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RAPID SOLID PHASE SYNTHESIS OF α-AMINO ACIDS

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Abstract : A solid phase synthetic method for amino acids is developed. The N-acetyl-dehydroalanine is quantitatively bound to Wang resin by the Mitsunobu method. Then a Michael addition is achieved on the double bond with different nucleophiles. Non proteinic N-acetyl-α-amino acids are obtained after cleavage from the resin by mild acid treatment (Scheme 1).

Recently solid phase synthesis of small molecules has emerged as an important tool in drug discovery [1]. Therefore efficient strategies for the development of novel solid phase synthetic methods continue to be of considerable interest in organic synthesis [2].

This report describes solid phase synthesis of α-amino acids by Michael addition of nucleophilic heterocycles on commercially available 2-acetamidoacrylic acid.

β-heterocyclic alanines such as pyrazolyl alanine, quisqualic acid have been isolated from several plant sources [3]; quisqualic acid isolated from *Quisqualis indica* possesses potent neuroexcitatory activities [4]; triazolylalanine is known as an important metabolite in plants of the fungicide Myclobutanil [5]; pyrazolylalanine was isolated from the pressed juice of *Citrullus vulgaris* and presents hypoglycemic properties [6].

Solid phase synthesis of α-amino acids has been described by alkylation of resin bound aldimine or ketimine esters of α-amino acids [7]. Few synthetic approaches on solid support have appeared concerning dehydroaminoacids [8].

We have investigated the following strategy for the synthesis of β-heterocyclic alanines as shown in scheme 1, Wang resin was selected because of an easy acidic cleavage, the starting material was N-protected by an acetyl group, convenient to obtain optically pure α-amino acids by enzymatic resolution using an amino acylase.

We have previously tested the solution synthesis of β-heterocyclic α-amino acids using the same strategy but the purification was very tedious because the starting material and the product presented the same solubility.

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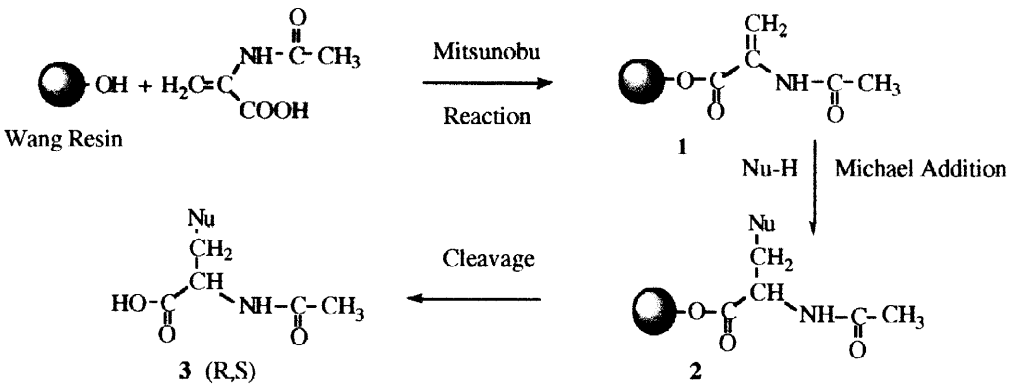

Scheme 1

Anchoring of N-acetamidoacrylic acid on the Wang resin by activation of the carboxylic acid function was unsuccessful (DCC/DMAP; DCI; BOP); the Mitsunobu reaction turned out to be the method.

For the second step concerning Michael addition of the nucleophile, several experimental conditions were tested and the results are reported in the Table. Two solvents were used (DMF and CH_3CN); CH_3CN yielded the best results.

The first experiment using 1,2,4-triazole as nucleophile was carried out using K_2CO_3 as base according to the experimental conditions described in [9] using 3 equivalents of nucleophile in the presence of 3 equivalents of base (entry 1) at 50-60°C. Only 7% yield was obtained, the starting material was recovered in the washings.

When the number of nucleophile equivalents (entries 6, 10, 15) was increased to 6, a maximum yield of 42% was reached with 6 equivalents.

Best yields were obtained at room temperature (entry 4).

In the presence of Cs_2CO_3 (3 equivalents) addition of triazole (6 equivalents) on acetamidoacrylic acid at room temperature in CH_3CN yielded N-acetyl-triazolylalanine after cleavage (50-54%) (entry 5). $KHCO_3$ and $CsHCO_3$ as bases were tried but without success, suggesting that the basicity of the mixture and the nature of the counter ion have a significant influence.

When the reaction time of the Michaël addition (2 to 4 days) was increased, the yield was not improved (43-45%). The expected product was recovered in the washings before the acidic cleavage suggesting that the product was cleaved from the resin in basic conditions.

When the nucleophile concentration was lowered (entry 7) yields were also improved (72-75%).

To optimize these experimental conditions, we chose 1,2,4-triazole because the isolation of the N-acetyl-triazolylalanine was easy.

Applying the optimized reaction conditions to pyrazole as nucleophile, N-acetyl-pyrazolylalanine was obtained in 54% yield (entry 11). If the nucleophile concentration (175 mM) and the temperature (RT) were decreased the yield was not improved. Only the N-acetyl-dehydroalanine was recovered in 98% yield.

The cleavage was achieved using 20% TFA in DCM.

Table : Experimental conditions

Entries	Nu-H	Michael addition				Reaction	Overall Yield
		Nu-H (eq)	Base (eq)	[Nu-H] (mM)	Temp °C	Time (days)	(%)
1 -	1,2,4-Triazole	3	3 K_2CO_3	70	50-60	2	5-10
2 -		6	3 K_2CO_3	140	50-60	2	40-42
3 -		6	6 K_2CO_3	140	50-60	2	31-34
4 -		6	3 K_2CO_3	140	25	2	75-78
5 -		6	3 Cs_2CO_3	140	25	2	50-54
6 -		6	3 K_2CO_3	70	50-60	2	65-68
7 -		6	3 K_2CO_3	70	25	2	72-75
8-	Pyrazole	3	3 K_2CO_3	70	50-60	2	2
9 -		10	3 K_2CO_3	220	50-60	2	14
10 -		15	3 K_2CO_3	350	50-60	2	10
11 -		15	6 K_2CO_3	350	50-60	2	54

The yields were based on the Wang resin initial loading (0.63 meq/g). The Mitsunobu reaction proceeded in quantitative yield as determined by elemental analysis. The solid support synthesis overall yield was evaluated after cleavage from the resin.

All racemic mixtures were enzymatically resolved by *Aspergillus genus* acylase [9].

A typical procedure is outlined as follow:

Mitsunobu reaction on the Wang resin:

2-acetamidoacrylic acid (5 eqs), triphenylphosphine and Wang resin or para-alcoxybenzylic resin (loading: 0.63 me/g) were dried in vacuo for 12 hr. Anhydrous THF was then added to the mixture which was magnetically stirred at room temperature. DEAD (diethyl azodicarboxylate) (5 eq) was added dropwise under argon. The mixture was stirred for 24 hr, the resin was washed several times with THF and DCM. The IR spectrum showed the ester band (1745 cm^{-1}) and the NH amide band (3390 cm^{-1}) as compared to the IR spectrum of the Wang commercial resin. Analysis: %N 0,998 calculated (1.10 and 1.07 found). The reaction was monitored by solid gel ^{13}C and ^{1}H NMR.

Michael addition:

The substituted resin was washed and swollen in CH_3CN. The resin was magnetically stirred with K_2CO_3 (3 eq) at room temperature. Then 6 equivalents of nucleophile dissolved in CH_3CN were added. The mixture was stirred for 48 hr and the resin washed with CH_3CN. The total volume of the mixture is 34 ml.

The reaction was monitored by solid gel ^{13}C and ^{1}H NMR by HRMAS technic on a Avance 400 MHz spectrometer with a HRMAS 4mm probe (1H-13C-2H-gradients). The samples **1** and **2** were swollen in DMF.

^{1}H HRMAS Sample **1**: 2.1 ppm (s, 3H); 5.8 and 6.4 ppm (dxd, 2H); 9.2 (s, NH).
^{1}H HRMAS Sample **2**: 1.9 ppm (s, 3H); 4.75 (s, 2H, CH_2N); 5.0 (CHα); 8 and 8.5 (two s, CH=N).

Cleavage from the resin

To avoid the hydrolysis of the N-acetyl group a mild condition of cleavage has been carried out in a mixture of 20% TFA in DCM for 30mn. The resin was washed with DCM and TFA removed by concentration in vacuo with methanol. The racemic N-acetylamino acids **3** were easily precipitated in MeOH/Et_2O system.

Enzymatic resolution [9]

The substrate **3** (2.5 mmoles) was dissolved in phosphate buffer with 5.10^{-4} M $CoCl_2$ (15 ml, 0.1 M; pH 7.2). Then the purified *Aspergillus genus* acylase (20mg) dissolved in buffer was added. The reaction was stirred at room temperature during 32h.

We described in this paper a novel strategy of synthesis on solid support of racemic N-acetyl-amino acids in acceptable yields. This technology is presently being used with different nucleophiles and is applicable for the combinatorial synthesis of non proteinic aminoacids. Moreover we are trying to synthesize dehydroaminoacids on solid support.

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