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GLYCOCONJUGATES OF OPIOID PEPTIDES - III+. A NOVEL REGIOSELECTIVE SYNTHESIS OF 6-*O*-PEPTIDYL-D-GLYCOPYRANOSES USING UNPROTECTED SUGARS

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Abstract – The primary hydroxyl groups of D-glucose and 2-acetamido-2-deoxy-D-glucose were selectively esterified by treating the free sugars with activated esters of peptides in the presence of imidazole, thus affording exclusively 6-*O*-peptidyl-D-glucopyranoses.

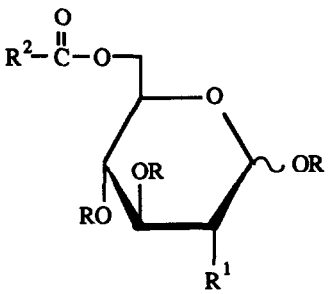
Enkephalins (Tyr-Gly-Gly-Phe-Leu/Met) are endogenous opioid peptides found to mediate a remarkable number of physiological functions[1]. In an effort to reduce the conformational flexibility of these peptides and thus to develop opioid receptor ligands with high selectivity, we recently reported synthesis and biological activity of several carbohydrate-enkephalin conjugates[2-4]. In a previous paper[4], we described the synthesis of enkephalin derivatives in which D-glucose has been linked to the opioid pentapeptide through the ester bond involving the carboxyl function at the *C*-terminal of the peptide and C-1 or C-6 hydroxyl group of the D-glucopyranose moiety. The synthesis of these compounds was carried out in a stepwise manner involving the coupling of suitably protected sugar derivatives to the corresponding peptides, followed by the removal of protecting groups. This method has certain disadvantages, *i.e.*, the necessity of introducing and subsequent removing of the protecting groups at the sugar moiety. As an atractive alternative, we decided to carry out the synthesis of the above carbohydrate esters by using the unprotected sugar molecule. We now report on a simple, straightforward procedure that allows the preparation of glycoconjugates in large quantities.

The strategy devised for the synthesis of the target compounds involved at first the construction of a glycosylated dipeptide. Attempts to prepare 6-*O*-(Boc-Phe-Leu)-D-glucopyranose (**1**) under the conditions used by Kochetkov and co-workers[5], who prepared 6-*O*-aminoacyl derivatives of neutral monosaccharides by condensing the unprotected carbohydrate with *N*-benzyloxycarbonyl amino acid in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC), failed. By reacting D-glucose and *N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-leucine (Boc-Phe-Leu-OH) in the presence of DCC we obtained the 6-*O*-dipeptidyl ester **1** after tedious purification in a very low yield (~15%). Therefore, an alternative synthesis, based on the imidazole-promoted reaction[6] of the unprotected sugar with the activated ester of *N*-acylamino acid, was designed. Thus, treatment of a solution of D-glucose (3 equiv.) in dry pyridine with 1 equiv. of pentachlorophenyl ester of *N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-leucine (Boc-Phe-Leu-OPCP) in the presence of imidazole (5 equiv.) afforded, exclusively, 6-*O*-acyl derivative **1** in 55% yield. The structure of **1**

+For parts I and II see Refs. 3 and 4.

was established from analytical and NMR data. In the ^{13}C NMR spectrum of **1**, the introduction of the dipeptidyl group at the C–6 hydroxyl group of D–glucopyranose moiety generated a downfield shift of this carbon atom (δ 61.9→65.1) typical for *O*–acylation effects in carbohydrates[7]. To test the applicability of this method, Boc–Phe–Met–OPCP was coupled under the same reaction conditions with D–glucose and 2–acetamido–2–deoxy–D–glucose to give the corresponding 6–*O*–acyl derivatives **2** and **3** in fairly good yields of 40% and 38%, respectively.

The 6–*O*–(Boc–Phe–Leu)–D–glucopyranose (**1**) was selectively deprotected at the *N*–terminal position with trifluoroacetic acid and then coupled with Boc–Tyr(Boc)–Gly–Gly–OPCP to give the glucosylated pentapeptide **4** in 68% yield. Removal of the protecting groups at the *N*–terminal tyrosyl residue was effected by trifluoroacetic acid and resulted in the target substance **5** which was chromatographically and analytically indistinguishable from 6–*O*–(Tyr–Gly–Gly–Phe–Leu)–D—glucopyranose prepared by the alternative synthetic route[4].



	R	R^1	R^2–CO–
1	H	OH	Boc–Phe–Leu–
2	H	OH	Boc–Phe–Met–
3	H	NHAc	Boc–Phe–Met–
4	H	OH	Boc–Tyr(Boc)–Gly–Gly–Phe–Leu–
5	H	OH	TFAxH–Tyr–Gly–Gly–Phe–Leu–
6	Ac	OAc	Boc–Tyr(Boc)–Gly–Gly–Phe–Leu–
7	Ac	OAc	TFAxH–Tyr–Gly–Gly–Phe–Leu–

Recent studies on the biological activity of carbohydrate–enkephalin conjugates[4] have shown that 1,2,3,4–tetra–*O*–acetyl–6–*O*–(Tyr–Gly–Gly–Phe–Leu)–β–D–glucopyranose (**7β**) is about three times more δ–receptor selective than [Leu^5]enkephalin. In the search of an alternative synthetic route to the compound **7β**, the partially protected glucosylated pentapeptide **4** was acetylated with acetic anhydride – pyridine either for 1 h at 0° or 24 h at room temperature. In both cases the corresponding peracetylated product **6** was obtained as a mixture of α– and β–anomers in the ratios 1:1 and 2.5:1, respectively. Finally, the peracetylated ester **6** was selectively deprotected at the *N*–terminal amino acid residue to yield the anomeric mixture (α:β=2:1) of the acetylated glucopeptide **7**.

In conclusion, the method described in this paper allows regioselective esterification of the primary hydroxyl groups in unprotected sugars, thus leading to monosaccharide–enkephalin conjugates of potential pharmacological interest.

EXPERIMENTAL

General – Optical rotations were measured with a Carl–Zeiss polarimeter. Capillary melting points were determined on a Tottoli (Büchi) apparatus and are reported uncorrected. N.m.r. spectra were obtained on a Jeol FX 90 Q FT spectrometer. Amino acid analyses were performed with a Biotronik LC 2000 automated analyser. Samples were hydrolysed for 24 h at 110° in sealed tubes with constant boiling HCl. Thin layer chromatography (t.l.c.) was performed on silica gel plates (Merck F_{254}). Column chromatography was performed on Merck 60, 70–230 mesh, silica gel or 230–400 mesh in the case of flash chromatography. Solvent system used: *a*, chloroform; *b*, chloroform–isopropyl alcohol (6:1); *c*, ethyl acetate; *d*, ethyl acetate–acetic acid (20:1); *e*, chloroform–ethyl alcohol–ethyl acetate–acetic acid–water (85:5:8:1:0.25).

***N*–*tert*–Butyloxycarbonyl–L–phenylalanyl–L–leucine pentachlorophenyl ester** was prepared from Boc–Phe–Leu–OH[8] by using the crystalline DCC–pentachlorophenol complex[9]; m.p. 138–140° (dichloromethane–diisopropyl ether), $[\alpha]_D^{22}$ –24° (c 1.57, *N,N*–dimethyl– formamide).

***N*–*tert*–Butyloxycarbonyl–L–phenylalanyl–L–methionine pentachlorophenyl ester** was prepared from Boc–Phe–Met–OH[8] as described above; m.p. 160–161° (ethyl acetate– diiso– propyl ether), $[\alpha]_D^{22}$ –19° (c 1.7, *N,N*–dimethylformamide).

***N*–*tert*–Butyloxycarbonyl–*O*–*tert*–butyloxycarbonyl–L–tyrosyl–glycylglycine.** To an ice–cold solution of Boc–Tyr(Boc)–OH[10] (12.5 g, 32.8 mmol) and DCC (6.75 g, 32.8 mmol) in *N,N*–dimethylformamide (DMF, 100 mL) *N*–hydroxysuccinimide (3.77 g, 32.8 mmol) was added. The reaction mixture was stirred for 1 h at 0° and 2 h at room temperature. The precipitated DCHU was filtered off and the filtrate added dropwise to the stirred slurry of glycyl– glycine (4.33 g, 32.8 mmol), 1M NaOH (33 mL) and $NaHCO_3$ (5.46 g, 65 mmol). The mixture was stirred for 1 h at room temperature and filtered. The ice–cold filtrate was acidified to pH 3.5 with 10% citric acid solution and extracted with chloroform (3x 100 mL). Combined extracts were washed with water (100 mL), dried (15 min. on sodium sulphate) and evaporated. The residue was crystallized from etanol–water to give product (11.2 g) as a white solid which was purified by stirring overnight with benzene (160 mL). Filtration afforded chromatographically homogeneous Boc–Tyr(Boc)–Gly–Gly–OH (8.6 g, 53%); m.p. 98–101°, $[\alpha]_D^{22}$ +6° (c 1.4, chloroform).

Anal. Calc. for $C_{23}H_{33}N_3O_9$: C, 55.74; H, 6.71; N, 8.48. Found: C, 55.79; H, 6.90; N, 8.41.

***N*–*tert*–Butyloxycarbonyl–*O*–*tert*–butyloxycarbonyl–L–tyrosyl–glycylglycine pentachlorophenyl ester** was synthesized from equimolar amounts of Boc–Tyr(Boc)–Gly–Gly–OH and DCC–pentachlorophenol complex in dichloromethane. After two recrystallizations from tetrahydrofuran–diisopropyl ether, the product (69% yield) had m.p. 184–185°, $[\alpha]_D^{22}$ +3° (c 2, tetrahydrofuran).

Anal. Calc. for $C_{29}H_{32}Cl_5N_3O_9$: C, 46.83; H, 4.33; N, 5.65. Found: C, 46.99; H, 4.16; N, 5.67.

6-*O*-(*N*-*tert*-Butyloxycarbonyl-L-phenylalanyl-L-leucyl)-D-glucopyranose (1). To a solution of D-glucose (1.62 g, 9 mmol) in dry pyridine (30 mL), Boc-Phe-Leu-OPCP (1.88 g, 3 mmol) and imidazole (1.02 g, 15 mmol) were added. After stirring overnight at room temperature, the solvent was evaporated and the residue purified by flash chromatography eluting succesively with solvents *a* and *b* affording product as a colorless syrup (1.16 g). Crystallization from dichloromethane-diisopropyl ether yielded pure **1** (0.9 g, 55%); m.p. 171–172°, $[\alpha]_D^{21}$ +28° (c 1.2, DMF); ^{13}C n.m.r. (DMF-*d*): δ 173.3 (Phe,Leu, CO), 156.3 (Boc, CO), 138.5 (Phe, Cγ), 129.9 (Phe, Cδ), 128.7 (Phe, Cε), 126.9 (Phe, Cζ), 97.8 (βGlc, C-1), 93.3 (αGlc, C-1), 65.1 (Glc, C-6), 56.4 (Phe, Cα), 51.4 (Leu Cα), 40.7 (Leu, Cβ), 38.5 (Phe, Cβ), 28.3 (Boc, CH_3), 25.0 (Leu, Cγ), 22.9, 21.7 (Leu, Cδ).

Anal. Calc. for $C_{26}H_{40}N_2O_{10}$: C, 57.76; H, 7.46; N, 5.18. Found: C, 57.68; H, 7.60; N, 5.32.

6-*O*-(*N*-*tert*-Butyloxycarbonyl-L-phenylalanyl-L-methionyl)-D-glucopyranose (2) Imidazole (340 mg, 5 mmol) and Boc-Phe-Met-OPCP (644 mg, 1 mmol) were added to a solution of D-glucose (540 mg, 3 mmol) in dry pyridine (20 mL) and the reaction mixture was stirred overnight at room temperature. The solvent was evaporated and the residue partitioned between ethyl acetate and ice-cold 10% citric acid solution. The organic layer was washed with water, dried, and evaporated. The residue was purified by column chromatography by using succesively solvents *c* and *d*. Crystallization from chloroform gave pure **2** (223 mg, 40%); m.p. 149–151°, $[\alpha]_D^{22}$ +48° (c 1, pyridine); ^{13}C n.m.r. (CD_3COOD + $CDCl_3$): δ 172.8, 171.0 (Phe, Met, CO), 155.9 (Boc, CO), 136.1 (Phe, Cγ), 128.7 (Phe, Cδ), 127.8 (Phe, Cε), 126.2 (Phe, Cζ), 95.9 (βGlc, C-1), 91.6 (αGlc, C-1), 63.6 (Glc, C-6), 55.2 (Phe, Cα), 51.0 (Met, Cα), 37.69 (Phe, Cβ), 30.4 (Met, Cβ), 29.6 (Met, Cγ), 27.8 (Boc, CH_3), 15.4 (Met, S-CH_3).

Anal. Calc. for $C_{25}H_{38}N_2O_{10}S$: C, 53.75; H, 6.85; N, 5.01; S, 5.74. Found: C, 53.56; H, 6.99; N, 5.21; S, 5.50.

2-Acetamido-2-deoxy-6-*O*-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-methionyl)-D-glucopyranose (3) Treatment of 2-acetamido-2-deoxy-D-glucose (884 mg, 4 mmol) with Boc-Phe-Met-OPCP (644 mg, 1 mmol) and imidazole (340 mg, 5 mmol) in dry pyridine (50 mL) and processing as described for **2**, gave 230 mg (38%) of **3**, m.p. 134–136° (chloroform-diisopropyl ether), $[\alpha]_D^{22}$ +25° (c 1.9, methanol); ^{13}C n.m.r. (CD_3SOCD_3): δ 172.1, 171.7 (Phe, Met, CO), 169.5 (NAc, CO), 155.3 (Boc, CO), 138.2 (Phe, Cγ), 129.3 (Phe, Cδ), 128.1 (Phe, Cε), 126.3 (Phe, Cζ), 90.8 (αGlcNAc, C-1), 78.1 (Boc, C-*quat.*), 71.1, 70.4, 69.3 (αGlcNAc, C-3,4,5), 64.6 (αGlcNAc, C-6), 55.6 (Phe, Cα), 54.2 (αGlcNAc, C-2), 50.9 (Met, Cα), 37.3 (Phe, Cβ), 30.9 (Met, Cβ), 29.5 (Met, Cγ), 28.2 (Boc, CH_3), 22.8 (NAc, CH_3), 14.7 (Met, S-CH_3).

Anal. Calc. for $C_{27}H_{41}N_3O_{10}S$: C, 54.08; H, 6.89; N, 7.01; S, 5.35. Found: C, 53.97; H 6.82, N, 7.10; S, 5.27.

6-*O*-(*N*-*tert*-Butyloxycarbonyl-*O*-*tert*-butyloxycarbonyl-L-tyrosyl-glycylglycyl-L-phenylalanyl-L-leucyl)-D-glucopyranose (4) Compound **1** (1080 mg, 2 mmol) was dissolved in 90% trifluoroacetic acid solution (10 mL) containing anisole (0.25 mL) and stirred at room temperature for 30 min. Addition of dry ether to the reaction mixture at 0° precipitated 6-*O*-(Phe-Leu)-D-glucopyranose trifluoroacetate salt which was collected by centrifugation and recrystallized from tetrahydrofuran-ether. To the obtained solid (984 mg, 1.77 mmol) dissolved in dioxane (20 mL), *N*-ethylmorpholine (0.22 mL) and Boc-Tyr(Boc)-Gly-Gly-OPCP (1320 mg, 1.77 mmol) were added. The reaction mixture was stirred overnight

at room temperature, the solvent evaporated and the crude product purified by flash chromatography using solvents *e* and *e*–isopropyl alcohol (4:1), succesively. The oily product (1114 mg, 68%) was crystallized from chloroform–diisopropyl ether to give pure **4**, m.p. 114–130°, $[\alpha]_D^{20}$ +6° (c 2, DMF); ^{13}C n.m.r. (CD_3OD + $CDCl_3$): δ 174.6, 173.4, 173.0, 171.8, 171.0 (Tyr, Gly_2, Gly_3, Phe, Leu, CO), 157.5 (Tyr, Cζ), 153.1, 151.0 (N,O–Boc, CO), 138.0 (Phe, Cγ), 135.8 (Tyr, Cγ), 131.1, 130.0, 129.2, 127.5 (Tyr, Phe, C–arom.), 122.0 (Tyr, Cε), 97.8 (βGlc, C–1), 93.6 (αGlc, C–1), 84.1, 80.8 (N,O–Boc, C–*quat.*), 65.4 (Glc, C–6), 57.3, 55.6 (Tyr, Phe, Cα), 52.0 (Leu, Cα), 43.6, 43.3 (Gly_2, Gly_3, CH_2), 41.1 (Leu, Cβ), 38.4, 37.9 (Tyr, Phe, Cβ), 28.6, 27.9 (N,O–Boc, CH_3), 25.5 (Leu, Cγ), 23.2, 21.8 (Leu, Cδ).

Anal. Calc. for $C_{44}H_{63}N_5O_{16}$: C, 57.57; H, 6.92; N, 7.63. Found: C, 57.39; H, 6.75; N, 7.35.

6–*O*–(L–Tyrosylglycylglycyl–L–phenylalanyl–L–leucyl)–D–glucopyranose trifluoroacetate salt (5) The protected ester **4** (100 mg, 0.11 mmol) was treated with 90% trifluoroacetic acid (5 mL) in the presence of anisole (0.2 mL) and stirred at room temperature for 30 min. After addition of diisopropyl ether at 0°, the precipitate was centrifuged off, and recrystallized from etanol—diisopropyl ether to give pure **5**, 83 mg (91%), m.p. 127–130°, $[\alpha]_D^{20}$ +27° (c 1.2, water); ^{13}C n.m.r. (D_2O): δ 174.1, 173.4, 171.7, 171.3, 170.6 (Tyr, Gly_2, Gly_3, Phe, Leu, CO), 156.2 (Tyr, Cζ), 137.0 (Tyr, Phe, Cγ), 131.6, 130.0, 129.5, 127.9, 126.1 (Tyr, Phe, C–arom.), 116.7 (Tyr, Cε), 98.9 (βGlc, C–1), 93.0 (αGlc, C–1), 65.0 (Glc, C–6), 55.3 (Tyr, Phe, Cα), 52.1 (Leu, Cα), 43.1 (Gly_2, Gly_3, CH_2), 40.2 (Leu, Cβ), 38.0, 36.8 (Tyr, Phe, Cβ), 25.1 (Leu, Cγ), 22.8, 21.8 (Leu, Cδ).

Amino acid ratios in acid hydrolysate: Gly, 1.99; Leu, 1.01; Phe, 1.02; Tyr, 0.94.

Anal. Calc. for $C_{36}H_{48}F_3N_5O_{14}$: C, 51.98; H, 5.82; N, 8.42. Found: C, 51.88; H, 6.06; N, 8.53.

1,2,3,4–Tetra–*O*–acetyl–6–*O*–(*N*–*tert*–butyloxycarbonyl–*O*–*tert*–butyloxy–carbonyl—L–tyrosylglycylglycyl–L–phenylalanyl–L–leucyl)–D–glucopyranose (6) A sample of **4** (246 mg, 0.27 mmol) was acetylated with pyridine–acetic anhydride (1:1, 20 mL) for 24 h at room temperature. The residue obtained upon evaporation was purified by flash chromatography (solvent *e*) to give **6** (255 mg, 87%) as a mixture of diastereoisomers (α:β=2.5:1). Crystallization from chloroform–petroleum ether afforded **6** as a solid (α:β=1.7:1), m.p. 147–148°, $[\alpha]_D^{27}$ +12° (c 1, chloroform). ^{1}H n.m.r. ($CDCl_3$): δ 6.29 (d, $J_{1,2}$ 3.5 Hz, H–1), 5.71 (d, $J_{1,2}$ 7.7 Hz, H–1).

Anal. Calc. for $C_{52}H_{71}N_5O_{20}$: C, 57.50; H, 6.59; N, 6.45. Found: C, 57.47; H, 6.83; N, 6.32.

Acetylation of **4** performed at 0° for 1 h afforded **6** (70%) with α:β anomeric ratio= 1:1.

1,2,3,4–Tetra–*O*–acetyl–6–*O*–(L–tyrosylglycylglycyl–L–phenylalanyl–L–leucyl)–D–glucopyranose trifluoroacetate salt (7) Compound **6** (217 mg, 0.2 mmol) was treated with 40% trifluoroacetic acid in dichloromethane (5 mL) for 20 min. at room temperature. The solvent was evaporated, the residue triturated with ether and dried to give pure **7** as a mixture of anomers (α:β=2:1), 160 mg (80%), m.p. (sintering 70°) 122–130°, $[\alpha]_D^{25}$ +31° (c 1.4, chloroform); ^{1}H n.m.r. (D_2O): δ 6.21 (d, $J_{1,2}$ 3.4 Hz, H–1), 5.81 (d, $J_{1,2}$ 8.0 Hz, H–1).

Anal. Calc. for $C_{44}H_{56}F_3N_5O_{18}$: C, 52.85; H, 5.64; N, 7.00. Found: C, 52.60; H, 5.57; N, 6.97.

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