Di-*tert*-butyl Dicarbonate: A Novel Reagent for the Efficient Synthesis of Dipeptides under Mild Conditions

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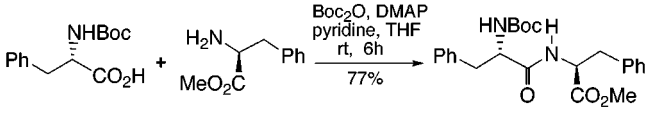
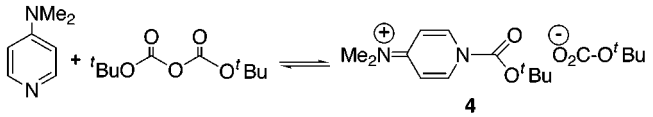
With the increasing importance of synthetic peptides in chemical and biomedical research, a variety of methods and reagents have been developed for peptide bond-forming reactions. Ideally, the reagent for such a reaction should allow rapid product formation under mild conditions and in good yield, not compromise the diastereomeric purity, avoid side reactions, and generate easily removable coproducts. Applicability to both solution- and solid-phase synthesis should be an additional criteria. Thus, development of new reagents and methods for peptide synthesis continues to be challenging and worthwhile. In the realm of amino acid/peptide research, di-*tert*-butyl dicarbonate (Boc-anhydride; Boc_2O) is an extensively used reagent for the clean and rapid introduction of acid-labile Boc-protecting group for the amine functionality.[1] It is also an efficient *tert*-butoxycarbonylating agent for alcohols, thiols, amines, and carbon nucleophiles.[2] Boc-anhydride has also been used for the conversion of amines to the corresponding isocyanates, carbamates, and urea derivatives.[3] Interestingly, Boc-anhydride has been found to be an effective reagent for the activation of carboxylic acid carbonyls toward nucleophilic addition, presumably via a mixed anhydride intermediate. This reaction has been utilized for the conversion of various carboxylic acids to symmetrical anhydrides, esters, and amino acid amides.[4] In a novel application of the above strategy, macrolactonization of ω-hydroxy acids has been performed using Boc-anhydride as the activating reagent.[5] Recent work from our laboratory has also demonstrated the utility of Boc-anhydride in cyclodehydration of *N*-acylamino acids into oxazoles and benzoxazinones.[6]

Scheme 1



1a **2a** **3a**

Scheme 2



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The above observations encouraged us to explore the possibility of using Boc_2O as a peptide-coupling reagent. It was reasoned that treatment of an N-protected amino acid with Boc_2O might result in the activation of the carboxylic carbonyl,[4a] aminolysis of which with a second amino acid will then culminate in the peptide bond formation. Accordingly, *N*-Boc-phenylalanine (**1a**; Scheme 1) was treated with Boc_2O in the presence of pyridine as a base and a catalytic amount of 4-(dimethylamino)-pyridine (DMAP). To the resulting solution was added methyl ester of phenylalanine (**2b**), and the reaction mixture was stirred at room temperature for 6 h. To our immense satisfaction, the product formed was found to be the expected dipeptide **3a** (77%), and its specific rotation was in good agreement with the reported value [**3a**; $[\alpha]_D = -13.5$ ($c = 1$, MeOH) (lit.[7] $[\alpha]_D = -13.8$ ($c = 1$, MeOH))]. The diastereomeric purity was further verified by HPLC analysis of **3a** and a diastereomeric mixture of (D,L)- and (L,L)-**3a** [prepared by coupling **2a** with racemic **1a**], which clearly showed **3a** to be free from any (D,L) isomer, thereby indicating the nonracemizing nature of the above coupling protocol.

The coupling reaction was found to be very sluggish in the absence of DMAP. This can be attributed to an initial formation of the highly reactive 1-*tert*-butoxycarbonyl-4-dimethylaminopyridinium *tert*-butyl carbonate (**4**; Scheme 2), which facilitates the nucleophilic addition of the amino acid carboxylate anion at the *tert*-butoxycarbonyl group of the pyridinium system **4**, consequently activating the amino acid carbonyl toward nucleophilic attack. The formation of the intermediate **4** by the reaction of Boc_2O and DMAP has been postulated and confirmed by Knölker et al.[3a] in a report of an efficient Boc_2O-mediated conversion of amines to the corresponding isocyanates.

Having achieved the initial objective of peptide bond formation using Boc_2O as the coupling reagent, we proceeded to study the generality and efficiency of this method. Thus, coupling of *N*-Boc-proline (**1b**) with **2b** (Table 1) under the described conditions uneventfully afforded the dipeptide **3b** in high yield. Specific rotation of **3b** [$[\alpha]_D = -50.4$ ($c = 1.4$, MeOH)] compared well with that of the same peptide [$[\alpha]_D = -51$ ($c = 1.4$, MeOH)], prepared using the more conventional reagents dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT). HPLC analysis of **3b** further confirmed its

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Table 1. Synthesis of Dipeptides 3 via Boc_2O-Mediated Coupling of the Amino Acid Derivatives 1 and 2

	N-Boc-amino acid **1**[a]	amino acid ester **2**[a]	dipeptide **3**	time (h)	yield (%)
b	Boc-Pro-OH	H_2N-Phe-OMe	Boc-Pro-Phe-OMe	4	91
c	Boc-Phe-OH	H_2N-Ala-OMe	Boc-Phe-Ala-OMe	6	74
d	Boc-Ala-OH	D-H_2N-Phg-OMe	Boc-Ala-D-Phg-OMe	5	75
e	Boc-Val-OH	D-H_2N-Phg-OMe	Boc-Val-D-Phg-OMe	5	81
f	N_α,N_ϵ-Boc-Lys-OH	H_2N-Ala-OMe	N_α,N_ϵ-Boc-Lys-Ala-OMe	7	67
g	Boc-Thr(OTBDMS)-OH	H_2N-Val-OMe	Boc-Thr(OTBDMS)-Val-OMe	6	79

[a] L-Amino acid, unless stated otherwise.

diastereomeric purity. A variety of amino acid combinations were similarly subjected to dipeptide formation affording the products **3c**−**g** (Table 1) in good yields and high diastereomeric purity.

In conclusion, the described reaction constitutes a convenient method for the synthesis of peptides under mild, nonracemizing reaction conditions. The method also eliminates some commonly encountered problems associated with peptide coupling, such as separation of product from reagent derived coproducts, racemization, poor recovery of product, high cost, instability of the reagent, etc. Incidentally, because of its extensive use for N-protection in amino acid/peptide related-chemistry, Boc_2O is already a familiar and commonly available reagent to researchers involved in this area of research, thus adding to its utility for the present method. It is hoped that the above reaction will be a useful addition to the existing methodologies for dipeptide formation. Further application toward synthesizing higher peptides and in solid-phase synthesis is currently under investigation.[8]

Experimental Section[9]

Typical Experimental Procedure. A room-temperature solution of the *N*-Boc-amino acid **1** (1 mmol), pyridine (1.1 mmol), and a catalytic amount of 4-(dimethylamino)pyridine (20 mg) in anhydrous THF (10 mL) under N_2 atmosphere was treated with a solution of di-*tert*-butyl dicarbonate (1 mmol) dissolved in anhydrous THF (2 mL) and the mixture stirred for 20−30 min. To this mixture was added in one lot a solution of the amino acid ester **2** (1.1 mmol) in anhydrous THF (3 mL), and stirring was continued at room temperature. After completion of reaction (TLC monitoring), solvent was removed under vacuum, and the residue was dissolved in ethyl acetate (50 mL) and washed sequentially with ice-cooled 10% aqueous HCl (2 × 20 mL), saturated aqueous $NaHCO_3$ solution (2 × 20 mL), and brine. Drying over anhydrous Na_2SO_4, removal of solvent under vacuum, and purification of the residue by silica gel column chromatography (hexane/ethyl acetate 9/1 → 5/1) afforded the pure product **3**. Enantiomeric purity of the products formed was verified by HPLC analysis. HPLC conditions: column, CHIRALCEL (OD); mobile phase, 10% *i*-PrOH in *n*-hexane; flowrate, 1 mL/min; UV detection at 225 nm.

3a: white solid; mp (CH_2Cl_2) 112−113 °C (lit.[7] mp 114−115 °C); HPLC retention time = 21.0 min; $[\alpha]_D = -13.5$ ($c = 1$, MeOH); IR (neat) 3340, 1744, 1696, 1665 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.4 (s, 9H), 3.02 (m, 4H), 3.66 (s, 3H), 4.28 (dd, $J = 6.4$, 12.4 Hz, 1H), 4.74 (dd, $J = 6.5$, 12.9 Hz, 1H), 4.94 (br s, 1H), 6.21 (br d, $J = 7.6$ Hz, 1H), 6.95 (m, 2H), 7.25 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 171.3, 170.8, 155.1, 136.5, 135.6, 129.8, 129.1, 128.5, 128.4, 127.0, 126.8, 80.0, 55.7, 53.2, 52.1, 38.2, 37.9, 28.2; MS (FAB^+) 427 (MH^+). Anal. Calcd for $C_{24}H_{30}N_2O_5$ (426.51): C, 67.58; H, 7.09; N, 6.57. Found: C, 67.89; H, 7.44; N, 6.96.

3b: white solid; mp (CH_2Cl_2) 65−66 °C; HPLC retention time = 21.6 min; $[\alpha]_D = -50.4$ ($c = 1.4$, MeOH); IR (neat) 3300, 1754, 1694, 1662 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48 (s, 9H), 1.74−2.2 (m, 4H), 3.05 (dd, $J = 7.3$, 13.6 Hz, 1H), 3.22 (dd, $J = 6.9$, 13.6 Hz, 1H), 3.34 (br s, 2H), 3.77 (s, 3H), 4.25 (br s, 1H), 4.86 (br s, 1H), 6.52 (br s, 1H), 7.22 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 171.7, 171.4, 155.8, 135.8, 128.9, 128.2, 126.7, 80.2, 60.2, 52.7, 51.9, 46.6, 37.8, 28.0, 23.6; HRMS (FAB^+) calcd for $C_{20}H_{28}N_2O_5$ 376.1998 (M^+), found 376.2007. Anal. Calcd for $C_{20}H_{28}N_2O_5$ (376.45): C, 63.81; H, 7.49: N, 7.44. Found: C, 64.19; H, 7.44; N, 7.78.

3c: white solid; mp (CH_2Cl_2) 94−95 °C (lit.[7] mp 98 °C); HPLC retention time = 16.8 min; $[\alpha]_D = -21.5$ ($c = 0.4$, MeOH); IR (neat) 3335, 1750, 1693, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (d, $J = 6.8$ Hz, 3H), 1.43 (s, 9H), 3.05 (m, 2H), 3.7 (s, 3H), 4.3 (m, 1H), 4.47 (m, 1H), 4.96 (br s, 1H), 6.34 (br d, $J = 7.2$ Hz, 1H), 7.23 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 172.3, 171.6, 155.2, 135.7, 129.1, 128.3, 126.9, 79.8, 53.1, 52.1, 50.0, 37.7, 28.1, 18.2; HRMS (FAB^+) calcd for $C_{18}H_{26}N_2O_5$ 350.1841 (M^+), found 350.1844. Anal. Calcd for $C_{18}H_{26}N_2O_5$ (350.42): C, 61.69; H, 7.48: N, 7.99. Found: C, 61.35; H, 7.82; N, 8.18.

3d: white solid; mp (CH_2Cl_2) 115−117 °C; HPLC retention time = 14.9 min; $[\alpha]_D = -125.1$ ($c = 1.2$, MeOH); IR (neat) 3298, 1750, 1719, 1663 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (d, $J = 8.2$ Hz, 3H), 1.41 (s, 9H), 3.67 (s, 3H), 4.24 (br s, 1H), 5.17 (br d, $J = 6.8$ Hz, 1H), 5.5 (br d, $J = 7.7$ Hz, 1H), 7.32 (br s, 6H); ^{13}C NMR ($CDCl_3$) δ 172.2, 171.1, 155.8, 136.2, 128.9, 128.5, 127.2, 127.1, 80.1, 56.4, 52.7, 28.2, 19.2; HRMS (FAB^+) calcd for $C_{17}H_{25}N_2O_5$ 337.1763 (MH^+), found 337.1773. Anal. Calcd for $C_{17}H_{24}N_2O_5$ (336.39): C, 60.70; H, 7.19: N, 8.32. Found: C, 60.35; H, 7.22; N, 8.68.

3e: white solid; mp (CH_2Cl_2) 91−93 °C; HPLC retention time = 11.9 min; $[\alpha]_D = -98.8$ ($c = 1$, MeOH); IR (neat) 3330, 1743, 1692, 1654 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9 (br t, $J = 11.7$ Hz, 6H), 1.44 (s, 9H), 2.3 (m, 1H), 3.74 (s, 3H), 3.97 (m, 1H), 5.07 (d, $J = 8.2$ Hz, 1H), 5.51 (d, $J = 7.2$ Hz, 1H), 7.08 (br d, $J = 6.3$ Hz, 1H), 7.34 (br s, 5H); ^{13}C NMR ($CDCl_3$) δ 171.2, 171.1, 155.8, 136.2, 128.8, 128.4, 127.2, 79.8, 59.5, 56.3, 52.6, 30.8, 28.2, 19.1, 17.5; MS (FAB^+) 365 (MH^+). Anal. Calcd for $C_{19}H_{28}N_2O_5$ (364.44): C, 62.62; H, 7.74: N, 7.68. Found: C, 62.98; H, 7.87; N, 8.05.

3f: viscous liquid; HPLC retention time = 17.5 min; $[\alpha]_D = -13.4$ ($c = 1$, MeOH); ^1H NMR ($CDCl_3$) δ 1.27−1.8 (m, 27H), 3.07 (m, 2H), 3.7 (s, 3H), 4.23 (m, 1H), 4.56 (br s, 1H), 5.07 (br d, $J = 8.2$ Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 173.1, 171.8, 156.1, 155.6, 79.9, 79.0, 54.1 52.3, 47.9, 39.9, 32.0, 29.5, 28.4, 28.3, 22.4, 18.0; EIMS 431 (M^+). Anal. Calcd for $C_{20}H_{37}N_3O_7$ (431.53): C, 55.67; H, 8.64: N, 9.74. Found: C, 55.98; H, 8.90; N, 10.11.

3g: white solid; mp (CH_2Cl_2) 64−66 °C; HPLC retention time = 7.6 min; $[\alpha]_D = -6.5$ ($c = 0.6$, MeOH); IR (neat) 3365, 1738, 1710, 1675 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.18 and 0.2 (2s, 6H), 0.95 (br s, 15H), 1.14 (d, $J = 6.4$ Hz, 3H), 1.48 (s, 9H), 2.14 (m, 1H), 3.74 (s, 3H), 4.11 (br s, 1H), 4.24 (m, 1H), 4.51 (m, 1H), 5.51 (br d, $J = 6.1$ Hz, 1H), 7.32 (br d, $J = 7.8$ Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 171.7, 169.9, 155.6, 79.8, 68.5, 58.6, 57.3, 51.7, 30.9, 28.2, 25.7, 18.9, 17.9, 17.8, 17.6, −4.8, −5.1; HRMS (FAB^+) calcd for $C_{21}H_{43}N_2SiO_6$ 447.2890 (MH^+), found 447.2885. Anal. Calcd for $C_{21}H_{42}N_2SiO_6$ (446.65): C, 56.47; H, 9.48; N, 6.27. Found: C, 56.75; H, 9.87; N, 6.60.

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Supporting Information Available: Copies of ^1H NMR spectra and HPLC chromatograms for dipeptide **3a** and its epimeric mixture. This material is available free of charge via the Internet at http://pubs.acs.org.

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