ORGANIC LETTERS

2004
Vol. 6, No. 22
4089−4092

Solid-Phase Combinatorial Synthesis of Peptide–Biphenyl Hybrids as Calpain Inhibitors[†,‡]

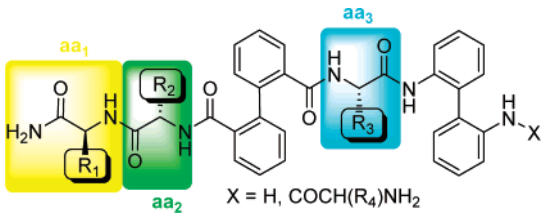
Ana Montero,[§,⊥] Fernando Albericio,*[,||] Miriam Royo,*[,⊥] and Bernardo Herradón*[,§]

Instituto de Química Orgánica General, C.S.I.C., Juan de la Cierva 3, 28006-Madrid, Spain, and Combinatorial Chemistry Unit and Barcelona Biomedical Research Institute, Barcelona Science Park, University of Barcelona, 08028 Barcelona, Spain

herradon@iqog.csic.es; mroyo@pcb.ub.es; albericio@pcb.ub.es

Received September 3, 2004

ABSTRACT



The combinatorial parallel synthesis of peptide-biphenyl hybrids on solid support using state of the art of peptide synthesis is reported. Key steps were the N to C addition of an amino moiety, hydrolysis of the methyl ester, and the absence of cross-linked compounds when the 2,2′-diamino-1,1′-biphenyl was incorporated. When tested for activity as calpain inhibitors, some of the compounds exhibited IC_{50} values in the nanomolar range.

Peptide-biphenyl hybrids **A** (Figure 1) are compounds in which peptides or single amino acids are bound to the 2- and 2′-positions of a biphenyl ring system.[1] Both peptides and biaryl compounds have been used as chiral catalysts,[2] as building blocks for the design of technological materials,[3] as biologically active compounds, and in other applications.[4] The combination of both structural fragments in a single molecule provides compounds with synergistic properties.[1,5]

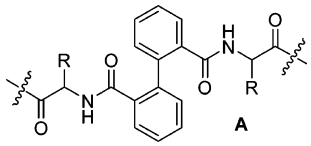


Figure 1. Generic structure of peptide-biphenyl hybrid **A**.

[†] Taken in part from the Ph.D. Thesis of A.M. (Universidad Autónoma, Madrid, 2004).

[‡] Abbreviations: CDI, carbonyldiimidazole; DIEA, *N*,*N*-diisopropylethylamine; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; DMF, *N*,*N*-dimethylformamide; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; MBHA, 4-methylbenzhydrylamine; PS, polystyrene; PyBOP, (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran. Amino acid symbols denote L-configuration.

[§] C.S.I.C.

[⊥] Combinatorial Chemistry Unit, Barcelona Science Park, University of Barcelona.

[||] Barcelona Biomedical Research Institute, Barcelona Science Park, University of Barcelona.

(1) (a) Brandmeier, V.; Feigel, M.; Bremer, M. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 486. (b) Weigand, C.; Feigel, M.; Landgrafe, C. *Chem. Commun.* **1998**, 679. (c) Mann, E.; Montero, A.; Maestro, M. A.; Herradón, B. *Helv. Chim. Acta* **2002**, *85*, 3624. (d) Amine, M.; Atmani, Z.; El Hallaoui, A. A.; Giorgi, M.; Pierrot, M.; Réglier, M. *Biorg. Med. Chem. Lett.* **2002**, *12*, 57.

(2) (a) Jarvo, E. R.; Miller, S. J. *Tetrahedron* **2002**, *58*, 2481. (b) McCarthy, M.; Guiry, P. J. *Tetrahedron* **2001**, *57*, 3809.

(3) (a) Voyer, N. *Top. Curr. Chem.* **1997**, *184*, 1. (b) Hartley, C. S.; Lazar, C.; Wand, M. D.; Lemieux, R. P. *J. Am. Chem. Soc.* **2002**, *124*, 13513.

(4) (a) Hajduk, P. J.; Bures, M.; Praestgaard, J.; Fesik, S. W. *J. Med. Chem.* **2000**, *43*, 3443. (b) Kutzki, O.; Park, H. S.; Ernst, J. T.; Orner, B. P.; Yin, H.; Hamilton, A. D. *J. Am. Chem. Soc.*, **2002**, *124*, 11838. (c) Wang, W.; Cai, M.; Xiong, C.; Zhang, J.; Trivedi, D.; Hruby, V. J. *Tetrahedron* **2002**, *58*, 7365. (d) Peukert, S.; Brendel, J.; Pirard, B.; Brüggemann, A.; Below, P.; Kleemann, H.-W.; Hemmerle, H.; Schmidt, W. *J. Med. Chem.* **2003**, *46*, 486. (e) Sheppard, G. S.; Kawai, M.; Craig, R. A.; Davidson, D. J.; Majest, S. M.; Bell, R. L.; Henkin, J. *Biorg. Med. Chem. Lett.* **2004**, *14*, 865.

10.1021/ol048216j CCC: $27.50
Published on Web 09/29/2004

Our previous research on the inhibition of calpain by peptide-biphenyl hybrids yielded several nanomolar inhibitors (Figure 2).[5a] To establish structure−activity relationships for peptide-biphenyl hybrids, a large variety of peptide-biphenyl hybrids is needed, and a solid-phase combinatorial synthetic strategy is desired.[6] Our target library includes compounds of the types **XXX** and **XXXX** (Figure 3), which exhibit structural similarity to the lead molecule **3**. The target compounds possess two common biphenyl fragments and either three or four amino acid residues that are used to generate molecular diversity.

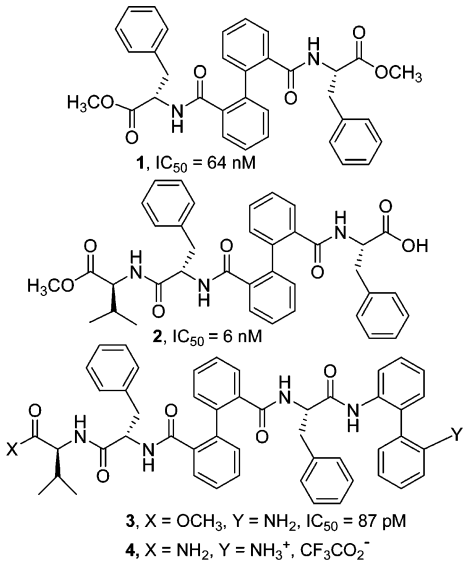


Figure 2. Structures and IC_{50} values of previously reported peptide-biphenyl hybrid calpain inhibitors **1**−**3** as well as the structure of target **4**.

The synthesis of peptide-biphenyl hybrids was previously carried out in solution using an efficient coupling reaction between a nucleophile (either an amino acid or a peptide derivative) and an electrophile [either conveniently activated 2,2′-biphenyl-1,1′-dicarboxylic acid or dibenzo[*c*,*e*]oxepine-5,7-dione (diphenic anhydride)]. The aforementioned strategy is not readily applicable to the solid phase, as it does not facilitate linkage to a solid support. Alternatively, a standard solid-phase peptide strategy that involves linkage of the first amino acid to the solid support through the C-terminal carboxylic acid and elongation of the chain through the N-terminus was probed.[7] In this case, however, when the biphenyl residue, which contains two carboxylic moieties, is incorporated, the chain reverses sense. Optimization of the solid-phase process was carried out for type **XXX** (**4**) and type **XXXX** (**13**) compounds. The synthesis is illustrated in Scheme 1.

Rink-MBHA-PS-resin (the loading of intial MBHA-PS resin was 0.7 mmol/g) was transformed into the solid-phase linked dipeptide **5** by standard methodology involving the appropriate Fmoc-removal [piperidine-DMF (1:4)] and coupling (DIPCDI/HOBt, 3 equiv each) to the corresponding *N*-Fmoc-amino acid (Val and Phe, sequentially, 3 equiv). All of the reactions involving resin-bound amines were monitored by Kaiser's test,[8] and when necessary, the coupling was repeated until a negative test result was obtained. The first biphenyl fragment was introduced by the reaction of **5** with diphenic anhydride in the presence of TEA to give the acid **6**. The free acid of **6** was activated with PyBOP/HOAt and then coupled with H-L-Phe-OMe to give **7**. The reactions involving resin-bound carboxylic acids were monitored by the malachite green test.[9] At this stage an aliquot of **7** was treated with TFA to give **8**, which had a purity of over 95% by HPLC-MS.[10] The next step, the hydrolysis of the methyl ester with LiOH in THF to afford the acid **9**, required extensive optimization.[11] Thus, the amount of LiOH, reaction temperature, and reaction time were all investigated in order to achieve a satisfactory result. This process was readily followed by HPLC/MS analysis of samples obtained after TFA cleavage of aliquots of **9**. Complete conversion and a purity of >95% of the acid **10** were obtained when the reaction was carried out with 25 equiv of LiOH, at 50 °C for 17 h.[11] The penultimate step of the synthesis was activation of the acid functionality of **9** with CDI (10 equiv) followed by addition of the rather poor nucleophile 2,2′-diamino-1,1′-biphenyl (5 equiv) to give the monoacylated product **11**. No diacylated cross-linked product was detected under these conditions. This absence can be attributed to the "pseudo-dilution phenomenon"[12] associated with the solid phase, as well as to the poor nucleophilicity of the amino group of compound **11**. Finally, TFA cleavage from the resin yields in the target molecule **4** (as the trifluoroacetate salt). Compound **4** was analyzed by HPLC-MS.[10] It is interesting to note that at room temperature, the chromatogram of the peptide-biphenyl **4** shows two close peaks at 9.61 and 9.73 min that coalesce (9.60 min) when the temperature is increased to 40 °C (Figure 4, Supporting Information). These two peaks may be attributed to the two possible atropisomers of compound **4** that have been also detected by ^{1}H NMR.[1c] The synthesis is very efficient, providing **4** in an overall yield of 75% for the 11 step sequence. [13]

The last coupling for the preparation of type **XXXX** involves the reaction of the poor nucleophile aromatic amine

(5) (a) Montero, A.; Mann, E.; Chana, A.; Herradón, B. *Chem. Biodiver.* **2004**, *1*, 442. (b) Montero, A.; Alonso, M.; Benito, E.; Chana, A.; Mann, E.; Navas, J. M.; Herradón, B. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2753. (c) Herradón, B.; Benito, E.; Chana, A.; Mann, E.; Montero, A. Spanish Patent Application 200301125; PCT Application ES2004/070034.

(6) Nicolaou, K. C.; Hanko, R.; Hartwig, W., Eds. *Handbook of Combinatorial Chemistry*; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2002

(7) Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Chemical Approaches to the Synthesis of Peptides and Proteins*; CRC Press: Boca Raton, FL, 1997.

(8) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 594.

(9) Attardi, M. E.; Portu, G.; Taddei, M. *Tetrahedron Lett.* **2000**, *41*, 7391.

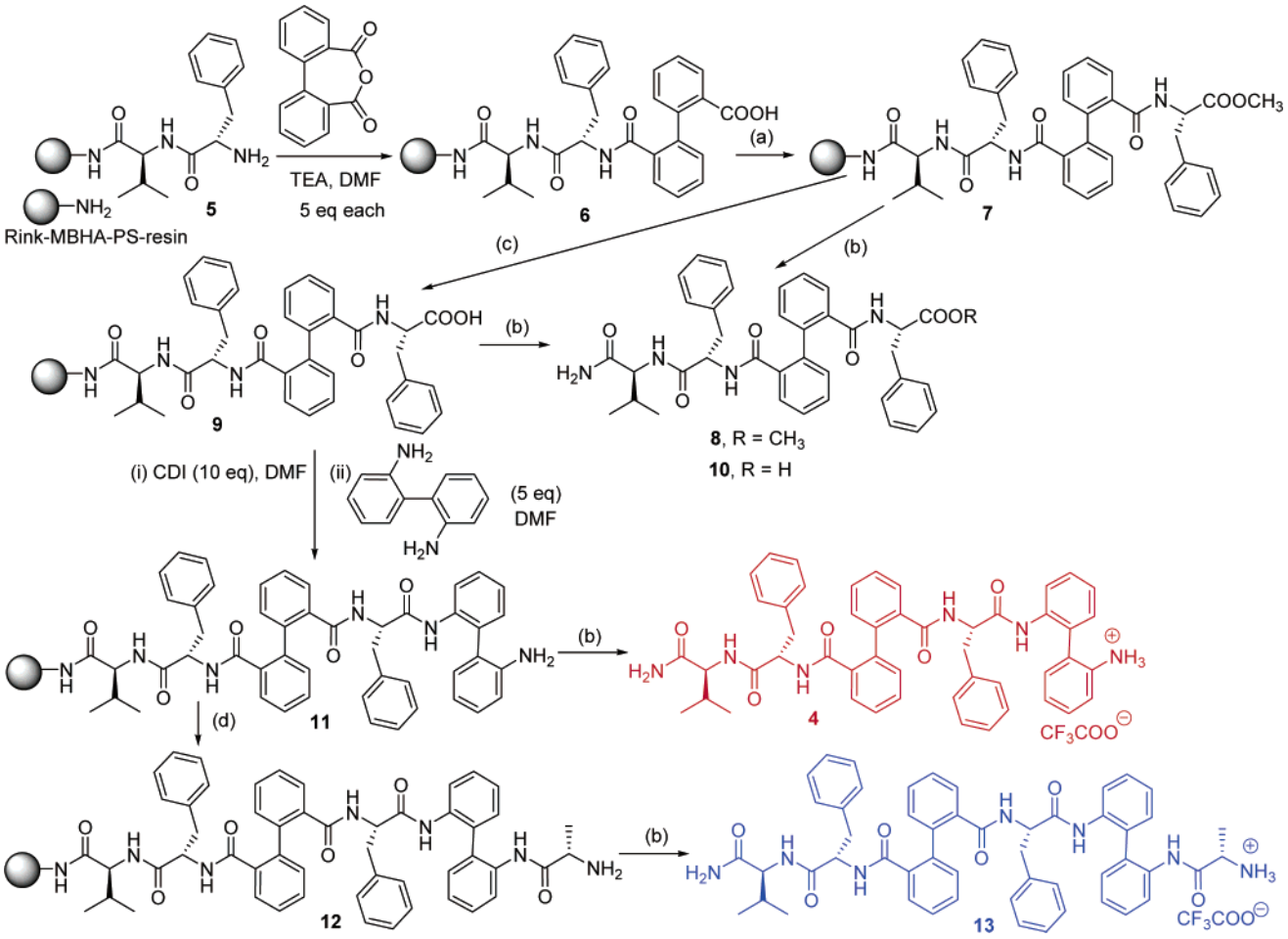
(10) HPLC analyses are included as Supporting Information.

(11) Cantel, S.; Desgranges, S.; Martinez, J.; Fehrentz, J.-A. *J. Peptide Sci.* **2004**, *10*, 326.

(12) Mazur, S.; Jayalekshmy, P. *J. Am. Chem. Soc.* **1979**, *101*, 677.

(13) Starting from 50 mg of resin (0.035 mmol of reacting amine) afforded 25.6 mg of **4** (93% purity).

Scheme 1. Solid-Phase Synthesis of Target Molecules **4** (as a Representative Example of Peptide-biphenyl Hybrids of Type **XXX**) and **13** (as a Representative Example of Peptide-biphenyl Hybrids of Type **XXXX**)[a]



[a] Reagents and conditions: (a) HCl H-Phe-OMe (3.0 equiv), PyBOP (3.0 equiv), HOAt (3.0 equiv), DIEA (6.0 equiv), DMF. (b) TFA−CH_2Cl_2 (95:5). (c) 2 N LiOH (25 equiv), THF−H_2O (7:3), 50 °C. (d) (i) symmetric *N*-Fmoc-amino acid anhydride (3.0 equiv), TEA (3.0 equiv), CH_2Cl_2; (ii) piperidine−DMF (1:4).

present in **11** and an N-protected amino acid derivative. Several procedures such as activation with DIPCDI/HOBt or use of the acid chloride were tested but proved to be unsatisfactory.[14] The best result was obtained using the symmetrical anhydride in CH_2Cl_2, providing **12** (Scheme 1). Finally, after removal of the *N*-Fmoc protecting group and cleavage from the resin, the peptide-biphenyl hybrid **13** was obtained as a trifluoroacetate salt.

The proof of concept of this methodology was undertaken by the generation of a small combinatorial library of compounds of the types **XXX** and **XXXX** using a parallel synthesis strategy. In addition to having studied the scope and limitation of the synthetic methodology, these experiments should test the utility of the semiautomatic system proposed for these and future libraries.

The peptide-biphenyl hybrids **XXX** and **XXXX** (Figure 3) possess three and four amino acids, respectively. Since previous results from one of our laboratories indicated that the peptide-biphenyl hybrids that are most active as calpain

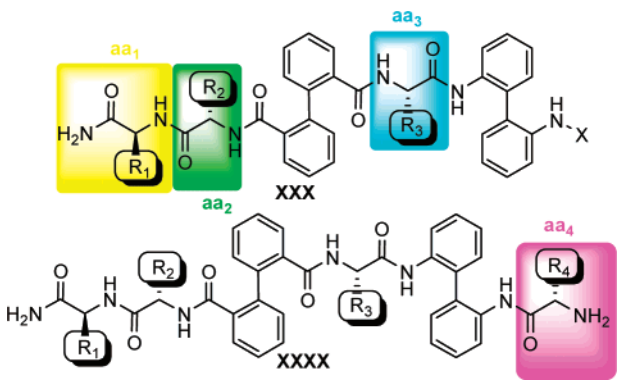


Figure 3. Generic structures of the target peptide-biphenyl hybrids **XXX** and **XXXX** with the sites (R_1−R_4) for generating molecular diversity highlighted. XXX and XXXX denote sublibraries containing three and four amino acids, respectively.

(14) While the reaction promoted by DIPCDI/HOBt was very slow, the acid chloride methods were not always the best methods for incorporating urethane-protected amino acids, because they led to the formation of the less reactive oxazolone. See ref 7.

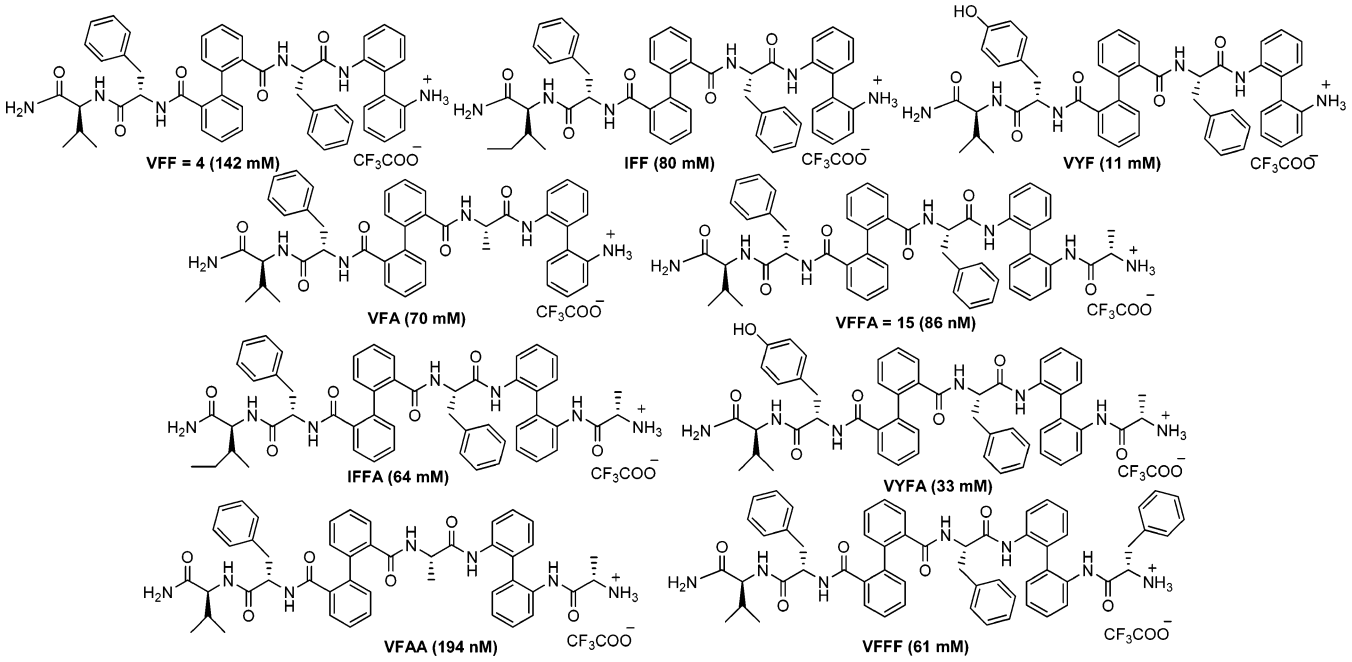


Figure 4. Structures and IC_{50} values of the compounds of the library of peptide-biphenyl hybrids. IC_{50} values were determined using a previously reported spectrofluorimetric method incorporating calpain I from porcine erythrocytes and fluorescence-labeled casein as a substrate.[5]

inhibitors are those with aromatic and hydrophobic amino acids, the following amino acids were selected: Val and Ile for position aa_1, Phe and Tyr for position aa_2, Phe and Ala for position aa_3, and Ala and Phe for position aa_4. Peptide-biphenyl hybrid libraries were prepared according to the synthetic procedure illustrated in Scheme 1. Figure 4 shows the structures of the peptide-biphenyl hybrids prepared on a solid phase.

It is worth mentioning that eight out of the nine compounds have purities higher than 90%; only compound **VYF** has a lower but still acceptable purity of 78%. The overall yields for the entire sequences (11 steps for compounds of type **XXX** and 13 steps for hybrids of type **XXXX**) ranged from 39 to 75%. Finally, the calpain inhibitory capacity of the peptide-biphenyl hybrids was determined in the manner previously reported.[5] As expected from our previous experience, all of the compounds in the library are calpain inhibitors, although it must be noted that when the biological activities of the parent compound **3** and the closest analogue **4** are compared, a significant reduction in activity is observed. This can be attributed to the subtle influence of the different functionalities at both ends of each molecule. On the other hand, two of the compounds of the library (**VFFA** and **VFAA**, Figure 4 and Table 1, Supporting Information) are potent calpain inhibitors, having IC_{50} values in the nanomolar range.

In summary, we have shown that peptide-biphenyl hybrids can be obtained via solid-phase methodology. Key steps of this protocol were the N to C addition of an amino moiety, the hydrolysis of the methyl ester, and the total absence of cross-linked compounds when the 2,2′-diamino-1,1′-biphenyl was incorporated. The interesting biological activity of these compounds has inspired an ongoing preparation of a large number of congeners.

Acknowledgment. This work was partially supported by MCyT (BQU2003-00089, 2002-02047, and 2001-2270) and Fundació “la Caixa” (02/162-02). We thank Mercedes Giner (IQOG, CSIC) for her assistance with the experiments on calpain inhibition.

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

OL048216J