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Convenient preparation of amino acid derivatives with two ^{13}C labels

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Abstract

An efficient preparation of doubly ^{13}C-labeled methyl nitroacetate and its application in the preparation of six doubly labeled amino acid derivatives are reported. This versatile precursor should allow the convenient preparation of many classes of natural and non-natural doubly labeled amino acids.

Keywords: amino acids; labeled compounds; conjugate addition.

The efficient incorporation of isotopic labels into amino acids is an important objective for a variety of applications. In particular, such compounds are extraordinarily useful in the study of metabolic and biosynthetic processes with a variety of techniques including NMR and mass spectrometry.[1] In addition, labeled amino acids have been used in structural and conformational studies of biomolecules employing solid state and solution NMR techniques.[2] Amino acids labeled with ^{13}C in the C-1 or C-2 positions are typically prepared by the functionalization of ^{13}C-labeled glycine,[3–5] although procedures involving the introduction of ^{13}C-labeled carbon monoxide[6] and ^{13}C-labeled carbon dioxide[7] have been reported. ^{13}C incorporation within the amino acid side chain is more readily accomplished via alkylation with labeled electrophiles[8] or by enzymatic methods.[9]

Methyl nitroacetate **1** (R=Me) is a versatile glycine synthetic equivalent in the preparation of non-natural amino acids (Fig. 1). Simple α-amino acids **2** may be prepared from **1** by direct alkylation,[10] by a Knoevenagel condensation/conjugate reduction sequence,[11] or by a Knoevenagel condensation/deconjugation sequence.[12] Compound **1** has effectively been used in the preparation of enantioenriched α,α-disubstituted amino acids **3** employing both enzymatic[13] and asymmetric catalytic[14] reactions. Our group recently reported the preparation of a variety of increasingly complex non-natural amino acids **4** and **5** utilizing **1** in a three-step Knoevenagel condensation/organozinc conjugate addition/α-alkylation sequence,[15] and a recent report from Feringa described an attractive asymmetric variant of this procedure.[16] Furthermore, **1** may be used in the preparation of a variety of heterocyclic structures[10,17] and

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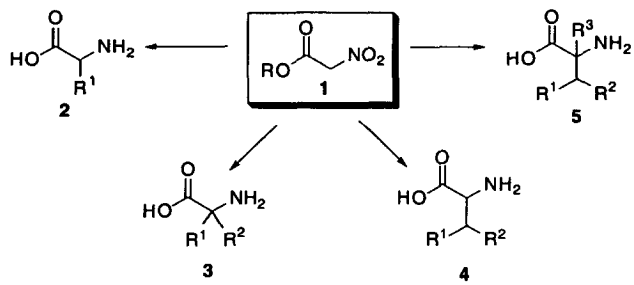

Figure 1.

may be linked to a Merrifield resin.[18] Given its utility in the above applications, we envisioned that the efficient preparation of doubly ^{13}C-labeled **1** would serve as an attractive precursor to numerous classes of doubly labeled amino acids.

Methyl nitroacetate is conveniently prepared by the tail to tail dimerization of nitromethane under basic conditions, followed by esterification with acidic methanol (Eq. 1).[19] Given the availability of $^{13}CH_3NO_2$ as a convenient ^{13}C source, we have utilized its dimerization in the preparation of $MeO_2{}^{13}C{}^{13}CH_2NO_2$ (**6**).[20] Compound **6** is readily prepared[21] from ^{13}C-nitromethane by a minor variation of the published procedure for unlabeled material.[22]

$$^{13}CH_3\text{-}NO_2 \xrightarrow[76\ \%]{KOH} \left[KO_2{}^{13}C{}^{13}CH{=}NO_2K \right] \xrightarrow[47\ \%]{H_2SO_4,\ CH_3OH} \underset{\mathbf{6}}{CH_3O_2{}^{13}C{}^{13}CH_2NO_2} \qquad (1)$$

Utilizing the Lehnert modification of the Knoevenagel condensation, which employs $TiCl_4$ in the presence of *N*-methylmorpholine in THF, highly electrophilic α,β-unsaturated nitroacetates were readily constructed from compound **6** (Table 1).[22] Aromatic and aliphatic aldehydes were tolerated in the sequence in moderate to good yield. Further functionalization of the unsaturated nitroacetates was accomplished with transmetalation-derived organozincs in THF (prepared in situ from a 1.5:1 ratio of organolithiums or Grignard reagents and zinc chloride) or by conjugate reduction[11] utilizing $NaBH_4$ in EtOH (Table 1). The organozinc conjugate additions proceeded in good to excellent yield at 0°C in THF to afford a 1:1 mixture of diastereomers. The conjugate reductions also proceeded in good yield at rt in EtOH or EtOH/THF to afford racemic alkylated methyl nitroacetates.

Reduction of the nitro moiety with Ra–Ni in EtOH under 60 psi of H_2 proceeded smoothly to produce the primary amine[23] which was derivatized directly with acetyl chloride and pyridine in CH_2Cl_2 to provide the protected amino acids in moderate to excellent yield (Table 1). Ethanol was the most effective solvent for the nitro reduction, however, ester exchange products were observed in small amounts in some instances in which R=H. We anticipate that this general protocol[24,25] should be widely applicable to the preparation of many classes of natural and non-natural doubly ^{13}C-labeled amino acids.

Acknowledgements

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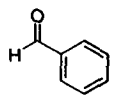
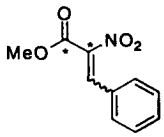
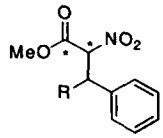
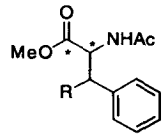
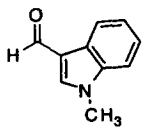
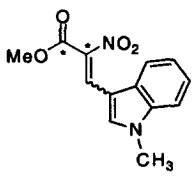
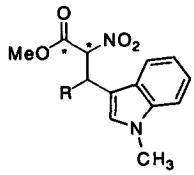
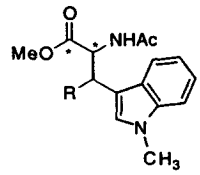
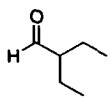
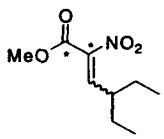
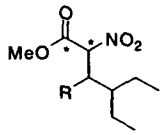
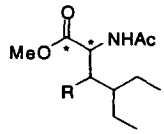
Table 1
Preparation of doubly labeled amino acid derivatives[24,25]

aldehyde	Knoevenagel product[a] (% yield)	functionalized nitroalkane[b] (% yield)	protected amino acid (% yield[c])
	 (79 %)	 (R = *n*-pr, 83 %) (R = H, 70 %)	 (R = *n*-pr, 78 %) (R = H, 82 %)
	 (55 %)	 (R = *n*-Bu, 92 %) (R = H, 76 %)	 (R = *n*-Bu, 90 %) (R = H, 84 %)
	 (50 %)	 (R = Ph, 72 %) (R = H, 84 %)	 (R = Ph, 78 %) (R = H, 52 %)

[a]Products were obtained and used as *E/Z* mixtures.
[b]Products were obtained and used as 1:1 diastereomeric mixtures.
[c]Two step yields for the nitro reduction and acetylation are reported. Products were analyzed to be 98 % doubly ^{13}C labeled and 2 % singly ^{13}C labeled by mass spectrometric determination.

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20. ^{13}C-Nitromethane may be prepared from ^{13}C-iodomethane upon treatment with $AgNO_2$ in 40–45% yield. Price per mmol based on a 1 g purchase from Aldrich Chemical Company is \$6.81 for ^{13}C-iodomethane and \$18.78 for ^{13}C-nitromethane. Thus, in our hands, there was ultimately a minimal cost advantage to using iodomethane.
21. Preparation of [1,2-$^{13}C_2$]-methyl nitroacetate (**6**). Caution: see Ref. 19 for a discussion of safety issues. ^{13}C-Nitromethane (99% ^{13}C, 401 mg, 0.350 mL, 6.46 mmol) was added dropwise by syringe drive to KOH (1.548 g, 27.59 mmol) in 1 mL of water over approximately 30 min. The reaction mixture was heated in an oil bath maintained at 130°C for 1 h, cooled to room temperature, filtered, washed with methanol, and dried overnight under vacuum to yield 450 mg (76%) of the dipotassium salt of nitroacetic acid. The salt (348 mg, 1.90 mmol) was ground into a fine powder using a mortar and pestle and weighed into a two neck 25 mL round bottomed flask fitted with a pressure equalizing dropping funnel and a thermometer. Methanol (11.5 mL, 285 mmol) was added, and the suspension was cooled to –9°C. H_2SO_4 (570 mg, 0.310 mL, 5.70 mmol) diluted with 3 mL of methanol was added dropwise over approximately 10 min, maintaining a temperature range of –9°C to –6°C. The reaction mixture was allowed to warm slowly to rt over 4 h, then filtered, and the solvent was removed under reduced pressure. The crude mixture was taken up in CH_2Cl_2, washed with water, dried, and the solvent removed in vacuo to yield crude **6** 107 mg (47%) as a yellow oil. The crude methyl nitroacetate was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.17 (dd, *J*=6.5, 149.0 Hz, 2H), 3.84 (d, *J*=4.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 162.7 (d, *J*=61.9 Hz), 76.3 (d, *J*=61.9 Hz), 53.9; IR (film) 1713, 1558 cm^{-1}; HRMS (EI) *m/e* calcd for $C_2H_2NO_3$ 90.0102, found 90.0097 ($(M-CH_3O)^+$).
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24. Knoevenegal condensation: the methyl nitroacetate (1 equiv.) and aldehyde (1.1 equiv.) in THF (0.5 M with respect to methyl nitroacetate) were added to a 0.1 M solution of $TiCl_4$ (2 equiv.) in THF at 0°C. A 1.0 M solution of *N*-methylmorpholine (4 equiv.) was then added by syringe over 2 h at 0°C. After consumption of starting material by TLC analysis (typically 18–24 h at rt), the reaction mixture was subjected to extractive workup (H_2O/Et_2O) followed by flash chromatography on SiO_2. 1,4 Additions: A 0.3–0.5 M solution of $ZnCl_2$ (2.5–3.0 equiv.) in THF was stirred at 0°C, and the organolithium or Grignard reagent (4.0–4.5 equiv.) was added by syringe followed by stirring for 0.1–0.25 h at 0°C. This solution was transferred by cannula to a 0.1–0.2 M solution of the unsaturated substrate (1.0 equiv.) at 0°C. After consumption of starting material by TLC analysis (typically 0.25–2.0 h at 0°C), the reaction mixture was subjected to extractive workup (NH_4Cl/NH_4OH pH=8 buffer/Et_2O) followed by flash chromatography on SiO_2. Conjugate reductions: $NaBH_4$ (5.0 equiv.) was added in one portion to a 0.01–0.03 M solution of the unsaturated substrate (1 equiv.) in EtOH or EtOH/THF at rt. After consumption of the starting material by TLC analysis (typically 0.1–0.25 h), the reaction mixture was subjected to extractive workup (saturated NH_4Cl/CH_2Cl_2) followed by flash chromatography on SiO_2. Reduction/acetylation of the nitro moiety: Ra–Ni (Raney® 2724 obtained from Grace Davison) in water was washed four times with EtOH and then stirred in 1 mL of EtOH. The nitro compound in 2 mL of EtOH was transferred to the reaction vessel containing the Ra–Ni suspension. The reaction mixture was flushed three times with H_2 and hydrogenated at 60 psi for 18–24 h at rt. The reaction mixture was filtered through Celite and the solvent removed in vacuo. The crude product was dissolved in CH_2Cl_2 (4 mL) and then acetyl chloride (3 equiv.) and pyridine (4 equiv.) were added. After stirring for 1–2 h, the reaction mixture was subjected to extractive workup ($NaHCO_3/CH_2Cl_2$) followed by flash chromatography on SiO_2.
25. Representative characterization data: [1,2-$^{13}C_2$]-methyl 2-(*N*-acetyl)amino-3-phenylhexanoate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22–7.35 (m, 3H), 7.09 (d, *J*=7.5 Hz, 2H), 5.95 (d, J=8.5 Hz, 1H)$_{\text{one isomer}}$, 5.65 (d, J=8.5 Hz, 1H)$_{\text{one isomer}}$, 4.92 (dddd, *J*=5.5, 6.5, 9.5, 142.5 Hz, 1H)$_{\text{one isomer}}$, 4.80 (ddt, *J*=6.5, 8.5, 144.5 Hz, 1H)$_{\text{one isomer}}$, 3.70 (d, *J*=4.0 Hz, 3H)$_{\text{one isomer}}$, 3.53 (d, *J*=4.0 Hz, 3H)$_{\text{one isomer}}$, 3.20 (m, 1H)$_{\text{one isomer}}$, 2.94 (sextet, *J*=6.5 Hz, 1H)$_{\text{one isomer}}$, 2.00 (s, 3H)$_{\text{one isomer}}$, 1.96 (s, 3H)$_{\text{one isomer}}$, 1.76–1.81 (m, 1H)$_{\text{one isomer}}$, 1.68–1.73 (m, 1H)$_{\text{one isomer}}$, 1.12–1.29 (m, 2H), 0.86 (t, *J*=7.0 Hz, 3H)$_{\text{one isomer}}$,

0.85 (t, J=7.0 Hz, 3H)$_{\text{one isomer}}$; ^{13}C NMR (125 MHz) δ 172.4 (d, J=61.9 Hz), 172.2 (d, J=61.9 Hz), 170.2, 169.7, 139.7, 139.4, 128.9, 128.6, 128.5, 128.3, 127.6, 127.5, 57.2 (d, J=61.9 Hz), 56.2 (d, J=61.9 Hz), 52.4, 52.1, 49.2 (d, J=33.1 Hz), 47.7 (d, J=34.1 Hz), 33.8, 33.7, 23.5, 23.4, 20.8 (d, J=2.8 Hz), 20.6 (d, J=3.8 Hz), 14.1; IR (film) 1701, 1654 cm^{-1}; HRMS (EI) *m/e* calcd for $C_{13}H_{16}O_2$ 206.1217, found 206.1218 ((M-C_2H_5NO)$^+$). [1,2-$^{13}C_2$]-Methyl 2-(*N*-acetyl)amino-3-phenylpropanoate. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23–7.30 (m, 3H), 7.08 (d, J=7.0 Hz, 2H), 5.93 (d, J=5.5 Hz, 1H), 5.36 (dquintet, J=6.5, 143.5 Hz, 1H), 3.72 (d, J=3.5 Hz, 3H), 3.06–3.18 (m, 2H), 1.98 (s, 3H); ^{13}C NMR (125 MHz) δ 172.3 (d, J=60.9 Hz), 171.6, 136.0, 129.5, 128.8, 127.4, 53.3 (d, J=61.8 Hz), 52.6, 38.1 (d, J=33.3 Hz), 23.4; IR (film) 1698, 1652 cm^{-1}; HRMS (EI) *m/e* calcd for $C_{12}H_{15}NO_3$ 223.1119, found 223.1124 (M$^+$).