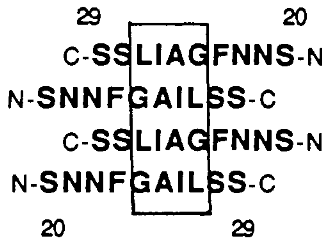


Figure 1. A crude model for a portion of the subunit antiparallel β-sheet of the IAPP 20–29 fibril which is consistent with the FTIR and ssNMR data. The enclosed section resembles the idealized antiparallel β-sheet.[4]

amide from the neighboring ^{12}C amides.[5] The effect of the substitution on the ^{12}C amide mode will depend on whether the substituted carbonyl was located in an extensively coupled (i.e., β-sheet) region of the amyloid. If the substituted amide is in the center of a β-sheet, one observes a decrease in the overall coupling of the ^{12}C amides, resulting in a decrease in the observed splitting and a shift of the intense amide I band to higher frequency. Eight analogues of IAPP 20–29 in which a single amide carbonyl was replaced with ^{13}C were synthesized[10] and analyzed in the film state by FTIR (see Table I).[11] The greatest decoupling effect was observed for analogue G24, where the ^{12}C amide peak absorbs at 1643 cm^{-1}, as compared to 1628 cm^{-1} in the natural abundance peptide (shift = +15 cm^{-1}; see Table I, column A). The magnitudes of the shifts in the ^{12}C amide absorption frequency indicate that residues G24 (+15 cm^{-1}), A25 (+9 cm^{-1}), I26 (+11 cm^{-1}), and L27 (+10 cm^{-1}) are located in the idealized β-sheet, whereas the termini of the peptide are not.

Analysis of each labeled peptide amyloid by ^{13}C cross-polarization magic angle spinning (CPMAS) solid-state NMR[12,13] revealed significant variations which are consistent with the model proposed above. Two structure-dependent variables were measured (see Table I).[13] The deviation from the "unstructured" chemical shift value[14b] (column C) and the line width (column D) are both related to structural rigidity.[14] According to each of these measurements, the region between G24 and L27 is more highly ordered than the termini of the peptide in the amyloid. The observed upfield chemical shifts (F23–L27) are typical of shifts measured for other β-sheets in the solid state.[14a]

The position of the ^{13}C amide I band for each analogue (column B) reflects the intrinsic properties of that carbonyl as well as the intermolecular dipole coupling with adjacent labeled amides in the β-sheet.[9] In order to experimentally eliminate the latter effect, each labeled peptide film was combined with five parts of the unlabeled peptide and analyzed by FTIR.[11] The observed shift in the ^{13}C band on "isotopic dilution", due to the loss of intermolecular coupling, was greatest for residues A25 (+9 cm^{-1}) and I26 (+9 cm^{-1}). The fact that intermolecular dipole coupling maximizes in the middle of the sequence is consistent with a crude model of the β-sheet in which residues A25 and I26 are proximal to one another on *both* sides of the antiparallel β-strand (see Figure 1).[15]

This model resembles the idealized cross-β fibril in the G24–L27 region.[4] The implication that this region is critical for amyloidogenesis may explain the fact that rodents, in which A25 of IAPP is substituted by proline, among other changes, do not form pancreatic amyloid.[8] Finally, the regularity of the IAPP 20–29 amyloid distinguishes it from the amyloid formed from a C-terminal fragment of the amyloid protein of AD (β34–42) which contains an unusual structural feature.[5,16]

Acknowledgment. We thank Mr. Charles E. Hines III for technical assistance and the NSF Biotechnology Process Engineering Center for funding his summer fellowship. Thanks are also due to Prof. Robert Griffin, in whose laboratories the ssNMR experiments were carried out, and to Mr. Ed. Takach (FABMS) and Ms. Pat Reilly (EM) for assistance. This work was supported by the National Institutes of Health (RO1-AG08470-02) and a Presidential Young Investigator Award from the NSF. Matching funds for the latter were generously provided by Merck, Pfizer, Hoechst-Celanese, General Electric, and ICI. P.L. is a 1991 Sloan Research Fellow, a Camille and Henry Dreyfus Teacher-Scholar, and the Firmenich Career Development Professor of Chemistry. M.A. thanks the Natural Sciences and Engineering Research Council of Canada for a postdoctoral fellowship.

Supplementary Material Available: The FTIR spectra discussed herein (4 pages). Ordering information is given on any current masthead page.

(10) Purity of the ^{13}C-labeled peptides was judged to be >95% by RPHPLC (Waters Delta-Pac C4 (3.9 × 30 cm), 83% H_2O/17% CH_3CN (0.1% TFA), R_V = 23 mL). Label position was verified by fast atom bombardment mass spectrometry [(M + H)$^+$ = 1050.5]. Labeled Asn analogues were not synthesized due to expense.

(11) Thin films were formed by evaporating a formic acid (88% in water) solution of the peptide on a CaF_2 plate. Evaporation from a solution of 2:1 hexafluoro-2-propanol (HFIP)/water afforded films of nonuniform thickness; hence this solvent was not commonly used. The IR spectra of these films were identical to the formic acid films with regard to band position; however, resolution was poor due to the variability of these films.

(12) Mehring, M. *High Resolution NMR in Solids*; Springer-Verlag: New York, 1983.

(13) NMR samples were prepared by diluting a filtered HFIP solution of the peptide with water. HFIP was removed under reduced pressure, and the aqueous solution was lyophilized. Magic angle spinning (MAS) spectra were collected on a home-built spectrometer operating at a ^1H frequency of 317.5 MHz and a ^{13}C frequency of 79.9 MHz. A home-built probe was used, with a stator and rotors from Doty Scientific Inc. (Columbia, SC). Typical ^1H and ^{13}C 90° pulse lengths were 3.2 and 4.0 μs, respectively. Recycle delays were 3 s, and the cross-polarization time was 2.0 ms. Spectra were recorded at room temperature and at spinning speeds of 2.5–3.0 kHz. Chemical shift values were referenced to external tetramethylsilane.

(14) (a) Saitô, H. *Magn. Reson. Chem.* **1986**, *24*, 835. (b) Howarth, O. W.; Lilley, D. M. J. *Prog. Nucl. Magn. Reson. Spectrosc.* **1978**, *12*, 1.

(15) Coupling between dipoles is related to the distance between the dipoles (*r*) and their relative orientation by a simple equation.[5,9]

(16) Spencer, R. G. S.; Halverson, K. J.; Auger, M.; McDermott, A. E.; Griffin, R. G.; Lansbury, P. T., Jr. *Biochemistry* **1991**, *30*, 10382.

Cycloisomerization for Atom Economy. Polycycle Construction via Tandem Transition Metal Catalyzed Electrocyclic Processes

Barry M. Trost* and Yian Shi

Department of Chemistry, Stanford University
Stanford, California 94305-5080

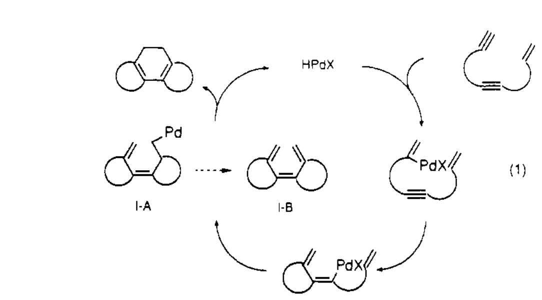
Received October 8, 1991

Biomimetic polyolefin cyclizations have proved to be a valuable strategy for polycyclizations.[1] Mediating such processes via intermediates complexed to transition metals may offer additional avenues for control.[2] Synthetic efficiency will be enhanced if polycyclization can be accomplished by simple isomerization of some acyclic polyunsaturated species to a polycycle, with any other reagent required only in catalytic amounts.[3] We envisioned the feasibility of a stereospecific polycycloisomerization of an enediyne catalyzed by palladium (eq 1), which requires a high level of chemoselectivity in the initial hydropalladation step.

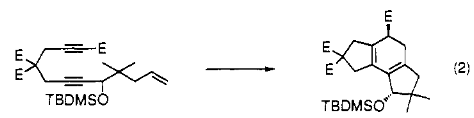
(1) Johnson, W. S.; Telfer, S. J.; Cheng, S.; Schubert, U. *J. Am. Chem. Soc.* **1987**, *109*, 2517. For reviews, see: Bartlett, P. A. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic: New York, 1984; Vol. 3, pp 341–409. Speckamp, W. N. *Recl. Trav. Chim. Pays-Bas* **1981**, *100*, 345. Johnson, W. S. *Bioorg. Chem.* **1976**, *5*, 51; *Acc. Chem. Res.* **1968**, *1*, 1.

(2) Zhang, Y.; Wu, G.; Agnel, G.; Negishi, E. *J. Am. Chem. Soc.* **1990**, *112*, 8590. Grigg, R.; Dorrity, M. J.; Malone, J. F.; Sridharan, V.; Sukirthalingam, S. *Tetrahedron Lett.* **1990**, *31*, 1343. Kucera, D. J.; Overman, L. E. *Abstracts of Papers*, 200th National Meeting of the American Chemical Society, Washington, DC, Aug. 1990; American Chemical Society: Washington, DC, 1990; ORGN 128. Carpenter, N. E.; Kucera, D. J.; Overman, L. E. *J. Org. Chem.* **1989**, *54*, 5846. Abelman, M. M.; Overman, L. E. *J. Am. Chem. Soc.* **1988**, *110*, 2328. Zhang, Y.; Negishi, E. I. *J. Am. Chem. Soc.* **1989**, *111*, 3454. For related studies that appeared after submission of this paper, see: Meyer, F. E.; deMeijere, A. *Synlett* **1991**, 777. Meyer, F. E.; Parsons, P. J.; de Meijere, A. *J. Org. Chem.* **1991**, *56*, 6487.

(3) Trost, B. M.; Shi, Y. *J. Am. Chem. Soc.* **1991**, *113*, 701.



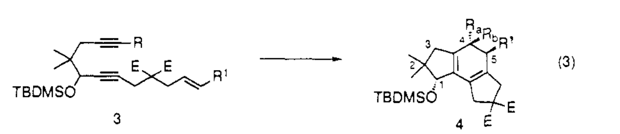
Enediyne **1**[4,5] serves as our test substrate. An approximately 0.1 M solution of **1** in benzene is added to a mixture of 2.5 mol % of $(dba)_3Pd_2\cdot CHCl_3$[6] and 10 mol % of triphenylphosphine (TPP). After the addition of 20 mol % of acetic acid, the reaction is warmed to 70 °C. Upon the disappearance of starting material, direct concentration and flash chromatography give a 75% yield of diastereomerically pure tricycle **2**,[5] tentatively assigned the stereochemistry depicted upon mechanistic considerations (eq 2).



1 2

E = CO_2CH_3

Using these standard conditions, except for a change of ligand where noted, a variety of ene-poly-ynes are cyclized. Either the initiator acetylene and/or the terminator olefin may bear substituents (eq 3, **3b–e**) or not (**3a**). The substituents may be ester,

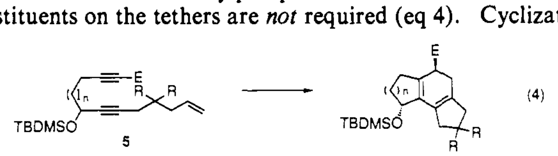


3 4

a) R = R¹ = H
b) R = E, R¹ = H
c) R = H, R¹ = Ph
d) R = E, R¹ = Ph
e) R = E, R¹ = CH₂OAc
f) R = CH₂OCH₃, R¹ = Ph

a) Ra = Rb = R¹ = H 57%
b) Rb = E, Ra = R¹ = H 75%
c) Ra = Rb = H, R¹ = Ph 69%
d) Ra = E, Rb = H, R¹ = Ph 90%
e) Ra = H, Rb = E, R¹ = CH₂OAc 61%
f) Ra = H, Rb = CH₂OCH₃, R¹ = Ph 70%

aryl, or alkyl. The insensitivity of the chemoselectivity of the hydropalladation step to acetylene substitution is remarkable. The choice of phosphine may be important. The cyclization of enediyne **3e** proceeds best with trifurylphosphine rather than TPP. Geminal substituents on the tethers are *not* required (eq 4). Cyclization



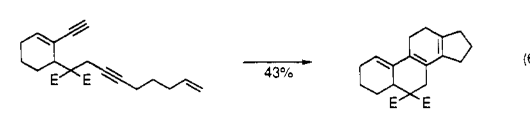
5 6

a) R = E n = 1 60%   b) R = H n = 2 57%

of **5b** also shows the extension to six-membered-ring formation even without the assistance of geminal substituents on either tether. The six-membered-ring formation allows extension of the process to a synthesis of the steroid nucleus with great variability of substitution pattern (eqs 5 and 6). For the cyclization of eq 6, tri-*o*-tolylphosphine is employed as the ligand.

7 8

a) R = R¹ = X = H 56%   b) R = E, R¹ = CH₃, X = OTBDMS 71%



9 10

The mechanism of this reaction does appear to involve triene I-B (eq 1). In several cyclizations, traces of this requisite triene can be detected by NMR spectroscopy. For example, in the cycloisomerization of **5b**, signals at δ 5.60 (d, J = 1.6 Hz, vinyl H), 5.24 (t, J = 2.8 Hz, CHOR), 5.20 (s), and 5.14 (s) (terminyl methylene), assignable to the triene intermediate, can be seen to build up and decay in time since the six-membered ring seems to slow the electrocyclization of the hexatriene. The fact that high diastereoselectivity is seen in all cases then derives from the rotoselectivity[7] of the hexatriene to cyclohexadiene isomerization. Molecular models reveal that the torsional selectivity depicted in **11** twists the 3,4-substituents such that X rotates away from the neighboring ring. The opposite triene rotation depicted in **12**

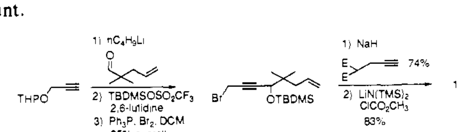
11 12

causes the twist of the 3,4-substituents to rotate X toward the neighboring ring, which should significantly enhance unfavorable nonbonded interactions and thereby be disfavored. The C_4–C_5 stereochemistry of **4e** and **4f** as Z derives from the 7.4 ± 0.1 Hz coupling of H_4–H_5; however, the stereochemistry of **4d** is assigned as E from the 11.2 Hz coupling constant, which indicates a diaxial orientation of these two hydrogens. While the stereochemistry of **4e** and **4f** is that expected from a disrotation of the E,E-triene, that of **4d** is not. Since the latter corresponds to the thermodynamically more stable isomer, it may derive from an easy epimerization α to the ester. Although cyclization of I-A (eq 1) is conceivable, apparently β-H insertion dominates. While further mechanistic work is clearly required, the synthetic efficiency of polycycle formation by cycloisomerization with high diastereoselectivity, despite the remoteness of the stereogenic centers, and chemoselectivity should make this methodology useful for complex synthesis. The results demonstrate the feasibility of high rotoselectivity of hexatrienes to cyclohexatrienes and raise the question concerning the factors that may influence such reactions.[8] The lack of effect of the nature of the substituents on the initiator acetylene provides great generality for the process.

Acknowledgment. We thank the National Science Foundation and the General Medical Sciences Institute of the National Institutes of Health for their generous support of our programs. Mass spectra were provided by the Mass Spectrometry Facility, University of California—San Francisco, supported by the NIH Division of Research Resources.

Supplementary Material Available: Characterization data for compounds **1–10** (5 pages). Ordering information is given on any current masthead page.

(4) The following sequence for the preparation of substrate **1** typifies the preparation of substrates. Full details on all routes will be reported in our full account.



(5) New compounds have been fully characterized spectroscopically. Elemental compositions have been established by combustion analysis and/or high-resolution mass spectrometry.

(6) Ukai, T.; Kawazura, H.; Ishii, Y.; Bonnet, J. J.; Ibers, J. A. *J. Organomet. Chem.* **1974**, *65*, 253.

(7) For consideration of rotoselectivity in 4-electron cases, see: Trost, B. M.; McDougal, P. G. *J. Org. Chem.* **1984**, *49*, 458. Kallel, E. A.; Wang, Y.; Spellmeyer, D. C.; Houk, K. N. *J. Am. Chem. Soc.* **1990**, *112*, 6759.

(8) For a review, see: Marvell, E. N. *Thermal Electrocyclic Reactions*; Academic: New York, 1980; Chapter 7.