[O$_2$] curve was calculated for N-meDPA. Good agreement with the experimental curve was obtained with one parameter, $k_3/(k_1 + k_3)$, adjusted for best fit (0.37).

The maximum in φ occurring in degassed solutions as the temperature is lowered (observed both in our work and by Bowen and Eland[2]) is a result of different temperature dependence of 610 yield and k_7. At room temperature, although 610 yield is high, the reversible decay (k_7) is fast, and φ is low. As the temperature is lowered, a relatively strong decrease in k_7 occurs $(E_A = 10 \text{ kcal.})$. The temperature-independent process (k_8) can then predominate and φ rises. At still lower temperatures the 610 yield drops also, and causes the final decrease in φ.

The nature of the process k_8 (0.28 sec.$^{-1}$ for N-meDPA), responsible for carbazole formation in degassed solutions at low temperatures, is of the greatest interest. We suggest that k_8 corresponds to a decomposition of 610 by a "tunneling" process, of the type discussed by Robinson[3] in connection with the problem of radiationless transitions in complex molecules. In this case, the "radiationless transition" would occur along the particular vibrational coordinate leading to splitting out of hydrogen[2] from 610 and would correspond to chemical reaction instead of return to the ground state. Alternatively, k_8 may be an internal conversion process, with very low activation energy, leading to a high vibrational level of the amine ground state and *subsequent* decomposition of the resulting hot molecule.[4] In condensed phase, one would expect that vibrational deactivation would be too rapid to permit efficient thermal decomposition of such a complex molecule and the tunneling mechanism is therefore favored. One may further conjecture that the two central hydrogens of 610[1] are in the *cis* configuration.

Experiments using deuterated DPA are in progress. A detailed report of these experiments will appear shortly.

(3) G. W. Robinson, *J. Mol. Spectry.*, **6**, 58 (1961).

(4) R. Srinavasan and J. C. Power, *J. Chem. Phys.*, **39**, 580 (1963); *J. Am. Chem. Soc.*, **85**, 1355 (1963).

DEPARTMENT OF CHEMISTRY   HENRY LINSCHITZ
BRANDEIS UNIVERSITY   KARL-HEINZ GRELLMANN
WALTHAM, MASSACHUSETTS

Solid Phase Peptide Synthesis. II. The Synthesis of Bradykinin

Sir:

Solid phase peptide synthesis was introduced recently[1,2] as a new approach to the preparation of polypeptides. This process, which was designed to speed and simplify peptide synthesis, depends on the attachment of the C-terminal amino acid residue to a solid particle, the stepwise addition of succeeding amino acids to the peptide chain, and finally the cleavage of the completed peptide from the solid support. The

(1) R. B. Merrifield, *Federation Proc.*, **21**, 412 (1962).

(2) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963).

feasibility of the idea was demonstrated by the synthesis of a tetrapeptide, which was shown to be identical with a sample made by conventional procedures.[2] More recently, a dipeptide has also been made by a related method.[3] It was recognized at the outset that, for the method to be of real value, it would be necessary to apply it to the preparation of larger peptides, and preferably to ones with biological activity. We wish to report here the successful application of the method to the synthesis of the nonapeptide hormone, bradykinin.

The structure of bradykinin was elucidated by Elliott, *et al.*,[4] and the peptide was synthesized by Boissonnas, *et al.*,[5] and by Nicolaides and De Wald.[6] The present synthesis followed the basic concept of solid phase peptide synthesis outlined previously,[2] but differed in several important details: (1) *t*-Butyloxy carbonyl (*t*-BOC) amino acids were used in place of benzyloxycarbonyl derivatives to permit milder conditions for the deacylation, and thus to decrease the loss of peptide from the resin. With this modification it was unnecessary to nitrate the resin. (2) The coupling steps were carried out in dimethylformamide (DMF) and an excess of each *t*-BOC amino acid was used. (3) The acetylation steps were eliminated. With these modifications, the total time required for each cycle was shortened to 4 hr., and the over-all yield of peptide was improved.

For the synthesis of bradykinin, *t*-BOC-nitro-L-arginine triethylammonium salt was coupled in ethanol with 10 g. of chloromethylcopolystyrene-divinylbenzene (2%) to give the ester. All of the remaining reactions were carried out in the reaction vessel described previously.[2] First, the acyl group was removed (1 *N* HCl–acetic acid, 25°, 30 min.), and the resulting hydrochloride was neutralized by washing with triethylamine in DMF. The free base was then coupled in DMF with an excess of *t*-BOC-L-phenylalanine by the aid of dicyclohexylcarbodiimide to give *t*-BOC-L-phenylalanyl-nitro-L-arginyl resin. Excess reagents and by-products were removed from the totally insoluble and easily filterable product by thorough washing with DMF, ethanol, and acetic acid. In exactly the same way the peptide chain was lengthened one amino acid at a time until the protected nonapeptide, *t*-BOC-nitro-L-arginyl-L-prolyl-L-prolyl-glycyl-L-phenylalanyl-O-benzyl-L-seryl-L-prolyl-L-phenylalanyl-nitro-L-arginyl resin was produced. The product contained 0.150 mmole of peptide per gram of resin, yield 83% based on the C-terminal nitroarginine residue. Therefore, the average retention of the peptide on the resin during each of the 8 deprotection steps was over 97%. The coupling reactions also were nearly quantitative at each step as indicated by the amino acid analysis of a hydrolysate of the resin-bound peptide. Amino acid ratios were: arg, 2.00; pro, 2.74; phe, 2.13; gly, 1.01; ser, 1.04. The peptide was cleaved from the resin in 75% yield with HBr in trifluoroacetic acid. The amount liberated was followed by measurement of the absorption of the soluble material at 268 mμ due to nitroarginine. Catalytic hydrogenation with palladium black in methanol–acetic acid gave a quantitative yield of free nonapeptide, based on the decrease in absorption at 268 mμ and a corresponding increase in the Sakaguchi reaction for arginine. The product was purified chromatographically on IRC-50 ion-exchange resin by gradient elution

(3) R. L. Letsinger and M. J. Kornet, *ibid.*, **85**, 3045 (1963).

(4) D. F. Elliott, G. P. Lewis, and E. W. Horton, *Biochem. Biophys. Res. Commun.*, **3**, 87 (1960).

(5) (a) R. A. Boissonnas, St. Guttmann, and P.-A. Jaquenoud, *Helv. Chim. Acta*, **43**, 1349 (1960); (b) St. Guttmann, J. Pless, and R. A. Boissonnas, *ibid.*, **45**, 170 (1962).

(6) E. D. Nicolaides and H. A. De Wald, *J. Org. Chem.*, **26**, 3872 (1961).

with aqueous acetic acid.[5b] Pure bradykinin (610 mg., 51% of the material liberated from the solid support) was obtained from the major Sakaguchi positive peak.

The product was homogeneous and indistinguishable from authentic bradykinin[7] by paper electrophoresis and paper chromatography (detected by ninhydrin and Sakaguchi reagents): R_{arg} 0.62 (0.1 M pyridine acetate, pH 5.0); R_{glu} 1.38 (formic acid–acetic acid–H_2O, 1.5:1:100, pH 2.1); R_f 0.50 (propanol–H_2O, 2:1); R_f 0.49 (sec-butyl alcohol–formic acid–H_2O, 100:16:16); R_f 0.26 (isoamyl alcohol–pyridine–H_2O, 35:35:30); $[\alpha]^{25}D$ −76.5° (c 1.37, 1 N acetic acid). Amino acid ratios were: arg, 1.90; pro, 2.71; phe, 2.04; gly, 1.00; ser, 1.01.

Anal. Calcd. for $C_{50}H_{73}O_{11}N_{15}\cdot3CH_3CO_2H$: C, 54.2; H, 6.9; N, 16.9. Found: C, 54.3; H, 6.9; N, 17.2.

The synthetic bradykinin possessed the full biological activity of the natural hormone. It was compared quantitatively with an authentic standard in the isolated rat uterus assay and in the rat duodenum assay. Over the range of 10^{-10} to 10^{-9} g./ml., the two preparations were equally active in both tests.

The over-all yield of biologically active bradykinin was 32%. The total time required for the synthesis starting with t-BOC amino acids and ending with chromatographically pure bradykinin was 8 days.

(7) Obtained through the courtesy of Dr. E. D. Nicolaides, Parke, Davis & Company, Ann Arbor, Michigan.
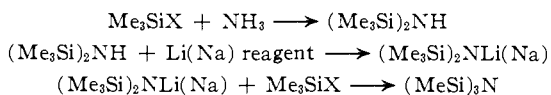
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NEW YORK 21, NEW YORK
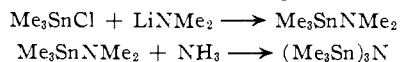RECEIVED NOVEMBER 25, 1963

Preparation of Tris(trimethylsilyl)- and Tris(trimethylstannyl)amines

Sir:

It is reported in the literature[1-3] that attempts to prepare tris(trimethylsilyl)amine directly by reaction of trimethylchlorosilane with ammonia failed, even at 500° with pyridine as solvent. Successful preparations of this material[2,3] and of the analogous tin compound, tris(trimethylstannyl)amine,[4] have required at least two steps, one of which involved the preparation of an N-lithio or N-sodio intermediate.

$$Me_3SiX + NH_3 \longrightarrow (Me_3Si)_2NH$$
$$(Me_3Si)_2NH + Li(Na) \text{ reagent} \longrightarrow (Me_3Si)_2NLi(Na)$$
$$(Me_3Si)_2NLi(Na) + Me_3SiX \longrightarrow (MeSi)_3N$$

The tin compound has been prepared as follows.

$$Me_3SnCl + LiNMe_2 \longrightarrow Me_3SnNMe_2$$
$$Me_3SnNMe_2 + NH_3 \longrightarrow (Me_3Sn)_3N$$

The purpose of this paper is to describe a simple, convenient, one-step synthesis of these materials by the use of the novel new reagent, lithium nitride (I).[5] It has been found that a slurry of lithium nitride in tetrahydrofuran (THF) will react with trimethylchlorosilane (IIa) and trimethylchlorostannane (IIb) to give the corresponding tris(trimethylmetallo)amine (IIIa,b)

(1) R. O. Sauer and R. H. Hasek, J. Am. Chem. Soc., **68**, 241 (1946).
(2) U. Wannagat and H. Niederprum, Angew. Chem., **71**, 574 (1959).
(3) J. Goubeau and J. Jimenez-Barbera, Z. anorg. allgem. Chem., **303**, 217 (1960).
(4) K. Jones and M. F. Lappert, Proc. Chem. Soc., 358 (1962).
(5) Commercially available from Foote Mineral Company, Philadelphia 44, Pennsylvania.

in good yield. Koenig and co-workers[6] reported that lithium nitride reacts "with organotin halides, which presumably reacted metathetically, since the lithium halide separated" but did not indicate the nature of the other product.

$$Li_3N + 3Me_3MCl \longrightarrow (Me_3M)_3N + LiCl$$
$$\quad I \qquad\qquad II \qquad\qquad\qquad III$$
a, M = Si; b, M = Sn
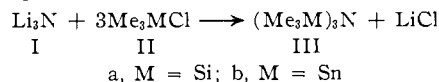
The general procedure used was as follows. To a slurry of 0.05 mole of I in 50 ml. of dry THF, maintained under an atmosphere of dry nitrogen, was added dropwise, with stirring, a solution of II, 0.15 mole in 50 ml. of THF, over a period of 1 hr. Care was exercised during the addition due to the extremely exothermic nature of the reaction. After the addition was completed the reaction mixture was heated at reflux for 2 hr. The major portion of the THF was removed by distillation, 150 ml. of petroleum ether (b.p. 30–60°) was added to the concentrate, and the mixture filtered to remove insoluble LiCl. The filtrate was concentrated and the residue distilled to give the desired product. The silyl compound (IIIa) was obtained in 72% yield and was identified by comparison of its infrared[3] and n.m.r.[7] spectra with values published in the literature and its elemental analysis and molecular weight. The stannyl compound (IIIb) was obtained in 59% yield and was identified by a comparison of its boiling point, 84° (.40 mm.), with the value published in the literature and by the determination of its molecular weight. Anal. Calcd: 505. Found: 515, 519. The n.m.r. of IIIb exhibited only a single peak, 11.0 c.p.s. downfield from tetramethylsilane (CCl_4 solution) consistent with its structure. No attempts were made to optimize the yields of these reactions.

It is expected that the reaction of lithium nitride with haloorganometallo compounds will be a general reaction and will be applicable to the preparation of a wide variety of other tris(organometallo)amines of the group III, IV, V, and VI elements. Work is proceeding along these lines.

(6) P. E. Koenig, et al., J. Org. Chem., **26**, 4777 (1961).
(7) H. Schmidbaur, J. Am. Chem. Soc., **85**, 2336 (1963).

POLYMER BRANCH W. L. LEHN
NONMETALLIC MATERIALS DIVISION
AIR FORCE MATERIALS LABORATORY
U. S. AIR FORCE
WRIGHT-PATTERSON AIR FORCE BASE, OHIO
RECEIVED DECEMBER 10, 1963

Large Salt Effects and Mechanism in Acetone and Ether[1,2]

Sir:

Salt effects on rate of ionization of organic substrates can become enormous in poorly ionizing solvents.[3] In this communication we call attention to the magnitude and specific pattern of such salt effects on ionization of p-methoxyneophyl p-toluenesulfonate[3] (ROTs) and the spirodienyl p-nitrobenzoate[4] (I-OPNB). Some mechanistic features of salt-promoted ionization are also discussed.

Salt effects on rate of ionization of ROTs in acetone,[3] measured by acid production, are moderately large. Addition of 0.05 M LiClO₄ increases the rate by a factor of 3.4. With I-OPNB first-order rate constants for production of HOPNB and tetralin show much greater

(1) Research supported by the National Science Foundation.
(2) Research sponsored by the U. S. Army Research Office (Durham).
(3) S. Winstein, S. Smith, and D. Darwish, J. Am. Chem. Soc., **81**, 5511 (1959).
(4) E. C. Friedrich and S. Winstein, Tetrahedron Letters, **No. 11**, 475 (1962).