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THE FUNGICIDAL BENZOTHIAZOLE METHOXYACRYLATES: SYNTHESIS, CONFORMATIONAL ANALYSIS AND FUNGICIDAL ACTIVITY[#]

Choon Sup Ra,[1*] Bon Young Jung,[2] and Gyoosoon Park[3*]

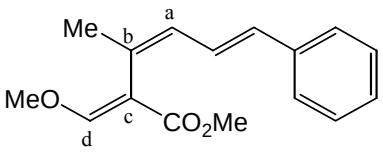
[1]Department of Chemistry and Institute of Natural Sciences, Yeungnam University, Gyongsan 712-749, Korea email: csra@yu.ac.kr

[2]LG Chemical Ltd./Research Park, Yusung-gu, Daejon 305-380, Korea

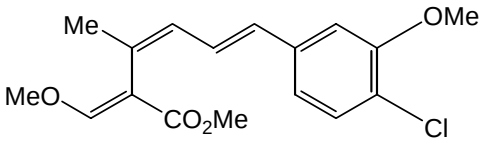
[3]Department of Chemistry, Kookmin University, Seoul 136-702, Korea

Abstract – Synthesis of a series of novel benzothiazole methoxyacrylate compounds (**3-4**) based on the antifungal natural product, Strobilurin A is described. Fungicidal activities of **3-4** against six representative fungal diseases and their comparative conformational analyses *vs* Strobilurin A are accounted.

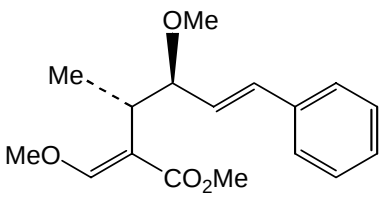
Strobilurins (**1**) and oudemansins (**2**) are antifungal metabolites which have been produced by various fungi such as *Strobilurus* and *Oudenmansiella* (Figure1).[1]



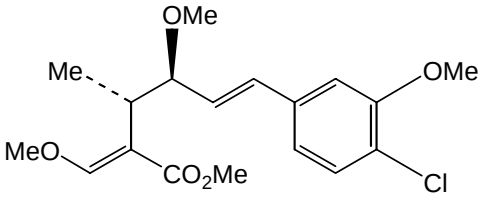
Strobilurin A, **1a**



Strobilurin B, **1b**



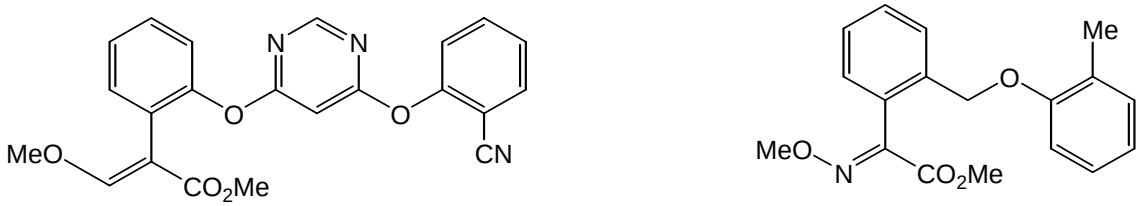
Oudemansin A, **2a**



Oudemansin B, **2b**

Figiure 1. Natural fungicidal MOA compounds: strobilurins and oudemansins.

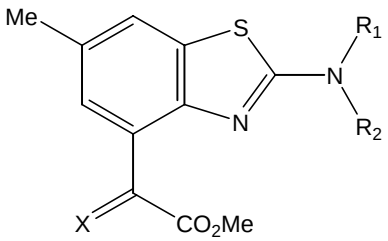
They show a potent fungicidal activity with a broad spectrum and inhibit mitochondrial respiration by blocking electron transport at the cytochrome bc_1 complex.[2] The following efforts by major industrial R & D groups to develop more active synthetic analogs have led to a new class of fungicides containing methyl β-methoxyacrylate (MOA) group.[3] Azoxystrobin by Zeneca Agrochemicals[3b] and Kresoxim-methyl by BASF[3c] are the first two MOA compounds recently introduced to the market (Figure 2).



Azoxystrobin (Zeneca) *Kresoxim-methyl* (BASF)

Figure 2. Commercial fungicides developed from natural leads: Azoxystrobin and Kresoxim-methyl.

The striking structural feature observed in the conformational analysis of the bioactive natural compound (**1a**) is the orthogonal geometry of two planar moieties: the phenylpentadienyl group and methyl β-methoxyacrylate function.[4] We previously examined the relationship between the calculated geometry of two moieties in **1a** and its several synthetic and their fungicidal activities. Energy profile to torsional angles (C_a-C_b-C_c-C_d, degree shown in compound (**1a**, Figure1.) of two moieties in highly active compounds was found to have a 'U' shape: the rotation of two planar moieties is quite restricted for a highly active compound.[5] The same structural feature has been observed in novel benzothiazole MOA compounds (**3-4**) we have synthesized. Here we describe their structural features, synthetic methods and their fungicidal activity.

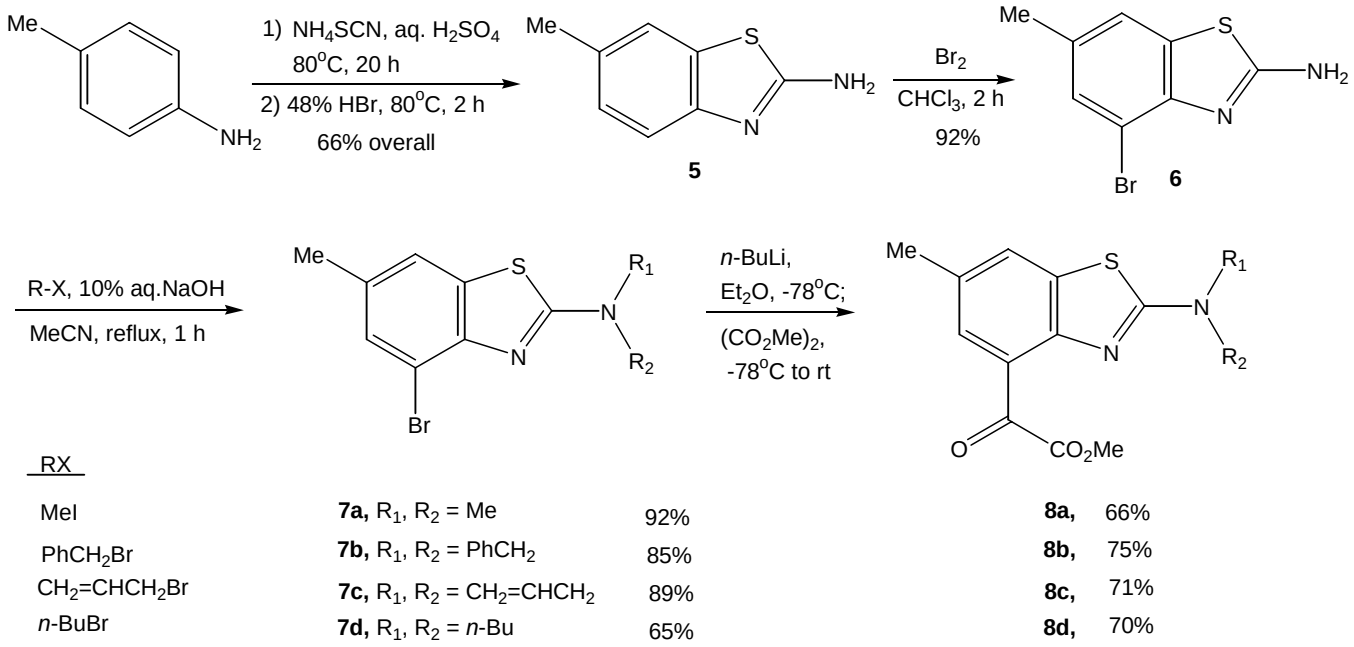


X = CHOMe, (E)/(Z)-**3**
X = NOMe, (E)/(Z)-**4**

Synthesis.

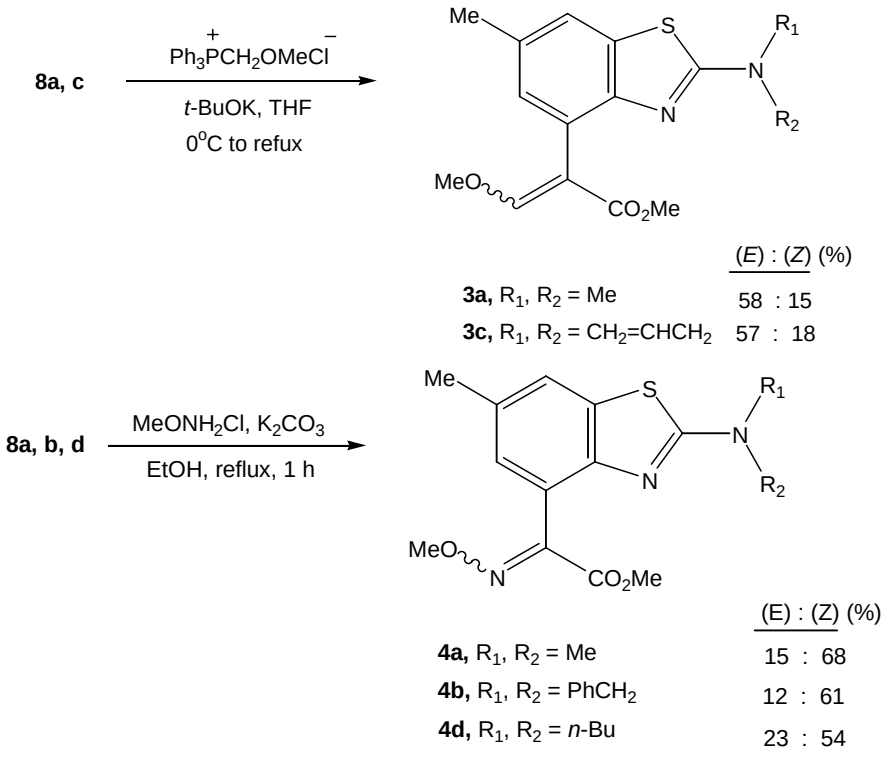
Synthetic process began with transformation of 4-toluidine into 2-amino-6-methylbenzothiazole by known procedure.[6] Treatment of 2-amino-6-methylbenzothiazole (**5**) with bromine gave

2-amino-4-bromo-6-methylbenzothiazole (**6**). Dialkylation of **6** with alkyl halides under a basic condition afforded *N*,*N*-disubstituted products (**7**). Treatment of lithium intermediates generated *via* a lithium-halogen exchange of **7** with dimethyl oxalate afforded keto esters (**8**) (Scheme 1).



Scheme 1

When the keto esters (**8**) were allowed to react with methoxymethyltriphenylphosphnium chloride in the presence of potassium *t*-butoxide, the desired benzothiazole MOA compounds (**3**) were obtained as two isomeric mixtures. The configurational assignment of products (**3**) can be made by comparing the chemical shifts of two distinct olefinic protons by the established method.[7] It predicts that the *cis*-protons vicinal to a carboxylic ester group in the methoxyacrylate moieties ((*E*)-isomers) resonate at a lower field than the *trans*-protons.[3a,4,8] Treatment of **8** with *O*-methylhydroxylamine afforded the corresponding *O*-methyloximes (**4**) as a mixture of two geometric isomers (Scheme 2). Assignment of the stereochemistry of each oxime was based on the previous industrial work. Zeneca chemists examined the *O*-methyloxime products from various α-keto esters containing hydrophobic aromatic systems to find out the more polar isomers on silica gel invariably correspond to (*E*)-isomers by X-Ray structural analysis.[9]



Scheme 2

Fungicidal acitivity and conformation analysis using *ab initio* method.

The fungicidal activity of benzothiazole MOA compounds was examined against the following six different diseases (*in vivo* preventative effect at 250 ppm): rice blast, RCB (*Pyricularia oryzae*); rice sheath blight, RSB (*Rhizoctonia solani*); cucumber gray mold, CGM (*Botrytis cineria*); tomato late blight, TLB (*Phytophothora infestans*); wheat leaf rust, WLR (*Puccinia recondita*); barley powdery mildew, BPM (*Erysiphe graminis f. sp. Hordei*) and they are given in Table 1. Most compounds maintain a high level of preventative activity against WLR and BPM.[10] As mentioned earlier, in (*E*)-isomers of **3** and **4** two planar moieties: the phenylpentadienyl group and methyl β-methoxyacrylate function obviously adopt the orthogonal geometry, which deems to be essential for the high bioactivity of these compounds.

Table 1. Fungicidal Activity[a] of Benzothiazole Compounds (**3-4**).

Compound	(*E*)/(*Z*)	RCB	RSB	CGM	TLB	WLR	BPM
3a	(*E*)	B	C	C	C	A	A
	(*Z*)	C	C	C	C	A	B
3c	(*E*)	B	C	C	C	A	B
	(*Z*)	C	C	C	C	A	B

4a	(*E*)	B	C	C	C	A	B
	(*Z*)	B	C	C	C	B	B
4b	(*E*)	B	C	C	B	B	A
	(*Z*)	B	C	C	B	B	A
4d	(*E*)	B	C	C	B	A	A
	(*Z*)	C	C	B	C	A	B

[a] Activity is expressed on the scale: A = 90-100%, B = 60-89%, C = 0-59% preventive effect.

The structural features of **3a** and **4a** are compared with Strobilurin A (**1a**). We investigated relative energies of these molecules varying the torsion angle between two moieties (phenylpentadiene / β-methoxyacrylate for **1a**, benzothiazole / β-methoxyacrylate for **3a** and benzothiazole / *O*-methyloxime for **4a**). All calculations are performed by HF/6-31G* method using Gaussian 98 program.[11] Molecules are fully optimized except frozen torsion angles (C_a-C_b-C_c-C_d (or N_d)) varying with 0^o to 180^o. The energy profiles of the molecules are shown in Figure 3. The energy profiles of **3a/4a-(*E*)** are found to have 'U' shapes, which are similar to a bioactive natural compound (**1a**).[5] The torsional energies for **3a/4a-(*E*)** are predicted to be 15-16 kcal/mol at 0^o and less than 1 kcal/mol between 60^o and 120^o. According to the optimized structures at HF/6-31G* for the lowest energy conformations, the torsion angles (<a-b-c-d) are shown by 78.13^o, 115.34^o, and 124.79^o for **1a-*scis***, **3a-(*E*)-*scis***, and **4a-(*E*)-*strans***, respectively (Figure 4).

Meanwhile, the shapes of energy profiles in **3a/4a-(*Z*)** are away from the 'U' shape, which can be ascribed to their geometric nature of the (*Z*)-isomer. Based upon the bioactivities being from the orthogonal geometry of two moieties, it would be expected that a high fungicidal activity is shown in **3a/4a-(*E*)** like Strobilurin A (**1a**) and a weak activity in their (*Z*)-isomers. The (*Z*)-isomer in target site in fungi, although it can not be verified, may be prone to have the optimum structure required for a high activity. The torsion angles (<a-b-c-d) in the lowest energy conformations are shown by 126.58^o and 146.49^o for **3a-(*Z*)-*scis*** and **4a-(*Z*)-*strans***, respectively. The torsion angles of these compounds are not much different from those of (*E*)-isomers. Therefore, these compounds may easily adopt the desired structure having conformations similar to **3a/4a-(*E*)** or Strobilurin A (**1a**) to give a bioactivity when located in the target organism.

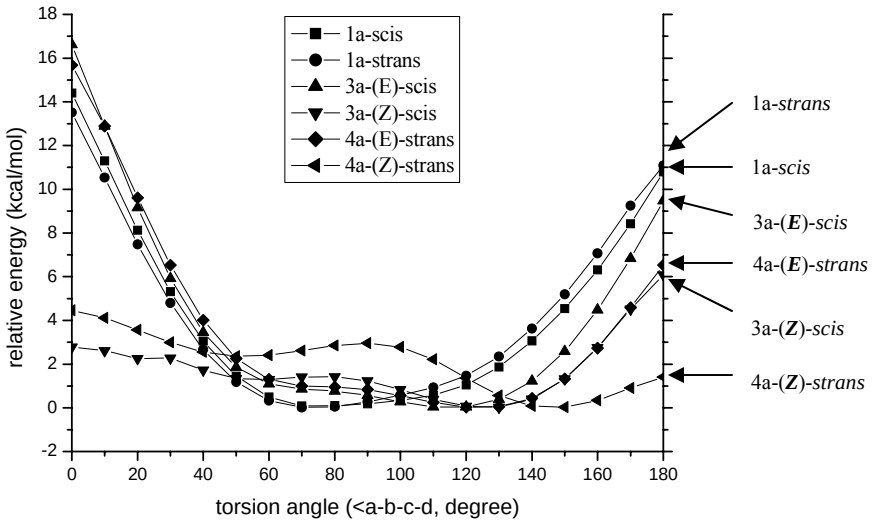
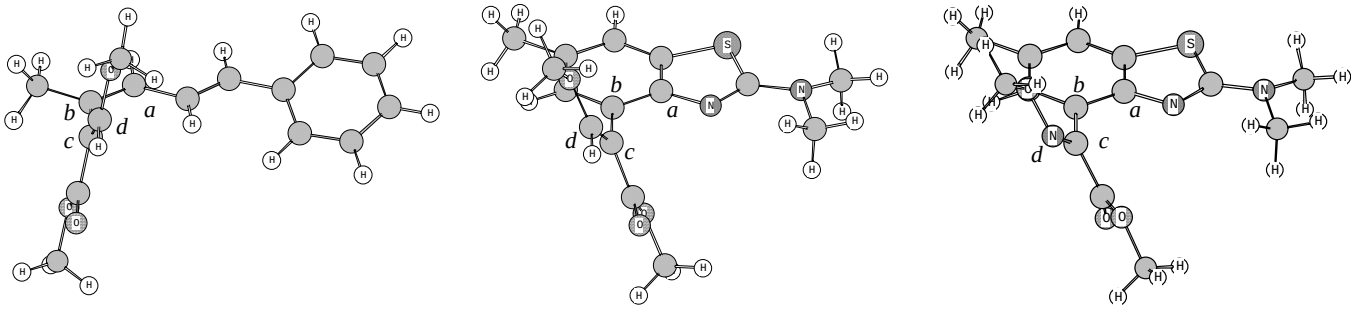


Figure 3. Energy profile to torsion angles (<a-b-c-d, degree) for **1a-*scis***, **1a-*strans***, **3a-(*E*)-*scis***, **3a-(*Z*)-*scis***, **4a-(*E*)-*strans*** and **4a-(*Z*)-*strans*** at HF/6-31G(d) levels. The relative energies (kcal/mol) are obtained from the lowest energies of the corresponding compounds.



(<a-b-c-d = 78.13°) (<a-b-c-d = 115.34°) (<a-b-c-d = 124.79°)

1a-*scis* **3a-(E)-*scis*** **4a-(E)-*strans***

Figure 4. The optimized structures for the lowest energy conformations of **1a-*scis***, **3a-(*E*)-*scis*** and **4a-(*E*)-*strans*** at HF/6-31G(d) levels.

EXPERIMENTAL

^{1}H NMR spectra were recorded in deuteriochloroform on a XEOL-270 spectrometer. The chemical shifts are reported in ppm (δ value) downfield from tetramethylsilane (TMS), which was used as an internal standard. Tetrahydrofuran and diethylether were distilled from sodium benzophenone ketyl prior to use. Dichloromethane and diisopropylamine were distilled from calcium hydride. All other solvents and reagents were used without furthur purification. Flash column chromatography was carried out on Merck silica gel 60 (70-230 mesh). TLC was carried out on silica gel GF_{254} precoated plates.

2-Amino-6-methylbenzothiazole (5). The stirred mixture of 4-toluidine (10.7 g, 99.9 mol) and ammonium thiocyanate (8.40 g, 110 mmol) in 27% aqueous sulfuric acid (20 mL) was heated at 85°C for 20 h, cooled, and treated with toluene (50 mL). After being heated at reflux for 1 h and the cooled mixture was neutralized to pH 7.5 to 8 by adding slowly 28% aqueous ammonium hydroxide. The resulting precipitate was filtered, washed sequentially with water and toluene, and dried under reduced pressure. 4-Methylphenylthiourea thus obtained was as a white solid (13.0 g, 78.5 %). To a hot (80°C) solution of 4-methyphenylthourea (8.30 g, 50.5 mol) in concentrated sulfuric acid (15 mL) was added 48% hydrobromic acid (0.5 g) slowly and heated at 80°C for 2 h. The reaction mixture was slowly introduced into cold water (100 mL) and the solution was then adjusted to pH 9 to 10 by adding 28% aqueous ammonium hydroxide. The whole mixture was stirred at 70°C for 1 h and cooled to rt. The product was extracted with dichloroethane (2 X 100 mL) and the combined organic layers were dried over anhydrous magnesium sulfate and concentrated to give **5** as a pale yellow solid (6.97 g, 84%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.40 (3H, s), 5.50 (2H, br s), 7.12 (1H, m), 7.38 (1H, d, J = 2.3 Hz), 7.43 (1H, d, J = 7.1 Hz).

2-Amino-4-bromo-6-methylbenzothiazole (6). To **5** (16.4 g, 99.9 mmol) in chloroform (500 mL) was added dropwise bromine (16.0 g, 100 mmol) in chloroform (15 mL) and the mixture was stirred at rt for 2 h. The reaction mixture was washed with 10% sodium hydroxide solution (100 mL) and then brine (100 mL) and the organic layer was dried over anhydrous magnesium sulfate and concentrated to give **6** as a pale yellow solid (22.4 g, 92%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.41 (3H, s), 5.94 (2H, br s), 7.31 (1H, s), 7.32 (1H, s).

2-(*N,N*'-Dimethyl)amino-4-bromo-6-methylbenzothiazole (7a). The stirred mixture of **6** (2.43 g, 10.0 mmol), sodium hydroxide (0.82 g, 20.5 mmol), and methyl iodide (2.88 g, 20.3 mmol) in acetonitrile (30 mL) was heated at reflux for 1 h. The reaction mixture was concentrated and the residue was partitioned between ether (2 X 100 mL) and water (50 mL). The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, and concentrated. The residual oil was purified by column chromatography on silica gel (elution with 10% ethyl acetate in hexane) furnished **7a** as a pale yellow solid (17.9 g, 66%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.35 (3H, s), 3.21 (6H, s), 7.30 (1H, s), 7.31 (1H, s).

Methyl [2-(*N,N*'-dimethyl)amino-6-methylbezothiazol-4-yl]oxoacetate (8a). A solution of **7a** (550 mg, 2.03 mmol) in ether (5 mL) was added dropwise to *n*-butyl lithium (1.26 mL as 2.5 M hexane) in ether (10 mL) at –78°C and the mixture was stirred for 30 min. Dimethyl oxalate (248 mg, 2.10 mmol) in ether (5 mL) was introduced dropwise and the temperature was slowly increased to rt during 1 h. The mixture was treated with saturated ammonium chloride solution and extracted with ether (3 X 15 mL). The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, concentrated, and subjected to column chromatography (elution with 20% ethyl acetate in hexane) to give **8a** as a pale yellow oil (435 mg, 77 %): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.41 (3H, s), 3.20 (6H, s), 3.96 (3H, s), 7.63 (1H, s), 7.74 (1H, s).

(*E*)- and (*Z*)-Methyl 2[2-(*N,N*'-dimethyl)amino-6-methylbezothiazol-4-yl]-3-methoxypropenoate ((*E*)-3a and (*Z*)-3a). To methoxymethyltriphenylphosphonium chloride (3.43 g, 10.0 mmol) in

tetrahydrofuran (30 mL) was added potassium *t*-butoxide (900 mg, 8.02 mmol) and the mixture was stirred at rt for 30 min before **8a** (1.4 g, 5.03 mmol) in tetrahydrofuran (10 mL) was introduced slowly. The reaction mixture was heated at reflux for 2 h and removed solvent under reduced pressure. The residue was partitioned between ethyl acetate (3 X 50 mL) and water (30 mL). The dried organic concentrate was subjected to column chromatography (elution with 25% ethyl acetate in hexane) to give two isomeric products as a colorless oil; a major product **(*E*)-3a** (894 mg, 58 %) and a minor product **(*Z*)-3a** (185 mg, 12 %). For **(*E*)-3a**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.37 (3H, s), 3.15 (6H, s), 3.68 (3H, s), 3.82 (3H, s), 7.05 (1H, s), 7.29 (1H, s), 7.52 (1H, s). For **(*Z*)-3a**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.38 (3H, s), 3.17 (6H, s), 3.71 (3H, s), 3.89 (3H, s), 6.88 (1H, s), 6.94 (1H, s), 7.30 (1H, s).

(*E*)- and (*Z*)-Methyl 2-[2-(*N,N'*-Diallylamino-6-methylbenzothiazol-4-yl)]-3-methoxypropenoate ((*Z*)-3c and (E)-3c). The same procedure as described above for the synthesis of **3a** was followed. For **(*E*)-3c** as a viscous oil (36% overall from **6**): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.37 (3H, s), 3.66 (3H, s), 3.84 (3H, s), 4.08 (4H, m), 5.22 (4H, m), 5.82 (2H, m), 7.01 (1H, s), 7.30 (1H, s), 7.52 (1H, s). For **(*Z*)-3c** as a viscous oil (11% overall from **6**): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.37 (3H, s), 3.71 (3H, s), 3.94 (3H, s), 6.88 (1H, s), 6.94 (1H, s), 7.30 (1H, s).

(*E*)- and (*Z*)-Methyl 2[2-(*N,N'*-Dimethyl)amino-6-methylbezothiazol-4-yl]-2-methoxyiminoacetate ((*Z*)-4a and (*E*)-4a). The stirred mixture of **8a** (280 mg, 1.01 mmol), methoxylamine hydrochloride (90 mg, 1.10 mmol), and potassium carbonate (80 mg, 0.58 mmol) in ethanol (1 mL) was heated at reflux for 1 h. After cooling, ethanol was evaporated under reduced pressure and the residue was partitioned between ethyl acetate (3 X 30 mL) and water (30 mL). The combined organic layers were dried over anhydrous magnesium sulfate and concentrated. The crude product was purified by column chromatography (elution with 30% ethyl acetate in hexane) to give two isomeric products. For a minor product **(*E*)-4a** as a viscous oil (32 mg, 15%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.39 (3H, s), 3.15 (6H, s), 3.92 (3H, s), 4.05 (3H, s), 7.43 (1H, s), 7.57 (1H, s). For a major product **(*Z*)-4a** as a viscous oil (211 mg, 68%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.40 (3H, s), 3.19 (6H, s), 3.88 (3H, s), 4.10 (3H, s), 7.42 (1H, s), 7.61 (1H, s).

(*E*)- and (*Z*)-Methyl 2[2-(*N,N'*-Dibenzyl)amino-6-methylbezothiazol-4-yl]-2-methoxyiminoacetate ((*Z*)-4b and (*E*)-4b). The same procedure as described above for the synthesis of **4a** was followed. For a minor product **(*E*)-4b** as a viscous oil (8% overall from **6**): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.34 (3H, s), 3.89 (3H, s), 4.06 (3H, s), 4.81 (4H, s), 7.30 (10H, m), 7.41 (1H, s), 7.58 (1H, s). For a major product **(*Z*)-4b** as a viscous oil (39% overall from **6**): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.36 (3H, s), 3.85 (3H, s), 4.08 (3H, s), 4.79 (4H, s), 7.30 (10H, m), 7.44 (1H, s), 7.60 (1H, s).

(*E*)- and (*Z*)-Methyl 2[2-(*N,N'*-Dibutyl)amino-6-methylbezothiazol-4-yl]-2-methoxyiminoacetate ((*Z*)-4d and (*E*)-4d). The same procedure as described above for the synthesis of **4a** was followed. For a minor product **(*E*)-4d** as a viscous oil (10% overall from **6**): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.95 (6H, t, J = 7.1 Hz), 1.51 (4H, m), 1.69 (4H, m), 2.35 (3H, s), 3.34 (4H, m), 3.69 (3H, s), 3.84 (3H, s), 7.01 (1H, s), 7.31 (1H, s).

For a major product **(*Z*)-4d** as a viscous oil (25% overall from **6**): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.96 (6H, t, J = 7.1 Hz), 1.50 (4H, m), 1.71 (4H, m), 2.33 (3H, s), 3.34 (4H, m), 3.67 (3H, s), 3.85 (3H, s), 6.95 (1H, s), 7.29 (1H, s).

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[#]Dedicated to Professor Leo A. Paquette on the occasion of his 70th birthday.

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