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Received April 16, 1987

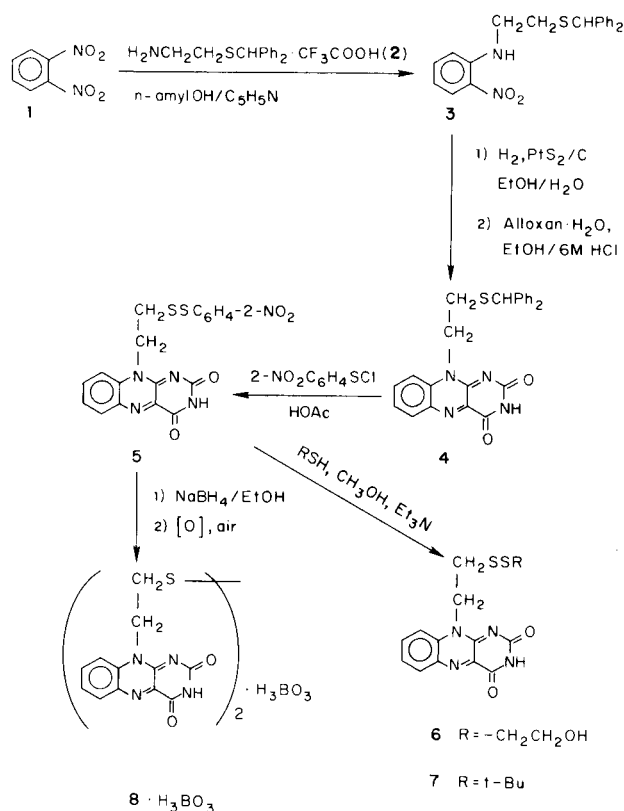
A facile, high yield route to *N*-10 flavin disulfides is described. The key feature is catalytic reduction of the nitro group to an amine function in an organic sulfide using platinum sulfide on charcoal.

J. Heterocyclic Chem., **25**, 241 (1988).

Flavins modified at the *N*-10 position are of interest because of their potential as models for the coenzymes FMN and FAD [2]. Several strategies to prepare *N*-10 alkyl-, aryl-, and hydroxyalkyl-substituted flavins have been developed, and there now exist numerous examples in the literature [3]. The corresponding sulfur-functionalized flavins, however, have received little attention; bis[2-(10-isalloxaziny)ethyl] disulfide, **8**, and its sulfonic acid derivative [4] are the only such compounds known to date. In conjunction with our research program directed towards the synthesis of model complexes for metal-flavin interactions in metalloflavoenzymes, we required a convenient supply of **8**. While preparing this compound according to the literature procedure [4], we found that the final step, a condensation reaction between alloxan monohydrate and 2-amino-*N*-2-mercaptoethylaniline (generated *in situ* from bis[2-(*N*-2-nitrophenyl)aminoethyl] disulfide using mossy zinc/acetic acid) in boric acid-acetic acid mixture, is sensitive to slight changes in reaction conditions and, as reported, requires a large excess of alloxan monohydrate (≈ 50 equivalents). Decreasing this ratio produced a mixture from which pure **8** could not be readily separated. This observation is not surprising considering that (i) the mercaptan that is being generated *in situ* upon reduction may cause competitive condensation reactions, (ii) alloxan monohydrate may condense in either of the two possible orientations recently recognized by us [5], and (iii) the resulting flavin mercaptan may undergo acid-catalyzed *N*-10 dealkylation, a phenomenon well characterized in flavin chemistry [6], or become involved in inter- or intramolecular addition reactions through nucleophilic attack of the thiol on electron-deficient carbon centers of the isalloxazine ring. In this paper we report a facile route which provides a general approach to *N*-10 flavin disulfides such as **5-8**.

The various reaction steps involved in the preparation of compounds **5-8** are shown in Scheme 1. The key to our approach is the diphenylmethyl (benzhydryl) group, which proved to be an excellent protecting agent for the thiol group of cysteamine (2-aminoethanethiol). The trifluoroacetic acid salt of 2-(diphenylmethylthio)ethylamine was prepared from 2-aminoethanethiol or the hydrochloride salt and diphenylcarbinol in trifluoroacetic acid following the method of Photaki *et al.* [7], with minor modifications.

This was allowed to react with 1,2-dinitrobenzene in *n*-amyl alcohol and pyridine to give 2-nitro-*N*-(2-diphenylmethylthio)ethylaniline (**3**) in 72-87% yield. Catalytic hydrogenation of **3** in ethanol-water at 60 psi, using platinum sulfide/charcoal catalyst, gave the diamino compound, which was condensed anaerobically with alloxan monohydrate in ethanol-hydrochloric acid (6*N*). Filtration, followed by washing of the residue with water, ethanol, and ether, gave satisfactorily pure 10-(2-diphenylmethylthio)ethylisalloxazine (**4**). An analytically pure sample of **4** was obtained by recrystallization from hot ethanol. Photaki *et al.* [7] have reported that the removal of the *S*-diphenylmethyl group from cysteine or cysteine peptides is best achieved by a mixture of trifluoroacetic acid and phenol (2.5%). Attempts to cleave the *S*-diphenylmethyl group from **4** under similar conditions or with mild heating in tri-



Scheme 1

fluoroacetic acid alone failed to produce 10-(2-mercaptoethyl)isoalloxazine or the disulfide **8**; instead, lumichrome was obtained as the major product. Degradation of isoalloxazines to alloxazines under acidic conditions has been demonstrated previously [6].

Selective displacement of the *S*-diphenylmethyl group in **4** was achieved by treatment with 2-nitrobenzenesulfonyl chloride in acetic acid [8]. The resulting solid was recrystallized from aqueous acetic acid to give pure 2-(10-isoalloxazinyl)ethyl 2-nitrophenyl disulfide (**5**). Reaction of **5** with excess mercaptan (*t*-butyl mercaptan or 2-mercaptoethanol) in methyl alcohol in the presence of triethylamine produced the mixed disulfides **6** and **7**, respectively. When **5** and 2-mercaptoethanol were reacted in a 1:1 molar ratio, a greenish-yellow solid was obtained that was sparingly soluble in trifluoroacetic acid. Although the ¹H nmr spectrum of a trifluoroacetic acid solution exhibited signals due to only **8**, all attempts to obtain analytically pure material *via* this route were unsuccessful. Instead, **8** was obtained (as the boric acid salt) from **5** by reduction with sodium borohydride in ethanol under nitrogen; formation of boric acid complexes of flavins has been noted previously by Ehrenberg [9].

The noteworthy features of the reaction route are: (i) it provides a general, high yield approach to bis(flavin) disulfide and to mixed flavin-alkyl or -aryl disulfides, and (ii) it utilizes reduction of the nitro group to an amine function in an organic sulfide, using platinum sulfide/charcoal catalyst. Variations of this route have been known for 30 years [10], but it appears to be relatively little utilized. (Another method available is transfer-hydrogenation using cyclohexane and palladium-charcoal catalyst [11], which could not be applied successfully to the flavin systems.)

EXPERIMENTAL

2-Aminoethanethiol and its hydrochloride salt, 2-nitrobenzenesulfonyl chloride, *t*-butyl mercaptan, and platinum sulfide/charcoal catalyst were purchased from Aldrich Chemical Co. Alloxan monohydrate, diphenylcarbinol, and 2-mercaptoethanol were obtained from Sigma, Matheson Coleman and Bell, and J. T. Baker Chemical Company, respectively. All solvents used were reagent grade. *n*-Amyl alcohol was dried over calcium sulfate and distilled before use. Pyridine was distilled from barium oxide. All operations during flavin syntheses were performed under low-intensity red light. Dinitrogen gas was purified and dried by passage over hot BASF catalyst R-3-11 and supported phosphorus pentoxide (Aquasorb), respectively. Flavin derivatives were dried *in vacuo* ($\approx 10^{-1}$ torr) at 70° for three days before elemental analysis.

Proton nmr spectra were recorded on a Varian EM-390 or Nicolet NTC-360 spectrometer using tetramethylsilane (TMS) as internal standard. Optical and mass spectra were obtained on a Cary 219 spectrophotometer and Finnigan-MAT 4600 spectrometer, respectively. Melting points reported were measured either on a Thomas Hoover or a Mel-Temp apparatus and are uncorrected.

Preparation of 2-(Diphenylmethylthio)ethylamine Trifluoroacetic Acid Salt (**2**).

(A) 2-Aminoethanethiol (21.73 g, 281.7 mmoles) and diphenylcarbinol

(52.0 g, 282 mmoles) were placed in a round-bottom flask, which had been degassed and flushed with nitrogen. To this trifluoroacetic acid (80 ml) was added gradually with constant stirring. An immediate exothermic reaction took place. The reaction solution was stirred for 3-4 hours and then concentrated to a viscous oil under reduced pressure. Addition of diethyl ether produced **2** as a white solid. This was filtered and recrystallized from hot chloroform/diethyl ether or from water, mp 124-126°, yield 92.6 g (92%); ¹H nmr (deuteriochloroform): δ 2.51 (2H, t, SCH₂), 2.78 (2H, t, NCH₂), 5.06 (1H, s, SCH), 6.97-7.43 (10H, m, aryl protons), 7.58 (3H, br, NH₃); ir (potassium bromide): ν (C=O) 1695 cm⁻¹; eims: m/e 243 (M⁺.CF₃COOH).

Anal. Calcd. for C₁₇H₁₈F₃N₂O₂S: C, 57.13; H, 5.08. Found: C, 57.28; H, 5.07.

(B) To a mixture of 2-aminoethanethiol hydrochloride (30.0 g, 264 mmoles) and diphenylcarbinol (48.7 g, 264 mmoles), trifluoroacetic acid (80 ml) was added with constant stirring. After work-up of the reaction mixture as described above, the solid obtained was fractionally crystallized from hot water to give **2** (less soluble) in 83% yield. The more soluble product was characterized as the hydrochloride salt of 2-(diphenylmethylthio)ethylamine, mp 164-166°, yield 4.3 g (5.8%); ¹H nmr (deuteriochloroform): δ 2.56 (2H, t, SCH₂), 2.88 (2H, t, NCH₂), 5.23 (1H, s, SCH), 6.93-7.53 (13H, m, aryl protons and NH₃); ¹H nmr (dimethylsulfoxide-d₆): δ 2.57 (t, SCH₂ and residual dimethylsulfoxide-d₆), 2.92 (2H, t, NCH₂), 5.40 (1H, s, SCH), 7.03-7.57 (10H, m, aryl protons), 8.12 (3H, br, NH₃); eims: m/e 243 (M⁺.HCl).

Preparation of 2-Nitro-*N*-(2-diphenylmethylthio)ethylaniline (**3**).

A solution of 1,2-dinitrobenzene (13.6 g, 80.9 mmoles) and **2** (52.7 g, 162 mmoles) in *n*-amyl alcohol (250 ml) and pyridine (70 ml) was refluxed for \approx 24 hours with constant stirring. The brown solution was concentrated under reduced pressure to a viscous oil. Isopropyl alcohol (= 250 ml) was added, and the mixture was stirred for 1 hour. The yellow-orange solid thus precipitated was filtered and recrystallized from hot isopropyl alcohol, mp 75-76°, yield 18.3 g (62%); ¹H nmr (deuteriochloroform): δ 2.68 (2H, t, SCH₂), 3.38 (2H, t, NCH₂), 5.21 (1H, s, SCH), 6.5-8.2 (15H, m, aryl protons and NH); eims: m/e 364 (M⁺).

An additional 10-15% of **3** was obtained from the mother liquor by column chromatography (silica gel; solvent system: ethyl acetate and hexane (1:1)); R_f (0.80), followed by recrystallization from isopropyl alcohol.

Anal. Calcd. for C₂₁H₂₀N₂O₂S: C, 69.21; H, 5.53; N, 7.68; S, 8.80. Found: C, 69.10; H, 5.54; N, 7.64; S, 8.72.

Preparation of 10-(2-Diphenylmethylthio)ethylisoalloxazine (**4**).

A suspension of compound **3** (10.0 g, 27.4 mmoles) and platinum sulfide/charcoal (1.5 g) in an ethanol-water mixture (175 ml/10 ml) was shaken with hydrogen at room temperature and 60 psi pressure until the supernatant appeared colorless. The hydrogenate was filtered under nitrogen directly into a suspension of alloxan monohydrate (30.7 g, 192 mmoles) in ethanol-6*N* hydrochloric acid (100 ml/10 ml), which had been thoroughly degassed and flushed with nitrogen. The mixture was immediately heated at 110-115° for 30 minutes and then allowed to stir at room temperature for 24 hours. The yellow-orange solid formed was filtered in air, washed with water (\approx 800 ml), cold ethanol (\approx 75 ml), and ether (\approx 25 ml), and recrystallized from hot ethanol, mp 261-263°, yield 6.2 g (51%); ¹H nmr (dimethylsulfoxide-d₆): δ 2.72 (2H, t, SCH₂), 4.68 (2H, t, NCH₂), 5.63 (1H, s, SCH), 7.03-8.13 (14H, m, aryl protons); ¹H nmr (trifluoroacetic acid): 3.33 (2H, t, SCH₂), 5.02 (2H, t, NCH₂), 5.25 (1H, s, SCH), 7.17-7.62, 8.05-8.60 (14H, m, aryl protons); eims: negative ion, m/e 440 (M⁻1); optical spectrum (dimethylformamide): λ , nm (ϵ M⁻¹cm⁻¹), 268 (32,400), 329 (7,070), 412 (sh), 438 (9,910), 465 (sh).

Anal. Calcd. for C₂₅H₂₀N₄O₂S: C, 68.16; H, 4.58; N, 12.72; S, 7.28. Found: C, 68.03; H, 4.61; N, 12.65; S, 7.24.

Preparation of 2-(10-Isoalloxazinyl)ethyl 2-Nitrophenyl Disulfide (**5**).

A suspension of compound **4** (1.0 g, 2.3 mmoles) and 2-nitrobenzenesulfonyl chloride (2.12 g, 11.4 mmoles) in glacial acetic acid (50 ml) was

stirred at 95° for 1 hour and then at room temperature for 4-5 hours. The reaction mixture was filtered. The residue collected was washed with ethanol (≈ 20 ml) and ether (≈ 15 ml), and recrystallized from aqueous acetic acid (80%) to give pure **5**, mp 246-248° dec, yield 0.78 g (80%); ¹H nmr (trifluoroacetic acid): δ 3.43 (2H, t, SCH₂), 5.40 (2H, t, NCH₂), 7.42-8.68 (8H, m, aryl protons); cims: positive ion, m/e 428 (M⁺ + 1); optical spectrum (dimethylformamide): λ , nm (ϵ , M⁻¹cm⁻¹), 268 (39,600), 334 (9,920), 412 (sh), 434 (10,600), 464 (sh).

Anal. Calcd. for C₁₈H₁₃N₅O₂S₂: C, 50.58; H, 3.07; N, 16.39; S, 15.00. Found: C, 50.94; H, 3.11; N, 16.20; S, 15.24.

Preparation of 2-(10-Isoalloxaziny)ethyl 2-Hydroxyethyl Disulfide (**6**).

Compound **5** (0.10 g, 0.23 mmole) was mixed with methanol (50 ml), and the resulting suspension was degassed and flushed with nitrogen. To this, triethylamine (0.5 ml) and 2-mercaptoethanol (0.41 g, 5.8 mmoles) were added. The reaction mixture was stirred at 55-60° for 1 hour and then at room temperature for 4 hours. After filtration, the solution was concentrated and ether was added. The yellow solid thus precipitated was filtered, washed with ether, and recrystallized from methanol/ether to give analytically pure **6**, mp 258-263°; yield 70 mg (73%); ¹H nmr (trifluoroacetic acid): δ 3.07 (2H, s, SCH₂CH₂OH), 3.43 (2H, t, SCH₂CH₂N), 4.17 (2H, t, OCH₂), 5.45 (2H, t, NCH₂), 8.06-8.73 (4H, m, aryl protons); cims: positive ion, m/e 351 (M⁺ + 1); optical spectrum (dimethylformamide): λ , nm (ϵ , M⁻¹cm⁻¹), 270 (31,400), 334 (6,820), 414 (sh), 438 (9,740), 465 (sh).

Anal. Calcd. for C₁₄H₁₄N₄O₃S₂: C, 47.99; H, 4.03; N, 15.99; S, 18.30. Found: C, 48.01; H, 4.05; N, 15.96; S, 18.20.

Derivative trifluoroacetate ester (prepared by heating **6** in trifluoroacetic acid), mp 268-273°; ¹H nmr (trifluoroacetic acid): δ 3.13 (2H, t, SCH₂CH₂O), 3.45 (2H, t, SCH₂CH₂N), 4.70 (2H, t, OCH₂), 5.40 (2H, t, NCH₂), 8.00-8.67 (4H, m, aryl protons); cims: positive ion, m/e 447 (M⁺ + 1); optical spectrum (dimethylformamide): λ , nm (ϵ , M⁻¹cm⁻¹), 270 (39,800), 330 (8,560), 416 (sh), 439 (12,130), 464 (sh).

Preparation of 2-(10-Isoalloxaziny)ethyl *t*-Butyl Disulfide (**7**).

Compound **5** (1.0 g, 2.3 mmoles) and *t*-butylthiol (1.3 ml, 11.5 mmoles) in methanol (50 ml) and triethylamine (1.0 ml) were allowed to react in the same manner as described above for **6**. The yellow suspension was filtered, and the residue was washed with methanol (≈ 30 ml), ethanol (≈ 15 ml), and ether (≈ 10 ml). The solid was recrystallized from ethanol to give orange microcrystals of ethanol-solvated **7**; the presence of ethanol in the stoichiometry indicated was confirmed by proton nmr, mp 265-270°, yield 0.72 g (80%); ¹H nmr (trifluoroacetic acid): δ 1.33 (9H, s, S-*t*-Bu), 3.43 (2H, t, SCH₂), 5.43 (2H, t, NCH₂), 8.13-8.67 (4H, m, aryl protons); cims: positive ion, m/e 363 (M⁺ + 1); optical spectrum (dimethylformamide): λ , nm (ϵ , M⁻¹cm⁻¹), 268 (30,700), 328 (6,920), 410 (sh), 437 (9,710), 464 (sh).

Anal. Calcd. for C₁₁H₁₆N₄O₂S₂·0.5 C₂H₆OH: C, 52.96; H, 5.49; N, 14.53; S, 16.63. Found: C, 52.98; H, 5.48; N, 14.50; S, 16.57.

Preparation of Bis[2-(10-Isoalloxaziny)ethyl] Disulfide (**8**) Boric Acid Salt.

To a thoroughly degassed and flushed suspension of compound **5** (1.0 g, 2.3 mmoles) in ethanol (50 ml), sodium borohydride (1.8 g) was added. The reaction mixture was stirred at room temperature for 8-9 hours and then was diluted with degassed distilled water (≈ 20 ml). The suspension was acidified anaerobically with degassed 6*N* hydrochloric acid to pH ≈ 4 . After standing at room temperature for 2 hours, the solid was filtered anaerobically, washed with water (≈ 100 ml), ethanol (≈ 25 ml), ether (≈ 25 ml), and then vacuum dried. Upon air oxidation and subsequent reprecipitation from trifluoroacetic acid/ether, the boric acid salt of **8** was obtained, mp 280-282°, yield 0.58 g (73%); ¹H nmr (trifluoroacetic acid): δ 3.53 (2H, t, SCH₂), 5.43 (2H, t, NCH₂), 8.03-8.73 (4H, m, aryl protons); cims: negative ion, m/e 546 ((M-H₃BO₃)⁻); optical spectrum (saturated solution in dimethylformamide): λ nm 270, 330, 412 (sh), 438, 464 (sh).

Anal. Calcd. for C₂₄H₁₈N₈O₄S₂·H₃BO₃: C, 47.38; H, 3.48; N, 18.42; S, 10.54; B, 1.78. Found: C, 47.58; H, 3.85; N, 18.30; S, 10.49; B, 1.37.

Acknowledgement.

This research was supported by grants to B.A.A. from the National Institute of Health (GM 32117 and GM 28636).

REFERENCES AND NOTES

- [1] Alfred P. Sloan Foundation Fellow, 1981-85.
- [2] Abbreviations: FMN, flavin mononucleotide or riboflavin-5-monophosphate; FAD, flavin adenine dinucleotide or riboflavin-adenosine-diphosphate.
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