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143. The Synthesis of [4-Carboranylalanine, 5-Leucine]-Enkephalin

(Including an Improved Preparation of *t*-Butoxycarbonyl-L-*o*-carboranylalanine, New Derivatives of L-Propargylglycine, and a Note on Melanotropic and Opiate Receptor Binding Characteristics)[1])

by **Jean-Luc Fauchère, Othmar Leukart, Alex Eberle,** and **Robert Schwyzer**

Institut für Molekularbiologie und Biophysik, Eidgenössische Technische Hochschule Zürich, CH-8093 Zürich

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Summary

The title compound, an analogue of [Leu^5]-enkephalin with L-*o*-carboranylalanine replacing L-phenylalanine in position 4, was prepared by fragment condensation. The analogue has a 3-fold higher affinity for rat brain opiate receptors in the [^{3}H]naloxone competition assay than natural [Leu^5]-enkephalin. Like [Leu^5]-enkephalin and N^{α}-acetyl-[Leu^5]-enkephalin, the *N*-terminal tripeptide fragment, H · Tyr-Gly-Gly · OH, had no melanotropic activity in the *Rana pipiens* frog skin assay.

A convenient, direct synthesis of methyl *t*-butoxycarbonyl-L-*o*-carboranylalaninate from methyl *t*-butoxycarbonyl-L-propargylglycinate is described, and the ^{13}C-NMR. spectra of L-*o*-carboranylalanine recorded. The procedure was extended to the preparation of BOC · Car-Leu · OMe from BOC · Pra-Leu · OMe. A number of new propargylglycine derivatives are reported.

Introduction. - L-*o*-Carboranylalanine (Car, *Fig. 1*) [6] is the first in a series of so-called 'fat' or 'super' amino acids comprising compounds such as L-adamantylalanine (Ada) [7], β-methylvaline (*t*-butylglycine, Bug), γ-methylleucine (neopentylglycine, Neo) [8]. Replacement of phenylalanine by Car in a chymotrypsin inhibitor [9] and in [Leu^5]-enkephalin [10] enhanced their respective enzyme and opiate receptor affinity 3-fold, despite the larger size of the Car side-chain. This may be

[1]) This work was supported by research grants of the *Swiss National Science Foundation* and the *Swiss Federal Institute of Technology*. Abbreviations are according to the IUPAC-IUB Commission on Biochemical Nomenclature [1] and *Houben-Weyl* [2]; Car represents L-*o*-carboranylalanine and Pra L-propargylglycine. A former statement in the summary of [3] that L-propargylglycine is a new amino acid is incorrect; only its derivatives with protecting groups suitable for peptide synthesis were new. The free amino acid had been described earlier as the racemate [4] and as the individual enantiomers [5].

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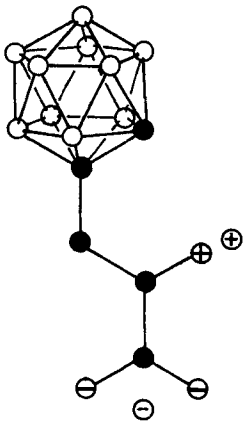


Fig. 1. *A diagrammatic representation of* o-*carboranylalanine* (**11**). The hydrogen atoms are omitted; ● represents carbon, ○ boron, ⊕ nitrogen, and ⊖ oxygen; ⊖ and ⊕ are charges of the zwitterion.

due to its greater lipophilicity (the *Hansch* π values are being examined [8]). We hope that the new concept of 'fat' or 'super' amino acids may help to distinguish between 'address' and 'message' sequences in peptide hormones and to produce agonists and antagonists with strong receptor affinity.

The first synthesis of Car [6] [11] utilized the addition of bisacetonitrilo decaborane [12] to the triple bond of methyl *N*-phthalyl-L-propargylglycinate [3] followed by ester hydrolysis and removal of the phthalyl group with boiling concentrated HCl. For use in peptide synthesis, the free amino acid was then either esterified or protected with a group like *t*-butoxycarbonyl.

This lengthy and rather awkward procedure was necessary for 2 reasons: (i) the carborane cage formation appeared to be drastically impaired by acidic protons of carboxy or monosubstituted carboxamide groups (*e.g.* in methyl *N*-acetyl-L-propargylglycinate). (ii) In the case of Car, the phthalyl group is useless for peptide synthesis, because 'mild' procedures for its removal, such as hydrazinolysis, destroy the carborane cage, and acid hydrolysis which leaves Car intact cleaves the peptide bonds.

This paper describes a more direct, improved synthesis of the peptide intermediate BOC · Car · OH which is feasible [11] probably because of the steric shielding of the NH proton by the bulky *t*-butoxycarbonyl group. The ^{13}C-NMR. spectrum of the free amino acid is also described *(Fig. 2)*.

[Car^4, Leu^5]-enkephalin, H · Tyr-Gly-Gly-Car-Leu · OH, was the first Car-containing hormone analogue ever prepared and biologically tested [10]. We now report in detail its synthesis and summarize the biological data (in the meantime, a number of Car analogues of this and of other hormones have been synthesized on an insoluble carrier [13]). In this context, the synthesis of BOC · Car-Leu · OMe (**19**) by direct addition of bisacetonitrilo decaborane to BOC · Pra-Leu · OMe (**14**) is also described in order to demonstrate the feasibility of the production of Car peptides from corresponding propargylglycine peptides, although the general applicability has not been proven. In addition, a number of new derivatives of L-propargylglycine were prepared but found unsuitable for successful reaction with decaborane.

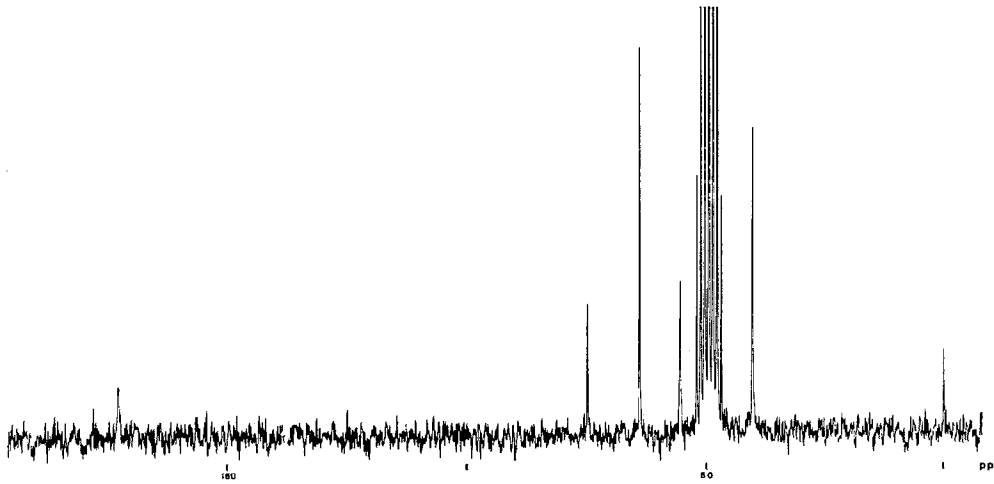


Fig. 2. *^{13}C-NMR. spectra of* L-o-*carboranylalanine* (**11**). The spectra at 25.14 MHz with proton noise decoupling were recorded on a *Varian* XL100 spectrometer using the *Fourier* Transform technique. The sample contained 45 mg of **11** dissolved in 1.5 ml of CD_3OD. The temperature was 22°. The chemical shifts are relative to TMS. 8400 Pulses were accumulated: pulse repetition 0.8 s. Internal lock on D of methanol CD_3. The signal assignment was made by a ^1H off-resonance experiment. δ (ppm): 172.2 (CO, *s*), 74.5 (C_γ, *s*), 63.6 (C_δ, *d*), 55.15 (C_α, *d*), 40.0 (C_β, *t*). The septet centered at 49.1 is from methanol. The multiplicity observed in the double resonance experiment is given in parentheses (*s* = singulet, *d* = doublet, *t* = triplet). The assignment of the doublets is based on the comparison with known values of the C_α resonance in natural amino acids as well as on electronegativity considerations (shift to low field by the strongly electron-deficient carboranyl residue). Coupling with the boron nuclei was not manifested in the spectra. The spectra were recorded in this institute (Laboratory of Prof. Dr. *K. Wüthrich*).

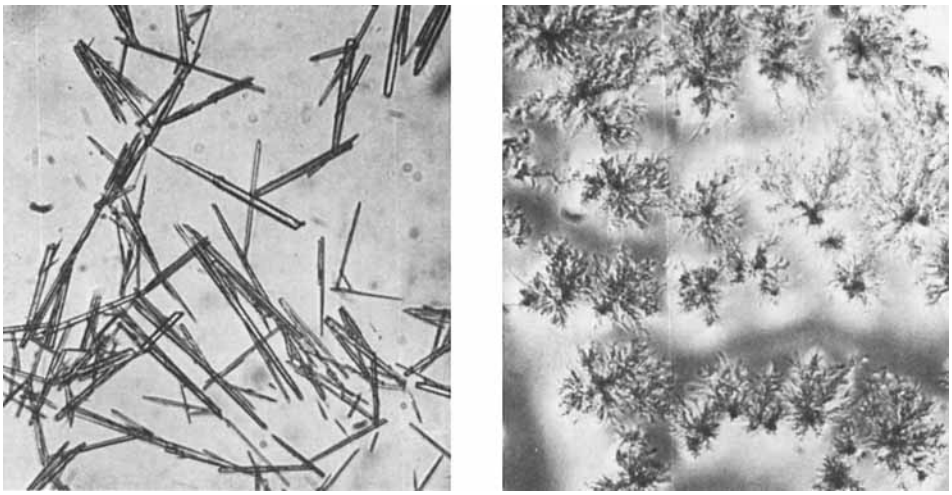
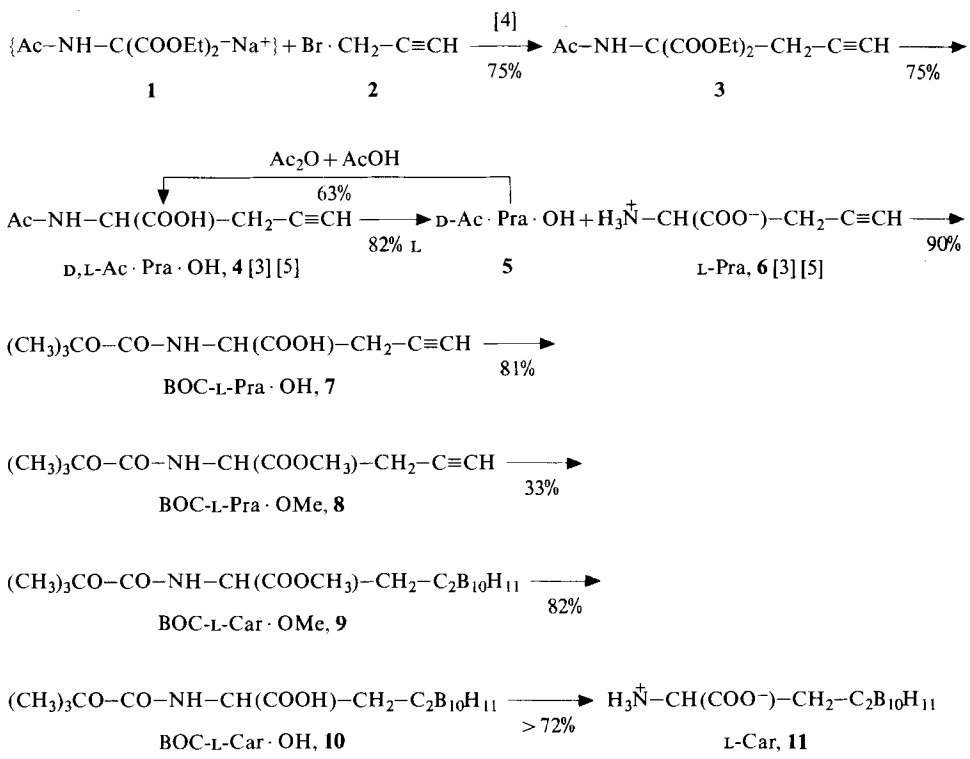


Fig. 3. *Crystals of BOC · Car · OH* (**10**) *and H · Car · OH* (**11**) (magnification 100× and 400× respectively)

Reports about an apparent melanotropic activity of enkephalin and enkephalin fragments [14] prompted us to investigate this matter as a side-line. We are not able to support these statements.

Improved synthesis of BOC · Car · OH *(Scheme 1)*: – The starting material (L-Pra, **6**), was obtained essentially as described earlier [3–5]. In order to increase the yield of **6**, the *N*-acetyl-D-propargylglycine (**5**) which was not hydrolyzed by swine kidney acylase I was racemized with acetic anhydride and reprocessed with the enzyme. BOC · Pra · OH (**7**) was easily produced in excellent yield with di-*t*-butyldicarbonate, the new reagent of *Moroder et al.* [15]. Its methyl ester, **8**, was prepared in good yield with caesium carbonate [16] and methyl iodide. The condensation of **8** with bisacetonitrilo decaborane [12] to BOC-L-Car · OMe (**9**) was carried out in boiling benzene. Because the carborane cage is apparently attacked by unidentified side-products of the reaction, it is essential to remove these immediately after the condensating step. Thus, a moderate yield of **9** was obtained. Despite a certain lability of the carborane in alkali, the methyl ester **9** was hydrolyzed in good yield to produce the desired BOC · Car · OH (**10**) as a nicely crystalline compound. A small sample was converted to the free amino acid **11** in order to further prove its identity.

Scheme 1



(The physical and analytical data of all compounds described in this paper are shown in *Tables 1–4*.)

The overall yield of BOC · Car · OH (**10**) from L-Pra (**6**) is 20%; with the phthalyl procedure [6] [11], it is about 9%. The new procedure also has the advantage of having one step less.

Miscellaneous Pra derivatives *(Scheme 2)*. – A number of new Pra derivatives were prepared for use in peptide synthesis and for condensation with decaborane. The *t*-butyl ester of L-Pra (HCl salt, **12**) was prepared from L-Pra and isobutylene according to well-known procedures of amino acid chemistry [2]. It can be used as a *C*-terminal element in peptide synthesis. The *p*-nitrophenylester of BOC · Pra · OH (**13**) can be used for *N*-terminal chain elongation. However, attempts to produce from it directly the active ester BOC · Car · ONp were unsuccessful (*p*-nitrophenylesters are usually not very stable during the silica gel chromatography that appeared necessary for purification). The dipeptide derivative **14** is readily prepared in a crystalline state; it is useful for the preparation of BOC · Car-Leu · OMe (**19**, *Scheme 3*). Finally, an *N*-disubstituted Pra methyl ester (**15b**) was prepared *via* **15** (and **15a**). It is a derivative of 4,5-diphenyl-4-oxazoline-2-one, abbreviated as OX-L-Pra · OH (**15**). The OX group was introduced by *Sheehan* [17], and we used it to replace the phthalyl group in an alternative Car synthesis [11]: OX · Car · OMe was, indeed, obtained in 80% yield from **15b** as a nicely crystallized compound, m.p. 79–80°, $[\alpha]_D^{20} = -47.5°$ ($c = 0.6$, EtOH). Alkaline hydrolysis produced the crystalline

Scheme 2

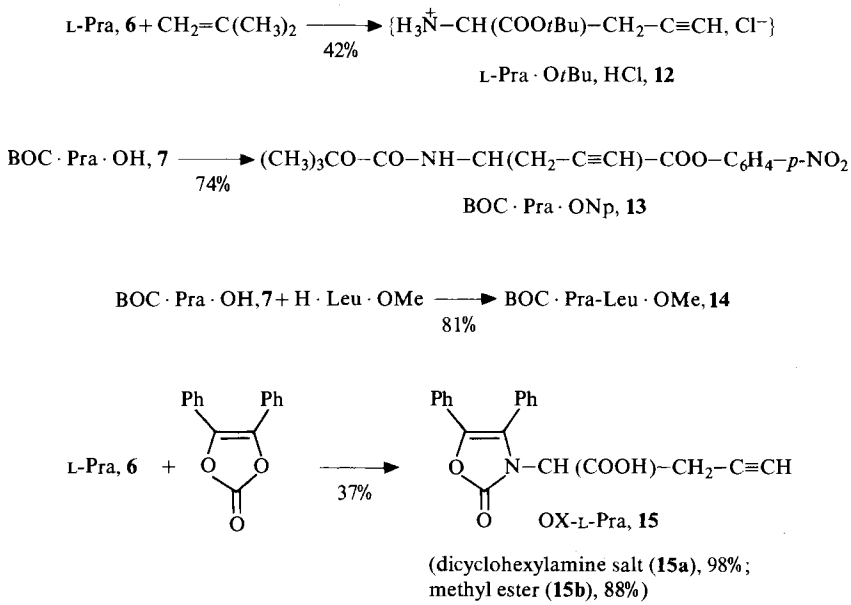


Table 1. *Thin-layer chromatography of Pra and Car derivatives* (Rf values)[a])

	HE 41[b])	CM 91[c])	CM 11[c])	EM 51[d])	CMA 9553[e])	BAW 411[f])	BAW 772[f])	BPAW 5112[g])	IWP 766[h])	BN 103[i])
3		0.60			0.43	0.85	0.78			
4						0.58	0.48			
6						0.26				
7					0.37		0.66	0.60		
8	0.33	0.75			0.55					
9	0.14		0.72				0.78			
10			0.51				0.67			
11							0.54		0.64	
12			0.72		0.20					
13	0.20			0.70	0.75[k])		0.80			
14		0.70			0.85					
15		0.70				0.70				
15b		0.70								
16							0.62	0.68		0.84
17							0.50			0.41
18								0.30[l])		
19				0.68	0.71		0.77			
20					0.35		0.67			
21		0.30					0.72		0.73	
22							0.70			
23							0.50		0.30	

[a]) Detected with I_2, ninhydrin, *Reindel-Hoppe* reagent, fluorescence quenching in UV., or other suitable means. Unless otherwise indicated, only one spot was observed on the *Merck* F524 silica gel plates. [b]) Hexane/ethyl acetate 4:1. [c]) $CHCl_3$/methanol 9:1 or 1:1. [d]) Ethyl acetate/methanol 5:1. [e]) $CHCl_3$/methanol/acetic acid 95:5:3. [f]) Butanol/acetic acid/water 4:1.1 or 72:7:21. [g]) Butanol/pyridine/acetic acid/water 50:12:12:25. [h]) 2-Propanol/water/pyridine 7:6:6. [i]) Butanol/aqueous ammonia (25%) 10:3. [k]) Slight decomposition. [l]) 0.25 in butanol/pyridine/acetic acid/water 42:24:4:30.

Table 2. *Physical data.* Aspect: s = solid, c = crystalline (A); solvent for crystallization (S); M.p. °[a]); specific rotation, $[\alpha]_D^{20}$, ($c = 1$, MeOH) unless noted.

	A	S	M.p.	$[\alpha]_D^{20}$deg.		A	S	M.p.	$[\alpha]_D^{20}$deg.
3	c	*i*-Pr_2O	91	0	**15**	c	EtOAc/pentane	175–183[c])	−43.2[l])
4	c	water	138–140[b])	0	**15a**	c	EtOH/*i*-Pr_2O	185–186	−17.5[m])
6	c	H_2O/acetone	230[c])	−35.0[d])	**15b**	oil	–	–	−49.2[n])
7[e])	c	2-PrOH/Et_2O	154–155	+41.1[f])	**16**	c	2-PrOH/*i*-Pr_2O	161–162	+14.4
8	oil	–	–	−5.2[g])	**17**	s	EtOAc/pentane	120–122	+13.8
9	c	MeOH/water	121–122	−30.2[h])	**18**	s	MeOH/Et_2O	145–147	+47.9
10	c	MeOH/water	158[c])	−22.6[i])	**19**	c	Et_2O/pentane	163	−40.4
11	c	water	220–224[c])[j])	−11.0[k])	**20**	c	2-PrOH/pentane	168–174[c])	–
12	c	4N HCl, EtOAc	195	−17.2	**21**	c	Et_2O/pentane	146–150	−21.2
13	c	*i*-Pr_2O/Et_2O	117	−47.8	**23**	s	HCOOH/ether		−2.0
14	c	EtOAc/pentane	100	−34.9					

[a]) Uncorrected; open capillary tube. [b]) [5]: 138–140°, [3]: 137–139°. [c]) With decomposition. [d]) $c = 1$, water. [e]) Dicyclohexylamine salt. [f]) $c = 1.3$, MeOH. [g]) $c = 3$, MeOH. [h]) $c = 0.7$, MeOH. [i]) $c = 0.86$, MeOH. [j]) [6]: 215–220°. [k]) $c = 1$, glacial acetic acid. [l]) $c = 0.63$, EtOH. [m]) $c = 0.4$, EtOH. [n]) $c = 2.0$, EtOH.

Table 3. *Analytical data* (microanalyses[a])): C, H, N, B (% Calc./% Found)

	C	H	N	B		C	H	N
7[b])					**15a**			
$C_{22}H_{38}N_2O_4$	66.97	9.71	7.10		$C_{32}H_{38}N_2O_4$	74.68	7.44	5.44
(393.6)	66.50	9.66	6.88		(514.7)	74.14	7.30	5.30
9					**15b**			
$C_{11}H_{27}B_{10}NO_4$	38.24	7.88	4.05	31.30	$C_{21}H_{17}NO_4$	72.61	4.93	4.03
(345.5)	38.40	7.74	4.12	31.03	(347.4)	72.45	5.11	4.10
10					**16**			
$C_{10}H_{25}B_{10}NO_4$	36.24	7.60	4.23	–	$C_{19}H_{27}N_3O_7$	55.74	6.65	10.26
(331.4)	36.12	7.68	4.20	–	(403.4)	55.81	6.81	9.95
11					**17**			
$C_5H_{17}B_{10}NO_2$	25.96	7.41	6.05	46.74[c])	$C_{18}H_{25}N_3O_7$	54.68	6.37	10.63
(231.3)	26.04	7.47	5.97	46.13[c])	(395.4)	54.78	6.48	10.41
12					**18**[d])			
$C_9H_{16}ClNO_2$	52.55	7.84	6.81		$C_{13}H_{18}ClN_3O_5$	47.07	5.47	12.67
(205.7)	52.40	7.81	6.78		(331.8)	46.91	5.31	12.42
13					**19**			
$C_{16}H_{18}N_2O_6$	57.48	5.43	8.38		$C_{17}H_{38}B_{10}N_2O_5$	44.51	8.35	6.11
(334.3)	57.51	5.39	8.34		(458.6)	44.65	8.32	6.29
14					**21**[d])			
$C_{17}H_{28}N_2O_5$	59.98	8.29	8.23		$C_{30}H_{53}B_{10}N_5O_5$	48.96	7.26	9.52
(340.4)	59.92	8.11	8.03		(735.9)	48.97	7.39	9.28
15					**23**			
$C_{20}H_{15}NO_4$	72.06	4.54	4.20		$C_{24}H_{44}B_{10}ClN_5O_7$	43.80	6.74	10.64
(333.3)	71.99	4.58	4.26		(658.2)	–	–	–

[a]) Performed in the Laboratorium für organische Chemie ETHZ *(D. Manser)* and in the Mikroanalytisches Laboratorium *Alfred Bernhard*, D-5251 Elbach über Engelskirchen (Prof. Dr. *H. Malissa & G. Reuter*); for C and H, boron-containing compounds combustion was effected in the presence of V_2O_5. [b]) Dicyclohexylamine salt. [c]) Corrected values from [6]. [d]) Amino acid analyses carried out in our institute in the laboratory of Prof. Dr. *H. Zuber* (Calc./Found); **18**: Tyr 1.0/1.0, Gly 2.0/2.2; **21**: Tyr 1.00/1.01, Leu 1.00/1.02, Gly 2.00/1.96, carboranylalanine is retained in the column (according to a control experiment).

Table 4. *IR. data.* Wavenumbers (cm^{-1}) of diagnostic absorption bands only[a])

	N–H	C≡C–H	B–H	C=O	other		N–H	C≡C–H	B–H	C=O	other
8	3420	3300		1745[b]) 1710[c])		**15**	–[e])	3300		1770[f])	3700– – 2200[g])
9	3410		2580	1740[b]) 1705[c])		**15b**		3300		1775[f])	
						19	3410		2580	1735[b]) 1690[c]) 1670[c])	
10	3410	–[d])	2580	1710[c])							
14	3405	3300		1735[b]) 1705[c]) 1690[c]) 1675[c])		**21**	3405		2570	1730[b]) 1690[c]) 1670[c])	3300[h])

[a]) Determined in $CHCl_3$ or Nujol. [b]) Ester. [c]) Amide. [d]) C≡C–H and ester C=O absent. [e]) N–H absent (disubstitution). [f]) Oxazoline C=O. [g]) COO–H. [h]) Tyrosine O–H.

OX · Car · OH, m.p. 183–185°, $[\alpha]_D^{20} = -9.3°$ ($c = 0.2$, EtOH) [11]. However, it proved impossible to remove the OX group by catalytic hydrogenolysis [17] or reduction with sodium in liquid ammonia [17] without destroying the carborane. Only drastic hydrolysis with HCl produced Car; liquid HF was ineffective. Hence, except for the yield in the decaborane addition step, there is no apparent advantage over the phthalyl group.

[Car^4, Leu^5]-enkephalin *(Scheme 3).* – This pentapeptide was synthesized by fragment condensation of the protected *N*-terminal tripeptide **17**, with the protected *C*-terminal dipeptide **20**, using dicyclohexylcarbodiimide. *Scheme 3* is self-explanatory.

The protected dipeptide **19** was also prepared by adding decaborane to the corresponding Pra dipeptide **14**. The first approach, using BOC · Car · OH (**10**), product **19** in 16% overall yield from Pra (**6**). With the second approach, it was 24%; however, the applicability of this approach to other peptides remains to be demonstrated.

Biological data. – For **23**, the binding to opiate receptors of rat brain has been studied by competition with [^{3}H]naloxone [10]. Its receptor affinity was 116 ± 18% relative to [Met^5]-enkephalin (100%); it was 3 times greater than that of its parent com-

Scheme 3

{H · Gly-Gly · OMe, HCl} + BOC · Tyr · OH ⟶ (75%) BOC · Tyr-Gly-Gly · OMe ⟶ (83%)
16

BOC · Tyr-Gly-Gly · OH ⟶ (91%) {H · Tyr-Gly-Gly · OH, HCl}
17 **18**

BOC · Car · OH, **10** + {H · Leu · OMe, HCl} ⟶ (83%) BOC · Car-Leu · OMe ⟶ (98%)
19

17
{H · Car-Leu · OMe, HCl} ⟶ (65%) BOC · Tyr-Gly-Gly-Car-Leu · OMe ⟶
20 **21**

BOC · Tyr-Gly-Gly-Car-Leu · OH ⟶ (85%) {H · Tyr-Gly-Gly-Car-Leu · OH, HCl}
22 (from **21**) **23**

BOC · Pra-Leu · OMe, **14** ⟶ (33%) BOC · Car-Leu · OMe, **19**

pound, [Leu^5]-enkephalin ($36 \pm 6\%$). This is comparable to the affinity increase for chymotrypsin in the compounds Z · Ala-Ala-Phe · OH ($K_{ass} = 1 \cdot 10^3\ l \cdot mol^{-1}$) and Z · Ala-Ala-Car · OH ($K_{ass} = 3.3 \cdot 10^3\ l \cdot mol^{-1}$) and may reflect increased lipophilic properties of the specifically 'binding' amino acid (Phe → Car).

The tripeptide H · Tyr-Gly-Gly · OH (**18**) was tested for its alleged melanotropic properties [14] using the skin of the leopard frog, *Rana pipiens* [18]. As in the case of [Leu^5]-enkephalin and N^{α}-acetyl-[Met^5]-enkephalin [19], no activity whatsoever was observed up to 0.5 mg/ml.

Experimental Part

General. Removal of solvents from dissolved products was carried out in a rotatory evaporator at reduced pressure (0.1–10 Torr) and low temperature. All solvent ratios are in volume parts. Product characteristics are displayed in *Tables 1–4.* Whenever well-known procedures of peptide chemistry or trivial intermediates were used, reference is made to *Houben-Weyl* [2] where experimental details are given or the literature is quoted. For general remarks on instrumentation and other general items, see [20].

Diethyl-α-acetamido-α-propargylmalonate (**3**) was prepared as described in the literature [4].

D,L-N-*Acetyl-α-propargylglycine* (**4**) [3] [5] was prepared by a modified procedure. A suspension of **3** (192 g, 0.75 mol) in 2.5N NaOH (550 ml) was stirred at 20° for 16 h. The mixture was then neutralized to pH 5.5 with conc. hydrochloric acid and the volume reduced to 200 ml by evaporation. After acidification to pH 2.5, the solution was heated to 100° for 2 h (decarboxylation). After cooling, the product was extracted into ethyl acetate and recrystallized first from this solvent, then from water: 87.3 g.

L-*Propargylglycine* (*Pra,* **6**) was obtained as described in the literature [3] [5], using swine kidney acylase I. The remaining *N*-acetyl-D-propargylglycine (**5**) was recovered by evaporating the mother liquors to dryness. It was racemized by heating for 30 min at 115° in acetic anhydride/glacial acetic acid 8:160, followed by evaporation and recrystallization from water.

t-*Butoxycarbonyl-L-propargylglycine* (**7**). A solution of **6** (6.5 g, 57.5 mmol) in dioxane/water (120 ml/60 ml) was treated at 20° with 1N NaOH (60 ml) followed by di-*t*-butyl-dicarbonate (13.8 g, 63.2 mmol) [15] at 0°. The mixture was kept at 20° for 30 min. Evaporation of the dioxane and extraction of the acid into ethyl acetate at pH 2 afforded 12 g of chromatographically pure **7** as an oil. The *dicyclohexylamine salt* was prepared in ether with an equimolar amount of dicyclohexylamine (11.6 ml, 57.5 mmol): 20.41 g of colourless crystals.

Methyl t-*butoxycarbonyl-L-propargylglycinate* (**8**). The dicyclohexylamine salt of **7** (1.97 g, 5 mmol) was dissolved in a cold 2-phase system consisting of ethyl acetate and 0.1M $KHSO_4/K_2SO_4$ (pH 2). The free acid, **7**, was extracted with ethyl acetate (3×) and obtained as a chromatographically pure oil by evaporation. Its solution in methanol/water 10:1 (20 ml) was treated with Cs_2CO_3 (1.62 g, 5 mmol) according to [16]. The white, solid caesium salt obtained after solvent evaporation was stirred for 16 h in dimethylformamide (DMF) (10 ml) containing one equiv. of methyl iodide (710 mg, 5 mmol). The mixture was then freed of CsI by filtration, evaporated, and the residue purified by dissolving in ethyl acetate, filtration, and evaporation: 925 mg of pure **8**.

Methyl t-butoxycarbonyl-L-O-*carboranylalaninate* (**9**). A solution of **8** (2.5 g, 11 mmol) in boiling benzene (250 ml) was treated with the bis-acetonitrile complex of decaborane [12] (2.5 g, 12.5 mmol). After 1 h an additional amount of the decaborane bis-acetonitrile complex (0.8 g, 4 mmol) was added to the almost transparent reaction mixture. The solvent was evaporated under reduced pressure at 20° (caution: the desired product is slightly volatile!) and the residue chromatographed *without delay* (!) on silica gel (120 g, column 3×40 cm) using $CHCl_3$ and disregarding the gas bubbles formed within the column (due to product degradation). The first 250 ml of eluate contained impurities only (undefined boron compounds with low Rf on TLC. in HE 41). The next 400 ml contained chromatographically pure **9**, and the last fractions (300 ml) a mixture of **9** with some unreacted **8**. The fractions were filtered and evaporated at 20° under reduced pressure, and **9** purified by crystallization from methanol/water: 1.25 g.

In other experiments, **9** was obtained by dissolving the product mixture (before chromatography) in ethanol, filtering from insoluble impurities and direct crystallization. The mother liquors were then chromatographed as above to obtain further quantities of **9**.

t-*Butoxycarbonyl*-L-o-*carboranylalanine* (**10**). A suspension of **9** (687 mg, 2 mmol) in a dioxane/0.2N aqueous KOH 1:1 (20 ml) was treated with more dioxane (about 10 ml) to obtain a clear solution. After 10–15 min at 20°, hydrolysis was complete (TLC. monitoring). The solution was then brought to pH 5 with 1N HCl and the dioxane evaporated. Water was added, the solution cooled with ice and acidified to pH 2.5, and the product extracted into ethyl acetate. Pure **10** was obtained by crystallization from methanol/water at 20° in an open flask (slow evaporation of some of the methanol): 540 mg.

L-o-*Carboranylalanine* (**11**). A solution of **10** (99 mg, 0.3 mmol) in cold trifluoroacetic acid (5 ml) was kept at 0° for 30 min. The solvent was then evaporated and the oily residue dissolved in water. Neutralization with ammonia to pH 7 precipitated the amino acid. Recrystallization from methanol/water afforded a first fraction of pure **11** (50 mg, 0.22 mmol).

t-*Butyl* L-*propargylglycinate hydrochloride* (**12**) was prepared according to the usual procedure [2]. A solution of **6** (1.13 g, 10 mmol), in dioxane (10 ml), conc. sulfuric acid (1.5 ml), and liquid isobutene (20 ml) afforded the ester, H · Pra · O*t*Bu, as an oil which was converted to the crystalline hydrochloride with 4N HCl in ethyl acetate: 865 mg.

p-*Nitrophenyl* N-t-*butoxycarbonyl*-L-*propargylglycinate* (**13**) was prepared by the usual procedure for *N*-protected amino acid *p*-nitrophenylesters [2]. A solution of **7** (prepared from 3.94 g, 10 mmol, of its dicyclohexylamine salt; see preparation of **8**) in ethyl acetate (50 ml), 4-nitrophenol (1.39 g, 10 mmol), and dicyclohexylcarbodiimide (2.26 g, 11 mmol) afforded 2.47 g of recrystallized **13**.

Methyl t-*butoxycarbonyl*-L-*propargylglycyl*-L-*leucinate* (**14**) was prepared from a solution of the dicyclohexylamine salt of **7** (4,40 g, 11,2 mmol), methyl L-leucinate hydrochloride (2.03 g, 11.2 mmol), and 1-hydroxybenzotriazole (2.26 g, 16.8 mmol) in DMF with dicyclohexylcarbodiimide (2.54 g, 12.3 mmol) according to standard procedures [2]: 3.08 g of recrystallized **14**.

N-*[(S)-1-Carboxybut-3-yn-1-yl]-4,5-diphenyl-4-oxazoline-2-one* (**15**) and its dicyclohexylamine salt (**15a**) were prepared from **6** and the cyclic carbonate of 1,2-diphenyl-vinylene-1,2-diol [17] according to the procedure described in [11].

N-*[(S)-1-Carbomethoxybut-3-yn-1-yl]-4,5-diphenyl-4-oxazoline-2-one* (**15b**) was prepared from **15** by esterification in methanolic HCl-solution according to [11].

Methyl N-t-*butoxycarbonyl*-L-*tyrosyl-glycyl-glycinate* (**16**) was prepared according to standard procedures [2] by condensation of BOC · Tyr · OH (1.41 g, 5 mmol) with {H · Gly-Gly · OMe, HCl} (0.91 g, 5 mmol) in a mixture of DMF (10 ml) and *N*-ethylmorpholine (0.63 ml, 5 mmol) using dicyclohexylcarbodiimide (1.13 g, 5.5 mmol) and 1-hydroxybenzotriazole (1.35 g, 10 mmol). The crude material was purified by chromatography on a silica gel column (2 × 70 cm) with $CHCl_3$/methanol 19:1 and gives 1.54 g pure **16** (after recrystallization).

N-t-*Butoxycarbonyl*-L-*tyrosyl-glycyl-glycine* (**17**) was obtained from **16** (1.23 g, 3 mmol) by saponification for 12 min at 20° with 1N NaOH (6 ml) in methanol/dioxane 1:1 (12 ml). The usual isolation procedure (evaporation, acidification, extraction with ethyl acetate *etc.*) afforded 0.98 g of pure **17**.

L-*Tyrosyl-glycyl-glycine hydrochloride* (**18**) was prepared from **17** (0.79 g, 2 mmol) by deprotection with 0.12N HCl in HCOOH for 15 min at 20° according to standard procedures [2]: 600 mg of pure **18**.

Methyl N-t-*butoxycarbonyl*-L-o-*carboranylalanyl*-L-*leucinate* (**19**). – *Procedure a.* BOC · Car · OH (**10**, 329.6 mg, 1 mmol), methyl L-leucinate hydrochloride (181.6 mg, 1 mmol), *N*-ethylmorpholine (0.11 ml, 1 mmol), and 1-hydroxybenzotriazole (226 mg, 1.1 mmol) were dissolved in this order in dry DMF (10 ml). The solution was cooled to 0°, treated with dicyclohexylcarbodiimide (226 mg, 1.1 mmol), and kept at 0 and 22° for 1 and 16 h, respectively. After addition of a few drops of glacial acetic acid, the dicyclohexylurea was filtered off and the solvent evaporated. The solid residue was dissolved in ethyl acetate and washed as usual to remove acidic and basic components. Evaporation and recrystallization yielded 380 mg of pure **19**.

Procedure b. A solution of BOC · Pra-Leu · OMe (**14**, 2.06 g, 6.05 mmol) and bisacetonitrilo decaborane [12] (1.36 g, 6.67 mmol) in dry benzene (80 ml) was refluxed for 2.5 h. After evaporation of the solvent from the yellow solution, the residue was *immediately* dissolved in $CHCl_3$ and chromatographed through silica gel (100 g), disregarding the formation of bubbles in the column. Development of the chromatogram in a manner similar to that used in the preparation of **9** yielded 510 mg of pure BOC · Car-Leu · OMe (**19**) and 915 mg of BOC · Pra-Leu · OMe (**14**), containing traces of **19**.

Methyl N-t-*butoxycarbonyl*-L-*tyrosyl-glycyl-glycyl*-L-o-*carboranylalanyl*-L-*leucinate* (**21**). BOC · Car-Leu · OMe (**19**, 420 mg, 0.92 mmol) were treated for 30 min at 22° with 1.2 equiv. of 0.1N HCl in HCOOH. The solvent was removed by evaporation and the residue crystallized from 2-propanol by adding pentane: 352 mg of pure **20**.

The condensation of **17** and **20** (0.5 mmol each) was carried out as described for the preparation of **19**. The crude product which still contained some dicyclohexylurea was purified by chromatography on silica gel. Elution with $CHCl_3$ removed dicyclohexylurea; $CHCl_3$/methanol 9:1 eluted pure **21** (240 mg after recrystallization).

L-*Tyrosyl-glycyl-glycyl*-L-o-*carboranylalanyl*-L-*leucine hydrochloride* (**23**). A solution of **21** (368 mg, 0.5 mmol) in 0.2N KOH in dioxane/water 1:1 (20 ml) was kept at 20° for 15 min. After neutralization to pH 7 with 0.1NHCl, the organic solvent was removed by evaporation and the intermediate acid **22** extracted into ethyl acetate. The solvent was evaporated and the product precipitated from methanol with ether. The BOC group was removed by treatment with 0.1N HCl in HCOOH (12 ml) for 20 min at 20°. The product was precipitated with ether and gathered by centrifugation: 280 mg of pure **23**.

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