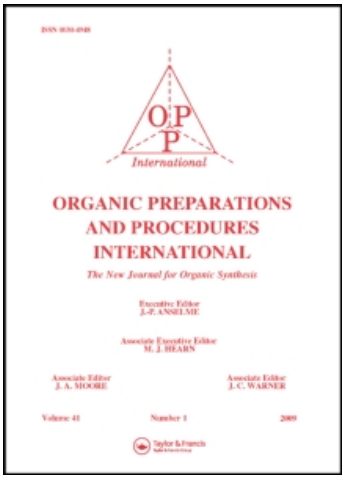

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A CONVENIENT SYNTHESIS OF (d1)-*o*-CARBOXY^{13}C-PHENYLALANINE

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A CONVENIENT SYNTHESIS OF (dl)-o-CARBOXY^{13}C-PHENYLALANINE

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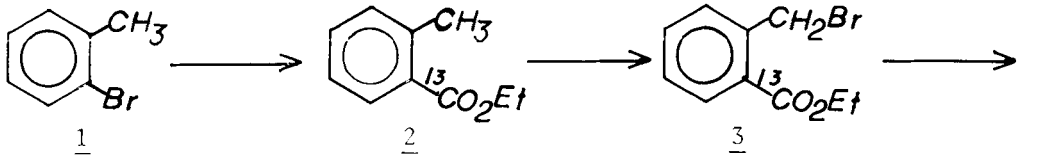
In the course of work which is aimed at the synthesis of several cyclic and bicyclic lactams, amino acids, and peptides a convenient synthesis of (dl)-o-carboxy^{13}C-phenylalanine was required. The target molecules serve as model compounds in the elucidation of the structure of peptides in solution via nuclear magnetic resonance spin-spin coupling constant data[1]. (dl)-o-Carboxyphenylalanine previously had been prepared by vigorous oxidation of N-benzoyl-(dl)-3-carboxy-1,2,3,4-tetrahydroisoquinoline[2] which was obtained from (dl)-phenylalanine and formaldehyde in concentrated hydrochloric acid. Upon repeated recrystallization a yield of only 39% was realized. This method was not suitable for a synthetic method which would permit facile and efficacious incorporation of a carbon-13 label at the o-carboxy group. We chose as starting material o-bromotoluene because it offered easy access to incorpo-

ration of a carbon-13 label via known $^{13}CO_2$ labeling techniques[3], and secondly provided a benzylic carbon atom for a subsequent S_N2 displacement reaction to complete the synthesis. The synthetic outline is given below.



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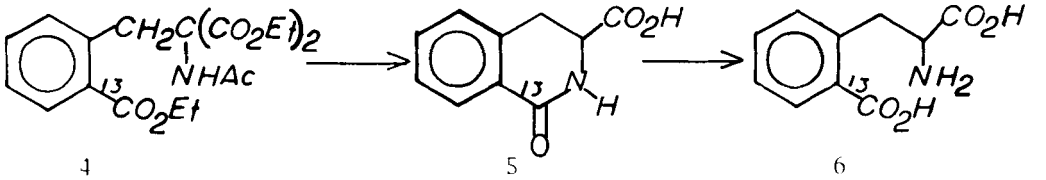

4 5 6

a) Mg° b) $^{13}CO_2$ c) H^+ d) p-TsOH, EtOH e) NBS, CCl_4, AIBN, 78° f) $AcNHC(CO_2Et)_2$, NaOEt g) H^+, heat

EXPERIMENTAL SECTION

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analysis were performed by Galbraith Laboratories, Knoxville, TN. and Mic-Anal Organic Microanalysis, Tucson, AZ. Satisfactory analysis (±0.4% of the calculated values) were obtained for all new compounds. ^1H and ^{13}C nmr were recorded on a JEOL FX-90Q FT NMR spectrometer. Mass spectra were determined on a Hewlett Packard 5995B GC/MS spectrometer. Infrared spectra were obtained on a Perkin-Elmer Model 1430 spectrophotometer.

Ethyl(^{13}C-carboxy)-o-toluate.- A solution of o-bromotoluene (1.71g., 10.0 mmol) in ether was added to magnesium metal (0.267g., 11.0 mmol) at a rate to maintain a rapid reflux. The reaction was kept under a positive pressure of nitrogen in flame-dried glassware. Upon complete addition the mixture was

allowed to stir an additional hour and transferred directly to a vacuum line and frozen in liquid nitrogen. Carbon dioxide (99 mole % C-13, 0.495 g., 11.0 mmol) was then added to the frozen mixture and allowed to thaw overnight at which time rapid stirring commenced. The mixture was acidified with 10% HCl, extracted with ether, dried over anhydrous magnesium sulfate and the ether removed by evaporation in vacuum. The light yellow solid of ^{13}C-carboxy-o-toluic acid, mp. 103-105°, lit.[4] 104-105°, weighed 1.21 g. (88%) and was used without further purification. The carboxylic acid was subsequently converted to ethyl^{13}C-carboxy-o-toluate in quantitative yield by refluxing with absolute ethanol in the presence of p-toluenesulfonic acid for 48 hrs. Distillation of the ester at reduced pressure (63°, 1.0mm) provided the pure ester with incorporation of the label which was verified by ^{13}C and ^{1}H nmr. ^{1}H nmr ($CDCl_3$ at 89.55 MHz): δ 1.35 (3H, t), 2.60 (3H, s), 4.30 (2H, q), 7.20 (3H, m), 7.85 (1H, m); ^{13}C nmr ($CDCl_3$ at 22.49MHz): δ 14.30 (aliphatic methyl), 21.72 (aromatic methylene), 60.62 (aromatic methyl), 125.68, 130.56, 131.64, 140.40 (aromatic carbons), 131.81 (2 aromatic carbons), 167.56 (carbonyl carbon).

Ethyl^{13}C-carboxy-α-bromo-o-toluate.- A mixture of 1.452 g. (8.80 mmol) of ethyl^{13}C-carboxy-o-toluate, 1.72 g. (9.70 mmol) of N-bromosuccinimide, and a catalytic amount of azobisisobutyronitrile (ca. 0.1 g.) was dissolved in 70 ml carbon tetrachloride in flame-dried glass apparatus. The mixture was kept at reflux under a nitrogen atmosphere for 3.5 hrs. The hot reaction mixture was filtered through Celite and the

Celite washed with hot carbon tetrachloride. The combined carbon tetrachloride filtrates were evaporated to a light yellow oil (2.15 g., 100% yield). Total conversion of the methyl group to the bromomethyl group was shown by the absence of an aromatic methyl group at δ 2.58 and the presence of a bromomethyl group at δ 4.90 in the ^{1}H nmr spectrum. ^{1}H nmr ($CDCl_3$ at 89.55 MHz): δ 1.35 (3H, t), 4.35 (2H, q), 4.90 (2H, s), 7.30 (3H, m), 7.90 (1H, m); ^{13}C nmr ($CDCl_3$ at 22.49 MHz): δ 14.19 (aliphatic methyl), 31.58 (aromatic methylene), 61.22 (aliphatic methylene), 128.44, 131.15, 132.29, 139.01 (aromatic carbons), 131.59 (2 aromatic carbons) 166.42 (carbonyl carbon).

(dl)-o-Carboxy^{13}C-phenylalanine.- Dry ethyl alcohol (30 ml) was added to a 100 ml flask equipped with a reflux condenser and two pressure equalizing addition funnels, the whole having been previously flame-dried; the reaction was carried out under a positive nitrogen pressure. To the ethanol was added 0.203 g. (8.80 mmol) of sodium metal. After complete dissolution of the metallic sodium, 1.91 g. (8.80 mmol) of diethyl-acetamidomalonate[5] in 15 ml ethanol was added over a period of 0.5 hr. After an additional 0.5 hr., 2.15 g. (8.80 mmol) of ethyl^{13}C-carboxy-α-bromo-o-toluate in 15 ml ethanol was added dropwise over a 10 min. period. The reaction mixture was then heated at reflux for 16 hrs. The ethanol was removed in vacuum and 10 ml of 6N HCl was added to the yellow oily residue (crude 4). This mixture was heated at reflux for 6 hrs., treated with activated carbon and filtered through Celite. The filtrate upon cooling overnight in the cold room deposited

a white solid (1.44 g., 78%), mp. 222-224°.
^{1}H nmr (DMSO-d_6 at 89.55 MHz): δ 3.26 (2H, m), 4.29 (1H, m), 7.34 (4H, m); ^{13}C nmr (DMSO-d_6 at 22.49 MHz): δ 30.40 (aliphatic methylene), 51.99 (aliphatic methine), 126.97 (2 aromatic carbons), 127.69, 128.86, 131.92, 136.46 (aromatic carbons), 164.41, 137.25 (carbonyl carbons); MS, M^+ Calcd 191.2 obsd 191.2.

Anal. Calcd for $C_{10}H_9NO_3$: C, 62.82; H, 4.75; N, 7.33
Found: C, 63.09; H, 4.56; N, 7.01

This product was identified as (dl)-3-carboxy-3,4-dihydro-1-^{13}C-isocarbostyril (5).

Concentration of mother liquors provided an additional crop of light yellow solid (0.40 g., 22%) which proved to be the desired (dl)-o-carboxy^{13}C-phenylalanine, mp. > 300°.
^{1}H nmr (DMSO-d_6 at 89.55 MHz): δ 2.53 (2H, s), 3.26 (2H, m), 4.29 (1H, m), 7.34 (4H, m); ^{13}C nmr (DMSO-d_6 at 22.49 MHz): δ 31.64 (aliphatic methylene), 53.63 (aliphatic methine), 126.39, 126.72, 127.58, 129.32, 131.48, 138.36 (aromatic), 164.37, 173.31 (carbonyl carbons); MS, M^+ not seen but parent ion m/e found to be 164 from loss (M-45) of the carboxyl.

Anal. Calcd for $C_{10}H_{11}NO_4$: C, 57.41; H, 5.30; N, 6.70
Found: C, 57.06; H, 5.39; N, 7.00

Additional (dl)-o-carboxy^{13}C-phenylalanine could be obtained by prolonged hydrolysis of the (dl)-3-carboxy-3,4-dihydro^{13}C-isocarbostyril with either 6N HCl or ethanolic NaOH[6]. A total yield of 78% of 6 was thus realized.

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