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TfNN^{15}N: A γ-^{15}N-Labeled Diazo-Transfer Reagent for the Synthesis of β-^{15}N-Labeled Azides

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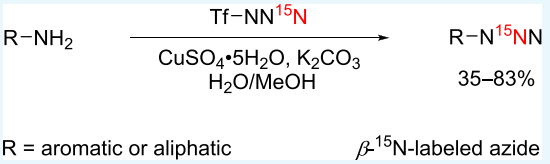
ABSTRACT: Azides are infrared (IR) probes that are important for structure and dynamics studies of proteins. However, they often display complex IR spectra owing to Fermi resonances and multiple conformers. Isotopic substitution of azides weakens the Fermi resonance, allowing more accurate IR spectral analysis. Site-specifically ^{15}N-labeled aromatic azides, but not aliphatic azides, are synthesized through nitrosation. Both ^{15}N-labeled aromatic and aliphatic azides are synthesized through nucleophilic substitution or diazo-transfer reaction but as an isotopomeric mixture. We present the synthesis of TfNN^{15}N, a γ-^{15}N-labeled diazo-transfer reagent, and its use to prepare β-^{15}N-labeled aliphatic as well as aromatic azides.



INTRODUCTION

Various spectroscopic techniques have been used to study the structures and dynamics of proteins. Fluorophores are widely used probes for studying changes in the protein structure.[1] However, the introduction of relatively large fluorophores significantly disturbs the native structure.

Infrared (IR) probes, such as CO,[2] CN,[3] and SCN,[4] which directly convey intramolecular bonding vibrations, are relatively small, thus minimizing native structure disturbance. IR probes have been used as site-specific probes of biomolecules because of their sensitivity to the local environment. However, IR spectral analysis of biomolecules is difficult because their IR signals often overlap with those of peptides. Therefore, IR probes with isotopic labels or a signal in the transparent window region between 1800 and 2500 cm^{-1} are used.[5]

Azides have considerable potential as vibration probes of biomolecules due to their IR absorption in the transparent window region of the spectrum.[6] In addition, the molar extinction coefficient of the azide probe is approximately 5−19 times larger than that of the CN probe. Therefore, azide probes may be used for low-concentration peptides or proteins. Azides are also used in site-specific "click chemistry".[7]

However, short vibrational lifetimes and Fermi resonance are disadvantages in the IR spectral analysis of azide.[8] In the presence of Fermi resonance, the IR absorption spectrum is complex, which hampers the spectral analysis for probing structural changes in proteins or surrounding solvents. Furthermore, whether the IR spectrum is complicated by Fermi resonance or multiple conformation is unclear.

Accidental Fermi resonance can be detected by FTIR absorption and 2DIR spectroscopies.[9] However, the complex spectra, due to Fermi resonance, are challenging to analyze. Generally, isotopic substitution overcomes spectral interference by Fermi resonance because its effect is reduced by increasing the energy difference between the fundamental and overtone (or combination) modes.[10]

Three synthetic routes are known for preparing ^{15}N-labeled azides (Scheme 1).[10,11] First, nucleophilic substitution reaction, wherein halides or good leaving groups are substituted with $^{15}N_3^-$, is the most commonly used method for the synthesis of ^{15}N-labeled aliphatic azides. This method was used by Brewer and co-workers to prepare azido isotopomers of 2′-azido-2′-deoxyuridine (dU-NNN) as a mixture of dU-^{15}NNN and dU-NN^{15}N.[11] Although there was a slight frequency red-shift of dU-^{15}NNN (2111 cm^{-1}) and dU-NN^{15}N (2089 cm^{-1}) relative to dU-NNN (2111 cm^{-1}), IR spectral analysis of the two-isotopomer mixture was still difficult because their IR spectra overlapped.

Second, nitrosation of aryl hydrazine with Na^{15}NO$_2$ is useful for the synthesis of site-specifically ^{15}N-labeled aromatic azides. This method was used by Brewer and co-workers to prepare azido isotopomers of 3-azidopyridine (PyrNNN) in a site-specific manner.[10] In the IR spectrum of PyrNNN, a complex band containing the Fermi resonance was observed at 2075−2150 cm^{-1}. The IR bands of Pyr^{15}NNN, PyrNN^{15}N, and PyrN^{15}NN were observed at 2121, 2080, and 2067 cm^{-1}, respectively. The IR spectrum of PyrNN^{15}N was still complex, due to Fermi resonance, but those of Pyr^{15}NNN and PyrN^{15}NN revealed one band. However, unlike aryl or carbonyl hydrazine, alkyl hydrazine has the limitation that it cannot be rearranged through nitrosation to produce azides.[12]

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Scheme 1. (a–d) Syntheses of Site-Specifically ^{15}N-Labeled Azides

(a) Nucleophilic substitution

$R-X_1 \xrightarrow{Na^{15}NNN} R-X_2$

R = aromatic or aliphatic

X_1 = leaving group

X_2 = ^{15}NNN or NN^{15}N

(b) Nitrosation

$R-NHNH_2 \xrightarrow[H^+]{Na^{15}NO_2} R-NN^{15}N$

R = aromatic

(c) Diazo-transfer

$Tf-Cl \xrightarrow{Na^{15}NNN} Tf-X_1 \xrightarrow{R-NH_2} R-X_2$

R = aromatic or aliphatic

X_1 = ^{15}NNN or NN^{15}N

X_2 = NNN or N^{15}NN

(d) This work

$Tf-NHNHBoc \xrightarrow[H^+]{Na^{15}NO_2} Tf-NN^{15}N \xrightarrow{R-NH_2} R-N^{15}NN$

R = aromatic or aliphatic

Finally, the diazo-transfer reaction of primary amines is an efficient method for the syntheses of both ^{15}N-labeled aromatic and aliphatic azides.[11,13] Diazo-transfer occurs via nucleophilic attack of amine on the azido group of the reagent at its terminal γ-N atom as suggested by Wong's mechanism.[14] Accordingly, the α-, β-, and γ-^{15}N-labeled diazo-transfer reagents furnish the unlabeled, γ-, and β-^{15}N-labeled azides, respectively. Brewer and co-workers synthesized azido isotopomers of trifluoromethanesulfonyl azides (TfNNN), a diazo-transfer reagent, as a mixture of Tf^{15}NNN and TfNN^{15}N (α- and γ-^{15}N-labeled ones) by the nucleophilic substitution reaction of trifluoromethanesulfonic anhydride (Tf_2O) with Na^{15}NNN.[11] That mixture was then used to prepare a mixture of dU-NNN and dU-N^{15}NN. The IR spectrum of dU-N^{15}NN (2069 cm^{-1}) exhibited a red-shift of 42 cm^{-1} from that of dU-NNN. Such frequency difference is greater than those for dU-^{15}NNN and dU-NN^{15}N. Therefore, the β-^{15}N-labeled azide modulated the accidental Fermi resonance occurring in the unlabeled azide by the largest frequency shift among the single-labeled azides, but its IR spectrum still overlapped with that of the unlabeled azide. Azido isotopomers of other diazo-transfer reagents such as imidazole-1-sulfonyl azide ($ImSO_2N_3$) and 2-azido-1,3-dimethylimidazolinium hexafluorophosphate (ADMP) were also synthesized as an isotopomeric mixture. A mixture of α- and γ-^{15}N-labeled azido isotopomers of $ImSO_2N_3$ was synthesized by nucleophilic substitution using Na^{15}NNN.[13] Recently, we found that a 1:1 mixture of α- and γ-^{15}N-labeled azido isotopomers of ADMP was synthesized by nitrosation of 1,3-dimethylimidazolidinone hydrazone with $Na^{15}NO_2$.[15] Such a mixture was also obtained by the nucleophilic substitution reaction of 2-chloro-1,3-dimethylimidazolinium chloride with Na^{15}NNN.

Taken together, site-specifically ^{15}N-labeled aromatic azides, but not aliphatic azides, can be synthesized through nitrosation. Both ^{15}N-labeled aromatic and aliphatic azides can be synthesized by nucleophilic substitution or diazo-transfer reaction but as an isotopomeric mixture (-^{15}NNN, -NN^{15}N- or -N^{15}NN, -NNN). That is, a synthetic method for preparing site-specifically ^{15}N-labeled aliphatic azides has not been established yet. In particular, β-^{15}N-labeled azides are demanded to facilitate the IR spectral analysis of the azide probe by decreasing the Fermi resonance effect. Herein, we report the synthesis of TfNN^{15}N, a γ-^{15}N-labeled diazo-transfer reagent, via nitrosation of $TfNHNH_2$ with $Na^{15}NO_2$, and its use to prepare β-^{15}N-labeled aliphatic as well as aromatic azides.

RESULTS AND DISCUSSION

A γ-^{15}N-labeled diazo-transfer reagent was designed based on TfN_3, an early model diazo-transfer reagent.[16] Like aryl or carbonyl hydrazine, sulfonyl hydrazine may rearrange to produce γ-^{15}N-labeled azides upon nitrosation with $Na^{15}NO_2$.[17]

Scheme 2. Synthesis of TfNN^{15}N 1

$Tf_2O \xrightarrow[Et_3N,\ CH_2Cl_2]{NH_2NHBoc} Tf-NHNHBoc$ **1‴**, 80% $\xrightarrow[Na^{15}NO_2,\ H_2O]{TFA,\ CH_2Cl_2} Tf-NN^{15}N$ **1**

TfNN^{15}N **1** (or TfNNN **1′**) was synthesized by nitrosation of in situ generated $TfNHNH_2$ **1″** with $Na^{15}NO_2$ (or $NaNO_2$) (Scheme 2). $TfNHNH_2$ could not be obtained upon treatment of Tf_2O with NH_2NH_2.[18,19] Instead, it was generated in situ from TfNHNHBoc **1‴**,[20,21] which was synthesized using Tf_2O and NH_2NHBoc. Hydrazine precursor **1‴** is a more stable and easier-to-use solid than its derived hydrazine **1″**. After the removal of Boc in **1‴** with trifluoroacetic acid (TFA), it was subjected without purification to direct reaction with $Na^{15}NO_2$ to afford the desired product **1**. This indicates that nitrosation occurs under acidic conditions without being severely affected by the cleavage product of Boc. TfNN^{15}N present in the organic layer (CH_2Cl_2) obtained through the work-up process was used without further purification for subsequent spectral analyses and diazo-transfer reactions because of its low boiling point.[22] Currently, the yields in the preparation of TfNN^{15}N are inconsistent. However, hydrazine is easily obtained via its precursor for TfN_3 but not for $ImSO_2N_3$.

The synthesis of TfNN^{15}N was confirmed by ^{15}N NMR and IR spectroscopies. Nitrosation usually occurs at the β-N of hydrazine, forming the γ-^{15}N-labeled azide. Occasionally, however, nitrosation occurs at the α-N of hydrazine, forming not only γ-^{15}N-labeled but also β-^{15}N-labeled azide.[23] The ^{15}N NMR spectrum of TfNN^{15}N shows only one peak at −139.08 ppm, confirming the synthesis of the γ-^{15}N-labeled azide via nitrosation at the β-N of hydrazine. The IR spectrum of TfNN^{15}N shows a strong, broad band at 2126 cm^{-1}, confirming the synthesis of the γ-^{15}N-labeled azide (Figure 1). The IR band of TfNNN appears at 2154 cm^{-1}, which is blue-shifted by approximately 28 cm^{-1} from that of TfNN^{15}N. Note that TfNN^{15}N and TfNNN show the shoulder peaks at 2155 and 2128 cm^{-1}, respectively, which arise from Fermi resonance. Although site-specific isotopic substitutions are confirmed through the observed frequency shift, accurate

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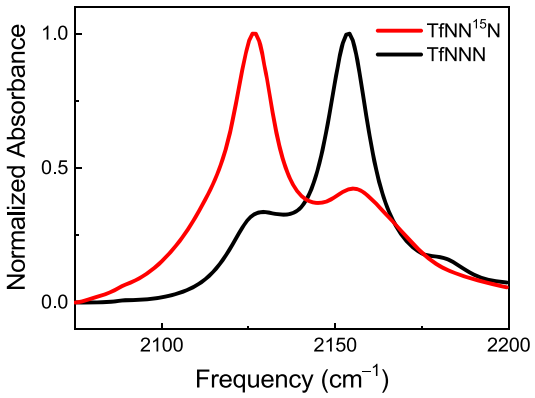
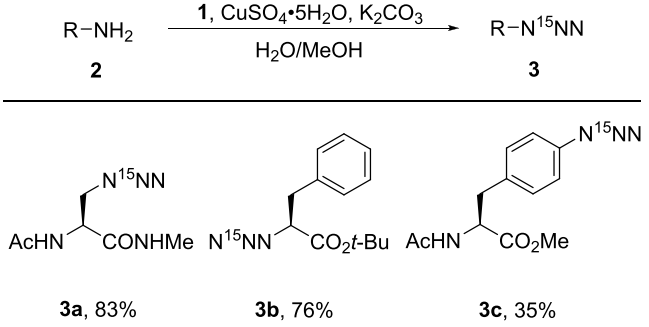


Figure 1. IR spectra of TfNN^{15}N **1** and TfNNN **1′** in CH_2Cl_2 at 20 °C.

analysis of the IR spectrum is difficult because of Fermi resonance.

Scheme 3. Syntheses of Azides 3 by Diazo-Transfer Reactions of Amines 2 with TfNN^{15}N 1



3a, 83% **3b**, 76% **3c**, 35%

With TfNN^{15}N in hand, we then explored the diazo-transfer reaction of various amines (Scheme 3). Three representative amines used were Ac-DAP-NHMe·TFA **2a**,[6a] H-Phe-O*t*Bu·HCl **2b**, and Ac-Phe(*p*-NH_2)-OMe **2c**, which are aliphatic amines bonded to primary and secondary carbons, and aromatic amines, respectively. Upon the reaction with TfNN^{15}N, they were converted to the β-^{15}N-labeled aliphatic and aromatic azides **3a**−**3c** in moderate yields.[24]

The syntheses of the β-^{15}N-labeled aliphatic and aromatic azides were confirmed by ^{1}H NMR, ^{13}C NMR, and IR spectroscopies. First, the ^{1}H NMR spectra of AlaN^{15}NN (Ac-Ala(N^{15}NN)-NHMe, **3a**) and AlaNNN (Ac-Ala(NNN)-NHMe,[6b] **3a′**) revealed that the splitting pattern of the signal for two H$^\beta$s, H$^{\beta1}$ and H$^{\beta2}$, at 3.45−3.80 ppm was different between **3a** and **3a′** (Figure 2). The signal for the two H$^\beta$s in **3a** and **3a′** is split into 16 and 8 peaks, respectively, which can be explained as follows. Each of the two H$^\beta$s exhibits a different signal, which appear at 3.73 and 3.53 ppm for **3a** and 3.72 and 3.54 ppm for **3a′**. Each of these two signals is further split into eight and four peaks for **3a** and **3a′**, which is due to the coupling of H$^{\beta1}$ with H$^{\beta2}$, H$^\alpha$, and β-^{15}N$^\beta$ for **3a** but with H$^{\beta2}$ and H$^\alpha$ for **3a′**. Thus, the ^{1}H NMR spectrum confirms the presence of β-^{15}N$^\beta$ in **3a**. The ^{13}C NMR spectrum of **3a** shows the ^2J and ^3J couplings of β-^{15}N$^\beta$ with adjacent C$^\beta$ (^2J(β-^{15}N$^\beta$,C$^\beta$) = 1.9 Hz) and C$^\alpha$ (^3J(β-^{15}N$^\beta$,C$^\alpha$) = 1.9 Hz), which also confirms the presence of β-^{15}N$^\beta$ in **3a**. The IR spectra of **3a** and **3a′** exhibit one band at 2061 and 2104 cm^{-1}, respectively (Figure 3). A red-shift of 43 cm^{-1} is due to replacing β-N$^\beta$ with β-^{15}N$^\beta$. Thus, the IR spectrum also confirms the presence of β-^{15}N$^\beta$ in **3a**. The same patterns were also observed in the ^{1}H NMR, ^{13}C NMR, and IR spectra of other azides **3b**, **3b′**, **3c**, and **3c′** (Figures S1−S6 of the Supporting Information): ^{1}H NMR spectra (500 MHz, $CDCl_3$) of **3b**, δ 3.91 (ddd, J = 8.5, 6.0, 5.0 Hz, 1H); **3b′**, δ 3.91 (dd, J = 8.0, 6.0 Hz, 1H); ^{13}C NMR spectra (125 MHz, $CDCl_3$) of **3b**, ^2J(β-^{15}N$^\alpha$,C$^\alpha$) = 1.9 Hz, ^3J(β-^{15}N$^\alpha$,C$^\beta$) = 1.8 Hz; **3c**, ^2J(β-^{15}N$^\gamma$,C$^\gamma$) = 2.8 Hz; IR spectra of **3b**, 2062 cm^{-1}; **3b′**, 2112 cm^{-1}; **3c**, 2090 cm^{-1}; **3c′**, 2143 cm^{-1}.

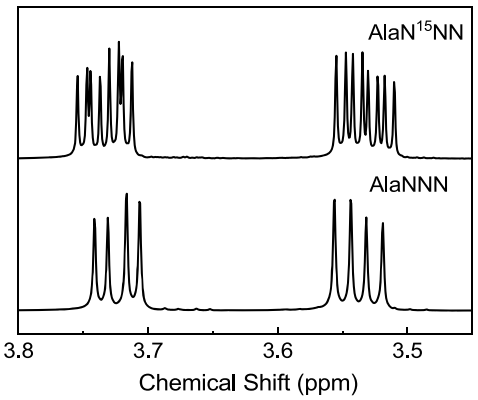


Figure 2. ^{1}H NMR spectra (500 MHz, $CDCl_3$) of AlaN^{15}NN **3a** and AlaNNN **3a′** in the β-proton region: **3a**, δ 3.73 (ddd, J = 12.4, 5.1, 3.6 Hz, 1H), 3.53 (ddd, J = 12.3, 6.3, 3.8 Hz, 1H); **3a′**, δ 3.72 (dd, J = 12.3, 4.8 Hz, 1H), 3.54 (dd, J = 12.3, 6.3 Hz, 1H).

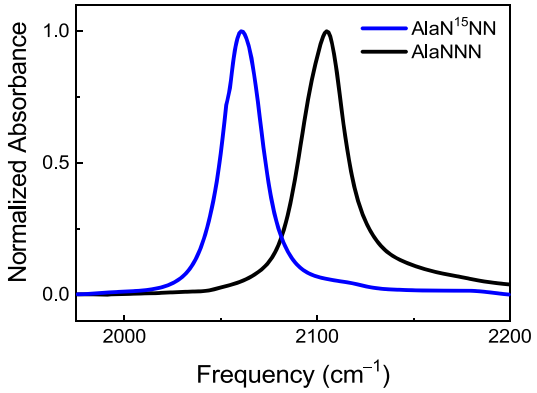


Figure 3. IR spectra of AlaN^{15}NN **3a** and AlaNNN **3a′** in DMF at 20 °C.

CONCLUSIONS

In conclusion, we synthesized TfNN^{15}N, a γ-^{15}N-labeled diazo-transfer reagent, via nitrosation of $TfNHNH_2$ with $Na^{15}NO_2$. We then demonstrated that it could be used to prepare both β-^{15}N-labeled aliphatic and aromatic azides. TfNN^{15}N is the first example of a site-specifically ^{15}N-labeled diazo-transfer reagent, which can provide site-specifically ^{15}N-labeled aliphatic azides for the first time. β-^{15}N-Labeled azides display a larger frequency red-shift than α- and γ-^{15}N-labeled azides compared to the unlabeled one. Thus, β-^{15}N-labeled azides render the IR spectral analysis of azide probes much easier because a more significant decrease in the Fermi resonance effect is attained.[25,26]

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EXPERIMENTAL SECTION

General. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Ascend 500 NMR spectrometer. ^{15}N NMR spectra were recorded on an Agilent DD2 700 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 $CDCl_3$) as an internal standard. ^{13}C NMR spectra are referenced to the solvent (^{13}C: $CDCl_3$, δ 77.00 ppm) as an internal standard. 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 **3** were dissolved in DMF at 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 **2b**, and Ac-*p*-amino-Phe-OMe **2c**) were purchased from BACHEM. Sodium nitrite (^{15}N, 98%+) was purchased from Cambridge Isotope Laboratories. TfNHNHBoc **1‴**[21] and Ac-Dap-NHMe·TFA **2a**[6a] were prepared as reported previously.

Preparation of γ-^{15}N-Labeled Trifluoromethanesulfonyl Azide (TfNN^{15}N, 1). To a cooled (0 °C) and stirred solution of TfNHNHBoc **1‴**[21] (2.38 g, 9.00 mmol) in CH_2Cl_2 (40 mL) was added trifluoroacetic acid (15 mL). After stirring at 0 °C for 1 h, a solution of $Na^{15}NO_2$ (931 mg, 13.3 mmol) in H_2O (10 mL) was added. After stirring at 0 °C for a further 1 h, the reaction mixture was treated with saturated aqueous Na_2CO_3 solution (200 mL). The organic layer was collected and used without further purification.

General Procedure for the Preparation of β-^{15}N-Labeled Azides 3. To a stirred solution of amine **2** (1.0 mmol) in H_2O/MeOH (1:2, 15 mL) were added $CuSO_4$·$5H_2O$ (10 mg, 40 μmol), K_2CO_3 (691 + x mg, 5.0 + n mmol),[a] and then a solution of TfNN^{15}N **1** (∼3 mmol) in CH_2Cl_2 (15 mL). After stirring vigorously at room temperature for 12 h, the reaction mixture was concentrated in vacuo. The residue was purified by flash chromatography to give azide **3**.

*Ac-Ala(N^{15}NN)-NHMe (**3a**).* Ac-Dap-NHMe·TFA **2a**[6a] (273 mg, 1.0 mmol) was treated according to the general procedure (flash chromatography, MeOH/CH_2Cl_2 = 1:50) to give **3a** (155 mg, 83%) 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 = 7.5 Hz, 1H), 4.62 (ddd, J = 7.6, 6.4, 5.1 Hz, 1H), 3.73 (ddd, J = 12.4, 5.1, 3.6 Hz, 1H), 3.53 (ddd, J = 12.3, 6.3, 3.8 Hz, 1H), 2.84 (d, J = 4.5 Hz, 3H), 2.06 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.55, 169.69, 52.24 (d, J = 1.9 Hz), 51.88 (d, J = 1.9 Hz), 26.43, 23.13; ^{15}N NMR (70 MHz, $CDCl_3$) δ −133.45; HRMS (FAB+) for $C_6H_{12}N_4{}^{15}NO_2$ (MH^+), calcd 187.0961, found 187.0966.

*N^{15}NN-Phe-OtBu (**3b**).* H-Phe-O*t*Bu·HCl **2b** (258 mg, 1.0 mmol) was treated according to the general procedure (flash chromatography, CH_2Cl_2/*n*-hexene = 1:6) to give **3b** (189 mg, 76%) as a colorless oil. TLC (CH_2Cl_2/*n*-hexene = 1:3) R_f = 0.32; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34−7.23 (m, 5H), 3.91 (ddd, J = 8.5, 6.0, 5.0 Hz, 1H), 3.13 (dd, J = 14.0, 6.0 Hz, 1H), 2.99 (dd, J = 14.0, 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 (d, J = 1.9 Hz), 37.55 (d, J = 1.8 Hz), 27.94; ^{15}N NMR (70 MHz, $CDCl_3$) δ −135.09; HRMS (FAB+) for $C_{13}H_{18}N_2{}^{15}NO_2$ (MH^+), calcd 249.1369, found 249.1371.

*Ac-Phe(p-N^{15}NN)-OMe (**3c**).* Ac-Phe(*p*-NH_2)-OMe **2c** (236 mg, 1.0 mmol) was treated according to the general procedure (flash chromatography, EtOAc/*n*-hexene = 1:1) to give **3c** (91.1 mg, 35%) as a yellow solid. TLC (EtOAc/*n*-hexene = 3:1) R_f = 0.41; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.08 (d, J = 8.0 Hz, 2H), 6.96 (d, J = 8.0 Hz, 2H), 5.93 (d, J = 7.0 Hz, 1H), 4.87 (q, J = 6.5 Hz, 1H), 3.74 (s, 3H), 3.14 (dd, J = 13.5, 6.0 Hz, 1H), 3.06 (dd, J = 14.0, 5.5 Hz, 1H), 1.99 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.93, 169.50, 138.97, 132.57, 130.59, 119.15 (d, J = 2.8 Hz), 53.11, 52.39, 37.29, 23.14; ^{15}N NMR (70 MHz, $CDCl_3$) δ −137.72; HRMS (FAB+) for $C_{12}H_{15}N_3{}^{15}NO_3$ (MH^+), calcd 264.1115, found 264.1110.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c04679.

NMR and IR spectra of compounds (PDF)

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Author Contributions

#H.-J.K. and S.G. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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ADDITIONAL NOTE

[a]Additional quantities (x, n) should be added when the reaction does not reach completion. For example, the reaction of TfNN[15]N **1** and Ac-Phe(p-NH_2)-OMe **2c** required x = 691 mg, n = 5.0 mmol.

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