Novel Polycyclic Guanidine Alkaloids from Two Marine Sponges of the Genus *Monanchora*

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Two marine sponges of the genus *Monanchora* (Poecilosclerida, Crambeidae) have been found to contain new polycyclic guanidine alkaloids bearing the (5,6,8*b*)-triazaperhydroacenaphthylene skeleton. Their structures have been determined by detailed spectroscopic analysis. Dehydrobatzelladine C (**1**) has been isolated from *M. arbuscula* and crambescidins 359 (**2**) and 431 (**3**) from *M. unguiculata*. The chemotaxonomic implications of these findings are discussed.

Since the isolation by Kashman et al.[1] of ptilomycalin A from a Caribbean sponge identified as *Ptilocaulis spiculifer* and from a Red Sea sponge identified as *Hemimycale* sp., several alkaloids possessing the unique (5,6,8*b*)-triazaperhydroacenaphthylene system have been reported from four other marine sponge samples: crambescidins 800, 816, 830, and 844; isocrambescidin 800; and crambidine; from several samples of the Mediterranean sponge *Crambe crambe*,[2−4] crambescidin 800; from a Brazilian specimen of *Monanchora arbuscula*,[5] ptilomycalin A, crambescidins 800 and 816, and batzelladines A−E from *Batzella* sp.,[6] collected in the Bahamas; and batzelladines F−I from another *Batzella* sp. collected in Jamaica.[7] Interestingly, Palagiano et al. reported recently the puzzling discovery of the same type of compounds (crambescidin 800, ptilomycalin A, celeromycalin, and fromiamycalin) from two New Caledonian starfish, *Celerina heffernani* and *Fromia monilis*.[8] Many of these cyclic guanidine derivatives show noteworthy biological activities (e.g., HIV gp120-human CD4-binding inhibition,[6] $p56^{lck}$-CD4 dissociation induction,[7] Ca^{2+} channel blocker activities,[2] cytotoxicity,[8] and antifungal and antimicrobial activities[3]).

It has been suggested by Van Soest et al.[9] that, because of similar morphological characters and identical secondary metabolite content, the above-mentioned sponges should eventually be united in the same genus. To evaluate the validity of this suggestion, the secondary metabolite content of another species of *Monanchora*, namely, *M. unguiculata*, was investigated. This led to the isolation of two new cyclic guanidine alkaloids bearing the (5,6,8*b*)-triazaperhydroacenaphthylene skeleton, crambescidin 359 (**2**) and crambescidin 431 (**3**). Moreover, a further cyclic guanidine alkaloid, dehydrobatzelladine C (**1**), was isolated from *M. arbuscula*. The structure determination of these new compounds, except the absolute configuration, is presented herein. These compounds occur naturally as the protonated guanidinium salts. This implies that the spectroscopic properties reported for **1**, **2**, and **3** are those of the protonated salts rather than those of the free bases.

Results and Discussion

In a recent paper,[10] we reported the isolation of crambescidin 800, ptilocaulin, and 8b-hydroxyptilocaulin from the methanolic extract of the sponge *M. arbuscula*, collected at Cat Cay Lagoon in the barrier reef of Belize. Further fractionation of this extract by Sephadex LH-20 chromatography, followed by reversed-phase preparative HPLC afforded compound **1** as a slightly brown gum. Its molecular formula was determined as $C_{27}H_{46}N_6O_2$ [$(M + H)^+$ at *m*/*z* 487.3711 by positive HRFABMS]. An intense fragment peak at *m*/*z* 373.2796 ($C_{22}H_{35}N_3O_2$) revealed the loss from the molecular ion of 113 Da, corresponding to a molecule of 4-guanidino-1-butene, also observed in batzelladine C[6] and resulting from a McLafferty rearrangement. This was confirmed by the presence of an intense peak at *m*/*z* 114 ($C_5H_{12}N_3$) corresponding to a protonated molecule of 4-guanidino-1-butene. Complete assignments of the ^1H and ^{13}C NMR spectra were performed using 1D and 2D NMR experiments ($^1H-^1H$ COSY, HMQC, HMBC, NOEDS) and are reported in Table 1. The molecule has eight degrees of unsaturation and, because the ^{13}C NMR data require one C=C double bond (δ_C 167.0 and 112.8), one ester carbonyl (δ_C 164.5), and three C=N bonds (δ_C 152.5, 158.8, and 181.4), it must contain three rings. The close relationship between the ^{13}C NMR and UV spectrum (λ_{max} at 206, 258, and 303 nm) of **1** and those of crambidine[2] immediately suggested that **1** possessed the same chromophore as the latter. In addition, the presence of the 4-guanidino−*n*-butyl chain was confirmed because the chemical shifts of the hydrogen and carbon atoms of this part of the molecule are nearly identical with those of its counterparts in crambescin A[11] and batzelladine C.[6] The presence of a pentyl side chain at C-15 and an heptyl side chain at C-8 was ascertained by NMR (Table 1) and MS data. In particular, the ^1H NMR spectrum of **1** contained a contiguous series of signals from the two allylic H-12 (δ 3.54 and 3.30) proton signals to the H-8 signal at δ 3.78, itself coupled to the first methylene (H_2-7) of the heptyl side chain (δ 1.84 and 1.64). On the other hand, a correlation between C-15 (δ_C 181.4) and the hydrogen atoms of the two first methylene groups of the pentyl side chain (H_2-16, δ 3.06) and the H_2C-17 methylene at δ 1.72 in the HMBC spectrum allowed us to locate the pentyl chain on the heterocyclic skeleton of **1**. The relative configuration of **1** could be established by NOE difference spectra, which

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Table 1. ^{1}H and ^{13}C NMR Data of Dehydrobatzelladine C (**1**) (600 and 150.87 MHz, CD_3OD, δ, J in Hz)

position	^{1}H	^{13}C
1	0.88, t, 6.5[a]	14.1[a]
2	1.33, m[b]	23.7[b]
3	1.32, m	33.0
4	1.32, m	30.5
5	1.32, m	30.5
6	1.46, m	26.0
7	1.84, m; 1.64, m	35.2
8	3.78, m	53.3
9α	1.58, q, 13.5	31.0
9β	2.58, dt, 13.5, 3.5	
10	4.58, m	63.2
11α	1.99, m[c]	30.2
11β	2.64, m[d]	
12α	3.54, br d, 8[e]	33.0
12β	3.30, m[e,f]	
13		167.0
14		112.8
15		181.4
16	3.06, t, 7[e]	38.5
17	1.72, m	29.0
18	1.38, m	32.8
19	1.36, m[b]	23.5[b]
20	0.90, t, 6.5[a]	14.3[a]
21		152.5
22		164.5
23	4.38, t, 6.4[e]	66.5
24	1.82, m	26.8
25	1.72, m	26.6
26	3.24, t, 7[e]	42.0
27		158.8

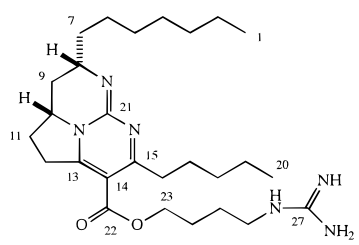
[a,b] Assignments may be interchanged. [c] Becomes t (J = 12 Hz) upon exchange of H_2-12. [d] Becomes dd (J = 12, 6 Hz) upon exchange of H_2-12. [e] Disappears slowly in CD_3OD. [f] Overlapped by solvent signal.

showed that the hydrogen atoms at C-10 and C-8 are in a cis relationship. All these arguments indicated structure **1** for the new compound. It follows that compound **1** only differs from batzelladine C[6] by the number and arrangement of C=N double bonds, and, accordingly, we have named the latter dehydrobatzelladine C.

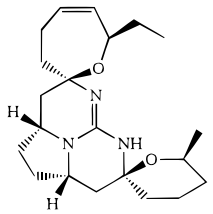
It is worth pointing out that the ^{1}H NMR spectrum of **1** in CD_3OD varies with time. After two or three hours, the signals of H_2-12 and H_2-16 (i.e., the hydrogen atoms allylic to the unsaturated heterocycle) disappeared almost completely due to exchange with deuterium. Moreover, when the sample was left for several days in CD_3OD, other signals were affected: the H_2-23 triplet of the ester moiety shifted from δ 4.38 to 3.58, whereas the triplet of the H_2-26 linked to the guanidine shifted slightly from δ 3.24 to 3.20. These transformations were interpreted as resulting from a transesterification of the ester function with CD_3-OD, releasing deuterated methyl ester **4** and free 4-guanidinobutan-1-ol. It is presumed that both the exchange of the allylic hydrogen atoms and the transesterification reaction in CD_3OD are catalyzed by the basicity of the guanidine moieties of **1**.

Freshly collected specimens of *M. unguiculata* were immediately immersed in ethanol and stored at room temperature. The ethanol extract was concentrated in vacuo to an aqueous suspension and then partitioned between water and dichloromethane. The dichloromethane-soluble materials exhibited toxic activities against nauplii of the brine shrimp *Artemia salina* (LD_{50} 2 mg/L).[12] Successive Si gel vacuum−liquid and flash column chromatographies followed by C_{18} reversed-phase chromatography led to the isolation of two new alkaloids, compounds **2** and **3**, the structures of which were elucidated on the basis of their spectral properties.

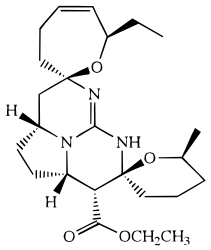
Compound **2** (crambescidin 359) gave a parent ion at m/z 359.2567 in the HREIMS appropriate for a molecular formula of $C_{21}H_{33}N_3O_2$ (calcd 359.2573). The ^{13}C NMR spectrum of **2** (Table 3) showed resolved peaks for all 21 carbon atoms, and HMQC experiments indicated that 32 out of the 33 hydrogen atoms were attached to carbons (3 C, 6 CH, 10 CH_2, and 2 CH_3). Two exchangeable protons at δ 9.95 and 9.63 in the ^{1}H NMR spectrum implied that **2** was N-protonated. The ^{13}C NMR spectrum of **2** (Table 3) also exhibited peaks characteristic of a guanidine group (δ_C 148.4) and of a disubstituted double bond (δ_C 133.7 and 129.7; δ 5.46 and 5.64). The absence of evidence for additional unsaturated functionality suggested that the remaining five unsaturations required by the molecular formula of **2** had to be accounted for by rings. The assignments of the signals of the ^{1}H and ^{13}C NMR spectra of **2** are presented in Tables 2 and 3. They are based upon the analysis of the 1D and 2D NMR spectra at highfield (^{1}H−^{1}H COSY, HMQC, HMBC, and NOEDS). Comparison of these data with those of acetylcrambescidin 800 (**5**)[2] clearly indicated that compounds **2** and **5** had the same pentacyclic guanidine moiety. The most significant difference in the ^{1}H NMR spectra of the two compounds was the replacement of the doublet at δ 2.94 in **5**, attributable to H-14, by two 1H signals at δ 2.16 and 1.42. Moreover, in the ^{13}C NMR spectrum of **2** the signal attributable to C-14 appeared at δ 39.7 rather than at δ 48.9, and in the HMBC spectrum of **2** were observed clear correlations between the H_2-14 signals and those of C-13 (δ_C 51.4) and C-15 (δ_C 80.1). These data led us to assign structure **2** to crambescidin 359. As far as we know, this is the first report of a ptilomycalin A-type derivative lacking the usual carboxy substituent at C-14.



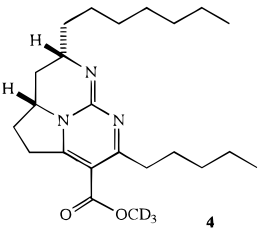
Dehydrobatzelladine C **1**



Crambescidin 359 **2**



Crambescidin 431 **3**



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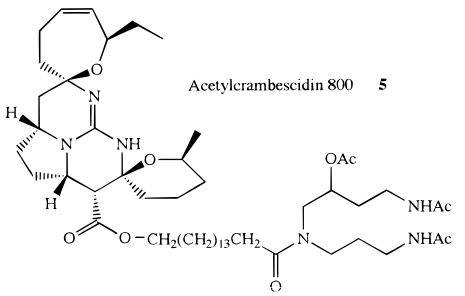

Acetylcrambescidin 800 **5**

Table 2. Comparison of the ^{1}H NMR Data of Crambescidin 359 (**2**), Crambescidin 431 (**3**), and Acetylcrambescidin 800 (**5**)[2] [600 MHz, $CDCl_3$, δ, J in Hz]

position	**2**	**3**	**5**[2]
H_3C-1	0.81, t, 7.3	0.80, t, 7	0.83, t, 7.2
H_2C-2	1.50, m; 1.42, m	1.48, m; 1.37, m	1.52, m; 1.48, m
HC-3	4.49, m	4.47, m	4.49, m
HC-4	5.46, br d, 11	5.41, dt, 11, 2, 2	5.49, dt, 10, 2, 2
HC-5	5.64, br dd, 11, 8	5.62, ddt,11,7,2,2 22	5.67, ddt,10,7,2,2
H_2C-6	2.30, m; 2.18, m[a]	2.27, m; 2.11, m	2.34, m; 2.16, m
H_2C-7α	2.48, m	2.46, m	2.54, m
H_2C-7β	1.88, m	1.86, m	1.92, m
H_2C-9α	1.28, dd, 13, 13	1.35, dd, 13, 13	1.40, dd, 12, 12
H_2C-9β	2.53, dd, 13, 5	2.50, dd, 13, 5	2.56, dd, 12, 4
HC-10	3.98, m	3.92, m	4.02, m
H_2C-11	2.27, m; 1.65, m	2.10, m; 1.60, m	2.27, m; 1.65, m
H_2C-12	2.27, m; 2.16, m[a]	2.22, m; 1.78, m	2.27, m; 1.80, m
HC-13	3.98, m	4.23, m	4.30, m
H_2C-14 or HC-14	2.16, m; 1.42, m	2.85, d, 5	2.94, d, 5
H_2C-16	1.75, m; 1.57, m	1.70, m; 1.60, m	not reported
H_2C-17	2.27, m; 1.75, m	2.25, m; 1.71, m	not reported
H_2C-18	1.65, m; 1.16, m	1.70, m; 1.15, m	not reported
HC-19	3.84, m	3.92, m	3.93, m
H_3C-20	1.03, d, 7	1.02, d, 6	1.05, d, 6.5
H_2C-23		4.08, m	4.12, m; 4.07, m
H_3C-24		1.24, t, 7	

[a] Assignments may be interchanged.

Table 3. Comparison of the ^{13}C NMR Data of Crambescidin 359 (**2**), Crambescidin 431 (**3**), and Acetylcrambescidin 800 (**5**)[2] [150.87 MHz, $CDCl_3$, δ]

position	**2**	**3**	**5**[2]
H_3C-1	10.2	10.1	10.1
H_2C-2	29.1	29.1	29.8
HC-3	70.9	70.8	71.0
HC-4	133.7	133.7	133.6
HC-5	129.7	129.8	129.8
H_2C-6	23.5	23.5	23.5
H_2C-7	37.0	36.8	37.0
C-8	83.5	83.6	83.7
H_2C-9	37.3	36.9	36.9
HC-10	53.0	53.8	53.9
H_2C-11	30.0	30.6	30.6
H_2C-12	23.5	26.8	27.0
HC-13	51.4	51.8	51.9
H_2C-14 or HC-14	39.7	49.8	48.9
C-15	80.1	80.7	80.7
H_2C-16	33.7	32.0	not reported[a]
H_2C-17	18.5	18.3	not reported[a]
H_2C-18	32.3	31.9	not reported[a]
HC-19	67.0	67.2	67.2
H_3C-20	21.5	21.4	21.4
C-21	148.4	148.9	148.8
O=C-22		168.2	168.8
H_2C-23		61.2	65.5
H_3C-24		14.2	

[a] The chemical shifts reported[3] for crambescidin 800 in CD_3OD are the following: H_2C-16, 32.8; H_2C-17, 18.1; H_2C-18, 32.1.

Compound **3** (crambescidin 431) possessed the molecular formula $C_{24}H_{37}N_3O_4$ as shown by HREIMS (M^+ at m/z 431.2780, calcd 431.2784). Comparison of the ^{1}H and ^{13}C NMR data (Tables 2 and 3) with those of acetylcrambescidin 800 (**5**)[2] clearly showed that both compounds possess the same pentacyclic guanidine skeleton. The only difference was the replacement of the signals attributable to the hydroxyspermidine moiety of **5** by signals characteristic of an ethyl group [δ 4.08 (2H) and 1.24 (3H); δ_C 61.2 and 14.2]. This suggested that compound **3** is formally the ethyl ester resulting from transesterification of crambescidin 800 or from esterification of the corresponding carboxylic acid by ethanol. This was supported by analysis of the 1D and 2D NMR spectra at highfield (^{1}H−^{1}H COSY, HMQC) that allowed the assignments presented in Tables 2 and 3. The relative stereochemistry for **3** was determined by NOE difference experiments. Irradiation of H_3-1 produced an enhancement to H-10, while irradiations of H_3-20 produced an enhancement to H-10 and H-13, of H-14 to H-13, of H-10 to H-13 and H_3-20, and of H-13 to H-14, H-10, and H_3-20. These observations clearly defined the relative stereochemistry of **3** as shown.

In conclusion, this work confirms the occurrence of polycyclic guanidine alkaloids in sponges of the genus *Monanchora* (Poecilosclerida, Crambeidae). Closely related compounds have been isolated from several Mediterranean samples of *Crambe crambe*[2−4] and from Caribbean samples of *Batzella* sp.[6,7] In addition, these compounds co-occur in the same sponges with biogenetically related crambescin- and ptilocaulin-type derivatives.[9,13] Thus, because of similar morphological characters (skeletal structure, spicule shape variation, subdivision into two spicule categories, rarity or absence of microscleres) and identical secondary metabolite contents, the above-mentioned sponges should eventually be united in one sponge genus, which, for priority reasons, has to be *Crambe*. This proposal is reinforced by the recent observation by one of us (R. V. S.) that the Bahamian specimen of *Batzella* sp. studied by Patil et al.[6] is, in fact, a specimen of the variable species *M. arbuscula*.[9]

Moreover, polycyclic guanidine alkaloids were also reported from sponges of the genera *Ptilocaulis* and *Hemimycale*.[1,14] But, in these cases, the identifications are questionable.[9] In addition, we assume that the occurrence of such alkaloids in New Caledonian asteroids[8] is most probably due to sequestration of these compounds from sponges, as several asteroids are known sponge predators. Interestingly, ptilocaulin-type derivatives have been recently reported from the Poecilosclerid sponge *Arenochalina mirabilis* (Mycalidae),[15] suggesting that the distribution of the polycyclic guanidine alkaloids may be wider than had been previously found.

Experimental Section

General Experimental Procedures. UV−vis spectra were recorded on a Philips PU 8700 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3OD at 600 and 150.87 MHz, respectively, using a Varian Unity 600 instrument. HREIMS and HRFABMS were performed on

a Micromass Autospec 3F instrument. Thin-layer chromatography (TLC) analyses were performed on 0.25-mm Polygram Si gel SILG/UV_{254} precoated plates (Macherey Nagel) and column chromatographies over Si gel (MN Kieselgel 0.04−0.063 mm), using the flash technique. HPLC separations were performed on a Waters LCM1 plus apparatus coupled to a Waters 996 photodiode array detector, using a Lichrospher 60 RP select B column (6 × 250 mm; 10 μm).

Biological Material. A specimen of *Monanchora arbuscula* was collected by snorkling from among *Rhizophora mangle* mangrove roots at a 0.5-m depth in Cat Cay Lagoon, Pelican Cays, off the coast of Belize. The sponge formed upright branches up to 10 cm high, 1 cm in diameter, with an undulating surface. Its color was red with a white pattern formed by superficial channels; skeleton, plumoreticulate, with irregular knotty spongin-enforced main fibers 45−120 μm in diameter with a core of 4−20 spicules, interconnected irregularly by fibers cored by 1−5 spicules. Between the fibers were abundant microscleres. At the surface the spicules fan out from the fibers and take up a tangential position. Fibers were cored by tylostyles and thin subtylostyles; at the surface there were only subtylostyles and microscleres. Spicule sizes: tylostyles 265−425 × 9−14 μm, subtylostyles 210−388 × 2−4 μm, anchorate−unguiferate isochelae 24−28 μm, reduced sigmoid chelae 12−15 μm; no spined microrhabds were observed in the preparations. A voucher fragment consisting of a 3.5-cm long branch is kept in the collections of the Zoological Museum of Amsterdam under reg. no. 10571.

A large specimen of *M. unguiculata* was collected by scuba during The Netherlands Indian Ocean Program 1992−93 at station 793, on the south side of St. Joseph's Atoll, Amirantes Islands (Republic of the Seychelles), at a depth of 10 m. It formed an extensive thin crust of 20 × 15 × 0.3 cm on a dead coral plate. The surface was smooth, and it had a characteristic white-gray pattern of superficial channels on a bright red background. Consistency was soft. The skeleton consists of columns of megascleres rising up from a basal spongin plate and fanning out at the surface. Basal spongin plate and spongin columns incorporate spicules as well as considerable amounts of foreign debris. Spicule sizes: tylostyles not clearly differentiated from subtylostyles, up to 360 × 10 μm, with sharp point; subtylostyles 210−340 × 2−6 μm, often with rounded or mucronate endings; strongly curved reduced unguiferate chelae 27−33 μm with 3−5 vesitigial teeth. This closely matches the original description of the type. A voucher specimen consisting of two encrusted coral fragments of 8 × 8 cm is kept in the Zoological Museum of Amsterdam under reg. no. 10406.

Extraction and Isolation. Samples of *M. arbuscula* collected at Cat Cay Lagoon (Belize) were stored in MeOH and extracted exhaustively with MeOH−CH_2Cl_2. The organic solvents were evaporated in vacuo, and the residual aqueous solution was successively extracted with hexane, CCl_4, and *n*-BuOH. The CCl_4 and *n*-BuOH extracts were combined and chromatographed on Sephadex LH-20 (eluent MeOH). The separations were monitored by TLC (eluent: lower phase of the mixture $CHCl_3$−MeOH−*i*-PrOH−H_2O 9:12:1:8; visualized by spraying with vanillin−conc H_2SO_4 and heating at 120 °C). This afforded crambescidin 800 (20 mg), ptilocaulin (43 mg), and 8b-hydroxyptilocaulin (3 mg).[5,10] In addition, one of the fractions contained a mixture of more polar compounds from which dehydrobatzelladine C (12 mg) was separated by HPLC (UV detection at 212 nm; eluent: MeOH−0.1% aqueous NH_4-Ac, 70:30).

Specimens of *M. unguiculata* (12.6 g dry wt) stored in EtOH were exhaustively extracted with MeOH−CH_2Cl_2. The extract was evaporated in vacuo and the residue (7.5 g) partitioned between water and CH_2Cl_2. The organic phase was evaporated to dryness in vacuo to obtain a gum (1.065 g) that was toxic against nauplii of the brine shrimp *A. salina* (LD_{50} 2 mg/L). This gum was flash chromatographed several times over Si gel columns using successive mixtures of hexane−acetone and CH_2Cl_2−MeOH as solvents to afford, after final purification on a Si gel RP_{18} column (eluent MeOH−H_2O), crambescidin 359 (**2**, 6 mg) and crambescidin 431 (**3**, 2.5 mg).

Dehydrobatzelladine C (1): slightly brown gum; UV (CH_3-OH) λ_{max} at 206 (8500), 258 (7500), 303 nm (1600); ^{1}H and ^{13}C NMR spectra, see Table 1; positive HRFABMS *m/z* 487.3711 (30) $(M + H)^+$; calcd for $C_{27}H_{47}N_6O_2$ 487.3760), 463 (75), 373.2796 (100; calcd for $C_{22}H_{35}N_3O_2$ 373.2729), 350 (68), 330 (27), 318 (15), 304 (21), 290 (14), 132 (13), 114 (47).

Crambescidin 359 (2): colorless gum; $[\alpha]_{577}$ −9° (*c* 0.18, CH_2Cl_2); ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3; HREIMS *m/z* 359.2567 (45; M^+; calcd for $C_{21}H_{33}N_3O_2$ 359.2573), 330 (90), 301 (45), 288 (50), 262 (100), 261 (80), 150 (21).

Crambescidin 431 (3): colorless gum; $[\alpha]_{577}$ 12° (*c* 0.19, CH_2Cl_2); ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3; HREIMS *m/z* 431.2780 (59; M^+; calcd for $C_{24}H_{37}N_3O_4$ 431.2784), 402 (90), 388 (45), 373 (40), 358 (100), 334 (85), 304 (52), 260 (42), 162 (84), 151 (65), 113 (64).

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References and Notes

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