Carboxy Terminus Coupling Using 1,1′-Carbonylbis(3-methylimidazolium triflate) (CBMIT) in the Presence of Cu(II) Salts

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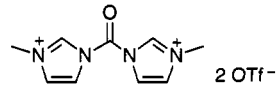
Introduction

Peptide bond formation is one of the most studied reactions in organic chemistry. Many methods have been developed, each with its own advantages and drawbacks.[1] Of major concern has been the degree to which the C-terminal amino acid residue is racemized in the course of coupling. For *N*-alkoxycarbonyl-blocked amino acids, racemization is not a major concern, and most of the well-known methods for coupling occur without racemization. The use of *N*-carbamate-blocked amino acids, however, limits peptide construction to linear growth from the *N*-terminus. This process, although well developed through automation and routinely applied to the synthesis of 20+ residue peptides, is not the most efficient method for construction of large peptides. The convergent and efficient coupling of multiresidue segments, wherein the C-terminal amino acid of necessity is *N*-acylated, would be a useful alternative to linear synthesis; however, *N*-acyl amino acids do racemize under the well-known conditions for amide bond formation.[2]

Recently, several articles have appeared describing segment couplings using *N*-acyl-blocked C-terminus dipeptides to form tripeptides with limited racemization.[3] These articles report peptide bond formation using several common carbodiimides (DCC, EDC, and DIC) in conjunction with HOBT and anhydrous Cu(II) salts, which has been reported earlier.[4] Use of HOBT alone gave small but expected amounts of racemization.[5] We obtained similar results using our newly developed bis-[4-(2,2-dimethyl-1,3-dioxolyl)methyl]carbodiimide (BDDC)[6] with HOBT and anhydrous $CuCl_2$.

Having described the usefulness of CBMIT[7] as an efficient amino acylating reagent, we sought to explore CBMIT's potential as a segment coupling reagent, where an *N*-acyl-blocked amino acid is the activated C-terminus. The advantages of using CBMIT as a coupling reagent include rapid preparation and reaction, neutral conditions, ease of isolation, and much diminished waste stream. Previous work in this laboratory[8] has determined that *N*-acyl-protected amino acids were racemized substantially during coupling with CBMIT alone. Because of the minimization of racemization afforded by $CuCl_2$ in conjunction with HOBT esters during segment coupling, we next investigated the effect of copper salts on racemization of *N*-methylimidazolium-activated *N*-acyl-blocked amino acids. We now present the results of some peptide segment couplings using 1,1′-carbonylbis-(3-methylimidazolium triflate) (CBMIT) in combination with anhydrous $CuCl_2$ and $Cu(OTf)_2$.



CBMIT

Results and Discussion

For our investigations we chose L,L-Z-Ala-Phe (**1**) and L,D-Z-Ala-Phe (**2**) as the *N*-acyl C-terminus peptide segments. This dipeptide has two advantages that made it an ideal chiral probe. First, we desired the couplings to be conducted with a C-terminus amino acid residue known to be sensitive to racemization. If racemization could be minimized with Phe as the C-terminus, then racemization with other less sensitive C-terminal amino acids such as Ala or Pro would assuredly be minimized or eliminated altogether. Second, the Z-Ala-Phe-AA-OR diastereomers L,L,X and L,D,X are easily distinguishable by ^{1}H NMR and quantitatively separable by HPLC, allowing for rapid, convenient, and accurate determination of diastereomeric ratios in the product mixtures. In the case of ^{1}H NMR, the methyl doublet of the Ala residue is shifted approximately 0.05 ppm upfield in the L,D isomers.[9] This allows rapid qualitative and quantitative determinations (using doping experiments) of diastereomer ratios to be conducted by NMR.

Gram quantities of both **1** and the L,D isomer **2** were produced and were determined by ^{1}H NMR and HPLC to be enantiomerically pure. Initial experiments were carried out using Gly-OBn·TsOH as the amine component, coupling with **1** to optimize the reaction conditions. Attempts to use the exact conditions of the earlier CBMIT article[7] were not successful, as nitromethane and DMF in the final reaction mixture resulted in large amounts of racemization. Use of THF as the reaction solvent proved to be more effective. The formation of CBMIT in THF, however, can be problematic due to polymerization caused by traces of triflic acid. The quality of the MeOTf is critical to the success of the reaction. In order to avoid the polymerization problem, CBMIT can be formed in nitromethane, after which the solvent is removed in vacuo and replaced with dry THF. When THF is the solvent for both formation of CBMIT and the coupling, the racemization results are the same, but yields are less predictable and varying amounts of poly-THF are formed, which is difficult to separate from the desired product. Initial couplings varied the amount of $CuCl_2$ and the reaction temperature. The best conditions in this limited study were 100 mol % of $CuCl_2$ and rt,

(1) Klausner, Y. S.; Bodanszky, M. *Synthesis* **1972**, 453.

(2) Some selected articles on carboxy terminus coupling are as follows: Bodanszky, M.; Klausner, Y. S.; Ondetti, M. A. *Peptide Synthesis*, 2nd ed.; Wiley: New York, 1976. Gross, E., Meienhofer, J., Eds. *The Peptides*; Academic Press: New York, 1979; Vols. 1 and 2. Thaler, A.; Seebach, D. *Helv. Chim. Acta* **1991**, *74*, 617. Bodanszky, M. *Pept. Res.* **1992**, *5*, 134. Bodanszky, M. *Principles of Peptide Synthesis, 2nd Ed.*; Springer-Verlag: Berlin, 1993.

(3) (a) Miyazawa, T.; Otomatsu, T.; Fukui, Y.; Yamada, T.; Kuwata, S. *Int. J. Pept. Protein Res.* **1992**, *39*, 308. (b) Miyazawa, T.; Otomatsu, T.; Fukui, Y.; Yamada, T.; Kuwata, S. *Int. J. Pept. Protein Res.* **1992**, *39*, 237.

(4) Higashiyama, T.; Ueda, H.; Nakahara, A. *Bull. Chem. Soc. Jpn.* **1968**, *41*, 3028.

(5) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.

(6) Gibson, F. S.; Park, M.; Rapoport, H. *J. Org. Chem.* **1994**, *59*, 7503.

(7) Saha, A. K.; Shultz, P.; Rapoport, H. *J. Am Chem. Soc.* **1989**, *111*, 4856.

(8) Under typical reaction conditions in THF, racemization was in the range of 4–6%, using L,L-Z-Ala-Phe as the *N*-acyl carboxy terminus segment.

(9) Halpern, B.; Chew, L. F.; Weinstein, B. *J. Am. Chem. Soc.* **1967**, *89*, 5051.

0022-3263/95/1960-2615$09.00/0

Table 1. Coupling of Z-Ala-Phe-OH with Various Amino Acid Esters Using CBMIT/$CuCl_2$, $Cu(OTf)_2$[a]

amino acid ester[b]	product	yield, %	racemization at Phe,[c] %
Gly-OBn	Z-Ala-Phe-Gly-OBn	81	<0.1
Ala-OMe	Z-Ala-Phe-Ala-OMe	79	<0.1
Phe-OBn	Z-Ala-Phe-Phe-OBn	84	<0.1
Val-OMe	Z-Ala-Phe-Val-OMe	73	0.2–0.3
Ile-Phe-OMe	Z-Ala-Phe-Ile-Phe-OMe	76	0.2–0.5
Sar-OMe	Z-Ala-Phe-Sar-OMe	64	1.1–1.3
Aib-OMe	Z-Al-Phe-Aib-OMe	54	3–3.5
N-MeLeu-OMe	Z-Ala-Phe-*N*-MeLeu-OMe	36	~6

[a] Couplings were carried out in THF at rt for 4 h. Both $CuCl_2$ and $Cu(OTf)_2$ were used and gave identical results. [b] In each example, 110 mol % of amino acid ester was used. [c] Refers to carboxy terminus Phe.

yielding L,L-Z-Ala-Phe-Gly-OBn in 81% yield, with less than 0.1% racemization to the L,D-diastereomer. Excess $CuCl_2$ decreased the yield, and reactions at 0 °C, rather than rt, were much slower and showed increased racemization. The same results were obtained when $Cu(OTf)_2$ was used. In all cases, 110 mol % of the amine component was used.

A series of peptide segment couplings were examined (Table 1) with increasingly more hindered amine components. The relatively unhindered Ala-OMe and Phe-OBn also gave little or no racemization. Val-OMe[10] and Ile-Phe-OMe produced a small amount of racemization, and a slightly decreased yield. Racemization increased substantially with Sar-OMe, Aib-OMe, and *N*-MeLeu-OMe, and the yield of product dropped significantly. These results indicate the sensitivity of the activated C-terminus *N*-methylimidazolium derivative to racemization. Long lifetimes produce increased racemization. Without the use of the more polar DMF and nitromethane, the reaction rate is lower, as is clear when comparing yields to those reported in the previous work.[7] Considering the sensitivity of Phe residues toward racemization, these results are promising in that the unhindered amines were able to couple without racemization. Less sensitive amino acids would be expected to couple without racemization, opening the possibility for many peptide segment couplings where the C-terminus amino acid does not have to be glycine. Furthermore, increasing the stoichiometry of the amine component should also decrease racemization.

Conclusion

Activation of the carboxy terminus of a peptide with CBMIT followed by reaction with unhindered amino acid esters in the presence of $CuCl_2$ or $Cu(OTf)_2$ gives peptide segment couplings with very little racemization. The reaction conditions are mild, effectively neutral, and produce innocuous byproducts easily removed by washing with dilute aqueous base and acid. The limited extent of epimerization observed in the coupled carboxy terminus compares quite favorably with other coupling protocols.[3,6]

Experimental Section

All peptide coupling reactions were carried out in oven-dried glassware under a nitrogen atmosphere. THF was distilled from sodium benzophenone ketyl; NMM and *N*-methylimidazole were distilled prior to use and stored under nitrogen. Anhydrous $CuCl_2$ and $Cu(OTf)_2$[11] were prepared by heating $CuCl_2 \cdot H_2O$ and $Cu(OTf)_2$ at 100 °C and 0.1 Torr for 24 h. CDI was recrystallized from benzene and dried under high vacuum before use. Methyl triflate was distilled immediately prior to use. All amino acid ester salts were dried at 100 °C and 0.1 Torr for 24 h. Nitromethane was freshly distilled from CaH_2 and stored under nitrogen. IR spectra were recorded as thin films with absorptions reported in reciprocal centimeters. ^1H NMR spectra were recorded at 300 MHz in $CDCl_3$ using TMS as an internal reference unless otherwise noted. All chemical shifts (δ) are reported in ppm and coupling constants are in hertz. Melting points are uncorrected. Final organic extracts were dried over Na_2SO_4. All HPLC data were obtained directly from crude reaction mixtures that had not been chromatographed or recrystallized using 230–400 mesh silica and detecting at 254 nm. HPLC percentages are measured against authentic doped standards. Final yields are for chromatographed or recrystallized material.

General Procedure for Peptide Segment Coupling Using CBMIT with Anhydrous $CuCl_2$ or $Cu(OTf)_2$. CDI (88 mg, 0.54 mmol) was placed in a dry 10 mL flask under a nitrogen atmophere with a magnetic stir bar and capped with a septum. Dry nitromethane (2 mL) was introduced by syringe, and the flask was cooled to 0 °C. Methyl triflate (122 μL, 200 mol %) was then added, and the reaction mixture was allowed to stir for 5 min. The nitromethane was removed in vacuo to give a white solid residue, which was redissolved/suspended in dry THF (6 mL), and recooled to 0 °C. $CuCl_2$ or $Cu(OTf)_2$ was added (100 mol %, 74 or 194 mg, respectively), and the mixture was stirred for 5 min. Z-Ala-Phe (L,L or L,D, 200 mg, 100 mol %) was added in one portion and the dipeptide dissolved rapidly, with gas evolution. After another 5 min of stirring, the dry amino acid ester salt was added (110 mol %) followed by NMM (66 μL, 110 mol %) or *N*-methylimidazole (110 mL%, 48 μL), and the reaction mixture was allowed to stir at rt for 4 h, by which time it had become homogeneous. The reaction solution was then diluted with 40 mL of EtOAc and washed with one 20 mL portion of 0.1 M H_3PO_4, followed by one 20 mL portion of aqueous saturated bicarbonate. The organic layer was dried, filtered, and evaporated to yield either a white solid or thick oil. This material was then redissolved in fresh EtOAc for HPLC determination of % racemization.

L,L-Z-Ala-Phe-Gly-OBn was prepared from L,L-Z-Ala-Phe and Gly-OBn·TsOH: yield, 81%; HPLC analysis (EtOAc/hexane, 1/1, 1 mL/min) indicated <0.1% racemization; mp 160–163 °C; $[\alpha]^{25}_D$ −43.1° (*c* 0.7, $CHCl_3$); ^1H NMR δ 7.48–7.21 (m, 15H), 6.65–6.58 (m, 2H), 5.27 (d, 1H, J = 6.6), 5.13 (s, 2H), 5.08 (d, 1H, J = 12), 5.00 (d, 1H, J = 12), 4.74 (dd, 1H, J = 7.2, 15), 4.16 (t, 1H, J = 6.7), 4.13–3.87 (m, 2H), 3.20–3.00 (m, 2H) 1.23 (d, 3H, J = 7). Anal. Calcd for $C_{28}H_{29}N_3O_6$: C, 67.3; H, 6.0; N,8.1. Found: C, 66.9; H, 6.0; N, 8.1.

L,D,L-Z-Ala-Phe-Ala-OMe was prepared from L,D-Z-Ala-Phe and Ala-OMe·HCl: yield, 79%, HPLC analysis (EtoAc/hexane, 1/1, 1 mL/min) indicated <0.1% racemization; mp 171–172 °C; $[\alpha]^{25}_D$ −5.0° (*c* 1.1, $CHCl_3$); ^1H NMR δ 7.40–7.20 (m, 10H), 6.97 (d, 1H, J = 7.1), 6.86 (d, 1H, J = 6.8), 5.65 (d, 1H, J = 7.0, 5.12–4.99 (m, 2H), 4.81–4.73 (m, 1H), 4.51–4.46 (m, 1H), 4.23–4.13 (m, 1H), 3.65 (*s*, 3H), 3.20–3.00 (m, 2H), 1.25 (d, 6H, J = 7.0). Anal. Calcd for $C_{24}H_{29}N_3O_6$: C, 63.3; H, 6.4; N, 9.2. Found: C, 62.9; H, 6.5; N, 9.2.

L,D,L-Z-Ala-Phe-Phe-OBn was prepared from L,D-Z-Ala-Phe and Phe-OBn·TsOH: yield, 84%; HPLC analysis (EtOAc/hexane, 2/3, 1/1, 1 mL/min) indicated <0.1% racemization; mp 172–173 °C; $[\alpha]^{25}_D$ −82.8° (*c* 1.8, $CHCl_3$); ^1H NMR δ 7.50–6.90 (m, 20H), 6.60 (d, 1H, J = 6.9), 6.55 (d, 1H, J = 7.0), 5.20 (d, 1H, J = 7.0), 5.15–5.00 (m, 4H), 4.89–4.80 (m, 1H), 4.70–4.64 (m, 1H), 4.15–4.05 (m, 1H), 3.11–2.95 (m, 4H), 1.19 (d, 3H, J = 7.0). Anal. Calcd for $C_{36}H_{37}N_3O_6$: C, 71.2; H, 6.1; N, 6.9. Found: C, 71.4; H, 6.4; N, 6.9.

L,L,L-Z-Ala-Phe-Val-OMe was prepared from L,L-Z-Ala-Phe and Val-OMe·HCl: yield, 73%; HPLC analysis (EtOAc/hexane, 1/1, 1 mL/min) indicated 0.2–0.3% racemization; recrystallization from THF/hexane gave pure L,L,L isomer, mp 154–155 °C; $[\alpha]^{25}_D$ −45.3° (*c* 0.45, EtOH); ^1H NMR δ 7.45–7.15 (m, 10H), 6.88 (d, 1H, J = 5.1), 6.77 (d, 1H, J = 6.0), 5.48 (d, 1H, J = 6.8),

(10) Sar = sarcosine, *N*-methylglycine; Aib = aminoisobutyric acid.

(11) Jenkins, C. L.; Kochi, J. K. *J. Am. Chem. Soc.* **1972**, *94*, 843.

5.10 (m, 2H), 4.75 (m, 1H), 4.43 (m, 1H), 4.30 (m, 1H), 3.69 (s, 3H), 3.05 (d, 2H, J = 6.4), 2.10–2.00 (m, 1H), 1.30 (d, 3H, J = 6.8), 0.9–0.8 (m, 6H). Anal. Calcd for $C_{26}H_{33}N_3O_6$: C, 64.6; H, 6.9; N, 8.7. Found: C, 64.4; H, 7.1; N, 8.3.

L,L,L,L-Z-Ala-Phe-Ile-Phe-OMe was prepared from L,L-Z-Ala-Phe and L,L-Ile-Phe-OMe: yield, 76%; HPLC analysis (EtOAc/hexane, 1/1, 1 mL/min) indicated 0.2–0.5% racemization; recrystallization from THF/hexane gave pure L,L,L,L isomer: mp 204–205 °C; $[\alpha]^{25}_D$ −45.3° (c 0.45, EtOH); ^{1}H NMR δ 8.15 (br s, 1H), 7.60–7.50 (br s, 1H), 7.51–7.50 (m, 15H), 6.20 (br s, 1H), 5.10–5.02 (m, 3H), 4.85–4.55 (m, 4H), 3.58 (s, 3H), 3.05–2.90 (m, 4H), 1.80–1.70 (m, 1H), 1.50–1.40 (m, 1H), 1.29 (d, 3H, J = 6.8), 1.15–1.00 (m, 1H), 0.87 (d, 3H, J = 6.6), 0.79 (t, 3H, J = 7.2). Anal. Calcd for $C_{36}H_{44}N_4O_7$: C, 67.1; H, 6.9; N, 8.7. Found: C, 67.4; H, 7.2; N, 8.8.

L,L-Z-Ala-Phe-Sar-OMe was prepared from L,L-Z-Ala-Phe and Sar-OMe·HCl; yield, 64%; HPLC analysis (EtOAc/hexane, 1/1, 1 mL/min) indicated 1.3–1.1% racemization. Anal. Calcd for $C_{24}H_{29}N_3O_6$: C, 63.3; H, 6.4; N, 9.2. Found: C, 63.0; H, 6.6; N, 9.1.

L,L-Z-Ala-Phe-Aib-OMe was prepared from L,L-Z-Ala-Phe and Aib-OMe: yield, 54%; HPLC analysis (EtOAc/hexane, 1/1, 1 mL/min) indicated 3–3.5% racemization. Anal. Calcd for $C_{25}H_{31}N_3O_6$: C, 63.9; H, 6.6; N, 8.9. Found: C, 63.7; H, 6.7; N, 8.9.

L,L-Z-Ala-Phe-*N*-MeLeu-OMe was prepared from L,L-Z-Ala-Phe and N-MeLeu-OMe·HCl: yield, 36%; HPLC analysis (EtOAc/hexane, 2/3, 1 mL/min) indicated ~6% racemization. Anal. Calcd for $C_{28}H_{37}N_3O_6$: C, 65.7; H, 7.3; N, 8.2. Found: C, 65.8; H, 6.9; N, 8.4.

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