find it to be a trimer.[4] The infrared spectrum indicates the presence of terminal and bridging isocyanide ligands (C≡N) stretch. However, there was no evidence of ligand inequivalence from low temperature ^1H NMR studies.

(4) M. Green, J. A. Howard, J. L. Spencer, and F. G. A. Stone, *J. Chem. Soc., Chem. Commun.*, 3 (1975), have now established the platinum analog to be a trimer with three bridging isonitrile ligands.

(5) The low field CH_3 proton NMR peak of relative intensity 27 is at τ 7.92 which is higher than the τ 6.23 value reported[6] for the bridging CH_3NC ligand in $(\eta^5\text{-}C_5H_5)_2Fe_2(CO)_2(NCCH_3)_2$. The two high field peaks at τ 9.59 and 9.37, respectively, are also higher than the reported[6] value of τ 7.48 for the terminal ligand in the aforementioned iron complex but are close to the value of τ 8.69 that we find for $Ni[CNC(CH_3)_3]_4$.

(6) R. D. Adams and F. A. Cotton, *J. Am. Chem. Soc.*, **95**, 6589 (1973).

(7) The first number in parentheses is the root mean squared estimated standard deviation of an individual datum. The second and third numbers, when given, are the average and maximum deviations from the average value, respectively.

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(9) S. G. Shore, in "Boron Hydride Chemistry", E. L. Muetterties, Ed., Academic Press, New York, N.Y., 1975, Chapter III.

(10) We found the reactivity of this cluster to be exceptional among the many "reactive" coordination and organometallic complexes we have studied. This complicated all handling and characterization (especially density and molecular weight) procedures.

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Solid Phase Peptide Synthesis by Oxidation-Reduction Condensation

Sir:

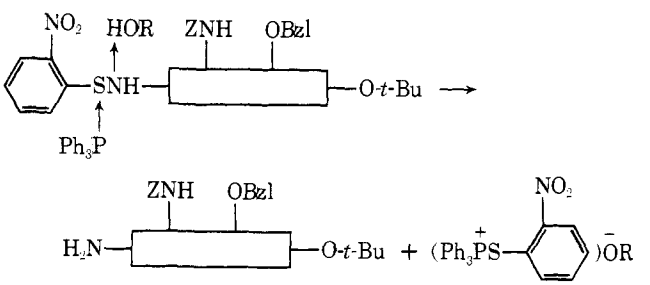
Several modifications on the solid-phase peptide synthesis toward fragment condensation[1] have been attempted to minimize erroneous sequences in the chain elongation from C-terminal amino acid to N-terminal amino acid (A-type elongation). On the other hand, there has been reported one instance[2] in which chain elongation via fragment condensation was carried out from N-terminal amino acid to C-terminal amino acid (B-type elongation) according to the azide method. However, some problems remained unsolved because of the limitations placed on solvents and of low yields in each coupling step. Recently it has been shown that the oxidation-reduction condensation can be successfully applied to the synthesis of LH-RH by B-type elongation via fragment condensation on a solid support.[3]

This communication reports a synthesis of ACTH(1-24) by the above mentioned method employing a new technique to monitor the amount of amino component in solution based on high pressure liquid chromatography. Furthermore, a novel, neutral cleavage technique of the *o*-nitrophenylsulfenyl (Nps-) protecting group was successfully employed in the preparation of peptide fragments.

The synthetic scheme for this method is shown in Figure 1.

In this strategy, the following precautions were taken: (1) the *tert*-butyl ester was selected for α-carboxyl protection since it is easily deprotected with trifluoroacetic acid; (2) the biologically active center was included in one fragment V in order to ensure the incorporation of this fragment which could be proved from the activity; (3) each fragment was designed to incorporate a uv absorbing moiety in order to detect any remaining fragment rapidly by way of high pressure liquid chromatography.

The syntheses of protected fragments were achieved stepwise in solution by oxidation-reduction condensation using the readily available benzyloxycarbonylamino acids except in the cases of the incorporation of *o*-nitrophenylsulfenyl (Nps-) amino acid residues which were coupled with *N,N'*-dicyclohexylcarbodiimide or by the active ester method. Selective removal of the Nps-protecting group from the protected fragments having benzyloxycarbonyl, benzyl ester, and *tert*-butyl groups was achieved under neutral conditions at room temperature through the phosphonium salt[4] by using triphenylphosphine and active hydrogen compounds such as phenol or water as shown in the following scheme.



Starting with 1.5 g of 2% cross-linked resin containing 0.046 mmol of *O*-benzylseryltyrosine,[5] coupling steps II and III were carried out with a three-fold excess of the added

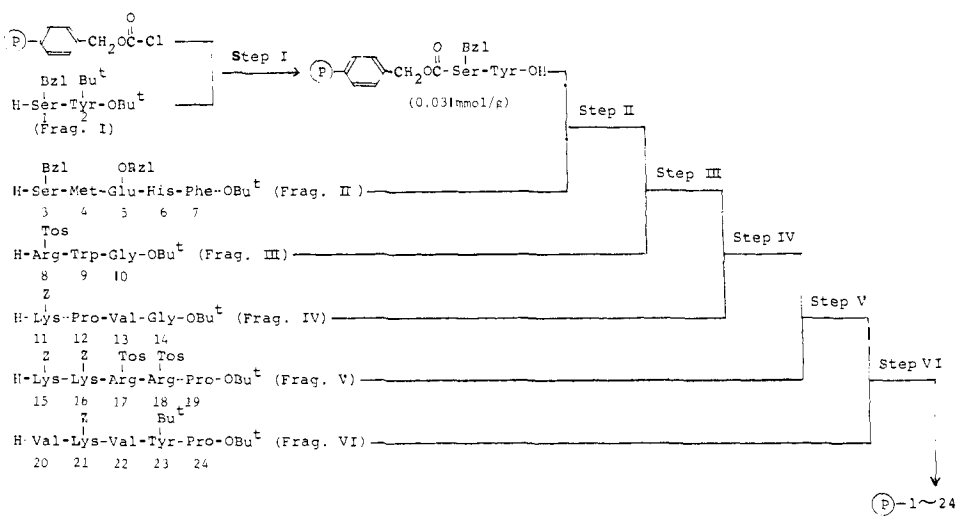


Figure 1. The scheme for the synthesis of ACTH(1-24).

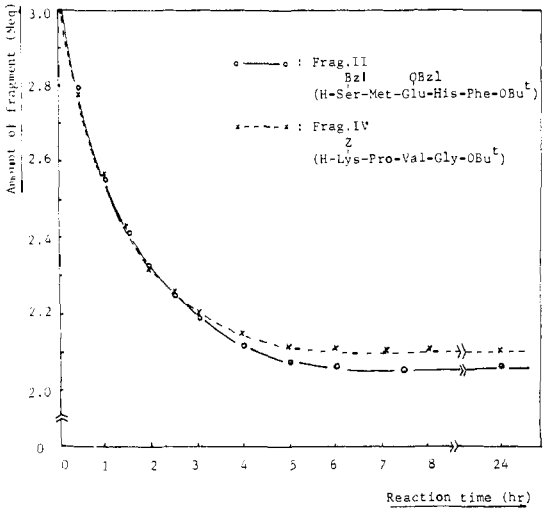


Figure 2. Example of monitoring results by high pressure liquid chromatography. Separation conditions: column, aminated Hitachi Gel 3010 (5 × 500 mm); carrier solvent, 0.1 *N* HCl–MeOH = 1:9; flow rate, 2.0 ml/min; temperature, ambient; detection, uv (240 nm). Preparation of sample solution: to 20 μl of reaction solution, add 20 μl of 0.2 *N* KOH–80% MeOH, mix and neutralize with 20 μl of 0.2 *N* HCl–80% MeOH and inject 10 μl of this sample solution. Every fragment gave the same retention time (about 2.5 min) and each sample could be analyzed within 15 min.

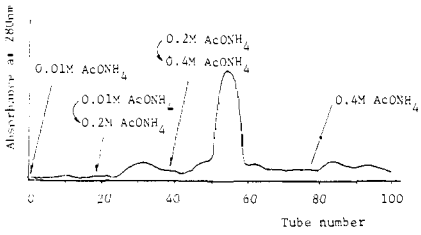


Figure 3. Carboxymethylcellulose chromatography of crude tetracosa peptide. Column: 1.0 cm i.d. × 55 cm length, 15 ml cut. The peptide was chromatographed in a gradient of $AcONH_4$ buffer at pH 6.7. The main peak in the gradient of 0.2 and 0.4 *M* $AcONH_4$ was rechromatographed in the same manner and desalted.

fragment and a 30-fold excess each of triphenylphosphine(Ph_3P), 2,2′-dipyridyl disulfide($(PyS)_2$) and 2-mercaptopyridine in CH_2Cl_2, 6 hr at −15° and overnight at room temperature in the presence of α-nitronaphthalene,[6] and general procedures[3] were followed.[7] Steps IV, V, and VI were carried out at room temperature in the absence of 2-mercaptopyridine since carboxyl ends as glycine and proline are free from racemization. During the coupling reactions, coupling rates were monitored by measuring the amount of fragments in solution by high pressure liquid chromatography with an aminated Hitachi Gel 3010.[8] Employing this technique, the remaining amino component in solution vs. time relation was plotted as shown in Figure 2.

The completion of the reaction was principally determined by noting the time at which the curve indicated no further consumption of the fragment. In addition coupling was repeated until no fragment uptake was detected and it was also confirmed by determination of the amino acid ratios of the resulting peptide resin.[9] The amino acid ratios of the resulting resin-tetracosapeptide after hydrolysis with propionic acid–12 *N* HCl (1:1 v/v)[10] were as follows: Ser, 1.92; Tyr, 2.34; Met, 1.08; Glu, 1.01; His, 0.98; Phe, 1; Arg, 2.87; Gly, 2.19; Lys, 3.71; Pro, 3.01; Val, 2.87. This result shows that fragment condensation proceeds efficiently with incomplete protection of side chains of amino acids by this new method.

The resin-tetracosapeptide was treated with HF[11] for 30 min at 0° in the presence of anisole and 2-mercaptopyridine.[12] The yield of this cleavage step was 83%. The peptide was converted into acetate form and chromatographed on carboxymethylcellulose[13] as shown in Figure 3 after gel filtration on Sephadex G-25 in 1 *N* AcOH. The pure ACTH(1–24) was obtained by precipitation from MeOH–AcOEt–petroleum ether and dried in vacuo over P_2O_5; 41 mg, 25% yield[14] from the initial resin-Ser(Bzl)-Tyr-OH and 43% yield from the liberated peptide from the resin: $[\alpha]^{20}_D$ −86.8° (*c* 0.5, 1% AcOH) (lit.[15] $[\alpha]^{22}_D$ −88.6 ± 2° (*c* 0.51, 1% AcOH)). Anal. Calcd for $C_{136}H_{210}O_{31}N_{40}S \cdot 7CH_3COOH \cdot 9H_2O$: C, 51.24; H, 7.33; N, 15.94; S, 0.91. Found: C, 51.19; H, 7.05; N, 15.93; S, 0.93. Amino acid ratios after complete enzymatic digestion[16] and acid hydrolysis[10] were in good agreement.[17]

The ACTH activity of this purified peptide[18] was assayed[19] by the ascorbic acid depletion test according to U.S.P. XVIII, and compared with the 2nd international standard it exhibited 96 (83–128) IU/mg which was identical activity with the literature.[15]

Thus, this new approach demonstrated that an oligopeptide as ACTH(1–24) is prepared in good yield by the simple purification procedure since the contamination of the similar peptides with small difference in sequence is eliminated.

The versatility of this approach, along with a combination of the advantages of the classical synthesis in solution, promises the new possibility of rapid preparation of pure peptides with long sequences and significantly expands the scope of the usual solid phase peptide synthesis.

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(4) T. Mukaiyama, M. Ueki, H. Maruyama, and R. Matsueda, *J. Am. Chem. Soc.*, **90**, 4490 (1968); M. Ueki, H. Maruyama, and T. Mukaiyama, *Bull. Chem. Soc. Jpn.*, **44**, 1108 (1971); T. Mukaiyama, H. Maruyama, and K. Inoue, Japan Patent, 673069. In a typical experiment, a five-fold excess each of triphenylphosphine and pentachlorophenol was added to 0.1 mmol of the Nps-protected fragment in 20 ml of MeOH and stirred at room temperature for 1 hr. The pure fragment with the free amino function was quantitatively obtained by gel filtration on Sephadex LH-20 in MeOH and drying in vacuo after evaporation of solvent.

(5) Prepared according to the method described in the literature.[3] Deprotection of the *tert*-butyl group was carried out with F_3CCOOH–CH_2Cl_2(1:1 v/v) containing 5% 2-mercaptoethanol.

(6) Internal standard for monitoring.

(7) Excess amounts of fragments in washing solvents were easily purified by gel filtration on Sephadex LH-20 in MeOH (the amounts of recovered fragments were 80–90% of theoretical value). A successive coupling of 1 g of new resin–dipeptide with the recovered fragments gave resin–tetracosapeptide with nearly the same amino acid ratios.

(8) We thank Dr. N. Takai of the Institute of Industrial Science, University of Tokyo, for providing us with aminated Hitachi Gel.

(9) The amount of the consumed fragment as shown from Figure 2 was generally smaller than the actual amount consumed in chain elongation since the initial peak height after preshaking the resin with the fragment was arbitrarily scaled as 3 mequiv neglecting the small amount of adsorbed fragment on the resin. This small amount of the adsorbed fragment incorporated into the peptide chain makes up the difference in the quantity consumed as shown in the graph and the required value of 1 mequiv. Amino acid analyses of the resulting peptide resin after washing out the residual fragment adsorbed on the resin with trifluroacetic acid–CH_2Cl_2 (1:1) proved the quantitative coupling yield in each step. After these analytical procedures the peptide resin was covered by the reaction with 10 equiv of diethylamine and 30 equiv each of Ph_3P and $(PyS)_2$ in CH_2Cl_2 at room temperature for 6 hr in each step to terminate any unreacted chain, if it is present at all.

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(14) The present yield seems to be superior to 8% yield in the stepwise synthesis of ACTH(1–19)[13] and this approach promises to be an economical method since excess fragments are recovered unchanged and can be reused.

(15) R. Schwyzer and H. Kappeler, *Helv. Chim. Acta*, **46**, 1150 (1963).
(16) Ap-M (Rohm & Haas) and Prolidase (Miles Laboratories) digestion after digestion with trypsin and chymotrypsin (Worthington Biochemical Co.) in Tris buffer, pH 8.5 at 37°.[13]
(17) Enzymatic digestion; Ser, 1.78; Tyr 1.98; Met, 1.02; Glu, 1.15; His, 0.98; Phe, 1.10; Arg, 3.41; Trp, 1; Gly, 1.98; Lys, 4.51; Pro, 2.94; Val, 3.26 and acid hydrolysis; Ser, 1.91; Tyr, 2.07; Met, 0.96; Glu, 1; His, 0.87; Phe, 1.07; Arg, 3.32; Gly, 2.18; Lys, 4.39; Pro, 3.28; Val, 3.14. This result shows that no detectable racemization has occurred during fragment condensation by the oxidation–reduction process.
(18) Thin-layer chromatography gave a single spot detected by Ehlrich, Pauly, Cl–tolidine, and ninhydrin reactions and uv absorption in 0.1 *N* NaOH was in good agreement with the literature.[15] High voltage electrophoresis gave a single spot in 0.5 *N* HCOOH–2*N* AcOH (1:1) (R_m, 0.72) and pyridine–AcOH–H_2O (10:0.4:90) (0.77) with lysine as reference.
(19) We were indebted to Dr. J. Serizawa and Mr. T. Yoshizaki of our laboratories.

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Mechanism of Anion Exchange of Alkyl Mercurials

Sir:

Anion exchange reactions of methylmercury halides and pseudo halides exhibit second-order kinetic behavior. NMR experiments have established that exchange in the magnetic environment of the methyl group in CH_3HgX-CH_3HgY mixtures involves transfer of the ligand (X and Y) on mercury. Cleavage of the covalent carbon–mercury bond has been excluded by the observation of ^2J ^{199}Hg-^1H coupling under anion exchange conditions.[1,2] Five mechanisms for this rapid anion or potential anion exchange may be considered (eq 1–5).[3-5]

$$RHgX + Y^- \rightleftharpoons RHg^{-}\begin{matrix}X\\Y\end{matrix} \rightleftharpoons RHgY + X^- \quad (1)$$

$$CH_3\overset{*}{H}g^+ + CH_3HgY \rightleftharpoons CH_3\overset{*}{H}gY + CH_3Hg^+ \quad (2)$$

$$CH_3\overset{*}{H}g^+\|X^- + CH_3HgY \rightleftharpoons CH_3\overset{*}{H}gY + CH_3Hg^+\|X^- \quad (3)$$

$$CH_3\overset{*}{H}g^+\|X^- + CH_3Hg^+\|Y^- \rightleftharpoons CH_3\overset{*}{H}g^+\|Y^- + CH_3Hg^+\|X^- \quad (4)$$

$$CH_3\overset{*}{H}gX + CH_3HgY \rightleftharpoons \left[CH_3\overset{*}{H}g\begin{matrix}X\\Y\end{matrix}HgCH_3\right] \rightleftharpoons CH_3\overset{*}{H}gY + CH_3HgX \quad (5)$$

1

Kinetic evidence[1] based upon an NMR study in aqueous medium of the exchange reaction of CH_3HgCN and OH^- supports a bimolecular anionic mechanism at pH greater than 10.3 (eq 1). A second mechanism was operating at a pH of 9 or below. By the process of eliminating other mechanisms, the exchange was attributed to a "direct" exchange as suggested by eq 5. An exchange process involving methylmercuric ion (eq 2) was ruled unlikely. A mechanism involving the bridged intermediate (**1**) was also preferred for CH_3HgCN-CH_3HgX (X = Cl, Br, I) anion exchange in DMF solvent.[2] In the latter study, pathways involving solvent-separated ions pairs (eq 4) were also considered but not rigorously excluded. One of the basic difficulties in excluding an ionic mechanism in these exchange reactions is the possibility of ionization of RHgX and a rapid diffusion controlled anion exchange involving ions (eq 1 and 2) or reactive solvent-separated ion pairs (eq 3 and 4).[3]

Table I. Activation Parameters for the Exchange of CH_3HgCN with CH_3HgX in DMF

Compound	X	$\Delta H^{\ddagger}$,[a,b]	$\Delta S^{\ddagger}$, eu	$\Delta G^{\ddagger}_{298°K}$[a,b]	$k_{2_{298°K}}$[c]
2a	Cl	11.6 ± .2	−18 ± 1	17.1	1.5
2b	OAc	12.1 ± .3	−16 ± 1	16.9	2.2
2c	Br	11.3 ± .2	−17 ± 1	16.3	5.8
2d	SCN	16.1 ± .3	−1 ± 1	16.3	6.1
2e	SCH_3	11.2 ± .2	−13 ± 1	15.2	38.
2f	SC_6H_5	13.0 ± .2	−7 ± 1	15.2	40.
2g	S-*p*-C_6H_5Cl	11.7 ± .2	−11 ± 1	15.1	45.
2h	$SC(CH_3)_3$	11.8 ± .3	−9 ± 1	14.6	98

[a] $\Delta H^{\ddagger}$ and $\Delta G^{\ddagger}$ are given in kilocalories per mole. [b] The error limits given are those derived from least-squares analysis. [c] Second-order rate constants $k(M^{-1}\ sec^{-1})$ were not measured at 298°K but were obtained by extrapolation of a least-squares plot of data obtained at least eight other temperatures. Correlation coefficients of >0.9977 were obtained for each plot. The rate constants were calculated assuming a bimolecular mechanism where rate = $(k_2/2)[CH_3HgX][CH_3HgCN]$ and the observed rate of exchange $1/\tau_{CH_3HgX} = (k_2/2)[CH_3HgCN]$.

We now report a series of NMR experiments involving CH_3HgCN-CH_3HgX anion exchange in DMF solvent. The principal objective of this study was to establish the effect of the ionic character of CH_3HgX on its anion exchange rate with CH_3HgCN. By keeping the CH_3HgCN common to each exchange system, the variation in $\Delta G^{\ddagger}$ should be attributable largely to an enthalpy change if an ionic mechanism obtains or if extensive Hg–X bond breaking is involved in the rate limiting step. However, our data establish that the trend observed for the rates of anion exchange is the opposite to that which would be anticipated on the basis of an ionic pathway involving ionization of CH_3HgX. We also report the first unequivocal evidence excluding an ionic process in the mercaptide anion exchange of RHgSR compounds. These data are consistent with a bridged intermediate or transition state such as **1** (eq 5).

The thermodynamic parameters for exchange, which are summarized in Table I, were obtained by the complete line shape analysis NMR method.[6] Our results are consistent with earlier studies that established that the C–Hg bond was not labile under exchange conditions.[1,2,7] NMR experiments with the CH_3HgCN-$CH_3HgSC_6H_5$ system at three different temperatures each at four different concentrations in DMF established that exchange proceeded by a second-order pathway.

The measured[8] association constants for the formation of CH_3HgX from CH_3Hg^+ and the anions chosen for this study vary over 13 orders of magnitude.[9] The complete ionization of the Hg–X bond has been shown to increase in the order $RS^- < CN^- < Br^- < SCN^- < Cl^- < OAc^-$.[9] The relatively fast rate of exchange for the highly covalent methylmercury mercaptides (**1e–h**) strongly argues against any of the above ionic mechanisms (eq 1–4) being involved in mercaptide anion exchange. The magnitude of the exchange rates we have measured completely exclude all exchange processes involving two solvent separated ion pairs (eq 4). The rate expression, rate = $k_2K_1K_3$·$[CH_3HgX][CH_3HgCN]$, consistent with this mechanism contains equilibrium constants (K_1 and K_3) for the dissociation of both CH_3HgX and CH_3HgCN. If the equilibrium constants (K_1) for the formation of solvent separated