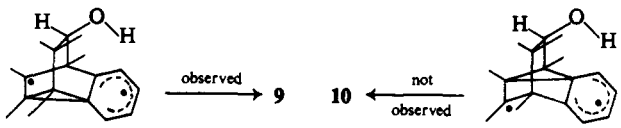
(4 H).[8] Reduction of ketone **8** with lithium aluminum hydride provided only the *anti* alcohol **6**.

In contrast with the anti alcohol **3**, *similar irradiation of the* syn *alcohol* **4**[4] *provided only a single alcohol*, **9**.[9] Alcohol **9**, mp 72–73°, shows in the infrared[7] a band at 3580 cm^{-1}, and its nmr spectrum contains three-proton singlets at τ 10.00, 9.02, 8.93, 8.83, 8.67, and 8.62, a broad one-proton signal, τ 6.53–6.73, and an aromatic multiplet, τ 2.88–3.13 (4 H). Oxidation of **9** with CrO_3–pyridine provided ketone **7**, while reduction of **7** with lithium aluminum hydride yields only the *syn* alcohol **9**.

Thus, whereas the acetone-sensitized irradiation of the *anti* alcohol **3** gave both possible isomers **5** and **6**, identical irradiation of the *syn* alcohol **4** gave a single product, **9**. No **10** was formed. The regiospecificity[10] in the latter rearrangement indicates a strong preference for intermediate **11** *vis-à-vis* **12**. No such preference is observed when the hydroxyl group is in the *anti* position. Possible factors which may contribute to the preference of **11** over **12** may be hydrogen bonding or charge-transfer interaction with the oxygen. Further studies to determine the nature of the interaction which controls the course of this photorearrangement are in progress.



11 **12**

Acknowledgment. Support of this research by a grant from the National Science Foundation is gratefully acknowledged.

(8) This ketone was identical with the product of acetone-sensitized irradiation of 1,3,3,4,7,8-hexamethyl-5,6-benzobicyclo[2.2.2]octa-5,7-dien-2-one: H. Hart and R. K. Murray, Jr., *Tetrahedron Letters*, in press.

(9) Irradiation to only 18% conversion of **4** gave a 97% yield of **9**; at 80% conversion, the yield of **9** was 68%.

(10) A. Hassner, *J. Org. Chem.*, **33**, 2684 (1968).

(11) National Institutes of Health Predoctoral Fellow at Michigan State University, 1967–1968.

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Studies on Polypeptides. XLII. Synthesis and Characterization of Seven Fragments Spanning the Entire Sequence of Ribonuclease T_1[1-3]

Sir:

For some time this laboratory has been engaged in studies aimed at a total synthesis of the proposed amino acid sequence of the enzyme ribonuclease T_1.[4] Reports[5,6] on the synthesis of materials possessing some ribonuclease A activity prompt us to disclose at this time the status of our investigations.

We have completed the synthesis and careful characterization of seven protected fragments spanning the entire sequence of the enzyme.

Ribonuclease T_1 contains only one residue each of lysine and arginine and no methionine; moreover, three of the four half-cystines are located in the N-terminal region. The presence in the enzyme of 12 glycine residues allows subdivision of the chain into a number of fragments of convenient size which C-terminate in glycine. It was for these reasons that we selected T_1 for synthetic studies.

Our plan of synthesis (Figure 1) involves construction of six fragments (A to F), each terminating with a protected hydrazide, and a C-terminal tetracosapeptide amide (G) followed by assembly of these component parts into the complete sequence by azide coupling steps. This approach is patterned according to a scheme proposed in 1952[7] except that *t*-butoxycarbonyl[8,9] rather than benzyloxycarbonylhydrazides were employed; the benzyloxycarbonylhydrazide of tyrosine was used in the construction of fragment A.

Preparation of fragments B–E involved conversion of the C-terminal benzyloxycarbonyl di- or tripeptides into the corresponding *t*-butoxycarbonylhydrazides, removal of the benzyloxycarbonyl function by hydrogenolysis, followed by stepwise elongation of the chains[10] with the desired benzyloxycarbonylamino acid N-hydroxysuccinimide[11] 2,4,5-trichlorophenyl[12] or *p*-nitrophenyl[13] esters. Benzyloxycarbonyl dipeptide azides were also used in some instances. The aspartic and glutamic acid side chains were protected with *t*-butyl esters.[14] The α-amino group of alanine-1 and the ϵ-amino group of lysine-41 were protected by formyl groups since it appears[15] that deamination of these residues does not destroy the catalytic activity of the enzyme. Similarly, since the C-terminal threonine residue can be removed with carboxypeptidase without loss of enzyme activity,[16] it was replaced by threonine amide. Fragment F was prepared by azide coupling of two protected peptides corresponding to positions 66–74 and 75–80, respectively. Three fragments corresponding to positions 95–104, 89–94, and 81–88 served to construct fragment G.

The presence of three cysteine residues in fragment A necessitated a different approach from that employed

(1) See R. Andreatta and K. Hofmann, *J. Amer. Chem. Soc.*, **90**, 7334 (1968), for paper XLI in this series.

(2) Supported by the U. S. Public Health Service and the Research Laboratories Edgewood Arsenal, Contract DA-18-035-AMC-307 (A). The opinions expressed in this communication are those of the authors and do not reflect endorsement by the contractor.

(3) The amino acids except glycine are of the L configuration. DMSO, dimethyl sulfoxide; DMF, dimethylformamide; TEA, triethylamine; TFA trifluoroacetic acid; O-*t*-Bu, *t*-butyl ester; EC ethylcarbamyl; F, formyl; TCP, 2,4,5-trichlorophenyl; X, *t*-butoxycarbonylhydrazide; Y, benzyloxycarbonylhydrazide; Z, benzyloxycarbonyl; AP-M, aminopeptidase M. See ref 1 for solvent systems used for tlc chromatograms.

(4) K. Takahashi, *J. Biol. Chem.*, **240**, PC4117 (1965).

(5) B. Gutte and R. B. Merrifield, *J. Amer. Chem. Soc.*, **91**, 501 (1969).

(6) R. Hirschmann, R. F. Nutt, D. F. Veber, R. A. Vitali, S. L. Varga, T. A. Jacob, F. W. Holly, and R. G. Denkewalter, *ibid.*, **91**, 507 (1969).

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(16) K. Takahashi, *ibid.*, **52**, 72 (1962).

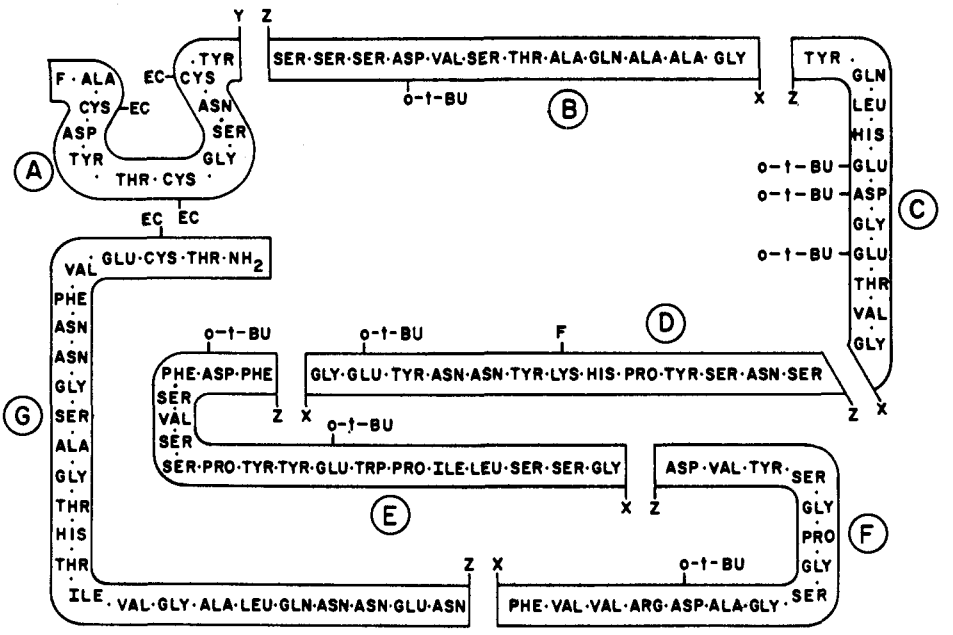

Figure 1.

for construction of the other fragments. Starting with the benzyloxycarbonylhydrazide of tyrosine the chain was elongated with TCP esters of the desired *t*-butoxycarbonylamino acids and terminated with the TCP ester of formylalanine.

Guttmann[17] introduced the ethylcarbamyl group for cysteine protection in peptide synthesis. He found this group stable to acid but labile to dilute sodium hydroxide, ammonium hydroxide, and liquid ammonia in methanol. We selected the ethylcarbamyl group for thiol protection since we have found that this group is readily cleaved by mercuric acetate or *p*-chloromeuribenzoate.

Intermediates in the synthesis of these subunits were shown to be homogeneous by elemental analysis, amino acid analysis of acid hydrolysates and AP-M digests, and chromatography in several solvent systems.

The following data serve to characterize the seven fragments.[18]

Fragment A (positions 1–11): mp 208–213°; $[\alpha]^{28}D$ −51.5° (*c* 1.12, DMF). *Anal.* Found: C, 48.9; H, 5.8; N, 15.0; S, 6.4. Amino acid ratios in 24-hr acid hydrolysate of HBr–TFA deblocked hydrazide: $Ala_{1.26}Asp_{2.30}Tyr_{1.70}Thr_{0.81}Ser_{0.90}Gly_{1.03}Cys_{2.87}$.

Fragment B (positions 12–23): mp 295–297° dec; $[\alpha]^{26}D$ −13.2° (*c* 0.92, DMSO); R_f^{I} 0.5; R_f^{III} 0.8. *Anal.* Found: C, 50.2; H, 7.0; N, 15.0; O, 28.0. Amino acid ratios in 24-hr acid hydrolysate: $Ser_{3.64}Asp_{1.01}Val_{1.00}Thr_{0.95}Ala_{3.00}Glu_{1.01}Gly_{0.99}$.

Fragment C (positions 24–34): mp 218–220° dec; $[\alpha]^{27}D$ −23.6° (*c* 1.72, DMF); R_f^{I} 0.7; R_f^{III} 0.8. *Anal.* Found: C, 56.5; H, 7.3; N, 13.4. Amino acid ratios in 24-hr acid hydrolysate of deblocked hydrazide: $Tyr_{0.92}Leu_{0.98}His_{1.00}Glu_{3.30}Asp_{0.98}Gly_{1.95}Thr_{0.90}Val_{0.97}$.

Fragment D (positions 35–47): mp 201–202° dec; $[\alpha]^{28}D$ −25.6° (*c* 1.63, DMF); R_f^{I} 0.5; R_f^{III} 0.8. *Anal.* Found: C, 54.9; H, 6.2; N, 15.1; O, 23.5. Amino acid ratios in 24-hr acid hydrolysate of decarbobenzoxylated *t*-butoxycarbonylhydrazide: $Ser_{1.87}Asp_{3.07}Tyr_{2.51}Pro_{1.09}His_{0.84}Lys_{0.99}Glu_{1.00}Gly_{0.99}$.

Fragment E (positions 48–65): mp 193–194° dec; $[\alpha]^{28}D$ −32.0° (*c* 2.78, DMF); R_f^{I} 0.9; R_f^{III} 0.9. *Anal.* Found: C, 59.3; H, 7.0; N, 11.9; O, 21.8. Amino acid ratios in 48-hr acid hydrolysate of deblocked peptide hydrazide: $Phe_{2.37}Asp_{1.15}Ser_{4.75}Val_{1.05}Pro_{1.99}Tyr_{1.73}Glu_{0.99}Ile_{0.97}Leu_{0.95}Gly_{1.04}$.

Fragment F (positions 66–80): mp 218–220° dec; $[\alpha]^{27}D$ −28.3° (*c* 0.95, DMSO); R_f^{I} 0.5; R_f^{III} 0.8. *Anal.* Found (for the acetate): C, 54.4; H, 6.9; N, 14.5. Amino acid ratios in 48-hr acid hydrolysate: $Asp_{2.10}Val_{2.99}Tyr_{0.70}Ser_{1.69}Gly_{3.06}Pro_{1.14}Ala_{1.07}Arg_{0.97}Phe_{0.98}$.

Fragment G (positions 81–104): amino acid ratios in 72-hr acid hydrolysate: $Asp_{5.25}Glu_{3.22}Leu_{1.03}Ala_{2.17}Gly_{3.06}Val_{1.99}Ile_{1.03}Thr_{2.67}His_{0.89}Ser_{0.82}Phe_{0.89}$.

The synthesis of three peptides corresponding to positions 1–11, 12–16, and 24–30, respectively, of ribonuclease T_1 has been reported by Izumiya, *et al.*[19]

Acknowledgment. The skillful technical assistance of Miss Judy Montibeller and Mrs. Elaine Gleeson is gratefully acknowledged.

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