2-Methyl-3-(trimethylsilyl)-4-decanol (**2a**; entry 1): ^{1}H NMR (CCl_4) −0.02 (s, 9 H), 0.66 (dd, 1 H, J = 3.0 and 3.3 Hz), 0.78 (br t, 3 H), 0.88 (d, 3 H, J = 7.0 Hz), 0.92 (d, 3 H, J = 7.0 Hz), 1.08–1.54 (m, 11 H), 1.7–2.14 (m, 1 H), 3.63–3.92 (m, 1 H); IR 3500 (m), 1248 (s), 836 (s); MS, m/e (relative intensity) 229 (M^+ − Me, 0.5), 226 (M^+ − H_2O, 0.5), 75 (100).

1-(Trimethylsilyl)-2-octanone (entry 1): ^{1}H NMR (CCl_4) 0.00 (s, 9 H), 0.84 (br t, 3 H), 1.03–1.63 (m, 8 H), 2.01 (s, 2 H), 2.19 (t, 2 H, J = 7.5 Hz); IR 1695 (s), 850 (s); MS, m/e (relative intensity) 200 (M^+, 11), 75 (62), 73 (100).

2-Methyl-3-(dibutylmethylsilyl)-4-decanol (**2b**; entry 2): ^{1}H NMR (CCl_4) −0.07 (s, 3 H), 0.36–0.68 (m, 5 H), 0.68–1.72 (m, 35 H), 3.63–3.89 (m, 1 H); IR 3504 (m), 1250 (m); MS, m/e (relative intensity) 310 (M^+ − H_2O, 0.3), 117 (100).

1-(Dibutylmethylsilyl)-2-octanone (entry 2): ^{1}H NMR (CCl_4) −0.07 (s, 3 H), 0.35–0.64 (m, 4 H), 0.70–1.03 (m, 9 H), 1.03–1.71 (m, 16 H), 1.97 (s, 2 H), 2.18 (t, 2 H, J = 7 Hz); IR 1698 (s); MS, m/e (relative intensity) 284 (M^+, 5), 227 (100).

(*Z*)-1-(Isopropoxydimethylsilyl)-1,2-epoxyoctane (a crude intermediate in entry 10): ^{1}H NMR (CCl_4) 0.11 (s, 6 H), 0.87 (br t, 3 H), 1.05–1.6 (m, including d at 1.11, J = 6 Hz, total 14 H), 2.22 (d, J = 5 Hz), 2.85–3.05 (m, 1 H), 4.07 (sep, 1 H, J = 6 Hz).

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Registry No. **1a**, 62427-09-2; **1b**, 111581-57-8; **1c**, 111581-58-9; **1d**, 111581-59-0; **1e**, 111581-60-3; **1f**, 111581-61-4; **2a**, 111581-62-5; **2b**, 111581-63-6; **2c**, 111581-66-9; **2d** (R′ = Et), 111581-68-1; **2d** (R′ = *i*-Pr), 111581-69-2; **2d** (R′ = *t*-Bu), 111581-70-5; **2e**, 111581-71-6; (*E*)-**3b**, 111581-64-7; (*Z*)-**3b**, 111581-65-8; (*E*)-**3c**, 111615-21-5; (*Z*)-**3c**, 111581-67-0; (*E*)-**3d**, 109786-60-9; (*Z*)-3d, 109786-61-0; (*E*)-**3e**, 111581-72-7; (*Z*)-**3e**, 111581-73-8; Pt-[[$(CH_2$=$CH)Me_2Si]_2O]_2$, 81032-58-8; *i*-PrMgCl, 1068-55-9; EtMgBr, 925-90-6; *t*-BuMgBr, 677-22-5; CuCN, 544-92-3; HMe_2SiCl, 1066-35-9; $HMeSiCl_2$, 75-54-7; 1-octyne, 629-05-0; 1-(dimethylsilyl)-1-octyne, 109786-62-1; (*Z*)-1-(dimethylsilyl)-1-octene, 111581-74-9; 2-methyl-3-decene, 78645-74-6.

Synthesis and Tubulin Binding of 4′-(1-Azi-2,2,2-trifluoroethyl)oncodazole, a Photolabile Analogue of Oncodazole

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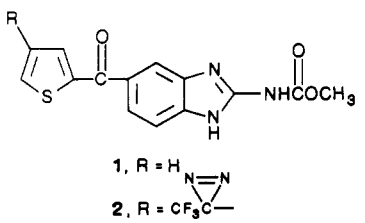
Oncodazole (**1**, methyl [5-(2-thienylcarbonyl)-1*H*-benzimidazol-2-yl]carbamate) is a tubulin binding agent[1] that has potent anthelmintic[2] and antifungal[3] activities and that has shown promise as an experimental antineoplastic agent.[4] The binding of tubulin by drugs that cause disruption or hyperstabilization of the mitotic apparatus is presently an area of great interest.[5] We became interested in characterizing the interaction of **1** with tubulin and for this required a photoaffinity analogue of **1**. Here we report a synthesis of the 4′-(1-azi-2,2,2-trifluoroethyl) analogue (**2**) of **1**.[6] The diazirine function was placed in the 4′-position on the basis of a systematic study of oncodazole derivatives that has shown this to be one of the positions on **1** that can be substituted with retention of biological activity. This compound, which is one of the few examples of a heterocyclic diazirine ring system and certainly one of the most highly functionalized, possesses both the requisite photolability ($t_{1/2}$ ~ 21 s upon irradiation at 350 nm in methanol) and the required tubulin binding affinity.



1, R = H

2, R = CF_3C(diazirine)

Results and Discussion

The synthesis of **2** was divided into three stages: (i) construction of the appropriate carbon skeleton, (ii) formation of the diazirine group, and (iii) formation of the benzimidazole carbamate group.

The synthesis began with 2,3,5-tribromothiophene as shown in Scheme I. Thiophene acid **3** was prepared from the tribromothiophene by sequential replacement of the 2-bromo with hydrogen, followed by replacement of the 5-bromo with carboxyl via a modification of a previously reported sequence.[7] Friedel–Crafts acylation of anisole with the acid chloride of **3** produced ketone **4**,[2] which was then protected as the ethylene ketal **5**. Metal–halogen exchange on **5** followed by reaction with *N*-(trifluoroacetyl)piperidine[6b] and removal of the protecting group gave the covalently hydrated diketone **6**. Reaction of **6** with nitric and sulfuric acids produced the appropriately substituted nitro derivative **7**. The electron-withdrawing power of the trifluoroacetyl group served to protect the reactive thiophene ring from nitration.

Next, the trifluoroacetyl group was converted into the 1-azi-2,2,2-trifluoroethyl group by the modification of known methods.[6b,8] Thus, diketone **7** was selectively oximated on the trifluoroacetyl carbonyl and the resultant mixture of syn and anti oximes **8** was converted to the oxime tosylates **9**. Selective oximation of the trifluoromethyl carbonyl in the presence of the diaryl ketone and the readily displaced aromatic methoxyl group required carefully controlled conditions employing 1.1 equiv of hydroxylamine. Reaction of **9** with ammonia in THF followed by oxidation of the intermediate diaziridine with *tert*-butyl hypochlorite gave diazirine **10**.

With the diazirine moiety completed the benzimidazole carbamate portion of the molecule was constructed next. The methoxyl group of **10** was displaced with ammonia in THF to produce **11**. The nitro group in **11** was selectively reduced with sodium hydrosulfite buffered with sodium

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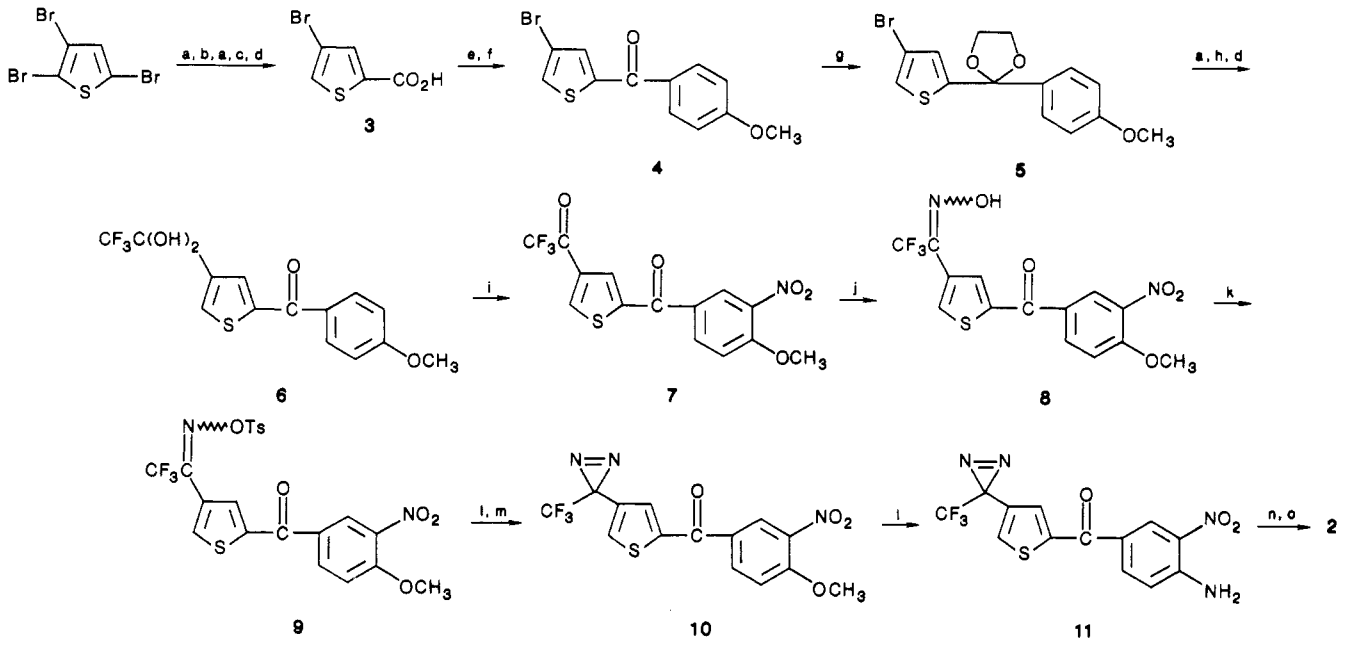
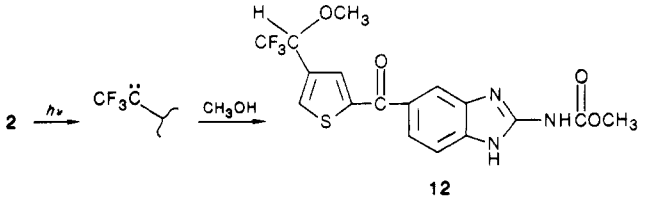
Scheme I[a]



[a] Reagents and conditions: (a) *n*-BuLi; (b) H_2O; (c) CO_2; (d) HCl; (e) $SOCl_2$; (f) C_6H_5OMe, $AlCl_3$; (g) $HOCH_2CH_2OH$, TsOH; (h) $CF_3C(ONC_5H_{10})$; (i) HNO_3, H_2SO_4, CH_2Cl_2; (j) $H_2NOH{\cdot}HCl$, C_5H_5N, EtOH; (k) TsCl, C_5H_5N; (l) NH_3, THF; (m) *t*-BuOCl, C_5H_5N, MeOH; (n) $Na_2S_2O_4$, $NaHCO_3$, H_2O/THF; (o) $CH_3SC(NCO_2CH_3)NHCO_2CH_3$, TsOH.

bicarbonate and the resultant diamine was converted immediately to **2** by reaction with bis(methoxycarbonyl)-*S*-methylisothiourea catalyzed by *p*-toluenesulfonic acid. Interestingly, the diazirine ring in **11** proved resistant to reduction under these conditions. Proton NMR and mass spectral data on **2** are consistent with the structure shown; however, acceptable elemental analysis data (see Experimental Section) could not be obtained. HPLC analysis of **2** showed it to be greater than 95% pure; attempts at further purification were unsuccessful.

As additional proof of structure, **2** was photolyzed in a methanol solution. Under these conditions a nearly quantitative yield of photoadduct was obtained. The ^1H NMR and mass spectral data for the product are consistent with structure **12**, the product of carbene insertion into the



12

solvent O–H bond. Adducts of this type have been shown by other investigators to be the major product of diazirine photolysis in methanol.[8]

In order to determine the relative affinities of **1** and photolabile analogue **2** for bovine brain tubulin, it became necessary to develop a new binding assay. The relatively high, micromolar dissociation constant of **1** from tubulin required that tubulin binding affinity be measured under true equilibrium conditions. Whereas an accurate dissociation constant for **1** had already been determined under equilibrium conditions by spectrophotometric techniques,[1c] this was clearly inappropriate for the photolabile analogue **2**. Therefore, relative affinities of **1** and **2** for bovine brain tubulin (expressed as IC_{50} values) were determined in a new assay procedure by competitive displacement of [^3H]oncodazole. Thus incubation of homogeneous bovine brain tubulin under equilibrium conditions with varying concentrations of **1** or **2** and [^3H]oncodazole as radioligand led to IC_{50} values of 5.7 ± 1.9 μM for **1** and 8.3 ± 3.0 μM for **2**. This experiment demonstrates similar affinities of **1** and **2** for bovine brain tubulin and illustrates a new equilibrium binding assay for antimitotic agents that bind tubulin at the site which binds benzimidazoles.

Experimental Section

Melting points are uncorrected. Elemental analyses and high field ^1H NMR, FT-IR, and mass spectra were obtained by the Analytical Department of Smith Kline & French Laboratories. IR spectra were obtained on Perkin-Elmer 780 Series infrared and Nicolet 20DXB FTIR spectrophotometers and are reported in cm^{-1}. ^1H NMR spectra were obtained with Varian EM-390, JEOL JNM-GX270, and Bruker WP 360 and AM 250 spectrometers and are reported as ppm values downfield from Me_4Si. Mass spectra were obtained on Varian-MAT CH-5 DF (DCI) and VG ZAB-1F (FAB) spectrometers. HPLC's were carried out on a 25-cm EM Hibar LiChrosorb Diol column using a Beckman Series 330 Isocratic System equipped with a Beckman 427 integrator and an LDC spectromonitor D variable wavelength detector set at 254 nm. The mobile phase was *n*-hexane–$CHCl_3$–MeOH–CH_3SO_3H (500:400:100:0.33 or 500:300:200:0.33). Photolyses were carried out in a Rayonet mini-photochemical reactor model RMR-500 manufactured by The Southern New England Ultraviolet Co. Flash chromatography was carried out according to Still.[9] 2,3,5-Tribromothiophene was purchased from Fairfield Chemical Co. [^3H]Oncodazole was prepared by the Department of Synthetic Chemistry, Smith Kline & French Laboratories.

4-Bromo-2-thiophenecarboxylic Acid (3). A solution of 80.21 g (0.25 mol) of 2,3,5-tribromothiophene in 800 mL of dry ether was cooled in a dry ice–acetone bath under an argon atmosphere, and then 100.0 mL (0.25 mol) of 2.5 *M* n-butyllithium in hexane was added slowly, below −70 °C. The reaction mixture was stirred in the cold for 5 min and then poured into cold H_2O. The organic layer was washed with H_2O, dried over $MgSO_4$, concentrated, and vacuum distilled at aspirator pressure to yield 47.90 g (79%) of 2,4-dibromothiophene: bp 81.5–96.5 °C (10 Torr).

A solution of 24.19 g (0.100 mol) of 2,4-dibromothiophene in 250 mL of dry ether was cooled in a dry ice–acetone bath under an argon atmosphere, and then 40.0 mL (0.100 mol) of 2.5 *M* n-butyllithium in hexane was added at −65 °C. The reaction

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mixture was stirred in the cold for 5 min and then poured onto a slurry of dry ice/ether. The slurry was stirred for several minutes, then H_2O was added, and the layers were separated. Acidification of the aqueous layer produced an oil that was extracted into CH_2Cl_2. The CH_2Cl_2 solution was dried over $MgSO_4$ and concentrated to 17.72 g of crude product which was recrystallized from H_2O to give 13.40 g (65%): mp 110–118.5 °C (lit.[10] mp 122–124 °C).

(4-Bromo-2-thienyl)(4-methoxyphenyl)methanone (4). Acid **3** (13.40 g, 0.0647 mol) was converted to the acid chloride by heating at reflux for 2 h with a mixture of 67 mL of $CHCl_3$ and 27 mL (0.37 mol) of thionyl chloride. The solution was concentrated to an oil, then dissolved in 67 mL of CH_2Cl_2, and added slowly with ice bath cooling to a mixture of anisole (7.70 g, 0.0712 mol) and $AlCl_3$ (9.49 g, 0.0712 mol) in 134 mL of CH_2Cl_2. The reaction mixture was stirred in the cold for 0.5 h followed by 0.5 h at room temperature and then cooled with an ice bath while H_2O was added slowly. The organic layer was washed first with H_2O and then with 5% $NaHCO_3$, then dried over $MgSO_4$, and concentrated to give 19.78 g (103%) of an oil which solidified upon standing and was sufficiently pure for use in the next step: ^1H NMR ($CDCl_3$) δ 3.83 (s, 3 H, OCH_3), 6.97 and 7.85 (4 H, A_2B_2 system), 7.53 (m, 2 H, thiophene H's).

An analytical sample of **4** was prepared by recrystallization from hexane: mp 90–91.5 °C (lit.[2] mp 88.2 °C).

Anal. Calcd for $C_{12}H_9BrO_2S$: C, 48.50; H, 3.05. Found: C, 48.67; H, 3.13.

(4-Bromo-2-thienyl)(4-methoxyphenyl)methanone Ethylene Ketal (5). A solution of 19.78 g of crude **4**, 40 mL of ethylene glycol, 300 mL of benzene, and 0.3 g of *p*-toluenesulfonic acid was heated at reflux for 89.5 h while H_2O was removed azeotropically. The benzene solution was washed first with 5% $NaHCO_3$ and then with H_2O and was dried over $MgSO_4$ and concentrated to 20.68 g of a dark oil. Flash chromatography (petroleum ether with a 33% to 75% CH_2Cl_2 gradient) afforded 14.27 g (65% based on **3**) of purified, colorless oil: ^1H NMR ($CDCl_3$) δ 3.77 (s, 3 H, OCH_3), 4.05 (m, 4 H, $O(CH_2)_2O$), 6.80 and 7.13 (d's, 2 H, thiophene H's), 6.87 and 7.47 (4 H, A_2B_2 system); IR (mineral oil), no C=O absorption.

Anal. Calcd for $C_{14}H_{13}BrO_3S$: C, 49.28; H, 3.84. Found: C, 48.97; H, 3.77.

(4-(Trifluoroacetyl)-2-thienyl)(4-methoxyphenyl)-methanone Hydrate (6). A solution of 14.00 g (0.041 mol) of **5** in 253 mL of dry ether was cooled in a dry ice–acetone bath under an argon atmosphere, and then 16.4 mL of 2.5 M *n*-butyllithium in hexane (0.041 mol) was added slowly. After 35 min in the cold, VPC analysis of a H_2O-quenched aliquot indicated incomplete metal–halogen exchange. An additional 1.64 mL (0.0041 mol) of *n*-butyllithium was added and 20 min later a solution of 9.29 g (0.0153 mol) of *N*-(trifluoroacetyl)piperidine[11] in 25 mL of ether was added slowly. After 20 min the cooling bath was removed and the reaction mixture was allowed to warm to 0 °C and poured into H_2O. The organic layer was washed with H_2O, dried over $MgSO_4$, and concentrated to yield 13.10 g (89%) of ketal-protected product as an amber oil.

The protecting group was removed by heating at reflux 11.92 g (0.0333 mol) of ketal with 414 mL of THF and 84 mL of 1 N HCl for 1 h. The cooled reaction mixture was diluted with H_2O, then dried over $MgSO_4$, and concentrated to 9.95 g (90%) of product as an amber oil.

A crystalline analytical sample of **6** was prepared by trituration with hexane: mp 120–122 °C dec; ^1H NMR (DMSO-d_6/D_2O) δ 3.91 (s, 3 H, OCH_3), 7.07 and 7.88 (4 H, A_2B_2 system), 7.71 and 7.99 (2 H, thiophene H's); mass spectrum (FAB), m/e 331 $(M + H)^+$; IR (mineral oil), 3400, 3250 (br), 1620 (diaryl ketone), no $C(O)CF_3$ absorption.

Anal. Calcd for $C_{14}H_{11}F_3O_4S$: C, 50.60; H, 3.34. Found: C, 51.04; H, 3.26.

(4-(Trifluoroacetyl)-2-thienyl)(3-nitro-4-methoxyphenyl)methanone (7). A solution of 10.91 g (0.0347 mol) of **6** in 230 mL of CH_2Cl_2 was cooled in an ice bath and 9.25 mL of concentrated H_2SO_4 and 2.23 mL of concentrated HNO_3 were slowly added successively. The reaction mixture was stirred at 0 °C for 1.5 h; then H_2O and ether were added. The organic layer was washed once with H_2O, twice with 5% $NaHCO_3$, then dried over $MgSO_4$, and concentrated to 10.53 g of an oily residue which was shown by VPC analysis to contain some starting material. The product was further nitrated with an additional 0.67 mL of concentrated HNO_3 as described above to yield 10.57 g of an oil. This oil was flash chromatographed, eluting with a 1:1 mixture of CH_2Cl_2 and hexane containing a 2.5% to 15% EtOAc gradient followed by 100% CH_2Cl_2 to yield 9.70 g (78%) of yellow solid: mp 89–92.5 °C; ^1H NMR ($CDCl_3$) δ 4.10 (s, 3 H, OCH_3), 7.27 and 8.15 (2 H, H_B and H_A of ABX system, J_{AB} = 8.8 Hz), 8.11 and 8.67 (2 H, thiophene H's), 8.44 (1 H, H_X of ABX system, J_{AX} = 2.2 Hz); mass spectrum (DCI, CH_4), m/e 360 $(M + H)^+$, 330, 290, 207, 180; IR (mineral oil), 1710 ($C(O)CF_3$), 1630 (diaryl ketone).

Anal. Calcd for $C_{14}H_8F_3NO_5S$: C, 46.80; H, 2.24; N, 3.90. Found: C, 46.82; H, 2.50; N, 4.25.

1-[2-(3-Nitro-4-methoxybenzoyl)-4-thienyl]-2,2,2-trifluoroethanone Oxime (8). A solution of 5.19 g (14.5 mmol) of **7**, 1.10 g (15.9 mmol) of hydroxylamine hydrochloride, 1.75 mL of pyridine, and 104 mL of absolute EtOH was heated at reflux for 3.5 h and then concentrated to dryness. The residue was dissolved in CH_2Cl_2, washed with H_2O, dried over $MgSO_4$, and concentrated to a foamy oil. This material was taken up in a 2% solution of ether in CH_2Cl_2 (an insoluble byproduct was removed by filtration) and flash chromatographed, eluting with CH_2Cl_2 with a 2% to 3% ether gradient to yield 0.59 g of starting material (**7**) along with 2.28 g (42%) of product (mixture of syn/anti isomers): mp 90–126 °C; ^1H NMR ($CDCl_3$) δ 4.08 (2s, 3 H, OCH_3), 7.24 and 7.25 (1 H, H_B of ABX system), 7.85, 8.00, 8.11 and 8.39 (2 H, thiophene H's), 8.11–8.17 (m, 1 H, H_A of ABX system), 8.43 and 8.46 (2d, 1 H, H_X of ABX system), 8.71 (s, 1 H, NOH, D_2O exchangeable); mass spectrum (DCl, CH_4), m/e 375 $(M + H)^+$, 359, 289, 222, 180; IR (mineral oil), 3300 (br), 1610 (diaryl ketone).

Anal. Calcd for $C_{14}H_9F_3N_2O_5S$: C, 44.93; H, 2.42; N, 7.48. Found: C, 45.30; H, 2.63; N, 7.50.

1-[2-(3-Nitro-4-methoxybenzoyl)-4-thienyl]-2,2,2-trifluoroethanone *O*-[(4-Methylphenyl)sulfonyl]oxime (9). A mixture of 3.46 g (9.24 mmol) of **8**, 2.64 g (13.9 mmol) of *p*-toluenesulfonyl chloride, and 69 mL of pyridine was heated in an oil bath at 80 °C under argon for 4 h and then concentrated to dryness and flash chromatographed, eluting with CH_2Cl_2 to yield 4.15 g (85%) of product (mixture of syn/anti isomers): mp 94–106 °C; ^1H NMR ($CDCl_3$) δ 2.48 (s, 3 H, CH_3), 4.10 (s, 3 H, OCH_3), 7.27 and 8.15 (2 H, H_B and H_A of ABX system, J_{AB} = 8.8 Hz), 7.41 and 7.92 (4 H, $A'_2B'_2$ system, $J_{A'B'}$ = 8.4 Hz), 8.01 and 8.43 (2 H, thiophene H's), 8.43 (1 H, H_X of ABX system, J_{AX} = 2.2 Hz); mass spectrum (DCI, CH_4), m/e 529 $(M + H)^+$, 359, 357, 289, 180, 155; IR (mineral oil), 1630 (diaryl ketone).

Anal. Calcd for $C_{21}H_{15}F_3N_2O_7S_2$: C, 47.73; H, 2.86; N, 5.30. Found: C, 48.01; H, 3.06; N, 5.31.

[4-(1-Azi-2,2,2-trifluoroethyl)-2-thienyl](3-nitro-4-methoxyphenyl)methanone (10). A solution of 0.830 g (1.57 mmol) of **9** in 5 mL of THF was placed in a heavy-walled glass tube and then cooled in a dry ice–acetone bath. After condensing approximately 5 mL of NH_3 into the reaction mixture, the tube was sealed and the cooling bath removed. The solution was stirred at ambient temperature for 1 h, cooled in dry ice–acetone, unsealed, and allowed to warm to room temperature. The reaction mixture was then taken up in CH_2Cl_2 and filtered; the filtrate was evaporated to dryness, redissolved in CH_2Cl_2, and washed twice with H_2O. The CH_2Cl_2 solution was dried over $MgSO_4$ and concentrated to 0.527 g (90%) of nearly pure (TLC) diaziridine: ^1H NMR ($CDCl_3$) δ 2.35 and 2.92 (dd, 2 H, NH's), 4.07 (s, 3 H, OCH_3), 7.25 and 8.12 (2 H, H_B and H_A of ABX system), 7.78 and 8.03 (2 H, thiophene H's), 8.38 (1 H, H_X of ABX system); mass spectrum (DCl, CH_4), m/e 374 $(M + H)^+$.

The above diaziridine was dissolved in 8.3 mL of MeOH, cooled in an ice bath, and treated successively with 0.171 mL (2.18 mmol) of pyridine and 0.185 mL (1.55 mmol) of *tert*-butyl hypochlorite. After stirring in the cold for 45 min, a 10% solution of sodium metabisulfite was added, and the mixture was extracted twice with CH_2Cl_2. The combined extracts were washed with H_2O, then dried over $MgSO_4$, and concentrated to 0.509 g (87%) of **10** as an oil.

An analytical sample of **10** was prepared by flash chromatography, eluting with a 3:1 mixture of CH_2Cl_2/petroleum ether: ^1H NMR ($CDCl_3$) δ 4.08 (s, 3 H, OCH_3), 7.27 and 8.12 (2 H, H_B and

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(11) Cockburn, W. F.; Bannard, R. A. B. *Can. J. Chem.* **1957**, *35*, 1289.

H_A of ABX system), 7.45 and 7.58 (2 H, thiophene H's), 8.40 (1 H, H_X of ABX system); mass spectrum (DCI, CH_4), m/e 372 $(M + H)^+$, 344, 327, 180; IR (mineral oil), 1640 (diaryl ketone).

Anal. Calcd for $C_{14}H_8F_3N_3O_4S$: C, 45.29; H, 2.17; N, 11.32. Found: C, 45.29; H, 2.08; N, 10.99.

[4-(1-Azi-2,2,2-trifluoroethyl)-2-thienyl](3-nitro-4-aminophenyl)methanone (11). A solution of 0.531 g (1.43 mmol) of **10** in 34 mL of THF was reacted in the dark with approximately an equal volume of NH_3 at room temperature for 70.5 h as described for **10**, yielding 0.496 g (97%) of product.

An analytical sample of **11** was prepared by flash chromatography, eluting with CH_2Cl_2: mp 117 °C dec; ^1H NMR ($CDCl_3$) δ 6.57 (br s, 2 H, NH_2), 6.94 and 7.94 (2 H, H_B and H_A of ABX system, J_{AB} = 8.8 Hz), 7.45 and 7.50 (2 H, thiophene H's), 8.75 (1 H, H_X of ABX system); mass spectrum (FAB), m/e 357 $(M + H)^+$; IR (mineral oil), 3450 and 3330 ($ArNH_2$), 1635 (diaryl ketone).

Anal. Calcd for $C_{13}H_7F_3N_4O_3S$: C, 43.83; H, 1.98; N, 15.73. Found: C, 43.91; H, 1.80; N, 15.78.

Methyl [5-[[4-(1-Azi-2,2,2-trifluoroethyl)-2-thienyl]-carbonyl]-1*H*-benzimidazol-2-yl]carbamate (2). A 5% solution of $NaHCO_3$ (10 mL) was added to a solution of 105 mg (0.295 mmol) of **11** in 10 mL of THF under an argon atmosphere. The mixture was stirred at room temperature while technical grade (85%) sodium hydrosulfite (211 mg, 1.03 mmol) was added in portions over a 20-min period. After stirring for an additional 10 min, the layers were separated; the organic layer was washed with saturated sodium chloride, filtered, and concentrated with mild heating to give the dark red diamine. The diamine was dissolved in 2.0 mL of MeOH, then treated with 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea[12] (63.8 mg, 0.309 mmol) and a catalytic amount of *p*-toluenesulfonic acid. The mixture was heated at reflux under argon for 5 min during which time a precipitate formed. After cooling to room temperature, the precipitate was filtered, washed with MeOH, and vacuum-dried to 40.0 mg (33%) of tan solid: mp >400 °C dec; ^1H NMR (DMSO-d_6) δ 3.79 (s, 3 H, OCH_3), 7.54 and 8.18 (2 H, thiophene H's), 7.55 and 7.66 (2 H, H_B and H_A of ABX system), 7.96 (1 H, H_X of ABX system); mass spectrum (FAB), m/e 410 $(M + H)^+$; IR (KBr), 1709 (carbamate), 1646 and 1632 (ketone and C=N).

Anal. Calcd for $C_{16}H_{10}F_3N_5O_3S$: C, 46.95; H, 2.46; N, 17.11. Found: C, 47.14; H, 2.43; N, 16.57.

Photolysis of 2. A 0.5 mM solution of **2** in MeOH (44 mL, 0.022 mmol) was irradiated for 20 min in a quartz vessel at room temperature, then concentrated to dryness, and vacuum-dried at 45 °C. HPLC analysis showed a new product with longer retention time than **2**: ^1H NMR (DMSO-d_6) δ 3.41 (s, 3 H, ether CH_3), 3.80 (s, 3 H, carbamate CH_3), 5.25 (q, 1 H, CF_3CH), 7.56 and 7.65 (2 H, H_B and H_A of ABX system), 7.71 and 8.19 (2 H, thiophene H's), 7.96 (1 H, H_X or ABX system); mass spectrum (DCl, NH_3), m/e 414 $(M + H)^+$, 398, 384.

The $t_{1/2}$ of **2** in MeOH was determined as follows: 50-μL aliquots of a 0.5 mM solution of **2** in MeOH were irradiated for 0, 20, 40 and 60 s; each aliquot was diluted with 250 μL of HPLC mobile phase [*n*-hexane–$CHCl_3$–MeOH–CH_3SO_3H (500:400:100:0.33) and 20 μL of this solution injected into the HPLC. A plot of log (area % **2**) vs time produced a slope of –0.0144; $t_{1/2}$ was calculated to be 21 s from the equation $t_{1/2}$ = 0.693/(–slope × 2.303).

Tubulin Competitive Equilibrium Binding Assay. An equilibrium binding assay was developed to determine the degree that an oncodazole analogue can compete with binding of [^{3}H]-**1** to tubulin. This assay was based on the gel partition equilibrium binding method of Hirose and Kano[13] as modified by Head et al.[1c] in their investigation of equilibrium binding of oncodazole to tubulin.

Bovine brain tubulin was utilized in these studies and was purified as described by Hamel and Lin.[14] In preparation for the binding assay 20 mg of purified tubulin was dialyzed for 2 h at 4 °C against 500 mL of 10 mM KH_2PO_4 buffer, pH 7.0 which contained 0.1 mM GTP and 12% DMSO ("PGD buffer"). After dialysis, 50-μL aliquots (containing 0.325 mg of tubulin) were added to polypropylene microfuge tubes which contained 50 mg of BioGel P-6 resin (BioRad Laboratories) previously equilibrated with 400 μL of PGD buffer. Solutions of [^{3}H]-**1** (ca. 3.66 × 10^{13} cpm/mol) and varying concentrations of oncodazole analogue were made in PGD buffer and aliquots (150 μL) were added to duplicate microtubes containing the tubulin and P-6 resin to give a final volume of 600 μL for all additions. The final concentration of [^{3}H]-**1** in the microtubes was 9.0 × 10^{-6} M and the final concentration of oncodazole analogue ranged from 10^{-8} M to 10^{-4} M. The tubes were each vortexed for 5 s and incubated at 30 °C for 20 min with 5 s of vortexing for every 5 min of incubation time. The microtubes were then placed in an Eppendorf microfuge and the resin was gently separated by a brief spin of the microfuge. A resin-free aliquot (75 μL) was removed from the supernatant of each tube and placed in a fresh microtube. Aliquots of 25 μL in duplicate were then added to minivials and counted with 5 mL of Aquasol (Dupont) in a Beckman Model 3801 scintillation counter. The binding of [^{3}H]-**1** to tubulin was determined from the radioactivity and calculated by the methods of Hirose and Kano.[13] Under these experimental conditions, [^{3}H]-**1**, in the absence of any competing analogue, will saturate ca. 50% of the tubulin. This amount of binding is taken as the maximal binding value for [^{3}H]-**1**. The concentration of the oncodazole analogue that reduces the binding of [^{3}H]-**1** to 50% of the maximal binding value is designated as the IC_{50} value for that analogue. The IC_{50} was calculated by a nonlinear least squares fit of the binding data to the equation binding/maximal binding = $1/(1 + 10^{x}/IC_{50})$ where x = log [oncodazole analogue].

Registry No. **2**, 111690-73-4; **2** (diamine precursor), 111690-68-7; **3**, 16694-18-1; **4**, 66938-33-8; **5**, 111690-61-0; **6**, 111690-62-1; **6** (ketal protected), 111690-69-8; **7**, 111690-63-2; (*E*)-**8**, 111690-64-3; (*Z*)-**8**, 111690-65-4; (*E*)-**9**, 111690-70-1; (*Z*)-**9**, 111690-71-2; **10**, 111690-66-5; **10** (diaziridine precursor), 111690-72-3; **11**, 111690-67-6; PhOMe, 100-66-3; 2,3,5-tribromothiophene, 3141-24-0; 2,4-dibromothiophene, 3140-92-9; 1,3-bis(methoxycarbonyl)-*S*-methylisothiourea, 34840-23-8; *N*-(trifluoroacetyl)-piperidine, 340-07-8.

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Stereochemistry and Conformation of 8-Aryl-1,5-diazabicyclo[3.2.1]octanes by 2D NMR Studies

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1,4-Diazacycloheptane reacts with benzaldehyde to give 8-phenyl-1,5-diazabicyclo[3.2.1]octane (**1a**) of undetermined stereochemistry (Scheme I).[1] Nair has reported the synthesis of several compounds possessing structure **1**.[2] While their sharp melting points indicated single stereoisomers, their stereochemistry could not be established with the available data. An X-ray structure for **1e** has been published[3] but without information on its melting point or method of preparation. Thus the stereochemical outcome of the reaction of Scheme I is unknown.

We have established the stereochemistry of the products of the reaction in Scheme I through the use of 2D NMR (NOESY) at 500 MHz. In addition, we have shown that the conformation of the C8–Ar bond in **1** depends on the nature of the aryl group. A mechanism is proposed to account for the formation of only one stereoisomer.

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