Synthesis and glycosidase inhibitory activity of noeurostegine†—a new and potent inhibitor of β-glucoside hydrolases‡

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A new, stable hemi-aminal nor-tropane christened noeurostegine was synthesised in 22 steps from levoglucosan and tested for inhibitory activity against glycoside hydrolases. Sweet almond and *Thermotoga maritima* β-glucosidases, coffee bean α-galactosidase, and *Asp. oryzae* β-galactosidase were inhibited in the low micromolar region but significant tightening of binding to K_i 50 nM for almond β-glucosidase was found to occur after pre-incubation. Yeast α-glucosidase and *E. coli* β-galactosidase were not inhibited at 1 mM.

Introduction

The medicinal potential of modulating glycoside hydrolase activity by small molecules is commonly recognised,[1] and valuable activity towards diseases like HIV, cancer, diabetes, influenza and lysomal storage disorders have been found.[2] Significant progress in design and synthesis of new glycoside hydrolase inhibitors have occurred and the field remains a highly active area of research. Among the most potent compounds targeting these enzymes are synthetic and naturally occurring piperidine carbohydrate mimics, referred to as iminosugars or azasugars depending on the position of the nitrogen atom.[3]

It has been shown that a large degree of transition state stabilisation during enzymatic glycoside hydrolysis occurs through the 2-OH of the substrate.[4–7] This stabilisation is exploited by one of the strongest current glycoside hydrolase inhibitors, noeuromycin (**1**, Fig. 1).[8] A disadvantage of this hemi-aminal inhibitor is its lack of stability for longer periods of time as it has been shown to undergo Amadori rearrangement.[8,9] Structurally similar, naturally occuring nor-tropane glycoside hydrolase inhibitor calystegine B_2 (**3**, Fig. 1),[10,11] however, has been shown to be stable, possibly due to the bicyclic structure preventing iminium ion formation (Bredt's rule).

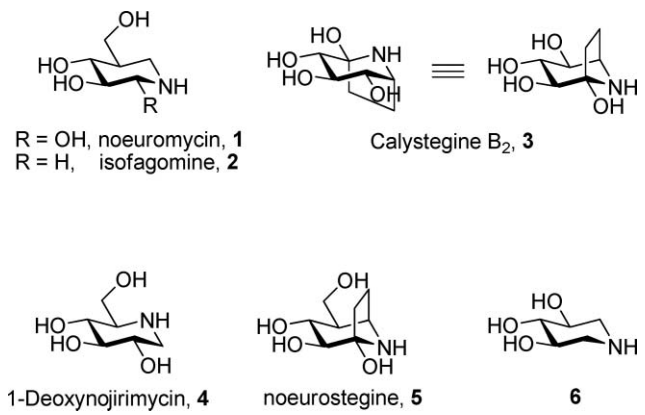

Fig. 1

Calystegine B_2 has recently been crystallographically shown to bind to *Thermotoga maritima* of glycoside hydrolase family 1 (*Tm*GH1) in a 'noeuromycin binding mode' rather than the opposite '1-deoxynojirimycin (**4**) binding mode' placing the nitrogen atom in the position expected to be occupied by the anomeric carbon of the substrate.[12] This allows for tight inhibitor binding between the hemi-aminal oxygen and the protein but places a hydroxyl group in a position occupied by the hydroxymethyl substituent of both a substrate glucoside and noeuromycin (**1**). We envisaged[13] that a hybrid of noeuromycin (**1**) and calystegine B_2 (**3**), being noeurostegine,† (**5**, Fig. 1) would inherit the potency of the former and stability of the latter inhibitor provided that the ethylene bridge can be accommodated by the enzyme active site. Changing a hydroxyl for a hydroxymethyl substituent has previously been shown to result in a dramatic increase in inhibitory potency for, *e.g.*, isofagomine (**2**),[14–16] being three orders of magnitude better than piperidine triol **6** (Fig. 1).[17]

Synthesis

Calystegine B_2 has been synthesised previously, but the elegant ring-closing metathesis route by Boyer/Hanna[18] and Skaanderup/Madsen[19] especially drew our attention for the synthesis of noeurostegine. However, we decided to introduce nitrogen as an azide instead of as a carbamate to achieve well resolved NMR spectra for easy structure elucidation. A known key intermediate **8**[20] was needed for this approach, which could be prepared in a streamlined approach from levoglucosan (**7**) in eight steps.[21] This sequence of reactions could be carried out on a multigram scale without the need for intermediate chromatographic purification.

Known anhydrosugar **8** underwent acid-promoted methanolysis followed by iodination to give halide **10** in 87% yield over two steps (Scheme 1).[22] Zinc-mediated fragmentation under sonication in THF–H_2O[23] resulted in clean conversion to aldehyde **11**, which was used directly in the following Barbier reaction without purification. This resulted in a separable diastereoisomeric

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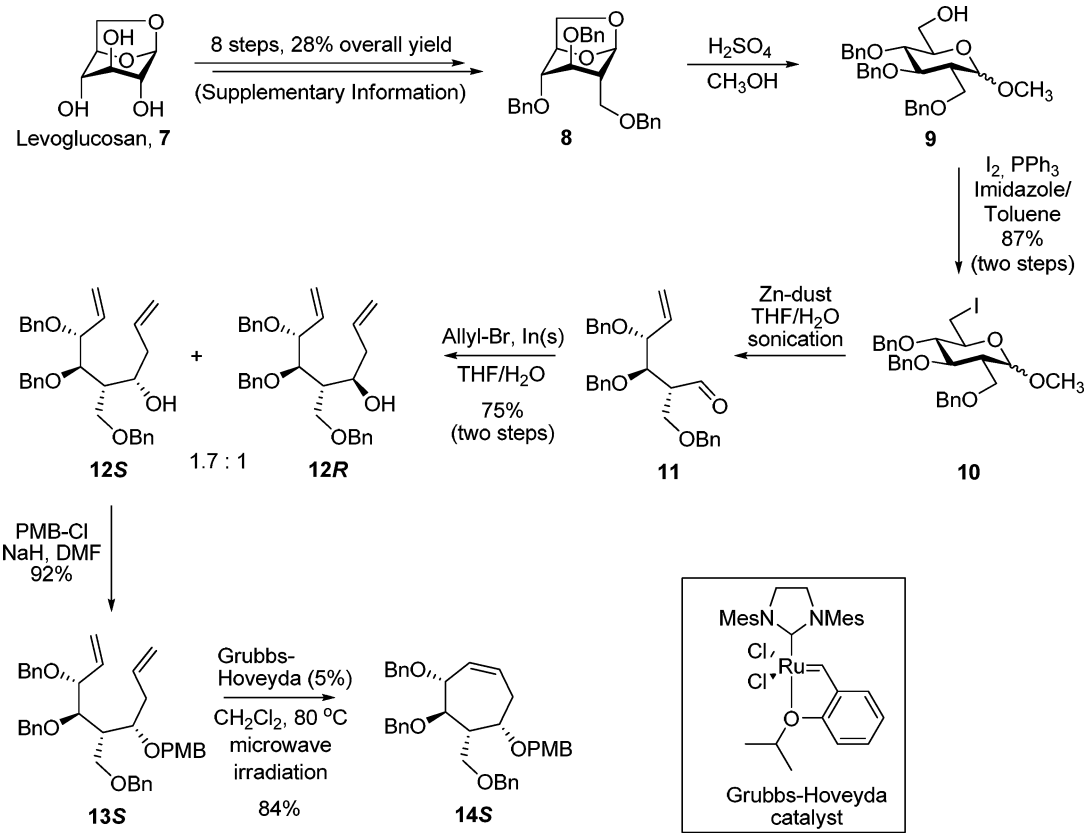
† We suggest the name *noeurostegine* for this new compound since it is a hybrid between *noeuro*mycin and caly*stegine* B_2.

‡ Electronic supplementary information (ESI) available: Experimental section, NMR spectra, and Michaelis–Menten and Hanes plots. See DOI: 10.1039/b918576c



Scheme 1 Synthesis of cycloheptene **14*S*** from anhydrosugar **8**.

1 : 1.7 mixture of homoallylic alcohols **12*R*/12*S***. Configuration was established by NMR analysis at a later stage. The alcohol function of the *S*-isomer was protected as a *p*-methoxybenzyl (PMB) ether (**13*S***). The following ring closing metathesis[24] was found to proceed smoothly to give cycloheptene **14*S*** in 84% yield but required that the homoallylic alcohol function was protected (Scheme 1).

The cycloheptene (**14*S***) was oxidized to an isomeric mixture of secondary alcohols by a standard hydroboration/oxidative work-up protocol in 86% yield. The desired regioisomer **16*S*** was found to be the major product as only one diastereoisomer in the ratio of 3 : 1.[25] The configuration of the undesired isomer (**15*S***) was not established, but was also found to be only one diastereoisomer. Alcohol **16*S*** was protected as a benzoyl ester with benzoyl chloride (BzCl) in pyridine to afford **17*S***, which was reacted with DDQ[26] to give **18*S*** in 85% over two steps (Scheme 2).

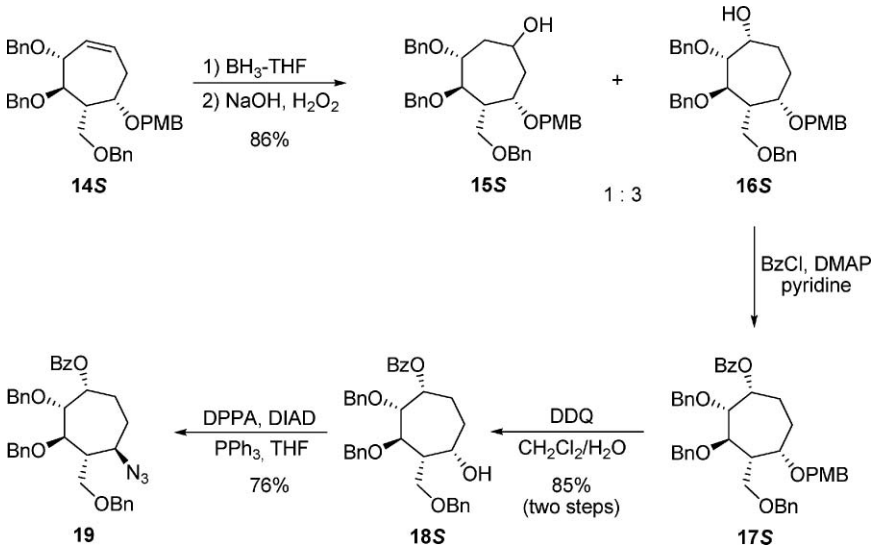
The free secondary alcohol of **18*S*** was substituted for azide to give **19** with inversion of configuration using diphenyl phosphonic azide (DPPA) under Mitsunobu conditions in 76% yield (Scheme 2). The benzoyl ester was removed by Zemplén deacylation[27] and the alcohol (**20**) oxidized by Dess–Martin periodinane[28] (DMP) to ketone **21** in 80% over two steps (Scheme 3).

In an attempt to convert azido ketone **21** directly into the desired deprotected noeurostegine **5**, only the azide and not the benzyl ethers was found to undergo reduction with H_2 over Pearlman's catalyst ($Pd(OH)_2/C$). As previously reported by others, we also found that cyclisation onto the keto functionality did not occur spontaneously, making it necessary to raise the pH and impeding product isolation.[29] Accordingly, we chose to spend a further synthetic step and found that Staudinger conditions[30] indeed caused clean and spontaneous cyclisation, as established by a hemi-aminal ^{13}C chemical shift of 92.9 ppm rather than a ketone resonance around 209 ppm. Catalytic hydrogenolysis over Pearlman's catalyst next yielded the desired noeuromycin/calystegine B_2-hybrid (Scheme 3).

It was next investigated whether the minor product from the Barbier reaction (Scheme 1) could also be used for the synthesis of noeurostegine **5**. Minor homoallylic alcohol **12*R*** was protected as its PMB-ether (**13*R***) and cyclised using the Hoveyda–Grubbs catalyst (Scheme 4).[24] Hydroboration/oxidation resulted in a separable 2 : 1 mixture of regioisomers favouring **16*R***. Again, only one diastereoisomer of the major isomer was formed, which, after purification, underwent benzoylation, PMB-ether cleavage with DDQ[26] and oxidation to ketone **23** with DMP in 90% yield over three steps. The ketone **23** was then reduced with lithium tri-*tert*-butoxyaluminium hydride to predominantly give alcohol **18*S*** in 85% yield.

Inhibition studies

To investigate hemi-aminal stability, noeurostegine (**5**) was dissolved in D_2O and left for 10 d at room temperature without any signs of degeneration by ^1H-NMR analysis. The nor-tropane **5** was then evaluated as an inhibitor of commercially available glycoside hydrolases and the values compared to known compounds.



Scheme 2 Synthesis of azido cycloheptane **19** from cycloheptene **14*S***.

Noeurostegine (**5**) was found to competitively inhibit α-galactosidase with an inhibition constant (K_i) of 2.5 μM being somewhat weaker than calystegine B_2 (K_i 0.86 μM).[31] Also *Asp. oryzae* β-galactosidase was inhibited by noeurostegine (**5**) in the micromolar region (K_i 23 μM) while *E. coli* β-galactosidase was not inhibited at 1 mM (Table 1).

Noeuromycin (**1**) is known to be a very powerful inhibitor of yeast α-glucosidase but neither calystegine B_2 (**3**)[31] nor noeurostegine (**5**) inhibit this enzyme at 1 mM. This could be due to the spatial requirement of the ethylene bridge making noeurostegine (**5**) too large for the enzyme active site.

β-Glycosidase from *Thermotoga maritima* (*Tm*GH1) was found to be inhibited strongly by noeurostegine (**5**) with an inhibition constant of 1.1 μM at enzyme optimum (K_i 0.14 μM at noeuromycin optimum)[32] (Table 1). This is slightly better than what has been observed for calystegine B_2 (**3**), which suggests that a hydroxymethyl substituent has a positive influence over a hydroxyl substituent in inhibitor binding to *Tm*GH1.

Initial experiments also established noeurostegine (**5**) as a low micromolar inhibitor (K_i 1.5 μM) of almond β-glucosidase. However, when measuring after pre-incubation of the enzyme with the inhibitor for 30 min, as described for calystegine B_2 (**3**),[31] a significant tightening of binding (K_i 50 nM) was observed. Slow onset of inhibition of sweet almond β-glucosidase by nitrogen containing carbohydrate mimics has previously been thoroughly described. We speculate that this slow binding process could be a result of slow almond β-glucosidase dynamics upon binding of the rigid inhibitor **5**. No such slow inhibition was observed for any other of the enzymes tested in this study.

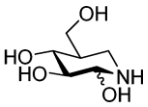
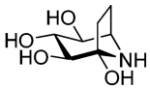
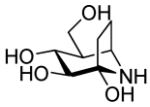
Conclusion

A new nor-tropane (noeurostegine, **5**, Fig. 1) has been synthesised in 22 steps from levoglucosan through a cycloheptene prepared by Hoveyda–Grubbs catalyst-mediated ring closing metathesis. We have demonstrated that the target compound possessing hydroxyl groups in the 2, 3, 4 and 6 positions corresponding to the enzyme substrates, is fully stable in aqueous solution for several days and furthermore is a potent, competitive inhibitor of almond and *Thermotoga maritima* β-glucosidase. Yeast α-glucosidase, however, was not inhibited at 1 mM, making noeurostegine a highly selective inhibitor, which can possibly be traced back to its congested bicyclic structure.

General experimental information

All reagents and enzymes, except otherwise stated were used as purchased without further purification. Levoglucosan was purchased from Carbosynth Ltd. and enzymes from Sigma-Aldrich, except *Tm*GH1, which was purchased from Megazyme. Oven dried glassware (*ca.* 120 °C) was used for reactions carried out under nitrogen or argon atmosphere. Solvents were dried according to standard procedures prior to use ('Purification of Laboratory Chemicals, 3rd Edition' D. D. Perrin, W. L. F. Armarego, 1988, Butterworth-Heinemann Ltd). Dichloromethane was dried by distillation over CaH_2, and diethyl ether was dried over sodium wire. THF was dried over sodium and distilled from benzophenone. Flash chromatography was performed with Merck silica 60 (230-400 mesh) as stationary phase and TLC was performed on silica-coated aluminium plates (Merck 60 F_{254}). TLC plates were first observed in UV-light and then visualised with ceric sulfate/ammonium molybdate in 10% H_2SO_4 stain or $KMnO_4$ stain. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) were recorded on a Varian Mercury 400 spectrometer. $CDCl_3$ (δ 7.26 ppm ($CHCl_3$) for proton and δ 77.16 ppm for carbon resonances) and D_2O (δ 4.79 ppm for proton) were used as internal references. Spectra were assigned based on *g*COSY, *g*HMQC, and DEPT-135 experiments. MS spectra were recorded on a Micromass LC-TOF instrument by using electrospray ionization (ESI). High resolution spectra were recorded with either of the following compounds as internal standard: (Boc-L-alanine: $C_8H_{15}NO_4Na$: 212.0899; BzGlyPheOMe: $C_{19}H_{20}N_2O_4Na$: 363.1321; BocSer(OBn)SerLeuOMe: $C_{25}H_{39}N_3O_8Na$: 532.2635; erythromycin: $C_{37}H_{67}NO_{13}Na$: 756.4510) Masses of standards and analytes are calculated and reported in Daltons for uncharged species. Melting points were measured on a Büchi B-540

Table 1 K_i values in nM at pH 6.8. Inhibition was found to be competitive

	 noeuromycin, **1**	 calystegine B_2, **3**	 noeurostegine, **5**
α-Galctosidase (Green coffee beans)	—	860[a,b]	2500
β-Galactosidase (*E. coli*)	—	—	$>10^6$
β-Galactosidase (*Asp. oryzae*)	—	—	23000
α-Glucosidase (Yeast)	22[b]	$>10^6$	$>10^6$
β-Glucosidase (*T. maritima*)	37	1250	140
	88[c]	4000[c]	1100[c]
β-Glucosidase (Sweet almonds)	69[b]	1200[a,b]	1500
			50[a]

[a] Pre-incubation (30 min). [b] pH-value unknown. [c] pH 5.8.

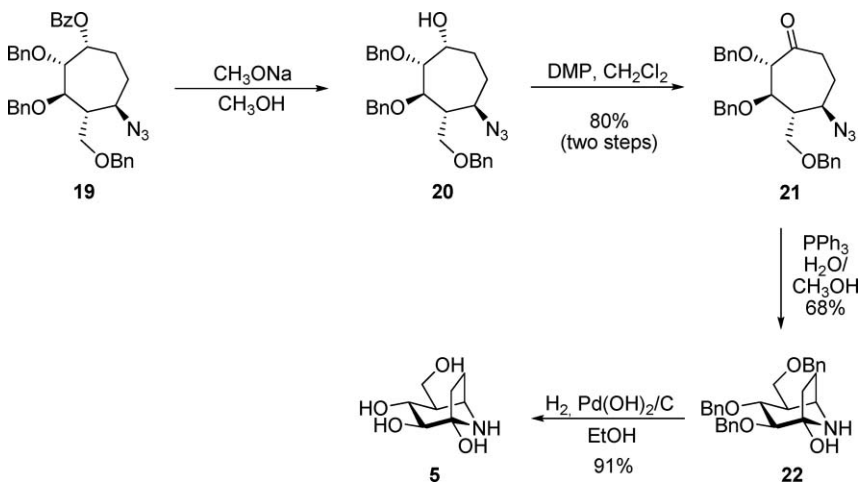
instrument and are uncorrected. Optical rotation was measured on a PE-241 polarimeter and reported in units of deg cm^2 g^{-1}. Concentrations are reported in g/100 mL. Sonication was conducted by use of a Branson 1510 ultrasonic bath. Microwave experiments were carried out on a Biotage Initiator (Biotage, Sweden). Reaction times listed refer to 'hold time' at the specified temperature.

Inhibition studies

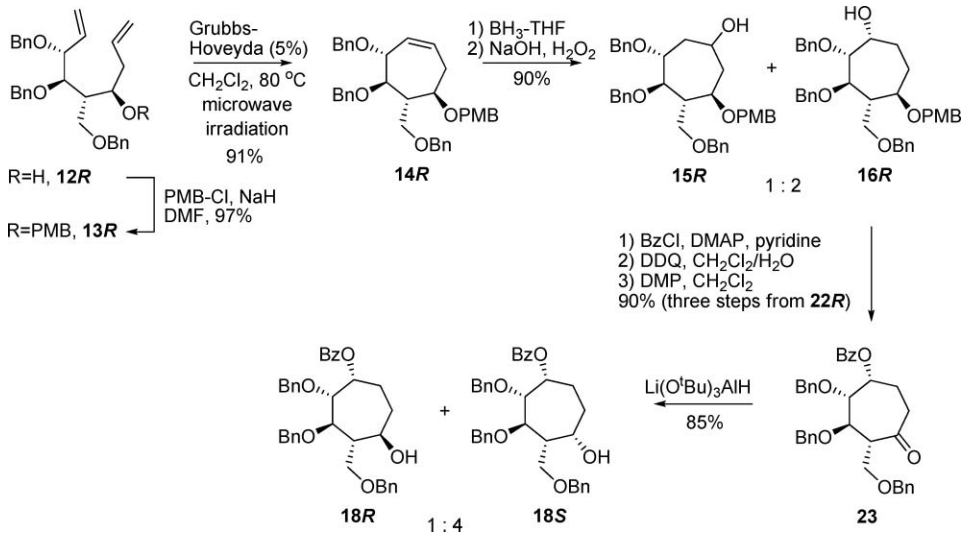
Inhibition constants (K_is) were determined by measuring initial rates (<10% of substrate conversion) using 2,4-dinitrophenyl, *o*-nitrophenyl or *p*-nitrophenyl glycosides (*p*-nitrophenyl β-D-glucopyranoside for almond β-glucosidase, *p*-nitrophenyl α-D-glucopyranoside for yeast α-glucosidase, *o*-nitrophenyl β-D-galactopyranoside for *E. coli* β-galactosidase, *p*-nitrophenyl β-D-galactopyranoside for *Asp. oryzae* β-galactosidase, *p*-nitrophenyl α-D-galactopyranoside for green coffee bean α-galactosidase, 2,4-dinitrophenyl β-D-glucopyranoside for *Tm*GH1 β-glucosidase) at six concentrations ranging from $\frac{1}{4}$–4 times K_M monitoring at 400 nm with $A < 1$ using a Varian Cary 100 Bio uv-vis spectrophotometer. Measurements were conducted in 50 mM phosphate buffer (pH 6.8 or pH 5.8) at 25 °C for 2 min. For experiments with pre-incubation inhibitor and enzyme were mixed 30 min prior to addition of substrate and monitoring of reaction rates. K_M and K'_M values were obtained by fitting to the equation $v = (V_{max}\cdot[S])/(K_M+[S])$ using the program Sigma Plot 11.0. Competitive inhibition was established from Hanes plots. K_i values were calculated as $K_i = [I]/((K_M'/K_M) - 1)$ having $[I] \approx K_i$. For Michaelis–Menten and Hanes plots, see the ESI.‡

Organic synthesis

Methyl 3,4-di-*O*-benzyl-2-*C*-benzyloxymethyl-2-deoxy-α/β-D-glucopyranoside (9). Anhydrosugar **8** (2.50 g, 5.59 mmol) was dissolved in dry methanol containing 10 v/v% sulfuric acid (70 mL) and the reaction mixture heated to 40 °C under a nitrogen atmosphere for 24 h. The reaction mixture was then poured into a saturated aq. solution of $NaHCO_3$ (150 mL) and the aqueous phase extracted with EtOAc (3 × 170 mL). The combined organic layers were washed with brine (2 × 200 mL), dried over Na_2SO_4, filtered and concentrated. The obtained anomeric mixture of **9** was used directly for the next reaction, but could be purified by flash column chromatography (pentane–EtOAc 5 : 1→4 : 1) to give the product **9** (2.47 g, 92%) as a 3 : 1 (determined by ^{13}C NMR) mixture of α/β anomers. LRMS (ES+) calcd. for $C_{29}H_{34}O_6Na$: 501.2, found 501.4. ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) α: 138.6, 138.4, 138.3 (ArC), 128.6-127.7 (m, ArC), 99.2 (C1), 79.6,



Scheme 3 Synthesis of noeurosegine **5** from cycloheptane **19** *via* Staudinger reduction.



Scheme 4 Synthesis sequence for the preparation of **18*S*** from **12*R***.

78.9, 75.1, 75.0, 73.2, 71.3, 67.9, 62.2, 55.1, 47.0, β: 138.7, 138.5, 138.4 (ArC), 128.6-127.7 (m, ArC), 102.0 (C1), 79.8, 79.2, 75.4, 75.3, 73.3, 68.4, 64.8, 62.3, 57.3, 48.8.

Methyl 3,4-di-*O*-benzyl-2-*C*-benzyloxymethyl-2,6-dideoxy-6-iodo-α/β-D-glucopyranoside (10). To a solution of α/β mixture **9** (2.47 g, 5.17 mmol) in dry toluene (100 mL) was added triphenylphosphine (3.40 g, 12.95 mmol, 2.5 eq), iodine (1.44 g, 5.65 mmol, 1.1 eq) and imidazole (0.71 g, 10.43 mmol, 2.0 eq) under a nitrogen atmosphere. The reaction mixture was heated to reflux for 90 min before the yellow mixture became colourless and turned clear. The reaction mixture was then cooled to rt and 5% aqueous sodium thiosulfate (50 mL) was added, and the organic layer washed with brine (2 × 50 mL). The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash column chromatography (pentane–EtOAc 20 : 1) to give the product **10** (2.64 g, 87%) as a 3 : 1 (determined by ^{13}C NMR) mixture of α/β anomers. NMR data was in accordance with previously reported data.[22b]

(2*R*,3*R*,4*R*)-3,4-Di-*O*-benzyl-2-*C*-benzyloxymethyl-2,5,6-trideoxy-hex-5-enal (11). To a solution of iodide **10** (0.56 g, 0.96 mmol) in THF (25 mL) was added pre-activated Zn dust[33] (0.69 g, 10.54 mmol, 11.0 eq) and water (2.8 mL). The resulting suspension was sonicated at 40 °C until TLC-analysis showed full conversion (1 h). The reaction mixture was then diluted with Et_2O (40 mL) and water (15 mL) and the resulting mixture filtered through a pad of Celite and the organic phase washed with water (15 mL), brine (15 mL), and dried over Na_2SO_4 and concentrated. The isolated aldehyde **11** (0.41 g) was used directly in the next reaction without further purification. ^1H crude NMR (400 MHz, $CDCl_3$): δ (ppm) 9.75 (s, 1 H, C*H*O), 7.35-7.23 (m, 15 H, ArH), 5.90 (ddd, 1 H, $J_{4,5} = 7.6$ Hz, $J_{cis} = 10.6$ Hz, $J_{trans} = 18.0$ Hz, H5), 5.32 (m, 2 H, H6), 4.69 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.57 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.56 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.45 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.39 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.28 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.05 (dd, 1 H, $J_{3,4} = 4.8$ Hz, $J_{2,3} = 6.0$ Hz, H3), 3.95 (dd, 1 H, $J_{3,4}$ = 4.8 Hz, $J_{4,5} = 7.6$ Hz, H4), 3.88 (dd, 1 H, $J_{2,7} = 6.0$ Hz, J_{gem} = 10.0 Hz, H7), 3.64 (dd, 1 H, $J_{2,7'} = 6.0$ Hz, $J_{gem} = 10.0$ Hz, H7′), 2.89 (dq, 1 H, $J_{1,2} = 1.2$ Hz, $J_{2,3;2,7;2,7'} = 6.0$ Hz, H2).

(3*R*,4*R*,5*S*,6*R*/*S*)-3,4-Di-*O*-benzyl-5-*C*-benzyloxymethyl-6-hydroxy-1,8-nonadiene (12). To a stirred solution of the intermediate aldehyde **11** (2.22 g, 5.16 mmol) in 50% aqueous THF (32 mL) was added indium dust (0.90 g, 7.84 mmol, 1.5 eq) and allyl bromide (1.10 mL, 13.0 mmol, 2.5 eq). The reaction mixture was stirred overnight at rt. before a saturated aq. solution of NH_4Cl was added and the aqueous phase neutralised with a saturated aq. solution of $NaHCO_3$. The resulting mixture was extracted with CH_2Cl_2 (4 × 80 mL) and the combined organic phases dried over $MgSO_4$ and concentrated. The crude product was purified by flash column chromatography (CH_2Cl_2) to give the product **12** (2.04 g, 75% (two steps)) as a 4 : 5 mixture of *R*- and *S*-diastereoisomers.

(3*R*,4*R*,5*S*,6*R*)-3,4-Di-*O*-benzyl-5-*C*-benzyloxymethyl-6-hydroxy-1,8-nonadiene (12*R*). Colourless oil (0.81 g, 30%). $[\alpha]_D^{20}$ −12 (*c* 1.0, $CHCl_3$). R_f (CH_2Cl_2) = 0.29. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.37-7.26 (m, 15 H, ArH), 5.98 (m, 1 H, H2), 5.72 (m, 1 H, H8), 5.35 (m, 2 H, H1), 5.02 (m, 2 H, H9), 4.76 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.64 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.52 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.43 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.33 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.32 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.10 (dd, 1 H, $J_{3,4} = 4.0$ Hz, $J_{2,3}$ = 7.6 Hz, H3), 3.86 (m, 2 H, H4, H10), 3.88 (dd, 1 H, $J_{5,10'} = 3.6$ Hz, $J_{gem} = 9.6$ Hz, H10′), 3.71 (m, 1 H, H6), 2.23 (m, 2 H, H7,H7′), 2.09 (qu, 1 H, $J_{4,5;5,6;5,10;5,10'} = 3.6$ Hz, H5). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 138.5, 138.1, 137.7 (ArC) 136.1 (C2), 135.4 (C8), 128.5-127.6 (m, ArC), 119.0 (C1), 116.9 (C9), 80.9 (C4), 80.7 (C3), 74.9 (O*C*H$_2$Ph), 73.5 (O*C*H$_2$Ph), 71.5 (C6), 70.4 (O*C*H$_2$Ph), 67.7 (C10), 43.5 (C7), 40.3 (C5). HRMS (ES+): calcd. for $C_{31}H_{36}O_4Na$: 495.2511, found 495.2512.

(3*R*,4*R*,5*S*,6*S*)-3,4-Di-*O*-benzyl-5-*C*-benzyloxymethyl-6-hydroxy-1,8-nonadiene (12*S*). Colourless oil (1.23 g, 45%). $[\alpha]_D^{20}$ −15 (*c* 1.0, $CHCl_3$). R_f (CH_2Cl_2–EtOAc 100 : 1) = 0.47. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.30-7.20 (m, 15 H, ArH), 5.76 (m, 2 H, H2, H8), 5.29-5.21 (m, 2 H, H1/H9), 5.05-5.00 (m, 2 H, H1, H9), 4.87 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.60 (d, 1 H,

J_{gem}= 11.6 Hz, OCH_2Ph), 4.56 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.43 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.37 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.32 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.05 (m, 1 H, H3), 3.88 (m, 1 H, H4), 3.82 (m, 1 H, H6), 3.68 (dd, 1 H, $J_{5,10}$ = 5.2 Hz, J_{gem} = 9.6 Hz, H10), 3.59 (dd, 1 H, $J_{5,10'}$ = 7.2 Hz, J_{gem} = 9.6 Hz, H10′), 2.66 (bs, 1 H, OH), 2.23 (m, 2 H, H7, H7′), 2.06 (m, 1 H, H5). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 138.7, 138.4 (ArC), 135.5, 135.2 (C2, C8), 128.4-127.6 (m, ArC), 119.4, 117.4 (C1, C9), 83.4 (C3), 80.3 (C4), 74.4 (OCH_2Ph), 73.3 (OCH_2Ph), 71.4 (C6), 70.6 (OCH_2Ph), 68.3 (C10), 44.3 (C5), 39.9 (C7). HRMS (ES+): calcd. for $C_{31}H_{36}O_4Na$: 495.2511, found 495.2523.

(3*R*,4*R*,5*S*,6*S*)-3,4-Di-*O*-benzyl-5-*C*-benzyloxymethyl-6-*O*-*p*-methoxybenzyl-1,8-nonadiene (13*S*). Sodium hydride (60%, 0.44 g, 11.08 mmol, 2.0 eq) was added to a cooled (0 °C) and stirred solution of **12*S*** (2.492 g, 5.28 mmol) in dry DMF (20 mL) under nitrogen atmosphere. After stirring for 10 min at 0 °C, *p*-methoxybenzyl chloride (1.44 mL, 10.6 mmol, 2.0 eq) was added. The reaction mixture was warmed to rt and stirred overnight before being quenched by addition of n-butylamine (6 mL). The reaction mixture was diluted with EtOAc (100 mL) before the organic layer was washed with water (3 × 70 mL), dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash column chromatography (heptane/EtOAc 30 : 1) to give the product **13*S*** (2.89 g, 92%) as a clear oil. $[\alpha]_D^{20}$ 4 (*c* 1.0, $CHCl_3$). R_f (pentane–EtOAc 16 : 1) = 0.38. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.30-7.13 (m, 17 H, ArH), 6.77-6.74 (m, 2 H, ArH), 5.78 (m, 2 H, H2, H8), 5.24 (m, 2 H, H1/H9), 4.99 (m, 2 H, H1/H9), 4.74 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.55 (d, 1 H, J_{gem}= 12.0 Hz, OCH_2Ph), 4.49 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.43 (d, 1 H, J_{gem}= 10.8 Hz, OCH_2Ph), 4.33 (d, 1 H, J_{gem}= 12.0 Hz, OCH_2Ph), 4.25 (d, 1 H, J_{gem}= 10.8 Hz, OCH_2Ph), 4.42, 4.39 (AB, 2 H, J_{AB} = 12.0 Hz, OCH_2Ph), 4.01 (m, 1 H, H3), 3.83 (dd, 1 H, J = 6.8 Hz, J = 2.7 Hz, H4), 3.76 (m, 1 H, H10), 3.72 (s, 3H, OCH_3), 3.63 (q, 1 H, $J_{5,6;6,7,6,7'}$ = 5.6 Hz, H6), 3.54 (dd, 1 H, $J_{5,10'}$ = 6.8 Hz, J_{gem} = 9.6 Hz, H10′), 2.35 (m, 2 H, H7,H7′), 2.15 (m, 1 H, H5). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 159.1, 139.7, 138.9, 138.7 (ArC), 135.9, 135.6 (C2, C8), 131.1 (ArC), 129.5-127.2 (m, ArC), 119.1, 116.9 (C1, C9), 113.8 (ArC), 83.7 (C3), 79.5 (C4), 77.9 (C6), 74.3, 73.1, 71.4, 70.8 (OCH_2Ph), 67.9 (C10), 55.4 (OCH_3), 43.5 (C5), 36.4 (C7). HRMS (ES+): calcd. for $C_{39}H_{44}O_5Na$: 615.3086, found 615.3075.

(3*R*,4*R*,5*S*,6*S*)-3,4-Di-*O*-benzyl-5-*C*-benzyloxymethyl-6-*O*-*p*-methoxybenzyl-cycloheptene (14*S*). Diene **13*S*** (0.79 g, 1.33 mmol) and Grubbs'–Hoveyda 2nd generation catalyst (CAS [301224-40-8], 16 mg, 0.026 mmol, 0.02 eq) were dissolved in freshly distilled CH_2Cl_2 (4 mL) in a sealed microwave vial. The reaction mixture was heated by microwave irradiation for 2 min (80 °C) after which the formed ethylene gas was released; the microwave irradiation was then continued for 10 min. The solvent was removed under reduced pressure and the remaining oil was purified by flash column chromatography (pentane–EtOAc 20 : 1) to give the desired cycloheptene **14*S*** as an oil (0.63 g, 84%). $[\alpha]_D^{20}$ 17 (*c* 1.0, $CHCl_3$). R_f (pentane–EtOAc 10 : 1) = 0.37. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.36-7.23 (m, 17 H, ArH), 6.86 (m, 2 H, ArH), 5.75 (m, 1 H, H2), 5.65 (m, 1 H, H1), 4.82 (d, 1 H, J_{gem}= 11.0 Hz, OCH_2Ph), 4.69 (s, 2 H, OCH_2Ph), 4.56 (d, 1 H, J_{gem}= 11.0 Hz, OCH_2Ph), 4.47 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.47 (s, 2 H, OCH_2Ph), 4.42 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.28 (m, 1 H, H3), 4.08 (m, 1 H, H6), 3.82 - 3.70 (m, 6 H, H4, H8, H8′, OCH_3), 2.42 (m, 2 H, H5, H7), 2.31 (m, 1 H, H7′). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 159.2, 139.0, 138.9, 138.8 (ArC), 132.4 (C2), 131.0 (ArC), 129.3-127.5 (m, ArC), 116.7 (C1), 113.8 (ArC), 81.6 (C3), 78.9 (C4), 73.8, 73.2 (OCH_2Ph), 73.0 (C6), 72.7, 70.5 (OCH_2Ph), 68.6 (C8), 55.4 (OCH_3), 48.5 (C5), 31.9 (C7). HRMS (ES+): calcd. for $C_{37}H_{40}O_5Na$: 587.2773, found 587.2772.

(1*R*/*S*,3*R*,4*R*,5*S*,6*S*)-3,4-Di-*O*-benzyl-5-*C*-benzyloxymethyl-6-*O*-*p*-methoxybenzyl-cycloheptane-1-ol (15*S*) and (1*R*,2*R*,3*R*,4*S*,5*S*)-2,3-di-*O*-benzyl-4-*C*-benzyloxymethyl-5-*O*-*p*-methoxybenzyl-cycloheptane-1-ol (16*S*). BH_3·THF complex (1 M solution in THF, 6.0 mL, 6.0 mmol, 2.0 eq) was added in a drop wise fashion to a stirred solution of **14*S*** (1.69 g, 3.00 mmol) in dry THF (60 mL) at 0 °C under an atmosphere of nitrogen. After stirring for 2 h at 0 °C, 2 M NaOH (6 mL) and 35% aqueous H_2O_2 (12 mL) were added. The reaction mixture was allowed to reach rt and stirred for additional 2 h before diluted with Et_2O (100 mL). The organic phase was washed with water (3 × 60 mL), brine (2 × 60 mL), dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash column chromatography (pentane–EtOAc 4 : 1→2 : 1→0 : 1) to give regioisomers **15*S*** and **16*S*** (1.50 g, 86%) as a 1 : 3 mixture which were easily separated.

(1*R*/*S*,3*R*,4*R*,5*S*,6*S*)-3,4-Di-*O*-benzyl-5-*C*-benzyloxymethyl-6-*O*-*p*-methoxybenzyl-cycloheptane-1-ol (15*S*). Colourless oil (0.45 g, 26%). $[\alpha]_D^{20}$ 29 (*c* 1.0, $CHCl_3$). R_f (pentane–EtOAc 2 : 1) = 0.18. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.35-7.20 (m, 17 H, ArH), 6.83 (m, 2 H, ArH), 4.71 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.62 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.57 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.52 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.50 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.45 (d, 1 H, J_{gem}= 12.4 Hz, OCH_2Ph), 4.40 (d, 1 H, J_{gem}= 12.4 Hz, OCH_2Ph), 4.37 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.13 (m, 1 H, H1), 4.08 (m, 1 H, H6), 3.84 (m, 2 H, H3, H4), 3.76 (s, 3 H, OCH_3), 3.73 (m, 1 H, H8), 3.61 (m, 1 H, H8′), 2.28 (m, 2 H, H5, H7), 2.15 (m, 1 H, H2), 1.96 (m, H1, H2′), 1.59 (m, 1 H, H7′). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 159.0, 138.8, 138.7, 138.6, 131.0 (ArC), 129.1-127.5 (ArC), 113.7 (ArC), 80.1, 79.8 (C3, C4), 73.6, 73.1 (OCH_2Ph), 71.8 (C6), 71.7, 70.9 (OCH_2Ph), 68.5 (C8), 64.1 (C1), 55.3 (OCH_3), 46.5 (C5), 40.1 (C7), 38.5 (C2). HRMS (ES+): calcd. for $C_{37}H_{42}O_6Na$: 605.2879, found 605.2885.

(1*R*,2*R*,3*R*,4*S*,5*S*)-2,3-Di-*O*-benzyl-4-*C*-benzyloxymethyl-5-*O*-*p*-methoxybenzyl-cycloheptane-1-ol (16*S*). Colourless oil (1.05 g, 60%). $[\alpha]_D^{20}$ 37 (*c* 1.0, $CHCl_3$). R_f (pentane–EtOAc 2 : 1) = 0.46. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.37-7.23 (m, 17 H, ArH), 6.87 (m, 2 H, ArH), 4.92 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.71 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.59 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.56 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.51 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.49 (s, 2 H, OCH_2Ph), 4.40 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 3.90 (m, 2 H, H3, H5), 3.81 (s, 3 H, OCH_3), 3.75 (m, 1 H, H1), 3.70 (m, 2 H, H8, H8′), 3.45 (m, 1 H, H2), 2.68 (bs, 1 H, OH), 2.42 (dq, 1 H, $J_{4,5}$ = 2.4 Hz, $J_{3,4;4,8;4,8'}$ = 6.8 Hz, H4), 2.03 (m, 1 H, H6), 1.83 (m, 1 H, H6′), 1.75 (m, 1 H, H7), 1.58 (m, 1 H, H7′). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 159.2, 138.6, 138.5, 138.4, 131.0 (ArC), 129.2-127.6 (m, ArC), 113.8 (ArC), 89.4 (C2) 79.8 (C3/C5), 75.0 (OCH_2Ph), 74.5 (C3/C5) 73.4, 73.3 (OCH_2Ph), 71.3 (C1), 70.8 (OCH_2Ph), 68.3 (C8), 55.4 (OCH_3), 46.0 (C4), 28.8

(C6), 26.8 (C7). HRMS (ES+): calcd. for $C_{37}H_{42}O_6Na$: 605.2879, found 605.2874.

(1*R*,2*R*,3*R*,4*S*,5*S*)-1-*O*-Benzoyl-2,3-di-*O*-benzyl-4-*C*-benzyloxymethyl-cycloheptane-5-ol (18*S*). To a stirred solution of alcohol **16*S*** (1.05 g, 1.80 mmol) in dry pyridine (10 mL) under an atmosphere of nitrogen was added benzoyl chloride (0.42 mL, 3.62 mmol, 2.0 eq) and a catalytic quantity of DMAP (0.02 g, 0.19 mmol, 0.1 eq). The reaction mixture was stirred at rt overnight before the reaction was quenched by addition of water (25 mL) in small portions. After stirring for 10 min the mixture was diluted with EtOAc (100 mL) and the organic phase washed with a 1 M hydrochloric acid (3 × 50 mL), saturated aq. $NaHCO_3$ (50 mL), brine (2 × 50 mL), dried over $MgSO_4$, filtered and concentrated. The resulting product **17*S*** (1.26 g) was dissolved in CH_2Cl_2 (23 mL) and H_2O (0.8 mL), and vigorously stirred with DDQ (0.47 g, 2.05 mmol, 1.1 eq) for 1 h. A saturated aq. solution of $NaHCO_3$ (100 mL) and CH_2Cl_2 (100 mL) were then added to the reaction mixture and the layers separated before the organic layer was washed with brine (3 × 100 mL), dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash column chromatography (pentane–EtOAc 10 : 1→5 : 1) to give the desired product **18*S*** (0.87 g, 85% (two steps)) as a colourless oil. $[\alpha]_D^{20}$ 68 (*c* 1.0, $CHCl_3$). R_f (pentane–EtOAc 4 : 1) = 0.34. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.01 (m, 2 H, *o*-Bz), 7.58-7.54 (m, 1 H, ArH), 7.44-7.40 (m, 3 H, ArH), 7.37-7.25 (m, 12 H, ArH), 7.18-7.16 (m, 2 H, ArH), 5.51 (m, 1 H, H1), 4.80 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.71 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.69 (d, 1 H, J_{gem}= 10.8 Hz, OCH_2Ph), 4.55 (d, 1 H, J_{gem}= 12.0 Hz, OCH_2Ph), 4.49 (d, 1 H, J_{gem}= 12.0 Hz, OCH_2Ph), 4.48 (d, 1 H, J_{gem}= 10.8 Hz, OCH_2Ph), 4.36 (m, 1 H, H5), 4.05 (t, 1 H, $J_{1,2;2,3}$ = 4.8 Hz, H2), 3.99 (dd, 1 H, $J_{4,8}$ = 6.4 Hz, J_{gem} = 8.8 Hz, H8), 3.89 (dd, 1 H, $J_{2,3}$ = 4.8 Hz, $J_{3,4}$ = 9.2 Hz, H3), 3.66 (dd, 1 H, $J_{4,8}$ = 3.6 Hz, J_{gem} = 8.8 Hz, H8′), 3.48 (bs, 1 H, OH), 2.32 (m, 1 H, H4), 2.24 (m, 1 H, H7), 1.97 (m, 3 H, H6, H6′, H7′). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 165.8 (C=O), 138.5, 138.0, 137.7 (ArC), 133.0 (ArC), 130.4 (ArC), 129.7-127.5 (m, ArC), 83.0 (C2), 78.4 (C3), 73.9, 73.6 (OCH_2Ph), 73.5 (C1), 73.1 (OCH_2Ph), 71.9 (C8), 70.0 (C5), 47.1 (C4), 30.2 (C6), 22.2 (C7). HRMS (ES+): calcd. for $C_{36}H_{38}O_6Na$: 589.2566, found 589.2565.

(1*R*,2*R*,3*R*,4*R*,5*R*)-5-Azido-1-*O*-benzoyl-2,3-di-*O*-benzyl-4-*C*-benzyloxymethyl-cycloheptane (19). To a cooled (0 °C) and stirred solution of alcohol **18*S*** (0.87 g, 1.54 mmol) in dry THF (10 mL) under an atmosphere of nitrogen were consecutively added PPh_3 (0.45 g, 1.70 mmol, 1.1 eq) and DIAD (0.35 mL, 1.69 mmol, 1.1 eq) in a drop wise fashion. After stirring for 10 min, DPPA (0.43 mL, 1.99 mmol, 1.3 eq) was added, and the reaction mixture allowed to reach rt and then stirred for 2 h. The reaction mixture was concentrated and the resulting residue purified by flash column chromatography (pentane–EtOAc 50 : 1→20 : 1) to give the desired product **19** (0.69 g, 76%) as a clear oil. $[\alpha]_D^{20}$ 52 (*c* 1.0, $CHCl_3$). R_f (pentane–EtOAc 9 : 1) = 0.53. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.05 (m, 2 H, *o*-Bz), 7.60 (m, 1 H, ArH), 7.46 (m, 2 H, ArH), 7.42-7.21 (m, 15 H, ArH), 5.44 (m, 1H, H1), 4.82 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.80 (d, 1 H, J_{gem}= 10.8 Hz, OCH_2Ph), 4.74 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.57 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.55 (d, 1 H, J_{gem}= 10.8 Hz, OCH_2Ph), 4.51 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 3.97 (m, 3 H, H8, H2, H5), 3.79 (m, 2 H, H8′, H3), 2.08 (m, 5 H, H4, H6, H6′, H7, H7′). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 165.9 (C=O), 138.6, 138.4, 138.2 (ArC), 133.0 (ArC), 130.4 (ArC), 129.7-127.5 (m, ArC), 83.5 (C2), 78.2 (C3), 74.7 (OCH_2Ph), 74.3 (C1), 74.3, 73.3 (OCH_2Ph), 68.5 (C8), 60.9 (C5), 48.4 (C4), 28.1, 24.6 (C6, C7). HRMS (ES+): calcd. for $C_{36}H_{37}N_3O_5Na$: 614.2631, found 614.2636.

(2*S*,3*R*,4*R*,5*R*)-5-Azido-2,3-di-*O*-benzyl-4-*C*-benzyloxymethyl-cycloheptanone (21). Sodium (0.04 g, 1.65 mmol, 1.4 eq) was dissolved in dry MeOH (10 mL) and the resulting solution added to **19** (0.69 g, 1.17 mmol) under an atmosphere of nitrogen. The reaction mixture was stirred overnight before being diluted with EtOAc (70 mL) and the organic phase washed with water (3 × 50 mL), dried over $MgSO_4$, filtered and concentrated to give crude **20**. Dess–Martin periodinane (0.86 g, 2.03 mmol, 1.6 eq) was added to **20** (0.61 g) dissolved in CH_2Cl_2 (10 mL). The reaction mixture was stirred at rt for 30 min and then diluted with Et_2O (50 mL) after which stirring was continued for 30 min. The resulting mixture was washed with a saturated aq. solution of $Na_2S_2O_3$ (3 × 50 mL), brine (2 × 50 mL), dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash column chromatography (pentane–EtOAc 20 : 1) to give the product **21** (0.46 g, 80% (two steps)) as colourless crystals. The crystals were recrystallised from CH_2Cl_2 and heptane. Mp 74.6-76.5 °C, $[\alpha]_D^{20}$ –0.3 (*c* 1.0, $CHCl_3$). R_f (pentane–EtOAc 9 : 1) = 0.36. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.30 – 7.16 (m, 15 H, ArH), 4.56 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.45 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.42 (d, 1 H, J_{gem}= 12.0 Hz, OCH_2Ph), 4.41 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.38 (d, 1 H, J_{gem}= 12.0 Hz, OCH_2Ph), 4.33 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.11 (d, 1 H, $J_{2,3}$ = 6.4 Hz, H2), 3.88 (t, 1 H, $J_{2,3;3,4}$ = 6.4 Hz, H3), 3.80 (dt, 1 H, $J_{5,6'}$ = 2.0 Hz, $J_{4,5;5,6}$ = 9.2 Hz, H5), 3.66 (dd, 1 H, $J_{4,8}$ = 3.6 Hz, J_{gem} = 9.2 Hz, H8), 3.55 (dd, 1 H, $J_{4,8'}$ = 5.6 Hz, J_{gem} = 9.2 Hz, H8′), 2.53 (m, 2 H, H7, H7′), 2.18 (m, 1 H, H6), 2.00 (m, 2 H, H6′, H4). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 209.3 (C=O), 138.3, 137.9, 137.0 (ArC), 128.7-127.8 (ArC), 87.9 (C2), 76.4 (C3), 73.2, 72.9, 72.9 (OCH_2Ph), 68.9 (C8), 61.2 (C5), 48.1 (C4), 37.6 (C7), 26.8 (C6). HRMS (ES+): calcd. for $C_{29}H_{31}N_3O_4Na$: 508.2212, found 508.2212.

(1*R*,2*S*,3*R*,4*R*,5*R*)-2,3-Di-*O*-benzyl-4-*C*-benzyloxymethyl-8-azabicyclo[3.2.1]octane-1-ol (22). PPh_3 (0.09 g, 0.32 mmol, 1.6 eq) was added to a stirred solution of ketone **21** (0.10 g, 0.21 mmol) in MeOH (5 mL) and H_2O (0.25 mL). The reaction mixture was stirred at 40 °C for 150 min. during which N_2 evolution was observed. The reaction mixture was concentrated and the crude product purified by flash column chromatography (EtOAc) to give the desired product **22** (0.064 g, 68%) as a colourless oil. $[\alpha]_D^{20}$ 26 (*c* 1.0, $CHCl_3$) R_f (EtOAc) = 0.16. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.40-7.21 (m, 15 H, ArH), 5.10 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.86 (d, 1 H, J_{gem}= 10.8 Hz, OCH_2Ph), 4.78 (d, 1 H, J_{gem} = 11.2 Hz, OCH_2Ph), 4.51 (d, 1 H, J_{gem}= 10.8 Hz, OCH_2Ph), 4.49 (d, 1 H, J_{gem}= 12.0 Hz, OCH_2Ph), 4.41 (d, 1 H, J_{gem}= 12.0 Hz, OCH_2Ph), 3.62 (dd, 1 H, $J_{4,8}$ = 3.2 Hz, J_{gem}= 9.6 Hz, H8), 3.56 (m, 2 H, H5, H2/H3), 3.34 (m, 2 H, H8′, H2/H3), 2.22 (m, 1 H, H7), 1.98 (m, 2 H, H4, H6), 1.72 (m, 1 H, H6′), 1.53 (m, 1 H, H7′). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 139.3, 138.7, 138.3 (ArC), 128.7-127.5 (m, ArC), 92.9 (C1) 88.4, 79.0 (C2, C3) 75.1, 74.6, 73.2 (OCH_2Ph), 68.6 (C8), 53.5 (C5), 49.7 (C4), 31.0 (C7), 23.5 (C6). HRMS (ES+): calcd. for $C_{29}H_{33}NO_4Na$: 482.2307, found 482.2306.

(1*R*,2*S*,3*R*,4*R*,5*R*)-4-(hydroxymethyl)-8-azabicyclo[3.2.1]octane-1,2,3-triol (5). Protected noeurostegine (**22**) (0.060 g, 0.12 mmol) was dissolved in 96% EtOH (3 mL) and flushed with N_2. Pearlmann's catalyst (37 mg) was added and the reaction mixture was stirred overnight under an atmosphere of H_2 (balloon) before the reaction mixture was then transferred directly to a flash column (silica), where the product was eluted with EtOAc–isopropanol–H_2O 1 : 1 : 1 to give the desired product **5** (21 mg, 91%) as a clear oil. $[\alpha]_D^{20}$ 26 (*c* 0.5, H_2O). R_f (5% aq. ammonium hydroxide in EtOH (99.9%)) = 0.13. ^{1}H NMR (400 MHz, D_2O): δ (ppm) 3.87 (dd, 1 H, J = 4.0 Hz, J = 11.6 Hz, H3), 3.63 (m, 1 H, H5), 3.56 (m, 2 H, H2, H8), 3.32 (m, 1 H, H8′), 2.04 (m, 2 H, H6, H6′), 1.92 (m, 1 H, H4), 1.78 (m, 1 H, H7), 1.61 (m, 1 H, H7′). ^{13}C NMR (100 MHz, D_2O): δ (ppm) 92.0 (C1) 78.2, 70.8, 59.8, 53.2, 49.0, 28.0, 22.0. HRMS (ES+): calcd. for $C_8H_{15}NO_4Na$: 212.0899, found 212.0890.

(3*R*,4*R*,5*S*,6*R*)-3,4-Di-*O*-benzyl-5-*C*-benzyloxymethyl-6-*O*-*p*-methoxybenzyl-1,8-nonadiene (13*R*). **13*R*** was prepared according to the procedure described for **13*S*** and obtained (2.21 g, 97%) as a clear oil. $[\alpha]_D^{20}$ 4 (*c* 1.0, $CHCl_3$). R_f (pentane–EtOAc 16 : 1) = 0.38. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.37-7.26 (m, 15 H, ArH), 7.23-7.20 (m, 2 H, ArH), 6.88-6.84 (m, 2 H, ArH), 5.86 (m, 2 H, H2, H8), 5.33 (m, 2 H, H1/H9), 5.06 (m, 2 H, H1/H9), 4.94 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.63 (d, 1 H, J_{gem}= 12.0 Hz, OCH_2Ph), 4.59 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.50 (d, 1 H, J_{gem}= 12.0 Hz, OCH_2Ph), 4.46 (d, 1 H, J_{gem}= 12.0 Hz, OCH_2Ph), 4.40 (d, 1 H, J_{gem}= 12.0 Hz, OCH_2Ph), 4.40 (s, 2 H, OCH_2Ph), 4.18 (t, 1 H, $J_{2,3;3,4}$ = 7.2 Hz, H3), 3.84 (m, 2 H, H4, H10), 3.82 (s, 3H, OCH_3), 3.64 (m, 2 H, H6, H10′), 2.43 (m, 2 H, H5, H7), 2.27 (m, 1 H, H7′). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 159.2, 139.4, 138.9, 138.8 (ArC), 136.1, 136.0 (C2, C8), 131.0 (ArC), 129.5-127.3 (ArC), 119.1, 116.6 (C1, C9) 113.8 (ArC), 83.4 (C3), 79.6 (C4), 78.3 (C6), 73.9, 73.0, 71.0, 70.7 (OCH_2Ph), 67.8 (C10), 55.4 (OCH_3), 42.2 (C5), 35.7 (C7). HRMS (ES+): calcd. for $C_{39}H_{44}O_5Na$: 615.3086, found 615.3080.

(3*R*,4*R*,5*S*,6*R*)-3,4-Di-*O*-benzyl-5-*C*-benzyloxymethyl-6-*O*-*p*-methoxybenzyl-cycloheptene (14*R*). **14*R*** as prepared according to the procedure described for **14*S*** and obtained (1.90 g, 91%) as an oil. $[\alpha]_D^{20}$ −24 (*c* 1.0, $CHCl_3$). R_f (pentane–EtOAc 10 : 1) = 0.45. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.37-7.26 (m, 15 H, ArH), 7.23-7.20 (m, 2 H, ArH), 6.88-6.84 (m, 2 H, ArH), 5.85 (m, 1 H, H2), 5.76 (m, 1 H, H1), 4.93 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.70 (s, 2 H, OCH_2Ph), 4.56 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.52 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.44 (d, 1 H, J_{gem}= 12.0 Hz, OCH_2Ph), 4.41 (d, 1 H, J_{gem}= 12.0 Hz, OCH_2Ph), 4.37 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.36 (m, 1 H, H3), 3.80 (s, 3 H, OCH_3) 3.78-3.74 (m, 3 H, H4, H8, H8′), 3.70 (dt, 1 H, $J_{6,7}$ = 2.4 Hz, $J_{5,6;6,7'}$ = 8.0 Hz, H6), 2.44 (m, 2 H, H7, H7′), 2.18 (m, 1 H, H5). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 159.2, 139.1, 139.0, 138.8 (ArC), 133.1 (C2), 130.8 (ArC), 129.5-127.5 (m, ArC), 126.7 (C1), 113.9 (ArC), 81.1 (C3), 78.9 (C4), 75.5 (C6), 74.7, 73.0, 72.7, 70.7 (OCH_2Ph), 68.6 (C8), 55.4 (OCH_3), 50.9 (C5), 31.3 (C7). HRMS (ES+): calcd. for $C_{37}H_{40}O_5Na$: 587.2773, found 587.2775.

(1*R*/*S*,3*R*,4*R*,5*S*,6*R*)-3,4-Di-*O*-benzyl-5-*C*-benzyloxymethyl-6-*O*-*p*-methoxybenzyl-cycloheptane-1-ol (15*R*) and (1*R*,2*R*,3*R*,4*S*,5*R*)-2,3-di-*O*-benzyl-4-*C*-benzyloxymethyl-5-*O*-*p*-methoxybenzyl-cycloheptane-1-ol (16*R*). Hydroboration was conducted as described for the formation of **15*S*** and **16*S*** and gave compound **15*R*** and **16*R*** (2.10 g, 90%) as a 2 : 1 mixture of regioisomers which were easily separated.

15*R* Colourless oil (0.77 g, 33%). $[\alpha]_D^{20}$ −28 (*c* 1.0, $CHCl_3$). R_f (pentane–EtOAc 2 : 1) = 0.28. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.35-7.20 (m, 17 H, ArH), 6.84 (m, 2 H, ArH), 4.66-4.40 (m, 8 H, OCH_2Ph), 3.98 (m, 2 H), 3.83 (m, 1 H), 3.80 (s, 3 H, OCH_3), 3.74 (m, 1 H), 3.59 (m, 1 H), 3.50 (m, 1 H), 2.44 (bs, 1 H), 2.36 (m, 1 H), 2.10 (m, 5 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 159.3, 139.0, 138.7, 138.7, 130.4 (ArC), 129.7-127.5 (m, ArC), 113.9 (ArC), 79.2, 78.2, 76.1, 73.0, 72.6, 71.6, 70.9, 70.5, 67.0, 55.4, 48.1, 38.6, 36.9. HRMS (ES+): calcd. for $C_{37}H_{42}O_6Na$: 605.2879, found 605.2881.

16*R* Colourless oil (1.33 g, 57%). $[\alpha]_D^{20}$ −2 (*c* 1.0, $CHCl_3$). R_f (pentane–EtOAc 2 : 1) = 0.60. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.38-7.25 (m, 17 H, ArH), 6.89 (m, 2 H, ArH), 4.92 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.83 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.63 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.59 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.54 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.46 (d, 1 H, J_{gem}= 12.0 Hz, OCH_2Ph), 4.39 (d, 1 H, J_{gem}= 12.0 Hz, OCH_2Ph), 4.37 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 3.82 (s, 3 H, OCH_3), 3.72 (m, 5 H, H1, H2, H3, H5, H8), 3.54 (dd, 1 H, $J_{4,8'}$ = 4.8 Hz, J_{gem} = 8.8 Hz, H8′), 3.36 (bs, 1 H, OH), 2.23 (m, 1 H, H4), 2.07 (m, 1 H, H6), 1.87 (m, 2 H, H6′,H7), 1.66 (m, 1 H, H7′). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 159.2, 138.7, 138.6, 138.6, 130.9 (ArC), 129.4-127.6 (m, ArC), 113.9 (ArC), 86.0 78.9, 75.1, 74.4, 73.1 (OCH_2Ph), 72.9, 70.3 (OCH_2Ph), 70.0 (C8), 55.4 (OCH_3), 48.8 (C4), 26.7(C6), 24.6 (C7). HRMS (ES+): calcd. for $C_{37}H_{42}O_6Na$: 605.2879, found 605.2876.

(2*R*,3*R*,4*R*,5*R*)-5-*O*-Benzoyl-3,4-di-*O*-benzyl-2-*C*-benzyloxymethyl-cycloheptanone (23). Benzoyl protection of **16*R*** was conducted as described for the formation of **17*S***. DDQ deprotection of the crude product was carried out as described for the formation of **18*S***. The resulting alcohol was oxidised directly using Dess–Martin periodinane as described for the formation of **21**. This gave the desired ketone **23** (1.17 g, 90% (three steps)) as an oil. $[\alpha]_D^{20}$ 10 (*c* 1.0, $CHCl_3$). R_f (pentane–EtOAc 5 : 1) = 0.46. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.99 (m, 2 H, *o*-Bz), 7.56 (m, 1 H, ArH), 7.44-7.40 (m, 2 H, ArH), 7.37-7.24 (m, 13 H, ArH), 7.19-7.17 (m, 2 H, ArH), 5.48 (m 1 H, H5), 4.78 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.75 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.73 (d, 1 H, J_{gem}= 11.6 Hz, OCH_2Ph), 4.55 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.48 (s, 2 H, OCH_2Ph), 4.07-3.97 (m, 3 H, H3, H4, H8), 3.75 (dd, 1 H, $J_{2,8'}$ = 4.8 Hz, J_{gem} = 8.8 Hz, H8), 3.08 (m, 1 H, H2), 2.77 (m, 1 H, H7), 2.67 (m, 1 H, H7′), 2.34 (m, 2 H, H6, H6′). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 209.5 (C=O), 165.7 (C=O, ester) 138.2, 138.1, 137.8 (ArC), 133.2 (ArC) 129.9 (ArC), 129.8-127.7 (m, ArC), 82.4 (C3/C4) 77.7 (C3/C4), 74.2 (OCH_2Ph), 73.4 (OCH_2Ph), 72.9 (C5), 69.6 (C8) 55.8 (C2), 39.0 (C7), 24.3 (C6). HRMS (ES+): calcd. for $C_{36}H_{36}O_6Na$: 587.2410, found 587.2405.

(1*R*,2*R*,3*R*,4*S*,5*S*)-1-*O*-Benzoyl-2,3-di-*O*-benzyl-4-*C*-benzyloxymethyl-cycloheptane-5-ol (18*S*). $Li(O^tBu)_3AlH$ (2.90 g, 11.42 mmol, 5.0 eq) was added to a stirred solution of ketone **23** (1.29 g, 2.29 mmol) in dry Et_2O (40 mL) at −78 °C. After 1 h the solution was allowed to warm to rt and stirred overnight before diluted with Et_2O (100 mL). The organic phase was then washed with 1 M HCl until acidic (2 × 50 mL) and the aqueous phase extracted with EtOAc (2 × 50 mL). The combined organic phases

were washed with a saturated aq. solution of $NaHCO_3$ (2 × 50 mL), brine (50 mL), dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash column chromatography (pentane–EtOAc 10 : 1→8 : 1→5 : 1) to give the product **18** (1.10 g, 85%) as a 1 : 4 mixture of *R*- and *S*-diastereomers. ^{1}H NMR data for **18*S*** (colourless oil (0.86 g, 66%)) was in accordance with data given above.

18*R*: Colourless oil (0.23 g, 18%) ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.00 (m, 2 H, *o*-Bz), 7.57-7.53 (m, 1 H, ArH), 7.43-7.17 (m, 17 H, ArH), 5.35 (m, 1 H, H1), 4.73 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.72 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.65 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.50 (s, 2 H, OCH_2Ph), 4.39 (d, 1 H, J_{gem}= 11.2 Hz, OCH_2Ph), 4.04-3.91 (m, 3 H, H2, H5, H8), 3.65 (m, 3 H, H3, H8′, OH), 2.32 (m, 1 H, H4), 2.05 (m, 1 H, H7) 1.96-1.86 (m, 3 H, H6, H6′, H7′). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 166.0 (C=O), 138.2, 138.1, 137.9 (ArC), 133.0 (ArC), 130.5 (ArC), 129.8 (ArC), 128.6-127.7 (m, ArC), 84.3, 78.6, 76.0, 74.5, 74.2, 73.6, 71.4, 70.6, 48.5, 30.5, 23.8. HRMS (ES+): calcd. for $C_{36}H_{38}O_6Na$: 589.2566, found 589.2568.

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