Pergamon

Tetrahedron Letters 40 (1999) 1203–1206

TETRAHEDRON LETTERS

Solid Phase Synthesis of Depsides and Depsipeptides

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Received 20 October 1998; accepted 30 November 1998

Abstract: A general solid phase methodology for the synthesis of depside and depsipeptide chains from optically active α-hydroxy acids and α-amino acids is reported. The automated preparation of depsides (97% yield per cycle) made up from the same enantiomer [i.e. H-[(*S*)-Man]$_8$-OH, **1**]†, by both enantiomers [i.e. H-[(*R*)-Man-(*S*)-Man]$_4$-OH, **2**], or by different hydroxy acids in the same chain [i.e. H-[(*S*)-Lac-(*S*)-Hiv]$_3$-OH, **3**], and of depsipeptides where α-amino and α-hydroxy acids are alternatively introduced such as depsipeptide **6** (Cbz-[(*S*)-Val-(*R*)-Man-(*R*)-Val-(*S*)-Lac]$_3$-OH), are presented. Cyclization of the open chain depsides leads to chiral macrocycles whose structures resemble those of crown ethers.

Keywords: Solid-phase synthesis; Depsipeptides

Solid phase synthesis has become a routine tool for the preparation of the three major classes of biopolymers: peptides,[1] nucleotides[2] and oligosaccharides.[3] Nevertheless, for another class of natural oligomers, namely depsipeptides and depsides, no general methodology has yet been described for their systematic solid phase preparation.

Depsides are formed exclusively by hydroxy acids as building blocks connected through ester bonds, while depsipeptides are composed of amino and hydroxy acids, with both amide and ester bonds being present. Many depsipeptides are bioactive ionophores and ion transporters. Representative examples are the fungal antibiotic valinomycin,[4] a cyclic depsipeptide formed by α-amino and α-hydroxy acids, and the onchidins,[5] cytotoxic macrocycles containing α- and β-amino acids and α- and β-hydroxy acids, which are isolated from a marine source.

As far as the depsides are concerned, a representative example is provided by the nactins,[4] isolated from various species of *Actinomyces*, which are cyclic ionophores built up by four units of an 8-hydroxy acid. An example of a depside derived from α-hydroxy acids is the methyl ester of hexa-α-hydroxyisovaleric acid, isolated from the lichen *Cladonia gonecha*.[6] The β-hydroxy acid 3-hydroxybutyric acid (3-HB) is the monomer of a series of synthetic cyclodepsides[7-9] and of a naturally occurring polymer present in procaryotic microorganisms.

Synthetic polymers and copolymers of some α-hydroxy acids are of commercial importance as biodegradable plastic materials[10] ideal for surgery and transplants.[11] The use of α-hydroxy acids for the structural modification of peptides and the preparation of peptidomimetics has been recently described.[12]

However, in spite of all those facts, no general solid phase procedure for the synthesis of oligomeric depside or depsipeptide chains has been presented. In fact, although many depsipeptide syntheses have been carried out by "solid phase methodology," only the peptide bonds were formed on the resin while the ester bonds were created in solution[13] or in conditions that do not require the use of protecting groups.[14] Recently, Davies et al. presented a protocol using TBDMS (*t*-butyldimethylsilyl) and Fmoc (9-fluorenylmethyloxycarbonyl) for

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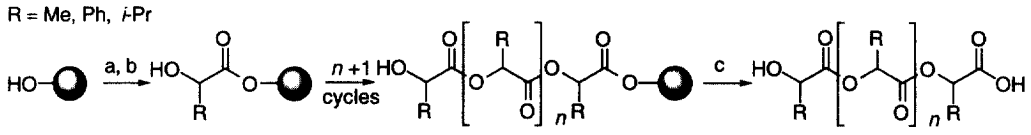
0040-4039/99/$ - see front matter
PII: S0040-4039(98)02566-0

protection of the hydroxy- and amino groups respectively,[15] but the relatively low yields reported (52% for a pentadepsipeptide containing two peptide- and two ester bonds) represent a limit for the synthesis of longer chains.

In this communication we present a general methodology for the automatic solid-phase synthesis of depsides and depsipeptides that allows the construction of long chains formed by α-hydroxy acids or combinations of α-hydroxy and α-amino acids, with high yield and total preservation of the stereochemistry of the repeating units.

The results are summarised in Scheme 1 and a brief account is described below.



a. THP-O-CH(R)-CO 2H, DIC/D MAP, THF, 2 h; b. TsOH, MeOH/ CH_2Cl_2 3:97, 2 h; c. TFA/CH_2Cl_2 1:1, 1 h

Scheme 1

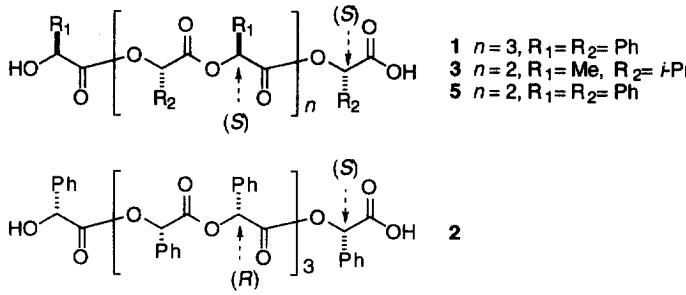
(a) Protection of the α-hydroxy group. After several attempts to find a protecting group that assures a high yield synthesis, we found that protection of the hydroxyl group of the hydroxy acids as tetrahydropyranyl ethers (THP) was the method of choice. Neither undesired deprotection nor racemization was observed[16] during coupling and deprotection steps. Protection of the hydroxy acid was achieved in solution by treatment with dihydropyran (1.4 eq.) and *p*-TsOH (0.02 eq.) in $CHCl_3$ at rt for one hour, affording the THP protected acid in a 74% yield. Deprotection of the resin-bound depside was carried out with *p*-TsOH (5 mg/mL) in CH_2Cl_2/MeOH (97:3), at rt for 2 h.

(b) Solid support, activation and coupling. Wang resin was chosen as the solid support. Linkage of the first THP-protected hydroxy acid to this resin can be easily achieved using the same conditions and reagents employed for the following couplings[17] and significantly simplifies the whole automatic process. The disconnection of the depside chain from the resin was accomplished with a 1:1 TFA/CH_2Cl_2 solution (1 h at rt). Under these conditions, the depside bonds remain untouched as shown by the absence of depside fragments in the solution (checked by HPLC).

(c) Monitoring of the depside bond forming and deprotection steps. To our knowledge, no simple test —similar to ninhydrin— for assessing the efficiency of coupling in depside bond formation has been described. We found that the scarcely known Pomonis test[18] can be used directly on the resin bound depside chain. It gives a deep purple colour when positive and we observed a negative test when the resin contained less than 1% free OH groups. Cleavage of the THP protecting group was monitored by GC of the deprotection solution.

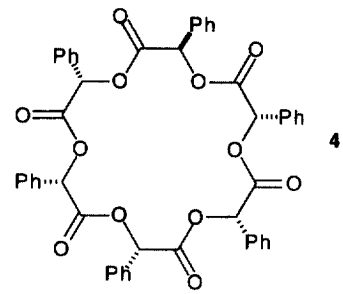
The versatility of this methodology is well illustrated by the automatic synthesis of depsides **1-3**, consisting of the same enantiomer [i.e. H-[(*S*)-Man]$_8$-OH, **1**], of both enantiomers [i.e. H-[(*R*)-Man-(*S*)-Man]$_4$-OH, **2**], or of different hydroxy acids in the same chain [i.e. H-[(*S*)-Lac-(*S*)-Hiv]$_3$-OH, **3**].[19]

Cleavage after each one of the synthetic cycles leading to **1**, **2** and **3**, and isolation by HPLC of the corresponding depsides showed that all the synthetic intermediates retain the configuration of the units incorporated in every step. Also the absence of other diastereoisomers has been proven in the case of the didepsides derived from mandelic acid by HPLC comparison with authentic samples.



1 $n = 3$, $R_1 = R_2 = Ph$
3 $n = 2$, $R_1 = Me$, $R_2 = i\text{-}Pr$
5 $n = 2$, $R_1 = R_2 = Ph$

2



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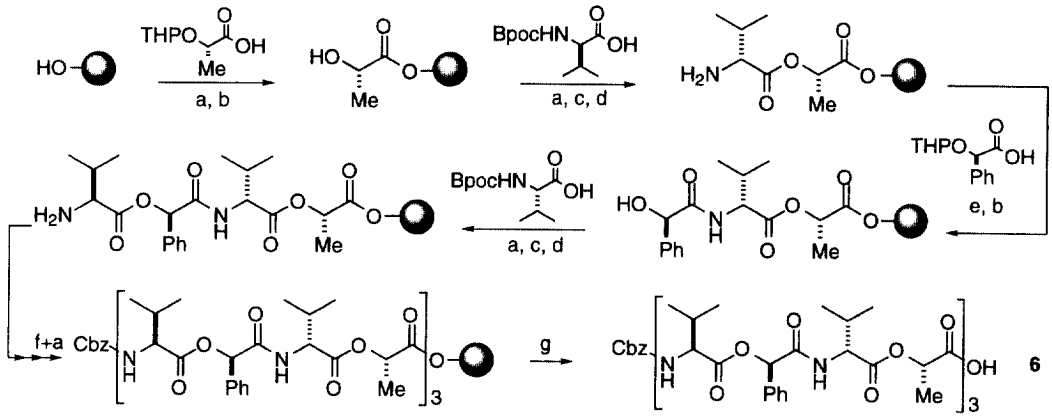
The overall yield of **1** (after HPLC isolation) was 74% (including cleavage from the resin). This corresponds to an average value of 97 % per cycle. The side products accounting for the remaining 3% per cycle were isolated by HPLC and identified as the (n+1) oligomer (1 %) and the depsides lacking one or more residues. Cyclization of these open chain depsides leads to a new class of chiral macrocycles. In this way, when hexadepside **5** was submitted to Mitsunobu reaction[20] (diethyl azodicarboxylate, triphenylphosphine), cyclohexadepside **4**, a chiral ester analogue of 12-crown-6, was obtained.

In order to extend this methodology to the preparation of depsipeptides, that is to say, the incorporation of both amino and hydroxy acids to the chain, Bpoc (2-(4-biphenylyl)-2-propyloxycarbonyl)[21] was selected as the protecting group for the α-amino groups. Bpoc-protected depsipeptides can be effectively cleaved under mild acidic conditions (0.5% TFA/CH_2Cl_2, 20 min at rt), without breaking the depside bonds or the linkage to the resin (checked by HPLC of the washes).

Scheme 2 illustrates the general procedure for the solid phase synthesis of depsipeptide **6** (Cbz-[(*S*)-Val-(*R*)-Man-(*R*)-Val-(*S*)-Lac]$_3$-OH, 12 coupling steps), which was obtained, after HPLC purification, in 63 % overall yield and optically pure form.[19]



(a) DIC 3 eq., DMAP 0.1 eq, THF, 2 h; (b) TsOH (5 mg/ml), MeOH:CH_2Cl_2(3:97), 2 h; (c) TFA:CH_2Cl_2 (0.5:99.5), 20 min; (d) (i-Pr)$_2$EtN:CH_2Cl_2 (5:95), 2 x 5 min; (e) DIC, THF, 2 h; (f) Cbz- L-Val; (g) TFA:CH_2Cl_2(1:1), 1 h.

Scheme 2

In conclusion, we present here a solid-phase methodology for the rapid, reliable and high yielding synthesis of stereochemically controlled chains of depsides and depsipeptides. It allows the preparation of peptidomimetics by introduction of hydroxy acids in peptide chains and opens the way to new chiral ionophores and to the preparation of combinatorial libraries.

Acknowledgements. This work was supported by grants from CICYT (MAR95-1933-CO2-O2 and PM95-0135) and XUGA (20910B96 and 20908B97).

References and Notes:

† Abbreviations for α–hyroxy acids and depsides are consistent with those used for α–amino acids and peptides in the nomenclature of peptides, with "H-" representing the hydroxy group and "-OH" representing the carboxy group: H-Hiv-OH = α–hyroxyisovaleric acid; H-Lac-OH = lactic acid; H-Man-OH = mandelic acid.

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(17) Depside coupling conditions: DIC (3 eq.), DMAP (0.1 eq.), THP-protected α-hydroxy acid (3 eq.), in THF (1 ml/100 mg resin), 2 h; washings (3 min each): 3 x 15 ml THF - 3 x 15 ml acetone - 3 x 15 ml CH_2Cl_2.

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