Tetrahedron 67 (2011) 58–68

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Brominated polyunsaturated lipids and their stereochemistry from the Chinese marine sponge *Xestospongia testudinaria*

Wei Jiang [a], Dong Liu [a], Zhiwei Deng [b], Nicole J. de Voogd [c], Peter Proksch [d], Wenhan Lin [a,*]

[a] *State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, PR China*
[b] *Analytical and Testing Center, Beijing Normal University, Beijing 100075, PR China*
[c] *National Museum of Natural History, PO Box 9517, 2300 RA Leiden, The Netherlands*
[d] *Institute of Pharmaceutical Biology and Biotechnology, Heinrich-Heine University, Duesseldorf D-40225, Germany*

ARTICLE INFO

Article history:
Received 23 June 2010
Received in revised form 1 November 2010
Accepted 9 November 2010
Available online 13 November 2010

Keywords:
Marine sponge
Xestospongia testudinaria
Brominated polyunsaturated lipids
Structural elucidation
Mosher reaction

ABSTRACT

Chemical examination of the Chinese marine sponge *Xestospongia testudinaria* led to the isolation of 39 new brominated polyunsaturated compounds, which were designated with the trivial names xestospongienes A–Z and Z_1–Z_{13}. The structures of these compounds were elucidated by analyzing extensive spectroscopic (IR, MS, 1D, and 2D NMR) data and the results from CD spectral assignment, chemical conversion, and Mosher reaction.

1. Introduction

Brominated polyunsaturated fatty acids are a family of unusual natural products that are widely distributed in marine algae, sponges, tunicates, anemones, and lichens, but they are rarely found in higher plants. Most brominated lipids possess one or more olefinic and/or acetylenic groups. Straight-chain acetylenic lipids differ in terms of chain length, number, and position of polyenynic bonds, and the presence of other functionalities. Brominated polyacetylenic compounds were reported to possess significant biological activities, including antifungal and antimicrobial effects,[1–4] HIV-1 integrase inhibition,[5,6] and cytotoxicity.[7] At least two species of sponge genus *Xestospongia* (Nepheliospongiidae) (*Xestospongia muta* and *Xestospongia testudinaria*) contained diverse brominated fatty acids,[8–12] in addition to alkaloids,[13–16] xestoquinones,[17,18] terpenoids,[19,20] and sterols.[21] Thus far, four brominated acetylenic acids have been isolated from the Australian sponge *X. testudinaria*,[3,12,22] along with two xestosterol esters containing brominated acetylenic moieties, which are the inhibitors of [^{3}H]DPCPX-bound rat-brain adenosine A_1 receptors.[9] In the course of our studies on the chemical diversity of sponges inhabited in different locations, the sponge *X. testudinaria* as a main specimen inhabited in Weizhou Island of the South China Sea was collected. Chemical examination of this specimen led to the isolation and structural elucidation of 39 new brominated polyunsaturated lipids, namely, xestospongienes A–Z and Z_1–Z_{13} together with two known methyl esters of brominated C_{18} acetylenic acids.

2. Results and discussion

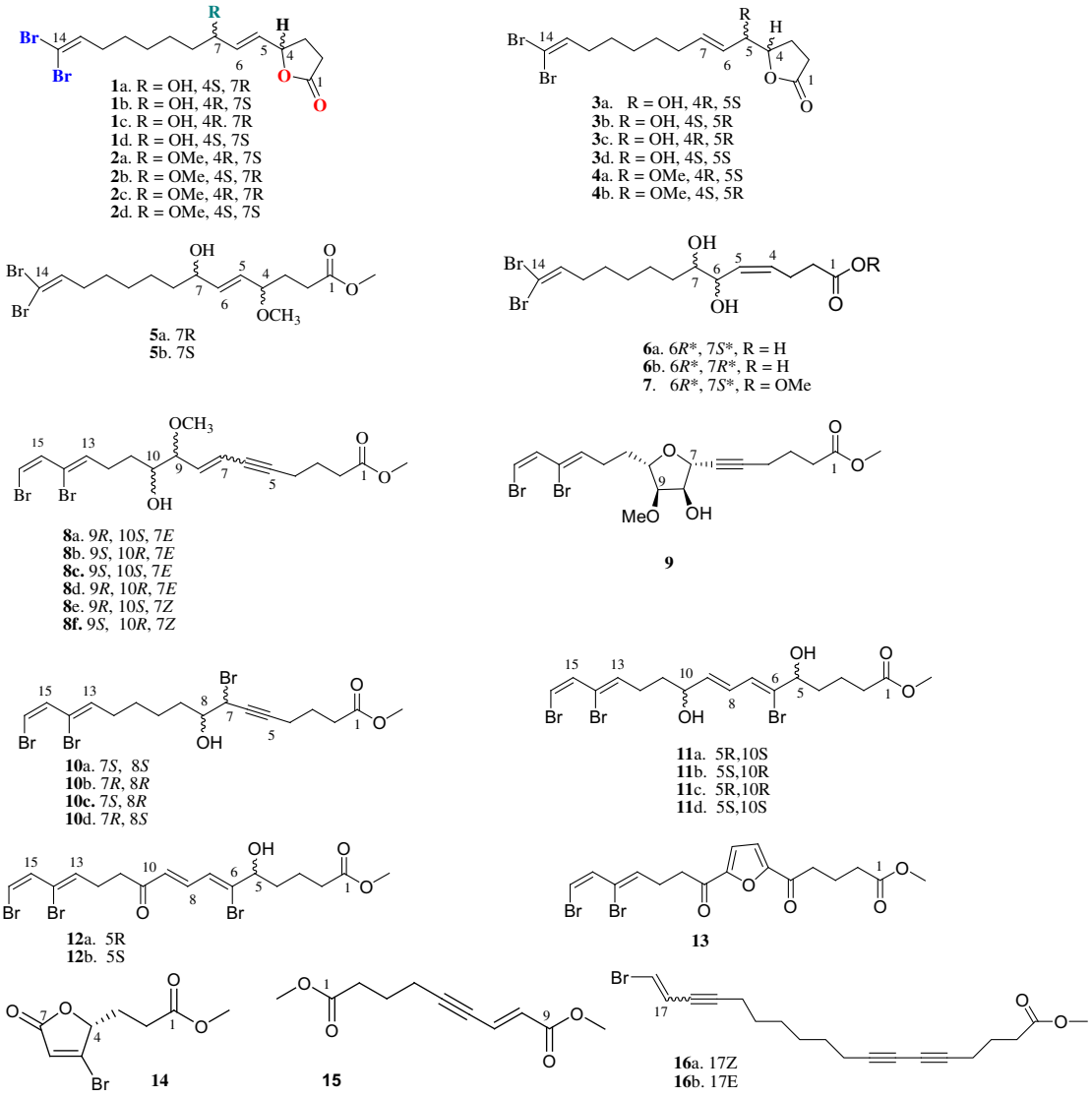
Repeated column chromatography of EtOAc fraction of sponge extract using reversed-phase and chiral-phase HPLC separation led to the isolation and characterization of 41 brominated polyunsaturated fatty acids.

Xestospongienes A-B (**1a**,**b**) were separated by the reversed-phase semi-preparative HPLC (ODS column) and followed by chiral HPLC on an AD-H column.

Xestospongiene A (**1a**) had a pseudomolecular ion at *m*/*z* 417 $[M+Na]^+$ in ESIMS. Its molecular formula ($C_{14}H_{20}Br_2O_3$) was determined by HRESIMS (*m*/*z* 416.9675), indicating 4° of unsaturation. The 1:2:1 isotopic distribution at *m*/*z* 415, 417, and 419 indicated the presence of two bromine atoms. IR absorptions at 3399 and 1772 cm^{-1} were attributed to hydroxy and lactone groups. The ^{13}C NMR and APT spectra exhibited 14 carbon resonances, including seven aliphatic methylenes ranging between δ_C 25.0–37.0; two oxymethines at δ_C 71.6 (CH, C-7) and 79.9 (CH, C-4); three olefinic carbons at δ_C 127.6 (CH, C-5), 136.5 (CH, C-6), and

* Corresponding author. Tel.: +8610 82806188; fax: +8610 82802724; e-mail address: whlin@bjmu.edu.cn (W. Lin).

0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.11.045



138.7 (CH, C-13); and a carbonyl carbon at δ_C 176.8 (qC, C-1). A quaternary carbon presented at δ_C 88.7 (qC, C-14) was characteristic of a terminal dibrominated olefinic carbon.[23] COSY and TOCSY correlations established the spin system of an alkyl chain from C-2 to C-13, in which two double bonds were found to be located at C-5/C-6 and C-13/C-14. The proton H-4 (δ_H 4.99, dt, J=6.3, 7.2 Hz) showed HMBC correlation to the carbonyl carbon C-1, indicating a γ-lactone to be linked to a terminus of the alkyl chain. Thus, C-7 was substituted by a hydroxy group, while the other terminus was ended by a dibromovinyl group. The large coupling constant $J_{H\text{-}5/H\text{-}6}$ (15.5 Hz) was assigned to 5*E* geometry. The absolute configuration of C-7 was established by a modified Mosher's method.[24] Treatment of **1a** with (*S*)- and (*R*)-MTPA-Cl yielded (*R*)- and (*S*)-MTPA esters. Analysis of their chemical shift differences ($\Delta\delta=\delta_S-\delta_R$) (Fig. 1 and Table 1) led to the assignment of (7*R*) configuration. The absolute configuration of the stereogenic center C-4 was further determined on the basis of comparing the experimental CD and computed CD spectra. The positive Cotton effect at 214 (+6.0) nm was observed for **1a** in experiment (Fig. 2), while that at 213 nm (+4.0) for (4*S*,7*R*)-**1a** (Fig. 3, blue line) and at 223 nm (+11.3) for (4*R*,7*R*)-**1a** (pink line) were calculated at B3LYP/aug-cc-pVDZ//B3LYP/6-31G(d) level.[25,26] The calculated CD curve of (4*S*,7*R*)-**1a** was more close to the experimental result, **1a** was thus assigned as (4*S*,7*R*)-configurations. In addition, the experimental CD data of **1a** were compatible to that of (4*S*)-marmelo lactones, which showed positive band near 215 nm due to the $n\rightarrow\pi^*$ transition.[27]

Fig. 1. $\Delta\delta$ ($\delta S-\delta R$) values (in ppm) of (*S*)- and (*R*)-MTPA esters of **1a**.

Table 1
$\Delta\delta$ ($\delta_S-\delta_R$) Values in ppm for the MTPA esters of **1a**–**d**, **3a**–**d**, and **5a**,**b** in $CDCl_3$

Position	1a	1b	1c	1d	3a	3b	3c	3d	5a	5b
H-2	−0.01	+0.02	−0.02	+0.02	+0.03	−0.03	−0.02	+0.03	−0.04	+0.04
					+0.07	−0.07	−0.03	+0.02	−0.03	+0.04
H-3	−0.05	+0.04	−0.03	+0.03	+0.03	−0.03	−0.09	+0.08	−0.04	+0.05
	−0.06	+0.05	−0.04	+0.04	+0.04	−0.04	−0.03	+0.03	−0.04	+0.04
H-4	−0.05	+0.06	−0.05	+0.05	+0.05	−0.05	−0.02	+0.02	−0.05	+0.06
H-5	−0.12	+0.05	−0.11	+0.11					−0.11	+0.11
H-6	−0.12	+0.10	−0.12	+0.12	−0.13	+0.14	+0.11	−0.21	−0.10	+0.10
H-7					−0.10	+0.09	+0.09	−0.07		
H-8	+0.04	−0.03	+0.05	−0.05	−0.05	+0.05	+0.02	−0.03	+0.06	−0.05
	+0.09	−0.04	+0.10	−0.11	−0.05	+0.04	+0.02	−0.03	+0.05	−0.06
H-9	+0.03	−0.05	+0.10	−0.10	−0.02	+0.02	+0.01	−0.02	+0.04	−0.04
H-10	+0.08	−0.07	+0.12	−0.10	−0.03	+0.03	+0.02	−0.02	+0.03	−0.03

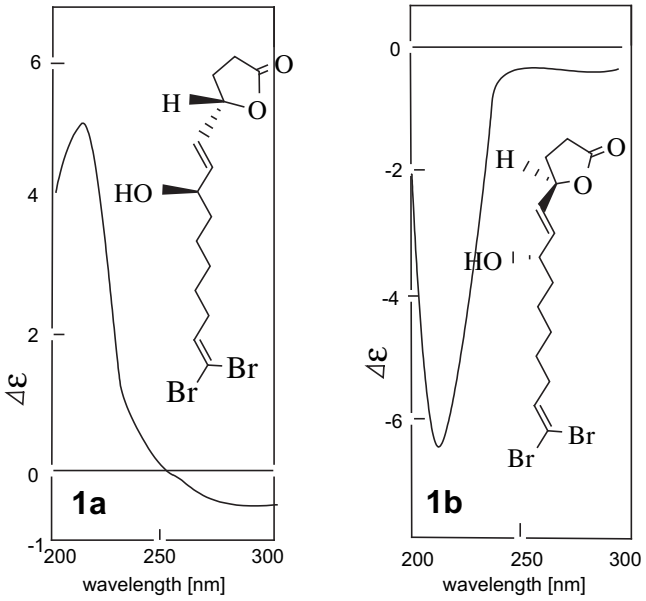


Fig. 2. Cotton effects of **1a** and **1b**.

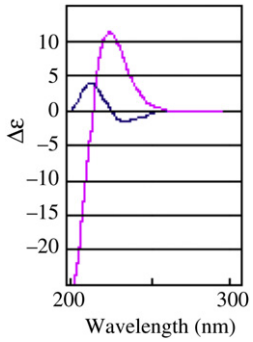


Fig. 3. Calculated CD for (4*S*,7*R*)-**1a** (blue line) and (4*R*,7*R*)-**1a** (pink line).

Xestospongiene B (**1b**) was determined to be an enantiomer of **1a** based on the duplicated NMR data (Tables 2 and 3) of the two compounds. This assignment was also supported by the observation of the negative Cotton effect at 214 nm, in contrast to that of **1a** (Fig. 2), reflecting (4*R*) configuration, and the optical rotation value (+20) of **1b** in contrast to that (−21.5) of **1a**. In addition, the ^{1}H NMR chemical shift differences of the Mosher esters of (*S*)-MTPA-**1b** and (*R*)-MTPA-**1b** (Table 1) allowed the assignment of (7*S*) configuration.

The ^{1}H and ^{13}C NMR spectra of the inseparable xestospongienes C (**1c**) and D (**1d**) were completely duplicated and were almost identical to those of **1a**. Analysis of the 2D NMR spectroscopic data indicated that **1c** and **1d** are the diastereomers of **1a**. Esterification of a mixture containing **1c**/**1d** using (*S*)-MPTA-Cl yielded two esters that were well separated by reversed-phase HPLC. The absolute configuration of C-7 in **1c** was determined to be (7*R*), whereas that of C-7 in **1d** was (7*S*), based on the chemical shift differences of their Mosher esters (Table 1). Thus, the stereogenic centers of C-4 in **1c** and **1d** were assumed to be (4*R*) and (4*S*), respectively.

Xestospongienes E (**2a**) and F (**2b**) were a pair of enantiomers with a ratio of 1:1 as detected by chiral HPLC (AD-H column). Their molecular formula of $C_{15}H_{22}Br_2O_3$ was determined by HRESIMS, whose molecular mass was 14 amu higher than that of **1**. The duplicated ^{1}H and ^{13}C NMR data of **2a** and **2b** were compatible to those of **1**, except for the presence of a methoxy group (δ_H 3.30, s; δ_C 56.5) and the downfield shifted C-7 (δ_C 81.2). These findings suggested a methoxy group to be linked to C-7. The HMBC correlations from the methoxy protons to C-7, and in turn, from H-7 (δ_H 3.60, ddd, J=6.1, 6.1, 6.8 Hz) to methoxy carbon, confirmed the position of methoxy linkage. Methylation of **1b** using MeI[28] yielded **2a** (Fig. 4), indicating that the configurations of C-4 and C-7 in **2a** were identical to those of **1b**. Thus, compound **2a** was a 7-methoxy derivative of **1b**. Following the same methylation protocol as used for **1b**, **1a** was converted to **2b**, leading to the assignment of (4*S*) and (7*R*) configurations of **2b**.

Xestospongienes G (**2c**) and H (**2d**) were separated in the ratio of 1:1 by chiral-phase HPLC. The duplicated NMR and MS data of **2c** and **2d** were almost identical to those of **2a** (Tables 2 and 3), suggesting **2c**/**2d** to be the diastereomers of **2a**. Methylation of **1c** to yield **2c** led to the assignment of (4*R*, 7*R*) configurations in **2c**. Accordingly, the stereogenic centers of **2d** were assigned to (4*S*) and (7*S*) configurations.

Xestospongiene I (**3a**) had the same molecular formula as **1** based on its HRESIMS data. The close similar NMR data of **3a** in comparison with those of **1** indicated **3a** to be an analogue of **1**. The presence of a γ-lactone in **3a** was evident from COSY correlations and the HMBC relationship between H-4 (δ_H 4.54, dt, J=3.2, 7.1 Hz) and a carbonyl carbon at δ_C 177.4. However, analysis of the COSY and HMQC relationships revealed that H-4 coupled to a vicinal oxymethine H-5 (δ_H 4.49, dd, J=3.2, 6.3 Hz) instead of an olefinic proton of **1**, while H-5 further correlated to olefinic proton H-6 (δ_H 5.44, dd, J=6.3, 15.5 Hz). These findings indicated that C-5 was hydroxylated, whereas a double bond was located at C-6/C-7. Analysis of 2D NMR data conducted to assign the remaining substructure to be identical to that of **1a**. The coupling constant $J_{H\text{-}6/H\text{-}7}$ (15.5 Hz) was indicative of 6*E* geometry. Based on the conventional CD rule for saturated lactones,[29] the positive Cotton effect of **3a** at 212 nm for the $n\rightarrow\pi^*$ transition of lactone was in accordance with (4*R*) configuration. This assignment was in opposite to that of **1a**–d,

Table 2
^{1}H NMR data of xestospongienes A–S (**1a**–**7**) in $CDCl_3$

No.	**1a**/**1b**	**1c**/**1d**	**2a**/**2b**	**2c**/**2d**	**3a**/**3b**	**3c**/**3d**	**4a**/**4b**	**5a**/**5b**	**6a**	**6b**	**7**
2	2.57, m 2.59, m	2.58, t (8.7)	2.59, t (8.7)	2.59, t (8.7)	2.55, m 2.70, m	2.60, m	2.46, m 2.60, m	2.41, t (7.5)	2.45, m 2.60, m	2.45, m 2.68, m	2.47, m
3	2.05, m 2.43, m	2.05, m 2.45, m	2.46, m 2.04, m	2.46, m 2.04, m	2.15, m 2.25, m	2.00, m 2.20, m	2.21, m	1.90, m	2.25, m 2.65, m	2.25, m 2.65, m	2.31, m 2.62, m
4	4.99, dt (6.3, 7.2)	4.99, ddd (6.3, 7.2, 7.2)	5.00, ddd (6.1, 6.1, 7.0)	5.00, ddd (6.1, 6.1, 7.0)	4.54, dt (3.2, 7.1)	4.48, ddd (6.0,6.9, 6.9)	4.49, ddd (3.0, 6.8, 6.8)	3.61, ddd (6.4, 6.4, 7.6)	5.60, ddd (6.1, 6.1, 11.0)	5.62, ddd (6.1, 6.1, 11.8)	5.60, ddd (6.1, 6.1, 11.0)
5	5.77, dd (6.3, 15.5)	5.77, dd (6.3, 15.5)	5.73, dd (7.0, 15.7)	5.73, dd (7.0, 15.7)	4.49, dd (3.2, 6.3)	4.12, dd (6.0,6.3)	3.80, dd (3.0,7.7)	5.52, dd (7.6, 15.6)	5.67, dd (8.2, 11.0)	5.50, dd (8.2, 11.8)	5.68, dd (8.8, 11.0)
6	5.87, dd (5.7, 15.5)	5.87, dd (5.7,15.5)	5.69, dd (6.8, 15.7)	5.69, dd (6.8, 15.7)	5.44, dd (6.3, 15.5)	5.52, dd (7.2, 15.5)	5.29, dd (7.7, 15.4)	5.73, dd, (6.3, 15.6)	4.53, dd (4.0,8.2)	4.41, (8.0,8.2)	4.45, dd (3.9, 8.8)
7	4.20, ddd (5.7, 6.3, 6.3)	4.20, ddd (5.7, 6.3, 6.3)	3.60, ddd (6.1, 6.1, 6.8)	3.60, ddd (6.1, 6.1, 6.8)	5.86, ddd (6.7, 6.7, 15.5)	5.86, ddd (6.7, 6.7, 15.5)	5.80, ddd (6.8, 6.8, 15.4)	4.16, ddd (6.3, 6.3, 6.3)	3.80, ddd (4.0, 7.0, 7.0)	3.52, ddd (6.5, 6.5, 8.0)	3.75, ddd (3.9, 6.2, 6.2)
8	1.56, m	1.56, ddd (3.6, 3.6, 6.3)	1.52, m 1.60, m	1.52, m 1.60, m	2.10, m	2.10, m	2.10, m	1.56, m	1.45, m	1.37, m 1.47, m	1.45, m
9	1.36, m	1.36, m	1.34, m	1.34, m	1.45, m	1.45, m	1.45, m	1.35, m	1.35, m	1.55, m 1.35, m	1.35, m
10	1.45, m 1.36, m	1.45, m 1.36, m	1.34, m	1.34, m	1.35, m	1.35, m	1.35, m	1.35, m	1.35, m	1.35, m	1.35, m 1.56, m
11	1.45, m	1.45, m	1.45, m	1.45, m	1.45, m	1.45, m	1.45, m	1.45, m	1.45, m	1.45, m	1.45, m
12	2.13, ddd (7.1, 7.1, 7.3)	2.13, td (7.1, 7.3)	2.12,ddd (7.2, 7.2, 7.2)	2.12, ddd (7.2, 7.2, 7.2)	2.15, m	2.15, m	2.10, m	2.12, ddd (7.2, 7.2, 7.2)	2.10, ddd (7.2, 7.3, 7.3)	2.10, ddd (7.2,7.3, 7.3)	2.11, ddd (7.3, 7.3, 7.3)
13	6.40, t (7.3)	6.40, t (7.3)	6.40, t (7.3)	6.40, t (7.3)	6.40, t (7.3)	6.40, t (7.3)	6.40, t (7.3)	6.41, t (7.2)	6.40, t (7.2)	6.40, t (7.2)	6.41, t (7.3)
OMe			3.30,s	3.30, s			3.31, s	3.29, s			3.70, s
OMe								3.70, s			

Table 3
^{13}C NMR data of xestospongienes A–S (**1a**–**7**) in $CDCl_3$

Position	**1a**/**1b**	**1c**/**1d**	**2a**/**2b**	**2c**/**2d**	**3a**/**3b**	**3c**/**3d**	**4a**/**4b**	**5a**/**5b**	**6a**	**6b**	**7**
1	176.8, C	176.9, C	176.7, C	176.9, C	177.4, C	177.0, C	177.7, C	173.9, C	177.3, C	177.3, C	174.0, C
2	28.5, CH_2	28.7, CH_2	28.5, CH_2	28.6, CH_2	28.6, CH_2	28.6, CH_2	28.4, CH_2	29.9, CH_2	33.2, CH_2	32.9, CH_2	33.3, CH_2
3	28.7, CH_2	28.9, CH_2	28.9, CH_2	28.9, CH_2	21.3, CH_2	23.8, CH_2	22.0, CH_2	30.5, CH_2	23.0, CH_2	23.0, CH_2	23.0, CH_2
4	79.9, CH	80.0, CH	80.0, CH	80.1, CH	82.3, CH	82.7, CH	81.6, CH	80.6, CH	132.6, CH	132.9, CH	132.6, CH
5	127.6, CH	127.7, CH	129.8, CH	129.6, CH	72.8, CH	75.1, CH	83.1, CH	130.3, CH	128.4, CH	129.6, CH	129.1, CH
6	136.5, CH	136.7, CH	134.4, CH	134.6, CH	126.3, CH	126.8, CH	124.9, CH	136.4, CH	69.9, CH	70.6, CH	69.9, CH
7	71.6, CH	71.7, CH	81.2, CH	81.1, CH	135.2, CH	136.0, CH	137.1, CH	72.1, CH	73.7, CH	73.0, CH	73.7, CH
8	37.0, CH_2	37.0, CH_2	35.2, CH_2	35.1, CH_2	32.2, CH_2	32.2, CH_2	32.2, CH_2	37.1, CH_2	31.3, CH_2	32.4, CH_2	31.9, CH_2
9	25.0, CH_2	25.0, CH_2	24.9, CH_2	24.9, CH_2	28.5, CH_2	28.4, CH_2	28.4, CH_2	25.1, CH_2	25.4, CH_2	25.3, CH_2	25.6, CH_2
10	28.8, CH_2	28.5, CH_2	28.8, CH_2	28.8, CH_2	28.4, CH_2	28.0, CH_2	28.7, CH_2	28.9, CH_2	28.9, CH_2	29.0, CH_2	29.1, CH_2
11	27.7, CH_2	27.7, CH_2	27.7, CH_2	27.7, CH_2	27.6, CH_2	27.6, CH_2	27.6, CH_2	27.7, CH_2	27.7, CH_2	27.6, CH_2	27.7, CH_2
12	32.9, CH_2	32.9, CH_2	32.9, CH_2	32.9, CH_2	32.9, CH_2	32.9, CH_2	32.9, CH_2	32.9, CH_2	32.9, CH_2	32.6, CH_2	32.9, CH_2
13	138.7, CH	138.7, CH	138.7, CH	138.7, CH	138.7, CH	138.7, CH	138.7, CH	138.7, CH	138.6, CH	138.6, CH	138.8, CH
14	88.7, C	88.7, C	88.7, C	88.7, C	88.7, C	88.7, C	88.7, C	88.7, C	88.7, C	88.7, C	88.6, C
OMe			56.5, CH_3	56.5, CH_3			56.9, CH_3	56.3, CH_3			51.8, CH_3
OMe								51.6, CH_3			

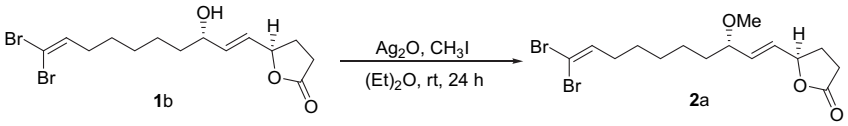


Fig. 4. Preparation of **2a** by methylation of **1b**.

in which a vinyl group attached to C-4. The configuration of C-5 was deduced to be (*S*) based on the chemical shift differences of the (*S*)- and (*R*)-Mosher esters of **3a** (Table 1).

The NMR and MS data of **3b** were identical to those of **3a**, indicating that **3b** is an enantiomer of **3a**. The negative Cotton effect at 212 nm for the $n\rightarrow\pi^*$ transition of the lactone carbonyl group of **3b** reflected (4*S*) configuration, while the ^{1}H NMR spectroscopic data of its Mosher esters determined (5*R*) configuration (Table 1).

Xestospongienes K (**3c**) and L (**3d**) were separated as a pair of enantiomers and were determined to be the diastereomers of **3a** based on 1D and 2D NMR spectroscopic data. The absolute configuration of C-5 in **3c** was determined to be (*R*) through the calculation of $\Delta\delta_H$ ($\delta_S-\delta_R$) values of its Mosher esters. The stereogenic center at C-4 was thus assumed to be *R*, as evident from the positive Cotton effect at 208 nm for the $n\rightarrow\pi^*$ transition. Thus, the negative Cotton effect at 212 nm of **3d** was indicative of (4*S*) configuration. Consequently, the configuration of C-5 in **3d** was assigned to (*S*) based on the chemical shift differences of its Mosher esters.

The almost duplicated NMR data of xestospongienes M (**4a**) and N (**4b**) suggested them to be a pair of stereoisomers, which were well separated in the ratio of 1:1 by chiral HPLC. They had same molecular formula, which was determined by HRESIMS and NMR data, and the molecular mass was 14 amu higher than that of **3**. Comparison of IR and NMR data revealed that **4a** and **4b** had a methoxy group at C-5, that was evident from the methoxy protons (δ_H 3.31, s) correlated to C-5 in HMBC spectrum. The coupling constant of H-5 in **4a**/**4b** was compatible to that of **3a**/**3b** rather than that of **3c**/**3d** (Table 2), implying the stereochemistry of **4a**/**4b** to be related to **3a**/**3b**. Since the methylated product of **3a** was identical to **4a** based on the same retention times in chiral HPLC (AD-H), the stereogenic centers in **4a** were thus determined to be (4*R*) and (5*S*) configurations. Accordingly, **4b** should be (4*S*) and (5*R*).

The ^{1}H and ^{13}C NMR spectroscopic data of xestospongiene O (**5a**) were similar to those of **1**, except for the presence of two methoxy resonances. A C_{14} alkyl straight-chain was established by 2D NMR (COSY, HMQC, and HMBC) analysis. One of the methoxy groups was positioned at C-4 as evident from the HMBC correlations between H-4 (δ_H 3.61, ddd, *J*=6.4, 6.4, 7.6 Hz) and a methoxy carbon at δ_C 56.3, and in turn, between MeO (δ_H 3.29) and C-4 (δ_C 80.6). The second methoxy group was confirmed to form a methyl ester at C-1 based on the HMBC correlation between the methoxy protons (δ_H 3.70) and a carbonyl carbon at δ_C 173.9. The remaining substructure from C-5 to C-14 was identical to that of **1**. The coupling constant $J_{H\text{-}5/H\text{-}6}$ (15.6 Hz) was indicative of 5*E* geometry. The absolute configuration of C-7 was determined to be (7*R*) by calculation of the $\Delta\delta_H$ ($\delta_S-\delta_R$) values of its Mosher esters. The configuration of C-4 remains undetermined.

Xestospongiene P (**5b**) was determined to be the stereoisomer of **5a** based on the 2D NMR and HRESIMS data. Calculation of the $\Delta\delta_H$ ($\delta_S-\delta_R$) values of the (*S*)- and (*R*)-MPTA esters of **5b** (Table 1) led to the assignment of (7*S*) configuration.

The molecular formula of xestospongiene Q (**6a**) was $C_{14}H_{22}Br_2O_4$ as determined by HRESIMS and NMR data, indicating 3° of unsaturation. IR absorption bands at 3412 and 1712 cm^{-1} suggested the presence of hydroxy and carbonyl groups. The ^{1}H and ^{13}C NMR spectroscopic data were closely related to those of **5**, except for the absence of methoxy group. Analysis of 2D NMR (COSY, HMQC, and HMBC) data resulted in a C_{14} fatty acid, in which a double bond was located at C-4/C-5, while C-6 and C-7 were hydroxylated. Thus, the structure of **6a** was in accordance with 14-dibromo-4,13-dien-6,7-dihydroxytetradecanoic acid. The coupling constant $J_{H\text{-}4/H\text{-}5}$ (11.0 Hz) was assigned to 4*Z* geometry, whereas $J_{H\text{-}6/H\text{-}7}$ (4.0 Hz) was attributed to *erythro* relationship of the vicinal diol.[30] Thus, the relative configurations of **6a** were assigned to 6*R** and 7*S**.

Comparison of the NMR data of xestospongiene R (**6b**) with those of **6a** indicated that **6b** was a stereoisomer of **6a**. The difference was found by the $J_{H\text{-}6/H\text{-}7}$ (8.0 Hz) value, which was in agreement with *threo* configuration of the vicinal diol.[30] Since the coupling constant $J_{H\text{-}5/H\text{-}6}$ was almost identical to that of **6a**, **6b** was assumed to be an 7-epimer of **6a**. Therefore, the relative configurations of **6b** were assigned to 6*R** and 7*R**.

Xestospongiene S (**7**) was determined to be a methyl ester of **6a** by comparing the NMR data of both compounds and based on the evidence that a methoxy group (δ_H 3.70, s; δ_C 51.8) showed HMBC correlation with the carbonyl carbon C-1 (δ_C 174.0). Accordingly, the relative configurations of **7** were assigned to 6*R** and 7*S**.

Xestospongiene T (**8a**) had a molecular formula of $C_{18}H_{24}Br_2O_4$ based on the HRESIMS data at *m*/*z* 484.9945 $[M+Na]^+$ (calcd 484.9936), implying 6° of unsaturation. IR absorption bands at 3378, 2367, and 1738 cm^{-1} suggested the presence of hydroxy, acetylenic, and carbonyl functionalities. The ^{13}C NMR spectrum exhibited a total of 18 carbon resonances, including two methoxys (δ_C 51.6, 56.8), six olefinic carbons for three double bonds, two oxymethines, two acetylenic carbons (δ_C 78.9, 90.4), and a carbonyl carbon (δ_C 173.6). The remaining signals were attributed to five alkyl methylenes. The 1D and 2D NMR spectroscopic data were indicative of a methyl ester of a C_{16} fatty acid, which was closely related to 14,16-dibromohexadeca-7,13,15-trien-5-ynoic acid as previously isolated from a sponge *Oceanapia* sp.[30] The difference lay in the presence of two oxymethines at δ_C 84.9 (C-9) and 72.5 (C-10), the positions of which were assigned by the COSY and HMQC relationships. The linkage of a methoxy group (δ_H 3.32, s; δ_C 56.8) to C-9 was confirmed by HMBC correlation. In addition, a terminal methyl ester was evident from the HMBC correlation between the methoxy protons (δ_H 3.71) and a carbonyl carbon at δ_C 173.6. Thus, C-10 was attached by a hydroxy group. The geometries of double bonds were assigned to 7*E*, 13*Z*, and 15*Z*, based on the NOESY correlations between H-13/H-15 and H-15/H-16 in association with the coupling constants $J_{H\text{-}7/H\text{-}8}$ (16.0 Hz) and $J_{H\text{-}15/H\text{-}16}$ (7.7 Hz). The absolute configuration at C-10 was determined to be (10*S*) based on the chemical shift differences of its Mosher esters. The $J_{H\text{-}9/H\text{-}10}$ (3.5 Hz) value was in accordance with *erythro* configuration of the vicinal protons H-9/H-10. Thus, C-9 was assigned to (*R*) configuration.

Xestospongiene U (**8b**) exhibited spectroscopic features (Tables 4 and 5) that were identical to those of **8a**, indicating a stereoisomer of the latter compound. The ^{1}H NMR data of its (*S*)- and (*R*)-MPTA esters (Table 6) were in agreement with (10*R*), while the similarity of $J_{H\text{-}9/H\text{-}10}$ value to that of suggested an 9,10-*erythro* configuration. Thus, C-9 was assumed to be (*S*) configuration.

The enantiomers, xestospongienes V (**8c**) and W (**8d**), were well separated by chiral HPLC method. They were assigned to the diastereomers of **8a** on the basis of 2D NMR data analysis. The coupling constant $J_{H\text{-}9/H\text{-}10}$ (7.6 Hz) of **8c** and **8d** was indicative of 9,10-*threo* configuration. The absolute configuration of C-10 was determined to be (*S*) for **8c** and (*R*) for **8d**, based on the chemical shift differences of their Mosher esters. Therefore, the chiral center C-9 was supposed to be (*S*) for **8c** and (*R*) for **8d**.

Table 4
^{1}H NMR data of xestospongienes T–Z, Z_1–Z_{10} (**8a**–**12b**)

No.	**8a**/**8b**[a]	**8c**/**8d**[a]	**8e**/**8f**[a]	**9**[a]	**10a**/**10b**[a]	**10c**/**10d**[a]	**11a**/**11b**[b]	**11c**/**11d**[b]	**12a**/**12b**[a]
2	2.48, t (6.9)	2.49, t (6.9)	2.49, t (7.4)	2.46, t (7.5)	2.46, t (7.3)	2.46, t (7.3)	2.38, t (7.1)	2.38, t (7.0)	2.40, m
3	1.88, dq (6.9, 6.9)	1.89, dq (6.9,6.9)	1.89, dq (6.9, 6.9)	1.87, tt (7.0,7.5)	1.88, dq (7.0, 7.0)	1.88, dq (7.0, 7.0)	1.62, m	1.65, m	1.84, m
4	2.42, t (6.9)	2.42, t (6.9)	2.44, td (1.3, 6.9)	2.33, dt (1.7, 7.0)	2.38, dt (1.3, 7.0)	2.38, dt (1.6, 7.0)	1.65, m 1.74, m	1.65, m 1.74, m	1.84, m
5							4.16, t (5.9)	4.16, m	4.29, t (5.4)
7	5.70, d (16.0)	5.72, dt (1.3, 15.9)	5.81, d (11.0)	4.39, dd (1.7, 4.5)	4.51, dd (1.3, 4.7)	4.61, dd (1.6, 1.7)	6.67, d (10.1)	6.67, d (10.2)	6.79, d (10.5)
8	5.95, dd (8.0, 16.0)	5.81, dd (7.9,15.9)	5.80, dd (8.1, 11.0)	4.15, dd (5.6, 4.5)	3.67, m	3.67, m	6.57, dd (10.1, 15.2)	6.57, m	7.44, dd (10.5, 15.6)
9	3.54, dd (3.8, 8.0)	3.36, dd (7.6,7.9)	4.17, dd (3.5, 8.1)	3.55, dd (5.6, 5.6)	1.57, m 1.75, m	1.57, m 1.75, m	5.94, dd (6.3, 15.2)	5.94, dd (6.4, 15.3)	6.30, d (15.6)
10	3.67, m	3.46, m	3.80, ddd (3.5, 6.3, 6.3)	3.78, ddd (6.5, 6.5, 5.6)	1.60, m 1.39, m	1.60, m 1.39, m	4.13, m	4.13, m	
11	1.53, m	1.49, m 1.60, m	1.54, m	1.78, dt (6.5, 7.7)	1.45, m 1.55, m	1.49, m	1.65, m	1.66, m	2.78, t (7.2)
12	2.10, m 2.22, m	2.25, m 2.14, m	2.11, m 2.22, m	2.20, dt (7.4, 7.7, 7.7)	2.08, ddd (6.9,6.9,7.6)	2.08, ddd (7.0,7.0,7.8)	2.13, ddd (5.0,5.0,7.6)	2.13, ddd (7.7, 7.7, 7.7)	2.40, td (7.2, 7.9)
13	6.11, t (7.8)	6.12, t (7.9)	6.12, t (8.2)	6.12, dd (7.4,7.5)	6.10, t (7.6)	6.10, t (7.8)	6.10, t (7.6)	6.11, t (7.7)	6.11, t (7.9)
15	6.73, d (7.7)	6.73, d (7.6)	6.74, d (7.6)	6.74, d (7.5)	6.73, d (7.6)	6.73, d (7.6)	6.80, d (7.6)	6.79, d (7.6)	6.75, d (7.6)
16	6.43, dd (1.0, 7.7)	6.4, dd (1.3, 7.6)	6.42, d (7.6)	6.43, d (7.5)	6.43, d (7.6)	6.43, d (7.6)	6.57, dd (10.1, 15.2)	6.57, dd (10.2, 15.3)	6.45, d (7.6)
OMe	3.71, s	3.71, s	3.72, s	3.71, s	3.70, s	3.72, s	3.68, s	3.68, s	3.71, s
OMe	3.32, s	3.32, s	3.35, s	3.47, s					

[a] Measured in $CDCl_3$.
[b] Measured in CD_3OD.

Table 5
^{13}C NMR data of xestospongienes T–Z, Z_1–Z_{10} (**8a**–**12b**)

Position	**8a**/**8b**[a]	**8c**/**8d**[a]	**8e**/**8f**[a]	**9**[a]	**10a**/**10b**[a]	**10c**/**10d**[a]	**11a**/**11b**[b]	**11c**/**11d**[b]	**12a**/**12b**[a]
1	173.6, C	173.5, C	173.0, C	174.0, C	173.5, C	173.5, C	174.2, C	174.24C	173.9, C
2	32.8, CH_2	32.9, CH_2	32.9, CH_2	32.8, CH_2	32.8, CH_2	32.8, CH_2	33.1, CH_2	33.09 CH_2	33.5, CH_2
3	23.8, CH_2	23.8, CH_2	23.9, CH_2	23.6, CH_2	23.5, CH_2	23.5, CH_2	20.5, CH_2	20.57 CH_2	20.4, CH_2
4	18.9, CH_2	18.9, CH_2	19.0, CH_2	18.3, CH_2	18.5, CH_2	18.5, CH_2	34.3, CH_2	34.28 CH_2	34.9, CH_2
5	90.4, C	90.7, C	94.8, C	86.3, C	89.0, C	89.4, C	75.4, C	75.40C	76.3, C
6	78.9, C	78.8, C	77.4, C	78.2, C	77.0, C	77.0, C	131.5, C	131.51C	140.9, C
7	115.1, CH	115.3, CH	115.2, CH	73.8, CH	45.8, CH	45.0, CH	127.4, CH	127.41CH	126.2, CH
8	137.7, CH	138.1, CH	137.4, CH	75.3, CH	74.6, CH	74.4, CH	126.7, CH	126.67 CH	138.6, CH
9	84.9, CH	85.8, CH	81.3, CH	84.2, CH	33.3, CH_2	33.5, CH_2	140.0, CH	140.04 CH	132.6, CH
10	72.5, CH	72.7, CH	72.5, CH	80.4, CH	25.2, CH_2	25.3, CH_2	70.9, CH	70.94 CH	198.8, C
11	30.9, CH_2	31.3, CH_2	30.8, CH_2	33.1, CH_2	28.4, CH_2	28.4, CH_2	35.3, CH_2	35.27 CH_2	38.7, CH_2
12	27.6, CH_2	27.4, CH_2	27.6, CH_2	27.6, CH_2	31.1, CH_2	31.1, CH_2	27.0, CH_2	27.03 CH_2	25.6, CH_2
13	136.1, CH	136.1, CH	136.3, CH	135.6, CH	136.5, CH	136.4, CH	135.6, CH	135.60 CH	134.7, CH
14	113.8, C	113.8, C	113.7, C	114.0, C	113.5, C	113.5, C	113.7, C	113.70C	114.6, C
15	130.9, CH	130.9, CH	130.9, CH	131.0, CH	131.0, CH	131.0, CH	130.9, CH	130.9 CH	130.9, CH
16	112.6, CH	112.6, CH	112.6, CH	112.9, CH	112.5, CH	112.5, CH	112.1, CH	112.1 CH	112.8, CH
OMe	51.6, CH_3	51.6, CH_3	51.7, CH_3	51.6, CH_3	51.7, CH_3	51.7, CH_3	50.6, CH_3	50.6 CH_3	51.7, CH_3
OMe	56.8, CH_3	56.7, CH_3	56.6, CH_3	58.4, CH_3					

[a] Measured in $CDCl_3$.
[b] Measured in CD_3OD.

Table 6
$\Delta\delta$ Values (δ_S–δ_R) in ppm for the MTPA esters of **8a**–**8f**, **10a**–**12b**

	8a[a]	**8b**[a]	**8c**[a]	**8d**[a]	**8e**[a]	**8f**[a]	**10a**[a]	**10b**[a]	**10c**[a]	**10d**[a]	**11a**[b]	**11b**[b]	**11c**[b]	**11d**[b]	**12a**[a]	**1**[a]
H-2	+0.01	−0.01	+0.01	−0.01	+0.01	−0.01	+0.04	−0.04	−0.01	+0.01	+0.09	−0.09	+0.18	−0.18	+0.05	−0.05
H-3	+0.01	−0.01	+0.01	−0.01	+0.01	−0.01	+0.03	−0.03	−0.02	+0.02	+0.04	−0.04	+0.13	−0.13	+0.12	−0.12
H-4	+0.01	−0.02	+0.02	−0.02	+0.02	−0.02	+0.07	−0.07	−0.02	+0.03	+0.05	−0.05	+0.10	−0.10	+0.06	−0.06
H-7	+0.05	−0.05	+0.10	−0.09	+0.07	−0.07	+0.03	−0.03	−0.03	+0.03	+0.02	−0.02	−0.16	+0.16	−0.12	+0.12
H-8	+0.09	−0.09	+0.07	−0.07	+0.04	−0.04					+0.07	−0.07	−0.15	+0.15	−0.05	+0.05
H-9	+0.07	−0.07	+0.07	−0.07	+0.07	−0.07	−0.10	+0.10	+0.07	−0.07	+0.02	−0.03	−0.17	+0.17	−0.19	+0.19
H-10							−0.10	+0.10	+0.06	−0.06						
							−0.07	+0.07	+0.06	−0.06						
H-11	−0.07	+0.07	−0.05	+0.05	−0.13	+0.12	−0.22	+0.21	+0.05	−0.05	−0.07	+0.07	+0.12	−0.12	−0.01	+0.01
	−0.08	+0.07	−0.12	+0.11	−0.12	+0.12	−0.20	+0.20	+0.07	−0.07	−0.06	+0.06	+0.12	−0.12		
H-12	−0.07	+0.07	−0.07	+0.07	−0.13	+0.13	−0.05	+0.05	+0.10	−0.10	−0.11	+0.11	+0.18	−0.18	−0.01	+0.01
	−0.06	+0.06	−0.16	+0.15	−0.15	+0.14	−0.05	+0.05	+0.10	−0.10	−0.10	+0.10	+0.20	−0.20		
H-13	−0.04	+0.04	−0.06	+0.06	−0.04	+0.04	−0.07	+0.07	+0.08	−0.08	−0.07	+0.07	+0.09	−0.09		
H-15	−0.03	+0.03	−0.04	+0.04	−0.03	+0.03	−0.02	+0.02	+0.04	−0.04	−0.14	+0.14	+0.17	−0.17		
H-16	−0.01	+0.01	−0.01	+0.01	−0.01	+0.01	−0.01	+0.01	+0.01	−0.01	−0.02	+0.02	+0.06	−0.06		

[a] Measured in $CDCl_3$.
[b] Measured in CD_3OD.

The enantiomers of xestospongienes X (**8e**) and Y (**8f**) were separated by chiral HPLC (AD-H). Analysis of 2D NMR spectroscopic data revealed that they are the geometrical isomers of **8a** and **8b**, respectively. The $J_{H\text{-}7/H\text{-}8}$ (11.0 Hz) value was consistent with 7*Z* geometry. The chiral carbon C-10 in **8e** was assigned to (10*S*) configuration in contrast to (10*R*) of **8f**, based on the ^{1}H NMR data of their Mosher esters. The coupling constant $J_{H\text{-}9/H\text{-}10}$ (3.5 Hz) of both **8e**/**8f** was indicative of an *erythro* configuration. Accordingly, the chiral center C-9 was supposed to be (*R*) for **8e** and (*S*) for **8f**.

The molecular formula of xestospongiene Z (**9**) was determined to be $C_{18}H_{24}Br_2O_5$ by HRESIMS (*m*/*z* 500.9892 $[M+Na]^+$, calcd 500.9883), indicating 6° of unsaturation. Comparison of NMR spectroscopic data revealed that the structure of **9** was partially related to that of **8** in respect of C-1 to C-6 and C-11 to C-16. The NMR spectra showed three olefinic protons H-16 (δ_H 6.43, d, *J*=7.5 Hz), H-15 (δ_H 6.74, d, *J*=7.5 Hz), and H-13 (δ_H 6.12, dd, *J*=7.4, 7.5 Hz); two acetylenic carbons at δ_C 86.3 and 78.2; five methylenes; and a methylate. COSY correlations unambiguously established the alkyl moieties from C-2 to C-4 and from C-7 to C-13 in the molecule, while COSY and HMQC data ascertained four oxygenated carbons from C-7 to C-10. The HMBC correlations from H-7 to C-10 (δ_C 80.4) and from H-10 to C-7 (δ_C 73.8) led to the assignment of a tetrahydrofuran ring between C-7 and C-10. The HMBC correlation from the methoxy protons at δ_H 3.47 (3H, s) to C-9 (δ_C 84.2) confirmed the linkage of a methoxy group at C-9. Additional HMBC correlation from the other methoxy protons at δ_H 3.71 (3H, s) to a carbonyl carbon at δ_C 174.0 indicated a terminus to be methylated. Thus, C-8 was substituted by a hydroxy group. Irradiation of H-7 leading to NOE enhancement of H-10 suggested H-7 and H-10 being oriented in same face. Irradiation of OMe at C-9 enhanced the integration of H-10, indicating that same orientations of OMe-9 and H-10. In addition, irradiation of H-9 led to the NOE enhancement of H-8, suggesting that H-8 and H-9 were oriented in the same face.

The enantiomers of xestospongienes Z_1 (**10a**) and Z_2 (**10b**) were determined to be the methyl esters of C_{16} fatty acid containing 7,14,16-Br-8-OH-13,15-diene-5-yn, as determined by 2D NMR analysis and comparison with the NMR data of relative analogues.[30] They were well separated by chiral HPLC chromatography. The *Z*-geometries of the double bonds at C-13/C-14 and C-15/C-16 were confirmed by NOESY cross-peaks. The absolute configuration at C-8 of **10a** was deduced to be (*S*) based on the $\Delta\delta_H$ ($\delta_S-\delta_R$) values of (*S*)- and (*R*)-MPTA esters, while C-8 of **10b** was assigned (*R*) configuration using the same method. The coupling constant $J_{H\text{-}7/H\text{-}8}$ (8.5 Hz) of both compounds led to the assignment of 7,8-*threo* configuration. Thus, the configuration of C-7 was assumed to be (*S*) for **10a** and (*R*) for **10b**.

The enantiomers of xestospongienes Z_3–Z_4 (**10c**,**d**) were well separated by chiral HPLC chromatography. The NMR data revealed that they shared the same gross structure as **10a** but differed due to the presence of 7,8*serythro* configuration instead of 7,8-*threo* configuration based on the coupling constant $J_{H\text{-}7/H\text{-}8}$ (3.5 Hz). Calculation of the $\Delta\delta_H$ ($\delta_S-\delta_R$) values of the (*S*)- and (*R*)-MPTA esters led to the assignment of (8*R*) to **10c** and (8*S*) to **10d**, respectively. Thus, the chiral center of C-7 in **10c** was in agreement with (*S*) in contrast to (*R*) of **10d**.

Xestospongienes Z_5–Z_8 (**11a**–**d**) were separated as optically pure compounds by the same protocol as used for **10** and were determined to be the stereoisomers representing two pairs of enantiomers. The 1D and 2D NMR data indicated that **11a** is a methyl ester of a C_{16} unsaturated fatty acid containing four double bonds. COSY cross-peaks established the residues from C-2 to C-5, C-7 to C-13, and C-15 to C-16, The connection of each moiety was determined by HMBC relationships. A terminal methyl ester was deduced by the HMBC correlation between C-1 (δ_C 174.2) and methoxy protons at δ_H 3.68 (3H, s). HMBC correlations from H-5 (δ_H 4.16, t, *J*=5.9 Hz) to C-6 (δ_C 131.5, qC) and C-7 (δ_C 127.4) and from H-16 (δ_H 6.57) to C-14 (δ_C 113.7, qC), together with its NMR data comparing with those of above analogues, led to the positioning of bromine atoms at C-6, C-14, and C-16, respectively. Additional COSY and HMBC correlations enabled the location of double bonds at C-6/C-7, C-8/C-9, C-13/C-14, and C-15/C-16. Thus, C-5 (δ_C 75.4) and C-10 (δ_C 70.9) were hydroxylated. The coupling constants $J_{H\text{-}8/H\text{-}9}$ (15.2 Hz) and $J_{H\text{-}15/H\text{-}16}$ (8.0 Hz), along with the NOESY correlations between H-5/H-7, H-7/H-9, H-13/H-15, and H-15/H-16 were indicative of 6*Z*, 8*E*, 13*Z*, and 15*Z* geometries. The absolute configurations of C-5 and C-10 were determined to be (5*R*) and (10*S*) based on the calculations of $\Delta\delta_H$ ($\delta_S-\delta_R$) values of its Mosher esters.

By employing Mosher's method and using the same protocol as for **11a**, the stereogenic centers of **11b** were determined to be in (5*S*) and (10*R*) configurations, whereas those of **11c** were in (5*R*) and (10*R*) configurations. These results were in contrast to (5*S*) and (10*S*) of **11d**.

The 1D and 2D NMR spectroscopic data in association with MS data indicated that xestospongienes Z_9–Z_{10} (**12a**,**b**) were a pair of stereoisomers that were structurally related to **11**. The difference was found by the presence of a ketone group in **12a** and **12**b, which was confirmed to be positioned at C-10 based on the COSY and HMBC correlations. The geometries of the double bonds in **12a** and **12b** were the same as those of **11** due to the similar *J* values of olefinic protons and NOE relationships. The difference between **12a** and **12b** was due to the configuration of C-5. The absolute configuration of C-5 in **12a** was determined to be (*R*) based on Mosher's method. Therefore, the absolute configuration of C-5 in **12b** was in accordance with (*S*), which was further confirmed by the ^{1}H NMR data of its Mosher esters.

The molecular formula of xestospongiene Z_{11} (**13**) was determined to be $C_{17}H_{18}Br_2O_5$, by HRESIMS (*m*/*z* 482.9413 $[M+Na]^+$, calcd 482.9413), indicating 8° of unsaturation. Analysis of 2D NMR data (COSY and HMBC) and *J* values of olefinic protons revealed that **13** contained a moiety of 14, 16-dibromodiene, corresponding to that of **12**. The presence of a furan ring was evident from the ^{13}C NMR data at δ_C 117.1 (2×CH), 153.1, and 153.3, along with HMBC correlations. ^{1}H–^{1}H COSY cross-peaks established the alkyl chains from H-2 to H-4 and from H-11 to H-13, while a terminal methyl ester was assigned by HMBC correlations. The HMBC correlations observed from H-3 and H-4 to a ketone at δ_C 189.2 and from H-3 to C-2 (δ_C 32.9) and C-1 (δ_C 175.0) indicated the location of a ketone at C-5. Moreover, the HMBC correlations from the furan protons H-7 and H-8 to δ_C 188.6 determined the second ketone group to be positioned at C-10. The connection of each subunit was realized by HMBC relationships. The 13*Z* and 15*Z* configurations of **13** were based on the NOE relationships between H-13/H-15 and H-15/H-16 and their coupling constants.

The HRESIMS data of xestospongiene Z_{12} (**14**) showed the pseudomolecular ions at *m*/*z* 270.9576, 272.9555 (1:1) $[M+Na]^+$, which corresponded to a formula of $C_8H_9BrO_4$ (calcd 270.9576) with 4° of unsaturation. IR absorptions at 1775, 1741, and 1605 cm^{-1} suggested the presence of olefinic and carbonyl functionalities. The APT and HMQC spectra assigned to eight carbons in the molecule and their corresponding protons, including two methylenes (δ_C 27.5, 28.5), one methine (δ_C 84.0), two olefinic carbons (δ_C 122.5, 150.1), two carbonyl carbons (δ_C 172.6, 169.8), and a methoxy group (δ_C 52.0). The ^{1}H–^{1}H COSY correlations between H-4 (δ_H 5.11, ddd, *J*=1.5, 1.6, 9.0 Hz)/H_2-3 (δ_H 1.89) and H_2-3/H_2-2 (δ_H 2.50, t, *J*=7.0 Hz) established a subunit from C-2 to C-4, while a long range COSY correlation between H-4 and H-6 (δ_H 6.36, d, *J*=1.6 Hz) was also observed. The HMBC relationships from H-6 to C-7 (δ_C 169.8) and C-4 (δ_C 84.0), and in turn, from H-4 to C-6 (δ_C 122.5) and C-7, ascertained an α,β-unsaturated-γ-lactone. A methyl ester at C-1 was evidenced by the HMBC correlation from MeO (δ_H 3.71), H_2-2, and H_2-3 to the carbonyl carbon at δ_C 172.6. Accordingly, the quaternary carbon C-5 was assumed to be positioned near a bromine atom. The absolute configuration of the stereogenic center C-4 was assigned on the basis of the sign of the positive Cotton effect of the $n\rightarrow\pi^*$ (252 nm) transition and the negative CE of the $\pi\rightarrow\pi^*$ (219 nm) transition, which correlated with (4*R*) configuration.[31]

Xestospongiene Z_{13} (**15**) had a molecular formula of $C_{11}H_{14}O_4$ as determined by HRESIMS (*m*/*z* 233.0783 $[M+Na]^+$, calcd 233.0784), implying 5° of unsaturation. The APT spectrum exhibited 11 carbon resonances, including two carbonyl carbons (δ_C 166.5, 173.4), two olefinic carbons (δ_C 129.3, 126.0), two methoxy carbons (δ_C 51.7, 51.8), three methylene carbons (δ_C 19.2, 23.5, 32.8), and two acetylenic carbons (δ_C 77.4, 99.2). The HMQC spectrum helped in assigning all the protons and their associated carbons. COSY correlations led to the establishment of a subunit from C-2 to C-4, while H-4 (δ_H 2.49, m) showed weak correlation to the olefinic proton H-7 (δ_H 6.77, td, *J*=2.9, 16.0 Hz). The HMBC interactions from H-8 (δ_H 6.20, d, *J*=16.0 Hz), H-7, and methoxy protons δ_H 3.77 (3H, s) to a carbonyl carbon at δ_C 166.5, and from H-7 and H-4 to two acetylenic carbons, together with the correlations of H-2, H-3, and the methoxy protons δ_H 3.71 (3H, s) to the second carbonyl carbon at δ_C 173.4, allowed the assignment of an unsaturated C_9 alkyl chain which contained a C-5/C-6 acetylenic bond and two terminal methyl esters. The *E* geometry of the double bond C-7/C-8 was deduced by $J_{H\text{-}7/H\text{-}8}$ (16.0 Hz) value.

Based on the NMR and MS spectroscopic analyses and comparison with the data presented in literature,[3,30] compounds **16a** and **16b** were identified as the methyl esters of xestospongic acid and its 17*Z* isomer, respectively, which were previously isolated from the marine sponge *Petrosia volcano* Hoshino.[3]

The isolated brominated polyunsaturated fatty acids could be classified into two subtypes, i.e., the terminal dibrominated pattern (**1**–**7**) and 14,16-dibromo-13,15-diene pattern (**8**–**13**). Brominated and unsaturated fatty acids are a group of unusual metabolites that may be used for chemotaxonomic purposes of sponge genera *Xestospongia* and *Petrosia* (Nepheliospongidae). The South China Sea is a new location of sponge *X. testudinaria*, which produces abundant brominated lipids that mainly contain C_{14}, C_{16}, and C_{18} chain-lengths. The similar brominated fatty acids obtained from the same specimen inhabiting in different locations suggest that these compounds may be of sponge origin rather than from associated algae or microorganisms. The naturally occurring enantiomers and diastereoisomers of the brominated fatty acids as described in this paper have rarely been reported from other sponges or from the same sponge inhabiting in other locations. Most brominated unsaturated fatty acids tend to be unstable, and succumb to oxidative, photolytic, or pH-dependent decomposition. The absence of methyl ester and ethoxy signals in the ^{1}H NMR spectrum of EtOH extract from the sponge suggested that the products with methyl esters or ethoxy groups may be the artifacts derived from separation process. Thus, the natural origins of **5** and **7**–**15** are assumed to be the carboxylic acids. However, methoxy substitution at the alkyl chain was suggested to originate naturally, and this was partly supported by the facts that **1** could not be converted to **2** but partly converted to methyl esters in acetic acid-containing MeOH after one week. The chiral-phase HPLC spectrum of crude extract informed that the enantiomers may be the metabolites originated from sponge.

The brominated polyunsaturated fatty acids are recognized to originate from precursors PKs or fatty acids, which follow the steps of primary and secondary metabolism.[32] During primary metabolism, saturated fatty acids are synthesized by multifunctional fatty acid synthase complexes. The vinyl and acetylenic groups are formed by the action of desaturases and acetylenases, while oxidation and bromination are generated by an enzyme, such as bromoperoxidase in the presence of hydrogen peroxide.[32]

In this paper, a semi-preparative HPLC protocol was established to separate diastereomers using RP-8 column, and enantiomers by chiral HPLC on AD-H column. All compounds showed weak cytotoxicity against the tumor cell lines HL-60 (human promyelocytic leukemia), BGC-823 (human gastric cancer), Bel-7402 (human hepatic carcinoma), and KB (human oral epithelium carcinoma).

3. Experimental

3.1. General experimental procedures

Optical rotations were measured using an Autopol III automatic polarimeter (Rudolph Research Co.). IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrometer. NMR spectra were measured on a Bruker Avance-500 FT 500 MHz NMR spectrometer using TMS as the internal standard. HRESIMS spectra were obtained on a PEQ-STAR ESI-TOF-MS/MS spectrometer, and EIMS spectra were recorded on a Bruker APEX II mass spectrometer. CD spectra were measured on a JASCO J-810 spectropolarimeter. Column chromatography was performed on Merck silica gel (200–300 mesh). The HF_{254} silica gel for TLC was provided by Sigma Co. Ltd. Sephadex LH-20 (18–110 μm) was obtained from Pharmacia Co., and ODS (50 μm) was provided by YMC Co. High performance liquid chromatography (HPLC) was performed on an Alltech 426 apparatus with a 3300-ELSD UV detector. A Kromasil prepacked column (ODS, 10 ×250 mm, for reversed-phase) and a chiral column (AD-H, 4.6 ×250 mm) were used in the HPLC separation.

3.2. Marine sponge

Sponge *X. testudinaria* was collected from the inner coral reef at a depth of around 10 m in Northern Bay (Weizhou Island), Guangxi Province, P.R. China, in June 2007, and the sample was immediately frozen after collection. The specimen was identified by de Voogd (National Museum of Natural History, The Netherlands). A voucher specimen (GW11) was deposited at State Key Laboratory of Natural and Biomimetic Drugs, Peking University.

3.3. Extraction and isolation

The frozen sponge (1.0 kg) was homogenized and extracted with EtOH. The concentrated extract was desalted by dissolving in MeOH to obtain a residue (70.0 g) that was further partitioned between H_2O and EtOAc. The EtOAc fraction (10.0 g) was subjected to VLC using a 200–300 mesh Si gel and eluted with a gradient of petroleum ether/acetone to collect the fraction (3.0 g, 5:1–1:1) that mainly contained brominated fatty acids as detected by ^{1}H NMR and LC–MS spectra. This fraction was subsequently applied to an ODS column and eluted with MeOH/H_2O (75%) to obtain five subfractions (SF-1 to SF-5). SF-1 (120.0 mg) was subjected to semi-preparative HPLC (RP-18 column, 67% MeOH in H_2O as the mobile phase) to yield **6a** (2.7 mg) and **6b** (2.7 mg), along with portions of SF-1–1 (22.0 mg), SF-1–2 (4.0 mg), SF-1–3 (4.5 mg), SF-1–4 (18.0 mg), and SF-1–5 (18.0 mg). SF-1–1 was separated by semi-preparative HPLC (RP-8 column, 57% ACN in H_2O as a mobile phase) to obtain the mixtures of **1c**/**1d** (10 mg) and **1a**/**1b** (10.0 mg). Compounds **1a** (5.0 mg) and **1b** (5.0 mg) were separated by chiral HPLC (AD-H column, 88% hexane in isopropanol as a mobile phase). A mixture containing **1c** and **1d**, which could not be separated by chiral HPLC, was converted to the (*R*)- and (*S*)-MTPA esters using (*S*)-MTPACl and (*R*)-MTPACl reagents, and then separated by HPLC (RP-8 column, 80% ACN in H_2O as a mobile phase) to obtain (*R*)-MTPA esters of **1c** (2.5 mg) and **1d** (2.5 mg), (*S*)-MTPA esters of **1c** (3.0 mg) and **1d** (2.0 mg). SF-1–4 (18.0 mg) and SF-1–5 (18.0 mg) were separated by HPLC (AD-H column) using a mobile phase of 82% hexane in isopropanol to obtain **11a** (8.0 mg), **11b** (8.0 mg), **11c** (8.0 mg), and **11d** (8.0 mg), respectively. SF-2 (100.0 mg) was separated by semi-preparative HPLC (RP-18 column, 72% MeOH in H_2O as a mobile phase) to obtain **7** (1.9 mg), together with portions of SF-2–1 (4.0 mg), SF-2–2 (28.0 mg), and SF-2–3 (14.0 mg). Semi-preparative HPLC (RP-8 column, 62% ACN in H_2O as the mobile phase) separation of SF-2–2 (38 mg) yielded two portions, of which portion A contained the enantiomers of **3a**/**3b** and portion B contained **3c**/**3d**. The isomers of **10a**/**10b**, **10c**/**10d**, and **12a**/**12b** were collected from SF-2–3, respectively.

HPLC (AD-H column, 90% hexane in isopropanol as a mobile phase) separation of portions A and B yielded **3a** (10.2 mg), **3b** (10.2 mg), **3c** (2.2 mg), and **3d** (2.2 mg). Compound **10a** (3.1 mg), **10b** (3.1 mg), **10c** (3.1 mg), **10d** (3.1 mg), **12a** (1.7 mg), and **12b** (1.7 mg) were separated by the same protocol as for **3a**–**d**. SF-3 (80.0 mg) was separated by semi-preparative HPLC (RP-18 column, 78% MeOH in H_2O as a mobile phase) to yield **9** (1.4 mg), together with the portions of SF-3–1 (19.0 mg), SF-3–2 (6.0 mg), SF-3–3 (5.0 mg), and SF-3–4 (13.0 mg). Semi-preparative HPLC on the RP-8 column (65% ACN in H_2O) followed by the AD-H column (90% hexane in isopropanol) resulted in the isolation of **8a** (5.4 mg) and **8b** (5.4 mg), **8c** (2.1 mg) and **8d** (2.1 mg) from SF-3–1, **8e** (2.4 mg) and **8f** (2.4 mg) from SF-3–2, and **5a** (2.0 mg) and **5b** (2.0 mg) from SF-3–4. SF-3–3 was separated by semi-preparative HPLC (RP-8 column, 70% ACN in H_2O as the mobile phase) followed by the AD-H column (90% hexane in isopropanol) yielded **2a** (2.7 mg) and **2b** (2.7 mg), **2c** (2.7 mg), and **2d** (2.7 mg). SF-4 (70.0 mg) was separated by semi-preparative HPLC on an RP-8 column (65% ACN in H_2O) followed by an AD-H column (90% hexane in isopropanol) yielded **4a** (5.6 mg), **4b** (7.3 mg), **13** (1.5 mg), **14** (1.0 mg), **15** (1.2 mg), **16a** (15.9 mg), and **16b** (15.9 mg), respectively.

*3.3.1. Xestospongiene A (**1a**).* Colorless oil; $[\alpha]_D^{20}$ −21.5 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3399, 2926, 2857, 1772, 1634, 1456, 1380, 1170, 1071 cm^{-1}; CD (MeOH) λ 211 nm ($\Delta\theta$ +6.00); ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{14}H_{20}Br_2O_3NH_4$ $[M+NH_4]^+$ 412.0117; found 412.0122, 414.0100, 416.0081 (1:2:1); HRMS (ESI): calcd for $C_{14}H_{20}Br_2O_3Na^+$ $[M+Na]^+$ 416.9676; found 416.9876, 418.9655, 420.9635 (1:2:1).

*3.3.2. Xestospongiene B (**1b**).* Colorless oil; $[\alpha]_D^{20}$ +20.0 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3399, 2926, 2857, 1772, 1634, 1456, 1380, 1170, 1071 cm^{-1}; CD (MeOH) λ 215 nm ($\Delta\theta$ −9.30); ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{14}H_{20}Br_2O_3NH_4$ $[M+NH_4]^+$ 412.0117; found 412.01222, 414.01002, 416.0081 (1:2:1).

*3.3.3. Xestospongienes C (**1c**) and D (**1d**).* Colorless oil; IR (KBr) ν_{max} 3399, 2926, 2857, 1772, 1634, 1456, 1380, 1170, 1071 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{14}H_{20}Br_2O_3NH_4$ $[M+NH_4]^+$ 412.0117; found 412.0116, 414.0096, 416.0072 (1:2:1).

*3.3.4. Xestospongiene E (**2a**).* Colorless oil; $[\alpha]_D^{20}$ +45.6 (*c* 0.4, MeOH); IR (KBr) ν_{max} 2926, 1779, 1622, 1455, 1374, 1165, 1093 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{15}H_{22}Br_2O_3NH4$ $[M+NH_4]^+$ 426.0274; found 426.0278, 428.0257, 430.0237 (1:2:1).

*3.3.5. Xestospongiene F (**2b**).* Colorless oil; $[\alpha]_D^{20}$ −44.3 (*c* 0.5, MeOH); IR (KBr) ν_{max} 2926, 1779, 1622, 1455, 1374, 1165, 1093 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{15}H_{22}Br_2O_3Na$ $[M+Na]^+$ 430.9828; found 430.9832, 432.9811, 434.9791 (1:2:1).

*3.3.6. Xestospongiene G (**2c**).* Colorless oil; $[\alpha]_D^{20}$ −10.5 (*c* 0.5, MeOH); IR (KBr) ν_{max} 2926, 1774, 1625, 1455, 1374, 1165, 1093 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{15}H_{22}Br_2O_3NH_4$ $[M+NH_4]^+$ 426.0274; found 426.0278, 428.0258, 430.0236 (1:2:1).

*3.3.7. Xestospongienes H (**2d**).* Colorless oil; $[\alpha]_D^{20}$ +11.0 (*c* 0.5, MeOH); IR (KBr) ν_{max} 2926, 1774, 1625, 1455, 1374, 1165 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{15}H_{22}Br_2O_3Na$ $[M+Na]^+$ 430.9828; found 430.9832, 432.9812, 434.9790 (1:2:1).

*3.3.8. Xestospongiene I (**3a**).* Colorless oil; $[\alpha]_D^{20}$ −5.2 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3420, 2925, 2854, 1771, 1670, 1626, 1458, 1363, 1188 cm^{-1}; CD (MeOH) λ 205 nm ($\Delta\theta$ −0.06), 212 nm ($\Delta\theta$ +0.16), 225 ($\Delta\theta$ +0.10); ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{14}H_{20}Br_2O_3NH_4$ $[M+NH_4]^+$ 412.0117; found 412.0118, 414.0096, 416.0076 (1:2:1).

*3.3.9. Xestospongiene J (**3b**).* Colorless oil; $[\alpha]_D^{20}$ +6.0 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3421, 2926, 2853, 1770, 1671, 1626, 1457, 1362, 1187, 1025 cm^{-1}; CD (MeOH) λ 208 nm ($\Delta\theta$ +0.25), 212 nm ($\Delta\theta$ −0.03), 220 ($\Delta\theta$ −0.05); ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{14}H_{20}Br_2O_3NH_4$ $[M+NH_4]^+$ 412.0117; found 412.0118, 414.0096, 416.0076 (1:2:1).

*3.3.10. Xestospongiene K (**3c**).* Colorless oil; $[\alpha]_D^{20}$ −17.6 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3420, 2925, 2854, 1771, 1670, 1626, 1458, 1363, 1188, 1026 cm^{-1}; CD (MeOH) λ 208 nm ($\Delta\theta$ +4.03), 223 nm ($\Delta\theta$ −1.47); ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{14}H_{20}Br_2O_3NH_4$ $[M+NH_4]^+$ 412.0117; found 412.0118, 414.0099, 416.0084 (1:2:1).

*3.3.11. Xestospongiene L (**3d**).* Colorless oil; $[\alpha]_D^{20}$ +16.6 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3421, 2924, 2854, 1771, 1670, 1626, 1458, 1363, 1188 cm^{-1}; CD (MeOH) λ 212 nm ($\Delta\theta$ −8.51), 226 nm ($\Delta\theta$ +2.04); ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{14}H_{20}Br_2O_3NH_4$ $[M+NH_4]^+$ 412.0117; found 412.0118, 414.0099, 416.0084 (1:2:1).

*3.3.12. Xestospongiene M (**4a**).* Colorless oil; $[\alpha]_D^{20}$ −7.7 (*c* 0.5, MeOH); IR (KBr) ν_{max} 2926, 2856, 1779, 1738, 1602, 1440, 1381, 1167, 1108, cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{15}H_{22}Br_2O_3NH_4$ $[M+NH_4]^+$ 426.0274; found 426.0275, 428.0250, 430.0232 (1:2:1).

*3.3.13. Xestospongienes N (**4b**).* Colorless oil; $[\alpha]_D^{20}$ +7.5 (*c* 0.5, MeOH); IR (KBr) ν_{max} 2926, 2856, 1779, 1738, 1602, 1440, 1381, 1167, 1108, 1067 cm^{-1}; ^1H and ^{13}C NMR data, sees Tables 1 and 2; HRMS (ESI): calcd for $C_{15}H_{23}Br_2O_3$ $[M+H]$+409.0084; found 409.0002, 410.9982, 412.9954 (1:2:1).

*3.3.14. Xestospongiene O (**5a**).* Colorless oil; $[\alpha]_D^{20}$+2.7 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3399, 2928, 2861, 1737, 1587, 1456, 1382, 1260, 1164 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{16}H_{26}Br_2O_4Na$ $[M+Na]^+$ 463.0090; found 463.0098, 465.0082, 467.0076 (1:2:1).

*3.3.15. Xestospongiene P (**5b**).* Colorless oil; $[\alpha]_D^{20}$ −3.0 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3398, 2928, 2861, 1737, 1587, 1456, 1382, 1260, 1164 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{16}H_{26}Br_2O_4Na$ $[M+Na]^+$ 463.0090; found 463.0098, 465.0082, 467.0076 (1:2:1).

*3.3.16. Xestospongiene Q (**6a**).* Colorless oil; $[\alpha]_D^{20}$ −5.5 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3412, 2931, 2859, 1712, 1562, 1410, 1162 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{14}H_{22}Br_2O_4Na$ $[M+Na]^+$ 434.9777; found 434.9775, 436.9742, 438.9720 (1:2:1).

*3.3.17. Xestospongiene R (**6b**).* Colorless oil; $[\alpha]_D^{20}$ −8.1 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3356, 2930, 2858, 1713, 1560, 1384, 1090 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{14}H_{22}Br_2O_4Na$ $[M+Na]^+$ 434.9777; found 434.9781, 436.9761, 438.9741 (1:2:1).

*3.3.18. Xestospongiene S (**7**).* Colorless oil; $[\alpha]_D^{20}$ −4.1 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3432, 2931, 2858, 1736, 1624, 1440, 1380, 1202, 1165 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRMS (ESI): calcd for $C_{15}H_{24}Br_2O_4Na$ $[M+Na]^+$ 448.9934; found 448.9929, 450.9910, 452.9891 (1:2:1).

*3.3.19. Xestospongiene T (**8a**).* Colorless oil; $[\alpha]_D^{20}$ −6.7 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3378, 2924, 2866, 2367, 1738, 1632, 1456,

1377, 1162 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{18}H_{24}Br_2O_4NH_4$ $[M+NH_4]^+$ 480.0380; found 480.0381, 482.0362, 484.0340 (1:2:1).

*3.3.20. Xestospongiene U (**8b**).* Colorless oil; $[\alpha]_D^{20}$ +5.4 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3378, 2924, 2866, 2367, 1738, 1632, 1456, 1377, 1162 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{18}H_{24}Br_2O_4Na$ $[M+Na]^+$ 484.9934; found 484.9935, 486.9916, 488.9894 (1:2:1).

*3.3.21. Xestospongiene V (**8c**).* Colorless oil; $[\alpha]_D^{20}$ −5.6 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3416, 3355, 2927, 2367, 1736, 1668, 1450, 1372, 1160, 1086 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{18}H_{24}Br_2O_4NH_4$ $[M+NH_4]^+$ 480.0380; found 480.0386, 482.0364, 484.0345 (1:2:1).

*3.3.22. Xestospongiene W (**8d**).* Colorless oil; $[\alpha]_D^{20}$ +4.8 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3416, 3355, 2927, 2367, 1736, 1668, 1450, 1372, 1160, 1086 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{18}H_{24}Br_2O_4Na$ $[M+Na]^+$ 484.9934; found 484.9939, 486.9918, 488.9898 (1:2:1).

*3.3.23. Xestospongiene X (**8e**).* Colorless oil; $[\alpha]_D^{20}$ −50.8 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3422, 2958, 2921, 2367, 1733, 1625, 1457, 1377, 1164, 1066 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{18}H_{24}Br_2O_4NH_4$ $[M+NH_4]^+$ 480.0380; found 480.0385, 482.0365, 484.0344 (1:2:1).

*3.3.24. Xestospongiene Y (**8f**).* Colorless oil; $[\alpha]_D^{20}$ +49.3 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3422, 2958, 2921, 2367, 1733, 1625, 1457, 1377, 1164, 1066 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{18}H_{24}Br_2O_4Na$ $[M+Na]^+$ 484.9934; found 484.9940, 486.9919, 488.9897 (1:2:1).

*3.3.25. Xestospongiene Z (**9**).* Colorless oil; $[\alpha]_D^{20}$ −2.5 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3331, 2928, 2855, 2241, 1736, 1646, 1450, 1375, 1217, 808 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{18}H_{24}Br_2O_5Na$ $[M+Na]^+$ 500.9883; found 500.9892, 502.9871, 504.9851 (1:2:1).

*3.3.26. Xestospongiene Z$_1$ (**10a**).* Colorless oil; $[\alpha]_D^{20}$ −12.4 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3446, 2928, 2857, 2233, 1734, 1634, 1438, 1373, 1319, 1163, 1061 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{17}H_{23}Br_3O_3NH_4$ $[M+NH_4]^+$ 529.9536; found 529.9544, 531.9522, 533.9503, 535.9486 (1:3:3:1).

*3.3.27. Xestospongiene Z$_2$ (**10b**).* Colorless oil; $[\alpha]_D^{20}$ +11.7 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3446, 2928, 2857, 2233, 1734, 1634, 1438, 1373, 1319, 1163 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{17}H_{23}Br_3O_3Na$ $[M+Na]^+$ 534.9090; found 534.9097, 536.9076, 538.9056, 540.9034 (1:3:3:1).

*3.3.28. Xestospongiene Z$_3$ (**10c**).* Colorless oil; $[\alpha]_D^{20}$ −3.0 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3422, 2938, 2857, 2232, 1735, 1647, 1594, 1436, 1373, 1318, 1224, 1165 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{17}H_{23}Br_3O_3NH_4$ $[M+NH_4]^+$ 529.9536; found 529.9543, 531.9523, 533.9502, 535.9482 (1:3:3:1).

*3.3.29. Xestospongiene Z$_4$ (**10d**).* Colorless oil; $[\alpha]_D^{20}$ +3.2 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3422, 2938, 2857, 2232, 1735, 1647, 1594, 1436, 1373, 1318, 1224, 1165 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{17}H_{23}Br_3O_3Na$ $[M+Na]^+$ 534.9090; found 534.9100, 536.9077, 538.9056, 540.9033 (1:3:3:1).

*3.3.30. Xestospongiene Z$_5$ (**11a**).* Colorless oil. $[\alpha]_D^{20}$ −12.8 (*c* 0.7, MeOH); IR (KBr) ν_{max} 3376, 2924, 1732, 1621, 1449, 1380, 1161, 1073 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{17}H_{23}Br_3O_4Na$ $[M+Na]^+$ 550.9039; found 550.9032, 552.9013, 554.8999, 556.8973 (1:3:3:1).

*3.3.31. Xestospongiene Z$_6$ (**11b**).* Colorless oil. $[\alpha]_D^{20}$ +11.3 (*c* 0.46, MeOH); IR (KBr) ν_{max} 3376, 2924, 1732, 1621, 1449, 1380, 1161, 1073 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{17}H_{23}Br_3O_4NH_4$ $[M+NH_4]^+$ 545.9485; found 545.9484, 547.9463, 549.9441, 551.9420 (1:3:3:1).

*3.3.32. Xestospongiene Z$_7$ (**11c**).* Colorless oil. $[\alpha]_D^{20}$ −36.4 (*c* 0.38, MeOH); IR (KBr) ν_{max} 3376, 2924, 1732, 1621, 1449, 1380, 1161, 1073 cm^{-1}. ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{17}H_{23}Br_3O_4NH_4$ $[M+NH_4]^+$ 545.9485; found 545.9493, 547.9473, 549.9453, 551.9434 (1:3:3:1).

*3.3.33. Xestospongiene Z$_8$ (**11d**).* Colorless oil. $[\alpha]_D^{20}$ +37.7 (*c* 0.42, MeOH); IR (KBr) ν_{max} 3376, 2923, 1732, 1621, 1447, 1380, 1162, 1088 cm^{-1}. ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{17}H_{23}Br_3O_4Na$ $[M+Na]^+$ 550.9039; found 550.9047, 552.9027, 554.9007, 556.8986 (1:3:3:1).

*3.3.34. Xestospongiene Z$_9$ (**12a**).* Colorless oil. $[\alpha]_D^{20}$ −21.3 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3342, 2923, 2217, 1734, 1440, 1371, 1164, 1060, 895 cm^{-1}. ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{17}H_{22}Br_3O_4$ $[M+H]^+$ 526.9063; found 526.9064, 528.9047, 530.9026, 528.9007 (1:3:3:1).

*3.3.35. Xestospongiene Z$_{10}$ (**12b**).* Colorless oil. $[\alpha]_D^{20}$ +20.0 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3342, 2923, 2217, 1734, 1440, 1371, 1164, 1060, 895 cm^{-1}. ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS (ESI): calcd for $C_{17}H_{21}Br_3O_4NH_4$ $[M+NH_4]^+$ 543.9328; found 543.9337, 545.9315, 547.9295, 549.9274 (1:3:3:1).

*3.3.36. Xestospongiene Z$_{11}$ (**13**).* Colorless oil. IR (KBr) ν_{max} 2954, 2921, 1737, 1685, 1564, 1378, 1205, 1030, 815 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ_H 2.46 (2H, t, *J*=6.8 Hz, H-2), 2.10 (2H, tt, *J*=6.8, 7.1 Hz, H-3), 3.02 (2H, t, *J*=7.1 Hz, H-4), 7.25 (1H, d, *J*=3.4 Hz, H-7), 7.25 (1H, d, *J*=3.4 Hz, H-8), 3.10 (2H, t, *J*=7.2 Hz, H-11), 2.50 (2H, td, *J*=7.2, 7.6 Hz, H-12), 6.15 (1H, t, *J*=7.6 Hz, H-13), 6.77 (1H, d, *J*=7.7 Hz, H-15), 6.47 (1H, d, *J*=7.7 Hz, H-16), 3.72 (3H, s, OMe). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 175.0 (s, C-1), 32.9 (t, C-2), 18.8 (t, C-3), 37.7 (t, C-4), 189.2 (s, C-5), 153.1 (s, C-6), 117.1 (d, C-7), 117.1 (d, C-8), 153.3 (s, C-9), 188.6 (s, C-10), 37.2 (t, C-11), 25.1 (t, C-12), 134.2 (d, C-13), 115.1 (s, C-14), 130.9 (d, C-15), 112.9 (d, C-16), 51.7 (q, OMe); HRMS (ESI): calcd for $C_{17}H_{18}Br_2O_5Na$ $[M+Na]^+$ 482.9413; found 482.9413, 484.9400, 486.9388 (1:2:1).

*3.3.37. Xestospongiene Z$_{12}$ (**14**).* Colorless oil. $[\alpha]_D^{20}$ −4.0 (*c* 0.50, MeOH); IR (KBr) ν_{max} 2921, 2853, 1775, 1741, 1605, 1442, 1257, 1158, 943 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ_H 2.50 (2H, t, *J*=7.0 Hz, H-2), 1.89 (2H, m, H-3), 5.11 (1H, ddd, *J*=1.5, 1.6, 9.0 Hz, H-4), 6.36 (1H, d, *J*=1.6 Hz, H-6), 3.71 (3H, s, OMe). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 172.6 (s, C-1), 28.5 (t, C-2), 27.5 (t, C-3), 84.0 (d, C-4), 150.1 (s, C-5), 122.5 (d, C-6), 169.8 (s, C-7), 52.0 (q, OMe); HRMS (ESI): calcd for $C_8H_9{}^{79}BrO_4Na$ $[M+Na]^+$ 270.9576; found 270.9576, 272.9555 (1:1).

*3.3.38. Xestospongiene Z$_{13}$ (**15**).* Colorless oil. IR (KBr) ν_{max} 2956, 2924, 1611, 1453, 1380, 1116, 817 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ_H 6.20 (1H, d, *J*=16.0 Hz, H-8), 6.77 (1H, dt, *J*=2.9, 16.0 Hz, H-7), 2.49 (2H, m, H-4), 1.93 (2H, m, H-3), 2.49 (2H, m, H-2), 3.77 (3H, s, OMe-9), and 3.71 (3H, s, OMe-1). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 166.5 (s, C-9), 129.3 (d, C-8), 126.0 (d, C-7), 77.4 (s, C-6), 99.2 (s, C-5), 19.2 (t, C-4), 23.5 (t, C-3), 32.8 (t, C-2), 173.4 (s, C-1), 51.7 (q, OMe-9),

51.8 (q, OMe-1); HRMS (ESI): calcd for $C_{11}H_{14}O_4Na$ $[M+Na]^+$ 233.0784; found 233.0783.

3.4. Preparation of the Mosher esters

Ten microliters of (*S*)-(+)-MTPA chloride were added to a stirred solution of 1.0 mg of the hydroxy compound in 0.3 mL dry pyridine. The mixture was stirred under N_2 at room temperature for 1 h, and the solvent was subsequently removed by blowing with N_2. The residue was separated by HPLC (RP-8) to obtain the (*R*)-(+)-MTPA ester.

As described for the *R* esters, 0.3 mg of the compound and 10 μL of (*R*) (−)-MTPA chloride were used to obtain the (*S*)-MTPA esters.

3.4.1. (S)-MTPA ester of ***1c****.* Colorless oil; $[\alpha]_D^{20}$ −11.0 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 2.57 (2H, dd, *J*=8.5, 9.0 Hz, H-2), 2.00 (1H, m, H-3a), 2.46 (1H, m, H-3b), 4.99 (1H, m, H-4), 5.84 (1H, dd, *J*=6.0, 16.0 Hz, H-5), 5.84 (1H, dd, *J*=6.5, 16.0 Hz, H-6), 5.51 (1H, m, H-7), 1.72 (1H, m, H-8a), 1.65 (1H, m, H-8b), 1.24 (2H, m, H-9), 1.38 (2H, m, H-10),1.38 (2H, m, H-11), 2.08 (2H, m, H-12), 6.40 (1H, t, *J*=7.3 Hz, H-13), 7.43 (3H, m), 7.52 (2H, m).

3.4.2. (S)-MTPA-ester of ***1d****.* Colorless oil; $[\alpha]_D^{20}$ +8.4 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 2.55 (2H, t, *J*=9.0 Hz, H-2), 1.96 (1H, m, H-3a), 2.43 (1H, m, H-3b), 4.94 (1H, m, H-4), 5.73 (1H, dd, *J*=6.0, 16.0 Hz, H-5), 5.72 (1H, dd, *J*=6.5, 16.0 Hz, H-6), 5.50 (1H, m, H-7), 1.77 (1H, m, H-8a), 1.76 (1H, m, H-8b), 1.34 (2H, m, H-9), 1.50 (2H, m, H-10), 1.34 (2H, m, H-11), 2.12 (2H, m, H-12), 6.40 (1H, t, *J*=7.3 Hz, H-13), 7.43 (3H, m), 7.53 (2H, m).

3.5. Methylation

A solution of **1b** (1.0 mg, 2.5 μmol) in Et_2O (2.0 mL) was added to MeI (3.1 mg, 21.8 μmoL), Drierite® (0.2 mg), and freshly prepared Ag_2O (0.3 mg). After 20 h of vigorous stirring at 20 °C, the reaction mixture was filtered and concentrated, and the residue was purified by silica gel chromatography (petroleum ether/acetone=4:1). This procedure yielded the methyl ether (1.1 mg), which was identical to **2a**. Compounds **2b**–**d** and **4a**,**b** were prepared by the same method as for **2a**.

Acknowledgements

This project was supported by grants from the National Hi-Tech Development Project (863 project) (No. 2006AA09Z446 and 2006DFA31100), NSFC (No.30930109), National Key Innovation Project (2009ZX09501-014), and the International Cooperation Projects of BMBF-MOST (2+2).

Supplementary data

IR, MS, CD, ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra for xestospongienes A–Z, and Z_1–Z_{13}. Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2010.11.045.

References and notes

1. Barrow, R. A.; Capon, R. J. *Aust. J. Chem.* **1994**, *47*, 1901–1918.
2. Hirsh, S.; Carmely, S.; Kashman, Y. *Tetrahedron* **1987**, *14*, 3257–3261.
3. Bourguet-Kondracki, M. L.; Rakotoarisoa, M. T.; Martin, M. T.; Guyot, M. *Tetrahedron Lett.* **1992**, *33*, 225–226.
4. Fusetani, N.; Li, H.; Tamura, K.; Matsuhaga, S. *Tetrahedron* **1993**, *49*, 1203–1210.
5. Patil, A. D.; Kokke, W. C.; Cochran, S.; Francis, T. A.; Tomszek, T.; Westley, J. W. *J. Nat. Prod.* **1992**, *55*, 1170–1177.
6. Lerch, M. L.; Harper, M. K.; Faulkner, D. J. *J. Nat. Prod.* **2003**, *66*, 667–670.
7. Li, Y.; Ishibashi, M.; Sasaki, T.; Kabayashi, J. *J. Chem. Res.* **1995**, 901–921.
8. Morinaka, B. I.; Skepper, C. K.; Molinski, T. F. *Org. Lett.* **2007**, *9*, 1975–1978.
9. Pham, N. B.; Butler, M. S.; Hooper, J. N. A.; Moni, R. W.; Quinn, R. J. *J. Nat. Prod.* **1999**, *62*, 1439–1442.
10. Brantley, S. E.; Molinski, T. F.; Preston, C. M.; DeLong, E. F. *Tetrahedron* **1995**, *51*, 7667–7672.
11. Li, Y.; Ishibashi, M.; Sasaki, T.; Kobayashi, J. *J. Chem. Res.* **1995**, *4*, 126–127.
12. Quinn, R. J.; Tucker, D. J. *Tetrahedron Lett.* **1985**, *26*, 1671–1672.
13. Kobayashi, M.; Chen, Y.; Aoki, S.; Yasuko, I.; Ishida, T.; Kitagawa, I. *Tetrahedron* **1995**, *51*, 3727–3736.
14. Calcul, L.; Longeon, A.; Mourabit, A. A.; Guyota, M.; Bourguet-Kondracki, M. L. *Tetrahedron* **2003**, *59*, 6539–6544.
15. Rodriguez, J.; Peters, B. M.; Kurz, L.; Schatzman, R. C.; McCarley, D.; Lou, L.; Crews, P. *J. Am. Chem. Soc.* **1993**, *115*, 10436–10437.
16. Gulavita, N. K.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 366–369.
17. Laurent, D.; Jullian, V.; Parenty, A.; Knibiehler, M.; Dorin, D.; Schmitt, S.; Lozach, O.; Lebouvier, N.; Frostin, M.; Alby, F.; Maurel, S.; Doerig, C.; Meijer, L.; Sauvain, M. *Bioorg. Med. Chem.* **2006**, *14*, 4477–4482.
18. Roll, D. M.; Scheuer, P. J. *J. Am. Chem. Soc.* **1983**, *105*, 6178–6179.
19. Coval, S. J.; Conover, M. A.; Mierzwa, R.; King, A.; Puar, M. S.; Phife, D. W.; Pai, J.; Burrier, R. E.; Ahn, H.; Boykow, G. C.; Patel, M.; Pomponi, S. A. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 605–610.
20. Northcote, P. T.; Andersen, R. J. *J. Nat. Prod.* **1987**, *50*, 1174–1177.
21. Gauvin, A.; Smadja, J.; Aknin, M.; Gaydou, E. M. *Biochem. Syst. Ecol.* **2004**, *32*, 469–476.
22. Quinn, R. J.; Tucker, D. J. *J. Nat. Prod.* **1991**, *54*, 290–294.
23. Keffer, J. L.; Plaza, A.; Bewley, C. A. *Org. Lett.* **2009**, *11*, 1087–1090.
24. Seco, J. M.; Martino, M.; Quinoa, E.; Riguera, R. *Org. Lett.* **2000**, *2*, 3261–3264.
25. Ren, J.; Jiang, J.; Li, L.; Liao, T.; Tian, R.; Chen, X.; Jiang, S.; Pittman, C. U., Jr.; Zhu, H. *Eur. J. Org. Chem.* **2009**, 3987–3991.
26. Goerigk, L.; Grimme, S. *J. Phys. Chem. A* **2009**, *113*, 767–776.
27. Nishida, Y.; Ohrui, H.; Meguro, H.; Mori, K. *Agric. Biol. Chem.* **1986**, *50*, 813–818.
28. Tsuda, M.; Toriyabe, Y.; Endo, T.; Kobayashi, J. *Chem. Pharm. Bull.* **2003**, *51*, 448–451.
29. Laurent, F.; Sbastien, R.; Patrice, C.; Janine, C. *Org. Lett.* **2007**, *9*, 2461–2464.
30. Okuda, T.; Harigara, S.; Kiyomoto, A. *Chem. Pharm. Bull.* **1964**, *12*, 504–506.
31. Wu, A.; Cremer, D.; Auer, A. A.; Gauss, J. *J. Phys. Chem. A* **2002**, *106*, 657–667.
32. Piazza, G. J.; Nuñez, A.; Foglia, T. A. *Lipids* **2003**, *38*, 255–261.