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Ingenamine Alkaloids Isolated from the Sponge *Xestospongia Ingens* : Structures and Absolute Configurations

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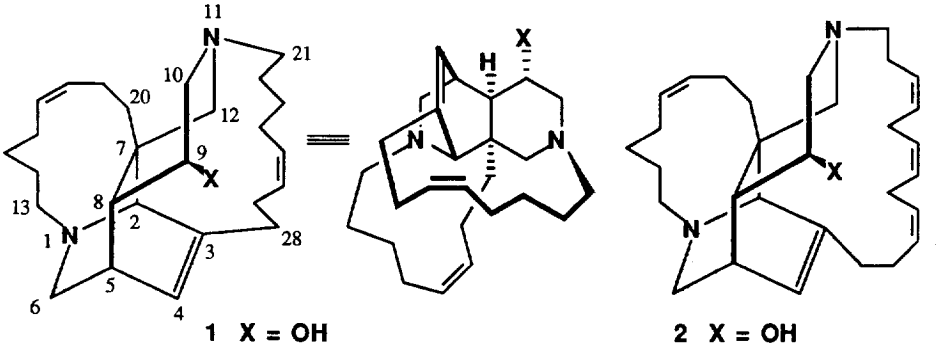
Abstract: Five new minor ingenamine alkaloids have been isolated from extracts of the sponge X. ingens collected in Papua New Guinea. The structures of the new metabolites were solved via spectroscopic analysis. Mosher ester methodology has been used to determine the absolute configurations of ingenamine (1), ingamine A (2) and ingenamine E (11). The results show that the 'ingenamine' alkaloids isolated from X. ingens are antipodal to the manzamines.

Ingenamine (**1**),[1] ingamine A (**2**) and ingamine B (**3**)[2] are metabolites of the sponge *Xestospongia ingens* that represent the first reported examples of the new 'ingenamine' family of cytotoxic sponge alkaloids. Keramaphidin B (**4**),[3] isolated from an *Amphimedon* sp., and xestocyclamines A and B[4,5], isolated from a *Xestospongia* sp., are additional 'ingenamine' alkaloids whose structures have also been described. Interestingly, the existence of the 'ingenamine' alkaloids was anticipated by Baldwin and Whitehead in their elegant proposal for the biogenetic origin of the manzamines (Scheme 1).[6] Their proposal suggested that a *bis*-3-alkyldihydropyridine macrocycle undergoes the biological equivalent of a [4 + 2] cycloaddition reaction to give an initial pentacyclic intermediate. Redox exchange between the two piperidine rings in the pentacyclic

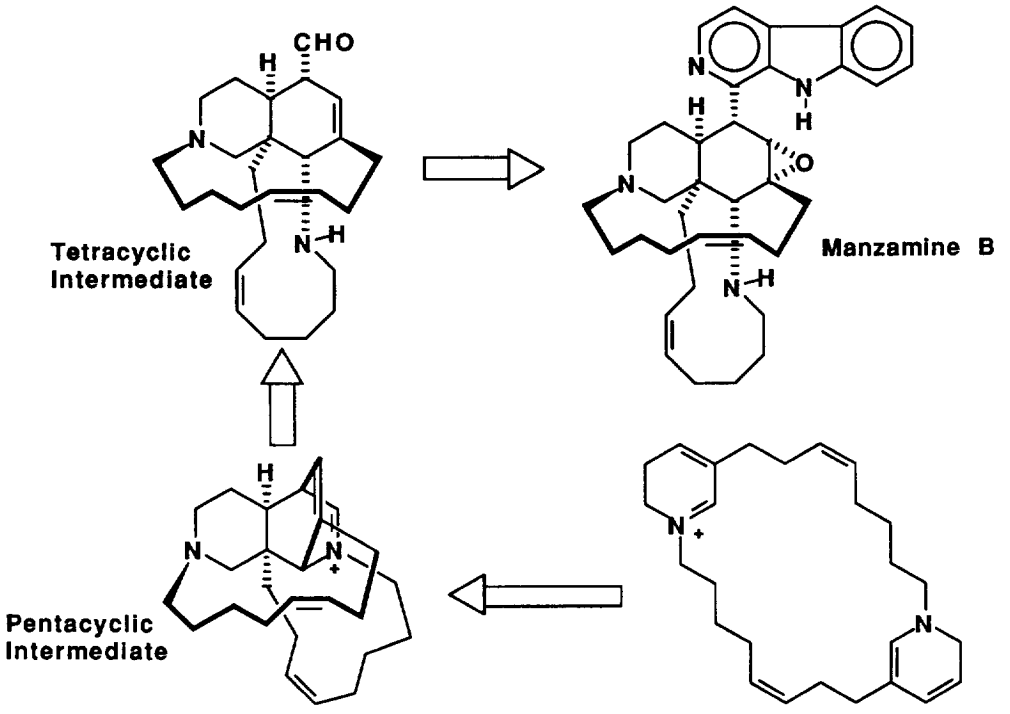


1 X = OH **2** X = OH

4 X = H **3** X = H

intermediate leads to an iminium salt that upon hydrolysis produces a new tetracyclic aldehyde intermediate. Condensation of the aldehyde with tryptamine generates the manzamine skeleton. Ingenamine (**1**), keramaphidin B (**4**) and xestocyclamine A have skeletons that are identical to the skeleton of the pentacyclic

intermediate in Baldwin and Whitehead's proposal, and ircinal B (**5**),[7] isolated from an *Amphimedon* sp., has the overall skeleton and aldehyde functionality present in their tetracyclic intermediate. The discovery of the ingenamines and the ircinals has provided strong support for Baldwin and Whitehead's proposed biogenetic pathway to the manzamines.

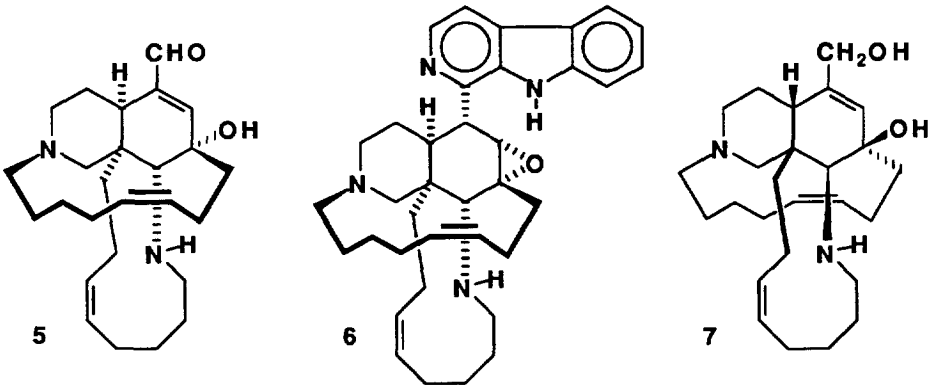


SCHEME 1

***bis*-3-Alkyldihydropyridine Macrocycle**

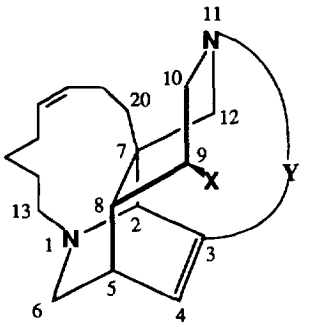
If the 'ircinal' and 'ingenamine' alkaloids are produced by the same biosynthetic manifold as the manzamines, as suggested by the Baldwin and Whitehead proposal, it is reasonable to expect that all three families of alkaloids would have the same absolute configurations. Indeed, ircinals A and B (**5**) were shown by chemical correlation to have the same configuration as manzamines A and B (**6**).[7] However, two recent reports suggest that the situation might be more complex than anticipated. Keramaphidin B (**4**), whose structure was determined by x-ray diffraction analysis, was reported to be a naturally occurring racemate,[3] and ircinols A and B (**7**) were found to be antipodal to the corresponding ircinals (e.g. **5**) and manzamines (e.g. **6**) obtained from the same sample of sponge.[8]

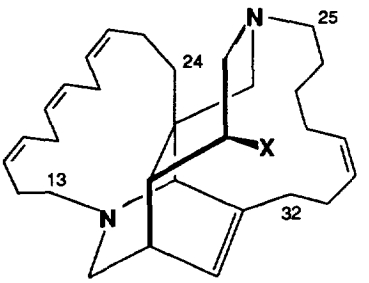
The Papua New Guinea sponge *X. ingens* is an extremely rich source of novel alkaloids.[1,2,9] We have undertaken a thorough examination of all the minor components present in the extracts of *X. ingens*, resulting



in the identification of five new alkaloids, ingenamines B (**8**) to F (**12**), whose structures are described below. Baldwin and Whitehead's proposal (Scheme 1) suggests that the ingenamine alkaloids are generated by the same biosynthetic manifold that produces the ircinols and the manzamines. In an attempt to shed further light on the stereochemical features of this interesting biosynthetic manifold, we have utilized Mosher ester methodology[10] to determine the absolute configurations of ingenamine (**1**), ingamine A (**2**) and ingenamine E (**11**). The results of the Mosher ester analyses are also presented below.

Examination of the NMR data (Tables 1 and 2) obtained for the minor *X. ingens* alkaloids revealed that ingenamines B, C and D had hydroxylated tricyclic cores (N1 to C12) and eight carbon N1 to C7 bridges that were identical to those previously identified in ingenamine (**1**).[1] The COSY, HMQC, HMBC and difference NOE data obtained for compounds **8**, **9** and **10** were in complete agreement with these partial assignments. Ingenamine B (**8**) gave a parent ion in the EIHRMS at m/z 410.3299 appropriate for a molecular formula of $C_{27}H_{42}N_2O$. Subtraction of the atoms present in the already identified tricyclic core and N1 to C7 bridge ($C_{18}H_{26}N_2O$) from the molecular formula of ingenamine B (**8**) indicated that the C3 to N11 bridge contained nine carbons and one alkene functionality. The ^{13}C NMR data obtained for **8** (Table 1) showed that all of the nine carbons in the bridge were aliphatic methylene or olefinic methines, which required a linear chain. HMBC, HMQC and COSY data located the olefinic functionality in the bridge at C27/C28, and the ^{13}C chemical shift of C26 (δ 26.3)[2,9] along with the observation of 10.8 Hz coupling between H27 and H28 indicated that the olefin had the Z configuration. Ingenamine C acetate (**9**) gave a parent ion in the EIHRMS at m/z 452.3406 appropriate for a molecular formula of $C_{29}H_{44}N_2O_2$, indicating that the parent metabolite, ingenamine C, was isomeric with ingenamine B (**8**). Analysis of the ^{13}C, COSY, HMQC and HMBC data for ingenamine C acetate (**9**) showed that it contained a linear nine carbon C3 to N11 bridge with a Z alkene between C24 and C25. Ingenamine D (**10**) gave a parent ion in the EIHRMS at m/z 422.3292 appropriate for a molecular formula of $C_{28}H_{42}N_2O$. Subtraction of the atoms present in the tricyclic core and N1 to C7 bridge ($C_{18}H_{26}N_2O$) from the molecular formula of ingenamine D (**10**) indicated that the C3 to N11 bridge contained ten carbons and two alkene functionalities. The ^{13}C NMR data for **10** once again indicated a linear ten carbon C3 to N11 bridge and the HMBC, HMQC and COSY data located the two alkenes between C24 and C25 and between C27 and C28. The chemical shifts of the C23 (δ 26.8), C26 (δ 26.9) and C29 (δ 25.8) allylic carbons were consistent with the Z configuration for both of the $\Delta^{24,25}$ and $\Delta^{27,28}$ alkenes.[2,9]

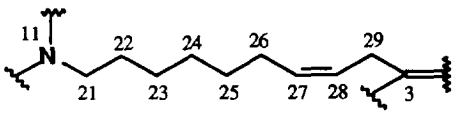




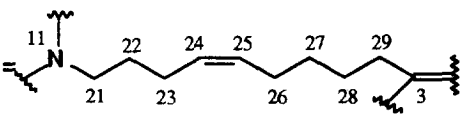
11 X= OH

12 X= H

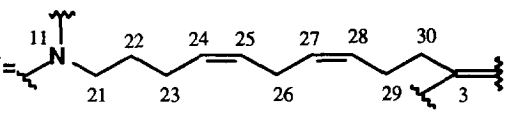
8 X= OH,Y =

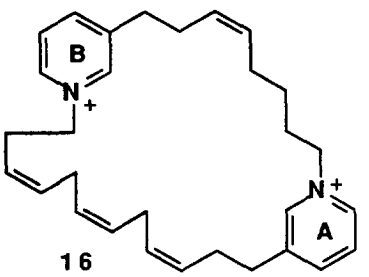


9 X= OAc,Y =



10 X= OH, Y=





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Ingenamine E (**11**) gave a parent ion at m/z 448.3458 in the EIHRMS appropriate for a molecular formula of $C_{30}H_{44}N_2O$. Examination of the NMR data for **11** (Tables 1 and 2) revealed that it contained a hydroxylated tricyclic core (N1 to C12) and eight carbon C3 to N11 bridge (C21 to C28 in ingenamine; C25 to C32 in ingenamine E) that was identical to that present in ingenamine (**1**). The COSY, HMQC, HMBC and difference NOE data obtained for compound **11** were in complete agreement with this partial assignment. Subtraction of the atoms present in the tricyclic core and C3 to N11 bridge ($C_{18}H_{26}N_2O$) from the molecular formula of ingenamine E (**11**) indicated that the N1 to C7 bridge contained twelve carbons. The ^{13}C NMR data obtained for ingenamine E (**11**) (Table 1) showed that the bridge contained six aliphatic methylene and six olefinic methine carbons, which required a linear chain with three alkene functionalities. HMBC, HMQC and COSY data identified $\Delta^{15,16}$, $\Delta^{18,19}$ and $\Delta^{21,22}$ alkenes and the ^{13}C chemical shifts of the C14 (δ 29.5), C17 (27.2), C20 (δ 26.8) and C23 (δ 22.9) allylic carbons were consistent with the Z configuration for all three alkenes.[2,9] Ingenamine F (**12**) gave a parent ion in the EIHRMS at m/z 432.3497 appropriate for a molecular formula of $C_{30}H_{44}N_2$ that differed from the molecular formula of ingenamine E (**11**) only by the absence of an oxygen atom. Comparison of the NMR data obtained for ingenamine F (**12**) (Tables 1 and 2) with the NMR data for ingamine B (**3**)[2] and keramaphidin B (**4**) (see Experimental) showed that ingenamine B (**12**) contained the same non-hydroxylated tricyclic core (N1 to C12) found in **3** and **4**. Further comparison of the ingenamine F (**12**) NMR data (Tables 1 and 2) with that obtained for ingenamine E (**11**) showed that the N1 to C7 and C3 to

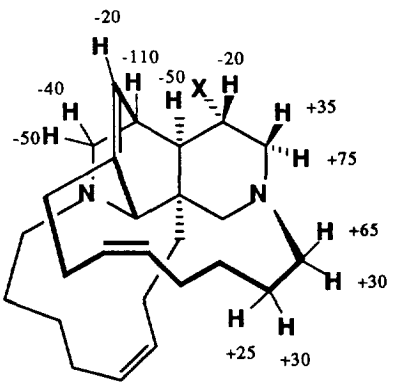
N11 bridges were identical in both molecules. The COSY, HMQC, HMBC and difference NOE data obtained for ingenamine F were completely consistent with the proposed structure **12**.

The methanol extract of *X. ingens* also yielded keramaphidin B (**4**), which was shown by NMR and mass spectrometric analysis (see Experimental) to have the same constitution and relative configuration as the original sample isolated from *Amphimedon*.[3] Interestingly, the keramaphidin B (**4**) isolated from *X. ingens* was optically active, having an $[\alpha]_D = +29.8^o$.

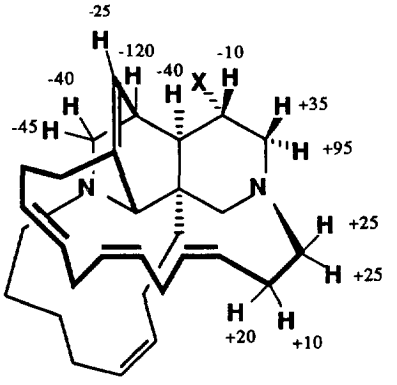
(R)- and (S)-Mosher's esters of ingenamine (**1**), ingamine A (**2**) and ingenamine E (**11**) were prepared according to literature procedures.[10,11] The (R)- and (S)-Mosher esters of cholesterol were prepared in parallel with each derivatization of **1**, **2** and **11** as a control for the chirality of the reagents. Detailed NMR analysis of the esters **13a**, **13b**, **14a**, **14b**, **15a** and **15b** using COSY, HMQC, HMBC and difference NOE experiments led to complete assignments of the ^{1}H NMR spectra of each derivative (see Experimental). The $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$ in Hz) for each proton were calculated (see Experimental) and plotted on the conformational representations of ingenamine-MTPA (**13**), ingamine A-MTPA (**14**) and ingenamine E-MTPA (**15**) shown in Figure 1.

Coupling constant analysis and difference NOE results demonstrated that the C7 to C12 piperidine ring in the Mosher esters **13**, **14**, and **15** was in a boat conformation with H9 and H12' occupying flagpole positions as in the parent compounds **1**, **2** and **11**. Examination of Dreiding models indicated that there are no steric impediments to the MTPA group adopting the 'ideal conformation' having the trifluoromethyl, ester carbonyl, and carbinol methine proton coplanar in derivatives **13**, **14** and **15**. As can be seen in Figure 1, when the MTPA plane contains the X (= MTPA) and H groups at C9 as required by the 'ideal conformation', the $\Delta\delta$ values for derivatives **13**, **14** and **15** are all positive on one side of the plane and negative on the other side. Following the empirical rule for analyzing the data as put forth by Ohtani et al.,[10] it is apparent that ingenamine (**1**), ingamine A (**2**) and ingenamine E (**11**) have the absolute configurations (2R, 5S, 7S, 8R, 9S) as shown for their MTPA derivatives **13**, **14** and **15** in Figure 1.

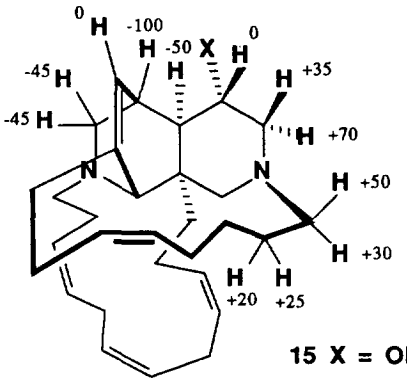
The suite of ingenamine alkaloids represented by ingenamine (**1**), ingamines A (**2**) and B (**3**), keramaphidin B (**4**), the xestocyclamines A and B, and ingenamines B (**8**) to F (**12**) illustrate some of the structural variations that are possible in this family. The only variation observed thus far in the central N1 to C12 tricyclic core is the presence or absence of a hydroxyl functionality at C9. Both the N1 to C7 and the C3 to N11 linear carbon bridges vary in length and position and degree of unsaturation. The greatest range of variation occurs in the C3 to N11 bridge which has eight carbons and one alkene in ingenamine (**1**), nine carbons and one alkene in ingenamines B (**8**) and C (i.e. **9**), ten carbons and two alkenes in ingenamine D (**10**) and twelve carbons and three alkenes in ingamines A (**2**) abd B (**3**). Only eight and twelve carbon N1 to C7 bridges have been identified. An interesting relationship exists between ingamine A (**2**) and ingenamine E (**11**) and between the corresponding non-hydroxylated analogs ingamine B (**3**) and ingenamine F (**12**). The N1 to C7 bridge in ingamines A (**2**) and B (**3**) is identical to the C3 to N11 bridge in ingenamines E (**11**) and F (**12**), and the C3 to N11 bridge in ingamines A (**2**) and B (**3**) is identical to the N1 to C7 bridge in ingenamines E (**11**) and F (**12**). Therefore, in principle ingamines A (**2**) and B (**3**) and ingenamines E (**11**) and F (**12**) can all arise from a common *bis*-3-alkylpyridine macrocyclic biogenetic precursor **16**. If a partially reduced ring A acts as the diene and a partially reduced ring B acts as the dieneophile in the biological [4 + 2] cyclization reaction proposed by Baldwin and Whitehead (Scheme 1) the skeleton of ingamines A (**2**) and B (**3**) is formed. Conversely, if a



13 X = OMTPA



14 X = OMTPA



15 X = OMTPA

Figure 1. $\Delta\delta = \delta_S - \delta_R$ (Hz) values for the Mosher esters of ingenamine, ingamine A and ingenamine E. The ^1H NMR data were recorded in CD_2Cl_2 at 500MHz.

partially reduced ring A acts as the dieneophile and a partially reduced ring B acts as a diene in the condensation reaction the ingenamine E (**11**) and F (**12**) skeleton is formed. Interestingly, the Mosher ester analysis has shown that the absolute configuration of the tricyclic central core of ingamine A (**2**) is identical to the absolute configuration of the central core of ingenamine E (**11**).

The absolute configurations of ingenamine (**1**), ingamine A (**2**) and ingenamine E (**11**) isolated from *X. ingens* are antipodal to the absolute configurations reported for manzamines A and B (**6**) and ircinals A and B (**5**). However, their configurations are identical to the absolute configurations recently reported for the ircinols A and B (**7**). The keramaphidin B (**4**) isolated by Kobayashi et al. from the same *Amphimedon* sp. that yielded ircinals A and B, manzamines A, B , G and H, and ircinols A and B was reported to be a naturally occurring racemate, while keramaphidin B (**4**) isolated from *X. ingens* in the current study was found to be optically active. The structural relationship between the ingenamine, ircinol, ircinal and manzamine alkaloids provides

compelling evidence for the Baldwin and Whitehead biogenetic proposal that they all arise from an achiral *bis*-3-alkyldihydropyridine macrocycle as shown in Scheme 1. It is clear, however, that there are two antipodal series of alkaloids formed via this pathway. One of the enantiomers of keramaphidin B (**4**) isolated from *Amphimedon* sp., the ircinals A and B (**5**), and the manzamines A and B (**6**) all belong to one configurational series. The other enantiomer of keramaphidin B (**4**), ircinols A and B (**7**), ingenamine (**1**), ingamine A (**2**) and ingenamine E (**11**) belong to the other configurational series. According to the Baldwin and Whitehead proposal, the chirality of these alkaloids is established by the biological equivalent of an intramolecular [4 + 2] cycloaddition reaction of an achiral *bis*-3-alkyldihydropyridine macrocycle. Therefore, it would appear that there are enantiomeric enzymes capable of catalyzing this intramolecular condensation.

Table 1: ^{13}C NMR data recorded in MeOH-d_4 at 125 MHz

Carbon no.	Ingenamine (1)	Ingenamine B (8)	Ingenamine C acetate (9)	Ingenamine D (10) [a]	Ingenamine E (11)	Ingenamine F (12) [b]
2	65.2	65.3	65.80	66.6	63.4	64.2
3	143.8	144.8	145.0	145.3	143.5	141.4
4	124.0	121.2	121.1	122.8	124.5	123.3
5	35.2	35.0	35.4	36.3	35.2	37.6
6	54.3	55.0	54.4	53.9	55.6	54.8
7	46.9	47.8	48.0	48.0	45.8	43.6
8	53.1	52.6	50.1	54.5	52.5	42.4
9	69.3	69.5	73.1	70.7	69.1	27.0
10	54.7	54.7	52.4	57.8	54.2	46.9
12	51.5	50.2	51.4	56.2	51.2	50.0
13	55.2	55.3	55.0	54.6	59.4	57.8
14	27.1	27.1	27.0	26.8	29.5	28.4
15	27.7	27.4	27.6	27.7	129.7	128.7
16	23.8	23.9	23.9	23.7	129.4	128.3
17	132.4	132.5	132.5	132.5	27.2	26.3
18	131.4	131.3	131.2	131.2	128.6	127.3
19	21.6	21.6	21.7	21.8	129.0	128.5
20	42.9	42.6	43.4	46.1	26.8	25.5
21	56.9	57.3	57.4	58.7	129.1	127.6
22	22.1	24.2	27.1	27.6	131.1	130.5
23	25.7	28.4	28.4	26.8	22.9	22.1
24	26.0	27.8	130.7	130.3	40.4	38.8
25	132.6	28.6	131.2	129.4	56.8	55.8
26	133.1	26.3	27.5	26.9	21.8	20.4
27	26.5	132.4	28.8	129.2	27.4	26.5
28	37.9	125.6	25.3	130.4	25.9	25.0
29		34.6	34.8	25.8	132.6	131.4
30			172.3	37.3	133.2	132.0
31			19.8		26.5	25.7
32					38.2	37.2

[a] Based on HMQC and HMBC. [b] Recorded in $CDCl_3$

Table 2: ^{1}H NMR data recorded in MeOH-d_4 at 500 MHz

Proton no.	Ingenamine (1)	Ingenamine B (8)	Ingenamine C acetate (9)	Ingenamine D (10)	Ingenamine E (11)	Ingenamine F (12) [a]
2	3.14, d(1.3)	3.12, bs	3.09, bs	3.06, bs	3.03, d(1.5)	2.90, bs
4	5.85 d(6.5)	5.90, d(6.3)	5.91, dd(6.5, 1.5)	5.95, bd(6.5)	5.89, d(6.6)	5.82, d(6.5)
5	2.64, m	2.70	2.42	2.68, m	2.62, m	2.18
6	2.84, dd(9.2, 1.9)	2.91, dd(9.1, 1.8)	2.88, dd(9.3, 1.8)	2.86 dd(9.3, 2.2)	2.99, dd(9.5, 1.9)	2.98, dd(9.3, 1.7)
6'	1.71, dd(9.2, 2.9)	1.86, dd(9.1, 2.6)	1.79, dd(9.3, 2.7)	1.80, dd(9.3, 2.6)	1.78, dd(9.5, 2.7)	1.68, dd(9.3, 2.5)
8	0.69 dd(10.1, 2.1)	0.77 dd(9.9, 2.0)	1.02, dd(9.7, 2.3)	0.92, dd(8.7, 2.8)	0.77, dd(9.9, 1.9)	1.00, dd(12.0, 4.5)
9	3.27, ddd(11.8, 10.1, 4.8)	3.32 ddd(11.9, 10.1, 4.7)	4.57, ddd(11.0, 9.7, 4.7)	3.41, ddd(14.0, 8.7, 5.0)	3.27, ddd(11.9, 10.1, 4.7)	1.41
9'						1.15, qd(12.5, 3.8)
10	2.61, dd(12.0, 4.8)	2.68, dd(12.2, 4.7)	2.70 dd(11.9, 4.7)	2.67	2.65, dd(12.2, 4.7)	2.86
10'	2.46, t(12.0)	2.57, t(12.2)	2.49, t(11.5)	2.13	2.53, t(12.0)	2.64, dt(12.5, 3.0)
12	2.26 d(10.8)	2.42, d(11.3)	2.38, d(11.2)	2.29, d(12.1)	2.44, d(11.3)	2.42
12'	1.98, d(10.8)	2.25, d(11.3)	2.26, d(11.2)	2.21, d(12.1)	2.02, d(11.3)	2.10, d(10.5)
13	2.97, td(12.6, 5.2)	2.99, td(12.6, 5.0)	2.93, td(12.4, 5.1)	2.92, td(12.4, 5.1)	2.49, td(10.7, 4.4)	2.42, td(10.8, 4.3)
13'	2.21, m	2.30, td(12.6, 4.2)	2.24	2.24	2.22, m	2.21
14	1.48	1.53	1.48	1.47	2.37	2.32
14'	1.27, m	1.28	1.27, m	1.27, bd(11.8)	2.14	2.09
15	1.58	1.60	1.58	1.58	5.37	5.34
15'	1.50	1.52	1.48	1.45		
16	2.41	2.40	2.40	2.39	5.52	5.50
16'	1.54	1.57	1.56	1.55		
17	5.63	5.64	5.63	5.62	2.88, dt(15.3, 7.4)	2.86
17'					2.78, m	2.73, dt(14.5, 7.3)
18	5.63	5.64	5.63	5.59	5.37	5.37
19	2.34	2.33	2.35	2.33	5.34	5.36
19'	1.73	1.78	1.74	1.68		
20	1.82	1.78	1.83, m	1.71	3.17, td(15.8, 7.5)	3.11, dt(16.0, 8.0)
20'	1.71	1.78	1.72	1.71	2.81, m	2.81
21	3.04, ddd(14.1, 8.2, 6.1)	2.99	2.83, ddd(13.8, 8.2, 3.9)	2.51	5.45	5.43
21'	2.19	2.47, dt(14.1, 5.0)	2.44	2.48		
22	1.64	1.50	1.68, m	1.58	5.48	5.55
22'	1.35	1.43	1.43, m	1.45		
23	1.48	1.39	2.25	2.24	2.34	2.23
23'	1.34	1.35	1.91, m	1.96	1.82	1.84, m
24	2.18	1.40	5.39, td(11.0, 4.8)	5.32	1.86	1.76, td(12.5, 3.3)
24'	1.95	1.40			1.59, bt(10.9)	1.66, td(12.5, 4.5)
25	5.22, tt(10.8, 2.9)	1.53	5.42, td(10.6, 5.5)	5.40	3.09, dt(14.0, 7.1)	3.09
25'		1.32			2.30	2.32
26	5.36, m	2.33	2.35	3.06	1.68, m	1.55, m
26'		1.79	1.78	2.53	1.37	1.40
27	2.31	5.54 , td(10.8, 4.4)	1.48	5.47	1.48, m	1.44
27'	2.08		1.48		1.36	1.32
28	2.35	5.67, td(10.8, 4.4)	1.79	5.49	2.20	2.11
28'	2.28		1.49		1.97, bd(14.4)	1.97
29		3.09, dd(18.1,10.7)	2.22	2.47	5.24, bt(10.6)	5.23, bt(10.5)
29'		2.72	2.22	2.12		
30				2.30	5.38	5.34
30'				2.00		
31			2.00, s		2.32	2.22
31'					2.06, m	2.09
32					2.38	2.34
32'					2.18	2.14

[a] Recorded in $CDCl_3$

EXPERIMENTAL

Specimens of *Xestospongia ingens* were collected by hand using SCUBA on reefs at depths of -15 to -20 m near Sek Point off Madang, Papua New Guinea in 1992. Freshly collected sponge were frozen on site and transported to Vancouver over dry ice. The sponge was identified by Dr. R van Soest. A voucher sample (ZMA 10701) has been deposited at the Zoologisch Museum, University of Amsterdam.

Extraction and Isolation of Ingenamine Alkaloids: Specimens (200 g, wet) of *Xestospongia ingens* were thawed and extracted exhaustively with MeOH (500 mL x 3, each about one day). The MeOH extract was filtered and concentrated *in vacuo* to give a dark brown aqueous suspension which was then diluted with H_2O to 300 mL and partitioned sequentially against hexanes (400 mL x 3) and EtOAc (400 mL x 3). The hexanes-soluble fraction (770 mg) was subjected to silica-gel flash chromatography using gradient elution (hexanes/EtOAc 1:9 to 1:1) to give three fractions: A (120 mg, mainly madangamine A[9]), B (290 mg) and C (200 mg) in sequence. Fractions B and C were found to contain mainly ingamine B (**3**) and ingamine A (**2**), respectively. The EtOAc-soluble fraction (650 mg) was chromatographed on a Sephadex LH-20 column using MeOH first, and then EtOAc:MeOH:H_2O 40:10:4 as the eluent to afford three fractions: fraction 1 (190 mg), consisting of mainly ingamines A (**2**) and B (**3**) plus xestocyclamine B; fraction 2 (440 mg), a very complex mixture of ingenamine type compounds; and fraction 3 (65 mg), mainly ingenamine (**1**) and the minor component ingenamine E (**11**). Recycling of fraction 3 via the same column (Sephadex LH-20, EtOAc/MeOH/H_2O 40:10:4) gave pure ingenamine (**1**) which was in a protonated form (20 mg). Ingenamine F (**12**) (4 mg) was obtained from fraction 2 (100 mg) by Sephadex LH-20 chromatography (EtOAc/MeOH/H_2O 40:5:2) followed by preparative silica-gel TLC (eluent: EtOAc/MeOH 75:25). Repeated fractionation of fractions 2 and 3 on normal-phase HPLC using an eluent of EtOAc/hexane modified by a small amount of iPr$_2$NH and/or MeOH gave ingenamine B (**8**) (25 mg) and keramaphidin B (**4**) (17 mg), a mixture of xestocyclamine B and ingenamine C (30 mg), ingenamine D (**10**) (1 mg), ingenamine E (**11**) (7 mg) and unprotonated ingenamine (**1**) (25 mg). The mixture of xestocyclamine B and ingenamine C was subjected to repeated recrystallization from MeOH to afford pure xestocyclamine B[5] (5.7 mg, colorless needles (acetonitrile:MeOH 4:1), mp 151-3°C). Acetylation of the residue from the crystallization mother liquor (23 mg) using 1 mL of pyridine and 1 mL of acetic anhydride at room temperature with stirring overnight and followed by a normal phase HPLC separation (eluent: hexane / EtOAc / iPr$_2$NH / MeOH 97.5:2.5:0.05:0.05) gave pure ingenamine C acetate (**9**) (7 mg).

Keramaphidin B (4): Amorphous white solid, $[\alpha]_D$ +29.8° (c 1.1; MeOH); HREIMS, $C_{26}H_{40}N_2$ (M+) m/z: 380.319 (ΔM -0.0 mmu); LREIMS m/z (formula, relative intensity); 380 ($C_{26}H_{40}N_2$, 76), 283 ($C_{19}H_{27}N_2$, 100), 217 ($C_{14}H_{21}N_2$, 25), 206 ($C_{14}H_{24}N$, 27), 192 ($C_{13}H_{22}N$, 62), 190 ($C_{13}H_{20}N_1$ 33), 188 ($C_{13}H_{18}N$, 45), 148 ($C_{10}H_{14}N$, 27), 134 ($C_9H_{12}N$, 29), 110 ($C_7H_{12}N$, 77), 93 (C_6H_7N, 38); ^{1}H NMR (MeOH-d_4, 500 MHz), δ: 0.98 (ddd, J = 12.5, 5.5, 2.1 Hz, H8), 1.23 (qd, J = 14.0, 4.1 Hz, H9'), 1.27 (m, H14'), 1.44 (m, H23'), 1.49 (m, H22'), 1.50 (m, H9), 1.50, (m, H15'), 1.52 (m, H23), 1.53 (m, H14), 1.56 (m, H16'), 1.61 (m, H15), 1.68 (dd, J = 9.2, 2.6 Hz, H6'), 1.73 (m, H22), 1.75 (2H, m, H20), 1.76 (m, H19'), 2.02 (bd, J = 15.2 Hz, H24'), 2.11 (m, H27'), 2.16 (d, J = 11.6 Hz, H12'), 2.21 (ddd, J = 12.5, 5.2, 1.2 Hz, H13'), 2.26 (m, H24), 2.30 (m, H5), 2.31 (m, H28'), 2.35 (m, H27), 2.38 (m, H19), 2.38 (m, H28), 2.41 (m, H16), 2.52 (ddd, J = 13.5, 7.5, 2.5 Hz, H21'), 2.70 (d, J = 11.6 Hz, H12), 2.88 (m, H10'), 2.89 (dd, J = 9.2, 1.9 Hz, H6), 2.97 (td, J = 13.5, 2.6 Hz, H10), 2.99 (td, J = 12.5, 5.2 Hz, H13), 3.18 (bs, H2), 3.24 (dt, J = 13.5, 7.5Hz, H21), 5.28(tt, J = 10.8, 2.8 Hz,

H25), 5.41 (m, H26), 5.65 (m, H17), 5.65(m, H18), 5.91 (d, J = 6.4 Hz, H4); ^{13}C NMR (MeOH-d_4, 125 MHz), δ: 20.87 (C22), 21.56 (C19), 23.79 (C16), 26.13 (C24), 26.47 (C27), 26.81 (C9), 27.06 (C14), 27.06 (C23), 27.47 (C15), 37.59 (C28), 38.76 (C5), 41.77 (C20), 44.06 (C8), 44.95 (C7), 48.79 (C10), 50.76 (C12), 54.31 (C6), 55.11 (C13), 56.88 (C21), 64.63 (C2), 125.0 (C4), 131.0 (C18), 132.6 (C25), 132.8 (C17), 133.4 (C26), 142.8 (C3).

Ingenamine B (8): obtained as a white powder; $[\alpha]_D$ = +22.4° (c 0.25, MeOH); EIHRMS M^+, m/z 410.3299 ($C_{27}H_{42}N_2O$, ΔM 0.2 mmu); EILRMS m/z (formula, relative intensity %), 410 ($C_{27}H_{42}N_2O$ 100), 393 ($C_{27}H_{41}N_2$ 30), 392 ($C_{27}H_{40}N_2$ 27), 391 ($C_{27}H_{39}N_2$ 37), 379 ($C_{26}H_{39}N_2$ 56), 367 ($C_{25}H_{37}NO$ 19), 295 ($C_{20}H_{27}N_2$ 61), 281 ($C_{19}H_{25}N_2$ 93), 231 ($C_{15}H_{23}N_2$ 30), 202 ($C_{14}H_{20}N$ 34), 190 ($C_{13}H_{20}N$ 24), 189 ($C_{13}H_{19}N$ 19), 188 ($C_{13}H_{18}N$ 20), 146 ($C_{10}H_{12}N$ 30), 132 ($C_9H_{10}N$ 33), 120 ($C_8H_{10}N$ 17), 106 (C_7H_8N 22), 93 (C_6H_7N 43), 79 (C_6H_7 19), 67 (C_5H_7 27), 55 (C_3H_5N 26); ^{1}H NMR (MeOH-d_4, 500 MHz) and ^{13}C NMR (MeOH-d_4, 125 MHz) are listed in Tables 1 and 2.

Ingenamine C acetate (9): obtained as a colorless glass; $[\alpha]_D$ = +41.6° (c 0.09, MeOH); EIHRMS M^+, m/z 452.3406 ($C_{29}H_{44}N_2O_2$, ΔM 0.3 mmu); EILRMS m/z (formula, relative intensity %), 452 ($C_{29}H_{44}N_2O_2$ 49), 409 ($C_{27}H_{41}N_2O$ 10), 393 ($C_{27}H_{41}N_2$ 100), 379 ($C_{26}H_{39}N_2$ 96), 297 ($C_{20}H_{29}N_2$ 36), 295 ($C_{20}H_{25}N_2$ 29), 283 ($C_{19}H_{27}N_2$ 26), 281 ($C_{19}H_{25}N_2$ 34), 190 ($C_{13}H_{20}N$ 22), 189 ($C_{13}H_{19}N$ 38) 188 ($C_{13}H_{18}N$ 29), 174 ($C_{12}H_{16}N$ 24), 162 ($C_{11}H_{16}N$ 22), 148 ($C_{10}H_{14}N$ 30), 134 ($C_9H_{12}N$ 37), 120 ($C_8H_{10}N$ 42), 107 (C_7H_9N 47), 106 (C_7H_8N 46), 93 (C_6H_7N 75), 79 (C_6H_7 40), 67 (C_5H_7 53), 55 (C_4H_7 33); ^{1}H NMR (MeOH-d_4, 500 MHz) and ^{13}C NMR (MeOH-d_4, 125 MHz) are listed in Tables 1 and 2.

Ingenamine D (10): obtained as a colorless glass; EIHRMS M^+, m/z 422.3292 ($C_{28}H_{42}N_2O$, ΔM -0.5 mmu); EILRMS m/z (formula, relative intensity %), 422 ($C_{28}H_{42}N_2O$ 47), 405 ($C_{28}H_{41}N_2$ 15), 391 ($C_{27}H_{39}N_2$ 40), 321 ($C_{22}H_{29}N_2$ 11), 307 ($C_{21}H_{27}N_2$ 17), 293 ($C_{20}H_{25}N_2$ 30), 216 ($C_{15}H_{22}N$ 27), 214 ($C_{15}H_{20}N$ 29), 188 ($C_{13}H_{18}N$ 31), 162 ($C_{11}H_{16}N$ 20), 148 ($C_{10}H_{14}N$ 19), 134 ($C_9H_{12}N$ 33), 120 ($C_8H_{10}N$ 26), 107 (C_7H_9N 29), 93 (C_6H_7N 100), 79 (C_6H_7 34), 67 (C_5H_7 43), 55 (C_4H_7 33); ^{1}H NMR (MeOH-d_4, 500 MHz) and 13C NMR (MeOH-d_4, 125 MHz) are listed in Tables 1 and 2.

Ingenamine E (11): obtained as colorless glass; $[\alpha]_D$ = -23.8° (c 0.062, MeOH); EIHRMS M^+, m/z 448.3458 ($C_{30}H_{44}N_2O$, ΔM 0.5 mmu); EILRMS m/z (formula, relative intensity %), 448 ($C_{30}H_{44}N_2O$ 100), 431 ($C_{30}H_{43}N_2$ 35), 417 ($C_{29}H_{41}N_2$ 34), 405 ($C_{28}H_{41}N_2$ 23), 335 ($C_{23}H_{31}N_2$ 26), 281 ($C_{19}H_{25}N_2$ 58), 267 ($C_{18}H_{23}N_2$ 51), 242 ($C_{17}H_{24}N$ 26), 217 ($C_{14}H_{21}N_2$ 31), 188 ($C_{13}H_{18}N$ 51), 148 ($C_{10}H_{14}N$ 18), 146 ($C_{10}H_{12}N$ 23), 134 ($C_9H_{12}N$ 25), 120 ($C_8H_{10}N$ 19), 107 (C_7H_9N 45), 93 (C_6H_7N 81), 79 (C_6H_7 39), 67 (C_5H_7 36), 55 (C_4H_7 27); ^{1}H NMR (MeOH-d_4, 500 MHz) and 13C NMR (MeOH-d_4, 125 MHz) are listed in Tables 1 and 2.

Ingenamine F (12): obtained as a colorless glass; $[\alpha]_D$ = -64.3° (c 0.062 MeOH); EIHRMS M^+, m/z 432.3497 ($C_{30}H_{44}N_2$, ΔM -0.8 mmu); EILRMS m/z (formula, relative intensity %), 432 ($C_{30}H_{44}N_2$ 92), 417 ($C_{29}H_{41}N_2$ 13), 391 ($C_{27}H_{39}N_2$ 20), 351 ($C_{24}H_{35}N_2$ 12), 337 ($C_{23}H_{33}N_2$ 27), 311 ($C_{21}H_{31}N_2$ 13), 297 ($C_{20}H_{29}N_2$ 21), 283 ($C_{19}H_{27}N_2$ 100), 217 ($C_{14}H_{21}N_2$ 31), 192 ($C_{13}H_{22}N$ 28), 190 ($C_{13}H_{20}N$ 17), 188 ($C_{13}H_{18}N$ 18), 174 ($C_{12}H_{16}N$ 10), 160 ($C_{11}H_{14}N$ 11), 110 ($C_7H_{12}N$ 53), 107 (C_7H_9N 35), 93 (C_6H_7N 35), 79 (C_6H_7 34), 67 (C_5H_7 36), 55 (C_4H_7 27); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) are listed in Tables 1 and 2.

Preparation and Purification of Mosher Esters.

To a solution of 3.5 mg of ingenamine (**1**) and 6 mg of 4-(dimethylamino)pryridine (DMAP) in 1 mL of methylene chloride was added 8 μL of (*R*)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) prepared according to the literature procedure.[10,11] After the mixture was stirred at room temperature for about 4 hours, 2 drops of triethylamine was added. The reaction mixture was allowed to stir overnight at room temperature during which time the solution gradually became pale yellow. Evaporation of the solvent under reduced pressure gave a residue which was washed by water to give the crude (*S*)-Mosher ester (5 mg). Further purification by normal phase HPLC (eluent: 90:10:0.1 hexane/EtOAc/*i*Pr$_2$NH) yielded pure (*S*)-MTPA ester of ingenamine (**13a**) (3.5 mg). Following the identical procedure with (*S*)-MTPA-Cl gave the (*R*)-Mosher ester of ingenamine (**13b**). The (R)- and (S)-Mosher esters of ingamine A (**14a** and **14b**) and ingenamine E (**15a** and **15b**) were prepared as described. The Mosher esters of ingamine A and ingenamine E were purified by normal phase HPLC (**14a** and **14b** eluent: 95:5:0.1 hexane/EtOAc/*i*Pr$_2$NH; **15a** and **15b** eluent: 92:8:0.1 hexane/EtOAc/*i*Pr$_2$NH).

(*S*)-MTPA-Ingenamine (13a): obtained as a white glass; ^{1}H NMR (CD_2Cl_2, 500 MHz) δ (*Δδ* in Hz, proton number) 3.09 (*-10*, H2), 5.80 (*-20*, H4), 2.20 (*-110*, H5), 2.74 (*-50*, H6), 1.63 (*-40*, H6'), 1.07 (*-50*, H8), 4.81 (*-20*, H9), 2.91 (*+35*, H10), 2.39 (*+75*, H10'), 2.29 (*0*, H12), 2.21 (*+15*, H12'), 2.85 (*-10*, H13), 2.23 (*0*, H13'), 1.44 (*0*, H14), 1.24 (*-15*, H14'), 1.58 (*-10*, H15), 1.37 (*-15*, H15'), 2.32 (*-10*, H16), 1.55 (*-15*, H16'), 5.64 (*0*, H17), 5.62 (*0*, H18), 2.27 (*-10*, H19), 1.61 (*-20*, H19'), 1.63 (*-25*, H20), 1.63 (*-25*, H20'), 2.77 (*+65*, H21), 2.24 (*+30*, H21'), 1.54 (*+25*, H22), 1.37 (*+20*, H22'), 1.52 (*+10*, H23), 1.33 (*0*, H23'), 2.07 (*+15*, H24), 2.01 (*0*, H24'), 5.42 (*0*, H25), 5.48 (*0*, H26), 2.24 (*+10*, H27), 2.20 (*-10*, H27'), 2.38 (*+10*, H28), 2.22 (*0*, H28'), 7.50 (ArH), 7.43 (ArH), 7.42 (ArH), 7.43 (ArH), 7.50 (ArH), 3.52 (MeO); HREIMS, $C_{36}H_{47}N_2O_3F_3$ (M^+) m/z: 612.3543 (ΔM 0.5 mmu).

(*R*)-MTPA-Ingenamine (13b): obtained as a white glass; ^{1}H NMR (CD_2Cl_2, 500 MHz) δ 3.11 (H2), 5.84 (H4), 2.42 (H5), 2.84 (H6), 1.70 (H6'), 1.17 (H8), 4.85 (H9), 2.84 (H10), 2.24 (H10'), 2.29 (H12), 2.18 (H12'), 2.87 (H13), 2.23 (H13'), 1.44 (H14), 1.27 (H14'), 1.60 (H15), 1.40 (H15'), 2.34 (H16), 1.58 (H16'), 5.64 (H17), 5.62 (H18), 2.29 (H19), 1.65 (H19'), 1.68 (H20), 1.68 (H20'), 2.64 (H21), 2.18 (H21'), 1.49 (H22), 1.33 (H22'), 1.50 (H23), 1.33 (H23'), 2.04 (H24), 2.01 (H24'), 5.42 (H25), 5.48 (H26), 2.22 (H27), 2.22 (H27'), 2.36 (H28), 2.22 (H28'), 7.49 (ArH), 7.43 (ArH), 7.42 (ArH), 7.43 (ArH), 7.49 (ArH), 3.52 (MeO); HREIMS, $C_{36}H_{47}N_2O_3F_3$ (M^+) m/z: 612.3544 (ΔM 0.5 mmu).

(*S*)-MTPA-Ingamine A (14a): obtained as a white glass; ^{1}H NMR (CD_2Cl_2, 500 MHz) δ (*Δδ* in Hz, proton number) 3.08 (*-10*, H2), 5.88 (*-25*, H4), 2.21 (*-120*, H5), 2.79 (*-45*, H6), 1.68 (*-40*, H6'), 1.09 (*-40*, H8), 4.77 (*-10*, H9), 2.85 (*+35*, H10), 2.74 (*+95*, H10'), 2.38 (*+10*, H12), 2.21 (*0*, H12'), 2.86 (*-10*, H13), 2.23 (*0*, H13'), 1.47 (*0*, H14), 1.26 (*0*, H14'), 1.59 (*0*, H15), 1.43 (*-20*, H15'), 2.30 (*-10*, H16), 1.58 (*0*, H16'), 5.64 (*0*, H17), 5.63 (*0*, H18), 2.23 (*-15*, H19), 1.66 (*-10*, H19'), 1.85 (*-10*, H20), 1.64 (*-30*, H20'), 2.58 (*+25*, H21), 2.54 (*+25*, H21'), 2.28 (*+20*, H22), 2.26 (*+10*, H22'), 5.35 (*0*, H23), 5.50 (*0*, H24), 2.93 (*+10*, H25), 2.73 (*-25*, H25'), 5.44 (*0*, H26), 5.44 (*0*, H27), 3.01 (*-10*, H28), 2.78 (*0*, H28'), 5.44 (*0*, H29), 5.49 (*0*, H30), 2.40 (*0*, H31), 2.20 (*-20*, H31'), 2.34 (*0*, H32), 2.05 (*0*, H32'), 7.51 (ArH), 7.43 (ArH), 7.42 (ArH), 7.43 (ArH), 7.51 (ArH), 3.53 (MeO); HREIMS, $C_{40}H_{51}N_2O_3F_3$ (M^+) m/z: 664.3850 (ΔM -0.2 mmu).

(*R*)-MTPA-Ingamine A (14b): obtained as a colorless glass; ^{1}H NMR (CD_2Cl_2, 500 MHz) δ 3.10 (H2), 5.93 (H4), 2.45 (H5), 2.88 (H6), 1.76 (H6'), 1.17 (H8), 4.79 (H9), 2.78 (H10), 2.55 (H10'), 2.36 (H12), 2.21 (H12'),

2.88 (H13), 2.23 (H13'), 1.47 (H14), 1.26 (H14'), 1.59 (H15), 1.47 (H15'), 2.32 (H16), 1.58 (H16'), 5.64 (H17), 5.63 (H18), 2.26 (H19), 1.68 (H19'), 1.87 (H20), 1.70 (H20'), 2.53 (H21), 2.49 (H21'), 2.24 (H22), 2.24 (H22'), 5.35 (H23), 5.50 (H24), 2.91 (H25), 2.78 (H25'), 5.44 (H26), 5.44 (H27), 3.03 (H28), 2.78 (H28'), 5.44 (H29), 5.49 (H30), 2.40 (H31), 2.24 (H31'), 2.34 (H32), 2.05 (H32'), 7.50 (ArH), 7.43 (ArH), 7.43 (ArH), 7.43 (ArH), 7.50 (ArH), 3.53 (MeO); HREIMS, $C_{40}H_{51}N_2O_3F_3$ (M+) m/z: 664.3852 (ΔM 0.1 mmu).

(*S*)-MTPA-Ingenamine E (15a): obtained as a colorless glass; ^1H NMR (CD_2Cl_2, 500 MHz) δ (Δδ in Hz, proton number) 2.98 (-10, H2), 5.85 (0, H4), 2.18 (-100, H5), 2.90 (-45, H6), 1.68 (-45, H6'), 1.13 (-50, H8), 4.81 (0, H9), 2.91 (+35, H10), 2.46 (+70, H10'), 2.31 (0, H12), 2.23 (0, H12'), 2.45 (-10, H13), 2.20 (-10, H13'), 2.29 (0, H14), 2.10 (-10, H14'), 5.37 (0, H15), 5.51 (0, H16), 2.84 (0, H17), 2.79 (0, H17'), 5.37 (0, H18), 5.38 (0, H19), 3.15 (0, H20), 2.78 (0, H20'), 5.43 (0, H21), 5.38 (-10, H22), 2.26 (-15, H23), 1.72 (-20, H23'), 1.73 (-45, H24), 1.44 (-40, H24'), 2.78 (+50, H25), 2.26 (+30, H25'), 1.54 (+20, H26), 1.38 (+25, H26'), 1.50 (0, H27), 1.32 (0, H27'), 2.06 (0, H28), 2.01 (0, H28'), 5.41 (0, H29), 5.44 (0, H30), 2.22 (0, H31), 2.22 (0, H31'), 2.34 (-10, H32), 2.12 (0, H32'), 7.50 (ArH), 7.43 (ArH), 7.43 (ArH), 7.43 (ArH), 7.50 (ArH), 3.53 (MeO); HREIMS, $C_{40}H_{51}N_2O_3F_3$ (M+) m/z: 664.38501 (ΔM -0.2 mmu).

(*R*)-MTPA-Ingenamine E (15b): obtained as a colorless glass; ^1H NMR (CD_2Cl_2, 500 MHz) δ 3.00 (H2), 5.85 (H4), 2.38 (H5), 2.99 (H6), 1.76 (H6'), 1.23 (H8), 4.81 (H9), 2.84 (H10), 2.32 (H10'), 2.31 (H12), 2.23 (H12'), 2.47 (H13), 2.22 (H13'), 2.29 (H14), 2.12 (H14'), 5.37 (H15), 5.51 (H16), 2.84 (H17), 2.79 (H17'), 5.37 (H18), 5.38 (H19), 3.15 (H20), 2.78 (H20'), 5.43 (H21), 5.40 (H22), 2.29 (H23), 1.76 (H23'), 1.82 (H24), 1.52 (H24'), 2.68 (H25), 2.20 (H25'), 1.50 (H26), 1.33 (H26'), 1.50 (H27), 1.32 (H27'), 2.06 (H28), 2.01 (H28'), 5.41 (H29), 5.44 (H30), 2.22 (H31), 2.22 (H31'), 2.36 (H32), 2.12 (H32'), 7.49 (ArH), 7.43 (ArH), 7.43 (ArH), 7.43 (ArH), 7.49 (ArH), 3.52 (MeO); HREIMS, $C_{40}H_{51}N_2O_3F_3$ (M+) m/z: 664.38435 (ΔM -0.8 mmu).

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