has been established which involves lyophobic and electrostatic binding. The ester in the resulting complex has been shown to undergo an "intramicellar" displacement, the rate of which is dependent upon the mole fraction of $A_T$ as $A_{10}^+$. The maximum first-order rate constants for ester disappearance ($\sim$0.06 to 0.2 $min^{-1}$) are comparable to those for the poorer substrates of esteratic enzymes. We believe our initial endeavors in this new field show sufficient promise to pursue the topic further.

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# Communications to the Editor

## Synthesis by the Merrifield Method of a Protected Nonapeptide Amide with the Amino Acid Sequence of Oxytocin[1]

*Sir:*

Since the synthesis of oxytocin was first accomplished by du Vigneaud, *et al.*,[2] several other syntheses have been reported,[3-12] all utilizing the same nonapeptide intermediate as was used in the original synthesis but, in some cases, with different protecting groups. With all of these approaches, in which the classical methods of peptide chemistry are employed, many weeks and, in some cases, months are required for the synthesis of the required protected nonapeptide amide intermediate and the over-all yields are low. Using the method of solid-phase peptide synthesis recently introduced by Merrifield,[13] a protected nonapeptide has been synthesized in high yield in a few days. Removal of the protecting groups followed by oxidation and purification yielded oxytocin.

The protected nonapeptide was synthesized in a stepwise manner beginning with 6 g of *t*-butyloxycarbonylglycyl resin containing 1.236 mmoles of glycine according to the general procedure of Merrifield,[13,14] with the following modifications. (1) Chloroform was used as a solvent for the triethylamine neutralization steps and for the washes immediately preceding and following these steps.[15] (2) Trifluoroacetic acid was used to remove the *t*-butyloxycarbonyl (Boc) group from the glutamine residue before the addition of the next protected amino acid residue.[16] (3) All coupling reactions were allowed to proceed for 4 hr. (4) The protected peptide was cleaved from the resin by ammonolysis.[17] Eight cycles of deprotection, neutralization, and coupling were carried out with appropriate Boc-amino acids,[18] producing the protected nonapeptide esterified to the resin. Boc-amino acids with protected side chains were S-Bzl-Cys and O-Bzl-Tyr. The final cysteine residue was added as the N-carbobenzoxy-S-benzyl (N-Z-S-Bzl) derivative. All coupling reactions to form peptide bonds were mediated by dicyclohexylcarbodiimide[19] in methylene chloride except those involving the carboxyl groups of Asn and Gln, which were allowed to react in dimethylformamide (DMF) as their nitrophenyl esters.[7b]

Following the coupling of the final residue, the dried resin weighed 7.24 g. The weight increase of 1.24 g represents the incorporation of 1.00 mmole of protected nonapeptide on the resin. This is 81% of the amount expected, based on the original glycine content of 1.236 mmoles of the esterified resin. Ammonolytic cleavage was effected as follows: the protected nonapeptide resin (2.5 g) was suspended in 85 ml of anhydrous methanol and the stirred suspension was bubbled with a stream of ammonia from a refluxing solution of ammonia, which contained sodium as a drying agent, at a temperature of $-5°$ for 2.5 hr with exclusion of moisture. Stirring at 4° was continued overnight and subsequently at 23° for 2 hr. The flask containing

(1) Supported by grants from the Medical Research Council of Canada and the Quebec Medical Research Council.

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(3) R. A. Boissonnas, S. Guttmann, P. A. Jaquenoud, and J. P. Watter, *Helv. Chim. Acta*, **38**, 1491 (1955).

(4) J. Rudinger, J. Honzl, and M. Zaoral, *Collection Czech. Chem. Commun.*, **21**, 202 (1956).

(5) L. Velluz, G. Amiard, J. Bartos, B. Goffinet, and R. Heymès, *Bull. Soc. Chim. France*, 1464 (1956).

(6) C. H. Beyerman, J. S. Bontekoe, and A. C. Koch, *Rec. Trav. Chim.*, **78**, 935 (1959).

(7) (a) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 2504 (1959); (b) *ibid.*, **81**, 5688 (1959).

(8) L. Kisfaludy, S. Dualsky, S. Bajusz, M. Low, A. Uskert, and K. Medzihradsky, Hungarian Patent 151,959 (1965).

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(10) S. Sakakibara and Y. Nobuhara, *Bull. Chem. Soc. Japan*, **38**, 120 (1965).

(11) I. Photaki, *J. Am. Chem. Soc.*, **88**, 2292 (1966).

(12) F. H. C. Stewart, *Australian J. Chem.*, **19**, 2361 (1966).

(13) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963); *Science*, **150**, 178 (1965).

(14) R. B. Merrifield, *Biochemistry*, **3**, 1385 (1964); G. R. Marshall and R. B. Merrifield, *ibid.*, **4**, 2394 (1965).

(15) J. M. Stewart, J. D. Young, E. Benjamini, M. Shimizu, and C. Y. Leung, *ibid.*, **5**, 3396 (1966).

(16) The author is indebted to Dr. V. du Vigneaud and Dr. H. Takashima for suggesting this modification which circumvents the problem of a chain-terminating reaction, possibly due to pyroglutamyl formation, which has been observed in exploratory experiments in both laboratories when the normal procedure for deprotection using 1 *N* hydrochloric acid–acetic acid was employed. The modified procedure, utilized in this synthesis, was as follows: the resin was washed with three 30-ml portions of dry glacial acetic acid and rinsed through with one 30-ml portion of trifluoroacetic acid. A 30-ml portion of trifluoroacetic acid was added and the suspension was rocked at room temperature for 15 min. After removing the trifluoroacetic acid the resin was washed with three 30-ml aliquots of dry glacial acetic acid and the subsequent neutralization and coupling were carried out in the usual manner.

(17) M. Bodanszky and J. T. Sheehan, *Chem. Ind.* (London), 1423 (1964).

(18) The abbreviations used for amino acids and protecting groups are those recommended by the IUPAC–IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **241**, 2491 (1966); *Biochemistry*, **5**, 1445, 2485 (1966).

(19) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

the mixture was connected to a water pump through two soda lime U-tubes and was evacuated with stirring for 2 hr to remove ammonia and methanol. The evaporation was completed with a vacuum pump for 3 additional hr.[20] The cleaved material was extracted with DMF (three 10-ml portions) and methanol (two 10-ml portions) and the resin was removed by filtration. The solvents were evaporated at 30° on a rotary evaporator and the residue was dried *in vacuo* over $P_2O_5$ to remove residual solvents. Upon trituration with 95% ethanol followed by washing with 95% ethanol and diethyl ether and further drying *in vacuo* over $P_2O_5$, the protected nonapeptide amide Z-Cys(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-$NH_2$ was obtained as a white amorphous powder, weight 355 mg, mp 246–248°, $[\alpha]^{22}D$ −50.5° (*c* 1, dimethylformamide). *Anal.* Calcd for $C_{72}H_{92}N_{12}O_{14}S_2$: C, 61.1; H, 6.53; N, 11.9. Found: C, 61.2; H, 6.82; N, 11.8.

The yield of the protected nonapeptide amide from the cleavage was 73% of the amount expected based on the increase in weight of the resin. The over-all yield based on the amount of glycine originally esterified to the resin was 59%. Amino acid analysis[21] gave: Asp, 1.00; Glu, 1.12; Pro, 0.90; Gly, 1.07; Ile, 1.02; Leu, 1.14; Tyr, 0.83; Bzl-Cys, 1.95; Cys, 0.07; $NH_3$, 3.1. The total time required for the synthesis and cleavage was 6 days. The protected nonapeptide (100 mg) was treated with sodium in liquid ammonia as described by du Vigneaud, *et al.*,[2] and the resulting free thiol groups were oxidized by treatment with an aqueous solution of potassium ferricyanide[22] to give a solution possessing a total of 15,900 IU of oxytocic activity.[23] Pure oxytocin (32.5 mg, 46%) was obtained from a lyophilizate of this solution by gel filtration on Sephadex G-15,[24] $[\alpha]^{22.5}D$ −24.0° (*c* 0.5, 1 *N* acetic acid). *Anal.* Calcd for $C_{43}H_{66}N_{12}S_2 \cdot CH_3COOH \cdot H_2O$: C, 49.80; H, 6.69; N, 15.49. Found: C, 49.93; H, 6.85; N, 15.37. Amino acid analysis gave: Asp, 1.00; Glu, 1.00; Pro, 1.10; Gly, 1.0; Cys, 1.80; Ile, 0.89; Leu, 1.00; Tyr, 0.85; $NH_3$, 3.0. When examined by thin layer and paper chromatography the product was shown to be homogeneous and to give the same $R_f$ values as those reported for oxytocin.[25] The material exhibited an oxytocic activity of ~430 IU/mg. This value may be increased by ~12% if the water and acetic acid content of the lyophilized product are taken into account. The over-all yield of biologically active oxytocin was 27%. The total time required for the synthesis starting with Boc-amino acids and ending with chromatographically pure oxytocin was 10 days.

(20) During the evacuation at the vacuum pump the methanolic ammonia was trapped by interposing between the flask containing the suspension and the vacuum pump a glass freeze-drying apparatus (Scientific Glass Apparatus Co., Inc., Bloomfield, N. J., catalog no. JD-9379) having a Dry Ice–acetone mixture in the central compartment and the central flask, containing 100 ml of a 1:1 mixture of 12 *N* HCl and glacial acetic acid, immersed in a Dry Ice–acetone bath at −80°.

(21) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, 30, 1190 (1958).

(22) V. du Vigneaud, G. Winestock, V. V. S. Murti, D. B. Hope, and R. D. Kimbrough, Jr., *J. Biol. Chem.*, **235**, PC64 (1960); D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *ibid.*, **237**, 1563 (1962).

(23) P. Holton, *Brit. J. Pharmacol.*, 3, 328 (1948); R. D. Munsick, *Endocrinology*, 66, 860 (1960).

(24) M. Manning and T. C. Wuu, unpublished data.

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The advantages of rapidity and efficiency which characterize the Merrifield method have been fully demonstrated in the extension of the method for use in the synthesis of the key intermediate required for the synthesis of oxytocin. Further application is being made toward the synthesis of protected nonapeptides required for the synthesis of lysine-vasopressin[26] and of analogs of oxytocin and lysine-vasopressin. It is also worthy of note that Beyerman[27] has carried out the solid-phase synthesis of the partially protected nonapeptide leading to 9-deamido-oxytocin[28] and Takashima, du Vigneaud, and Merrifield[29] have utilized the solid-phase technique for the synthesis of deamino-oxytocin.[22]

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(26) During the preparation of this report the approach outlined herein has been used to synthesize in 60% yield (based on the amount of glycine originally esterified to the resin) an analytically pure protected nonapeptide amide with the amino acid sequence of lysine-vasopressin: Z-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Z)-Gly-$NH_2$, mp 228–231°, $[\alpha]^{22.5}D$ −42° (*c* 1, dimethylformamide). An aliquot (100 mg) of this material upon reduction with sodium in liquid ammonia[2] and subsequent oxidation with potassium ferricyanide[22] gave a solution possessing a total of 15,000 units of pressor activity: "The Pharmacopeia of the United States of America," 16th revision, Mack Publishing Co., Easton, Pa., 1960, p 793.

(27) H. C. Beyerman, C. A. M. Doers-Boonekamp, W. J. Van Zoest, and D. Van Den Berg, "Peptides," H. C. Beyerman, A. Van de Linde, and W. M. Van Den Brink, Ed., North Holland Publishing Co., Amsterdam, 1967, p 117.

(28) B. M. Ferrier and V. du Vigneaud, *J. Med. Chem.*, **9**, 55 (1966).

(29) Personal communication from Drs. Herbert Takashima, Vincent du Vigneaud, and Bruce Merrified.

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## The Stereochemistry and Inversion of Trivalent Oxygen

*Sir:*

The most succinct example of a unimolecular thermal reorganization is the pyramidal inversion of molecules that possess lone pairs. The product is either enantiomeric, diastereomeric, or equivalent to the starting material. Nearly all previous studies have focused on the inversion properties of nitrogen,[1] although other atoms have received some attention.[2,3] We have initiated a general program to broaden the scope of knowledge in this field to include other atoms. In the present communication and the next,[4] we wish to report the first observation of the thermal stereomutation of oxygen and of arsenic.

Oxygen must necessarily be trivalent for inversion studies. Although the planar pyrilium salts are clearly

(1) (a) For a very complete review, see G. Binsch, "Topics in Stereochemistry," Vol. III, N. L. Allinger and E. L. Eliel, Ed., Interscience Publishers, Inc., New York, N. Y., in press; (b) for calculations of inversion barriers, see G. W. Koeppl, D. S. Sagatys, G. S. Krishnamurthy, and S. I. Miller, *J. Am. Chem. Soc.*, **89**, 3396 (1967).

(2) Sulfur: (a) D. R. Rayner, E. G. Miller, P. Bickart, A. J. Gordon, and K. Mislow, *ibid.*, **88**, 3138 (1966); (b) P. Haake and P. C. Turley, *ibid.*, **89**, 4611, 4617 (1967); (c) E. W. Abel, R. P. Bush, F. J. Hopton, and C. R. Jenkins, *Chem. Commun.*, 58 (1966); (d) D. Darwish and G. Tourigny, *J. Am. Chem. Soc.*, **88**, 4303 (1966).

(3) Phosphorus: (a) J. B. Lambert and D. C. Mueller, *ibid.*, **88**, 3669 (1966); (b) L. Horner and H. Winkler, *Tetrahedron Letters*, 461 (1964).

(4) J. B. Lambert and G. F. Jackson, III, *J. Am. Chem. Soc.*, **90**, 1350 (1968).