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Reactive desorption electrospray ionization mass spectrometry (DESI-MS) of natural products of a marine alga

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Abstract

Presented here is the optimization and development of a desorption electrospray ionization mass spectrometry (DESI-MS) method for detecting natural products on tissue surfaces. Bromophycolides are algal diterpene-benzoate macrolide natural products that have been shown to inhibit growth of the marine fungal pathogen *Lindera thalassiae*. As such, they have been implicated in antimicrobial chemical defense. However, the defense mechanisms are not yet completely understood. Precise detection of these compounds on algal tissue surfaces under ambient conditions without any disruptive sample processing could shed more light onto the processes involved in chemical defense of marine organisms. Conventional DESI-MS directly on algal tissue showed relatively low sensitivity for bromophycolide detection. Sensitivity was greatly improved by the addition of various anions including Cl^- , Br^- , and CF_3COO^- into the DESI spray solvent. Chloride adduction gave the highest sensitivity for all assayed anions. Density functional optimization of the bromophycolide anionic complexes produced during DESI supported this observation by showing that the chloride complex has the most favorable binding energy. Optimized DESI protocols allowed the direct and unambiguous detection of bromophycolides, including A, B, and E, from the surface of untreated algal tissue.

Keywords

Desorption electrospray ionization; Mass spectrometry; Direct analysis; Natural products

Introduction

Over the past 4 years, efforts toward performing mass spectrometry (MS) experiments under ambient environmental conditions have been steadily intensifying. The ability to record mass spectra under such conditions has made many materials now amenable to direct MS analyses, enabling new research avenues. Ambient MS techniques do not require sample preparation thereby allowing rapid analysis times and preserving the innate chemical information to a maximum. Several ambient MS ionization techniques have been described. Desorption electrospray ionization (DESI) [1–9], direct analysis in real time [10], desorption atmospheric pressure chemical ionization [2, 9, 11], electrospray assisted laser desorption ionization [11], atmospheric solids analysis probe [12], infrared laser-assisted desorption electrospray ionization [13], and flowing afterglow atmospheric pressure glow discharge ionization [14, 15] are some of the recently developed techniques that have generated considerable interest.

DESI, a widely investigated ambient ionization technique, gained popularity because of its simple operating nature and electrospray-like instrumentation. DESI allows probing the surface of solids, liquids, frozen solutions, and adsorbed gases using MS. DESI ion sources are simple and can accommodate a variety of sample sizes and shapes. DESI can be readily connected to any mass spectrometer equipped with an atmospheric pressure interface and provides high sensitivity, short analysis time, and the capability to desorb and ionize a range of different analytes, including small organic compounds, peptides, proteins, and other chemical species [3, 6, 7, 16–18]. Since its discovery, DESI has been successfully utilized in many types of applications, including forensics [6, 19, 20], imaging [2, 21–24], explosives detection [3, 6, 25], pharmaceutical analysis [1, 8, 9, 20, 26–28], study of natural products [6, 29], polymer characterization [30, 31], proteomics [6, 16, 32], metabolomics [6, 33], and glycomics [18].

In DESI, a pneumatically assisted electrospray plume is continuously sprayed towards the sample, forming a thin, micron-sized solvent film on its surface. Formation of this film allows rapid liquid–solid extraction of analyte molecules off the sample. Continuous flow of the electrospray plume dynamically replaces the existing solvent film, generating analyte-containing secondary droplets. The secondary droplets are propelled towards the inlet of the mass spectrometer [34]. In most cases, DESI does not require sample preparation.

To date, the ability of DESI-MS to probe the soft tissue of aquatic plants, in particularly algae, has not been investigated. Algae are a large and diverse group of simple, autotrophic organisms. They are important ecologically as food for herbivorous fishes and invertebrates and as habitat for many other plants and animals. A recent investigation into the marine red algal species, *Callophycus serratus*, found this algae to produce bromine-containing secondary metabolites [35, 36]. These compounds, particularly the macrolide-containing bromophycolides, have shown promise as cytotoxic agents with potential for pharmaceutical drug candidacy.

The present investigation reports the application of DESI-MS to detect these macrolides directly on the surface and interior of the algae soft tissue. Reactive DESI approaches were investigated to improve ionization efficiency and detection limits. Finally, in order to gain more insight on the species formed during the reactive DESI process, the relative gas phase

stabilities of different bromophycolide complexes were compared via ab initio computational calculations.

Experimental

Materials and reagent

All reagents were used as received without additional purification. High-purity high-performance liquid chromatography (HPLC)-grade methanol 99.9+% (Honeywell Burdick & Jackson, Muskegon, MI, USA) was used for all DESI and reactive DESI experiments. Ultrapure water ($18.2 \text{ M}\Omega \text{ cm}^{-1}$) was obtained from a Nanopure purification unit (Barnstead, San Jose, CA, USA). Ammonium chloride (Sigma-Aldrich, St. Louis, MO, USA), hydrobromic acid (Mallinckrodt Baker Inc., Phillipsburg, NJ), and trifluoroacetic acid (Fisher Scientific, Fair Lawn, NJ) were used for reactive DESI experiments. Pure bromophycolides A, B, and E (chemical structures shown in Scheme 1) were isolated from the Fijian red alga, *C. serratus*, following previously reported procedures [35, 36]. Algal samples were collected in February 2008 off Yanuca Island in Beqa Lagoon, Fiji ($18^{\circ}23'57'' \text{ S}$, $177^{\circ}57'59'' \text{ E}$). Samples were preserved in 10% v/v formalin/sea water and stored at room temperature. Samples were rinsed in ultrapure water and allowed to air dry prior to analysis.

DESI-MS—DESI experiments were performed using a home-built DESI ion source [37]. Briefly; the DESI source consisted of a joystick-controlled motorized microscope XY stage (Prior Scientific, Rockland, MA, USA) for automatic and manual sample positioning in the x - y and z coordinates, respectively. The sprayer was mounted on a MicroBlock 3-axis positioner (Thorlabs, Newton, NJ, USA) for manual adjustment of the emitter position in the x - y - z coordinates with respect to the capillary inlet and fitted with a high-precision rotation mount (Thorlabs) for manual adjustment of the spray impact angle. For reagent-free DESI experiments, various spray solvent compositions including 100% MeOH, 100% CH_3CN , 50:50 MeOH/ H_2O , and $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ were tested. One hundred percent MeOH gave the best signal for all bromophycolides and was thus chosen for all subsequent experiments. For reactive DESI, various additives including NH_4Cl (100 μM), HBr (10 μM), and CF_3COOH (0.001% v/v=135 μM) were added to the spray solvent (MeOH) at the concentrations in parenthesis unless stated otherwise. The spray solvent flow rate was 5 $\mu\text{L}/\text{min}$. The nebulizer gas pressure was set at 110 psi and the spray solution electrically charged externally to -3 kV . The spray-to-surface distance was 1–2 mm, and the spray impact angle was set to 55° . Bromophycolide working solutions were prepared in MeOH, and 10 μL , 1 mg/mL of each standard were deposited onto polytetrafluoroethylene (PTFE) surfaces and air dried prior to analysis. For the analyses of *C. serratus* algal sample, small pieces of tissue were affixed to glass slides using double-sided tape and analyzed by exposing different positions of their surfaces to the DESI spray plume. All experiments were performed on an LCQ DECA XP^+ ion trap mass spectrometer (Thermo Finnigan, San Jose CA, USA) operated in negative ion mode. Data was collected via the Xcalibur software (version 2.0, Thermo Finnigan). The ion transfer capillary was held at 300°C . The instrument was set to collect spectra in automatic gain mode for an ion trap injection time of 200 ms at two microscans per spectrum for a total acquisition time of 10 s/spectrum.

Theoretical methods

Geometries for bromophycolides A, B, and E were optimized at the B3LYP-D/6–31+G* level of theory, where the “–D” denotes the inclusion of empirical terms to compensate for the inability of the B3LYP functional to account for dispersion interactions. The empirical dispersion terms developed by Grimme [38] were used in this work. The complexes of bromophycolide A, B, and E with Cl^- , Br^- , and CF_3COO^- were fully optimized at the

B3LYP-D/6-31+G* level of theory. Binding energies were computed at the B3LYP-D/6-31+G* level of theory using a Lebedev grid with 302 angular points for each of 100 radial points. This grid was chosen to avoid errors related to numerical integration. Counterpoise correction was applied to all binding energies according to the scheme of Boys and Bernardi [39]. Geometries of bromophycolide A, B, and E dimers were optimized at the B3LYP/6-31+G* level of theory. All computations were performed using QChem 3.1 [40] and Jaguar 5.5 [41].

Results and discussion

Twenty different natural products including bromophycolides (A–I) and debromophycolide A have thus far been isolated from the marine alga, *C. serratus*, the most abundant being bromophycolide A (39.7 mg; 0.80% plant dry mass) and B (6.1 mg; 0.11% plant dry mass) [35, 36, 42]. These compounds were previously isolated by liquid–liquid extraction in MeOH or MeOH/CH₂Cl₂ solvent mixtures, followed by HPLC separation [43]. Except for debromophycolide A which contains no bromine, all other bromophycolides identified from this alga contain two to three bromine atoms, resulting in unique mass spectrometric isotopic signatures. The presence of bromines has been shown to be vital for their moderate anticancer and antimicrobial activities [35]. Furthermore, bromophycolides have also been observed to be very effective antifungal agents, especially against the marine pathogenic fungus *Lindra thalassiae*, with IC₅₀ values for each bromophycolide at or below whole tissue natural concentrations [43].

The higher abundance of bromophycolides A and B (depicted in Scheme 1) relative to other bromophycolides in extracts [43] suggested a higher chance for their detection from algal tissue by DESI-MS, and as such, they were chosen for the optimization experiments together with the less abundant bromophycolide E (Scheme 1). Analysis of pure bromophycolides was explored in both positive and negative ion modes. However, ionization and detection of bromophycolides was significantly more efficient in the latter, presumably due to the presence of a phenolic acid functionality in their macrolide structure in which the conjugate phenolate anion is electronically stabilized by resonance. No appreciable ionization was observed in positive ion mode even in the presence of various concentrations of organic acids or various cations such as Na⁺ and NH₄⁺ which, when added to the spray solution, could potentially enhance ionization efficiency by cationization [17]. As such, all subsequent experiments were performed in negative ion mode.

Figure 1 shows the DESI-MS, MS², and MS³ spectra of purified bromophycolides deposited on PTFE substrates and interrogated with a solution of neat MeOH. Figure 1a shows the DESI spectrum of bromophycolide A. The spectrum consists of two major isotopic clusters, with base peaks at *m/z* 665 and *m/z* 1,329. These clusters correspond to the deprotonated monomer and dimer species of bromophycolide A, respectively. The insert in Fig. 1a shows the isotopic distribution of the deprotonated bromophycolide A monomer together with the theoretical isotopic pattern based on its elemental formula. As seen in the insert, bromophycolide A shows a unique isotopic distribution consistent with the presence of three bromine atoms in its structure [35, 36], matching the theoretical distribution. Deprotonation of bromophycolide A is predicted to preferentially occur on the phenyl hydroxyl group due to the ability of the phenyl ring to stabilize the negative charge. Deprotonation at this site was calculated to be about 38 kcal mol^{−1} more favorable than at the macrolide hydroxyl group. The structure of the deprotonated bromophycolide A calculated at the B3LYP-D/6-31+G* level of theory is shown in Fig. 2a.

The structural connectivity of the deprotonated bromophycolide A monomer can be further evaluated by performing sequential ion trap MS^{*n*} experiments by collision-induced

dissociation (CID). Figure 1b shows the MS² spectrum of the deprotonated bromophycolide A monomer, which gives rise to a fragment ion cluster with a base peak at m/z 583 corresponding to the elimination of HBr from its precursor. Inserted in Fig. 1b is the MS³ spectrum generated from CID analysis of the ion cluster at m/z 583 which shows intense fragment ion clusters with base peaks at m/z 503 and m/z 421, corresponding to the elimination of one HBr and two HBr molecules, respectively. MS⁴ analysis of the cluster at m/z 503 generated the ion at m/z 421 as the predominant species in the spectrum, corresponding to the elimination of HBr from its precursor ion (data not shown). The sequential elimination of HBr indicates a lability of the C–Br bonds in the precursor ion molecule, presumably due to their relatively longer bond lengths compared to all other bonds in this molecule [36]. MS⁵ analysis of the isotopic cluster centered at m/z 665 generated ions at m/z 377 (loss of CO₂) and m/z 359 (loss of H₂O, Figure S-1a, Electronic Supplementary Material).

Bromophycolide B is a constitutional isomer of bromophycolide A, containing a 16-membered macrolide within a diterpene-benzoate framework as opposed to a 15-membered macrolide for bromophycolide A [36]. Figure 1c shows the DESI-MS spectrum for pure bromophycolide B, which shows isotopic clusters with base peaks at m/z 665 and 1,329 corresponding to the deprotonated monomer and dimer, respectively. Shown as insert in Fig. 1c is the isotopic distribution of the deprotonated monomer which is almost identical to that obtained for bromophycolide A, thus verifying their isomeric relationship. Mass spectrometric distinction between isomeric species can sometimes be accomplished via MS^{*n*} analysis [44] but, as shown in Fig. 1d and S-1b, stepwise CID analysis (up to MS⁵) of bromophycolide B led to elimination of the same species as for bromophycolide A at each stage, thus eliminating this possibility. Figure 1e shows the DESI-MS spectrum of bromophycolide E which revealed isotopic clusters with base peaks at m/z 583 and 1,167, corresponding to the deprotonated bromophycolide E monomer and dimer, respectively. MS² analysis of the isotopic ion cluster with a base peak at m/z 583 generated a cluster with a base peak at m/z 503 due to the elimination of one HBr molecule and a second peak corresponding to the completely debrominated species at m/z 421 resulting from the elimination of two HBr molecules from the precursor ions (Fig. 1f).

The potentially low abundance of bromophycolides on the algal tissue surface necessitates an appreciably sensitive detection means. As shown above, the most abundant bromophycolides, A and B, produced the deprotonated dimer as the base peak in their DESI spectra (Fig. 1). Decustering of such dimers by in-source CID could lead to an increase in the abundance of the deprotonated monomer thus improving the sensitivity for the detection of bromophycolides [45, 46]. In-source CID experiments were performed by varying the direct current (DC) voltage offset of the ion source ion transfer octopole between 0 and 60 V. This resulted in an overall decrease in the signal intensity of the deprotonated bromophycolide A dimer and only a slight increase in the intensity of the deprotonated monomer ion, with a maximum at 40 V. Higher voltages resulted in the concomitant formation of the fragment at m/z 583 (Figure S-2). Even at the maximum DC offset investigated (60 V), an appreciable signal was still observed for the dimer ion, indicating a significant stability. This stability can be attributed to a strong hydrogen bond between the phenyl hydroxyl group of one bromophycolide molecule and the deprotonated phenyl hydroxyl group of the other, contributing roughly 30 kcal mol⁻¹ to the stability as demonstrated by theoretical studies at the B3LYP/6-31+G* level of theory (Fig. 2b). Additionally, the stabilization due to dispersion interaction between the two bromophycolide units can be larger than 10 kcal mol⁻¹.

Performing the analysis in the reactive DESI mode can be an alternative means of improving sensitivity by improving ionization efficiency or increasing ion survival during transport

through the atmospheric pressure interface. In reactive DESI, the spray solvent is mixed with a reagent that binds with the analyte [4, 17, 47–49]. Addition of halides to the spray solvent has been found beneficial, improving ionization of target analytes such as explosives [5]. In this study, various anions including: Cl^- , Br^- , and CF_3COO^- were explored as additives to investigate their effect on the various bromophycolide signals. Results from the reactive analysis of bromophycolide A standards deposited onto PTFE with various anions are shown in Fig. 3. When ammonium chloride was added to the DESI spray solvent (Fig. 3a), the DESI spectrum showed signals at m/z 701 and 1,365, corresponding to $([\text{bromophycolide A} + \text{Cl}]^-)$ and $([2 \text{ bromophycolide A} + \text{Cl}]^-)$, respectively. A peak at m/z 1,329 corresponding to the deprotonated bromophycolide A dimer was also observed. The identity of the peak at m/z 701 was verified by DESI- MS^2 (Fig. 3b), where the noncovalently bound chloride was eliminated as HCl to give the ion at m/z 665 corresponding to deprotonated bromophycolide A. The addition of a low concentration of hydrobromic acid to the DESI spray solvent resulted to peaks at m/z 745 and 1,411 assigned to the complexes of bromide with bromophycolide A monomer and dimer, respectively (Fig. 3c). DESI- MS^2 analysis of the ion at m/z 745 generated predominantly the ion at m/z 665 corresponding to deprotonated bromophycolide A via loss of HBr (Fig. 3d). Peaks at m/z 779 and 1,443 corresponding to bromophycolide A monomer and dimer adducts with trifluoroacetate were observed when trifluoroacetic acid was added to the DESI spray solvent. A peak at m/z 1,329 corresponding to the deprotonated bromophycolide A dimer was also observed in the spectrum (Fig. 3e). The identity of the peak at m/z 779 was verified by DESI- MS^2 analysis, giving the deprotonated bromophycolide A monomer by elimination of a trifluoroacetic acid molecule (Fig. 3f).

Figure 4 shows the B3LYP-D optimized structures of bromophycolide A, together with its Cl^- , Br^- and CF_3COO^- adducts. Similar structures for bromophycolide B and E are shown in Figures S-4 and S-5. Density functional optimizations of the bromophycolide complexes with Cl^- , Br^- , and CF_3COO^- showed in all cases hydrogen bonding between the respective anions and the hydroxyl group on the phenolic ring. The preference for binding to the phenolic hydroxyl over the macrolide ring hydroxyl group is due, in part, to delocalization over the phenyl ring of the partial negative charge transferred from the anion, as indicated by Mulliken charge analysis. Another reason for the preference for binding to the phenolic hydroxyl is that anion binding to the macrolide ring hydroxyl disrupts the intramolecular hydrogen bonding between this hydroxyl and the neighboring macrolide ring bromine atom (Fig. 4a). This gives rise to a less stable conformation thereby making anion binding to this site less favorable by roughly 10 kcal mol^{-1} (data not shown). Also, hydrogen bond lengths resulting from anion binding to the phenyl hydroxyl were about $0.2 \text{ \AA}$ shorter for complexes with Cl^- and CF_3COO^- compared to similar complexes where anion binding occurs at the macrolide hydroxyl group. Similarly, for the Br^- complexes, hydrogen bond lengths for the phenyl hydroxyl were shorter by about $0.1 \text{ \AA}$ (data not shown). Table 1 presents the binding energies for the various bromophycolide–anion complexes together with their corresponding intermolecular hydrogen bond lengths. In general, chloride complexes showed the highest binding energies and bromide complexes the lowest. The former were observed to show greater charge delocalization compared to the bromide complexes, this factor probably accounting for the higher binding energies. This observation is consistent with the higher sensitivity that was obtained by reactive DESI-MS when chloride was added to the spray solvent (*vide infra*). In the case of the CF_3COO^- complex, despite the anion oxygen atoms showing two favorable interactions with the phenolic hydrogen atom of the bromophycolide, the anion tends to retain more of its negative charge which might account for their slightly lower binding energies compared to the chloride complexes.

Figure 5 shows the effect of the DESI spray solvent chloride concentration on the intensity of the bromophycolide A adduct (m/z 710) and the deprotonated dimer (m/z 1,329). The

intensity of the chloride adduct increases with the concentration of chloride, reaching a maximum at approximately 100 μM NH_4Cl . Above 100 μM NH_4Cl , the intensity of both species decreases, possibly due to increased analyte suppression effects as the electrolyte concentration increases. When HBr and CF_3COOH were each added to the DESI spray, the optimum concentration for the formation of bromide and trifluoroacetate adducts with bromophycolide A were 10 and 135 μM , respectively.

The S/N of all the investigated bromophycolide anion adducts obtained by reactive DESI together with those of their corresponding deprotonated counterparts obtained by reagentless DESI are as shown in Figure S-3. At the optimized concentration of each of the investigated anions, the chloride adducts gave the highest S/N. This results in S/N gains for the chloride adduct of 10 $\times$, 19 $\times$, and 7 $\times$ for bromophycolide A, B, and E, respectively, when compared to the corresponding deprotonated species. The high S/N for the chloride adducts is probably due to the comparably smaller size and high electronegativity of the chloride anion. The presence of chloride in the spray solvent also somewhat inhibits the formation of the deprotonated dimer species potentially resulting in the improvement of the signal for the monomer adduct. The limit of detection values (S/N 3) for all the investigated species are shown in Table 2. The addition of chloride into the DESI spray clearly results in a very sensitive approach potentially allowing the direct and rapid detection for bromophycolides on the alga tissue surface.

The application of the above protocols was then evaluated for the direct detection of bromophycolides on the surface of untreated *C. serratus* samples. No appreciable bromophycolide signals were observed when analysis was performed by scanning the 400–1,500 m/z range. This is probably due to the fact that trapping within such a wide m/z range effectively results in a low number of bromophycolide species trapped in the quadrupole ion trap mass analyzer. Analysis was then performed in the 550–750 m/z range, and signals corresponding to bromophycolide species were unambiguously observed in this case (Fig. 6). The peak at m/z 701 was assigned as the chloride adduct of bromophycolides A and/or B. This assignment is consistent with the theoretical isotopic distribution of these species and with MS/MS experiments (data not shown). However, there could also be some contribution from the less abundant bromophycolides D and H, which have the same elemental formula [35, 43]. An intense peak was also observed at m/z 619, and this was assigned as the chloride adduct of bromophycolide E, consistent with its theoretical mass and isotopic distribution (data not shown). Some minute peaks were also observed at m/z 665 and 583, corresponding to [bromophycolide A(B)–H] $^-$ and [bromophycolide E–H] $^-$, respectively. The bromophycolide signal intensity was not homogeneous throughout the sample surface, with some regions showing no signal whatsoever. However, when the surface of the alga was scraped with a razor blade resulting in the exposure of underlying internal tissue, bromophycolide signals also were readily observed in all cases (data not shown), suggesting an endogenous origin for these bromophycolides. This phenomenon requires further investigations, which are underway.

Conclusions

DESI-MS has been shown to be a sensitive and effective approach for the direct surface detection of antimicrobial bromophycolides on the marine alga *C. serratus*. Sensitivity enhancement for the detection of various bromophycolide standards was achieved by the addition of various anions including Cl^- , Br^- , and CF_3COO^- into the DESI spray solvent. The formation of chloride complexes resulted in the highest signals with S/N gains of 10 $\times$, 19 $\times$, and 7 $\times$ for bromophycolide A, B, and E, respectively. Computational calculations indicated that formation of complexes occurred through intermolecular hydrogen bonding interaction between the anion and the phenolic hydroxyl group in the bromophycolide.

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Biography

Facundo M. Fernández has been an Assistant Professor of Chemistry at the Georgia Institute of Technology since January 2004. He has recently won the NSF CAREER award, ASMS Research award, SACP Starter Grant award, and the 3M Nontenured Faculty Grant award. His current research interests include the study and development of new ionization methods for analytical mass spectrometry, mass spectrometry imaging, and ion mobility gas-phase separations. He is also interested in the application of these technologies to metabolomics.

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Fig. 1.

Conventional DESI-MS, MS², and MS³ spectra of pure bromophycolides (10 μ L, 1 mg/mL) deposited on PTFE substrates and interrogated with a neat MeOH spray solution: **a** mass spectrum of bromophycolide A, **b** corresponding MS² spectrum of the precursor ion at m/z 665 (the *insert* in **b** shows the MS³ spectrum of the precursor ion at m/z 583); **c** mass spectrum of bromophycolide B, **d** corresponding MS² spectrum of the precursor ion at m/z 665 (the *insert* in **c** shows the MS³ spectrum of the precursor ion at m/z 665); **e** mass spectrum of bromophycolide E, and **f** corresponding MS² spectrum of the precursor ion at m/z 583

Fig. 2. Geometries for **a** deprotonated bromophycolide A and **b** [2M-H]⁻ complex of neutral and deprotonated bromophycolide A optimized at the B3LYP/6-31+G* level of theory

Fig. 3. Reactive DESI-MS and MS² spectra of bromophycolide A standards (10 μ L, 1 mg/mL), deposited on PTFE surfaces and interrogated with spray solutions of various anions dissolved in MeOH; **a** 100 μ M NH₄Cl with **b** corresponding MS² spectrum of the precursor ion at m/z 701; **c** 10 μ M HBr, with **d** corresponding MS² spectrum of the precursor at m/z 745; and **e** 0.001% v/v (135 μ M) CF₃COOH with **f** corresponding MS² spectrum of the ion at m/z 779

Fig. 4. Optimized geometries for: **a** bromophycolide A and its complexes with **b** Cl^- , **c** Br^- , and **d** CF_3COO^- optimized at the B3LYP-D/6-31+G* level of theory

Fig. 5.

Effect of chloride ion concentration on the intensity of the [bromophycolide A+Cl]⁻ and [2 bromophycolide A-H]⁻ ions. The intensity values were normalized to that of the maximum value. For all experiments, 10 μL, 1 mg/mL bromophycolide A were deposited and allowed to air dry prior to analysis

Fig. 6.
DESI spectrum obtained from directly interrogating the surface of the Fijian red alga *C. serratus* with a spray solution of 100 μ M NH_4Cl in MeOH

Scheme 1.

Chemical structure of bromophycolides identified from the Fijian red alga *C. serratus*

Table 1

Binding energies for various bromophycolide–anion complexes obtained from geometry optimizations at the B3LYP-D/6-31+G* level of theory

Analyte (M)	<u>Binding energy kcal⁻¹ mol⁻¹ (H-bond length/Å)</u>		
	[M+Br] ⁻	[M+CF ₃ COO] ⁻	[M+Cl] ⁻
Bromophycolide A	32.7 (2.09)	36.2 (1.57)	37.1 (1.92)
Bromophycolide B	30.5 (2.10)	34.1 (1.58)	34.9 (1.93)
Bromophycolide E	31.9 (2.11)	35.2 (1.58)	36.2 (1.94)

The intermolecular hydrogen bond lengths are indicated in parenthesis

Table 2

Limits of detection (S/N 3) for various bromophycolides obtained by monitoring different species by DESI-MS in negative ion mode

Analyte (M)	Detection limit (pmol/mm ²)			
	[M-H] ^{-a}	[M+Br] ^{-b}	[M+CF ₃ COO] ^{-b}	[M+Cl] ^{-b}
Bromophycolide A	85	80	42	8
Bromophycolide B	146	78	55	8
Bromophycolide E	66	61	75	10

^aReagentless DESI

^bReactive DESI where various anions including Br⁻, CF₃COO⁻, and Cl⁻ were added to the spray solution