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Facile Enantioselective Synthesis of (S)-5-(2-Methyl-1,2-dicarba-closo-dodecaborane(12)-1-yl)-2-aminopentanoic Acid (L-MeCBA) Using the Bislactim Ether Method

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The interest in the design and synthesis of Boron-rich substances is presently thriving. They are indispensable reagents for the new electron microscopic techniques electron energy loss spectroscopy (EELS) and electron spectroscopic imaging (ESI) [1] and for boron neutron capture therapy (BNCT) [2]. Both, the electron microscopic techniques and the BNCT demand high topical concentrations of boron. Therefore, carborane-cluster containing building blocks such as amino acids are promising synthetic targets. The first carborane-bearing amino acid described was D,L-carboranylalanine (Car) [3]. Recently, the preparation of a more attractive carborane containing amino acid [(R,S)-5-(2-methyl-1,2-dicarba-closo-dodecaborane(12)-1-yl)-2-aminopentanoic acid (D,L-MeCBA] was described [4] and used for peptide synthesis [4, 5].

We report here on a convenient and highly efficient enantioselective synthesis of L-MeCBA [6] proceeding via the bislactim ether method [7].The bislactim ether of cyclo(D-Val-Gly) [8] **1** was regiospecifically metallated by butyllithium for generating the anion **2**. Since alkylation with the CH-acidic 3-carboranylpropyl-iodide only led to a cyclisation product [μ-1,2-trimethylene-1,2-dicarba-closo-decaborane(12)] and no alkylation products could be isolated, a terminal protecting group at the carborane cage seemed to be necessary. However, alkylation of the metallated bislactim ether with the alkyliodide **3** [4] proceeded in high yield giving **4a** and a small portion of its diastereomer **4b** (ratio 94:6, gravimetrically; 88% de). The isolation of pure diastereomers was readily achieved by flash chromatography on silica gel. Mild hydrolysis with 0.5N HCl led to the hydrochlorides of the methyl esters of D-valine and L-MeCBA **5** [ee > 98%, determined by ^{1}H NMR spectroscopy using Eu(hfc)$_3$]. Differences in the solubility of the obtained methyl ester hydrochlorides enabled their easy separation: The o-carborane cage has a high effect on the hydrophobicity of the molecule; the side chain of carboranylalanine is reported to be 1000 times more hydrophobic than that of valine [9]. As previously described no racemisation occurred during the mild hydrolysis of the bislactim ether adduct C7]. Finally, L-MeCBA methyl ester **5** was hydrolysed to the free L-carboranyl amino acid **6**. Since difficulties occurring during elemental analyses of boron compounds containing nitrogen are well known [4], all new compounds being prepared were characterized by various spectroscopic methods.

The application of the bislactim ether method proved to be a short and convenient way for the synthesis of the enantiomerically pure carboranyl amino acid **6** which was obtained in good overall yield (68%) and high enantiomeric purity (ee > 98%, determined by chiral-phase hplc). The facile availability of even large amounts of the boron-rich building block described opens an entry to the generation of carboranyl oligomers applicable to the BNCT-treatment of cancer and the design of probes for ESI/EELS.

Current work is concerned with the incorporation of the carboranyl amino acid produced into different kinds of peptides exploiting the well established methods of peptide synthesis.

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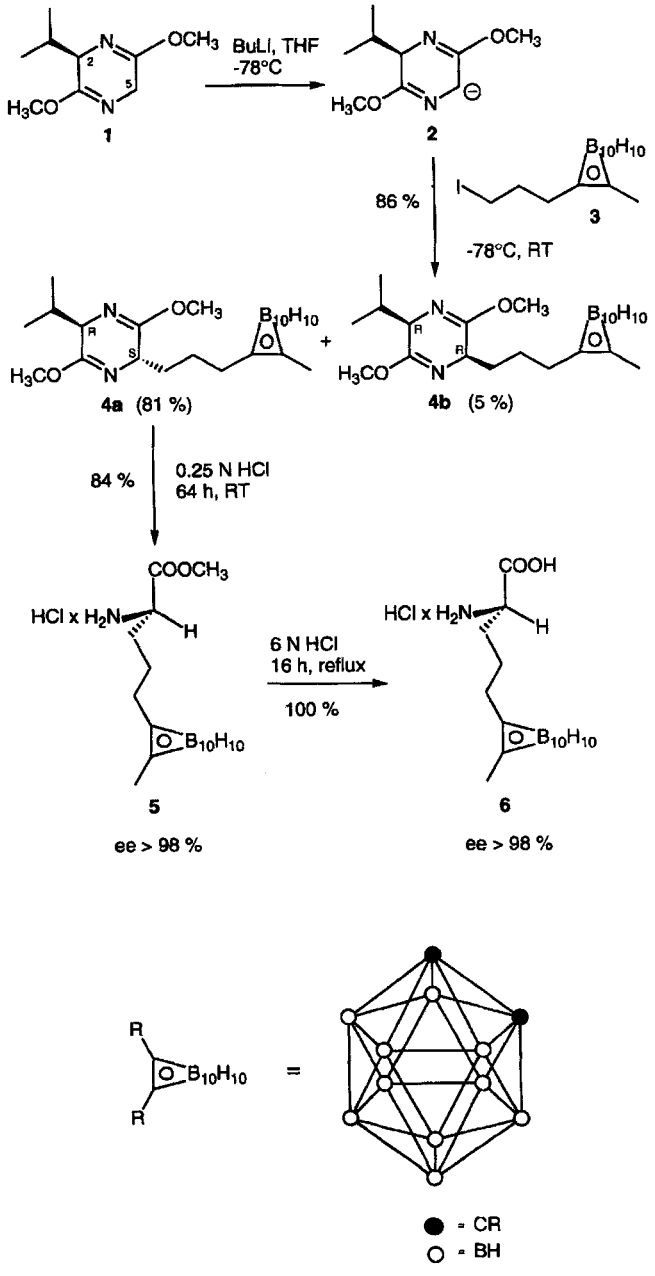
Experimental

All reagents and solvents were reagent grade and purchased from commercial sources. TLC was performed on silica gel precoated aluminium sheets 60 F_{254} (Merck) and on silica gel precoated IB2 Baker-flex sheets (Baker). ^{1}H NMR, ^{13}C NMR and IR spectra were obtained using a Bruker 300 MHz spectrometer and a Perkin Elmer Infrared Spectrometer 283, respectively. The optical activities of the compounds were

determined by using a Perkin Elmer Polarimeter 241 MC. HPLC measurements were performed on a Kontron instrument. The MS data were obtained from an AEI MS 3074/ Kratos DS 55 (GC-MS) and a Finnigan MAT 900 (FAB-MS), respectively.



Scheme 1

(2R,5S)-2,5-Dihydro-2-isopropyl-3,6-dimethoxy-5-[2-methvl-1,2-dicarba-closo-dodecaborane(12)-1-yl]-propylpyrazine (**4a**):

2.35 ml Butyllithium in hexane (2.5 M; 5.9 mmol) were injected dropwise under stirring into a solution of the bislactim ether **1** (5.6 mmol in THF at –78 °C). Stirring was continued for 20 min (formation of 2). Then a solution of the carboranyliodide **3** (5.6 mmol in 10 ml THF) was added and stirring continued at –78 °C for 16 h. After addition of acetic acid (5.5 mmol in 1 ml THF), the mixture was warmed to room temp. and then poured into icecold water. After separation, the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were dried with magnesium sulfate, and the solvent was removed in vacuo. Product purification and separation of the diastereomers were performed by flash chromatography [CH_2Cl_2/PE (9:1); petroleum ether boiling range: 90–100 °C; R_f(2R,5S): 0.7, R_f (2R,5R): 0.3, R_f(educts: 0.95 and 0.05). TLC detection: 2.5% $PdCl_2$ in 1N HCl, Et_2O]. 1,73 g of **4a** (81%) and 0.12 g of its (2R,5R)-diastereomer **4b** (5%) were obtained. 300 MHz ^{1}H NMR of **4a** was void of diastereomer signals.

4a: Crystalline colourless solid; $[a]_D^{20} = -5.1°$ (c = 0.864 in $CHCl_3$). ^{1}H NMR, ^{1}H COSY (300 MHz, $CDCl_3$): δ = 0.68 and 0.71 (d, 3H, -CH($C\underline{H}_3$)$_2$), 1.03 and 1.06 (d, 3H, CH($C\underline{H}_3$)$_2$), 1.2–3.1(10H, BH), 1.50–1.64 (m, 2H, CH_2-$C\underline{H}_2$-CH_2), 1.62–1.72 and 1.80–1.90 (2 × m, 2 × 1H, CH-$C\underline{H}_2$-CH_2), 1.99 (s, 3H, C_{carb}-$C\underline{H}_3$), 2.18–2.23 (m, 2H, $C\underline{H}_2$-$C_{Carb.}$), 2.22–2.32 (m, 1H, (CH_3)$_2$C$\underline{H}$), 3.67 and 3.70 (2 × s, 2 × 3H, 2 × OCH_3), 3.95–4.02 (m, 2 × 1H, (CH_3)$_2$CH-C$\underline{H}$ and C$\underline{H}CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.64 and 19.06 (($\underline{C}H_3$)$_2$CH), 23.07 (C_{Carb}-$\underline{C}$H3), 25.11 (CH_2-$\underline{C}H_2$-CH_2), 31.86 ((CH_3)$_2\underline{C}$H), 33.46 (CH-$\underline{C}H_2$-CH_2), 35.08 ($\underline{C}H_2$-$C_{Carb.}$), 52.44 (2×OCH_3), 54.75 (C_5), 60.89 (C_2), 74.56 $\underline{C}_{Carb}$-CH_3), 78.26 (CH_2-$\underline{C}_{Carb}$), 163.31 and 163.95 (C_3 and C_6). IR (KBr, cm^{-1}): 2950 (m, ν-H_3C(-$C_{Carb.}$)), 2870 (m, ν-CH_3C(-O)), 2580 (s, ν(BH)), 1690 (s, ν-C=N), 1465 (m), 1440 (m), 1385 and 1375 (w, δ-CH_3)), 1300 (m), 1240 and 1005 (s, νC–O), 1195 (m), 1140 (w), 1120 (m), 765 (m), 725(m). GC-MS m/z calcd. 382.5 ($C_{15}H_{34}B_{10}N_2O_2$), observed clusters of peaks centered around 382 (M^+), 367 (M^+–CH_3), 339 (M^+– $CH(CH_3)_2$) and 183 (M^+–$(CH_2)_3C_2B_{10}H_{10}CH_3$).

(2R,5R)-2,5-Dihydro-2-isopropyl-3,6-dimethoxy-5-[2-methyl-1,2-dicarba-closo-dodecaborane(12)- 1-yl]-propylpyrazine (**4b**):

Significant differences in spectroscopic data compared with **4a**: $[\alpha]_D^{20} = -24.2°$ (c = 1.05 in $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.71 and 0.74 (d, 3 H, -CH($C\underline{H}_3$)$_2$), 1.06 and 1.09 (d, 3H, CH($C\underline{H}_3$)$_2$), 2.02 (s, 3H, $C_{Carb.}$-$C\underline{H}_3$), 3.64 and 3.67 (2 × s, 2 × 3H, 2 × OCH3). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 17.36 and 19.49 (($\underline{C}H_3$)$_2$CH), 26.12 (CH_2-$\underline{C}H_2$-CH_2), 31.09 ((CH_3)$_2\underline{C}$H), 34.51(CH-$\underline{C}H_2$-CH,).

(S)-5-(2-Methyl-1,2-dicarba-closo-dodecaborane(12)-1-yl)-2-aminopentanoic acid methyl ester hydrochloride [L-MeCBA-OMe(×HCl)] (**5**):

1.5 g (3.9 mmol) **4a** was dissolved in 1.2 ml diethyl ether. 15.7 ml 0.25N HCl were added under rapid stirring, continued at room temp. for 64 h. The very fine white solid was filtered off, washed twice with cold water and with petroleum ether and dried (1.06 g **5**; 84% yield). Determination of enantiomeric purity by ^{1}H NMR with Eu(hfc)$_3$ (300 MHz, (CD_3)$_2$CO): ΔΔδ(OCH_3) 0.05 ppm (**5**: Eu(hcf)$_3$, 1:0.25), ee> 98%.

5: White powder; TLC [$CHCl_3$/MeOH (9:1)], R_f 0.4. $[\alpha]_D^{20}$ = +23.4° (c = 0.896 in MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ =1.2–3.1 (10H, BH), 1.6–1.8 (m, 2H, γ-CH_2), 1.8–2.0 (m,

2H, β-CH_2), 2.10 (s, 3H, Carb.–CH_3), 2.32–2.40 (t, 2H, δ–CH_2), 3.86 (s, 3H, OCH_3), 4.08–4.14 (t, 1H, α-CH). ^{13}C NMR (75 MHz, CD_3OD): δ = 23.53 (Carb.–CH_3), 26.35 (γ-CH_2), 30.79 (β-CH_2,), 35.39 (δ-CH_2), 53.51 (α-CH), 53.73 (OCH_3), 76.93 and 79.29 (2 × $C_{Carb.}$), 170.59 (COOMe). IR (KBr, cm^{-1}): 3000 (s, broad, ν-NH_3^+), 2590 (s, ν-BH), 2400 (s, broad), 2000 (s, broad), 1760 (s, n-C=O), 1600 (m), 1520 (m, d-NH_3^+), 1450 (m), 1380 (m), 1290 (m), (m, n-C–O), 1250 (m, ν-CO), 1200 (w), 1150 (m), 1110 (m), 1020 (m), 730 (m). FAB-MS m/z calcd. 288.4 ($M+H^+$, $C_9H_{26}B_{10}NO_2$), observed clus- ters of peaks centered around 288.5 ($M+H^+$) and 228.4 ($M+H^+$–$HCOOCH_3$).

(S)-5-(2-Methyl-1,2-dicarba-closo-dodecaborane(12)-1-yl)-2-aminopentanoic acid (L-MeCBA(×HCl)) (6):

The suspension of 0.94 g (2.9 mmol) **5** in 23 ml 6N HCl was refluxed 16 h. The fine white solid was filtered off, washed with cold 1N HCl, dried and quantitatively washed off from the sintered glass funnel with warm methanol. After concentration, 0.89 g dry **6** were recovered (yield 100%). Control of enantiomeric purity was performed with Fmoc derivatives by chiral-phase hplc (ChiraDex® Gamma, 250×4 mm, Merck): 25 mM acetate buffer pH 4.4/acetonitrile (50:50), flow: 1.2 ml/min, detection: uv absorption at 280 nm. R_t: L isomer 70 min; R_t(D isomer) 75 min (control experiment); no D isomer detectable in Fmoc derivative of **6** (ee > 98%).

6: White powder. TLC [ethyl acetate/n-butanol/HAc/H_2O (10:6:2:2)], R_f 0.6 .$[a]_D^{20}$ = +23.2° (c = 0.854). ^{1}H HMR (300 MHz, CD_3OD): δ = 1.2–3.1 (10H, BH), 1.65–1.80 (m, 2H, γ-CH_2), 1.8–2.0 (m, 2H, β-CH_2), 2.10 (s, 3H, Carb.–CH_3), 2.32–2.42 (t, 2H, δ-CH_2), 3.95–4.02 (t, 1H, α-CH). ^{13}C NMR (75 MHz, CD_3OD): δ = 23.53 (Carb.–CH_3), 26.32 (γ-CH_2), 30.81 (β-CH_2), 35.48 (δ-CH_2), 53.44 (α-CH), 76.89 and 79.34 (2 × $C_{carb.}$), 171.42 (COOH). IR (KBr, cm^{-1}): 3000 (s, broad, ν-NH_3^+ and ν–OH), 2590 (s, ν-BH), 2400 (w, broad), 1950 (w, broad), 1750 (s, ν-C=O), 1600 (m, broad), 1500 (m, δ-NH_3^+, 1460 (m), 1420 (w), 1380 (w), 1220 (m, ν-C–O), 1150 (w), 1100 (w), 1020 (m), 730 (m). FAB-MS m/z calcd. 274.4 ($M+H^+$, $C_8H_{24}B_{10}NO_2$), observed clusters of peaks centered around 274.4 ($M+H^+$) and 228.4 ($M+H^+$–HCOOH).

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