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Synthetic pseudopterosin analogues: A novel class of antiinflammatory drug candidates

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ABSTRACT

The synthesis and in vivo anti-inflammatory activity of a series of pseudopterosin analogues are presented. Synthetic tricyclic catechol aglycons with different substitution patterns were monofucosylated or -xylosylated. Anti-inflammatory activity was conserved over a wide range of structural modifications. The most active synthetic compound **33** reduced phorbol myristate acetate (PMA)-induced inflammation in the mouse ear by 72% at 50 μg/ear. This corresponds to 80% of the activity of natural pseudopterosin A.

1. Introduction

Current nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenases and thus the biosynthesis of prostaglandins.[1–3] While being efficient for short term pain relief and sporadic treatment of inflammation, their therapeutic use for long term inflammatory diseases such as rheumatism is problematic due to gastrointestinal side effects as well as adverse effects on blood formation and the function of liver and kidney.[2] Therefore, there is new interest in drug lead structures with anti-inflammatory and analgesic activities based on alternative molecular mechanisms and novel structures.

Such a series of compounds are the pseudopterosins A–L. These compounds are produced by the Caribbean Sea whip *Pseudopterogorgia elisabethae* (*Octocorallia*, *Cnidaria*) and were discovered by Fenical and co-workers during the years 1986–1990 (Fig. 1).[4–7]

Additional representatives of the pseudopterosins and closely related compounds have later been isolated by several groups at different collection sites in the Caribbean Sea, and the rich and diverse production of diterpene glycosides by *P. elisabethae* appears highly specific to the geographic location of collection.[8–15]

The pharmacologically most well studied congeners, pseudopterosins A and E (PsA and PsE), show potent analgesic and anti-inflammatory activities when applied topically or systemically, but only minor (PsA) to no (PsE, up to >300 mg/kg) toxicity in acute assays.[7] This is important since positive results in anti-inflammatory assays often go along with undesired cytotoxicity.

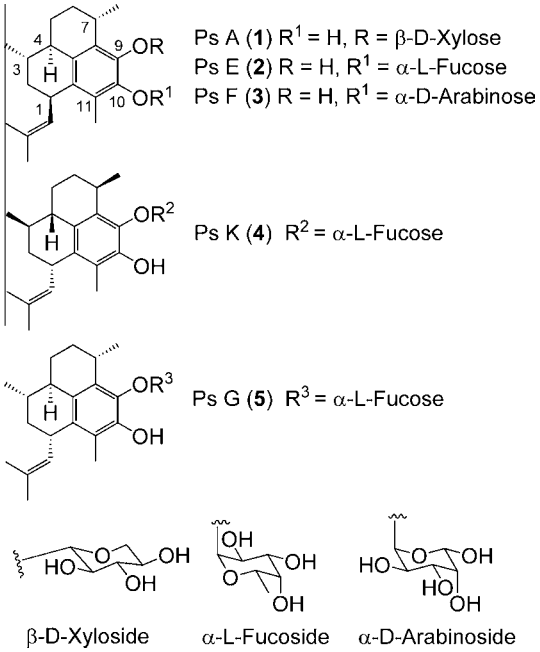

Figure 1. Antiinflammatory and analgesic pseudopterosins A, E, F, K and G isolated from *Pseudopterogorgia elisabethae*.[4–7]

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Other pseudopterosins showed, in addition to their anti-inflammatory properties, anti-bacterial,[12] anti-tuberculosis, anti-viral and cancer cell cytotoxicity activities.[11] By using the laser Doppler blood flow evaluation method, Dayan et al. recently demonstrated the clinical efficacy of pseudopterosins to suppress subcutaneous inflammation induced by ethyl nicotinate.[16] The in vivo anti-inflammatory activity of pseudopterosins P–U rich extracts of *P. elisabethae* collected at the Providencia and San Andrés Islands (Colombia) has been demonstrated by Correa et al.[17] The extracts were active at 500 μg/ear in the mouse ear assay and myeloperoxidase levels at the inflammation site were reduced down to basal levels.

In vitro experiments, conducted in order to better understand the pharmacological mode of action of the pseudopterosins, showed that these natural products reduce eicosanoid production without inhibiting the enzymatic activity of phospholipase A_2 and cyclooxygenase.[18] Furthermore, pseudopterosin A (PsA, **1**) was also shown to stabilize cell membranes[19] and trigger the release of calcium from intracellular stores.[20] Interestingly, this latter observation supports an earlier hypothesis about the pseudopterosins acting as structural mimics of phosphatidylinositol.[21] Pretreatment with Pertussis toxin (PTX) blocked the activity of PsA in functional assays conducted in the ciliate *Tetrahymena thermophila* suggesting that pseudopterosins may act via a G-protein coupled receptor related mechanism.[20] Furthermore, competitive binding experiments conducted in human embryonic kidney cells (HEK-293) show that PsA selectively binds to adenosine A_{2A} and A_3 receptors with positive cooperativity. PsA was capable of displacing specific antagonists for the A_{2A} and A_3 receptors with IC_{50} values equal to 13 and 4.1 μM, respectively.[22]

In an experiment combining pharmacology and chemical ecology, it was shown that pseudopterosins block an inducible oxidative burst in the symbiotic dinoflagellate (*Symbiodinium* sp.) isolated from the tissues of *P. elisabethae*.[23]

Some fundamental results on structure–activity relationship have been obtained from semisynthetic modifications of the natural products. Hydrolytic removal of the carbohydrate moiety from PsA yielded a catechol aglycon that rapidly oxidized to the corresponding *o*-benzoquinone, but was stable in its monomethylated or monoacetylated form. None of these aglycon-like structures showed any anti-inflammatory activity.[13,24] A glycosidase resistant *C*-glycoside of PsA methyl ether has been prepared by Zhong et al. and was shown to have a similar biological activity profile as PsA and its methyl ether, including in vivo anti-inflammatory activity.[25] These findings strongly suggest that the intact diterpene glycoside is needed for a pseudopterosin to express its biological effects. Finally, after completion of the herein presented study, the synthesis of six simplified analogues of the bicyclic seco-pseudopterosins and their in vitro bioactivity was presented by Tanis et al.[26] The compounds were also able to inhibit phagocytosis in *T. thermophila* and to competitively bind to the adenosine receptor A_{2A} in human embryonic kidney cells. The importance of the second non-glycosylated hydroxyl function for the observed biological activity was demonstrated.

A series of non-natural pseudopterosin A ethers and esters prepared from the natural compounds were found to exhibit anti-inflammatory and analgesic properties as well as activity against lymphoma type cancers.[24,27,28] These compounds were also shown to inhibit degradation of cartilage in bovine articular cartilage explants[28] and effectively promote wound-healing.[29] Due to the excellent anti-inflammatory activity of the contained pseudopterosins and their ability to restrict allergic responses in the skin, organic extracts of harvested *P. elisabethae* from the Islands of the Bahamas are commercially used in skin care preparations.[30] However, in spite of the remarkable ability of the sea whip to regrow after harvesting, this natural resource would not sustain a large scale pharmaceutical exploitation. A possible production of pseudopterosins by means of biotechnology was suggested by Kerr and co-workers based on their studies of the biosynthesis of these metabolites.[31–33]

The pseudopterosins have attracted much attention among the organic synthetic community, and total syntheses of pseudopterosins A and E or their aglycons have been reported by Broka,[34] Corey,[21,35–37] Kocienski[38,39] and Harrowven.[40,41] In addition, Schmalz[42] and others have developed innovative methodologies towards the synthesis of the hexahydrophenalene skeleton of the pseudopterosins.[43,44] Surprisingly, none of these achievements in organic synthesis has been exploited for the preparation and pharmacological evaluation of synthetic pseudopterosin analogues. Herein, we report the synthesis and in vivo anti-inflammatory activity of a series of pseudopterosin analogues, synthesized via a new and flexible synthetic scheme.

2. Results and discussion

2.1. Chemistry

For the design of our synthetic hexahydrophenalene aglycons, we decided to focus on variations of the C(7) and C(11) substituents, as well as the nature and site of attachment of the carbohydrate moiety. By omitting the C(1) and C(3) substituents, we would simplify the synthesis and reduce the number of emerging stereoisomers. As outlined in Scheme 1, Grignard addition of **7** to dimethoxy α-tetralone **6**, followed by hydrogenation and acetal hydrolysis afforded aldehyde **9**.

This pivotal intermediate was oxidized to carboxylic acid **10**, which was subjected to intramolecular Friedel–Crafts acylation yielding ketone **11**. A two-step reduction with sodium borohydride followed by catalytic hydrogenation led to the unsubstituted tricyclic catechol dimethyl ether **17** (49% overall yield from **6**). Alternatively, arylketone **11** could have been reduced to **17** by direct hydrogenation in the presence of acid.

Additional methyl substituents at C(7) were introduced via Wittig olefination of **9** to yield **12**. Friedel–Crafts cyclization of **12** in the presence of Eaton's reagent[45] led to a 2:3 mixture of the *anti* and *syn*-diastereomers **16a**/**b**, which could be separated by semi-preparative silica gel HPLC. The two compounds showed marked differences in the ^{1}H NMR coupling pattern of H–C(7). The coupling patterns were compared to the corresponding dihedral angles in calculated lowest energy conformers (semiempirical PM3 method, see Section 4) to assign the indicated relative configurations. The cyclization of carbinol **14**, obtained in three steps from **9**, yielded the C(7) dimethylated aglycon **15**.

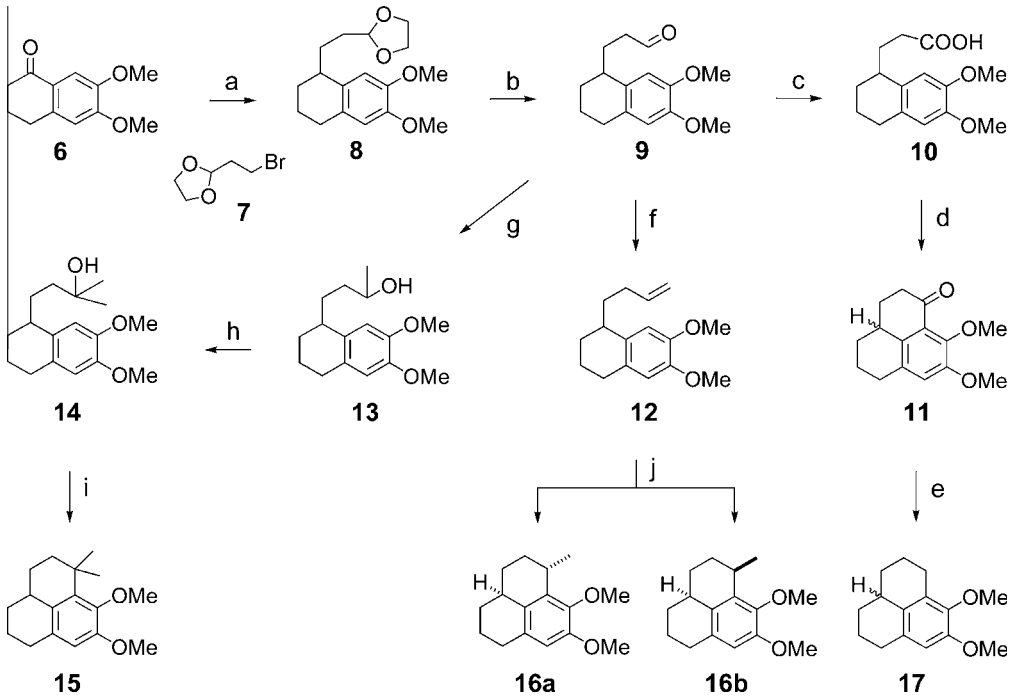
A more direct approach to **16**, starting from dimethoxyphenyl acetic acid (**18**), features a tandem cyclization of **21** (Scheme 2).

This route would offer the possibility to introduce substituents at C(1) via α-alkylation of **18** and at C(3) via the use of a 1-substituted analogue of epoxide **20**.

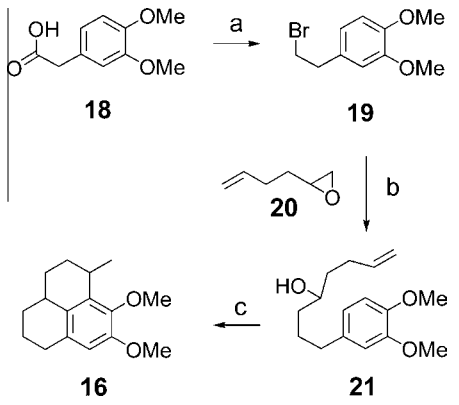
Aglycons substituted at C(11) with a methyl or a more lipophilic trifluoromethyl group were prepared from bromide **22** through a Pd-catalyzed cross-coupling reaction with Me_3Al[46] and a Cu-catalyzed thermal exchange reaction with trifluoro acetic anhydride,[47] respectively (Scheme 3).

All aglycones were deprotected with BBr_3 to liberate the oxidation-labile catechols, which were immediately monofucosylated with L-fucose or xylosylated with D-xylose to yield compounds **27–36** as outlined in Schemes 4 and 5 and Table 1.

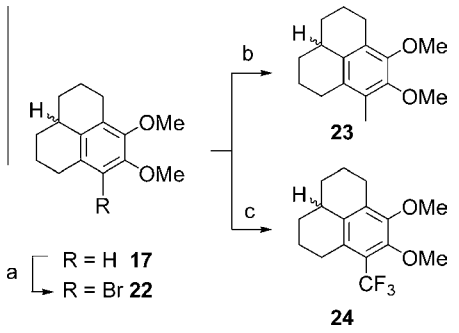
The fucosyl donor **25** was formed in situ by reacting persilylated L-fucose with TMS-I according to the method of Uchiyama and Hindsgaul.[48] After deprotection with K_2CO_3/MeOH, α-fucosides were obtained exclusively. This remarkable stereoselectivity is more likely due to neighbouring group participation from the *O*-TMS-



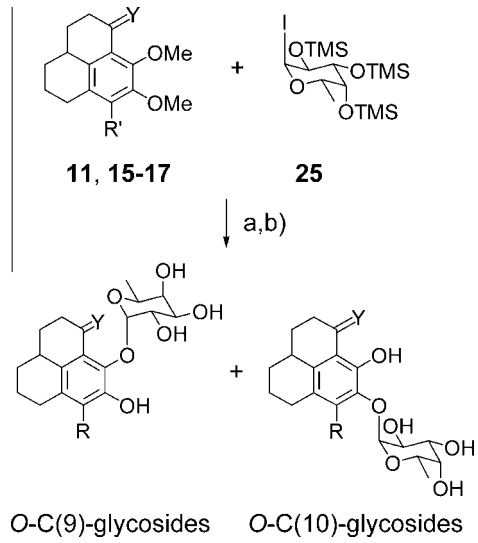
Scheme 1. Total synthesis of simplified pseudopterosin aglycons (all compounds racemic). Reagents and conditions: (a) (i) **7**, Mg, THF; (ii) H_2, Pd/C (72%, two steps); (b) HCl, THF/H_2O; (c) $AgNO_3$, KOH (76%, two steps); (d) MsOH/P_2O_5 (96%); (e) (i) $NaBH_4$; (ii) H_2, Pd/C (94%); (f) CH_2=PPh_3 (56%); (g) MeMgI; (h) (i) TPAP; (ii) MeMgI (54%, three steps); (i) MsOH/P_2O_5 (84%); (j) MsOH/P_2O_5 (91%), then FC on SiO_2.



Scheme 2. Tandem cyclization route to aglycon **16**. Reagents and conditions: (a) (i) $LiAlH_4$; (ii) PBr_3; (b) (i) Mg, THF; (ii) CuI, **20** (45%); (c) SiO_2, MsOH/P_2O_5 (40%).



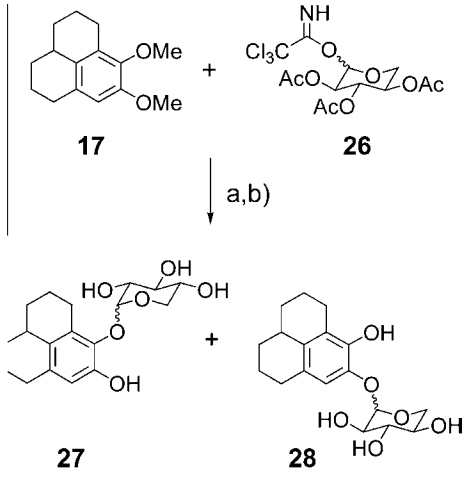
Scheme 3. Introduction of substituents at C(11). Reagents and conditions: (a) NBS (84%); (b) cat. Pd^0, Me_3Al, dioxane, reflux (81%); (c) F_3C–C(O)ONa, CuI, NMP, 170 °C (82%).



Scheme 4. Deprotection of aglycons and L-fucosylation. Reagents: (a) BBr_3; (b) (i) 2,6-di-*tert*-butyl pyridine; (ii) MeOH. For yields and isomer ratios see Table 1.

groups rather than to the anomeric effect alone. As a result of the racemic nature of the aglycons, the final glycosides were obtained as likely inseparable mixtures of two diastereomers (ca. 1:1).

The regioselectivity of the glycosylation reaction with respect to the two C(9) and C(10) hydroxyl groups depended on the substitution pattern of the aglycon and the nature of the glycosyl donor. Ketone **11**, as well as the C(7)-substituted aglycones **15** and **16**, yielded exclusively *O*–C(10) fucosides. From **17**, mixtures of *O*–C(9) and C(10)-fucosides were formed, which could be separated by semipreparative SiO_2–HPLC. Unfortunately, this was not the case for the regioisomeric mixtures obtained from aglycones **23** and **24**.



Scheme 5. Deprotection of aglycons and D-xylosylation. Reagents: (a) BBr_3; (b) (i) TMSOTf; (ii) K_2CO_3, MeOH. For yields and isomer ratios see Table 1.

Table 1
Glycosylation of deprotected aglycons **11** and **15–24** with L-fucose and D-xylose

Entry	S.M.	Glycosyl donor	Yield (%)	Glycosylation at *O*–C(9):*O*–C(10)	Product(s)
1	**17**	**26**	57	1:2 (separated)	**27**, **28**
2	**17**	**25**	51	1:3 (separated)	**29**, **30**
3	**11**	**25**	25	<1:>99	**31**
4	**15**	**25**	49	<1:>99	**32**
5	**16a**	**25**	39	<1:>99	**33**
6	**16b**	**25**	50	<1:>99	**34**
7	**23**	**25**	67	3:2 (not separated)	**35**
8	**24**	**25**	21	1:1 (not separated)	**36**

Finally, D-xylosylation of tricycle **17** yielded xylosides **27** and **28**, which were again separated by SiO_2–HPLC. Both xylosides were obtained as inseparable 1:1 mixtures of α- and β-anomers, leading, together with the C(4) chiral center, to the presence of four diastereomers (Scheme 5).

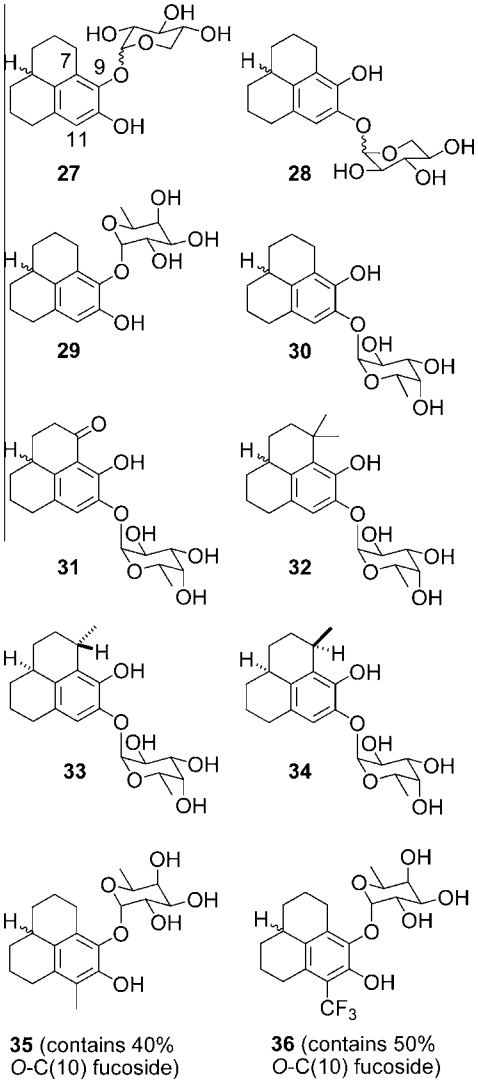


Figure 2. Pseudopterosin analogues **27–36**.

2.2. Anti-inflammatory activity

The synthetic pseudopterosin analogues were tested for their ability to reduce PMA-induced mouse ear edema when administered topically in a single-dose study (25 μg/ear in acetone solution, test series 1–3). An additional dose–response study was carried out with compounds **30** and **33** (series 4). Pseudopterosin A was included in test series 1 and 3 as a reference compound. With one exception, all compounds showed statistically significant reduction of edema relative to the PMA control means (p <0.05, paired Student's t-test) (Fig. 2 and Table 2).

The exception was ketone **31**, which proved to be completely inactive. Besides a general adverse effect of a polar hydrogen acceptor group at C(7), an enhanced chemical reactivity related to the acidity of the HO–C(9) group (vinylogous carboxylic acid) or to the presence of tautomeric quinone-methide structures may be the cause for this lack of activity.

The reproducibility of the results for the active compounds is exemplified with two single dose measurements for compound **33** at 25 μg/ear in series 3 and 4, giving average edema reductions of 58% and 53%, respectively, and no significant difference between the two assessments (t = 0.07, paired Student's t-test). Due to the relatively high variance of the single dose experiments, only limited conclusions can be drawn about potency trends of the active synthetic compounds.

When comparing compounds **27–30** derived from the unsubstituted aglycon **17**, no significant differences were observed between D-xylosides and L-fucosides as well as between *O*–C(9) and *O*–C(10) glycosides. On the contrary, PsA (**1**), a *O*–C(9) xyloside, was reported to be more potent (ED_{50} = 8–15 μg/ear) in reducing mouse-ear edema than PsE (**2**), a *O*–C(10) fucoside, (ED_{50} = 38–41 μg/ear), in two independent dose–response studies.[13,18] Furthermore, the recently isolated natural product iso-PsE, a *O*–C(9) L-fucoside, showed higher potency (ED_{50} = 27 μg/ear) than PsE.[8] These results indicate that with the natural diterpene aglycons, a small, but significant beneficial effect on potency is exerted by D-xyloside over L-fucoside and *O*–C(9) over *O*–C(10) glycosidation.

A comparison of subgroups revealed no statistically significant differences in edema reduction between fucosides **30** and **32–36** (paired t-test, p = 0.05). Thus, the addition of substituents at either C(7) or C(11) does not strongly alter the activity of the compounds. Compound **33**, which was the only synthetic analogue which did not show a significantly lower activity than the natural product PsA (t-test, p = 1.36), was measured at three different dosages (12.5, 25 and 50 μg/ear). The dose response of edema reduction proved to be significant (p <0.05) and the comparison with PsA (obtained from an independent dose–response study[18]) is shown

Table 2
Reduction of PMA-induced (2 μg/ear) mouse ear edema by compounds **27–36**

Compound	Dosage (μg/ear)	Mean Δm ± SD (mg)	Control group mean Δm ± SD (mg)	Edema inhibition (%)
27[e]	25	9.4 ± 3.7[a]	15.0 ± 2.0	−37
28[e]	25	8.2 ± 3.8[a]	15.0 ± 2.0	−44
29[e]	25	10.7 ± 3.1[b]	14.1 ± 5.3	−24
30[e]	25	9.3 ± 3.0[b]	14.1 ± 5.3	−34
30[e]	50	6.1 ± 3.1[c]	16.0 ± 2.0	−62
31	25	15.5 ± 2.2[b]	14.1 ± 5.3	10
32[e]	25	9.9 ± 3.6[c]	16.0 ± 2.0	−38
33[e]	25	6.7 ± 2.2[c]	16.0 ± 2.0	−58
34[e]	25	8.1 ± 1.8[c]	16.0 ± 2.0	−50
35[e]	25	8.1 ± 2.2[c]	16.0 ± 2.0	−49
36[e]	25	8.8 ± 1.7[c]	16.0 ± 2.0	−45
33[e,f]	12.5	8.6 ± 1.3[d]	13.9 ± 2.4	−38
33[e,f]	25	6.6 ± 1.7[d]	13.9 ± 2.4	−53
33[e,f]	50	4.0 ± 1.6[d]	13.9 ± 2.4	−72

The compounds were tested in four series and applied in acetone. Δm refers to the weight difference between treated (PMA plus test compound) and untreated ear (vehicle only). The reference compound pseudopterosin A (PsA) showed edema inhibition of −70 ± 2% at 25 μg/ear. For test details see experimental section.
[a–d] Test series 1–4.
[e] Statistically significant difference relative to control (p <0.05). Paired student t-test.
[f] ED_{50} equal to 24 μg/ear. Estimates based on least square regression analysis.

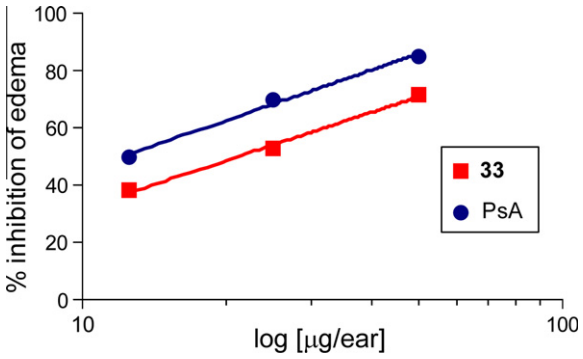


Figure 3. Dose–response curves for inhibition of PMA-induced mouse ear edema upon topical administration of **33** (ED_{50} = 24 μg/ear) in comparison to pseudopterosin A (PsA, ED_{50} = ca. 12 μg/ear) measured in an earlier study.[18]

in Figure 3. On the other hand, the increase of edema reduction from 33% to 62% upon doubling the dose of fucoside **30** from 25 to 50 μg/ear proved to be not significant (p = 0.17).

3. Conclusions

In the present study, a flexible and efficient synthetic scheme has been devised for the preparation of 10 simplified anti-inflammatory pseudopterosin analogues. Structural modifications include the degree of substitution of the hexahydrophenalene core, different relative C(4,7) configurations as well as variations of the sugar moiety and the site of glycosidation. The presented route would allow for the preparation of multigram amounts required for additional biological testing.

A variation of the synthetic scheme is presented in which additional substituents at C(1) and C(3) could be introduced.

All final compounds were tested as mixtures of diastereomers due to the racemic nature of the aglycons. Stereochemically homogenous compounds would have been accessible only with homochiral aglycons. We anticipate that the anti-inflammatory activity of this class of compounds would be only slightly influenced by stereochemical features alone.

All compounds with the exception of ketone **31** proved to be active in the mouse-ear assay, and no statistically significant potency differences could be identified among the compounds.

The stable levels of in vivo anti-inflammatory activity across a wide array of different Pseudopterosin related structures strongly suggests that glycosides from even more simplified, more easily accessible aglycons would satisfy the minimal pharmacophore requirements for the unique mode of action of this substance class.

In that sense, the present study, together with the study by Tanis et al.,[26] can be regarded as a promising start into the exploration of the anti-inflammatory potential of a wider diversity of monoglycosylated, electron-rich catechols, a theme expressed by *P. elisabethae* and its production of the pseudopterosins.

4. Experimental section

4.1. Mouse-ear edema assay[49]

Compounds were topically applied in acetone to the inside pinnae of the ears of mice in a solution containing the edema-causing irritant, phorbol 12-myristate 13-acetate (PMA). Mice were briefly anesthetized with halothane and PMA alone (2 μg/ear) or in combination with various dilutions of test compound, were applied to the left ears (five mice per treatment group) and acetone was applied to all right ears. After 3 h and 20 min incubation, the mice were euthanized, the ears removed and a 6 mm biopsy was taken from the center of the ear and immediately weighed. Edema was measured by subtracting the weight of the right ear (acetone control) from the weight of the left ear (treated). Results were recorded as % decrease (inhibition) or % increase (potentiation) in edema relative to the PMA control group edema.

4.2. Chemistry

4.2.1. General methods

All reactions were carried out under Argon with magnetic stirring unless otherwise specified. Reaction temperatures refer to the bath, 'RT' designates 24 ± 2 °C. Solvents used were as follows: 2,2,4-trimethylpentane (TMP), heptane, dichloromethane (CH_2Cl_2) and ethyl acetate (EtOAc) were HPLC-grade, THF was dried by distillation from Na. Evaporation of solvents was carried out under vacuum at 40 °C in a rotary evaporator; column flash chromatography (FC) was performed on SiO_2 (Merck 9385). Semipreparative HPLC was carried out using Rainin DYNAMAX-60 Å SiO_2-columns (250 × 10 mm) with refractive index detection. With this setup, all final products **27–36** were purified to a chemical purity of

>95% (sum of the indicated stereoisomer mixtures). *Physical and spectroscopic analysis of new compounds.* $[\alpha]_D$: *Autopol 3* polarimeter. ^{1}H and all 2D NMR experiments at 300 MHz; ^{13}C NMR at 100 MHz, solvent indicated. NMR chemical shifts are reported in ppm relative to residual $CHCl_3$ (^{1}H: 7.26 ppm, ^{13}C: 77.23 ppm) or acetone-d_6 (^{1}H: 2.05 ppm, ^{13}C: 29.8 ppm). IR spectra were recorded on a Hewlett–Packard 1600 FTIR spectrometer; absorption band intensity is indicated as vs (very strong), s (strong), m (medium), w (weak), vw (very weak).

4.2.2. 2-(2-(6,7-Dimethoxy-1,2,3,4-tetrahydronaphthalen-1-yl)ethyl)-1,3-dioxolane (8)

Magnesium turnings (310 mg, 12.8 mmol, 2.5 equiv) were suspended in THF (4 mL) and an iodine crystal was added. Neat 2-(2-bromoethyl)-1,3-dioxolane (10% of a total of 1.50 mL, 12.8 mmol, 2.5 equiv) was introduced and the mixture stirred for 20 min at RT. After this time decolorization indicated initiation of the reaction. The remaining bromide was added as a solution in THF (5 mL) at such a rate as to keep the temperature below 40 °C. Additional THF (5 mL) was added and the solution was left to stand at RT for 2 h, then cooled to −50 °C. 6,7-Dimethoxy-1-tetralone (**6**, 1.05 g, 5.1 mmol, 1 equiv) was added dropwise via cannula as a solution in THF (7 mL). After warming to RT and stirring for 14 h, the solution was poured on ice water and extracted with TMP–EtOAC (1:1). The organic layer was washed with 1 N aq HCl, then 1 N aq $NHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and the solvents removed under vacuum. The crude oil obtained (1.6 g) was dissolved in EtOH (30 mL), Pd/C (100 mg) was added, the flask filled with H_2 (3 × vacuum/H_2) and the solution was stirred under 1 atm of H_2 (balloon) during 14 h. After filtration over SiO_2 (rinsing with EtOAc), removal of the solvents under vacuum and purification by silica flash chromatography (hexane/EtOAc 1:4), dioxolane **8** (1.04 g, 70%) was isolated as a colorless oil along with 110 mg (10%) of starting material. IR (film, NaCl): 2932s, 1609w, 1513vs, 1254vs, 1118m, 853w, 798vw. ^{1}H NMR (400 MHz, $CDCl_3$): 6.69 (s, 1H), 6.54 (s, 1H), 4.87 (t, J = 4.4, 1H), 3.96–3.93 (m, 2H), 3.85–3.81 (m, 8H), 2.77–2.70 (m, 1H), 2.69–2.63 (m, 2H), 1.87–1.75 (m, 4H), 1.74–1.63 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): 146.8 (2C), 132.5 (C), 128.8 (C), 111.5 (CH), 111.4 (CH), 104.5 (CH), 64.7 (CH_2), 64.6 (CH_2), 55.8 (CH_3), 55.6 (CH_3), 36.8 (CH), 31.4 (CH_2), 30.5 (CH_2), 29.1 (CH_2), 27.3 (CH_2), 19.8 (CH_2). MS (EI, 70 eV): 292 (18, M^+), 204 (97), 191 (100), 160 (20), 73 (20).

4.2.3. 3-(6,7-Dimethoxy-1,2,3,4-tetrahydronaphthalen-1-yl)propanal (9)

Dioxolane **8** (827 mg, 2.82 mmol) was dissolved in THF (10 mL) and 1 N aq HCl (10 mL) was added. After stirring for 4 h at RT the solution was diluted with EtOAC–hexane (1:1), the organic phase separated and washed with 1 N aq K_2CO_3 solution and brine. Drying over Na_2SO_4 and evaporation of solvent under vacuum yielded analytically pure aldehyde **9** (701 mg, 98%) as a colorless oil, which was carried on to the following transformations without further purification. IR: 2929m, 2832m, 1719s, 1609w, 1510vs, 1251vs, 1216vs, 1117vs. ^{1}H NMR (400 MHz, $CDCl_3$): 9.78 (t, J = 1.6, 1H), 6.68 (s, 1H), 6.55 (s, 1H), 3.84 (s, 3H), 3.82 (s, 3H), 2.70–2.77 (m, 1H), 2.66 (q, J = 5.1, 2H), 2.50 (td, J = 7.6, 1.5, 2H), 2.00–2.09 (m, 1H), 1.77–1.89 (m, 3H), 1.64–1.72 (m, 1H), 1.55–1.62 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 202.0 (CH), 146.8 (C), 146.8 (C), 131.4 (C), 128.8 (C), 111.5 (CH), 111.2 (CH), 55.6 (CH_3), 55.4 (CH_3), 41.3 (CH_2), 36.2 (CH), 28.9 (CH_2), 28.2 (CH_2), 27.1 (CH_2), 19.6 (CH_2).

4.2.4. 3-(6,7-Dimethoxy-1,2,3,4-tetrahydronaphthalen-1-yl)propanoic acid (10)

A solution of $AgNO_3$ (520 mg, 3.06 mmol, 2.2 equiv) in H_2O (3 mL) was added to the solution of aldehyde **9** (346 mg, 1.39 mmol) in MeOH (6 mL). After cooling to 0 °C, 7 N aq KOH (3 mL) was introduced dropwise, upon which a black precipitate was formed. The mixture was warmed to 35 °C for 30 min, and then filtered carefully and the filter paper was rinsed with 1 N aq KOH. The filtrate was twice washed with CH_2Cl_2, then the aqueous layer was acidified to pH 1 with concd aq HCl and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and the solvent removed under vacuum. After purification by silica flash chromatography (EtOAc/TMP 3:2), acid **10** (287 mg, 78%) was isolated as a crystalline solid, mp = 98 °C. IR (film, NaCl): 3000–2400 br, 2933s, 1707vs, 1610w, 1514vs, 1255vs, 1218s, 1117s, 1078w. ^{1}H NMR (400 MHz, $CDCl_3$): 6.69 (s, 1H), 6.55 (s, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 2.76 (hext, J = 4.5, 1H), 2.71-2.65 (m, 2H), 2.51–2.38 (m, 2H), 2.12–2.02 (m, 1H), 1.92–1.79 (m, 3H), 1.74–1.60 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 165.0 (C), 154.6 (C), 146.9 (C), 131.6 (C), 128.9 (C), 111.6 (CH), 111.3 (CH), 56.0 (CH_3), 55.8 (CH_3), 36.6 (CH), 31.7 (CH_2), 31.5 (CH_2), 31.1 (CH_2), 29.3 (CH_2), 27.4 (CH_2). MS (EI, 70 eV): 264 (100, M^+), 235 (65), 215 (64), 204 (77). HRMS (FAB): ($C_{15}H_{20}O_4$) calcd 264.1362, found 264.1368.

4.2.5. 8,9-Dimethoxy-2,3,3a,4,5,6-hexahydro-1*H*-phenalen-1-one (11)

Carboxylic acid **10** (280 mg, 1.06 mmol) was stirred with Eaton's reagent[45] (1.5 mL) at 45 °C for 80 min. The solution was poured on ice water and extracted with EtOAc. The organic layer was washed with water and brine, then dried over Na_2SO_4. After evaporation of the solvent under vacuum, 250 mg (96%) of analytically pure ketone **11** was isolated. IR (film, NaCl): 2931s, 1686vs, 1476s, 1257vs. ^{1}H NMR (400 MHz, $CDCl_3$): 6.84 (s, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 2.80 (d, J = 3.6, 1H), 2.78 (m, 1H), 2.71 (dd, J = 5.4, 1.8, 1H), 2.60 (d, J = 6.0, 1H), 2.56 (d, J = 5.4, 1H), 2.06–1.97 (m, 3H), 1.80–1.58 (m, 2H), 1.32 (qd, J = 12.3, 2.4, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 198.4 (C), 151.8 (C), 148.0 (C), 135.9 (C), 131.8 (C), 126.8 (C), 117.8 (CH), 61.5 (CH_3), 56.3 (CH_3), 40.6 (CH_2), 36.2 (CH), 30.7 (CH_2), 30.5 (CH_2), 29.4 (CH_2), 22.6 (CH_2). MS (FAB): 269 (100, $[M+Na]^+$), 247 (59, $[M+H]^+$). HRMS (FAB): ($C_{15}H_{18}O_3$) calcd 264.1334, found 264.1340.

4.2.6. 7,8-Dimethoxy-2,3,3a,4,5,6-hexahydro-1*H*-phenalene (17)

Ketone **11** (162 mg, 0.66 mmol) was dissolved in MeOH/CH_2Cl_2 1:1 (15 mL) and $NaBH_4$ (60 mg, 1.59 mmol, 2.4 equiv) was added. After stirring at RT overnight, excess reagent was quenched with 1 N aq HCl. The mixture was extracted with EtOAc, the organic layer washed with brine, then dried over Na_2SO_4 and the solvents were removed under vacuum. The crude oil was dissolved in a 1:1 mixture of 5 N aq HCl and THF (15 mL). After stirring for 1 h at RT and workup described as above, the crude yellow oil was dissolved in EtOH (10 mL) and hydrogenated under 1 atm of H_2 in the presence of Pd/C (20 mg). The crude product obtained after filtration and concentration under vacuum was purified by silica flash chromatography to yield 144 mg (94%) of **17** as a colorless oil. IR (film, NaCl): 2925vs, 2854s, 1598m, 1484vs, 1312m, 802w. ^{1}H NMR (400 MHz, $CDCl_3$): 6.52 (s, 1H), 3.83 (s, 3H), 3.78 (s, 3H), 2.90 (ddd, J = 17.4, 6.3, 2.4, 1H), 2.78 (dd, J = 8.7, 4.5, 2H), 2.73–2.62 (m, 1H), 2.50 (t, J = 11.7, 1H), 2.01–1.83 (m, 4H), 1.82–1.65 (m, 2H), 1.36–1.20 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 150.0 (C), 144.2 (C), 131.6 (C), 130.5 (C), 130.3 (C), 109.8 (CH), 59.9 (CH_3), 55.7 (CH_3), 36.6 (CH), 30.8 (CH_2), 30.6 (CH_2), 29.6 (CH_2), 23.7 (CH_2), 23.1 (CH_2), 22.6 (CH_2). MS (EI, 70 eV): 232 (80, M^+), 204 (77), 201 (100, $[M-OCH_3]^+$). HRMS (FAB): ($C_{15}H_{20}O_2$) calcd 232.1463, found 232.1470.

4.2.7. 1-(But-3-enyl)-6,7-dimethoxy-1,2,3,4-tetrahydronaphthalene (12)

The suspension of methyltriphenyl-phosphonium bromide (810 mg, 2.27 mmol, 1.3 equiv) in THF (10 mL) was treated

dropwise with BuLi (purchased from Aldrich Inc., 1.6 N in hexane, 1.4 mL, 2.24 mmol) at 0 °C. The orange suspension was stirred for another 40 min at 0 °C, then a solution of aldehyde **9** (441 mg, 1.78 mmol) in THF (5 mL) was added dropwise via cannula. After warming to RT and stirring for 3 h, the mixture was diluted with TMP and washed with water and brine. Drying over Na_2SO_4, evaporation of the solvents under vacuum, followed by purification by silica flash chromatography yielded 245 mg (56%) of **12** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): 6.69 (s, 1H), 6.58 (s, 1H), 5.90 (ddt, J = 17.1, 10.3, 6.6, 1H), 5.08 (dd, J = 17.1, 1.7, 1H), 5.01 (d, J = 10.0, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 2.80–2.68 (m, 3H), 2.32–2.07 (m, 2H), 1.92–1.61 (m, 6H). MS (EI, 70 eV): 232 (80, M^+), 204 (77), 201 (100, $[M-OCH_3]^+$). HRMS (FAB): ($C_{15}H_{20}O_2$) calcd 232.1463, found 232.1470.

4.2.8. 8,9-Dimethoxy-1-methyl-2,3,3a,4,5,6-hexahydro-1*H*-phenalene (16)

Olefin **12** (247 mg, 1.00 mmol) was stirred with Eaton's reagent (1.5 mL) in a closed vial at 48 °C for 30 min. Workup as described for ketone **11** and purification by silica flash chromatography (EtOAc/TMP 1:9) yielded 223 mg (90%) of **16** as a colorless oil. ^{1}H NMR analysis revealed the presence of two diastereomers in a 2:3 ratio. IR (film, NaCl): 2924vs, 1595w, 1481vs, 833w, 800w. MS (EI, 70 eV): 246 (100, M^+), 235 (65), 215 (64), 204 (77).

Further separation of diastereomers was performed with 40 mg of the mixture via semipreparative HPLC (EtOAc/TMP 98:2, 4 mL/min) to yield compound **16a** (15.4 min, 12 mg) and **16b** (16.2 min, 12 mg) along with a mixed fraction (11 mg). The assignment of the two diastereomers was made based on the appearance of H–C(7) as a quintet in the ^{1}H NMR spectrum of **16a** and as a sextet in **16b**, indicating that the dihedral angle to one H-atom of CH_2(6) in **16a** was close to 90°. A modeling study in silico (semiempirical, PM3[50]) of the relatively rigid tricycles revealed for the lowest energy conformers *H*,*H*-dihedral angles between H(7) and CH_2(6) of 67° and 49° for the 4R^*,7S^*-diastereomer, and 6° and 108° for the 4R^*,7R^*-diastereomer. Accordingly, the 4R^*,7R^* relative configuration was assigned to **16a**. A similar procedure was successfully applied in sesquiterpene conformational analysis by Milosavljevic et al.[51]

4.2.8.1. (1R^*,3aR^*)-8,9-Dimethoxy-1-methyl-2,3,3a,4,5,6-hexahydro-1*H*-phenalene (16a). ^{1}H NMR (400 MHz, $CDCl_3$): 6.52 (s, 1H), 3.82 (s, 6H), 3.21 (quint, J = 6.6, 1H), 2.79–2.76 (m, 2H), 2.51–2.42 (m, 1H), 1.93-1.67 (m, 5H), 1.59–1.47 (m, 2H), 1.37–1.24 (m, 1H), 1.21 (d, J = 7.2, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 150.5 (C), 144.9 (C), 135.6 (C), 132.2 (C), 129.7 (C), 110.5 (CH), 60.8 (CH_3), 55.8 (CH_3), 37.4 (CH), 30.9 (CH_2), 30.7 (CH_2), 29.8 (CH_2), 28.4 (CH), 25.9 (CH_2), 23.2 (CH_2), 23.1 (CH_3).

4.2.8.2. (1R^*,3aS^*)-8,9-Dimethoxy-1-methyl-2,3,3a,4,5,6-hexahydro-1*H*-phenalene (16b). ^{1}H NMR (400 MHz, $CDCl_3$): 6.53 (s, 1H), 3.83 (s, 3H), 3.81 (s, 3H), 3.28 (hext, J = 7.0, 1H), 2.77 (dd, J = 8.4, 4.0, 2H), 2.53–2.45 (m, 1H), 2.10–2.02 (sym. m, 1H), 1.98–1.86 (m, 3H), 1.79–1.66 (m, 1H), 1.52–1.43 (m, 1H), 1.26 (d, J = 7.0, 3H), 1.28–1.14 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 150.6 (C), 145.0 (C), 136.1 (C), 131.6 (C), 131.4 (C), 110.2 (CH), 60.7 (CH_3), 59.8 (CH_3), 35.2 (CH), 31.4 (CH_2), 30.9 (CH_2), 30.2 (CH_2), 30.0 (CH_2), 28.3 (CH), 23.8 (CH_3), 22.9 (CH_2).

4.2.9. 8,9-Dimethoxy-1,1-dimethyl-2,3,3a,4,5,6-hexahydro-1*H*-phenalene (15)

Aldehyde **9** (205 mg, 0.82 mmol) in Et_2O (2 mL) was treated with freshly prepared 1 N MeMgI in Et_2O (1.2 mL, 1.2 mmol, 1.5 equiv) at RT. After stirring overnight, the mixture was poured on ice and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4 and the solvents were evaporated under vacuum. Silica flash chromatography of the resultant product (EtOAc/TMP 1:2) yielded 152 mg (70%) of alcohol **13**. The product (0.58 mmol) was dissolved in CH_2Cl_2 (2 mL). *N*-Methylmorpholin-*N*-oxide (1140 mg, 1.20 mmol, 2.1 equiv) and powdered MS4 Å (280 mg) were added, followed after 15 min by TPAP (12 mg, 0.033 mmol, 6 mol %). After 2 h the mixture was filtered over SiO_2 (rinsing with CH_2Cl_2/10% EtOAc). After removal of the solvents under vacuum, the pure ketone **12** (139 mg, 91%) was dissolved in Et_2O (2 mL) and the solution was added dropwise to the ice-cold 1 N solution of MeMgI in Et_2O (2.2 mL, 2.2 mmol, 3.4 equiv). After 30 min the cooling bath was removed and the mixture stirred for an additional 3 h, then cooled back to 0 °C and quenched by dropwise addition of 1 N aq HCl (pH 6). The mixture was extracted with EtOAc, the organic layer washed with water and brine and dried over Na_2SO_4. After evaporation of the solvent under vacuum, crude **14** (138 mg, 94%) was obtained, which was then stirred with Eaton's reagent (1 mL) for 20 min at 45 °C. Workup as described for the preceding step, followed by silica flash chromatographic purification (EtOAc/TMP 1:9) yielded 108 mg (84%, 51% from aldehyde **9**) of aglycon **15** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): 6.53 (s, 1H), 3.84 (s, 3H), 3.82 (s, 3H), 2.80–2.76 (m, 2H), 2.47-2.39 (m, 1H), 1.92–1.83 (m, 2H), 1.77–1.60 (m, 3H), 1.46 (s, 3H), 1.43–1.36 (m, 1H), 1.32 (s, 3H), 1.30–1.20 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 143.6 (C), 141.3 (C), 131.6 (C), 131.3 (C), 130.4 (C), 110.7 (CH), 60.5 (CH_3), 55.6 (CH_3), 42.7 (C), 38.4 (CH), 35.5 (CH_2), 31.1 (CH_2), 30.9 (CH_2), 30.0 (CH_2), 29.5 (CH_2), 28.1 (CH_3), 22.7 (CH_3). MS (EI, 70 eV): 260 (52, M^+), 245 (100, $[M-CH_3]^+$), 204 (34). HRMS (FTMS-MALDI): ($C_{17}H_{24}O_2$) calcd 260.1776, found 260.1777.

4.2.10. 1-(3,4-Dimethoxyphenyl)oct-7-en-4-ol (21)

A solution of bromide **19** prepared according to Bradsher and Hunt[52] (580 mg, 2.37 mmol) in dry THF (2 mL) was added dropwise to a suspension of Mg (105 mg, 4.32 mmol, 1.8 equiv) in THF (1 mL), containing a small iodine crystal. After complete addition, the solution was heated to 60 °C for 1 h, after which the suspension became turbid and the iodine color disappeared. The Grignard solution was then added dropwise at −30 °C to the slurry of CuI (81 mg, 0.43 mmol, 0.18 equiv) in THF (1 mL). After stirring for 10 min at this temperature, 1,2-epoxy-5-hexene (**20**) was added and stirring continued at 0 °C for 1 h. After quenching with water, 1 N aq HCl-solution was added to dissolve all the mg and the mixture extracted with EtOAc. The organic extracts were washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The product was purified by silica flash chromatography (trimethylpentane/EtOAc 3:1 to 2:1) to yield 242 mg of compound **21** (45%). ^{1}H NMR (400 MHz, $CDCl_3$): 6.81–6.76 (m, 1H), 6.74–6.67 (m, 2H), 5.82 (ddt, J = 17.0, 10.1, 6.2, 1H), 5.03 (dd, J = 17.0, 1.7, 1H), 4.97 (d, J = 10.0, 1H), 3.82 (s, 3H), 3.71 (s, 3H), 3.35 (quint, J = 7.1, 3H), 2.57 (t, J = 6.8, 2H), 1.67–1.42 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): 148.8, 147.1, 138.6, 135.1, 120.2, 114.8, 111.7, 111.2, 71.3, 55.9, 55.8, 37.0, 36.5, 35.5, 30.1, 27.7. HRMS (FTMS-MALDI): ($C_{16}H_{24}O_3$) calcd 264.1725, found 264.1725.

4.2.11. 8,9-Dimethoxy-1-methyl-2,3,3a,4,5,6-hexahydro-1*H*-phenalene (16)

Preparation by double cyclization of **21**. Precursor **21** (24 mg, 0.09 mmol) was absorbed on SiO_2 (1.29 g) and the resulting powder was placed in a dropping funnel and added during 20 min to Eaton's reagent (3 mL) stirring at 45 °C (controlled slow addition of the powder could conveniently be effected by using the vibrations caused by the magnetic stirring bar in the oil bath knocking against the bottom of the flask). The thick suspension was stirred for 3 h at 45 °C. Workup as described for ketone **11**, and silica flash chromatographic purification (EtOAc/TMP 1:9), yielded 9 mg (40%)

of **16** as a colorless oil which illustrated NMR characteristics identical to the mixture of **16a/b**.

4.2.12. 7-Bromo-8,9-dimethoxy-2,3,3a,4,5,6-hexahydro-1*H*-phenalene (22)

Tricycle **17** (120 mg, 0.52 mmol) was dissolved in DMF (4 mL) and NBS (140 mg, 0.78 mmol, 1.5 equiv) was added. After stirring at RT for 20 h under exclusion of light, the solution was diluted with TMP and the organic layer washed with water, followed by drying over Na_2SO_4 and concentration under vacuum to yield bromide **22** (147 mg, 91%) as a slightly yellow crystalline solid, mp = 56 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.87 (s, 3H), 3.84 (s, 3H), 2.87 (td, J = 17.2, 6.6, 2H), 2.73–2.56 (m, 2H), 2.52 (sym. m, 1H), 2.07–1.86 (m, 4H), 1.82–1.64 (m, 2H), 1.28 (sym. m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 149.1 (C), 147.8 (C), 135.7 (C), 131.6 (C), 129.8 (C), 117.5 (C), 60.3 (CH_3), 60.1 (CH_3), 37.3 (CH), 30.7 (CH_2), 30.3 (CH_2), 30.2 (CH_2), 23.5 (CH_2), 22.9 (CH_2), 22.1 (CH_2). MS (EI, 70 eV): 312 (97, $[M-^{81}Br]^+$), 310 (100, $[M-^{79}Br]^+$), 231 (73, $[M-Br]^+$). HRMS (FTMS-MALDI): ($C_{15}H_{19}BrO_2$) calcd (^{79}Br) 310.0568, found 310.0577.

4.2.13. 7,8-Dimethoxy-9-methyl-2,3,3a,4,5,6-hexahydro-1*H*-phenalene (23)

Bromide **22** (34 mg, 0.11 mmol) was dissolved in dioxane (1.5 mL) together with $Pd(PPh_3)_4$ (10 mg, 9 μmol, 7 mol %) and Me_3Al (1 N in toluene, 0.40 mL, 0.4 mmol, 3.2 equiv). The resulting solution was heated to reflux for 3 h, then cooled and the solvent removed under vacuum. The crude product was absorbed on SiO_2 and purified by flash chromatography (EtOAc/heptane 1:9) to yield aglycon **23** (22 mg, 82%) as a colorless viscous oil. IR (film, NaCl): 2925vs, 2825m, 1461s, 1089s, 948w, 799w, 721w, 696w, 542m. ^{1}H NMR (300 MHz, $CDCl_3$): 3.82 (s, 3H), 3.81 (s, 3H), 2.92–2.84 (m, 1H), 2.78–2.65 (m, 2H), 2.62–2.46 (m, 2H), 2.12 (s, 3H), 2.06–1.86 (m, 4H), 1.38–1.21 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): 148.6 (C), 148.1 (C), 133.8 (C), 131.9 (C), 130.6 (C), 127.5 (C), 60.4 (CH_3), 60.0 (CH_3), 37.4 (CH), 30.7 (CH_2), 29.8 (CH_2), 27.5 (CH_2), 23.6 (CH_2), 23.2 (CH_2), 22.6 (CH_2), 11.9 (CH_3). HRMS (FAB): ($C_{16}H_{22}O_2$) calcd 246.1620, found 246.1629.

4.2.14. 7,8-Dimethoxy-9-trifluoromethyl-2,3,3a,4,5,6-hexahydro-1*H*-phenalene (24)

Bromide **22** (40 mg, 0.13 mmol) was dissolved in NMP (2 mL) and sodium trifluoroacetate (138 mg, 1.01 mmol, 7.8 equiv) and CuI (107 mg, 0.72 mmol, 5.6 equiv) were added. The resulting suspension was heated to 170 °C for 4.5 h. After washing with 1 N aq HCl, extraction with TMP, washing of the organic layer with brine, drying over Na_2SO_4, and concentration under vacuum, purification by silica flash chromatography (EtOAc/TMP 1:9) yielded product **24** (32 mg, 82%) as a colorless oil. IR (film, NaCl): 2939s, 2861s, 1578m, 1462vs, 1415vs, 1287s, 1118s, 1035m, 705w. ^{1}H NMR (300 MHz, $CDCl_3$): 3.83 (s, 3H), 3.79 (s, 3H), 2.93–2.78 (m, 2H), 2.75 (t, J = 6.6, 2H), 2.46 (hept, J = 5.9, 1H), 1.98–1.78 (m, 4H), 1.78–1.63 (m, 2H), 1.31–1.18 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 149.8 (C), 149.1 (C), 136.0 (C), 135.4 (C), 132.0 (C), 125.0 (d, $^1J_{C-F}$ = 274), 119.6 (d, $^2J_{C-F}$ = 27), 61.3 (CH_3), 60.0 (CH_3), 36.0 (CH), 30.2 (CH_2), 29.5 (2 CH_2), 23.5 (CH_2), 22.2 (CH_2), 21.4 (CH_2). MS (EI, 70 eV): 300 (100, M^+), 281 (19, $[M-F]^+$), 257 (43), 231 (69, $[M-CF_3]^+$).

4.2.15. Xylosides 27 and 28

Aglycon **17** (70 mg, 0.343 mmol) was deprotected according to procedure A (see below) and dissolved in CH_2Cl_2 (12 mL). Triacetyl-L-xylose trichloroacetimidate **26** (128 mg, 0.343 mmol, 1 equiv), prepared according to Kinzy,[53] was added, followed by dry molecular sieves 4 Å (100 mg). The mixture was stirred for 15 min at RT then cooled to −10 °C and TMSOTf (170 μL of 0.1 M solution in CH_2Cl_2, 0.017 mmol, 5 mol %) was added dropwise. The mixture was stirred at −10 °C for 30 min, then water was added. The mixture was diluted with CH_2Cl_2, the phases were separated, the organic layer washed with brine and dried over Na_2SO_4. After removal of the solvent, 195 mg of a crude oil were obtained, of which 70 mg were subjected to semipreparative SiO_2–HPLC (TMP/EtOAc 3:1, 4 mL/min) to yield tri-*O*-acetyl-**28** (22.7 mg, 40%, HPLC 18.9 min) and tri-*O*-acetyl-**27** (9.8 mg, 17%, HPLC 21.8 min), both as mixtures of four diastereomers (epimers at C(4) as well as 1:1 mixtures of anomers). Deprotection of both regioisomers was effected by stirring in methanolic K_2CO_3 solution at RT for 30 min. The resulting glycosides were purified as described in procedure A.

4.2.15.1. 5-Hydroxy-2,3,7,8,9,9a-hexahydro-1*H*-phenalen-4-yl-D-xylopyranoside (27). $[\alpha]_D$ = −27 (*c* 0.20, EtOH). IR (film, NaCl): 3313br, 2832s, 1456s, 1288m, 980m. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.63 (s, 0.5H), 8.21 (s, 0.5H), 6.18 (s, 1H), 5.72 (d, J = 2.9, 0.5H), 5.61 (d, J = 3.0, 0.5H), 5.28 (d, J = 4.7, 1H), 5.21 (d, J = 4.9, 1H), 4.48 (d, J = 7.0, 0.5H), 4.46 (d, J = 7.4, 0.5H), 3.84 (m, 1H), 3.43–3.37 (m, 1H), 3.29–3.14 (m, 3H), 2.80–2.74 (m, 1H), 2.68–2.61 (m, 2H), 2.50–2.32 (m, 2H), 2.01–1.80 (m, 4H), 1.59–1.56 (m, 2H), 1.23–1.15 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD): 146.7, 146.2, 134.5, 134.4, 127.6, 121.5, 117.9 117.7, 107.8, 107.6, 78.1, 75.5, 75.1, 71.3, 67.5, 38.7, 32.4, 32.3, 30.0, 25.8, 24.5, 24.4. MS (FAB^+): 359 (61, $[M+Na]^+$), 204 (100 $[M-Xylose]^+$). HRMS (FAB^+): ($C_{18}H_{24}O_6Na$) calcd 359.1471, found 359.1463.

4.2.15.2. 4-Hydroxy-2,3,7,8,9,9a-hexahydro-1*H*-phenalen-5-yl-D-xylopyranoside (28). $[\alpha]_D$ = −35 (*c* 0.25, EtOH). IR (film, NaCl): 3325br, 2825s, 1475s, 1288m, 980m, 825w, 773m. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.01 (s, 0.5H), 7.98 (s, 0.5H), 6.56 (s, 1H), 5.83 (d, J = 3.5, 0.5H), 5.83 (d, J = 3.6, 0.5H), 5.14 (d, J = 4.8, 1H), 5.07 (d, J = 5.2, 1H), 4.44 (d, J = 7.2, 0.5H), 4.43 (d, J = 7.6, 0.5H), 3.75 (dd, J = 11.4, 5.4, 1H), 3.38–3.31 (m, 1H), 3.26–3.13 (m, 3H), 2.74–2.65 (m, 1H), 2.53–2.57 (m, 2H), 2.49–2.37 (m, 2H), 1.98-1.78 (m, 4H), 1.67–1.59 (m, 2H), 1.22–1.10 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD): 146.9, 146.8, 134.7, 134.6, 127.4, 121.7, 118.1 118.0, 107.5, 107.4, 78.4, 75.1, 75.0, 71.2, 67.6, 38.7, 32.7, 32.5, 30.2, 25.9, 24.8, 24.6. MS (FAB^+): 359 (65, $[M+Na]^+$), 204 (100 $[M-Xylose]^+$). HRMS (FAB^+): ($C_{18}H_{24}O_6Na$) calcd 359.1471, found 359.1459.

4.2.16. General procedure A: Deprotection and fucosylation of aglycones

BBr_3 (1 N in CH_2Cl_2, 0.60 mL, 0.60 mmol, 3.0 equiv) was added dropwise to the solution of dimethoxyaglycon (0.20 mmol) in CH_2Cl_2 (3.0 mL) at −20 °C. The solution was warmed to −5 °C over 1 h, then poured on ice cold water and extracted with EtOAc. The organic layer was washed thoroughly with water and brine, dried over Na_2SO_4 and the solvent removed under vacuum at RT. The free catechol was immediately dissolved in CH_2Cl_2 (4 mL) and 2,6-di-*t*-butylpyridine (135 μl, 0.60 mmol, 3.0 equiv) was added at RT, followed by the solution of α-tri-*O*-TMS-fucosyl-2-iodide[48] in CH_2Cl_2 (0.3 N, 2.00 mL, 3.0 equiv). After stirring overnight at RT, MeOH (2 mL) was added and the solution stirred for additional 30 min. The solution was treated with K_2CO_3 (10 mg), stirred for 30 min and the resulting suspension filtered. The crude product obtained after concentration under vacuum was purified by silica flash chromatography (eluent: EtOAc/acetone 95:5). The product containing fractions were collected and purified by semipreparative silica HPLC (EtOAC/acetone 97:3) for final purification (purity >95%) with concomitant separation of regioisomers where indicated.

4.2.17. Fucosides 29 and 30

Aglycon **17** (40 mg, 0.17 mmol) was deprotected and fucosylated according to general procedure **A** to yield 7 mg (12%) of *O*–C(9) fucoside **29** and 24 mg (40%) of *O*–C(10) fucoside **30**, both as white solids. Both compounds are 1:1 mixtures of epimers at C(4), which have been designated as α and β.

4.2.17.1. 5-Hydroxy-2,3,7,8,9,9a-hexahydro-1*H*-phenalen-4-yl 6-deoxy α-L-galactopyranoside (29). HPLC 10.2 min. Mp 203 °C (decomp.). $[\alpha]_D = -45$ (*c* 0.20, EtOH). IR (film, NaCl): 3325br, 2924s, 1732m, 1475m, 1073s, 998m, 954s. ^{1}H NMR (300 MHz, $(CD_3)_2CO$): δ 8.96 (s, 0.5H, phenolic OH), 8.87 (s, 0.5H, phenolic OH), 6.39 (s, 1H), 5.84 (br. d, *J* ~8, 1H), 4.94 (br s, 0.5H, anomeric H), 4.90 (d, *J* = 2.7, 0.5H, anomeric H), 4.46 (quint, *J* = 6.1, 1H), 4.09–4.04 (m, 1H), 4.00 (br s, 2H), 3.84 (br s, 1H), 3.81 (t, *J* = 3.8, 1H), 3.16–3.05 (m, 1H), 2.94-2.86 (m, 1H), 2.67 (dd, *J* = 8.6, 4.8, 2H), 2.44–2.36 (m, 1H), 1.94–1.80 (m, 4H), 1.76–1.58 (m, 2H), 1.28 (d, *J* = 6.6, 1.5H, CH_3), 1.27 (d, *J* = 6.6, 1.5H, CH_3), 1.25–1.16 (m, 2H). ^{13}C NMR (100 MHz, $(CD_3)_2O$): 146.0, 143.4, 133.8, 130.8, 130.6, 129.2, 114.5, 105.5, 72.9, 70.9, 70.4, 68.9, 68.7, 37.6, 37.4, 25.4, 24.7, 24.0, 23.9, 23.8, 23.5, 17.3, 17.2. MS (ESI neg.): 349 (100, $[M-H]^-$). HR-MS (FAB^+): ($C_{19}H_{26}O_6Na$) calcd 373.16271, found 373.16150.

4.2.17.2. 4-Hydroxy-2,3,7,8,9,9a-hexahydro-1*H*-phenalen-5-yl 6-deoxy α-L-galactopyranoside (30). HPLC 12.7 min. Mp 177–178 °C (decomp.). $[\alpha]_D = -113$ (*c* 0.40, EtOH). IR (film, NaCl): 3369br, 2923s, 2853s, 1611w, 1482s, 1073s, 848w, 826w, 767w. ^{1}H NMR (300 MHz, acetone-d_6): δ 7.88 (s, 0.5H, phenolic OH-α), 7.86 (s, 0.5H, phenolic OH-β), 6.67 (s, 1H), 5.10 (br s, 1H), 5.00 (d, *J* = 3.4, 0.5H, anomeric H-α), 4.99 (d, *J* = 3.4, 0.5H, anomeric H-β), 4.26 (qd, *J* = 6.6, 2.9, 1H), 4.07–4.01 (m, 1H), 4.00–3.89 (m, 2H), 3.79 (s, 2H), 2.85-2.72 (m, 1H), 2.68 (dd, *J* = 8.7, 4.8, 2H), 2.61-2.36 (m, 2H), 2.00–1.81 (m, 4H), 1.76–1.59 (m, 2H), 1.28 (d, *J* = 6.6, 3H), 1.25–1.15 (m, 2H). ^{13}C NMR (100 MHz, $(CD_3)_2O$): 144.9 (C), 144.8 (C), 144.1 (C), 134.2 (C), 127.0 (C), 117.9 (CH), 117.7 (CH), 103.6 (CH), 72.9 (CH), 71.1 (CH), 70.0 (CH), 68.3 (CH), 37.7 (CH), 31.6 (CH_2), 31.5 (CH_2), 29.6 (CH_2), 24.3 (CH_2), 23.9 (CH_2), 23.3 (CH_2), 16.9 (CH_3). MS (ESI^-): 349 (100, $[M-H]^-$). FT-HRMS: ($C_{19}H_{26}O_6Na$) calcd 373.1627, found: 373.1628.

4.2.18. 9-Hydroxy-2,3,3a,4,5,6-hexahydro-1*H*-phenalen-1-on-8-yl 6-deoxy α-L-galactopyranoside (31)

Ketone **11** (58 mg, 0.24 mmol) was deprotected and fucosylated according to general procedure **A** to yield fucoside **27** (21 mg, 25%) as a crystalline solid (1:1 mixture of C(4)-epimers, α and β). $[\alpha]_D = -31$ (*c* 0.25, EtOH). IR (film, NaCl): 3423vs (br), 2972m, 1638m, 1379w, 1161w, 1129w, 953w. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.75 (s, 0.5H, phenolic OH-α), 12.74 (s, 0.5H, phenolic OH-β), 7.12 (s, 1H), 5.37 (d, *J* = 3.4, 1H, anomeric), 4.75 (dd, *J* = 8.9, 6.0, 1H), 4.72 (d, *J* = 6.4, 1H), 4.54 (d, *J* = 4.5, 1H), 4.12–4.05 (m, 1H), 3.82–3.69 (sym. m, 3H), 3.56 (t, *J* = 3.2, 1H), 2.85-2.59 (m, 4H), 2.05-1.89 (m, 3H), 1.72–1.56 (m, 2H), 1.29 (q, *J* = 11.9, 1H), 1.02 (d, *J* = 6.4, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): 206.4 (C), 152.5 (s, α), 152.4 (s, β), 143.2 (s, α), 143.1 (s, β), 136.6 (s, α), 136.5 (s, β), 125.6 (CH), 125.5 (d, α), 125.4 (d, β), 72.9 (CH), 116.0 (C), 99.6 (d, α), 99.5 (d, β), 71.5 (CH), 69.4 (CH), 67.8 (CH), 67.3 (CH), 35.3 (CH), 30.2 (CH_2), 29.8 (CH_2), 28.0 (CH_2), 27.9 (CH_2), 22.2 (CH_2), 16.5 (CH_3). HRMS: ($C_{19}H_{24}O_7Na$) calcd 387.1420, found: 387.1421.

4.2.19. 4-Hydroxy-3,3-dimethyl-2,3,7,8,9,9a-hexahydro-1*H*-phenalen-5-yl 6-deoxy α-L-galactopyranoside (32)

Aglycon **15** (17 mg, 0.065 mmol) was deprotected and fucosylated according to general procedure **A** to yield product **32** (12 mg, 49%) as a colorless solid (HPLC 8.8 min) (1:1-mixture of epimers at C(4), α and β). $[\alpha]_D = -109$ (*c* 0.35, EtOH). IR (film, NaCl): 3370br, 2922vs, 2855m, 1602w, 1453m, 1264m, 1081s, 960m, 1034m, 982w, 958w, 825w, 738w. ^{1}H NMR (300 MHz, acetone-d_6): δ 7.86 (s, 0.5H, phenolic OH-α), 7.84 (s, 0.5H, phenolic OH-β), 6.68 (br s, 0. 0.5H), 6.67 (s, 0.5H), 5.13–5.07 (m, 1H), 4.94 (d, *J* = 3.7, 1H, anomeric), 4.24 (quint, *J* = 6.0, 1H), 4.06–3.87 (m, 3H), 3.77 (s, 2H), 2.66 (dd, *J* = 8.0, 5.6, 2H), 2.43–2.30 (m, 1H), 1.90–1.58 (m, 6H), 1.43 (s, 3H), 1.30 (s, 3H), 1.26 (d, *J* = 6.6, 3H), 1.39–1.20 (m, 1H), 1.17 (t, *J* = 7.0, 1H). ^{13}C NMR (acetone-d_6): 146.3, 146.2, 144.9, 134.5, 134.4, 131.1, 127.2, 118.6, 118.2, 103.8, 103.7, 72.8, 71.7, 69.8, 68.3, 43.1, 39.2, 35.7, 31.8, 30.4, 28.8, 27.8, 27.7, 23.4, 16.8. MS (neg. ES): 377 (100, $[M-H]^-$). HRMS (FT-MALDI): ($C_{21}H_{30}O_6Na$) calcd 401.1935, found 401.1920.

4.2.20. (3*R**,9a*R**)-4-Hydroxy-3,3-dimethyl-2,3,7,8,9,9a-hexahydro-1*H*-phenalen-5-yl 6-deoxy α-L-galactopyranoside (33)

Aglycon **16a** (12 mg, 0.049 mmol) was deprotected and fucosylated according to general procedure **A** to yield product **33** (9 mg, 50%) as a colorless solid (HPLC 10.0 min). NMR-analysis indicated the presence of a 1:1-mixture of epimers at C(4) (α and β). $[\alpha]_D = -93$ (*c* 0.35, EtOH). IR (film, NaCl): 3361br, 2922s, 2856m, 1608w, 1350m, 1285m, 1080s, 960w, 840m, 738w. ^{1}H NMR (300 MHz, acetone-d_6): δ 7.89 (s, 0.5H, phenolic OH-α), 7.83 (s, 0.5H, phenolic OH-β), 6.66 (s, 1H), 5.14 (d, *J* = 5.1, 0.5H), 5.06 (d, *J* = 5.1, 0.5H), 4.99 (d, *J* = 3.7, 0.5H, anomeric H-α), 4.94 (d, *J* = 3.4, 0.5H, anomeric H-β), 4.21 (q, *J* = 6.4, 1H), 4.06–3.86 (m, 3H), 3.77 (br s, 2H), 3.14 (quint, *J* = 6.1, 1H), 2.64 (dd, *J* = 8.1, 4.2, 2H), 2.47–2.33 (m, 1H), 1.92–1.75 (m, 4H), 1.74–1.58 (m, 1H), 1.46–1.04 (m, 2H), 1.26 (d, *J* = 6.4, 1.5H), 1.26 (d, *J* = 6.6, 1.5H), 1.21 (d, *J* = 6.8, 3H). ^{13}C NMR (DMSO-d_6): 143.0, 142.9, 142.8, 142.6, 132.7, 127.7, 127.6, 125.3, 114.7, 114.5, 101.4, 101.0, 71.4, 69.4, 68.1, 67.2, 62.1, 35.4, 35.1, 31.0, 30.8, 30.7, 30.6, 30.1, 30.0, 28.8, 27.9, 27.7, 22.5, 22.4, 16.6. MS (ESI^-): 363 (100, $[M-H]^-$). HRMS (MALDI): ($C_{20}H_{28}O_6Na$) calcd 387.1784, found 387.1797.

4.2.21. (3*R**,9a*S**)-4-Hydroxy-3,3-dimethyl-2,3,7,8,9,9a-hexahydro-1*H*-phenalen-5-yl 6-deoxy α-L-galactopyranoside (34)

Aglycon **16b** (12 mg, 0.049 mmol) was deprotected and fucosylated according to general procedure **A** to yield product **34** (7 mg, 39%) as a colorless solid (HPLC 9.3 min). NMR-analysis indicated the presence of only O–C(10)-fucosylated product (1:1-micture of epimers at C(4)). $[\alpha]_D = -185$ (*c* 0.25, EtOH). IR (film, NaCl): 3375br, 2925vs, 2858m, 1608w, 1479s, 1296m, 1082vs, 960m, 837m, 738m. ^{1}H NMR (300 MHz, acetone-d_6): δ 7.90 (s, 0.5H, phenolic OH-α), 7.87 (s, 0.5H, phenolic OH-β), 6.66 (br s, 1H), 5.15 (br s, 0.5H), 5.11 (br s, 0.5H), 5.00 (d, *J* = 3.2, 0.5H, anomeric H-α), 4.94 (d, *J* = 3.2, 0.5H, anomeric H-β), 4.25 (quint, *J* = 6.5, 1H), 4.10–3.86 (m, 3H), 3.77 (s, 2H), 3.15 (quint, *J* = 7.1, 1H), 2.65 (dd, *J* = 7.6, 5.6, 2H), 2.47–2.34 (m, 1H), 1.92-1.60 (m, 6H), 1.48 (q, *J* = 12.5, 1H), 1.27 (d, *J* = 6.1, 1.5H), 1.26 (d, *J* = 6.6, 1.5H), 1.16 (d, *J* = 6.8, 3H), 2H). ^{13}C NMR (acetone-d_6): 142.9, 142.61, 142.57, 142.47, 130.8, 127.6, 125.8, 115.1, 114.8, 101.6, 101.0, 71.4, 69.5, 68.2, 67.3, 67.2, 38.9, 36.7, 30.4, 30.0, 28.7, 27.5, 27.4, 25.5, 22.8, 21.5, 21.4, 16.7. MS (ESI^-): 363 (100, $[M-H]^-$). HRMS (MALDI): ($C_{20}H_{28}O_6Na$) calcd 387.1784, found 387.1791.

4.2.22. 5-Hydroxy-6-methyl-2,3,7,8,9,9a-hexahydro-1*H*-phenalen-4-yl 6-deoxy α-L-galactopyranoside (35)

Aglycon **19** (8 mg, 0.033 mmol) was deprotected and fucosylated according to general procedure **A** to yield product **21** (8 mg, 67%) as a colorless solid (HPLC 8.6 min). NMR-analysis indicates the presence of a mixture of O–C(9) and O–C(10) glycosides, ca. 1:1, both present as C(4) epimers. $[\alpha]_D = -78$ (*c* 0.35, EtOH). IR (film, NaCl): 3341vs (br), 2922vs, 2855m, 1602vw, 1452m,

1308w, 1165w, 1081vs, 953m, 834w, 737w. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.04, 9.00, 8.95, 8.93 (each s, 0.5H, phenolic OH), 5.85 (br s, 1H), 4.92–4.80 (m, 1H), 4.51–4.38 (m, 1H), 4.10 (br s, 1H), 3.99 (s, 2H), 3.83 (d, J = 4.6, 2H), 2.76–2.55 (m, 3H), 2.55–2.34 (m, 2H), 2.10 (d, J = 4, 2H), 1.99 (s, 3H), 2.00–1.75 (m, 2H), 1.75–1.57 (m, 2H), 1.34–1.08 (m, 2H), 1.26 (d, J = 6.6, 3H). MS (ESI$^-$): 363 (100, [M−H]$^-$). HRMS (MALDI): ($C_{20}H_{28}O_6Na$) calcd 387.1778, found: 387.1763.

4.2.23. 5-Hydroxy-6-trifluoromethyl-2,3,7,8,9,9a-hexahydro-1*H*-phenalen-4-yl 6-deoxy α-L-galactopyranoside (36)

Aglycon **20** (20 mg, 0.07 mmol) was deprotected and fucosylated according to general procedure **A** to yield fucoside **36** (6 mg, 20%) as a glass (HPLC 9.4). The product constitutes in an inseparable mixture of *O*–(C-9) and *O*–C(10)-regioisomers, ca. 1:1, each as C(4)-epimeric mixtures. $[\alpha]_D = -87$ (c 0.25, EtOH). IR (film, NaCl): 3307br, 2930s, 2864w, 1585vw, 1449s, 1303m, 1094br s, 1015m, 951m, 668m. ^{1}H NMR (400 MHz, (CD_3OD): δ 4.95–4.87 (m, 1H), 4.46 (quint, J = 6.4, 0.6H), 4.34 (q, J = 6.4, 0.4H), 4.02–3.89 (m, 2H), 3.79 (dd, J = 13.2, 2.0, 1H), 3.02–2.94 (m, 1H), 2.90–2.55 (m, 3H), 2.54–2.42 (sym. m, 1H), 2.01–1.85 (m, 4H), 1.82–1.63 (m, 4H), 1.29 (d, J = 6.8, 0.9H), 1.28 (d, J = 6.4, 0.6H), 1.24 (d, J = 5.6, 0.9H) 1.23 (d, J = 6.4, 0.6H). MS (ESI$^-$): 417 (100, [M−H]$^+$). HRMS (MALDI-FTMS): ($C_{20}H_{25}F_3O_6Na$) calcd 441.1495 found: 441.1506.

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