Preparation of [1,2,3,4,5-$^{13}C_5$]-5-Amino-4-oxopentanoic Acid (ALA) − Design of a Synthetic Scheme to Prepare Any ^{13}C- and ^{15}N-Isotopomer with High Isotopic Enrichment

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Keywords: Biosynthesis / Isotopic labelling / NMR spectroscopy / Amino acids

5-Amino-4-oxopentanoic acid (5-aminolevulinic acid) is a precursor in the biosynthesis of the biologically active porphyrins such as chlorophyll, bacteriochlorophyll, heme, etc. These systems are central in photosynthesis, oxygen transport, electron transport, etc. In this paper we describe a simple scheme to prepare any isotopomer of 5-aminolevulinic acid in a few steps in high yield. Using a similar scheme, levulinic acid can now also be prepared in any isotopomeric form.

Introduction

5-Amino-4-oxopentanoic acid (5-aminolevulinic acid, ALA; Figure 1) is an early intermediate in the biosynthesis of essential porphyrin derivatives.[1] The essential cofactor chlorophyll is built up from eight ALA building blocks. The photochemical and photophysical properties of chlorophyll bound in membrane protein complexes in the chloroplasts of plants and cyanobacteria are the basis of photosynthesis, in which the energy of solar light is used to reduce CO_2 to energy-rich organic molecules while simultaneously oxidizing water to oxygen and four protons. This process provides the material and energy requirements for the whole biosphere. It also directly and indirectly delivers the energy and material needed for the worldwide economy. Through the interactions between the chlorophyll cofactors in antenna pigments in plants, algae and cyanobacteria, light energy is harvested and transported as electronic excitons to two so-called photosynthetic reaction centers, namely, photosynthetic reaction center II (PSII) and photosynthetic reaction center I (PSI). Electronic excitation causes PSII to generate the oxidation potential to convert water in the oxygen-evolving complex in eight steps into O_2 and 4 H^+. The reduction equivalents also produced by PSII are directed to PSI and, upon electronic excitation, are used to drive the Calvin cycle to convert CO_2 into energy-rich organic molecules. Because of the preeminent role of oxygenic photosynthesis in the biosphere and human economy, it is the focus of intense international research.

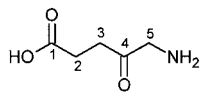

Figure 1. Structure and numbering of 5-amino-4-oxopentanoic acid (ALA)

To obtain a real understanding of how the ground state and the electronic excited state properties of the chlorophyll molecules in PSII and PSI are tuned by their binding in the protein, it is clear that structural and functional information on each carbon atom in the chlorophyll complex has to be obtained at the atomic level without perturbation. The technique of choice is to study reaction centers highly enriched with ^{13}C by solid-state ^{13}C NMR techniques.

In principle there are three ways to isotopically enrich C atoms in the photosynthetic reaction center (RC). First, the photosynthetic organism can be grown on media containing 99% $^{13}CO_2$. This indeed leads to RC with all the carbon atoms fully enriched. However, these systems do not give the structural information we need because of severe spectral overlap. The second method would be to exchange the normal-abundance bacteriochlorophyll for fully ^{13}C-enriched bacteriochlorophyll. This method works with many of the other cofactors, but not with bacteriochlorophylls because the bacteriochlorophylls are bound by covalent linkages to histidine residues in the protein which cannot be broken without denaturing the protein complex. The third method is to grow the bacteria in media that contain highly ^{13}C-enriched biosynthetic intermediates.

Recently we published the first solid-state ^{13}C NMR study of the RC highly enriched with eight ^{13}C atoms in the bacteriochlorophylls which we obtained by growing *Rhodobacter sphaeroides* in commercially available 99% 4-^{13}C-en-

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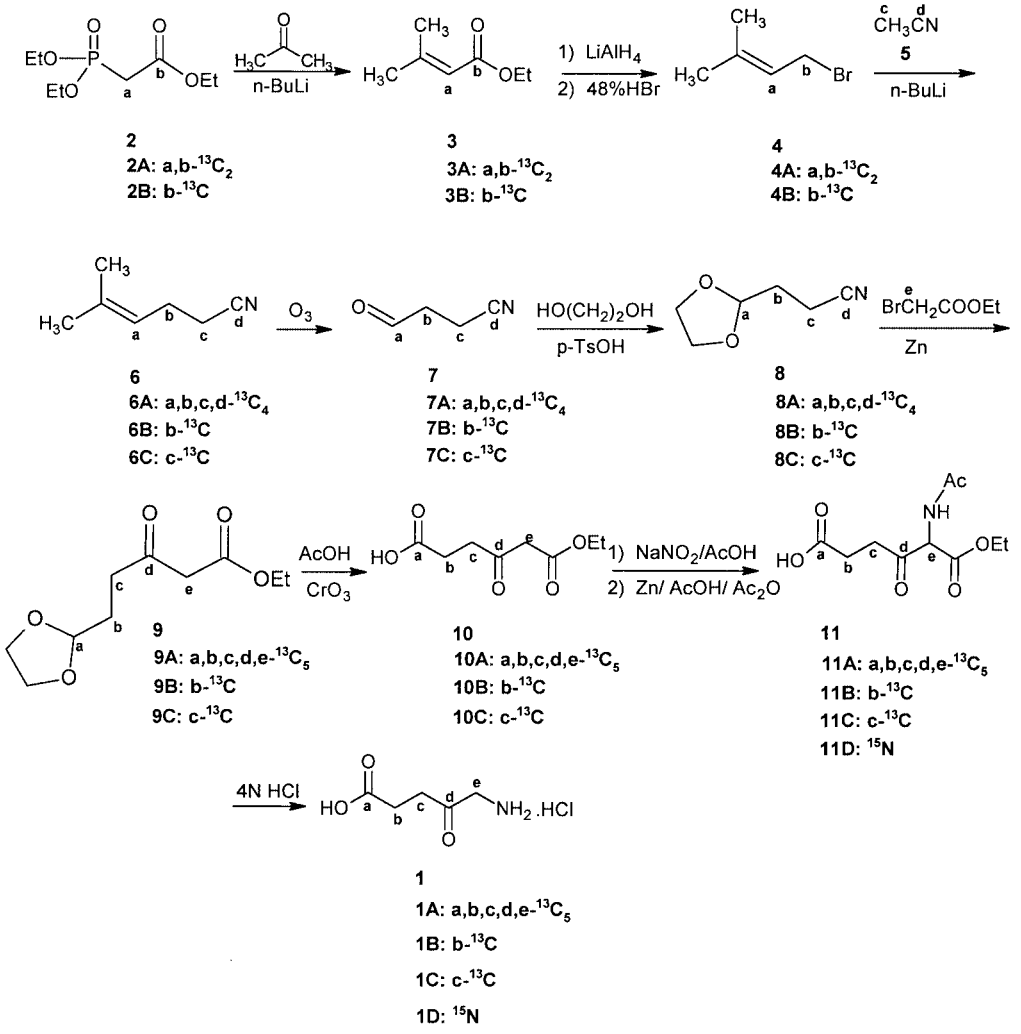
  DOI: 10.1002/ejoc.200300408



Scheme 1. Synthesis of 5-aminolevulinic acid in any isotopomeric form starting from triethyl phosphorylacetate (**2**); small letters indicate the ^{13}C-enriched labeled carbon atoms in the synthesis of ALA (**1**), [1,2,3,4,5-$^{13}C_5$]ALA (**1A**), [2-^{13}C]ALA (**1B**), [3-^{13}C]ALA (**1C**), and [^{15}N]ALA (**1D**)

riched ALA.[2] It was very rewarding that we established that the two chlorophylls in the so-called special pair show different interactions with the protein. This has motivated us to prepare the individually ^{13}C-enriched 1,2,3,4,5-$^{13}C_5$-ALA by a novel synthetic scheme (Scheme 1) that allows the efficient preparation of any site-directed ^{13}C- and ^{15}N-isotopomer of ALA and which is the subject of this study. Previously published schemes are unable to give access to all isotopomers of ALA.[3−11]

Access to ^{13}C-enriched ALAs may be similarly important in the study of biological systems involving porphyrin ligands, which play a role in electron transport, redox reactions, catalytic processes, etc. ALA is also used as a porphyrin precursor in a human photodynamic anticancer therapy. Using these isotopically enriched ALAs and mass spectrometry techniques, all metabolic products involved in this therapy may be monitored at the undisturbed physiological or therapeutic level in the individual patient, just as we recently pioneered with other groups for β-carotene uptake and metabolism in human nutrition.[12,13]

Results and Discussion

Scheme 1 indicates the steps that lead to ALA labeled at any position with ^{13}C and ^{15}N up to the 100% level. The steps were first optimized by reactions with reagents incorporating isotopes in their natural abundance. The first step starting from ethyl 2-(diethylphosphoryl)acetate (**2**) via ethyl 3-methyl-2-butanoate (**3**) into 4-bromo-2-methyl-2-butene (**4**) has already been described by our group.[14] Treatment of the anion of acetonitrile (**5**) at −70 °C with bromide **4** by an S_N2 reaction resulted in 5-methyl-4-hexenenitrile (**6**). Ozonolysis of compound **6** at −60 °C in CH_2Cl_2 yielded 3-cyanopropanal (**7**) in good yield. Protection of the aldehyde function in **7** as glycol acetal **8** and subsequent reaction with the organozinc derivative of a bromoacetic ester (Blaise reaction[15]) gave ethyl 5-(1,3-dioxolan-2-yl)-3-oxopentanoate (**9**). Deprotection of the acetal group and subsequent oxidation of the aldehyde group in **9** with chromium trioxide in acetic acid yielded the 1-ethyl ester **10** of 3-oxohexanedioic acid.

Treatment of ester **10** with sodium nitrite in acetic acid gave the 2-oximino derivative which was further converted into the 2-acetamido derivative **11** by reductive acetylation of the oxime in 4 N aqueous HCl, yielding the HCl salt **1** of 5-aminolevulinic acid. After optimization of this scheme, we prepared [1,2,3,4,5-$^{13}C_5$]ALA (**1A**) starting from ethyl [1,2-$^{13}C_2$]-2-(diethylphosphoryl)acetate (**2A**) and [2-^{13}C]ALA (**1B**) starting from ethyl [1-^{13}C]-2-(diethylphosphoryl)acetate (**2B**); [3-^{13}C]ALA (**1C**) was prepared via [2-^{13}C]acetonitrile (**5**) and [^{15}N]ALA (**1D**) was prepared using $Na^{15}NO_2$ in the conversion of **10** into **11D**. Except for [2-^{13}C]ALA (**1B**) all other singly labeled ALAs are commercially available.

All the analytical properties of **1A**, **1B**, **1C**, and **1D** are in agreement with authentic ALA. ^{1}H NMR and ^{13}C NMR spectroscopy allowed us to establish the position and the amount of isotope enrichment in the prepared compounds.

Figure 2 depicts the significant regions of the ^{1}H NMR spectrum (600 MHz, D_2O) of the uniformly labeled [1,2,3,4,5-$^{13}C_5$]ALA (I), as well as the spectrum of the same sample recorded with ^{1}H{^{13}C}-decoupling (II) and, for comparison, the spectrum of natural-abundance ALA (III). In Figure 2 (I), signals in the 2−3-ppm region are apparently due to coupling between 2-C and 2-H ($^1J_{C,H}$ = 127.5 Hz) and 3-C and 3-H ($^1J_{C,H}$ = 128.6 Hz) and signals in the 3.5−4.5-ppm region are apparently due to coupling between 5-C and 5-H ($^1J_{C,H}$ = 143.5 Hz). From the spectrum it is clear that all hydrogen-bonded carbon atoms have a very high ^{13}C incorporation and show the expected ^{1}H signals for ALA. Figure 2 (IV and V)show the ^{1}H NMR spectra of [2-^{13}C]ALA and [3-^{13}C]ALA which are also consistent with a very high ^{13}C incorporation in positions 2 and 3, respectively. Figure 2 (VI) shows the ^{1}H NMR spectrum of [^{15}N]ALA, and the expected ^{1}H signals are observed to be quite similar to those of Figure 2 (III; 5-ALA). The coupling ($^1J^{15}_{N\text{-}H}$ = 74.4 Hz) of ^{15}N and H of the NH_2 functional group of [^{15}N]ALA is observed in the region of δ = 8 ppm (see Expt. Sect.) and the ^{15n} signal is observed

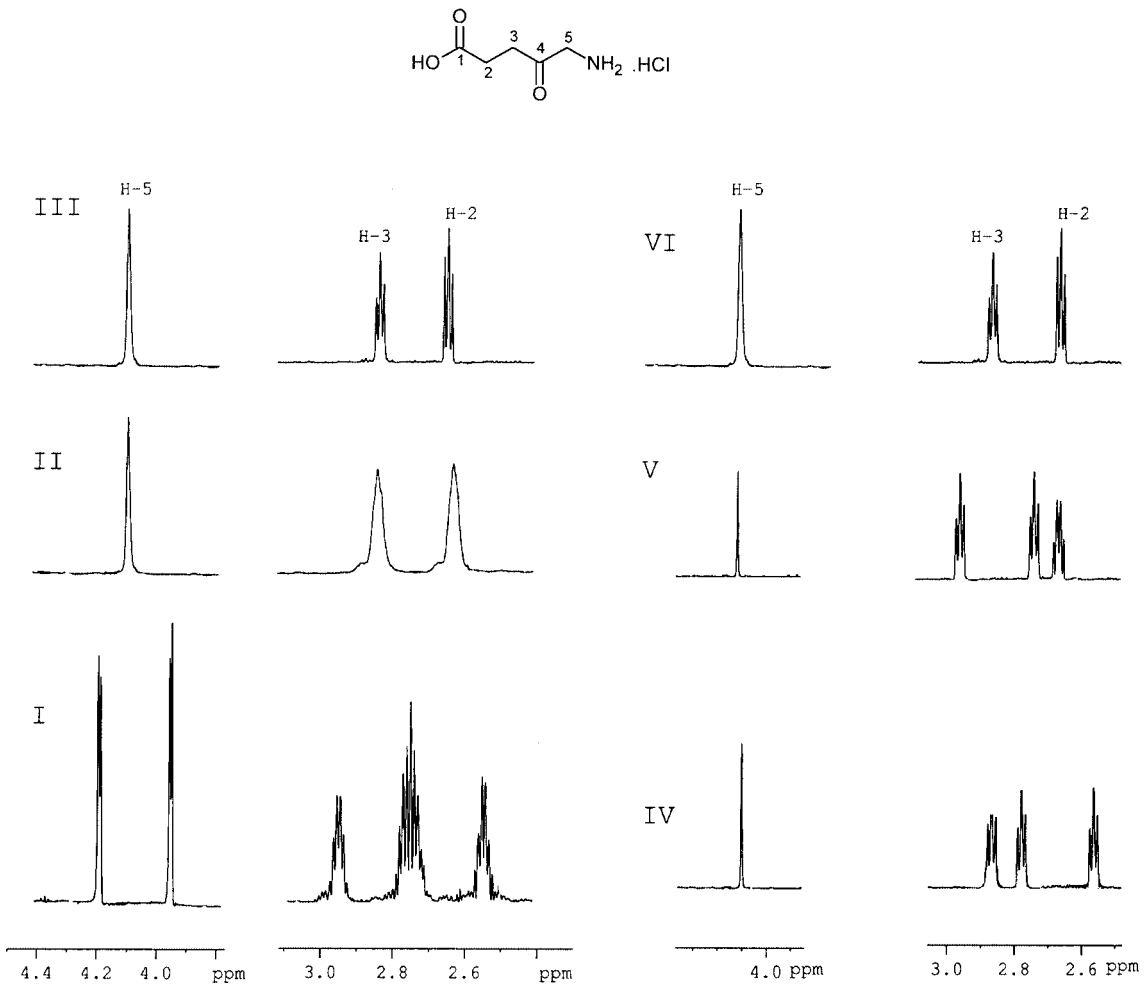


Figure 2. Significant regions of the ^{1}H NMR spectrum (600 MHz, D_2O) of [1,2,3,4,5-$^{13}C_5$]ALA (I), {^{13}C}-decoupled [1,2,3,4,5-$^{13}C_5$]ALA (II), natural-abundance ALA (III), [2-^{13}C]ALA (IV), [3-^{13}C]ALA (V), and [^{15}N]ALA (VI)

at δ = 27.72 ppm. The observed resonances and coupling constants for the ^{1}H NMR spectrum of [1,2,3,4,5-$^{13}C_5$]ALA (**1A**) are shown in Table 1.

Table 1. ^{1}H NMR data of [1,2,3,4,5-$^{13}C_5$]-5-Aminolevulinic acid hydrochloride (**1A**)

δ [ppm]	-H	Multiplicity	*J* [Hz]	Intensity
4.09	5-	dd	$^1J_{C\text{-}H}$ = 143.5 $^2J_{C\text{-}C}$ = 4.05	2
2.85	3-	dm	$^1J_{C\text{-}H}$ = 128.6	2
2.65	2-	dm	$^1J_{C\text{-}H}$ = 127.5	2

In Figure 3 the ^{13}C NMR spectra (150 MHz, D_2O) of the isotopically enriched ALA are reproduced. Figure 3 (I) shows that all carbon atoms are highly enriched. In Figure 3 (II) of [^{15}N]-ALA the coupling ($^1J_{^{13}C\text{-}^{15}N}$ = 7.32 Hz) between 5-C and ^{15}N can be seen. Figure 3 (III) shows non-enriched ALA for comparison. The other ^{13}C-enriched ALAs show the high intensity of the enriched atoms at the expected frequencies. The observed resonances and coupling constants for the ^{13}C NMR spectrum of [1,2,3,4,5-$^{13}C_5$]ALA (**1A**) are shown in Table 2.

Table 2. ^{13}C NMR data of [1,2,3,4,5-$^{13}C_5$]-5-Aminolevulinic acid hydrochloride (**1A**)

δ [ppm]	C-	Multiplicity	*J* [Hz]
204.5	-4	t	$^1J_{C\text{-}C}$ = $^1J_{C\text{-}C}$ = 38.09
177.6	-1	d	$^1J_{C\text{-}C}$ = 54.20
47.56	-5	dd	$^1J_{C\text{-}C}$ = 39.55 $^2J_{C\text{-}C}$ = 17.58
35.03	-3	td	$^1J_{C\text{-}C}$ = $^1J_{C\text{-}C}$ = 39.55 $^2J_{C\text{-}C}$ = 17.57
27.67	-2	dd	$^1J_{C\text{-}C}$ = 54.20 $^1J_{C\text{-}C}$ = 38.06

Before we arrived at the synthetic strategy depicted in Scheme 1 we also tried possible alternative approaches to the synthesis of ALA starting from ethyl propenoate (**12**; Scheme 2). Preparation of the complete set of isotopomers of ethyl propenoate (**12**) from simple starting materials has been described earlier by our group.[16,17] 1,4-Addition of KCN to ethyl propenoate (**12**) leads to ethyl 3-cyanopro-

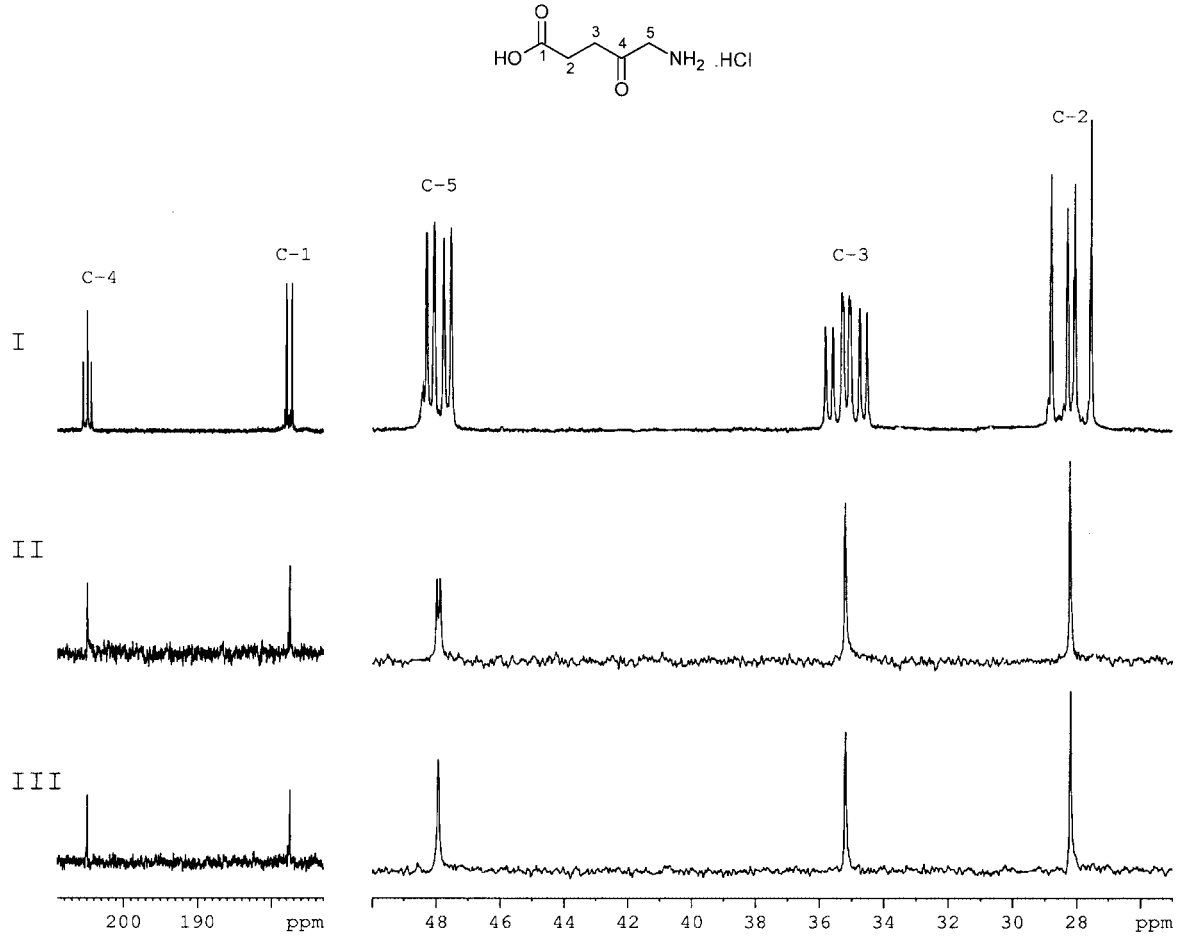
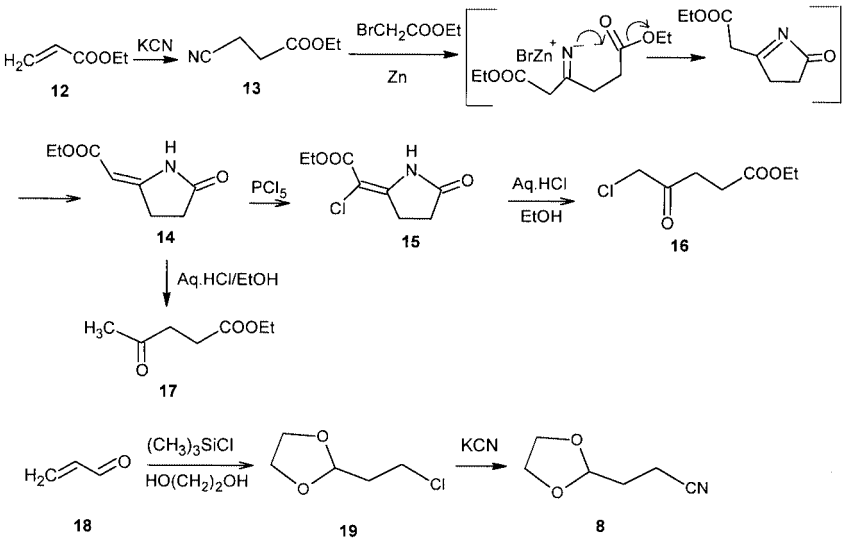


Figure 3. Significant regions of the ^{13}C NMR spectrum (75 MHz, D_2O) of [1,2,3,4,5-$^{13}C_5$]ALA (I), [^{15}N]ALA (II), and natural-abundance ALA (III)



Scheme 2. Synthesis of 5-chlorolevulinic acid (**16**) and ethyl levulinate (**17**) starting from ethyl propenoate (**12**); synthesis of 3-(1′,3′-dioxolan-2′-yl)propanenitrile (**8**) from acrolein (**18**)

panoate (**13**) in high yield. It is known that the zinc ester enolate (Blaise reaction) selectively attacks the nitrile function without attacking the ester part. However, the intermediate imino nitrogen anion attacks the ester function forming a five-membered ring compound to afford ethyl (2-oxo-3,4-dihydro-2*H*-pyrrol-5-yl)acetate which is converted into ethyl (2*Z*)-(5-oxopyrrolidin-2-ylidene)acetate (**14**; Scheme 2), which is more stable than the corresponding (*E*) isomer as a result of intramolecular hydrogen bonding between the NH and carbonyl groups. The analytical data for compound **14** are in complete agreement with those published in the literature.[18,19] In principle, this could be a useful result because compound **14** has the full carbon skeleton of ALA which could possibly be converted into ALA by simple reaction steps. Nitrosation of compound **14** could lead to the introduction of a nitrogen function in the vinylic position, which on catalytic reduction and subsequent hydrolysis might lead to ALA. However, nitrosation of **14** did not lead to useful products. Subsequently, we tried various electrophilic chlorination reactions; the best turned out to be treatment with PCl_5, which led to the chloro-substituted product **15** in good yield (85%). Subsequent treatment with aqueous HCl gave ethyl 5-chlorolevulinoate (**16**) in 60% yield, which upon Gabriel reaction with phthalilimide leads to ALA in low yield. This reaction could not be sufficiently optimized to give acceptable yields. Furthermore, HCl-catalyzed hydrolysis of ethyl (2*Z*)-(5-oxopyrrolidin-2-ylidene)-acetate (**14**) gave ethyl levulinic acid ester in good yield. Ethyl levulinate, which is also a biologically and chemically important molecule, is now accessible in any isotopomeric form.

Acrolein (**18**) can be easily converted into 2-(2-chloroethyl)-1,3-dioxolane[20] (**19**) in high yield. It is known that 3-(1′,3′-dioxolan-2′-yl)propanenitrile (**8**) can be prepared by smooth displacement of chloride with KCN in warm H_2O. Using Scheme 2, only ALA can be prepared with ^{13}C introduced in positions 4 and 5 and ^{15}N on the amino group. The bottleneck is the lack of a simple preparation of the full cassette of all the isotopomers of the very reactive and highly volatile propenal (**18**). Ethyl propenoate (**12**) is available in all isotopomeric forms. However, we did not succeed in converting the ester function to the aldehyde function of propenal (**18**) in acceptable yields. Scheme 1 indicates how compound **8** can be efficiently converted into any isotopomers of ALA.

Conclusion

In this paper we describe a synthetic scheme to prepare any isotopically labeled 5-amino-4-oxopentanoic acid (ALA). This is demonstrated by preparing and characterizing [1,2,3,4,5-$^{13}C_5$]ALA (**1A**) and singly isotopically labeled [2-^{13}C]ALA (**1B**), [3-^{13}C]ALA (**1C**), and [^{15}N]ALA (**1D**). A Blaise reaction of 3-cyanopropanal acetal with the zinc enolate of ethyl bromoacetate gave the synthon with the full carbon skeleton of ALA which could easily be converted into any isotopomer of ALA in a few steps in high yield. Earlier we tried in a similar way to use a Blaise reaction with ethyl 3-cyanopropanoate to obtain a synthon with the full carbon skeleton of ALA. However, in this case we were unable to reach ALA in an acceptable yield. But this synthon could easily be converted into any ^{13}C labeled isotopomer of the ethyl ester of levulinic acid.

Experimental Section

General: All experiments were carried out under dry nitrogen, unless aqueous conditions were used. Tetrahydrofuran (THF) and diethyl ether were distilled from benzophenone ketyl. Saturated solutions of $NaHCO_3$ and NH_4Cl refer to saturated solutions of the salt in water. Brine refers to a saturated solution of NaCl in water. Reactions were monitored using thin-layer chromatography (TLC) on Merck F254 silica gel 60 aluminum sheets, 0.2 mm; spots were

visualized by treatment with an oxidizing spray [$KMnO_4$ (2 g) and $NaHCO_3$ (4 g) in water (100 mL)]. Column chromatography was performed on Merck silica gel 60. ^1H NMR spectra were recorded with a Bruker WM-300 instrument with tetramethylsilane (TMS: $\delta = 0.00$ ppm) as internal standard. ^1H noise-decoupled ^{13}C spectra were recorded with a Bruker AM-600 instrument at 150 MHz and a Bruker WM-300 instrument at 75 MHz with chloroform ($\delta = 77.0$ ppm) as internal standard. Mass spectra were recorded with a Finnigan MAT 900 spectrometer equipped with a direct insertion probe (DIP) or with a Finnigan MAT 700-TSQ instrument equipped with a custom-made electrospray interface (ESI). The experimental conditions are given for the unlabeled compounds. For labeled compounds, only the changes relative to the corresponding unlabeled compounds are given. [1,2-$^{13}C_2$]Bromoacetic acid, [1-^{13}C]bromoacetic acid [2-^{13}C]bromoacetic acid, [1,2-$^{13}C_2$]acetonitrile, [2-^{13}C]acetonitrile, and $Na^{15}NO_2$ were purchased from Cambridge Isotope Laboratories Inc.,USA. All other chemicals were purchased from Aldrich Fluka or Across Chimica.

Ethyl 2-(Diethylphosphoryl)acetate (2): Triethyl phosphite (6.00 g, 36 mmol) was added to ethyl bromoacetate (5.07 g, 30 mmol), prepared according to a literature procedure.[21] The mixture was heated at 130−140 °C for 3 h, then allowed to cool to room temperature. The product was purified by chromatography on silica gel (ethyl acetate/hexane, 80:20) to give **2** (6.33 g, 28 mmol, 93%) as a light-yellow oil. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 4.18$ (m, 6 H, O*CH*$_2$CH$_3$), 2.96 (d, $^2J_{P\text{-}H} = 21.62$ Hz, 2 H, CH_2), 1.35 (m, 9 H, OCH$_2$*CH*$_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 165.1$ (C=O), 62.05, 61.94, 60.86, 33.74 (d, $^1J_{C,P} = 134.7$ Hz, CH_2), 15.76, 15.61, 13.41 ppm.

Ethyl [1,2-$^{13}C_2$]-2-(Diethylphosphoryl)acetate (2A): Similarly, ethyl [1,2-$^{13}C_2$]bromoacetate (5.07 g, 30 mmol) yielded **2A** (6.33 g, 28 mmol, 93%). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 4.18$ (m, 6 H, O*CH*$_2$CH$_3$), 2.91 (ddd, $^1J_{C,H} = 129.7$, $^2J_{P\text{-}H} = 21.63$, $^2J_{C,H} = 7.21$ Hz, 2 H, CH_2), 1.35 (m, 9 H, OCH$_2$*CH*$_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 165.6$ (dd, $^1J_{C\text{-}C} = 58.60$, $^2J_{C,P} = 5.86$ Hz, C=O), 33.74 (dd, $^1J_{C,P} = 134.7$, $^1J_{C\text{-}C} = 58.6$ Hz, CH_2) ppm.

Ethyl [1-^{13}C]-2-(Diethylphosphoryl)acetate (2B): Similarly, ethyl [1-^{13}C]bromoacetate (3.86 g, 23 mmol) yielded **2B** (4.72 g, 21 mmol, 91%). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 4.18$ (m, 6 H, O*CH*$_2$CH$_3$), 2.93 (dd, $^2J_{P\text{-}H} = 21.63$, $^2J_{C,H} = 7.21$ Hz), 1.35 (m, 9 H, OCH$_2$*CH*$_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 165.6$ (d, $^2J_{C,P} = 5.86$ Hz, C=O) ppm.

Ethyl 3-Methyl-2-butanoate (3): *n*BuLi (18.75 mL of a 1.6 M solution in hexane, 30 mmol) was added through a syringe to ethyl 2-(diethylphosphoryl)acetate (**2**) (6.30 g, 28 mmol), dissolved in dry tetrahydrofuran (100 mL) at −70 °C. Acetone (5.80 g, 100 mmol) was added to the anion of the phosphonate. The mixture was stirred at −50 °C for 30 min, then at room temperature for 2 h. The reaction was quenched by addition of a saturated NH_4Cl solution (50 mL). The aqueous layer was extracted with diethyl ether (3 × 100 mL) and the organic fractions were collected, dried with $MgSO_4$, and filtered. Evaporation of the solvent in vacuo yielded **3** (3.25 g, 25 mmol, 89%) as a light-yellow oil. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.67$ (m, 1 H, C=*CH*), 4.16 (q, $^3J_{H,H} = 7.21$ Hz, 2 H, O*CH*$_2$CH$_3$), 2.17 [d, $^4J_{H,H} = 1.37$ Hz, (*Z*)-CH$_3$], 1.89 [d, $^4J_{H,H} = 1.01$ Hz, (*E*)-CH$_3$], 1.27 (t, $^3J_{H,H} = 7.21$ Hz, OCH$_2$*CH*$_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 165.4$ (C=O), 155.41 (*C*=CH), 115.5 (C=*CH*), 58.79 (CH_2), 26.74 (CH_3), 19.08 (CH_3), 14.68 (CH_3) ppm.

Ethyl [1,2-$^{13}C_2$]-3-Methyl-2-butanoate (3A): Similarly, ethyl [1,2-$^{13}C_2$]-2-(diethylphosphoryl)acetate (**2A**) (6.30 g, 28 mmol) yielded **3A** (3.25 g, 25 mmol, 89%). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.66$ (dm, $^1J_{C,H} = 160.9$ Hz), 4.15 (qd, $^3J_{H,H} = 7.21$, $^3J_{C,H} = 3.09$ Hz), 2.17 (d, $^3J_{C,H} = 4.46$ Hz), 1.88 (d, $^3J_{C,H} = 5.84$ Hz), 1.27 (t, $^3J_{H,H} = 7.21$ Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 166.3$ (d, $^1J_{C\text{-}C} = 76.17$ Hz), 115.5 (d, $^1J_{C\text{-}C} = 76.17$ Hz) ppm.

Ethyl [1-^{13}C]-3-Methyl-2-butanoate (3B): Similarly, ethyl [1-^{13}C]-2-(diethylphosphoryl)acetate (**2B**) (4.72 g, 21 mmol) yielded **3B** (2.32 g, 18 mmol, 86%). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.67$ (m, CH), 4.15 (qd, $^3J_{H,H} = 7.21$, $^3J_{C,H} = 3.09$ Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 166.1$ (C=O) ppm.

1-Bromo-3-methyl-2-butene (4): A solution of ethyl 3-methyl-2-butanoate (**3**) (3.25 g, 25 mmol) was added to a stirred suspension of $LiAlH_4$ (1.42 g, 37.5 mmol) in dry tetrahydrofuran (150 mL) at 0 °C. The mixture was stirred at 0 °C for 15 min and at room temperature for a further 30 min. Na_2SO_4 (20 g) mixed with water (5 g) was added to the mixture in portions at 0 °C. After stirring the mixture at room temperature for 30 min, the solid mass was filtered off. The residue was thoroughly washed with diethyl ether (3 × 50 mL), and the eluents were collected and combined. Evaporation of the solvent in vacuo yielded 3-methyl-2-buten-1-ol (2 g, 23 mmol, 91%) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.39$ (tm, $^3J_{H,H} = 8.21$ Hz, 1 H, C=*CH*), 4.08 (br. s, 2 H, CH_2), 1.74 (CH_3), 1.67 (CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 135.1 (*C*=CH), 123.2 (C=*CH*), 58.46 (CH_2), 25.07 (CH_3), 17.16 (CH_3) ppm. HBr (30 mL of 48% solution) was added to 3-methyl-2-buten-1-ol (2 g, 23 mmol) in dichloromethane (75 mL) at 0 °C. Stirring was continued at 0 °C for 90 min with the exclusion of light, then CH_2Cl_2 (50 mL) and $MgSO_4$ (ca. 10 g) were added to the mixture. The aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL). The CH_2Cl_2 layers were combined, dried with $MgSO_4$, and filtered. The solvent was evaporated in vacuo to yield 1-bromo-3-methyl-2-butene (**4**) (2.90 g, 19 mmol, 83%) as a colorless oil. The product was used without further purification. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.57$ (tm, $^3J_{H,H} = 8.21$ Hz, 1 H, CH), 4.03 (d, $^3J_{H,H} = 8.58$ Hz, 2 H, CH_2), 1.78 (s, 3 H, CH_3), 1.73 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 139.4$ (*C*=CH), 120.2 (C=*CH*), 29.10 (CH_2), 25.22 (CH_3), 16.96 (CH_3) ppm.

[1,2-$^{13}C_2$]-1-Bromo-3-methyl-2-butene (4A): Similarly, $LiAlH_4$ (1.42 g, 37.5 mmol) in dry tetrahydrofuran (150 mL) was added at 0 °C to a solution of ethyl [1,2-$^{13}C_2$]-3-methyl-2-butanoate (**3A**) (3.25 g, 25 mmol) and further worked up as described above to yield [1,2-$^{13}C_2$]-3-methyl-2-buten-1-ol (2 g, 23 mmol, 91%) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.39$ (dm, $^1J_{C,H} = 153.1$ Hz, CH), 3.97 (dm, $^1J_{C,H} = 142.1$ Hz, CH_2), 1.75 (d, $^3J_{C,H} = 5.83$ Hz, CH_3), 1.66 (d, $^3J_{C,H} = 4.47$ Hz, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 122.6$ (d, $^1J_{C\text{-}C} = 46.84$ Hz), 58.29 (d, $^1J_{C\text{-}C} = 48.34$ Hz) ppm. HBr (30 mL of 48% solution) was added at 0 °C to a solution of [1,2-$^{13}C_2$]-3-methyl-2-buten-1-ol (2.00 g, 23 mmol) in dichloromethane (75 mL) and worked up to yield [1,2-$^{13}C_2$]-1-bromo-3-methyl-2-butene (**4A**) (2.90 g, 19 mmol, 83%) as a colorless oil. The product was used without further purification. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.21$ (dm, $^1J_{C,H} = 142.1$ Hz, CH), 3.63 (ddd, $^1J_{C,H} = 161.5$, $^2J_{C,H} = 8.59$, $^2J_{H,H} = 4.12$ Hz, CH_2), 1.79 (d, $^3J_{C,H} = 6.18$ Hz, CH_3), 1.72 (d, $^3J_{C,H} = 6.05$ Hz, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 119.7$ (d, $^1J_{C\text{-}C} = 48.34$ Hz), 28.66 (d, $^1J_{C\text{-}C} = 48.34$ Hz) ppm.

[1-^{13}C]-1-Bromo-3-methyl-2-butene (4B): Similarly, ethyl [1-^{13}C]-3-methyl-2-butanoate (**3B**) (2.32 g, 18 mmol) yielded [1-^{13}C]-3-methyl-2-buten-1-ol (1.31 g, 15 mmol, 83%). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.41$, 3.75 (dd, $^1J_{C,H} = 141.7$, $^2J_{H,H} = 7.55$ Hz, CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 58.79$ ppm. [1-^{13}C]-3-

Methyl-2-buten-1-ol (1.31 g, 15 mmol) was further converted into [1-^{13}C]-1-bromo-3-methyl-2-butene (**4B**) (1.69 g, 11.26 mmol, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.55 (m, CH), 3.65 (dd, $^1J_{C,H}$ = 153.1, $^3J_{H,H}$ = 8.59 Hz, CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 29.25 ppm.

5-Methyl-4-hexenenitrile (6): *n*BuLi (12.8 mL of a 1.6 M solution in hexane, 20.5 mmol) was added through a syringe to a solution of acetonitrile (**5**) (0.86 g, 20 mmol) in dry tetrahydrofuran (50 mL) at −70 °C. After stirring the mixture at −70 °C for 15 min, 1-bromo-3-methyl-2-butene (**4**) (2.90 g, 19 mmol) in THF (25 mL) was added. The mixture was stirred at −50 °C for 30 min and at room temperature for a further 2 h. Saturated NH_4Cl solution (50 mL) was added, the aqueous phase was extracted with diethyl ether (3 × 100 mL), the organic fractions were collected, dried with $MgSO_4$, and filtered. Evaporation of the solvent in vacuo yielded **6** (1.92 g, 17 mmol, 85%) as a light-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.14 (m, CH), 2.35 (br. s, CH_2CH_2), 1.73 [(*Z*)-CH_3], 1.66 [(*E*)-CH_3] ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 134.8 (*C*=CH), 119.7 (C=*CH*), 118.2 (CN), 25.01 (CH_3), 23.52 (CH_2CH_2CN), 17.07 (CH2CH_2CN) ppm.

[1,2,3,4-$^{13}C_4$]-5-Methyl-4-hexenenitrile (6A): Similarly, [1,2-$^{13}C_2$]acetonitrile (**5**) (0.86 g, 20 mmol) and [1,2-$^{13}C_2$]-1-bromo-3-methyl-2-butene (**4A**) (2.90 g, 19 mmol) yielded **6A** (1.92 g, 17 mmol, 85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.10 (dm, $^1J_{C,H}$ = 143.1 Hz), 2.35 (dm, $^1J_{C,H}$ = 131.5 Hz), 1.73 (d, $^3J_{C,H}$ = 6.87 Hz), 1.64 (d, $^3J_{C,H}$ = 4.80 Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 119.2 (dm, $^1J_{C-C}$ = 43.94 Hz), 117.2 (m, CN), 22.74 (dd, $^1J_{C-C}$ = 57.13, $^1J_{C-C}$ = 32.21 Hz), 17.95 (dd, $^1J_{C-C}$ = 43.94, $^1J_{C-C}$ = 45.41 Hz) ppm.

[3-^{13}C]-5-Methyl-4-hexenenitrile (6B): Similarly, **4B** (1.69 g, 11.26 mmol), acetonitrile (0.50 g, 12 mmol) and *n*BuLi (7.8 mL, 12.5 mmol) yielded **6B** (1.05 g, 9.53 mmol, 85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.12 (m, CH), 2.35 (m, CH_2), 2.34 (dm, $^1J_{C,H}$ = 131.5 Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 17.07 ppm.

[2-^{13}C]-5-Methyl-4-hexenenitrile (6C): Similarly, **4** (3.72 g, 25 mmol), [1-^{13}C]acetonitrile (**5**) (1.05 g, 25 mmol) and *n*BuLi (16.25 mmol, 26 mmol) yielded **6C** (2.31 g, 21 mmol, 84%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.12 (m, CH), 2.35 (m, CH_2), 2.34 (dm, $^1J_{C,H}$ = 131.5 Hz, CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.55 ppm.

4-Oxobutanenenitrile (7): A solution of **6** (1.92 g, 17 mmol) in CH_2Cl_2 (150 mL) was ozonolysed at −60 °C until the solution turned pale blue. The mixture was stirred with dimethyl sulfide (2.33 g, 37.5 mmol) at room temperature for 2 h. After evaporation of the solvent, the crude product 3-cyanopropanal[22] (**7**) (1.35 g, 12 mmol, 71%) was obtained which was further used without purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.78 (s, 1 H, CHO), 2.98 (t, $^3J_{H,H}$ = 6.5 Hz, 2 H, CH_2CH_2CN), 2.64 (t, $^3J_{H,H}$ = 6.52 Hz, 2 H, CH_2CH_2CN) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 196.97 (C=O), 118.2 (CN), 38.27 (CH_2CH_2CN), 9.46 (CH_2CH_2CN) ppm.

[1,2,3,4-$^{13}C_4$]-4-Oxobutanenenitrile (7A): Similarly, **6A** (1.92 g, 17 mmol) was ozonolysed to yield crude **7A** (1.35 g, 12 mmol, 71%), which was further used without purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.78 (ddd, $^3J_{C,H}$ = 3.43, $^2J_{C,H}$ = 28.15, $^1J_{C,H}$ = 151.0 Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 196.22 (d, $^1J_{C-C}$ = 36.62 Hz), 118.2 (t, $^1J_{C-C}$ = 55.66 Hz, CN), 38.27 (t, $^1J_{C-C}$ = 36.63 Hz), 9.66 (dd, $^1J_{C-C}$ = 57.12, $^2J_{C-C}$ = 36.63 Hz) ppm.

[3-^{13}C]-4-Oxobutanenenitrile (7B): Similarly, **6B** (1.05 g, 9.53 mmol) was ozonolysed to yield **7B** (0.58 g, 7 mmol, 73%) as a light-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.79 (d, $^3J_{C,H}$ = 3.44 Hz, 1 H, CHO), 2.93, 2.64 (dt, $^1J_{C,H}$ = 132.5 Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 38.27 (d, $^1J_{C-C}$ = 44.80 Hz), 9.43 ppm.

[2-^{13}C]-4-Oxobutanenenitrile (7C): Similarly, **6C** (2.31 g, 21 mmol) was ozonolysed to yield **7C** (1.26 g, 15 mmol, 71%). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 38.41 ppm.

3-(1′,3′-Dioxolan-2′-yl)propanenitrile (8): A solution of 3-cyanopropanal (**7**) (1.35 g, 12 mmol) in dichloromethane (50 mL) was mixed with ethylene glycol (1.24 g, 20 mmol), *p*TsOH (0.50 g), and heated silica gel 60 (2.50 g). The mixture was refluxed for 2 h, filtered and the residue was washed with dichloromethane (3 × 20 mL). The solvent was removed in vacuo. The crude product was purified by column chromatography (ethyl acetate/hexane, 80:20; R_f = 0.70) to yield **8** (1.08 g, 8.24 mmol, 69%) as a slightly yellow oil.^{1}H NMR (200 MHz, $CDCl_3$,): δ = 4.98 (t, $^3J_{H,H}$ = 4.81 Hz, CH), 3.97 (m, 2 H, OCH_2), 3.89 (m, 2 H, OCH_2), 2.46 (t, $^3J_{H,H}$ = 7.21 Hz, CH2CH_2CN), 2.04 (td, $^3J_{H,H}$ = 7.21, $^3J_{H,H}$ = 3.77 Hz, CH_2CH_2CH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 118.7 (CN), 101.2 (CH), 64.62 (OCH_2CH_2O), 28.75 (CH_2), 10.59 (CH_2) ppm.

[1,2,3,2′-$^{13}C_4$]-3-(1′,3′-Dioxolan-2′-yl)propanenitrile (8A): Similarly, **7A** (1.35 g, 12 mmol) yielded **8A** (1.08 g, 8.24 mmol, 69%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.98 (dm, $^1J_{C,H}$ = 171.17, $^3J_{H,H}$ = 7.3, $^4J_{H,H}$ = 2.76 Hz, CH), 3.99 (m, 2 H, CH_2O), 3.91 (m, 2 H, CH_2O), 2.48 (dm, $^1J_{C,H}$ = 173.6 Hz, 2 H, CH_2CH_2CN), 2.05 (dm, $^1J_{C,H}$ = 169.8 Hz, CH_2CH_2CN) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 119.4 (d, $^1J_{C-C}$ = 57.13 Hz, CN), 101.70 (dd, $^1J_{C-C}$ = 43.94, $^2J_{C-C}$ = 4.40 Hz, CH), 28.57 (dd, $^1J_{C-C}$ = 43.94, $^1J_{C-C}$ = 35.16 Hz, CH_2CH_2CN), 10.84 (dd, $^1J_{C-C}$ = 57.13, $^1J_{C-C}$ = 35.16 Hz, CH_2CN) ppm.

[3-^{13}C]-3-(1′,3′-Dioxolan-2′-yl)propanenitrile (8B): Similarly, **7B** (0.58 g, 7 mmol) yielded **8B** (0.64 g, 5 mmol, 71%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.98 (td, $^3J_{H,H}$ = 6.62, $^2J_{C,H}$ = 6.34, CH), 4.00 (m, CH_2O), 3.90 (m, CH_2O), 2.44 (m, CH_2CN), 2.03 (dtd, $^1J_{C,H}$ = 127.4, $^3J_{H,H}$ = 7.33, $^3J_{H,H}$ = 3.85 Hz, CH-CH_2CH_2CN) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.69 ppm.

[2-^{13}C]-3-(1′,3′-Dioxolan-2′-yl)propanenitrile (8C): Similarly, **7C** (1.26 g , 15 mmol,) yielded **8C** (1.53 g, 12 mmol, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.99 (td, $^3J_{H,H}$ = 7.21, $^3J_{C,H}$ = 3.78 Hz, CH), 4.00 (m, CH_2O), 3.90 (m, CH_2O), 2.48 (dt, $^1J_{C,H}$ = 135.6, $^3J_{C,H}$ = 7.42 Hz, CH_2CN), 2.05 (m, CHCH_2CH_2CN) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 10.56 ppm.

Ethyl 5-(1′,3′-Dioxolan-2′-yl)-3-oxopentanoate (9): Ethyl [2-^{13}C]bromoacetate (4 drops) was added to a stirred mixture of activated Zn (2.00 g, 30 mmol) in THF (50 mL) at 80−90 °C under N_2. After stirring for 15 min, a solution of **8** (1.05 g, 8 mmol) in THF (20 mL) was added. This mixture was stirred for a further 15 min, a solution of ethyl bromoacetate (3.36 g, 20 mmol) in THF (50 mL) was added dropwise over a period of 30−45 min, and stirring was continued at 80−90 °C for a further 2−3 h. The mixture was allowed to cool to room temperature and quenched with 50% K_2CO_3 (10 mL). Water (25 mL) and THF (50 mL) were added and the mixture was stirred rapidly for 30 min. The clear solution was decanted and the residue was washed with THF (6 × 50 mL). Under reduced pressure the volume of the solvent was reduced to 100 mL and it was treated with 10% HCl (10 mL) and stirred at room temp. for 5−10 min. The mixture was successively washed with brine, 5% Na_2CO_3 and brine, dried with $MgSO_4$, and the solvents were evaporated to yield **9** as a light yellow-brown oil (1.50 g, 85%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.92 (t, $^3J_{H,H}$ = 4.11 Hz, CH), 4.21 (q, $^3J_{H,H}$ = 7.21 Hz, OCH_2CH_3), 3.93 (m, 2 H, OCH_2),

3.87 (m, 2 H, OCH_2), 3.47 (s, 2 H, CH_2), 2.68 (t, 2 H, CH_2CH_2CH), 1.99 (td, $^3J_{H,H}$ = 4.11 Hz, CH_2CH_2CH), 1.28 (t, $^3J_{H,H}$ = 7.21 Hz, OCH_2CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 201.4 (C=O) 166.5 (C=O), 102.3 (CH), 64.42, 60.74, 48.66, 36.16, 26.85, 13.51 ppm.

Ethyl [2,3,4,5,2′-$^{13}C_5$]-5-(1′,3′-Dioxolan-2′-yl)-3-oxopentanoate (9A): Similarly, **8A** (1.05 g, 8 mmol), activated Zn (2.00 g, 30 mmol) and ethyl [2-^{13}C]bromoacetate (3.36 g, 20 mmol) yielded **9A** (1.50 g, 85%). ^1H NMR (200 MHz, $CDCl_3$): δ = 4.92 (dm, $^1J_{C,H}$ = 174.7 Hz, 1 H, CH), 4.18 (q, $^3J_{H,H}$ = 7.12 Hz, 2 H, OCH_2CH_3), 3.93 (m, 2 H, OCH_2), 3.84 (m, 2 H, OCH_2), 3.47 (dd, $^1J_{C,H}$ = 130.4, $^2J_{C,H}$ = 5.84 Hz, 2 H, CH_2), 2.68 (dm, $^1J_{C,H}$ = 126.6 Hz, 2 H, CH_2CH_2CH), 1.99 (dm, $^1J_{C,H}$ = 131.7 HZ, 2 H, CH_2CH_2CH), 1.28 (t, 3 H, OCH_2CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 201.4 (t, $^1J_{C-C}$ = $^1J_{C-C}$ = 39.55 Hz, C=O),) 103.4 (dd, $^1J_{C-C}$ = 43.94, $^2J_{C-C}$ = 5.86 Hz, CH), 49.45 (dd, $^1J_{C-C}$ = 38.08, $^2J_{C-C}$ = 14.65 Hz, CH_2), 36.28 (tdd, $^1J_{C-C}$ = $^1J_{C-C}$ = 35.16, $^2J_{C-C}$ = 5.85, $^2J_{C-C}$ = 13.91 Hz), 26.79 (ddd, $^1J_{C-C}$ = $^1J_{C-C}$ = 43.95, $^2J_{C-C}$ = 5.86 Hz) ppm.

Ethyl [5-^{13}C]-5-(1′,3′-Dioxolan-2′-yl)-3-oxopentanoate (9B): Similarly, **8B** (0.64 g, 5 mmol) yielded **9B** (0.87 g, 4 mmol, 80%). ^1H NMR (200 MHz, $CDCl_3$): δ = 1.99 (dm, $^1J_{C-H}$ = 131.7 Hz, CH_2CH_2CH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 26.88 ppm.

Ethyl [4-^{13}C]-5-(1′,3′-dioxolan-2′-yl)-3-oxopentanoate (9C): Similarly, **8C** (1.53 g, 12 mmol) yielded **9C** (2.17 g, 10 mmol, 83%). ^1H NMR (200 MHz, $CDCl_3$): δ = 2.68 (dt, $^1J_{C-H}$ = 126.6, $^3J_{H-H}$ = 7.56 Hz, CH_2CH_2CH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 201.8 (d, $^1J_{C-C}$ = 41.05 Hz, C=O), 36.16 (CH_2CH_2CO) ppm.

α-Ethoxycarbonyl-β-oxoadipic Acid (10): CrO_3 (3.50 g) was added to a solution of **9** (1.50 g, 6.78 mmol) in 50% AcOH in H_2O (40 mL). The reaction mixture was stirred at room temp. for 16 h. The mixture was extracted with diethyl ether (6 × 100 mL), washed with brine, and dried with $MgSO_4$. The solvent was evaporated under reduced pressure to yield **10** as a light-yellowish oil (1.00 g, 87%) which crystallized after standing at 0 °C for 2 d. ^1H NMR (300 MHz, $CDCl_3$): δ = 4.22 (q, $^3J_{H-H}$ = 6.86 Hz, 2 H, OCH_2CH_3), 3.51 (s, 2 H, CH_2), 2.88 (t, $^3J_{H-H}$ = 6.18 Hz, 2 H, CH_2), 2.66 (t, $^3J_{H-H}$ = 6.52 Hz, 2 H, CH_2), 1.28 (t, $^3J_{H-H}$ = 7.21 Hz, 3 H, OCH_2CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 200.4 (C=O), 177.5 (C=O), 166.5 (C=O), 60.97 (OCH_2CH_3), 48.57 (CH_2), 36.54 (CH_2), 27.12 (CH_2), 13.45 (OCH_2CH_3) ppm.

[2,3,4,5,6-$^{13}C_5$]-α-Ethoxycarbonyl-β-oxoadipic Acid (10A): Similarly, **9A** (1.50 g, 6.78 mmol) yielded **10A** (1.00 g, 87%). ^1H NMR (300 MHz, $CDCl_3$): δ = 4.22 (q, $^3J_{H-H}$ = 6.86 Hz, 2 H, OCH_2CH_3), 3.51 (dd, $^1J_{C-H}$ = 130.4, $^2J_{C-H}$ = 6.52 Hz, CH_2), 2.88 (m, $^1J_{C-H}$ = 126.6 Hz, CH_2), 2.66 (m, $^1J_{C-H}$ = 128.6 Hz, CH_2), 1.28 (t, $^3J_{H-H}$ = 7.21 Hz, 3 H, OCH_2CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 200.2 (t, $^1J_{C-C}$ = $^1J_{C-C}$ = 39.55 Hz, C=O), 177.2 (d, $^1J_{C-C}$ = 57.13 Hz, C=O), 48.39 (dd, $^1J_{C-C}$ = 52.74, $^2J_{C-C}$ = 13.19 Hz, CH_2), 36.66 (td, $^1J_{C-C}$ = 39.55, $^2J_{C-C}$ = 13.18 Hz, CH_2), 27.32 (dd, $^1J_{C-C}$ = 55.66, $^1J_{C-C}$ = 38.08 Hz, CH_2) ppm.

[5-^{13}C]-α-Ethoxycarbonyl-β-oxoadipic Acid (10B): Similarly, **9B** (0.87 g, 4 mmol) yielded **10B** (0.57 g, 3 mmol, 75%). ^1H NMR (300 MHz, $CDCl_3$): δ = 4.22 (q, $^3J_{H-H}$ = 6.86 Hz, 2 H, OCH_2CH_3), 3.51 (s, CH_2), 2.88 (t, $^3J_{H-H}$ = 6.18 Hz, 2 H, CH_2), 2.66 (m, $^1J_{C-H}$ = 128.6 Hz, 2 H, CH_2), 1.28 (t, $^3J_{H-H}$ = 7.21 Hz, 3 H, OCH_2CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.12 ppm.

[4-^{13}C]-α-Ethoxycarbonyl-β-oxoadipic Acid (10C): Similarly, **9C** (2.17 g, 10 mmol) yielded **10C** (1.49 g, 7.88 mmol, 79%). ^1H NMR (300 MHz, $CDCl_3$): δ = 4.22 (q, $^3J_{H-H}$ = 6.86 Hz, 2 H, OCH_2CH_3), 3.51 (s, CH_2), 2.88 (m, $^3J_{C-H}$ = 128.6 Hz, 2 H, CH_2), 2.66 (t, $^3J_{H-H}$ = 6.52 Hz, 2 H, CH_2), 1.28 (t, $^3J_{H-H}$ = 7.21 Hz, 3 H, OCH_2CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 36.54 ppm.

5-(Acetylamino)-6-ethoxy-4,6-dioxohexanoic Acid (11): A solution of $NaNO_2$ (0.48 g, 7 mmol) in H_2O (10 mL) was added dropwise (45−60 min) to a cooled (0 °C) and stirred solution of **10** (1.00 g, 5.18 mmol) in glacial acetic acid (10 mL). The mixture was stirred at 0−5 °C for 4 h and then at room temp. for 18 h. The mixture was extracted with ethyl acetate (3 × 50 mL), the combined organic extracts were collected, washed with brine, and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield 6-ethoxy-5-oximino-4,6-dioxohexanoic acid (0.95 g, 4.27 mmol, 82%) as a light-yellow crystalline solid. ^1H NMR (300 MHz, $CDCl_3$): δ = 11.05 (br., COOH), 4.39 (q, $^3J_{H-H}$ = 6.8 Hz, 2 H, CH_2), 3.13 (t, 2 H, CH_2), 2.72 (t, 2 H, CH_2), 1.34 (t, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 193.2 (C=O), 177.7 (C=O), 161.1 (C=O), 149.5 (C=N), 62.00, 31.79, 26.76, 13.40 ppm. Zn dust (2.09 g, 32 mmol) was added in portions to a solution of 6-ethoxy-5-oximino-4,6-dioxohexanoic acid (0.95 g, 4.27 mmol), acetic anhydride (2.04 g, 20 mmol), and acetic acid (10 mL) over a period of 1 h. The mixture was stirred at room temp. for 18−24 h, filtered, and the residue was washed with ethyl acetate (3 × 20 mL). The solvent was evaporated under reduced pressure to yield **11** (0.90 g, 3.59 mmol, 84%) as a light-brown oil. ^1H NMR (200 MHz, $CDCl_3$): δ = 10.50 (br., COOH), 7.56 (br., NH), 5.27 (d, $^3J_{H-H}$ = 6.86 Hz, CH-NH), 4.21 (q, $^3J_{H-H}$ = 7.20 Hz, OCH_2CH_3), 3.02 (t, 2 H, CH_2), 2.75 (m, 2 H, CH_2), 2.06 (s, 3 H, CH_3), 1.29 (t, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 197.9 (C=O) 173.5 (C=O) 169.8 (C=O) 160.0 (C=O), 60.60, 60.48, 25.84, 25.00, 21.42, 13.48 ppm.

[1,2,3,4,5-$^{13}C_5$]-5-(Acetylamino)-6-ethoxy-4,6-dioxohexanoic Acid (11A): Similarly, **10A** (1.00 g, 5.18 mmol) yielded [1,2,3,4,5-$^{13}C_5$]-6-ethoxy-5-oximino-4,6-dioxohexanoic acid (0.95 g, 4.27 mmol, 82%) as a light-yellow crystalline solid. ^1H NMR (300 MHz, $CDCl_3$): δ = 4.39 (q, $^3J_{H-H}$ = 6.87 Hz, OCH_2CH_3), 3.11 (dm, $^1J_{C-H}$ = 137.4 Hz, CH_2), 2.72 (dm, $^1J_{C-H}$ = 137.4 Hz, CH_2), 1.34 (t, $^3J_{H-H}$ = 6.86 Hz, OCH_2CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 192.8 (dd, $^1J_{C-C}$ = 62.53, $^1J_{C-C}$ = 42.48 Hz, C=O), 177.8 (d, $^1J_{C-C}$ = 57.12 Hz, COOH), 149.5 (dd, $^1J_{C-C}$ = 61.52, $^2J_{C-C}$ = 11.72 Hz, C=N), 31.81 (td, $^1J_{C-C}$ = $^1J_{C-C}$ = 38.09, $^2J_{C-C}$ = $^2J_{C-C}$ = 11.73 Hz, CH_2), 26.74 (dd, $^1J_{C-C}$ = 55.67, $^1J_{C-C}$ = 38.09 Hz, CH_2) ppm. Zn dust (2.09 g, 32 mmol) was added in portions to a solution of [1,2,3,4,5-$^{13}C_5$]-6-ethoxy-5-oximino-4,6-dioxohexanoic acid (0.95 g, 4.27 mmol), acetic anhydride (2.04 g, 20 mmol), and acetic acid (10 mL) over a period of 1 h. The mixture was stirred at room temp. for 18−24 h, filtered, and the residue washed with ethyl acetate (3 × 20 mL). The solvent was evaporated under reduced pressure to yield **11A** (0.90 g, 3.59 mmol, 84%) as a light-brown oil. ^1H NMR (200 MHz, $CDCl_3$): δ = 10.50 (br., COOH), 7.56 (br., NH), 5.27 (d, $^3J_{H-NH}$ = 6.86 Hz, CH-NH), 4.21 (q, $^3J_{H-H}$ = 7.20 Hz, OCH_2CH_3), 3.02 (t, 2 H, CH_2), 2.75 (m, 2 H, CH_2), 2.06 (s, 3 H, CH_3), 1.29 (t, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 172.33 (dd, $^1J_{C-C}$ = $^1J_{C-C}$ = 45.41, $^2J_{C-C}$ = 4.3 Hz, C=O), 160.0 (dd, $^1J_{C-C}$ = 36.63 Hz, $^2J_{C-C}$ = 4.3 Hz, C=O), 101.58 (d, $^1J_{C-C}$ = 43.94 Hz), 25.28 (dm, $^1J_{C-C}$ = 43.41 Hz), 25.01 (dm, $^1J_{C-C}$ = 44.31 Hz) ppm.

[2-^{13}C]-5-(Acetylamino)-6-ethoxy-4,6-dioxohexanoic Acid (11B): Similarly, **10B** (0.57 g, 3 mmol) yielded [2-^{13}C]-6-ethoxy-5-oximino-4,6-dioxohexanoic acid (0.54 g, 2.5 mmol, 83%). ^1H NMR (300 MHz, $CDCl_3$): δ = 11.05 (br., COOH), 4.39 (q, 2 H, CH_2, $^3J_{H-H}$ = 6.8 Hz), 3.13 (m, 2 H, CH_2), 2.72 (dm, $^1J_{C-H}$ = 131.5 Hz,

2 H, CH_2), 1.34 (t, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.79 ppm. [2-^{13}C]-6-Ethoxy-5-oximino-4,6-dioxohexanoic acid (0.54 g, 2.50 mmol) yielded **11B** (0.49 g, 2 mmol, 80%). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.01 ppm.

[3-^{13}C]-5-(Acetylamino)-6-ethoxy-4,6-dioxohexanoic Acid (11C): Similarly, **10C** (1.49 g, 7.88 mmol) yielded [3-^{13}C]-6-ethoxy-5-oximino-4,6-dioxohexanoic acid (1.42 g, 6.50 mmol, 82%). ^1H NMR (300 MHz, $CDCl_3$): δ = 11.05 (br., COOH), 4.36 (q, $^3J_{H\text{-}H}$ = 6.8 Hz, OCH_2CH_3), 3.13 (dm, $^1J_{C\text{-}H}$ = 141.1 Hz, 2 H, CH_2), 2.72 (m, 2 H, CH_2), 1.34 (t, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 31.76 ppm. [3-^{13}C]-6-Ethoxy-5-oximino-4,6-dioxohexanoic acid (1.42 g, 6.50 mmol) yielded **11C** (1.35 g, 5.50 mmol, 85%). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.28 ppm.

[^{15}N]-5-(Acetylamino)-6-ethoxy-4,6-dioxohexanoic Acid (11D): A solution of $Na^{15}NO_2$ (1.00 g, 14.28 mmol) in H_2O (15 mL) was added dropwise (45−60 min) to a cooled (0 °C) and stirred solution of **10** (2.00 g, 10.63 mmol) in glacial acetic acid (10 mL). The mixture was worked up as for **11A** to yield [^{15}N]-6-ethoxy-5-oximino-4,6-dioxohexanoic acid (1.94 g, 8.90 mmol, 82%) as a light-yellow crystalline solid. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 192.68 (d, $^2J_{C\text{-}^{15}N}$ = 11.72 Hz, C=O) ppm. Zn dust (4.57 g, 70 mmol) was added in portions to a solution of [^{15}N]-6-ethoxy-5-oximino-4,6-dioxohexanoic acid (1.94 g, 8.90 mmol), acetic anhydride (5.10 g, 50 mmol), and acetic acid (25 mL) over a period of 1 h. The mixture was worked up as for **11A** to yield **11D** (1.84 g, 7.50 mmol, 84%) as a light-brown substance.

5-Aminolevulinic Acid Hydrochloride (1): A solution of **11** (0.90 g, 3.59 mmol) in 4 N HCl (10 mL) was refluxed for 3−3.5 h. A pinch of charcoal (Norit) was added to it, the mixture was filtered and the residue was washed with 2 N HCl (2 × 5 mL). The clear pale solution was concentrated under reduced pressure and the residue was crystallized to yield the colorless hydrochloride of aminolevulinic acid (**1**) (0.38 g, 2.20 mmol, 73%). ^1H NMR (600 MHz, D_2O): δ = 4.07 (s, 2 H, $CO\mathit{CH}_2NH_2$), 2.83 (t, $^3J_{H\text{-}H}$ = 6.26 Hz, 2 H, CH_2), 2.64 (t, $^3J_{H\text{-}H}$ = 6.26 Hz, 2 H, CH_2) ppm. ^{13}C NMR (150 MHz, D_2O): δ = 204.5(C=O), 177.21 (C=O), 47.64 (CH_2), 34.88 (CH_2), 27.91 (CH_2) ppm. FAB-MS (3-nitrobenzyl alcohol): *m*/*z* = 132 [MH^+ − HCl].

[1,2,3,4,5-$^{13}C_5$]-5-Aminolevulinic Acid Hydrochloride (1A): Similarly, **11A** (0.90 g, 3.59 mmol) yielded **1A** (0.38 g, 2.20 mmol, 73%), the colorless hydrochloride of aminolevulinic acid. ^1H NMR (600 MHz, D_2O): δ = 4.09 (dd, $^1J_{C\text{-}H}$ = 143.5, $^2J_{C\text{-}C}$ = 4.12 Hz, CH_2), 2.85 (dm, $^1J_{C\text{-}H}$ = 128.6 Hz, CH_2), 2.65 (dm, $^1J_{C\text{-}H}$ = 127.5 Hz, CH_2) ppm. ^{13}C NMR (150 MHz, D_2O): δ = 204.5 (t, $^1J_{C\text{-}C}$ = $^1J_{C\text{-}C}$ = 38.09 Hz, C=O), 177.6 (d, $^1J_{C\text{-}C}$ = 54.20 Hz, COOH), 47.56 (dd, $^1J_{C\text{-}C}$ = 39.55, $^2J_{C\text{-}C}$ = 17.58 Hz, CH_2), 35.03 (td, $^1J_{C\text{-}C}$ = $^1J_{C\text{-}C}$ = 39.55, $^2J_{C\text{-}C}$ = 17.57 Hz, CH_2), 27.67 (dd, $^1J_{C\text{-}C}$ = 54.20, $^2J_{C\text{-}C}$ = 38.06 Hz, CH_2) ppm. MS (ESI): *m*/*z* = 137 [MH^+ − HCl].

[2-^{13}C]-5-Aminolevulinic Acid Hydrochloride (1B): Similarly, **11B** (0.49 g, 2 mmol) in 4 N HCl (5 mL) yielded **1B** (0.28 g, 1.70 mmol, 77%) as a slightly yellow crystalline salt. ^1H NMR (600 MHz, D_2O): δ = 4.09 (s, CH_2), 2.85 (dm, $^2J_{C\text{-}H}$ = 6.52 Hz CH_2), 2.67 (dt, $^1J_{C\text{-}H}$ = 131.8, $^3J_{H\text{-}H}$ = 6.18 Hz, CH_2) ppm. ^{13}C NMR (150 MHz, D_2O): δ = 27.96 ppm. MS (ESI): *m*/*z* = 133 [MH^+ − HCl].

[3-^{13}C]-5-Aminolevulinic Acid Hydrochloride (1C): Similarly, **11C** (1.35 g, 5.50 mmol) in 4 N HCl (10 mL) yielded **1C** (0.76 g, 4.50 mmol, 82%) as a slightly yellow crystalline salt. ^1H NMR (600 MHz, D_2O): δ = 4.05 (s, CH_2), 2.83 (dt, $^1J_{C\text{-}H}$ = 129.4, $^3J_{H\text{-}H}$ = 5.83 Hz, CH_2), 2.64 (dm, $^2J_{C\text{-}H}$ = 6.52 Hz, CH_2) ppm. ^{13}C NMR (150 MHz, D_2O): δ = 204.8 (d, $^1J_{C\text{-}C}$ = 42.19 Hz), 47.97 (d, $^2J_{C\text{-}C}$ = 16.96 Hz), 34.85 ppm. FAB-MS (3-nitrobenzyl alcohol): *m*/*z* = 133 [MH^+ − HCl].

[^{15}N]-5-Aminolevulinic Acid Hydrochloride (1D): Similarly, **11D** (1.84 g, 7.50 mmol) in 4 N HCl (15 mL) yielded **1D** (1.02 g, 6.04 mmol, 81%) as a slightly yellow crystalline salt. ^1H NMR (600 MHz, D_2O): δ = 4.07 (s, 2 H, $CO\mathit{CH}_2NH_2$), 2.84 (t, $^3J_{H\text{-}H}$ = 6.10 Hz, 2 H, CH_2), 2.65 (t, $^3J_{H\text{-}H}$ = 6.10 Hz, 2 H, CH_2), 8.01 (br. d, $^{15}NH_2$, $^1J^{15}_{N\text{-}H}$ = 74.41 Hz) ppm. ^{13}C NMR (150 MHz, D_2O): δ = 204.5, 177.0, 47.75 (d, $^1J_{C\text{-}^{15}N}$ = 7.32 Hz), 34.97, 27.99 ppm. ^{15}N NMR (30 MHz, H_2O/D_2O, 90:10, saturated solution of NH_4NO_3 as an external standard): δ = 27.72 ppm. FAB-MS (3-nitrobenzyl alcohol): *m*/*z* = 133 [MH^+ − HCl].

Ethyl 3-Cyanopropanoate (13): A solution containing ethyl acrylate (**12**) (10.01 g, 100 mmol) in acetic acid (6.60 g, 110 mmol) was stirred at 0 °C while KCN (8.14 g, 125 mmol), dissolved in a mixture of EtOH (40 mL) and H_2O (20 mL), was added dropwise. The mixture was stirred at 0 °C for 3 h and then at room temp. for 24 h. Water (10 mL) was added to the mixture and the pH was adjusted with 2.5 N HCl to approximately 7. The mixture was extracted with diethyl ether (3 × 50 mL). The combined organic extracts were washed with brine and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a light-brown oil (8.09 g, 64%). Purification of the oil by column chromatography on silica gel 60 (eluted with ethyl acetate/hexane, 8:2; R_f = 0.55) yielded a light-yellow oil (7.50 g, 59%). ^1H NMR (200 MHz, $CDCl_3$): δ = 4.15 (q, $^3J_{H\text{-}H}$ = 6.8 Hz, 2 H, CH_2), 2.67 (br. t, 4 H, CH_2-CH_2), 1.32 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$,): δ = 117.9 (CN), 169.4 (C=O), 60.74, 29.36, 13.51, 12.35 ppm.

Ethyl (2*Z*)-(5-Oxopyrrolidin-2-ylidene)acetate (14): Ethyl bromoacetate (4 drops) was added to a stirred mixture of Zn (16.34 g, 250 mmol) and THF (150 mL) at 80−90 °C under N_2. After 15 min of stirring, a solution of **13** (6.36 g, 50 mmol) in THF (50 mL) was added. After stirring for a further 15 min, a solution of ethyl bromoacetate (33.40 g, 200 mmol) in THF (50 mL) was added dropwise over a period of 30−45 min, and stirring was continued at 80−90 °C for a further 2 h. The mixture was allowed to cool at room temp. and quenched with 50% K_2CO_3 (50 mL); H_2O (150 mL) and THF (150 mL) were added and the mixture stirred rapidly for 30 min. The clear solution was decanted and the residue was washed with THF (3 × 200 mL). Under reduced pressure the volume of the solvent was reduced to 100 mL, it was treated with 10% HCl (10 mL) and stirred at room temp. for 3 h. The mixture was extracted with CH_2Cl_2 (3 × 25 mL). The organic fractions were collected, washed with 5% $NaHCO_3$ and brine and dried with $MgSO_4$. The solvent was evaporated under reduced pressure (crude product 8.00 g, 94%), purified by column chromatography (silica gel 60; ethyl acetate/hexane, 8:2) to yield **14** (5.65 g, 67%). ^1H NMR (200 MHz, $CDCl_3$): (*E*) isomer: δ = 9.00 (br., 1 H, NH), 5.37 (m, 1 H, CH), 4.17 (q, $^3J_{H\text{-}H}$ = 7.21 Hz, 2 H, CH_2), 3.26 (m, 2 H, CH_2), 2.61 (m, 2 H, CH_2), 1.27 (t, $^3J_{H\text{-}H}$ = 7.21 Hz, 3 H, CH_3) ppm; (*Z*) isomer: δ = 9.80 (NH), 5.00 (CH), 4.18, 2.89, 2.54, 1.28 ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.7 (C=O), 166.8 (C=O), 157.8 (CN), 92.59, 59.05, 28.43, 25.51, 13.86 ppm; (*Z*) isomer: δ = 176.7, 167.5, 156.8, 89.59, 59.31, 27.14, 25.51, 13.80 ppm.

Ethyl 2-Chloro-(5-oxopyrrolidin-2-ylidene)acetate (15): PCl_5 (3.74 g, 18 mmol) was added to a stirred solution of **14** (2.19 g, 13 mmol) in dry toluene (20 mL). The mixture was stirred at room temp. for 30 min. The mixture was extracted with diethyl ether (4 × 100 mL).

The combined organic extracts were washed with brine and dried with $MgSO_4$. The solvent was evaporated under reduced pressure to yield a light-purple solid which was purified by column chromatography (silica gel 60; ethyl acetate/hexane, 8:2; R_f = 0.65) to yield **15** (1.64 g, 62%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 9.51 (br., 1 H, NH), 4.30 (q, $^3J_{H-H}$ = 7.21 Hz, 2 H, CH_2), 2.95 (m, 2 H, CH_2), 2.62 (m, 2 H, CH_2), 1.38 (t, $^3J_{H-H}$ = 7.2 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 176.7 (C=O), 163.8 (C=O), 153.7(C-N), 95.77 (C-Cl), 61.27, 27.35, 26.44, 13.72 ppm.

Ethyl 5-Chloro-4-oxopentanoate (16): Compound **15** (0.42 g, 2 mmol) was dissolved in EtOH (20 mL) and 37% HCl (7.50 mL) and refluxed for 6 h. The mixture was extracted with diethyl ether (3 × 25 mL). The combined organic extracts were washed with 5% $NaHCO_3$, brine and dried with $MgSO_4$. The solvent was evaporated under reduced pressure to yield **16** as a light-brown oil (0.20 g, 55%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.16 (s, 2 H, CH_2), 4.12 (q, 2 H, CH_2CH_3), 2.89 (t, 2 H, CH_2), 2.65 (t, 2 H, CH_2), 1.16 (t, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 200.56 (C=O), 171.5 (C=O), 60.19, 45.53, 33.68, 27.47, 13.54 ppm.

Ethyl 4-Oxopentanoate (17): A solution of **14** (0.85 g, 5 mmol) in EtOH (15 mL) and 7 N HCl (10 mL) was refluxed for 4 h. The mixture was treated with H_2O (50 mL), ether (50 mL), and 0.5 N HCl (10 mL) and stirred well. The mixture was extracted with diethyl ether (3 × 50 mL). The combined organic extracts were washed with brine and dried with $MgSO_4$. The solvent was evaporated under reduced pressure to yield **17** as a light-yellowish brown oil (0.50 g, 70%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.15 (q, $^3J_{H-H}$ = 7.21 Hz, 2 H, CH_2), 2.75 (t, 2 H, CH_2), 2.56 (t, 2 H, CH_2), 2.20 (s, 3 H, CH_3), 1.26 (t, $^3J_{H-H}$ = 7.21 Hz, 2 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 205.9 (C=O), 171.9 (C=O), 59.87, 29.16, 27.35, 13.51 ppm.

2-(2-Chloroethyl)-1,3-dioxolane (19): Trimethylsilyl chloride (21.71 g, 200 mmol) was added to a cooled (0 °C) and stirred solution of acrolein (**18**; 8.40 g, 150 mmol) in CH_2Cl_2 (50 mL). To the mixture was added a solution of ethylene glycol (9.31 g, 150 mmol) in CH_2Cl_2 (50 mL). The mixture was stirred at 0 °C for 15 min and at room temp. for 15 min, then refluxed for 4 h. The solvent was evaporated to yield a brown oil (20.01 g, 98%) which was purified by column chromatography (silica gel 60; ethyl acetate/hexane, 8:2; R_f = 0.52) to yield **19** as a light-yellow oil (14.04 g, 69%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 5.02 (t, $^3J_{H-H}$ = 4.12 Hz, 1 H, CH), 3.88−3.98 (m, 4 H, OCH_2CH_2O), 3.64 (t, 2 H, ClCH_2CH$_2$), 2.08 (td, 2 H, CH_2CH_2CH), ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 36.37, 39.08, 64.36 (2 C), 101.29 (CH) ppm.

Acknowledgments

This investigation was supported by the Netherlands Foundation for Chemical research (SON) with financial aid from the Netherlands Organization for Scientific research (NOW). The authors wish to thank F. Lefeber and C. Erkelens for recording the NMR spectra and H. Peters (Free University, Amsterdam, The Netherlands) for recording mass spectra.

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Received July 1, 2003
Early View Article
Published Online October 30, 2003

