the derivatization reaction. (*R*)-(−)-*O*-Acetylmandelate esters were prepared in the same manner as described.

Fermentation Methods. *B. cereus* UI-1477 (University of Iowa, College of Pharmacy Culture Collection), and *S. griseus* ATCC 10137 were used as biocatalysts.[25] Cultures were grown according to a standard two-stage fermentation protocol in 125-mL DeLong culture flasks holding 25 mL each of a soybean meal–glucose culture medium.[39,41] Cultures were incubated at 27 °C on New Brunswick Scientific Model G-25 Gyrotory incubator shakers operating at 250 rpm.

Hydroxylations of 1,4-Cineole with *B. cereus*. (a) Preparative-scale incubations were conducted with 16 125-mL flasks, each of which received 42 mg of 1,4-cineole substrate. After 24 h, TLC analysis indicated that the substrate had been completely converted into hydroxy-1,4-cineole products. The hydroxy-1,4-cineoles were isolated by exhaustive methylene chloride extraction (4 × 500 mL) of the combined cultures. The organic solvent was carefully removed under vacuum to leave a residue of 985 mg. The concentrated extract containing volatile cineoles was purified over a column of $ZnSO_4$–silica gel (100 g, 3 × 30 cm) eluted with hexane–ethyl acetate (4:1) to afford 158 mg (21.3%) of pure (GC) 2-*endo*-hydroxy-1,4-cineole and 28 mg (3.8%) of 2-*exo*-hydroxy-1,4-cineole. These compounds were subjected to derivatization as described, and the resulting (*S*)-(+)-*O*-acetylmandelate esters were purified by column chromatography before being examined by ^1H NMR at 360 MHz. The 2-*endo*-hydroxy-1,4-cineole ester was found to be exclusively **10a**, while the 2-*exo*-hydroxy-1,4-cineole ester was identified as exclusively **4a**.

Kinetics of 1,4-Cineole Hydroxylation. This incubation with *B. cereus* was conducted as described.[25] Duplicate flasks containing 42 mg of 1,4-cineole (**1**) were harvested at 24, 48, and 72 h following substrate additions. Cultures were extracted exhaustively with three 25-mL portions of dichloromethane; extracts were combined and dried over anhydrous sodium sulfate and analyzed by GC to show that each sample contained 14 mg (30%) of hydroxylated cineoles of which 85% was 2-*endo*-hydroxy-1,4-cineoles and 14% was 2-*exo*-hydroxy-1,4-cineoles. Each of the 24-, 48-, and 72-h samples were subjected to derivatization with (*S*)-(+)-*O*-acetylmandelic acid, and the proportions of diastereomeric esters **6a** and **10a** were determined by 360-MHz ^1H NMR analysis to be 2.2% and 97.8%, 2.3% and 97.7%, and 3.0 and 97%, respectively.

Hydroxylations of 1,4-Cineole with *S. griseus*. *S. griseus* were grown in 550 mL of medium containing 970 mg of unlabeled 1,4-cineole. TLC analysis indicated that the substrate had been consumed at 72 h at which time the cultures were combined and extracted four times with 150 mL of dichloromethane. The extracts were combined, dried over anhydrous sodium sulfate, and concentrated to a dark oil, with a composition of *endo*- (6%), *exo*- (3%), and 8-hydroxy-1,4-cineole (18%) similar to that observed before.[25] The extract was subjected to column chromatography over silica gel containing 10% zinc sulfate with hexane–ethyl acetate (4:1) as solvent to give 12 mg (1.2%) of pure 2-*endo*-hydroxy-1,4-cineoles and 5 mg (0.5%) of pure 2-*exo*-hydroxy-1,4-cineoles. These samples were esterified with (*S*)-(+)-*O*-acetylmandelic acid and the diastereomeric ester mixtures **6a** and **10a**, and **4a** and **8a** were determined by NMR analysis (Table I).

Acknowledgment. We are grateful for a research grant provided by Shell Development Co., Modesto, CA, in support of this work.

Supplementary Material Available: ^{13}C NMR chemical shifts for (*S*)-(+)-*O*-acetylmandelate esters of enantiomeric *endo*-hydroxy-1,4-cineoles (**6a** and **11a**) (1 page). Ordering information is given on any current masthead page.

(41) Goswami, A.; Schaumberg, J. P.; Duffel, M. W.; Rosazza, J. P. *J. Org. Chem.* **1987**, *52*, 1500.
(42) Betts, R. B.; Walters, D. E.; Rosazza, J. P. *J. Med. Chem.* **1974**, *17*, 599–603.

Synthesis of 3,6-Dichloro-4-(2-chloro-1,1-dimethylethyl)pyridazine

Ronald E. Hackler,* Barry A. Dreikorn, George W. Johnson, and David L. Varie

Lilly Research Laboratories, A Division of Eli Lilly and Company, Greenfield, Indiana 46140

Received June 10, 1988

3,6-Dichloro-4-(2-chloro-1,1-dimethylethyl)pyridazine (**3**) is a systemic plant fungicide whose synthesis by several routes is described. Free-radical alkylations of 3,6-dichloropyridazine (**1**) gave either the 4-*tert*-butyl derivative **2** or the alcohol 3,6-dichloro-β,β-dimethyl-4-pyridazineethanol (**24**). Pyridazine **2** must be subjected to a free-radical chlorination, which resulted in other products, but alcohol **24** could be smoothly converted to **3**. Alternatively, the pyridazine ring was constructed with the side chain preattached by utilizing lactone intermediates **10** or **18**. The lactone **10** with hydrazine yielded the ether **11**, and chlorination with ether cleavage proceeded to **3**. Lactone **20** could not be transformed to **3**.

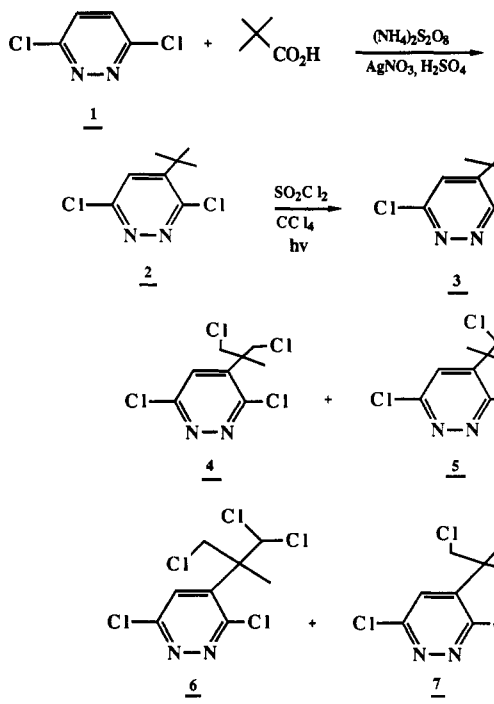
3,6-Dichloro-4-(2-chloro-1,1-dimethylethyl)pyridazine (**3**) is a systemic fungicide that controls several pathogenic *Phycomycetes* organisms in plants.[1] It was originally synthesized in our laboratories by a free-radical chlorination of **2** using sulfuryl chloride. We describe in this paper a diversity of approaches to the synthesis of **3**. One can begin with the dichloropyridazine **1** and attach the side chain, or the pyridazine ring can be formed with the side chain already attached.

Free-Radical Chlorination of 2. 3,6-Dichloro-4-alkylpyridazines such as **2** are made by a free-radical alkylation of 3,6-dichloropyridazine (**1**) with a silver-catalyzed oxidation of carboxylic acids to generate the alkyl radicals.[2] The use of pivalic acid leads to a high yield of **2**, resulting from attack of the *tert*-butyl radical on the pyridazine ring. When chloropivalic acid was used in this alkylation reaction, however, no **3** was observed. We assume the chloro-*tert*-butyl radical underwent homolytic cleavage to isobutene and chlorine radicals. This observation led to our chlorination of the *tert*-butyl group directly in order to synthesize **3**.

Random chlorination of the *tert*-butyl group of **2** does not become a problem until significant amounts of **3** are present. When the chlorination of **2** was stopped at

(1) Dow, W. C.; Johnson, G. W.; Arnold, W. R. Eur. Pat. Appl. 208431, 1987.
(2) (a) Samaritoni, J. G. *Org. Prep. Proc. Intl.* **1988**, *20*, 117. (b) Samaritoni, J. G. U.S. Pat. 4628088, 1986.

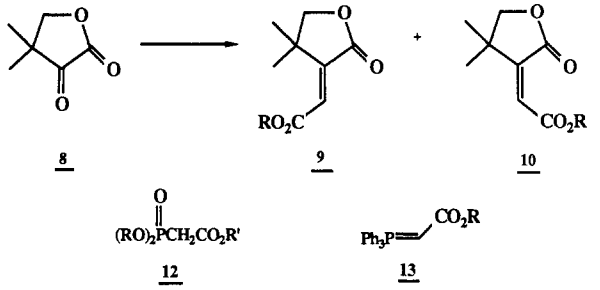
0022-3263/88/1953-5704$01.50/0



20–23% completion, **3** was the only significant product, and it could be separated by chromatography while **2** was recycled. Allowing the chlorination to proceed beyond this percentage resulted in the formation of the additional products **4–7**.

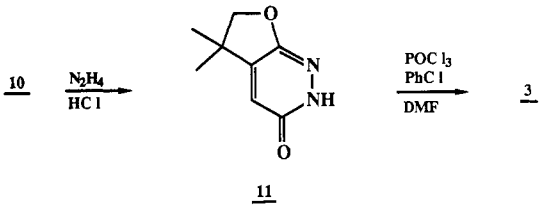
Lactones as Intermediates to Substituted Pyridazines. Seeking to avoid the random chlorination, we looked for methods to synthesize **3** in which the pyridazine ring is formed with a side chain preattached. The keto lactone **8** emerged as an easily prepared[3,4] intermediate, which permits the hydroxy-*tert*-butyl group to be masked as a lactone.

The isomeric ester lactones **9** and **10** are formed by Wittig reactions,[5] but we soon learned that only the *Z* isomer **10** could be used for pyridazine ring formation. Use of the Wadsworth–Emmons modified Wittig reagents **12** gave *Z*:*E* ratios that were very dependent upon the size of R′. When R and R′ were both methyl, the *E* isomer predominated by 9:1. When R was ethyl, and R′ was *tert*-butyl, the *Z* isomer predominated by 4:1.

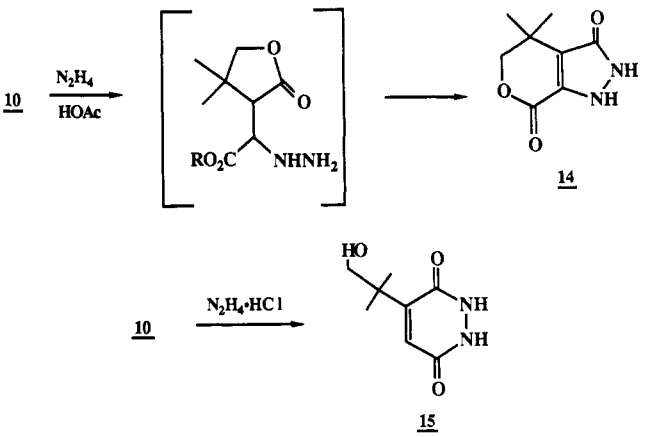


These isomers were separated by chromatography on silica gel in dichloromethane, with the *E* isomer being eluted first. The ^{1}H NMR spectrum for the *E* isomer displayed lower field shifts for both the olefinic proton and the gem-dimethyl group. The structural assignments were confirmed by NOE studies.

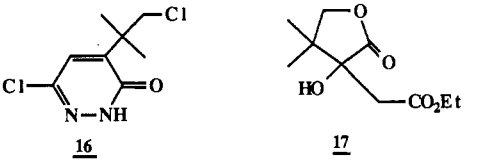
Reaction of **8** with triphenylphosphorane Wittig reagents **13** gave a preponderance of the *Z* isomer **10**. We observed a *Z*:*E* ratio of 9:1 when R was methyl, even higher when R was ethyl, and a literature report[5] states that no *E* isomer **9** is formed when R is *tert*-butyl.



Conversion of **10** to **11** was accomplished in 52% yield with hydrazine in aqueous hydrochloric acid and required the more acidic conditions. When hydrazine in acetic acid was employed, the major product was **14**, apparently the result of Michael addition to the double bond, opening of the five-membered lactone, and formation of a six-membered lactone. An oxidation step is required to complete the conversion, and it is presumed to be an air-oxidation. In addition to formation of **14**, we have also observed small yields of **15**. No attempts were made to optimize the yield of **15**, but it was formed by the use of hydrazine hydrochloride in water with no excess acid.

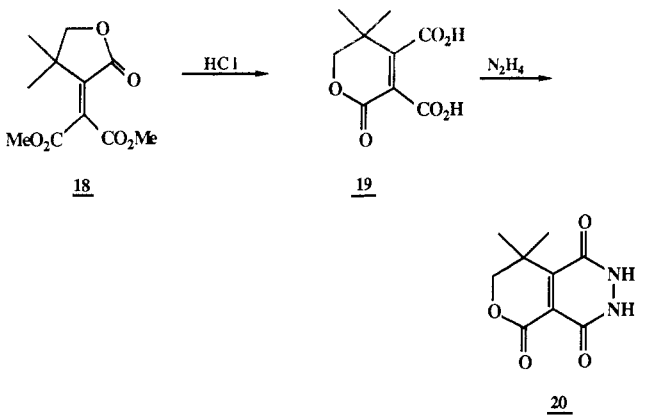


Compound **11** was transformed to the desired product **3** in 50% yield by the use of phosphorus oxychloride in chlorobenzene with DMF catalysis. In addition, the partially chlorinated product **16** was isolated in 15% yield. Lactone **8** underwent a smooth Reformatsky reaction to form **17**, but we were unable to convert **17** to pyridazine derivatives. Dehydration of **17** gave predominately **9** and very little of the required *Z* isomer **10**.



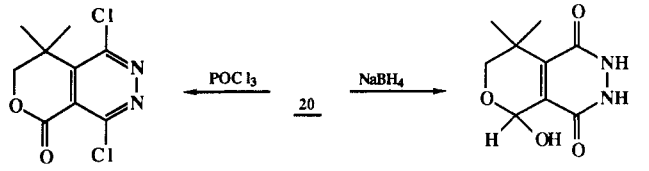
We then sought to avoid the inefficiency and ambiguity resulting from the possible presence of the useless isomer **9**. We turned to the diester **18**, made by a literature procedure[6] from **8**. Treatment of **18** with hydrazine in aqueous hydrochloric acid resulted first in a buildup of the intermediate diacid **19**,[6] which was further transformed to a 70% yield of product **20**. No conditions could be found for decarboxylation in this reaction, and isolated **20** proved to be remarkably resistant to hydrolysis, ammonolysis, or decarboxylation.

(3) Ojima, I.; Kogure, T.; Yoda, Y. *Org. Synth.* **1985**, *63*, 18.
(4) Fizet, C. *Helv. Chim. Acta* **1982**, *65*, 2024.
(5) Falsone, G.; Spur, B.; Erdmann, M. *Arch. Pharm. (Weinheim)* **1983**, *316*, 530.
(6) Falsone, G.; Spur, B. *Justus Liebigs Ann. Chem.* **1979**, 923.



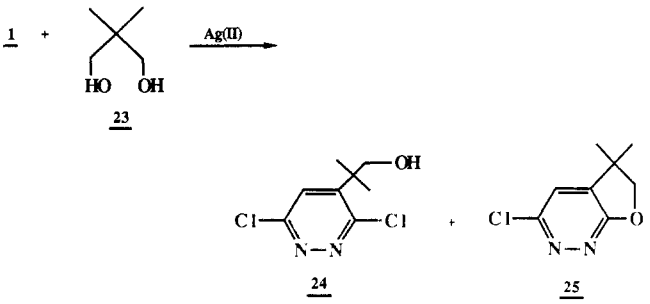
18 19 20

Treatment of **20** with phosphorus oxychloride produced the dichloro derivative **21**, but we were never able to convert **20** or **21** to **3**. Treatment of **20** with sodium borohydride led to a 68% yield of **22**, but it also was resistant to further transformations to **3**.



21 20 22

Addition of the Hydroxy-*tert*-butyl Radical to 1. We then considered a modification of the radical alkylation of 3,6-dichloropyridazine **1**, which would give us a functionalized *tert*-butyl group, which could be transformed to chloro-*tert*-butyl. Treatment of 2,2-dimethyl-1,3-propanediol **23** with silver(II) (generated from acidic silver(I) nitrate and ammonium persulfate)[7] in the presence of **1** gave alcohol **24** and ether **25** in 66% and 11% yields, respectively. Although hydroxyalkyl radicals have been previously generated, we are unaware of their use to alkylate heterocyclic ring systems.

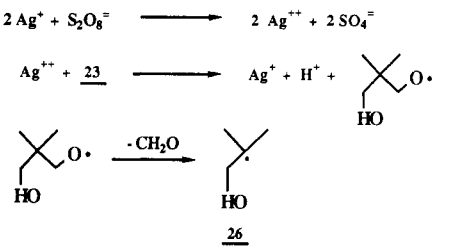


1 + 23 → 24 + 25

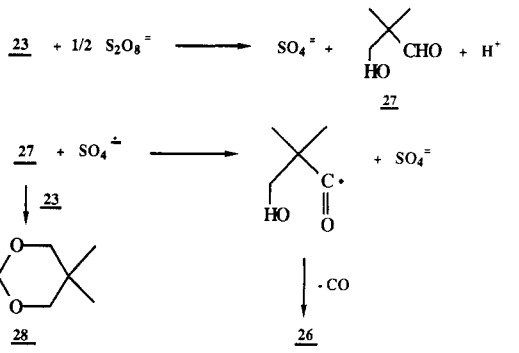
Alcohol **24** was obtained in 55–60% isolated yield by recrystallization of product mixtures of **24** and **25** from toluene. The optimum yields of **24** were obtained by using 1 equiv of trifluoroacetic acid and 0.5–1.0 equiv of silver nitrate in water. While control experiments showed that both acid and silver nitrate accelerate the rate of hydrolysis of **1**, omission of an acid or use of less than 0.5 equiv of silver nitrate gave 20–30% yields of recovered **1**.

The formation of the necessary radical **26** for the alkylation reaction is presumed to occur via the known[8] fragmentation of an alkoxy radical (Scheme I). Omission

Scheme I

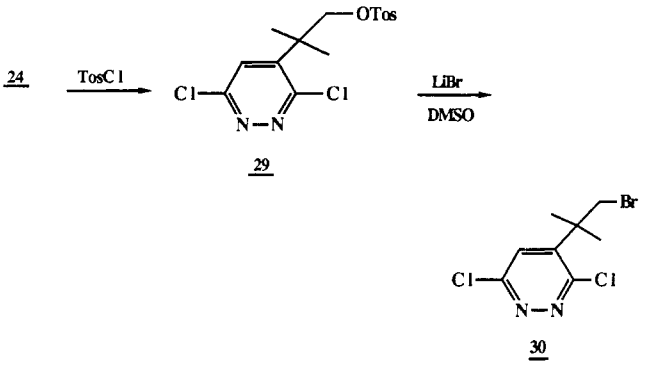


Scheme II



of silver nitrate from the reaction mixture gave **24** in only 10% yield, along with 3% cyclic ether **25** and acetal **28**. The formation of **28** presumably involves the intermediacy of 3-hydroxy-2,2-dimethylpropionaldehyde **27**, and suggests that in the non-silver-catalyzed reaction, radical **26** may form via an oxidative decarbonylation of **27**[9] (Scheme II). Indeed, reaction of an aqueous solution of aldehyde **27** with persulfate in the presence of **1** and the absence of silver gave an 80:20 mixture of **24** and **25** in 40% yield.

Alcohol **24** was treated with thionyl chloride/pyridine to give the desired product **3** in 76% yield. The tosylate **29** could be prepared from **24** in 80% yield. Treatment of **29** with lithium bromide in DMSO afforded bromo analogue **30** in 91% yield.



24 29 30

Conclusion

The free radical alkylation of **1** followed by chlorination of the *tert*-butyl group is a short route to compound **3**, but the chlorination is plagued by products of random substitution. The Wittig chemistry gives the product most unambiguously, but requires stereochemical control of the lactone ester. The bis(ester lactone) gives the pyridazine ring in high yield, but leaves one with an intermediate that cannot be further transformed. Finally, the novel addition of the hydroxy-*tert*-butyl radical to **1** gives an intermediate

(7) Anderson, J. M.; Kochi, J. K. *J. Am. Chem. Soc.* **1970**, *92*, 1651.
(8) (a) Clerici, A.; Minisci, F.; Ogawa, K.; Surzur, J.-M. *Tetrahedron Lett.* **1978**, 1149. (b) Caronna, T.; Citterio, A.; Grossi, L.; Minisci, F.; Ogawa, K. *Tetrahedron* **1976**, *32*, 2741. (c) Ledwith, A.; Russell, P. J.; Sutcliffe, L. H. *J. Chem. Soc., Perkin Trans. 2* **1973**, 630.

(9) Caronna, T.; Galli, R.; Malatesta, V.; Minisci, F. *J. Chem. Soc. C* **1971**, 1747 and references cited therein.

24, which is readily converted to 3 or the bromo analogue 30.

Experimental Section

Melting points were determined on a Mel-Temp open capillary melting point apparatus and are uncorrected. ^1H NMR spectra were recorded at 60, 80, 90, or 250 MHz. ^{13}C NMR spectra were recorded at 75 MHz. Preparative chromatography was done with a Waters Associates Prep LC/System 500 using PrepPak-500 silica cartridges.

Radical Chlorination of 4-*tert*-Butyl-3,6-dichloropyridazine (2). The radical chlorination reactions were run in CCl_4 with a 250-W infrared reflector lamp, positioned such that the desired temperature was maintained by the heat of the lamp.

Preparation of 3,6-Dichloro-4-(2-chloro-1,1-dimethylethyl)pyridazine (3). A solution of 2 (100 g, 0.49 mol), benzoyl peroxide (0.025 g), and sulfuryl chloride (65.8 g, 0.49 mol) in CCl_4 (250 mL) was stirred under N_2 and irradiated at 58 °C for 1.5 h. ^1H NMR analysis of an aliquot indicated the presence of 76% 2, 22% 3, and 2% 4. Concentration under vacuum afforded a tan oil (110 g), which was subjected to preparative chromatography with 10:1 heptane–ethyl acetate. Eluted first was starting material 2 (67 g), followed by a broad band containing 3 and 4. Removal of the front portion of this band left 3 (22.8 g) as an oil, which solidified. Recrystallization from heptane gave 3 as a white, crystalline solid, mp 64–5 °C: ^1H NMR ($CDCl_3$) δ 7.48 (s, 1 H), 4.03 (s, 2 H), and 1.58 (s, 6 H); IR (KBr) 3050, 2980, 1560, 1392, 1374, 1334, 1285, 1160, 1145, 1122, 1087, 738 cm^{-1}. Anal. Calcd for $C_8H_9Cl_3N_2$: C, 40.11; H, 3.79; N, 11.70. Found: C, 39.92; H, 3.72; N, 11.44.

3,6-Dichloro-4-[2-chloro-1-(chloromethyl)-1-methylethyl]pyridazine (4). Irradiation was carried out as above with 2 (5.0 g, 0.024 mol), benzoyl peroxide (0.025 g), and SO_2Cl_2 (6.6 g, 0.049 mol) in CCl_4 (100 mL) for 6 h. Additional SO_2Cl_2 (6.6 g) was added, and the reaction was continued for another 7 h, at which time ^1H NMR analysis showed no 2 remaining, 4 predominating over 3 by a ratio of 6:1, with other products present in smaller amounts. Concentration yielded a tan oil (8.7 g), which was chromatographed as above. Pure fractions of 4 were combined and recrystallized from heptane to give white crystals (0.82 g), mp 92–4 °C: ^1H NMR ($CDCl_3$) δ 7.4 (s, 1 H), 4.2 (d, 2 H, J = 11 Hz), 3.85 (d, 2 H, J = 11 Hz), 1.7 (s, 3 H). Anal. Calcd for $C_8H_8Cl_4N_2$: C, 35.07; H, 2.94; N, 10.22. Found: C, 35.34; H, 3.00; N, 10.48.

3,6-Dichloro-4-(2,2-dichloro-1,1-dimethylethyl)pyridazine (5) and 3,6-Dichloro-4-[2,2-dichloro-1-(chloromethyl)-1-methylethyl]pyridazine (6). Irradiation was carried out as above with 2 (50 g, 0.24 mol), benzoyl peroxide (0.025 g), and SO_2Cl_2 (59 mL, 0.73 mol) in CCl_4 (125 mL) for 19 h. Additional SO_2Cl_2 (59 mL) was added, and the reaction continued for 6 h, at which time ^1H NMR analysis showed no 2 remaining, but all of the other products could be observed. Peaks at δ 6.72 and 6.8 were indicative of the presence of 5 and 6. Concentration under vacuum yielded a tan oil (87 g), which was purified by preparative chromatography. Compound 6 was the first product eluted from the column. Recrystallization from heptane gave a white, crystalline solid (1.26 g), mp 82–85 °C: ^1H NMR ($CDCl_3$) δ 7.4 (s, 1 H), 6.72 (s, 1 H), 4.50 (d, J = 11 Hz, 1 H), 3.77 (d, J = 11 Hz, 1 H), 1.85 (s, 3 H). Anal. Calcd for $C_8H_7Cl_5N_2$: C, 31.15; H, 2.29; Cl, 57.47; N, 9.08. Found: C, 31.23; H, 2.26; Cl, 57.73; N, 9.28. Further elution yielded a broad band containing 7 in the forward edge and 5 in the trailing edge. Appropriate cuts yielded 3.8 g of a solid, which was recrystallized from heptane to give 5 as white crystals (1.0 g), mp 57–8 °C: ^1H NMR ($CDCl_3$) δ 7.4 (s, 1 H), 6.8 (s, 1 H), 1.7 (s, 6 H). Anal. Calcd for $C_8H_8Cl_4N_2$: C, 35.07; H, 2.94; Cl, 51.76; N, 10.22. Found: C, 34.98; H, 2.89; Cl, 51.66; N, 10.11.

3,6-Dichloro-4-[2-chloro-1,1-bis(chloromethyl)ethyl]pyridazine (7). From the above chromatography was obtained an oil (5.5 g) consisting of combined fractions containing 3, 4, and 7. This oil was irradiated as above in CCl_4 (30 mL), benzoyl peroxide (0.01 g), and SO_2Cl_2 (9.3 g) for 24 h. Additional SO_2Cl_2 (9.3 g) was added, and the reaction was continued for another 24 h. Concentration under vacuum gave a tan oil (6.3 g), which was purified by chromatography, eluting with CH_2Cl_2 to give an oil (2.3 g) containing 7. A second chromatography with 10:1 heptane–ethyl acetate yielded a white solid 7 (0.22 g), mp 111–112 °C: ^1H NMR ($CDCl_3$) δ 7.3 (s, 1 H), 4.2 (s, 6 H). Anal. Calcd for $C_8H_7Cl_5N_2$: C, 31.15; H, 2.29; Cl, 57.47; N, 9.08. Found: C, 31.00; H, 2.22; Cl, 57.67; N, 9.35.

Preparation of Ester Lactones 9 and 10. The general procedure using phosphonoacetates is typified by the methyl ester, and conventional Wittig reactions using triphenylphosphoranes 13 followed closely published procedures.[5]

(*E*)- and (*Z*)-(Dihydro-4,4-dimethyl-2-oxo-3(2*H*)-furanylidene)acetic Acid, Methyl Ester (9 and 10). Method A. NaH (50%) in mineral oil (0.96 g, 0.02 mol) was washed with hexane before stirring in THF (25 mL) at 0 °C and adding dropwise trimethylphosphonoacetate (3.24 g, 0.02 mol) in THF (25 mL). Stirring was continued for 30 min before the lactone[3,4] 8 (2.56 g, 0.02 mol) was added. The solution was allowed to warm to room temperature, concentrated under vacuum, and partitioned between CH_2Cl_2 (75 mL) and H_2O (25 mL). The organic layer was dried ($MgSO_4$) and concentrated under vacuum to a white solid (3.5 g). ^1H NMR shows a 9:1 ratio of 9 to 10. Recrystallization from hexane gave 9 (R = Me, 2.3 g, 62%), mp 102–4 °C (reported[5] mp 102 °C). Flash chromatography on silica gel in CH_2Cl_2 produced additional 9 (0.6 g) and then *Z* isomer 10 (0.3 g), mp 29–31 °C (reported[5] mp 32–33 °C).

Method B. The lactone 8 (7.0 g, 0.055 mol) and methyl (triphenylphosphoranylidene)acetate (13, R = Me; 18.3 g, 0.055 mol) were stirred at room temperature in dry THF (100 mL) overnight. The solution was concentrated under vacuum, most of the triphenylphosphine oxide was crystallized from EtOAc–hexane (1:1), and the filtrate was chromatographed on silica gel in CH_2Cl_2, giving 9 (R = Me, 0.8 g, 8%) and 10 (R = Me, 8.0 g, 79%). NMR data and isomer assignments may be found in the earlier publication.[5] Additional confirmation of the isomer assignments was found in an NOE experiment in which the geminal dimethyl peaks were irradiated with a much greater response on the olefin proton of 10 than the corresponding proton in 9.

(*E*)- and (*Z*)-(Dihydro-4,4-dimethyl-2-oxo-3(2*H*)-furanylidene)acetic Acid, Ethyl Ester (9 and 10). Method A. Reaction as above with triethylphosphonoacetate (5.6 g, 0.025 mol) and 8 (3.2 g, 0.025 mol) led to a mixture of isomers, which were separated as above.

Compound 9 (R = Et) was obtained as white crystals (2.1 g, 42%) mp 48–49 °C: ^1H NMR ($CDCl_3$) δ 6.8 (s, 1 H), 4.26 (q, 2 H), 4.06 (s, 2 H), 1.46 (s, 6 H), and 1.33 (t, 3 H). Anal. Calcd for $C_{10}H_{14}O_4$: C, 60.59; H, 7.12. Found: C, 60.30; H, 7.24.

Compound 10 (R = Et) was obtained as white crystals (1.0 g, 20%), mp 39–41 °C: ^1H NMR ($CDCl_3$) δ 6.2 (s, 1 H), 4.3 (q, 2 H), 4.03 (s, 2 H), 1.27 (s, 6 H), and 1.34 (t, 3 H). Anal. Found: C, 60.53; H, 7.07.

Method B. Ethyl (triphenylphosphoranylidene)acetate (34.5 g, 0.099 mol) and lactone 8 (12.7 g, 0.099 mol) in THF (300 mL) as above yielded 10 (R = Et, 16.8 g, 86%). A small amount of 9 (R = Et) was present, but not obtained pure.

(*E*)- and (*Z*)-(Dihydro-4,4-dimethyl-2-oxo-3(2*H*)-furanylidene)acetic Acid, *tert*-Butyl Ester (9 and 10). Reaction as above with *tert*-butyl (diethylphosphono)acetate[10] (6.3 g, 0.025 mol) and 8 (3.2 g, 0.025 mol) led to a mixture of isomers separated as above.

Compound 9 (R = *t*-Bu) was obtained as white crystals (0.7 g, 12%), mp 42–3 °C: ^1H NMR $CDCl_3$ δ 6.7 (s, 1 H), 4.03 (s, 2 H), 1.50 (s, 9 H), and 1.41 (s, 6 H). Anal. Calcd for $C_{12}H_{18}O_4$: C, 63.70; H, 8.02. Found: C, 63.94; H, 7.93.

Compound 10 (*R* = *t*-Bu) was obtained as white crystals (3.5 g, 62%), mp 69–72 °C: ^1H NMR ($CDCl_3$) δ 6.2 (s, 1 H), 3.98 (s, 2 H), 1.48 (s, 9 H), and 1.22 (s, 6 H).

Tetrahydro-3-hydroxy-4,4-dimethyl-2-oxo-3-furanacetic Acid, Ethyl Ester (17). Lactone 8 (3.2 g, 0.025 mol) and ethyl bromoacetate (3.7 g, 0.022 mol) were dissolved in benzene (4 mL) and ether (1 mL), and a portion was added to powdered Zn (1.6 g, 0.025 g-atom). The mixture was heated until exotherm began, and then the heat was removed and the remainder of the solution of reactants was added dropwise at such a rate as to maintain reflux. Reflux was maintained for 30 min after addition, and then

(10) Erion, M. D.; Walsh, C. T. *Biochemistry* **1987**, *26*, 3417.

the reaction mixture was cooled in an ice bath, and 10% H_2SO_4 (10 mL) was cautiously added. The layers were separated, and the organic layer was washed with 5% H_2SO_4 (2 × 2.5 mL). The combined acid layers were extracted with ether (10 mL), and then the combined organic layers were washed with 10% Na_2CO_3 (10 mL) and H_2O (5 mL) before drying ($MgSO_4$) and removing the solvent under vacuum to leave a clear oil (3.6 g, 76%): ^1H NMR ($CDCl_3$) δ 5.35 (s, OH), 4.25 (q, 2 H), 4.23 (d, 1 H), 3.86 (d, 1 H), 2.52 (s, 2 H), 1.31 (t, 3 H), 1.12 (s, 3 H), and 1.02 (s, 3 H); MS (FAB), *m/z* 217. Anal. Calcd for $C_{10}H_{16}O_5$: C, 55.55; H, 7.46. Found: C, 55.61; H, 7.23.

1,2,4,5-Tetrahydro-4,4-dimethylpyrano[3,4-*c*]pyrazole-3,7-dione (14). The methyl ester **10** (4.3 g, 0.023 mol), 85% $N_2H_4 \cdot H_2O$ (6 mL), and acetic acid (25 mL) were stirred and heated under reflux overnight. The solvent was removed under vacuum, and H_2O (25 mL) was added. Product **14** was collected as a white solid (2.0 g, 47%), mp 250–252 °C: ^1H NMR (DMSO-d_6) δ 12.80 and 10.15 (exchangeable NH's), 4.12 (s, 2 H), and 1.22 (s, 6 H); ^{13}C NMR (DMSO-d_6) δ 23.3 (Me_2), 30.6 (CMe_2), 79.5 (CH_2), 114.2, 128.0, 156.2, 158.3; MS, *m/z* 182. Anal. Calcd for $C_8H_{10}N_2O_3$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.97; H, 5.54; N, 15.15.

1,2-Dihydro-4-(2-hydroxy-1,1-dimethylethyl)-3,6-pyridazinedione (15). The ethyl ester **10** (1.1 g, 0.0056 mol), $N_2H_4 \cdot HCl$ (0.82 g, 0.012 mol), and H_2O (15 mL) were stirred and heated under reflux overnight. The product mixture was cooled and extracted with EtOAc, and the extract was dried ($MgSO_4$) and concentrated to a small volume. The mixture was cooled, and white crystals of **15** were collected (0.6 g, 58%), mp 231 °C: ^1H NMR (DMSO-d_6) δ 11.7 and 10.6 (NH's), 6.73 (s, 1 H), 3.61 (s, 2 H), and 1.19 (s, 6 H); MS, *m/z* 184. Anal. Calcd for $C_8H_{12}N_2O_3$: C, 52.17; H, 6.57; N, 15.21. Found: C, 51.92; H, 6.69; N, 15.14.

5,6-Dihydro-5,5-dimethylfuro[2,3-*c*]pyridazin-3(2*H*)-one (11). The ethyl ester **10** (2.5 g, 0.013 mol), $N_2H_4 \cdot HCl$ (2.0 g, 0.029 mol), and 3 N HCl (40 mL) were stirred and heated under reflux overnight. The mixture was evaporated to a small volume, and a white solid was collected and recrystallized from H_2O to give white crystals (1.1 g, 52%), mp 199–202 °C: ^1H NMR (DMSO-d_6) δ 9.75 (NH), 6.77 (s, 1 H), 4.24 (s, 2 H), and 1.34 (s, 6 H); MS (FAB) 167 (M + 1). Anal. Calcd for $C_8H_{10}N_2O_2$: C, 57.82; H, 6.07; N, 16.85. Found: C, 57.59; H, 5.97; N, 16.73.

Chlorination of 11 To Give 3 and 16. The pyridazinone **11** (0.65 g, 0.0039 mol), $POCl_3$ (4 mL), PhCl (15 mL), and DMF (10 drops) were stirred and heated under reflux for 4 h. The solvent was removed under vacuum, and the product was partitioned between CH_2Cl_2 and H_2O. The organic layer was dried ($MgSO_4$), and the solvent was removed under vacuum to leave a semisolid (0.88 g). This was chromatographed on silica gel in CH_2Cl_2, eluting **3** (0.36 g, 50%), identical with that obtained previously, and **16** as a white solid (0.12 g, 15%), mp 189–192 °C: ^1H NMR (DMSO-d_6) δ 13.16 (s, 1 H), 7.27 (s, 1 H), 4.1 (s, 2 H), and 1.31 (s, 6 H). Anal. Calcd for $C_8H_{10}Cl_2N_2O$: C, 43.46; H, 4.56; N, 12.67. Found: C, 43.49; H, 4.51; N, 12.47.

2,3,7,8-Tetrahydro-8,8-dimethyl-5*H*-pyrano[3,4-*d*]pyridazine-1,4,5-trione (20). Lactone[6] **18** (4.84 g, 0.02 mol) and $N_2H_4 \cdot HCl$ (5.48 g, 0.08 mol) were added to 3 N HCl (50 mL) and stirred and heated under reflux for 5 h. The mixture was cooled, and the solid was collected, washing with H_2O (3 × 10 mL). Vacuum drying gave yellow crystals of **20** (2.74 g, 65%), mp 244–6 °C: IR (KBr) 3160, 1710, 1649, 1608, 1553, 1420, 1397, 1030 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.5–11.5 (s, 2 H), 4.20 (s, 2 H), 1.34 (s, 6 H). Anal. Calcd for $C_9H_{10}N_2O_4$: C, 51.43; H, 4.80; N, 13.15. Found: C, 51.20; H, 4.65; N, 13.15.

1,4-Dichloro-7,8-dihydro-8,8-dimethyl-5*H*-pyrano[3,4-*d*]pyridazin-5-one (21). To PhCl (30 mL) were added hydrazide **20** (3.17 g, 0.015 mol), $POCl_3$ (4.2 mL, 0.045 mol), and DMF (1.2 mL, 0.015 mol). The gummy mass was heated with stirring at 130 °C for 17 h. The solution was cooled to 25 °C, and H_2O (30 mL) was added. The mixture was stirred for 10 min (temperature increased to 40 °C), and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers were washed with H_2O (30 mL), brine (30 mL), and saturated $NaHCO_3$ (30 mL), dried (Na_2SO_4), and evaporated under vacuum to give black crystals (2.59 g). Flash chromatography on silica gel (400 g) with 40% EtOAc in hexane gave white crystals of **21** (1.74 g, 47%), mp 141–3 °C: IR (KBr) 3480, 2970, 1753, 1521, 1466, 1410, 1382, 1372, 1315, 1233, 1227, 1114, 1078, 1048 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.27 (s, 2 H), 1.58 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 154.2, 154.1, 147.6, 124.1, 75.9, 35.1, 21.7. Anal. Calcd for $C_9H_8Cl_2N_2O_2$: C, 43.75; H, 3.42; N, 11.26. Found: C, 43.75; H, 3.26; N, 11.34.

2,3,7,8-Tetrahydro-5-hydroxy-8,8-dimethyl-5*H*-pyrano[3,4-*d*]pyridazine-1,4-dione (22). The trione **20** (10.8 g, 0.051 mol) was stirred in MeOH (100 mL) and H_2O (100 mL) as sodium borohydride was added in small portions until the yellow color had disappeared (about 3 g, 0.088 mol). The solvent was removed under vacuum, the mixture was made acidic with 2 N HCl, and the white solid was collected, washing with cold EtOAc. The solid was recrystallized from H_2O (7.3 g, 67%), mp 340 °C dec: ^1H NMR (DMSO-d_6) δ 11.8 and 10.95 (NH's), 6.8 (OH), 5.50 (s, 1 H), 3.86 (d, 1 H), 3.27 (d, 1 H), and 1.20 (s, 6 H); MS, *m/z* 211 (M − 1). Anal. Calcd for $C_9H_{12}N_2O_4$: C, 50.94; H, 5.70; N, 13.20. Found: C, 51.11; H, 5.72; N, 13.38.

3,6-Dichloro-β,β-dimethyl-4-pyridazineethanol (24). To a stirred solution of 2,2-dimethylpropanediol (**23**) (22.9 g, 0.22 mol) in H_2O (100 mL) were added 3,6-dichloropyridazine (**1**) (14.9 g, 0.1 mol) and CF_3CO_2H (9.2 mL, 0.12 mol). The mixture was heated to 35 °C, and solid $AgNO_3$ (17.0 g, 0.1 mol) was added. A solution of ammonium persulfate (39.9 g, 0.175 mol) in H_2O (75 mL) was added dropwise over 8 min during which time the reaction temperature increased to 80 °C. The mixture was cooled to 25 °C, and CH_2Cl_2 (100 mL) was added. The mixture was filtered to remove silver salts, and the cake was washed with CH_2Cl_2 (50 mL). The layers of the filtrate were separated, and the aqueous layer was washed with CH_2Cl_2 (50 mL). The combined organic layers were washed with H_2O (2 × 100 mL), dried (Na_2SO_4), and evaporated under vacuum to leave a tacky yellow solid. The solid was dissolved in toluene (50 mL) at 95 °C and filtered. The filtrate was cooled to 0 °C, and the product **24** was collected and washed with cold toluene (20 mL). Vacuum drying gave white crystals (12.96 g, 58%), mp 134–6 °C: IR (KBr) 3364, 1558, 1371, 1353, 1307, 1169, 1142, 1097, 1054 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.60 (s, 1 H), 4.40 (s, 1 H), 4.00 (s, 2 H), 1.48 (s, 6 H). Anal. Calcd for $C_8H_{10}Cl_2N_2O$: C, 43.46; H, 4.56; N, 12.67. Found: C, 43.75; H, 4.48; N, 12.67. The mother liquor was flash chromatographed on silica gel (400 g) with hexane–EtOAc (60:40) as eluant to obtain a white solid (3.62 g). ^1H NMR ($CDCl_3$) showed this to be a 60:40 mixture (8% and 11% yield) of **24** and **25**.

3-Chloro-5,6-dihydro-5,5-dimethylfuro[2,3-*c*]pyridazine (25). The above mixture of **24** and **25** (1.7 g) was flash chromatographed on silica gel (400 g) with hexane–EtOAc (7:3) as eluant. Compound **24** (1.1 g) was obtained first, followed closely by a tacky white solid (0.52 g). Recrystallization from hexane–EtOAc (1:1) gave **25** as large white plates, mp 143–5 °C: IR (KBr) 3090, 2965, 1464, 1422, 1390, 1358, 1174, 1064 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.2 (s, 1 H), 4.40 (s, 2 H), and 1.43 (s, 6 H). Anal. Calcd for $C_5H_5ClN_2O$: C, 52.02; H, 4.91; N, 15.18. Found: C, 52.49; H, 5.10; N, 15.23.

3,6-Dichloro-β,β-dimethyl-4-pyridazineethanol (24) from Alcohol 23 and No Silver Catalysis. To a stirred solution of 2,2-dimethylpropanediol (**23**) (10.4 g, 0.10 mol) in H_2O (80 mL) were added 3,6-dichloropyridazine (**1**) (3.0 g, 0.02 mol), ammonium persulfate (22.8 g, 0.10 mol), and concentrated H_2SO_4 (1.4 mL, 0.025 mol). The mixture was heated to 100 °C for 2 h. After cooling, the mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were washed with 1 N NaOH (50 mL) and brine (50 mL), dried (Na_2SO_4), and evaporated under vacuum to obtain a yellow oil. The oil was dissolved in toluene (15 mL) and cooled to −20 °C to give a white solid (0.30 g), which was shown by ^1H NMR analysis to be a 90:10 mixture of **24** and **25**. The mother liquor was concentrated under vacuum and flash chromatographed on silica gel (100 g) with hexane–EtOAc (7:3). A white solid (0.84 g) was obtained and recrystallized from hexane to give crystalline **28**,[11] mp 61–63 °C: IR (KBr) 3345, 2981, 2971, 2958, 2862, 1394, 1109, 1046 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.22 (1 H, s), 3.64 (1 H, d, *J* = 11 Hz), 2.94 (1 H, t, *J* = 6 Hz), 1.18 (3 H, s), 0.94 (6 H, s), 0.72 (3 H, s); ^{13}C NMR ($CDCl_3$) δ 107.8, 77.3, 69.5, 39.0, 30.3, 22.9, 21.7, 19.9. Anal. Calcd for $C_{10}H_{20}O_3$: C, 63.80; H, 10.71; O, 25.49. Found: C, 63.62; H, 10.58; O, 25.49. A later fraction from the

(11) Ko, K.; Kunimoto, S.; Shimono, Y.; Yamaguchi, T. Japanese Patent, 16,867, 1966; *Chem. Abstr.* **1967**, *66*, 10943.

column contained a white solid (0.25 g), which was shown by ^1H NMR to be a 70:30 mixture of **24** and **25**. The total yields of **24** and **25** were 0.45 g (10%) and 0.10 g (3%), respectively.

3,6-Dichloro-β,β-dimethyl-4-pyridazineethanol (24) from Aldehyde and No Silver Catalysis. To an 80 °C solution of concentrated H_2SO_4 (0.33 mL, 0.006 mol) in H_2O (10 mL) were added 3-hydroxy-2,2-dimethylpropionaldehyde (**27**) (2.55 g, 0.025 mol) and **1** (0.75 g, 0.005 mol). A solution of ammonium persulfate (5.7 g, 0.025 mol) in H_2O (15 mL) was added dropwise over 10 min. Gas evolution and a temperature increase to 100 °C accompanied the addition. After 1 h at 90 °C, TLC showed **1** still present, so additional **27** (0.51 g, 0.005 mol) and ammonium persulfate (1.1 g, 0.005 mol) were added, and the reaction mixture was stirred at 90 °C for 1 h. The reaction mixture was cooled and extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers were washed with H_2O (20 mL) and saturated $NaHCO_3$ (20 mL), dried (Na_2SO_4), and evaporated to an orange oil. Flash chromatography on silica gel (100 g) with 70/30 hexane–EtOAc gave a white solid (0.43 g, 40%), which was shown by ^1H NMR spectroscopy to be an 80/20 mixture of **24** and **25**.

3,6-Dichloro-4-(2-chloro-1,1-dimethylethyl)pyridazine (3). To a stirred solution of alcohol **24** (11.05 g, 0.05 mol), dry pyridine (5.4 mL, 0.067 mol), and toluene (60 mL) was added $SOCl_2$ (4.9 mL, 0.067 mol) over 1 min. The solution was heated at 75 °C under N_2 for 16 h and cooled to 25 °C; 1 N HCl (30 mL) and toluene (20 mL) were added. The layers were separated, the toluene layer was washed with H_2O (30 mL) and brine (30 mL) and dried (Na_2SO_4), and the toluene was removed under vacuum to leave yellow crystals (9.62 g). Recrystallization from *i*-PrOH gave white crystals of **3** (8.57 g, 76%, 2 crops), mp 68–70 °C, identical with that obtained earlier.

3,6-Dichloro-β,β-dimethyl-4-pyridazineethanol, 4-Methylbenzenesulfonate Ester (29). T a stirred slurry of *p*-toluenesulfonyl chloride (10.48 g, 0.055 mol) in dry pyridine (5 mL) was added a solution of **24** (11.05 g, 0.050 mol) in pyridine (40 mL) over 10 min. The reaction mixture was stirred under N_2 at 25 °C for 17 h. CH_2Cl_2 (50 mL) was added, and the mixture was cooled to 0 °C. Concentrated HCl (45 mL) was added dropwise, while the temperature was kept below 25 °C. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (50 mL). The combined organic layers were washed with H_2O (2 × 50 mL) and brine (50 mL) and dried over Na_2SO_4. Solvent evaporation gave a light yellow oil (17.76 g), which crystallized upon standing. Recrystallization from *i*-PrOH (65 mL) gave **29** as white crystals (14.95 g, 80%), mp 111–113 °C: IR (KBr) 2920, 1595, 1560, 1485, 1358, 1350, 1310, 1190, 1146, 978, 847, 818 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.59 (dd, J_1 = 9 Hz, J_2 = 2 Hz, 2 H), 7.36 (s, 1 H), 7.28 (dd, J_1 = 9 Hz, J_2 = 2 Hz, 2 H), 4.34 (s, 2 H), 2.44 (s, 3 H), 1.45 (s, 6 H). Anal. Calcd for $C_{15}H_{16}Cl_2N_2O_3S$: C, 48.01; H, 4.30; N, 7.46; S, 8.54. Found: C, 48.09; H, 4.31; N, 7.47; S, 8.74.

3,6-Dichloro-4-(2-bromo-1,1-dimethylethyl)pyridazine (30). Oven-dried LiBr (13.92 g, 0.16 mol) and **29** (37.5 g, 0.10 mol) were added to dry DMSO (100 mL). The mixture was heated at 110 °C under N_2 for 2 h and cooled to 25 °C. H_2O (100 mL) was added dropwise, and a solid was collected and washed with H_2O (3 × 50 mL). Vacuum drying at 45 °C gave 27.74 g (97%). Recrystallization from *i*-PrOH (55 mL) gave white crystals (25.92 g, 91%) of **30**, mp 86.5–88.5 °C: IR (KBr) 1560, 1390, 1372, 1354, 1310, 1248, 1158, 1140, 1107 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.48 (s, 1 H), 3.95 (s, 2 H), 1.63 (s, 6 H). Anal. Calcd for $C_8H_9BrCl_2N_2$: C, 33.84; H, 3.19; Br, 28.14; Cl, 24.97; N, 9.86. Found: C, 33.89; H, 3.10; Br, 29.12; Cl, 25.43; N, 9.80.

Acknowledgment. We thank George E. Babbitt for conducting the NOE experiments.

Total Synthesis of (–)-Neplanocin A

Victor E. Marquez,*[†] Mu-Ill Lim,[†,§] Christopher K.-H. Tseng,[†] Anica Markovac,[‡] Matthew A. Priest,[‡] M. Sami Khan,[‡] and Bashir Kaskar[‡]

Laboratory of Medicinal Chemistry, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, and Ash-Stevens, Inc., 5861 John C. Lodge Freeway, Detroit, Michigan 48202

Received June 29, 1988

An efficient synthesis of neplanocin A, which is easily adaptable for the preparation of other cyclopentenyl containing nucleoside isosteres, has been developed. This enantioselective synthesis provides control of two of the three chiral centers of neplanocin A by constructing the carbocyclic ring portion of the molecule (compound **10a**) from optically pure D-ribonolactone (**9**). The last chiral center is constructed by the regiospecific reduction of the intermediate cyclopentenone **10a** to the α allylic alcohol **8a**, followed by the inversion of this center to the required β stereochemistry by S_N2 displacement with LiN_3 or the sodium salt of 6-chloropurine. The resulting cyclopentenyl azide (**15**) and the cyclopentenyl purine (**17a**) were both converted to (–)-neplanocin A.

Carbocyclic nucleosides are biologically interesting materials that sometimes display important antitumor or antiviral activities.[1–4] Because of the absence of a true glycosidic bond, carbocyclic nucleosides are chemically more stable and not subject to the action of the enzymes that cleave this linkage in conventional nucleosides.[1–4] A remarkable change in the biological activity of some of these pseudonucleosides occurs when the cyclopentane ring is modified into a cyclopentene ring. This structural change is usually accompanied by an increase in the biological potency and specificity of the unsaturated compounds when compared to the corresponding saturated carbocyclic analogues. Such phenomenon was first observed after the isolation and evaluation of neplanocin A, which proved to have superior antitumor and antiviral properties compared with its saturated counterpart aristeromycin (Figure 1).[5–8] Since the isolation of neplanocin

[†] National Institutes of Health.
[‡] Ash-Stevens, Inc.
[§] Present address: Clairol, Inc., Research Laboratories, 2 Blachley Road, Stamford, CT 06902.

(1) Marquez, V. E.; Lim, M.-I. *Med. Res. Rev.* **1986**, *6*, 1.
(2) Goodchild, J. *Top. Antibiot. Chem.* **1982**, *6*, 99.
(3) Buchanan, J. G.; Wightman, R. H. *Top. Antibiot. Chem.* **1982**, *6*, 229.
(4) Montgomery, J. A. *Med. Res. Rev.* **1982**, *2*, 271.