FTICR-MS and LC-UV/MS-SPE-NMR Applications for the Rapid Dereplication of a Crude Extract from the Sponge *Ianthella flabelliformis*

Cherie A. Motti,* Marnie L. Freckelton, Dianne M. Tapiolas, and Richard H. Willis

Australian Institute of Marine Science, Australia 4810

Received September 8, 2008

Dereplication of a methanolic extract of the marine sponge *Ianthella flabelliformis* using FTICR-MS accurate mass determination and MS^n techniques enabled rapid and unambiguous detection of a new compound among a plethora of known compounds. Isolation of this compound and the known 19-deoxy analogue using the hyphenated technique LC-UV/MS-SPE-NMR was undertaken, and the structures were confirmed, from a single chromatographic run, as 19-hydroxyaraplysillin-I N^{20}-sulfamate (**1**) and araplysillin-I N^{20}-sulfamate (**2**).

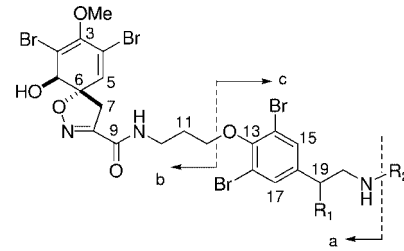
In our continued search for new herbicides from marine organisms that selectively inhibit the rate-limiting enzyme of the C_4 acid cycle, pyruvate phosphate dikinase (PPDK),[1] attention was focused on the methanolic extract of *Ianthella flabelliformis* from Queensland, Australia. *Ianthella* is a well-researched genus, with over 50 compounds recorded in MarinLit by 2008.[2] With this in mind it is important to rapidly identify, early in the discovery process, those components within the extract that are known. This process is referred to as dereplication.

Large-scale collection of marine organisms for the discovery process may lead to possible adverse ecological effects.[3] Several methods are now available that enable detailed structural information to be obtained directly from small quantities of crude extract, making them useful for dereplication while minimizing ecological impacts. FTICR-MS is a powerful method for structural characterization of compounds within crude extracts. It is capable of obtaining accurate exact mass measurements for elemental formula determination and ultrahigh resolution over a large mass range (*m*/*z* 200−2000) for component separation at multiple stages of MS (MS^n). All this can be achieved on nanogram quantities in a single mass spectrum without any LC separation and in a fraction of the time usually required for large-scale isolation.[4−7]

The relatively new hyphenated technique LC-UV/MS-SPE-NMR has also been successfully employed in the dereplication of crude extracts and enables the rapid isolation and structure elucidation of novel compounds from crude extracts in a single step. Solid-phase extraction (SPE) is used as an interface between chromatography, UV, MS, and NMR analysis.[8] The main advantages of this technique are that a single analyte eluted from the HPLC column can be concentrated in the NMR flow cell and that automated trappings to the same SPE cartridge from multiple chromatographic runs are possible, enabling a substantial increase in the amount of material available for the automated 1D and 2D NMR measurements.[9] Sample requirement is minimal (sub milligram), and no fractionation of the extract is required prior to analysis.

In this paper we report the accelerated dereplication of the PPDK active methanolic extract of the marine sponge *I. flabelliformis* using a combination of FTICR-MS techniques[10] and the subsequent isolation of two compounds, 19-hydroxyaraplysillin-I N^{20}-sulfamate (**1**) and the known araplysillin-I N^{20}-sulfamate (**2**),[11] using LC-UV/MS-SPE-NMR.[12]

ESI FTICR-MS of the methanolic extract using negative ion detection showed a series of signals between *m*/*z* 1000 and 1200 with isotope envelopes indicative of brominated compounds.

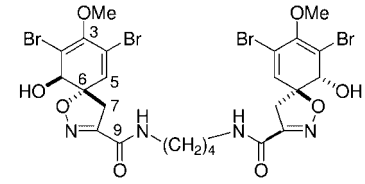


1 R_1=OH, R_2= SO_3Na

2 R_1=H, R_2=SO_3Na

3 R_1=H, R_2=H

a, b and c represent ESI FTICR-MS/MS fragments



4

Comparison of exact mass measurements with literature data indicated the presence of bastadins 5, 6, 9, 10, and 11, or corresponding isomers, in the crude extract (Table S1, Supporting Information).[13] Two signals were also detected at lower mass, a 1:4:6:4:1 quintet centered at *m*/*z* 811.7772 $[M - H]^-$ and a second 1:4:6:4:1 quintet centered at *m*/*z* 795.7817 $[M - H]^-$ (Figure 1). The observed mass difference between the monoisotopic peak ($^{79}Br_4$) and the +2 peak ($^{79}Br_3$:^{81}Br) of Δ = 1.9976 and 1.9988 Da, respectively, and the fact that the isotope envelopes were quintets were indicative of the presence of four bromine atoms in both molecules. The observed mass difference between the two isotope envelopes of 15.9955 Da suggested that the heavier of the two molecules was an oxygenated analogue of the lighter one.

While accurate mass measurement can indicate the molecular formula, it does not provide structural information; hence multiple stage ESI FTICR-MS experiments were undertaken on the crude extract. The most abundant ion from each isotope distribution (*m*/*z* 796 and 812) was isolated for tandem (MS^n) analysis. Isolation of the single ion from the isotope envelope means that each of the isolated parent ions represents a compound with four bromines with the precise distribution $^{79}Br_2$:$^{81}Br_2$. Both isolated ions were subjected to sustained off-resonance irradiation (SORI) collision-induced

* To whom correspondence should be addressed. Tel: 617 4753 4143. Fax: 617 47725852. E-mail: C.Motti@aims.gov.au.

10.1021/np800562m CCC: $40.75 Published 2009 by the American Chemical Society
Published on Web 02/10/2009

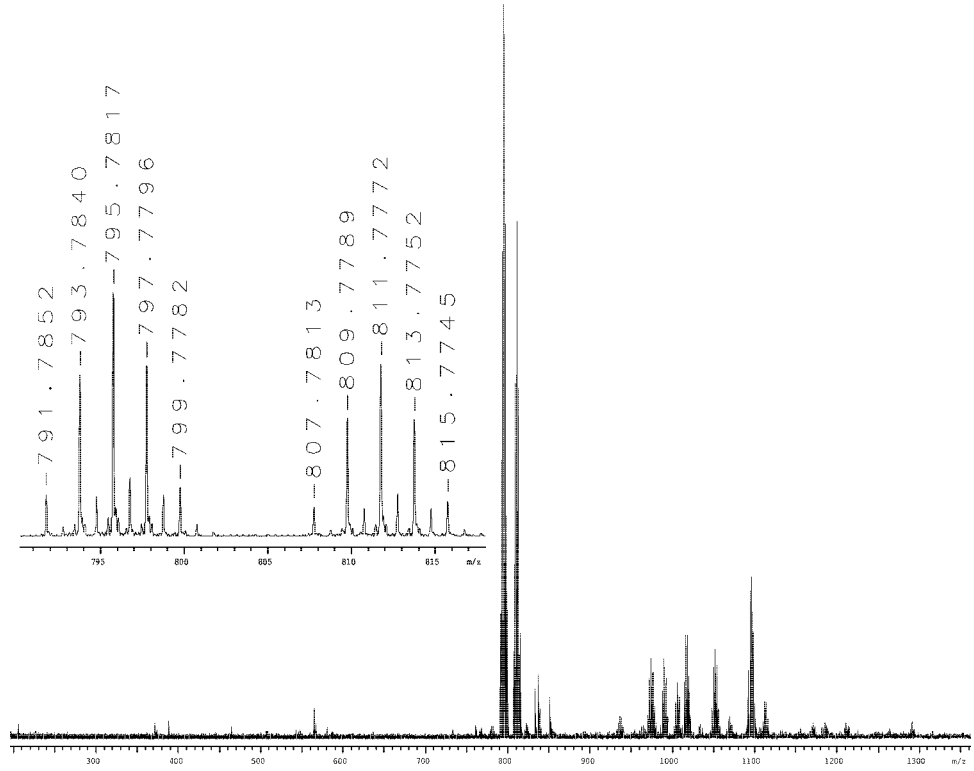


Figure 1. Accurate mass determination of the two brominated isotope envelopes for **1** (*m*/*z* 807.7813) and **2** (*m*/*z* 791.7852) observed in the methanolic extract of *I. flabelliformis*.

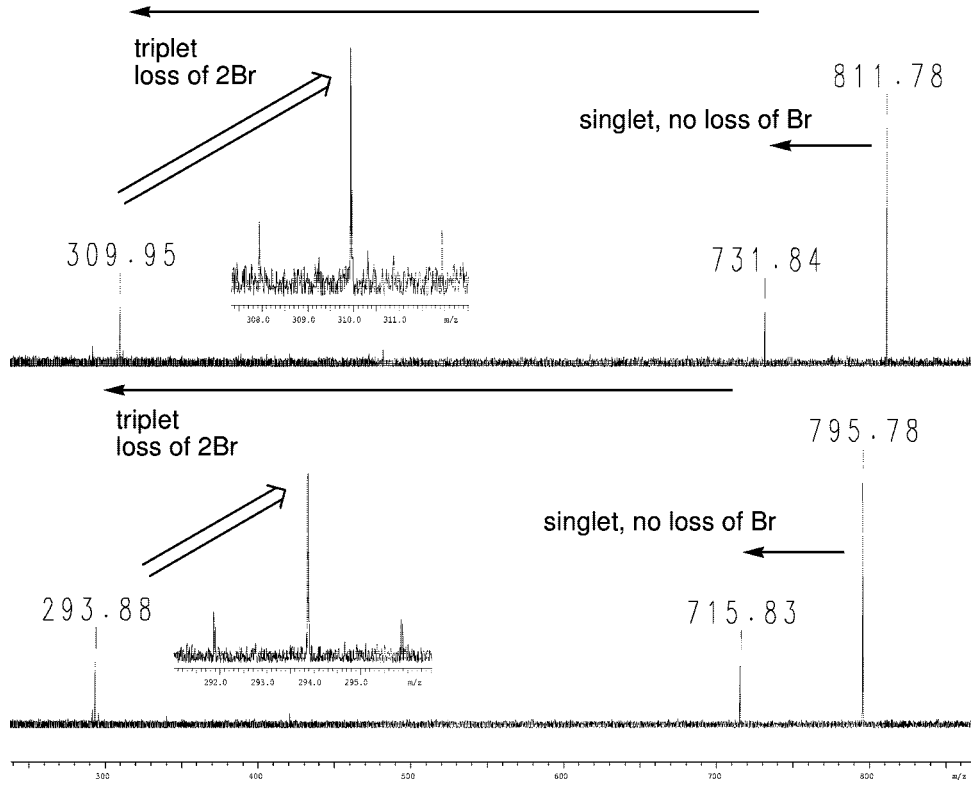


Figure 2. Sustained off-resonance irradiation (SORI) collision-induced dissociation (CID) of the isolated most abundant signals ($^{79}Br_2$:$^{81}Br_2$) *m*/*z* 796 and 812.

dissociation (CID), resulting in preserved singlets ($^{79}Br_2$:$^{81}Br_2$) at *m*/*z* 716 and 732, respectively (Figure 2).[5] This indicated that the loss of 80 was not a result of a loss of bromine (as the neutral loss of HBr, a typical MS fragment of brominated metabolites), which would have caused a change in the isotope distribution from a singlet ($^{79}Br_2$:$^{81}Br_2$) to a symmetrical doublet ($^{79}Br_2$:^{81}Br and ^{79}Br:$^{81}Br_2$), since the loss of HBr could include either ^{79}Br or ^{81}Br with equal probability. Each parent ion also produced a triplet fragment centered at *m*/*z* 294 and 310, respectively (Figure 2), representative of fragments with only two bromine atoms ($^{79}Br_2$, ^{79}Br:^{81}Br, and $^{81}Br_2$) and an overall formula corresponding to $C_8H_8O_{(1\text{ or }2)}NBr_2^-$ (fragment c).

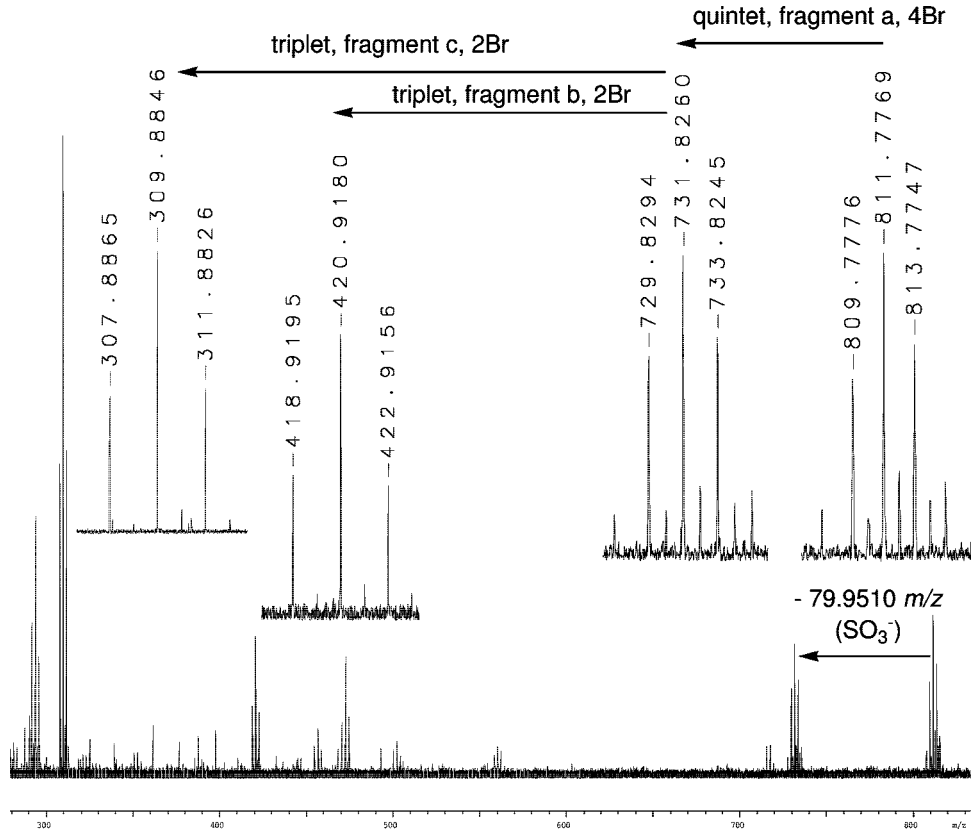


Figure 3. On-resonance CID of the isotope envelope centered at *m/z* 812.

On-resonance CID of both the 796 and 812 isotope envelopes in the crude extract preserved the isotopic distribution of any fragment ions generated and enabled accurate mass measurement of the daughter ions. In both instances the isotopic distribution did not change after an initial loss of 80 Da was observed, further supporting the notion that there was no loss of bromine (Figure 3). The other typical neutral loss fragment with *m/z* 80 is SO_3, a facile loss from compounds containing sulfate or sulfamate groups. The expected mass difference resulting from a loss of HBr is 79.9262 Da, and that for a loss of SO_3 is 79.9574 Da. The observed mass difference was 79.9510 Da, providing further evidence that the fragment lost was indeed SO_3.[14] Three stages of MS using on-resonance CID on the fragment ion 716 produced a triplet at 294 (fragment c) and at 421 (fragment b, formula $C_{13}H_{13}O_4N_2Br_2^-$), both fragments containing only two bromines. This, and the pattern of the isotopic distributions, confirms that the two compounds each have four bromine atoms and one SO_3 group and that the extra oxygen in the heavier molecule is definitely in fragment c.

With the knowledge that both compounds contain four bromines and one SO_3, the monoisotopic mass of 807.7813 ($[M - H]^-$) supported a molecular formula of $C_{21}H_{22}Br_4N_3O_9S^-$, while the monoisotopic mass of 791.7852 ($[M - H]^-$) supported a molecular formula of $C_{21}H_{22}Br_4N_3O_8S^-$.

The higher mass compound was found to be new after searching MarinLit,[2] while the molecular formula of the lower mass compound corresponded to araplysillin-I N^{20}-sulfamate (**2**) on the basis of the ESI FTICR-MS data. An off-line HPLC method was developed, and one fraction (t_R 11.0−15.0 min) was found to be active in the PPDK assay with 10.1% of control based on equivalent dose. Subsequent fractionation of 400 μg of the crude methanolic extract using the automated LC-UV/MS-SPE-NMR technique was undertaken.

The ^{1}H NMR spectrum obtained for peak 1 (t_R 11.5 min), trapped using MS as the detection mode and triggers set at *m/z* 796 and 812 ($[M - H]^-$), revealed the presence of a single compound, **1**, with a corresponding *m/z* 812 isotope envelope. The ^{1}H NMR spectrum obtained for peak 2 (t_R 12.5 min) showed signals corresponding to two compounds, **1** (*m/z* 812) and **2** (*m/z* 796), in a ratio of 1:4. On the basis of the integration of these two relatively minor peaks at λ 220 nm and the assumption that they have similar extinction coefficients, **1** represented approximately 6% and **2** approximately 4% of the UV-active components, corresponding to approximately 24 and 16 μg, respectively.

The ^{1}H NMR data of **1** (in CD_3CN) closely aligned with those of the known compound araplysillin-I (**3**).[15] The ^{1}H NMR (Table 1) showed the following signals: δ_H 6.43 (s, 1H, ethylenic proton), δ_H 4.15 (m, 1H, H-C-OH), δ_H 3.72 (s, 3H, OMe), and an AB system δ_H 3.71 (d, 1H, J = 18.3 Hz) and δ_H 3.08 (d, 1H, J = 18.3 Hz) characteristic of the spirocyclic oxazoline ring in araplysillin-I (**3**).[15] A second set of signals, δ_H 7.60 (s, 2H), were attributable to the equivalent aromatic protons on the dibromophenoxy chain. There were, however, some differences between the ^{1}H NMR spectra of **1** and **3**, in particular, the chemical shifts for H-19 and H-20 (Table 1). We observed downfield shifts of the signals for H-19 (δ_H 2.74 [t, CD_3OD] in **3** to δ_H 4.71 [m, 1H, CD_3CN] in **1**) and for H-20 (δ_H 2.95 [bt, CD_3OD] in **3** to δ_H 3.07 and 3.20 [dd, 2H, J = 14.3 and 2.9 Hz, CD_3CN] in **1**), indicating the presence of a hydroxyl group at C-19 in **1**.

The ^{1}H NMR spectrum of **2** showed the characteristic signals of a spirocyclo oxazoline ring.[15] The upfield shift of H-19, δ_H 2.87 (t, 2H, J = 7.0 Hz), compares well with that observed in **3** (δ_H 2.74, CD_3OD) and clearly indicates C-19 is not hydroxylated.

The 1D NMR data obtained for **1** and **2** confirmed the usefulness of the LC-UV/MS-SPE-NMR experiments for analysis of the crude extract and the accelerated identification of two of its constituents. Although the resolution of the HPLC peaks was not complete, the 1D NMR and LRMS data obtained on a single trapping and (−)-ESI FTICR-MS of the crude extract were sufficient for preliminary assignment of the first peak as **1** and the second peak as **2** based on comparisons with literature values for **3**.

Table 1. NMR Spectroscopic Data (600 MHz) for 19-Hydroxyaraplysillin-I N^{20}-Sulfamate (**1**)

pos[a]	δ_C, mult.[c]	δ_H (J in Hz)[b]	δ_H (J in Hz)[c]	COSY[c]	HMBC[c]
1	73.5, CH	4.15, m	3.92, dd (8.1, 0.9)	1-OH, 5	1-OH, 5, 7a, 7b
2	121.2, qC				1, 5
3	147.0, qC				1, 5, OMe
4	113.3, qC				1-OH, 1, 5, 7b
5	131.4, CH	6.43, s	6.59, d (0.9)	1	1, 7a, 7b
6	90.4, qC				1-OH, 1, 5, 7a, 7b
7	39.4, CH_2	3.71, d (18.3)	3.62, d (18.4)	7b	1, 5
		3.08, d (18.3)	3.22, d (18.4)	7a	
8	154.8, qC				7a, 7b,
9-CO	159.0, qC				7a, 7b, 9-NH, 10
9-NH			8.58, t (5.8)	10	
10	36.2, CH_2	3.55, t (6.8)	3.40, m	9-NH, 11	9-NH, 11, 12
11	29.5, CH_2	2.12, m	1.99, p (6.8)	10, 12	10, 12
12	71.1, CH_2	4.07, t (6.2)	3.96, t (6.6)	11	H10, H11
13	151.1, qC				12, 15, 17
14, 18	117.5, qC				15, 17
15, 17	130.5, CH	7.60, s	7.56, s	19	17, 19
16	144.0, qC				15, 17, 19-OH, 19, 20
19	70.6, CH	4.71, m	4.66, m	19-OH, 15, 17, 20	15, 17, 20
20	51.6, CH_2	3.20, dd (14.3, 2.9)	2.90, m	19	19-OH, 19
		3.07, dd (14.3, 2.9)			
OMe	59.7, CH_3	3.72, s	3.64, s		
1-OH			6.38, d (8.1)	1	
19-OH			5.76, d (4.3)	19	
20-NH			6.97, br		

[a] Numbering for the structure of **1** is similar to that used for **4**.[11] [b] Spectra were recorded in CD_3CN using a flow cell. [c] Spectra were recorded in DMSO-d_6 using a 1.7 mm tube.

2D NMR spectra of **1** in DMSO-d_6 were obtained off-line and confirmed all structural assignments made from 1D ^{1}H NMR spectra. The ^{1}H−^{1}H COSY spectrum of **1** revealed the presence of a 1,3-disubstituted propane moiety (cross-peaks of H-10/H-11 and H-11/H-12) and a 1,2-disubstituted ethane moiety (cross-peaks of H-19/H-20 and H-19/19-OH). The AB system at δ_H 3.08/3.71 (d, 2H, J = 18.3 Hz) was assigned as an isolated methylene group adjacent to a π-electron system. The H−C correlations obtained by the HSQC and HMBC experiments for **1** are summarized in Table 1, revealing the presence of a 1,2,3,5,5,6-hexasubstituted 1,3-cyclohexadiene and a 1,2,3,5-tetrasubstituted phenyl group. The positions of the oxygen substituents were determined to be δ_C 73.5 (CH, C-1), 147.0 (qC, C-3), 90.4 (qC, C-6), 71.1 (CH_2, C-12), 151.1 (qC, C-13), 70.6 (CH, C-19), and 59.7 (CH_3, OMe).

The relatively upfield shifts of the quaternary sp^2 carbons of δ_C 121.2 (C-2), 113.3 (C-4), and 117.5 (C-14/C-18) indicated the location of the four bromine atoms at these carbons in **1**.[16] The HMBC correlations of OMe/C-3 and H-12/C-13 indicated the connectivity between C-3 and OMe and between C-12 and C-13 via ether bonds. The HMBC correlations of H-10/C-9 revealed the connectivity between C-9 and C-10 via a nitrogen atom, which was confirmed by a N-HMBC correlation observed between 9-N/H-10 and H-11. The presence of a spiroisoxazoline structure was confirmed by the observation of a N-HMBC correlation between 8-N/H-7a and H-7b.

Although no correlations were observed from the 20-NH proton into the rest of the molecule and from the fact that this signal integrated for only one proton and that the signals due to 1-OH, 9-NH, and 19-OH were confirmed, it was deduced that the SO_3 group must be attached to the amine at C-20.

The geometry of the vicinal oxygens in the spiroisoxazoline moiety of **1** was established as *trans* by comparison with data for aerothionin (**4**),[17] in particular, the resonances of δ_H 4.15 (H-1), 6.43 (H-5), and 3.71/3.08 (H-7a/b) (acquired in DMSO-d_6), which are agreeable with those of δ_H 4.12, 6.34, and 3.80/3.03 in **4**.[17] A selective gradient ROESY experiment established a dipolar coupling between δ_H 6.38 (1-OH) and 3.62 (H-7a), as was reported for **2**, confirming the assigned geometry.[11,17] The geometry of **2** was assigned as depicted on the basis of the chemical shifts of H-1, H-5, and H-7a/b and the observed ROESY correlation between δ_H 6.38 (1-OH) and 3.62 (H-7a).

The measured optical rotation for **2** of −72 is suggestive that it is the enantiomer of the reported **2** (+100).[11] The peak containing **2** was, however, not entirely pure, and as only a very small amount was available for measurement of the optical rotation, the 1*S**,6*R** configuration for **2** is a tentative assignment at this time. Likewise, the measured optical rotation for **1** of −69 is suggestive that **1** has the same configuration as **2** isolated in this study, so the 1*S**,6*R** configuration for **1** is again only a tentative assignment. The limited amount of sample obtained for **1** also meant the configuration at C-19 was not determined.

Both **1** and **2** were found to be nonselective inhibitors of the pyruvate phosphate dikinase (PPDK) coupled enzyme assay[1a] (13% and 2.3% of control at 435 μg/mL, respectively). Given our interest in finding selective inhibitors of PPDK, no further assessment was carried out.

The ESI FTICR-MS techniques described here allow rapid and unambiguous detailed structural information about individual constituents to be derived from a complex crude extract without prior fractionation. The high-resolution and routine accurate mass capabilities of the ESI FTICR-MS instrument adds another dimension to the dereplication of crude extracts, where known and new compounds in the extracts can be readily identified by assignment of ion formulas and the observation of MS/MS fragments that can be rationalized from the structure of known compounds.

Subsequent application of the hyphenated LC-UV/MS-SPE-NMR technique resulted in the mass-targeted trapping of two chromatographic peaks and their preliminary identification by 1D NMR in CD_3CN. The structures were fully elucidated using off-line 1D and 2D NMR in DMSO-d_6. The advantage of using both FTMS and LC-UV/MS-SPE-NMR methods in the early stages of the discovery process is that they can interrogate crude extracts with minimal preparation or fractionation, providing detailed information of the individual components and identifying those that may be of interest in terms of novel structures, making them valuable tools in biodiscovery research programs.

Experimental Section

General Experimental Procedures. Solvents were HPLC grade Omnisolv methanol (MeOH), Burdick and Jackson dichloromethane (DCM), J. T. Baker acetonitrile (CH_3CN), and milli-Q filtered water.

Deuterated solvents CD_3CN (D 99.96%) and DMSO-d_6 (D 99.9%) were sourced from Cambridge Isotope Laboratories.

Optical rotations were measured using a Jasco P-1020 spectropolarimeter, light source Na, λ 589 nm, and cell length 100 mm.

Sponge Material. The sponge was collected, by dredging at a depth of 30−42 m, from Shelburne Bay, Queensland, Australia (11.42.04′ S, 143.39.05′ E) in 1994 under GBRMPA Permit G88/354 and identified as *Ianthella flabelliformis* (phylum Porifera, class Demospongiae, order Verongida, family Ianthellidae). A voucher specimen (AIMS-16704) is stored at AIMS, Townsville, Queensland, Australia.

Freeze-dried *I. flabelliformis* (∼1 g) was exhaustively extracted with DCM followed by MeOH. After filtration and evaporation of solvents the extract was redissolved in MeOH for LC-MS (10 mg/mL) and ESI FTICR-MS (0.2 mg/mL in MeOH) analyses.

FTICR-MS Instrumentation. FTICR-MS measurements were performed on an unmodified Bruker BioAPEX 47e mass spectrometer equipped with an Analytica model 103426 (Branford, CT) electrospray ionization (ESI) source in negative mode. Direct infusion of the sample (0.2 mg/mL in MeOH) was carried out using a Cole Palmer 74900 syringe pump at a rate of 100 μL/h. The instrument was calibrated using a methanolic solution of CF_3COONa (0.1 mg/mL MeOH).

The ESI FTICR-MS^2 experiments were performed by initially isolating either the entire isotope envelope or the most abundant ion within the isotope envelope with correlated sweeps. The selected parent ions were selectively activated using either on-resonance excitation collision-induced dissociation (CID) or sustained off-resonance irradiation (SORI) excitation CID.[5]

LC-UV/MS-SPE-NMR Instrumentation. Separation was achieved using an Agilent 1100 liquid chromatograph equipped a quaternary solvent delivery pump, degasser, column oven (25 °C), and autosampler, an Agilent PDA UV detector connected to a Knauer K120 Wellchrom pump for post column water delivery, a Bruker Esquire3000 ion trap mass spectrometer with an Apollo ESI ion source operating in negative mode, and a Spark Prospekt 2 solid-phase extraction (SPE) device.

A solution of the methanolic extract (10 mg/mL MeOH) was injected (40 μL, 400 μg), without further purification, onto a Phenomenex Luna phenyl hexyl HPLC column (5 μm, 250 mm × 4.60 mm i.d.) and eluted with CH_3CN in H_2O (10% at 0 min rising linearly to 100% at 30 min) at a flow rate of 1 mL/min. The chromatography was monitored at λ 200 and 254 nm and *m/z* 796 and 812 (negative ion detection). The intensity of the *m/z* values was used to define the thresholds to trigger SPE trapping. The SPE cartridges were dried and the analytes eluted with CD_3CN directly into the 1 mm flow cell. CD_3CN was chosen because it has become a standard solvent for HPLC-SPE-NMR experiments owing to its low viscosity, good solubility properties, and minimal effects resulting from solvent suppression.[18]

^{1}H NMR spectra in LC-UV/MS-SPE-NMR mode were obtained with a Bruker Avance 600 MHz spectrometer equipped with a 5 mm CPTXI inverse ^{1}H−^{13}C/^{15}N Z-gradient cryogenically cooled probe (at 600 MHz for ^{1}H detected experiments and at 150 MHz for ^{13}C spectra) and a 60 μL active volume 3 mm flow cell, operated at 300 K with ^{1}H chemical shifts referenced to the solvent signal of CD_3CD at 1.96 ppm. ^{1}H NMR spectra were recorded using a multiple presaturation 1D NOESY pulse sequence.

1D and 2D NMR spectra were obtained off-line in a 1.7 mm NMR tube, operating at 300 K, and the ^{1}H and ^{13}C chemical shifts were referenced to the solvent signals of DMSO-d_6, 2.50 and 39.5 ppm, respectively. COSY and HSQC spectra were acquired in phase-sensitive mode; HMBC spectra (optimized for J_{CH}, 8 Hz or J_{NH}, 10 Hz) were acquired with gradient selection. Selective gradient 1D ROESY spectra were acquired with a continuous wave spinlock pulse length of 500 ms.

Assay. Inhibition of PPDK by HPLC fractions and by compounds **1** and **2** was performed as described previously.[1]

19-Hydroxyaraplysillin-I N^{20}-sulfamate (1): solid; $[\alpha]_D^{23.7}$ −69 (*c* 0.0024, MeOH); UV (CH_3CN/H_2O) λ_{max} nm 206, 222(sh), 283; ^{1}H and ^{13}C NMR (DMSO-d_6 and CD_3CN) data, see Table 1; (−)-ESI FTICR-MS *m/z* 807.7813 (calcd for $C_{21}H_{22}Br_4N_3O_9S^-$, 807.7816).

Araplysillin-I N^{20}-sulfamate (2):[11] solid; $[\alpha]_D^{23.7}$ −72 (*c* 0.0016, MeOH); UV (CH_3CN:H_2O) λ_{max} nm 206, 222(sh), 283; ^{1}H and ^{13}C NMR (DMSO-d_6 and CD_3CN) data, see Table S2 Supporting Information; (−)-ESI FTICR-MS *m/z* 791.7886 (calcd for $C_{21}H_{22}Br_4N_3O_8S^-$, 791.7866).

Acknowledgment. Collection of this sponge was made possible by the access and benefit sharing arrangements between AIMS and the Australian Commonwealth Government. The authors are grateful to those AIMS staff, both past and present, involved in the collection of the sponge, to J. Doyle for the biological testing, and to P. Berquist for the identification of the specimen as *I. flabelliformis*.

Supporting Information Available: Detailed description of FTMS and LC-UV/MS-SPE-NMR analyses. Table listing known bastadins and corresponding isomers. Table of NMR spectroscopic data for **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Doyle, J. R.; Burnell, J. N.; Haines, D. S.; Llewellyn, L. E.; Motti, C. A.; Tapiolas, D. M. *J. Biomol. Screen.* **2005**, *10*, 67–75. (b) Haines, D. S.; Burnell, J. N.; Doyle, J. R.; Llewellyn, L. E.; Motti, C. A.; Tapiolas, D. M. *J. Agric. Food Chem.* **2005**, *53*, 3856–3862. (c) Motti, C. A.; Bourne, D. G.; Burnell, J. N.; Doyle, J. R.; Haines, D. S.; Liptrot, C. H.; Llewellyn, L. E.; Ludke, S.; Muirhead, A.; Tapiolas, D. M. *Appl. Environ. Microbiol.* **2007**, *73*, 1921–1927. (d) Motti, C. A.; Bourguet-Kondracki, M.-L.; Longeon, A.; Doyle, J. R.; Llewellyn, L. E.; Tapiolas, D. M.; Yin, P. *Molecules* **2007**, *12*, 1376–1388.

(2) MarinLit, University of Canterbury, New Zealand, 2008.

(3) Hunt, B.; Vincent, A. C. J. *Ambio* **2006**, *35*, 57–64.

(4) Vu, H.; Pham, N. B.; Quinn, R. J. *J. Biomol. Screen.* **2008**, *13*, 265–275.

(5) McDonald, L. A.; Barbieri, L. R.; Carter, G. T.; Kruppa, G.; Feng, X.; Lotvin, J. A.; Siegel, M. M. *Anal. Chem.* **2003**, *75*, 2730–2739.

(6) Feng, X.; Siegel, M. M. *Anal. Bioanal. Chem.* **2007**, *389*, 1341–1363.

(7) Cummins, L. L.; Chen, S.; Blyn, L. B.; Sannes-Lowery, K. A.; Drader, J. J.; Griffey, R. H.; Hofstadler, S. A. *J. Nat. Prod.* **2003**, *66*, 1186–1190.

(8) Seger, C.; Sturm, S. *LC GC Eur.* **2007**, *20*, 587–597.

(9) (a) Lambert, M.; Stærk, D.; Hansen, S. H.; Sairafianpour, M.; Jaroszewski, J. W. *J. Nat. Prod.* **2005**, *43*, 771–775. (b) Christophoridou, S.; Dais, P.; Tseng, L.-H.; Spraul, M. *J. Agric. Food Chem.* **2005**, *53*, 4667–4679.

(10) Asamoto, B. *FT-ICR/MS: Analytical Applications of Fourier Transform Ion Cyclotron Resonance Mass Spectrometry*; VCH Publishers, Inc: New York, 1991.

(11) Rogers, E. W.; Molinski, T. F. *J. Nat. Prod.* **2007**, *70*, 1191–1194, Note: Araplysillin-I N^9-sulfamate is misnamed in this reference and should read araplysillin-I N^{20}-sulfamate.

(12) Clarkson, C.; Sibumb, M.; Mensenb, R.; Jaroszewski, J. W. *J. Chromatogr., A* **2007**, *1165*, 1–9.

(13) (a) Kazlauskas, R.; Lidgard, R. O.; Murphy, P. T.; Wells, R. J.; Blount, J. F. *Aust. J. Chem.* **1981**, *34*, 765–786. (b) Pordesimo, E. O.; Schmitz, F. J. *J. Org. Chem.* **1990**, *55*, 4704–4709. (c) Butler, M. S.; Lim, T. K.; Capon, R. J.; Hammond, L. S. *Aust. J. Chem.* **1991**, *44*, 287–296. (d) Dexter, A. F.; Garson, M. J. *J. Nat. Prod.* **1993**, *56*, 782–786. (e) Park, S. K.; Jurek, J.; Carney, J. R.; Scheuer, P. *J. Nat. Prod.* **1994**, *57*, 407–410. (f) Franklin, M. A.; Penn, S. G.; Lebrilla, C. B.; Lam, T. H.; Pessah, I. N.; Molinski, T. F. *J. Nat Prod.* **1996**, *59*, 1121–1127, and references therein.

(14) Yi, L.; Dratter, J.; Wang, C.; Tunge, J. A.; Desaire, H. *Anal. Bioanal. Chem.* **2006**, *386*, 666–674.

(15) Longeon, A.; Guyot, M.; Vacelet, J. *Experientia* **1990**, *46*, 548–550.

(16) Okamoto, Y.; Ojika, M.; Kato, S.; Sakagami, Y. *Tetrahedron* **2000**, *56*, 5813–5818.

(17) McMillan, J. A.; Paul, I. C.; Goo, Y. M.; Rinehart, K. L.; Krueger, W. C.; Pschigoda, L. M. *Tetrahedron Lett.* **1981**, *22*, 39–42.

(18) Lambert, M.; Stærk, D.; Hansen, S. H.; Jaroszewskil, J. W. *Magn. Reson. Chem.* **2005**, *43*, 771–775.

NP800562M