African Journal of Pure and Applied Chemistry Vol. 3 (6), pp. 108-115, June 2009
Available online at http://www.academicjournals.org/AJPAC
ISSN 1996 - 0840 © 2009 Academic Journals

Full Length Research Paper

Selective synthesis applying amino acids with basic side chains as peptide precursors

Jeanne L. Kuhler*, Robey T. Brooks, Whitney K. Helms, Monica Hodge, Krystal D. Holly, Brandi L. Inman, Oluwaseun A. Koriko, Rimpal R. Patel and Tiffany R. Shorter

Department of Physical Science, Goodwyn Hall, Room 309 O, P.O. Box 244023, Auburn University, Montgomery, AL 36124.

Accepted 5 June, 2009.

Synthesis of protected amino acid derivatives has long been established as one of the most useful methods for peptide coupling, with applications in studies involving receptor-binding affinities and in protein stereochemical studies to elucidate enzymatic mechanisms. Amino acid esters incorporating glycine, proline, phenylalanine and related methyl esters have often been synthesized due to their usefulness, and the lysine derivative named N_a - Benzyloxycarbonyl - N_e - *tert*- butyloxycarbonyl - L - lysin - (*N* - hydroxy-succinimidester) has also been used extensively as a coupling agent for the formation of peptide bonds and to facilitate the synthesis of endorphins and enzyme inhibitors. However, efficient syntheses have been rare, and therefore a novel, multi-step sequence involving the selective addition and subsequent removal of several common protecting groups has recently been completed. This novel method was facilitated via the use of a sterically hindered base and a Dean-Stark trap to decrease the time required for complete cyclization. Reaction progress was monitored using thin layer chromatography, and all products have been analytically characterized using nuclear magnetic resonance spectroscopy and infrared spectroscopy. Enantioselectivity has been maintained for both of the amino acids arginine and lysine.

Key words: Protecting groups, amino acids, peptides, lactams.

INTRODUCTION

Synthesis of amino acid active esters protected at the nitrogen atom of an amino acid (*N*-protected amino acid derivatives) has become widely recognized as one of the most useful methods to cause the activetion necessary for peptide coupling (Giralt et al. 1990; Riniker et al., 1993). These *N*-protected amino acid derivatives have been important for the synthesis of peptides for studies involving receptor-binding affinities and for protein stereochemical studies to elucidate enzymatic mechanisms.

Reports of syntheses of amino acid esters et al., 1991; Furlan et al., 1998). The target incorporating glycine, proline, phenylalanine and related methyl esters have been widespread (Ishizu compounds of this report, N_a-Benzyloxycarbonyl - N_e - *tert* - butyloxycarbonyl - L - lysin - (*N* - hydroxy-succinimidester) (6) (Figure 1) and N_a – Benzyloxycarbonyl - N_e - *tert* - butyloxycarbonyl - L - arginin - (*N* - hydroxy-succinimidester)(22) (Figure 3) have been used extensively as coupling agents for the formation of peptide bonds (Hiskey et al., 1975) and for application in the synthesis of endorphins and peptide factors (Moroder et al., 1994), analogs of somatostatin (Allen et al., 1988), enzyme inhibitors (Sawayama et al., 1989) and in structure activity

*Corresponding author. Email: jkuhler@aum.edu

Abbreviations: **^{1}H NMR;** Proton Nuclear Magnetic ,Resonance spectroscopy, **^{13}C NMR;** Carbon Nuclear Magnetic Resonance spectroscopy, **IR;** Infrared Radiation spectroscopy, **GC-MS;** Gas Chromatography-Mass Spectrometry, **THF;** Tetrahydrofuran (common solvent medium for reactions), **CBz-Cl;** Benzyloxycarbonyl chloride, a common protecting group, **$(BOC)_2O$;** di-*tert*-butyldicarbonate, a common protecting group, **DMAP;** dimethylaminopyridine, a common base used as a catalyst, **TEA;** triethylamine, a common base, **Pd/C;** palladium on carbon, a common catalyst.

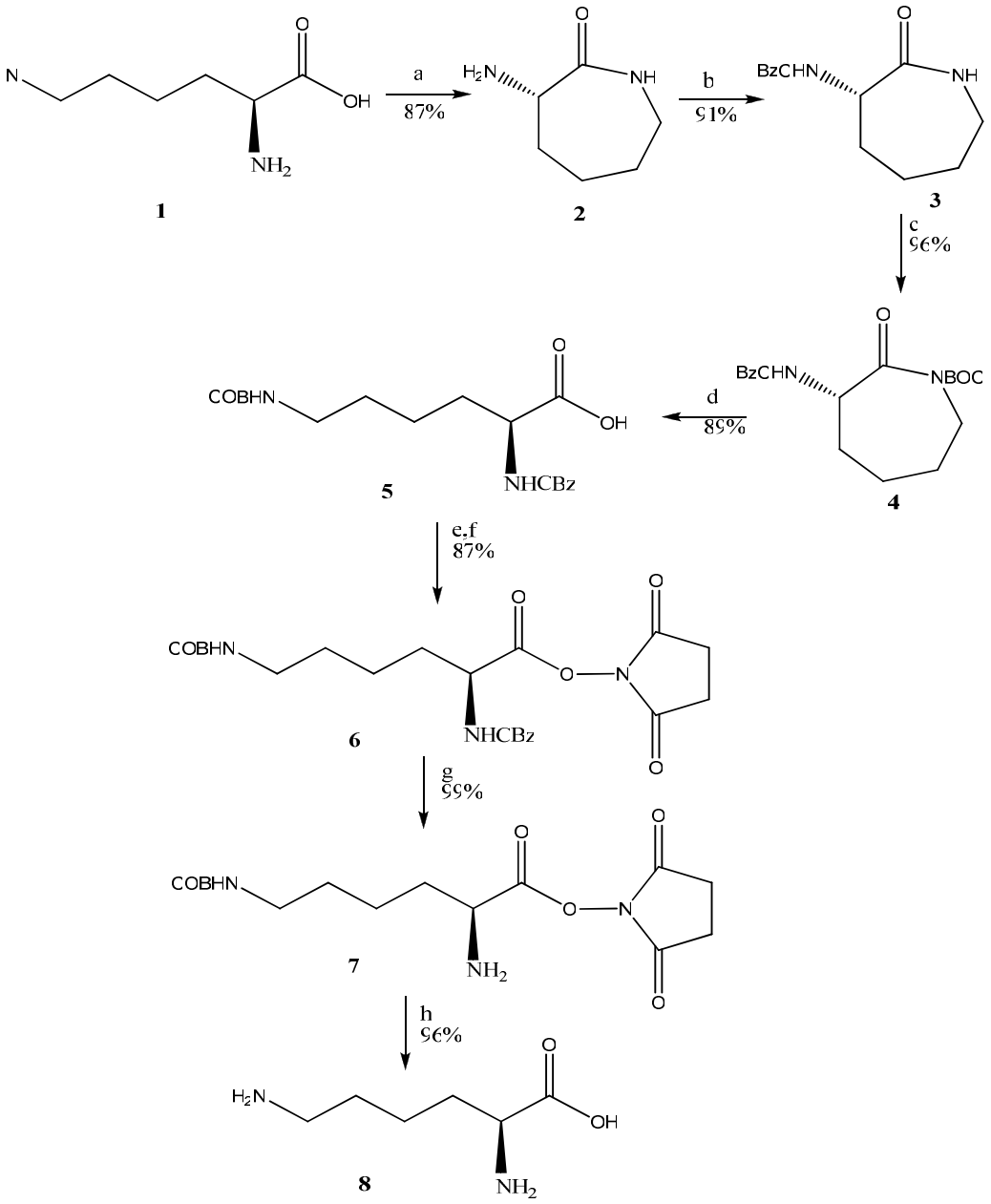

Figure 1. Synthetic steps to protect and deprotect L - (+) - lysine.
a) 1,3-Bis(trimethylsilyl)urea, dicyclohexylamine, THF, reflux; b) CBz-Cl, saturated $NaHCO_3$, THF; c) $(BOC)_2O$, DMAP, THF; d) 1N LiOH, aqueous THF; e) Trimethylacetyl chloride, TEA, THF; f) *N* – hydroxysuccinimide, THF; g) Pd/C, H_2, Ethyl Acetate; h) aqueous HCl.

relationship studies of hormones (Moroder et al., 1979).

MATERIALS AND METHODS

General experimental materials

Reagents and solvents were used as received from commercial vendors and no attempts were made to purify or dry these components further. Thin layer chromatography was performed using 1" x 3" Analtech GF 350 silica gel plates with fluorescent indicator. Visualization of TLC plates was made by observation in iodine vapors. The proton and carbon magnetic resonance spectra were recorded on a Bruker AC 300 MHz Nuclear Magnetic Resonance Spectrometer, using either $CDCl_3$ or CD_3OD as the solvent with tetramethylsilane as an internal reference. Melting points were obtained using an electrothermal melting point apparatus and are uncorrected. Infrared spectra (IR) were obtained as KBr pellets and obtained on a Perkin-Elmer Spectrum 1000 FT-Infrared Spectrophotometer. Low resolution mass spectroscopic analyses were performed on a Shimadzu QP - 5000 GC/Mass Spectrometer (CI, methane) by direct insertion.

Synthetic methods

(S) - (α) - Amino - (α) - caprolactam (2): To a 100 mL 3 necked flask equipped with a magnetic stir bar, a water cooled reflux condenser, and a Dean-Stark trap was added L - (+) - lysine hydrochloride (1) (5.00 g, 27.38 mmol). THF (50 mL) was added and the stirrer was started. A solution of 1,3-bis(trimethylsilyl)urea (8.40 g, 41.07 mmol) dissolved in THF (20 mL) was added in one portion, followed by the addition of dicyclohexylamine (2.50 mL, 13.69 mmol) via syringe. The 3 necked flask was placed in an electric heating mantle and the mixture was heated at reflux for 50 min, until thin layer chromatography (TLC) (silica gel plate eluted with 1: 1 Ethyl Acetate: Hexanes; Iodine visualization) indicated the complete consumption of the lysine. The solution was cooled to ambient temperature and was poured into a 100 mL separatory funnel. The solution was washed with saturated aqueous sodium chloride (1 x 10 mL), water (2 x 15 mL), and 0.1 N hydrochloric acid (1 x 15 mL). The organic layer and the interfacial precipitates were dried over anhydrous sodium sulfate, filtered, and the solvent was evaporated under reduced pressure conditions to afford an 87% yield of (S) - (α) - Amino - (ε) - caprolactam (2) (3.06 g) as a white solid. Mp: 97 - 101°C. R_f 0.49 (1:1 Ethyl Acetate: Hexanes). ^{1}H NMR (300 MHz, CD_3OD): δ 7.19 (br s, 2 H), 5.12 (d, J = 11.1 Hz, 1 H), 3.58 (s, 1 H), 3.15 (m, 2 H), 1.49 - 1.14 (m, 6 H). ^{13}C NMR (75 MHz, CD_3OD): δ 178.26, 52.82, 41.01, 33.72, 28.17, 27.94. IR (KBr): 3448, 3321, 2929, 1676, 1155 cm^{-1}; CI Mass Spectrum (methane) m/z 129 (M + 1). $(\alpha)_D^{25}$ = - 25.2° (c = 1.00, CH_3OH). Anal. Calcd. For $C_6H_{12}N_2O$: C, 56.23; H, 9.44; N, 21.86. Found: C, 56.61; H, 9.52; N, 21.51.

(S) - *N* - (α) - Benzyloxycarbonyl - (ε) - caprolactam (3): To a 100 mL round bottom flask equipped with a magnetic stir bar was added (S) - (α) - Amino - (ε) - caprolactam (2) (0.75 g, 5.85 mmol) and THF (30 mL). Saturated sodium bicarbonate (10 mL) was added to adjust the pH to 8. Benzyl chloroformate (0.84 mL, 5.85 mmol) was added via syringe. The solution was stirred at room temperature for 15 min. Then the mixture was poured into a 100 mL separatory funnel and washed with saturated sodium chloride solution (1 x 5.0 mL) and water (2 x 5.0 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and the solvent was removed under reduced pressure to afford a 91% yield of (S) - *N* - (α) – Benzyloxycarbonyl - (ε) - caprolactam (3) (1.39 g) as a white solid. mp: 142 - 146°C. R_f 0.88 (1:1 Ethyl Acetate: Hexanes). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72 (m, 1H), 7.32 (s, 5 H), 7.01 (d, 1H, J = 11.1 Hz), 6.21 (s, 1 H), 5.01 (s, 2 H), 3.08 (m, 2 H), 2.19-1.26 (m, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 186.75, 172.05, 154.72, 140.89, 134.85, 128.13, 127.95, 127.61, 126.52, 69.24, 45.79, 31.34, 28.79. CI Mass Spectrum (methane) m/z 262 (M^+); IR (KBr): 1776, 1686, 1134, 791, 750 cm^{-1}. Anal Calcd. For $C_{14}H_{18}N_2O_3$: C, 64.11; H, 6.92; N, 10.68. Found: C, 64.38; H, 7.11; N, 10.37.

(S) - *N* - (3) - Benzyloxycarbonyl - *N* -(1) - *tert* - butyloxycarbonyl - (ε) - caprolactam (4): To a 100 mL round bottom flask equipped with a magnetic stir bar was added (S) - *N* - (α) - Benzyloxycarbonyl - (ε) - caprolactam (3) (0.50 g, 1.92 mmol) and DMAP (0.0370 g, 0.303 mmol). THF (30 mL) was added. Di-*tert*-butyldicarbonate (0.132 g, 0.605 mmol) in THF (20 mL) was added to the solution. The solution was stirred at room temperature for 20 min and then was poured into a separatory funnel and washed with saturated sodium chloride solution (1 x 5.0 mL) and water (2 x 5.0 mL). The organic layer was dried, filtered, and evaporated to afford a 96% yield of (S) - *N* - (3) - Benzyloxycarbonyl - *N* - (1) - *tert*-butyloxycarbonyl - (ε) - caprolactam (4) (0.67 g) as a pale yellow solid. R_f 0.47 (1: 4 Ethyl Acetate: Hexanes). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34 (s, 5 H), 5.11 (s, 2 H), 3.92 (m, 1 H), 3.62 - 3.30 (m, 2 H), 1.86 - 1.52 (m, 5 H), 1.51 (s, 9 H), 1.37 - 1.08 (m, 2 H). CI Mass Spectrum (methane) m/z 362 (M^+); IR (KBr): 3448, 3102, 1778, 1762, 1688, 1455, 1122, 1091, 699 cm^{-1}. Anal. Calcd. For $C_{19}H_{26}N_2O_5$: C, 62.97; H, 7.23; N, 7.73. Found: C, 63.05; H, 7.37; N, 7.52.

***N_a* - Benzyloxycarbonyl - *N_e*- *tert* -butyloxycarbonyl - L - lysine (5):** To a 100 mL round-bottom flask equipped with a magnetic stir bar and containing aqueous THF (70 mL) was added (S) - *N* - (3) - Benzyloxycarbonyl - *N* - (1) *tert*-butyloxycarbonyl - (ε) - caprolactam (4) (0.38 g, 1.04 mmol).

Lithium hydroxide (1.0 N, 3.0 mmol, 3.0 mL) was added and the solution was stirred at room temperature for 15 min until TLC analysis (1:1 Ethyl Acetate: Hexanes) indicated the complete disappearance of starting material. The solution was poured into a 100 mL separatory funnel and washed with saturated sodium chloride solution (1 x 5.0 mL) and water (2 x 5.0 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and the THF was removed under reduced pressure to afford an 89% yield of *N_a* - Benzyloxycarbonyl - *N_e*- *tert* - butyloxycarbonyl - L - lysine (5) (0.35 g) as a clear oil which solidified to a pale yellow solid. R_f 0.28 (1: 1 Ethyl Acetate: Hexanes). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34 (s, 5 H), 5.11 (s, 2 H), 4.70 (s, 1 H), 4.12 (m, 1 H), 3.62 - 3.32 (m, 2 H), 1.86 - 1.09 (m, 7 H), 1.51 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.69, 137.27, 128.51, 128.37, 127.61, 126.94, 68.67, 66.43, 55.42, 31.18, 27.76, 26.28, 25.47. CI Mass Spectrum (methane) m/z 380 (M^+); IR (KBr): 3504 - 3102, 2931, 1741, 1726, 1650, 1292, 1292, 1021, 768, 698 cm^{-1}. Anal. Calcd. For $C_{19}H_{28}N_2O_6$: C, 59.99; H, 7.42; N, 7.36. Found: C, 60.22; H, 7.50; N, 7.28.

***N_a* - (3) - Benzyloxycarbonyl - *N_e*- (1) - *tert* - butyloxycarbonyl - L -0lysin - (*N* - hydroxysuccinimide ester) (6):** To a 100 mL round-bottom flask equipped with a magnetic stir bar and capped with a rubber septum was added *N_a* - Benzyloxycarbonyl - *N_e* - *tert* - butyloxycarbonyl - L -lysine (5) (0.26 g, 0.69 mmol) and THF (40 mL). Triethylamine (0.10 mL, 0.69 mmol) was added via syringe. Trimethylacetyl chloride (0.09 mL, 0.69 mmol) was added and the solution was stirred for 10 min at room temperature. *N*-hydroxysuccinimide (0.079 g, 0.69 mmol) was added, which caused a white precipitate to form, and the solution was stirred at room temperature for 10 min. The solution was poured into a 100 mL separatory funnel and washed with saturated sodium chloride solution (1 x 5.0 mL) and water (2 x 5.0 mL). The organic layer was collected, dried over anhydrous sodium sulfate, filtered, and the solvent removed under reduced pressure to yield a clear syrup, which solidified to afford an 92% yield of *N_a* - (3) - Benzyloxycarbonyl - *N_e* - (1) - *tert* - butyloxycarbonyl - L -lysin - (*N* - hydroxysuccinimide ester) (6) (0.301 g) as a pale yellow solid. Crystallization using isopropyl alcohol and hexanes afforded an 87% yield of *N* - (3) - Benzyloxycarbonyl - *N* - (1) - *tert*-butyloxycarbonyl - L - lysin - *N* - hydroxysuccinimide ester (6) (0.286 g) as white crystals. Mp: 96 - 100ºC. R_f 0.30 (1:1 Ethyl Acetate: Hexanes). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35 (s, 5 H), 5.12 (s, 2 H), 4.69 (s, 1 H), 4.11 (m, 1 H), 3.52 - 3.30 (m, 2 H), 2.80 (s, 4 H), 1.77 - 1.22 (m, 7 H), 1.42 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.14, 169.05, 155.45, 137.08, 133.04, 128.14, 127.96, 127.38, 68.39, 66.20, 55.24, 38.09, 30.09, 27.52, 26.86, 26.67, 26.05, 25.37, 25.24. CI Mass Spectrum (methane) m/z 478 (M^+); IR (KBr): 3422, 2934, 1809, 1776, 1735, 1727, 1687, 1213, 1071, 750, 697 cm^{-1}. $(\alpha)_D^{25}$ = + 15.1°(c = 1.00, $CHCl_3$). Anal. Calcd. For $C_{23}H_{31}N_3O_8$: C, 57.85; H, 6.54; N, 8.80. Found: C, 57.96; H, 6.88; N, 8.61.

***N_a* - (3) - Amino - *N_e* -1 - *tert* - butyloxycarbonyl - L - lysin - (*N* - hydroxysuccinimide ester) (7):** To a 100 mL flask charged with *N_a* - (3) - Benzyloxycarbonyl - *N_e* - (1) - *tert* - butyloxycarbonyl - L - lysin - (*N* - hydroxysuccinimide ester) (6) (0.280 g, 0.583 mmol) was added 5% Palladium on carbon (0.05 g) and ethyl acetate (50 mL). The flask was evacuated and purged with nitrogen 3 times, then filled with hydrogen (30 psi). The reaction was monitored by thin layer chromatography (TLC) (2:3 Ethyl Acetate: Hexanes; Iodine visualization) and after 10 min the reaction was complete. The contents of the flask were filtered through a pad of Celite and the filtrate was concentrated to afford a 99% yield (0.255 g) of *N_a* - (3) - Amino - *N_e* - 1 - *tert* - butyloxycarbonyl - L - lysin - (*N* - hydroxysuccinimide ester) (7) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.67 (s, 2 H), 4.10 (m, 1 H), 3.48 - 3.30 (m, 2 H), 2.79 (s, 4 H), 1.75 - 1.19 (m, 7H), 1.41 (s, 9 H).

L – (+) - lysine (8): To a 100 mL round bottom flask equipped with a magnetic stir bar was added *N_a* - (3) - Amino - *N_e* - 1 - *tert* - butyloxycarbonyl - L - lysin - (*N* - hydroxysuccinimide ester) (7) (0.355 g, 1.04 mmol). A 0.1 N solution of hydrochloric acid (20 mL) was added and the stirrer was started. The reaction was monitored by thin layer chromatography (TLC) (3:2 Ethyl Acetate: Hexanes; Iodine visualization) and after stirring for 10 min, the reaction was complete. The solution was filtered to afford a 96% yield (0.145 g) of L - (+) - lysine (8) as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 4.05 (t, 1 H, J = 6.0 Hz), 3.02 (t, 2 H, J = 7.1 Hz), 1.99 (m, 2 H), 1.71 (t, 2 H, J = 7.2 Hz), 1.52 (m, 2 H). CI Mass Spectrum (methane) m/z 147 (M^+ + H); $(\alpha)_D^{25}$ = + 10.2º (c = 1.00, H_2O).

(R) - (α) - Amino - (ε) - caprolactam (10): To a 100 mL 3 necked flask equipped with a mechanical stirrer, a water cooled reflux condenser, and a Dean-Stark trap was added D - (-) - lysine hydrochloride (9) (5.00 g, 27.38 mmol). THF (80 mL) was added and the stirrer was started. A solution of 1,3 - bis(trimethylsilyl)urea (8.40 g, 41.07 mmol) dissolved in THF (20 mL) was added in one portion, followed by the addition of dicyclohexylamine (2.50 mL, 13.69 mmol) via syringe. The 3 necked flask was placed in an electric heating mantle and the mixture was heated at reflux for 50 min, until thin layer chromatography (TLC) (silica gel plate eluted with 1: 1 Ethyl Acetate: Hexanes; iodine visualization) indicated the complete consumption of the lysine. The solution was cooled to ambient temperature and was poured into a 100 mL separatory funnel. The solution was washed with saturated aqueous sodium chloride (1 x 10 mL), water (2 x 15 mL), and 0.1 N hydrochloric acid (1 x 15 mL). The organic layer and the interfacial precipitates were dried over anhydrous sodium sulfate, filtered, and the solvent was evaporated under reduced pressure conditions to afford an 88% yield of (R) - (α) - Amino - (ε) - caprolactam (10) (3.09 g) as a white solid. Mp: 97 - 101º C. R_f 0.49 (1:1 Ethyl Acetate: Hexanes). ^{1}H NMR (300 MHz, CD_3OD): δ 7.16 (br s, 2 H), 5.10 (d, J = 11.1 Hz, 1 H), 3.55 (s, 1 H), 3.11 (m, 2 H), 1.50 - 1.13 (m, 6 H). ^{13}C NMR (75 MHz, CD_3OD): δ 178.14, 52.37, 40.98, 33.62, 28.09, 27.52. IR (KBr): 3447, 3322, 2929, 1677, 1154 cm^{-1}; CI Mass Spectrum (methane) m/z 129 (M + 1). $(\alpha)_D^{25}$ = + 25.4º (c = 1.00, CH_3OH). Anal. Calcd. For $C_6H_{12}N_2O$: C, 56.23; H, 9.44; N, 21.86. Found: C, 56.58; H, 9.59; N, 21.64.

(R) - *N* - (α) - Benzyloxycarbonyl - (ε) - caprolactam (11): To a 100 mL round bottom flask equipped with a magnetic stir bar was added (R) - (α) - Amino - (ε) - caprolactam (10) (0.75 g, 5.85 mmol) and THF (30 mL). Saturated sodium bicarbonate (10 mL) was added to adjust the pH to 8. Benzyl chloroformate (0.84 mL, 5.85 mmol) was added via syringe. The solution was stirred at room temperature for 15 min. Then the mixture was poured into a 100 mL separatory funnel and washed with saturated sodium chloride solution (1 x 5.0 mL) and water (2 x 5.0 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and the solvent was removed under reduced pressure to afford an 89% yield of (R) - *N* - (α) - Benzyloxycarbonyl - (ε) - caprolactam (11) (2.73 g) as a white solid. Mp: 142-145ºC. R_f 0.88 (1:1 Ethyl Acetate: Hexanes). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71 (m, 1 H), 7.30 (s, 5 H), 6.99 (d, 1 H, J = 11.1 Hz), 6.19 (s, 1 H), 4.98 (s, 2 H), 3.07 (m, 2 H), 2.18 - 1.24 (m, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 186.55, 172.04, 154.69, 140.77, 134.81, 128.11, 127.92, 127.59, 126.49, 69.22, 45.71, 31.31, 28.79. CI Mass Spectrum (methane) m/z 262 (M^+); IR (KBr): 1774, 1688, 1132, 790, 751 cm^{-1}. Anal. Calcd. For $C_{14}H_{18}N_2O_3$: C, 64.11; H, 6.92; N, 10.68. Found: C, 64.27; H, 7.09; N, 10.41.

(R) - *N* - (3) - Benzyloxycarbonyl - *N* - (1) - *tert* - butyloxycarbonyl - (ε) - caprolactam (12): To a 100 mL round bottom flask equipped with a magnetic stir bar was added (R) - *N* - (α) - Benzyloxycarbonyl - (ε) - caprolactam (11) (1.95 g, 7.45 mmol) and DMAP (0.080 g, 0.65 mmol). THF (30 mL) was added. Di - *tert*- butyldicarbonate (1.43 g, 6.56 mmol) in THF (20 mL) was added to the solution. The solution was stirred at room temperature for 10 min and then was poured into a 100 mL separatory funnel and washed with saturated sodium chloride solution (1 x 5.0 mL) and water (2 x 5.0 mL). The organic layer was dried, filtered, and evaporated to afford an 88% yield of (R) - *N* - (3) - Benzyloxycarbonyl - *N* - (1) - *tert* - butyloxycarbonyl - (ε) - caprolactam (12) (2.53 g) as a pale yellow solid. R_f 0.47 (1:4 Ethyl Acetate: Hexanes). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33 (s, 5 H), 5.10 (s, 2 H), 3.90 (m, 1 H), 3.61 - 3.29 (m, 2 H), 1.82 - 1.52 (m, 5 H), 1.50 (s, 9 H), 1.39 - 1.10 (m, 2 H). CI Mass Spectrum (methane) m/z 362 (M^+); IR (KBr): 3449, 3102, 1776, 1764, 1687, 1452, 1121, 1094, 701 cm^{-1}. Anal. Calcd. For $C_{19}H_{26}N_2O_5$: C, 62.97; H, 7.23; N, 7.73. Found: C, 63.11; H, 7.31; N, 7.56.

***N_a* - Benzyloxycarbonyl - *N_e*- *tert* - butyloxycarbonyl - D - lysine (13):** To a 100 mL round bottom flask equipped with a magnetic stir bar and containing aqueous THF (70 mL) was added (R) - *N* - (3) - Benzyloxycarbonyl - *N* - (1) - *tert* - butyloxycarbonyl - (ε) - caprolactam (12) (2.41 g, 6.65 mmol). Lithium hydroxide (1.0 N, 3.0 mmol, 3.0 mL) was added and the solution was stirred at room temperature for 15 min until TLC analysis (1:1 Ethyl Acetate: Hexanes) indicated the complete disappearance of starting material. The solution was poured into a 100 mL separatory funnel and washed with saturated sodium chloride solution (1 x 5.00 mL) and water (2 x 5.00 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and the THF was removed under reduced pressure to afford an 85% yield of *N_a* – Benzyloxycarbonyl - *N_e*- *tert* - butyloxycarbonyl - D - lysine (13) (2.16 g) as a clear oil which solidified to a pale yellow solid. R_f 0.28 (1:1 Ethyl Acetate: Hexanes). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32 (s, 5 H), 5.08 (s, 2 H), 4.65 (s, 1 H), 4.09 (m, 1 H), 3.63-3.30 (m, 2 H), 1.85 - 1.07 (m, 7 H), 1.50 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.58, 137.23, 128.49, 128.32, 127.59, 126.90, 68.72, 66.42, 55.38, 31.14, 27.72, 26.24, 25.45. CI Mass Spectrum (methane) m/z 380 (M^+); IR (KBr): 3505 - 3104, 2930, 1742, 1726, 1650, 1293, 1023, 769, 699 cm^{-1}. Anal. Calcd. For $C_{19}H_{28}N_2O_6$: C, 59.99; H, 7.42; N, 7.36. Found: C, 60.16; H, 7.53; N, 7.29.

***N_a* - (3) - Benzyloxycarbonyl - *N_e* - (1) - *tert* - butyloxycarbonyl - D - lysin - (N - hydroxysuccinimide ester) (14):** To a 100 mL round bottom flask equipped with a magnetic stir bar and capped with a rubber septum was added *N_a* - Benzyloxycarbonyl - *N_e* - *tert* - butyloxycarbonyl - D - lysine

(13) (1.22 g, 3.21 mmol) and THF (50 mL). Triethylamine (0.47 mL, 3.35 mmol) was added via syringe. Trimethylacetyl chloride (0.42 mL, 3.35 mmol) was added and the solution was stirred for 10 min at room temperature. *N*-hydroxysuccinimide (0.386 g, 3.35 mmol) was added, which caused a white precipitate to form, and the solution was stirred at room temperature for 10 min. The solution was poured into a separatory funnel and washed with saturated sodium chloride solution (1 x 5.0 mL) and water (2 x 5.0 mL). The organic layer was collected, dried over anhydrous sodium sulfate, filtered, and the solvent removed under reduced pressure to yield a clear syrup, which solidified to afford a 90% yield of *N* - (3) - Benzyloxycarbonyl - *N* - (1) - *tert*- butyloxycarbonyl - D –lysin - *N* - hydroxysuccinimide ester (14) (1.34 g) as a pale yellow solid. Crystallization using isopropyl alcohol and hexanes afforded an 87% yield of N_a - (3) - Benzyloxycarbonyl - N_e - (1) - *tert* - butyloxycarbonyl - D - lysin - *N* - hydroxysuccinimide ester (14) (1.24 g) as white crystals. Mp: 95 - 98°C. R_f 0.30 (1:1 Ethyl Acetate: Hexanes). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34 (s, 5 H), 5.11 (s, 2 H), 4.68 (s, 1 H), 4.10 (m, 1 H), 3.50 - 3.32 (m, 2 H), 2.78 (s, 4 H), 1.75 - 1.21 (m, 7 H), 1.41 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.12, 169.03, 155.43, 137.01, 133.02, 128.12, 127.94, 127.37, 68.37, 66.18, 55.22, 38.05, 30.06, 27.48, 26.82, 26.63, 26.02, 25.33, 25.22. CI Mass Spectrum (methane) m/z 478 (M^+); IR (KBr): 3421, 2933, 1808, 1778, 1732, 1728, 1687, 1211, 1069, 749, 698 cm^{-1}. $(\alpha)_D^{25}$ = -16.3º (c = 1.00, $CHCl_3$). Anal. Calcd. For $C_{23}H_{31}N_3O_8$: C, 57.85; H, 6.54; N, 8.80. Found: C, 57.91; H, 6.82; N, 8.65.

***N_a* - (3) - Amino - *N_e* - 1 - *tert* - butyloxycarbonyl - D - lysin - (*N* - hydroxysuccinimide ester) (15):** To a 100 mL flask charged with N_a - (3) - Benzyloxycarbonyl - N_e- (1) - *tert* - butyloxycarbonyl - D - lysin - (*N* - hydroxysuccinimide ester) (14) (0.50 g, 1.04 mmol) was added 5% Palladium on carbon (0.050 g) and ethyl acetate (40 mL). The flask was evacuated and purged with nitrogen 3 times, then filled with hydrogen (30 psi). The reaction was monitored by thin layer chromatography (TLC) (2:3 Ethyl Acetate: Hexanes; Iodine visualization) and after 1.5 h the reaction was judged to be complete. The contents of the flask were filtered through a pad of Celite and the filtrate was concentrated to afford a 93% yield (0.34 g) of N_a - (3) - Amino - N_e -1 - *tert* - butyloxycarbonyl - D - lysin - (N - hydroxysuccinimide ester) (15) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.66 (s, 2 H), 4.10 (m, 1 H), 3.46 - 3.28 (m, 2 H), 2.77 (s, 4 H), 1.75 - 1.20 (m, 7 H), 1.41 (s, 9 H).

D - (-) - Lysine (16): To a 100 mL round bottom flask equipped with a magnetic stir bar was added N_a - (3) - Amino - N_e - 1 - *tert* - butyloxycarbonyl - D - lysin - (*N* - hydroxysuccinimide ester) (15) (0.34 g, 0.98 mmol). A solution of 0.1 N hydrochloric acid (10 mL) was added and the stirrer was started. The reaction was monitored by thin layer chromatography (TLC) (3:2 Ethyl Acetate: Hexanes; Iodine visualization) and after stirring for 6.0 h, the reaction was complete. The solution was filtered to afford an 87% yield (0.125 g) of D - (-) - lysine (16) as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 4.03 (t, 1 H, J = 6.0 Hz), 2.97 (t, 2 H, J = 7.1 Hz), 1.93 (m, 2 H), 1.68 (t, 2 H, J = 7.2 Hz), 1.48 (m, 2 H). CI Mass Spectrum (methane) m/z 147 (M^+ + H); $(\alpha)_D^{25}$ = -12.8º (c = 1.00, H_2O).

RESULTS AND DISCUSSION

As shown in the first step shown in Figure 1, L - (+) - Lysine was cyclized to the ε-caprolactam intermediate, (S) - (α) - Amino - (α) - caprolactam (2), in order to cause pronounced differentiation between the two amino groups of lysine.

Previous syntheses of the target compound, N_a - benzyloxycarbonyl - N_e - *tert* - butyloxycarbonyl - L - lysin - (*N* - hydroxy-succinimidester) (6) (Pearson et al., 1996) have not used this ε-caprolactam as an intermediate and required lengthy purification steps, such as flash column chromatography, after each intermediate step, causing this type of multi-step synthesis to be problematic in the past. The use of the ε-caprolactam intermediate in this new synthetic route has caused pronounced differentiation between the two amino groups of lysine and arginine to facilitate benzyloxycarbonyl protection of the primary (α) amino and subsequent *tert*-butyloxycarbonyl protection of the secondary (ε) amide group, with higher yields and less racemization.

Literature precedence has shown that no significant amount of racemization occurs when cyclization occurs via an exo-trigonal ring closure (Baldwin et al., 1976) using hexamethyldisilazane. The use of either hexamethyldisilazane or 1,3 - Bis (trimethylsilyl) urea (Klebe, 1970) is necessary to prevent racemization, but literature precedence has shown that cyclization to produce the ε-caprolactam (2) in Figure 1 or the ε-caprolactam (10) in Figure 2 using either of these reagents can take up to two days for completion. Therefore, Table 1 summarizes the results of the optimization studies using 1, 3-Bis (trimethylsilyl) urea and hexamethyldisilazane.

In addition, a Dean-Stark trap was used to facilitate the removal of water from the reaction, and dicyclohexylamine was used as a bulky base for general base catalysis resulting in complete cyclization within a 50 min reflux instead of the 48 h previously reported. Figure 3 shows the same methodology applied to the selective protection sequence of L - (+) -Arginine, thus illustrating this general method can be successfully applied to the selective protection of basic amino acids with polar side chains. Deprotection steps are currently in progess.

Conclusions

The common benzyloxycarbonyl, *tert* – butyloxycarbonyl and succinimide protecting groups have been applied in a multi-step sequence to synthesize optically pure enantiomers based on the amino acids lysine and arginine. Use of dicyclohexylamine as a bulky base for general base catalysis and the use of a Dean-Stark trap decreased the time required for complete cyclization to the ε-caprolactam intermediate. Optical rotation, ^{1}H NMR, ^{13}C NMR, FTIR, GC-MS, and elemental analysis have been used to analyze the purity and optical activity. The results indicate that significant epimerization does not occur. Therefore, the retention of enantiomeric purity and one-pot synthetic sequence indicate that this method

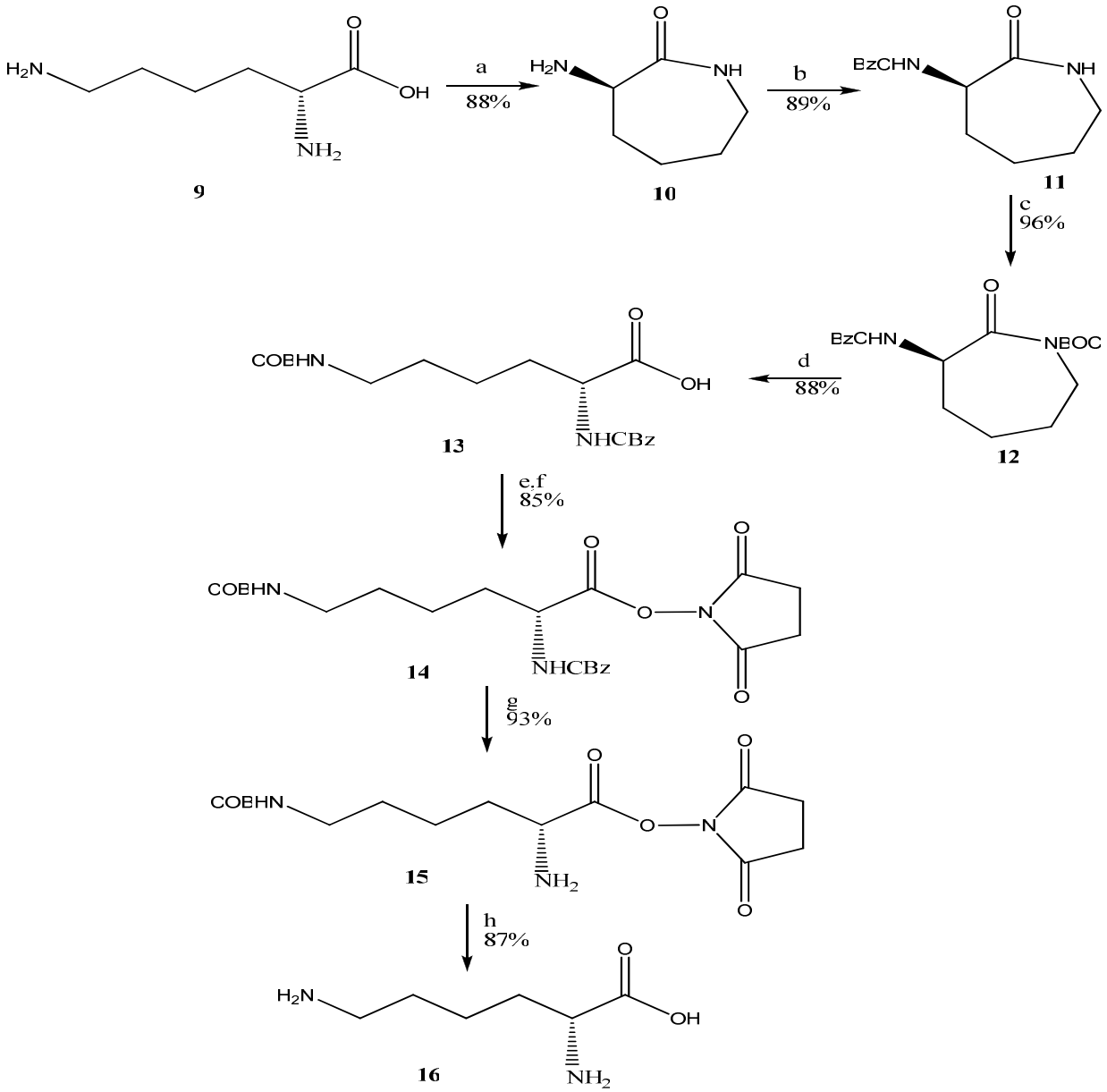

Figure 2. Steps to selectively protect and deprotect D - (-) - lysine.
(a) 1,3-Bis(trimethylsilyl)urea, dicyclohexylamine, THF, reflux; (b) CBz-Cl, saturated $NaHCO_3$, THF (c) $(BOC)_2O$, DMAP, THF (d) 1N LiOH, aqueous THF; (e) Trimethylacetyl chloride, TEA, THF; (f) N – hydroxysuccinimide, THF; (g) Pd/C, H_2, Ethyl acetate; (h) aqueous HCl.

Table 1. Effectiveness of 1, 3-Bis (trimethylsilyl) urea**.**

Reagent / Solvent	Equivalents of reagent used	Duration of reflux	% Yield of product (6)
1,3-Bis(trimethylsilyl)urea/Toluene	1.0	2 h	88%
1,3-Bis(trimethylsilyl)urea / Toluene	1.5	1 h	91%
1,3-Bis(trimethylsilyl)urea / THF	1.0	50 min	83%
1,3-Bis(trimethylsilyl)urea / THF	1.5	50 min	87%
HMDS / Toluene	1.5	5 h	85%
HMDS / THF	1.5	5 h	81%

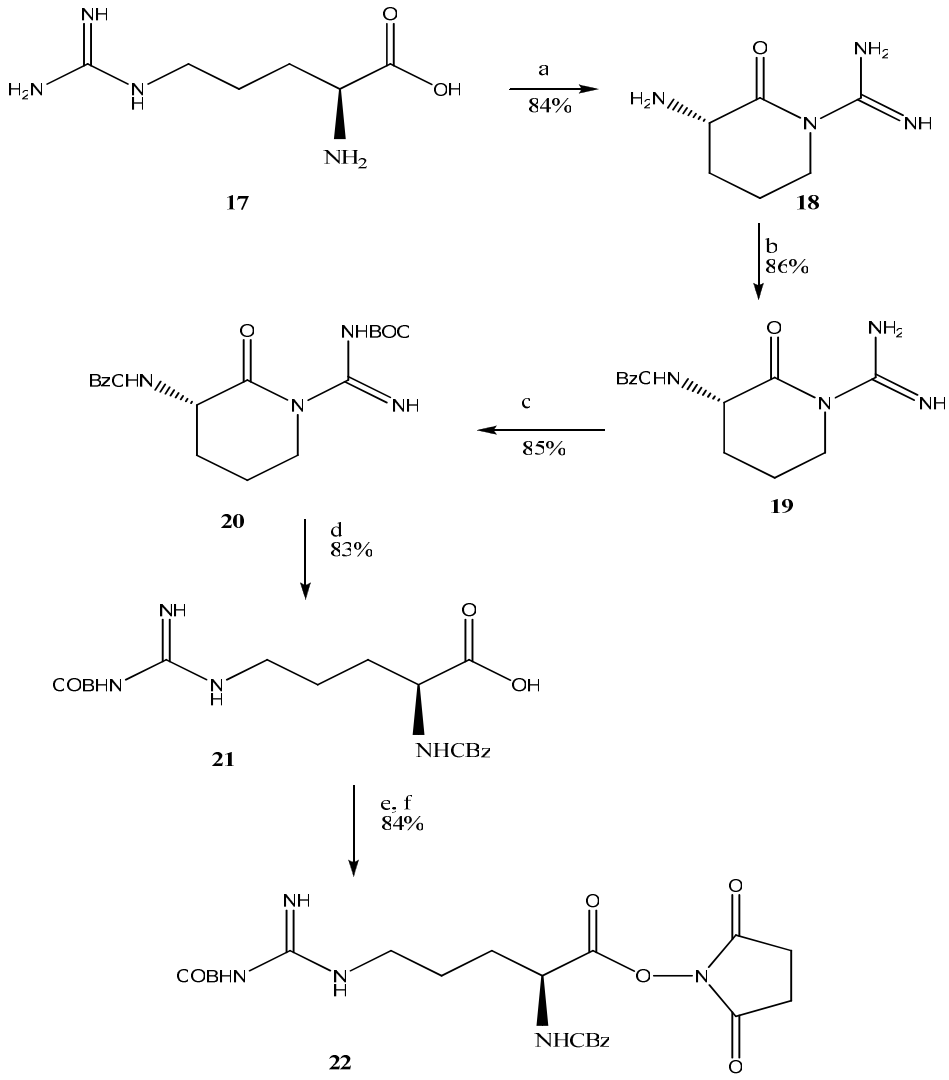

Figure 3. Steps to selectively protect L - (+) - arginine.
(a) 1,3-Bis(trimethylsilyl)urea, dicyclohexylamine, THF, reflux; (b) CBz-Cl, saturated $NaHCO_3$, THF (c) $(BOC)_2O$, DMAP, THF (d) 1N LiOH, aqueous THF; (e) Trimethylacetyl chloride, TEA, THF; (f) N – hydroxysuccinimide, THF.

provides a useful learning experience for undergraduates.

ACKNOWLEDGEMENT

Funding from the Auburn Research Council is gratefully acknowledged.

REFERENCES

Allen MC, Wade RJ (1988). Synthesis of somatostatin analogs resistant to the action of trypsin. Int. J. Peptide Protein Res. 32: 89-97.

Baldwin JE, Christie MA, Haber SB, Kruse LI (1976). Stereospecific synthesis of penicillins. Conversion from a peptide precursor. J. American Chemical Society 98: 3045-3047.

Furlan RE, Mata G, Mascaretti OA (1998). Efficient, non-acidolytic method for the selective cleavage of N-Boc amino acid and peptide phenacyl esters linked to a polystyrene resin. J. Chemical Society, Perkin Transactions 1: Organic and Bio-Organic Chem. (2): 355-358.

Giralt E, Andreu D (1990). Peptides. Escom Science Publishers, Barcelona, Spain.

Hiskey RG, Vunnam CLIRR (1975). Sulfur-containing polypeptides. XVIII. Unambiguous synthesis of parallel and antiparallel isomers of some biscystine peptides. J. Organic Chem. (40): 3697-3707.

Ishiz TA, Fujii S, Noguchi S (1991). Conformational studies of Cyclo(L-Phe-L-Pro-Gly-L-Pro)2 by ^{13}C nuclear magnetic resonance. Chemical & Pharmaceutical Bulletin 39: 1617-1619.

Klebe JF (1970). Silyl-proton exchange reactions. Acc. Chem. Res. 3: 299-301.

Moroder LE, Jaeger F, Drees M, Gemeiner S, Knof H, Stelzel P, Thamm D, Bataille S, Domschke S (1979). The influence of secretin, glucagon and other peptides, of amino acids, prostaglandin endoperoxide analogues and diazepam on the level of adenosine 3',5'-cyclic monophosphate in neuroblastoma x glioma hybrid cells. J. Neurochem. (32): 1495-500.

Moroder LR, Romano R (1994). Synthesis, conformational and biological properties of lipophilic derivatives of gastrin and

cholecystokinin peptides. Pure Appl. Chem. (66): 2111-14.
Pearson DA, Lister-James J, McBride WJ, Wilson DM, Martel LJ (1996). Somatostatin receptor-binding peptides labeled with technicium-99m. Chemistry and initial biological studies. J. Med. Chem. (7): 1361-1371.
Riniker BA, Florsheimer H, Fretz P, Siever B, Kamber B (1993). A General Strategy for the Synthesis of Large Peptides: The Combined Solid-Phase and Solution Approach. Tetrahedron (49): 9307-9320.
Sawayama TM, Tsukamoto T, Sasagawa K, Nishimura R, Yamamoto T, Deguchi K, Takeyama K, Hosoki K (1989). Angiotensin-converting enzyme inhibitors: Synthesis and structure-activity relationships of potent N-benzyloxycarbonyl tripeptide inhibitors. Chemical and Pharmaceutical Bulletin 37: 2417-22.