

# Synthesis and Antifungal Activity of Novel Bis(dithiocarbamate) Derivatives of Glycerol

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Three bis(dithiocarbamate) derivatives of glycerol were obtained in 30–95% yields by reaction of a dithiocarbamic acid salt with 1,3-dichloro-1,3-dideoxyglycerol. The *in vitro* antifungal activity of the new compounds was evaluated against *Alternaria brassicae*, *Pseudocercospora herpotrichoides*, *Septoria nodorum*, and *Phytophthora cinnamomi*. Some of the compounds displayed greater activity than commercial fungicides. Bis(*N,N*-diethylthiocarbamoyl)-1,3-dideoxyglycerol (**4**) was found to maintain its pesticidal activity up to 24 days in open field tests against *A. brassicae*.

**Keywords:** Fungicides; bis(1,3-dithiocarbamoyl)-1,3-dideoxyglycerol; *in vitro* antifungal activity; *in vivo* antifungal activity; open field test

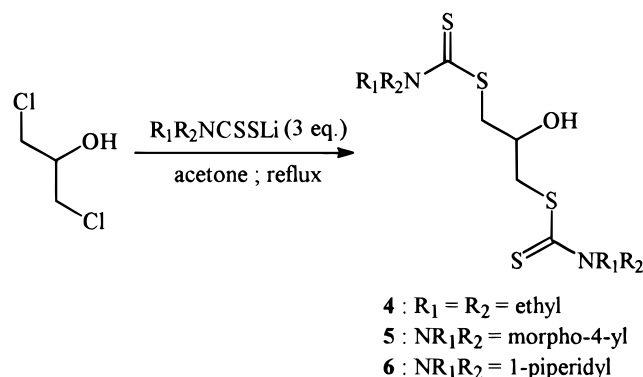
Dithiocarbamates are important as all-purpose fungicides used for the treatment of soil and seed and as foliar sprays for the control of a large variety of diseases affecting several types of crops. They are commonly separated into two classes: (i) dialkyldithiocarbamates including thiurame sulfide such as Ziram (zinc dimethyldithiocarbamate); (ii) alkylene bis(dithiocarbamates) and their oxidation products, such as Nabam (disodium ethylenebis(dithiocarbamate)) (Thorn et al., 1962). A problem associated with such salts is the possibility of their containing or releasing trace toxic elements or compounds (Woodrow et al., 1995; Buzasi-Gyorfy, 1992). Also, it is possible that such compounds may not be used to their full advantage due to the problems of transport in the plant. Therefore, we have investigated an alternative approach involving bis(dithiocarbamic esters) of glycerol which are not associated with metal ions and can act as propesticides.

The derivatization of known pesticides to produce novel propesticides having, for example, greater residual effectiveness, continues to be a fruitful area of development (Fahmy et al., 1981; Mallipudi et al., 1994). Another strategy has been to derivatize carbamates with molecules such as carbohydrates, amino acids, plant hormones, and organic acids. These molecules have relatively high polarity (Ziegler et al., 1975) and are phloem mobile. Such derivatives have been shown to increase plant systemic activity (Jojima et al., 1983). By a similar approach, we have synthesized a series of novel dithiocarbamic esters possessing the hydrophilic moiety glycerol and two substituted dithiocarbamoyl groups following Scheme 1.

## MATERIALS AND METHODS

**General.** Technical grade of Carbendazime (**I**), Maneb (**II**), and 1,3-dichloro-1,3-dideoxyglycerol obtained from a commercial source (Aldrich) were used as starting material after purification by recrystallization from appropriate solvents or by distillation. Melting points were measured with an electrothermal melting point apparatus and were uncorrected. <sup>1</sup>H (at 300.13 MHz) and <sup>13</sup>C (at 75.47 MHz) NMR spectra were recorded on a Bruker AM 300 spectrometer. Deuteriochloroform (99.8% atom enriched, Aldrich) was used as NMR solvent

**Scheme 1**



throughout unless otherwise stated. <sup>1</sup>H and <sup>13</sup>C NMR chemical shifts were reported in  $\delta$  values based on the internal reference tetramethylsilane. Column chromatography was carried out with a Matrex silica gel 60 Å (70–230 mesh, Merck).

**Synthesis of *N,N*-Diethylthiocarbamic Acid Lithium Salt (**1**).** The synthesis of **1** was accomplished by adding dropwise CS<sub>2</sub> (10.6 g, 0.14 mol) to a solution of *N,N*-diethylamine (10.0 g, 0.14 mol) in acetone (112 mL) at 8 °C. The mixture was stirred for 10 min, and LiOH·H<sub>2</sub>O (5.9 g, 0.14 mol) was added. Stirring was continued to ensure complete salt formation (15 min). The solution was filtered to remove solids. The organic filtrate was concentrated under reduced pressure. The crude product was recrystallized from dioxane to yield 18.2 g (86% yield) of **1**. The structure was confirmed by spectral analyses.

**Synthesis of Morpho-4-yl- (**2**) and 1-Piperidyl- (**3**) Dithiocarbamic Acid Lithium Salts.** The above method applied to morpholine and piperidine yielded the corresponding morpho-4-yl- (**2**) and 1-piperidylthiocarbamic lithium salt (**3**) in 97 and 96% yield, respectively.

**Synthesis of Bis-1,3-*S*-(*N,N*-diethylthiocarbamoyl)-1,3-dideoxyglycerol (**4**).** The synthesis of **4** was accomplished as shown in Scheme 1 adding dropwise **1** (3.6 g, 23.2 mmol) to a stirred solution of 1,3-dichloro-1,3-dideoxyglycerol (1.0 g, 7.8 mmol) in 43 mL of acetone. Upon completion of the addition, the reaction mixture was stirred at 56 °C for 1 h. The mixture was filtered and the solvent removed by distillation under reduced pressure to yield a viscous oil. This oily residue was treated with ether–hexane (1:1) and washed twice with water and dried over anhydrous sodium sulfate.

**Table 1. Physicochemical Data for the Bis(dithiocarbamates) 4–6**

compd	MW (g·mol <sup>-1</sup> )	mp (°C)	NMR data
<b>4</b>	354.62	syrupe liquid	<sup>1</sup> H δ 3.58 (dd 2H, <i>J</i> <sub>1,1'</sub> <i>J</i> <sub>3,3'</sub> 14.2 Hz, H-1-3); 3.45 (dd 2H, <i>J</i> <sub>1,2</sub> <i>J</i> <sub>2,3</sub> 4.5 Hz, H-1'-3'); 4.07 (m 1H, <i>J</i> <sub>1,2</sub> <i>J</i> <sub>2,3'</sub> 6.9 Hz, H-2); 3.86 (q 4H, <i>J</i> <sub>CH<sub>2</sub>,CH<sub>3</sub></sub> 7.1 Hz, NCH <sub>2</sub> ); 3.66 (q 4H, <i>J</i> <sub>CH<sub>2</sub>,CH<sub>3</sub></sub> 7.1 Hz, NCH <sub>2</sub> ); 1.17 (t 6H, CH <sub>3</sub> ); 1.12 (t 6H, CH <sub>3</sub> ); <sup>13</sup> C δ 42.4 (2C C1-3); 70.2 (1C C2); 195.5 (2CCS); 49.9 (2C NCH <sub>2</sub> ); 47.0 (2C NCH <sub>2</sub> ); 12.5 (2C CH <sub>3</sub> ); 11.5 (2C CH <sub>3</sub> )
<b>5</b>	382.59	136–138	<sup>1</sup> H δ 3.79 (dd 2H, <i>J</i> <sub>1,1'</sub> <i>J</i> <sub>3,3'</sub> 14.2 Hz, H-1-3); 3.57 (dd 2H, <i>J</i> <sub>1,2</sub> <i>J</i> <sub>2,3</sub> 4.7 Hz, H-1'-3'); 4.21 (m 1H, <i>J</i> <sub>1,2</sub> <i>J</i> <sub>2,3'</sub> 7.2 Hz, H-2); 4.29 (4H, NCH <sub>2</sub> ); 3.97 (4H, NCH <sub>2</sub> ); 3.73 (4H, CH <sub>2</sub> p); <sup>13</sup> C δ 42.2 (2C C1-3); 70.0 (1C C2); 197.5 (2C CS); 51.0 (4C NCH <sub>2</sub> ); 66.2 (4C OCH <sub>2</sub> )
<b>6</b>	378.64	122–124	<sup>1</sup> H δ 3.58 (dd 2H, <i>J</i> <sub>1,1'</sub> <i>J</i> <sub>3,3'</sub> 14.1 Hz, H-1-3); 3.43 (dd 2H, <i>J</i> <sub>1,2</sub> <i>J</i> <sub>2,3</sub> 4.5 Hz, H-1'-3'); 4.06 (m 1H, <i>J</i> <sub>1,2</sub> <i>J</i> <sub>2,3'</sub> 6.9 Hz, H-2); 4.12 (4H, NCH <sub>2</sub> ); 3.78 (4H, NCH <sub>2</sub> ); 1.55 (12H, CH <sub>2</sub> ); <sup>13</sup> C δ 42.4 (2C C1-3); 70.0 (1C C2); 195.3 (2C CS); 53.3 (2C NCH <sub>2</sub> ); 51.5 (2C NCH <sub>2</sub> ); 25.6 (2C CH <sub>2</sub> m); 24.1 (4C CH <sub>2</sub> p)

**Table 2. Percent Growth Inhibition of *A. brassicae* Caused by the Bis(dithiocarbamates) 4–6**

compd	concn (ppm)	days			
		4	7	14	21
<b>4</b>	50	58	58	54	37
	20	58	45	32	8
	2	35	6	4	0
<b>5</b>	50	49	29	28	5
	20	53	55	37	16
	2	35	16	15	0
<b>6</b>	50	49	42	34	8
	20	51	29	23	0
	2	25	13	6	0
<b>I</b>	50	38	23	23	0

The crude product was purified by column chromatography on silica gel eluted with hexane–acetone (85:15) to give 2.6 g (95%) of **4** (physicochemical data are reported in Table 1).

**Synthesis of Bis-1,3-*S*(morpho-4-ylidithiocarbamoyl)-1,3-dideoxyglycerol (5) and Bis-1,3-*S*(1-piperidylidithiocarbamoyl)-1,3-dideoxyglycerol (6).** The above method applied to lithium salts **2** and **3** yielded the corresponding bis-[1,3-*S*(morpho-4-ylidithiocarbamoyl)]- (**5**) and bis[1,3-*S*(1-piperidylidithiocarbamoyl)]-1,3-dideoxyglycerol (**6**) in 42 and 30% yield, respectively (corresponding physicochemical are reported in Table 1).

**Stock Cultures.** The fungi *Alternaria brassicae*, *Pseudocercospora herpotrichoides*, *Septoria nodorum*, and *Phytophthora cinnamomi* were used as test organisms. Cultures were obtained from the Institut National de la Recherche Agronomique (INRA Paris, France) and the Service Régional de la Protection des Végétaux de Picardie (SRPV Picardie, France) and maintained on potato dextrose agar (PDA) at 18 °C in a phytotron room. Fungal growth plugs were cut using a 4.5 cm cork borer and transferred from stock plates to fresh agar biweekly to maintain actively growing fungi.

**Radial Fungal Growth Assays.** *In vitro* assays were conducted on PDA at 18 °C in continuous light into phytotron room. Prior to the PDA being poured into plastic petri plates (100 × 15 mm), both fungicide solutions and solvent were added to the molten PDA (50 °C) and mixed on a rotary shaker for 2 min. Agar was poured into five control and five test plates for each of the conditions used. Bis(dithiocarbamates) **4–6** and commercial fungicides **I** and **II** were tested against all four fungi at concentrations of 50, 20, and 2 ppm in dimethyl sulfoxide (DMSO). **I**, usually associating with *Fluizilazole* Such Punch (**III**) for treatment of *Alternaria* and *Cercospora* diseases (Index Phytosanitaire, 1993), was used for *in vitro* carbamic reference against *A. brassicae* and *P. herpotrichoides*. **II**, known for fungicide activity against *Septoria* and *Phytophthora* diseases, was selected for reference with *S. nodorum* and *P. cinnamomi*. Small samples of each fungi (30 mm diameter) were taken from the outer margin of fresh stock culture plates and transferred to the center of each medium. Measurements were taken three times daily over a period of 4–21 days, noting the distance from the edge of the fungal plug to the edge of the actively growing fungus. Measurements ceased when fungal growth reached the edge of the DMSO control plates.

**In Vivo Assay in an Open Field.** Compound **4** was tested at concentration of 8.46 mol·ha<sup>-1</sup>, which is the concentration chosen for preliminary investigations. The results from this

study were compared with those obtained from a parallel trial in which **III** (100 g·ha<sup>-1</sup>) and Kidan (Iprodione) (**IV**) (500 g·ha<sup>-1</sup>) were used in commercially available formulation. **III** and **IV** were imposed as national homologation references for fungicides against *A. brassicae* of colza (Regnault, 1983; Bourdin and al., 1995). Different parcels of colza (45 m<sup>2</sup>) in an open field were treated with four repetitions of **4**, **III**, and **IV**, respectively. After 3 days, all the treated parcels and the four control parcels were artificially contaminated with *A. brassicae*. Measurements were taken 24 days after the treatment, noting for each condition the number of spots per silique per parcel over a total of 50 siliques.

## RESULTS AND DISCUSSION

In unpublished work, we demonstrated that 1-*S*(*N,N*-diethylidithiocarbamoyl)-1-deoxy-D,L-glycerol and other dithiocarbamic ester derivatives of itols and carbohydrates showed modest inhibition of growth of *A. brassicae* at different concentrations. The synthesis of glycerol derivatives permitted the introduction of more than one dithiocarbamic ester into a molecule. It was envisaged that a multiple graft of dithiocarbamoyl groups on a low molecular weight polyol leads to an enhanced activity. Thus dithiocarbamic salts were converted into bis(dithiocarbamic esters) of glycerol following Scheme 1, using very mild conditions.

The growth inhibitory effect of **4–6** derivatives was tested *in vitro* on PDA plates using DMSO as solvent. This solvent was used since the bis(dithiocarbamates) **4–6** are only weakly soluble in most other common solvents and it is well established that DMSO can be used in pesticide bioassays (Stratton, 1985). Commercial dithiocarbamic compounds were used to inhibit the growth of a number of fungal species. Four species were chosen for this study on the basis of the potential economical advantage of successful treatments of crops such as colza, corn, and potato. Antifungal activity of **4–6** was compared to that of the commercial **I** and **II** products. All inhibition data were normalized as percentage inhibition compared to the control plates using only DMSO. The three bis(dithiocarbamates) **4–6** did not cause statistically significant growth inhibition of *P. herpotrichoides* and *P. cinnamomi* at the three concentrations tested (nontoxic at 2 ppm; growth inhibition < 20% at 50 ppm). We also examined the effect of **4–6** compared to **I** for *A. brassicae* (Table 2) and **II** for *S. nodorum* (Table 3). The results noted in Table 2 showed that **4–6**, in concentrations of 50 and 20 ppm, were more antifungal than the commercial dithiocarbamate **I** at 50 ppm. The results described in Table 3 show that **4–6** had good antifungal activity against *S. nodorum* at 50, 20, and 2 ppm. At the same concentration range, **4–6** showed higher activity than commercial dithiocarbamate **II** at 50 ppm during the 21 day observation.

Compound **4** was selected for an *in vivo* assay in an open field of colza contaminated by *A. brassicae*. This

**Table 3. Percent Growth Inhibition of *S. nodorum* Caused by the Bis(dithiocarbamates) 4–6**

compd	concn (ppm)	days			
		4	7	14	21
<b>4</b>	50	55	57	67	62
	20	55	58	66	62
	2	50	58	58	53
<b>5</b>	50	50	59	62	61
	20	50	56	64	60
	2	50	63	64	58
<b>6</b>	50	27	42	42	29
	20	29	35	43	25
	2	29	32	50	45
<b>II</b>	50	36	29	23	5

bioassay was also conducted with the two commercial fungicides **III** and **IV**. The trial showed that **4** maintained its antifungal activity at level of 30% contaminated surface reduction, using a nonoptimized formulation. This result is lower than those obtained for **III** (62%) and **IV** (57%); however, these were measured using an optimized formulation.

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