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PAPER

^{15}N-labeled ionic probe attachment mass spectrometry of carbon clusters†

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An ionization method that uses metal-complex-based ionization probes, malonic acid 3-[2,6-bis(4,4-dimethyloxazolin-2-yl)pyridin-4-yloxy]propyl ethyl ester (EM-TMpybox) and potassium *N*-{3-[2,6-bis(4,4-dimethyloxazolin-2-yl)pyridine-4-yloxy]propyl} aminoacetate (Sar-TMpybox), was developed for isotope ratio analysis and the effective ionization of unsubstituted carbon clusters. The preparation of Sar-TMpybox and EM-TMpybox and their applications in cold-spray ionization mass spectrometry are reported. A probe applicable to a substituted fullerene is also demonstrated.

Introduction

Mass spectrometry (MS) is an essential tool for characterizing various compounds. Not only is MS used for the analysis of molecular weights, it is also one of the most sensitive detection methods for biological, chemical, pharmaceutical, and environmental substances. With regard to recent advances in ionization technology for MS, soft ionization, such as electrospray ionization (ESI),[1] matrix-assisted laser desorption/ionization (MALDI),[2] and chemically modified ionization using ammonium salts, the so-called "MS probe" or "tag" has been investigated. The MS probe is basically used to increase the relative abundance of ions formed from a compound[3] and for isotope labeling[4] in the analysis of higher-order structures of biomolecules. Previously, we have reported MS probes that can donate plural charges contained in their metal charged sites to the target compound in order to analyze biomolecules. Furthermore, the isotopic shift was detected easily by the probes using ^{14}N- and ^{15}N-pybox–La complexes **3** (^{14}N–**3** and ^{15}N–**3**).[5] Recently, the synthesis and analysis of complex fullerene derivatives have been widely conducted.[6] MS measurement is often troublesome because large and stable carbon clusters are usually difficult to ionize by soft ionization techniques. As regards previous observations of the ionization of carbon clusters by MS, limited reports, such as the electron ionization (EI) MS of a C_{60}/C_{70} mixture by Luffer and Schram[7] and the high-energy collision-induced dissociation (CID) of singly and multiply charged C_{60} and C_{70} by Young *et al.*[8] and Doyle and Ross,[9] are known. Therefore, we planned to analyze carbon clusters with our ionic probe, which would make it possible to adopt soft ionization in order to establish facile and effective MS measurement in carbon cluster chemistry. By comparing the isotopic shifts of ^{15}N-labeled and non-labeled fullerenes, the precise elucidation of the complex structure will be realized. This is the first example of the observation of ^{15}N labeling isotopic shift and ionization using an MS probe for carbon clusters. Our probe is comprised of three functional parts: a charged site, a linker, and an anchoring site (Fig. 1), as reported previously.[10] We attempted to modify fullerenes by means of the Prato reaction[11] and the Bingel–Hirsch reaction.[12] In the Prato reaction, an unstable azomethine ylide obtained from sarcosine and aldehyde reacts with the carbon clusters. In the Bingel–Hirsch reaction, the enolate of diethyl bromomalonate reacts with the carbon clusters. Therefore, we designed two new probes that have diethyl malonate (EM-TMpybox **1**) and sarcosinate (Sar-TMpybox **2**) as anchoring sites (Fig. 1). In this paper, we describe the synthesis of the new probes and their application to carbon clusters and the analysis of their isotopic shifts using ^{14}N– and ^{15}N–**3** in soft ionization conditions by means of cold-spray ionization mass spectrometry (CSI-MS).[13]

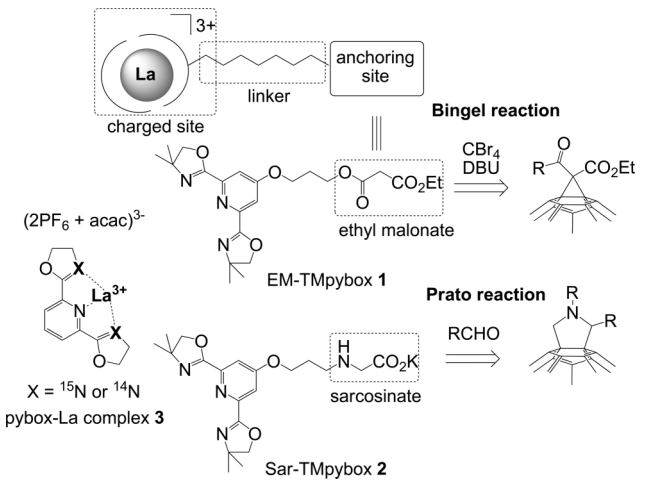

Fig. 1 The construction of the ionic probe.

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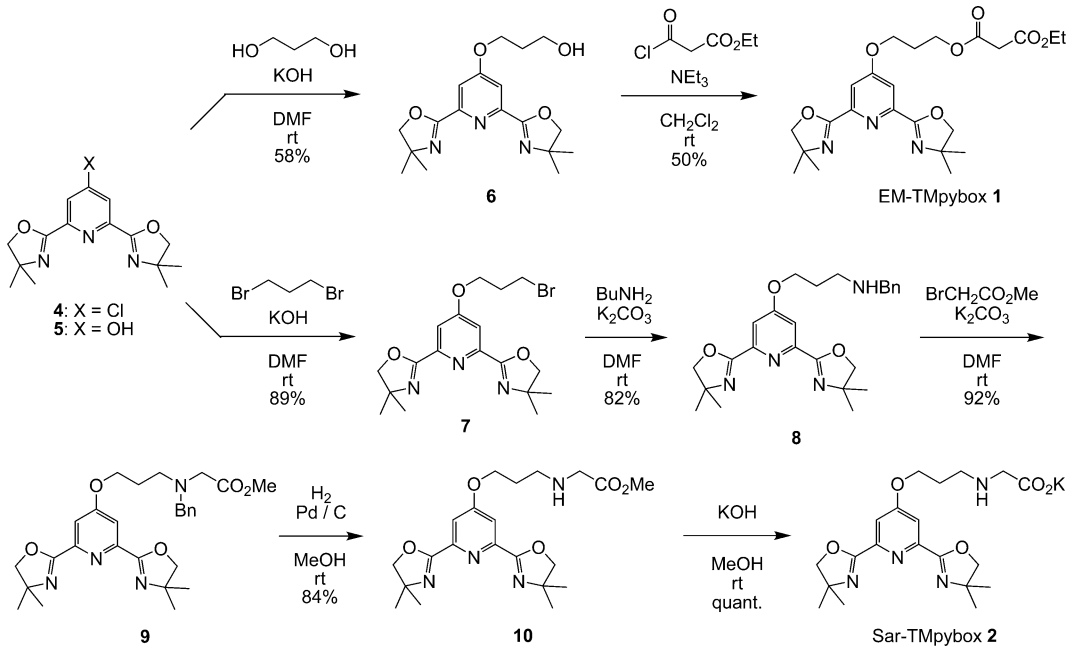
† Electronic supplementary information (ESI) available: Multiply charged ionization of fullerenes C_{60} and C_{70} using Sar-TMpybox **2**, CSI mass spectra of fullerenes having some probes **2** and ^{1}H and ^{13}C NMR spectra of **1**, **2**, **6**–**10**, and **12**. See DOI: 10.1039/c0ob00887g



Scheme 1 The synthesis of EM-TMpybox **1** and Sar-TMpybox **2**.

Results and discussion

MS probes **1** and **2** were synthesized as follows. EM-TMpybox was prepared by nucleophilic aromatic substitution of 4-Cl-pybox **4** with 1,3-propanediol and conversion of the hydroxyl group on **6** into ethyl malonate with ethyl 3-chloro-3-oxo-propionate. Alkylation of 4-hydroxypybox **5** with 1,3-dibromopropane afforded the bromide **7** in 89% yield. Amine **9** was obtained by conversion of the bromide into benzylamine **8** with $BnNH_2$, alkylation into **9**, and removal of the benzyl group. Finally, the desired Sar-TMpybox **2** was obtained by hydrolysis of methyl ester **10** (Scheme 1). At first, EM-TMpybox **1** was attached to fullerene C_{60} in the presence of DBU and CBr_4 at room temperature. Probe **1** was reacted with C_{60} and singly charged ions of C_{60} having 1–4 probes **1** attached were detected (Fig. 2). However, the excess amount of DBU prevented the formation of the La complex (**3** + **1**) with the desired charges and it was difficult to remove the DBU salt. Next, Sar-TMpybox **2** was attached to fullerenes C_{60} and C_{70}. To promote the reaction of the probe with the fullerenes, a suspension of probe **1**, benzaldehyde,[14] and fullerene in toluene was refluxed for 24 h. After the reaction mixture was filtered, toluene was removed *in vacuo*. The obtained residue was washed with Et_2O. The mixture of fullerene derivative and ^{14}N– or ^{15}N–**3** was stirred in MeCN. This solution diluted with MeCN was then applied to CSI-MS measurement.[15] Using the excess probe **2** in the modification reaction, all of the fullerenes should react.

Probe **2** was reacted with C_{60} and singly charged ions of C_{60} having 1–6 probes **2** attached were detected (see ESI†).[16] Multiply charged ions $[C_{60} + (^{14}N–\mathbf{3} + \mathbf{2}) + acac^-]^{2+}$ (m/z 812), $[C_{60} + 2(^{14}N–\mathbf{3} + \mathbf{2}) + 2acac^-]^{4+}$ (m/z 632), and $[C_{60} + 3(^{14}N–\mathbf{3} + \mathbf{2}) + 3acac^-]^{6+}$ (m/z 572) were detected by the formation of charged sites coupled with ^{14}N–**3** including La^{3+} (Fig. 3a). One of the three ions at the charged site was cancelled out by an $acac^-$. In the case of ^{15}N–**3**, C_{60}, to which the ^{15}N-labeled probes were attached, this was detected as multiply charged ions $[C_{60} + (^{15}N–\mathbf{3} + \mathbf{2}) + acac^-]^{2+}$ (m/z 813), $[C_{60} + 2(^{15}N–\mathbf{3} + \mathbf{2}) + 2acac^-]^{4+}$ (m/z 633), and $[C_{60} + 3(^{14}N–\mathbf{3} + \mathbf{2}) + 3acac^-]^{6+}$ (m/z 573), as observed in the non-labeled ones with a definite isotopic shift of m/z 1 (Fig. 3b). Compared with the other

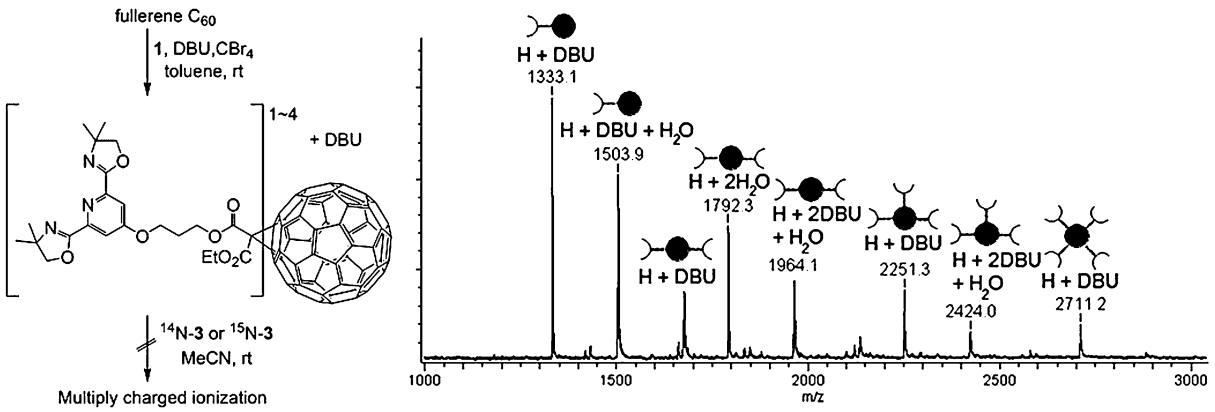


Fig. 2 The CSI mass spectrum of **1** binding fullerene C_{60}.

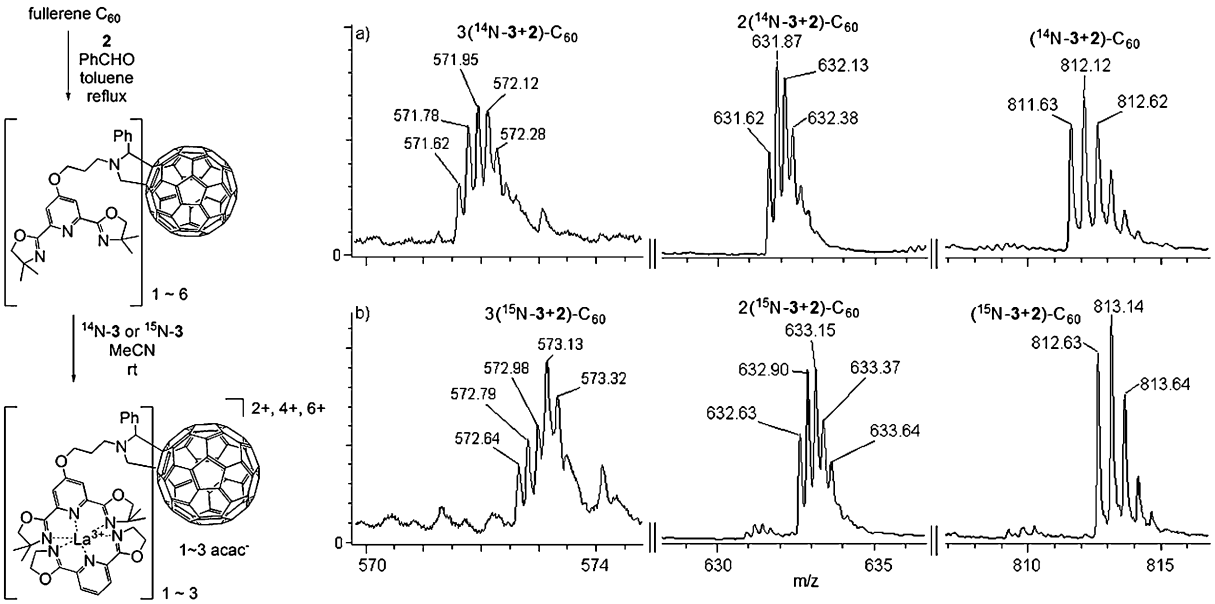


Fig. 3 CSI mass spectra of a) (^{14}N–**3** + **2**)–C_{60} and b) (^{15}N–**3** + **2**)–C_{60}.

multiply charged ions, $[C_{60} + 3(^{14}N–\mathbf{3} + \mathbf{2}) + 3acac^-]^{6+}$ was weak because the relative amount of the fullerenes is definitely small in the case of the three probes that are attached. Although the C_{60} derivatives with more than three probes **2** were observed, multiply charged ions were not detected for these species because of the difficulty in forming the charged site due to the steric hinderance and/or the electronic repulsion of the pybox–La complexes.

In the case of C_{70}, ions of C_{70} with 1–5 probes **2** attached were detected (see ESI†). Similar to C_{60}, multiply charged ions $[C_{70} + (^{14}N–\mathbf{3} + \mathbf{2}) + acac^-]^{2+}$ (*m*/*z* 872), $[C_{70} + 2(^{14}N–\mathbf{3} + \mathbf{2}) + 2acac^-]^{4+}$ (*m*/*z* 662), and $[C_{70} + 3(^{14}N–\mathbf{3} + \mathbf{2}) + 3acac^-]^{6+}$ (*m*/*z* 592) were detected by the formation of the charged site coupled with ^{14}N–**3** including La^{3+} (Fig. 4a). In the case of ^{15}N–**3**, (^{15}N–**3** + **2**)–C_{70} was detected as $[C_{70} + (^{15}N–\mathbf{3} + \mathbf{2}) + acac^-]^{2+}$ (*m*/*z* 873), $[C_{70} + 2(^{15}N–\mathbf{3}+\mathbf{2}) + 2acac^-]^{4+}$ (*m*/*z* 663), and $[C_{70} + 3(^{15}N–\mathbf{3} + \mathbf{2}) + 3acac^-]^{6+}$ (*m*/*z* 593) with a definite isotopic shift of *m*/*z* 1 compared to the non-labeled ones (Fig. 4b).

Finally, this method was adopted for (1,2-methano-fullerene C_{60})-61 carboxylic acid[17] as a substituted C_{60}. As complex fullerene derivatives possessing various functional groups have been prepared only quite recently,[6] we examined the ionic probe attachment ionization of commercially available fullerene derivatives. In the case of fullerenes with a carboxyl group as the functional group, BrAc-TMpybox **11** was attached to the carboxyl group by the method previously reported by us.[10] The probe (^{14}N–**3** + **11**)-bound C_{60} derivative was detected as a doubly charged ion $[M + acac^-]^{2+}$ (*m*/*z* 817) (Fig. 5a). In the case of the (^{15}N–**3** + **11**)-C_{60} derivative, a doubly charged ion $[M + acac^-]^{2+}$ (*m*/*z* 818) was observed as well (Fig. 5b). The isotopic shift of *m*/*z* 1 was also observed in these spectra.

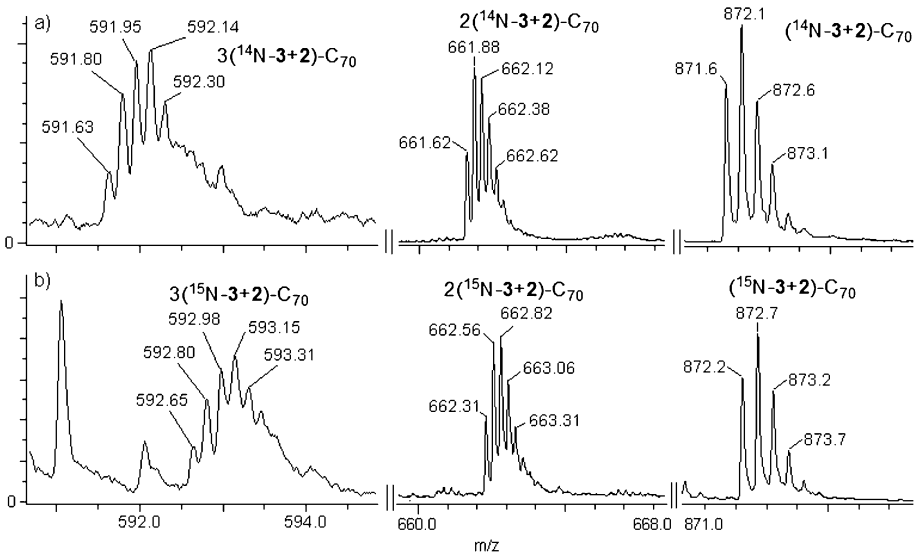


Fig. 4 CSI mass spectra of a) (^{14}N–**3** + **2**)–C_{70} and b) (^{15}N–**3** + **2**)–C_{70}.

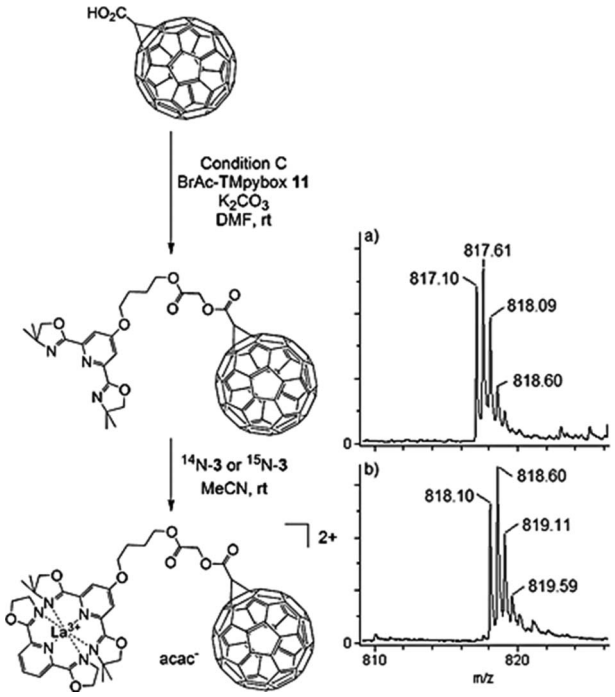


Fig. 5 CSI mass spectra of a) the (^{14}N–**3** + **11**)-C_{60} derivative and b) the (^{15}N-**3** + **11**)-C_{60} derivative.

Conclusions

The preparation of a new ionic probe, Sar-TMpybox **2**, and the development of an effective and reliable ionization method using this probe, together with the isotopic labeling of unsubstituted fullerenes C_{60} and C_{70}, were reported. By using the probe BrAc-TMpybox **11**, ^{14}N– and ^{15}N–**3** isotopic shifts and multiply charged ions were clearly observed in CSI-MS, in the case of the substituted fullerene, (1,2-methano-fullerene C_{60})-61 carboxylic acid. This method was proven to be effective for ^{15}N-labeled multiply charged ionization of stable fullerenes. We found that halving the number of the observed charge state indicates the number of the attached probe. The doubly charged ion is preferably observed in the pybox complex formed from the probe–complex **3** species by taking a single counter anion. Further application of the charged probes and the ionization of more complex large carbon clusters, such as carbon nanotubes, are under consideration.

Experimental

General

All melting points were measured on a Yanaco MP-500D and are uncorrected. Infrared spectra were recorded on a JASCO FT/IR 6300 spectrophotometer with an ATR (attenuated total reflectance) system. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ unless otherwise stated, on a JEOL JNM-ECP 400 and a Bruker 400 MHz with tetramethylsilane (TMS) as the internal reference. CSI mass spectra were recorded on a JEOL JMS-T100LC mass spectrometer equipped with a cold-spray ion source. NH plates (Fuji Silysia Chemical, Ltd., No. TO80817) were used for TLC. For column chromatography, NH silica gel (Fuji Silysia Chemical Ltd., particle size 100–200 mesh, No. IO61280 and 200–350 mesh, No. HU80502) was used. All anhydrous solvents were purchased from Wako Pure Chemical. Fullerenes C_{60}, C_{70}, and (1,2-methano-fullerene C_{60})-61-carboxylic acid were purchased from Aldrich.

Synthesis of 4-(3-bromo-prop-1-yloxy)-2,6-bis(4,4-dimethyloxazolin-2-yl)pyridine (7)

Under N_2, after a suspension of **5** (550 mg, 1.90 mM) and powdered KOH (345 mg, 6.16 mM) in DMF (5 mL) was stirred for 10 min at room temperature, 1,3-dibromopropane (1.2 mL, 11.8 mM) was added. After this was stirred at room temperature for 2 h, the mixture was poured into H_2O. The mixture was extracted with ethyl acetate and the organic layer was washed with H_2O and brine, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (*n*-hexane–ethyl acetate = 1 : 1) to give **7** (692 mg, 89%) as colorless prisms. mp 132–133 °C. IR (ATR, cm^{-1}): 1641. ^{1}H NMR (400 MHz) δ: 1.39 (12H, s), 2.34 (2H, quin., J = 6.0), 3.58 (2H, t, J = 6.0), 4.21 (4H, s), 4.28 (2H, t, J = 6.0 Hz), 7.71 (2H, s). ^{13}C NMR (100 MHz) δ: 28.5, 29.3, 31.9, 66.0, 68.0, 79.9, 112.2, 148.7, 148.7, 161.0, 165.7. HRCSIMS m/z 432.0932 (calcd for $C_{18}H_{24}{}^{79}BrN_3NaO_3$: 432.0899) and 434.0880 (calcd for $C_{18}H_{24}{}^{81}BrN_3NaO_3$: 434.0878). Anal. calcd for $C_{18}H_{24}BrN_3O_3$: C, 52.69; H, 5.90; N, 10.24. Found: C, 52.69; H, 5.99; N, 10.20.

Synthesis of *N*-benzyl-3-[2,6-bis(4,4-dimethyloxazolin-2-yl)pyridine-4-yloxy]propyl-1-amine (8)

Under N_2, a suspension of **7** (1.16 g, 2.82 mM), K_2CO_3 (2.24 g, 16.2 mM), and $BnNH_2$ (0.80 mL, 7.32 mM) in DMF (5 mL) was stirred at room temperature for 24 h. This reaction mixture was poured into H_2O and extracted with ethyl acetate. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (*n*-hexane : acetone = 6 : 1) to give **8** (1.00 g, 82%) as a colorless oil. IR (ATR, cm^{-1}): 3446, 1642. ^{1}H NMR (400 MHz) δ: 1.39 (12H, s), 1.99 (2H, quin., J = 6.5 Hz), 2.80 (2H, t, J = 6.5 Hz), 4.20 (4H, s), 4.21 (2H, t, J = 6.5 Hz), 7.22–7.32 (5 H, m, overlapped with $CHCl_3$), 7.69 (2H, s). ^{13}C NMR (100 MHz) δ: 28.5, 29.5, 45.8, 54.1, 67.1, 68.0, 79.8, 112.3, 127.1, 128.2, 128.5, 140.4, 148.6, 161.1, 166.0. HRCSIMS m/z 437.2543 (calcd for $C_{25}H_{33}N_4O_3$: 437.2553).

Synthesis of methyl *N*-benzyl-*N*-{3-[2,6-bis(4,4-dimethyloxazolin-2-yl)pyridine-4-yloxy]prop-1-yl}aminoacetate (9)

Under N_2, a suspension of **8** (392 mg, 0.90 mM), K_2CO_3 (623 mg, 4.50 mM), and methyl α-bromoacetate (0.17 mL, 1.80 mM) in DMF (3 mL) was stirred at room temperature for 12 h. This reaction mixture was poured into H_2O and extracted with ethyl acetate. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (*n*-hexane–ethyl acetate = 1 : 3) to give **9** (419 mg, 92%) as a colorless oil. IR (ATR, cm^{-1}): 1737, 1640. ^{1}H NMR (400 MHz) δ: 1.40 (12H, s), 1.93 (2H, quin., J = 6.4 Hz), 2.81 (2H, t, J = 6.4 Hz), 3.34 (2H, s), 3.69 (3H, s), 3.78 (2H, s), 4.15 (2H, t, J = 6.4 Hz), 7.17–7.30 (5 H, m, overlapped with $CHCl_3$), 7.63 (2H, s). ^{13}C NMR (100 MHz) δ: 27.3, 28.5, 49.9, 51.4, 54.4, 58.5, 66.5, 68.0, 79.8, 112.3, 127.3, 128.4, 129.0, 138.8,

148.5, 161.1, 166.0, 171.9. HRCSIMS *m/z* 509.2733 (calcd for $C_{28}H_{37}N_4O_5$: 509.2764).

Synthesis of methyl *N*-{3-[2,6-bis(4,4-dimethyloxazolin-2-yl)pyridine-4-yloxy]prop-1-yl}aminoacetate (10)

A solution of **9** (1.09 g, 2.14 mM) in MeOH (10 mL) was hydrogenated over 10% Pd/C (519 mg) under a hydrogen atmosphere at room temperature for 8 h. After removal of the catalyst by filtration through a Celite pad, the filtrate was evaporated *in vacuo*. The residue was purified by column chromatography (*n*-hexane : acetone = 4 : 1) to give **10** (751 mg, 84%) as colorless oil. IR (ATR, cm^{-1}): 3437, 1738, 1641. ^1H NMR (400 MHz) δ: 1.39 (12H, s), 1.98 (2H, quin., J = 6.5 Hz), 2.79 (2H, t, J = 6.5 Hz), 3.42 (2H, s), 3.74 (3H, s), 4.20 (4H, s), 4.23 (2H, t, J = 6.5 Hz), 7.70 (2H, s). ^{13}C NMR (100 MHz) δ: 28.5, 29.4, 46.0, 50.8, 51.9, 66.8, 68.0, 79.8, 112.2, 148.5, 161.0, 166.0, 172.9. HRCSIMS *m/z* 419.2335 (calcd for $C_{21}H_{31}N_4O_5$: 419.2294).

Synthesis of potassium *N*-{3-[2,6-bis(4,4-dimethyloxazolin-2-yl)pyridine-4-yloxy]prop-1-yl}aminoacetate (2)

Powdered KOH was continuously added to a solution of **10** (751 mg, 1.79 mM) in MeOH (10 mL) with stirring until **10** disappeared. Then, the mixture was purified directly by NH column chromatography (200–350 mesh, EtOH–MeOH = 10 : 1) to give **2** (1.10 g, quant.) as a colorless powder. mp >300 °C. IR (ATR, cm^{-1}): 3378, 1646. ^1H NMR (400 MHz, CD_3OD) δ: 1.36 (12H, s), 2.04 (2H, quin, J = 6.5 Hz), 2.79 (2H, t, J = 6.5 Hz), 3.16 (2H, s), 4.24 (4H, s), 4.25 (2H, t, J = 6.5 Hz), 7.63 (2H, s). ^{13}C NMR (100 MHz, CD_3OD) δ: 27.0, 28.4, 45.8, 52.6, 67.1, 67.7, 79.6, 112.1, 148.0, 161.4, 166.7, 177.0. HRCSIMS *m/z* 443.1741 (calcd for $C_{20}H_{28}KN_4O_5$: 443.1697).

Synthesis of 3-[2,6-bis-(4,4-dimethyl-4,5-dihydro-oxazol-2-yl)-pyridin-4-yloxy]-propan-1-ol (6)

Under N_2, after a suspension of **4** (1.0 g, 3.3 mM) and powdered KOH (947 mg, 17 mM) in DMF (20 mL) was stirred for 30 min at 70 °C, 1,3-propanediol (0.3 mL, 4.2 mM) was added. After this was stirred at 70 °C for 2 h, the mixture was poured into H_2O. The mixture was extracted with CH_2Cl_2 and the organic layer was washed with H_2O and brine, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by NH column chromatography (*n*-hexane–ethyl acetate = 1 : 1 to 0 : 1) to give **6** (661 mg, 58%) as colorless prisms. mp 93–94 °C. IR (ATR, cm^{-1}): 3226, 1673. ^1H NMR (400 MHz) δ: 1.40 (12H, s), 2.06 (2H, quin., J = 6.0 Hz), 3.84 (2H, t, J = 6.0 Hz), 4.21 (4H, s), 4.28 (2H, t, J = 6.0 Hz), 7.70 (2H, s). ^{13}C NMR (100 MHz) δ: 28.4, 31.6, 59.2, 65.9, 67.9, 79.8, 112.2, 148.4, 161.0, 165.9. HRCSIMS *m/z* 348.1929 (calcd for $C_{18}H_{26}N_3O_4$: 348.1923). Anal. calcd for $C_{18}H_{25}N_3O_4+H_2O$: C, 59.16; H, 7.45; N, 11.50. Found: C, 58.89; H, 7.39; N, 11.41.

Synthesis of malonic acid 3-[2,6-bis-(4,4-dimethyl-4,5-dihydro-oxazol-2-yl)-pyridin-4-yloxy]-propyl ester ethyl ester (1)

Under N_2, into a solution of **6** (493 mg, 1.42 mM) and NEt_3 (1.0 mL, 7.17 mM) was stirred ethyl 3-chloro-3-oxo-propionate (0.55 mL, 4.30 mM) in DMF (5 mL) for 10 min at room temperature, and then 1,3-dibromopropane (1.2 mL, 11.8 mM) was added. After this was stirred under reflux for 2 h, the mixture was poured into H_2O. The mixture was extracted with ethyl acetate and the organic layer was washed with H_2O and brine, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (*n*-hexane–ethyl acetate = 1 : 1) to give **1** (330 mg, 50%) as colorless prisms. mp 96–97 °C. IR (ATR, cm^{-1}): 1746, 1727, 1632. ^1H NMR (400 MHz) δ: 1.24 (3H, t, J = 7.3 Hz), 1.36 (12H, s), 2.14 (2H, quin., J = 6.2 Hz), 3.37 (2H, s), 4.18 (4H, s), 4.18 (2H, t, J = 6.2 Hz), 4.18 (2H, q, J = 7.3 Hz), 4.30 (2H, t, J = 6.2 Hz), 7.67 (2H, s). ^{13}C NMR (100 MHz) δ: 14.2, 28.2, 28.5, 41.6, 61.7, 64.9, 68.0, 79.8, 112.2, 148.6, 161.0, 165.7, 166.5. HRCSIMS *m/z* 484.2084 (calcd for $C_{23}H_{31}N_3NaO_7$: 484.2060). Anal. calcd for $C_{23}H_{31}N_3O_7$: C, 59.91; H, 6.87; N, 9.11. Found: C, 59.86; H, 6.77; N, 9.10.

Synthesis of 5-[2,6-bis-(4,4-dimethyl-4,5-dihydro-oxazol-2-yl)-pyridin-4-yloxy]-pentan-2-one (12)

A solution of 4-benzyloxy-2,6-bis-(4,4-dimethyl-4,5-dihydro-oxazol-2-yl)-pyridine[5] (1.0 g, 2.65 mM) in degassed dry MeOH (10 mL) was hydrogenated over 10% Pd/C (326 mg) under a H_2 atmosphere at room temperature for 4 h. After removal of the catalyst by filtration through a Celite pad, the filtrate was evaporated *in vacuo*. The residue was washed with Et_2O to give crude **5**. Under N_2, 5-chloro-2-pentanone (0.6 mL, 5.26 mM) was added to a mixture of crude **5** and K_2CO_3 (1.1 g, 8.07 mM) in DMF (5 mL). The whole was stirred at room temperature for 24 h. The reaction was quenched with H_2O and extracted with CH_2Cl_2. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by NH column chromatography (200–350 mesh, *n*-hexane–$CHCl_3$ = 5 : 1–2 : 1) to give colorless oil **12** (20 mg, 2.0%). ^1H NMR (400 MHz) δ: 1.37 (12H, s), 2.06 (2H, quin, J = 6.6 Hz), 2.16 (3H, s), 2.62 (2H, t, J = 6.6 Hz), 4.12 (2H, t, J = 6.6 Hz), 4.18 (4H, s), 7.64 (2H, s). ^{13}C NMR (100 MHz) δ: 22.9, 28.5, 30.1, 39.5, 67.7, 68.0, 79.8, 112.2, 148.5, 161.0, 165.9, 207.7. HRCSIMS *m/z* 374.2072 (calcd for $C_{20}H_{28}N_3O_4$: 374.2080).

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16 The probe **12** (3-[2,6-bis-(4,4-dimethyl-4,5-dihydro-oxazol-2-yl)-pyridin-4-yloxy]-propan-1-ol) having a carbonyl group as an anchoring site was also synthesized. However, it was not reacted with fullerene C_{60} and glycine.

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