

# **iso-PsE: A New Pseudopterosin with Anti-inflammatory and Potential Wound Healing Properties**

Christophe Hoarau,<sup>†</sup> Daniel Day,<sup>††</sup> Claudia Moya,<sup>††</sup> Guang Wu,<sup>†</sup> Abdul Hackim,<sup>†</sup>  
Robert S. Jacobs,<sup>††</sup> and R. Daniel Little<sup>†\*</sup>

<sup>†</sup> Department of Chemistry and Biochemistry

&

<sup>††</sup> Department of Ecology, Evolution and Marine Biology

University of California, Santa Barbara

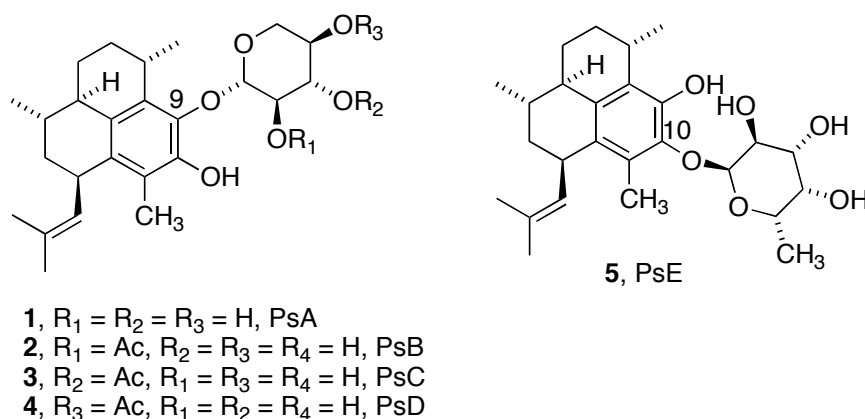
Santa Barbara, CA 93106

## **Abstract**

We report the characterization and several of the pharmacological properties of a new pseudopterosin that was isolated from extracts of air-dried *Pseudoptero-gorgia elisabethae*. The substance, whose structure was ascertained using a combination of degradation, semi-synthesis, and x-ray analysis, proved to be the positional isomer of pseudopterosin E wherein the sugar (L-fucose) is appended to C-9 rather than C-10. The name “iso-PsE” is proposed. In addition to the anti-inflammatory activity that is characteristic of many of the pseudopterosins, iso-PsE was shown to bind competitively to adenosine receptors A<sub>2A</sub> and A<sub>3</sub> obtained from human embryonic kidney cells (HEK-293). Their modulation has been shown to accelerate wound healing.

**Introduction.** The pseudopterosins represent a well-known class of marine natural products that are isolated from the gorgonian coral, *Pseudoptero-gorgia elisabethae*.<sup>1</sup> Readily identifiable by their tricyclic diterpene core and pendant sugar, they have attracted much attention as a result of their significant anti-inflammatory and wound healing properties,<sup>2</sup> as well as their synthetically challenging molecular architecture.<sup>3</sup> Figure 1 illustrates five members of the class, pseudopterosins A-D (**1-4**) differing from one another by the degree of acetylation of the equatorially positioned D-xylose sugar unit appended to C-9, while pseudopterosin E (PsE; **5**) finds an axially oriented L-fucose unit appended to C-10. PsE (**5**) was first reported by Fenical and co-workers;<sup>4</sup> Corey and Carpino recorded its first total synthesis in 1989.<sup>5</sup> Herein we report the isolation and characterization of a new pseudopterosin, its anti-inflammatory activity and binding properties toward several adenosine receptors.

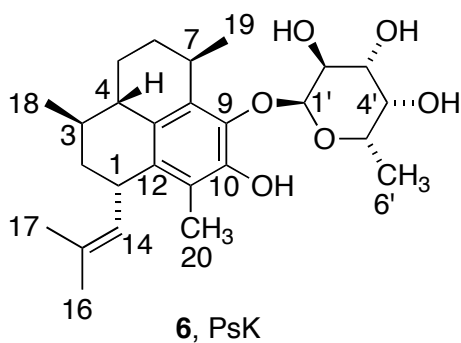
**Figure 1.** Structure of pseudopterosins A-E



In conjunction with our efforts to identify the pseudopterosin pharmacophore, we isolated the principle component of dry feathers of *Pseudoptero-gorgia elisabethae* that were harvested from a Caribbean collection field. Spectral data was recorded following a saponification to remove any acetate units that were appended to the sugar. The proton and carbon NMR spectra strongly suggested that the product was pseudopterosin K (**6**; PsK; Table 1).<sup>3,4,6</sup> Identifying features

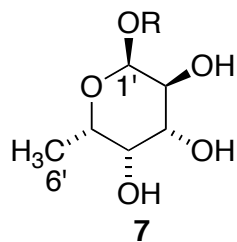
included the presence of three doublets centered at 1.02, 1.12, and 1.25 ppm corresponding to the methyl groups appended to C-3, C-7, and C-6' respectively, and three methyl singlet signals appearing at 1.65, 1.74, and 1.98 ppm corresponding, respectively, to the two allylic methyl groups and the methyl group appended to the aromatic ring. In addition to the signals corresponding to the tricyclic core, the proton and carbon signals for the sugar residue are listed in Table 1. Clearly the agreement between the literature values and those recorded for the substance we isolated is excellent.

**Table 1.** Observed chemical shift data compared with the literature values for PsK (**6**).<sup>3</sup> The proton data was recorded at 400 MHz, carbon at 75 MHz.



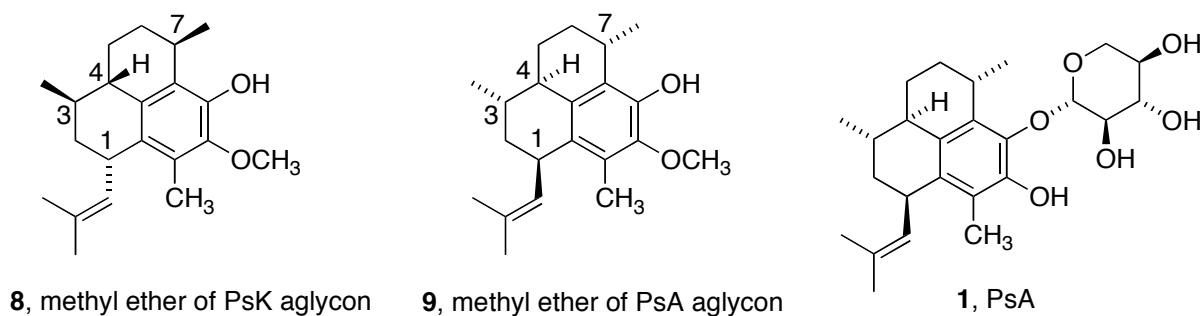
carbon number	chemical shift	
	this study	literature
1	35.7	35.7
2	39.6	39.5
3	30.2	30.2
4	43.0	42.8
5	27.3	27.2
6	30.5	30.2
7	28.3	28.1
8	133.3	133.2
9	142.6	142.2
10	145.4	145.1
11	121.3	121.2
12	135.0	135.1
13	129.9	130.0
14	130.0	129.6
15	128.9	128.8
16	17.8	17.6
17	25.9	25.6
18	21.2	20.9
19	24.3	23.8
20	11.0	10.8

position	chemical shift (ppm)	
	<sup>1</sup> H – this study; literature	<sup>13</sup> C – this study; literature
1'	5.06; 5.03	103.7; 103.4
2'	3.90; 3.89	69.1; 69.4
3'	4.15; 4.11	70.7; 70.6
4'	4.15; 4.11	72.4; 72.4
5'	4.34; 4.32	67.8; 67.6
6'	1.24; 1.22	16.4; 16.2



Treatment of the substance with potassium carbonate and methyl iodide in acetone served to alkylate the phenolic hydroxyl group and produce the corresponding methyl ether.<sup>3</sup> Fortunately the proton NMR spectrum displayed a clear separation of the vinyl and anomeric protons, thereby allowing the measurement of the coupling constant between the anomeric proton and its neighbor at C-2'. The result,  $J = 3.5$  Hz, is clearly consistent with that for an axial orientation of the tricyclic core that is appended to C-1', and with fucose being the sugar unit.

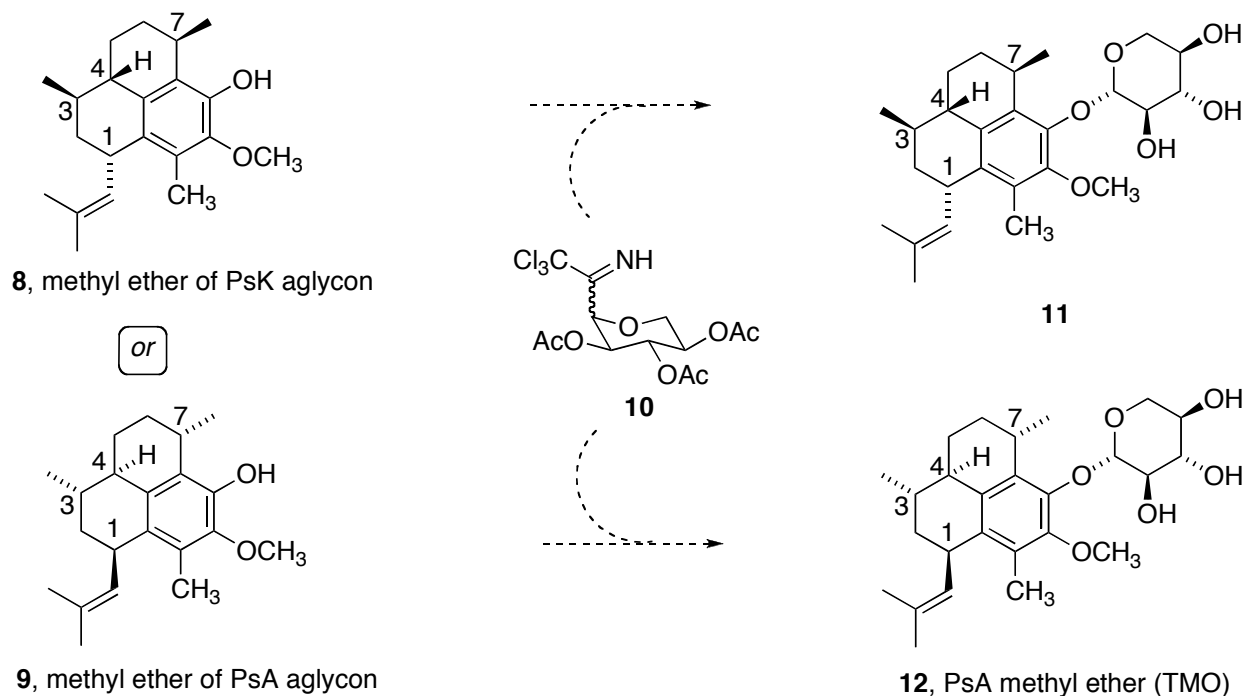
Next, the sugar was removed using 1N HCl in methanol by following a well-defined protocol,<sup>3</sup> and the purified sample of the resulting aglycon was analyzed spectroscopically. The proton and carbon NMR spectral data proved, within experimental error, to be identical to that reported for the aglycon methyl ether derived from PsK (**6**).<sup>3</sup> We cautiously concluded, therefore, that we were dealing with **8** rather than **9**, the latter possessing the enantiomeric tricyclic core that is present in PsA (**1**).



**Figure 2.** Possible structures for the aglycon methyl ether

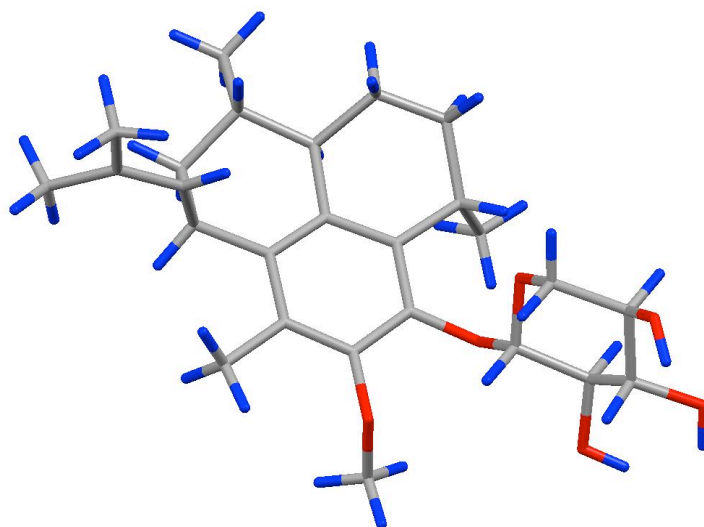
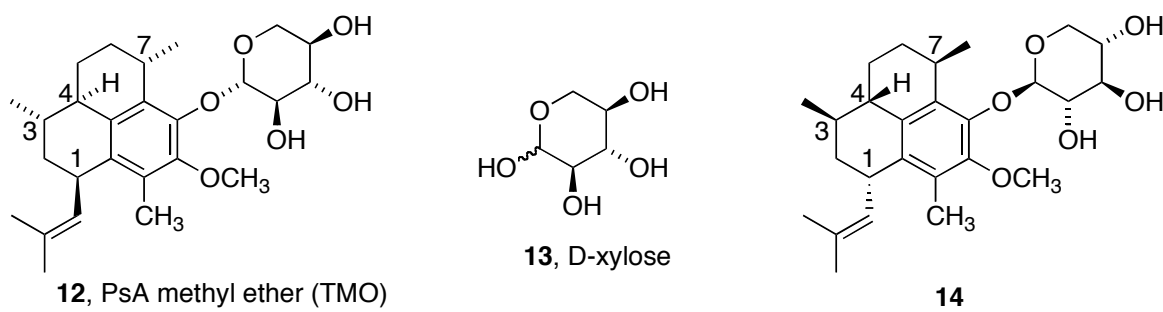
To determine whether this was the case, we elected to convert the aglycon to a glycoside by coupling it to an enantiomerically pure sugar. To facilitate product identification, we selected D-xylose (**13**) as the sugar since the coupling of it to aglycon **9** would afford pseudopterosin A methyl ether (**12**, TMO), a substance that has been fully characterized spectroscopically. Coupling to the alternative formulation of the aglycon, *viz.* to **8**, would lead to the diastereomeric structure **11**. As outlined in Scheme 1, 2,3,4-tri-O-acetyl- $\beta$ -D-xylopyranosyl trichloroacetimidate (**10**) derived from enantiomerically pure D-xylose (**13**), was successfully and efficiently coupled to the aglycon ( $\text{BF}_3$  etherate, 4Å molecular sieves,  $-78^\circ\text{C}$ ; 91%) and the acetate units were removed ( $\text{K}_2\text{CO}_3$ , MeOH, room temperature; 95%) to afford an adduct, either **11** or **12**.<sup>7</sup>

**Scheme 1.** Possible outcomes of the reaction of the aglycon methyl ether with the D-xylose-derived trichloroacetimidate **10**



Fortunately, the product crystallized and we were able to perform an X-ray analysis to determine its structure. Analysis of the data revealed the enantiomeric structures **12** and **14** as

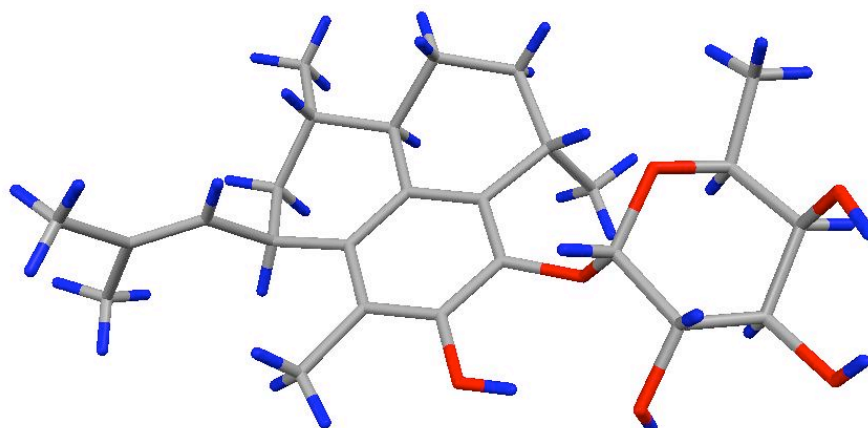
the only options. Knowing, however, that the configurations at each of the stereogenic centers in the sugar portion of the structure must match those found in D-xylose, and that the coupling reaction did nothing to change those centers, we are compelled to conclude that the adduct is TMO (**12**) rather than **14**.



**Figure 3.** Top: Enantiomeric structures **12** and **14**. The structure of D-xylose is also illustrated to facilitate the comparison. Bottom: X-ray structure corresponding to semi-synthetic TMO. For ease of visualization against the white background, the hydrogens are shown in blue.

It follows, therefore, that the substance isolated from the dry coral is a new pseudopterosin that it is composed of an axially oriented fucose sugar unit appended to C-9, and a tricyclic core that is of the PsA variety. The difference between it and PsE (**5**) is the point of attachment of the

sugar residue. We call the new substance, therefore, iso-PsE (**5**). A purified sample of the material crystallized upon standing at room temperature, and an x-ray analysis was performed. The result, illustrated in Figure 4, fully corroborates our conclusions.



**Figure 4.** Top: Structure of iso-PsE (**15**) and PsE (**5**) for comparison. Bottom: X-ray derived structure of iso-PsE (**15**). Once again, the hydrogens are shown in blue.

**Pharmacological activity of iso-PsE (15).** It has been known for many years that the pseudopterosins possess anti-inflammatory,<sup>1</sup> analgesic,<sup>1</sup> and antibiotic properties.<sup>8</sup> We have found that iso-PsE (**15**; ED<sub>50</sub> = 27 μg/ear) exhibits local anti-inflammatory activity that is characteristic of PsA (**1**; ED<sub>50</sub> = 8 μg/ear) and TMO (**12**; ED<sub>50</sub> = 22 μg/ear). Thus, its concurrent application or pretreatment blocks phorbol myristate acetate (PMA) induced inflammation when

applied in 25  $\mu$ L acetone to the inner pinnae of a mouse ear. Local application of these marine natural products also inhibits neutrophil infiltration at the application site as determined by measuring the accompanying decrease in myeloperoxidase activity.<sup>9</sup>

Inhibition of neutrophil infiltration is a key feature of the unique anti-inflammatory activities of the pseudopterosins in that they lack significant activity on eicosanoid biosynthesis but appear to act, in part, by blocking the release of pro-inflammatory mediators from neutrophils and macrophages.<sup>9b</sup> Recent reports described a role of neutrophil infiltration in wound healing,<sup>2, 10</sup> while recent clinical reports, although debated, have emphasized an inflammatory role of neutrophil infiltration and degranulation during injury.<sup>10</sup>

At the cellular level iso-PsE (**15**), as well as PsA (**1**) and TMO (**12**), significantly decrease basal levels of phagocytosis in cultured *Tetrahymena* cells.<sup>11</sup> In cultured human embryonic kidney cells (HEK-293) expressing several G-protein coupled receptors, isotope binding studies indicate that iso-PsE (**15**) displays a significant degree of selectivity toward adenosine A<sub>2A</sub> and A<sub>3</sub> receptors when tested at concentrations above 5  $\mu$ M. At these concentrations iso-PsE (**15**), although markedly less potent than adenosine analogs,<sup>12, 13</sup> followed binding kinetics similar to the adenosine standards. The A<sub>2A</sub> receptors have been shown to operate through a G-protein coupled receptor (GPCR) pathway and modulation of these receptors have shown positive effects in the acceleration of wound healing.<sup>14</sup>

**Closing remarks.** Currently studies are underway to examine effects of iso-PsE (**15**) in functional assays in cells expressing adenosine receptors. Because of its abundance and high degree of purity, iso-PsE (**15**) serves as a lead compound for our continued semi-synthetic efforts.

**Acknowledgements.** We are pleased to acknowledge the U. S. Army Medical Research Program (Grant number W81XWH-06-1-0089) for its support of this research. We are also grateful to Professor David Marten of Westmont University for obtaining the APT-DEPT spectrum of iso-PsE (**15**).

**Supporting Information Available:** Supporting information (12 pages) for iso-PsE (**15**;  $^1\text{H}$ ,  $^{13}\text{C}$ , APT-DEPT, HSQC) and iso-PsE O-methyl ether ( $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT, HSQC), is available free of charge *via* the Internet at <http://pubs.acs.org>.

### Experimental section

**Isolation.** A collection of *Pseudopterogorgia elisabethae* feathers was obtained from Lipo Chemical Inc. (Paterson, New Jersey). The air-dried collection was allowed to dry further under vacuum desiccation for several weeks. The feathers were then cut into small pieces approximately 0.5 inch in length and subsequently ground to a dry powder. The powder was extracted three times with  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  (1:1 by volume) with filtering between rinsing to produce a clear, dark-tan liquid. The combined organic extracts were concentrated in vacuo to afford a dark green oil.

**Saponification.** Methanol (20 mL) was added to 2.8 g of the crude extract in order to dissolve all methanol-soluble compounds. The resulting solution was then added to a 100 mL round-bottomed flask in which 2.8 g (50 mmol) of KOH had been dissolved in 100 mL of methanol. The solution was stirred at room temperature for 4 h after which 40 g of ice-water was added. The mixture was then poured into a 1L beaker containing 200 g of ice-water and acidified with 15% HCl until reaching a pH of 7.6 at which point a yellow/green precipitate began to form. The resulting mixture was transferred to a separatory funnel, extracted three times with dichloromethane, washed with saturated aqueous  $\text{NaHCO}_3$ , and dried using  $\text{NaSO}_4$ .

The organic layer was concentrated in vacuo to yield a dark-green oil (632 mg, 23%) corresponding to a mixture of ca. 10% PsA (**1**) and 90% iso-PsE (**15**). The mixture was allowed to stand overnight in 95% MeOH/water leading to the formation of a whitish-yellow pseudocrystal.<sup>15</sup>

Iso-PsE (**15**) that was used in the pharmacological studies and for its spectral characterization was further purified using reverse phase HPLC: Denali 120-5 C18 column, 250 mm x 4.6 mm; UV detection at 280 nm; mobile phase ranging from 60/40 (v/v) acetonitrile/H<sub>2</sub>O to 100/0 acetonitrile/ H<sub>2</sub>O over a period of 15 min, with a subsequent hold at 100/0 acetonitrile/ H<sub>2</sub>O for 30 min; 1mL/min flow rate; retention time 16.70 min.

Iso-PsE (**15**) was very slowly crystallized from the mixture described above. Thus, 2 mg of the material was placed in a clear 20 mL glass scintillation vial and was solubilized using methanol (2 mL) and two drops of deionized water. The resulting solution was shaken to afford a clear yellow/brown solution. A polypropylene cap was used to cover (not seal) the vial, and the solution was allowed to sit at room temperature for ~6 months to afford clear (primitive orthorhombic) white crystals of iso-PsE (**15**) from a bright yellow mother liquor. These crystals were used for the x-ray analysis.

**i-PsE (15).** <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.02 (3H, d, J = 6.0), 1.05-1.09 (1H, m), 1.11 (3H, d, J = 7.3), 1.24 (3H, d, J = 6.7), 1.36-1.43 (1H, m), 1.50-1.57 (1H, m), 1.59-1.62 (1H, m), 1.63-1.69 (1H, m), 1.65 (3H, s), 1.74 (3H, s), 1.94-1.99 (1H, m), 1.96 (3H, s), 2.05-2.13 (2H, m), 3.39 (1H, m), 3.58 (1H, d, J = 9.2), 3.91 (1H, s), 4.02 (1H, br s, OH), 4.15 (2H, apparent s), 4.34 (1H, q, J = 6.5), 4.65 (1H, br s, OH), 5.06 (1H, s), 5.09 (1H, d, J = 9.2), 5.66 (1H, br s, OH), 8.70 (1H, br s, OH); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 11.0 (C-20), 16.4 (C-6'), 17.8 (C-16), 21.2 (C-18), 24.3 (C-19), 25.9 (C-17), 27.3 (C-7), 28.3 (C-5), 30.2 (C-3), 30.5 (C-6), 35.7 (C-1), 39.6 (C-

11/17/07 3:56 PM

2), 43.0 (C-4), 67.8 (C-5'), 69.1 (C-2'), 70.7 (C-3'), 72.4 (C-4'), 103.7 (C-1'), 121.3 (C-11), 128.9 (C-15), 129.9 (C-13), 130.0 (C-14), 133.3 (C-8), 135.0 (C-12), 142.6 (C-9), 145.4 (C-10).

HRMS (positive ion electrospray in CH<sub>3</sub>CN) *m/z* found 469.2540, calcd 469.2560 for C<sub>26</sub>H<sub>38</sub>O<sub>6</sub>Na.

**i-PsE O-Methyl Ether (i-PsE-OMe).** <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.05 (3H, d, *J* = 6.0), 1.08-1.11 (1H, m), 1.20 (3H, d, *J* = 7.0), 1.38 (3H, d, *J* = 6.6), 1.40-1.47 (1H, m), 1.56-1.67 (1H, m), 1.66-1.70 (1H, m), 1.70-1.76 (1H, m), 1.68 (3H, d, *J* = 1.1), 1.75 (3H, d, *J* = 1.3), 2.02 (1H, m), 2.05 (3H, s), 2.12-2.18 (2H, m), 2.69 (1H, br s, OH), 3.20 (1H, br s, OH), 3.59 (1H, ddd, *J*<sub>1</sub> = 6.8, *J*<sub>2</sub> = 4.5, *J*<sub>3</sub> = 2.2), 3.69 (1H, m), 3.74 (3H, s), 3.83 (1H, dt, *J*<sub>1</sub> = 10.1, *J*<sub>2</sub> = 3.4), 3.97 (1H, d, *J*<sub>1</sub> = 2.3), 4.07 (1H, dd, *J*<sub>1</sub> = 10, *J*<sub>2</sub> = 3.2), 4.43 (1H, q, *J* = 6.4), 4.99 (1H, d, *J* = 10.4), 5.06 (1H, Me<sub>2</sub>C=CH), 5.11 (1H, d, *J* = 3.5, *H* at anomeric carbon); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 11.2, 16.7, 17.9, 21.2, 23.7, 25.9, 27.6, 28.1, 30.0, 30.6, 35.9, 39.5, 43.7, 61.6, 68.1, 69.7, 71.6, 71.8, 104.8, 127.8, 129.5, 130.5, 134.4, 135.4, 135.5, 147.8, 148.5. HRMS (positive ion electrospray in CH<sub>3</sub>CN) *m/z* found 483.2730, calcd 483.2717 for C<sub>27</sub>H<sub>40</sub>O<sub>6</sub>Na.

**Conversion of the aglycon methyl ether derived from the natural source to the acetimidate coupled product** (note Scheme 1). To a round-bottomed flask containing a mixture of tri-*O*-acetyl- $\alpha$ -D-xylose chloroacetimidate (0.1 mmol, 42.0 mg, 1.3 equiv) and the aglycone *O*-methyl ether derived from the natural source (24.0 mg, 0.076 mmol, 1 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL, 0.08 M) at room temperature, was added 4 Å molecular sieve (76.0 mg, 1.0 g/mmol). The mixture was stirred for 15 min at room temperature before cooling to -78 °C. BF<sub>3</sub>-OEt<sub>2</sub> (2  $\mu$ L) was then added and the mixture was stirred at that temperature for 30 min. The reaction was quenched using saturated aqueous NaHCO<sub>3</sub>, and the resulting solution was allowed to warm to room temperature before being diluted with EtOAc. The mixture was filtered through a plug of

Na<sub>2</sub>SO<sub>4</sub>/Celite, and the solution was concentrated in vacuo. The residue was chromatographed on silica gel with a gradient ranging from 5 up to 10% EtOAc/hexanes ( $R_f = 0.08$  at 5% EtOAc/hexanes), to afford the product in a 91% yield (40.5 mg, 0.071 mmol).

The acetate units were removed immediately. In a typical run, 58.0 mg (0.101 mmol, 1.0 equiv) of the glycosylation product dissolved in 1.3 mL of MeOH, was treated with K<sub>2</sub>CO<sub>3</sub> (2 mg, 0.014 mmol, ~0.10 equiv) at room temperature. The resulting mixture was stirred at that temperature for 2 hours until the reaction, as monitored by TLC (70% EtOAc/hexanes), was complete. The solvent was removed in vacuo, brine was added, and the aqueous layer was extracted twice using EtOAc. The solvent was removed in vacuo and the residue was chromatographed on silica gel using 70% EtOAc/hexanes to provide TMO (**12**) in > 90% yield ( $R_f = 0.23$ , 70% EtOAc/hexanes). Comparison of the proton and carbon NMR spectral data,<sup>1b</sup> as well as the result of an x-ray crystallographic analysis confirmed the fact that the material isolated in this manner was TMO (**12**).

---

#### References and notes

- <sup>1</sup> (a) Look, S. A.; Fenical, W.; Jacobs, R. S.; Clardy, J. *Proc. Natl. Acad. Sci. USA*, **1986**, *83*, 6238-6240. (b) Look, S. A.; Fenical, W.; Matsumoto, G. K.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5140-5145. (c) Fenical, W. *Trends Biotechnol.* **1997**, *15*, 339-341. (d) Coleman, A. C.; Kerr, R. G. *Tetrahedron*, **2000**, *56*, 9569-9574.
- <sup>2</sup> Montesinos, M. C.; Gadangi, P.; Longaker, M.; Sung, J.; Levine, J.; Nilsen, D.; Reibman, J.; Li, M.; Jiang, C-K.; Hirschhorn, R.; Recht, P. A.; Ostad, E.; Levin, R. I.; Cronstein, B. N. *J. Exp. Med.* **1997**, *186* (9), 1615-1620.

- 
- <sup>3</sup> (a) Harrowven, D. C.; Tyte, M. J. *Tetrahedron Lett.* **2004**, *45*(10), 2089-2091. (b) Chow, R.; Kocienski, P. J.; Kuhl, A.; LeBrazidec, J-Y.; Muir, K.; Fish, P. *J. Chem. Soc., Perkin Trans. 1* **2001**, *19*, 2344-2355. (c) Lazerwith S. E.; Johnson T. W.; Corey E. J. *Org. Lett.* **2000**, *2*(15), 2389-92. (d) Majdalani, A.; Schmalz, H. G. *Synlett* **1997**, *11*, 1303-1305. (e) Buszek, K. R.; Bixby, D. L. *Tetrahedron Lett.* **1995**, *36*(50), 9129-32. (f) McCombie, S. W.; Cox, B.; Ganguly, A. K. *Tetrahedron Lett.* **1991**, *32*(19), 2087-90. (g) Ganguly, A. K.; McCombie, S. W.; Cox, B.; Lin, S.; McPhail, A. T. *Pure Appl. Chem.* **1990**, *62*(7), 1289-91. (h) Broka, C. A.; Chan, S.; Peterson, B. *J. Org. Chem.* **1988**, *53*(7), 1584-6.
- <sup>4</sup> Roussis, V.; Wu, Z.; Fenical, W.; Strobel, S. A.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1990**, *55*(16), 4916-22.
- <sup>5</sup> Corey, E. J.; Carpino, P. *J. Am. Chem. Soc.* **1989**, *111*(14), 5472-4.
- <sup>6</sup> Kocienski, P. J.; Pontiroli, A.; Qun, L. *J. Chem. Soc., Perkin Trans. 1* **2001**, *19*, 2356-2366.
- <sup>7</sup> Pletcher, J. M.; McDonald, F. E. *Org. Lett.* **2005**, *7*(21), 4749-4752.
- <sup>8</sup> Ata, A.; Win, Hla Y.; Holt, D.; H., Paul; S., Edward P.; Jayatilake, G. S. *Helv. Chim. Acta* **2004**, *87*(5), 1090-1098.
- <sup>9</sup> (a) Luedke, E. (1990). The Identification and characterization of the Pseudopterosins: Anti-inflammatory agents isolated from the gorgonian coral *Pseudopterogorgia elisabethae*. Ph.D. Dissertation Thesis. University of California, Santa Barbara. (b) Mayer, A. M. S.; Jacobson, P. B.; Fenical, W.; Jacobs, R. S.; Glaser, K. B. *Pharm. Lett.* **1998**, *62*, 401-407. (c) Moya, C. E., Jacobs, R. S. *The FASEB Journal* **2007**, *21* (5) part 1, A427.
- <sup>10</sup> Dovi, J. V.; He, L.; DiPietro, L. A. *J. Leukocyte Bio.* **2003**, *73*, 448-455.
- <sup>11</sup> Moya, C. E.; Jacobs, R. S. *Comparative Biochemistry and Physiology Part C*, **2006**, *143*, 436-443.

---

<sup>12</sup> For example, the adenosine structural analog called NECA displays an IC<sub>50</sub> toward the A<sub>2A</sub> receptor of 36 nM, while IB-MECA displays an IC<sub>50</sub> toward the A<sub>3</sub> receptor of 1.2 nM.

<sup>13</sup> The *in vitro* pharmacology studies were conducted by the drug development company, Cerep, located in Paris, France.

<sup>14</sup> Montesinos, M. C.; Gadangi, P.; Longaker, M.; Sung, J.; Levine, J.; Nilsen, D.; Reibman, J.; Li, M.; Jiang, C-K.; Hirschhorn, R.; Recht, P. A.; Ostad, E.; Levin, R. I.; Cronstein, B. N. *J. Exp. Med.* **1997**, *186* (9), 1615-1620.

<sup>15</sup> A pseudocrystal is defined to be a substance that appears to be a crystal but lacks crystalline diffraction. In the present instance, a yellow crystal-like substance formed when the recrystallization occurred too quickly (e.g. overnight), leading to the co-crystallization of the two pseudopterosins in their original proportions.