JOC*Note*

Syntheses of Regioisomerically Pure 5- or 6-Halogenated Fluoresceins

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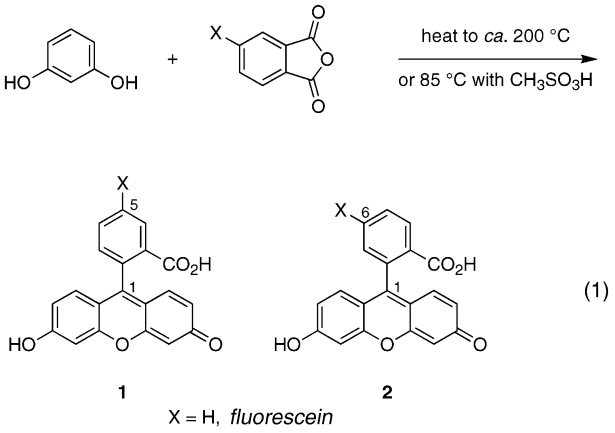
Abstract: Three routes to regioisomerically pure 5- and 6-iodofluoresceins or 5- and 6-bromofluoresceins are described. The first, shown in Scheme 1, involves diazotization/iodination of the corresponding aminofluoresceins. In the second approach (Scheme 2) a mixture of regioisomeric fluoresceins was prepared, and the 5-bromo isomers were isolated as the ring closed diacetates **9b** and **11** by fractional crystallization. Scheme 3 shows an approach to sulfonic acid derivatives **3** and **4** of 5-iodofluorescein. This is the most convenient procedure of the three, and it is particularly useful as sulfofluoresceins have more favorable water solubility characteristics than fluoresceins that lack the sulfonic acid group.

Fluorescein is one of the most common dyes used for fluorescence detection.[1] It has a high quantum yield, some water solubility, and is easily prepared in one step from 1,3-dihydroxybenzene and phthalic anhydride (reaction 1). Preparation of functionalized fluoresceins via



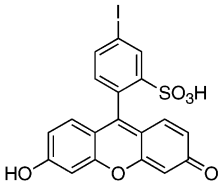
(1)

1 **2**

X = H, *fluorescein*

analogous routes with substituted phthalic anhydrides is, however, complicated by the formation of regioisomers, as illustrated in reaction 1. In some cases, especially those where the X-substituent is a polar one like CO_2H or NO_2, it has been possible to separate the two isomers via fractional crystallization,[2−5] but if the substituent is less polar then separation of the regioisomeric forms becomes more difficult.

A project in our laboratory on syntheses of through-bond energy transfer cassettes[6,7] led us to explore syntheses of the 5- or 6-halofluoresceins (**1** and **2**, X = I or Br) that could be valuable substrates in various metal-catalyzed coupling reactions. These 5- or 6-halofluoresceins have not been reported previously. Described here are routes to a series of these compounds that can be used to produce the products in gram quantities. This methodology has also been extended to the first syntheses of the halogenated fluorescein sulfonic acids **3** and **4**. Sulfonic acid derivatives of fluorescein are coveted in the area of fluorescence labeling since they tend to have similar, excellent, quantum yields, but greater water solubilities than fluorescein, particularly at neutral pH.[8,9]



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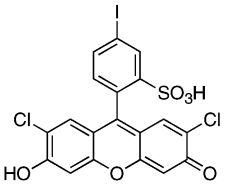

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Regioisomerically pure 5- and 6-aminofluoresceins **5** and **6** are commercially available (Fluka), but they are extremely expensive. For preparative work it is better to make them in gram amounts via the known route that involves condensation of 1,3-dihydroxybenzene with 3-nitrophthalic acid, separation of the isomers via fractional crystallization,[2] then reduction of the nitro group.[10] The corresponding diacylated forms **7** and **8** are also available via the same route but with an extra step: treatment with acetic anhydride.[10,11] Scheme 1 shows how compounds **5** and **6** were used to obtain the corresponding iodofluorescein derivatives **1a**, **2a**, **9a**, and **10a** via diazotization and treatment with potassium iodide; these compounds were isolated via flash chromatography.

For the 5-bromofluorescein derivative **9b** we found a more direct route than the diazotization procedure (Scheme 2a). This involved formation of 5- and 6-bromofluoresceins as a mixture of two regioisomers then, without separation, acylation to the ring-closed form and recrystallization several times from acetic anhydride. The substitution pattern of the 5- and 6-bromofluorescein diacetate samples from the fractional recrystallization procedure was assigned by comparison of ^{1}H NMR spectra of 5-bromo isomer with an authentic sample

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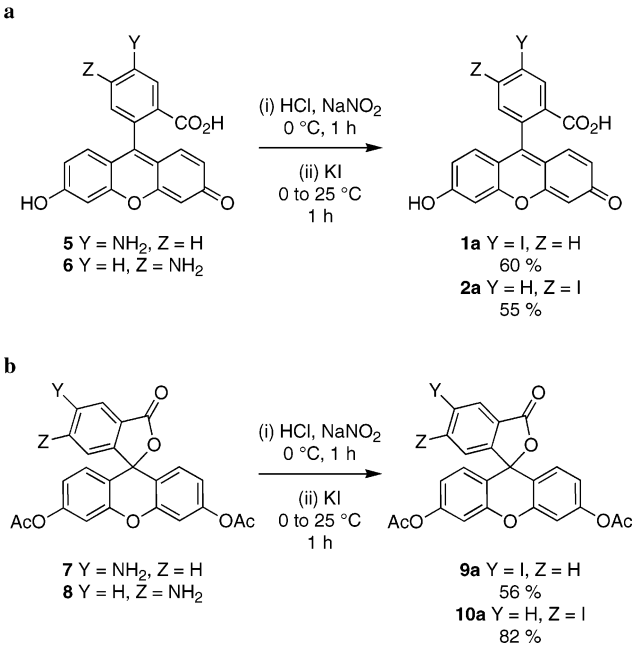
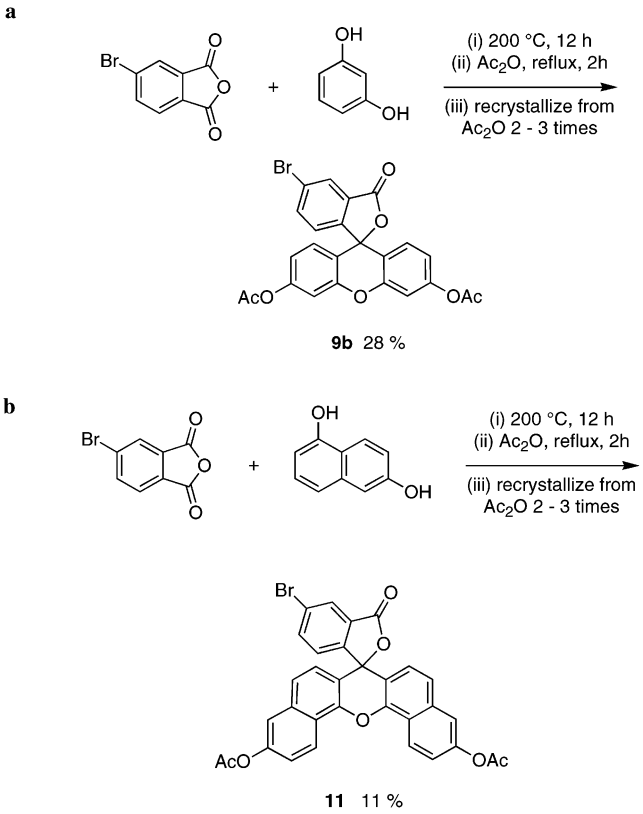
SCHEME 1. Preparation of Iodofluorescein Derivatives via Diazotization

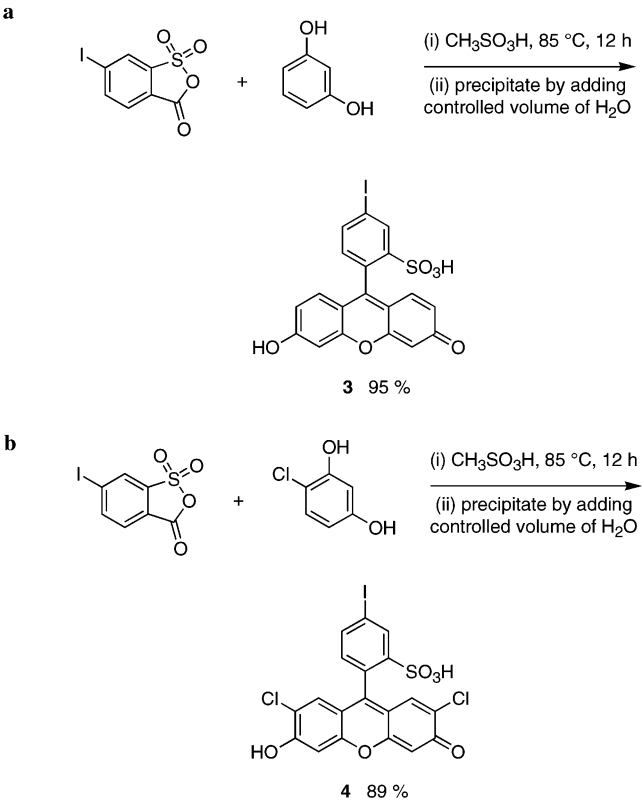


SCHEME 2. Preparation of 5-Bromofluorescein Derivatives via Direct Condensation Reactions



prepared from regioisomerically pure 5-aminofluorescein, using the diazotization route as shown in Scheme 1 (thus, 5-aminofluorescein **5** was diazotized and treated with CuBr, instead of using KI for iodofluorescein, to give regioisomerically pure 5-bromofluorescein). The starting materials used in the diazotization route shown in Scheme 1 are so expensive that most researchers would

SCHEME 3. Preparation of 5-Iodosulfofluorescein Derivatives via Direct Condensation Reactions



want to prepare them, so the route to the 5-bromo derivative in Scheme 2a is more direct. Further, no chromatography is required and the method gives direct access to gram quantities of product. NMR analyses of the mother liquors from the recrystallizations show them to contain a mixture of the 5- and 6- bromo isomers, but with the 6-bromo compound representing about 85% of that material. The reaction shown in Scheme 2b was performed to illustrate that the process could be applied to form at least one other 5-bromonaphthofluorescein derivative, compound **11**. The low chemical yields in these reactions must be evaluated in the context of the experimental convenience of the procedure, and by the accessibility of the starting materials.

Formation of regioisomers is not a problem when substituted 2-sulfobenzoic anhydrides[12,13] are used as substrates for formation of sulfofluoresceins. Scheme 3 illustrates this for the formation of 5-iodo derivatives. The products precipitate from the reaction conditions in a high state of purity when a limited quantity of water is added to the reaction mixture. This is the most direct access to fluorescein derivatives that are substituted with iodine or bromine atoms that we have discovered to date.

The syntheses delineated in this Note provide practical routes to regioisomerically pure bromo- and iodofluoresceins in gram amounts. Future applications from these laboratories will demonstrate how these can be applied in useful Suzuki and Sonogashira coupling reactions to obtain fluorescent donor−acceptor cassettes.

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Experimental Section

5-Iodofluorescein (1a). 5-Aminofluorescein (1.00 g, 2.88 mmol) was suspended in 10 mL of 12 N HCl and 5 g of ice was added. The mixture was cooled and stirred in an ice/water bath. Sodium nitrite (0.25 g, 3.60 mmol) in 5 mL of water was added dropwise over 2 min. The mixture was stirred for 30 min at 0 °C, then potassium iodide (4.78 g, 28.7 mmol) in 8 mL of water was added dropwise over 2 min with vigorous stirring at 0 °C. The cooling bath was removed and the stirring was continued for a further 1 h at 25 °C. The reaction mixture was extracted three times with 25% *i*PrOH/$CHCl_3$. The combined organic extracts were washed with saturated aqueous $Na_2S_2O_3$ then dried with Na_2SO_4 and concentrated in vacuo. The resulting residue was adsorbed onto silica and purified by flash chromatography eluting with 10% MeOH/CH_2Cl_2 to afford 0.79 g (60% yield) of 5-iodofluorescein as an orange solid. Mp 323−325 °C dec; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.17 (br, 2H), 8.28 (dd, J = 1.5, 0.5 Hz, 1H), 8.09 (dd, J = 8.0, 1.5 Hz, 1H), 7.07 (dd, J = 8.0, 0.5 Hz, 1H), 6.67 (d, J = 2.4 Hz, 2H), 6.60 (d, J = 8.5 Hz, 2H), 6.54 (dd, J = 8.5, 2.4 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.3, 159.7, 152.0, 151.8, 144.1, 133.2, 129.3, 128.5, 126.2, 112.8, 109.1, 102.4, 96.3, 83.4; MS (ESI-TOF) m/z 457 $(M - H)^+$; HRMS (ESI) calcd for $C_{20}H_{11}IO_5$ 458.9729 $(M + H)^+$, found 458.9701.

6-Iodofluorescein (2a). This compound was prepared following the procedure described above for compound **1a** except that 6-aminofluorescein was used. Yield 55%. Mp 322−324 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.12 (br, 2H), 8.04 (dd, J = 8.0, 1.4 Hz, 1H), 7.72 (dd, J = 8.0, 0.6 Hz, 1H), 7.66 (d, J = 0.7 Hz, 1H), 6.65 (d, J = 1.8 Hz, 2H), 6.59−6.52 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.1, 159.5, 154.0, 151.7, 139.1, 132.6, 129.1, 126.2, 125.6, 112.7, 109.0, 104.4, 102.5, 82.9; MS (ESI-TOF) m/z 457 $(M - H)^+$. Anal. Calcd for $C_{20}H_{11}IO_5 \cdot H_2O$: C, 50.44; H, 2.75. Found: C, 50.46; H, 2.57.

5-Iodofluorescein Diacetate (9a). 5-Aminofluorescein diacetate (5.00 g, 11.60 mmol) was suspended in 10 mL of 12 N HCl and 7.5 g of ice was added. The mixture was cooled and stirred in an ice/water bath. Sodium nitrite (0.96 g, 13.9 mmol) in 40 mL of water was added dropwise. The mixture was stirred for 30 min at 0 °C then potassium iodide (19.26 g, 116.0 mmol) in 60 mL of water was added dropwise over 2 min with vigorous stirring at 0 °C. The cooling bath was removed and stirring continued for a further 1 h at 25 °C. The reaction mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with saturated aqueous $Na_2S_2O_3$ then dried with Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by flash chromatography eluting with CH_2Cl_2 to afford 3.50 g (56% yield) of 5-iodofluorescein diacetate as a white solid. Mp 222−223 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (dd, J = 1.5, 0.6 Hz, 1H), 7.98 (dd, J = 8.1, 1.5 Hz, 1H), 7.10 (t, J = 1.2 Hz, 2H), 6.95 (dd, J = 8.1, 0.6 Hz, 1H), 6.83 (d, J = 1.2 Hz, 4H), 2.30 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.1, 167.6, 152.5, 152.4, 151.8, 144.3, 134.5, 129.1, 128.5, 126.0, 118.2, 115.9, 110.8, 95.6, 82.2, 21.4; MS (ESI + TOF) m/z 543 $(M + H)^+$.

6-Iodofluorescein Diacetate (10a). This compound was prepared following the procedure described above for compound **9a** except that 6-aminofluorescein diacetate was used. Yield 82%. Mp 182−183 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (dd, J = 8.0, 1.5, Hz, 1H), 7.74 (dd, J = 8.0, 0.5 Hz, 1H), 7.55 (dd, J = 1.5, 0.5 Hz, 1H), 7.11−7.10 (m, 2H), 6.86−6.85 (m, 4H), 2.32 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.1, 168.7, 154.6, 152.5, 151.7, 139.8, 133.5, 129.1, 126.6, 125.7, 118.2, 116.0, 110.8, 103.5, 81.5, 21.4; MS (ESI+TOF) m/z 543 $(M + H)^+$.

5-Bromofluorescein Diacetate (9b). A mixture of 4-bromophthalic anhydride (17.50 g, 77.00 mmol) and resorcinol (17.00 g, 154.0 mmol) was heated at 200 °C with stirring for 12 h. After cooling, a solid product was collected and ground in a mortar to give 30.0 g of a mixture of 5- and 6-bromofluorescein. This crude mixture of isomers was used without any purification for acylation. Thus, 30.0 g of the crude bromofluorescein mixture in 120 mL of acetic anhydride was refluxed for 3 h, and slowly cooled to room temperature to induce recrystallization. Pale yellow crystals formed and were collected on a glass funnel, then washed with cold acetic anhydride and ethyl alcohol, successively. After drying under reduced pressure, 9.6 g of bromofluorescein diacetate was obtained, in which the ratio of 5- and 6-isomers was 10:1 (^{1}H NMR). The mother liquor was concentrated to around half the volume with warming under reduced pressure, then allowed to stand for approximately 15 h after which time pale yellow crystals formed. These were isolated via filtration and dried under reduced pressure to give 2.0 g of bromofluorescein diacetate, in which the ratio of 5- and 6-isomers was 5:1. The first and second crops of crystals were combined, dissolved in 30 mL of hot acetic anhydride, and recrystallized again. After filtering and washing with cold acetic anhydride and ethyl alcohol, 10.4 g (28% yield) of 5-bromofluorescein diacetate was obtained, in which the ratio of 5- and 6-isomers was 20:1. Further recrystallization gave regioisomerically pure 5-bromofluorescein diacetate. Mp 228−229 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.17 (dd, J = 1.8, 0.6 Hz, 1H), 7.81 (dd, J = 8.1, 1.8 Hz, 1H), 7.11 (m, 2H), 7.09 (dd, J = 8.1, 0.6 Hz, 1H), 6.84 (m, 4H), 2.33 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 168.8, 167.4, 152.2, 151.5, 151.4, 138.3, 128.8, 128.1, 125.6, 124.2, 117.9, 115.7, 110.5, 81.8, 21.1; MS (APCI) m/z 495/497 $(M + H)^+$. Anal. Calcd for $C_{24}H_{15}BrO_7$: C, 58.20; H, 3.05. Found: C, 58.36; H, 2.98.

For purification of 6-bromofluorescein diacetate, the mother liquors from the recrystallization of 5-isomer in acetic anhydride were combined and concentrated to dryness under reduced pressure. Ethanol (150 mL) was added to the solid residue, and the resulting suspension was warmed to dissolve the solid materials, then cooled slowly to induce recrystallization. After filtration and washing with cold ethanol, 4.3 g (11% yield) of 6-bromofluorescein diacetate was obtained, in which the ratio of 5- and 6-isomers was 1:10. Repeated recrystallization from ethanol (2×) gave regioisomerically pure 6-bromofluorescein diacetate. Mp 146−147 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.90 (dd, J = 8.1, 0.6 Hz, 1H), 7.77 (dd, J = 8.1, 1.5 Hz, 1H), 7.34 (dd, J = 1.5, 0.6 Hz, 1H), 7.11 (m, 2H), 6.86 (m, 4H), 2.33 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.8, 168.1, 154.5, 152.2, 151.4, 133.7, 130.6, 128.8, 127.4, 126.5, 124.8, 117.9, 115.7, 110.5, 81.1, 21.1; MS (APCI) m/z 495/497 $(M + H)^+$. Anal. Calcd for $C_{24}H_{15}BrO_7$: C, 58.20; H, 3.05. Found: C, 58.22; H, 3.12.

5-Bromonaphthofluorescein Diacetate (11). This compound was prepared in a manner similar to **9b** as described above, except with 1,6-naphthalenediol (Aldrich) instead of resorcinol. Recrystallization from CH_2Cl_2 and hexane, followed by recrystallization from hot acetic anhydride gave regioisomerically pure 5-bromonaphthofluorescein diacetate with 11% yield. Mp 300 °C dec; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.77 (d, J = 9.0 Hz, 2H), 8.25 (d, J = 1.5 Hz, 1H), 7.77 (dd, J = 8.1, 1.5 Hz, 1H), 7.62 (m, 2H), 7.53−7.49 (m, 4H), 6.98 (d, J = 8.1 Hz, 1H), 6.88 (d, J = 9.0 Hz, 2H), 2.41 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.1, 167.9, 152.6, 150.2, 146.3, 138.3, 135.1, 128.1, 125.6, 124.4, 124.1, 123.9, 123.8, 122.0, 121.8, 118.8, 111.6, 83.1, 21.2; MS (APCI) m/z 595/597 $(M + H)^+$.

5-Iodosulfofluorescein (3). 4-Iodo-2-sulfobenzoic anhydride (0.50 g, 1.61 mmol) and resorcinol (0.36 g, 3.22 mmol) were weighed into a 10-mL round-bottomed flask. Methanesulfonic acid (3 mL) was added in one portion and the mixture was heated at 90 °C under N_2 for 22 h. After cooling to room temperature, the red solution was poured into water (20 mL) and filtered. The solid was dissolved into 10 mL of 10% NaOH solution and filtered. The filtrate was carefully acidified with concentrated HCl. An orange solid formed, was collected by filtration, and dried (0.76 g, 95% yield). Mp >400 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.21 (d, J = 1.8 Hz, 1H), 7.98 (dd, J = 7.9, 1.8 Hz, 1H), 7.44 (d, J = 9.2 Hz, 2H), 7.33 (d, J = 2.2 Hz, 2H), 7.18 (dd, J = 9.2, 2.2 Hz, 2H), 7.11 (d, J = 7.9 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.9, 167.4, 158.6, 148.2, 137.6, 136.1, 134.8, 131.1, 128.0, 119.5, 117.1, 101.8, 97.1; MS (ESI-TOF) m/z 493 $(M - H)^+$.

2′,7′-Dichloro-5-iodosulfofluorescein (4). This compound was prepared following the procedure described above for compound **3** except that 2,4-dihydroxychlorobenzene was used.

Yield 89%. Mp >400 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.25 (d, J = 1.4 Hz, 1H), 7.92 (dd, J = 8.0, 1.4 Hz, 1H), 7.05 (d, J = 8.0 Hz, 1H), 6.92 (s, 2H), 6.75 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3, 155.1, 151.5, 148.2, 138.0, 136.1, 131.8, 129.8, 128.4, 125.0, 115.9, 103.4, 96.4; MS (ESI-TOF) m/z 561 $(M - H)^+$. Anal. Calcd for $C_{19}H_9Cl_2IO_6S \cdot 3H_2O$: C, 36.97; H, 2.45, S, 5.20. Found: C, 37.27; H, 2.49, S, 5.35.

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Supporting Information Available: Procedure to obtain 4-iodo-2-sulfobenzoic anhydride, and ^{1}H and ^{13}C NMR spectra for **1a**, **2a**, **3**, **4**, **9a**, **9b**, **10a**, and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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