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USE OF AN ALLYLIC ANCHOR GROUP AND OF ITS PALLADIUM CATALYZED HYDROSTANNOLYTIC CLEAVAGE IN THE SOLID PHASE SYNTHESIS OF PROTECTED PEPTIDE FRAGMENTS

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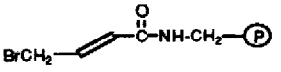
Abstract : The allylic handle $-O-CH_2-CH=CH-CH_2-O-CH_2-CO-$ has been used in the synthesis of protected peptide fragments on aminomethyl polystyrene. The palladium-catalyzed hydrostannolytic cleavage of the peptide fragments from the resin occurs under very mild conditions.

In the past recent years, we and others have emphasized the potentialities of the allyl and allyloxycarbonyl groups, which can be removed under very specific and mild conditions using homogeneous palladium catalysis, for the protection of carboxylic acids, phenols, alcohols and amines[1]. In particular, the allyloxycarbonyl group has been used successfully for the temporary protection of the α-amino group of amino acids during peptide synthesis, either in liquid phase[2] or on support[1].

By extension, it could be expected that the allyl methodology would soon find application in the design of new linking agents for use in solid phase peptide synthesis. Indeed, Kunz[3] recently described the synthesis of several oligopeptides on aminomethyl resin functionalized with the 4-bromocrotonyl handle **1**.



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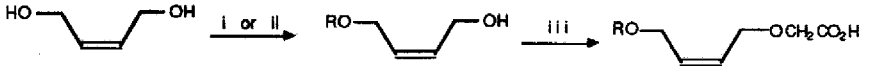
A more recent report[4] relative to peptide synthesis on allyl functionalized cellulose disks prompts us to disclose our own results.

Like Blankemeyer-Menge and Frank[4] we started with the inexpensive cis 2-butene-1,4-diol **2** for the elaboration of the allylic handle. **2** was sequentially converted to the monotrityl derivative **3**-a or mono-THP derivative **3**-b and alkylated with bromoacetic acid, according to eq. 1, to give **4**-a, **4**-b.



2 **3-a,b** **4-a,b** [1]

a : R = Trt ; b : R = THP

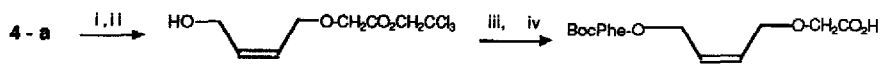
i) TrtCl (0.2 equiv.), Et_3N (0.4 equiv.), DAMP (0.01 equiv.)[5]

ii) DHP (0.75 equiv.), TsOH (0.05 equiv.), CH_2Cl_2 r.t., 2h

iii) NaH (1.1 equiv.), $BrCH_2CO_2Na$ (0.9 equiv., from $BrCH_2CO_2H$ and NaH, THF, r.t. 2h then 60°C 4h

With 4-a and 4-b in hand, three methods were tested. The first one involved prior attachment of the first aminoacid (AA) to the handle and subsequent anchorage of the AA-handle fragment to the resin. Thus 4-a was converted to the Boc-Phe derivative **5** by the sequence of reactions of scheme I, which utilizes the trichloroethyl group for temporary protection of the handle carboxylic function.

Scheme I



5

i) CCl_3CH_2OH, DCC, DMAP, CH_2Cl_2 ; ii) dilute HCl ;
iii) BocPheOH, DCC, DMAP, CH_2Cl_2 ; iv)[8] $Zn/THF/NH_4^+AcO^-/H_2O$, pH 7.2, 45 min., r.t.

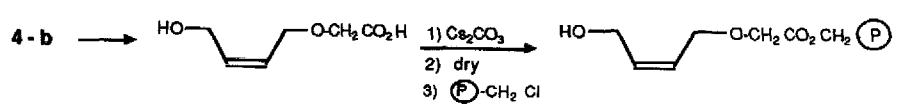
The Boc-Phe-handle derivative **5** was anchored on aminomethyl resin (DCC, HOBT, CH_2Cl_2). After capping (Ac_2O, Et_3N)[7] the sequence Boc-Val-Gly-Phe-support was built using the standard Boc-strategy (Boc-cleavage by CF_3CO_2H/CH_2Cl_2 and coupling with Boc-AA by DCC-HOBT)[7]. Palladium-catalyzed hydrostannolytic cleavage[8] gave the N-Boc, O-tributylstannyl peptide derivative **6** fully characterized by NMR. The tripeptide **7** was finally obtained in 90% overall yield (based on aminomethyl substitution of the resin), by acidic hydrolysis of **6** under mild conditions[9]. Semi-quantitative tlc analysis confirmed a minimum purity of > 95%. The HPLC profile of peptide **7** is reproduced in fig. 1.

Boc-Val-Gly-PheO-R (**6** : R = $SnBu_3$; **7** : R = H)

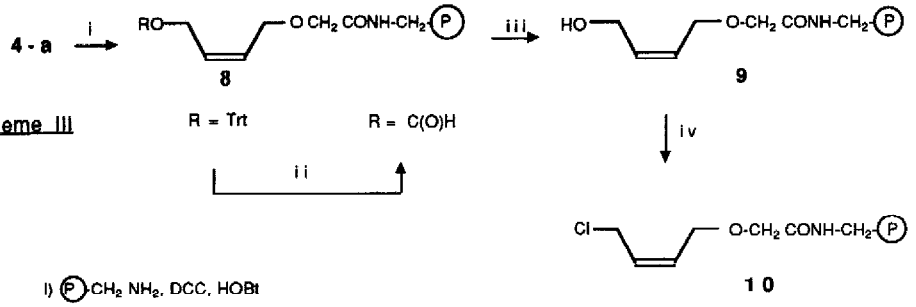
6,7

In the second method, the THP group of 4-b was removed by acid hydrolysis and the resulting hydroxyacid directly condensed on chloromethyl resin by the Gisin method[7,10] (scheme II).

Scheme II



In the third method, the trityl derivative **4**-a was condensed on aminomethyl resin. The hydroxy protecting group was then liberated by treatment with formic acid followed by cleavage of the resulting formate ester with hydrazine hydrate (scheme III, i-iii).



Scheme III

i) P-CH_2 NH_2, DCC, HOBt
ii) HCO_2H/CH_2Cl_2, (3/2), 3h, r.t.
iii) NH_2 NH_2,H_2O / DMF
iv) TsCl, DMAP, CH_2Cl_2, 16h, r.t.

Attachment of the first amino-acid to **9** by standard DCC/DMAP method proved to be difficult, possibly owing to steric hindrance induced by the cis geometry of the butene-diol unit. Therefore, the hydroxy group was converted to chloride by action of TsCl/DMAP in CH_2Cl_2[11]. Volhard analysis gave a chloride content of 0,43 meq/g resin (80% functionalization based on starting amino substitution), which could not be improved by repeating the reaction.

Capping of the residual active sites was carried out with PhCOCl/EtN(iPr)$_2$[12] Boc-Tyr(Dcb) was attached to the handle **10** by the Gisin method[7,10]. The assembly of the model peptide **11** was performed by the standard Boc-methodology. **11** was finally obtained after palladium-catalyzed hydrostannolytic cleavage from the resin followed by conversion of the resulting tin carboxylate to carboxylic acid[13]. The HPLC profile of the crude peptide **11** is represented on fig. 2.

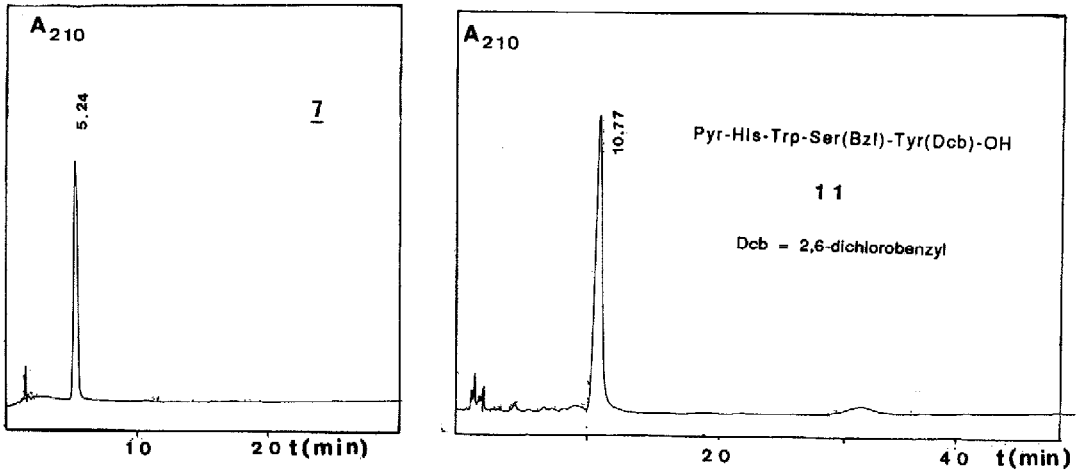


Fig. 1, 2 : HPLC profiles of crude peptides **7** and **11**. Orpegen column C18, 5 µ, 80 A. Isocratic mode 40% CH_3CN in triethylammonium phosphate buffer. pH : 3.0 ; flow rate : 1.2 mL/min.

Notes and References

(1) Dangles,O. ; Guibé,F. ; Balavoine,G. ; Lavielle,S. ; Marquet,A. J.Org.Chem., 1987, 52, 4984 and references therein.

(2) Kunz,H. ; Waldmann,H. Ang.Chem.Int.Ed.Engl., 1984, 23, 436 ; Kunz,H. ibid, 1987, 26, 294.

(3) Kunz,H. ; Dombo,R. Ang.Chem.Int.Ed.Engl., 1988, 100, 711.

(4) Blankemeyer-Menge,B ; Frank,R. Tetrahedron Lett., 1988, 29, 5871.

(5) In this reaction, the inexpensive 2-butene-1,4-diol is used in large excess over the tritylating agent to ensure total conversion of trityl chloride and exclusive formation of the monotrityl derivative of the diol. In this way, **2** being very soluble in water, **3**-a is obtained in a very good state of purity by simple extractive work-up.

(6) Just,G. ; Grozinger,K. Synthesis, 1976, 457.

(7) Stewart,J.M. ; Young,J.D. Solid Phase Peptide Synthesis, 2nd edition, Pierce Chemical Company, Rockford (Ill.), 1984.

(8) To a suspension of 1.2 g of resin in CH_2Cl_2 (30 mL) containing 15 mg (0.02 mmol,ca 0.04 equiv. based on "allyl" substitution of the resin) of $PdCl_2(PPh_3)_2$, Bu_3SnH (450 mg, 1.5 mmol, 3 equiv.) was slowly added, under argon atmosphere and with magnetic stirring over a period of 5 min. Stirring was pursued for 20 min at room temperature. The resin was filtered and washed twice with CH_2Cl_2.

(9) **6** was converted to **7** by shaking in a two-phase system AcOEt/HCl 1N for 5 min. **7** was then freed from tributyltin by-products by repeated dissolution in AcOEt and precipitation with Et_2O. Yield : 80%.

(10) Gisin,B.F. Helv.Chim.Acta, 1973, 56, 1476.

(11) Our initial purpose was to introduce the p-toluenesulfonyl group on the handle but when the free hydroxy group is reacted with TsCl a double sulfonylation-SN_2 displacement reaction takes place :

$$ROH + TsCl \xrightarrow{B} [RO\text{-}Ts + BH^+Cl^-] \longrightarrow RCl + TsO^-BH^+.$$

No sulfonylester group could be detected by infra-red analysis of the resin.

(12) Use of Ac_2O/Et_3N for capping resulted in some quaternarization (ca 20% according to Volhard titration) of Et_3N by the chloroallyl handle.

(13) The tributyltin carboxylate was converted to the free acid by dissolving in DMF/HCl 1N 1/1 (v/v). After 5 min, the aqueous DMF solution was neutralized with 1N aqueous sodium hydroxide and concentrated. Sodium chloride was precipitated by addition of diethyl ether. The filtrate was evaporated in vacuo and the oily residue triturated in AcOEt to yield the crude peptide as an off-white powder. Final purification was achieved by column chromatography on silica-gel (butanol/acetic acid/water : 10/1/2.5 as the eluent). Yield 70% (based on allyl substitution of the resin). Positive FAB mass spectrometry : MH^+ m/e 951(^{35}Cl + ^{35}Cl), 953(^{35}Cl + ^{37}Cl).

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