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Synthesis of Peptides Using Palladium Promoted Selective Removal of Allyloxycarbonyl Protecting Groups in Aqueous Medium

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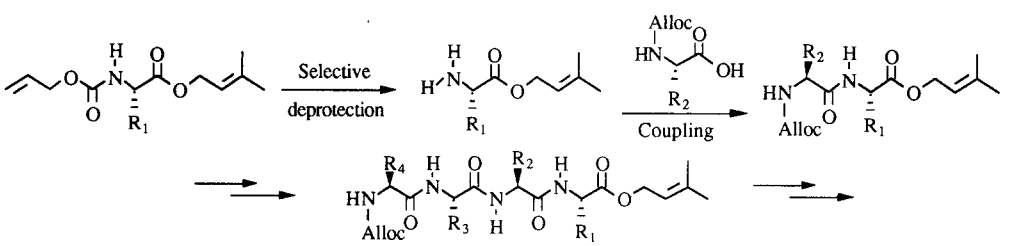
Abstract : Peptides can be easily synthesized according to a stepwise strategy in solution without any purification. The selective removal of allyloxycarbonyl protecting groups catalyzed by a water-soluble palladium catalyst constitutes the key step of the peptide chain elongation.

The assembly of peptides using conventionnal methods of organic chemistry is seriously limitated by purification, analytical and characterization steps for each intermediate of the peptide chain. In the early 1960's Merrifield[2] proposed a new strategy of synthesis on a solid support which proved to be very efficient for the rapid preparation of medium-sized peptides. Nevertheless, the final product is sometimes contaminated by impurities that can't be eliminated, resulting in a poor homogeneity of the peptide. Some of these problems have been circumvented by using continuous solution techniques, which may be used with Boc and Fmoc strategies[3]. Rhône-Poulenc introduced a powerful technology for the automated large scale synthesis of peptides in solution phase avoiding purification operations, known as the SAPPHO technology[4]. Having recently developed an efficient methodology for the chemoselective deprotection of N-allyloxycarbonyl-O-dimethylallyl-α-aminoesters using the water-soluble palladium catalyst generated *in situ* from $Pd(OAc)_2$ and TPPTS, in the presence of diethylamine as allyl acceptor[5]. We apply these conditions for the synthesis of peptides.(Scheme 1)



Scheme 1

As both the zerovalent palladium catalytic system and the volatile N-allyldiethylamine by-product are easily removed by simple aqueous work-up and evaporation, affording very clean crude deprotected products, we aimed at assembling the peptide by repetition of the key sequence : selective cleavage of the terminal allylcarbamate / peptide coupling, without any intermediate purification. In this paper we wish to report some applications of this

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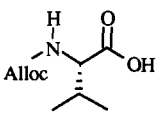
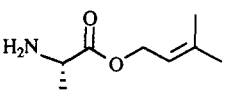
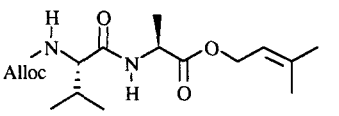
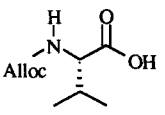
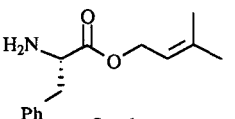
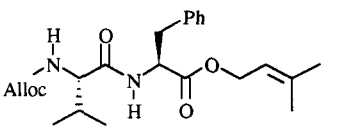
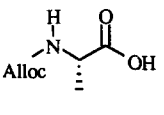
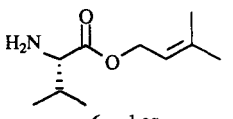
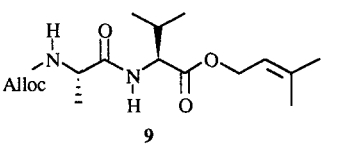
methodology in the preparation of tri and tetrapeptides.

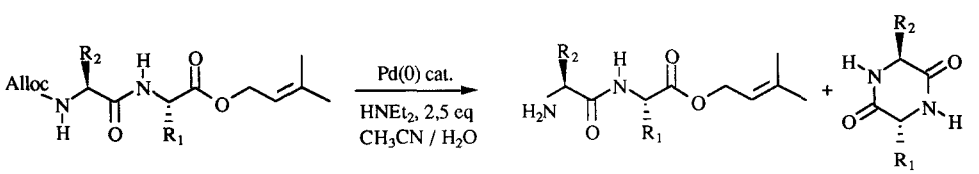
We first studied the formation of dipeptides in order to find the best conditions for the coupling step and different reagents were compared (Table 1). Using the well-known DCC / HOBT[6] system, dimethylallyl N-(allyloxycarbonyl)-L-valyl-L-alalinate **7** was obtained in 71% yield (entry 1). Nevertheless, as the N,N-dicyclohexylurea by-product could not be separated from the product by simple filtration, purification by flash chromatography was required. When the reaction was carried out in the presence of either TBTU[7] or the cyclic propylphosphonic anhydride PPA[8], it was possible to eliminate the by-products by either acidobasic or simple aqueous treatments, to provide very clean crude dipeptides **8** and **9** (entries 2 and 3).

Entry	N-protected aminoacid	O-protected aminoacid	Coupling agent	Dipeptide	Yld (%)
1	 **1** 1 eq	 **4** 1.05 eq	HOBT 1.5 eq DCC 1.5 eq	 **7**	71
2	 **1** 1 eq	 **5** 1 eq	(a) TBTU 1 eq (b) PPA 1.3 eq	 **8**	77 82
3	 **2** 1 eq	 **6** 1 eq	(a) TBTU 1 eq (b) PPA 1.3 eq	 **9**	66 68

Conditions : $(iPr)_2NEt$ 2.1 to 3 eq; DMF; 0°C -->RT.

Table 1

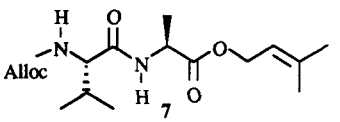
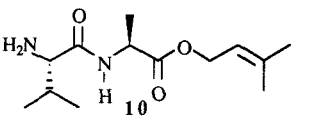
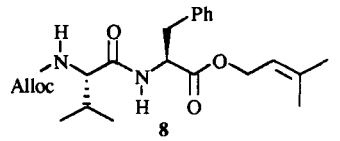
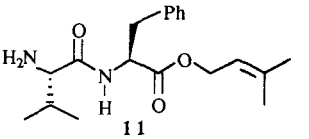
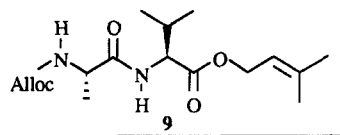
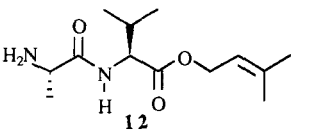
According to our strategy, the following step consisted in the selective deprotection of the terminal amine moiety of the dipeptides. This reaction must be carefully controlled as the deprotected amino group can react with the terminal ester to produce diketopiperazine derivatives[9] (Scheme 2).



Scheme 2

In the present work, we were pleased to find that, in the presence of molar 0.5% of the water soluble palladium catalyst and 2.5 equivalents of diethylamine, the terminal allylcarbamate was selectively removed without any formation of the undesired cyclized by-product (Table 2). Within 20 to 30 minutes, all the dipeptides were deprotected with good to quantitative yields.

After completion, simple aqueous work-up allowed elimination of the catalytic system to give very clean crude products. As a result, the key sequence, peptide coupling / selective deprotection, could be realized without purification.

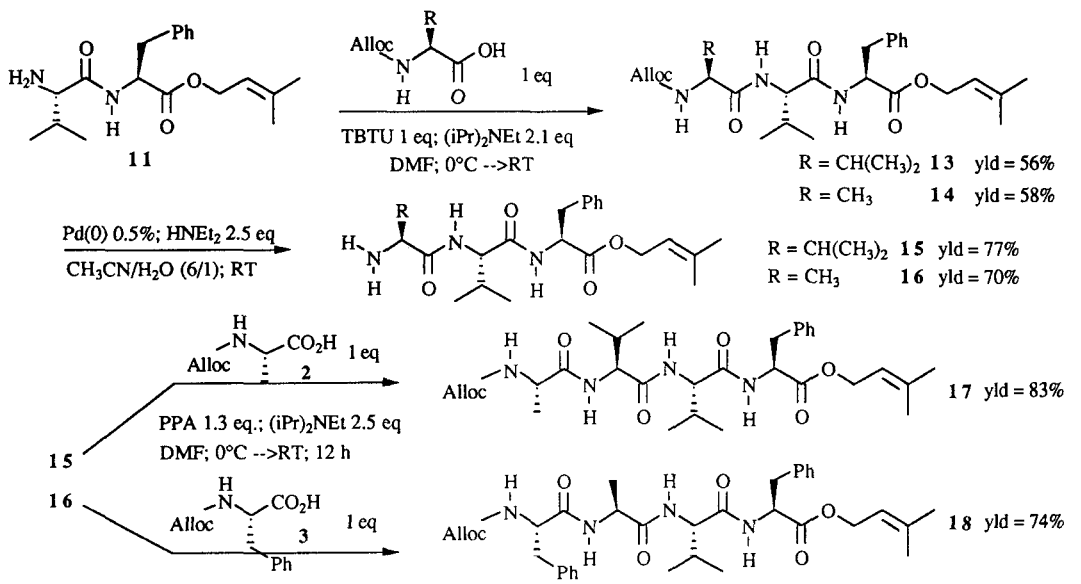
Entry	Dipeptide	Product	Time (min)	Yld(%)
1	 **7**	 **10**	20	100
2	 **8**	 **11**	30	83
3	 **9**	 **12**	20	88

Conditions : $Pd(OAc)_2$ / TPPTS (1/2) mol 0.5%; $HNEt_2$ 2.5 eq; CH_3CN / H_2O (6/1); RT

Table 2

The synthesis of tri and tetrapeptides was then achieved according to this methodology, and one example of such peptide chain elongation is given in Scheme 3. L-valyl-L-phenylalanyl-O-dimethylallyl was coupled with N-allyloxycarbonyl-L-valine or N-allyloxycarbonyl-L-alanine, in the presence of TBTU as the coupling reagent, with 56 and 58% yield respectively. As previously noted, no purification was required after acidobasic treatment and extraction, affording pure crude tripeptides **13** and **14** wich were directly engaged in the deprotection step under aqueous conditions. The terminal amine moiety was rapidly and selectively regenerated in good yields. The fourth aminoacid was then assembled using PPA, to produce the two tetrapeptides **17** and **18**, in 83 and 74% yield respectively[10]. As the length of the peptide increases, its water-solubility decreases so that better yields are obtained due to less loss of material during the aqueous washings to eliminate the DMF, solvent of the coupling step.

As a conclusion, we developed a rapid methodology for the preparation of peptides in solution using the selective removal of an allylcarbamate in the presence of a substituted allylic ester, catalyzed by the water-soluble $Pd(OAc)_2$ / TPPTS catalytic system. This technique proved to be efficient for the synthesis of tetrapeptides that can be used as fragments for the assembly of larger molecules. As no purification is required throughout the chain elongation, these conditions should find industrial applications since the coupling and deprotection steps can be quickly carried out in large scale.



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References and notes :

1 Abbreviations used in this paper. DCC : 1,3-dicyclohexylcarbodiimide; dimethylallyl : 3-methylbut-2-enyl; HOBT : 1-hydroxybenzotriazole; PPA : tripropylphosphoric anhydride; TBTU : 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TPPTS : triphenylphosphinotrisulfonate sodium salt.

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10 Data for compound **17**. ^{1}H NMR (DMSO-d_6, 200 MHz) : 8.37 (1H, d, ^{3}J = 7.6 Hz, NH); 7.74 (1H, d, ^{3}J = 7.9 Hz, NH); 7.70 (1H, d, ^{3}J = 8.3 Hz, NH); 7.45 (1H, d, ^{3}J = 9.2 Hz, NH); 7.22 (5H, m, Harom); 5.90 (1H, m, HC=); 5.28 (1H, dm, ^{3}J = 14.6 Hz, H_2C=); 5.17 (1H, m, HC=); 5.16 (1H, dm, ^{3}J = 11.0 Hz, H_2C=); 4.49 (1H, m, NHCH); 4.46 (4H, m, CH_2allyl); 4.19 (2H, m, NHCH); 4.08 (1H, m, NHCH); 2.98 and 2.91 (2H, 2d, ^{3}J = 5.4 and 7.2 Hz, CH_2Ph); 1.91 (2H, m, $CHMe_2$); 1.69 and 1.62 (6H, 2s, H_3C=); 1.17 (3H, d, ^{3}J = 7.0 Hz, CH_3); 0.79 (12H, m, CH_3). ^{13}C NMR (DMSO-d_6, 50 MHz) : 173.30, 172.03, 171.67, 171.49, 154.25, 139.36, 137.85, 134.49, 129.83, 129.09, 127.39, 119.33, 117.86, 65.25, 62.03, 58.90, 58.35, 57.99, 54.22, 37.50, 31.79, 31.37, 26.31, 20.07, 19.92, 18.92, 18.84, 18.68, 16.26. DCI / NH_3 : 587 $(M + H)^+$. IR : 3297, 1734, 1696, 1663, 1635, 1558, 1539, 1258, 1227, 1199. $[\alpha]_D^{20}$ = - 10 (c = 0.85, DMSO). F = 244°C. Data for compound **18**. ^{1}H NMR (DMSO-d_6, 200 MHz) : 8.39 (1H, d, ^{3}J = 7.4 Hz, NH); 8.19 (1H, d, ^{3}J = 7.3 Hz, NH); 7.67 (1H, d, ^{3}J = 9.1 Hz, NH); 7.43 (1H, d, ^{3}J = 8.6 Hz, NH); 7.29-7.22 (10H, m, Harom); 5.80 (1H, m, HC=); 5.19 (1H, dm, ^{3}J = 11.6 Hz, H_2C=); 5.16 (1H, m, HC=); 5.11 (1H, d, ^{3}J = 10.0 Hz, H_2C=); 4.48 (2H, d, ^{3}J = 7.2 Hz, ^{3}J = 10 Hz, OCH_2); 4.36 (2H, d, ^{3}J = 6.6 Hz, CH_2allyl); 4.41-4.28 (2H, m, NHCH); 4.21 (1H, q, ^{3}J = 7.0 Hz, NHCH); 4.19 (1H, m, NHCH); 2.97 (4H, m, CH_2Ph); 1.91 (1H, m, $CHMe_2$); 1.69 and 1.62 (6H, 2s, H_3C=); 1.18 (3H, d, ^{3}J = 7.0 Hz, CH_3); 0.82 (3H, d, ^{3}J = 5.5 Hz, CH_3); 0,79 (3H, ^{3}J = 7.0 Hz ^{3}J = 6.6 Hz). ^{13}C NMR (DMSO-d_6, 50 MHz) : 172.04, 171.61, 171.41, 171.06, 155.97, 138.73, 138.46, 137.26, 133.76, 129.46, 129.28, 128.45, 128.27, 126.76, 119.11, 118.69, 116.50, 64.52, 61.40, 57.26, 56.40, 53.71, 48.49, 37.53, 36.88, 31.13, 25.66, 19.32, 18.33, 18.04. DCI / NH_3 : 635 $(M + H)^+$: IR : 3293, 1734, 1696, 1650, 1636, 1540, 1294, 1255. $[\alpha]_D^{20}$ = - 4 (c = 0.81, DMF). F = 193°C.

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