Structure Correction and Synthesis of the Naturally Occurring Benzothiazinone BMY 40662

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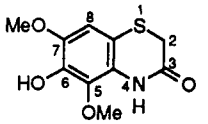
In the course of joint efforts of the National Cancer Institute and Bristol-Myers aimed at the large-scale fermentative production of the anticancer agent esperamycin A_1, a number of byproducts was generated.[1] One of those side products was designated BMY 40662 and assigned structure 1, largely on the basis of a variety of NMR experiments. Our continuing interest[2] in the synthesis of naturally occurring heterocycles coupled with a desire to validate the structure assignment led us to undertake the synthesis of 1.



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The synthesis (Scheme I) commenced with electrophilic thiocyanation[3] of commercially available 2,6-dimethoxyphenol (2) to give thiocyanate 3. Attempts to introduce the ring nitrogen of 1 by nitrating 3 failed, perhaps because of side reactions entailing, in part, competitive oxidation to a benzoquinone. The corresponding acetate 4 of 3, however, underwent the desired nitration to give 5. The yield of 5 leaves much room for improvement, but for reasons soon to be apparent, no efforts were directed to that objective. Reduction of 5 with neutral dithiothreitol[4] produced thiophenol 6. Attempts to isolate 6 were frustrated by formation of the dimeric disulfide 8. However, trapping of 6 *in situ* with methyl α-bromoacetate gave 7 in good overall yield. Reaction of 7 with $SnCl_2$/HCl then not only reduced the nitro group but also induced cyclization and deacetylation, giving 1 directly.[5]

The NMR (^1H and ^{13}C) spectra and melting point for synthetic 1 are similar (see Table I) to those reported for BMY 40662, but differences are, nevertheless, apparent.

Scheme I

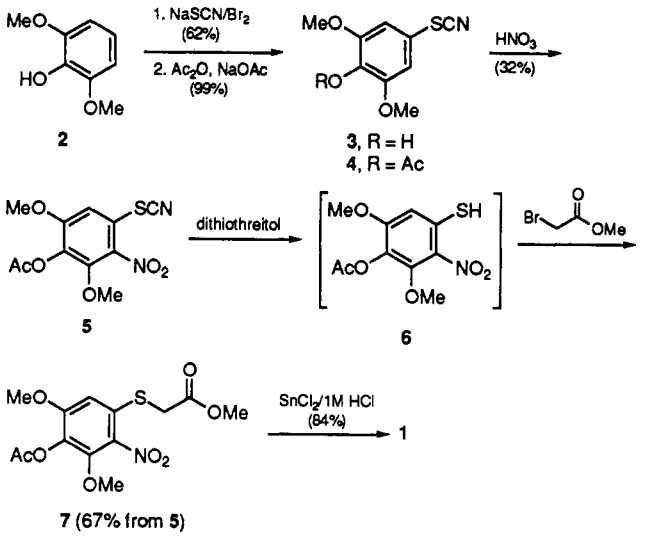


Table I

	1	BMY 40662[1]	10
mp (°C)	128–130	149–152	174–175
solubility in $CHCl_3$	soluble	insoluble	insoluble
^1H NMR (300 MHz, DMSO-d_6)	3.35	3.34	3.34
	3.71	3.73	3.72
	3.73	3.74	3.73
	6.67	6.44	6.42
	8.90	8.62	8.62
	9.63	10.25	10.23
^{13}C NMR (75.43 MHz, DMSO-d_6)	29.92	28.81	28.77
	56.24	55.95	55.96
	60.19	59.72	59.67
	105.96	97.72	97.69
	108.21	105.31	105.27
	124.97	129.14	129.10
	137.12	135.38	135.34
	138.38	144.23	144.19
	144.36	147.76	147.73
	164.85	164.74	164.66
UV/vis λ_{max}^{MeOH} nm (log ε)	212 (4.10)	212 (4.29)	210 (4.13)
	236 (4.09)	239 (4.23)	236 (4.07)
	292 (3.42)	295 (3.64)	294 (3.49)
$\lambda_{max}^{MeOH+NaOH}$	248 (4.14)	262 (4.10)	264 (3.95)
	296 (3.62)	312 (3.81)	314 (3.69)

The UV/vis spectra (Table I) of 1 and BMY 40662 are virtually identical in neutral methanol, but in alkaline methanol they are at variance. Unfortunately, we were unable to determine whether the discrepancies were real or instrumental/typographical because we could not obtain an authentic sample of BMY 40662 or copies of its spectra.

Nonetheless, the accumulation of discord in the specifics led to the working hypothesis that BMY 40662 does not possess structure 1. Careful reconsideration of all of the data in the original report[1] identified a number of inconsistencies and errors.[6] The reexamination of data suggested that 9 and 10 were among alternative structures for BMY 40662 not incontrovertibly excluded by the

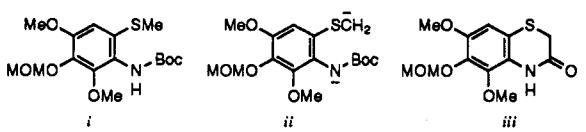
(1) Beutler, J. A.; Chmurny, G. N.; Clark, P.; Metral, C. J.; Roman, J.; Forenza, S. *J. Antibiot.* **1990**, *43*, 107–109.

(2) For earlier work see: Kelly, T. R.; Walsh, J. J. *J. Org. Chem.* **1992**, *57*, 6657-6658 and ref 3 cited therein.

(3) Wood, J. L. In *Organic Reactions*; Wiley: New York, 1967; Vol III, pp 240–266.

(4) Witkop, B.; Nagamachi, T.; Fourrey, J. L.; Torrence, P. F.; Waters, J. A. *J. Med. Chem.* **1974**, *17*, 403–406 (see ref 29 therein).

(5) In an earlier route to 1 we were once able to convert i to iii (presumably via ii) by reaction with *n*-BuLi in the presence of DABCO (Wakefield, B. J. *Organolithium Methods*; Academic Press: New York, 1988; pp 34, 36, 38). Unfortunately, the conversion of i to iii could never be repeated. Compound i was made from 2 by successive reactions with (i) Br_2, (ii) MOM-Cl, NaOH, (iii) *n*-BuLi; $Me_3SiCH_2N_3$; aq workup, (iv) Boc_2O, (v) *t*-BuLi; MeSSMe. Hydrolysis (conc HCl in MeOH, 50 °C) of a sample of iii gave 1.



i *ii* *iii*

(6) For example, the text in ref 1 states the C-8 proton "is coupled to all other the aromatic carbon signals except the methoxy-substituted carbon at 148 ppm, thus defining it as *para* to this methoxyl group," but the tabulation of data assigns the 148 ppm peak to C-7, not C-5, and the same data table lists coupling between the C-8 proton and *all six* aromatic carbons.

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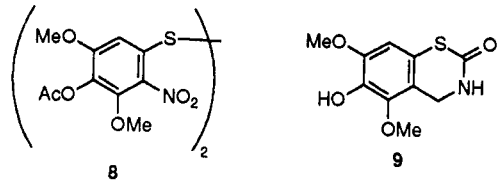
evidence provided[7] in the original structure report.[1] We tentatively set **9** aside as a possible structure since the IR carbonyl stretching frequency (1680 cm^{-1}) reported for BMY 40662 is substantially different from that (1620 cm^{-1}) reported[8] for **11**, the closest analog we located.



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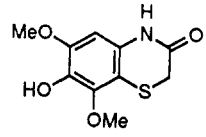
9



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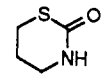

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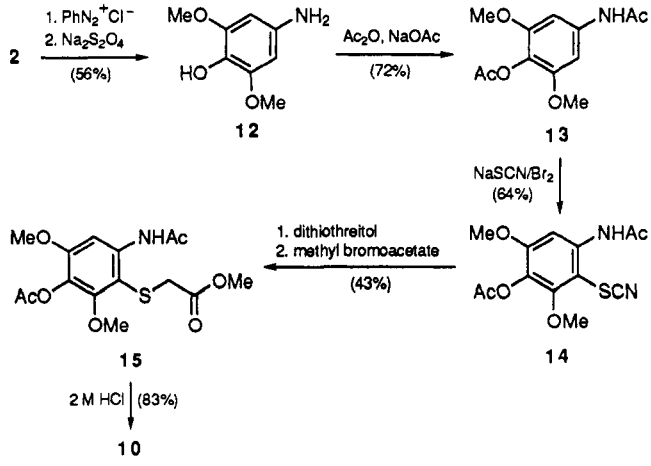
Accordingly, the synthesis of **10** was undertaken (Scheme II). Aminophenol **12** is a known compound,[9] available in

Scheme II



two known steps by diazonium coupling of phenol **2** with phenyldiazonium chloride followed by reduction. The bisacetyl derivative **13** was then converted to thiocyanate **14** which was reduced. As before, the resulting thiophenol species was trapped *in situ* to give **15**.[10] Treatment of **15** with 2 N HCl then cleaved the two acetyl groups and induced lactamization, giving **10** directly. As evidenced by the data given in Table I, the physical and spectral properties of BMY 40662 are in much closer agreement with those of **10** than **1**. Our inability to secure a sample or original spectra of BMY 40662 makes direct comparison of synthetic and natural materials impossible. Nonetheless, based on the congruity of the spectral data tabulated for them in Table I, we submit that the structure of BMY 40662 should be revised to **10**.

Experimental Section[11]

2,6-Dimethoxy-4-thiocyanophenol (3). 2,6-Dimethoxyphenol (**2**, 20.0 g, 130 mmol, Aldrich) and sodium thiocyanate (37.0 g, 456 mmol) were dissolved in methanol (200 mL). To this solution was added dropwise over 5 min through a dropping funnel at 0 °C a solution made by mixing 10.0 mL of bromine with 70 mL of a saturated solution of NaBr in methanol. The resulting mixture was stirred at 0–5 °C for 1 h. Water (150 mL) was added, and the methanol was evaporated on a rotary evaporator under aspirator vacuum. The aqueous phase was extracted with CH_2Cl_2 (3 × 300 mL), and the combined organic phases were dried ($MgSO_4$) and then reduced *in vacuo* to give an orange oil. The crude mixture was separated by flash column chromatography on a 4- × 5.5-in. column, eluting with 1:1 Et_2O/petroleum ether to give **3** (17.1 g, 81.0 mmol, 62%) as a pale yellow solid: ^1H NMR ($CDCl_3$) δ 3.93 (6 H, s), 5.74 (1 H, s), 6.81 (2 H, s); MS *m/z* (rel intensity) 212 (11), 211 (100, M^+), 196 (33); IR (KBr) ν 3343 (br), 2951, 2147, 1596 cm^{-1}. An analytical sample was obtained as white needles, mp 73–75 °C, after recrystallization from 1:1 Et_2O/petroleum ether. Anal. Calcd for $C_9H_9NO_3S$: C, 51.17; H, 4.29; N, 6.63. Found: C, 50.97; H, 4.39; N, 6.72.

2,6-Dimethoxy-4-thiocyanophenyl Acetate (4). To a stirred solution of 2,6-dimethoxy-4-thiocyanophenol (**3**, 14.2 g, 67.2 mmol) in acetic anhydride (100 mL) was added anhydrous sodium acetate (6.00 g, 73.0 mmol) in one portion at room temperature. The resulting suspension was stirred at room temperature for 15 h under nitrogen. The reaction mixture was quenched by adding water (100 mL) and stirred for a further 1 h to destroy excess acetic anhydride. The reaction mixture was extracted with CH_2Cl_2 (3 × 150 mL), and the combined organic phases were washed with water (200 mL) and brine (200 mL). The organic phase was dried ($MgSO_4$) and concentrated to give **4** (16.8 g, 66.3 mmol, 99%) as a yellow solid which was ordinarily used without further purification: ^1H NMR ($CDCl_3$) δ 2.34 (3 H, s), 3.85 (6 H, s), 6.77 (2 H, s); MS *m/z* (rel intensity) 253 (5, M^+), 212 (11), 121 (100); IR (KBr) ν 2944, 2360, 2161, 1769, 1596 cm^{-1}. An analytical sample was obtained as white plates, mp 65–66 °C, after recrystallization from 1:1 Et_2O/petroleum ether. Anal. Calcd for $C_{11}H_{11}NO_4S$: C, 52.17; H, 4.38; N, 5.53. Found: C, 51.93; H, 4.25; N, 5.38.

2,6-Dimethoxy-3-nitro-4-thiocyanophenyl Acetate (5). To a stirred solution of 2,6-dimethoxy-4-thiocyanophenyl acetate (**4**, 2.34 g, 9.24 mmol) in acetic anhydride (10 mL) was added, dropwise over 20 min, 90% fuming nitric acid (1.95 g, 27.8 mmol) at 0 °C. The reaction mixture was then warmed to room temperature and stirred for a further 1 h before being poured into ice/water (100 mL), giving a yellow precipitate. The yellow solid was collected and purified by flash column chromatography on a 2.75- × 9-in. column, eluting with 1:1 CH_2Cl_2/petroleum ether, to give **5** (0.87 g, 2.92 mmol, 32%) as a white solid; ^1H NMR ($CDCl_3$) δ 2.39 (3 H, s), 3.97 (3 H, s), 3.99 (3 H, s), 7.21 (1 H, s); MS *m/z* (rel intensity) 298 (7, M^+), 257 (11), 256 (100); IR (KBr) ν 2957, 2167, 1775, 1583, 1523 cm^{-1}. An analytical sample was obtained as pale yellow rhombic crystals, mp 113–115 °C, after recrystallization from Et_2O. Anal. Calcd for $C_{11}H_{10}N_2O_6S$: C, 44.30; H, 3.38; N, 9.39. Found: C, 44.26; H, 3.24; N, 9.33.

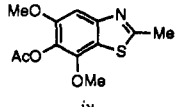
Methyl [(4-Acetoxy-3,5-dimethoxy-2-nitrophenyl)thio]acetate (7). To a stirred solution of dithiothreitol (1.31 g, 8.49 mmol) in methanol (120 mL) was added 2,6-dimethoxy-3-nitro-4-thiocyanophenyl acetate (**5**, 1.20 g, 4.02 mmol) in one portion at room temperature. The reaction mixture was stirred for 24 h under nitrogen. The solvent was evaporated and the residue was dissolved in anhydrous DMF (60 mL), followed by addition of methyl bromoacetate (10.0 mL, 119 mmol). This solution was heated at 90–95 °C for 1 h under nitrogen and then cooled to room temperature. The mixture was diluted with water (60 mL) and extracted with ethyl acetate (3 × 100 mL). The combined

(7) Permutations in methylation patterns of **1** or **10** were regarded as less likely alternatives since the 2D NMR[1] data appeared[6] to indicate that unsubstituted and OH-bearing carbons in the aromatic ring of BMY 40662 possessed a *meta* relationship.

(8) Grisley, D. W., Jr.; Szabo K. *Synthesis* **1972**, 318.

(9) Ettel, V.; Hebky, J. *Collect. Czech. Chem. Commun.* **1950**, *15*, 639–652.

(10) A byproduct is the benzothiazole **iv** which is very difficult to separate from **15**. Longer reaction times during the reduction step led to more of **iv** being produced at the ultimate expense of **15**. Mp and ^1H NMR ($CDCl_3$) data for **iv**: 102–104 °C, δ 2.39 (3 H, s), 2.80 (3 H, s), 3.89 (3 H, s), 3.99 (3 H, s), 7.27 (1 H, s).



iv

(11) For general experimental procedures see: Kelly, T. R.; Bridger, G. J.; Zhao, C. *J. Am. Chem. Soc.* **1990**, *112*, 8024–8034.

organic phases were dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by flash column chromatography on a 1.25- × 14-in. column, eluting with 5:1 CH_2Cl_2/petroleum ether to give 7 (0.94 g, 2.7 mmol, 67%) as a thick yellow oil which eventually solidified; ^1H NMR ($CDCl_3$) δ 2.37 (3 H, s), 3.65 (2 H, s), 3.75 (3 H, s), 3.90 (3 H, s), 3.91 (3 H, s), 7.06 (1 H, s); MS *m/z* (rel intensity) 345 (8, M^+), 304 (13), 303 (100), 272 (7); IR (KBr) ν 2951, 1775, 1736, 1583, 1530 cm^{-1}. An analytical sample was obtained as yellow crystals, mp 84–86 °C, after recrystallization from Et_2O. Anal. Calcd for $C_{13}H_{15}NO_8S$: C, 45.22; H, 4.38; N, 4.06. Found: C, 45.17 H, 4.42; N, 3.99.

5,7-Dimethoxy-6-hydroxy-2*H*-1,4-benzothiazin-3(4*H*)-one (1). A stirred mixture of methyl [(4-acetoxy-3,5-dimethoxy-2-nitrophenyl)thio]acetate (7, 392 mg, 1.13 mmol), tin(II) chloride dihydrate (1.5 g, 6.6 mmol), ethanol (10 mL), and 1 M HCl (20 mL) was heated at reflux for 21 h. After being cooled to room temperature, the reaction mixture was neutralized with saturated $NaHCO_3$ and the ethanol was evaporated. The aqueous mixture was extracted with ethyl acetate (3 × 100 mL), and the combined organic layers were dried ($MgSO_4$) and evaporated to give 1 (229 mg, 0.95 mmol, 84%) as a white solid; ^1H NMR ($CDCl_3$) δ 3.41 (2 H, s), 3.87 (3 H, s), 3.95 (3 H, s), 5.59 (1 H, br s), 6.56 (1 H, s), 7.88 (1 H, br s); MS *m/z* (rel intensity) 243 (6), 242 (13), 241 (100, M^+), 226 (54), 198 (16), 182 (2), 156 (4), 68 (3); IR (KBr) ν 3339, 3142 (br), 2938, 1666, 1588 cm^{-1}. See Table I for additional data. An analytical sample was obtained as reddish beige crystals, mp 128–130 °C, after recrystallization from CH_2Cl_2. Anal. Calcd for $C_{10}H_{11}NO_4S$: C, 49.78; H, 4.60; N, 5.81. Found: C, 49.66 H, 4.45; N, 6.06.

4-(Acetylamino)-2,6-dimethoxyphenyl Acetate (13). To a stirred solution of 4-amino-2,6-dimethoxyphenol[9] (12, 8.20 g, 48.5 mmol) in acetic anhydride (125 mL) was added anhydrous NaOAc (8.0 g, 98 mmol) in one portion at room temperature and the resulting mixture was stirred at room temperature, for 16 h under nitrogen. Water (125 mL) was added to the reaction mixture, and stirring was continued for a further 1 h to destroy excess acetic anhydride. The reaction mixture was then extracted with CH_2Cl_2 (3 × 100 mL), and the combined extracts were washed with water (150 mL) and brine (150 mL). After drying ($MgSO_4$), evaporation *in vacuo* afforded 13 (8.86 g, 35.0 mmol, 72%) as a white solid: ^1H NMR ($CDCl_3$) δ 2.14 (3 H, s), 2.34 (3 H, s), 3.78 (6 H, s) 6.79 (2 H, s), 7.34 (1 H, br s); MS *m/z* (rel intensity) 253 (12, M^+), 211 (100), 169 (82), 154 (27); IR (KBr) ν 3374, 2945, 1729, 1694, 1616 cm^{-1}. An analytical sample was obtained as white crystals, mp 158–159 °C, after recrystallization from 5:1 ethyl acetate/petroleum ether. Anal. Calcd for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 57.02; H, 6.02; N, 5.49.

4-(Acetylamino)-2,6-dimethoxy-3-thiocyanophenyl Acetate (14). 4-(Acetylamino)-2,6-dimethoxyphenyl acetate (13, 7.78 g, 30.7 mmol) and sodium thiocyanate (17.4 g, 215 mmol) were dissolved in methanol (200 mL). To this solution was added dropwise over 15 min through a dropping funnel at 0 °C a solution made by mixing 5.7 mL of bromine with 50 mL of a saturated solution of NaBr in methanol. The resulting mixture was stirred at 0–5 °C for a further 10 min. The ice bath was removed, and the reaction mixture was stirred for a further 45 min. Water (100 mL) was added, and the methanol was evaporated. The aqueous phase was extracted with CH_2Cl_2 (3 × 300 mL), and the combined organic extracts were dried ($MgSO_4$) and then reduced *in vacuo* to give a yellow solid. The solid was separated by flash column chromatography on a 3.75- × 6-in. column, eluting with 1:1 ethyl acetate/petroleum ether to give 14 (6.13 g, 19.8 mmol, 64%) as a pale yellow solid: ^1H NMR ($CDCl_3$) δ 2.29 (3 H, s), 2.36 (3 H, s), 3.87 (3 H, s), 3.99 (3 H, s), 7.89 (1 H, br s), 7.95 (1 H, s); MS *m/z* (rel intensity) 310 (13, M^+), 268 (69), 226 (100), 211 (31); IR (KBr) ν 3220, 2952, 2158, 1764, 1645, 1595 cm^{-1}. An analytical sample was obtained as pale yellow crystals, mp 223–224 °C, after recrystallization from 1:1 ethyl acetate/Et_2O. Anal. Calcd for $C_{13}H_{14}N_2O_5S$: C, 50.32; H, 4.55; N, 9.03. Found: C, 50.24; H, 4.50; N, 8.91.

Methyl [[5-Acetoxy-2-(acetylamino)-4,6-dimethoxyphenyl]thio]acetate (15). To a stirred solution of dithiothreitol (115 mg, 0.745 mmol) in methanol (10 mL) was added (4-acetylamino)-2,6-dimethoxy-3-thiocyanophenyl acetate (14, 153 mg, 0.493 mmol) in one portion at room temperature. The reaction mixture was stirred for 30 min under nitrogen.[10] The methanol was evaporated, the residue was dissolved in anhydrous DMF (10 mL), and then methyl bromoacetate (0.50 mL, 6.0 mmol) was added in one portion. The reaction mixture was heated at 90–95 °C for 1 h. After being cooled to room temperature, the reaction mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (2 × 30 mL). The combined organic extracts were washed with water (30 mL) and dried ($MgSO_4$). The solvent was evaporated, and the residue was purified by preparative TLC (1000-μm plate, 98:2 CH_2Cl_2/methanol) to give 15 (76 mg, 0.21 mmol, 43%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 2.28 (3 H, s), 2.34 (3 H, s), 3.51 (2 H, s), 3.66 (3 H, s), 3.85 (3 H, s), 3.87 (3 H, s), 8.02 (1 H, s), 9.30 (1 H, br s); MS m/z (rel intensity) 357 (25, M^+), 315 (100), 272 (30); IR (Nujol) ν 3346, 2945, 1764, 1736, 1694, 1595, 1518 cm^{-1}. Anal. Calcd for $C_{15}H_{19}NO_7S$: C, 50.41; H, 5.36; N, 3.92. Found: C, 50.08; H, 5.55; N, 3.79.

6,8-Dimethoxy-7-hydroxy-2*H*-1,4-benzothiazin-3(4*H*)-one (BMY 40662, 10). A stirred mixture of methyl [[5-acetoxy-2-(acetylamino)-4,6-dimethoxyphenyl]thio]acetate (15, 44 mg, 0.12 mmol), 2 M HCl (5 mL), and methanol (2 mL) was heated at reflux for 16 h under nitrogen. After being cooled to room temperature, the reaction mixture was neutralized with saturated $NaHCO_3$ and extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated to give a white solid. The solid was purified by preparative TLC (1000-μm plate, 96:4 CH_2Cl_2/methanol) to give 10 (24 mg, 0.10 mmol, 83%) as a white solid. An analytical sample was obtained as colorless needles, mp 174–175 °C, after recrystallization from 3:97 methanol/CH_2Cl_2. For NMR and UV/vis spectra see Table I: MS *m/z* (rel intensity) 243 (6), 242 (13), 241 (100, M^+), 226 (28), 198 (12), 182 (2), 156 (17), 68 (13); IR (KBr)[12] ν 3462 (br sh), 3202 (br), 3118, 3013, 1652, 1614, 1500, 1315, 1245, 1112, 1008, 900, 817, 788, 736, 496 cm^{-1}. Anal. Calcd for $C_{10}H_{11}NO_4S$: C, 49.78; H, 4.60; N, 5.81. Found: C, 49.85 H, 4.35; N, 5.75.

(12) There are some differences between this IR spectrum of 10 and that reported[1] for BMY 40662. In the absence of an authentic sample of BMY 40662 or its spectra, we cannot explain the difference in the IR spectra, but the data for 10 and BMY 40662 in Table I are in such close agreement that we are convinced of their identity.