Synthesis of a Paramagnetic Myeloperoxidase Substrate with Superoxide Dismutase Mimetic Activity

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Background

Myeloperoxidase (MPO) is an enzyme contained within azurophilic granules of neutrophils and is released as a result of neutrophil degranulation upon activation

- Reacts with H_2O_2 first to generate oxidized enzyme
- Cl^- and 5-hydroxytryptamide are common substrates
- Products are involved in cellular inflammation signaling pathways
- Level in arteries has been shown to correlate with adverse effects

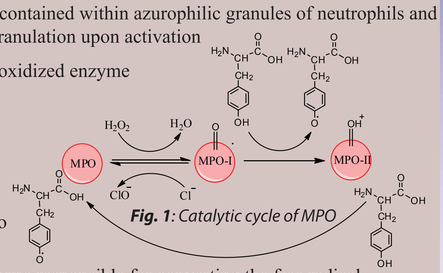


***Fig. 1**: Catalytic cycle of MPO*

Superoxide Dismutase (SOD) is an enzyme responsible for converting the free radical O_2^- into H_2O_2 and water

- O_2^- is generated in the mitochondria during normal respiratory processes or generated by NADPH oxidase during phagocytosis
- O_2^- is associated with oxidative damage and $[O_2^-]$ correlates with infarction and ischemia
- M40401 is a Mn(II) macrocycle with SOD activity exceeding the native enzyme
- Mn(II) center allows for use as contrast agent in MRI

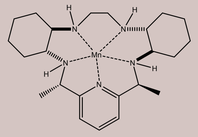


***Fig. 2**: M40401*

Magnetic Resonance Imaging (MRI) is an emerging noninvasive imaging technique capable of generating high-resolution images of three dimensional anatomical structure

- Charged, spinning ^1H nuclei interact with magnetic fields to generate signals that can be viewed as magnetic moments using external magnetic field gradients
- Electronic interactions from paramagnetic species alter the signal strength from local protons to generate additional contrast
- Some paramagnetic species are attached to a substrate that interacts with an enzyme, allowing MR images to display local enzymatic activity as changes in contrast

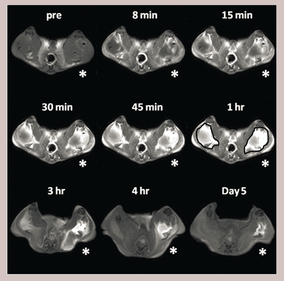


***Fig. 3**: MR imaging of a mouse femoral muscle at 3T with implanted Matrigel and MPO/GOX mixture. T1-weighted images are shown post IV injection of bis-HTrp-DTPA(Gd) depicting MPO/GOX enhancement as a function of time in the left femoral muscle region (indicated with asterisk) while the contralateral side implanted with Matrigel served as the control. The signal intensities from all time points were normalized to pre-contrast slice and are thus directly comparable [REF 2].*

Click Chemistry

Defined as a subset of reactions that are *easy to perform*. Some criteria include:

- High yield, wide scope, compatibility with non-toxic solvents, available from readily available starting materials, and purification without chromatography

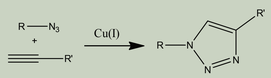
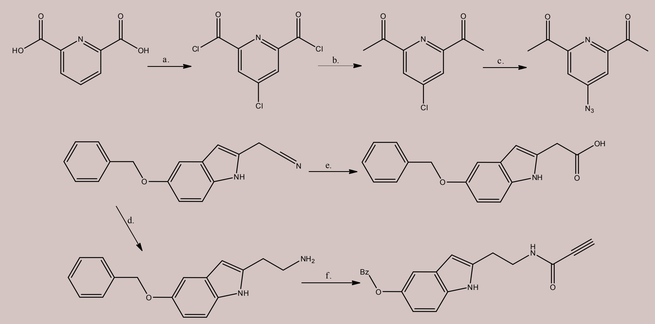


***Fig. 4**: Cu(I) catalyzed Huisgen 1,3 dipolar (3+2) cycloaddition, a click chemistry reaction*

References

[1] Riley D P. Chem. Rev. 1999, 99: 2573-2587
[2] Shazeeb M, Xie Y, Gupta S, Bogdanov A Jr. *Mol Imaging*. 2012, 11: 433-443.
[3] Kolb H C, Finn M G, Sharpless K B. *Angew. Chem. Int. Ed*. 2001, 40: 2004–2021.
[4] Teyssot M-L et al. *Eur. J. Org. Chem.*, 2010, 3507-3515.

Methods



***Scheme 1**: Synthesis of intermediates for click reaction. (**a**) (i) 30% H_2O_2, Na_2WO_4 (0.6 mol-%), 16h (ii) $(COCl)_2$, CH_2Cl_2, 72h. (**b**) (i) Meldrum's acid (2 eq), pyridine (4 eq), CH_2Cl_2, 2h (ii) H_2O/HOAc (2:1), reflux, 12h. (**c**) NaN_3 (10 eq), DMF, 50 °C, 16h. (**d**) $LiAlH_4$, THF (**e**) 20% aq. KOH, reflux, 16h. (**f**) propiolic acid (1.5 eq), HBTU (1.5 eq), Et_3N (2.5 eq), DMF, 2h.*

Azide and alkyne intermediates were synthesized and reacted in MeCN using CuI as a catalyst

- Analysis on reaction was inconclusive, and TLC showed very little new product
- Literature analysis pointed to a problem with heterocycles coordinating and scavenging free Cu(I) catalyst
- A better catalyst system was identified that includes Cu(I) bound to an N-heterocyclic carbene (NHC), 1,3-Bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene (SIMes), and secondary ligand 4,7-dichloro-1,10-phenanthroline

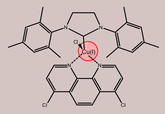
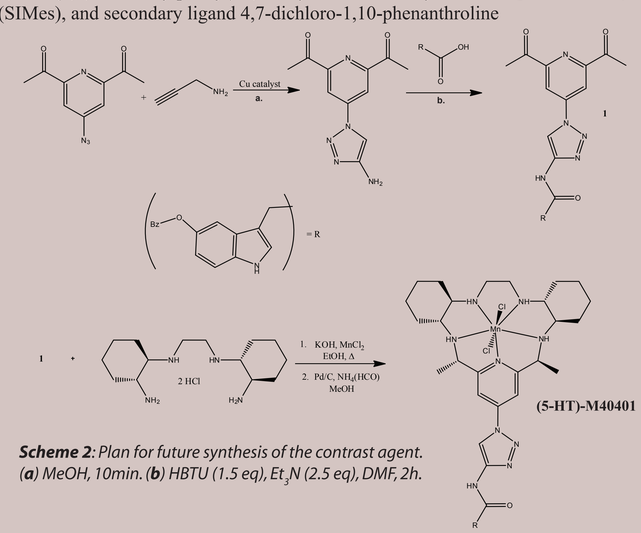


***Fig. 5**: Cu(I) catalyst*



***Scheme 2**: Plan for future synthesis of the contrast agent. (**a**) MeOH, 10min. (**b**) HBTU (1.5 eq), Et_3N (2.5 eq), DMF, 2h.*

Synthesis of the contrast agent (5-HT)-M40401 will hopefully proceed over the summer

- Catalyst will be synthesized in 2 steps from commercially available reagents
- Deprotection of benzyl group will conveniently take place during macrocycle reduction in the presence of Pd/C and ammonium formate
- Properties of contrast agent will be tested both *in vitro* and *in vivo*

Acknowledgements

I would like to thank my on-campus advisor Professor Drew Brodeur for giving me assistance whenever he could and for (trying to) keep me caught up with everything.

Above all, I would like to thank Professor Alexei Bogdanov for making this project possible and everyone in his molecular imaging lab for offering me their experienced guidance with any lab procedures and for staying patient with me throughout all of my mistakes.

Results

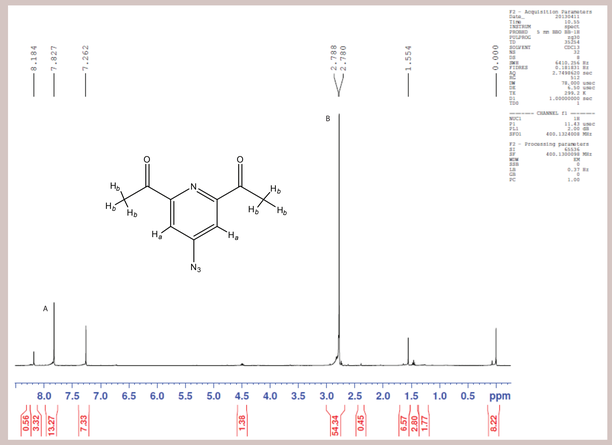


***Fig. 6**: ^1H NMR spectrum of 4-azido-2,6-diacetylpyridine ($CDCl_3$). Remaining reactant 4-chloro-2,6-diacetylpyridine can bee seen as a small peak at 8.18 ppm.*

1) We obtained two click chemistry intermediates, i.e. 4-azido-2,6-diacetylpyridine and 2-(2-propiolamidoethyl)-1H-indol-5-yl benzoate, in quantities sufficient for characterization.
2) We identified potential improvements that can be made to increase yields. The meldrum's acid step in the 4-chloro-2,6-diacetylpyridine synthesis that resulted in low yields will be replaced with a Grignard reaction of 4-chloropyridine-2,6-dicarboxamide.
3) We plan to replace the poorly reactive propiolamidoethylindole product with propargylamine for the click reaction, which can then be joined to an indoleacetic acid by a condensation reaction. This is expected to increase yields of 4-substituted 2,6-diacetylpyridine.

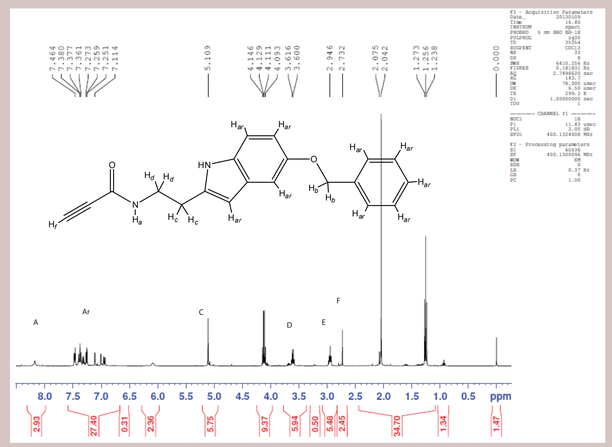


***Fig. 7**: ^1H NMR spectrum of 2-(2-propiolamidoethyl)-1H-indol-5-yl benzoate ($CDCl_3$). Residual solvent peaks (EtOAc) can bee seen at 4.12, 2.04, and 1.26 ppm.*

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