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Synthesis of [3-^{13}C]-, [4-^{13}C]- and [11-^{13}C]-porphobilinogen

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[4-^{13}C]-porphobilinogen 1a, [3-^{13}C]-porphobilinogen 1b and [11-^{13}C]-porphobilinogen 1c are prepared from [1-^{13}C]-3-(tetrahydropyran-2′-yloxy)-propionaldehyde 2a, methyl [4-^{13}C]-4-nitrobutyrate 3b and [1-^{13}C]-isocyanoacetonitrile 5c, respectively. The building blocks 2, 3 and 5 can be prepared efficiently in any isotopomeric form. Via base-catalyzed condensation of these building blocks porphobilinogen can be enriched with ^{13}C and ^{15}N stable isotopes at any position and combination of positions.

Keywords: phorphobilinogen isotopomers; isotopic labeling; cyanopyrrole

Introduction

Tetrapyrrole molecules such as heme, chlorophyll and vitamin B_{12} play essential roles in life processes.[1] An early intermediate in their biosynthesis is 5-amino-4-oxopentanoic acid (5-aminolevulinic acid, ALA), which undergoes asymmetric condensation with another ALA to give porphobilinogen (Figure 1) as next intermediate.

We have published the preparation of [1,2,3,4,5-$^{13}C_5$]-5-amino-4-oxopentanoic acid (ALA) via a synthetic route that gives access to any isotopomer of ALA.[2] The addition of these labeled biosynthetic precursors to a suitable organism would enable the production of a large variety of site-directed labeled natural tetrapyrroles. In general, isotopically labeled (bacterio)-chlorophyll can be obtained by growing the microorganisms, which produce the required cofactor on the synthetic media supplemented with an isotopically enriched precursor ALA or porphobilinogen to study its roles in the living system at the atomic level without perturbation. Very recently, primary radical pair in the reaction center of membrane fragments of *Heliobacillus mobilis and Rhodobacter sphaeroides* that have been grown on media containing [4-^{13}C]-ALA have been characterized by the ^{13}C photo-CIDNAP MAS NMR.[3,4]

Porphobilinogen, the next unique biosynthetic precursor, will give a much better control of the final labeling pattern of (bacterio)chlorophyll and other essential tetrapyrrole systems in the study of tetrapyrrole biosynthesis because only four porphobilinogen molecules are incorporated in contrast to the eight molecules of ALA.

The preparation of [11-^{13}C]-porphobilinogen has been reported and its biosynthetic incorporation (enzymic transformation) together with the [2,11-$^{13}C_2$]-isotopomer of porphobilinogen into uroporphyrinogens I and III through a transient-free intermediate, pre-uroporphyrinogen (hydroxymethylbilane), produced by porphobilinogen deaminase (uroporphyrinogen I synthetase) via ^{13}C-NMR spectroscopic studies has been described.[5,6] In addition, [3,5-$^{13}C_2$]-porphobilinogen obtained via asymmetric condensation of [4-^{13}C]-aminolevulinic acid has been studied by Raman spectroscopy to probe the structure and mechanism of porphobilinogen synthase.[7] The synthetic routes used in the preparation of porphobilinogen did not allow the preparation of all possible stable labeled (^{13}C, ^{15}N) isotopomers. However, it is possible to synthesize isotopomers of porphobilinogen based on the base-promoted condensation[8] of labeled α-acetoxynitro compounds with labeled isocyanoacetonitrile (Scheme 1).

3-(Tetrahydropyran-2′-yloxy)-propionaldehyde **2** is condensed with methyl 4-nitrobutyrate **3** in an aldol-type condensation (Henry reaction) to give α-acetoxynitro compound, which condenses with isocyanoacetonitrile **5** to give the 5-cyanopyrrole **7**, the full carbon skeleton of porphobilinogen **1**. This strategy using the isotopically enriched building blocks **2**, **3** and **5** allows in a simple and convergent way to the preparation of isotopically labeled porphobilinogen at any position and combination of positions.

Results and discussion

In Scheme 1 it is shown that porphobilinogen **1** was prepared from 3-(tetrahydropyran-2′-yloxy)-propionaldehyde **2**, methyl 4-nitrobutyrate **3** and isocyanoacetonitrile **5**. For the preparation of porphobilinogen isotopomers labeled at different positions, we have used synthetic route shown in Scheme 2, which allows the building blocks **2**, **3** and **5** to be prepared in any possible ^{13}C- and ^{15}N-enriched forms. The conversions were first optimized through reactions using building blocks in non-labeled forms. After that we have selected the isotopically enriched building blocks **2a**, **3b** and **5c** to prepare porphobilinogen isotopomers **1a**, **1b** and **1c**, respectively (Figure 1).

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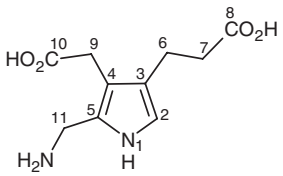
The ^{13}C-labeled bromoacetic acid, cyanoacetic acid, ethyl cyanoacetate and 3-hydroxypropionitrile **12** were prepared from ^{13}C-labeled acetic acid **9** according to literature procedures.[9–11] The hydroxyl function of 3-hydroxypropionitrile **12** (3.56 g, 50 mmol) was first protected with dihydropyrane via acid-catalyzed reaction to afford 3-(tetrahydropyran-2′-yloxy)-propionitrile (6.98 g, 91%). Subsequent DIBAL-H reduction of the nitrile function afforded 3-(tetrahydropyran-2′-yloxy)-propionaldehyde **2** as a light-yellow oil (6.02 g, 86%). All the reactions described above were carried out in stoichiometric amounts to obtain reasonable yields, which mean that Scheme 2 allows us to prepare product **2** in any stable ^{13}C- and ^{15}N-enriched forms.



1

1a: [4-^{13}C]

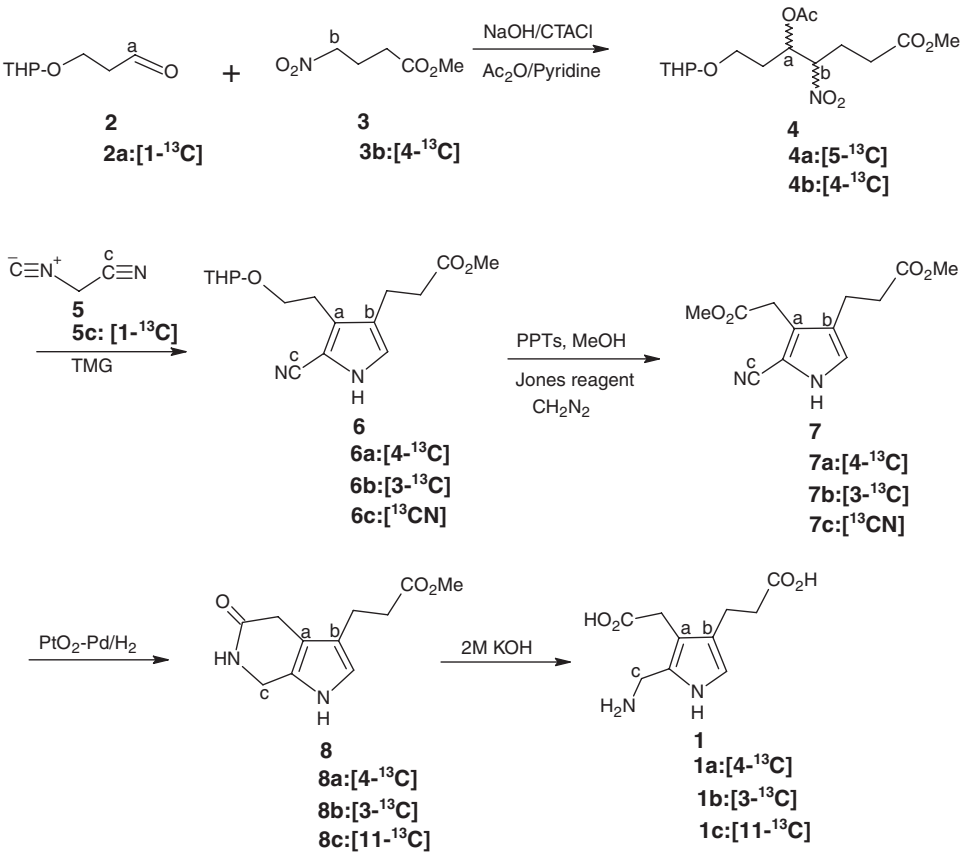
1b: [3-^{13}C]

1c: [11-^{13}C]

Figure 1. Structure and numbering of porphobilinogen **1** and its highly enriched isotopomers **1a**, **1b** and **1c** whose preparations are described in this paper.

For the preparation of methyl 4-nitrobutyrate **3** we have used 3-hydroxypropionitrile **12** as the starting material. 3-Hydroxypropionitrile **12** (3.56 g, 50 mmol) was treated with 48% aqueous hydrobromic acid, which converted the nitrile function into the carboxylic function, and simultaneously the hydroxyl group was substituted by bromine to afford 3-bromopropionic acid. Treatment of 3-bromopropionic acid in methanol gave methyl bromopropionate **13**, which was treated further with an excess of the anion of nitromethane to afford methyl 4-nitrobutyrate **3**. Via the synthetic route shown in Scheme 2 it is also possible to label methyl 4-nitrobutyrate **3** at positions 1, 2, 3 and 4 with ^{13}C to afford porphobilinogen **1** with ^{13}C enrichment at positions C-3, C-6, C-7 and C-8. We have optimized the synthetic route shown in Scheme 2 using stoichiometric amounts of **13** and **14** to yield methyl 4-nitrobutyrate **3** (29% after purification). Similarly, 4-^{13}C-labeled methyl 4-nitrobutyrate **3b** as one of the building blocks for isotope-enriched porphobilinogen **1b** was prepared from commercially available ^{13}C-nitromethane **14**. The isotope enrichment of building blocks **2a** and **5c** can easily be accomplished from the least expensive ^{13}C-potassium cyanide **10** to prepare **1a** and **1c**, respectively.

Via Strecker synthesis, the reaction of NH_4Cl (4.21 g, 78 mmol), two equivalents of HCHO (35%) and KCN (5.07 g, 78 mmol) afforded *N*-methyleneamino acetonitrile **17**[12] (2.91 g, 55%). After treatment of **17** with concentrated H_2SO_4 amino acetonitrile hydrosulfate was isolated as a colorless solid with the recovery of an additional equivalent of formaldehyde as diethoxy methane. Formylation of amino acetonitrile **18** and subsequent dehydration give isocyanoacetonitrile **5** (80%), which is used



2 **2a:[1-^{13}C]**

3 **3b:[4-^{13}C]**

4 **4a:[5-^{13}C]** **4b:[4-^{13}C]**

5 **5c: [1-^{13}C]**

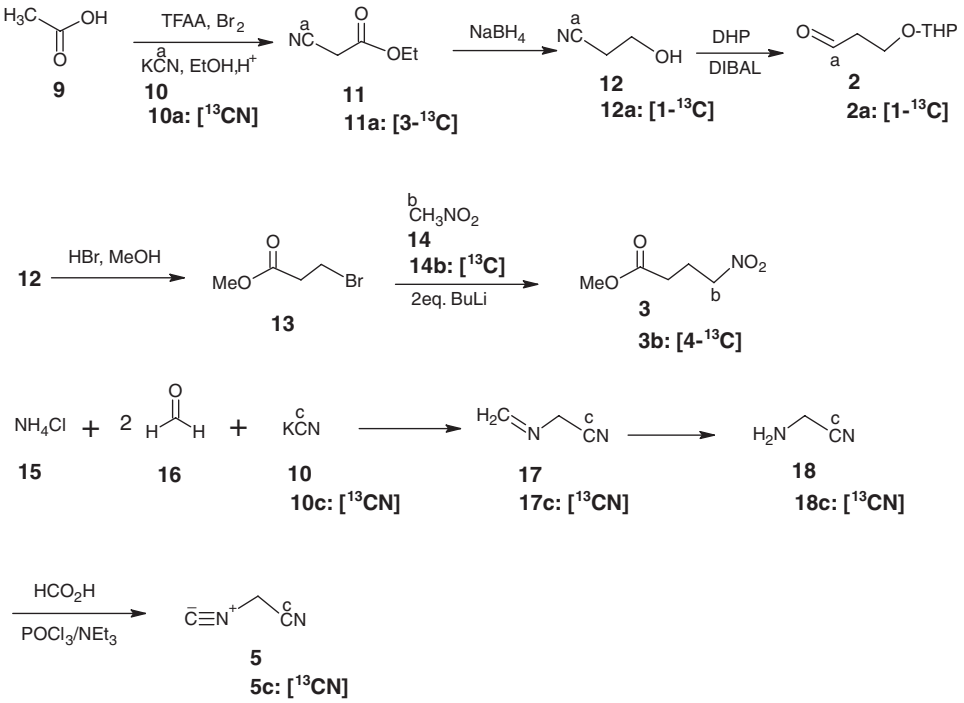
6 **6a:[4-^{13}C]** **6b:[3-^{13}C]** **6c:[^{13}CN]**

7 **7a:[4-^{13}C]** **7b:[3-^{13}C]** **7c:[^{13}CN]**

8 **8a:[4-^{13}C]** **8b:[3-^{13}C]** **8c:[11-^{13}C]**

1 **1a:[4-^{13}C]** **1b:[3-^{13}C]** **1c:[11-^{13}C]**

Scheme 1. Synthesis of [4-^{13}C]-porphobilinogen **1a**, [3-^{13}C]-porphobilinogen **1b** and [11-^{13}C]-porphobilinogen **1c** from [1-^{13}C]-3-(tetrahydropyran-2′-yloxy)-propionaldehyde **2a**, methyl [4-^{13}C]-4-nitrobutyrate **3b** and [1-^{13}C]-isocyanoacetonitrile **5c**, respectively.



Scheme 2. Synthesis of building blocks [1-^{13}C]-3-(tetrahydropyran-2′-yloxy)-propionaldehyde **2a**, methyl [4-^{13}C]-4-nitrobutyrate **3b** and [1-^{13}C]-isocyanoacetonitrile **5c**.

further without purification. The strategy shown in Scheme 2 allows us to prepare building blocks **2**, **3** and **5** with ^{13}C isotope enrichment at any position and combination of positions.

As outlined in Scheme 1, reaction of methyl 4-nitrobutyrate **3** (2.95 g, 20 mmol) and 3-(tetrahydropyran-2′-yloxy)-propionaldehyde **2** (3.16 g, 20 mmol) in the presence of base and phase transfer catalyst afforded methyl 5-hydroxy-4-nitro-7-(tetrahydropyran-2′-yloxy)-heptanoate as a mixture of stereoisomers. Acetylation of the hydroxyl function in acetic anhydride afforded methyl 5-acetoxy-4-nitro-7-(tetrahydropyran-2′-yloxy)-heptanoate **4** as a light-yellow oil (2.45 g, 70%). Base (tetramethylguanidine, TMG)-catalyzed condensation of methyl 5-acetoxy-4-nitro-7-(tetrahydropyran-2′-yloxy)-heptanoate **4** and isocyanoacetonitrile **5** yielded 5-cyano-3-(methoxycarbonylethyl)-4-(tetrahydropyran-2′-yloxyethyl)-pyrrole **6** as a yellow oil (82%). The tetrahydropyrane function is deprotected in the presence of acid to give the corresponding alcohol, which is subsequently oxidized with Jones reagent to the carboxylic acid and further methylated to afford 5-cyano-3-(methoxycarbonylethyl)-4-(methoxycarbonylmethyl)-pyrrole **7**. The nitrile function of pyrrole **7** is reduced to the amine by Pd-black/PtO_2-catalyzed hydrogenation. The simultaneous cyclization of the free amino group with methyl ester function present at position 4 afforded a colorless solid of porphobilinogen lactam methyl ester **8**. Porphobilinogen **1** was obtained after basic hydrolysis of a solution of porphobilinogen lactam methyl ester **8**. Similarly, using [1-^{13}C]-3-(tetrahydropyran-2′-yloxy)-propionaldehyde **2a**, methyl [4-^{13}C]-4-nitrobutyrate **3b** and [1-^{13}C]-isocyanoacetonitrile **5c** as building blocks afforded [4-^{13}C]-porphobilinogen **1a**, [3-^{13}C]-porphobilinogen **1b** and [11-^{13}C]-porphobilinogen **1c**, respectively. Porphobilinogen **1a**–**c** were obtained in 7–15% overall yield based on product **4** (Scheme 1).

The exact masses of the compounds **1a**–**c** were determined by using double focus mass spectrometry in which masses corresponding to the $[M-NH_2]^+$ of the parent molecules **1a**–**c** are obtained corresponding to the molecular formula $^{13}C_1C_9H_{14}N_2O_4$. The isotopomers **1a**, **1b** and **1c** showed the isotopic enrichments of 99, 98 and 98%, respectively. No isotopic dilution or scrambling has occurred during the synthesis. The chemical shifts are in agreement with the literature values for the unlabeled porphobilinogen **1**.[8] The $^3J_{H,C}$ couplings of the porphobilinogen (4.9 and 3.5 Hz) are lower than that of the unsubstituted pyrrole ($^3J_{H,C} = 7.1$ Hz) due to presence of the substituents.[13] The proton attached to position 11 in porphobilinogen **1c** appears as doublet ($^1J_{H,C}$) at 3.83 ppm with a coupling constant of 141 Hz. Similarly, the ^{13}C-NMR chemical shifts of porphobilinogen **1a**–**c** were compared with the natural abundance chemical shifts of porphobilinogen **1**. The coupling constants of $^1J_{C,C}$ (49–68 Hz) and $^2J_{C,C}$ (1–5 Hz) are measured in porphobilinogen **1a**–**c**. The intense peaks arising from C-4 at 117.5 ppm in **1a**, C-3 at 121.5 ppm in **1b** and C-11 at 34.2 ppm at **1c** reveal high ^{13}C incorporation at the expected positions without any ^{13}C scrambling.

Experimental section

General

Reactions were monitored by using thin layer of chromatography (on Merck F254 silica gel 60 aluminum sheets, 0.2 mm: spots were visualized by treating with an oxidizing spray (2 g of $KMnO_4$ and 4 g of $NaHCO_3$ in 100 mL of water)). Column chromatography was performed on Merck silica gel 60. ^{1}H-NMR spectra were recorded on Bruker WM-300 or Bruker AM-600 with tetramethylsilane (TMS: $\delta = 0.00$ ppm) as an internal standard. ^{1}H noise-decoupled ^{13}C spectra were recorded on Bruker WM-300 at 75 MHz or Bruker AM-600 at 150 MHz. Mass spectra were recorded on a Finnigan MAT 900 double focus spectrometer (Finnigan MAT, San Jose, CA, USA). All experiments were carried out under dry nitrogen. Tetrahydrofuran (THF) and diethyl ether

were distilled from benzophenone ketyl. ^{13}C-enriched (99%) chemicals ($K^{13}CN$, $^{13}CH_3NO_2$) were purchased from Cambridge Isotope Laboratories, USA. All chemicals were purchased from Aldrich Fluka or Acros Chimica. The experimental conditions are given for the unlabeled compounds. For the labeled compounds, only the changes relative to the corresponding unlabeled compounds are given.

3-(Tetrahydropyran-2′-yloxy)-propionaldehyde (2)

To a solution of 3-hydroxypropionitrile **12** (3.56 g, 50 mmol) in CH_2Cl_2 (100 mL) was added 2,3-dihydropyrane (5.05 g, 60 mmol) at room temperature. To the mixture was added pyridinium *p*-toluenesulfonate (P*p*Ts) (1.76 g, 7 mmol) and stirred for 48 h at room temperature. The reaction mixture was quenched by the addition of H_2O (100 mL) and extracted with CH_2Cl_2 (2 × 200 mL). The organic solutions were combined, washed with saturated $NaHCO_3$, H_2O and saturated NaCl and dried over $MgSO_4$. The solution was filtered and the solvent was evaporated *in vacuo* to obtain the product 3-(tetrahydropyran-2′-yloxy)-propionitrile as a light-yellow oil (6.98 g, 91%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.51–1.91 (m, 6H, 3 × CH_2), 2.65 (t, $^3J_{H,H}$ = 6.7 Hz, 2H, CH_2), 3.53–3.60 (m, 1H, CH_2), 3.65 (dt, $^2J_{H,H}$ = 10 Hz, $^3J_{H,H}$ = 6.0 Hz, 1H, CH_2), 3.89 (m, 1H, CH_2), 3.93 (dt, $^2J_{H,H}$ = 10 Hz, $^3J_{H,H}$ = 6 Hz, 1H, CH_2), 4.65 (t, $^3J_{H,H}$ = 3.3 Hz, 1H, CH) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 18.18 (2 × CH_2), 25.12 (CH_2), 30.12 (CH_2), 61.90 (CH_2), 62.01(CH_2), 98.62 (CH), 117.9 (CN) ppm. HRMS calculated for $C_8H_{13}NO_2$, $[M-H]^+$: 154.0868; found: 154.0864.

To a cold solution (−50°C) of 3-(tetrahydropyran-2′-yloxy)-propionitrile (6.98 g, 45 mmol) in ether (100 mL) was added DIBAL-H (1 M in *n*-hexane, 50 mL, 50 mmol). The reaction mixture was stirred for 2 h at −20°C and stirred overnight at room temperature. To the mixture was added 1 M citric acid (150 mL) and extracted with ether (2 × 200 mL). The organic solutions were combined, washed with saturated $NaHCO_3$, H_2O and saturated NaCl and dried over $MgSO_4$. The solution was filtered and the solvent was evaporated *in vacuo* to obtain the product 3-(tetrahydropyran-2′-yloxy)-propionaldehyde **2** as a light-yellow oil (6.02 g, 86%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.51–1.91 (m, 6H, 3 × CH_2), 2.69 (m, bdt, $^3J_{H,H}$ = 1.9 Hz, 2 × $^3J_{H,H}$ = 6.1 Hz, 2H, CH_2), 3.55 (m, 1H, CH_2), 3.76 (dt, $^2J_{H,H}$ = 10 Hz, $^3J_{H,H}$ = 6.1 Hz, 1H, CH_2), 3.84 (m, 1H), 4.10 (bdt, $^2J_{H,H}$ = 10 Hz, $^3J_{H,H}$ = 6.1 Hz, 1H, CH_2), 4.63 (bt, $^3J_{H,H}$ = 3.5 Hz, 1H, CH), 9.81 (t, $^3J_{H,H}$ = 1.9 Hz, CHO) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 19.21 (CH_2), 25.21 (CH_2), 30.32 (CH_2), 43.71 (CH_2), 62.12 (CH_2), 63.61 (CH_2), 98.62 (CH), 201.1 (CHO) ppm.

[1-^{13}C]-3-(tetrahydropyran-2′-yloxy)-propionaldehyde (2a)

Similarly, [1-^{13}C]-3-hydroxypropionitrile **12a** (1.58 g, 22 mmol) was treated to afford [1-^{13}C]-3-(tetrahydropyran-2′-yloxy)-propionitrile (3.15 g, 92%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.51–1.91 (m, 6H, 3 × CH_2), 2.65 (dt, $^2J_{H,C}$ = 10.2 Hz, $^3J_{H,H}$ = 6.3 Hz, 2H, CH_2), 3.53–3.60 (m, 1H, CH_2), 3.66 (ddt, $^2J_{H,H}$ = 10 Hz, $^3J_{H,C}$ = 6.2 Hz, $^3J_{H,H}$ = 6.3 Hz, 1H, CH_2), 3.89 (m, 1H, CH_2), 3.93 (ddt, $^2J_{H,H}$ = 10 Hz, $^3J_{H,C}$ = 6.2 Hz, $^3J_{H,H}$ = 6.3 Hz, 1H, CH_2), 4.68 (t, $^3J_{H,H}$ = 3.3 Hz, 1H, CH) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 18.85 (d, $^1J_{H,C}$ = 57.6 Hz, CH_2), 19.0 (CH_2), 25.12 (CH_2), 30.12 (CH_2), 62.0 (d, $^2J_{H,C}$ = 3.0 Hz, CH_2), 62.01 (CH_2), 98.82 (CH), 117.9 (^{13}C-labeled, intense peak, CN) ppm. HRMS calculated for $^{13}C_1C_7H_{13}NO_2$, $[M-H]^+$: 155.0902; found: 155.0896.

Similarly, [1-^{13}C]-3-(tetrahydropyran-2′-yloxy)-propionitrile (3.15 g, 20 mmol) was reduced with DIBAL-H to afford [1-^{13}C]-3-(tetrahydropyran-2′-yloxy)-propionaldehyde **2a** as a light-yellow oil (2.55 g, 80%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.51–1.91 (m, 6H, 3 × CH_2), 2.69 (dq, $^2J_{H,C}$ = 6.1 Hz, $^3J_{H,H}$ = 1.9 Hz, 2 × $^3J_{H,H}$ = 6.3 Hz, 2H, CH_2), 3.55 (m, 1H, CH_2), 3.77 (ddt, $^2J_{H,H}$ = 10.2 Hz, $^3J_{H,C}$ = 6.2 Hz, $^3J_{H,H}$ = 6.1 Hz, $^3J_{H,C}$ = 6.0 Hz, 1H, CH_2), 3.84 (m, 1H, CH_2), 4.10 (ddt, $^2J_{H,H}$ = 10.2 Hz, $^3J_{H,H}$ = 6.2 Hz, $^3J_{H,C}$ = 6.0 Hz, 1H, CH_2), 4.63 (bt, $^3J_{H,H}$ = 3.5 Hz, 1H, CH), 9.81 (dt, $^1J_{H,C}$ = 173.3 Hz, $^3J_{H,H}$ = 1.9 Hz, CHO) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 19.2 (CH_2), 25.12 (CH_2), 30.31 (CH_2), 43.7 (d, $^1J_{C,C}$ = 39.1 Hz, CH_2), 62.1 (d, $^2J_{C,C}$ = 1.7 Hz, CH_2), 63.6 (CH_2), 98.82 (CH), 201.1 (^{13}C-labeled, intense peak, CHO) ppm. HRMS calculated for $^{13}C_1C_7H_{14}O_3$, $[M-H]^+$: 158.0898; found: 158.0897.

Methyl 3-bromopropionate (13)

3-Hydroxypropionitrile **12** (3.55 g, 50 mmol) was refluxed with HBr (48% aqueous solution, 40 mL) for 4 h. The mixture was diluted with H_2O (50 mL) and then extracted with ether (2 × 100 mL). The solvent was evaporated *in vacuo* to yield a colorless solid of 3-bromopropionic acid (6.25 g, 82%). The product was esterified with MeOH (25 mL) in CH_2Cl_2 (100 mL) by refluxing 18 h using Dean stark trap. To the mixture was added H_2O (150 mL) and then extracted with CH_2Cl_2 (2 × 200 mL). The organic solutions were combined, washed with saturated $NaHCO_3$, H_2O and saturated NaCl and dried over $MgSO_4$. The solution was filtered and the solvent was evaporated to yield a light-yellow oil of methyl 3-bromopropionate **13** (6.75 g, 81%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.94 (t, $^3J_{H,H}$ = 6.7 Hz, 2H, CH_2), 3.59 (t, $^3J_{H,H}$ = 6.7 Hz, 2H, CH_2), 3.74 (s, 3H, OCH_3) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 25.80 (CH_2), 37.30 (CH_2), 51.92 (OCH_3), 170.8 (C = O) ppm.

Methyl 4-nitrobutyrate (3)

To a cold solution (−80°C) of nitromethane **14** (1.52 g, 25 mmol) in THF (100 mL) and HMPA (25 mL) was added *n*-BuLi (17 mL of a 1.6 M solution in hexane, 27 mmol) dropwise. After stirring the mixture for 1 h, the temperature was allowed to rise to −60°C and methyl 3-bromopropionate **13** (4.16 g, 25 mmol) in THF (50 mL) was added to it. The reaction mixture was stirred for 24 h below −15°C and the reaction was quenched by the addition of acetic acid (10 mL). To the mixture was added H_2O (150 mL) and then extracted with ether (2 × 200 mL). The organic solutions were combined, washed with saturated $NaHCO_3$, H_2O and saturated NaCl and dried over $MgSO_4$. The solution was filtered and the solvent was evaporated to yield the product (1.52 g, 41%). The product was purified by column chromatography (silicagel 60: ether/petroleum ether, 4:1) to yield **3** as a light-yellow oil (1.05 g, 29%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.32 (dt, $^3J_{H,H}$ = 6.7 Hz, $^3J_{H,H}$ = 7.0 Hz, 2H, CH_2), 2.49 (t, $^3J_{H,H}$ = 7.0 Hz, 2H, CH_2), 3.70 (s, 3H, OCH_3), 4.50 (t, $^3J_{H,H}$ = 6.7 Hz, 2H, CH_2) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 22.20 (CH_2), 30.03 (CH_2), 51.72 (OCH_3), 74.20 (CH_2), 172.2 (C = O) ppm.

Methyl [4-^{13}C]-4-nitrobutyrate (3b)

Similarly, [^{13}C]-nitromethane **14b** (1.55 g, 25 mmol) and 3-bromopropionate **13** (4.16 g, 25 mmol) afforded **3b** as a light-yellow oil (1.01 g, 27%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.32 (ddt, $^3J_{H,H}$ = 6.7 Hz, $^3J_{H,H}$ = 7.0 Hz, $^2J_{C,H}$ = 5.3 Hz, 2H, CH_2), 2.48 (dt, $^3J_{H,H}$ = 7 Hz, $^3J_{C,H}$ = 4.9 Hz, 2H, CH_2), 3.70 (s, 3H), 4.50 (dt, $^3J_{H,H}$ = 6.7 Hz, $^1J_{C,H}$ = 146 Hz, 2H, OCH_3) ppm. ^{13}C-NMR (75 MHz,

$CDCl_3$): δ = 22.20 (d, $^1J_{C,C}$ = 35.6 Hz, CH_2), 30.03 ($^2J_{C,C}$ = 0.5 Hz, CH_2), 51.72 (OCH_3), 74.20 (^{13}C-labeled, intense peak, CH_2), 172.2 (d, $^3J_{C,C}$ = 3.0 Hz, C = O) ppm.

Methyl 5-acetoxy-4-nitro-7-(tetrahydropyran-2′-yloxy)-heptanoate (4)

To a cold solution (0°C) of methyl 4-nitrobutyrate **3** (2.95 g, 20 mmol) was added aqueous NaOH (4%, 50 mL), followed by cetyltrimethylammonium chloride (2.5 mL, 5 mmol). During vigorous stirring 3-(tetrahydropyran-2′-yloxy)-propionaldehyde **2** (3.16 g, 20 mmol) was added dropwise. The emulsion was stirred overnight. To the mixture was added saturated NaCl (100 mL) and then extracted with ethyl acetate (2 × 200 mL). The organic solutions were combined, washed with saturated NaCl and dried over $MgSO_4$. The solution was filtered and the solvent was evaporated. The product was purified by column chromatography (silicagel 60: ethyl acetate/hexane, 1:4 and 1:1) to yield methyl 5-hydroxy-4-nitro-7-(tetrahydropyran-2′-yloxy)-heptanoate as a light-yellow oil (4.12 g, 68%). ^{1}H-NMR (300 MHz, $CDCl_3$): a mixture of stereoisomers: δ = 1.51−1.71 (m, 4H), 1.71–1.91 (m, 3H), 1.92 (m, 1H, CH_2), 2.21 (m, 4H), 3.56 (m, 1H), 3.65 (m, 1H), 3.69 (s, 6H, 2 × OCH_3), 3.89 (m, 1H), 3.98 (m, 1H), 4.23 (m, 1H), 4.47–4.75 (m, 2H) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 19.4, 19.6, 19.8, 19.9, 22.5, 23.6, 23.7, 24.8, 24.9, 25.1, 29.7, 29.9, 30.3, 30.6, 51.8 (2 × OCH_3), 62.4, 64.7, 64.8, 65.1, 70.3, 70.8, 71.2, 71.6, 90.6, 91.5, 91.6, 99.1, 99.2, 99.6, 172.3 (C = O), 172.5 (C = O) ppm.

To a cold solution (0°C) of alcohol (methyl 5-hydroxy-4-nitro-7-(tetrahydropyran-2′-yloxy)-heptanoate) (3.04 g, 10 mmol) in CH_2Cl_2 (45 mL) was added pyridine (1.2 mL, 15 mmol) followed by dropwise addition of acetic anhydride (2.3 mL, 25 mmol). The reaction mixture was stirred overnight. To the mixture was added 10% $NaHCO_3$ (50 mL) and extracted with CH_2Cl_2 (2 × 100 mL). The organic solutions were combined, washed with 0.2 M HCl, H_2O and saturated NaCl and dried over $MgSO_4$. The solution was filtered and the solvent was evaporated. The product was purified by column chromatography (silicagel 60: ethyl acetate/*n*-hexane, 1:3) to yield methyl 5-acetoxy-4-nitro-7-(tetrahydropyran-2′-yloxy)-heptanoate **4** as a light-yellow oil (2.45 g, 71%) ^{1}H-NMR (300 MHz, $CDCl_3$): a mixture of stereoisomers: δ = 1.41−1.61 (m, 4H), 1.62−2.01 (m, 4H), 2.06 (s, 6H, 2 × OAc,), 2.15−2.52 (m, 4H), 3.31−3.43 (m, 1H), 3.55 (m, 1H), 3.69 (s, 6H, 2 × OCH_3), 3.89 (m, 2H), 3.98 (m, 2H), 4.56 (m, 1H), 4.81−4.93 (m, 1H), 5.40−5.50 (m, 1H) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 19.1, 19.2, 19.5, 20.5, 20.6, 24.7, 24.8, 24.9, 25.2, 29.6, 29.7, 29.9, 30.1, 30.2, 30.5, 30.6, 30.7, 51.8 (2 × OCH_3), 61.8, 62.0, 62.5, 62.6, 62.7, 63.0, 70.3, 70.4, 70.7, 70.8, 88.0, 88.7, 98.4, 98.6, 99.2, 99.3, 169.5 (C = O), 171.9 (C = O) ppm.

Methyl [5-^{13}C]-5-acetoxy-4-nitro-7-(tetrahydropyran-2′-yloxy)-heptanoate (4a)

Similarly, methyl 4-nitrobutyrate **3** (1.48 g, 10 mmol) and [1-^{13}C]-3-(tetrahydropyran-2′-yloxy)-propionaldehyde **2a** (1.57 g, 10 mL) afforded methyl 5-hydroxy-4-nitro-7-(tetrahydropyran-2′-yloxy)-heptanoate as a light-yellow oil (2.15 g, 71%). ^{1}H-NMR (300 MHz, $CDCl_3$): a mixture of stereoisomers: δ = 1.51−1.71 (m, 4H), 1.71−1.91 (m, 4H), 1.92 (m, 1H), 2.21 (m, 1H), 2.11−2.61 (m, 4H), 3.56−3.65 (m, 1H), 3.61−3.71 (m, 1H), 3.69, 3.70 (s, 6H, 2 × OCH_3), 3.90 (m, 1H), 4.12 (m, 1H), 4.27−4.35 (dm, $^1J_{H,C}$ = 75 Hz, 1H), 4.56 (m, 2H) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 19.4, 19.5, 19.8, 19.9, 24.9, 25.0 (d, $^2J_{C,C}$ = 2.5 Hz, CH_2), 25.1, 29.7, 30.4, 30.6, 32.4 (d, $^1J_{C,C}$ = 38 Hz, CH_2), 51.8, 51.9 (OCH_3), 62.5, 62.6, 63.2, 63.4, 64.5, 64.8, 64.9, 65.2 (d, $^2J_{C,C}$ = 2.4 Hz, CH_2), 70.4, 70.9, 71.3, 71.8 (^{13}C-labeled, intense peak, C-5), 90.6, 91.5, 91.6 (d, $^1J_{C,C}$ = 39 Hz, C-4), 99.1, 99.2, 99.6, 172.3 (C = O), 172.5 (C = O) ppm.

Similar conversion of methyl 5-hydroxy-4-nitro-7-(tetrahydropyran-2′-yloxy)-heptanoate (2.12 g, 7 mmol) yielded **4a** as a light-yellow oil (1.67 g, 68%). ^{1}H-NMR (300 MHz, $CDCl_3$): a mixture of stereoisomers: δ = 1.41−1.61 (m, 4H), 1.62–2.01 (m, 4H), 2.06 (s, 2 × OCH_3, 6H), 2.15–2.52 (m, 4H), 3.31–3.43 (m, 1H), 3.55 (m, 1H), 3.69 (s, 2 × OCH_3, 6H), 3.74–3.95 (m, 2H), 4.56 (m, 1H), 4.814.93 (m, 1H), 5.40–5.50 (dm, $^1J_{H,C}$ = 150 Hz, 1H) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 19.2, 19.3, 19.4, 19.5, 20.7, 24.8, 24.9, 25.3, 29.6, 29.7, 29.8, 30.3, 30.4, 30.5, 30.7, 30.6 (d, $^1J_{C,C}$ = 57 Hz, C-6), 51.9, 61.9, 62.1, 62.4, 62.5, 62.7, 62.8, 63.1, 70.4, 70.5, 70.7, 70.8 (^{13}C-labeled, intense peak, C-5), 88.0, 88.7, 98.5, 98.7, 99.3, 99.4, 169.6, 169.7 (d, $^2J_{C,C}$ = 2.1 Hz, C = O), 172.0, 172.1, 172.2 (C = O) ppm. HRMS calculated for $^{13}C_1C_{14}H_{25}NO_8$, $[M-H]^+$: 347.1535; found: 347.1550.

Methyl [4-^{13}C]-5-acetoxy-4-nitro-7-(tetrahydropyran-2′-yloxy)-heptanoate (4b)

Similarly, methyl [4-^{13}C]-4-nitrobutyrate **3b** (1.48 g, 10 mmol) and 3-(tetrahydropyran-2′-yloxy)-propionaldehyde **2** (1.57 g, 10 mL) afforded afforded methyl 5-hydroxy-4-nitro-7-(tetrahydropyran-2′-yloxy)-heptanoate as a light-yellow oil (2.15 g, 71%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.51–1.71 (m, 4H), 1.71–1.91 (m, 4H), 2.01 (m, 1H), 2.11–2.61 (m, 4H), 3.56–3.65 (m, 1H), 3.61–3.71 (m, 1H), 3.69, 3.70 (s, 2 × OCH_3, 6H), 3.90 (m, 1H), 4.12 (m, 1H), 4.27–4.35 (m, 1H), 4.56 (2 × t, $^3J_{H,H}$ = 3.06 Hz, 2H), 4.61 (dm, $^1J_{H,C}$ = 150 Hz, 1H) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 19.3, 19.4, 19.8, 19.9, 23.6, 23.7 (d, $^1J_{C,C}$ = 37 Hz, C-3), 25.0, 25.1, 25.2, 25.3, 29.7, 29.9, 30.3, 30.6, 30.7, 32.4, 51.8, 51.9 (OCH_3), 62.4, 62.5, 63.2, 64.5, 64.8, 64.9, 65.37 (d, $^3J_{C,C}$ = 3.6 Hz), 70.4, 70.9, 71.3, 71.7 (d, $^3J_{C,C}$ = 39 Hz, C-5), 90.6, 91.5, 91.6 (^{13}C-labeled, intense peak, C-4), 99.1, 99.2, 99.5, 99.7, 172.3, 172.5 (d, $^3J_{C,C}$ = 3.2 Hz, C = O) ppm. HRMS calculated for $^{13}C_1C_{12}H_{23}NO_7$, $[M-H]^+$: 305.1430; found: 305.1440.

Similar conversion yielded **4b** as a light-yellow oil (1.65 g, 68%). ^{1}H-NMR (300 MHz, $CDCl_3$): a mixture of stereoisomers: δ = 1.41–1.61 (m, 4H), 1.62–2.01 (m, 4H), 2.06, 2.09 (2 × s, 3H, OAc), 2.15–2.52 (m, 4H), 3.31–3.43 (m, 1H), 3.55 (m, 1H), 3.69, 3.70 (s, 2 × OCH_3, 6H), 3.80 (m, 1H), 3.90 (m, 1H), 4.56, 4.58 (2 × t, $^3J_{H,H}$ = 3.0 Hz, 1H), 4.78–4.93 (dm, $^1J_{H,C}$ = 150 Hz, 1H), 5.40–5.50 (m, 1H) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 19.2, 19.3, 19.4, 19.5, 20.6, 20.7, 20.8, 20.9, 24.7, 24.8, 25.2, 29.6, 29.7, 29.9, 30.0, 30.2, 30.4, 30.5, 30.6, 30.7, 51.8, 51.9 (OCH_3), 61.9, 62.1, 62.4, 62.5, 62.7, 63.2 (d, $^3J_{C,C}$ = 2.7 Hz, C-7), 70.3, 70.4, 70.7, 70.8 (d, $^1J_{C,C}$ = 39 Hz, C-5), 88.0, 88.7 (^{13}C-labeled, intense peak, C-4), 98.5, 98.7, 99.3, 99.4, 169.7, 169.9 (CO), 172.0, 172.1, 172.2 (d, $^3J_{C,C}$ = 3.4 Hz, C = O) ppm. HRMS calculated for $^{13}C_1C_{14}H_{25}NO_8$, $[M-H]^+$: 347.1535; found: 347.1550.

Isocyanoacetonitrile (5)

To a solution of amino acetonitrile **18** (1.12 g, 20 mmol) in CH_2Cl_2 (50 mL) was added dicyclohexylcarbodiimide (4.25 g, 20 mmol) and cooled to −50°C. A solution of formic acid (one equivalent, 7.5 mL, 20 mmol) in CH_2Cl_2 (15 mL) was added to the mixture dropwise (within 1 h) and continued to be stirred at 0°C for 6 h. The mixture was filtered, evaporated and purified by column chromatography (silicagel 60: ethyl acetate) to afford

N-formylamino acetonitrile as a pale-yellow/green oil (1.17 g, 70%). ^{1}H-NMR (300 MHz, D_2O): $\delta = 4.30$ (s, 2H, CH_2), 8.23 (s, CHO) ppm. ^{13}C-NMR (75 MHz, D_2O): $\delta = 27.20$ (CH_2), 117.7 (CN), 165.4 (CHO) ppm.

To a cold solution (−50°C) of *N*-formylamino acetonitrile (1.17 g, 14 mmol) in CH_2Cl_2 (50 mL) was added Et_3N (3.02 g, 30 mmol), followed by addition of $POCl_3$ (2.01 mL, 20 mmol). After stirring for 10 min at −30°C, the reaction mixture was stirred for 10 min at room temperature. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and poured in 20% Na_2CO_3 (100 mL). The organic layer was separated and dried over $MgSO_4$. The solution was filtered and the solvent was evaporated at 0°C and dark brown crude isocyanoacetonitrile **5** (0.74 g, 80%) was used immediately.

[1-^{13}C]-isocyanoacetonitrile (5c)

Similarly, [1-^{13}C]-amino acetonitrile **18c** (1.65 g, 29 mmol) afforded [1-^{13}C]-*N*-formylamino acetonitrile (1.54 g, 64%). ^{1}H-NMR (300 MHz, D_2O): $\delta = 4.30$ (d, $^{2}J_{H,C} = 8.4$ Hz, 2H, CH_2), 8.23 (s, CHO) ppm. ^{13}C-NMR (75 MHz, D_2O): $\delta = 27.01$ (d, $^{1}J_{C,C} = 61.7$ Hz, CH_2), 117.7 (^{13}C-labeled, intense peak, CN), 165.4 (CHO) ppm. Similar conversion of [1-^{13}C]-*N*-formylamino acetonitrile (1.54 g, 18 mmol) afforded [1-^{13}C]-isocyanoacetonitrile **5c** (0.82 g, 68%).

5-Cyano-3-(methoxycarbonylethyl)-4-(tetrahydropyran-2′-yloxyethyl)-pyrrole (6)

A solution of methyl 5-acetoxy-4-nitro-7-(tetrahydropyran-2′-yloxy)-heptanoate **4** (3.47 g, 10 mmol) in THF (50 mL) was added to isocyanoacetonitrile **5** (0.72 g, 11 mmol) at room temperature. To the mixture was added TMG (3.7 mL, 30 mmol) and stirred at 0°C for 30 min and at room temperature for 90 min. The reaction was quenched by the addition of H_2O (100 mL) and extracted with EtOAc (2 × 100 mL). The organic solution was washed with H_2O and saturated NaCl and dried over $MgSO_4$. The solution was filtered and the solvent was evaporated. The product was purified by column chromatography (silicagel 60: ethyl acetate/*n*-hexane, 1:3) to yield 5-cyano-3-(methoxycarbonylethyl)-4-(tetrahydropyran-2′-yloxyethyl)-pyrrole **6** as a yellow oil (2.51 g, 82%). ^{1}H-NMR (300 MHz, $CDCl_3$): $\delta = 1.41$–1.61 (m, 4H), 1.62–1.91 (m, 2H), 2.59 (t, $^{3}J_{H,H} = 7.5$ Hz, 2H), 2.78 (t, $^{3}J_{H,H} = 7.5$ Hz, 2H), 2.89 (t, $^{3}J_{H,H} = 6.9$ Hz, 2H), 3.50 (m, 1H), 3.58 (dt, $^{2}J_{H,H} = 9.6$ Hz, $^{3}J_{H,H} = 6.9$ Hz, 1H), 3.68 (s, OCH_3), 3.80 (m, 1H), 3.89 (dt, $^{2}J_{H,H} = 9.6$ Hz, $^{3}J_{H,H} = 6.9$ Hz, 1H), 4.62 (bt, $^{3}J_{H,H} = 3.5$ Hz, 1H), 6.73 (d, $^{3}J_{H,H} = 2.9$ Hz, 1H), 9.59 (bs, NH) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): $\delta = 19.3$, 20.0, 25.3, 25.4, 30.4, 34.6, 51.6 (OCH_3), 62.6, 67.0, 98.8, 99.8 (C-5), 114.4 (CN), 121.3 (C-2), 122.6 (C-3), 130.9 (C-4), 173.5 (C = O) ppm.

[4-^{13}C]-5-cyano-3-(methoxycarbonylethyl)-4-(tetrahydropyran-2′-yloxyethyl)-pyrrole (6a)

A solution of methyl [5-^{13}C]-5-acetoxy-4-nitro-7-(tetrahydropyran-2′-yloxy)-heptanoate **4a** (3.48 g, 10 mmol) in THF (50 mL) was added to isocyanoacetonitrile **5** (0.72 g, 11 mmol) at room temperature to yield [5-^{13}C]-5-cyano-3-(methoxycarbonylethyl)-4-(tetrahydropyran-2′-yloxyethyl)-pyrrole **6a** as a yellow oil (2.51 g, 82%). ^{1}H-NMR (300 MHz, $CDCl_3$): $\delta = 1.41$–1.61 (m, 4H), 1.62–1.91(m, 2H), 2.59 (t, $^{3}J_{H,H} = 7.5$ Hz, 2H), 2.78 (dt, $^{3}J_{H,C} = 3.7$ Hz, $^{3}J_{H,H} = 7.5$ Hz, 2H), 2.89 (dt, $^{2}J_{H,C} = 6.4$ Hz, $^{3}J_{H,H} = 6.9$ Hz, 2H), 3.50 (m, 1H), 3.58 (ddt, $^{2}J_{H,H} = 9.6$ Hz, $^{3}J_{H,H} = 6.9$ Hz, $^{3}J_{H,C} = 4.1$ Hz, 1H), 3.68 (s, OCH_3), 3.80 (m, 1H), 3.89 (ddt, $^{2}J_{H,H} = 9.6$ Hz, $^{3}J_{H,H} = 6.9$ Hz, $^{3}J_{H,C} = 4.1$ Hz, 1H), 4.62 (bt, $^{3}J_{H,H} = 3.5$ Hz, 1H), 6.68 (dd, $^{3}J_{H,H} = 2.9$ Hz, $^{3}J_{H,C} = 6.9$ Hz, 1H), 9.40 (bs, NH) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): $\delta = 19.3$, 20.0 (d, $^{2}J_{C,C} = 2.9$ Hz, CH_2), 25.3, 25.4 (d, $^{1}J_{C,C} = 48$ Hz, CH_2), 30.4, 34.6 (d, $^{3}J_{C,C} = 2.0$ Hz, CH_2), 51.6 (OCH_3), 62.6, 67.0 (d, $^{2}J_{C,C} = 1.7$ Hz, CH_2), 98.8, 99.9 (d, $^{1}J_{C,C} = 73$ Hz, C-5), 114.4 (d, $^{2}J_{C,C} = 4.6$ Hz, CN), 121.3 (d, $^{2}J_{C,C} = 2.4$ Hz, C-2), 122.6 (d, $^{1}J_{C,C} = 54$ Hz, C-3), 130.9 (^{13}C-labeled, intense peak, C-4), 173.5 (C = O) ppm. HRMS calculated for $^{13}C_1C_{15}H_{22}N_2O_4$, $[M]^+$: 307.1613; found: 307.1625.

[3-^{13}C]-5-cyano-3-(methoxycarbonylethyl)-4-(tetrahydropyran-2′-yloxyethyl)-pyrrole (6b)

A solution of methyl [4-^{13}C]-5-acetoxy-4-nitro-7-(tetrahydropyran-2′-yloxy)-heptanoate **4b** (3.48 g, 10 mmol) in THF (50 mL) was added to isocyanoacetonitrile **5** (0.72 g, 11 mmol) at room temperature to yield [4-^{13}C]-5-cyano-3-(methoxycarbonylethyl)-4-(tetrahydropyran-2′-yloxyethyl)-pyrrole (**6b**) as a yellow oil (2.51 g, 82%). ^{1}H-NMR (300 MHz, $CDCl_3$): $\delta = 1.41$–1.61 (m, 4H), 1.62–1.91 (m, 2H), 2.58 (dt, $^{3}J_{H,H} = 7.5$ Hz, $^{3}J_{H,C} = 4.0$ Hz, 2H), 2.78 (dt, $^{2}J_{H,C} = 7.0$ Hz, $^{3}J_{H,H} = 7.5$ Hz, 2H), 2.88 (dt, $^{3}J_{H,C} = 4.3$ Hz, $^{3}J_{H,H} = 6.9$ Hz, 2H), 3.50 (m, 1H), 3.58 (dt, $^{2}J_{H,H} = 9.6$ Hz, $^{3}J_{H,H} = 6.9$ Hz, 1H), 3.68 (s, OCH_3), 3.80 (m, 1H), 3.89 (dt, $^{2}J_{H,H} = 9.6$ Hz, $^{3}J_{H,H} = 6.9$ Hz, 1H), 4.62 (bt, $^{3}J_{H,H} = 3.5$ Hz, 1H), 6.69 (dd, $^{2}J_{H,C} = 6.8$ Hz, $^{3}J_{H,H} = 3.0$ Hz, 1H), 9.40 (bs, NH) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): $\delta = 19.3$, 20.0 (d, $^{1}J_{C,C} = 49$ Hz, CH_2), 25.3, 25.4 (d, $^{2}J_{C,C} = 2.0$ Hz, CH_2), 30.4, 34.6 (d, $^{2}J_{C,C} = 2.0$ Hz, CH_2), 51.6 (OCH_3), 62.6, 67.0 (d, $^{3}J_{C,C} = 2.0$ Hz, CH_2), 98.8, 99.8 (d, $^{2}J_{C,C} = 6.5$ Hz, C-5), 114.4 (d, $^{3}J_{C,C} = 5.1$ Hz, CN), 121.3 (d, $^{1}J_{C,C} = 54$ Hz, C-2), 122.6 (^{13}C-labeled, intense peak, C-3), 130.9 (d, $^{1}J_{C,C} = 54$ Hz, C-4), 173.5 (d, $^{3}J_{C,C} = 3.7$ Hz, C = O) ppm. HRMS calculated for $^{13}C_1C_{15}H_{22}N_2O_4$, $[M]^+$: 307.1613; found: 307.1617.

5-[^{13}C-cyano]-3-(methoxycarbonylethyl)-4-(tetrahydropyran-2′-yloxyethyl)-pyrrole (6c)

A solution of methyl 5-acetoxy-4-nitro-7-(tetrahydropyran-2′-yloxy)-heptanoate **4** (3.47 g, 10 mmol) was added to [1-^{13}C]-isocyanoacetonitrile **5c** (0.72 g, 11 mmol) at room temperature to yield 5-[^{13}C-cyano]-3-(methoxycarbonylethyl)-4-(tetrahydropyran-2′-yloxyethyl)-pyrrole **6c** as a yellow oil (2.51 g, 82%). ^{1}H-NMR (300 MHz, $CDCl_3$): $\delta = 1.41$–1.61 (m, 4H), 1.62–1.91 (m, 2H), 2.59 (t, $^{3}J_{H,H} = 7.5$ Hz, 2H), 2.78 (t, $^{3}J_{H,H} = 7.5$ Hz, 2H), 2.89 (t, $^{3}J_{H,H} = 6.9$ Hz, 2H), 3.50 (m, 1H), 3.58 (dt, $^{2}J_{H,H} = 9.6$ Hz, $^{3}J_{H,H} = 6.9$ Hz, 1H), 3.68 (s, OCH_3), 3.80 (m, 1H), 3.89 (dt, $^{2}J_{H,H} = 9.6$ Hz, $^{3}J_{H,H} = 6.9$ Hz, 1H), 4.62 (bt, $^{3}J_{H,H} = 3.5$ Hz, 1H), 6.69 (dd, $^{3}J_{H,H} = 2.9$ Hz, $^{4}J_{H,C} = 2.5$ Hz, 1H), 9.03 (bs, NH) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): $\delta = 19.3$, 20.0, 25.3, 25.4, 30.4, 34.6, 51.6 (OCH_3), 62.6, 67.0, 98.8, 99.9 (d, $^{1}J_{C,C} = 100$ Hz, C-5), 114.4 (^{13}C-labeled, intense peak, CN), 121.3 (d, $^{3}J_{C,C} = 3.4$ Hz, C-2), 122.6 (d, $^{3}J_{C,C} = 3.1$ Hz, C-3), 130.9 (d, $^{2}J_{C,C} = 5.1$ Hz, C-4), 173.5 (C = O) ppm. HRMS calculated for $^{13}C_1C_{15}H_{22}N_2O_4$, $[M]^+$: 307.1613; found: 307.1617.

5-Cyano-3-(methoxycarbonylethyl)-4-(methoxycarbonylmethyl)-pyrrole (7)

To a solution of 5-cyano-3-(methoxycarbonylethyl)-4-(tetrahydropyran-2′-yloxyethyl)-pyrrole **6** (2.44 g, 8 mmol) in MeOH (20 mL) was added P*p*Ts (2.10 g 8 mmol) and stirred for 4 days at room temperature. The solvent was removed *in vacuo* and

the residue was dissolved in H_2O and EtOAc. The mixture was extracted with EtOAc (2×100 mL). The organic solution was washed with H_2O and saturated NaCl and dried over $MgSO_4$. The solution was filtered and the solvent was evaporated. The product was purified by column chromatography (silicagel 60: ethyl acetate/*n*-hexane, 1:1) to yield 5-cyano-4-(methoxycarbonylethyl)-3-(hydroxyethyl)-pyrrole as a colorless solid (1.51 g, 85%). ^{1}H-NMR (300 MHz, acetone-d_6): $\delta = 2.58$ (t, $^3J_{H,H} = 7.5$ Hz, 2H), 2.76 (t, $^3J_{H,H} = 7.3$ Hz, 2H), 2.80 (t, $^3J_{H,H} = 7.2$ Hz, 2H), 3.62 (s, OCH_3), 3.70 (dt, $^3J_{H,H} = 7.2$ Hz, $^3J_{H,H} = 5.6$ Hz, 2H), 3.88 (bt, $^3J_{H,H} = 7.2$ Hz, 1H), 6.90 (d, $^3J_{H,H} = 2.9$ Hz, 1H), 10.87 (bs, NH) ppm. ^{13}C-NMR (75 MHz, acetone-d_6): $\delta = 20.8$, 29.3, 35.2, 51.6 (OCH_3), 62.8, 100.5 (C-5), 114.9 (CN), 122.5 (C-2), 123.5 (C-3), 131.2 (C-4), 173.6 (C = O) ppm.

To a cold solution (0°C) of 5-cyano-4-(methoxycarbonylethyl)-3-(hydroxyethyl)-pyrrole (1.51 g, 7 mmol) in acetone (100 mL) was added Jones reagent (2.6 M, 4 mL, 10 mmol) dropwise over the period of 90 min. To the reaction mixture was added isopropanol (20 mL) and stirred for 30 min at room temperature. The mixture was filtered through celite and washed with acetone (100 mL). The crude acid was dissolved in ether/ethyl acetate mixture (10 mL, 1:1) and treated with ethereal diazomethane at 0°C. The mixture was stirred for 2 h, solvent evaporated and the residue was purified by column chromatography (silicagel 60: ethyl acetate/*n*-hexane, 1:3) to yield 5 - cyano-3-(methoxycarbonylethyl)-4-(methoxycarbonylmethyl)-pyrrole **7** as a light-yellow oil (0.51 g, 30%). ^{1}H-NMR (300 MHz, $CDCl_3$): $\delta = 2.58$ (t, $^3J_{H,H} = 7.3$ Hz, 2H), 2.74 (t, $^3J_{H,H} = 7.3$ Hz, 2H), 3.63 (s, 2H), 3.67 (s, OCH_3), 3.73 (s, OCH_3), 6.72 (d, $^3J_{H,H} = 3.0$ Hz, 1H), 9.64 (bs, NH) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): $\delta = 20.0$, 30.2, 34.3, 51.7 (OCH_3), 52.2 (OCH_3), 100.7 (C-5), 113.8 (CN), 121.8 (C-2), 122.8 (C-3), 125.6 (C-4), 171.1 (C = O), 173.6 (C = O) ppm.

[4-^{13}C]-5-cyano-3-(methoxycarbonylethyl)-4-(methoxycarbonylmethyl)-pyrrole (7a)

Similarly, [4-^{13}C]-5-cyano-3-(methoxycarbonylethyl)-4-(tetrahydropyran-2′-yloxyethyl)-pyrrole **6a** (2.45 g, 8 mmol) yielded 5-cyano-4-(methoxycarbonylethyl)-3-(hydroxyethyl)-pyrrole (1.51 g, 85%). ^{1}H-NMR (300 MHz, acetone-d_6): $\delta = 2.58$ (t, $^3J_{H,H} = 7.3$ Hz, 2H), 2.76 (t, $^3J_{H,H} = 7.3$ Hz, 2H), 2.80 (dt, $^2J_{H,C} = 6.1$ Hz, $^3J_{H,H} = 7.2$ Hz, 2H), 3.62 (s, OCH_3), 3.70 (ddt, $^3J_{H,H} = 7.2$ Hz, $^3J_{H,H} = 5.6$ Hz, $^3J_{H,C} = 4.1$ Hz, 2H), 3.81 (bt, $^3J_{H,H} = 5.6$ Hz, 1H), 6.90 (dd, $^3J_{H,C} = 6.9$ Hz, $^3J_{H,H} = 2.9$ Hz, 1H), 10.85 (bs, NH) ppm. ^{13}C-NMR (75 MHz, acetone-d_6): $\delta = 20.8$, 29.3 (d, $^1J_{C,C} = 48$ Hz, CH_2), 35.2, 51.6 (OCH_3), 62.8 (d, $^2J_{C,C} = 2.0$ Hz, CH_2), 100.5 (d, $^1J_{C,C} = 72$ Hz, C-5), 114.9 (d, $^2J_{C,C} = 5.3$ Hz, CN), 122.5 (d, $^2J_{C,C} = 2.5$ Hz, C-2), 123.5 (d, $^1J_{C,C} = 54$ Hz, C-3), 131.2 (^{13}C-labeled, intense peak, C-4), 173.6 (C = O) ppm. HRMS calculated for $^{13}C_1C_{10}H_{14}N_2O_3$, $[M]^+$: 223.1038; found: 223.1025.

Similarly, 5-cyano-4-(methoxycarbonylethyl)-3-(hydroxyethyl)-pyrrole (1.35 g, 6 mmol) afforded **7a** (0.51 g, 34%). ^{1}H-NMR (300 MHz, $CDCl_3$): $\delta = 2.58$ (bt, $^3J_{H,H} = 7.3$ Hz, 2H), 2.74 (dt, $^3J_{H,H} = 7.3$ Hz , $^3J_{H,C} = 4.0$ Hz, 2H), 3.63 (d, $^2J_{H,C} = 6.5$ Hz, 2H), 3.67 (s, OCH_3), 3.73 (s, OCH_3), 6.72 (dd, $^3J_{H,C} = 3.0$ Hz, $^3J_{H,H} = 6.8$ Hz, 1H), 8.96 (bs, NH) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): $\delta = 20.8$ (d, $^2J_{C,C} = 2.8$ Hz, CH_2), 30.2 (d, $^1J_{C,C} = 50$ Hz, CH_2), 34.3 (d, $^3J_{C,C} = 1.8$ Hz, CH_2), 51.7 (OCH_3), 52.2 (OCH_3), 100.7 (d, $^1J_{C,C} = 74$ Hz, C-5), 113.8 (d, $^2J_{C,C} = 5.1$ Hz, CN), 121.8 (d, $^2J_{C,C} = 2.8$ Hz, C-2), 122.8 (d, $^1J_{C,C} = 56$ Hz, C-3), 125.6 (^{13}C-labeled, intense peak, C-4), 171.1 (d, $^2J_{C,C} = 2.9$ Hz), 173.5 (C = O) ppm. HRMS calculated for $^{13}C_1C_{11}H_{14}N_2O_4$, $[M]^+$: 251.0987; found: 251.0964.

[3-^{13}C]-5-cyano-3-(methoxycarbonylethyl)-4-(methoxycarbonylmethyl)-pyrrole (7b)

Similarly, [3-^{13}C]-5-cyano-3-(methoxycarbonylethyl)-4-(tetrahydropyran-2′-yloxyethyl)-pyrrole **6b** (2.45 g, 8 mmol) yielded 5-cyano-4-(methoxycarbonylethyl) - 3 - (hydroxyethyl) - pyrrole (1.35 g, 76%). ^{1}H-NMR (300 MHz, acetone-d_6): $\delta = 2.58$–2.62 (m, 2H), 2.76–2.80 (m, 2H), 2.80 (dt, $^3J_{H,H} = 7.2$ Hz, $^3J_{H,C} = 4.3$ Hz, 2H), 3.62 (s, OCH_3), 3.70 (bdt, $^3J_{H,H} = 7.2$ Hz, $^3J_{H,H} = 5.6$ Hz, 2H), 3.86 (bt, $^3J_{H,H} = 5.6$ Hz, 1H), 6.90 (dd, $^2J_{H,C} = 7.1$ Hz, $^3J_{H,H} = 2.9$Hz, 1H), 10.89 (bs, NH) ppm. ^{13}C-NMR (75 MHz, acetone-d_6): $\delta = 20.8$ (d, $^1J_{C,C} = 49$ Hz, CH_2), 29.3 (d, $^2J_{C,C} = 2.0$ Hz, CH_2), 35.2 (d, $^2J_{C,C} = 1.5$ Hz, CH_2), 51.6 (OCH_3), 62.8 (d, $^3J_{C,C} = 1.0$ Hz, CH_2), 100.5 (d, $^2J_{C,C} = 6.7$ Hz, C-5), 114.9 (d, $^3J_{C,C} = 4.9$ Hz, CN), 122.5 (d, $^1J_{C,C} = 73$ Hz, C-2), 123.5 (^{13}C-labeled, intense peak, C-3), 131.2 (d, $^1J_{C,C} = 54$ Hz, C-4), 173.6 (d, $^3J_{C,C} = 3.8$ Hz, C = O) ppm. HRMS calculated for $^{13}C_1C_{10}H_{14}N_2O_3$, $[M]^+$: 223.1038; found: 223.1023.

Similarly, 5-cyano-4-(methoxycarbonylethyl)-3-(hydroxyethyl)-pyrrole (1.35 g, 6 mmol) afforded **7b** (0.48 g, 32%). ^{1}H-NMR (300 MHz, $CDCl_3$): $\delta = 2.58$ (bdt, $^3J_{H,H} = 7.4$ Hz, $^3J_{H,C} = 4.4$ Hz, 2H), 2.74 (bdt, $^2J_{H,C} = 7.2$ Hz , $^3J_{H,H} = 7.2$ Hz, 2H), 3.63 (d, $^3J_{H,C} = 4.2$ Hz, 2H), 3.67 (s, OCH_3), 3.73 (s, OCH_3), 6.72 (dd, $^2J_{H,C} = 6.8$ Hz, $^3J_{H,H} = 3.0$ Hz, 1H), 8.76 (bs, NH) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): $\delta = 20.8$ (d, $^1J_{C,C} = 49$ Hz, CH_2), 30.2 (d, $^2J_{C,C} = 2.0$ Hz, CH_2), 34.2 (d, $^2J_{C,C} = 1.6$ Hz, CH_2), 51.7 (OCH_3), 52.2 (OCH_3), 100.7 (d, $^2J_{C,C} = 1.9$ Hz, C-5), 113.8 (d, $^3J_{C,C} = 4.8$ Hz, CN), 121.8 (d, $^1J_{C,C} = 80$ Hz, C-2), 122.8 (^{13}C-labeled, intense peak, C-3), 125.6 (d, $^1J_{C,C} = 56$ Hz, C-4), 171.1 (d, $^3J_{C,C} = 1.3$ Hz, C = O), 173.5 (d, $^3J_{C,C} = 3.5$ Hz, C = O) ppm. HRMS calculated for $^{13}C_1C_{11}H_{14}N_2O_4$, $[M]^+$: 251.0987; found: 251.0963.

[^{13}CN]-5-cyano-3-(methoxycarbonylethyl)-4-(methoxycarbonylmethyl)-pyrrole (7c)

Similarly, [^{13}CN]-5-cyano-3-(methoxycarbonylethyl)-4-(tetrahydropyran-2′-yloxyethyl)-pyrrole **6c** (2.45 g, 8 mmol) yielded [^{13}CN]-5-cyano-4-(methoxycarbonylethyl)-3-(hydroxyethyl)-pyrrole (1.40 g, 79%). ^{1}H-NMR (300 MHz, acetone-d_6): $\delta = 2.58$ (t, $^3J_{H,H} = 7.3$ Hz, 2H), 2.76 (t, $^3J_{H,H} = 7.3$ Hz, 2H), 2.80 (t, $^3J_{H,H} = 7.2$ Hz, 2H), 3.62 (s, OCH_3), 3.70 (dt, $^3J_{H,H} = 7.2$ Hz, $^3J_{H,H} = 5.6$ Hz, 2H), 3.88 (bt, $^3J_{H,H} = 5.6$ Hz, 1H), 6.90 (dd, $^3J_{H,H} = 2.9$ Hz, $^4J_{H,C} = 2.1$ Hz, 1H), 10.87 (bs, NH) ppm. ^{13}C-NMR (75 MHz, acetone-d_6): $\delta = 20.8$, 29.3, 35.2, 51.6 (OCH_3), 62.8, 100.5 (d, $^1J_{C,C} = 99$ Hz, C-5), 114.9 (^{13}C-labeled, intense peak, CN), 122.5 (d, $^3J_{C,C} = 3.2$ Hz, C-2), 123.5 (d, $^3J_{C,C} = 5.0$ Hz, C-3), 131.2 (d, $^2J_{C,C} = 5.3$ Hz, C-4), 173.6 (C = O) ppm. HRMS calculated for $^{13}C_1C_{10}H_{14}N_2O_3$, $[M]^+$: 223.1038; found: 223.1022.

Similarly, [^{13}CN]-5-cyano-4-(methoxycarbonylethyl)-3-(hydroxyethyl)-pyrrole (1.40 g, 6.3 mmol) afforded **7c** (0.65 g, 41%). ^{1}H-NMR (300 MHz, $CDCl_3$): $\delta = 2.58$ (bt, $^3J_{H,H} = 7.3$ Hz, 2H), 2.74 (t, $^3J_{H,H} = 7.3$ Hz, 2H), 3.63 (s, 2H), 3.67 (s, OCH_3), 3.73 (s, OCH_3) 6.72 (dd, $^3J_{H,H} = 2.8$ Hz, $^4J_{H,C} = 2.0$ Hz, 1H), 8.87 (bs, NH) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): $\delta = 20.0$, 30.2 (d, $^3J_{C,C} = 0.7$ Hz, CH_2), 34.3, 51.7 (OCH_3), 52.2 (OCH_3), 100.7 (d, $^1J_{C,C} = 101$ Hz, C-5), 113.8 (^{13}C-labeled, intense peak, CN), 121.8 (d, $^3J_{C,C} = 3.6$ Hz, C-2), 123.3 (d, $^3J_{C,C} = 5.0$ Hz, C-3), 125.6 (d, $^2J_{C,C} = 5.1$ Hz, C-4), 171.1 (d, $^4J_{C,C} = 0.8$ Hz, C = O), 173.5 (C = O) ppm. HRMS calculated for $^{13}C_1C_{11}H_{14}N_2O_4$, $[M]^+$: 251.0987; found: 251.0964.

Porphobilinogen lactam methyl ester (8)

To a solution of **7** (0.51 g, 2 mmol) in EtOH (50 mL) Pd-black (0.15 g, 1.4 mmol) and PtO_2 (0.37 g, 1.6 mmol) were added to the

reaction mixture and hydrogenated in a Parr apparatus at 3.5 bar for 48 h. After releasing the pressure the mixture was heated for 10 min at (50°C). The mixture was filtered over glass fiber paper (Whatmann) and concentrated *in vacuo* to yield a colorless solid of porphobilinogen lactam methyl ester **8** (0.26 g, 58%). ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 2.50$, 3.13 (t, $^3J_{H,H} = 3.2$ Hz, 2H), 3.56 (s, OCH_3), 4.24 (dt, $^3J_{H,H} = 1.9$ Hz, $^5J_{H,H} = 3.2$ Hz, 2H), 6.45 (d, $^3J_{H,C} = 7.2$ Hz, 1H), 7.69 (bs, NH), 10.29 (bs, NH) ppm. ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 20.3$ (CH_2), 29.0 (CH_2), 34.5 (CH_2), 40.0 (CH_2), 51.2 (OCH_3), 110.6 (C-4), 115.2 (C-2), 117.8 (C-3), 119.9 (C-5), 169.5 (C = O), 173.1 (C = O) ppm. HRMS calculated for $^{13}C_1C_{10}H_{14}N_2O_3$, $[M]^+$: 222.0953; found: 222.1004.

[4-^{13}C]-porphobilinogen lactam methyl ester (8a)

Similarly, **7a** (0.51 g, 2 mmol) afforded **8a** (0.39 g, 87%). ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 2.50$, 3.13 (dt, $^2J_{H,C} = 6.0$ Hz, $^5J_{H,H} = 3.2$ Hz, 2H, CH_2), 3.56 (s, OCH_3), 4.24 (dt, $^3J_{H,H} = 2.0$ Hz, $^5J_{H,H} = 3.2$ Hz, 2H), 6.45 (dd, $^3J_{H,C} = 7.2$ Hz, $^3J_{H,H} = 2.1$ Hz, 1H), 7.67 (bs, NH), 10.29 (bd, $^3J_{H,C} = 2.9$ Hz, NH) ppm. ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 20.3$ (d, $^2J_{C,C} = 2.9$ Hz, CH_2), 29.0 (d, $^1J_{C,C} = 46$ Hz, CH_2), 34.5 (d, $^3J_{C,C} = 1.7$ Hz, CH_2), 40.1 (CH_2), 51.2 (OCH_3), 110.6 (^{13}C-labeled, intense peak, C-4), 115.2 (d, $^2J_{C,C} = 4.4$ Hz, C-2), 117.8 (d, $^1J_{C,C} = 66$ Hz, C-3), 119.9 (d, $^1J_{C,C} = 55$ Hz, C-5), 169.5 (d, $^2J_{C,C} = 1.8$ Hz, C = O), 173.1 (C = O) ppm. HRMS calculated for $^{13}C_1C_{10}H_{14}N_2O_3$, $[M]^+$: 223.1038; found: 223.1033.

[3-^{13}C]-porphobilinogen lactam methyl ester (8b)

Similarly, **7b** (0.48 g, 1.9 mmol) afforded **8b** (0.35 g, 82%). ^{1}H-NMR (600 MHz, DMSO-d_6): $\delta = 2.50$, 3.13 (dt, $^3J_{H,C} = 1.5$ Hz, $^5J_{H,H} = 3.2$ Hz, 2H), 3.56 (s, OCH_3), 4.24 (dt, $^3J_{H,H} = 1.9$ Hz, $^5J_{H,H} = 3.2$ Hz, 2H), 6.44 (dd, $^2J_{H,C} = 7.4$ Hz, $^3J_{H,H} = 2.1$ Hz, 1H), 7.67 (bs, NH), 10.29 (bd, $^3J_{H,H} = 2.1$ Hz, NH) ppm. ^{13}C-NMR (150 MHz, DMSO-d_6): $\delta = 20.3$, 29.0 (d, $^2J_{C,C} = 2.2$ Hz, CH_2), 34.5 (d, $^2J_{C,C} = 1.6$ Hz, CH_2), 40.0 (d, $^3J_{C,C} = 2.6$ Hz, CH_2), 51.2 (OCH_3), 110.6 (d, $^1J_{C,C} = 55$ Hz, C-4), 115.2 (d, $^1J_{C,C} = 69$ Hz, C-2), 117.8 (^{13}C-labeled, intense peak, C-3), 119.9 (d, $^2J_{C,C} = 4.0$ Hz, C-5), 169.5 (d, $^3J_{C,C} = 3.5$ Hz, C = O), 173.1 (d, $^3J_{C,C} = 3.7$ Hz, C = O) ppm. HRMS calculated for $^{13}C_1C_{10}H_{14}N_2O_3$, $[M]^+$: 223.1038; found: 223.1038.

[11-^{13}C]-porphobilinogen lactam methyl ester (8c)

Similarly, **7c** (0.55 g, 2.2 mmol) afforded **8c** (0.45 g, 79%). ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 2.50$, 3.13 (t, $^5J_{H,H} = 3.2$ Hz, 2H), 3.69 (s, OCH_3), 4.24 (ddt, $^1J_{H,C} = 141$ Hz, $^3J_{H,H} = 1.9$ Hz, $^5J_{H,H} = 3.2$ Hz, 2H), 6.45 (d, $^3J_{H,H} = 2.1$ Hz, 1H), 7.69 (bs, NH), 10.29 (bs, NH) ppm. ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 20.3$, 29.0, 34.5, 40.0 (^{13}C-labeled, intense peak, CH_2), 51.2 (OCH_3), 110.6 (d, $^2J_{C,C} = 2.0$ Hz, C-4), 115.2 (C-2), 117.8 (C-3), 119.9 (d, $^1J_{C,C} = 53$ Hz, C-5), 169.5 (C = O), 173.1 (C = O) ppm. HRMS calculated for $^{13}C_1C_{10}H_{14}N_2O_3$, $[M]^+$: 223.1038; found: 223.1026.

Porphobilinogen (1)

A solution of porphobilinogen lactam methyl ester **8** (0.33 g, 1.5 mmol) in aqueous 2 M KOH (3 mL) was stirred in dark for 4 days at room temperature. A total of 6 M acetic acid was used to make pH 6–7 and kept at 0°C for 3 h. The solid was filtered off and washed with water and acetone to yield porphobilinogen **1** as a colorless solid (0.20 g, 59%). ^{1}H-NMR (600 MHz, DMSO-d_6): $\delta = 2.35$ (t, $^3J_{H,H} = 8.0$ Hz, 2H), 2.54 (t, $^3J_{H,H} = 8.0$ Hz, 2H), 3.10 (s, 2H,), 3.83 (s, 2H), 6.40 (d, $^3J_{H,H} = 2.3$ Hz, 1H), 10.6 (bs, 1H, NH) ppm. ^{13}C-NMR (150 MHz, $CDCl_3$): $\delta = 20.7$ (CH_2), 34.2 (CH_2), 34.6 (CH_2), 34.7 (CH_2), 113.9 (C-2), 117.5 (C-4), 121.5 (C-3), 122.0 (C-5), 174.7 (C = O), 175.0 (C = O) ppm. HRMS calculated for $C_{10}H_{14}N_2O_4$, $[M-NH_2]^+$: 210.0766; found: 210.0751.

[4-^{13}C]-porphobilinogen (1a)

Similarly, **8a** (0.36 g, 1.6 mmol) was worked out to afford [4-^{13}C]-porphobilinogen **1a** as a colorless solid (0.15 g, 42%). ^{1}H-NMR (600 MHz, DMSO-d_6): $\delta = 2.35$ (dt, $^3J_{H,C} = 3.3$ Hz, $^3J_{H,H} = 8.0$ Hz, 2H), 2.54 (dt, $^3J_{H,C} = 3.3$ Hz, $^3J_{H,H} = 8.0$ Hz, 2H), 3.10 (d, $^2J_{H,C} = 6.3$ Hz, 2H), 3.83 (d, $^3J_{H,C} = 3.2$ Hz, 2H), 6.40 (dd, $^3J_{H,C} = 6.6$ Hz, $^3J_{H,H} = 2.3$ Hz, 2H), 10.6 (bd, $^3J_{H,C} = 3.5$ Hz, NH) ppm. ^{13}C-NMR (150 MHz, DMSO-d_6): $\delta = 20.7$ (d, $^2J_{C,C} = 2.7$ Hz, CH_2), 34.2 (d, $^2J_{C,C} = 3.5$ Hz, CH_2), 34.7 (d, $^1J_{C,C} = 46$ Hz, CH_2), 34.7 (CH_2), 113.9 (d, $^2J_{C,C} = 3.8$ Hz, C-2), 117.5 (^{13}C-labeled, intense peak, C-4), 121.5 (d, $^1J_{C,C} = 54$ Hz, C-3,) 122.0 (d, $^1J_{C,C} = 70$ Hz, C-5), 174.7 (C = O), 175.0 (d, $^2J_{C,C} = 3.8$ Hz, C = O) ppm. HRMS calculated for $^{13}C_1C_9H_{14}N_2O_4$, $[M-NH_2]^+$: 211.0800; found: 211.0809.

[3-^{13}C]-porphobilinogen (1b)

Similarly, **8b** (0.36 g, 1.6 mmol) was worked out to afford [3-^{13}C]-porphobilinogen **1b** as a colorless solid (0.18 g, 50%). The crude mixture was lypholyzed and purified in HPLC (Alltech Atima RP C18, 5 μm (10 × 250 mm); 5–15% CH_3CN: H_2O–0.1% HCO_2H; flow rate 5.00 mL/min; detection at $\lambda = 225$ nm; $R_t = 13.5$) to afford a colorless solid of **1b** (0.11 g, 48%). ^{1}H-NMR (600 MHz, DMSO-d_6): $\delta = 2.35$ (dt, $^3J_{H,C} = 3.3$ Hz, $^3J_{H,H} = 8.0$ Hz, 2H), 2.54 (dt, $^2J_{H,C} = 6.6$ Hz, $^3J_{H,H} = 8.0$ Hz, 2H), 3.10 (d, $^3J_{H,C} = 4.1$ Hz, 2H), 3.83 (s, 2H), 6.40 (dd, $^2J_{H,C} = 6.6$ Hz, $^3J_{H,H} = 2.3$ Hz, 2H), 10.5 (bd, $^3J_{H,C} = 4.9$ Hz, NH) ppm. ^{13}C-NMR (150 MHz, DMSO-d_6): $\delta = 20.7$ (d, $^1J_{C,C} = 49$ Hz, CH_2), 34.2 (CH_2), 34.5 (CH_2), 34.7 (CH_2), 113.9 (d, $^1J_{C,C} = 68$ Hz, C-2), 117.5 (d, $^1J_{C,C} = 54$ Hz, C-4), 121.5 (^{13}C-labeled, intense peak C-3), 122.0 (d, $^2J_{C,C} = 4$ Hz, C-5), 174.7 (C = O), 175.0 (C = O) ppm. HRMS calculated for $^{13}C_1C_9H_{14}N_2O_4$, $[M-NH_2]^+$: 211.0800; found: 211.0796.

[11-^{13}C]-porphobilinogen (1c)

Similarly, **8c** (0.36 g, 1.6 mmol) was worked out to afford [11-^{13}C]-porphobilinogen **1c** as a colorless solid (0.21 g, 57%). ^{1}H-NMR (600 MHz, DMSO-d_6): $\delta = 2.35$ (t, $^3J_{H,C} = 7.5$ Hz, 2H), 2.54 (t, $^3J_{H,H} = 7.6$ Hz, 2H), 3.10 (s, 2H), 3.83 (d, $^1J_{H,C} = 141.0$ Hz, s, 2H), 6.40 (d, $^3J_{H,H} = 2.3$ Hz, 2H), 10.6 (bs, NH) ppm. ^{13}C-NMR (150 MHz, DMSO-d_6): $\delta = 20.7$ (CH_2), 34.2 (^{13}C-labeled, intense peak, CH_2), 34.7 (d, $^3J_{C,C} = 2$ Hz, CH_2), 34.7 (CH_2), 113.9 (C-2), 117.5 (d, $^2J_{C,C} = 3$ Hz, C-4), 121.5 (d, $^3J_{C,C} = 2$ Hz, C-3,) 122.0 (d, $^1J_{C,C} = 56$ Hz, C-5), 174.7 (C = O), 175.0 (C = O) ppm. HRMS calculated for $^{13}C_1C_9H_{14}N_2O_4$, $[M-NH_2]^+$: 211.0800; found: 211.0802.

Conclusions

The novel ^{13}C-enriched porphobilinogens [3-^{13}C]- and [4-^{13}C]-porphobilinogen together with the known [11-^{13}C]-porphobilinogen have been prepared in reasonable overall yields using simple starting materials that are commercially available in isotopically labeled form. The isotope incorporation of the target product is in agreement within the experimental error with those of the starting materials. This shows that during the synthesis no isotopic dilution or scrambling has taken place. All possible isotopomers of building blocks **2**, **3** and **5** and

porphobilinogen **1** in high isotopic enrichment forms are now accessible via the synthetic routes described in this paper.

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