Bis(BOC) Amino Acid Fluorides as Reactive Peptide Coupling Reagents[1]

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Largely as a result of the work of Ragnarsson and associates,[2] protected *N,N*-bis(BOC) α-amino acids, potentially important synthetic intermediates which incorporate urethane protection of both available α-NH bonds, have routinely become available. It was pointed out that such double protection, precluding oxazolone formation, could be advantageous with regard to potential racemization problems. On the other hand, the presence of a second inductively electron-withdrawing carbonyl function might be expected to enhance any component of racemization due to α-H exchange.[3]

Unfortunately, as noted previously bis(BOC) amino acids, presumably because of severe steric constraints, undergo coupling reactions very sluggishly by ordinary procedures (active ester, mixed anhydride, or carbodiimide)[2b] with the result that the activated component may be subject to an increased risk of racemization. The question of racemization was not explicitly examined in the earlier work. A second consequence of the low reactivity of the bis(BOC) amino acids was encountered during applications of these derivatives to the synthesis of a simple model peptide, leucine enkephalin. In a stepwise synthesis, the final step involving reaction of the free tetrapeptide ester **1** with $(BOC)_2$Tyr(Bn)-ONp (**2a**) gave mainly a hydantoin (70%) rather than the desired protected pentapeptide.[2b] Best results (37–50% of the

H-Gly-Gly-Phe-Leu-OBn
1

$(BOC)_2$Tyr(Bn)-X
2a, X = ONp
b, X = F

pentapeptide) were obtained by the DCC/HOSu technique. By itself DCC gave only the corresponding *N*-acylurea. Application of the mixed anhydride technique using isobutyl chloroformate to the coupling of $(BOC)_2$Phe-OH and H-Leu-OBn gave up to 30% of the side product isobutyloxycarbonyl leucine benzyl ester.

Because ordinary BOC amino acids can be converted to stable acid fluorides which are highly reactive coupling agents,[4] it was deemed worthwhile to examine the synthesis of the bis(BOC) analogs. Key bis(BOC) amino acid fluorides[5] required for the synthesis of leucine enkephalin have been synthesized and have indeed been shown to be more suitable coupling agents than those previously examined. In particular, in the last step of the synthesis of leucine enkephalin, which involved coupling of the free tetrapeptide ester **1** with $(BOC)_2$Tyr(Bn)-F (**2b**), none of the undesired hydantoin was detected and the protected pentapeptide was obtained in 60% yield.

In order to address the question of racemization during the coupling of ordinary bis(BOC) amino acid fluorides, $(BOC)_2$Phe-F and its D-enantiomer were each separately coupled with alanine methyl ester to give the protected dipeptide esters **3a** and **3b**, respectively. In these two

$(BOC)_2$Phe-Ala-OMe
3a, LL-
b, DL-

C_6H_5CO-Phe-Ala-OMe
4a, LL-
b, DL-

coupling reactions, none of the DL- or LL-diastereomers, respectively, were formed (<1% according to the ^{1}H NMR criterion).[6] The assay for racemization was preceded by deblocking of the bis(BOC) residues and subsequent benzoylation to give **4** (see Experimental Section). This result also confirms that no significant racemization accompanies conversion of mono-BOC amino acid esters to the corresponding bis-BOC derivatives, an everpresent risk in view of the need for the strong organic base DMAP as a necessary catalyst in the synthesis.[2] In contrast, under the same conditions the more sensitive[8] amino acid ester BOC-Phg-OBn and its enantiomer both suffered near total loss of configuration upon conversion to the bis(BOC) derivatives. Attempts in this case to substitute DIEA for DMAP were unsuccessful, no bis(BOC) derivative being formed. Thus for proteinogenic amino acids, general conversion to the chiral bis(BOC) amino acids and their fluorides is to be expected although caution should be exercized in the case of amino acids bearing substituents which might enhance the acidity of the α-hydrogen atom.

Experimental Section

General. Mono-BOC amino acid ester precursors were prepared as described earlier, using benzyl esters in all cases except that of tyrosine in which the allyl ester was used. Formation of bis(BOC) derivatives followed the earlier method[2] except that infrared spectroscopy was used to follow the course of the reaction (disappearance of the NH absorption near 3345 cm^{-1}) in addition to TLC. If complete conversion had not occurred after 5–6 h, it was found expedient to evaporate the reaction mixture to dryness and redissolve the residue in CH_3CN prior to charging with additional $(BOC)_2O$. Deblocking of the allyl

(1) The abbreviations used for amino acids follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1971**, *247*, 997). Other abbreviations are as follows: TFA = trifluoroacetic acid, BOC = *tert*-butyloxycarbonyl, DCC = dicyclohexylcarbodiimide, DMAP = 4-(dimethylamino)pyridine, DIEA = diisopropylethylamine, HOSu = *N*-hydroxysuccinimide, NCA = *N*-carboxyanhydride, Phg = α-phenylglycine.

(2) (a) Gunnarsson, K.; Grehn, L.; Ragnarsson, U. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 400. (b) Gunnarsson, K.; Ragnarsson, U. *Acta Chem. Scand.* **1990**, *44*, 944.

(3) Review: Kemp, D. S. In *The Peptides. Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic: New York, 1979; p 315. For examples of the much greater tendency toward racemization of phthaloyl amino acid derivatives which are structurally related to the bis(BOC) analogs in the presence of triethylamine see (a) Liberek, B. *Tetrahedron Lett.* **1963**, 1103. (b) Anderson, G. W.; Callahan, F. M.; Zimmerman, J. E. *Acta Chim. Hung.* **1965**, *44*, 51.

(4) Carpino, L. A.; Mansour, E. M. E.; Sadat-Aalaee, D. *J. Org. Chem.* **1991**, *56*, 2611.

(5) While this study was underway, the group of Wakselman described the synthesis of a number of bis(BOC) amino acid fluorides and demonstrated the high reactivity of the phenylalanine derivative toward certain pyrrolyl anions under conditions where the corresponding BOC-Phe-NCA was unreactive. See Savrda, J.; Wakselman, M. *J. Chem. Soc. Chem. Commun.* **1992**, 812.

(6) The method used has been described previously.[7] In the case of **4a** the two ^{13}C side bands, theoretically 0.55% each ($J_{^{13}C-H}$ = 148 Hz) of the LL-methoxy peak (δ 3.7) were clearly visible, whereas at the position of absorption expected for the DL-diastereomer **4b** (δ 3.6) the spectral trace did not rise above the baseline.

(7) (a) Carpino, L. A.; Chao, H.-G.; Nowshad, F.; Shroff, H. *J. Org. Chem.* **1988**, *53*, 6139. (b) Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. *J. Org. Chem.* **1983**, *48*, 77.

(8) Compare Carpino, L. A. *J. Org. Chem.* **1988**, *53*, 875.

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Table I. Bis(BOC) Amino Acid Fluorides

dipeptide	yield, %[a]	mp, °C	$[\alpha]_D$, deg [t, °C]	^{1}H NMR ($CDCl_3$), δ	mol formula	anal. data, calcd (found) C	H	N
$(BOC)_2$Gly-F	80	42–4		1.56 (s, 18, CMe_3), 4.58 (d, 2, CH_2)	$C_{12}H_{20}FNO_5$	51.98 (51.93)	7.27 (7.14)	5.05 (4.95)
$(BOC)_2$Phe-F	80.4[a]	36–8	−169.5 (c 1, EtOAc) [24]	1.47 (s, 18, CMe_3), 3.25–3.46 (m, 2, CH_2), 5.42 (m, 1, α-CH), 7.30 (s, 5, aryl)	$C_{19}H_{26}FNO_6$	62.11 (62.11)	7.13 (7.16)	3.81 (4.08)
$(BOC)_2$-D-Phe-F	70[b]	36–8	+165 (c 1, EtOAc) [24]	1.47 (s, 18, CMe_3), 3.25–3.46 (m, 2, CH_2), 5.42 (m, 1, α-CH), 7.30 (s, 5, aryl)				
$(BOC)_2$Tyr(Bn)-F	65[c]	oil	−38.2 (c 1, EtOAc) [24][d]	1.46 (s, 18, CMe_3), 3.2 (m, 2, β-CH_2), 5.07 (s, 2, CH_2Ar), 5.30 (m, 1, α-CH), 7.00 (dd, 4, Tyr), 7.42 (m, 5, aryl)	$C_{26}H_{32}FNO_6$	65.96 (65.87)	6.77 (6.68)	2.96 (3.18)

[a] A trace of the corresponding Leuchs anhydride was formed: mp 101–2 °C, lit.[5] mp 102–4 °C, $[\alpha]^{24}_D$ = +124° (c, 1, EtOAc), IR (KBr) 1873, 1806, 1731 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ 1.66 (s, 9, CMe_3), 3.45 (t, 2, CH_2Ar), 4.95 (m, 1, α-CH), 7.31 (m, 5, aryl). [b] A trace of the corresponding Leuchs anhydride was formed: mp 102–3 °C, $[\alpha]^{24}_D$ = −129.4° (c, 1, EtOAc); IR (KBr) 1873, 1806, 1731 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ 1.65 (s, 9, CMe_3), 3.45 (t, 2 CH_2Ar), 4.95 (m, 1, α-CH), 7.31 (m, 5, aryl). [c] A trace of the corresponding Leuchs anhydride was formed: mp 128–130 °C, $[\alpha]^{24}_D$ = +105.5° (c, 1, EtOAc); IR (KBr) 1875, 1808, 1734 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ 1.62 (s, 9, CMe_3), 3.4 (two m, β-CH_2), 4.85 (m, 1, α-CH), 5.04 (s, 2, CH_2Ar), 6.95 (dd, 4, Tyr), 7.42 (m, 5, aryl). Anal. Calcd for $C_{22}H_{24}NO_6 \cdot 0.5\ H_2O$: C, 64.70; H, 6.13; N, 3.43. Found: C, 64.48; H, 5.87; N, 3.36. [d] In this case the rotation was determined at λ_{589} rather than the sodium D-line.

ester of $(BOC)_2$Tyr(Bn)-OH via tris(triphenylphosphine)rhodium gave the acid in only 15–20% yield (reported yield[2b] 48%).

General Method for the Preparation of Bis(BOC) Amino Acid Fluorides. To a stirred solution of the amino acid (2 mmol) in 5 mL of dry CH_2Cl_2 and 162 μL (2 mmol) of pyridine under N_2 was added 900 μL (10 mmol) of cyanuric fluoride at −30 to −20 °C. The course of the reaction was followed by TLC (EtOAc–hexane, 3/1) and found to be complete after 30–45 min. After stirring at −30 to −20 °C for a total of 1 h, crushed ice was added along with an additional 10 mL of CH_2Cl_2. The organic layer was extracted with 10 mL of ice-cold water and dried ($MgSO_4$), and the solvent was removed *in vacuo*. The residue was chromatographed[9] on flash grade silica gel using as eluent EtOAc–hexane (3/1). Alternatively, the crude residue was treated with hexane and cooled in a refrigerator which caused the separation of small and variable amounts of the corresponding Leuchs anhydride which was filtered. The filtrate was evaporated and the residue purified by column chromatography as noted above. The resulting oil was used as such or recrystallized from hexane. All bis(BOC) amino acid fluorides showed their characteristic carbonyl absorption at 1846–1851 cm^{-1}. See Table I for other characterization data.

$(BOC)_2$Tyr(Bn)-Gly-Gly-Phe-Leu-OBn. To a stirred solution of 1.27 g (3.2 mmol) of H-Leu-OBn·TsOH and 0.83 g (1.1 mL, 3.2 mmol) of DIEA in 7.5 mL of CH_2Cl_2 there was added a solution of 1.30 g (3.4 mmol) of $(BOC)_2$Phe-F in 7.5 mL of CH_2Cl_2 over a period of 60 s at room temperature. The mixture was stirred for a total of 2 h (TLC using EtOAc/hexane 3/1 showed reaction to be complete in 50–60 min) and transferred to a separatory funnel with an additional 10 mL of CH_2Cl_2, and the solution was washed three times each with 1 M $KHSO_4$, 10% Na_2CO_3, and saturated NaCl. Drying ($MgSO_4$) and evaporation gave 1.77 g (96.4%) of the dipeptide ester as a yellow oil which was crystallized from hexane to give 1.47 g (80%) of the ester: mp 61–2 °C, lit.[2b] mp 62.5–63 °C), $[\alpha]^{26}_D$ = −65.9° (c, 1, MeOH), lit.[2b] $[\alpha]_D$ −68° (c, 0.98, MeOH). The protected dipeptide ester (1.14 g, 2 mmol) was treated with 5 mL of TFA at 0 °C for 1 h. After evaporation of TFA the residue was dissolved in a mixture of 75 mL of EtOAc and 25 mL of 1 M $NaHCO_3$ solution. The organic layer was washed with 1 M $NaHCO_3$ solution and saturated NaCl solution, dried ($MgSO_4$), and evaporated to give 0.74 g of the free dipeptide ester as an oil which was acylated by $(BOC)_2$Gly-F as described for the corresponding dipeptide. The protected tripeptide [yield 75%, mp 104–5 °C, lit.[2b] mp 107–107.5 °C, $[\alpha]^{27}_D$ = −16.8° (c, 1, MeOH), lit.[2b] $[\alpha]_D$ = −16.4° (c, 1, MeOH)] was converted in the same way to the corresponding protected tetrapeptide [yield 83.3%, mp 134–5 °C, lit.[2b,10] mp 135–7 °C, $[\alpha]^{27}_D$ = −18.2° (c, 1, MeOH), lit.[2b] $[\alpha]_D$ = −17.9° (c, 1.01, MeOH)] and then to the protected pentapeptide, the crude product being obtained as a white foam (90.5%), which by TLC showed three spots, one of which corresponded to unreacted $(BOC)_2$Tyr(Bn)-F. Purification by column chromatography using CH_2Cl_2–acetone (70/30) as eluent gave the pure protected pentapeptide in 61.6% yield as a thick oil: IR (NaCl) 1752, 1698, 1645 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ 0.92 (d, 6, $CH(CH_3)_2$), 1.38 (s, 18, CMe_3), 1.65 (m, 3, CH_2CH), 3.15 (m, 2, β-CH_2), 4.02 (d, 2, CH_2 of Gly), 4.1 (d, 2, CH_2 of Gly), 4.6 (m, 1, α-CH), 4.8 (m, 1, α-CH), 5.0 and 5.1 (two s, 4, CH_2Ar), 6.8 (t, 1, NH), 6.9–7.1 (dd, 4, Tyr), 7.15 and 7.6 (m, 18, aryl, NH); MS (FAB/3-NBA/GLYC/TFA) *m/z* 736.4 (-$(BOC)_2$ + 3H)$^+$; MS (LD/TOF) *m/z* 958.8 (M + Na)$^+$, 974.8 (M + K)$^+$. Catalytic deblocking (Pd/C/H_2) followed by TFA deblocking gave free leucine enkephalin, identified by coinjection with an authentic sample; t_R 7.98 min, flow rate = 1 mL/min, isocratic 30% CH_3CN, 70% H_2O, 0.1% TFA, Waters Z-Module Radial Compression, C_{18} column, 0.8 × 10 cm, 10 μm. Neither the crude nor the purified material showed any ^{1}H-NMR absorption at δ 1.6, a position characteristic of the hydantoin which is the major product formed on acylation of the tetrapeptide by means of $(BOC)_2$Tyr(Bn)-ONp.[2b]

$(BOC)_2$Phe-Ala-OMe (3a). A solution of 0.367 g (1 mmol) of $(BOC)_2$Phe-F in 1 mL of dry CH_2Cl_2 was added to a stirred solution of H-Ala-OMe·HCl (0.140 g, 1 mmol) and DIEA (0.342 mL, 2 mmol) in 2 mL of dry CH_2Cl_2. The mixture was stirred for 2 h at room temperature, washed three times each with 10% $KHSO_4$, 10% $NaHCO_3$, and saturated NaCl, dried ($MgSO_4$), and evaporated. Recrystallization of the residue from ether–hexane gave 0.35 g of (78%) of the dipeptide ester: mp 70–72 °C, $[\alpha]^{24}_D$ = −96.9° (c, 1, MeOH); IR (KBr) 3283 cm^{-1} (NH); 1735, 1692, 1659 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ 1.39 (s, 18, CMe_3), 1.41 (d, 3, $CHCH_3$), 3.35 (two m, 2, β-CH_2), 3.75 (s, 3, CH_3O), 4.65 and 5.0 (two m, 2, α-CH), 6.4 (d, 1, NH), 7.25 (m, 5, aryl). Anal. Calcd for $C_{23}H_{34}N_2O_7$: C, 61.32; H, 7.61; N, 6.21. Found: C, 61.40; H, 7.78; N, 6.21.

$(BOC)_2$-D-Phe-Ala-OMe (3b). The DL-dipeptide ester was prepared as described for the LL-diastereomer in 75% yield as white crystals: mp 130–131 °C; $[\alpha]^{24}_D$ = +53.5° (c, 1, MeOH); IR (KBr) 3319 cm^{-1} (NH), 1755, 1694, 1649 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ 1.38 (s, 18, CMe_3), 1.42 (d, 3, $CHCH_3$), 3.35 (m, 2, β-CH), 3.74 (s, 3, CH_3O), 4.65 and 5.0 (two m, 2, α-CH), 6.5 (d, 1, NH), 7.25 (m, 5, aryl). Anal. Calcd for $C_{23}H_{34}N_2O_7$: C, 61.32; H, 7.61; N, 6.21. Found: C, 61.12; H, 7.67; N, 6.16.

(9) In spite of their reactivity toward amino acid esters, the bis(BOC) amino acid fluorides can generally be chromatographed without difficulty on silica gel. The hydrolysis of mono- and bis(BOC) glycine fluorides is easily followed by ^{1}H NMR spectroscopy. The mono derivative, BOC-Gly-F in DMF-d_7 containing a small amount of water was allowed to stand at room temperature. The acid fluoride (δ 4.2 dd) was slowly converted to the acid (δ 3.8 d). After 4 days the ratio of the two peak areas stabilized as the water present in the solvent was consumed. No further change occurred within 2 days. In contrast, when a sample of $(BOC)_2$Gly-F was added to an aliquot of the same DMF-d_7 there was no change in the ratio of acid fluoride (δ 4.74 d) to residual acid present (δ 4.3 s) over a period of 7 days.

Racemization Test. Due to overlap of the ^{1}H-NMR signals of the alanine α-Me doublets and the *tert*-butyl singlets as well as overlap of the methyl ester peaks of the two diastereomeric dipeptides **3a** and **3b**, it was not possible to determine the extent of racemization by direct examination of the NMR spectra. Since clean separation of the diagnostic peaks is observed for the corresponding benzoyl dipeptides esters **4a** and **4b**,[11] the two BOC residues were removed and replaced by a benzoyl function. The crude sample of **3a** obtained by acylation of alanine methyl ester as described above was treated with 2.5 mL of TFA at 0 °C for 1 h. The TFA was evaporated *in vacuo* and the residue dissolved in a mixture of 35 mL of EtOAc and 15 mL of 1 M $NaHCO_3$. The organic layer was washed twice with 10-mL portions each of 1 M $NaHCO_3$ and saturated NaCl. Drying ($MgSO_4$) and removal of solvent gave 0.2 g (80%) of the free dipeptide ester as an oil which was dissolved in 2 mL of dry CH_2Cl_2 containing 0.13 mL of DIEA. To the solution there was added 0.11 mL of benzoyl chloride in 1 mL of dry CH_2Cl_2 at room temperature over a period of 60 s. The mixture was stirred for 1 h, treated with 5 mL of CH_2Cl_2, and extracted with 5-mL portions each of 1 M $KHSO_4$, 1 M $NaHCO_3$, and saturated NaCl. After drying ($MgSO_4$), removal of solvent *in vacuo* gave 0.21 g (74.2%) of the crude *N*-benzoyl dipeptide methyl ester: mp 135–137 °C; ^{1}H-NMR ($CDCl_3$) δ 1.35 (d, 3, CH_3CH), 3.2 (t, 2, β-CH_2), 3.7 (s, 3, CH_3O), 4.5 (m, 1, α-CH), 5.0 (m, 1, α-CH), 6.6 (d, 1, NH), 7.0 (d, 1, NH), 7.25–7.7 (m, 10, aryl). Examination of the ^{1}H-NMR spectra in both the alanine α-Me region and the methyl ester region showed that no significant amount of the DL-diastereomer was present (<1%) in the crude sample. The ^{1}H-NMR absorption positions for the other diastereomer were determined by analogous coupling to DL-H-Ala-OMe (δ 1.25 (d, CH_3CH, LD-), 1.35 (d, CH_3CH, LL-), 3.7 (s, CH_3O, LL-), 3.6 (s, CH_3O, LD-).

(10) We are indebted to Professor Ragnarsson for an authentic sample.
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