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

Xiaheng Zhang\textsuperscript{1}, Yu Zhou\textsuperscript{2}, Jianping Zuo\textsuperscript{2} & Biao Yu\textsuperscript{1}

Periploside A is a pregnane hexasaccharide identified from the Chinese medicinal plant \textit{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 Sinaj\textsuperscript{\textregistered}'s and Crich's protocol for the formation of orthoester and acetal glycosides, respectively. The 2-deoxy-\textbeta-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.
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 configuration comprising an unusual quaternary anomeric carbon was overlooked but drawn in a significant in mice models 8–10, that validates the folkloric to their activities6, remains ambiguous in both the chemical tert

**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→4)-cymarosyl linkage between

**Figure 1 | Periploside A 1 and the retrosynthetic analysis.** Shadowed 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-anomer14–17; 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-glycosyloxypyrylum or α-trilate intermediate might get invoked18. The β-(1→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 choice15,17. After furnishing the cymarosyl trisaccharide 5, its coupling with the terminal digitolose 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 anomic 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 3β-silyloxy-pregnene-17α,20x-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-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 Sinay’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 Sinay’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-0-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)23. 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)2, p-TsOH, RT, 93% for (13); 91% (for 15); (d) Bu2SnO, MeOH, reflux; (e) BnBr, DMF, CsF, RT; (f) LiOH, THF, H2O, RT; 87% (for three steps); (g) Mel, 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%; β/α = 4/1; (j) Ag(DPAH)2, CH3CN, H2O, 0 °C to RT, 95%; (k) α-cyclopropylethynylbenzoic acid, EDCI, DMAP, 4 Å MS, CH2Cl2, RT, 99%; DMAP, 4,4-dimethylaminopyridine; DPAH, bis(hydrogen dipicolinate); EDCI, N-ethyl-N’-(3-dimethylaminopropyl)carbodiimide hydrochloride; MS, molecular sieves; TBAF, tetrabutylammonium fluoride.
(Pd(OH)\textsubscript{2}/C, H\textsubscript{2}, room temperature (RT)) led to cleavage of the glycosidic linkages, testifying the vulnerability of the cymarosyl-\(\beta\)\-(1→4)-linkage. Thus, Et\textsubscript{3}N was added to sequester any nascent proton; the hydrogenolysis proceeded sluggishly, nevertheless, leading to the desired trisaccharide \(\text{5}\) in a satisfactory 93% yield at 50 °C for 2 days.

Given the acid lability of the cymarosyl-\(\beta\)\-(1→4)-linkage, it was not fully surprising to find that glycosylation of trisaccharide acceptor \(\text{5}\) with digitoxyl donor \(\text{4}\) in the presence of Ph\textsubscript{3}PAuOTf led to considerable cleavage of the trisaccharide. Ph\textsubscript{3}PAuOTf was found to be more stable towards moisture (thus less acidic)\textsuperscript{32}, therefore, it catalysed the condensation of \(\text{5}\) and \(\text{4}\) smoothly. However, in spite of the presence of 2-\(\text{O}\)-acetyl group in donor \(\text{4}\), the glycosylation led to anomeric mixtures of the coupled tetrasaccharide. Under optimized conditions (0.2 equiv Ph\textsubscript{3}PAuOTf, toluene, 5 Å MS, −50 °C to RT), tetrasaccharide \(\text{20}\) was obtained in 93% yield with \(\beta/\alpha\) ratio of 4:1, which was separable on silica gel column chromatography. Removal of the anomeric MP group in tetrasaccharide \(\text{20}\) with CAN was not successful under a variety of conditions, resulting unavoidably in degradation of the cymarosyl-\(\beta\)\-(1→4)-linkages. Fortunately, a mild oxidizing agent, Ag(DPAH), could achieve this task (CH\textsubscript{3}CN/H\textsubscript{2}O, 0 °C to RT) to afford smoothely the corresponding hemicetal (95%) (ref. 28), which was then subjected to the formation of \(\text{ortho-cyclopropylethynylbenzoate 2}\) (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\textsuperscript{29,30}, transacetalation of sugar lactones with sugar 4-hydroxyl-3-methyoxymethyl ether derivatives\textsuperscript{31}, and intramolecular hydrogen atom transfer reactions of formyl acetal linked disaccharides\textsuperscript{21,32,33}. However, no coupled FABO products have ever been detected, that prompted us to focus our attention on the planned Crich–Sinay approach (Fig. 3). Thus, starting from \(\text{D-glucal}\), that is, addition with PhSeCl to provide 2-phenylseleno-\(\text{D-mannopyranose 22}\), transglycosylation of pregnane diol with \(\text{22}\) in 85% (96%, b.r.s.m.) yield and \(\beta/\alpha\) ratio of 3:1. The erosion of 1,2-\(\text{trans-selectivity}\) in the glycosylation with a donor (that is, \(\text{27}\)) equipped with an iodide at \(\text{C2}\) 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 Et\textsubscript{3}N), so as to avoid the decomposition of the acid-labile FABO motif. Reductive removal of the bromide and iodide in \(\text{28}\) under the conventional radical conditions (AlBN/\text{BuSnH}, toluene, 80 °C) led to decomposition of the substrate. Et\textsubscript{3}B could initiate the radical reaction at RT\textsuperscript{44}, which thus enabled the reduction of \(\text{28}\) with \text{BuSnH} to proceed smoothly to afford the corresponding deoxydisaccharide (99%). Subsequent removal of the 4′-\(\text{O}-\text{benzoyl group provided the desired preganone 20-O-disaccharide 29}\) (99%).

The expected challenge in stereoselective construction of the 2-deoxy-\(\beta\)-cymarosyl-\((1\rightarrow4)-\text{linkage in the union of tetrasaccharide 2 and pregnane disaccharide 29}\) was further manifest 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) of donor \(\text{10}\) to a mixture of the acceptor \(\text{11}\) and promoter NIS/\(\text{TiO}\text{H}\) at −30 °C and increased the equivalent of acceptor \(\text{11}\) (to 5.0 equiv), acetal glycoside \(\text{24}\) was obtained in a satisfactory 75% yield. The 4′-\(\text{O}-\text{acetyl and 4′-O-benzoyl group in 24}\) were removed simultaneously in the presence of MeONa in MeOH, providing diol \(\text{9}\) (93%), which was ready for Sinay’s orthoester formation.

Thus, 2′-seleno-disaccharide \(\text{9}\) was subjected to oxidation (Na\textsubscript{2}O\textsubscript{4}, NaHCO\textsubscript{3}, MeOH/CH\textsubscript{2}Cl\textsubscript{2}/H\textsubscript{2}O, RT) to provide the selenoxide cleanly, subsequent \(\text{syn-elimination and intramolecular cyclization of the resultant ketene acetal}\) (A) took place sluggishly under forced conditions (vinyl acetate/toluene/DIPA, 140 °C), leading to an orthoester product \(\text{25}\) in a moderate 20% yield for 12 h. By carefully screening the reaction conditions, we finally managed to obtain \(\text{25}\) in an excellent 85% yield under microwave irradiation (145 °C, 20 min). The addition of the 4′-hydroxyl group onto \(\text{C1′}\) of the ketene acetal in \(\text{A}\) could proceed from both the top and the bottom faces, thus two diastereoisomers should be provided. Surprisingly, only one isomer \(\text{25}\) 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 \(\text{25}\)). However, in the previous synthesis of the Sinay-type five- and six-membered orthoesters, cyclization of the incipient ketene acetal was found to favour formation of the axial \(\alpha\)-linkage, so as to maximize the anomeric effect\textsuperscript{22–24} or to follow the trajectory of addition onto Woerpel’s low-energy conformer of the dioxaocarbentium intermediate\textsuperscript{42,43}. Determination of the configuration of the quaternary \(\text{C1′}\) in \(\text{25}\) was not possible by spectroscopic methods, especially without comparison to its epimer, and attempts at acquisition of a single crystal of \(\text{25}\) or its derivatives for X-ray diffraction analysis were unsuccessful. Therefore, this problem was not solved until completion of the total synthesis.

Synthesis of \(\text{Cl−epi-periploside A 31}\). Temporary protection of the 4′-\(\text{OH in 25 with benzoyl group led to 26 (99%)}, which was subjected to selective cleavage of the anomeric MP group (Ag(DPAH)), CH\textsubscript{3}CN, H\textsubscript{2}O, 90%) and subsequent formation of trifluoroacetimide (95%) to afford the desired donor \(\text{27}\) (Fig. 3). Glycosylation of pregnane diol \(\text{7}\), which was prepared from hydroxynadrost-5-en-17-one in three steps (see Supplementary Methods and Supplementary Fig. 59), with disaccharide imidate \(\text{27}\) proceeded smoothly under the catalysis of TBSOTf (0.1 equiv) in the presence of 5 Å MS at −78 °C in CH\textsubscript{2}Cl\textsubscript{2}, affording pregnane 20-O-disaccharide \(\text{28}\) in 85% (96%, b.r.s.m.) yield and \(\beta/\alpha\) ratio of 3:1. The erosion of 1,2-\(\text{trans-selectivity}\) in the glycosylation with a donor (that is, \(\text{27}\)) equipped with an iodide at \(\text{C2}\) 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 Et\textsubscript{3}N), so as to avoid the decomposition of the acid-labile FABO motif. Reductive removal of the bromide and iodide in \(\text{28}\) under the conventional radical conditions (AlBN/\text{BuSnH}, toluene, 80 °C) led to decomposition of the substrate. Et\textsubscript{3}B could initiate the radical reaction at RT\textsuperscript{44}, which thus enabled the reduction of \(\text{28}\) with \text{BuSnH} to proceed smoothly to afford the corresponding deoxydisaccharide (99%). Subsequent removal of the 4′-\(\text{O}-\text{benzoyl group provided the desired pregnane 20-O-disaccharide 29 (99%)\).
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 intermediate. Nevertheless, the HOTf generated after glycosylation, before being quenched by the isochromen-4-yl gold(I) complex, degraded the FABO linkage. On the basis of this rational, we introduced a hindered base, that is, 2,4,6-tri-isopropylphenyltriﬂate, 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. In fact, the condensation of 2 and 29 proceeded...
smoothly in the presence of Ph₃PAuOTf (0.8 equiv) and TTBP (1.5 equiv) in CH₂Cl₂ 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 thiourea and pyridine-buffered HF-pyridine, respectively, furnishing the target pregnane hexasaccharide 31 cleanly (91%, two steps).

The ¹H and ¹³C 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 acetal linked ketene acetal 32. In addition, the adjacent C⁴, C⁷ and C⁹ are also in disparate chemical shifts (C⁴: 81.5 versus 79.2 p.p.m., C⁷: 37.9 versus 36.7 p.p.m., C⁹: 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 (periploside A is the 3'-epimer of periploside A). 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 33 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 CH₃CN, 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 I₂, 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-α-mannopyranoside 22 was converted into the fluoride (with DAST), which was then coupled with sugar acceptor 32 (see Supplementary Methods) under the action of SnCl₂ 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 Sinaï conditions (NaIO₄, NaHCO₃, MeOH/CH₂Cl₂/H₂O, RT; then vinyl acetate/toluene/DIPA, microwave 140 °C, 40 min). Although purification of 34 on silica gel required the addition of 1% Et₃N 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 PhSHCH₂OH onto ketene acetal 34 was first attempted in CDCl₃ 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) SnCl₂, Et₂O, 4 Å MS, 0 °C to RT; 85% (for two steps); (c) NaIO₄, NaHCO₃, MeOH, CH₂Cl₂, H₂O, RT, 99%; (d) vinyl acetate, toluene, DIPA, Mw, 140 °C, 40 min, 92%; (e) EISCH₂OH, DCC, Mw, 10 min, 110 °C, 81%; (f) H₂NNH₂ - H₂O, pyridine, HOAc, 0 °C to RT, 92%; (g) BSP, Tf₂O, DTBP, 5 Å MS, Et₂O, −114 °C, 64% (for 37), 18% (for 38), BSP, 1-benzenesulfonyl 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 Et₃SCH₂OH, toluene, RT, 95%; (37-membered FABO product cation (readily undergo decomposition to the glycosyl oxocarbenium hydroxyl group to Table 2), such as MeOTf/TTBP, DMTST/TTBP, and CuBr₂/CO accounted for the transient glycosyloxymethyl strong promoter which can activate the thioacetal under milder conditions might be able to allow the transient glycosyloxymethyl decomposition. Using Crich’s conditions (Tf₂O/BSP/TTBP, CH₂Cl₂) and formaldehyde; intramolecular addition of the proximal accharide affects the glycosidation transition state of the disaccharide. The conformational restraint provided by the FABO motif 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 anomer 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, CH₂CN, 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                      | CC50 (µM) | IC50 (µM) | SI  |
|-------------------------------|-----------|-----------|-----|
| Periploside A (1)             | 2.10 ± 0.32 | 0.17 ± 0.04 | 12   |
| Cy'epi-periploside A (31)     | 1.58 ± 0.19 | 0.41 ± 0.15 | 3.9  |
| Cy"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.

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 NaOAc (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 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 a-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 layers were washed with brine, dried over Na2SO4 and concentrated. The crude glycosyl fluoride was azeotroped with toluene (3 x 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). Disopropylamine (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 60 °C and concentrated. The residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc = 1:1) to afford 25 (83 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 NaOAc (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 glycosyl fluoride was azeotroped with toluene (3 x 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). Disopropylamine (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% EtN) 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 Et3NHCH2OH (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% EtN) 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 8 °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% EtN) to afford 36 (90 mg, 92%) as a colourless syrup.

Synthesis of FABO disaccharide 37. To a solution of 36 (19 mg, 0.024 mmol) in EtO (3 ml) were added BSA (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, TfO (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 (113 mg, 64%) and the five-membered orthoester 38 (3.0 mg, 18%) as colourless syrups.
Synthesis of FABO disaccharide trifluoroacetimide 6. To a solution of p-methoxymethyl glycoside 37 (36 mg, 0.049 mmol) in CH2Cl2/H2O (3 ml/3 ml) was added Ag(DFAPH1)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, 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, 0.041 mmol) 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 Davyasil silica (pH = 7.0, petrolether/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 −8 °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/CH2Cl2/EtOAc = 5:1) to afford the corresponding α,β-dicarbonyl (16.5 mg, 0.019 mmol) as a white solid (see Supplementary Methods). To a solution of this compound (15.6 mg, 0.035 mmol) in CH2Cl2 (3 ml) was added NaOMe (0.14 mmol) at RT. After stirring for 30 min at this temperature, the mixture was filtered. The filtrate was washed with saturated NaHCO3 solution and brine, respectively, and was then washed with water and then dried over Na2SO4 and concentrated. The residue was purified by flash chromatography (CHCl3/MeOH = 1:1) to afford 3 (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/CH2Cl2/EtOAc = 2:1) to afford 3 (14.0 mg, 97%) as a white solid.

Synthesis of pregnane hexasaccharide 40. To a solution of tetrascarcarid orchio-cyclopropylphenylbenzoate 2 (22.3 mg, 0.025 mmol), pregnane disaccharide 3 (8.0 mg, 0.011 mmol) and 2,4,6-tri-tert-butylypyrimidine (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 30 °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 Periploide A. A 1. To a solution of 40 (4.7 mg, 3.2 µmol) in pyridine/ EtOH (1.0 ml/1.0 ml) was added thylose (10.0 mg, 0.013 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 (CH3Cl/McOH = 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 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 (CH3Cl/McOH = 30:1) to afford periploide A (1) (3.8 mg, 93%) as a white foam.

References
1. Oshima, Y., Hirota, T. & Hikino, H. Periploides A, B and C, and steroidal glycosides of Periploca sepium root bark. Heterocycles 26, 2093–2098 (1987).
2. Itokawa, H. et al. Studies on chemical constituents of antitumor fraction from Periploca sepium. II. Structures of new pregnane glycosides, periploides A, B and C. J. Chem. Pharm. Bull. 36, 982–987 (1988).
3. Itokawa, H. et al. Studies on chemical constituents of antitumor fraction from Periploca sepium. III. Structures of new pregnane glycosides, periploides D, E, L and M. J. Chem. Pharm. Bull. 36, 2084–2089 (1988).
4. Itokawa, H. et al. Studies on chemical constituents of antitumor fraction from Periploca sepium. V. Structures of new pregnane glycosides, periploides J, K, F and O. Chem. Pharm. Bull. 36, 4441–4446 (1988).
5. Feng, J. Q. et al. Immunosuppressive pregnane glycosides from Periploca sepium and Periploca forestii. Phytochemistry 69, 2716–2723 (2008).
disaccharide model promoted by alkoxyl radicals. Conformational and stereochemical requirements. Org. Lett. 9, 1785–1788 (2007).
34. Nicolaou, K. C. et al. Total synthesis of apopolixin: construction of enantioselectively pure fragments. J. Am. Chem. Soc. 125, 15433–15442 (2003).
35. Lian, G., Zhang, X. & Yu, B. Thioglycosides in carbohydrate research. Carbohydr. Res. doi:10.1016/j.carres.2014.06.009 (in the press).
36. Sasaki, M. & Tachibana, K. An efficient and stereoselective synthesis of the nephrigenoside core structure. Tetrahedron Lett. 32, 6873–6876 (1991).
37. Crich, D. & Smith, M. 1-Benzenesulfinyl piperidine/trifluoromethanesulfonic anhydride: a potent combination of shelf-stable reagents for the low-temperature conversion of thioglycosides to glycosyl triflates and for the formation of diverse glycosidic linkages. J. Am. Chem. Soc. 123, 9015–9020 (2001).
38. Fugedi, P. & Garegg, P. J. A novel promoter for the efficient construction of 1,2-trans linkages in glycoside synthesis, using thioglycosides as glycosyl donors. Carbohydr. Res. 119, C9–C12 (1986).
39. Ishiwata, A., Sakurai, A., Nishimiya, Y., Tsuda, S. & Ito, Y. Synthetic study and structural analysis of the antifreeze agent xylomannan from Upis ceramboidea. J. Am. Chem. Soc. 133, 19524–19535 (2011).
40. Konradsson, P., Udodong, U. E. & Fraser-Reid, B. Iodonium promoted reactions of disarmed thioglycosides. Tetrahedron Lett. 31, 4313–4316 (1990).
41. Schmidt, R. R. & Toepfer, A. Glycosylation with highly reactive glycosyl donors: efficiency of the inverse procedure. Tetrahedron Lett. 32, 3353–3356 (1991).
42. Yang, M. T. & Woerpel, K. A. The effect of electrostatic interactions on conformational equilibria of multiply substituted tetrahydropropyloxocarbenium ions. J. Org. Chem. 74, 545–553 (2009).
43. Chamberland, S., Ziller, J. W. & Woerpel, K. A. Structural evidence that alkoxyl substituents adopt electronically preferred pseudoaxial orientations in six-membered ring dioxocarbenium ions. J. Am. Chem. Soc. 127, 5322–5323 (2005).
44. Miura, K. et al. Triethylborane-induced hydrodehalogenation of organic halides by tin hydrides. Bull. Chem. Soc. Jpn 62, 143–147 (1989).
45. Zhu, Y. & Yu, B. Characterization of the isochromen-4-yl-gold(I) intermediate in the gold(I)-catalyzed glycosidation of glycosyl ortho-alkynylbenzoates and enhancement of the catalytic efficiency thereof. Angew. Chem. Int. Ed. 50, 8329–8332 (2011).
46. Nie, S., Li, W. & Yu, B. Total synthesis of nucleoside antibiotic A201A. J. Am. Chem. Soc. 136, 4157–4160 (2014).
47. Cook, A. F. & Maichuk, D. T. Use of chloroacetic anhydride for the protection of nucleoside hydroxyl groups. J. Org. Chem. 35, 1940–1943 (1970).
48. Adam, W. & Wang, X. E/Z Isomerization, solvolysis, addition, and cycloaddition reactions of (E)-tert-butyketene methyl tert-butyldimethylsilyl acetal. J. Org. Chem. 56, 7244–7250 (1991).
49. Sawada, D. & Ito, Y. A new method for formacetal linkage formation: protection of alcohols, phenols and carboxylic acids. Tetrahedron Lett. 42, 2501–2504 (2001).

Acknowledgements
This work is financially supported by the National Natural Science Foundation of China (21432012 and 91213301), the Ministry of Sciences and Technology of China (2012ZX09502-002) and the E-Institute of Shanghai Municipal Education Commission (E09013). We are grateful to Professor Wei-Min Zhao for providing an authentic sample of the natural product periploside A and to Professor Tian-Xiang Li for providing a photo of the plant.

Author contributions
X.Z. and B.Y. conceived the synthetic route. X.Z. conducted the synthetic work. Y.Z. and Ito, Y. Synthetic study and structural analysis of the antifreeze agent xylomannan from Upis ceramboidea.

Additional information
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

How to cite this article: Zhang, X. et al. Total synthesis of periploside A, a unique pregnane hexasaccharide with potent immunosuppressive effects. Nat. Commun. 6:5879 doi: 10.1038/ncomms6879 (2015).