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Efficient and Inexpensive Synthesis of ^{15}N-Labeled 2-Azido-1,3-dimethylimidazolinium Salts Using $Na^{15}NO_2$ Instead of $Na^{15}NNN$

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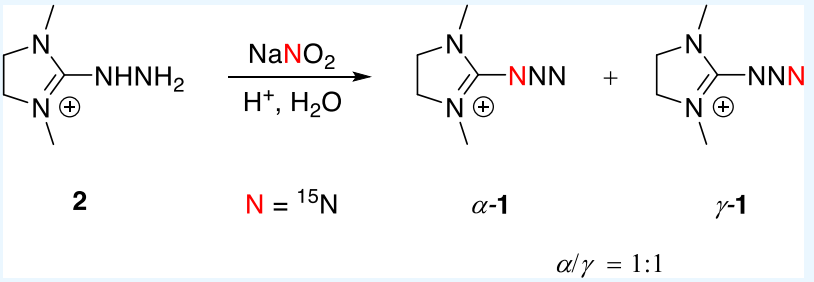

ABSTRACT: ^{15}N-Labeled azides are important probes for infrared and magnetic resonance spectroscopy and imaging. They can be synthesized by reaction of primary amines with a ^{15}N-labeled diazo-transfer reagent. We present the synthesis of ^{15}N-labeled 2-azido-1,3-dimethylimidazolinium salts **1** as a ^{15}N-labeled diazo-transfer reagent. Nitrosation of 1,3-dimethylimidazolinium-2-yl hydrazine (**2**) with $Na^{15}NO_2$ under acidic conditions gave **1** as a 1:1 mixture of α- and γ-^{15}N-labeled azides, α- and γ-**1**, rather than γ-**1** alone. The isotopomeric mixture thus obtained was then subjected to the diazo-transfer reaction with primary amines **3** to afford azides **4** as a 1:1 mixture of β-^{15}N-labeled azides β-**4** and unlabeled ones **4′**. The efficient and inexpensive synthesis of **1** as a 1:1 mixture of α- and γ-**1** using $Na^{15}NO_2$ instead of $Na^{15}NNN$ facilitates their wide use as a ^{15}N-labeled diazo-transfer reagent for preparing ^{15}N-labeled azides as molecular probes.

INTRODUCTION

Organic azides have found extensive use in organic syntheses including click chemistry.[1,2] Their use has been extended to infrared (IR) probes of protein structure and dynamics.[3] Azides have received much attention due to their strong IR absorption signal in the transparent window (1800−2500 cm^{-1}) free of native signals.[3a] However, the complicated line shape of their IR spectrum often arises from the Fermi resonance and multiple conformations, hampering its accurate spectral analysis.[4] The accidental Fermi resonance can be modulated to be less pronounced by isotopic substitution.[5,6] Recently, ^{15}N-labeled azides have attracted attention as magnetic resonance imaging (MRI) agents.[7] This is because they exhibit long-lasting hyperpolarization lifetimes and are more practical and effective than other ^{15}N-labeled MRI agents. ^{15}N-Labeled MRI reagents enable the acquisition of valuable information inaccessible by commonly used ^{13}C-based agents.

The synthesis of ^{15}N-labeled azides was achieved using three common reagents: $Na^{15}NNN$, $Na^{15}N^{15}N^{15}N$, and $Na^{15}NO_2$.[5−9] $Na^{15}N^{15}N^{15}N$ is too expensive to be used for making ^{15}N-labeled azides.[5c,7] The relatively inexpensive $Na^{15}NNN$ gives a 1:1 mixture of α- and γ-^{15}N-labeled azides (-^{15}NNN and -NN^{15}N) when used in the nucleophilic substitution reaction of halides or good leaving groups.[5a] $Na^{15}NNN$ was used to synthesize a 1:1 mixture of α- and γ-^{15}N-labeled diazo-transfer reagents, which were then reacted with primary amines to give a 1:1 mixture of unlabeled and β-^{15}N-labeled azides (-NNN and -N^{15}NN), respectively, as suggested by Wong's mechanism.[8,10] $Na^{15}NO_2$, which is less expensive than $Na^{15}NNN$, gives ^{15}N-labeled azides when used in the nitrosation of hydrazine under acidic conditions. The nitrosation of phenylhydrazine with $Na^{15}NO_2$ forms a mixture of minor β- and major (93−98%) γ-^{15}N-labeled azides (-N^{15}NN and -NN^{15}N),[9] whereas that of 3-pyridylhydrazine produces γ-^{15}N-labeled azide alone (Scheme 1a).[5b]

Recently, we developed γ-^{15}N-labeled trifluoromethanesulfonyl (triflyl) azide (TfNN^{15}N), a useful diazo-transfer reagent for preapring β-^{15}N-labeled azide.[6] This reagent was synthesized via nitrosation of in situ generated $TfNHNH_2$ with $Na^{15}NO_2$ under acidic conditions. However, TfNN^{15}N has the disadvantages of poor stability and safety. To overcome the physicochemical drawbacks of triflyl azide, we explored other diazo-transfer reagents, where their azido group can also

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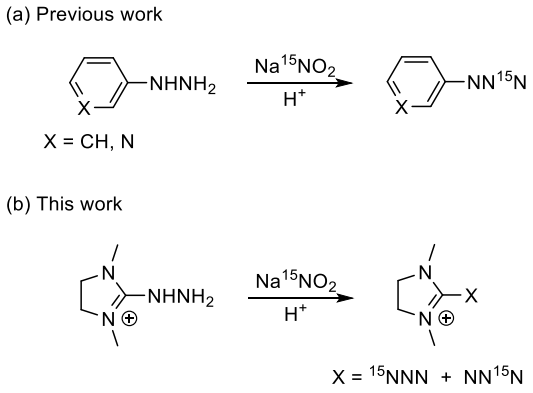
Scheme 1. Syntheses of ^{15}N-Labeled Azides by Nitrosation of Aryl (a) and 1,3-Dimethylimidazolinium-2-yl (b) Hydrazines with $Na^{15}NO_2$ under Acidic Conditions



be ^{15}N-labeled in a site-specific manner.[11] 2-Azido-1,3-dimethylimidazolinium salt is known to possess much better physicochemical properties than triflyl azide.[11,12]

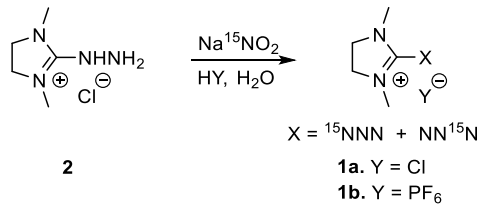
Here, we report the synthesis of ^{15}N-labeled 2-azido-1,3-dimethylimidazolinium salts **1** as a ^{15}N-labeled diazo-transfer reagent. This reagent was synthesized via nitrosation of 1,3-dimethylimidazolinium-2-yl hydrazine (1,3-dimethylimidazolidin-2-one hydrazone hydrochloride or 2-hydrazino-1,3-dimethylimidazolinium chloride (HDMC, **2**)) with $Na^{15}NO_2$ under acidic conditions (Scheme 1b). Unlike triflyl azide, however, 1,3-dimethylimidazolinium-2-yl azide (2-azido-1,3-dimethylimidazolinium chloride (ADMC) or hexafluorophosphate (ADMP)) obtained was found to be ^{15}N-labeled equally at either the α or γ positions rather than only the γ one in the azido group. The efficient and inexpensive synthesis of **1** as a 1:1 mixture of α- and γ-^{15}N-labeled azides using $Na^{15}NO_2$ instead of $Na^{15}NNN$ makes them a feasible ^{15}N-labeled diazo-transfer reagent for preparing ^{15}N-labeled azides as molecular probes.

RESULTS AND DISCUSSION

To prepare **2**, 2-chloro-1,3-dimethylimidazolinium chloride (CDMC, **2′**) was reacted with hydrazine monohydrate in MeCN containing K_2CO_3.[12a] Nitrosation of **2** with $Na^{15}NO_2$ under acidic conditions gave **1** as a 1:1 mixture of α- and γ-^{15}N-labeled azides, α- and γ-**1** (**1a** and **1b** depending on the type of acid employed) (Scheme 2).

The synthesis of **1** as a 1:1 mixture of α- and γ-**1** rather than γ-**1** alone was confirmed by IR and ^{13}C NMR spectroscopies. The IR spectra show strong bands at 2130 and 2155 cm^{-1} for **1a**, 2130, 2157, and 2171 cm^{-1} for **1b**, and 2176 cm^{-1} for unlabeled ADMP **1b′** (Figure 1a). Thus, the IR spectra of the azido group in **1** are red-shifted upon ^{15}N labeling. However,

Scheme 2. Synthesis of ^{15}N-Labeled ADMC 1a and ADMP 1b by Nitrosation of HDMC 2 with $Na^{15}NO_2$ under Acidic Conditions



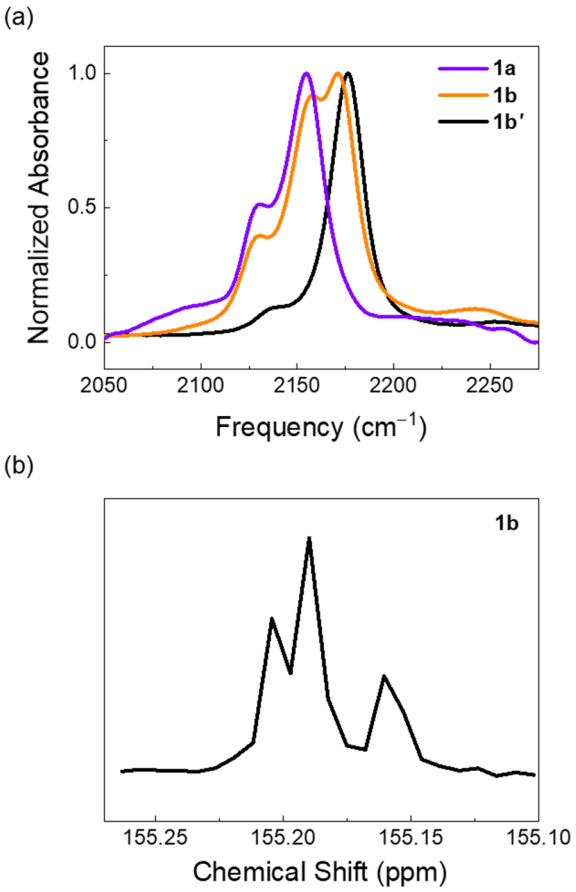


Figure 1. (a) IR spectra of ^{15}N-labeled ADMC **1a**, ^{15}N-labeled ADMP **1b**, and unlabeled ADMP **1b′** in DMF at 20 °C. (b) Inverse-gated ^{13}C NMR spectrum (150 MHz, DMSO-d_6) of **1b** in the C2 region.

the azido IR spectra become complicated by such isotopic substitution. Similar frequency shift and line shape change between the singly ^{15}N-labeled and unlabeled azides were observed for phenylazide[9b] and 3-azidopyridine:[5b] Ph^{15}NNN at 2116 cm^{-1}, PhNN^{15}N at 2068, 2085, and 2116 cm^{-1}, and PhNNN at 2095 and 2128 cm^{-1}; Py^{15}NNN at 2121 cm^{-1}, PyNN^{15}N at 2080 cm^{-1}, and PyNNN at 2097 and 2133 cm^{-1}. The complicated line shapes of phenylazide and 3-azidopyridine arise from the Fermi resonance. Thus, it cannot be ruled out the possibility that those of **1** also arise from Fermi resonance. Unlike phenylazide and 3-azidopyridine, however, **1** exhibits complex IR spectra, possibly due to other reasons as well. First of all, the complex IR spectra of **1** can result mainly from their composition as an isotopomeric mixture, which is evidenced by ^{13}C NMR spectra, as described below. Obviously, the different line shape between **1a** and **1b** is due to the different counteranion in them, and thus, the salt effect is somehow responsible for their complicated line shape.

The inverse-gated ^{13}C NMR spectrum of **1b** shows that the signals for the C2 atom appear at 155.19, 155.18, and 155.15 ppm (Figure 1b). It can be analyzed as a mixture of two distinct signals: a singlet at 155.18 ppm and a doublet ($^1J(\alpha$-$^{15}N^2,^{13}C^2)$ = 6.6 Hz) at 155.17 ppm, with an intensity ratio of 1:1. The signal at 155.18 ppm can be attributed to γ-**1b**, whereas that at 155.17 ppm can be attributed to α-**1b**. These assignments are supported by comparing the ^{13}C NMR spectra of **1b** with those of **1b′** and **1b″** (data not shown).

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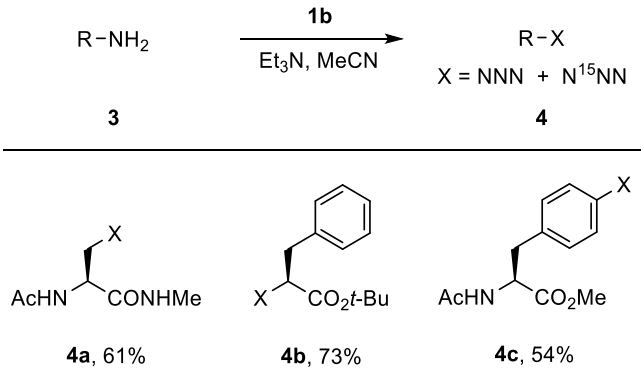
Here, **1b″** is an authentic 1:1 mixture of α- and γ-**1b**, which was prepared by nucleophilic substitution reaction of 2-chloro-1,3-dimethylimidazolinium hexafluorophosphate (CDMP, **2″**) with $Na^{15}NNN$.[12b] As a result, nitrosation of **2** with $Na^{15}NO_2$ under acidic conditions gave a 1:1 mixture of α- and γ-**1** rather than γ-**1** alone. Confident that the ^{13}C NMR spectrum of **1b** had been suitably assigned, we assigned the IR spectra of **1a** and **1b** by comparison with that of **1b′**. The IR spectra of **1a** and **1b** can be analyzed as a mixture of two major bands at 2130 and 2155 (or 2157) cm^{-1}. The IR band at 2130 cm^{-1} is attributed to γ-**1**, whereas that at 2155 (or 2157) cm^{-1} is attributed to α-**1**.[6] The additional IR band at 2171 cm^{-1} in **1b** is attributed to the Fermi resonance and/or salt effect, which made no difference in the signal pattern for the C2 atom between **1a** and **1b** in the ^{13}C NMR spectra.

With **1b** in hand, we then explored the diazo-transfer reaction of three representative primary amines: aliphatic amines bonded to primary (Ac-DAP-NHMe·TFA **3a**)[13] and secondary (H-Phe-O*t*Bu·HCl **3b**) carbons, and aromatic amine (Ac-Phe(p-NH_2)-OMe **3c**) (Scheme 3). Upon the reaction with **1b**, they were converted to azides **4** as a 1:1 mixture of β-^{15}N-labeled azides β-**4** and unlabeled ones **4′**, which were formed by the reaction of amines **3** with γ- and α-**1b**, respectively, as suggested by Wong's mechanism.[10] This result again supports that **1b** was a 1:1 mixture of α- and γ-**1b** rather than γ-**1b** alone when it was prepared via nitrosation of **2** with $Na^{15}NO_2$ under acidic conditions.

Scheme 3. Synthesis of Azides 4 by Diazo-Transfer Reactions of Amines 3 with ^{15}N-Labeled ADMP 1b



4a, 61% **4b**, 73% **4c**, 54%

The synthesis of **4** as a 1:1 mixture of β-**4** and **4′** rather than β-**4** alone was confirmed by IR and ^1H NMR spectroscopies. The IR spectra show strong bands at 2062 and 2105 cm^{-1} for **4a** and at 2105 cm^{-1} for **4a′**, where **4a** and **4a′** were prepared from **3a** using **1b** and **1b′**, respectively (Figure 2a). The IR spectrum of **4a** was assigned by comparison with that of **4a′**. The IR spectrum of **4a** can be analyzed as a mixture of two bands at 2062 and 2105 cm^{-1}, with an intensity ratio of 1:1. The IR band of **4a** at 2062 cm^{-1} is attributed to β-**4a**, whereas that at 2105 cm^{-1} is attributed to **4a′**.[6] This result indicates that **4a** was a 1:1 mixture of β-**4a** and **4a′**. The same results were also observed in the IR spectra of **4b** and **4c** (Figure S1 of the Supporting Information).

The ^1H NMR spectra show that the splitting pattern of the signal for two H^{β}s, $H^{\beta 1}$ and $H^{\beta 2}$, at 3.45−3.80 ppm differs between **4a** and **4a′**, which were prepared from **3a** using **1b** and **1b′**, respectively (Figure 2b). The signal for the two H^{β}s in **4a** and **4a′** is split into 24 and 8 peaks, respectively. The ^1H NMR spectrum of **4a** in the β-proton region was assigned by comparison with that of **4a′**. The 24-peak signal in **4a** can be analyzed as a mixture of 16- and 8-peak signals, with an intensity ratio of 1:1. The 16-peak signal is attributed to β-**4a**, whereas the 8-peak signal is attributed to **4a′**.[6] This result indicates that **4a** was a 1:1 mixture of β-**4a** and **4a′**. The same results were also observed in the ^1H NMR spectra of **4b** (Figure S2 of the Supporting Information).

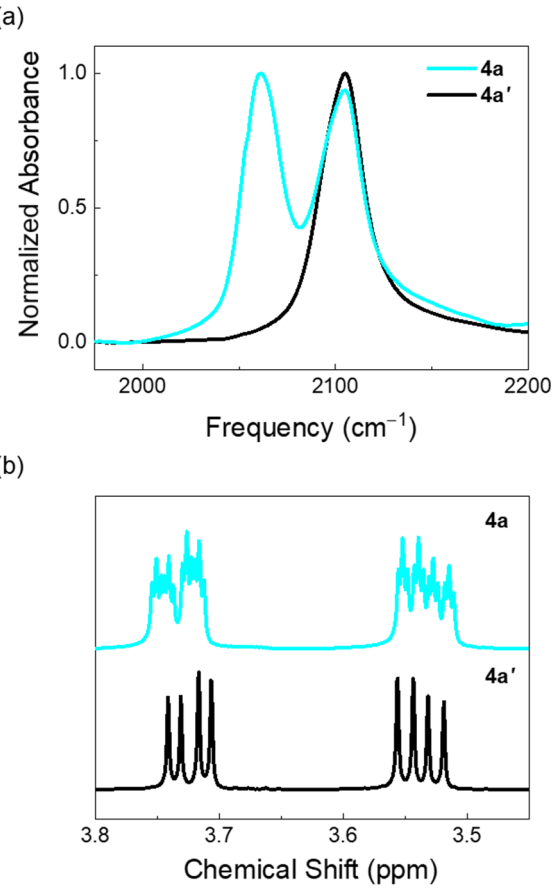


Figure 2. (a) IR spectra of Ac-AlaX-NHMe (X = NNN + $N^{15}NN$, **4a**) and Ac-AlaNNN-NHMe **4a′** in DMF at 20 °C. (b) ^1H NMR spectra (500 MHz, $CDCl_3$) of **4a** and **4a′** in the β-proton region. Here, **4a** and **4a′** were prepared by a diazo-transfer reaction of **3a** with **1b** and **1b′**, respectively.

The synthesis of **4** as a 1:1 mixture of β-**4** and **4′** rather than β-**4** alone was also confirmed by mass spectrometry. The mass spectra of **4** show a peak for each of β-**4** and **4′**, with a relative intensity ratio of nearly 1:1, albeit slightly higher for β-**4**: β-**4a**/**4a′** = 100:95.3, β-**4b**/**4b′** = 100:92.4, and β-**4c**/**4c′** = 100:92.5 (Figure S3 of the Supporting Information). This result again supports that **1b** was a nearly 1:1 mixture of α- and γ-**1b**, albeit slightly higher for γ-**1b**.

CONCLUSIONS

In conclusion, we synthesized **1**, a ^{15}N-labeled diazo-transfer reagent, as a 1:1 mixture of two isotopomers α- and γ-**1** rather than γ-**1** alone, via nitrosation of hydrazine **2** with $Na^{15}NO_2$ under acidic conditions. A similar result could be obtained by the nucleophilic substitution reaction of halide **2′** or **2″** with $Na^{15}NNN$, which is more expensive than $Na^{15}NO_2$. The synthesis of **1** as a 1:1 mixture of α- and γ-**1** using $Na^{15}NO_2$ instead of $Na^{15}NNN$ is efficient and inexpensive, enabling their wide use as a ^{15}N-labeled diazo-transfer reagent for preparing

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^{15}N-labeled azides as molecular probes. Further experimental and computational studies are awaited to delineate a mechanism for the formation of **1** as such an equimolar isotopomeric mixture via nitrosation of **2** with $Na^{15}NO_2$ under acidic conditions.

EXPERIMENTAL SECTION

General. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova 500 NMR spectrometer. Inverse-gated ^{13}C NMR spectra were recorded on a Bruker Avance 600 NMR spectrometer. ^{15}N NMR spectra were recorded on a Bruker Ascend 500 NMR spectrometer. Chemical shifts (δ) and coupling constants (J) are reported in parts per million (ppm) and hertz (Hz), respectively. ^{1}H NMR spectra are referenced to TMS (0.03% v/v tetramethylsilane in $CDCl_3$ and DMSO-d_6) as an internal standard. ^{13}C NMR spectra are referenced to the solvent (^{13}C: $CDCl_3$, δ 77.00 ppm; DMSO-d_6, δ 39.50 ppm) as an internal standard. ^{15}N NMR spectra are referenced to CH_3NO_2 as an external standard (neat, δ 381.78 ppm). High-resolution mass spectra (HRMS) were recorded on a JEOL JMS-700 mass spectrometer using the fast atom bombardment (FAB) technique. IR spectra were measured on a Bruker VERTEX 70 FTIR spectrometer equipped with a HgCdTe detector. The samples **1** and **4** were dissolved in DMF at a concentration of 0.3 M. IR spectra were measured with a frequency resolution of 1 cm^{-1} in 12 scans using a CaF_2 cell (2 mm thickness) confined with a Teflon spacer (25 μm thickness). Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} precoated plates (0.25 mm thickness, Merck, Darmstadt). Flash chromatography was carried out on silica gel 60 (230−400 mesh, Merck). Reagent-grade chemicals were purchased from Sigma-Aldrich, Alfa Aesar, and TCI, and used as received, unless otherwise specified. Amino acids (H-DAP(Boc)-OMe·HCl, H-Phe-O*t*Bu·HCl **3b**, and Ac-*p*-amino-Phe-OMe **3c**) were purchased from BACHEM. Sodium nitrite (^{15}N, 98%+) and sodium azide (1-^{15}N, 98%+) were purchased from Cambridge Isotope Laboratories. 1,3-Dimethylimidazolidin-2-one hydrazone dihydrochloride (**2**·HCl),[12a] 2-chloro-1,3-dimethylimidazolinium hexafluorophosphate (**2″**),[12b] 2-azido-1,3-dimethylimidazolinium hexafluorophosphate (**1b′**),[12b] and Ac-Dap-NHMe·TFA **3a**[13] were prepared as reported previously.

Preparation of 1a. To a cooled (0 °C) and stirred solution of **2**·HCl (2.21 g, 11.0 mmol) in 1 N aqueous HCl (20 mL) was slowly added a solution of $Na^{15}NO_2$ (1.00 g, 14.3 mmol) in H_2O (7 mL). After being stirred at 0 °C for 2 h, the reaction mixture was concentrated in vacuo. The residue was filtered through Celite 545, washed with MeCN (150 mL), and the filtrate was concentrated in vacuo. The residue was dissolved in MeCN (10 mL), and Et_2O (150 mL) was added. After sonication for 5 min, the Et_2O layer was removed, and the remaining layer was dried in vacuo to give **1a** (2.66 g, 100%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.06 (s, 4H), 3.27 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.54, 49.61, 34.26;[a] ^{15}N NMR (50 MHz, $CDCl_3$) δ 237.93, 92.13; HRMS (FAB+) for $C_5H_{10}N_4{}^{15}N$ ($M^+ - Cl^-$), calcd 141.0907, found 141.0906.

[a]The ^{13}C NMR spectrum of **1a** shows the significant overlap of the signals for α- and γ-**1a** in the C2 region at 156.54 ppm.

Preparation of 1b. To a cooled (0 °C) and stirred solution of **2**·HCl (2.21 g, 11.0 mmol) in H_2O (90 mL) were added HPF_6 (60% w/w aqueous solution, 4.0 mL, 27.1 mmol) and then slowly a solution of $Na^{15}NO_2$ (1.00 g, 14.3 mmol) in H_2O (20 mL). After being stirred at 0 °C for 2 h, the reaction mixture was concentrated in vacuo. The residue was filtered through Celite 545, washed with MeCN (150 mL), and the filtrate was concentrated in vacuo. The residue was dissolved in MeCN (10 mL), and Et_2O (150 mL) was added. The precipitate was collected by filtration and dissolved in MeCN (10 mL), and EtOAc (250 mL) was added. After being stirred at room temperature for 30 min, the precipitate was collected by filtration to give **1b** (3.29 g, 100%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.79 (s, 4H), 3.06 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.15, 155.14 (d, J = 6.7 Hz), 48.72, 32.82; inverse-gated ^{13}C NMR (150 MHz, DMSO-d_6) δ 155.18 (0.5C), 155.17 (d, J = 6.6 Hz, 0.5C), 48.73 (2C), 32.82 (2C); ^{15}N NMR (50 MHz, DMSO-d_6) δ 237.99, 91.94; HRMS (FAB+) for $C_5H_{10}N_4{}^{15}N$ ($M^+ - PF_6^-$), calcd 141.0907, found 141.0909.

Preparation of 1b″.[12b] To a cooled (0 °C) and stirred solution of **2″** (3.06 g, 11.0 mmol) in MeCN (15 mL) was added $Na^{15}NNN$ (1.00 g, 15.1 mmol). After being stirred at 0 °C for 30 min, the reaction mixture was filtered through Celite 545, washed with MeCN (150 mL), and the filtrate was concentrated in vacuo. The residue was dissolved in MeCN (10 mL), and Et_2O (150 mL) was added. The precipitate was collected by filtration to give **1b″** (2.34 g, 74%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.79 (s, 4H), 3.06 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.15, 155.14 (d, J = 6.7 MHz), 48.72, 32.83; HRMS (FAB+) for $C_5H_{10}N_4{}^{15}N$ ($M^+ - PF_6^-$), calcd 141.0907, found 141.0908.

General Procedure for Preparation of 4. To a stirred solution of **1b** (435 mg, 1.52 mmol) and amine **3** (1.00 mmol) in MeCN (20 mL) was added Et_3N (0.69 mL, 4.95 mmol).[b] After being stirred under Ar at room temperature for 3 h, the reaction mixture was concentrated in vacuo. The residue was purified by flash chromatography to give **4**.

[b]To increase the yield of **4a**, a solution of amine **3a** in MeCN (5 mL) was added to a solution of **1b** and Et_3N in MeCN (15 mL).

*Ac-AlaX-NHMe (X = NNN + N^{15}NN, **4a**).* Ac-Dap-NHMe·TFA **3a** (273 mg, 1.00 mmol) was treated according to the general procedure (flash chromatography, MeOH/CH_2Cl_2 = 1:50) to give **4a** (115 mg, 62%) as a white solid. TLC (MeOH/CH_2Cl_2 = 1:15) R_f = 0.38; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.69 (brs, 1H), 6.63 (d, J = 7.3 Hz, 1H), 4.62 (ddd, J = 7.6, 6.3, 5.3 Hz, 1H), 3.73 (ddd, J = 12.4, 5.0, 3.7 Hz, 0.5H), 3.73 (dd, J = 12.2, 4.9 Hz, 0.5H), 3.53 (ddd, J = 12.2, 6.4, 3.7 Hz, 0.5H), 3.53 (dd, J = 12.5, 6.4 Hz, 0.5H), 2.84 (d, J = 4.9 Hz, 3H), 2.06 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.56, 169.69, 52.24, 51.89, 26.43, 23.12; ^{15}N NMR (50 MHz, $CDCl_3$) δ 246.63; HRMS (FAB+) for $C_6H_{12}N_5O_2$ (MH^+), calcd 186.0991, found 186.0996, $C_6H_{12}N_4{}^{15}NO_2$ (MH^+), calcd 187.0961, found 187.0954.

*X-Phe-OtBu (X = NNN + N^{15}NN, **4b**).* H-Phe-O*t*Bu·HCl **3b** (258 mg, 1.00 mmol) was treated according to the general procedure (flash chromatography, CH_2Cl_2/*n*-hexane = 1:6) to give **4b** (182 mg, 73%) as a colorless oil. TLC (CH_2Cl_2/*n*-hexane = 1:3) R_f = 0.32; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (t, J = 7.2 Hz, 2H), 7.26 (t, J = 7.6 Hz, 1H), 7.24 (d, J = 7.9 Hz, 2H), 3.91 (ddd, J = 8.2, 5.8, 4.9 Hz, 0.5H), 3.91 (dd, J = 8.2, 5.8 Hz, 0.5H), 3.13 (dd, J = 14.0, 5.8 Hz, 1H), 2.99 (dd, J = 13.7, 8.5 Hz, 1H), 1.45 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.99, 136.18, 129.26, 128.56, 127.11, 83.00, 63.60, 37.55, 27.94; ^{15}N NMR (50 MHz, $CDCl_3$) δ 245.42; HRMS (FAB+) for $C_{13}H_{18}N_3O_2$ (MH^+), calcd 248.1399, found

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248.1391, $C_{13}H_{18}N_2{}^{15}NO_2$ (MH^+), calcd 249.1369, found 249.1370.

*Ac-p-X-Phe-OMe (X = NNN + N^{15}NN, **4c**).* Ac-*p*-amino-Phe-OMe **3c** (236 mg, 1.00 mmol) was treated according to the general procedure (flash chromatography, EtOAc/*n*-hexane = 1:1) to give **4c** (143 mg, 54%) as a yellow solid. TLC (EtOAc/*n*-hexane = 3:1) R_f = 0.41; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.08 (d, J = 8.5 Hz, 2H), 6.96 (d, J = 8.5 Hz, 2H), 5.93 (d, J = 7.3 Hz, 1H), 4.87 (dt, J = 7.6, 5.8 Hz, 1H), 3.74 (s, 3H), 3.14 (dd, J = 14.0, 5.8 Hz, 1H), 3.06 (dd, J = 14.0, 5.5 Hz, 1H), 2.00 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.93, 169.51, 138.98, 132.57, 130.59, 119.15, 119.15 (d, J = 1.8 Hz), 53.11, 52.39, 37.29, 23.14; ^{15}N NMR (50 MHz, $CDCl_3$) δ 242.74; HRMS (FAB+) for $C_{12}H_{15}N_4O_3$ (MH^+), calcd 263.1144, found 263.1137, $C_{12}H_{15}N_3{}^{15}NO_3$ (MH^+), calcd 264.1115, found 264.1118.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c07147.

NMR, IR, and mass spectra of compounds (PDF)

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Notes

The authors declare no competing financial interest.

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