(3). To a suspension of 10.71 g (100 mmol) of 3-fluoro-DL-alanine in 40 ml of 90% methanol was added a solution of 5.61 g (100 mmol) of potassium hydroxide in 20 ml of 90% MeOH followed by the addition of a solution of 10.1 g (100 mmol) of 2,4-pentanedione in 60 ml of methanol. The mixture was refluxed for 20 min and concentrated in vacuo. Recrystallization from 2-propanol (300 ml) yielded 17.6 g (78%) of product.

Anal. Calcd for $C_8H_{11}NO_3FK$: C, 42.27; H, 4.87; N, 6.16; F, 8.35. Found: C, 42.30; H, 4.88; N, 6.23; F, 8.11.

^1H NMR (D_2O): δ 1.97 (s, 3, CH_3), 2.02 (s, 3, CH_3), 4.4 (m, 1, CH, $J_{H-F} \approx 31$ Hz), 4.8 (m, 2, CH_2F, $J_{H-F} \approx 46$ Hz).

Resolution of 3-fluoro-DL-alanine was performed starting from potassium *N*-(1-methyl-2-acetylvinyl)-3-fluoro-DL-alaninate.

To a solution of 22.72 g (100 mmol) of potassium *N*-(1-methyl-2-acetylvinyl)-3-fluoro-DL-alaninate (3) in 200 ml of methanol was added 40.5 g (102.5 mmol) of quinine hydrochloride dihydrate. The mixture was heated under reflux for a period of 1 h. Application of the same method as described above for the separation and isolation gave 3-fluoro-D-alanine, $[\alpha]^{25}D$ −10.4°, and 3-fluoro-L-alanine, $[\alpha]^{25}D$ +10.4°, in 54.2 and 64% yield, respectively.

Resolution of DL-Alanine. Application of the same method gave quinine *N*-(1-methyl-2-acetylvinyl)-L-alaninate in 93% yield, mp 142–143 °C, $[\alpha]^{25}D$ −73.8°.

Anal. Calcd for $C_{28}H_{37}N_3O_5 \cdot \frac{1}{2}H_2O$: C, 66.64; H, 7.59; N, 8.33. Found: C, 66.45; H, 7.80; N, 8.10.

L-Alanine was obtained from the crystalline enamine–quinine salt as described above for the fluoroalanine in 76% yield, $[\alpha]^{25}D$ +12.8° (*c* 5%, 5 N HCl) (lit.[7] +13°).

Registry No.—DL-**1**, 16652-37-2; D-**1**, 35455-20-0; L-**1**, 35455-21-1; **2**, 123-54-6; **3**, 60526-14-9; **4a**, 60526-16-1; **4b**, 60526-18-3; quinine, 130-95-0; potassium hydroxide, 1310-58-3; DL-alanine, 302-72-7; quinine *N*-(1-methyl-2-acetylvinyl)-L-alaninate, 60526-20-7; L-alanine, 56-41-7.

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Some Novel, Acid-Labile Amine Protecting Groups[1]

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Under certain circumstances the lability of the *t*-Boc protecting group to 50% aqueous acetic acid is a shortcoming during the synthesis of large polypeptides in solution. The somewhat more stable 1-methylcyclobutyloxycarbonyl protecting group has been found to overcome this problem, but still is sufficiently acid labile to be useful as a temporary protecting group. Selected small ring carbamates exhibiting varying degrees of acid lability have also been prepared and evaluated. During cleavage in trifluoroacetic acid partial isomerization of the protecting group was observed in the case of *N*-cyclopropylcarbinyloxycarbonyl phenylalanine. The significance of this observation for the design of protecting groups is discussed. The effect of added nucleophile on the rate of protecting group removal for three selected N-protected phenylalanine derivatives has also been studied, and the implications of this effect in peptide synthesis are noted.

In the synthesis[2] of some large peptide fragments of ribonuclease S-protein using *tert*-butyloxycarbonyl (*t*-Boc) for temporary protection of α-amino nitrogen, substantial undesired loss of this protecting group was occasionally encountered during purification by gel filtration in 50% aqueous acetic acid. For example, after the purification of the synthetic N-terminal *t*-Boc eicosapeptide ribonuclease fragment 21–40 the loss of about 3–7% of the protecting group was demonstrated.[3] This result is consistent with the reported[4] half-life of *t*-Boc glycine ethyl ester of 10 days in 60% aqueous acetic acid at 22–25 °C, even if one considers that N-terminal *t*-Boc peptides are more stable to acid, presumably due to the fact that the terminal urethane group in N-carbamoylated peptides is less basic than that of the N-protected amino acid.[5]

To avoid undesired loss of the *t*-Boc group we undertook the search for an acid-labile protecting moiety more stable than *t*-Boc in 50% acetic acid, yet readily and completely removable by relatively mild acid treatment. At the same time we sought to avoid both introduction of a new asymmetric center and significant reduction of the solubility of the protected peptides by a new protecting group.

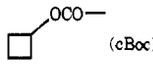
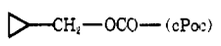
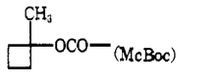
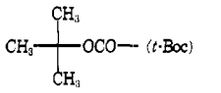
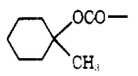
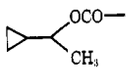
Some years ago Blaha and Rudinger[6] demonstrated a direct correlation of alkylcarbamate stability with the rates of ethanolysis of the corresponding *p*-toluenesulfonates. We were forced to adopt a more empirical approach, owing mainly to the lack of solvolytic rate data on derivatives of *tert*-butyl alcohol required to establish a relationship to the *t*-Boc group. Using published solvolytic data[7–11] as a rough guide to the selection of synthetic targets, we prepared and studied several derivatives of L-phenylalanine (see Chart I) in the hope that they would comprise a series of graded stability.

The alkoxycarbonyl amino acids were prepared by conversion of the corresponding alcohols into chloroformates by reaction with phosgene. The chloroformates were allowed to react with either phenylalanine in a dilute solution of sodium bicarbonate or with phenylalanine methyl ester in chloroform solution in the presence of triethylamine.

Rates of removal of the various protecting groups were compared by measuring the liberation of amino acid or dipeptide ester in either trifluoroacetic acid or formic acid. Trifluoroacetic acid was appropriate for study of the more stable protecting groups, formic acid for study of the more labile groups (see Table I).

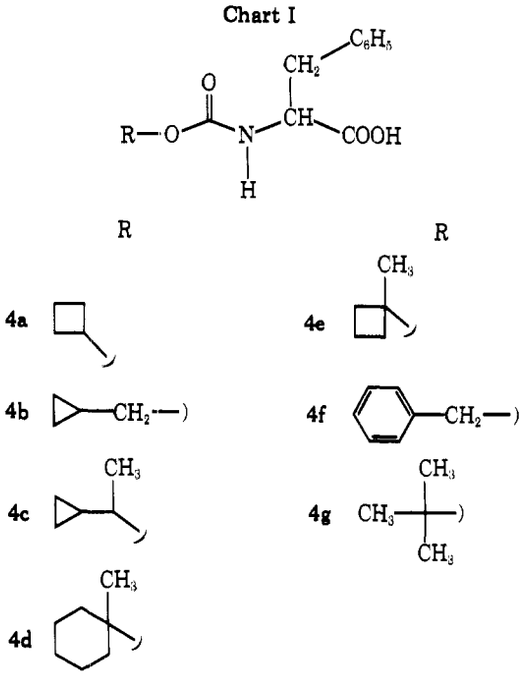
The data suggested that the 1-methylcyclobutyloxycar-

Table I. Acid-Mediated Cleavage of Various N-Protecting Groups of Phenylalanine and Phenylalanyl–Alanine Methyl Ester

X	Compd	$t_{1/2}$ (X-Phe), min (25 °) TFA	HCOOH	$t_{1/2}$ (X-Phe-Ala-OCH_3), min (25 °C) TFA	HCOOH
$C_6H_5CH_2$—OCO— (Cbz)	4f	300			
 (cBoc)	4a	>300[c]			
 (cPoc)	4b	40		~50[c] (57282-36-7)[d]	
 (McBoc)	4e	2		3 (59602-95-8)[d]	180
 (t-Boc)	4g	1[a,b]	4	<2[a] (15136-29-5)[d]	10
	4d	1[a]	2		
	4c		1.5		

[a] Complete cleavage observed. [b] Direct NMR probe of the reaction of 4g in deuteriotrifluoroacetic acid demonstrated the generation of nearly 1 equiv of *tert*-butyl trifluoroacetate, as evidenced by a characteristic methyl group signal at δ 1.63 ppm.[12a] In this medium, therefore, a continuous source of the *tert*-butyl cation is present, lending support to the rationale for the use of nucleophilic scavengers during protecting group removal in TFA.[12b] [c] Determined by TLC after solvent removal. [d] Registry no.

Chart I



bonyl (McBoc) protecting group would be sufficiently stable in 50% acetic acid to be useful in gel filtration, yet labile enough to be removable under mild acidic conditions. In accord with this prediction, after 48 h in 50% acetic acid more than 99% of McBoc phenylalanine (4e) remained unchanged; whereas under the same conditions amino acid was liberated from *t*-Boc phenylalanine (4g) to the extent of about 10–15%.[13] Complete removal of McBoc was achieved by treatment of 4e with trifluoroacetic acid at 25 °C for 30 min.

We are not recommending that the McBoc protecting group should generally replace the widely used *t*-Boc group in peptide synthesis. We do regard McBoc as appropriate for protection of the amino termini of large peptides of limited solubility, where the favorable solvent properties of 50% aqueous acetic acid have proved useful for purification procedures.

In the course of these studies we noted that removal of the cPoc protecting group of **4b** proceeded to the extent of only about 65% in the time indicated as sufficient for completion based on kinetic data obtained during initial stages of the cleavage. The dilemma was resolved when we found that the relatively acid-stable cyclobutyloxycarbonyl derivative **4a** comprised about 40% of the remaining "unreacted" starting material (which extrapolates to approximately 20% of cBoc phenylalanine at zero remaining cPoc isomer).[14] This observation points out that certain urethane-protected derivatives are potentially susceptible to rearrangement to more stable isomers. The known isobornyloxycarbonyl group[15] is a possible case in point. Is such instances the initial rate of generation of free amine could give misleading information about the utility of such a temporary protecting group. Furthermore, in synthetic operations more vigorous conditions or extended reaction times required for complete removal of a rearranged (more stable) protecting group would risk unwanted concomitant exposure of other protected functionality.

In view of the expected rapid rate of decarboxylation of carbamates after solvent separation,[16] we believe that the observed urethane isomerization is likely to have occurred via internal return. That is, rearrangement is thought to have taken place in advance of solvent separation along the reaction pathway.[17,18]

We have also noted that the relative rates of removal of two protecting groups will be affected by the presence or absence of inert diluents, and by nucleophiles. Nucleophilic scavengers are often employed in peptide synthesis to trap unwanted cations generated during the removal of various protecting groups.[12b,19] The effect of any compound introduced into the reaction medium on the rate of an S_N1 process should reflect its contribution to the overall polarity of the solvent system; whereas the effect on the rate of an S_N2 process should be related to the nucleophilicity of the added component. Thus, addition of either methylene chloride or dimethyl sulfide *decreases* the rate of solvolytic removal of the *t*-Boc protecting group [presumably a reflection of the destabilizing effect of diminished solvent polarity on carbonium ion (*tert*-butyl) intermediates]. On the other hand, the nucleophilic dimethyl

Table II. Relative Half-Times ($t_{1/2}$) for Removal of N-Protection

Substrate	$t_{1/2}$, min		
	Formic acid	4:1 formic acid/DMS[a]	4:1 formic acid/CH_2Cl_2[c]
t-Boc-Phe (4g)	4	26	21
	TFA[b]	4:1 TFA/DMS	4:1 TFA/benzene
Cbz-Phe (4f)	300	60	600
cPoc-Phe (4b)	40	110	110

[a] DMS = dimethyl sulfide. [b] TFA = trifluoroacetic acid. [c] Benzene is not miscible with HCOOH.

Table III

Compd	Yield,[a] %	Mp, °C	^{1}H NMR, δ_{Me_4Si} ($CDCl_3$), ppm	TLC R_f (system)	Anal., % Calcd	Found
4a	75	121–123	7.21 (s, 5 H, phenyl) 1.7–2.3 (broad d, 6 H, cyclobutyl) 4.7 (m, 1 H, cyclobutyl) methine)	0.75 (D) 0.38 (E)	C 61.76 H 6.66 N 5.14 ($C_{14}H_{17}NO_4 \cdot {}^1/_2H_2O$)	61.66 6.46 5.10
4b	57	105–107	7.20 (s, 5 H phenyl) 0.1–1.1 (m, 5 H, cyclopropyl) 3.78 (d, J = 6 Hz, 2 H, —OCH_2—)	0.72 (D) 0.36 (E)	C 61.76 H 6.66 N 5.14 ($C_{14}H_{17}NO_4 \cdot {}^1/_2H_2O$)	61.87 6.41 5.18
4c		91–93.5	7.23 (s, 5 H, phenyl) 1.25 (d, J = 7 Hz, 3 H, CH_3) 4.22 (m, 1 H, —CHO—)	0.80 (B)	C 64.96 H 6.91 N 5.05 ($C_{15}H_{19}NO_4$)	65.09 6.74 5.20
4d		Oil	7.16 (s, 5 H, phenyl) 1.40 (s, 3 H, CH_3)	0.60 (D)		
4e		60–62	7.18 (s, 5 H, phenyl) 1.6–2.0 (envelope, 6 H, cyclobutyl) 1.47 (s, 3 H, CH_3)	0.71 (B)	C 64.96 H 6.91 N 5.05 ($C_{15}H_{19}NO_4$)	64.65 7.05 5.13

[a] Based on phenylalanine.

sulfide enhances the rate of cleavage of the Cbz protecting group, whereas an inert, nonpolar solvent reduces the rate of Cbz cleavage (see Table II). Thus, the presence of dimethyl sulfide as scavenger clearly reduces the selectivity of protecting group removal in peptides containing both *t*-Boc and Cbz.[3] The distinctly different behavior of these two protecting groups must be related to a difference in their respective mechanisms for acidolysis (i.e., S_N1 vs. S_N2, respectively). The data in Table II suggest that with respect to mechanism of solvolysis cPoc is more closely related to *t*-Boc than to Cbz.

Development of the relatively stable McBoc protecting group should allow one to take advantage of the favorable solvent properties of aqueous acetic acid in working with large peptides. The other urethane protecting groups reported may be of value in organic syntheses where gradations of greater or lesser lability are sought. Finally, mechanistic relationships must be considered whenever two acid-labile protecting groups are chosen for use in a synthesis, where at some point one group is to be selectively removed in the presence of the other.

Experimental Section[20]

Capillary melting points were determined on a Thomas-Hoover apparatus and are reported uncorrected. Thin layer chromatograms were developed on silica gel (Quantum Industries, Q-1 plates), and components were visualized by either *tert*-butylhypochlorite–KI reagents[21] or by ninhydrin reagent. Systems used in TLC were as follows: EtOAc–pyridine–HOAc-H_2O, 5:5:1:3 (A); $CHCl_3$–CH_3OH–H_2O–HOAc, 80:20:2:1 (B); $CHCl_3$–CH_3OH, 9:1 (C); $CHCl_3$–OH–H_2O–HOAc, 85:15:1.5:1 (D); EtOAc–HOAc–isooctane–H_2O, 7:2:7:10 upper layer (E). Amino acids were all of the L configuration.

Alkoxycarbonyl Phenylalanine Derivatives (4a–e). Two methods of preparation were used, depending upon the stability of the precursor alkoxycarbonyl chloride in question.

Method A (More Stable Derivatives: 4a, 4b). A solution of 20 mmol of either cyclobutanol (Columbia Organic Chemicals, 97%) or cyclopropylcarbinol (Aldrich) in 10 ml of dry ether was treated at 0 °C with 40–60 mmol of phosgene gas. The excess phosgene was allowed to evaporate by slow nitrogen purge at 20–25 °C overnight; and finally, any residual phosgene was removed by evacuation at 0 °C under water aspirator pressure to give in 80–90% crude yield a nearly colorless liquid, ir λ_{max} (film) 1770 cm^{-1} (COCl), which was used without further purification.

Thus, a solution of 8.0 mmol of phenylalanine in 40 ml of 1.0 M $NaHCO_3$ was treated portionwise under vigorous stirring with either cBoc or cPoc chloride, using TLC (system A) to confirm disappearance of phenylalanine. After an additional 0.5 h, 2.5 N HCl was added dropwise to bring the reaction mixture to pH 3, and the resulting solid was isolated by filtration, washed with water, dried in vacuo, and crystallized from hot ethyl acetate/hexane (see Table III).

Method B (Less Stable Derivatives: 4c, 4d, 4e). A solution of about 40 mmol of phosgene in 20 ml of dry benzene was prepared at 0 °C. Then a mixture of 20 mmol of either 1-methylcyclohexyl alcohol (Aldrich), 1-cyclopropylethanol (K & K), or 1-methylcyclobutyl alcohol[22] and 20 mmol of pyridine in 20 ml of benzene was added over a period of about 20 min. After 2 h the reaction vessel was fitted with a reflux condenser connected to a water aspirator via a tube filled with Drierite and then carefully evacuated at 0 °C to remove excess phosgene, and the filtrate was concentrated in vacuo to a volume of ca. 5–10 ml, maintaining the temperature at 0 °C. Thus prepared, the sample, owing to its instability,[23] was used immediately without purification in the next step.

A sample of 0.60 g of phenylalanine methyl ester hydrochloride[24] suspended in chloroform was treated with 1 equiv of triethylamine ("pH 8" as measured on moistened narrow range indicator papers). The solution of alkoxycarbonyl chloride was added portionwise at 0 °C with stirring, alternating with addition of triethylamine to maintain "pH 8". After 2 h the reaction was quenched by addition of 5% $NaHCO_3$. Additional chloroform was added, and the organic phase was washed with 50% saturated NaCl, 0.2 N H_2SO_4, and 50% saturated NaCl (two portions), and dried over anhydrous Na_2SO_4. Solvent removal gave a colorless oil, R_f >0.90 (systems B, C), which was subjected to saponification without further purification.

The sample of N-protected phenylalanine methyl ester in 50% aqueous methanol was maintained at pH 12 using 1 N NaOH. The progress of the reaction was monitored by TLC, and complete saponification required about 46 h. The reaction mixture was then adjusted to pH 6 using 0.2 N H_2SO_4, the solvent was almost completely

removed under reduced pressure, and dilute $NaHCO_3$ was added. The aqueous layer was washed with two portions of ether. After acidification (concentrated H_2SO_4 to pH 3), the product was extracted into ethyl acetate. The organic extract was washed with two portions of saturated NaCl and dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo to give product, which was purified by crystallization or column chromatography, as appropriate (Table III).

cPoc Phenylalanine (4b) *N*-Hydroxysuccinimide Ester. A solution of 531 mg (2.02 mmol) of cPoc phenylalanine and 233 mg (2.01 mmol) of *N*-hydroxysuccinimide in 3.0 ml of dry, peroxide-free THF, cooled to 0–5 °C, was treated with 0.48 g (15% excess) of DCC. The reaction mixture was stored overnight at 0–5 °C, then filtered to remove precipitated dicyclohexylurea. The solvent was removed under reduced pressure to give an oily residue, which slowly crystallized upon the addition of isopropyl alcohol. This crude product was isolated by filtration and recrystallized once from isopropyl alcohol to give 305 mg (42% yield) of colorless needles, mp 135–135.5 °C.

Anal. Calcd for $C_{18}H_{20}N_2O_6$: C, 59.99; H, 5.59; N, 7.77. Found: C, 59.71; H, 5.75; N, 7.80.

cPoc Phenylalanyl-Alanine Methyl Ester. A solution of 241 mg (0.67 mmol) of cPoc phenylalanine *N*-hydroxysuccinimide ester and 120 mg of L-alanine methyl ester hydrochloride[24] in 10 ml of methylene chloride was treated with triethylamine ("pH 7.6–8.0" as measured on moistened narrow range indicator papers). After 6 h the mixture was transferred to a separatory funnel, and the solution was washed successively with portions of 5% $NaHCO_3$, 50% saturated NaCl, 0.2 N H_2SO_4, and 50% saturated NaCl (twice), and dried over anhydrous Na_2SO_4. The solvent was removed to give a solid residue, which was recrystallized from hot ethyl acetate–hexane to give 207 mg (85% yield) of fluffy, white needles, mp 147–147.5 °C. Amino acid analysis after acid hydrolysis: Ala, 1.00, Phe 1.00.

Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 61.86; H, 7.15; N, 8.17.

***t*-Boc Phenylalanyl-Alanine Methyl Ester.** A solution of 732 mg (2.02 mmol) of *tert*-butyloxycarbonyl phenylalanine *N*-hydroxysuccinimide (Cyclo Chemicals) and 342 mg of L-alanine methyl ester hydrochloride[24] in 30 ml of methylene chloride was treated with triethylamine ("pH 7.5–8.0" as measured on moistened narrow range indicator papers). After 6 h the reaction mixture was processed as described in the preceding experiment to afford a solid which was recrystallized from ethyl acetate–hexane, giving 353 mg (48% yield) of fluffy needles, mp 108–109 °C. Amino acid analysis after acid hydrolysis: Ala, 1.00; Phe 1.00.

Anal. Calcd for $C_{18}H_{22}N_2O_5$: C, 61.70; H, 7.48; N, 8.00. Found: C, 61.57; H, 7.68; N, 8.07.

1-Methylcyclobutyloxycarbonyl (McBoc) Phenylalanyl-Alanine Methyl Ester. To a solution of 140 mg of McBoc phenylalanine (**4e**) and 83 mg of alanine methyl ester hydrochloride[24] in 4 ml of acetonitrile was added 80 μl of triethylamine (ca. 1 equiv), followed by a solution of 119 mg of DCC in 2 ml of acetonitrile. After 20 h at 25 °C, the excess DCC was destroyed by the addition of 5 drops of 50% acetic acid, and workup proceeded in a standard fashion, with crystallization from hot ethyl acetate–hexane giving 130 mg of dipeptide, mp 113.5–115 °C. Amino acid analysis after acid hydrolysis: Phe, 1.00; Ala, 1.03.

Anal. Calcd for $C_{19}H_{26}N_2O_5$: C, 62.96; H, 7.23; N, 7.73. Found: C, 62.83; H, 7.14; N, 7.66.

Studies of the Cleavage Rates of Amine Protecting Groups. A sample of the protected amino acid or peptide was dissolved in the solvent mixtures indicated in Tables I and II. Except where noted in the tables, the quantity of amino group released was measured by the colorimetric assay developed by Burton,[25a] based on the 2,4,6-trinitrobenzenesulfonic acid–sulfite procedure reported by Fields.[25b] Thus, aliquots were removed at various time intervals and quenched by direct transfer to the reagent solution in a cuvette for uv analysis. Infinity values were determined after at least 10 half-lives.

Relative Stability of McBoc Phenylalanine vs. *t*-Boc Phenylalanine in 50% Acetic Acid. Samples of 5.6 mg of solid McBoc phenylalanine (**1**) and 5.5 mg of *t*-Boc phenylalanine (**2**) were each dissolved in 0.10 ml of 50% acetic acid, determining amino group released after 48 h: A (**1**), 0.01; A (**2**), 0.15 (ca. 10–15% loss of *t*-Boc).

Isolation of cBoc Phenylalanine from Trifluoroacetolysis of cPoc Phenylalanine (4b). A sample of 147 mg of cPoc phenylalanine was dissolved in 3.0 ml of anhydrous TFA at 23–24 °C and allowed to stand for 17 h (calculated as ca. 25 half-lives based on study of kinetics). The reaction mixture was then transferred to 12 ml of ethyl acetate, and the resulting solution was washed three times with water and once with saturated NaCl, and dried over anhydrous $MgSO_4$. It was then evaporated and flushed three times with benzene, then twice with $CHCl_3$, to remove residual TFA, affording after dry column chromatography (system E, 5:2:9:10, silica gel) an oil which deposited fluffy crystals, mp 121–122.5 °C (mixture with authentic **4a**, mp 121.5–123.5 °C) from ethyl acetate/hexane, upon seeding with cBoc phenylalanine (**4a**). Additional crystalline solid recovered upon concentration of the mother liquor was shown to contain no cPoc phenylalanine (**4b**) by NMR, δ Me_4Si ($CDCl_3$) 3.12 (doublet, J = 7 Hz, 2 H, benzylic), 1.6–2.4 (broad envelope, 6 H, cyclobutyl), 4.88 ppm (triplet, J = 9 Hz, 1 H, cyclobutyl methine), indicative of almost exclusively cBoc isomer.[14]

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Registry No.—**4a,** 60538-78-5; **4b,** 57282-35-6; **4c,** 57282-38-9; **4d,** 47187-53-1; **4e,** 59602-94-7; **4f,** 1161-13-3; **4g,** 13734-34-4; cyclobutanol, 2919-23-5; cyclopropylcarbinol, 2516-33-8; phenylalanine, 673-06-3; 1-methylcyclohexyl alcohol, 590-67-0; 1-cyclopropylethanol, 765-42-4; 1-methylcyclobutyl alcohol, 20117-47-9; cPOC phenylalanine *N*-hydroxysuccinimide ester, 57282-37-8; *N*-hydroxysuccinimide, 6066-82-6; L-alanine methyl ester HCl, 2491-20-5; *tert*-butyloxycarbonyl phenylalanine *N*-hydroxysuccinimide, 3674-06-4.

References and Notes

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(13) Note from Table I confirmation of the enhanced stability of peptide derivatives compared with amino acid derivatives.[4]
(14) No excess of signal was detectable within the region expected for the olefinic protons of the isomeric allylcarbinyl derivatives.
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