Synthesis of New DNA Gyrase Inhibitors: Application of the DMSO Oxidation to the Conversion of the Amine into the Imine

Yoshikazu Jinbo*, Hirosato Kondo, Masahiro Taguchi, Fumio Sakamoto, and Goro Tsukamoto

New Drug Research Laboratories, Kanebo Ltd., 5-90, Tomobuchi-cho, 1-Chome, Miyakojima-ku, Osaka 534, Japan

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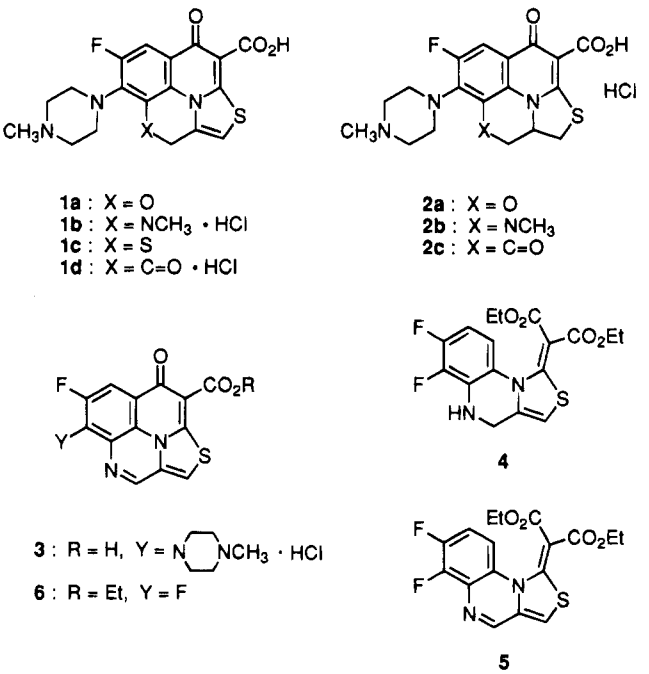
A novel type of pyridonecarboxylic acid with a planar thiazolopyrazine-incorporated tetracyclic skeleton, 4,9*b*-diaza- 8,9*b*-dihydro-6-fluoro-5-(4-methyl-1-piperazinyl)-8-oxo-1-thia- 1*H*-cyclopenta-[*cd*]phenalene-9-carboxylic acid (**3**), was prepared. Compound **3** was a potent inhibitor of DNA gyrase and exhibited attractive antibacterial activity against both Gram-positive and Gram-negative bacteria. In the course of the synthetic studies, we have found a new oxidation method of the amine **4** to the imine **5** with DMSO activated by trifluoroacetic anhydride or oxalyl chloride. The DMSO/trifluoroacetic anhydride method gave the byproduct **8**, an unusual (methylthio)methyl adduct. The DMSO/oxalyl chloride method gave the byproducts **9** and **10**, a (methylthio)methyl ester, and a halogen adduct, respectively. The mechanisms of these oxidations are also discussed.

Quinolone antibacterials are DNA gyrase inhibitors[1] and are useful therapeutic agents. A large number of analogues has been synthesized.[2] However, their activity against some pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), is not sufficient for clinical usage.[3] We have recently reported the synthesis of tetracyclic pyridonecarboxylic acids with a planar thiazole ring (**1a–d**[4,5]) and with a nonplanar thiazolidine ring (**2a–c**[6]). Compounds **1a**, **1b**, and **2a** exhibited attractive antibacterial activity against the bacteria, including MRSA strains. In the course of our study of tetracyclic quinolone antibacterials, we next focused on the other planar tetracyclic pyridone carboxylic acid **3** and extensively studied a new oxidation method for the conversion of the amine **4** into the imine **5** (Chart 1).

Useful oxidation procedures[7] of alcohols to yield ketones with DMSO activated by oxalyl chloride, trifluoroacetic anhydride, acetic anhydride, or dicyclohexylcarbodiimide have been reported. Surprisingly, the oxidation of amines to imines using the activated DMSO has not yet been reported. We have succeeded in finding a useful method for the conversion of an amine into an imine using the activated DMSO reagent.

In this paper, we describe the synthesis of a new type of DNA gyrase inhibitor (**3**) with a planar tetracyclic structure, using the DMSO oxidation of the amine **4** to the imine **5** as the key step. We also provide some information concerning the mechanism of the oxidation.

Chart 1



Results and Discussion

The tetracyclic compound **6** is an important intermediate in designing compound **3**. Compound **6** could be achieved by cyclization of the tricyclic imine **5**, using an acid catalyst. The desirable imine **5** could be obtained from an amine **4** by the oxidation, which is a key step in our synthesis.

Oxidation of the Amine 4 to the Imine 5. Jorgensen et al.[8] have reported a procedure for the conversion of an amine to an imine, which is characterized by the HNO-elimination of *N*-nitroso compounds under pyrolytic conditions. We also obtained the imine **5** using this method, but the yield was insufficient. Accordingly, the amine **4** was allowed to react with $NaNO_2$ and 2 N HCl to give the *N*-nitroso compound **7** (72%), which was heated in toluene to afford the imine **5** (33%) (Scheme 1).

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(2) (a) Radl, S.; Bouzard, D. *Heterocycles* **1992**, *34*, 2143–2177.

(3) Inoue, Y.; Kondo, H.; Taguchi, M.; Jinbo, Y.; Sakamoto, F.; Tukamoto, G. *J. Med. Chem.* **1994**, *37*, 586–592.

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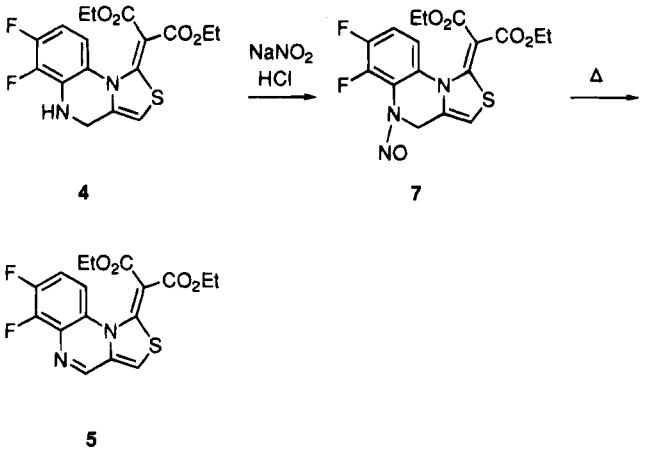
Scheme 1



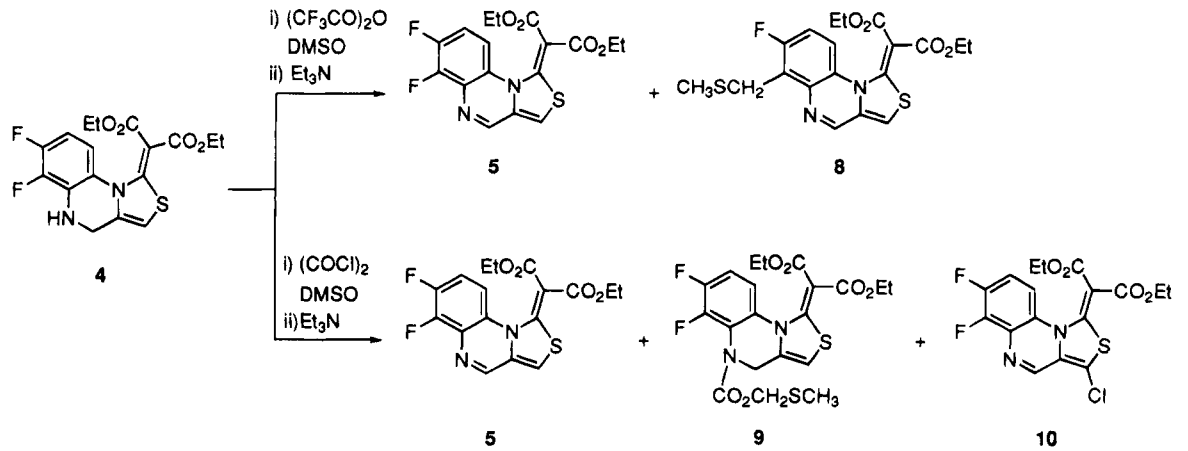
We investigated the oxidation of the amine **4** to the imine **5** with DMSO activated by suitable electrophiles, which were similar to those used in the oxidizing procedures of alcohols to ketones.

Firstly, the amine **4** was oxidized with DMSO/trifluoroacetic anhydride to give the imine **5** and the byproduct **8** (Scheme 2). The amine **4** was allowed to react with 2.5 equiv of DMSO, activated by 1.6 equiv of trifluoroacetic anhydride, at −65 °C in dichloromethane. After 1.4 h, an unstable intermediate was observed by TLC and the reaction mixture was treated with triethylamine at −50 °C to yield the imine **5** (48%) and the byproduct **8** (30%). The ^{1}H-NMR of **5** revealed two signals at δ 7.44 (s, 1 H) and δ 8.47 (s, 1 H), corresponding to the two vinyl protons, and no signal corresponding to allylic methylene protons. The MS spectrum showed m/z = 380 (M^+). The ^{1}H-NMR of **8** revealed two signals at δ 7.32 (s, 1 H) and δ 8.46 (s, 1 H), corresponding to the two vinyl protons, and two signals at δ 4.15 (d, J = 2 Hz, 2 H) and δ 2.13 (d, J = 1 Hz, 3 H), indicating the presence of a new methylene and a new methyl group, respectively. Also, the two signals at δ 7.05 (dd, J = 8.5, 9.5 Hz, 1 H) and δ 7.78 (dd, J = 5, 9.5 Hz, 1 H) suggested a monofluorine substitution, based on the couplings of the protons. The MS spectrum of **8** showed m/z = 422 (M^+). These data supported the suggested structures of **5** and **8**.

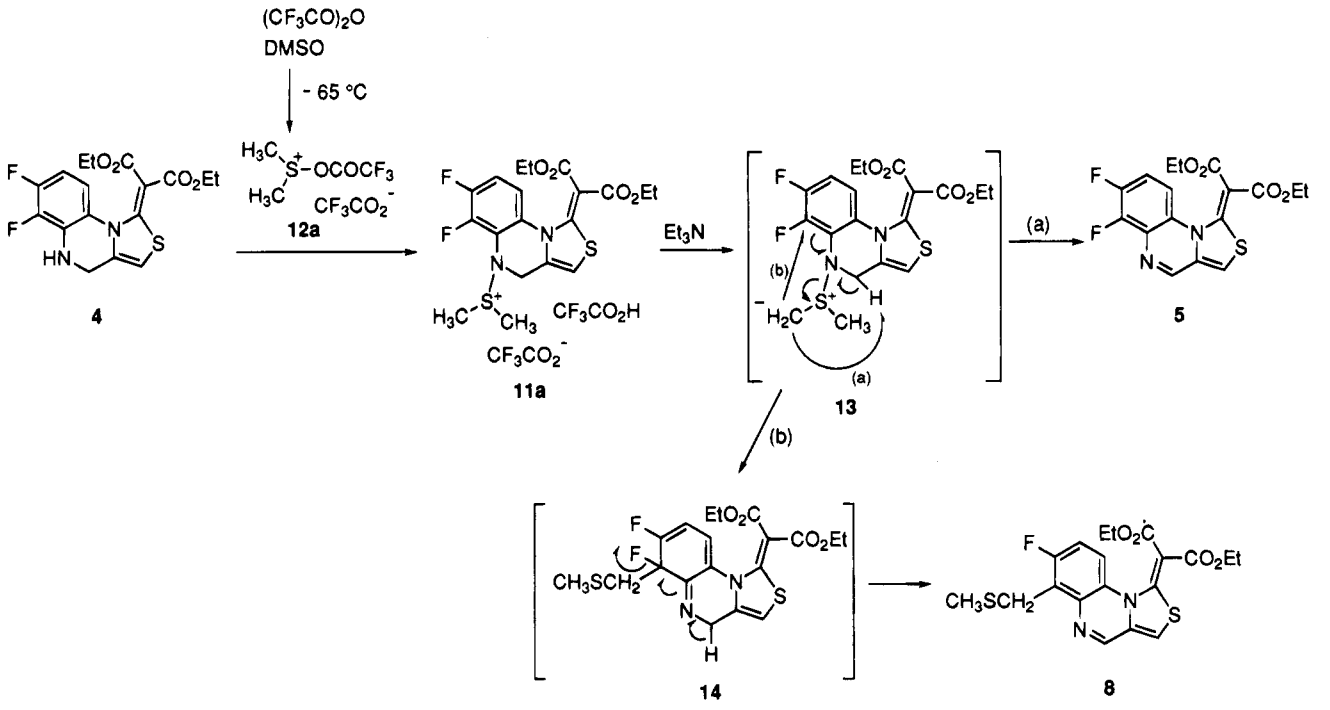
The amine **4** was also oxidized with DMSO/oxalyl chloride. The amine **4** was allowed to react with 3.0 equiv of DMSO, activated by 1.65 equiv of oxalyl chloride at −65 °C in dichloromethane. After 1.5 h, an unstable intermediate was again observed by TLC and the reaction mixture was treated with triethylamine at −60 °C to give **5** (35%) and the byproducts **9** (23%) and **10** (11%) (Scheme 2). The ^{1}H NMR of **9** revealed two signals at δ 3.96 (d, J = 16 Hz, 1 H) and δ 5.48 (d, J = 16 Hz, 1 H), corresponding to the allylic methylene protons, and three signals at δ 5.09 (d, J = 12 Hz, 1 H), δ 5.42 (d, J = 12 Hz, 1 H), and δ 2.28 (s, 3 H), indicating the presence of the new methylene and methyl group, respectively. The ^{13}C NMR revealed three signals at δ 152.6, δ 15.3, and δ 72.0, corresponding to the new carbonyl, methyl, and methylene carbon, respectively. The MS spectrum showed m/z = 486 (M^+). The IR spectrum of **9** was characterized by the presence of a $NCO_2CH_2SCH_3$ moiety at 1730 cm^{-1}. The ^{1}H-NMR of **10** revealed a signal at δ 8.51 (s, 1 H), corresponding to the new vinyl protons, and no signal corresponding to the allylic methylene protons. The MS spectrum showed m/z = 414 (M^+). These data supported the suggested structures of **9** and **10**.

Reaction Mechanism. The oxidation of **4** with DMSO/trifluoroacetic anhydride: In order to clarify the reaction mechanism, we tried to isolate the intermediate observed by TLC. The amine **4** was allowed to react with 1.8 equiv of DMSO, activated by 1.6 equiv of trifluoroacetic anhydride, at −65 °C in dichloromethane. After 1.5 h, the solution was evaporated and the residue was recrystallized twice from dry dichloromethane/diisopropyl ether to give an azasulfonium salt **11a** (76%). The ^{1}H-NMR of **11a** revealed a signal at δ 3.36 (s, 6 H), indicating the presence of the two new methyl groups. The ^{13}C NMR were characterized by two signals at δ 160.7 (q, J = 38 Hz) and δ 115.8 (q, J = 289 Hz), corresponding to the two carbons of trifluoroacetic acid. The signal appearing at δ 30.2 (s) was due to the new methyl carbon. The analyses for C, H, N were within ±0.2% of theoretical values of $C_{23}H_{11}F_8N_2O_8S_2$. On the basis of these data, the structure of dimethylazasulfonium ditrifluoroacetate **11a** was established. The treatment of isolated **11a** with triethylamine at −68 °C in dichloromethane gave **5** (42%), **8** (27%), and **4** (16%). From these results, the mechanism of oxidation of amine **4** with DMSO activated by trifluoroacetic anhydride may be illustrated, as shown in Scheme 3. The DMSO "activator" **12a**,[9] obtained from DMSO and trifluoroacetic anhydride, reacts with **4** to give the azasulfonium salt **11a** as the intermediate. The azasulfonium salt **11a** is treated with triethylamine to form the ylide **13**, followed by a spontaneous reaction (paths a and b) to give the imine **5** and the byproduct **8**. The nucleophilic center of the ylide **13** attacks the

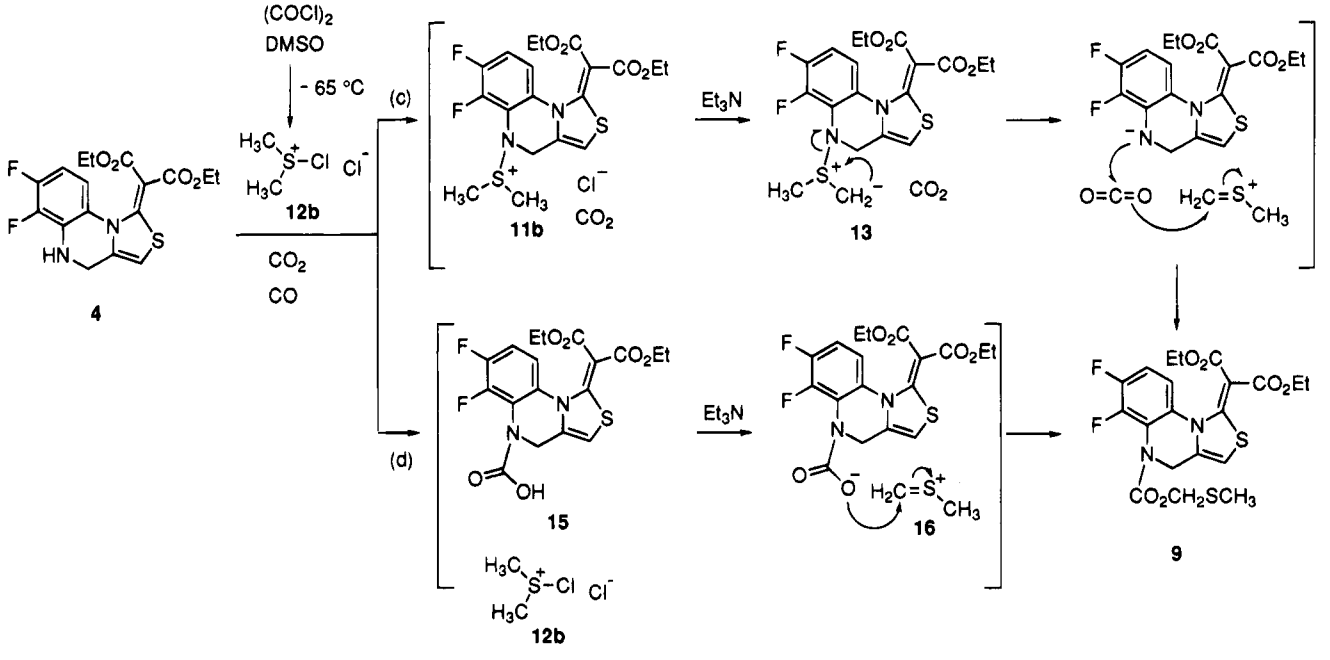
Scheme 2



Scheme 3



Scheme 4



hydrogen atom of the α-position of the nitrogen atom, and the elimination of dimethyl sulfide leads to **5** (path a). The intermolecular attack on the nucleophilic center of the ylide **13**, in a Sommelet–Hauser-type rearrangement,[10] affords **14** followed by the elimination of hydrogen fluoride to give the byproduct **8** (path b).

For the oxidation of **4** with DMSO/oxalyl chloride, the plausible mechanisms of the formation of the byproduct **9** from the amine **4** can proceed via two routes (pathways c and d) (Scheme 4). The reaction of the amine **4** with the DMSO "activator" **12b**[11] gives the putative azasulfonium salt **11b** and is followed by treatment with triethylamine to form the ylide **13**. The byproduct **9** is yielded by an insertion of a carbon dioxide, resulting from DMSO and oxalyl chloride, during a Pummerer-type rearrangement[9] of the ylide **13** (path c). The reaction of the amine **4** with carbon dioxide generates the carbamic acid **15**, which reacts[12] with the reactive cationic **16** formed by treatment of the DMSO "activator" **12b** with triethylamine to give **9** (path d). In both pathways, the carbon dioxide in the reaction system participates, and its removal would prevent the occurrence of the byproduct **9**. The bubbling of argon gas before the addition of

(9) Omura, K.; Sharma, A. K.; Swern, D. *J. Org. Chem.* **1976**, *41*, 957–962.

(10) (a) Hiraki, Y.; Kamiya, M.; Tanikaga, R.; Ono, N.; Kaji, A. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 447–452. (b) Gassman, P. G.; Gruetzmacher, G. D. *J. Am. Chem. Soc.* **1974**, *96*, 5487–5495.

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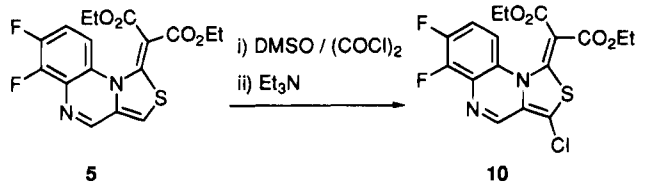
(12) Ho, T.-L. *Synth. Commun.* **1979**, *9*, 267–270.

Scheme 5



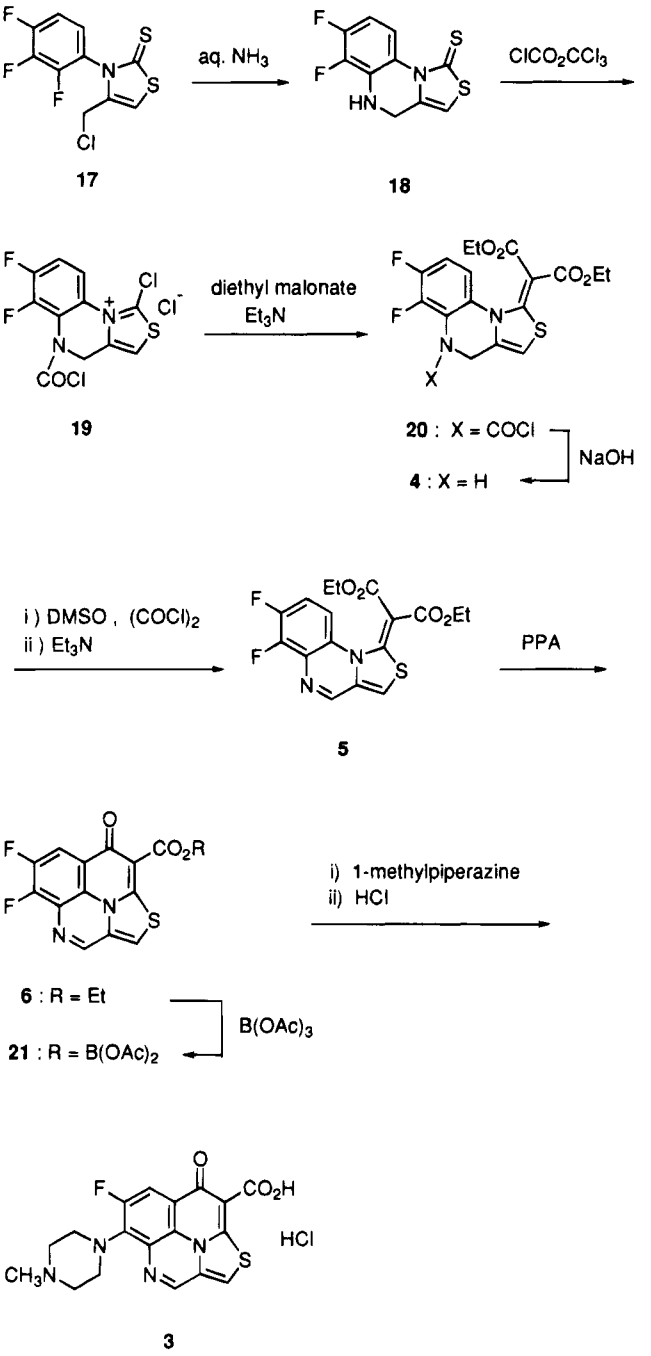
5 10

the amine **4** produced **5**, **10**, and **9** in 58%, 19%, and about 4% yields, respectively. Also, the bubbling of argon gas before the treatment with triethylamine produced **5** and **10** in 52% and 27% yields, respectively, with **9** occurring as only a trace. From these results, it seems likely that the byproduct **9** was formed by path c. On the other hand, the addition of silver trifluoroacetate before the triethylamine treatment gave **5** and **8**, which were the same products of the oxidation of amine **4** with DMSO/trifluoroacetic anhydride, in 25% and 15% yields, respectively, and **9** was not obtained. This result suggests that the counteranion of **11b** was exchanged[10b] by silver trifluoroacetate to give **11a**, which gave **5** and **8** upon treatment with triethylamine. The formation of the byproduct **9** from **4** would depend upon the nature of the counteranion of the dimethylazasulfonium salt **11**.

The formation of the byproduct **10** is interpreted to occur by the chlorination of the imine **5** with the DMSO "activator" **12b**. To the best of our knowledge, however, a chlorination reaction with DMSO/oxalyl chloride has not been reported. The imine **5** was allowed to react with 3.0 equiv of DMSO, activated by 2.0 equiv of oxalyl chloride, at low temperatures (−70 °C) in dichloromethane. After being stirred for 2 h, the reaction mixture was treated with triethylamine at −50 °C to yield **10** (76%) (Scheme 5). Before the treatment with triethylamine, the imine **5** could only be observed on analytical TLC plates. This observation unequivocally indicates that the byproduct **10** was generated by a chlorination of the imine **5** with an excess of the DMSO "activator" **12b** and triethylamine. We are in the progress of obtaining more data on the mechanism of the conversion of the imine **5** to **10**.

Synthesis of the New DNA Gyrase Inhibitor 3. Compound **17**[2] was treated with aqueous ammonia in acetonitrile to give the tricyclic compound **18** in a 56% yield. Treatment of compound **18** with trichloromethyl chloroformate in toluene yielded the iminium chloride **19** as a moisture-sensitive precipitate, which was allowed to react with diethyl malonate/triethylamine in acetonitrile to give **20** (61%). Compound **20** was hydrolyzed with NaOH in acetone/H_2O to afford **4** (85%). Oxidation of the amine **4** with DMSO/oxalyl chloride under the condition of argon gas bubbling gave the imine **5** in a 58% yield. The imine **5** was cyclized in polyphosphoric acid to give the tetracyclic pyridone carboxylic acid ester **6** in a 57% yield. The ester **6** was reacted with triacetoxyborane[13] in acetic anhydride to give the borate complex **21** (50%). The borate complex **21** readily reacted with 1-methylpiperazine in DMSO and was then treated with HCl (2 N) to give the final product **3** in a 54% yield (Scheme 6). Compound **3** was a strong DNA gyrase inhibitor. The antibacterial activity of **3** was potent, as compared to **1b**, against both Gram-positive and Gram-negative bacteria. Further studies of derivatives of **3** might create useful antibacterial agents and DNA gyrase inhibitors.

Scheme 6



17 18 19 20 : X = COCl 4 : X = H 5 6 : R = Et 21 : R = B(OAc)2 3

Experimental Section

Melting points were determined with a Buchi capillary melting point apparatus, Model 535; all melting points are uncorrected. The ^1H and ^{13}C NMR spectra were measured on a Bruker AM-300 spectrometer, with TMS or 3-(trimethylsilyl)-3-propanesulfonic acid sodium salt as an internal reference in a solution of $CDCl_3$, DMSO-d_6, or D_2O. IR spectra were recorded with a Hitachi IR 270-50 infrared spectrometer. Mass spectral measurements were obtained on a Hitachi M-80B. Elemental analyses were performed with a Yanagimoto CHN-CORDER MT-3, and all analytical values were within ±0.4% of the calculated theoretical values.

Oxidation of Diethyl (6,7-Difluoro-1*H*,4*H*-thiazolo[3,4-*a*]quinoxalin-1-ylidene)malonate (4) with the Procedure

(13) Fujiwara, T.; Tsurumi, H.; Sato, Y. Japan. Patent 60-75489, 1985; *Chem. Abstr.* **1986**, *105*, 153293j.

Using DMSO/Trifluoroacetic Anhydride. Trifluoroacetic anhydride (0.24 mL, 1.70 mmol) was added dropwise to a solution of DMSO (0.19 mL, 2.68 mmol) in CH_2Cl_2 (4 mL) at −65 °C. The reaction mixture was stirred for 15 min at the same temperature, the amine **4** (406 mg, 1.06 mmol) was added, and the reaction mixture was stirred at −60 °C for 1.4 h. Triethylamine (0.74 mL, 5.31 mmol) was added dropwise, and the reaction mixture was stirred at −50 °C for 50 min. The reaction mixture was diluted with $CHCl_3$, washed with water, dried over $MgSO_4$, and evaporated in vacuo. The residue was chromatographed through a flash silica gel column (eluted with $CHCl_3$) to give two fractions. Each fraction was evaporated in vacuo, and the resulting residues were precipitated with diisopropyl ether to afford diethyl (7-fluoro-6-[(methylthio)methyl]-1*H*-thiazolo[3,4-*a*]quinoxalin-1-ylidene)-malonate (**8**) (136 mg, 30%) as yellow crystals and diethyl (6,7-difluoro-1*H*-thiazolo[3,4-*a*]quinoxalin-1-ylidene)malonate (**5**) (195 mg, 48%) as yellow crystals, respectively. **8** (diisopropyl ether): mp 119.8−121.8 °C; IR (KBr) 1684, 1636, 1477, 1455, 1419 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.14 (t, J = 7 Hz, 6 H), 2.13 (d, J = 1 Hz, 3 H), 4.08 (q, J = 7 Hz, 4 H), 4.15 (d, J = 2 Hz, 2 H), 7.05 (dd, J = 8.5, 9.5 Hz, 1 H) 7.32 (s, 1 H), 7.78 (dd, J = 5, 9.5 Hz, 1 H), 8.46 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.1, 15.6, 26.3 (d, J_{CCCF} = 2 Hz), 60.5, 91.3, 113.4, 114.1 (d, J_{CCF} = 24.9 Hz), 115.4 (d, J_{CCCF} = 9.5 Hz), 125.2 (d, J_{CCF} = 16.8 Hz), 126.7 (d, J_{CCCCF} = 3.1 Hz), 132.0, 136.2 (d, J_{CCCF} = 6.4 Hz), 147.4, 158.4 (d, J_{CF} = 248.3 Hz), 165.5, 165.9. Anal. Calcd for $C_{19}H_{19}F_1N_2O_4S_2$: C, 54.01; H, 4.53; N, 6.63. Found: C, 53.94; H, 4.57; N, 6.71. **5** (diisopropyl ether/ $CHCl_3$): mp 191−193 °C; IR (KBr) 1662, 1627, 1505, 1417 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.18 (t, J = 7 Hz, 6 H), 4.11 (q, J = 7 Hz, 4 H), 7.14 (dt, J = 8, 9 Hz, 1 H) 7.44 (s, 1 H), 7.58 (ddd, J = 2.5, 4.5, 9.5 Hz, 1 H), 8.47 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.1, 60.6, 92.2, 110.9 (dd, J_{CCCCF} = 5.4 Hz, J_{CCCF} = 6.7 Hz), 114.8, 115.0 (d, J_{CCF} = 19.1 Hz), 126.7 (d, J_{CCCF} = 3.0 Hz), 128.4 (q, J_{CCCF}, J_{CCF} = 7.9 Hz), 131.6, 145.5 (dd, J_{CCF} = 13.5 Hz, J_{CF} = 257.3 Hz), 148.3 (dd, J_{CCF} = 11.5 Hz, J_{CF} = 250.3 Hz), 165.3, 165.7; MS m/z 380 (100). Anal. Calcd for $C_{17}H_{14}F_2N_2O_4S$: C, 53.68; H, 3.71; N, 7.36. Found: C, 53.77; H, 3.65; N, 7.29.

Oxidation of the Amine 4 with the Procedure Using DMSO/Oxalyl Chloride. Method A. DMSO (0.28 mL, 3.95 mmol) was added dropwise to a solution of oxalyl chloride (0.19 mL, 2.18 mmol) in CH_2Cl_2 (5 mL) at −65 °C. The reaction mixture was stirred for 15 min at the same temperature, the amine **4** (500 mg, 1.31 mmol) was added, and the reaction mixture was stirred at −60 °C for 1.5 h. Triethylamine (1.00 mL, 7.17 mmol) was added dropwise, and the reaction mixture was stirred at −60 °C for 50 min. The reaction mixture was diluted with $CHCl_3$, washed with water, dried over $MgSO_4$, and evaporated in vacuo. The residue was chromatographed through a flash silica gel column (eluted with $CHCl_3$) to give three fractions. Each fraction was evaporated in vacuo, and the resulting residues were precipitated with diisopropyl ether to afford diethyl (3-chloro-6,7-difluoro-1*H*-thiazolo[3,4-*a*]quinoxalin-1-ylidene)malonate (**10**) (58 mg, 11%) as dark yellow crystals, diethyl (6,7-difluoro-5-[[[(methylthio)methyl]oxy]carbonyl]-1*H*,4*H*-thiazolo[3,4-*a*]quinoxalin-1-ylidene)malonate (**9**) (146 mg, 23%) as yellow crystals, and **5** (171 mg, 35%), respectively. **10** (diisopropyl ether/$CHCl_3$): mp 225−233 °C dec; IR (KBr) 1704, 1640, 1506, 1482, 1437 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.18 (t, J = 7 Hz, 6 H), 4.10 (q, J = 7 Hz, 4 H), 7.12 (dt, J = 8, 9 Hz, 1 H) 7.42 (ddd, J = 2, 4.5, 9.5 Hz, 1 H), 8.51 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.1, 61.0, 93.6, 110.6 (t, J_{CCCCF}, J_{CCCF} = 5.8 Hz), 115.3 (d, J_{CCF} = 19.1 Hz), 121.0, 126.3 (d, J_{CCCF} = 3.0 Hz), 127.0, 128.4 (q, J_{CCCF}, J_{CCF} = 10.5 Hz), 145.7 (dd, J_{CCF} = 13.7 Hz, J_{CF} = 258.6 Hz), 146.5, 148.3 (dd, J_{CCF} = 11.5 Hz, J_{CF} = 250.4 Hz), 162.5, 165.4; MS m/z 416 (35), 414 (100). Anal. Calcd for $C_{17}H_{13}ClF_2N_2O_4S$: C, 49,22; H, 3.16; N, 6.75. Found: C, 49.34; H, 3.26; N, 6.69. **9** (diethyl ether/$CHCl_3$): mp 150.1−152.1 °C; IR (KBr) 1730, 1698, 1649, 1514, 1493, 1452 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.0−1.3 (m, 6H), 2.28 (s, 3 H), 3.95 (d, J = 16 Hz, 1 H), 3.9−4.2 (m, 4 H), 5.09 (d, J = 11.5 Hz, 1 H), 5.42 (d, J = 11.5 Hz, 1 H), 5.54 (d, J = 16 Hz, 1 H), 6.59 (t, J = 1 Hz, 1 H), 7.12 (dt, J = 8, 9.5 Hz, 1 H), 7.39 (ddd, J = 2, 4.5, 9.5 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.2, 15.3, 44.2, 60.4, 72.0, 90.5, 105.0, 113.8, 114.9 (d, J_{CCF} = 18.9 Hz), 121.6 (d, J_{CCF} = 12.7 Hz), 130.4, 136.2, 145.3 (dd, J_{CCF} = 14.5 Hz, J_{CF} = 271.8 Hz), 148.4 (dd, J_{CF} = 250.7 Hz, J_{CCF} = 11.3 Hz), 152.6, 165.4, 165.9; MS m/z 486 (75), 61 (100). Anal. Calcd for $C_{20}H_{20}F_2N_2O_6S_2$: C, 49.38; H, 4.14; N, 5.76. Found: C, 49.40; H, 4.01; N, 5.83.

Method B (Bubbling of Argon Gas before Addition of 4). DMSO (0.28 mL, 3.95 mmol) was added dropwise to a solution of oxalyl chloride (0.19 mL, 2.18 mmol) in CH_2Cl_2 (5 mL) under bubbling of argon gas at −65 °C, and the reaction mixture was stirred under bubbling of argon gas at the same temperature for 20 min. The amine **4** (500 mg, 1.31 mmol) was added, and the reaction mixture was stirred at −60 °C for 1.3 h. Triethylamine (1.00 mL, 7.17 mmol) was added dropwise, and the reaction mixture was stirred at −50 °C for 2.2 h. The reaction mixture was diluted with $CHCl_3$, washed with water, dried over $MgSO_4$, and evaporated in vacuo. The residue was chromatographed through a flash silica gel column (eluted with $CHCl_3$) to give three fractions. The first and third fractions were evaporated in vacuo, and the resulting residues were precipitated with diisopropyl ether to afford **10** (119 mg, 19%) and **5** (206 mg, 52%), respectively. The second fraction was evaporated in vacuo to give a mixture of **9** and **8** (36 mg, the yields of **9** and **8** were about 4% and 1% based on ^{1}H NMR, respectively).

Method C (Bubbling of Argon Gas before Treatment of Triethylamine). DMSO (0.22 mL, 3.10 mmol) was added dropwise to a solution of oxalyl chloride (0.15 mL, 1.72 mmol) in CH_2Cl_2 (4 mL) at −65 °C and the reaction mixture was stirred at the same temperature for 20 min. The amine **4** (400 mg, 1.05 mmol) was added, the reaction mixture was stirred at −60 °C for 1 h, and argon gas was bubbled in the reaction mixture at the same temperature for 30 min. Triethylamine (0.72 mL, 5.17 mmol) was added dropwise, and the reaction mixture was stirred at −60 °C for 1 h. The reaction mixture was diluted with $CHCl_3$, washed with water, dried over $MgSO_4$, and evaporated in vacuo. The residue was chromatographed through a flash silica gel column (eluted with $CHCl_3$) to give two fractions. Each fraction was evaporated in vacuo, and the resulting residues were precipitated with diisopropyl ether to afford **10** (119 mg, 27%) and **5** (206 mg, 52%), respectively.

Method D (Treatment of Silver Trifluoroacetate). DMSO (0.15 mL, 2.11 mmol) was added dropwise to a solution of oxalyl chloride (0.10 mL, 1.15 mmol) in CH_2Cl_2 (3 mL) at −65 °C. The reaction mixture was stirred at the same temperature for 20 min, the amine **4** (267 mg, 0.698 mmol) was added, and the reaction mixture was stirred at −60 °C for 1.5 h. Silver trifluoroacetate was added, and the reaction mixture was stirred at −60 °C for 20 min. Triethylamine (1.00 mL, 7.17 mmol) was added dropwise, and the reaction mixture was stirred at −60 °C for 1 h. The reaction mixture was diluted with $CHCl_3$, washed with water, dried over $MgSO_4$, and evaporated in vacuo. The residue was chromatographed through a flash silica gel column (eluted with $CHCl_3$) to give two fractions. Each fraction were evaporated in vacuo, and the resulting residues were precipitated with diisopropyl ether to afford **8** (44 mg, 15%) and a mixture of **5** and **4** (109 mg, the yields of **5** was 25% based on the ^{1}H NMR), respectively.

Isolation of [1-[Bis(ethoxycarbonyl)methylene]-6,7-difluoro-1*H*,4*H*-thiazolo[3,4-*a*]quinoxalin-5-yl]dimethylsulfonium Bis(trifluoroacetate) (11a). Trifluoroacetic anhydride (0.20 mL, 1.42 mmol) was added dropwise to a solution of DMSO (0.11 mL, 1.54 mmol) in dry CH_2Cl_2 (3 mL) at −65 °C. The reaction mixture was stirred at the same temperature for 20 min, the amine **4** (328 mg, 0.858 mmol) was added, and the reaction mixture was stirred at −55 °C for 1.4 h. The reaction mixture was evaporated in vacuo at rt, and the residue was recrystallized twice from dry CH_2Cl_2/diisopropyl ether to afford **11a** (439 mg, 76%) as pale yellow crystals: mp 96.9−99.2 °C; IR (KBr) 1781, 1688, 1641, 1511, 1488, 1471 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.27 (t, J = 7 Hz, 6 H), 3.36 (s, 6 H), 4.09 (m, 4 H), 4.91 (br, 2 H), 6.94 (t, J = 1 Hz), 7.27 (dt, J = 8.5, 9.5 Hz, 1 H), 7.43 (ddd, J = 2, 4, 9.5 Hz, 1 H), 17.60 (br, s); ^{13}C NMR ($CDCl_3$) δ 14.2, 30.2, 49.0, 61.0, 92.4, 108.0, 114.8 (t, $J_{CCCF, CCCCF}$ = 4.7 Hz), 115.8 (q, J_{CF} = 289.5 Hz, CF_3), 117.9 (d, J_{CCF} = 18.8 Hz), 120.8 (d, J_{CCF} = 12.2 Hz), 133.1, 133.5 (d,

J_{CCCCF} = 3.2 Hz), 145.9 (dd, J_{CCF} = 14.8 Hz, J_{CF} = 256.1 Hz), 148.1 (dd, J_{CCF} = 11.2 Hz, J_{CF} = 254.0 Hz), 160.7 (q, J_{CCF} = 37.8 Hz, CCF_3), 164.7, 166.0. Anal. Calcd for $C_{23}H_{22}F_8N_2O_8S_2$: C, 41.20; H, 3.31; N, 4.18. Found: C, 41.21; H, 3.26; N, 4.35.

Treatment of 11a with Triethylamine. Triethylamine (0.16 mL, 1.15 mmol) was added dropwise to a solution of compound **11a** (220 mg, 0.329 mmol) in dry CH_2Cl_2 at −60 °C, and the reaction mixture was stirred at the same temperature for 1 h. The reaction mixture was diluted with $CHCl_3$, washed with water, dried over $MgSO_4$, and evaporated in vacuo. The residue was chromatographed through a flash silica gel column (eluted with $CHCl_3$) to give **8** (38 mg, 27%) and a mixture of **5** and **4** (72 mg, the yield of **5** and **4** were 42% and 16% based on the ^1H NMR, respectively).

Diethyl (6,7-Difluoro-5-nitroso-1*H*,4*H*-thiazolo[3,4-*a*]quinoxalin-1-ylidene)malonate (7). To a solution of the amine **4** (1.14 g, 2.98 mmol) in dioxane was added HCl (2 N, 8 mL) and a solution of $NaNO_2$ (479 mg, 6.85 mmol) in water (3 mL) at 5 °C, and the reaction mixture was stirred at the same temperature for 1.5 h. The precipitates were collected by filtration, washed with water, and recrystallized from $CHCl_3$/diisopropyl ether to afford **7** (888 mg, 72%) as yellow crystals: mp 158−163 °C dec; IR (KBr) 2250, 1707, 1704, 1646, 1517, 1481, 1463 cm^{-1}; ^1H-NMR ($CDCl_3$) as the mixture of stereoisomer of nitroso group at 4 position (**7** and **7′**; 7:3) δ 1.17 (t, J = 7 Hz, 4.2 H, 2 CH_3 of **7**), 1.0−1.3 (br, 1.8 H, 2 CH_3 of **7′**), 3.9−4.2 (m, 4 H), 4.9−5.2 (br, 1.7 H), 6.31 (d, J = 16.5, 0.3 H), 6.63 (t, J = 1.5 Hz, 0.7 H, =CHS of **7**), 6.73 (t, J = 1.5 Hz, 0.3 H, =CHS of **7′**), 7.2−7.4 (m, 1 H), 7.44 (ddd, J = 2, 4, 9.5 Hz, 0.3 H), 7.50 (ddd, J = 2, 4, 9.5 Hz, 0.7 H). Anal. Calcd for $C_{17}H_{15}F_2N_3O_5S$: C, 49.63; H, 3.68; N, 10.21. Found: C, 49.45; H, 3.76; N, 10.01.

Diethyl (6,7-Difluoro-1*H*-thiazolo[3,4-*a*]quinoxalin-1-ylidene)malonate (5). A solution of compound **7** (1.04 g, 2.53 mmol) in toluene (20 mL) was heated at 110−115 °C for 6.5 h. The reaction mixture was filtrated, the filtrate was concentrated in vacuo, and the residue was chromatographed on silica gel with $CHCl_3$ as eluate. The resulting precipitates were washed with diethyl ether to give **5** (318 mg, 33%).

6,7-Difluoro-1*H*,4*H*-thiazolo[3,4-*a*]quinoxaline-1-thione (18). A mixture of compound **17** (20.0 g, 67.6 mmol) and 36% aqueous ammonia solution (88 mL) in CH_3CN (197 mL) was stirred at 45 °C for 16 h. Triethylamine (19.9 mL, 0.143 mol) was added, and the reaction mixture was refluxed for 23 h. After the reaction mixture was cooled, the precipitates were collected and washed with CH_3CN to give **18** (9.78 g, 56%) as yellow crystals: mp 211−218 °C dec; IR (KBr) 3316, 1520, 1491 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 4.21 (m, 2 H), 4.49 (br, 1 H), 6.41 (t, J = 1 Hz, 1 H), 6.74 (dt, J = 8.5, 9.5 Hz, 1 H), 7.26 (s, 1 H), 9.45 (ddd, J = 2.5, 5, 9.5 Hz, 1 H). Anal. Calcd for $C_{10}H_6F_2N_2S_2$: C, 46.86; H, 2.36; N, 10.93. Found: C, 46.92; H, 2.45; N, 10.91.

Diethyl (5-(Chlorocarbonyl)-6,7-difluoro-1*H*,4*H*-thiazolo[3,4-*a*]quinoxalin-1-ylidene)malonate (20). A mixture of compound **18** (20.0 g, 0.078 mol) and trichloromethyl chloroformate (13.1 mL, 0.109 mol) in dry toluene (194 mL) was stirred at 80 °C for 18 h to yield compound **19** as a precipitate, which was not isolated because of its moisture-sensitivity. The supernatant solution was removed by decantation, and the precipitate was washed with dry toluene by decantation. A solution of diethyl malonate (16.25g, 0.101 mol) in dry CH_3CN (60 mL) was added to the precipitate, and triethylamine (25.1 mL, 0.179 mol) was then added dropwise. After being stirred at room temperature for 35 min, the reaction mixture was poured in ice−water and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried over $MgSO_4$, and evaporated in vacuo. The residue was washed with diisopropyl ether to afford **20** (23.6 g, 68%) as yellow crystals: mp 196−202 °C dec; IR (KBr) 1745, 1738, 1695, 1646, 1514, 1449 cm^{-1}; ^1H-NMR (acetone-d_6) as the mixture of stereoisomers of chlorocarbonyl group at the 4 position (**20** and **20′**; 13:7) δ 0.9−1.4 (m, 6 H), 3.5−4.2 (m, 4 H), 4.38 (d, J = 16 Hz, 0.65 H , NCH_2 of **20**), 4.65 (d, J = 17Hz, 0.35 H, NCH_2 of **20′**), 5.62 (d, J = 16Hz, 0.65 H, NCH_2 of **20**), 5.81 (d, J = 17Hz, 0.35 H, NCH_2 of **20′**) 7.09 (s, 0.65 H, =CHS of **20**), 7.13 (s, 0.35 H, =CHS of **20′**), 7.4−7.6 (m, 2 H). Anal. Calcd for $C_{18}H_{15}ClF_2N_2O_5S$: C, 48.60; H, 3.40; N, 6.30. Found: C, 48.26; H, 3.34; N, 6.26.

Diethyl (6,7-Difluoro-1*H*,4*H*-thiazolo[3,4-*a*]quinoxalin-1-ylidene)malonate (4). A mixture of compound **20** (5.20 g, 0.0119 mol) and aqueous NaOH (1 N, 24.7 mL, 0.0247 mol) in acetone (25 mL) was stirred for 70 min at rt. HCl (2 N, 12.4 mL) was added, and the reaction mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was precipitated with diisopropyl ether to yield **4** (3.84 g, 85%) as yellow crystals. **4** (recrystallized from chloroform/diisopropyl ether): mp 173−176 °C; IR (KBr) 3366, 1680, 1634 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 1.16 (t, J = 7 Hz, 6 H), 3.96 (q, J = 7 Hz, 4 H), 4.23 (d, J = 1 Hz, 2 H), 6.53 (t, J = 1 Hz, 1 H), 6.68 (dt, J = 8, 9.5 Hz, 1 H) 7.33 (ddd, J = 2.5, 4.5, 9.5 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.1, 43.7, 60.1, 89.2, 104.4, 107.1 (d, J_{CCF} = 19.0 Hz), 113.4 (dd, J_{CCCF} = 4 Hz, J_{CCF} = 7.7 Hz), 125.5 (t, $J_{CCCF,\ CCCCF}$ = 3 Hz), 130.1 (d, J_{CCF} = 13.2 Hz), 136.5, 140.3 (dd, J_{CCF} = 15.5 Hz, J_{CF} = 241.4 Hz), 148.0 (dd, J_{CCF} = 10.6 Hz, J_{CF} = 248.5 Hz), 166.1. Anal. Calcd for $C_{17}H_{16}F_2N_2O_4S$: C, 53.40; H, 4.22; N, 7.33. Found: C, 53.28; H, 4.12; N, 7.24.

Ethyl 4,9*b*-Diaza-5,6-difluoro-8,9*b*-dihydro-8-oxo-1-thia-1*H*-cyclopenta[*cd*]phenalene-9-carboxylate (6). A mixture of the imine **5** (1.16 g, 3.06 mol) and polyphosphoric acid (11.9 g) was stirred at 100−105 °C for 1 h. Ice−water was added, and the reaction mixture was dissolved and extracted with five 150-mL portions of $CHCl_3$. The combined organic phases were washed with water, dried over $MgSO_4$, and evaporated in vacuo. The residue was suspended with DMSO (17 mL) and stirred at 90−95 °C for 15 min. After the mixture was cooled, the precipitates were filtrated to give **6** (587 mg, 57%) as yellow crystals. **6** (recrystallized from DMSO/EtOH): mp > 280 °C; IR (KBr) 1712, 1580, 1507, 1474, 1448 cm^{-1}; ^1H-NMR (DMSO-d_6) δ 1.34 (t, J = 7 Hz, 3 H), 4.33 (q, J = 7 Hz, 2 H), 8.03 (dd, J = 7.5, 10.5 Hz, 1 H), 8.41 (s, 1 H), 9.08 (s, 1 H). Anal. Calcd for $C_{15}H_8F_2N_2O_3S$: C, 53.89; H, 2.41; N, 8.38. Found: C, 53.67; H, 2.55; N, 8.14.

Diacetoxy (4,9*b*-Diaza-5,6-difluoro-8,9*b*-dihydro-8-oxo-1-thia-1*H*-cyclopenta[*cd*]phenalene-9-carboxylate)borane (21). A mixture of **6** (494 mg, 1.48 mmol) and triacetoxyborane (419 mg, 2.23 mmol) in acetic anhydride (3 mL) was heated at 90−95 °C for 65 min. The precipitates were collected by filtration and washed with acetic anhydride and isopropyl ether to afford **21** (321 mg, 50%) as pale yellow crystals: mp > 280 °C; IR (KBr) 1739, 1717, 1691, 1513, 1477 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.93 (s, 6 H), 8.51 (dd, J = 7.5, 10 Hz, 1 H), 9.08 (s, 1 H), 9.42 (s, 1 H). Anal. Calcd for $C_{17}H_9BF_2N_2O_7S$: C, 47.03; H, 2.09; N, 6.45. Found: C, 46.98; H, 2.16; N, 6.46.

4,9*b*-Diaza-8,9*b*-dihydro-6-fluoro-5-(4-methyl-1-piperazinyl)-8-oxo-1-thia-1*H*-cyclopenta[*cd*]phenalene-9-carboxylic Acid Hydrochloride (3). A mixture of **21** (340 mg, 0.783 mmol) and 1-methylpiperazine (0.43 mL, 3.915 mmol) in DMSO (3 mL) was stirred at 50−60 °C for 2 h. DMSO was removed under reduced pressure, HCl (2 N, 6 mL) was added, and the mixture was stirred for 20 min at room temperature. The precipitates were collected by filtration and recrystallized from HCl (1 N) to afford **3** (180mg, 54%) as pale yellow crystals: mp > 280 °C dec; IR (KBr) 1695, 1613, 1574, 1483 cm^1; ^1H NMR (D_2O) δ 3.06 (s, 3 H), 3.4−3.6 (m, 2 H), 3.6−3.8 (m, 4 H), 3.9−4.1 (m, 2 H), 7.09 (d, J = 12.5 Hz, 1 H), 7.37 (d, J = 12.5 Hz, 1 H), 8.13 (s, 1 H), 8.67 (s, 1 H). Anal. Calcd for $C_{18}H_{16}ClFN_4O_3S{\cdot}0.5H_2O$: C, 50.06; H, 3.97; N, 12.97. Found: C, 49.91; H, 3.89; N, 12.89.

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