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Synthesis and SOD activity of manganese complexes of substituted pyridino pentaaza macrocycles that contain axial auxiliary

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ABSTRACT

New manganese(II) complexes of substituted pyridino pentaaza macrocyclic ligands were prepared. The amino-, carboxy-, or other functional groups were placed in the vicinity of the axial position of the metal complex. Their SOD-like activity was determined by cytochrome *c* assay and compared with one another. The activities of pyridine analogs (**12a–b** and **13)** and *m*-substituted analogs (**12c** and **12j**) were similar and significantly better than that of the standard compound M-40403. The most potent compound was an *o*-aminobenzoyl derivative **12i**, while the *o*-carboxybenzoyl analog **12d** was the lowest active compound.

Reactive oxygen species (ROS), such as the superoxide radical anion ($O_2^{-\cdot}$) and hydrogen peroxide (H_2O_2), can be generated from cellular metabolism in aerobic organisms. The imbalance between ROS production and elimination is implicated in many diseases and aging, while the overproduced ROS have been shown to oxidize various biomolecules, including DNA, proteins, and lipids, which can cause various forms of damage to cells and tissues. Therefore, antioxidant materials have been considered as therapeutic agents for a variety of disorders, including ischemia-reperfusion injuries,[1] arthritis,[2] stroke,[3] Parkinson's disease,[4] ALS (Lou Gehrig's disease),[5] and cancer,[6] in which ROS play a significant role. So far, many mimetics of antioxidant enzymes such as superoxide dismutases (SODs) or catalases have been developed[7] and tested in vivo.[8] Such catalytic mimetics of antioxidant enzymes include manganese(III) and iron(III) porphyrin complexes,[9] manganese(II) complexes of penta-azamacrocycles,[10] manganese(II) complexes that centered on tripodal ligands,[11] manganese(III) salen complexes,[12,8c] and the tetra-aza[14]annulene-Fe(III) complex.[13]

For the Mn(II) complexes of pentaaza macrocycles, Riley et al. prepared a series of C-substituted 1,4,7,10,13-pentaazacyclopentadecane ([15]aneN$_5$) and their corresponding Mn(II) complexes as their dichlorocomplexes, [Mn([15]aneN$_5$)Cl$_2$].[14] Methyl and fuged cycloalkyl substituents on carbon or pyridino derivatives of the core structure, [15]aneN$_5$, possess high SOD catalytic activity. In particular, one of the optimized complexes, M-40403 as shown in Figure 1, showed in vivo antitumor,[15] antiarthritis,[10a] and pain-relieving[16] activities. Recently, M-40403 has been granted orphan drug designation for the prevention of radiation or chemotherapyÙinduced oral mucositis in cancer patients.

In this Letter, an efficient synthesis and manganese complexes of new pyridino pentaaza macrocycles that have aminophenyl substitutent on the pyridine ring (Fig. 1) are described. Their SOD-like activity was determined via cytochrome *c* assay and compared with that of the standard compound, M-40403.

From the conformational analysis of the new ligand, it was expected that the functional group on the ortho position of the phenyl auxiliary could be positioned over the plane of the Mn complex via the rotation of biaryl linkage. It was also expected that the catalytic cycle of the superoxide dismutation would be influenced by the functional group, that is, attached to the R.

The synthesis of the macrocyclic ligands started with the di(*p*-methoxybenzyl (PMB)) protected *trans*-cyclohexyldiamine **1**.[17] Two *N*-tosylaziridines were added to the diamine **1** via a ring-opening reaction in order to produce the bistosyl derivative **2** in

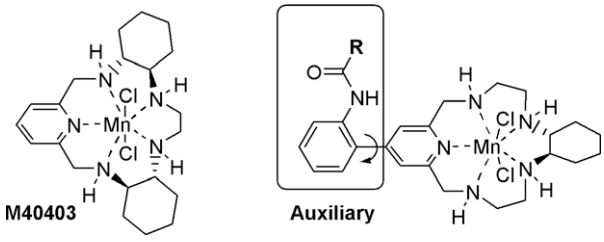

Figure 1. Structures of M-40403 and new manganese complexes of pentaaza macrocyclic derivatives.

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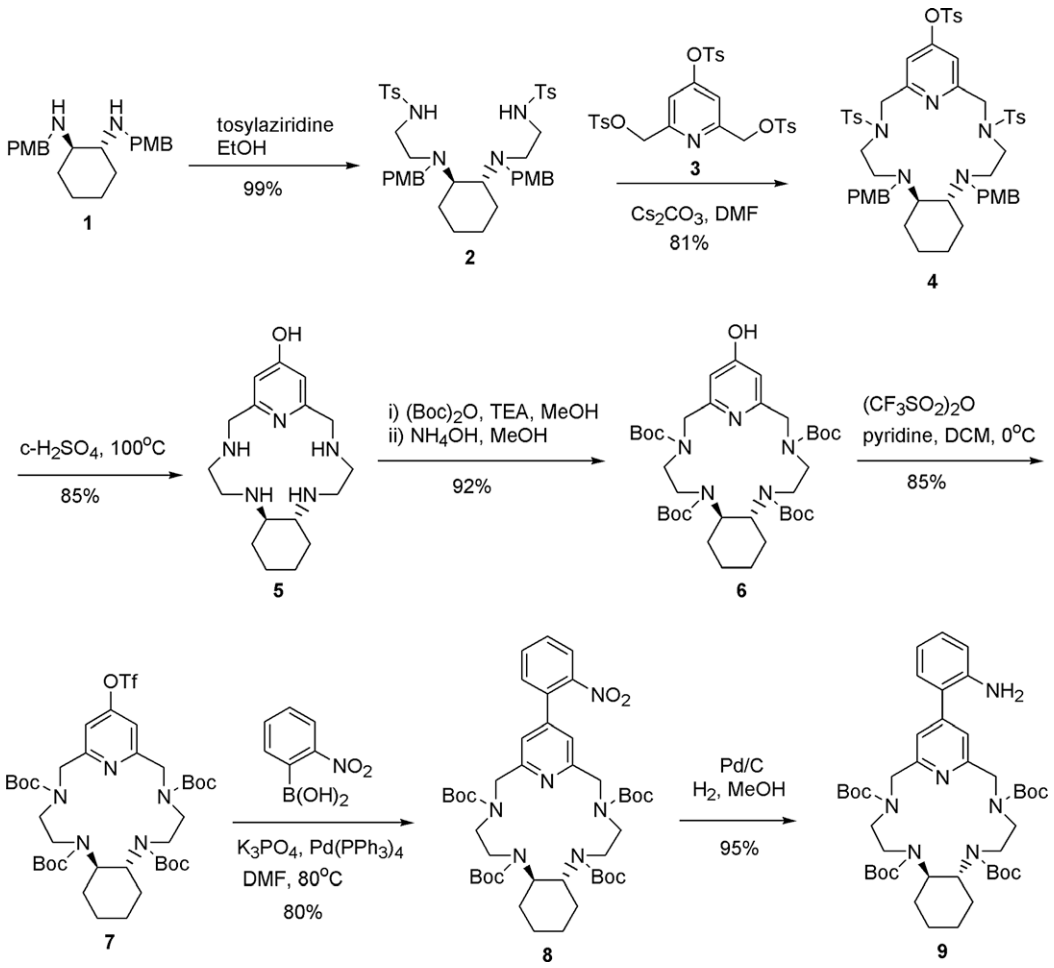
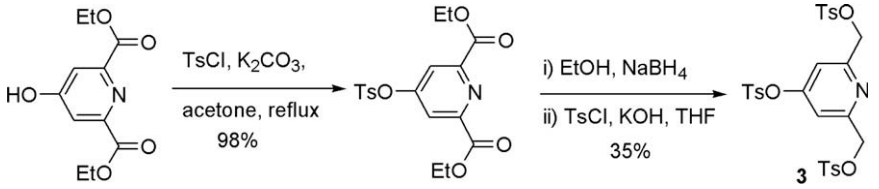


Scheme 1. Synthesis of protected pentaaza macrocyclic derivative **9**.



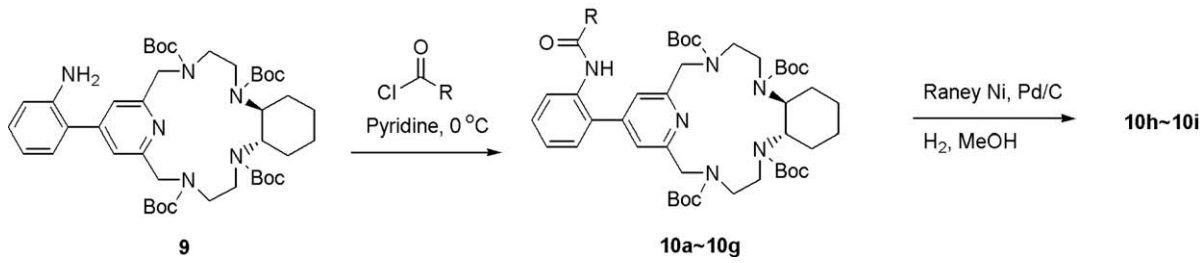
Scheme 2. Synthesis of tritosylated pyridine derivative.

excellent yield. The cyclization of the bistosyl amide **2** with the tritosylated pyridine derivative **3** was carried out under cesium carbonate in dimethylformamide with an 81% yield. This type of successful macrocyclization that can provide pentaaza macrocyclic ligands was previously reported.[18]

The tritosylated pyridine derivative **3** was prepared, as shown in Scheme 2. The diester of the commercially available chelidamic acid[19] was first tosylated with a 98% yield, and then the product was reduced with sodium borohydride and ditosylated without purification in order to produce the tritosylated pyridine derivative **3**.

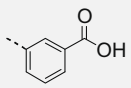
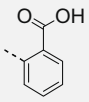
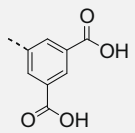
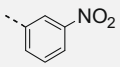
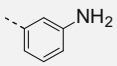
Following the macrocyclization of **2** as shown in Scheme 1, both the PMB and tosyl protecting groups of the macrocycle **3** were removed under sulfuric acid to provide the desired macrocycle (85%). Four Boc groups were introduced on the aliphatic nitrogen atoms via whole protection of the four secondary amines and a pyridinol followed by selective removal of the Boc group on the pyridinolic oxygen, providing the macrocycle **6** with a 92% yield. Replacing the PMB and tosyl protecting groups with the Boc is necessary for mild and convenient procedure at the later deprotection step. The pyridinol derivative **6** was triflated with trifluoromethanesulfonic anhydride and the triflate **7** was cross coupled to 2-nitrophenylboronic acid via Suzuki reaction[20] to provide the biaryl analog **8**. The nitro group in **8** was then converted to amino group under the Pd/C hydrogenation condition. The direct transformation of the triflate **7** to the amine **9** by a Suzuki reaction, which was coupled with a 2-aminophenylboronic acid pinacol ester, was problematic in the purification step.

Derivatization of the amine **9** was performed to incorporate various pyridine, carboxylic acid, and amine moieties in the pentaaza-macrocyclic ligand (Scheme 3 and Table 1). For the preparation of the *m*,*m*′-dicarboxybenzanilide **10e**, a low yield (36%) was obtained due to the sluggish hydrolysis of the diacid chloride, which is an intermediate from the reaction with a benzene triacid chloride. The nitrobenzanilide derivatives **10f–g** were further reduced to the corresponding aminobenzoyl derivatives **10h–i** by using a mixture of Raney Ni and Pd/C as a catalyst. Other conditions, like Raney

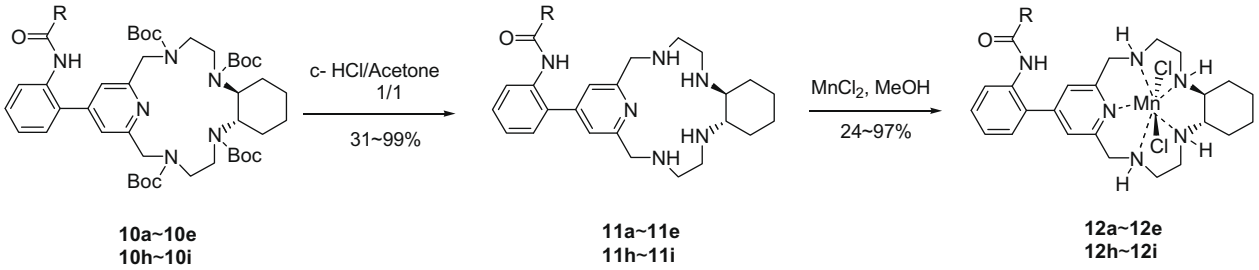


Scheme 3. Derivatization of the protected pentaaza macrocyclic ligand **9**.

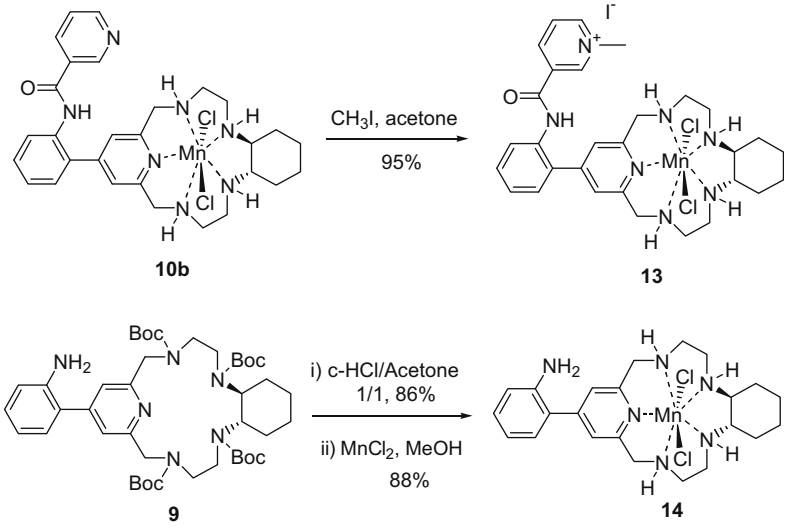
Table 1
Isolated yields for the derivatization of **9**

Product	**10a**	**10b**	**10c**[a]	**10d**[b]	**10e**[c]	**10f**	**10g**	**10h**	**10i**
R									
Yield (%)	77	96	72	77	36	95	87	94	88

[a] Isophthaloyl dichloride was used in the reaction and the remaining acid chloride was treated with 0.5 N NaOH solution.
[b] Instead of acid chloride, phthalic anhydride was used in the coupling reaction.
[c] Benzene 1,3,5-triacid chloride was used and the remaining diacid chloride was hydrolyzed with 0.5 N NaOH solution.



Scheme 4. Boc deprotection and metal complexation of the ligands.



Scheme 5. Syntheses of the aminophenyl and *N*-methyl pyridinium complexes.

Ni, Pd/C or Raney Ni–$NaBH_4$,[21] were not efficient for these substrates because they provided a mixture of both the desired product and partially reduced hydroxyamino intermediate.

The four Boc protecting groups of the pentaaza macrocycles **10a–e** and **10h–i** were removed under the HCl/acetone condition to give new ligands, **11a–e** and **11h–i**, with 31–99% yields (Scheme 4). The products were then complexed with $MnCl_2$ in MeOH, which afford new SOD enzyme mimetics, **12a–e** and **12h–i**, that contained various auxiliary positioned over the plane of the Mn complex.

Table 2
SOD-like activities of new compounds

Compound	SOD activity[a] IC_{50}, μM
M-40403	2.7 (0.6)
12a	0.58 (0.14)
12b	0.63 (0.09)
12c	0.58 (0.02)
12d	4.7 (1.3)
12e	0.94 (0.26)
12h	0.38 (0.01)
12i	0.59 (0.07)
13	0.63 (0.07)
14	2.2 (0.5)

[a] Values are averages of duplicate determinations with pH 7.8, standard deviation is given in parentheses.

The new SOD mimic that contains *N*-methyl pyridinium auxiliary **13** was also prepared from the nicotinoyl derivative **10b** via N-methylation (Scheme 5). For the picolinyl derivative **10a**, the corresponding *N*-methyl analog was not obtained successfully. Since some of the *N*-alkylpyridinium analogs of manganese(III) porphyrins that bore positive charges in close proximity to the metal site have been known to possess potent catalytic SOD-like activity,[22] the effects of the charged *N*-methyl pyridinium group on its SOD-like activity were explored. The aminophenyl analog **14** was also prepared from the intermediate **9** by a similar procedure as shown in Scheme 4, in order to verify the effect of aminophenyl substitution on its activity.

The new manganese complexes prepared in this study were tested for their SOD-like activity and the results are given in Table 2.

The SOD-like activity of the new complexes was measured by an indirect method, as described by McCord and Freidovich,[23] which used cytochrome *c* as an electron acceptor. Superoxide anions were generated by a xanthine–xanthine oxidase system. The possible interference with the manganese complexes was examined by following the rate of urate formation at 290 nm in the absence of cyt *c*. The complexes did not interfere with the reaction of xanthine with xanthine oxidase. Among the compounds tested in this study, the *o*-aminobenzoyl analog **12h** exhibited the best SOD-like activity, which possessed about sevenfold lower IC_{50} than the standard compound M-40403. Other compounds such as two pyridine analogs **12a–b**, *m*-carboxybenzoyl analog **12c**, *m*-aminobenzoyl analog **12i**, and *N*-methyl pyridinium analog **13** also showed significantly better activities than M-40403. The lowest SOD-like activity was observed for the *o*-carboxybenzoyl analog **12d**, which was in sharp contrast to the best activity of the *o*-aminobenzoyl derivative **12h**. For the simple aminophenyl analog **14**, a slightly better activity was obtained in comparison to that of M-40403.

In summary, new manganese complexes of pentaaza macrocyclic ligands were prepared that bore several functional groups in the vicinity of the metal site. Their activities were compared with one another. The SOD-like activities of pyridine analogs, **12a–b** and **13**, and *m*-substituted analogs, **12c** and **12i**, were similar and better than that of the standard compound M-40403. The most potent compound was an *o*-aminobenzoyl derivative **12h**, while the *o*-carboxybenzoyl analog **12d** was the compound that had the lowest activity. Further investigation is needed to understand the effects of the aminobenzoyl group on the catalytic cycle of superoxide dismutation.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.037.

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