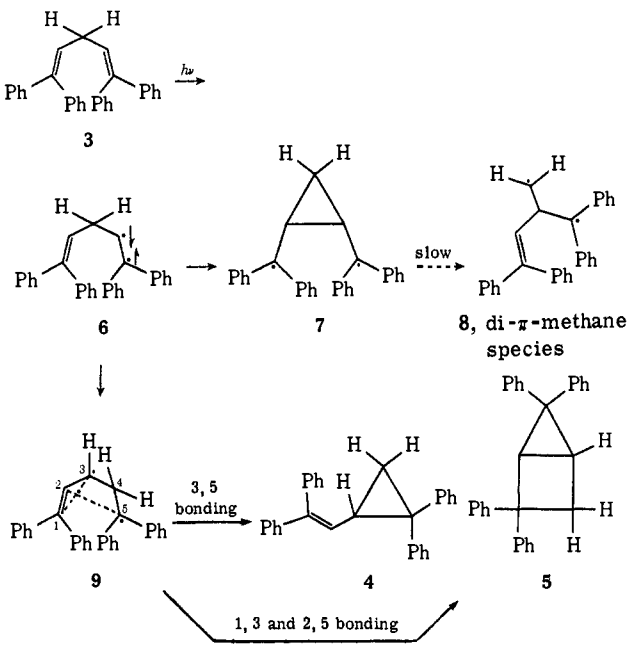
That the situation was more complex became apparent from irradiation of the dideuterio derivative **3b**[5] of the tetraphenyldiene **3a**. A simple di-π-methane mechanism of **3b** would afford vinylcyclopropane product **4** with deuteriums at C-2 and C-1′ and the two aliphatic hydrogens at C-3. Instead, nmr analysis indicated minimally the product consisted of 90% **4b** with single hydrogens at C-2 and C-3, a product not expected from the di-π-methane rearrangement of **3**. This means that, to the extent that an undetected di-π-methane process occurs at all, its efficiency of formation from the excited singlet must be even lower than that for **4** and is estimated as $\phi \leq 0.0002$ corresponding to $k_r \leq 1.2 \times 10^9$ sec^{-1}.

Thus, the central substitution of the dimethyltetraphenyldiene **1** seems essential for an efficient di-π-methane rearrangement.

It is seen that vinylcyclopropane **4b** arises from 1,2-excited state sigmatropic rearrangement of a central hydrogen atom as depicted in Chart I. The normal

Chart I. Mechanisms of Rearrangement of the Tetraphenyldiene **3**



di-π-methane route involves putting odd electron density on a primary carbon (note species **8**) and is inhibited,[11] accounting for its low rate. The inhibition of the usually facile di-π-methane rearrangement by diminished central substitution suggests that ring opening of species **7** plays a role in determining the excited state rate.[12] The reaction which does occur is the 1,2-hydrogen sigmatropic shift[13] leading to species **9**. 3,5-Bonding then leads directly to vinylcyclopropane product **4** while a unique 2,5- plus 1,3-bonding process affords housane **5**.[14] Overall this is $2_\sigma + 2_\pi + 2_\pi$ and could be concerted. Hence, one mechanism accounts for both unexpected products.

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(11) Di-π-methane systems involving phenyl migrations seem able to overcome this inhibition because of regeneration of aromaticity in this step. For examples note: (a) G. W. Griffin, A. F. Marcantonio, H. Kristinsson, R. C. Peterson, and C. S. Irving, *Tetrahedron Lett.*, 2951 (1965); (b) S. Hixson, *J. Amer. Chem. Soc.*, **94**, 2507 (1972).

(12) A conformational contribution to the central methyl effect is a possibility presently under study.

(13) Similar 1,2-hydrogen shifts have been observed in other systems.[11a]

(14) Previous studies[15] have indicated that vinylcyclopropane to housane interconversions do occur; this would fit the observed labeling. However, housane **5** is a primary photoproduct of the photolysis of tetraphenyldiene **3** since its quantum yield of formation is constant in runs varying from 2 to 8% conversion.

(15) H. Kristinsson and G. S. Hammond, *J. Amer. Chem. Soc.*, **89**, 5970 (1967).

Howard E. Zimmerman,* James A. Pincock
Chemistry Department, University of Wisconsin
Madison, Wisconsin 53706
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Bridging of Peptides to Solid Supports through the Dinitrophenylene Moiety. Bidirectional Extension of Peptide Chains[1-3]

Sir:

We wish to report on a method of peptide synthesis which permits the bidirectional (NH_2 and COOH directed) elaboration of a peptide chain starting with an amino acid anchored by its side chain *via* a thiol-labile linkage[4] to a solid support.[5]

The principle of the method is illustrated in Scheme I by the synthesis of thyrotropin-releasing hormone (TRH).[6-8] In brief, the histidine residue, bound to a solid support through an N^{im}-dinitrophenylene bridge, served as a point of departure from which the peptide chain was extended in the COOH and NH_2 terminal directions to yield resin-bound TRH. The hormone was liberated from the resin by treatment in DMF solution with 2-mercaptoethanol—an unusually mild treatment for the removal of a peptide from its polymeric support.

Boc-Glycine, esterified with chloromethylpolystyrene–2% divinylbenzene resin (5 g, 0.28 mequiv of glycine/g of esterified resin),[9] was converted to the free base. The esterified resin was washed with $CHCl_3$ and then suspended in 25 ml of $CHCl_3$ containing a large molar excess (5 g) of 1,5-difluoro-2,4-dinitrobenzene (FFDNB).[10] During the next 5 hr, a total of 0.2 ml of Et_3N was added in three portions to the agitated suspension; 2 hr later the yellow, ninhydrin-negative[12]

(1) Supported in part by U. S. Public Health Service Grants AM-10080 and AM-13567 and by the Atomic Energy Commission.

(2) Abbreviations follow the rules of the IUPAC-IUB Commission on Biochemical Nomenclature in *Biochem. J.*, **126**, 773 (1972).

(3) Analytical grade solvents were further purified prior to use: DMF was distilled *in vacuo*, stirred for 24 hr at 23° with (*Z*)-Leu-ONp, redistilled *in vacuo* under nitrogen, and stored over Linde 4A Molecular Sieve. Pyridine was distilled from NaOH pellets and triethylamine from ninhydrin. In addition, $CHCl_3$ and MeOH were distilled. Each "washing" procedure entailed the use of three 25-ml portions of the solvents in question; the solvents are cited in the order they were used.

(4) S. Shaltiel, *Biochem. Biophys. Res. Commun.*, **29**, 178 (1967).

(5) R. B. Merrifield, *J. Amer. Chem. Soc.*, **85**, 2149 (1963); *Science*, **150**, 178 (1965).

(6) A. V. Schally, T. W. Redding, C. Y. Bowers, and J. F. Barrett, *J. Biol. Chem.*, **244**, 4077 (1969).

(7) J. Bøler, F. Enzmann, K. Folkers, C. Y. Bowers, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, 37, 705 (1969).

(8) R. Burgus, T. F. Dunn, D. Desiderio, and R. Guillemin, *C. R. Acad. Sci.*, **269**, 1870 (1969).

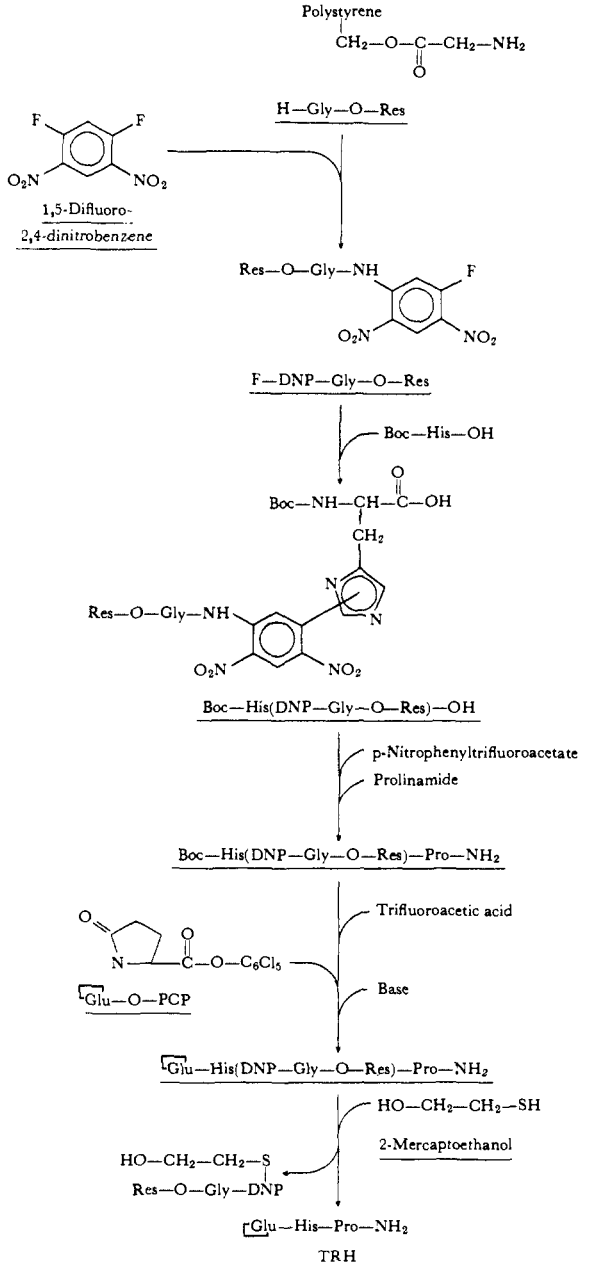
(9) R. B. Merrifield, *Biochemistry*, 3, 1385 (1964).

(10) The use of appropriate concentrations of FFDNB in the reaction mixture was successfully applied by Zahn and Meienhofer[11] in the selective preparation of monofunctional, bifunctional, or mixed bifunctional amino acid derivatives.

(11) H. Zahn and J. Meienhofer, *J. Makromol. Chem.*, **26**, 126 (1958).

(12) E. Kaiser, R. L. Colescott, C. D. Bossinger, and P. I. Cook, *Anal. Biochem.*, **34**, 595 (1970).

Scheme I. Synthesis of Thyrotropin Releasing Hormone (TRH)



polymer derivative was collected by filtration and washed with $CHCl_3$ and DMF. The *N*-[5-fluoro-2,4-dinitrophenyl]glycine resin ester (F-DNP-Gly-O-Res) was suspended in 25 ml of DMF containing 2.8 mmol of Boc-His-OH[13] and the mixture was shaken for 22 hr with the addition of 0.18 ml of Et_3N in three portions over the first 16 hr. The substituted resin was then washed[3] successively with DMF, EtOH, DMF, EtOH, and Et_2O prior to drying *in vacuo*. The Boc-His(DNP-Gly-O-Res)-OH (0.22 mequiv of Boc-His-OH/g of esterified resin[14,15]) was washed with pyridine, suspended in 25 ml of dry pyridine containing 1.0 g of TFA-ONP,[16] and shaken in suspension for 30 min. The reaction mixture was washed with DMF, then suspended in 25 ml of DMF containing 2.8 mequiv of H-Pro-NH_2·HCl[17] and 0.09 ml of Et_3N. After 5 hr, 0.09 ml of Et_3N was added and shaking was continued for 36 hr. Analysis of a sample removed from the resin by treatment with 2-mercaptoethanol[14] revealed that the major product contained two trace contaminants; the ratio of ninhydrin-active components of the acid hydrolysate of the crude product was His, 0.96, Pro, 1.00, ammonia, 1.1. No residual Boc-His-OH was detected in this material.[18] After washing with AcOH and TFA the Boc group was removed from Boc-His(DNP-Gly-O-Res)-Pro-NH_2 by shaking the substituted polymer as a suspension in 25 ml of TFA for 30 min.[21] The solvent was removed by filtration and the residue washed successively with AcOH, EtOH, and DMF. The deprotected dipeptide derivative was resuspended in 25 ml of DMF containing 2.8 mmol of pentachlorophenyl pyrrolidonecarboxylate[22] and 1.4 mmol of *N*-methylmorpholine. After 20 hr the ninhydrin-negative resin was washed with DMF, EtOH, AcOH, EtOH, and Et_2O, and then dried *in vacuo*. The resulting powder was suspended in 40 ml of DMF containing 2.0 ml of 2-mercaptoethanol and 100 μl of Et_3N for 9 hr[23] and then washed with DMF. The filtrate and washings were concentrated to an oil under reduced pressure The residue was taken up in 2.0 ml of methanol and added dropwise to a 30-ml bath of Et_2O. Chromatography on silica gel G with 95% EtOH as solvent revealed that the precipitated crude product was comprised of one major component (identical with authentic TRH), along with two trace contaminants.[24]

Purification of the crude product was achieved by column chromatography on silica gel G (30 × 0.9 cm) with $CHCl_3$–MeOH (7:3, v/v) as eluent, followed by partition chromatography[25] on a Sephadex G-25 column (56.5 × 2 cm) with *n*-BuOH–95% EtOH–pyridine–AcOH–H_2O (40:10:10:4:64, v/v/v/v/v) (R_f 0.13) as solvent; yield 248 mg (49% based on the degree of Gly substitution of H-Gly-O-Res) of chromatographically homogeneous TRH,[26] $[\alpha]^{26}D$ −69.7° (*c* 0.9, H_2O, (lit.[22] $[\alpha]^{25}D$ −65.5° (*c* 1, H_2O)); $[\alpha]^{26}D$ −72.0 (*c* 0.7)

(13) B. O. Hanford, T. A. Hylton, K.-T. Wang, and B. Weinstein, *J. Org. Chem.*, 33, 4251 (1968).

(14) A portion of the peptide–resin was subjected to thiolysis in order to test for the effectiveness of attachment to and removal from the substituted polymer of Boc-His-OH. Generally, thiolysis for analytical purposes was accomplished by incubating approximately 10 mg of the resin for 3 hr with 0.5 ml of DMF in the presence of 0.5 μl of Et_3N and 10 μl of 2-mercaptoethanol. Aliquots (20 μl) of the thiolysis solution were withdrawn at set time intervals from the reaction mixture and taken to dryness *in vacuo* for qualitative and quantitative analyses.

(15) The Boc-His-OH was identified by chromatographic comparison with authentic material and His was measured quantitatively by amino acid analysis following acid hydrolysis.

(16) S. Sakakibara and N. Inuka, *Bull. Chem. Soc. Jap.*, 37, 1231 (1964); 38, 1979 (1965).

(17) D. Hamer and J. P. Greenstein, *J. Biol. Chem.*, 193, 81 (1951).

(18) Samples were chromatographed in duplicate on silica gel G with 95% EtOH and visualized by Pauly reagent[19] and chlorine–starch–iodide.[20]

(19) H. Pauly, *Hoppe-Seyler's Z. Physiol. Chem.*, 94, 288 (1915).

(20) H. N. Rydon and P. W. G. Smith, *Nature (London)*, 169, 922 (1952).

(21) H. Kappeler and R. Schwyzer, *Helv. Chim. Acta*, 43, 1453 (1960).

(22) G. Flouret, *J. Med. Chem.*, 13, 843 (1970).

(23) We have since found that the thiolysis is completed after a 2–3-hr period, and that a longer incubation time increases non-peptide contaminants in the crude product without improving yields.

(24) A sample of the crude product, subjected to acid hydrolysis and then amino acid analysis, gave the following molar ratios: Glu, 1.00; His, 1.03; Pro, 1.00; ammonia, 1.03.

(25) D. Yamashiro, *Nature (London)*, 201, 76 (1964).

(26) TRH was tested on silica gel G plates developed with the following solvent systems (all v/v): 95% EtOH; $CHCl_3$–MeOH (7:3); MeOH–$CHCl_3$ (2:3)[22]; MeOH–$CHCl_3$ (2:1)[22]; $CHCl_3$–MeOH–38% AcOH (3:2:1)[22]; $CHCl_3$–MeOH–NH_4OH (60:45:20)[22]; and BuOH–EtOAc–AcOH–H_2O (1:1:1:1).[22]

NAcOH) (lit.[27] $[\alpha]^{25}D$ −69.2 (*c* 1, *N* AcOH)). Amino acid analysis of an acid-hydrolyzed sample gave the following ratios: Glu, 1.04; His, 1.00; Pro, 0.96; ammonia, 1.01. The biological activity of the synthetic TRH compared favorably to the standard preparation used by Bowers, *et al.*, when tested in the T_3-TRH assay in mice.[28] Doses of 3, 9, and 18 ng of the synthetic TRH raised the ^{125}I level in the blood by 3981, 4144, and 6668 cpm, respectively; identical doses of standard gave values of 3432, 4322, and 5871 cpm. An acid–saline control experiment gave a value of Δ cpm of 145.

The dinitrophenylene group played a dual function in the synthesis of TRH; it served both as a protection for the imidazole nucleus of histidine and as a bridge between the histidine and the solid support. The known reactions of cysteinyl and tyrosyl derivatives with fluoro-2,4-dinitrobenzene to yield thiol-sensitive S-DNP and O-DNP derivatives[5] suggests that the dinitrophenylene-attachment method may be extended to the solid-phase synthesis of cysteinyl- and tyrosyl-containing peptides.

The dinitrophenylene bridging of cysteine, tyrosine, and histidine residues to solid supports promises a useful set of resin-bound amino acids from which peptide chains can be extended bidirectionally. The versatility of such a system, along with the mild conditions required for cleavage of the peptide from the resin, significantly expands the scope of the "side-chain attachment method."[29,30]

Acknowledgment. We are grateful to Dr. C. Y. Bowers and Mrs. G. Reynolds of the Tulane University School of Medicine, New Orleans, La., for bioassay, and to Dr. G. Flouret of Northwestern Medical School for supplying us with a sample of highly purified TRH for chromatographic comparison with synthetic TRH. Thanks are also due to Mrs. I. Mintz for amino acid analyses.

(27) D. Gillessen, F. Piva, H. Steiner, and R. O. Studer, *Helv. Chim. Acta*, **54**, 1335 (1971).
(28) C. Y. Bowers, A. V. Schally, F. Enzmann, J. Bøler, and K. Folkers, *Endocrinology*, **86**, 1143 (1970).
(29) L. Yu. Sklyarov and I. V. Shaskova, *Zh. Obshch. Khim.*, **39**, 2779 (1969).
(30) J. Meienhofer and A. Trzeciak, *Proc. Nat. Acad. Sci. U. S.*, **68**, 1006 (1971).

* Address correspondence to this author at the Department of Physiology, Mount Sinai School of Medicine, New York, N. Y. 10029.

J. D. Glass, I. L. Schwartz, Roderich Walter*
Mount Sinai School of Medicine
City University of New York
New York, New York 10029

Brookhaven National Laboratory
Upton, New York 11973

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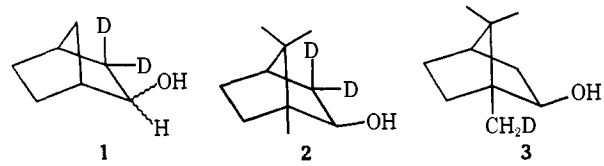
An Unusual Stereospecific Elimination of Water in the Mass Spectra of Bicyclo[2.2.1]heptan-2-ols

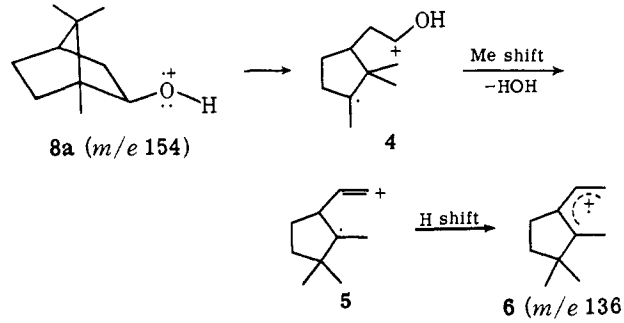
Sir:

The exact means by which water is lost from alcohols when subjected to electron impact has been the subject of several investigations.[1-3] Prior to this work, deuterium labeling studies have established that loss of water does not occur by a 1,2 elimination. For example, Green and coworkers[2] have demonstrated that both a very stereospecific cis-1,4 elimination and a nonstereospecific 1,3 elimination of water occur in cyclohexanol. Another example is that of the isomeric 3,3-dideuterionorbornanols (**1**), exo and endo, which do not lose HOD on electron impact.[3]



1 **2** **3**

In contrast to these findings, 3,3-dideuterioisoborneol (**2**) loses HOD approximately 50% of the time on electron impact.[4] The mass spectrum of deuterated isoborneol **3** showed that the remaining 50% water loss involves the C-10 methyl group.[4] This latter way of losing water is equivalent to the solution chemistry dehydration of isoborneol to camphene. At the time, we explained the 1,2 water elimination (involving carbons 2 and 3 of the bicyclic skeleton) by evoking a postulate of Bieman's[5] that dehydration could occur after fragmentation



8a (*m/e* 154) **4** **5** **6** (*m/e* 136)

The results reported herein, however, demonstrate that the mechanism **8a** → **6** is not correct and that a uniquely different type of dehydration is occurring.

Camphor was stereospecifically deuterated to give ketones **7b** and **7c**.[6] Under the conditions employed,[7] the ketones were contaminated by some d_0 and d_2 ketones. The structural assignments of **7b** and **7c**, which rely on the nmr spectra of the hydride reduction products, were in agreement with that reported in the literature.[6] Lithium aluminum hydride reduction of the ketones gave a 9:1 mixture of deuterated isoborneol–borneol, which were separated by preparative gas chromatography.

(1) H. Budzkiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, San Francisco, Calif., 1964, Chapter 2.
(2) M. M. Green, R. J. Cook, J. M. Schwab, and R. B. Roy, *J. Amer. Chem. Soc.*, **92**, 3076 (1970), and references therein.
(3) K. Humski and L. Klasinc, *J. Org. Chem.*, **36**, 3057 (1971); (b) H. Kwart and T. A. Blazer, *ibid.*, **35**, 2726 (1970); (c) K. Biemann, "Mass Spectrometry Organic Chemical Applications," McGraw-Hill, New York, N. Y., 1962, pp 108–110.
(4) D. R. Dimmel and J. Wolinsky, *J. Org. Chem.*, **32**, 410 (1967).
(5) Reference 3c, p 95.
(6) (a) A. F. Thomas, R. A. Schneider, and J. Meinwald, *J. Amer. Chem. Soc.*, **89**, 68 (1967); (b) A. F. Thomas and B. Willhalm, *Tetrahedron Lett.*, 1309 (1965).
(7) The conditions that we found were best for the production of the desired monodeuterated camphor were 1 part **7a** (or **7d**): 80 parts D_2O (or H_2O); catalytic amount of base in sufficient dioxane to make the solution homogeneous and stirring at room temperature for 48 hr. Analysis of the deuterium content was accomplished by comparing the molecular ion region of the mass spectra of the ketones.