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Preparation of 2-(2-Cyanoethyl)sulfanyl-1*H*-isoindole-1,3-(2*H*)-dione and Related Sulfur-Transfer Agents

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Abstract: The title compound **3** and 4-[(2-cyanoethyl)sulfanyl]morpholine-3,5-dione **12** are both conveniently prepared in good yield from 2-cyanoethyl disulfide, which itself is readily prepared in one step from *S*-(2-cyanoethyl)isothiouronium chloride **4**. In the same way, dimethyl and diphenyl disulfides are converted into 2-methylsulfanyl- and 2-phenylsulfanyl-1*H*-isoindole-1,3-(2*H*)-diones **8a** and **8b**, respectively, also in good yields.
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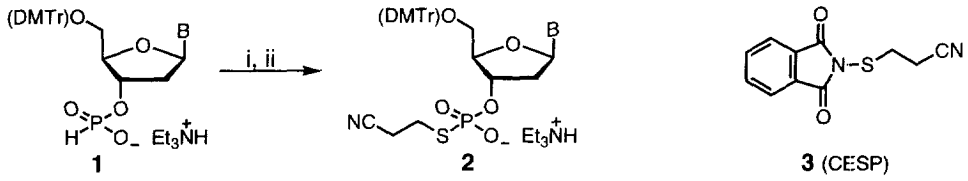
In the past few years, there has been considerable interest in the large scale synthesis of phosphorothioate analogues[1,2] of oligonucleotides. A number of specific sequences have shown promise as chemotherapeutic agents, and are presently undergoing clinical trials[3]. This has led to a demand for relatively large quantities particularly of oligodeoxyribonucleotide phosphorothioates which have been prepared almost exclusively by solid-phase synthesis[4]. Our belief that solid-phase synthesis is unlikely ultimately to be the best method for the preparation of really large quantities of oligonucleotides has led us to re-examine the phosphotriester approach to the synthesis of oligonucleotide phosphorothioates in solution[5,6].

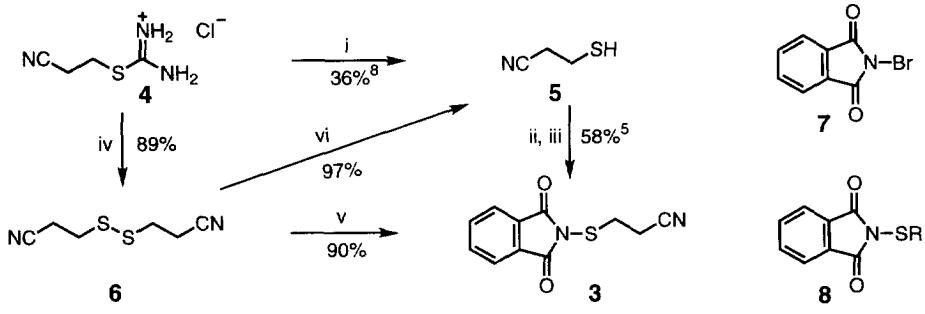


DMTr = 4,4'-dimethoxytrityl

Scheme 1 *Reagents and conditions*: i, **3**, 4-methylmorpholine, Me_3SiCl, CH_2Cl_2, room temp.; ii, aq. $Et_3NH^+ HCO_3^-$.

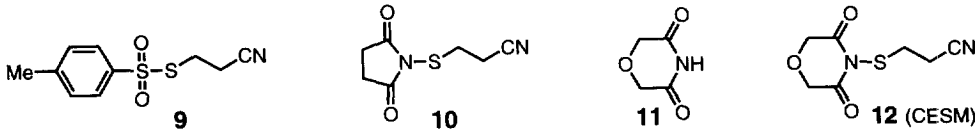
In the course of these studies, we have demonstrated[5,6] that triethylammonium salts of 5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyribonucleoside 3'-*S*-(2-cyanoethyl) phosphorothioates **2** are valuable synthetic intermediates. These building blocks can readily be prepared and usually in very high yield by treating the corresponding 3'-*H*-phosphonates[7] **1** with 2-(2-cyanoethyl)sulfanyl-1*H*-isoindole-1,3-(2*H*)-dione (CESP)[5] **3** in the presence of chlorotrimethylsilane and 4-methylmorpholine in dichloromethane solution at room temperature (Scheme 1). The key sulfur-transfer agent **3**, which is a stable crystalline solid, was prepared[5] in 58% yield (Scheme 2, steps ii-iii) by treating 3-mercaptopropanonitrile[8] **5** first with chlorine in dichloromethane solution and then with phthalimide and

triethylamine in DMF solution. Unfortunately, the literature preparation[8] of 3-mercaptopropanonitrile **5** (Scheme 2, step i), which involves the reaction between the easily accessible *S*-(2-cyanoethyl)isothiouronium chloride[8] **4** and aqueous sodium hydroxide, proceeds only in very modest yield. We now report a much improved preparation of CESP **3**, following a procedure which might well prove to be generally applicable in the preparation of related sulfur-transfer reagents.

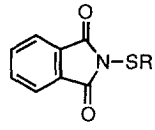


Scheme 2 *Reagents and conditions*: i, 3 M aq. NaOH, 0°C; ii, Cl_2, CH_2Cl_2, 0°C; iii, phthalimide, Et_3N, DMF, 0°C; iv, $NaBO_3 \cdot 4H_2O$, NaOH, H_2O, CH_2Cl_2, 0°C; v, Br_2, phthalimide, C_5H_5N, MeCN, 0°C; vi, Zn powder, 2 M - aq. HCl, 40°C.

If 3-mercaptopropanonitrile **5** is generated in the usual way[8] (*i.e.* by treating the *S*-alkylisothiouronium salt **4** with aqueous sodium hydroxide) in the presence of sodium perborate in a two phase (dichloromethane - water) system (Scheme 2, step iv), it undergoes *in situ* oxidative dimerization. Di-(2-cyanoethyl) disulfide **6** can then be isolated from the products as a crystalline solid in 89% yield. Alkyl- and aryl-sulfanyl derivatives **8** of phthalimide may conveniently be prepared from disulfides[9] (such as **6**) as well as from thiols[10] (such as **5**). Thus both dialkyl and diaryl disulfides react readily[9] with *N*-bromophthalimide **7** to give the corresponding alkyl- and aryl-sulfanyl derivatives **8**. In the case of a valuable disulfide, it is desirable that both sulfur atoms should be incorporated into the alkyl- or aryl-sulfanyl derivative **8**. For this reason, it is better to use a mixture of bromine, phthalimide and base rather than *N*-bromophthalimide **7** (see below). Thus when di-(2-cyanoethyl) disulfide **6** (1.0 mol. equiv.) was allowed to react with bromine (*ca.* 1.15 mol. equiv.) and phthalimide (*ca.* 1.9 mol. equiv.) in pyridine - acetonitrile solution at 0°C, CESP **3** was obtained in 90% isolated yield. This represents *ca.* 80% overall yield, based on the isothiouronium salt **4**, compared with the *ca.* 20% overall yield that was obtained by the previously reported route[5]. If, for some other purpose, 3-mercaptopropanonitrile **5** itself is required, it is best prepared (in 97% yield; Scheme 2, step vi) by reducing the disulfide **6** with zinc powder and hydrochloric acid.



It occurred to us that reagents other than CESP **3** might be equally useful or indeed perhaps even more effective sulfur-transfer agents in the preparation of nucleotide building blocks such as **2**. Brill showed[11] that the toluene-4-thiosulfonate ester **9** could transfer a (2-cyanoethyl)sulfanyl residue to a putative phosphite triester. The latter compound **9** is presumably therefore also potentially useful in the conversion of protected nucleoside *H*-phosphonates **1** into the corresponding *S*-(2-cyanoethyl) phosphorothioates **2**. Although we have so far been unable to develop a satisfactory preparation of *N*-[(2-cyanoethyl)sulfanyl]succinimide **10**, we have found that when di-(2-cyanoethyl) disulfide **6** was treated with bromine and morpholine-3,5-dione[12] **11** in pyridine - acetonitrile solution at *ca.* 0°C, 4-[(2-cyanoethyl)sulfanyl]morpholine-3,5-dione (CESM) **12** was obtained and isolated as a stable crystalline solid in 82% yield. Preliminary experiments have indicated that CESM **12** is an excellent sulfur-transfer agent. Morpholine-3,5-dione **11** is easily prepared in good yield (see Experimental) by fusing the readily available diglycolic acid with ammonium carbonate.



8 **a**; R = Me
b; R = Ph
c; R = 4-Cl·C_6H_4

The present procedure, that is the reaction between a dialkyl disulfide, a very slight excess of bromine and nearly 2 mol. equiv. of phthalimide in pyridine - acetonitrile solution appeared to us to be particularly suitable for the preparation of 2-methylsulfanyl-1*H*-isoindole-1,3(2*H*)-dione[13] **8a**, another useful sulfur-transfer agent[14]. Very surprisingly, although this compound was first referred to by Harpp and Back[13] twenty-five years ago, we were unable to find experimental details for its preparation in the literature. As methanethiol is an extremely volatile compound (b.p. 6°C) with a very disagreeable odour, dimethyl disulfide (b.p. 109°C) is clearly a much more convenient starting material for the preparation of compound **8a**. Indeed, when dimethyl disulfide was allowed to react with bromine and phthalimide under the conditions used in the preparation of CESP **3**, 2-methylsulfanyl-1*H*-isoindole-1,3(2*H*)-dione **8a** was obtained and isolated as a crystalline solid in 82% yield. Similarly, when diphenyl disulfide, bromine and phthalimide were allowed to react together in pyridine - acetonitrile solution at room temperature, 2-phenylsulfanyl-1*H*-isoindole-1,3(2*H*)-dione[9,10] **8b** was obtained and isolated as a crystalline solid in 91% yield. In their paper on the reaction between disulfides and *N*-bromo-imides, Büchel and Conte reported[9] that the benzylsulfanyl compound **8**, R = $PhCH_2$ was obtained in 80% yield when one mol. equiv. each of dibenzyl disulfide and *N*-bromophthalimide **7** were allowed to react together. In this reaction, one half of the dibenzyl disulfide was converted, presumably *via* benzenemethanesulfenyl bromide, into benzyl bromide and sulfur. It seems likely that the reaction between dimethyl disulfide and *N*-bromophthalimide **7** would follow the same stoichiometry and the same course, and that only one-half of the dimethyl disulfide would be converted into the methylsulfanyl compound **8a**. Büchel and Conte also reported[9] that diphenyl disulfide reacted with two mol. equiv. of *N*-bromophthalimide **7** to give the phenylsufanyl derivative **8b** in 90% yield. Presumably 1 mol. equiv. of elemental bromine was also formed as a by-product. The formation of such undesirable by-products is avoided when the present procedure is followed.

When diaryl disulfides are not commercially available, the present procedure can be modified to accommodate arenethiols and presumably also alkanethiols as starting materials. However, double the quantity of bromine is then

required. Thus, when phthalimide (1.0 mol. equiv.), 4-chlorothiophenol (1.05 mol. equiv.) and bromine (1.2 mol. equiv.) were allowed to react together in pyridine - acetonitrile solution at room temperature, 2-(4-chlorophenyl)sulfanyl-1*H*-isoindole-1,3(2*H*)-dione[10] **8c** was obtained and isolated as a crystalline solid in 87% yield. We believe that this procedure is more convenient than that originally described by Behforouz and Kerwood[10], which involved the reaction between an arenesulfenyl chloride (generated *in situ* from the arenethiol and chlorine), phthalimide and triethylamine.

EXPERIMENTAL

Mps are uncorrected. ^1H and ^{13}C NMR spectra were measured, unless otherwise stated, at 360.1 and 90.6 MHz, respectively, with a Bruker AM 360 spectrometer; tetramethylsilane was used as an internal standard. *J* Values are given in Hz. Merck silica gel 60 F_{254} pre-coated plates (Art 5715) were used for TLC. Acetonitrile, pyridine and 2,6-lutidine were dried by heating, under reflux, with calcium hydride for 3-5 h, and were then distilled.

Di-(2-cyanoethyl) disulfide 6

Dichloromethane (400 ml), followed by sodium perborate tetrahydrate (44.1 g, 0.286 mol) were added to a cooled (ice-water bath), stirred solution of *S*-(2-cyanoethyl)isothiouronium chloride[8] **4** (83.0 g, 0.50 mol) in water (500 ml). A solution of sodium hydroxide (30.0 g, 0.75 mol) in water (250 ml) was then added dropwise over 30 min to the cooled, stirred reaction mixture. After a further period of 5 h, the layers were separated and the aqueous layer was extracted with dichloromethane (3 x 50 ml). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The residual solid was crystallized from methanol to give *di-(2-cyanoethyl) disulfide* **6** as colourless needles (38.5 g, 89%) (Found : C, 41.92; H, 4.74; N, 16.19. Calc. for $C_6H_8N_2S_2$: C, 41.83; H, 4.68; N, 16.26%), m.p. 47-48°C (lit.[15] m.p. 49-51°C); δ_H [$CDCl_3$] 2.91 (4 H, m), 3.02 (4 H, m); δ_C [$CDCl_3$] 17.1, 32.5, 119.2.

3-Mercaptopropanonitrile 5

Zinc powder (13.1 g, 0.20 g.atom) was added gradually over 30 min to a stirred mixture of di-(2-cyanoethyl) disulfide **6** (17.2 g, 0.10 mol) and 2M-hydrochloric acid (250 ml) at 40°C. After a further period of 30 min, the cooled products were extracted with dichloromethane (4 x 50 ml). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give *3-mercaptopropanonitrile* **5** (16.9 g, 97%) as a colourless liquid, b.p. 70°C/12 mmHg; δ_H [$CDCl_3$] 1.83 (1 H, t, *J* 8.6), 2.71 (2 H, m), 2.80 (2 H, m); δ_C [$CDCl_3$] 20.6, 22.9, 118.2.

2-(2-Cyanoethyl)sulfanyl-1*H*-isoindole-1,3(2*H*)-dione 3

Di-(2-cyanoethyl) disulfide **6** (9.03 g, 52.4 mmol) and phthalimide (14.7 g, 0.10 mol) were dissolved in hot pyridine (40 ml) and acetonitrile (50 ml), and the stirred solution was cooled (ice-water bath). A solution of bromine (9.6 g, 3.10 ml, 60 mmol) in acetonitrile (60 ml) was added dropwise with stirring over 1 h. Water (200 ml) was then added dropwise over 30 min to the cooled products. After a further period of 2 h at *ca.* 0°C, the products were filtered to give *2-(2-cyanoethyl)sulfanyl-1*H*-isoindole-1,3(2*H*)-dione* **3** (21.0 g, 90%). Recrystallization of this material from ethanol gave colourless needles (Found : C, 57.13; H, 3.69; N, 11.98. Calc. for $C_{11}H_8N_2O_2S$: C, 56.88; H, 3.47; N, 12.06%), m.p. 162-164°C; δ_H [$(CD_3)_2SO$] 2.82 (2 H, t, *J* 6.8), 3.11 (2 H, t, *J* 6.8), 7.88 - 7.95 (4 H, m); δ_C[$(CD_3)_2SO$] 18.1, 33.9, 119.2, 123.4, 131.9, 134.7, 168.1.

Morpholine-3,5-dione 11

Diglycolic acid (26.9 g, 0.20 mol) and ammonium carbonate (19.2 g, 0.20 mol) were powdered and thoroughly mixed together. The mixture, contained in a round-bottomed flask, was heated at 230°C in an atmosphere of argon. After 5 h, the homogeneous liquid obtained was cooled to *ca.* 70°C, and water (200 ml) was added. The resulting solution was stirred with activated carbon (1.0 g) for 10 min and then filtered. The residue was washed with hot water (20 ml). The filtrate and washings were cooled to *ca.* 0°C to give *morpholine-3,5-dione* **11** as colourless crystals (15.7 g, plus a second crop of 4.0 g after concentrating the original mother liquors to *ca.* 20 ml; total yield 19.7 g, 85%). Recrystallization of this material from ethanol gave colourless needles [Found : C, 41.79; H, 4.38; N, 12.17. Calc. for $C_4H_5NO_3$: C, 41.75; H, 4.38; N, 12.17%], m.p. 141-142°C (lit.[12] m.p. 143-145°C); δ_H [$(CD_3)_2SO$] 4.25 (4 H, s), 11.40 (1 H, br); δ_C [$(CD_3)_2SO$] 66.3, 171.0.

4-[(2-Cyanoethyl)sulfanyl]morpholine-3,5-dione 12

A solution of bromine (4.82 g, 1.55 ml, 30.2 mmol) in dichloromethane (20 ml) was added dropwise over 30 min to a stirred, cooled (ice-water bath) mixture of di-(2-cyanoethyl) disulfide (4.51 g, 26.2 mmol), morpholine-3,5-dione (5.75 g, 50.0 mmol), 2,6-lutidine (17.4 ml, 0.15 mol) and dichloromethane (20 ml). The cooled reactants were then stirred for a further period of 1.5 h. Ice-cold methanol (50 ml) was then added dropwise over 30 min. The precipitated *4-[(2-cyanoethyl)sulfanyl]morpholine-3,5-dione* **12** (8.23 g, 82%) was collected by filtration. Recrystallization of this material from ethyl acetate gave colourless needles (Found : C, 41.96; H, 4.01; N, 14.16. $C_7H_8N_2O_3S$ requires : C, 41.99; H, 4.03; N, 13.99%), m.p. 121-122°C; δ_H [$(CD_3)_2SO$] 2.77 (2 H, t, *J* 6.9), 3.10 (2 H, t, *J* 6.9), 4.49 (4 H, s); δ_C [$(CD_3)_2SO$] 17.9, 33.0, 68.1, 119.4, 170.8.

2-Methylsulfanyl-1*H*-isoindole-1,3(2*H*)-dione 8a

Dimethyl disulfide (2.50 g, 2.39 ml, 26.5 mmol) and phthalimide (7.35 g, 50 mmol) were dissolved in hot pyridine (20 ml) and acetonitrile (25 ml), and the stirred solution was cooled (ice-water bath). A solution of bromine (4.8 g, 1.55 ml, 30.0 mmol) in acetonitrile (30 ml) was added dropwise over 1 h. After a further period of 1 h, water (100 ml) was added dropwise over 30 min to the stirred, cooled reactants. The products were allowed to stand for 1 h and were then filtered. The residue was washed with ice-cold methanol to give *2-methylsulfanyl-1*H*-isoindole-1,3(2*H*)-dione* **8a** (7.9 g, 82%). Recrystallization of this material from methanol gave colourless needles (Found : C, 55.99; H, 3.53; N, 7.03. $C_9H_7NO_2S$ requires : C, 55.95; H, 3.65; N, 7.25%), m.p. 175-176°C; δ_H [$(CD_3)_2SO$] 2.46 (3 H, s), 7.89 (4 H, m); δ_C [$(CD_3)_2SO$] 21.7, 123.4, 131.8, 134.7, 167.7.

2-Phenylsulfanyl-1*H*-isoindole-1,3(1*H*)-dione 8b

Diphenyl disulfide (11.46 g, 52.5 mmol) and phthalimide (14.7 g, 0.10 mol) were dissolved in hot pyridine (40 ml) and acetonitrile (50 ml), and the stirred solution was cooled to room temperature. A solution of bromine (9.6 g, 3.10 ml, 60 mmol) in acetonitrile (50 ml) was then added dropwise over 1 h. After a further period of 2 h, water (100 ml) was added dropwise over 30 min. The products were cooled (ice-water bath) for 30 min, and then filtered to give *2-phenyl-1*H*-isoindole-1,3(2*H*)-dione* **8b** as pale yellow crystals (Found : C, 65.58; H, 3.32; N, 5.35. Calc. for $C_{14}H_9NO_2S$: C, 65.87; H, 3.55; N, 5.49%), m.p. 160-161°C (lit.[10] m.p. 160-161°C); δ_H [$(CD_3)_2SO$] 7.29 (1 H, m), 7.36 (4 H, m), 7.92 (2 H, m), 7.98 (2 H, m); δ_C [$(CD_3)_2SO$] 123.8, 126.1, 127.6, 129.4, 131.5, 135.1, 136.0, 167.4.

2-(4-Chlorophenyl)sulfanyl-1*H*-isoindole-1,3(2*H*)-dione 8c

4-Chlorothiophenol (15.19 g, 0.105 mol) and phthalimide (14.70 g, 0.10 mol) were dissolved in hot pyridine (40 ml) and acetonitrile (50 ml), and the stirred solution was cooled to room temperature. A solution of bromine (19.2 g,

6.19 ml, 0.12 mol) in acetonitrile (50 ml) was then added dropwise over 30 min. After a further period of 2 h, methanol (200 ml) was added dropwise over 30 min. The products were cooled (ice-water bath) for 30 min, and then filtered to give *2-(4-chlorophenyl)sulfanyl-1*H*-isoindole-1,3(2*H*)-dione* **8c** as pale yellow crystals (25.2 g, 87%). Recrystallization of this material from methanol gave pale yellow crystals (Found : C, 57.95; H, 2.53; N, 4.76. Calc. for $C_{14}H_8ClNO_2S$: C, 58.04; H, 2.78; N, 4.83%), m.p. 173-174°C (lit.[10] m.p. 179-181°C); δ_H [$(CD_3)_2SO$] 7.40 (4 H, m), 7.93 (2 H, m), 7.98 (2 H, m); δ_C [$(CD_3)_2SO$] 123.9, 128.0, 129.3, 131.7, 132.3, 135.2, 167.4.

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