# REGIOSELECTIVE SYNTHESIS OF

# (E)-2-[$^{2}$H or $^{3}$H]-5-SUCCINIMIDO-4-OXO-PENT-2-ENOIC ACID

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## SUMMARY

The synthesis of highly enriched C-2 deuteriated and tritiated (E)-5-succinimido-4-oxo-pent-2-enoic acid for use in enzymatic reduction is described. The starting materials 3-methoxycarbonyl-[3-$^{2}$H]propionyl chloride and 3-methoxycarbonyl-[3-$^{3}$H]propionyl chloride were prepared in high yield by regioselective deuteriation or tritiation of monomethyl succinate. The synthetic route involved regioselective bromination of 5-succinimidolaevulinic acid followed by dehydrobromination.

Key Words: Regioselective, Deuterium, Tritium, Bromination, Dehydrobromination

In the course of our studies on chlorophyll biosynthesis we required δ-aminolaevulinic acid (ALA) stereospecifically isotopically labelled at C-2 (1). It was hoped to establish the required chirality by enzymatic reduction of an α β-unsaturated C-2 tritiated derivative (2c) with Clostridium kluyerii in $D_2O$. This organism has been shown to reduce some α β-unsaturated acids with a high degree of stereospecificity [1].

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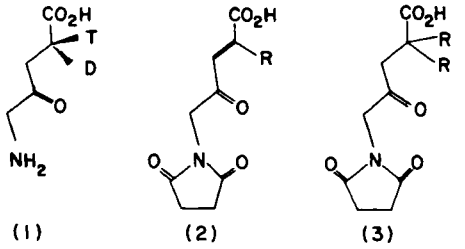
0362-4803/85/070649-08$01.00

Received September 25, 1984
Revised November 12, 1984

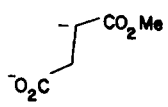


(1) (2) (3)

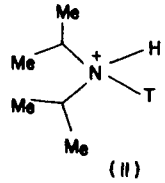
a, R=H; b, R=D; c, R=T.

Although 5-succinimido-4-oxo-pent-2-enoic acid (2), hitherto an unknown compound, proved not to be a substrate for the reducing enzyme system, we wish to report its synthesis in labelled form, which was based, in part, on the route of Neuberger and Scott [2] for the preparation of ALA. Our synthesis (Scheme 1), involved regioselective deuteriation or tritiation of monomethyl succinate (4a) and made use of regioselective bromination of 5-succinimido-laevulanic acid (3).

Treatment of (4a) with 2.0 eq. of MeONa in MeOD led through the regio-specific generation of the bis-anion (I) to the deuteriated compound (4b), which contained approximately 1.3 atom/mole of deuterium. Although this method is convenient and effective for hydrogen deuterium exchange, it is not practical for high level incorporation of tritium, which is present at tracer levels. A different procedure was therefore designed for the incorporation of tritium. The bis-anion (I) was formed by reaction of (4a) with two equivalents of LDA, generated *in situ* from n-butyl lithium and diisopropyl amine, and quenched with $CF_3CO_2T$, generated from trifluoroacetic anhydride and tritiated water. Tritium incorporation yield was significantly higher if before the quenching most of the regenerated diisopropylamine was removed by distillation of the solvent at -20 °C in vacuum. We assume that diisopropylamine competes with the bis-anion for the added proton to form the diisopropylammonium cation (II), which once formed would quench the bis anion by preferential transfer of $H^+$ rather than $T^+$, due to the tritium isotope effect.

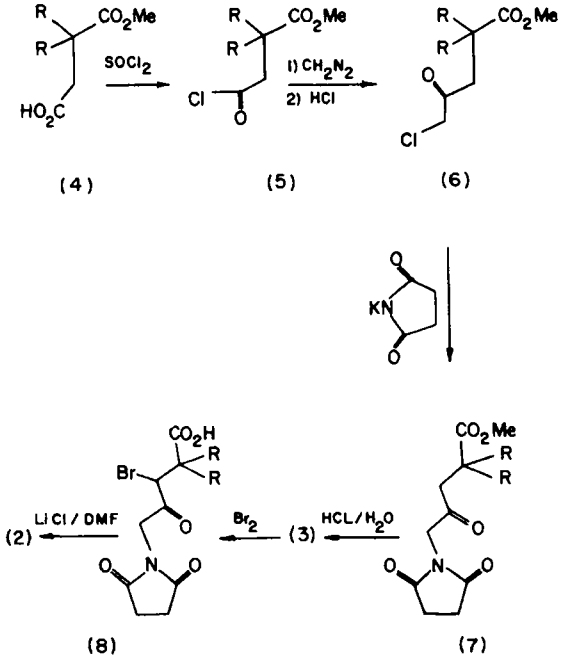


(I)



(II)

(4b) and (4c) were converted into (3b) and (3c) respectively by the adoption of the published method [2] (Scheme 1) without any detectable loss of label, as determined by NMR spectroscopy in the case of deuterium labelling and by measurements of specific activity in the case of tritium labelling. The acids (3a), (3b) and (3c) were quantitatively brominated exclusively at C-3 by the slow addition of one equivalent of bromine in glacial acetic acid with concentrated hydrochloric acid catalyst at 55 °C. The selective bromination at C-3 is probably due to the fact that position-5 is largely hindered by the bulky



(4) (5) (6)

(8) (7)

a, R=H; b, R=D; c, R=T

Scheme 1

succinimido group. The 3-bromo acids (8a), (8b) and (8c) were dehydrobrominated by heating with 3 equiv. of LiCl in DMF at 100 °C.

The NMR spectrum of (2a) showed clearly the trans-stereochemistry (J=16 Hz for the vinylic protons). The NMR of (2b) showed that deuterium remains in position-2: the high-field part of the vinylic AB quartet had diminished to about 0.2H, while the down-field part still corresponded to 1H, but collapsed to a singlet, broadened by deuterium coupling. An expected isotope effect was observed during the dehydrobromination step: more prolonged heating with LiCl in DMF were required in the case of the deuteriated 3-bromoacid (3b) than in the case of the unlabelled compound (3a); (2b) had a $^2H$ content of 80 atom %, significantly higher than [1.3/2 x 100] = 65 atom% in (8b).

## EXPERIMENTAL SECTION

Radioactive counting involved the use of a Packard liquid scintillation counter, model 526, using [$^3H$]-hexadecane as internal standard. $^1H$ NMR spectra were recorded on a Varian T-60 instrument; chemical shifts are reported in parts per million downfield from internal tetramethylsilane; data are presented as follows: chemical shift (multiplicity, number of protons, coupling constants in hertz). Unless otherwise noted the NMR solvent was $CDCl_3$. IR spectra were recorded on a Perkin-Elmer Model 298 spectrometer. Melting points (Pyrex capillary) are uncorrected. Distillations were bulb-to-bulb distillations performed with a Kugelrohr oven at the temperature and pressure indicated. Microanalysis was performed by the Microanalytical Laboratory of the University Chemical Laboratories, Cambridge, England.

3-Methoxycarbonyl-[3-$^2H$]propionylchloride (5b). Methyl hydrogen succinate (4a) (4g, 30 mmole) was added to a 1.7 M solution of sodium methoxide in deuteriated methanol [prepared from sodium (1.382g, 60 matom) and methanol-$d_1$ (35 ml, 99.5 + atom %D)]. The mixture was stirred under argon at room temperature for 14h. Hydrochloric acid (3M, 25 ml) was added and the resulting solution was extracted with chloroform (6 x 25 ml), the combined extracts were dried ($MgSO_4$) and the solvent was evaporated to give an oil which solidified on

standing (4b) (3.2g, 80%); NMR δ 10.9 (br. s, 1, $-CO_2H$), 3.72 (s, 3, $-OCH_3$), 2.70 (br. s, 2.7, $-CH_2-CH_2-$, i.e. 1.3H replaced by deuterium). 2g of this product were dissolved in thionyl chloride (5 ml) and kept for 3h at 40 °C. The excess of thionyl chloride was removed by evaporation, the residue distilled in vacuum and (5b) (2.05 g, 90%) was collected as a fraction boiling at 51-53 °C at 1 mm Hg. NMR δ 3.75 (s, 3, $-OCH_3$), 3.23 (br. s, 2, $-CHD-CH_2-COCl$), 2.5-2.9 (m, 0.7, $-CHD-CH_2-$).

3-Methoxycarbonyl [3-$^3$H] propionyl chloride (5c). Lithium diisopropylamide was prepared by slow addition of a 1.7 M solution of butyl lithium in hexane (23.5 ml, 40 mmole) to diisopropylamine (5.7 ml, 40 mmole) in dry THF (70 ml) under argon. The reaction mixture was cooled so that the temperature did not rise above 4 °C. The resulting solution was stirred for 20 min at 4 °C, and then cooled to -78 °C. (4a), (2.64 g, 20 mmole) dissolved in 10 ml of THF was added to the solution giving a white precipitate. After 1 h at -78 °C the temperature was allowed to rise to about -20 °C and the solvent (70 ml) was distilled out at 0.1 mm Hg. Dry THF (50 ml) was added to the mixture, 50 ml of solvent were distilled out again, and this operation was repeated three more times. The combined distilled solvent contained 37 mmole of diisopropylamine, as established by titration with 0.2 N hydrochloric acid. The reaction mixture was solidified by cooling in liquid nitrogen and tritiated trifluoroacetic acid, $CF_3CO_2T$, was added by vacuum transfer. [The $CF_3CO_2T$ was obtained by vacuum transfer of water enriched with tritium (200 mCi, 80 mg, 4.4 mmole) into trifluoroacetic anhydride (935 mg, 4.4 mmole)]. The reaction mixture was allowed to warm up to about -40 °C and radioinactive trifluoroacetic acid (8 g, 70 mmole) was added. Most of the solvent was removed by evaporation, the residue poured into water (30 ml) and extracted with diethyl ether (5 x 30 ml). The combined ether extracts were washed with 10 ml water, dried, and the solvent evaporated affording a yellow oil (4c) (2.2 g). This oil was dissolved in thionyl chloride (10 ml) and kept for 3 h at 40 °C, resulting in gas evolution during the first 20-30 min. The excess of thionyl chloride was removed by evaporation, the

remaining dark liquid was diluted with radioinactive acid chloride (5a) (6g) and distilled in vacuum. The fraction boiling at 51-53 °C at 1 mm Hg (7.2 g) was collected. Its NMR spectrum was identical to that of pure (5a) and liquid scintillation counting showed the product to contain 69.7 mCi (35% incorporation of tritium, based on tritiated water). The specific activity of (5c) was 1.45 mCi/mmole.

5-Succinimido-4-oxo[2-$^2$H]pentanoic acid (3b). This was prepared from (5b) by the published procedure [2] in 45% overall yield. Yields and NMR spectroscopic data of the intermediate products are given below:

a) The acid chloride (5b) (1.45 g) was converted into Methyl-5-chloro-4-oxo-[2-$^2$H]pentanoate (6b), 0.95 g, 60%; b.p. 68-70 °C, 0.4 mm Hg; NMR δ 4.18 (s, 2, Cl-$CH_2$-CO-), 3.72 (s, 3, -$OCH_3$), 2.90 (br. s, 2, -CO-$CH_2$-CHD), 2.8-2.5 (m, 0.7, -$CH_2$-CHD-$CO_2$Me).

b) The foregoing chloreketone (6b) (0.8 g) was reacted with succinimide and anhydrous potassium carbonate in DMF to give Methyl-5-succinimido-4-oxo-[2-$^2$H]pentanoate (7b), 0.9 g, 82%; m.p. 74-75 °C; NMR δ 4.36 (s, 2, -N-$CH_2$-CO-), 3.70 (s, 3, -$OCH_3$), 2.7 (br. s, 6.7, succinimido-4H and -$CH_2$-CHD-).

c) The foregoing (7b) (0.6 g) was hydrolysed to give 5-succinimido-4-oxo-[2-$^2$H]pentanoic acid (3b), 500 mg, 89%; m.p. 124-126 °C; NMR ($(CD_3)_2CO$) δ 4.36 (s, 2, N-$CH_2$-CO-), 2.85 (br. s, 2, -$CH_2$-CHD-), 2.80 (s, 4, succinimido), 2.65 (m, 0.7, -$CH_2$-CHD-$CO_2$H).

5-Succinimido-4-oxo-[2-$^3$H]pentanoic acid (3c). This was prepared from (5c) in 47% overall yield as described above for the deuteriated compound: m.p. 124-126 °C; NMR identical to that of (3a). Liquid scintillation counting showed the specific activity to be 1.47 mCi/mmole. This indicates that there was no loss of tritium during the conversion of (5c) into (3c).

3-Bromo-5-succinimido-4-oxo-pentanoic acid (8a). The acid (3a) (1.28 g, 6 mmole) was dissolved in glacial acetic acid (10 ml) containing concentrated hydrochloric acid (0.1 ml), and bromine (0.31 ml, 6.1 mmole) was added to the solution over 1 h at 50-60 °C. Most of the acetic acid was removed by

evaporation and the residue crystallised on standing. Trituration with diethyl ether (50 ml) afforded pale yellow crystals (1.62 g, 92%) m.p. 147-149 °C; N.M.R. ($(CD_3)_2CO$) δ 10.1 (broad s, 1, $CO_2H$), 5.05 (t, 1, -CHBr-$CH_2$-), 4.85 (d, 1), 4.56 (d, 1, -N-$CH_2$-CO-CHBr-, $J_{AB}$ = 18 Hz), 3.56-2.84 (m, 2, -CHBr-$CH_2$-, $J_{AB}$ = 18 Hz, $J_{AX}$ = $J_{BX}$ =7.5 Hz), 2.83 (s, 4, succinimido).

(E)-5-succinimido-4-oxo-pent-2-enoic acid (2a). The bromo acid (8a) (1.25g, 4.3 mmole) from the previous experiment was dissolved in dry DMF (15 ml) containing lithium chloride (550 mg, 13 mmole) and the solution was heated at 100 °C for 2h. About 10 ml of the DMF was removed by evaporation and the remaining dark yellow solution was poured into chloroform (50 ml), and dried ($MgSO_4$). The solvent was evaporated to a yellow oil which solidified on trituration with chloroform/hexane (700 mg. 77%). Recrystallisation from acetone/toluene gave white needles, m.p. 176.5-178 °C (decomposition begins at 167 °C); NMR ($(CD_3)_2CO$) δ 10.4 (br. s, 1, $-CO_2H$), 7.18 (d, 1), 6.90 (d, 1, $J_{AB}$ = 16 Hz, vinylic protons), 4.65 (s,2, -N-$CH_2$-CO-), 2.85 (s, 4, succinimido); IR γ 1770 (weak), 1710, 1170 $cm^{-1}$. Anal. Calcd. for ($C_9H_9NO_5$): C, 51.19; H, 4.30; N, 6.63. Found: C, 50.92; H, 4.36; N, 6.68%.

3-Bromo-5-succinimido-4-oxo-[2-$^2$H]pentanoic acid (8b). This was prepared by reaction of (3b) (250 mg, 1.16 mmole) with bromine (0.06 ml, 1.16 mole) as described for the preparation of (8a). Pale yellow crystals were obtained (310 mg, 91%); m.p. 148-149 °C; NMR δ $(CD_3)_2CO$ 10.1 (br. s, 1, $-CO_2H$), 5.05 (br. s, 1, -CHBr-CHD-), 4.85 (d, 1), 4.56 (d, 1, -N-$CH_2$-CO-, $J_{AB}$= 18 Hz), 3.56-2.80 (m, 0.7, -CHBr-CHD-), 2.83 (s, 4, succinimido).

(E)-5-succinimido-4-oxo-[2-$^2$H]pent-2-enoic acid (2b). This was prepared from the foregoing (8b) (200 mg, 0.68 mmole) as described for the preparation of (2a), except the heating of the reaction mixture was carried out for 7h. White needles were obtained (75 mg, 52%); m.p. 175-177 °C (decomposition begins at 167 °C); NMR δ $(CD_3)_2CO$ 10.2 (br. s, 1, $-CO_2H$), 7.18 (1H, d, $J_{AB}$ = 16 Hz and broad s, -CH=CD(H)-$CO_2H$), 6.90 (d, 0.2, $J_{AB}$ = 16 Hz, -CH=CD(H)-$CO_2H$), 4.65 (s, 2, -N-$CH_2$-CO-), 2.85 (s, 4, succinimido).

3-Bromo-5-succinimido-4-oxo-[2-$^{3}$H]pentanoic acid (8c). This was prepared from (3c) (500 mg), 2.32 mmole, 1.47 mCi/mmole) as described above for the preparation of (8b): (630 mg, 92%); m.p. 148-149 °C; NMR identical to that of (8a); specific activity 1.44 mCi/mmole.

(E)-5-succinimido-4-oxo-[2-$^{3}$H]pent-2-enoic acid (2c). This was prepared from the foregoing (8c) (400 mg, 1.36 mmole, 1.44 mCi/mmole) as described for the preparation of (2b). White needles were obtained (160 mg, 54%); m.p. 175-177 °C (decomposition begins at 167 °C); NMR identical to that of (2a); specific activity 1.12 mCi/mmole.

Acknowledgement:

The authors wish to thank Professor A.R. Battersby, F.R.S. for his suggestions and interest in this work.

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