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# Some of the amino acid chemistry going on in the Laboratory of Amino Acids, Peptides and Proteins

*Review Article*

**S. Bouifraden, C. Drouot, M. El Hadrami, F. Guenoun, L. Lecointe, N. Mai, M. Paris, C. Pothion, M. Sadoune, B. Sauvagnat, M. Amblard, J. L. Aubagnac, M. Calmes, P. Chevallet, J. Daunis, C. Enjalbal, J. A. Fehrentz, F. Lamaty, J. P. Lavergne, R. Lazaro, V. Rolland, M. L. Roumestant, Ph. Viallefont, Y. Vidal,** and **J. Martinez**

Universités Montpellier II et I, Montpellier, France

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**Summary.** Some of the chemistry of amino acids going on in our laboratory (Laboratoire des Amino acides Peptides et Protéines) is described as well as some mass spectrometry methodology for their characterization particularly on solid supports. Several aspects are presented including: (i) the stereoselective synthesis of natural and unnatural amino acids using 2-hydroxypinan-3-one as chiral auxiliary; (ii) the stereoselective synthesis of natural and unnatural amino acids by deracemization of $\alpha$-amino acids *via* their ketene derivatives; (iii) the synthesis of $\alpha$-aryl-$\alpha$-amino acids *via* reaction of organometallics with a glycine cation; (iv) the diastereoselective synthesis of glycosyl-$\alpha$-amino acids; (v) the synthesis of $\beta$-amino acids using $\alpha$-aminopyrrolidinopiperazinediones as chiral templates; (vi) the reactivity of urethane-N-protected N-carboxyanhydrides. To characterize natural and non natural amino acids through their immonium ions by mass spectrometry, some methodology is also described.

**Keywords:** $\alpha$-Amino acids – $\beta$-Amino acids – $\alpha$-Aryl-$\alpha$-amino acids – Chiral auxiliary – Deracemization – Glycosyl-$\alpha$-amino acids – 2-Hydroxypinan-3-one – Immonium ions – Urethane N-protected N-carboxyanhydrides

**Abbreviations:** Boc, *tert*-butyloxycarbonyl; BOP, benzotriazol-1-yl-oxytris(dimethylamino) phosphonium hexafluorophosphate; CAD, Collision Activated Dissociation; DCC, N,N′-dicyclohexylcarbodiimide; DIEA, diisopropylethylamine; DMAP, dimethylaminopyridine; FAB, Fast Atom Bombardment; HOBt, 1-hydroxybenzotriazole; H.P., 2-hydroxypinan-3-one; NMM, N-methylmorpholine; PEG, polyethylene glycol; SIMS, Secondary Ion

Mass Spectrometry; TEA, triethylamine; UNCAs, urethane N-protected N-carboxyanhydrides; Z-, Benzyloxycarbonyle.

## Introduction

Since many years, we have been involved in the chemistry of amino acids and peptides, both in solution and on solid support. We report here on some of the chemistry that was recently developed in our laboratory, concerning: (i) the stereoselective synthesis of natural and unnatural amino acids using 2-hydroxypinan-3-one as chiral auxiliary or (ii) by deracemization of $\alpha$-amino acids *via* their ketene derivatives; (iii) the synthesis of $\alpha$-aryl-$\alpha$-amino acids *via* reaction of organometallics with a glycine cation; (iv) the diastereoselective synthesis of glycosyl-$\alpha$-amino acids; (v) the synthesis of $\beta$-amino acids using $\alpha$-aminopyrrolidinopiperazinediones as chiral templates; (vi) the reactivity of urethane-N-protected N-carboxyanhydrides. However, we have developed some methodology in order to characterize natural and unnatural amino acids through their immonium ions by mass spectrometry. This methodology could be applied on solid supports.

## Stereoselective synthesis of $\alpha$-amino acids by use of 2-hydroxypinan-3-one as chiral auxiliary

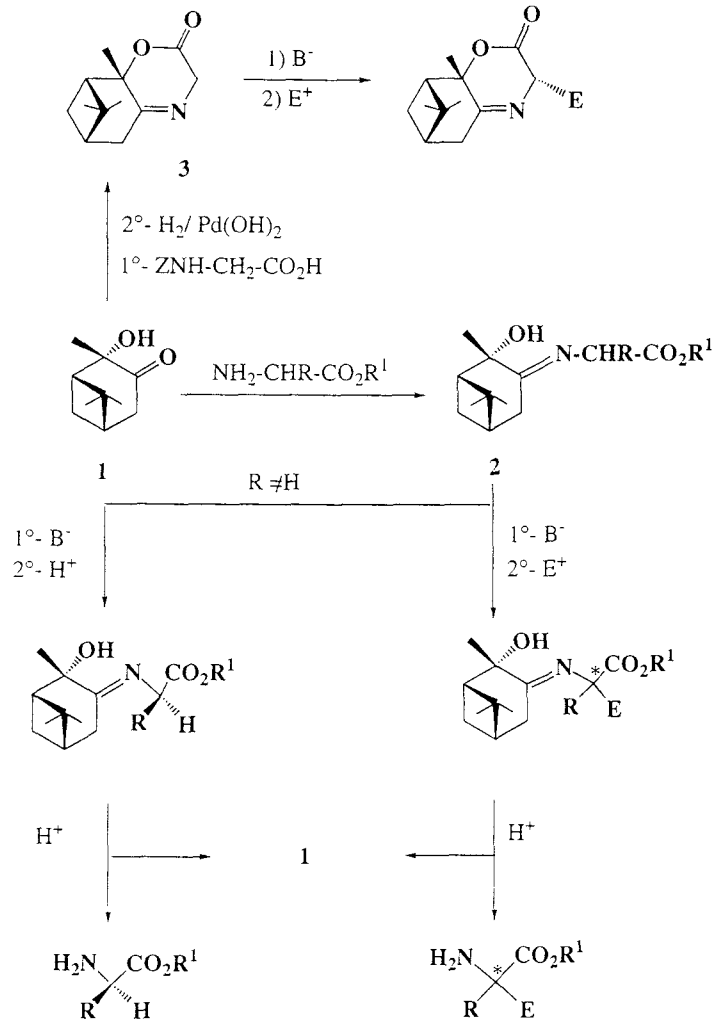
Several strategies have been explored for the enantioselective synthesis of $\alpha$-amino acids using 2-hydroxypinan-3-one as chiral auxiliary (Carlson and Pierce, 1971; Krishnamurthy et al., 1997). This chiral auxiliary first used by the group of Yamada (Oguri et al., 1978) was adopted later on by other groups (Minowa et al., 1984; Solladie-Cavallo et al., 1989).

From 2-hydroxypinan-3-one (H.P) **1**, Schiff bases **2** were prepared in good yields by reaction with $\alpha$-amino esters in refluxing benzene or toluene containing boron trifluoride etherate. From the same chiral auxiliary, the oxazinone **3** was obtained by reaction of 2-hydroxypinan-3-one with Z-glycine in the presence of dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) followed by the cleavage of the amino protecting group and spontaneous cyclisation (El Achqar et al., 1988).

We have studied the reactivity of these two systems and we summarize here our results.

## Reactivity of chiral Schiff bases 2

For several years, we have been engaged in the study of reactions of lithiated Schiff bases with electrophiles. We have been able to prepare several optically pure mono and disubstituted $\alpha$-amino acids (Bajgrowicz et al., 1983; Chaari et al., 1991; Jenhi et al., 1991; Tabcheh et al., 1991; Tabcheh et al., 1992; Hoarau et al., 1996; Jacob et al., 1997; Receveur et al., 1995, 1998), imino acids (Bajgrowicz et al., 1986) and functionalized amino acids (El Achqar et al., 1988; Tabcheh et al., 1992). The method was extended to the asymmetric




**Scheme 1**

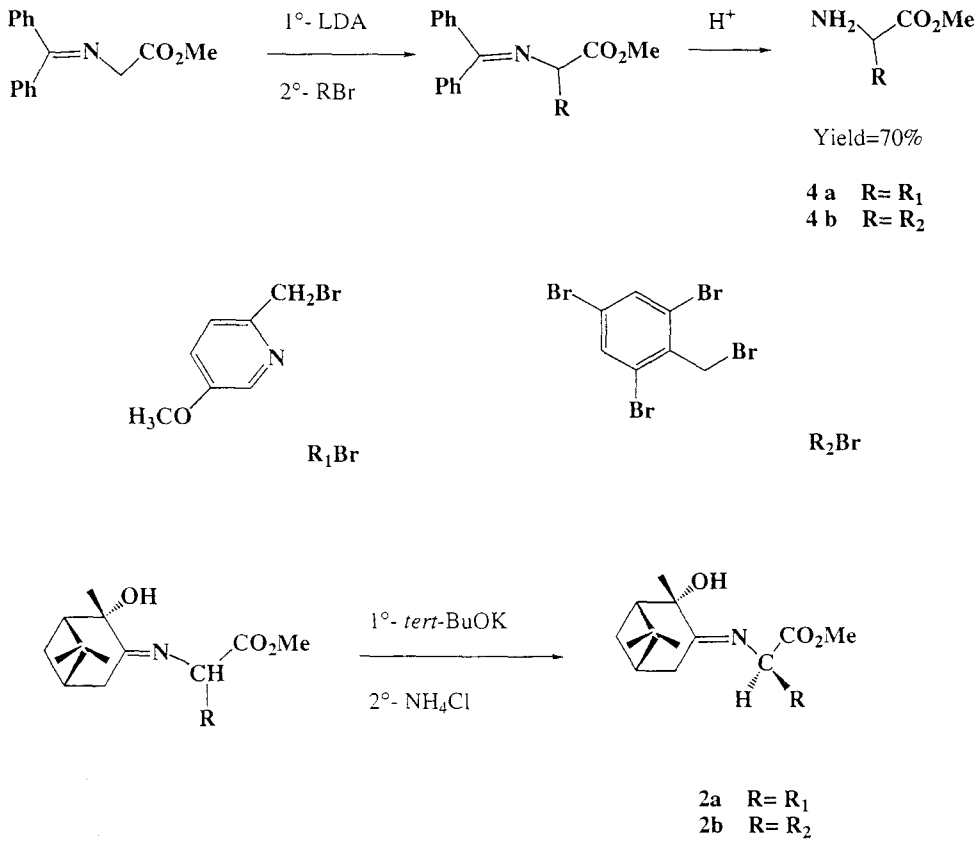
synthesis of aminophosphonic acids (Jacquier et al., 1988; Ouazzani et al., 1991; Alami et al., 1992) and of constituents of natural products (Jacquier et al., 1984), including leucinostatine (El Hadrami et al., 1991).

Recently we have used 2-hydroxypinan-3-one as chiral auxiliary in diastereoselective protonation of Schiff bases **2**. Amongst the different reported methods (Williams, 1989; Duthaler, 1994) for the synthesis of $\alpha$-amino acids in enantiomerically pure form, asymmetric protonation of enolates has not been studied in detail, although it is a very efficient method allowing conversion of a racemic compound into the desired enantiomer. For deprotonation of chiral Schiff bases several bases have been tested (LDA, LHMDS, *tert*-BuOK, KHMDS); *tert*-BuOK appeared to be the most suitable. Protonation was attempted by different proton sources ($H_2O$, $CH_3CO_2H$, $NH_4Cl/H_2O$). The best results have been obtained using a saturated solution of $NH_4Cl$ (Yields 92 to 96%; d.e 79 to >98%) (Tabcheh et al., 1998). This

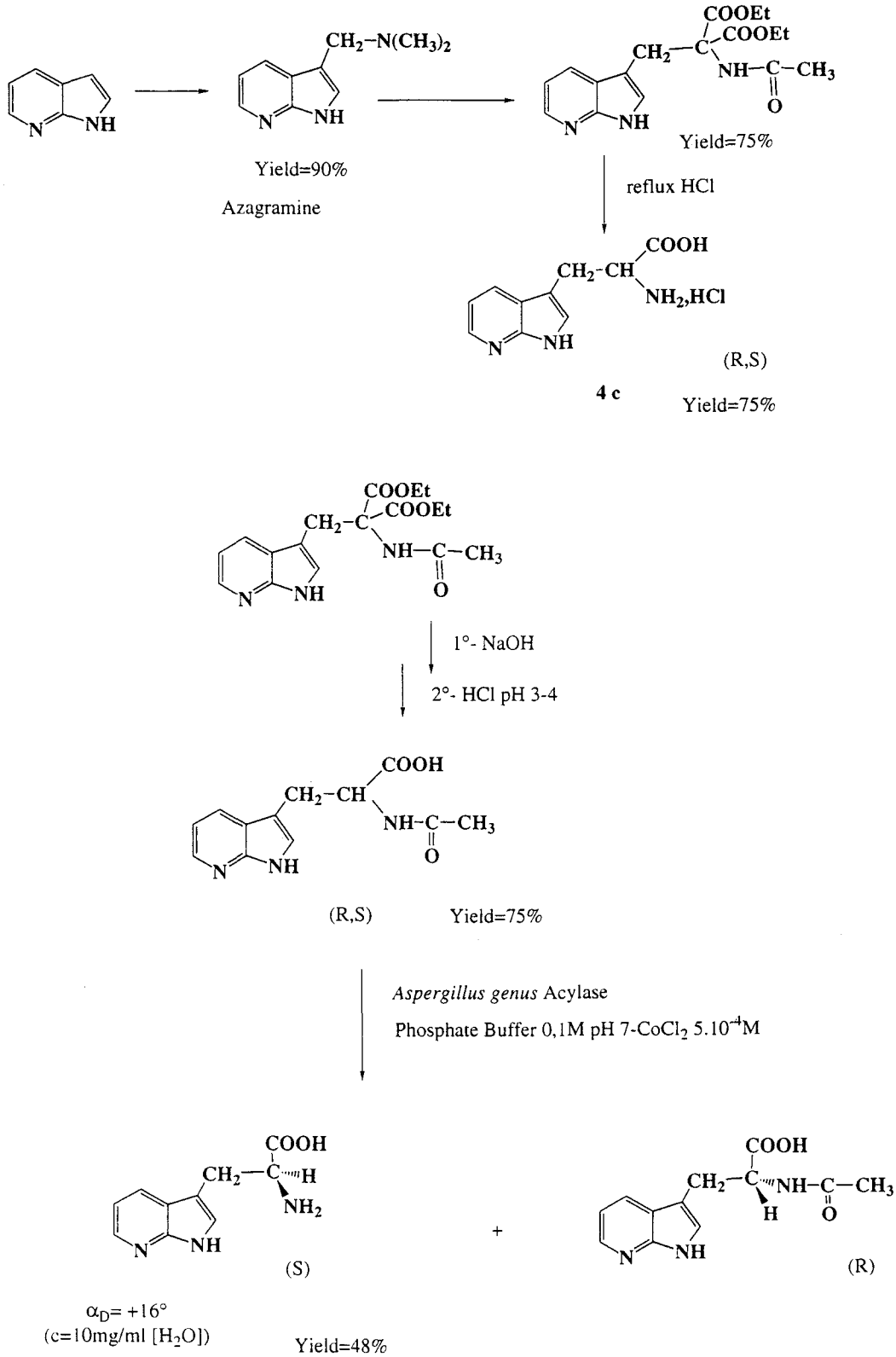
study has been extended to unnatural $\alpha$-amino esters particularly to the synthesis of azatryptophane which is a potential fluorescent probe, azatyrosine which is known as an antibiotic and anticarcinogenic compound and to tribromo- and trichlorophenylalanine which are interesting precursors for the labelling of biologically active peptides.

Racemic aminoesters (azatyrosine, tribromophenylalanine methyl esters **4a**,**4b**) were obtained by alkylation of methyl N-diphenylmethylene glycinate with the corresponding bromides easily prepared from commercially available materials (Scheme 2).

Racemic azatryptophane methyl ester **4c** was prepared by reaction of azagramine on diethyl N-acetylaminomalonate in the presence of NaOH in refluxing toluene using Robison's method (Robison and Robison, 1955). From these racemic amino esters, reaction with 2-hydroxypinan-3-one yielded the chiral Schiff bases which were deprotonated by *tert*-BuOK in THF and reprotonated by a saturated solution of $NH_4Cl$ (Scheme 2). Under these conditions azatyrosine and tribromophenyl alanine Schiff bases **2a** and **2b** were obtained in good yields and excellent d.e. (>98%), whereas 7-azatryptophane was prepared only in 66% d.e.. This last compound was synthesized enantiomerically pure by enzymatic resolution of the N-acetyl derivative by *Aspergillus genus* acylase (Scheme 3).




**Scheme 2**


CH2–N(CH3)2
Yield=90%
Azagramine
COOEt
COOEt
CH2
NH–C–CH3
O
Yield=75%
reflux HCl
COOH
CH2-CH
NH2,HCl
(R,S)
4 c
Yield=75%
1°- NaOH
2°- HCl pH 3-4
(R,S)
Yield=75%
Aspergillus genus Acylase
Phosphate Buffer 0,1M pH 7-CoCl2 5.10-4M
(S)
αD= +16°
(c=10mg/ml [H2O])
Yield=48%
+
(R)

**Scheme 3**

## Reactivity of the 1,4-oxazinone 3

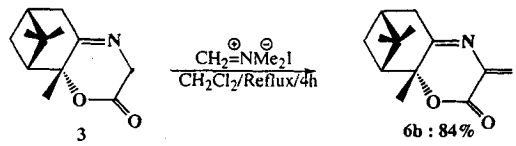
We have previously described that alkylation of the rigid, 1,4-oxazinone **3** using *tert*-BuOK as base allowed the synthesis of amino acids in high enantiomeric excess (El Achqar et al., 1988). Moreover the potassium enolate of **3** reacted rapidly with aldehydes (acetaldehyde, propionaldehyde, benzaldehyde) at −78°C to afford the dehydro compound **6** resulting from the spontaneous dehydration of the intermediate aldols. NMR spectra showed that only the Z configuration was present. These dehydro compounds allowed the synthesis of cyclopropanic $\alpha$-amino acids by cycloaddition with diazomethane (Alami et al., 1991) or by reaction with Corey's ylide (Calmes et al., 1996a).

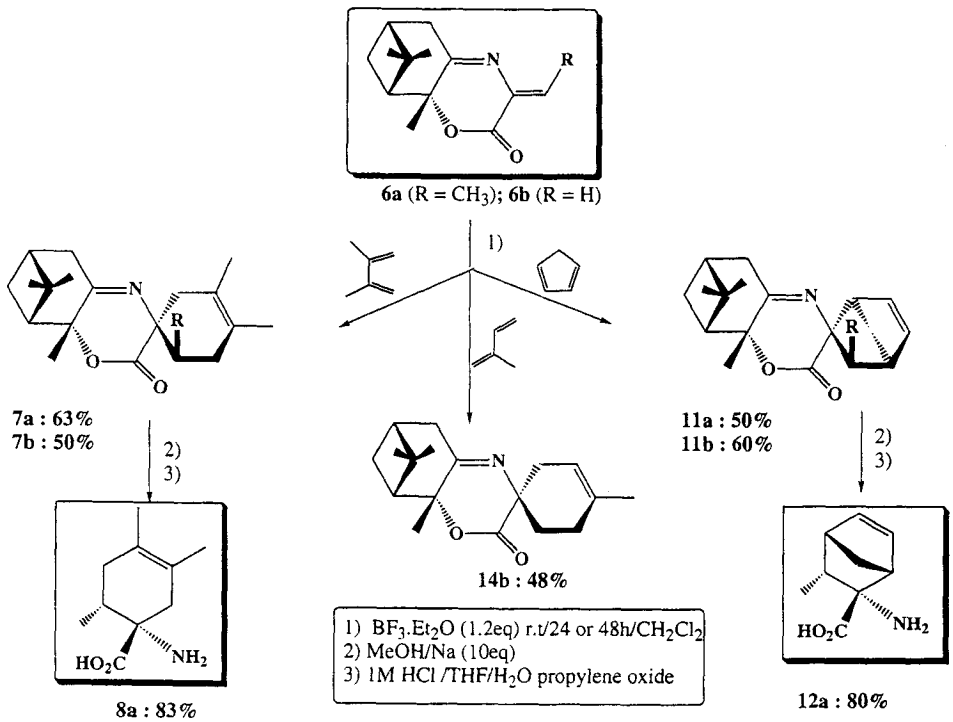
We describe here our results concerning Diels-Alder reactions on two dehydro compounds **6a** (R = Me) and **6b** (R = H) obtained by reaction of Eschenmoser's salt on oxazinone **3** in $CH_2Cl_2$ in 84% yield. For cycloaddition reactions, among the Lewis acids that were tested ($TiCl_4$, $Et_2AlCl$, $BF_3/Et_2O$) $BF_3/Et_2O$ produced the best results; in the presence of $TiCl_4$ **6a** was isomerised (Z to E). The Diels-Alder reaction of **6a** with 2,3-dimethyl butadiene (8 eq) in $CH_2Cl_2$ in the presence of $BF_3/Et_2O$ at room temperature yielded the cyclo adduct **7a** in 63% yield (Scheme 4). Simultaneous cleavage of both ester and imine functions by 6M HCl (one step procedure) was unsuccessful and led to degradation products. Cleavage of the ester function by MeONa (10 eq) in refluxing MeOH followed by treatment of the imine with 1M HCl (two step procedure) led to the amino acid **8a** in 83% yield. After hydrogenation in the presence of $Pd(OH)_2/C$ the cycloadduct **7a** yielded a 1/1 mixture of the two isomers $\mathbf{9a_1}$ and $\mathbf{9a_2}$ that were easily separated by column chromatography. Configuration of $\mathbf{9a_1}$ was established by X-ray diffraction (Chiaroni et al., 1994). The S configuration of the spiranic carbon confirmed as expected, that the diene approached the opposite face to the gem dimethyl group. The two amino acids $\mathbf{10a_1}$ and $\mathbf{10a_2}$ were obtained in 90% yield after treatment of $\mathbf{9a_1}$ and $\mathbf{9a_2}$ with 6M HCl in refluxing THF followed by neutralisation with propylene oxide (Scheme 4).

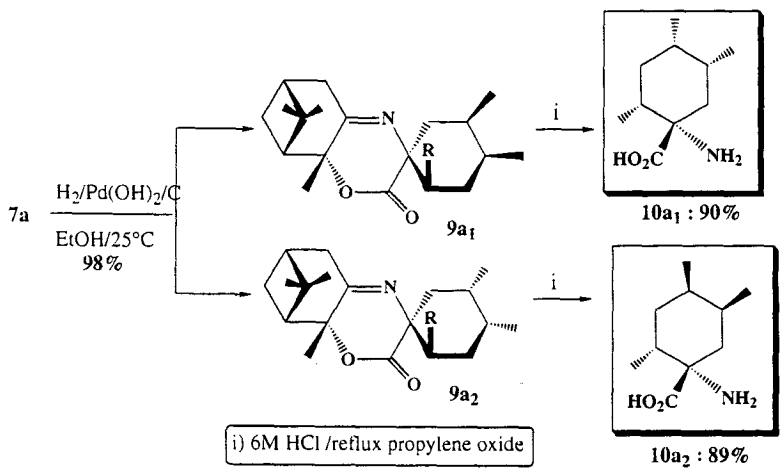
Under the same reaction conditions cyclopentadiene led to the cycloadduct **11a** in 50% yield. Its configuration was established by analysis of $^1H$ NMR spectra indicating an exo approach. Cleavage by a two step procedure yielded the amino acid **12a** (80%). Catalytic hydrogenation of **11a** on palladium hydroxide followed by cleavage using the one step procedure led to the bicyclic amino acid **13a** in 85% yield. Starting from the dehydro compound **6b** Diels Alder reaction afforded under the same conditions the cyclo adducts **7b**, **11b**, **14b** in respectively 50%, 60%, 48% yield.

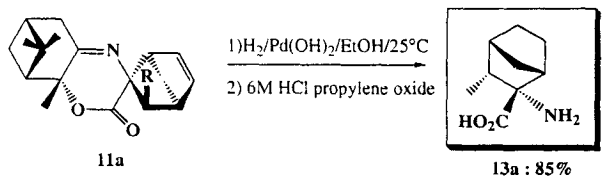
To our knowledge the dehydro compounds **6a** and **6b** are the first dienophiles inducing a total stereoselectivity with acyclic or cyclic dienes allowing an easy access to cyclic $\alpha$-amino acids in enantiomerically pure form.

Finally we have studied the reaction of organocuprates on compound **6b** (Scheme 5). Dimethyl, dibutyl and diphenyl cuprates (4 eq) in $THF/Et_2O$ (3/1) at −78°C led to the 1,4 addition products in respectively 73, 57 and 50% yield. In each case, only one stereoisomer was detected by $^1H$ NMR in $C_6D_6$

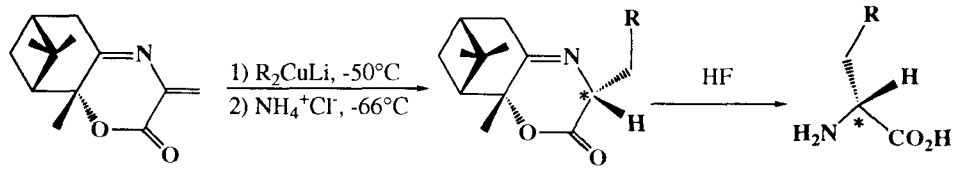

$CH_2=\overset{\oplus}{N}Me_2\ \overset{\ominus}{I}$
$CH_2Cl_2$/Reflux/4h
3
6b : 84%


6a (R = $CH_3$); 6b (R = H)
1)
7a : 63%
7b : 50%
2)
3)
8a : 83%
14b : 48%
11a : 50%
11b : 60%
12a : 80%
1) $BF_3.Et_2O$ (1.2eq) r.t/24 or 48h/$CH_2Cl_2$
2) MeOH/Na (10eq)
3) 1M HCl /THF/$H_2O$ propylene oxide


7a
$H_2$/Pd(OH)$_2$/C
EtOH/25°C
98%
$9a_1$
$9a_2$
i
$10a_1$ : 90%
$10a_2$ : 89%
i) 6M HCl /reflux propylene oxide


11a
1)$H_2$/Pd(OH)$_2$/EtOH/25°C
2) 6M HCl propylene oxide
13a : 85%

**Scheme 4**




**6b**

**R : $CH_3$, Bu, Ph**

| | $CH_3$ | Bu | Ph |
|---|---|---|---|
| *Yield (%)* | 73 | 57 | 50 |

**Scheme 5**

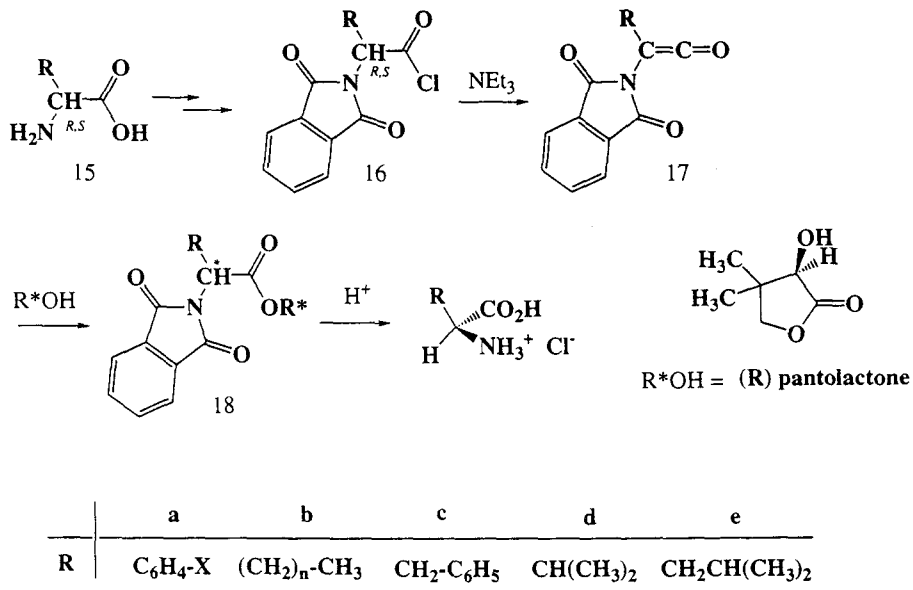
showing that the organocuprate attacked the less shielded face opposite to the gem dimethyl group. This strategy will allow the synthesis of enantiomerically pure mono and disubstituted $\alpha$-amino acids.

## Stereoselective synthesis of amino acid derivatives through asymmetric transformation of racemic $\alpha$-amino acids *via* their ketenes

Amino acid asymmetric transformation (deracemization) usually results from the stereoselective protonation of the corresponding prochiral enolates. Successful examples of this methodology has been described in many papers. On the other hand less has been published concerning the asymmetric transformation of a racemic amino acid *via* a prochiral aminoketene. The reaction consists of the base catalyzed stereoselective addition of an alcohol to a prochiral N-protected aminoketene affording the corresponding aminoester as the diastereopure isomer (Scheme 6).

In order to prevent intramolecular reaction, it is necessary to fully protect aminoketenes on the nitrogen. These aminoketenes cannot be isolated due to their instability (except in the case of the very hindered *tert*-leucine), but they are easily generated *in situ* from amino acids, generally *via* acid chlorides, mixed anhydrides or $\alpha$-chloro acid chlorides, and they are mainly used in [2 + 2] cycloadditions with imines to afford amino-$\beta$-lactams (Tidwell, 1995).

The only two groups that have investigated the stereoselective addition of an alcohol to aminoketene are the group of Pracejus, thirtyfive years ago, and more recently the group of Hegedus. Pracejus et al. (Pracejus, 1959, 1960; Pracejus and Winter, 1964, 1966) noted that, in the presence of a chiral tertiary amine, addition of an achiral alcohol to a phthalylamino ketene afforded the corresponding phthalylamino ester in modest diastereoisomeric excess, depending on the nature of the chiral base and of the temperature. Hegedus (1995) has studied the stereoselective addition at room temperature of achiral alcohols to chirally derivatized chromium-aminoketene complexes generated by photolysis of the corresponding chromium aminocarbene com-




| | a | b | c | d | e |
|---|---|---|---|---|---|
| R | $C_6H_4$-X | $(CH_2)_n$-$CH_3$ | $CH_2$-$C_6H_5$ | $CH(CH_3)_2$ | $CH_2CH(CH_3)_2$ |

**Scheme 6**

plexes. Although high diastereoselectivities were generally obtained, this method suffers from long irradiation times and sometimes poor yields of aminocarbene precursors.

We have explored the use of chiral alcohols (Calmes et al., 1996b, 1997a,b), added to the prochiral N-diprotected aminoketene for stereoselective induction. This approach is interesting because there is a wide range of commercially available, efficient and inexpensive chiral alcohols. The addition of alcohol has to be base-catalysed since otherwise the reaction time was very long and the yield often poor. In a subsequent step, the N and O protecting groups were cleaved under non racemizing conditions like in the enolate methodology.

Some chiral alcohols known to be efficient chiral auxiliaries, [e.g. (*R*)-N-methyl-3-hydroxysuccinimide, (*1R,2R,5R*)-2-hydroxy-3-pinanone, (S,S)-pseudoephedrine and (*R*)-pantolactone], and solvents ($CS_2$, toluene, chlorobenzene, dichloromethane, THF, acetonitrile) were tested. (*R*)-Pantolactone and THF were used in all subsequent experiments because they provided the best selectivities. In all cases the amine function of the amino acid was protected with the phthalyl group and the ketene was obtained by action of a tertiary amine on the corresponding acid chloride, the later resulting from reaction of the N-phthalyl amino acid with oxalyl chloride. The results showed that three classes of amino acids can be considered according to their side chain: aryl, unbranched alkyl or branched alkyl derivatives (Table 1). In the case of aryl N-phthalylamino acids the aromatic ring stabilizes the ketene. Upon treatment with triethylamine, the ketene formation was carried out at −78°C, and (*R*)-pantolactone was then added at the same temperature (method 1). The pantolactonyl ester **18a** with the *R,R* configuration was

**Table 1.** Yield and configuration of the $C_\alpha$ of phthalylamino acid pantolactonyl esters

| | R | T°(C) | yield (%) | *d.e* (%) | $C_\alpha$Config |
|---|---|---|---|---|---|
| Method 1 | $C_6H_4X$ X = H, F | −78 | 96 | 98 | R |
| | | 0 | 63 | 48 | R |
| | $(CH_2)_n$-$CH_3$ n = 0 à 3 | 0 or 20 | 90 | 72–94 | S |
| | $CH_2$-$C_6H_5$ | −78 | 55 | 27 | S |
| | | 0 | 98 | 71 | S |
| Method 2 | | 20 | 90 | 80 | S |
| | $CH(CH_3)_2$ | −78 | 0 | – | – |
| | | 0 | 70 | 42 | S |
| | | 20 | 73 | 22 | S |
| | $CH_2CH(CH_3)_2$ | −78 | 39 | 22 | S |
| | | 0 | 69 | 33 | S |
| | | 20 | 89 | 31 | S |

obtained in 98% *d.e* and in 96% yield (Table 1). When the reaction was performed at higher temperatures, both the diastereoselectivity and the chemical yield dropped drastically.

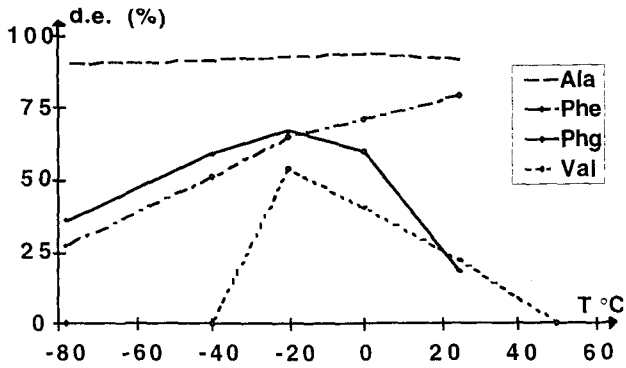
When alkyl N-phthalyl amino acids were treated in the same way, the corresponding (*S,R*)-esters were obtained in modest yield with 70% diastereoselectivity in the best cases. When leucine and the hindered amino acids *tert*-leucine and valine were used, their corresponding acid chlorides were unable to react with pantolactone or to afford the corresponding ketene.

To improve both the chemical yields and the stereoselectivities, we have modified the experimental conditions and added triethylamine and (*R*)-pantolactone simultaneously at a higher temperature to the acid chloride solution. Under these conditions, the ketene reacted as soon as it was formed (method 2). We have noted that when phenylalanine, phenylglycine and valine derivatives were used, the diastereoselectivity was dependent on the temperature, in agreement with Scharf's observation (Scharf et al., 1991). This was not the case for the alanine derivative (Scheme 7).

For unbranched amino acids **15b** and **15c** a temperature between −20 and 0°C seemed more efficient. The esterification was very fast and went to completion after about 1 hour (4 to 5h were necessary at −78°C), the diastereoselectivity of the esters increasing in the 72–94% range. Unfortunately in the case of the branched amino acids **15d** and **15e**, both the diastereoselectivity and the yield remained modest.

Surprisingly, for all the branched or unbranched alkyl amino acids which were investigated, the dominant diastereoisomeric ester had always the *S,R* configuration instead of the *R,R* configuration obtained in the case of aryl amino acid esters.

We tried to study conditions improving the diastereoselectivity in the cases of branched amino acids, particularly by replacing triethylamine by bases of various bulkiness or basicity. Indeed, it has been previously reported that diastereoisomeric excesses of aryl propionic esters, resulting from the stereoselective addition of the alcohol to the corresponding ketene, are base-




**Scheme 7**

dependent (Salz and Rüchard, 1982; Bellucci et al., 1988; Larsen et al., 1989; Senanayake et al., 1994; Calmes et al., 1994).

We have shown that in the case of the hindered valine derivative **17d**, the diastereoselectivity is very much dependent on the tertiary amine (Table 2). Indeed, the use of the less hindered trimethylamine (Table 2, entry 1) led to an improvement in both the chemical yield and the *d.e.* of the corresponding pantolactonyl ester **18d** (Scheme 6). Surprisingly, the generated stereogenic center has the *R*-configuration in place of the *S*-configuration found when triethylamine was used (Table 2, entry 6). With slightly bulkier bases (Table 2, entries 3 and 4), the percentage of the *R,R* diastereoisomer decreased and that of the *S,R* diastereoisomer increased. When too bulky or too basic bases were used (Table 2, entries 7 and 8), we observed a drastic drop in *d.e.* and no selectivity could be detected.

Finally we introduced additives into the reaction mixture before the addition of the tertiary base and (*R*)-pantolactone. The influence of such salts has been widely studied in the case of asymmetric alkylation of enolates (Seebach,

**Table 2.** Base dependence of the diastereoselectivity of N-phthalyl valine pantolactonyl ester **18d**

| Entries | Base | Yield (%) | *d.e.* % of esters | $C_\alpha$ Config |
|---|---|---|---|---|
| 1 | $N(CH_3)_3$ | 85 | 75 | *R* |
| 2 | quinuclidine | 90 | 72 | *R* |
| 3 | $N(CH_3)_2C_2H_5$ | 84 | 36 | *R* |
| 4 | $N(C_2H_5)_2CH_3$ | 83 | 11 | *R* |
| 5 | N-methyl morphline | 77 | 5 | *S* |
| 6 | $N(C_2H_5)_3$ | 80 | 42 | *S* |
| 7 | DIEA | 71 | 0 | *rac* |
| 8 | DBU | 88 | 0 | *rac* |
| 9 | $N(CH_3)_3$/LiCl | 96 | 80 | *R* |
| 10 | quinuclidine/LiCl | 97 | 67 | *R* |
| 11 | quinuclidine/LiCL −30°C | 96 | 85 | *R* |
| 12 | $N(C_2H_5)_3$/LiCl | 97 | 40 | *R* |

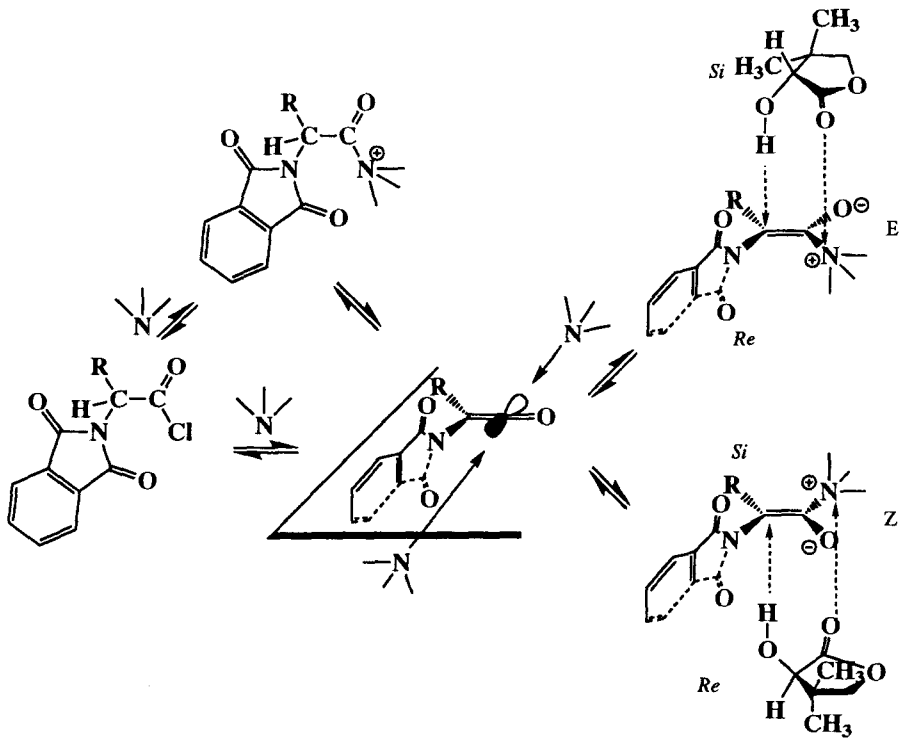
Solvent: THF; temperature: 0°C.

1988; Rück, 1995). In our case only LiCl modified the course of the reaction (Table 2, entries 9–12). Whatever the base used, the reaction was clean, and the esterification fast. Surprisingly with triethylamine as catalyst (entry 12) the newly generated stereogenic center was the inverse of that resulting from the same reaction carried out in the absence of LiCl (entry 6).

Several possible mechanisms of base-catalyzed addition of alcohols to ketenes have been proposed in the literature (Pracejus, 1960; Pracejus and Kohl, 1969; Jähme and Rüchardt, 1981; Satchell and Satchell, 1975, Chaumette, 1996; Calmes et al., 1996b, 1997a,b), but none of them can explain all our results.

After formation of the prochiral N-phthalylaminoketene (Scheme 8), from the corresponding acid chloride probably *via* the acylammonium salt, the attack of the tertiary amine on the $C_\beta$ may occur in either half-plane of the ketene inducing formation of E or Z enolate-like species. The E/Z ratio is dependent on the steric hindrance of both the tertiary amine and the R side chain. This is certainly the determining step of the stereoselective reaction.

In a second step, each E or Z species may react stereoselectively with (*R*)-pantolactone, from above or below the plane, to afford the corresponding *R,R* or *S,R* ester. The (*R*)-pantolactone approach could occur with less steric hindrance. The pantolactone electron-rich carboxylate could be close to the ammonium moiety, while the bulky gem-dimethyl group could be near the oxygen of the enolate (the less sterically hindered group), the pantolactone ring being directed away from the enolate. Thus, the (*R*)-pantolactone ap-




**Scheme 8**

proach may take place *via* the *Si* face of the E enolate, yielding the *R,R* ester, and *via* the *Re* face of the Z enolate yielding the *S,R* configuration.

In the case of aryl N-phthalylaminoglycine ketenes **17a**, like for diphenylketene (Gong et al., 1991), both the $C_\beta$ substitutions cannot be coplanar. We believe the phthalylamino group turns around the $C_\beta$-N link, allowing the amine to approach in the corresponding half-plane with formation of the E zwitterion. This possibility may be extended to the case of 2-arylpropionic ketenes: the amine approach occurred in the half-plane containing the less hindered methyl group with formation of the Z zwitterion which, after addition of (*R*)-pantolactone according to the same mechanism, led to (*R*)-2-arylpropionic acids (Salz and Rüchard, 1982; Bellucci et al., 1988; Larsen et al., 1989; Senanayake et al., 1994; Calmes et al., 1994).

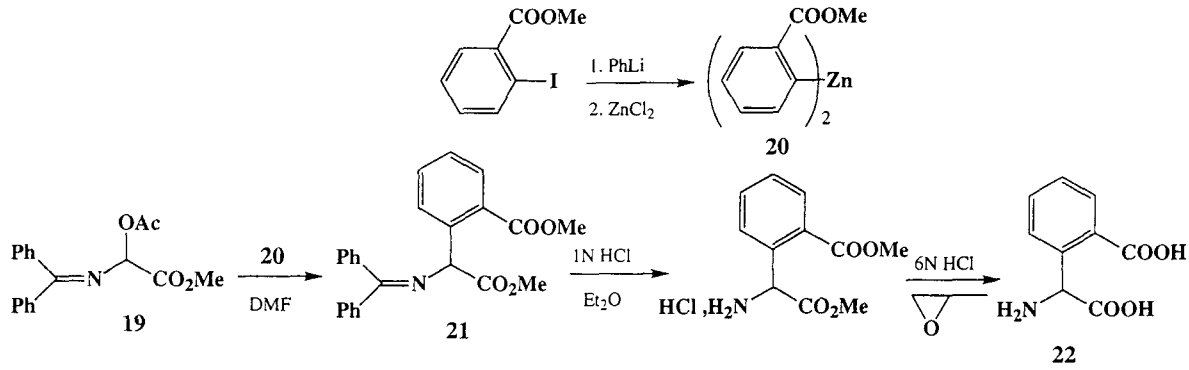
In the case of alkyl phthalyl aminoglycine ketenes **17b-e**, when the R alkyl group is not very bulky the amine approach occurred in the R containing half-plane yielding the Z zwitterion. When R becomes too bulky the amine approach is very dependent on both the R and amine protection. In the last case, addition of LiCl favoured the formation of the E zwitterion, as already reported for enolates (Seebach, 1988; Galiano-Roth et al., 1991; Humphrey et al., 1994; Myers et al., 1994; Rück, 1995; Majewski et al., 1995).

In conclusion, the asymmetric transformation of a racemic amino acid involving a prochiral aminoketene proved to be a simple and convenient possible alternative to the popular methodology using stereoselective protonation of enolates. However, except for aryl glycines, reaction may take place with high stereoselectivity at room temperature.

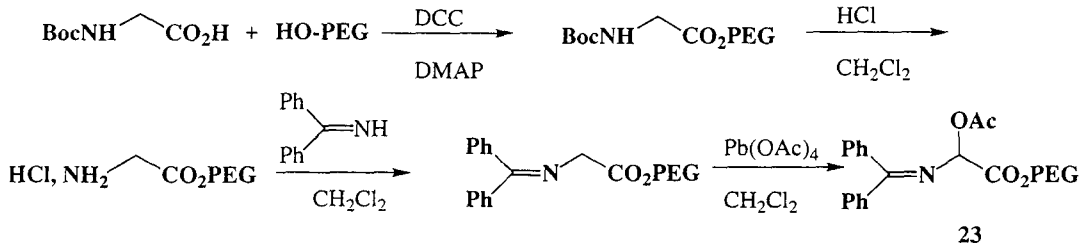
## Synthesis of $\alpha$-aryl $\alpha$-amino acids via reaction of organometallics with a glycine cation equivalent

$\alpha$-Aryl $\alpha$-amino acids (Williams and Hendrix, 1992) are an important class of molecules found in many biologically active compounds such as antibiotics derived from Penicillin (Morin and Gorman, 1982). Moreover, it has been shown also that conformationally well-defined analogues of glutamic acid such as carboxyphenylglycines are important pharmacological tools for the characterization of metabotropic receptors (Watkins and Collingridge, 1994).

To synthesize functionalized aryl glycines such as 2-carboxyphenylglycine **22**, we have developed a general method starting from a glycine cation equivalent such as Schiff base **19** (O'Donnell and Polt, 1982; O'Donnell et al., 1985) and a functionalized aryl organometallic (Scheme 9). The metal of choice tolerant for a wide variety of functional groups is zinc (Klement et al., 1996) and so far to our knowledge no reaction involving arylzinc derivatives and a glycine cation equivalent had been published. We have found that after halogen-lithium exchange and transmetalation with $ZnCl_2$ aryl halides reacted with Schiff base **19** to yield the corresponding protected $\alpha$-aryl $\alpha$-aminoesters (Lamaty et al., 1997). As an illustration of this method, the synthesis of 2-carboxyphenylglycine (2-CPG) is reported herein (Scheme 9).




**Scheme 9**

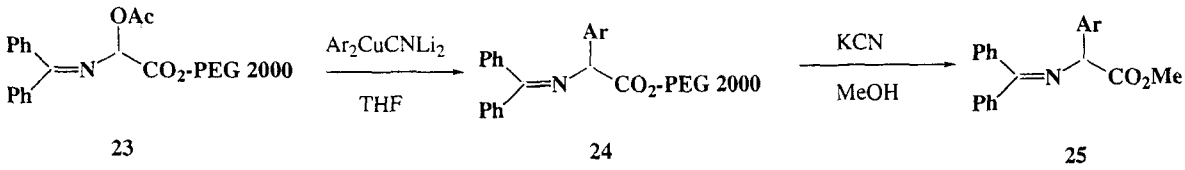



**Scheme 10**

Methyl 2-iodobenzoate was treated with PhLi in THF at −100°C and then with $ZnCl_2$ to yield the diarylzinc **20** which was reacted with Schiff Base **19** in DMF. The protected aminoester **21** was obtained in 65% yield after column chromatography. Sequential hydrolyses yielded 2-CPG **22** (Evidente et al., 1986). It is noteworthy to mention that a polar solvent such as DMF is necessary to allow completion of the reaction. In THF, no reaction was observed.

Since the reaction of diarylzincs with a glycine cation equivalent was clean and efficient (no by-products were obtained during the course of the reaction), we decided to study this reaction in liquid phase using a soluble polymeric support such as polyethylene glycol (PEG) (Gravert and Janda, 1997; Sauvagnat et al., 1998). This method combined the strategical features of solution and solid phase methods and thus might be useful in combinatorial chemistry. The synthesis was carried out in homogeneous solution while purification was performed by precipitation of the polymer. A glycine cation equivalent anchored to the two hydroxyl groups of a PEG with an average molecular weight of 2000 was synthesized as described in Scheme 10.

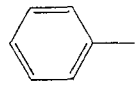
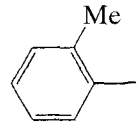
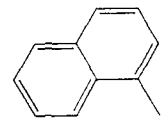
Unfortunately when **23** was treated with diarylzincs, no reaction took place even in DMF. Probably the oxygen atoms of PEG had the same Zn chelating effect as the oxygens of THF when the reaction was performed in solution (Frakland, 1859; Markies et al., 1992).




23 24 25

**Scheme 11**

**Table 3.** Synthesis of arylglycines

| Ar– |  |  |  |
|---|---|---|---|
| Product | **25a** | **25b** | **25c** |
| Yield (%) of **25** from **23** | 74 | 76 | 65 |

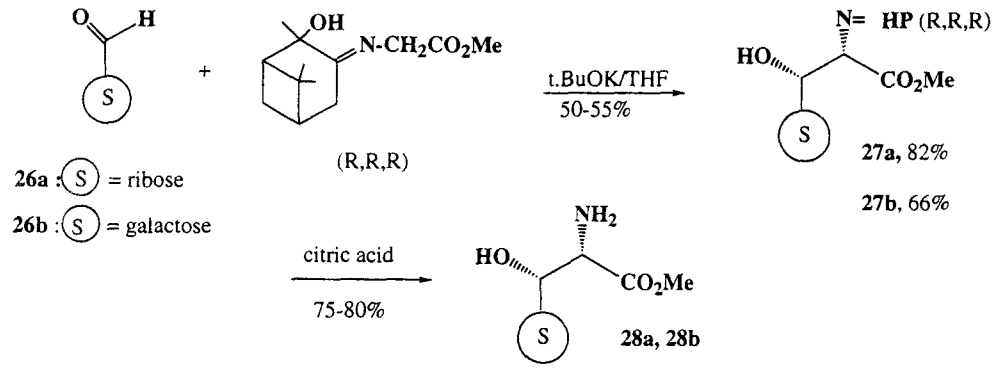
Since higher order cyanocuprates react in THF solution with Schiff base **19** (O'Donnell and Falmagne, 1985), we tried this reaction on the supported Schiff base **23**. In this case we obtained the corresponding supported $\alpha$-aryl $\alpha$-aminoesters **24** (Scheme 11 and Table 3). The $\alpha$-aryl $\alpha$-aminoesters **24** could either be used for further reaction on solid support such as peptide synthesis after N-deprotection with TFA (Mutter and Bayer, 1980) or released from the polymer *via* transesterification with methanol (Zhu and Hegedus, 1995; Moore and McMaster, 1978).

## Diastereoselective synthesis of glycosyl-$\alpha$-amino acids and glycopeptide derivatives

O- and N-Glycopeptides constitute an important class of compounds widely distributed among living organisms. We have performed the synthesis of glycosyl-$\alpha$-amino acids and of some glycopeptide derivatives in which the sugar and the amino acid moiety are joined by a C-C bond.

In the first part of our study we have synthesized glycosyl-$\beta$-hydroxy-$\alpha$-amino esters. $\beta$-hydroxy-$\alpha$-amino acids are important compounds because they are constituents of biologically active peptides (Kurokawa and Ohfune, 1986), precursors of antibiotic $\beta$-lactams (Miller, 1986) and enzymatic inhibitors (Nakatsuka et al., 1981).

The glycine enolates prepared from the Schiff bases of (*R*, *R*, *R*) 2-hydroxypinan-3-one (Oguri et al., 1978) were condensed on the two sugar aldehydes **26a** and **26b** (Scheme 12). A mixture of two diastereoisomers was obtained. The major product was isolated after chromatography on silica-gel; **27a** arising from the ribose **26a**, and **27b** coming from **26b** represented

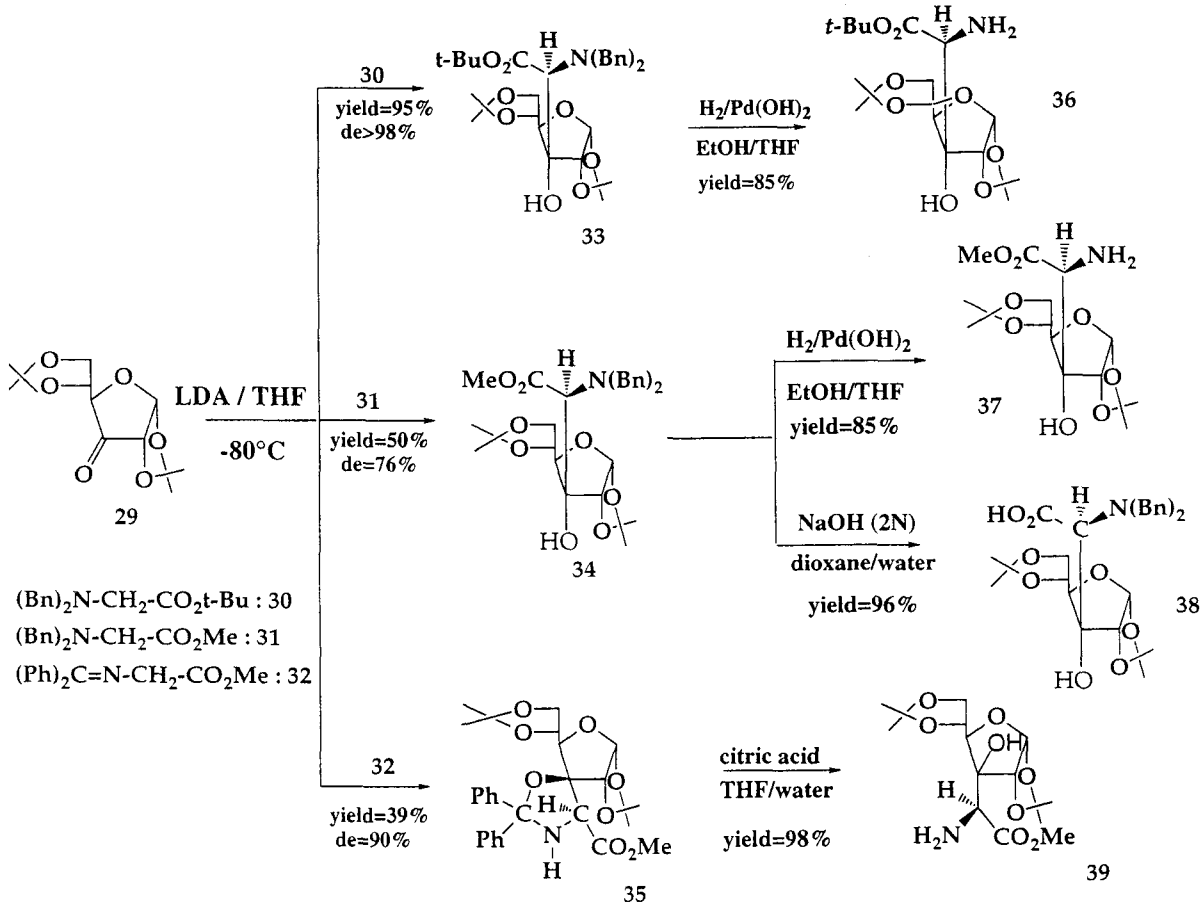



**Scheme 12**

respectively 82% and 66% of the mixture. The stereochemistry of these compounds was determined from crystallographic analysis on $\beta$-hydroxy-$\alpha$-amino esters **28a** and **28b** obtained after citric acid catalyzed hydrolysis of **27** (El Hadrami et al., 1993). In this case, a double asymmetric induction was observed. We have previously shown that alkylation of Schiff bases prepared from 2-hydroxypinan-3-one took place with excellent diastereoselectivity (El Achqar et al., 1988).

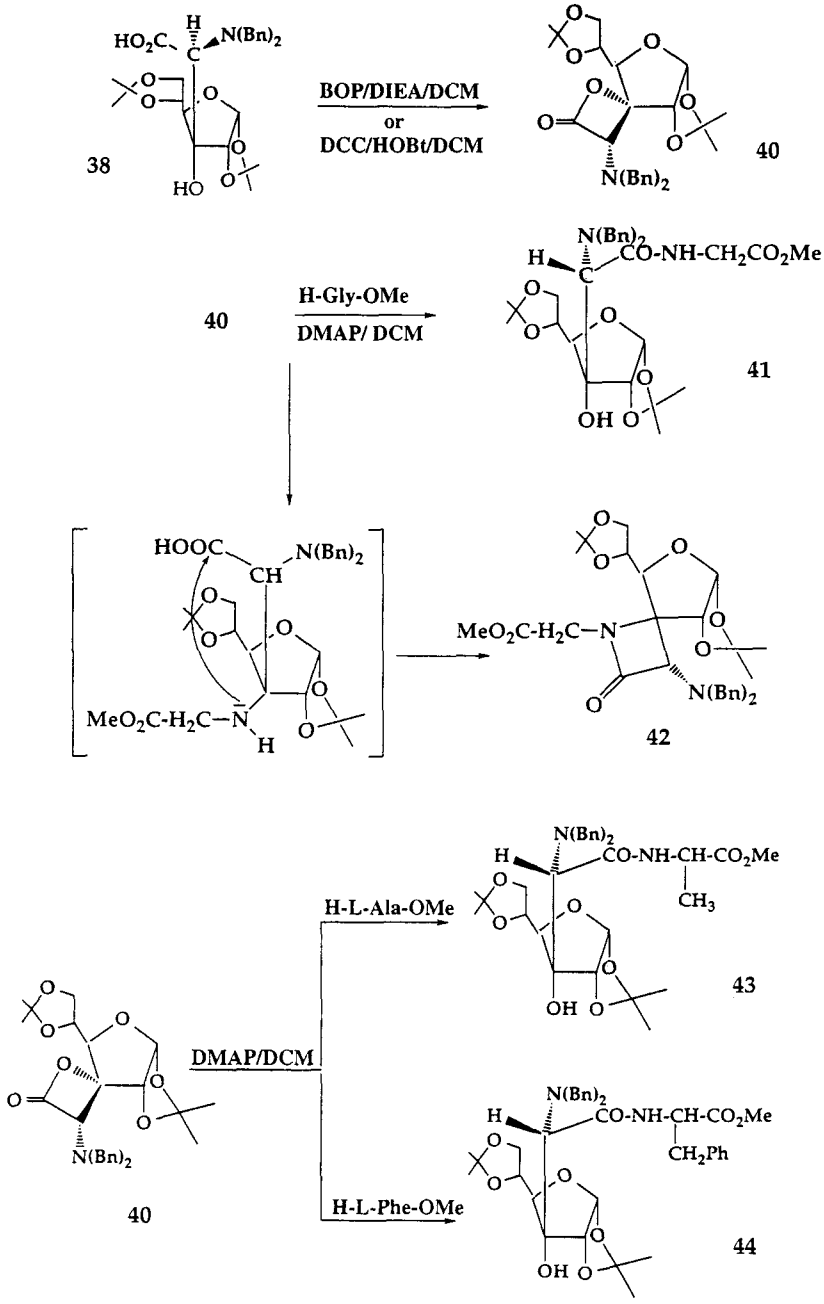
Various glycine enolates (prepared from aminoesters **30**, **31**, and **32** by treatment with LDA in THF at $-80°C$ were reacted with the carbonyl group of 1,2:5,6-di-O-isopropylidene-$\alpha$-D-ribohexofuranos-3-ulose **29** (Scheme 13). Starting from compound **30**, the reaction took place in excellent yield and with total diastereoselectivity implying a two-fold control of chirality. The afforded compound **33** contained the aminoester residue in the exo position. The structure and the stereochemistry of **33** were assigned from the spectral data and from the X-ray diffraction pattern. In the case of compound **31**, the reaction occurred with lower yield and with reduced diastereoselectivity. The major isolated diastereoisomer **34** had a stereochemistry identical to that of compound **33**. This stereochemistry was deduced from the X-ray diffraction pattern of the spirolactone derivative **40**. The enolate of the Schiff base **32** afforded the spirooxazolidine **35**. The stereochemistry of compound **35** was confirmed by X-ray analysis. Hydrogenolysis of compounds **33** and **34** in the presence of palladium hydroxide in a mixture of THF/ethanol proceeded in nearly quantitative yield affording the glycosylated aminoesters **36** and **37** with a free amino group (Scheme 13). Treatment of compound **33** with neat trifluoroacetic acid yielded a mixture of compounds. However, the corresponding glycosyl-amino acid **38** was obtained by saponification (dioxane/NaOH) of the methyl ester **34**. The glucofuranos-3-yl-aminoester **39** with a free amino group was obtained by hydrolysis of compound **35** in the presence of 15% citric acid.

Several glycosyl-$\alpha$-amino esters (or acids) of controlled chirality were obtained. After coupling with properly protected amino acid derivatives they afforded glycopeptides (Bouifraden et al., 1997a,b). However, under standard coupling conditions [BOP (Castro et al., 1975) or DCC/HOBt] the glycosyl-




**Scheme 13**

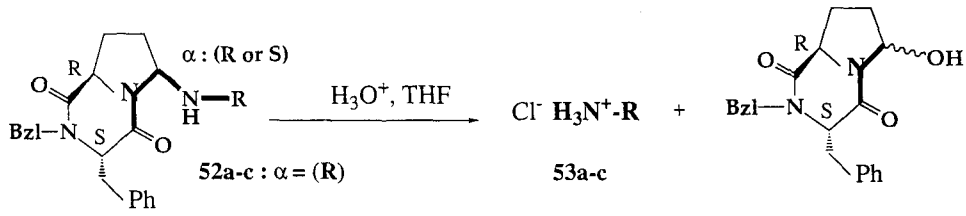
amino acid **38** showed a strong tendency to readily cyclize as the lactone derivative **40**. In fact, when **38** was treated with BOP in the presence of DIEA in dichloromethane the spirolactone **40** was obtained in high yield (Scheme 14). When the spirolactone **40** was treated with H-Gly-OMe, H-L-Ala-OMe or H-L-Phe-OMe, in the presence of DMAP, the corresponding glycopeptides **41**, **43** and **44** were obtained. When glycine methyl ester was reacted with **40**, two compounds were isolated. Compound **41** resulted from the O-acyl opening of the spirolactone **40** (50% yield). The spirolactame **42** (20% yield) resulted from the O-alkyl cleavage of the spirolactone **40** followed by an intramolecular cyclisation between the carboxylic function and the amino group of the intermediate (Scheme 14). When alanine and phenylalanine methyl esters were used, compounds **43** (90% yield) and **44** (77% yield) were obtained respectively, resulting exclusively from the cleavage of the O-acyl bond (Scheme 14). In the last case the phenylalanine was epimerized (9% of the diastereosiomer was detected) due to the presence of DMAP in the reaction conditions.




**Scheme 14**

## Synthesis of β-amino acids via asymmetric Mannich reaction using α-amino-pyrrolidinopiperazinediones as chiral template

Besides their α-analogues, β-amino acids have not deserved the same effort for their asymmetric synthesis (Juaristi, 1997) although they represent an important class of biomolecules.




**Scheme 15**

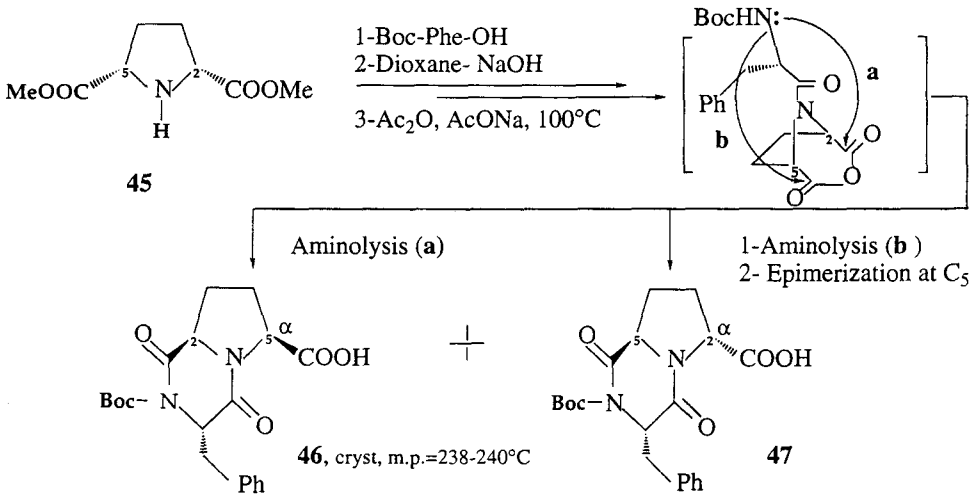
Optically active amino compounds can be obtained by asymmetric amino group transfer from amines grafted on a chiral auxiliary (Knupp and Fram, 1984). However just a few methods allow a smooth cleavage as well as a good template recovery (Kunz et al., 1997, Matsumura and Tomita, 1994).

Using the same approach, we have performed a new synthesis of an optically pure $\beta$-amino acid using an asymmetric Mannich reaction on an imine obtained from a bicyclic pyrrolidino-diketopiperazine containing an $\alpha$-acylaminoamine moiety (Scheme 15). This chiral auxiliary was designed for the following reasons: (i) The $\alpha$-acylaminoamine moiety can be smoothly cleaved (dilute acid hydrolysis) yielding the newly formed amine as well as the chiral template (Scheme 15). This auxiliary can be easily regenerated by azide substitution followed by reduction already performed with a closely related piperidine derivative (Matsumura and Tomita, 1994). (ii) The carbonyl group of the acylated amine is favourably situated near the imine nitrogen and is supposed to produce a strong interaction between the oxygen atom and the metal ($Zn^{++}$) of the Lewis acid catalyst (Scheme 18). In that way, an increase of stereoselectivity can be expected. (iii) The diketopiperazine is a simple cyclic peptide, easily prepared from available $\alpha$-amino acids.

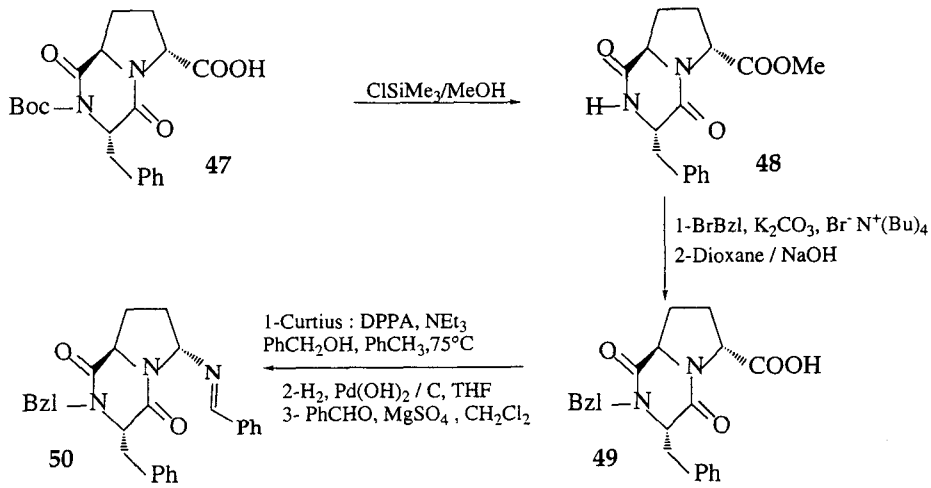
The only questionable point for synthesis of the auxiliary resides in the preparation of the optically pure functionalized proline residue described in Scheme 16.

It should be pointed out that starting from the easily available (cis) 2,5 dimethoxycarbonyl pyrrolidine **45** (Guenoun et al., 1997), it was possible to obtain after purification the optically pure (trans) compound **47** by the intramolecular aminolysis (path b) followed by a total spontaneous epimerization of the prolyl $\alpha$-center of the diketopiperazine ring. This inversion to the more stable trans ring has been already observed (Lucente et al., 1980, Cerrini et al., 1984). Indeed, the postulated structure **47** has been confirmed by 250 MHz $^1$H NMR analysis and compared to that of the epimer compound **46** whose absolute configuration was deduced from X-ray crystallography (Guenoun et al., 1997).

In our preliminary study, compound **46** was chosen as the starting auxiliary for the preparation of the methyl ester of (S)-$\alpha$-dimethyl $\beta$-amino phenyl propionic acid. However, it appeared that the (trans) pyrrolidino isomer **47** led to a better template: the resulting amine was more stable and could be conveniently handled and submitted to column chromatography purification (vide infra).



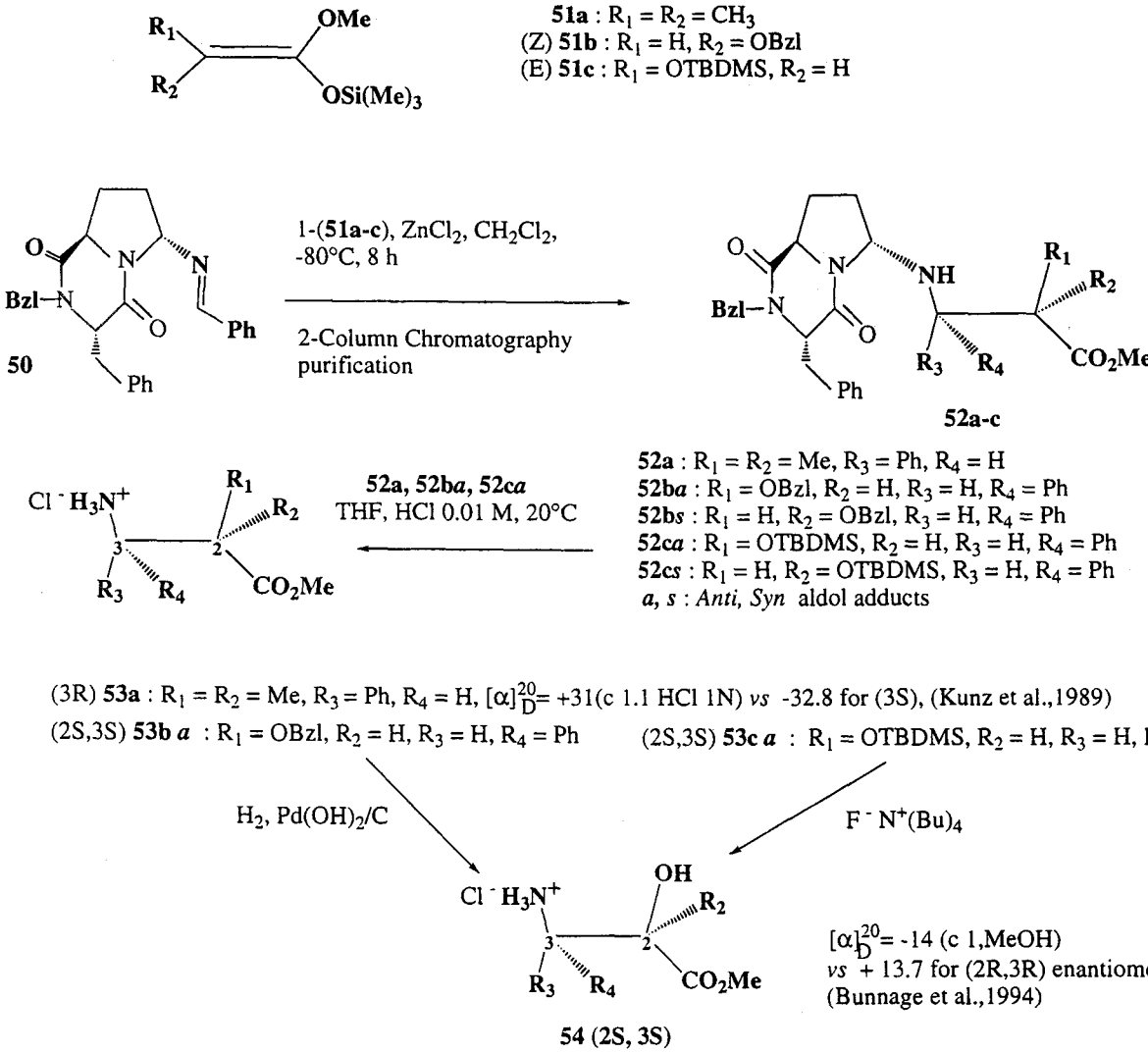

**Scheme 16**




**Scheme 17**

From the carboxylic derivative **47**, the imine **50** was obtained *via* a modern version of the Curtius reaction (Ninomiya et al., 1974) applied on the N-benzylated bicyclic acid **49** (Scheme 17). The resulting Z-protected amine (not depicted) was hydrogenated and immediately transformed into the desired phenyl aldimine. This imine **50** was further subjected to the silyl ketene acetal addition in presence of $ZnCl_2$ as Lewis acid at $-80°C$ in $CH_2Cl_2$.

This asymmetric Mannich reaction was performed with the 3 different ketene silyl acetals **51a-c** (Scheme 18): **51a** and (Z)-**51b** were prepared respectively from methyl isobutyrate or methyl benzyloxyacetate (Fischer and Gohlke, 1933) by deprotonation with lithium diisopropylamide in THF at $-80°C$ followed by silylation with trimethylsilyl chloride (Ainsworth et al., 1972, Slougui et al., 1982). **51c** was prepared in essentially (E)-form using




**Scheme 18**

lithium 2,2,6,6-tetramethylpiperidide as sterically hindered base (Hattori and Yamamoto, 1993). After hydrolysis, the raw material was purified by chromatography on silica gel column. It was found that the final secondary amine with the trans geometry of the pyrrolidino ring is more stable than the previously used cis-isomer (Guenoun et al., 1997). Indeed, we anticipated an opposite configuration (*3R*) for the new asymmetric $\beta$ carbon. Only one compound was eluted in 70% yield after purification from the Mannich reaction of **51a** whereas two compounds were obtained from **51b** and **51c** in respectively: 1.9/1 (**52ba**/**52bs**) and 6/1 (**52ca**/**52cs**) proportions.

The main adducts **52a**, **52ba** and **52ca** were subjected to smooth acid hydrolysis, yielding the corresponding esters in almost quantitative yields. The phenylisoserine derivatives were further O-deprotected either by

hydrogenolysis of **53b*a*** [$Pd(OH)_2/C$ catalyst] or by $F^-N^+Bu_4$ cleavage for **53c*a***.

The aminoester (3R)-**53a** was obtained in 70% yield based on the imine **50** with a good optical purity: $[\alpha]_D^{20}$ + 31 (cl.1 HCl 1N) *vs* −32.8 for the (3S)-isomer (Kunz and Schanzenbach, 1989). As expected and in accordance with the result already obtained with the epimeric imine (Guenoun et al., 1997), the attack of the entering ketone acetal **53a** proceeded preferentially by the *Si* face. In contrary, the two other ketene acetal derivatives bearing an oxygen atom attacked from the other side (*Re* face).

The pronounced influence of the oxygen atom in the $\beta$ position of the ketene acetals (**53b** or **53c**) can be explained by its chelating property towards the $Zn^{++}$ ion leading to the stabilization of the transition complex. Similarly, a cis stereoselectivity was observed during the condensation of titanium enolates of 2-pyridylthioesters bearing oxygen containing groups (Annunziata et al., 1994) whereas trans $\beta$-lactams were always formed in presence of bulky, oxygen-free groups. Additionnally, the reaction of N-silyl imines with silyl ketene acetals in the presence of $ZnI_2$ produced the cis $\beta$-lactams only when an OR substituent was present in the starting ketene acetals (Colvin et al., 1988).

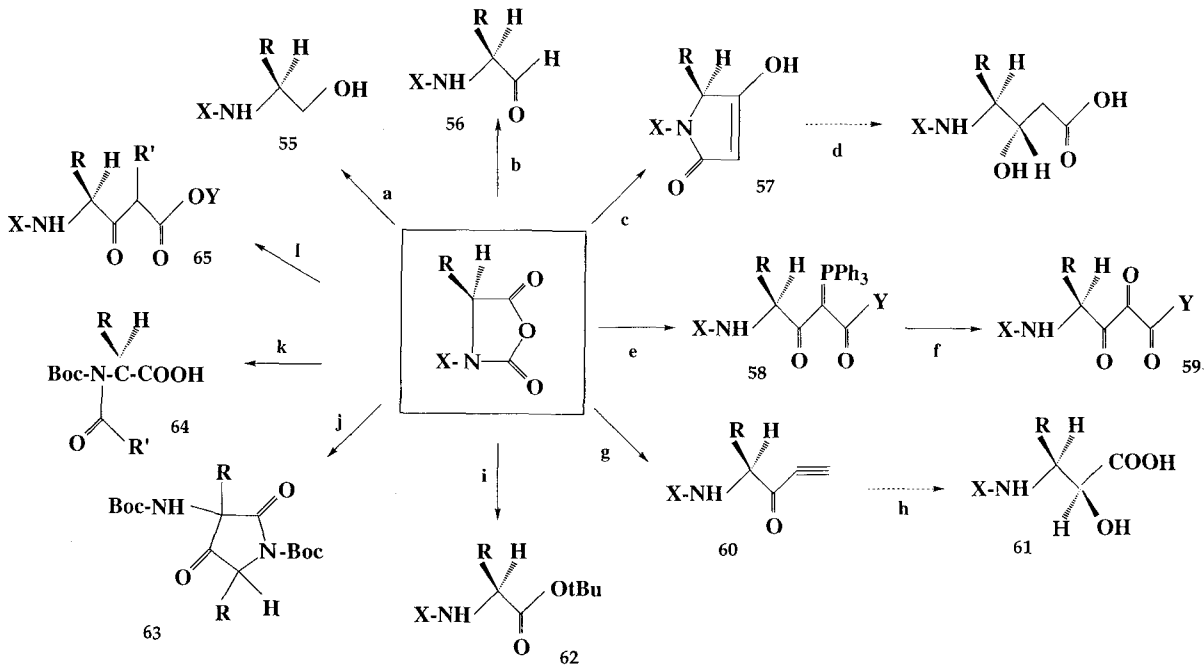
It is also noteworthy that beside a complete diastereoselectivity resulting from exclusive *Re* face attack, the major adduct is always the anti (*2S,3S*) isomer whatever the geometry of the silyl ketene acetal (**53b** or **53c**). Nevertheless, **53c** giving the best anti selectivity: 6/1 vs 1.9/1, is thus more interesting in increasing the yield of the optically pure methyl ester of (−)*2S,3S* phenylisoserine **54** [showing the same absolute value of rotatory power than its (+)*2R,3R* enantiomer (Bunnage et al., 1994) (Scheme 18)]. This useful intermediate of Taxotere preparation was used (Denis et al., 1994) for the esterification of 10-desacetyl baccatin III which hopefully takes place with complete epimerization at the $C_2$ center of the lateral chain.

This new chiral auxiliary easily prepared has been successfully used for the synthesis of optically pure $\beta$-amino esters and, obviously, will be valuable in other asymmetric amino transfer reactions.

## Synthesis of amino acid derivatives from N-protected carboxyanhydrides (UNCAs)

The use of N-protected carboxyanhydrides (UNCAs) has been widely described for the synthesis of peptides, either in solution (Rodriguez et al., 1992) or on solid support (Fuller et al., 1990; Xue and Naider, 1993). We have investigated the reactivity of UNCAs towards various nucleophiles (Scheme 19).

UNCAs can be reduced at room temperature in a few minutes by sodium borohydride in dimethylethylene glycol in the presence of water to produce N-protected $\beta$-amino alcohols **55** (Fehrentz et al., 1994a) in almost quantitative yields. When bulky hydrides were used at lower temperatures, they led to N-protected $\alpha$-amino aldehydes **56** (Fehrentz et al., 1994b): lithium tri-*tert*-butoxyaluminium hydride at −10°C or lithium tris[(3-ethyl-3-




**Scheme 19.** *a* $NaBH_4$, $H_2O$, DME, RT; *b* $AlLiH(OtBu)_3$, THF, O°C; *c* Melddrum's acid, THF, TEA, RT then AcOEt, Δ; *d* $NaBH_4$ then NaOH 1N; *e* $Ph_3P$ = COOY; *f* [O]; *g* $C_2HMgBr$, THF, −60°C; *h* $NaBH_4$, $CeCl_3$-$7H_2O$, MeOH, −78°C then DHP, PPTS then $NaIO_4$, $RuCl_3$, $CH_3CN/CCl_4$, $H_2O$ then $H_3O$+; *i* tert-butanol, $KHCO_3$, 72 h, 45°C; *j* DBU, THF; *k* R'MgBr, THF, 0°C; *l* LiR'CH-COOY, THF, −78°C

pentyl)oxy]aluminium hydride at 0°C in THF as solvent could smoothly reduce UNCAs to yield the corresponding aldehydes. These two reductive reactions to prepare alcohols and aldehydes derivatives are compatible with a broad set of side-chain and N-protections and are free from racemization.

UNCAs are reactive enough to promptly react with Meldrum's acid in the simple presence of a tertiary amine (triethylamine TEA, N,N-diisopropylethylamine DIEA, N-methylmorpholine NMM, etc.) (Fehrentz et al., 1994c). As described (Jouin et al., 1987), the obtained intermediates were cyclized to produce the corresponding enantiomerically pure tetramic acid derivatives **57**, which yielded by diastereoselective reduction the corresponding (4S,SS)-N-protected urethane-4-hydroxy-pyrrolidin-2-ones. The statine analogues were obtained after hydrolysis. UNCAs were also able to react with malonic acid derivatives. The reaction of UNCAs with benzyl ethyl malonate in the presence of sodium hydride easily lead to γ-N-benzyloxycarbonylamino-β-oxodicarboxylic esters in good yields. Their hydrogenolytic deprotection in the presence of 4-dimethylaminopyridine, followed by decarboxylation produced also the tetramic acid derivatives.

UNCAs efficiently react with phosphoranes to yield the keto phosphoranes **58** in excellent yields (Fehrentz et al., 1995). These compounds can produce by subsequent oxidation with oxone or [bis(acetoxy)-iodo]-benzene

the vicinal tricarbonyl derivatives **59**. These latter are potential inhibitors of serine proteases (Wasserman et al., 1992) or starting materials for the synthesis of natural compounds (Wasserman et al., 1993).

Reaction of ethynylmagnesium bromide with UNCAs at −60°C afforded the corresponding keto-acetylenic compounds **60** with acceptable yields (Audin et al., 1998). Reduction of the carbonyl moiety by sodium borohydride in the presence of lanthanoid chlorides yielded the propargylic alcohol with *syn* diastereoselectivity and good yields. After protection of the hydroxyl group, the propargylic ethers were converted to the norstatine derivatives **61** as previously described (Kourtal and Paris, 1996).

Treatment of UNCAs with primary and secondary alcohols at room temperature produced the corresponding esters. Under these mild conditions, *tert*-butanol was unable to react with UNCAs. The presence of a base and/or molecular sieves and higher temperature reaction in *tert*-butanol as solvent allowed the obtention of the corresponding *tert*-butyl esters **62**. The desired *tert*-butyl esters were prepared with 60–70% yield in the presence of potassium hydrogenocarbonate (1.25 equivalent) at 45°C in 45 hours (Chevallet et al., 1995). This method appeared to be available for the preparation of Z- or Boc- protected amino acid *tert*-butyl esters and proceeded without detectable racemization.

In the presence of a base in aprotic medium, UNCAs led to N-urethane protected-3,5-dialkyl-3-amino pyrrolidine-2,4-diones **63** (Pothion et al., 1996; Leban and Colson, 1996). Consequently the choice of the base should be carefully considered to optimize yields and avoid side products when UNCAs are used in peptide synthesis or in reactions with slow reactivity nucleophiles.

The reactivity of Grignard reagents with UNCAs did not lead as expected to the corresponding keto derivatives as major products. We observed that depending on the way of addition of the organometallic compounds, the reaction proceeded differently (Pothion et al., 1997):

- When the UNCA was added to the Grignard reagent we obtained a mixture of five different compounds which were identified as the corresponding N-urethane protected amino acid, keto compound, ester, disubstituted alcohol and N-acyl derivatives.
- When the organometallic reagent was added to the UNCA, we also obtained a mixture of the same products but in different proportions, the major one corresponding to the N-urethane protected N-acyl amino acid derivative **64**.

This reaction showed the particular reactivity of UNCAs towards some Grignard reagents.

N-protected $\gamma$-amino-$\beta$-ketoesters **65** can be easily prepared from the corresponding UNCAs by reaction with lithium enolates in good yields. These compounds are precursors of statine derivatives. In the case of the lithium enolate of ethyl acetate, the $\gamma$-amino-$\beta$-ketoesters derivatives (statones) were obtained (Paris et al., 1996). These N-protected $\gamma$-amino-$\beta$-ketoesters can produce statine derivatives i) as a mixture of diastereoisomers by reduction with non chiral agents or ii) as pure diastereoisomers by chiral reduction of

the corresponding ketones as described by Nishi et al. (1988) in the presence of Wilkinson catalyst. When the lithium enolates were prepared from alkylated ethyl acetate, N-protected $\alpha$–alkyl–$\gamma$-amino-$\beta$-ketoesters can be prepared in good yields from UNCAs. These compounds are particularly interesting because they are precursors of 2-alkylated statine derivatives which could be incorporated into peptide chains to yield potential enzyme inhibitors with a side chain in the S'1 subsite of the enzyme.

As shown in these experiments, UNCAs are very reactive intermediates, which are useful in peptide synthesis but also in chemistry of amino acid derivatives. They are interesting intermediates which can yield to N-protected $\beta$-amino alcohols, N-protected $\alpha$-amino aldehydes, N-protected tetramic acid derivatives (precursors of statine residues), vicinal tricarbonyl derivatives, N-protected ketoacetylenic compounds (precursors of norstatine residues), N-protected *tert*-butyl esters, pyrolidin-2,4-diones, N-protected-N-acyl amino acid derivatives and N-protected $\gamma$-amino-$\beta$-ketoesters alkylated or not in the $\alpha$-position. All these derivatives are important intermediates in the synthesis of potentially biologically active molecules or can be used as building blocks in combinatorial chemistry.

All these reactions with UNCAs are generally performed without racemization of the amino acid derivatives, in good yields and in a very simple and efficient way. They are the expression of the high reactivity and simple use of UNCAs which undoubtedly will lead to various other attractive intermediates.

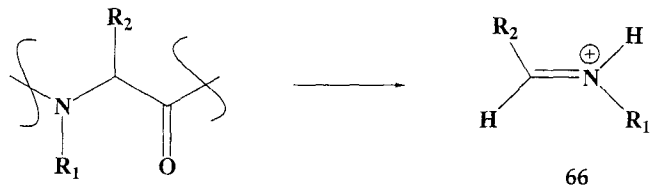
## Use of immonium ions to identify proteinogenic and non proteinogenic amino acids in solution-phase or solid-phase peptide synthesis

The Fast Atom Bombardment (FAB) mass spectrometry (Barber et al., 1981) is particularly well adapted to the characterization of polar compounds such as peptides (Biemann and Martin, 1987). Moreover, FAB is the analytical method of choice in the case of structurally modified amino acids, particularly to identify N- and C-alkylated residues (Schwarz and Eckart, 1986; Schwarz et al., 1986) as well as peptoids (Heerma et al., 1996 and 1997).

The FAB mass spectrum of a linear peptide can be divided in three distinctive parts providing each complementary analytical information:

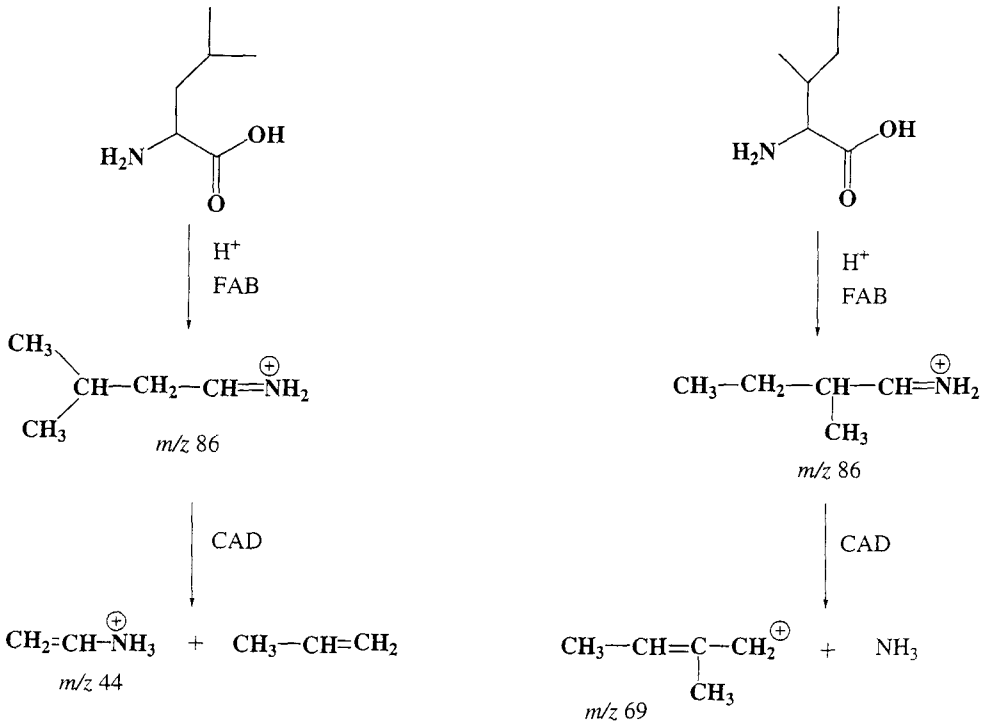
- The protonated molecular ion providing the molecular weight of the studied compound.
- Fragments ions featuring the N- and C-terminal sequences (Roepstorff and Fohlman, 1984)
- Immonium ions **66** (Scheme 20) which differ from the amino acid residue by the loss of CO and the addition of one hydrogen. These ions are very abundant in the low mass area of the spectrum and enable the amino acid identification (Falick et al., 1993) except for the isomeric residues leucine and isoleucine.

We have shown in the laboratory (Aubagnac et al., 1985) that isobaric leucine and isoleucine can be distinguished by the use of tandem mass



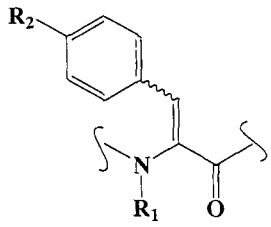
66

**Scheme 20**



**Scheme 21**

spectrometry (McLafferty, 1983). This bidimentional technique is undertaken to identify a selected ion by colliding it with a neutral gas giving rise to a spectrum of characteristic daughter fragment ions. The Collision Activated Dissociation (CAD) applied respectively to leucine and isoleucine immonium ions at *m/z* 86 lead to CAD spectra where the major fragment ion was in the case of leucine the ion at *m/z* 44 issued from the McLafferty rearrangement loosing propene and in the case of isoleucine the ion at *m/z* 69 formed by the loss of ammonia (Scheme 21). Such criterion to distinguish leucine from isoleucine was thereafter extented to norleucine and to peptides containing these residues (Heerma and Bathelt, 1986; Heerma et al., 1994) and was proven to be general as these results were reproduced by low energy CAD on a triple quadrupole mass spectrometer (Huslt, 1996). More recently, we applied the same method to identify non proteinogenic phenylalanine based




| | $R_1$ | $R_2$ |
|---|---|---|
| **67** ΔPhe | H | H |
| **68** $NCH_3$ ΔPhe | $CH_3$ | H |
| **69** $NCH_3$ ΔTyr($OCH_3$) | $CH_3$ | $OCH_3$ |

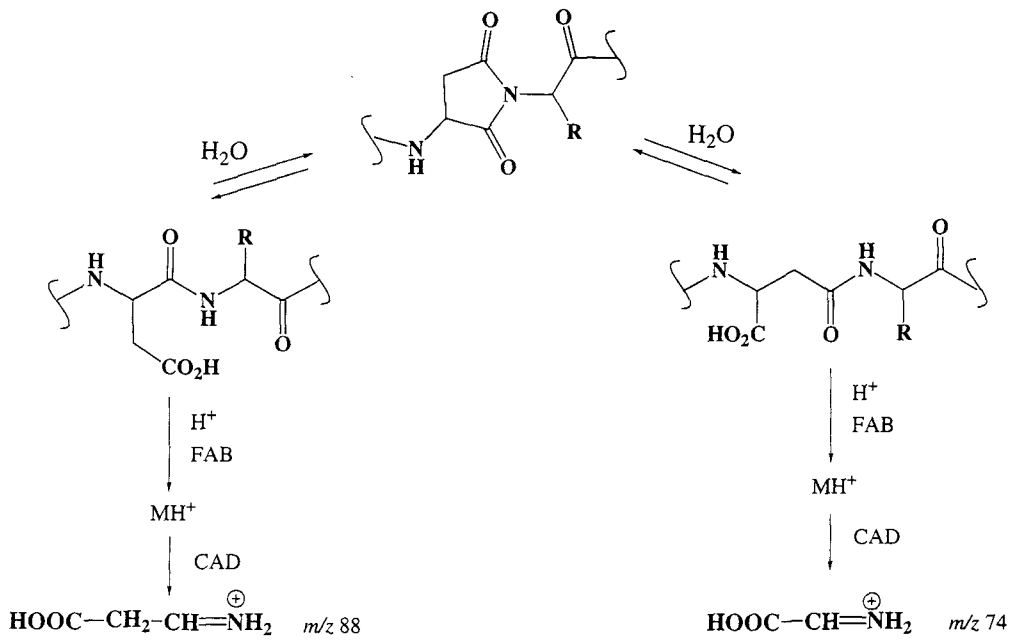
| Residue | Peptides | Immonium Ion | CAD |
|---|---|---|---|
| **67** | Boc-Leu-ΔPhe-Gly-$OCH_3$ | 118 | *m/z* 91 and *m/z* 89 |
| **68** | Boc-Leu-$NCH_3$ΔPhe-Gly-$OCH_3$ | 132 | *m/z* 91 and *m/z* 89 |
| | Tentoxine (cyclic tetrapeptide) | | *m/z* 116 and *m/z* 117 |
| **69** | Boc-$NCH_3$Ala-Leu-$NCH_3$ΔTyr($OCH_3$)-Gly-$OCH_3$ | 162 | *m/z* 89 and *m/z* 121 |
| | H-$NCH_3$Ala-Leu-$NCH_3$ΔTyr($OCH_3$)-Gly-OH | | *m/z* 146 and *m/z* 147 |

**Scheme 22**

amino acids (Aubagnac et al., 1996a) (Scheme 22). N-Methylated residues were evidenced by the loss of $CH_3^\circ$ and $CH_4$ as described in the literature (Schwarz and Eckart, 1986). The dehydrophenylalanine residue was evidenced by the presence of the ion at *m/z* 89 next to the tropylium ion at *m/z* 91 which is characteristic of the benzyl side-chain. The aromatic substitution of one hydrogen by a methoxy group implied a 30 Daltons displacement of the tropylium ion from *m/z* 91 to *m/z* 121.

Furthermore, α-aspartic acid and β-aspartic acid were also distinguished in the laboratory (Aubagnac et al., 1996c) by tandem mass spectrometry to detect any unwanted isomerization *via* a cyclic intermediate (Scheme 23) during peptide synthesis. Such differentiation has been previously studied according to other criteria (Biemann and Scoble, 1987; Lloyd et al., 1988). CAD spectra of the protonated molecules exhibited clear differences. First, the immonium ions at *m/z* 88 and *m/z* 74 were abundant in the case of α-aspartic acid and β-aspartic acid containing dipeptides, respectively. Besides, each protonated structure lost a molecule of water according to a fragmentation pathway which implies the carbonyl function either in the α or β position, the subsequent fragmentations being characteristic of each structure.

Finally, we have studied the abundance of immonium ions in Secondary Ion Mass Spectrometry (SIMS) Mass Spectrometry (Benninghoven et al., 1987) to follow step-by-step solid-phase peptide synthesis (Merrifield, 1963). Indeed, standard spectroscopic methods to identify organic compounds such as $^1H$ NMR, $^{13}C$ NMR and FAB or electrospray (ESI) mass spectrometry require prior dissolution of the studied structure in an appropriate organic solvent. Therefore these techniques could only be used for solution synthesis or at the end of the solid-phase synthesis after chemical cleavage of the structures from the support. Noteworthy, other analytical methods such as




**Scheme 23**

FT-IR (Yan et al., 1995), CP-MAS NMR (Lippens et al., 1997) are also studied for the same purposes. Static SIMS mass spectrometry was used in the laboratory to follow supported peptide syntheses with both Boc/Merrifield (Aubagnac et al., 1996b) and Fmoc/Sheppard (Aubagnac et al., 1997) strategies. Only a small area of a single bead was subjected to bombardment of energetic ions without the need for a matrix which means that this non destructive technique could be used to follow multistep syntheses, in particular for the analytical control of supported library (Gallop et al., 1994). At each step of the synthesis, the SIMS mass spectrum contained on one hand the immonium ions corresponding to each constituent amino acid (Pro m/z 70; Phe m/z 120; . . .) and on the other hand ions characteristic of the present protecting groups (Boc m/z 57; Fmoc m/z 179).

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**Authors' address:** Dr. Jean Martinez, UMR 5810, Université Montpellier II, Place E. Bataillon, F-34095 Montpellier Cédex 05, France,
Fax 04 67 41 20 17, E-mail: martinez@pharma.univ-montp 1.fr

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