Use of Dimethyldioxirane for the Oxidation of 1,2-Dithiolan-3-ones to 1-Oxides or 1,1-Dioxides. Preparation of 3*H*-1,2-Benzodithiol-3-one 1,1-Dioxide (Beaucage Sulfurizing Reagent)

Vicente Marchán,[a] Mariona Gibert,[b] Angel Messeguer,[b] Enrique Pedroso,[a] Anna Grandas*[a]

[a] Departament de Química Orgànica, Facultat de Química, Universitat de Barcelona, Martí i Franquès 1-11, E-08028 Barcelona, Spain
E-mail: grandas@admin.qo.ub.es
[b] Departament de Química Orgànica Biològica, CID-CSIC, Jordi Girona Salgado 18-26, E-08034 Barcelona, Spain

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Abstract: Quantitative oxidation of 3*H*-1,2-benzodithiol-3-one to 3*H*-1,2-benzodithiol-3-one 1,1-dioxide (Beaucage sulfurizing reagent) is achieved by reaction of the dithiolanone with a fourfold molar excess of dimethyldioxirane for 2–4 hours at room temperature. A one- or twofold molar excess of reagent affords the 1-oxide as the main product.

Key words: oligonucleotides, phosphorothioates, sulfurization, dimethyldioxirane, Beaucage reagent

The modification of oligonucleotide phosphodiester linkages by substituting sulfur for one of the nonbridging oxygen atoms has been regularly used over the past few years to obtain nuclease-resistant nucleic acid fragments. Phosphorothioate oligonucleotides are able to effectively hybridize with RNA and to intervene in gene expression both by translation arrest and by RNase H-mediated RNA cleavage.[1] These analogues are charged molecules that, to a certain extent, succeed in penetrating cells.

The synthesis of phosphorothioate oligonucleotide analogues can be carried out using either the H-phosphonate or the phosphite triester approaches.[2] In the first case the assembly of the molecule is followed by a sulfurizing step that transforms all H-phosphonate diesters into phosphorothioates.[3,4] The phosphite triester methodology allows the oligonucleotide chain to be assembled more efficiently, but low levels of phosphodiester contamination may be obtained depending on the substrate, the reaction conditions and the sulfurizing reagent used.[5–12] However, this methodology offers the advantage that phosphodiester bonds can, if desired, be selectively modified to thioates at certain positions.

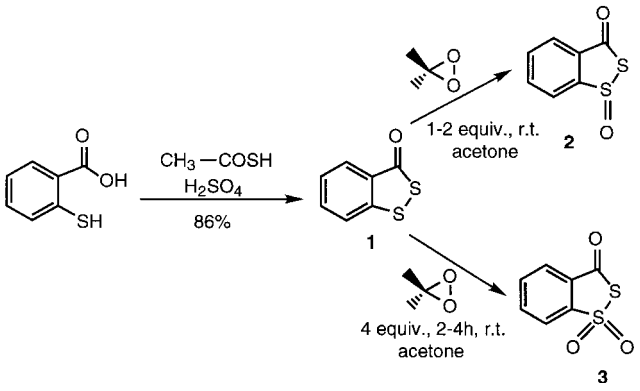
Of the many reagents described for sulfurizing phosphite triester groups[5,7–18] besides sulfur, only tetraethylthiuram disulfide (TEDT)[16] and 3*H*-1,2-benzodithiol-3-one 1,1-dioxide (Beaucage reagent)[14] are commercially available. The latter allows sulfurization to be carried out in considerably shorter reaction times, but it is much more expensive and its synthesis is not straightforward. We wish to report on an improved oxidation procedure for the preparation of this sulfurizing reagent.

The precursor of the Beaucage reagent, 3*H*-1,2-benzodithiol-3-one **(1)**, can be obtained by reaction between 2-mercaptobenzoic acid and thiolacetic acid under acidic conditions, following essentially the described procedure.[19,20] However, in our hands, attempts to oxidize dithiolanone **1** to dithiolanone 1,1-dioxide **3** with trifluoroperoxyacetic acid[19] or 3-chloroperoxybenzoic acid[21] did not give the expected result, since the oxidation afforded a very complex reaction mixture in which the 1-oxide **2** was the main product. We then considered the possibility of replacing the peroxyacid by potassium peroxymonosulfate (Oxone™) or dimethyldioxirane, which have been used to oxidize sulfides, sulfoxides, polysulfides, and episulfides.[22–27]

Attempts to prepare 3*H*-1,2-benzodithiol-3-one 1,1-dioxide **(3)** by oxidation of **1** with potassium peroxymonosulfate (50–100-fold molar excess) were unsuccessful. The reaction was attempted in mixtures of acetonitrile/H_2O or dioxane/H_2O, and in two-phase systems such as ethyl acetate/H_2O or dichloromethane/H_2O in the presence of 18-crown-6. Less than 20% of **3** was detected by gas chromatography in all cases.

Preliminary experiments carried out to determine optimum oxidation conditions with dimethyldioxirane showed that treatment of 3*H*-1,2-benzodithiol-3-one **(1)** with a one- or twofold molar excess of dimethyldioxirane for 2 hours afforded 3*H*-1,2-benzodithiol-3-one 1-oxide **(2)** as the main product, as shown by gas chromatography. When the reaction was carried out with a threefold molar excess of dimethyldioxirane, 1,1-dioxide **3** was formed in 74% yield after 2 hours, but prolonging the reaction time did not allow **3** to be formed quantitatively.

Complete conversion of dithiolanone **1** to the 1,1-dioxide **3** was accomplished by treatment with a fourfold molar excess of dimethyldioxirane (70–90 mM solution) for 2–4 hours at room temperature. Both the molar excess and



the concentration of dimethyldioxirane are important for the quantitative transformation of the dithiolanone **1** into the 1,1-dioxide **3**, since a 95:5 mixture of **3** and **2** was obtained when the reaction was carried out with a ~40 mM solution of dimethyldioxirane. The preparation of 3*H*-1,2-benzodithiol-3-one 1,1-dioxide might be scaled-up by concentration of the dimethyldioxirane solutions.[28]

In summary, treatment with one- or twofold molar excesses of dimethyldioxirane is a convenient alternative for oxidation to the 1-oxide, whereas larger excesses of reagent allow the 1,1-dioxide to be obtained. This procedure, useful for laboratory gram-scale preparations, allows quantitative oxidation to be achieved, whilst the large scale oxidation of 3*H*-1,2-benzodithiol-3-one to the 1,1-dioxide with H_2O_2/trifluoroacetic acid has been reported[20] to take place in 49% yield.

3*H*-1,2-Benzodithiol-3-one (1)

On completion of the reaction between 2-mecaptobenzoic acid (5 g, 32.4 mmol) and thiolacetic acid (4.7 mL, 65.7 mmol) in the presence of H_2SO_4 (50 mL) (cf. ref 19), the dark crude mixture was poured onto ice and the precipitate filtered and washed with water. $CHCl_3$ (70 mL) and sat. aq $NaHCO_3$ (80 mL) were then added to the crude solid. The mixture was stirred for 1 h at r.t., filtered and washed with $CHCl_3$ and $NaHCO_3$, and these filtrates were combined with the previous ones. No *3H*-1,2-benzodithiol-3-one was obtained from additional treatment of the solid with hot $CHCl_3$ (reflux, 1.5 h). The organic and aqueous phases were separated and the aqueous layer was washed with $CHCl_3$. The combined organic extracts were washed with $NaHCO_3$ and water, dried ($MgSO_4$), filtered and treated with activated charcoal pellets. Crude 3*H*-1,2-benzodithiol-3-one **1** was obtained as a crystalline dark yellow solid after filtration and evaporation of the solvent; yield: 4.54 g (86%); mp 73–74 °C (lit.[20] mp 73–74 °C). The product was recrystallized from EtOH (66% overall synthesis and purification yield); TLC ($CHCl_3$): R_f = 0.63.

^{13}C NMR ($CDCl_3$, 50 MHz): δ = 125.2 (CH), 126.2 (CH), 127.8 (CH), 129.6 (Cq), 134.0 (CH), 148.8 (Cq), 194.2 (CO).

EI-MS: *m/z* (%) = 69 (14), 76 (94), 96 (36), 104 (35), 140 (38), 168 (M) (100), 169 (M + 1) (13), 170 (M + 2) (13).

Control of the Oxidation Reaction by Gas Chromatography

GC conditions (Hewlett–Packard 5890A): Tracer TRB-5 column (30 m), 7 °C/min gradient from 100 °C to 250 °C, injector temperature: 280 °C, detector temperature: 300 °C; t_R: 3*H*-1,2-benzodithiol-3-one **(1)** 9.0 min, 3*H*-1,2-benzodithiol-3-one 1-oxide **(2)** 11.6 min, 3*H*-1,2-benzodithiol-3-one 1,1-dioxide **(3)** 11.4 min. **3** partially decomposes under the analysis conditions, the area of main peak amounting ~88% of the total area (authentic sample of commercial origin).

3*H*-1,2-Benzodithiol-3-one 1-Oxide (2)

This was the main product when the oxidation of **1** was attempted with one- or twofold molar excess of dimethyldioxirane; TLC ($CHCl_3$): R_f = 0.54.

^{13}C NMR ($CDCl_3$, 50 MHz): δ = 126.9 (CH), 128.0 (CH), 131.1 (Cq), 133.1 (CH), 135.6 (CH), 151.9 (Cq), 190.2 (CO).

GC-EI-MS: *m/z* (%) = 69 (33), 76 (61), 96 (20), 104 (22), 136 (100), 184 (M) (21), 185 (M + 1) (2), 186 (M + 2) (2).

3*H*-1,2-Benzodithiol-3-one 1,1-Dioxide (3)

Dimethyldioxirane was prepared following described procedures,[29,30] by addition of potassium peroxymonosulfate (monopersulfate triple salt or Oxone™) to an acetone/aq $NaHCO_3$ mixture. The dimethyldioxirane/acetone solution was distilled at reduced pressure (~150 Torr) and kept dry over $MgSO_4$ at –20 °C. The dioxirane concentration (70–90 mM) was determined by addition to an aliquot of aq KI and AcOH and titration of the iodine formed with $Na_2S_2O_3$ soln.

A solution of 3*H*-1,2-benzodithiol-3-one **(1)** (600 mg, 3.56 mmol) in acetone (HPLC grade, 15 mL) was added, dropwise over 20 min to 74 mM dimethyldioxirane in acetone (200 mL, 14.8 mmol) at r.t. The mixture was magnetically stirred at r.t. and its progress was monitored by TLC and GC. The conversion of 3*H*-1,2-benzodithiol-3-one **(1)** into 3*H*-1,2-benzodithiol-3-one 1,1-dioxide **(3)** was quantitative in 2–4 h, as checked by comparison with a commercial sample. The solvent was eliminated under reduced pressure, and the product was dried by coevaporation with anhyd MeCN. The crude product (slightly yellow, mp 96–97 °C) was dissolved in CH_2Cl_2 and filtered, and solid **3** was obtained by the addition of hexanes. Elimination of the solvent under reduced pressure afforded the target product **3** as a white crystalline solid; mp 98.5–99.5 °C (lit.[20] mp 102.5–103 °C, lit.[21] mp 98–99 °C). TLC ($CHCl_3$): R_f = 0.77.

IR ($CHCl_3$): ν = 898, 1159, 1334, 1720 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.88 (t, 1H), 7.99 (t, 1H), 8.05 (d, 1H), 8.13 (d, 1H).

^{13}C NMR ($CDCl_3$, 75 MHz): δ = 121.8 (CH), 125.5 (CH), 130.0 (Cq.), 134.3 (CH), 136.3 (CH), 148.4 (Cq), 182.9 (C=O).

EI-MS: *m/z* (%) = 69 (28), 76 (73), 96 (8), 104 (33), 108 (40), 136 (100), 200 (M) (29), 201 (M + 1) (3), 202 (M + 2) (39).

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