



## <sup>15</sup>N-D-labeled ionic probes for mass spectrometry

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### ARTICLE INFO

#### Article history:

Received 19 July 2011

Accepted 6 August 2011

Available online 16 August 2011

#### Keywords:

Cold-spray ionization mass spectrometry

Isotope shift

Bis(oxazolinyl)pyridine

### ABSTRACT

An effective <sup>15</sup>N- and deuterium (D)-labeled 2,6-bis(oxazolin-2-yl)pyridine (pybox)–La complex based probe ionization method that produces a distinct isotopic shift was developed. The distinct isotopic shift was detected by using the newly synthesized <sup>15</sup>N-D-labeled pybox complexes. Moreover, O-[3-(tetramethylpybox)-propyl]-hydroxylamine (oxime–TMpybox) was prepared for attachment to the carbonyl group of the target molecule. Distinct isotopic shifts and multiple charged ions were detected for various compounds having amino, thiol, carboxyl, and carbonyl groups and fullerenes, using the TMpybox ionic probe series in cold-spray ionization mass spectrometry.

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### 1. Introduction

Bis(oxazolinyl)pyridine tridentate ligands were first developed by Nishiyama et al. for enantioselective organometallic catalysis,<sup>1</sup> and have been used to generate chiral Lewis acid catalysts with transition metals and lanthanides. Recently, chiral mass spectrometry (MS) was reported to be a new approach for stereochemical analysis because of its speed and simplicity.<sup>2</sup> In particular, using an isotope-labeled pybox–metal complex, Arakawa et al. reported an excellent method for the determination of heterochirality, which uses electrospray ionization (ESI)-MS.<sup>3</sup> Isotope

labeling is highly useful for precise structure analysis as well as trace analysis in MS.<sup>4</sup>

Previously, we developed MS probes, including NHS–TMpybox **1**, Mal–TMpybox **2**, BrAc–TMpybox **3**, and Sar–TMpybox **4**. These probes can donate multiple charges contained in their respective metal charged sites to the target compound in order to analyze large complex molecules, including biomolecules,<sup>5</sup> and large carbon clusters, such as fullerenes,<sup>6</sup> in soft ionization conditions (Fig. 1). These ionic probes enable us to obtain <sup>15</sup>N-labeled compounds by complexation with <sup>15</sup>N-labeled pybox–La complex, as exemplified by (1+6), thereby allowing comparison of the isotopic

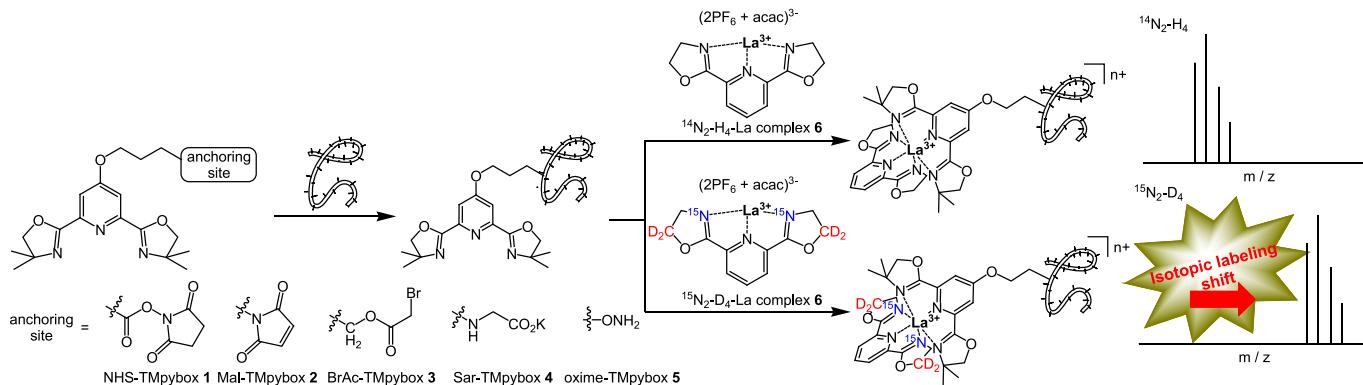


Fig. 1. Ionic probe attachment ionization mass spectrometry.

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shifts between labeled and non-labeled ionic probe attached biomolecules and fullerenes.<sup>6,7</sup> However, the isotopic shift induced by <sup>15</sup>N labeling was not distinct because the difference was only 0.7–1.3 u. Therefore, we prepared <sup>15</sup>N<sub>2</sub>-D<sub>4</sub>-labeled pybox–La

complex **6** ( $^{15}\text{N}_2\text{-D}_4\text{-6}$ ) in an attempt to observe the extended isotopic shift clearly. We also prepared a new MS probe, oxime-TMpybox **5**, which can react with the carbonyl group of the target molecule. Various carbonyl compounds are important for human physiology and a major concern in environmental chemistry. Testosterone, an important hormone, shows low ionization efficiency because of its steroid moiety.<sup>8</sup> In this paper, we describe the preparation of the  $^{15}\text{N}_2\text{-D}_4$ -labeled ionic probes, including oxime-TMpybox **5**, which can selectively react with the carbonyl group, for isotope-labeled MS in soft ionization conditions by using cold-spray ionization MS (CSI-MS).<sup>9</sup>

## 2. Results and discussion

Oxime-TMpybox **5** and  $^{15}\text{N}_2\text{-D}_4\text{-6}$  were prepared as follows (Scheme 1). Pybox **9** was obtained by alkylation of 4-hydroxypybox **7**,<sup>5</sup> which was prepared from chelidamic acid in six steps in 32% yield, with phthalimide **8**.<sup>10</sup> Treatment of **9** with hydrazine monohydrate gave oxime-TMpybox **5** in quantitative yield. After ethyl ester **10**, which was prepared from pyridine 2,6-dicarbonyl dichloride and glycine- $^{15}\text{N}$  ethyl ester in 77% yield,<sup>5</sup> was reduced with  $\text{NaBD}_4$  and  $\text{LiCl}$  to furnish diol **11** in 79% yield,<sup>3</sup> diol **11** containing two hydroxyl groups was converted into dichloride **12** in 76% yield. Finally,  $^{15}\text{N}_2\text{-D}_4\text{-6}$  was obtained by construction of an oxazoline ring and complexation with La. Synthesized probe **5** and  $^{15}\text{N}_2\text{-D}_4\text{-6}$  were then introduced to testosterone (TS) and cinnamaldehyde (CA), and CSI-MS measurement was conducted to confirm the specific probe attachment of **5** and the  $^{15}\text{N}$  and D isotope labeling.<sup>11</sup>

5-Bound molecules were prepared as follows (method E). After a pyridine solution of target carbonyl compound and **3** was stirred at 80 °C, the solution containing the derivative was poured into a MeCN solution of  $^{14}\text{N}_2\text{-H}_4$ - or  $^{15}\text{N}_2\text{-D}_4$ -**6**.

The CSI mass spectra of  $^{14}\text{N}_2\text{-H}_4$ -,  $^{15}\text{N}_2\text{-H}_4$ -, and  $^{15}\text{N}_2\text{-D}_4$ -carbonyl compounds are shown in Fig. 2. In the case of ( $^{14}\text{N}_2\text{-H}_4\text{-6+5}$ )- and ( $^{15}\text{N}_2\text{-H}_4\text{-6+5}$ )-bound CA, doubly charged ions of  $[\text{M+acac}^-]^{2+}$  ( $m/z$ : 465 and 466) were detected, respectively (Fig. 2a and b). Similarly, the ion of  $[\text{M+acac}^-]^{2+}$  ( $m/z$ : 468) was detected in ( $^{15}\text{N}_2\text{-D}_4\text{-6+5}$ )-bound CA (Fig. 2c). This isotope-labeled ion was partially exchanged from 'D' to 'H' ( $m/z$ : 467.4;  $^{15}\text{N}_2\text{-H}_2\text{-D}_2$  and  $m/z$ : 467.9;  $^{15}\text{N}_2\text{-H}_1\text{-D}_3$ ). In the case of ( $^{14}\text{N}_2\text{-H}_4\text{-6+5}$ )- and ( $^{15}\text{N}_2\text{-H}_4\text{-6+5}$ )-

bound TS, doubly charged ions of  $[\text{M+acac}^-]^{2+}$  ( $m/z$ : 543 and 544) were detected, respectively (Fig. 2d and e). Similar to CA, the isotope-labeled ion  $[\text{M+acac}^-]^{2+}$  ( $m/z$ : 546), together with the partially exchanged composition from 'D' to 'H' ( $m/z$ : 545.5,  $^{15}\text{N}_2\text{-H}_2\text{-D}_2$  and  $m/z$ : 546.0,  $^{15}\text{N}_2\text{-H}_1\text{-D}_3$ ), was detected. Compared with  $^{14}\text{N}_2\text{-H}_4$ - and  $^{15}\text{N}_2\text{-D}_4$ -bound carbonyl compounds, the isotopic shift of  $m/z$  ca. 3.0, corresponding to the increase in mass induced

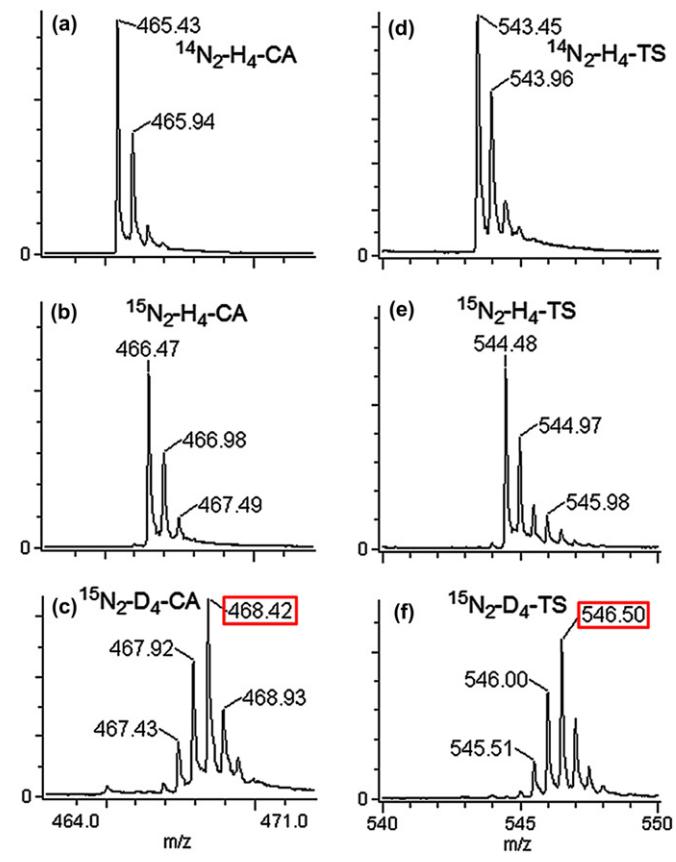
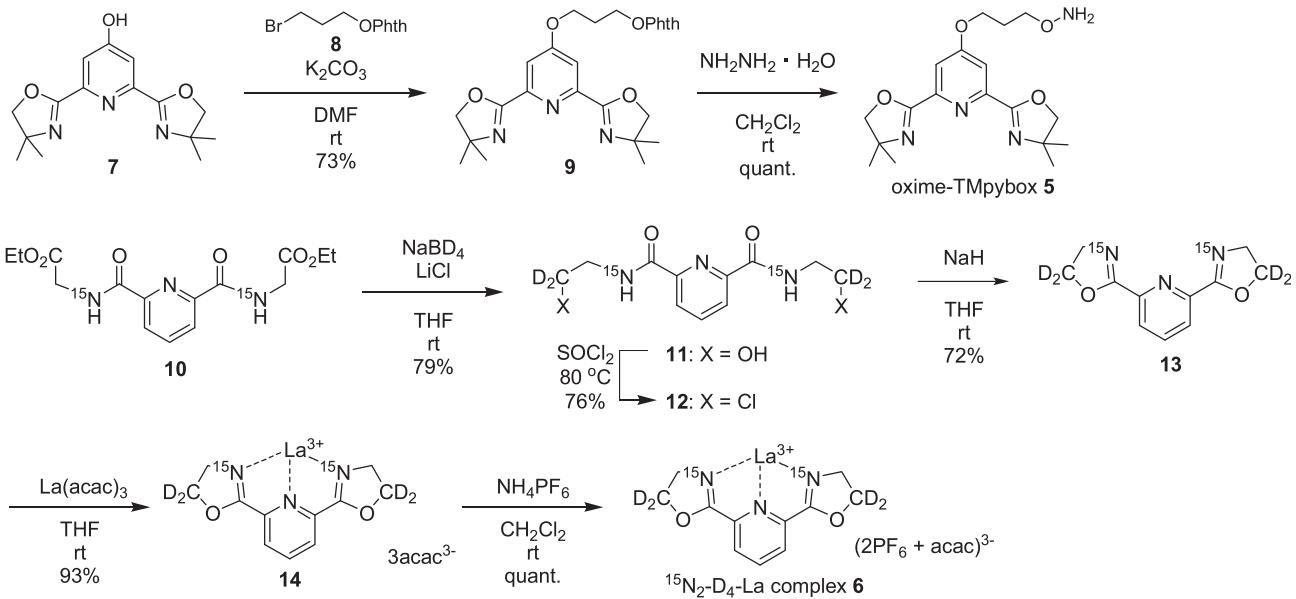


Fig. 2. CSI mass spectra of (a) ( $^{14}\text{N}_2\text{-H}_4\text{-6+5}$ )-CA, (b) ( $^{15}\text{N}_2\text{-H}_4\text{-6+5}$ )-CA, (c) ( $^{15}\text{N}_2\text{-D}_4\text{-6+5}$ )-CA, (d) ( $^{14}\text{N}_2\text{-H}_4\text{-6+5}$ )-TS, (e) ( $^{15}\text{N}_2\text{-H}_4\text{-6+5}$ )-TS, and (f) ( $^{15}\text{N}_2\text{-D}_4\text{-6+5}$ )-TS.



Scheme 1. Preparation of oxime-TMpybox **5** and  $^{15}\text{N}_2\text{-D}_4\text{-6}$ .

by two  $^{15}\text{N}$  and four D atoms on an ion with two charges, was detected. Compared with these mass spectra, extended isotopic shifts were observed in  $^{15}\text{N}_2\text{-D}_4\text{-6}$ .

In order to induce multiple charged ions and observe the isotopic shift by  $^{15}\text{N}$  and D labeling, we applied this method to various compounds [lysine (Lys), arginine, (Arg), cysteine (Cys), bradykinin (BK), pepstatin A (PA), and fullerene ( $\text{C}_{60}$ )].

The CSI mass spectra of  $^{14}\text{N}_2\text{-H}_4\text{-}$  and  $^{15}\text{N}_2\text{-D}_4\text{-}$  bound amino acids (Lys, Arg, and Cys) are shown in Fig. 3.  $^{15}\text{N}_2\text{-D}_4\text{-}$  amino acids, which were prepared by method B<sup>5</sup> using NHS-TMpybox 1 and Mal-TMpybox 2, exhibited doubly charged ions  $[\text{M}+\text{acac}^-]^{2+}$  ( $m/z$ : 482, 496, and 504) (Fig. 3). The isotopic shift of  $m/z$  ca. 3.0, which corresponded to the increase in mass induced by two  $^{15}\text{N}$  and four D atoms on an ion with two charges, was detected. Next, the CSI mass spectra of  $^{14}\text{N}_2\text{-H}_4\text{-}$  and  $^{15}\text{N}_2\text{-D}_4\text{-}$  bound BK and those of  $^{14}\text{N}_2\text{-H}_4\text{-}$

(5+6). Moreover, we prepared useful ionic probes that can react with major functionalities in biomolecules, such as amino, carboxyl, carbonyl, and thiol groups. The biomolecules to which these  $^{15}\text{N}_2\text{-D}_4\text{-}$  labeled ionic probes are attached were easily ionized in soft conditions and exhibited distinct isotopic shifts. We believe that this method is useful for proteome analysis and isotope trace experiments as well as the structure determination of large complex molecules by deducing the mass-to-charge ratio in MS.

#### 4. Experimental section

##### 4.1. General

All melting points were measured on a Yanaco MP-500D and are uncorrected. Infrared spectra were recorded on a JASCO FT/IR 6300

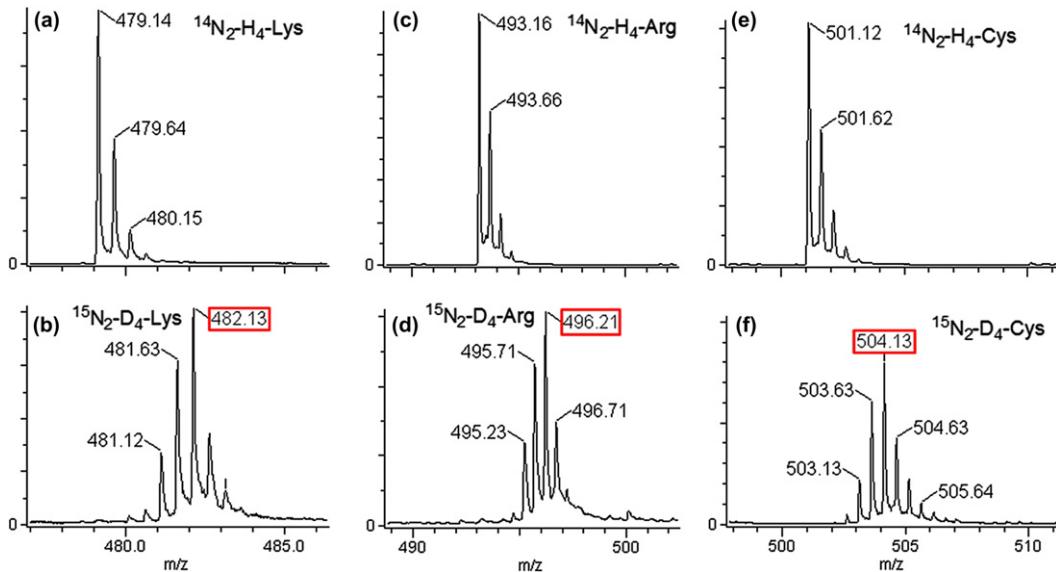


Fig. 3. CSI mass spectra of (a) ( $^{14}\text{N}_2\text{-H}_4\text{-6+1}$ )-Lys, (b) ( $^{15}\text{N}_2\text{-D}_4\text{-6+1}$ )-Lys, (c) ( $^{14}\text{N}_2\text{-H}_4\text{-6+1}$ )-Arg, (d) ( $^{15}\text{N}_2\text{-D}_4\text{-6+1}$ )-Arg, (e) ( $^{14}\text{N}_2\text{-H}_4\text{-6+2}$ )-Cys, (f) ( $^{15}\text{N}_2\text{-D}_4\text{-6+2}$ )-Cys.

$\text{H}_4\text{-}$  and  $^{15}\text{N}_2\text{-D}_4\text{-}$  bound PA are shown in Fig. 4. In the case of  $^{14}\text{N}_2\text{-H}_4\text{-}$  and  $^{15}\text{N}_2\text{-D}_4\text{-}$  PA, which were prepared by method C<sup>7</sup> using BrAc-TMpybox 3, doubly charged ions of  $[\text{M}+\text{acac}^-]^{2+}$  ( $m/z$ : 771 and 774) (Fig. 4a and b, respectively) were observed, respectively, in the CSI mass spectra. For the same reason, the isotopic shift of  $m/z$  ca. 3.0 was detected. In the case of  $^{14}\text{N}_2\text{-H}_4\text{-}$  and  $^{15}\text{N}_2\text{-D}_4\text{-}$  BK, which were prepared by method B<sup>5</sup> using NHS-TMpybox 1, triply charged ions of  $[\text{M}+\text{acetylacetone}]^{3+}$  ( $m/z$ : 624 and 626) (Fig. 4c and d, respectively) were observed, respectively, in the CSI mass spectra. The isotopic shift of  $m/z$  ca. 2.0, which corresponded to the increase in mass induced by two  $^{15}\text{N}$  and four D atoms on an ion with three charges, was detected. Finally, the CSI mass spectra of  $^{14}\text{N}_2\text{-H}_4\text{-}$  and  $^{15}\text{N}_2\text{-D}_4\text{-C}_{60}$ , which were prepared by method D<sup>6</sup> using Sar-TMpybox 4, are shown in Fig. 5. Using  $^{14}\text{N}_2\text{-H}_4\text{-6}$ , multiple charged ions  $[(^{14}\text{N}_2\text{-H}_4\text{-6+4})\text{-C}_{60}+\text{acac}^-]^{2+}$  ( $m/z$ : 812) and  $[(2^{14}\text{N}_2\text{-H}_4\text{-6+4})\text{-C}_{60}+2\text{acac}^-]^{4+}$  ( $m/z$ : 632) were detected (Fig. 5a). In the case of  $^{15}\text{N}_2\text{-D}_4\text{-6}$ ,  $^{15}\text{N}_2\text{-D}_4$ -labeled probes attached to  $\text{C}_{60}$  were detected as multiple charged ions  $[(^{15}\text{N}_2\text{-D}_4\text{-6+4})\text{-C}_{60}+\text{acac}^-]^{2+}$  ( $m/z$ : 815) and  $[(2^{15}\text{N}_2\text{-D}_4\text{-6+4})\text{-C}_{60}+2\text{acac}^-]^{4+}$  ( $m/z$ : 635) as observed in the non-labeled ones with a definite isotopic shift of  $m/z$  ca. 3.0 (Fig. 5b).<sup>12</sup>

#### 3. Conclusions

We succeeded in synthesizing  $^{15}\text{N}_2\text{-D}_4$ -labeled pybox-La complex 6 and oxime-TMpybox 5 to obtain five ionic probes, isotope-labeled metal complexes (1+6), (2+6), (3+6), (4+6), and

spectrophotometer with an ATR (attenuated total reflectance) system. Unless otherwise stated,  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ , and  $^{31}\text{P}$  NMR spectra were recorded in  $\text{CDCl}_3$  on a JEOL JNM-ECP 400 and a Bruker 400 MHz with tetramethylsilane (TMS) as internal reference. CSI

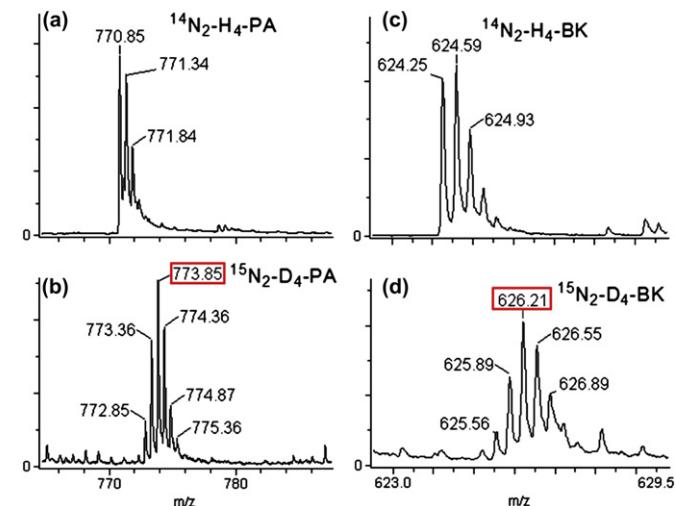


Fig. 4. CSI mass spectra of (a) ( $^{14}\text{N}_2\text{-H}_4\text{-6+3}$ )-PA, (b) ( $^{15}\text{N}_2\text{-D}_4\text{-6+3}$ )-PA, (c) ( $^{14}\text{N}_2\text{-H}_4\text{-6+1}$ )-BK, (d) ( $^{15}\text{N}_2\text{-D}_4\text{-6+1}$ )-BK.

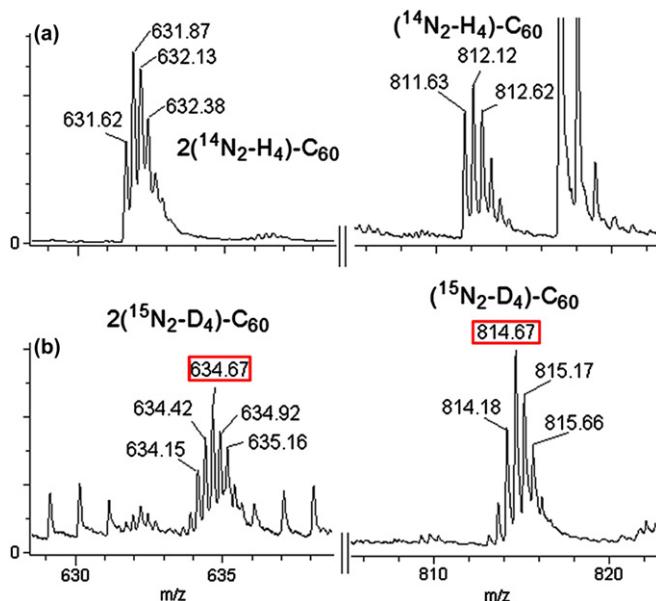


Fig. 5. CSI mass spectra of (a)  $(^{14}\text{N}_2\text{-H}_4\text{-6+4})\text{-C}_60$  and (b)  $(^{15}\text{N}_2\text{-D}_4\text{-6+4})\text{-C}_60$ .

mass spectra were recorded on a JEOL JMS-T100LC mass spectrometer equipped with a cold-spray ion source. Silica gel 60 F<sub>254</sub> TLC plates (Merck No. 5715) and NH plates (Fuji Silysia Chemical, Ltd., No. TO80817) were used for TLC. For column chromatography, silica gel 60 (spherical, particle size 63–210  $\mu\text{m}$ , Kanto Chemical, No. 37565-84 for neutral) and NH silica gel (Fuji Silysia Chemical Ltd., particle size 200–350 mesh, No. HU80502) were used. CombiFlash Companion (Teledyne Isco) was used for reversed-phase flash column chromatography. Eyela FDU-1200 was used as the vacuum freeze dryer. TS, BK, and all anhydrous solvents were purchased from Wako Pure Chemical. DMSO was purchased from Merck. NaBD<sub>4</sub> (90%, 98 atm % D) was purchased from Aldrich. Fullerene C<sub>60</sub> was purchased from TCI.

**4.1.1. Synthesis of *N*-3-bromopropoxy-phthalimide (8).** Under Ar, a solution of *N*-hydroxyphthalimide (2.01 g, 12.3 mmol), 1,3-dibromopropane (2.5 mL, 24.5 mmol), and NEt<sub>3</sub> (3.3 mL, 23.7 mmol) in dry DMF (30 mL) was stirred at room temperature for 18 h. The reaction was quenched with H<sub>2</sub>O and extracted with ethyl acetate. The organic layer was washed with H<sub>2</sub>O and brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated in vacuo. The residue was purified by SiO<sub>2</sub> column chromatography (*n*-hexane/ethyl acetate=5:1) to give **8** (2.30 g, 66%) as a colorless solid. Mp 71–72 °C. IR (ATR, cm<sup>-1</sup>): 1725. <sup>1</sup>H NMR (400 MHz)  $\delta$ : 2.32 (2H, q, *J*=6.0 Hz), 3.72 (2H, t, *J*=6.0 Hz), 4.37 (2H, t, *J*=6.0 Hz), 7.75–7.79 (2H, m), 7.83–7.88 (2H, m). <sup>13</sup>C NMR (100 MHz)  $\delta$ : 29.3, 31.5, 76.1, 123.6, 128.9, 134.6, 163.6. HRESIMS *m/z* 305.9704 (calcd for C<sub>11</sub>H<sub>10</sub><sup>79</sup>BrNNaO<sub>3</sub>: 305.9742) and 307.9685 (calcd for C<sub>11</sub>H<sub>10</sub><sup>81</sup>BrNNaO<sub>3</sub>: 307.9721). Anal. Calcd for C<sub>11</sub>H<sub>10</sub>BrNO<sub>3</sub>: C, 46.50; H, 3.55; N, 4.93. Found: C, 46.59; H, 3.28; N, 4.96.

**4.1.2. Synthesis of 2,6-bis(4,4-dimethyloxazolin-2-yl)-4-(3-phthalimidooxy-propoxy)pyridine (9).** Under Ar, a mixture of 4-hydroxypyridine **7** (1.44 g, 4.98 mmol), phthalimide **8** (2.24 g, 7.90 mmol), and K<sub>2</sub>CO<sub>3</sub> (2.21 g, 16.0 mmol) in dry DMF (20 mL) was stirred at room temperature for 15 h. The reaction was quenched with H<sub>2</sub>O and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was washed with H<sub>2</sub>O and brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated in vacuo. The residue was washed with a mixed solvent of *n*-hexane/ethyl acetate=1:1 to give **9** (1.78 g, 73%) as a colorless solid. Mp 191–192 °C. IR (ATR, cm<sup>-1</sup>): 1725, 1641. <sup>1</sup>H NMR (400 MHz)  $\delta$ : 1.39

(12H, s), 2.27 (2H, t, *J*=6.0 Hz), 4.20 (4H, s), 4.39 (2H, t, *J*=6.0 Hz), 4.41 (2H, t, *J*=6.0 Hz), 7.72 (2H, s), 7.75–7.78 (2H, m), 7.82–7.85 (2H, m). <sup>13</sup>C NMR (100 MHz)  $\delta$ : 28.0, 28.4, 64.7, 67.9, 74.6, 79.7, 112.2, 123.6, 128.9, 134.6, 148.5, 160.9, 163.6, 165.7. HRESIMS *m/z* 515.1909 (calcd for C<sub>26</sub>H<sub>28</sub>N<sub>4</sub>NaO<sub>6</sub>: 515.1907). Anal. Calcd for C<sub>26</sub>H<sub>28</sub>N<sub>4</sub>O<sub>6</sub>: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.21; H, 5.62; N, 11.38.

**4.1.3. Synthesis of O-[3-{2,6-bis(4,4-dimethyloxazolin-2-yl)-pyridin-4-yloxy}-propyl]-hydroxylamine (5).** Hydrazine monohydrate (0.40 mL, 2.76 mmol) was added to a solution of **9** (300 mg, 0.83 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and the whole mixture was stirred at room temperature for 30 min and then filtered through a Celite pad. The filtrate was concentrated in vacuo to give **5** (482 mg, 72%) as a yellowish powder. This product was used without further purification. Mp 58–63 °C. IR (ATR, cm<sup>-1</sup>): 3371, 1643. <sup>1</sup>H NMR (400 MHz)  $\delta$ : 1.38 (12H, s), 2.08 (2H, q, *J*=6.0 Hz), 3.80 (2H, t, *J*=6.0 Hz), 4.19 (4H, s), 4.20 (2H, t, *J*=6.0 Hz), 5.43 (2H, br s), 7.67 (2H, s). <sup>13</sup>C NMR (100 MHz)  $\delta$ : 28.0, 28.4, 65.6, 67.9, 71.7, 79.7, 112.1, 148.4, 161.0, 165.9. HRESIMS *m/z* 385.1867 (calcd for C<sub>18</sub>H<sub>26</sub>N<sub>4</sub>NaO<sub>4</sub>: 385.1852).

**4.1.4. Synthesis of <sup>15</sup>N<sup>2</sup><sup>15</sup>N<sup>6</sup>-bis(1,1-dideutero-2-hydroxyethyl)-2,6-pyridine-dicarboxamide (11).** Under Ar, to a dry THF (30 mL) solution of **10** (1.08 g, 3.17 mmol) and LiCl (1.00 g, 23.6 mmol) was added NaBD<sub>4</sub> (846 mg, 20.2 mmol) at room temperature. After the whole mixture was stirred at room temperature for 12 h, MeOH and acetone were added at 0 °C and the whole mixture was evaporated in vacuo. The residue was purified by reversed-phase flash column chromatography (H<sub>2</sub>O/MeCN=100:0 to 50:50) to obtain colorless powder **11** (647 mg, 79%). Mp 164–168 °C. IR (ATR, cm<sup>-1</sup>): 3386, 3274, 1657, 1519. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$ : 3.58 (4H, s), 8.14 (1H, t, *J*=8.2 Hz), 8.27 (2H, d, *J*=8.2 Hz). <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD)  $\delta$ : 41.6 (d, *J*<sub>CN</sub>=10 Hz), 59.9 (t, *J*<sub>CD</sub>=22 Hz), 124.3, 139.0, 148.7 (d, *J*<sub>CN</sub>=7 Hz), 164.8 (d, *J*<sub>CN</sub>=18 Hz). <sup>15</sup>N NMR (40 MHz, CD<sub>3</sub>OD)  $\delta$ : 102.0. HRCSIMS *m/z* 282.1110 (calcd for C<sub>11</sub>H<sub>11</sub>H<sub>4</sub><sup>14</sup>N<sub>2</sub>NaO<sub>4</sub>: 282.1152).

**4.1.5. Synthesis of <sup>15</sup>N<sup>2</sup><sup>15</sup>N<sup>6</sup>-bis(1,1-dideutero-2-chloroethyl)-2,6-pyridine-dicarboxamide (12).** Under Ar, a SOCl<sub>2</sub> (8 mL) solution of alcohol **11** (613 mg, 2.36 mmol) was stirred under reflux for 1 h. After cooling to room temperature, SOCl<sub>2</sub> was azeotroped with toluene. The residue was diluted with CH<sub>2</sub>Cl<sub>2</sub> and the organic layer was washed with H<sub>2</sub>O and brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated in vacuo. The residue was purified by column chromatography (*n*-hexane/ethyl acetate=1:2) to obtain **12** (408 mg, 76%) as a colorless powder. Mp 145–147 °C. IR (ATR, cm<sup>-1</sup>): 3344, 3303, 1650, 1507. <sup>1</sup>H NMR (400 MHz)  $\delta$ : 3.87 (4H, d, *J*=6.0 Hz), 8.07 (1H, t, *J*=8.0 Hz), 8.09 (1H, t, *J*=6.0 Hz), 8.32 (1H, t, *J*=6.0 Hz), 8.38 (1H, d, *J*=8.0 Hz). <sup>13</sup>C NMR (100 MHz)  $\delta$ : 41.1 (d, *J*<sub>CN</sub>=11 Hz), 43.6 (t, *J*<sub>CD</sub>=21 Hz), 125.3, 139.2, 148.5 (d, *J*<sub>CN</sub>=8.0 Hz), 163.6 (d, *J*<sub>CN</sub>=17 Hz). <sup>15</sup>N NMR (40 MHz)  $\delta$ : 97.6. HRCSIMS *m/z* 318.0429 (calcd for C<sub>11</sub>H<sub>9</sub>H<sub>4</sub><sup>23</sup>Cl<sub>2</sub><sup>34</sup><sup>35</sup>Cl<sub>2</sub><sup>14</sup>N<sub>2</sub>NaO<sub>2</sub>: 318.0474), 320.0407 (calcd for C<sub>11</sub>H<sub>9</sub>H<sub>4</sub><sup>23</sup>Cl<sub>2</sub><sup>37</sup>Cl<sub>2</sub><sup>14</sup>N<sub>2</sub>NaO<sub>2</sub>: 320.0445), 322.0381 (calcd for C<sub>11</sub>H<sub>9</sub>H<sub>4</sub><sup>23</sup>Cl<sub>2</sub><sup>37</sup>Cl<sub>2</sub><sup>14</sup>N<sub>2</sub>NaO<sub>2</sub>: 322.0415).

**4.1.6. Synthesis of 2,6-bis(5,5-dideutero-oxazolin-2-yl)-<sup>15</sup>N<sub>2</sub>-pyridine (13).** Under Ar, to a dry THF (5 mL) solution of dichloride **12** (507 mg, 1.71 mmol) was added NaH (60%, 217 mg, 5.41 mmol) at room temperature. After the whole mixture was stirred at room temperature for 1 h, the mixture was filtered. After the filtrate was evaporated in vacuo, the residue was purified by NH column chromatography (*n*-hexane/acetone=1:1) to obtain **13** (274 g, 72%) as a brownish powder. Mp 234–236 °C. IR (ATR, cm<sup>-1</sup>): 1647, 1623, 1567. <sup>1</sup>H NMR (400 MHz)  $\delta$ : 4.12 (4H, s), 7.89 (1H, t, *J*=8.0 Hz), 8.17 (2H, d, *J*=8.0 Hz). <sup>13</sup>C NMR (100 MHz)  $\delta$ : 54.9 (d, *J*<sub>CN</sub>=4 Hz), 68.0 (t, *J*<sub>CD</sub>=22 Hz), 125.5 (d, *J*<sub>CN</sub>=1 Hz), 137.4, 146.7 (d, *J*<sub>CN</sub>=8 Hz), 163.5. <sup>15</sup>N

NMR (40 MHz)  $\delta$ : 221.0. HRCSIMS  $m/z$  246.0904 (calcd for  $C_{11}H_7H_4^{14}N^{15}N_2NaO_2$ ; 246.0941).

**4.1.7. Synthesis of tris(acetylacetonato)[2,6-bis(5,5-dideutero-oxazolin-2-yl)- $^{15}N_2$ -pyridine]lanthanum (III) (14).** Under Ar, after a mixture of **13** (103 mg, 0.46 mmol) and La(acac)<sub>3</sub> (353 mg, 0.81 mmol) in THF (5 mL) was stirred at room temperature for 24 h, it was filtered to give **14** (282 mg, 93%) as a colorless powder. This product was used without further purification. Mp 223–225 °C. IR (ATR, cm<sup>-1</sup>): 1645, 1591, 1575, 1509. <sup>1</sup>H NMR (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>)  $\delta$ : 1.66 (18H, s), 4.32 (4H, s), 5.15 (3H, s), 7.89 (2H, d,  $J$ =8.0 Hz), 8.00 (1H, t,  $J$ =8.0 Hz). <sup>13</sup>C NMR (100 MHz, CD<sub>2</sub>Cl<sub>2</sub>)  $\delta$ : 27.5, 53.7, 69.9, 99.7, 124.9, 139.3, 145.1, 163.7, 187.8. <sup>15</sup>N NMR (40 MHz, CD<sub>2</sub>Cl<sub>2</sub>)  $\delta$ : 224.4. HRCSIMS  $m/z$  560.0973 (calcd for  $C_{26}H_{28}H_4^{139}La^{14}N^{15}N_2O_8$ –acac; 560.0999).

**4.1.8. Synthesis of acetylacetonato(dihexafluorophosphine)[2,6-bis(5,5-dideutero-oxazolin-2-yl)- $^{15}N_2$ -pyridine]lanthanum (III) (6).** Under Ar, a suspension of **14** (245 mg, 0.34 mmol) and NH<sub>4</sub>PF<sub>6</sub> (376 mg, 2.31 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was stirred at room temperature for 24 h. The reaction mixture was filtered to give  $^{15}N_2$ –D<sub>4</sub>–**6** (435.7 mg, quant.) as a colorless powder. This product was used without further purification. Mp >300 °C. IR (ATR, cm<sup>-1</sup>): 1644, 1575, 1508, 1409. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 1.77 (6H, s), 3.97 (4H, s), 5.35 (1H, s), 8.08 (3H, s). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 27.9, 54.6, 68.7, 94.8, 126.4, 140.0, 145.6, 162.8, 188.8. <sup>31</sup>P{<sup>1</sup>H} NMR (162 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : -143.6 (heptet,  $J$ =711 Hz). <sup>15</sup>N NMR (40 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 224.2. HRCSIMS  $m/z$  342.0766 (calcd for  $C_{16}H_{18}F_{12}^{139}LaN^{15}N_2O_4P_2$ +**13** ( $C_{11}H_7H_4^{14}N^{15}N_2O_2$ )–2PF<sub>6</sub>; 342.0798).

## Acknowledgements

This work was supported by JST and a Grant-in-Aid for Scientific Research on Innovation Areas 'Emergence in Chemistry' (20111007) from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT).

## Supplementary data

<sup>1</sup>H and <sup>13</sup>C NMR spectra of **5**, **8**, **9**, **11**, **12**, **13**, **14** and mass spectrometry of  $[3(^{15}N_2\text{-D}_4\text{-}6\text{+}5)\text{+}C_{60}\text{+}3\text{acac}^-]^{6+}$ ,  $[3(^{14}N_2\text{-H}_4\text{-}6\text{+}5)\text{+}C_{60}\text{+}3\text{acac}^-]^{6+}$ . Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2011.08.013.

## References and notes

- (a) Nishiyama, H.; Kondo, M.; Nakamura, T.; Itoh, K. *Organometallics* **1991**, *10*, 500–508; (b) Motoyama, Y.; Kurihara, O.; Murata, K.; Aoki, K.; Nishiyama, H. *Organometallics* **2000**, *19*, 1025–1034.
- (a) Speranza, M. *Int. J. Mass Spectrom.* **2004**, *232*, 277–317; (b) Filippi, A.; Giardini, A.; Piccirillo, S.; Speranza, M. *Int. J. Mass Spectrom.* **2000**, *198*, 137–163; (c) Sawada, M. *Mass Spectrom. Rev.* **1997**, *16*, 73–90; (d) Tao, W. A.; Cooks, R. G. *Anal. Chem.* **2003**, *75*, 25A–31A.
- Sato, H.; Suzuki, Y.; Takai, Y.; Kawasaki, H.; Arakawa, R.; Shizuma, M. *Chem. Lett.* **2010**, *39*, 564–566.
- (a) Sawada, M.; Takai, Y.; Yamada, H.; Yamaoka, H.; Azuma, T.; Fujioka, T.; Kawai, Y.; Tanaka, T. *Chem. Commun.* **1998**, 1569–1570; (b) Shizuma, M.; Imamura, H.; Takai, Y.; Yamada, H.; Takeda, T.; Takahashi, S.; Sawada, M. *Chem. Lett.* **2000**, 1292–1293; (c) Shizuma, M.; Kadoya, Y.; Takai, Y.; Yamada, H.; Takeda, T.; Arakawa, R.; Takahashi, S.; Sawada, M. *J. Org. Chem.* **2002**, *67*, 4795–4807; (d) Sawada, M.; Takai, Y.; Yamada, H.; Hirayama, S.; Kaneda, T.; Tanaka, T.; Kamada, K.; Mizooku, T.; Takeuchi, S.; Ueno, K.; Hirose, K.; Tobe, Y.; Naemura, K. *J. Am. Chem. Soc.* **1995**, *117*, 7726–7736.
- (a) Ito, F.; Nakamura, T.; Yorita, S.; Danjo, H.; Yamaguchi, K. *Tetrahedron Lett.* **2009**, *50*, 6252–6255; (b) Ito, F.; Yamaguchi, K. *Heterocycles* **2010**, *81*, 895–990.
- Ito, F.; Yamaguchi, K. *Org. Biomol. Chem.* **2011**, *9*, 2674–2679.
- Ito, F.; Ando, S.; Iuchi, M.; Nakamura, T.; Yorita, S.; Yamaguchi, K. *Org. Biomol. Chem.* **2010**, *8*, 4408–4413.
- Suzuki, Y.; Taniji, N.; Ikeda, C.; Honda, A.; Ookubo, K.; Citterio, D.; Suzuki, K. *Anal. Sci.* **2004**, *20*, 475–482.
- CSI condition is suited for the analysis of biomolecules and metal complexes; (a) Sakamoto, S.; Fujita, M.; Kim, K.; Yamaguchi, K. *Tetrahedron* **2000**, *56*, 955–964; (b) Yamaguchi, K. *J. Mass Spectrom.* **2003**, *38*, 473–490.
- Rathore, K.; Vyas, R.; Talesara, G. L. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **2005**, *44B*, 2166–2170.
- CSI mass spectra were recorded on a JEOL JMS-T100LC time of flight mass spectrometer equipped with a cold-spray ion source. This setup makes it possible to conduct measurements at low temperatures. Measurement conditions were as follows: sprayer temperature: -20 to 20 °C; needle voltage: +1500 to 2500 V; ring lens voltage: +5 to 10 V; orifice 1 voltage: +20 to 50 V; orifice 2 voltage: +5 to 10 V; concn ca. 0.5 mM.
- Although  $[3(^{15}N_2\text{-D}_4\text{-}6\text{+}5)\text{+}C_{60}\text{+}3\text{acac}^-]^{6+}$  ( $m/z$  575) was detected, the ion intensity was as small as a noise (see Supplementary data).