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Synthesis and Acidity Constants of $^{13}CO_2H$-Labelled Mono and Dipyrrole Carboxylic Acids. pK_a from ^{13}C-NMR

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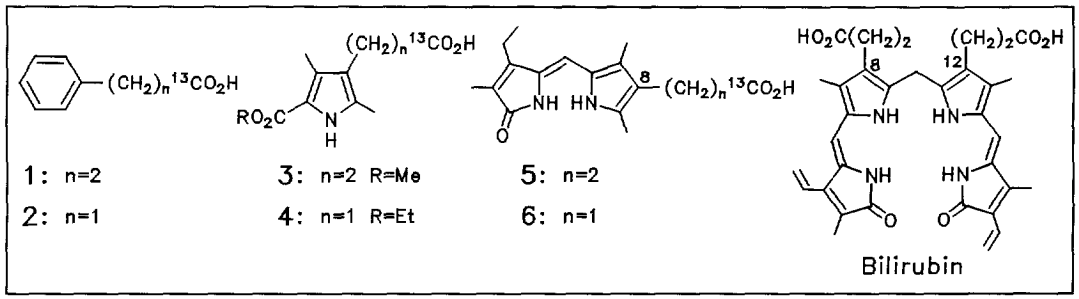
Abstract: Six monocarboxylic acids were prepared highly enriched with ^{13}C in their CO_2H groups, and their pK_a values were determined at low concentrations (10^{-4}-10^{-5} *M*) in H_2O and in H_2O-$(CD_3)_2SO$ mixtures by analysis of pH-dependent $^{13}CO_2H$ NMR chemical shifts. Plots of the variation of $CO_2H(CO_2^-)$ ^{13}C-NMR chemical shift *vs* pH gave a typical titration curve from which pK_a's for [1-^{13}C]-phenylpropionic (**1**) and [1-^{13}C]-phenylacetic (**2**) acids were determined to be 4.60 and 4.16 respectively in H_2O, and 4.67 and 4.31 respectively in H_2O-27% vol $(CD_3)_2SO$. Bilirubin analogs, xanthobilirubic acid (**5**) and nor-xanthobilirubic acid (**6**) were determined to have pK_a values of 4.76 and 4.64 respectively in H_2O-27% vol $(CD_3)_2SO$, and extrapolated to pK_a values ~4.62 and 4.51 in H_2O.

INTRODUCTION

Twenty-five years ago, Hagen and Roberts[1] reported on the ^{13}C-NMR chemical shifts of simple short chain *n*-alkanoic acids and their tetramethylammonium salts in aqueous solution, observing an ~5 ppm deshielding of the CO_2H resonance upon ionization. Although these findings suggested that ^{13}C-NMR might be applied to the determination of carboxylic acid acidity constants (pK_a), only a few investigations have been reported,[2] and those used natural isotopic abundance ^{13}C-NMR for high concentrations (0.04-0.05 *M*) of acetic, propionic and butyric acids to determine pK_a values quite close to those found by classical emf titrations.[2] However, the method was limited to water-soluble acids. Limited solubility was a somewhat lesser consideration in the application of natural isotopic abundance ^{13}C-NMR to protonation studies of amino acids,[3] determination of their pK_a,[2-4] and a detailed analysis of microscopic acid dissociation constants.[5,6]

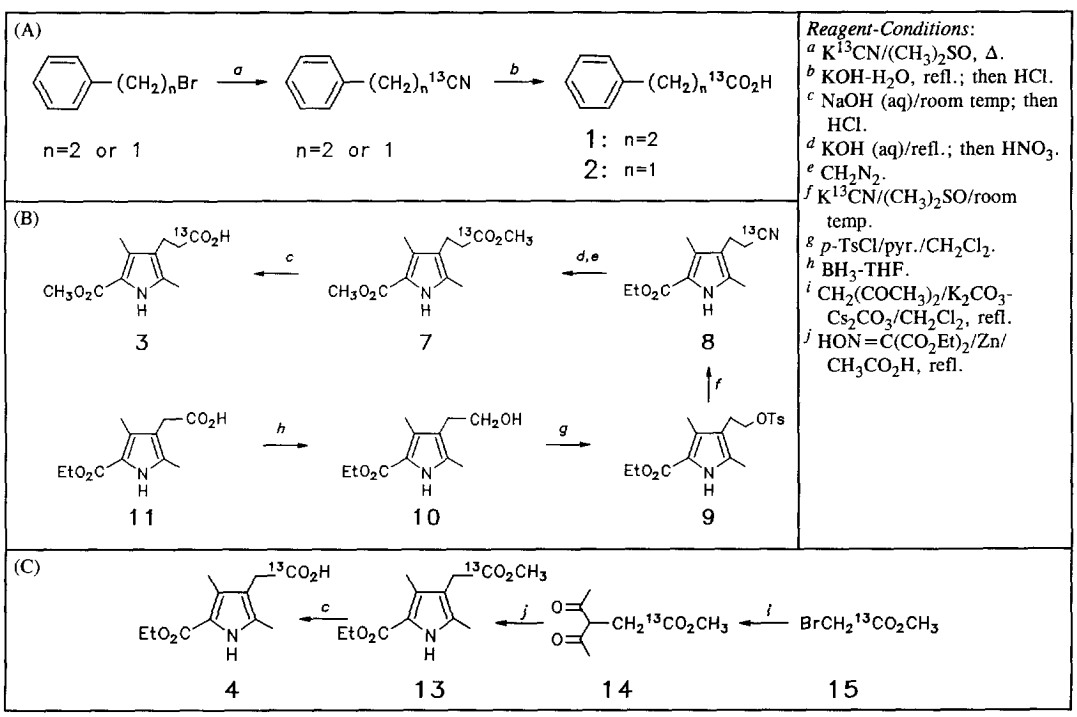
The main limitations associated with the use of ^{13}C-NMR to determine carboxylic acid pK_a lie principally with the water insolubility of some of the most interesting carboxylic acids and the insensitivity of the method in natural isotopic abundance ^{13}C-NMR. In the following we show how these limitations might be overcome by (*i*) the use of $(CD_3)_2SO$ as an organic co-solvent and (*ii*) the use of ^{13}C-enriched ($^{13}CO_2H$-labelled) acids, which allows pK_a determinations at the 10^{-5} *M* concentration level. Our ultimate goal is to determine the pK_a values for bilirubin, the yellow pigment of jaundice and a dicarboxylic acid whose reported pK_a values are controversial, ranging from ~5 to ~8.[7,8] For the study reported here, we focus on several ^{13}C-labelled monocarboxylic acid dipyrrinone analogs of bilirubin (**5** and **6**) and their monopyrrole components (**3** and **4**), and phenyl analogs (**1** and **2**) whose pK_a values have been determined previously by emf methods.



RESULTS AND DISCUSSION

Synthesis. Syntheses of **1** and **2** were straightforward (Scheme 1A). Carbon-13 was introduced as $K^{13}CN$ by nucleophilic displacement of bromide from 2-phenethyl bromide and benzyl bromide, followed by hydrolysis to afford 90% ^{13}C-enriched β-phenylpropionic acid (**1**) and phenylacetic acid (**2**), respectively. Similarly, $K^{13}CN$ was the label source in the synthesis (Scheme 1B) of monopyrrole acid **3**. The starting material, 3,5-dimethyl-2-ethoxycarbonyl-1*H*-pyrrole-4-acetic acid ethyl ester (**12**),[9] was selectively saponified to give **11** in 96% yield. Diborane reduction of **11** gave alcohol **10** (86% yield), which was converted to its tosylate (**9**) in 82% yield. Reaction of **9** with 99% ^{13}C-enriched $K^{13}CN$ to afford nitrile **8** in 95% yield. Base-catalyzed hydrolysis gave the homologated pyrrole diacid, which was isolated as its dimethyl ester (**7**) following treatment with diazomethane. Selective saponification of **7** gave **3** in 86% yield.

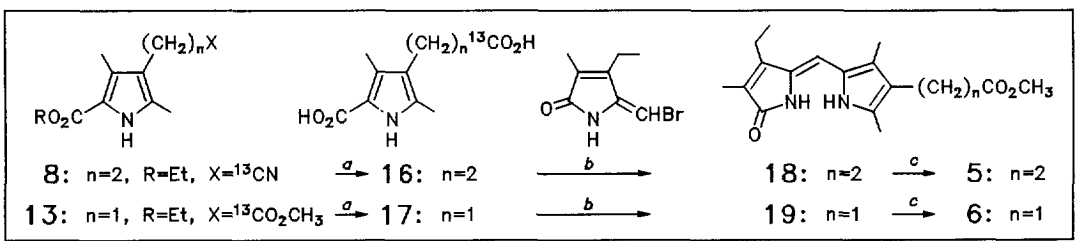
Synthetic Scheme 1



Synthesis of **4**, the nor-analog of **3**, was accomplished by a Fischer pyrrole synthesis. Pentane-2,4-dione was alkylated in 91% yield with the methyl ester of [1-^{13}C]-bromoacetic acid (99% ^{13}C-enriched), and the product (**14**) was condensed with diethyl oximinomalonate using zinc and acetic acid to afford **13** (55%). Selective saponification of **13** gave **4** in 92% yield.

^{13}C-Labelled dipyrrinones **5** and **6** were prepared from labelled monopyrroles **8** and **13**, respectively, as shown in Scheme 2. Monopyrrole **8** was converted to diacid **16**, which was condensed with 5-bromomethylene-4-ethyl-3-methyl-2-oxo-2,5-dihydropyrrole[10] in refluxing methanol to give labelled methyl xanthobilirubinate **18** in 67% yield. Saponification of **18** afforded the acid (**5**). Similarly, monopyrrole diester **13** was converted to **6**.

Synthetic Scheme 2

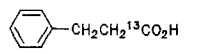
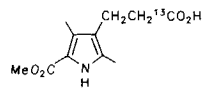
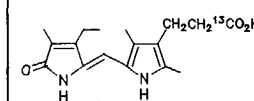
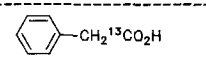
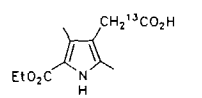
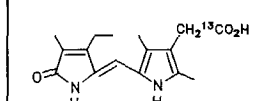


[a] NaOH (aq.) + $NaNO_3$/refl.; then cool and add HNO_3-$NaNO_3$. [b] CH_3OH, refl. [c] NaOH-CH_3OH/refl.; then HCl.

Acid and Anion δ_{CO_2H} and $\delta_{CO_2^-}$. The "titration shift" noted earlier for carboxylic acids in water,[1,11] the downfield shift in the ^{13}C-NMR of a carboxylic acid resonance upon deprotonation has been suggested to be approximately equal to the acid's pK_a value.[11] It can be detected in aqueous solutions of **1-6** (Δ of Table 1) but is not much evident in the dipolar, aprotic solvent (d_6-dimethylsulfoxide, $(CD_3)_2SO$) or in the nonpolar solvent $CDCl_3$. However, it can be seen in H_2O-$(CD_3)_2SO$ solutions. With increasing mole percent $(CD_3)_2SO$, the titration shift in **1-4** diminishes, with major changes occurring as the mole percent of $(CD_3)_2SO$ approaches and exceeds that of H_2O. Although δ_{CO_2H} decreases fairly linearly with increasing percent $(CD_3)_2SO$, $\delta_{CO_2^-}$ does not. It decays rapidly and nonlinearly for $(CD_3)_2SO > 64\%$ to nearly the same value as δ_{CO_2H} in 100% $(CD_3)_2SO$. In $CDCl_3$ solvent, Δ is also small and variable, with δ_{CO_2H} and $\delta_{CO_2^-}$ typically lying between the corresponding values in pure H_2O and pure $(CD_3)_2SO$.

The origin of the titration shift is not well understood. Clearly, there is an electric field change at the carboxyl carbon upon deprotonation of the carboxyl. Solvation effects, the nature of the counterion and the tightness of the ion pair are expected to play a role in determining the shielding at the carboxylate carbon and hence the size of the titration shift. However, solvation of both the acid and the anion species must play a role. And differences in solvation of carboxylic acids upon transfer from one solvent to another may be large.[12] In fact, large upfield shifts in δ_{CO_2H} and $\delta_{CO_2^-}$ are found (Table 1) in transferring from H_2O (dipolar, protic) to $(CD_3)_2SO$ (dipolar, aprotic) or $CDCl_3$ (nonpolar) solvents. The greatest solvent shift is found in transferring from H_2O to $(CD_3)_2SO$, with $\delta_{CO_2^-}$ shifting much more than δ_{CO_2H}. The former is apparently more sensitive to solvation effects, the nature of the cation and the tightness of the ion pair. This may be detected in transferring from n-Bu_4N^+ cation to Cs^+ cation (Table 1). In H_2O, where the carboxylate ions are hydrogen bonded to solvent, the ion pair is probably well-separated by solvent, and $\delta_{CO_2^-}$ is independent of n-Bu_4N^+ or Cs^+ cation. In $(CD_3)_2SO$, the ions are not hydrogen bonded to solvent but are solvated by

TABLE 1. Solvent Dependence of ^{13}C-NMR Chemical Shifts[a] of $^{13}CO_2H$-Labelled Propionic and Acetic Acid Analogs and Their Tetra-*n*-butylammonium Carboxylate Anions

Compound	H_2O			H_2O-9% $(CD_3)_2SO$			H_2O-27% $(CD_3)_2SO$			H_2O-64% $(CD_3)_2SO$			$(CD_3)_2SO$			$CDCl_3$		
	δ_{CO_2H}	$\delta_{CO_2^-}$	Δ^b	δ_{CO_2H}	$\delta_{CO_2^-}$	Δ^b	δ_{CO_2H}	$\delta_{CO_2^-}$	Δ^b	δ_{CO_2H}	$\delta_{CO_2^-}$	Δ^b	δ_{CO_2H}	$\delta_{CO_2^-}$	Δ^b	δ_{CO_2H}	$\delta_{CO_2^-}$	Δ^b
 1	177.1	181.6 (181.5)[c]	4.5	176.9	181.3	4.4	176.7	180.9	4.3	175.0	178.4	3.4	173.7	173.5 (174.4)[c]	-0.2	174.6	176.8 (172.3)[c]	2.2
 3	177.4	181.8 (181.7)[c]	4.4	177.2	181.4	4.2	176.6	180.8	4.2	175.3	178.9	3.6	173.9	174.0 (176.3)[c]	0.1	175.6	177.4	0.8
 5	177.1[d]	181.9 (181.9)[c]	4.8	176.9	181.6	4.7	176.8	180.9	4.1	175.4	178.8	3.4	173.7	174.0 (175.5)[c]	0.3	178.4	178.0	-0.4
 2	176.1	180.1 (179.9)[c]	4.0	175.9	179.7	3.8	175.4	178.9	3.5	173.9	176.7	2.8	172.6	171.8 (172.5)[c]	-0.8	175.8	175.0 (171.0)[c]	-0.8
 4	176.1	180.1 (180.0)[c]	4.0	175.7	179.8	4.1	175.4	179.1	3.7	174.0	177.0	3.0	172.8	173.7 (173.2)[c]	0.9	174.7	175.1	0.4
 6	176.3[d]	180.3 (180.4)[c]	4.0	175.9	180.0	4.1	175.5	179.3	3.8	174.1	177.2	3.0	172.9	173.8 (173.0)[c]	0.9	173.6	175.9	2.3

[a] $(CD_2)_2SO$ was used as an external reference (δ 39.50) in aqueous and $CDCl_3$ solvents at 25°C, with δ reported in ppm downfield from $(CH_3)_4Si$. [b] $\delta_{CO_2^-}$ minus δ_{CO_2H}. [c] Cesium salt.
[d] Extrapolated value.

dipolar solvent molecules. The influence of cation type on $\delta_{CO_2^-}$ is evident, presumably due to a somewhat tighter ion pair in $(CD_3)_2SO$ than in H_2O. The greatest sensitivity to change in cation type is observed in $CDCl_3$ solvent, which is expected to solvate ions only very poorly, leaving a tight ion pair.

Interestingly, for the same cation, the sets of $\delta_{CO_2^-}$ values for the propionic acid type (**1**, **3** and **5**) and for the acetic acid type (**2**, **4** and **6**) are nearly identical in a given solvent (Table 1), except **5** in $CDCl_3$. An entirely similar behavior is seen for δ_{CO_2H}, with **5** in $CDCl_3$ the exception.

Acid pK_a Determination. For aqueous solvents, including those containing dimethylsulfoxide co-solvent, the acid deprotonation equilibrium may be expressed in simplified form as:

$$R\text{-}CO_2H + H_2O \rightleftarrows R\text{-}CO_2^- + H_3O^+ \quad (1)$$

where,

$$K_a = \frac{[H_3O^+]\,[R\text{-}CO_2^-]}{[H_2O]\,[R\text{-}CO_2H]} \quad (2)$$

Since $[H_2O]$ is in very high concentration relative to the concentrations of the species in equilibrium, it is taken to be unity. Thus, when $[R\text{-}CO_2H] = [R\text{-}CO_2^-]$, $pK_a = pH$. As the equilibrium is driven from R-CO_2H to R-CO_2^- (or vice-versa), the observed ^{13}C-NMR chemical shift (δ_{obs}) varies between that of the pure acid (δ_{CO_2H}) to that of the pure anion ($\delta_{CO_2^-}$) according to the relationship:

$$\delta_{obs} = N_{CO_2H}\,\delta_{CO_2H} + N_{CO_2^-}\,\delta_{CO_2^-} \quad (3)$$

where N_{CO_2H} and $N_{CO_2^-}$ are the mole fractions of acid and anion, respectively. When $N_{CO_2H} = N_{CO_2^-}$ (or $[RCO_2H] = [RCO_2^-]$ of equation (2)), then

$$\delta_{obs} = 1/2\,(\delta_{CO_2H} + \delta_{CO_2^-}) \quad (4)$$

and under those conditions, pK_a=pH. Since pH can be measured accurately with a good pH meter, pK_a values can thus be determined easily from ^{13}C-NMR chemical shifts, δ_{obs}, δ_{CO_2H} and $\delta_{CO_2^-}$. The latter two are constant in a given solvent system and fall at the flat extremes of standard sigmoidal titration curves obtained by plotting δ_{obs} *vs* pH, as in Fig. 1. From curves such as these, one can easily read the half equivalence point (satisfying equation 4) and thus the pK_a=pH of the acid. Although the presence of $(CD_3)_2SO$ clearly affects the titration curves of Fig. 1, displacing them downward with increasing $(CD_3)_2SO$, the shapes and the pK_a values are relatively invariant. The data suggest that dimethylsulfoxide can be a useful co-solvent for determining pK_a.

Interestingly, over the range of H_2O-$(CD_3)_2SO$ solvent mixtures used, from 0% to 64% (vol) $(CD_3)_2SO$, the pK_a values of a given acid vary by only ~0.2 pK units (Table 2). This is perhaps due to the fact that an aqueous solution 64% (vol) in dimethylsulfoxide is mainly water, some 69 mole % water, and equimolar solutions are not reached until the vol % dimethylsulfoxide reaches ~80%. However, as mole ratio of dimethylsulfoxide exceeds that of water, the pK values apparently rise steeply to ~10-11. Previously, it had been shown that the pK's of acetic acid and benzoic acid in pure $(CH_3)_2SO$ are 11.4 and 10.0 (respectively) *vs* 4.76 and 4.20 in H_2O.[13] Nevertheless, when the solvent is mainly water (*e.g.*, < 64 vol % dimethylsulfoxide), aqueous dimethylsulfoxide appears to be quite useful for determining carboxylic acid pK_a's, as shown in the following.

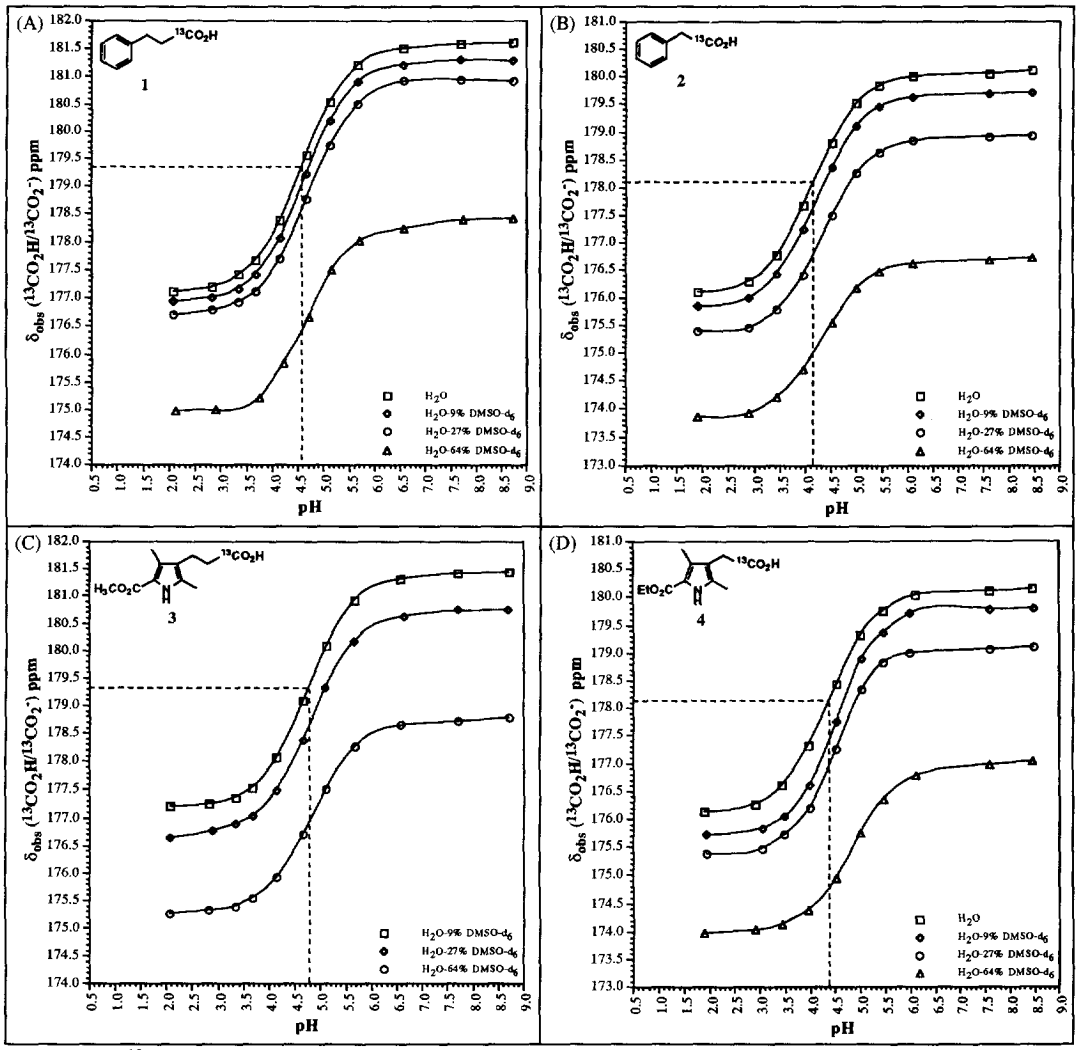
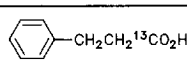
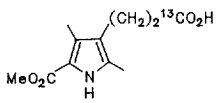
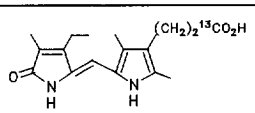
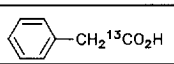
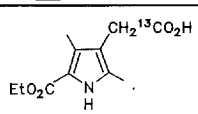
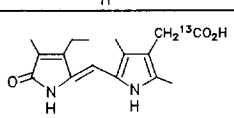


FIGURE 1. ^{13}C-NMR titration curves showing the solvent and pH-dependent behavior of δ_{obs} for the carboxyl group resonance in 90-99% enriched ($^{13}CO_2H$): (A) hydrocinnamic acid (**1**), (B) phenylacetic acid (**2**), (C) monopyrrole propionic acid **3**, and (D) monopyrrole acetic acid **4**. The data are taken for 10^{-4}-10^{-5} *M* acid solutions in 0.1 *M* buffers containing various (vol/vol) percents $(CD_3)_2SO$ in H_2O at 25°C.

Plots of pK_a *vs* log % (vol) $(CD_3)_2SO$ give good straight line behavior, as shown in Fig. 2, and the pK_a's found by extrapolation to 100% H_2O are in excellent agreement with those determined independently in water (Table 3). These observations might be important in determining pK_a's of acids with limited water solubility, because they indicate that one may extrapolate a pK_a (in H_2O) from pK_a's determined in aqueous dimethylsulfoxide solutions. Dipyrrinones **5** and **6** are just such examples. They are too insoluble in water to determine pK_a's directly by ^{13}C-NMR, but they are soluble in dimethylsulfoxide and dissolve in H_2O-$(CD_3)_2SO$ solutions where the vol % $(CD_3)_2SO$ is > 20%. In H_2O-$(CD_3)_2SO$ solutions, their titration curves (Fig. 3) are found to lie in propionic or acetic acid-type groupings: **5** with propionic acids **1** and **3**; **6** with

acetic acids **2** and **4**. As might be anticipated, the titration curve of **5** is closer to that of monopyrrole **3** than to **1**, and the titration curve of **6** is closer to that of monopyrrole **4** than to **2**. If one assumes that the slope of a plot of pK_a *vs* log vol % $(CD_3)_2SO$ for **6** would be parallel to that of **4** in Fig. 2, then the pK_a of **6** at any point will be the same as that of **4**, adjusted for the displacement of the parallel lines. One may thus estimate a pK_a in 100% H_2O of ~4.62 for **6** and ~4.51 for **5** using the data of Table 2.

TABLE 2. Comparison of pK_a Values[a] of Labelled Carboxylic Acids in H_2O-$(CD_3)_2SO$ Mixtures.

Carboxylic Acid	pK_a in Aqueous $(CD_3)_2SO$ Vol % (Mole %) $(CD_3)_2SO$						Lit. pK_a in H_2O
	0 (0)	1.8 (0.5)	3.6 (1.0)	9 (2.5)	27 (8.6)	64 (31)	
1: 	4.59	4.60	4.61	4.63	4.68	4.72	4.66[b]
3: 	4.68	4.70	4.72	4.78	4.82	4.84	NA
5: 	*Insol*	*Insol*	*Insol*	4.66	4.76	4.83	NA
2: 	4.16	4.20	4.22	4.25	4.31	4.35	4.31
4: 	4.37	4.39	4.41	4.48	4.50	4.85	NA
6: 	*Insol*	*Insol*	*Insol*	4.49	4.64	4.93	NA

[a] Determined from pH-dependent ^{13}C-NMR data of Fig. 1. [b] From emf measurements and tabulated in Serjeant, E.D.; Dempsey, B. *Ionization Constants of Organic Acids in Aqueous Solution*. Pergamon Press, Oxford, U.K., **1979**.

Although one can apparently extrapolate pK_a nicely to 100% H_2O from pK_a values in H_2O-dimethylsulfoxide, extrapolation to pK in 100% dimethylsulfoxide is less clear. Extrapolation of straight-line plots of pK_a *vs* log vol % $(CD_3)_2SO$ (Fig. 2) predict pK values far below those expected (pK ~10-11)[13] for 100% dimethylsulfoxide, and the plots curve upward as the solvent changes from mainly H_2O to mainly dimethylsulfoxide. The departure from linearity is already evident for **4** at 50% vol $(CD_3)_2SO$, but it is not clear why **4** should exhibit nonlinear behavior in the 27-64 vol % range of $(CD_3)_2SO$ when **1**-**3** do not.

CONCLUDING COMMENTS

In solvents where carboxylic acids and their carboxylate ions exhibit different ^{13}C-NMR chemical shifts for CO_2H and CO_2^-, pK_a's may be determined from ^{13}C-NMR spectroscopy. With 90-99% ^{13}C-enrichment in the CO_2H group, the method becomes sensitive to 10^{-5} *M* concentrations. For water-insoluble acids, dimethylsulfoxide may be added to improve aqueous solubility, and pK_a's may be extrapolated to 100% water. In fact, pK_a values in aqueous dimethylsulfoxide (up to 27 vol %) are consistently slightly higher but differ

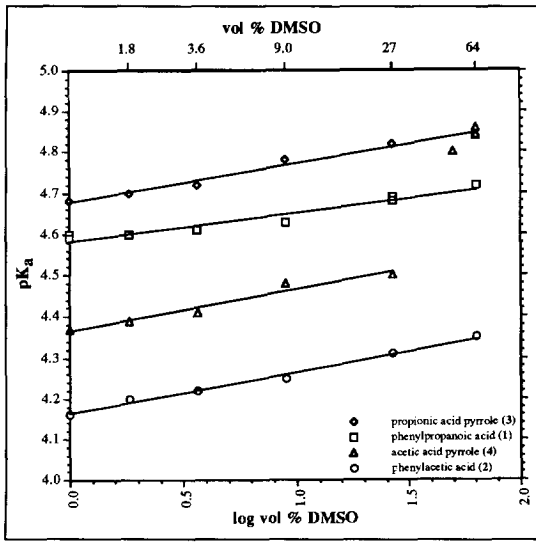


FIGURE 2. Plots of pK_a *vs* log vol % $(CD_3)_2SO$ for carboxylic acids **1-4**. The pK_a values are from Fig. 1. The graphs show, but do not include the pK_a values for 100% H_2O.

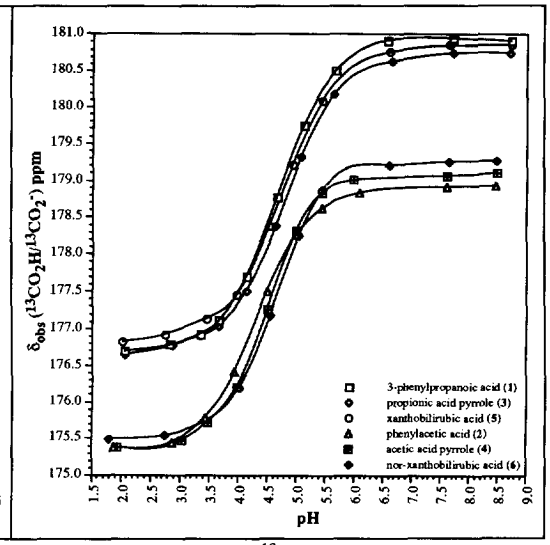
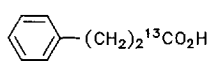
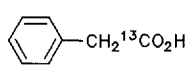
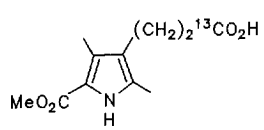
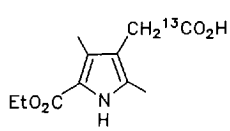


FIGURE 3. Comparison of ^{13}C-NMR titration curves for **1-6** in H_2O 27 vol % $(CD_3)_2SO$ solutions. The concentrations of **1-6** are 10^{-4}-10^{-5} *M* in 0.1 *M* aq. buffers at 25°C.

TABLE 3. Least Squares Fit Linear Equations for the Plots of pK_a *vs* log vol % $(CD_3)_2SO$ and Comparison of Extrapolated and Independently-Determined pK_a Values for Acids **1-4**.

Acid	Least Squares Line[a]	Extrapolated pK_a[b]	Observed pK_a[c]
1: 	y = 0.084x + 4.56[d]	4.56	4.59
2: 	y = 0.094x + 4.17[e]	4.17	4.16
3: 	y = 0.096x + 4.68[f]	4.68	4.68
4: 	y = 0.101x + 4.36[g]	4.36	4.37

[a] y = pK_a value; x = log vol % $(CD_3)_2SO$. [b] Extrapolated to 100% H_2O. [c] Experimental value in H_2O (Table 1). [d] r^2 = 0.96. [e] r^2 = 0.98. [f] r^2 = 0.97. [g] r^2 = 0.93, does not include data points for >27% vol $(CD_3)_2SO$.

by less than 0.2 pK units from pK_a's in 100% water. These results are important for they suggest that ^{13}C-NMR can be used to determine pK_a's of carboxylic acids that have only a very limited aqueous solubility. Further work is underway involving dicarboxylic acid pK_a's, including that of the biologically important natural product bilirubin.

EXPERIMENTAL

General Procedures. Infrared (IR) spectra were recorded on a Perkin-Elmer 1610 FT spectrophotometer. Nuclear magnetic resonance (NMR) spectra were determined in $CDCl_3$ or $(CD_3)_2SO$ on a Varian 500 MHz Unity plus spectrometer or a General Electric QE-300 300 MHz spectrometer and are reported in δ (ppm) downfield from $(CH_3)_4Si$. pH measurements were determined on a model 811 Orion Research microprocessor pH/millivolt meter. GC-MS analyses were carried out on a Hewlett-Packard GCMS Model 5890A ion selective detector equipped with a DB-1 (100% dimethylpolysiloxane) column. Melting points were determined on a Thomas-Hoover Uni-Melt capillary apparatus and are uncorrected. All solvents were reagent grade obtained from Fisher. Deuterated chloroform and dimethylsulfoxide were from Cambridge Isotope Laboratories. Labelled potassium cyanide ($K^{13}CN$, 90% and 99% ^{13}C-enriched) and [1-^{13}C]-bromoacetic acid (99% ^{13}C-enriched) were obtained from Cambridge Isotope Laboratories. 2-Bromoethylbenzene, benzylbromide, 2,4-pentanedione, pyridine, *p*-toluenesulfonyl chloride, and 1M BH_3-THF were obtained from Aldrich and used as received. Analytical thin layer chromatography (TLC) was carried out on J.T. Baker silica gel IB-F plates (125 μm layer). Aspirator flash chromatography was carried out on Woelm silica gel F, thin layer chromatography grade. Combustion analyses were performed by Desert Analytics, Tucson, AZ.

Sample Preparation. NMR samples were prepared in NMR tubes by adding standard aliquots of a stock solution of acid or its tetra-*n*-butylammonium salt or cesium salt to aqueous buffers. The stock solutions were prepared as 6-8 x 10^{-3} *M* solutions in either H_2O or in $(CD_3)_2SO$. Buffered solutions were 0.1 *M* acetate (for pH ~3.2-6.8) and 0.1 M Tris (for pH > ~6.8). At pH < 3.2, either 0.1 *M* acetic acid, or 0.1 *M* acetic acid-HCl, or 0.2 *M* HCl were used (non-buffered). Phosphate buffers (0.1 *M*) were used to compare δ_{CO_2H}/$\delta_{CO_2^-}$ values derived from 0.1 *M* Tris buffer. No difference was detected at the same pH. For the concentrations of 3-phenylpropionic acid used (~10^{-4} *M*) no difference in $\delta_{CO_2^-}$ could be detected at pH 7.4 in 0.1 *M* and 1.0 *M* Tris buffer. Ten to eleven simple solutions were prepared in NMR tubes at various pH for use in a complete titration curve.

1. Aqueous solutions were prepared by adding 50 μL of a 6-8 x 10^{-3} *M* stock solution of acid or its salt dissolved in deionized H_2O to 500 μL of buffer.

2. Aqueous dimethylsulfoxide solutions were prepared by adding an aliquot of a 6-8 x 10^{-3} *M* stock solution of acid or its salt in $(CD_3)_2SO$ to an aliquot of buffer:

Final vol % $(CD_3)_2SO$	μL Aliquot Stock Solution	Vol. Buffer (μL)
1.8	10	540
3.6	20	530
9	50	500
27	50	400 + 100 μL $(CD_3)_2SO$
64	50	200 + 300 μL $(CD_3)_2SO$

Final sample concentrations ranged from 8 x 10^{-4} *M* to 2 x 10^{-5} *M*, with δ_{obs} for the CO_2H/CO_2^- group being independent of concentration in this range. Compounds with only a limited solubility in the NMR solution, *e.g.*, at low pH, were run more dilute.

NMR measurements of δ_{obs} for CO_2H/CO_2^- were carried out on a Varian Unity Plus 500 MHz spectrometer. The instrument settings and parameters were: frequency 125.706 MHz; spectral width 28,368.8 Hz; acquisition time 2.000 sec; relaxation time 0.000 sec; pulse width 5.0 μsec; decouple ^{1}H; high power 40; decoupler continuously on; Waltz 16 modulated; double precision acquisition; line broadening 1.8 Hz, number of acquisitions varied depending on sample concentration and % ^{13}C-label; and temperature 25°C. Titration curves for 3-phenylpropionic acid run at 37°C in 64% vol $(CD_3)_2SO$ were shifted downward but gave essentially the same pK_a value: 4.72 (25°C) and 4.73 (37°C). To each sample was added a sealed mp capillary insert filled with 50 μL of $(CD_3)_2SO$ that was used as the lock and external reference to standardize all samples to an independent of environment reference.

[1-^{13}C]-3-Phenylpropanoic acid (hydrocinnamic acid-[$^{13}CO_2H$]) (**1**). In a 25 mL Erlenmeyer flask equipped with a magnetic stir bar was placed K^{13}CN (359 mg, 5.42 mmol, 90% ^{13}C-enriched) in $(CH_3)_2SO$ (15 mL) and heated to 75°C while 2-bromoethylbenzene (775 μL, 1.03 g, 5.59 mmol) was added and reaction allowed to stir at 75°C for 3 h. The reaction mixture was then poured into water (30 mL), and the resulting emulsion was extracted with CH_2Cl_2. The extract was washed with water then saturated aqueous NaCl, and dried over anhyd. $MgSO_4$. After filtering, concentrating to dryness afforded an orange liquid. This was then suspended in 1 *M* NaOH (20 mL) and heated to reflux for 48 h. The reaction was then cooled in an ice bath and acidified by the addition of conc. HCl. The resulting yellow tan ppt. was collected by filtration, washed with ice cold water, and dried *in vacuo* to give the acid as a tan solid (578 mg, 3.82 mmol, 70%). It had mp 47-49°C (Lit.[14] nonlabeled mp 48.5°C); ^{1}H-NMR ($CDCl_3$) δ: 2.69 (2H, dt, $^3J_{HH}$=7.7 Hz and $^2J_{CH}$= 7.2 Hz, $CH_2CH_2{}^{13}CO_2H$), 2.96 (2H, dt, $^3J_{HH}$=7.7 Hz and $^3J_{CH}$=3.8 Hz, $CH_2CH_2{}^{13}CO_2H$), 7.23-7.33 (5H, m, Ar 5 x CH), 9.61 (1H, brs, $^{13}CO_2H$) ppm; ^{13}C-NMR ($CDCl_3$) δ: 177.30 (C=O) ppm (8 scans); ^{13}C-NMR 500 MHz (decoupled, $(CD_3)_2SO$) δ: 30.29 (C_3 CH_2), 35.17 (d, $^1J_{CC}$=55 Hz, C_2 CH_2), 125.91 (*para* CH), 128.17 (2 x *ortho* CH), 128.24 (2 x *meta* CH), 140.82 (d, $^3J_{CC}$=2.9 Hz), 173.68 (d, $^1J_{CC}$=55 Hz, C=O) ppm.

[1-^{13}C]-Phenylacetic acid (**2**). In a 100 mL round bottom flask equipped with a magnetic stir bar was dissolved K^{13}CN (358 mg, 5.41 mmol, 90% ^{13}C enriched) in water (5 mL) and $(CH_3)_2SO$ (10 mL) and heated to 50°C, upon which benzyl bromide (650 μL, 5.45 mmol) was added and reaction allowed to stir at 60°C for 4 h. To this mixture was then added 40% NaOH (20 mL), and it was heated to reflux for 18 h. The reaction was then cooled in an ice bath and acidified by the addition of conc. HCl. The clear solution was extracted with CH_2Cl_2, dried over anhyd. $MgSO_4$, filtered, and concentrated to dryness to afford a white solid (414 mg, 3.02 mmol, 56%). It had mp 74-76°C (Lit.[15] 76-76.5°); ^{1}H-NMR 500 MHz ($(CD_3)_2SO$) δ: 3.53 (2H, d, $^2J_{CH}$=7.7 Hz, $CH_2{}^{13}CO_2H$), 7.17-7.47 (5H, m, Ar 5 x CH), 12.32 (1H, brs, $^{13}CO_2H$) ppm; ^{13}C-NMR 500 ($(CD_3)_2SO$) δ: 172.64 (C_1 C=O) ppm (8 scans); ^{13}C-NMR 500 MHz (decoupled, $(CD_3)_2SO$) δ: 40.74 (d, $^1J_{CC}$=55 Hz, C_3 CH_2), 126.49 (*para* CH), 128.17 (2 x *meta* CH), 129.31 (d, $^3J_{CC}$=1.43 Hz, 2 x *ortho* CH), 135.06 (d, $^2J_{CC}$=2.4 Hz, C_3 C), 172.64 (d, $^1J_{CC}$=55 Hz, C_1 C=O) ppm.

3,5-Dimethyl-2-ethoxycarbonyl-1*H*-pyrrole-4-acetic acid ethyl ester (**12**).[16] In a 3 L 3-neck, round bottom flask equipped with a mechanical stirrer, thermometer, and reflux condenser was placed ethyl-3-acetyl-4-oxopentanoate[17] (102 g, 0.550 mol), diethyl oximinomalonate[18] (118 g, 0.622 mol), and anhyd. sodium acetate (101 g, 1.23 mol) in acetic acid (750 mL). The reaction mixture was heated to 90°C, then zinc dust (100 g, 1.53 g atoms) was added in small portions so that the reaction temperature did not exceed 95°C (controlled by air cooling, approx. addition time was 2.5 h). After all the zinc was added, the pale green reaction mixture was heated at reflux for 24 h. The (now) pale yellow reaction was filtered while hot and quenched by pouring the hot mixture into 2 L of ice-water and placed at 4°C overnight to complete the precipitation. The resulting white precipitate was collected, dried, and then crystallized from hot ethanol to afford the desired pyrrole as a white solid (61.0 g, 0.241 mol, 44%). It had mp 93-95°C; ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, ^{3}J=7.1 Hz, chain OCH_2CH_3), 1.34 (3H, t, ^{3}J=7.1 Hz, OCH_2CH_3), 2.24 (3H, s, C_3 CH_3), 2.28 (3H, s, C_5 CH_3), 3.37 (2H, s, $C_4{}^1$ CH_2), 4.13 (2H, q, ^{3}J=7.1 Hz, chain OCH_2CH_3), 4.29 (2H, q, ^{3}J=7.1 Hz, -OCH_2CH_3), 9.13 (1H, brs, NH) ppm; ^{13}C-NMR ($CDCl_3$) δ: 10.46 (chain OCH_2CH_3), 11.31 (OCH_2CH_3), 14.00 ($C_3{}^1$ CH_3), 14.35 ($C_5{}^1$ CH_3), 30.07 ($C_4{}^1$ CH_2), 59.57 (chain -OCH_2CH_3), 60.46 (-OCH_2CH_3), 114.34 (C_5), 116.91 (C_3), 127.36 (C_4), 130.87 (C_2), 162.21 ($C_2{}^1$ C=O), 171.63 ($C_4{}^2$ C=O) ppm.

3,5-Dimethyl-2-ethoxycarbonyl-1*H*-pyrrole-4-acetic acid (**11**). In a 500 mL round bottom flask equipped with a magnetic stir bar was dissolved 3,5-dimethyl-2-ethoxycarbonyl-1*H*-pyrrole-4-acetic acid ethyl ester (**12**) (10.0 g, 39.5 mmol) in ethanol (200 mL). To this solution was added NaOH (1.64 g, 41.0 mmol)

and water (15 mL). This clear solution was then allowed to stir at room temperature for 28 h. After this time more water (50 mL) was added and the ethanol was removed *in vacuo* to give a clear solution. This was further diluted with water (500 mL) and acidified by addition of 1*M* HCl. The resulting white precipitate was collected by filtration and dried to give the pyrrole acid as a white solid (8.52 g, 37.9 mmol, 96%). It had mp 194-196°C; IR (film) ν: 3307, 2986, 1707, 1672, 1451, 1277, 1219, 1093 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.34 (3H, t, ^{3}J=7.1 Hz, OCH_2CH_3), 2.23 (3H, s, C_3 CH_3), 2.28 (3H, s, C_5 CH_3), 3.41 (2H, s, C_4^1 CH_2), 4.31 (2H, q, ^{3}J=7.1 Hz, -OCH_2CH_3), 9.29 (1H, brs, NH) ppm; ^{13}C-NMR ($CDCl_3$) δ: 10.86 (-OCH_2CH_3), 11.45 (C_3^1 CH_3), 14.50 (C_5^1 CH_3), 29.83 (C_4^1 CH_2), 59.97 (-OCH_2CH_3), 113.85 (C_5), 117.45 (C_3), 127.64 (C_4), 131.45 (C_2), 162.17 (C_2^1 C=O), 177.31 (C_4^2 C=O) ppm.

Anal. Calcd. for $C_{11}H_{15}NO_4$ (225): C, 58.64; H, 6.72; N, 6.22.
Found: C, 58.74; H, 6.54; H, 6.12.

3,5-Dimethyl-2-ethoxycarbonyl-4-(2-hydroxyethyl)-1*H*-pyrrole (10). In a 500 mL round bottom flask equipped with a magnetic stir bar and N_2 inlet was placed 3,5-dimethyl-2-ethoxycarbonyl-1*H*-pyrrole-4-acetic acid (**11**) (11.6 g, 45.4 mmol) dissolved in dry tetrahydrofuran (THF) (100 mL). This was cooled to -20°C where 1 M BH_3-THF (55.0 mL, 55.0 mmol) was added dropwise over a 1 h period. The (now) white suspension was then allowed to slowly warm to room temperature where it was allowed to stir under N_2 for 23 h. The reaction was quenched by the addition of water (10.0 mL, 555 mmol) to give an orange solution which was acidified by the addition of 1*M* HCl (50 mL) and concentrated to give a two phase system. This was then extracted with ethyl acetate which was back extracted into 5% $NaHCO_3$ (3 x 50 mL). The combined bicarbonate extracts were acidified to give unreacted starting acid (1.64 g, 6.40 mmol, 14%). The ethyl acetate extracts were then washed with water (2 x 50 mL) and saturated aqueous NaCl (1 x 50 mL) then dried over anhyd. $MgSO_4$, filtered and concentrated to dryness to afford the alcohol as a white solid (8.22 g, 38.9 mmol, 86%). It had mp 121-123°C; IR (film) ν: 3318, 3234, 2931, 1675, 1453, 1439, 1272, 1170, 1101 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.34 (3H, t, ^{3}J=7.1 Hz, -OCH_2CH_3), 2.23 (3H, s, C_3 CH_3), 2.27 (3H, s, C_5 CH_3), 2.65 (2H, t, ^{3}J=6.8 Hz, CH_2CH_2OH), 3.65 (2H, t, ^{3}J=6.8 Hz, CH_2CH_2OH), 4.28 (2H, q, ^{3}J=7.1 Hz, OCH_2CH_3), 8.92 (brs, 1H, NH) ppm; ^{13}C-NMR ($CDCl_3$) δ: 10.62 (OCH_2CH_3), 11.44 (C_3^1 CH_3), 14.43 (C_5^1 CH_3), 27.51 (C_4^1 CH_2), 59.71 (C_4^2 CH_2), 62.69 (-OCH_2CH_3), 117.17 (C_5), 117.42 (C_3), 127.40 (C_4), 130.69 (C_2), 161.73 (C_2^1 C=O) ppm.

Anal. Calcd. for $C_{11}H_{17}NO_3$ (211): C, 62.52; H, 8.12; N, 6.63.
Found: C, 62.79; H, 7.91; N, 6.65.

3,5-Dimethyl-2-ethoxycarbonyl-4-(2-*p*-toluenesulfonyloxyethyl)-1*H*-pyrrole (9). In a 250 mL round bottom flask equipped with a magnetic stir bar and drying tube was dissolved 3,5-dimethyl-2-ethoxycarbonyl-4-(2-hydroxyethyl)-1*H*-pyrrole (**10**) (7.40 g, 35.1 mmol) in CH_2Cl_2 (175 mL). Pyridine (5.60 mL, 5.48 g, 69.2 mmol) was then added, and the solution was cooled in an ice bath to 5°C then *p*-toluenesulfonyl chloride (10.0 g, 52.6 mmol) was added over a 1 h period at 5°C. Intermittent warming to 10°C was required in order to prevent precipitation from occurring. The reaction was then allowed to stir at 5°C for 64 h. This clear brown solution was then washed with 1 *M* HCl (3 x 100 mL). The combined aqueous washings were back extracted with CH_2Cl_2, and the organic layers were added to the organic layer remaining after the HCl washings. The combined organic layers were washed with 1 *M* Na_2CO_3 (3 x 75 mL), water (1 x 100 mL), and dried over anhyd. $MgSO_4$. After filtration, the solvents were evaporated to afford a brown solid, which was crystallized from hot ethyl acetate:hexane to give the desired tosylate as a light red-brown solid (10.5 g, 28.9 mmol, 82%). It had mp 126-128°C; IR (film) ν: 3304, 2983, 2957, 2924, 1661, 1444, 1356, 1273, 1188, 1105, 951, 773, 664 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.35 (3H, t, ^{3}J=7.1 Hz, OCH_2CH_3), 2.11 (3H, s, C_3 CH_3), 2.14 (3H, s, tosylate CH_3), 2.41 (3H, s, C_5 CH_3), 2.71 (2H, t, ^{3}J=7.1 Hz, CH_2CH_2OTs), 3.99 (2H, t, ^{3}J=7.1 Hz, CH_2CH_2OTs), 4.28 (2H, q, ^{3}J=7.1 Hz, OCH_2CH_3), 7.25 (2H, d, ^{3}J=8.1 Hz, tosylate

CH), 7.65 (2H, d, ^3J=8.1 Hz, tosylate CH), 9.00 (1H, brs, NH) ppm; ^{13}C-NMR ($CDCl_3$) δ: 10.32 (-OCH_2CH_3), 11.27 (C_3^1 CH_3), 14.50 (C_5^1 CH_3), 21.50 (tosylate CH_3), 23.96 (C_4^1 CH_2), 59.71 (C_4^2 CH_2), 69.76 (-OCH_2CH_3), 115.48 (C_5), 117.02 (C_3), 127.62 (tosylate CH), 129.58 (tosylate CH), 128.93 (C_4), 130.80 (C_2), 132.83 (tosylate -CCH_3), 144.53 (tosylate -OC-), 161.63 (C_2^1 C=O) ppm.

Anal. Calcd. for $C_{18}H_{23}NO_5S$ (365): C, 59.16; H, 6.35; N, 3.84.
Found: C, 59.12; H, 6.43; N, 3.76.

3,5-Dimethyl-2-ethoxycarbonyl-1*H*-pyrrole-4-[3-^{13}C]-propionitrile (8).[19] In a 50 mL Erlenmeyer flask equipped with a magnetic stir bar was dissolved K^{13}CN (0.363 g, 5.49 mmol, 99% ^{13}C-enriched) in $(CH_3)_2SO$ (20 mL). To this was added 3,5-dimethyl-2-ethoxycarbonyl-4-(2-*p*-toluenesulfonyloxyethyl)-1*H*-pyrrole (**9**) (2.00 g, 5.49 mmol) in a single portion, and the reaction was allowed to stir at room temperature for 19 h. To this (now) orange solution was added water (100 mL) to precipitate out the product which was cooled in an ice bath, collected by filtration, washed with ice cold water, and dried to afford the nitrile as a pale yellow solid (1.15 g, 5.20 mmol, 95%). It had mp 134-136°C (Lit.[19] mp 134°C); IR (film) ν: 3312, 3131, 2986, 2919, 2195, 1676, 1458, 1443, 1400, 1275, 1225, 1174, 1096, 770, 721 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.35 (3H, t, ^3J=7.1 Hz, OCH_2CH_3), 2.27 (3H, s, C_3 CH_3), 2.28 (3H, s, C_5 CH_3), 2.44 (2H, dt, $^3J_{HH}$=7.1 Hz, $^2J_{CH}$= 9.3 Hz, $CH_2CH_2^{13}CN$), 2.75 (2H, dt, $^3J_{HH}$=7.1 Hz, $^3J_{CH}$=6.1 Hz, CH_2CH_2-^{13}CN), 4.31 (2H, q, ^3J=7.1 Hz, OCH_2CH_3), 8.59 (brs, 1H, NH) ppm; ^{13}C-NMR ($CDCl_3$) δ: 119.32 (C_4^3 ^{13}CN) ppm (8 scans). ^{13}C-NMR (decoupled, $CDCl_3$) δ: 10.48 (OCH_2CH_3), 11.47 (C_3^1 CH_3), 14.52 (C_5^1 CH_3), 18.66 (C_4^1 CH_2), 20.35 (C_4^2 CH_2), 59.81 (OCH_2CH_3), 117.39 (C_3), 117.94 (C_4), 119.48 (d, J_{CC}=55 Hz, C_4^3 ^{13}CN), 130.22 (C_2), 161.58 (C_2^1 C=O) ppm.

3-5-Dimethyl-2-methoxycarbonyl-1*H*-pyrrole-4-[3-^{13}C]-propionic acid methyl ester (7). In a 250 mL round bottom flask equipped with a magnetic stir bar, Teflon sleeve and reflux condenser was placed 3,5-dimethyl-2-ethoxycarbonyl-1*H*-pyrrole-4-[3-^{13}C]propionitrile (**8**) (5.58 g, 26.5 mmol) in ethanol (75 mL) and water (75 mL). To this was added KOH (20 g, 357 mmol) and heated at reflux for 40 h. The (now) orange solution was concentrated to dryness to afford a light pink solid. This was then taken up in a solution of $NaNO_3$:water (1:2 w/v) (50 mL) and cooled to -12°C, in a -60°C dry ice/acetone bath, where the insoluble material was filtered off. The tan solution was cooled down to -12°C and, with vigorous stirring, acidified by the rapid addition of conc. HNO_3 (18.0 mL, 25.2 g, 400 mmol) such that the temperature did not raise above -10°C. The resulting pink suspension was then collected by filtration, washed with ice cold water, and dried *in vacuo* to give the diacid as a lavender solid (4.60 g, 20.8 mmol). This was then taken up in CH_3OH (40 mL) and esterified by the addition of an excess solution of ethereal CH_2N_2, as indicated by a persistent yellow color. The solution was evaporated to dryness, taken up in $CHCl_3$ and submitted to flash chromatography. The $CHCl_3$ solution was deposited on a column of silica gel flash (2.5 x 6.5 cm diameter) and with water aspirator vacuum pre-eluted with $CHCl_3$ then eluted with $CHCl_3$:CH_3OH (10:1) to remove a tan band which was collected and concentrated to dryness to afford the pyrrole as a tan solid (4.89 g, 20.4 mmol, 77%). It had mp 102-104°C; ^{1}H-NMR ($CDCl_3$) δ: 2.22 (3H, s, C_3 CH_3), 2.27 (3H, s, C_5 CH_3), 2.44 (2H, dt, $^3J_{HH}$=7.8 Hz and $^2J_{CH}$=7.3 Hz, $CH_2CH_2^{13}CO_2CH_3$), 2.70 (2H, dt, $^3J_{HH}$=7.8 Hz and $^3J_{CH}$=3.8 Hz, $CH_2CH_2^{13}CO_2CH_3$), 3.67 (3H, d, $^3J_{CH}$=3.8 Hz, propionic OCH_3), 3.82 (3H, s, OCH_3), 8.72 (1H, brs, NH) ppm; ^{13}C-NMR ($CDCl_3$) δ: 173.36 (C_4^3 C=O) ppm (8 scans). ^{13}C-NMR (decoupled, $CDCl_3$) δ: 10.24 (C_3^1 CH_3), 11.18 (C_5^1 CH_3), 19.41 (C_4^1 CH_2), 34.32 (d, $^1J_{CC}$=55 Hz, C_4^2 CH_2), 50.69 (d, $^3J_{CC}$=2.3 Hz, propionic -OCH_3), 51.38 (OCH_3), 116.60 (C_3), 119.81 (C_4), 126.88 (C_5), 161.83 (C_5^1 C=O), 173.33 (d, $^1J_{CC}$=55 Hz, C_4^3 C=O) ppm.

Anal. Calcd for $C_{12}H_{17}NO_4$ (239): C, 60.22; H, 7.17; N, 5.86.
Found: C, 60.14; H, 7.05; N, 5.79.

3,5-Dimethyl-2-methoxycarbonyl-1*H*-pyrrole-4-[3-^{13}C]-propionic acid (3). In a 25 mL round bottom flask equipped with a magnetic stir bar was placed 3,5-dimethyl-2-methoxycarbonyl-1*H*-pyrrole-4[3-^{13}C]propionic acid methyl ester (**7**) (201 mg, 0.838 mmol) in CH_3OH (4 mL). To this was added 1*M* NaOH (1 mL) and the solution was allowed to stir at room temperature for 18 h. Excess CH_3OH was removed using a rotovap and the residue was diluted with water (4 mL). This was then cooled in an ice bath and acidified by the addition of 1 M HCl (1.5 mL) to give a light pink precipitate which was collected by filtration and washed with ice cold water to afford the mono-acid as a light pink solid (162 mg, 0.717 mmol, 86%). It had mp 177-179°C; ^{1}H-NMR ($CDCl_3$) δ: 2.16 (3H, s, C_3 CH_3), 2.27 (3H, s, C_5 CH_3), 2.52 (2H, dt, $^3J_{HH}$=7.4 Hz and $^2J_{CH}$=7.2 Hz, $CH_2CH_2^{13}CO_2H$), 2.83 (2H, dt, $^3J_{HH}$=7.4 Hz and $^3J_{CH}$=4.4 Hz, $CH_2CH_2^{13}CO_2H$), 3.83 (3H, s, OCH_3), 8.88 (1H, brs, NH) ppm; ^{13}C-NMR ($CDCl_3$) δ: 173.94 (C=O) ppm (8 scans); ^{13}C-NMR 500 MHz (decoupled, $CDCl_3$) δ: 10.30 (C_3^1 CH_3), 10.77 (C_5^1 CH_3), 19.20 (C_4^1 CH_2), 34.78 (d, $^1J_{CC}$=55 Hz, C_4^2 CH_2), 50.32 (-OCH_3), 115.53 (C_3), 119.42 (C_4), 125.97 (C_5), 130.62 (C_2), 161.08 (C_2^1 C=O), 173.94 (d, $^1J_{CC}$=55 Hz, C_4^2 C=O) ppm.

2-Carboxy-3,5-dimethyl-1*H*-pyrrole-4-[3-^{13}C]propionic acid (16).[20] In a 100 mL round bottom flask equipped with a magnetic stir bar and reflux condenser (with Teflon sleeve) was placed 3,5-dimethyl-2-ethoxycarbonyl-1*H*-pyrrole-4-[2-^{13}C]propionitrile (**8**) (1.11 g, 5.02 mmol) in ethanol (25 mL). To this solution was added KOH (1.51 g, 26.9 mmol) in water (25 mL). The mixture was then heated to reflux for 45 h, then cooled to room temperature and concentrated to dryness to afford a tan solid. The flask was cooled while adding a solution of $NaNO_3$:water (1:2 w/v) (35 mL), then cooled to -13°C. To this mixture was added a cold solution of HNO_3 (1.3 mL, 1.82 g, 28.9 mmol) in $NaNO_3$:water (1:2, w/v) (9 mL) dropwise so that the temperature did not rise above -10°C. A lavender precipitate resulted, and this was collected by filtration and washed with ice cold water to afford the di-acid as a lavender solid (459 mg, 2.16 mmol, 43%). It had ^{1}H-NMR ($(CD_3)_2SO$) δ: 2.09 (3H, s, C_3 CH_3), 2.14 (3H, s, C_5 CH_3), 2.26 (2H, dt, $^3J_{HH}$=7.1 Hz and $^2J_{CH}$=7.3 Hz, $CH_2CH_2^{13}CO_2H$), 2.49 (2H, dt, $CH_2CH_2^{13}CO_2H$, under $(CD_2H)_2SO$ peak), 10.95 (1H, brs, NH), 11.92 (2H, brs, 2 x -CO_2H) ppm; ^{13}C-NMR ($(CD_3)_2SO$) δ: 174.07 (C_4^3 C=O) (32 scans) ppm.

[8^3-^{13}C]-Xanthobilirubic acid methyl ester (18).[20] In a 25 mL round bottom flask equipped with a magnetic stir bar and reflux condenser was placed 2-carboxy-3,5-dimethyl-1*H*-pyrrole-4-[3-^{13}C]propionic acid (**16**) (381 mg, 1.78 mmol) and 5-bromomethylene-4-ethyl-3-methyl-2-oxo-2,5-dihydropyrrole[20] (390 mg, 1.81 mmol) in dry CH_3OH (10 mL). The solution was swept with N_2 and heated to reflux for 1.5 h. After cooling to -30°C, the resulting precipitate was collected by filtration and washed with CH_3OH (-30°C) to give a yellow-green solid. The solid was recrystallized from $CHCl_3$ — CH_3OH and the crystals were collected at -30°C, and washed with CH_3OH (-30°C) to afford the dipyrrinone as yellow crystals (380 mg, 1.20 mmol, 67%). It had mp 214-216°C (Lit.[21] 217-220°C, non-labeled); ^{1}H-NMR ($CDCl_3$) δ: 1.17 (3H, t, $^3J_{HH}$=7.5 Hz, CH_2CH_3), 1.96 (3H, s, C_7 CH_3), 2.14 (3H, s, C_9 CH_3), 2.41 (3H, s, C_2 CH_3), 2.46 (2H, dt, $^3J_{HH}$=7.2 Hz and $^2J_{CH}$=7.8 Hz, $CH_2CH_2CO_2CH_3$), 2.52 (2H, q, $^3J_{HH}$=7.5 Hz, CH_2CH_3), 2.72 (2H, dt, $CH_2CH_2^{13}CO_2CH_3$, $^3J_{HH}$=7.2 Hz and $^3J_{CH}$=3.5 Hz), 3.69 (s, 3H, OCH_3), 6.13 (s, 1H, C_5 H), 10.32 (1H, brs, NH pyrrole), 11.23 (1H, brs, NH lactam) ppm; ^{13}C-NMR ($CDCl_3$) δ: 173.72 (C_8^3 C=O) ppm (8 scans).

[8^3-^{13}C]-Xanthobilirubic acid (5). In a 25 mL round bottom flask equipped with a magnetic stir bar and reflux condenser was placed ester **18** (380 mg, 1.20 mmol) in CH_3OH (8 mL) and 1*M* NaOH (10 mL), and the mixture was heated to reflux for 18 h. The resulting dark orange solution was cooled to room temperature, and excess CH_3OH was removed *in vacuo* to give a yellow suspension. The solid was collected by filtration, directly suspended in 10% HCl and allowed to stir for 6 h. The resulting yellow green suspension was then collected by filtration and washed with ice cold water to afford dipyrrinone **5** as a yellow solid (163 mg, 0.538 mmol, 45%). It had mp 268-270°C (dec) (Lit.[22] 280-281°C); ^{1}H-NMR ($(CD_3)_2SO$) δ:

1.07 (3H, t, $^3J_{HH}$=7.5 Hz, OCH_2CH_3), 1.76 (3H, s, C_7 CH_3), 2.01 (3H, s, C_9 CH_3), 2.16 (3H, s, C_2 CH_3), 2.25 (2H, q, $^3J_{HH}$=7.5 Hz, OCH_2CH_3), 2.54 (4H, m, $CH_2CH_2{}^{13}CO_2H$, under $(CD_2H)_2SO$ peak), 5.92 (1H, s, C_5 CH), 9.76 (1H, s, NH pyrrole), 10.27 (1H, s, NH lactam), 12.04 (1H, s, -$^{13}CO_2H$) ppm; ^{13}C-NMR ($(CD_3)_2SO$) δ: 174.46 ($C_8{}^3$ C=O) ppm (8 scans); ^{13}C-NMR 500 MHz (decoupled, $(CD_3)_2SO$) δ: 8.05 ($C_3{}^2$ CH_3), 9.20 ($C_7{}^1$ CH_3), 10.96 ($C_2{}^1$ CH_3), 14.83 ($C_9{}^1$ CH_3), 17.14 ($C_3{}^1$ CH_2), 19.44 ($C_8{}^1$ CH_2), 34.92 (d, $^1J_{CC}$=54 Hz, $C_8{}^2$ CH_2), 97.59 (C_5 CH), 118.72 (d, C_8, $^3J_{CC}$=3.3 Hz), 121.64 (C_7), 122.56 (C_6), 122.61 (C_2), 127.23 (C_4), 129.39 (C_9), 147.16 (C_3), 171.86 (C_1 C=O), 173.68 (d, $^1J_{CC}$=54 Hz, $C_8{}^3$ C=O,) ppm.

[1-^{13}C] Bromoacetic acid methyl ester (15). In a 250 mL round bottom flask equipped with a magnetic stir bar was dissolved [1-^{13}C]-bromoacetic acid (4.95 g, 35.4 mmol, 99% ^{13}C enriched) in ether (20 mL). To this stirred solution was added ethereal CH_2N_2 (6.96 g nitrosomethyl urea in 70 mL of 40% KOH and 70 mL ether), and the solution was allowed to stir for 10 min. The yellow solution was then concentrated to dryness to afford the product as a clear liquid (5.45 g, 35.4 mmol, 100%). It had ^{1}H-NMR ($CDCl_3$) δ: 3.78 (3H, d, $^3J_{CH}$=3.8 Hz, OCH_3), 3.84 (2H, d, $^2J_{CH}$=4.7 Hz, CH_2) ppm; ^{13}C-NMR ($CDCl_3$) δ: 167.69 (C=O) ppm (8 scans).

Methyl 3-acetyl-4-oxo-[1-^{13}C]-pentanoate (14).[23] In a 100 mL round bottom flask equipped with a magnetic stir bar and reflux condenser was placed [1-^{13}C]-bromoacetic acid methyl ester (**15**) (5.45 g, 35.4 mmol) and 2,4-pentanedione (3.60 g, 36.0 mmol) in CH_2Cl_2 (50 mL). To this solution was added K_2CO_3 (5.00 g, 36.2 mmol) and Cs_2CO_3 (500 mg, 10% of K_2CO_3), and the mixture was heated to reflux for 12 h. The salts were removed by filtration, washed with CH_2Cl_2, then concentrated *in vacuo* to remove CH_2Cl_2 and excess dione, leaving the product (**14**) as a pale yellow liquid (5.58 g, 32.2 mmol, 91%). It had ^{1}H-NMR ($CDCl_3$) δ^{keto}: 2.27 (6H, s, 2x $COCH_3$), 2.89 (2H, dd, $^3J_{HH}$=7.3 Hz and $^2J_{CH}$=7.4 Hz, $CHCH_2{}^{13}CO_2CH_3$), 3.68 (3H, d, $^3J_{CH}$=3.8 Hz, OCH_3), 4.17 (1H, dt, $^3J_{HH}$=6.8 Hz and $^3J_{CH}$=3.1 Hz, $CHCH_2{}^{13}CO_2CH_3$), δ^{enol}: 2.16 (3H, s, $COHCH_3$), 2.27 (2H, s, $COCH_3$), 3.28 (2H, d, $^3J_{CH}$=7.5 Hz, $=CCH_2{}^{13}CO_2CH_3$), 3.72 (3H, d, $^3J_{CH}$=3.9 Hz, OCH_3) ppm; ^{13}C-NMR ($CDCl_3$) δ: 171.70 (s) ppm (8 scans).

3,5-Dimethyl-2-ethoxycarbonyl-1*H*-pyrrole-4-[1-^{13}C]-acetic acid methyl ester (13).[16,24] In a 250 mL 3-neck round bottom flask equipped with a magnetic stir bar, thermometer, and reflux condenser was placed methyl-3-acetyl-4-oxo-[1-^{13}C]-pentanoate (**14**) (5.58 g, 32.2 mol), diethyl oximinomalonate[18] (6.30 g, 33.3 mmol), and anhyd. sodium acetate (5.50 g) in glacial acetic acid (45 mL). The reaction mixture was heated to 60°C, and zinc dust (5.50 g, 84.1 mg atoms) was added in small portions so that the reaction temperature did not exceed 70°C (controlled by air cooling, approx. addition time was 40 min). After all the zinc was added, the pale green reaction mixture was heated to reflux for 14 h. Analysis of the reaction mixture by GC-MS showed one peak. The resulting pale yellow reaction was filtered hot to remove unreacted zink and quenched by pouring into ice and water (80 mL). After cooling overnight at 5°C to complete the precipitation, the resulting solid was collected by filtration, washed with a small amount of ice cold water, and dried to afford the pyrrole as a white solid (4.26 g, 17.7 mmol 55%). It had mp 109-110°C (non-labeled 107-108°C); ^{1}H-NMR ($CDCl_3$) δ: 1.34 (3H, t, $^3J_{HH}$=7.1 Hz, OCH_2CH_3), 2.23 (3H, s, C_3 CH_3), 2.28 (3H, s, C_5 CH_3), 3.37 (2H, d, $^2J_{CH}$=7.6 Hz, $C_4{}^1$ CH_2), 3.62 (3H, d, $^3J_{CH}$=3.9 Hz, OCH_3), 4.28 (2H, q, $^3J_{HH}$= 7.1 Hz, OCH_2CH_3), 8.68 (1H, brs, NH) ppm; ^{13}C-NMR ($CDCl_3$) δ: 172.22 ($C_4{}^2$ C=O) ppm (8 scans).

3,5-Dimethyl-2-ethoxycarbonyl-1*H*-pyrrole-4-[1-^{13}C]-acetic acid (4). In a 25 mL round bottom flask equipped with a magnetic stir bar was placed 3,5-dimethyl-2-ethoxycarbonyl-1*H*-pyrrole-4-[1-^{13}C]acetic acid methyl ester (**13**) (201 mg, 0.791 mmol) in ethanol (10 mL). To this stirred solution was added 1*M* NaOH (1.00 mL, 1.00 mmol), and stirring was continued at room temperature for 23 h. Excess ethanol was removed *in vacuo*, and the resulting solution was cooled in an ice bath. Acidified by the addition of conc. HCl to

give a white precipitate, which was collected by filtration and washed with ice cold water to afford acid **4** as a white solid (75.2 mg, 0.333 mmol, 42%). It had mp 195-196°C; ^{1}H-NMR ($CDCl_3$) δ: 1.34 (3H, t, ^{3}J= 7.1 Hz, OCH_2CH_3), 2.24 (3H, s, C_3 CH_3), 2.28 (3H, s, C_5 CH_3), 3.41 (2H, d, $^2J_{CH}$=7.5 Hz, $C_4{}^1$ CH_2), 4.28 (2H, q, ^{3}J=7.1 Hz, OCH_2CH_3), 8.79 (1H, brs, NH) ppm; ^{13}C-NMR ($CDCl_3$) δ: 176.22 (s) ppm (8 scans); ^{13}C-NMR 500 MHz (decoupled, $(CD_3)_2SO$) δ: 10.36 (OCH_2CH_3), 10.83 ($C_3{}^1$ CH_3), 14.51 ($C_5{}^1$ CH_3), 29.61 (d, $^1J_{CC}$=55 Hz, $C_4{}^1$ CH_2), 58.72 (-OCH_2CH_3), 114.53 (C_5), 115.88 (C_3), 126.42 (C_4), 131.37 (C_2), 160.76 ($C_2{}^1$ C=O), 172.81 (d, $^1J_{CC}$=55 Hz, $C_4{}^2$ C=O) ppm.

5-Carboxy-2,4-dimethyl-1*H*-pyrrole-3-[1-^{13}C]-acetic acid (17).[23] In a 250 mL round bottom flask equipped with a magnetic stir bar and reflux condenser was dissolved 3,5-dimethyl-2-ethoxycarbonyl-1*H*-pyrrole-4-[1-^{13}C]acetic acid methyl ester (**13**) (2.00 g, 8.33 mmol) in ethanol (20 mL). After adding NaOH (1.65 g, 41.4 mmol) and a solution of $NaNO_3$:water (1:2 w/v) (10 mL), the resulting solution was heated to reflux for 3 h. Then it was allowed to cool to room temperature and ethanol removed *in vacuo*. The resultant tan solution was cooled in a dry ice-acetone bath to -15°C, then a solution of $NaNO_3$:water (1:2 w/v) (20 mL) and HNO_3 (4.1 mL) was added dropwise such that the temperature remained below -10°C. The resulting light tan precipitate was collected by filtration, washed with ice cold water, and dried to give diacid **17** as a light tan solid (1.52 g, 7.67 mmol, 92%). It had ^{1}H-NMR ($(CD_3)_2SO$) δ: 2.09 (3H, s, C_4 CH_3), 2.12 (3H, s, C_2 CH_3), 3.21 (2H, d, $^2J_{CH}$=7.5 Hz, $C_3{}^1$ CH_2), 11.06 (1H, s, NH), 11.98 (2H, brs, 2 x CO_2H) ppm; ^{13}C-NMR ($(CD_3)_2SO$) δ: 172.96 ($C_3{}^2$ C=O) ppm (8 scans).

[8^3-^{13}C]-Nor-xanthobilirubic acid methyl ester (19).[23] In a 100 mL round bottom flask equipped with a magnetic stir bar and reflux condenser was placed the 5-carboxy-2,4-dimethyl-1*H*-pyrrole-3-[2-^{13}C]acetic acid (**17**) (1.52 g, 7.67 mmol) and 5-bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole[20] (1.67 g, 7.70 mmol) in CH_3OH (60 mL) and water (1 mL). This reaction was swept with N_2, and the solution was heated to reflux for 15 h. After cooling to -50°C, the resulting precipitate was collected by filtration and washed with CH_3OH (-50°C) to give the dipyrrinone as a yellow solid (1.59 g, 5.24 mmol, 68%). It had mp 229-231°C (Lit.[23] 240°C (dec)); ^{1}H-NMR ($CDCl_3$) δ: 1.17 (3H, t, $^3J_{HH}$=7.5 Hz, CH_2CH_3), 1.94 (3H, s, C_7 CH_3), 2.14 (3H, s, C_9 CH_3), 2.42 (3H, s, C_2 CH_3), 2.56 (2H, q, $^3J_{HH}$=7.5 Hz, CH_2CH_3), 3.40 (2H, d, $^2J_{CH}$=7.4 Hz, $C_8{}^1$ CH_2), 3.67 (3H, d, $^3J_{CH}$=3.7 Hz, OCH_3), 6.14 (1H, s, C_5 CH), 10.41 (1H, brs, NH pyrrole), 11.23 (1H, brs, NH lactam) ppm; ^{13}C-NMR ($CDCl_3$) δ: 172.45 ($C_8{}^2$ C=O) ppm (8 scans).

[8^3-^{13}C]-Nor-xanthobilirubic acid (6). In a 25 mL round bottom flask equipped with a magnetic stir bar and reflux condenser was placed ester **19** (116 mg, 0.384 mmol) in CH_3OH (3 mL) and 1*M* NaOH (7 mL), and the mixture was heated to reflux for 3 h. The resulting dark orange solution was cooled to room temperature, and excess CH_3OH was removed *in vacuo* to give an orange solution. This was cooled in an ice bath and directly acidified with conc. HCl. A resulting yellow green suspension was then collected by filtration and washed with ice cold water to afford dipyrrinone **6** as a yellow solid (111 mg, 0.383 mmol, 99%). It had mp 290-293°C (non-labeled 289-292°C); ^{1}H-NMR ($(CD_3)_2SO$) δ: 1.07 (3H, t, $^3J_{HH}$=7.5 Hz, CH_2CH_3), 1.77 (3H, s, C_7 CH_3), 1.99 (3H, s, C_9 CH_3), 2.16 (3H, s, C_2 CH_3), 2.52 (2H, q, CH_2CH_3, under $(CD_2H)_2SO$ peak), 3.22 (2H, d, $^2J_{CH}$=7.4 Hz, $CH_2{}^{13}CO_2H$), 5.93 (1H, s, C_5 CH), 9.78 (1H, s, NH pyrrole), 10.36 (1H, s, NH lactam), 12.03 (1H, s, 1H -$^{13}CO_2H$) ppm; ^{13}C-NMR ($(CD_3)_2SO$) δ: 172.89 ($C_8{}^2$ C=O) ppm (8 scans); ^{13}C-NMR 500 MHz (decoupled, $(CD_3)_2SO$) δ: 8.05 ($C_3{}^2$ CH_3), 9.33 ($C_7{}^1$ CH_3), 11.02 ($C_9{}^1$ CH_3), 14.82 ($C_2{}^1$ CH_3), 17.14 ($C_3{}^1$ CH_2), 29.87 (d, $^1J_{CC}$=55 Hz, $C_8{}^1$ CH_2), 97.55 (C_5 CH), 113.79 (d, $^3J_{CC}$=2.4 Hz, C_6), 121.66 (C_2), 122.80 (d, $^3J_{CC}$=4.3 Hz, C_9), 124.16 (C_7), 127.46 (C_4), 130.28 (d, $^2J_{CC}$=1.4 Hz, C_8), 147.19 (C_3), 171.88 (C_1 C=O), 172.94 (d, $^1J_{CC}$=55 Hz, $C_8{}^2$ C=O) ppm.

Anal. Calcd for $C_{16}H_{20}N_2O_3$ (288): C, 66.63; H, 7.00; N, 9.72.
Found: C, 66.67; H, 7.00; N, 9.58.

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