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Amino
Acids

Urethane protected amino acid N-carboxyanhydrides and fluorides (U-NCAs and U_2AAFs)

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Summary. In order to obtain peptide analogues containing a central pyrrolide bond, as potential mechanism-based inhibitors of the HIV-1 proteinase, activated derivatives of amino acids were required. Treatment of a N,N-*bis*(Boc) amino acid pyridinium salt with cyanuric fluoride in dichloromethane furnished the corresponding *bis*(Boc) amino acid fluoride (Boc_2AAF). Use of the Vilsmeier reagent in acetonitrile, instead of the cyanuric fluoride, led to a N-Boc amino acid N-carboxyanhydride (Boc-NCA). From a mixed N-Z,N-Boc amino acid salt a N-Z,N-Boc amino acid fluoride and a Z-NCA were respectively obtained. The very sensitive Young test showed that during the coupling of the N-benzoyl-L-Leucine N-carboxyanhydride or the N-benzoyl N-Boc-L-leucyl fluoride with ethyl glycinate the degrees of racemization were weak. Owing to the electronegativity and the small size of the fluorine atom, the *bis*(urethane) amino acid fluorides are efficient acylating agents for amines and pyrrole anions.

Keywords: Amino acids – N,N-bis(alkoxycarbonyl) amino acid fluorides – N-alkoxycarbonyl amino acid N-carboxy anhydrides – N-aminoacyl pyrroles – *tert*-Butoxycarbonyl group participation

Introduction

In a synthetic scheme for the preparation of potential inhibitors of the HIV-1 protease incorporating a substituted 2-pyrrole carboxylic acid residue as a functionalized proline mimic, we came upon the problem of acylating a pyrrole anion using an activated derivative of an amino acid possessing easily removable N-protecting groups and devoid of an exchangeable NH hydrogen. Owing to the great current interest in the chemistry of urethane protected amino acid N-carboxyanhydrides and halides (U-NCAs and UAAXs) we planned to use such type of reagents for that purpose. During this study we realized the synthesis of the Boc- and Z-NCAs by a new route, as well as the preparation of the unknown N,N-*bis*(urethane) amino acid fluorides (U_2AAFs).

Objective and background

HIV produces a small dimeric aspartyl proteinase which specifically cleaves the polyprotein precursors encoding the structural proteins and enzymes of the virus. This proteolytic activity is absolutely required for the production of mature infectious virions and is therefore one of the most interesting targets for synthetic inhibitors, in an approach to the therapy of AIDS (Meek, 1992; Huff, 1991). As the selectivity of the inhibition is a crucial factor for the efficiency of an enzyme inhibitor, we have been particularly interested in the rare ability of the HIV-1 proteinase to cleave substrates having a phenylalanine or a tyrosine at P_1 and a proline residue at P_1' position (Schechter and Berger notation (Schechter and Berger, 1967)). Indeed, few proteinases can efficiently cleave a peptide bond before an iminoacid (Yaron and Naider, 1993). We propose the synthesis of substrate analogs of the Ser-Gln-Asn-Tyr-Pro-Ile-Val sequence which spans the p17–p24 cleavage site of the Pr 55 ^{gag} human immunodeficiency virus polyprotein. These compounds of general formula P_n.. P_2-Phe-[A]-P_2'.. P_n', possessing a phenylalanine-"functionalized proline mimic" central segment could behave as competitive inhibitors, affinity labels, "suicide" substrates or prodrugs. Peptides containing a dehydropipecolic acid derivative or a substituted *ortho-* or *meta*-aminobenzoyl residue, as substitutes of the prolyl P_1' residue, which constitute latent α-eneiminium or quinoniminium methide functions, have already been prepared (Mazaleyrat et al., 1992; Xie et al., 1992).

If we consider a 2-pyrrole carboxylic acid derivative as a proline substitute, selective enzymic hydrolysis of the pyrrolide bond between phenylalanine and a pyrrole having a good leaving group X can result in the formation of an azafulvenium ion (Jones et al., 1977), an α-eneiminium electrophile (Fig. 1). Then, this electrophile can react with an enzyme active site nucleophile Nu(E) leading to its inactivation ("suicide" mechanism). A. D. Abell and J. C. Litten have recently proposed an analogous scheme for inhibition of the HIV-1 protease (Abell and Litten, 1992).

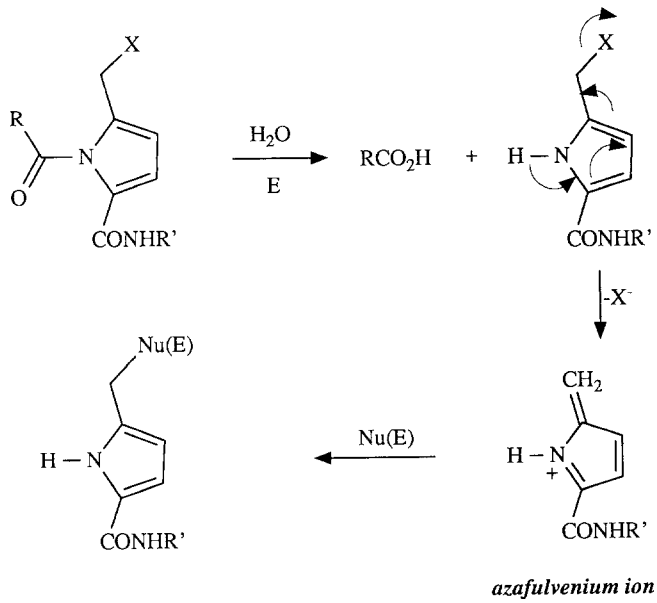


Fig. 1. Postulated mechanism of inactivation with a pyrrole derivative

Our first attempts to react a N-protected aminoacid with the weakly nucleophilic 2-pyrrole carboxylic acid esters, under either the mixed anhydride or

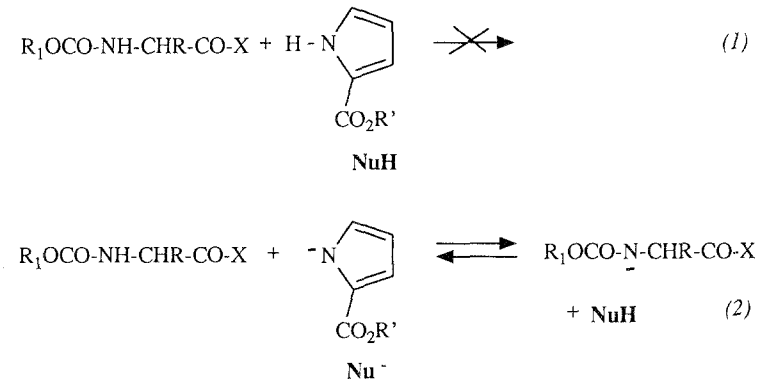


Fig. 2. Acylation of neutral or anionic pyrrole derivatives with N-monoprotected amino acids

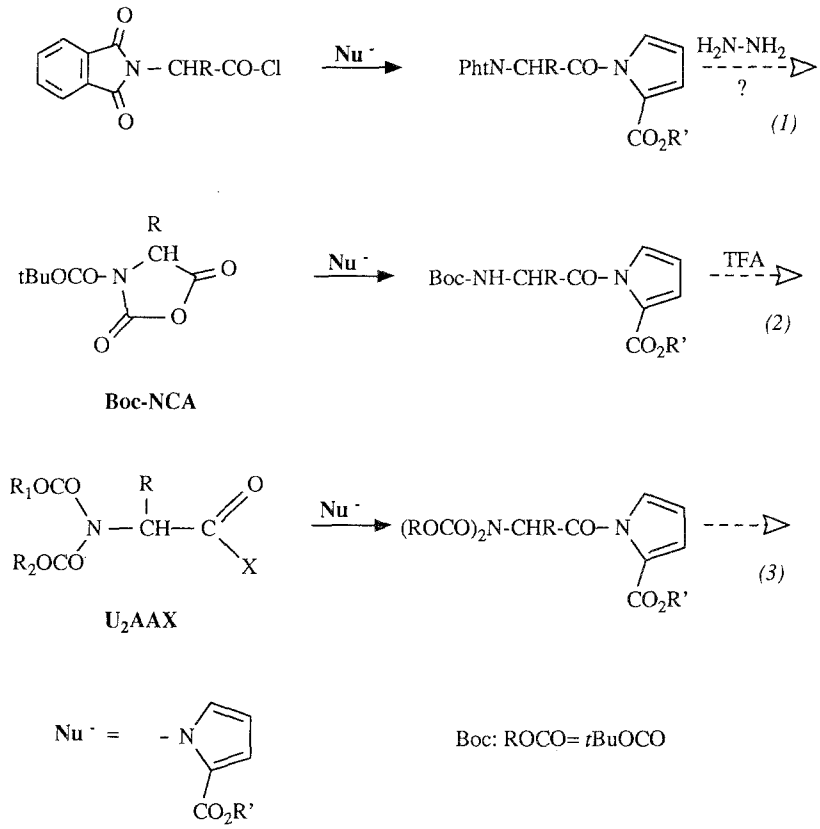


Fig. 3. Acylation of anionic pyrrole derivatives with N,N-disubstituted amino acids

the DCC/HOBt methods, were unsuccessful (Fig. 2, eq 1). Acylation of the corresponding pyrrole anion was also inefficient. In the last case, a possible explanation for this lack of reactivity, could be a proton abstraction from the carbamate NH, leading to the unreactive protonated nucleophile (Fig. 2, eq 2). To avoid this proton transfer, we thaught of a bifunctional protecting group such as the phtaloyl one. However, the pyrrolide bond, which is much more

reactive than a classical amide one (Menger and Donohue, 1973; Lee et al., 1983), will not survive in one of the subsequent elongation steps, the cleavage of the protecting group with hydrazine, an α-nucleophile (Fig. 3, eq 1). We considered then the use of a Boc-NCA or a N,N-*bis*(alkoxycarbonyl) amino acid halide together with the possible protecting group removal with an acid (Fig. 3, eq 2 and 3).

Amino acid N-carboxy anhydrides (Leuchs anhydrides, NCAs) are usualy prepared from aminoacids and phosgene or a phosgene substitute. They are not very stable and have mainly been used for the synthesis of polyaminoacids (Blacklock et al., 1987; Wilder and Mobashery, 1992). Many attempts have been made to prepare N-protected NCAs. Recently, W. D. Fuller and M. Goodman have succeeded in the preparation of the stable N-urethane protected NCAs, the U-NCAs, by acylation of NCAs in the presence of N-methyl morpholine (Fig. 4). The U-NCAs are activated internal mixed anhydrides. They are highly soluble in different organic solvents and can be used at high concentration for difficult coupling reactions. The only coproduct during their fast aminolysis is carbon dioxide. In solid phase synthesis the monitoring is therefore very easy

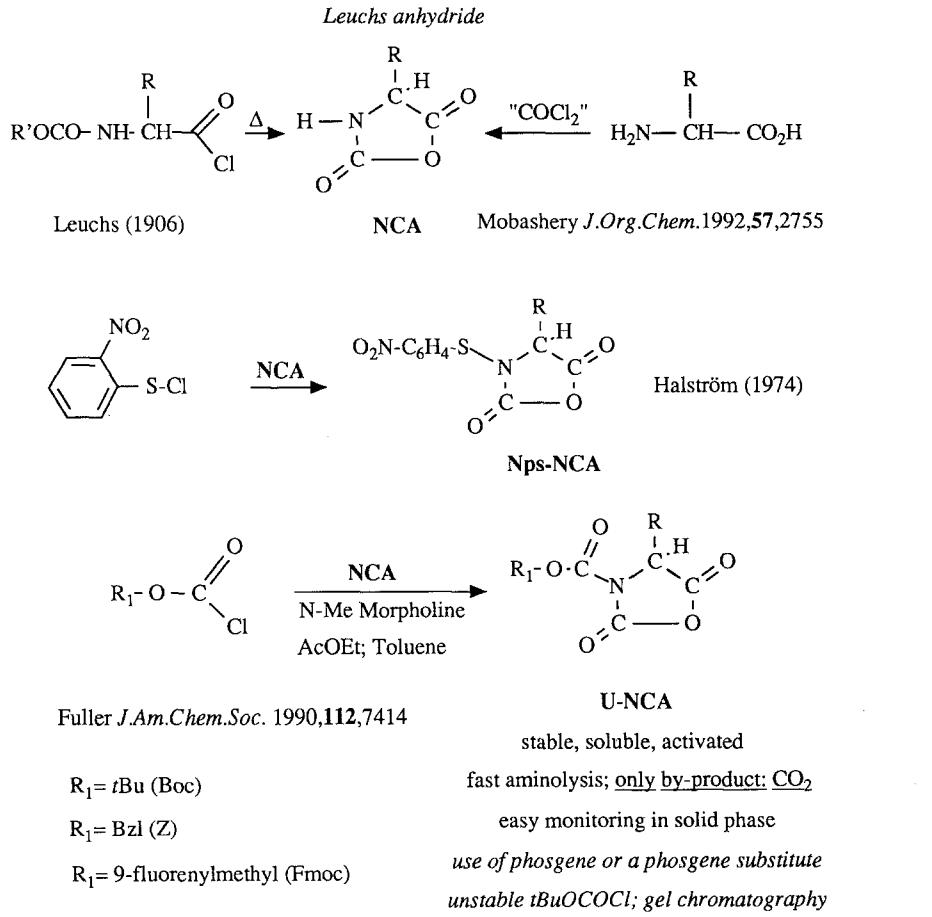


Fig. 4. Synthesis of unprotected and protected amino acid N-carboxy anhydrides (NCAs and U-NCAs)

(Xue and Naider, 1993). However, gel chromatography is necessary to obtain analytical pure U-NCAs. For the synthesis of the Boc-NCAs, use of the unstable *tert*-butyl chlorocarbonate is also necessary (Fuller et al., 1990).

As the Leuchs anhydrides, the N-protected amino acid halides have been known as coupling reagents since the earliest days of peptide synthesis (Wieland and Bodansky, 1991). Mainly due to the L. A. Carpino's group, a renewed interest in the preparation of this type of reagents is observed (Fig. 5). The necessary basic trialkylamine coreagent used for the coupling of the N-Fmoc amino acid chlorides causes immediate conversion to the corresponding oxazolones which are more slugghish in their further conversion. Moreover, in the case of the trifunctional amino acids, *tert*-butyl type protecting groups (Boc, *tert*-butyl ester or ether) cannot be present (Carpino et al., 1986). These limitations are overcome in the case of the corresponding fluorides. Furthermore, owing to the electronegativity and the small size of the fluor atom, the N-Fmoc-, Z- and Boc-amino acid fluorides have a high reactivity for the coupling of hindered amino acids (Carpino et al., 1991; Bertho et al., 1991).

We knew also from the work of the U. Ragnarsson group that by using di-*tert*-butyl dicarbonate and 4-dimethylaminopyridine (Boc_2O/DMAP) it is possible to *tert*-butoxycarbonylate Boc- or Z-amino acid esters (Fig. 6, eq 2) (Gunnarsson et al., 1988; Gunnarsson and Ragnarsson, 1990). This reaction, as well as that of amides (Fig. 6, eq 1) (Flynn et al., 1983; Davidsen et al., 1991), probably involves the formation of a 1-*tert*-butoxycarbonyl-4-dimethylaminopyridinium ion intermediate. We have previously prepared water-soluble salts of that type

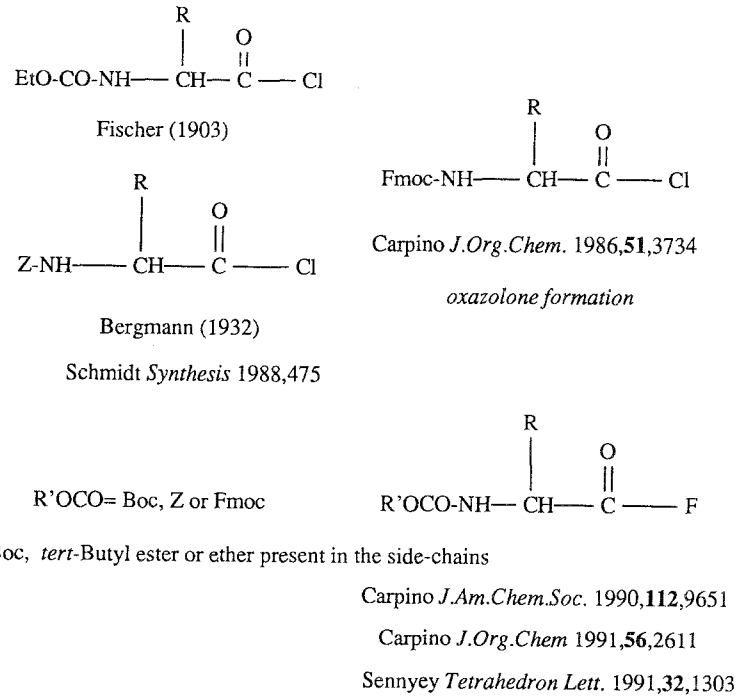
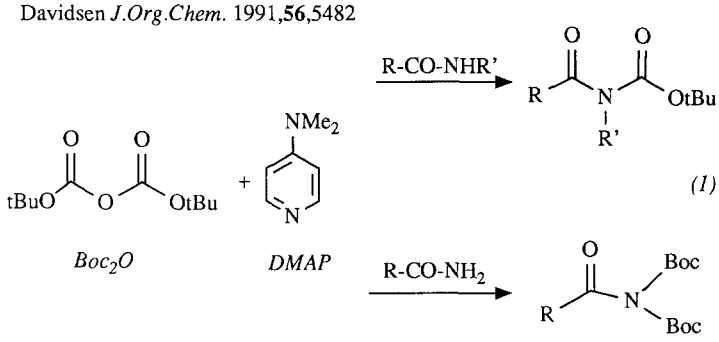


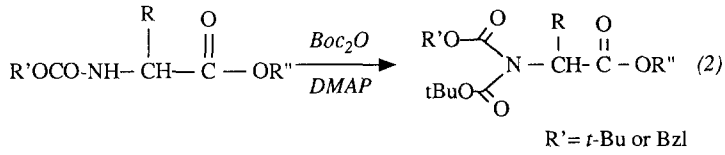
Fig. 5. Structures of some N-substituted amino acid halides

Grieco *J.Org.Chem.* 1983,**48**,2424

Davidsen *J.Org.Chem.* 1991,**56**,5482



Ragnarsson *Acta Chem. Scand.* 1990,**44**,944



Guibé-Jampel *Synthesis* 1977,772

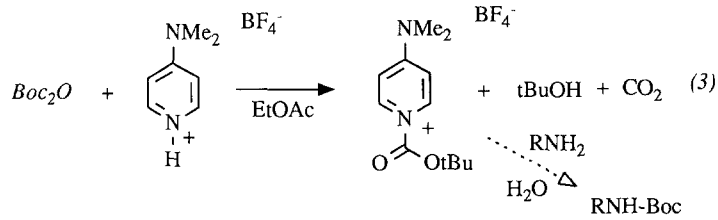


Fig. 6. *tert*-butyloxycarbonylation of amides, carbamates and amines

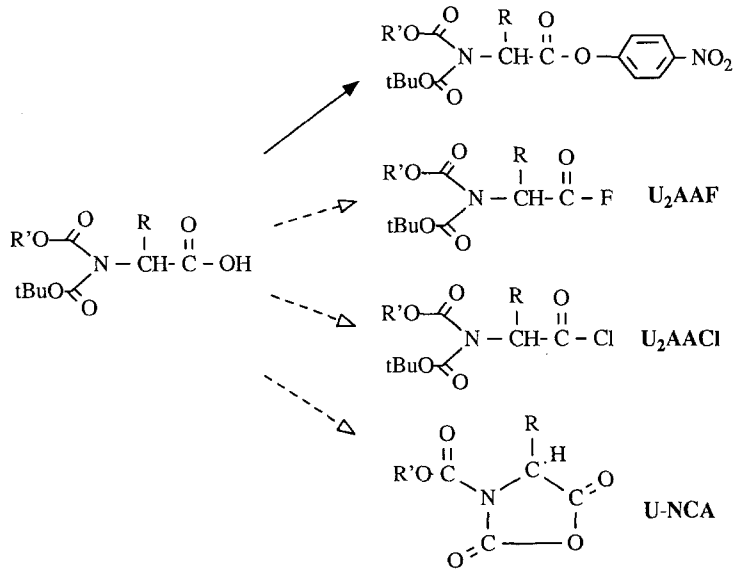


Fig. 7. Activation of a N,N-*bis*(alkoxycarbonyl)amino acid

and used them for the *tert*-butoxycarbonylation of amines in aqueous media (Fig. 6, eq 3) (Guibé-Jampel and Wakselman, 1977).

For coupling the N,N-*bis*(alkoxycarbonyl) amino acids, K. Gunnarsson and U. Ragnarsson have used the DCC/HOBt or the *para*-nitrophenyl ester methods (Gunnarsson and Ragnarsson, 1990). We wondered whether the unknown *bis*-(alkoxycarbonyl) amino acid halides (U_2AAXs) could be prepared or if an intramolecular reaction could lead to the U-NCAs (Fig. 7).

Results

Experimentally, treatment of a N,N-*bis*-Boc amino acid pyridinium salt with cyanuric fluoride at low temperature gives the corresponding fluoride (Fig. 8, eq 1). This fluoride is accompanied by a small amount of a U-NCA, and the percentage of this by-product increases with the temperature (Savrda and Wakselman, 1992). On the other hand, treatment of the *bis*-Boc amino acid salt with the Vilsmeier reagent induces the formation of the U-NCA with a good yield (Fig. 8, eq 2). The N,N-*bis*-Boc amino acid chloride, cyanuric ester or imidoyl ester are possible intermediates for this intramolecular reaction, but not the fluoride which does not spontaneously cyclize (Savrda and Wakselman, 1992). These U-NCAs are obtained with a high degree of purity without gel chromatography purification (Table 1).

The optical rotations of the U-NCAs prepared by our method were often higher than that reported by Fuller. We checked that no epimerization (racemization) occurred during both the formation and the aminolysis of our U-NCAs, using the very sensitive Young test (Table 2).

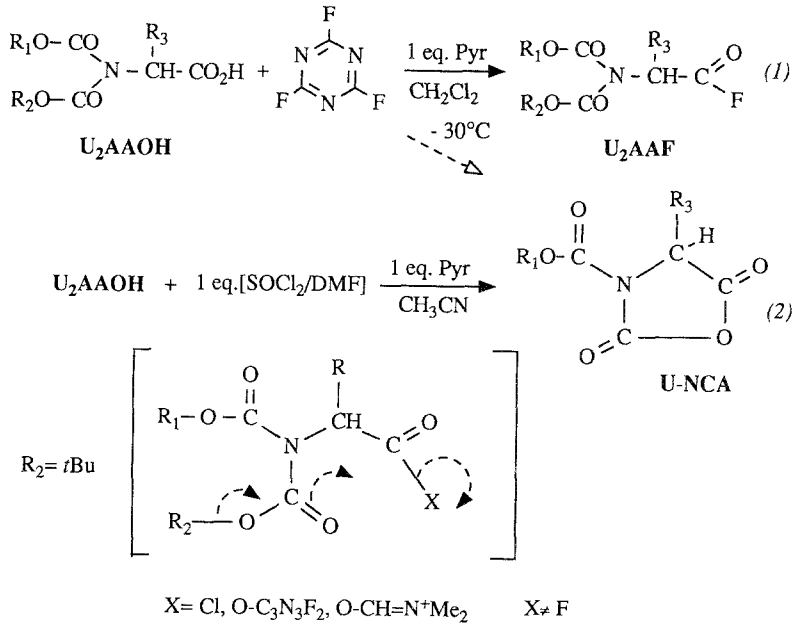
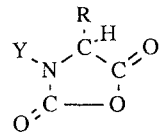


Fig. 8. Reactions of a N,N-*bis*(alkoxycarbonyl)amino acid with cyanuric fluoride and Vilsmeier reagent

Table 1. Characteristics of the U-NCAs

N-Protected Aminoacid N-Carboxyanhydrides



= Y-AA-NCA

Y-AA-NCA	m.p. °C	yield%	$[\alpha]_D^{25}$ (1.8;THF)	Fuller m.p. °C	Fuller yield%	Fuller $[\alpha]_D^{25}$ (1.8;THF)
Boc-Gly-NCA	147-149	72	-	-	-	-
Boc-Phe-NCA	102-104	92	+ 120.1	-	-	-
Boc-Ala-NCA	101-103	80	+ 56.9	103-104	65	+ 21.6
Boc-Val-NCA	117-119	86	+ 59.7	-	-	-
Boc-Ser(OBzl)-NCA	99-101	89	+ 52.3	98-99.5	52	+ 47.2
Boc-Asp(OBzl)-NCA	106-108	82	+ 35.1	-	-	-
Z-Gly-NCA	130-132	87	-	-	-	-
Z-Phe-NCA	105-106	75	+ 138.8	105-106	48	+ 127.6
Bz-Leu-NCA	134-136	85	+ 272.3	-	-	-

Table 2. Assays for racemization. Young test of the RCO-NCAs

Bz-L-Leu-NCA + NR_3 + HCl·H-Gly-OEt ⟶ Bz-Leu-Gly-OEt + CO_2 + HCl·NR_3

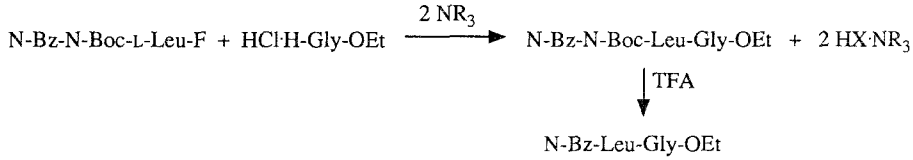
	solvent	NR_3	Bz-Leu-Gly-OEt Yield %	Bz-Leu-Gly-OEt e.e. % (L)
i	CH_2Cl_2	$N(Et)_3$	83	72.9
ii	CH_2Cl_2	N-Me Morpholine	85	96.9
iii	DMF	N-Me Morpholine	92	98.2

Numerous U-NCAs are now commercialy available. Our method seems particularly appropriate for the synthesis, with a great degree of optical and chemical purities, of the Boc- and the Z-NCAs derivatives of unnatural amino acids.

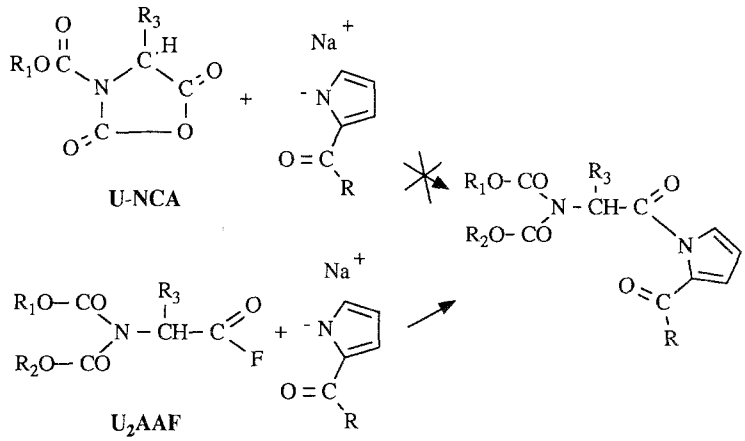
The preparation and the aminolysis of the *bis*-(alkoxycarbonyl) amino acid fluoride is also racemization free because the coupling of N-benzoyl-N-Boc-L-leucyl fluoride with ethyl glycinate hydrochloride, in the presence of two equivalents of base, followed by the trifluoracetolysis of the Boc group, gave the N-Bz-Leu-Gly-OEt dipeptide with a good optical rotation (Table 3).

The N-Boc N-carboxy aminoacid anhydrides proved to be unreactive towards anions of pyrrole-2-carboxylic acid esters. However, the coupling of the N,N-*bis*(alkoxycarbonyl) amino acid fluorides efficiently gave analogs of a di-

Table 3. Assays for racemization. Young test of the $(RCO)_2AAFs$



	solvent	NR_3	Bz-Leu-Gly-OEt	
			Yield %	e.e. % (L)
i	CH_2Cl_2	$N(Et)_3$	60	95.2
ii	CH_2Cl_2	N-Me Morpholine	67	91.2
iii	DMF	N-Me Morpholine	60	96.0



R_1, R_2= tBu, $C_6H_5CH_2$; R= Obzl, OtBu, H

Fig. 9. Synthesis of amino acid pyrrolides

peptide having an internal pyrrolide bond and easily removable N-protecting groups (Fig. 9). For the coupling of hindered amino acids such as α-amino-isobutyric acid, Boc-PheNCA is more reactive than Boc-PheF (Spencer, 1992); however Fmoc-AibF is more reactive than Fmoc-AibNCA (Wenschuh et al., 1993). Application of the here described chemistry for the synthesis of potential HIV-1 PR inhibitors is under study.

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