Synthesis of Carboranyl Phenylalanine for Potential Use in Neutron Capture Therapy of Melanoma

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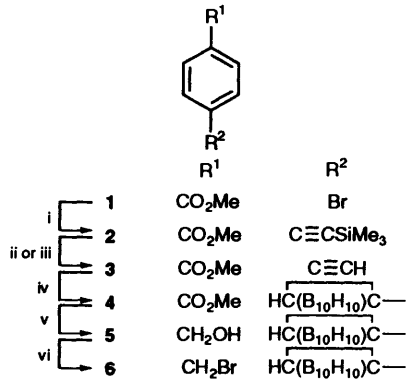
A phenylalanine derivative incorporating the 1,2-dicarba-*closo*-dodecaborane(12) cage has been synthesised by the alkylation of diethylformamidomalonate with benzyl bromide [containing the 1,2-dicarba-*closo*-dodecaborane(12) cage at the *para*-position] in the presence of sodium ethoxide followed by the removal of the protecting groups by hydrolysis and decarboxylation. The *closo*-carborane species was converted into the anionic *nido*-form to produce a more water-soluble form of the compound.

Neutron capture therapy (NCT) for cancer is based on the use of a boron-10 labelled compound which can localise preferentially in the tumour cells, there to be activated by neutron irradiation, producing cytotoxic short-range alpha and lithium particles.[1] The successful use of *p*-boronophenylalanine (BPA) as the agent for the delivery of boron to melanoma for NCT is based on it being incorporated with tyrosine as a precursor for melanin synthesis.[2] Ideally for the patient undergoing NCT, the boron concentration in the tumour should reach 20 to 30 ppm,[3] and for BPA this level can be achieved by the administration of very high doses. However, the procedure would be improved by a compound with the same affinity for the tumour but with a larger boron content. Consequently a new generation of boronated amino acids are being synthesised to take advantage of the unique bonding characteristics of boron and its ability to form cluster compounds containing up to 12 boron atoms in a compact structure. Following this approach, we have synthesised a 1,2-dicarba-dodecaboranyl derivative of phenylalanine for potential use in NCT of melanoma and other cancers.†

Results and Discussion

One of the most useful general syntheses of amino acids is the Sorensen process in which the phthalimidomalonic ester is the central reagent. The desired amino acid side-chain is introduced by alkylation at the α-carbon, after which the resulting product is subjected to hydrolysis and decarboxylation to give the amino acid. Modifications to this procedure, involving the use of acetamidomalonic ester[4] or formamidomalonic ester[5] have been reported for the preparation of several amino acids.

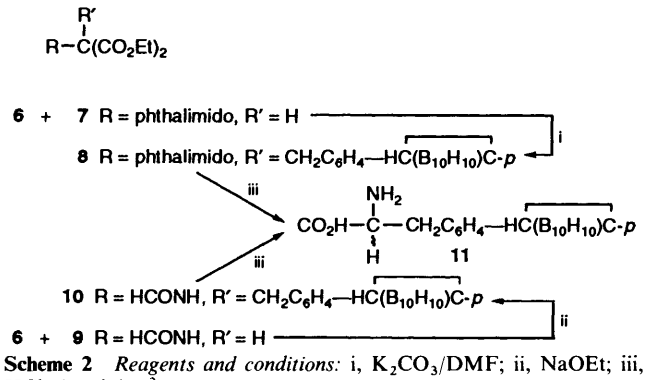
The addition of the boron cluster to an aromatic residue is most readily achieved by reaction of decaborane with an acetylene derivative.[6] The *closo*-carborane structure so obtained is relatively stable except to alkaline conditions when it can be converted into the *nido* form.[7] The common procedure for the introduction of the acetylenic substituent onto an aromatic nucleus is the Stephen–Castro coupling reaction between an aryl iodide and a protected cuprous acetylide in pyridine at reflux.[8] However, removal of the protecting groups often requires many steps and/or strongly alkaline conditions.[9] The Wittig reaction is possible, but requires more steps. Therefore, a new procedure was used, involving the condensation of methyl *p*-bromobenzoate **1** with ethynyltrimethylsilane in the presence of triethylamine, triphenylphosphine and palladium acetate in tetrahydrofuran, followed by removal of the silane group under very mild conditions with tetrabutylammonium fluoride to give methyl *p*-ethynylbenzoate **3**.[10] We found that use of tetrahydrofuran as solvent gave a better yield compared to the original procedure in which triethylamine acted as the solvent. Addition of decaborane to a solution of **3** in benzene in the presence of acetonitrile yielded methyl *p*-1,2-dicarba-*closo*-dodecaboranylbenzoate **4**. Reduction of **4** with lithium aluminium hydride and treatment of the resulting benzyl alcohol derivative **5** with phosphorus tribromide in pyridine gave the benzyl bromide derivative **6**.



Scheme 1 *Reagents and conditions:* i, $HC{\equiv}CSiMe_3$, NEt_3, Ph_3P/$Pd(OAc)_2$, THF, reflux, 4 h; ii, Bu_4NF/THF, 20 min; iii, K_2CO_3/MeOH, 3 h, room temp.; iv, decaborane, MeCN/benzene, reflux, 62 h; v, $LiAlH_4$/THF; vi, PBr_3/pyridine, benzene/0 °C

Diethyl phthalimidomalonate **7** was prepared by condensation of potassium phthalimide with ethyl bromomalonate at 110–120 °C. Initial reaction of **6** with **7** in the presence of sodium hydride was unsuccessful. However, this condensation was finally achieved in good yield by refluxing the mixture of **6** and **7** in dimethylformamide and anhydrous potassium carbonate.[11] The hydrolysis and decarboxylation of **8** to form *p*-dicarba-*closo*-dodecaboronophenylalanine **11** went smoothly. However the final product was contaminated with the side-product phthalic acid and, therefore, required chromatography. In order to eliminate chromatography from the final step, diethyl formamidomalonate **9** was used instead of **7**. This was readily prepared by nitrosation of diethyl malonate with sodium nitrite in acetic acid, followed by reduction with zinc and formic acid. Reaction of **6** with **9** in the presence of sodium ethoxide furnished **10** in good yield. On treatment of **10** with hydro-

† The work described in this paper was reported by us at the 5th International Symposium on Neutron Capture Therapy in Sept. 1992. At this time the synthesis of carboranylphenylalanine(11) was also reported by Wyzlie and Soloway using a different route. Their work has been communicated in *Tetrahedron Lett.*, 1992, **33**, 7489.



Scheme 2 *Reagents and conditions:* i, K_2CO_3/DMF; ii, NaOEt; iii, HCl (6 mol dm^{-3})

chloric acid (6 mol dm^{-3}) and acetic acid the final compound **11** was obtained pure.

All products and intermediates in the synthesis were examined by low and high resolution mass spectrometry, ^1H NMR and IR spectroscopy. The introduction of the carborane cage into a molecule can be identified by particular features of their spectra. In the IR the B–H stretch at *ca.* 2600 cm^{-1} is relatively strong, while the low-resolution chemical ionization mass spectrum shows a cluster of peaks around the expected mass number when boron of natural abundance (81.2% ^{11}B) is used. In the ^1H NMR spectrum, however, the hydrogens attached to boron in the carborane cage appear as a broad unresolved band in the region 1.3–3.2 ppm.

As is common for amino acids, the solubility of **11** in aqueous solutions of neutral pH was extremely low, in fact much lower than for BPA. The use of alkali was able to overcome the solubility problem, but the improvement may be caused by conversion into the anionic *nido*-carborane with loss of one boron atom. This conversion was taken to completion by reaction with potassium hydroxide in absolute ethanol,[7] to produce the potassium salt of *p-nido*-carboranylphenylalanine, which is readily soluble in water and thus more suitable for biological testing. The introduction of the negative charge in the *nido*-carborane cage resulted in the upfield shift of all protons in the NMR spectrum. However, the effect was much more significant in the case of the carborane proton for which the shift was from 5.20 ppm (*closo*) to 2.13 ppm (*nido*).

Experimental

General.—Thin-layer chromatography was carried out on Merck Art. 5554 sheets, precoated with Keiselgel 60 F_{254} of 0.25 mm thickness. M.p.s are uncorrected and were determined on a Gallenkamp melting point apparatus. Mass spectra were obtained either by chemical ionisation at low resolution on a Finnegan 6110-9500 TSQ system or at high resolution on an AEI-MS9 upgraded to Kratos MS50 configuration. Mass numbers for carborane-containing compounds were calculated on the basis of the $^{10}B_2{}^{11}B_8$ isotopomer. IR spectra were recorded on a Perkin-Elmer 580B spectrophotometer. NMR spectra were obtained in $CDCl_3$ at 300 MHz (Varian) with a chemical shift quoted on the δ scales relative to $CHCl_3$ (δ 7.26 ppm) unless stated otherwise.

Light petroleum is the fraction with b.p. 60–80 °C. All solutions in water immiscible solvents which had been in contact with water were dried over magnesium sulfate before evaporation at reduced pressure on a Buchi rotary evaporator.

Methyl 4-[1,2-*Dicarba*-closo-*dodecaboran*(12)-1-*yl*]*benzoate* **4**.—A mixture of methyl 4-ethynylbenzoate **3** (6.0 g), acetonitrile (19.5 cm^3) and decaborane(14) (7.2 g) in benzene (300 cm^3) was stirred at reflux for 62 h under nitrogen. The resulting mixture was concentrated under reduced pressure to give a pale yellow oil which was purified by column chromatography on silica (eluent hexane–ethyl acetate, 5:1) to give the *title compound* (6.9 g, 66%) as a white solid, m.p. 100–105 °C (Found: M^+, 278.2282. $C_{10}H_{18}B_{10}O_2$ requires *M*, 278.2265); ν(Nujol)/cm^{-1} 2590, 1722, 1462 and 1377; δ 3.94 (3 H, s, CO_2CH_3), 4.02 (1 H, b, CH proton in the carborane cage) and 7.54–8.01 (4 H, 2 × d, ArH).

4-[1,2-*Dicarba*-closo-*dodecaboran*(12)-1-*yl*]*phenylmethanol* **5**.—Lithium aluminium hydride (900 mg) was added carefully to freshly distilled tetrahydrofuran (THF; 100 cm^3) under nitrogen. To this a solution of **4** (4.5 g) in THF (18 cm^3) was added dropwise over a 2 h period. The mixture was stirred overnight at room temperature by which time starting material was absent as judged by TLC. The THF was evaporated and the residue cooled in ice and diluted with water (12 cm^3) followed by 10% aqueous NaOH (12 cm^3). The product was extracted with ether, and the extract washed with water and concentrated. The residue was chromatographed on silica and the colourless oil solidified after 24 h at 4 °C to give the *title compound* (3.4 g, 84%), m.p. 68–70 °C (Found: M^+, 250.2358. $C_9H_{18}B_{10}O$ requires *M*, 250.2359); ν(Nujol)/cm^{-1} 2595, 1462 and 1377; δ 3.96 (1 H, b, CH proton in the carborane cage), 4.72 (2 H, CH_2OH) and 7.33–7.50 (4 H, 2 × d, ArH).

1-[p-(α-*Bromo*)*tolyl*]-1,2-*dicarba*-closo-*dodecaborane*(12) **6**.—Pyridine (0.19 g) was added to a cooled solution of phosphorus tribromide (freshly distilled; 0.96 g) in dry benzene (1 cm^3). A solution of **5** (2.5 g) in dry benzene (1 cm^3) was added to the cooled mixture at a rate designed to keep the temperature < 0 °C. Following the addition, stirring was continued for a further 1 h after which the cooling bath was allowed to warm up to room temperature. The mixture was stored at room temperature for 48 h after which the solvent was evaporated and the residue dissolved in dichloromethane. This solution was then washed with water and brine, evaporated and chromatographed on silica. Recrystallisation from aqueous ethanol afforded the *title product* (4.4 g, 78%), m.p. 118–120 °C (Found: M^+, 312.1529, $C_9H_{17}B_{10}Br$ requires M^+, 312.1511); ν(Nujol)/cm^{-1} 2577, 1462 and 1377; δ 3.95 (1 H, CH proton in the carborane cage), 4.51 (2 H, CH_2Br) and 7.35–7.48 (4 H, 2 × d, ArH).

Diethyl p-[1,2-*Dicarba*-closo-*dodecaboran*(12)-1-*yl*]*benzyl*-(*phthalimido*)*malonate* **8**.—A mixture of *p*-carboranylbenzyl bromide **6** (1.6 g) with diethyl phthalamidomalonate **7** (1.55 g) and anhydrous potassium carbonate (385 mg) in dimethylformamide (14 cm^3) was stirred under nitrogen at room temperature for 1 h and then heated to 160 °C for 1.5 h. The dimethylformamide was distilled off under reduced pressure and the residue triturated with crushed ice and extracted with dichloromethane. The extract was dried and evaporated and the residue was purified by chromatography on silica. Recrystallisation from ethanol yielded the *title compound* (2.3 g, 84%), m.p. 137–139 °C (Found: M^+, 537.3109. $C_{24}H_{31}B_{10}NO_6$ requires M^+, 537.3150); ν(Nujol)/cm^{-1} 2586, 1730, 1462 and 1377; δ 1.27 (6 H, t, 2 × $CO_2CH_2CH_3$), 3.77 (2 H, s, $CH_2C_6H_5$), 3.81 (1 H, b, CH proton in the carborane cage), 4.31 (4 H, m, 2 × $CO_2CH_2CH_3$), 7.09–7.21 (4 H, 2 × d, ArH) and 7.71 (4 H, m, ArH).

Diethyl p-[1,2-*Dicarba*-closo-*dodecaboran*(12)-1-*yl*]*benzyl*-(*formamido*)*malonate* **10**.—Diethyl formamidomalonate **9** (1.19 g) was added to a solution of sodium (150 mg) in dry absolute alcohol (25 cm^3) and this was followed by compound **6** (2.0 g) and a few crystals of sodium iodide. The mixture was stored at room temperature for 2 days, heated to reflux for 2.5 h and then evaporated to dryness. The residue was dissolved in ether and

the solution washed with water and evaporated. The residue was purified by chromatography and then recrystallised from ethanol to furnish the *title product* (2.4 g, 87%), m.p. 178–180 °C (Found: M^+, 435.3077. $C_{17}H_{29}B_{10}NO_5$ requires M^+, 435.3037) ν(Nujol)/cm^{-1} 2571, 1753, 1651, 1462 and 1377; δ 1.30 (6 H, t, 2 × $CO_2CH_2CH_3$), 3.68 (2 H, s, $CH_2C_6H_4$), 3.93 (1 H, br, CH, proton in the carborane cage), 4.29 (4 H, m, 2 × CO_2CH_2-CH_3), 6.70 (1 H, br, NH), 6.99–7.39 (4 H, 2 × d, ArH) and 8.19 (1 H, s, CHO).

4-[1,2-*Dicarba*-closo-*dodecaboran*(12)-1-*yl*]*phenylalanine* **11**.—The penultimate compound **10** (1.0 g) was taken up in hydrochloric acid (6 mol dm^{-3}; 7 cm^3) and the mixture allowed to reflux. After 20 min, acetic acid (3 cm^3) was introduced and reflux continued for 4 h. The mixture was evaporated to dryness and the white residue crystallised from aqueous ethanol to yield **11** (600 mg, 89%), m.p. 230 °C (decomp.) (Found: M^+, 307.2498. $C_{11}H_{21}B_{10}NO_2$ requires M^+ 307.2572) ν(Nujol)/cm^{-1} 2593, 1649, 1582, 1513, 1462 and 1377; δ(CD_3OD) 3.02–3.09 (dd, 1 H, $CH_2C_6H_4$), 3.25–3.27 (dd, 1 H, $CH_2C_6H_4$), 3.79–3.84 [dd, 1 H, $CH(NH_2)CO_2H$], 5.20 (1 H, b, CH in the carborane cage) and 7.30–7.55 (4 H, 2 × d, ArH).

Conversion of the closo *Amino Acid* **10** *into its Anionic* nido *Derivative*.—The amino acid **11** (100 mg) was added to a solution of potassium hydroxide (50 mg) in absolute ethanol (2 cm^3) under nitrogen. The mixture was stirred at room temperature for 1 h, refluxed for 2 h and then cooled. Ethanol (1.5 cm^3) was added to the mixture which became turbid and was then filtered (no residue). Carbon dioxide was bubbled through the filtrate and the milky suspension was filtered. The filtrate was evaporated to dryness to give the potassium salt as a white solid (106 mg, 87%); δ(CD_3OD) 2.14 (1 H, br, CH in the carborane cage), 2.66–2.73 (dd, 1 H, $CH_2C_6H_5$), 3.03–3.47 (dd, 1 H, $CH_2C_6H_4$), 3.45 [br, 1 H, $CH(NH_2)CO_2H$], 6.98–7.13 (4 H, 2 × d, ArH); *m/z* (observed) cluster of peaks centred around *m/z* 298 [FAB–MS (negative ion) calc. for $C_{11}H_{21}B_9NO_2$: 297].

Acknowledgements

This project was supported by The National Health and Medical Research Council of Australia.

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Paper 2/06728E
Received 21*st December* 1992
Accepted 10*th February* 1993