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A convenient synthesis of a chlorobenzothiophenyl-indole-based inhibitor of bacterial cystathionine γ-lyase

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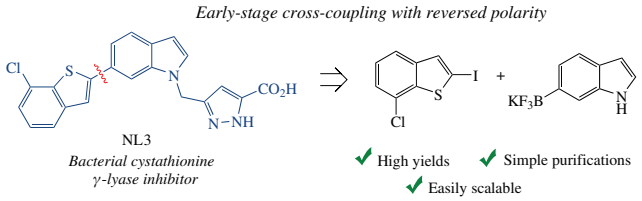
A convenient and efficient synthesis of 3-{[6-(7-chlorobenzo[*b*]thiophen-2-yl)-1*H*-indol-1-yl]methyl}-1*H*-pyrazole-5-carboxylic acid (NL3), which is currently among the most active and promising bacterial cystathionine γ-lyase (bCSE) inhibitors, has been developed. It is based on shifting the key stage of [Pd]-catalyzed cross-coupling of the indole and benzothiophene counterparts to the beginning of the synthetic scheme, with the polarity reversal of the components being coupled, to give 6-(7-chlorobenzo[*b*]thiophen-2-yl)-1*H*-indole as the key intermediate. The STD NMR method was used to estimate the NL3 compound obtained in the optimized synthesis as a ligand to saCSE (the main producer of H_2S in pathogenic *S. aureus*).



Keywords: antibacterial compounds, indole-based inhibitors, NL3, bCSE, antibiotics potentiation, benzo[*b*]thiophenes, indoles, cross-coupling.

There is an ever-growing problem of antibiotic resistance, which leads to whole classes of antibiotics ceasing to be efficient over their lifetime in therapy. One of the possible solutions to overcome this inactivation is to combine the known antibiotic drugs with compounds-potentiators that synergistically target specific enzymes in bacteria, thus lowering the dosage of antibiotic and possibly even eliminating the mechanism of resistance. For example, the drug-associated damage in bacteria *via* oxidative stress can be enhanced additionally by blocking the bacterial H_2S-producing enzymes and, as a result, the production of H_2S and glutathione. Among such enzymes, cystathionine γ-lyase (CSE) was described as an additional drug target for further investigation and drug development. The desired drug candidates should be selective inhibitors of this enzyme in bacterial pathogens (bCSE) and possess low activity against the human version of the enzyme (hCSE).[1,2] It was shown that some indole-based molecules could be applied as potentiators for enhancement of the antibiotic drugs against pathogenic bacterial microorganisms, including resistant strains.[3] Further development of this approach requires simple and efficient methods for the syntheses of these molecules and their analogues.[4,5]

Currently, molecules based on 6-substituted indole derivatives that feature good selectivity and low toxicity, including [2-(6-bromo-1*H*-indol-1-yl)acetyl]glycine (NL1), 5-[(6-bromo-1*H*-indol-1-yl)methyl]-2-methylfuran-3-carboxylic acid (NL2), and 3-{[6-(7-chlorobenzo[*b*]thiophen-2-yl)-1*H*-indol-1-yl]-methyl}-1*H*-pyrazole-5-carboxylic acid (NL3), are most efficient and promising for optimization (Figure 1).[3] Recently,[6] we developed a methodology for the gram-scale syntheses of

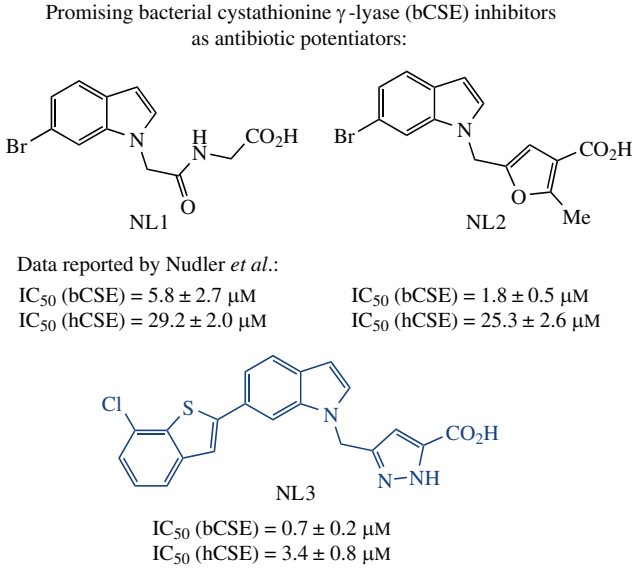


Figure 1 Inhibitors of bacterial cystathionine γ-lyase (bCSE).[3]

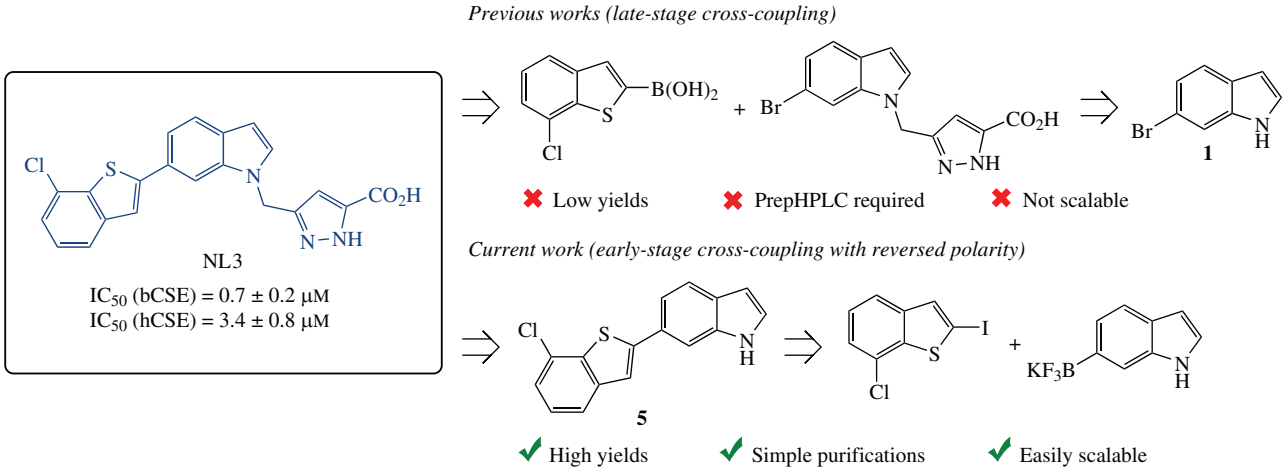
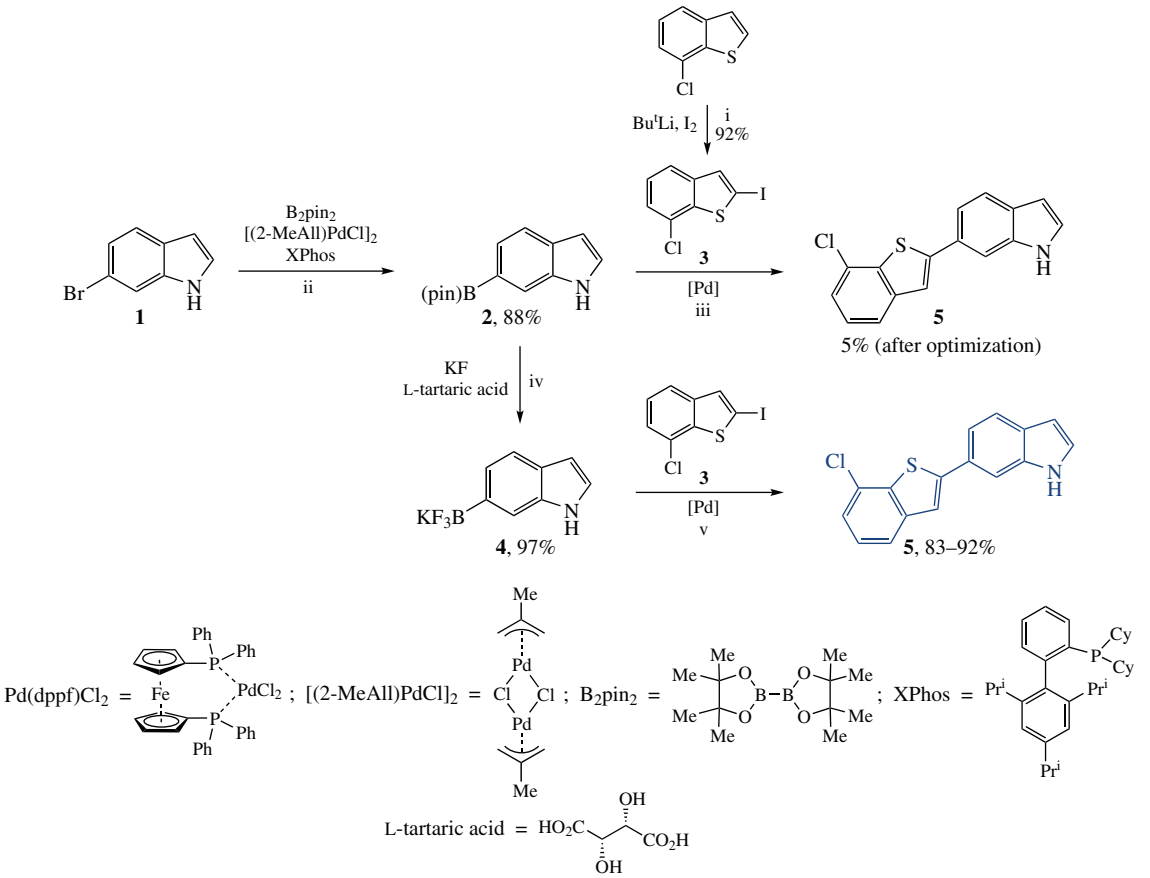


Figure 2 Synthetic routes to the bCSE inhibitor NL3.

these bCSE inhibitors, namely NL1, NL2 and NL3. However, in the case of NL3 that is the most active inhibitor, the problem of the last key cross-coupling step, which is very poorly reproducible and does not allow the product to be obtained in good yields, has not been solved to date.

The previously reported[3,4,6] syntheses of NL3 inhibitor are based on the use of 6-bromoindole as the starting reagent and are quite laborious. The final stage of Pd-catalyzed cross-coupling is characterized by a number of side processes and occurs poorly due to the low reactivity of the bromine or iodine atom in combination with the pyrazole substituent that strongly interferes with the process (Figure 2). As a result, the yield at the last key stage is 16% or even less, and preparative HPLC is required to isolate pure NL3 inhibitor from the reaction mixture, which further reduces the yield and results in poor reproducibility and infeasibility of scaling up above 50–70 mg.[4,6]

In this work, we developed a simpler and better scalable synthesis of NL3 inhibitor by shifting the step of cross-coupling the indole and benzothiophene moieties to the very beginning of the scheme (before the assembly of the pyrazole moiety in NL3). This makes it possible to increase their reactivity considerably and get rid of the adverse effect of pyrazole, as well as by reversing the polarities of the components being combined by cross-coupling (see Figure 2). Moreover, we performed a qualitative test of the inhibitory activity of the resulting NL3 against saCSE (a special case of bCSE isolated from *Staphylococcus Aureus*) using STD NMR.

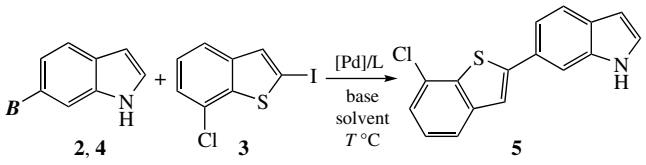


Scheme 1 *Reagents and conditions*: i, Bu^{t}Li (1.6 M in hexane, 1.5 equiv.), –78 °C, 5 h, THF, then I_2 (1.5 equiv.), THF, –78 → 20 °C, overnight; ii, B_2pin_2 (1.05 equiv.), $[(2\text{-MeAll})PdCl]_2$ (1 mol%), XPhos (4 mol%), KOAc (3 equiv.), 2-MeTHF, 80 °C; iii, $[(2\text{-MeAll})PdCl]_2$ (2.5 mol%), XPhos (10 mol%), Na_2CO_3 (3 equiv.), 2-MeTHF, H_2O, 80 °C; iv, KF (4 equiv.), L-tartaric acid (2.05 equiv.), MeCN, MeOH, THF, 25 °C; v, $Pd(dppf)Cl_2$ (5 mol%), Na_2CO_3 (3 equiv.), CH_2Cl_2, toluene, EtOH, H_2O, 100 °C.

Preliminary optimization of the cross-coupling[7–11] step (Scheme 1) was performed using 6-boronylindole **2** with (pin)B group as the nucleophilic component, which is easy to obtain by 6-bromoindole **1** borylation with $B_2(pin)_2$. Extensive optimization of the reaction parameters showed that acceptable yields of the target product in the cross-coupling of **2** with 7-chloro-2-iodobenzothiophene **3** could not be achieved by simply varying the reaction conditions (Pd source/ligand/base/solvent) (Scheme 2). In this way, Na_2CO_3 was chosen as starting base, and toluene/EtOH/H_2O mixture was chosen as the starting solvent to ensure high temperature in the reaction mixture (100 °C), while EtOH is necessary to improve solubility of the reagents in toluene. Under these conditions Pd(dppf)Cl_2 as the catalyst gave completely no reaction proceed (Table 1, entry 1). Catalysts $Pd(PPh_3)_4$ or [(2-MeAll)PdCl]$_2$ with XPhos or SPhos as the ligands allowed to get traces of the target product detected by GC (entries 2–4). Switching the solvent to MeTHF/H_2O (MeTHF is 2-methyltetrahydrofuran) along with lowering the reaction temperature to 80 °C according to MeTHF boiling point allowed to increase the yield to as high as 5% (entry 5). Further variation of base (K_3PO_4, Cs_2CO_3, Bu^{t}OK, PhOK) or solvent (dioxane/water, DMF, DMAc) at 100 °C did not gave any detectable product.

At the same time, the most important factors affecting the efficiency of the cross-coupling of indole-boronic derivatives involve substituents on the boron atom and the type of the protecting group on the nitrogen atom.[12] In this case, the best way to simplify the synthetic scheme was to get rid of protecting groups on the nitrogen atom at all and use a free NH-group. Thus, converting the pinacolboronate moiety in **2** to (indol-6-yl)-trifluoroborate **4** allowed us to significantly increase the yield in the cross-coupling reaction to 83% without specific conditions (see Scheme 1, Table 1, entry 6). Further scaling of the reaction to 2.5 mmol of benzothiophene **3** makes it possible to increase the yield up to 92%.

The thus developed novel synthetic scheme allowed us to easily obtain 6-(7-chlorobenzo[*b*]thiophen-2-yl)-1*H*-indole **5** in considerable amounts. Compound **5** proved to be a very convenient building block for the assembly of the NL3 inhibitor. This approach may be employed in the future to conveniently vary the heterocyclic moiety at position 6 of the indole ring for the development of new efficient inhibitors of bacterial cystathionine γ-lyase.



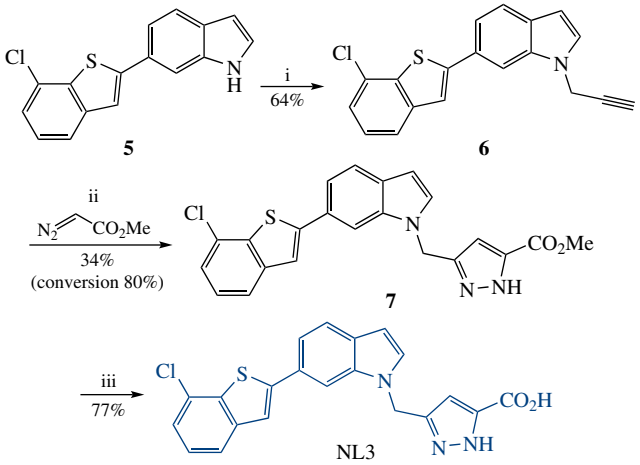
Scheme 2 *Reagents and conditions*: see Table 1.

Table 1 Optimization of the Pd-catalyzed cross-coupling step (selected data).[a]

Entry	***B***	[Pd]/L	Yield (%)[b]
1	Bpin (**2**)	Pd(dppf)Cl_2	no reaction
2	Bpin (**2**)	$Pd(PPh_3)_4$	trace[c]
3	Bpin (**2**)	[(2-MeAll)PdCl]$_2$/XPhos	trace[c]
4	Bpin (**2**)	[(2-MeAll)PdCl]$_2$/SPhos	trace[c]
5	Bpin (**2**)	[(2-MeAll)PdCl]$_2$/XPhos[d]	5
6	BF_3K (**4**)	Pd(dppf)Cl_2	83

[a] Reagents and conditions: 0.1 mmol scale, **2** or **4** (1.5 equiv.), [Pd]/L (5 mol%), Na_2CO_3 (3 equiv.), toluene, EtOH, H_2O, 100 °C, sealed tube, 4 h. [b] Isolated yields. [c] Trace is *ca.* 1% detected by GC. [d] MeTHF/H_2O as the solvent, 80 °C.

After the cross-coupling step giving compound **5** has been accomplished, the remaining pyrazole moiety in NL3 may be further assembled. Compound **5** is poorly soluble even in polar solvents, so we were worried that due to this fact problems would be encountered in the further synthetic stages. However, those concerns did not prove true, and the previously developed part of the synthetic route for the assembly of the 3-methylpyrazole-5-carboxylic acid moiety based on 6-bromoindole[6] was found to be applicable to access compound **6** as well (Scheme 3). The synthetic chain involves three steps: alkylation with propargyl bromide,[13,14] [3+2]-cycloaddition of the diazo ester at the triple bond to form the pyrazole,[4] followed by hydrolysis of the carboxylic ester. Due to the much bulkier heterocyclic moiety than in bromoindole, compound **5** and the subsequent intermediates are less reactive, thus requiring more drastic reaction conditions, while excellent yields are not ensured (however, they are still higher than in the previously published article). On the other hand, the isomeric selectivity of the reactions is higher. In fact, no allene side product is formed at the alkylation stage with propargyl bromide, that used to be a serious problem in the original synthesis.



Scheme 3 *Reagents and conditions*: i, NaH (1.3 equiv.), DMSO, 2 h, then $HC{\equiv}CCH_2Br$ (1.3 equiv.), 3 h; ii, $N_2{=}CHCO_2Me$ (2 equiv.), toluene–pyridine (5 : 1), 100 °C, 8 h; iii, KOH (9 equiv.), MeOH/THF/H_2O (2 : 1 : 1), 40 °C, 8 h, then HCl/H_2O (10%), pH 1.

At the stage of diazo ester cycloaddition, almost nothing of side regio-isomer of pyrazole is formed (>15 : 1), and that is also a significant improvement. In the previously published method, the similar step was significantly complicated during the purification. The by-product isomer used to be very difficult to separate from the target one at the initial isomers' ratio of 5 : 1. As for the last hydrolysis stage, it occurred smoothly and without problems. Thus, by changing the sequence of stages and preliminary preparation of the activated boron substrate for the cross-coupling reaction, we obtained the target inhibitor NL3 in a good yield and with the possibility of scaling up the synthesis in the future. The structure of NL3 synthesized by the new procedure is confirmed by ^1H, ^{13}C, ^{15}N 1D and 2D NMR spectroscopy and HRMS spectral data, that matches with the previously reported samples.[3,4,6]

Next, the ability of the resulting NL3 sample to inhibit recombinant bacterial cystathionine γ-lyase (saCSE from *Staphylococcus aureus*)[3] was experimentally tested using NMR spectroscopy. For this purpose, we used Saturation Transfer Difference (STD) NMR experiments,[15–17] which proved to be well suitable for very low protein concentrations, including unstable samples. Taking into consideration the strong binding and a reported small constant for this inhibitor (~0.7 μM),[3]

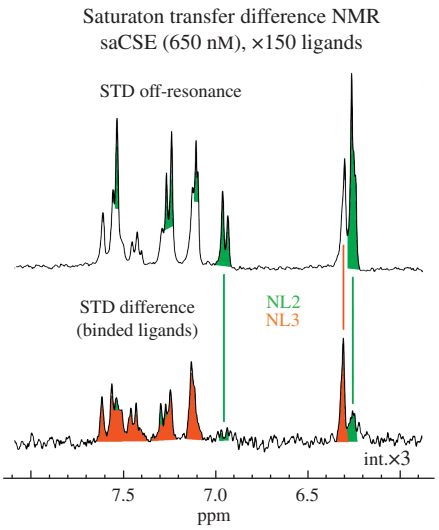


Figure 3 Competitive binding of NL3 and NL2 inhibitors with bacterial saCSE protein (650 nM) using STD NMR spectra.

experiments were performed with a very dilute sample of saCSE protein using a concentration as small as 650–800 nM. Binding was estimated at a qualitative level (full quantitative titration was not performed), which is sufficient for the testing purposes stated. The STD experiments clearly demonstrate the efficient inhibition of saCSE protein; moreover, the parameters of the experiments fit in the range of the previously reported constants for the NL3 inhibitor. In addition, STD NMR experiments on competitive binding of two inhibitors, NL3 and a less active reference inhibitor NL2, were performed (Figure 3). The superior inhibitory ability of NL3 in comparison with NL2 was shown and the data matched quite well with the values previously reported[3] by the Nudler team, with an almost 3-fold difference between the values for NL3 and NL2.

To sum up, a convenient and efficient synthesis of 3-{[6-(7-chlorobenzo[*b*]thiophen-2-yl)-1*H*-indol-1-yl]methyl}-1*H*-pyrazole-5-carboxylic acid (NL3), which is among the most promising inhibitors of bacterial cystathionine γ-lyase (bCSE), has been accomplished. The suggested synthetic method allowed us to considerably increase the yield of 6-(7-chlorobenzo[*b*]-thiophen-2-yl)-1*H*-indole as the key intermediate building block. Main crucial findings in the synthesis involved a shift of the key step of [Pd]-catalyzed cross-coupling of the indole and benzothiophene moieties to the beginning of the synthetic chain, and the additional reversal of polarity of the components that are coupled. The binding of the synthesized NL3 potentiator with saCSE (the main producer of H_2S in pathogenic *S. aureus*) has been qualitatively confirmed by STD NMR spectra. The developed method would be important for obtaining new sets of NL3-based bCSE inhibitors in the further screening of active potentiators.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.02.030.

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