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Total synthesis of periploside A, a unique pregnane hexasaccharide with potent immunosuppressive effects

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Periploside A is a pregnane hexasaccharide identified from the Chinese medicinal plant *Periploca sepium*, which features a unique seven-membered formyl acetal bridged orthoester (FABO) motif and potent immunosuppressive activities. Here, we show the synthesis of this molecule in a total of 76 steps with the longest linear sequence of 29 steps and 9.2% overall yield. The FABO motif is constructed via a combination of Sinaÿ's and Crich's protocol for the formation of orthoester and acetal glycosides, respectively. The 2-deoxy- β -glycosidic linkages are assembled stereoselectively with judicious choice of the glycosylation methods. The epimer at the spiro-quaternary carbon in the FABO motif has also been elaborated in a stereo-controlled manner. This epimer, as well as the synthetic analogues bearing the FABO motif, retain largely the inhibitory activities of periploside A against the proliferation of T-lymphocyte, indicating the importance of the chemical connection of the FABO motif to their immunosuppressive activity.

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Periploside A (or Periplocoside E) is the prototypical member of a group of pregnane glycosides isolated from *Periploca sepium* and *P. forrestii* (Asclepiadaceae), which features a unique seven-membered formyl acetal bridged orthoester (FABO) linkage between two sugar units^{1–7}. This natural product shows immunosuppressive activities, that is, it inhibits the ConA-induced T cell proliferation as potently as rapamycin and cyclosporin A while showing reasonably low toxicity ($IC_{50} = 0.64 \mu\text{M}$ and $CC_{50} = 10.1 \mu\text{M}$)^{5,6}. The immunosuppressive effects of periploside A has also been found significant in mice models^{8–10}, that validates the folkloric reputation of the plant as a traditional Chinese medicine for rheumatoid arthritis¹¹. Such autoimmune diseases as rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus and Crohn's disease, are notoriously malignant and refractory, thus efficacious new immunosuppressant drugs have long been a quest¹².

The FABO motif in periplosides, which has been found critical to their activities⁶, remains ambiguous in both the chemical connection and stereochemistry for over two decades. In 1987, Hikino *et al.* reported the first structural assignment of periploside A, in that the connection of the orthoester was proposed on the basis of extensive chemical degradation and spectroscopic analysis¹. However, the stereochemistry of the quaternary anomeric carbon was overlooked but drawn in a configuration comprising an unusual α -(1 \rightarrow 4)-glycosidic linkage (**1a**). Shortly afterwards, Itokawa *et al.*^{2–4} suggested a novel peroxy linkage (**1b**) for periplosides (and named as periplocosides), as misled by a conventional colour reaction of peroxides, although all the analytical data of periplocoside E were found identical to those of the previous periploside A (ref. 3). This peroxide structure was then accepted till 2011 (refs 5,6),

Zhao and coworkers acquired an X-ray diffraction of a single crystal of a periploside congener, confirming the originally assigned FABO connection⁶. Unfortunately, the stereochemistry of the spiro-quaternary carbon remained incorrect until we examined it carefully during our journey toward the synthesis of periploside A (ref. 7).

Herein, we report the first total synthesis of periploside A (**1**), employing total of 76 steps of transformations from glucal, methyl α -D-glucopyranoside and dehydroepiandrosterone, with the longest linear sequence of 29 steps and in 9.2% overall yield. The stereoselective construction of the FABO motif with either the natural or unnatural configuration at the anomeric spiro-quaternary center is achieved via alternative combination of Crich's protocol for acetal glycoside synthesis and Sinay's protocol for glycosyl orthoester formation. The 2-deoxy- β -glycosidic linkages are synthesized by glycosylation with trifluoroacetimidate donors (**6/27**) equipped with 2-iodide, with digitoxosyl *ortho*-alkynylbenzoate donor (**8**) installed with bulky TBDPS group on the remote 3,4-OH, and with cymarosyl *ortho*-alkynylbenzoate donor (**2**) under the promotion of Ph_3PAuOTf /TTBP. In addition, a preliminary structure–activity relationship of the synthetic periplosides against the proliferation of T-lymphocyte is provided, indicating the importance of the chemical connection of the FABO motif to the activities.

Results

Retrosynthetic analysis. Given the big size and linear structure of the target molecule, a convergent synthesis was desired¹³. Thus, periploside A (**1**) was disconnected into two fragments of similar complexity, that is, tetrasaccharide donor **2** and pregnane FABO disaccharide **3** (Fig. 1). The (1 \rightarrow 4)-cymarosyl linkage between

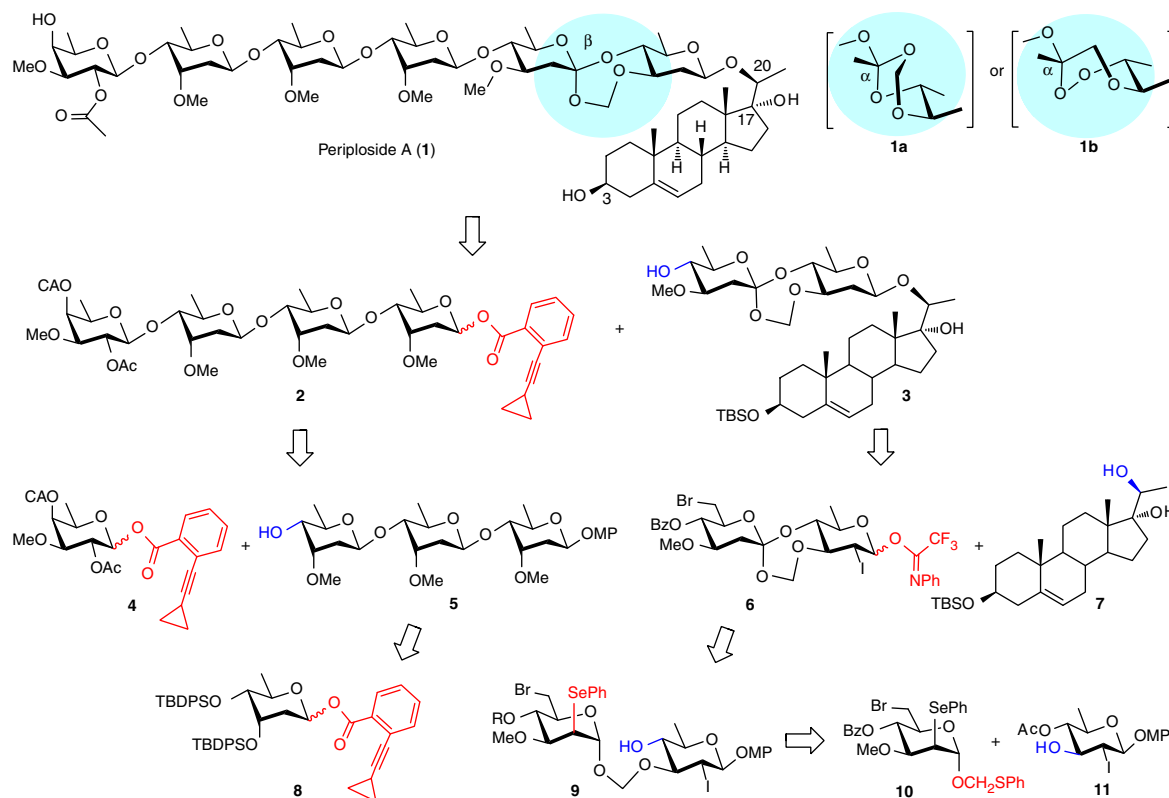


Figure 1 | Periploside A **1 and the retrosynthetic analysis.** Shaded in pale blue are the FABO motifs that have been previously assigned; highlighted in red are leaving groups in donors and in blue are hydroxyl groups in acceptors. Ac, acetyl; CA, chloroacetyl; MP, 4-methoxyphenyl; TBDPS, *tert*-butyldiphenylsilyl; TBS, *tert*-butyldimethylsilyl.

the tetrasaccharide unit and **3** would be difficult to construct in favour of formation of the thermodynamically unfavored β -anomer^{14–17}; the glycosylation protocol with *ortho*-alkynylbenzoates as leaving groups (as in **2**) under the catalysis of a gold(I) complex could address this problem, in that substitution via an α -glycosyloxypyrylium or α -triflate intermediate might get invoked¹⁸. The β -(1 \rightarrow 4)-cymarosyl linkages in tetrasaccharide **2**, however, could be built stereoselectively under the influence of a substituent at the axial 3-OH of a digitoxosyl donor, which could subsequently be converted into the required methyl group. In this respect, we had screened carefully during the synthesis of digitoxin and gordonoside F and found gold(I)-catalysed glycosylation with digitoxosyl *ortho*-cyclopropylethynylbenzoate equipped with two bulky TBDPS groups at 3,4-OH (that is, **8**) to be an optimal choice^{15,17}. After furnishing the cymarose trisaccharide **5**, its coupling with the terminal digitalose unit could be realized with donor **4**, in that the formation of the β -linkage should be secured by participation of the 2-*O*-acetyl group; and the 4-*O*-chloroacetyl group was selectively removable afterwards. The *p*-methoxyphenyl (MP) group was employed throughout (as in **5**, **9** and **11**) as the anomeric protecting group, which could remain intact before cleavage and subsequently converting the saccharides into donors (for example, **2** and **6**).

Uncertainty lies in the synthesis of FABO disaccharide **3**. Condensation of 3 β -silyloxy-pregnene-17 α ,20 α -diol **7** with disaccharide trifluoroacetimidate **6**, one of the most reliable type of glycosylation donors¹⁹, which was equipped with an equatorial iodide at C2 (ref. 20), would lead to the desired C20-*O*- β -glycoside **3** in a regio- and stereo- selective manner. However, few clues occur in the literatures, which could lead to the construction of a disaccharide precursor consisting of the FABO linkage. The most promising approach turned out to be a combination of Crich's protocol to form the formyl acetal glycosidic linkage

(from a thiomethyl glycoside donor such as **10**; ref. 21) and Sinaý's protocol to synthesize the anomeric orthoester via a ketene acetal intermediate (derived from a 2-selenoglycoside such as **9**; refs 22–24). However, the tolerance of the formyl acetal (in **9**) towards the strong conditions required for Sinaý's oxidation–elimination–cyclization sequence and the stereochemistry in the formation of the seven-membered spiro-orthoester were beyond estimation.

Synthesis of tetrasaccharide donor 2. The desired digitalosyl and digtoxosyl *ortho*-cyclopropylethynylbenzoates **4** and **8** were prepared in a robust manner from D-fucose (13 steps and 37% overall yield) (see Supplementary Methods) and methyl α -D-glucopyranoside (10 steps and 47% overall yield)¹⁵, respectively. Subjection of **8** to glycosidation with *p*-methoxyphenol under optimized conditions (0.1 equivalent (equiv) Ph₃PAuNTf₂, toluene, 4 Å molecular sieves (MS), –40 °C) led to β -glycoside **12** as the sole anomer in an excellent 95% yield (Fig. 2). Removal of the 3,4-di-*O*-TBDPS group followed by selective acetylation of the axial 3-OH via orthoester formation/hydrolysis provided glycoside **13** bearing a free 4-OH (92%, two steps)²⁵. Similar transformations were then applied to extend monosaccharide **13** to trisaccharide **17**, in that the glycosidation of **8** with monosaccharide acceptor **13** and disaccharide acceptor **15** led to the corresponding coupled 2-deoxy- β -saccharides nearly quantitatively. Trisaccharide 3'',4''-diol **17** was then subjected to a tin-mediated selective benzylation on the equatorial 4''-OH (ref. 26); cleavage and scramble of the acetyl groups on the axial hydroxyl groups were detected, nevertheless, exposure of the resultant mixture to LiOH afforded **18** in excellent yield (87%, three steps). The three axial hydroxyl groups in **18** were then methylated (99%). Unexpectedly, hydrogenolysis of the 4''-*O*-benzyl group in trisaccharide **19** under conventional conditions

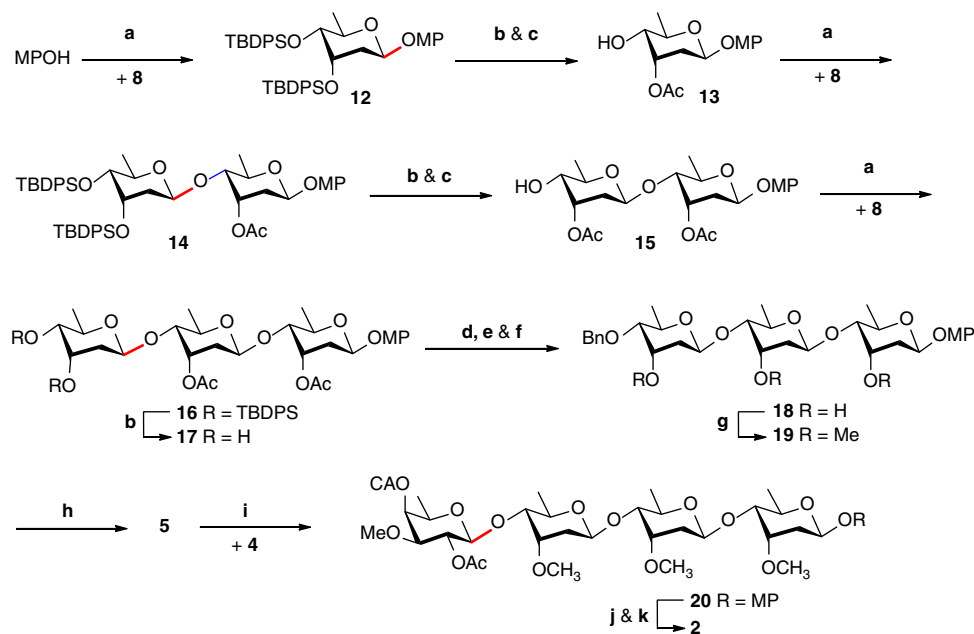


Figure 2 | Synthesis of tetrasaccharide donor 2. Highlighted in red are the nascent glycosidic bonds. (a) Ph₃PAuNTf₂ (0.1 equiv), toluene, 4 Å MS, –40 °C; 95% and β only (for **12**); 99% and β only (for **14** and **16**); (b) TBAF, THF, 0 °C to RT; 99% (from **12**); 94% (from **14**); 96% (from **16**); (c) CH₃(OMe)₃, *p*-TsOH, RT, 93% for (**13**); 91% (for **15**); (d) ⁿBu₂SnO, MeOH, reflux; (e) BnBr, DMF, CsF, RT; (f) LiOH, THF, H₂O, RT; 87% (for three steps); (g) MeI, NaH, DMF, 0 °C to RT, 99%; (h) Pd(OH)₂/C, H₂ (1 atm), Et₃N, EtOAc, MeOH, 50 °C, 93%; (i) Ph₃PAuNTf₂ (0.2 equiv), toluene, 5 Å MS, –50 °C to RT, 93%, $\beta/\alpha = 4/1$; (j) Ag(DPAH)₂, CH₃CN, H₂O, 0 °C to RT, 95%; (k) *o*-cyclopropylethynylbenzoic acid, EDCl, DMAP, 4 Å MS, CH₂Cl₂, RT, 99%. DMAP, 4,4-dimethylaminopyridine; DPAH, bis(hydrogen dipicolinate); EDCl, *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride; MS, molecular sieves; TBAF, tetrabutylammonium fluoride.

(Pd(OH)₂/C, H₂, room temperature (RT)) led to cleavage of the glycosidic linkages, testifying the vulnerability of the cymarosyl-β-(1 → 4)-linkage. Thus, Et₃N was added to sequester any nascent proton; the hydrogenolysis proceeded sluggishly, nevertheless, leading to the desired trisaccharide **5** in a satisfactory 93% yield at 50 °C for 2 days.

Given the acid lability of the cymarosyl-β-(1 → 4)-linkage, it was not fully surprising to find that glycosylation of trisaccharide acceptor **5** with digitalosyl donor **4** in the presence of Ph₃PAuOTf led to considerable cleavage of the trisaccharide. Ph₃PAuNTf₂ was found to be more stable towards moisture (thus less acidic)²⁷, therefore, it catalysed the condensation of **5** and **4** smoothly. However, in spite of the presence of 2-*O*-acetyl group in donor **4**, the glycosylation led to anomeric mixtures of the coupled tetrasaccharide. Under optimized conditions (0.2 equiv Ph₃PAuNTf₂, toluene, 5 Å MS, −50 °C to RT), tetrasaccharide **20** was obtained in 93% yield with β/α ratio of 4:1, which was separable on silica gel column chromatography. Removal of the anomeric MP group in tetrasaccharide **20** with CAN was not successful under a variety of conditions, resulting unavoidably degradation of the cymarosyl-β-(1 → 4)-linkages. Fortunately, a mild oxidizing agent, Ag(DPAH)₂, could achieve this task (CH₃CN/H₂O, 0 °C to RT) to afford smoothly the corresponding hemiacetal (95%) (ref. 28), which was then subjected to the formation of *ortho*-cyclopropylethynylbenzoate **2** (99%) (see Supplementary Methods).

Attempts at construction of the FABO motif. We have tried a number of approaches to the construction of the FABO disaccharide motif, those include condensation of sugar lactones with vicinal diols in the presence of formaldehyde or its surrogates^{29,30}, transesterification of sugar lactones with sugar 4-hydroxyl-3-methoxymethyl ether derivatives³¹, and intramolecular hydrogen atom transfer reactions of formyl acetal linked disaccharides^{21,32,33}. However, no coupled FABO products have ever been detected, that prompted us to focus our attention on the planned Crich–Sinaÿ approach (Fig. 3). Thus, starting from *D*-glucal triacetate, 3-*O*-methyl-6-bromo-glucal **21** and *p*-methoxyphenyl 2,6-dideoxy-2-iodo-glucoside **11** were prepared in seven steps (66% overall yield) and 13 steps (18% overall yield), respectively (see Supplementary Methods). Glucal **21** was converted into the desired phenylthiomethyl 2-phenylseleno-α-*D*-mannopyranoside **10** in three steps (45%), that is, addition with PhSeCl to provide 2-phenylseleno-*D*-mannopyranose **22**, conversion of the lactol into fluoride with DAST, and subsequent glycosidation with PhSCH₂OH in the presence of SnCl₂ (refs 23,34). Condensation of **10** with sugar acceptor **11** under Crich's conditions (NIS, TfOH, CH₂Cl₂, −30 °C) did lead to the desired acetal glycoside **24**, albeit in only a moderate yield of ~30%; the major product turned out to be the α-glycoside **23** (~65%). A wide variety of the promoters for glycosidation of thioglycosides³⁵ were screened in the present coupling (see Supplementary Table 1), including NBS/TfOH³⁶, Tf₂O/BSP/TTBP³⁷, DMTST/TTBP³⁸, MeOTf³⁹ and NIS/AgOTf⁴⁰. However, the yield of acetal glycoside **24** was not improved. These results indicate that the transient glycosyloxy-methyl cation (generated from **10**) decomposes favourably to the glycosyl oxocarbenium species (and a molecule of formaldehyde); capture of the oxocarbenium intermediate by the incoming hydroxyl group (in **11**) leads to disaccharide **23**. Adjustment of the substituting groups in donor **10**, that is, removal of the 6-bromide and/or replacement of the 4-*O*-benzoyl group with benzyl group also failed to improve the yields of the corresponding acetal glycosides in condensation with **11**. Gratifyingly, when we applied the inverse procedure⁴¹ (addition

of donor **10** to a mixture of the acceptor **11** and promoter NIS/TfOH at −30 °C) and increased the equivalent of acceptor **11** (to 5.0 equiv), acetal glycoside **24** was obtained in a satisfactory 75% yield. The 4-*O*-acetyl and 4'-*O*-benzoyl group in **24** were removed simultaneously in the presence of MeONa in MeOH, providing diol **9** (93%), which was ready for Sinaÿ's orthoester formation.

Thus, 2'-seleno-disaccharide **9** was subjected to oxidation (NaIO₄, NaHCO₃, MeOH/CH₂Cl₂/H₂O, RT) to provide the selenoxide cleanly, subsequent *syn*-elimination and intramolecular cyclization of the resultant ketene acetal (**A**) took place sluggishly under forced conditions (vinyl acetate/toluene/DIPA, 140 °C), leading to an orthoester product (**25**) in a moderate 20% yield for 12 h. By carefully screening the reaction conditions, we finally managed to obtain **25** in an excellent 85% yield under microwave irradiation (145 °C, 20 min). The addition of the 4-hydroxyl group onto C1' of the ketene acetal in **A** could proceed from both the top and the bottom faces, thus two diastereoisomers should be provided. Surprisingly, only one isomer (**25**) was isolated under various conditions. We had expected that the diastereoselectivity bestowed by the two sugar units could lead to formation of the native configuration in the natural product (opposite to that drawn in **25**). However, in the previous synthesis of the Sinaÿ-type five- and six-membered orthoesters, cyclization of the incipient ketene acetals was found to favour formation of the axial α-linkage, so as to maximize the anomeric effect^{22–24} or to follow the trajectory of addition onto Woerpel's low-energy conformer of the dioxocarbenium intermediate^{42,43}. Determination of the configuration of the quaternary C1' in **25** was not possible by spectroscopic methods, especially without comparison to its epimer, and attempts at acquisition of a single crystal of **25** or its derivatives for X-ray diffraction analysis were unsuccessful. Therefore, this problem was not solved until completion of the total synthesis.

Synthesis of C1''-epi-periploside A (31). Temporary protection of the 4'-OH in **25** with benzoyl group led to **26** (99%), which was subjected to selective cleavage of the anomeric MP group (Ag(DPAH)₂, CH₃CN, H₂O, 90%) and subsequent formation of trifluoroacetimidate (95%) to afford the desired donor **27** (Fig. 3). Glycosylation of pregnane diol **7**, which was prepared from hydroxyandrost-5-en-17-one in three steps (see Supplementary Methods and Supplementary Fig. 59), with disaccharide imidate **27** proceeded smoothly under the catalysis of TBSOTf (0.1 equiv) in the presence of 5 Å MS at −78 °C in CH₂Cl₂, affording pregnane 20-*O*-disaccharide **28** in 85% (96%, b.r.s.m.) yield and β/α ratio of 3:1. The erosion of 1,2-*trans*-selectivity in the glycosylation with a donor (that is, **27**) equipped with an iodide at C2 could be contributed to the presence of the FABO motif, which might cause conformational restraint for the neighbouring participation. Notably, the present reaction must be quenched at −78 °C (with Et₃N), so as to avoid the decomposition of the acid-labile FABO motif. Reductive removal of the bromide and iodide in **28** under the conventional radical conditions (AIBN/Bu₃SnH, toluene, 80 °C) led to decomposition of the substrate. Et₃B could initiate the radical reaction at RT⁴⁴, which thus enabled the reduction of **28** with Bu₃SnH to proceed smoothly to afford the corresponding deoxydisaccharide (99%). Subsequent removal of the 4'-*O*-benzoyl group provided the desired pregnane 20-*O*-disaccharide **29** (99%).

The expected challenge in stereoselective construction of the 2-deoxy-β-cymarosyl-(1 → 4)-linkage in the union of tetrasaccharide **2** and pregnane disaccharide **29** was further manifested by the fact that both the 2,6-dideoxyglycosidic linkages and the FABO motif were shown to be extremely labile toward acid. Thus, we screened carefully the coupling conditions, including the gold(I)

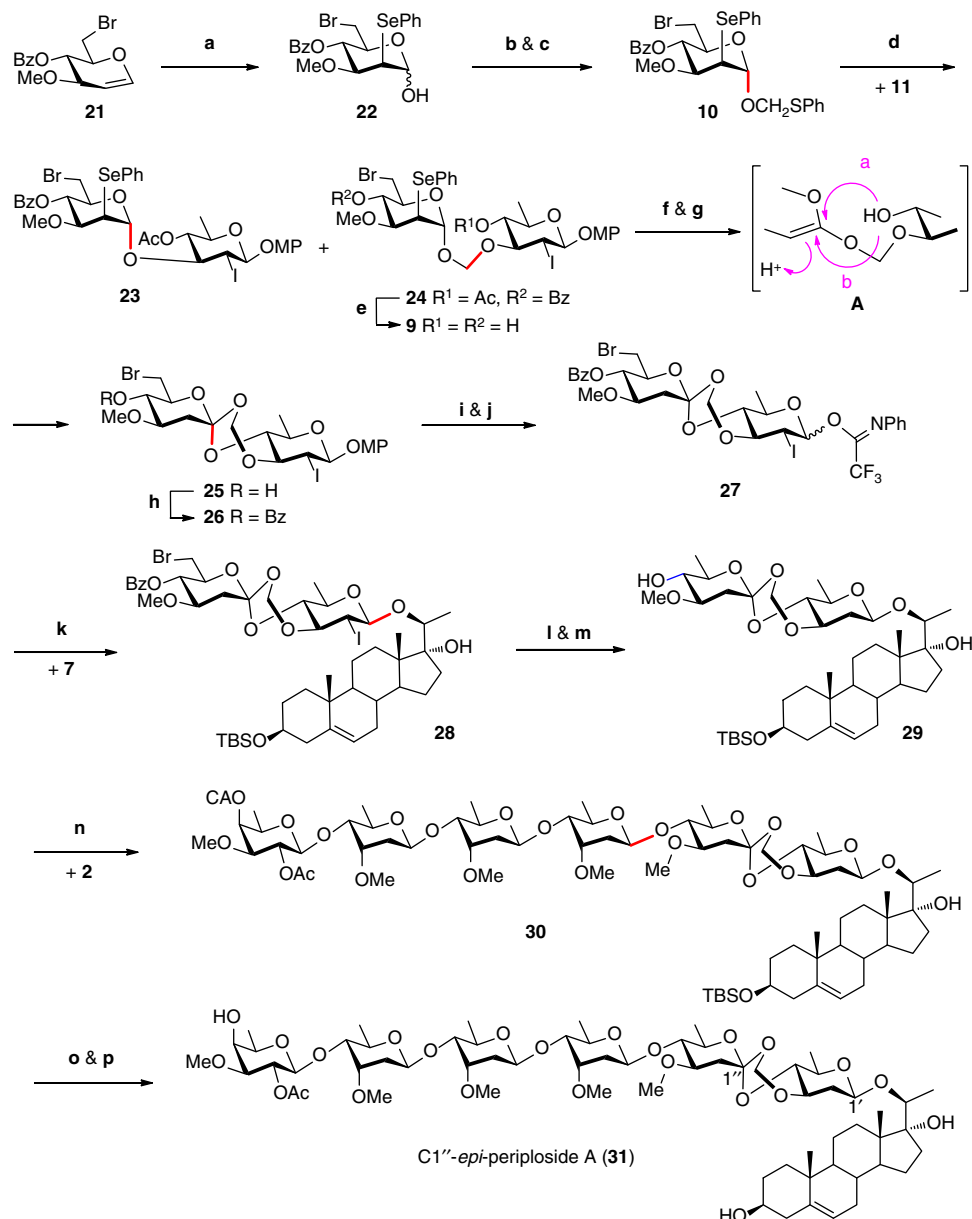


Figure 3 | Construction of the FABO motif and synthesis of C1''-epi-periploside A (31). Highlighted in red are the nascent glycosidic bonds. (a) PhSeCl, CH₃CN, -40 °C, 78%; (b) DAST, THF, -30 °C to RT; (c) PhSCH₂OH, SnCl₂, CH₂Cl₂, 4 Å MS, -40 °C to RT; 58% (for two steps); (d) NIS, TfOH, CH₂Cl₂, -30 °C, 75% (for **24**), 20% (for **23**); (e) MeONa, MeOH, CH₂Cl₂, RT, 93%; (f) NaIO₄, NaHCO₃, MeOH, CH₂Cl₂, H₂O, RT, 99%; (g) vinyl acetate, toluene, DIPA, Mw, 145 °C, 20 min, 85%; (h) BzCl, Et₃N, DMAP, CH₂Cl₂, 0 °C to RT, 99%; (i) Ag(DPAH)₂, CH₃CN, H₂O, 0 °C to RT, 90%; (j) *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride, Cs₂CO₃, CH₂Cl₂, RT, 95%; (k) TBSOTf, CH₂Cl₂, 5 Å MS, -78 °C, 85%, β/α = 3/1; (l) Et₃B, Bu₃SnH, toluene, RT, 97%; (m) MeONa, MeOH, CH₂Cl₂, RT, 99%; (n) Ph₃PAuOTf (0.8 equiv), TTBP, CH₂Cl₂, 4 Å MS, -20 °C to -10 °C, 64% (87% b.r.s.m.), β/α = 2.1/1; (o) thiourea, pyridine, EtOH, 80 °C; (p) HF·py, pyridine, THF, 0 °C to RT; 91% (for two steps). DAST, (diethylamino)sulfur trifluoride; DIPA, diisopropylamine; TBSOTf, *tert*-butyldimethylsilyl trifluoromethane sulfonate; Mw, microwave; TTBP, 2,4,6-tri-*tert*-butylpyrimidine.

catalyst, solvent and temperature. When the coupling of **2** and **29** was conducted in the presence of Ph₃PAuNTf₂ (0.2 equiv), the coupled hexasaccharide was obtained in good yield (50%) but in favour of the thermodynamically more stable α-anomer (β/α ~ 1:3). With Ph₃PAuOTf as the catalyst, however, the reaction of **2** and **29** provided a messy mixture. Careful isolation led to identification of a pentasaccharide lactone, which was derived from cleavage of the FABO linkage in the coupled hexasaccharide (**30**). Encouragingly, the originally formed cymarosyl-(1 → 4)-linkage in the resultant pentasaccharide lactone was found in the required β configuration, implying that the

glycosylation reaction took place via a glycosyl α-triflate intermediate¹⁸. Nevertheless, the HOTf generated after glycosylation, before being quenched by the isochromen-4-yl gold(I) complex⁴⁵, degraded the FABO linkage. On the basis of this rationale, we introduced a hindered base, that is, 2,4,6-tri-*tert*-butylpyrimidine (TTBP), into the present Ph₃PAuOTf-catalysed glycosylation to rest the incipient HOTf, so as to avoid the cleavage of the FABO linkage. The sequester of the HOTf also retarded the gold(I) catalytic cycle, therefore, requiring almost equivalent of the gold complex to drive the reaction to completion^{45,46}. In fact, the condensation of **2** and **29** proceeded

smoothly in the presence of Ph_3PAuOTf (0.8 equiv) and TTBP (1.5 equiv) in CH_2Cl_2 at -10°C , leading to the coupled hexasaccharide **30** in a satisfactory yield of 64% (87%, b.r.s.m.) and β/α ratio of 2.1:1. Finally, removal of the terminal CA group and TBS group was achieved with thiourea⁴⁷ and pyridine-buffered HF·pyridine, respectively, furnishing the target pregnane hexasaccharide **31** cleanly (91%, two steps).

The ^1H and ^{13}C NMR spectra of **31** are similar to those of the authentic periploside A (**1**) (see Supplementary Figs 60 and 61). However, discrepancies occur for the signals from the formyl acetyl CH_2 residue (88.4 p.p.m. for the ^{13}C signal in **31** versus 86.4 p.p.m. in **1**; 4.93 and 4.82 p.p.m. for the ^1H signals in **31** versus 5.13 and 4.74 p.p.m. in **1**) (see Supplementary Table 3). In addition, the adjacent $\text{C}4'$, $\text{C}2''$ and $\text{C}3''$ are also in disparate chemical shifts ($\text{C}4'$: 81.5 versus 79.2 p.p.m.; $\text{C}2''$: 37.9 versus 36.7 p.p.m.; $\text{C}3''$: 77.4 versus 78.3 p.p.m., in **31** and **1**, respectively). These discrepancies indicate that the synthetic **31** is indeed the epimer of periploside A (**1**) with the opposite configuration at the quaternary $\text{C}1''$. The problem of diastereoselectivity in the previous formation of the FABO motif is thus addressed.

Construction of the FABO motif of the natural configuration.

The addition of the hydroxyl group onto the ketene acetal in **A** proceeded exclusively from the α face, leading to the FABO disaccharide with the unnatural configuration (**9**→**25**). The presence of a nucleophile capable of interception of the oxocarbenium species developed from the ketene acetal might lead to a thermodynamically favored α -intermediate; substitution of the intermediate *in situ* by the 4-hydroxyl group would then proceed from the β face to provide the FABO disaccharide with the natural configuration. Therefore, we tried the elimination/cyclization (from **9**) in various nucleophilic solvents, including CH_3CN , 1,4-dioxane, THF, DME, DMF and MeOH, under varied

temperatures; however, the only FABO disaccharide identified was **25**. Addition of nucleophilic additives, such as 4-dimethylaminopyridine (DMAP), LiBr, NaBr and NaI, was also found futile. The presence of an electrophilic reagent, such as NIS, NBS and I_2 , might convert the ketene acetal into a 1,2-halonium intermediate, which could then be attacked by the 4-hydroxyl group to give the FABO disaccharide. These attempts were again unsuccessful.

The failure in formation of the seven-membered orthoester in the desired stereochemistry forced us to construct the orthoester before cyclization of the formyl acetal (Fig. 4). Thus, 2-phenylseleno-D-mannopyranoside **22** was converted into the fluoride (with DAST), which was then coupled with sugar acceptor **32** (see Supplementary Methods) under the action of SnCl_2 to give α -disaccharide **33** (85%). Gratifyingly, after intensive attempts, we managed to obtain the ketene acetal **34** in excellent yield (91%) from disaccharide **33** via selenoxide formation and *syn*-elimination under the modified Sinay conditions (NaIO_4 , NaHCO_3 , $\text{MeOH}/\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$, RT; then vinyl acetate/toluene/DIPA, microwave 140°C , 40 min). Although purification of **34** on silica gel required the addition of 1% Et_3N in the eluent to prevent hydrolysis, the purified ketene acetal **34** remained stable at -20°C for several days. To the best of our knowledge, this is the first time a ketene acetal linked disaccharide (that is, **34**) being isolated. Noteworthy is the decisive role played by the 3-O-Lev group (in **33**–**35**), which facilitated the procurement of the ketene acetal disaccharide as well as the selective removal afterwards; in contrast, analogues of **33** bearing an electron-donating group (for example, TBS) at the 3-OH led to decomposed monosaccharide derivatives under identical conditions.

Addition of PhSCH_2OH onto ketene acetal **34** was first attempted in CDCl_3 at 50°C (ref. 48), the starting ketene acetal decomposed largely in 12 h, with the desired orthoester being isolated in low yield (20%) as a single α -isomer. By raising the

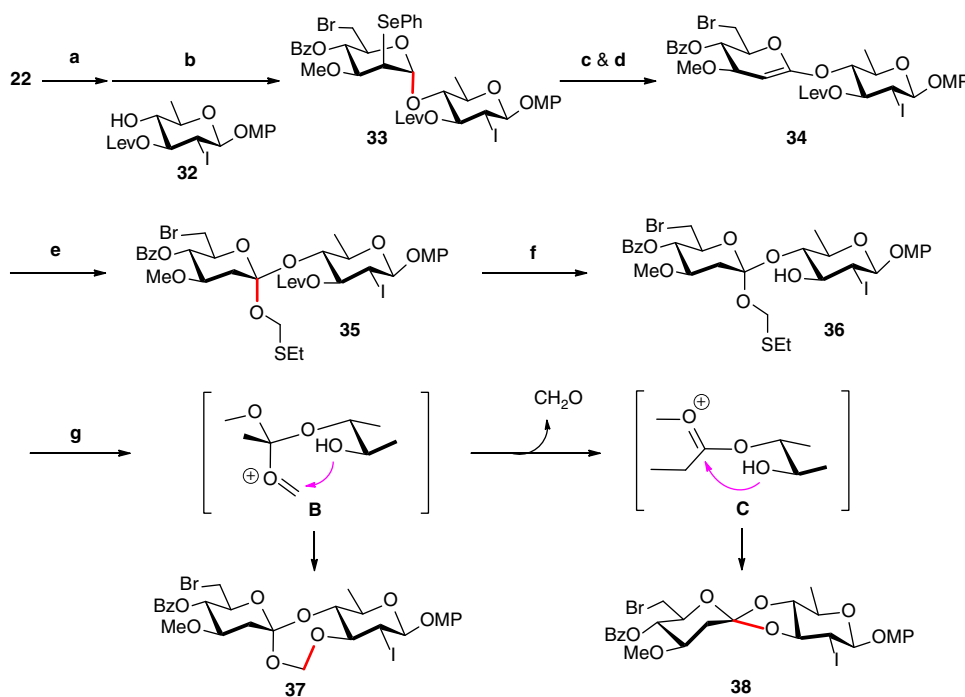


Figure 4 | Construction of the FABO motif with the natural configuration. Highlighted in red are the nascent glycosidic bonds. (a) DAST, THF, -30°C to RT; (b) SnCl_2 , Et_2O , 4 Å MS, 0°C to RT; 85% (for two steps); (c) NaIO_4 , NaHCO_3 , MeOH , CH_2Cl_2 , H_2O , RT, 99%; (d) vinyl acetate, toluene, DIPA, Mw, 140°C , 40 min, 92%; (e) EtSCH_2OH , DCCl_3 , Mw, 10 min, 110°C , 81%; (f) $\text{H}_2\text{NNH}_2 \cdot \text{H}_2\text{O}$, pyridine, HOAc, 0°C to RT, 92%; (g) BSP, Tf_2O , DTBP, 5 Å MS, Et_2O , -114°C , 64% (for **37**), 18% (for **38**). BSP, 1-benzenesulfinyl piperidine; DTBP, 2,6-di-*tert*-butylpyridine; Lev, levuloyl; Mw, microwave.

temperature and shortening the reaction duration (CDCl₃, microwave 100 °C, 1 h), the addition of **34** and PhSCH₂OH led to the desired orthoester in a good 70% yield but in a moderate diastereoselectivity of 1.4/1. Modification of the reaction conditions by the addition of acidic promoters (*p*-TsOH or PPTS) or variation of solvent (toluene, CH₂Cl₂ or CHCl₃) failed to improve the yield or the diastereoselectivity. Fortunately, replacement of PhSCH₂OH with the more nucleophilic EtSCH₂OH, the addition with ketene acetal **34** under the optimal conditions (CDCl₃, microwave 110 °C, 10 min) furnished the desired orthoester **35** in 81% yield as a single α -isomer. It should be noted that the replacement of CDCl₃ with CHCl₃ or toluene led to lower yields of **35** (<65%). Selective removal of the 3-*O*-Lev group in **35** was achieved with H₂NNH₂·H₂O to provide orthoester **36** (92%).

Treatment of ethylthiomethyl glycoside **36** with the mild promoters for glycosidation of thioglycosides (see Supplementary Table 2), such as MeOTf/TTBP, DMTST/TTBP, and CuBr₂/Bu₄NBr⁴⁹, failed to afford the seven-membered FABO derivative **37**; instead, the five-membered orthoester **38** was isolated in high yield (>80%) as a mixture of the two diastereoisomers. These results indicate again (*cf.*, **10**→**23/24**) that the glycosyloxymethyl cation (**B**) generated via activation of the thioacetal could readily undergo decomposition to the glycosyl oxocarbenium (**C**) and formaldehyde; intramolecular addition of the proximal hydroxyl group to **C** led to the five-membered orthoester. Thus, a strong promoter which can activate the thioacetal under milder conditions might be able to allow the transient glycosyloxymethyl cation **B** to be captured by the hydroxyl group before decomposition. Using Crich's conditions (Tf₂O/BSP/TTBP, -60 °C, CH₂Cl₂)³⁷, however, the five-membered orthoester **38** was again obtained nearly quantitatively as a mixture of the diastereoisomers. Encouragingly, when lowering the reaction temperature to -78 °C, a trace amount of the desired seven-membered FABO product **37** was detected. Optimizing along this direction, we finally managed to obtain the desired **37** in a

satisfactory 64% yield in the presence of Tf₂O/BSP/DTBP in Et₂O at -114 °C, whereas the five-membered orthoester **38** was isolated in 18% yield as a single diastereoisomer. Comparison of the NMR data of the present FABO disaccharide **37** with those of the previous **26** supported that we had fixed the desired configuration by the present approach.

Completion of the synthesis of periploside A (1). The transformations previously developed for the synthesis of C1''-*epi*-periploside A (**26**→**31**, eight steps, 28% overall yield) were applied to the synthesis of periploside A (Fig. 5). Thus, FABO disaccharide **37** was subjected to selective cleavage of the anomeric MP group (with Ag(DPAH)₂, 91%) and conversion into the trifluoroacetimidate donor **6** (90%). Coupling of pregnane diol **7** with donor **6** under the similar conditions as that for **7**+**27**→**28** (85%, β/α =3:1) led to the corresponding disaccharide **39** in a similar yield (87%) but much higher β selectivity (β/α =6.1:1). This result supports the previous assumption that the conformational restraint provided by the FABO motif affects the glycosidation transition state of the disaccharide. The bromide and iodide in **39** were removed cleanly with Et₃B/Bu₃SnH (95%); subsequent cleavage of the 4'-*O*-benzoyl group (NaOMe, HOME, 96%) provided the desired disaccharide acceptor **3**. Condensation of **3** with tetrasaccharide donor **2** under the optimized conditions for **29**+**2**→**30** (64%, β/α =2.1:1) afforded hexasaccharide **40** in a higher 80% (92%, *b.r.s.m.*) yield and similar β/α ratio of 2:1. Finally, the terminal CA and TBS groups were removed successfully with thiourea and pyridine-buffered HF-pyridine, respectively, furnishing the target periploside A (**1**) (93%, two steps). Gratifyingly, all the analytical data of **1** are in full agreement with those obtained for the natural product¹ (see Supplementary Table 3).

The immunosuppressive activities of the synthetic compounds. The synthetic periploside A (**1**) showed similar toxicity and

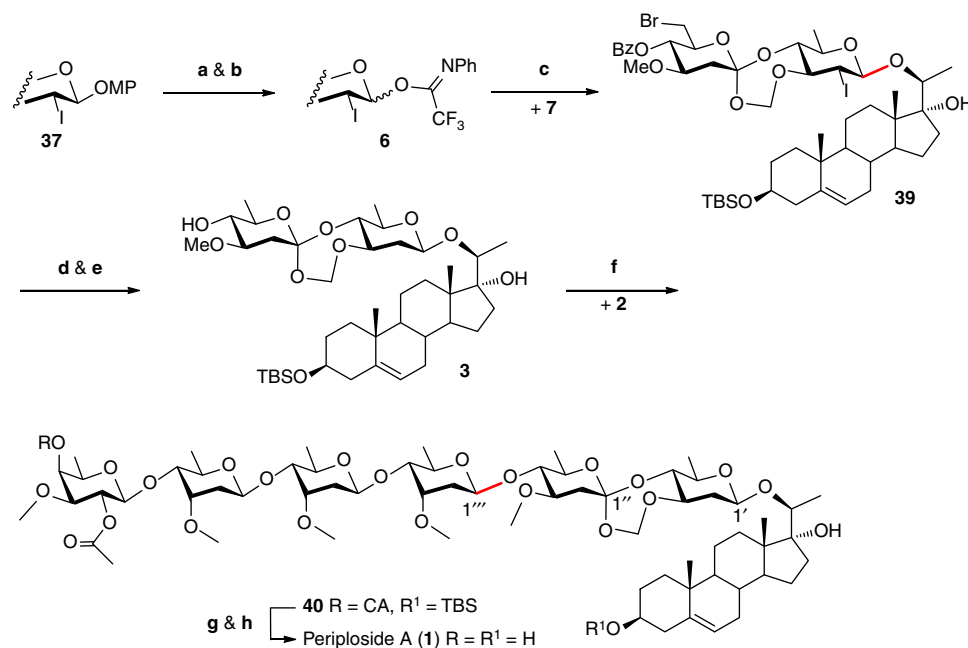


Figure 5 | Completion of the total synthesis of periploside A (1). Highlighted in red are the nascent glycosidic bonds. (a) Ag(DPAH)₂, CH₃CN, H₂O, 0 °C, 91%; (b) *N*-phenyl-trifluoroacetimidoyl chloride, Cs₂CO₃, CH₂Cl₂, RT, 90%; (c) TBSOTf, CH₂Cl₂, 5 Å MS, -78 °C, 87%, β/α =6.1/1; (d) Et₃B, Bu₃SnH, toluene, RT, 95%; (e) MeONa, MeOH, CH₂Cl₂, RT, 96%; (f) Ph₃PAuOTf (0.8 equiv), TTBP, CH₂Cl₂, 4 Å MS, -10 °C, 80%, β/α =2/1; (g) thiourea, pyridine, EtOH, 80 °C; (h) HF·py, pyridine, THF, 0 °C to RT, 93% (for two steps).

Table 1 | Inhibitory activity of periploside A and analogues against T-lymphocyte proliferation*.

Compound	CC ₅₀ (μM)	IC ₅₀ (μM)	SI
Periploside A (1)	2.10 ± 0.32	0.17 ± 0.04	12
C1''-epi-periploside A (31)	1.58 ± 0.19	0.41 ± 0.15	3.9
C1'''-epi-periploside A (41)	11.40 ± 3.66	1.96 ± 0.43	5.8
Disaccharide 42	12.80 ± 0.52	6.20 ± 3.20	2.1
Disaccharide 43	27.70 ± 6.61	3.70 ± 2.25	7.5

*See Supplementary Methods and Supplementary Figs 62 and 63 for details.

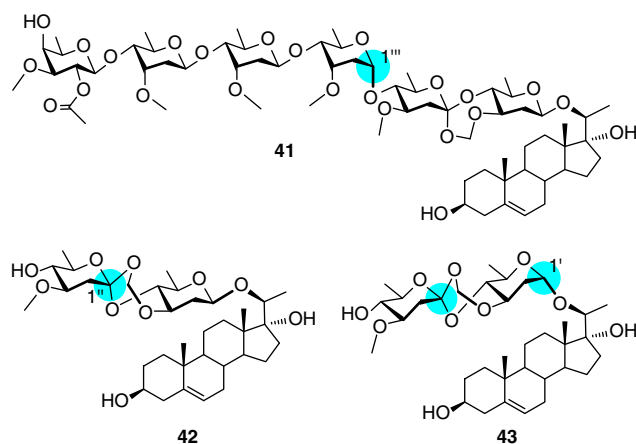


Figure 6 | Synthetic analogues of periplosides. Shaded in pale blue are the carbon centres with opposite configurations to those in the nature product.

activity as the natural product against T-lymphocyte and their proliferation induced by ConA^{5,6}. The C1''-epimer (**31**) which has the opposite configuration at the FABO motif as in periploside A was also found active (IC₅₀ = 0.41 μM), although being ~2.4-fold less potent than the natural product (Table 1). The C1'''-epimer of periploside A (**41**) was also active (IC₅₀ = 1.96 μM) (Fig. 6). Interestingly, disaccharide **42**, with the tetrasaccharide in **31** being truncated, still had certain activity (IC₅₀ = 6.20 μM); and its α-anomer **43** even showed a higher activity (IC₅₀ = 3.70 μM). Previous assays have demonstrated that reductive cleavage of the formyl acetal in the FABO motif in periploside A abolished the activity completely⁶. Taken together, it demonstrates that the chemical connection of the FABO motif, rather than the stereochemistry, has a critical role in the immunosuppressive activities of periplosides. The capability of releasing a formylaldehyde might be taken into account in the future researches into the mechanism of action of this unique type of compounds.

Methods

General methods. All the reactions were carried out under nitrogen or argon with anhydrous solvents in flame-dried glassware, unless otherwise noted. All the glycosylation reactions were performed in the presence of 4 or 5 Å MS, which were flame-dried immediately before use in the reaction under high vacuum. Glycosylation solvents were dried using a solvent purification system and used directly without further drying. The chemicals used were reagent grade as supplied, except where noted. For details of the synthetic procedures and the characterization data of compounds, see Supplementary Methods. For ¹H and ¹³C NMR spectra of the compounds prepared in this study, see Supplementary Figs 1–58.

Synthesis of acetal glycoside 24. To a solution of sugar alcohol **11** (620 mg, 1.47 mmol) in CH₂Cl₂ (5 ml) was added acid-washed 3 Å MS at RT. After stirring

for 30 min at RT, NIS (142 mg, 0.63 mmol) and TfOH (2.6 μl, 0.029 mmol) were added to the mixture at –30 °C, followed by the addition of a solution of phenylthiomethyl glycoside **10** (183 mg, 0.29 mmol) in CH₂Cl₂ (3 ml) via a syringe pump. The reaction mixture was stirred at –30 °C for 1 h. When TLC showed the donor **10** had been consumed, saturated aqueous NaHCO₃ was added at 0 °C. The mixture was filtered. The filtrate was washed with a solution of Na₂O₃ and brine, respectively, and was then dried over Na₂SO₄ and concentrated. The residue was purified by flash chromatography (petroleum ether/CH₂Cl₂/EtOAc = 8:1:1) to afford **24** (206 mg, 75%) and glycoside **23** (53 mg, 20%) as syrups.

Synthesis of FABO disaccharide 25. To a solution of acetal glycoside **9** (187 mg, 0.24 mmol) in MeOH/CH₂Cl₂/H₂O (3 ml/2 ml/1 ml) were added NaIO₄ (507 mg, 2.37 mmol) and NaHCO₃ (159 mg, 1.90 mmol) at RT. After stirring for 3 h, the mixture was diluted with CH₂Cl₂, washed with saturated NH₄Cl solution and brine, respectively, and was then dried over Na₂SO₄ and concentrated. The resultant selenoxide was azeotroped with toluene (3 × 5 ml) and dried under high vacuum for 2 h to afford a white solid (190 mg, 99%). The above selenoxide (125 mg, 0.155 mmol) was dissolved in toluene (6 ml). Diisopropylamine (3 ml) and vinyl acetate (6 ml) were added, and the reaction was conducted under microwave at 145 °C for 20 min. The mixture was cooled to RT and concentrated. The residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc = 1.5:1) to afford **25** (83 mg, 85%) as a colourless syrup.

Synthesis of α-disaccharide 33. To a solution of lactol **22** (603 mg, 1.21 mmol) in THF (10 ml) was added DAST (0.44 ml, 3.63 mmol) at –30 °C. After stirring for 1.5 h while warming to RT, a saturated NaHCO₃ solution was added slowly to the mixture. The resulting mixture was extracted with CH₂Cl₂. The combined organic layer was washed with brine, dried over Na₂SO₄ and concentrated. The crude glycosyl fluoride was azeotroped with toluene (3 × 5 ml). After drying under high vacuum for 2 h, the above product was dissolved in Et₂O (10 ml), 4 Å MS (1.2 g) and sugar alcohol **32** (330 mg, 0.69 mmol) were added and the reaction mixture was stirred at 0 °C for 30 min. SnCl₂ (235 mg, 1.24 mmol) was added in one portion and the reaction mixture was allowed to warm to RT and stirred for 4 h. The mixture was quenched with Et₃N (1 ml) and filtered. The solution was diluted with EtOAc and washed with water. The water layer was extracted with EtOAc twice. The combined organic layer was washed with saturated NaHCO₃ solution and brine, respectively, and was then dried over Na₂SO₄ and concentrated. The residue was purified by flash chromatography (petroleum ether/EtOAc = 4:1) to afford **33** (560 mg, 85%) as a colourless syrup.

Synthesis of ketene acetal 34. To a solution of **33** (248 mg, 0.26 mmol) in MeOH/CH₂Cl₂/H₂O (3 ml/2 ml/1 ml) were added NaIO₄ (552 mg, 2.58 mmol) and NaHCO₃ (173 mg, 2.06 mmol) at RT. After stirring for 12 h at RT, the mixture was diluted with CH₂Cl₂, and washed with saturated NH₄Cl solution and brine, respectively. The organic layer was dried over Na₂SO₄ and concentrated. The crude selenoxide was azeotroped with toluene (3 × 5 ml) and dried under high vacuum for 2 h to afford a colourless syrup (251 mg, 99%). The above selenoxide (104 mg, 0.11 mmol) was dissolved in toluene (2 ml). Diisopropylamine (1 ml) and vinyl acetate (2 ml) were added. The reaction was conducted under microwave at 140 °C for 40 min. The mixture was cooled to RT and concentrated. The residue was purified by flash chromatography (petroleum ether/EtOAc = 5:1, containing 1% Et₃N) to afford **34** (85 mg, 92%) as a colourless syrup.

Synthesis of orthoester 35. To a solution of **34** (75 mg, 0.093 mmol) in CDCl₃ (3 ml) was added EtSCH₂OH (0.05 ml) at RT. The reaction was conducted under microwave at 110 °C for 10 min. The mixture was cooled to RT and concentrated. The residue was purified by flash chromatography (petroleum ether/EtOAc = 5:1, containing 1% Et₃N) to afford **35** (67 mg, 81%) as a colourless syrup.

Synthesis of orthoester 36. To a solution of **35** (110 mg, 0.120 mmol) in pyridine/HOAc (3 ml/2 ml) was added H₂NNH₂·H₂O (0.10 ml, 1.60 mmol) at 0 °C. After stirring at RT for 5 h, the mixture was diluted with CH₂Cl₂, and washed with ice water and then with a saturated NaHCO₃ solution and brine, respectively. The organic layer was dried over Na₂SO₄ and concentrated. The residue was purified by flash chromatography (petroleum ether/EtOAc = 5:1, containing 1% Et₃N) to afford **36** (90 mg, 92%) as a colourless syrup.

Synthesis of FABO disaccharide 37. To a solution of **36** (19.1 mg, 0.024 mmol) in Et₂O (3 ml) were added BSP (8.2 mg, 0.036 mmol), 2,6-di-*tert*-butylpyridine (16.0 μl, 0.072 mmol) and 5 Å MS at RT. After stirring at –114 °C (liq. N₂-EtOH) for 20 min, Tf₂O (6.0 μl, 0.036 mmol) was added to the mixture. The mixture was stirred at –114 °C for 1 h, and was then warmed to RT and filtered. The filtrate was washed with a saturated aqueous NaHCO₃ solution and brine, respectively, and was then dried over Na₂SO₄ and concentrated. The residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc = 5:1) to afford **37** (11.3 mg, 64%) and the five-membered orthoester **38** (3.0 mg, 18%) as colourless syrups.

Synthesis of FABO disaccharide trifluoroacetimidate 6. To a solution of *p*-methoxyphenyl glycoside **37** (36 mg, 0.049 mmol) in CH₂CN/H₂O (3 ml/3 ml) was added Ag(DPAH)₂ (79 mg, 0.17 mmol) at 0 °C. After stirring for 30 min at this temperature, the mixture was filtered. The filtrate was diluted with CH₂Cl₂, washed with saturated NaHCO₃ solution and brine, respectively, and was then dried over Na₂SO₄ and concentrated. The residue was purified by flash chromatography (petroleum ether/EtOAc = 3:1) to yield the corresponding hemiacetal (28 mg, 91%) as a colourless syrup. To a solution of the hemiacetal (29 mg, 0.046 mmol) in CH₂Cl₂ (3 ml) were added Cs₂CO₃ (75 mg, 0.23 mmol) and *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride (14 μl, 0.14 mmol) at RT. After stirring for 3 h, the mixture was filtered. The filtrate was evaporated in vacuo to give a residue, which was subjected to chromatography on Davisil silica (pH = 7.0, petroleum ether/EtOAc, 5:1) to give **6** (33 mg, 90%) as a colourless syrup. This compound was used directly without further characterization.

Synthesis of pregnane β-disaccharide 39. To a solution of imidate **6** (33.0 mg, 0.041 mmol) and pregnane diol **7** (15.6 mg, 0.035 mmol) in CH₂Cl₂ (3 ml) was added 5 Å MS at RT. After stirring at -78 °C for 30 min, TBSOTf (1.2 μl, 0.0052 mmol) was added to the mixture. After stirring for 6 h at this temperature, Et₃N was added to quench the reaction. The resulting mixture was filtered. The filtrate was evaporated in vacuo to give a residue, which was purified by flash chromatography (petroleum ether/CH₂Cl₂/EtOAc = 10:5:1) to afford **39** (27.6 mg, 75%) and its α-anomer (4.5 mg, 12%) as white solids.

Synthesis of pregnane disaccharide 3. To a solution of **39** (21.0 mg, 0.020 mmol) in toluene (2 ml) were added Bu₃SnH (32 μl, 0.12 mmol) and Et₃B (12 μl, 0.012 mmol) at 0 °C. After stirring for 1 h at RT, the mixture was concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/EtOAc = 5:1) to afford the corresponding 2',6''-deoxy derivative (16.5 mg, 97%) as a white solid (see Supplementary Methods). To a solution of this compound (16.5 mg, 0.019 mmol) in CH₂Cl₂/MeOH (1.5 ml/1.5 ml) was added NaOMe (20 mg, 0.37 mmol) at RT. After stirring for 40 h, the mixture was filtered through silica gel. The filtrate was evaporated in vacuo to give a residue, which was purified by flash chromatography (petroleum ether/EtOAc = 2:1) to afford **3** (14.0 mg, 97%) as a white solid.

Synthesis of pregnane hexasaccharide 40. To a solution of tetrasaccharide *ortho*-cyclopropylethynylbenzoate **2** (22.3 mg, 0.025 mmol), pregnane disaccharide **3** (8.0 mg, 0.011 mmol) and 2,4,6-*tert*-butylpyrimidine (TTBP) (4.0 mg, 0.016 mmol) in CH₂Cl₂ (2 ml) was added 4 Å MS at RT. After stirring for 30 min at -20 °C, a solution of PPh₃AuOTf in CH₂Cl₂ (0.05 ml, 0.1 M) was added to the mixture. The mixture was stirred for 2 h while warming to -10 °C, then another portion of PPh₃AuOTf in CH₂Cl₂ (0.05 ml, 0.1 M) was added to the mixture. After stirring for 4 h at -10 °C, Et₃N was added to quench the reaction. The resulting mixture was filtered and concentrated. The residue was purified by flash chromatography (petroleum ether/CH₂Cl₂/EtOAc = 1:1:1) to afford **40** (8.3 mg, 53%), its α-anomer (4.2 mg, 27%) as white foams, and recovered **3** (1.0 mg, 13%).

Synthesis of Periploside A 1. To a solution of **40** (4.7 mg, 3.2 μmol) in pyridine/EtOH (1.0 ml/1.0 ml) was added thiourea (10 mg, 0.13 mmol) at RT. After stirring for 2 h at 80 °C, the mixture was concentrated in vacuo to give a residue, which was purified by flash chromatography (CHCl₃/MeOH = 30:1) to afford a colourless syrup. The syrup was dissolved in THF/pyridine (1.5 ml/0.75 ml). HF-py (70% HF in pyridine, 0.10 ml) was added dropwise at 0 °C. After stirring for 40 h at RT, a saturated NaHCO₃ solution was added slowly to the mixture at RT. The resulting mixture was diluted with CH₂Cl₂, washed with saturated NaHCO₃ solution and was then extracted with CH₂Cl₂ twice. The combined organic layer was washed with brine, dried over Na₂SO₄ and concentrated. The residue was purified by flash chromatography (CHCl₃/MeOH = 30:1) to afford periploside A (**1**) (3.8 mg, 93%) as a white foam.

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Author contributions

X.Z. and B.Y. conceived the synthetic route. X.Z. conducted the synthetic work. Y.Z. and J.Z. conducted bioassay. B.Y. and X.Z. wrote the manuscript.

Additional information

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