Application of hexafluoroacetone as protecting and activating reagent in solid phase peptide and depsipeptide synthesis

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Dedicated to Professor Eusebio Juaristi on the occasion of his 55th birthday
(received 29 Apr 05; accepted 03 May 05; published on the web 05 May 05)

Abstract
Hexafluoroacetone-protected/activated hydroxy acids [2,2-bis(trifluoromethyl)-1,3-dioxolan-4-ones] represent recoverable and reusable monomers for the solid phase synthesis of depsipeptides. The reactivities of HFA-protected/activated malic acid and its C^{α}-methylated analog citramalic acid toward resin bound amino acids were studied and are compared herein. The potential of HFA-protected/activated amino acids [2,2-bis(trifluoromethyl)-1,3-oxazolidin-5-ones] such as Phe, Leu, MeLeu, Pro and Tic as pre-activated monomers for solid phase peptide synthesis was also investigated.

Keywords: Hexafluoroacetone, bidentate reagents, citramalic acid, tetrahydroisoquinoline carboxylic acid

Introduction

Peptides and depsipeptides are important classes of bioactive compounds ubiquitous in nature, and their monomers, amino and hydroxy acids, are key low molecular weight components in the natural chiral pool. Synthetic analoging of peptides and depsipeptides for pharmacological development demands readily accessible, structurally diverse monomers as well as a suitable methodology for the incorporation of these monomers into peptides or depsipeptides.

The coupling of two amino acids generally requires four steps: protection, activation, peptide bond formation and deprotection. Additional protection and deprotection steps are necessary when multifunctional monomers are involved.[1] Orthogonally protected, homochiral monomers should ideally be prepared in a minimum of high-yielding, chemo- and site-selective

steps. The use of bidentate protecting/activating groups, whereby protection and activation occur in tandem, and subsequent coupling and deprotection also occur simultaneously, simplifies peptide synthesis by halving the total number of steps required for each coupling. However, the application of these reagents is often limited, as exemplified in the case of phosgene, that reacts with the 1-carboxylic group and the α-amino group of an amino acid to give *N*-carboxy anhydrides (NCA's). These cyclic anhydrides react quickly with nucleophiles such as amino acid esters, however peptide bond formation is accompanied by rapid deprotection of the amino group. The resulting free amine can compete as an acyl acceptor and consequently form oligomers. An additional *N*-protection, giving UNCA's (urethane-protected NCA's), prevents oligomerization but the advantage of a short synthetic pathway is lost due to additional protection and deprotection of the α-amino function.[2] Likewise, the requirement of a additional *N*-protecting and deprotection steps makes the formaldehyde-route a four step procedure.[3] Recently, dichlorodimethylsilane was evaluated for the simultaneous protection and activation of certain α-amino acids. While use of this strategy in the reaction of aspartic acid with primary amines afforded α-amides in excellent yield, it gave unsatisfactory results for the synthesis of the dipeptide aspartame.[4]

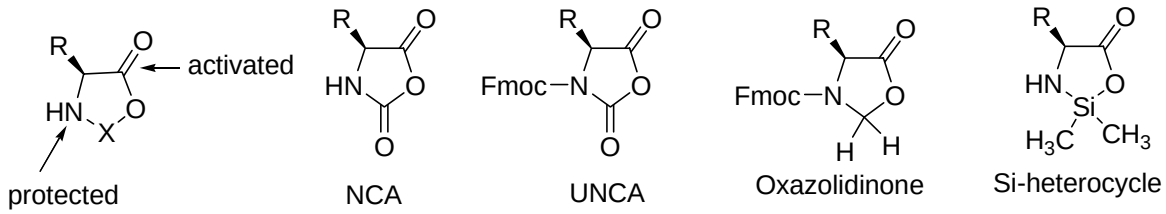
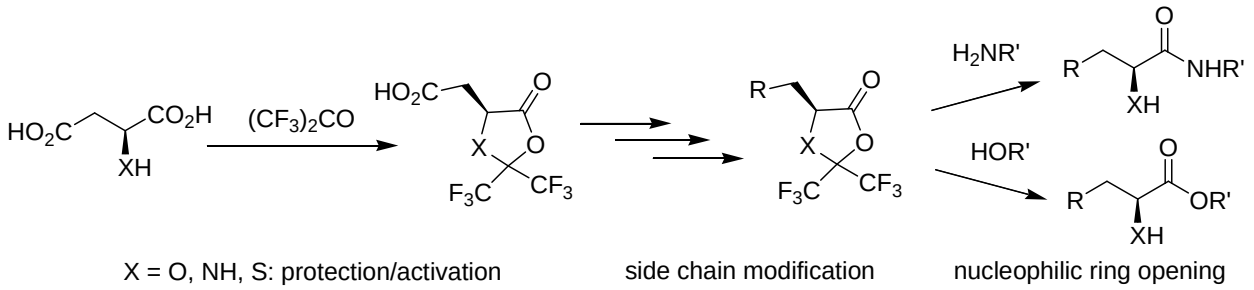


Figure 1. Bidentate protected/activated amino acids.

Hexafluoroacetone (HFA) has been demonstrated by our group to be a versatile bidentate protecting/activating reagent for α-amino, α-hydroxy, and α-mercapto acids. The α-functionality and 1-carboxylic group of these compounds heterocyclize in one step. The 1-carboxylic group is activated, as a lactone, towards nucleophiles while the α-functionality is protected from electrophiles. Nucleophilic attack gives rise to the corresponding 1-carboxyl derivatives and concomitantly the α-amino function is deblocked. This was demonstrated by the stereoconservative synthesis of esters, amides, peptides, azapeptides and hydroxamic acids in solution. Furthermore, the HFA-route is advantageous in that it tolerates unprotected carboxylic groups in the side chain. Selective modification of these carboxyl groups provides access to numerous rare, natural and non-natural building blocks as well as enantiomerically pure glycoconjugates.[5]

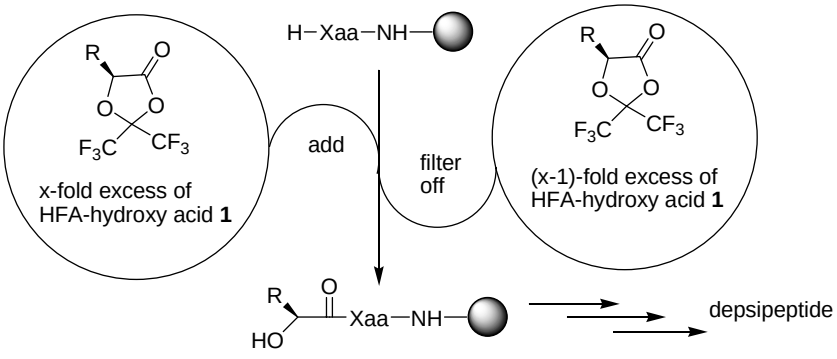


Scheme 1. The hexafluoroacetone-route for the preparation of side-chain modified amino, hydroxy and mercapto acid derivatives.

Solid phase synthesis (SPS) is the strategy of choice for the preparation of small and medium size peptides because nearly every sequence can be assembled with standard reaction procedures. SPS reaction cycles are generally much faster than analogous solution syntheses. In order to drive each reaction to completion, a four- to ten-fold excess of reagents is typically added to the resin. For laboratory scale runs, this excess material is normally non-recoverable and non-reusable. Consequently, SPS may be excluded for reactions involving very expensive monomers for purely economic reasons, regardless of its utility.[6]

HFA-protected/activated hydroxy acids[7] **1** were recently described by our group as valuable building blocks for solid-phase depsipeptide synthesis. These acids derivatives are highly soluble in most common organic solvents and their solutions are stable over a long time. Moreover, couplings with these building block proceed racemization-free in suitable solvents, such as THF, yielding products of high purity. The progress of these reactions can be monitored by ^{19}F-NMR spectroscopy. In solid phase protocols, excess **1** is easily recoverable and reusable. From these findings it was concluded that HFA-hydroxy acids **1** are valuable monomers for solid phase depsipeptide synthesis, especially for the incorporation of precious α–hydroxy acids in depsipeptides on laboratory or bulk production scales.[8]

These findings encouraged us to explore the synthetic utility of other HFA building blocks such as α-branched HFA-hydroxy acids and HFA-amino acids for solid phase synthesis.



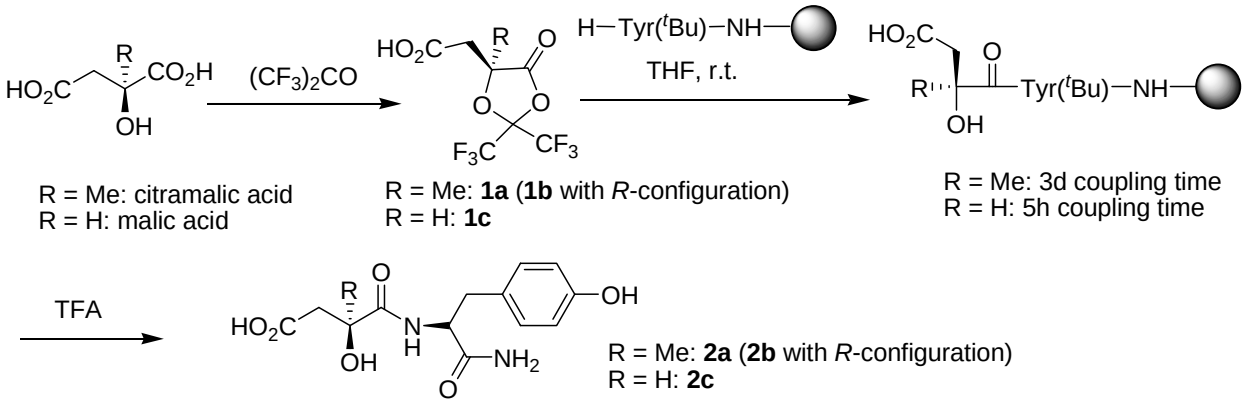
Scheme 2. HFA-hydroxy acids are recoverable and reusable building blocks for SPS.

Results and Discussion

Reactivity of HFA-citramalic acid on solid-phase

Conformationally constrained α-amino acids such as Aib (α-aminoisobutyric acid) and (α-Me)Val (C^{α}-methyl-valine) are strong inducers of β-turns and 3_{10}/α-helices. They are excellent tools for the construction of rigid spacers, templates and catalysts. The incorporation of their hydroxy-analogs [hydroxyisobutyric acid (Hib) and C^{α}-hydroxy-C^{α}-methylisovaleric acid (αMe)Hyv] induce similar effects on the conformational behavior of the resulting depsipeptides.[9] Citramalic acid is a C^{α}-hydroxy-C^{α}-methyldicarboxylic acid. Reaction with hexafluoroacetone yields exclusively HFA-citramalic acid **1a**, in which the 1-carboxylic group is activated. This compound was demonstrated to be a valuable building block for a large number of HFA-C^{α}-methylated hydroxy acids.[10] Here is reported the direct incorporation of **1a** into depsipeptides on solid-phase.

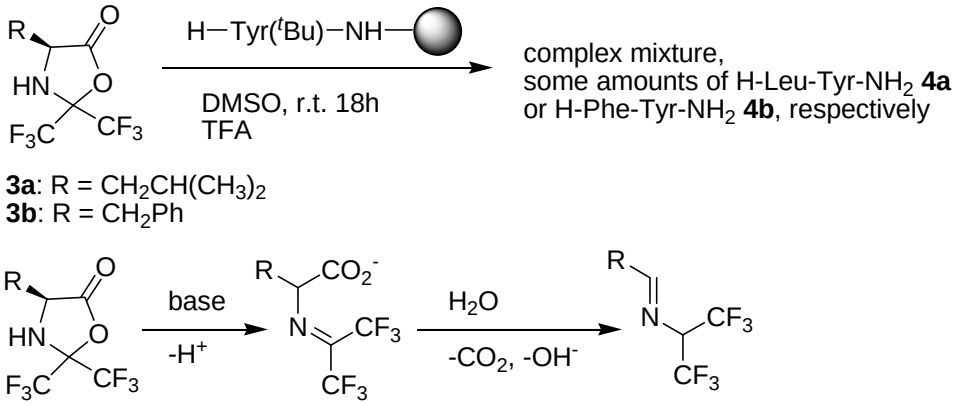
A four-fold excess of **1a** dissolved in THF was added to H-Tyr(^{t}Bu)-Rink-MBHA-resin. The progress of the reaction was monitored with the Kaiser (ninhydrin) test. After three days coupling time, the test gave a negative result and the product was cleaved with 95% TFA from the resin and lyophilized. HPLC-MS and ^{1}H-NMR confirmed that the desired product **2a** was formed, and its purity was determined by HPLC to be 89%. The same reaction was also performed with HFA-*D*-citramalic acid **1b** to give depsipeptide **2b**, with no evidence of cross-contamination observed. The HPLC retention times for **2a** and **2b** are distinguishable and only minor amounts (<1%) of the other diastereomer was detected (see experimental part). Thus, despite the long reaction time, racemization was negligible. In contrast, HFA-malic acid **1c**, which does not bear a methyl group at the C^{α}, readily reacts under the same conditions within 5 hours to yield depsipeptide **2c** in 94 % purity (HPLC). It can thus be concluded that the dramatic difference of the reaction time (*i.e,* 72 h *versus* 5 h) is caused by the extra α-methyl group present in citramalic acid.



Scheme 3. Coupling of HFA-citramalic and HFA-malic acid on solid-phase.

The reactivity of HFA-amino acids 3 on solid-phase

In order to evaluate the synthetic potential of HFA-amino acids analogous to that of HFA-hydroxy acids as acyl donors in SPPS, a four-fold excess of HFA-Leu **3a** and HFA-Phe **3b** were reacted in DMSO with H-Tyr(^{t}Bu)-Rink-MBHA-resin for 18h. DMSO was chosen as the solvent because it is non-volatile and it slightly accelerated aminolytic cleavage in previous experiments as compared to THF, although it can induce some racemization.[8] After 18 h the resins were washed with DMSO and DCM and the products were cleaved from the resin with 95% TFA and lyophilized.[11] HPLC-MS and ^{1}H-NMR showed a complex mixture of products, in which the presence of H-Phe-Tyr-NH_2 (**4a**) and H-Leu-Tyr-NH_2 (**4b**) could be detected by HPLC-MS.[12] These experiments show that in the case of HFA-amino acids **3a** and **3b**, side reactions compete with nucleophilic ring opening at the free terminal amines of solid-phase bound amino acids. Efforts to improve the reaction by base addition or heating are futile. Firstly, coupling proceeds with deprotection of the α-amino group, which can subsequently react with HFA-building blocks to yield oligomers, as in the case with NCA's (see above). Secondly, addition of bases or the nucleophile itself may cause deprotonation of the NH moiety in HFA-Leu and HFA-Phe, resulting in fragmentation of the heterocyclic system.[13]

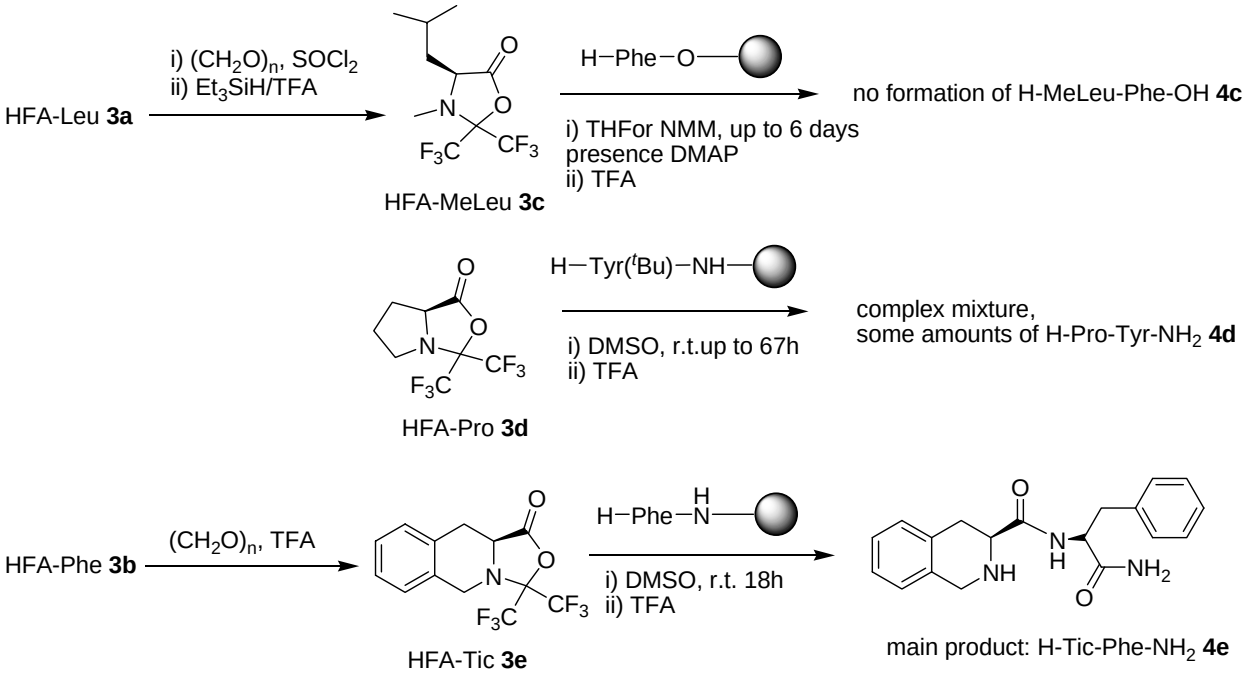


Scheme 4. Solid-phase peptide synthesis using HFA-amino acids and base-induzed fragmentation of HFA-amino acids.

The aforementioned limitations were not considered relevant for *N*-substituted HFA-amino acids **3c-e**, because fragmentation of the heterocycle cannot be induced by abstraction of the NH-proton. HFA-*N*-methyl amino acids can be prepared from the corresponding HFA-amino acids in a one-pot procedure.[14] Since *N*-methyl amino acids are rather precious building blocks, an SPPS methodology combining the HFA-route with the possibility of recovering the added excess was sought. HFA-MePhe **3c** dissolved in THF or NMM was added in a 4-fold excess to H-Phe-O-Wang resin. However, even using DMAP and prolonged reaction times (up to 6 days), no H-MePhe-Phe-OH **4c** could be detected in the product by HPLC-MS. HFA-Pro **2d** reacts similarly

to **2a** and **2b**. Although product **4d** was identified in the complex product mixture, prolonged reaction times (up to 67 h) did not drive the reaction to completion.

Tetrahydroisoquinoline-3-carboxylic acid (Tic) represents a conformationally constrained cyclic Phe analogue and is widely used for incorporation into cyclic peptides for structure-activity-relationship (SAR) studies.[15] Its HFA-derivative HFA-Tic **3e** is easily accessible from HFA-Phe **3b** *via* a Pictet-Spengler reaction.[16] HFA-Tic **3e** was found to be a suitable carboxyl-activated building block for SPS. After 16 h reaction of a four-fold excess of **3e** in THF with H-Phe-MBHA Rink amide resin, a negative ninhydrin test was observed. The resin was washed, and the product was cleaved with 95% TFA and then precipitated with ether. A single major product with 91 % purity (HPLC) was detected and identified by HPLC-MS and ^{1}H-NMR as H-Tic-Phe-NH_2 **4e**.



Scheme 5. Reactions of *N*-substituted HFA-amino acids on solid-phase.

Conclusions

HFA-hydroxy acids **1** can be directly incorporated into depsipeptides on solid-phase using general and straight-forward methods. C^{α}-methyl hydroxy acids like citramalic acid **1a** and **1b** also undergo coupling, but much slower than their counterpart malic acid **1c**. This finding demonstrates that the use of hexafluoroacetone as a bidentate reagent is an interesting alternative to conventional coupling/activation protocols. On the other hand, the use of HFA-amino acids **3**

as carboxyl-activated monomers for solid phase peptide synthesis is limited. One exception is the case of HFA-Tic **3e**, which afforded the dipeptide H-Tic-Phe-NH_2 **4e** in good yield.

Experimental Section

General Procedures. Analytical apparatus: HPLC analytic use a HPLC Waters 1525, an automatic injector 717 plus and a detector UV-Vis Waters 2487. The column Nucleosil C18 (250 x 4 mm) was run with acetonitrile (0.036% TFA) and water millipore (0.045% TFA). Datas were managed with Breeze v3.20 software. In semipreparative scale, the HPLC used was the model Waters 600, the automatic injector Waters 2700, the detector UV-Vis Waters 2487. Samples were collected with the Waters Fraction Collector II. The column Symmetry C18 (100 x 30 mm) was run with acetonitrile (0.05% TFA) and water milipore (0.1% TFA). Datas were managed with MassLynux 3.5 software. HPLC-Mass data were obtained from a collection of following modules: HPLC Waters Alliance 2795, detector UV-Vis 2487, mass detector Electrospray ZQ. The column Symmetry 200 C18 (150 x 3.9 mm) was run with acetonitrile (0.07% formic acid) and water milipore (0.1% formic acid). Datas were managed with MassLynux 4.0 software. Compounds used: Citramalic acid, malic acid, phenylalanine, leucine and proline were purchased from Acros. 4-Dimethylamino pyridine (DMAP) were purchased from Fluka, Fmoc-Tyr(^{t}Bu)-OH from Advanced Chemtech, solvents from SDS, and TFA from Fluorochem. This compounds have analisis grade. Solvents were used without prior drying. Hexafluoroacetone-trihydrate is a honorous gift from the former Hoechst AG. HFA-hydroxy acids **1a-c** and HFA-amino acids **3a-c** were obtained from malic acid, citramalic acid, leucine, proline and phenylalanine according to standard conditions.[17]

General procedure: Synthesis of depsipeptide amides 3a-c and peptide amide 4e
A sample of the resin loaded with the *N*-terminal deprotected amino acid was swollen in the solvent. The corresponding HFA-building blocks (4 equiv) dissolved in the minimum of the given solvent was added. Termination of reaction was detected by the Kaiser test that was performed according to reported procedures.[18] Then, the resin was washed 3 times with solvent and DCM each, filtered to dryness and treated with the acid coctail (for Rink amide MBHA resin 95% TFA, for Wang resin TFA / DCM 1 : 1) for one hour. After evaporation of TFA, the residues were dissolved in water/acetonitrile and lyophilized to give depsipeptide amides **3a-c** as white powders. Peptide amide **4e** was obtained by precipitation with diethyl ether.

Compound characterization
***N*$^{\alpha}$-[(2*S*)-2-Carboxymethyl-2-hydroxy-2-methyl]acetyl-tyrosine-amide (H-Cit-Tyr-NH_2) (2a).** From dioxolanone **1a** (solvent: THF). **HPLC** (100% H_2O to 90% ACN, 15 min, r.t.): t_r = 7,36 min. **HPLC-MS** (100% H_2O to 90% ACN, 15 min, r.t.): t_r =3,75 min., m/z = 311 $(M+H)^+$ (calculated: M = 310 $gmol^{-1}$). **^{1}H-NMR** (400 MHz, acetone-d_6): δ (ppm) = 1.34 (3H, s), 2.75

(2H, m), 3.02 (2H, d, J = 6.4 Hz), 4.53 (1H, m), 6.73 (2H, d, J = 8.4 Hz), 7.09 (2H, d, J = 8.4 Hz).

***N*$^{\alpha}$-[(2*R*)-2-Carboxymethyl-2-hydroxy-2-methyl]acetyl-tyrosine-amide (H-*D*-Cit-Tyr-NH$_2$) (2b).** From dioxolanone **1b** (THF). **HPLC** (100% H_2O to 90% ACN, 15 min, r.t.): t_r = 7,30 min. **HPLC-MS** (100% H_2O to 90% ACN, 15 min, r.t.): t_r = 4,18 min., m/z = 311 $(M+H)^+$ (calculated: M = 310 gmol^{-1}). **^{1}H-NMR** (400 MHz, acetone-d_6): δ (ppm) = 1.08 (3H, s), 2.40 (1H, m), 2.83 (2H, m), 3.07 (1H, m), 4.41 (1H, m), 6.60 (2H, d, J = 7.9 Hz), 7.09 (2H, d, J = 8.2 Hz).

***N*$^{\alpha}$-[(2*S*)-2-Carboxymethyl-2-hydroxy]acetyl-tyrosine-amide (H-Mal-Tyr-NH$_2$) (2c).** From dioxolanone **1c** (THF). **HPLC** (100% H_2O to 90% ACN, 15 min, r.t.): t_r = 6,87 min. **HPLC-MS** (100% H_2O to 90% ACN, 15 min, r.t.): t_r = 3,82 min., m/z = 297 $(M+H)^+$ (calculated: M = 296 gmol^{-1}). **^{1}H-NMR** (400 MHz, acetone-d_6): δ (ppm) = 2.31 (1H, m), 2.64 (1H, m), 2.83 (1H, m), 2.93 (1H, m), 4.29 (1H, m), 4.47 (1H, m), 6.60 (2H, d, J = 8. 5 Hz), 6.9 (2H, d, J = 8.4 Hz).

***N*$^{\alpha}$-[(3*S*)-3-(1,2,3,4-Tetrahydroisoquinolyl)]carbonyl-phenylalanine-amide trifluoroacetate (H-Tic-Phe-NH$_2$ * TFA) (4e).** From oxazolidinone **3e** (THF). **HPLC** (95% H_2O to 95% ACN, 15 min, r.t.): t_r = 7,68 min broad. **HPLC-MS** (95% H_2O to 95% ACN, 15 min, r.t.): t_r = 4,25 min., m/z = 324.6 $(M+H)^+$ (calculated: M = 323.4 gmol^{-1}). **^{1}H-NMR** (400 MHz, methanol-d_4): δ (ppm) = 2.75 (1H, m), 2.95-3.13 (2H, m), 3.16 (1H, m), 3.85 (1H, m), 4.10 (2H, br. m), 4.45 (1H, m), 6.94-7.03 (9H, m).

Compound **4e** also was prepared from phenylalanine amide (2 eq.) and **3e** in solution (2-propanol). After 16 h, the precipitate was filtered off and recrystallized from water. After addition of one drop of TFA to a solution of product in methanol-d_4, an ^{1}H-NMR spectrum identical with the solid-phase product was obtained.

Acknowledgements

J.S. thanks the VW Foundation for providing a grant for this project. This work was partially supported by Ministerio de Ciencia y Tecnología (BQU 2003-00089), Generalitat de Catalunya [Grups Consolidats (2001 SGR 0047) and CERBA], Pharma Mar Ltd., and the Barcelona Science Park.

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