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Computationally Guided Organometallic Chemistry: Preparation of the Heptacyclic Pyrazine Core of Ritterazine N

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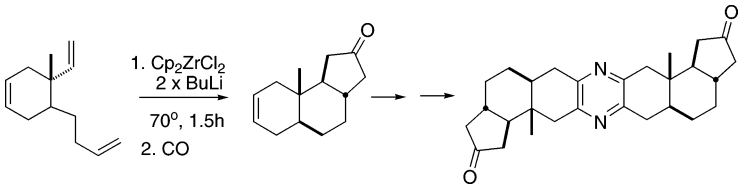
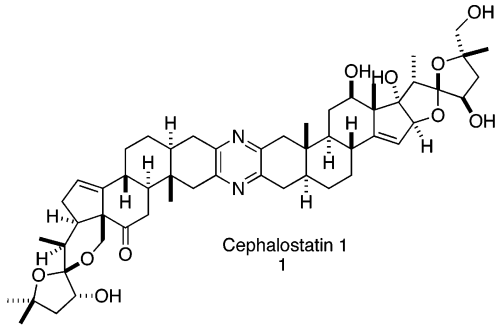


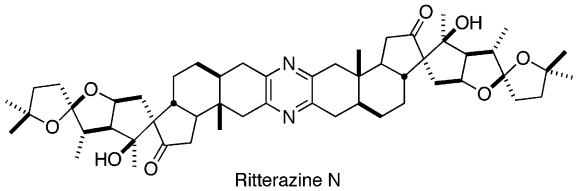
Diels−Alder cycloaddition of **10** followed by Wittig homologation and intramolecular diene cyclozirconation of the resulting triene under equilibrating conditions led to the tricyclic 6−6−5 ketone **5** with high diastereocontrol. The derived α-azido ketone **16** cyclized efficiently to the heptacyclic pyrazine core of ritterazine N.

Introduction

The cephalostatins, represented by cephalostatin 1 (**1**),[1] and ritterazines, represented by ritterazine N (**2**), comprise a family of 45 structurally unprecedented marine products with selective cytotoxicity against human tumors. The cephalostatins, from the Indian Ocean hemichordate *Cephalodiscus gilchristi*, and the ritterazines, from the Japanese marine tunicate *Ritterella tokioka*, are clearly related (ritterazine K, for example, contains[2] the "left half" of cephalostatin 7), although identical alkaloids have not yet been found in both species. Isolated from *C. gilchristi*, cephalostatin **1** exhibits remarkable cytotoxic activity[3] against P388 murine leukemia cells with IC_{50} values of $10^{-4}-10^{-6}$ ng/mL. Ritterazine B treated HL-60 cells become multinucleated, leading at 20 nM to apoptosis by a novel mechanism. Ritterazine N shows the same profile, but at near-micromolar concentrations. It is particularly exciting that this family of alkaloids induces apoptosis in apoptosis-resistant malignant cell lines.



Cephalostatin 1
1



Ritterazine N
2

These compounds are isolated in only microgram quantities from natural sources. If their potent physiological activity is to be pursued, they must be prepared by synthesis. Fuchs and coworkers have completed much elegant work toward the synthesis of the cephalostatins and ritterazines from steroid precursors.[4]

(1) For the isolation and activity of the ritterazines and cephalostatins, see: (a) Pettit, G. R.; Tan, R.; Xu, J.; Ichihara, Y.; Williams, M. D.; Boyd, M. R. *J. Nat. Prod.* **1998**, *61*, 955. (b) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1997**, *62*, 4484.

(2) Ganesan, A. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 611.

(3) For the physiological activity of the cephalostatins and ritterazines, see: (a) Komiya, T.; Fusetani, N.; Matsunaga, S.; Kubo, A.; Kaye, F. J.; Kelley, M. J.; Tamura, K.; Yoshida, M.; Fukuoka, M.; Nakagawa, K. *Cancer Chemother. Pharm.* **2003**, *51*, 202. (b) Dirsch, V. M.; Mueller, I. M.; Eichhorst, S. T.; Pettit, G. R.; Kamano, Y.; Inoue, M.; Xu, J. P.; Ichihara, Y.; Wanner, G.; Vollmar, A. M. *Cancer Res.* **2003**, *63*, 8869. (c) Mueller, I. M.; Dirsch, V. M.; Rudy, A.; Lopez-Anton, N.; Pettit, G. R.; Vollmar, A. M. *Mol. Pharm.* **2005**, *67*, 1684.

(4) For leading references to the synthesis of the fused 6−5 cephalostatins and ritterazines, see: (a) Li, W.; Fuchs, P. L. *Org. Lett.* **2003**, *5*, 2849. (b) Flessner, T.; Jautelat, R.; Scholz, U.; Winterfeldt, E. *Prog. Chem. Org. Nat. Prod.* **2004**, *87*, 1. (c) Tietze, L. F.; Krahnert, W. R.; *Chem. Eur. J.* **2002**, *8*, 2116. No work has yet been reported toward the spiro 5-5 ritterazines.

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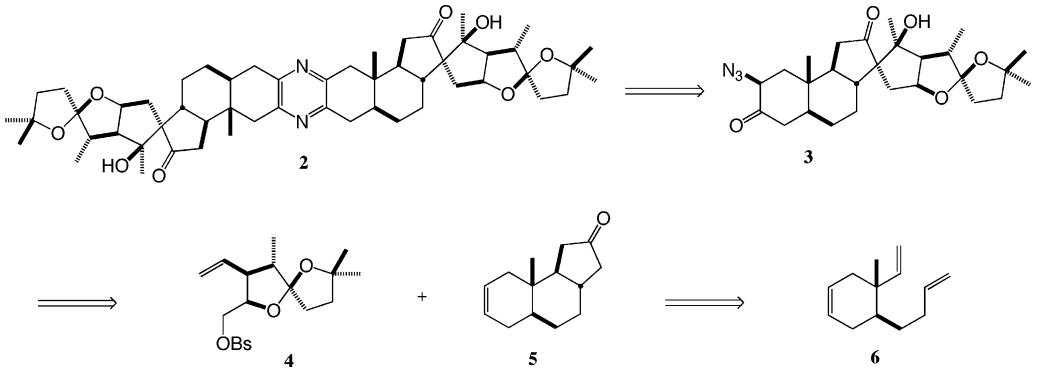
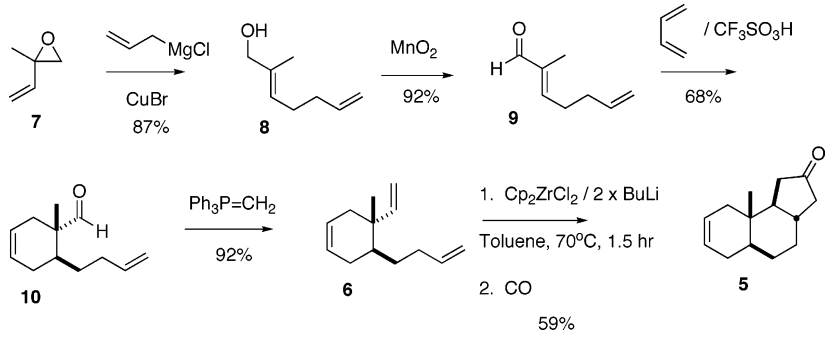
SCHEME 1



SCHEME 2



We report the preparation of the symmetrical heptacyclic core of the spiro 6−6−5−5 ritterazines.

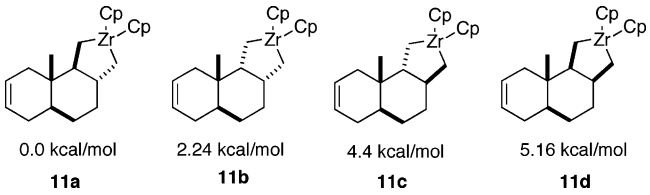
Results and Discussion

Retrosynthetic Analysis. We envisioned (Scheme 1) that the symmetrical pyrazine **2** might be prepared by reductive dimerization of the azido ketone **3**. The ketone would be available by convergent coupling of the sulfonate **4** with the tricyclic ketone **5**. It was particularly encouraging that computational studies on the four diastereomeric zirconacycles derived from **6** led to the prediction that the equilibrating cyclozirconation should proceed with substantial stereocontrol. We report the preparation of the ketone **5**, by cyclozirconation[5,6] of the triene **6**, and the dimerization of **5** to the heptacyclic pyrazine core of ritterazine N **2**.

Preparation of Triene 6. Diels−Alder cycloaddition (Scheme 2) of the aldehyde derived from **8**[7] to butadiene delivered the aldehyde **10**. This set the relative configuration of the A−B ring system. Another advantage of this approach is that it installs the Δ-2 alkene (steroid numbering) needed for pyrazine construction. Wittig methylenation of **10** then provided the triene **6**.

Computational Analysis. The four diastereomers of the zirconacycle (Scheme 3) will be equilibrating[5,6] under the conditions of the reaction. The cyclization is readily reversible, even though the equilibrium lies toward the cyclized product. To predict diastereoselectivity, it was important to evaluate the relative thermodynamic stability of the diastereomeric zirconacycles. ZINDO calculations indicated that zirconacycle **11a** (which would give tricyclic ketone **5**, Scheme 2) was more stable than its nearest competitor by 2.24 kcal/mol.

SCHEME 3



Oxygenation of the Zirconacycle. We initially ran the cyclozirconation of **6** (Scheme 4) at room temperature. After 3 h, the intermediate zirconacycles were converted to the diols by exposure to molecular oxygen. We recovered a dominant kinetic product **12c** from this reaction, corresponding to intermediate zirconacycle **11c** (Scheme 3). The structure of diol **12c** was confirmed by X-ray analysis. A minor diol **12b** was also isolated. On reacting the triene **6** with zirconocene dichloride and 2 equiv of butyllithium, heating for 4 h at 70 °C, and then oxygenating, we recovered a new dominant diol, derived from the equilibrated zirconacycle **11a**. This was assigned the structure **12a**.

Time Course of the Cyclozirconation. An HPLC assay was developed for the benzoates of **11a**−**c**. To determine the optimum time and temperature for equilibration of the zirconacycles, a time and temperature study was performed (Figure 1). After 1 h at room temperature, the **13a**/**13b**/**13c** ratio was 10:25:65. After an additional 1 h at 55 °C, the ratio was

(5) For the development of intramolecular diene cyclozirconation, including computational analysis, see: (a) Nugent, W. A.; Taber, D. F. *J. Am. Chem. Soc.* **1989**, *111*, 6435. (b) Taber, D. F.; Louey, J. P.; Wang, Y.; Nugent, W. A.; Dixon, D. A.; Harlow, R. L. *J. Am. Chem. Soc.* **1994**, *116*, 9457.

(6) For the development of Zr-based intramolecular diene cyclocarbonylation, including computational analysis, see: (a) Negishi, E.; Miller, S. R. *J. Org. Chem.* **1989**, *54*, 6014. (b) Taber, D. F.; Wang, Y. *J. Am. Chem. Soc.* **1997**, *119*, 22. (c) Taber, D. F.; Zhang, W.; Campbell, C. L.; Rheingold, A. R.; Incarvito, C. D. *J. Am. Chem. Soc.* **2000**, *122*, 4813. (d) Both ZINDO and molecular mechanics were used as implemented on a Tektronix CAChe workstation.

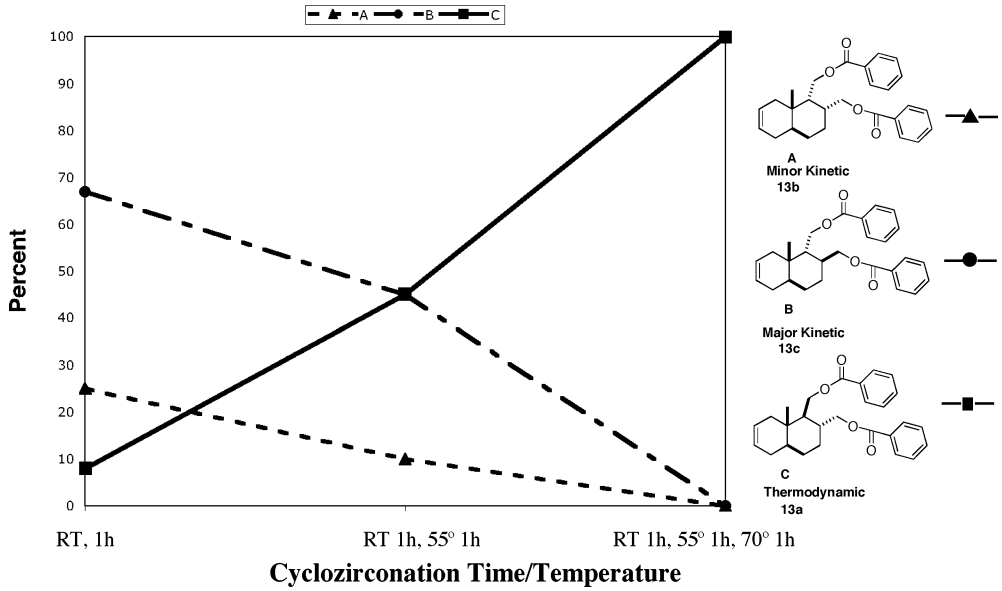
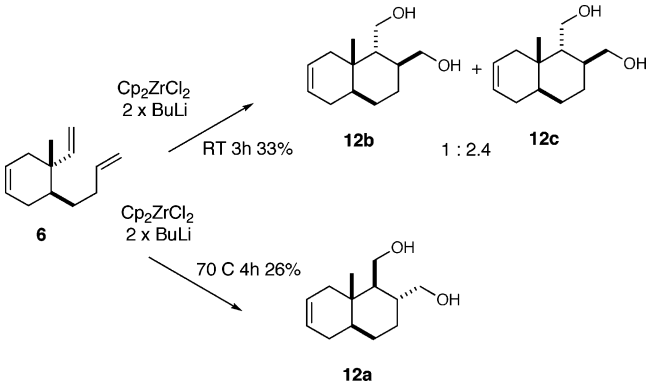


FIGURE 1. Time course of the cyclozirconation.

45:10:45. After a third hour at 70 °C, **13a** was the single dominant product.

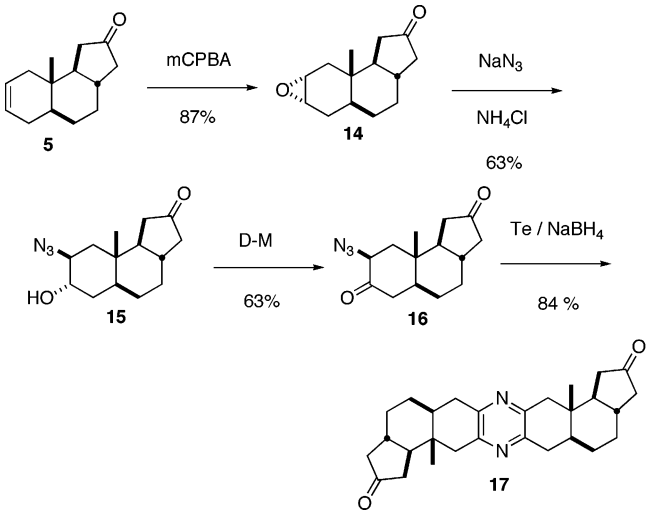
These results indicated that the reaction is complete after 3 h total and that there was no need to heat it past 2 h at 70 °C in order for the zirconacycles to fully equilibrate. This helped to improve our yields, since significant degradation was observed on prolonged heating. Using the results from the time and temperature study, the cyclozirconation of **6** was maintained at 70 °C for 1.5 h to equilibrate the zirconacycles. The resulting solution was exposed[6] to carbon monoxide, and a single dominant ketone **5** was formed.

SCHEME 4



Preparation of the Heptacyclic Pyrazine. Before proceeding with the preparation of **4** (Scheme 1), we thought it wise to explore the prospective azido ketone dimerization (Scheme 5). As expected from the steroid precedent,[8] epoxidation of **5** proceeded to give **14** as a single major diastereomer. Opening of the epoxide[9] with NaN_3 also proceeded to give a single product **14** from the preferred diaxial opening of the epoxide. Oxidation then gave ketone **15**.

SCHEME 5



In the past, such azido ketones have been reduced to the dimeric pyrazines using phosphines.[10] These reactions did not work well with our substrate. We were pleased to observe, however, that reduced Te[11] efficiently dimerized **16** to give **17**. This is the first preparation of the heptacyclic pyrazine core of the 6−6−5−5 ritterazines.

Conclusion

We expect that the key Diels−Alder/Wittig/diene cyclozirconation approach illustrated here, supported by ZINDO calculations, will be a general strategy for the stereocontrolled construction of polycarbacyclic target structures. The tricyclic ketone **5** may become a useful "truncated steroid" platform for drug discovery.

(7) (a) Cahiez, C.; Alexakis, A.; Normant, J. F. *Synthesis* **1978**, 528. (b) Johnston, B. D.; Oehlschlager, A. C. *Can. J. Chem.* **1984**, *62*, 2148.

(8) Forcellese, M. L.; Calvitti, S.; Camerini, E.; Martucci, I.; Mincione, E. *J. Org. Chem.* **1985**, *50*, 2191.

(9) Campbell, M. M.; Craig, R. C.; Boyd, A. C.; Gilbert, I. M.; Logan, R. T.; Redpath, J.; Roy, R. G.; Savage, D. S.; Sleigh, T. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2235.

(10) Heathcock, C. H.; Smith, S. C. *J. Org. Chem.* **1994**, *59*, 6828.

(11) (a) Suzuki, H.; Kawaguchi, T.; Takaoka, K. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 665. (b) Jeong, J. U.; Sutton, S. C.; Kim, S.; Fuchs, P. L. *J. Am. Chem. Soc.* **1995**, *117*, 10157.

Experimental Section

Cyclic Aldehyde 10. To a stirred suspension of activated manganese dioxide (123.0 g, 1.418 mol) and dry CH_2Cl_2 (284 mL) was added allylic alcohol **8** neat (20.0 g, 142 mmol) dropwise over 30 min. The residual allylic alcohol was rinsed into the reaction mixture with 5 mL of CH_2Cl_2. After 48 h, the mixture was filtered through Celite using an additional 3 × 300 mL of CH_2Cl_2. The organic extract was concentrated, and the aldehyde was used crude in the next reaction: TLC R_f (PE/MTBE = 9/1) = 0.47; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (1H, s), 6.48−6.52 (1H, t, J = 7.25), 5.77−5.87 (1H, m), 5.01−5.10 (2H, m), 2.44−2.49 (2H, m), 2.24−2.30 (2H, m), 1.75 (3H, s); ^{13}C NMR δ u 28.3, 32.4, 115.9, 139.7; d 9.4, 137.1, 153.8, 195.3; IR (film) 3350, 3079, 2979, 2925, 2818, 2762, 2711, 1688, 1642, 1442, 1404, 1379, 1359, 1320, 1251, 1188, 1068, 992, 915, 859, 824, 788, 641; LRMS *m*/*z* (rel intensity) 123 (6), 109 (41), 106 (13), 95 (71), 67 (54), 55 (100), 41 (44); HRMS calcd for $C_8H_{12}O$ 125.096640, obsd 125.096525.

The aldehyde (7.16 g, 51.1 mmol) was dissolved in dry CH_2Cl_2, and methylene blue (10 mg) was added. The reaction flask was then cooled to −30 °C. Butadiene (21.7 mL) was condensed using a coldfinger condensor, taken up in cold CH_2Cl_2 (118 mL), and added to the reaction mixture. Triflic acid in CH_2Cl_2 (12 mL, 6.0 mmol) was added dropwise at −10 °C over 30 min. The reaction mixture was warmed to room temperature and stirred for an additional 5 h. The reaction mixture was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The combined organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the aldehyde **10** (6.18 g, 68% yield) as a colorless oil: TLC R_f (PE/MTBE = 9/1) = 0.63; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.44 (1H, s), 5.63−5.81 (3H, m), 4.95−5.04 (2H, m), 2.17−2.34 (3H, m), 1.89−2.01 (2H, m), 1.66−1.71 (2H, m), 1.24−1.32 (2H, m), 0.99(3H, s); ^{13}C NMR δ u 27.2, 30.2, 31.8, 32.0, 48.4, 115.2; d 14.0, 35.4, 123.6, 126.0, 138.4, 206.4; IR (film) 3076, 3027, 2976, 2921, 2835, 2689, 1728, 1640, 1454, 1433, 1189, 994, 912, 752; LRMS *m*/*z* (rel intensity) 178 (3), 163 (16), 149 (54), 145 (17), 135 (40), 121 (30), 107 (73), 93 (100), 79 (90), 67 (63), 55 (39), 41 (39); HRMS calcd for $C_{12}H_{18}O$ 178.135765, obsd 178.136210.

Triene 6. To methyltriphenylphosphonium bromide (17.98 g, 50.33 mmol) and potassium *tert*-butoxide (5.28 g, 47.19 mmol) was added THF (125 mL). The reaction mixture was stirred at room temperature for 30 min, and then aldehyde **10** (5.60 g, 31.46 mmol) in THF (10 mL) was added dropwise over 15 min. After an additional 30 min, the reaction mixture was partitioned between diethyl ether and, sequentially, saturated aqueous NH_4Cl and brine. The combined organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the triene **6** (5.09 g, 92% yield) as a colorless oil: TLC R_f (PE/ CH_2Cl_2 = 9.5/0.5) = 0.83; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.66−5.86 (4H, m), 4.95−5.06 (4H, m), 2.08−2.28 (3H, m), 1.92−1.98 (1H, m), 1.67−1.76 (2H, m), 1.59−1.63 (1H, m), 1.46−1.50 (1H, m), 1.06−1.12 (1H, m), 0.96 (3H, s); ^{13}C NMR δ u 28.4, 30.0, 32.2, 38.7, 39.1, 111.3, 114.5; d 16.6, 40.1, 125.4, 125.9, 139.3, 149.0; IR (film) 3079, 3023, 2974, 2918, 2880, 2833, 1823, 1639, 1432, 1413, 1373, 1350, 1327, 1225, 1188, 1001, 909, 875, 783; LRMS *m*/*z* (rel intensity) 176 (<1), 161 (6), 147 (6), 134 (13), 122 (21), 107 (33), 93 (43), 81 (100), 77 (17), 67 (16), 55 (10), 41 (14); HRMS calcd for $C_{13}H_{20}$ 176.156501, obsd 176.155848.

Ketone 5. Zirconocene dichloride (992 mg, 3.4 mmol) was added to a 50 mL round-bottomed flask. Triene **6** (500 mg, 2.8 mmol) in toluene (8 mL) was added to the flask. The reaction mixture was cooled to −78 °C, and *n*-BuLi was added (2.17 M in hexanes, 3.10 mL) via syringe. The reaction was warmed to room temperature over 15 min and then was heated to 70 °C for 1.5 h. The reaction was cooled to room temperature, and then 8 mL of THF was added. CO was bubbled through the reaction mixture for 45 min. The reaction was stirred under a CO atmosphere for 24 h. Glacial acetic acid (1.18 mL) was added to the reaction mixture. The reaction mixture was partitioned between EtOAc and a 1:1 mixture of 5% aqueous H_2SO_4 and saturated aqueous Na_2SO_4. The combined organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the ketone **5** (337 mg, 59% yield) as a colorless oil: TLC R_f (PE/MTBE = 8/2) = 0.53; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.60−5.65 (2H, m), 2.36−2.42 (1H, dd, J = 6 Hz, J = 17 Hz), 2.17−2.23 (1H, dd, J = 6 Hz, J = 17 Hz), 1.93−2.03 (4H, m), 1.75−1.82 (4H, m), 1.47−1.59 (3H, m), 1.22−1.36 (2H, m), 0.81 (3H, s); ^{13}C NMR δ u 28.9, 29.8, 32.1, 34.1, 39.4, 40.0, 46.3, 218.4; d 11.0, 37.5, 41.3, 54.6, 125.3, 126.3; IR (film) 3467, 3019, 2961, 2914, 2852, 1744, 1651, 1445, 1431, 1406, 1384, 1366, 1201, 1153, 989, 912; LRMS *m*/*z* (rel intensity) 204 (61), 189 (7), 176 (6), 162 (13), 150 (41), 122 (100), 107 (27), 93 (41), 79 (30), 67 (10), 53 (9), 41 (12); HRMS calcd for $C_{14}H_{20}O$ 204.151415, obsd 204.151768.

Epoxide 14. To a stirred solution of ketone **5** (37 mg, 0.18 mmol) and CH_2Cl_2 (1.2 mL) was added *m*-CPBA (43 mg, 0.25 mmol). After 30 min, the reaction mixture was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The combined organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the epoxide **14** (34 mg, 87% yield) as a colorless oil: TLC R_f (CH_2Cl_2/acetone = 9.5/0.5) = 0.43; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.23 (1H, s), 3.14−3.16 (1H, m), 2.36−2.39 (1H, m), 2.17−2.24 (1H, dd, J = 7 Hz, J = 17 Hz), 1.90−2.02 (3H, m), 1.77−1.83 (3H, m), 1.48−1.59 (5H, m), 1.18−1.31 (2H, m), 0.83 (3H, s); ^{13}C NMR δ u 28.6 (2), 31.9, 33.3, 38.8, 39.2, 46.1, 217.9; d 12.5, 36.2, 37.5, 50.6, 52.5, 54.2; IR (film) 3053, 2921, 2850, 1741, 1604, 1448, 1406, 1380, 1320, 1265, 1169, 1144, 984, 898, 801, 737; LRMS *m*/*z* (rel intensity) 220 (41), 205 (37), 192 (100), 187 (13), 174 (14), 162 (27), 145 (31), 122 (91), 107 (63), 93 (80), 79 (61), 67 (33), 55 (30), 41 (38); HRMS calcd for $C_{14}H_{20}O_2$ 220.146330, obsd 220.145760.

Azido Alcohol 15. To a stirred solution of epoxide **14** (146.4 mg, 0.67 mmol) in an 8:1 methanol/water solution (3.35 mL) was added NaN_3 (130 mg, 2.0 mmol). After 48 h at 80 °C (bath), the reaction mixture was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The combined organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the azido alcohol **15** (111 mg, 63% yield) as a colorless oil: TLC R_f (CH_2Cl_2/acetone = 9.5/0.5) = 0.20; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.96 (1H, s), 3.81−3.83 (1H, m), 2.36−2.42 (1H, dd, J = 7 Hz, J = 17 Hz), 2.21−2.27 (1H, dd, J = 7 Hz, J = 17 Hz), 1.90−2.06 (3H, m), 1.79−1.89 (4H, m), 1.67−1.72 (2H, m), 1.44−1.51 (1H, m), 1.40−1.42 (3H, m), 1.28−1.37 (1H, m), 1.07 (3H, s); ^{13}C NMR δ u 28.1, 31.4, 32.1, 35.2, 36.9, 39.4, 46.1, 218.4; d 12.0, 36.9, 38.5, 55.1, 61.3, 68.5; IR (film) 3429, 2921, 2102, 1738, 1447, 1254, 1169, 1025, 902.

Azido Ketone 16. To a stirred solution of the azido alcohol **15** (71 mg, 0.27 mmol) in CH_2Cl_2 (1.8 mL) at room temperature was added Dess−Martin periodinane (228 mg, 0.54 mmol). After an additional 20 min, the reaction mixture was partitioned between CH_2Cl_2 and a 1:1 mixture of saturated aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$. The organic extract was dried (Na_2SO_4) and concentrated. The residue was adsorbed onto silica gel and chromatographed to afford the azido ketone **16** (44 mg, 63% yield) as a colorless oil: TLC R_f (CH_2Cl_2/acetone = 9.5/0.5) = 0.62; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.08−4.11 (1H, m), 2.41−2.55 (3H, m), 2.24−2.30 (1H, m), 1.81−2.11 (6H, m), 1.45−1.62 (3H, m), 1.20−1.29 (1H, m) 1.16 (1H, m), 1.04 (3H, s); ^{13}C NMR δ u 28.6, 31.4, 35.1, 39.5, 41.6, 43.3, 206.2, 216.8; d 12.8, 37.0, 43.5, 45.7, 54.7, 63.7; IR (film) 2922, 2102, 1740, 1444, 1404, 1261, 1187, 1147, 1101, 1015, 746; HRMS calcd for $C_{14}H_{20}O_2N_3$ (M + H) 262.155552, obsd 262.156570.

Pyrazine 17. Sodium hydrogen telluride was prepared by heating powdered tellurium (75 mg, 0.59 mmol) and $NaBH_4$ (52 mg, 1.38 mmol) to 75 °C in ethanol (2.2 mL) for 1 h. To the resulting dark red solution was added azido ketone **16** (59.4 mg, 0.23 mmol) in ethanol (0.6 mL) with stirring. The color instantly turned black. The mixture was stirred open to the air overnight. The ethanol was

removed under high vacuum, and then the reaction mixture was adsorbed directly onto silica gel and chromatographed to afford the heptacyclic pyrazine core **17** (41 mg, 84% yield) as a pale yellow solid: mp 185 °C dec; TLC R_f (CH_2Cl_2/acetone = 9.5/0.5) = 0.34; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.83−2.84 (2H, m) 2.61−2.75 (6H, m), 2.40−2.46 (2H, dd, J = 7 Hz, J = 17 Hz), 2.28−2.34 (2H, dd, J = 7 Hz, J = 17 Hz), 1.78−2.11 (12 H, m), 1.67−1.70 (2H, m), 1.44−1.50 (2H, m), 1.21−1.35 (2H, m), 0.84 (6H, s); ^{13}C NMR δ u 28.8, 32.1, 35.3 (2), 39.8, 46.4 (2), 148.7, 149.0, 218.0; d 11.6, 37.5, 41.9, 54.4; IR (film) 2920, 2854, 1741, 1446, 1398, 1158, 1016, 914, 733; HRMS calcd for $C_{28}H_{37}N_2O_2$ 433.285504, obsd 433.284413.

Acknowledgment. We thank the NIH (GM42056) for financial support of this work and J. V. Mitten for preliminary experiments. This study is dedicated to E. I. Negishi and his students, pioneers of reductive cyclozirconation.

Supporting Information Available: General experimental details, preparation of **8** and **12a**−**c**, HPLC details, and spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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