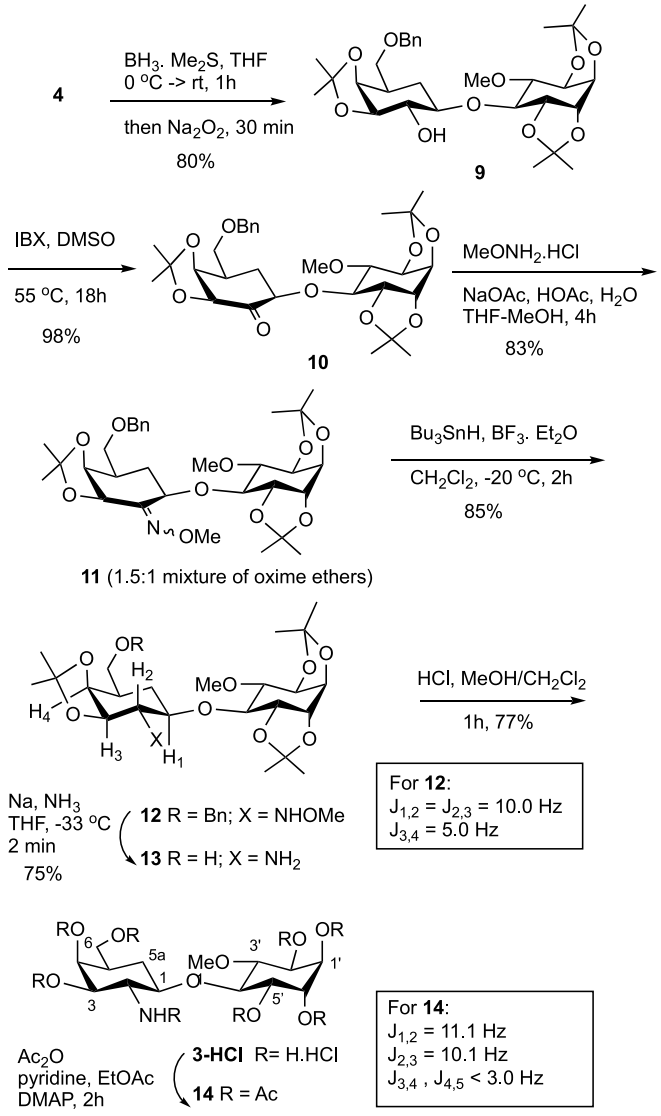
Scheme 1. Synthesis of cyclic enol ether 4.

The next transformation was the introduction of the 2-amino substituent. To this end, we envisaged stereoselective reduction on a 2-oxime derivative [37]. Thus hydroboration-oxidation on **4** provided alcohol **9** as a single diastereomer (Scheme 2). Then oxidation of **9** using IBX provided ketone **10**, which was treated with *O*-methyl hydroxylamine to provide oxime **11** as a 1.5:1 mixture of isomers. Attempted reduction of **11** with standard hydride reducing agents gave low yield of the desired product [38,39]. Optimal conditions were exposure of **11** to Bu_3SnH and $BF_3.OEt_2$ in anhydrous CH_2Cl_2 at −20 °C, which provided a single methoxyamine **12** in 83% yield [40]. This reaction proceeded smoothly at −20 °C, but elevation of the temperature to 0 °C led to low yields due to reductive opening of the 3,4-*O*-isopropylidene, and lower stereoselectivity in the reduction of the oxime. The stereochemistry in the newly formed "glycone" residue was based on the absolute configuration in the acid precursor **6** and vicinal $J_{H,H}$ coupling constants. Thus $J_{1,2} = J_{2,3} = 10.0$ and $J_{3,4} = 5.0$ Hz supported the *β-galcto* type motif in **12**.

Treatment of **12** under Birch reduction conditions effected removal of the benzyl protecting group and N–O reduction to give **13**. Exposure of **13** to a mixture of HCl in methanol and dichloromethane provided carba–INS–2 as the hydrochloride salt **3-HCl**. For characterization purposes this material was transformed to the peracetylated derivative **14**, by treatment with acetic anhydride in a mixture of ethyl acetate and pyridine. The structures of **3-HCl** and **14** were confirmed by 1D and 2D ^1H and ^{13}C NMR experiments and HRMS (Table 1 and Supporting Information).

3. Conclusion

The synthesis of a carba galactosamine-chiro inositol pseudo disaccharide, a mechanistic probe for studying glucose metabolism, was accomplished from a lyxose derived chiron and commercially available D-pinitol. The key steps in this synthesis were a pivitol oxocarbenium ion cyclization on a D-pinitol derived enol ether-thioacetal precursor, and a highly stereoselective reduction of a 2-oximino carbagalactoside. Applications of this synthetic approach to carba-disaccharides of β-GalNAc and other classes of 2-amino-2-deoxy pyranosides, and evaluation of the protein phosphatase activating properties of the title compound are in progress.



Scheme 2. Transformation of 4 to carba–INS–2.

4. Experimental section

4.1. General methods

Solvents were purified by standard procedures or used from commercial sources as appropriate. Petroleum ether refers to the fraction of petroleum ether boiling between 40 and 60 °C. Unless otherwise stated thin layer chromatography (TLC) was done on 0.25 mm thick precoated silica gel 60 (HF-254, Whatman) aluminium sheets and flash column chromatography (FCC) was performed using Kieselgel 60 (32–63 mesh, Scientific Adsorbents). Elution for FCC usually employed a stepwise solvent polarity gradient, correlated with TLC mobility. Chromatograms were observed under UV (short and long wavelength) light, and/or were visualized by heating plates that were dipped in a solution of ammonium (VI) molybdate tetrahydrate (12.5 g) and cerium (IV) sulfate tetrahydrate (5.0 g) in 10% aqueous sulfuric acid (500 mL), or a solution of 20% sulfuric acid in ethanol. NMR spectra were recorded using Varian Unity Plus 500 and Bruker Ultra Shield Plus 600 MHz instruments, in $CDCl_3$ or C_6D_6 and D_2O solutions. Unless otherwise stated residual $CHCl_3$ and C_6H_6 or HOD were used as internal standards respectively (δ_H 7.27, 7.16, 4.80 and δ_C 77.2, 128.4 ppm). Chemical shifts are quoted in ppm relative to tetramethysilane (δ_H 0.00) and coupling constants (J) are given in Hertz. First order approximations are employed throughout. High resolution mass spectrometry was performed on an Agilent 6520 Q-TOF instrument.

Table 1
NMR data for carba–INS–2-HCl (3-HCl) and carba–INS–2-OAc (14).

	carba–INS–2-HCl (3-HCl) – (D_2O)		carba–INS–2-OAc (14) – (C_6D_6)	
# H/C	^{1}H (600 MHz) ppm [multiplicity, J (Hz)]	^{13}C (125 MHz) ppm	^{1}H (600 MHz) ppm [multiplicity, J(Hz)]	^{13}C (125 MHz) ppm
1	3.73 (dt, 4.7, 10.9)	79.7	3.82 (m)	78.0
2	3.27 (t, 9.3)	57.2	4.29 (bq, 11.6)	54.3
3	3.66 (dd, 2.6, 11.0)	71.4	5.08 (bd, 10.0)	72.7
4	4.03 (bs)	68.8	5.60 (bs)	68.1
5	1.71 (m)	38.6	1.50 (m)	35.3
5a	1.38 (dt, 11.8, 12.7) 2.08 (dt, 3.7, 12.7)	27.3	1.54 (m) 1.89 (m)	29.1
6	3.53 (m) 3.63 (dd, 7.9, 10.7)	62.5	3.69 (dd, 5.3, 11.2) 4.00 (dd, 8.9, 10.9)	63.5
1'*	3.94 (m)	72.0	5.71 (dd, 3.2, 5.8)	68.5
2'*	3.76 (dd, 2.7, 10.0)	71.8	5.59 (dd, 3.0, 8.6)	71.8
3'*	3.28 (t, 9.3)	82.6	3.66 (t, 7.9)	81.5
4'*	3.53 (m)	81.6	3.97 (t, 7.9)	76.6
5'*	3.89 (dd, 2.7, 9.8)	70.9	5.65 (dd, 2.5, 7.9)	71.8
6'*	3.94 (m)	72.1	5.76 (bs)	68.1
OMe	3.55 (s)	61.3	3.40 (s)	61.2
NH	ND	–	4.96 (bs)	–
CH_3CO	–	–	1.54 (s), 1.60 (s), 1.68 (s), 1.69 (s), 1.76 (s), 1.79 (s), 1.84 (s), 1.96 (s)	20.3, 20.5 20.6, 20.8 20.9, 21.2 23.7
C=O	–	–	–	169.4, 169.6 169.8, 170.2 170.3, 170.4 170.6, 170.8

For **3-HCl**, peak assignments for nuclei pairs 3'/4', 2'/5', 1'/6' may be interchanged.

4.2. (3aS,4S,5R,5aR,8aR,8bS)-5-methoxy-2,2,7,7-tetramethylhexahydrobenzo[1,2-d:3,4-d']bis([1,3]dioxole)-4-yl 4-(benzyloxy)-3-((2R,4S,5S)-2-methyl-5-(phenylthio)-1,3-dioxolan-4-yl) butanoate **(8)**

DCC (0.048 g, 0.23 mmol) was added at 0 °C to a mixture of **6** [29] (77.2 mg, 0.19 mmol), **7** [32] (68.2 mg, 0.25 mmol) and DMAP (7.0 mg, 0.058 mmol) in dry dichloromethane (2 mL). The reaction mixture was warmed to rt and stirred for 6 h. The mixture was then diluted with ether and filtered. The filtrate was successively washed with 0.1 N aqueous HCl and brine, dried (Na_2SO_4), filtered, and evaporated in *vacuo*. FCC of the residue afforded **8** (110 mg, 87%) as colorless oil; R_f = 0.60 (15% ethyl acetate: petroleum ether). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.36 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.47 (s, 6H, 2xCH_3), 1.58 (s, 3H, CH_3), 2.55 (m, 2H, 3, 3a), 2.78 (m, 1H, 3a)), 3.22 (dd, J = 7.4, 11.4 Hz, 1H. 3'), 3.47 (s, 3H, OCH_3), 3.65 (m, 2H, CH_2-4), 4.26 (m, 3H, 2, 2', 5'), 4.38 (m, 2H, 1', 6'), 4.52 (ABq, J = 12.0 Hz, $\Delta\delta$ = 0.04 ppm, 2H, $PhCH_2$), 5.07 (dd, J = 8.2, 11.4 Hz, 1H, 4'), 5.48 (d, J = 6.6 Hz, 1H, 1), 7.22–7.41 (m, 8H, Ph), 7.45–7.53 (m, 2H, Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.4, 25.6, 25.8, 27.4, 25.6, 27.9, 32.3, 38.5, 59.7, 69.5, 72.3,

73.1, 75.8, 76.4, 78.8, 80.0, 80.6, 87.1, 109.5, 109.8, 110.9, 127.2, 127.5, 127.6, 128.3, 128.9, 131.3, 131.5, 134.6, 138.3, 171.7; HRMS (ESI) *m/z* calcd for $C_{35}H_{46}O_{10}NaS$ (M + Na) + 681.2703; found 681.2684.

4.3. (3aR,4S,5S,5aR,8aR,8bR)-4-((5-(benzyloxy)-4-((4S,5S)-2,2-dimethyl-5-(phenylthio)-1,3-dioxolan-4-yl)pent-1-en-2-yl)oxy)-5-methoxy-2,2,7,7-tetramethylhexahydrobenzo[1,2-d:3,4-d']bis([1,3]dioxole) **(5)**

To a solution of **8** (1.10 g, 1.70 mmol), and pyridine (0.5 mL) in an anhydrous mixture of toluene: THF (30:15 mL), was added, under an argon atmosphere and at −78 °C, Tebbe reagent (10.0 mL, 0.5 M in THF). The reaction mixture was warmed to rt and stirred at this temperature for 1 h. The mixture was then slowly poured into a solution of 1 M aqueous NaOH at 0 °C, and the resulting suspension extracted with ether. The combined organic phase was washed with brine, dried (Na_2SO_4), filtered and concentrated in *vacuo*. FCC of the residue afforded **5** (1.04 g, 94%) as a light yellow oil; $R_f = 0.80$ (basic alumina, 20% ethyl acetate: petroleum ether); IR (film) cm^{-1} 1673. ^1H NMR (500 MHz, C_6D_6) δ 1.16 (s, 6H, CH_3), 1.41 (s, 3H, CH_3), 1.46 (2s, 6H, 2x CH_3), 1.52 (s, 3H, CH_3), 2.42 (dd, 1H, $J = 8.6$, 13.8 Hz, 1H, 3a), 2.65–2.80 (m, 2H, 3, 3a), 3.13–3.22 (m, 1H, 3'), 3.48 (s, 3H, OCH_3), 3.65 (dd, $J = 7.1$, 9.4 Hz, 1H, 4), 3.75 (dd, $J = 3.8$, 9.3 Hz, 1H, 4), 4.05 (m, 2H, 2xIns-H), 4.15 (m, 3H, 3xIns-H), 4.22 (d, $J = 1.5$ Hz, 1H, C═CH), 4.37 (ABq $\Delta\delta = 0.07$, $J = 9.9$ Hz, 2H, $PhCH_2$), 4.60 (d, $J = 1.5$ Hz, 1H, C═CH), 4.63 (dd, $J = 5.3$, 6.4 Hz, 1H, 2), 5.88 (d, $J = 6.6$ Hz, 1H, 1), 7.89–7.38 (m, 8H, Ph), 7.56–7.67 (m, 2H, Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.7, 25.8, 26.3, 28.0, 28.3, 28.3, 34.2, 39.7, 60.2, 70.5, 73.7, 77.3, 77.4, 78.9, 79.2, 79.5, 81.9, 82.1, 87.0, 88.0, 109.6, 111.3, 127.3, 127.9, 128.2, 128.7, 128.9, 129.0, 128.9, 129.5, 129.5, 129.5, 131.6; HRMS (ESI) *m/z* calcd for $C_{36}H_{48}O_9NaS$ (M + Na) + 679.2908, found 679.2928.

4.4. (3aR,4S,5S,5aR,8aR,8bR)-4-(((7R,7aS)-7-((benzyloxy)methyl)-2,2-dimethyl-3a,6,7,7a-tetrahydrobenzo[d][1,3]dioxol-5-yl)oxy)-5-methoxy-2,2,7,7-tetramethylhexahydrobenzo[1,2-d:3,4-d']bis([1,3]dioxole) **(4)**

A mixture of **5** (100 mg, 0.15 mmol), 2,6-di-tert-butyl-4-methylpyridine (370 mg, 1.8 mmol), and freshly activated, powdered 4 Å molecular sieves (300 mg) in anhydrous CH_2Cl_2 (5 mL), was stirred for 15 min, at rt, under atmosphere of argon, then cooled to 0 °C. Methyl triflate (0.17 ml, 1.5 mmol) was then introduced, and the mixture warmed to rt, and stirred for an additional 18 h, at which time, triethylamine (0.3 mL) was added. The mixture was diluted with ether, washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered and evaporated in *vacuo*. FCC of the residue provided **4** (55 mg, 71%) as light yellow oil; $R_f = 0.55$ (basic alumina, 20% ethyl acetate: petroleum ether); $[\alpha]_{23}^{D}$: +34 (c 0.30C_6H_6). IR (film) cm^{-1} 1668. ^1H NMR (500 MHz, C_6D_6) δ 1.17 (s, 3H, CH_3), 1.18 (3, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 2.15 (m, 1H, 5), 2.33 (dd, $J = 5.3$, 16.2 Hz. 1H, 5a), 2.45 (ddt, $J = 1.9$, 11.6, 16.2 Hz, 1H, 5a), 3.28 (dd, $J = 7.3$, 11.0 Hz, 1H, 3'/4'), 3.43 (dd, $J = 7.8$, 8.8 Hz, 1H, 6), 3.46 (s, 3H, OCH_3), 3.60 (dd, $J = 7.0$, 8.8 Hz, 1H, 6), 4.06 (t, $J = 7.2$ Hz, 1H, 3'/4'), 4.10–4.25 (m, 5H, H4, 1′, 2′, 5′, 6'), 4.31 (bs, 2H, $PhCH_2$), 4.71 (m, 1H, 3), 5.25 (bt, $J = 2.6$ Hz, 1H, 2), 7.04–7.29 (m, 5H, Ph); ^{13}C NMR (125 MHz, C_6D_6) δ 25.6, 25.7, 27.5, 27.9, 28.2, 28.3, 28.9, 38.0, 60.3, 72.7, 73.5, 73.5, 75.4, 77.5, 77.7, 78.1, 79.2, 79.5, 82.0, 98.5, 109.1, 109.6 (two peaks), 128.2, 128.7, 128.6, 128.7, 139.7, 156.7. HRMS (ESI) *m/z* calcd for $C_{30}H_{42}O_9Na$ (M + Na) + 569.2721, found 569.2702.

4.5. (3aR,4S,5R,7R,7aS)-7-((benzyloxy)methyl)-5-(((3aR,4S,5S,5aR,8aR,8bR)-5-methoxy-2,2,7,7-tetramethylhexahydrobenzo[1,2-d:3,4-d']bis([1,3]dioxole)-4-yl)oxy)-2,2-dimethylhexahydrobenzo[d][1,3]dioxol-4-ol **(9)**

$BH_3.Me_2S$ (0.45 mL, 1 M solution in THF, mmol, 0.45 mmol) was added at 0 °C to a solution of **4** (0.60 mg, 0.11 mmol) in anhydrous THF (3 mL) under an atmosphere of argon. The mixture was warmed to rt and stirred for an additional 1 h. At that time the solution was recooled to 0 °C and treated with a mixture of 3 N NaOH (1 mL) and 30% aqueous H_2O_2 (1 mL) for 30 min. The solution was then diluted with ether and washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered and evaporated under reduced pressure. FCC of the crude product afforded **9** (48 mg, 80%) as a clear gum; $R_f = 0.17$ (30% ethyl acetate: petroleum ether); IR (film) cm^{-1} 3488. ^1H NMR (500 MHz, $CDCl_3$) δ1.29 (s, 6H, 2xCH_3), 1.33 (s, 3H, CH_3), 1.49 (m, 1H, 5a), 1.54 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 1.57 (s, 3H), 2.05 (m, 1H, 5a), 2.15 (m, 1H, 5), 3.10 (dd, $J = 7.0$, 11.2 Hz, 1H, 3′), 3.34 (m, 1H, 1), 3.53 (m, 2H, 2′, 6), 3.57 (s, 3H, OCH_3), 3.65 (t, $J = 9.3$ Hz, 1H, 2), 3.78 (dd, $J = 7.5$, 9.0 Hz, 1H, 6), 3.93 (dd, $J = 5.0$, 7.6 Hz, 1H, 3), 4.19 (t, $J = 6.7$ Hz, 1H, 4′), 4.21–4.39 (m, 4H. 4, 1′, 5′, 6'), 4.49 (s, 2H, Ph CH_2), 7.35 (m, 5H, Ph); ^{13}C NMR (125 MHz, C_6D_6) δ 25.0, 25.4, 26.4, 27.3, 27.8, 28.3, 28.5, 35.1, 59.8, 71.4, 73.1, 74.1, 76.3, 76.4, 76.7, 76.8, 78.3, 78.7, 79.7, 80.3, 81.6, 82.1, 83.6, 109.2, 109.5, 110.1, 127.1, 127.4, 127.5, 128.4, 138.6; HRMS (ESI) *m/z* calcd for $C_{30}H_{43}O_{10}Na$ (M + Na) + 587.2826, found 587.2815.

4.6. (5R,7R,7aS)-7-((benzyloxy)methyl)-5-(((3aR,4S,5S,5aR,8aR,8bR)-5-methoxy-2,2,7,7-tetramethylhexahydrobenzo[1,2-d:3,4-d']bis([1,3]dioxole)-4-yl)oxy)-2,2-dimethyltetrahydrobenzo[d][1,3]dioxol-4(3aH)-one **(10)**

IBX (60 mg, 0.20 mmol) was added to the solution of **9** (43 mg, 0.076 mmol) in DMSO (1 ml). The mixture was heated at 55 °C over 18 h, then diluted with saturated aqueous $NaHCO_3$ and extracted with ether. The organic phase was dried (Na_2SO_4) and evaporated in *vacuo*. Purification of the residue by FCC provided **10** (42 mg, 98%) as the colorless oil; $R_f = 0.79$ (30% ethyl acetate: petroleum ether); IR (film) cm^{-1} 1748. ^1H NMR (500 MHz, C_6D_6) δ 1.18 (s, 3H, CH_3), 1.19 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.50 (s, 1H, CH_3), 1.92 (apparent q, $J = 12.7$ Hz, 1H, 5a), 2.00–2.11 (m, 1H, 5), 2.28 (m, 1H, 5a), 3.30 (dd, $J = 7.1$, 8.8 Hz, 1H, 3'), 3.50 (dd, J = 6.5, 11.7, 1H, 6), 1H), 3.52 (m, 2H, 6, Ins-H), 3.56 (s, 3H, OCH_3), 3.99 (d, $J = 5.0$ Hz, 1H, 3), 4.05–4.18 (m, 4H, 1, 4, Ins-H), 4.30 (m, 2H, 2xIns-H), 4.27 (ABq $\Delta = 0.07$ ppm, $J = 12.2$ Hz, 2H, $PhCH_2$), 4.50 (t, $J = 6.9$ Hz, 1H, Ins-H), 7.07–7.33 (m, 10H, Ph); ^{13}C NMR (125 MHz, C_6D_6) δ 25.5, 25.8, 26.8, 27.8, 28.2, 28.3, 31.9, 36.1, 60.5, 71.3, 73.7, 77.6, 78.0, 78.1, 79.7, 80.0, 80.3, 81.7, 81.8, 82.2, 109.8, 109.8, 110.3, 128.1, 128.9, 139.4, 204.5; HRMS (ESI) *m/z* calcd for $C_{30}H_{42}O_{10}Na$ (M + Na) + 585.2670, found 585.2676.

4.7. (5R,7R,7aS)-7-((benzyloxy)methyl)-5-(((3aR,4S,5S,5aR,8aR,8bR)-5-methoxy-2,2,7,7-tetramethylhexahydrobenzo[1,2-d:3,4-d']bis([1,3]dioxole)-4-yl)oxy)-2,2-dimethyltetrahydrobenzo[d][1,3]dioxol-4(3aH)-one O-methyl oxime **(11)**

To a solution of **10** (17 mg, 0.30 mmol) in a 1:1 mixture THF/MeOH (1.5 mL), was added a solution of O-methyl hydroxylamine hydrochloride (30 mg, 4.39 mmol) and NaOAc (32 mg, 4.82 mmol) in water (0.75 mL). The pH of the solution was adjusted to 4.5 by addition of glacial acetic acid, and the mixture stirred at rt for 4 h. The solution was then diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ and water, dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure to give **11** as a 2:1 mixture of oxime O-methyl ethers (15 mg, 83%) as a clear film; $R_f = 0.65$ (20% ethyl acetate: petroleum ether); ^1H

NMR (600 MHz, $CDCl_3$), major isomer, δ 1.25 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.55 (m, buried under H_2O peak, 1H, 5), 1.78 (m, 1H, 5a), 2.05 (m, 1H, 5a), 3.22 (dd, $J = 7.0$, 11.1 Hz, 1H, Ins-H), 3.30 (dd, $J = 5.8$, 9.2 Hz, 1H, 6), 3.48 (m, partially buried, 1H, 6), 3.50 (s, 3H, OCH_3), 3.68 (dd, $J = 6.1$, 11.2 Hz, 1H, Ins-H), 3.83 (s, 3H, $NOCH_3$), 4.05 (m, 3H, 3xIns-H), 4.25 (t, $J = 6.1$ Hz, 1H, Ins-H), 4.38 (t, $J = 9.2$ Hz, 1H, 1), 4.45 (m, 3H, 4, $PhCH_2$), 5.42 (dd, 1H, $J = 7.6$, 0.8 Hz, 3), 7.25–7.40 (m, 5H, Ph); ^{13}C NMR (125 MHz, $CDCl_3$), mixture, δ 23.8, 23.9, 24.2, 25.0, 25.1, 25.3, 25.5, 25.6, 25.8, 27.7, 27.7, 28.0, 29.1, 35.3, 35.8, 60.5, 60.7, 62.1, 62.3, 66.4, 67.3, 67.7, 70.6, 70.9, 73.2, 74.0, 74.6, 75.7, 76.6, 77.8, 78.6, 78.7, 79.5, 80.5, 81.2, 109.5, 109.7, 109.9, 110.1, 127.6, 127.6, 128.4, 138.4, 153.2; HRMS (EI) *m/z* calcd for $C_{31}H_{46}NO_{10}$ (M + H) + 592.3116, found 592.3122.

4.8. *N-((3aR,4S,5R,7R,7aS)-7-((benzyloxy)methyl)-5-(((3aR,4S,5S,5aR,8aR,8bR)-5-methoxy-2,2,7,7-tetramethylhexahydrobenzo[1,2-d:3,4-d']bis([1,3]dioxole)-4-yl)oxy)-2,2-dimethylhexahydrobenzo[d][1,3]dioxol-4-yl)-O-methylhydroxylamine* ***(12)***

To a mixture of **11** (14 mg, 0.024 mmol), and Bu_3SnH (0.026 mL, 0.096 mmol) in CH_2Cl_2 (2 mL) at −20 °C, was added $BF_3.OEt_2$ (0.005 mL, 0.033 mmol). The reaction was stirred at −20 °C for 2 h, then diluted with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic phase was dried (Na_2SO_4) and concentrated in *vacuo*. FCC of the residue provided compound **12** (12 mg, 85%) as a clear film; $R_f = 0.62$ (35% ethyl acetate: petroleum ether). ^{1}H NMR (600 MHz, C_6D_6) δ 1.21 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.58 (s, 3H, CH_3), 1.80 (apparent q, $J = 12.4$ Hz, 1H, $5a_{ax}$), 2.10 (m, 1H, 5), 2.48 (dt, $J = 3.6$, 12.4 Hz, 1H, $5a_{eq}$), 3.04 (t, $J = 10.0$ Hz, 1H, 2), 3.23 (dd, 1H, $J = 6.3$, 10.4 Hz, 1H, 3′), 3.59 (s, 3H, OCH_3), 3.61 (t, $J = 12.5$ Hz, 1H, 6) 3.74 (s, 3H, OCH_3), 3.75 (m, 1H, 6), 3.89 (m, 1H, 4′), 4.05 (dt, $J = 3.8$, 11.2 Hz, 1H, 1), 4.12 (t, J = . 6.7 Hz, 1H, 2′), 4.27 (m, 4H, 4, 1′, 5′, 6`), 4.43 (dd, $J = 5.0$, 8.5 Hz, 1H, 3), 4.46 (s, 2H, $PhCH_2$) 7.15–7.40 (m, 5H, Ph); ^{13}C NMR (125 MHz, C_6D_6) δ 25.3, 25.6, 27.2, 27.9, 28.2, 29.1, 30.3, 30.4, 36.7, 59.7, 63.3, 70.3, 72.6, 73.5, 75.0, 76.2, 76.8, 77.7, 77.8, 79.4, 80.4, 80.5, 83.3, 109.1, 109.6, 109.9, 128.2, 128.4, 128.6, 139.8; HRMS (ESI) *m/z* calcd for $C_{31}H_{48}NO_{10}$ (M + H) + 594.3272, found 594.3283.

4.9. *((3aS,4R,6R,7S,7aR)-7-amino-6-(((3aR,4S,5S,5aR,8aR,8bR)-5-methoxy-2,2,7,7-tetramethylhexahydrobenzo[1,2-d:3,4-d']bis([1,3]dioxole)-4-yl)oxy)-2,2-dimethylhexahydrobenzo[d][1,3]dioxol-4-yl)methanol* ***(13)***

Liquid NH_3 (ca 1 mL) was condensed into a solution of **12** (10 mg, 0.02 mmol) in THF (1 mL) at −78 °C under an atmosphere of argon. Sodium was then slowly added to the solution until the blue color was persistent for 2 min. After warming to rt, the reaction was quenched with solid NH_4Cl, and filtered. The organic phase was concentrated in *vacuo*. FCC of the residue afforded **13** (6 mg, 75%) as a clear film; $R_f = 0.62$ (10% MeOH: ethyl acetate). ^{1}H NMR (500 MHz, $C_6D_6 - D_2O$) δ 0.98 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.40 (m, 1H, 5), 1.61 (q, $J = 12.8$ Hz, 1H, 5a), 1.93 (dt, $J = 3.7$, 12.6 Hz, 1H, 5a), 2.93 (t, $J = 9.8$ Hz, 1H, 2), 2.97 (dd, $J = 6.9$, 11.0 Hz, 1H, 3′), 3.30 (s, 3H, OCH_3), 3.39 (dt, $J = 2.7$, 10.0 Hz, 1H, 1), 3.45 (dd, $J = 5.2$, 10.7 Hz, 1H, 6), 3.49 (dd, $J = 5.9$, 10.7 Hz, 1H, 6), 3.59 (m, 1H, 4′), 3.73 (m, 1H, 3), 3.76 (t, $J = 4.3$ Hz, 1H, 4), 3.90 (t, $J = 6.4$ Hz, 1H, 2′), 3.99 (m, 3H, 1′, 5′, 6`); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.8, 25.0, 26.3, 27.4, 27.6, 28.2, 28.5, 37.4, 59.2, 59.8, 64.5, 75.1, 77.0 (2C), 79.0, 79.5, 79.7, 81.2, 81.5, 82.3, 108.9, 109.0, 109.2; HRMS (ESI) *m/z* calcd for $C_{23}H_{39}NO_9$ (M + H) + 474.2698, found 474.2697.

4.10. *(1S,2S,3S,4S,5R,6R)-5-(((1R,2R,3R,4S,5R)-2-amino-3,4-dihydroxy-5-(hydroxymethyl)cyclohexyl)oxy)-6-methoxycyclohexane-1,2,3,4-tetraol hydrochloride* ***(3-HCl)***

A mixture of 5% (v/v) acetyl chloride in methanol (0.2 mL) was added to a solution of **13** (2.4 mg, 0.005 mmol) in CH_2Cl_2 (1 mL) at rt. The reaction mixture was stirred at this temperature for 1 h, then evaporated in vacuo at rt. The residue was purified by preparative reversed-phase HPLC (XBridge® Prep C-18, 5 μm OBD™ 19 × 150 mm; 5–95% gradient of acetonitrile in water; flow rate: 20 mL/min), with automated MS fraction monitoring, to give **3-HCl** (1.5 mg, 77%) as an amorphous powder; $[\alpha]_{23}^{D}$: +132 (c 0.15H_6O). ^{1}H/^{13}C NMR (Table 1). HRMS (EI) *m/z* calcd for $C_{14}H_{28}NO_9$ (M + H) + 354.1758, found 354.1769.

4.11. *(1R,2R,3R,4R,5S,6S)-5-(((1R,2S,3R,4S,5R)-2-acetamido-3,4-diacetoxy-5-(acetoxymethyl)cyclohexyl)oxy)-6-methoxycyclohexane-1,2,3,4-tetrayl tetraacetate* ***(14)***

A mixture of **3-HCl** (ca 1 mg), 3:1 pyridine: ethyl acetate (0.4 mL), acetic anhydride (0.1 mL) and DMAP (1 mg) was stirred at rt for 2 h. Methanol (0.05 mL) was then added to the reaction and the volatiles removed under reduced pressure. FCC of the residue afforded **14** as a thin film; $R_f = 0.10$ (ethyl acetate). ^{1}H/^{13}C NMR (Table 1). HRMS (EI) *m/z* calcd for $C_{30}H_{43}NO_{17}Na$ (M + Na) + 712.2423, found 712.2424.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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