of an N-terminus blocking group at S_4 through S_6 can have major effects on catalytic efficiency and rate-limiting steps.

Together with previous studies,[1,3-10] the results of this investigation indicate that substrate structural features regulate an important component of the catalytic apparatus possessed by serine proteases: the coupling and effective operation of the catalytic triad. This may represent an example of a more general phenomenon in which interactions between the enzyme and nonreacting portions of the substrate not only pay the energetic cost of catalysis[23] but also enforce one of several mechanistic alternatives available to the enzyme.[3,10,24]

Supplementary Material Available: Table giving the analysis of proton inventories for the hydrolyses of acyl-enzymes of serine proteases and figures showing the 16 proton inventories discussed in the text (18 pages). Ordering information is given on any current masthead page.

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Communications to the Editor

Studies on Tumor Promoters.[1] The First Synthesis of the Phorbol Skeleton

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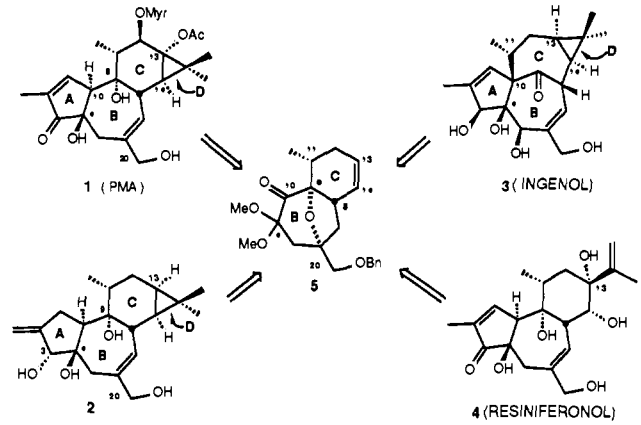
The mechanism of carcinogenesis has been the focus of global research for most of this century.[3] The early view of a unifactorial relationship between carcinogen and tumor formation was expanded to accommodate a multistage mechanism in the 1930s when it was demonstrated that several noncarcinogenic compounds, now referred to as tumor promoters, amplified the effect of carcinogens on mouse skin. The most potent of these compounds was later shown to be phorbol myristate acetate (**1**, PMA; Scheme I).[3] More recently, the major PMA receptor was identified as protein kinase C (PKC)[4]—a ubiquitous enzyme of great organomedicinal interest because of its critical role in hormonal signal transduction in cells. We describe herein the first synthesis of a stereochemically complete tigliane skeleton in the form of phorboid **2**. This compound is a designed hybrid of the tigliane and ingenane promoters which was formulated to probe systematically our recently proposed pharmacophore model for PKC activation[1a] and to define the structural requirements for diterpene recognition at the PKC regulatory domain.[1,5]

Our program goals necessitated the development of a divergent synthetic strategy in order to service studies on the tigliane, ingenane, and daphnane promoters (**1**, **3**, and **4**, respectively).[6] The

Scheme I



strategy which best met this objective called for the construction of a BC ring system (e.g., **5**) common to two families (**1** and **4**) and convertible to the third (**3**) through rearrangement (C11 to C10). Subsequent attachment of the A ring to C4 and C10 in **5** and the D ring to C13 and C14 or an isopropenyl to C13 would then provide access to all three skeleta. The seemingly superfluous oxygen bridge in **5** was incorporated in this plan to provide internal protection of the C-9 oxygen and to convert the otherwise flexible seven-membered B-ring and cyclohexenyl C-ring into a conformationally and facially biased tricyclic system capable of guiding the genesis of key stereocenters. A further attribute of this plan was the potential ready availability of the key intermediate **5** through the intramolecular Diels-Alder reaction of the triene **9** (Scheme II).

The execution of the above strategy, initially entailing the synthesis of **9**, started with the hetero-Diels-Alder reaction of 2-methoxybutadiene[7a] and ethyl glyoxalate,[7b] from which only one pyran product was obtained (**6**,[8] 60%). Introduction of the diene

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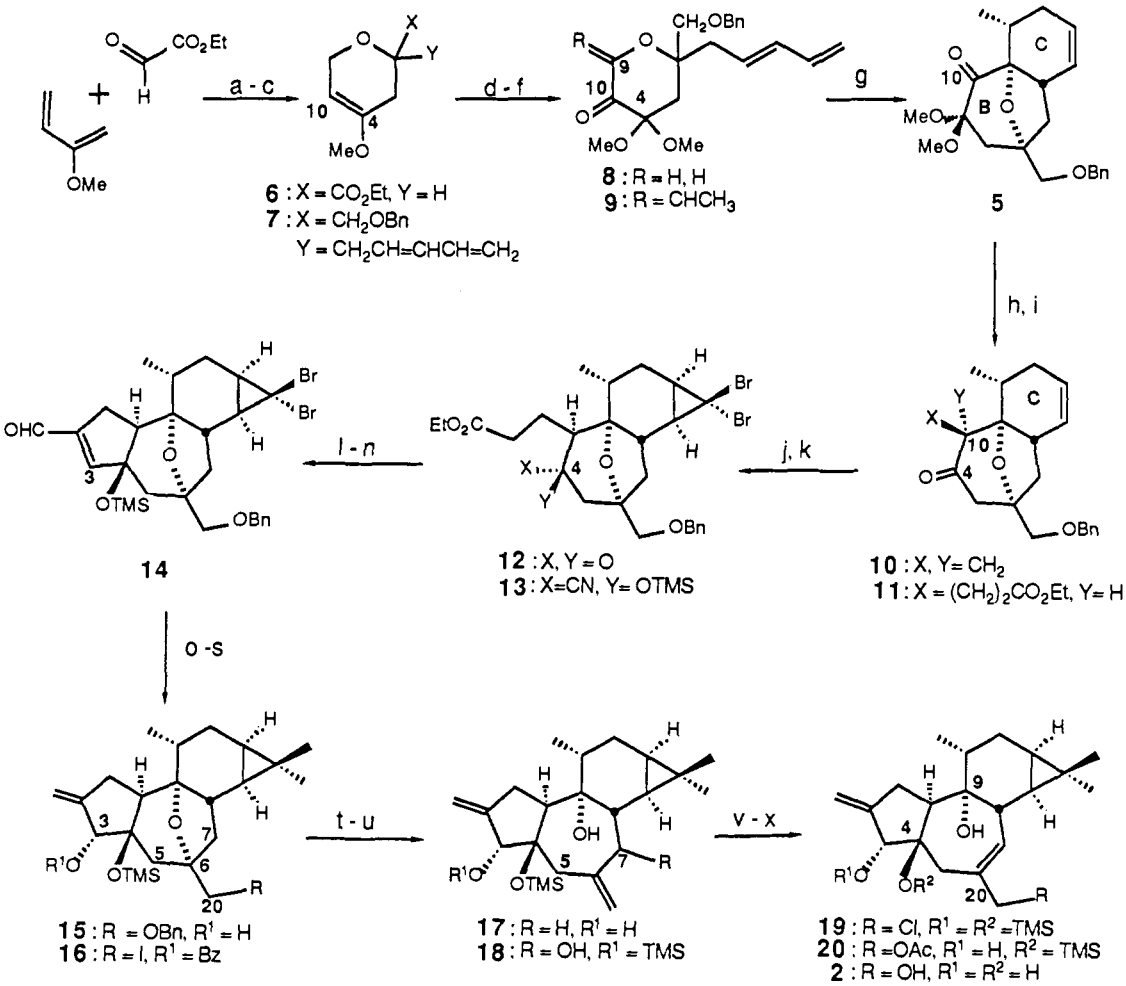
Scheme II[a]



[a] (a) 110 °C, toluene. (b) (i) LDA/THF, −78 °C; CH_2=CHCH=$CHCH_2Br$, −78 → 0 °C; (ii) LAH/THF, 0 °C. (c) BnBr, $(Bu)_4NI$, NaH/THF, room temperature. (d) (i) *m*-CPBA/MeOH, 0 °C; (ii) $(COCl)_2$, Me_2SO/CH_2Cl_2, −60 °C; Et_3N. (e) $LiN(TMS)_2$, LiBr/THF, −78 °C; CH_3CHO, −78 °C. (f) (i) MsCl, Et_3N/CH_2Cl_2; (ii) DBU/THF. (g) 145 °C/xylene. (h) (i) Ph_3P=CH_2/toluene, 105 °C; (ii) $(COOH)_2$, SiO_2/CH_2Cl_2. (i) (i) CH_2=C(OTBS)OEt, ZnI_2/CH_2Cl_2; (ii) HF/CH_3CN. (j) PhHgCBr_3/benzene, 80 °C. (k) TMSCN, ZnI_2/CH_2Cl_2. (l) DIBAH/toluene, −78 → 0 °C. (m) $(COCl)_2$, Me_2SO/CH_2Cl_2, −60 °C; Et_3N. (n) $Bn_2NH_2CF_3CO_2$/benzene. (o) DIBAH/toluene, −78 °C. (p) $Me_2CuCNLi_2$/ether, −20 °C; MeI. (q) (i) *o*-NO_2PhSeCN, Bu_3P/pyridine; (ii) H_2O_2/THF. (r) (i) Bz_2O, DMAP, Et_3N/CH_2Cl_2; (ii) ZnI_2/TMSCN. (s) (i) Tf_2O, pyridine/CH_2Cl_2; (ii) Bu_4NI/HMPA. (t) *t*-BuLi/THF, −78 °C. (u) (i) TMS-Im/CH_2Cl_2; (ii) SeO_2, $(CH_3)_3COOH$/CH_2Cl_2, 0 °C. (v) $SOCl_2/Et_2O$, 0 °C. (w) KOAc–TMEDA, AgOAc/CH_3CN. (x) TBAF/THF.

subunit required in **9** was achieved by treatment of **6** with LDA and 1-bromo-2,4-pentadiene, which after LAH reduction and protection of the resultant C-20 alcohol gave ether **7**[9] (82% for three steps). Selective oxidation of **7** with *m*-CPBA in methanol[10] afforded epimeric C-10 alcohols which upon Swern oxidation[11] were converted to keto ketal **8** (65% for two steps), thereby setting the stage for introduction of the ethylidene group at C-9. Unfortunately, crossed-aldol condensation of acetaldehyde with the enolate of **8** proved difficult. However, when lithium bromide was added to the enolate solution,[12] the reaction proceeded smoothly and gave only two aldol products. These alcohols were dehydrated by sulfonylation followed by DBU elimination which produced the key trienone **9** as a single isomer. Gratifyingly, brief heating of **9** provided, within the limit of detection (≤1%), only the desired cycloadduct **5** (52% for four steps).[8,13] The exo-selectivity of this process can be attributed to the steric congestion

(8) Satisfactory NMR, IR, and low-resolution mass spectra and combustion or exact mass analysis were obtained for all new compounds. Partial physical data of **5**, **14**, and **2**: **5**, ^{1}H NMR ($CDCl_3$) δ 7.35–7.28 (br s, 1 H), 5.71–5.64 (m, 1 H), 5.53–5.48 (m, 1 H), 4.63 (s, 2 H), 3.54 (s, 2 H), 3.26 (s, 3 H), 3.20 (s, 3 H), 2.74–2.67 (m, 1 H), 2.50 (dd, 1 H, *J* = 9.8, 12.3 Hz), 2.42 (dd, 1 H, *J* = 0.8, 13.5 Hz), 2.33 (dt, 1 H, *J* = 6.5, 12.9 Hz), 2.17 (d, 1 H, *J* = 13.5 Hz), 1.99–1.85 (m, 2 H), 1.55 (ddd, 1 H, *J* = 1.2, 4.6, 10.7 Hz), 0.98 (d, 3 H, *J* = 6.6 Hz); IR (CCl_4) 3020, 2980, 2945, 2845, 1740, 1460, 1120, 1100, 1040 cm^{-1}; mp 72–74 °C. **14**, ^{1}H NMR ($CDCl_3$) δ 9.78 (s, 1 H), 7.35 (br s, 5 H), 7.06 (s, 1 H), 4.66 (d, 1 H, *J* = 12.3 Hz), 4.60 (d, 1 H, *J* = 12.3 Hz), 3.52 (d, 1 H, *J* = 10.3 Hz), 3.48 (d, 1 H, *J* = 10.3 Hz), 3.3–3.07 (m, 1 H), 2.85 (dd, 1 H, *J* = 6.5, 11.9 Hz), 2.42 (dd, 1 H, *J* = 6.8, 15.9 Hz), 2.34 (dd, 1 H, *J* = 10.8, 15.9 Hz), 2.24–2.17 (m, 1 H), 2.07 (d, *J* = 13.4 Hz), 2.01 (dd, 1 H, *J* = 6.8, 10.8 Hz), 1.97 (d, 1 H, *J* = 13.4 Hz), 1.94–1.87 (m, 2 H), 1.59 (dd, 1 H, *J* = 5.55, 11.9 Hz), 1.50 (dd, 1 H, *J* = 3.1, 10.6 Hz), 1.44 (dt, 1 H, *J* = 4.2, 13.4 Hz), 0.96 (d, 3 H, *J* = 7.2 Hz); IR (CCl_4) 2960, 2910, 2860, 1680, 1455, 1255, 1030, 905 cm^{-1}. **2**, ^{1}H NMR (CCl_3) δ 5.75 (br d, 1 H, *J* = 7.5 Hz), 5.31 (s, 1 H), 5.20 (s, 1 H), 4.18 (dd, 1 H, *J* = 3.6, 12.0 Hz), 4.10 (dd, 1 H, *J* = 5.2, 12.0 Hz), 3.86 (br s, 1 H), 2.92 (d, 1 H, *J* = 15.7 Hz), 2.77 (br t, 1 H, *J* = 5.0 Hz), 2.66 (dd, 1 H, *J* = 8.2, 15.6 Hz), 2.55 (dd, *J* = 9.6, 15.6 Hz), 2.44 (d, 1 H, *J* = 15.7 Hz), 2.41 (dd, 1 H, *J* = 8.2, 9,6 Hz), 2.292 (m, 1 H), 2.08 (br s, 1 H), 1.87 (t, 1 H, *J* = 7.5 Hz), 1.45 (ddd, 1 H, *J* = 1.4, 7.6, 15.0 Hz), 1.28 (t, 1 H, *J* = 15.0 Hz), 1.09 (s, 1 H), 1.07 (s, 3 H), 1.02 (s, 3 H), 1.01 (d, 3 H, *J* = 7.5 Hz), 0.81 (dd, 1 H, *J* = 7.5, 15 Hz), 0.68 (t, 1 H, *J* = 7.5 Hz).

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(13) The protecting group of **5** was replaced by methyl ether (i) ZnI_2–TMSCN, (ii) NaH, MeI/DME and its spectroscopic data were shown to be identical with that of the same compound which was made from the compound whose structure was confirmed by X-ray crystallography: Keenan, R. M. Ph.D. Thesis, Stanford University, 1986.

between the diene and C-4 methoxy group which would arise in the endo transition state.

The next phase of our synthesis called for the introduction of the A and D target rings to the B and C rings, respectively, of **5**. Toward this end, **5** was converted to **10** by using an olefination and hydrolysis sequence[14] (91%). The hetero-Diels-Alder reaction of enone **10** with the ketene acetal of ethyl acetate in the presence of zinc iodide gave a single ortholactone which, in accord with the intended stereoinductive influence of its tricyclic subunit, underwent sterically and stereoelectronically controlled protonation at C-10 to provide a single keto ester **11** (72% for two steps). Benefiting similarly from the biases inherent in the tricyclic core of **11**, introduction of the D ring by using Seyferth's reagent[15] proceeded exclusively through addition to the less hindered, convex face of the C ring and gave *gem*-dibromocyclopropane **12** (92%). Finally, C-4, the fourth stereocenter, designed to arise under the guidance of the key tricycle, was indeed set through kinetically controlled addition of cyanide[16] to the sterically less encumbered face of ketone **12**, producing **13** (72%) with 92-95% isomeric selectivity. DIBAH reduction of both the nitrile and ester groups followed by Swern oxidation gave a dialdehyde from which **14**[8] was formed by base-catalyzed intramolecular aldol condensation[17] (31.5% for three steps). Completion of the tigliane carbon network and introduction of the desired C-3 oxygen as required for our pharmacophore model were achieved through a four-step conversion of **14** to **15**[20] (DIBAH reduction, higher order cuprate substitution of halide,[18] and allylic transposition,[19] 73% for four steps).

The final phase of our synthesis required cleavage of the ether bridge in **15** and introduction of the desired B-ring allylic alcohol. Accordingly, protection of the C-3 alcohol in **15** as its benzoyl ester and deprotection of the C-20 benzyl ether (ZnI_2 in TMSCN) produced the corresponding primary alcohol (64% for two steps) which was converted into iodide **16** by a two-step procedure. *tert*-Butyllithium treatment of the iodide resulted in cleavage of the ether at C-6 and cleanly gave tetracyclic compound **17** (51% for three steps). Protection of the C-3 alcohol and SeO_2 oxidation[21] of the B-ring exocyclic olefin produced a single isomeric allylic alcohol **18**. The remarkable regioselectivity of this allylic oxidation reflects in part the greater steric hindrance at C-5 relative to C-7. Transposition of the allylic functionality in **18** was accomplished by thionyl chloride in the presence of propylene oxide as an acid scavenger. Treatment of the resultant chloride **19** with silver acetate and potassium acetate-TMEDA complex in acetonitrile gave **20** (50% for four steps). Finally, hydrolysis of protecting groups provided phorboid **2** (>70%, oil).[8]

Phorboid **2** is the first compound possessing the complete tigliane skeleton and stereochemistry to be prepared through total synthesis. It incorporates seven of the eight stereocenters of PMA and the C-4, C-9, and C-20 functionalities putatively required for biological activity. However, unlike PMA but like the ingenane promoters, it is devoid of oxygenation at C-12 and C-13 and possesses A-ring functionalities required for conformational rigidity and for the attachment of lipophilic groups. The above strategy and the availability of phorboid **2** and its derivatives open new opportunities for the investigation of carcinogenesis at the molecular level. Pharmacological and further synthetic studies are in progress.

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Matrix Isolation of the First Silanediimine, *N,N'*-Bis(trimethylsilyl)silanediimine

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Studies of multiple bonding between silicon and nitrogen[1-3] have recently culminated in the isolation of several kinetically stable silanimines.[1] However, the existence of the silanediimine structure (RN=Si=NR),[4] containing one more double bond than the related silanimine ($R_2Si{=}NR$), has yet to be unambiguously demonstrated. In this paper, we present data from UV spectroscopy and trapping experiments that provide compelling evidence for the formation of the first silanediimine, *N,N'*-bis(trimethylsilyl)silanediimine.

Photolysis (254 nm) of $(Me_3Si)_2Si(N_3)_2$[5] (**1**) in a mixture of Me_3SiOMe and methylcyclohexane at room temperature initially gives **2**, believed to result from addition of the alkoxysilane[6] to the silanimine formed from a 1,2-trimethylsilyl migration, eq 1. Further photolysis of this solution produces **3**, the formation of which is attributed to a second migration-addition step.[7,8]

$$(Me_3Si)_2Si(N_3)_2 \xrightarrow{h\nu} [Me_3SiSi(N_3){=}NSiMe_3] \xrightarrow{Me_3SiOMe} \underset{\mathbf{1}}{}$$

$$Me_3SiSi(OMe)(N_3)N(SiMe_3)_2 \xrightarrow{h\nu} \underset{\mathbf{2}}{}$$

$$[(Me_3Si)_2NSi(OMe){=}NSiMe_3] \xrightarrow{Me_3SiOMe} [(Me_3Si)_2N]_2Si(OMe)_2 \quad (1)$$

$$\underset{\mathbf{3}}{}$$

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(5) Diazide **1** was synthesized from $(Me_3Si)_2SiCl_2$ and NaN_3 in refluxing toluene and purified by vacuum distillation [55-57 °C (0.05 mm Hg)]. ^1H NMR ($CDCl_3$, 200 MHz) δ 0.28 (s); IR (neat) 3430, 2970, 2910, 2130, 2115, 1295, 1250, 835 cm^{-1}; HRMS (30 eV, EI) calcd for $C_6H_{18}Si_3N_6$ 258.0897, found 258.0901.
(6) Alkoxysilanes undergo addition across silicon-nitrogen double bonds. See: Elsheikh, M.; Pearson, N.; Sommer, L. *J. Am. Chem. Soc.* **1979**, *101*, 2491-2492. Wiberg, N.; Preiner, G. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 362-363.
(7) Compound **2** was isolated by preparative GC as a colorless liquid: ^1H NMR (C_6D_6, 270 MHz) δ 3.32 (s, 3 H), 0.25 (s, 18 H), 0.20 (s, 9 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 49.58, 4.85, -0.96; IR (neat) 3420, 2950, 2900, 2840, 2140, 1260, 1080, 955, 900, 850 cm^{-1}; HRMS (30 eV, EI) calcd for $C_9H_{27}Si_4N_4O$ ($M^+ - 15$) 319.1262, found 319.1265. Compound **3** was isolated in the same manner as a waxy solid (mp 139.0-142.5 °C): ^1H NMR (C_6D_6, 270 MHz) δ 3.30 (s, 6 H), 0.34 (s, 36 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 49.72, 5.23; IR (neat) 2950, 2900, 2840, 1270, 1255, 1120, 950, 910, 865, 850 cm^{-1}; HRMS (EI, 30 eV) calcd for $C_{13}H_{39}H_2O_2Si_5$ ($M^+ - 15$) 395.1858, found 395.1849. Anal. $(C_{14}H_{42}N_2O_2Si)_5$ C, H, N.

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