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Motualevic acids and analogs: Synthesis and antimicrobial structure–activity relationships

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ABSTRACT

Synthesis of the marine natural products motualevic acids A, E, and analogs in which modifications have been made to the ω-brominated lipid (*E*)-14,14-dibromotetra-deca-2,13-dienoic acid or amino acid unit are reported, together with antimicrobial activities against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Enterococcus faecium*, and vancomycin-resistant *Enterococcus*.

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Starting with Fleming's discovery of penicillin in the 1920's, the discovery and development of new antibiotics was marked by unmatched success in the area of drug discovery and remained at the fore for decades.[1] Numerous classes of antibacterial agents were discovered from Nature, and many of those are in clinical use today. The expansion of antibiotic research reached its peak by the middle of the last century, then paused for four decades before approval of oxazolidinone linezolid in 2000[2] and the lipopeptide daptomycin in 2003.[3] Nonetheless, a variety of infectious diseases persist as true global health burdens,[4] and infections associated with drug resistant organisms[5] such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* spp. (VRE) continue to rise. To help alleviate this problem, development of new antibiotics that function with unique mechanisms of action and can escape the resistance mechanisms used by drug resistant bacteria are needed. Combined studies involving discovery and synthesis of new antimicrobial natural products can be valuable in this area.[6a,b]

Recently we reported the isolation, structure elucidation and antibacterial properties of motualevic acids A–E (e.g., **1a**–**c** and **8**) from the marine sponge *Siliquariaspongia* sp.[7] These compounds contain an unusual ω-brominated lipid (*E*)-14,14-dibromotetra-deca-2,13-dienoic acid, where motualevic acids A–D are its glycyl conjugates and motualevic acid E the free acid (Fig. 1). Antimicrobial assays revealed that motualevic acid A (**1a**) inhibited the growth of *S. aureus* (SA) and MRSA at low micromolar concentrations, while motualevic acid E (**8**) showed greatly diminished activity, effective against SA only at much higher concentrations (50 μg/disk). Interestingly, the naturally occurring amides **1b** and **1c** and the *Z* isomer of **1a** also exhibited greatly diminished antimicrobial activity providing some insight into the structure–activity relationships for this series. To obtain a more general picture of the chemical features necessary for antimicrobial activity, we have synthesized motualevic acids A and E together with a number of analogs and tested their effects on growth of several bacterial strains, the results of which are reported here.

Synthesis of motualevic acid A (**1a**) began with monoprotection of commercially available and inexpensive 1,9-nonanediol with TBDPSCl (Scheme 1). Conversion of the remaining hydroxyl group to α,β-unsaturated ester **5** was accomplished by an oxidation-Wittig reaction sequence.[8] Successive reductions of the double bond

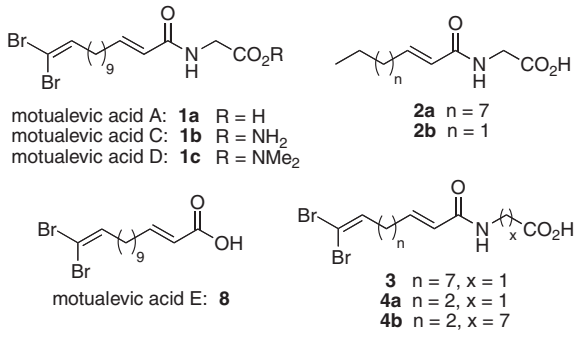

Figure 1. Examples of motualevic acids and synthetic analogs.

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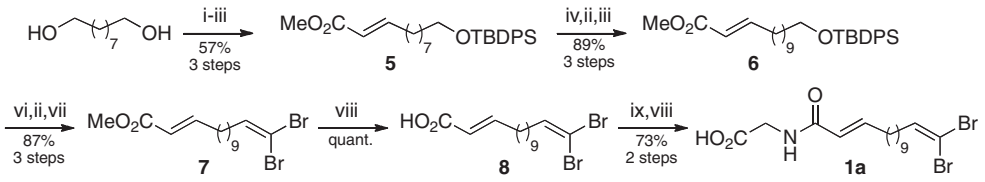
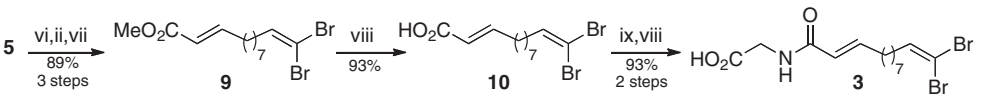
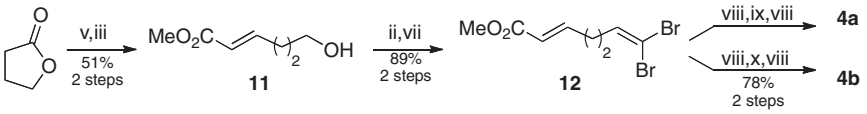
Scheme 1



Scheme 2



Scheme 3



Schemes 1–3. Reagents and conditions: (i) TBDPSCl, imidazole, THF, 0 °C, 5 h; (ii) Dess–Martin periodinane, DCM, rt, 0.5–1 h; (iii) $(C_6H_5)_3P{=}CHCO_2CH_3$, toluene, 80 °C, 4 h; (iv) pd/C. H_2, rt, 0.5 h; (v) DIBAL-H, toluene, −70 °C, 4 h; (iv) TBAF, THF, rt, O/N; (vii) PPh_3, CBr_4, CH_2Cl_2, 0 °C to 15 °C, 1 h; (viii) 1 M NaOH, THF, rt, on; (ix) glycine methylester hydrochloride, EDC, HOBt, DMF, 0 °C to rt, 4–6 h; (x) caprylic acid methyl ester, EDC, HOBt, DMF, 0 °C to rt, 4–6 h.

and ester by catalytic hydrogenation and treatment with DIBAL-H, followed by a second two-carbon Wittig olefination afforded α,β-unsaturated ester **6**. Fluoride-induced cleavage of the silyl-protecting group, followed by oxidation and a Corey–Fuchs *gem*-dibromo olefination[9] yielded the vinyl *gem*-dibromo olefin **7**.[10] Saponfication of **7** provided motualevic acid E (**8**), that was in turn coupled to glycine methyl ester using standard coupling reagents (EDC, HOBT); and saponification of the methylester completed the total synthesis of motualevic acid A (**1**) in 31% overall yield (1 mmol scale).[11] Analytical data for synthetic motualevic acids A and E[7] were identical to those of the natural products.

With motualevic acid E (**8**) in hand, we synthesized a range of additional conjugates **1d**–**n**, where amino acids or diamines were chosen to explore the effects of charge, hydrophobicity, and flexibility in this region of the molecule. Acid **8** was activated with HOBt and coupled to respective amino acid methyl esters, followed by hydrolysis to give **1d**–**m**; or coupled to 2-amino-*N*,*N*,*N*-trimethylethaneammonium chloride to give quaternary ammonium **1n** (Table 1).

To explore the effects of fatty acid chain length and composition on antimicrobial activity, we synthesized glycyl conjugates of the α,β-unsaturated C6, C7 and C12 acids, with and without the terminal dibromovinyl olefin. Knowing that the *Z* configuration at C2–C3 diminished activity, (*E*)-didec-2-enoic acid was synthesized starting from decanal using the same homologation approach employed for synthesis of **1** according to published procedures.[12,13] Coupling of the synthetic (*E*)-didec-2-enoic acid or commercially available (*E*)-hex-2-enoic acid to glycine provided alkenes **2a**–**b**. Similarly, the protected C11 α,β-unsaturated ester **5** was converted to the C12 *gem*-dibromoolefin **9** after deprotection, oxidation and Corey–Fuchs reaction conditions (Scheme 2). Saponification and coupling to glycine provided analog **3**, whose fatty acid unit is two carbons shorter than natural product **1**. As shown in Scheme 3, the C7 analog (*E*)-7,7-dibromohex-2,6-dienoic acid present in analogs **4a**–**b** was prepared starting with commercially available γ-butyrolactone. Reduction with DIBAL-H provided the hemiacetal, and a Wittig reaction afforded the α,β-unsaturated ester **11**.[14] Oxidation of **11** to its aldehyde followed by Corey–Fuchs *gem*-dibromo olefination afforded **12**, and subsequent saponification, coupling to glycyl or caprylic acid methyl ester, and saponification gave analogs **4a**–**b**.

Antimicrobial susceptibility testing was carried out using agar disk diffusion and microbroth dilution assays using Clinical Laboratory Standards Institute guidelines as described in our previous work.[7] Initial screening was performed using the solid agar assays, and for compounds exhibiting zones of inhibition of at least 8–10 mm at 25 μg/disk, microbroth dilution assays were also performed.

Prior to synthesizing the suite of amino acid conjugates, compounds designed to investigate the antimicrobial effects of fatty acid chain length and composition were tested first. As shown in Table 1, the composition of the fatty acid was critical to the antimicrobial activity, and any changes that were made to the dienoic acid in this study diminished or abolished activity. Thus glycyl conjugates **2a** and **2b**, both of which lack the terminal dibromoolefin and possess shorter alkyl chains (C12 and C6, respectively) were inactive. Surprisingly, activity was diminished by at least fivefold for analog **3** that is almost identical to motualevic acid A differing only by deletion of an ethylene unit. Compounds **4a** and **4b** were constructed both to test whether a truncated dienoic acid containing a terminal dibromoolefin could substitute for the natural C14 dienoic acid, and whether total chain length was sufficient for antimicrobial activity and, in the case of **4b**, whether position of the amide mattered. Truncated glycyl conjugate **4a** was inactive. Modest activity was observed for the more conservative analog **4b**, but zones of inhibition were hazy. Thus, modification of motualevic acid A within the fatty acid portion of the molecule is detrimental to antibacterial activity and led us to construct all other conjugates from motualevic acid E (**8**).

Subsequent to our original report describing antimicrobial activities of motualevic acids A–E toward SA and MRSA, these compounds were further screened for effects on EF and VRE. Similar to

Table 1
In vitro antimicrobial activities of compounds **1**–**4** and **8**[a]

Compound(s)	SA	MRSA
1a	10	10
1b–**c**, **2a**–**b**, **4a**	na	na
4b	25	25
3	50	50
8	50	na

[a] Determined by agar disk diffusion assays; values are in μg/disk and represent zones of inhibition of 8–11 mm; na (not active at 50 μg/disk).

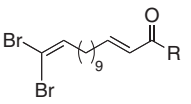
the anti-staphylococcal activities, motualevic acid A inhibited the growth of EF and VRE with similar potency (Table 2). Thus, amino acid/amine conjugates **1d**–**n** were tested against these four bacterial strains.

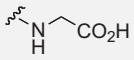
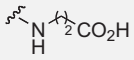
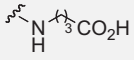
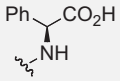
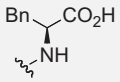
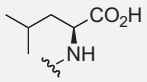
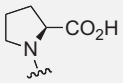
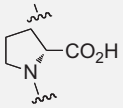
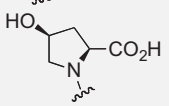
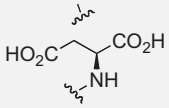
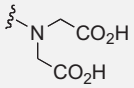
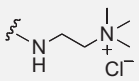
Slight differences in potency were observed in disk diffusion assays for analogs **1d** and **1e**, conjugates of β-alanine and 3-aminopropanoic acid, respectively; but in liquid culture both analogs inhibited the growth of SA and MRSA with MIC values around 3 μg/mL, an improvement over **1a**; and inhibited EF and VRE with MIC values of 8.5–12 μg/mL, similar to **1a**. Two aromatic amino acid conjugates **1f** and **1g** were prepared by coupling **8** to phenylglycine or phenylalanine, respectively. Surprisingly, these analogs showed very different potencies where MICs for **1f** averaged 20 μg/mL over the four strains, while values for **1g** ranged from 2.9 to 6.3 μg/mL and showed good activity toward VRE. The leucine conjugate **1h** proved to be the most potent of all analogs, and inhibited the growth of SA and MRSA with MICs less than 3 μg/mL. To test the effects of rigidity of the amino acid unit on activity, several proline-containing analogs were prepared. The antimicrobial profile for **1i** (L-proline) was almost identical to motualevic acid A; and somewhat surprisingly, its enantiomer **1j** showed reduced activity for all strains but VRE. The hydroxyproline conjugate showed only modest activity in disk diffusion assays and was not tested further.

The structure–activity profile for natural motualevic acids showed that the presence of a free carboxylic acid was essential for antimicrobial activity. This prompted us to synthesize aspartic acid- and diacetic acid conjugates **1l** and **1m**, analogs with a charge of −2. In disk diffusion and microbroth dilution assays both were found to be inactive against all strains in both formats. Finally, mindful of the properties of quaternary ammonium compounds as general antiseptics, we prepared the 2-amino-*N*,*N*,*N*-trimethylethanaminium conjugate **1n**. Similar to the leucine analog **1h**, **1n** showed improved potency toward all strains relative to **1a**, with MIC_{50} values of 2.4–5.2 μg/mL.

In summary, we have synthesized the antibacterial marine natural products motualevic acids A and E, together with analogs designed for structure-function relationships within the fatty acid

Table 2
Antimicrobial data for conjugates **1a** and **1d**–**n**

8 → *coupling* (Amine (R), HOBt, EDC) → 

	R	(Disk diffusion[a]) microbroth dilution[b]			
		SA	MRSA	EF	VRE
1a		(10) 11	(10) 9.3	(25) 9.3	(10) 12
1d		(10) 3.6	(10) 3.3	(25) 11	(10) 12
1e		(25) 2.5	(25) 2.4	(10) 11	(10) 8.5
1f		(25) 17	(25) 21	(10) 25	(10) 22
1g		(na) 5.0	(50) 4.4	(5) 6.3	(5) 2.9
1h		(25) 2.3	(25) 2.9	(10) 6.4	(5) 3.7
1i		(25) 10	(10) 9.0	(25) 10	(5) 12
1j		(25) 18	(25) 19	(25) 15	(10) 11
1k		(na) nt	(25) nt	(25) nt	(25) nt
1l		(na) na	(na) na	(na) na	(na) na
1m		(50) na	(50) na	(na) na	(na) na
1n		(10) 2.4	(10) 4.2	(25) 3.7	(25) 5.2

[a] Results for disk diffusion assays are shown in parentheses in μg/disk and correspond to values giving 8–10 mm zones of inhibition.
[b] MIC_{50} values reported as μg/mL. Standard deviations averaged 25%; na = not active at 50 μg/disk or higher; nt = not tested. Bacterial strains included *Staphylococcus aureus* (SA), methicillin-resistant *S. aureus* (MRSA), *Enterococcus faecium* (EF), and vancomycin-resistant *Enterococcus* (VRE).

and amino acid units. This work has shown that the ω-brominated lipid (*E*)-14,14-dibromotetra-deca-2,13-dienoic acid present in all of the natural products is required for low micromolar antimicrobial activity, replacement of glycine by more hydrophobic amino acids (with the exception of phenylglycine) increases potency, and introduction of polar amino acids or more than one negative charge abrogates activity.

Compounds **1a**, **1g**, **1h**, and **1n** were tested for cytotoxicity toward a control mammalian cell line (BSC1); each exhibited mild toxicity only when treated at 100 μg/mL or higher. Interestingly, survival assays showed that the proline analogs **1i** and **1j** along with motualevic acid E are bacteriostatic to SA and MRSA, while analogs **1d**–**h**, **1n** and motualevic acid A are bactericidal, each at 2–5× their MICs. Together these results indicate that motualevic acid A analogs with improved antibacterial potency can be constructed, and that their mode of antibacterial activity can be altered with composition. These analogs provide starting points for synthesis of motualevic acid probes that may be useful in studying their mechanism/s of action in more detail.

Acknowledgments

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12. While preparing this manuscript a paper reporting the synthesis of several motualevic acids was published where Sudhakar and co-workers followed a similar strategy: Sudhakar, G.; Kadam, V. D.; Reddy, V. V. N. *Tetrahedron Lett.* **2010**, *51*, 1124.
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