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Synthesis of ethoxy-linked pseudo-disaccharides incorporating a crown ether macrocycle and lectin recognition☆

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Abstract

Six ethoxy-linked pseudo-disaccharides incorporating a non-reducing D-galactopyranosyl residue and a glucose bearing an 18-crown-6-ether were synthesized by the trichloracetimidate method. Deprotection left four novel structures from which two were tested against the galactose-specific lectin *Kluyveromyces bulgaricus*. Their minimum inhibitory concentration (MIC) was in the same order of magnitude as compared to previously prepared oligosaccharides without spacer. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Sugar crown ethers; Pseudo-disaccharides; Trichloracetimidates; Ozonolysis; Galactosylation; *Kluyveromyces bulgaricus*; Lectin recognition

1. Introduction

In Part 1 of this series [1], we reported the synthesis of di- and trisaccharides bearing an 18-crown-6-ether macrocycle using a trichloracetimidate as donor and secondary alcohol groups as acceptors. Assuming that an increase in the distance between the galactose residue and the macrocycle would be beneficial to the recognition of the galactose unit by a specific lectin, we now report the synthesis of pseudo-disaccharides, having a two-carbon tether between the two sugar residues, by a similar method. Two possibilities have been explored according to the position of the ethylene glycol spacer: (i) at C-1 of the glycose unit bearing the crown ether (Scheme 1(A)); (ii) at C-4 of this unit (Scheme 1(B)).

2. Results and discussion

The allyl group, easily introduced by alkylation and cleaved by ozone [2], was used throughout this study as the common precursor of the two-carbon spacer. The synthesis of allyl D-glucopyranoside crown ethers is summarised in Scheme 2.

Protected crown ethers **3** and **4** were obtained by usual methods [3] from allyl

☆ Lectin-targeted crown ethers, Part 2. For Part 1, see Ref. [1]. Part of the University Thesis of B. Dumont-Hornebeck (Nancy, 1997), presented in part at the 18th International Carbohydrate Symposium, Milan, 21–26 July, 1996.

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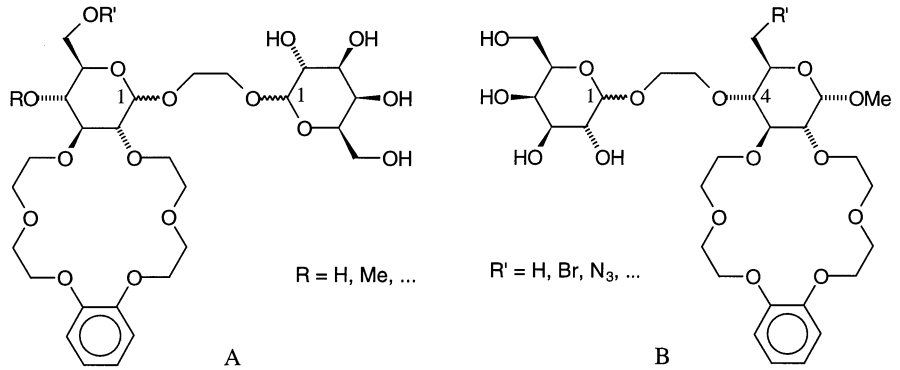
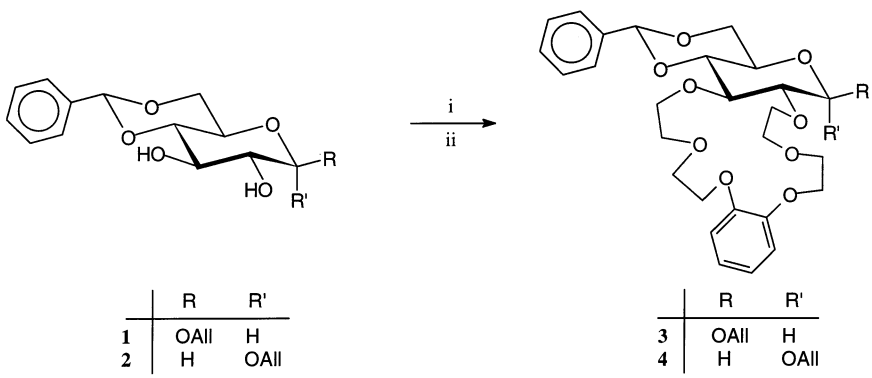


Scheme 1.

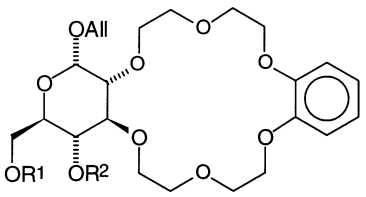
D-glycosides **2** and **1** in 89 and 64% yield, respectively in two steps.

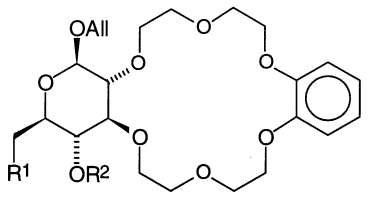
Two methyl groups were easily introduced in **5** under phase-transfer catalysis conditions (Me_2SO_4, 50% aqueous NaOH, room temperature) to afford **6** in 96% yield (Scheme 3). Curiously, mono-alkylation at C-4 via a classical protection/O-alkylation/deprotection sequence failed in our hands to give the target alcohol **9** in good yield; a major degradation product (not further investigated) was obtained at the deprotection step and (*p*-TsOH, MeOH) in addition to **9** as the minor product ($\sim$ 35%). Nevertheless, the same sequence applied in the β-series gave the monomethyl ether **12** from which the 6-azido-6-deoxy sugar **14** could be prepared by tosylation and subsequent nucleophilic displacement by sodium azide (38% overall yield from **3**). Finally, the allyl group underwent ozonolysis at low temperature and the intermediate ozonide was reduced in situ with sodium borohydride to give the acceptor **15** in 69% yield. Optimisation of the ozonolysis step was achieved within 1 h at -10 °C in the same solvents on allyl glycoside **6** to afford **17** almost quantitatively (Scheme 4).

The synthesis of **18** and **19** has been reported [1]. The preferred strategy to synthesize 6-amino-crown ethers was to introduce the amine-functionality after the glycosylation step of the 6-bromo or 6-azido precursors. The 6-azido-6-deoxy sugar **21** could be obtained in two steps from **18** after quantitative 4-O-alkylation in a two-phase system (allyl bromide, 50% aqueous NaOH) at room temperature (Scheme 5). In this case, the crown ether could play both the role of substrate and phase-transfer catalyst. Nucleophilic displacement of the bromide in **20** by sodium azide in *N*,*N*-dimethylformamide around 130 °C also afforded **21** in excellent yield, but a more polar by-product appeared if the reaction time exceeded 1 h. The ^{1}H NMR spectrum of this by-product showed the absence of allylic signals between 5.1 and 5.9 ppm and its EIMS$^+$ spectrum (70 eV) displayed a molecular peak at m/z 509 $[M]^{\bullet+}$ and another intense peak at m/z



Scheme 2. (i) $(ClCH_2CH_2)_2O$, 50% aqueous NaOH, Bu_4NHSO_4; (ii) catechol, K_2CO_3, *n*-BuOH.





	R1	R2
5	H	H
6	Me	Me
7	Tr	H
8	Tr	Me
9	H	Me

	R1	R2
10	OTr	H
11	OTr	Me
12	OH	Me
13	OTs	Me
14	N_3	Me

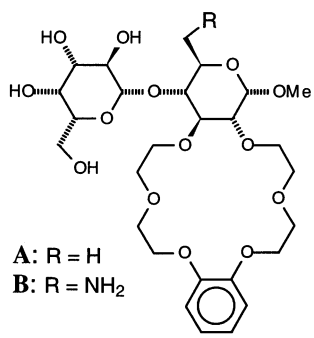
Scheme 3.

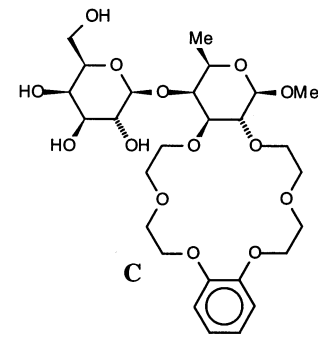
481 $[M-28]^{\bullet+}$. As a similar reaction has been reported by Lamberth and Bednarski on allyl glycosides bearing an azide at C-2 [4], we postulated that an intramolecular cycloaddition took place when the azido-sugar was heated over too long a period and that, at least, a nitrogen molecule was likely to be extruded to produce an aziridine. As for **21**, the allyl ether **23** was isolated by the phase-transfer reaction of **22** with allyl bromide. Monosubstituted glycol ethers **24**, **25** and **26** were isolated after an in situ ozonolysis/reduction sequence of the corresponding 4-*O*-allyl precursors **23**, **20** and **21**, respectively. Alternatively, the 6-deoxysugar **24** could be obtained by quantitative reduction of **25** with lithium aluminium hydride. The primary alcohol **15** was glycosylated with D-galactose trichloracetimidates to give the ethoxy-linked $(1\rightarrow1)$-disaccharide **27** in 55% yield (Scheme 6).

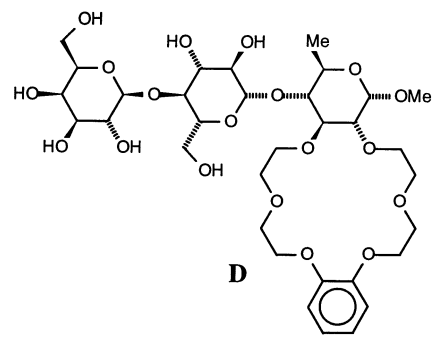
Boron trifluoride diethyl etherate was preferred to trimethylsilyl triflate (Me_3SiOTf) as catalyst since better yield and selectivity had previously been observed with this Lewis acid used in a two-fold excess with crown ethers as acceptors [1]. In the same manner, glycols **16**, **17**, **24**, **25** and **26** were reacted as donors to yield pseudo-disaccharides **28**, **29**, **30**, **35**, **36** and **37** (Scheme 7).

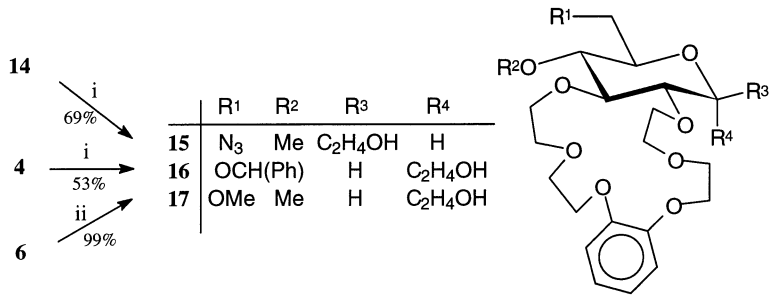
However, the cleavage of the benzylidene acetal in **28** to yield mainly **29** after only 2 h of reaction at room temperature was unexpected. Judging from the ^1H NMR coupling constants, only a β-glycosidic linkage could be ascertained in all these galactosides in opposition to a preceding result obtained with a primary alcohol as acceptor. Classical Zemplén deacetylation afforded the deprotected carriers **32**, **33**, **34** and **38** quantitatively from their respective precursors **28**, **31**, **30** and **35**. For unclear reasons, the reduction of the azide **37** failed to yield the corresponding amine (Scheme 7, R = Ac, $R^1 = NH_2$) even in a Paar reactor under 5 MPa hydrogen pressure in the presence of 10% palladium on charcoal.

The in vitro flocculation inhibition capabilities of oligosaccharides **A**, **B**, **C** and **D** prepared in Part 1 of this series [1] toward the yeast *Kluyveromyces bulgaricus* (Kb CWL1) lectin [5,6] are compared with those of the presently synthesized galactosides **34** and **38** in Table 1.









Scheme 4. (i) O_3, THF–MeOH, −75 °C, then $NaBH_4$, −75 °C → rt; (ii) O_3, THF–MeOH, −10 °C, then $NaBH_4$, −10 °C → rt.

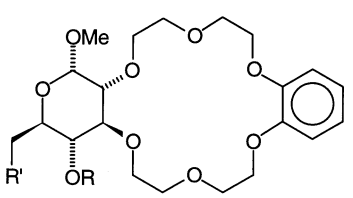
These biological results altogether gave conspicuous proof of the in vitro activity of the non-reducing D-galactose moiety after the glycosylation step with acceptors bearing a crown ether (compounds **A**, **B**, **C**, **D** [7,8]) or with a functionalised monoether of ethylene glycol (**34** and **38**). More precisely, the incorporation of an intermediate sugar as hydrophilic spacer in compound **D** does not really change the recognition by the lectin. The presence of a free amine group at C-6 of the acceptor slightly increased the minimum inhibitory concentration (MIC) of the disaccharide **B**. However, one must admit that the primary amine group is mainly protonated in the acetate buffer (pH ~ 4.5) used in all tests, and that the MIC of **B** may be different at a physiological pH (~ 7.2). The best results were measured with compound **C** incorporating two galactose units without spacer and with compound **34**. Yet these differences are small and cannot be considered as significant improvements.

In summary, four simple models (compounds **32**, **33**, **34** and **38**) incorporating a two-carbon spacer between two hexopyranosides, have been synthesised in good yields by a convergent approach using the trichloroacetimidate method from D-glucose and D-galactose. A unique β-glycosidic linkage was observed in the main isolated reaction products. Deprotected compounds **34** and **38** gave similar results to oligosaccharides **A**, **B**, **C** and **D** [1] when tested in vitro towards a galactose-specific lectin. Taking all our results into account, it is strongly suggested that the lectin isolated from *K. bulgaricus* recognises the non-reducing D-galactose residue of these six compounds in a very similar manner.

3. Experimental

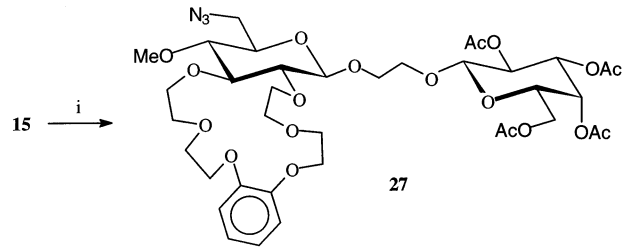
Synthesis of crown ethers and general methods and materials.—The syntheses of D-galactose trichloracetimidate and crown ethers **A**, **B**, **C** and **D** have been described in Part 1 of this series [1]. Due to shortage of products, elemental analysis has not been performed.

Allyl [2,3-b](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-4,6-O-benzylidene-2,3-dideoxy-β-D-glucopyranoside (**3**).—To a stirred dispersion of allyl 4,6-*O*-benzylidene-D-glucopyranosides [9] (31.5 g, 102 mmol) and n-Bu_4NHSO_4 (69.3 g, 2.0 equiv) in bis(2-chloroethyl)ether (300 mL) were added 50% aq NaOH (300 mL) below 10 °C. The two-phase system was vigorously stirred below 15 °C for 6 h, the reaction being monitored by TLC (EtOAc). The reaction mixture was then partitioned between chilled water (800 mL) and CH_2Cl_2 (500 mL). The aq phase was extracted with CH_2Cl_2 (3 × 200 mL), the organic phases were combined, washed with water (2 × 100 mL), dried over $MgSO_4$, concentrated under reduced pressure, and the excess of reagent finally removed in vacuo. Chromatography with 9:1 n-hexane–EtOAc successively yielded allyl 4,6-*O*-benzylidene-



	R	R'
18	H	Br
19	H	N_3
20	All	Br
21	All	N_3
22	H	H
23	All	H
24	C_2H_4OH	H
25	C_2H_4OH	Br
26	C_2H_4OH	N_3

Scheme 5.



Scheme 6. (i) D-Galactose trichloracetimidates (2 equiv), crushed activated 4 Å MS, CH_2Cl_2, rt, 30 min, then $Et_2O{\cdot}BF_3$ (2 equiv), rt, 20 h.

2,3-*O*-bis[2-(2-chloroethoxy)ethyl]-β-D-glucopyranoside (6.52 g, 12.5%) and allyl 4,6-*O*-benzylidene-2,3-*O*-bis[2-(2-chloroethoxy)ethyl]-α-D-glucopyranoside (19.0 g, 36.4%) as colourless gums pure enough for the next step. From condensation of allyl 4,6-*O*-benzylidene-2,3-*O*-bis[2-(2-chloroethoxy)ethyl]-β-D-glucopyranoside (4.00 g, 7.67 mmol) with pyrocatechol (1.69 g, 2 equiv) according to Ref. [10], after 24 h of reflux in *n*-BuOH, usual work-up and chromatography with 1:1 EtOAc–*n*-hexane, the crown ether **3** was obtained (3.856 g, 90%) as a white solid: mp 123–125 °C; $[\alpha]_D$ −42.0° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.42 (m, 2 H, H-2, -6, Ar), 7.4–7.3 (m, 3 H, H-3,-4, -5, Ar), 6.9 (bs, 4 H, catechol), 5.92 (dddd, 1 H, OCH_2–C*H*=CH_2), 5.52 (s, 1 H, Hb), 5.33 (dq, 1 H, J_{trans} 17.0 Hz, OCH_2–CH=CH*H*), 5.21 (dq, 1 H, J_{cis} 10.5 Hz, $J_{gem} \leq 1.5$ Hz, OCH_2–CH=C*H*H), 4.48 (d, 1 H, H-1), 4.42–3.62 (m, 20 H, 9 × O*CH*$_2$, H-3, -6′), 3.55 (bt, 1 H, J_{3-4} 8.0 Hz, J_{4-5} 9.0 Hz, H-4), 3.53 (dd, 1 H, J_{5-6} 9.0 Hz, $J_{6-6'}$ 14.0 Hz, H-6), 3.35 (m, 1 H, H-5), 3.27 (t, 1 H, J_{1-2} 7.8 Hz, J_{2-3} 8 Hz, H-2); EIMS: *m*/*z* 558 $[M]^{\bullet+}$.

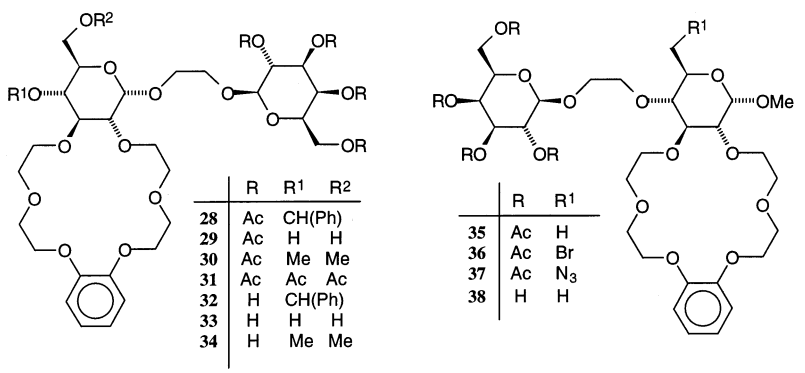
Table 1
Comparative minimum inhibitory concentrations (MICs) of D-glucose, D-galactose, methyl β-D-galactopyranoside, lactose, pseudo-disaccharides **34**, **38** and disaccharide derivatives **A**–**D** towards Kb CWL1 [a]

Carbohydrate	MIC (mM) [b]
D-Glucose	Negative
D-Galactose	3.50
Methyl β-D-galactopyranoside	1.75
Lactose	1.25
A [1]	2.65
B [1]	3.75
C [1]	2.10
D [1]	2.65
34	2.00
38	2.50

[a] The lectin activity was 3.4 AU/μg of protein (1 UA → inhibition capacity of a 0.5 mg/mL lectin solution towards a given yeast cell suspension).

[b] The MIC is the lowest concentration that totally inhibits flocculation (mean value of three measurements).

Allyl [2,3-b](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-4,6-O-benzylidene-2,3-dideoxy-α-D-glucopyranoside (**4**).—Cyclisation of allyl 4,6-*O*-benzylidene-2,3-*O*-bis[2-(2-chloroethoxy)ethyl]-α-D-glucopyranoside (6.0 g, 11.5 mmol) with pyrocatechol (2.53 g, 2 equiv) afforded, after 24 h of reflux in *n*-BuOH, usual work-up and chromatography with 1:1 EtOAc–*n*-hexane, the crown-ether **4** (4.495 g, 70%) as a white solid: mp 153–155 °C; $[\alpha]_D$ +38.6° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.47 (m, 2 H, Ar), 7.35 (m, 2 H, Ar), 7.23 (d, 1 H, Ar), 6.88 (bs, 4 H, catechol), 5.92 (dddd, 1 H, OCH_2–C*H*=CH_2), 5.52 (s, 1 H, Hb), 5.33 (dq, 1 H, J_{trans} 17.0 Hz, OCH_2–CH=CH*H*), 5.22 (dq, 1 H, J_{cis} 10.5 Hz, $J_{gem} \leq 1.5$ Hz, OCH_2–CH=C*H*H), 4.98 (d, 1 H, H-1), 4.28–3.75 (m, 21 H, 9 × O*CH*$_2$, H-3,



Scheme 7.

-5, -6′), 3.7 (dd, 1 H, J_{5-6} 3.5 Hz, $J_{6-6'}$ 10.0 Hz, H-6), 3.55 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9 Hz, H-4), 3.48 (dd, 1 H, J_{1-2} 3.7 Hz, J_{2-3} 9.5 Hz, H-2); EIMS: m/z 558 $[M]^{\bullet+}$.

Allyl [2,3-b](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-2,3-dideoxy-α-D-glucopyranoside (**5**).—Crown ether **4** (2.00 g, 3.58 mmol) was dissolved in AcOH (60 mL) at 90 °C and distilled water (40 mL) was then dropped onto the soln at the same temperature. The reaction was monitored by TLC (EtOAc) and was completed after 3 h. The solvents were evaporated under reduced pressure and the remaining gum dissolved in CH_2Cl_2 (100 mL), washed by aq satd $NaHCO_3$ (15 mL) and twice by water (10 mL). The isolated organic phase was then dried over $MgSO_4$ and concentrated under reduced pressure to yield **5** (1.63 g, > 99%) as a colourless homogeneous gum pure enough for the next step: $[\alpha]_D$ + 71.6° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$–D_2O): δ 6.88 (bs, 4 H, catechol), 5.9 (dddd, 1 H, OCH_2–C*H*=CH_2), 5.31 (dq, 1 H, J_{trans} 17.0 Hz, OCH_2–CH=CH*H*), 5.2 (dq, 1 H, J_{cis} 10.0 Hz, $J_{gem} \leq$ 1.5 Hz, OCH_2–CH=C*H*H), 4.94 (d, 1 H, H-1), 4.37–3.61 (m, 20 H, 9 × O*CH*$_2$, H-6′,-5), 3.57 (t, 1 H, J_{4-5} 8.5 Hz, H-4), 3.45 (t, 1 H, $J_{3-4} \sim$ 8.0 Hz, H-3), 3.44 (dd, 1 H, J_{5-6} 9.0 Hz, $J_{6-6'}$ 14.0 Hz, H-6), 3.4 (dd, 1 H, J_{1-2} 3.8 Hz, J_{2-3} 8.5 Hz, H-2); EIMS: m/z 470 $[M]^{\bullet+}$.

Allyl [2,3-b](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-2,3-dideoxy-4,6-di-O-methyl-α-D-glucopyranoside (**6**).—To a soln of **5** (0.174 g, 0.369 mmol) in toluene (10 mL) were added 10 mL of 50% aq NaOH and Me_2SO_4 (1 mL) under the fume board. The resulting mixture was magnetically stirred for 20 h at room temperature (rt) and then quenched with chilled water (40 mL). The isolated aq phase was extracted with CH_2Cl_2 (3 × 50 mL) and the combined organic phases washed with water (2 × 10 mL), dried over $MgSO_4$, and concentrated under reduced pressure to yield the crown ether **6** (0.176 g, 96%) as a white amorphous solid: mp 81–83 °C; $[\alpha]_D$ + 68.1° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.88 (bs, 4 H, catechol), 5.9 (dddd, 1 H, OCH_2–C*H*=CH_2), 5.3 (dq, 1 H, J_{trans} 17.0 Hz, OCH_2–CH=CH*H*), 5.18 (dq, 1 H, J_{cis} 10.0 Hz, $J_{gem} \leq$ 1.5 Hz, OCH_2–CH=C*H*H), 4.96 (d, 1 H, H-1), 4.23–3.7 (m, 20 H, 9 × O*CH*$_2$, H-6, -6′), 3.63 (m, 2 H, H-3, -5), 3.54 (s, 3 H, O*CH*$_3$ on C-4), 3.39 (s, 3 H, O*CH*$_3$ on C-6), 3.38 (d, 1 H, J_{1-2} 3.7 Hz, J_{2-3} 9.5 Hz, H-2), 3.25 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9.5 Hz, H-4); EIMS: m/z 498 $[M]^{\bullet+}$.

Allyl [2,3-b](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-2,3-dideoxy-6-O-trityl-α-D-glucopyranoside (**7**).—To a stirred solution of diol **5** (1.63 g, 3.47 mmol) in toluene (10 mL) and pyridine (1.0 mL) was added trityl chloride (1.45 g, 1.5 equiv) and the mixture heated to a gentle reflux. The reaction was monitored by TLC (EtOAc) and stopped after 10 h, allowed to cool, concentrated to a gum, which was dissolved in CH_2Cl_2 (50 mL) to give, after usual work-up and chromatography on deactivated silica gel (1% NEt_3) with 1:2 EtOAc–*n*-hexane, the protected alcohol **7** (1.53 g, 60%) as a white foam: mp 63–65 °C; $[\alpha]_D$ + 37.0° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.47 (d, 6 H, Ar), 7.34–7.18 (m, 9 H, Ar), 6.88 (bs, 4 H, catechol), 5.96 (dddd, 1 H, OCH_2–C*H*=CH_2), 5.33 (dq, 1 H, J_{trans} 17.0 Hz, OCH_2–CH=CH*H*), $J_{gem} \leq$ 1.5 Hz, OCH_2–CH=C*H*H), 5.22 (dq, 1 H, J_{cis} 10.0 Hz, 5.01 (d, 1 H, H-1), 4.29–3.7 (m, 19 H, 8.5 × O*CH*$_2$, H-4, -5), 3.6 (t, 1 H, $J_{3-4} \sim$ 9.0 Hz, H-3), 3.57 (d, 1 H, OCH*H*), 3.45 (dd, 1 H, J_{1-2} 3.5 Hz, J_{2-3} 9.0 Hz, H-2), 3.37 (dd, 1 H, $J_{5-6'}$ 3.5 Hz, H-6′), 3.3 (dd, 1 H, J_{5-6} 5.0 Hz, $J_{6-6'}$ 9.5 Hz, H-6), 2.8 (bs, 1 H, OH); EIMS: m/z 712 $[M]^{\bullet+}$.

Allyl [2,3-b](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-2,3-dideoxy-4-O-methyl-6-O-trityl-α-D-glucopyranoside (**8**).—A soln of Me_2SO_4 (1 mL, 8.5 equiv) in toluene (5 mL) was vigorously stirred for 5 min at rt with 50% aq NaOH (10 mL) under the fume board. Then **7** (0.89 g, 1.24 mmol) was added and the mixture stirred again for 20 h. Chilled water (100 mL) was added and the organic phase was extracted with CH_2Cl_2 (3 × 50 mL). The organic extracts were combined and washed with water (3 × 20 mL), dried ($MgSO_4$), concentrated in vacuo, and purified by elution with 1:1 EtOAc–*n*-hexane through basic alumina to yield **8** (0.85 g, 95%) as a pale yellow gum: $[\alpha]_D$ + 40.3° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.46 (d, 6 H, Ar), 7.33–7.2 (m, 9 H, Ar), 6.87 (bs, 4 H,

catechol), 5.9 (dddd, 1 H, OCH_2–C*H*=CH_2), 5.19 (dq, 1 H, J_{cis} 10.0 Hz, $J_{gem} \leq 1.5$ Hz, OCH_2–CH=C*H*H), 4.92 (d, 1 H, H-1), 4.24–4.02 (m, 7 H, $3.5 \times OCH_2$), 4.0–3.7 (m, 13 H, $5.5 \times OCH_2$, H-5, -6′), 3.66 (t, 1 H, H-3), 3.57 (s, 3 H, OCH_3), 3.38 (dd, 1 H, J_{5-6} 3.0 Hz, $J_{6-6'}$ 11.5 Hz, H-6), 3.36 (dd, 1 H, J_{1-2} 3.5 Hz, J_{2-3} 9.5 Hz, H-2), 3.22 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9.0 Hz, H-4); EIMS: *m*/*z* 726 $[M]^{\bullet+}$.

*Allyl [2,3-*b*](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-2,3-dideoxy-4-*O*-methyl-α-*D*-glucopyranoside* (**9**).—A soln of **8** (0.65 g, 0.89 mmol) and *p*-TsOH (17 mg, 0.1 equiv) in MeOH (80 mL) was magnetically stirred at rt. The reaction, monitored by TLC (EtOAc), was stopped after 24 h by addition of $NaHCO_3$ (0.15 g, 2.0 equiv). The mixture was stirred again for 15 min, concentrated under reduced pressure to a gum, which was dissolved in CH_2Cl_2 (50 mL), washed twice with water (15 mL), dried ($MgSO_4$), concentrated in vacuo and purified by chromatography (2:1 EtOAc–*n*-hexane) on silica gel to yield **9** (0.16 g, 35%) as a homogeneous gum: $[\alpha]_D$ +78.3° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.88 (bs, 4 H, catechol), 5.89 (dddd, 1 H, OCH_2–C*H*=CH_2), 5.3 (dq, 1 H, J_{trans} 17.0 Hz, OCH_2–CH=CH*H*), 5.19 (dq, 1 H, J_{cis} 10.0 Hz, $J_{gem} \leq 1.5$ Hz, OCH_2–CH=C*H*H), 4.93 (d, 1 H, H-1), 4.23–3.65 (m, 21 H, $9 \times OCH_2$, H-5, -6, -6′), 3.64 (t, 1 H, H-3), 3.55 (s, 3 H, OCH_3), 3.34 (dd, 1 H, J_{1-2} 3.6 Hz, J_{2-3} 9.0 Hz, H-2), 3.21 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9.0 Hz, H-4), 1.9 (bs, 1 H, OH); EIMS: *m*/*z* 484 $[M]^{\bullet+}$.

*Allyl [2,3-*b*](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-2,3-dideoxy-6-*O*-trityl-β-*D*-glucopyranoside* (**10**).—The same procedure was used as described for **5** except for the reaction time, which was only 2 h. From crown ether **3** (0.844 g, 1.51 mmol) was obtained the corresponding diol (0.75 g, 99%) as a homogeneous gum pure enough for the next step. The same procedure of tritylation and purification was used as described for **7** except for reaction time, which was 14 h. From 0.71 g of the starting material was obtained 0.97 g (92%) of trityl ether **10** as a pale yellow foam: mp < 50 °C; $[\alpha]_D$ −13.6° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.46 (d, 6 H, Ar), 7.33–7.2 (m, 9 H, Ar), 6.88 (bs, 4 H, catechol), 5.96 (dddd, 1 H, OCH_2–C*H*=CH_2), 5.33 (dq, 1 H, J_{trans} 17.2 Hz, OCH_2–CH=CH*H*), 5.2 (dq, 1 H, J_{cis} 10.0 Hz, $J_{gem} \leq 1.5$ Hz, OCH_2–CH=C*H*H), 4.37 (d, 1 H, H-1), 4.25–3.6 (m, 20 H, $9 \times OCH_2$, H-4, -5), 3.57 (t, 1 H, J_{3-4} 9.0 Hz, H-3), 3.4–3.3 (m, 2 H, H-6, -6′), 3.24 (dd, 1 H, J_{1-2} 7.0 Hz, $J_{2-3} \sim 8.5$ Hz, H-2), 2.9 (bs, 1 H, OH); EIMS: *m*/*z* 712 $[M]^{\bullet+}$.

*Allyl [2,3-*b*](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-2,3-dideoxy-4-*O*-methyl-6-*O*-trityl-β-*D*-glucopyranoside* (**11**).—The same procedure was used as described for **8** except for the reaction time, which was 14 h. From the alcohol **10** (0.94 g, 1.3 mmol) was obtained, after usual work-up and chromatography on deactivated silica gel (1:1 EtOAc–*n*-hexane), the crown ether **11** (0.79 g, 83%) as a pale yellow gum: $[\alpha]_D$ −11.5° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.55–7.4 (m, 6 H, Ar), 7.33–7.2 (m, 9 H, Ar), 6.9 (s, 4 H, catechol), 5.99 (dddd, 1 H, OCH_2–C*H*=CH_2), 5.35 (dq, 1 H, J_{trans} 17.0 Hz, OCH_2–CH=CH*H*), 5.22 (dq, 1 H, J_{cis} 10.0 Hz, $J_{gem} \leq 1.5$ Hz, OCH_2–CH=C*H*H), 4.43 (ddd, 1 H, OC*H*H), 4.36 (d, 1 H, H-1), 4.3–3.7 (m, 18 H, H-5, $8.5 \times OCH_2$), 3.62 (bt, 1 H, $J_{3-4} \sim 9.0$ Hz, H-3), 3.5–3.13 (m, 6 H, OCH_3, H-4, -6, -6′), 3.07 (dd, 1 H, J_{1-2} 7.0 Hz, J_{2-3} 9.0 Hz, H-2); EIMS: *m*/*z* 726 $[M]^{\bullet+}$.

*Allyl [2,3-*b*](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-2,3-dideoxy-4-*O*-methyl-β-*D*-glucopyranoside* (**12**).—The same procedure was used as described for **9** except for the reaction time, which was 14 h. From the trityl ether **11** (0.74 g, 1.02 mmol) the crown ether **12** (0.254 g, 50%) was obtained after usual work-up and chromatography on silica gel (1:2 EtOAc–*n*-hexane) as a pale yellow gum: $[\alpha]_D$ −20.8° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.89 (bs, 4 H, catechol), 5.91 (dddd, 1 H, OCH_2–C*H*=CH_2), 5.3 (dq, 1 H, J_{trans} 17.0 Hz, OCH_2–CH=CH*H*), 5.18 (dq, 1 H, J_{cis} 10.0 Hz, $J_{gem} \leq 1.5$ Hz, OCH_2–CH=C*H*H), 4.43 (ddd, 1 H, OC*H*H), 4.37 (d, 1 H, J_{1-2} 7.3 Hz, H-1), 4.3–3.73 (m, 19 H, $8.5 \times OCH_2$, H-6, -6′), 3.69 (ddd, 1 H, J_{4-5} 10.0 Hz, J_{5-6} 4.5 Hz, $J_{5-6'}$ 8.0 Hz, H-5), 3.55 (s, 3 H, OCH_3), 3.33 (t, 1 H, J_{3-4} 9.0 Hz, H-3), 3.23–3.12 (m, 2 H, $J_{2-3} \sim 8$ Hz, H-2, -4), 1.7 (bs, 1 H, OH); EIMS: *m*/*z* 484 $[M]^{\bullet+}$.

Allyl [2,3-b](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-2,3-dideoxy-4-O-methyl-6-O-tosyl-β-D-glucopyranoside (**13**).—To a stirred soln of the alcohol **12** (0.23 g, 0.464 mmol) in abs pyridine (10 mL) was added *p*-TsCl (0.27 g, 3 equiv) and the mixture was heated to 100 °C under argon. The reaction was monitored by TLC (EtOAc) and cooled after 13 h, concentrated to a gum, which was dissolved in CH_2Cl_2 (50 mL) and washed with HCl (0.1 N, 20 mL), satd $NaHCO_3$ (10 mL), and water (10 mL) to afford, after elution through neutral alumina (EtOAc), the crown ether **13** (0.30 g, 99%) as a colourless gum: $[\alpha]_D$ −15.3° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.78 (d, 2 H, Ar), 7.32 (d, 2 H, Ar), 6.89 (bs, 4 H, catechol), 5.85 (dddd, 1 H, OCH_2–C*H*=CH_2), 5.27 (dq, 1 H, J_{trans} 17.0 Hz, OCH_2–CH=CH*H*), 5.16 (dq, 1 H, J_{cis} 10.0 Hz, $J_{gem} \leq 1.5$ Hz, OCH_2–CH=C*H*H), 4.25 (d, 1 H, H-1), 4.3–3.65 (m, 20 H, 9 × O*C*H_2, H-6, -6′), 3.48 (s, 3 H, O*C*H_3), 3.31 (m, 1 H, J_{4-5} 10, $J_{5\text{-}6}$ 2.0 Hz, $J_{5-6'}$ 5.5 Hz, H-5), 3.28 (t, 1 H, H-3), 3.1 (bt, 1 H, J_{1-2} 7.5 Hz, J_{2-3} 9.0 Hz, H-2), 3.07 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9 Hz, H-4), 2.45 (s, 3 H, ArCH_3); EIMS: *m*/*z* 638 $[M]^{\bullet+}$.

Allyl 6-azido-[2,3-b](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-4-O-methyl-2,3,6-trideoxy-β-D-glucopyranoside (**14**).—To a stirred soln of the tosylate **13** (0.28 g, 0.432 mmol) in DMF (10 mL) was added NaN_3 (56 mg, 2 equiv) and the resulting mixture immediately heated to 150 °C. The reaction was monitored by TLC (EtOAc) — the azido-sugar producing a typical brownish colour after being sprayed with dil H_2SO_4 and heated to around 250 °C — and was allowed to cool after 7 h. The solvent was evaporated in vacuo and the residue dissolved in CH_2Cl_2 (50 mL) and washed with water (3 × 10 mL) to afford after rapid chromatography on neutral alumina ($CH_2Cl_2 \rightarrow$ EtOAc) the crown ether **14** (0.22 g, 99%) as a pale yellow wax: mp < 50 °C; $[\alpha]_D$ −40.5° (*c* 1, $CHCl_3$); IR (NaCl) *ν* 2099 cm^{-1} (N_3); ^{1}H NMR ($CDCl_3$): δ 6.88 (bs, 4 H, catechol), 5.9 (dddd, 1 H, OCH_2–C*H*=CH_2), 5.3 (dq, 1 H, J_{trans} 17.0 Hz, OCH_2–CH=CH*H*), 5.17 (dq, 1 H, J_{cis} 10.0 Hz, $J_{gem} \leq 1.5$ Hz, OCH_2–CH=C*H*H), 4.35 (ddd, 1 H, OC*H*H), 4.34 (d, 1 H, H-1), 4.25–3.7 (m, 18 H, 8.5 × O*C*H_2, H-6′), 3.52 (s, 3 H, O*C*H_3), 3.39 (dd, 1 H, H-6), 3.34 (m, 1 H, J_{5-6} 4.0 Hz, $J_{5-6'}$ 6.5 Hz, H-5), 3.31 (t, 1 H, H-3), 3.19 (dd, 1 H, J_{1-2} 7.5 Hz, J_{2-3} 9.0 Hz, H-2), 3.07 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9 Hz, H-4); EIMS: *m*/*z* 509 $[M]^{\bullet+}$.

(2-Hydroxy-ethyl) 6-azido-[2,3-b](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-4-O-methyl-2,3,6-trideoxy-β-D-glucopyranoside (**15**).—A 1:9 O_3–O_2 mixture was bubbled through a stirred soln of crown ether **14** (0.20 g, 0.39 mmol) in 1:1 THF–MeOH (20 mL) cooled between −70 and −78 °C. The ozonolysis was monitored by TLC (EtOAc) and stopped by streaming pure O_2 into the solution after 3 h. Two equiv of $NaBH_4$ (30 mg) were then added and the resulting stirred solution allowed to warm to rt. The reduction was also monitored by TLC (EtOAc) and stopped by evaporation of the solvents after 24 h. The residue was dissolved in CH_2Cl_2 (50 mL), washed with water (2 × 10 mL), dried over $MgSO_4$, and chromatographed on a silica gel column with EtOAc to yield **15** (0.140 g, 69%) as a colourless gum: $[\alpha]_D$ −31.0° (*c* 1, $CHCl_3$); IR (NaCl) *ν* 2100 cm^{-1} (N_3); ^{1}H NMR ($CDCl_3$): δ 6.95–6.83 (m, 4 H, catechol), 4.35 (d, 1 H, H-1), 4.25–3.7 (m, 20 H, 10 × O*C*H_2), 3.53 (s, 3 H, O*C*H_3), 3.5–3.4 (m, 2 H, $J_{6-6'}$ 13.0 Hz, H-6, -6′), 3.35 (m, 1 H, J_{5-6} 5.5 Hz, $J_{5-6'}$ 3.0 Hz, H-5), 3.34 (t, 1 H, H-3), 3.26 (t, 1 H, J_{1-2} 7.5 Hz, J_{2-3} 8.0 Hz, H-2), 3.08 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 8.0 Hz, H-4), 1.65 (bs, 1 H, OH); EIMS: *m*/*z* 527 $[M]^{\bullet+}$.

(2-Hydroxy-ethyl) [2,3-b](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-4,6-O-benzylidene-2,3-dideoxy-α-D-glucopyranoside (**16**).—The same procedure was used as for the synthesis of **15** except for the reaction time, which was 8 h for the ozonolysis and 12 h for the reduction steps. From **3** (0.15 g, 0.27 mmol) the alcohol **16** (79 mg, 53%) was isolated as a colourless wax, after chromatography on silica gel with 1:1 EtOAc–*n*-hexane and finally with 4:1 EtOAc–EtOH. Mp < 50 °C; $[\alpha]_D$ +39.4° (*c* 1, $CHCl_3$) ^{1}H NMR ($CDCl_3$): δ 7.5–7.41 (m, 2 H, Ar), 7.4–7.3 (m, 3 H, Ar), 6.89 (bs, 4 H, catechol), 5.52 (s, 1 H, Hb), 4.99 (d, 1 H, H-1), 4.3–3.67 (m, 23 H,

10 × OCH_2, H-3, -6, -6′), 3.61 (m, 1 H, J_{5-6} 2.5 Hz, $J_{5-6'}$ 7.5 Hz, H-5), 3.53 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9.5 Hz, H-4), 3.46 (dd, 1 H, J_{1-2} 3.5 Hz, J_{2-3} 9.0 Hz, H-2), 1.9 (bs, 1 H, OH); EIMS: m/z 562 $[M]^{\bullet+}$.

*(2-Hydroxy-ethyl) [2,3-*b*](11,12-benzo-1,4,-7,10,13,16-hexaoxacyclooctadeca-11-ene)-2,3-dideoxy-4,6-di-*O*-methyl-α-*D*-glucopyranoside* (**17**).—The same procedure was used as for the synthesis of **16** except for the temperature, which was only −10 °C and for the reaction time, which was respectively, 1 h for the ozonolysis and 5 h for the reduction. From **7** (0.155 g, 0.31 mmol), the crude alcohol **17** (0.16 g, > 99%) was obtained as a homogeneous colourless gum, following usual work-up (but without chromatography): $[\alpha]_D$ +58° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.88 (bs, 4 H, catechol), 4.92 (d, 1 H, H-1), 4.2–3.72 (m, 22 H, 10 × OCH_2, H-5, -6′), 3.7–3.6 (m, 2 H, H-3, -6), 3.51 (s, 3 H, OCH_3), 3.38 (s, 3 H, OCH_3), 3.36 (dd, 1 H, J_{1-2} 3.5 Hz, J_{2-3} 9.5 Hz, H-2), 3.18 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9.5 Hz, H-4), 1.23 (bs, 1 H, OH); EIMS: m/z 502 $[M]^{\bullet+}$.

*Methyl 4-*O*-allyl-[2,3-*b*](11,12-benzo-1,4,-7,10,13,16-hexaoxacyclooctadeca-11-ene)-6-bromo-2,3,6-trideoxy-α-*D*-glucopyranoside* (**20**).—A soln of the crown ether **18** (0.55 g, 1.08 mmol) in toluene (10 mL) and allyl bromide (1 mL, ~10 equiv) was vigorously stirred for 14 h at rt with 50% aq NaOH (10 mL) under an efficient fume board. Chilled water (80 mL) was added and the organic phase extracted with CH_2Cl_2 (3 × 50 mL). The organic extracts were combined and washed with water (3 × 20 mL), dried ($MgSO_4$), concentrated in vacuo, and purified by chromatography on silica gel to yield **20** (0.58 g, > 99%) as a colourless gum: $[\alpha]_D$ +71.6° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.87 (bs, 4 H, catechol), 5.90 (dddd, 1 H, OCH_2–C*H*=CH_2), 5.24 (dq, 1 H, J_{trans} 17.0 Hz, OCH_2–CH=CH*H*), 5.14 (dq, 1 H, J_{cis} 10.5 Hz, $J_{gem} \leq 1.5$ Hz, OCH_2–CH=C*H*H), 4.37 (dd, 1 H, *J* 5.5 Hz, J_{gem} 12.5 Hz, OC*H*H), 4.80 (d, 1 H, H-1), 4.22–3.78 (m, 18 H, 7.5 × OCH_2, H-6′, -7, -7′), 3.73 (m, 1 H, H-5), 3.65 (t, 1 H, H-3), 3.57 (dd, 1 H, J_{5-6} 6 Hz, $J_{6-6'}$ 11.5 Hz, H-6), 3.4 (s, 3 H, OCH_3), 3.38 (dd, 1 H, J_{1-2} 3.5 Hz, J_{2-3} 9.0 Hz, H-2), 3.31 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9.0 Hz, H-4); EIMS: m/z 546 $[^{79}BrM]^{\bullet+}$ and 548 $[^{81}BrM]^{\bullet+}$.

*Methyl 4-*O*-allyl-6-azido-[2,3-*b*](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-2,3,6-trideoxy-α-*D*-glucopyranoside* (**21**)

Method A. The same procedure and reagents were used as for the synthesis of **20**. From the crown ether **19** [1] (0.558 g, 1.25 mmol) the azide **21** (0.60 g, 98%) was obtained as a colourless wax, after usual work-up and chromatography on silica gel (1:1 → 9:1 EtOAc–*n*-hexane): $[\alpha]_D$ +73.5° (*c* 1, $CHCl_3$); IR (NaCl): ν 2100 cm^{-1} (N_3); ^{1}H NMR ($CDCl_3$): δ 6.87 (bs, 4 H, catechol), 5.87 (dddd, 1 H, OCH_2–C*H*=CH_2), 5.23 (dq, 1 H, J_{trans} 17.0 Hz, OCH_2–CH=CH*H*), 5.13 (dq, 1 H, J_{cis} 10.5 Hz, $J_{gem} \leq 1.5$ Hz, OCH_2–CH=C*H*H), 4.77 (d, 1 H, H-1), 4.36–3.77 (m, 17 H, 8.5 × OCH_2), 4.325 (dd, 1 H, *J* 5.5 Hz, J_{gem} 12.5 Hz, OC*H*H), 3.62 (t, 1 H, H-3), 3.73 (m, 1 H, H-5), 3.5 (dd, 1 H, $J_{5-6'}$ 2.0 Hz, H-6′), 3.42 (s, 3 H, OCH_3), 3.39 (dd, 1 H, J_{1-2} 3.5 Hz, J_{2-3} 9.0 Hz, H-2), 3.37 (dd, 1 H, J_{5-6} 6.0 Hz, $J_{6-6'}$ 13.5 Hz, H-6), 3.27 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9 Hz, H-4); EIMS: m/z 509 $[M]^{\bullet+}$, 481 $[M-N_2]^{\bullet+}$.

Method B. To a stirred soln of **20** (1.0 g, 1.826 mmol) in DMF (80 mL) was added NaN_3 (0.18 g, 1.5 equiv) and the solution heated to 130 °C. The reaction was monitored by TLC (1:1 EtOAc–*n*-hexane), and was complete after 1 h. The solvent was evaporated, the residue dissolved in CH_2Cl_2 (100 mL), washed twice with water (20 mL), dried over $MgSO_4$, and concentrated under reduced pressure. Chromatography of the residue (3:2 EtOAc–*n*-hexane) yielded the azide **21** (0.92 g, > 99%) as a colourless wax identical in all aspects to the previous compound obtained from **19**.

*Methyl 4-*O*-allyl-[2,3-*b*](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-2,3,6-trideoxy-α-*D*-glucopyranoside* (**23**).—The same procedure and reagents were used as for the synthesis of **21**. From the crown ether **22** (0.75 g, 1.75 mmol) after 24 h of reaction, following the usual work-up and chromatography on silica gel (2:1 EtOAc–*n*-hexane) the crown ether **23** (0.74 g, 90%) was obtained as a colourless gum: $[\alpha]_D$ +62.3° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.89 (4 H, catechol), 5.92

(dddd, 1 H, OCH_2–C*H*=CH_2), 5.26 (dq, 1 H, J_{trans} 17.0 Hz, OCH_2–CH=CH*H*), 5.14 (dq, 1 H, J_{cis} 10.5 Hz, $J_{gem} \leq 1.5$ Hz, OCH_2–CH=C*H*H), 4.72 (d, 1 H, H-1), 4.33 (dd, 1 H, *J* 5.5 Hz, J_{gem} 12.5 Hz, OC*H*H), 4.23–3.63 (m, 18 H, 8.5 × OCH_2, H-5), 3.59 (t, 1 H, H-3), 2.37 (s, 3 H, OCH_3), 2.85 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9.0 Hz, H-4), 2.36 (dd, 1 H, J_{1-2} 3.5 Hz, J_{2-3} 9.0 Hz, H-2), 1.24 (d, 3 H, J_{5-6} 6.0 Hz, H-6); EIMS: *m*/*z* 509 $[M]^{\bullet+}$.

*Methyl [2,3-*b*](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-4-*O*-(2-hydroxy-ethane-1-yl)-2,3,6-trideoxy-α-*D*-glucopyranoside* (**24**)

Method A. The same procedure and reagents were used as for the synthesis of **17** except for the reaction time, which was respectively 2 h for the ozonolysis and 14 h for the reduction. From **23** (0.56 g, 1.19 mmol) the crown ether **24** (0.56 g, 99%) was obtained as colourless crystals after the usual work-up but without chromatography: mp 57–59 °C (EtOAc); $[\alpha]_D$ +55.0° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.85 (bs, 4 H, catechol), 4.69 (d, 1 H, H-1), 4.25–3.65 (m, 21 H, 10 × OCH_2, OH), 3.62 (m, H-5), 3.58 (t, H-3), 3.39 (dd, 1 H, J_{1-2} 3.5 Hz, J_{2-3} 9.0 Hz, H-2), 3.36 (s, 3 H, OCH_3), 3.0 (bt, 1 H, $J_{3-4} \sim 9.0$ Hz, J_{4-5} 9.5 Hz, H-4), 1.25 (d, 3 H, J_{5-6} 6.0 Hz, H-6); EIMS: *m*/*z* 472 $[M]^{\bullet+}$.

Method B. A soln of the crown ether **25** (0.30 g, 0.544 mmol) (vide infra) in THF (5 mL) was dropped over 10 min onto a stirred suspension of $LiAlH_4$ (83 mg, 4 equiv) in THF (5 mL) at 0 °C under argon. After addition, the temperature was raised up to rt under stirring. The reaction was monitored by TLC (EtOAc) and stopped after 45 min at rt by slow addition of EtOAc (1 mL). Hydrolysis of the hydride excess was completed with satd aq Na_2SO_4 (0.5 mL), the suspension filtered through a sintered glass and the remaining salts rinsed twice with THF (25 mL). The solvents were eliminated under reduced pressure and the residue dissolved in CH_2Cl_2 (50 mL), washed twice with water (10 mL), dried over $MgSO_4$, and concentrated to a colourless gum, which crystallised slowly on standing. Yield: 0.247 g (99%) of white crystals identical in all aspects to the above glycol **24**.

*Methyl [2,3-*b*](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-6-bromo-4-*O*-(2-hydroxy-ethane-1-yl)-2,3,6-trideoxy-α-*D*-glucopyranoside* (**25**).—The same procedure was used as for the synthesis of **17** except for the reaction time, which was 3 h for the ozonolysis and 4 h for the reduction (TLC with 2:1 EtOAc–*n*-hexane). From the allyl ether **20** (0.64 g, 1.16 mmol), following usual work-up (but without chromatography), the crude glycol **25** (0.65 g, >99%) was obtained as a colourless gum: $[\alpha]_D$ +59.7° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.9 (bs, 4 H, catechol), 4.82 (d, 1 H, H-1), 4.27–3.65 (m, 22 H, 10 × OCH_2, H-5, -6), 3.6 (dd, 1 H, J_{5-6} 5.0 Hz, $J_{6-6'}$ 11.5 Hz, H-6′), 3.43 (s, 3 H, OCH_3), 3.42 (dd, 1 H, J_{1-2} 3.5 Hz, J_{2-3} 9 Hz, H-2), 3.36 (t, 1 H, J_{3-4} 8.5 Hz, H-3), 3.29 (t, 1 H, J_{4-5} 6 Hz, H-4), 1.65 (s, 1 H, OH); EIMS: *m*/*z* 550 $[^{79}BrM]^{\bullet+}$ and 552 $[^{81}BrM]^{\bullet+}$.

*Methyl 6-azido-[2,3-*b*](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-4-*O*-(2-hydroxy-ethane-1-yl)-2,3,6-trideoxy-α-*D*-glucopyranoside* (**26**).—The same procedure was used as for the synthesis of **15** except for the reaction time, which was 14 h (TLC with 2:1 EtOAc–*n*-hexane). From the allyl ether **21** (0.40 g, 0.785 mmol) the glycol **26** (0.25 g, 61%) was obtained, following usual work-up and chromatography (2:3 EtOAc–*n*-hexane), as a colourless gum: $[\alpha]_D$ +64.2° (*c* 1, $CHCl_3$); IR (NaCl): *ν* 2098 cm^{-1} (N_3); ^{1}H NMR ($CDCl_3$): δ 6.89 (bs, 4 H, catechol), 4.79 (d, 1 H, H-1), 4.25–3.65 (m, 22 H, 10 × OCH_2, H-5, -6′), 3.61 (t, 1 H, H-3), 3.51 (dd, 1 H, J_{5-6} 3 Hz, $J_{6-6'}$, 13 Hz, H-6), 3.4 (s, 3 H, OCH_3), 3.39 (dd, 1 H, J_{1-2} 3.5 Hz, J_{2-3} 9 Hz, H-2), 3.31 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9.5 Hz, H-4), 2.05 (s, 1 H, OH); EIMS: *m*/*z* 513 $[M]^{\bullet+}$.

*Ethyl-2-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyloxy) 6-azido-[2,3-*b*](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-4-*O*-methyl-2,3,6-trideoxy-β-*D*-glucopyranoside* (**27**).—To a soln of the acceptor **15** (0.10 g, 0.194 mmol) and D-galactose trichloracetimidate (0.19 g, 2 equiv) in CH_2Cl_2 (10 mL) were added crushed molecular sieves (~1 g, activated at 105 °C and stored in vacuo over P_2O_5) and the suspension magnetically stirred under Ar at rt for 30 min. $BF_3{\cdot}OEt_2$ (50 μL, 2 equiv) in CH_2Cl_2 (10 mL) was then dropped

over a period of 15 min. The reaction was monitored by TLC (1:1 EtOAc–*n*-hexane) and stopped after 20 h by addition of $NaHCO_3$ (90 mg, 4 equiv). The suspension was filtered and the filtrate washed with water (2×20 mL), dried with $MgSO_4$, and concentrated under reduced pressure. Chromatography of the residue on a silica gel column (1:1 EtOAc–*n*-hexane) yielded the target compound **27** (0.116 g, 55%) as a colourless gum: $[\alpha]_D$ +7.7° (*c* 0.5, $CHCl_3$); IR (NaCl): *v* 2099 cm^{-1} (N_3); ^1H NMR ($CDCl_3$): δ 6.9 (bs, 4 H, catechol), 5.36 (d, 1 H, H-4 Gal), 5.17 (t, 1 H, H-2 Gal), 4.99 (dd, 1 H, J_{2-3} 10.5 Hz, J_{3-4} 3.5 Hz, H-3 Gal), 4.52 (d, 1 H, J_{1-2} 8.0 Hz, H-1 Gal), 4.31 (d, 1 H, H-1 Glc), 4.27–3.65 (m, 24 H, $10 \times OCH_2$, H-6′ Glc, H-5, -6, -6′ Gal), 3.52 (s, 3 H, OCH_3), 3.45–3.3 (m, 2 H, H-5, -6 Glc), 3.29 (t, 1 H, H-3 Glc), 3.12 (t, 1 H, J_{1-2} 8.0 Hz, J_{2-3} 9.0 Hz, H-2 Glc), 3.06 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9.0 Hz, H-4 Glc), 2.15–1.9 (4 s, each 3 H, $4 \times$ OAc); EIMS: *m*/*z* 843 $[M]^{\bullet+}$.

*Ethyl-2-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyloxy) [2,3-*b*](11,12-benzo-1,4,7,-10,13,16-hexaoxacyclooctadeca-11-ene)-4,6-*O*-benzylidene-2,3-dideoxy-α-*D*-glucopyranoside* (**28**).—The same procedure and work-up were used as for the synthesis of **27** except for the reaction time, which was 2 h. From the acceptor **16** (0.16 g, 0.284 mmol) the target compound **28** (60 mg, 22%) was first recovered as a colourless gum after HPLC on a silica gel column (20 mm i.d., 1:2 EtOAc–*n*-hexane): $[\alpha]_D$ +28.8° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.5–7.3 (m, 5 H, Ar), 6.9 (bs, 4 H, catechol), 5.5 (s, 1 H, Hb), 5.36 (d, 1 H, J_{4-5} 3.0 Hz, H-4 Gal), 5.18 (t, 1 H, H-2 Gal), 5.0 (dd, 1 H, J_{2-3} 10.0 Hz, J_{3-4} 3.5 Hz, H-3 Gal), 4.96 (d, 1 H, H-1 Glc), 4.55 (d, 1 H, J_{1-2} 8.0 Hz, H-1 Gal), 4.3–3.6 (m, 26 H, $10 \times OCH_2$, H-5, -6, -6′ Glc, H-5, -6, -6′ Gal), 3.54 (t, 1 H, J_{4-5} 9.0 Hz 9 Hz, H-4 Glc), 3.52 (t, 1 H, J_{3-4} 9.5 Hz, H-3 Glc), 3.45 (dd, 1 H, J_{1-2} 3.5 Hz, J_{2-3} 9.5 Hz, H-2 Glc), 2.2–1.9 (4 s, each 3 H, $4 \times$ OAc); EIMS: *m*/*z* 892 $[M]^{\bullet+}$; then, with 1:1 EtOAc–*n*-hexane as eluent, ethyl-2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy) [2,3 - *b*](11,12 - benzo - 1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-2,3-dideoxy-α-D-glucopyranoside (**29**) (0.10 g, 44%) was isolated as a colourless gum: $[\alpha]_D$ +15.4° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.9 (bs, 4 H, catechol), 5.36 (d, 1 H, J_{4-5} 3.0 Hz, H-4 Gal), 5.15 (t, 1 H, H-2 Gal), 5.0 (dd, 1 H, J_{2-3} 10.5 Hz, J_{3-4} 3.5 Hz, H-3 Gal), 4.9 (d, 1 H, J_{1-2} 3.75 Hz, H-1 Glc), 4.5 (d, 1 H, J_{1-2} 8.0 Hz, H-1 Gal), 4.3–3.55 (m, 27 H, $10 \times OCH_2$, H-3, -4, -6, -6′ Glc, H-5, -6, -6′ Gal), 3.53 (dd, 1 H, J_{1-2} 3.0 Hz, J_{2-3} 7.5 Hz, H-2 Glc), 3.4 (m, 1 H, J_{5-6} 2.5 Hz, J_{4-5} 9.0 Hz, $J_{5-6'}$ 6.0 Hz, H-5 Glc), 2.65 (bs, 2 H, OH), 2.2–1.9 (4 s, each 3 H, $4 \times$ OAc); LSIHRMS: calcd for $C_{36}H_{52}O_{20}Na$ 827.2950, found *m*/*z* 827.2956.

*Ethyl-2-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyloxy) [2,3-*b*](11,12-benzo-1,4,7,-10,13,16-hexaoxacyclooctadeca-11-ene)-2,3-dideoxy-4,6-di-*O*-methyl-α-*D*-glucopyranoside* (**30**).—The same procedure and work-up were used as for the synthesis of **27** except for the reaction time, which was 22 h. From **7** (0.15 g, 0.298 mmol), after chromatography on a silica gel column (1:1 EtOAc–*n*-hexane), the target compound **30** (0.12 g, 47%) was obtained as a colourless gum: $[\alpha]_D$ +39.3° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.89 (bs, 4 H, catechol), 5.35 (d, 1 H, J_{4-5} 3.5 Hz, H-4 Gal), 5.15 (t, 1 H, H-2 Gal), 4.98 (dd, 1 H, J_{2-3} 10.0 Hz, J_{3-4} 3.5 Hz, H-3 Gal), 4.87 (d, 1 H, H-1 Glc), 4.52 (d, 1 H, J_{1-2} 8.0 Hz, H-1 Gal), 4.2–3.65 (m, 24 H, $10 \times OCH_2$, H-6′ Glc, H-5, -6, -6′ Gal), 3.68 (dd, 1 H, J_{5-6} 6.5 Hz, $J_{6-6'}$ 14.0 Hz, H-6 Glc), 3.56 (m, 1 H, H-5 Glc), 3.55 (t, 1 H, J_{2-3} 9.5 Hz, H-3 Glc), 3.5 (s, 3 H, OCH_3), 3.37 (s, 3 H, OCH_3), 3.35 (dd, 1 H, J_{1-2} 3.7 Hz, H-2 Glc), 3.21 (bt, 1 H, J_{3-4} 9.0 Hz, H-4 Glc), 2.2–1.9 (4 s, each 3 H, $4 \times$ OAc); EIMS: *m*/*z* 832 $[M]^{\bullet+}$.

*Ethyl-2-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyloxy) 4,6-di-*O*-acetyl-[2,3-*b*](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-2,3-dideoxy-α-*D*-glucopyranoside* (**31**).—From **29** (0.10 g, 0.124 mmol) after reaction with Ac_2O (1 mL) for 24 h at rt in pyridine (10 mL) and a catalytical amount of 4-DMAP, usual work-up, and chromatography (1:1 EtOAc–*n*-hexane) on a silica gel column, the peracetylated derivative **31** (0.110 g, ~ 99%) was isolated as a colourless gum: $[\alpha]_D$ +32.8° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.88 (bs, 4 H, catechol), 5.38 (d, 1 H, J_{4-5} 3.0 Hz, H-4 Gal), 5.17 (t, 1 H, H-2 Gal), 5.01 (dd, 1 H, J_{2-3} 10.0 Hz, J_{3-4} 3.5 Hz, H-3 Gal), 4.97 (t, 1 H, J_{4-5} 9.0 Hz, H-4 Glc), 4.95 (d, 1 H, H-1

Glc), 4.52 (d, 1 H, J_{1-2} 8.0 Hz, H-1 Gal), 4.25 (dd, 1 H, $J_{5-6'}$ 4.5 Hz, H-6′ Glc), 4.2–3.7 (m, 24 H, 10 × OCH_2, H-5 Glc, H-5, -6, -6′ Gal), 4.03 (dd, 1 H, J_{1-2} 2.0 Hz, $J_{6-6'}$ 12.5 Hz, H-6 Glc), 3.67 (t, 1 H, J_{3-4} 8.5 Hz, H-3 Glc), 3.45 (dd, 1 H, J_{1-2} 3.0 Hz, J_{2-3} 8.5 Hz, H-2 Glc), 2.2–1.9 (m, 18 H, 6 × OAc); EIMS: *m*/*z* 802 $[M]^{\bullet+}$.

*Ethyl-2-(β-*D*-galactopyranosyloxy) [2,3-*b*]-(11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-4,6-*O*-benzylidene-2,3-dideoxy-α-*D*-glucopyranoside* (**32**).—To a stirred soln of **28** (40 mg, 44.8 μmol) in MeOH (10 mL) was added MeONa (25 mg) at rt. The reaction was monitored by TLC (95:5 EtOAc–EtOH) and was stopped after 24 h by addition of Dowex H^+ beads (~ 0.5 mL). The suspension was stirred again for 10 min and filtered to yield, after evaporation of solvents, the galactoside **32** (28 mg, > 99%) as a pale yellow gum: $[\alpha]_D$ + 18.1° (*c* 0.2, CH_3CN); ^{1}H NMR selected values (D_2O): δ 7.53–7.4 (m, 5 H, Ar), 7.03 (bs, 4 H, catechol), 5.63 (s, 1 H, Hb), 5.17 (d, 1 H, H-1 Glc), 4.48 (d, 1 H, J_{1-2} 8.4 Hz, H-1 Gal), 3.55 (dd, 1 H, J_{1-2} 3.5 Hz, J_{2-3} 9.5 Hz, H-2 Glc); ^{13}C NMR (1:1 CD_3CN–D_2O): δ 149.0 (C-1, -2 catechol), 137.7 (C-1 Bz), 130.2 (C-4 Bz), 129.4 (C-2, -3 Bz), 127.1, 122.6 (C-4, -5 catechol), 114.6 (C-3, -6 catechol), 103.8 (C-1 Gal), 102.2 (O*C*HO), 97.9 (C-1 Glc), 81.9 (C-4 Glu), 80.1 (C-5 Gal), 78.5 (C-2 Glc), 75.9 (C-3 Glc), 73.8 (C-3 Gal), 72.4 (O*C*H_2), 71.6 (C-2 Gal), 71.1, 71.0, 70.6, 70.1, 70.0 (O*C*H_2), 69.6 (C-4 Gal), 69.3 (O*C*H_2), 67.8 (O*C*H_2 spacer), 63.2 (C-5 Glc), 61.8 (C-6 Gal), 61.6 (C-6 Glc); LSIHRMS: calcd for $C_{35}H_{48}O_{16}Na$ 747.2840, found *m*/*z* 747.2846; t_{ret} (HPLC) 10.7 min (4:1 water–MeCN).

*Ethyl-2-(β-*D*-galactopyranosyloxy) [2,3-*b*]-(11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-2,3-dideoxy-α-*D*-glucopyranoside* (**33**).—The same procedure was used as for the synthesis of **32** except for the reaction time, which was 6 h. From **31** (0.10 g, 0.11 mmol), after the same work-up, the deprotected galactoside **33** (72 mg, 86%) was obtained as a colourless wax: $[\alpha]_D$ + 27.2° (*c* 0.2, CH_3CN); ^{1}H NMR (1:1 CD_3CN–D_2O): δ 7.03 (bs, 4 H, catechol), 5.15 (d, 1 H, H-1 Glc), 4.4 (d, 1 H, J_{1-2} 7.0 Hz, H-1 Gal), 4.29–3.57 (m, 28 H, 10 × OCH_2, H-3, -5, -6, -6′ Glc, H-3, -5, -6, -6′ Gal), 3.6 (dd, 1 H, J_{1-2} 3.5 Hz, J_{2-3} 9.5 Hz, H-2 Glc), 3.49 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9.0 Hz, H-4 Glc); ^{13}C NMR (1:1 CD_3CN–D_2O): δ 148.4 (C-1, -2 catechol), 122.6 (C-4, -5 catechol), 114.0 (C-3, -6 catechol), 103.7 (C-1 Gal), 96.4 (C-1 Glc), 80.9 (C-5 Gal), 78.8 (C-2 Glc), 75.9 (C-3 Glc), 72.65 (C-4 Glc), 72.1 (O*C*H_2), 71.6 (C-4 Gal), 71.3, 70.7 (O*C*H_2), 70.6 (C-3 Gal), 69.8 (O*C*H_2), 69.5 (C-2 Gal), 69.3, 69.1, 68.6 (O*C*H_2), 67.3 (O*C*H_2 spacer), 61.8 (C-6 Gal), 61.4 (C-6 Glc); LSIHRMS: calcd for $C_{28}H_{44}O_{16}Na$ 659.2527, found *m*/*z* 659.2525; t_{ret} (HPLC) 7.1 min (4:1 water–MeCN).

*Ethyl-2-(β-*D*-galactopyranosyloxy) [2,3-*b*]-(11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-2,3-dideoxy-4,6-di-*O*-methyl-α-*D*-glucopyranoside* (**34**).—The same procedure was used as for the synthesis of **32**. From **30** (0.10 g, 0.12 mmol) the deprotected galactoside **34** (77 mg, 96%) was obtained after the same work-up as a colourless gum: $[\alpha]_D$ + 21.5° (*c* 0.4, CH_3CN); ^{1}H NMR (1:1 CD_3CN–D_2O): δ 7.0 (bs, 4 H, catechol), 5.06 (d, 1 H, H-1 Glc), 4.73 (dd, 1 H, J_{2-3} 10.5 Hz, H-2 Gal), 4.35 (d, 1 H, J_{1-2} 8.5 Hz, H-1 Gal), 4.21–3.52 (m, 29 H, 10 × OCH_2, H-3, -5, -6, -6′ Glc, H-3, -4, -5, -6, -6′ Gal), 3.48 (s, 3 H, OCH_3), 3.41 (dd, 1 H, J_{1-2} 3.9 Hz, J_{2-3} 10.0 Hz, H-2 Glc), 3.33 (s, 3 H, OCH_3), 3.17 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9.5 Hz, H-4 Glc); ^{13}C NMR (1:1 CD_3CN–D_2O): δ 149.3 (C-1, -2, catechol), 115.1 (C-3, -6, catechol), 112.7 (C-4, -5, catechol), 103.9 (C-1 Gal), 96.9 (C-1 Glc), 82.0 (C-5 Gal), 80.2 (C-2, -4 Glc), 76.0 (C-3 Glc), 73.9 (C-5 Glc), 73.1, 71.8 (O*C*H_2 crown), 71.45, 71.2 (O*C*H_2 crown), 71.7 (C-4 Gal), 70.5 (C-3 Gal), 70.4, 70.1, 69.7 (O*C*H_2 crown), 69.6 (C-2 Gal), 69.3 (O*C*H_2 crown), 67.5 (O*C*H_2 spacer), 61.9 (C-6 Glc), 61.2 (C-6 Gal), 61.1 (O*C*H_3), 59.4 (O*C*H_3); LSIHRMS: calcd for $C_{30}H_{48}O_{16}Na$ 687.2840, found *m*/*z* 687.2849; (HPLC) 7.8 min (73:27 water–MeCN).

*Methyl [2,3-*b*](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-4-*O*-[2-(2,3,-4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyloxy)-ethane-1-yl]-2,3,6-trideoxy-α-*D*-glucopyranoside* (**35**).—The same procedure was used as for the synthesis of **28**. From **24** (0.30 g, 0.634 mmol) the galactoside **35** (0.255 g, 50%) was

obtained after chromatography on silica gel (1:1 → 2:1 EtOAc–*n*-hexane) as a colourless gum: $[\alpha]_D$ +31.3° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.90 (bs, 4 H, catechol), 5.39 (d, 1 H, J_{4-5} 3.0 Hz, H-4 Gal), 5.21 (dd, 1 H, H-2 Gal), 5.01 (dd, 1 H, J_{2-3} 10.0 Hz, J_{3-4} 3.0 Hz, H-3 Gal), 4.73 (d, 1 H, H-1 Glc), 4.52 (d, 1 H, J_{1-2} 8.0 Hz, H-1 Gal), 4.22-3.6 (m, 24 H, 10 × OCH_2, H-5, -6,-6′ Glc, H-5, -6, -6′ Gal), 3.56 (t, 1 H, H-3 Glc), 3.38 (s, 3 H, OCH_3), 3.37 (dd, 1 H, J_{1-2} 3.5 Hz, J_{2-3} 9.5, H-2 Glc), 2.91 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9.5 Hz, H-4 Glc), 2.2–1.9 (4 s, each 3 H, 4 × OAc), 1.25 (d, 3 H, J_{5-6} 6.5 Hz, H-6 Glc); LSIHRMS: calcd for $C_{37}H_{54}O_{17}Na$ 825.3157, found *m*/*z* 825.3163.

*Methyl [2,3-*b*](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-6-bromo-4-*O*-[2-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyloxy)-ethane-1-yl]-2,3,6-trideoxy-α-*D*-glucopyranoside* (**36**).—The same procedure was used as for the synthesis of **27** except for the reaction time, which was 4 h. From **25** (0.37 g, 0.671 mmol) the galactoside **36** (0.255 g, 43%) was obtained after chromatography on silica gel (1:1 EtOAc–*n*-hexane) as a colourless gum: $[\alpha]_D$ +37.6° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.87 (bs, 4 H, catechol), 5.36 (d, 1 H, J_{4-5} 3.5 Hz, H-4 Gal), 5.19 (dd, 1 H, H-2 Gal), 4.98 (dd, 1 H, J_{2-3} 10.0 Hz, J_{3-4} 3.5 Hz, H-3 Gal), 4.8 (d, 1 H, H-1 Glc), 4.49 (d, 1 H, J_{1-2} 8.0 Hz, H-1 Gal), 4.22–3.73 (m, 25 H, 10 × OCH_2, H-6, -6′ Glc, H-5, -6, -6′ Gal), 3.67 (m, 1 H, J_{5-6} 7.0 Hz, $J_{5-6'}$ 2.5 Hz, H-5 Glc), 3.61 (t, 1 H, H-3 Glc), 3.4 (s, 3 H, OCH_3), 3.37 (dd, 1 H, J_{1-2} 3.0 Hz, J_{2-3} 9.0 Hz, H-2 Glc), 3.28 (t, 1 H, $J_{3-4} \sim J_{4-5}$ 9.0 Hz, H-4 Glc), 2.2–1.9 (4 s, each 3 H, 4 × OAc); EIMS: *m*/*z* 880 $[^{79}BrM]^{\bullet+}$ and 882 $[^{81}BrM]^{\bullet+}$.

*Methyl 6-azido-[2,3-*b*](11,12-benzo-1,4,7,-10,13,16-hexaoxacyclooctadeca-11-ene)-4-*O*-[2-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyloxy)-ethane-1-yl]-2,3,6-trideoxy-α-*D*-glucopyranoside* (**37**).—The same procedure was used as for the synthesis of **27** except for the reaction time, which was 4 h. From **26** (0.23 g, 0.448 mmol) the galactoside **37** (0.214 g, 57%) was obtained after chromatography on silica gel (1:1 → 2:1 EtOAc–*n*-hexane) as a colourless gum: $[\alpha]_D$ +50.4° (*c* 1, $CHCl_{3)}$); IR (NaCl): *v* 2099 cm^{-1} (N_3); ^1H NMR ($CDCl_3$): δ 6.87 (bs, 4 H, catechol), 5.37 (d, 1 H, J_{4-5} 3.5 Hz, H-4 Gal), 4.99 (dd, 1 H, H-2 Gal), 4.9 (dd, 1 H, J_{2-3} 10.0 Hz, J_{3-4} 3.5 Hz, H-3 Gal), 4.79 (d, 1 H, H-1 Glc), 4.47 (d, 1 H, J_{1-2} 8.0 Hz, H-1 Gal), 4.24–3.62 (m, 25 H, 10 × OCH_2, H-5, -6′, -5, -6, -6′), 3.57 (t, 1 H, H-3 Glc), 3.44 (dd, 1 H, J_{5-6} 2.5 Hz, $J_{6-6'}$ 12.5 Hz, H-6 Glc), 3.4 (s, 3 H, OCH_3), 3.32 (t, 1 H, H-4 Glc), 3.23 (dd, 1 H, J_{1-2} 3.5 Hz, J_{2-3} 8.5 Hz, H-2 Glc), 2.2–1.9 (4 s, each 3 H, 4 × OAc); EIMS *m*/*z* 843 $[M]^{\bullet+}$, 816 $[M-N_2+H]^+$.

*Methyl [2,3-*b*](11,12-benzo-1,4,7,10,13,16-hexaoxacyclooctadeca-11-ene)-4-*O*-[2-(β-*D*-galactopyranosyloxy)-ethane-1-yl]-2,3,6-trideoxy-α-*D*-glucopyranoside* (**38**).—The same procedure was used as for the synthesis of **32** except for the reaction time, which was 5 h. From the precursor **35** (0.11 g, 0.137 mmol) the deprotected galactoside **38** (87 mg, 99%) was obtained after usual work-up as a colourless gum: $[\alpha]_D$ +43.1° (*c* 0.5, CH_3CN); ^1H NMR (D_2O): δ 7.0 (bs, 4 H, catechol), 4.86 (d, 1 H, H-1 Glc), 4.35 (d, 1 H, H-1 Gal), 4.2–3.55 (m, 24 H, 10 × OCH_2, H-3, -5, -6, -6′ Gal), 3.65 (m, 1 H, H-5 Glc), 3.58 (d, 1 H, $J_{3-4} \sim J_{4-5}$ 3.5 Hz, H-4 Gal), 3.49 (t, 1 H, $J_{1-2} \sim J_{2-3}$ 8.0 Hz, H-2 Gal), 3.488 (t, 1 H, H-3 Glc), 3.43 (dd, 1 H, J_{1-2} 3.0 Hz, J_{2-3} 10.0 Hz, H-2 Glc), 3.35 (s, 3 H, OCH_3), 3.03 (t, 1 H, J_{3-4} 9.0 Hz, J_{4-5} 10.0 Hz, H-4 Glc), 1.26 (d, 3 H, J_{5-6} 6.5 Hz, CH_3); ^{13}C NMR (1:1 D_2O–CD_3CN): δ 149.25, 149.2 (C-1, -2, catechol), 122.8 (C-4, -5, catechol), 115.05, 114.9 (C-3, -6, catechol), 103.9 (C-1 Gal), 97.9 (C-1 Glc), 84.8 (C-5 Glc), 81.8 (C-5 Gal), 80.4 (C-2 Glc), 76.0 (C-3 Glc), 73.9 (C-4 Glc), 73.0, 72.9 (OCH_2), 71.9 (C-4 Gal), 71.5, 71.3, 70.15, 70.1 (OCH_2), 69.8 (C-3 Gal), 69.6, 69.55 (OCH_2), 67.4 (C-2 Gal), 62.1 (OCH_2), 61.9 (C-6 Gal), 55.6 (OCH_3), 17.9 (C-6 Glc); FABMS: calcd for $C_{29}H_{46}O_{15}$ 634, found *m*/*z* 635 $[M+H]^+$, 495 $[M-C_6H_{10}O_5+Na]^+$, 379 $[M-C_{11}H_{18}O_8+Na]^+$; LSIHRMS: calcd for $C_{29}H_{46}O_{15}Na$ 657.2734, found *m*/*z* 657.2730; t_{ret} (HPLC) 6.9 min (73:27 water–MeCN).

Micro-organisms and culture conditions.—The flocculent yeast strain used throughout the present study was *K. bulgaricus* (ATCC 96631). The growth of the yeast was performed in a liquid medium composed of 4.0% D-glucose, 0.4% bactopeptone (Difco, MI,

USA), 0.1% KH_2PO_4, 0.02% $MgSO_4 \cdot 7H_2O$, and 0.02% $CaCl_2$ (Prolabo, France). The cells were cultured under aerobic conditions at 20 °C in a 2 L capacity fermentor containing 1.5 L of medium. The aeration was 20 L/h and the agitation 220 rpm.

Extraction of the galactose-specific lectin Kb CWL1.—The D-galactose lectin Kb CWL1 was extracted from whole cells harvested at the beginning of the stationary growth phase according to Al-Mahmood et al. [5]. After growth in the 2 L fermentor, *K. bulgaricus* cells were harvested by centrifugation at 3000*g* for 10 min at 4 °C, washed with Helm's buffer (150 mM CH_3CO_2Na, 3.75 mM $CaCl_2$, 3.0 mM NaN_3) at pH 5.0, treated with a 0.2 M D-galactose solution and extensively washed with 0.01 M phosphate buffer (pH 7.0). Cells were then suspended at a concentration of 4% (w/v) in PBS buffer (137 mM NaCl, 2.7 mM KCl, 8.0 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.5 $MgCl_2$, 1.0 mM $CaCl_2$) at pH 7.4 supplemented with 5 mM EDTA and incubated at 37 °C for 90 min under moderate stirring. After centrifugation at 3000*g* for 10 min, the supernatant containing the lectin was collected and dialysed at 4 °C for at least 48 h against distilled water, then freeze dried. The residual flocculating activity of the yeast was assessed in Helm's acetate buffer.

Flocculation inhibition tests.—Non-flocculating yeast cells obtained after lectin extraction were washed with distilled water, then with 10 mM EDTA aqueous solution, and twice with distilled water. Washed cells (about 2.5×10^6 cells in 1 mL) were suspended in 10 mL Helm's acetate buffer (pH 4.5). Inhibition of flocculation was assayed in glass tubes by mixing 150 mL of a two-fold serial dilution of oligosaccharidic derivatives in Helm's acetate buffer with 150 mL of a lectinic solution titre 4. The mixture was incubated for 60 min at rt after mild agitation, then 150 mL of an EDTA deflocculated yeast cell suspension were added. After 1 h of incubation at rt, yeast flocculation was estimated by visual reading or microscope observation. The MIC of the flocculation corresponds to the tube in which flocculation is visible. The control of inhibition was achieved in the same way, but with D-galactose, methyl β-D-galactoside and lactose [11].

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