Total synthesis and structure of the ramoplanin A1 and A3 aglycons: Two minor components of the ramoplanin complex

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Ramoplanin is a potent antibiotic, first disclosed in 1984, that acts by inhibiting bacterial cell-wall biosynthesis. The original ramoplanin complex was shown to consist of a mixture of three closely related compounds, ramoplanin A1–A3, of which ramoplanin A2 is the most abundant. The structure of ramoplanin A2 was unambiguously established first through a series of extensive spectroscopic studies, allowing complete stereochemical assignments and subsequently providing a minor reassignment of the side-chain double-bond stereochemistry and, most recently, through total synthesis of authentic material. Here we report the total syntheses of the aglycons of the minor components of the ramoplanin complex, A1 and A3, which unambiguously establish their structure and provide an expected structural revision for the lipid side-chain double-bond stereochemistry.

Ramoplanin is a lipoglycodepsipeptide with potent antibacterial activity that was isolated from the fermentation broth of *Actinoplanes* sp. ATCC 33076 as a mixture of three closely related compounds, **1**–**3**, of which **2** is the most abundant (Fig. 1) (1, 2). Although less extensively studied, the enduracidins represent closely related antibiotics (3, 4), and the uncharacterized antibiotic janiemycin has been reported to bear an amino acid composition and biological properties that suggest it represents an additional member of this class of natural products (5). The ramoplanin complex is 2–10 times more active than vancomycin against Gram-positive bacteria (6) and exhibits a distinct mode of action (7–12), and the ramoplanin A2 aglycon is equally or slightly more potent than the corresponding natural product in antimicrobial assays (13). Ramoplanin has been shown to inhibit bacterial cell-wall biosynthesis by binding and sequestering lipid intermediates I and II (II > I), thereby preventing the intracellular glycosyltransferase (MurG) and the more accessible extracellular transglycosylase-catalyzed steps of the peptidoglycan assemblage. As a consequence of its unique mechanism of action, cross-resistance with existing antibiotics including vancomycin or the β-lactams has not been observed. Ramoplanin is currently in phase III clinical trials for the oral treatment of intestinal vancomycin-resistant *Enterococcus faecium* and in phase II trials for nasal methicillin-resistant *Staphylococcus aureus*.

Five years after the report of its isolation, the initial structure of ramoplanin was disclosed in 1989. It was established that compounds **1**–**3** differ only in the acyl group attached to the Asn-1 N terminus (14–17), and the stereochemistry of the two double bonds in the three different acyl groups was assigned as cis-cis (14). In 1991, the structure of a closely related natural product, ramoplanose (**4**), was disclosed by Williams and coworkers (18), whose composition was identical to ramoplanin A2 with the exception of the branched mannose trisaccharide attached at Hpg^{11} and the stereochemistry of the lipid side chain (cis,trans- vs. cis,cis-7-methyloctadi-2,4-enoic acid). Soon thereafter, the stereochemistry of the 7-methyloctadi-2,4-enoic acid side chain of ramoplanin A2 was also revised to cis-trans by Kurz and Guba (19) in studies that served to establish the Hpg^{6} and

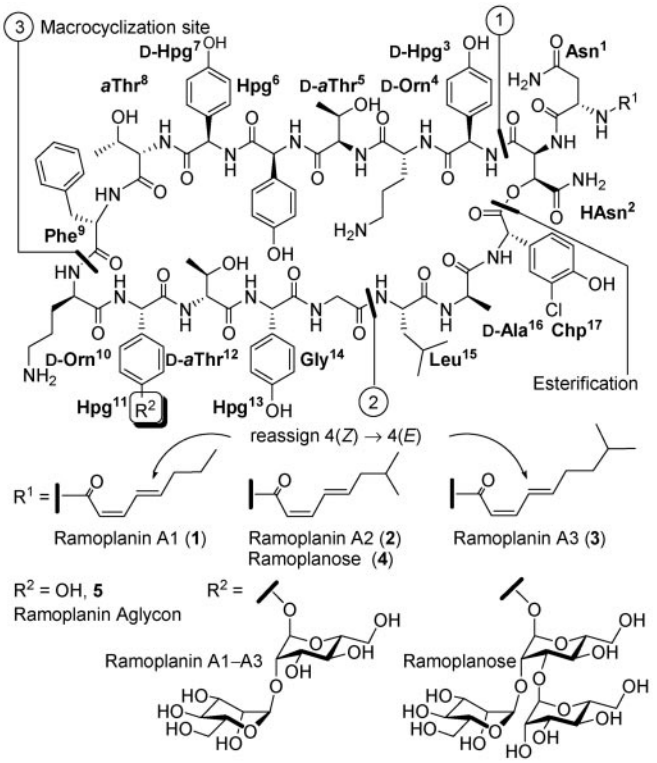

Fig. 1. Structure of the ramoplanins.

Hpg^{7} absolute stereochemistry and provided the three dimensional, solution-phase conformation of the natural product. Recently, we described the total synthesis of the ramoplanin A2 aglycon (**5**) and confirmed the revised structure of ramoplanin A2 (20, 21). Key to the strategic planning of the approach was the introduction of the lipid side chain onto the fully functionalized cyclic depsipeptide core, thereby potentially providing direct access to all natural aglycons from a common, late-stage intermediate. Thus, three key subunits composed of residues 3–9 (a heptapeptide), 10–14 (a pentapeptide), and 1, 2, and 15–17 (a depsipentapeptide) were sequentially coupled and cyclized in a solution-phase, convergent synthesis of the 49-membered depsipeptide core **6a**. The indicated coupling sites were chosen to maximize the convergency of the synthesis, including that of the three subunits, to prevent late-stage opportunities for racemization of carboxylate-activated phenylglycine-derived residues and to enlist β-sheet preorganization of an acyclic macrolac-

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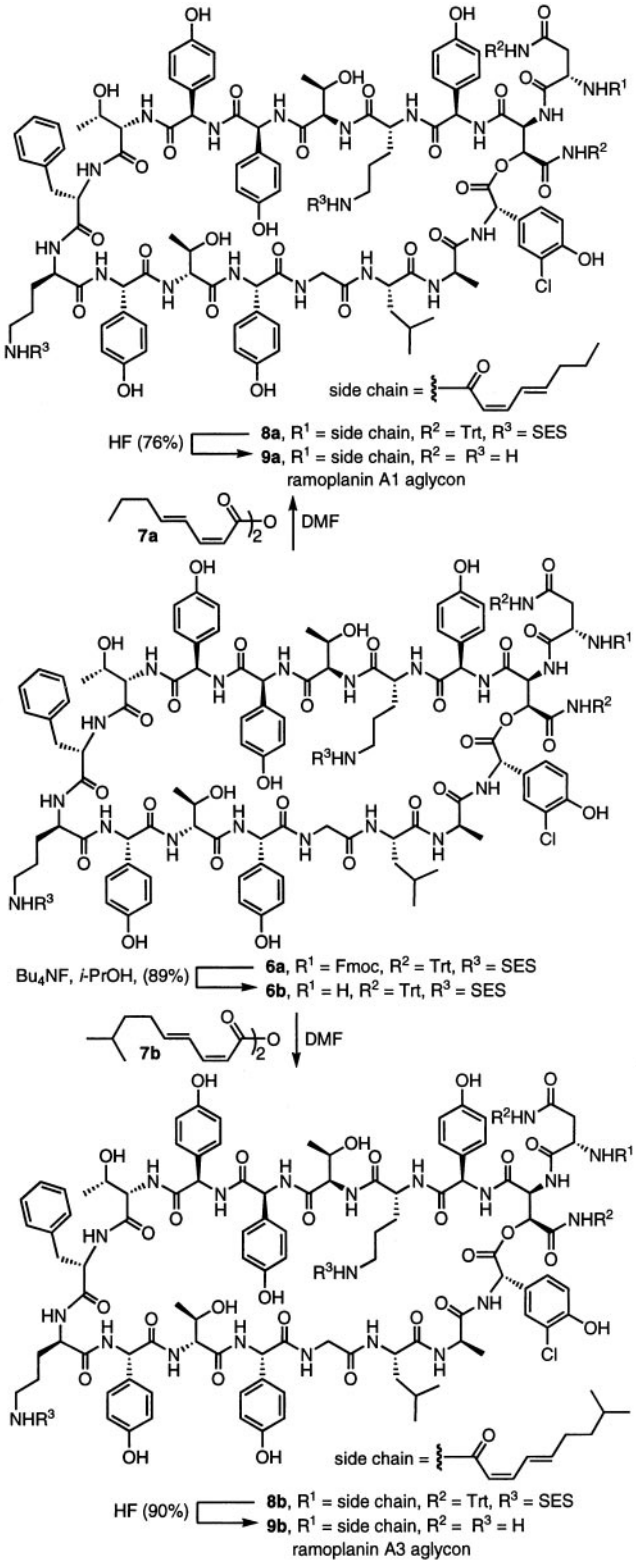
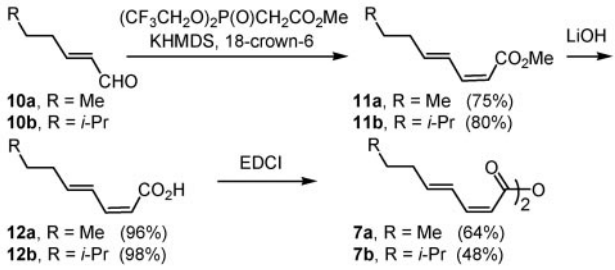


Scheme 1. Synthesis of the ramoplanin A1 and A3 aglycons.

tamization substrate to facilitate ring closure. Here we report the further implementation of this approach in the total syntheses of the ramoplanin A1 and A3 aglycons, which confirm an expected structural revision of the lipid side-chain stereochemistry of ramoplanins A1 and A3.



Scheme 2. Synthesis of the lipid side chains.

Methods

Total Synthesis of Ramoplanin A1 and A3 Aglycons. Full details of the synthetic work and full characterization of all intermediates and final products, (2*Z*,4*E*)-2,4-octadienoic acid (**12a**), (2*Z*,4*E*)-8-methyl-2,4-nonadienoic acid (**12b**), (2*Z*,4*E*)-octadienoic acid anhydride (**7a**), (2*Z*,4*E*)-8-methyl-2,4-nonadienoic acid anhydride (**7b**), **8a**, **8b**, ramoplanin A1 aglycon (**9a**), and ramoplanin A3 aglycon (**9b**), are provided in *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site.

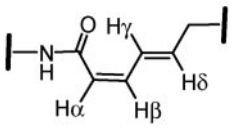
Results and Discussion

The syntheses of the ramoplanin A1 and A3 aglycons were accomplished by acylation introduction of the side chains onto the cyclic depsipeptide core of **6b** (Scheme 1). Thus, removal of the fluorenylmethoxycarbonyl (Fmoc) protecting group from **6a** (20–23) [8 eq of Bu_4NF, 10 eq of *i*-PrOH, dimethylformamide (DMF), 25°C, 1 h, sonication], the advanced synthetic intermediate prepared en route to the ramoplanin A2 aglycon (**5**) (20–23), followed by treatment of the resulting free amine with the anhydride **7a** (3.0 eq, DMF, 25°C, 14 h) provided the protected aglycon **8a**. Global deprotection using HF (HF, anisole, 0°C, 90 min) furnished the ramoplanin A1 aglycon (**9a**, 76%). Similarly, Fmoc deprotection, acylation of the free amine with anhydride **7b** (3.0 eq, DMF, 25°C, 14 h) to provide **8b**, and HF global deprotection provided the ramoplanin A3 aglycon (**9b**, 90%). Notably, Fmoc deprotection enlists conditions introduced and developed for ramoplanin that avoid competitive depsipeptide cleavage by β-elimination that was observed with typical base-catalyzed Fmoc deprotections (21). Similarly, the Orn-4 and Orn-10 2-(trimethylsilyl)ethylsulfonyl (SES) deprotections upon treatment with HF used conditions first introduced and developed to avoid competitive depsipeptide cleavage under strongly basic conditions (24).

The lipid side-chain carboxylic acid anhydrides **7a**/**7b** were prepared as shown in Scheme 2. The (*E*)-α,β-unsaturated aldehydes **10a**/**10b**[†] were converted to the (2*Z*,4*E*)-conjugated esters **11a**/**11b** by using the Still–Gennari modification of the Horner–Wadsworth–Emmons olefination (25). Hydrolysis of **11a**/**11b** followed by the treatment of the resulting acids (**12a**/**12b**) with 0.5 eq of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride yielded the carboxylic acid anhydrides **7a**/**7b**.

For comparison, the authentic ramoplanin A1 and A3 aglycons were obtained from the natural ramoplanin complex by deglycosidation (HF) followed by HPLC purification (26). The ^{1}H NMR spectroscopic data of the synthetic ramoplanin A1 and A3 aglycons (**9a**/**9b**) were in complete agreement with the authentic compounds. Although the H^{β} and H^{γ} proton signals of the lipid side chains are not clearly resolved in the ^{1}H NMR

[†](*E*)-2-hexenal (**10a**) was purchased from Aldrich, and (*E*)-6-methyl-2-heptenal (**10b**) was prepared from 4-methyl-1-pentanol via (*i*) pyridinium chlorochromate (PCC) oxidation (82%); (*ii*) $(EtO)_2POCH_2CO_2Et$, NaH (85%); (*iii*) diisobutylaluminum hydride (96%); and (*iv*) MnO_2 (80%).



Compound	$J_{\alpha\beta}$	$J_{\gamma\delta}$
A1 Aglycon	11.8 Hz	14.9 Hz
A2 Aglycon	11.4 Hz	15.3 Hz
A3 Aglycon	11.3 Hz	15.4 Hz

Fig. 2. Diagnostic coupling constants.

spectra because of partial coincidence with other resonances, $J_{\alpha\beta}$ and $J_{\gamma\delta}$ can be measured directly from the remaining two olefin proton signals, H^{α} and H^{δ}, in the spectra. Their values are not only very similar in ramoplanins A1–A3 (Fig. 2), but the coupling constants of ≈11.3–11.8 Hz and ≈14.9–15.4 Hz for $J_{\alpha\beta}$ and $J_{\delta\gamma}$, respectively, define a cis stereochemistry for the C^{α}–C^{β} double bonds and a revised trans stereochemistry for C^{γ}–C^{δ} double bonds.

Conclusions

Total syntheses of the aglycons of two minor components of the ramoplanin complex, ramoplanins A1 and A3, were achieved from the orthogonally protected synthetic cyclic depsipeptide core **6**, and the stereochemistry of the lipid side chains of these compounds was established to be cis,trans (2*Z*,4*E*).

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