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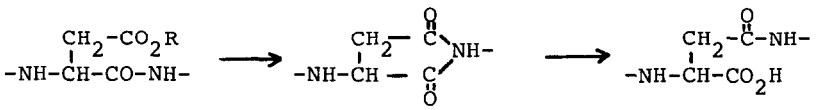
CYCLOHEXYL ESTER AS A NEW PROTECTING GROUP FOR ASPARTYL PEPTIDES TO MINIMIZE ASPARTIMIDE FORMATION IN ACIDIC AND BASIC TREATMENTS

James P. Tam,* Tai-Wai Wong, Mark W. Riemen, Foe-S. Tjoeng and R. B. Merrifield

The Rockefeller University, New York, New York 10021

Summary: Boc-Asp(OcHex) and Boc-Glu(OcHex) were synthesized and used in solid phase peptide synthesis. cHex ester is stable to TFA, cleavable by HF and minimizes aspartimide formation and α,β-rearrangement of aspartyl peptides in acid and particularly in base.

The major side reaction during the synthesis of peptides containing aspartyl sequences such as Asp-Gly, Asp-Ser and Asp-His is aspartimide formation.[1-4] Subsequently during aqueous exposure, the imide opens to give mainly the β-isomer. Since many peptide hormones, biologically active factors, enzymes, and toxins contain these sequences, their syntheses often pose a difficult problem. We wish to report the design and development of a new protecting group for aspartyl peptides that minimizes aspartimide formation during either acidic or basic treatment.

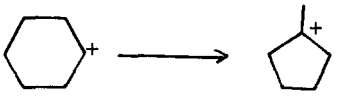


aspartimide β-peptide (major)

Based on mechanistic analyses of aspartimide formation, our effort was centered on the search for a protecting group that can fulfill the following criteria: (1) provide steric hindrance to intramolecular aminolytic attack of the ester by the amide nitrogen in acidic and basic media (2) stable to TFA treatment but cleavable in strong acid (3) the carbenium ion produced by cleavage of the protecting group should be stable and non-reactive to recapture by the peptide. The first two criteria are necessitated by the repeated acidic and basic treatment in the normal procedure of peptide synthesis. An acid-stable but sterically hindered protecting group should offer a starting point for the development of a group to prevent imide formation. The last criterion

is often ignored in the design of new protecting groups. It can be achieved if the carbenium ion rearranges to a more stable ion while still within the solvent cage. Thus, the carbenium ion becomes inactivated before it can be recaptured by the reactive moieties of the peptide. This concept in protecting group design has been recently explored in a new protecting group for the phenolic moiety of Tyr.[5]

Of the many protecting groups we have examined, the cyclohexyl (chex) ester[6] seems best to fulfill these requirements. The secondary cyclohexyl ester is more acid stable and more sterically hindered than the primary benzyl ester. Furthermore, the secondary cHex carbenium ion was shown by Olah and Lukas[7] to rearrange in strong anhydrous acid to a more stable tertiary methylcyclopentyl cation. This rearrangement meets our last requirement of a self-inactivated carbenium ion.



Boc-Asp(OcHex)-OH (m.p. 93-95° C) was most conveniently prepared through a two step synthesis, in 85% yield as indicated in scheme 1. A water soluble carbodiimide (wsc[1]) was used as the coupling reagent in order to allow ready removal of the N-acyl urea by-product. A catalytic amount of 4-(dimethylamino)pyridine (DMAP) was necessary to accelerate the reaction.[8]

$$\text{Boc-Asp-OBzl} \xrightarrow{a} \text{Boc-Asp(OcHex)-OBzl} \xrightarrow{b} \text{Boc-Asp(OcHex)-OH}$$

Scheme 1 a. Wsc(1eq), cHex-OH(3eq), DMAP(10 mol%), CH_2Cl_2, 0° C, 1h. b. H_2, 5% $Pd/BaSO_4$, 95% EtOH, 17h.

The cHex ester is an order of magnitude more stable than the corresponding benzyl ester in 50% TFA/CH_2Cl_2. It is quantitatively cleaved by HF or methanesulfonic acid in 1.0h at 0° C. It is also stable to mild saponification, hydrazinolysis, and hydrogenation.

Table I

Aspartimide Formation From

Boc-Glu(OBzl)-Asp(OR)-Gly-Thr(Bzl)-O-CH_2-Polystyrene Resin

in Acid and Base Treatments

Condition			Product (%)[a]					
			1 R=Bzl			2 R=cHex		
Reagent	Temp (°C)	Time (h)	alpha	beta	imide	alpha	beta	imide
HF	0	0.5	87.5	0	12.5	95.6	0	4.4
HF	-20	0.5	97.2	0	2.8	99.2	0	0.8
TEA[b]	20	24.0	0.0	0	100.0[c]	86.0	0	14.0
DIEA[b]	20	24.0	49.0	0	51.0	99.7	0	0.3

a. Ninhydrin positive compounds were analyzed on a Beckman 120B amino acid analyzer AA-15 column (0.9x54 cm) with pH 3.20 citrate buffer at 59° C. Elution times were 49 min for β-peptide (<0.05% detected), 70 min for the α-peptide and 130 min for the aspartimide. See reference 3 for details. b. Followed by HF cleavage, 0° C, 0.5h. The values (B in %) due to base treatment are corrected according to the equation $B = 100(T-A)/(1-A)$, where T = total mole fraction imide observed and A = mole fraction imide due to HF cleavage. c. Work up in dilute base (5% aqueous Et_3N) gave 85% β-peptide and 15% α-peptide.

To test the aspartimide formation, we used a model peptide, Glu-Asp-Gly-Thr which had been shown previously to give extensive imide formation when Bzl esters were used as protecting groups.[3] Two synthetic peptides (1 and 2), with either cHex or Bzl ester protecting groups on Asp were synthesized using the solid phase method. As shown in Table 1, upon HF treatment at 0° C, peptide 2 gave only 4.4% imide as compared to 12.5% in 1. More significantly, peptide 2 gave an almost negligible amount of imide (0.8%) at -20° C. When peptides 1 and 2 were treated with 5% trialkylamine in CH_2Cl_2 for 24 h, the Asp(OcHex)-containing peptide 2 gave dramatically less imide than the corresponding Asp(OBzl)-containing peptide 1 in both DIEA and TEA. Thus in a normal peptide synthesis with 10 min DIEA neutralization cycles, Asp(OcHex) would give <0.002% of imide per step. This is a significant reduction compared to 0.34% per step calculated for the Bzl ester. These data also show that DIEA is more desirable in peptide synthesis than TEA for minimization of imide formation.[10]

We have also explored the use of cHex for Glu peptides. Boc-Glu(OcHex)-OH (m.p. 135-136° C, DCHA salt) was synthesized in 82% overall yield as indicated in scheme 1. Our preliminary results have indicated it possesses chemical properties similar to those of Boc-Asp(OcHex). The usefulness of the cHex ester in preventing side reactions[11] related to Glu peptides is currently under investigation.

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1. Boc - tertbutoxycarbonyl, Bzl - benzyl, cHex - cyclohexyl, Asp - aspartic acid, Glu - glutamic acid, Gly - glycine, Thr - threonine, TFA - trifluoroacetic acid, DIEA - diisopropylethylamine, TEA - triethylamine, wsc(water soluble carbodiimide) - 1-ethyl-3-(-3-dimethylaminopropyl)-carbodiimide hydrochloride, DMAP - 4(dimethylamino)pyridine.

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