Molecular Diversity, **4:** 191–197, 1998.
KLUWER/ESCOM

Synthesis of peptidomimetics using a polymer-bound Boc-linker

Thomas Redemann, Holger Bandel & Günther Jung*
Institut für Organische Chemie, Auf der Morgenstelle 18, D-72076 Tübingen, Germany

Received 19 April 1999; Accepted 15 July 1999

Key words: α-hydroxy-β-amino-aldehyde, amino acid homologation, Boc-resin, 2-hydroxy-1,3-diamines, γ-hydroxy-vinylsulfone, peptidomimetics

Summary

Boc-resin-bound α-hydroxy-β-amino-aldehydes are accessible starting from N-terminally bound amino acid esters by using Dondoni's C_1-homologation reaction sequence. The conversion of these synthons to two different peptide mimetics – 2-hydroxy-1,3-ethyl-diamines and γ-hydroxy-δ-amino-vinyl sulfones – has been investigated. The successful transfer of the complex α-amino acid homologation reaction sequence into solid-phase chemistry demonstrates the potentials of the Boc-resin for synthesis of peptidomimetics.

Introduction

Combinatorial libraries of peptidomimetics, which incorporate isosteres to mimic the transition state of amide bond hydrolysis, have proven to be a versatile and successful tool in the search for novel enzyme inhibitors [1]. Most of these inhibitors have been prepared following a common synthetic strategy. Specific isosteric moieties like hydroxyethylene, diol, α-ketoamide or phosphonate have been built in using solid phase peptide synthesis [2] or, alternatively, were attached to the solid support via the functionality of the isosteric core, e.g. the hydroxy group of a hydroxyethylamine or diol structure [3]. Such building blocks can then be functionalized in both directions of a peptide chain.

We focused on a different, versatile strategy, in which isosteric moieties are synthesised directly on solid support by C-terminal modification of amino acids. For this approach we have investigated a solid-phase equivalent of the Boc protecting group as solid support [4], since various synthetic strategies for amino acid or dipeptide analogues are based on the C-terminal modification of Boc-amino acids [5]. Besides its mild cleavage conditions, the *tert*-alkoxycarbonyl anchor group (abbreviated as 'Boc') offers also a broad stability range, especially against strong nucleophiles like lithiumorganyles.

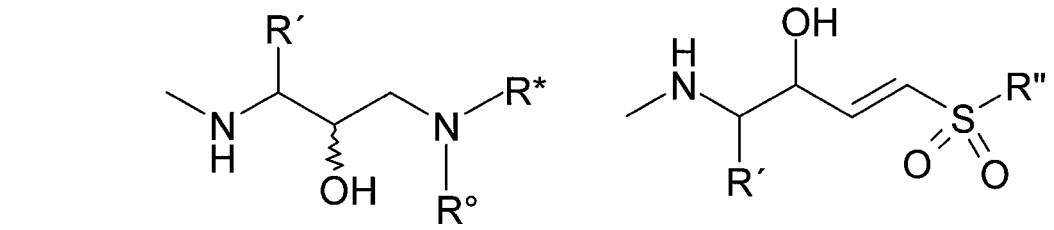
Our solid-phase synthetic route on the Boc-resin allows the conversion of N-terminal resin-bound amino acids to their corresponding α-hydroxy-β-amino-aldehydes by C_1-elongation. This route – based on thiazolyl-lithium as masked formyl equivalent – was developed by Dondoni's group in solution-phase chemistry. A number of dipeptide analogs and other bioactive compounds are accessible via this versatile synthon [6]. We present their transformation to hydroxyethylene peptide isosteres via reductive amination [7] and the Horner–Emmons reaction with sulfonyl diethyl phosphonates [8], which leads to a novel core structure, γ-hydroxy-vinylsulfones [9].

Results and discussion

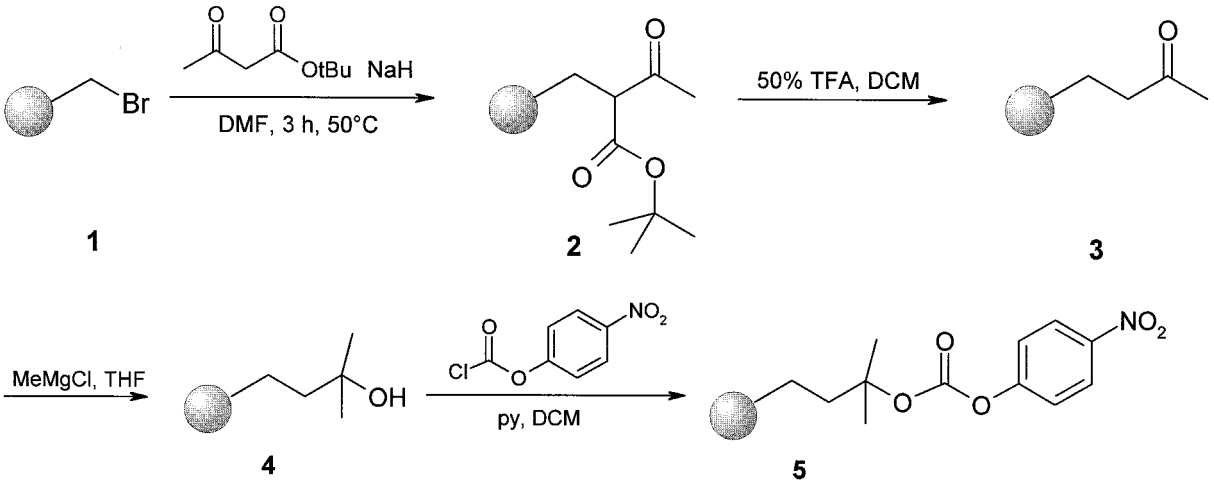
Boc-resin synthesis

The resin-bound Boc linker was synthesized via a resin-bound tertiary alcohol group followed by its conversion to an activated carbonate derivative. Earlier work included the preparation of a resin-bound (*tert*-alkoxycarbonyl) hydrazine for the synthesis of C-terminal peptide hydrazines starting from chloromethylated resin [10]. Instead of the chloromethylated Merrifield resin, the more reactive (bromo-methyl)-

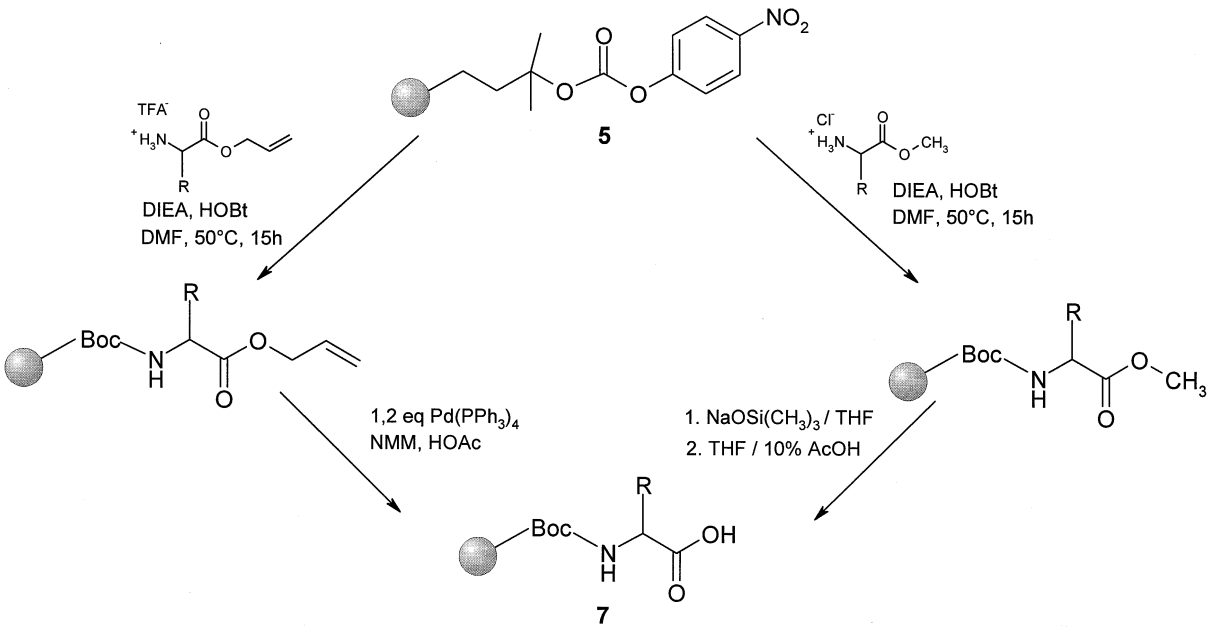
* To whom correspondence should be addressed. E-mail: guenther.jung@uni-tuebingen.de.



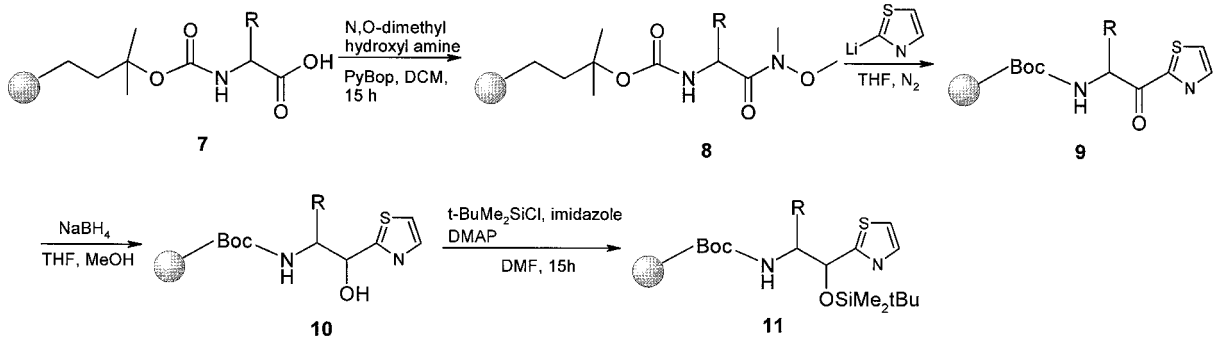
Scheme 1. 2-Hydroxy-1,3-diaminopropane and δ-amino-γ-hydroxyvinylsulfone cores.



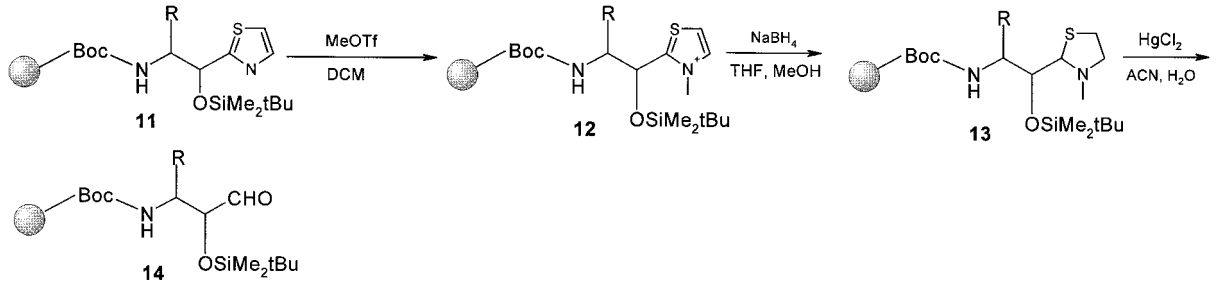
Scheme 2. Synthesis of the *tert*-alkyl-*p*-nitrophenyl-carbonate resin.



Scheme 3. Loading of the resin-bound active carbonate with amino acid esters and synthesis of Boc-resin-bound amino acids.



Scheme 4. Modification of Boc-resin-bound amino acids to protected β-1-(thiazolyl)-aminoalcohols.



Scheme 5. Dondoni degradation sequence.

PS/DVB resin **1** (resin loading 0.95 mmol/g) was substituted with *tert*-butyl acetoacetate in DMF at 50 °C for 3 h with NaH as base [11]. After ester cleavage and decarboxylation with 50% TFA/DCM, the intermediate methyl ketone **3** was reacted with 5 equiv methyl magnesium chloride for 1 h at 0 °C to give the tertiary alcohol **4**. The active *tert*-alkyl-*p*-nitrophenyl-carbonate resin **5** was prepared by reaction with *p*-nitrophenyl chloroformate, pyridine in DCM, 0 °C (Scheme 2). The *tert*-alkyl-*p*-nitrophenyl carbonate is more reactive than other carbonates (e.g. alkoxy-carbonyl imidazoles) and stable for longer storage. The reaction sequence was followed by FT-ATR-IR spectroscopy [12] and gel phase NMR spectroscopy [13].

Loading of the resin

The *tert*-alkyl-*p*-nitrophenyl-carbonate resin **5** shows only low reactivity due to its steric hindrance. Therefore several attempts to load the resin with amino acid esters failed at room temperature. But at elevated temperature (50 °C) and prolonged reaction times (15 h) the loading with amino acid esters can be carried out in a convenient way (Scheme 3). First, we investigated amino acid allyl esters, which were prepared in a fast two step synthesis from Boc-amino acids and allyl alcohol [14]. The loading proceeds nicely with 5 equiv of crude, unpurified TFA salt of amino acid allyl esters, 5 equiv DIEA, 1 equiv HOBt in DMF at 50 °C.

The conversion can be followed on bead by FT-ATR-IR spectroscopy. The carbonate absorbance at 1760 cm^{-1} disappeared while the absorbances of the carbamate linkage at 1715 cm^{-1} and of the ester group at 1735 cm^{-1} increased. The Boc-resin-bound amino acid allyl esters can be cleaved under Pd-catalysis using $Pd(PPh_3)_4$ in NMM/HOAc/$CHCl_3$ (2:1:37), N_2-atmosphere [15].

The Boc-resin can also be loaded with amino acid methyl ester hydrochlorides, in DMF at 50 °C in the presence of DIEA, HOBt. The methyl ester group was saponified with 1 M $NaOSiMe_3$ in THF [16]. The intermediate silyl ester is immediately hydrolyzed by washing the resin with THF/MeOH.

Via these two approaches polymer-tethered Boc-amino acids **7** are accessible with an average resin loading of 0.7 mmol/g. Other attempts using unprotected amino acids and N,O-bis-(trimethylsilyl)-acetamide to form an intermediate silyl ester [17] were unsuccessful and the coupling was incomplete.

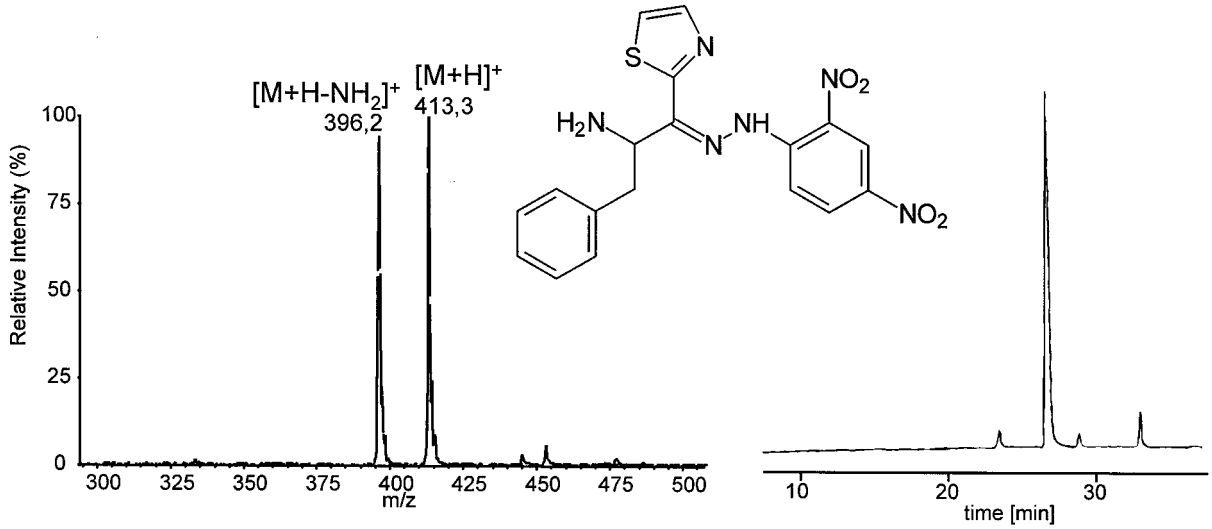


Figure 1. ES-MS and RP-HPLC chromatogram of 2,4-dinitrophenylhydrazone of 1-amino-2-phenyl ethyl-thiazolyl ketone (10–100% ACN/H_2O (1% TFA) in 45 min).

Table 1. Purities (%) of the products of the reductive amination to 2-hydroxy-1,3-diamines

Aldehyde of	Tryptamine	4-Methyl benzylamine	Piperonyl amine	Adamantyl amine
Phe	70	80	71	64
Val	69	80	60	53
Leu	64	65	64	55
Ile	65	78	70	69
Lys(Z)	63	60	62	58

C_1-chain elongation

β-Amino-α-hydroxy aldehydes are accessible in a multistep solid phase strategy starting from N-terminally polymer-tethered amino acids (Schemes 4 and 5). Following the amino ketone route of Dondoni, the carboxy group was transferred to the Weinreb amide **8** using N,O-dimethyl hydroxylamine hydrochloride (10 equiv) and PyBop (10 equiv) in DCM with DIEA. The hydroxamate reacted with thiazolyllithium (5 equiv) in THF, –30 to 0 °C, 30 min under nitrogen atmosphere to thiazolylketone **9**. The lithium organyl was freshly prepared under inert conditions [18]. The C_1-elongation was followed on bead by FT-ATR-IR spectroscopy. The Weinreb amide has a characteristic absorption at 1665 cm^{-1}, which shifts to the ketone absorption band at 1690 cm^{-1}. The ketones were also characterized as their dinitrophenylhydrazone derivatives (Figure 1).

Racemic β-1-(thiazolyl)-aminoalcohols **10** were obtained by reduction with 0.1 M $NaBH_4$ in THF/MeOH (4:1). After protection of the secondary alcohol as *tert*-butyldimethylsilylether (with TBDMS-Cl, imidazole, DMAP), the thiazol moiety in **11** could be converted to the aldehyde in a three-step demasking protocol (Scheme 5).

Finally an N-methyl thiazolium cation was generated by methylation with 5 equiv methyl triflate in DCM, at 0 °C, inert atmosphere for 2 h. The reduction to the corresponding methyl-thiazolidine was carried out with sodium borohydride at 0 °C. The final and most crucial step was the thiazolidine hydrolysis to the α-hydroxy aldehyde under heavy-metal catalysis. The best results could be obtained by using 3 equiv $HgCl_2$ in acetonitrile/water (4:1). In contrast to $CuCl_2$, $HgCl_2$ is soluble in organic solvents like acetonitrile, and compatible with the Boc-linker. The demasking reaction sequence is difficult to mon-

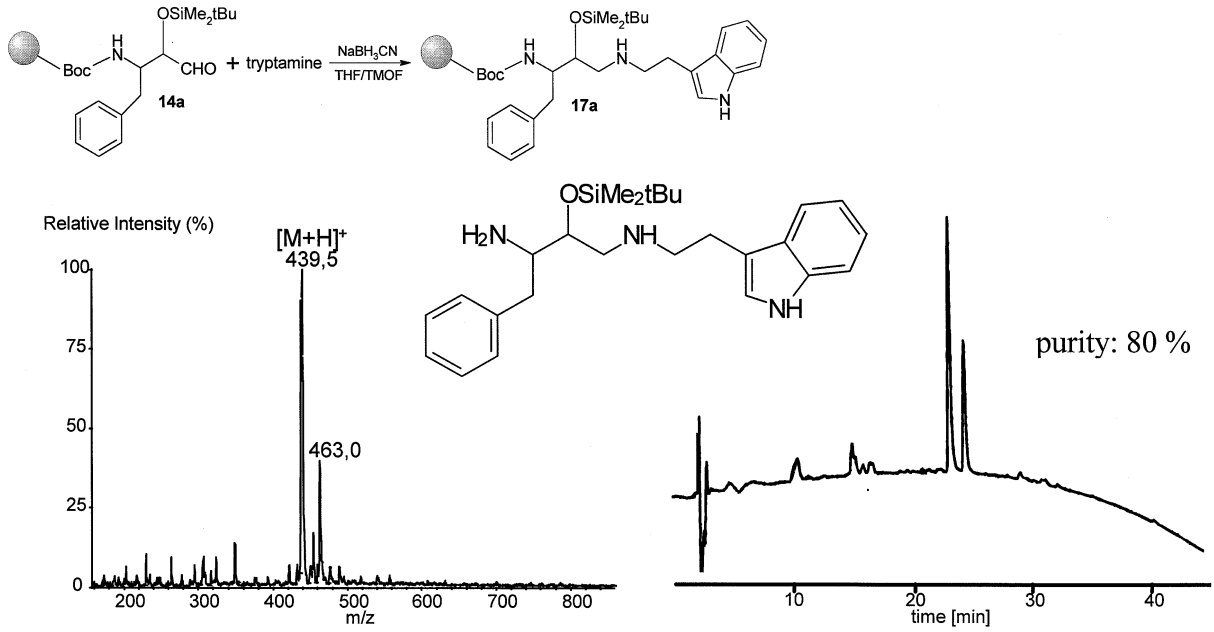
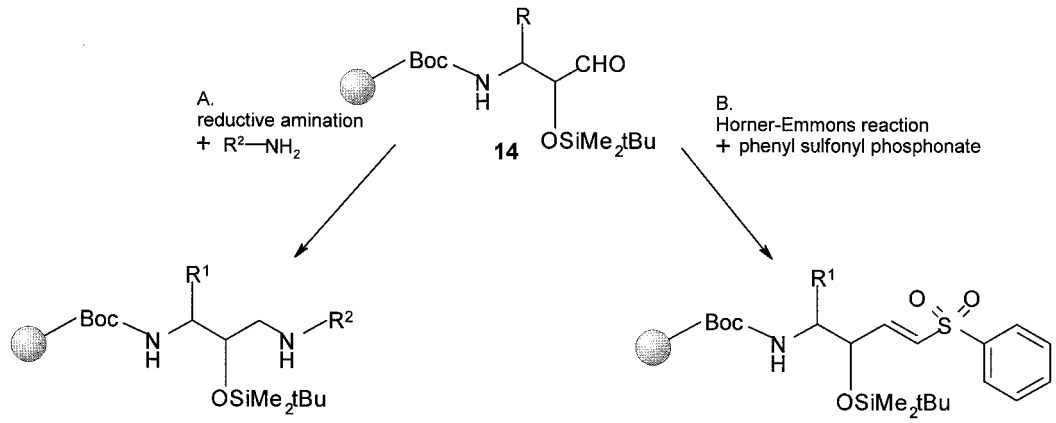


Figure 2. Reductive amination with tryptamine: MS of crude diastereomeric diamines; RP-HPLC, 10–100% ACN/H_2O (0.1% TFA) in 45 min. The sum of the two major HPLC peaks corresponds to 80%.



Scheme 6. Derivatisation of the resin-bound α-hydroxy-β-amino-aldehydes to 2-hydroxy-1,3-diaminopropanes and δ-amino-γ-hydroxy vinylsulfones.

Table 2. Analysis of the products of the Horner–Emmons reaction

Amino acid	Overall purity HPLC (218 nm, %)	Overall yield (%)	ES-MS[a] ($M + H^+$) (amu)
Phe	89	65	431/317
Val	90	66	383/269
Leu	86	68	397/283
Ile	90	63	397/283
Lys(Z)	93	64	546/432

[a] Protected/unprotected vinyl sulfone.

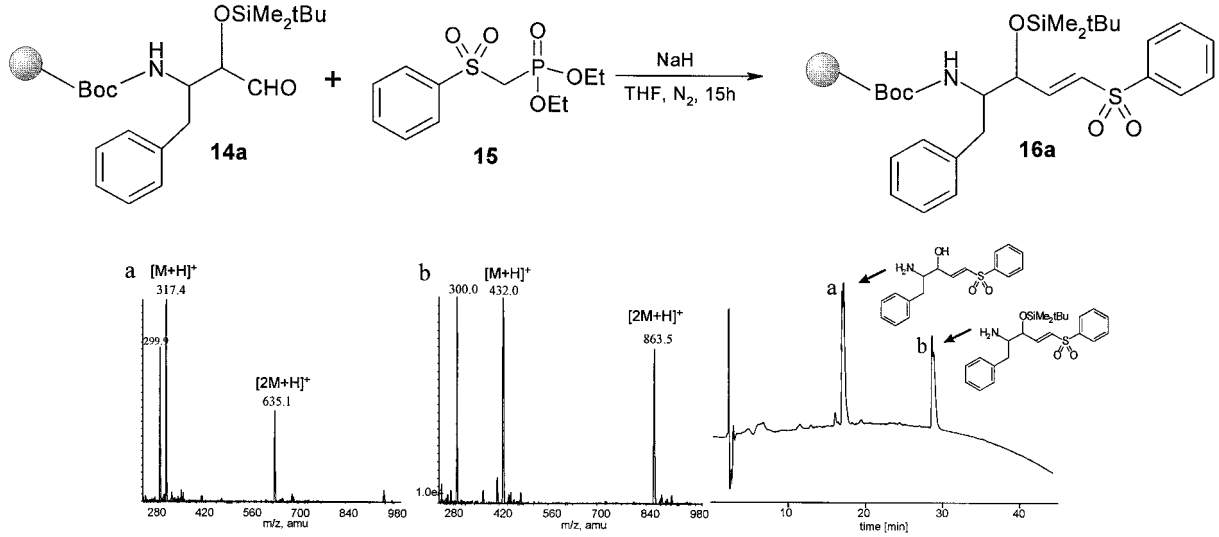


Figure 3. Horner–Emmons reaction of 3-amino-2-(*tert*-butyl-dimethylsilyl)-2-hydroxy-4-phenyl-butanal **14a** with phenyl-sulfonyl-phosphonate. LC-MS analysis of the crude product; RP-HPLC-chromatogram, 10–100% ACN/H_2O (0.1% TFA) in 45 min.

itor by off-bead analysis, since the intermediates and the final products, α-hydroxy-β-amino-aldehydes, are unstable. The successful transformation onto solid phase could be demonstrated by FT-ATR-IR on-bead analysis [12] and ^{1}H-NMR spectroscopy [19].

Synthesis of 2-hydroxy-1,3-diamines and γ-hydroxy-vinylsulfones

Finally Boc-resin bound α-hydroxy-β-amino-aldehydes were derivatized to prove the success of the C_1-chain elongation (Scheme 6) on two examples.

(A) Diamino alcohols are obtained via reductive amination with primary amines. The resin bound aldehyde was treated twice with 10 equiv amine in the presence of sodium cyano borohydride in TMOF/THF (1/1) [20]. Figure 2 shows the results of the reaction with tryptamine as an example. The purities of 4 aliphatic amines combined with 5 aldehydes are reasonable (Table 1). The overall yield from all 20 compounds is 55%. The lower average purities and yields of the final diamino alcohols is due to the reductive amination step, since the derivatisation by Horner–Emmons reaction leads to products with excellent average purities (Table 2). In contrast to published results [6c], the HPLC analysis of the diamino alcohol shows that the C_1-elongation proceeds with low stereoselectivity (Figure 2). The final products were cleaved from the solid support using DCM/TFA (1/1) for 1 h.

(B) The aldehyde function of the resin-bound α-hydroxy-β-amino-aldehyde can be converted to a γ-hydroxy-vinylsulfone in a Horner–Emmons reaction with sulfone phosphonates, which is a novel access to C-terminal modifications of α-amino acids (Figure 3).

We investigated this reaction using phenyl-sulfonyl phosphonate **15**, which was synthesized in solution. 10 equiv of **15** was added to the resin bound aldehyde in the presence of NaH in THF, 15 h at room temperature to yield **16a** (Figure 3).

Table 2 shows the analytical results after cleavage from the solid support. Two double peaks were always observed with LC-MS, which is explained by partial hydrolysis of the silyl protected hydroxy function of the diastereomeric products.

Conclusions

A new Boc resin was synthesized in a four-step reaction sequence. The linker group contains no labile functionalities like benzylethers [4a], which may limit the applicability of the Boc resin. The *p*-nitrophenyl-(*tert*-alkoxy) carbonate resin can be loaded with commercially available amino acid methyl or allyl esters, which are saponified by $NaOSiMe_3$ or $Pd(PPh_3)_4$, respectively. Thus polymer-bound N-Boc-amino acids are accessible for solid-phase synthesis by means of a convenient loading protocol.

In summary, the successful transfer of Dondoni's thiazole based α-amino acid homologation to solid-phase chemistry demonstrates the broad scope of the Boc-linker for peptidomimetic synthons. β-Amino-α-hydroxy-aldehydes have been synthesized by a solid phase protocol for the first time and were converted to peptide and amino acid analogues.

Both cores, 2-hydroxy-1,3-diamines and α-hydroxy-vinyl sulfones, are accessible in good yield and purity. The excellent purities and yields are encouraging to continue the work on this novel building block. Combinatorial synthesis of peptidomimetic libraries exploring these synthetic strategies is ongoing in our group.

Acknowledgements

The work was supported by BMBF grant 03D003707 and the DFG Graduiertenkolleg 'Chemie in Interphasen'.

References

1. Dolle, R.E., Mol. Div., 2 (1996) 223–236.
2. a. Pognan, F., Saucier, J.M., Paoletti, C., Kaczmarek, L., Nantka-Namirski, P., Nordarski, M. and Peczynska-Czoch, W., Biochem. Pharmacol., 44 (1992) 2149–2168.
   b. Thomson, L.A. and Ellmann, J.A., Chem. Rev., 96 (1996) 555–600.
   c. Früchtel, J.S. and Jung, G., Angew. Chem., 108 (1996) 19–45.
   d. Jung, G. (Ed.), *Peptide and Nonpeptide Libraries*, Wiley-VCH, New York, NY, 1996.
3. Kick, E.K. and Ellmann, J.A., J. Med. Chem., 38 (1995) 1427–1435.
4. a. Hernandez, A.S. and Hodges, J.C., J. Org. Chem., 62 (1997) 3153–3157.
   b. Léger, R., Yen, R., She, M.W., Lee, V.J. and Hecker, S.J., Tetrahedron Lett., 39 (1998) 4171–4174.
5. a. Jones, J., *The Chemical Synthesis of Peptides*, Clarendon Press, Oxford, 1991.
   b. Jurczak, J. and Golebiowski, A., Chem. Rev., 89 (1989) 149–164.
6. a. Dondoni, A., Fantin, G., Fognalolo, M. and Pedrini, P., J. Org. Chem., 55 (1990) 1439–1446.
   b. Dondoni, A., Marra, A. and Perrone, D., J. Org. Chem., 58 (1993) 275–277.
   c. Dondoni, A. and Perrone, D., Aldrichimica Acta, 30 (1997) 35–46.
7. West, M.L. and Fairlie, D.P., Trends Pharmacol. Sci., 16 (1995) 67–74.
8. Wadsworth, W.S., Org. Reactions, 25 (1977) 77–85.
9. References for vinylsulfones:
   a. Otto, H.-H. and Schirmeister, T., Chem. Rev., 97 (1997) 133–171.
   b. Palmer, J., Rasnick, D., Klaus, J. and Brömme, D., J. Med. Chem., 38 (1995) 3193–3196.
   c. Brömme, D., Klaus, J., Okamoto, K., Rasnick, D. and Palmer, J., Biochem. J., 315 (1996) 85–89.
   d. Bogyo, M., McMaster, J.S., Gactynska, M., Tortorella, D., Goldberg, A.L. and Ploegh, H., Proc. Natl. Acad. Sci. USA, 94 (1997) 6629–6634.
   e. Bogyo, M., Shin, S., McMaster, J. and Ploegh, H.L., Chem. Biol., 5 (1998) 307–320.
10. Wang, S.S., J. Org. Chem., 40 (1975) 1235–1240.
11. Tietze, L.F. and Eichler, T., *Reaktionen und Synthesen im Organisch-chemischen Praktikum*, Thieme Verlag, Stuttgart, 1981.
12. Haap, W., Redemann, T., Früchtel, J. and Jung, G., Glas, Industrie, Technik, 4 (1997) 97–103.
13. Redemann, T., Dissertation, Universität Tübingen, 1997, pp. 66–107.
14. Monforts, F.P. and Schwartz, U.M., Liebigs Ann. Chem. (1991) 709–715.
15. a. Kates, S.A., Sole, N.A., Johnson, C.R., Hudson, D., Barany, G. and Albericio, F., Tetrahedron Lett. 34 (1993) 1549–1555.
16. Laganis, E.D. and Chenard, B.L., Tetrahedron Lett., 25 (1984) 5831–5838.
17. Dressmann, B.A., Spangle, L.A. and Kaldor, S.W., Tetrahedron Lett., 37 (1996) 937–940.
18. Brauer, G., *Handbuch der Präparativen Anorganischen Chemie*, Ferdinand Enke Verlag, Stuttgart, 1995.
19. Pursch, M., Schlotterbeck, G., Tseng, L.-H., Albert, K. and Rapp, W., Angew. Chem., 35 (1996) 2867–2871.
20. Look, G.C., Murphy, M.M., Campell, D.A. and Gallop, M.A., Tetrahedron Lett., 36 (1995) 2937–2945.