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Synthesis of Spiro Carba-Sugars by Ring-Closing Metathesis

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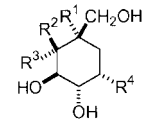
Dedicated to Prof. Rafael Suau on the occasion of his 60th birthday

Keywords: Carba-sugars / Ring-closing metathesis / Glycosidase inhibitors

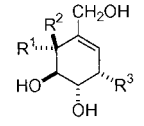
Six conformationally restricted carba-sugars have been synthesized by ring-closing metathesis of a readily available diallyl keto derivative of (–)-quinic acid. The rich functionality of the resulting spiro ketone was exploited for the diastereoselective synthesis of various spiro carba-sugars: four of them polyhydroxylated and two aminopolyhydroxylated. The results of the testing of these compounds against various commercially available glycosidases (amyloglicosidase, α- and β-galactosidase, α- and β- glucosidase, α-mannosidase, trehalase and neuraminidase) are provided.

Introduction

Carba-sugars (pseudo-sugars[1]), carbocyclic analogues of monosaccharides in which the endocyclic oxygen of the latter is replaced by a methylene group, have attracted considerable interest as inhibitors of glycosidase enzymes. These enzymes are involved in numerous biological processes, and glycosidase inhibitors have enormous potential for the treatment of many diseases, including cancer, diabetes and viral infections, through their alteration of the glycosylation or catabolism of glycoproteins or their blocking of the recognition of specific sugars.[2,3] Naturally occurring carba-sugars include polyhydroxycarbocycles such as pseudo-α-galactose,[4] streptol[4,5] and MK7067,[6] as well as aminopolyhydroxycarbocycles such as validamine,[7] valiolamine[7] and valienamine.[7]



Pseudo-α-galactose: $R^1 = R^3 = H$; $R^2 = R^4 = OH$
Validamine: $R^1 = R^2 = H$; $R^3 = OH$; $R^4 = NH_2$
Valiolamine: $R^1 = R^3 = OH$; $R^2 = H$; $R^4 = NH_2$



Streptol: $R^1 = R^3 = OH$; $R^2 = H$
MK7067: $R^1 = H$; $R^2 = R^3 = OH$
Valienamine: $R^1 = OH$; $R^2 = H$; $R^3 = NH_2$

In the past decade, much has been learned about the mechanisms of glycosidases, their interactions with their substrates, and the structures of enzyme–inhibitor complexes.[8] Because they can bear pharmacophoric groups with well defined spatial orientations, carba-sugars could be useful probes of the structural determinants involved in the molecular recognition of glycosidases. As part of our ongoing research directed toward the synthesis of structurally diverse carba-sugar entities,[9] we became interested in the synthesis of a variety of conformationally restricted carba-sugar analogues (spiro carba-sugars) that might be usable to obtain important information about key structural features of receptor ligand complexes. Here we disclose the synthesis of the six novel conformationally restricted spiro carba-sugars **3**–**8** (Scheme 1), four of which are polyhydroxylated (**3**, **6**, **7** and **8**) and two aminopolyhydroxylated (**4** and **5**). Our approach involves modification of a common key intermediate obtained by ring-closing metathesis from diallyl ketone **2**, easily prepared from (–)-quinic acid (**1**). The rich functionality present in this key intermediate was exploited for the synthesis of diverse analogues, study of which may provide important information about which structure features of the analogues are important for inhibitory activity. The results of the testing of these compounds against various commercially available glycosidases (amyloglicosidase, α- and β-galactosidase, α- and β-glucosidase, α-mannosidase, trehalase and neuraminidase) are provided.

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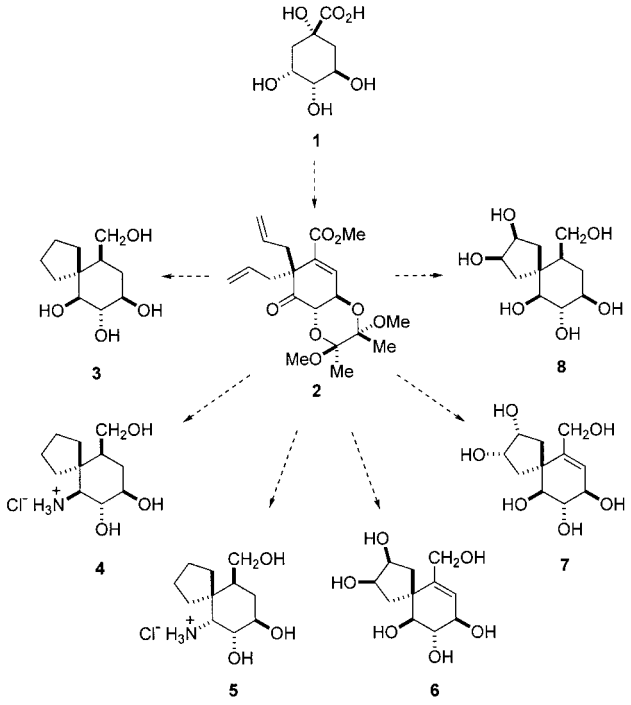
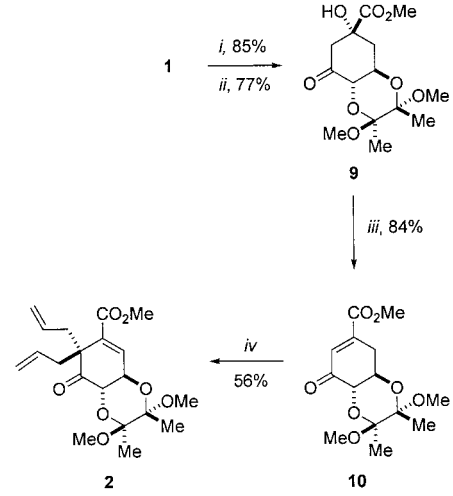


Scheme 1.

Results and Discussion

The strategy used to synthesize compounds **3**–**8** involved the initial preparation of diallyl ketone **2**. Our first approach consisted of the synthesis of diallyl ketone **2** via ketone **10**,[10] which was prepared in three steps from (–)-quinic acid (**1**) by previously reported methods (Scheme 2).[10,11] Treatment of ketone **10** with various bases (NaH, LDA, DBU, KHMDS) in THF or DMF at –78 °C and subsequently with allyl bromide afforded the desired diallyl derivative, the structure of which was confirmed by X-ray crystallography (Figure 1).[12] We found that the best reaction conditions involved the addition of a solution of the ketone in DMF to a solution of KHMDS (0.5 M in toluene) in DMF (2:1, w/w). Under these conditions diallyl ketone **2** was obtained in 56% yield (47% overall yield from **9**).

Having observed that ketone **9** easily undergoes β-elimination reactions without any need for conversion of the tertiary hydroxy group into a good leaving group, we investigated the synthesis of diallyl ketone **2** by addition of allyl bromide to **10** generated in situ. We found that treatment of ketone **9** with KHMDS and then with an excess of allyl bromide afforded a 30% yield of diallyl ketone **2** (Scheme 3). The low yield is probably due to product hydrolysis caused by the potassium hydroxide generated during the β-elimination step. This problem was avoided by using trimethylsilyloxy ketone **11**,[11c] which afforded cleaner reaction mixtures than had been obtained from ketones **9** and **10**, plus a better overall yield of diallyl ketone **2** (65% overall yield from **9**).



Scheme 2. Reagents and conditions: *i*) $(MeCO)_2$, $CH(OMe)_3$, MeOH, CSA, Δ. *ii*) PDC, MS 4 Å, DCM. *iii*) Ac_2O, Py, room temp. *iv*) 1. KHMDS, DMF, –78 °C; 2. allyl bromide, –78 °C → –60 °C.

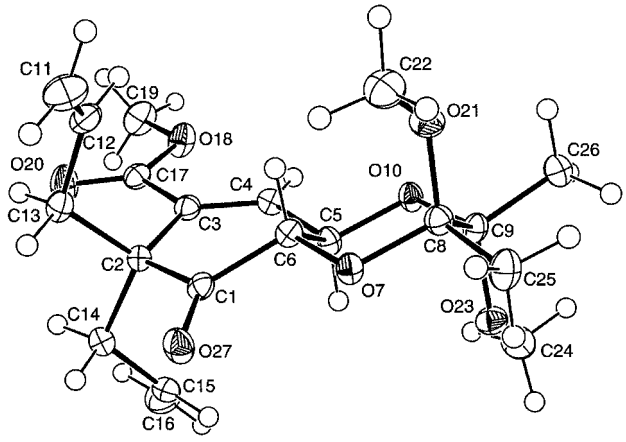
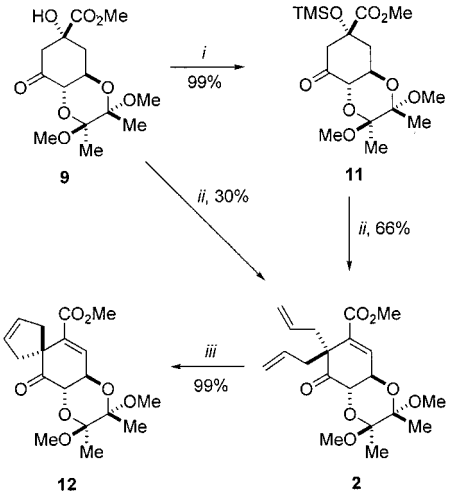


Figure 1. X-ray structure of **2**.



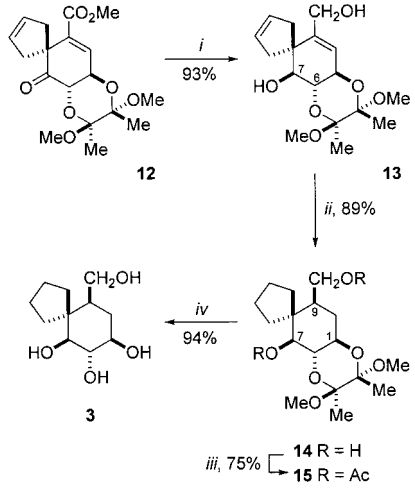
Scheme 3. Reagents and conditions: *i*) TMSCl, HMDS, Py, room temp. *ii*) 1. KHMDS, DMF, –78 °C; 2. allyl bromide, –78 °C → –60 °C. *iii*) 5% 2nd generation Grubbs′ catalyst, DCM, Δ.

Ring closing metathesis of diallyl ketone **2** in the presence of a Grubbs catalyst afforded the desired spiro ketone **12** (Scheme 3). Use of 15% of first-generation Grubbs catalyst provided a 90% yield of spiro ketone **12** in 4 hours, while use of only 5% of second-generation Grubbs catalyst afforded an almost quantitative yield of spiro ketone **12** in a reaction time reduced to 15 min.

The rich functionality of spiro ketone **12** was then exploited for the synthesis of a wide range of analogues. Briefly, reduction of the C=C double bonds and replacement of the C7 carbonyl moiety with a hydroxy or an amino group afforded target compounds **3**, **4** and **5**, while compounds **6**, **7** and **8** were obtained by diastereoselective *cis*-dihydroxylation of the cyclopentene double bond, reduction of the cyclohexene double bond (**8** only), and reduction of the C7 carbonyl.

Synthesis of Compound 3

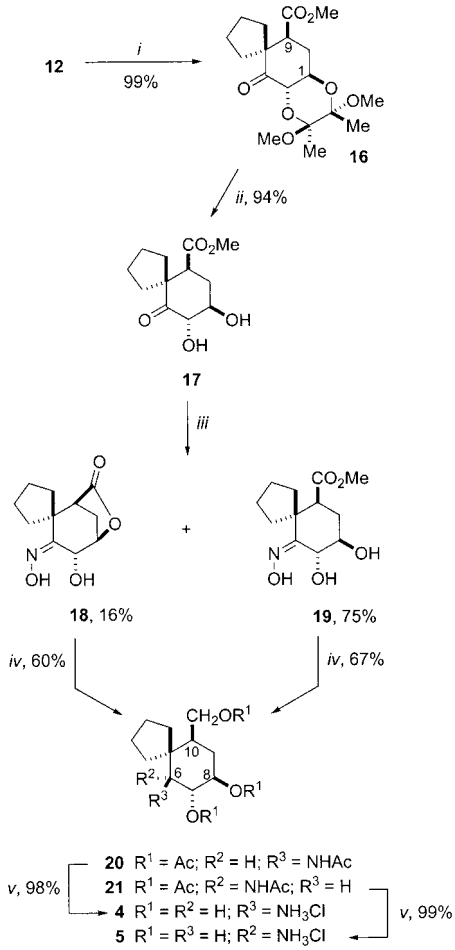
Compound **3** was obtained by successive reduction of the exocyclic and endocyclic double bonds of keto ester **12** (Scheme 4). Reduction of **12** with DIBALH at –78 °C afforded an excellent 93% yield of the single diastereoisomer **13**, the ^{1}H NMR spectrum of which shows H7 to be axial ($J_{6,7}$ = 10.9 Hz). Catalytic hydrogenation of **13** afforded the saturated diol **14** as the sole diastereoisomer, its stereochemistry being confirmed by NOE experiments on the acetylated derivative **15** (obtained by treatment of **14** with acetic anhydride): irradiation of H1 enhanced the signals of H7 (8%) and H9 (7%). Finally, deprotection of **14** with aqueous trifluoroacetic acid gave spiro tetraol **3** in an excellent 94% yield.



Scheme 4. Reagents and conditions: *i*) DIBALH, THF, –78 °C. *ii*) H_2, 10% Pd-C (cat.), EtOH, room temp. *iii*) Ac_2O, Et_3N, DMAP, DCM, 0 °C to room temp. *iv*) TFA/H_2O (20:1), room temp.

Synthesis of Compounds 4 and 5

An amino group was introduced at C7 by reduction of the appropriate oximes **18** and **19** (Scheme 5). Catalytic hydrogenation of dialkene **12** afforded an almost quantitative yield of the single diastereomer **16**, the stereochemistry of which was confirmed by NOE experiments in which irradiation of H1 enhanced the signal of H9 (6%). However, treatment of **16** with hydroxylamine hydrochloride and various combinations of base (NaOAc·3H_2O, K_2CO_3, Na_2CO_3), solvent (MeOH, CH_3CN, EtOH, dioxane) and reaction temperature always afforded mainly starting material. Reasoning that this poor reactivity might be due to conformational impediments, we removed the *trans*-diol protecting group of **16** by treatment with aqueous trifluoroacetic acid. Treatment of the resulting ketone **17** with hydroxylamine hydrochloride and NaOAc·3H_2O in ethanol now afforded a chromatographically separable mixture of the oxime carbolactone **18** (16%) and the oxime methyl ester **19** (75%).



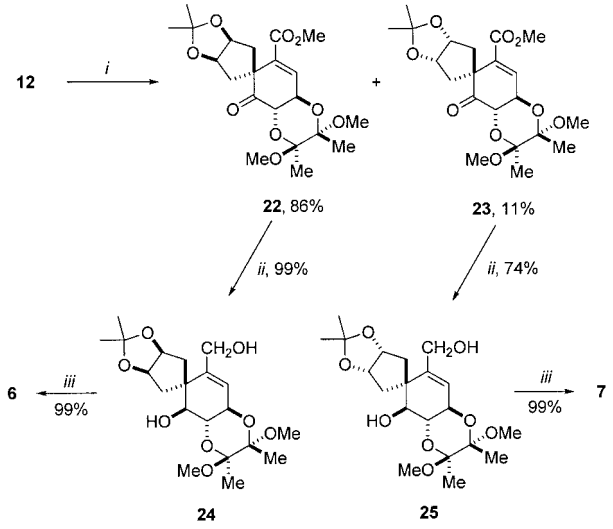
Scheme 5. Reagents and conditions: *i*) H_2, 10% Pd-C (cat.), EtOH, room temp. *ii*) TFA/H_2O (20:1), room temp. *iii*) NH_2OH·HCl, NaOAc·3H_2O, EtOH, room temp. *iv*) 1. LAH, THF, 100 °C; 2. Ac_2O, Δ. *v*) HCl (2 M), Δ.

Treatment of **18** and **19** with a number of reducing agents, Lewis acids and reaction temperatures reduced the lactone group of **18** and the methyl ester of **19**, but not the oxime group. However, the use of a large excess of LAH at 100 °C followed by heating at reflux in Ac_2O eventually afforded the corresponding acetyl derivative as a chromatographically separable mixture of diasteroisomers **20** and **21**;

the diaxial coupling constant between H6 and H7 ($J_{6,7}$ = 10.6 Hz) supports the structure of **20**. Finally, acid hydrolysis of **20** and **21** gave the desired amino carba-sugars **4** and **5** in 98% and 99% yields, respectively. The stereochemistry of **4** was confirmed by NOE experiments in which irradiation of H8 enhanced the signals of H6 (6%) and H10 (4%) (**4** is also consistent with the diaxial coupling constant between H6 and H7, $J_{6,7}$ = 10.4 Hz).

Synthesis of Compounds 6 and 7

Selective *cis*-dihydroxylation of the cyclopentene moiety of **12** by treatment with catalytic osmium tetraoxide, followed by 2,2-dimethoxypropane, gave a chromatographically separable mixture of two isopropylideneacetals **22** (86%) and **23** (11%) (Scheme 6).



Scheme 6. Reagents and conditions: *i*) 1. OsO_4 (cat.), NMO, dioxane/H_2O, 0 °C, 2. $Me_2C(OMe)_2$, Me_2CO, CSA, Δ. *ii*) DIBALH, THF, –78 °C. *iii*) TFA/H_2O (50%), room temp.

Reduction of **22** with DIBAL-H at –78 °C afforded an almost quantitative yield of a single diastereomer, the stereochemistry of which was confirmed by NOE experiments (Figure 2),[13,14] whilst hydrolysis of the acetal groups with aqueous trifluoroacetic acid gave spiro analogue **6**.

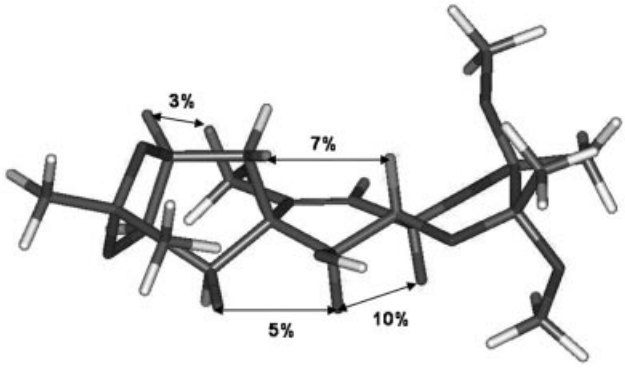


Figure 2. Results of NOE experiments with compound **24**.

Compound **7** was obtained similarly to **23**, its stereochemistry being confirmed by NOE experiments on diol **25** (Figure 3).[13,14]

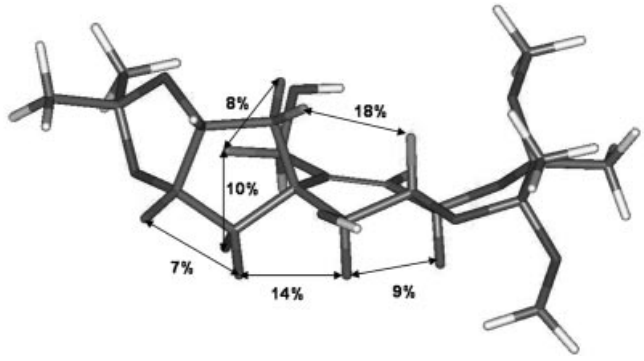
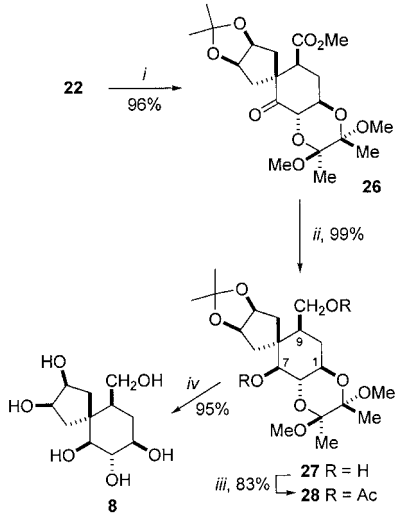


Figure 3. Results of NOE experiments with compound **25**.

Synthesis of Compound 8

Spiro carba-sugar **8** was synthesized from the *cis*-dihydroxylated spiro compound **22** (Scheme 7). Catalytic hydrogenation of the α,β-unsaturated ester **22** mainly afforded the saturated ester **26** (96%), which upon subsequent diastereoselective reduction with DIBAL-H was transformed into the single diastereomer **27**. The stereochemistry of **27** was confirmed by NOE experiments on its acetylated derivative **28** (obtained by treatment of **27** with acetic anhydride): irradiation of H1 enhanced the signals of H7 (9%) and H9 (6%). Finally, deprotection of diacetal **27** with aqueous trifluoroacetic acid gave spiro carba-sugar **8** in 95% yield.



Scheme 7. Reagents and conditions: *i*) H_2, 10% Pd-C (cat.), EtOH, room temp. *ii*) DIBALH, THF, –78 °C. *iii*) Ac_2O, Et_3N, DMAP, DCM, 0 °C to room temp. *iv*) TFA/H_2O (50%), room temp.

Glycosidase Inhibition Results

The six spiro carbasugars **3**–**8** were tested for their inhibitory properties against a wide range of commercially available glycosidases: amyloglicosidase (*Aspergillus niger*), α-galactosidase (green coffee beans), β-glucosidase (almonds),

Table 1. Tested glycosidases and assay conditions.[a]

Glycosidases	pH	Buffer	Substrate
Amyloglicosidase (*Aspergillus niger*)	4.5	phosphate/citrate	PNP-α-D-Glu
α-Gal (green coffee beans)	6.5	phosphate	PNP-α-D-Gal
β-Glc (almonds)	5	phosphate/citrate	PNP-β-D-Glu
β-Gal (bovine liver)	7.3	phosphate	PNP-β-D-Gal
α-Man (jack beans)	4.5	phosphate/citrate	PNP-α-D-Man
β-Gal (*E. coli*)	7.3	phosphate	ONP-β-D-Gal
α-Glc (bakers' yeast)	6.8	phosphate	PNP-α-D-Glu
Trehalase (porcine kidney)	5.7	phosphate	D-trehalose
Neuraminidase (*Vibrio cholera*)	5	acetate	2-O-PNP-α-D-*N*-AcNeu

[a] α-Gal = α-galactosidase, β-Glc = β-glucosidase, β-Gal = β-galactosidase, α-Man = α- mannosidase, α-Glc = α-glucosidase. PNP = *p*-nitrophenyl. ONP = *o*-nitrophenyl. AcNeu = acetylneuraminic acid.

β-galactosidase (bovine liver), α-mannosidase (jack beans), β-galactosidase (*E. coli*), α-glucosidase (bakers' yeast), trehalase (porcine kidney) and neuraminidase (*Vibrio cholera*). Table 1 summarizes the assay conditions, under which all enzymes tested showed satisfactory activities. The enzymatic reactions were carried out at 37 °C for 20 min with chromogenic nitrophenyl glycosides as substrates, with the exception of trehalase, for which D-trehalose was employed, with subsequent quenching by addition of potassium carbonate (1 M). The extent of the reaction was calculated from the absorbance of the released phenolate, at 400 nm for *p*-nitrophenyl substrates and at 420 nm for *o*-nitrophenyl β-D-galactoside, and in the case of trehalase by measurement of the amount of D-glucose derived from trehalase hydrolysis with a reagent for reducing sugars. The K_i values were estimated, as a first approach, with a competitive inhibition model [$K_i = K_M$ [I]/((v_o/v_i) – 1)(K_M + [S])], where [I] is the inhibitor concentration, v_o is the initial velocity in absence of inhibitor, v_i is the initial velocity in the presence of inhibitor and [S] is the substrate concentration. Initial inhibitor screening was done at K_M and 2.5 K_M concentrations and for compounds showing significant inhibition under these conditions, the apparent competitive inhibition constants were determined more closely. The results are summarized in Table 2.

Table 2. Inhibition results (K_M and K_i values in mM).[a]

			K_i					
Entry	Glycosidases	K_M	3	4	5	6	7	8
1	Amyloglicosidase (*Aspergillus niger*)	3.0	NI	NI	NI	NI	NI	NI
2	α-Gal (*Green coffee beans*)	1.0	NI	NI	NI	NI	NI	NI
3	β-Glc (*Almonds*)	3.5	NI	NI	NI	NI	NI	NI
4	β-Gal (*Bovine liver*)	2.5	NI	**1.2**	NI	NI	NI	NI
5	α-Man (*Jack beans*)	1.6	NI	NI	NI	NI	NI	NI
6	β-Gal (*E. coli*)	0.1	NI	**0.032**	NI	0.84	0.75	NI
7	α-Glc (*Bakers yeast*)	0.25	NI	**0.092**	NI	NI	NI	NI
8	Trehalase (*Porcine kidney*)	4.0	6.4	NI	NI	NI	NI	NI
9	Neuraminidase (*Vibrio cholera*)	1.6	NI	NI	NI	NI	NI	NI

[a] NI = no inhibition or less than 10% was observed at [I] >2.5 K_M.

Compounds **3**, **5**, **6**, **7** and **8** showed no significant activity against the tested enzymes. However, amino derivative **4** proved to be an inhibitor of β-galactosidase from bovine liver (entry 4, K_i = 1.2 mM), β-galactosidase from *E. coli* (entry 6, K_i = 32 μM), and α-glucosidase from bakers' yeast (entry 7, K_i = 92 μM) with inhibition constants approximately 2 to 3 times lower than the corresponding K_M values under these conditions.

Conclusions

Six conformationally restricted carba-sugars have been synthesized. The spirocyclic framework of these compounds was constructed by ring-closing metathesis from compound **2**, a readily available diallyl keto derivative of (–)-quinic acid. The rich functionality of the resulting spiro ketone **12** was exploited for diastereoselective synthesis of spiro carba-sugars. Inhibition studies of these compounds against various commercially available glycosidases showed amino spiro carba-sugar **4** to be a moderate inhibitor of β-galactosidase.

Saturation Transfer Difference (STD) NMR experiments combined with docking studies for determining the binding conformation of the inhibitor **4** in the active site of β-galactosidase are underway. These studies should provide an insight into which structure features of **4** are responsible for the observed inhibition activity and also which structural modifications should be necessary for improvement of the observed activity.

Experimental Section

General Remarks: All starting materials and reagents were commercially available and were used without further purification. FT-IR spectra were recorded as NaCl plates or KBr discs. $[a]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR spectra (250, 300 and 500 MHz) and ^{13}C NMR spectra (63, 75 and 125 MHz) were measured in deuterated solvents. *J* values are given in Hertz. NMR assignments were made by a combination of 1D, COSY, DEPT-135 and NOE experiments.

Glycosidase Assay: The enzyme stocks were diluted before use with the corresponding buffer (Table 1) and stored on ice. Glycosidases (with the exception of trehalase) were assayed by the end-point method, by measurement of the amount of hydrolyzed product as estimated from the UV absorption of the released phenol (as the phenolate at basic pH), at 400 nm for *p*-nitrophenolate and at 420 nm for *o*-nitrophenolate. Each assay was initiated by addition of the enzyme to a mixture of the corresponding substrate (concentration around K_M), the inhibitor (concentrations between 0.2–10 mM) and buffer in a total volume of 50 μL. The reaction mixture was incubated at 37 °C for 20 min and was then quenched and

brought to basic pH by dilution with of Na_2CO_3 (1 M, 350 μL), with the exceptions of α-glucosidase from bakers' yeast and β-galatosidase from *E. coli*, where only 50 μL were used. The amount of enzyme in each assay was adjusted to produce less than 20% hydrolysis of the initial substrate concentration.

Trehalase was assayed by measurement of the amount of released D-glucose, as estimated by the Somogyi–Nelson (molybdenum blue) method[15] from the UV absorption at 540 nm. The reaction mixture was made up in a total volume of 50 μL and incubated at 37 °C for 20 min, as in the cases of the other enzymes, but was quenched in this case by addition of Somogyi reagent. The resultant mixture was heated at 100 °C for 15 min, allowed to cool to room temperature and then treated with Nelson reagent (50 μL) and diluted with water (450 μL). Finally, absorbance at 540 nm was measured and compared with a calibration curve for D-glucose.

Diallyl Ketone 2. Method A (from ketone 11): A solution of the ketone **11**[11] (2.2 g, 5.59 mmol) in dry DMF (17 mL) was added dropwise at –78 °C to a solution of KHMDS (0.5 M in toluene, 34 mL, 16.77 mmol) in dry DMF (17 mL) and the mixture was stirred for 2 h at this temperature. Allyl bromide (2.5 mL, 28.9 mmol) was added and the resultant reaction mixture was stirred for 1 h at –60 °C. Saturated aqueous NH_4Cl and diethyl ether were added and the resultant mixture was allowed to warm to room temperature. The organic layer was separated, and the aqueous phase was extracted twice with diethyl ether. All the combined organic layers were washed with diluted HCl, dried (anh. Na_2SO_4), filtered and evaporated to give a light orange oil, which was purified by flash chromatography, with elution with diethyl ether/hexanes (30%) to afford diallyl ketone **2** (1.41 g, 66%) as a colourless oil that solidifies on standing: m.p. 87–88 °C (ethanol). $[\alpha]_D^{20}$ = +133.3 (c = 1.50, in $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.08 (d, J = 1.3 Hz, 1 H), 5.67–5.35 (m, 2 H), 5.03–4.90 (m, 4 H), 4.40 (d, J = 10.8 Hz, 1 H), 4.34 (dd, J = 10.8 and 1.3 Hz, 1 H), 3.75 (s, 3 H), 3.22 (s, 3 H), 3.18 (s, 3 H), 3.01 (dd, J = 13.6 and 5.9 Hz, 1 H), 2.93 (dd, J = 13.45 and 6.7 Hz, 1 H), 2.58 (dd, J = 13.45 and 8.4 Hz, 1 H), 2.34 (dd, J = 13.6 and 9.4 Hz, 1 H), 1.37 (s, 3 H), 1.32 (s, 3 H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 204.3 (C), 165.0 (C), 140.2 (CH), 133.5 (CH), 133.1 (CH), 131.6 (C), 118.8 (CH_2), 118.7 (CH_2), 100.3 (C), 99.8 (C), 74.5 (CH), 66.0 (CH), 57.5 (C), 52.0 (CH_3), 48.2 (CH_3), 48.2 (CH_3), 43.4 (CH_2), 39.6 (CH_2), 17.7 (CH_3), 17.6 (CH_3) ppm. IR (NaCl): $\tilde{\nu}_{max}$ = 1735, 1718 cm^{-1}. MS (CI): m/z = 381 [M + H]$^+$. HRMS calcd. for $C_{20}H_{29}O_7$ [M + H]$^+$: 381.1913; found 381.1918. Elemental analysis (%) calcd for $C_{20}H_{28}O_7$ (380.43): C 63.13, H 7.42; found C 63.15, H 7.46.

Method B (from ketone 10): A solution of the ketone **10**[10] (200 mg, 0.67 mmol) in dry DMF (2 mL) was added dropwise at –78 °C to a solution of KHMDS (0.5 M in toluene, 4 mL, 2.01 mmol) in dry DMF (2 mL) and the mixture was stirred for 0.5 h at this temperature. Allyl bromide (0.23 mL, 2.68 mmol) was added and the resultant reaction mixture was stirred for 1 h at –60 °C. Saturated aqueous NH_4Cl and diethyl ether were added and the resultant mixture was allowed to warm to room temperature. The organic layer was separated, and the aqueous phase was extracted twice with diethyl ether. All the combined organic layers were washed with diluted HCl, dried (anh. Na_2SO_4), filtered and evaporated to give a light orange oil, which was purified by flash chromatography, with elution with diethyl ether/hexanes (30%), to afford diallyl ketone **2** (142 mg, 56%).

Spiro Ester 12: A stirred solution of the diallyl ether **2** (208 mg, 0.55 mmol) and Grubbs' 2nd generation catalyst (25 mg, 0.03 mmol) in dry DCM (30 mL) was heated at reflux under inert atmosphere for 15 min, then cooled and concentrated under reduced pressure. The residue was purified by flash chromatography, with elution with diethyl ether/hexanes (40%) to yield the spiro enone **12** as a white foam (192 mg, 99%): $[\alpha]_D^{20}$ = +101.4 (c = 1.46, in $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 6.93 (d, J = 1.7 Hz, 1 H), 5.75 (m, 1 H), 5.48 (m, 1 H), 4.74 (d, J = 10.4 Hz, 1 H), 4.55 (dd, J = 10.4 and 1.7 Hz, 1 H), 3.74 (s, 3 H), 3.26 (s, 3 H), 3.24 (s, 3 H), 3.08 (m, 2 H), 2.40 (m, 2 H), 1.40 (s, 3 H), 1.34 (s, 3 H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 202.4 (C), 165.1 (C), 136.9 (CH), 135.0 (C), 130.6 (CH), 125.7 (CH), 100.6 (C), 100.0 (C), 72.6 (CH), 67.3 (CH), 55.0 (C), 52.0 (CH_3), 48.4 (CH_3), 48.1 (CH_3), 47.4 (CH_2), 40.2 (CH_2), 17.6 (CH_3), 17.5 (CH_3) ppm. IR (NaCl): $\tilde{\nu}_{max}$ = 1740, 1722 cm^{-1}. MS (CI): m/z = 353 [M + H]$^+$. HRMS calcd. for $C_{18}H_{25}O_7$ [M + H]$^+$: 353.1600; found 353.1599.

Spiro Diol 13: DIBALH (ca. 1.0 M solution in hexanes, 2.8 mL, 2.8 mmol) was added under argon and at –78 °C to a stirred solution of the keto ester **12** (100 mg, 0.28 mmol) in dry THF (3 mL). The resultant solution was stirred at –78 °C for 1 h. Water was then added and the mixture was allowed to warm to room temperature. The mixture was then acidified with HCl (10%) and extracted with diethyl ether (×3). The combined organic layers were dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography, with elution with diethyl ether/hexanes (70%) to yield the diol **13** (85 mg, 93%) as white prisms: m.p. 118–119 °C (hexane). $[\alpha]_D^{20}$ = +168.1 (c = 1.26, in $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 5.69–5.61 (m, 2 H), 5.58 (d, J = 2.0 Hz, 1 H), 4.26 (dd, J = 8.9 and 2.0 Hz, 1 H), 4.02 (br. s, 1 H), 3.80 (d, J = 10.9 Hz, 1 H), 3.58 (dd, J = 10.9 and 8.9 Hz, 1 H), 3.26 (s, 3 H), 3.25 (s, 3 H), 3.00 (m, 1 H), 2.53 (m, 3 H), 2.11 (m, 1 H), 1.34 (s, 3 H), 1.32 (s, 3 H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 145.8 (C), 129.8 (CH), 129.6 (CH), 118.1 (CH), 100.1 (C), 73.5 (CH), 70.6 (CH), 67.9 (CH), 61.3 (CH_2), 50.1 (C), 47.9 (CH_3), 47.8 (CH_3), 41.8 (CH_2), 40.0 (CH_2), 17.8 (2×CH_3) ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3478, 3294 cm^{-1}. MS (CI): m/z = 295 [M – MeOH + H]$^+$. HRMS calcd. for $C_{16}H_{23}O_5$ [M – MeOH + H]$^+$: 295.1545; found 295.1536. Elemental analysis (%) calcd for $C_{17}H_{26}O_6$ (326.38): C 62.54, H 8.03; found C 62.51, H 8.08.

Spiro Diol 14: A suspension of the alkene **13** (21 mg, 0.06 mmol) and 10% palladium on carbon (5 mg) in ethanol (1.5 mL) was shaken under hydrogen atmosphere at room temperature for 1 h. The mixture was filtered through Celite and the residue was washed with ethanol. The filtrate and washings were evaporated under reduced pressure to yield a colourless oil, which was purified by flash chromatography, with elution with acetone/hexanes (40%), to yield diol **14** (19 mg, 89%) as a colourless oil: $[\alpha]_D^{20}$ = +171.7 (c = 0.99, in $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 3.72 (dd, J = 10.6 and 3.6 Hz, 1 H), 3.42 (m, 1 H), 3.27 (m, 3 H), 3.12 (s, 3 H), 3.10 (s, 3 H), 1.89 (ddd, J = 12.6, 4.5 and 3.3 Hz, 1 H), 3.42 (m, 1 H), 1.56–1.16 (m, 6 H), 1.09 (s, 3 H), 1.12 (s, 3 H), 1.05 (m, 3 H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 99.6 (C), 99.4 (C), 76.9 (CH), 73.7 (CH), 68.1 (CH), 63.3 (CH_2), 49.4 (C), 47.9 (CH_3), 47.8 (CH_3), 45.3 (CH), 35.8 (CH_2), 29.9 (CH_2), 28.4 (CH_2), 27.0 (CH_2), 26.9 (CH_2), 17.8 (CH_3) and 17.8 (CH_3) ppm. IR (NaCl): $\tilde{\nu}_{max}$ = 3419 cm^{-1}. MS (CI): m/z = 299 [M – MeOH + H]$^+$. HRMS calcd. for $C_{16}H_{27}O_5$ [M – MeOH + H]$^+$: 299.1858; found 299.1860.

Spiro Diacetate 15: Triethylamine (20 μL, 0.14 mmol), acetic acid anhydride (12 μL, 0.12 mmol) and DMAP (0.1 equivalents) were added under inert atmosphere at 0 °C to a stirred solution of the diol **14** (18 mg, 54.5 μmol) in dry DCM (1 mL) . The resultant solution was stirred at room temperature for 6 h. DCM was added, and then water. The organic layer was separated and the aqueous phase was extracted twice with DCM. All the combined organic

extracts were dried (anhydrous Na_2SO_4) and filtered, and the solvents were evaporated. The obtained residue was purified by flash chromatography, with elution with diethyl ether/hexanes (25%), to yield diacetate **15** (17 mg, 75%) as a colourless oil which solidifies on standing: $[\alpha]_D^{20}$ = +123.9 (*c* = 1.50, in $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 4.83 (d, *J* = 10.4 Hz, 1 H), 4.11 (dd, *J* = 3.8 and 11.25 Hz, 1 H), 3.71 (dd, *J* = 11.25 and 9.5 Hz, 1 H), 3.51 (ddd, *J* = 10.0, 12.3 and 4.7 Hz, 1 H), 3.38 (dd, *J* = 10.4 and 10.0 Hz, 1 H), 3.10 (s, 3 H), 3.07 (s, 3 H), 1.90 (s, 3 H), 1.88 (s, 3 H), 1.72 (ddd, *J* = 12.8, 4.7 and 4.4 Hz, 1 H), 1.63–1.27 (m, 9 H), 1.15 (q, *J* = 12.6 Hz, 1 H), 1.07 (s, 3 H), 1.03 (s, 3 H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 171.1 (C), 170.4 (C), 99.5 (C), 99.4 (C), 77.4 (CH), 71.6 (CH), 67.9 (CH), 64.9 (CH_2), 49.3 (C), 47.8 (CH_3), 47.4 (CH_3), 41.5 (CH), 35.4 (CH_2), 29.8 (CH_2), 28.1 (CH_2), 28.1 (CH_2), 26.8 (CH_2), 20.9 (2 × CH_3) and 17.7 (2 × CH_3) ppm. IR (NaCl): $\tilde{\nu}_{max}$ = 1741 cm^{-1}. MS (CI): *m*/*z* = 383 [*M* – MeOH + H]$^+$. HRMS calcd. for $C_{20}H_{31}O_7$ [*M* – MeOH + H]$^+$: 383.2070; found 383.2063.

Spiro Tetrol 3: A solution of the bis(methoxy acetal) **14** (34 mg, 0.10 mmol) in TFA/H_2O (20:1 v/v, 1 mL) was stirred at room temperature for 15 min. The solvent was removed under reduced pressure and the crude reaction was purified by flash chromatography, with elution with ethyl acetate to yield 21 mg of tetrol **3** (94%) as a colourless oil: $[\alpha]_D^{20}$ = +24.1 (*c* = 1.90, in MeOH). ^{1}H NMR (250 MHz, CD_3OD): δ = 5.07 (dd, *J* = 14.8 and 5.2 Hz, 1 H), 4.51 (dd, *J* = 14.8 and 12.6 Hz, 1 H), 3.47–3.36 (m, 1 H), 3.10 (m, 2 H), 1.50–1.37 (m, 2 H), 1.28–0.97 (m, 4 H), 0.88–0.53 (m, 5 H) ppm. ^{13}C NMR (63 MHz, CD_3OD): δ = 80.4 (CH), 78.6 (CH), 73.0 (CH), 70.6 (CH_2), 50.8 (C), 42.2 (CH), 36.6 (CH_2), 34.3 (CH_2), 29.4 (CH_2), 27.8 (CH_2), 27.6 (CH_2) ppm. IR (NaCl): $\tilde{\nu}_{max}$ = 3398 cm^{-1}. MS (CI): *m*/*z* = 217 [*M* + H]$^+$. HRMS calcd. for $C_{11}H_{21}O_4$ [*M* + H]$^+$: 217.1440; found 217.1436.

Spiro Ester 16: A suspension of the alkene **12** (48 mg, 0.14 mmol) and 10% palladium on carbon (10 mg) in ethanol (2 mL) was shaken under hydrogen atmosphere at room temperature for 1 h. The mixture was filtered through Celite and the residue was washed with ethanol. The filtrate and washings were evaporated under reduced pressure to yield a colourless oil, which was purified by flash chromatography with elution with diethyl ether/DCM (10%) to yield the saturated ester **16** (48 mg, 99%) as a colourless oil: $[\alpha]_D^{20}$ = +112.7 (*c* = 1.48, in $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 4.43 (d, *J* = 10.9 Hz, 1 H), 3.60 (m, 1 H), 3.57 (s, 3 H), 3.11 (s, 3 H), 3.06 (s, 3 H), 2.35 (m, 2 H), 2.20 (m, 1 H), 2.09 (m, 1 H), 1.88 (dt, *J* = 13.6 and 4.3 Hz, 1 H), 1.29 (m, 6 H), 1.18 (s, 3 H), 1.09 (s, 3 H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 203.3 (C), 172.3 (C), 100.3 (C), 99.3 (C), 74.5 (CH), 68.1 (CH), 57.8 (C), 52.0 (CH_3), 48.3 (CH_3), 47.9 (CH_3), 46.3 (CH), 32.8 (CH_2), 31.0 (CH_2), 29.9 (CH_2), 26.4 (CH_2), 25.6 (CH_2), 17.6 (CH_3), 17.5 (CH_3) ppm. IR (NaCl): $\tilde{\nu}_{max}$ = 1726 cm^{-1}. MS (CI): *m*/*z* = 325 [*M* – MeOH + H]$^+$. HRMS calcd. for $C_{17}H_{25}O_6$ [*M* – MeOH + H]$^+$: 325.1651; found 325.1646.

Spiro Diol 17: A solution of the bis(methoxy acetal) **16** (846 mg, 2.38 mmol) in TFA/H_2O (20:1 v/v, 1 mL) was stirred at room temperature for 15 min. The solvent was removed under reduced pressure and the crude residue was purified by flash chromatography, with elution with acetone/hexanes (40%), to yield diol **17** (541 mg, 94%) as a colourless oil: $[\alpha]_D^{25}$ = –11.8 (*c* = 2.70, in $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 4.17 (dd, *J* = 9.6 and 3.0 Hz, 1 H), 3.75 (d, *J* = 3.7 Hz, 1 H), 3.58 (s, 3 H), 3.34 (m, 1 H), 3.31 (br. s, 1 H), 2.38–2.18 (m, 3 H), 1.98 (m, 2 H), 1.32 (m, 6 H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 208.6 (C), 172.2 (C), 78.7 (CH), 73.8 (CH), 56.9 (C), 52.1 (CH_3), 45.8 (CH), 32.0 (CH_2), 31.4 (CH_2), 31.0 (CH_2), 26.1 (CH_2), 25.4 (CH_2) ppm. IR (NaCl): $\tilde{\nu}_{max}$ = 3418, 1733, 1714 cm^{-1}. MS (CI): *m*/*z* = 225 [*M* – H_2O + H]$^+$; HRMS calcd. for $C_{12}H_{17}O_4$ [*M* – H_2O + H]$^+$: 225.1127; found 225.1128.

Spiro Oximes 18 and 19: Hydroxylamine hydrochloride (278 mg, 4.00 mmol) and sodium acetate trihydrate (1.12 g, 8.20 mmol) were added to a stirred solution of the ketone **17** (484 mg, 2.00 mmol) in ethanol (10 mL). The resultant solution was stirred at room temperature for 48 h. DCM was added, and then water. The organic layer was separated and the aqueous phase was extracted twice with DCM. All the combined organic extracts were dried (anhydrous Na_2SO_4) and filtered, and the solvents were evaporated. The obtained residue was purified by flash chromatography, with elution with diethyl ether to yield oxime **19** (386 mg, 75%) and oxime carbolactone **18** (72 mg, 16%), both as white solids.

Data for Carbolactone 18: M.p. 167–168 °C. $[\alpha]_D^{20}$ = –19.1 (*c* = 1.50, in CH_3COCH_3). ^{1}H NMR (250 MHz, CD_3OD): δ = 5.12 (dd, *J* = 4.1 and 1.4 Hz, 1 H), 4.63 (ddd, *J* = 5.5, 4.1 and 0.9 Hz, 1 H), 2.64 (d, *J* = 12.4 Hz, 1 H), 2.38 (dd, *J* = 5.5 and 0.9 Hz, 1 H), 2.34–2.08 (m, 3 H), 1.86–1.63 (m, 6 H) ppm. ^{13}C NMR (63 MHz, CD_3OD): δ = 179.6 (C), 160.2 (C), 79.5 (CH), 61.7 (CH), 52.6 (C), 48.8 (CH), 41.0 (CH_2), 35.4 (CH_2), 29.5 (CH_2), 25.6 (CH_2), 25.3 (CH_2) ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3324, 3254, 1746 cm^{-1}. MS (CI): *m*/*z* = 208 [*M* – H_2O + H]$^+$. HRMS calcd. for $C_{11}H_{14}O_3N$ [*M* – H_2O + H]$^+$: 208.0974; found 208.0971. Elemental analysis (%) calcd for $C_{11}H_{15}NO_4$ (225.24): C 58.66, H 6.71, N 6.22; found C 58.35, H 6.39, N 6.26.

Data for Methyl Ester 19: M.p. 103–104 °C (CH_2Cl_2). $[\alpha]_D^{20}$ = +47.1 (*c* = 1.05, in CH_3COCH_3). ^{1}H NMR (250 MHz, CD_3OD): δ = 4.93 (d, *J* = 5.6 Hz, 1 H), 3.96 (m, 1 H), 3.72 (s, 3 H), 2.75 (t, *J* = 6.4 Hz, 1 H), 2.40–2.25 (m, 3 H), 1.96 (c, *J* = 7.1 Hz, 1 H), 1.86–1.51 (m, 6 H) ppm. ^{13}C NMR (63 MHz, CD_3OD): δ = 177.1 (C), 159.6 (C), 72.0 (CH), 70.2 (CH), 52.1 (CH_3), 51.5 (C), 50.2 (CH), 38.9 (CH_2), 34.6 (CH_2), 31.3 (CH_2), 25.1 (CH_2), 25.0 (CH_2) ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3203, 1729 cm^{-1}. MS (CI): *m*/*z* = 258 [*M* + H]$^+$. HRMS calcd. for $C_{12}H_{20}O_5N$ [*M* + H]$^+$: 258.1341; found 258.1343. Elemental analysis (%) calcd for $C_{12}H_{19}NO_5$ (257.28): C 56.02, H 7.44, N 5.44; found C 56.36, H 7.20, N 5.18.

Reduction of Oxime 19: A solution of the oxime **19** (179 mg, 0.70 mmol) in dry THF (10 mL) was added to a stirred suspension of LAH (800 mg, 21.05 mmol) in dry THF (10 mL). The resultant suspension was heated at 100 °C for 12 h. After the mixture had cooled to room temperature, ethyl acetate (15 mL), NaOH (5 mL, 10%) and water (2 mL) were added. The mixture was filtered through Celite and the residue was washed with methanol. The filtrate and washings were evaporated under reduced pressure. The obtained residue was dissolved in acetic anhydride (8 mL) and heated under reflux for 6 h. The crude product was redissolved in a mixture of saturated $NaHCO_3$ and DCM. The organic layer was separated and the aqueous layer was extracted twice with DCM. All the combined organic extracts were dried (anh. Na_2SO_4), filtered and concentrated to give an oil, which was purified by flash chromatography, with elution with ethanol/DCM (5%), to yield acetamides **20** (98 mg, 37%) and **21** (80 mg, 30%) both as foams.

Data for 20: $[\alpha]_D^{20}$ = –10.6 (*c* = 1.50, in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.53 (d, *J* = 10.1 Hz, 1 H), 4.91 (m, 1 H), 4.81 (dd, *J* = 10.6 and 10.0 Hz, 1 H), 4.19 (dd, *J* = 10.8 and 3.6 Hz, 1 H), 4.07 (t, *J* = 10.6 Hz, 1 H), 3.80 (dd, *J* = 10.8 and 9.4 Hz, 1 H), 2.21 (m, 1 H), 2.05 (s, 3 H), 2.01 (s, 3 H), 2.00 (s, 3 H), 1.94 (s, 3 H), 1.91–1.45 (m, 8 H), 1.35–1.20 (m, 2 H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 171.9 (C), 171.1 (C), 170.3 (C), 169.9 (C), 74.3 (CH), 71.6 (CH), 64.7 (CH_2), 58.2 (CH), 49.2 (C), 41.8 (CH), 35.0 (CH_2), 30.5 (CH_2), 28.1 (CH_2), 28.0 (CH_2), 26.9 (CH_2), 23.3

(CH_2), 21.0 (CH_2), 20.9 (CH_2), 20.8 (CH_2) ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3373, 3295, 1742, 1661 cm^{-1}. MS (CI): *m/z* = 384 [*M* + H]$^+$. HRMS calcd. for $C_{19}H_{30}O_7N$ [*M* + H]$^+$: 384.2022; found 384.2025.

Data for 21: $[\alpha]_D^{20}$ = +13.3 (*c* = 1.08, in $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 5.83 (d, *J* = 10.2 Hz, 1 H), 5.06 (dd, *J* = 10.5 and 3.9 Hz, 1 H), 4.98 (ddd, *J* = 10.5, 10.8 and 4.8 Hz, 1 H), 4.30 (dd, *J* = 10.5 and 3.9 Hz, 1 H), 4.22 (dd, *J* = 10.8 and 3.6 Hz, 1 H), 3.75 (dd, *J* = 10.8 and 9 Hz, 1 H), 2.17 (m, 1 H), 2.04 (s, 3 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.96 (s, 3 H), 1.85 (m, 1 H), 1.63–1.23 (m, 9 H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 176.0 (C), 175.7 (C), 175.5 (C), 175.3 (C), 71.4 (CH), 69.0 (CH), 64.4 (CH_2), 54.3 (CH), 46.8 (C), 36.3 (CH), 32.4 (CH_2), 29.1 (CH_2), 28.4 (CH_2), 23.9 (CH_2), 22.9 (CH_2), 20.4 (CH_3), 18.0 (CH_3), 17.8 (CH_3), 17.8 (CH_3) ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3383, 3328, 1742, 1655 cm^{-1}. MS (CI): *m/z* = 384 [*M* + H]$^+$. HRMS calcd. for $C_{19}H_{30}O_7N$ [*M* + H]$^+$: 384.2022; found 384.2028.

Reduction of Oxime 18: The reaction was carried out as for oxime **19**: oxime **18** (70 mg, 0.31 mmol) in THF (5 mL)/LAH (350 mg, 9.33 mmol) in THF (5 mL)/Ac_2O (3 mL). Amide **20** (42 mg, 35%) and amide **21** (30 mg, 25%) were obtained.

Spiro Amine Hydrochloride 4: A solution of the amide **20** (49 mg, 0.13 mmol) in HCl (2 M, 6 mL) was heated under reflux for 12 h. After the system had cooled to room temperature, the solvent was evaporated. The obtained residue was redissolved in water and washed with diethyl ether (×2). The aqueous extract was lyophilized to afford amine hydrochloride **4** (32 mg, 98%) as a colourless foam: $[\alpha]_D^{20}$ = +24.7 (*c* = 1.14, in H_2O). ^1H NMR (250 MHz, D_2O): δ = 3.76 (dd, *J* = 11.0 and 3.3 Hz, 1 H), 3.49 (ddd, *J* = 11.3, 9.1 and 4.8 Hz, 1 H), 3.34 (m, 2 H), 3.04 (d, *J* = 10.4 Hz, 1 H), 2.10 (ddd, *J* = 13.0, 3.3 and 4.8 Hz, 1 H), 1.85 (m, 1 H), 1.62–1.46 (m, 8 H), 1.21 (dt, *J* = 13.0 and 11.3 Hz, 1 H) ppm. ^{13}C NMR (63 MHz, D_2O): δ = 74.1 (CH), 72.4 (CH), 63.0 (CH), 62.2 (CH_2), 47.5 (C), 45.1 (CH), 35.2 (CH_2), 32.6 (CH_2), 27.8 (CH_2), 26.8 (CH_2), 26.4(CH_2) ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3267, 3356 cm^{-1}. MS (CI): *m/z* = 216 [*M* – HCl + H]$^+$. HRMS calcd. for $C_{11}H_{22}O_3N$ [*M* – HCl + H]$^+$: 216.1600; found 216.1599.

Spiro Amine Hydrochloride 5: The reaction was carried out as for compound **4**. Amide **21** (40 mg, 0.10 mmol) and HCl (6 mL, 2 M) were combined to produce a mixture. Compound **5** was obtained as a colourless foam (26 mg, 99%): $[\alpha]_D^{20}$ = +1.7 (*c* = 1.10, in H_2O). ^1H NMR (300 MHz, D_2O): δ = 3.70 (dd, *J* = 9.9 and 4.2 Hz, 1 H), 3.60 (dd, *J* = 11.1 and 3.6 Hz, 1 H), 3.47 (td, *J* = 11.1 and 4.8 Hz, 1 H), 3.25 (dd, *J* = 11.1 and 9.0 Hz, 1 H), 3.16 (d, *J* = 4.2 Hz, 1 H), 2.02 (dt, *J* = 12.0 and 3.9 Hz, 1 H), 1.68–1.31 (m, 9 H), 1.13 (q, *J* = 12.3 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 70.9 (CH), 68.8 (CH), 62.5 (CH), 62.1 (CH_2), 46.9 (C), 40.3 (CH), 34.8 (CH_2), 32.3 (CH_2), 31.4 (CH_2), 25.9 (CH_2), 25.8 (CH_2) ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3389 and 2952 cm^{-1}. MS (CI): *m/z* = 216 [*M* – HCl + H]$^+$. HRMS calcd. for $C_{11}H_{22}O_3N$ [*M* – HCl + H]$^+$: 216.1600; found 216.1597.

Spiro Acetals 22 and 23: Freshly made aqueous osmium tetraoxide solution (0.12 M, 1.3 mL) was added at 0 °C to a stirred solution of the alkene **12** (359 mg, 1.02 mmol) and NMO (143 mg, 1.22 mmol) in dioxane/water (1:1, 9 mL). After the mixture had been stirred for 3 h, ethyl acetate was added, and then saturated Na_2SO_3. The reaction mixture was stirred for 20 min. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. All combined organic layers were dried (anh. Na_2SO_4), filtered and concentrated in vacuo. The crude residue was redissolved in acetone (10 mL), and 2,2-dimethoxypropane (0.7 mL, 5.7 mmol) and camphorsulfonic acid (15 mg, 0.06 mmol) were added. The resultant mixture was heated under reflux for 3 h and allowed to cool to room temperature, and K_2CO_3 was then added. The solvent was removed under reduced pressure and the residue was partitioned between diethyl ether and water. The organic layer was separated and the aqueous phase was extracted twice. The combined organic layers were dried (anh. Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash chromatography, with elution with THF/hexanes (25%) to yield acetal **22** (373 mg, 86%) as white prisms and acetal **23** (46 mg, 11%) as a colourless oil.

Data for 22: M.p. 160–161 °C (hexane). $[\alpha]_D^{20}$ = +139.6 (*c* = 1.08, in $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 6.83 (d, *J* = 1.7 Hz, 1 H), 4.90 (m, 1 H), 4.68 (dt, *J* = 1.6 and 6.0 Hz, 1 H), 4.60 (d, *J* = 10.6 Hz, 1 H), 4.38 (dd, *J* = 10.6 and 1.7 Hz, 1 H), 3.61 (s, 3 H), 3.11 (s, 3 H), 3.07 (s, 3 H), 2.74 (dd, *J* = 15.1 and 4.0 Hz, 1 H), 2.34 (dd, *J* = 15.4 and 6.3 Hz, 1 H), 1.85 (dd, *J* = 15.4 and 1.3 Hz, 1 H), 1.71 (dd, *J* = 8.2 and 15.1 Hz, 1 H), 1.35 (s, 3 H), 1.19 (s, 3 H), 1.12 (s, 3 H), 1.06 (s, 3 H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 201.1 (C), 165.0 (C), 136.6 (CH), 135.7 (C), 111.4 (C), 100.6 (C), 100.1 (C), 83.8 (CH), 82.5 (CH), 73.0 (CH), 67.6 (CH), 58.3 (C), 52.1 (OCH_3), 48.4 (CH_3), 48.0 (CH_3), 45.1 (CH_2), 38.8 (CH_2), 27.2 (CH_3), 25.4 (CH_3), 17.5 (CH_3), 17.5 (CH_3) ppm. IR (KBr): $\tilde{\nu}_{max}$ = 1747, 1715 cm^{-1}. MS (CI): *m/z* = 427 [*M* + H]$^+$. HRMS calcd. for $C_{21}H_{31}O_9$ [*M* + H]$^+$: 427.1968; found 427.1988. Elemental analysis (%) calcd for $C_{21}H_{30}O_9$ (426.46): C 59.13, H 7.09; found C 59.08, H 7.09.

Data for 23: $[\alpha]_D^{20}$ = +27.3 (*c* = 2.30, in $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 6.96 (d, *J* = 1.8 Hz, 1 H), 4.92 (m, 1 H), 4.60 (d, *J* = 10.9 Hz, 1 H), 4.45 (c, *J* = 7.0 Hz, 1 H), 4.38 (dd, *J* = 10.9 and 1.8 Hz, 1 H), 3.65 (s, 3 H), 3.11 (s, 3 H), 3.08 (s, 3 H), 2.67 (dd, *J* = 14.3 and 6.8 Hz, 1 H), 2.10 (m, 2 H), 1.75 (dd, *J* = 14.3 and 7.3 Hz, 1 H), 1.21 (s, 3 H), 1.19 (s, 3 H), 1.13 (s, 3 H), 1.09 (s, 3 H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 205.1 (C), 164.6 (C), 139.0 (CH), 132.1 (C), 113.3 (C), 100.6 (C), 100.0 (C), 81.6 (CH), 80.3 (CH), 71.9 (CH), 66.8 (CH), 60.1 (C), 52.0 (CH_3), 48.5 (CH_3), 48.1 (CH_3), 41.8 (CH_2), 38.0 (CH_2), 30.2 (CH_3), 27.7 (CH_3), 25.3 (CH_3), 17.5 (CH_3) ppm. IR (NaCl): $\tilde{\nu}_{max}$ = 1730 cm^{-1}. MS (CI): *m/z* = 427 [*M* + H]$^+$. HRMS calcd. for $C_{21}H_{31}O_9$ [*M* + H]$^+$: 427.1968; found 427.1961.

Spiro Diol 24: DIBAL-H (7 mL, 7 mmol, ca 1.0 M solution in heptane) was added under argon and at –78 °C to a stirred solution of the keto ester **22** (152 mg, 0.36 mmol) in dry THF (4 mL). The resultant solution was stirred at –78 °C for 1 h. Water was then added and the mixture was allowed to warm to room temperature. The mixture was then acidified with 5% H_2SO_4 and extracted with ethyl acetate (×3). The combined organic layers were dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residue was purified by flash chromatography, with elution with acetone/hexanes (40%) to yield diol **24** (143 mg, 99%) as white prisms: m.p. 190–191 °C (diethyl ether). $[\alpha]_D^{20}$ = +89.9 (*c* = 1.05, in $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 5.58 (d, *J* = 1.8 Hz, 1 H), 4.68 (m, 2 H), 4.22 (dd, *J* = 9.2 and 1.8 Hz, 1 H), 4.00 (br. s, 2 H), 3.77 (d, *J* = 10.6 Hz, 1 H), 3.50 (dd, *J* = 10.6 and 9.2 Hz, 1 H), 3.27 (s, 3 H), 3.23 (s, 3 H), 2.49 (dd, *J* = 15.3 and 2.0 Hz, 1 H), 2.22 (dd, *J* = 15.2 and 6.7 Hz, 1 H), 2.05 (dd, *J* = 15.2 and 3.5 Hz, 1 H), 1.87 (dd, *J* = 15.3 and 6.8 Hz, 1 H), 1.54 (s, 3 H), 1.33 (s, 3 H), 1.30 (s, 3 H), 1.29 (s, 3 H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 143.6 (C), 122.2 (CH), 111.6 (C), 100.1 (C), 100.0 (C), 82.0 (CH), 81.6 (CH), 73.2 (CH), 71.1 (CH), 67.5 (CH), 63.5 (CH_2), 54.1 (C), 47.9 (CH_3), 47.8 (CH_3), 41.1(CH_2), 37.8 (CH_2), 27.3 (CH_3), 25.1 (CH_3), 17.8 (CH_3), 17.8 (CH_3) ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3497 cm^{-1}. MS (CI): *m/z* = 401 [*M* + H]$^+$. HRMS calcd. for $C_{20}H_{33}O_8$ [*M* + H]$^+$: 401.2175; found 401.2188.

Spiro Polyol 6: A solution of the bis(methoxy acetal) **24** (84 mg, 0.21 mmol) in TFA/H_2O (1.5 mL, 50%) was stirred at room tem-

perature for 1 h. The solvent was removed under reduced pressure and the crude mixture was diluted with ethyl acetate and water. The organic layer was separated and the aqueous layer was washed with ethyl acetate (3 × 3 mL). The aqueous extract was freeze-dried to afford polyol **6** as a white foam (51 mg, 99%): m.p. 105–106 °C. $[a]_D^{20}$ = –3.3 (*c* = 1.54, in EtOH). ^{1}H NMR (250 MHz, D_2O): δ = 5.58 (br. s, 1 H), 4.25–4.08 (m, 5 H), 3.53 (m, 2 H), 2.25 (dd, *J* = 14.3 and 6.9 Hz, 1 H), 2.07 (dd, *J* = 15.1 and 3.6 Hz, 1 H), 1.92–1.79 (m, 2 H) ppm. ^{13}C NMR (63 MHz, D_2O): δ = (ppm) 143.1 (C), 123.0 (CH), 75.0 (CH), 74.6 (CH), 73.8 (CH), 73.5 (CH), 71.8 (CH), 61.0 (CH_2), 48.3 (C), 37.8 (CH_2), 37.5 (CH_2) ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3361 cm^{-1}. MS (CI): *m*/*z* = 246 [*M* + H]$^+$. HRMS calcd. for $C_{11}H_{19}O_6$ [*M* + H]$^+$: 247.1182; found 247.1183.

Spiro Diol 25: The reaction was carried out as for compound **24**. Keto ester **23** (46 mg, 0.11 mmol), DIBAL-H (2.3 mL) and THF (1 mL) were combined to produce a mixture. Chromatographic eluent: THF/hexanes (30%). Compound **25** as white needles (32 mg, 74%): m.p. 194–195 °C (diethyl ether). $[a]_D^{20}$ = +109.6 (*c* = 1.08, in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.66 (br. d, *J* = 0.9 Hz, 1 H), 4.77 (m, 1 H), 4.68 (m, 1 H), 4.23 (s, 2 H), 4.19 (m, 1 H), 3.61 (d, *J* = 10.9 Hz, 1 H), 3.55 (dd, *J* = 10.9 and 8.5 Hz, 1 H), 3.26 (s, 3 H), 3.25 (s, 3 H), 2.48 (br. s, 1 H), 2.34 (dd, *J* = 14.6 and 7.2 Hz, 1 H), 2.23 (dd, *J* = 14.6 and 4.9 Hz, 1 H), 2.00 (dd, *J* = 14.6 and 7.0 Hz, 1 H), 1.77 (dd, *J* = 14.6 and 4.1 Hz, 1 H), 1.52 (s, 3 H), 1.33 (s, 3 H), 1.32 (s, 3 H), 1.30 (s, 3 H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 142.6 (C), 122.9 (CH), 111.4 (C), 100.2 (C), 100.1 (C), 82.1 (CH), 81.9 (CH), 75.7 (CH), 69.7 (CH), 67.6 (CH), 62.8 (CH_2), 54.2 (C), 47.9 (CH_3), 47.8 (CH_3), 41.7 (CH_2), 39.2 (CH_2), 27.7 (CH_3), 25.1 (CH_3), 17.7 (2 × CH_3) ppm. IR (NaCl): $\tilde{\nu}_{max}$ = 3391 cm^{-1}. MS (CI): *m*/*z* = 401 [*M* + H]$^+$. HRMS calcd. for $C_{20}H_{33}O_8$ [*M* + H]$^+$: 401.2175; found 401.2174.

Spiro Polyol 7: The reaction was carried out as for compound **6**. Acetal **25** (38 mg, 0.10 mmol) and TFA/H_2O (50%, 1.5 mL) were combined to produce a mixture. Compound **7** was obtained as a white foam (24 mg, 99%): $[a]_D^{20}$ = +6.4 (*c* = 0.98, in EtOH). ^{1}H NMR (250 MHz, D_2O): δ = 5.65 (d, *J* = 1.0 Hz, 1 H), 4.46 (d, *J* = 14.1 Hz, 1 H), 4.39–4.20 (m, 4 H), 3.60 (m, 2 H), 2.28 (dd, *J* = 14.5 and 6.3 Hz, 1 H), 2.18 (dd, *J* = 14.5 and 5.8 Hz, 1 H), 2.02 (dd, *J* = 14.5 and 6.0 Hz, 1 H), 1.74 (dd, *J* = 14.5 and 5.9 Hz, 1 H) ppm. ^{13}C NMR (63 MHz, D_2O): δ = 143.2 (C), 124.1 (CH), 77.1 (CH), 74.3 (CH), 73.7 (CH), 73.5 (CH), 72.1 (CH), 62.2 (CH_2), 47.9 (C), 38.9 (CH_2), 38.7 (CH_2) ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3377 cm^{-1}. MS (CI): *m*/*z* = 247 [*M* + H]$^+$. HRMS calcd. for $C_{11}H_{19}O_6$ [*M* + H]$^+$: 247.1182; found 247.1189.

Spiro Ester 26: A suspension of the alkene **22** (127 mg, 0.30 mmol) and palladium on carbon (10%, 10 mg) in ethanol (3 mL) was shaken under hydrogen atmosphere at room temperature for 1 h. The mixture was filtered through Celite and the residue was washed with ethanol. The filtrate and washings were evaporated under reduced pressure to yield a colourless oil, which was purified by flash chromatography, with elution with diethyl ether/DCM (10%) to yield saturated ester **26** (123 mg, 96%) as a foam: $[a]_D^{20}$ = +84.0 (*c* = 1.09, in $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 4.67 (d, *J* = 10.5 Hz, 1 H), 4.45 (m, 2 H), 3.58 (m, 1 H), 3.58 (s, 3 H), 3.11 (s, 3 H), 3.05 (s, 3 H), 2.70 (br. d, *J* = 15.3 Hz, 1 H), 2.38 (dd, *J* = 15.3 and 5.4 Hz, 1 H), 2.46 (dd, *J* = 13.3 and 3.7 Hz, 1 H), 2.03 (m, 2 H), 1.87 (ddd, *J* = 13.6, 4.9 and 1.2 Hz, 1 H), 1.42 (dd, *J* = 15.3 and 7.1 Hz, 1 H), 1.18 (s, 3 H), 1.14 (s, 3 H), 1.10 (s, 3 H), 1.01 (s, 3 H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 200.5 (C), 171.8 (C), 110.4 (C), 100.2 (C), 99.5 (C), 80.0 (CH), 79.9 (C), 74.0 (CH), 69.4 (CH), 58.6 (C), 52.0 (CH_3), 47.9 (CH_3), 47.8 (CH_3), 46.4 (CH), 37.3 (CH_2), 36.2 (CH_2), 29.3 (CH_2), 25.6 (CH_3), 24.3 (CH_3), 17.4 (2 × CH_3) ppm. IR (NaCl): $\tilde{\nu}_{max}$ (NaCl): 1732 cm^{-1}. MS (CI): *m*/*z* = 413 [*M* – CH_4 + H]$^+$. HRMS calcd. for $C_{20}H_{29}O_9$ [*M* – CH_4 + H]$^+$: 413.1812; found 413.1808.

Spiro Diol 27: DIBAL-H (2.8 mL, 2.8 mmol, ca. 1.0 M solution in heptane) was added under argon and at –78 °C to a stirred solution of the keto ester **26** (118 mg, 0.28 mmol) in dry THF (3 mL). The resultant solution was stirred at –78 °C for 1 h. Ethyl acetate and 5% H_2SO_4 was then added. The mixture was warmed to room temperature and extracted with ethyl acetate (× 3). The combined organic layers were dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residue was purified by flash chromatography, with elution with acetone/hexanes (40%) to yield diol **27** (110 mg, 99%) as a colourless oil that solidifies on standing: $[a]_D^{20}$ = +29.2 (*c* = 1.90, in CH_3COCH_3). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 4.44 (m, 2 H), 3.41 (m, 2 H), 3.25 (m, 2 H), 3.12 (d, *J* = 10.1 Hz, 1 H), 3.12 (s, 3 H), 3.08 (s, 3 H), 2.75 (br. s, 1 H), 2.08 (dd, *J* = 15.3 and 4.1 Hz, 1 H), 1.81 (dd, *J* = 15.3 and 5.8 Hz, 1 H), 1.74 (m, 3 H), 1.58 (dd, *J* = 15.2 and 7.3 Hz, 1 H), 1.33 (s, 3 H), 1.24 (m, 1 H), 1.11 (s, 3 H), 1.07 (s, 6 H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 111.6 (C), 99.5 (C), 99.3 (C), 82.4 (CH), 80.8 (CH), 74.3 (CH), 73.5 (CH), 67.8 (CH), 63.6 (CH_2), 52.2 (C), 47.9 (CH_3), 47.8 (CH_3), 45.0 (CH), 40.5 (CH_2), 31.9 (CH_2), 29.1 (CH_2), 27.6 (CH_3), 25.5 (CH_3), 17.7 (2 × CH_3) ppm. IR (NaCl): $\tilde{\nu}_{max}$ = 3465 cm^{-1}. MS (CI): *m*/*z* = 387 [*M* – CH_4 + H]$^+$. HRMS calcd. for $C_{19}H_{31}O_8$ [*M* – CH_4 + H]$^+$: 387.2019; found 387.2018.

Spiro Diacetate 28: Triethylamine (30 μL, 0.19 mmol), acetic acid anhydride (15 μL, 0.15 mmol) and DMAP (2 mg) were added under inert atmosphere at 0 °C to a stirred solution of the diol **27** (30 mg, 0.07 mmol) in dry DCM (1 mL). The resultant solution was stirred at room temperature for 6 h. DCM was added, and then water. The organic layer was separated and the aqueous phase was extracted twice with DCM. All the combined organic extracts were dried (anhydrous Na_2SO_4) and filtered, and the solvents were evaporated. The obtained residue was purified by flash chromatography, with elution with acetone/hexanes (40%) to yield diacetoxy compound **28** (30 mg, 83%) as a colourless oil: $[a]_D^{20}$ = +93.5 (*c* = 1.05, in CH_3COCH_3). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 4.85 (d, *J* = 10.4 Hz, 1 H), 4.51 (m, 2 H), 3.95 (dd, *J* = 11.0, 4.4 Hz, 1 H), 3.69 (dd, *J* = 11.0, 8.0 Hz, 1 H), 3.59 (m, 1 H), 3.35 (t, *J* = 10.1 Hz, 1 H), 3.20 (s, 3 H), 3.18 (s, 3 H), 2.10 (s, 3 H), 2.05 (s, 3 H), 1.98–1.77 (m, 1 H), 1.59 (m, 1 H), 1.47 (s, 3 H), 1.24 (s, 6 H), 1.21 (s, 3 H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 170.7 (C), 170.4 (C), 111.6 (C), 99.5 (C), 99.4 (C), 81.8 (CH), 80.4 (CH), 74.1 (CH), 71.2 (CH), 67.6 (CH), 64.8 (CH_2), 51.8 (C), 47.8 (CH_3), 47.4 (CH_3), 42.0 (CH), 40.1 (CH_2), 33.2 (CH_2), 28.7 (CH_2), 28.1 (CH_3), 25.3 (CH_3), 20.9 (CH_3), 20.8 (CH_3), 17.6 (2 × CH_3) ppm. IR (NaCl): $\tilde{\nu}_{max}$ (NaCl): 1741 cm^{-1}. MS (CI): *m*/*z* = 471 [*M* – CH_4 + H]$^+$. HRMS calcd. for $C_{23}H_{35}O_{10}$ [*M* – CH_4 + H]$^+$: 471.2230; found 471.2228.

Spiro Polyol 8: A solution of the bis(methoxy acetal) **27** (37 mg, 0.1 mmol) in TFA/H_2O (1 mL, 50%) was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the crude mixture was diluted with ethyl acetate and water. The organic layer was separated and the aqueous layer was washed with ethyl acetate (3 × 3 mL). The aqueous extract was freeze-dried to afford polyol **8** (22 mg, 95%) as a colourless oil: $[a]_D^{20}$ = +25.9 (*c* = 0.95, in EtOH). ^{1}H NMR (250 MHz, D_2O): δ = 3.67 (m, 2 H), 3.50 (dd, *J* = 10.9 Hz and 3.5 Hz, 1 H), 3.19 (m, 2 H), 2.98 (m, 2 H), 1.81 (m, 2 H), 1.51 (dd, *J* = 14.6 and 5.7 Hz, 2 H), 1.32 (m, 2 H), 0.96 (c, *J* = 12.3 Hz, 1 H) ppm. ^{13}C NMR (63 MHz, D_2O): δ = 77.9 (CH), 76.8 (CH), 74.1 (CH), 73.0 (CH), 71.8 (CH), 62.1 (CH_2), 47.0 (C), 42.7 (CH), 38.8 (CH_2), 32.4 (CH_2), 30.7 (CH_2) ppm. IR

(NaCl): $\tilde{\nu}_{max}$ (NaCl): 3362 cm^{-1}. MS (CI): *m/z* = 249 $[M + H]^+$. HRMS calcd. for $C_{11}H_{21}O_6$ $[M + H]^+$: 249.1338; found 249.1342.

Supporting Information (see also the footnote on the first page of this article): ^1H NMR, ^{13}C NMR and DEPT spectra of compounds **3**–**8**.

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[12] CCDC-268721 (for **2**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[13] The 3D minimum energy structures of **24** and **25** were generated by use of Gaussian 98W,[14] and their energies were minimized with AM1.

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