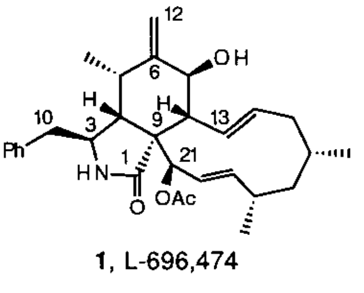
ON THE CHEMISTRY OF THE 18-DEOXYCYTOCHALASIN H, HIV-1 PROTEASE INHIBITOR, L-696,474. 2:[1] NOVEL RING ANNULATIONS

B. Moon Kim,* James P. Guare,* and Steven M. Pitzenberger

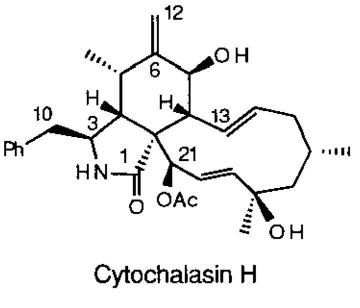
Department of Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486, U.S.A.

Abstract - Unprecedented annulation of the 11-membered ring portion of 18-deoxycytochalasin H, a 3μM inhibitor of HIV-1 protease, has been observed furnishing a novel tetracyclic cytochalasin (**5**). Subsequent desilylation of **5** provided yet another set of new tetracyclic cytochalasin derivatives.

The 18-deoxycytochalasin H, L-696,474 (**1**), was isolated from the fermentation broth of a bark-inhabiting ascomycete, *Hypoxylon fragiforme* and shown to competitively inhibit HIV-1 protease with an IC_{50} of 3 μM.[2] Since most of the known peptidomimetic HIV-1 protease inhibitors, though extremely potent in *in vitro* assays, exhibit poor bioavailability profiles in animal pharmacokinetic studies,[3] the discovery of this unique nonpeptidyl cytochalasin lead for HIV-1 protease proved to be very stimulating. As part of a program aimed at developing novel HIV-PR inhibitors, we embarked on the structure-activity studies of this abundantly produced cytochalasin via systematic modification of its ring scaffold.



1, L-696,474

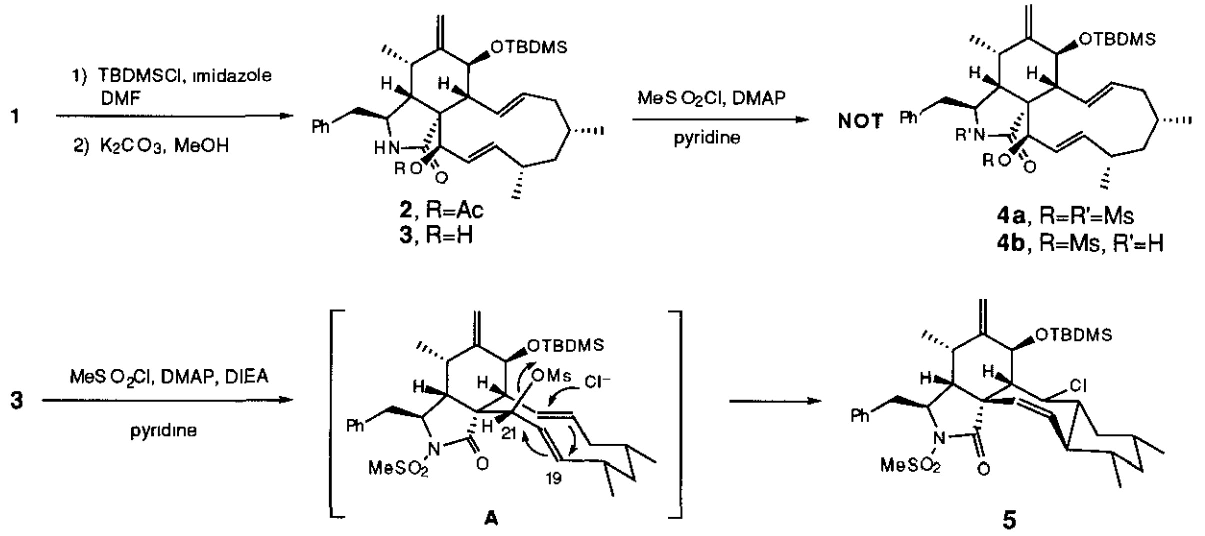


Cytochalasin H

The structures of cytochalasins are characterized by an isoindolone core fused to a macrocycle. Much effort has been devoted to the syntheses of various cytochalasins[4] due to their interesting biological activity.[5] Our initial focus was on the modification of the 11-membered unsaturated ring portion of **1**. During the course of this investigation, a series of unprecedented ring annulations of the 11-membered ring of **1** were discovered and these annulation reactions comprise the subject of this note.

According to Scheme 1, the 7-hydroxyl group of **1** was protected as the t-butyldimethylsilyl ether (**2**) in 95% yield. Hydrolysis of the C(17)-acetyl group of **2** (K_2CO_3, MeOH, 15 h, room temperature, ~75%) yielded the allylic alcohol (**3**), which is unstable at room temperature due to its tendency to undergo retroaldol-type fragmentation.[6] In order to ascertain if the allylic acetyl group at C(21) was needed for the observed inhibitory activity, we pursued the corresponding allylic displacement on the C(21) alcohol. However, when compound (**3**) was subjected to the usual methanesulfonylation conditions ($MeSO_2Cl$, DMAP, pyridine, room temperature), the reaction was rather slow and after addition of excess methanesulfonyl chloride and diisopropylethylamine, a compound was obtained (82% isolated yield) that proved not to be either of the expected mesylates (**4a**) or (**4b**) by ^{1}H and ^{13}C nmr.

Scheme 1



Extensive spectroscopic analysis of this compound including ^{1}H COSY, 1D difference NOE and HETCOR revealed a novel tetracyclic structure (**5**) incorporating a bicyclo[5.4.0]undecene system in place of the 11-

membered ring with chloride at C(13) as shown in Scheme 1.[7] The presence of the chlorine was also corroborated by ms analysis and the Beilstein's test.[8] The equatorial orientation of the chloride at C(13) was deduced from two large trans-diaxial couplings from C(13)H to vicinal methines at C(8) and C(14) as well as an NOE between C(13)H and C(7)H. The new ring juncture at C(14) and C(19) was identified as *trans* on the basis of a large coupling constant (~11 Hz) between C(14)H and C(19)H. Figure 1 illustrates several diagnostic 1D NOE correlations that were observed.

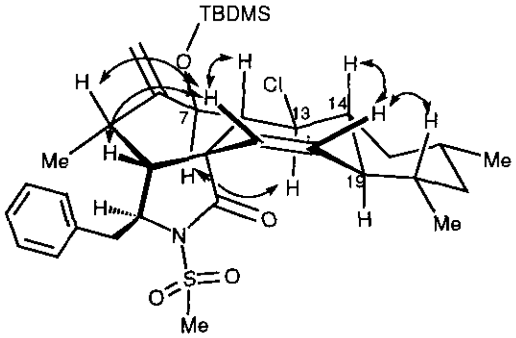
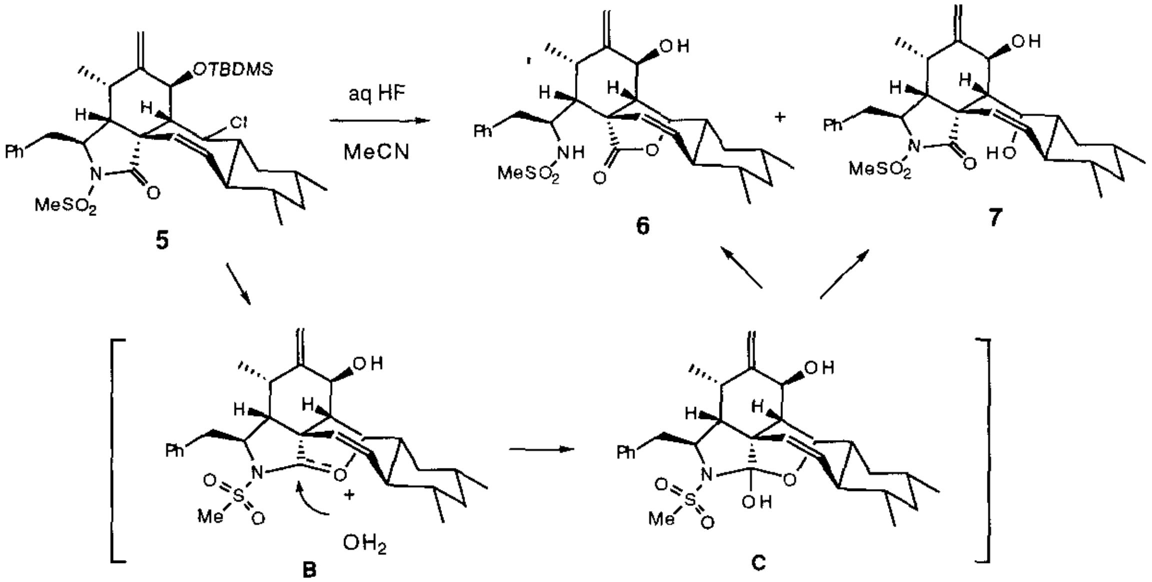


Figure 1. Selected 1D NOE Correlations of Structure (**5**)

As depicted in the figure A in Scheme 1, the mechanism of this trans-annular cyclization is believed to involve, after formation of the C(21)-OMs, an attack of chloride ion on the C(13)-C(14) double bond which in turn cyclizes to the parallel C(19)-C(20) double bond with concomitant elimination of the mesylate at C(21) in an S_N2' fashion. The basic nature of the reaction conditions should exclude S_N1 type demesylation at C(21) followed by olefin cascade and capture of the chloride ion at C(13). It is particularly noteworthy that this transannulation of the 11-membered ring to form a bicyclo[5.4.0]undecene system represents the reversal of the fragmentations of bicyclo[5.4.0]undecanones employed in other laboratories in attempts to prepare the 11-membered rings of various cytochalasin derivatives.[9]

The outcome of the deprotection step of the C(7)O-*tert*-butyldimethylsilyl group of the tetracyclic cytochalasin (**5**) was also intriguing. Treatment of **5** with aq HF in acetonitrile at ambient temperature yielded two products (~1.2:1, 92% combined yield), which were separated by flash chromatography (10% EtOAc-hexane). The faster eluting compound (**6**) exhibited ir absorption at 1765 cm^{-1}, typical of a 5-membered lactone, while the later eluting compound (**7**) showed regular amide carbonyl absorption (1693 cm^{-1}). Examination of the two products by ^1H nmr COSY spectra coupled with the IR spectral data revealed another interesting rearrangement involving the lactam carbonyl oxygen as depicted in Scheme 2.[10] The oxygen attached to the C(13) appears to adopt a pseudoaxial position in both structures based on the coupling data for the C(13) methine proton (a singlet in **6** and a dd (10.8[11] and 2.8 Hz) in **7**). This strongly suggests the involvement of the lactam carbonyl oxygen in the displacement of the chloride. Although an understanding of the exact nature of the mechanism requires further investigation, the two products are presumed to be formed through a common intermediate such as C which would be derived from the oxonium ion intermediate B.

Scheme 2



In summary, a novel tetracyclic cytochalasin structure incorporating a cycloheptene ring was prepared through a ring annulation reaction of the 11-membered ring portion of a cytochalasin analog derived from L-696,474 (**1**). Subsequent desilylation in the presence of aq HF facilitated another set of rearrangements to provide two novel tetracyclic cytochalasin derivatives (**6**) and (**7**).

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3. For a review on HIV-1 protease inhibitors, see: J. R. Huff, *J. Med. Chem.*, **1991**, *34*, 2305.
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6. First observed by B. D. Dorsey, Merck Research Laboratories, private communication. The methanolysis was monitored by hplc (reverse phase, C_{18}, μ-bondapack, 95/5 to 5/95 water-acetonitrile) and upon disappearance of the starting material, it was worked up immediately.
7. Physical data for compound (**5**): ^{1}H nmr (CDCl$_3$, 400 MHz) -0.11 (3H$_{TBDMS}$, s), 0.06 (3H$_{TBDMS}$, s), 0.57 (H$_{11}$, d, *J*=7.0 Hz), 0.70 (H$_{17\beta}$, q, *J*=~11.8 Hz), 0.78 (9H$_{TBDMS}$, s), ~0.78 (H$_{15\alpha}$, obscured), 0.90 (3H$_{16\text{-Me}}$, d, *J*=6.4 Hz), 0.91 (3H$_{18\text{-Me}}$, d, *J*=6.3 Hz), 1.24 (H$_{18}$, m), 1.40 (H$_{16}$, m), 1.58 (H$_{14}$, qd, *J*=~10, ~3 Hz), 1.61 (H$_{17\alpha}$, m), 2.25 (H$_{19}$, tdd, *J*=~10.8, 5.2, 2.0 Hz), 2.27 (H$_8$, dd, *J*=10.5, 4.3 Hz), 2.44 (H$_4$, d, *J*=8.4 Hz), 2.65 (H$_{15\beta}$, dq, 13.1, ~2.5 Hz), 2.83 (H$_5$, pentet, *J*=~7.3 Hz), 2.89 (H$_{10A}$, dd, *J*=13.3, 11.2 Hz), 3.16 (3H$_{SO2Me}$, s), 3.52 (H$_{10B}$, dd, *J*=13.1, 3.8 Hz), 4.15 (H$_7$, d, *J*=4.4 Hz), 4.26 (H$_3$, dd, *J*=11.5, 3.9 Hz), 4.77 (H$_{12A}$, d, *J*=3.2 Hz), 4.96 (H$_{12B}$, d, *J*=2.5 Hz), 5.04 (H$_{13}$, dd, *J*=10.6, 9.3 Hz), 5.65 (H$_{21}$, dd, *J*=11.5, 1.9 Hz), 5.75 (H$_{20}$, dd, *J*=11.4, 5.0 Hz), 7.26 (2H$_{10\text{-Ph-}o}$, d, *J*=~7 Hz), 7.27 (H$_{10\text{-Ph-}p}$, t, *J*=~7 Hz), 7.35 (2H$_{10\text{-Ph-}m}$, t, *J*=~7 Hz,); ^{13}C NMR (100 MHz) -4.9 (Si-C), -4.1 (Si-C), 12.2 (11), 18.1 (Si-$\underline{C}$(CH$_3$)$_3$), 20.7 (18-Me), 22.6 (16-Me), 26.0 (Si-C($\underline{C}$H$_3$)$_3$), 28.8 (5), 31.4 (16), 38.6 (18), 41.0 (2-SO$_2$CH$_3$), 41.5 (10), 42.1 (15), 42.5 (19), 43.1 (17), 50.9 (4), 52.5 (9), 55.0 (8), 58.0 (3), 74.1 (13), 80.3 (7), 109.8 (12), 127.1 (10-Ph-*p*), 128.9 (2C, 10-Ph-*m*),

129.7 (2C, 10-Ph-*o*), 133.5 (21), 136.8 (10-Ph-*i*), 142.4 (20), 174.3 (1); LRFABms (M+1) 646, 610, 588, 568, 199 (100).

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9. (a) D. A. Clark and P. L. Fuchs, *J. Am. Chem. Soc.*, **1979**, *101*, 3567, (b) S. G. Pyne, D. C. Spellmeyer, S. Chen, and P. L. Fuchs, *J. Am. Chem. Soc.*, **1982**, *104*, 5728.

10. Physical data for compound (**6**): ir (cm^{-1}) 3565, 3307, 1765, 1334, 1133; Diagnostic ^{1}H nmr (CDCl$_3$, 400 MHz) 1.94 (H$_8$, d, *J*=10.0 Hz), 1.99 (H$_4$, dd, *J*=10.3, 4.6 Hz), 2.81 (3H$_{SO2Me}$, s), 4.01 (H$_{SO2NH}$, d, *J*=10.4 Hz), 4.22 (H$_7$, ddd, *J*=10.0, 1.7, 1.6 Hz), 4.61 (H$_{13}$, s), 4.76 (H$_3$, dddd, *J*=10.3, 10.3, 4.5, 4.0 Hz), 4.98 (H$_{12A}$, dd, *J*=2.1, 1.1 Hz), 5.05 (H$_{12B}$, dd, *J*=~1.8, ~1.0 Hz), 5.35 (H$_{20}$, dd, *J*=11.7, 3.6 Hz), 5.41 (H$_{21}$, dd, *J*=11.8, 2.2 Hz); LRFABms (M+H)=514; HRFABms calcd for $C_{29}H_{40}NO_5S$: 514.2627; obsd: 514.2622; Physical data for compound **7**: Ir (cm^{-1}) 3561, 3304 (br), 1693, 1362, 1174; Diagnostic ^{1}H nmr (CDCl$_3$, 400 MHz) 1.99 (H$_8$, dd, J=9.8, 2.8 Hz), 2.07 (H$_{19}$, m), 2.43 (H$_4$, dd, *J*=6.4, 1.6 Hz), 2.66 (H$_5$, pentet, *J*=6.4 Hz), 2.87 (H$_{10A}$, dd, *J*=12.9, 11.1 Hz), 3.21 (3H$_{SO2Me}$, s), 3.55 (H$_{10B}$, dd, *J*=13.0, 3.4 Hz), 4.05 (H$_3$, ddd, *J*=11.0, 3.6, 1.5 Hz), 4.16 (H$_{13}$, dd, *J*=10.8, 2.8 Hz), 4.44 (H$_7$, dd, *J*=9.8, 3.3 Hz), 4.96 (H$_{12B}$, br d, *J*=~1.2 Hz), 5.14 (H$_{12A}$, br s), 5.18 (-OH$_{13}$, d, *J*=10.7 Hz), 5.43 (H$_{21}$, dd, *J*=12.5, 2.8 Hz), 5.80 (H$_{20}$, dd, *J*=12.4, 3.3 Hz); LRFABms (M+1)=514; HRFABms calcd for $C_{29}H_{40}NO_5S$: 514.2627; obsd: 514.2626.

11. This unusually large coupling constant is due to the coupling between -OH$_{13}$ and C(13)H. Intramolecular hydrogen bonding of the C(13) alcohol proton to the C(1) carbonyl oxygen appears to place the alcohol proton trans to the equatorial C(13)H, hence the large coupling constant.

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