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Anthelmintic PF1022A: stepwise solid-phase synthesis of a cyclodepsipeptide containing *N*-methyl amino acids

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ABSTRACT

Cyclodepsipeptides of the enniation-, PF1022-, and verticilide-family represent a diverse class of highly interesting natural products with respect to their manifold biological activities. However, until now no stepwise solid-phase synthesis has been accomplished due to the difficult combination of *N*-methyl amino acids and hydroxycarboxylic acids. We report here the first stepwise solid-phase synthesis of the anthelmintic cyclooctadepsipeptide PF1022A based on an Fmoc/THP-ether protecting group strategy on Wang-resin. The standard conditions of our synthesis allow an unproblematic adaption to an automated peptide synthesizer.

1. Introduction

Depsipeptides are defined as heterodetic peptides with at least one ester bond. While most depsipeptides contain only few, usually irregular ester groups, depsipeptides with alternating ester bonds are less common. Examples (Fig. 1) of these highly symmetric natural products comprise PF1022A (**1**), verticilide (**3**), bassianolide (**4**), valinomycin (**5**), and the enniatins (**6**–**9**).[1] These regular cyclodepsipeptides share several characteristic structural features, among those the occurrence of (*R*)-hydroxycarboxylic acids and *N*-methyl amino acids.

Natural depsipeptides, in particular cyclodepsipeptides, are of increasing interest for pharmaceutical research because of their wide range of biological activities (Fig. 1).[2] For instance, valinomycin (**5**) a well known potassium-selective ionophore was recently reported to be the most potent agent against severe acute respiratory-syndrome coronavirus (SARS-CoV).[3] Verticilide (**3**), a cyclooctadepsipeptide isolated from a *Verticilium* sp. shows a strong and selective inhibiting activity on ryanodine binding in insects.[4] Bassianolide (**4**), another cyclooctadepsipeptide, and the enniatins (**6**–**9**), a large family of cyclohexadepsipeptides, display diverse biological activities, including antibiotic, antifungal, insecticidal, antiproliferative, and cell migration inhibitory activities.[5] The 24-membered cyclooctadepsipeptide PF1022A (**1**), a metabolite of *Mycelia sterilia* (Rosselinia sp.), originally isolated from leaves of *Camellia japonica* has been established as a resistance-breaking anthelmintic with low toxicity in animals (Fig. 1).[6,7] Other cyclodepsipeptides act by selective ion transport through cellular membranes.

Despite the high potential of cyclodepsipeptides for drug discovery, only very limited screening and structure–activity data have been reported until now, probably due to the difficult access to these compounds. The combination of amino acids and bulky hydroxycarboxylic acids represents a formidable challenge for a solid-phase synthesis, which is on the other hand a prerequisite for the preparation of larger compound collections needed for biological screening. The stepwise assembly of such compounds is expected to be particularly difficult when *N*-methyl amino acids are involved.[8] Due to the increased steric hindrance couplings need more time with the consequence that dioxomorpholine formation and epimerization can become the predominant side-reactions.[9–11] The difficulties, resulting from the stepwise assemblage of *N*-methyl amino acids have been impressively demonstrated in several cyclosporine syntheses.[12,13]

Although advanced reagents, such as BMTB and DFET have been developed and applied for the coupling of sterically hindered *N*-methyl amino acids, high-yielding ester bond assemblage on solid support remains a synthetic problem.[14,15] Besides standard reagents, such as DIC/DMAP alsotriphosgene and hexafluoroacetonides of α-hydroxycarboxylic acids have been employed for the construction of depsipeptides.[16–18]

Abbreviations: ACN, acetonitrile; Boc, *tert*-butyloxycarbonyl; BOPCl, *N*,*N*′-bis(2-oxo-3-oxazolidinyl)phosphinic chloride; DCM, dichloromethane; DEAD, diethylazodicarboxylate; DHP, 3,4-dihydro-2*H*-pyrane; DIC, *N*,*N*′-diisopropylcarbodiimide; DIEA, diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide; DMSO, dimethylsulfoxide; EDCI, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide methiodide; Fmoc, 9-Fluorenyl-methoxycarbonyl; HATU, *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(7-azabenzo-triazol-1-yl)uroniumhexa-fluorophosphate; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxy-benzotriazole; MeOH, methanol; TEA, triethylamine; TFA, trifluoro acetic acid; THF, tetrahydrofuran; THP, tetrahydropyranyl; TPP, triphenylphosphane; *p*-TsOH, *para*-toluenesulfonic acid.

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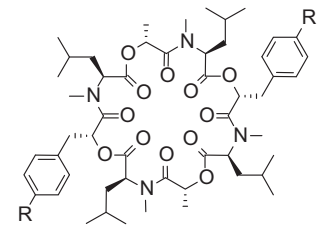
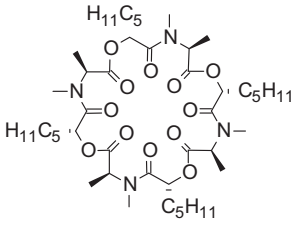
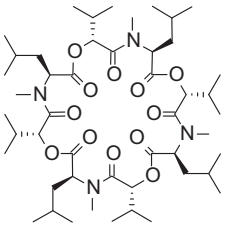


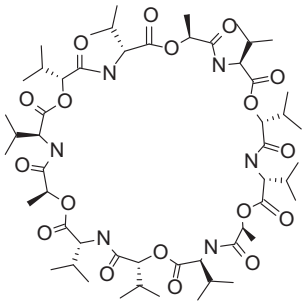
PF1022A (**1**):R = H
emodepside (**2**):R =



Verticilide (**3**)



Bassianolide (**4**)



Valinomycine (**5**)

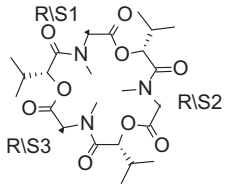


Fig. 1. Naturally occurring cyclodepsipeptides.

Usually, the ester bonds are either assembled beforehand in solution and only the peptide bonds are formed on the resin or the hydroxycarboxylic acids are introduced without any protection.[19,20] Only a few procedures have been published for stepwise solid-phase depsipeptide syntheses, the most impressive one comprises a twenty-four step total synthesis of the cyclododecadepsipeptide valinomycine (**5**), including the on-resin formation of six ester bonds.[21–23] However, no stepwise solid-phase syntheses of depsipeptides containing *N*-methyl amino acids have been accomplished until now. In this paper we wish to report a solid-phase total synthesis of the cyclooctadepsipeptide PF1022A in which all ester- and amide-bonds are formed on the support.

2. Results and discussion

2.1. Protecting group strategy and preparation of building blocks

In principle, the most frequently used Fmoc- and Boc-protecting group strategies should be applicable also for the solid-phase synthesis of depsipeptides. However, both of them have their own problems when ester bonds are involved. The basic conditions needed for the removal of the Fmoc-group may cause a partial ester cleavage, diketopiperazine formation and enhanced racemization of the chiral hydroxy acids. On the other hand, Fmoc-protecting schemes are well established for standard supports, such as the Wang-resin and can be easily adapted on a peptide synthesizer. Diketopiperazine formation is drastically reduced or even suppressed under the strong acidic cleavage conditions used for the cleavage of Boc protecting groups. However, activated ester bonds as those from PhLac for instance, may be affected, too. Additionally, the resins compatible with Boc-protecting, mainly the Kaiser-oxime and the PAM-resin are significantly more expensive and have lower loadings compared to Wang-resin.

Silyl ethers and acetyl groups have been employed in solid-phase syntheses as protecting groups for the OH-function of hydroxy acids.[24] While acetyl groups are cleaved under strong basic conditions, bearing again the risk of racemization, silyl ethers appear generally unsuited for the coupling of *N*-methyl amino acids and hydroxy acids due to the sterical shielding of the bulky silyl residue. For these reasons we chose the THP-protecting group, which is cleaved under mild acidic conditions, compatible even with the Boc-protecting group.[25] Fmoc protected *N*-methyl leucine and THP protected hydroxy acids were prepared according to standard literature procedures (Scheme 1).[26–30]

2.2. Solid-phase synthesis of PF1022A

First we used the Kaiser-oxime resin, which we had already applied successfully in the course of a didepsipeptide segment synthesis of PF1022A (**1**) and emodepside (**2**). However, in the considerably longer stepwise synthesis of PF1022A, deletion- and failure-sequences accumulated, accompanied by premature cleavages from the resin, which resulted in an inseparable mixture of products after six or seven couplings.

In a second attempt we chose the Wang-resin, being well aware of the potential problems arising from the basic cleavage conditions of the Fmoc-group. Since the strongly solvating DMF has been shown to be an unfavorable solvent in critical couplings with respect to yield, diketopiperazine formation and degree of racemization we used the less solvating THF, in which all reagents were soluble and the Wang-resin still showed good swelling properties.[14,31] After each coupling step a small portion of the resin was cleaved and the product analyzed by HPLC-MS. The results of our optimization experiments are summarized in Table 1.

As expected, the coupling-yields were strongly dependent on the residue used as the first building block, on the coupling reagents and conditions. THP-PhLac **18**, which was used as the anchoring-residue due to its lower tendency to form