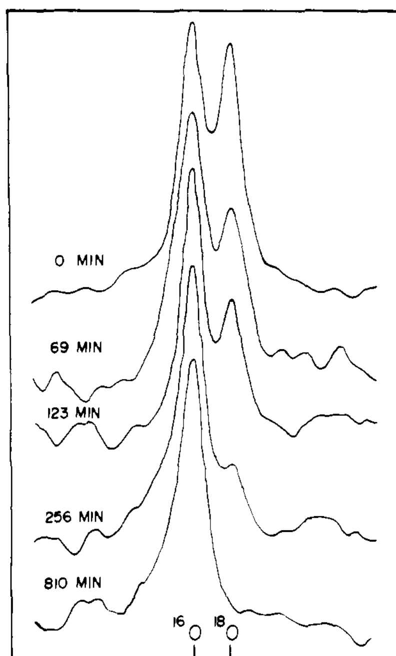

Figure 2. The acid-catalyzed exchange of the hydroxyl oxygen of *tert*-butyl alcohol as measured by ^{13}C isotope shift NMR. ^{18}O-Labeled *tert*-butyl alcohol was dissolved in 0.16 N HCl solution made up using normal $[^{16}O]$water. The loss of ^{18}O from the C-1 position of the alcohol was measured as a function of time. By 810 min no further change was measureable. The ^{13}C spectra were recorded on a Varian CFT-20 instrument at 65 ± 2 °C.

time, 45° pulse angle, and 8K data block were used to record the spectra in the Fourier transform mode. The exponential weighting for the free induction decay was 1.0 Hz and the resolution was 0.50 Hz. The shift separation was verified on Nicolet 150-MHz and Nicolet 360-MHz NMR instruments. Figure 1 illustrates the ^{18}O-isotope effect on the hydroxyl carbon resonance position. It shows ^{18}O-labeled *tert*-butyl alcohol diluted with known amounts of $[^{16}O]$-*tert*-butyl alcohol. The upfield shift is 0.035 ppm in water. The separation of the ^{13}C resonance positions was 0.7 Hz at 20 MHz, 1.32 Hz at 37.7 MHz, and 3.17 Hz at 90 MHz.

The utility of the ^{18}O-isotope shift effect on carbon was demonstrated by following the kinetics of the loss of label from a sample of $[^{18}O]$-*tert*-butyl alcohol. The reaction mixture for the exchange experiment contained 0.16 N HCl and 1.88 M $[^{18}O]$-*tert*-butyl alcohol in 3.3-mL total volume. The variable-temperature probe on the CFT-20 was equilibrated at 65 ± 2 °C for 1 h, while $[^{18}O]$-*tert*-butyl alcohol and glass-distilled water made acidic with HCl were equilibrated separately in a water bath at 65 °C for 30 min. The reaction was begun by addition of the $[^{18}O]$-*tert*-butyl alcohol to the acid solution. Figure 2 shows the ^{13}C NMR spectra of the hydroxyl carbon at five times during the course of the reaction. The disappearance of the ^{18}O-labeled signal is readily detectable and quantitative changes are easily measured.

^{13}C NMR spectra such as those shown in Figure 2 were resolved using a Du Pont 310 curve resolver set for Lorentzian curves. A kinetic plot of quantitative data for the exchange experiment gave a pseudo-first-order rate constant of 5.18×10^{-5} s^{-1}, in excellent agreement with the literature value[13] of 5.33×10^{-5} s^{-1} obtained by conventional mass spectrometric methods.

Thus, we have demonstrated the ^{18}O-isotope shift upon ^{13}C NMR spectra and provided an example of its use. The technique should be widely applicable. The present study employed natural abundance spectra and a Fourier transform instrument operating at 20 MHz for ^{13}C, so that instruments of very high magnetic field strength are not necessary for kinetic studies providing that curve resolution or electronic integration are employed. However, since the quantitative data obtained from the resolved spectra closely approximated those obtainable by peak-height measurements, it seems probable that the greater separation available with instruments of higher field strength should permit even more convenient quantitation. Further gains in sensitivity (thus providing in effect the possibility of following more rapid exchange reactions) can be achieved by measurements with ^{13}C-enriched compounds. Typical among the uses to which these procedures should be applicable may be mentioned the measurement of oxygen exchange accompanying both enzymatic and nonenzyme-catalyzed reactions of carbonyl and carboxyl group derivatives.

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Synthesis of Thymosin α_1

Sir:

Thymosin α_1, an acetyl octacosapeptide, isolated from calf thymus gland by Goldstein et al.[1] was reported to exhibit biological activities that are important for the development of thymus-dependent lymphocytes (T cells) in man and in animals.[2] Its amino acid sequence was determined to be[3] Ac-Ser-Asp-Ala-Ala-Val-Asp-Thr-Ser-Ser-Glu-Ile-Thr-Thr-Lys-Asp-Leu-Lys-Glu-Lys-Lys-Glu-Val-Val-Glu-Glu-Ala-Glu-Asn. In this communication, a solution synthesis[4] of thymosin α_1 is described. The synthetic product was found to be identical with the natural material, confirming the proposed structure of the polypeptide hormone.

As outlined in Scheme I, Boc-Ile-Thr(Bzl)-Thr(Bzl)-Lys(Z)-OH (**3**) was treated with 4 N HCl in THF to remove the Boc group and the ensuing tetrapeptide hydrochloride salt was coupled with Boc-Val-Asp(OBzl)-Thr(Bzl)-Ser(Bzl)-Ser(Bzl)-Glu(OBzl)-HNNH$_2$ (**2**) by the azide method[5] to give the protected decapeptide Boc-Val-Asp(OBzl)-Thr(Bzl)-Ser(Bzl)-Ser(Bzl)-Glu(OBzl)-Ile-Thr(Bzl)-Thr(Bzl)-Lys-(Z)-OH (**7**); 76.2%; mp 268–271 °C. Anal. $C_{106}H_{133}N_{11}O_{24}$. C, H, N. Amino Acid Anal. Asp$_{1.05}$, Ser$_{1.88}$, Glu$_{1.09}$, Thr$_{2.93}$, Val$_{0.98}$, Ile$_{1.01}$, Lys$_{1.06}$. Azide coupling between Ac-Ser(Bzl)-Asp(OBzl)-Ala-Ala-HNNH$_2$ (**1**) and **7** after removal of Boc group from **7** with TFA provided the protected N-terminal acetyl tetradecapeptide Ac-Ser(Bzl)-Asp(OBzl)-Ala-Ala-Val-Asp(OBzl)-Thr(Bzl)-Ser(Bzl)-Ser(Bzl)-Glu(OBzl)-

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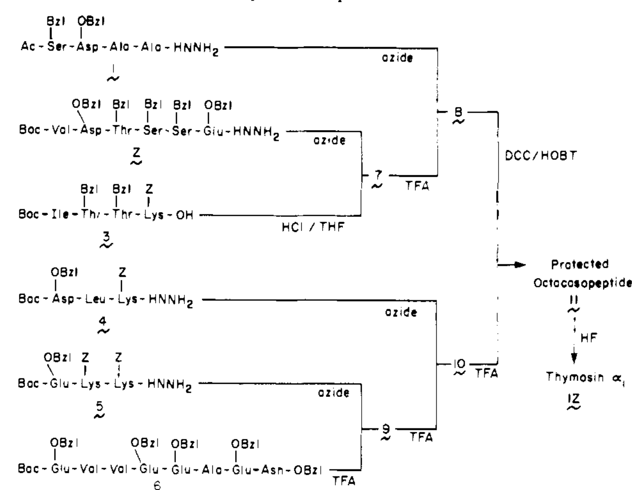
Scheme I. Synthesis of Thymosin α_1



Ile-Thr(Bzl)-Thr(Bzl)-Lys(Z)-OH (**8**): 89.4%; mp 297-298 °C; $[\alpha]^{25}_D$ +6.37° (*c* 1, Me_2SO). Anal. $C_{130}H_{159}N_{15}O_{30}$, C, H, N. Amino Acid Anal. $Asp_{1.97}$, $Ser_{2.96}$, $Glu_{1.08}$, $Ala_{1.85}$, $Val_{0.90}$, $Ile_{0.97}$, $Lys_{1.05}$, $Thr_{2.98}$.

Deblocking of Boc group with TFA from Boc-Glu(OBzl)-Val-Val-Glu(OBzl)-Glu(OBzl)-Ala-Glu(OBzl)-Asn-OBzl (**6**) provided the corresponding TFA octapeptide salt which was then condensed with Boc-Glu(OBzl)-Lys(Z)-Lys(Z)-$HNNH_2$ (**5**) via azide procedure to afford the protected undecapeptide Boc-Glu(OBzl)-Lys(Z)-Lys(Z)-Glu(OBzl)-Val-Val-Glu(OBzl)-Glu(OBzl)-Ala-Glu(OBzl)-Asn-OBzl (**9**) in good yield: 91.6%; mp 312-314 °C; $[\alpha]^{25}_D$ −13.68° (*c* 1, Me_2SO). Anal. $C_{117}H_{146}N_{14}O_{29}$, C, H, N. Amino Acid Anal. $Asp_{1.00}$, $Glu_{5.28}$, $Ala_{1.03}$, $Val_{1.78}$, $Lys_{1.90}$. Treatment of **9** with TFA and coupling of the resultant undecapeptide TFA salt with the tripeptide azide derived from Boc-Asp(OBzl)-Leu-Lys(Z)-$HNNH_2$ (**4**) yielded the protected C-terminal tetradecapeptide Boc-Asp(OBzl)-Leu-Lys(Z)-Glu(OBzl)-Lys(Z)-Lys(Z)-Glu(OBzl)-Val-Val-Glu(OBzl)-Glu(OBzl)-Ala-Glu(OBzl)-Asn-OBzl (**10**): 87.4%; mp 326-327 °C; $[\alpha]^{25}_D$ −15.71° (*c* 1, Me_2SO). Anal. $C_{148}H_{186}N_{18}O_{36}$, C, H, N. Amino Acid Anal. $Asp_{2.00}$, $Glu_{5.10}$, $Ala_{1.00}$, $Val_{1.93}$, $Leu_{1.03}$, $Lys_{2.89}$.

The final coupling of N-terminal tetradecapeptide **8** and C-terminal tetradecapeptide **10** was achieved by the DCC-HOBT procedure.[6] Thus **10** was treated with TFA for removal of Boc group from the α-amino function and the tetradecapeptide TFA salt (69.35 g) obtained was allowed to react with the acetyl tetradecapeptide active ester derived from the reaction of 8 (59.27 g) with DCC and HOBT in a solvent mixture of DMF and Me_2SO. The fully protected acetyl octacosapeptide Ac-Ser(Bzl)-Asp(OBzl)-Ala-Ala-Val-Asp(OBzl)-Thr(Bzl)-Ser(Bzl)-Ser(Bzl)-Glu(OBzl)-Ile-Thr(Bzl)-Thr-(Bzl)-Lys(Z)-Asp(OBzl)-Leu-Lys(Z)-Glu(OBzl)-Lys(Z)-Lys(Z)-Glu(OBzl)-Val-Val-Glu(OBzl)-Glu(OBzl)-Ala-Glu(OBzl)-Asn-OBzl (**11**) was obtained in satisfactory yield: 96.7 g (77.3%); mp 330 °C dec. Anal. $C_{273}H_{335}N_{33}O_{63}$, C, H, N. Amino Acid Anal. $Asp_{4.03}$, $Thr_{2.84}$, $Ser_{2.87}$, $Glu_{6.12}$, $Ala_{3.00}$, $Val_{2.89}$, $Ile_{0.98}$, $Leu_{0.97}$, $Lys_{4.02}$.

The fully protected thymosin α_1 (**11**) was then treated with anhydrous HF in the presence of anisole[7] to remove all the protecting groups. Typically, 10 g of **11** was mixed with 15 mL of anisole and stirred with 100 mL of HF at 0 °C for 30 min. The crude material obtained was then purified on a DEAE-Sephadex A-25 column (0.05 M Tris-HCl buffer, pH 8.0, linear gradient of NaCl 0-0.35 M) followed by gel filtration on a Sephadex G-10 column to give thymosin α_1 (**12**) as a white amorphous powder: 0.56 g (9.2%).[8] Amino Acid Anal. $Asp_{4.08}$, $Thr_{2.90}$, $Ser_{3.05}$, $Glu_{5.97}$, $Ala_{3.00}$, $Val_{3.04}$, $Ile_{0.98}$, $Leu_{1.00}$, $Lys_{4.02}$ (average recovery, 95%). The product migrated as a single spot[9] on acrylamide gel isoelectric focusing (pH 3.5-9.5) and on high voltage silica gel thin-layer electrophoresis (pH 1.9 and pH 5.6), indistinguishable from the natural thymosin α_1. The tryptic peptide maps[10] from synthetic and natural thymosin α_1 were also identical. Synthetic **12** shows activities equivalent to the natural compound in the MIF (macrophage migration inhibitory factor),[1] E-Rosette,[1] and other assays.

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Supplementary Material Available: Elemental analyses of compounds **1-11** (2 pages). Ordering information is given on any current masthead page.

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(3) Abbreviations used followed the recommendations of IUPAG-IUB Commision on Biochemical Nomenclature, *J. Biol. Chem.* **1972**, *247*, 977–983. Other abbreviations used follow: Ac, acetyl; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; HOBT, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid; THF, tetrahydrofuran; Tris, tris(hydroxymethyl)aminomethane; Z, benzyloxycarbonyl.

(4) See for example, Finn, F. M.; Hofmann, K. "The Proteins". Third ed., Neurath, H., Hill, R. L., and Boeder, C. L., Ed.; Academic Press: New York, N. Y., 1976; Vol. II.

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(8) The low yield in this step is probably due to side reactions occurring during HF treatment (Cf. the following. Tzougraki C., et al. *J. Am. Chem. Soc.* **1978**, *100*, 6248–6249. Atherton, E., et al. *J. Chem. Soc., Chem. Commun.* **1977**, 819–821. Yamashiro, D.; Li, C. H. *J. Am. Chem. Soc.* **1978**, *100*, 5174–5179. Feinberg, F. S.; Merrifield, R. B. *ibid.* **1975**, *97*, 3485–3496. Sano, S.; Kawanishi, S. *ibid.* **1975**, *97*, 3480–3484.) combined with the loss of material during the chromatographic purification procedures. Other deprotection processes such as hydrogenolysis (Pd catalyst at hydrogen pressures as high as 2500 psi), sodium–liquid ammonia reduction, and acidolysis with methanesulfonic acid, trifluoromethanesulfonic acid, and hydrobromic acid in acetic acid were investigated. None of these experiments gave better results, presumably owing to the extreme insolubility of the fully protected thymosin α_1 (**11**) in the solvents commonly used in peptide chemistry. For discussions on the general problems associated with deblocking protecting groups from fully protected larger polypeptides, see, for example, Finn, F. M.; Hofmann, K. ref 4, pp 105–253.

(9) The mixture of natural and synthetic thymosin α_1 migrated as a single spot in all the systems tested.

(10) To obtain the tryptic peptide map, thymosin α_1 was digested with TPCK-trypsin (100:1 ratio, pH 9.0 sodium borate buffer, 25 °C, 15 h), acidified to pH 2, and spotted on a silica gel TLC plate (Merck F-254). Electrophoresis (pH 1.9, 3000 V, 35 min) was carried out for the first dimension and thin-layer chromatography in *n*-BuOH-EtOAc-HOAc-H_2O (1:1:1:1) was carried out for the second dimension. The color was developed with ninhydrin or with chlorine-tolidine reagent.

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Synthesis and Chemistry of *cis*- and *trans*-2,3-Divinylthiirane

Sir:

In recent years, there has been a considerable interest in the thermal rearrangements of divinylcyclopropanes and their heteroanalogues. Next to *cis*-1,2-divinylcyclopropane,[1] the Cope rearrangement of *cis*-2,3-divinyloxirane[2] and *cis*-2,3-divinylaziridine[3] (eq 1) has been investigated in detail. Simi-

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