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Obscuraminols, new unsaturated amino alcohols from the tunicate *Pseudodistoma obscurum*: structure and absolute configuration

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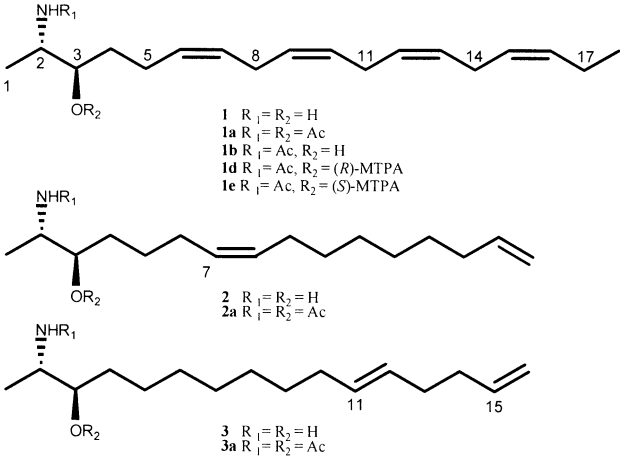
Abstract—The study of the ascidian *Pseudodistoma obscurum* from Tarifa Island (Cádiz, Spain) has led to the characterization of six new unsaturated 2-amino-3-ol compounds, the obscuraminols A-F. Five of them, the obscuraminols B–F, were isolated as their corresponding diacetyl derivatives. Their structures were established by spectroscopic analysis, their relative configurations by NOEDS study of oxazolidinone derivatives, and their absolute configurations by application of Mosher's method to *N*-acetyl derivatives.

Ascidians from the genus *Pseudodistoma* have led to the isolation of two different kinds of cytotoxic alkaloids. The first group is represented by amino alcohols either cyclic as the piperidine alkaloids isolated from *P. kanoko*[1–3] and *P. megalarva*,[4] or linear as those isolated from a species of *Pseudodistoma* collected from the South African coast[5] and the crucigasterins from *P. crucigaster*.[6] This group of compounds also includes the C-14 triene amines isolated from *P. novaezelandiae*.[7] The second group of metabolites isolated from this genus is tryptophan-derived compounds as the arborescidins and eudistomin V isolated from *P. arborescens*[8] and *P. aureum*,[9] respectively.

As a part of our project directed towards the search for pharmacologically active compounds from marine ascidians of the southern coast of Spain we have examined specimens of the tunicate *Pseudodistoma obscurum* (Pérès, 1959). Specimens of *P. obscurum* were collected by hand using SCUBA from Tarifa Island (Cádiz, Spain) in May 1996 and immediately frozen. The frozen material was lyophilized and extracted with methanol following the Kupchan method.[10] The $CHCl_3$ extract resulted to be the more cytotoxic against the tumor cell lines of mouse lymphoma P-388, human lung carcinoma A-549 and human colon carcinoma HT-29 (IC_{50}=0.5, 0.2, and 0.5 μg/mL, respectively). Column chromatography of a portion of the $CHCl_3$ extract and bioassay-guided isolation allowed us to obtain the more active fraction against the mentioned tumor cell lines. Further purification of this fraction using different chromatographic conditions led to the isolation of obscuraminol A (**1**) and a complex mixture of minor compounds which could not be separated. ^{1}H NMR spectrum of both the mixture and obscuraminol A (**1**) revealed enough evidences for the presence of hydrochloride salts providing the clue for the difficulty of separating them. Furthermore, addition of silver nitrate to the mixture and obscuraminol A (**1**) produced, in both cases, an immediate precipitate indicative of hydrochloride salts. Mild acetylation of the remaining portion of the $CHCl_3$ extract yielded a crude that was separable by HPLC into seven compounds: six new *N*-acetyl-*O*-acetylaminoalcohols (**1a**–**6a**) and the diacetyl derivative **7a** of the known compound xestoaminol C (**7**).[11]



Keywords: marine metabolites; biologically active compounds; amino alcohols.

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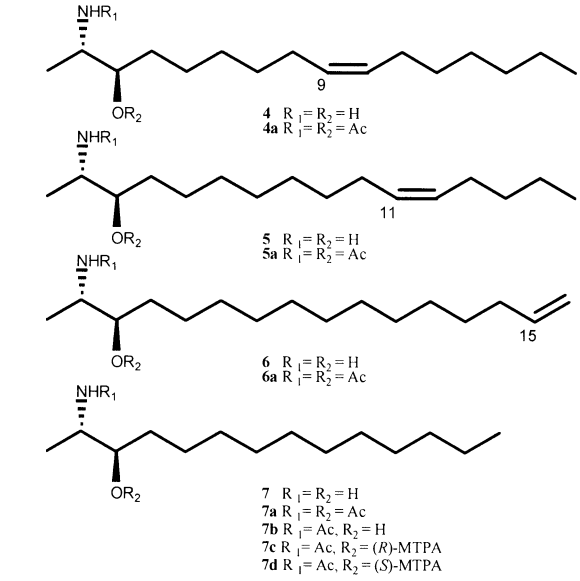
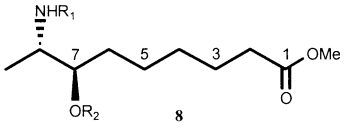
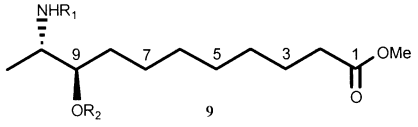
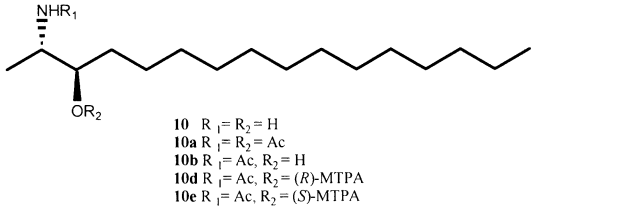
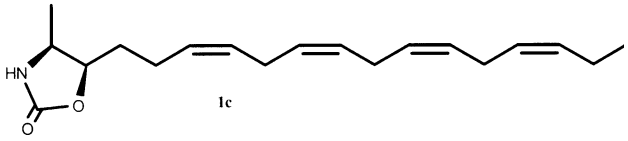
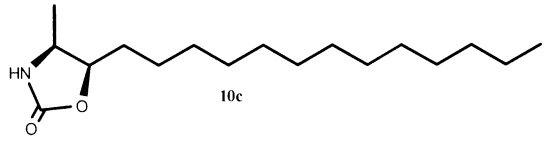












Obscuraminol A (**1**) was isolated as an optically active colorless oil. The molecular formula, $C_{18}H_{31}NO{\cdot}HCl$, was obtained from the HRCI mass measurement and required four degrees of unsaturation for this hydrochloride. A signal on the ^1H NMR spectrum at δ 5.34 (8H, m) assigned to eight olefinic protons was correlated in the COSY spectrum to the signals at δ 2.79 (6H, m), 2.22 (2H, m) and 2.05 (2H, m) indicating a sequence of four methylene interrupted double bonds. The signal at δ 2.05 was additionally coupled with a triplet at δ 0.96 (3H, t, J=7.5 Hz) indicating that the double bonds system bore an ethyl terminus. The ^{13}C NMR signals at δ 25.6 (t, 2C) and 25.5 (t) assigned to three *bis*-allylic methylene carbons indicated the *cis* geometry for all methylene interrupted double bonds.[12] On the other hand, the allylic protons signal at δ 2.22 was additionally coupled to the methylene proton signals at δ 1.45 (1H, m) and 1.36 (1H, m). The remaining signals of the ^1H NMR spectrum included a doublet at δ 1.28 (3H, d, J=6.8 Hz) which was correlated in the COSY spectrum with a signal at δ 3.41 (1H, m) assigned to the proton of a methine bearing nitrogen. This proton signal at δ 3.41 was additionally coupled to a signal at δ 3.97 (1H, m) due to a methine proton geminal to an hydroxyl group indicating the presence of a 2-amino-3-hydroxy unit which gave rise to the ^{13}C NMR signals at δ 70.0 (d, C-3), 51.9 (d, C-2) and 12.0 (q, C-1). All these spectral evidences defined the structure of (6*Z*,9*Z*,12*Z*,15*Z*)-2-aminooctadeca-6,9,12,15-tetraen-3-ol for obscuraminol A (**1**). Furthermore, obscuraminol A (**1**) was converted into its diacetyl derivative **1a** by treatment with Ac_2O in pyridine to confirm the proposed structure for the natural compound. Thus, the ^1H NMR of **1a** contained two singlets at δ 2.09 and 1.94 integrating for three protons each, assigned to the methyls of the two acetyl groups whereas the H-2 and H-3 signals were downfield shifted in **1a** to δ 4.16 (1H, dqd, J=8.5, 6.9 and 3.2 Hz) and δ 4.84 (1H, ddd, J=9.3, 4.3 and 3.2 Hz) (Table 1). These spectral features fully confirmed the presence of the 2-amino-3-hydroxy functionalities in the structure of obscuraminol A (**1**).

The relative configuration of obscuraminol A (**1**) was defined by a series of NOEDS experiments in the oxazolidinone **1c** that was formed by treatment of **1** with 1,1′-carbonyldiimidazole. Irradiation of H-2 signal caused enhancements of the H-3 (8.2%) and Me-1 (3.0%) signals indicating a configuration in oxazolidinone **1c** that requires an *erythro* configuration of C-2 and C-3 in obscuraminol A (**1**).

The absolute configuration of obscuraminol A (**1**) was determined by application of Mosher's method[13] to the *N*-acetyl derivative of obscuraminol A (**1b**) obtained by conventional acidic methanolysis at room temperature of diacetyl obscuraminol A (**1a**). The (*R*)- and (*S*)-MTPA esters **1d** and **1e** were obtained by treatment of **1b** with (*R*)- and (*S*)-MTPA acids, respectively. Positive $\Delta\delta$ ($\delta_S-\delta_R$) values were found for protons H-4, H-5, H-8 and H-11 while negative $\Delta\delta$ values were found for Me-1, H-2 and acetamido group protons (Fig. 1). Following the MTPA rules these data indicated and *R* configuration for C-3 and therefore an absolute configuration 2*S*,3*R* for obscuraminol A (**1**).

Diacetyl obscuraminol B (**2a**) had the molecular formula $C_{20}H_{35}NO_3$ as indicated by HRCIMS. The two signals at δ 2.09 (3H, s) and 1.94 (3H, s) together with those at δ 4.83 (1H, ddd, J=8.5, 4.9 and 3.1 Hz) and 4.15 (1H, dqd, J=8.5, 6.7 and 3.1 Hz) and the presence in a DEPT spectrum of three methine and one methylene signals in the olefinic region, indicated that **2a** was the diacetyl derivative of a 2-aminohexadecadien-3-ol. A methylene carbon signal at δ 114.5 (t) (Table 2) was correlated in the HMQC spectrum with the olefinic proton signals at δ 4.95 (1H, brd, J=10.1 Hz) and 5.02 (1H, ddt, J=17.2, 3.5 and 1.5 Hz). These signals were coupled to an olefinic proton signal at δ 5.82 (1H, m) which was additionally coupled to an allylic methylene signal at δ 2.10 (2H, m) indicating a terminal

Table 1. [1] HMR data recorded in $CDCl_3$ for the diacetyl derivatives of metabolites of *Pseudodistoma obscurum* (assignments were aided by COSY, LR COSY and HMQC experiments)

	1a	**2a**[a]	**3a**[a]	**4a**	**5a**	**6a**
1	1.09 (d, 7.0)	1.09 (d, 6.8)	1.08 (d, 6.8)	1.08 (d, 7.2)	1.08 (d, 7.2)	1.09 (d, 6.8)
2	4.16 (dqd, 8.5, 6.9, 3.2)	4.15 (dqd, 8.5, 6.7, 3.1)	4.15 (dqd, 8.5, 6.8, 3.1)	4.14 (dqd, 8.5, 6.9, 3.1)	4.14 (dqd, 8.5, 6.9, 3.1)	4.16 (dqd, 8.5, 6.8, 3.1)
3	4.84 (ddd, 9.3, 4.3, 3.2)	4.83 (ddd, 8.5, 4.9, 3.1)	4.82 (ddd, 8.8, 4.9, 3.1)	4.83 (ddd, 8.4, 4.9, 3.1)	4.83 (ddd, 8.4, 4.9, 3.1)	4.83 (ddd, 8.8, 4.9, 3.1)
4	1.66 (m)	1.56 (m)	1.55 (m)	1.56 (m)	1.56 (m)	1.56 (m)
	1.56 (m)	1.49 (m)	1.49 (m)	1.48 (m)	1.48 (m)	1.50 (m)
5	2.09 (m)	1.27 (m)	1.30 (m)	1.29 (m)	1.29 (m)	1.25 (m)
6	5.34 (m)	2.10 (m)	1.30 (m)	1.29 (m)	1.29 (m)	1.25 (m)
7	5.34 (m)	5.36 (m)	1.30 (m)	1.29 (m)	1.29 (m)	1.25 (m)
8	2.82 (m)[b]	5.36 (m)	1.30 (m)	2.01 (m)[b]	1.29 (m)	1.25 (m)
9	5.34 (m)	2.01 (m)	1.30 (m)	5.33 (m)	1.29 (m)	1.25 (m)
10	5.34 (m)	1.27 (m)	1.96 (m)	5.33 (m)	2.01 (m)[b]	1.25 (m)
11	2.82 (m)[b]	1.27 (m)	5.37 (m)[b]	1.99 (m)[b]	5.33 (m)	1.25 (m)
12	5.34 (m)	1.27 (m)	5.34 (m)[b]	1.29 (m)	5.33 (m)	1.25 (m)
13	5.34 (m)	1.27 (m)	2.04 (m)	1.29 (m)	1.99 (m)[b]	1.25 (m)
14	2.79 (m)[b]	2.10 (m)	2.08 (m)	1.29 (m)	1.29 (m)	2.02 (m)
15	5.34 (m)	5.82 (m)	5.81 (m)	1.29 (m)	1.29 (m)	5.80 (m)
16	5.34 (m)	5.02 (ddt, 17.2, 3.5, 1.5)	5.00 (ddt, 17.0, 1.9, 1.6)	0.88 (t, 6.9)	0.89 (t, 6.9)	4.99 (ddt, 17.1, 2.2, 1.7)
		4.95 (brd, 10.1)	4.94 (ddt, 10.2, 2.1, 1.6)			4.92 (ddt, 10.1, 2.2, 1.3)
17	2.05 (m)					
18	0.97 (t, 7.5)					
NH	5.86 (brd, 8.0)	5.82 (m)	5.84 (brd, 8.1)	5.82 (brd, 7.7)	5.82 (brd, 7.7)	5.81 (m)
MeCONH-	1.94 (s)	1.94 (s)	1.94 (s)	1.94 (s)	1.94 (s)	1.94 (s)
MeCOO-	2.09 (s)	2.09 (s)	2.08 (s)	2.09 (s)	2.09 (s)	2.09 (s)

[a] Assignments were aided by HMBC experiment.
[b] Values with the same superscript in the same column may be interchanged.

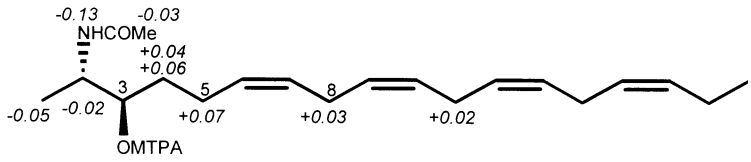


Figure 1. Chemical shifts differences (Δδ) between the MTPA derivatives of **1b**.

Table 2. ^{13}C NMR data recorded in $CDCl_3$ for the diacetyl derivatives of metabolites of *Pseudodistoma obscurum* (assignments were aided by HMQC experiments). Assignments[a] were aided by HMBC experiment. Values[b–d] with the same superscript in the same column may be interchanged

	1a	**2a**[a]	**3a**[a]	**4a**	**5a**	**6a**
1	14.9 (q)	14.7 (q)	14.7 (q)	14.7 (q)	14.7 (q)	14.7 (q)
2	47.6 (d)	47.5 (d)	47.5 (d)	47.5 (d)	47.5 (d)	47.6 (d)
3	76.3 (d)	76.9 (d)	76.9 (d)	76.8 (d)	76.8 (d)	77.0 (d)
4	31.1 (t)	31.2 (t)	31.2 (t)	31.2 (t)	31.2 (t)	31.2 (t)
5	23.3 (t)	25.6 (t)	25.5 (t)	25.5 (t)	25.5 (t)	25.6 (t)
6	127.8 (d)	26.6 (t)	29.5 (t)[b]	29.7 (t)[b]	29.7 (t)[b]	29.6 (t)[b]
7	128.9 (d)	130.3 (d)[b]	29.3 (t)[b]	29.5 (t)[b]	29.5 (t)[b]	29.6 (t)[b]
8	25.5 (t)[b]	128.9 (d)[b]	29.3 (t)[b]	27.2 (t)[c]	29.1 (t)[b]	29.5 (t)[b]
9	128.6 (d)[c]	27.2 (t)	29.0 (t)[b]	130.1 (d)[d]	28.9 (t)[b]	29.5 (t)[b]
10	128.4 (d)[c]	29.6 (t)[c]	32.5 (t)	129.5 (d)[d]	27.1 (t)[c]	29.4 (t)[b]
11	25.5 (t)[b]	29.3 (t)[c]	130.8 (d)[c]	27.0 (t)[c]	129.9 (d)[d]	29.3 (t)[b]
12	128.3 (d)	29.3 (t)[c]	129.4 (d)[c]	29.3 (t)[b]	129.7 (d)[d]	29.1 (t)[b]
13	128.0 (d)	29.1 (t)[c]	32.0 (t)	29.0 (t)[b]	27.0 (t)[c]	28.9 (t)[b]
14	25.5 (t)[b]	33.8 (t)	33.8 (t)	31.7 (t)	31.9 (t)	33.8 (t)
15	126.9 (d)	138.5 (d)	138.5 (d)	22.6 (t)	22.3 (t)	139.3 (d)
16	132.0 (d)	114.5 (t)	114.4 (t)	14.1 (q)	14.0 (q)	114.1 (t)
17	20.5 (t)					
18	14.2 (q)					
***Me*CONH-**	23.4 (q)	23.5 (q)	23.5 (q)	23.4 (q)	23.4 (q)	23.5 (q)
***Me*COO-**	21.0 (q)	21.1 (q)	21.1 (q)	21.1 (q)	21.1 (q)	21.1 (q)
Me*CO*NH-	169.3 (s)	169.2 (s)	169.3 (s)	169.3 (s)	169.3 (s)	169.3 (s)
Me*COO*-	171.5 (s)	171.5 (s)	171.5 (s)	171.5 (s)	171.5 (s)	171.6 (s)

double bond in the structure of **2a**. The presence of a *cis*-disubstituted olefin was indicated by a signal in the ^1H NMR at δ 5.36 (2H, m) which was coupled to two methylene proton signals at δ 2.01 (2H, m) and 2.10 (2H, m) which were, in turn, correlated in the HMQC experiment with the carbon signals at δ 27.2 (t) and 26.6 (t), respectively. The location of this disubstituted double bond was established by a careful study of the COSY and HMBC spectra. The allylic methylene signal at δ 2.10 (2H, m) showed a long range correlation in the COSY spectrum with the proton signals at δ 1.49 (1H, m) and 1.56 (1H, m) assigned to the C-4 protons. Furthermore, these protons were additionally coupled to a methylene signal at δ 1.27 (2H, m) whose carbon signal at δ 25.6 (t) exhibited a three bond correlation in the HMBC spectrum with the olefinic proton signal at δ 5.36. These data defined the presence of a Δ^7 double bond and therefore a structure of (7*Z*)-2-acetamido-3-acetoxyhexadeca-7,15-diene for the diacetyl derivative of obscuraminol B (**2a**).

Compound **3a** was another component isolated from the acetylation mixture of the natural material. The molecular formula $C_{20}H_{35}NO_3$, obtained by HRCIMS, indicated that **3a** was an isomer of **2a** previously discussed. The ^{13}C NMR spectrum of **3a** exhibited the signals at δ 138.5 (d), 130.8 (d), 129.4 (d) and 114.4 (t) assigned to a disubstituted and a terminal double bond. The location of the disubstituted double bond at Δ^{11} was established upon observation in the COSY spectrum of the correlation between two allylic methylene proton signals at δ 2.08 (2H, m, H-14) and 2.04 (2H, m, H-13). The *trans* configuration of this double bond was defined by the downfield resonances at δ 32.0 (t, C-13) and 32.5 (t, C-10) for the allylic carbon signals. Thus, the structure of (11*E*)-2-acetamido-3-acetoxyhexadeca-11,15-diene was proposed for the diacetyl derivative of obscuraminol C (**3a**).

Diacetyl obscuraminol D (**4a**) was isolated from the acetylation mixture as a colorless oil. The analysis of the spectroscopic data and the molecular formula $C_{20}H_{37}NO_3$, deduced from its HRCIMS, clearly indicated that **4a** was the diacetyl derivative of a monounsaturated C_{16} member of the obscuraminol family. The ^{13}C NMR signals at δ 27.2 (t) and 27.0 (t) assigned to two allylic methylene carbons, indicated the *cis* geometry for the double bond. The location of the double bond was established by identification of the methyl ester **8** obtained by treatment of **4a** with periodic acid and a catalytic amount of ruthenium (III) chloride hydrate[14] and further methylation with $TMSCHN_2$. Both the NMR and HRMS data of the methyl ester **8** allowed us to characterize it as the methyl 8-acetamido-7-acetoxynonanoate (**8**) and thus to define a Δ^9 double bond in **4a**. This chemical degradation result led us to propose the structure (9*Z*)-2-acetamido-3-acetoxyhexadec-9-ene for the diacetyl derivative of obscuraminol D (**4a**).

Diacetyl obscuraminol E (**5a**) was obtained as a mixture with the above discussed diacetyl obscuraminol D (**4a**) since no chromatographic conditions which could separate this mixture of compounds **4a/5a** were found. Four signals

of allylic methylene carbons in the ^{13}C NMR spectrum at δ 27.2 (t), 27.1 (t), 27.0 (t, 2C) indicated that both components shared the *Z* geometry in one double bond. Furthermore, the common spectral features together with the molecular ion and the fragmentations observed in the low resolution MS indicated that **5a** and **4a** were isomers which differed in the location of the double bond. In order to determine the location of the double bond in **5a** the same methodology as described above was used on the mixture of diacetyl obscuraminol D (**4a**) and E (**5a**). Treatment of the **4a**/**5a** mixture with periodic acid and a catalytic amount of ruthenium (III) chloride hydrate[14] and further methylation yielded a mixture of two methyl esters, **8** and **9**, that were separated using HPLC. Spectroscopic analysis of these methyl esters using both NMR and HRMS allowed us to identify **9** as the methyl 10-acetamido-9-acetoxyundecanoate. This result indicated the structure of (11*Z*)-2-acetamido-3-acetoxyhexadec-11-ene for the diacetyl derivative of obscuraminol E (**5a**).

Obscuraminol F (**6**) was isolated as its diacetyl derivative (**6a**) from the acetylation mixture as an amorphous solid. The molecular formula of **6a**, $C_{20}H_{37}NO_3$, obtained from the HRMS, indicated that it was an isomer of the compounds **4a** and **5a**. However, the ^{13}C NMR signals at δ 139.3 (d) and 114.1 (t) evidenced the presence of a monosubstituted double bond which gave rise to the ^{1}H NMR signals at δ 5.80 (1H, m, H-15), 4.99 (1H, ddt, *J*=17.1, 2.2 and 1.7 Hz, H-16a) and 4.92 (1H, ddt, *J*=10.1, 2.2 and 1.3 Hz, H-16b). These HRMS and NMR evidences led to propose the structure of 2-acetamido-3-acetoxyhexadec-15-ene for the diacetyl derivative of obscuraminol F (**6a**).

The study of the relative configuration of obscuraminols B–F (**2**–**6**) implied the transformation of the diacetyl derivatives **2a**–**6a** into the compound **10a** by hydrogenation of each compound **2a**–**6a** with $Pd(OH)_2/C$ under H_2 atmosphere. Each of the hydrogenated products obtained exhibited identical spectroscopic data and the same optical rotation value, indicating that they shared an identical structure and the same configuration at C-2 and C-3 centers. Treatment of **10**, obtained by acidic methanolysis of the hydrogenation compound **10a** at 90°C, with 1,1′-carbonyldiimidazole led to oxazolidinone **10c** which allowed unambiguous assignment of the relative configuration at C-2 and C-3 by NOEDS. Irradiation of H-2 signal caused enhancements on H-3 (5.0%) and Me-1 (2.0%) signals indicating a configuration in **10c** consistent with an *erythro* configuration at C-2 and C-3 in the obscuraminols B–F (**2**–**6**). Furthermore, their absolute configurations were elucidated by application of Mosher's method[13] to the *N*-acetyl derivative **10b** obtained by acidic methanolysis at room temperature of the hydrogenation compound **10a**. The (*R*)- and (*S*)-MTPA esters **10d** and **10e** were obtained by treatment of **10b** with (*R*)- and (*S*)-MTPA acids, respectively. Positive

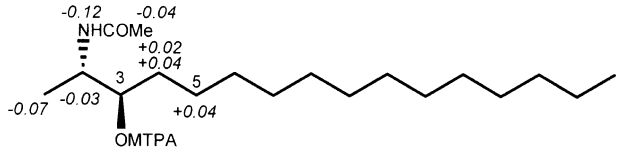
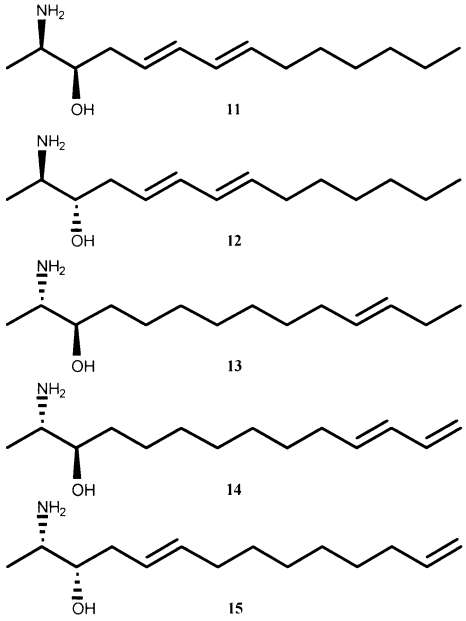


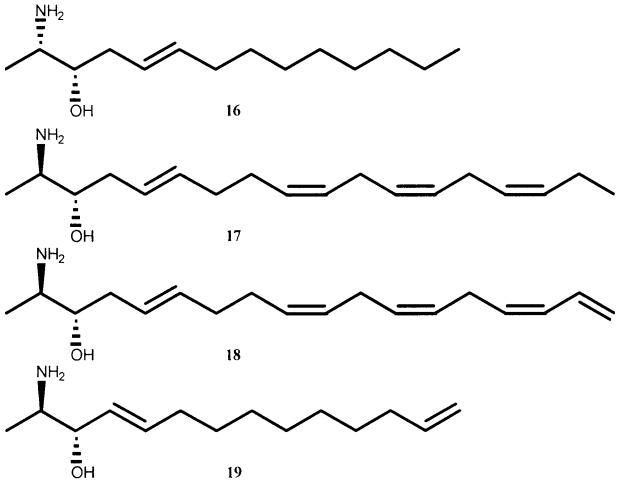
Figure 2. Chemical shifts differences (Δδ) between the MTPA derivatives of **10b**.

Δδ ($\delta_S-\delta_R$) values were found for H-4 and H-5 while negative Δδ values were found for Me-1, H-2 and acetamido group protons (Fig. 2). Following the MTPA rules these data indicated and *R* configuration for C-3 and therefore an absolute configuration 2*S*,3*R* for obscuraminols B–F (**2**–**6**).

A survey among marine natural products literature reveals that the configuration of 2-amino-3-ol compounds is controversial. These amino alcohols comprise compounds **11** and **12** described by Gulavita and Scheuer[15] and the xestoaminols A–C (**13**, **14**, **7**) isolated by Jiménez and Crews[11] from two different specimens of sponges belonging to the *Xestospongia* genus. In addition, ascidians from *Pseudodistoma* genus have also been prolific in the production of 2-amino-3-ol compounds yielding the metabolites **15**–**19**.[5,6] The absolute configuration of the compounds **11**,**12** was initially assigned as 2*S*,3*R* by chemical degradation to alanine that was identified as the L- isomer by HPLC analysis. However, it was later proposed an opposite absolute configuration of **11**,**12** based on total synthesis of both enantiomers of **11** and **12**[16] since the synthetic 2*R*-isomers exhibited the same sign of optical rotation than the natural compounds. It was also suggested that this result implied an opposite absolute configuration for xestoaminols A–C (**13**, **14**, **7**) whose absolute configuration had been proposed by comparison with **11** and **12**. Finally, the absolute configuration of compounds **17**–**19** was assigned by chemical degradation to (3*S*,4*R*)-3-hydroxy-4-aminopentanoic acid.

The minor component of the acetylation mixture was characterized by means of its spectroscopic data as the 2-acetamido-3-acetoxytetradecane (**7a**). Acidic methanolysis of **7a** led to a compound identical in all respects, including the optical rotation to xestoaminol C (**7**) and therefore compound **7a** was the diacetyl xestoaminol C. Because of the controversy existing about the absolute configuration of this group of compounds, we decided to determine the absolute configuration of xestoaminol C (**7**) by application





of Mosher's method[13] to the *N*-acetyl derivative of xestoaminol C (**7b**) as we had applied similarly to the obscuraminols. Compound **7b**, obtained by acidic methanolysis at room temperature of diacetyl xestoaminol C (**7a**), was treated with (*R*)- and (*S*)-MTPA acids to give (*R*)- and (*S*)-MTPA esters **7c** and **7d**, respectively. Positive $\Delta\delta$ ($\delta_S-\delta_R$) values were found for protons H-4 and H-5 while negative $\Delta\delta$ values were found for Me-1, H-2 and acetamido group (Fig. 3). Following the MTPA rules these data indicated an *R* configuration for C-3. These data unambiguously established an absolute configuration 2*S*,3*R* on the stereogenic centers C-2 and C-3 in **7b**, and thus on xestoaminol C (**7**).

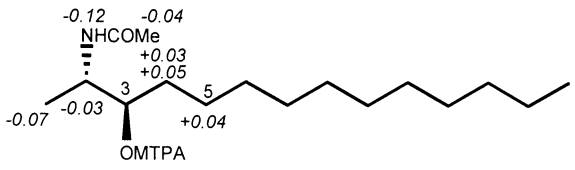


Figure 3. Chemical shifts differences ($\Delta\delta$) between the MTPA derivatives of **7b**.

As it has been proposed by Gulavita and Scheuer,[15] the absolute configuration determined for the 2-amino-3-ol components present in *P. obscurum* requires L-alanine and the corresponding fatty acids as precursors involved in the biogenesis of these compounds **1**–**7**.

The obscuraminols A–F (**1**–**6**) were active enough to be isolated using cytotoxicity guided fractionation. However, only obscuraminol A (**1**) could be tested in its natural form, being the remaining compounds tested as their diacetyl derivatives (**2a**–**6a**). Nonetheless, they are only mildly active compounds since no activity higher than 1 μg/mL was encountered either for obscuraminol A (**1**) or the acetyl derivatives **2a**–**6a** against the tumor cell lines of mouse lymphoma P-388, human lung carcinoma A-549 and human colon carcinoma HT-29.

1. Experimental

1.1. General

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Genesis Series FT IR Mattson spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on a Varian Unity 400 spectrometer using $CDCl_3$ or C_6D_6 as solvents. Proton chemical shifts were referenced to the residual $CHCl_3$ or benzene signals at δ 7.26 and 7.15 respectively. ^{13}C NMR spectra were referenced to the central peak of $CDCl_3$ or C_6D_6 at δ 77.0 and 128.0 respectively. ^{1}H-^{1}H-COSY, LR COSY, HMQC and HMBC (9.0 Hz) were performed using standard VARIAN pulse sequences. Assignments marked with the same superscript may be interchanged. Low resolution mass spectra were recorded on a Finnigan Voyager GC8000^{top} spectrometer. High resolution chemical ionization mass spectra were recorded on a VG Autospec spectrometer. Column chromatography was carried out using Merck Silica gel 60 (70–230 mesh). HPLC separations were performed on a LaChrom-Hitachi apparatus equipped with LiChrosorb RP-18 (Merck) and LiChrosorb Si 60 (Merck) columns using a differential refractometer RI-71. All solvents were spectral grade or were distilled from glass prior to use.

1.2. Collection, extraction, and isolation procedure

The tunicate *Pseudodistoma obscurum* (67.23 g dry weight) was collected by hand using SCUBA at Tarifa Island in 1996 and kept frozen until its extraction. The frozen material was lyophilized and extracted with methanol. The water content (% v/v) of the methanol extract was adjusted before sequentially partitioning against n-hexane (10% H_2O), CCl_4 (20% H_2O), $CHCl_3$ (40% H_2O) and n-BuOH (100% H_2O). A portion of the bioactive extract from the $CHCl_3$ (3.8 g) layer (IC_{50} 0.5, 0.2, 0.5 μg/mL against P-388, A-549 and HT-29, respectively) was subjected to SiO_2 column chromatography using solvents of increasing polarities from $CHCl_3$/MeOH (99:1) to CH_3OH. The most active fractions were further chromatographed to obtain a mixture of unstable compounds whose purification attempts were unsuccesful and only the major compound obscuraminol A (**1**) was isolated. A ^{1}H NMR spectrum of the total mixture showed a broad signal centered about 6 ppm which appeared to be due to ammonium protons. This was confirmed by addition of silver nitrate (5 mL 0.1 M $AgNO_3$ in 9:1 CH_3OH/H_2O) to 21 mg of the mixture, which produced a precipitate of silver chloride. This precipitate was soluble in ammonium hydroxide and reprecipitated upon acidification with nitric acid. The mixture was acetylated to facilitate separation and prevent decomposition.

1.2.1. Obscuraminol A (1). Colorless oil; $[\alpha]_D^{20}$ +5.0° (*c* 0.14, CH_3OH); IR (film) 3500–3100, 2929, 1608, 1515, 1401, 1054, 718 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.58 (brs, 3H, NH), 5.34 (m, 8H, H-6, H-7, H-9, H-10, H-12, H-13, H-15 and H-16), 3.97 (m, 1H, H-3), 3.41 (m, 1H, H-2), 2.79 (m, 6H, H-8, H-11 and H-14), 2.22 (m, 2H, H-5), 2.05 (m, 2H, H-17), 1.45 (m, 1H, H-4a), 1.36 (m, 1H, H-4b), 1.28 (d, *J*=6.8 Hz, 3H, H-1), 0.96 (t, *J*=7.5 Hz, 3H, H-18); ^{13}C NMR ($CDCl_3$) δ 132.0 (d, C-16), 128.9 (d, C-7), 128.7[a] (d, C-9), 128.6[a] (d, C-10), 128.4 (d, C-12), 128.1 (d, C-13), 127.8 (d, C-6), 127.0 (d, C-15), 70.0 (d, C-3), 51.9 (d, C-2), 32.8 (t, C-4), 25.6[b] (t, C-8), 25.6[b] (t, C-11), 25.5[b] (t, C-14), 23.6 (t, C-5), 20.6 (t, C-17), 14.3 (q, C-18), 12.0 (q,

C-1); HRCIMS[+] *m/z* 278.2474 $(M\text{-}Cl)^+$, $C_{18}H_{32}NO$ requires *m/z* 278.2484; *m/z* 260.2355 $(M\text{-}Cl\text{-}H_2O)^+$, $C_{18}H_{30}N$ requires *m/z* 260.2378.

1.3. Acetylation of obscuraminols mixture

A portion of the bioactive $CHCl_3$ extract (1.2 g) was treated with pyridine (2.0 mL) and distilled acetic anhydride (3.0 mL) at room temperature for 3 h. The reaction mixture was evaporated under reduced pressure and the residue partitioned between water and EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to yield an oil (754 mg). Final purification of the compounds was achieved on repeated HPLC separations ($CH_3OH/CH_3CN/H_2O$ 3:4:1.5 to 3:4:3) to yield in order of elution pure diacetyl obscuraminol A (**1a**, 141.0 mg), diacetyl obscuraminol B (**2a**, 15.5 mg), diacetyl obscuraminol C (**3a**, 12.0 mg), diacetyl xestoaminol C (**7a**, 5.7 mg), diacetyl obscuraminol D (**4a**, 27.0 mg), a mixture (1:1) of diacetyl obscuraminols D and E (**4a** and **5a**, 115.0 mg) and diacetyl obscuraminol F (**6a**, 16.0 mg).

1.3.1. Diacetyl obscuraminol A (1a). Colorless oil; $[\alpha]_D^{24}$ −23.3° (*c* 0.78, CH_3OH); IR (film) 3310 (br), 2932, 1738, 1657, 1548, 1373, 1238, 721 cm^{-1}; ^1H NMR (Table 1); ^{13}C NMR (Table 2); EIMS (70 eV) *m/z* (rel. int.) 361 (0.3), 301 (9), 242 (20), 173 (17), 133 (18), 93 (33), 86 (100); HRCIMS[+] *m/z* 362.2692 $(M+H)^+$, $C_{22}H_{36}NO_3$ requires *m/z* 362.2695.

1.3.2. Diacetyl obscuraminol B (2a). Colorless oil; $[\alpha]_D^{24}$ −21.1° (*c* 0.65, CH_3OH); IR (film) 3285 (br), 2926, 1742, 1657, 1557, 1373, 1238, 970, 911 cm^{-1}; ^1H NMR (Table 1); ^{13}C NMR (Table 2); EIMS (70 eV) *m/z* (rel. int.) 277 (6), 236 (13), 218 (2), 194 (9), 140 (3), 86 (100); HRCIMS[+] *m/z* 338.2702 $(M+H)^+$, $C_{20}H_{36}NO_3$ requires *m/z* 338.2695.

1.3.3. Diacetyl obscuraminol C (3a). Amorphous solid; $[\alpha]_D^{24}$ −24.5° (*c* 0.83, CH_3OH); IR (film) 3284 (br), 2926, 1742, 1657, 1557, 1372, 1238, 968, 910 cm^{-1}; ^1H NMR (Table 1); ^{13}C NMR (Table 2); EIMS (70 eV) *m/z* (rel. int.) 337 (0.4), 277 (13), 236 (18), 218 (4), 196 (4), 140 (4), 86 (100); HRCIMS[+] *m/z* 338.2687 $(M+H)^+$, $C_{20}H_{36}NO_3$ requires *m/z* 338.2695.

1.3.4. Diacetyl obscuraminol D (4a). Colorless oil; $[\alpha]_D^{24}$ −21.6° (*c* 0.44, CH_3OH); IR (film) 3285 (br), 2926, 1742, 1657, 1557, 1372, 1238, 724 cm^{-1}; ^1H NMR (Table 1); ^{13}C NMR (Table 2); EIMS (70 eV) *m/z* (rel. int.) 340 (1.3), 339 (6), 279 (20), 222 (9), 220 (20), 208 (5), 149 (7), 86 (100); HRCIMS[+] *m/z* 340.2862 $(M+H)^+$, $C_{20}H_{38}NO_3$ requires *m/z* 340.2852.

1.3.5. Diacetyl obscuraminol E (5a). Colorless oil; ^1H NMR (Table 1); ^{13}C NMR (Table 2); EIMS (70 eV) *m/z* (rel. int.) 340 (0.6), 339 (3), 279 (15), 250 (5), 236 (7), 220 (6), 86 (100).

1.3.6. Diacetyl obscuraminol F (6a). Amorphous solid; $[\alpha]_D^{24}$ −19.2° (*c* 0.71, CH_3OH); IR (film) 3297 (br), 2921, 1730, 1642, 1547, 1371, 1239, 970, 914 cm^{-1}; ^1H NMR (Table 1); ^{13}C NMR (Table 2); EIMS (70 eV) *m/z* (rel. int.) 340 (1), 339 (1), 279 (14), 238 (6), 236 (5), 220 (5), 129 (40), 86 (100); HRCIMS[+] *m/z* 340.2843 $(M+H)^+$, $C_{20}H_{38}NO_3$ requires *m/z* 340.2852.

1.3.7. Diacetyl xestoaminol C (7a). Amorphous solid $[\alpha]_D^{24}$ −21.8° (*c* 0.40, CH_3OH); IR (film) 3290 (br), 2926, 1742, 1657, 1557, 1372, 1238 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.80 (brd, *J*=7.4 Hz, 1H, NH), 4.83 (ddd, *J*=8.7, 5.0, 3.1 Hz, 1H, H-3), 4.15 (dqd, *J*=8.5, 6.8, 3.1 Hz, 1H, H-2), 2.09 (s, 3H, *Me*COO-), 1.95 (s, 3H, *Me*CONH-), 1.57 (m, 1H, H-4a), 1.50 (m, 1H, H-4b), 1.25 (m, 18H, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12 and H-13), 1.09 (d, *J*=6.8 Hz, 3H, H-1), 0.88 (t, *J*=6.8 Hz, 3H, H-14); ^{13}C NMR ($CDCl_3$) δ 171.6 (s, Me*CO*O-), 169.3 (s, Me*CO*NH-), 77.0 (d, C-3), 47.6 (d, C-2), 31.9 (t, C-12), 31.3 (t, C-4), 29.6[a] (t, C-6), 29.6[a] (t, C-7), 29.5[a] (t, C-8), 29.4[a] (t, C-9), 29.4[a] (t, C-10), 29.3[a] (t, C-11), 25.6 (t, C-5), 23.5 (q, *Me*CONH-), 22.7 (t, C-13), 21.1 (q, *Me*COO-), 14.7 (q, C-1), 14.1 (q, C-14); EIMS (70 eV) *m/z* (rel. int.) 314 (0.7), 253 (3), 194 (7), 129 (44), 86 (100).

1.4. Oxidative cleavage and methylation of diacetyl obscuraminol D (4a)

To a solution of diacetyl obscuraminol D (**4a**, 4.0 mg, 0.012 mmol) in 3.5 mL of the solvent system CCl_4:CH_3CN:H_2O (1:1:1.5) were added with vigorous stirring periodic acid (18.4 mg, 0.081 mmol) and a catalytic amount of ruthenium (III) chloride hydrate at room temperature. After 3.5 h the reaction was extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The resulting residue was filtered through a celite column to yield 7.0 mg of cleavage product. The crude product (7.0 mg) was treated with trimethylsilyldiazomethane 2.0 M (60 μL, 0.12 mmol) in CH_3OH (1 mL) for 2.5 h at room temperature. The solvent was taken to dryness and the residue chromatographed by HPLC on reversed phase mode ($CH_3OH/CH_3CN/H_2O$ 1:1:2) to obtain 1.3 mg (0.005 mmol) of pure methyl (7*R*,8*S*)-8-acetamido-7-acetoxynonanoate (**8**).

1.4.1. Methyl (7*R*,8*S*)-8-acetamido-7-acetoxynonanoate (8). Colorless oil; $[\alpha]_D^{20}$−25.7° (*c* 0.14, CH_3OH); IR (film) 3290 (br), 2923, 1742, 1657, 1548, 1373, 1239, 1170 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.77 (brd, *J*=8.3 Hz, 1H, NH), 4.83 (ddd, *J*=8.7, 4.9, 3.2 Hz, 1H, H-7), 4.15 (dqd, *J*=8.4, 6.9, 3.2 Hz, 1H, H-8), 3.66 (s, 3H, *Me*O-), 2.30 (t, *J*=7.5 Hz, 2H, H-2), 2.09 (s, 3H, *Me*COO-), 1.95 (s, 3H, *Me*CONH-), 1.61 (m, 3H, H-3a, H-3b and H-6a), 1.51 (m, 1H, H-6b), 1.32 (m, 4H, H-4 and H-5), 1.09 (d, *J*=6.8 Hz, 3H, H-9); EIMS (70 eV) *m/z* (rel. int.) 288 (1.2), 256 (9), 194 (7), 129 (19), 86 (100); HRCIMS[+] *m/z* 288.1792 $(M+H)^+$, $C_{14}H_{26}NO_5$ requires *m/z* 288.1811.

1.5. Oxidative cleavage and methylation of diacetyl obscuraminols D (4a) and E (5a)

To a solution of a 1:1 mixture of diacetyl obscuraminol D and E (**4a/5a**, 10.3 mg, 0.030 mmol) was applied the same experimental procedure described previously yielding 2.6 mg (0.009 mmol) of methyl (7*R*,8*S*)-8-acetamido-7-acetoxynonanoate (**8**) and 2.1 mg (0.007 mmol) of methyl (9*R*,10*S*)-10-acetamido-9-acetoxyundecanoate (**9**).

1.5.1. Methyl (9*R*,10*S*)-10-acetamido-9-acetoxyundecanoate (9). Colorless oil; $[\alpha]_D^{20}$ −20.8° (*c* 0.12, CH_3OH); IR (film) 3290 (br), 2923, 1742, 1657, 1538, 1373, 1251, 1171 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.78 (brd, *J*=7.7 Hz, 1H, NH), 4.83 (ddd, *J*=8.8, 4.9, 3.1 Hz, 1H, H-9), 4.15 (dqd, *J*=8.5, 6.7, 3.0 Hz, 1H, H-10), 3.66 (s, 3H, *Me*O-), 2.30 (t, *J*=7.5 Hz, 2H, H-2), 2.10 (s, 3H, *Me*COO-), 1.95 (s, 3H, *Me*CONH-), 1.60 (m, 3H, H-3a, H-3b and H-8a), 1.51 (m, 1H, H-8b), 1.29 (m, 8H, H-4, H-5, H-6 and H-7), 1.09 (d, *J*=6.8 Hz, 3H, H-11); EIMS (70 eV) *m/z* (rel. int.) 316 (0.4), 284 (3), 255 (3), 129 (31), 86 (100); HRCIMS[+] *m/z* 316.2122 $(M+H)^+$, $C_{16}H_{30}NO_5$ requires *m/z* 316.2124.

1.6. Hydrogenation of diacetyl obscuraminols B–F (2a–6a)

A solution of diacetyl obscuraminol B (**2a**, 3.8 mg, 0.011 mmol) in EtOAc (3 mL) and catalytic amount of 20% $Pd(OH)_2/C$ was stirred under an atmosphere of H_2 for 6 h at room temperature. The catalyst was removed by filtration and the solvent evaporated to yield 2.8 mg (0.008 mmol) of pure hydrogenated compound **10**a.

1.6.1. Compound 10a. Amorphous solid; $[\alpha]_D^{20}$ −27.8° (*c* 0.21, CH_3OH); IR (film) 3291 (br), 2917, 1732, 1651, 1552, 1372, 1240 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.81 (brd, *J*=8.4 Hz, 1H, NH), 4.83 (ddd, *J*=8.7, 5.0, 3.1 Hz, 1H, H-3), 4.15 (dqd, *J*=8.5, 6.8, 3.1 Hz, 1H, H-2), 2.09 (s, 3H, *Me*COO-), 1.95 (s, 3H, *Me*CONH-), 1.56 (m, 1H, 4a), 1.50 (m, 1H, H-4b), 1.25 (m, 22H, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, H-14 and H-15), 1.09 (d, *J*=7.0 Hz, 3H, H-1), 0.87 (t, *J*=6.8 Hz, 3H, H-16); ^{13}C NMR ($CDCl_3$) δ 171.6 (s, Me*CO*O-), 169.3 (s, Me*CO*NH-), 76.9 (d, C-3), 47.6 (d, C-2), 31.9 (t, C-14), 31.2 (t, C-4), 29.6[a] (t, C-6), 29.6[a] (t, C-7), 29.6[a] (t, C-8), 29.6[a] (t, C-9), 29.5[a] (t, C-10), 29.4[a] (t, C-11), 29.4[a] (t, C-12), 29.3[a] (t, C-13), 25.6 (t, C-5), 23.5 (q, *Me*CONH-), 22.7 (t, C-15), 21.1 (q, *Me*COO-), 14.7 (q, C-1), 14.1 (q, C-16); EIMS (70 eV) *m/z* (rel. int.) 342 (0.5), 281 (5), 222 (12), 129 (65), 86 (100); HRCIMS[+] *m/z* 342.3008 $(M+H)^+$, $C_{20}H_{40}NO_3$ requires *m/z* 342.3035.

Application of this procedure to 3.5 mg (0.010 mmol) of diacetyl obscuraminol C (**3a**), 4.3 mg (0.013 mmol) of diacetyl obscuraminols D (**4a**) and E (**5a**) and 3.5 mg (0.010 mmol) of diacetyl obscuraminol F (**6a**) yielded 2.4 mg (0.007 mmol), 3.8 mg (0.011 mmol) and 2.7 mg (0.008 mmol), respectively, of hydrogenated products **10a**.

1.7. Relative configuration

Natural obscuraminol A (**1**, 4.3 mg, 0.014 mmol) was treated with 1,1′-carbonyldiimidazole (4.1 mg, 0.025 mmol) in CH_2Cl_2 (2 mL) and DMF (100 μL). The solution was stirred under N_2 atmosphere at 0° for 19 h. The reaction was treated with water and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and taken to dryness yielding 1.9 mg (0.006 mmol) of pure obscuraminol A oxazolidinone (**1c**).

1.7.1. Obscuraminol A oxazolidinone (1c). ^1H NMR ($CDCl_3$) δ 5.39 (m, 8H, H-6, H-7, H-9, H-10, H-12, H-13, H-15 and H-16), 5.24 (brs, 1H, NH), 4.58 (ddd, *J*=10.2, 7.4, 3.8 Hz, 1H, H-3), 3.91 (dq, *J*=6.8, 6.8 Hz, 1H, H-2), 2.83 (m, 6H, H-8, H-11 and H-14), 2.30 (m, 1H, H-5a), 2.23 (m, 1H, H-5b), 2.08 (td, *J*=7.5, 7.5 Hz, 2H, H-17), 1.84 (m, 1H, H-4a), 1.56 (m, 1H, H-4b), 1.17 (d, *J*=6.5 Hz, 3H, H-1), 0.97 (t, *J*=7.5 Hz, 3H, H-18); ^{13}C NMR (C_6D_6) δ 132.1[a] (d, C-16), 129.5[a] (d, C-7), 128.9[a] (d, C-9), 128.7[a] (d, C-10), 128.6[a] (d, C-12), 128.0[a] (d, C-13), 127.6[a] (d, C-6), 127.2[a] (d, C-15), 78.4 (d, C-3), 53.3 (d, C-2), 29.2 (t, C-4), 26.0[b] (t, C-8), 25.9[b] (t, C-11), 25.9[b] (t, C-14), 23.8 (t, C-5), 20.9 (t, C-17), 15.4 (q, C-1), 14.4 (q, C-18); EIMS (70 eV) *m/z* (rel. int.) 232 (0.3), 215 (0.6), 188 (2), 91 (74), 79 (100), 67 (72).

1.7.2. Methanolysis of 10a. Compound **10a** (8.4 mg, 0.025 mmol) was treated with 1N HCl in 75% aqueous CH_3OH (5 mL) at 90 °C for 16.5 h. The reaction mixture was neutralized with NaOH 1N (5.4 mL), extracted with EtOAc, dried over Na_2SO_4 and evaporated under reduced pressure obtaining 7.6 mg of deacetylated compound **10**.

1.7.3. Oxazolidinone 10c. Compound **10** was treated with 1,1′-carbonyldiimidazole (8.4 mg, 0.052 mmol) in CH_2Cl_2 (2 mL) and DMF (100 μL) with stirring at 0°C for 10 h under N_2 atmosphere. Then, the mixture was allowed to warm up to room temperature, quenched by addition of water (4 mL), extracted with CH_2Cl_2 and purified (SiO_2, $CHCl_3$ to $CHCl_3/CH_3OH$ 9:1) obtaining 3.8 mg (0.013 mmol) of pure oxazolidinone **10c**.

1.7.4. 10c. ^1H NMR (C_6D_6) δ 5.72 (brs, 1H, NH), 3.93 (ddd, *J*=9.2, 7.3, 4.3 Hz, 1H, H-3), 2.99 (dq, *J*=6.9, 6.5 Hz, 1H, H-2), 1.32 (m, 24H, H-4, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, H-14 and H-15), 0.91 (t, *J*=6.5 Hz, 3H, H-16), 0.52 (d, *J*=6.5 Hz, 3H, H-1); ^{13}C NMR (C_6D_6) δ 159.5 (s, -NHCOO-), 79.5 (d, C-3), 50.7 (d, C-2), 32.3 (t, C-14), 30.1[a] (t, C-4), 30.1[a] (t, C-6), 30.1[a] (t, C-7), 30.1[a] (t, C-8), 30.0[a] (t, C-9), 29.9[a] (t, C-10), 29.8[a] (t, C-11), 29.7[a] (t, C-12), 29.3[a] (t, C-13), 26.3 (t, C-5), 23.1 (t, C-15), 15.4 (q, C-1), 14.3 (q, C-16); EIMS (70 eV) *m/z* (rel. int.) 284 (0.3), 268 (3), 224 (5), 128 (7), 114 (49), 88 (100).

1.8. Methanolysis of diacetyl xestoaminol C (7a)

Treatment of 1.6 mg (0.005 mmol) of diacetyl xestoaminol C (**7a**) in the same way as described above gave 3.0 mg of reaction mixture, which was purified (SiO_2, $CHCl_3/CH_3OH$ 7:3) yielding 0.6 mg (0.003 mmol) of pure xestoaminol C (**7**): $[\alpha]_D^{20}$=+5.0° (*c* 0.06, CH_3OH).

1.9. Absolute configuration

1.9.1. Preparation of *N*-acetyl obscuraminol A (1b), 10b and *N*-acetyl xestoaminol C (7b). Diacetyl obscuraminol A (**1a**, 12.1 mg, 0.034 mmol) was treated with 1N HCl in 75% aqueous CH_3OH (2.5 mL) and stirred at room temperature for 20 h. After the usual workup the crude product was purified by HPLC ($CH_3OH/CH_3CN/H_2O$ 3:4:3) yielding 7.0 mg (0.022 mmol) of pure *N*-acetyl obscuraminol A (**1b**). **Compound 10a** (4.2 mg, 0.012 mmol) and diacetyl xestoaminol C (**7a**, 3.5 mg, 0.011 mmol) were hydrolyzed

under the same conditions and purified to give 2.4 mg (0.008 mmol) and 2.1 mg (0.008 mmol) of monoacyl derivatives **10b** and **7b**, respectively.

1.9.2. *N*-acetyl obscuraminol A (1b). ^{1}H NMR ($CDCl_3$) δ 5.75 (brd, *J*=6.8 Hz, 1H, NH), 5.38 (m, 8H, H-6, H-7, H-9, H-10, H-12, H-13, H-15 and H-16), 4.01 (dqd, *J*=8.0, 6.9, 2.7 Hz, 1H, H-2), 3.65 (td, *J*=6.6, 2.7 Hz, 1H, H-3), 2.84[a] (m, 4H, H-8 and H-11), 2.81[a] (m, 2H, H-14), 2.25 (m, 1H, H-5a), 2.17 (m, 1H, H-5b), 2.08 (m, 2H, H-17), 1.99 (s, 3H, *Me*CONH-), 1.46 (m, 2H, H-4), 1.10 (d, *J*=6.9 Hz, 3H, H-1), 0.97 (t, *J*=7.5 Hz, 3H, H-18).

1.9.3. 10b. ^{1}H NMR ($CDCl_3$) δ 5.79 (brd, *J*=6.8 Hz, 1H, NH), 4.01 (m, 1H, H-2), 3.64 (m, 1H, H-3), 1.99 (s, 3H, *Me*CONH-), 1.46 (m, 1H, H-4a), 1.38 (m, 1H, H-4b), 1.25 (m, 22H, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, H-14, and H-15), 1.09 (d, *J*=6.8 Hz, 3H, H-1), 0.97 (t, *J*=7.0 Hz, 3H, H-16).

1.9.4. *N*-acetyl xestoaminol C (7b). ^{1}H NMR ($CDCl_3$) δ 5.79 (brd, *J*=6.9 Hz, 1H, NH), 4.01 (m, 1H, H-2), 3.64 (m, 1H, H-3), 1.99 (s, 3H, *Me*CONH-), 1.46 (m, 1H, H-4a), 1.39 (m, 1H, H-4b), 1.25 (m, 18H, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12 and H-13), 1.09 (d, *J*=6.8 Hz, 3H, H-1), 0.97 (t, *J*=6.9 Hz, 3H, H-14).

1.9.5. Synthesis of (*R*)-MTPA ester 1d. *N*-acetyl obscuraminol A (**1b**, 3.7 mg, 0.012 mmol) was treated with CH_2Cl_2 solutions of *N*,′-dicyclohexylcarbodiimide (26.0 mg, 0.126 mmol in 1.0 mL), *N*,*N*-dimethylaminopyridine (2.8 mg, 0.023 mmol in 0.5 mL) and (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid (6.2 mg, 0.027 mmol in 0.5 mL) and the mixture stirred at room temperature for 16 h. Evaporation of the solvent under reduced pressure yielded a residue that was purified by HPLC ($CHCl_3$/CH_3OH 99.5:0.5) to obtain 4.2 mg (0.008 mmol) of (*R*)-MTPA ester **1d**: ^{1}H NMR ($CDCl_3$) (selected data, assignments aided by a COSY experiment) δ 5.36 (m, 8H, H-6, H-7, H-9, H-10, H-12, H-13, H-15 and H-16), 5.35 (m, 1H, NH), 5.16 (ddd, *J*=8.5, 4.9, 3.0 Hz, 1H, H-3), 4.23 (dqd, *J*=8.4, 6.8, 3.0 Hz, 1H, H-2), 2.80[a] (m, 4H, H-11 and H-14), 2.74[a] (dd, *J*=6.3, 6.3 Hz, 2H, H-8), 2.07 (m, 2H, H-17), 2.04 (m, 2H, H-5), 1.89 (s, 3H, *Me*CONH-), 1.72 (m, 1H, H-4a), 1.59 (m, 1H, H-4b), 1.08 (d, *J*=7.0 Hz, 3H, H-1), 0.97 (t, *J*=7.5 Hz, 3H, H-18).

1.9.6. Synthesis of (*S*)-MTPA ester 1e. Treatment of *N*-acetyl obscuraminol A (**1b**, 3.3 mg, 0.010 mmol) with CH_2Cl_2 solutions of *N*,*N*′-dicyclohexylcarbodiimide (23.0 mg, 0.111 mmol in 0.75 mL), *N*,*N*-dimethylaminopyridine (2.5 mg, 0.020 mmol in 0.5 mL) and (*S*)-α-methoxy-α-trifluoromethylphenylacetic acid (5.0 mg, 0.021 mmol in 0.75 mL) as described above (rt., 16 h) yielded 3.1 mg (0.006 mmol) of (*S*)-MTPA ester **1e**: ^{1}H NMR ($CDCl_3$) (selected data, assignments aided by a COSY experiment) δ 5.37 (m, 8H, H-6, H-7, H-9, H-10, H-12, H-13, H-15 and H-16), 5.22 (brd, *J*=8.7 Hz, 1H, NH), 5.16 (ddd, *J*=7.8, 5.8, 2.7 Hz, 1H, H-3), 4.21 (dqd, *J*=8.6, 6.8, 2.7 Hz, 1H, H-2), 2.82[a] (m, 2H, H-11), 2.80 (m, 2H, H-14), 2.77[a] (dd, *J*=6.4, 6.4 Hz, 2H, H-8), 2.11 (m, 2H, H-5), 2.07 (m, 2H, H-17), 1.86 (s, 3H, *Me*CONH-), 1.78 (m, 1H, H-4a), 1.63 (m, 1H, H-4b), 1.03 (d, *J*=6.8 Hz, 3H, H-1), 0.97 (t, *J*=7.6 Hz, 3H, H-18).

1.9.7. Synthesis of (*R*)-MTPA ester 10d. Treatment of **10b** (1.3 mg, 0.004 mmol) with CH_2Cl_2 solutions of *N*,*N*′-dicyclohexylcarbodiimide (5.3 mg, 0.026 mmol in 0.75 mL), *N*,*N*-dimethylaminopyridine (1.0 mg, 0.008 mmol in 0.5 mL) and (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid (5.0 mg, 0.021 mmol in 0.75 mL) as described above (rt., 46 h) yielded 1.8 mg (0.003 mmol) of (*R*)-MTPA ester **10d**: ^{1}H NMR ($CDCl_3$) (selected data, assignments aided by a COSY experiment) δ 5.33 (brd, *J*=8.0 Hz, 1H, NH), 5.12 (ddd, J=8.3, 5.4, 3.0 Hz, 1H, H-3), 4.22 (m, 1H, H-2), 1.89 (s, 3H, *Me*CONH-), 1.63 (m, 1H, H-4a), 1.52 (m, 1H, H-4b), 1.25 (m, 2H, H-5), 1.25 (m, 20H, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, H-14 and H-15), 1.08 (d, *J*=6.9 Hz, 3H, H-1), 0.88 (t, *J*=6.8 Hz, 3H, H-16).

1.9.8. Synthesis of (*S*)-MTPA ester 10e. Treatment of **10b** (1.0 mg, 0.003 mmol) with CH_2Cl_2 solutions of *N*,*N*′-dicyclohexylcarbodiimide (4.9 mg, 0.024 mmol in 0.75 mL), *N*,*N*-dimethylaminopyridine (1.1 mg, 0.009 mmol in 0.5 mL) and (*S*)-α-methoxy-α-trifluoromethylphenylacetic acid (4.0 mg, 0.017 mmol in 0.75 mL) as described above (rt., 46 h) yielded 1.3 mg (0.002 mmol) of (*S*)-MTPA ester **10e**: ^{1}H NMR ($CDCl_3$) (selected data, assignments aided by a COSY experiment) δ 5.21 (brd, *J*=8.4 Hz, 1H, NH), 5.12 (ddd, J=8.0, 5.8, 2.3 Hz, 1H, H-3), 4.19 (m, 1H, H-2), 1.85 (s, 3H, *Me*CONH-), 1.67 (m, 1H, H-4a), 1.54 (m, 1H, H-4b), 1.29 (m, 2H, H-5), 1.25 (m, 20H, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, H-14 and H-15), 1.01 (d, *J*=7.0 Hz, 3H, H-1), 0.88 (t, *J*=6.8 Hz, 3H, H-16).

1.9.9. Synthesis of (*R*)-MTPA ester 7c. Treatment of *N*-acetyl xestoaminol C (**7b**, 2.0 mg, 0.007 mmol) with CH_2Cl_2 solutions of *N*,*N*′-dicyclohexylcarbodiimide (12.4 mg, 0.060 mmol in 0.75 mL), *N*,*N*-dimethylaminopyridine (1.8 mg, 0.015 mmol in 0.5 mL) and (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid (11.6 mg, 0.049 mmol in 0.75 mL) as described above (rt., 22 h) yielded 0.8 mg (0.002 mmol) of (*R*)-MTPA ester **7c**: ^{1}H NMR ($CDCl_3$) (selected data, assignments aided by a COSY experiment) δ 5.33 (brd, *J*=8.4 Hz, 1H, NH), 5.12 (ddd, J=8.1, 5.3, 2.8 Hz, 1H, H-3), 4.22 (m, 1H, H-2), 1.89 (s, 3H, *Me*CONH-), 1.63 (m, 1H, H-4a), 1.51 (m, 1H, H-4b), 1.26 (m, 2H, H-5), 1.25 (m, 16H, H-6, H-7, H-8, H-9, H-10, H-11, H-12 and H-13), 1.08 (d, *J*=6.8 Hz, 3H, H-1), 0.88 (t, *J*=6.8 Hz, 3H, H-14).

1.9.10. Synthesis of (*S*)-MTPA ester 7d. Treatment of *N*-acetyl xestoaminol C (**7b**, 1.9 mg, 0.007 mmol) with CH_2Cl_2 solutions of *N*,*N*′-dicyclohexylcarbodiimide (11.8 mg, 0.060 mmol in 0.75 mL), *N*,*N*-dimethylaminopyridine (1.8 mg, 0.015 mmol in 0.5 mL) and (*S*)-α-methoxy-α-trifluoromethylphenylacetic acid (6.2 mg, 0.026 mmol in 0.5 mL) as described above (rt., 22 h) yielded 0.6 mg (0.001 mmol) of (*S*)-MTPA ester **7d**: ^{1}H NMR ($CDCl_3$) (selected data, assignments aided by a COSY experiment) δ 5.21 (brd, *J*=8.7 Hz, 1H, NH), 5.12 (ddd, J=8.0, 5.0, 3.0 Hz, 1H, H-3), 4.19 (m, 1H, H-2), 1.85 (s, 3H, *Me*CONH-), 1.68 (m, 1H, H-4a), 1.54 (m, 1H, H-4b), 1.30 (m, 2H, H-5), 1.25 (m, 16H, H-6, H-7, H-8, H-9, H-10,

H-11, H-12 and H-13), 1.01 (d, J=6.8 Hz, 3H, H-1), 0.88 (t, J=6.7 Hz, 3H, H-14).

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