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Methodology for the Preparation of 2-Argininylbenzothiazole

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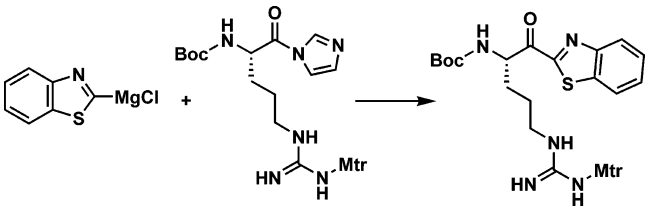

An efficient process to produce kilogram quantities of a key argininylbenzo[*d*]thiazole intermediate was developed for the preparation of the tryptase inhibitor RWJ-56423. A variety of activated arginine esters and benzo[*d*]thiazole nucleophiles were evaluated as coupling partners. Our work led to the selection and optimization of an argininyl imidazolide ester and benzothiazol-2-yl MgCl nucleophile. This paper focuses on the preparation, use, and stability of the benzothiazol-2-yl Grignard reagents.

RWJ-56423[1] (**1**) is a selective, potent, small-molecule inhibitor of human mast cell tryptase having therapeutic potential for treating allergic or inflammatory disorders.[2] This synthetic pharmacologic agent is an argininyl-tryptase inhibitor which shows selectivity over other serine proteases such as kallikrein, factor Xa, and chymotrypsin.[3]

Structurally, RWJ-56423 (**1**) is a dipeptide containing derivatized arginine and L-proline amino acids. The arginine residue bears both a benzo[*d*]thiazole heterocycle and an L-proline residue which has both an *N*-acetyl and 4-hydroxyl group attached.

An early approach to synthesize RWJ-56423 (**1**) was to use ornithine as a precursor for arginine (Scheme 1). Coupling of Boc-ornithine ester **3** with the derivatized L-proline amino acid **2** using classical coupling conditions gave **4** (Scheme 1). The subsequent preparation of acid **5** and the Weinreb[4] amide **6** was successful; however, attempting to react this amide with 2-lithiobenzothiazole gave none of the desired product **7** but rather the loss of the *N*-acetyl group on the proline residue.

As a consequence of this result, we made three modifications to this approach: first we decided to introduce the proline residue last in the synthesis to avoid interference from the *N*-acetylproline function; second, we used the Grignard reagent (benzothiazol-2-ylmagnesium halide) rather than the lithium analogue to conduct the reaction at a more practical temperature; third, we used protected arginine-activated esters rather than ornithine as an arginine precursor. Thus, we selected three suitable activated arginine esters (**11a**, **11b**, and **12**) to react with different benzothiazol-2-ylmagnesium halides to prepare the 2-argininylbenzo[*d*]thiazole **13**.[5] Compound **13** can then be converted to RWJ-56423 (**1**). The benzothiazol-2-ylmagnesium halides (**9a** and **9b**) were prepared by reacting benzo[*d*]thiazole **8** with excess Grignard reagents such as EtMgBr, EtMgCl, or *t*-BuMgCl at 0−5 °C. The reaction conditions and the results obtained from coupling the benzothiazol-2-ylmagnesium halides with the selected activated esters are summarized in Table 1. The reaction of the thiolester **11a**[6] with benzothiazol-2-ylmagnesium bromide (**9a**) gave the ketone **13** in moderate yield (63%, Table 1, entry 1) on a 400 g laboratory scale. However, upon scale up of the reaction to 2 kg, the yield dropped to 25% (Table 1, entry 2). We observed the solution of benzothiazol-2-ylmagnesium bromide change to a thick suspension with prolonged stirring. This suggested that a lesser amount of benzothiazol-2-ylmagnesium bromide was in solution on scale up and thereby caused lower yields for the subsequent coupling reaction. The outcome of the coupling of imidazolide **11b** with benzothiazol-2-ylmagnesium halides was dependent on the Grignard reagent being used. Experimental results showed that treatment of **11b** with **9a** resulted in isolation of a 71% yield of product **13** (Table 1, entry 3) on a 50 g laboratory scale, compared to 90% yield when **9b** was used (Table 1, entry 4) on a 2 kg scale. During the formation of **9a** by reacting **8** with EtMgBr in ether, the product formed as a heavy precipitate, making it difficult to determine the amount of Grignard reagent available for the reaction. On the other hand, the benzothiazol-2-yl MgCl Grignard reagent **9b** formed by reacting **8** with EtMgCl in THF was soluble in the reaction solvent. These results demonstrated a noticeable advantage for the use of the chloro Grignard reagent **9b** over the bromo analogue **9a**. However, further scale-up using **9b** gave a lower yield (40%, Table 1, entry 5) of product **13**. The lactam (**12**) was also tested

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SCHEME 1. Original Synthetic Strategy To Prepare RWJ-56423

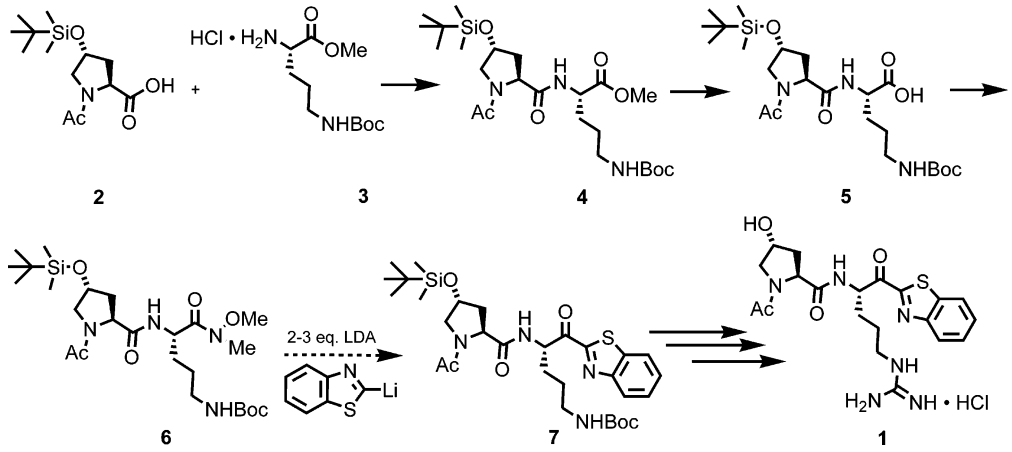
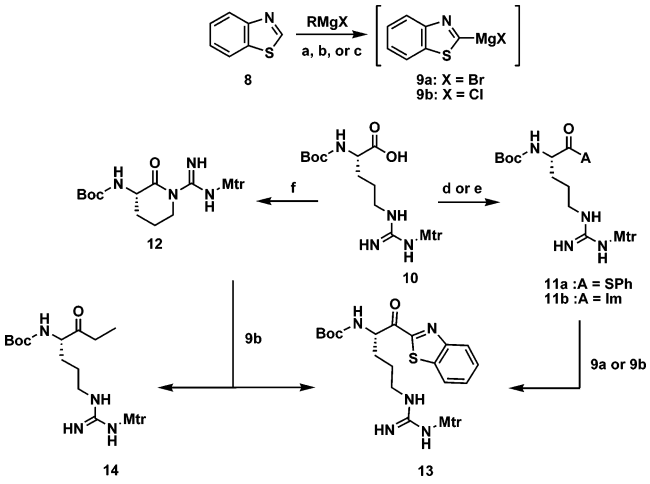


TABLE 1. Preparation of 2-Arginylbenzothiazole 13[a]



entry	Grignard reagent	method of prepn	activated ester	method of prepn	scale	yield (%)
1	**9a**	EtMgBr (a)	**11a**	d	400 g	63
2	**9a**	EtMgBr (a)	**11a**	d	2 kg	25
3	**9a**	EtMgBr (a)	**11b**	e	50 g	71
4	**9b**	EtMgCl (b)	**11b**	e	2 kg	90
5	**9b**	EtMgCl (b)	**11b**	e	4 kg	40
6	**9b**	EtMgCl (b)	**12**	f	5 g	95
7	**9b**	EtMgCl (b)	**12**	f	50 g	75
8	**9b**	*t*-BuMgCl (c)	**11b**	e	10 kg	80

[a] Reagents and conditions: (a) THF, 10 °C, EtMgBr in Et_2O, 10−15 min; (b) THF, 10 °C, EtMgCl in THF, 10−15 min; (c) THF, 3−5 °C, *t*-BuMgCl in THF, 10−15 min; (d) Ph_2POCl, Et_3N, PhSH, 0 °C, 15 min; (e) CDI, THF, rt, 30 min; (f) CDI, DMF, TEA, rt, 30 min.

in the reaction with benzothiazol-2-yl MgCl **9b**. Surprisingly, this reaction produced the desired ketone **13** in excellent yield on a 5 g scale (95%, Table 1, entry 6). However, scaling up the reaction to 50 g (Table 1, entry 7) resulted in a lower (75%) conversion to product **13**. Increasing the equivalents of EtMgCl compared to benzo[*d*]thiazole resulted in the consumption of **12**; however, this also resulted in the formation of ethyl ketone **14** as a byproduct.

The results obtained from the reactions listed in Table 1 point to one major observation; regardless of which activated ester is used, the large-scale reactions resulted in compromised yields. We continued process development to evaluate the following critical parameters: addition rate, exclusion of moisture, elimination of light, and order of addition. On laboratory scale (50−100 g), we were unable to identify the cause of our problematic scale-up issues because yields and purities were greater than 90%. Attention was then turned to a careful examination of the stability of the Grignard reagents **9a** and **9b**.

Stability studies of benzothiazol-2-yl MgBr (**9a**) and benzothiazol-2-yl MgCl (**9b**) were carried out at 0 °C under nitrogen (Figure 1). Reacting **8** with either EtMgBr or EtMgCl prepared the desired Grignard reagent (**9a** or **9b**). Aliquots were taken in either 15 min or 1 h intervals of the initial reaction time and immediately quenched into dilute acid. Based upon the weight percent analysis of the aliquots taken, the amount of benzo[*d*]-thiazole was determined over a 6 h period. The mostly insoluble **9a** showed no change in the amount of benzo[*d*]thiazole over time. On the other hand, the soluble benzothiazol-2-yl MgCl (**9b**) showed decreased amounts of benzo[*d*]thiazole over time. After 6 h at 0 °C, only 70% of active **9b** remained, and the disappearance of 30% of **9b** was associated with the formation of a yellow viscous oily residue. On the other hand, additional studies showed that the reverse addition of up to 80% of the benzo[*d*]thiazole /THF solution into EtMgCl/THF afforded a stable solution of **9b** for at least 1 h at 10 °C without the formation of the yellow viscous oily residue. However, the addition of excess benzo[*d*]thiazole/THF solution into the solution of EtMgCl resulted in the immediate formation of the yellow viscous oily residue observed previously. This result suggested that the presence of excess benzo[*d*]thiazole catalyzes the decomposition of **9b** to the yellow oily residue, while excess EtMgCl impedes its formation.[7] In addition to that, we noticed that prolonged stirring of the **9b** solution in the presence of air introduced by opening of the reaction flask caused its faster decomposition. The isolation, purification, and characterization of the yellow oily residue was later determined to be the benzo-[*d*]thiazole trimer **15**. The decomposition to the benzo[*d*]thiazole

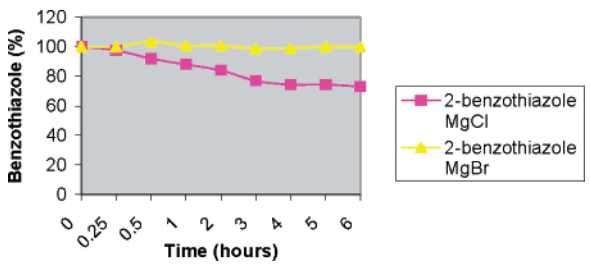
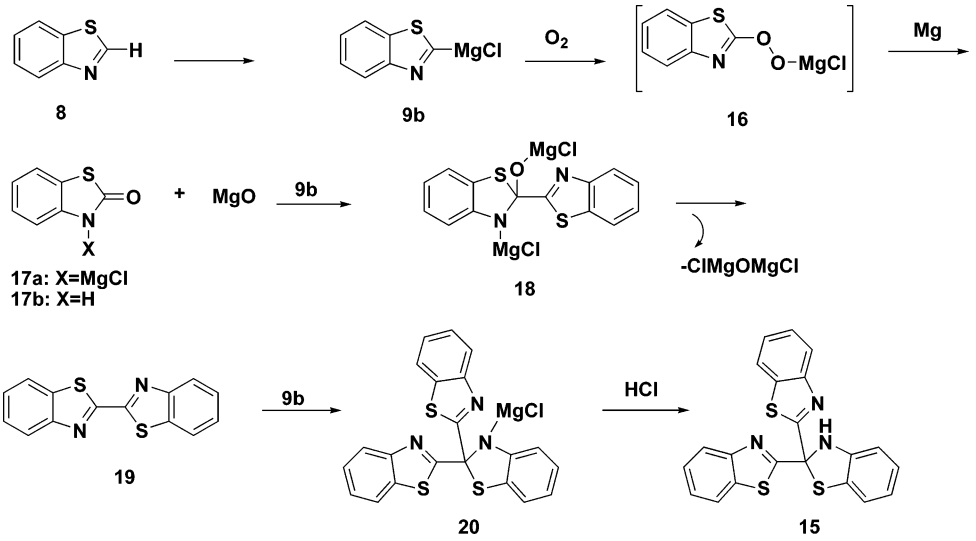
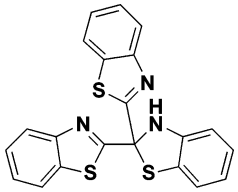


FIGURE 1. Comparative stability of 2-benzothiazole Grignards.

SCHEME 2. Mechanism for the Formation of Benzo[*d*]thiazole Trimer (15) in the Presence of Oxygen and Magnesium



trimer explained the lowered yields on large-scale compared to the laboratory scale reactions.



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The solution for this decomposition problem was to maintain excess EtMgCl during the formation of **9b**. However, excess EtMgCl reacts with **11a**, **11b**, and **12** and gives rise to undesired side products. It is at this point that we replaced EtMgCl with the more sterically hindered *t*-BuMgCl to prepare **9b**. The *t*-BuMgCl is unreactive toward the imidazolide (**11b**). An excess of *t*-BuMgCl could be used without concern of a side reaction with imidazolide **11b**. With this new procedure, Boc-Arg-Mtr ketone (**13**) could be successfully prepared on a 10 kg scale in 80% yield using 4 equiv of benzo[*d*]thiazole and 12 equiv of *t*-BuMgCl in a predictable reproducible manner (Table 1, entry 8). As a safety precaution, the reaction mixture was heavily blanketed with inert argon due to the pyrophoric nature of *t*-BuMgCl. The argon gas also minimizes any oxygen absorption and subsequent benzo[*d*]thiazole degradation.

This modification provided a reliable and scaleable process in preparing the key intermediate argininyl-benzo[*d*]thiazole **13** to meet the RWJ-56423 drug substance demand. However, one issue remained: we still needed to determine the mechanism for the formation of the benzo[*d*]thiazole trimer.

It was proposed that oxygen played a key role in the formation of the benzo[*d*]thiazole trimer (**15**), regardless to the order of addition. The benzothiazol-2-yl MgCl formation in which oxygen was rigorously excluded via an inert atmosphere of nitrogen and a 1:1.1 ratio of EtMgCl/benzo[*d*]thiazole resulted in 100% recovered benzo[*d*]thiazole with no formation of the trimer (**15**) following an acid quench. However when the same experimental conditions were explored in an open reaction vessel, a significant amount of trimer was observed. These results showed that the exclusion of air was an uncontrolled critical parameter on large scale. The Grignard reaction was being compromised by air entering the large-scale reactors. Air dissolved in the THF solvent or EtMgCl reagent solution prior to use are potential sources for oxygen getting in the reactor.

In the presence of oxygen, two competitive pathways are in effect: (1) the nucleophilic acylation of activated Boc-Arg-Mtr with benzothiazol-2-yl MgCl which leads to product and (2) an oxygen quench of benzothiazol-2-yl MgCl which leads to its degradation (formation of the trimer). LC/MS analysis of the reaction mixtures showed evidence that benzothiazolone (**17a**, Scheme 2) and benzo[*d*]thiazole dimer (**19**, Scheme 2) are along the reaction pathway to form benzo[*d*]thiazole trimer (**15**). To confirm this finding, we subjected commercially available benzothiazolone (**17b**) to benzothiazol-2-yl MgCl (**9b**) under the same reaction conditions. The *in situ* product matched the benzo[*d*]thiazole trimer (**15**) by HPLC retention times and LC/MS exact mass. Floating particulates in the Grignard reagent were observed, which were thought to be residual magnesium metal leftover from the Grignard reagent formation. In preparing benzothiazol-2-yl MgCl (**9b**), magnesium metal was added. As a result, the formation of the benzo[*d*]thiazole trimer (**15**) was facilitated. The Grignard reagent was not at any time filtered before use.

The proposed mechanism (Scheme 2) reflects the essential role of oxygen and magnesium in the formation of the benzo[*d*]thiazole trimer (**15**). Following deprotonation of benzo[*d*]thiazole (**8**) with EtMgCl, benzothiazol-2-yl MgCl (**9b**) is oxidized to benzo[*d*]thiazole peroxide (**16**) by oxygen. Magnesium then reduces the peroxide to benzothiazolone (**17a**). Nucleophilic addition of benzothiazol-2-yl MgCl (**9b**) to benzothiazolone (**17a**) results in the metalated dimer **18**.

The metalated dimer (**18**) aromatizes to dimer (**19**) followed by the nucleophilic addition of a second equivalent of benzothiazol-2-yl MgCl (**9b**) to form the metalated trimer (**20**). Quenching the reaction mixture with acidic water then leads to benzo[*d*]thiazole trimer (**15**).

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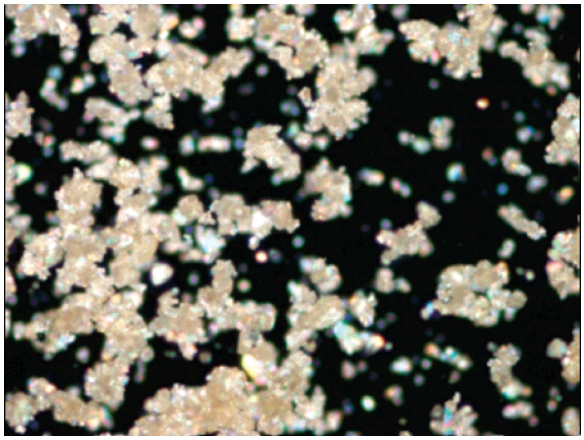

FIGURE 2. Photomicrograph of crystalline benzo[*d*]thiazole trimer (**15**).

The benzo[*d*]thiazole trimer[8] was isolated and slowly crystallized from dichloromethane and pentane. The trimer was isolated as orange crystalline clusters (Figure 2) in the absence of any apparent extended conjugation present in its molecular structure. Evidence of a possible tautomer was not apparent in our spectroscopic data analysis as a plausible explanation for the orange-colored benzo[*d*]thiazole trimer.

When the initial optimization of the coupling of various activated acids with benzothiazol-2-yl magnesium halide **9a** and **9b** failed to yield ketone **13** reproducibly on large scale, an investigation into the parameters leading to the troublesome benzo[*d*]thiazole trimer process impurity **15** was undertaken. Atmospheric oxygen, metallic magnesium, and excess benzothiazol-2-yl MgCl were shown as interdependent critical parameters in enabling the unexpected degradation pathway to benzo[*d*]thiazole trimer (**15**). Generating benzothiazol-2-yl MgCl (**9b**) with excess *t*-BuMgCl and carefully excluding oxygen from the reaction vessel minimized the trimer (**15**) byproduct. Utilizing the optimized procedure to ketone **13**, the demand for drug substance (**1**) was readily met in additional synthetic campaigns.

Experimental Section

[(1*S*)-1-(2-Benzothiazolylcarbonyl)-4-[[imino[[(4-methoxy-2,3,6-trimethylphenyl)sulfonyl]amino]methyl]amino]butyl]carbamic Acid 1,1-Dimethylethyl Ester (13). To a slurry of 1,1′-carbonyldiimidazole (6.48 g, 40 mmol) in THF (23 mL) at rt was added a solution of Boc-Arg (Mtr)-OH (**10**, 18.00 g, 33.3 mmol) in THF (54 mL) over 2 min via cannula. The resulting clear light yellow imidazolide solution (**11b**) was stirred at rt for 5 min and then concentrated to ca. 50 mL in vacuo for 30 min. In a separate reaction vessel was added a solution of benzo[*d*]thiazole (55.70 g, 400 mmol) in THF (100 mL) to a solution of *t*-BuMgCl (200 mL, 400 mmol) in THF (100 mL) at 4 °C over 2 h. After the addition, the internal temperature of the resulting benzothiazol-2-yl MgCl (**9b**) solution was maintained between 6 and 8 °C while stirring continued for 10 min. The imidazolide solution (**11b**) was transferred into an addition funnel and subsequently added into the benzothiazol-2-yl MgCl (**9b**) solution over 25 min. The resulting dark reddish solution was stirred at 10 °C for 15 min. The reaction mixture was transferred into a cold (5−10 °C) mixture of 2 M HCl (300 mL, 600 mmol) and EtOAc (150 mL) while stirring continued. The resulting brown mixture was stirred for 5 min in a cool water bath (7−10 °C). The layers were separated, and the aqueous layer was extracted with EtOAc (150 mL). The combined brownish organic layers were sequentially washed with saturated aq $NaHCO_3$ (150 mL), water (150 mL), and brine (150 mL). The organics were subsequently dried over $MgSO_4$ (20 g) for 2 h. The crude mixture was filtered, concentrated to ca. 140 mL in vacuo, and transferred to an addition funnel. The crude mixture was added in a steady stream to a vigorously stirred mixture of heptane (600 mL) and EtOAc (60 mL). After the mixture was stirred for 15−30 min, the solids were collected by vacuum filtration, washed with EtOAc/heptane (1/4) (100 mL × 3), and air-dried overnight (ca. 16 h) to yield the title compound **13** as a tan solid in 80% yield: mp 227.22 °C; rotamers observed ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.38−1.46 (m, 9 H) 1.64−1.76 (m, 2 H) 2.05−2.13 (m, 4 H) 2.57−2.61 (m, 3 H) 2.65−2.70 (m, 3 H) 3.20−3.32 (m, 1 H) 3.51 (s, 1 H) 3.80−3.84 (m, 4 H) 5.55−5.66 (m, 2 H) 6.19 (s, 2 H) 6.50 (s, 1 H) 7.53−7.60 (m, 2 H) 7.96−7.99 (m, 1 H) 8.17−8.21 (m, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ11.9 (s,1C), 18.26, 18.34 (s, 1C), 24.1 (s, 1C), 25.38, 25.47 (s, 1C), 28.3 (s, 3C), 31.4 (s, 1C), 40.68, 40.75 (s, 1C), 55.2, 55.4 (s, 1C), 80.6 (s, 1C), 111.6 (s, 2C), 122.4 (s, 1C), 124.7 (s, 1C), 125.9 (s, 1C), 127.3 (s, 1C), 128.2 (s, 1C), 129.2 (s, 1C), 133.7 (s, 1C), 134.6 (s, 1C), 136.6 (s, 1C), 137.2 (s, 1C), 138.6 (s, 1C), 153.4 (s, 1C), 156.2 (s, 1C), 158.3 (s, 1C), 193.20, 193.26 (s, 1C); MH^+ 604.3. Anal. Calcd for $C_{28}H_{37}N_5O_6S_2$: C, 55.70; H, 6.18; N, 11.60; O, 15.90; S, 10.62. Found: C, 55.52; H, 6.39; N, 11.33; S, 10.35. In cases where EtMgCl Grignard was used instead of *t*-BuMgCl, benzo[*d*]thiazole trimer (**15**) was isolated as an orange solid (33% yield): mp 194−212 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.76 (td, J = 7.5, 1.1 Hz, 1H), 6.84 (dd, J = 7.5, 1.1 Hz, 1H), 7.01 (td, J = 7.5, 1.1 Hz, 1H), 7.19 (dd, J = 7.5, 1.1 Hz, 1H), 7.44−7.56 (m,4H), 7.95 (dd, J = 8.2, 1.1 Hz, 2H), 8.15 (dd, J = 8.2, 1.1 Hz, 2H), 8.70 s,1H, NH); ^{13}C (100 MHz, DMSO-d_6) δ 78.9 (s, 1C), 110.3 (d, 1C), 120.2 (d, 1C), 121.2 (d, 1C), 122.4 (d, 2C), 122.8 (s, 1C), 123.0 (d, 2C), 125.6 (d, 2C), 126.3 (d, 1C), 126.5 (d, 2C), 135.2 (s, 2C), 145.0 (s, 1C), 153.1 (s, 2C), 174.7 (s, 2C); MH^+ 404.1. Anal. Calcd for $C_{21}H_{13}N_3S_3$: C, 62.50; H, 3.25; N, 10.41; S, 23.84. Found: C, 62.25; H, 3.25; N, 10.41; S, 23.84.

2-(Benzo[d]thiazol-2-yl)-2,3-dihydro-2,2′-bibenzo[*d*]thiazole (15). To a solution of **8** (2.6 g, 19.2 mmol) in 2 mL of THF was added 2 M EtMgCl (9.72 mL, 19.4 mmol) while the reaction temperature was maintained between 0 and 5 °C. Solution **8** was allowed to stir for 10 min at 0−5 °C. In a separate flask, EtMgCl (5.1 mL, 10.2 mmol) was added to a solution of benzothiazol-2-one (**17b**) (1.40 g, 9.25 mmol) in 3 mL of THF while the reaction temperature was maintained between 0 and 5 °C. Solution **17b** was allowed to stir for 10 min. Solution **8** was transferred via cannula to solution **17b** while the reaction temperature was maintained between 0 and 5 °C. The reaction temperature was allowed to warm to rt. The *in situ* product was identified by HPLC and LC/MS as the trimer **15**.

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, DEPT, MS, and elemental analysis and X-ray crystal structure. This material is available free of charge via the Internet at http://pubs.acs.org.

JO7019543

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