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A New Strategy for Solid-Phase Depsipeptide Synthesis Using Recoverable Building Blocks

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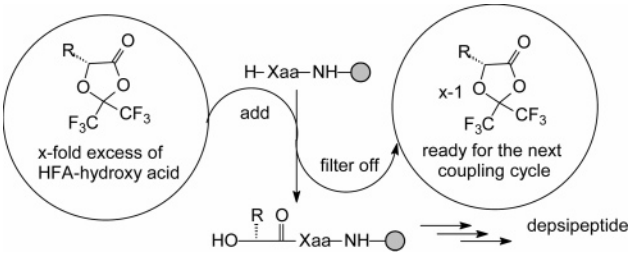
ABSTRACT



The technique of choice for synthesis of small-scale depsipeptides is on a solid support. However, if expensive monomers have to be incorporated, solid-phase synthesis can quickly turn out to be unattractive because of its low atom economy. Herein, we describe a new type of recoverable and reuseable α-hydroxy acid building block for solid-phase synthesis and its application in the synthesis of a number of small cyclic depsipeptides.

Depsipeptides are oligomers consisting of hydroxy and amino acids linked together by ester and amide bonds. Naturally occurring depsipeptides exhibit interesting biological activities, including antibacterial, antiviral, antifungal, antiinflammatory, and other properties. However, their greatest future therapeutic potential will most probably be cancer treatment.[1] Furthermore, depsipeptides can be branching units for building dendrimers[2] and possess the capacity to be nonviral vectors for drug delivery.[3] Finally, incorporation of hydroxy acids into strategically positions on peptidic backbones is a useful tool for evaluation of the role of individual hydrogen bonds for secondary structure stability.[4]

Solid-phase synthesis (SPS) is the strategy of choice for small- and medium-scale peptide synthesis because almost every peptide sequence can be built with standard reaction procedures. Every synthetic cycle is performed in a very short time, compared to conventional solution synthesis. Since the evaluation of physiological properties of synthetic depsipeptides is a newly emerging and rapidly growing research area, solid-phase protocols for depsipeptide synthesis would be highly attractive for creation of libraries in future. A literature search for depsipeptide synthesis revealed that a broad range of protocols are available, such as synthesis on solid phase,[5] in solution,[6] and combinations[7] of these strategies. Ether, ester, carbonate and silica protection of the hydroxylic group

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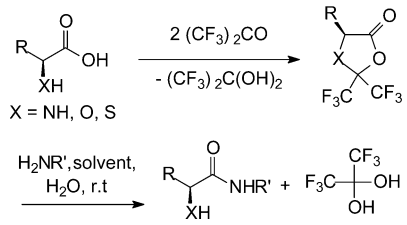
are employed.[8] However, commercial sources of hydroxy acids are limited and *O*-terminal protected hydroxy acids are not available. Hydroxy acids as constituents of naturally occurring depsipeptides are characterized by high structural diversity. Apart from a few hydroxy analogues of proteinogenic amino acids (lactic acid, hydroxyisovaleric acid, leucic acid, isoleucic acid and phenyllactic acid), complex and synthetically challenging architectures are common. Thus, hydroxy acids are, in comparison to amino acids, rather expensive monomers, often difficult to obtain. In this context, the main drawback of solid-phase protocols is undoubtedly the low atom economy compared to that of solution-phase synthesis.[9] To drive each reaction step to completion, normally a 4- up to 10-fold excess of building blocks, activation reagents and additives are added to the resin. Normally, the added excess is neither recovered nor reused in laboratory scale runs. Therefore, when highly expensive hydroxy acid monomers are involved, solid-phase synthesis can become unattractive because high costs overrule all other advantages. In the following we report on a new solid-phase protocol where the excess of the activated hydroxy acid monomer can be easily recovered and reused.

Hexafluoroacetone is a bidentate protecting/activating reagent for α-functionalized carboxylic acids such as amino, hydroxy and mercapto acids. In one reaction step, the α-functionality and the adjacent carboxylic group undergo a heterocyclization process yielding a lactone, in which the carboxylic group is activated and the α-functionality is protected. These five-membered heterocycles are stable compounds and they can be prepared in multigram-scale. The lactones represent activated esteres, which yield on nucleophilic attack carboxylic acid derivatives already at room temperature. Concomitantly, the α-functionality is deblocked. This derivatization/deprotection proceeds with a broad range of nucleophiles. Until recently, this strategy was applied for solution synthesis of low-molecular-weight derivatives such as esters, amides, peptides, and hydroxamic acids. With respect to conventional protocols, in which protection, activation, coupling and deprotection are performed in separate steps, the HFA strategy saves two reaction steps.[10] However, if the product does not crystallize spontaneously, a separation from the hexafluoroacetone hydrate formed as byproduct can be laborious and cause low yields. Application of HFA-lactones in solid-phase synthesis would overcome this disadvantage and connect synergistically two straightforward techniques.

Scheme 1. Hexafluoroacetone as Bidentate Protecting/Activating Reagent



In a first series of experiments we studied whether HFA-hydroxy acids undergo nucleophilic ring opening with solid-phase-bound *N*-terminal deprotected amino acids. Key criteria for the value of the new approach are chiral integrity of the products, yield, and reaction time. As model compounds we choose HFA-D-mandelic acid **1a**, because of its high sensitivity to racemization, analogous to phenylglycine. Four equivalents of **1a** were reacted in different solvents with H-Tyr(O^{t}Bu)-Rink-MBHA-resin. The progress of the reaction was monitored with Kaiser's ninhydrin test (check for free NH_2). Once a negative test is obtained, the resin was washed with the corresponding solvent in order to remove all hexafluoroacetone hydrate and the excess of starting material. The product was cleaved from the resin with 95% TFA and lyophilized. HPLC-MS and ^{1}H NMR confirmed that the desired product **2a** was formed. The ^{19}F NMR spectra show only the signal of trifluoroacetate, indicating that the OH-group is deprotected. As expected, reaction time and degree of racemization are solvent dependent. Dissociation of the α-proton, which is more acidic in mandelic acid than in other hydroxy acids because of the electron-withdrawing effect of the phenyl group, is favored in polar solvents. In DMSO, after 4.5 h reaction time, 18% of the diastereomer **2b** was detected. In DMF the reaction proceeds more slowly (24 h), and again the tendency of racemization is high (11% **2b**).[11] On the other hand, in the apolar solvents DCM, chloroform and toluene, despite long reaction times (up to 50 h) the reaction proceeds practically without racemization (<1% **3b**). Finally, we found that THF is the solvent of choice. The reaction time is short (5 h) and racemization can be neglected (< 1%). The lactone is activated at the right degree to react readily with amino groups but not with hydroxy groups under solid-phase conditions, and therefore oligomerization was not observed.[12]

In the next series of experiments we demonstrated that the added excess of HFA-hydroxy acids can be recovered and reused conveniently. Since carboxyl activation is an

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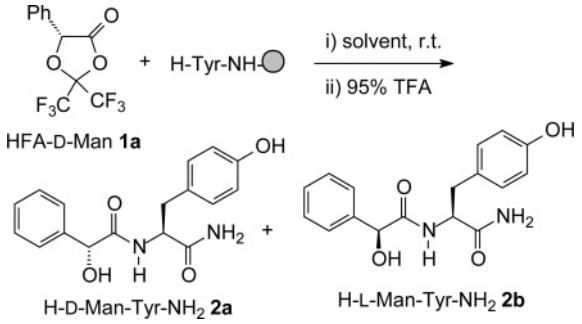
(11) The di-depsipeptide **3b** as reference was synthesized from HFA-*S*-Man **1b**. Both diastereomeres **3a** and **3b** have distinct retention times on HPLC.

(12) Products resulting from nonselective multi-incorporation were detected by addition of catalytic amounts of DMAP to the reaction mixture and could be identified by HPLC-MS.

Scheme 2. Solid-Phase Coupling of HFA-Hydroxy Acids

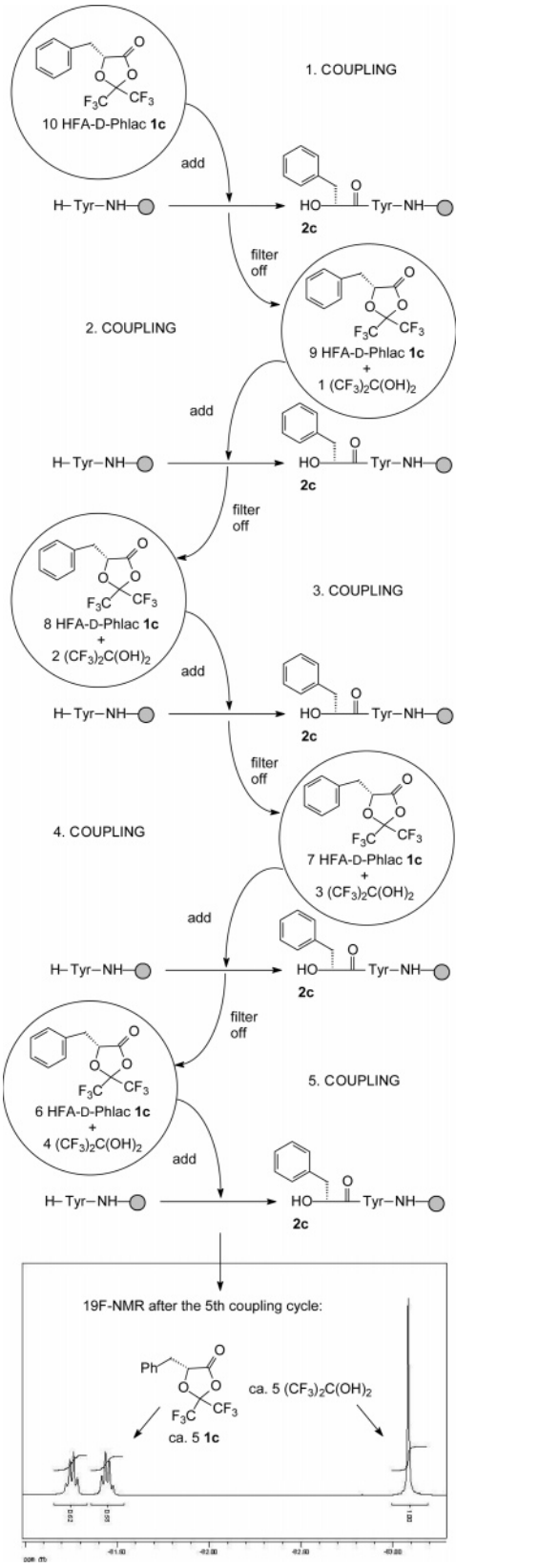


intrinsic property of the HFA compounds, no addition of activation reagents and additives are required. H-Tyr(OtBu)-Rink-MBHA-resin was treated with a 10-fold excess of HFA-D-phenyllactic acid **1c** in THF. After the reaction was complete, the resin was filtered off, and the filtrate was evaporated, redissolved and reused four more times. After the fifth coupling cycle, ^{19}F NMR spectroscopy showed the signal of HFA-hydrate formed as byproduct in each coupling turn. Compound **1c** and HFA-hydrate show approximately the same integration, indicating a consumption of ca. 50% of the HFA compound **1c** in agreement with the theoretically estimated amount. The presence of HFA-hydrate do not showed any negative influence on subsequent couplings. All five samples of di-depsipeptide **2c** showed a HPLC purity of 98−99% with amounts of the diastereomer H-L-Phlac-Tyr-NH_2 (**2d**) less than 1%.[13]

Finally, the compatibility of the new method with the Fmoc/^{t}Bu strategy on solid phase was demonstrated by synthesizing five-membered cyclodepsipeptides **4a**−**c**. The linear precursors **3a**−**c** were synthesized on the Wang resin: the two hydroxy acid units were incorporated via HFA derivatives according to the above-described method, and the three Fmoc-AAs were esterificated with the solid-phase-bound hydroxy groups using Steglich activation (DIC/DMAP).[14] Structural diversity was achieved by incorporation of different hydroxy acids in the fourth position. After cleavage from the resin, the linear precursors **3a**−**c** were obtained in purities between 57% and 92% (HPLC). After HPLC purification, cyclization was performed under high dilution conditions with DIC/HOAt/DIEA to give cyclodepsipeptides **4a**−**c** in 28−31% yield and 95−98% purity (HPLC).

In conclusion, HFA-hydroxy acids **1** are activated monomers that work in solution as well as in solid phase. They are excellently soluble in the most common organic solvents. Their solutions are stable over a long time. Progress of reactions can be monitored by ^{19}F NMR spectroscopy. Couplings proceed racemization-free in suitable solvents, yielding products in a high purity. In solid-phase protocols, added excess of HFA-hydroxy acids is easily recoverable and reuseable. From these findings we conclude that HFA-hydroxy acids **1** are valuable coupling reagents for solid-

Scheme 3. Recovery and Reutilization of HFA-Hydroxy Acids in a Solid-Phase Protocol



(13) Analogously to ref 11, the di-depsipeptide **3d** as reference was synthesized from HFA-*S*-Phlac **1d**. Both diastereomeres **3c** and **3d** have distinct retention times on HPLC.

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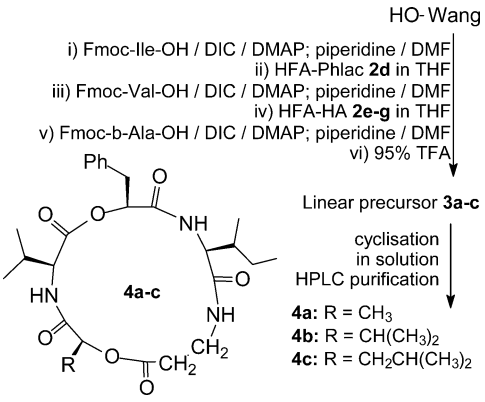
Scheme 4. Compatibility of the HFA Group with the Fmoc Strategy: Synthesis of Cyclodepsipeptides



phase depsipeptide synthesis. Future possible applications can be the incorporation of precious and/or laborious α-hydroxy acids in depsipeptides in laboratory scale or bulk production.[15]

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Supporting Information Available: Experimental procedures and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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