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Synthesis and characterization of well-defined L-lactic acid-caprolactone co-oligomers and their rhenium (I) and technetium(I) conjugates

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ABSTRACT

Staring from L-lactide and ε-caprolactone, the corresponding lactic-caprolactone cooligomer with hydroxyl and carboxylic acid groups were synthesized. These oligomers were connected with chelating groups through a long chain tether, ready for transition metal binding. Coordination of organometallic rhenium(I) and technetium(I) complexes generated the conjugates in high yield and short time, satisfying the requirements for short-lived radiopharmaceuticals in clinical applications. A reasonable pharmacophore model has been established to guide the design of well-defined lactic acid oligomer for nuclear medicine.

1. Introduction

Biodegradable polymers are degraded in the environment by the action of naturally occurring microorganisms. The conjugates of biodegradable polymers with radiopharmaceutical agents would allow new insight into their bio-distribution and controlled release. The coordination and characterization of *fac*-$[M(CO)_3]^+$ conjugate complexes would benefit their applications in SPECT image or radio-therapy. Biodegradable nature of these conjugates would also alleviate environmental contamination for un-used radiopharmaceuticals [1].

Aliphatic polyesters are the most known and studied biodegradable polymers, including poly(lactide) (PLA) and poly(caprolactone) (PCL). PLA is prepared from renewable resources and commercially available from a variety of manufacturers [2]. PCL is a FDA-approved biodegradable polymer and has been used in various medical and pharmaceutical applications, including controlled drug delivery, implants, and surgical dressings [3].

Preparation of monodisperse co-oligomers of poly(lactide)-poly(caprolactone) (PLA-PCL) would enable a wide range of structural studies. A series of blends of biodegradable polymers using PCL and PLA were prepared by the variation of the mass fraction across the composition range [4–7]. Traditionally, PLA and PCL have been prepared via the ring opening polymerization of L-lactide and ε-caprolactone using a catalyst, such as stannous octanoate. Significant effort has been devoted to the development of length-controlled polymerization process in recent years [8,9]. Although there is growing academic and industrial interest in PLA, PCL, or their copolymers (PLA-PCL), the synthesis of well-defined PLA or PCL oligomers have been scarcely reported [10,11].

Technetium-99m (140 keV (89%), $T_{1/2}$ = 6.02 h) complexes present a major synthetic challenge with respect to designing radiopharmaceuticals that have suitable in vivo stabilites. Alberto et al developed a particularly stable and kinetically inert $[M(H_2O)_3(CO)_3]^+$ core ($M = {}^{99m}Tc, {}^{186/188}Re$) reagent [12,13], which can be used to form complexes with various ligands, including the bis(picolyl)amine moiety [14–16]. Saatchi have synthesized length-controlled PLA by ring-opening polymerization of L-lactide and connected with rhenium(I) for medicinal applications [17,18]. Previously, we reported the synthesis of oligomeric (monomer to heptamer) lactic acids, and their conjugates with a chelating agent through a short oligoethylene oxide spacer, which can bind with a radioactive transition metal ion [19,20]. Here, we report the synthesis of molecularly defined LA-CL co-oligomers, their conjugates with rhenium(I) and ^{99m}Tc(I). We intended to develop these

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complexes as new agents for applications in SPECT image or chemotherapy.

2. Experimental

2.1. Materials and methods

All of the starting materials and reagents were commercially available and used directly without further purification. High resolution mass spectra were obtained on a Thermo-MAT95XP mass spectrometer using electron impact ionization. Matrix assisted laser desorption/ionization (MALDI TOF-HRMS) was carried out on a Thermo Finnigan Bioanalysis DYNAMO TOF Mass Spec Spectrometer. NMR spectra were recorded on a Bruker Avance 400 or 500 MHz spectrometer. IR spectra were recorded on an Avataar 370 FT-IR spectrometer (250–4000 cm^{-1}). The elemental analyses were conducted using Elementar Analysen-System GmbH (Germany) vario EL III. High performance liquid chromatography (HPLC) analyses of the Re and ^{99m}Tc complexes were performed on a hypersil BDS C-18 reversed-phase column (5 μm, 60% CH_3CN/40% H_2O, flow rate 1.0 mL/min), using a dionex P680 system equipped with a tunable absorption detector and a PDA-100 photodiode-array detector.

2.2. Chemical synthesis

2.2.1. Synthesis of L-lactic acid-caprolactone co-oligomers

*2.2.1.1. Synthesis of 6-hydroxyhexanoic acid (**H2**).* ε-Caprolactone (22.8 g, 0.20 mol), H_2O (300 mL), and sodium hydroxy (NaOH) (8.4 g, 0.21 mol) were added in a round bottom flask. This reaction mixture was stirred at room temperature overnight. The clear reaction mixture was then extracted with ethyl acetate (EtOAc) (4 × 50 mL), and the organic phase was, dried by anhydrous sodium sulphate (Na_2SO_4), to give the product **H2** 26.0 g (yield 98%) as a clear white solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.7 (b, CH_2O**H**, 1H), 3.55 (t, J = 6.2 Hz, C**H**$_2$OH, 2H), 2.3 (t, J = 7.2 Hz, C**H**$_2$$CO_2$H, 2H), 1.6 (m, HOC$H_2$(C**H**$_2$)$_3$$CH_2CO_2$H, 6H). IR (KBr) ν: 3260, 2912, 2849, 1600, 1256, 583 cm^{-1}.

*2.2.1.2. Synthesis of benzyl 6-hydroxyhexanoate (**H3**).* **H2** (13.20 g, 0.10 mol), triethylamine (30.3 g, 0.30 mol), and dichloromethane (CH_2Cl_2, 60 mL) were added in a round bottom flask. Benzyl bromide (BnBr, 12.2 g, 0.12 mol) in 40 mL CH_2Cl_2 was dropped into the mixture, and the solution was stirred overnight at room temperature. Water (100 mL) was added to the reaction mixture, and the solution was extracted with CH_2Cl_2 (2 × 100 mL). The organic layer was dried over Na_2SO_4, filtered, and the crude product was purified via silica gel flash column chromatography to give **H3** 18.89 g (yield 85%) as colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.30–7.38 (m, Ar, 5H), 5.12 (s, CO_2C**H**$_2$Ph, 2H), 3.63 (t, J = 6.5 Hz, C**H**$_2$OH, 2H), 2.38 (t, J = 6.5 Hz, C**H**$_2$$CO_2$Bn, 2H), 1.45–1.65 (m, HOCH_2(C**H**$_2$)$_3$$CH_2CO_2$Bn, 6H). IR (KBr) ν: 3180, 2992, 2840, 1650, 1126, 638 cm^{-1}.

*2.2.1.3. Synthesis of 6-(tert-Butyldimethyl)siloxyhexanoic benzyl ester (**H4**).* **H3** (31.62 g, 0.12 mol), imidazole (10.30 g, 0.15 mol), and dimethylformamide (DMF, 20 mL) were added in a round bottom flask. *tert*-Butyldimethylsilyl chloride (TBDMSCl, 22.65 g, 0.15 mmol) was added into the mixture, which was stirred overnight at room temperature under dinitrogen atmosphere. The resulting mixture was poured into a separation funnel containing 150 mL of saturated aqueous $NaHCO_3$, and the product was extracted with hexane (3 × 150 mL). The organic phase was dried over Na_2SO_4, filtered, and the crude product was purified via silica gel flash column chromatography, to give **H4** 26.88 g (yield 80%) as colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.30–7.38 (m, Ar, 5H), 5.10 (s, CO_2C**H**$_2$Ph, 2H), 3.61 (t, J = 6.5 Hz, C**H**$_2$OH, 2H), 2.35 (t, J = 6.5 Hz, C**H**$_2$$CO_2$Bn, 2H), 1.45–1.65 (m, CH_2(C**H**$_2$)$_3$$CH_2$, 6H), 0.88 (s, (C**H**3)$_3$CSi, 9H), 0.09 (s, (C**H**$_3$)$_2$Si, 6H). IR (KBr) ν: 2991, 2802, 1555, 1326, 1107, 618 cm^{-1}. ESI-HRMS: calcd for $C_{19}H_{33}O_3Si$ $[M + H]^+$ 337.2199, found 337.2107.

*2.2.1.4. Synthesis of 6-(tert-Butyldimethyl)siloxyhexanoic acid (**H5**).* Pd–C (10 wt %, 0.88 g) was added into a solution of **H4** (3.36 g, 10 mmol) in ethyl acetate (50 mL), and the reaction mixture was stirred for 2 h at room temperature under dihydrogen. The resulting mixture was filtered through celite, and the cake was washed with 100 mL of ethyl acetate. The combined filtrate was concentrated under reduced pressure to give **H5** 2.34 g (yield 95%) as clear and colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 3.56 (t, J = 6.5 Hz, C**H**$_2$OSi, 2H), 2.31 (t, J = 7.5 Hz, C**H**$_2$$CO_2$H, 2H), 1.30–1.55 (m, SiOCH_2(C**H**$_2$)$_3$$CH_2CO_2$H, 6H), 0.84 (s, (C**H**$_3$)$_3$CSi, 9H), 0.05 (s, (C**H**$_3$)$_2$Si, 6H). IR (KBr) ν: 3201, 2908, 1675, 1542, 1126, 1102, 713 cm^{-1}. ESI-HRMS: calcd for $C_{12}H_{26}O_3Si$ $[M + H]^+$, 246.1651; found, 246.1649.

*2.2.1.5. Synthesis of 6-[(S)-2-((S)-2-(tertbutyldimethylsilyloxy)propanoyloxy)propanoate] hexanoic benzyl ester (**L5**).* **L4** (5.52 g, 20 mmol), EDC (4.20 g, 22 mol, 1.1 eq), and DMAP (2.68 g, 22 mmol, 1.1 eq) were dissolved in CH_2Cl_2 (50 mL). **H3** (4.44 g, 20 mmol) was added into the mixture, and the solution was stirred at room temperature for 10 h. The reaction mixture was then added into H_2O (50 mL), and the product was extracted with additional CH_2Cl_2 (2 × 50 mL). The combined organic layer was dried over Na_2SO_4, filtered, and the crude product was purified via silica gel flash column chromatography, to give **L5** 7.20 g (yield 75%) as colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.25 (b, Ar, 5H), 5.12–5.30 (m, CO_2C**H**$_2$Ph and CO_2C**H**(CH_3)CO_2, 3H), 4.38 (q, J = 6.8 Hz, SiOC**H**(CH_3)CO, 1H), 4.10 (t, J = 6.6 Hz, COOC**H**$_2$(CH_2)$_3$, 2H), 2.35 (t, J = 8.6 Hz, C**H**$_2$$CO_2$Bn, 2H), 1.56–1.65 (m, OCH_2(C**H**$_2$)$_3$$CH_2CO_2$Bn, 6H), 1.42–1.50 (m, OCH(C**H**$_3$)CO_2CH(C**H**$_3$)COO, 6H), 0.93 (s, (C**H**$_3$)$_3$CSi, 9H), 0.11 (s, (C**H**$_3$)$_2$Si, 3H), 0.09 (s, (C**H**$_3$)$_2$Si, 3H). IR (KBr) ν: 2986, 1765, 1231, 1103, 1055, 743 cm^{-1}. ESI-HRMS: calcd for $C_{25}H_{41}O_7Si$ $[M + H]^+$ 481.2622, found 481.2496.

*2.2.1.6. Synthesis of 6-[(S)-2-((S)-2-(tertbutyldimethylsilyloxy)propanoyloxy)propanoate] hexanoic acid (**L6**).* The mono-protected, acid-functionalized LA-CL-COOH, **L6**, was prepared using the general procedure described above for deprotection of benzyl ester **L5** by hydrogenolysis (Pd–C/H2). The combined filtrates were concentrated under reduced pressure to give **L6** (95% yield) as colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.10 (q, J = 6.8 Hz, CO_2C**H**(CH_3)CO_2, 1H), 4.40 (q, J = 6.8 Hz, SiOC**H**(CH_3)CO, 1H), 4.11 (t, J = 6.5 Hz, COOC**H**$_2$(CH_2)$_3$, 2H), 2.35 (t, J = 7 Hz, C**H**$_2$$CO_2$H, 2H), 1.58–1.65 (m, OCH_2(C**H**$_2$)$_3$$CH_2CO_2$H, 6H), 1.38–1.50 (m, OCH(C**H**$_3$)CO_2CH(C**H**$_3$)COO, 6H), 0.93 (s, (C**H**$_3$)$_3$CSi, 9H), 0.11 (s, (C**H**$_3$)$_2$Si, 3H), 0.09 (s, (C**H**$_3$)$_2$Si, 3H). IR (KBr) ν: 2916, 1815, 1301, 1213, 1156, 723 cm^{-1}. ESI-HRMS: calcd for $C_{18}H_{34}O_7SiNa$ $[M + Na]^+$ 413.1971, found 413.1690.

*2.2.1.7. Synthesis of 6-[(S)-2-((S)-2- hydroxypropanoyloxy) propanoate] hexanoic benzyl ester (**L7**).* The mono-protected, hydroxyl-functionalized LA-CL-OH, **L7**, was prepared using the general procedure described above for deprotection of TBDMS group of the protected Monomer, **L5** (TBAF/THF). The crude product was purified via silica gel flash column chromatography, to give **L7** (yield 75%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.25 (b, Ar, 5H), 5.12–5.30 (m, CO_2C**H**$_2$Ph and CO_2C**H**(CH_3)CO_2, 3H), 4.38 (q, J = 6.8 Hz, SiOC**H**(CH_3)CO, 1H), 4.10 (t, J = 6.6 Hz, COOC**H**$_2$(CH_2)$_3$, 2H), 2.35 (t, J = 8.6 Hz, C**H**$_2$$CO_2$Bn, 2H), 1.56–1.65 (m, OCH_2(C**H**$_2$)$_3$$CH_2CO_2$Bn,

6H), 1.42–1.50 (m, OCH(C**H**$_3$)CO$_2$CH(C**H**$_3$)COO, 6H), 0.93 (s, (C**H**$_3$)$_3$CSi, 9H), 0.11 (s, (C**H**$_3$)$_2$Si, 3H), 0.09 (s, (C**H**$_3$)$_2$Si, 3H). IR (KBr) ν: 3206, 3012, 1745, 1531, 1130, 1089, 813 cm^{-1}. ESI-HRMS: calcd for $C_{19}H_{27}O_7$ $[M + H]^+$ 367.1757, found 367.1901.

*2.2.1.8. Synthesis of LA-CL dimer (**L8**).* The doubly protected LA-CL dimer, **L8**, was prepared using the general procedure described above for esterification of LA-CL-COOH (**L6**) and LA-CL-OH (**L7**). The crude product was purified via silica gel flash column chromatography, to yield **L8** (50% yield) as colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.25 (b, Ar, 5H), 5.12–5.30 (m, CO$_2$C**H**$_2$Ph and CO$_2$C**H**(CH$_3$)CO$_2$, 5H), 4.38 (q, J = 6.8 Hz, SiOC**H**(CH$_3$)CO, 1H), 4.10 (m, COOC**H**$_2$(CH$_2$)$_3$, 4H), 2.35 (m, C**H**$_2$CO$_2$Bn, 4H), 1.56–1.65 (m, OCH$_2$(C**H**$_2$)$_3$CH$_2$CO$_2$Bn, 12H), 1.42–1.50 (m, OCH(C**H**$_3$)CO$_2$CH(C**H**$_3$)COO, 12H), 0.93 (s, (C**H**$_3$)$_3$CSi, 9H), 0.11 (s, (C**H**$_3$)$_2$Si, 3H), 0.09 (s, (C**H**$_3$)$_2$Si, 3H). IR (KBr) ν: 2896, 1695, 1230, 1112, 1046, 734 cm^{-1}. ESI-HRMS: calcd for $C_{37}H_{59}O_{13}Si$ $[M + H]^+$ 739.3725, found 739.3710.

*2.2.2. Synthesis of 6-(N-dipicoylaminoethyl)hexylamine (**N4**)*

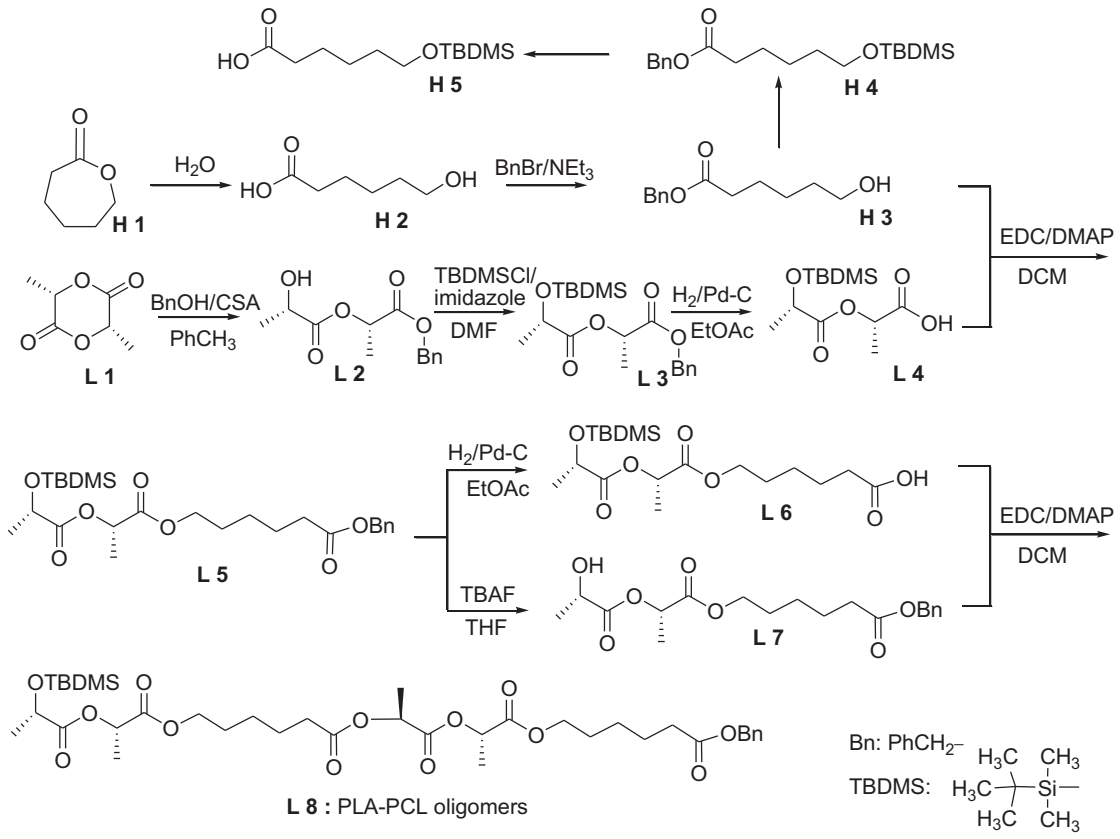
Compound **N2**–**N3** were synthesized according to our previously published procedure with slight modification [20,21]. Pd–C (10 wt %, 0.40 g) was added to a solution of **N3** (1.99 g, 5 mmol) in absolutely methanol (20 mL), and the reaction mixture was stirred for 2 h at room temperature under dihydrogen. The resulting mixture was filtered and the cake was washed with 20 mL of ethyl acetate. The combined filtrate was concentrated under reduced pressure to give **N4** 1.41 g (yield 98%) as pale red oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.50 (d, J = 4.1 Hz, Py-σH, 2H), 7.63 (dt, J = 7.7 Hz, J = 1.7 Hz, Py-γH, 2H), 7.52 (d, J = 7.8 Hz, Py-βH, 2H), 7.12 (t, J = 5.2 Hz, Py-δH, 2H), 3.79 (s, –N(C**H**$_2$)$_2$, 4H), 2.62 (d, J = 7.1 Hz, C**H**$_2$NCH$_2$, 2 H), 2.52 (t, J = 7.3 Hz, CONCH$_2$C**H**$_2$, 2 H), 1.75 (s, C**H**$_2$CH$_2$CH$_2$NH$_2$, 2H), 1.50–1.56 (m, C**H**$_2$N**H**$_2$, 4H), 1.20–1.29 (m, (C**H**$_2$)$_2$(CH$_2$)$_2$NH$_2$, 4 H); IR (KBr): 2924, 2028, 1917 cm^{-1}; EI-HRMS: calcd for $C_{18}H_{26}N_4$ 298.2157, found 298.2157.

2.2.3. Synthesis of the conjugates between lactic acid and tricarbonyl rhenium complex

*2.2.3.1. Synthesis of hexamer-alkyl-tri (**C1**).* A mixture of lactic acid hexamer (0.56 g, 1.0 mmol), EDC (0.21 g, 1.1 mmol, 1.1 eq), and HOBt (0.147 g, 1.1 mmol, 1.1 eq), were dissolved in CH_2Cl_2 (50 mL). **N4** (0.30 g, 1.0 mmol) was added into the mixture, which was stirred at room temperature for 10 h. The crude product was added into H_2O (50 mL), the product was extracted with additional CH_2Cl_2 (2 × 50 mL). The organic layer was dried over Na_2SO_4, filtered, and the crude product was purified via silica gel flash column chromatography to give **C1** 0.63 g (yield 75%) as colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.51 (d, J = 4.0 Hz, Py-σH, 2H), 7.65 (t, J = 7.5 Hz, Py-γH, 2H), 7.52 (d, J = 8.0 Hz, Py-βH, 2H), 7.15 (t, J = 6.0 Hz, Py-δH, 2H), 6.18 (t, J = 5.5 Hz, CON**H**CH$_2$, 1H), 5.13–5.23 (m, CO[OC**H**(CH$_3$)CO]$_5$N, 5H), 4.38 (q, J = 6.0 Hz, SiOC**H**(CH$_3$)CO, 1H), 3.80 (s, –N(C**H**$_2$)$_2$, 4H), 3.11–3.25 (m, NHC**H**$_2$CH$_2$, 2H), 2.53 (t, J = 7.3 Hz, C**H**$_2$N(CH$_2$)$_2$, 2H), 1.43–1.57 (m, Si[OCH(C**H**$_3$)CO]$_6$N and NHCH$_2$C**H**$_2$(CH$_2$)$_2$C**H**$_2$CH$_2$N, 22H), 1.23–1.28 (m, NH(CH$_2$)$_2$(C**H**$_2$)$_2$(CH$_2$)$_2$N, 4H), 0.90 (s, (C**H**$_3$)$_3$CSi, 9H), 0.11 (s, (C**H**$_3$)$_2$Si, 3H), 0.083 (s, (C**H**$_3$)$_2$Si, 3H). ^{13}C NMR ($CDCl_3$): δ: 173.53 (**C**O$_2$N, 1C), 170.68 (**C**O$_2$C, 1C), 169.98 (**C**O$_2$C, 1C), 169.85 (**C**O$_2$C, 1C), 169.58 (**C**O$_2$C, 1C), 168.62 (**C**O$_2$C, 1C), 159.96 (Py-αC, 2C), 148.92 (Py-σC, 2C), 136.41 (Py-γC, 2C), 122.90 (Py-βC, 2C), 121.91 (Py-δC, 2C), 71.80 (O**C**H$_2$CH$_2$N, 1C), 69.76 (CO$_2$**C**H(CH$_3$)CO$_2$, 1C), 69.13 (CO$_2$**C**H(CH$_3$)CO$_2$, 1C), 68.65 (CO$_2$**C**H(CH$_3$)CO$_2$, 1C), 68.46 (CO$_2$**C**H(CH$_3$)CO$_2$, 1C), 67.97 (CO$_2$**C**H(CH$_3$)CON, 1C), 60.43 (Py(**C**H$_2$)$_2$, 2C), 54.31 (CH$_2$**C**H$_2$N, 1C), 39.30 (CONH**C**H$_2$CH$_2$O, 1C), 29.25 (**C**H$_2$CH$_2$N, 1C), 26.98 (**C**H$_2$CH$_2$CH$_2$N, 1C), 26.92 (CONCH$_2$CH$_2$**C**H$_2$, 1C), 26.63 (CONCH$_2$**C**H$_2$, 1C), 25.68 ((**C**H$_3$)$_3$CSi, 3C), 21.22 (SiOCH(**C**H$_3$)CO, 1C), 18.27 ((CH$_3$)$_3$**C**Si, 1C), 17.79 (CO$_2$CH(**C**H$_3$)CO$_2$, 1C), 16.72 (CO$_2$CH(**C**H$_3$)CO$_2$, 1C), 16.70 (CO$_2$CH(**C**H$_3$)CO$_2$, 1C), 16.63 (CO$_2$CH(**C**H$_3$)CO$_2$, 2C), –4.92 ((**C**H$_3$)$_2$Si, 1C), –5.30 ((**C**H$_3$)$_2$Si, 1C). ESI-HRMS: calcd for $C_{42}H_{65}N_4O_{12}Si$ $[M + H]^+$ 845.4368, found 845.3624.

*2.2.3.2. Synthesis of HO-hexamer-alkyl-tri (**C2**).* Acetic acid (0.5 g, 8.3 mmol) was added into a solution of **C1** (0.34 g, 0.4 mmol) in THF (2 mL). To this solution was added TBAF (1.0 M solution in THF, 0.25 g, 0.8 mmol), and the solution was stirred for 48 h at room temperature. The reaction mixture was added into aqueous $NaHCO_3$ (40 mL), and the crude product was extracted with additional EtOAc (2 × 40 mL). The combined organic layer was washed with H_2O and saline, and dried over Na_2SO_4, filtered, and the crude product was purified via silica gel flash column chromatography, to give **C2** 0.24 g (yield 82%) as a light yellow oil. IR (KBr) ν: 2921, 1605, 1503, 1238, 833, 764 cm^{-1} ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.52 (d, J = 4.7 Hz, Py-σH, 2H), 7.68 (t, J = 7.6 Hz, Py-γH, 2H), 7.55 (d, J = 7.8 Hz, Py-βH, 2H), 7.16 (t, J = 6.1 Hz, Py-δH, 2H), 6.22 (t, J = 5.5 Hz, CON**H**CH$_2$, 1H), 5.12–5.23 (m, CO[OC**H**(CH$_3$)CO]$_5$N, 5H), 4.38 (q, J = 6.8 Hz, SiOC**H**(CH$_3$)CO, 1H), 3.82 (s, –N(C**H**$_2$)$_2$, 4H), 3.14–3.24 (m, NHC**H**$_2$CH$_2$, 2H), 2.54 (t, J = 7.1 Hz, C**H**$_2$N(CH$_2$)$_2$, 2H), 1.44–1.58 (m, Si[OCH(C**H**$_3$)CO]$_6$N and NHCH$_2$C**H**$_2$(CH$_2$)$_2$C**H**$_2$CH$_2$N, 22H), 1.25 (b, NH(CH$_2$)$_2$(C**H**$_2$)$_2$(CH$_2$)$_2$N, 4H); ^{13}C NMR ($CDCl_3$): δ: 175.20 (**C**O$_2$N, 1C), 170.63 (**C**O$_2$C, 1C), 169.73 (**C**O$_2$C, 1C), 169.68 (**C**O$_2$C, 1C), 169.64 (**C**O$_2$C, 1C), 168.62 (**C**O$_2$C, 1C), 159.23 (Py-αC, 2C), 148.71 (Py-σC, 2C), 136.75 (Py-γC, 2C), 123.17 (Py-βC, 2C), 122.14 (Py-δC, 2C), 71.79 (O**C**H$_2$CH$_2$N, 1C), 69.80 (CO$_2$**C**H(CH$_3$)CO$_2$, 1C), 69.19 (CO$_2$**C**H(CH$_3$)CO$_2$, 1C), 68.96 (CO$_2$**C**H(CH$_3$)CO$_2$, 1C), 68.88 (CO$_2$**C**H(CH$_3$)CO$_2$, 1C), 66.68 (CO$_2$**C**H(CH$_3$)CON, 1C), 60.00 (Py(**C**H$_2$)$_2$, 2C), 54.28 (CH$_2$**C**H$_2$N, 1C), 39.29 (CONH**C**H$_2$CH$_2$O, 1C), 29.20 (**C**H$_2$CH$_2$N, 1C), 26.85 (**C**H$_2$CH$_2$CH$_2$N, 1C), 26.73 (CONCH$_2$CH$_2$**C**H$_2$, 1C), 26.56 (CONCH$_2$**C**H$_2$, 1C), 20.50 (HOCH(**C**H$_3$)CO, 1C), 17.79 (CO$_2$CH(**C**H$_3$)CO$_2$, 1C), 16.72 (CO$_2$CH(**C**H$_3$)CO$_2$, 1C), 16.69 (CO$_2$CH(**C**H$_3$)CO$_2$, 1C), 16.65 (CO$_2$CH(**C**H$_3$)CO$_2$, 1C), 16.64 (CO$_2$CH(**C**H$_3$)CO$_2$, 1C). ESI-HRMS: calcd for $C_{36}H_{51}N_4O_{12}$ $[M + H]^+$ 731.3498, found 731.3499.

*2.2.3.3. Synthesis of HO-hexamer-alkyl-tri-Re(CO)$_3$ (**C3**).* **C2** (73.0 mg, 0.10 mmol) was dissolved in 2 mL methanol. $(Et_4N)_2[Re(CO)_3Br_3]$ (85 mg, 0.11 mmol) was added into the mixture, and the solution was stirred for 30 min at room temperature under dinitrogen. The reaction mixture was evaporated to dryness, and the residue was purified via silica gel flash column chromatography, to yield **C3** 90 mg (90% yield) as a light yellow solid. The sample for element analysis was recrystallized from $CHCl_3$ and hexane. IR (KBr) ν: 2921, **2030**, **1920**, 1511, 1238, 772 cm^{-1} ^{1}H NMR (CD_3OD, 400 MHz) δ: 8.85 (d, J = 5.4 Hz, Py-σH, 2H), 7.94 (td J_1 = 8.0 Hz, J_2 = 1.1 Hz, Py-γH, 2H), 7.56 (d, J = 7.8 Hz, Py-βH, 2H), 7.36 (t, J = 6.6 Hz, Py-δH, 2H), 5.13–5.22 (m, CO[OC**H**(CH$_3$)CO]$_4$OCH(CH$_3$), 4H), 5.06 (q, OC**H**(CH$_3$)CON, J = 5.0 Hz, 1H), 4.86 (s, –N(C**H**$_2$), 2H), 4.32 (q, J = 5.0 Hz, HOC**H**(CH$_3$)CO, 1H), 3.80 (s, –N(C**H**$_2$), 2H), 3.20–3.30 (m, NHC**H**$_2$CH$_2$, 2H), 1.97 (t, J = 7.1 Hz, C**H**$_2$N(CH$_2$)$_2$, 2H), 1.42–1.62 (m, Si[OCH(C**H**$_3$)CO]$_6$N and NHCH$_2$C**H**$_2$(CH$_2$)$_2$C**H**$_2$CH$_2$N, 22H), 0.82–0.91 (m, NH(CH$_2$)$_2$(C**H**$_2$)$_2$(CH$_2$)$_2$N, 4H); ^{13}C NMR ($CDCl_3$): δ: 197.56 (*fac*-Re(CO)$_3$, 3C), 176.04 (**C**O$_2$N, 1C), 172.94 (**C**O$_2$C, 1C), 171.71 (**C**O$_2$C, 1C), 171.68 (**C**O$_2$C, 1C), 171.47 (**C**O$_2$C, 1C), 171.11 (**C**O$_2$C, 1C), 162.51 (Py-αC, 2C), 153.43 (Py-σC, 2C), 141.92 (Py-γC, 2C), 127.17 (Py-βC, 2C), 124.93 (Py-δC, 2C), 72.90(O**C**H$_2$CH$_2$N, 1C), 72.13 (CO$_2$**C**H(CH$_3$)CO$_2$, 1C), 71.24 (CO$_2$**C**H(CH$_3$)CO$_2$, 1C), 70.89 (CO$_2$**C**H(CH$_3$)CO$_2$, 1C), 70.60 (CO$_2$**C**H(CH$_3$)CO$_2$, 1C), 70.18 (CO$_2$**C**H(CH$_3$)CON, 1C), 69.10 (Py(**C**H$_2$)$_2$, 2C), 67.83 (CH$_2$**C**H$_2$N, 1C), 40.25 (CONH**C**H$_2$CH$_2$O, 1C), 30.37 (**C**H$_2$CH$_2$N, 1C), 27.75 (**C**H$_2$CH$_2$CH$_2$N, 1C), 27.64 (CONCH$_2$CH$_2$**C**H$_2$, 1C), 26.49 (CONCH$_2$**C**H$_2$, 1C), 20.91 (HOCH(**C**H$_3$)CO, 1C), 18.42 (CO$_2$CH(**C**H$_3$)CO$_2$, 1C), 17.38 (CO$_2$CH(**C**H$_3$)CO$_2$, 1C), 17.35 (CO$_2$CH(**C**H$_3$)CO$_2$, 1C), 17.32 (CO$_2$CH(**C**H$_3$)CO$_2$, 1C), 17.25 (CO$_2$CH(**C**H$_3$)CO$_2$, 1C). Elemental Analysis: Calcd for $[C_{39}H_{50}N_4O_{15}Re]Br \bullet 2CHCl_3$: C 37.31, H 3.97, N 4.24; found: C 37.61, H 3.83, N 3.80. MALDI/DHB-HRMS: calcd for $C_{39}H_{50}O_{15}N_4^{185}Re$, 999.28425.

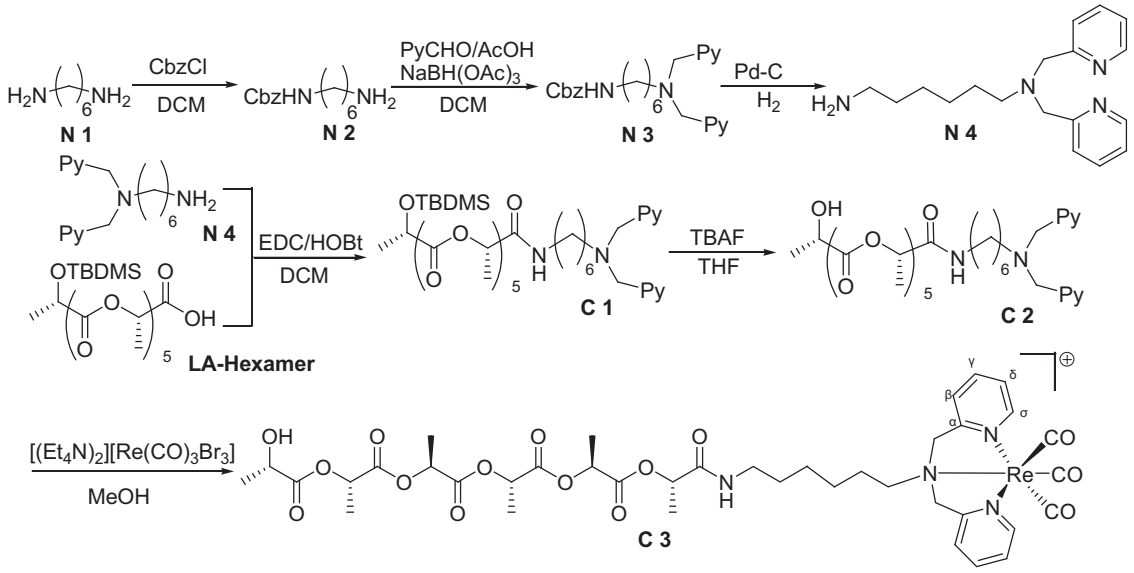


Scheme 1. Synthetic scheme for well-defined L-lactic acid-caprolactone co-oligomers.

2.3. Radio-labeling of ^{99m}Tc for SPECT imaging

2.3.1. Radio-synthesis of the conjugate $^{99m}Tc(CO)_3$-lactic acid

For radio-synthesis of the conjugate $^{99m}Tc(CO)_3$-lactic acid: $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared as reported before [22,23], with radiochemical purity of <95%, as determined by radio-HPLC. An aqueous $[^{99m}Tc(CO)_3(H_2O)_3]^+$ solution (0.2 mL) and **C2** (10^{-4} M, 100 μL) were added into a 5-mL serum vial. The reaction mixture was heated at 42 °C for 30 min, and the progress of the reaction was monitored by radio-TLC. After the mixture was cooled to room temperature, the radiotracer was purified by HPLC using the same conditions as those for the analysis of **C2** and **C3**. The radioactivity of the eluate was counted and calibrated against the standard solutions.



Scheme 2. Synthetic scheme for $Re(CO)_3$-coordinated lactide conjugate.

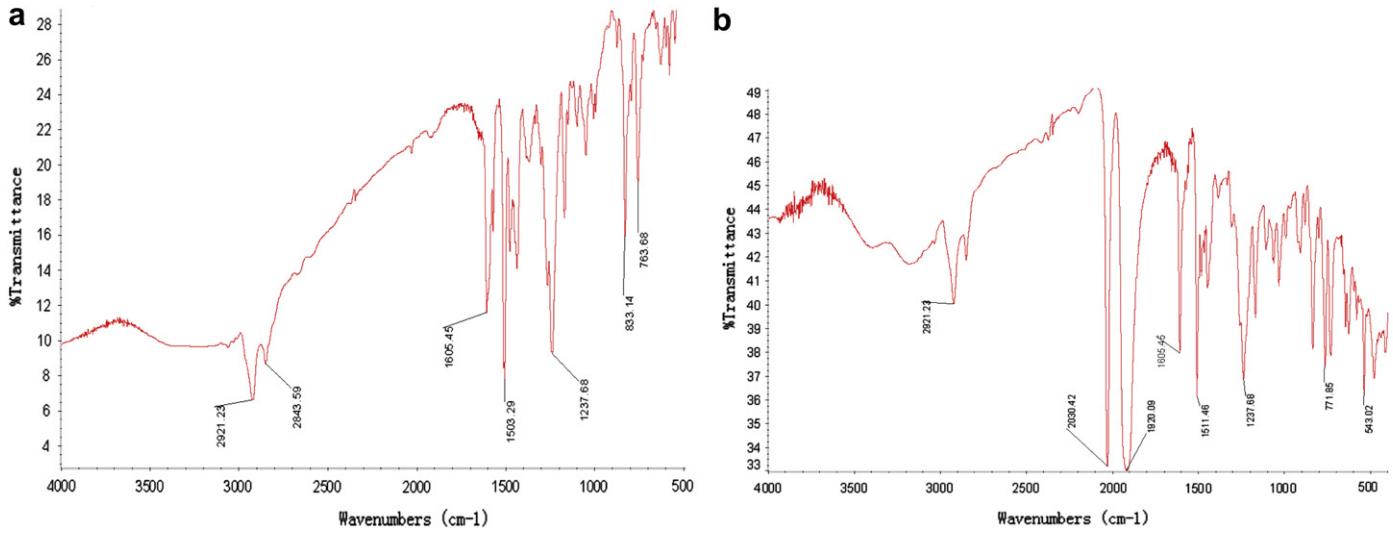


Fig. 1. IR spectra of **C2** (left) and its corresponding $[Re(CO)_3]^+$-conjugate **C3** (right).

2.3.2. In vitro stability of the conjugate $^{99m}Tc(CO)_3$-lactic acid

The stability of the conjugate $^{99m}Tc(CO)_3$-lactic acid was studied by measuring the radiochemical purity using radio-HPLC at different time intervals after preparation. The complex was added to a test tube with PBS. The mixture was incubated by shaking the test tube at 37 °C in an incubation apparatus. The radiochemical purity was measured at 30 min and 1, 2, 4, and 8 h by radio-HPLC.

3. Result and discussion

3.1. Synthesis of L-lactic acid-caprolactone co-oligomers

The synthesis of LA, CL and LA-CL oligomers relies on the availability of orthogonal protecting groups for carboxylic acid and hydroxyl group (Scheme 1). Benzyl (Bn) ester and t-butyldimethylsily (TBDMS) ether were selected to protect carboxylic and hydroxyl group [20]. Starting from L-lactide (**L1**) and ε-caprolactone (**H1**), the corresponding dimer **L2** and monomer **H2** were obtained in quantitative yields by continuous extraction of the base-catalyzed ring opening reactions. Initial studies using benzyl alcohol (BnOH) as protect group of **H2** gave low chemical yield [10]. The carboxylic acid-protected **H3** was prepared instead from **H2** by treatment with $BnBr/NEt_3$. Reaction of **H3** with TBDMSCl led to **H4** in high yield, with both hydroxyl and carboxylic acid group protected at this stage. Hydrogenolysis of **H4** gave the carboxylic acid CL **H5** almost quantitatively.

The coupling of the orthogonally protected LA dimers **L4** and CL monomer **H3** in the presence of EDC and DMAP proved to be a high yielding procedure affording the LA-CL oligomer, **L5**, with minimal side products (Scheme 1). The prowess of orthogonal protecting groups is shown in the synthesis of **L6** and **L7** from the same **L5**. The comboxylic acid group was released by hydrogenolysis to generate **L6**, and the hydroxy group was activated by treatment with tetra-*n*-butyl ammonium fluoride (TBAF) in the presence of acetic acid to give **L7**. The coupling of carboxylic acid and alcohol group using the same procedure to synthesize **L5** gave the LA-CL dimer **L8**. Following the protocols established in Scheme 1, each of the molecularly defined LA-CL oligomers could be synthesized.

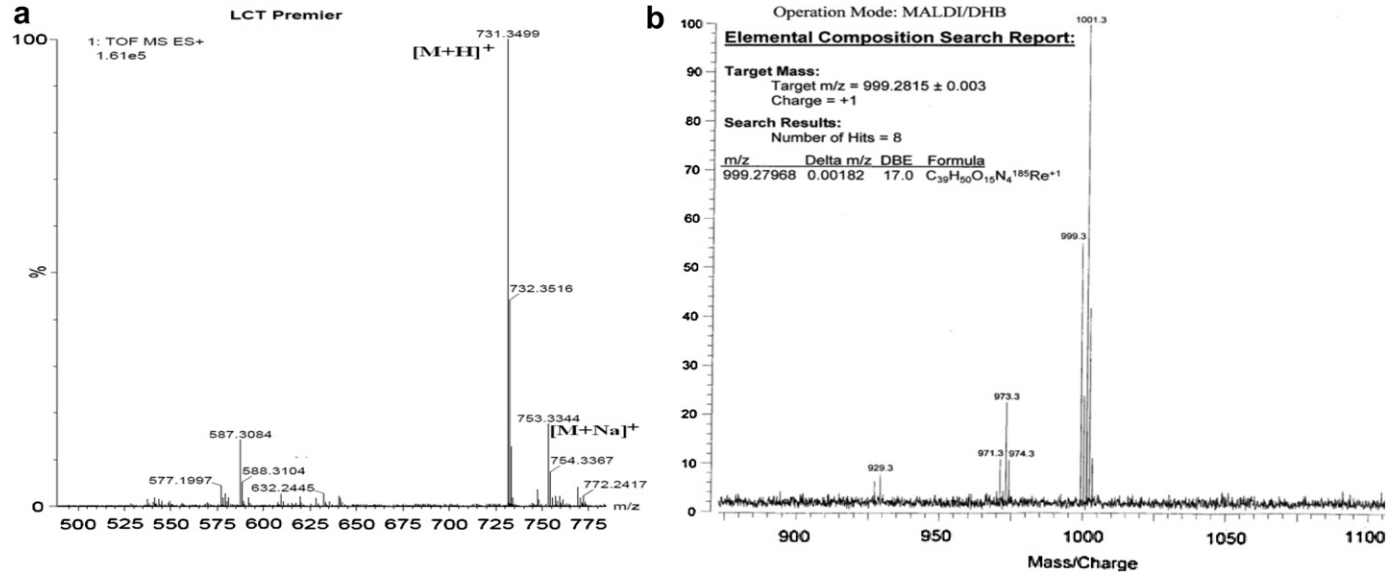


Fig. 2. (a) ESI-HRMS analysis of **C2**, and (b) MALDI/DHB-HRMS analysis of **C3**.

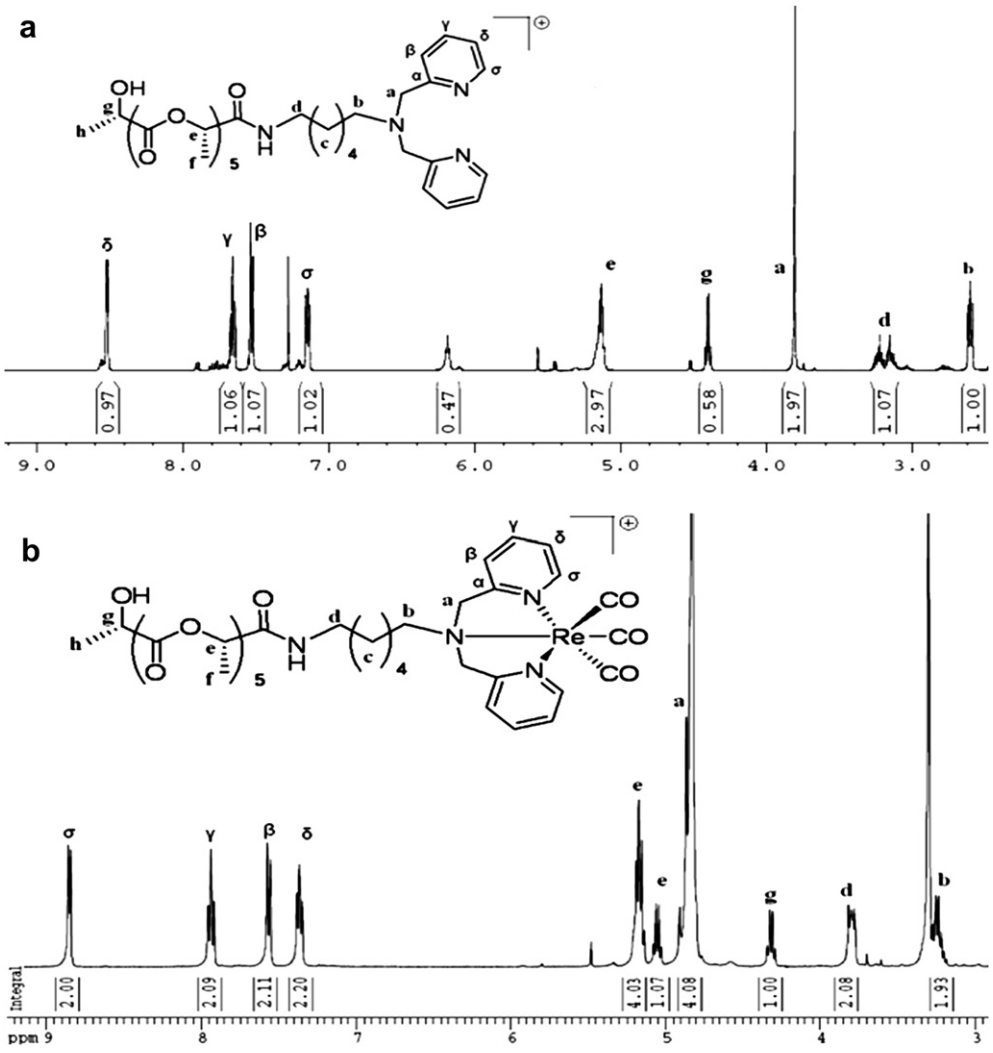


Fig. 3. ^{1}H NMR analysis of compound (a) **C2**, and (b) **C3**.

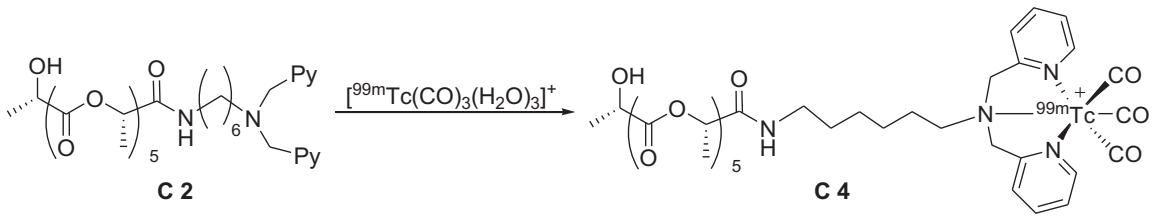
3.2. Synthesis and characterization of conjugates between lactic acid and $Re(CO)_3$ complexes

3.2.1. Synthesis of the conjugates

The chemical structure of the chelate, and the structure and length of the spacer, greatly affect the radiolabeling efficiency in the complex formation step. There are a number of reports on the preparation of tridentate chelating systems with variable spacer groups comprising terminal primary amine [20]. Here, we present a new procedure to synthesize tridentate chelating compound **N4** (Scheme 2). Starting from mono-Cbz-protected diaminohexane **N2**, the two py groups in **N3** were introduced by in situ imine formation with 2-pyridinecarbaldehyde and reduction with sodium borohydride triacetate. Cbz-deprotection of **N3** was achieved by hydrogenolysis (Pd/C, H2), leading to compound **N4**. The coupling of the free amine in **N4** and the free carboxylic acid in lactic acid hexamer [19,20] by EDC/HOBt gave intermediate **C1**. Deprotection of TBDMS groups generated **C2** in good yield. The rhenium(I) species was introduced using tricarbonyl rhenium complex ($[Et_4N]_2[Re(CO)_3Br_3]$) prepared from $[Bu_4N][ReO_4]$ [24]. To illustrate the stability of the conjugate, the crude product **C3** was purified via silica gel flash chromatography without any sign of decomposition.

3.2.2. Characterization of conjugate

A number of unique feature of the conjugates in spectroscopy have been observed.^{1}H NMR spectra of the hydroxyl-protected oligomers showed unique resonances for three methyl groups



Scheme 3. Radio-synthesis of ^{99m}Tc-lactic acid conjugate.

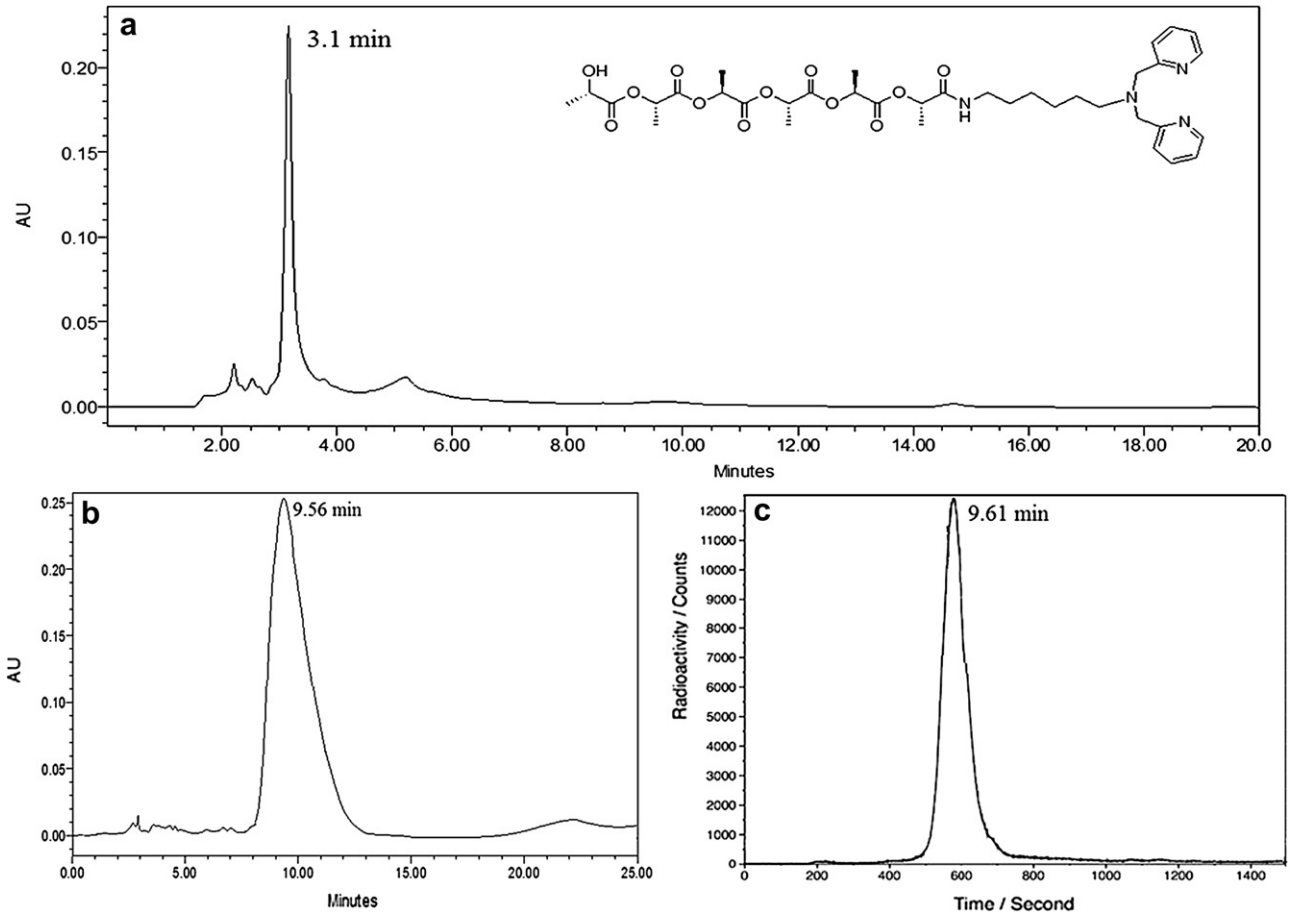


Fig. 4. HPLC analyses of compounds **C2** (a. UV detector) **C3** (b. UV detector) and **C4** (c. γ detector).

around TBDMS group region with chemical shifts at 0.90, 0.11, and 0.08 ppm. The small quartet (J = 6.0 Hz) at 4.38 ppm, due to the unique CH group next to TBDMS group (or HO group in the end of the chain), also functioned as a quantitative reference peak to monitor the number of repeating units. The analytical data (^{1}H NMR, ^{13}C NMR, and ESI-HRMS) for **C1** and **C2** are given in the electronic supporting information Figs. S3–S6.

Infrared spectroscopy of the conjugates showed unique features of $[Re(CO)_3]^+$ with strong band and intense absorption at 1920 and 2030 cm^{-1}, which were attributed to ν(C–O) of the fac-$Re(CO)_3^+$ units (Fig. 1). These absorption bands are significantly blue shifted compared with the starting material ($[Et_4N]_2[Re(CO)_3Br_3]$) (1871, 1998 cm^{-1}) [25].

MALDI/DHB mass spectrometry of the conjugates and their precursors shows the fingerprint of oligomer chain (Fig. 2) [26]. Inductively coupled plasma-mass spectrometry (ICP-MS) measured the actual amount of rhenium in the conjugates (Fig. S8). The isotope pattern of rhenium complex matches the simulation signal.

^{1}H and ^{13}C NMR spectra of the rhenium complex **C3** and its precursor **C2** show that the rhenium core is an electron-withdrawing group, making the chemical shift moving down-field (Fig. 3). The ^{13}C NMR spectra of the rhenium complexes **C3** also showed three carbonyl peaks in the range 197.0–198.0 ppm (Fig. S7).

3.3. HPLC analysis of the conjugate ^{99m}Tc-lactic acid

The radiochemical conjugate of technetium(I) complex was synthesized following the same procedure as that for Rhenium (I) complex (Scheme 3). The radiochemical purity of the crude ^{99m}Tc-lactic acid conjugate **C4** (Fig. 4) exceeded 90%. The conjugate and its precursor were well separated, and the conjugate was purified by semi preparative HPLC.

3.4. In vitro stability of ^{99m}Tc-lactic acid conjugate

The radio-conjugate, **C4**, showed no degradation in PBS solution over 8 h at room temperature (Fig. 5). Decomposition or dissociation of the complexes to either $[^{99m}Tc(CO)_3]^+$ or other side products was not observed for all of the complexes under the conditions used.

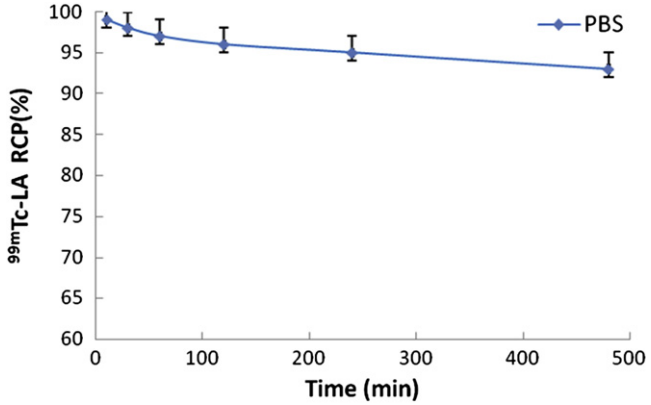


Fig. 5. In vitro PBS stability of ^{99m}Tc-lactic acid conjugate.

4. Conclusion

We have prepared molecularly defined LA-CL oligomers with terminal functional groups of hydroxyl and carboxylic acid. These oligomers were connected with chelating groups through a long chain tether, ready for transition metal binding. Coordination of organometallic rhenium(I) and technetium(I) complexes generated the conjugates in high yield and short time, satisfying the requirements for short-lived radiopharmaceuticals in clinical applications. These conjugates are stable in PBS at RT, ready for SPECT image or radio-therapy study. The availability of these well-defined oligomers of LA-CL radiochemical conjugates allows new insight into the drug distribution and controlled release in future studies.

Acknowledgment

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jorganchem.2012.06.001.

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