Preparation of Nonsymmetrical *p*-Benzoquinone Diimines for Evaluation as Protein Cleavage Reagents

Thomas J. Holmes, Jr.,* and Richard G. Lawton

Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota 55455, and Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109

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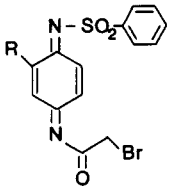
In an effort to improve the yield, selectivity, and mildness of reaction conditions employed in protein cleavage with bisalkylating quinone diimines, a series of analogous reagents (**1a–h**) were prepared. These novel N^1,N^4 nonsymmetrically substituted benzoquinone diimines were designed to maintain steric parameters, which had been recognized as necessary in the cleavage reaction, while varying the electronic nature of the 2-substituent in order to observe effects upon the cleavage process. A number of synthetic strategies were necessary to arrive at this desired series of compounds. A comparison of relative efficiencies of protein cleavage for certain members of this series is presented elsewhere.

As we have previously reported, 2-methyl-N^1-(phenylsulfonyl)-N^4-(bromoacetyl)benzoquinone diimine (**1a**) is



1 (a–h)

a, R = CH_3
b, R = CH_3 ; Br = H
c, R = H
d, R = H ; Br = H
e, R = CN
f, R = OCH_3
g, R = CF_3
h, R = Cl

a useful reagent for selective chemical cleavage of proteins at cysteine residues under relatively mild conditions.[1] The detailed preparation of this reagent (**1a**), as well as the preparation of alternative reagents of this type (**1b–h**), is described herein. The relative merits of these reagents with respect to sulfhydryl reactivity and protein cleavage are discussed elsewhere.[2] The development of **1a** as a useful reagent for selective protein cleavage is based not only upon observed reactivity but also upon ease of synthesis and handling and reasonable shelf life. A discussion of the 2-cyanobenzoquinone diimine (**1e**), which could not be prepared by these methods, is included to illustrate the limitations of these methods.

Investigation of these nonsymmetrical N^1-sulfonyl-N^4-acyl ring-substituted benzoquinone diimines confirms and extends early observations of Adams and co-workers regarding the chemistry of this class of compounds.[3] Syntheses of these nonsymmetrical benzoquinone diimines required differentiation of the amino groups in the *p*-phenylenediamine system, while allowing variation of the ring substituent (R) to investigate steric and electronic effects on sulfhydryl reactivity. These constraints limited to some extent the evaluation of variously substituted benzoquinone diimines and necessitated the development of several strategies for incorporation of a variety of C-2 substituents.

In the simplest case, where the 2-substituted-4-nitroaniline starting materials were readily available (R = H, CH_3, CN), syntheses of the desired benzoquinone diimines (**1a–e**) could be approached as shown in Scheme I. Formation of the benzenesulfonanilides **2a–c** occurred readily upon treatment of the nitroanilines with benzenesulfonyl chloride in pyridine. Reduction of the benzenesulfonanilides **2a–c** with aqueous sodium dithionite according to Fieser's procedure[4] was improved by the addition of methanol as a cosolvent. Only 2′-cyano-4′-aminobenzenesulfonanilide (**3c**), which may form in an internal salt, required concentration of the largely aqueous reaction mixture and continuous extraction (EtOAc) to recover the product in a reasonable yield (80%). Solvents for acylation of the N^4-amino function were varied; however, in all cases only weak bases (sodium acetate, sodium bromoacetate, solid sodium bicarbonate) and room temperature were employed in these reactions. The use of sodium bromoacetate in acetonitrile provided particularly clean and reproducible conditions for acylation with bromoacetyl bromide, compared to the chloroform/sodium bicarbonate mixture that had been employed initially. The resulting nonsymmetrical derivatives (**4a–h**) were only sparingly soluble in chloroform, unlike the benzoquinone diimines. In spite of this limited solubility, chloroform (dry; EtOH removed) proved to be a suitable solvent for oxidation to the desired benzoquinone diimines (**1a–h**) as discussed further below.

An alternative route to the nonsymmetrically substituted *p*-phenylenediamine derivatives is also shown in Scheme I. Where the 2-substituted-4-nitroanilines were not available commercially (R = OCH_3 and CF_3), access to the nonsymmetrical *p*-phenylenediamine system could be gained via regioselective nitration of the 2′-substituted benzenesulfonanilides, which were prepared from the aniline derivatives (*o*-anisidine and 2-aminobenzotrifluoride). Reduction of the *p*-nitro function proceeded smoothly with sodium dithionite; and the resulting amines could be acylated under mild conditions. Oxidation of these nonsymmetrical derivatives (**4f,g**) to the desired benzoquinone diimines (**1f,g**) with lead tetraacetate in chloroform proceeded in very good yields (84–90%).

Yet another approach to the introduction of a 2-substituent in this series of compounds involved regioselective addition to an unsubstituted benzoquinone diimine (**1c**). This regioselective addition of HCl was first reported by Adams, who observed in similar systems that 1,4-addition of chloride ion occurs at the ring position meta to the most basic nitrogen since this position is presumably "activated" by protonation of the carboxamide nitrogen.[5] We subsequently observed that Lewis acid catalyzed addition of methanol (BF_3·Et_2O) to this same benzoquinone diimine did *not* show this regioselectivity (an equal mixture of the

(1) Holmes, T. J., Jr.; Lawton, R. G. *J. Am. Chem. Soc.* **1977**, *99*, 1984–1986.
(2) Holmes, T. J., Jr.; Lawton, R. G. *Intl. J. Pept. Prot. Res.*, in press.
(3) Adams, R.; Reifschneider, W. *Bull. Soc. Chim. Fr.* **1958**, 23–65.
(4) L. F. Fieser, K. L. Williamson, Eds. "Organic Experiments", 3rd ed.; H. C. Heath and Co.: Lexington, MA, 1975; pp 285–290.
(5) Adams, R.; Colgrove, R. S. *J. Am. Chem. Soc.* **1954**, *76*, 3584–3587.

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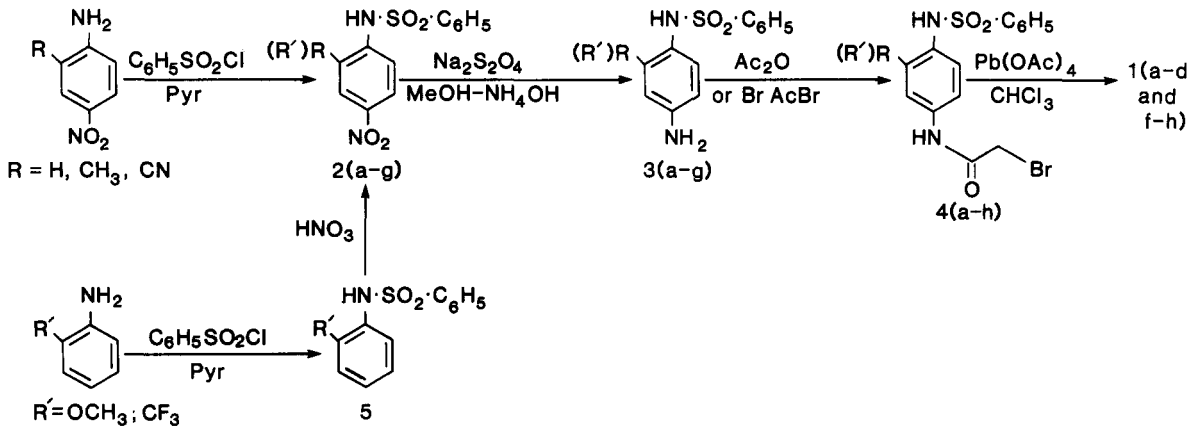
Scheme I

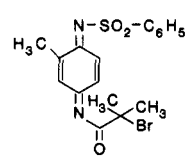


2- and 3-methanol adducts resulted). Likewise, sulfhydryl addition (*n*-BuSH/$CHCl_3$/TEA) to the non-brominated, nonsubstituted benzoquinone diimine **1d** was not regioselective.

Although the unsubstituted benzoquinone diimine (**1c**) could not be isolated in crystalline form, conversion of the crude oil to the stable HCl adduct (**4h**) proceeded in satisfactory yield (75%). Rapid chromatography, which enabled purification of the sensitive 2-chlorobenzoquinone diimine **1h**, was not attempted with the brominated unsubstituted benzoquinone diimine **1c**. In contrast, the non-brominated, unsubstituted benzoquinone diimine **1d** was successfully crystallized, although it was unstable to prolonged storage (darkening within days to intractable materials). Once crystallized, the yellow benzoquinone diimines were generally stable to room-temperature storage over several months. Partially decomposed samples could be repurified by recrystallization (ether/chloroform).

The utility of the various 2-substituted benzoquinone diimines as practical reagents for protein cleavage was limited somewhat by their difficulty of synthesis and purification and stability under normal storage conditions. Thus, of the benzoquinone diimines that have been synthesized for this purpose so far, 2-methyl-N^1-(phenylsulfonyl)-N^4-(bromoacetyl)benzoquinone diimine (**1a**) appears most satisfactory with regard to these criteria and is available as Cyssor I through Sigma Chemical Co., although both the 2-methoxy (**1f**) and 2-(trifluoromethyl) (**1g**) benzoquinone diimines are only slightly less attractive (based on lower synthetic yield). As discussed elsewhere, these derivatives (**1a,f,g**) appear to be equivalent with respect to sulfhydryl addition.[2] However, the 2-chlorobenzoquinone **1h**, which appears to show some advantage in the protein cleavage reaction (heating unnecessary),[2] is isolated in significantly lower yield and with some difficulty (requires chromatographic purification prior to crystallization) and is observed to decompose upon standing within weeks at 5 °C. As described previously,[1] the nonsubstituted benzoquinone diimine **1c,d** and the non-brominated benzoquinone diimines **1b,d** were not useful in the cleavage reaction, and furthermore, were prepared in considerably lower yield. The 2-cyanobenzoquinone diimine **1e** could not be prepared via the usual oxidation method employed ($Pb(OAc)_4$/chloroform). Under these conditions, acetate substitution occurred in the ring position adjacent to the cyano function. Whether this substitution occurred by direct transfer from $Pb(OAc)_4$ or by nucleophilic attack of released acetic acid was not determined. Alternate conditions for oxidation to the 2-cyanobenzoquinone diimine **1e** were not investigated.

To complete this description of novel benzoquinone diimines that were synthesized for their possible application to the chemical cleavage of proteins, we should mention the following derivatives (**6** and **7**). Compound **6** was



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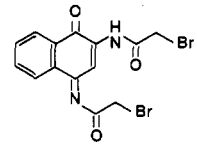

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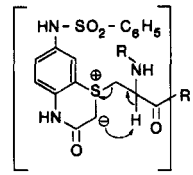

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designed to test the hypothesis that β-elimination in the cleavage reaction might be assisted by ylide formation as illustrated by the hypothetical intermediate **8**. Unfortunately, although sulfhydryl addition (*N*-acetylcysteine) did occur to **6** as expected, no bromide displacement was observed upon heating (NMR/MS spectra correspond to adduct) and no amide bond cleavage was observed by thin-layer chromatography. These results parallel those reported for the non-brominated, 2-methylbenzoquinone diimine **1b**.[1]

Similarly, no nucleophilic reaction of 1-butanethiol was observed with the bis(bromoacetyl)naphthoquinone imine **7**, which was prepared by a modification of Fieser's procedure for the bisacetyl derivative.[6] This particular compound (**7**) of markedly lower oxidation potential and increased steric constraint (as compared to the previously discussed benzoquinone diimines) showed only reduction upon prolonged heating with 1-butanethiol. Therefore, this type of compound (**7**) was pursued no further for application to protein cleavage.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton magnetic resonance spectra were obtained on a Varian Associates T-60 instrument. Mass spectra were obtained on an Associated Electrical Industries MS-902. Infrared spectra were obtained on either a Perkin-Elmer 237 or Perkin-Elmer 437 spectrophotometer. Ultraviolet spectra were obtained on a Cary-14 spectrophotometer. Satisfactory elemental analyses were obtained for all new compounds (except where noted) and performed by Spang Microanalytical Laboratory, Ann Arbor, MI. All reactions were routinely monitored by thin-layer chromatography utilizing chloroform, or 5% methanol in chloroform, on silica gel adsorbent.

Preparation of 2′-Methyl-4′-nitrobenzenesulfonanilide (2a) and Similar Compounds (2b,c; 5a,b). To 30.4 g (0.2 mol) of 2-methyl-4-nitroaniline (Aldrich) dissolved in 100 mL of pyridine was added dropwise with ice-bath cooling 30 mL (42 g, 0.24 mol) of benzenesulfonyl chloride. After the reaction mixture was stirred at room temperature for 13 h, 40 mL of water was added dropwise, followed by 100 mL of 2 N hydrochloric acid.

(6) Fieser, L. F.; Fieser, M. *J. Am. Chem. Soc.* **1934**, *56*, 1565–1578.

The large amount of yellow solid produced was partitioned into 2 L of chloroform, and this extract was then washed successively with 2 N hydrochloric acid (3 × 500 mL), saturated potassium bicarbonate solution (1 × 400 mL), and saturated sodium chloride solution (1 × 400 mL). The chloroform solution was dried over anhydrous sodium sulfate prior to removal of the solvent in vacuo. recrystallization of this yellowish solid from acetone provided 41 g (70% yield) of slightly off-white crystals; mp 160–162 °C (lit.[7] mp 157–159 °C); ^{1}H NMR (acetone-d_6) δ 2.3 (s, 3), 7.4–8.2 (m, 8), 9.0 (br s, 1); IR (KBr) 1590, 1515, 1340, 1165, 1090 cm^{-1}.

4′-Nitrobenzenesulfonanilide (2b): 83% yield; mp 136–138 °C (lit.[8] mp 139 °C; ^{1}H NMR ($CDCl_3$) δ 7.27 (d, J = 8 Hz, 2), 8.13 (d, J = 8 Hz, 2), 7.30–8.10 (m, 5); IR ($CHCl_3$) 1595, 1525, 1350, 1165, 1100 cm^{-1}.

2′-Cyano-4′-nitrobenzenesulfonanilide (2c) (reaction mixture heated at 85 °C for 36 h; acidified mixture extracted with EtOAc): 95% yield; mp 325–327 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.26–8.16 (m, 7), 8.27 (d, J = 3 Hz, 1); IR (KBr) 1300, 1130, 1080 cm^{-1}; MS, m/e 303 (M^+·), 141, 77 (base).

2′-Methoxybenzenesulfonanilide (5a) (addition of petroleum ether (40–60 °C) to the concentrated chloroform extract induced crystallization of the product): 100% yield; mp 88–89 °C (lit.[9] mp 89 °C); ^{1}H NMR ($CDCl_3$) δ 3.53 (s, 3), 6.6–7.9 (m, 10); IR ($CHCl_3$) 1500, 1450, 1400, 1350, 1250, 1170 cm^{-1}.

2′-(Trifluoromethyl)benzenesulfonanilide (5b) (reaction mixture heated to 85 °C for 8 h; addition of petroleum ether (30–60 °C) to the concentrated chloroform extract induced crystallization of the product): 88% yield; mp 76–78 °C; ^{1}H NMR ($CDCl_3$) δ 6.8–8.0 (m, 10); IR ($CHCl_3$) 1320, 1170, 1120 cm^{-1}.

Preparation of 2′-(Trifluoromethyl)-4′-nitrobenzenesulfonanilide (2e) and Similarly 2d. A slight modification of established procedures was used in this nitration.[10] To 30 g (0.1 mol) of 2′-(trifluoromethyl)benzenesulfonanilide suspended in 100 mL of 20% aqueous acetic acid at room temperature with rapid stirring was added 10 mL (0.2 mol) of fuming nitric acid (sp gr = 1.6) in small portions. As there was no evidence of reaction, this mixture was heated on a steam bath. Practically all of the solid dissolved upon heating, and gradually a yellow color developed in the solution. After the reaction mixture was heated for 24 h, the cooled reaction solution was added to 200 mL of water, and small volume of saturated sodium chloride solution was added. This mixture was extracted with ethyl acetate (2 × 150 mL), which was then washed with saturated sodium chloride solution and dried over anhydrous sodium sulfate. Removal of the solvent in vacuo provided 30.6 g (90% yield) of the crude desired product. Recrystallization of this material from a mixture of ethyl acetate, benzene, and petroleum ether (30–60 °C) provided the pure 2′-(trifluoromethyl)-4′-nitrobenzenesulfonanilide (**2e**); mp 124.5–125.5 °C; NMR (acetone-d_6) δ 7.5–8.0 (m, 7), 8.33 (br s, 1); IR ($CHCl_3$) 1600, 1350, 1285, 1170, 1120 cm^{-1}.

2′-Methoxy-4′-nitrobenzenesulfonanilide (2d) (nitration was only 80% complete (NMR) after 20 h; the reaction mixture was worked up, and the crude product was reduced and purified as the amine): ^{1}H NMR (Me_2SO-d_6) δ 3.84 (s, 3), 7.4–8.0 (m, 8), 10.3 (br s, 1); IR (KBr) 1580, 1510, 1335, 1170 cm^{-1}.

Preparation of 2′-Methyl-4′-aminobenzenesulfonanilide (3a) and Similarly 3b–e. The reduction procedure of Fieser[4] was modified by the addition of methanol as a cosolvent. To 20 g (0.07 mol) of 2′-methyl-4′-nitrobenzenesulfonanilide suspended in 215 mL of methanol was added in portions with ice-bath cooling 40 g (0.21 mol) of sodium dithionite (technical grade) dissolved in 200 mL of 7% ammonium hydroxide solution. A residual light yellow coloration was discharged by adding 0.5 g of solid sodium dithionite. After being stirred for 30 min, the reaction mixture was refrigerated for 1 h; then the white solid precipitate was filtered with suction and washed with water. This solid was reprecipitated from dilute hydrochloric acid solution by neutralization with saturated potassium bicarbonate solution to provide 14.2 g (77% yield) of pinkish white crystals: mp 148–149 °C (lit.[11] mp 147 °C); ^{1}H NMR ($CDCl_3$) δ 1.87 (s, 3), 3.6 (br s, 2), 6.16–7.0 (m, 3), 7.33–7.83 (m, 5); IR (KBr) 3375, 1500, 1320 cm^{-1}.

4′-Aminobenzenesulfonanilide (3b): 77% yield; mp 171–174 °C (lit.[12] mp 171–172 °C); ^{1}H NMR ($CDCl_3$) δ 6.40 (d, J = 8 Hz, 2), 6.74 (d, J = 8 Hz, 2), 7.60 (br s, 5); IR (KBr) 3280–3480, 1630, 1500, 1325, 1160, 1100 cm^{-1}.

2′-Cyano-4′-aminobenzenesulfonanilide (3c): 80% yield (required continuous extraction of neutralized, concentrated aqueous filtrate for recovery; only 30% isolated directly by filtration); mp 197–199 °C; ^{1}H NMR (Me_2SO-d_6) δ 5.6 (br s, 2), 6.5–6.8 (m, 3), 7.5 (br s, 5); IR (KBr) 3300, 3395, 2215, 1160 cm^{-1}; MS, m/e 273 (M^+), 132 (base; $M^+ - C_6H_5SO_2$).

2′-Methoxy-4′-aminobenzenesulfonanilide (3d): 73% yield; mp 174–176 °C; ^{1}H NMR ($CDCl_3$) δ 3.37 (s, 3), 3.16–3.85 (vbr s, 2), 6.0 (d, J_X = 2 Hz, 1), 6.2 (dd, J_{AB} = 10 Hz, J_X = 2 Hz, 1), 6.46 (br s, 1), 7.16–7.75 (m, 6); IR ($CHCl_3$) 3340, 1620, 1510, 1340, 1165 cm^{-1}.

2′-(Trifluoromethyl)-4′-aminobenzenesulfonanilide (3e): 60% yield by filtration (continuous extraction could have been employed); mp 138–140 °C; ^{1}H NMR (acetone-d_6) δ 5.16 (br s, 2), 6.67–7.83 (m, 8); IR ($CHCl_3$) 3400, 1630, 1340, 1165, 900 cm^{-1}.

Preparation of 2′-Methyl-4′-(bromoacetamido)benzenesulfonanilide (4a) and Similarly 4b–g. To 5.24 g (20 mmol) of 2′-methyl-4′-aminobenzenesulfonanilide (**3a**) and 2.2 g (22 mmol) of finely divided potassium bicarbonate suspended in 80 mL of chloroform (dry, ethanol removed) at 0 °C was added dropwise over 50 min 2.1 mL (24 mmol, 4.8 g) of bromoacetyl bromide dissolved in 20 mL of chloroform. After stirring for 24 h at room temperature, starting material was observed to be present by thin-layer chromatography; therefore, another 1.0 mL (12 mmol) of bromoacetyl bromide was added dropwise to the reaction mixture. Within 30 min no amine could be detected. After 50 mL of 2 N hydrochloric acid was added dropwise to the reaction mixture, the pinkish solid (6.7 g) was removed by filtration. The chloroform filtrate was washed successively with 2 N hydrochloric acid (1 × 300 mL), saturated potassium bicarbonate solution (1 × 300 mL), and saturated sodium chloride solution (1 × 200 mL) and then dried over anhydrous sodium sulfate. Removal of the solvent in vacuo provided an additional 0.6 g of white crystals (total yield of 96%). Recrystallization of these solid fractions from acetone and petroleum ether gave pure **4a**: mp 155–157 °C; ^{1}H NMR (acetone-d_6) δ 2.03 (s, 3), 4.03 (s, 2), 7.0–7.9 (m, 8), 8.22 (br s, 1), 9.42 (br s, 1); IR (KBr) 3300, 3115, 1670, 1545, 1330, 1160 cm^{-1}; UV (EtOH) 265 nm (ε 16 000); MS, m/e 384 and 382 (M^+), 243 and 241 ($M^+ - C_6H_5SO_2$), 121 (base).

2′-Methyl-4′-acetamidobenzenesulfonanilide (4b). Acetylation of **3a** with acetic anhydride was carried out in glacial acetic acid by using anhydrous sodium acetate as the base. The product (**4b**) was isolated as a precipitate by dilution of the reaction mixture with water: 93% yield; mp 170–171 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.00 (s, 3), 2.08 (s, 3), 6.8–7.8 (m, 8); UV (EtOH) 255 nm (ε 19 000).

4′-(Bromoacetamido)benzenesulfonanilide (4c): 81% yield; 167–168.5 °C; ^{1}H NMR (Me_2SO-d_6) δ 4.0 (s, 2), 7.0 (d, J = 8 Hz, 2), 7.2–7.8 (m, 7); IR (KBr) 3350, 3250, 1675, 1320, 1150 cm^{-1}; MS, m/e 370 and 368 (M^+), 229 and 227 ($M^+ - C_6H_5SO_2$), 107 (base).

4′-Acetamidobenzenesulfonanilide (4d). The hydrochloride salt of 4′-aminobenzenesulfonanilide was acetylated in aqueous solution with acetic anhydride by utilizing sodium acetate as a base. The product (**4d**) was isolated as a solid precipitate: 99% yield; mp 149–150 °C; ^{1}H NMR (acetone-d_6) δ 2.03 (s, 3), 7.0–8.0 (m, 9); IR (KBr) 3340, 3260, 1675, 1330, 1160 cm^{-1}; MS, m/e 290 (M^+), 149 ($M^+ - C_6H_5SO_2$), 107 (base).

2′-Cyano-4′-(bromoacetamido)benzenesulfonanilide (4e). This bromoacetylation procedure was an improvement over that previously employed. To 3.75 g (14 mmol) of 2′-cyano-4′-aminobenzenesulfonanilide (**3c**) and 2.3 g (14 mmol) of sodium bromoacetate suspended in dry acetonitrile (80 mL) was added dropwise 1.3 mL (14 mmol, 2.8 g) of bromoacetyl bromide with good stirring over 10 min. After stirring at room temperature for 24 h, 80 mL of water was added, and the stirring was continued

(7) Jansen, R. *Z. Farben-Ind.* **1910**, *12*, 109–110.
(8) Morgan, G. T.; Micklethwait, F. M. G. *J. Chem. Soc.* **1905**, *87*, 80–87.
(9) Aktien-Gesellschaft fur Anilin-Fabrikation (German Patent), *Chem. Zentr.* **1905I**, 416.
(10) Aktien-Gesellschaft fur Anilin-Fabrikation (German Patent), *Chem. Zentr.* **1905II**, 1207.
(11) Nietzki, R. *Chem. Ber.* **1877**, *10*, 1157–1159.
(12) Schreiber, R. S.; Shriner, R. L. *J. Am. Chem. Soc.* **1935**, *57*, 1306–1311.

for 1 h. At that time, 30 mL of 2 N hydrochloric acid was added, and the white precipitate was removed by filtration (3.7 g). The filtrate was then partitioned between ethyl acetate (250 mL) and 2 N hydrochloric acid (200 mL). After the ethyl acetate extract was washed with saturated potassium bicarbonate solution (200 mL) and saturated sodium chloride solution (200 mL), the extract was dried over anhydrous sodium sulfate and the solvent was removed in vacuo to provide an additional small amount of white solid (73% total yield). Recrystallization of these materials from ethyl acetate and petroleum ether (30–60 °C) provided the desired bromoacetamide in pure form: mp 185–186 °C; ^{1}H NMR (Me_2SO-d_6) δ 4.10 (s, 2), 7.0 (d, J = 8 Hz, 1), 7.56–7.80 (m, 6), 8.0 (d, J = 3 Hz, 1); IR (KBr) 2215, 1680, 1320, 1160 cm^{-1}; MS, m/e 395 and 393 (M^+, 1:1), 254 and 252 ($M^+ - C_6H_5SO_2$), 132 (base).

2′-Methoxy-4′-(bromoacetamido)benzenesulfonanilide (4f). This material was prepared by using chloroform as a solvent and suspended anhydrous sodium carbonate as the base: 65% yield; mp 152–153 °C; ^{1}H NMR (acetone-d_6) δ 3.50 (s, 3), 4.01 (s, 2), 6.97–7.83 (m, 8), 8.0 (br s, 1); IR (KBr) 1650, 1610 cm^{-1}; UV (EtOH) 265, 295 nm (ϵ 13 600 and 12 000); MS, m/e 400 and 398 (M^+, 1:1), 259 and 257 ($M^+ - C_6H_5SO_2$), 137 (base).

2′-(Trifluoromethyl)-4′-(bromoacetamido)benzenesulfonanilide (4g). This material was prepared by using acetonitrile/sodium bromoacetate as described for **4e** above: 83% yield; mp 162–164 °C; ^{1}H NMR (acetone-d_6) δ 4.05 (s, 2), 7.33–7.9 (m, 7), 8.03 (d, J = 3 Hz, 1), 8.3 (br s, 1), 9.7 (br s, 1); IR (KBr) 3280, 3120, 1680 cm^{-1}; UV (EtOH) 270 nm (ϵ 17 400); MS, m/e 438 and 436 (M^+, 1:1), 297 and 295 ($M^+ - C_6H_5SO_2$), 175 (base).

Preparation of 2′-Chloro-4′-(bromoacetamido)benzenesulfonanilide (4h). On the basis of previously observed regiospecificity of hydrochloric acid addition to benzoquinone diimines of this type,[5] the following reaction was carried out. To 7.7 g (21 mmol) of crude N^1-(phenylsulfonyl)-N^4-(bromoacetyl)benzoquinone diimine (**1c**) dissolved in 60 mL of glacial acetic acid was added all at once 4.0 mL (48 mmol) of concentrated hydrochloric acid dissolved in 12 mL of acetic acid. After being stirred for 1 h at room temperature, the reaction mixture was poured into 200 mL of water. The resulting suspension was extracted with chloroform (2 × 200 mL). The chloroform extract was subsequently washed with saturated potassium bicarbonate solution (2 × 150 mL) followed by saturated sodium chloride solution (1 × 150 mL). After being dried over anhydrous sodium sulfate, the solvent was removed to provide a dark brown oil, which solidified upon trituration with hot ethyl acetate. The addition of petroleum ether (30–60 °C) to the dark ethyl acetate solution provided a small amount of additional solid product (total solid 6.2 g; 75% yield). Recrystallization of this material from a mixture of acetone, chloroform, and petroleum ether (30–60 °C) gave the desired product (**4h**) in a pure state: mp 158–160 °C; ^{1}H NMR ($CDCl_3$) δ 4.0 (s, 2), 7.2–7.9 (m, 8), 8.7 (br s, 1); IR ($CHCl_3$) 1685, 1520, 1500, 1170 cm^{-1}; UV (EtOH): 265 nm (ϵ 24 000); MS, m/e 402, 404, 406, (M^+, 1.0:1.3:0.4), 261, 263, 265 ($M^+ - C_6H_5SO_2$, 1.0:1.3:0.3), 143 (32% base), 141 (base).

Preparation of 2-Methyl-N^1-(phenylsulfonyl)-N^4-(bromoacetyl)benzoquinone Diimine (1a) and Similarly 1b–h. A modification of Adams' procedure[3] is employed in this oxidation as well as in other similar oxidations. To 9.6 g (25 mmol) of 2′-methyl-4′-(bromoacetamido)benzenesulfonanilide (**4a**) suspended in 125 mL of chloroform (dry, ethanol removed) was added all at once 12.0 g (25 mmol) of finely divided, dry lead tetraacetate. After efficiently stirring the suspension for 2 h at room temperature, the insoluble lead salts were removed by filtration. To the filtrate was added 60 mL of petroleum ether (90–100 °C), and the volume was reduced to approximately 70 mL in vacuo. Upon cooling on dry ice, a large quantity of yellow crystals formed. Further removal of solvent followed by cooling on dry ice provided additional crops of yellow solid (total weight 9.3 g; 97% yield). Recrystallization from a large volume of ether or from a mixture of chloroform and ether provided pure benzoquinone diimine **1a**: mp 118–120 °C; ^{1}H NMR ($CDCl_3$) δ 2.03 (d, J = 1 Hz, 3), 4.03 (s, 2), 6.7–7.0 (m, 2), 7.33–8.07 (m, 6); IR ($CHCl_3$) 1680, 1590, 1320, 1160, 1090 cm^{-1}; UV (EtOH) 292 nm (ϵ 23 000); MS, m/e same as reduced form **4a**.

2-Methyl-N^1-(phenylsulfonyl)-N^4-acetylbenzoquinone Diimine (1b). Addition of diethyl ether to the concentrated filtrate followed by cooling on dry ice promoted formation of orange-yellow crystals (74% yield). Recrystallization of this product from ether gave pure benzoquinone diimine **1b**: mp 87–89 °C; ^{1}H NMR ($CDCl_3$) δ 2.04 (d, J = Hz, 3), 2.29 (s, 3), 6.68–7.0 (m, 6); IR ($CHCl_3$) 1692, 1590, 1320 cm^{-1}; UV (EtOH) 265, 300 nm (ϵ 13 000, 16 000).

N^1-(Phenylsulfonyl)-N^4-(bromoacetyl)benzoquinone Diimine (1c). Attempted crystallization of this benzoquinone diimine by adding petroleum ether (90–100 °C) and reducing the volume of solution led only to the isolation of a dark brown oil. Attempted crystallization from ether was also unsuccessful. Since both thin-layer chromatography (high R_f) and NMR spectra supported the presence of the desired product, this material was utilized in subsequent reactions (HCl addition to provide **4h**, as described above). Oxidations in ether and acetic acid were no more successful. ^{1}H NMR ($CDCl_3$) δ 4.1 (s, 2), 6.9–8.3 (m, 9); IR ($CHCl_3$) 1690, 1600, 1450, 1330, 1160, 1095 cm^{-1}.

N^1-(Phenylsulfonyl)-N^4-acetylbenzoquinone Diimine (1d). The filtrate was diluted by addition of 40 mL of petroleum ether (90–100 °C) and the volume reduced to approximately 40 mL by evaporation on a rotary evaporator. Upon cooling, brown crystals as well as tar-like impurities were deposited. Recrystallization from ether provided 765 mg (44% yield) of the yellow crystalline benzoquinone diimine **1d**, which decomposed upon standing: mp 112–115 °C; ^{1}H NMR ($CDCl_3$) δ 2.3 (s, 3), 6.8–8.2 (m, 9); IR (KBr) 1700, 1600, 1500, 1300, 1200 cm^{-1}.

Attempted Preparation of 2-Cyano-N^1-(phenylsulfonyl)-N^4-(bromoacetyl)benzoquinone Diimine (1e) from 2′-Cyano-4′-(bromoacetamido)benzenesulfonanilide. This benzoquinone diimine **1e** could *not* be prepared under the usual oxidative conditions. The only identifiable products isolated in small amounts were the oxidized and reduced 3′-acetoxy derivatives, which were isolated by column chromatography (0–1% ethanol/chloroform, silica gel) and identified by ^{1}H NMR and mass spectrometry, as follows.

2-Cyano-3-acetoxy-N^1-(phenylsulfonyl)-N^4-(bromoacetyl)benzoquinone Diimine: ^{1}H NMR ($CDCl_3$) δ 2.16 (s, 3), 4.03 (s, 2), 7.4–8.4 (m, 7); MS, m/e 451 and 449 (M^+), 409 and 407 ($M^+ - CH_3CO$).

2′-Cyano-3′-acetoxy-4′-(bromoacetamido)benzenesulfonanilide: ^{1}H NMR ($CDCl_3$/10% Me_2SO-d_6) δ 2.40 (s, 3), 3.94 (s, 2), 7.0–8.0 (m, 7); MS, m/e 453 and 451 (M^+), 411 and 409 ($M^+ - CH_3CO$), 395 and 393 ($M^+ - CH_3CO_2$), 132 (base).

2-Methoxy-N^1-(phenylsulfonyl)-N^4-(bromoacetyl)benzoquinone Diimine (1f). The addition of an equal volume of ether to the concentrated filtrate followed by cooling on dry ice provided an initial crop of yellow crystals. Removal of the solvent entirely provided a brown residue, which crystallized upon trituration with ether. Crude benzoquinone diimine **1f** was obtained in 90% yield. Rapid suction filtration of this material in chloroform solution through a thick bed of silica gel followed by recrystallization from ether provided pure benzoquinone diimine **1f**: mp 141–143 °C; ^{1}H NMR ($CDCl_3$) δ 3.74 (s, 3), 4.03 (s, 2), 5.99 (d, J_{BX} = 2 Hz, 1), 6.76 (dd, J_{AB} = 10 Hz, 1), 7.33–8.0 (m, 5); IR ($CHCl_3$) 1680, 1580, 1450, 1325, 1160 cm^{-1}; UV (EtOH) 260, 280 nm (ϵ 10 500, 8000).

2-(Trifluoromethyl)-N^1-(phenylsulfonyl)-N^4-(bromoacetyl)benzoquinone Diimine (1g). A large amount of ether was added to the concentrated filtrate, and the mixture was cooled on dry ice for 1–2 h. The bright yellow crystals that formed were removed by filtration, and the filtrate was reduced to dryness in vacuo to provide more yellow solid. These solids were combined and dissolved in ether (some insoluble lead salts were removed by filtration). The ether solution was cooled on dry ice to induce formation of orange-yellow crystals of pure benzoquinone diimine **1g**, which were removed by filtration (84% yield): mp 86–88 °C; ^{1}H NMR ($CDCl_3$) δ 4.09 (s, 2), 6.85–8.16 (m, 8); IR ($CHCl_3$) 1695, 1603, 1160 cm^{-1}; UV (EtOH) 260 nm (ϵ 12 400); MS, m/e small 434, 436 (M^+) overlapping 436, 438 of reduced form (expected reduction in mass spectrometer).

2-Chloro-N^1-(phenylsulfonyl)-N^4-(bromoacetyl)benzoquinone Diimine (1h). After filtration, the volume of the chloroform solution was reduced and several volumes of ether were added. Cooling on dry ice promoted formation of a large amount of yellow crystals. Further reduction of the volume and addition of ether provided additional crude product (total 67% yield). After washing a chloroform solution of this material rapidly with suction

through a thick bed of silica gel and subsequent recrystallization from ether, the desired benzoquinone diimine (**1h**) was obtained in a pure state: mp 110–112 °C; ^{1}H NMR ($CDCl_3$) δ 4.09 (s, 2), 6.97 (dd, J_{AB} = 10 Hz, 1), 7.16 (d, J_{AX} = 2 Hz, 1), 8.12 (d, J_{AB} = 10 Hz, 1), 7.50–8.16 (m, 5); IR ($CHCl_3$) 1690, 1590, 1450 cm^{-1}; UV (EtOH) 265 nm (ϵ 9000).

Preparation of 2′-Methyl-4′-(2-bromo-2-methylpropionamido)benzenesulfonanilide (Reduced 6). To a rapidly stirred suspension of 5.2 g (20 mmol) of 2′-methyl-4′-aminobenzenesulfonanilide (**3a**) and 2.0 g (20 mmol) of finely divided, anhydrous sodium carbonate in 300 mL of chloroform (dry, ethanol removed) was added dropwise 3.5 mL (20 mmol, 4.6 g) of 2-bromo-2-methylpropionyl bromide (Eastman Kodak) dissolved in 10 mL of chloroform. After stirring at room temperature for 3 h, 30 mL of 2 N hydrochloric acid was added, and the reaction mixture was transferred to a separatory funnel. After addition of additional 100 mL of chloroform and another 100 mL of 2 N hydrochloric acid, the layers were separated. The chloroform solution was subsequently washed with saturated potassium bicarbonate solution (1 × 100 mL) and saturated sodium chloride solution (1 × 100 mL) and then dried over anhydrous sodium sulfate. Removal of the solvent in vacuo provided 8.0 g (95%) of a semisolid material that solidified upon standing. Recrystallization of this material from ethyl acetate and petroleum ether provided the desired product in a pure state: mp 138–140 °C; NMR (acetone-d_6) δ 1.93 (s, 3), 2.01 (s, 6), 7.06 (d, J = 9 Hz, 1), 7.30–7.83 (m, 7), 8.3 (br s, 1), 9.0 (br s, 1); NMR ($CDCl_3$) δ 1.93 (s, 3), 2.10 (s, 6), 7.1–7.8 (m, 8). IR ($CHCl_3$) 3380, 1680, 1165 cm^{-1}; UV (EtOH) 265 nm (ϵ 14 000); MS, m/e 412 and 410 ($M^+ - C_6H_5SO_2$, 1:1).

Preparation of 2-Methyl-N^1-(phenylsulfonyl)-N^4-(2-bromo-2-methylpropionyl)benzoquinone Diimine (6). To 6.8 g (16.5 mmol) of 2′-methyl-4′-(2-bromo-2-methylpropionamido)benzenesulfonanilide (described above) dissolved in 75 mL of chloroform (dry, ethanol removed) was added all at once 8.0 g (17 mmol) of dry, finely divided lead tetraacetate with rapid stirring. After stirring at room temperature for 30 min, the insoluble lead salts were removed by filtration. The filtrate volume was reduced to approximately 50 mL, and petroleum ether (90–100 °C), approximately 25 mL, was added until the solution clouded. Cooling on dry ice induced crystallization of the desired product as bright yellow crystals. Subsequent crops were obtained by reducing the volume of the mother liquor (total yield 5.9 g, 85%). Recrystallization from ether by cooling on dry ice provided pure benzoquinone diimine **6**: mp 128–130 °C; NMR ($CDCl_3$) δ 2.01 (s, 6), 2.06 (d, J = 1 Hz, 3), 6.8–7.1 (m, 2), 7.4–8.15 (m, 6); IR ($CHCl_3$) 1683, 1590 cm^{-1}; UV (EtOH) 275 nm (ϵ 15 000).

Preparation of 2-(Bromoacetamido)-N^4-(bromoacetyl)naphthoquinone Imine (7). To 2.1 g (10 mmol) of 2-amino-N^4-naphthoquinone iminium hydrochloride (prepared according to Fieser's method[6]) suspended in dry acetonitrile along with 4.8 g (30 mmol) of sodium bromoacetate was added dropwise 2.3 mL (4.0 g, 23 mmol) of bromoacetyl bromide (Aldrich). The orange-red suspension was stirred at room temperature for 72 h, after which time the red solid material was removed by filtration. Refrigeration of the filtrate induced formation of a bright yellow crystalline product. Subsequent addition of water to the acetonitrile mother liquor yielded additional crystalline product (total product recovered, 1.4 g, 33%). Recrystallization of this product from hot ethyl acetate provided pure naphthoquinone imine **7**: mp 187–188.5 °C; NMR (acetonitrile-d_3) δ 4.13 (s, 2), 4.29 (s, 2), 7.67–8.33 (m, 5); IR (KBr) 1690, 1650, 1580, 1490, 1320 cm^{-1}; MS, m/E 412, 414, 416 (M^+, ratio approximately 1:2:1), 214 (base).

Registry No. **1a**, 62442-86-8; **1b**, 62442-88-0; **1c**, 86785-28-6; **1d**, 86785-29-7; **1f**, 86785-30-0; **1g**, 86785-31-1; **1h**, 86785-32-2; **2a**, 86785-33-3; **2b**, 1829-81-8; **2c**, 86802-67-7; **2d**, 65680-53-7; **2e**, 86785-34-4; **3a**, 86785-35-5; **3b**, 5466-91-1; **3c**, 86785-36-6; **3d**, 82565-49-9; **3e**, 86785-37-7; **4a**, 86785-38-8; **4b**, 86822-01-7; **4c**, 86785-39-9; **4d**, 27022-75-9; **4e**, 86785-40-2; **4f**, 86785-41-3; **4g**, 86785-42-4; **4h**, 86785-43-5; **5a**, 21226-32-4; **5b**, 86802-68-8; **6**, 86785-44-6; **7**, 86785-45-7; benzenesulfonyl chloride, 98-09-9; 2-methyl-4-nitroaniline, 99-52-5; bromoacetyl bromide, 598-21-0; 4′-aminobenzenesulfonanilide hydrochloride, 86785-46-8; sodium bromoacetate, 1068-52-6; 2-cyano-3-acetoxy-N'-(phenylsulfonyl)-N^4-(bromoacetyl)benzoquinone diimine, 4377-73-5; 2′-cyano-3′-acetoxy-4′-(bromoacetamido)benzenesulfonanilide, 86785-47-9; 2-bromo-2-methylpropionyl bromide, 20769-85-1; 2′-methyl-4′-(2-bromo-2-methylpropionamido)benzenesulfonanilide, 86785-48-0; 2-amino-N^4-naphthoquinone iminum hydrochloride, 5438-85-7.

Studies on the Syntheses of Heterocyclic Compounds and Natural Products. 1000. Double Enamine Annelation of 3,4-Dihydro-1-methyl-β-carboline and Isoquinoline Derivatives with 6-Methyl-2-pyrone-3,5-dicarboxylates and Its Application for the Synthesis of (±)-Camptothecin†

Masataka Ihara, Kazuharu Noguchi, Tatsushi Ohsawa, and Keiichiro Fukumoto

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980, Japan

Tetsuji Kametani*

Institute of Medicinal Chemistry, Hoshi University, Ebara, 2-4-41, Shinagawa-ku, Tokyo 142, Japan

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6-Methyl-2-pyrone-3,5-dicarboxylates **8** and **9** were prepared by the reaction of dimethyl (methoxymethylene)malonate and acetoacetates in the presence of sodium hydride followed by an acid treatment. Reaction of the pyrones **8** and **9** with 3,4-dihydro-1-methylisoquinolines **10** and **11** and β-carboline (**16**) produced the tetra- (**14** and **15**) and pentacyclic compounds (**17** and **18**) by a double annelation. 14-(*tert*-Butoxycarbonyl)-12b,2-(epoxyetheno)-1,2,3,6,7,12b-hexahydro-3-(methoxycarbonyl)-13-methylindolo[2,3-*a*]quinolizidin-4-one (**18**), prepared by the above double enamine annelation method, was converted into the acetate **34** which previously had been transformed into (±)-camptothecin (**1a**) in two steps.

Utilizing the enamine character of 1-methyl-3,4-dihydroisoquinolines and 1-methyl-3,4-dihydro-β-carbolines, we developed an efficient synthesis of benzo- and indolo[*a*]quinolizidine derivatives by reactions with α,β-unsaturated esters.[1] Application of this method led us to the

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