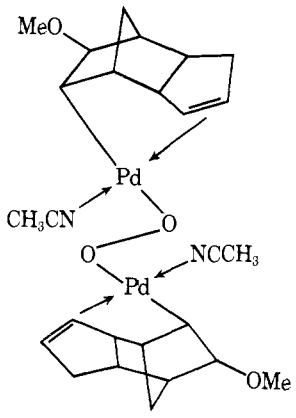

6

covered by addition of ether: mp >150 °C dec; IR (KBr) ν(C—O) 1093, ν(C≡N) 2220 cm^{-1}; Anal. Calcd for $C_{26}H_{36}O_4N_2Pd_2$: C, 47.79; H, 5.51; N, 4.29. Found: C, 48.51; H, 5.48; N, 4.56. These results and the nature of superoxide

$$[(\text{RO-DiCp})\text{PdCl}]_2 \xrightarrow[-\text{Cl}^-]{O_2^-} (\text{RO-DiCp})\text{Pd}\begin{matrix}\text{OO}\cdot\\ \text{Cl}\end{matrix}\text{Pd}(\text{RO-DiCp})$$

(nucleophilic displacement)

$$\xrightarrow[-O_2]{O_2^-} (\text{RO-DiCp})\text{Pd}\begin{matrix}\text{OO}^-\\ \text{Cl}\end{matrix}\text{Pd}(\text{RO-DiCp})$$

(electron transfer)

$$\xrightarrow[-\text{Cl}^-]{} (\text{RO-DiCp})\text{Pd}\begin{matrix}\text{O}\\ \text{O}\end{matrix}\text{Pd}(\text{RO-DiCp}) \qquad (3)$$

(nucleophilic displacement)

ion reported[9] suggest the scheme shown (eq 3) for the reaction to form dioxygen complexes. Both nucleophilic displacement[10] and electron transfer[11] involving O_2^- have been reported by several authors. The preparation method for dioxygen complexes reported here is quite simple and seems to be applicable for other transition metal systems. In addition, the dioxygen complexes having olefinic ligands may serve as good models for catalytic oxidation of hydrocarbons. Work along this line is now in progress.

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(3) Starting palladium complexes, 1 and **2**, were prepared by the method reported by J. K. Stille and R. A. Morgan, *J. Am. Chem. Soc.*, **88**, 5135 (1966).
(4) CH_2Cl_2 is the best solvent for both starting complexes, **1** and **2**, and dioxygen complexes, **3** and **4**. In spite of the poor solubility of KO_2, the best yields of dioxygen complexes were obtained using CH_2Cl_2 as solvent. Addition of 18-crown-6 accelerated the reaction, but difficulty arose in clean recovery of dioxygen complexes. Me_2SO, the best solvent for KO_2, was strongly coordinated to palladium and gave no reaction products.
(5) Beilstein test was also negative.
(6) The M-O_2-M system, where dioxygen serves as bidentate to each of the two metal atoms, was formerly estimated for the synthetic oxygen carrying chelates such as the Co-O_2-Co system.[12] However, this is the first case for the noble metal-dioxygen complexes. X-ray analysis is now proceeding for the precise structure.
(7) Melting point and ^1H NMR and IR spectra for the recovered materials were completely identical with those for **1** and **2**, respectively. The results of elemental analysis were also satisfactory.
(8) Hydrogen peroxide (~60% of stoichiometric amount) was detected in the decomposition of **4** (0.25 mmol) in benzene-MeOH solution by iodometry. Incorporation of oxygen in the complexes **3** and **4** was further confirmed by the decomposition of **3** and **4** under oxygen-free conditions which gave various oxygenated products depending on the conditions.
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(C_2)-Dioxa-C_{20}-octaquinane, a Heterocyclic Trisecododecahedrane

Sir:

Any tactical elaboration of the pentagonal dodecahedrane molecule must involve the successful incorporation of 20 methine units by means of 30 carbon-carbon bonds into an all-cis network of 12 polyfused cyclopentane rings. If the synthetic approach proceeds stepwise with the proper deployment of spherical topology, then it becomes likely that certain penultimate intermediates will be attained which are more highly strained than the target compound due to enhanced levels of nonbonded interaction. A case in point is the as yet unknown triseco hydrocarbon **1**, where three sets of endo hydrogen atom pairs are seen to be directed into the highly crowded molecular interior. We describe here an efficient

1 **2**

synthesis of **2** where two oxygen atoms have replaced pivotal methylene groups within such a framework. Although the impact of this structural change upon reduction of steric strain and/or molecular distortion must await the preparation of **1** and tandem physical measurements, the title compound (**2**), as constituted, represents the most advanced polyquinane structure known to this time.[1] Consequently, the present work provides evidence sufficient to justify the belief that **1** and related molecules where the spherical contour is highly developed should be amenable to conventional synthesis.

Previously, we reported the one-step preparation of diester **3a**[2] and diacid **3b**[3] by domino Diels-Alder addition of dimethyl acetylenedicarboxylate to 9,10-dihydrofulvalene[4] and their conversion to the C_2 symmetric bislactone **4**.[5] In gaining access to **2**, methodology has been developed for selective fission of the central bond in **4** and subsequent contraction of the heterocyclic rings, while strictly avoiding the serious complication of transannular bond formation so often witnessed with these molecules. To this end, **4** was reduced with lithium aluminum hydride in the tetrahydrofuran at 0 °C. Although the product of kinetic control was the endo,endo bislactol, rapid equilibration to **5a**, mp 252-255 °C (90%),[6] was seen upon dissolution in $CDCl_3$ or recrystallization from acetone. When admixed with freshly distilled thionyl chloride, **5a** was converted quantitatively to the highly reactive chloro ether **5b**, whose axial symmetry was substantiated by its 10-line ^{13}C NMR spectrum.[7] As expected, **5b** proved to be moisture sensitive, treatment with water or prolonged exposure to the atmosphere returning **5a**. Analogously, methanolysis of **5b** afforded the methyl acetal **5c**.[8]

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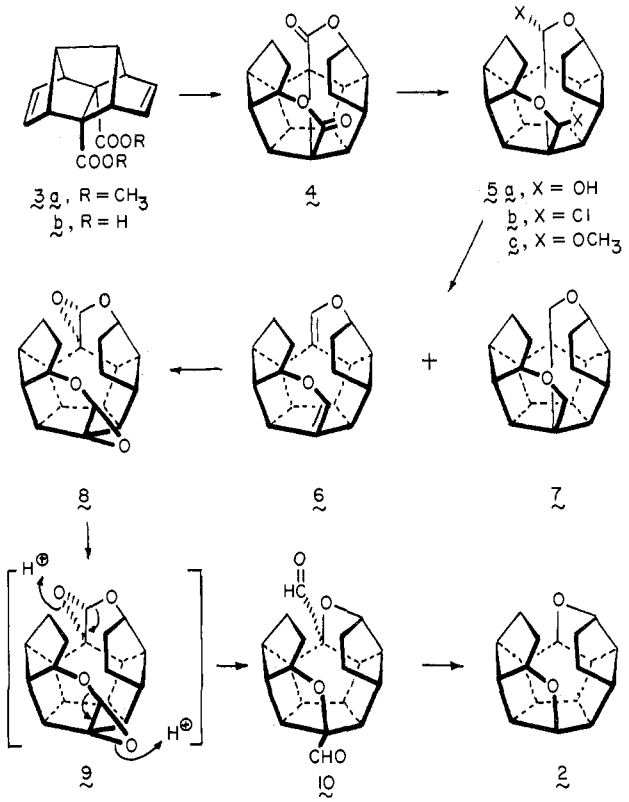
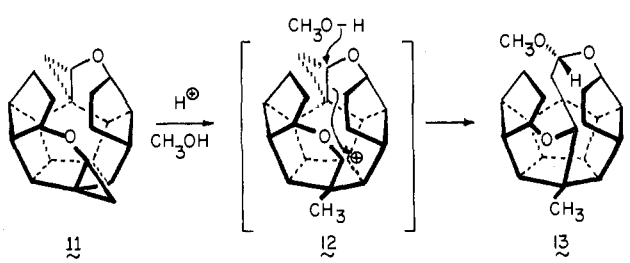


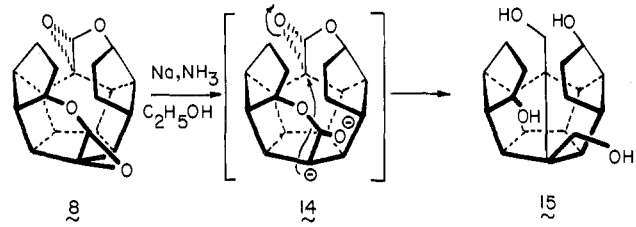
Reductive cleavage of the central bond in **5b** was brought about with sodium in liquid ammonia at −33 °C. This procedure gave rise to a mixture of the bisdihydropyran **6** (93%) and the saturated ether **7** (7%), the separation of which on silica gel furnished **6** in 80% isolated yield. Structural assignment to **6**. mp 116–118 °C, follows unambiguously from its spectra.[9]

At this stage, extrusion of the trigonal carbon atoms α to oxygen in the six-membered rings was undertaken. The sequence began by epoxidation of **6** with *m*-chloroperbenzoic acid to give α-epoxy ether **8** (67%), mp 197–200 °C dec.[10] In the presence of a wide range of acidic reagents, **8** was quickly isomerized to the bisaldehyde **10,** mp 195–200 °C dec. For preparative purposes, filtration of a CH_2Cl_2 solution of **8** through a silica gel column served as a convenient method of gaining access to this octaquinane system (44%). The relative simplicity of its ^1H ($(CDCl_3)$ δ 9.66 (s, 2), 4.70 (m, 2), and 3.60–1.40 (m, 18)) and ^{13}C NMR spectra ($(CDCl_3)$ 205.51, 103.43, 92.53, 63.50 (2 C), 61.72, 57.95, 49.20, 34.15, and 29.73 ppm) confirm the retention of a molecular C_2 axis.

The formation of **10** is regarded as the likely result of epoxide opening *toward the tertiary cation center* with concurrent or subsequent 1,2 oxygen migration as shown in **9**. This behavior contrasts in a striking way with the response of biscyclopropyl derivative **11,** mp 181–186 °C dec,[11] to electrophilic ring cleavage. In a reaction typical of its chemistry, **11** underwent isomerization in acidic methanol with cleavage of the cyclopropane ring *toward oxygen* to give oxonium ion **12** which suffers transannular bonding and solvent capture to deliver **13,** mp 141 °C.[12]



The above facts should not leave the impression that **8** is not guilty of transannular infractions. Perhaps the most blatant example is found in its conversion to tetraol **15,** mp 268–272 °C dec, when reduced with sodium in liquid ammonia containing ethanol. That the strained internal σ bond had been



introduced was established spectroscopically[13] and by independent preparation of **15** from **5a** under identical conditions.

The final step of the synthesis involved decarbonylation of **10.** Puzzlingly, many early attempts to perform this transformation according to traditional procedures (and to decompose the corresponding di-*tert*-butyl perester as well) afforded no material which could be characterized as **2.** However, success was achieved upon irradiation (275-W sun lamp) of an intimate mixture of **10,** acetophenone, benzyl mercaptan, and ethyl benzoate at 140 °C under argon for several hours.[14] The product, which could be isolated in yields as high as 90%, was obtained as soft, white crystals: mp 156–158 °C (methanol); ^1H NMR ($CDCl_3$) δ 4.55–4.0 (m, 4) and 3.3–1.5 (br m, 18); ^{13}C NMR 91.08, 90.30, 62.68, 62.29, 61.36, 56.22, 49.57, 34.08, and 28.84 ppm. When the yields of **2** were somewhat lower, the balance of the reaction mixture proved to be the monoaldehyde.

An X-ray crystal structure analysis of **2** is planned. It is not yet clear whether a similar ring contraction sequence can be usefully applied to the synthesis of **1.** Notwithstanding, several strategies aimed at the preparation of this interesting companion hydrocarbon are now being implemented.

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(6) ^1H NMR ($CDCl_3$) δ 5.96 (d, J = 3 Hz, 2), 4.07 (br s, 2), and 2.68–1.48 (br m, 20). Satisfactory analyses (combustion and mass spectrometric) have been obtained for all new compounds reported herein.
(7) ^{13}C NMR ($CDCl_3$) 95.50 (d), 71.76 (d), 59.46 (d), 55.44 (s), 53.44 (d), 49.83 (d), 44.32, 43.76, 37.50 (t), and 24.33 (t) ppm.
(8) ^1H NMR ($CDCl_3$) δ 5.40 (s, 2), 3.88 (br s, 2), 3.41 (s, 6), and 2.75–1.47 (m, 18); ^{13}C NMR ($CDCl_3$) 99.98 (d), 68.71 (d), 58.24 (d), 55.25 (d), 53.50 (d), 50.44 (s), 49.66 (d), 44.46, 44.20, 38.29, and 24.57 ppm.
(9) ^1H NMR ($CDCl_3$) δ 6.22 (br s, 2), 4.56–4.20 (m, 2), 3.57–2.34 (m, 10), and 2.34–1.25 (m, 8); ^{13}C NMR ($CDCl_3$) 139.12 (d), 119.80 (s), 75.91 (d), 58.51 (d), 53.66 (d), 48.59 (d), 47.96 (d), 44.70 (d), 39.04 (t), and 28.76 (t) ppm.
(10) ^1H NMR ($CDCl_3$) δ 5.83 (s, 2), 4.05 (m, 2), and 3.30–1.43 (m, 18); ^{13}C NMR ($CDCl_3$) 81.27 (d), 71.03 (d), 62.18 (s), 53.45 (d), 48.38 (d), 47.30 (d), 45.79 (d), 45.09 (d), 38.29 (t), and 21.84 (t) ppm.
(11) ^{13}C NMR ($CDCl_3$) 68.14, 60.54, 57.57, 52.50, 48.83, 47.37, 47.10, 38.63, 21.85, 20.93, and 9.12 ppm.
(12) ^1H NMR ($CDCl_3$) δ 4.79 (br s, 1), 4.44 (dd, J = 9.4 and 4.4 Hz, 1), 4.06 (br s, 2), 3.34 (s, 3), 2.74–1.33 (br m, 20), and 1.08 (s, 3).
(13) ^{13}C NMR (py-d_5) 74.96 (d), 59.72 (2C, d and t), 59.23 (d), 54.81 (s), 50.30 (d), 48.11 (d), 42.38 (d), 35.83 (t), and 23.01 (t) ppm.
(14) This procedure is an adaptation of that described earlier by J. D. Berman, J. H. Stanley, W. V. Sherman, and S. G. Cohen, *J. Am. Chem. Soc.*, **85,**

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(15) Postdoctoral Fellow of the Science Research Council, 1975–1977.
(16) National Science Foundation Graduate Fellow, 1972–1975.

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Enzyme-Assisted Semisynthesis of Human Insulin

Sir:

We wish to describe herein the use of trypsin as a catalyst for peptide-bond formation with special reference to the semisynthesis of human insulin. Conversion of porcine insulin into human insulin, which differs from porcine hormone by a single amino acid residue in position B30, has been attempted by Ruttenberg[1] and by Overmeier and Geiger.[2] In these attempts a derivative of deoctapeptide-(B23–B30)-insulin (DOI), derived from porcine hormone by tryptic digestion,[3] was coupled with DCC to a synthetic octapeptide corresponding to the sequence B23–B30 of human insulin. These chemically conducted couplings, however, seem to be of little use for simple and inexpensive production of pure insulin, because of a number of difficulties involved and low yields.[2,4] Now, we have found that, under certain conditions, trypsin can catalyze the coupling between DOI and human octapeptide to lead to the production of human insulin in a moderate yield.

Trypsin catalysis of peptide-bond formation has been little investigated except for such a special case as in a modified soybean trypsin inhibitor.[5] We thus studied the following coupling reaction in homogeneous systems:[6]

$$\text{Boc-X-OH} + \text{H-Val-OBu}^t \overset{\text{E}}{\rightleftharpoons} \text{Boc-X-Val-OBu}^t + \text{H}_2\text{O}$$

X = Lys or Arg; E = trypsin

The coupling was performed with a large excess of the amine component (H-Val-OBu^t) so as to make a substantial shift of equilibrium to the right. The optimum pH for these trypsin-catalyzed syntheses was found to be near 6.5, as seen in Figure 1. The equilibrium of the systems also depends on the concentration of water. Table I clearly shows that within a certain limit the addition of an organic solvent, such as DMF or Me_2SO, to the reaction medium remarkably increases peptide formation.[8] The effect is much greater than that expected from the decrease in water concentration.[9] The addition of organic solvent was also effective in increasing the solubility of both carboxyl (C) and amine (N) components, another important factor which determines the extent of peptide formation. These results prompted us to develop the procedure for semisynthesis of human insulin.

Table I. Effect of Concentration of Organic Solvent on the Yield of Boc-X-Val-OBu^t in Trypsin-Catalyzed Coupling of Boc-X-OH with H-Val-OBu^t (X = Lys or Arg)[a]

X	solvent added	concn of solvent, %	yield of Boc-X-Val-OBu^t, %
Lys	none	0	15
Lys	methanol	10	21
		20	30
		30	39
		40	49
		50	42
Lys	dimethylformamide	10	23
		20	34
		30	49
		40	65
		50	81
		60	79
		70	0
Lys	dimethyl sulfoxide	20	33
		40	68
Lys	tetrahydrofuran	20	30
		40	0
Arg	dimethylformamide	10	20
		20	42
		30	47
		50	75

[a] [Boc-X-OH] = 0.034 M, [H-Val-OBu^t] = 0.34 M, [E] = 1.89 mg/mL, pH 6.5, 25 °C, 20 h.

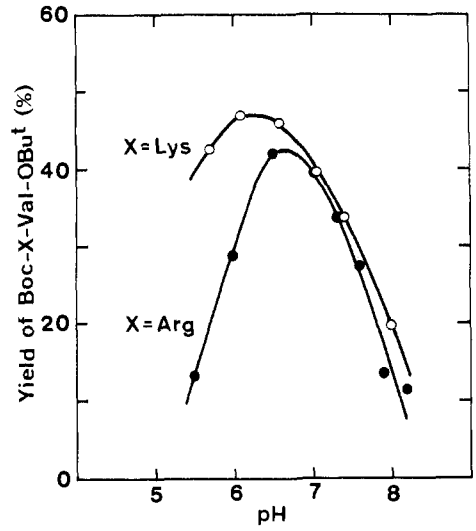

Figure 1. Effect of pH on the yield of Boc-X-Val-OBu^t in trypsin-catalyzed coupling of Boc-X-OH with H-Val-OBu^t- (X = Lys or Arg).[7] [Boc-X-OH] = 0.046 M (X = Lys) or 0.054 M (X = Arg), [H-Val-OBu^t] = 0.49 M (X = Lys) or 0.50 M (X = Arg), [E] = 1.96 mg/mL, [DMF] = 20%, 25 °C, 20 h. The progress curves of the reaction (not shown) revealed that it came to equilibrium within 20 h. The term yield used here represents the extent of peptide formation in such an equilibrium state.

Zinc-free porcine insulin[10] was digested with TPCK-treated trypsin[11] at pH 9–9.5[12] and the resulting DOI was acylated with Boc-N_3, as described for insulin,[13] to give $N^{\alpha A1}$,$N^{\alpha B1}$-$(Boc)_2$-DOI quantitatively. Human octapeptide was synthesized in the form of H-Gly-Phe-Phe-Tyr-Thr-Pro-Lys(R_1)-Thr(R_2)-OR_2 (I, R_1 = Boc, R_2 = H; II, R_1 = Boc, R_2 = Bu^t) by the conventional solution method, in which much care was taken to minimize the danger of racemization.[14] The C and N components thus obtained were coupled in a typical experiment as follows.

To a solution of $(Boc)_2$-DOI (100 mg) and octapeptide (I, 200 mg) in a mixture of DMF and 0.25 M Tris buffer (1:1 by volume, 1.2 mL) was added TPCK-trypsin[11] and the reaction mixture (pH 6.5) was incubated at 37 °C for 20 h. The enzyme (10 mg) was added in three portions at time: 0, 2, and 6 h. The concentrations of $(Boc)_2$-DOI and octapeptide were 16.3 and 163 mM, respectively (N/C ratio = 10), and that of enzyme 0.35 mM. At the end of the reaction, LC showed that 58% of the DOI had been converted into insulin. The entire material was applied to a Sephadex LH-20 column and eluted with DMF–0.5 M acetic acid (1:1). The $(Boc)_3$-insulin formed and the $(Boc)_2$-DOI which remained uncoupled were emerged as a single peak. The octapeptide which had been present in a large excess came out as the second peak and was isolated in pure state after rechromatography (160 mg). The material in the first peak was treated for deprotection with TFA at 0 °C for 60 min in the presence of anisole. The product was then chromatographed on a column of Sephadex G-50 (superfine) with 0.5 M acetic acid as eluant. From one of two major peaks

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