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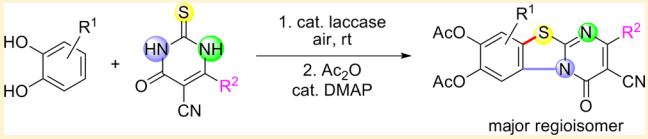
Laccase-Catalyzed Domino Reaction between Catechols and 6-Substituted 1,2,3,4-Tetrahydro-4-oxo-2-thioxo-5-pyrimidinecarbonitriles for the Synthesis of Pyrimidobenzothiazole Derivatives

Heba T. Abdel-Mohsen, Jürgen Conrad, and Uwe Beifuss*

Bioorganische Chemie, Institut für Chemie, Universität Hohenheim, Garbenstrasse 30, D-70599 Stuttgart, Germany

Supporting Information

ABSTRACT: The laccase-catalyzed domino reaction between catechols and 6-substituted 1,2,3,4-tetrahydro-4-oxo-2-thioxo-5-pyrimidinecarbonitriles using aerial O_2 as the oxidant delivers new pyrimidobenzothiazole derivatives. The complete structure elucidation of the ring-proton deficient heterocyclic products and the unambiguous determination of the regioselectivity of the reactions have been achieved by extended NMR spectroscopic methods including HSQMBC, super long-range HMBC, and ^{15}N measurements.



INTRODUCTION

Since there are a large number of biologically active heterocycles, their selective and efficient preparation is at the core of organic synthesis.[1] In view of the growing importance of enzyme-catalyzed transformations in organic synthesis, it is surprising that the number of methods for the synthesis of hetereocycles based on enzyme-catalyzed transformations is rather limited.[2] Among the most well-known methods are the lipase-catalyzed double bond epoxidation[3] and the generation of lactones using Baeyer–Villiger monooxygenases.[4] More recently, we became interested in the laccase-catalyzed transformations. Laccases are multicopper oxidases that are produced by fungi, plants, and prokaryotes, can be easily isolated and purified, and can be obtained commercially.[5] Laccases catalyze the oxidation of numerous substrates using aerial oxygen as the oxidant.[6] Employing laccase-mediator systems, the substrate range of laccases can be extended considerably.[6d,e,7] The use of laccase-catalyzed transformations is not restricted to simple oxidations such as the oxidation of alcohols to aldehydes,[8] the oxidative coupling of thiols to disulfides[9] or the aromatization of 1,4-dihyropyridines to the corresponding pyridines.[10] They can also be combined with chemical transformations like the Diels–Alder reaction[11] or 1,4-additions to new domino processes. Thus, the laccase-catalyzed oxidation of catechols and hydroquinones can be combined with the reaction of the resulting *o*- or *p*-benzoquinones with *C*-,[12] *N*-,[6c,13] and *S*-nucleophiles.[14] Using this approach, we have been able to demonstrate that the laccase-catalyzed oxidation of catechols in the presence of 1,3-dicarbonyls allows the synthesis of a number of *O*-heterocycles[12f–h,j,k] such as 6-substituted 7,8-dihydroxybenzofuro[3,2-*c*]pyridin-1(2*H*)-ones, 10-substituted 8,9-dihydroxybenzofuro[3,2-*c*]quinolin-6(5*H*)-ones, and 8,9-dihydroxy-5-thiocoumestans.[12g] Apart from applications in organic synthesis, laccases play also an important role in polymer chemistry. They have been used for the polymerization of numerous phenolics[15] as well as the degradation of lignin.[16]

Benzothiazoles exhibit a wide range of interesting pharmacological properties.[17] Pyrimidobenzothiazole derivatives, for example, are known for their antiallergic,[18] antibacterial,[19] and antifungal activities.[20] Some pyrimidobenzothiazoles have been demonstrated to have a high affinity to the benzodiazepine receptor.[21] This is why a number of methods have been developed for the synthesis of this heterocyclic skeleton. They include the condensation of 2-aminobenzothiazoles with acetylene carboxylic acids[22] and acetylene dicarboxylic acid derivatives,[21a] 2-aminofumarates,[21a] and *β*-ketoesters.[23] Pyrimidobenzothiazoles can also be obtained by a three-component reaction of 2-aminobenzothiazoles with aldehydes and *β*-ketoesters.[24] The amidines of 2-aminobenzothiazoles have also been employed as substrates for the synthesis of pyrimidobenzothiazoles.[25] In summary, the number of approaches to the pyrimidobenzothiazoles is rather limited. This is why the development of new methods for their preparation is highly desirable. In view of the fact that so far no enzyme-catalyzed synthesis of the pyrimidobenzothiazole skeleton has been developed, we wondered whether it is possible to develop a laccase-catalyzed synthesis of this heterocyclic system that is based on the combination of a laccase-catalyzed oxidation of catechols to *o*-benzoquinones and its reaction with thioxo-5-pyrimidinecarbonitriles.

RESULTS AND DISCUSSION

Here we report on the laccase-catalyzed synthesis of pyrimidobenzothiazoles by reaction of catechol (**1a**) and 3-methylcatechol (**1b**) with 6-substituted 1,2,3,4-tetrahydro-4-oxo-2-thioxo-5-pyrimidinecarbonitriles **5a–f** using aerial oxygen as the oxidant.

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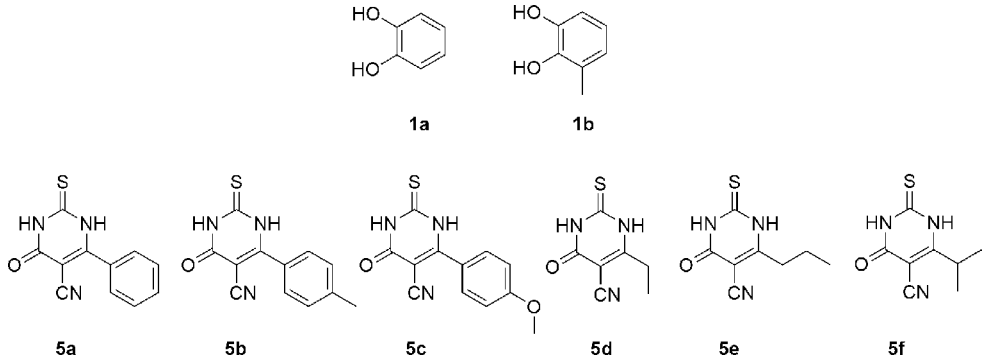
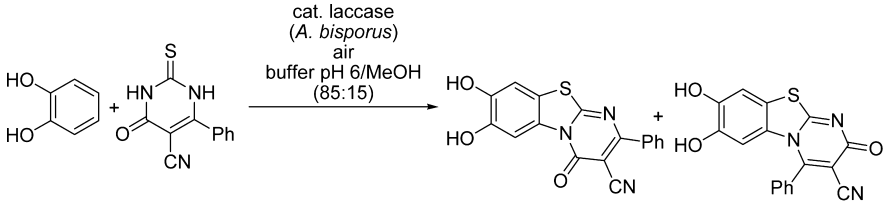


Figure 1. Substrates for the laccase-catalyzed domino reactions.

Table 1. Laccase-Catalyzed Domino Reaction between Catechol (1a) and 1,2,3,4-Tetrahydro-4-oxo-6-phenyl-2-thioxo-5-pyrimidinecarbonitrile (5a) under Different Conditions



entry	laccase (U)	1a:5a	buffer (mL)[a]	T (°C)	time (h)	6a:7a[b]	yield 6a + 7a (%)[c]
1	120	1:1[d]	80	22	24	75:25	84[g]
2	120	1.25:1[e]	80	22	12	72:28	95
3	60	1.25:1[e]	80	22	18	74:26	93
4	30	1.25:1[e]	80	22	24		traces
5	60	1.25:1[e]	30	22	15	71:29	95
6	120	1.25:1[f]	60	22	15	74:26	95
7	60	1.25:1[e]	30	50	10	75:25	90
8		1.25:1[e]	30	22	24		

[a]Phosphate buffer (0.2 M) was used. [b]Ratio determined from the ^{1}H NMR spectrum of the reaction mixture of **6a** and **7a**. [c]Yields refer to crude yields. [d]0.50 mmol of **1a** and 0.50 mmol of **5a** were reacted. [e]0.63 mmol of **1a** and 0.50 mmol of **5a** were reacted. [f]1.25 mmol of **1a** and 1 mmol of **5a** were reacted. [g]Traces of **5a** were present in the crude product.

In addition, we present a NMR method that allows the unequivocal structure elucidation of the pyrimidobenzothiazoles obtained. It is based on ^{1}H−^{15}N HMBC NMR correlations at natural abundance and experimental ^{1}H−^{13}C long-range coupling constants in addition to super long-range ^{1}H−^{13}C HMBC correlations, as well as calculated ^{13}C chemical shifts for the structure elucidation of aromatic ring-proton deficient heterocyclic compounds whose chemical structures only hardly could be assessed by standard NMR methods.

The 6-substituted 1,2,3,4-tetrahydro-4-oxo-2-thioxo-5-pyrimidinecarbonitriles **5a−f** required for this study were synthesized by three-component reactions between aromatic and aliphatic aldehydes **2a−f**, methyl cyanoacetate (**3**), and thiourea (**4**) in the presence of equimolar amounts of K_2CO_3 in methanol according to the procedure of Kambe et al. (Figure 1).[26]

In a first experiment, 0.50 mmol of catechol (**1a**) and 0.50 mmol of 1,2,3,4-tetrahydro-4-oxo-6-phenyl-2-thioxo-5-pyrimidinecarbonitrile (**5a**) were reacted in a mixture of phosphate buffer (0.2 M, pH 6) and MeOH (85:15 v/v) for 24 h at room temperature using a commercially available laccase from *Agaricus bisporus* (120 U, 6 U/mg) as the catalyst and air as the oxidant (Table 1, entry 1). After acidic workup, 84% of a 75:25 mixture (^{1}H NMR) of the regioisomers **6a** and **7a** was isolated. Since the crude product still contained traces of **5a** (TLC), the reaction was repeated using a slight excess of **1a** (Table 1, entry 2). After 12 h, 95% of a 72:28 mixture (^{1}H NMR) of the two regioisomers **6a** and **7a** was obtained. Further modifications of the experimental procedure with respect to the amount of catalyst and buffer, reaction temperature, and reaction time revealed that the best result was achieved when 0.63 mmol of **1a** and 0.50 mmol of **5a** were reacted in the presence of 60 U laccase in 30 mL of phosphate buffer pH 6 and 5.5 mL of MeOH at room temperature under air for 15 h (Table 1, entry 5). Under these conditions **6a** and **7a** were formed in 95%. The reaction could be also run on a 1 mmol scale (Table 1, entry 6). A control experiment demonstrated that without the laccase the transformation did not take place (Table 1, entry 8).

Apart from the two cyclization products **6a** and **7a**, no other products were obtained. It should be noted that none of the two possible noncyclic 1,4-addition products **I** and **II** were isolated (Figure 2).

It is assumed that the reaction sequence starts with the laccase-catalyzed oxidation of catechol (**1a**) to *o*-benzoquinone (**8a**) (Scheme 1). This is followed by 1,4-addition of the nucleophilic S atom of the 4-oxo-2-thioxo-5-pyrimidinecarbonitrile **5a** to deliver the two tautomers **9A** and **9B** as intermediates. Subsequent oxidation of **9A** or **9B** produces the tautomeric benzoquinones **10A** and **10B**, respectively. Depending on which of the *N* atoms acts as nucleophile, the intramolecular 1,4-addition gives either the type I product **6a** or

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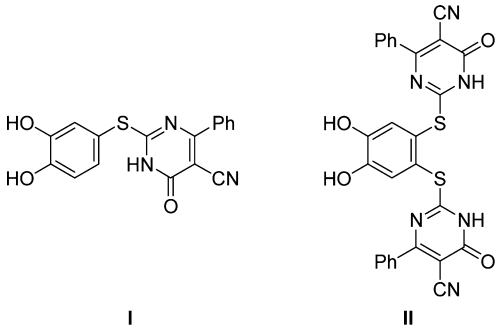


Figure 2. Possible noncyclic 1,4-addition products of the reaction between **1a** and **5a**.

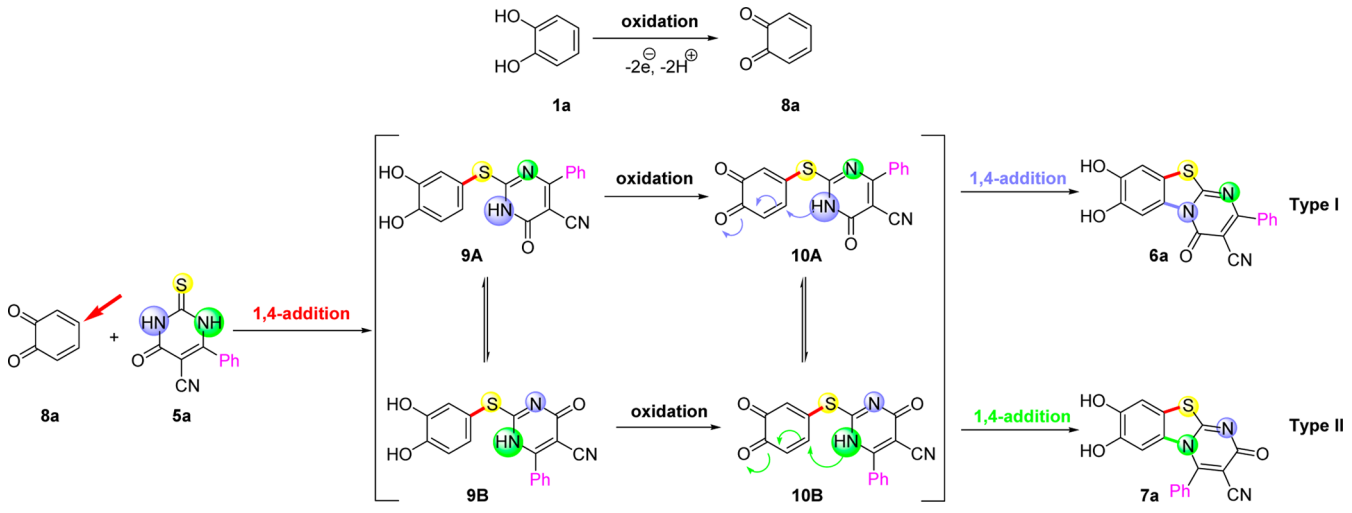
the type II product **7a**. The finding that the type I product **6a** was formed in excess indicates that *N*-3 (marked in blue) of tautomer **10A** is more reactive than *N*-1 (marked in green) of **10B**. This is supported by intermolecular nucleophilic substitution reactions of different *S*-alkylated/benzylated thioxopyrimidines with halides.[27]

Using the mixture of **6a** and **7a**, the structure elucidation of **6a** and **7a** proved to be difficult. This is why we tried to separate the two regioisomers. However, due to their high polarity and their low solubility even in polar solvents, they could not be separated. To increase their solubility, the pyrimidobenzothiazoles **6a** and **7a** were transformed into the corresponding diacetates **11a** and **12a**. For this purpose, the mixture of **6a** and **7a** was reacted with an excess of acetic anhydride in the presence of catalytic amounts of dimethylaminopyridine (DMAP) in pyridine[28] to deliver 77% of a mixture of **11a** and **12a** (Scheme 2). The two regioisomeric diacetates **11a** and **12a** could be separated by flash chromatography on silica gel to deliver the analytically pure heterocycles **11a** and **12a** in 50% and 8%, respectively.

Then, the protocol developed for the reaction between **1a** and **5a** was applied to the transformations of catechol (**1a**) with 1,2,3,4-tetrahydro-4-oxo-2-thioxo-5-pyrimidinecarbonitriles **5b–f** carrying different aryl or alkyl groups at the 6-position (Table 2). First of all, in all cases the corresponding pyrimidobenzothiazole derivatives **6** and **7** were formed exclusively. Again, none of the 1,4-addition products **I** and **II** could be detected. The yields were in the range of 83–96%, and the ratio of the two regioisomers ranged from 51:49 to 92:8. Obviously, the ratio was strongly affected by the substituent at C-6 of the 4-oxo-2-thioxo-5-pyrimidinecarbonitriles **5b–f**. With aromatic substituents, such as the 4-methylphenyl group in **5b** and the 4-methoxyphenyl group in **5c**, the ratios of **6**:**7** were similar to the ratio observed in the reaction between **1a** and **5a** (Table 2, entries 2 and 3). When the ethyl-substituted 4-oxo-2-thioxo-5-pyrimidinecarbonitrile **5d** was reacted, nearly equal amounts of the isomers **6d** and **7d** were formed (Table 2, entry 4). A similar result was observed in the transformation between the *n*-propyl-substituted 4-oxo-2-thioxo-5-pyrimidinecarbonitrile **5e** and **1a** (Table 2, entry 5). However, when the isopropyl-substituted 4-oxo-2-thioxo-5-pyrimidinecarbonitrile **5f** was used as a substrate, the regioisomer **6f** was formed in excess over the regioisomer **7f**; the ratio amounted to 92:8 (Table 2, entry 6). It is assumed that the ratio of the two isomers depends on the steric demand of the C-6 substituent of the 4-oxo-2-thioxo-5-pyrimidinecarbonitriles **5a–f**. Considering **10A** and **10B** as intermediates of the domino reaction, it is expected that sterically more demanding C-6 substituents favor the formation of type I products **6**. This is supported by the finding that with sterically less demanding unbranched alkyl groups as in **5d** and **5e**, the regioisomers **6** and **7** are formed in comparable amounts. As expected, the amount of **6** increases when sterically more demanding aryl groups or the isopropyl group are attached to C-6. The mixtures of the regioisomers **6b–f** and **7b–f** were also difficult to separate. In order to separate the isomers, the mixtures were transformed into the corresponding diacetates with yields ranging from 68 to 85% (Table 3). After separation by flash chromatography on silica gel, the individual isomers **11b–f** and **12b,d,e** were obtained in analytically pure form.

In order to study the influence of substituents on the *o*-benzoquinone moiety on the yield and the selectivity of the pyrimidobenzothiazole synthesis, 3-methylcatechol (**1b**) was reacted with different 6-substituted 1,2,3,4-tetrahydro-4-oxo-2-thioxo-5-pyrimidinecarbonitriles **5a–f** under the conditions developed for the laccase-initited domino reaction between catechol (**1a**) and **5a–f** (Table 1, entry 5 and Table 2). It is remarkable that in most cases only two out of four possible products could be detected (^{1}H NMR), namely, the type IA products **13a–f** and the type IB products **14a–f** (Table 4, Scheme 3).

Scheme 1. Possible Mechanism for the Reaction of Catechol (1a) with 1,2,3,4-Tetrahydro-4-oxo-6-phenyl-2-thioxo-5-pyrimidinecarbonitrile (5a)



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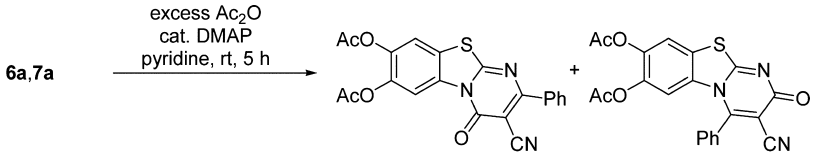
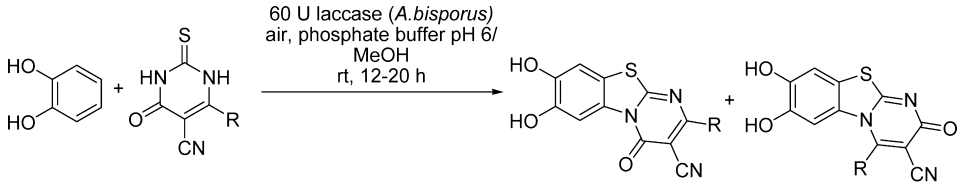
Scheme 2. Acetylation of the Mixture of 6a and 7a



11a (50%) **12a** (8%)

Table 2. Laccase-Catalyzed Domino Reaction between Catechol (1a) and 6-Substituted 1,2,3,4-Tetrahydro-4-oxo-2-thioxo-5-pyrimidinecarbonitriles 5a–f[a]

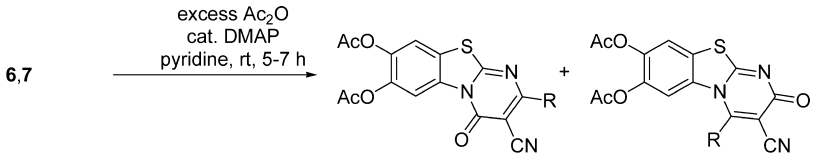


1a **5a-f** **6a-f** **7a-f**

entry	5	R	6,7	6:7	yield 6 + 7 (%)[b]
1	a	C_6H_5	a	71:29[c], 77:23[d]	95
2	b	4-CH_3-C_6H_4	b	76:24[c], 68:32[d]	96
3	c	4-CH_3O-C_6H_4	c	66:34[c,d]	90
4	d	C_2H_5	d	53:47[c], 57:43[d]	83
5	e	C_3H_7	e	51:49[c], 55:45[d]	84
6	f	$CH(CH_3)_2$	f	92:8[c,d]	92

[a]Experiments were carried out using 0.63 mmol of **1a** and 0.50 mmol of **5a–f**. [b]Yields refer to crude yields. [c]Ratio determined from ^{1}H NMR spectrum of the reaction mixture of **6** and **7**. [d]Ratio determined from ^{13}C NMR spectrum of the reaction mixture of **6** and **7**.

Table 3. Acetylation of 6,7



11 **12**

entry	6,7	R	11,12	yield 11 + 12 (%)[a]	yield 11 (%)[b]	yield 12 (%)[b]
1	a	C_6H_5	a	77	50	8
2	b	4-CH_3-C_6H_4	b	81	46	23
3	c	4-CH_3O-C_6H_4	c	77	45	*c*
4	d	C_2H_5	d	76	35	16
5	e	C_3H_7	e	85	38	36
6	f	$CH(CH_3)_2$	f	68	45	*c*

[a]Yields refer to crude yield of the acetylation step. [b]Yields refer to isolated products after flash chromatography. [c]**12c** and **12f** Could not be isolated in pure form.

The yields were between 78% and 93%, and the ratio of the two isomers ranged from 73:27 to 83:17.

It is assumed that the reaction starts with the laccase-catalyzed oxidation of 3-methylcatechol (**1b**) to **8b** followed by a nucleophilic 1,4-attack of the thiol group of **5a–f** at either C-4 or C-5 of *o*-methyl benzoquinone (**8b**) (Scheme 3). Attack at C-5 results in the formation of a tautomeric mixture of **15A** and **15B**. After oxidation to **16A** and **16B**, respectively, the intramolecular 1,4-addition delivers the corresponding type IA products **13** and type IIA products **17**, respectively. In contrast, the nucleophilic addition of **5** at C-4 of **8b** would give **18A** and **18B**, which after oxidation to **19A** and **19B**, respectively, and final intramolecular 1,4-addition would produce type IB products **14** and type IIB products **20**, respectively. In accordance with previous results regarding the nucleophilic 1,4-addition of *C*- and *S*-nucleophiles to *o*-methyl benzoquinones,[12f,g,j,29] it was no surprise that the 1,4-addition of **5a–f** at C-5 of **8b** is preferred over the 1,4-addition at C-4 of **8b**. The observation that **15A**/**15B** selectively delivers the type IA products **13** may be attributed to (a) the higher reactivity of *N*-3 (marked in blue) in comparison to *N*-1 (marked in green) and (b) the steric interactions between the CH_3 group of the *o*-benzoquinone moiety and the R group in **16B**. It should be mentioned that the oxidation/intramolecular 1,4-addition sequence starting from **18A**/**18B** results in the formation of the type IB products **14**. Type IIB products **20** could not be detected. The formation of type IB products **14** may be due to the fact that *N*-3 (marked in blue) in **19A** is more reactive than *N*-1 (marked in green) (Scheme 3).

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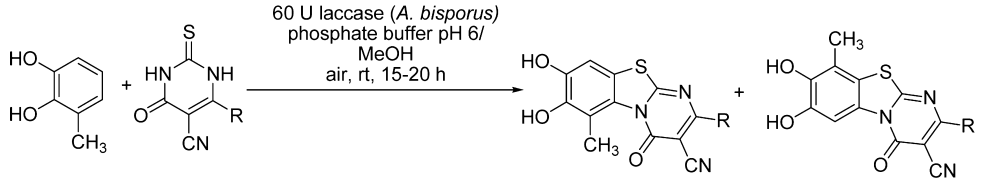
Table 4. Laccase-Catalyzed Domino Reaction between 3-Methylcatechol (1b) and 6-Substituted 1,2,3,4-Tetrahydro-4-oxo-2-thioxo-5-pyrimidinecarbonitriles 5a−f

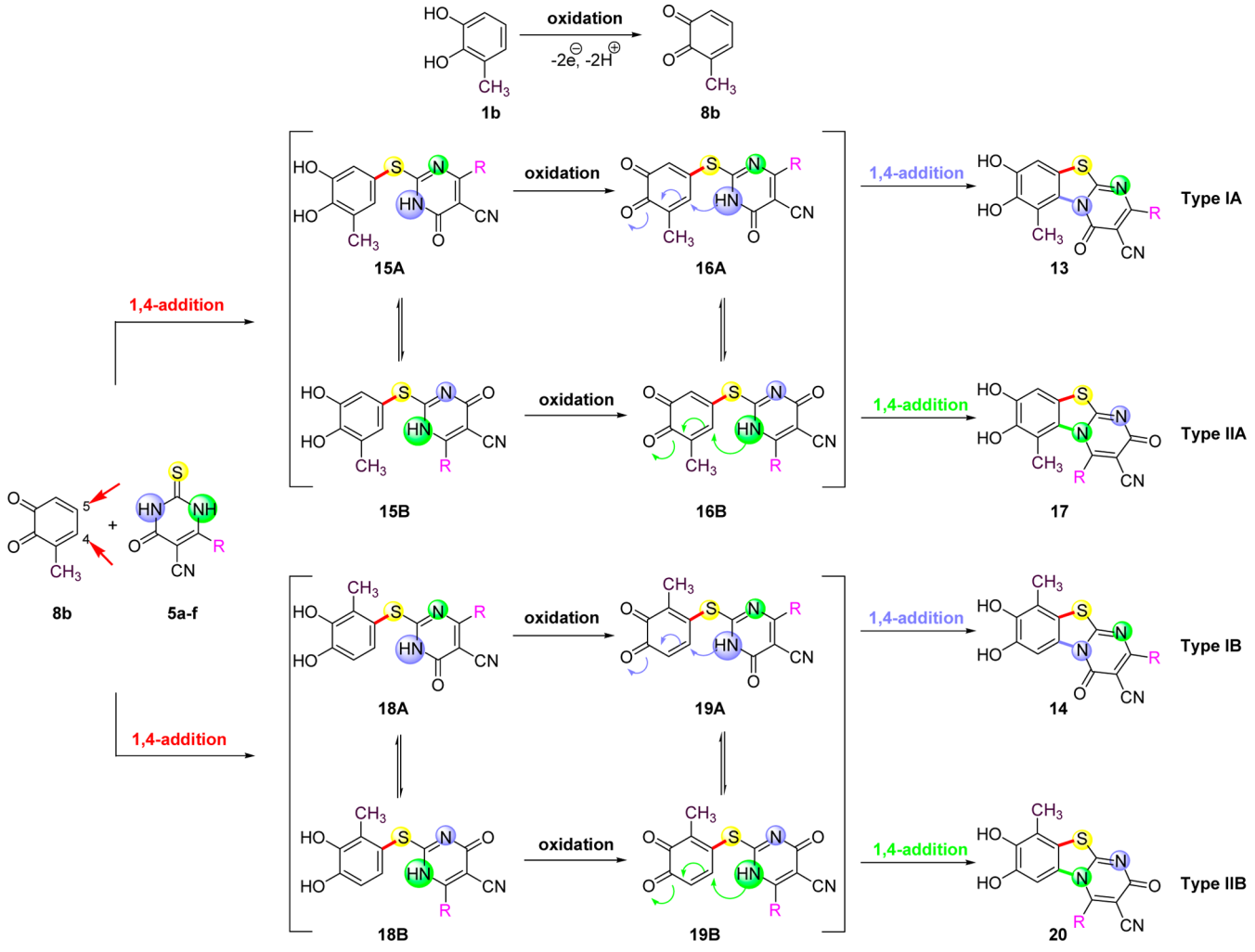


1b **5a-f** **13a-f** **14a-f**

entry	5	R	13,14	13:14[a]	yield 13 + 14 (%)[b]
1	a	C_6H_5	a	overlapped peaks	91
2	b	4-CH_3-C_6H_4	b	82:18	93
3	c	4-CH_3O-C_6H_4	c	overlapped peaks	89
4	d	C_2H_5	d	80:20[c]	82
5	e	C_3H_7	e	83:17[c]	78
6	f	$CH(CH_3)_2$	f	73:27	85

[a]Ratio determined from ^{1}H NMR spectrum of the reaction mixture of **13** and **14**. [b]Yields refer to crude yields. [c]The ^{1}H NMR spectra of **13d/14d** and **13e/14e** revealed the presence of traces of a third compound of unknown structure.

Scheme 3. Possible Mechanism for the Reaction of 3-Methylcatechol (1b) with 6-Substituted 1,2,3,4-Tetrahydro-4-oxo-2-thioxo-5-pyrimidinecarbonitriles 5a−f



Since we did not succeed in separating the highly polar isomeric pyrimidobenzothiazoles **13** and **14**, they were reacted with acetic anhydride and catalytic amounts of DMAP to yield mixtures of the corresponding diacetates **21a−f** and **22a−f** with yields between 70% and 78% (Table 5). Despite an increase in solubility, the diacetates **21a−f** and

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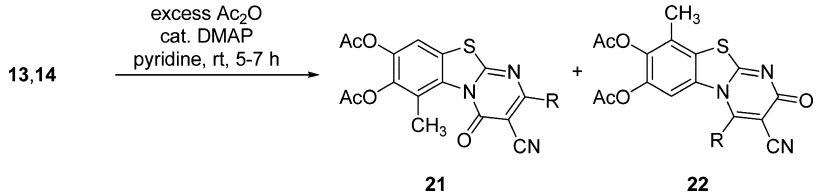
Table 5. Acetylation of 13,14



entry	13,14	R	21,22	yield 21 + 22 (%)[a]	21:22	yield 21 + 22 (%)[b]
1	**a**	C_6H_5	**a**	78	90:10[c]	53
2	**b**	4-CH_3-C_6H_4	**b**	76	84:16[c]	43
3	**c**	4-CH_3O-C_6H_4	**c**	70	87:13[c,d]	53
4	**d**	C_2H_5	**d**	72	85:15[c,d]	41
5	**e**	C_3H_7	**e**	76	87:13[c], 81:19[d]	57
6	f	$CH(CH_3)_2$	**f**	75	77:23[c,d]	58

[a]Yields refer to crude yield. [b]Yields refer to isolated yield of the mixtures of **21** and **22**. [c]Ratio determined from ^{13}C NMR spectrum of the mixture of **21** and **22** after column chromatography. [d]Ratio determined from ^{1}H NMR spectrum of the mixture of **21** and **22** after column chromatography.

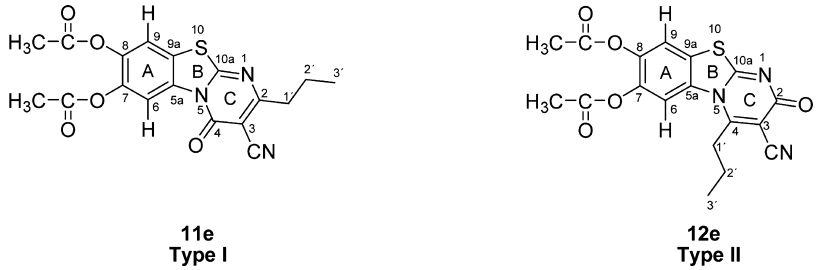


Figure 3. Structures of the two regioisomers **11e** and **12e**.

22a–f could not be separated by TLC or column chromatography.

Structure Elucidation by NMR. Preliminary analysis of the ^{1}H NMR spectra of **11,12** and **21,22**, respectively, revealed that the laccase-catalyzed reactions between catechols **1** and 6-substituted 1,2,3,4-tetrahydro-4-oxo-2-thioxo-5-pyrimidinecarbonitriles **5a–f** produces two regioisomeric pyrimidobenzothiazoles **6,7** and **13,14**, respectively (Tables 2 and 4). However, their structural identification was challenging since single crystals for X-ray analysis could not be obtained so far. The limited solubility of the acetylated reaction products **11,12** and **21,22** in almost all NMR solvents did not allow insensitive $^{13}C–^{13}C$ and/or $^{13}C–^{15}N$ NMR correlation spectroscopy at natural abundance to unambiguously assign the complete heterocyclic skeleton of the compounds. Moreover, all possible tricyclic derivatives suffer from a lack of aromatic protons in such a way that classical NMR methods (^{1}H, selective NOE/ROE, standard gHMBC) can provide only partial information for assignment purposes but not sufficient information for resolving regioselectivity issues. As an example, the two regioisomers **11e** and **12e** (Figure 3) possess only 2 aromatic protons in ring A and none in rings B and C (Figure 3).

This raised the question whether there is any NMR-based solution to differentiate between the two regioisomers without tedious derivatization and/or degradation reactions. Unfortunately, NOE experiments with the major isomer **11e** displayed no NOE enhancements at all between the propyl protons and the aromatic protons of ring A. This is due to the fact that their spatial distance is greater than 5 Å. A differentiation of the isomers by comparison of the ^{13}C chemical shifts of the C rings of **11e** (δ 172.6, 166.0, 158.1, 93.5 ppm) and **12e** (δ 166.6, 162.3, 160.4, 98.6 ppm) was also impossible since (i) the values were of the same order of magnitude, (ii) the ^{13}C chemical shifts could not easily be assigned by, e.g., standard HMBC, and (iii) there were no ^{13}C NMR data of similar structures in the literature available. The latter holds true also for ^{15}N chemical shift considerations. Nevertheless, we found a fast and reliable solution for the unequivocal determination of the regioselectivity by indirect detection of $^{1}H–^{15}N$ long-range gHMBC correlations, which could be recorded in 24–40 h without cryo probe technology despite the limited solubility of the compounds (ca. 10–50 mg/mL). The underlying idea is this: if we detect $^{1}H–^{15}N$ long-range correlations with two different ^{15}N chemical shifts (irrespective of the value itself), one of them originating from a ^{3}J and ^{4}J correlation of the two aromatic protons of ring A and the other from a ^{3}J correlation of the propyl protons, then the structure of the major regioisomer has to be **11e** (Figure 4 A). However, if the $^{1}H–^{15}N$ gHMBC spectrum displays long-range correlations of the aromatic protons of ring A and the propyl protons to only one (= the same) ^{15}N chemical shift, then the structure of the minor regioisomer has to be **12e** (Figure 4 B). Using this approach, both the C-2/C-4 substitution issue and the regioselectivity of the right part of the molecule (ring C) of all regioisomers **11** and **12** as well as **21** could be unequivocally determined.

The next question was how to prove the connectivity between rings A and C of the heterocyclic isomers. In case of the bisacetylated reaction products **21** and **22** this was a particularly difficult problem since both of them have only a single aromatic proton. A prerequisite for solving this problem was a fully assigned carbon skeleton with the focus on the quaternary carbons C-10a, C-4, C-5a, and C-9a. In the standard $^{1}H–^{13}C$ gHMBC (optimized for J_{CH} = 8 Hz) of, for example, **11e** each of the latter two at δ 122.2 and 132.8 (or vice versa) ppm showed

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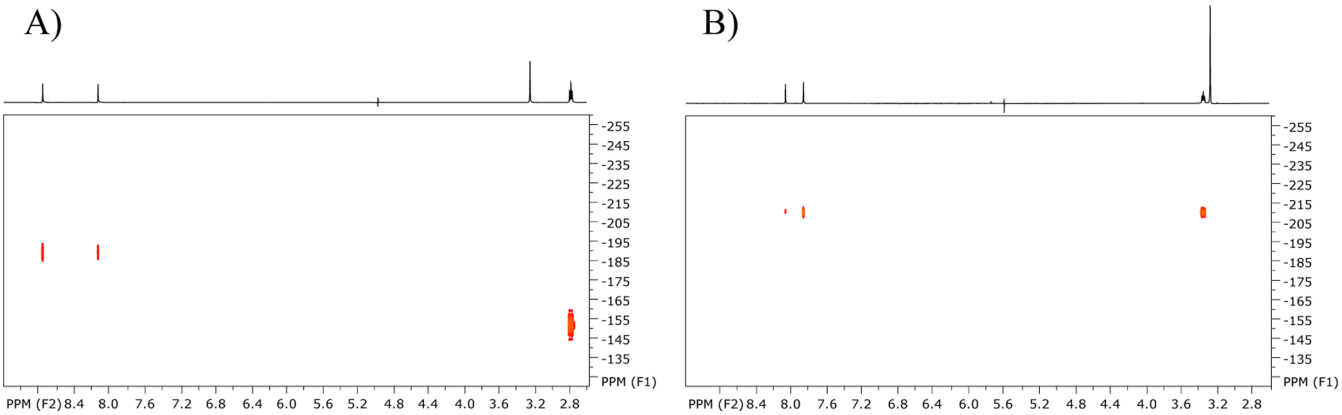

Figure 4. Part of the $^{1}H-^{15}N$ gHMBC spectrum of **11e** (A) and **12e** (B) at natural abundance.

two $^{1}H-^{13}C$ long-range correlations to both aromatic ring A protons at δ 8.77 and 8.14 ppm. This precludes any assignment of the respective positions (Figures 5, 6A). However, a gHMBC pulse sequence modified according to Seto et al.[30] enabled the detection of weak $^{1}H-^{13}C$ super long-range correlations ($^{4}J_{CH}$, $^{5}J_{CH}$, or even higher) in lieu of only $^{2}J_{CH}$ and $^{3}J_{CH}$ couplings. Thus, the aromatic proton at δ 8.14 ppm exhibits two additional $^{1}H-^{13}C$ super long-range correlations to the quaternary carbons at δ 158.1 and 166.0 ppm, whereas the aromatic proton at δ 8.77 ppm showed two additional $^{1}H-^{13}C$ super long-range correlations to two quaternary carbons at δ 158.1 and C-3 at δ 93.5 ppm. On the basis of a ^{5}J coupling between 6-H and C-3 rather than a ^{6}J coupling (between 9-H and C-3) that can hardly be observed, the proton at δ 8.77 ppm was assigned as 6-H and the proton at δ 8.14 ppm as 9-H (Figures 5, 6B).

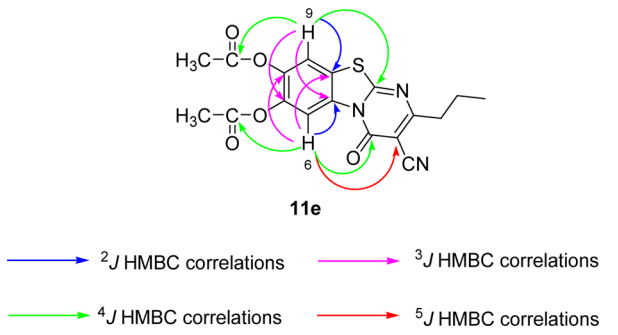

Figure 5. Important ^{2}J, ^{3}J, ^{4}J, and ^{5}J HMBC correlations from 6-H and 9-H of **11e**.

Evaluation of the experimental $^{1}H-^{13}C$ long-range coupling constants (absolute values) between 9-H and C-5a (^{3}J = 9.3 Hz) and C-9a (^{2}J = 3.0 Hz) as well as between 6-H and C-5a (^{2}J = 4.2 Hz) and C-9a (^{3}J = 8.8 Hz) allowed the assignment of the carbons C-5a and C-9a at δ 132.8 and 122.2 ppm, respectively, since $^{3}J_{CH}$ coupling constants (absolute values) in aromatic ring systems are significantly greater than $^{2}J_{CH}$ and $^{4}J_{CH}$ coupling constants (Figure 7).[12g,31]

In order to support the experimental assignments, we computed the ^{13}C NMR chemical shifts by DFT quantum mechanical DFT calculations at the DFT GIAO mPW1PW91/6-311+G(2d,p)//mPW1PW91/6-31G(d) level of theory[32] and found that the calculated values are in good agreement with the experimental data for C-5a (δ calcd 132.3, exptl 132.8 ppm) and C-9a (δ calcd 124.9, exptl 122.2 ppm). The remaining quaternary carbons C-4 and C-10a were assigned on the basis of computationally calculated ^{13}C chemical shifts of C-4 (δ calcd 158.3, exptl 158.1 ppm) and of C-10a (δ calcd 166.6, exptl 166.0 ppm), as the observed super long-range $^{1}H-^{13}C$ HMBC correlations of δ 158.1 and 166.0 ppm didn't allow an unambiguous assignment of C-4 and C-10a.

The structures of the minor isomers **12a,b,d,e** were elucidated and fully assigned in the same way as described for compounds **11a–f**. Fortunately, strong ROEs between the aromatic proton 6-H and the protons of the side chain at C-4 in the minor compounds **12a,b,d,e** confirmed our concept of the differentiation between the

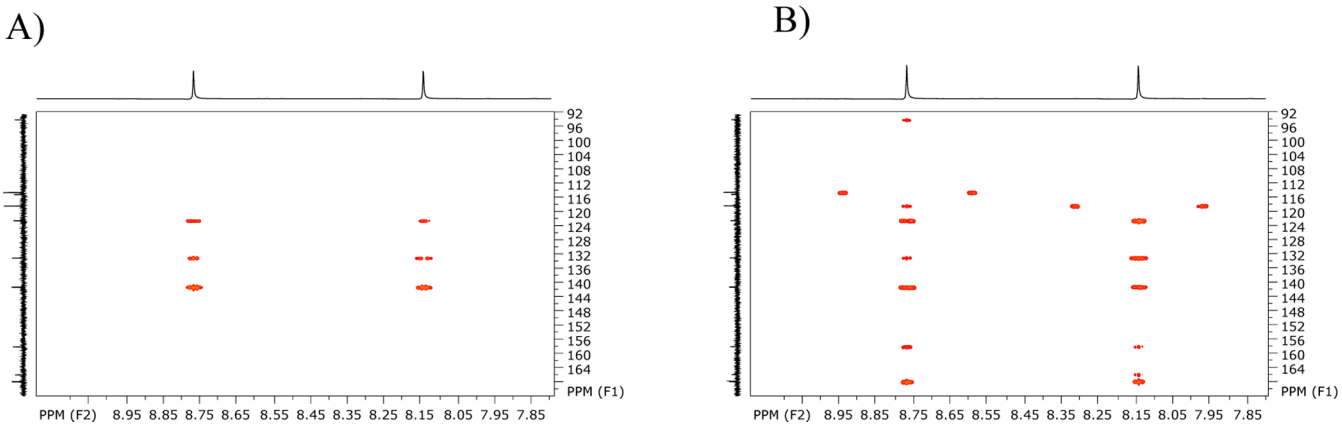

Figure 6. Part of the normal (A) and the super long-range (B) $^{1}H-^{13}C$ gHMBC spectrum of **11e**.

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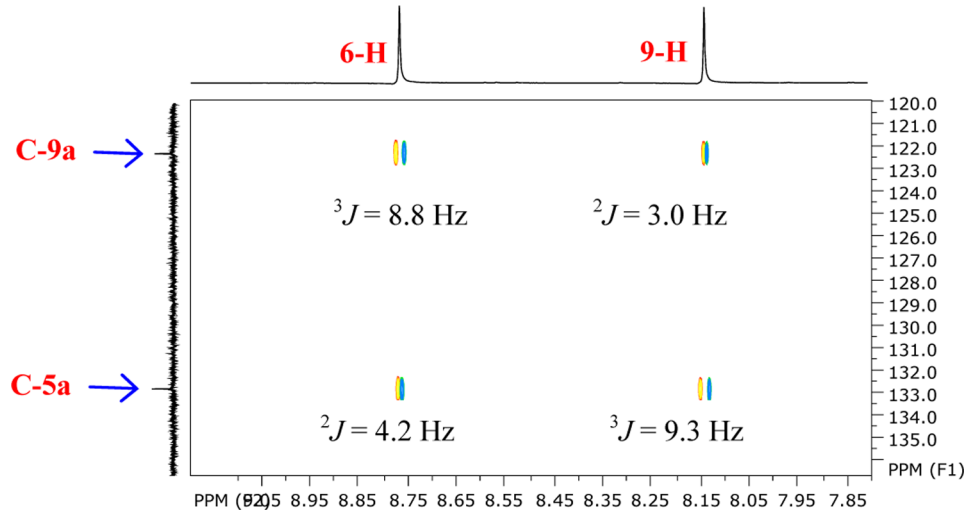

Figure 7. Part of the HSQMBC spectrum of **11e**.

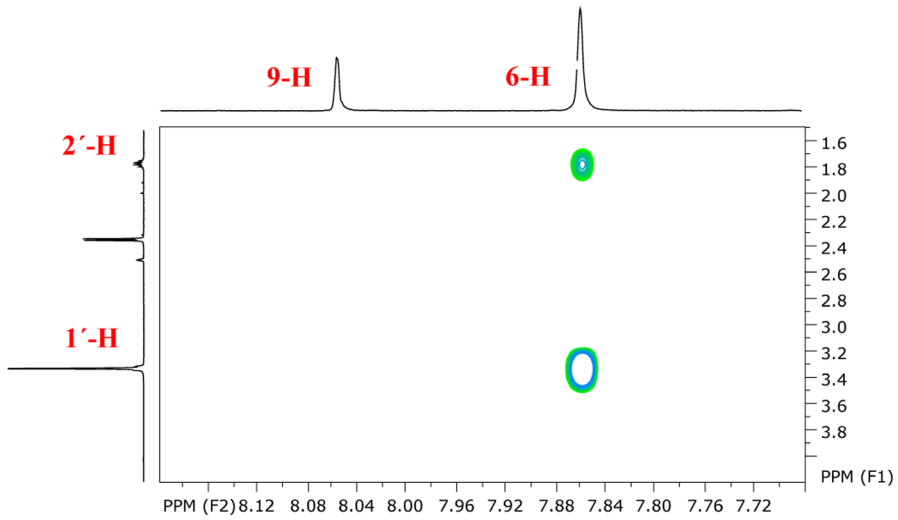

Figure 8. Part of the ROESY spectrum of **12e**.

regioisomers **11** and **12** based on the presence of one or two indirectly detectable ^{15}N chemical shifts (Figure 8).

Again, the calculated ^{13}C chemical shifts are in a good agreement with the experimental data: **12e** C-10a (δ calcd 164.5, exptl 166.6 ppm), C-2 (δ calcd 158.4, exptl 162.3 ppm), C-4 (δ calcd 160.7, exptl 160.4 ppm), C-5a (δ calcd 130.7, exptl 132.8 ppm), C-9a (δ calcd 125.6, exptl 121.8 ppm). Interestingly, in compounds **12a,b** a strong shielding of the aromatic proton 6-H (e.g δ 5.54 ppm in **12a**) induced by the ring current effect of the aryl moiety indicate the spatial proximity between 6-H and the phenyl substituent at C-4 (Figure 9). Moreover, the aryl groups caused ^{13}C chemical shift differences of ca. 7 ppm of C-2 in compounds **11a−c** compared to **11d−f** and ca. 4 ppm of C-4 in compounds **12a,b** compared to **12d,e**. The ^{13}C chemical shift calculations of C-2 (δ calcd 166.1, exptl 165.6 ppm) of, e.g., the phenyl-substituted compounds **11a** and C-4 (δ calcd 156.1, exptl 156.7 ppm) of, e.g., **12a** also support this observation.

In the reactions of the unsymmetrical catechol **1b** with **5a−f**, theoretically four different regioisomers can be obtained (Scheme 3). However, the ^{1}H NMR spectra of the crude products exhibited in most cases a mixture of two regioisomers. Acetylation of the crude products **13,14** did not help in the separation of the two isomers but caused a remarkable increase in solubility. Let us consider the regioisomers obtained from the reaction of **1b** with **5e**: here the whole molecule is represented by a single aromatic proton (Figure 10).

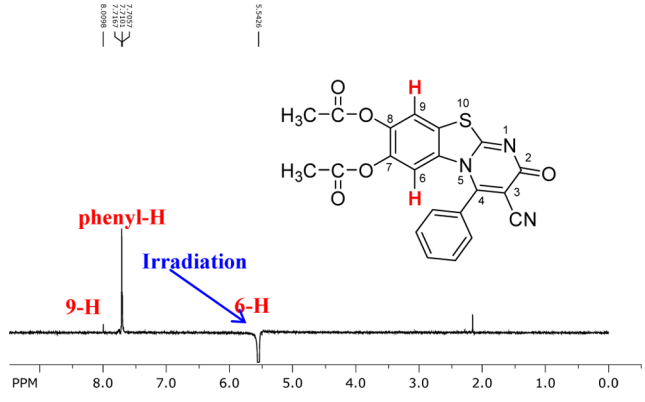

Figure 9. Selective 1D DPFG NOESY spectrum of **12a**.

In the ^{1}H−^{15}N gHMBC spectrum of the mixture of **21e,22e** the major isomer **21e** showed long-range correlations with two

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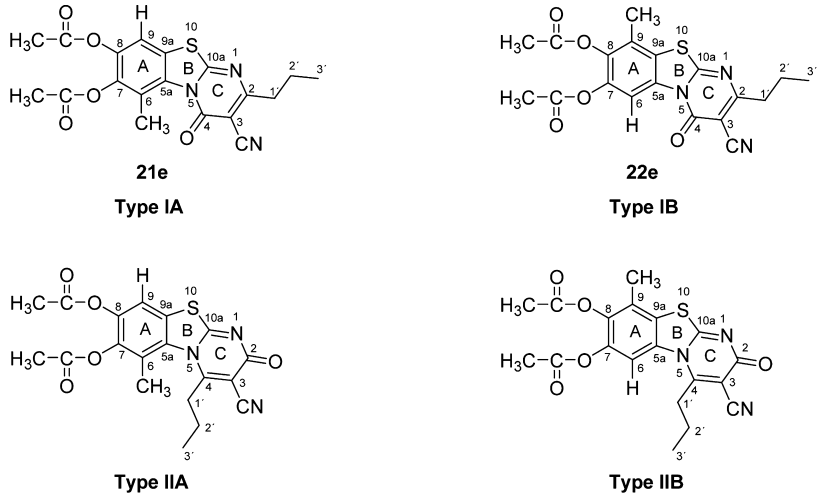

Figure 10. Structures of the four possible regioisomers obtained from the reaction of **1b** with **5e**.

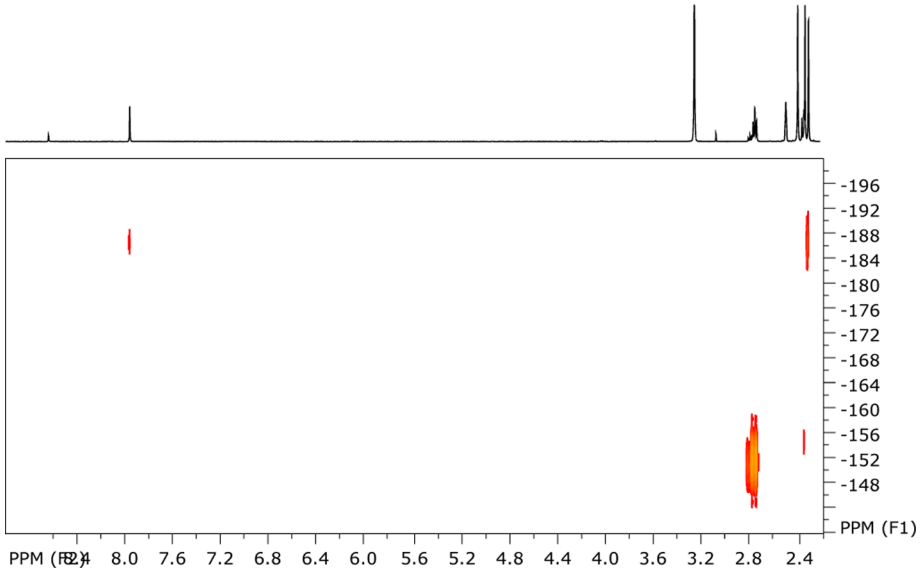

Figure 11. Part of the $^{1}H-^{15}N$ gHMBC spectrum of the mixture of **21e** and **22e**.

different ^{15}N chemical shifts indicating either a structure of type IA or of type IB (Figure 11). Unfortunately, due to the low concentration in the mixture no $^{1}H-^{15}N$ long-range correlations could be observed for the minor isomer **22e**.

The question of whether the minor isomer has the same arrangement in ring C as the major one or the alternative arrangement of type IIA or type IIB products could be answered as follows: As previously observed for compounds **11** and **12**, the ^{13}C chemical shift of the quaternary ring C carbon directly bonded to the side chain differ significantly in size ($\Delta\delta \approx 10$ ppm) depending on type I/II regioselectivity, e.g., δ 172.6 ppm (C-2) in **11e** and δ 160.4 ppm (C-4) in **12e**. Thus, the unambiguous identification (gHMBC) of the ring C carbon to which the side chain is attached and comparison of its ^{13}C chemical shift with the above values provides another possibility for a type I/II differentiation in the case of missing $^{1}H-^{15}N$ long-range correlations. In the $^{1}H-^{13}C$ gHMBC spectrum of the mixture **21e,22e** $^{2}J_{CH}$ and $^{3}J_{CH}$ long-range correlations between the propyl protons 1′-H and 2′-H and a ring C carbon at δ 172.1 ppm (= C-2) confirmed structure type I for the major isomer **21e**. Similarly, $^{2}J_{CH}$ and $^{3}J_{CH}$ HMBC correlations of the propyl protons 1′-H and 2′-H of **22e** with a ring C carbon at δ 172.6 ppm (C-2) establish structure type I in lieu of type II for which a ^{13}C chemical shift of $\delta \approx 160$ ppm (C-4) would be expected (Figure 12).

The remaining problem of the differentiation between the structures **21e** and **22e** was unequivocally solved by analysis of the experimental $^{1}H-^{13}C$ long rang coupling constants (absolute values) between the aromatic ring A proton and the quaternary carbons C-5a and C-9a attached to N at $\delta \approx 132$ ppm and S at $\delta \approx 123$ ppm, respectively. In the HSQMBC spectrum the aromatic A ring proton of the major isomer **21e** at δ 7.97 ppm revealed a J_{CH} coupling constant of 9.5 Hz to C-5a at δ 131.9 ppm (= $^{3}J_{CH}$ coupling) as well as a J_{CH} coupling constant of 3.3 Hz to C-9a at δ 123.1 ppm (= $^{2}J_{CH}$ coupling); this is why it was assigned as 9-H (type IA product). In contrast, J_{CH} coupling constants of 3.8 Hz to C-5a at δ 132.0 ppm (= $^{2}J_{CH}$ coupling) and 9.2 Hz to C-9a at δ 122.4 ppm (= $^{3}J_{CH}$ coupling) were observed for the minor isomer **22e**, establishing a type IB structure as well as position 6-H for the aromatic A ring

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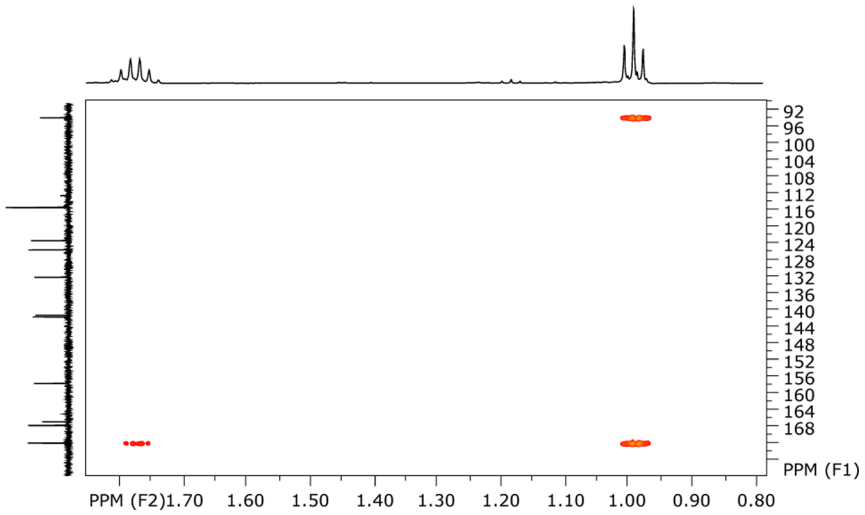

Figure 12. Part of $^{1}H-^{13}C$ gHMBC spectrum of the mixture of **21e** and **22e**.

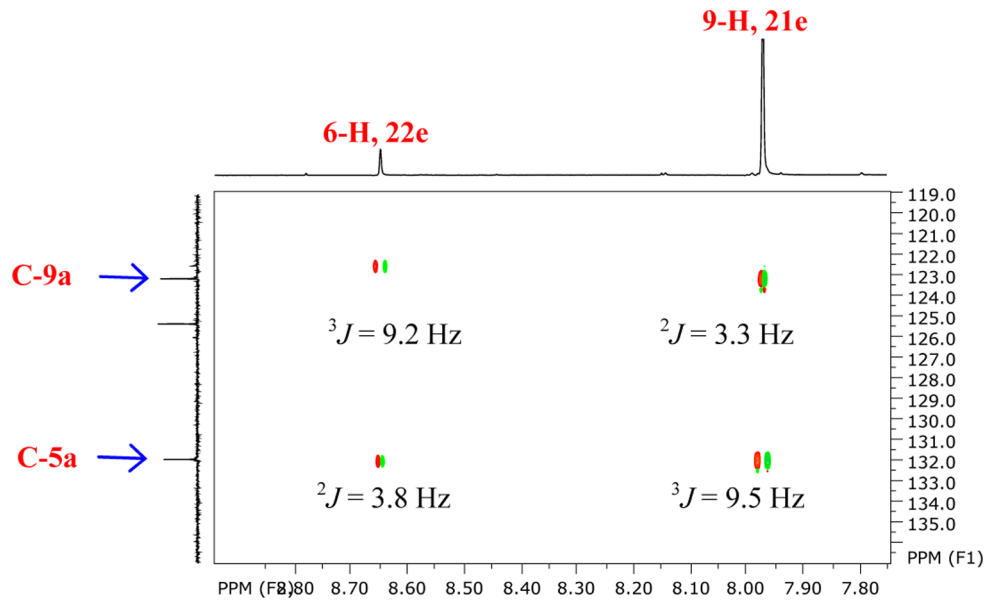

Figure 13. Part of the HSQMBC spectrum of the mixture of **21e** and **22e**.

proton at δ 8.65 ppm (Figure 13). The remaining full NMR assignment of the molecules was carried out as described for compounds **11** and **12**.

CONCLUSION

In summary, we have developed a method for the synthesis of pyrimidobenzothiazole derivatives by a laccase-catalyzed domino reaction between catechols and 6-substituted 1,2,3,4-tetrahydro-4-oxo-2-thioxo-5-pyrimidinecarbonitriles. The method makes use of aerial oxygen as an oxidant to initiate the domino reaction, which can be interpreted as a domino oxidation/intermolecular 1,4-addition/oxidation/intramolecular 1,4-addition. The transformations can be performed under mild conditions and deliver the corresponding pyrimidobenzothiazoles with yields up to 96%. The regioselectivity of the process depends both on the structure of the catechols and the structure of the substituents of the 6-substituted 1,2,3,4-tetrahydro-4-oxo-2-thioxo-5-pyrimidinecarbonitriles. Complete structure elucidation of the regioisomeric ring-proton deficient pyrimidobenzothiazoles, which could hardly be assessed by standard NMR methods, was achieved and was based on $^{1}H-^{15}N$ HMBC NMR correlations at natural abundance and experimental $^{1}H-^{13}C$ long-range coupling constants in addition to super long-range $^{1}H-^{13}C$ HMBC correlations, as well as calculated ^{13}C chemical shifts.

EXPERIMENTAL SECTION

General Remarks. All commercially available reagents were used without further purification. Laccase from *A. bisporus* (6 U/mg, ASA Spezialenzyme GmbH, Wolfenbüttel) is commercially available. Solvents used for extraction and purification were distilled prior to use. Thin-layer chromatography (TLC) was performed on TLC silica gel 60 F_{254}. Reaction temperatures are reported as bath temperatures. Products were purified either by crystallization or by flash column chromatography on silica gel. Melting points were recorded on a melting point apparatus with open capillary tubes and are uncorrected. IR spectra were measured using ATR insturment. UV−vis spectra were recorded with a spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded at (500/125 and 300/75 MHz) in DMSO-d_6 at room temperature or at 40 °C. The ^{1}H and ^{13}C chemical shifts were referenced to residual solvent signals at δ_H = 2.49 and δ_C = 39.5 (DMSO-d_6) relative

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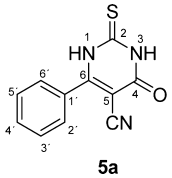
to TMS. Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), sex (sextet), sep (septet), m (multiplet), and br (broad). 1D (^{1}H, ^{13}C) and 2D NMR (COSY, ROESY, gHSQCAD, gHMBCAD) measurements were performed using standard pulse sequences. HSQMBC parameters: sw = 4000−6000 Hz, sw1 = 8000−12000 Hz, np = 8192, fn = 16384, jnxh = 8, ni = 128−512, nt = 16−64, d1 = 1s, 40 °C. Linear prediction in f1. Super long-range band selective gHMBC parameters: sw = 5000 Hz, sw1 = 11000−13000 Hz, np = 2048, fn = 4096, jnxh = 1 Hz, ni = 64−128, d1 = 1s, nt = 24−96, 40 °C. Linear prediction in f1. ^{15}N chemical shifts were indirectly determined using ^{1}H−^{15}N gc2HMBC (50.7 MHz). ^{1}H−^{15}N gc2HMBC (Chempack 4.1) parameter: sw = 5000 Hz, sw1 = 10136 Hz, np = 4096, fn = 8192, jnxh = 3 Hz, ni = 32−64, d1 = 1s, nt = 600−1200, 40 °C. Nitromethane (100 μL) in DMSO-d_6 (25 μL) in a capillary was used as an external standard (δ = 0 ppm). Copies of the NMR spectra were prepared using SpinWorks.[33] Mass spectra and high resolution mass spectra were recorded using either EI method or ESI method. The intensities are reported as percentages relative to the base peak (I = 100%).

Computational Studies. All calculations reported in this paper were performed within density functional theory, using the Gaussian 03 package.[32d] ^{13}C NMR chemical shifts of selected compounds **11a**, **11e**, **12a** and **12e** were calculated as follows: the rigid structures were optimized with the MM2 force field implemented in Chem3D Pro.[34] In the second step, the optimized structures were subsequently reoptimized at the AM1 level followed by the RHF/3-21G level and finally by the B3LYP/6-31G(d) level of theory within the Gaussian 03 package. In the final step, the gas-phase ^{13}C NMR chemical shifts of the reoptimized geometries were computed at the mPW1PW91/6-311+G(2d,p)//mPW1PW91/6-31G(d) level of theory. The references TMS and benzene for the MSTD approach according to Sarotti and Pellegrinet[32e] were computed in the same manner as for the aforementioned selected compounds. Theoretical ^{13}C NMR chemical shifts (δ_a) were derived by the following equation: $\delta_a = \sigma_{ref} - \sigma_a + \delta_{ref}$ where σ_{ref} and σ_a are the calculated NMR isotropic magnetic shielding tensors of the reference compound and carbon a of the compound of interest: σ_{TMS} = 186.965 and $\sigma_{benzene}$ = 54.4127 at the mPW1PW91/6-311+G(2d,p)// mPW1PW91/6-31G(d) level; δ_{ref} represents the chemical shift of the reference compound δ_{TMS} = 0 ppm; $\delta_{benzene}$ = 128.5 ppm (benzene plus TMS at 125 MHz). An HP Compaq with a 2.39 GHz processor and 2 GB of RAM was used for the calculations.

General Procedure I for the Synthesis of 6-Substituted 1,2,3,4-Tetrahydro-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (5a−f).[26] An oven-dried 10-mL vial with a magnetic stir bar was charged with a mixture of an aldehyde **2** (1 mmol), thiourea (**3**) (1 mmol, 76 mg), methyl cyanoacetate (**4**) (1 mmol, 99 mg), and K_2CO_3 (1 mmol, 138 mg) in methanol (2.5 mL). The vial was sealed, and the reaction mixture was left stirring at 80 °C for 7 h. After completion of the reaction, the potassium salt of **5a−f** that precipitates during the reaction was collected and washed with methanol. The crude solid was stirred in water at ∼80 °C until a clear solution was obtained. After cooling, the solution was acidified with acetic acid, and stirring was continued for 30 min. The deposited precipitate was collected to give solid crude product, which was further purified by recrystallization from methanol to yield **5a−f**.

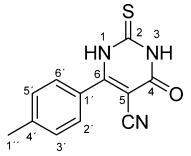
*1,2,3,4-Tetrahydro-4-oxo-6-phenyl-2-thioxo-5-pyrimidinecarbonitrile (**5a**).*



5a

According to the general procedure I, a mixture of benzaldehyde (**2a**) (106 mg, 1 mmol), thiourea (**3**) (76 mg, 1 mmol), methyl cyanoacetate (**4**) (99 mg, 1 mmol), and K_2CO_3 (138 mg, 1 mmol) was reacted for 7 h. Purification gave 1,2,3,4-tetrahydro-4-oxo-6-phenyl-2-thioxo-5-pyrimidinecarbonitrile (**5a**) as a white solid in 36% yield (83 mg, 0.36 mmol): mp 298−300 °C (lit.[26] 300−302 °C); R_f = 0.26 (CH_2Cl_2/EtOAc = 4:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.53−7.61 (m, 3H, 3′-H, 4′-H and 5′-H), 7.63−7.68 (m, 2H, 2′-H and 6′-H), 13.16 (s, 2H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 85.1, 118.9, 127.9, 128.2, 130.0, 137.7, 162.5, 167.3, 183.0; MS (EI, 70 eV) m/z (%) 229 (100) [M]$^+$, 201 (12).

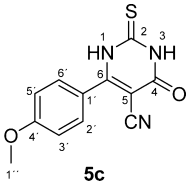
*1,2,3,4-Tetrahydro-6-(4-methylphenyl)-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (**5b**).*



5b

According to the general procedure I, a mixture of 4-methylbenzaldehyde (**2b**) (120 mg, 1 mmol), thiourea (**3**) (76 mg, 1 mmol), methyl cyanoacetate (**4**) (99 mg, 1 mmol), and K_2CO_3 (138 mg, 1 mmol) was reacted for 7 h. Purification gave 1,2,3,4-tetrahydro-6-(4-methylphenyl)-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (**5b**) as a white solid in 35% yield (86 mg, 0.35 mmol): mp 289−291 °C (lit.[26] 290−291 °C); R_f = 0.28 (CH_2Cl_2/EtOAc = 4:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.39 (s, 3H, 1″-H), 7.36 (d, ^3J (2′-H, 3′-H) = 8.1 Hz, ^3J (5′-H, 6′-H) = 8.1 Hz, 2H, 3′-H and 5′-H), 7.57 (d, ^3J (2′-H, 3′-H) = 8.1 Hz, ^3J (5′-H, 6′-H) = 8.1 Hz, 2H, 2′-H and 6′-H), 13.08 (s, 2H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.1, 90.2, 115.0, 126.6, 128.7, 129.0, 142.4, 158.7, 161.1, 176.4; MS (EI, 70 eV) m/z (%) 243 (68) [M]$^+$, 195 (100), 167 (24), 118 (28).

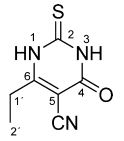
*1,2,3,4-Tetrahydro-6-(4-methoxyphenyl)-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (**5c**).*



5c

According to the general procedure I, a mixture of 4-methoxybenzaldehyde (**2c**) (136 mg, 1 mmol), thiourea (**3**) (76 mg, 1 mmol), methyl cyanoacetate (**4**) (99 mg, 1 mmol), and K_2CO_3 (138 mg, 1 mmol) was reacted for 7 h. Purification gave 1,2,3,4-tetrahydro-6-(4-methoxyphenyl)-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (**5c**) as a white solid in 26% yield (67 mg, 0.26 mmol): mp 280−282 °C (lit.[26] 280−281 °C); R_f = 0.30 (CH_2Cl_2/EtOAc = 4:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.84 (s, 3H, 1″-H), 7.10 (d, ^3J (2′-H, 3′-H) = 8.9 Hz, ^3J (5′-H, 6′-H) = 8.9 Hz, 2H, 3′-H and 5′-H), 7.65 (d, ^3J (2′-H, 3′-H) = 8.9 Hz, ^3J (5′-H, 6′-H) = 8.9 Hz, 2H, 2′-H and 6′-H), 13.07 (s, 2H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 55.3, 84.3, 113.3, 119.2, 129.6, 130.0, 160.9, 162.6, 166.3, 182.4.

*1,2,3,4-Tetrahydro-6-ethyl-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (**5d**).*



5d

According to the general procedure I, a mixture of propionaldehyde (**2d**) (58 mg, 1 mmol), thiourea (**3**) (76 mg, 1 mmol), methyl cyanoacetate (**4**) (99 mg, 1 mmol), and K_2CO_3 (138 mg, 1 mmol) was reacted for 7 h. Purification gave 1,2,3,4-tetrahydro-6-ethyl-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (**5d**) as a white solid in 25% yield (45 mg, 0.25 mmol): mp 257−259 °C (lit.[26] 258−259 °C); R_f = 0.30 (CH_2Cl_2/EtOAc = 4:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (t, ^3J (1′-H, 2′-H) = 7.5 Hz, 3H, 2′-H), 2.56 (q, ^3J (1′-H, 2′-H) = 7.5 Hz, 2H, 1′-H), 13.03 (s, 2H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.4, 25.5, 90.0, 114.0, 158.3, 166.8, 176.2; MS (EI, 70 eV) m/z (%) 181 (100) [M]$^+$, 123 (32), 56 (48).

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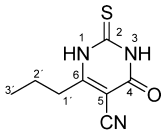
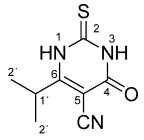
*1,2,3,4-Tetrahydro-4-oxo-6-propyl-2-thioxo-5-pyrimidinecarbonitrile (**5e**).*



5e

According to the general procedure I, a mixture of butyraldehyde (**2e**) (72 mg, 1 mmol), thiourea (**3**) (76 mg, 1 mmol), methyl cyanoacetate (**4**) (99 mg, 1 mmol), and K_2CO_3 (138 mg, 1 mmol) was reacted for 7 h. Purification gave 1,2,3,4-tetrahydro-4-oxo-6-propyl-2-thioxo-5-pyrimidinecarbonitrile (**5e**) as a white solid in 25% yield (49 mg, 0.25 mmol): mp 276−278 °C (lit.[26] 260−261 °C); R_f = 0.50 (CH_2Cl_2/EtOAc = 4:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.93 (t, ^3J (2′-H, 3′-H) = 7.5 Hz, 3H, 3′-H), 1.64 (sex, ^3J (1′-H, 2′-H) = 7.5 Hz, ^3J (2′-H, 3′-H) = 7.5 Hz, 2H, 2′-H), 2.54 (t, ^3J (1′-H, 2′-H) = 7.5 Hz, 2H, 1′-H), 13.02 (br, 2H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.3, 21.3, 33.5, 90.6, 114.2, 158.3, 165.3, 176.2; MS (EI, 70 eV) m/z (%) 195 (100) $[M]^+$, 180 (29) and 137 (12); HRMS calcd for $C_8H_9N_3OS$ (195.0466), found 195.0444.

*1,2,3,4-Tetrahydro-6-isopropyl-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (**5f**).*

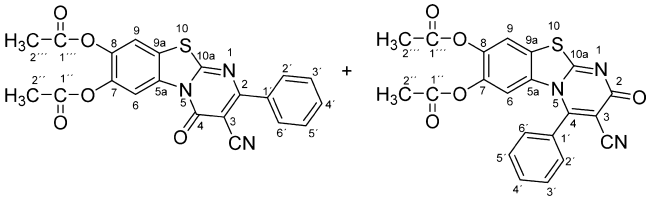


5f

According to the general procedure I, a mixture of isobutyraldehyde (**2f**) (72 mg, 1 mmol), thiourea (**3**) (76 mg, 1 mmol), methyl cyanoacetate (**4**) (99 mg, 1 mmol), and K_2CO_3 (138 mg, 1 mmol) was reacted for 7 h. Purification gave 1,2,3,4-tetrahydro-6-isopropyl-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (**5f**) as a white solid in 32% yield (62 mg, 0.32 mmol): mp 250−252 °C; R_f = 0.40 (CH_2Cl_2/EtOAc = 4:1); UV (MeCN) λ_{max} (log ε) 204 (4.31), 215 (4.20), 271 (4.15), 312 nm (4.28); IR (ATR) $\tilde{\nu}$ 3256 (NH), 3155 (NH), 2981 (CH), 2936 (CH), 2226 (CN), 1676 (C═O), 1546, 1202 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29 (d, ^3J (1′-H, 2′-H) = 7.0 Hz, 6H, 2′-H), 3.03 (sept, ^3J (1′-H, 2′-H) = 7.0 Hz, 1H, 1′-H), 12.77 (s, 1H, NH), 13.05 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 18.8, 32.2, 89.2, 114.1, 158.5, 169.4, 176.4; MS (EI, 70 eV) m/z (%) 195 (100) $[M]^+$, 180 (29), 137 (12); HRMS calcd for $C_8H_9N_3OS$ (195.0466), found 195.0439.

General Procedure II for the Laccase-Catalyzed Domino Reaction. A 100-mL round-bottomed flask with a magnetic stir bar was charged with a solution or suspension of the catechol **1** (0.63 mmol) and the 6-substituted 1,2,3,4-tetrahydro-4-oxo-2-thioxo-5-pyrimidinecarbonitrile **5** (0.50 mmol) in hot methanol (3−7 mL). Phosphate buffer (0.2 M, pH 6, 30 mL) and laccase from *A. bisporus* (10 mg, 6 U/mg) were added, and the mixture was left stirring under air at room temperature overnight (12−20 h). The reaction mixture was acidified with 2 M HCl to pH 4. The precipitated product was filtered with suction using a Buchner funnel. The filter cake obtained was washed with aq NaCl (15%, 20 mL) and dried to give the crude products **6,7** or **13,14**. The obtained mixture of isomers **6,7** or **13,14** was placed in an oven-dried 10-mL vial and charged with acetic anhydride (255 mg, 2.50 mmol) and DMAP (6 mg, 0.05 mmol). The vial was sealed, evacuated, and backfilled with argon, and then freshly distilled pyridine (2 mL) was added. The reaction mixture was stirred at room temperature for 5−7 h followed by neutralization with 2 M HCl (12 mL). The precipitated product was filtered, washed with water, and dried to give the acetylated solid crude products **11,12** or **21,22**, which were further purified by flash chromatography over silica gel using the eluent system (petroleum ether/CH_2Cl_2 = 1:1 → CH_2Cl_2/EtOAc = 1:2).

*3-Cyano-7,8-diacetyloxy-4H-2-phenyl-pyrimido[2,1-b]benzothiazol-4-one (**11a**) and 3-Cyano-7,8-diacetyloxy-2H-4-phenyl-pyrimido[2,1-b]benzothiazol-2-one (**12a**).*



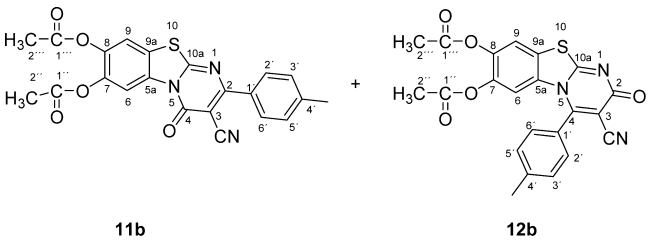
11a **12a**

According to the general procedure II, a suspension of catechol (**1a**) (69 mg, 0.63 mmol), 1,2,3,4-tetrahydro-4-oxo-6-phenyl-2-thioxo-5-pyrimidinecarbonitrile (**5a**) (115 mg, 0.50 mmol), methanol (5.5 mL), phosphate buffer (0.2 M, pH 6, 30 mL), and laccase from *A. bisporus* (10 mg, 6 U/mg) was stirred for 15 h. Workup gave a mixture of **6a** and **7a** in 95% yield (159 mg, 0.48 mmol). The obtained crude product was reacted with acetic anhydride (255 mg, 2.50 mmol) and DMAP (6 mg, 0.05 mmol) in pyridine (2 mL) under argon for 5 h. Workup gave the acetylated mixture of **11a** and **12a** in 77% (153 mg, 0.37 mmol). The obtained mixture was separated by column chromatography to give 3-cyano-7,8-diacetyloxy-4*H*-2-phenyl-pyrimido[2,1-*b*]benzothiazol-4-one (**11a**) as a white solid in 50% yield (100 mg, 0.24 mmol) and 3-cyano-7,8-diacetyloxy-2*H*-4-phenyl-pyrimido[2,1-*b*]benzothiazol-2-one (**12a**) as a pale yellow solid in 8% yield (15 mg, 0.04 mmol).

*3-Cyano-7,8-diacetyloxy-4H-2-phenyl-pyrimido[2,1-b]benzothiazol-4-one (**11a**).* Mp 262−264 °C; R_f = 0.24 (CH_2Cl_2/EtOAc = 20:1); UV (MeCN) λ_{max} (log ε) 230 (4.39), 254 (4.31), 300 (4.20), 369 nm (4.24); IR (ATR) $\tilde{\nu}$ 3113 (CH), 2935 (CH), 2217 (CN), 1780 (C═O), 1763 (C═O), 1672 (C═O), 1536, 1503, 1460, 1174 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.35 (s, 3H, 2″-H or 2‴-H), 2.36 (s, 3H, 2″-H or 2‴-H), 7.58−7.66 (m, 3H, 3′-H, 4′-H and 5′-H), 8.00−8.02 (m, 2H, 2′-H and 6′-H), 8.17 (s, 1H, 9-H), 8.82 (s, 1H, 6-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.15 (C-2″ or C-2‴), 20.20 (C-2″ or C-2‴), 91.5 (C-3), 114.3 (C-6), 115.6 (CN), 118.1 (C-9), 122.5 (C-9a), 128.6 (C-3′ and C-5′), 128.7 (C-2′ and C-6′), 131.8 (C-1′), 132.7 (C-5a), 134.7 (C-4′), 141.2 (C-7), 141.3 (C-8), 158.9 (C-4), 165.6 (C-2), 165.8 (C-10a), 167.9 (C-1‴), 168.1 (C-1″); ^{15}N NMR (indirectly determined, 50.7 MHz, DMSO-d_6) δ −191 (N-5); MS (ESI) m/z (%) 442 (100) $[M + Na]^+$, 437 (30), 420 (58) $[M + 1]^+$; HRMS calcd for $C_{21}H_{13}N_3O_5SNa$ (442.0468), found 442.0474.

*3-Cyano-7,8-diacetyloxy-2H-4-phenyl-pyrimido[2,1-b]benzothiazol-2-one (**12a**).* Mp 157−159; R_f = 0.28 (CH_2Cl_2/EtOAc = 2:1); UV (MeCN) λ_{max} (log ε) 208 (4.47), 234 (4.44), 267 (4.47), 306 nm (3.94); IR (ATR) $\tilde{\nu}$ 3052 (CH), 2231 (CN), 1770 (C═O), 1650 (C═O), 1608, 1594, 1515 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.14 (s, 3H, 2″-H), 2.27 (s, 3H, 2‴-H), 5.51 (s, 1H, 6-H), 7.70−7.71 (m, 5H, phenyl H), 8.02 (s, 1H, 9-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.8 (C-2″), 20.1 (C-2‴), 99.4 (C-3), 111.6 (C-6), 114.1 (CN), 118.7 (C-9), 121.8 (C-9a), 128.3 (C-2′ and C-6′), 129.2 (C-4′), 129.8 (C-3′ and C-5′), 131.9 (C-1′), 132.8 (C-5a), 140.2 (C-7 or C-8), 140.3 (C-7 or C-8), 156.6 (C-4), 162.3 (C-2), 166.2 (C-10a), 167.4 (C-1″), 167.9 (C-1‴); ^{15}N NMR (indirectly determined, 50.7 MHz, DMSO-d_6) δ −210.53 (N-5); MS (ESI) m/z (%) 442 (100) $[M + Na]^+$, 337 (11); HRMS calcd for $C_{21}H_{13}N_3O_5SNa$ (442.0468), found 442.0459.

*3-Cyano-7,8-diacetyloxy-4H-2-(4-methyl)phenyl-pyrimido[2,1-b]benzothiazol-4-one (**11b**) and 3-Cyano-7,8-diacetyloxy-2H-4-(4-methyl)phenyl-pyrimido[2,1-b]benzothiazol-2-one (**12b**).*



11b **12b**

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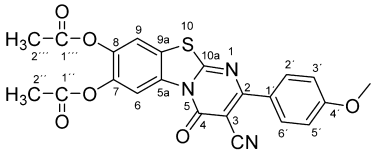
According to the general procedure II, a suspension of catechol (**1a**) (69 mg, 0.63 mmol), 1,2,3,4-tetrahydro-6-(4-methylphenyl)-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (**5b**) (122 mg, 0.50 mmol), methanol (7 mL), phosphate buffer (0.2 M, pH 6, 30 mL), and laccase from *A. bisporus* (10 mg, 6 U/mg) was stirred for 12 h. Workup gave a mixture of **6b** and **7b** in 96% (168 mg, 0.48 mmol). The obtained crude product was reacted with acetic anhydride (255 mg, 2.50 mmol) and DMAP (6 mg, 0.05 mmol) in pyridine (2 mL) under argon for 5 h. After workup the acetylated mixture of **11b** and **12b** was isolated in 81% (169 mg, 0.39 mmol). The obtained mixture was separated by column chromatography to give 3-cyano-7,8-diacetyloxy-4*H*-2-(4-methyl)phenyl-pyrimido[2,1-*b*]benzothiazol-4-one (**11b**) as a white solid in 46% yield (96 mg, 0.22 mmol) and 3-cyano-7,8-diacetyloxy-2*H*-4-(4-methyl)phenyl-pyrimido[2,1-*b*]benzothiazol-2-one (**12b**) as a white solid in 23% yield (48 mg, 0.11 mmol).

*3-Cyano-7,8-diacetyloxy-4H-2-(4-methyl)phenyl-pyrimido[2,1-b]-benzothiazol-4-one (**11b**).* Mp 246−248 °C; R_f = 0.44 (CH_2Cl_2/EtOAc = 20:1); UV (MeCN) λ_{max} (log ε) 233 (4.40), 252 (4.33), 312 (4.34), 368 nm (4.29); IR (ATR) $\tilde{\nu}$ 3112 (CH), 2217 (CN), 1779 (C═O), 1764 (C═O), 1672 (C═O), 1532, 1480 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 2.34 (s, 3H, 2″-H or 2‴-H), 2.35 (s, 3H, 2″-H or 2‴-H), 2.38 (s, 3H, 4′-CH_3), 7.42 (d like, ^3J (2′-H, 3′-H) = 8.1 Hz, ^3J (5′-H, 6′-H) = 8.1 Hz, 2H, 3′-H and 5′-H), 7.94 (d like, ^3J (2′-H, 3′-H) = 8.1 Hz, ^3J (5′-H, 6′-H) = 8.1 Hz, 2H, 2′-H and 6′-H), 8.15 (s, 1H, 9-H), 8.78 (s, 1H, 6-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.2 (C-2″ or C-2‴), 20.3 (C-2″ or C-2‴), 21.0 (4′-CH_3), 90.9 (C-3), 114.2 (C-6), 115.8 (CN), 118.1 (C-9), 122.5 (C-9a), 128.9 (C-2′ and C-6′), 129.3 (C-3′and C-5′), 131.8 (C-1′), 132.7 (C-5a), 141.2 (C-7), 141.3 (C-8), 142.4 (C-4′), 159.0 (C-4), 165.3 (C-2), 165.6 (C-10a), 168.0 (C-1‴), 168.1 (C-1″); ^{15}N NMR (indirectly determined, 50.7 MHz, DMSO-d_6) δ −190.63 (N-5), −156.72 (N-1); MS (ESI) m/z (%) 456 (100) $[M + Na]^+$, 434 (10) $[M + 1]^+$; HRMS calcd for $C_{22}H_{15}N_3O_5SNa$ (456.0625), found 456.0616.

*3-Cyano-7,8-diacetyloxy-2H-4-(4-methyl)phenyl-pyrimido[2,1-b]-benzothiazol-2-one (**12b**).* Mp 236−238; R_f = 0.36 (CH_2Cl_2/EtOAc = 2:1); UV (MeCN) λ_{max} (log ε) 211 (4.51), 234 (4.46), 266 (4.49), 306 nm (3.98); IR (ATR) $\tilde{\nu}$ 3112 (CH), 2217 (CN), 1779 (C═O), 1763 (C═O), 1672 (C═O), 1531, 1516, 1479 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 2.15 (s, 3H, 2″-H), 2.27 (s, 3H, 2‴-H), 2.47 (s, 3H, 4′-CH_3), 5.56 (s, 1H, 6-H), 7.53 (d like, ^3J (2′-H, 3′-H) = 8.0 Hz, ^3J (5′-H, 6′-H) = 8.0 Hz, 2H, 3′-H and 5′-H), 7.59 (d like, ^3J (2′-H, 3′-H) = 8.0 Hz, ^3J (5′-H, 6′-H) = 8.0 Hz, 2H, 2′-H and 6′-H), 8.02 (s, 1H, 9-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.8 (C-2″), 20.0 (C-2‴), 20.9 (4′-CH_3), 99.2 (C-3), 111.7 (C-6), 114.1 (CN), 118.5 (C-9), 121.7 (C-9a), 126.2 (C-1′), 128.1 (C-2′ and C-6′), 130.2 (C-3′and C-5′), 132.7 (C-5a), 140.1 (C-7 or C-8), 140.2 (C-7 or C-8), 142.1 (C-4′), 156.8 (C-4), 162.3 (C-2), 166.1 (C-10a), 167.4 (C-1″), 167.8 (C-1‴); ^{15}N NMR (indirectly determined, 50.7 MHz, DMSO-d_6) δ −211.14 (N-5); MS (ESI) m/z (%) 456 (100) $[M + Na]^+$, 434 (10) $[M + 1]^+$; HRMS calcd for $C_{22}H_{15}N_3O_5SNa$ (456.0625), found 456.0614.

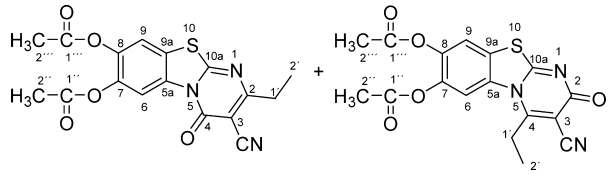
*3-Cyano-7,8-diacetyloxy-4H-2-(4-methoxy)phenyl-pyrimido-[2,1-b]benzothiazol-4-one (**11c**).*



11c

According to the general procedure II, a suspension of catechol (**1a**) (69 mg, 0.63 mmol), 1,2,3,4-tetrahydro-6-(4-methoxyphenyl)-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (**5c**) (130 mg, 0.50 mmol), methanol (7 mL), phosphate buffer (0.2 M, pH 6, 30 mL), and laccase from *A. bisporus* (10 mg, 6 U/mg) was stirred for 18 h. Workup gave a mixture of **6c** and **7c** in 90% (165 mg, 0.45 mmol). The obtained crude product was reacted with acetic anhydride (255 mg, 2.50 mmol) and DMAP (6 mg, 0.05 mmol) in pyridine (2 mL) under argon for 5 h. After workup the acetylated mixture of **11c** and **12c** was isolated in 77% (156 mg, 0.35 mmol). The obtained mixture was separated by column chromatography to give 3-cyano-7,8-diacetyloxy-4*H*-2-(4-methoxy)phenyl-pyrimido[2,1-*b*]benzothiazol-4-one (**11c**) as a white solid in 45% yield (91 mg, 0.20 mmol): mp 210−212 °C; R_f = 0.46 (CH_2Cl_2/EtOAc = 10:1); UV (MeCN) λ_{max} (log ε) 240 (4.40), 340 nm (4.51); IR (ATR) $\tilde{\nu}$ 3112 (CH), 2924 (CH), 2851 (CH), 2216 (CN), 1782 (C═O), 1765 (C═O), 1671 (C═O), 1602, 1532, 1516 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 2.34 (s, 3H, 2″-H or 2‴-H), 2.35 (s, 3H, 2″-H or 2‴-H), 3.86 (s, 3H, 4′-OCH_3), 7.14 (d like, ^3J (2′-H, 3′-H) = 9.0 Hz, ^3J (5′-H, 6′-H) = 9.0 Hz, 2H, 3′-H and 5′-H), 8.07 (d like, ^3J (2′-H, 3′-H) = 8.5 Hz, ^3J (5′-H, 6′-H) = 8.5 Hz, 2H, 2′-H and 6′-H), 8.14 (s, 1H, 9-H), 8.78 (s, 1H, 6-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.17 (C-2″ or C-2‴), 20.22 (C-2″ or C-2‴), 55.5 (4′-OCH_3), 89.9 (C-3), 114.1 (C-6), 114.2 (C-3′ and C-5′), 116.0 (CN), 118.1 (C-9), 122.4 (C-9a), 126.6 (C-1′), 130.9 (C-2′ and C-6′), 132.7 (C-5a), 141.1 (C-7), 141.3 (C-8), 159.1 (C-4), 162.4 (C-4′), 164.6 (C-2), 165.4 (C-10a), 168.0 (C-1‴), 168.1 (C-1″); ^{15}N NMR (indirectly determined, 50.7 MHz, DMSO-d_6) δ −191.24 (N-5), −154.96 (N-1); MS (ESI) m/z (%) 472 (100) $[M + Na]^+$, 450 (17) $[M + 1]^+$; HRMS calcd for $C_{22}H_{15}N_3O_6SNa$ (472.0574), found 472.0565.

*3-Cyano-7,8-diacetyloxy-4H-2-ethyl-pyrimido[2,1-b]-benzothiazol-4-one (**11d**) and 3-Cyano-7,8-diacetyloxy-2H-4-ethyl-pyrimido[2,1-b]benzothiazol-2-one (**12d**).*



11d **12d**

According to the general procedure II, a solution of catechol (**1a**) (69 mg, 0.63 mmol), 1,2,3,4-tetrahydro-6-ethyl-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (**5d**) (91 mg, 0.50 mmol), methanol (3 mL), phosphate buffer (0.2 M, pH 6, 30 mL), and laccase from *A. bisporus* (10 mg, 6 U/mg) was stirred for 18 h. Workup gave a mixture of **6d** and **7d** in 83% (120 mg, 0.42 mmol). The obtained crude product was reacted with acetic anhydride (255 mg, 2.50 mmol) and DMAP (6 mg, 0.05 mmol) in pyridine (2 mL) under argon for 5 h. Workup gave the acetylated mixture of **11d** and **12d** in 76% (118 mg, 0.32 mmol). The obtained mixture was separated by column chromatography to give 3-cyano-7,8-diacetyloxy-4*H*-2-ethyl-pyrimido[2,1-*b*]benzothiazol-4-one (**11d**) as a white solid in 35% yield (54 mg, 0.15 mmol) and 3-cyano-7,8-diacetyloxy-2*H*-4-ethyl-pyrimido[2,1-*b*]benzothiazol-2-one (**12d**) as a white solid in 16% yield (25 mg, 0.07 mmol).

*3-Cyano-7,8-diacetyloxy-4H-2-ethyl-pyrimido[2,1-b]-benzothiazol-4-one (**11d**).* Mp 258−260 °C; R_f = 0.30 (CH_2Cl_2/EtOAc = 20:1); UV (MeCN) λ_{max} (log ε) 206 (4.44), 222 (4.51), 342 (4.28), 356 nm (4.34); IR (ATR) $\tilde{\nu}$ 3105 (CH), 2939 (CH), 2223 (CN), 1787 (C═O), 1768 (C═O), 1677 (C═O), 1538, 1463, 1188 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 1.28 (t, ^3J (1′-H, 2′-H) = 7.5 Hz, 3H, 2′-H), 2.34 (s, 3H, 2″-H or 2‴-H), 2.35 (s, 3H, 2″-H or 2‴-H), 2.83 (q, ^3J (1′-H, 2′-H) = 8.0 Hz, 2H, 1′-H), 8.14 (s, 1H, 9-H), 8.77 (s, 1H, 6-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 11.6 (C-2′), 20.15 (C-2″ or C-2‴), 20.19 (C-2″ or C-2‴), 29.5 (C-1′), 92.8 (C-3), 114.2 (C-6), 114.7 (CN), 118.1 (C-9), 122.2 (C-9a), 132.8 (C-5a), 141.1 (C-7), 141.2 (C-8), 158.1 (C-4), 166.2 (C-10a), 167.9 (C-1‴), 168.0 (C-1″), 173.8 (C-2); ^{15}N NMR (indirectly determined, 50.7 MHz, DMSO-d_6) δ −189.94 (N-5), −152.41 (N-1); MS (ESI) m/z (%) 394 (100) $[M + Na]^+$, 372 (50) $[M + 1]^+$, 330 (20); HRMS calcd for $C_{17}H_{13}N_3O_5SNa$ (394.0468), found 394.0473.

*3-Cyano-7,8-diacetyloxy-2H-4-ethyl-pyrimido[2,1-b]-benzothiazol-2-one (**12d**).* Mp 224−226 °C; R_f = 0.22 (CH_2Cl_2/EtOAc = 2:1); UV (MeCN) λ_{max} (log ε) 213 (4.33), 235 (4.44), 262 (4.52), 306 nm (4.05); IR (ATR) $\tilde{\nu}$ 3120 (CH), 2978 (CH), 2230 (CN), 1773 (C═O), 1650 (C═O), 1602, 1498, 1183 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 1.35 (t, ^3J (1′-H, 2′-H) = 7.6 Hz, 3H, 2′-H), 2.33 (s, 3H, 2″-H or 2‴-H), 2.34 (s, 3H, 2″-H or 2‴-H), 3.34 (q, ^3J (1′-H, 2′-H) = 7.5 Hz, 2H, 1′-H), 7.92 (s, 1H, 6-H), 8.05 (s, 1H, 9-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 11.3 (C-2′), 20.3 (C-2″ or

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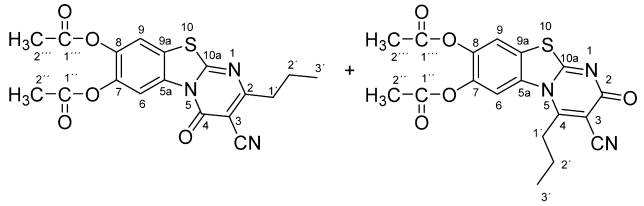
C-2‴), 20.5 (C-2″ or C-2‴), 25.7 (C-1′), 97.8 (C-3), 113.3 (C-6), 114.7 (CN), 118.5 (C-9), 121.8 (C-9a), 132.8 (C-5a), 140.6 (C-7), 141.4 (C-8), 162.2 (C-4), 162.4 (C-2), 166.5 (C-10a), 168.1 (C-1‴), 168.2 (C-1″); ^{15}N NMR (indirectly determined, 50.7 MHz, DMSO-d_6) δ −211.01 (N-5); MS (ESI) m/z (%) 394 (100) $[M + Na]^+$, 372 (5) $[M + 1]^+$; HRMS calcd for $C_{17}H_{13}N_3O_5SNa$ (394.0468), found 394.0458.

*3-Cyano-7,8-diacetyloxy-4H-2-propyl-pyrimido[2,1-b]-benzothiazol-4-one (**11e**) and 3-Cyano-7,8-diacetyloxy-2H-4-propyl-pyrimido[2,1-b]benzothiazol-2-one (**12e**).*



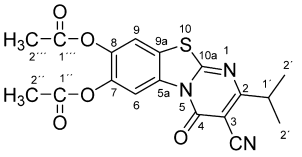
11e **12e**

According to the general procedure II, a solution of catechol (**1a**) (69 mg, 0.63 mmol), 1,2,3,4-tetrahydro-4-oxo-6-propyl-2-thioxo-pyrimidine-5-carbonitrile (**5e**) (98 mg, 0.50 mmol), methanol (3 mL), phosphate buffer (0.2 M, pH 6, 30 mL), and laccase from *A. bisporus* (10 mg, 6 U/mg) was stirred for 20 h. Workup gave a mixture of **6e** and **7e** in 84% yield (127 mg, 0.42 mmol). The obtained crude product was reacted with acetic anhydride (255 mg, 2.50 mmol) and DMAP (6 mg, 0.05 mmol) in pyridine (2 mL) under argon for 7 h. Workup gave the acetylated mixture of **11e** and **12e** in 85% yield (138 mg, 0.36 mmol). The obtained mixture was separated by column chromatography to give 3-cyano-7,8-diacetyloxy-4*H*-2-propyl-pyrimido[2,1-*b*]benzothiazol-4-one (**11e**) as a white solid in 38% yield (62 mg, 0.16 mmol) and 3-cyano-7,8-diacetyloxy-2*H*-4-propyl-pyrimido[2,1-*b*]benzothiazol-2-one (**12e**) as a white solid in 36% yield (58 mg, 0.15 mmol).

*3-Cyano-7,8-diacetyloxy-4H-2-propyl-pyrimido[2,1-b]-benzothiazol-4-one (**11e**).* Mp 243−245 °C; R_f = 0.22 (CH_2Cl_2/EtOAc = 20:1); UV (MeCN) λ_{max} (log ε) 206 (4.34), 223 (4.41), 342 (4.17), 356 nm (4.24); IR (ATR) $\tilde{\nu}$ 3123 (CH), 2965 (CH), 2937 (CH), 2878 (CH), 2224 (CN), 1786 (C═O), 1768 (C═O), 1676 (C═O), 1537, 1486, 1462 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.97 (t, ^3J (2′-H, 3′-H) = 8.0 Hz, 3H, 3′-H), 1.77 (sex, ^3J (1′-H, 2′-H) = 7.5 Hz, ^3J (2′-H, 3′-H) = 7.5 Hz, 2H, 2′-H), 2.34 (s, 3H, 2″-H or 2‴-H), 2.35 (s, 3H, 2″-H or 2‴-H), 2.78 (t, ^3J (1′-H, 2′-H) = 7.5 Hz, 2H, 1′-H), 8.14 (s, 1H, 9-H), 8.77 (s, 1H, 6-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 13.2 (C-3′), 20.15 (C-2″ or C-2‴), 20.19 (C-2″ or C-2‴), 20.7 (C-2′), 37.9 (C-1′), 93.5 (C-3), 114.2 (C-6), 114.8 (CN), 118.0 (C-9), 122.2 (C-9a), 132.8 (C-5a), 141.1 (C-7), 141.2 (C-8), 158.1 (C-4), 166.0 (C-10a), 167.9 (C-1‴), 168.1 (C-1″), 172.6 (C-2); ^{15}N NMR (indirectly determined, 50.7 MHz, DMSO-d_6) δ −189.56 (N-5), −151.61 (N-1); MS (ESI) m/z (%) 408 (100) $[M + Na]^+$, 386 (11) $[M + 1]^+$; HRMS calcd for $C_{18}H_{15}N_3O_5SNa$ (408.0625), found 408.0637.

*3-Cyano-7,8-diacetyloxy-2H-4-propyl-pyrimido[2,1-b]-benzothiazol-2-one (**12e**).* Mp 258−260 °C; R_f = 0.24 (CH_2Cl_2/EtOAc = 2:1); UV (MeCN) λ_{max} (log ε) 213 (4.34), 235 (4.44), 262 (4.53), 306 nm (4.05); IR (ATR) $\tilde{\nu}$ 3110 (CH), 2936 (CH), 2229 (CN), 1776 (C═O), 1647 (C═O), 1598, 1496, 1426, 1186 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.04 (t, ^3J (2′-H, 3′-H) = 7.5 Hz, 3H, 3′-H), 1.76 (sex, ^3J (1′-H, 2′-H) = 8.0 Hz, ^3J (2′-H, 3′-H) = 8.0 Hz, 2H, 2′-H), 2.33 (s, 3H, 2″-H or 2‴-H), 2.34 (s, 3H, 2″-H or 2‴-H), 3.30 (t, ^3J (1′-H, 2′-H) = 7.5 Hz, 2H, 1′-H), 7.86 (s, 1H, 6-H), 8.05 (s, 1H, 9-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 13.0 (C-3′), 19.9 (C-2′), 20.3 (C-2″ or C-2‴), 20.5 (C-2″ or C-2‴), 33.6 (C-1′), 98.6 (C-3), 113.2 (C-6), 115.0 (CN), 118.4 (C-9), 121.8 (C-9a), 132.8 (C-5a), 140.5 (C-7), 141.2 (C-8), 160.4 (C-4), 162.3 (C-2), 166.6 (C-10a), 168.12 (C-1‴), 168.14 (C-1″); ^{15}N NMR (indirectly determined, 50.7 MHz, DMSO-d_6) δ −210 (N-5); MS (ESI) m/z (%) 408 (100) $[M + Na]^+$, 386 (5) $[M + 1]^+$; HRMS calcd for $C_{18}H_{15}N_3O_5SNa$ (408.0625), found 408.0618.

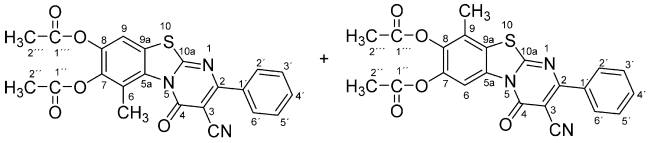
*3-Cyano-7,8-diacetyloxy-4H-2-isopropyl-pyrimido[2,1-b]-benzothiazol-4-one (**11f**).*



11f

According to the general procedure II, a solution of catechol (**1a**) (69 mg, 0.63 mmol), 1,2,3,4-tetrahydro-6-isopropyl-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (**5f**) (98 mg, 0.50 mmol), methanol (3 mL), phosphate buffer (0.2 M, pH 6, 30 mL), and laccase from *A. bisporus* (10 mg, 6 U/mg) was stirred for 20 h. Workup gave a mixture of **6f** and **7f** in 92% yield (139 mg, 0.46 mmol). The obtained crude product was reacted with acetic anhydride (255 mg, 2.50 mmol) and DMAP (6 mg, 0.05 mmol) in pyridine (2 mL) under argon for 5 h. Workup gave a mixture of **11f** and **12f** in 68% (121 mg, 0.31 mmol), which was separated by column chromatography to give 3-cyano-7,8-diacetyloxy-4*H*-2-isopropyl-pyrimido[2,1-*b*]benzothiazol-4-one (**11f**) as a white solid in 45% yield (80 mg, 0.21 mmol): mp 239−241 °C; R_f = 0.39 (CH_2Cl_2/EtOAc = 10:1); UV (MeCN) λ_{max} (log ε) 223 (4.50), 239 (4.28), 341 (4.26), 355 nm (4.34); IR (ATR) $\tilde{\nu}$ 3114 (CH), 2972 (CH), 2221 (CN), 1771 (C═O), 1685 (C═O), 1541, 1491, 1465, 1188 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.27 (d, ^3J (1′-H, 2′-H) = 6.8 Hz, 6H, 2′-H), 2.35 (s, 6H, 2″-H and 2‴-H), 3.27 (sept, ^3J (1′-H, 2′-H) = 6.8 Hz, 1H, 1′-H), 8.13 (s, 1H, 9-H), 8.76 (s, 1H, 6-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.29 (C-2″ or C-2‴), 20.33 (C-2″ or C-2‴), 20.5 (C-2′), 34.3 (C-1′), 92.2 (C-3), 114.3 (C-6), 114.8 (CN), 118.1 (C-9), 122.4 (C-9a), 132.9 (C-5a), 141.15 (C-7), 141.24 (C-8), 158.4 (C-4), 166.6 (C-10a), 168.1 (C-1‴), 168.2 (C-1″), 177.0 (C-2); ^{15}N NMR (indirectly determined, 50.7 MHz, DMSO-d_6) δ −189.24 (N-5), −155.35 (N-1); MS (ESI) m/z (%) 408 (39) $[M + Na]^+$, 403 (39) $[M + NH_4]^+$, 386 (67) $[M + 1]^+$; HRMS calcd for $C_{18}H_{19}N_4O_5S$ (403.1071), found 403.1069.

*3-Cyano-7,8-diacetyloxy-6-methyl-4H-2-phenyl-pyrimido[2,1-b]-benzothiazol-4-one (**21a**) and 3-Cyano-7,8-diacetyloxy-9-methyl-4H-2-phenyl-pyrimido[2,1-b]benzothiazol-4-one (**22a**).*



21a **22a**

According to the general procedure II, a suspension of 3-methylcatechol (**1b**) (78 mg, 0.63 mmol), 1,2,3,4-tetrahydro-4-oxo-6-phenyl-2-thioxo-5-pyrimidinecarbonitrile (**5a**) (115 mg, 0.50 mmol), methanol (5.5 mL), phosphate buffer (0.2 M, pH 6, 30 mL), and laccase from *A. bisporus* (10 mg, 6 U/mg) was stirred for 18 h. Workup gave a mixture of **13a** and **14a** in 91% yield (160 mg, 0.46 mmol). The obtained crude product of **13a** and **14a** was reacted with acetic anhydride (255 mg, 2.50 mmol) and DMAP (6 mg, 0.05 mmol) in pyridine (2 mL) under argon for 5 h. Workup gave the acetylated mixture of **21a** and **22a** in 78% yield (155 mg, 0.36 mmol). The obtained mixture was purified by column chromatography to give a mixture of 3-cyano-7,8-diacetyloxy-6-methyl-4*H*-2-phenyl-pyrimido-[2,1-*b*]benzothiazol-4-one (**21a**) and 3-cyano-7,8-diacetyloxy-9-methyl-4*H*-2-phenyl-pyrimido[2,1-*b*]benzothiazol-4-one (**22a**) as a pale yellow solid in 53% yield (105 mg, 0.24 mmol): mp 229−231 °C; R_f = 0.30 (CH_2Cl_2/EtOAc = 20:1); UV (MeCN) λ_{max} (log ε) 204 (4.52), 232 (4.40), 259 (4.27), 274 (4.20), 304 (4.21), 375 nm (4.20); IR (ATR) $\tilde{\nu}$ 3101 (CH), 2970 (CH), 2226 (CN), 1761 (C═O), 1681 (C═O), 1539, 1504, 1370 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) of **21a** δ 2.34 (s, 3H, 2‴-H), 2.35 (overlapped, 3H, 6-CH_3), 2.41 (s, 3H, 2″-H), 7.59−7.64 (m, 2H, 3′-H and 5′-H), 7.65−7.67 (m, 1H, 4′-H), 7.99 (s, 1H, 9-H), 8.00−8.05 (m, 2H, 2′-H and 6′-H); ^{13}C NMR (125 MHz, DMSO-d_6) of **21a** δ 16.5 (6-CH_3), 19.8 (C-2″), 20.18 (C-2‴), 91.0 (C-3), 115.4 (C-9), 115.9 (CN), 123.4 (C-9a), 125.4 (C-6),

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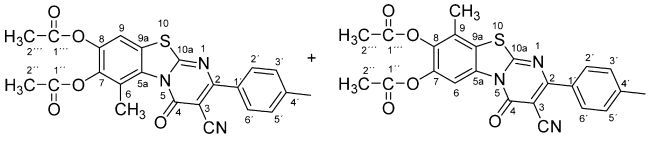
128.63 (C-3′ and C-5′), 128.8 (C-2′ and C-6′), 131.9 (C-5a), 132.0 (C-4′), 134.2 (C-1′), 141.2 (C-7), 141.6 (C-8), 158.9 (C-4), 165.2 (C-2), 166.6 (C-10a), 167.8 (C-1″), 167.9 (C-1‴); ^{15}N NMR (indirectly determined, 50.7 MHz, DMSO-d_6) of **21a** δ −187.44 (N-5); ^{1}H NMR (500 MHz, DMSO-d_6) of **22a** δ 2.35 (overlapped, 3H, 2″-H), 2.37 (s, 3H, 9-CH_3), 2.40 (s, 3H, 2‴-H), 7.59−7.64 (m, 2H, 3′-H and 5′-H), 7.65−7.67 (m, 1H, 4′-H), 8.00−8.05 (m, 2H, 2′-H and 6′-H), 8.69 (s, 1H, 6-H); ^{13}C NMR (125 MHz, DMSO-d_6) of **22a** δ 14.6 (9-CH_3), 19.8 (C-2‴), 20.21 (C-2″), 91.8 (C-3), 112.2 (C-6), 115.5 (CN), 122.7 (C-9a), 126.0 (C-9), 128.59 (C-3′ and C-5′), 128.8 (C-2′ and C-6′), 131.8 (C-5a), 132.0 (C-4′), 134.6 (C-1′), 140.0 (C-8), 141.9 (C-7), 158.9 (C-4), 164.8 (C-2), 165.5 (C-10a), 167.76 (C-1‴), 168.1 (C-1″); MS (ESI) *m*/*z* (%) 456 (100) $[M + Na]^+$, 434 (11) $[M + 1]^+$; HRMS calcd for $C_{22}H_{15}N_3O_5SNa$ (456.0625), found 456.0616.

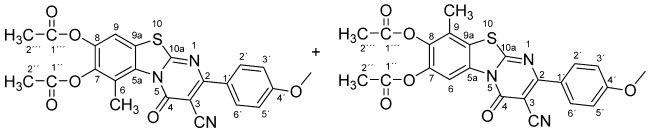
*3-Cyano-7,8-diacetyloxy-6-methyl-4H-2-(4-methyl)phenyl-pyrimido[2,1-b]benzothiazol-4-one (**21b**) and 3-Cyano-7,8-diacetyloxy-9-methyl-4H-2-(4-methyl)phenyl-pyrimido[2,1-b]-benzothiazol-4-one (**22b**)*



21b **22b**

According to the general procedure II, a suspension of 3-methylcatechol (**1b**) (78 mg, 0.63 mmol), 1,2,3,4-tetrahydro-6-(4-methylphenyl)-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (**5b**) (122 mg, 0.50 mmol), methanol (7 mL), phosphate buffer (0.2 M, pH 6, 30 mL), and laccase from *A. bisporus* (10 mg, 6 U/mg) was stirred for 15 h. Workup gave a mixture of **13b** and **14b** in 93% yield (170 mg, 0.47 mmol). The obtained crude product of **13b** and **14b** was reacted with acetic anhydride (255 mg, 2.50 mmol) and DMAP (6 mg, 0.05 mmol) in pyridine (2 mL) under argon for 5 h. Workup gave the acetylated mixture of **21b** and **22b** in 76% (159 mg, 0.36 mmol). The obtained mixture was purified by column chromatography to give a mixture of 3-cyano-7,8-diacetyloxy-6-methyl-4*H*-2-(4-methyl)-phenyl-pyrimido[2,1-*b*]benzothiazol-4-one (**21b**) and 3-cyano-7,8-diacetyloxy-9-methyl-4*H*-2-(4-methyl)phenyl-pyrimido[2,1-*b*]-benzothiazol-4-one (**22b**) as a pale yellow solid in 43% yield (90 mg, 0.20 mmol): mp 257−259 °C; R_f = 0.40 (CH_2Cl_2/EtOAc = 20:1); UV (MeCN) λ_{max} (log ε) 234 (4.38), 259 (4.23), 315 (4.26), 376 nm (4.12); IR (ATR) $\tilde{\nu}$ 3115 (CH), 2940 (CH), 2159 (CN), 1770 (C═O), 1688 (C═O), 1539, 1485 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) of **21b** δ 2.33 (s, 3H, 6-CH_3), 2.34 (s, 3H, 2‴-H), 2.40 (overlapped, 3H, 2″-H), 2.41 (s, 3H, 4′-CH_3), 7.41 (d like, ^3J (2′-H, 3′-H) = 8.0 Hz, ^3J (5′-H, 6′-H) = 8.0 Hz, 2H, 3′-H and 5′-H), 7.96 (d like, ^3J (2′-H, 3′-H) = 8.5 Hz, ^3J (5′-H, 6′-H) = 8.5 Hz, 2H, 2′-H and 6′-H), 7.98 (s, 1H, 9-H); ^{13}C NMR (125 MHz, DMSO-d_6) of **21b** δ 16.5 (6-CH_3), 19.9 (C-2″), 20.19 (C-2‴), 20.97 (4′-CH_3), 90.4 (C-3), 115.1 (C-9), 116.0 (CN), 123.4 (C-9a), 125.3 (C-6), 128.8 (C-2′ and C-6′), 129.2 (C-3′ and C-5′), 131.3 (C-1′), 131.9 (C-5a), 141.2 (C-7), 141.6 (C-8), 142.5 (C-4′), 158.7 (C-4), 165.0 (C-2), 166.4 (C-10a), 167.8 (C-1″), 167.9 (C-1‴); ^{15}N NMR (indirectly determined, 50.7 MHz, DMSO-d_6) of **21b** δ −187.50 (N-5), −154.41 (N-1); ^{1}H NMR (500 MHz, DMSO-d_6) of **22b** δ 2.35 (s, 3H, 2″-H), 2.37 (s, 3H, 9-CH_3), 2.40 (overlapped, 3H, 2‴-H), 2.41 (s, 3H, 4′-CH_3), 7.41 (d like, ^3J (2′-H, 3′-H) = 8.0 Hz, ^3J (5′-H, 6′-H) = 8.0 Hz, 2H, 3′-H and 5′-H), 7.94 (d like, ^3J (2′-H, 3′-H) = 8.5 Hz, ^3J (5′-H, 6′-H) = 8.5 Hz, 2H, 2′-H and 6′-H), 8.67 (s, 1H, 9-H); ^{13}C NMR (125 MHz, DMSO-d_6) of **22b** δ 14.6 (9-CH_3), 19.9 (C-2‴), 20.22 (C-2″), 20.95 (4′-CH_3), 91.2 (C-3), 112.2 (C-6), 115.7 (CN), 122.6 (C-9a), 126.0 (C-9), 128.8 (C-2′ and C-6′), 129.2 (C-3′ and C-5′), 131.2 (C-1′), 131.7 (C-5a), 139.9 (C-8), 141.9 (C-7), 142.4 (C-4′), 159.0 (C-4), 164.6 (C-2), 165.3 (C-10a), 167.78 (C-1‴), 168.1 (C-1″); MS (ESI) *m*/*z* (%) 470 (100) $[M + Na]^+$, 448 (11) $[M + 1]^+$; HRMS calcd for $C_{23}H_{17}N_3O_5SNa$ (470.0781), found 470.0777.

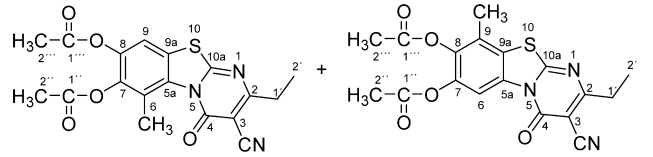
*3-Cyano-7,8-diacetyloxy-6-methyl-4H-2-(4-methoxy)phenyl-pyrimido[2,1-b]benzothiazol-4-one (**21c**) and 3-Cyano-7,8-diacetyloxy-9-methyl-4H-2-(4-methoxy)phenyl-pyrimido[2,1-b]-benzothiazol-4-one (**22c**).*



21c **22c**

According to the general procedure II, a suspension of 3-methylcatechol (**1b**) (78 mg, 0.63 mmol), 1,2,3,4-tetrahydro-6-(4-methoxyphenyl)-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (**5c**) (130 mg, 0.50 mmol), methanol (7 mL), phosphate buffer (0.2 M, pH 6, 30 mL), and laccase from *A. bisporus* (10 mg, 6 U/mg) was stirred for 18 h. Workup gave a mixture of **13c** and **14c** in 89% (170 mg, 0.45 mmol). The obtained crude product of **13c** and **14c** was reacted with acetic anhydride (255 mg, 2.50 mmol) and DMAP (6 mg, 0.05 mmol) in pyridine (2 mL) under argon for 7 h. Workup gave the acetylated mixture of **21c** and **22c** in 70% yield (146 mg, 0.32 mmol). The obtained mixture was purified by column chromatography to give a mixture of 3-cyano-7,8-diacetyloxy-6-methyl-4*H*-2-(4-methoxy)-phenyl-pyrimido[2,1-*b*]benzothiazol-4-one (**21c**) and 3-cyano-7,8-diacetyloxy-9-methyl-4*H*-2-(4-methoxy)phenyl-pyrimido[2,1-*b*]-benzothiazol-4-one (**22c**) as a yellow solid in 53% (110 mg, 0.24 mmol): mp 214−216 °C; R_f = 0.50 (CH_2Cl_2/EtOAc = 20:1); UV (MeCN): λ_{max} (log ε) 238 (4.30), 342 nm (4.39); IR (ATR) $\tilde{\nu}$ 2937 (CH), 2114 (CN), 1773 (C═O), 1687 (C═O), 1602, 1537, 1480 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) of **21c** δ 2.30 (s, 3H, 6-CH_3), 2.33 (s, 3H, 2‴-H), 2.40 (s, 3H, 2″-H), 3.81 (s, 3H, 4′-OCH_3), 7.13 (d like, ^3J (2′-H, 3′-H) = 8.5 Hz, ^3J (5′-H, 6′-H) = 8.5 Hz, 2H, 3′-H and 5′-H), 7.96 (s, 1H, 9-H), 8.08 (d like, ^3J (2′-H, 3′-H) = 8.5 Hz, ^3J (5′-H, 6′-H) = 8.5 Hz, 2H, 2′-H and 6′-H); ^{13}C NMR (125 MHz, DMSO-d_6) of **21c** δ 16.5 (6-CH_3), 19.8 (C-2″), 20.18 (C-2‴), 55.5 (4′-OCH_3), 89.2 (C-3), 114.12 (C-3′ and C-5′), 115.1 (C-9), 116.3 (CN), 123.3 (C-9a), 125.2 (C-6), 126.0 (C-1′), 130.9 (C-2′ and C-6′), 131.9 (C-5a), 141.2 (C-7), 141.5 (C-8), 158.8 (C-4), 162.5 (C-4′), 164.2 (C-2), 166.1 (C-10a), 167.8 (C-1″), 167.9 (C-1‴); ^{15}N NMR (indirectly determined, 50.7 MHz, DMSO-d_6) of **21c** δ −189.58 (N-5), −155.30 (N-1); ^{1}H NMR (500 MHz, DMSO-d_6) of **22c** δ 2.34 (s, 3H, 2″-H), 2.35 (s, 3H, 9-CH_3), 2.39 (s, 3H, 2‴-H), 3.81 (s, 3H, 4′-OCH_3), 7.13 (d like, ^3J (2′-H, 3′-H) = 8.5 Hz, ^3J (5′-H, 6′-H) = 8.5 Hz, 2H, 3′-H and 5′-H), 8.08 (d like, ^3J (2′-H, 3′-H) = 8.5 Hz, ^3J (5′-H, 6′-H) = 8.5 Hz, 2H, 2′-H and 6′-H), 8.64 (s, 1H, 6-H); ^{13}C NMR (125 MHz, DMSO-d_6) of **22c** δ 14.6 (9-CH_3), 19.8 (C-2‴), 20.20 (C-2″), 55.5 (4′-OCH_3), 90.1 (C-3), 112.1 (C-6), 114.07 (C-3′ and C-5′), 115.9 (CN), 122.5 (C-9a), 125.96 (C-9), 126.4 (C-1′), 130.9 (C-2′ and C-6′), 131.8 (C-5a), 139.8 (C-8), 141.9 (C-7), 159.0 (C-4), 162.4 (C-4′), 164.4 (C-2), 166.1 (C-10a), 167.75 (C-1‴), 168.0 (C-1″); MS (ESI) *m*/*z* (%) 486 (100) $[M + Na]^+$; HRMS calcd for $C_{23}H_{17}N_3O_6SNa$ (486.0730), found 486.0736.

*3-Cyano-7,8-diacetyloxy-2-ethyl-6-methyl-4H-pyrimido[2,1-b]-benzothiazol-4-one (**21d**) and 3-Cyano-7,8-diacetyloxy-2-ethyl-9-methyl-4H-pyrimido[2,1-b]benzothiazol-4-one (**22d**).*



21d **22d**

According to the general procedure II, a solution of 3-methylcatechol (**1b**) (78 mg, 0.63 mmol), 1,2,3,4-tetrahydro-6-ethyl-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (**5d**) (91 mg, 0.50 mmol), methanol (3 mL), phosphate buffer (0.2 M, pH 6, 30 mL), and laccase from *A. bisporus* (10 mg, 6 U/mg) was stirred for 18 h. Workup gave a mixture of **13d** and **14d** in 82% (124 mg, 0.41 mmol). The obtained crude product of **13d** and **14d** was reacted with acetic anhydride (255 mg, 2.50 mmol) and DMAP (6 mg, 0.05 mmol) in pyridine (2 mL) under argon for 7 h.

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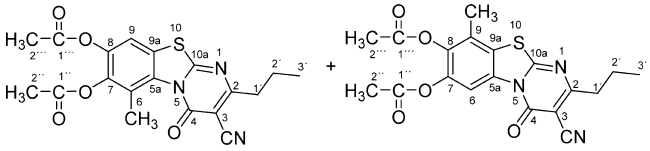
Workup gave the acetylated mixture of **21d** and **22d** in 72% yield (114 mg, 0.30 mmol). The obtained mixture was purified by column chromatography to give a mixture of 3-cyano-7,8-diacetyloxy-2-ethyl-6-methyl-4*H*-pyrimido[2,1-*b*]benzothiazol-4-one (**21d**) and 3-cyano-7,8-diacetyloxy-2-ethyl-9-methyl-4*H*-pyrimido[2,1-*b*]benzothiazol-4-one (**22d**) as a pale yellow solid in 41% yield (65 mg, 0.17 mmol): mp 212−214 °C; R_f = 0.26 (CH_2Cl_2/EtOAc = 10:1); UV (MeCN) λ_{max} (log ε) 208 (4.31), 226 (4.30), 362 nm (4.12); IR (ATR) $\tilde{\nu}$ 2943 (CH), 2223 (CN), 1764 (C═O), 1701 (C═O), 1541, 1451, 1169 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) of **21d** δ 1.27 (t, ^3J (1′-H, 2′-H) = 7.5 Hz, 3H, 2′-H), 2.29 (s, 3H, 6-CH_3), 2.33 (s, 3H, 2‴-H), 2.39 (overlapped, 3H, 2″-H), 2.79 (q, ^3J (1′-H, 2′-H) = 7.5 Hz, 2H, 1′-H), 7.97 (s, 1H, 9-H); ^{13}C NMR (125 MHz, DMSO-d_6) of **21d** δ 11.7 (C-2′), 16.7 (6-CH_3), 20.0 (C-2″), 20.32 (C-2‴), 29.2 (C-1′), 92.6 (C-3), 115.1 (CN), 115.3 (C-9), 123.3 (C-9a), 125.4 (C-6), 132.06 (C-5a), 141.2 (C-7), 141.6 (C-8), 157.9 (C-4), 167.3 (C-10a), 168.0 (C-1″), 168.1 (C-1‴), 173.4 (C-2); ^{15}N NMR (indirectly determined, 50.7 MHz, DMSO-d_6) of **21d** δ −186.65 (N-5), −152.66 (N-1); ^1H NMR (500 MHz, DMSO-d_6) of **22d** δ 1.28 (t, ^3J (1′-H, 2′-H) = 7.5 Hz, 3H, 2′-H), 2.34 (s, 3H, 2″-H), 2.35 (s, 3H, 9-CH_3), 2.39 (overlapped, 3H, 2‴-H), 2.82 (q, ^3J (1′-H, 2′-H) = 7.5 Hz, 2H, 1′-H), 8.63 (s, 1H, 6-H); ^{13}C NMR (125 MHz, DMSO-d_6) of **22d** δ 11.8 (C-2′), 14.7 (9-CH_3), 20.0 (C-2‴), 20.35 (C-2″), 29.6 (C-1′), 93.2 (C-3), 112.3 (C-6), 114.8 (CN), 122.6 (C-9a), 126.1 (C-9), 132.08 (C-5a), 139.9 (C-8), 141.8 (C-7), 158.3 (C-4), 165.3 (C-10a), 167.97 (C-1‴), 168.3 (C-1″), 173.9 (C-2); MS (ESI) *m*/*z* (%) 408 (100) $[M + Na]^+$, 386 (11) $[M + 1]^+$; HRMS calcd for $C_{18}H_{15}N_3O_5SNa$ (408.0625), found 408.0631.

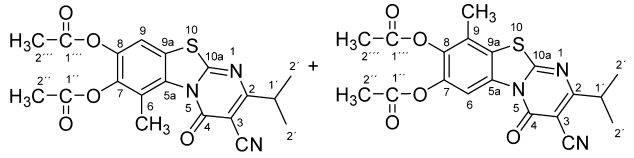
*3-Cyano-7,8-diacetyloxy-6-methyl-4H-2-propyl-pyrimido[2,1-b]-benzothiazol-4-one (**21e**) and 3-Cyano-7,8-diacetyloxy-9-methyl-4H-2-propyl-pyrimido[2,1-b]benzothiazol-4-one (**22e**).*



21e **22e**

According to the general procedure II, a solution of 3-methylcatechol (**1b**) (78 mg, 0.63 mmol), 1,2,3,4-tetrahydro-4-oxo-6-propyl-2-thioxo-5-pyrimidinecarbonitrile (**5e**) (98 mg, 0.50 mmol), methanol (3 mL), phosphate buffer (0.2 M, pH 6, 30 mL), and laccase from *A. bisporus* (10 mg, 6 U/mg) was stirred for 20 h. Workup gave a mixture of **13e** and **14e** in 78% yield (124 mg, 0.39 mmol). The obtained crude product of **13e** and **14e** was reacted with acetic anhydride (255 mg, 2.50 mmol) and DMAP (6 mg, 0.05 mmol) in pyridine (2 mL) under argon for 7 h. Workup gave the acetylated mixture of **21e** and **22e** in 76% yield (120 mg, 0.30 mmol). The obtained mixture was purified by column chromatography to give a mixture of 3-cyano-7,8-diacetyloxy-6-methyl-4*H*-2-propyl-pyrimido[2,1-*b*]benzothiazol-4-one (**21e**) and 3-cyano-7,8-diacetyloxy-9-methyl-4*H*-2-propyl-pyrimido[2,1-*b*]-benzothiazol-4-one (**22e**) as a pale yellow solid in 57% yield (90 mg, 0.23 mmol): mp 194−196 °C; R_f = 0.47 (CH_2Cl_2/EtOAc = 10:1); UV (MeCN) λ_{max} (log ε) 209 (4.44), 226 (4.43), 362 nm (4.25); IR (ATR) $\tilde{\nu}$ 3092 (CH), 2974 (CH), 2878 (CH), 2225 (CN), 1773 (C═O), 1705 (C═O), 1548, 1171 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) of **21e** δ 0.99 (t, ^3J (2′-H, 3′-H) = 7.5 Hz, 3H, 3′-H), 1.77 (sex, ^3J (1′-H, 2′-H) = 7.5 Hz, ^3J (2′-H, 3′-H) = 7.5 Hz, 2H, 2′-H), 2.30 (s, 3H, 6-CH_3), 2.33 (s, 3H, 2‴-H), 2.39 (overlapped, 3H, 2″-H), 2.76 (t, ^3J (1′-H, 2′-H) = 7.6 Hz, 2H, 1′-H), 7.97 (s, 1H, 9-H); ^{13}C NMR (125 MHz, DMSO-d_6) of **21e** δ 13.3 (C-3′), 16.6 (6-CH_3), 19.8 (C-2″), 20.17 (C-2‴), 20.5 (C-2′), 37.4 (C-1′), 93.3 (C-3), 115.0 (CN), 115.1 (C-9), 123.1 (C-9a), 125.3 (C-6), 131.9 (C-5a), 141.2 (C-7), 141.5 (C-8), 157.7 (C-4), 167.0 (C-10a), 167.78 (C-1″), 167.8 (C-1‴), 172.1 (C-2); ^{15}N NMR (indirectly determined, 50.7 MHz, DMSO-d_6) of **21e** δ −187.63 (N-5), −152.45 (N-1); ^1H NMR (500 MHz, DMSO-d_6) of **22e** δ 0.98 (t, ^3J (2′-H, 3′-H) = 7.6 Hz, 3H, 3′-H), 1.77 (sex, ^3J (1′-H, 2′-H) = 7.5 Hz, ^3J (2′-H, 3′-H) = 7.5 Hz, 2H, 2′-H), 2.34 (s, 3H, 2″-H), 2.35 (s, 3H, 9-CH_3), 2.39 (overlapped, 3H, 2‴-H), 2.79 (t, ^3J (1′-H, 2′-H) = 7.2 Hz, 2H, 1′-H), 8.65 (s, 1H, 6-H); ^{13}C NMR (125 MHz, DMSO-d_6) of **22e** δ 13.2 (C-3′), 14.6 (9-CH_3), 19.8 (C-2‴), 20.19 (C-2″), 20.6 (C-2′), 37.8 (C-1′), 93.8 (C-3), 112.1 (C-6), 114.7 (CN), 122.4 (C-9a), 126.0 (C-9), 132.0 (C-5a), 139.8 (C-8), 141.8 (C-7), 158.1 (C-4), 165.0 (C-10a), 167.8 (C-1‴), 168.0 (C-1″), 172.6 (C-2); MS (ESI) *m*/*z* (%) 422 (100) $[M + Na]^+$, 400 (11) $[M + 1]^+$; HRMS calcd for $C_{19}H_{17}N_3O_5SNa$ (422.0781), found 422.0784.

*3-Cyano-7,8-diacetyloxy-2-isopropyl-6-methyl-4H-pyrimido[2,1-b]benzothiazol-4-one (**21f**) and 3-Cyano-7,8-diacetyloxy-2-isopropyl-9-methyl-4H-pyrimido[2,1-b]benzothiazol-4-one (**22f**).*



21f **22f**

According to the general procedure II, a suspension of 3-methylcatechol (**1b**) (78 mg, 0.63 mmol), 1,2,3,4-tetrahydro-6-isopropyl-4-oxo-2-thioxo-5-pyrimidinecarbonitrile (**5f**) (98 mg, 0.50 mmol), methanol (3 mL), phosphate buffer (0.2 M, pH 6, 30 mL), and laccase from *A. bisporus* (10 mg, 6 U/mg) was stirred for 20 h. Workup gave a mixture of **13f** and **14f** in 85% yield (135 mg, 0.43 mmol). The obtained crude product of **13f** and **14f** was reacted with acetic anhydride (255 mg, 2.50 mmol) and DMAP (6 mg, 0.05 mmol) in pyridine (2 mL) under argon for 5 h. Workup gave the acetylated mixture of **21f** and **22f** in 75% yield (128 mg, 0.32 mmol). The obtained mixture was purified by column chromatography to give a mixture of 3-cyano-7,8-diacetyloxy-2-isopropyl-6-methyl-4*H*-pyrimido[2,1-*b*]benzothiazol-4-one (**21f**) and 3-cyano-7,8-diacetyloxy-2-isopropyl-9-methyl-4*H*-pyrimido[2,1-*b*]benzothiazol-4-one (**22f**) as a white solid in 58% yield (99 mg, 0.25 mmol): mp 227−229 °C; R_f = 0.27 (CH_2Cl_2/EtOAc = 20:1); UV (MeCN) λ_{max} (log ε) 209 (4.43), 224 (4.41), 359 nm (4.23); IR (ATR) $\tilde{\nu}$ 2978 (CH), 2932 (CH), 2221 (CN), 1778 (C═O), 1698 (C═O), 1540, 1496, 1188 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) of **21f** δ 1.27 (d like, ^3J (1′-H, 2′-H) = 6.5 Hz, 6H, 2′-H), 2.30 (s, 3H, 6-CH_3), 2.33 (s, 3H, 2‴-H), 2.39 (overlapped, 3H, 2″-H), 3.25 (sept, ^3J (1′-H, 2′-H) = 6.0 Hz, 1H, 1′-H), 8.00 (s, 1H, 9-H); ^{13}C NMR (125 MHz, DMSO-d_6) of **21f** δ 16.7 (6-CH_3), 20.0 (C-2″), 20.35 (C-2‴), 20.39 (C-2′), 34.0 (C-1′), 92.0 (C-3), 115.0 (CN), 115.2 (C-9), 123.3 (C-9a), 125.4 (C-6), 132.1 (C-5a), 141.2 (C-7), 141.6 (C-8), 158.0 (C-4), 167.6 (C-10a), 167.99 (C-1″), 168.1 (C-1‴), 176.5 (C-2); ^{15}N NMR (indirectly determined, 50.7 MHz, DMSO-d_6) of **21f** δ −186.67 (N-5), −156.01 (N-1); ^1H NMR (500 MHz, DMSO-d_6) of **22f** δ 1.27 (d like, ^3J (1′-H, 2′-H) = 6.5 Hz, 6H, 2′-H), 2.34 (s, 3H, 2″-H), 2.35 (s, 3H, 9-CH_3), 2.39 (overlapped, 3H, 2‴-H), 3.25 (sept, ^3J (1′-H, 2′-H) = 6.0 Hz, 1H, 1′-H), 8.63 (s, 1H, 6-H); ^{13}C NMR (125 MHz, DMSO-d_6) of **22f** δ 14.7 (9-CH_3), 20.0 (C-2‴), 20.35 (C-2″), 20.5 (C-2′), 34.3 (C-1′), 92.6 (C-3), 112.3 (C-6), 114.7 (CN), 122.6 (C-9a), 126.1 (C-9), 132.1 (C-5a), 139.9 (C-8), 141.9 (C-7), 158.5 (C-4), 165.6 (C-10a), 167.97 (C-1‴), 168.3 (C-1″), 176.9 (C-2); MS (ESI) *m*/*z* (%) 422 (100) $[M + Na]^+$, 400 (13) $[M + 1]^+$; HRMS calcd for $C_{19}H_{17}N_3O_5SNa$ (422.0781), found 422.0784.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Full characterization of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: ubeifuss@uni-hohenheim.de.

Notes

The authors declare no competing financial interest.

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