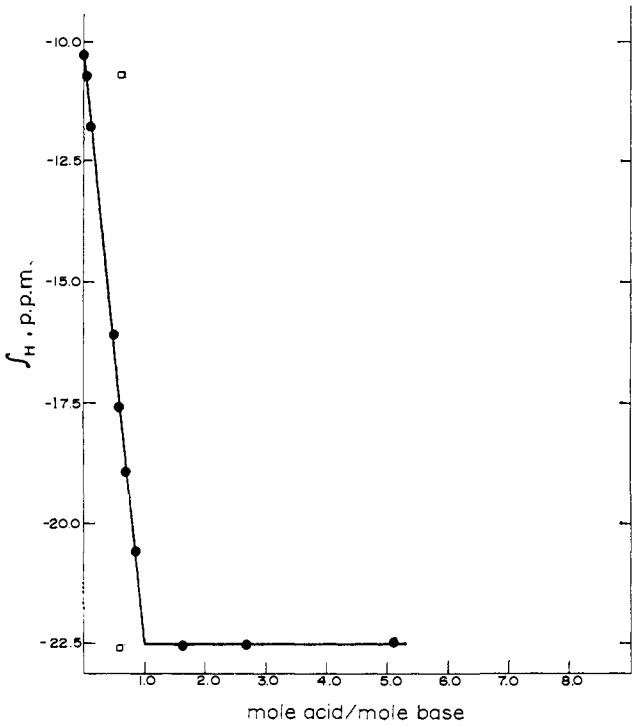

Fig. 2.—Demonstration of 1–1 donor–acceptor complex between boron trichloride and FBN in methylene chloride solution: ●, at 40 Mc. and 25°; □, at 56 Mc. and ~−20°.

More definitive evidence for the formation of the 1–1 complex has been obtained with FBN using as acid BCl_3. Figure 2 plots (in the same manner as Fig. 1) the results obtained (under the same conditions) for this system. The experimental points in Fig. 2 satisfactorily follow the theoretical curve for a 1–1 complex with infinite formation constant. The data are only fitted by such a formation constant with values exceeding $5 \times 10^3\ M^{-1}$. A conclusive additional result is the resolution of the hybrid fluorine signal for the solution of $a/b = 0.59$ into the two component signals of appropriate relative intensities for complexed and uncomplexed FBN (*cf.* Fig. 2) which is observed when the spectrum is taken at about −20° and 56 Mc.[7] These observations also indicate that the 1–1 complex is not appreciably dissociated to ions under our conditions.[8]

The acid BBr_3 with FBN gives a plot at 25° which is identical in form with that of Fig. 2. With the stronger base, fluorocyanodurene[9] ($a/b \cong 0.5$), the resolved signals for uncomplexed and complexed base are observed at 25° (and 40 Mc.) with this acid ($\Delta_{complex}$ = −13.0 p.p.m.). The acid BF_3 with FBN at 25° gives a curve which is intermediate in form between that of Fig. 1 and 2. The data are well fitted by a 1–1 formation constant of 76 ± 20 M^{-1}.[6] The acid B_2Cl_4 with FBN in a/b = 1.0 to 3.3 gives a constant limiting shift for what appears to be a 1–1 complex. At a/b of less than unity, material precipitated from the methylene chloride and no signals could be observed. The acid $B(CH_3)_3$ with FBN gives very small shifts to lower field even at an a/b ratio of 20 and −20°.

All solutions have been prepared in a vacuum line at 10^{-6} mm. Accurately weighed samples of FBN were introduced to the line from ampoules and transferred into the n.m.r. sample tube. All other components were measured as vapors in calibrated constant volume manometers, and transfers were made through appropriate mercury float valves. Condensation into the n.m.r. sample tube was made with liquid nitrogen baths, and the tube was sealed off under vacuum. Weighing of *p*-fluorophenylboron dichloride was achieved by distillation into tubes fitted with magnetic breaker seals.

Table I summarizes values of $\Delta_{complex}$ observed for FBN in methylene chloride solution at 25° for a series of acceptors. Listed for comparison are the heats of dissociation, ΔH_D, for the corresponding pyridine complexes obtained by Brown, *et al.*,[10] in nitrobenzene solution. The shifts and heats are clearly parallel. The formation constants for the FBN complexes obtained in this work are also listed in Table I. We believe the results given in Table I clearly confirm the potential of the method. Work is in progress involving an extensive series of acceptors with FBN and other *p*-fluorophenyl-labeled bases.

(7) Obtained with a Varian A-56–60 spectrometer, Pittsburgh airport. We are indebted for the assistance of Mr. Jerry Holcomb.

(8) We are indebted to Professor R. S. Drago for pointing out this possibility.

(9) Kindly supplied by Professor G. Illuminati, Rome, Italy.

(10) H. C. Brown and R. R. Holmes, *J. Am. Chem. Soc.*, **78**, 2173 (1956); H. C. Brown and D. Gintis, *ibid.*, **78**, 5378 (1956).

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The Synthesis of an Octapeptide Corresponding to a Sequence around the "Reactive Serine" of Chymotrypsin

Sir:

Recent investigations have shown that the "active site" of many proteolytic enzymes includes a "reactive serine" residue. In particular the sequence Gly-Asp-Ser-Gly is found around this serine in chymotrypsin, trypsin, and elastase.[1] Syntheses of this aspartyl tetrapeptide and the analogous glutamyl compound have been reported previously.[2,3] We report here the synthesis of these tetrapeptide sequences with end groups so blocked that further extension of the peptide chain may be readily accomplished. We then describe the synthesis of the octapeptide sequence Gly-Asp-Ser-Gly-Gly-Pro-Leu-Val, which has been shown[4] to comprise residues 193 through 200 of bovine chymotrypsinogen A.

The crystalline octapeptide I (with terminal amino and carboxyl groups blocked) was synthesized[5] according to the scheme shown in Chart I.[6] All peptide bonds were formed using Woodward's reagent (N-ethyl-5-phenylisoxazolium 3′-sulfonate)[7] except for the syn-

(1) J. A. Cohen, R. A. Oosterbaan, H. S. Jansz, and F. Berends, *J. Cellular Comp. Physiol.*, **54** (Suppl. 1), 231 (1959); F. Sanger, *Proc. Chem. Soc.*, 76 (1963).

(2) H. Kienhuis, A. van de Linde, J. P. J. van der Holst, and A. Verweij, *Rec. trav. chim.*, **80**, 1278 (1961).

(3) A. van der Linde, H. Kienhuis, A. Verweij, and J. P. J. van der Holst, *ibid.*, **80**, 1305 (1961).

(4) B. S. Hartley, *Nature*, **201**, 1284 (1964).

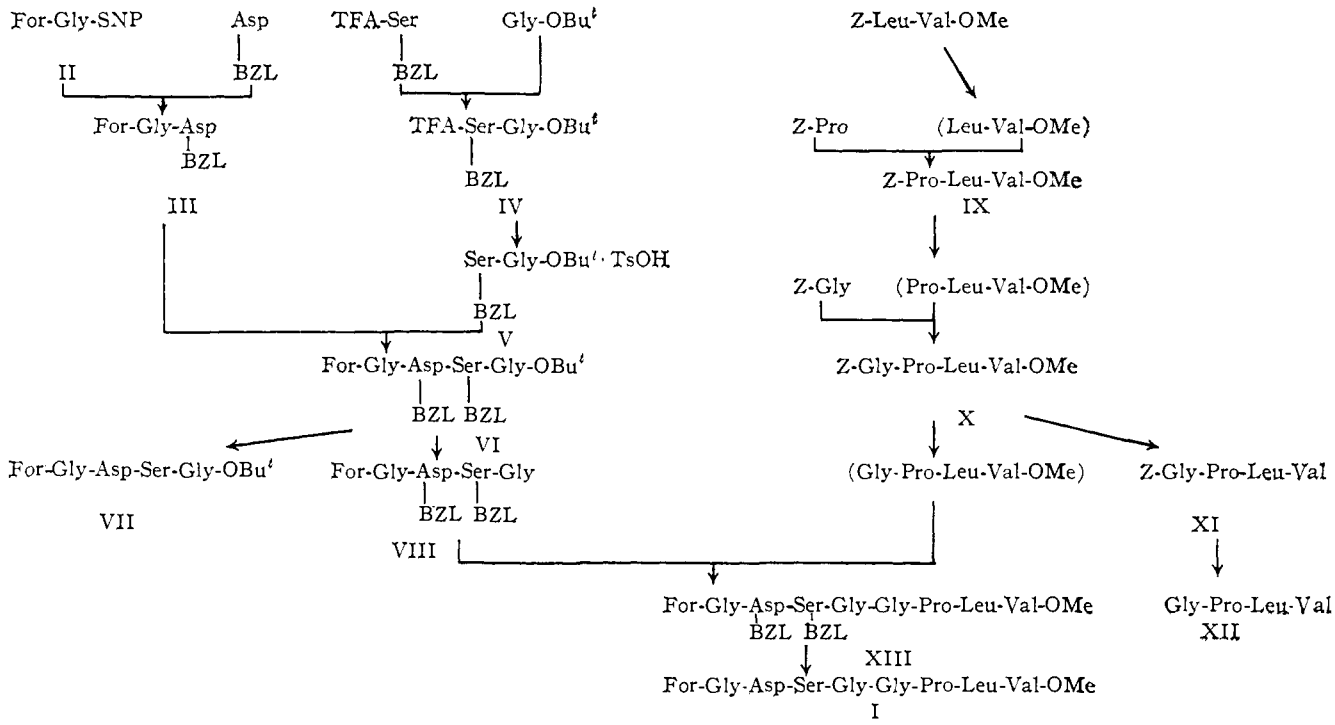
(5) Satisfactory analyses and spectral data were obtained for all crystalline compounds described here. Melting points were uncorrected. $[\alpha]_D$ refers to about 0.5% solution in methanol at 23–25°.

(6) The nomenclature adopted is that recommended at the Fifth European Symposium on Peptides, Oxford, 1962; see "Peptides, Proceedings of the Fifth European Symposium, Oxford, Sept., 1962," G. T. Young, Ed., Pergamon Press, Oxford, 1963, p. 261. "For-" denotes "formyl-"; (a) after a Roman numeral refers to the glutamyl analog.

(7) R. B. Woodward, R. A. Olofson, and H. Mayer, *J. Am. Chem. Soc.*, **83**, 1010 (1961).

thesis of III and the analogous glutamyl compound. The peptide bonds in these compounds were formed using the *p*-nitrothiophenolate ester. In every step the homogeneity of the compounds was ascertained by appropriate chromatographic techniques. The octapeptide synthesis was designed so that the final peptide formation step involved the establishment of a Gly-Gly bond between two tetrapeptides, thus minimizing racemization at this point. The optical purity of each of these tetrapeptides was determined by enzymic methods (see below).

CHART I



N-Formylglycine *p*-nitrothiophenolate (II), m.p. 135° (prepared by condensation of N-formylglycine[8] with *p*-nitrothiophenol using dicyclohexylcarbodiimide) was treated with β-benzyl-L-aspartate[9] in tetrahydrofuran–aqueous sodium hydroxide at pH 7–8 to give N-formylglycyl-β-benzyl-L-aspartate (III), m.p. 170°, $[\alpha]_D$ +6.7°. N-Trifluoroacetyl-O-benzyl-L-serine[10] was condensed with glycine *t*-butyl ester hydrochloride[11] yielding N-trifluoroacetyl-O-benzyl-L-serylglycine *t*-butyl ester (IV, oil). The trifluoroacetyl group was removed with hydrazine in refluxing ethanol, and the product O-benzyl-L-serylglycine *t*-butyl ester was isolated as the toluene-*p*-sulfonic acid salt (V), m.p. 111.5–112° $[\alpha]_D$ +10°. Condensation of the dipeptides V and III yielded the blocked tetrapeptide N-formylglycyl-β-benzyl-L-aspartyl-O-benzyl-L-serylglycine *t*-butyl ester (VI), m.p. 122–123°, $[\alpha]_D$ −18°. Selective removal of blocking groups from VI was carried out as follows: Hydrogenation over palladium–charcoal in aqueous ethanol at 55° resulted in debenzylation giving the terminal-blocked tetrapeptide N-formylglycyl-α-L-aspartyl-L-serylglycine *t*-butyl ester (VII), m.p. 125–127°, $[\alpha]_D$ −36°. The *t*-butyl ester of VI was removed by short treatment with anhydrous trifluoroacetic acid giving N-formylglycyl-β-benzyl-L-aspartyl-O-benzyl-L-serylglycine (VIII), m.p. 213–214° dec., $[\alpha]_D$ −10°.

The analogous peptides of the glutamyl series were similarly synthesized. Reaction of N-formylglycine *p*-nitrothiophenolate (II) with γ-benzyl-L-glutamate[12] yielded N-formylglycyl-γ-benzyl-L-glutamate (IIIa), m.p. 147–148°, $[\alpha]_D$ +0.3°, which was then condensed with the dipeptide V to give the blocked tetrapeptide N-formylglycyl-γ-benzyl-L-glutamyl-O-benzyl-L-serylglycine *t*-butyl ester (VIa), m.p. 125.5–126°, $[\alpha]_D$ −11°. Hydrogenation of VIa, as in the case of VI, resulted in N-formylglycyl-α-L-glutamyl-L-serylglycine *t*-butyl ester (VIIa), m.p. 143–146°. Removal of *t*-butyl ester from VIa was carried out using toluene-*p*-sulfonic acid in anhydrous benzene, yielding N-formylglycyl-γ-benzyl-L-glutamyl-O-benzyl-L-serylglycine (VIIIa), m.p. 177.5–178°.

N-Benzyloxycarbonyl-L-leucyl-L-valine methyl ester[13] was hydrogenated over palladium–charcoal at room temperature to remove the benzyloxycarbonyl group, and the resulting product was condensed with N-benzyloxycarbonyl-L-proline to give N-benzyloxycarbonyl-L-prolyl-L-leucyl-L-valine methyl ester (IX), m.p. 126–127°, $[\alpha]_D$ −92°. Hydrogenation of IX and condensation with N-benzyloxycarbonylglycine afforded N-benzyloxycarbonylglycyl-L-prolyl-L-leucyl-L-valine methyl ester (X, oil) characterized as the corresponding carboxylic acid XI, m.p. 135–136°, $[\alpha]_D$ −90°.

Hydrogenation of X gave the corresponding peptide ester which was then condensed with N-formylglycyl β-benzyl-L-aspartyl-O-benzyl-L-serylglycine (VIII) giving the fully blocked octapeptide N-formylglycyl-β-benzyl-L-aspartyl-O-benzyl-L-serylglycylglycyl-L-

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(9) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

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(11) E. Tashner, A. Chimiak, B. Bator, and T. Sokolowska, *Ann.*, **646**, 134 (1961).

(12) W. E. Hanby, S. G. Waley, and J. Watson, *J. Chem. Soc.*, 3239 (1950).

(13) E. L. Smith, D. H. Spackman, and W. J. Polglase, *J. Biol. Chem.*, **199**, 801 (1952).

prolyl-L-leucyl-L-valine methyl ester (XIII, oil). Upon removal of benzyl groups from XIII by hydrogenation at 55° over palladium–charcoal, there was obtained the terminal-blocked octapeptide I, N-formylglycyl-α-L-aspartyl-L-serylglycylglycyl-L-prolyl-L-leucyl-L-valine methyl ester, m.p. 192–193°, $[\alpha]_D$ −84°; amino acid ratios in acid hydrolysate $gly_{3.12}asp_{1.03}ser_{0.94}pro_{0.97}leu_{0.98}val_{0.94}$.

The optical purity of certain of the peptides synthesized was determined using appropriate enzyme systems. The dipeptide V was completely hydrolyzed by leucine aminopeptidase[14]; under similar conditions the corresponding DL-peptide, m.p. 95–97°, was only partially hydrolyzed. The tetrapeptide XII, m.p. 219° dec. (prepared by hydrogenation of XI), was converted completely to its component amino acids by a mixture of leucine aminopeptidase and prolidase.[14,15] Finally, the tetrapeptide VIII was completely hydrolyzed by carboxypeptidase-A.

The octapeptide I and various intermediate compounds are being investigated for possible esterase and peptidase activity. In addition, compound I serves as an intermediate for more extensive syntheses, including its incorporation into macromolecular polypeptide systems containing other amino acids involved in proteolytic enzyme action.

Acknowledgment.—We are pleased to acknowledge the support of this work by U. S. Public Health Service Grant No. AM-07300 and by the Office of the Surgeon General, Department of the Army. Mr. Richard Maine provided competent technical assistance. We thank Dr. Elizabeth R. Simons for participation in the enzymic work.

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σ-Bonded Alkyl Compounds of Niobium and Tantalum. Trimethyldichloroniobium and Trimethyldichlorotantalum[1]

Sir:

Recent developments in the organometallic chemistry of the elements of group V-A have been confined mainly to arene complexes. To this date, there have been no reports of σ-bonded alkyl compounds of these metals, although many attempts to prepare them have been described.[2] Accordingly, we wish to report the first successful syntheses of alkyl derivatives of niobium and tantalum. Trimethyldichloroniobium, $(CH_3)_3NbCl_2$, and trimethyldichlorotantalum, $(CH_3)_3TaCl_2$, have been prepared by the low temperature exchange of methyl groups and chlorine between dimethylzinc and the pentachlorides of niobium and tantalum, respectively.

In a typical experiment, 7.45 mmoles of $NbCl_5$ was sublimed *in vacuo* and treated with 18.26 mmoles of $(CH_3)_2Zn$ in 15 ml. of pentane. The vessel containing the $NbCl_5$ and pentane was cooled to −78°, and the $(CH_3)_2Zn$ was admitted in small portions because of the exothermic character of the reaction. A precipitate (probably $ZnCl_2$) was observed immediately following addition of the first portion of $(CH_3)_2Zn$. After each addition the reaction mixture was warmed nearly to room temperature and agitated. After the final addition of $(CH_3)_2Zn$, all volatiles were removed at room temperature; the $(CH_3)_3NbCl_2$ was trapped at −36°. The yield was 12.7% based on $NbCl_5$. Trimethyldichlorotantalum was prepared in an identical manner. All manipulations were carried out in a high vacuum system.

Trimethyldichloroniobium forms golden yellow crystals which sublime readily under vacuum at room temperature. However, the compound will darken and release methane when left at room temperature for several hours. It appears to be indefinitely stable at −78°.

Trimethyldichlorotantalum forms pale yellow crystals of similar volatility. This compound, however, appears to be much more thermally unstable than the niobium analog. The thermal stability of both compounds was found to be adversely affected by small quantities of impurities. Both compounds are highly reactive toward water and air.

The formulas of the new compounds were established by hydrolyzing freshly prepared samples *in vacuo* with aqueous KOH, measuring the resultant CH_4 directly by means of a Sprengel pump, and determining niobium and tantalum gravimetrically as the pentoxides. The chloride was also determined gravimetrically. The CH_4 was subsequently shown to be quantitatively pure by means of infrared and mass spectroscopy. For each compound, all analytical data were determined independently on the same weighed sample.

Anal. Calcd. for $(CH_3)_3NbCl_2$: CH_3, 21.59; Nb, 44.47; Cl, 33.94. Found: CH_3, 21.6; Nb, 44.6; Cl, 34.0.

Anal. Calcd. for $(CH_3)_3TaCl_2$: CH_3, 15.19; Ta, 60.93; Cl, 23.88. Found: CH_3, 14.8; Ta, 63.0; Cl, 23.6.

In addition, samples of the new compounds were subjected to slow hydrolysis by exposure to the air, and the residues were analyzed spectrographically. No significant quantities of zinc or other extraneous metals were found to be present.

Attempts to obtain the molecular weights by gas density and vapor pressure depression measurements have not been successful because of the instability of these compounds under the experimental conditions used.

The mass spectrum of the niobium compound is indicative of $(CH_3)_3NbCl_2$ monomer, although parent peaks (mass 208, 210, 212) were not observable at an ionizing voltage of 70 e.v. Major fragments are the $[(CH_3)_2NbCl_2]^+$ ions at masses 193, 195, and 197 (relative abundances of 57, 37, and 6%, respectively).

Proton nuclear magnetic resonance spectra were obtained for $(CH_3)_3NbCl_2$ at −10° in CCl_4 containing a trace of $(CH_3)_4Si$. All spectra were obtained with a Varian A-60 spectrometer. Trimethyldichloroniobium exhibits a peak (line width 0.6 c.p.s.) 29.8 c.p.s. upfield from $(CH_3)_4Si$ which is characteristic for protons on a

(1) This paper presents results of one phase of research carried out at the Jet Propulsion Laboratory, California Institute of Technology, under Contract No. NAS7-100, sponsored by the National Aeronautics and Space Administration.

(2) F. A. Cotton, *Chem. Rev.*, **55**, 551 (1955).