

89. [5-Leucin]enkephalin-Related Glycoconjugates: Structurally Novel Agents Effective against HIV-1

by Štefica Horvat*, Lidija Varga, and Jaroslav Horvat

Department of Organic Chemistry and Biochemistry, 'Ruder Bošković' Institute,
P.O.B. 1016, 41001 Zagreb, Yugoslavia

and Andreas Pfützner, Haryadi Suhartono, and Helga Rübsamen-Waigmann

Chemotherapeutisches Forschungsinstitut Georg-Speyer-Haus, Paul-Erlich-Str. 42–44, D-6000 Frankfurt 70

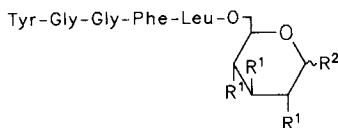
(24. IV. 91)

A series of [Leu⁵]enkephalin-related glycoconjugates with an ester-, ether-, or amide-type linkage were synthesized and evaluated for antiviral activity against HIV-1 in a cell-culture system using peripheral blood lymphocytes. All tested glycoconjugates exhibited a certain antiviral activity which was significantly higher than the activity of the parent peptide compound itself. These results indicate that synthetic glycoconjugates of opioid peptides are good candidates for the development of anti-HIV agents.

Introduction. – The enkephalins [1], endogenous opioid peptides (Tyr-Gly-Gly-Phe-Leu/Met), function as neurotransmitters or neuromodulators in the central nervous system and as transmitters in the periphery [2]. In the past few years, accumulating evidence indicates that enkephalins modulate numerous immune functions [3] and as such are important tools for therapy of immunodeficiency states and neoplastic diseases. Indeed several recent studies have shown that [Met⁵]enkephalin elicited a temporary enhancement of selected immune response in HIV positives or AIDS patients [4] [5].

Recently, we discovered that introduction of a carbohydrate moiety into [Leu⁵]enkephalin considerably influenced the biological activity of the parent peptide compound [6] [7]. In addition, variations in the type of linkage and sugar moiety led to marked consequences on opioid receptor selectivity. This observation and the urgent need for a new class of compounds that have potent anti-HIV activity and low toxicity and act through a different mode of action stimulated us to prepare a series of [Leu⁵]enkephalin glycoconjugates. The synthesis and anti-HIV activity of these compounds are reported herein.

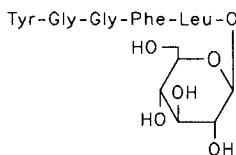
Chemistry. – Glycoconjugates in which a carbohydrate moiety is linked to [Leu⁵]enkephalin either through an ester (**1–4**), ether (**5**), or amide (**6–8**) bond were prepared. The synthetic pathways for the preparation of compounds **1–5** have been published previously [6–8]. The synthesis of [Leu⁵]enkephalin related *N*-glycoconjugates **6–8** was carried out in a stepwise manner as illustrated in the *Scheme* using *unprotected* or minimally protected amino sugars. As starting amino-monosaccharides, benzyl 6-amino-6-deoxy- β -D-glucopyranoside (**9**), 2-amino-2-deoxy-D-glucopyranose (**10**), and β -D-glucopyranosylamine (**11**) were used. The first step of the synthesis involved coupling of the *N*-protected dipeptide Boc-Phe-Leu-OH with the amino group of the corresponding sugar by using



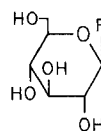
1 R¹ = OH, R² = OH

2 (β-D-anomer) R¹ = OH, R² = OBzl

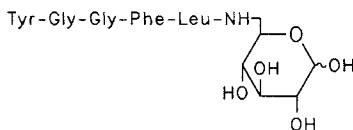
3 (β-D-anomer) R¹ = R² = OAc



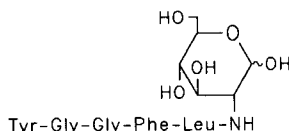
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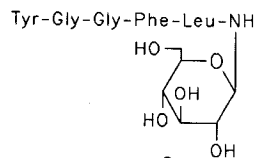
5 R = Tyr-Gly-Gly-Phe-Leu-OH



6



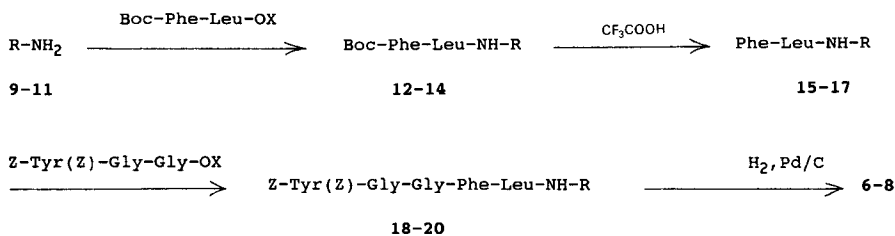
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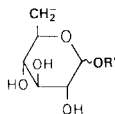
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either active-ester or mixed-anhydride activation procedures. Regioselectively acylated derivatives **12–14** were then deprotected at the N-terminal position using CF₃COOH, and the resulting dipeptide derivatives **15–17** were coupled with the activated tripeptide obtained from Z-Tyr(Z)-Gly-Gly-OH. Removal of the protecting groups at the N-terminal tyrosine residue in **18–20** by catalytic hydrogenation, followed by gel chromatography (*Sephadex G-15* column, 1% AcOH/H₂O) gave pure compounds **6–8**. The parent peptide Tyr-Gly-Gly-Phe-Leu was obtained by solid-phase synthesis (*HYCRAM*TM resin, Boc strategy) [9].

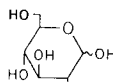
Scheme



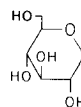
R:



R' = PhCH₂ and β-D for **9, 12, 15, and 18**
R' = H and α/β-D for **6**



α/β-D for **10, 16, 19, and 7**
α-D for **13**



β-D for **11, 14, 17, 20, and 8**

Biological Results. – The antiviral activities against HIV-1 of compounds **1–8**, tested in a cell-culture system by using peripheral blood lymphocytes, are listed in the *Table*. All [Leu⁵]enkephalin glycoconjugates expressed a certain antiviral activity which is significantly higher than the activity of the parent peptide Tyr-Gly-Gly-Phe-Leu. Neither compound, however, was capable of providing full protection to the cells against HIV infection. The activity of HIV-1 reverse transcriptase in the culture supernatant demonstrated the release of particle-bound enzyme, even at drug concentrations of 100 μM . Glycoconjugates **1–8** proved to be nontoxic under test conditions up to concentrations of 140 μM .

Table. Effect of [Leu⁵]enkephalin Derivatives on HIV-1 Infectivity of T-Lymphocytes^{a)}

	Cytopathic effect ^{b)} (% RT activity) ^{a)}				
	25 μM^c	50 μM^c	75 μM^c	100 μM^c	140 μM^c
1	++ (85)	++ (77)	+ (n.d.)	+ (72)	+ (n.d.)
2	+++ (100)	++ (85)	+ (n.d.)	+ (85)	+ (75)
3	+++ (100)	++ (82)	+ (n.d.)	+ (79)	+ (n.d.)
4	+++ (100)	++ (79)	+ (n.d.)	+ (75)	+ (60)
5	+++ (100)	++ (90)	+ (n.d.)	+ (79)	+ (n.d.)
6	++ (93)	++ (85)	+ (n.d.)	+ (84)	+ (n.d.)
7	++ (93)	++ (87)	+ (n.d.)	+ (72)	+ (64)
8	+++ (100)	++ (86)	+ (n.d.)	+ (75)	+ (67)
[Leu ⁵]enkephalin	+++ (100)	+++ (100)	+++ (100)	++ (97)	++ (94)
Controls: positive			+++ (100)		
negative			– (< 1)		

^{a)} The assay is described in detail in [10]. The antiviral activity of the compounds was determined on day 4 after virus infection by screening for the syncytia formation (cytopathic effect, CPE) and quantitatively by measuring reverse transcriptase (RT) activity in the culture supernatant. Each treatment was carried out in duplicate, and the results were averaged.

^{b)} Scoring of syncytia formation was based on graded level of no apparent syncytia ('–') to > 30 syncytia ('++++') in a single microscopic field.

^{c)} Concentration of the [Leu⁵]enkephalin derivative.

From the standpoint of structure *vs.* activity, no significant dependence of antiviral activity on the type of carbohydrate-peptide linkage or sugar compound could be observed. Possible reasons of altered anti-HIV activity of the glycopeptides studied could be increased stability of the conjugates in the cells, or a facilitated transport through the cell membrane. The effect of the sugar moiety remains to be elucidated, however. In conclusion, these results show, that glycopeptides should be investigated further as an interesting class of biomolecules for the development of anti-HIV drugs.

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Experimental Part

General. See [7]. For determination of antiviral activity, see [10]. Syntheses of **1–4** [7] [8] and **5** [6] have been described previously.

General Procedure for the Syntheses of Glycopeptides 6–8. To a chilled soln. (-15°) of Boc-Phe-Leu-OH (1 mmol) in THF (10 ml), *N*-methylmorpholine (1 mmol) and isobutyl chloroformate (1 mmol) were added. The resulting mixture was stirred for 2 min, and a cool soln. of the corresponding aminosugar **9**, **10**, or **11** (1 mmol) in THF/H₂O 1:1 (10 ml) was added. The mixture was stirred for 10 min at -15° and overnight at r.t. Evaporation of THF and addition of H₂O (10 ml) gave the corresponding glycodipeptide **12**, **13**, and **14** in 46, 79, and 59% yield, resp.

To a soln. of **12**, **13**, or **14** (0.3 mmol) in CH₂Cl₂ (3 ml), CF₃COOH (1.5 ml) was added and stirred for 30 min at r.t. Addition of (*i*-Pr)₂O (30 ml) to the mixture and centrifugation gave pure trifluoroacetate salt of **15**, **16**, and **17** in 93, 82, and 75% yield, resp.

To a soln. of Z-Tyr(Z)-Gly-Gly-OH (0.2 mmol) in THF (5 ml) at -15° , *N*-methylmorpholine (0.2 mmol) was added, followed by isobutyl chloroformate (0.2 mmol). The mixture was stirred for 2 min at -15° , a soln. of the trifluoroacetate salt of **15**, **16**, or **17** (0.2 mmol) and *N*-methylmorpholine (0.2 mmol) in THF/H₂O 1:1 (5 ml) added, and the mixture stirred for additional 20 h at r.t. The solvent was evaporated and the residue purified by FC (CHCl₃/MeOH 4:1): pure **18** (29%), **19** (24%), or **20** (51%).

The protected glycoconjugate **18**, **19**, or **20** (0.15 mmol) was dissolved in EtOH/AcOH/H₂O 4:1:1 (30 ml) and hydrogenated in the presence of 10% Pd/C (300 mg) for 24 h. The catalyst was filtered off, the filtrate evaporated, and the residue purified on a Sephadex G-15 column (1% AcOH/H₂O). Fractions containing product were lyophilized yielding **6** (76%), **7** (77%), and **8** (94%), resp.

Benzyl 6-{N-[(tert-Butyloxy)carbonyl]-L-phenylalanyl-L-leucylamino}-6-deoxy-β-D-glucopyranoside (12). M.p. 198–199°. $[\alpha]_D^{20} = -40$ ($c = 1.0$, MeOH). ¹³C-NMR (90 MHz, CD₃OD): 22.4, 23.8 (CH₃(5.2), CH₃(5'.2)); 26.0 (CH(4.2)); 28.9 ((CH₃)₃C); 39.1, 41.9, 42.4 (CH₂(3.1), CH₂(3.2), C(6')); 53.4 (CH(2.2)); 58.5 (CH(2.1)); 72.1, 73.2, 75.3, 76.0, 77.0 (C(2'), C(3'), C(4'), C(5'), PhCH₂); 81.0 ((CH₃)₃C); 103.7 (C(1')); 127.9–139.3 (12 arom. C, Ph–C(3.1), PhCH₂); 157.8 (*t*-BuOCO); 174.4, 175.2 (C(1.1), C(1.2)).

2-{N-[(tert-Butyloxy)carbonyl]-L-phenylalanyl-L-leucylamino}-2-deoxy-α-D-glucopyranose (13). M.p. 159–162°. $[\alpha]_D^{23} = +21.7$ ($c = 2.0$, DMF). ¹³C-NMR (90 MHz, (D₆)DMSO): 21.8, 23.5 (CH₃(5.2), CH₃(5'.2)); 24.1 (CH(4.2)); 28.2 ((CH₃)₃C); 38.7, 42.2 (CH₂(3.1), CH₂(3.2)); 51.0, 54.5, 55.9, 61.3, 70.6, 71.4, 72.2 (C(2'), C(3'), C(4'), C(5'), C(6'), CH(2.1), CH(2.2)); 78.3 ((CH₃)₃C); 90.8 (C(1')); 126.3–138.4 (6 arom. C, Ph–C(3.1)); 155.4 (*t*-BuOCO); 171.4, 172.3 (C(1.1), C(1.2)).

N-{N-[(tert-Butyloxy)carbonyl]-L-phenylalanyl-L-leucyl}-β-D-glucopyranosylamine (14). M.p. 192–194°. $[\alpha]_D^{21} = -31.2$ ($c = 1.0$, MeOH). ¹³C-NMR (90 MHz, CD₃OD): 22.1, 23.9 (CH₃(5.2), CH₃(5'.2)); 25.9 (CH(4.2)); 28.9 ((CH₃)₃C); 39.1, 42.3 (CH₂(3.1), CH₂(3.2)); 53.4 (CH(2.2)); 57.4 (CH(2.1)); 62.9 (C(6')); 71.6, 74.1, 79.0, 80.0, 80.9 (C(2'), C(3'), C(4'), C(5'), (CH₃)₃C); 81.6 (C(1')); 127.9–138.9 (6 arom. C, Ph–C(3.1)); 157.8 (*t*-BuOCO); 174.7, 175.6 (C(1.1), C(1.2)).

Benzyl 6-Deoxy-6-(L-phenylalanyl-L-leucylamino)-β-D-glucopyranoside Trifluoroacetate (15·CF₃COOH). M.p. 147–149°. $[\alpha]_D^{21} = -51.7$ ($c = 3.0$, MeOH). ¹³C-NMR (90 MHz, (D₆)DMSO): 21.9, 22.9 (CH₃(5.2), CH₃(5'.2)); 24.1 (CH(4.2)); 37.3, 41.1 (CH₂(3.1), CH₂(3.2), C(6')); 50.8, 51.2 (CH(2.1), CH(2.2)); 66.4, 69.5, 71.9, 73.5, 74.3 (C(2'), C(3'), C(4'), C(5'), PhCH₂); 102.1 (C(1')); 126.1–138.0 (12 arom. C, Ph–C(3.1), PhCH₂); 170.5, 172.1 (C(1.1), C(1.2)).

2-Deoxy-2-(L-phenylalanyl-L-leucylamino)-D-glucopyranose Trifluoroacetate (16·CF₃COOH). M.p. 122–125°. $[\alpha]_D^{24} = +15$ ($c = 1.5$, MeOH). ¹³C-NMR (90 MHz, (D₆)DMSO): 21.9, 23.4 (CH₃(5.2), CH₃(5'.2)); 24.2 (CH(4.2)); 51.5, 53.5, 54.6, 61.4, 70.5, 71.4, 72.3 (C(2'), C(3'), C(4'), C(5'), C(6'), CH(2.1), CH(2.2)); 91.1 (C(1'), α-D); 95.5 (C(1'), β-D); 127.3–135.0 (6 arom. C, Ph–C(3.1)); 167.7, 171.9 (C(1.1), C(1.2)).

N-(L-Phenylalanyl-L-leucyl)-β-D-glucopyranosylamine Trifluoroacetate (17·CF₃COOH). M.p. 176°. $[\alpha]_D^{22} = +4$ ($c = 1.25$, DMF). ¹³C-NMR (90 MHz, (D₆)DMSO): 21.6, 23.4 (CH₃(5.2), CH₃(5'.2)); 24.1 (CH(4.2)); 51.2, 53.4 (CH(2.1), CH(2.2)); 61.1 (C(6')); 70.1, 72.4, 77.7, 78.7 (C(2'), C(3'), C(4'), C(5')); 79.8 (C(1')); 127.2–134.9 (6 arom. C, Ph–C(3.1)); 167.9, 172.1 (C(1.1), C(1.2)).

Benzyl 6-[N,O-Bis((benzyloxy)carbonyl)-L-tyrosylglycylglycyl-L-phenylalanyl-L-leucylamino]-6-deoxy-β-D-glucopyranoside (18). M.p. 199–200°. $[\alpha]_D^{20} = -25.8$ ($c = 2.25$, AcOH). ¹³C-NMR (90 MHz, (D₆)DMSO): 21.8, 23.0 (CH₃(5.5), CH₃(5'.5)); 25.2 (CH(4.5)); 51.1 (CH(2.5)); 54.1, 56.3 (CH(2.1), CH(2.4)); 65.4, 69.6, 71.9, 73.6, 74.3, 76.5 (C(2'), C(3'), C(4'), C(5'), PhCH₂OCOO–C₆H₄–C(3.1), PhCH₂OCO–NH(2.1), PhCH₂); 102.3 (C(1')); 120.8 (2 CH, PhCH₂OCOO–C₆H₄–C(3.1)); 127.7–138.1 (27 arom. C, PhCH₂OCOO–C₆H₄–C(3.1), PhCH₂OCO–NH(2.1), Ph–C(3.4), PhCH₂); 149.3, 153.1, 155.9 (PhCH₂OCOO–C₆H₄–C(3.1),

$\text{PhCH}_2\text{OCOO}-\text{C}_6\text{H}_4-\text{C}(3.1)$, $\text{PhCH}_2\text{OCO}-\text{NH}(2.1)$; 168.6, 169.0, 170.7, 171.8, 172.1 (C(1.1), C(1.2), C(1.3), C(1.4), C(1.5)).

2-{N,O-Bis[(benzyloxy)carbonyl]-L-tyrosylglycylglycyl-L-phenylalanyl-L-leucylamino}-2-deoxy-D-glucopyranose (19). M.p. 200–202°. $[\alpha]_D^{25} = +31.2$ ($c = 1.25$, DMF). $^{13}\text{C-NMR}$ (90 MHz, $(\text{D}_6)\text{DMSO}$): 21.8, 23.2 ($\text{CH}_3(5.5)$, $\text{CH}_3(5'.5)$); 24.2 ($\text{CH}(4.5)$); 51.5 ($\text{CH}(2.5)$); 54.2, 54.6 ($\text{CH}(2.1)$, $\text{CH}(2.4)$); 61.4, 65.5, 69.9, 71.3, 71.5, 72.3 (C(2'), C(3'), C(4'), C(5'), C(6'), $\text{PhCH}_2\text{OCOO}-\text{C}_6\text{H}_4-\text{C}(3.1)$, $\text{PhCH}_2\text{OCO}-\text{NH}(2.1)$); 90.8 (C(1'), α -D); 95.5 (C(1'), β -D); 120.8 (2 CH, $\text{PhCH}_2\text{OCOO}-\text{C}_6\text{H}_4-\text{C}(3.1)$); 123.1–137.9 (21 arom. C, $\text{PhCH}_2\text{OCOO}-\text{C}_6\text{H}_4-\text{C}(3.1)$, $\text{PhCH}_2\text{OCO}-\text{NH}(2.1)$, $\text{Ph}-\text{C}(3.4)$); 149.4, 153.1, 155.9 ($\text{PhCH}_2\text{OCOO}-\text{C}_6\text{H}_4-\text{C}(3.1)$, $\text{PhCH}_2\text{OCOO}-\text{C}_6\text{H}_4-\text{C}(3.1)$, $\text{PhCH}_2\text{OCO}-\text{NH}(2.1)$); 168.7, 169.1, 170.7, 171.9, 172.2 (C(1.1), C(1.2), C(1.3), C(1.4), C(1.5)).

N-{N,O-Bis[(benzyloxy)carbonyl]-L-tyrosylglycylglycyl-L-phenylalanyl-L-leucyl}- β -D-glucopyranosylamine (20). M.p. 178–179°. $[\alpha]_D^{25} = -16.5$ ($c = 1.0$, DMF). $^{13}\text{C-NMR}$ (90 MHz, $(\text{D}_6)\text{DMSO}$): 21.4, 23.3 ($\text{CH}_3(5.5)$, $\text{CH}_3(5'.5)$); 24.1 ($\text{CH}(4.5)$); 51.0 ($\text{CH}(2.5)$); 54.3, 56.1 ($\text{CH}(2.1)$, $\text{CH}(2.4)$); 61.0 (C(6')); 65.4, 69.8, 70.1, 72.4, 77.5, 78.7 (C(2'), C(3'), C(4'), C(5'), $\text{PhCH}_2\text{OCOO}-\text{C}_6\text{H}_4-\text{C}(3.1)$, $\text{PhCH}_2\text{OCO}-\text{NH}(2.1)$); 79.8 (C(1')); 120.8 (2 CH, $\text{PhCH}_2\text{OCOO}-\text{C}_6\text{H}_4-\text{C}(3.1)$); 126.3–137.7 (21 arom. C, $\text{PhCH}_2\text{OCOO}-\text{C}_6\text{H}_4-\text{C}(3.1)$, $\text{PhCH}_2\text{OCO}-\text{NH}(2.1)$, $\text{Ph}-\text{C}(3.4)$); 149.3, 153.1, 156.0 ($\text{PhCH}_2\text{OCOO}-\text{C}_6\text{H}_4-\text{C}(3.1)$, $\text{PhCH}_2\text{OCOO}-\text{C}_6\text{H}_4-\text{C}(3.1)$, $\text{PhCH}_2\text{OCO}-\text{NH}(2.1)$); 169.6, 169.8, 170.8, 171.8, 172.4 (C(1.1), C(1.2), C(1.3), C(1.4), C(1.5)).

6-Deoxy-6-(L-tyrosylglycylglycyl-L-phenylalanyl-L-leucylamino)-D-glucopyranose (6). $[\alpha]_D^{25} = +19$ ($c = 2.0$, H_2O). Amino-acid ratios in acid hydrolysate: Tyr 0.97, Gly 1.91, Phe 1.00, Leu 0.92. $^{13}\text{C-NMR}$ (90 MHz, D_2O): 21.8, 22.9 ($\text{CH}_3(5.5)$, $\text{CH}_3(5'.5)$); 25.0 ($\text{CH}(4.5)$); 36.8, 37.8 ($\text{CH}_2(3.1)$, $\text{CH}_2(3.4)$); 40.8 ($\text{CH}_2(3.5)$, C(6')); 43.2 ($\text{CH}_2(2.2)$, $\text{CH}_2(2.3)$); 53.3 ($\text{CH}(2.5)$); 55.4, 55.8 ($\text{CH}(2.1)$, $\text{CH}(2.4)$); 70.5, 71.9, 72.4, 73.4, 74.8, 76.3 (C(2'), C(3'), C(4'), C(5')); 92.9 (C(1'), α -D); 96.8 (C(1'), β -D); 116.7 (2 CH, $\text{C}_6\text{H}_4-\text{C}(3.1)$); 126.3–136.9 (9 arom. C, $\text{C}_6\text{H}_4-\text{C}(3.1)$, $\text{Ph}-\text{C}(3.4)$); 156.0 (1 C, $\text{C}_6\text{H}_4-\text{C}(3.1)$); 170.7, 171.7, 171.9, 173.6, 174.9 (C(1.1), C(1.2), C(1.3), C(1.4), C(1.5)).

2-Deoxy-2-(L-tyrosylglycylglycyl-L-phenylalanyl-L-leucylamino)-D-glucopyranose (7). $[\alpha]_D^{23} = +32.8$ ($c = 0.5$, H_2O). Amino-acid ratios in acid hydrolysate: Tyr 1.00, Gly 1.99, Phe 1.17, Leu 1.22. $^{13}\text{C-NMR}$ (90 MHz, D_2O): 21.7, 23.0 ($\text{CH}_3(5.5)$, $\text{CH}_3(5'.5)$); 24.9 ($\text{CH}(4.5)$); 36.8, 37.7 ($\text{CH}_2(3.1)$, $\text{CH}_2(3.4)$); 40.7 ($\text{CH}_2(3.5)$); 43.2 ($\text{CH}_2(2.2)$, $\text{CH}_2(2.3)$); 53.3 ($\text{CH}(2.5)$); 54.9 (C(2'), α -D); 55.4, 55.8 ($\text{CH}(2.1)$, $\text{CH}(2.4)$); 57.7 (C(2'), β -D); 61.5 (C(6')); 70.9, 71.1 (C(3'), C(4'), α -D); 71.4 (C(4'), β -D); 72.5 (C(5'), α -D); 75.0 (C(3'), β -D); 76.8 (C(5'), β -D); 91.7 (C(1'), α -D); 95.6 (C(1'), β -D); 116.7 (2 CH, $\text{C}_6\text{H}_4-\text{C}(3.1)$); 126.2–136.9 (9 arom. C, $\text{C}_6\text{H}_4-\text{C}(3.1)$, $\text{Ph}-\text{C}(3.4)$); 156.0 (1 C, $\text{C}_6\text{H}_4-\text{C}(3.1)$); 170.7, 171.8, 171.9, 173.5, 175.0 (C(1.1), C(1.2), C(1.3), C(1.4), C(1.5)).

N-(L-Tyrosylglycylglycyl-L-phenylalanyl-L-leucyl)- β -D-glucopyranosylamine (8). $[\alpha]_D^{23} = +10.8$ ($c = 1.5$, H_2O). Amino-acid ratios in acid hydrolysate: Tyr 1.00, Gly 1.94, Phe 1.19, Leu 1.07. $^{13}\text{C-NMR}$ (90 MHz, D_2O): 21.4, 23.2 ($\text{CH}_3(5.5)$, $\text{CH}_3(5'.5)$); 25.0 ($\text{CH}(4.5)$); 36.8, 37.7 ($\text{CH}_2(3.1)$, $\text{CH}_2(3.4)$); 40.5 ($\text{CH}_2(3.5)$); 43.0, 43.1 ($\text{CH}_2(2.2)$, $\text{CH}_2(2.3)$); 53.2 ($\text{CH}(2.5)$); 55.3, 55.8 ($\text{CH}(2.1)$, $\text{CH}(2.4)$); 61.4 (C(6')); 70.1, 72.5, 77.2, 78.4 (C(2'), C(3'), C(4'), C(5')); 80.3 (C(1')); 116.7 (2 CH, $\text{C}_6\text{H}_4-\text{C}(3.1)$); 126.6–136.9 (9 arom. C, $\text{C}_6\text{H}_4-\text{C}(3.1)$, $\text{Ph}-\text{C}(3.4)$); 156.0 (1 C, $\text{C}_6\text{H}_4-\text{C}(3.1)$); 170.6, 171.8, 171.9, 173.7, 176.0 (C(1.1), C(1.2), C(1.3), C(1.4), C(1.5)).

REFERENCES

- [1] J. Hughes, T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan, H. R. Morris, *Nature (London)* **1975**, 258, 577.
- [2] 'Opioid Peptides: Molecular Pharmacology, Biosynthesis and Analysis', NIDA Research Monographs, Eds. R. S. Rapaka, G. Barnett, and R. L. Hawks, US Government Print Office, Washington, DC, 1986, Vol. 70.
- [3] G. B. Stefano, *Prog. Neurobiol. (Oxford)* **1989**, 33, 149.
- [4] N. P. Plotnikoff, J. Wybran, N. F. Nimeh, G. C. Miller, *Psychopharmacol. Bull.* **1986**, 22, 695.
- [5] K. M. Zurich, C. H. Kirkpatrick, *J. Clin. Immunol.* **1988**, 89, 95.
- [6] L. Varga, Š. Horvat, C. Lemieux, P. W. Schiller, *Int. J. Pept. Protein Res.* **1987**, 30, 371.
- [7] J. Horvat, Š. Horvat, C. Lemieux, P. W. Schiller, *Int. J. Pept. Protein Res.* **1988**, 31, 499.
- [8] Š. Horvat, J. Horvat, D. Kantoci, L. Varga, *Tetrahedron* **1989**, 45, 4579.
- [9] H. Kunz, B. Dombo, *Angew. Chem. Int. Ed.* **1988**, 27, 711.
- [10] L. Biesert, H. Suhartono, I. Winkler, C. Meichsner, M. Helsberg, G. Hewlett, V. Klimetzek, K. Mölling, E. Schrunner, H. D. Brede, H. Rübsamen-Waigmann, *AIDS* **1988**, 2, 449.