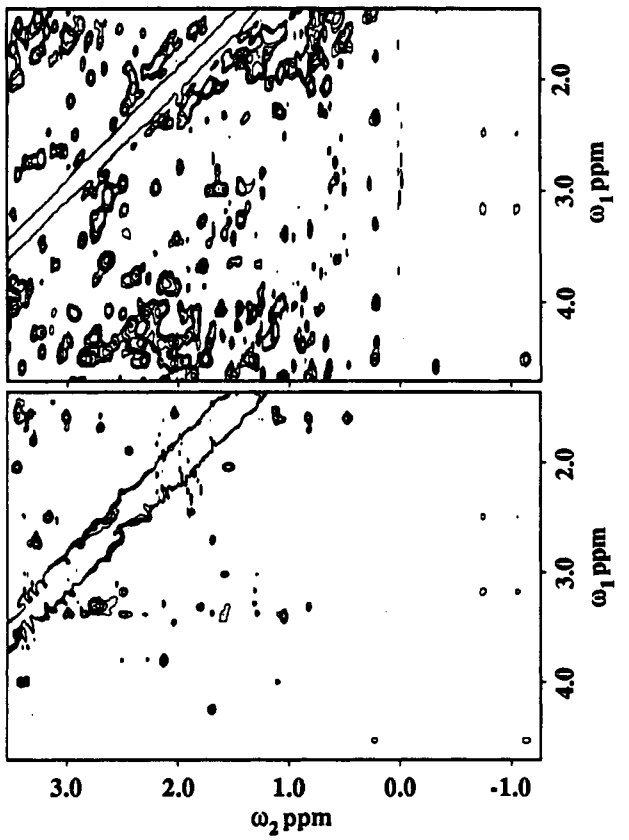

Figure 4. Top panel: section of 500-MHz ^1H NOESY spectrum of FKBP/FK506 complex recorded in D_2O, 25 mM phosphate buffer, pH 5.0 (uncorrected). Bottom panel: corresponding section of 500-MHz ^{13}C-filtered ^1H NOESY spectrum of uniformly ^{13}C-labeled KFBP/unlabeled FK506 complex recorded in D_2O, 25 mM phosphate buffer, pH 5.0 (uncorrected). Only intraligand crosspeaks are observed.

of FKBP as previously described[8] resulted in typical yields of 10 mg of protein per liter of growth media.

The utility of receptor labeling is demonstrated in Figure 3, which shows in the top panel a 1D spectrum of unlabeled FKBP/FK506 complex and in the bottom panel a ^{13}C-filtered spectrum of ^{13}C-FKBP/FK506 complex. In contrast to the unfiltered spectrum, the filtered spectrum shows base-line resolution of individual resonances of the bound ligand without the additional signals of the receptor.[10] The filtering allows tentative identification (based on chemical shifts), even in a 1D spectrum, of the 3 methoxy methyl signals (marked with asterisks), the 2 vinyl methyl signals (marked with daggers), and the 3 remaining methyl signals (marked with pound signs). The spectral simplification is equally dramatic in Figure 4, which shows the aliphatic regions of unfiltered and ^{13}C-filtered 2D-NOESY spectra of the ^{13}C-FKBP/FK506 complex. The unfiltered spectrum could not be unambiguously assigned in this region due to extensive spectral overlap with aliphatic resonances of FKBP. In particular, intraligand, intrareceptor, and intermolecular NOEs could not be differentiated unambiguously. In contrast, the filtered spectrum shown contains only NOEs between protons of FK506 and is thus more easily interpretable. Following complete assignment, the NOEs can then be analyzed along with coupling constant information obtained from ^{13}C-filtered 2D-COSY spectra to determine the bound conformation of this ligand without necessitating structure determination of the entire complex. Although complete assignment is currently in progress, simple inspection of the pattern of crosspeaks to the high-field signals ($\delta < 0$ ppm) confirms our previous identification of these signals as belonging to the pipecolinyl ring of FK506.[11] These assignments also provide further evidence for a single (>95%, given the sensitivity limits of NMR) bound conformer (amide bond rotamer) of FK506, in contrast to the unbound drug, which exists as a 3:1 cis:trans mixture. This is consistent with previous NMR investigations of ^{13}C-[C_8, C_9]-FK506.[12]

In conclusion, we have demonstrated a convenient alternative to previously published methods employing isotope-edited NMR to study receptor–ligand complexes. The approach produces spectra of comparable quality to other methods but is simpler to employ in cases of large or complicated ligands not amenable to isotopic labeling through synthetic chemistry. The use of biosynthetically labeled receptor should have general utility in studies of both the structural and dynamic properties of receptor–ligand complexes.

Acknowledgment. We thank Martin Karplus for fostering research into receptor–ligand interactions at Harvard. A generous gift of ^{13}C-labeled glucose from Pfizer, Inc., is gratefully acknowledged. This research was supported by the National Institute of General Medical Sciences (GM-38627). M.K.R. and T.J.W. gratefully acknowledge National Science Foundation Predoctoral Fellowships. S.W.M. was supported by a grant awarded to Professor Martin Karplus (GM-30804).

(10) Note that many of the low-field peaks in the filtered spectrum represent incompletely exchanged amide protons of the protein. Since these are not attached to the filtering nucleus (^{13}C), they are not purged from the spectrum.

(11) Wandless, T. J.; Michnick, S. W.; Rosen, M. K.; Karplus, M.; Schreiber, S. L. *J. Am. Chem. Soc.* **1991**, *113*, 2339–2341.
(12) Rosen, M. K.; Standaert, R. F.; Galat, A.; Nakatsuka, M.; Schreiber, S. L. *Science* **1990**, *248*, 863.

Synthesis of Macrocyclic Homopropargylic Alcohols through Intramolecular S_E' Addition of Allenylstannanes and Their Subsequent Conversion to 2,5-Furanocycles

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Summary: The 2,5-furanocyclododecenes **16**, **20**, and **24** have been prepared by a new route involving intramolecular S_E' addition of propargylic stannanes **3**, **6**, and **9** then exposure of the derived allenones **14**, **19**, and **23** to AgNO$_3$–CaCO$_3$ in aqueous acetone at room temperature.

In recent years, a number of interesting 2,5-furano macrocyclic diterpenes have been isolated from marine

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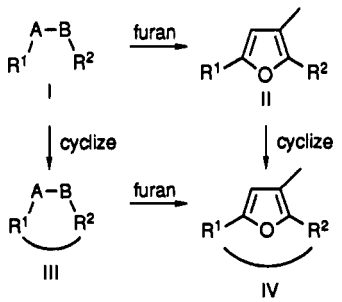
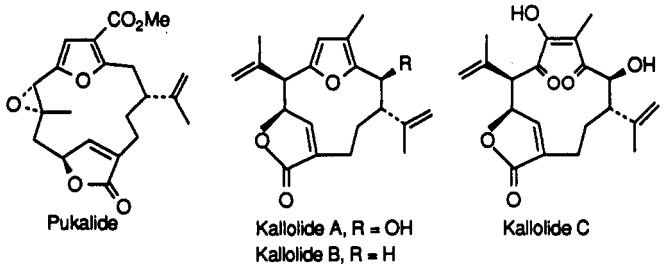


Figure 1. Strategy for the synthesis of 2,5-furano macrocyclic products.

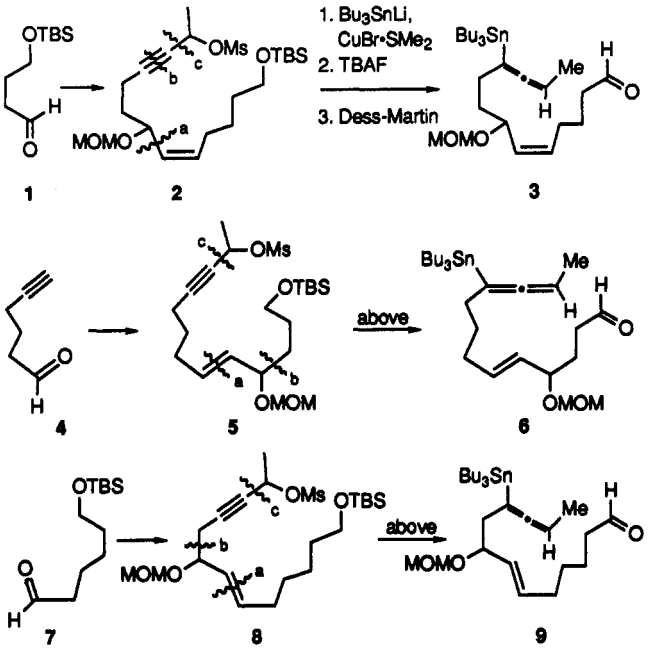
sources.[1] These can be grouped into two structural types: (1) the 14-membered furanocembranolides, as exemplified by pukalide,[1a-e] and (2) the 12-membered furanocycles, of which kallolide A and B are representative.[1f,g] The latter



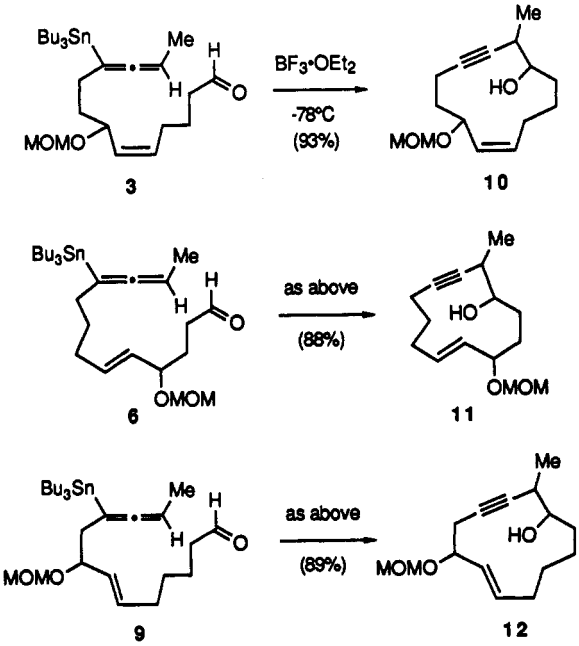
belong to a new class of diterpenoids for which the name pseudopterane has been suggested. Kallolide A possesses potent antiinflammatory activity comparable to that of indomethacin. Kallolide C may be viewed as an oxidation product of kallolide A.

Central to the development of synthetic routes to such compounds is the issue of ring closure. In view of the ready availability of suitable furan precursors, it is tempting to formulate a strategy involving elaboration and ultimate cyclization of the intact furanoid system (Figure 1, I → II → IV).[2] However, because of ring strain the cyclization step would expectedly be problematical, especially in the case of the pseudopteranes. For this reason we have been developing an alternative strategy in which macrocyclization preceeds furan formation (Figure 1, I → III → IV). In this approach, the initial cyclization can proceed without undue ring strain, and the aromatic resonance energy of the furan can help to overcome the inherent ring strain imposed during formation of the bridged furanocycle. In this paper, we describe an application of that strategy employing a novel method for the synthesis of macrocyclic homopropargylic alcohols and their further elaboration to 2,5-furanocycle prototypes of the pseudopteranes.

The propargylic mesylates **2**, **5**, and **8** were prepared from aldehydes **1**, **4**, and **7** by straightforward well-precedented methodology.[3] Addition of the reagent derived from Bu_3SnLi and $CuBr{\cdot}SMe_2$ afforded, in each case, only the S_N2' allenyl stannanes free of S_N2 propargylic isomer.[4] These were further elaborated, as mixtures of diastereomers, to the aldehydes **3**, **6**, and **9**.



Upon treatment with $BF_3{\cdot}OEt_2$ in CH_2Cl_2 at −78 °C each of the three propargylstannanyl aldehydes **3**, **6**, and **9** efficiently cyclized to the respective homopropargylic cyclododecenols **10**, **11**, and **12**, respectively. In all cases the



cyclization products were obtained as mixtures of diastereoisomers. Oxidation of propargylic alcohols **10** with the Dess–Martin periodinane reagent[5] afforded a nearly

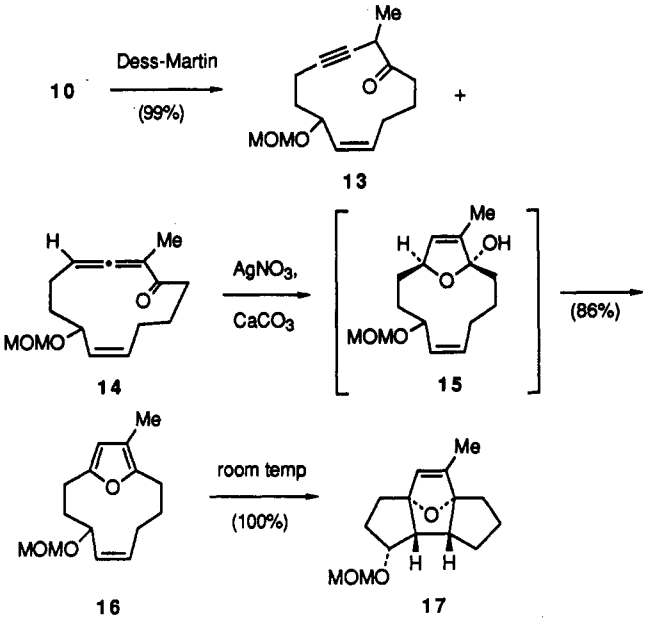
(1) (a) Pukalide: Missakian, M. G.; Burneson, B. J.; Scheuer, P. J. *Tetrahedron* **1975**, *31*, 2513. (b) Lophotoxin: Fenical, W.; Okeeda, R. K.; Bandurraga, M. M.; Culver, P.; Jacobs, R. S. *Science* **1981**, *212*, 1512. (c) Rubifolide: Williams, D.; Andersen, R. J.; Van Duyne, C. D.; Clardy, J. *J. Org. Chem.* **1987**, *52*, 332. (d) Corralodiolide: D'Ambroseo, M.; Fabbri, D.; Guerriero, A.; Pietra, F. *Helv. Chim. Acta* **1987**, *70*, 63. (e) Bipinnatins: Wright, A. E.; Bunes, N. S.; Schulte, G. K. *Tetrahedron Lett.* **1989**, *30*, 3491. (f) Pseudopterolide: Bandunaga, M. M.; Fenical, W.; Donovan, S. F.; Clardy, J. *J. Am. Chem. Soc.* **1982**, *104*, 6463. (g) Kallolides: Look, S. A.; Burch, M. T.; Fenical, W.; Qui-tai, Z.; Clardy, J. *J. Org. Chem.* **1985**, *50*, 5741.

(2) For examples of this approach, see: (a) Marshall, J. A.; Nelson, D. J. *Tetrahedron Lett.* **1988**, *29*, 741. (b) Marshall, J. A.; Robinson, E. D. *J. Org. Chem.* **1990**, *55*, 3451. (c) Paquette, L. A.; Rayner, C. M.; Doherty, A. M. *J. Am. Chem. Soc.* **1990**, *112*, 4078.

(3) 1 → 2: (a) $LiC{\equiv}C(CH_2)_4OLi$, (b) Ac_2O, Et_3N, (c) TBAF, (d) Lindlar, H_2, (e) Swern, (f) Ph_3P, CBr_4, (g) K_2CO_3, (h) TBSCl, Im, (i) MOMCl, *i*-Pr_2NEt, (j) BuLi, CH_3CHO, (k) MsCl, Et_3N. 4 → 5: (a) $Ph_3P{=}CHCO_2Me$, (b) DIBAH, (c) Swern, (d) $TBSO(CH_2)_3MgBr$, (e) MOMCl, *i*-Pr_2NEt, (f) *n*-BuLi, CH_3CHO, (g) MsCl, Et_3N. 7 → 8: (a) $Ph_3P{=}CHCO_2Me$, (b) DIBAH, (c) Swern, (d) $HC{\equiv}CCH_2Br$, Mg, (e) MOMCl, *i*-Pr_2NEt, (f) BuLi, CH_3CHO, (g) MsCl, Et_3N.

(4) Marshall, J. A.; Wang, X-j. *J. Org. Chem.* **1990**, *55*, 6246.

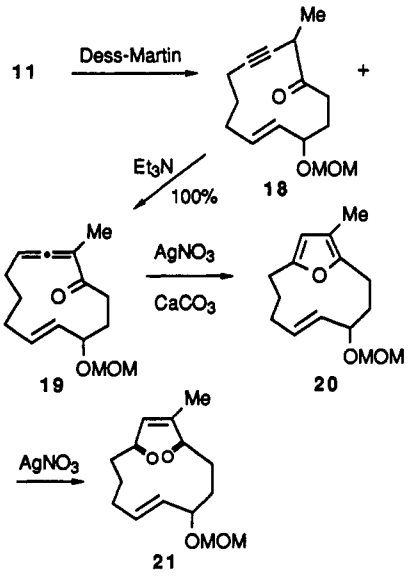
1:1 mixture of propargylic and allenic ketones **13** and **14** as mixtures of diastereoisomers. When exposed to $AgNO_3$



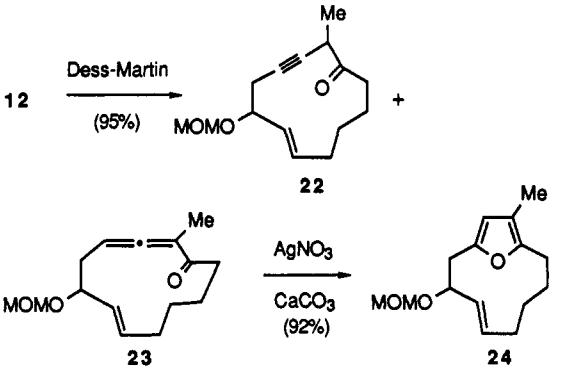
and $CaCO_3$ in aqueous acetone this mixture gave rise to a polar and two nonpolar products after 8 h at room temperature.[6] After 36 h only one of the nonpolar products was present. The polar product could not be isolated, but it is assumed to be the hydrofuran **15** by analogy with related acyclic allenone systems.[6] One of the nonpolar products was identified as the bridged furan **16**. The second and ultimate final product after prolonged reaction (86% yield) was shown to be the intramolecular Diels–Alder adduct **17**.[7] The cyclization of **16** to **17** took place quantitatively on standing in $CDCl_3$ (NMR tube) overnight.

Dess–Martin oxidation[5] of propargylic alcohols **11** proceeded in 95% yield to a nearly 1:2 mixture of diastereomeric propargylic ketones **18** and only a trace amount of the allenone **19**. Upon treatment with $AgNO_3$ and $CaCO_3$ in aqueous acetone ketone **18** was slowly (52 h) converted to a single polar product whose spectral characteristics were consistent with the ene dione structure **21**. During the course of this reaction only a faint spot attributable to the presumed precursor, furan **20**, could be detected by TLC analysis. On the other hand, the allenic ketone **19**, obtained in quantitative yield by treatment of **18** with Et_3N, cyclized to the bridged furan **20** in 95% yield within 4 h when exposed to 0.2 equiv of $AgNO_3$·$CaCO_3$ in aqueous acetone. Furan **20** was slowly converted to ene dione **21** upon treatment with an excess of this reagent. Dione **21** is a prototype of the kallolide C system.

Oxidation of alcohol **12** afforded a nearly 3:1 mixture of allenone **23** and propargylic ketone **22** in 95% yield.



This mixture smoothly cyclized to furan **24**, a prototype of kallolide A/B, upon exposure to $AgNO_3$ and $CaCO_3$ in aqueous acetone.



The present findings illustrate the potential of BF_3·OEt_2-promoted intramolecular additions of allenylstannanes to aldehydes as a promising new method for macrocyclization.[8] They also show that the resulting homopropargylic alcohols can be efficiently converted to 2,5-furanocycle prototypes of pseudopterane natural products. It is noteworthy that furan **18**, though sufficiently strained to undergo facile internal Diels–Alder cyclization under the mild conditions of furan formation, is nonetheless accessible by this methodology.

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Supplementary Material Available: Experimental procedures and ^1H NMR spectra for all intermediates and products and selected ^{13}C NMR spectra (39 pages). Ordering information is given on any current masthead page.

(5) Dess, P. M.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4156.
(6) Marshall, J. A.; Wang, X-j. *J. Org. Chem.* **1991**, *56*, 960.
(7) For additional examples of intramolecular Diels–Alder cycloadditions of macrocyclic trienes to tricyclic olefins see: Bérubé, G.; Deslongchamps, P. *Tetrahedron Lett.* **1987**, *28*, 5255. The present example is remarkable in view of the poor dienophilic reactivity of isolated double bonds and the poor dienic character of furans.
(8) Tius has shown that acetylide anions undergo intramolecular addition to aldehydes to afford cembranoid propargylic alcohols. Tius, M.; Culligham, J. M. *Tetrahedron Lett.* **1989**, *30*, 3749.