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Efficient synthesis of a new bifunctional Cu(I) chelator

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ABSTRACT

Herein we report an efficient eight-step synthesis of N^1-(3-(methylthio)propyl)-2-((3-(methylthio)propyl)thio)benzene-1,4-diamine (**1**) from 6-nitrobenzo[*d*]thiazole. Incorporation of a nitro rather than a hydroxyl functionality off the aromatic ring serves to enhance conversion during thia- and aza-conjugate additions. Ultimate reduction of the nitro substituent affords a bifunctional Cu(I) chelate in gram-scale quantities for subsequent sensing applications.

As the third most abundant metal in biology, copper is intimately involved in the transport and activation of molecular oxygen.[1] This role is due in large part to its facile redox nature. However, the ability to access multiple oxidation states also renders this metal potentially toxic, with an increase in oxidative and nitrosative stress being linked with cancer[2] and neurodegeneration.[3] Monitoring this metal in a biological context is therefore necessary to elucidate the relationship between disruptions in metal-ion homeostasis and pathophysiology. The most attractive method for doing so is through the design and development of ligands that result in a measurable change in fluorescence upon metal-ion binding.[4]

Fahrni and co-workers have developed a series of fluorescent pyrazolines exhibiting increased fluorescence in the presence of Cu(I) through inhibition of photoinduced electron transfer (PET) (Fig. 1).[5] Chelation of Cu(I) serves to lower the ligand's HOMO, thereby deactivating the PET process and restoring fluorescence. The authors quickly accessed these probes by first synthesizing a bifunctional N_1S_3 ligand (**2**) that then underwent an aldol condensation with 4-acetylbenzonitrile. Subsequent condensation with one of five fluoro-substituted phenylhydrazine derivatives afforded the corresponding N_1S_3-functionalized pyrazolines (**3**). This ligand is extremely selective for Cu(I) over other interfering metal ions given the soft sulfur donors[6] and the propensity of this ligand to form a tetrahedral coordination environment, thus stabilizing Cu(I) relative to Cu(II).[5] Despite their elegant synthesis, these probes suffered from poor solubility in aqueous media in addition to high-energy excitation (346–391 nm) and emission profiles (432–486 nm) unsuitable for biological imaging.

To improve tissue penetration, Chang and coworkers recently reported a near-infrared fluorescent sensor for monitoring biological Cu(I) also using PET.[7] They achieved this by developing a similar bifunctional N_1S_3 ligand (**4**) that was then covalently attached to cyanine **7** to afford **5** (Fig. 1). Although this account demonstrated *in vivo* visualization of labile Cu(I), these 'turn-on' approaches toward metal-ion monitoring are incapable of providing quantitative measurements. Thus, sensors capable of modulating the ratio of multiple emission bands (ratiometric) are preferred in that they generate quantitative information.[8] However, few ratiometric sensors for copper exist, while most suffer from poor water solubility[9] and irreversible sensing.[10]

Using a modular motif, we previously demonstrated ratiometric fluorescent metal-ion sensing for quantifying Cu(II) concentrations in environmental water samples.[11–13] This was achieved by copolymerizing a bifunctional ligand with poly(*N*-isopropylacrylamide) (PNIPAm) and a fluorophore pair capable of engaging in Förster resonance energy transfer (FRET).[12,13] Temperature-responsive PNIPAm undergoes a structural transformation (random coil to collapsed globule) at 32 °C, known as its lower critical solution temperature (LCST). This temperature may be systematically raised and lowered by increasing hydrophilic and hydrophobic interactions, respectively.[14] For instance, incorporating a charge-neutral ligand results in LCST depression, whereas metal-ion binding introduces charge that causes the polymer to expand when temperature is held constant. This change in shape results in modulation of FRET efficiency, which may then be correlated with metal concentration (Fig. 2).[12,13] The highly modular nature of this

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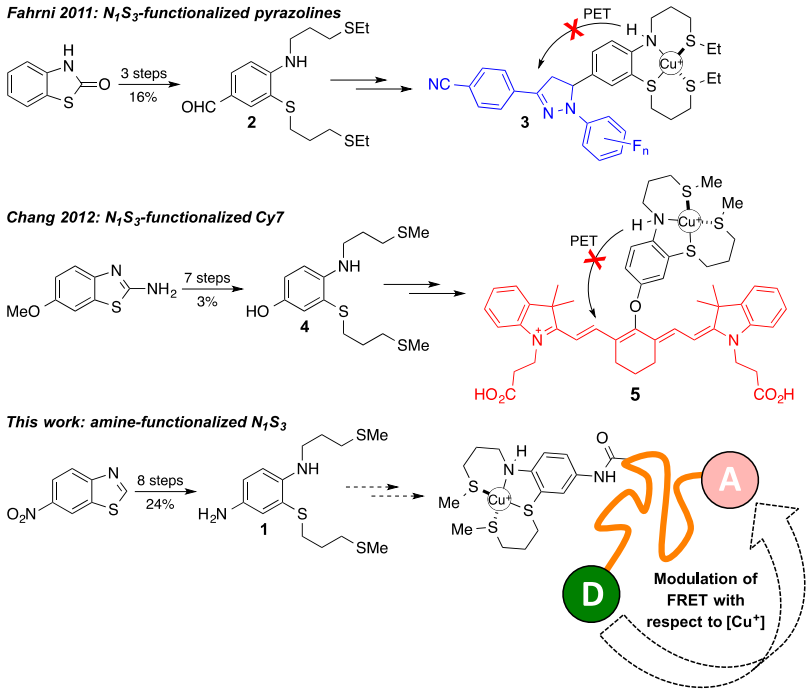


Figure 1. Bifunctional N_1S_3 ligands and their incorporation within sensing scaffolds for fluorescently monitoring Cu(I).

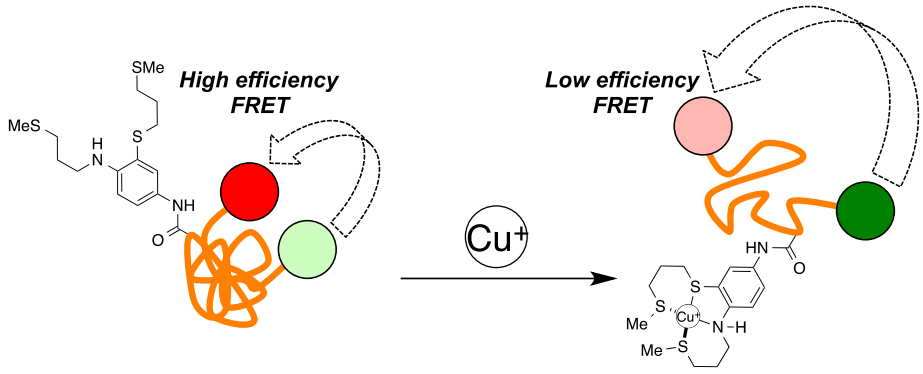
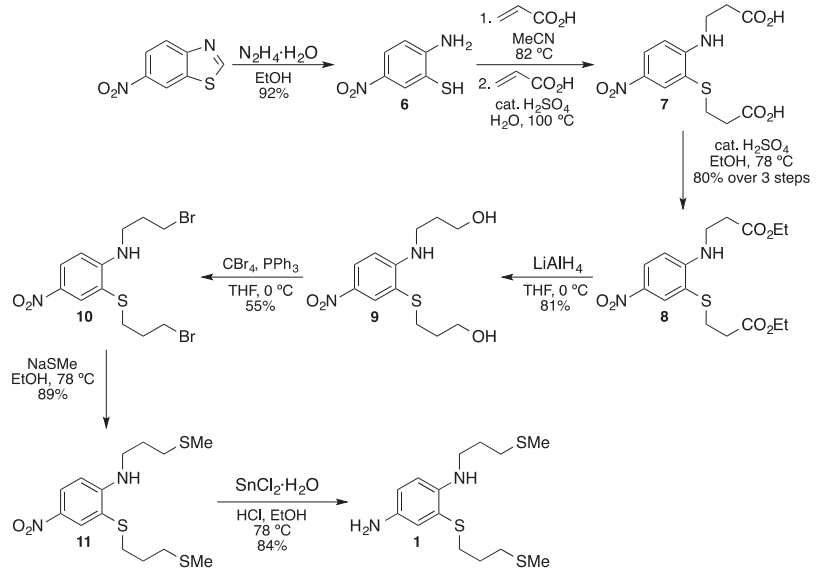


Figure 2. Temperature-responsive PNIPAm incorporating a charge-neutral ligand exists as a collapsed globule above its LCST. This macromolecular structure facilitates facile energy transfer between a donor and acceptor fluorophore pair. Binding of metal ions by this ligand, however, introduces charge along the polymer scaffold, thereby elevating the LCST. This results in polymer expansion and a concomitant decrease in energy transfer. By physically separating metal recognition from fluorescence transduction we have demonstrated ratiometric sensing in a modular fashion.

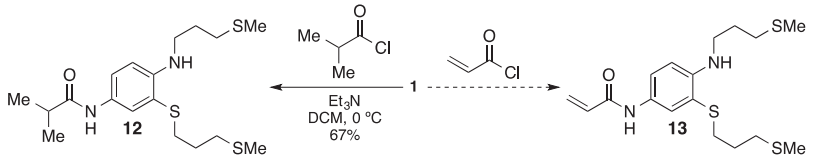
sensor permits selective sensing of the desired metal through rational design of the bifunctional ligand only.

Given our modular sensing platform, we proceeded to design a ligand similar to **4** that instead incorporated a polymerizable acrylamide for ratiometric sensing of Cu(I). Unfortunately, synthesis of **4** suffered from low yields throughout, especially during conjugate addition and Fischer esterification (Fig. 1). This observation is likely attributed to undesirable dialkylation of the anilino nitrogen as previously suggested.[5] Fahrni and co-workers initially overcame this obstacle by using benzothiazolinone to simultaneously mask the nitrogen's free valency and lower its pK_a. However, despite efficient monoalkylation, hydrolysis of the carbonyl and concomitant S-alkylation in a single step was hampered by a 24% yield. We therefore proposed starting with 6-nitrobenzo[*d*]thiazole as a means to deactivate dialkylation during aza-conjugate addition. Further, this functionality would allow easy access to the desired acrylamide for subsequent incorporation within our indicator design. To our delight, this resulted in an 80% yield over three steps as shown in Scheme 1 versus the previously reported 20% for the analogous reactions done in two steps during the synthesis of **4**.

Our synthesis commenced with deprotection of 6-nitrobenzo[*d*]thiazole by treatment with hydrazine monohydrate to afford *o*-aminothiophenol **6**,[15] which was carried on immediately to minimize disulfide formation (Scheme 1). In the event that substantial material was converted to the disulfide, addition of stoichiometric amounts of dithiothreitol was sufficient to generate the free thiol in situ during the subsequent thia-conjugate addition.[16] Thia- and aza-conjugate additions with acrylic acid were initially attempted in a single step, though this method proved unsuccessful. While the thia-conjugate addition proceeded smoothly in refluxing MeCN to afford a mixture of product and unreacted acrylic acid, the aza-conjugate addition required H_2SO_4 as an additive in refluxing H_2O. Upon cooling, pure diacid **7** precipitated out of solution as a yellow solid and was used without



Scheme 1. Efficient synthesis of a new amine-functionalized Cu(I) chelator.



Scheme 2. Model (**12**) and proposed copolymerizable compound (**13**), the latter of which will be incorporated within a water-soluble copolymer designed to fluorescently sense bioavailable Cu(I).

further purification. Fischer esterification of this material in refluxing EtOH afforded diester **8** upon workup in 80% yield over three steps. This material was then reduced to the corresponding diol (**9**) in 81% yield with $LiAlH_4$ in anhydrous THF. Conversion of **9** to **10** in the presence of CBr_4 and PPh_3 was achieved in 55% yield, followed by nucleophilic displacement of the corresponding alkyl bromides with NaSMe to yield dithioether **11** in 89% yield. Reduction of the nitro functionality with $SnCl_2{\cdot}H_2O$ under acidic conditions generated the desired bifunctional N_1S_3 ligand (**1**) in 84% yield.

Comparison of the above eight-step synthesis with the previously reported seven-step synthesis of **4** demonstrates an increase in the overall yield of a bifunctional Cu(I) chelator by eightfold (Fig. 1). Though nearly twice as long as the synthesis of **2**, our approach to inhibiting overalkylation of the anilino nitrogen afforded increased yields throughout. A number of intermediates were also accessed along the way with potential for tuning the indicating range of our sensor (**7** and **9**). Moreover, the bifunctional handle has been replaced with an aromatic amine, though the N_1S_3 moiety remains unchanged from **4**. As previously stated, our group is interested in converting this amine handle into an acrylamide (**13**) for subsequent copolymerization within a temperature-responsive polymer that functions as a fluorescent metal-ion indicator (Scheme 2).[11–13] This acrylamide functionality is necessary to ensure random distribution within our PNIPAm copolymer owing to similar kinetics of polymerization. To mimic the electronics experienced following copolymerization of target acrylamide **13**, we have synthesized model ligand **12**. Characterization of the metal-binding abilities of **12** by potentiometric/spectrophotometric titrations and cyclic voltammetry is currently underway. Further, **1** is predicted to display a heightened affinity for Cu(I) relative to **2** and **4** due to the increased electron-donating tendency of the amino functionality, thus yielding greater sensitivity necessary for biological sensing applications.

To conclude, we have designed and synthesized a new bifunctional ligand selective for Cu(I) over other interfering metal ions based upon previously reported N_1S_3 ligands. The overall yield was increased substantially (eightfold) relative to the account of Chang and coworkers by incorporating an electron-withdrawing nitro functionality to minimize dialkylation during aza-conjugate addition. Further, this strategy afforded enhanced yields throughout when compared to the approach taken by Fahrni and co-workers. Work is currently underway to demonstrate the sensing capability of this ligand following incorporation within a temperature-responsive polymer capable of ratiometrically sensing Cu(I) through macromolecular changes in conformation.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures and ^1H and ^{13}C NMR spectra) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.04.079.

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