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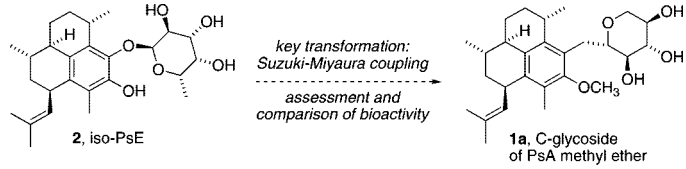
Synthesis and an Evaluation of the Bioactivity of the *C*-Glycoside of Pseudopterosin A Methyl Ether

Wei Zhong,[†] Claudia Moya,[‡] R. S. Jacobs,[‡] and R. Daniel Little*,[†]

Department of Chemistry and Biochemistry and Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, California 93106

little@chem.ucsb.edu

Received June 30, 2008



The Suzuki−Miyaura cross-coupling protocol was applied to the synthesis of **1a**, the *C*-glycoside analogue of PsA methyl ether. This marks the first construction of a *C*-glycoside for this class of marine natural products, thereby offering an opportunity to compare its bioactivity to the natural substances. Its activity profile resembled that of PsA (**1**) and PsA *O*-methyl ether (**1b**) when assayed for its anti-inflammatory activity and its ability to inhibit phagocytosis. We conclude that the intact structure is present when a pseudopterosin expresses its anti-inflammatory and phagocytosis inhibitory properties and that they are, therefore, not likely to be prodrugs. Results show that **1a** is an effective binding agent toward the A_{2A} and A_3 adenosine receptors, displaying IC_{50} values of 20 and 10 μM, respectively.

Introduction

The pseudopterosins, a class of diterpene glycosides isolated from the marine octocoral *Pseudopterogorgia elisabethae*,[1−3] show potent anti-inflammatory and wound healing properties.[4,5] Pseudopterosin A (PsA, **1**; Figure 1), a potent inhibitor of phorbol myristate acetate (PMA)-induced topical inflammation in mice,[6] stabilizes cell membranes,[4] prevents the release of prostaglandins and leukotrienes from zymosan-stimulated murine macrophages, and inhibits degranulation of human polymorphonuclear leukocytes[7] and also inhibits phagosome formation in *Tetrahymena* cells.[8] Its C-10 *O*-methyl ether, **1b**, offers a promising treatment for contact dermatitis.[9]

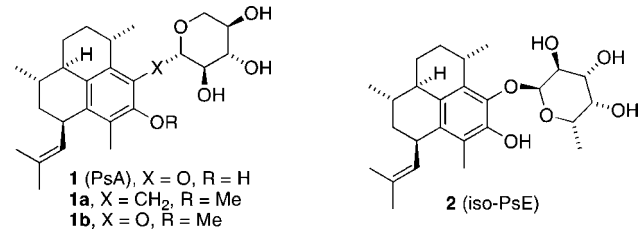


FIGURE 1. Structures of PsA (**1**), the target structure **1a**, and iso-PsE (**2**).

To explore the role of the *O*-glycoside linkage in the expression of PsA biological activity, a synthetic route that would provide access to *C*-glycoside analogues of the natural products was required. *C*-Glycosides often display biochemical profiles similar to those of their oxygenated relatives. We reasoned that the properties of this glycoside might be altered if hydrolytic stability is important to the maintenance of bioactivity since cleavage of the sugar by a glycosidase enzyme is not possible. On the other hand, should cleavage be required to deliver the active form of the drug then the presence of the carbon linkage ought to have deleterious consequences.

[†] Department of Chemistry and Biochemistry.
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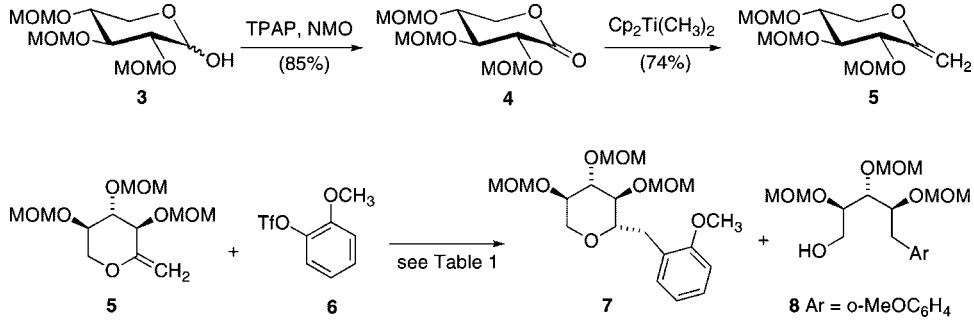
10.1021/jo801432t CCC: $40.75
Published on Web 08/19/2008

SCHEME 1. Assembly of Enol Ether Coupling Partner 5 and the Suzuki−Miyaura Coupling Protocol Applied to a Model System



Iso-PsE (**2**), a new pseudopterosin with anti-inflammatory and potential wound healing properties, was discovered recently.[10] We envisioned that it could serve as a lead structure for the synthesis of PsA-OMe *C*-glycoside (**1a**) since its aglycon is identical to that of PsA (**1**). The plan, therefore, was to remove the sugar from **2** and to replace it by the *C*-glycoside analog of D-xylose. Herein we report the first synthesis of a pseudopterosin *C*-glycoside, PsA-OMe *C*-glycoside (**1a**), via a generalizable sequence of transformations and provide an assessment of its pharmacology.

Results and Discussion

Model Study. To achieve our objectives we examined the Suzuki−Miyaura cross-coupling protocol as the key transformation in our synthetic scheme.[11−15] A model coupling reaction between the enol ether derived from 2,3,4-tri-*O*-methoxmethyl-D-xylopyranoside, **5**, and 2-methoxyphenyl triflate **6** was explored (Scheme 1). Coupling partner **5** was prepared via the oxidation of MOM-protected D-xylose structure **3** using TPAP and NMO,[16] followed by treatment of the resulting lactone **4** with freshly prepared Petasis' reagent.[17] With olefin **5** and triflate **6** in hand,[18] we examined the Suzuki−Miyaura coupling. Our initial attempts resulted in the formation of the acyclic product **8** rather than the desired adduct **7**. This outcome is consistent with observations made by Link and co-workers who noted the formation of an acyclic byproduct in the coupling of aryl bromides and triflates with the glucose-derived enol ether analogue of **5**.[11] Since xylose is a more reactive sugar than glucose, the formation of acyclic byproduct was, therefore, not entirely unexpected.

Our attempts to optimize the coupling reaction are summarized in Table 1. In each case, the cross-coupling was performed at room temperature using 1.0 equiv of the enol ether and 0.1 equiv of the catalyst $PdCl_2$(dppf). As illustrated by entries 1 and 2, attempts to form the alkylborane by using >2 equiv of 9-BBN at an elevated temperature resulted in the formation of the acyclic byproduct **8**, while no reaction occurred at 0 °C (entry 3). In contrast, when the olefin was stirred or sonicated in the presence of 1.5 equiv of 9-BBN at room temperature, the desired *C*-glycoside **7** was formed in acceptable yields (74% when stirred and 64% when sonicated); no acyclic byproduct was produced.

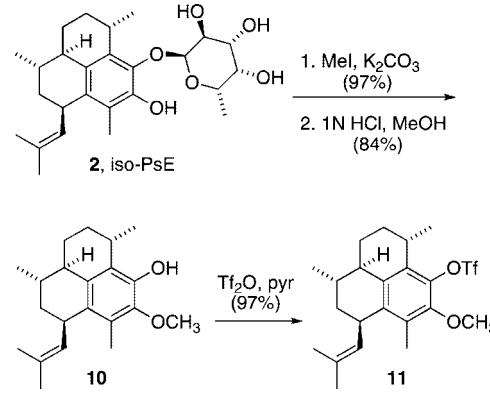
TABLE 1. Optimization of Suzuki−Miyaura Cross-Coupling Reaction

entry	9-BBN (equiv)	hydroboration conditions [a]	quenching agent[b]	triflate[c] (equiv)	comment
1	2.6	70 °C, 5 h	K_3PO_4	2	byproduct **8** only
2	2.5	60 °C, 2 h	$NaHCO_3$	1	byproduct **8** only
3	1.2	0 °C, 5 h	$NaHCO_3$	0.5	no reaction
4	1.5	0 °C to rt, 2 h with sonication	$NaHCO_3$	0.5	**7**, 64%
5	1.5	0 °C to rt, 2 h	$NaHCO_3$	0.5	**7**, 74%

[a] 0.5 M 9-BBN in THF. [b] 1 M aqueous solution. [c] 0.1 equiv of $PdCl_2$(dppf) and **6** in DMF.

Synthesis of the Target *C*-Glycoside 1a. We applied the knowledge and experience gained from the model studies to the synthesis of *C*-glycoside **1a** using the cross coupling of aryl triflate **11** and enol ether **5**. A short, efficient preparation of triflate **11** was achieved from iso-PsE (**2**), as shown in Scheme 2. Thus, methylation of iso-PsE (**2**), followed by acidic hydrolysis resulted in the formation of PsA methyl ether aglycon **10** in an 81% yield overall.[19] Treatment of **10** with triflic

SCHEME 2. Formation of Triflate Coupling Partner 11



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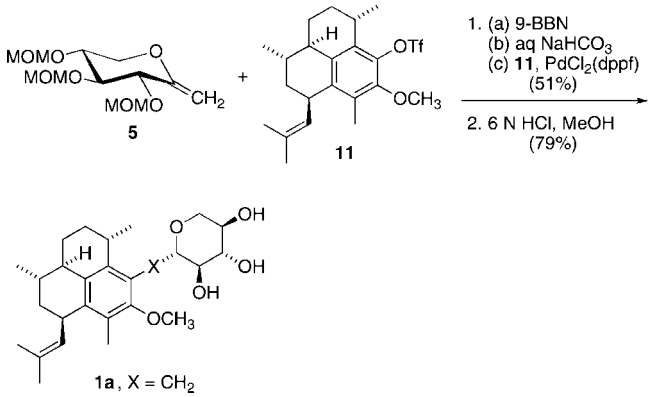
anhydride and pyridine resulted in the isolation of 97% of the required triflate **11**.

Unlike the coupling of **5** and triflate **6**, the room temperature reaction of triflate **11** with the hydroboration adduct derived from **5** did not lead to coupling, presumably because **11** is significantly more hindered than **6**. On the other hand, the addition of triflate **11** and $PdCl_2$(dppf) in DMF at room temperature to the hydroboration adduct obtained from **5** and 9-BBN followed by stirring overnight at 80 °C resulted in the formation of desired *C*-glycoside in a 51% yield. The MOM protecting groups were efficiently removed by using 6 N aqueous HCl in MeOH, thereby revealing the *C*-glycoside of PsA methyl ether, structure **1a** (Scheme 3).

SCHEME 3. Suzuki−Miyaura Coupling Leading to the Formation of the *C*-Glycoside 1a



Biological Activity of PsA O-Me *C*-Glycoside (1a). We assessed the anti-inflammatory activity and the ability of **1a** to inhibit phagocytosis in *Tetrahymena* since these characteristics are hallmarks of the pseudopterosins. Should the pseudopterosins be prodrugs that require a glycosidase enzyme to deliver the tricyclic aglycon core as the active species, then the *C*-glycoside **1a** ought to inhibit the enzymic process and the activity should be lost (Figure 2).

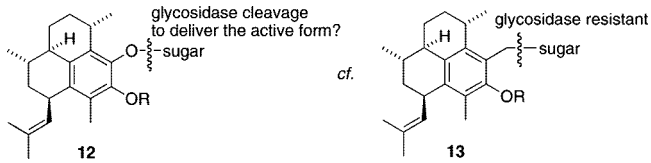


FIGURE 2. Are the pseudopterosins prodrugs?

As shown in Figure 3, PsA O-Me *C*-glycoside (**1a**) inhibits phorbol myristate acetate (PMA) induced inflammation in mouse ears in a dose-dependent manner ($N = 7$). The ED_{50} is 17 μg/ear with a Hill coefficient of 1.3 (correlation coefficient, $R^2 = 0.968$) and is statistically significant ($p < 0.01$). This value is of the same order of magnitude as that measured for iso-PsE (**2**; 27 μg/ear) and PsA-OMe (**1b**; 22 μg/ear) and is not significantly greater than it is for PsA (**1**; 8 μg/ear).

Figure 4 illustrates that **1a** is also capable of inhibiting the incidence of phagocytosis in *Tetrahymena* cells in a dose-dependent manner ($N = 7$). Its ED_{50} is 4 μM with a Hill coefficient of 1 (correlation coefficient, $R^2 = 0.931$) and is statistically significant ($p < 0.01$). In comparison, the ED_{50} value for PsA (**1**)-inhibited phagocytosis is 6 μM, while that for the methyl ether **1b** is 10 μM (data not shown).

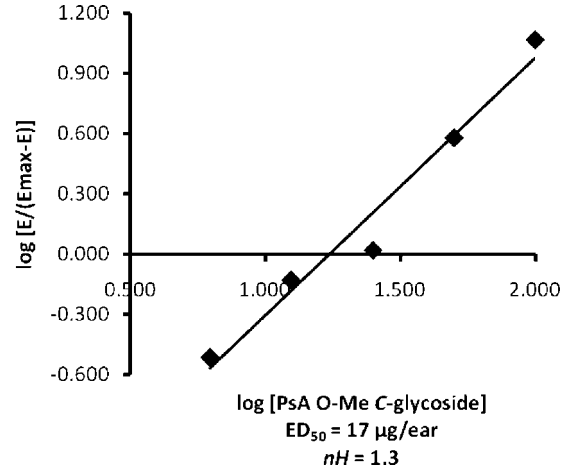


FIGURE 3. Inhibition of mouse ear edema.

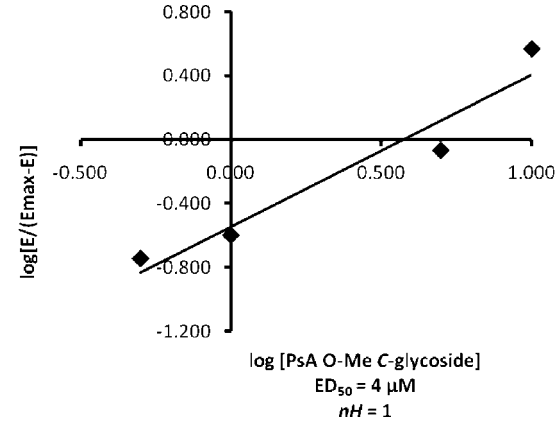
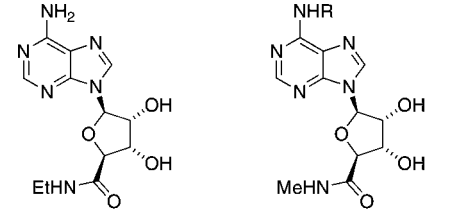


FIGURE 4. Inhibition of phagocytosis in *Tetrahymena* cells.

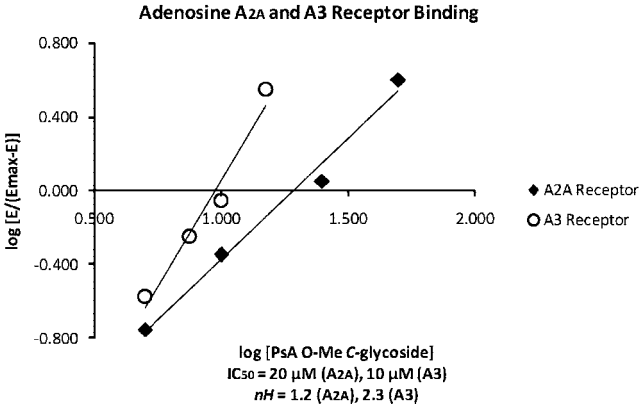
Since the pseudopterosins have demonstrable wound healing properties and because the involvement of adenosine receptors has been implicated in wound healing,[4,5] we elected to assess the ability of the C-glycoside **1a** to bind to adenosine receptors A_{2A} and A_3. Adenosine receptors are ubiquitously distributed throughout the body and are of great interest as therapeutics for a growing number of conditions.[20] In addition to wound healing, the therapeutic potential for compounds that modulate adenosine receptors includes hepato-protective, cardiac antiarrhythmic, anxiety disorders, neuroprotective, antitumor, and anti-inflammatory uses and as antihyperalgesic agents.[21]

The ability of **1a** to bind to cultured human embryonic kidney cells (HEK-293) in competition with specific adenosine receptor binding agents 5′-(*N*-ethylcarboxamido)adenosine (NECA, **14**) and IB-MECA (**15**) was examined.[22] Like the pseudopterosins, these compounds consist of a flat aromatic structural feature that is linked to a sugar residue.



The experiment demonstrated that the *C*-glycoside binds to the A_{2A} receptor in a cooperative manner with a Hill coefficient,

TABLE 2. Binding Data for Compounds 1, 1a, and 2 toward the Adenosine Receptors A_{2A} and A_3



compd	receptor	IC_{50} (μM)	Hill coefficient	ref compd
C-glycoside (**1a**)	A_{2A}	20	1.2	NECA (**14**)
PsA (**1**)	A_{2A}	13	1	NECA (**14**)
iso-PsE (**2**)	A_{2A}	13	1	NECA (**14**)
C-glycoside (**1a**)	A_3	10	2.3	IB-MECA (**15**)
PsA (**1**)	A_3	4.1	2.2	IB-MECA (**15**)
iso-PsE (**2**)	A_3	6.6	2.5	IB-MECA (**15**)

n(H), of 1.2. It competitively inhibited the binding of the reference compound, NECA (**14**), to the receptor with an IC_{50} equal to 20 μM and a binding constant, K_i, of 16 μM. The *C*-glycoside **1a** also binds the A_3 receptor in a cooperative manner with a Hill coefficient of 2.3. In this instance, it competitively inhibited the binding of IB-MECA (**15**), the reference compound, with an IC_{50} equal to 10 μM and a K_i value of 6 μM. From this data, it is clear that **1a** binds with a 2-fold greater affinity to the A_3 receptor compared to the A_{2A} receptor. These results are summarized graphically; the Table 2 provides a comparison between **1a** and the pseudopterosins PsA (**1**) and iso-PsE (**2**).

Concluding Remarks. We have successfully synthesized the *C*-glycoside analogue of PsA methyl ether, **1a**, using a Suzuki−Miyaura cross-coupling of an alkylborane generated in situ from enol ether **5** with the aryl triflate **11** derived from iso-PsE (**2**). Since the bioactivity of **1a** is maintained despite the presence of the C-linked sugar, we *conclude that the intact structure is present when a pseudopterosin expresses its anti-inflammatory and phagocytosis inhibitory properties* and, therefore, that *for these indications, the pseudopterosins are not prodrugs*. In addition, the *C*-glycoside is an effective binding agent toward adenosine receptors A_{2A} and A_3.

Experimental Section

2,3,4-Tri-*O*-methoxmethyl-D-xylopyranoside Lactone (4). To a solution of 2,3,4-tri-*O*-methoxmethyl-α/β-D-xylopyranosides (2.40 g, 8.5 mmol), NMO (1.49 g, 12.73 mmol), and freshly activated powdered 4 Å molecular sieves (4.26 g) in CH_2Cl_2 (17 mL) was added TPAP (152 mg, 0.43 mmol) at room temperature under argon. The resulting mixture was stirred for 1 h and then diluted with CH_2Cl_2 (50 mL). The mixture was washed with aqueous saturated Na_2SO_3 solution (100 mL), brine (100 mL), and aqueous saturated $CuSO_4$ (100 mL). The organic layer was dried over $MgSO_4$, filtered, and then concentrated to dryness. The residue was purified by column chromatography over silica gel (hexane/ethyl acetate, 2:1, v/v) to afford the desired product **4** (2.02 g, 7.21 mmol, 85%) as a colorless oil: R_f 0.40 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 4.82 (d, J = 6.6, 1H), 4.71−4.60 (m, 5H), 4.33−4.26 (m, 3H), 3.94 (dd, J = 2.0, 1H), 3.85 (d, J = 7.4, 1H), 3.35 (s, 3H), 3.33 (s, 3H), 3.30 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.4, 95.9, 95.8, 94.7, 78.6, 73.4, 73.3, 66.4, 55.8, 55.6; ESI-MS found 303.1053, $C_{11}H_{20}O_8Na^+$ calcd 303.1050.

2,3,4-Tri-*O*-methoxmethyl-D-xylopyranoside Olefin (5). To a solution of 2,3,4-tri-*O*-methoxmethyl-D-xylopyranoside lactone (**4**, 0.45 g, 1.61 mmol) in dry toluene (8 mL) was added freshly made Petasis' reagent ($Cp_2Ti(CH_3)_2$) in dry toluene (0.66 g in 1.5 mL, 3.12 mmol) dropwise to give a red solution. The resulting mixture was stirred in the dark at 70 °C for 18 h. The black solution was cooled and poured into hexanes (50 mL). The solution was filtered through Celite to remove the resulting precipitate and then concentrated to dryness in vacuo. The residue was purified by column chromatography over silica gel (hexane/ethyl acetate, 6:1, v/v) to afford desired product **5** (0.31 g, 1.11 mmol, 74%) as a colorless oil: R_f 0.25 (hexane/ethyl acetate, 3:1, v/v); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.79−4.62 (m, 6H), 4.48 (t, J = 0.8, 1.6, 1H), 4.10 (dd, J = 4.0, 10.4, 1H), 4.03 (d, J = 6.8, 1H), 3.79−3.52 (m, 4H), 3.40 (s, 3H), 3.39 (s, 3H), 3.34 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 155.9, 96.7, 96.4, 95.0, 93.6, 79.5, 75.8, 74.4, 67.5, 55.6, 55.5, 55.2; ESI-MS found 301.1251, $C_{12}H_{22}O_7Na^+$ calcd 301.1258.

***C*-Glycoside 7.** To a dried flask containing olefin **5** (94 mg, 0.34 mmol) under argon was added 0.5 M 9-BBN in THF (1.02 mL, 0.51 mmol, 1.5 equiv) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. Aqueous $NaHCO_3$ (1 M, 1.22 mL) was then added, and the resulting solution was allowed to stir for 15 min. $PdCl_2$(dppf) (28 mg, 0.034 mmol, 0.1 equiv) and **6** (44 mg, 0.17 mmol, 0.5 equiv) in DMF (2 mL) were added dropwise. The resulting mixture was stirred in the dark at room temperature for 18 h and then poured into Et_2O (10 mL). The organic layer was washed with H_2O (10 mL) and brine (10 mL). The aqueous layer was extracted with ether (3 × 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to dryness in vacuo. The residue was purified by column chromatography over silica gel (hexane/ethyl acetate, 5:1, v/v) to afford the *C*-glycoside **7** (49 mg, 0.13 mmol, 74%) as a colorless oil: R_f 0.60 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.26−7.17 (m, 2 H), 6.94−6.84 (m, 2 H), 5.02−4.63 (m, 6 H), 4.05−3.97 (dd, 1 H, J = 4.8, 11.4), 3.83 (s, 3 H), 3.62−3.56 (m, 2 H), 3.50 (s, 3 H), 3.45 (s, 3 H), 3.44 (m, 3 H), 3.31 (s, 3 H), 3.06 (m, 1 H), 2.52 (dd, 1 H, J = 9.4, 10.0); ESI-MS found 409.1841, $C_{19}H_{30}O_8Na^+$ calcd 409.1833.

Acyclic Byproduct 8. To a dried flask containing olefin **5** (100 mg, 0.36 mmol) under argon was added 0.5 M 9-BBN in THF (1.80 mL, 0.9 mmol, 2.5 equiv). The reaction mixture was allowed to reflux at 60 °C and stirred for 2 h. Aqueous $NaHCO_3$ (1 M, 1.07 mL) was then added and the mixture allowed to stir for 15 min. $PdCl_2$(dppf) (30 mg, 0.036 mmol, 0.1 equiv) and **6** (92 mg, 0.36 mmol, 1.0 equiv) in DMF (4 mL) were added dropwise. The resulting mixture was stirred in the dark at room temperature for 18 h and then poured into Et_2O (20 mL). The organic layer was washed with H_2O (20 mL) and brine (20 mL). The aqueous layer was extracted with ether (3 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to dryness in vacuo. The residue was purified by column chromatography over silica gel (hexane/ethyl acetate, 2:1, v/v) to afford adduct **8** (74 mg, 0.19 mmol, 53%) as a colorless oil: R_f 0.15 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.26−7.12 (m, 2 H), 6.89−6.80 (m, 2 H), 5.82−5.65 (m, 1 H), 5.36−5.28 (m, 2 H), 4.92−4.54 (m, 8 H), 4.31 (t, 1 H, J = 7.0, 7.4), 3.80 (s, 3 H), 3.79−3.72 (m, 4 H),

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(22) These studies were performed by Cerep, Inc., Seattle, WA, and Paris, France.

3.43 (s, 3 H), 3.38 (s, 3 H), 3.36 (s, 3 H), 2.77−2.65 (m, 1 H), 1.98 (br, 1 H); ESI-MS found 411.1978, $C_{19}H_{32}O_8Na^+$ calcd 411.1989.

Iso-PsE Methyl Ether Aglycon (10). To a solution of iso-PsE (**1b**, 0.25 g, 0.56 mmol) in dry acetone (30 mL) were added MeI (0.105 mL, 1.68 mmol) and K_2CO_3 (0.235 g, 1.68 mmol). The resulting mixture was refluxed at 60 °C for 24 h and then cooled to room temperature. The solution was concentrated to dryness in vacuo, followed by the addition of H_2O (20 mL). The aqueous layer was extracted with $CHCl_3$ (3 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to dryness in vacuo. The residue was purified by column chromatography over silica gel (CH_2Cl_2/MeOH, 20:1, v/v) to afford the desired product (0.25 g, 0.54 mmol, 97%) as a white solid. To a solution of iso-PsE methyl ether (0.25 g, 0.54 mmol) in MeOH (30 mL) was added 1 N aqueous HCl (24 mL). The resulting mixture was stirred at 50 °C for 4 h and then cooled to room temperature. The solution was concentrated to dryness in vacuo, followed by the addition of H_2O (20 mL). The aqueous layer was extracted with $CHCl_3$ (3 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to dryness in vacuo. The residue was purified by column chromatography over silica gel (hexane/ethyl acetate, 20:1, v/v) to afford desired product **10** (143 mg, 0.45 mmol, 84%) as a white solid: R_f 0.50 (hexane/ethyl acetate, 10:1, v/v); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.68 (s, 1 H), 5.15 (td, 1 H, J = 1.6, 9.2), 3.76 (s, 3 H), 3.61 (td, 1 H, J = 3.6, 9.6), 3.36 (dd, 1 H, J = 6.8, 15.6), 2.22−2.13 (m, 3 H), 2.10 (s, 3 H), 2.02 (m, 1 H), 1.77 (d, 3 H, J = 1.2), 1.70 (d, 3 H, J = 1.0), 1.67−1.39 (m, 4 H), 1.29 (d, 3 H, J = 7.0), 1.21−1.12 (dd, 1 H, J = 6.2, 11.0), 1.08 (d, 3 H, J = 6.0). ^{1}H NMR spectral data was the same as that reported in the literature.[17]

Iso-PsE Methyl Ether Aglycon Triflate (11). To a solution of iso-PsE methyl ether aglycon (**10**, 64 mg, 0.20 mmol) in dry dichloromethane (2 mL) was added dry pyridine (92 μL, 1.17 mmol) under argon at room temperature. Tf_2O (95 μL, 0.56 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 1 h. H_2O (5 mL) was then added, and the resulting mixture was extracted with dichloromethane (3 × 5 mL). The combined organic layers were washed with 1 N HCl (10 mL), H_2O (10 mL), and brine (10 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness in vacuo. The residue was purified by column chromatography over silica gel (hexane/ethyl acetate, 30:1, v/v) to afford the desired product **11** (88 mg, 0.20 mmol, 97%) as a colorless oil: R_f 0.50 (hexane/ethyl acetate, 15:1, *v/v*). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.11 (td, 1 H, J = 1.2, 9.2), 3.74 (s, 3 H), 3.65 (d, 1 H, J = 8.4), 3.38−3.29 (m, 1 H), 2.32−2.18 (m, 2 H), 2.12 (s, 3 H), 2.06−1.96 (m, 2 H), 1.77 (s, 3 H), 1.70 (s, 3 H), 1.66−1.52 (m, 4 H), 1.24 (d, 3 H, J = 7.2), 1.08 (d, 3 H, J = 5.6); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 147.4, 139.4, 139.1, 134.7, 133.4, 131.1, 129.1, 128.5, 121.9, 61.0, 41.9, 39.0, 35.8, 30.1, 29.0, 27.5, 26.9, 25.6, 22.3, 20.8, 17.7, 11.2; ESI-MS found 469.1647, $C_{22}H_{29}O_4F_3SNa^+$ calcd 469.1631.

MOM-Protected Iso-PsE Methyl Ether *C*-Glycoside. To a dried flask containing olefin **5** (61 mg, 0.22 mmol) under argon was added 0.5 M 9-BBN in THF (0.66 mL, 0.33 mmol, 1.5 equiv) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. Aqueous $NaHCO_3$ (1 M, 0.8 mL) was then added and the mixture allowed to stir for 15 min. $PdCl_2$(dppf) (20 mg, 0.024 mmol, 0.1 equiv) and **11** (38 mg, 0.085 mmol) in DMF (1 mL) were added dropwise. The resulting mixture was stirred in the dark at 80 °C for 18 h and then poured into Et_2O (10 mL). The organic layer was washed with H_2O (10 mL) and brine (10 mL). The aqueous layer was extracted with ether (3 × 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to dryness in vacuo. The residue was purified by column chromatography over silica gel (hexane/ethyl acetate, 6:1, v/v) to afford the desired product (25 mg, 0.043 mmol, 51%) as a colorless oil: R_f 0.30 (hexane/ethyl acetate, 3:1, v/v); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.22 (d, 1 H, J = 9.2), 5.07 (d, 1 H, J = 9.6), 4.91−4.62 (m, 5 H), 4.07−3.90 (m, 2 H), 3.70 (s, 3 H), 3.64−3.54 (m, 3 H), 3.49 (s, 3 H), 3.45 (s, 3 H), 3.44 (m, 3 H), 3.40−3.36 (m, 2 H), 3.31 (s, 3 H), 3.00 (m, 1 H), 2.81 (dd, 1 H, J = 10.0, 14.0), 2.31−2.22 (m, 2 H), 2.10 (s, 3 H), 1.76 (s, 3 H), 1.69 (s, 3 H), 1.64−1.45 (m, 2 H), 1.32−1.22 (m, 2 H), 1.18 (d, 3 H, J = 7.4), 1.06 (d, 3 H, J = 5.0); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 155.2, 142.1, 137.6, 132.4, 130.0, 129.4, 126.5, 126.3, 98.8, 98.1, 97.2, 83.0, 81.4, 80.1, 77.8, 68.9, 60.4, 56.6, 56.2, 55.4, 40.1, 39.2, 35.9, 31.4, 29.5, 28.9, 27.7, 26.6, 25.7, 22.6, 21.2, 17.6, 11.4; ESI-MS found 599.3574, $C_{33}H_{52}O_8Na^+$ calcd 599.3554.

Iso-PsE Methyl Ether *C*-Glycoside (1a). To a solution of MOM-protected starting material (25 mg, 0.043 mmol) in MeOH (1 mL) was added 6 N aqueous HCl (0.75 mL). The reaction mixture was stirred at room temperature for 18 h, and then concentrated to dryness in vacuo. The residue was purified by column chromatography over silica gel (CH_2Cl_2/MeOH, 25:1, v/v) to afford the desired target structure **1a** (15 mg, 0.034 mmol, 79%) as a white solid: R_f 0.52 (CH_2Cl_2/MeOH, 20:1, v/v); ^{1}H NMR (CD_3OD, 400 MHz) δ 5.09 (d, 1 H, J = 9.2), 3.69 (dd, 1 H, J = 2.4, 8.8), 3.62 (m, 1 H), 3.60 (s, 3 H), 3.48−3.38 (m, 2 H), 3.27−3.17 (m, 6 H), 3.09 (t, 1 H, J = 8.8), 2.88 (t, 1 H, J = 10.8), 2.63 (dd, 1 H, J = 9.6, 14.0), 2.27−2.11 (m, 2 H), 2.02 (s, 3 H), 1.81−1.76 (m, 1 H), 1.73 (s, 3 H), 1.65 (s, 3 H), 1.63−1.53 (m, 4 H), 1.12−1.08 (m, 1 H), 1.07 (d, 3 H, J = 7.2), 1.00 (d, 3 H, J = 5.6); ^{13}C NMR (CD_3OD, 100 MHz) δ 156.4, 143.3, 138.7, 133.6, 131.5, 131.1, 127.9, 127.2, 83.2, 79.9, 77.0, 71.9, 71.3, 60.9, 42.1, 40.6, 37.2, 32.7, 30.7, 30.3, 29.3, 28.4, 26.1, 22.3, 21.7, 17.9, 11.8; ESI-MS found 467.2776, $C_{27}H_{40}O_5Na^+$ calcd 467.2768.

Measurement of Phagocytic Activity in *Tetrahymena thermophila* Cells. The effect of drugs on phagosome formation in *Tetrahymena* cells was measured by visualizing newly formed phagosomes containing India ink by light microscopy. For the experiment, cells were washed twice with 10 mM HEPES buffer by centrifuging at 450*g* for 5 min. The pellet was then resuspended in 10 mM HEPES at pH 7.4. The final cell concentration for each experimental treatment was 250000 cells/mL in a total volume of 4 mL. Cell suspensions were placed in 13 × 100 mm test tubes in a 25 °C water bath and allowed to acclimate for 45 min to 1 h. Drugs were prepared at the desired concentrations in 0.4 mL volumes. For control samples, 0.4 mL of buffer and vehicle were added to the incubation mixture. In order to visualize the newly formed phagosomes, 0.45 mL of diluted India ink (1:25, v/v) was added to each of the test drug volumes. The experiment was started when the drug/ink mixture was added to the *Tetrahymena* cells and was terminated after 10 min when 500 μL of cell suspension (approximately 125,000 cells/mL) was removed and fixed in 100 μL of formalin solution. A minimum of 100 cells from each treatment was examined for the incidence of phagosome formation under light microscopy (40 x magnifications). Phagocytic activity was assessed by calculating the ratio of cells with food vacuoles compared to the cells with no food vacuoles.

Inhibition of Inflammation: In Vivo Studies. 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, PMA alone (2 μg/ear) or in combination with various dilutions of test compoundwas applied to the left ears (5 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. The ears were then flash frozen and stored in liquid nitrogen for myeloperoxidase activity studies. 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.

In Vitro Binding Studies of *C*-Glycoside 1a to Adenosine Receptors. These studies were performed by Cerep, Inc.[22] A_3 receptor/binding affinity of the C-glycoside to the A_3 receptor was measured in human recombinant (HEK-293 cells). The A_3 specific radiolabeled agonist, [^{125}I]AB-MECA, and the specific A_3 agonist, IB-MECA, were used to estimate the binding affinity of the *C*-glycoside to the receptor. A_{2A} receptor/binding affinity of the *C*-glycoside to the A_{2A} receptor was measured in human recombinant (HEK-293 cells). The A_{2A} specific radiolabeled agonist [^{3}H]CGS 21680 and the specific A_{2A} agonist NECA were used to estimate the binding affinity of the *C*-glycoside to the receptor. Binding affinity studies of one dose of the *C*-glycoside were also performed for A_1 receptors (expressed in CHO cells) and the A_{2B} receptor.

Acknowledgment. We gratefully acknowledge the U.S. Army Medical Research Program (Grant No. W81XWH-06-1-0089) for its support of this research.

Supporting Information Available: ^{1}H and ^{13}C NMR data for compounds **4**, **5**, **11**, the tris-MOM ether precursor of **1a**, and **1a** and the ^{1}H NMR spectral data for **7** and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO801432T