A Study on Some Copper(II) Complexes of Polyaza Cryptands and Planar Macrocycles—Synthesis, Characterization and Superoxide Dismutase Activity

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Two kinds of macrocyclic copper(II) complexes were synthesized. One of them is composed of copper(II) cryptates of ligands L^1—L^4 which are condensation products of 5-R-2-methoxy-1,3-phenyldialdehyde (R = OCH_3, L^1) with tris(2-aminoethyl)amine and 5-R-2-methothoxy-1, 3-phenyldialdehyde (R = CH_3, L^3) with tris(2-aminopropyl)amine as well as their reduced products of L^1 and L^3 (L^2 and L^4). The other is composed of two-dimensional macrocyclic copper(II) complexes of ligands L^5—L^8 of condensation products of diethylene triamine with 4-R-1-methoxy-2,6-phenyldialdehyde (R = Cl, Br, CH_3, OCH_3). Therelationship between their structures and superoxide dismutase (SOD) activity was investigated. The results can provide some clues to the synthesis of SOD mimics.

Keywords Cryptate, macrocyclic complex, copper(II), superoxide dismutase, mimic

Introduction

It was found that copper complexes exhibit antiinflammatory, anticancer and anticonvulsant activities in animal models.[1-3] One possible mechanism of actions of some complexes involves scavenging of the superoxide anion.[2b,4,5] Though the copper(II) complexes of various amino acids,[6] peptides,[7] salicylate,[2] and other acyclic ligands[8,9] show a relatively high superoxide dismutase (SOD) activity, their uses as SOD mimics *in vivo* are limited, because many co-existing substances in cells are able to compete coordination with copper(II) ions as ligands. So synthesis of thermodynamically and kinetically stable complexes has attracted much attention.[4,10] Although some macrocyclic copper(II) complexes as SOD mimics have been synthesized,[11,12] they exhibit better stability than that of acyclic complexes, but until now systematic studies have not been reported yet. Recently various copper(II) cryptate have been successfully synthesized,[13] but only a few of them have been studied as SOD mimics.[14] Therefore we synthesized two kinds of macrocyclic complexes. One of them is composed of copper(II) cryptates (complexes **1**—**4**, Fig. 1) of ligands L^1—L^4 which are condensation products of 5-R-2-methoxy-1, 3-phenyldialdehyde (R = OCH_3, L^1) with tris(2-aminoethyl)amine (tren) and 5-R-methoxy-1,3-phenyldiadehyde (R = CH_3, L^3) with tris(2-aminopropyl)amine (trpn) as well as their reduced products of L^1 and L^3 (*ie*. L^2 and L^4). The other is composed of two-dimentional macrocylclic complexes (**5**—**8**, Fig. 1) of condensation products of diethylenetriamine with 4-R-1-methoxy-2, 6-phenyldialdehyde (R = Cl, Br, CH_3, OCH_3). In order to investigate the relationship between structures of cryptates and SOD activity, four binuclear copper(II) cryptates were synthesized. We assayed their SOD activity by photoreduction of riboflavin in the presence and absence of bovine serum albumin. In study of the first kind of cryptates, a quantitative relationship between SOD activity and the substituents at arenes was

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found. In study of the second kind of cryptates, influence of coordination situation of copper(II) center, cavity size of cryptates and length of bridge chains on SOD activity was clarified. The experimental results show that it is possible to synthesize good SOD mimics by changing cavity size of cryptates, length of bridge chains and substituents at arenes.

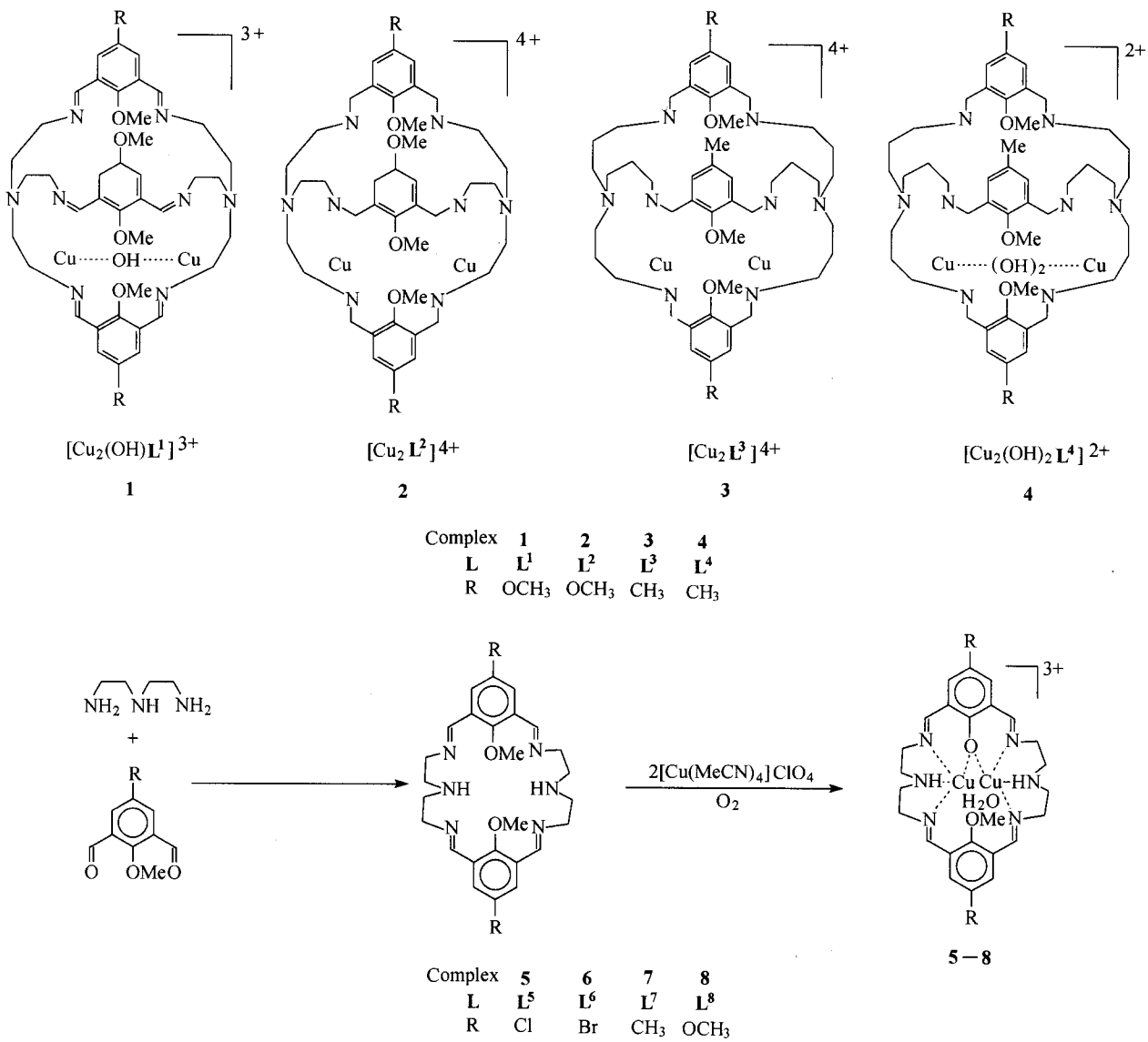

Fig. 1 Chemical structures of complexes.

Experimental

Materials

Tris(3-aminopropyl)amine was prepared with a modified method;[15] its purity was over 99%, as identified by conductimetric titration. 5-R-methoxy-1,3-phenyldialdehydes (R = CH_3, OCH_3) were prepared according to the literature method.[16] Nitroblue tetrazolium (NBT), L-methionine (B.R.), KH_2PO_4 (A.R.) and K_2HPO_4 (A.R.) were recrystallized twice in deionized water. Riboflavin was analyzed according to the literature method.[17] Cu_2Zn_2SOD was prepared according to the literature method,[18] with a specific activity of 3000 units/mg.

Physical measurements

IR spectra were recorded on a Nicolet 170 SXFT spectrometer (KBr disc). Magnetic susceptibility was determined with a Cahn-2000 magnetic balance. Solution electrical conductivity was measured by a BSD-A digital conductometer, in the MeCN solution at 23℃. ^{1}H NMR was performed on a Varian FT-80 NMR spectrometer (TMS as the internal reference).

Assay of superoxide dismutase activity

The activity was assayed by photoreduction of riboflavin.[19,9] The solutions, containing riboflavin (3.4×10^{-6} mol·dm^{-3}), methionine (0.01 mol·dm^{-3}), nitroblue tetrazolium chloride (NBT) (4.66×10^{-3} mol·dm^{-3}), phosphate buffer (0.05 mol·dm^{-3}) (pH = 7.8) and the complexes of various concentrations, were illuminated by a fluorescent lamp with a constant light intensity at 25℃. The optical absorbances (A) of the solution at 560 nm were measured with various illumination periods (t). Each solution was measured in two parallel tests. Inhibitation percentage (f%) was calculated according to a reported method.[19] The activity was defined as the necessary concentration to inhibit 50% reduction of NBT.

Syntheses of ligands

L^1·H$_2$O To a solution of 2,5-dimethoxy-1,3-phenyldialdehyde (1.164 g, 6 mmol) in 100 mL of methanol was added dropwise (*ca*. 6 h) a solution of tren (0.584 g, 4 mmol) in 100 mL of methanol. After concentrating the resultant solution to one half of the initial volume, **L^1** precipited out as yellow powder. It was filtered, washed with MeOH and recrystallized in CH_3Cl to give **L^1**·H$_2$O (1.34 g, 1.71 mmol) in yield of 88%. ^{1}H NMR ($CDCl_3$, 80 MHz) δ: 2.85 (s, 12H, CH_2N), 3.30 (s, 12H, $CH_2N=$), 3.69 (s, 18H, OCH_3), 7.55—7.26 (m, 6H, PhH); IR (KBr) ν: 3140 (br, OH), 2992, 2935, 2827 (m, CH), 1633 (s, C = N) cm^{-1}; Anal. calcd for $C_{42}H_{56}N_8O_7$: C 64.28, H 7.14, N 14.28; found C 64.35, H 7.08, N 14.18.

L^2·8HBr·6H$_2$O To a solution of **L^1**·H$_2$O (0.784 g, 1 mmol) in 100 mL of MeOH was added excessive $NaBH_4$ for reduction of C = N bond in **L^1**. Methanol was distilled off on a rotary evaporator and the residue was dissolved in 10 mL of water. The resulting solution was extracted with four 50 mL of portions of CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4. After bubbling HBr gas into the CH_2Cl_2 solution, **L^2**·8HBr·6H$_2$O was obtained as white powder. It was filtered, washed with MeOH and dried to give **L^2**·8HBr·6H$_2$O (1.3 g, 0.85 mmol) in yield of 84%. ^{1}H NMR ($CDCl_3$, 80 MHz) δ: 2.80 (m, 12H, $CH_2N=$), 3.10 (m, 12H, CH_2-N =), 3.65 (m, 18H, OCH3), 4.23 (m, 12H, $PhCH_2$), 7.05 (m, 6H, Ph-H); IR (KBr) ν: 3450 (br, OH), 3350 (m, NH), 2900 (m, CH) cm^{-1}; Anal. cacld for $C_{42}H_{86}N_8O_{14}Br_8$: C 32.88, H 5.65, N 7.30, Br 41.5; found C 32.68, H 5.31, N 7.59, Br 41.7.

L^3 **L^3** was obtained by condensation of trpn (0.75 g, 4 mmol) with 5-methyl-2-methoxy-1,3-phenyldialdehyde (1.07 g, 6 mmol) by the same synthesis procedure as **L^1**, the product **L^3** was obtained as pale yellow solid (1.37 g, 1.7 mmol) in yield of 85%; IR (KBr) ν: 2939 (m, CH), 2824 (w, CH), 1633 (s, C = N) cm^{-1}; Anal. cacld for $C_{48}H_{66}N_8O_3$: C 71.82, H 8.22, N 13.92; found C 71.68, H 8.51, N 14.35.

L^4·3H$_2$O Cryptand **L^4** was prepared by reduction of Ag(I) cryptate [Ag$_2$**L^3**](ClO$_4$)$_2$ which was prepared using the previous method.[16] To the suspension of [Ag$_2$**L^3**](ClO$_4$)$_2$·4H$_2$O (1.29 g, 1 mmol) in 100 mL of absolute methanol was added 1.5 g of $NaBH_4$ powder in batches. After reaction the mixture was filtered, and then the filtrate was dried on a rotary evaporator. The solid obtained was dissolved in 25 mL of water and the aqueous solution was extracted with four 100 mL of portions of CH_2Cl_2. The extract was dried over anhydrous Na_2SO_4. **L^4**·3H$_2$O (0.7 g, 0.81 mmol) in yeild of 80% was obtained by distilling off CH_2Cl_2 of the extract. IR (KBr) ν: 3366 (m, NH) cm^{-1}; Anal. cacld for $C_{48}H_{78}N_8O_3 \cdot 3H_2O$: C 66.36, H 9.68, N 12.91; found C 66.53, H 9.81, N 13.32.

L^5—L^8 The macrocyclic ligands **L^5—L^8** were prepared (Fig. 1) by condensation of diethylenetriamine with 4-R-methoxy-2,6-phenyldialdehyde (R = Cl, Br, CH_3, OCH_3) according to the procedure described previously.[20] The data of elemental analysis and of IR, ^{1}H

NMR spectra are in agreement with those reported.[20]

Syntheses of complexes

$[Cu_2(OH)\mathbf{L}^1](ClO_4)_3\cdot H_2O$ (**1**) To a solution of $Cu(ClO_4)_2\cdot 6H_2O$ (0.741 g, 2 mmol) in 20 mL of absolute ethanol was added dropwise a 60 mL of solution of $\mathbf{L}^1$ (0.784 g, 1 mmol) in 60 mL of CH_3CN-CH_3Cl (2∶1), after stirring for 1 h at room temperature, the solution was filtered. MeOH (60 mL) was added into the filtrate, the green precipitate was separated, washed with MeOH, and dried in reduced presure to give $[Cu_2(OH)\mathbf{L}^1](ClO_4)_3\cdot H_2O$ (1.0 g, 0.81 mmol) in yield of 83%. UV-Vis (MeCN) λ_{max}: 280 (logε 4.1), 345 (logε 4.1), 450 (logε 3.1), 630 (logε 2.4) nm; Λ_m (MeCN, 296 K): 371 $S\cdot cm^2\cdot mol^{-1}$; μ/perCu (293 K): 1.73 μB; IR (KBr) ν: 3556 (br, OH), 1601 (s, C = N) cm^{-1}; Anal. cacld for $C_{42}H_{57}N_8O_{20}Cl_3Cu_2$: C 41.07, H 4.64, N 9.12, Cu 10.42; found C 40.79, H 4.67, N 9.20, Cu 10.65.

$[Cu_2\mathbf{L}^2](ClO_4)_4\cdot 8H_2O$ (**2**) To a 5 mL of aqueous solution of $\mathbf{L}^2\cdot 8HBr\cdot 6H_2O$ (0.31 g, 0.2 mmol) which was adjusted to pH 6 using NaOH (0.1 mol/L), was added a solution of excessive $Cu(ClO_4)_2\cdot 6H_2O$ (0.185 g. 0.5 mmol) in 5 mL of MeOH; the green precipitate was separated while the solution was being stirred. It was treated just as complex **1** to give complex **2** (0.21 g, 0.14 mmol) in yield of 74%. UV-Vis (MeCN) λ_{max}: 215 (logε 4.5), 278 (logε 4.0), 700 (logε 2.2) nm; Λ_m (MeCN, 296 K): 520 $S\cdot cm^2\cdot mol^{-1}$. μ/perCu (293 K): 1.73 μB; IR (KBr) ν: 3250—3408 (br, NH, OH) cm^{-1}; Anal. cacld for $C_{42}H_{82}N_8O_{30}Cl_4Cu_2$: C 34.83, H 5.67, N 7.74, Cu 8.85, H_2O 9.95; found C 34.74, H 5.76, N 7.68, Cu 9.10, H_2O 9.12.

$[Cu_2\mathbf{L}^3](ClO_4)_4\cdot H_2O$ (**3**) The precusor complex $[Cu(trpn)_3](ClO_4)_2$ was obtained according to literature.[22] To 80 mL of MeOH solution of $[Cu(trpn)_3](ClO_4)_2$ (1.373 g, 3.05 mmol) was titrated 5-methyl-2-methoxy-1, 3-phenyldialdehyde (0.814 g, 4.52 mmol) in 80 mL of absolute methanol. After the solution was refluxed for 1 h, complex **3** (1.6 g, 1.2 mmol) was obtained as green powder in yield of 80%. UV-Vis (MeCN) λ_{max}: 278 (logε 3.8), 310 (logε 4.0), 402 (logε 4.0), 606 (logε 2.3) nm; Λ_m (MeCN, 296 K): 547 $S\cdot cm^2\cdot mol^{-1}$; μ/perCu (293 K): 1.87 μB; Anal. cacld for $C_{48}H_{68}N_8O_{20}Cl_4Cu_2$: C 42.82; H 5.06, N 8.33, Cu 9.51; found C 42.81, H 5.30, N 7.99, Cu 9.49.

$[Cu_2(OH)_2\mathbf{L}^4(ClO_4)_2\cdot 2CH_3OH\cdot H_2O$ (**4**) To a stirred solution of $\mathbf{L}^4\cdot 3H_2O$ (0.2 g, 0.23 mmol) in 10 mL of H_2O was added dropwise an aqueous solution of excessive $Cu(ClO_4)_2\cdot 6H_2O$ (0.4 g, 1.08 mmol); the solution was adjusted to pH 7—8 with NaOH (0.1 mol/L) to make complex **4** precipite. After being filtered and recrystallized in MeOH-MeCN (1∶1), complex **4** (1.96 g, 1.6 mmol) was obained as green needle in yield of 70%. UV-Vis (MeCN) λ_{max}: 220 (logε 4.3), 276 (logε 309), 700 (logε 2.3) nm; Λ_m (MeCN, 296 K): 286 $S\cdot cm^2\cdot mol^{-1}$; μ/perCu (293 K): 1.74 μB; IR (KBr) ν: 3250—3420 (br, NH, OH) cm^{-1}; Anal. cacld for $C_{50}H_{90}N_8O_{16}Cl_2Cu_2$: C 47.73, H 7.16, N 8.91, Cu 10.18; found C 47.63, H 7.52, N 8.99, Cu 10.32.

Complexes **5**—**8** They were prepared according to the same method[20] (Fig. 1): macrocyclic ligand ($\mathbf{L}^5$—$\mathbf{L}^8$) (1 mmol), in CH_2Cl_2-CH_3Cl (2∶1) (100 mL) was added to a deoxygenated 50 mL of $CHCl_3$ solution of $[Cu(MeCN)_4]ClO_4$ (2 mmol). After dioxygen bubbled through this solution for several hours, the copper(II) complex was precipitated as a green powder in yield of 70—78%. IR (KBr) ν: 1630—1640 (s, C = N), 3200—3500 (br, OH, NH), 1546—1595 (s, C—O) cm^{-1}; Λ_m. (MeCN, 293 K): 362—406 $S\cdot cm^2\cdot mol^{-1}$; μ/perCu (293 K): 1.68—1.98 μ_B. Complex **5**, Anal. cacld for $C_{25}H_{29}N_6O_{14}Cl_5Cu_2\cdot H_2O$: C 31.3, H 3.2, N 8.8, Cu 13.2; found C 30.7, H 3.8, N 8.9, Cu 13.2. Complex **6**, Anal. cacld for $C_{25}H_{29}N_6O_{14}Br_2Cl_3\cdot 2H_2O$: C 28.15, H 3.12, N 7.88, Cu 11.91; found C 27.68, H 3.12, N 7.96, Cu 12.39. Complex **7**, Anal. cacld for $C_{27}H_{35}O_{14}N_6Cl_3Cu_2\cdot 2H_2O$: C 34.61, H 4.19, N 8.96, Cu 13.56; found C 34.59, H 4.49, N 8.98, Cu 13.61. Complex **8**, Anal. cacld for $C_{27}H_{35}O_{16}N_6Cl_3Cu_2\cdot 2H_2O$: C 33.46, H 4.06, N 8.64, Cu 13.11; found C 33.62, H 4.21, N 8.72, Cu 13.29.

Results and discussion

Syntheses and characterization

Cryptands $\mathbf{L}^1$ and $\mathbf{L}^3$ could be obtained through a non-templated condensation of tren or trpn with phenyldialdehyde. $\mathbf{L}^3$ could not be reduced directly by $NaBH_4$ because of its poor solubility in common solvents, but $[Ag_2\mathbf{L}^3(ClO_4)_2$ has better solubility than $\mathbf{L}^3$ in MeOH. Therefore, $\mathbf{L}^3$ was replaced by its Ag(I) cryptate in the reduction of C = N. Similarly, pure cryptate $[Cu_2\mathbf{L}^3](ClO_4)_2$ could not be obtained by treatment of $\mathbf{L}^3$ with copper(II) or by templated condensation, but was accessible by using the modified synthesis method, which was effective and had scarcely been reported. In IR spectra, the reduced cryptands ($\mathbf{L}^2 \cdot 8HBr \cdot 6H_2O$ and $\mathbf{L}^4$) have no C = N absorption peaks around 1640 cm^{-1}, but they display ν (NH) around 3400 cm^{-1}, confirming the reduction of the C = N bond, which are in agreement with the results of elemental analysis.

Molar conductivities in MeCN solution of cryptates **2** and **3** indicate that both are 1:4 electrolyte and have no anion bridging two copper ions. Cryptates **1** and **4** are 1:3 and 1:2 electrolytes respectively.[23] The hydroxyl anions often enter the cryptates as bridging group during formation of the complexes depending on synthesized conditions.[20,24] Therefore, in complexes **1** and **2**, there are one or two hydroxyl anions bridging two copper ions.

The effective magnetic moments of copper(II) in binuclear complexes are in agreement with d^9 electron configuration.

Macrocyclic complexes **5** and **6** were characterized by us as reported in our previous papers.[20,21] They are a series of binuclear copper(II) complexes not only with phenolato and water bridges but also with different arene substituents.

SOD *activity of the cryptates*

The SOD activities of cryptates were studied according to the photoreduction method. The tested copper (II) complexes inhibited the reduction of NBT, which depended on complex concentrations. The activity data in Table 1 were obtained by plotting the percentage of inhibiting NBT reduction against concentration of complexes (Fig. 2). In addition, data of Cu_2Zn_2SOD and $Na_2[Cu(sal)_2]$ (sal = salcylate) were also presented for comparion. From Table 1 it can be seen that complexes **1** and **4** containing one or two hydroxyl bridges display lower activities than complexes **2** and **3**. The latter had no hydroxyl bridges and the coordination number of copper(II) center was not saturated. So the remaining coordination empty site was receptive to $\cdot O_2^-$. Therefore they displayed high activities. This is similar to the reactions in which the nature metal enzymes, such as superoxide dismutase, ligninase and so on, activate catalytically small molecules in solutions.[25]

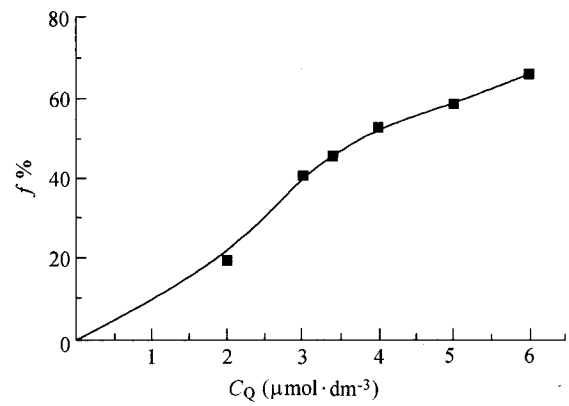

Fig. 2 A plot of inhibitation percentage (f%) *vs*. concentrations of complex **2** $[Cu_2\mathbf{L}^2](ClO_4)_4$.

Table 1 Superoxide dismutase activity of copper(II) cryptates tested by photoreduction

Cryptate	Activity (μmol·dm⁻³)	Relative activity (%)
1	46.2	0.13
2	3.65	1.65
3	10.2	0.59
4	51.2	0.12
$Na_2[Cu(sal)_2]$	14.8	0.41
Cu_2Zn_2SOD	0.06	100

We selected cryptates **2** and **3** for examining their activities in bovine serum ablumin (BSA) which is a competitive physiological copper(II) chelator. Plotting of units of activity vs concentrations of BSA is shown in Fig. 3. It shows that the activity of cryptate **3** is constant until the concentration of BSA increases to 0.5 mg/mL. Even if BSA concentration increases to 1.0 mg/mL, cryptate 3 keeps 63% of the original activity. This implies that it remained intact within cells in the solution of 0.5 mg/mL BSA. Inversely, cryptate **2** and

$Na_2[Cu(sal)_2]$ behave differently. This may be due to the cavity size of cryptate and flexibility of the ligand.

In order to investigate the effect of substituents on the macrocyclic arene, we examined the activities of macrocyclic complexes **5**—**8** which had similar structures except when they were with different arene substituents. Their activities are close to each other (Table 2). In complexes **5**—**8** a water molecule was linked weakly to two copper(II) centers as a bridging ligand. In solution it was easily removed, and the resulting empty site favored the reaction of $\cdot O_2^-$. Therefore their activities were close to those of cryptates **2** and **3**.

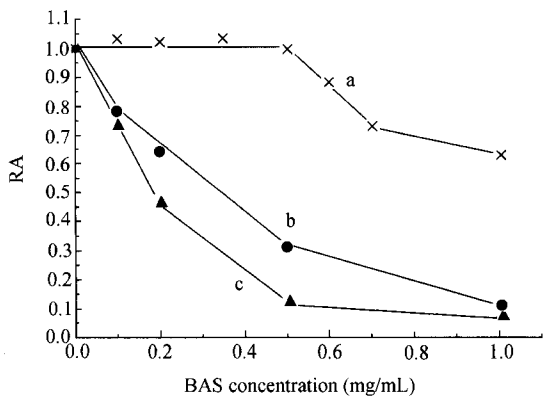

Fig. 3 Effects of bovine serum albumin (BSA) on SOD activity of copper complexes (a) complex **3**; (b) complex **2**; (c) $Na_2[Cu(sal)_2]$. RA: relative activity.

Table 2 Superoxide dismutase activity of two-dimensional macrocyclic complexes and substituent constants σ_p in arenes

Substituent	Cl	Br	CH_3	OCH_3
Activity ($\mu mol \cdot dm^{-3}$)	9.03	8.60	9.90	10.5
Relative activity (%)	0.67	0.70	0.61	0.57
σ_p	0.23	0.23	−0.17	−0.27

The activities of these four complexes with different arene substituents followed the sequence: Cl > Br > CH_3 > OCH_3, which was just the same as that of the *para*-substituent constant σ_p.[26] By plotting the relative activity γ *vs*. the corresponding σ_p, good liner fitting with the equation $\log\gamma = \rho\sigma_p + \log\gamma^0$ was obtained, with $\log\gamma^0 = -0.21$ ($\gamma^0 = 0.63$) and $\rho = 0.16$, where γ^0 stands for the activity of the complex without any substituents and ρ stands for the reaction constant which depended on the type of reactions, experimental conditions and the susceptibility of reaction on electron density at the site of reaction. The linearity implies that change of the electron-density on the ligand can be transmitted directly to copper centers; and the positive ρ value implies that catalytic dismutation of $\cdot O_2^-$ is favored by low electron density of copper center.

The above mentioned influence of substituents on activity is of guiding significance for design and synthesis of macrocyclic complexes (cryptates and planar macrocyclic complexes) used as SOD mimics.

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