# Synthesis of Extended Chromophores Derived From 5,5-Dimethyloxyluciferin by Barton–Kellogg Methodology

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**Abstract:** An approach to the synthesis of modified methyl ether-protected dimethyloxyluciferin derivatives is presented, focusing on the enlargement of the π-system. This was achieved by introducing an alkene bridge at the 4-position of the thiazoline moiety to link the two chromophoric substructures. The resulting derivatives show strong absorbing properties over a wide range of the visible spectrum. Also, the fluorescence properties of these novel dimethyloxyluciferin derivatives are unique and might lead to new photophysical studies and bioanalytical applications.

**Key words:** chromophores, heterocycles, Barton–Kellogg reaction, fluorophores, luciferins

Bioluminescent molecules, especially those of the class derived from the firefly *Photinus pyralis* (firefly luciferins, known also as just 'luciferins'), are of great interest in relation to biochemical research[1] and to medical applications,[2] especially in the field of highly sensitive bioanalytics. A wide variety of well-studied derivatives have been prepared that contain the parent firefly luciferin moiety as a core.[3] McElroy and co-workers synthesized a series of several derivatives (Figure 1) with many structural variations, including D,L-homoluciferin (**1**), methylluciferin (**2**), 6′-aminoluciferin (**3**), and 5′-chloroluciferin (**4**) among others, and they tested them as inhibitors or substrates for firefly luciferase.[4]

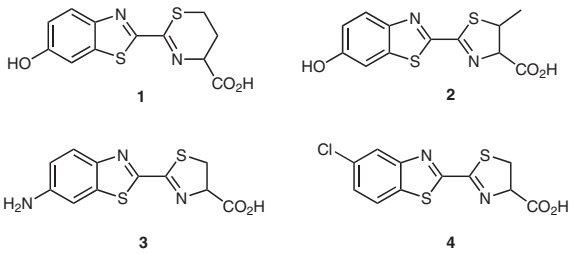
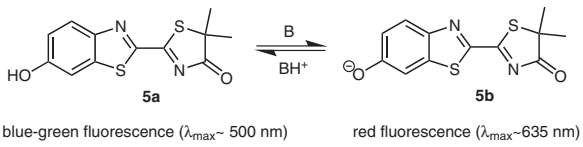



**Figure 1** Luciferin derivatives synthesized by McElroy and co-workers[4]

McCutcheon et al. prepared a series of benzoimidazole- and imidazole-containing luciferin analogues,[5a] and further research has been conducted by using naphthalene and quinoline to replace the benzothiazole moiety of luciferin.[5b] All those compounds show light emission with a broad range of quantum yields when incubated with firefly luciferase (EC 1.13.12.7). A different approach to modifying similar bioluminescent systems involves the mutation of luciferase enzymes from the firefly and related organisms.[6]

One oxyluciferin representative that has been well investigated is 2-(6-hydroxy-1,3-benzothiazol-2-yl)-5,5-dimethyl-1,3-thiazol-4-(5*H*)-one (**5a**; Scheme 1), which can act as an inhibitor of the luciferin–luciferase bioluminescent system.[7,8] More interestingly, this compound shows a bright greenish-yellow fluorescence in its neutral phenolic form **5a**, but when the phenolic moiety is deprotonated (phenolate **5b**), the chromophore emits light in the red region of the visible spectrum.[7] Phenol **5a** itself is a potent chromophore as a result of the partially double-bond character of the C2–C2′ bond connecting the two heterocyclic moieties; this leads to an almost planar structure for the molecule, permitting efficient light absorption.[9]




**Scheme 1** Phenol and phenolate form of dimethyloxyluciferin **5**

To the best of our knowledge, there are no reports of studies on structural modifications of dimethyloxyluciferin that have examined the effects of enlarging the conjugated π-system at the 4-position of the thiazoline moiety on the chromophoric and fluorophoric properties of the molecule. One report deals with the olefination of 5,5-dimethylluciferin but, unfortunately, no analytic data are given for any of the synthesized compounds.[10] We could, therefore, draw no correlations between our work and any work reported in the literature.

We have carried out some related research on 2-substituted oxazoline-4-thiones as precursors in a Barton–Kellogg olefination.[11] These potent fluorophoric materials were later used to determine the rate constants of the chemiexcitation step in the chemiluminescence of peroxyoxalate.[12]

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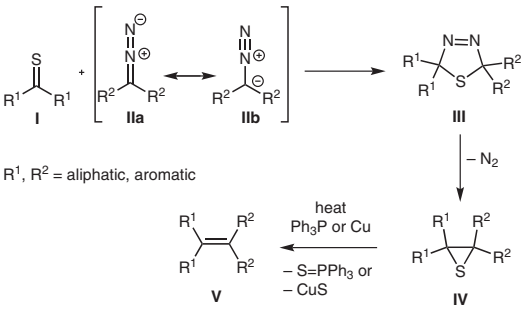
The thiazoline ring of 2-(6-methoxy-1,3-benzothiazol-2-yl)-5,5-dimethyl-1,3-thiazol-4(5*H*)-one (**6**), the methyl-protected derivative of **5a**, was the starting point for our investigations on the enlargement of the conjugated π-system. Various approaches for transforming the carbonyl group at the 4-position of the thiazoline ring were investigated. More-direct approaches using the McMurry, Knoevenagel, and Horner–Wadsworth–Emmons methodologies failed (Scheme 2). When the McMurry reaction was performed by using titanium tetrachloride and zinc powder as a reducing agent[13] and adamantan-2-one (**7**) as the ketone coupling partner, the reaction led to the isolation of symmetric adamantylideneadamantane as the only identifiable product in the reaction mixture. The desired product **8** (R = adamantylidene) was not obtained.

When we attempted a Horner–Wadsworth–Emmons olefination[14] with phosphonate **9** and lithium diisopropylamide as the base in tetrahydrofuran as solvent, monitoring by thin-layer chromatography (TLC) showed no consumption of the starting material; this was confirmed by the recovery of ether **6** and by NMR spectroscopic analysis. An attempted Knoevenagel condensation with diethyl malonate (**10**) in basic methanol gave similar results.

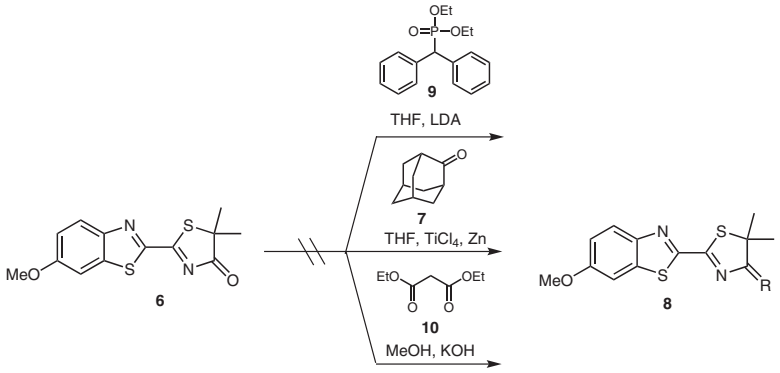
The low reactivity of the 4-thiazoline carbonyl group towards nucleophiles prompted us to use stronger nucleophiles, such as organomagnesium compounds.[15] Ether **6** did indeed react when the Grignard reagent isopropylmagnesium bromide (**11**) was added to a solution of the compound in tetrahydrofuran, but the nucleophilic Grignard species did not add at the expected 4-position of the thiazoline ring, but instead reacted at the 2-position to give a racemic mixture of thiazolidines **12** (Scheme 3). The formation of these unexpected structures was confirmed by spectroscopy and by x-ray crystallography.[15]

These results prompted us to consider a different olefination strategy. The need to transform the carbonyl group of ether **6** into a more reactive derivate led us to the Barton–Kellogg reaction.[16] This olefination method (Scheme 4) employs a thiocarbonyl compound **I**, that reacts by 1,3-dipolar cycloaddition with a diazo derivate **II** to form an unstable 1,3,4-thiadiazole **III**. After extrusion of nitrogen and the formation of a thiirane **IV**, the resulting intermediate can be desulfurized by using triphenylphosphine or copper powder to give the corresponding olefin **V** in high yield.[17]

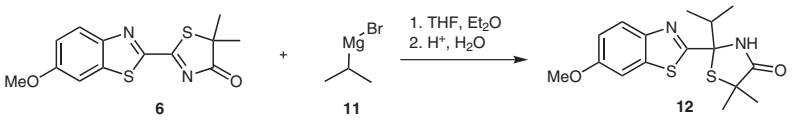



**Scheme 4** Barton–Kellogg methodology employing a thiocarbonyl compound **I** and a diazo compound **II** to obtain an olefin

The reaction of ether **6** with Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulfide] in refluxing xylene gave 2-(6-methoxy-1,3-benzothiazol-2-yl)-5,5-dimethyl-1,3-thiazole-4(5*H*)-thione (**13**) as a dark-yellow crystalline solid in yields of up to 65%. This transformation led to a strong downfield shift of approximately 41 ppm in the $^{13}C$ NMR signal of the ring carbon in the 4-position of the thiazoline. No rearrangement reaction of the type described by Koltai et. al for 2,5,5-triaryl-2-thiazolin-4-ones was observed.[18]

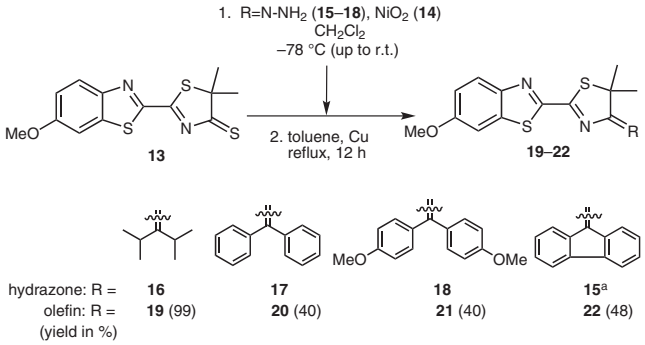



**Scheme 2** Various attempted approaches to olefination of methyl ether **6**




**Scheme 3** A Grignard reaction of methyl ether **6** leads to the formation of a racemic mixture of thiazolidines **12**

We then treated thione **13** with a series of diazo compounds generated in one of two ways (Scheme 5). The first method that we used was the oxidation of the corresponding hydrazone **16–18**. For this reaction, good results were obtained by treating the hydrazones with nickel peroxide (**14**) at low to ambient temperatures (–78 °C to r.t.) in an inert solvent.[19] 9-Diazo-9*H*-fluorene, however, could only be generated by using a variation of the Bamford–Stevens method, through treatment of the tosylhydrazone of 9*H*-fluoren-9-one **15** with aqueous potassium hydroxide in 1,4-dioxane.[20] All the diazo compounds reacted promptly with thione **13** with release of nitrogen. The resulting thiiranes were immediately desulfurized to form yellow- to red-colored olefins **19–22** in medium to excellent yields (Scheme 5).




**Scheme 5** Synthesis of olefin derivatives **19–22** from thione **13**. [a] For **15**, the tosylhydrazone was treated with aq KOH in 1,4-dioxane.

Olefins **19–22** showed some interesting features; for example, the benzothiazole and the thiazoline moieties are almost coplanar to one another. The connecting carbon atoms C1 and C2 are separated by 1.45 Å (Table 1), a value that is intermediate between that of a C–C single bond and that of a C=C double bond. The thiazoline ring shows a small distortion in its planar geometry caused by the $sp^3$-hybridized carbon and the bulky sulfur atom. The substituents on the newly generated olefin moiety do not show comparable behavior. The isopropyl groups of olefin **19** are oriented so that the hydrogen at the isopropyl carbon (C15) points toward the methyl groups of the thiazoline ring, thereby minimizing the repulsion between these bulky groups (Figure 2).

**Table 1** Structure Parameters for Luciferin Derivatives from X-ray Crystal Structure Analyses

| Compound | Bond length (Å) C1–C2 | C13–C14 | Torsion angle[a] (°) |
|---|---|---|---|
| **19** | 1.454(2) | 1.352(2) | –1.8(2) |
| **20** | 1.459(2) | 1.353(2) | –0.4(2) |
| **22** | 1.4465(19) | 1.372(2) | –4.5(2) |

[a] Torsion angle S2–C1–C2–N1.

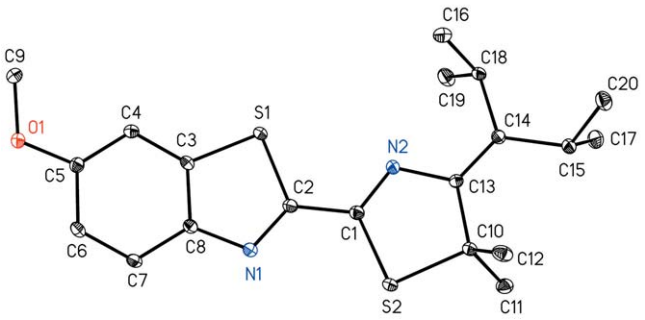



**Figure 2** Molecular structure of olefin derivative **19**. The ellipsoids represent a probability of 40%. Hydrogen atoms are omitted for the sake of clarity.

In olefin **20**, the two phenyl rings connected to the olefinic moiety are twisted out of the general plane of the molecule, blocking conjugation between these moieties (Figure 3). One of the phenyl rings is oriented almost parallel to the C10–C11–C12 plane, preventing closer contact with the methyl groups on the thiazoline ring. The other phenyl ring is twisted out of the olefin plane that is generated by the two $sp^2$ carbon atoms C13 and C14. The torsion angle C13–C14–C21–C26 of –38.9(2)° shows that the phenyl ring participates only slightly to the π-conjugation system of the molecule. Although the influence for the conjugation effects of the phenyl ring is weak, it cannot, however, be neglected.

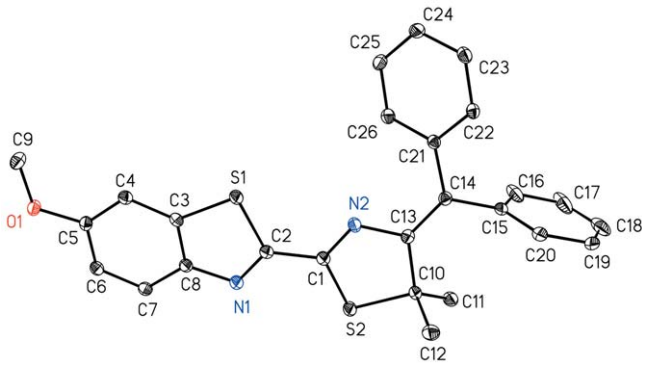



**Figure 3** Molecular structure of olefin **20**. The ellipsoids represent a probability of 40%. Hydrogen atoms are omitted for the sake of clarity.

Substitution at the 4-position, as in olefin **21**, produces significant changes in the UV/visible absorption and emission spectra. Unfortunately, the crystal structure of olefin **21** could not be determined and, therefore, a complete comparison of structures was not possible at this point.

Olefin **22**, however, occupied an exceptional position in this series. The whole molecule, with its large fluorenyl moiety, is almost completely planar (Figure 4). The fluorenyl moiety shows only a slight twist of –9.9(3)° in relation to the olefinic plain C10–C13–C14–C15. Despite this small deviation, the aromatic fluorenyl moiety was included in the conjugated π-system of the luciferin, result-



ing in a large bathochromic shift in its UV/visible absorption maximum in comparison to that of the starting luciferin **6**.

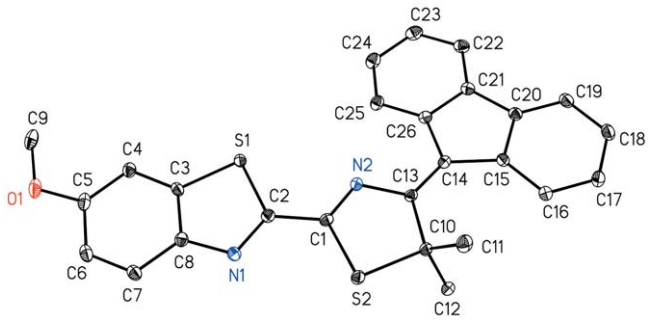



**Figure 4** Molecular structure of **22**. The ellipsoids represent a probability of 40%. Hydrogen atoms are omitted for the sake of clarity.

The UV/visible data for the derivatives showed some interesting properties (Figure 5 and Table 2). The UV/visible spectrum of olefin **19** in tetrahydrofuran contained a broad absorption plateau from 337 to 388 nm (29674 to 25773 $cm^{-1}$) with small maxima, possibly due to vibrational changes in the molecular geometry during the excitation process. In the plateau region, the absorption coefficient was very high (log ε > 4.0).

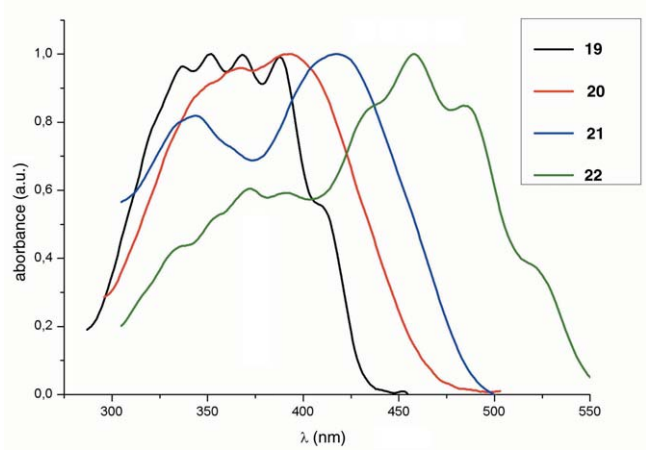



**Figure 5** Normalized UV/visible spectra of olefin derivatives **19**–**22** in tetrahydrofuran

**Table 2** Excitation and Emission Maxima with Stokes Shifts and Quantum Yields for Solutions of the Synthesized Olefins

| Compd | $\lambda_{max\ (ex)}$[a] (nm) | $\lambda_{max\ (em)}$[a] (nm) | Stokes shift ($cm^{-1}$) | Quantum yield[b] (%) |
|---|---|---|---|---|
| **19** | 337–388 | 466 | 4314 | 5.9 |
| **20** | 368–393 | 533 | 6683 | 3.8 |
| **21** | 344–417 | 548 | 5733 | 1.0 |
| **22** | 458 | 595 | 5027 | 0.02 |

[a] In THF.
[b] In EtOH.

Olefin **20** in tetrahydrofuran showed two absorption bands with maxima at 368 and 393 nm (27174 and 25445 $cm^{-1}$) that almost completely overlapped one another; the logarithmic absorption coefficients again exceeded 4. Olefin **21** in tetrahydrofuran also showed two absorption maxima, in this case at 344 and 417 nm (29070 and 23981 $cm^{-1}$); these bands did not show as strong an overlap as those of **20**. The methoxy groups affect the position of the absorption bands, even if the aromatic rings do not fully participate in the conjugated π-system, as shown in the crystal structure of **20**. The absorption maximum of **21** at 344 nm is hypsochromically shifted by 1896 $cm^{-1}$ and its maximum at 417 nm is bathochromically shifted by 1464 $cm^{-1}$ with respect to the maxima of **20**.

Olefin **22** shows the most-structured absorption bands of all the derivatives that we investigated. An absorption plateau reaching from 372 to 409 nm (26882 to 24450 $cm^{-1}$) was observed in tetrahydrofuran; furthermore, the absorption maximum of **22** in tetrahydrofuran occurred at 458 nm (21834 $cm^{-1}$), so that the compound showed an intense red coloration.

The fluorophoric properties of the olefins, measured in tetrahydrofuran, are shown in Figure 6 and Table 2. Olefin **19** showed a fluorescence maximum at 466 nm with a shoulder at 437 nm. Compared with **19**, olefins **20** and **21** showed bathochromically shifted emission maxima at 533 nm (18762 $cm^{-1}$) and 548 nm (18248 $cm^{-1}$), respectively. The fluorescence spectrum of **21** was more distinct than that of **20** in that it showed two shoulders at 516 nm and 598 nm. Compound **22** had the most-structured fluorescence spectrum. A small emission maximum was present at 555 nm, and the most bathochromically shifted maximum, in comparison with **19**, was found at 595 nm (16807 $cm^{-1}$). The compound also presented two shoulders at 529 nm and at 645 nm.

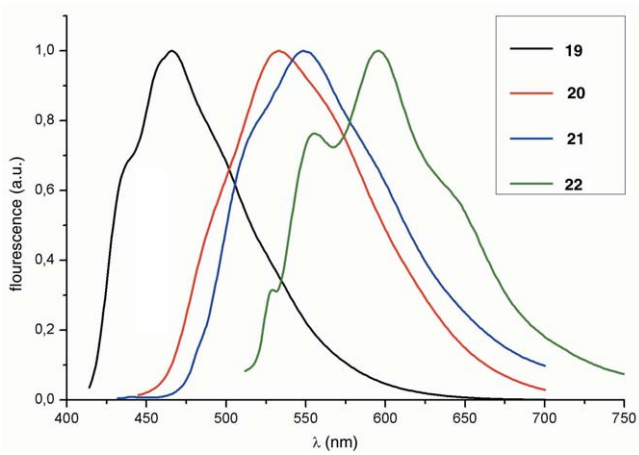
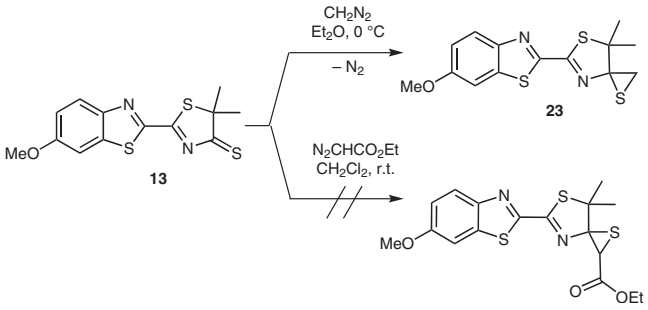



**Figure 6** Normalized fluorescence spectra of olefin derivatives **19**–**22** recorded in tetrahydrofuran

We also investigated the reactions of two other diazo compounds: diazomethane and ethyl diazoacetoacetate. These compounds did not show a comparable reactivity towards olefin formation (Scheme 6). Diazomethane re-

acted with thione **13** in diethyl ether at 0 °C with instantaneous visible extrusion of nitrogen, leading to an intensely red-colored solid that was not soluble in tetrahydrofuran, dimethyl sulfoxide, acetone, *N*,*N*-dimethylformamide, or chloroform. Investigation of the dried compound by elemental analysis suggested that the thiirane compound **23** was the main product; mass spectrometry did not lead to a conclusive result, and NMR studies could not be conducted because of the poor solubility of the compound. Further treatment of the product with copper powder in refluxing toluene resulted in complete degradation of the compound; therefore, no methylene derivative of the dimethyloxyluciferin series could be obtained or probed. In contrast to the above observations, no reaction of thione **13** with ethyl diazoacetoacetate in dichloromethane could be observed. TLC monitoring of the reaction mixture showed that both starting materials were present throughout, and no possible product was observed.




**Scheme 6** Attempted reactions of thione **13** with diazomethane or ethyl diazoacetoacetate

In conclusion, a synthetic methodology based on the Barton–Kellogg olefination (the reaction of a diazo compound with a thione), capable of forming very sterically hindered alkenes, allowed the preparation and isolation of four new luciferin derivatives. These compounds contain an alkene bridge at the 4-position of the thiazoline moiety of dimethyloxyluciferin, forming an expanded luciferin-like π-system that might be useful in color-modulated bioluminescence assays. The photophysical parameters of the four new substances in organic media showed a broad distribution of UV/visible absorption and emission maxima, permitting tuning of those characteristics. The fluorescence quantum yields are also reported. Trends in these parameters follow the electronic behavior of the extended π-systems, which show unique features that are correlated to the spatial arrangement of the two chromophoric systems involved. The applicability of those compounds in bioanalytical assays will be evaluated in future works.

All reagents were purchased from commercial sources. Nickel peroxide (**14**), 2-(6-methoxy-1,3-benzothiazol-2-yl)-5,5-dimethyl-1,3-thiazole-4(5*H*)-one (**6**), (1-isopropyl-2-methylpropylidene)hydrazine (**17**), [bis(4-methoxyphenyl)methylene]hydrazine (**18**), and 9-diazo-9*H*-fluorene were synthesized according to known literature procedures. Solvents were purified according to standard methods.[21] Spectroscopic-grade solvents were used in UV/vis and fluorescence measurements. The progress of reactions was monitored by TLC (0.2 mm Merck silica gel plates 60 $F_{254}$). $^1$H and $^{13}$C NMR spectra (in $CDCl_3$ or DMSO-$d_6$) were recorded on Bruker Avance 250 or 400 spectrometers. IR spectra were recorded by using an IR Affinity-1 (Shimadzu). Mass spectra were recorded on a VG Trio-2000 quadrupole mass spectrometer. Elemental analyses (CHNOS) were performed by using Vario EL III equipment. Melting points (uncorrected) were measured on a Büchi B-545 apparatus. UV/vis absorption and fluorescence spectra were recorded on a Thermo/UNICAM UV 500 spectrophotometer and on a JASCO FP-6500 spectrofluorimeter, respectively.

*Crystal Structure Determination:* The intensity data for the compounds were collected on a Nonius Kappa CCD diffractometer by using graphite-monochromated Mo-Kα radiation. Data were corrected for Lorentz and polarization effects, but not for absorption effects.[22,23] The structures were solved by direct methods (SHELXS [24]) and refined by full-matrix least squares techniques against $Fo^2$ (SHELXL-97 [24]). The hydrogen atoms of **20** and **22** were located by difference Fourier synthesis and refined isotropically. The hydrogen atoms of **19** were included at calculated positions with fixed thermal parameters. All nonhydrogen atoms were refined anisotropically.[23] XP (Siemens Analytical X-ray Instruments, Inc.) was used for structure representations.

Lawesson's reagent, (diphenylmethylene)hydrazine (**17**), and ethyl diazoacetoacetate were obtained commercially. Nickel peroxide (**17**), diazomethane, 2-(6-methoxy-1,3-benzothiazol-2-yl)-5,5-dimethyl-1,3-thiazole-4(5*H*)-one (**6**), (1-isopropyl-2-methylpropylidene)hydrazine (**16**), [bis(4-methoxyphenyl)methylene]hydrazine (**18**), and 9*H*-9-diazo-9*H*-fluorene were synthesized according to known literature procedures.[25–30]

**2-(6-Methoxy-1,3-benzothiazol-2-yl)-5,5-dimethyl-1,3-thiazole-4(5*H*)-thione (13)**

A mixture of ketone **6** (10.0 mmol) and Lawesson's reagent (8.0 mmol) in dry xylene (50 mL) was refluxed under $N_2$ for 4 h. When the starting material was completely consumed (TLC), the mixture was cooled to r.t. and filtered through a short column of silica gel (3 cm), which was subsequently washed with excess toluene. The organic phases were combined and concentrated, and the resulting solid was crystallized (heptane) to give a golden solid; yield: 2.0 g (65%); mp 193–194 °C (heptane).

IR (ATR): 3429, 1603, 1507, 1454, 1214, 918, 867, 827 $cm^{-1}$.

$^1$H NMR (250 MHz, DMSO-$d_6$): δ = 8.08 (d, $^3J$ = 9.1 Hz, 1 H), 7.40 (d, $^4J$ = 2.4 Hz, 1 H), 7.19 (dd, $^3J$ = 9.1 Hz, $^4J$ = 2.4 Hz, 1 H), 3.93 (s, 3 H), 1.81 (s, 6 H).

$^{13}$C NMR (63 MHz, DMSO-$d_6$): δ = 236.8, 186.3, 160.2, 157.0, 149.1, 140.2, 126.0, 118.5, 103.3, 73.1, 55.9, 31.7.

MS (EI): *m/z* (%) = 310 (53), 308 (100), 293 (56), 275 (53), 250 (15), 217 (36), 208 (57), 190 (30), 165 (12), 147 (14).

Anal. Calcd for $C_{13}H_{12}N_2OS_3$: C, 50.62; H, 3.92; N, 9.08; S, 31.19. Found: C, 50.62; H, 3.85; N, 8.98; S, 30.85.

UV/Vis ($CHCl_3$): $\lambda_{max}$ (log ε) = 281 (3.64), 420 (4.49) nm.

**Olefinations of 2-(6-Methoxy-1,3-benzothiazol-2-yl)-5,5-dimethyl-1,3-thiazole-4(5*H*)-thione (13); General Procedure**

A mixture of the appropriate hydrazone (4.0 mmol or 8.0 mmol for the aliphatic hydrazone) and $NiO_2$ (**14**; 33.0 mmol) in anhyd $CH_2Cl_2$ (50 mL) was stirred at 0 °C for 2 h under $N_2$ (–78 °C for 1 h for the aliphatic hydrazone). The mixture was filtered under inert conditions by using standard Schlenk techniques to give an intensely colored solution. A soln of thione **13** (1.0 mmol) in anhyd $CH_2Cl_2$ (20 mL) was added at 0 °C. The stirred mixture was allowed to warm to r.t. and kept at that temperature for additional 3 h. The solvent was then removed in a rotary evaporator and the remaining solid was mixed with copper powder, taken up in dry toluene (20 mL), and refluxed for 12 h. The mixture was then cooled to r.t., filtered, and concentrated to give a crude product that was purified by crystallization or by column chromatography.

**2-[4-(1-Isopropyl-2-methylpropylidene)-5,5-dimethyl-4,5-dihydro-1,3-thiazol-2-yl]-6-methoxy-1,3-benzothiazole (19)**
Yellow crystals; yield: 0.37 g (99%); mp 202–203 °C (heptane–EtOAc).

IR (ATR): 3447, 2959, 2925, 2866, 1601, 1496, 1266, 1219, 864 $cm^{-1}$.

$^1H$ NMR (250 MHz, DMSO-$d_6$): δ = 7.97 (d, $^3J$ = 9.0 Hz, 1 H), 7.31 (d, $^4J$ = 2.5 Hz, 1 H), 7.08 (dd, $^3J$ = 9.0 Hz, $^4J$ = 2.5 Hz, 1 H), 3.88 (s, 3 H), 3.04 (sept, $^3J$ = 6.8 Hz, 1 H), 2.40–2.60 (m, 1 H), 1.83 (s, 6 H), 1.36 (d, $^3J$ = 6.9 Hz, 6 H), 1.12 (d, $^3J$ = 7.0 Hz, 6 H).

$^{13}C$ NMR (63 MHz, DMSO-$d_6$): δ = 160.6, 158.7, 155.1, 154.3, 150.4, 148.4, 137.8, 124.8, 116.0, 103.9, 62.2, 55.8, 31.0, 23.2, 22.0, 20.8, 19.0.

MS (EI): *m/z* (%) = 376 (38), 375 (84), 374 (100), 359 (72), 341 (26), 331 (34), 208 (20), 43 (14).

Anal. Calcd for $C_{20}H_{26}N_2OS_2$: C, 64.13; H, 7.00; N, 7.48; S, 17.12. Found: C, 64.27; H, 7.11; N, 7.50; S, 17.09.

UV/vis ($CHCl_3$): $\lambda_{max}$ (log ε) = 337 (4.24), 351 (4.26), 368 (4.26), 388 (4.26) and 412 (4.00, sh) nm.

Crystal data:[31] $C_{20}H_{26}N_2OS_2$, $M_r$ = 374.55 g·mol$^{-1}$, colorless prism, size 0.05 × 0.05 × 0.04 $mm^3$, triclinic, space group $P\bar{1}$; $a$ = 8.1086(3), $b$ = 8.1910(3), $c$ = 17.0767(7) Å, α = 98.671(2), β = 90.205(2), γ = 119.001(2)°, $V$ = 976.78(6) Å$^3$, $T$ = –140 °C, $Z$ = 2, $\rho_{calcd}$ = 1.273 g·cm$^{-3}$, μ (Mo-Kα) = 2.83 $cm^{-1}$, F(000) = 400, 6336 reflections in $h$(–10/10), $k$(–10/10), $l$(–22/21), measured in the range 2.91° ≤ θ ≤ 27.53°, completeness $\theta_{max}$ = 96.9%, 4370 independent reflections, $R_{int}$ = 0.0201, 3854 reflections with $F_o > 4\sigma(F_o)$, 233 parameters, 0 restraints, $R1_{obs}$ = 0.0405, $wR2_{obs}$ = 0.0894, $R1_{all}$ = 0.0491, $wR2_{all}$ = 0.0974, GOOF = 1.027, largest difference peak and hole: 0.364/–0.269 e Å$^{-3}$.

**2-[4-(Diphenylmethylene)-5,5-dimethyl-4,5-dihydro-1,3-thiazol-2-yl]-6-methoxy-1,3-benzothiazole (20)**
Yellow crystals; yield: 0.18 g (40%); mp 182–183 °C (heptane).

IR (ATR): 3057, 3004, 2966, 1595, 1491, 1266, 1221, 1122, 1020, 923, 760, 699 $cm^{-1}$.

$^1H$ NMR (250 MHz, $CDCl_3$): δ = 8.01 (d, $^3J$ = 9.0 Hz, 1 H), 7.20–7.46 (m, 11 H), 7.12 (dd, $^3J$ = 9.0 Hz, $^4J$ = 2.5 Hz, 1 H), 3.89 (s, 3 H), 1.56 (s, 6 H).

$^{13}C$ NMR (63 MHz, $CDCl_3$): δ = 161.2, 159.8, 159.0, 157.7, 148.3, 141.7, 139.2, 138.2, 137.0, 131.0, 130.8, 127.7, 127.6, 127.2, 127.0, 125.0, 116.5, 103.7, 63.2, 55.8, 30.9.

MS (EI): *m/z* (%) = 444 (9), 443 (18), 442 (63), 409 (100), 408 (78), 244 (40), 234 (56), 208 (48), 165 (59), 121 (20), 59 (48).

Anal. Calcd for $C_{26}H_{22}N_2OS_2$: C, 70.56; H, 5.01; N, 6.33; S, 14.49. Found: C, 70.86; H, 5.02; N, 6.48; S, 14.38.

UV/vis (THF): $\lambda_{max}$ (log ε) = 367 (4.29) and 393 (4.31) nm.

Crystal data:[31] $C_{26}H_{22}N_2OS_2$, $M_r$ = 442.58 g·mol$^{-1}$, light-yellow prism, size 0.06 × 0.05 × 0.04 $mm^3$, monoclinic, space group $P2_1/c$, $a$ = 16.3282(3), $b$ = 8.1573(1), $c$ = 17.2187(3) Å, β = 104.615(1)°, $V$ = 2219.22(6) Å$^3$, $T$ = –130 °C, $Z$ = 4, $\rho_{calcd}$ = 1.325 g·cm$^{-3}$, μ (Mo-Kα) = 2.61 $cm^{-1}$, F(000) = 928, 12857 reflections in $h$(–21/21), $k$(–10/10), $l$(–22/17), measured in the range 2.78° ≤ θ ≤ 27.44°, completeness $\theta_{max}$ = 99.5%, 5052 independent reflections, $R_{int}$ = 0.0260, 4488 reflections with $F_o > 4\sigma(F_o)$, 368 parameters, 0 restraints, $R1_{obs}$ = 0.0389, $wR2_{obs}$ = 0.0958, $R1_{all}$ = 0.0455, $wR2_{all}$ = 0.1010, GOOF = 0.987, largest difference peak and hole: 0.411/–0.437 e Å$^{-3}$.

**2-[4-(9*H*-Fluoren-9-ylidene)-5,5-dimethyl-4,5-dihydro-1,3-thiazol-2-yl]-6-methoxy-1,3-benzothiazole (22)**
Red crystals; yield: 0.21 g (48%); mp 220–221 °C (heptane).

IR (ATR): 3051, 2964, 2930, 1602, 1584, 1488, 1440, 1264, 1220, 1139, 822, 808, 724 $cm^{-1}$.

$^1H$ NMR (250 MHz, $CDCl_3$): δ = 8.83 (d, $^3J$ = 7.2 Hz, 1 H), 8.02 (d, $^3J$ = 7.9 Hz, 1 H), 7.90 (d, $^3J$ = 9.0 Hz, 1 H), 7.54–7.70 (m, 2 H), 7.10–7.40 (m, 5 H), 7.03 (dd, $^3J$ = 9.0 Hz, $^4J$ = 2.4 Hz, 1 H), 3.80 (s, 3 H), 2.26 (s, 6 H).

$^{13}C$ NMR (63 MHz, $CDCl_3$): δ = 165.2, 164.7, 159.5, 159.1, 148.7, 142.0, 139.8, 138.8, 138.7, 135.4, 131.1, 129.6, 128.0, 127.7, 126.4, 126.3, 125.4, 120.1, 118.9, 117.2, 103.8, 65.1, 56.0, 28.3.

MS (EI): *m/z* (%) = 440 (52), 425 (76), 234 (62), 208 (56), 202 (100), 191 (60), 165 (38).

Anal. Calcd for $C_{26}H_{20}N_2OS_2$: C, 70.88; H, 4.58; N, 6.36; S, 14.56. Found: C, 70.65; H, 4.57; N, 6.32; S, 14.76.

UV/vis (THF): $\lambda_{max}$ (log ε) = 336 (4.11, sh), 372 (4.26), 391 (4.25), 435 (4.40), 458 (4.48), 484 (4.40), 522 (4.04, sh) nm.

Crystal data:[31] $C_{26}H_{20}N_2OS_2$, $M_r$ = 440.56 g·mol$^{-1}$, red prism, size 0.06 × 0.05 × 0.04 $mm^3$, monoclinic, space group Cc, $a$ = 11.0168(3), $b$ = 16.3502(4), $c$ = 11.6237(2) Å, β = 92.584(1)°, $V$ = 2091.61(8) Å$^3$, $T$ = –140 °C, $Z$ = 4, $\rho_{calcd}$ = 1.399 g·cm$^{-3}$, μ (Mo-Kα) = 2.77 $cm^{-1}$, F(000) = 920, 6369 reflections in $h$(–13/14), $k$(–19/21), $l$(–14/15), measured in the range 2.89° ≤ θ ≤ 27.47°, completeness $\theta_{max}$ = 99.5%, 4326 independent reflections, $R_{int}$ = 0.0192, 4276 reflections with $F_o > 4\sigma(F_o)$, 361 parameters, 2 restraints, $R1_{obs}$ = 0.0254, $wR^2_{obs}$ = 0.0644, $R1_{all}$ = 0.0259, $wR^2_{all}$ = 0.0651, GOOF = 1.057, Flack parameter 0.03(4), largest difference peak and hole: 0.191/–0.206 e Å$^{-3}$.

**2-{4-[Bis(4-methoxyphenyl)methylene]-5,5-dimethyl-4,5-dihydro-1,3-thiazol-2-yl}-6-methoxy-1,3-benzothiazole (21)**
Bright yellow crystals; yield: 0.20 g (40%); mp 184–185 °C (heptane).

IR (ATR): 3046, 2960, 2834, 1602, 1509, 1490, 1448, 1266, 1243, 1224, 1180, 1115, 1026, 918, 828, 804 $cm^{-1}$.

$^1H$ NMR (250 MHz, $CDCl_3$): δ = 8.00 (d, $^3J$ = 9.0 Hz, 1 H), 7.31–7.40 (m, 3 H), 7.22–7.28 (m, 2 H), 7.11 (dd, $^3J$ = 9.0 Hz, $^4J$ = 2.4 Hz, 1 H), 6.91 (d, $^3J$ = 8.5 Hz, 2 H), 6.84 (d, $^3J$ = 8.8 Hz, 2 H), 3.89 (s, 3 H), 3.85 (s, 3 H), 3.82 (s, 3 H), 1.55 (s, 6 H).

$^{13}C$ NMR (63 MHz, $CDCl_3$): δ = 160.1, 160.0, 159.0, 158.9, 158.8, 156.8, 148.4, 138.1, 136.4, 134.7, 132.5, 132.0, 131.7, 124.9, 116.5, 113.1, 112.6, 103.8, 63.5, 55.8, 55.2, 31.0.

MS (EI): *m/z* (%) = 502 (36), 487 (66), 469 (36), 304 (28), 294 (84), 280 (72), 236 (48), 208 (100), 193 (32), 165 (32), 152 (36), 59 (48).

Anal. Calcd for $C_{28}H_{26}N_2O_3S_2$: C, 66.91; H, 5.21; N, 5.57; S, 12.76. Found: C, 67.15; H, 5.60; N, 5.65; S, 12.69.

UV/vis (THF): $\lambda_{max}$ (log ε) = 343 (4.21) and 418 (4.29) nm.

**2-Isopropyl-2-(6-methoxy-1,3-benzothiazol-2-yl)-5,5-dimethyl-1,3-thiazolidin-4-one (12)**
For preparation and additional analytical data, see Würfel et al.[15]

Pale yellow crystals; yield: 1.43 g (70%); mp 130–132 °C (heptane–EtOAc).

$^1H$ NMR (250 MHz, DMSO-$d_6$): δ = 9.57 (s, 1 H), 7.84 (d, $^3J$ = 9.0 Hz, 1 H), 7.66 (d, $^4J$ = 2.5 Hz, 1 H), 7.08 (dd, $^3J$ = 9.0 Hz, $^4J$ = 2.5 Hz, 1 H), 3.82 (s, 3 H), 2.71 (m, $^3J$ = 6.7 Hz, 1 H), 1.48 (s, 6 H), 0.98 (m, 6 H).

$^{13}C$ NMR (63 MHz, DMSO-$d_6$): δ = 178.1, 178.0, 176.0, 157.8, 148.4, 136.5, 123.8, 116.2, 105.3, 70.8, 70.7, 56.2, 51.4, 37.0, 30.4, 28.8, 17.9, 17.8.

MS (EI): *m/z* (%) = 337 (38) [M + 1], 336 (8), 293 (100), 191 (40), 43 (20).

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