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A Novel Protecting/Activating Strategy for β-Hydroxy Acids and Its Use in Convergent Peptide Synthesis†

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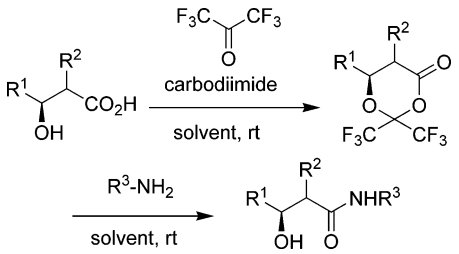

β-Hydroxy acids were reacted with hexafluoroacetone and carbodiimides to give carboxy-activated six-membered lactones in good yields. On reaction with amines, the corresponding amides were obtained. We demonstrate the following applications of this protecting/activating strategy: preparation of carboxamides in solution and on solid phase (both normal and reverse mode); recovery and reuse of the excess material in solid-phase synthesis; and convergent solid-phase peptide synthesis (CSPPS) with peptide segments bearing *C*-terminal Ser or Thr with very low levels of epimerization (<1%, HPLC).

Introduction

Amide and peptide bond formation are key reactions in the synthesis of peptides and other molecules of biological interest. A large number of reagents for the dehydrative coupling of carboxylic acids and amines are known.[1] However, some reagents offer additional advantages under certain conditions. We have recently reported the use of hexafluoroacetone (HFA) as a bidentate protecting/activating reagent in the synthesis of α-hydroxy acids and depsipeptides.[2] Thus, malic acid **1** reacts with HFA to give the 5-membered lactone **2**. These lactones react with nucleophiles like amines to give the corresponding carboxamides **3** (Scheme 1). In addition to the formation of an amide bond, this approach offers several advantages over conventional strategies: (i) a very short (minimal) reaction sequence (2 steps, because carboxyl-activation/OH-protection and amide formation/OH-deprotection proceed concomitantly); (ii) site-selectivity in the reaction with dicarboxylic acids like **1** (exclusively 5-membered lactones are formed); (iii) stability of the activated lactone, thereby allowing isolation, storage, and recovery of excess in solid-phase peptide synthesis (SPPS); and (iv) physical properties (good solubility in organic solvents, easy

† CSPPS, convergent solid-phase peptide synthesis; DCC, dicyclohexylcarbodiimide; DCM, dichloromethane; DIC, diisopropylcarbodiimide; DIEA, *N*,*N*′-diisopropyl ethyl amine; DKP, diketopiperazine; DMAP, 4-*N*,*N*′-dimethylaminopyridine; DMSO, dimethylsulfoxide; DMF, *N*,*N*′-dimethylformamide; EDC*HCl, *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride; Et_2O, diethylether; HFA, hexafluoroacetone; SPPS, solid-phase peptide synthesis; THF, tetrahydrofuran.

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(2) Spengler, J.; Böttcher, C.; Albericio, F.; Burger, K. *Chem. Rev.* **2006**, *106*, 4728−4746.

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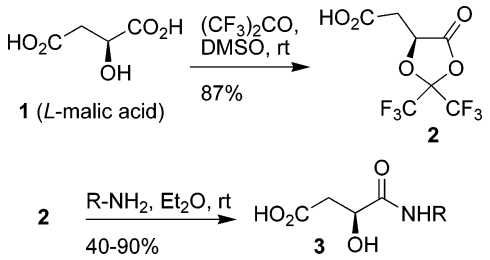
SCHEME 1. Site-Selective Derivatization of Malic Acid Using HFA



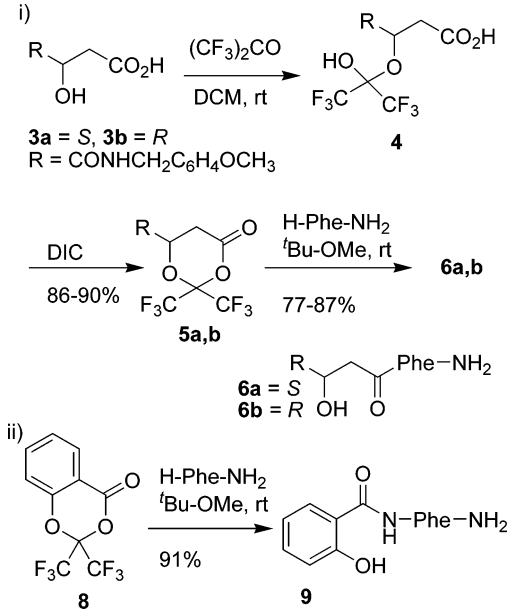
detection by ^{19}F-NMR) of the activated species.[3] However, the application of the HFA-strategy in solid-phase synthesis is limited to α-hydroxy acids. The corresponding 5-memberd aza- and thiaheterocycles derived from α-amino and α-mercapto acids are not generally suited for solid-phase protocols.[4] The *β*-hydroxy acid unit represents a common structural motif of naturally occurring compounds. However, on reaction of HFA with *β*-hydroxy acid, the formation of the corresponding 6-membered lactones does not proceed spontaneously, in contrast to 5-membered lactones. Here we discuss the synthesis and fields of application of the cyclic lactones derived from HFA and *β*-hydroxy acids.[5]

Results and Discussion

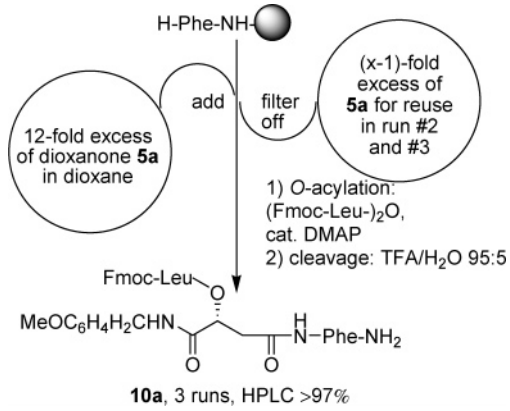
Malic acid amides **3** reacted with HFA to give semiketals **4**. A rapid cyclization to provide 2,2-bis(trifluoromethyl)-1,3-dioxan-4-ones **5** was achieved by addition of a carbodiimide (DIC) as carboxy-activating agent.[6] The enantiomers **5a** and **5b** were isolated in 86 and 90% yield from the L- and D-malic acid amides **3a** and **3b**, respectively. On reaction of a solution of **5** in *tert*-butyl methyl ether with H-Phe-NH$_2$, the depsipeptides **6a**,**b** precipitated and were obtained in 77 and 87% yield, respectively (Scheme 2, i).[7] Analogously, the conformationally more rigid salicylic acid **7** gave lactone **8** and peptide **9** in comparable yields (Scheme 2, ii).[8] In general, we found this heterocyclization to be a robust protocol that proceeds with quantitative conversion and gives isolated yields typically between 70 and 90%. The six-membered activated lactones are, like their 5-membered counterparts, storable under exclusion of moisture.

The dioxanones **5** can be used as activated building blocks in SPPS. Due to their stability, added excess material can be recovered and reused. Thus, a solution of a 12-fold excess of **5** in dioxane was added to H-Phe-NH-Rink amide-MBHA-resin. After approximately 4 h, the coupling was complete (negative ninhydrin test) and the resin was washed with dioxane. The filtered and evaporated solution was reused twice for couplings with new portions of resin. The diasteromer **10a** (from **5a**) was

SCHEME 2. Carbodiimide-Mediated Formation of 6-Membered Lactones



SCHEME 3. Reuse and Recovery of Dioxanone 5a in Solid-Phase Peptide Synthesis



obtained with >97% HPLC purity in all three runs after acylation of the 3-hydroxy group with Fmoc-Leu-OH and cleavage with TFA-H$_2$O (95:5). Comparison with the diastereomer **10b** (from **5b**) by analytical HPLC revealed that no epimerization occurs. Some 3-hydroxycarboxylic acids, such as statines, are very expensive, and therefore, the option for easy recovery of unreacted excess of activated material by simple filtration can make this protocol attractive (Scheme 3).

The 6-membered lactones were also generated on solid phase. Resin **11a**, obtained from H-Phe-NH-Rink Amide-MBHA-resin and **2**, was rinsed several times with a solution of HFA in THF.[9] A 5-fold excess of DIC was then added. After 2 h, the activated resin **11b** was washed (DMF, then DCM) and dried. The dioxanone **12a** was obtained after cleavage with TFA-H$_2$O (95:5) in >80% HPLC-purity. Samples of resin **11b** were treated with DMF-solutions of 5 equiv of *p*-methoxy-benzylamine and

(3) Albericio, F.; Burger, K.; Ruíz-Rodríguez, J.; Spengler, J. *Org. Lett.* **2005**, *7*, 597−600.

(4) Albericio, F.; Burger, K.; Cupido, T.; Ruiz, J.; Spengler, J. *Arkivoc* **2005**, *6*, 191−199.

(5) A preliminary account of this work was presented at the 29th European Peptide Symposium, September 3−6, 2006, Gdansk, Poland. *J. Pept. Sci.* **2006**, *12, suppl. S*, 119.

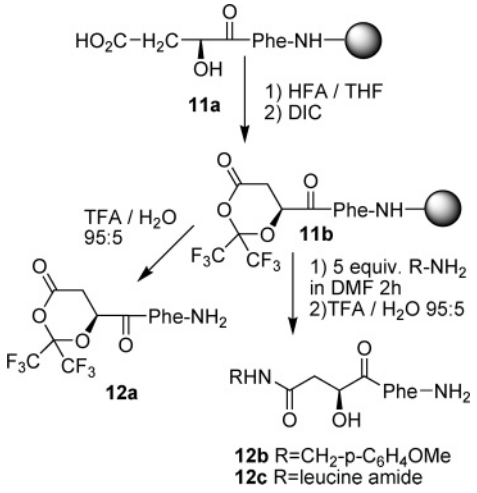
(6) The semiketal can be observed in the ^{19}F-NMR spectra. Its signal at −81 ppm (s) disappears and two quartets are observed.

(7) The reactivity of the 6-membered lactones is comparable to that of the 5-membered lactones. Thus, primary and secondary amides, hydrazides, and esters can be obtained on reaction with the corresponding nucleophiles. See ref 2.

(8) **8** was prepared from salicylic acid **7** with HFA/DCC in 74% yield.

(9) Gaseous (anhydrous) hexafluoroacetone is soluble in organic solvents (DCM < ethyl acetate ≤ THF, ca. 15 g per 100 g). Its solutions can be stored in a well-closed bottle at −20 °C. At room temperature, a slow reaction of HFA with THF takes place. **CAUTION**: hexafluoroacetone is volatile and very toxic; therefore, all operations must be performed in an efficient fumehood with properly skin and eye protection.

SCHEME 4. Synthesis of Amides on Solid-Phase in Reverse Mode



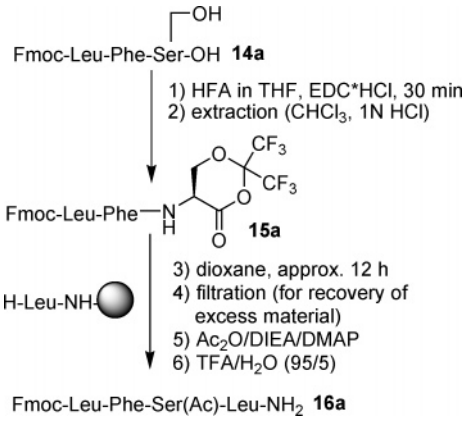
H-Leu-NH_2, respectively. After 2 h, cleavage and HPLC analysis showed no presence of **12a**, which indicates a rapid nucleophilic ring-opening of the resin **11b**. Products **12b** and **12c** were obtained in 81 and 89% HPLC purity, respectively. Because the dipeptide Mal-Phe-NH_2 (from unreacted resin **11a**) was not detected in the products **12a**−**c**, it is suggested that activation to **11b** proceeds quantitatively. These findings demonstrate that this activating strategy could be useful for reverse-mode solid-phase synthesis (Scheme 4).[10]

This protecting/activating strategy, when applied to the proteinogenic amino acids Ser and Thr, required special conditions, because the corresponding dioxanones were found to be sensitive to epimerization. Thus, enantiomerically pure **13** was obtained after dropwise addition of a solution of Boc-Ser-OH in HFA/THF into a stirred suspension of excess (3 equiv) of EDC*HCl in HFA/THF.[11] After 30 min of reaction, product **13** was extracted with $CHCl_3$, and the organic phase was washed with 1 N HCl. This crude product was chirally more stable (ca. 1% epimerization after 48 h standing at rt when dissolved in dioxane) than the recrystallized **13** (8% epimerization after 12 h at rt, but less than 1% if stored at −20 °C when dissolved in dioxane).[12] We suppose that the HCl traces from the washing procedure prevent dissociation of the proton from the chiral center, which bears two electron-withdrawing substituents. Thus, to prevent epimerization, it is advisable to perform coupling reactions of HFA-activated compounds derived from 2-amino 3-hydroxy carboxylic acids with the crude product (Scheme 5).

The activation protocol developed for Ser can be readily used in convergent solid-phase peptide synthesis (CSPPS) for the coupling of peptide segments bearing Ser or Thr in the *C*-terminal position. From a practical viewpoint, it is advantageous that HFA and EDC*HCl be used in excess, because otherwise it would be difficult to deliver stoechiometric amounts, when couplings are performed on a milligram scale. Thus, the peptide segment Fmoc-Leu-Phe-Ser-OH **14a** was activated as

SCHEME 5. Activation of Ser Requires Special Conditions

SCHEME 6. Protocol for CSPPS Using the HFA-Strategy



described above. The activated species **15a** was dissolved in a minimum of dioxane and added to H-Leu-NH-Rink Amide-ChemMatrix resin ($f = 0.15$ mmol/g).[13] The coupling required a considerably longer time than for monomers (8−16 h, to obtain a negative ninhydrin test). After acetylation of the OH-group and cleavage from the resin, the peptide Fmoc-Leu-Phe-Ser-(Ac)-Leu-NH_2 **16a** was obtained with less than 1% epimerization.[14] Similar results were found for the preparation of Fmoc-Leu-Phe-Thr(Ac)-Leu-NH_2 **16c** from Fmoc-Leu-Phe-Thr-OH **14c** (Scheme 6).

Recently, the "depsipeptide technique" and the use of pseudoproline building blocks were applied to overcome the problem of epimerization during coupling of peptide segments bearing *C*-terminal Ser and Thr.[15] The HFA-strategy addresses two more challenges associated with CSPPS. First, the trifluoromethyl groups significantly enhance the solubility of the activated peptide in organic solvents. Thus, a higher stationary concentration at the resin can be achieved. Second, the recovery and reuse of the excess of activated building block was found feasible under carefully controlled conditions (demonstrated with **15b**, see Supporting Information).[16]

Finally, peptides of the type Fmoc-Xaa-Leu-Phe-Ser-OH **17** (Xaa represents an amino acid with an unprotected side-chain

(10) Thieriet, N.; Guibé, F.; Albericio, F. *Org. Lett.* **2000**, *2*, 1815−1817.

(11) Revealed by the comparison of the HPLC spectra of H-Ser(Ac)-Leu-NH_2 and H-DSer(Ac)-Leu-NH_2, which were prepared on solid phase. When other carbodiimides, like DIC and DCC, or stoechiometric amounts of EDC*HCl were used, epimerization was found. Similar results were found for Fmoc-Ser-OH and Boc-Thr-OH. See Supporting information for data.

(12) Chromatography on silica caused >20% epimerization.

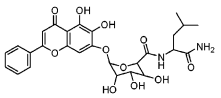
(13) García-Martin, F.; Quintanar-Audelo, M.; García-Ramos, Y.; Cruz, L. J.; Furic, R.; Côté, S.; Gravel, C.; Tulla-Puche, J.; Albericio, F. *J. Comb. Chem.* **2006**, *8*, 213−220.

(14) It can be clearly distinguished by HPLC from the diastereomer **16b** (Fmoc-Leu-Phe-DSer(Ac)-Leu-NH_2) by HPLC analysis.

(15) (a) Coin, I.; Doelling, R.; Krause, E.; Bienert, M.; Beyermann, M.; Sferdean, C. D.; Carpino, L. A. *J. Org. Chem.* **2006**, *71*, 6171−6177. (b) Yoshiya, T.; Sohma, Y.; Kimura, T.; Hayashi, Y.; Kiso, Y. *Tetrahedron Lett.* **2006**, *47*, 7905−7909. (c) Keller, M.; Wohr, T.; Dumy, P.; Patiny, L.; Mutter, M. *Chem.−Eur. J.* **2000**, *6*, 4358. (d) Cupido, T.; Tulla-Puche, J.; Spengler, J.; Albericio, F. *Curr. Opin. Drug Discovery Dev.* **2007**, *10*, 768−783.

(16) The activated peptide segments epimerize slowly in the presence of the (basic) amino groups of the resin. In an experiment, about 1% epimerization was found for the first and the second coupling cycle (the 2nd performed with recovered material), but 5% epimerization was found after the 3rd coupling. Furthermore, diketopiperazine formation diminishes the content of activated segment.

TABLE 1. Products of CSPPS in the Presence of Several Functional Groups

Educts **17** (Xaa, functionality)	Compounds **18** identified in the crude product mixture (% HPLC-purity at λ = 220 nm)
17a (Asp, CO_2H)	Fmoc-Asp-Leu-Phe-Ser-Leu-NH_2, **18a** (48%)
17b (Thr, OH)	Fmoc-Thr-Leu-Phe-Ser-Leu-NH_2, **18b** (56%)
17c (Tyr, OH)	Fmoc-Tyr-Leu-Phe-Ser-Leu-NH_2, **18c** (71%)
17d baicalin	 baicalin-Leu-NH_2, **18d** (79%)
17e (Asn, $CONH_2$)	Fmoc-Asn-Leu-Phe-Ser-Leu-NH_2, **18e** (18%) [Fmoc-Asn-Leu-Phe-Ser-Leu-NH_2 + HFA], **18e'** (44%)
17f (Arg, guanidino)	[Fmoc-Arg-Leu-Phe-Ser-Leu-NH_2 + 2HFA-H_2O], **18f'** (63%)
17g (Met, SCH_3)	Fmoc-Met-Leu-Phe-Ser-Leu-NH_2, **18g** (24%) Fmoc-Met(O)-Leu-Phe-Ser-Leu-NH_2, **18g'** (32%)

functionality) were activated and coupled with H-Leu-NH-Rink Amide-ChemMatrix resin as described above (Scheme 6). Table 1 summarizes the products (Fmoc-Xaa-Leu-Phe-Ser-Leu-NH_2 **18**) obtained after cleavage from the solid support and identified by HPLC-MS. The coupling proceeded site-selectively in the presence of unprotected carboxylic groups in the side-chain (Table 1, **17a** to **18a**). The formation of semiketals with alcoholic or phenolic OH groups was reversible by further aqueous treatment (Table 1, **17b** to **18b** and **17c** to **18c**). Analogously, the sugar-carboxylic acid baicalin **17d** was coupled without any OH-protection scheme on solid phase to give **18d**. The primary carboxamide of Asn (**17e**) appears to add HFA because a mixture of desired product **18e** and a product **18e′** with a mass 166 units higher was identified. However, **18e′** could not be transformed to **18e** by aqueous treatment. The product **18f** obtained from the Arg-containing peptide **17f** suggests that a reaction of the guanidino group takes place with 2 equiv of HFA. The Met-peptide **17g** oxidized partially under the experimental conditions (Table 1, **18g′**).[17] Thus, this activation method tolerates unprotected carboxylic acids, alcohols, and phenols.

Conclusions

Here we show that the protection/activation of β-hydroxy acids with HFA is a robust protocol that can be used for the site-selective carboxy-derivatization of a wide range of structurally diverse molecules that contain β-hydroxy acids: malic acid, sugar carboxylic acids, salicylic acid, the amino acids Ser and Thr, peptides with *C*-terminal Ser or Thr-residues, and functionalized resins. Several unprotected functional groups (secondary amide, carboxy, alcoholic and phenolic OH) are tolerated. No *O*-acylation was observed during the amide bond formation in solution or on solid phase. In CSPPS, epimerization of peptide segments bearing *C*-terminal Ser or Thr was reduced to <1% by appropriate choice of solvent and carbodiimide. The stability of the 6-membered lactones also allows easy recovery of unreacted excess of activated material in solid-phase protocols.

Experimental Section

Synthesis of 2,2-Bis(trifluoromethyl)-1,3-dioxan-4-ones 5 and 8. The β-hydroxy acid (2 mmol) was stirred in DCM (25 mL) under an atmosphere of hexafluoroacetone in a flask equipped with a dry ice condenser and a valve (**CAUTION!**). In a slight exothermic reaction, the hydroxy acid goes into solution (for salicylic acid, THF was used as solvent). Then, 1.1 equiv (2.2 mmol) of carbodiimide was added. After 1 h stirring, the precipitated urea was filtered off and the solvent was evaporated. The dioxanones were obtained analytically pure after flash chromatography or recrystallization. They are stable when stored under exclusion of moisture.[18]

Activation of Peptide Segments Using Hexafluoroacetone Solution on a Milligram Scale. Typical procedure: The peptide (0.02 mmol) was dissolved in a solution of HFA in THF (1 mL, ca. 15 g HFA in 100 mL THF) (**CAUTION!**). Then, EDC*HCl (15 mg, 0.08 mmol) was added and suspended by ultrasonification. After 30 min shaking, the reaction mixture was quenched with 1 N HCl (3 mL) and the product was extracted with chloroform (2 × 1 mL). The organic phase was dried over $MgSO_4$ and evaporated. The residue can be used directly for solid-phase couplings.

Solution-Phase Protocol for the Preparation of Amides 6 and 9. Typical procedure: H-Phe-NH_2 (177 mg, 0.71 mmol) in acetone (1 mL) was added to a solution of dioxanone (0.65 mmol) in *tert*-butyl methyl ether (2 mL). The resulting suspension was stirred until complete conversion of dioxanone (TLC, approximately 1 h). *tert*-Butyl methyl ether (20 mL) was then added, and the suspension was stirred vigorously for 12 h. The amides were obtained after filtration and drying.

Solid-Phase Protocol for Coupling and Recovery of 2,2-Bis-(trifluoromethyl)-1,3-dioxan-4-ones. Typical procedure: The resin (0.02 mmol) was pre-swollen in dioxane for 30 min. After removal of the solvent, dioxanone (0.2 mmol) in dioxane (required volume to cover the resin) was added and left with occasional stirring until the reaction was complete (negative ninhydrin test). The resin was then washed with dioxane (0.5 mL) and the filtrates were collected in a flask. After evaporation of the solvent, the recovered excess of dioxanone can be reused. Activated peptide segments were coupled and the excess was recovered analogously. Storage at −20 °C is recommended.

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Supporting Information Available: General procedures, compound characterization and copies of NMR and HPLC spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Peptides containing His and Trp gave complex mixtures.

(18) For the preparation of compound **13**, see Supporting Information.