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Synthesis and evaluation of galacto-noeurostegine and its 2-deoxy analogue as glycosidase inhibitors

Stéphane Salalone, a,§ Lise L. Clement, a Agnete H. Viuff, b Ole Juul Andersen, a,b Frank Jensen a and Henrik H. Jensen a

An epimer of the known glycosidase inhibitor noeurostegine, galacto-noeurostegine, was synthesised in 21 steps from levoglucosan and found to be a potent, competitive and highly selective galactosidase inhibitor of Aspergillus oryzae β-galactosidase. Galacto-noeurostegine was not found to be an inhibitor of green coffee bean α-galactosidase, yeast α-glucosidase and E. coli β-galactosidase, whereas potent but non-competitive inhibition against sweet almond β-glucosidase was established. The 2-deoxy-galacto-noeurostegine analogue was also prepared and found to be a less potent inhibitor of the same enzymes.

Introduction

The combined family of imino- and azasugars represent the most important class of carbohydrate mimics, owing to their remarkable biological activities. Initialy studied for their properties as glycosidase inhibitors, they have also been found to be potent inhibitors of glycolytransferases, metallotransferases and nucleoside-processing enzymes, making them valuable bioactive compounds against a wide range of diseases.

A few years ago, our group designed a new nor-tropane carbohydrate mimic named noeurostegine (3), as a hybrid between naturally occurring calystegine B2 (1) and the synthetic noeuromycin (2) (Figure 1). As a result of the ethylene bridge, both noeurostegine and calystegine B2 are stable hemiaminals contrary to noeuromycin, which over time will undergo Amadori rearrangement. The increased inhibitory potency of noeuromycin compared to calystegine B2 against a series of glycosidases, however, is partially a result of the presence of a hydroxymethyl substituent absent from calystegine B2. We were able to demonstrate that for enzymes that tolerate the ethylene bridge, increased inhibitory power, to the level of noeuromycin, was obtained with the hybrid compound noeurostegine compared to calystegine B2. We have furthermore shown that noeurostegine is a potent inhibitor of glucocerebrosidase, making it a potentially valuable compound against Gaucher’s disease. In addition, a uronic acid analogue (4) of noeurostegine has been prepared, which has been found to exhibit very powerful and selective inhibition of E. coli β-glucuronidase, making it a potentially useful candidate in cancer therapy in combination with irinotecan. Given the successful results achieved so far with the noeurostegines, we decided to embark on the synthesis of the galacto-configured congener, galacto-noeurostegine (5).

Several calystegines are known, but none of the naturally occurring nortropane hemiaminals have a substitution pattern which has 1,3-diaxial interactions with the ethylene bridge. Boyer et al. as well as Skaanderup and Madsen have attempted to make synthetic calystegines with this 1,3-diaxial interaction and found these to be unable to cyclise into the bicyclic form or exist in an equilibrium between nortropane and aminoacyloheptanone. Accordingly, it was unclear whether the bicyclic synthetic target 5 could be destabilised to such an extent by unfavourable diaxial interaction to cause it to only exist in its monocyclic cycloheptanone structure.

In addition to making galacto-noeurostegine (5) and testing it for inhibition of a panel of glycosidases, we furthermore planned to synthesise the 2-deoxy analogue (6) to explore the importance of this OH-group for binding. Previously, a hydroxyl group in this particular position has been found to contribute greatly to inhibitor-glycosidase binding.

Results and discussion

For the preparation of the target azasugars we chose a strategy previously successful to us. The starting point of our synthesis was the known alcohol 7 which is easily accessible from commercially available levoglucosan. To eventually reach the galacto-configuration, an early epimerisation at the 3-
position was envisaged. Hence the inverted alcohol 8 was the first target (Scheme 1). Triflation of alcohol 7 followed by nitrite-mediated substitution according to the Lattrell-Dax method was attempted, unfortunately leading to full recovery of the starting material. This lack of reactivity can be explained by the absence of a neighbouring ester group in an equatorial position, as described by Ramström et al. Furthermore, it was hypothesised that the steric hindrance of the bottom face resulted in a nitrite ion attack on the sulfur atom of the triflate rather than on C-3, giving rise to the initial alcohol with liberation of nitrosyl triflate. Consequently, it was decided to use the steric hindrance to our advantage and invert the stereochemistry in a two-step oxidation-hydride reduction process. Initially, Swern conditions were attempted to prepare ketone 9. However, the yields were not reproducible and the by-product 10, resulting from \( \beta \)-H elimination, was always isolated, albeit in lesser amount when \( \text{Et}_3\text{N} \) was replaced with DIPEA. Dess-Martin oxidation was also attempted but the \( \textit{in situ} \) liberation of acetic acid led to isomerisation of the double bond (11, not described).

Finally, the use of PCC prevented any undesired rearrangements and gave ketone 9, the hydride reduction of which furnished the inverted alcohol 8 (Scheme 2). Ozonolysis of the alkene followed by reductive work-up gave the diol 12 which was benzylated to give the protected anhydroallopyranoside 13 (31% yield from 7). Noteworthy, this synthetic sequence could be carried out on multigram scale without any purification of the different intermediates, only a single column chromatography was performed after the last step to give a clean compound 13. This anhydroosugar (13) then underwent acid-promoted methanolysis, giving alcohol 14 (85%) as a 3:1 \( \alpha/\beta \) mixture of anomers. Both anomers were isolated once for full characterisation, otherwise they were iodinated together under Garegg’s conditions, affording halides 15 in 94% yield (\( \alpha/\beta \) 3:1). Similarly, the anomeric mixture was used in the following zinc-mediated fragmentation, under sonication, to give a unique hexenal 16 which was subsequently allylated in a Barbier-type reaction, affording a separable 3:2 diastereoisomeric mixture of homoallylic alcohols 17R/17S. Our previous experience in the synthesis of \( \text{gluco} \)-configured noeurostegine (3) encouraged us to protect the homoallylic alcohol before performing the metathesis reaction, however, all of our attempts to form a PMB ether were unfruitful, once more probably because of steric hindrance. Gratifyingly, unlike their \( \text{gluco} \) counterparts, the ring closing metathesis could be performed without the need for protection of the homoallylic alcohol moieties and each diastereomer gave its corresponding cycloheptene in 78% yield. The undesired diastereomer (18R) was then efficiently converted to its epimer (18S) after PCC oxidation (19) and lithium tri-tert-butoxyaluminium hydride reduction (82% over two steps). The hydroboration-oxidation sequence of cycloheptene 18S gave a 3:1 mixture of regioisomers in favour of the desired one, as expected from previous work. Both regioisomers were found as single diastereomers and their configurations were determined by X-ray analysis, either directly for diol 21, which crystallised spontaneously after prolonged standing at -20 °C, or by formation of the bis-(3,5-dinitrobenzoate) derivative 22 for diol 20 (Figure 2).

Taking advantage of the lack of reactivity of the C-5 alcohol observed previously, we were able to regioselectively benzylate the C-1 alcohol in a good 85% yield, as confirmed by COSY correlations of H-1 and H-2 (23) (Scheme 3). Unfortunately, substitution of the free alcohol by an azide under Mitsunobu conditions resulted in only 35% yield of the expected azide 25, the elimination side reaction was predominant and cycloheptene 24 was obtained in 57% yield. Displacement of an activated alcohol under the form of a mesylate or a triflate was also largely in favour of the elimination process (not shown). Debenzylation (26) and subsequent Dess-Martin oxidation uneventfully lead to the key intermediate 27. In order to observe whether the 1,3-diaxial interactions are detrimental for cyclisation, it was decided to perform a sequential deprotection. To this end, the azide moieties of 27 was first reduced in the presence of pyridine so as to inhibit any benzyl ether hydrogenolysis. Pleasantly, a single compound was observed by TLC analysis and 28 was obtained in good yield (89%). Proton NMR in deuterated chloroform showed only broad signals (not shown) and while the spectrum was much better resolved in deuterated methanol, some signals were still unusually broad. These first observations were clearly in favour of a cyclised form and definitive evidence was obtained from carbon NMR. Indeed, while an experiment performed at 303 K showed no remaining trace of ketone (\( \delta_c \) 205.1 ppm), the hemiaminal signal (\( \delta_c \) 92.2 ppm) could eventually be observed with a cold experiment performed at 253 K (Figure 3). The final deprotection was performed using Degussa type Pd/C and \( \text{galacto} \)-noeurostegine (5) was isolated in quantitative yield.
Unexpectedly, NMR experiments showed that unlike its protected analogue 28 or its glucos epimer (3), galactonoeroestegine (5) existed as a mixture of monocyclic (5m) and bicyclic forms (5b) (ca. 2:3). Intrigued by this phenomenon we performed MP2/aug-cc-pVDZ/wb97xd/pcseg-1 26-29 quantum mechanical optimisations of both structures of benzylated and unprotected glucos- and galactonoeroestegines using the IEPFCM 30 solvent models of chloroform and water for the protected and unprotected analogues, respectively. Consistently with what was observed experimentally, the bicyclic structures of protected glucos- and galactonoeroestegines were more stable than the open structures by 27 and 23 kJ/mol, respectively. These energy differences were attributed to more favourable π-π interactions between the phenyl rings in the closed forms. As for the lower stability of galactoso compared to glucos-noeroestegine, it was attributed to 1,3-diaxial interactions. Regarding the deprotected compounds, once again, the bicyclic structures were more stable than the monocyclic ones; however the energy difference was lower for galactonoeroestegine (6 kJ/mol) than for

Figure 3. 13C NMR spectra recorded in CD3OD of compound 28 at 303K (blue) and 253K (red).

Scheme 2. Synthesis of diol 20 and bis-(3,5-dinitrobenzoate) 22 from anhydrosugar 7.
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noeurostegine (3) (12 kJ/mol), also probably due to the diaxial interactions, which could account for the existence of an equilibrium in the case of galacto-noeurostegine (5).

Finally, we turned our attention to the synthesis of the 2-deoxy analogue of 5 (6) (Scheme 3). Ketone 27 was stereospecifically reduced to provide the 1,4-trans azido alcohol 29 (97%), which was in turn submitted to mesylation conditions to afford compound 30 (96%). Once more, the final deprotection was performed sequentially and gave first the protected derivative 31 in 70% yield, then the deprotected target 6 (75%), which unlike 5 cannot undergo ring opening to a monocyclic form.

Inhibition studies

Compounds 5 and 6 were evaluated as inhibitors against commercially available glycoside hydrolases (Table 1). The ability of compound 5 to bind in both its monocyclic (5m) and bicyclic (5b) forms was expected to make precise analysis of the binding difficult. To the best of our knowledge only very few amino-cycloheptanes have been evaluated as glycosidase inhibitors and only weak or no inhibition has been found, but several azepanes have been shown to inhibit these enzymes, which demonstrates that it is possible to accommodate the enlarged ring structure of 5m.

Galacto-noeurostegine (5) was found to be an extremely potent inhibitor (K_i 18 nM) of sweet almond β-glucosidase but contrary to the gluco-configured congener (3), inhibition was established to be non-competitive. This particular enzyme is indeed known to be potently inhibited by azasugars otherwise designed to target galactosidases (iso-galacto-fagomine K_i 0.097 μM; aza-galacto-fagomine K_i 0.13 μM) but inhibition for these was competitive as opposed to that found for 5. In this case, as is often found for sweet almond β-glucosidase, inhibition was found to have a slow onset and required pre-incubating conditions (30 min) for tight binding. Measuring without pre-incubating conditions did not show micromolar inhibition.

2-Deoxy galacto-noeurostegine (6) was, contrary to 5, found to be a competitive inhibitor, but 100-fold weaker against almond β-glucosidase compared to iso-galacto-fagomine with the only structural difference being the ethylene bridge. This structural motif is otherwise tolerated for noeurostegine (3, K_i 50 nM), which is equipotent to noeumycin (K_i 69 nM).
Yeast α-glucosidase was also tested for inhibition but neither 5 nor 6 were found to bind to this enzyme, in accordance with data for other azasugars designed to target galactosidases (Table 1).

Galacto-noeurostegine (5) was found to be a highly potent inhibitor of β-galactosidase from Aspergillus oryzae, with a $K_i$ value of 31 nM, being of equal inhibitory potency as galactonoeuromycin. Also, the 2-deoxy analogue (6) inhibited this enzyme in the sub-micromolar range albeit 33-fold less than iso-galacto-fagomine. Interestingly, amine iso-galacto-fagomine is 9-fold more potent against this enzyme compared to hemiaminal galactonoeuromycin, while hemiaminal 5 was 4-fold more potent against this enzyme than amine 6. Both galacto-noeurostegine (5) and its 2-deoxy analogue (6) were also tested as inhibitors of E. coli β-galactosidase and coffee bean α-galactosidase, but found to be inactive. In the case of the latter galactosidase this is particularly interesting since both calystegine B$_2$ (1) and noeurostegine (3) are reasonable competitive inhibitors of this enzyme ($K_i$ 0.86 µM and 2.5 µM, respectively). This means that a binding mode exists where the ethylene bridge for both 1 and 3 can be accommodated. If this binding mode places the N-atom of these compounds in the place of the anomeric carbon of the enzyme substrate, then 5 and 6 would also be expected to be inhibitors of this α-galactosidase, which is not the case. This could suggest that 1 and 3 then bind in a deoxynojirimycin binding mode with the N-atom in place of the endocyclic O-atom of the substrate, with the ethylene bridge on the other face of the ring. One would then expect 1-deoxy-nojirimycin to be an inhibitor of this enzyme, which indeed has been found to be the case ($K_i$ 23 µM). In many cases, α-glycosidases are more potently inhibited by iminosugars bearing an N-atom in place of the endocyclic O-atom of the substrate, while calystegine B$_2$ (1) binds with its N-atom in place of the anomeric carbon in Thermotoga maritima β-glycosidase.

Table 1: $K_i$ values in µM of galacto-noeurostegine (5) and 2-deoxy-galacto-noeurostegine (6) as well as similar azasugars.

<table>
<thead>
<tr>
<th>$K_i$ (µM)</th>
<th>galacto-noeurostegine (5)</th>
<th>2-deoxy-galacto-noeurostegine (6)</th>
<th>noeurostegine (3)</th>
<th>galacto-noeurostegine</th>
<th>iso-galacto-fagomine</th>
<th>calystegine B$_2$ (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Glucosidase (almonds)</td>
<td>0.018$^{ab}$</td>
<td>120</td>
<td>0.05$^{a,6}$</td>
<td>n.d.</td>
<td>0.097$^{38}$</td>
<td>1.2$^{4,11}$</td>
</tr>
<tr>
<td>α-Glucosidase (yeast)</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000$^6$</td>
<td>n.d.</td>
<td>&gt;2000$^{39}$</td>
<td>&gt;1000$^{41}$</td>
</tr>
<tr>
<td>β-Galactosidase (Asp. oryzae)</td>
<td>0.031</td>
<td>0.13</td>
<td>23$^6$</td>
<td>0.035$^{34}$</td>
<td>0.004$^{40}$</td>
<td>n.d.</td>
</tr>
<tr>
<td>β-Galactosidase (E. coli)</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000$^6$</td>
<td>0.397$^{34}$</td>
<td>0.200$^{4,33}$</td>
<td>n.d.</td>
</tr>
<tr>
<td>α-Galactosidase (green coffee beans)</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>2.5$^6$</td>
<td>0.742$^{34}$</td>
<td>50$^{33}$</td>
<td>0.86$^{4,41}$</td>
</tr>
</tbody>
</table>

$^a$Preincubated 30 min; $^b$Non-competitive; $^c$IC$_{50}$ value; $^d$measured on racemic compound; n.d.: not determined

Conclusions

Inspired by the structure of calystegine B$_2$ (1) and noeurostegine (3), we have prepared galacto-noeurostegine (5) in 21 steps from levoglucosan. This compound is a stable nor-tropane hemiaminal, which has unfavourable 1,3-diaxial interactions between the ethylene bridge and a ring substituent. As a consequence of this steric repulsion an equilibrium mixture of monocyclic (5m) and bicyclic (5b) structures were obtained.

We have also presented the synthesis of the 2-deoxy version of galacto-noeurostegine (6) to investigate the influence of the 2-OH group on glycosidase inhibition.

Both compounds, 5 and 6, were evaluated as glycosidase inhibitors and found to only inhibit Aspergillus oryzae of the three galactosidases tested. Inhibition of this enzyme, however, was in the low nanomolar region for both compounds. Potent inhibition of sweet almond β-glucosidase was also established and found both to be slow and non-competitive for galactonoeuromystegine (5). Generally, hemiaminal 5 was found to be more potent than amine 6, emphasising the importance of a hydroxyl group in the 2-position.

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Experimental section

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Abbreviations

Aq.: aqueous; DCM: dichloromethane; DIAD: disopropyl azodicarboxylate; DPEA: N,N-diisopropylethylamine; DMAP: 4-(dimethylamino)pyridine; DMF: dimethylformamide; DMP: Dess-Martin periodinane; DPPA: diphenyl phosphoryl azide; Hept: heptanes; HRMS: high resolution mass spectrometry; ImH: imidazole; PCC: pyridinium chlorochromate; Pent: pentane; PMB: para-methoxybenzyl; r.t.: room temperature; THF: tetrahydrofuran; )): sonication.

General information

Commercially available chemicals were purchased from Sigma-Aldrich and used without further purification. All reactions with air- and moisture sensitive compounds were conducted in oven (ca. 120 °C) or flame-dried glassware under an argon atmosphere. DCM, THF and toluene were dried over aluminium oxide via a Braun solvent purification system. Methanol and pyridine were dried over molecular sieves (4 Å). EtO was dried over a sodium wire. DME was purchased from Sigma-Aldrich.

TLC analysis was carried out on silica-coated aluminium foil (Merek Kieselgel 60 F254). Solvents: heptanes; HRMS: high resolution mass spectrometry; ImH: imidazole; PCC: pyridinium chlorochromate; Pent: pentane; PMB: para-methoxybenzyl; r.t.: room temperature; THF: tetrahydrofuran; )): sonication.

Inhibition studies

Commercially available chemicals were purchased from Sigma-Aldrich and used without further purification. All reactions with air- and moisture sensitive compounds were conducted in oven (ca. 120 °C) or flame-dried glassware under an argon atmosphere. DCM, THF and toluene were dried over aluminium oxide via a Braun solvent purification system. Methanol and pyridine were dried over molecular sieves (4 Å). EtO was dried over a sodium wire. DME was purchased from Sigma-Aldrich.

Inhibition constants (Kᵢ) were determined by measuring initial rates (<10% substrate conversion) using nitrophenyl glycosides (p-nitrophenyl β-D-galactopyranoside for β-galactosidase from Asp orzaye, o-nitrophenyl β-D-galactopyranoside for β-galactosidase from E. coli, p-nitrophenyl β-D-glucopyranoside for almon β-glucosidase, p-nitrophenyl α-D-glucopyranoside for yeast α-glucosidase and p-nitrophenyl α-D-galactopyranoside for green coffee beans α-galactosidase) at six concentrations ranging from 4𝐾ᵢ to 4 𝐾ᵢ monitoring at 400 nm with 𝐴<1 using a Varian Cary 100 Bio UV-vis spectrophotometer. Measurements were conducted in 50 mM sodium phosphate buffer (pH 6.9) at 25 °C for 2 min. For experiments with pre-incubation, enzyme and inhibitor were mixed 30 min prior addition of substrate. 𝐾ᵢ and 𝐾ᵢ values were obtained by fitting to the equation 𝑣= (𝑉ₘₐₓ[S])/(𝐾ₘₐₙ+[S]) using the program GraphPad Prism 6. Competitive inhibition was established from Hanes plots. 𝑘ᵢ values were calculated as 𝑘ᵢ= [I]/([Kᵢ/Kᵢ-1]) having [I]=𝐾ᵢ.

Synthetic procedures

1.6-Anhydro-4-0-benzyl-2-deoxy-2-(E/Z)-prop-1′-eny1-β-D-ribo-hexo-pyranos-3-ulos (9). To a solution of the known compound 7' (14.49 g, 52.0 mmol) in dry DCM (200 mL) and under an argon atmosphere, was added silica gel (63–200 μm, 25 g) then PCC (22.42 g, 104 mmol, 2.0 eq.) at r.t. After 3 hours of stirring, additional PCC (5.61 g, 26.0 mmol, 0.5 eq.) was added. The reaction was left to stir for 30 min before it was filtered on Celite then transferred to cold EtO (150 mL). The organic phase was washed with water (1 × 100 mL), dried over MgSO₄, filtered and concentrated to give the crude ketone 8 as a yellow oil, which was used directly for the next step without further purification. Rᵢ (Pent/EtOAc 5:1) 0.47. 1H-NMR (CDCl₃, 400 MHz): δH 7.38-7.28 (m, 5H, ArH), 5.81-5.70 (m, 1H, HCH=CH), 5.67-5.58 (m, 1H, HIC=CH), 5.53 (br s, 1H, H1 major), 5.49 (br s, 1H, H1 minor), 4.79 (d, 1H, J 4.6 Hz, H5) 4.60 (d, 1H, Jgem 11.8 Hz, OCH3Ph,HPh major), 4.57 (d, 1H, Jgem 10.9 Hz, OCH3Ph,HPh minor), 4.40 (d, 1H, Jgem 11.8, OCH3Ph,HPh major), 4.35 (d, 1H, Jgem 10.9 Hz, OCH3Ph,HPh minor), 3.86–3.79 (m, 1H, H6a), 3.73 (dd, 1H, J6a,6b 8.1 Hz, J6b,6a 1.1 Hz, H6b minor), 3.70 (dd, 1H, J6a,6b 8.1 Hz, J6b,6a 1.1 Hz, H6b major), 3.60 (d, 1H, J5,5,6a=CH 9.4 Hz, H2 minor), 3.52 (s, 1H, H4), 3.19 (d, 1H, J5,5,6a=CH 8.6 Hz, H2 major), 1.75 (dd, 3H, J9a,9b 8.5 Hz, J9a,9b 1.7 Hz, CH31 minor) 1.70 (dd, 3H, J9a,9b 6.3 Hz, J9a,9b 1.4 Hz, CH31 major).

13C-NMR (CDCl₃, 100 MHz): δC 202.6 (C=O major), 202.3 (C=O minor), 136.8 (Ar), 131.3 (C=C major), 129.8 (C=C minor), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.2 (C=C major), 122.4 (C=C minor), 104.1 (C1 major), 103.8 (C1 minor), 81.2 (C4 minor), 81.0 (C4 major), 76.3 (C5 minor), 76.2 (C5 major), 71.4 (OCH3Ph,HPh minor), 71.3 (OCH3Ph,HPh minor), 65.4 (C6 minor), 65.3 (C6 major), 62.0 (C2 major), 57.0 (C2 minor), 18.1 (CH31 major), 13.6 (CH31 minor). HRMS (ESI): Calcd. for C39H31O8Na: 597.2103; found 597.2101.

1.6-Anhydro-4-0-benzyl-2-deoxy-2-(E/Z)-prop-1′-eny1-β-D-allopyranoside (8). To a solution of crude ketone 9 in dry MeOH (500 mL), cooled to -15 °C and under an argon atmosphere, NaBH4 (3.93 g, 104 mmol, 2.0 eq.) was added in a portionwise manner. The reaction mixture was stirred for 10 min before it was diluted with DCM (500 mL) and quenched with hydrochloric acid (1 M, 150 mL). The organic phase was washed with water (100 mL) and brine (100 mL), dried over MgSO₄, filtered and concentrated to give the crude alcohol 8 (8.88 g) as a colourless oil which was pure enough to be used in the next step without purification. Rᵢ (DCM/EtOAc 5:1) 0.53. 1H-NMR (CDCl₃, 400 MHz): δH 7.41-7.27 (m, 5H, ArH), 5.86-5.76 and 5.70-5.58 (m, 2H, HIC=CH), 5.37 (d, 1H, J2,3 2.3 Hz, H1 major), 5.34 (d, 1H, J1,2 2.2 Hz, H1 minor), 4.81 (d, 1H, Jgem 12.1 Hz, OCH3Ph,HPh major), 4.79 (d, 1H, Jgem 11.9 Hz, OCH3Ph,HPh minor), 4.67-4.60 (m, 2H, OCH3Ph and H5), 4.04 (bs, 1H, H3 minor), 3.96 (bs, 1H, H3 major), 3.70-3.75 (m, 2H, H6a and H6b), 3.60-3.57 (m, 1H, H4), 3.20-3.13 (m, 1H, H2 minor), 2.75-2.69 (m, 1H, H2 major), 2.41 (bs, 1H, OH minor), 2.33 (bs, 1H, OH major), 1.74-1.71 (m, 3H, CH32 major), 1.66 (dd, 3H, J9a,9b 6.8 Hz, J9b,9a 1.8 Hz, CH31 minor). 13C-NMR (CDCl₃, 100 MHz): δC 138.1 (Ar), 132.1 (C=C minor), 130.4 (C=C minor), 128.6 (Ar), 127.9 (Ar), 127.6 (Ar), 126.2 (C=C major), 124.9 (C=C, C minor), 103.9 (C1 major), 103.3 (C1 minor), 77.6 (C4 minor), 77.5 (C4 major), 73.4 (C5 major), 73.3 (C5 minor), 72.3 (OCH3Ph,HPh major), 72.2 (OCH3Ph major), 71.4 (OCH3Ph major).
65.5 (C6), 64.2 (C3 minor), 63.9 (C3 major), 52.0 (C2 minor), 45.6 (C2 major), 18.4 (CH3 major), 13.5 (CH3 minor). HRMS (ESI): Calcd. for C19H29O3Na: 299.1259; found 299.1254.

1.6-Anhydro-4-0-benzyl-1,2-dideoxy-2-(E/Z)-prop-1'-eny1-D-erythro-hex-1-enopyranos-3-uloside (10).  1H-NMR (CDCl3, 400 MHz): δH 7.43-7.29 (m, 6H, H-1, H-2), 6.24-6.15 (m, 1H, CH-CH2-o), 6.01-5.91 (m, 1C-CH2), 5.83-5.75 (m, 1H, CH-CH2-oH), 5.12 (d, 1H, Jgem 11.3 Hz, OCH3PhH-Ph major), 5.12 (d, 1H, Jgem 11.3 Hz, OCH3PhH-Ph major), 4.72 (d, 1H, Jgem 11.3 Hz, OCH3PhH-Ph minor), 4.69 (d, 1H, Jgem 11.3 Hz, OCH3PhH-Ph minor), 4.36 (dt, Jgem 12.0 Hz, Jsa 6.2 Hz, 1H, H-4 major), 4.30 (dt, Jgem 12.2 Hz, Jsa 5.5 Hz, 1H, H-4 minor), 4.23 (d, Jgem 12.2 Hz, H-4 minor), 4.19 (d, Jgem 12.2 Hz, 1H, H-4 major), 3.98-3.85 (m, 2H, H6a, H6b), 2.17 (br s, 1H, OH), 1.80 (dd, 3H, Jsa 6.6 Hz, Jgem 1.4 Hz, CH3-oH major), 1.74 (dd, 3H, Jsa 6.9 Hz, Jgem 1.6 Hz, CH3-oH minor), 1.53 (C-O minor), 193.1 (C-O major), 160.3 (C1 minor), 158.6 (C1 major), 137.4 (Ar), 128.6 (Ar), 128.5 (Ar), 128.1 (C8 minor), 126.8 (C8 major), 120.6 (C7 major), 119.0 (C7 minor), 115.5 (C2 major), 113.9 (C2 minor), 81.7 (C5 minor), 81.6 (C5 major), 74.6 (OCH3Ph), 73.6 (C4), 61.2 (C6), 18.9 (CH3 major), 14.8 (CH3 minor). HRMS (ESI): Calcd. for C19H21O4Na: 275.1283; found 275.1281.

1.6-Anhydro-4-0-benzyl-2-deoxy-2-hydroxymethyl-β-D-allopyranoside (12). Ozone was bubbled through a solution of crude alcohol 8 (8.88 g, 32.1 mmol) in EtOAc (96%, 140 mL) at 0 °C. After 2 hours of bubbling at 0 °C a stream of oxygen, followed by a stream of nitrogen was passed through the solution. NaBH4 (9.72 g, 257 mmol, 8.0 eq.) was then slowly added to the reaction mixture at 0 °C which was then stirred for 2 hours at r.t. before it was quenched by slow addition of Amberlite IR 120 H+ (620 mL) followed by stirring for 30 min. The reaction mixture was filtered and the Amberlite was washed with methanol. The resulting filtrate was concentrated to give the crude product diol 12 as a yellow oil (7.34 g), which was used directly in the next step without further purification. Rf (Pent/EtOAc 4:1) 0.22. 1H-NMR (CDCl3, 400 MHz): δH 7.42-7.50 (m, 5H, ArH), 5.56 (d, 1H, Jgem 11.8 Hz, H-3), 4.85 (d, 1H, Jgem 11.7 Hz, OCH3PhHPh), 4.71-4.68 (m, 1H, H5), 4.63 (d, 1H, Jgem 11.7 Hz, OCH3PhHPh), 4.21-4.16 (m, 1H, H3), 3.97 (dd, 1H, Jgem 11.2 Hz, Jsa 5.8 Hz, H7a), 3.86 (dd, 1H, Jgem 11.2 Hz, Jsa 5.7 Hz, H7b), 3.73-3.68 (m, 2H, H6), 3.65-3.62 (m, 1H, H4), 2.87 (bs, 1H, OH), 2.41-2.31 (m, 1H, H2), 1.62 (bs, OH). 13C-NMR (CDCl3, 100 MHz): δC 128.9 (2C, Ar), 128.4 (Ar), 127.9 (2C, Ar), 102.6 (C1), 76.7 (C4), 73.1 (C5), 72.3 (OCH3Ph), 65.1 (C6), 64.7 (C3), 60.3 (C7), 48.0 (C2). HRMS (ESI): Calcd. for C19H24O5Na: 289.1052; found 289.1051.

Methyl 3,4-di-O-benzyl-2-benzoxymethyl-2-deoxy-β-D-allopyranoside (14). A solution of compound 13 (4.91 g, 11.0 mmol) dissolved in 10% H2SO4 in dry MeOH (140 mL) under an argon atmosphere and stirred at 40 °C overnight. The solution was then slowly transferred to a saturated NaHCO3 solution (300 mL) and extracted with DCM (3 x 200 mL). The combined organic layers were dried (MgSO4) filtered and concentrated. The crude product was purified by flash column chromatography (Pent/EtOAc 80:20=60:40) to afford alcohol 14 (4.47 g, 9.34 mmol, 85%, α:β:3) as a colourless oil. HRMS (ESI): Calcd. for C23H23O4Na: 469.1991; found 469.1988.

Methyl 3,4-di-O-benzyl-2-benzoxymethyl-2-deoxy-α/β-D-allopyranoside (15). To a solution of alcohol 14 (11.93 g, 24.9 mmol) in dry toluene (400 mL) under an argon atmosphere, were successively added imidazole (4.24 g, 62.3 mmol, 2.5 eq.), iodine (9.48 g, 37.4 mmol, 1.5 eq.) and PPh3 (16.36 g, 62.4 mmol, 2.5 eq.). The reaction mixture was heated under reflux for 1 h 40 min then cooled down to r.t. before an aqueous solution of sodium thiosulfate (10%, 120 mL) was added. The reaction mixture was diluted with toluene (500 mL) before the two layers were separated and the organic layer washed with water (100 mL) and brine (100 mL) then dried (MgSO4)
and concentrated. The residue was purified by flash column chromatography (Pent/EtOAc 94:6→88:12) to give the iodinated compound 15 (13.8 g, 23.5 mmol, 94%, α/β 3:1) as a colourless oil. HRMS (ES⁻): Calcd. for C\textsubscript{28}H\textsubscript{33}O\textsubscript{14}Na\textsubscript{2}: 467.2198; found 467.2200.

(1R,5R,6S,7S)-5,6-Di-O-benzyl-7-C-benzoxymethyl-cycloheptene-3-endo-1-ol (18R). To a solution of diene 17 (6.12 g, 12.95 mmol) in dry toluene (220 mL), under an argon atmosphere, was added Hoveyda-Grubbs 2\textsuperscript{nd} generation catalyst (330 mg, 0.53 mmol, 0.04 eq) before the reaction mixture was heated to 80 °C. After 2 h 30 min of stirring the reaction mixture was cooled down to r.t. and then concentrated. The residue was purified by flash chromatography (Pent/EtOAc 85:15) to give the alkene 18R (4.48 g, 10.07 mmol, 78%) as a colourless oil. R\textsubscript{t} (Hpt/EtOAc 4:1) 0.18. [α]\textsubscript{D}\textsuperscript{22} = +54.5° (c 1.0, CHCl\textsubscript{3}). 1\textsuperscript{H}-NMR (CDCl\textsubscript{3}, 400 MHz): δ\textsubscript{H} 7.38-7.26 (m, 15 H, ArH), 5.91-5.82 (m, 2 H, H2, H8), 5.38-5.27 (m, 2 H, H1 or H9), 5.06-5.02 (m, 2 H, H1 or H9), 4.51 (d, 1 H, J=11.2 Hz, OCH\textsubscript{3}Ph), 4.63 (d, 1 H, J=11.2 Hz, OCH\textsubscript{3}Ph), 4.54 (d, 1 H, J=11.2 Hz, OCH\textsubscript{3}Ph), 4.43 (d, 1 H, J=12.0 Hz, OCH\textsubscript{3}Ph), 4.39 (d, 1 H, J=12.0 Hz, OCH\textsubscript{3}Ph), 4.35 (d, 1 H, J=11.9 Hz, OCH\textsubscript{3}Ph), 4.02-3.97 (m, 2 H, H4, H7), 3.92 (bt, 1 H, J=12.0 Hz, H5), 3.91 (bt, 1 H, J=5.4 Hz, H6), 3.52-3.45 (m, 2 H, H10a, H10b), 3.41 (br s, 1 H, OH), 2.39-2.33 (m, 1 H, H3a), 2.21-2.13 (m, 1 H, H3b), 2.12-2.05 (m, 1 H, H5). 13\textsuperscript{C}-NMR (CDCl\textsubscript{3}, 100 MHz): δ\textsubscript{C} 138.4 (Ar), 138.2 (Ar), 136.1 (C8), 135.7 (C5), 128.5-127.7 (m, 15 C, Ar), 120.0 (C9), 114.8 (C1), 81.6 (C6), 80.8 (C7), 74.9 (OCH\textsubscript{3}Ph), 73.2 (OCH\textsubscript{3}Ph), 70.2 (OCH\textsubscript{3}Ph), 70.0 (C4), 66.8 (C10), 42.9 (C5), 39.7 (CH3). HRMS (ES⁻): Calcd. for C\textsubscript{51}H\textsubscript{46}O\textsubscript{20}Na\textsubscript{2}: 495.2511; found 495.2513.

(4R,5S,5S,7R)-6,7-Di-O-benzyl-5-C-benzoxymethyl-nona-1,8-dien-4-ol (17). To a solution of crude aldehyde 16 (12.67 g) as a colourless oil, which was used without further purification in the next reaction. R\textsubscript{t} (Pent/EtOAc 9:1) 0.44. 1\textsuperscript{H}-NMR (CDCl\textsubscript{3}, 400 MHz): δ\textsubscript{H} 9.83 (d, 1 H, J=2.1 Hz, H1), 7.44-7.22 (m, 15 H, ArH), 5.93-5.80 (m, 4 H, OCH\textsubscript{3}Ph), 5.45-5.33 (m, 2 H, H6a, H6b), 4.69 (d, 1 H, J=11.4 Hz, OCH\textsubscript{3}Ph), 4.68 (d, 1 H, J=11.9 Hz, OCH\textsubscript{3}Ph), 4.55 (d, 1 H, J=11.4 Hz, OCH\textsubscript{3}Ph), 4.49-4.44 (m, 2 H, OCH\textsubscript{3}Ph), 4.41 (d, 1 H, J=11.9 Hz, OCH\textsubscript{3}Ph), 4.07-3.97 (m, 2 H, H3, H4), 3.78 (dd, 1 H, J=9.3 Hz, J=5.8 Hz, H7a), 3.70 (dd, 1 H, J=9.3 Hz, J=5.8 Hz, H7b), 3.02-2.93 (m, 1 H, H2). 13\textsuperscript{C}-NMR (CDCl\textsubscript{3}, 100 MHz): δ\textsubscript{C} 202.3 (C1), 138.0 (3C, Ar), 135.4 (C5), 128.5-127.7 (m, 15 Ar), 119.9 (C6), 80.3 (C3 or C4), 80.0 (C3 or C4), 73.4 (OCH\textsubscript{3}Ph), 73.4 (OCH\textsubscript{3}Ph), 70.6 (OCH\textsubscript{3}Ph), 66.3 (C7), 53.0 (C2). HRMS (ES⁻): Calcd. for C\textsubscript{32}H\textsubscript{36}O\textsubscript{14}Na\textsubscript{2}: 453.2042; found 453.2040.

(4R,5S,5S,7R)-6,7-Di-O-benzyl-5-C-benzoxymethyl-nona-1,8-dien-4-ol (17). To a solution of crude aldehyde 16 (12.67 g) in THF/H\textsubscript{2}O (1:1 v/v, 290 mL) was added allyl bromide (6.3 mL, 73.1 mmol, 2.5 eq.) and indium dust (4.65 g, 40.5 mmol, 1.4 eq.). The reaction mixture was stirred overnight at r.t. before a saturated NH\textsubscript{4}Cl solution (150 mL) was added. After 5 minutes of stirring a saturated NaHCO\textsubscript{3}aq. solution (150 mL) was added before the resulting mixture was extracted with DCM (4 x 200 mL). The combined organic layers were dried (MgSO\textsubscript{4}), filtered and concentrated and the residue purified by flash chromatography (Pent/EtOAc 95.5:→88:12) to afford the diene 17 (11.03 g, 23.3 mmol, 81% over two steps, R\textsubscript{f} 3.2) as a colourless oil. A second column chromatography (Pent/EtOAc 9:1) was necessary to fully separate both diastereomers.
An aliquot was purified by column chromatography (Pent/EtOAc 86:14) to give the alkyne 18S (1.70 g, 82 mmol, 78%) as a colourless oil. 

To a cooled solution of 17S (2.32 g, 9.11 mmol) in dry toluene (25 mL), under an argon atmosphere, was added silica gel (63–200 µm, 4 g) then PCC (2.46 g, 0.84 mmol, 23%) as a colourless oil, then diol 20 (0.39 g, 0.84 mmol, 23%) as a colourless oil. 

To a cooled solution of diol 20 (129.6 mg, 0.28 mmol) in dry DCM (3 mL), under an argon atmosphere, was added 4-toluenesulfonic acid (0.23 g, 1.62 mmol, 5.7 eq.) at r.t. After 4 hours of stirring the reaction mixture was cooled down to r.t. and then concentrated. The residue was purified by flash column chromatography (Pent/EtOAc 1:1, then EtOAc 100%) to give first the diol 21 (0.19 g, 0.41 mmol, 63%) as a colourless oil, then diol 21 (0.39 g, 0.84 mmol, 23%) as a colourless oil. 

Finally, to a cooled solution of ketone 19 (2.46 g, 5.57 mmol) in dry EtO (23 mL) at 78 °C under argon atmosphere. After 1 h of stirring the cold bath was removed and the reaction was stirred for another 2 h at r.t. The reaction mixture was diluted with EtO (230 mL), washed successively with HCl (1 M, 2 × 3 mL), a saturated NaHCO₃ aq. solution (30 mL) and brine (30 mL). The organic phase was dried (MgSO₄) and concentrated before the residue was purified by flash chromatography (Pent/EtOAc 85:15) to give alkyne 18S (2.04 g, 4.59 mmol, 82%) as a yellow oil.

An aliquot was purified by column chromatography to serve as an analytical sample (Pent/EtOAc 85:15). 

To a solution of diol 20 (275 mg, 0.59 mmol) in dry DCM (5 mL), under an argon atmosphere, were successively added Et₃N (0.41 mL, 2.94 mmol, 5.0 eq.) and DMAP (15 mg, 0.12 mmol, 2 eq.) and 3,5-dinitrobenzoyl chloride (544 mg, 2.36 mmol, 4.0 eq.). The reaction mixture was stirred at r.t. overnight before water (2 mL) was added and the DCM was removed under vacuum. The residue was taken up in EtOAc (30 mL) and washed with a saturated NaHCO₃ aq. solution (5 mL) and brine (5 mL). The organic layer was dried over MgSO₄, filtered and concentrated to give a crude ketone 19 (2.46 g) as a yellow oil. This was used directly for the next step without further purification. An aliquot was purified by column chromatography to serve as a sample for analytical sample (Pent/EtOAc 85:15). 

To a solution of diol 20 (275 mg, 0.59 mmol) in dry DCM (5 mL), under an argon atmosphere, were successively added Et₃N (0.41 mL, 2.94 mmol, 5.0 eq.) and DMAP (15 mg, 0.12 mmol, 2 eq.) and 3,5-dinitrobenzoyl chloride (544 mg, 2.36 mmol, 4.0 eq.). The reaction mixture was stirred at r.t. overnight before water (2 mL) was added and the DCM was removed under vacuum. The residue was taken up in EtOAc (30 mL) and washed with a saturated NaHCO₃ aq. solution (5 mL) and brine (5 mL). The organic layer was dried over MgSO₄, filtered and concentrated to give a crude ketone 19 (2.46 g) as a yellow oil. This was used directly for the next step without further purification. An aliquot was purified by column chromatography to serve as a sample for analytical sample (Pent/EtOAc 85:15). 

To a solution of diol 20 (275 mg, 0.59 mmol) in dry DCM (5 mL), under an argon atmosphere, were successively added Et₃N (0.41 mL, 2.94 mmol, 5.0 eq.) and DMAP (15 mg, 0.12 mmol, 2 eq.) and 3,5-dinitrobenzoyl chloride (544 mg, 2.36 mmol, 4.0 eq.). The reaction mixture was stirred at r.t. overnight before water (2 mL) was added and the DCM was removed under vacuum. The residue was taken up in EtOAc (30 mL) and washed with a saturated NaHCO₃ aq. solution (5 mL) and brine (5 mL). The organic layer was dried over MgSO₄, filtered and concentrated to give a crude ketone 19 (2.46 g) as a yellow oil. This was used directly for the next step without further purification. An aliquot was purified by column chromatography to serve as a sample for analytical sample (Pent/EtOAc 85:15). 

To a solution of diol 20 (275 mg, 0.59 mmol) in dry DCM (5 mL), under an argon atmosphere, were successively added Et₃N (0.41 mL, 2.94 mmol, 5.0 eq.) and DMAP (15 mg, 0.12 mmol, 2 eq.) and 3,5-dinitrobenzoyl chloride (544 mg, 2.36 mmol, 4.0 eq.). The reaction mixture was stirred at r.t. overnight before water (2 mL) was added and the DCM was removed under vacuum. The residue was taken up in EtOAc (30 mL) and washed with a saturated NaHCO₃ aq. solution (5 mL) and brine (5 mL). The organic layer was dried over MgSO₄, filtered and concentrated to give a crude ketone 19 (2.46 g) as a yellow oil. This was used directly for the next step without further purification. An aliquot was purified by column chromatography to serve as a sample for analytical sample (Pent/EtOAc 85:15).
To a solution of diol 20 (1.43 g, 3.00 mmol) in dry DCM, under an argon atmosphere, were added Et₂N (0.86 mL, 6.17 mmol, 2.0 eq.) and DMAP (78 mg, 0.64 mmol, 0.2 eq.). The reaction mixture was cooled to 0 °C before benzyl chloride was added (0.50 mL, 4.31 mmol, 1.4 eq.) and the ice-bath was removed. After 1 h of stirring at r.t. water was added (5 mL) and the mixture was partially evaporated to remove the DCM. EtOAc was added (150 mL) and the organic layer was washed with hydrochloric acid (1M, 2 × 20 mL), a saturated NaHCO₃ solution (2 × 20 mL) and brine (2 × 20 mL). The organic phase was dried (MgSO₄), filtered and concentrated before the residue was purified by flash column chromatography (Pent/EtOAc 80:20) to afford the ester 23 (1.52 g, 2.68 mmol, 86%) as a yellow oil. Rₜ (Hept/EtOAc 7:3) 0.32. [α]ทดลอง 75 +56.3 (c 1.0, CHCl₃).

(15S,2R,3S,4S,5S)-1-O-Benzoyl-2,3-di-O-benzyl-4-C-benzoyloxy-methyl-cycloheptan-5-ol (23). To a solution of diol 20 (1.43 g, 3.00 mmol) in dry DCM, under an argon atmosphere, were added Et₂N (0.86 mL, 6.17 mmol, 2.0 eq.) and DMAP (78 mg, 0.64 mmol, 0.2 eq.). The reaction mixture was cooled to 0 °C before benzyl chloride was added (0.50 mL, 4.31 mmol, 1.4 eq.) and the ice-bath was removed. After 1 h of stirring at r.t. water was added (5 mL) and the mixture was partially evaporated to remove the DCM. EtOAc was added (150 mL) and the organic layer was washed with hydrochloric acid (1M, 2 × 20 mL), a saturated NaHCO₃ solution (2 × 20 mL) and brine (2 × 20 mL). The organic phase was dried (MgSO₄), filtered and concentrated before the residue was purified by flash column chromatography (Pent/EtOAc 80:20) to afford the ester 23 (1.52 g, 2.68 mmol, 86%) as a yellow oil. Rₜ (Hept/EtOAc 7:3) 0.32. [α]ทดลอง 75 +56.3 (c 1.0, CHCl₃).
the reaction mixture was diluted with Et<sub>2</sub>O (100 mL) before a saturated aq. solution of NaHCO<sub>3</sub> (20 mL) followed by a saturated Na<sub>2</sub>SO<sub>4</sub> aq. solution (20 mL) were added and the reaction mixture was stirred for an additional 15 min. The phases were separated and the organic phase washed with brine (20 mL), the aq. phase back extracted with DCM (10 mL) and the combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated. The residue was purified by flash column chromatography (Pent/EtOAc 86:14) to give the ketone 27 (1.18 g, 2.43 mmol, 91%) as a colourless oil. R<sub>f</sub> (Hept/EtOAc 80:20) 0.35. [α]<sup>29</sup> +17.8 (c 1.0, CHCl<sub>3</sub>). 1H-NMR (CDCl<sub>3</sub>, 400 MHz): δ<sub>j</sub> 7.41-7.24 (m, 13H, Ar), 7.19-7.16 (m, 2H, ArH), 4.87 (d, 1H, J<sub>gem</sub> 11.8 Hz, OCH<sub>2</sub>Ph), 4.77 (d, 1H, J<sub>gem</sub> 11.1 Hz, OCH<sub>2</sub>Ph), 4.48-4.42 (m, 3H, OCH<sub>2</sub>Ph, H2), 3.78 (m, 3H, OCH<sub>2</sub>Ph), 3.71 (dd, 1H, J<sub>gem</sub> 9.0 Hz, J<sub>gem</sub> 4.7 Hz, H8a), 3.48 (pt, 1H, J<sub>gem</sub> 10.8 Hz, J<sub>gem</sub> 6.5 Hz, H5), 2.67-2.60 (m, 1H, H7a), 2.55-2.47 (m, 1H, H7b), 2.21-2.14 (m, 1H, H6a), 2.06-1.96 (m, 2H, H4, H6b). 13C-NMR (CDCl<sub>3</sub>, 100 MHz): δ<sub>r</sub> 205.1 (C1), 137.0 (2C, Ar), 136.9 (Ar), 127.6-126.8 (m, 15C, Ar), 87.6 (C2), 72.8 (OCH<sub>2</sub>Ph), 71.6 (OCH<sub>2</sub>Ph), 68.1 (C8), 59.9 (C5), 48.4 (C4), 37.1 (C7), 27.3 (C6). HRMS (ESI): Calcd. for C<sub>29</sub>H<sub>33</sub>N<sub>4</sub>O<sub>4</sub>Na: 508.2212; found 508.2210.

(1R,2S,3R,4S,5R)-2,3-Di-O-benzyl-4-C-benzyloxyethyl-8-azabicyclo[3.2.1]octane-1-ol (29). A solution of ketone 27 (413 mg, 0.85 mmol) at -20 °C in dry MeOH (8.5 mL) was added NaBH<sub>4</sub> (49 mg, 1.30 mmol, 1.5 eq.). The reaction mixture was stirred for 1 h before it was diluted with DCM (30 mL) and quenched with hydrochloric acid (1 M, 10 mL). The organic phase was washed with water (10 mL) and brine (10 mL) before the aq. phase was back extracted with DCM (10 mL). The combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated to give the crude alcohol 29 (401 mg, 0.82 mmol, 97%) as a colourless oil which was used in the next step without purification. R<sub>f</sub> (Hept/EtOAc 80:20) 0.29. [α]<sup>29</sup> +48.5 (c 1.0, CHCl<sub>3</sub>). 1H-NMR (CDCl<sub>3</sub>, 400 MHz): δ<sub>j</sub> 7.40-7.17 (m, 15H, ArH), 4.89 (d, 1H, J<sub>gem</sub> 11.4 Hz, OCH<sub>2</sub>Ph), 4.76 (d, 1H, J<sub>gem</sub> 11.9 Hz, OCH<sub>2</sub>Ph), 4.63 (d, 1H, J<sub>gem</sub> 11.9 Hz, OCH<sub>2</sub>Ph), 4.47 (br d, 2H, OCH<sub>2</sub>Ph, HPh), 3.74 (br s, 2H, OCH<sub>2</sub>Ph, HPh), 3.29 (dd, 1H, J<sub>gem</sub> 4.7 Hz, J<sub>gem</sub> 3.2 Hz, H2), 2.55-2.47 (m, 1H, H6a), 2.00-1.93 (m, 1H, H7a), 1.78-1.65 (m, 3H, H4, H6b, H7b). 13C-NMR (CDCl<sub>3</sub>, 100 MHz): δ<sub>r</sub> 138.5 (Ar), 138.2 (2C, Ar), 129.2-127.8 (m, 15C, Ar), 83.5 (C2), 80.1 (C3), 75.7 (OCH<sub>2</sub>Ph), 73.2 (OCH<sub>2</sub>Ph), 70.3 (C8), 68.9 (C1), 61.2 (C5), 45.6 (C4), 26.2 (C7), 24.2 (C6). HRMS (ESI): Calcd. for C<sub>29</sub>H<sub>33</sub>N<sub>4</sub>O<sub>4</sub>Na: 510.2369; found 510.2371.

(1R,2S,3R,4S,5R)-5-Azido-2,3-di-O-benzyl-4-C-benzyloxyethyl-cycloheptan-1-ol (30). A solution of alcohol 29 (511 mg, 1.05 mmol) at 0 °C in dry Et<sub>2</sub>O (6.3 mL) under an argon atmosphere, was added Et<sub>3</sub>N (0.29 mL, 2.08 mmol, 2.0 eq.) followed by methanesulfonyl chloride (0.12 mL, 1.55 mmol, 1.5 eq.). The reaction mixture was stirred at r.t. for 50 min before it was diluted with DCM (30 mL) and washed with a 5% NaHCO<sub>3</sub> aq. solution (10 mL) and brine (10 mL). The aq. phase was back extracted with DCM (5 mL) before the combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated. The residue was purified by flash column chromatography (Pent/EtOAc 80:20) to give the mesylate 30 as a colourless oil (572 mg, 1.01 mmol, 96%). R<sub>f</sub> (Hept/EtOAc 80:20) 0.25. 1H-NMR (CDCl<sub>3</sub>, 400 MHz): δ<sub>j</sub> 7.38-7.29 (m, 15H, ArH), 5.24-5.20 (m, 1H, H1), 4.96 (d, 1H, J<sub>gem</sub> 11.4 Hz, OCH<sub>2</sub>Ph), 4.70 (s, 2H, OCH<sub>2</sub>Ph), 4.50 (d, 1H, J<sub>gem</sub> 12.0 Hz, OCH<sub>2</sub>Ph), 4.46 (d, 1H, J<sub>gem</sub> 11.4 Hz, OCH<sub>2</sub>Ph), 4.38 (d, 1H, J<sub>gem</sub> 12.0 Hz, OCH<sub>2</sub>Ph), 3.43 (br s, 1H, H3), 3.63 (dd, 1H, J<sub>gem</sub> 9.1 Hz, J<sub>gem</sub> 4.1 Hz, H8a), 3.54-3.49 (m, 2H, H8b, H2), 3.47-3.42 (m, 1H, H5), 2.90 (s, 3H, SO<sub>2</sub>CH<sub>3</sub>), 2.65-2.57 (m, 1H, H6a), 2.23-2.16 (m, 1H, H7a), 1.99-1.92 (m, 1H, H7b), 1.80-1.70 (m, 2H, H6b, H4). 13C-NMR (CDCl<sub>3</sub>, 100 MHz): δ<sub>r</sub> 138.8 (Ar), 138.2 (Ar), 137.4 (Ar), 128.7-127.6 (m, 15C, Ar), 83.6 (C2), 79.9 (C1), 76.5 (C3), 73.9
(1S,2R,3S,4R,5R)-2,3-Di-O-benzyl-4-(hydroxymethyl)-8-azabicyclo[3.2.1]octane (6). A solution of mesylate 30 (355 mg, 0.73 mmol) and pyridine (30 µL, 0.37 mmol, 0.5 eq.) in MeOH (4 mL) was degassed and then flushed with nitrogen. Pearlman’s catalyst (20% Pd(OH)$_2$/C, 186 mg) was added and the reaction mixture was stirred overnight under a hydrogen atmosphere (balloon). The reaction mixture was filtered through Celite, concentrated and then co-evaporated with toluene. The residue was taken up in EtOAc (20 mL) and washed with a 5% NaHCO$_3$ aq. solution (2 x 5 mL) and brine (5 mL). The organic phase was dried over MgSO$_4$, filtered and concentrated before the residue was purified by flash column chromatography (DCM/MeOH 95:5-94:6) to afford protected 2-deoxy-galacto-nectoseitigene 31 (228 mg, 514 µmol, 70%) as a colourless oil. $R_f$ (DCM/MeOH 94:6) 0.28, [α]$_D^{25}$ $-5.9$ (c 1.0, CHCl$_3$). $^1$H-NMR (CD$_3$OD, 400 MHz): $\delta_{H}$ 7.38-7.20 (m, 15H, ArH), 4.87 (d, under DHO signal, 1H, OCH$_2$Ph), 4.57 (d, 1H, $J_{gem}$ 12.0 Hz, OCH$_2$Ph), 4.62 (d, 1H, $J_{gem}$ 12.0 Hz, OCH$_2$Ph), 4.44-4.34 (3H, 3H, OCH$_2$Ph, OCH$_2$Ph), 3.98 (t, 1H, $J$ 3.9 Hz, H3), 3.65-3.63 (m, 1H, H2), 3.60-3.58 (1H, H1), 3.52-3.44 (m, 2H, H8a, H8b), 3.40-3.38 (m, 1H, H5), 2.55-2.49 (m, 1H, H7a), 2.18-2.08 (m, 2H, H4, H6a), 1.66-1.53 (m, 2H, H6b, H7b). $^{13}$C-NMR (CD$_3$OD, 100 MHz): $\delta_{C}$ 140.8 (Ar), 139.7 (Ar), 124.1-128.2 (m, 15C, Ar), 80.6 (C2), 77.3 (C3), 76.8 (OCH$_2$Ph), 74.2 (OCH$_2$Ph), 72.2 (OCH$_2$Ph), 70.1 (C8), 57.3 (C1), 56.2 (C5), 46.1 (C4), 25.8 (C7), 25.1 (C6). HRMS (ES$^+$): Calcd. for C$_{32}$H$_{41}$NO$_4$H: 444.2548; found 444.2548.

Notes and references

18. The absolute configurations of the newly-formed stereogenic centres were determined at a later stage in the synthesis by X-ray analysis of 22.


42  C. S. Poulsen, R. Madsen, *J. Org. Chem.* 2002, **67**, 4441-4449. Zinc dust was activated and dried immediately before use: zinc dust (5 g) in 1 M aq. HCl (50 mL) was stirred at r.t. for 20 min, and then filtered and washed with H₂O and Et₂O. Finally, the zinc was dried under high vacuum with a heat gun.