Pergamon

Tetrahedron Letters, Vol. 35, No. 50, pp. 9379-9382, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)02106-6

Asymmetric Syntheses of Novel Amino Acids and Peptides from Acylnitroso-Derived Cycloadducts

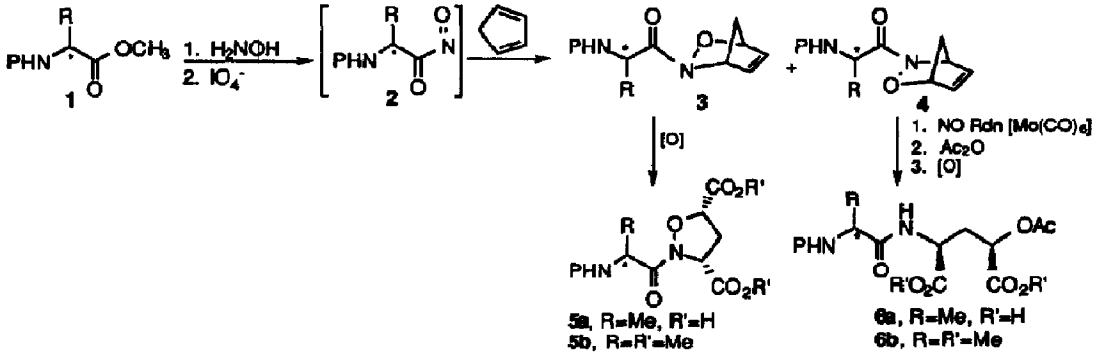
Allen R. Ritter and Marvin J. Miller*

Department of Chemistry and Biochemistry
University of Notre Dame
Notre Dame, IN 46556 USA

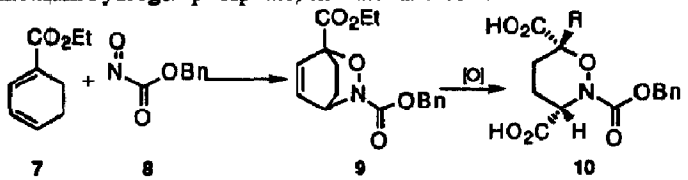
Abstract: Oxidative cleavage of cycloadducts of amino acid-derived acylnitroso compounds produces peptides in which the carbon framework of the new carboxy terminal amino acid was generated from cyclopentadiene.

Methods for efficient synthesis of both natural and unnatural amino acids and peptides have received, and continue to receive, tremendous attention.[1] In particular, knowledge about enzyme structure and function has prompted the development of novel peptide-based and related peptidomimetic pharmaceutical agents, including inhibitors of renin[2] and HIV-1 protease.[3] A large number of both solid and liquid phase synthetic methods have been developed for the preparation of peptides from appropriately protected and activated constituent amino acids.[4] The utility of these now classical and often automated methods depends on their efficiency and the extent to which racemization of the amino acid components in the coupling is prevented. A process that allows the simultaneous formation of a peptide bond with the generation of a new optically pure amino acid would provide an alternative approach to the synthesis of peptide building blocks. In early studies, reactions of optically active α-amino acid esters with phthalimido-containing ketenes provided dipeptides with up to 70% diastereoselection during formation of the new amino acid component.[5] More recently, Hegedus reported that irradiation of optically active chromium-aminocarbene complexes in the presence of optically pure amino acid esters gave dipeptides with very high (>97%) diastereoselectivity.[6] Herein, we report that proper manipulation of optically pure oxazines derived from Diels-Alder reactions of amino acid-based acylnitroso compounds provides effective routes to novel highly functionalized peptides in which the carbon framework of the new C-terminal amino acid residue originates from the diene. Specific targets included novel proline analogs, as in **5**, and hydroxy amino diacids related to **6**. The proline analog in peptide **5** also may be considered a conformationally restricted form of glutamate[7] and may be useful in the study of excitatory amino acid receptors,[8] metal chelation,[9] or as a peptide component to invoke unique structure and/or activity to a peptide.[10] α-Hydroxy acids related to that present in peptide **6** are important components of a number naturally-occurring products including siderophores (microbial iron chelators) such as pseudobactin,[11] ornibactin[12] and the alterobactins.[13] Synthetic access to analogs of these important iron-binding amino acids is anticipated to provide artificial siderophores and drug conjugates needed for further studies of microbial iron transport-mediated drug delivery.[14]

We previously reported details for the hydroxaminolysis of N-protected amino acids esters **1**, subsequent periodate-mediated oxidation to acylnitroso derivatives **2** and *in situ* cycloaddition reactions with cyclopentadiene to provide diastereomeric oxazines **3** and **4**.[15] While the diastereoselectivity of the hetero Diels-Alder reactions was modest and varied only slightly depending on the amino acid side chain (i.e., 50% de for R=Me, 60% for R=iPr),[15] the diastereomeric products were readily separated chromatographically or by direct recrystallization.[16] Consequently, cycloadducts **3** and **4** can be obtained in optically pure form suitable for use in a variety of asymmetric syntheses. For example, oxidative cleavage of the double bond and reduction of the N-O bond of cycloadducts **3** or **4** was anticipated to release various forms of the masked amino acid generated by the Diels-Alder reaction. Thus, oxidation of the alkene of **3** would produce dipeptide **5** containing a novel cyclic amino acid structurally similar to proline or a conformationally restricted glutamate. N-O reduction prior to alkene oxidation would generate a peptide (**6**) with a γ-hydroxyglutamate residue.



Similar methodology was utilized in Baldwin's racemic synthesis of tabtoxin precursors **10** [17] In that study, oxidation of acyl-nitroso cycloadduct **9** was accomplished using permanganate and a phase transfer catalyst (tetrabutylammonium hydrogen phosphate) in water and benzene.[18]



7 **8** **9** **10**

Initial attempts to oxidize cycloadduct **3** (P=Boc, R=CH_3), derived from L-alanine, to alanylproline analog **5** used permanganate,[19] periodate,[20] or ruthenium tetroxide.[21] Following the referenced procedure in each case, none of the desired product was isolated. However, use of ruthenium(III) chloride with periodate provided diacid **5a** in 94% yield. When the reaction was complete, the biphasic solution was saturated with sodium chloride and extracted repeatedly with ethyl acetate to isolate the diacid. Reaction of diacid **5a** with diazomethane gave diester **5b** (79% from **3**) which was readily purified.

Alternatively, $Mo(CO)_6$-induced reduction of cycloadduct **4**[15] followed by protection of the liberated hydroxyl group as the acetate and oxidation with Sharpless' catalytic ruthenium tetroxide procedure[20] gave dipeptide **6a**. Since all attempts to recrystallize the isolated diacid were unsuccessful, it also was esterified by reaction with diazomethane to give dimethyl ester **6b** (83% yield from **4**).[22] The diester was purified by silica gel chromatography.[23]

The newly formed amino acid in peptide **5**, derived from cycloadduct **3**, has the (R) configuration at the α-center, corresponding to a D-amino acid, whereas starting with diastereomeric cycloadduct **4** provides peptides with the new C-terminal amino acids having the (S) configuration corresponding to the "natural" L-amino acids, as in **6**. Thus, either optical form (D or L) of the new amino acids are readily available by this methodology.

Acknowledgments: We gratefully acknowledge support of this research by the NIH. ARR also gratefully acknowledges support from Nieuwland and Lubrizol Fellowships at the University of Notre Dame. We sincerely appreciate the mass spectral data obtained by Dr. Bruce Plashko of this department.

References and Notes:

1. (a) Williams, R. M. in *Synthesis of Optically Active α-Amino Acids*, Pergamon: Oxford, **1989**; vol. 7. (b) Williams, R. M. *Aldrichimica Acta* **1992**, *25*, 11.
2. Greenlee, W. J. *J. Med. Res. Rev.* **1990**, *10*, 173.
3. (a) Ghosh, A. K.; McKee, S. P.; Thompson, W. J. *J. Org. Chem.* **1991**, *56*, 6500. (b) Dunn, B. M.; Kay, *J. Antiviral Chem.* **1990**, *1*, 3.
4. (a) *The Peptides-Analysis, Synthesis, Biology*; Meienhofer, J.; Undenfriend, S. Eds.; Academic: New York, **1987**. (b) Bodanszky, M. *Principles of Peptide Synthesis*; Springer-Verlag: New York, **1983**. (c) Bodanszky, M. *The Practice of Peptide Synthesis*; Springer-Verlag: New York, **1984**.
5. Winter, S.; Pracejus, H. *Chem. Ber.* **1966**, *99*, 151.
6. Miller, J. R.; Pulley, S. R.; Hegedus, L. S.; DeLombaert, S. *J. Am. Chem. Soc.* **1992**, *114*, 5602.
7. For synthesis and studies of related competitive glutamate transport inhibitors (L-2,4-pyrrolidine dicarboxylate) see: (a) Bridges, R. J.; Lovering, F. J.; Humphrey, J. M.; Stanley, M. S.; Blakely, T. N.; Cristofaro, M.; Chamberlin, A. R. *BioMed Chem. Lett.* **1993**, *3*, 115. (b) Bridges, R.; Stanley, M. S.; Anderson, M. W.; Cotman, C.; Chamberlin, A. R. *J. Med. Chem.* **1991**, *34*, 717.
8. (a) Krogsgaard-Larson, P. in *Comprehensive Medicinal Chemistry*; Hansch, C. Ed.; Pergamon: New York, **1990**; vol. 3, p. 493. (b) Tamura, T. N.; Matsushita, Y.; Iwama, T.; Harada, S.; Kishimoto, S.; Itoh, K. *Chem. Pharm. Bull.* **1991**, *39*, 1199.
9. (a) Rich, D. H. in *Comprehensive Medicinal Chemistry* Pergamon Press, New York, **1990**, vol. 2, p.391. (b) Hong, W.; Dong, L.; Cai, Z.; Titmas, R. "Synthesis and N- and C- Terminal Extension of Peptidyl α,α-Difluoroalkyl Ketones," *Tetrahedron Lett.* **1992**, *33*, 741.
10. (a) Olson, G. L.; Voss, M. E.; Hill, D. E.; Kahn, M.; Madison, V. S.; Cook, C. M. *J. Am. Chem Soc.* **1990**, *112*, 323. (b) Kahn, M.; Wilke, S.; Chen, B.; Fujita, K. *J. Am. Chem. Soc.* **1988**, *110*, 1638.
11. Kolasa, T.; Miller, M. J. *J. Org. Chem.*, **1990**, *55*, 4246 and references therein.
12. Stephan, H.; Freund, S.; Meyer, J.-M.; Winkelmann, G.; Jung, G. *Liebigs Ann. Chem.* **1993**, 43-48.
13. Reid, R. T.; Live, D. H.; Faulkner, D. J.; Butler, A. *Nature* **1993**, *366*, 455.
14. (a) Miller, M. J. *Chem. Rev.* **1989**, *89*, 1563. (b) Miller, M. J ; Malouin, F. *Accts. Chem. Res.* **1993**, *26*, 241 and references therein.
15. Ritter, A. R.; Miller, M. J. *J. Org. Chem.* **1994**, *59*, 4602.
16. Lensky, S.; Miller, M. J. unpublished results.
17. Baldwin, J. E.; Bailey, P. D.; Gallagher, G.; Singleton, K. A.; Wallace, P. M. *J. Chem. Soc., Chem. Comm.* **1983**, 1049.
18. Starks, C. M. *J. Am. Chem. Soc.* **1971**, *93*, 195.
19. Lee, D. G.; Chang, V. S. *J. Org. Chem.* **1978**, *43*, 1532.
20. (a) Kalsi, P. S.; Kaur, P. P.; Singh, J.; Chabra, B. *Chem. Ind.* **1987**, 394. (b) Ireland, R. E.; Norbeck, D. W.; Mandel, G. S.; Mandel, N. S. *J. Am. Chem. Soc.* **1985**, *107*, 3285.
21. Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936.
22. For a recent alternative synthesis of γ-hydroxy glutamic acid see: Passerat, N.; Bolte, J. *Tetrahedron Lett.* **1987**, *28*, 1277.
23. Representative experimental and characterization data includes: ***N*-Boc-L-alanyl-δ-oxo-γ-(*S*)-carboxy-L-proline (5a, R'=H).** A 1:1:2 mixture of carbon tetrachloride, acetonitrile and water (35 mL total volume) was charged with 546 mg (2.04 mmol) of **3** (prepared as described in reference 15). The mixture was charged with 1.79 g of sodium periodate (8.4 mmol, 210 mol%) and then with 42 mg of ruthenium trichloride hydrate (0.204mmol, 10 mol%). The mixture turned black almost instantly, and was stirred at RT. After 10 min, TLC analysis indicated that no starting material remained. A new more polar spot appeared and gave a positive aldehyde test (purpald - Aldrich). Another 20 mg of ruthenium

trichloride was added to the reaction mixture which was then stirred for an additional 1.5 h. The reaction flask was charged with several grams of sodium chloride and with ethyl acetate. The aqueous layer of the reaction mixture was washed with ethyl acetate until TLC indicated that no more product was being extracted into the organic layer. Magnesium sulfate drying, filtration and concentration gave a brown oil which was dissolved in ammonia and water and concentrated again to give a viscous oil. After several codistillations with chloroform, 640 mg (94%) of an off-white solid was obtained. R_f=0.29 (20% MeOH, 77% CH_2Cl_2, 3% AcOH); MS (CI, isobutane) *m/e* calcd for $C_9H_{11}N_2O_7$ (M^+-O^tBu): 259.0566, found 259.0562; 333 (MH^+); FAB, 333 (MH^+); EI, 259 (27), 217 (18), 161 (100), 116 (90). ***N*-Boc-L-alanyl-δ-oxo-γ-(*S*)-carboxy-L-proline dimethyl ester (5b, R'=Me).** An ethereal slurry of **5a** was cooled in ice water and charged slowly with freshly prepared diazomethane in ether until the yellow color of the diazomethane persisted. The diester was isolated by silica gel chromatography after an acetic acid quench and saturated sodium bicarbonate workup. R_f=0.23 (50:50-hexanes:ethyl acetate); IR (neat) 3390, 2980, 1750, 1705, 1215, 1170 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.45 (d, *J*=7.2 Hz, 3H), 2.78 (ddd, *J*=4.5, 6.3, 12.9 Hz, 1H), 2.93 (ddd, *J*=8.1, 9.3, 12.9 Hz, 1H), 3.76 (s, 3 H), 3.81 (s, 3H), 4.75 (m, 1H), 4.81 (t, *J*=7.2 Hz, 1H), 4.99 (dd, *J*=4.2, 9.6 Hz, 1H), 5.11 (bd, *J*=7.8 Hz, 1 H); ^{13}C NMR δ 18.3, 28.2, 34.9, 47.0, 52.7, 52.9, 56.5, 77.6, 79.7, 155.3, 168.3, 169.4, 174.2; MS (CI, isobutane) *m/e* calcd for $C_{11}H_{15}N_2O_7$ (M^+-O^tBu): 287.0879, found 287.0882; 361 (MH^+, 45), 305 (100), 261 (20), 246 (20), 217 (25), 190 (80). **1(*R*)-O-Acetyl-4(*S*)-*N*[*N*-Boc-L-alanyl]aminocyclopent-2-ene.** A 2:1 pyridine/methylene chloride solution (8 mL) of 196 mg (0.73 mmol) of allylic alcohol derived from the $Mo(CO)_6$ reduction of 4 (R=Me)[15] was charged with 200 mol% of acetic anhydride (150 mg, 0.15mL) and was stirred overnight. The reaction mixture was diluted with ethyl acetate and 15 mL of 1N HCl was added. The organics were separated and washed with 1N HCl until no pyridine remained in the organic layer. After drying over magnesium sulfate, filtration and concentration, chromatography on silica gel gave 196 mg of an oil which was recrystallized from ethyl acetate and hexanes (88% yield): mp 86-88°C; $[\alpha]_D^{20}$ -147.3° (c=0.0093, $CHCl_3$); R_f=0.52 (ethyl acetate); IR (neat) 3305, 2965, 1715, 1655, 1520, 1240 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (d, *J*=6.9 Hz, 3H), 1.44 (s, 9H), 1.52 (dt, *J*=4.5, 14.4 Hz, 1H), 2.05 (s, 3H), 2.85 (dt, *J*=7.5, 14.4 Hz, 1H), 4.19 (m, 1H), 4.92 (dt, *J*=4.8, 7.8 Hz, 1H), 5.39 (d, *J*=4.8 Hz, 1H), 5.55 (dd, *J*=4.5, 7.5 Hz, 1H), 5.96 (apparent s, 2H), 6.79 (m, 1H); ^{13}C NMR δ 18.3, 21.0, 28.2, 38.0, 49.8, 52.7, 77.4, 79.8, 132.5, 136.2, 155.4, 170.4, 172.0; MS *m/e* calcd for $C_{13}H_{21}N_2O_3$ (M^+-OAc): 253.1552, found 253.1560; 313 (MH^+, 20), 253 (100), 197 (45), 179 (30), 144 (80); Anal. calcd for $C_{15}H_{24}N_2O_5$: C, 57.68; H, 7.74; N, 8.97; found: C, 57.85; H, 7.95; N, 8.90. ***N*-Boc-L-alanyl-γ-(*R*)-acetoxy-L-glutamic acid (6a, R'=H).** The allylic acetate derived from 4 (R=Me) was dissolved in 8 mL of a 1:1:2 mixture of carbon tetrachloride, acetonitrile and water respectively. Solid sodium periodate (412 mg, 1.93 mmol, 410 mol%) was then added followed by 10 mg (0.047 mmol) of ruthenium trichloride hydrate. The mixture was stirred at rt. Within 15 min no starting material remained. A new spot with R_f=0.27 (100% ethyl acetate) was observed on TLC analysis. This slowly gave way to a new permanganate positive spot with R_f=0.25 (25% methanol in methylene chloride with a trace of acetic acid). When the intermediate spot was gone, the reaction mixture was saturated with sodium chloride and was washed with 4 x 30 mL of ethyl acetate, after which time TLC analysis revealed that no product was in the extract. The combined organics were dried over magnesium sulfate, filtered, and concentrated to give an oil. ^1H NMR (1:1$CDCl_3$:DMSO-d_6) δ 1.33 (d, *J*=7.2 Hz, 3H), 1.44 (s, 9H), 2.09 (s, 3H), 2.29 (m, 1H), 2.47 (ddd, *J*=3.6, 6.3, 14.4Hz, 1H), 4.17 (m, 1H), 4.58 (dd, *J*=6.0, 12.6Hz, 1H), 5.05 (dd, *J*=9.9, 3.6 Hz, 1H), 6.15 (d, *J*=6.9Hz, 1H), 7.56 (d, *J*=6.9Hz, 1H), 9.92 (bs, 2 H); ^{13}C NMR (1:1, $CDCl_3$:DMSO-d_6) δ 17.6, 19.7, 27.5, 31.9, 48.3, 49.4, 68.1, 78.5, 154.5, 169.2, 170.5, 171.8, 172.2; MS *m/e* calcd for $C_{11}H_{16}N_2O_9$ (M^+-C_4H_8): 320.0856, found 320.0858. **Dimethyl *N*-Boc-L-alanyl-γ-(*R*)-acetoxy-L-glutamate dipeptide (6b, R'=Me).** An ethereal solution of **6a** (100 mg, 0.28mmol) was charged with freshly prepared diazomethane in ether until the yellow color persisted. The reaction was quenched with acetic acid. After saturated bicarbonate workup, drying, filtration and concentration, the resulting clear oil was chromatographed on silica gel using 1:1 ethyl acetate-hexanes as eluent. The diester was isolated as an oil: R_f=0.19 (50:50-hexanes:ethyl acetate); IR (neat) 3340, 2980, 1750, 1717, 1675, 1508, 1368, 1223 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38 (d, *J*=6.9 Hz, 3 H), 1.45 (s, 9 H), 2.11 (s, 3 H), 2.35 (ddd, *J*=4.2, 10.5, 14.7 Hz, 1 H), 2.57 (ddd, *J*=3.6, 6.6, 14.7 Hz, 1 H), 3.74 (s, 3 H), 3.78 (s, 3 H), 4.19 (m, 1 H), 4.70 (m, 1 H), 5.10 (d, J = 4.2 Hz, 1 H), 5.14 (d, J = 3.3 Hz, 1 H), 6.97 (d, J = 6.3 Hz, 1 H); ^{13}C NMR δ 18.1, 20.4, 28.2, 32.4, 49.1, 50.2, 52.5, 68.2, 80.2, 155.3, 169.8, 169.9, 171.6, 172.6; MS (EI) *m/e* calcd for $C_{17}H_{28}N_2O_9$: 404.1795, found 404.1798; 404 (M^+), 348 (8), 218 (11), 144 (36), 114 (25), 88 (35), 57 (60), 44 (100).

(*Received in USA* 13 *June* 1994; *revised* 1 *August* 1994; *accepted* 21 *October* 1994)