Pergamon

Tetrahedron Letters, Vol. 38, No. 5, pp. 883-886, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(96)02431-8

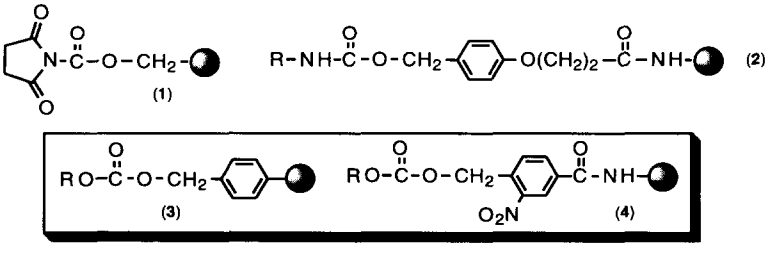
Active Carbonate Resins for Solid-Phase Synthesis Through the Anchoring of a Hydroxyl Function. Synthesis of Cyclic and Alcohol Peptides[1]

Jordi Alsina, Cristina Chiva, Marta Ortiz, Francesc Rabanal, Ernest Giralt, and Fernando Albericio*

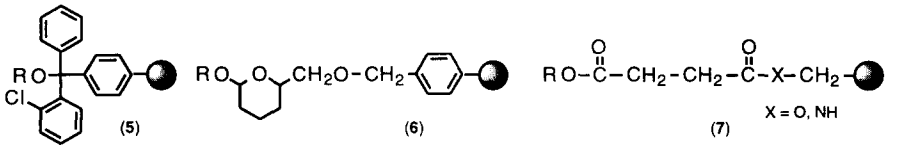
Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona, Spain.

Abstract: *N,N'*-Disuccinimidyl carbonate (DSC) has been successfully used for the efficient conversion of 4-hydroxymethylpolystyrene and 4-hydroxymethyl-3-nitrobenzamido (Nbb) resins into active carbonate resins, which are suitable for the incorporation of molecules *via* a hydroxyl function. This methodology has been applied to the preparation of the growth hormone inhibitor, Sandostatin.

The solid-phase synthesis (SPS) of both biomolecules, such as peptides[2] and oligonucleotides,[3] and small organic molecules[4] is based upon the anchoring of the first building block to the solid support through a *handle*.[5] Traditionally, the first step to SPS of linear peptides and oligonucleotides is the anchoring of a carboxylic or phosphoric acid to a hydroxyl or amino resin to give an ester or amide, which will render the biomolecule in the form of an acid or primary amide, following detachment from the resin at the end of the synthesis. The requirement for an extension to the solid-phase approach for the preparation of other classes of biomolecules, such as cyclic peptides or those which contain other functional groups at the *C*-terminus (aldehydes, secondary and tertiary amides, alcohols, etc.), and small organic molecules has provoked the development of new *handles* based upon a different method to anchor the first building block. Thus, the R(PAL) handle[6] allows the SPS of secondary amide peptides, while the BAL handle[7] provides a more general approach that involves preparation of peptides through a peptide backbone anchoring, allowing the synthesis of cyclic peptides and those with a modification at the *C*-terminal end. Furthermore, hydroxymethyl resins have been activated with DSC[8] and other carbonic acid derivatives[9] to give active carbonate resins (**1**), which allow the anchoring of amino groups (**2**) such as those from the α-amino acids, ε-amino function of Lys or Orn side-chains, monoprotected alkyldiamines, etc. These resins have been used for the SPS of peptides in the $N \rightarrow C$ terminal direction,[9a-c] of cyclic peptides through side-chain anchoring of Lys/Orn,[8] and of libraries of small molecules.[9d-g] In this comunication, an extension of the active carbonate resin (**1**) for the anchoring of hydroxyl functions of alcohols or phenols is reported (resins **3** and **4**).



To the best of our knowledge, hydroxyl functions have been only anchored to the solid supports through: (i) chlorotrityl (ClTrt)-linkers (**5**),[10] which are labile to dilute acid conditions (< 1% TFA) and therefore are compatible with bases and nucleophiles, but not with acids; (ii) tetrahydropyranyl (THP)-based linkers (**6**),[11] which are labile to acid conditions [PPTS at 60 °C or TFA–H_2O (95:5)] and therefore exhibit a similar range of compatibilities to ClTrt-resins; and (iii) hemi-succinates-linkers (**7**),[12] which are cleavable by base hydrolysis and, in principle, are compatible with Fmoc and Boc chemistries, but require a two step cleavage-deprotection protocol. Furthermore, phenol groups have also been attached to hydroxymethyl-*p*-alkoxybenzyl-resins through a Mitsunobu reaction.[13] The reaction of DSC[14] with 4-hydroxymethyl-PS-resin[15] and 4-hydroxymethyl-Nbb-PS-resin[16] afford the corresponding active carbonates, which can react smoothly with compounds containing hydroxyl functions (Boc-*O*-benzyl-threoninol [Boc-Thr(Bzl)-oh], Boc-Ser-OAllyl, and Boc-Tyr-OAllyl)[17] to afford the corresponding carbonates (**3** and **4**). Interestingly, the same sequence of reactions carried out with 4-hydroxymethylphenylacetamido-resin (PAM-resin)[18] did not form the corresponding active carbonate (1-10%). This result corroborates that side-chain anchoring of Boc-Asp(OH)-OFm to the 4-hydroxy-PAM-resin occurs with very low yields (<5%) using DIPCDI-DMAP, CDI, and DEAD-PPh_3 based methods.[19] Furthermore, the carbonate resin similar to **3** or **4**, but obtained from 4-hydroxymethylphenoxypropanamide-resin (PAC-resin)[20] is not completely stable to piperidine-DMF (1:4) (5% cleavage of alcohol and 35% of phenol, for 3x1 min + 1x10 min of treatment) and therefore is not suitable for use in Fmoc-based SPS.



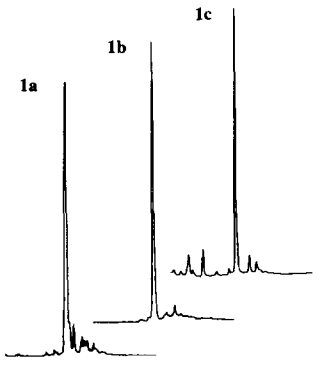
(5) (6) (7)

Active carbonate resins were prepared as described previously[8] by reaction of the corresponding hydroxymethyl resins with 10 equiv of DSC in DMF in the presence of 1 equiv of DMAP for 2 h at 25 °C under Ar atmosphere. Incorporation of the hydroxyl compound was carried out with 10 equiv of Boc-Thr(Bzl)-oh, Boc-Ser-OAllyl, and Boc-Tyr-OAllyl in the presence of 0.2 equiv of DMAP in DMF for 17 h at 25 °C under Ar atmosphere. Resins were washed with DMF and CH_3OH, and treated with CH_3OH in the presence of 0.2 equiv of DMAP (30 min) to methylate any unreacted succinimidyl carbonate. Yields were 65-70% for Boc-Tyr-OAllyl and 45-60% for Boc-Thr(Bzl)-oh and Boc-Ser-OAllyl as calculated by amino acid analysis.[21] The use of either a smaller amount of DMAP (0.1 equiv) or a shorter reaction time rendered lower yields.

The stability of these resins in the presence of base was examined by treatment of a peptidyl support with DIEA–CH_2Cl_2 (1:19) for 60 min (corresponding to 20 cycles of neutralization after TFA treatment for Boc removal) at 25 °C. No release of the peptide was detected during the base wash. Cleavage and concomitant removal of Bzl side-chain protecting groups from resin **3** was carried out by either HF–anisole (9:1) for 1 h at 0 °C or TFMSA–TFA–thioanisole–ethanedithiol–anisole (5:85:5:3:2) for 2 h at 25 °C with yields >95%. Release of protected peptides from resin **4** was conducted by photolysis at 350 nm in TFE–CH_2Cl_2 (2:8) for 16 h, as described previously for Nbb-resins.[16]

As models to illustrate this strategy, *cyclo*(Leu-Phe-Gly-Gly-Tyr), *cyclo*(Leu-Phe-Gly-Gly-Ser), *cyclo*(Glu-Ala-Ala-Arg-D-Phe-Pro-Glu-Asp-Asn-Ser), *cyclo*(Glu-Ala-Ala-Arg-D-Phe-Pro-Glu-Asp-Asn-Tyr) and the disulfide containing peptide Sandostatin [*cyclo*(H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-oh) were synthesized. The first two sequences were assembled on an active carbonate-O-Nbb-PS-resin, and the others on an active carbonate-O-PS-resin. For the preparation of cyclic peptides, the first protected amino derivatives

(Boc-Ser-OAllyl or Boc-Tyr-OAllyl) were incorporated onto the resins *via* the hydroxyl side-chain according to the method described above, followed by manual stepwise synthesis in the $C \rightarrow N$ direction according to a standard Boc/Bzl protocol.[22] After elongation of the peptide chain, a sample of the peptidyl resin was treated with HF–anisole (for **3**) or photolyzed (for **4**), and the purity by HPLC was >95% in each case. Allyl removal was carried out with $Pd(PPh_3)_4$ in DMSO–THF–0.5 N aqueous HCl–morpholine (2:2:1:0.1),[23] for 150 min at 25 °C. Following N^{α}-Boc removal, PyAOP/HOAt/DIEA (5:5:10)-mediated cyclization[24] was carried out in DMF for 2h (the ninhydrin test was negative) at 25 °C. After final cleavage of the anchoring linkage and removal of the side-chain protecting groups, if applicable, the crude product was purified by semi-preparative HPLC to give the correct ES-MS.



Synthesis of Sandostatin was carried out as described above using Acm, Bzl, ClZ, and For for side-chain protection of Cys, Thr, Lys, and D-Trp, respectively. After elongation of the peptide chain, an aliquot of the peptide resin was treated with HF-anisole (9:1) and the purity of the crude bis-Acm-For-peptide was analyzed by HPLC (Fig. 1a). Formation of the disulfide bridge was carried out on the solid-phase[25] in HOAc–H_2O (8:2) by the addition of iodine (10 equiv/Acm) for 40 min at 25 °C. The peptide resin was washed with DMF, saturated aqueous ascorbic acid solution, DMF, CH_2Cl_2, treated with TFA–CH_2Cl_2 (4:6) (1x1 min + 1x20 min), and washed with CH_2Cl_2. The release of the cyclic peptide was carried out with HF-anisole (9:1) (Fig. 1b). Finally, removal of the For group of Trp was accomplished by 10 min treatment with piperidine–DMF (1:1). The reaction was quenched with HOAc and the solution was evaporated to dryness (Fig. 1c). All products were characterized by either ES- or FAB-MS and amino acid analysis.

In conclusion, carbonate resins are compatible with Boc/Bzl strategy and are cleaved either by HF or photolysis. The supports are suitable for the anchoring of building blocks through a hydroxyl function, allowing the synthesis of "head-to-tail" cyclic peptides and other alcohol peptides, such as the relevant pharmacologically interesting compound Sandostatin. Finally, this new methodology can be useful for the preparation of small molecular libraries.[26]

Acknowledgments: This work was partially supported by CICYT (PB95-1131) and Generalitat de Catalunya [Grup Consolidat (1995SGR 494) i Centre de Referència en Biotecnologia]. J.A. is a recipient of a pre-doctoral fellowship (Ministerio de Educación y Ciencia, Spain). The comments of Dr. Steven A. Kates (PerSeptive Biosystems, USA) are gratefully appreciated.

References and Notes

1. Abbreviations not defined in the text: Acm, acetamidomethyl; Boc, *tert.*-butyloxycarbonyl; BAL, backbone amide linker; Bzl, benzyl; CDI, carbonyl imidazole; CI-MS, chemical ionization mass spectrometry; ClZ, chlorobenzyloxycarbonyl; DIEA, *N,N*-diisopropylethylamine; DEAD, diethyl azodicarboxylate; DIPCDI, *N,N'*-diisopropylcarbodiimide; DMAP, *N,N*-dimethyl-4-aminopyridine; DMF, *N,N*-dimethylformamide; DMSO, dimethylsulfoxide; ES-MS, electrospray mass spectrometry; Fmoc, 9-fluorenylmethyloxycarbonyl; For, formyl; HOAc, acetic acid; HOAt, 7-aza-1-hydroxybenzotriazole; HOBt, 1-hydroxybenzotriazole; HPLC, high performance liquid chromatography; PPTS, pyridinium *p*-toluensulfonate; PS, polystyrene; PyAOP, 7-aza-benzotriazol-1-yl-oxy-tris(pyrrolidino)-phosphonium hexafluorophosphate; R(PAL), tris(alkoxybenzyl) *N*-alkylamide; Su, succinimidyl; *t*Bu, *tert.*-butyl; TFA, trifluoroacetic acid; TFMSA, trifluoromethanesulfonic acid; THF, tetrahydrofuran; Thr-oh, threoninol. Amino acid symbols denote L-configuration unless indicated otherwise.
2. Lloyd-Williams, P.; Albericio, F.; Giralt, E. In *Chemical Approaches to the Synthesis of Peptides and Proteins*; CRC: Boca Raton, FL, 1997.
3. *Oligonucleotides and Analogues. A Practical Approach* (Eckstein, F., ed.) IRL Press: Oxford, 1991.
4. Hermkens, P.H.H.; Ottenheijm, H.C.J.; Rees, D. *Tetrahedron* **1996**, *52*, 4527-4554.

5. *Handles* are defined as bifunctional spacers, or linkers, which incorporate on one end features of a selectively removable protecting group and contain a second end which serves to achieve the required anchoring to the solid support as a separate chemical step. Barany, G.; Albericio, F. In *Peptides. Chemistry, Structure and Biology. Proceedings of the Thirteenth American Peptide Symposium* (Hodges, R.S.; Smith, J.A., eds.) ESCOM: Leiden, The Netherlands 1994; pp 1078-1079.
6. Songster, M. F.; Vágner, J.; Barany, G. *Lett. Pept. Sci.* **1995**, *2*, 265-270.
7. Jensen, K.J.; Songster, M.F.; Vágner, J.; Alsina, J.; Albericio, F.; Barany, G. In *Peptides: Chemistry, Structure and Biology, Proceedings of the Fourteenth American Peptide Symposium* (Kaumaya, P.T.P.; Hodges, R.S., eds.) Mayflower Scientific Ltd.: England, 1996; pp. 30-32.
8. Alsina, J.; Rabanal, F.; Giralt, E.; Albericio, F. *Tetrahedron Lett.* **1994**, *35*, 9633-9636.
9. (a) Letsinger, R.L.; Kornet, M.J. *J. Am. Chem. Soc.* **1963**, *85*, 3045-3046; (b) Felix, A.M.; Merrifield, R.B. *J. Am. Chem. Soc.* **1970**, *92*, 1385-1391. (c) Matsueda, R.; Maruyama, H.; Kitazawa, E.; Takahagi, H.; Mukaiyama, T. *Bull. Chem. Soc. Japan* **1973**, *46*, 3240-3247; and more recently (d) Hauske, J.R.; Dorff, P. *Tetrahedron Lett.* **1995**, *36*, 1589-1592; (e) Kaljuste, K.; Undén, A. *Tetrahedron Lett.* **1995**, *36*, 9211-9214; (f) Dressman, B.A.; Spangle, L.A.; Kaldor, S.W. *Tetrahedron Lett.* **1996**, *37*, 937-940; (g) Kaljuste, K.; Undén, A. *Tetrahedron Lett.* **1996**, *37*, 3031-3034; Marsh, I.R.; Smith, H.; Bradley, M. *Chem. Commun.* pp. 941-942; (h) Aviño, A.; Güimil-Garcia, R.; Albericio, F.; Mann, M.; Wilm, M.; Neubauer, G.; Eritja, R. *Bioorg. Med. Chem.* **1996**, in press.
10. (a) Barlos, K.; Chatzi, O.; Gatos, D.; Stavropoulos, G. *Int. J. Peptide Protein Res.* **1991**, *37*, 513-520; (b) Wenschuh, H.; Beyermann, M.; Haber, H.; Seydel, J. K.; Krause, E.; Bienert, M.; Carpino, L. A.; El-Faham, A.; Albericio, F. *J. Org. Chem.* **1995**, *60*, 405-410.
11. Thompson, L.A.; Ellman, J.A. *Tetrahedron Lett.* **1994**, *35*, 9333-9336.
12. (a) Swistok, J.; Tilley, J.W.; Danho, W.; Wagner, R.; Mulkerins, K. *Tetrahedron Lett.* **1989**, *30*, 5045-5048; (b) Neugebauer, W.; Escher, E. *Hel. Chim. Acta* **1989**, *72*, 1319-1323; (c) Romanovskis, P.; Spatola, A.F. In *Peptides 1996. Proccedings of the Twenty Fourth European Peptide Symposium* (Epton, R. ed.) Mayflower Scientific Ltd.: England, 1997, in press.
13. Richter, L.S.; Gadek, T.R. *Tetrahedron Lett.* **1994**, *35*, 4705-4706.
14. Ogura, H.; Kobayashi, T.; Shimizu, K.; Kawabe, K.; Takeda, K. *Tetrahedron Lett.* **1979**, *49*, 4745-4746.
15. 4-Hydroxymethyl-PS-resin was obtained from Advanced ChemTech (Louisville, KY); nominal loading: 1.35 mmol/g.
16. 4-Hydroxymethyl-Nbb-PS-resin was prepared from 4-hydroxymethyl-3-nitrobenzoic acid [(a) Giralt, E.; Albericio, F.; Pedroso, E.; Granier, C.; van Rietschoten, J. *Tetrahedron* **1982**, *38*, 1193-1208; (b) Barany, G.; Albericio, F. *J. Am. Chem. Soc.* **1985**, *107*, 4936-4942; (c) Lloyd-Williams, P.; Gairí, M.; Albericio, F.; Giralt, E. *Tetrahedron* **1991**, *47*, 9867-9880] and MBHA-resin, and the loading was 0.56 mmol/g.
17. Boc-Thr(Bzl)-oh was obtained from Advanced ChemTech (Louisville, KY). Boc-Ser-OAllyl and Boc-Tyr-OAllyl were obtaining by reacting commercially available Boc-Ser/Tyr-OH (1 equiv) with allyl bromide (25 equiv) in the presence of DIEA (1 equiv) for 21 h at 25 °C. The reaction mixture was diluted with EtOAc, extracted with 10% Na_2CO_3 and brine solution, followed by drying over $MgSO_4$, and concentration *in vacuo*. Boc-Ser-OAllyl (95% yield) obtained as an oil and Boc-Tyr-OAllyl (92% yield) obtained as a solid gave a single peak in HPLC and were characterized by CI-MS.
18. Mitchell, A. R.; Erickson, B. W.; Ryabtsev, M. N.; Hodges, R. S.; Merrifield, R. B. *J. Am. Chem. Soc.* **1976**, *98*, 7357-7362.
19. Valero, M-L., Giralt, E.; Andreu, D. *Tetrahedron Lett.* **1996**, *37*, 4229-4232.
20. Albericio, F.; Barany, G. *Int. J. Peptide Protein Res.* **1985**, *26*, 92-97.
21. Amino acid hydrolysis was carried out after the incorporation of a second protected amino acid in the sequence, since Tyr and Ser can be destroyed during the acid hydrolysis, and Thr-oh was not detected in the amino acid autoanalyzer.
22. Glu and Asp side-chains were protected with cHex, Tos was used for Arg, and Asn was used without side-chain protection. A standard protocol was as follows: Boc group removal with TFA–CH_2Cl_2 (1:2) (1x1+1x20 min), CH_2Cl_2 (5x30 sec), DIEA–CH_2Cl_2 (1:19) (3x1 min), CH_2Cl_2 (5x30 sec), amino acid coupling (30 min) with Boc-amino acids (5 equiv), HOBt (5 equiv) and DIPCDI (5 equiv) in DMF, and DMF washing (5x30 sec).
23. (a) Lloyd-Williams, P.; Jou, G.; Albericio, F.; Giralt. E. *Tetrahedron Lett.* **1991**, *32*, 4207-4210. (b) Kates, S.A.; Daniels, S.B.; Albericio, F. *Anal. Biochem.* **1993**, *212*, 303-310.
24. (a) Carpino, L.A. *J. Am. Chem. Soc.* **1993**, *115*, 4397-4398; (b) Carpino, L.A.; El-Faham, A.; Minor, C.A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, pp. 201-203.
25. (a) García-Echeverría, C.; Albericio, F.; Pons, M.; Barany, G.; Giralt, E. *Tetrahedron Lett.* **1989**, *30*, 2441-2444; (b) Albericio, F.; Hammer, R. P.; García-Echeverría, C.; Molins, M. A.; Chang, J. L.; Munson, M.; Pons, M.; Giralt, E.; Barany, G. *Int. J. Peptide Protein Res.* **1991**, *37*, 402-413.
26. Ellman, J. A. *Acc. Chem. Res.* **1996**, *29*, 132-143.

(*Received in UK* 13 *November* 1996; *revised* 6 *December* 1996; *accepted* 13 *December* 1996)