Pergamon

Tetrahedron Letters 40 (1999) 2749–2752

TETRAHEDRON LETTERS

# Solid-phase Synthesis of *N,N'*-Unsymmetrically Substituted Ureas : Application to the Synthesis of Carbaza Peptides

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Received 19 January 1999; accepted 5 February 1999

**Abstract.**

*The synthesis of Boc- or Fmoc-monoprotected propylenediamine derivatives is reported starting from N-protected α-amino acids. The introduction of these building blocks on solid support via the formation of a urea moiety leads to a new pseudopeptide family ($C^{\alpha}$-$CH_2$-$CH_2$-$N^{\alpha}$(R)-CO-NH-$C^{\alpha}$). Two carbonylating reagents, i.e N,N'-carbonyldiimidazole and triphosgene, as well as different coupling procedures, have been tested to optimize the Boc and Fmoc solid-phase synthesis of a model peptide incorporating this isosteric replacement.* 

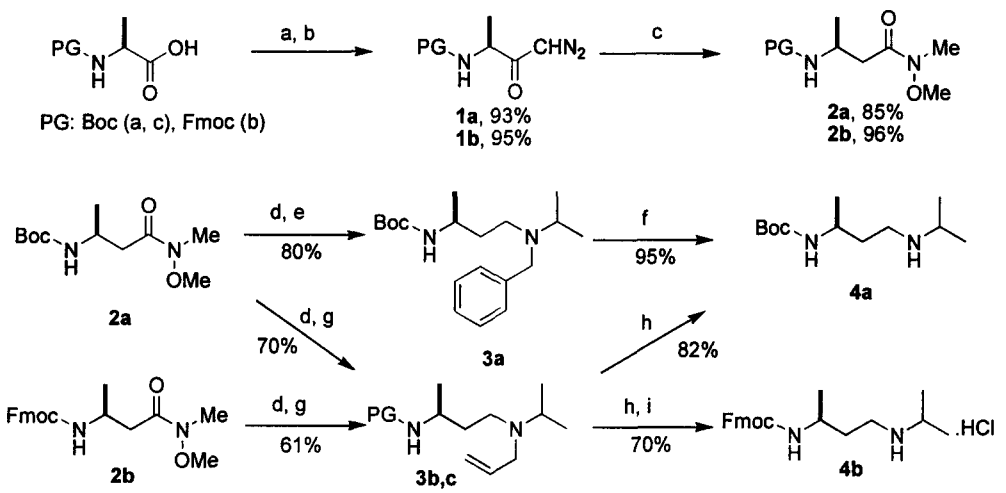
*Keywords: Peptide analogues/mimetics; Solid-phase synthesis; Isocyanates; Ureas.*

Isosteric peptide bond replacement has been extensively explored in the design of bioactive peptide analogues. As an example, the carba ($CH_2$-$CH_2$) junction has been claimed to be an excellent mimic of the peptide bond [1]. On the other hand, substitution of a nitrogen for the α-CH in one or several amino acid residues has led to attractive pseudopeptides named azapeptides [2]. Initial immunological studies in our laboratory have shown that the introduction of carba Ψ[$CH_2$-$CH_2$] or aza modifications in an antigenic peptide leads to highly immunogenic compounds (to be published). This observation tempted us to combine these two modifications and thus to develop the new motif -XaaΨ[$CH_2$-$CH_2$]AzXbb- according to Spatola's nomenclature [3]. In this paper, we report the Boc and Fmoc solid-phase synthesis of a pseudopeptide analogue in which a carba bond is substituted for an amide, and a nitrogen for the contiguous α-carbon. We propose to name the resulting peptide analogue a carbaza peptide.

The synthesis of carbaza peptides can be considered as the synthesis of a trisubstituted urea involving a monoprotected propylenediamine. In the last few years, solid-phase synthesis of substituted ureas has received a growing attention. The most usual strategy for urea formation involves the reaction of a polymer-supported amine with an activated carbamate or an isocyanate [4]. In the present work, we followed the much less frequently used alternative route [5,6], in which the polymer-supported activated carbamate or isocyanate is obtained from *N,N'*-carbonyldiimidazole or triphosgene as carbonylating reagents for solid-phase incorporation of the carbaza moiety [7,8].

0040-4039/99/$ - see front matter 
*PII:* S0040-4039(99)00288-9

We decided to introduce the carbaza modification at the Ala-Val position of the model peptide (H-Lys-Ala-Val-Tyr-Asn-Phe-Ala-Thr-Nle-$NH_2$) derived from the natural cytolytic T-cell epitope 33-41 of the lymphocytic choriomeningitis virus protein. Synthesis of the key intermediates, the Boc- or Fmoc-protected propylenediamine **4a** or **4b**, mimicking the cognate dipeptide sequence Ala-Val, was first undertaken (Scheme 1). *N*-protected *β*-amino *N,O*-dimethylhydroxamates **2a**, **2b** were obtained by direct Wolff rearrangement of the corresponding diazo ketone [9], and subsequent reduction with $LiAlH_4$ afforded the desired *β*-amino aldehydes in satisfactory yields [10,11]. In a first attempt, Boc-protected *β*-homoalaninal was reacted without further purification in a reductive amination step with isopropylamine in the presence of sodium triacetoxyborohydride [12]. Unfortunately, the reaction led to a mixture composed of the desired dialkylated amine and the trialkylated by-product. Similar results were observed with sodium cyanoborohydride independently of the nature of the solvent used.




Scheme 1.

(a) 1.1 equiv. iBuOCOCl/NMM, THF, -25°C, 1h; (b) $CH_2N_2/Et_2O$, r.t., 2 h; (c) 1.5 equiv. HN(OMe)Me.HCl/2 equiv. $Et_3N$/THF filtrate, 3 equiv. $Et_3N$, 0.15 equiv. $C_6H_5CO_2Ag$, THF, -25°C to r.t. in 2 h; (d) 3 equiv. $LiAlH_4$, THF, -30°C, 1 h; (e) 1.1 equiv. *N*-isopropylbenzylamine/1.4 equiv. $NaBH(OAc)_3$, DCE, r.t., 3 h; (f) $H_2$, 10% Pd/C in MeOH; (g) 1.1 equiv. *N*-isopropylallylamine/1.4 equiv. $NaBH(OAc)_3$, DCE, r.t., 3 h; (h) 0.05 equiv. $Pd(dba)_2$/DPPB (1:1)/2 equiv. 2-mercaptobenzoic acid/$CH_2Cl_2$, r.t., 2 h; (i) HCl.

In order to suppress the formation of the trialkylated amine, reductive amination was performed using a monoprotected secondary amine. Allyl and benzyl protecting groups were chosen for their compatibility with the general scheme of synthesis. The benzyl group is suitable for the Boc strategy, and the conditions for allyl amine deprotection using palladium are compatible with both Boc and Fmoc chemistries [13]. Reductive amination of Boc- and Fmoc-protected *β*-amino aldehydes performed in the presence of allyl- and benzyl-protected isopropylamine gave the expected compounds **3a,b,c** in good yields (60-80%). Afterwards, selective *N*-deprotection steps using classical catalytic hydrogenation for compound **3a** or allyl deprotection for compounds **3b** and **3c** gave the desired monoprotected diamine **4a** and **4b** respectively. All these compounds were purified by silica gel chromatography (except for **4b**) and were characterized by mass spectrometry and $^1H$ NMR analysis [14]. In the case of compound **4b**, purification was performed by precipitation of its hydrochloride salt in an etheral solution of HCl, thus allowing a prolonged storage without cleavage of the Fmoc group by the free secondary amine.

The Boc- and Fmoc-monoprotected diamine **4a** and **4b** were then introduced during the solid-phase assembly of the target peptide and the coupling conditions of **4a** on solid supported amines activated with triphosgene or *N,N'*-carbonyldiimidazole were studied in detail. We first showed that 30 min were sufficient for the formation

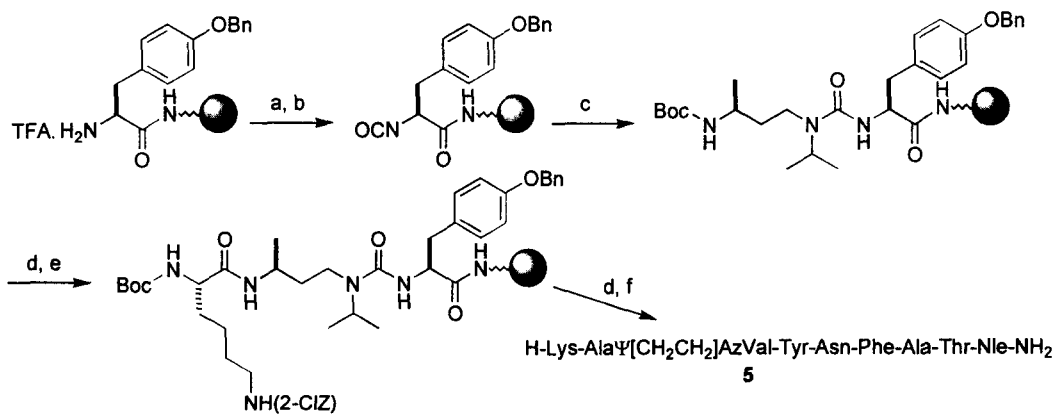
of the activated carbamate or isocyanate on the solid support and then, several pseudopeptide syntheses were performed in parallel by using classical Boc procedures with varying coupling times for the Boc-protected diamine **4a** (entries 1-6, Table 1).

Table 1. Coupling conditions of 4a and 4b on solid-supported amines.

| Entries | Strategy | Carbonylating reagent | Coupling time | Hydantoin[a] | Expected pseudopeptide[a] | Overall yield[b] |
|---|---|---|---|---|---|---|
| 1 | Boc | *N,N'*-carbonyldiimidazole | 1 h | / | 13 % | 6 % |
| 2 | Boc | " | 12 h | / | 38 % | 30 % |
| 3 | Boc | " | 72 h | / | 45 % | 35 % |
| 4 | Boc | Triphosgene | 1 h | 6 % | 40 % | 35 % |
| 5 | Boc | " | 12 h | 10 % | 40 % | 32 % |
| 6[c] | Boc | " | 1 h | 3 % | 45 % | 40 % |
| 7 | Fmoc | " | 12 h | / | 56 % | 35 % |

[a] According to RP-HPLC of the crude lyophilized material.
[b] Products were purified by semi-preparative RP-HPLC and gave satisfactory RP-HPLC, MS results.
[c] In this case, no base was added for the coupling of the monoprotected diamine **4a**. See also Scheme 2.

The peptide chains were assembled on a MBHA resin (0.63 mequiv./g) and elongated up to the Tyr residue. After neutralization of the trifluoroacetate salt with DIEA, the resins were washed several times with dry $CH_2Cl_2$ and then 10 equiv. of *N,N'*-carbonyldiimidazole or 3.3 equiv. of triphosgene were added with 10 equiv. of DIEA for 30 min. After being washed several times with dry $CH_2Cl_2$, the resins were allowed to react with a solution of 5 equiv. of compound **4a** and 5 equiv. of DIEA in $CH_2Cl_2$ for different coupling times. After several washings with $CH_2Cl_2$ and DMF, the peptide elongation was achieved in standard manner. Each batch of peptide resin was cleaved using a TMSOTf/TFA/anisole mixture [15]. HPLC analysis of the different crude products showed that in every case the expected pseudopeptide **5** (Rt = 11.74 min, linear gradient of A (0.1% TFA in $H_2O$) and B (0.08 % TFA in MeCN), 5-65% B in 20 min) was present as identified by MALDI-TOF spectrometry. With *N,N'*-carbonyldiimidazole as a carbonylating reagent (entries 1-3, Table 1), the total amount of by-products was reduced with increasing coupling time, and the expected compound was finally obtained in 35 % yield (entry 3, Table 1). We assume that the main by-products observed on the HPLC profiles were essentially due to the unreacted activated carbamate which then reacted during the final elongation step of the peptide.




Scheme 2. Optimized Boc solid-phase synthesis of the carbaza peptide analogue **5**.
(a) 10 equiv. DIEA in $CH_2Cl_2$, 10 min; (b) 3.3 equiv. triphosgene/10 equiv. DIEA in $CH_2Cl_2$, 30 min; (c) 5 equiv. of **4a** in $CH_2Cl_2$, 1 h; (d) Deprotection step with TFA; (e) Coupling of BocLys(2-ClZ)OH using BOP/HOBt/DIEA procedure; (f) Cleavage with TMSOTf/TFA/anisole.

Prolongation of the coupling times when triphosgene was used led not to an improvement in the purity of the target peptide but rather to the formation of significant amounts of hydantoin (entries 4-5, Table 1). Indeed, Quibell *et al.* have reported that the addition of a base in the presence of a resin-bound peptide isocyanate may induce the formation of hydantoin [6]. Finally, the best overall result was obtained by on-resin reaction with triphosgene followed by a 1 hour coupling of the monoprotected diamine **4a** without addition of a base (Scheme 2 and entry 6, Table 1). This approach was also evaluated in Fmoc chemistry on a Rink amide MBHA resin (0.6 mequiv./g) using the Fmoc-monoprotected diamine propylene hydrochloride salt **4b**. After Fmoc deprotection, several washings with dry $CH_2Cl_2$ and on-resin activation with triphosgene, 5 equiv. of **4b** were added and 5 equiv. of DIEA were used to regenerate the free amine. The mixture was allowed to react overnight. After final elongation and TFA cleavage, the crude material was analyzed by HPLC and mass spectroscopy. The crude product contained 56 % of the expected pseudopeptide **5** and HPLC purification led to 25 mg of pure peptide (>99 %) (entry 7, Table 1).

In summary, by using benzyl or allyl as a temporary *N*-protecting group, we have found that *N*-monoprotected propylene diamines are efficiently prepared either from Boc or Fmoc protected α-amino acids. The introduction of these building blocks onto solid support *via* urea formation leads to a new family of pseudopeptides that we propose to name carbaza peptides. In our hands, triphosgene is found to be a more efficient carbonylating reagent than *N,N'*-carbonyldiimidazole for the polymer supported synthesis of *N,N'*-unsymmetrically substituted ureas. We anticipate that the use of monoprotected commercially available primary amines will make it possible to mimic most of the α-amino acid side chains and, most importantly, will provide access to molecular diversity *via* carbaza motif-containing libraries.

## References and Notes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[14] All compounds gave satifactory $^1$H NMR and MALDI-TOF MS data. **1b**. Yellow solid. $^1$H NMR (250 MHz, $CDCl_3$) δ 1.36 (d, 3H, J = 7.1 Hz), 4.24 (t, 1H, J = 6.6 Hz), 4.43 (m, 1H), 4.46 (d, 2H, J = 7.1 Hz), 5.31 (s, 1H), 5.42 (bb, 1H), 7.27-7.45 (m, 4H), 7.61 (d, 2H, J = 6.6 Hz), 7.78 (d, 2H, J = 7.5 Hz). **2b**. White solid. $^1$H NMR (250 MHz, $CDCl_3$) δ 1.26 (d, 3H, J = 6.6Hz), 2.5-2.8 (m, 2H, J = 5.0 Hz, J = 15.7 Hz), 3.16 (s, 3H), 3.65 (s, 3H), 4.10 (m, 1H), 4.18 (t, 1H, J = 6.8 Hz), 4.32 (d, 2H, J = 6.8 Hz), 5.70 (bb, 1H), 7.22-7.39 (m, 4H), 7.57 (d, 2H, J = 7.3 Hz), 7.78 (d, 2H, J = 6.8 Hz). **3b**. White solid. $^1$H NMR (250 MHz, $CDCl_3$) δ 0.94-1.03 (2d, 6H, J = 6.6 Hz), 1.18 (d, 3H, J = 6.4 Hz), 1.38-1.74 (2m, 2H), 2.37-2.66 (2m, 2H), 2.91-3.16 (m, 3H), 3.77 (m, 1H), 4.21 (t, 1H, J = 6.8 Hz), 4.37 (d, 2H, J = 6.8 Hz), 5.06-5.29 (m, 2H), 5.72-5.92 (m, 1H), 6.77 (bb, 1H), 7.25-7.42 (m, 4H), 7.61 (d, 2H, J = 6.9 Hz), 7.59 (d, 2H, J = 7.2 Hz); MALDI-TOF MS: m/z 279.2 ($M+H^+$). **4b**. White solid (Hydrochlorid salt). $^1$H NMR (250 MHz, $CDCl_3$) δ 1.24 (d, 3H, J = 6.1 Hz), 1.39-1.47 (2d, 6H, J = 6.5 Hz), 1.65-2.08 (2m, 2H), 2.88-3.08 (2m, 2H), 3.26 (m, 1H), 3.71-3.92 (m, 1H), 4.20 (m, 1H), 4.39 (m, 2H), 5.23 (bb, 1H), 7.27-7.41 (m, 4H), 7.59 (d, 2H, J = 6.9 Hz), 7.60 (d, 2H, J = 6.8 Hz); MALDI-TOF MS: m/z 353.4 ($M+H^+$).

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