Acknowledgment. This work was supported by a grant from the Office of Naval Research. FT-NMR spectra were obtained with equipment funded in part by NIH Grant 1 S10 RR01458-01A1. L.E.F. acknowledges a faculty summer fellowship from the donors of the Petroleum Research Fund, administered by the American Chemical Society. A.W.C. thanks the A. P. Sloan and Dreyfus Foundations for support in the form of fellowships and Eli Lilly and Co. for support in the form of a granteeship.

Supplementary Material Available: Experimental details for the syntheses of **3** and **5** (4 pages). Ordering information is given on any current masthead page.

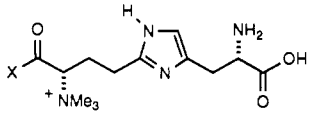
Synthesis of Diphthamide: The Target of Diphtheria Toxin Catalyzed ADP-Ribosylation in Protein Synthesis Elongation Factor 2

David A. Evans* and Kristin M. Lundy

Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138

Received November 20, 1991

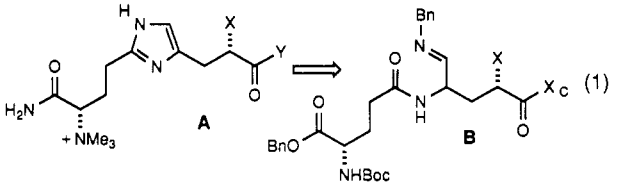
Diphtheria toxin (DT) expresses its cytotoxicity by inhibiting protein synthesis. Mechanistically, this toxin effects a single ADP-ribosylation of the critical enzyme, protein synthesis elongation factors 2 (EF-2), at a unique amino acid residue, thus terminating the translocation step of translation.[1] The gross structure of this targeted amino acid constituent of EF-2, initially referred to as amino acid X and later as diphthamide, was proposed by Bodley and co-workers from NMR and mass spectral studies of the hydrolysis products from ADP-ribosylated EF-2, ribosyldiphthamide and diphthine.[2] Biosynthetic labeling experiments support the proposed structure and reveal that the side chain of this elaborated histidine derivative is derived from methionine.[3] Diphthamide is the most complex posttranslationally modified amino acid known to date.



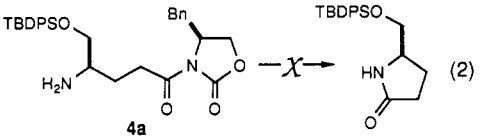
Diphthamide X = NH_2
Diphthine X = OH

The purpose of this communication is to describe the first syntheses of diphthamide and diphthine, which was prepared for direct comparison to the natural amino acid. A synthesis plan was developed which united the two carboxylic acid side chains prior to the construction of the imidazole nucleus through intermediates such as B (eq 1).[4] This plan afforded the flexibility of introducing the second amino-bearing stereocenter (X = H or NH_2) either before or after the imidazole construction.

The synthesis was initiated from D-pyroglutamic acid ethyl ester (**1**),[5] which was transformed to the *tert*-butyldiphenylsilyl-protected (TBDPS-protected) imide **2** in 77% overall yield (Scheme I). Peroxide-mediated hydrolysis of **2**[6] followed by its subsequent mixed anhydride acylation[7] with 4(*S*)-benzyloxazolidone[8] afforded imide **4** in 89% yield. After removal of the *N*-Boc protecting group, amine **4a** was acylated with mixed anhydride **6**,[9] derived from L-glutamic acid, to provide **7** in 89% yield. This transformation is noteworthy in that the potentially damaging intramolecular acylation of **4a** was not observed (eq 2).



(1)



(2)

In preparation for the construction of the imidazole nucleus, **7** was desilylated (HF–pyr, 13 h, 25 °C), the derived primary alcohol was transformed to the aldehyde,[10] and the *N*-benzylimine B (X = H) was formed (1.0 equiv of $BnNH_2$, $MgSO_4$, CH_2Cl_2, 1 h, 25 °C) without purification of intermediates. Cyclocondensation of this intermediate to imidazole **8** was effected by a modified Lee reaction (1.5 equiv of Ph_3P, 1.5 equiv of C_2Cl_6, 3.0 equiv of Et_3N, MeCN, 14 h, 35 °C) in an overall yield of 70% from **7**.[11] Our chiral enolate azidation methodology[12] was then employed to incorporate the α-amino moiety with the requisite (*S*) configuration. Unfortunately, the diastereoselection in the azidation of imide **8** was only moderate (76:24); nonetheless, the desired diastereomer **9** was isolated in 62% yield. Reduced diastereoselectivity was also observed in the analogous azidation of the C_2 unsubstituted imidazole imide, which was further transformed to L-histidine.[13] It is tentatively concluded that the imidazole moiety in these reactions is partially disrupting the chelated enolate and thus the reaction diastereoselectivity.

As a consequence of our collaborative interest in evaluating diphthamide amides as substrates for diphtheria toxin catalyzed ribosylation,[14] we selected *N*-acetyldiphthamide methyl ester **13** as the first target for synthesis. Treatment of **9** with thiolacetic acid (neat, 4 h, 25 °C)[15] afforded the *N*-acetamide, which was transformed to **10** via Boc removal (TFA) and reductive methylation (CH_2O, $NaBH_3CN$) in 86% overall yield. In the final steps of the synthesis, it was found that the benzyl ester in **10** could

(1) (a) Balestrieri, C.; Giovane, A.; Quagliuolo, L. *Adv. Exp. Med. Biol. (Adv. Post-Transl. Modif. Proteins Aging)* **1988**, *231*, 627–632. (b) Ward, W. H. J. *Trends Biochem. Sci.* **1987**, *12*, 28–31.

(2) Purification and properties: (a) Bodley, J. W.; Dunlop, P. C.; Van Ness, B. G. *Methods Enzymol.* **1984**, *106*, 378–387. (b) Van Ness, B. G.; Howard, J. B.; Bodley, J. W. *J. Biol. Chem.* **1980**, *255*, 10717–10720. Structure: (c) Van Ness, B. G.; Howard, J. B.; Bodley, J. W. *J. Biol. Chem.* **1980**, *255*, 10710–10716. (d) Bodley, J. W.; Upham, R.; Crow, F. R.; Tomer, K. B.; Gross, M. L. *Arch. Biochem. Biophys.* **1984**, *230*, 590–593. Recent review: Bodley, J. W.; Veldman, S. A. In *ADP-Ribosylating Toxins and G Proteins: Insights into Signal Transduction*; Moss, J., Vaughan, M., Eds.; American Society for Microbiology: Washington, DC, 1990; Chapter 2 and references therein.

(3) (a) Dunlop, P. C.; Bodley, J. W. *J. Biol. Chem.* **1983**, *258*, 4754–4758. (b) Chen, J.-Y. C.; Bodley, J. W. *J. Biol. Chem.* **1988**, *263*, 11692–11696. (c) Moehring, J. M.; Moehring, T. J. *Methods Enzymol.* **1984**, *106*, 388–395. (d) Moehring, T. J.; Danley, D. E.; Moehring, J. M. *Mol. Cell. Biol.* **1984**, *4*, 642–650.

(4) Preliminary studies in this laboratory demonstrated that diphthamide is not amenable to synthesis via conventional nucleophilic substitution at the C-2 position of the imidazole due to the poor nucleophilicity of the C-2 anion.

(5) (a) Silverman, R. B.; Levy, M. A. *J. Org. Chem.* **1980**, *45*, 815–818. (b) Amstutz, R.; Ringdahl, B.; Karlén, B.; Roch, M.; Jenden, D. J. *J. Med. Chem.* **1985**, *28*, 1760–1765.

(6) Evans, D. A.; Britton, T. C.; Ellman, J. A. *Tetrahedron Lett.* **1987**, *28*, 6141–6144.

(7) For a representative procedure for this reaction, see: Evans, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1989**, *111*, 1063–1072.

(8) Gage, J. R.; Evans, D. A. *Org. Synth.* **1989**, *68*, 77–82.

(9) Intermediate **5** is commercially available from BaChem or may be synthesized according to the following procedure: Pawelczak, K.; Krzyzanowski, L.; Rzeszotarska, B. *Org. Prep. Proced. Int.* **1985**, *17*, 416–419.

(10) Mancuso, A. J.; Swern, D. *Synthesis* **1981**, 165–185. This oxidation was carried out with diisopropylethylamine.

(11) The supplementary material should be consulted for a detailed description of this procedure. For a similar α-acylamino ketimine cyclization, see: Engel, N.; Steglich, W. *Liebigs Ann. Chem.* **1978**, 1916–1927.

(12) Evans, D. A.; Britton, T. C.; Ellman, J. A.; Dorow, R. L. *J. Am. Chem. Soc.* **1990**, *112*, 4011–4030.

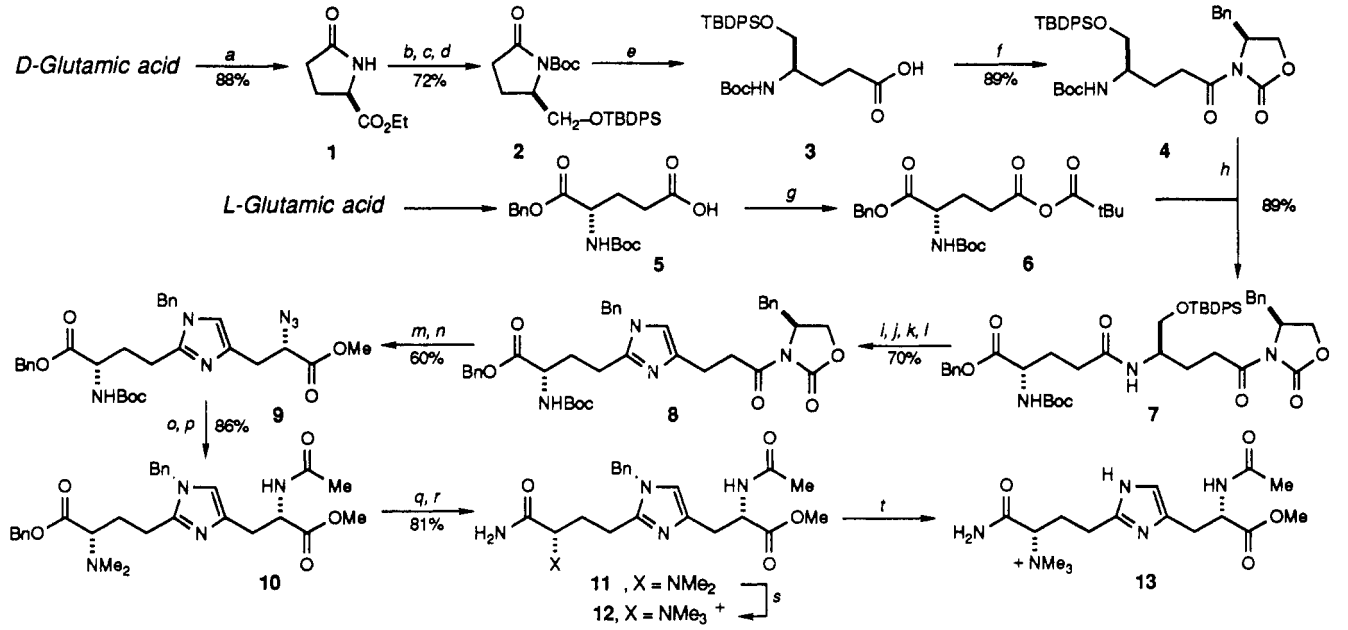
(13) The stereochemistry was assigned by comparison of the MTPA amides of both diastereomers with those derived from L-His and by analogy to the sense of induction seen in ref 12.

(14) This project is part of an ongoing collaboration with John Collier and co-workers at the Harvard Medical School.

(15) Rosen, T.; Lico, I. M.; Chu, D. T. W. *J. Org. Chem.* **1988**, *53*, 1580–1582.

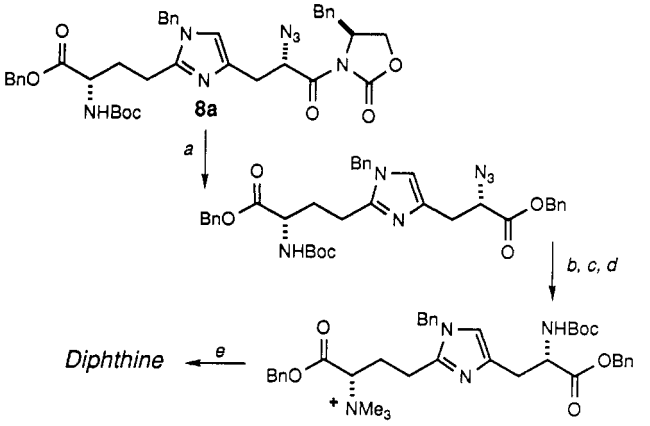
0002-7863/92/1514-1495$03.00/0

Scheme I[a]



[a] $SOCl_2$, EtOH; KOH; 150 °C. [b] $LiBH_4$. [c] TBDPSCl, Et_3N, DMAP. [d] BOC_2O, Et_3N, DMAP. [e] LiOOH. [f] *t*-BuCOCl, Et_3N; XpLi. [g] *t*-BuCOCl, Et_3N. [h] TFA; **6**, Et_3N. [i] HF-Pyr. [j] Swern. [k] $BnNH_2$. [l] PPh_3, C_2Cl_6, Et_3N. [m] KHMDS; trisyl-N_3; HOAc. [n] MeOH. [o] AcSH. [p] TFA; CH_2O, $NaCNBH_3$. [q] H_2, Pd/C, EtOH/H_2O. [r] *t*-BuCOCl, Et_3N; NH_3. [s] MeI. [t] H_2 (50 psi), Pd black, HOAc/H_2O.

Scheme II[a]



[a] BnOH, imidazole. [b] TFA; CH_2O, $NaCNBH_3$. [c] $SnCl_2$; BOC_2O, $NaHCO_3$. [d] MeI. [e] H_2 (50 psi), Pd black, HOAc/H_2O.

be selectively hydrogenolyzed (10% Pd/C, 9:1 EtOH/H_2O, 15 psi of H_2, 15 h) without concomitant removal of the imidazoyl benzyl moiety and that the derived acid could be transformed to the primary amide **11** in good yield via the derived mixed pivaloyl anhydride. In the next step, selective methylation of the dimethylamino nitrogen, in the presence of the imidazole ring, was achieved with excess methyl iodide (MeOH, 48 h, 25 °C). The final N-debenzylation of **12** was achieved with Pd black (4:1 HOAc/H_2O, 50 psi of H_2, 2-3 days).

In addition, (*S*,*S*)- and (*S*,*R*)-diphthine were prepared from the *S* azido imide **8a** and the enantiomeric (*R*) azido imide **8b**, respectively (Scheme II).[16] All of the diastereomers were distinguishable by NMR, suggesting that no epimerization had occurred in the synthesis of the amino acids. Bodley has demonstrated that the synthetic and natural diphthine coeluted during amino acid hydrolysis.[17]

Future publications will report the synthesis of other diphthamide diastereomers and the associated studies with diphtheria toxin.

Acknowledgment. Support has been provided by the National Institutes of Health. The NIH BRS Shared Instrument Grant Program (1-S10-RR04870) and the NSF (Grant CHE88-14019) are acknowledged for providing NMR facilities. We are grateful to Professors R. J. Collier (Harvard Medical School) and J. W. Bodley (University of Minnesota Medical School) for their involvement in this collaboration.

Supplementary Material Available: Complete experimental procedures as well as spectral and analytical data for all compounds (10 pages). Ordering information is given on any current masthead page.

(16) For simplicity, only the *S*,*S* products are shown.

(17) Bodley, J. W.; Donovan, M., University of Minnesota, unpublished results.

Interception of a Thermally Generated Biradical by Intramolecular Hydrogen Atom Transfer

Thomas H. Peterson and Barry K. Carpenter*

Department of Chemistry, Baker Laboratory
Cornell University, Ithaca, New York 14853-1301

Received November 25, 1991

Thermally generated, presumably singlet, biradicals are arguably among the most elusive of reactive intermediates. While triplet biradicals and certain classes of specially stabilized singlet biradicals can be directly detected[1] or trapped in bimolecular reactions,[2] it has hitherto been difficult to find reactions that could

(1) Examples include the following: (a) Herman, M. S.; Goodman, J. L. *J. Am. Chem. Soc.* **1988**, *110*, 2681. (b) Adam, W.; Grabowski, S.; Wilson, R. M.; Hannemann, K.; Wirz, J. *J. Am. Chem. Soc.* **1987**, *109*, 7572. (c) Dowd, P.; Chang, W.; Paik, Y. H. *J. Am. Chem. Soc.* **1987**, *109*, 5284. (d) Zilm, K. W.; Merrill, R. A.; Greenberg, M. M.; Berson, J. A. *J. Am. Chem. Soc.* **1987**, *109*, 1567. (e) Kelley, D. F.; Mazur, M. R.; Rentzepis, P. M.; Berson, J. A. *J. Am. Chem. Soc.* **1982**, *104*, 3764. (f) Doubleday, C., Jr. *Chem. Phys. Lett.* **1982**, *85*, 65. (g) Closs, G. L.; Miller, R. J. *J. Am. Chem. Soc.* **1981**, *103*, 3586. (h) Kaupp, G.; Teufel, E.; Hopf, H. *Angew. Chem.* **1979**, *91*, 232. (i) Muller, J. F.; Muller, D.; Dewey, H. J.; Michl, J. *J. Am. Chem. Soc.* **1978**, *100*, 1629. (j) Closs, G. L.; Doubleday, C. E. *J. Am. Chem. Soc.* **1973**, *95*, 2735.

0002-7863/92/1514-1496$03.00/0