# Amphilactams A−D: Novel Nematocides from Southern Australian Marine Sponges of the Genus *Amphimedon*

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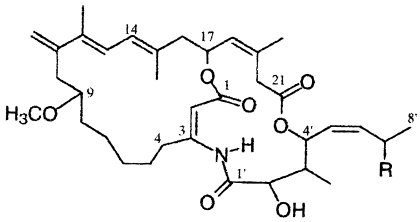
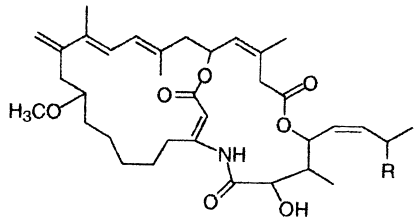
Bioassay-directed fractionation of the ethanol extracts of two *Amphimedon* spp. collected during trawling operations in the Great Australian Bight yielded four new macrocyclic lactone/lactams, amphilactams A−D (**1**−**4**). The amphilactams possess potent in vitro nematocidal properties, and their structures were assigned on the basis of detailed spectroscopic analysis and comparison with synthetic model compounds. The amphilactams feature both carbon skeletons and an enamino lactone/lactam moiety unprecedented in the natural products literature.

During the course of our ongoing investigations into marine metabolites with agrochemical potential, we have screened >1000 southern Australian and Antarctic marine sponges for in vitro nematocidal activity. Among the earliest leads detected in this screening program were extracts derived from two *Amphimedon* spp., collected at different locations in the Great Australian Bight. The crude ethanol extract of these sponges inhibited larval development of the nematode *Haemonchus contortus* ($LD_{50}$ = 4.0 and $LD_{99}$ = 130 $\mu$g/mL, respectively). In both cases, the bioactive agents partitioned into the $CH_2Cl_2$-soluble fraction and displayed common $^1$H NMR characteristics. These observations encouraged us to believe that both sponges contained identical and/or closely related bioactive agents. Bioassay-directed fractionation of both extracts yielded four new marine metabolites, amphilactams A−D (**1**−**4**), which represent a unique class of bicyclic macrocycles containing a novel enamino lactone/lactam moiety with selective nematocidal activity. This report describes the isolation, structure elucidation, and biological evaluation of these novel marine metabolites.




(**1**) R = H
(**2**) R = $CH_3$




(**3**) R = H
(**4**) R = $CH_3$

## Results and Discussion

The decanted crude aqueous ethanol extract of both *Amphimedon* sponges were concentrated separately in vacuo and triturated with $CH_2Cl_2$. The in vitro nematocidal activity partitioned into the $CH_2Cl_2$-soluble fractions, and this material was further resolved by silica solid-phase extraction and HPLC to yield a total of four pure nematocides, amphilactams A−D (**1**−**4**). The *Amphimedon* sp (F79979) collected in 1994 returned amphilactams A (**1**) and B (**2**), while the *Amphimedon* sp (F79980) collected in 1995 returned amphilactams A (**1**), C (**3**) and D (**4**).

Amphilactam A (**1**) was isolated as a stable oil with a molecular formula (M + Na, $C_{35}H_{51}NO_7Na$, $\Delta$ 1.7 mmu) requiring 11 degrees of unsaturation. Initially characterized by NMR in $CDCl_3$, $^1$H NMR spectra acquired in benzene-$d_6$ provided better dispersion of important resonances. The $^1$H NMR (benzene-$d_6$) spectrum for **1** revealed resonances consistent with three olefinic methyls ($\delta$ 1.60, 1.73, and 1.80), a primary methyl ($\delta$ 0.94), a secondary methyl ($\delta$ 0.82), an oxymethyl ($\delta$ 3.20), and an exchangeable amide/lactam proton ($\delta$ 10.03). Additional resonances were observed in the $^1$H and $^{13}$C NMR (benzene-$d_6$) spectra for a 1,1-disubstituted double bond ($^1$H, $\delta$ 4.81 and 5.08; $^{13}$C, 114.8 and 134.0 ppm), a *Z* 1,2-disubstituted double bond ($^1$H, $\delta$ 5.25 and 5.54, $J$ = 10.7 Hz; $^{13}$C, 127.4 and 137.0 ppm), and a trisubstituted double bond ($^1$H, $\delta$ 5.26; $^{13}$C, 124.5 and 142.6 ppm), as well as a 1,1,4,4-tetrasubstituted conjugated diene ($^1$H, $\delta$ 6.40 and 6.61; $^{13}$C, 122.8, 126.1, 135.6, and 145.3 ppm). The $^{13}$C NMR spectra also revealed resonances consistent with three lactone/lactam carbonyls (167.8, 170.8, and 176.1 ppm) and four oxymethine carbons (69.8, 71.2, 72.0,

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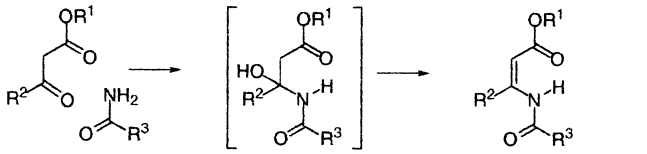
**Table 1. NMR Data (Benzene-$d_6$) for Amphilactam A (1)[a]**

| no. | $^{13}$C $\delta$ | $^{1}$H $\delta$ | m | $J$ (Hz) | COSY | TOCSY | gHMBC $^{1}$H to $^{13}$C | NOESY[b] |
|---|---|---|---|---|---|---|---|---|
| 1 | 176.1 | | | | | | | |
| 2 | 89.5 | 4.78 | s | | | $H_b$5 | C3, C4 | |
| 3 | 158.6 | | | | | | | |
| 4 | 40.9 | 2.30 | m | | | | | |
| | | 2.52 | m | | | | | |
| 5 | 27.1 | 1.10 | m | | | | | |
| | | 1.65 | m | | | | | |
| 6 | 27.9 | 2.42 | m | | | | | |
| 7 | 36.4 | 1.25 | m | | | | | |
| | | 1.50 | m | | | | | |
| 8 | 25.7 | 1.40 | m | | H9 | | | |
| 9 | 77.1 | 4.11 | m | | $H_a$10, $H_b$10, H8 | | | H13 |
| 10 | 39.0 | 2.15 | m | | H9 | | | |
| | | 2.84 | m | | H9 | | | |
| 11 | 134.0 | | | | | | | |
| 12 | 145.3 | | | | | | | |
| 13 | 122.8 | 6.61 | d | 11.4 | H14 | 15-C$H_3$ | C12, 12-C$H_3$ | H9, 15-C$H_3$ |
| 14 | 126.1 | 6.40 | d | 11.4 | H13 | 12-C$H_3$ | C16, 15-C$H_3$ | H18, 12-C$H_3$ |
| 15 | 135.6 | | | | | | | |
| 16 | 47.0 | 2.43 | m | | H17 | | | |
| | | 2.85 | m | | H17 | | | |
| 17 | 71.2 | 5.94 | ddd | 10.7, 10.7, 3.2 | $H16_a$, $H16_b$, $H_{18}$ | | | |
| 18 | 124.5 | 5.26 | d | 10.7 | H17 | | C20, 19-C$H_2$ | H14, 19-C$H_3$ |
| 19 | 142.6 | | | | | | | |
| 20 | 33.7 | 1.65 | m | | | | | |
| | | 2.45 | m | | | | C21 | |
| 21 | 170.8 | | | | | | | |
| NH | | 10.03 | br s | | | | | |
| 1′ | 167.8 | | | | | | | |
| 2′ | 69.8 | 3.87 | d | 10.6 | H3′ | | 3′-C$H_2$ | |
| 3′ | 43.3 | 1.40 | m | | H2′, H4′, 3-C$H_3$ | | | |
| 4′ | 72.0 | 5.66 | dd | 10.7, 10.7 | H3′, H5′ | H6′, 3′-C$H_3$ | C6′ | H8′, 3-C$H_3$ |
| 5′ | 127.4 | 5.25 | dd | 10.7, 10.7 | H4′, H6′ | $H_b$7′, H8′, 3′-C$H_3$ | C7′ | |
| 6′ | 137.0 | 5.54 | ddd | 10.7, 10.7, 7.5 | H5′, $H_a$7′, $H_b$7′ | H4′, H8′ | C4′ | |
| 7′ | 21.8 | 2.15 | m | | H6′, H8′ | | C6′, C8′ | |
| | | 2.40 | m | | H6′, H8′ | H5′ | C5′, C6′, C8′ | |
| 8′ | 14.1 | 0.94 | dd | 7.4, 7.4 | $H_a$7′, $H_b$7′ | H5′, H6′ | C6′, C7′ | |
| −O$CH_3$ | 54.6 | 3.20 | s | | | | C9 | |
| 11-$CH_2$ | 114.8 | 4.81 | s | | $H_b$11 | | C10, C11 | |
| | | 5.08 | s | | $H_a$11 | | | 12-C$H_3$ |
| 12-$CH_3$ | 18.5 | 1.73 | s | | | H14 | C11, C12, C13 | $H_b$11, H14 |
| 15-$CH_3$ | 14.4 | 1.80 | s | | | H13 | C14, C15, C16 | H13 |
| 19-$CH_3$ | 23.9 | 1.60 | s | | | | C18, C19, C20 | H8′, H18 |
| 3′-$CH_3$ | 8.7 | 0.82 | d | 6.8 | H3′ | H4′ | C2′, C3′ | |

[a] NMR data is reported in benzene-$d_6$ due to improved dispersion compared to $CDCl_3$. Assignments are supported by DEPT 135, DEPT 90, and gHMQC experiments. [b] NOESY experiment run in $CDCl_3$.

and 77.1 ppm). Perhaps the most intriguing feature of the $^{13}$C NMR data for amphilactam A (**1**) were the shielded and deshielded resonances for a trisubstituted double bond ($^{1}$H, $\delta$ 4.78; $^{13}$C, 89.5 and 158.6 ppm). From the analysis presented above, it became clear that amphilactam A (**1**) was a highly functionalized bicyclic macrocycle.

Analysis of the $^{1}$H−$^{1}$H COSY and $^{1}$H−$^{13}$C gHMBC NMR data for amphilactam A (**1**) (see Table 1) revealed connectivity sequences from C8 through C20 and from C1′ through C8′ (see the structure diagram). Although significant overlap occurred between $^{1}$H NMR resonances for $H_2$4 through $H_2$7, given their chemical shifts and other considerations listed above, the connectivity sequence could be extended from C4 through C20. The isolated nature of the $^{1}$H NMR spin system for $H_2$20 together with its chemical shift ($\delta$ 1.65 and 2.45) permitted assignment of C21 as one of the two remaining carbonyl carbons. $^{1}$H−$^{15}$N gHMBC NMR analysis of amphilactam A (**1**) revealed correlations from H2 to the amide/lactam *N*H. Consequently, the chemical shifts associated with C2 and C3 could be attributed to an acylated enamino lactone, as indicated in the structure diagram. In an attempt to provide supporting spectroscopic evidence for this novel functional group, the model compound **5** was prepared in excellent yield in a one-step condensation and dehydration of methyl 3-oxobutanoate with acetamide (see Figure 1). That this reaction yielded only the Z isomer (as determined by the deshielded H-bonded character of the N*H* resonance: see below) was attributed to intramolecular H bonding in the condensation product prior to loss of water. Selected NMR data for **5** ($^{1}$H, NH, $\delta$ 11.10; H 2, $\delta$ 4.75; $^{13}$C, C2, 95.5 ppm; C3, 155.8 ppm) was a good match with that for the relevant structural unit in amphilactam A (**1**) ($^{1}$H, NH, $\delta$ 10.03; H2, $\delta$ 4.78; $^{13}$C, C2, 89.5 ppm; C3, 158.6 ppm). Prompted by the ease with which the model compound **5** was prepared, we successfully applied this methodology to the preparation the




(**5**) $R^1 = R^2 = R^3 = CH_3$

(**6**) $R^1 = C_8H_{17}$, $R^2 = R^3 = C_7H_{15}$

**Figure 1.**

**Table 2. NMR Data (Benzene-$d_6$) for Amphilactam C (3)[a]**

| no. | $^{13}$C $\delta$ | $^{1}$H $\delta$ | m | $J$ (Hz) | DQCOSY | gHMBC $^{1}$H to $^{13}$C |
|---|---|---|---|---|---|---|
| 1 | 176.6 | | | | | |
| 2 | 92.2 | 5.08 | s | | | |
| 3 | 158.8 | | | | | |
| 4 | 40.8 | 2.35 | m | | | |
| | | 2.50 | m | | | |
| 5 | 28.1 | 1.62 | m | | | |
| 6 | 30.2 | 1.30 | m | | | |
| 7 | 27.8 | 1.60 | m | | | |
| 8 | 26.3 | 2.45 | m | | H9 | |
| 9 | 77.1 | 4.11 | m | | $H_2$8, $H_a$10, Hb10 | |
| 10 | 39.1 | 2.15 | m | | H9 | C12 |
| | | 2.86 | m | | H9 | C12 |
| 11 | 133.9 | | | | | |
| 12 | 145.4 | | | | | |
| 13 | 122.9 | 6.62 | d | 11.1 | H14 | C12, C14, 12-$CH_3$ |
| 14 | 126.1 | 6.43 | d | 11.1 | H13 | C13, 15-$CH_3$ |
| 15 | 135.9 | | | | | |
| 16 | 47.3 | 2.42 | m | | H17 | |
| | | 2.82 | m | | | |
| 17 | 70.5 | 5.99 | ddd | 10.8, 10.8, 3.3 | $H_a$16, H18 | |
| 18 | 125.0 | 5.32 | d | 10.8 | H17 | C20, 19-$CH_3$ |
| 19 | 142.0 | | | | | |
| 20 | 33.8 | 1.60 | m | | | |
| | | 2.40 | m | | | C21 |
| 21 | 170.9 | | | | | |
| NH | | 6.80 | br s | | | |
| 1′ | 166.4 | | | | | |
| 2′ | 69.8 | 3.86 | d | 10.1 | | |
| 3′ | 43.3 | 1.40 | m | | H4′, 3′-$CH_3$ | |
| 4′ | 72.0 | 5.66 | dd | 10.2,10.2 | H3′, H5′ | C21, C3′ |
| 5′ | 127.4 | 5.26 | dd | 10.7, 10.2 | H4′, H6′ | C7′ |
| 6′ | 137.1 | 5.55 | ddd | 10.7, 10.7, 7.6 | H5′, $H_a$7′, $H_b$7 | C4′ |
| 7′ | 21.8 | 2.15 | m | | H6′, H8′ | |
| | | 2.38 | m | | H6′, H8′ | |
| 8′ | 14.1 | 0.94 | dd | 7.5, 7.5 | $H_a$7′, $H_b$7′ | C6′, C7′ |
| $-OCH_3$ | 54.6 | 3.21 | s | | | C9 |
| 11-$CH_2$ | 114.6 | 4.81 | s | | | C10, C11 |
| | | 5.08 | s | | | C10, C11 |
| 12-$CH_3$ | 14.3 | 1.72 | s | | | C11, C12, C13 |
| 15-$CH_3$ | 18.3 | 1.82 | s | | | C14, C15, C16, C17 |
| 19-$CH_3$ | 23.9 | 1.62 | s | | | C18, C19, C20 |
| 3′-$CH_3$ | 8.7 | 0.80 | d | 6.9 | H3′ | C2′, C3′, C4′ |

[a] Assignments supported by DEPT 135, DEPT 90, and gHMQC experiments.

more lipophilic analogue **6**. Thus, condensation of octyl 3-oxodecanoate with octylamide (see Figure 1) returned a modest yield of **6** with no trace of the undesired *E* stereoisomer (see below) or other side products. Although no attempt was made to optimize this reaction (other than to replace benzene with toluene in order to raise the reaction temperature), it should be noted that the reaction proceeded cleanly with excellent recovery of **6** plus unreacted starting materials. Selected NMR data for **6** ($^{1}$H, NH, $\delta$ 11.44; H10, $\delta$ 5.04; $^{13}$C, C10, 95.4 ppm; C11, 160.1 ppm) compared very favorably with amphilactam A (**1**). The deshielded character of the lactam NH resonance in amphilactam A (**1**) ($\delta$ 10.03) and the amide NH resonances in model compounds **5** ($\delta$ 11.10) and **6** ($\delta$ 11.44) was deemed indicative of intramolecular H bonding to the C1 carbonyl (see the structure diagrams). This observation revealed both the *Z* $\Delta^{2,3}$ stereochemistry in amphilactam A (**1**) and closure of the macrocyclic lactone and lactam rings as shown (alternative ring closures did not accommodate the required H bonding). NOESY analysis of amphilactam A (**1**) (see Table 1) established a *Z* $\Delta^{18,19}$, *E* $\Delta^{12,13}$, *E* $\Delta^{14,15}$ stereochemistry and revealed significant correlations from the 12-$CH_3$ to $H_b$11 and 14 H, and from 14H to the 18H, also from 13- to 9H and 15-$CH_3$. These observations required that $\Delta^{12,13}$, $\Delta^{14,15}$, and $\Delta^{18,19}$ adopt a coplanar conformation with 13 H oriented into the cavity of the macrocyclic ring. The complete structure for amphilactam A (**1**) is assigned as shown, less relative stereochemistry about C9, C17, C2′, C3′, and C4′.

Amphilactam B (**2**) was isolated as a stable oil with a molecular formula (M + Na, $C_{36}H_{53}NO_7Na$, $\Delta$ 3.3 mmu) requiring 11 degrees of unsaturation. NMR comparisons confirmed that amphilactam B (**2**) was the homologue of amphilactam A (**1**) as indicated. Most significantly, an $^{1}$H NMR comparison confirmed that the primary methyl resonance in **1** ($\delta$ 0.94) was replaced by diastereotopic isopropyl methyl resonances in **2** ($\delta$ 0.87 and 1.09). Likewise, the doublet of doublet of doublet multiplicity for the 6′H resonance in **1** was replaced by a doublet of doublet multiplicity in **2**.

Amphilactam C (**3**) was isolated as a stable oil with a molecular formula (M + Na, $C_{35}H_{51}NO_7Na$, $\Delta$ 7.2 mmu) isomeric to that of amphilactam A (**1**). The NMR data for **3** (see Table 2) proved very similar to that for **1**, with the notable exception of the $^{1}$H NMR resonance for the lactam NH. The lactam NH in amphilactam C (**3**) resonated significantly upfield ($\delta$ 6.80) of that for the NH resonance in amphilactam A (**1**) ($\delta$ 10.03), consistent with absence of H bonding to the C1 carbonyl. Thus amphilactam C (**3**) was assigned as the *E* $\Delta^{2,3}$ stereoisomer of amphilactam A (**1**).

Amphilactam D (**4**) was isomeric to amphilactam B (**2**), and its structure was assigned by analogy with the preceding amphilactams.

The amphilactams were isolated with the assistance of bioassay-directed fractionation techniques, monitoring in vitro nematocidal activity against free-living stages of the parasitic nematode *Haemonchus contortus*. Fractions containing amphilactams accounted for all the nematocidal activity detected in crude *Amphimedon* extracts. Pure amphilactams A−D (**1**−**4**) were available in sufficient amounts to quantify their in vitro $LD_{99}$ activities as 7.5, 47, 8.5, and 0.39 $\mu$g/mL, respectively. The amphilactams inhibit larval development at the L1 stage but exhibit little or no activity against nematode eggs. The toxicity is rapid, with dead larvae apparent within a few hours of hatching, but does not appear to involve paralysis. The level of in vitro activity, particularly for amphilactams A, C, and D, is comparable to that of existing commercial anthelmintics, such as levamisole and closantel, and was considered sufficient to merit in vivo evaluation. The structure−activity relationships drawn from the limited range of natural amphilactams provides a contradictory pattern. Comparison of amphilactams A and C suggests that the $\Delta^{2,3}$ geometry of the unique enamino lactone/lactam moiety plays no role in modulating nematocidal activity. However, the corresponding homologues amphilactams B and D revealed a 100-fold increase in potency in progressing from the *E* to *Z* $\Delta^{2,3}$ geometry. A lack of nematocidal activity for the model compounds **5** and **6** supports the notion that the overall conformation of the bicyclic system is critical for nematocidal activity. Study of a more extensive selection of analogues than is currently available is required to more fully understand the structure−activity relationships within this class. The amphilactams were inactive in a broad range of antibacterial and antifungal chemotherapeutic screens. Further characterization of the chemotherapeutic profile of the amphilactams is currently in progress and will be reported elsewhere.

To the best of our knowledge, the amphilactams represent a novel structural motif in both terrestrial and marine natural products chemistry and join a select group of sponge metabolites known to possess in vitro nematocidal properties.[1−15]

## Experimental Section

**General Experimental Procedures.** The nematocidal bioassay has been described in an earlier paper.[16]

**Collection, Extraction and Isolation.** The two *Amphimedon* specimens were collected during trawling operations (depth ~50 m) in the Great Australian Bight in October 1994 and July 1995 (19.3 and 15.6 g dry weight, respectively, Museum of Victoria registry nos. F79979 and F79980, respectively). [A common description is as follows: growth form, macrobenthic, fixed directly to the substrate, lobate-irregular; texture, firm, harsh, barely compressible; surface, opaque, optically smooth, hispid; color, dark purple-brown on deck, dark gray in EtOH; oscules, conspicuous with a raised membranous lip, scattered; spicules, oxeas (curved, fusiform 125−128−135 × 5−8.5 $\mu$m); ectosome, a dense multispicular layer of paratangential oxeas barely protruding through the surface collagen which is darkly pigmented; choanosome, densely spiculose but spongin is dominant forming a close and regular reticulation of ascending multispicular tracts, becomine plumose bundles at the surface, and unispicular connecting tracts.] On the basis of these taxonomic criteria, the two samples were considered to belong to the same *Amphimedon* species.

The specimens were frozen at −18 °C and transported to the laboratory where they were diced and steeped in EtOH. The decanted extracts were concentrated in vacuo and partitioned into $CH_2Cl_2$-soluble and -insoluble fractions. The $CH_2Cl_2$ soluble fractions were subjected to silica solid-phase extraction (10% gradient elution from petroleum to EtOAc) and HPLC (2 mL/min 40% EtOAc/petroleum ether through a Phenomenex 5 $\mu$m silica 250 × 10 mm column) to return consolidated yields of amphilactam A (**1**) (74.4 mg, 0.21%), amphilactam B (**2**) (11.2 mg, 0.03%), amphilactam C (**3**) (8.1 mg, 0.02%), and amphilactam D (**4**) (2.8 mg, 0.01%).

**Amphilactam A (1):** stable yellow oil; $[\alpha]_D$ +11.8° (*c* 0.35 in $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ 270 ($\epsilon$ 44 800); IR $\nu_{max}$ ($CHCl_3$) 3610, 3420, 1730, 1680, 1630 $cm^{-1}$; $^1H$ NMR (400 MHz, benzene-$d_6$) see Table 1; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 9.89 (brs, NH), 7.99 (brs, OH), 6.38 (d, $J$ = 11.0 Hz, H13), 6.22 (d, $J$ = 11.0 Hz, H14), 5.61 (ddd, $J$ = 10.9, 10.9, 7.3 Hz, H6′), 5.48 (ddd, $J$ = 10.2, 10.2, 2.7, H17), 5.38 (dd, $J$ = 9.9, 9.9 Hz, H4′), 5.22 (s, 11-$CH_a$), 5.00 (s, 11-$CH_b$), 5.21 (dd, $J$ = 9.9, 9.6 Hz, H5′), 5.09 (d, $J$ = 9.9 Hz, H18), 4.98 (s, H2), 3.88 (m, H9), 3.61 (d, $J$ = 10.3 Hz, H2′), 3.39 (s, $OCH_3$), 2.85 (m, $H_2$5, $H_a$-10), 2.54 (m, $H_a$16), 2.52 (m, $H_2$4), 2.49 (m, $H_2$8), 2.44 (m, $H_a$-20), 2.28 (m, $H_b$16), 2.24 (m, $H_b$ 10, $H_a$7′), 2.12 (m, $H_b$7′), 1.92 (s, 12-$CH_3$), 1.79 (s, 15-$CH_3$), 1.73, (s, 19-$CH_3$), 1.73 (m, $H_a$7), 1.67 (m, $H_b$20), 1.58 (m, H3′), 1.53 (m, $H_a$6), 1.26 (m, $H_b$6), 0.98 (dd, $J$ = 7.5, 7.5 Hz, H8′), 0.93 (m, $H_b$7), 0.80 (d, $J$ = 6.9 Hz, 3′-Me); $^{13}C$ NMR (100 MHz, benzene-$d_6$) see Table 1; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 177.3 (s, C1), 171.5 (s, C21), 167.3 (s, C1′), 157.4 (s, C3), 144.8 (s, C12), 142.2 (s, C19), 137.2 (d, C6′), 134.8 (s, C15), 133.7 (s, C11), 126.4 (d, C5′), 125.6 (d, C14), 123.5 (d, C18), 122.0 (d, C13), 114.3 (t, 11-$CH_2$), 90.5 (d, C2), 76.6 (d, C9), 71.9 (d, C4′), 70.7 (d, C17), 69.6 (d, C2′), 55.3 (q, $CO_2CH_3$), 46.4 (t, C16), 43.0 (d, C3′), 40.4 (t, C4), 38.6 (t, C10), 35.8 (t, C6), 33.2 (t, C20), 27.7 (t, C8), 27.1 (t, C7), 26.0 (t, C5), 23.8 (q, 19-$CH_3$), 21.4 (t, C7′), 18.0 (15-$CH_3$), 14.4 (q, 12-$CH_3$), 13.9 (q, C8′), 8.4 (q, 3′-$CH_3$); ESIMS (25 kV) *m*/*z* 620 (M + Na, 100); HRESIMS *m*/*z* 620.3580 (calcd for $C_{35}H_{51}NO_7$-Na 620.3563).

**Amphilactam B (2):** stable yellow oil; $[\alpha]_D$ +8.5° (*c* 0.38 in $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ 280 ($\epsilon$ 18 800); IR $\nu_{max}$ ($CHCl_3$) 3610, 3430, 1730, 1710, 1640 $cm^{-1}$; $^1H$ NMR (400 MHz, benzene-$d_6$) $\delta$ 10.03 (br s, NH), 6.60 (d, $J$ = 11.0 Hz, H13), 6.40 (d, $J$ = 11.2 Hz, H14), 5.93 (ddd, $J$ = 10.8, 10.8, 2.9 Hz, H17), 5.65 (dd, $J$ = 10.1, 10.1 Hz, H4′), 5.38 (dd, $J$ = 10.5, 10.5 Hz, H6′), 5.26 (d, $J$ = 9.9 Hz, H18), 5.14 (dd, $J$ = 10.5, 10.1 Hz, H5′), 5.08 (s, 11-$CH_b$), 4.81 (s, 11-$CH_a$), 4.78 (s, H2), 4.11 (m, H9), 3.87 (d, $J$ = 10.8 Hz, H2′), 3.21 (s, $OCH_3$), 2.95 (m, H7′), 2.89

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(m, $H_b$10), 2.82 (m, $H_b$20), 2.80 (m, $H_b$16), 2.50 (m, $H_b$8), 2.49 (m, $H_a$8), 2.44 (m, $H_a$16), 2.13 (m, $H_a$10), 1.80 (s, 15-$CH_3$), 1.73 (s, 12-$CH_3$), 1.68 (m, $H_b$4), 1.61 (m, $H_a$20), 1.60 (s, 19-$CH_3$), 1.54 (m, $H_a$4), 1.50 (m, $H_2$ 5, $H_2$6), 1.40 (m, H3′), 1.15 (m, H7), 1.09 (d, $J$ = 6.6 Hz, $H_3$8′), 0.87 (d, $J$ = 6.6 Hz, 7′-$CH_3$), 0.83 (d, $J$ = 6.7 Hz, 3′-$CH_3$); $^{13}$C NMR (100 MHz, benzene-$d_6$) $\delta$ 175.9 (s, C1′), 170.7 (s, C21), 167.9 (s, C1), 158.7 (s, C3), 145.3 (s, C12), 142.6 (d, C18), 142.6 (s, C19), 142.6 (d, C6′), 135.7 (s, C15), 134.0 (s, C11), 126.1 (d, C14), 125.4 (d, C5′), 122.8 (d, C13), 114.8 (t, 11-$CH_2$), 89.4 (d, C2), 77.0 (d, C9), 72.3 (d, C4′), 71.2 (d, C17), 69.9 (d, C2′), 54.4 (q, $CO_2CH_3$), 47.0 (t, C16), 43.1 (d, C3′), 40.9 (t, C8), 39.0 (t, C10), 36.4 (t, C7), 33.8 (t, C20), 27.9 (d, C7′), 27.7 (t, C6), 27.1 (t, C5), 25.6 (t, C4), 23.9 (q, 19-$CH_3$), 23.0 (q, C8′), 22.8 (q, 7′-$CH_3$), 18.3 (q, 15-$CH_3$), 14.4 (q, 12-$CH_3$), 8.9 (q, 3′-$CH_3$); ESIMS (25 kV) $m/z$ 634 (M + Na, 20); HRESIMS $m/z$ 634.3719 (calcd for $C_{36}H_{53}NO_7Na$ 634.3686).

**Amphilactam C (3):** stable yellow oil; $[\alpha]_D$ +17.3° ($c$ 0.48 in $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ 280 ($\epsilon$ 14 000); IR $\nu_{max}$ ($CHCl_3$) 3610, 3360, 1750, 1680, 1640 cm$^{-1}$; $^1$H NMR (400 MHz, benzene-$d_6$) see Table 2; $^{13}$C NMR (100 MHz, benzene-$d_6$) see Table 2; ESIMS (25 kV) $m/z$ 620 (M + Na, 100); HRESIMS $m/z$ 620.3563 (calcd for $C_{35}H_{51}NO_7Na$ 620.3491).

**Amphilactam D (4):** stable yellow oil; $[\alpha]_D$ +2.5° ($c$ 0.03 in $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ 270 ($\epsilon$ 3050); IR $\nu_{max}$ ($CHCl_3$) 3610, 3420, 1730, 1680, 1640 cm$^{-1}$; $^1$H NMR (400 MHz, benzene-$d_6$) $\delta$ 6.61 (d, $J$ = 10.8 Hz, H13), 6.42 (d, $J$ = 10.8 Hz, H14), 5.98 (ddd, $J$ = 10.0, 10.0, 2.6 Hz, H17), 5.95 (br s, NH), 5.65 (dd, $J$ = 10.1, 10.1 Hz, H4′), 5.39 (dd, $J$ = 10.7, 10.7 Hz, H6′), 5.32 (d, $J$ = 10.0 Hz, H18), 5.16 (t, $J$ = 10.7, 10.1 Hz, H5′), 5.08 (s, 11-$CH_b$), 4.92 (s, H2), 4.80 (s, 11-$CH_a$), 4.12 (m, H9), 3.87 (br d, $J$ = 9.0 Hz, H2′), 3.20 (s, $OCH_3$), 2.73−2.88 (m, $H_b$10, $H_b$16, $H_b$20, H7′), 2.37−2.51 (m, $H_2$8, $H_a$16), 2.09 (m, $H_a$10), 1.81 (s, 15-$CH_3$), 1.71 (s, 12-$CH_3$), 1.62 (s, 19-$CH_3$), 1.22−1.44 (m, $H_2$4, $H_2$5, $H_2$6, $H_2$7, $H_a$20, H3′), 1.10 (d, $J$ = 6.4 Hz, H8′), 0.87 (d, $J$ = 6.5 Hz, 7′-$CH_3$), 0.81 (d, $J$ = 6.9 Hz, 3′-$CH_3$); $^{13}$C NMR (100 MHz, benzene-$d_6$) $\delta$ 176.5 (s, C1′), 170.7 (s, C21), 166.4 (s, C1), 157.3 (s, C3), 145.4 (s, C12), 142.7 (d, C18), 142.5 (s, C19), 142.1 (d, C6′), 135.9 (s, C15), 133.9 (s, C11), 126.1 (d, C14), 125.4 (d, C5′), 122.9 (d, C13), 114.8 (d, 11-$CH_2$), 92.1 (d, C2), 77.1 (d, C9), 72.3 (d, C4′), 70.6 (d, C17), 69.8 (d, C2′), 54.5 (q, $CO_2CH_3$), 47.3 (t, C16), 43.2 (d, C3′), 40.9 (t, C8), 39.3 (t, C10), 36.4 (t, C7), 33.8 (t, C20), 28.0 (t, C7′), 27.9 (t, C6), 27.7 (t, C5), 26.3 (t, C4), 23.9 (q, 19-$CH_3$), 23.0 (q, C8′), 22.8 (q, 7′-$CH_3$), 18.3 (q, 15-$CH_3$), 14.4 (q, 12-$CH_3$), 8.9 (q, 3′-$CH_3$); ESIMS (25 kV) $m/z$ 634 (M + Na, 15); HRESIMS $m/z$ 634.3693 (calcd for $C_{36}H_{53}NO_7Na$ 634.3719).

**Formation of Model Compound 5.** To a solution of methyl 3-oxobutanoate (2 g, 0.017 mol) in dry benzene were added acetamide (2 g, 0.034 mol) and TsOH (10 mg), and the mixture was left to reflux under $N_2$ for 12 h. The reaction mixture was then filtered, concentrated in vacuo, diluted with $H_2O$ (50 mL), and extracted into ether (3 × 50 mL), during which time the product **5** crystallized as colorless crystals (2.49 g, 93%): mp 37−39 °C; IR $\nu_{max}$ ($CHCl_3$) 3680, 3350, 1710, 1680, 1650 cm$^{-1}$; $^1$H NMR (300 MHz, benzene-$d_6$) $\delta$ 11.10 (s, NH), 4.75 (s, H2), 3.35 (s, $-CO_2CH_3$), 2.19 (s, 3-$CH_3$), 1.54 (s, $H_3$5); $^{13}$C NMR (75 MHz, benzene-$d_6$) $\delta$ 169.6, 168.0 (2s, C1, C5), 155.8 (s, C3), 95.5 (d, C2), 50.5 (q, $-CO_2CH_3$), 24.4, 21.7 (2q, C5, 3-$CH_3$); ESIMS $m/z$ 158 (M + H, 20); HRESIMS $m/z$ 180.0633 (calcd for $C_7H_{12}NO_3Na$ 180.0631).

**Formation of Model Compound 6.** To a solution of octyl 3-oxodecanoate (230 mg, 0.08 mmol) in dry toluene were added octanamide (1.1 g, 0.8 mmol) and TsOH (7 mg, 0.04 mmol), and the mixture was refluxed under $N_2$ for 72 h. The reaction mixture was then filtered, concentrated in vacuo, diluted with $H_2O$ (50 mL), and extracted into ether (3 × 50 mL). The ethereal phase was then washed with brine (100 mL), dried with $MgSO_4$, and concentrated in vacuo to yield a crude product (200 mg) that was subjected to silica chromatography (5% EtOAc/petroleum ether, Chromatatron) followed by Sephadex LH-20 chromatography (MeOH) to yield **6** (56.7 mg, 17%: not optimized) as a stable colorless oil: UV ($CHCl_3$) $\lambda_{max}$ 280 ($\epsilon$ 8470); IR $\nu_{max}$ ($CHCl_3$) 3700, 3670, 1710, 1660 cm$^{-1}$; $^1$H NMR (300 MHz, benzene-$d_6$) $\delta$ 11.44 (s, NH), 5.04 (s, H10), 4.09 (br t, $J$ = 6.6 Hz, $H_2$8), 2.82 (br t, $J$ = 7.3 Hz, $H_2$20), 2.07 (t, $J$ = 7.3 Hz, $H_2$12), 1.18 (m, $H_2$2, $H_2$3, $H_2$4, $H_2$5, $H_2$6, $H_2$7, $H_2$13, $H_2$14, $H_2$15, $H_2$16, $H_2$17, $H_2$21, $H_2$22, $H_2$23, $H_2$24, $H_2$25), 0.87 (m, $H_3$1, $H_3$18, $H_3$26); $^{13}$C NMR (75 MHz, benzene-$d_6$) $\delta$ 170.9, 169.8 (s, C9, C19), 160.1 (s, C11), 95.4 (d, C10), 64.1 (t, C8), 38.4, 34.7, 32.1, 32.0, 29.6, 29.5, 29.4, 29.3, 29.3, 28.9, 26.3, 25.4, 23.0, 22.9 (t, C2, C3, C4, C5, C6, C7, C13, C14, C15, C16, C17, C20, C21, C22, C23, C24, C25), 14.3 (q, C1, C18, C26); ESIMS (25 kV) $m/z$ 424 (M + H, 15), 446 (M + Na, 10), 462 (M + K, 15).

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**Supporting Information Available:** $^1$H and $^{13}$C NMR data for compounds **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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