

## Research Article

# Synthesis, Novel Crystal Structure, and $\beta$ -Amyloid Binding Property of Re(I) (tricarbonyl)<sup>+</sup> EHIDA Analogue

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A neutral compound  $\text{Re}(\text{CO})_3(\text{L})$  (L: 2-((2-(2,6-diethylphenylamino)-2-oxoethyl)(2-ethoxy-2-oxoethyl)amino)acetic acid, an IDA analogue) has been synthesized and evaluated for in vitro imaging probes of  $\beta$ -amyloid ( $A\beta$ ) aggregates. Results of X-ray measurement of  $\text{Re}(\text{CO})_3(\text{L})$  demonstrated that the coordination mode of  $\text{Re}(\text{CO})_3(\text{L})$  was different from that of classical Re/Tc(I) (tricarbonyl)-IDA analogues; the structure of  $\text{Re}(\text{CO})_3(\text{L})$  was confirmed by means of infrared spectrum, HPLC-UV, TOF MS, and X-ray measurements (Cambridge Crystallographic Data Centre number is 732731): monoclinic  $P2_1/c$ ,  $a = 15.6636$  (12) Å,  $b = 10.9360$  (8) Å,  $c = 27.756$  (2) Å,  $\alpha = 90.000$  (0)°,  $\beta = 90.783$  (5)°,  $\gamma = 90.000$  (0)°, and  $Z = 8$ . The binding affinity for  $\beta$ -amyloid plaques was assessed by in vitro binding assay using preformed synthetic  $A\beta_{(1-40)}$  aggregates. The neutral compound  $\text{Re}(\text{CO})_3(\text{L})$  showed binding affinity to  $A\beta$  aggregates at micromolar level by fluorescence spectroscopy, and this work will encourage for further exploration of imaging agents labeled by  $^{99m}\text{Tc}(\text{CO})_3^+$  center as probes for  $\beta$ -amyloid plaques in vivo.

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## 1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by dementia, cognitive impairment, and memory loss, and so far the only definitive confirmation of AD is universally accepted by histopathologic examination of extracellular amyloid plaques comprised of amyloid-beta ( $A\beta$ ) aggregates and intracellular numerous neurofibrillary tangles in the postmortem brain; furthermore, formation of  $A\beta$  aggregates in the brain became the hallmark feature on early pathogenesis of AD before another alternative hypothesis emerged [1]. Several compounds have been selected to be radiolabeled and used in the noninvasive detection of  $A\beta$  aggregates by imaging techniques of positron emission tomography (PET) and single photon emission-computed tomography (SPECT); in addition, these representative  $A\beta$ -detectors were derivatives of congo red, thioflavin, stilbene, and DDNP (6-dialakylamino-2-naphthylidene), such as  $^{11}\text{C}$ -PIB,  $^{125}\text{I}$ -TZDM,  $^{11}\text{C}$ -SB-13, and  $^{18}\text{F}$ -FDDNP [2]. However, the progress of SPECT imaging agents labeled by technetium-99m center fell behind, because of their low rates of penetration through the blood-brain barrier (BBB) [3]. Iminodiacetic acid (IDA) derivatives EHIDA

labeled by  $^{99m}\text{Tc}(\text{CO})_3$  has been studied on physicochemical properties and biological evaluation [4, 5], but the structure of technetium(I)-tricarbonyl EHIDA has not been identified. In this paper, we reported the synthesis of  $\text{Re}(\text{CO})_3(\text{L})$  (L is esterified EHIDA), and the novel crystal structure of this complex was determined by X-ray measurement: the coordination mode of  $\text{Re}(\text{CO})_3(\text{L})$  was different from that of classical Re/Tc(I) (tricarbonyl)-IDA derivatives. The neutral compound  $\text{Re}(\text{CO})_3(\text{L})$  showed binding affinity to  $A\beta$  aggregates in vitro at micromolar level by fluorescence spectroscopy.

## 2. Experimental

**2.1. Materials and Preformed Synthetic  $A\beta_{(1-40)}$  Aggregates.**  $A\beta_{(1-40)}$  peptide was purchased from Shang Hai "Supermed" Trade Limited Company.  $\text{Re}(\text{CO})_5\text{Br}$ , Thioflavin T, Phosphate Buffer Solution (PBS, pH = 7.4), and EDTA were purchased from Sigma-Aldrich Chemical Company. Other chemicals were all purchased from Beijing Chemical Reagents Company. EHIDA was synthesized according to the previous method [6].

TABLE 1: Summary of crystal data of  $\text{Re}(\text{CO})_3(\text{L})$ .

$\text{Re}(\text{CO})_3(\text{L})$	
Empirical formula	$\text{C}_{21}\text{H}_{19}\text{N}_2\text{O}_8\text{Re}$
Formula weight	613.58
Temperature	296 (2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, $P2(1)/c$
<i>Unit cell dimensions</i>	
$A$ (Å)	15.6636 (12)
$B$ (Å)	10.9360 (8)
$C$ (Å)	27.756 (2)
$\alpha$ (°)	90.000 (0)
$\beta$ (°)	90.783 (5)
$\gamma$ (°)	90.000 (0)
Unit cell Volume (Å <sup>3</sup> )	4754.1 (6)
$Z$ , Calculated density	8, 1.715 mg/m <sup>3</sup>
Absorption coefficient (mm <sup>-1</sup> )	5.158 mm <sup>-1</sup>
$F(000)$	2384
Crystal size	$0.34 \times 0.07 \times 0.05$ mm
Completeness to $\theta = 25.46$	99.5%
Goodness-of-fit on $F^2$	1.007
Final $R$ indices [ $I > 2\sigma(I)$ ]; $R_1, wR_2$	0.0324, 0.0729
$R$ indices (all data); $R_1, wR_2^a$	0.0619, 0.0838

$$^a R_1 = \frac{\sum(|F_o| - |F_c|)}{\sum |F_o|}; wR_2 = \left[ \frac{\sum [w(F_o^2 - F_c^2)^2]}{\sum [w(F_o^2)]} \right]^{1/2}.$$

*Formation of  $A\beta_{(1\sim 40)}$  aggregates.*  $A\beta_{(1\sim 40)}$  aggregates (Figure 1(a)) were prepared according to the method published previously [7]. 1 mg of brief  $A\beta_{(1\sim 40)}$  and 20  $\mu\text{L}$  EDTA solution (1 mM) were dissolved in 2 mL PBS (pH = 7.4), and the mixture was mixed with a magnetic stir bar (300 rpm) for 72 hours at 37°C to result in a visibly cloudy solution. The production of  $A\beta_{(1\sim 40)}$  aggregates was confirmed by a Jeol 100CX-transmission electron microscope (purchased from JEOL USA, Inc.). Additional test for the formation of  $A\beta_{(1\sim 40)}$  aggregates was also performed using Thioflavine T (2  $\mu\text{M}$ ) by fluorescence spectroscopy (excitation wavelength: 450 nm, scan range was from 460 to 600 nm) [8]; there was an obvious enhance of fluorescence intensity of Thioflavine T at the emission wavelength of 485 nm when 5  $\mu\text{L}$  solution of  $A\beta_{(1\sim 40)}$  aggregates was added to the 500  $\mu\text{L}$  solution of Thioflavine T (2  $\mu\text{M}$ ) (Figure 1(b)).  $A\beta_{(1\sim 40)}$  aggregates were used immediately after preparation.

*2.2. Syntheses and X-Ray Measurement.* EHIDA (2, 2'-(2-(2,6-diethylphenylamino)-2-oxoethylazanediyl)diacetic acid) was synthesized according to the previous method [6], and the structure of EHIDA has been confirmed by using melting point mensuration, IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, and Mass Spectrometry (data did not be shown). 200  $\mu\text{mol}$   $\text{Re}(\text{CO})_5\text{Br}$  reacted with the equimolar EHIDA in the refluxing mixture of 50 mL ethanol and 0.1 mL  $\text{H}_2\text{SO}_4$  (4 mol/L) for 48 hours, then the reaction solution was washed by saturated  $\text{NaHCO}_3$  solution and the target product was extracted by ethyl acetate, and after evaporating the ethyl acetate, the yellowish solid

TABLE 2: Selected bond distances (Å) and bond angles (°) for the neutral complex:  $\text{Re}(\text{CO})_3(\text{L})$ .

Ligand	$\text{Re}(\text{CO})_3(\text{L})$
$\text{Re}_1\text{-C}_{\text{CO}}$	1.896 (7), 1.871 (6), 1.912 (6)
$\text{Re}_2\text{-C}_{\text{CO}}$	1.891 (7), 1.904 (7), 1.918 (7)
$\text{Re}_1\text{-O}_{\text{carboxyl}}$	2.115 (4), 2.160 (4)
$\text{Re}_2\text{-O}_{\text{carboxyl}}$	2.109 (4), 2.174 (4)
$\text{Re}_1\text{-N}$	2.254 (4)
$\text{Re}_2\text{-N}$	2.266 (4)
$\text{C}(2)\text{-Re}(1)\text{-C}(1)$	87.9 (3)
$\text{C}(2)\text{-Re}(1)\text{-C}(3)$	87.0 (3)
$\text{C}(1)\text{-Re}(1)\text{-C}(3)$	90.0 (3)
$\text{C}(2)\text{-Re}(1)\text{-O}(4)$	97.8 (2)
$\text{C}(1)\text{-Re}(1)\text{-O}(4)$	172.1 (2)
$\text{C}(3)\text{-Re}(1)\text{-O}(4)$	95.7 (2)
$\text{C}(2)\text{-Re}(1)\text{-O}(6)$	175.6 (2)
$\text{C}(1)\text{-Re}(1)\text{-O}(6)$	93.2 (2)
$\text{C}(3)\text{-Re}(1)\text{-O}(6)$	97.2 (2)
$\text{O}(4)\text{-Re}(1)\text{-O}(6)$	80.68 (15)
$\text{C}(2)\text{-Re}(1)\text{-N}(1)$	100.4 (2)
$\text{C}(1)\text{-Re}(1)\text{-N}(1)$	95.6 (2)
$\text{C}(3)\text{-Re}(1)\text{-N}(1)$	170.8 (2)
$\text{O}(4)\text{-Re}(1)\text{-N}(1)$	78.01 (15)
$\text{O}(6)\text{-Re}(1)\text{-N}(1)$	75.23 (14)
$\text{C}(22)\text{-Re}(2)\text{-C}(23)$	86.2 (3)
$\text{C}(22)\text{-Re}(2)\text{-C}(24)$	88.1 (3)
$\text{C}(23)\text{-Re}(2)\text{-C}(24)$	86.5 (3)
$\text{C}(22)\text{-Re}(2)\text{-O}(12)$	98.9 (2)
$\text{C}(23)\text{-Re}(2)\text{-O}(12)$	174.3 (2)
$\text{C}(24)\text{-Re}(2)\text{-O}(12)$	96.2 (2)
$\text{C}(22)\text{-Re}(2)\text{-O}(14)$	174.3 (2)
$\text{C}(23)\text{-Re}(2)\text{-O}(14)$	94.1 (2)
$\text{C}(24)\text{-Re}(2)\text{-O}(14)$	97.7 (2)
$\text{O}(12)\text{-Re}(2)\text{-O}(14)$	80.57 (15)
$\text{C}(22)\text{-Re}(2)\text{-N}(3)$	99.1 (2)
$\text{C}(23)\text{-Re}(2)\text{-N}(3)$	99.1 (2)
$\text{C}(24)\text{-Re}(2)\text{-N}(3)$	171.2 (2)
$\text{O}(12)\text{-Re}(2)\text{-N}(3)$	77.70 (15)
$\text{O}(14)\text{-Re}(2)\text{-N}(3)$	75.20 (15)

precipitated. The product was recrystallized in the mixture of dichloromethane/ethanol (4 : 1) for one week to obtain  $\text{Re}(\text{CO})_3(\text{L})$  crystal. Results of X-ray measurement of  $\text{Re}(\text{CO})_3(\text{L})$  were obtained (Tables 1 and 2). IR (KBr): high bands of CO (2036.6, 1922.3, 1903.0  $\text{cm}^{-1}$ , very strong) and additional bands for the CO-group of the amide (1633.0  $\text{cm}^{-1}$ , strong),  $\text{CH}_3$  group (3062.9, 2969.3  $\text{cm}^{-1}$ , medium). TOF MS<sup>+</sup> ( $m/z$ ): 619.0880, 621.0872; calcd for  $\text{C}_{21}\text{H}_{25}\text{N}_2\text{O}_8^{185/187}\text{Re}$ ,  $M = 619.0, 621.0$ .

The X-ray measurement of  $\text{Re}(\text{CO})_3(\text{L})$  was carried out on a CCD area X-ray detector. The Mo  $K\alpha$  radiation (wavelength = 0.71073 Å) was used. The data were

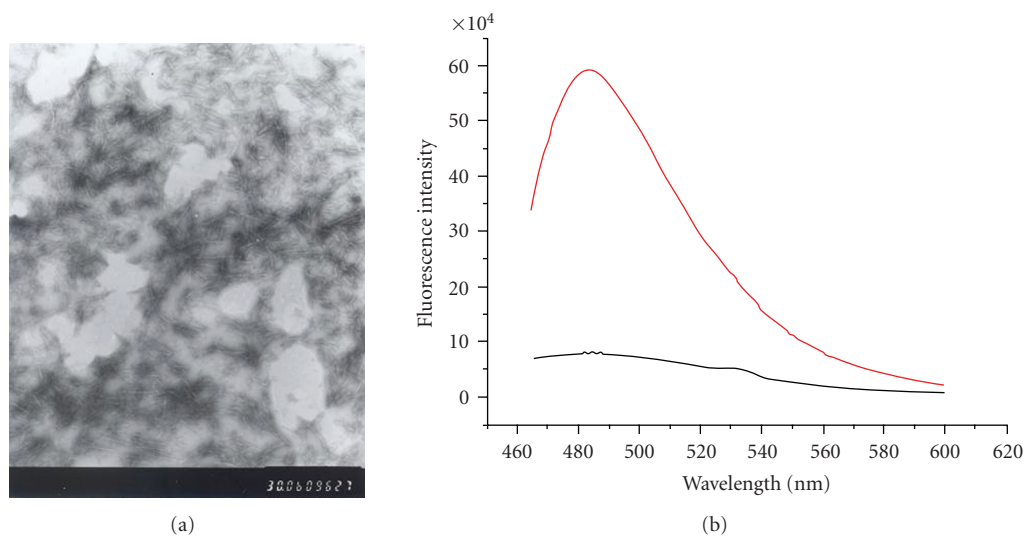


FIGURE 1: (a) Observation of  $A\beta_{(1-40)}$  aggregates by transmission electron microscope (b) Formation of  $A\beta_{(1-40)}$  aggregates using Thioflavine T by fluorescence spectroscopy red line: fluorescence intensity of the mixture of 5  $\mu\text{L}$  solution of  $A\beta_{(1-40)}$  aggregates and 500  $\mu\text{L}$  solution of Thioflavine T (2  $\mu\text{M}$ ); black line: fluorescence intensity of 500  $\mu\text{L}$  solution of Thioflavine T (2  $\mu\text{M}$ ) only.

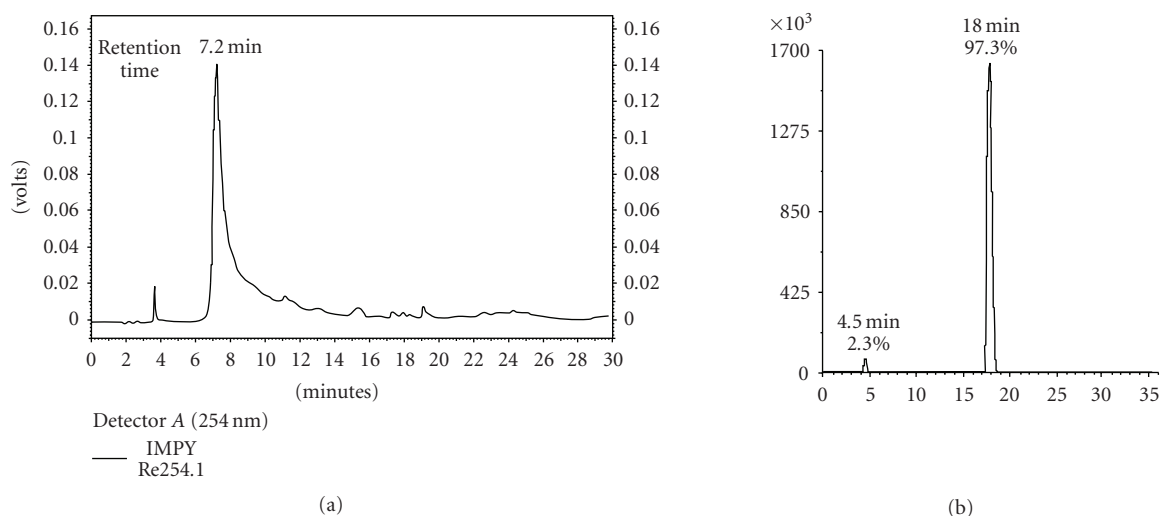


FIGURE 2: HPLC retention times of  $\text{Re}(\text{CO})_3(\text{L})$  (a) and  $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{EHIDA})$  (b).

collected by SADABS (Siemens Area Detector Absorption correction program). The nonhydrogen atoms were refined anisotropically, whereas the hydrogen atoms were placed in the calculated positions. The atomic scattering factors were taken from [9, Tables 6.1.1.4 and 4.2.6.8]. Details of the X-ray measurements and crystal data for  $\text{Re}(\text{CO})_3(\text{L})$  are given in Tables 1 and 2. Copies of the data can be obtained on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: (+44) 1223-336-033; E-mail: deposit@ccdc.cam.ac.uk; Internet: <http://www.ccdc.cam.ac.uk/conts/retrieving.html>). The CCDC number of  $\text{Re}(\text{CO})_3(\text{L})$  is 732731.

**2.3. HPLC-UV Analysis and UV Visible of  $\text{Re}(\text{CO})_3(\text{L})$  and EHIDA.** HPLC-UV analysis was carried out with an Alltima RP C-18 column ( $250 \times 4.6 \text{ mm}^2$ , 5  $\mu\text{m}$ ) by using an Shi-

madzu System with SCL-10Avp HPLC pump system and UV detector. The analysis gradient was (a): 0.1% trifluoroacetic acid in water, (b): 0.1% trifluoroacetic acid in acetonitrile: 0 to 20 minutes, from 100% (a) to 100% (b); 20 to 30 minutes, 100% (b). The flow rate was 0.9 mL/min. The excitation wavelength was 254 nm. Results were displayed in Figure 2. UV-visible absorption spectra of the ethanol solution of  $\text{Re}(\text{CO})_3(\text{L})$  and EHIDA were recorded, with excitation wavelength scanning from 220 to 360 nm at room temperature. Results were in Figure 3.

**2.4. Binding Affinity to  $A\beta_{(1-40)}$  Aggregates by Fluorescence Spectroscopy.** Fresh solution of 0.1 mM  $\text{Re}(\text{CO})_3(\text{L})$  was diluted to obtain a final concentration range of 19 ~ 24  $\mu\text{M}$   $\text{Re}(\text{CO})_3(\text{L})$  in 500  $\mu\text{L}$  PBS (pH 7.4). 5  $\mu\text{L}$  solution

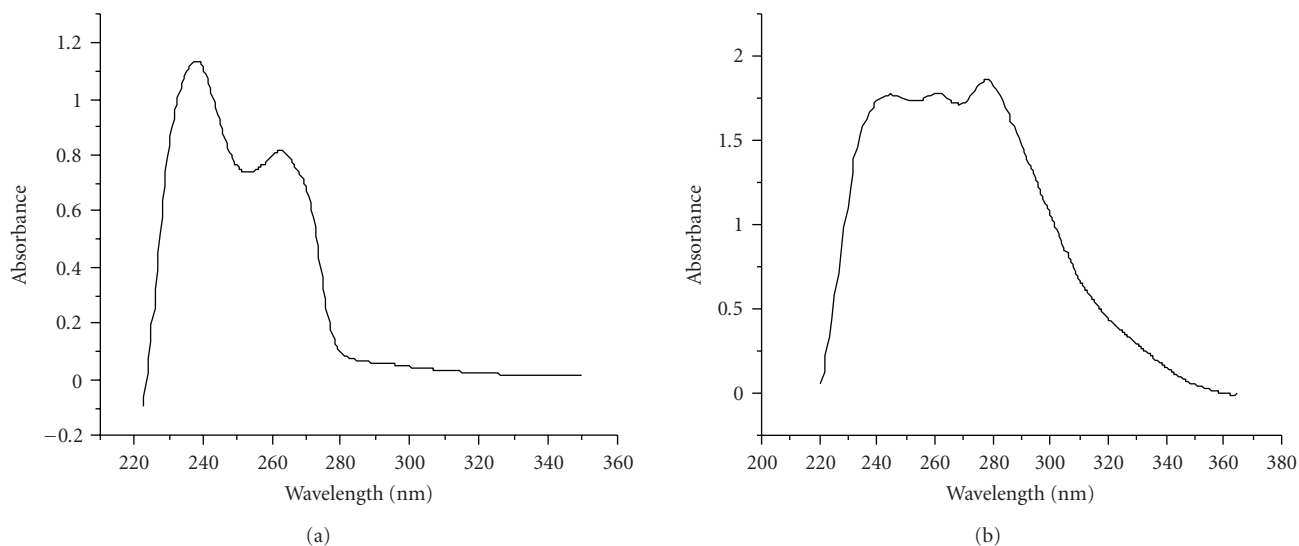


FIGURE 3: Ultraviolet Spectrum of the EHIDA (a) and  $\text{Re}(\text{CO})_3(\text{L})$  (b).

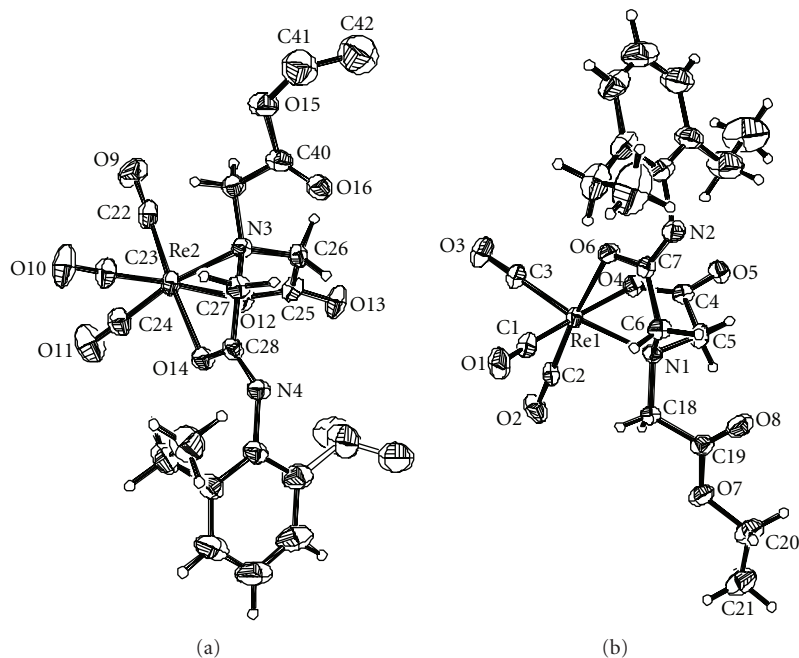


FIGURE 4: ORTEP drawing of the  $\text{Re}(\text{CO})_3(\text{L})$ .

of  $A\beta_{(1-40)}$  aggregates was added to 500  $\mu\text{L}$  PBS solution of  $\text{Re}(\text{CO})_3(\text{L})$  (the concentrations were 19, 20, 21, 22, 23, 24  $\mu\text{M}$ , resp.), and the mixed solution was incubated for 1 minute at room temperature before measuring the fluorescence intensity (slit-width was 2 nm). Excitation wavelength was 320 nm, and a 350 ~ 600 nm scan range was performed. All the data were performed in triplicate. The linear relationship between the concentrations ( $\text{Re}(\text{CO})_3(\text{L})$  only and  $\text{Re}(\text{CO})_3(\text{L})$  incubated with  $A\beta_{(1-40)}$  aggregates) and the integration of corresponding fluorescence intensity was analysed by Microcal Origin 6.0. Results were shown in Figure 6.

### 3. Results and Discussion

**3.1. Comparison of HPLC Analysis between  $\text{Re}(\text{CO})_3(\text{L})$  and  $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{EHIDA})$ .** The retention time of  $\text{Re}(\text{CO})_3(\text{L})$  (7.2 minutes) was not similar to that of  $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{EHIDA})$  (18.0 minutes) [5] (shown in Figure 2), which is speculated due to the change of lipophilicity of the ligand from EHIDA to esterification form. Esterified EHIDA (L) was more lipophilic than EHIDA; so the retention times of  $\text{Re}(\text{CO})_3(\text{L})$  and  $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{EHIDA})$  were different; however, the difference of properties of the  $\text{Re}(\text{CO})_3^+$  core and  $^{99\text{m}}\text{Tc}(\text{CO})_3^+$  core may also lead to the different retention times.

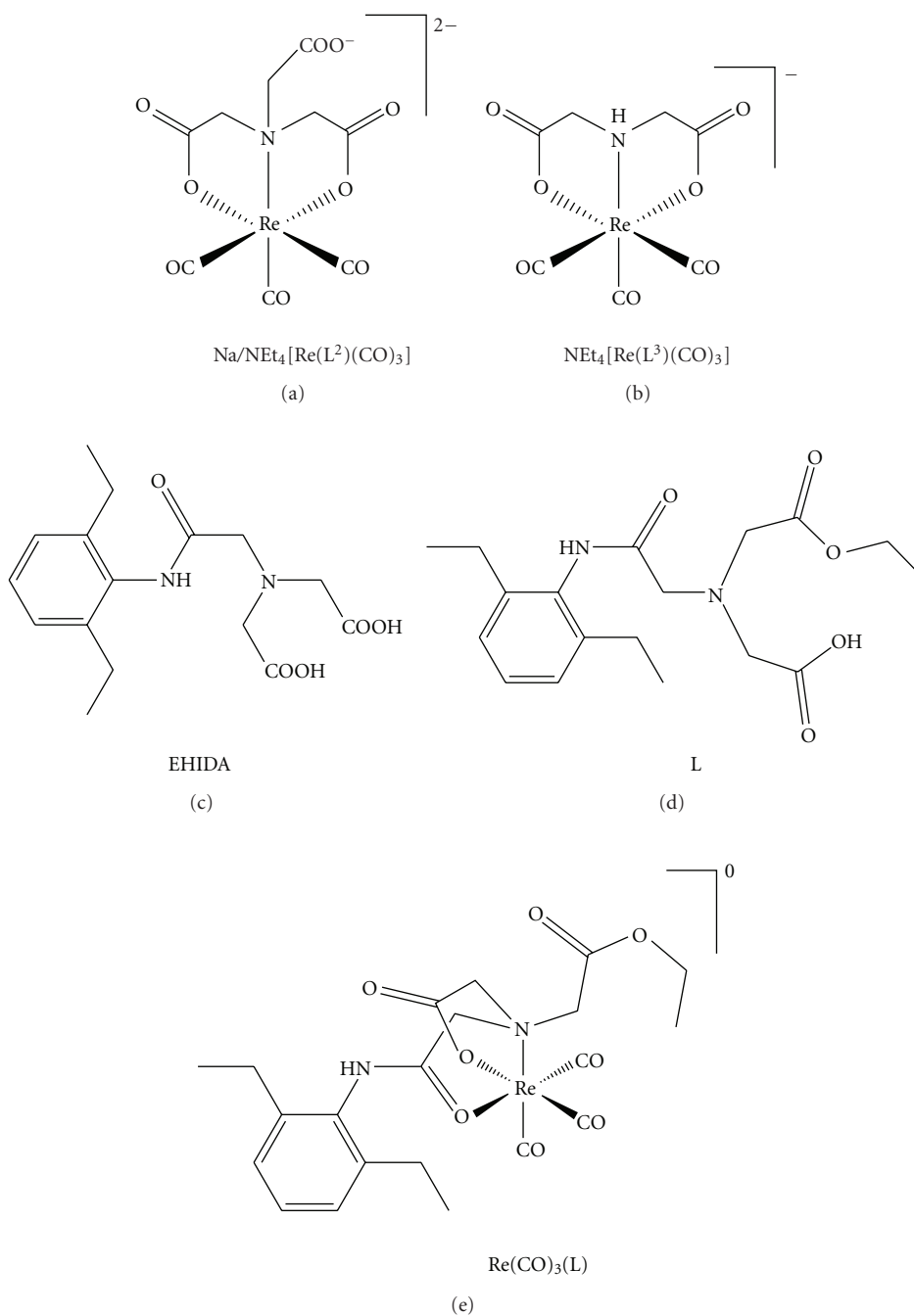


FIGURE 5: Structures of  $\text{Na/NEt}_4[\text{Re}(\text{L}^2)(\text{CO})_3]^{2-}$ ,  $\text{NEt}_4[\text{Re}(\text{L}^3)(\text{CO})_3]^{-}$  [11] EHIDA, L, and  $\text{Re}(\text{CO})_3(\text{L})$ .

**3.2. UV Visible of  $\text{Re}(\text{CO})_3(\text{L})$  and EHIDA.** The UV-visible absorption spectra of the complexes are illustrated in Figure 3. Upon EHIDA, the visible intraligand ( $\pi-\pi^*$ ) bands at 240 nm and 265 nm were due to the fact that these two bands are of mainly bpy-localized in nature. In comparison, the additional visible MLCT ( $d-\pi^*$ ) band of  $\text{Re}(\text{CO})_3(\text{L})$  at 280 nm was clearly associated with the coordinational  $-\text{C}=\text{O}$  group on comparison with the UV spectra of the analogue  $\text{Re}(\text{I})(\text{tricarboxyl})^+$  complexes [10] without the  $-\text{CH}_2-\text{C}=\text{O}$  group.

**3.3. Description of the Structure of  $\text{Re}(\text{CO})_3(\text{L})$ :** The neutral crystal of  $\text{Re}(\text{CO})_3$ . Selected distances and bond angles of the neutral crystal of  $\text{Re}(\text{CO})_3(\text{L})$  are listed in Table 2, and the molecular views are presented in Figure 4. From the ORTEP drawing of the  $\text{Re}(\text{CO})_3(\text{L})$ , it can be known that there were two different structures of  $\text{Re}(\text{CO})_3(\text{L})$  in one crystal cell.

The IDA derivatives often coordinate with  $\text{Re/Tc}(\text{I})$  (tricarboxyl) $^+$  centers via classic coordination manner, which is that each oxygen atom of the two carboxyl groups and one N atom combine to the empty orbit in

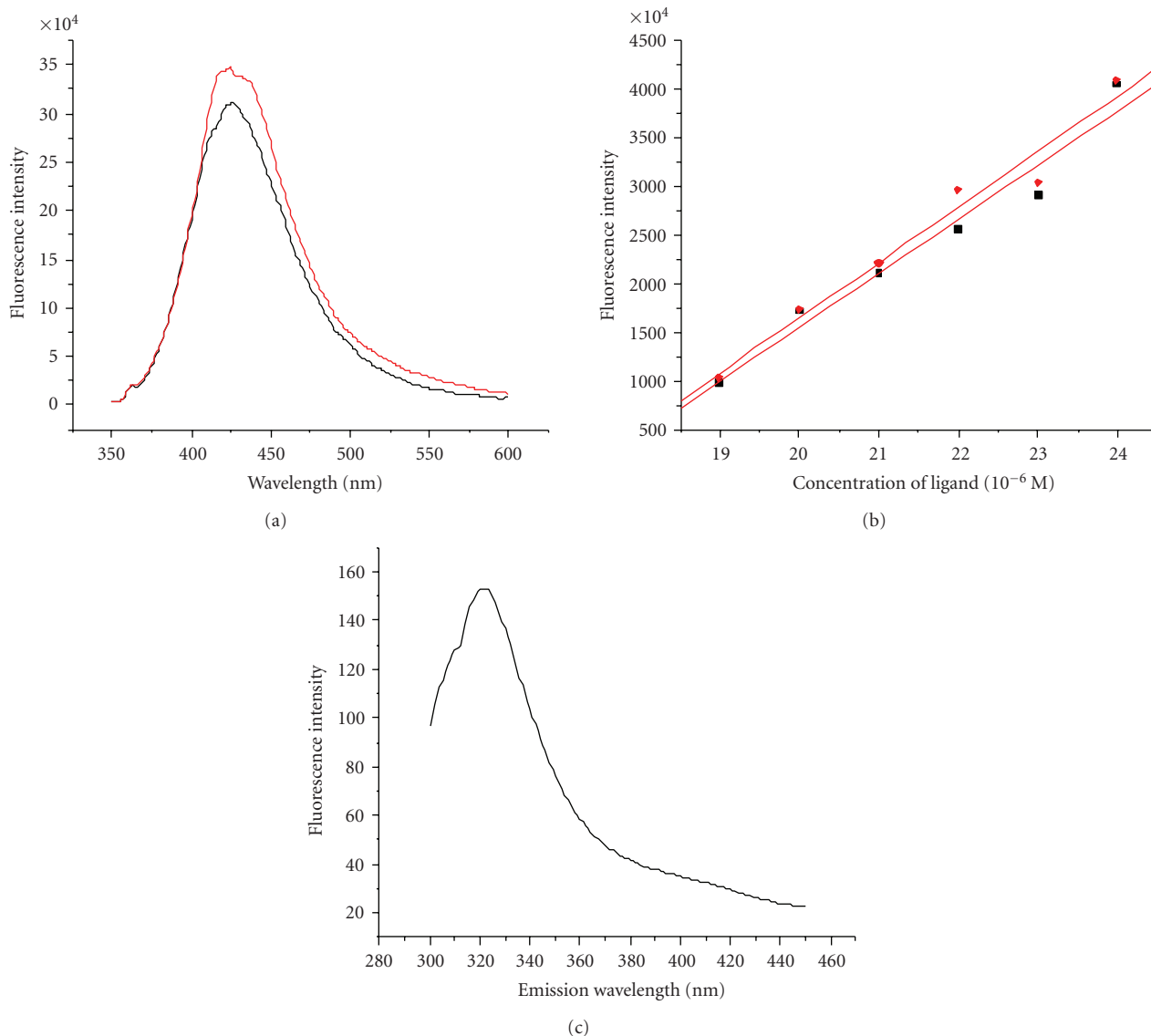


FIGURE 6: (a) The fluorescence intensity of  $\text{Re}(\text{CO})_3(\text{L})$  (20  $\mu\text{M}$ ) (black line) and  $\text{Re}(\text{CO})_3(\text{L})$  (20  $\mu\text{M}$ ) incubated with  $\text{A}\beta_{(1-40)}$  aggregates (red line) at the emission wavelength of 425 nm. (b) Equation (1): the nether line and Equation (2): the upper line. (c) The fluorescence intensity of EHIDA (0.4 mM) and emission wavelength (excitation wavelength is 280 nm).

the rhenium or technetium [11], oppositely, our research of the crystal structure of  $\text{Re}(\text{CO})_3(\text{L})$  demonstrated that oxygen atom of carbonyl has more stronger coordination ability than the carboxyl group, so that an oxygen atom in the carbonyl group, an oxygen atom in the carboxyl group, and the N atom coordinated with the  $\text{Re}(\text{CO})_3^+$  core in the molecular of  $\text{Re}(\text{CO})_3(\text{L})$ , and as a result, 2-((2-(2,6-diethylphenylamino)-2-oxoethyl)(2-ethoxy-2-oxoethyl)amino)acetic acid (L or Esterified EHIDA) reacted with  $\text{Re}(\text{CO})_5\text{Br}$  resulting in neutral but not negative  $\text{Re}(\text{CO})_3(\text{L})$  (Figure 5).

**3.4. Studies on Binding  $\text{A}\beta_{(1-40)}$  Aggregates In Vitro.** The fluorescence intensity of the mixture of  $\text{A}\beta_{(1-40)}$  aggregates and  $\text{Re}(\text{CO})_3(\text{L})$  can be significantly stronger than

the fluorescence intensity of  $\text{Re}(\text{CO})_3(\text{L})$  only when the concentration of  $\text{Re}(\text{CO})_3(\text{L})$  was more than 19  $\mu\text{M}$ ; for example, when 500  $\mu\text{L}$   $\text{Re}(\text{CO})_3(\text{L})$  (20  $\mu\text{M}$ ) was added to the solution of  $\text{A}\beta_{(1-40)}$  aggregates, there was an obvious enhance of fluorescence intensity at the emission wavelength of 425 nm (Figure 6(a)), oppositely, different from the visible fluorescence spectroscopy of EHIDA at 320 nm (Figure 6(c)). Upon excitation there occurs an intramolecular charge transfer of  $\text{Re}(\text{CO})_3(\text{L})$  between the  $-\text{NH}-\text{C}=\text{O}$  group and rhenium, and then back charge transfer will be impossible. Upon addition of  $\text{A}\beta_{(1-40)}$  aggregates, there was a visible enhance of fluorescence intensity at 425 nm, with no disturb of emission wavelength. Considering the breakage of the system of conjugated bonds due to the reorientation of the benzene ring and amide group of  $\text{Re}(\text{CO})_3(\text{L})$  with respect

to one another in PBS solution, the enhance of fluorescence intensity was mainly because of the breakage of the system of conjugated bonds being inhabited by the microsurrrounding of  $\beta$ -amyloid aggregates when  $A\beta_{(1-40)}$  aggregates was added [12]; as a result, an increased MLCT of  $\text{Re}(\text{CO})_3(\text{L})$  then occurred. However, the dissociation constant of Thioflavin T equaled  $2 \mu\text{M}$  [8], lower than that of  $\text{Re}(\text{CO})_3(\text{L})$ , because of there is no obvious enhance of fluorescence intensity when the concentration of  $\text{Re}(\text{CO})_3(\text{L})$  was little than  $19 \mu\text{M}$ . Generally, probes which bind characteristically to  $\beta$ -amyloid fibrils have more than one conjugation system and electron-donating groups such as  $\text{Me}_2\text{N}$ -,  $\text{MeNH}$ -,  $\text{MeO}$ -, and  $\text{HO}$ - in their molecular skeletons [13], opposite to the structure of  $\text{Re}(\text{CO})_3(\text{L})$ , indicative of a nonclassical intercalation of the complex binding to the  $A\beta_{(1-40)}$  aggregates. The rather good linear relationship between the concentrations of  $\text{Re}(\text{CO})_3(\text{L})$  and fluorescence intensity (equation (1), Figure 6(b)) was obtained by Microcal Origin 6.0:

$$Y = -9.51095 \times 10^7 + 5.53571 \times 10^6 X$$

$$(R: 0.98151, \text{SD}: 2.2583 \times 10^6, \quad (1)$$

$$N: 6, P: 5.09878 \times 10^{-4}),$$

where  $Y$  is the fluorescence intensity;  $X$  is the concentrations, from 19 to  $24 \mu\text{M}$ ;  $R$  is the linear correlation coefficient; SD is the standard error.

When binding to  $\beta$ -amyloid aggregates, there was also a great linear relationship between the concentrations of  $\text{Re}(\text{CO})_3(\text{L})$  and fluorescence intensity (equation (2), Figure 6(b)):

$$Y = -9.74219 \times 10^7 + 5.69714 \times 10^6 X$$

$$(R: 0.98544, \text{SD}: 2.05598 \times 10^6, \quad (2)$$

$$N: 6, P: 3.16429 \times 10^{-4}),$$

where  $Y$  is the fluorescence intensity;  $X$  is the concentrations of  $\text{Re}(\text{CO})_3(\text{L})$ , from 19 to  $24 \mu\text{M}$ ;  $R$  is the linear correlation coefficient; SD is the standard error.

#### 4. Conclusion

In conclusion, the synthesis and X-ray measurement of  $\text{Re}(\text{CO})_3(\text{L})$  was successfully completed, providing a novel crystal structure which was not similar to that of classical  $\text{Re}/\text{Tc}(\text{I})(\text{tricarboxyl})^+$  IDA derivatives. Evaluation of its binding affinity to  $A\beta_{(1-40)}$  aggregates by the fluorescence method demonstrated that the binding characteristic was at micromolar level, which suggested that the structural modification should be achieved for future exploration of  $^{99\text{m}}\text{Tc}(\text{CO})_3^+$  core labeled EHIDA derivatives as imaging agents for  $A\beta_{(1-40)}$  plaques in vivo, and this work will encourage for further exploration of probes for  $\beta$ -amyloid plaques.

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#### References

- [1] J. Hardy and D. J. Selkoe, "The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics," *Science*, vol. 297, no. 5580, pp. 353–356, 2002.
- [2] A. Nordberg, "PET imaging of amyloid in Alzheimer's disease," *Lancet Neurology*, vol. 3, no. 9, pp. 519–527, 2004.
- [3] Z.-P. Zhuang, M.-P. Kung, C. Hou, K. Ploessl, and H. F. Kung, "Biphenyls labeled with technetium 99 m for imaging  $\beta$ -amyloid plaques in the brain," *Nuclear Medicine and Biology*, vol. 32, no. 2, pp. 171–184, 2005.
- [4] D. D. Djokić, D. L. Janković, L. L. Stamenković, and I. Pirmettis, "Chemical and biological evaluation of technetium (I) tricarbonyl complexes with EHIDA and DPD. 99 m (I) complexes of EHIDA and DPD," *Nuclear Medicine Review*, vol. 7, no. 1, pp. 1–5, 2004.
- [5] Y. Yang, J. X. Zhang, J. J. Wang, and L. Zhu, "Two novel procedures of preparation for  $[\text{Tc}(\text{CO})_2(\text{NO})]^{2+}$  labeled by EHIDA and its biodistribution," *Journal of Radioanalytical and Nuclear Chemistry*, vol. 273, no. 1, pp. 31–36, 2007.
- [6] H. L. Chia, S. Fumagalli, F. Peter, A. G. Solco, and S. Birsfelden, "Synthesis and quality control of 99mTc-diethyl-IDA (EHIDA)," *Nucl. Compact*, vol. 9, no. 3, p. 86, 1978.
- [7] W. E. Klunk, R. F. Jacob, and R. P. Mason, "Quantifying amyloid  $\beta$ -peptide ( $A\beta$ ) aggregation using the congo red- $A\beta$  (CR- $A\beta$ ) spectrophotometric assay," *Analytical Biochemistry*, vol. 266, no. 1, pp. 66–76, 1999.
- [8] H. LeVine III, "Thioflavine T interaction with synthetic Alzheimer's disease  $\beta$ -amyloid peptides: detection of amyloid aggregation in solution," *Protein Science*, vol. 2, no. 3, pp. 404–410, 1993.
- [9] D. T. Cromer and J. T. Waber, *International Tables for X-Ray Crystallography*, vol. 4, Kynoch Press, Birmingham, UK, 1974.
- [10] R. Czerwieniec, A. Kapturkiewicz, J. Lipkowski, and J. Nowacki, " $\text{Re}(\text{I})(\text{tricarboxyl})^+$  complexes with the 2-(2-pyridyl)-N-methyl-benzimidazole, 2-(2-pyridyl)benzoxazole and 2-(2-pyridyl)benzothiazole ligands-syntheses, structures, electrochemical and spectroscopic studies," *Inorganica Chimica Acta*, vol. 358, no. 9, pp. 2701–2710, 2005.
- [11] N. Marti, B. Spingler, F. Breher, and R. Schibli, "Comparative studies of substitution reactions of Rhenium(I) dicarbonylnitrosyl and tricarbonyl complexes in aqueous media," *Inorganic Chemistry*, vol. 44, no. 17, pp. 6082–6091, 2005.
- [12] E. S. Voropai, M. P. Samtsov, K. N. Kaplevskii, et al., "Spectral properties of thioflavin T and its complexes with amyloid fibrils," *Journal of Applied Spectroscopy*, vol. 70, no. 6, pp. 868–874, 2003.
- [13] H. F. Kung, C.-W. Lee, Z.-P. Zhuang, M.-P. Kung, C. Hou, and K. Plössl, "Novel stilbenes as probes for amyloid plaques," *Journal of the American Chemical Society*, vol. 123, no. 50, pp. 12740–12741, 2001.