Carbohydrate Research, 89 (1981) 229–236
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

SYNTHESIS OF A GLYCOTRIPEPTIDE AND A GLYCOSOMATOSTATIN CONTAINING THE 3-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-L-SERINE RESIDUE*

SOLANGE LAVIELLE†§, NICOLAS C. LING§, ROBERT SALTMAN‡, AND ROGER C. GUILLEMIN§
Salk Institute for Biological Studies, Laboratories for Neuroendocrinology, La Jolla, California 92037, and Department of Chemistry, University of California, San Diego, La Jolla, California 92093 (U.S.A.)
(Received February 12th, 1980; accepted for publication in revised form, July 1st, 1980)

ABSTRACT

3-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(*tert*-butyloxycarbonyl)-L-serine was synthesized and condensed by the solid-phase procedure to give the sequence Gly-[β-D-Glc*p*NAc-(1→3)-Ser]-Ala-OH and β-D-Glc*p*NAc-(1→3)-Ser-13-somatostatin. The synthetic glycopeptides appeared homogeneous on t.l.c. and l.c. examination and showed the correct amino acid composition and 2-amino-2-deoxy-D-glucose content. The structure of Gly-[β-D-Glc*p*NAc-(1→3)-Ser]-Ala-OH was further confirmed by mass spectrometry of the *N*-acetyl permethyl derivative, and by n.m.r. spectroscopy.

INTRODUCTION

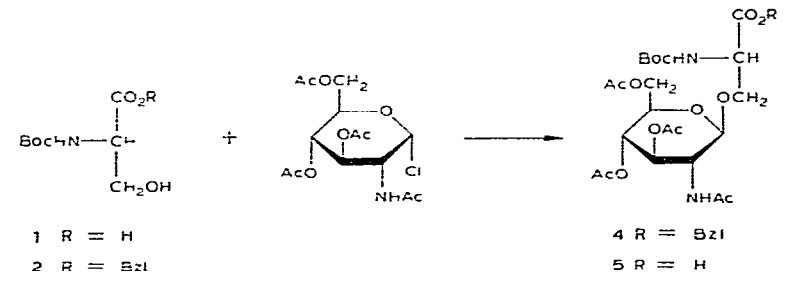
In naturally occurring glycoproteins, the carbohydrate residue has been found attached mainly to asparagine, serine, or threonine residues. In a previous paper[1] we described the synthesis of two glycopeptides, β-D-Glc*p*-(1→4)-Asn-Phe-Phe-Trp-Lys-OH and β-D-Glc*p*NAc-(1→4)-Asn-Phe-Phe-Trp-Lys-OH, and recently[2] reported the synthesis and biological activity of two glycosomatostatins (CHO-Asn-5)-SS, containing a β-D-glucopyranosyl or a 2-acetamido-2-deoxy-β-D-glucopyranosyl moiety coupled to the asparagine residue.

---

*This research was supported by grants from the National Institute of Arthritis, Metabolism, and Digestive Diseases (AM-18811-05) and the National Heart Institute (HD-09690-05), National Institutes of Health, and from the William Randolph Hearst Foundation. The n.m.r. spectra (220 MHz) were obtained at the UCSD n.m.r.–m.s. research source facility supported by a grant (RR-00 708) from the National Institutes of Health.
†Post-doctoral Fellow, 1977–1979, from the Délégation Générale à la Recherche Scientifique et Technique (D.G.R.S.T., France). To whom all correspondence should be addressed; present address: Laboratoire de Chimie Organique Biologique, Université P. et M. Curie (Tour 44-45), F-75230 Paris (France).
§Salk Institute for Biological Studies.
‡University of California.

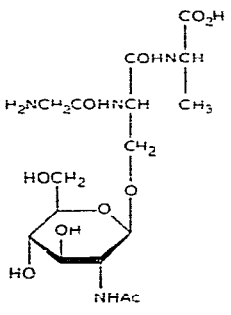
0008-6215/81/0000–0000/$ 02.50,

The remaining potential carbohydrate attachment positions in somatostatin[3], Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH are the two threonine (Thr-10, Thr-12) and the serine (Ser-13) residues. For the L-seryl–glycosylamine linkage, so far only the 2-acetamido-2-deoxy-D-galactopyranosyl residue has been found[4]. As a first attempt to synthesize by solid-phase procedure *O*-glycosylated peptides, we report herein the synthesis of a model tripeptide, Gly-[*β*-D-Glc*p*NAc-(1→3)-Ser]-Ala-OH (**6**) and a glycosomatostatin, *β*-D-GlcNAc*p*-(1→3)-Ser-13-somatostatin (**7**), containing a 2-acetamido-2-deoxy-*β*-D-glucopyranosyl residue coupled to the serine residue.

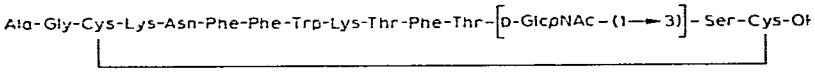


1 R = H
2 R = Bzl

4 R = Bzl
5 R = H



6



7

RESULTS AND DISCUSSION

Synthesis of 3-*O*-*β*-D-glycosyl-L-serine having an *N*-benzyloxycarbonyl protecting group has been reported[5,7]. To introduce a glycosylserine derivative into a peptide by a solid-phase procedure[8], an acid-labile, *N*-protecting group, such as the *N*-*tert*-butyloxycarbonyl group, that will not be cleaved during the hydrogenolysis of the benzyl ester protecting group and yet be readily removed by an anhydrous acid, such as trifluoroacetic acid, was required. As the benzyl ester **2** of *N*-(*tert*-butyloxycarbonyl)-L-serine (**1**) was not commercially available, it was prepared by benzylation of the potassium salt of **1** with benzyl bromide*. The Koenigs–Knorr condensation[9,10]

*The *N*-*tert*-butyloxycarbonyl-L-threonine benzyl ester was obtained by the same method in 72% yield, m.p. 74–76°, $[\alpha]_D^{23}$ −20.1° (*c* 2, methanol).

of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[11] (**3**) in the presence of mercuric cyanide, as described by Garg and Jeanloz[5], gave **4** with an average yield of 25 to 30%. This low yield, as compared with that obtained for the *N*-benzyloxycarbonyl derivative[5], may be explained by the presence of the *tert*-butyloxycarbonyl group, which was partially hydrolyzed by the hydrogen chloride generated[12] during the coupling reaction**. The mass spectrum confirmed the structure of the compound formed, and n.m.r. data at 220 MHz for the solution in dimethyl sulfoxide established the β-D configuration ($J_{1,2}$ 8 Hz). The material obtained was then hydrogenated with 10% palladium-on-charcoal to afford the acid **5**.

Synthesis of the glycopeptides Gly-[β-D-Glc*p*NAc-(1→3)-Ser]-Ala-OH (**6**) and β-D-Glc*p*NAc-(1→3)-Ser-13-somatostatin (**7**) by the solid-phase procedure[8], involved steps that have already been described[1,2,13]. Coupling of *N*-(*tert*-butyloxycarbonyl)-L-alanine and *N*-(*tert*-butyloxycarbonyl)-*S*-(*p*-methoxybenzyl)-L-cysteine to the chloromethylated resin was performed by the Monahan and Gilon procedure[14]. Appropriate (*N*-*tert*-butyloxycarbonyl)amino acids were coupled in the presence of dicyclohexylcarbodiimide (DCC) for 2 h, except for the glycosyl-L-serine **5**, which was coupled by the DCC–1-hydroxybenzotriazole method[15], and L-asparagine as its *p*-nitrophenyl ester overnight. After cleavage of the peptide from the resin by hydrofluoric acid[16,17], glycopeptide **6** was first purified by partition chromatography[16] on Sephadex G-25 fine, and then *O*-deacetylated by a saturated solution of ammonia in methanol, a procedure that minimizes β-elimination[4]. The deprotected glycopeptide was finally purified by partition chromatography[18] to yield a homogeneous product on t.l.c. The amino acid and 2-acetamido-2-deoxy-D-glucose content of **6** were in agreement with the theoretical values, and its structure was confirmed by mass spectroscopy after *N*-acetylation with an equimixture of acetic anhydride and [$^2H_6$]acetic anhydride, and permethylation[19] (Fig. 1). The β-D configuration was further ascertained by 220-MHz, n.m.r. spectroscopy on the solution in D_2O ($J_{1,2}$ 8 Hz).

The glycosylated analog of somatostatin β-D-Glc*p*NAc-(1→3)-Ser-13-somatostatin (**7**) was synthesized by the same procedure. After cleavage of the peptide from the resin with hydrofluoric acid, the peptide solution was diluted with water and oxidized by the ferricyanide method, as described by Rivier *et al.*[20]. After successive purification steps, the sugar moiety was *O*-deacetylated, and the glycopeptide finally purified by partition chromatography. The amino acid and 2-amino-2-deoxy-D-glucose composition of **7** was in agreement with the theoretical values. The glycosomatostatin were found to be homogeneous in t.l.c. and ~97% pure in l.c.; no somatostatin was detected in the product by l.c. analysis.

**On t.l.c. of the crude product, at least four spots could be detected by u.v. light: Two of these spots were detected by the ninhydrin reagent, whereas the remaining two were detected by the ninhydrin reagent after previous spraying with trifluoroacetic acid.

EXPERIMENTAL

General methods. — Amino acid analyses and determination of the 2-acetamido-2-deoxy-D-glucose content were performed after hydrolysis with 4M methanesulfonic acid for 20 h at 110°, in evacuated sealed tubes. Ascending t.l.c. on silica gel was performed on precoated silica gel 60 plates (Merck, 0.25-mm thick). The solvent systems were: (*A*) 1:4 ethyl acetate–chloroform; (*B*) 4:1:5 (upper phase) 1-butanol–acetic acid–water; (*C*) 5:3:11 (upper phase) 1-butanol–pyridine–0.1% acetic acid; (*D*) 1:1 (upper phase) 1-butanol–0.1M acetic acid; and (*E*) 6:4:1:9 1-butanol–pyridine–acetic acid–water (all v/v). High-pressure liquid chromatography (l.c.) was performed with a Waters Associates Model 204, liquid-chromatography system equipped with two M-6000 A pumps, a 660 solvent programmer, a Schoeffel variable u.v. detector, a Spectra Physics "Minigrator", and a Linear instrument 456 recorder; separation was accomplished in a 25 × 0.45-cm column filled with a Zorbax ODS, in isocratic mode with the indicated percent composition of acetonitrile in buffer *F* or buffer *G*, at a flow rate of 1.5 mL/min and 0.1 absorbance full-scale at 210 nm. Buffer *F* was 0.25M triethylammonium phosphate, pH 3.00 (refs. 21 and 22), and buffer *G* was 0.01M triethylammonium phosphate, pH 3.00; 10–20 μg of compound were injected. 100-MHz n.m.r. spectra were recorded with a JEOL JNM-PS-100 spectrometer, tetramethylsilane being the internal reference, and 220-Hz n.m.r. spectra obtained in the Fourier transform (F.t.) mode with a HR-220 spectrometer fitted with a Nicolet TT-100 system; spectra were accumulated into 8k points by use of a 45° pulse of 20 μs to cover a spectrum width of ±1250 Hz with quadrature-phase detection. Homonuclear spin-decoupling experiments were carried out in the FT mode with a home-built, double-irradiation apparatus assembled from a 73-MHz crystal oscillator coupled with a 10-MHz oscillator in a Syntest synthesizer, and tripled up to 220-MHz frequencies. The samples (4.0 mg) were dissolved in 0.5 mL of the appropriate solvent. The solvents D_2O (99.98%) and [$^2H_6$]dimethyl sulfoxide (99.5%) were purchased from Diaprep and Merck, respectively. For deuterated water, the chemical shifts are given relative to sodium 3-(trimethylsilyl)-[$^2H_4$]*propionate* (Wilmad Glass Co., Buexa, NJ 08310), and for ($^2H_6$)dimethyl sulfoxide the reference compound was tetramethylsilane.

Mass spectra of the glycopeptide derivatives were recorded with a Varian Mat CH-5 single-focusing mass-spectrometer with the direct-inlet system at an ionization current of 300 μA, an energy of 70 eV, and an acceleration voltage of 3 kV.

N-(tert-*Butyloxycarbonyl*)-L-*serine benzyl ester* (**2**). — To a solution of *N*-(*tert*-butyloxycarbonyl)-L-serine (Vega-Fox, Biochemicals Div., Newbery Energy Corp., Tucson, AZ 85719) (8.12 g, 40 mmol) in dimethyl sulfoxide (42 mL) was added of M ethanolic potassium hydroxide (42 mL, 1.05 equiv.). After 5 min at room temperature, benzyl bromide (9.52 mL, 80 mmol, 2 equiv.) was added, and the mixture was stirred for 15 h at room temperature. After addition of dichloromethane (300 mL), the solution was washed with water (7 ×), dried (magnesium sulfate), and the solvent evaporated. The yellowish syrup (10.75 g) was further purified by chromatography

on a silica gel column (300 g, *A*) to yield **2** (8.02 g, 68%), which crystallized on addition of petroleum ether and was recrystallized from diethyl ether–petroleum ether, m.p. 69–70°, $[\alpha]_D^{23}$ −18.9° (*c* 2, methanol); t.l.c.: R_F 0.68 (*A*); l.c.: (iso., 44% acetonitrile in buffer *G*), 10.4 min, purity 99%; ^{1}H-n.m.r. {100-MHz, [^{2}H]chloroform}: δ 7.28 (s, 5 H, $C_6H_5CH_2$), 5.58 (d, 1 H, $J_{NH\alpha,\alpha}$ 8 Hz), 5.15 (s, 2 H, $C_6H_5CH_2$), 4.38 (m, 1 H, CH_α), 3.89 (m, 2 H, $CH_{2\beta}$), 2.92 (m, 1 H, *OH*), and 1.38 (s, 9 H, *tert*-butyl of Boc).

Anal. Calc. for $C_{15}H_{21}NO_5$: C, 61.02; H, 7.12; N, 4.76. Found: C, 60.84; H, 7.21; N, 4.91.

O-(*2-Acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl*)-N-(tert-*butyloxycarbonyl*)-L-*serine benzyl ester*[6,7] (**4**). — A solution of **2** (2.95 g, 10 mmol) in benzene (125 mL) was dried by azeotropic distillation (25 mL of benzene removed), and then mercuric cyanide (3.04 g, 12 mmol) and benzene (25 mL) were added, and the mixture was further dried by distilling off benzene (25 mL). 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**4**) was added (4.40 g, 12 mmol), and the mixture was stirred for 18 h under reflux, and then kept for 24 h at room temperature. The dark-orange solution was evaporated and the residue extracted with dichloromethane. The extract was washed with 10% potassium iodide and dried (magnesium sulfate). After evaporation, the residual oil (6.07 g) was purified by chromatography on a silica gel column (300 g; solvent *A*) to afford **4** (1.7 g) as a slightly yellow oil, which crystallized on addition of diethyl ether (yield 27%), and was recrystallized from dichloromethane–diethyl ether, m.p. 137–138°, $[\alpha]_D^{23}$ −12.1° (*c* 1, chloroform): t.l.c.: R_F 0.44 (*A*); l.c. (iso., 48% acetonitrile in buffer *G*), 8.7 min, min. purity 99%; ^{1}H-n.m.r. [220 MHz, ($^2H_6$) dimethyl sulfoxide]: δ 7.93 (d, 1 H, *J* 10 Hz, N*H*Ac), 7.35 (s, 5 H, C_6H_5), 6.90 (d, 1 H, $J_{NH_1,H\text{-}1}$ 8 Hz, N*H*Boc), 5.12 (2 H, $J_{A,B}$ 14 Hz, $CH_2C_6H_5$), 5.07 (t, 1 H, *J* 10 Hz, *H*-3 of Glc); 4.84 (t, 1 H, *J* 10 Hz, *H*-4 of Glc), 4.67 (d, 1 H, *J* 8 Hz, *H*-1 of Glc), 4.28 (m, 1 H, *H*-1′ of Ser), 4.18 (1 H, $J_{A,B}$ 12, *J* 4 Hz, *H*-6 of Glc), 4.01 (1 H, $J_{A,B}$ 12, *J* 2 Hz, *H*-6′ of Glc), 3.84 (m, 1 H, *H*-5 of Glc), 3.75 (m, 2 H, H_2-2′ of Ser), 3.71 (m, 1 H, *H*-2 of Glc), 2.00 (s, 3 H, $COCH_3$), 1.97 (s, 3 H, $COCH_3$), 1.91 (s, 3 H, $COCH_3$), 1.76 (s, 3 H, $COCH_3$), and 1.37 (s, 9 H, Boc); m.s.: *m/z* 564 (1.1), 550 (1.7), 523 (1.9), 488 (1.8), 330 (52), 270 (7), 210 (31), 168 (31), 150 (49), and 91 (100).

Anal. Calc. for $C_{29}H_{40}N_2O_{13}$: C, 55.77; H, 6.41; N, 4.49. Found: C, 55.60; H, 6.54; N, 4.64.

O-(2-Acetamido-*3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl*)-N-(tert-*butyloxycarbonyl*)-L-*serine* (**5**). — A solution of **4** in 1:9 (v/v) water–ethanol (250 mL) was hydrogenated in the presence of 10% palladium-on-charcoal (0.250 g) for 5 h at atmospheric pressure and room temperature. After filtration through a Celite column, the filtrate was evaporated at 40° to yield a white, amorphous powder (1.01 g, 90%), m.p. 157–159°, $[\alpha]_D^{23}$ +3° (*c* 1, chloroform); t.l.c.: R_F 0.52 (*B*), 0.56 (*C*), and 0.37 (*D*).

Anal. Calc. for $C_{22}H_{34}N_2O_{13}$: C, 49.44; H, 6.37; N, 5.24. Found: C, 49.26; H, 6.19; N, 5.29.

L-*Glycyl*-[*3*-O-(*2-acetamido-2-deoxy-β*-D-*glucopyranosyl*)-L-*seryl*]-L-*alanine* (**6**).

— Coupling of *N*-(*tert*-butyloxycarbonyl)-L-alanine to a chloromethylated resin (0.9 meq. Cl/g, Lab Systems, Inc., San Mateo, CA 94401) was performed by the Monahan and Gilon procedure[14] to give a compound containing 0.23 mmol of L-alanine/g of resin. Coupling of the other *N*-(*tert*-butyloxycarbonyl)amino acids to 2 g of the *N*-(*tert*-butyloxycarbonyl)-L-alanine–resin was accomplished manually according to the previously described procedure[1], by use of 0.5 mmol of **5** per gram of resin in 1:3 (v/v) *N*,*N*-dimethylformamide–dichloromethane plus 1 equiv. of 2M DCC and 1.5 equiv. of 1-hydroxybenzotriazole for 6 h, and 1 mmol of *N*-(*tert*-butyloxycarbonyl)-L-glycine per gram of resin in dichloromethane plus 1 equiv. of 2M DCC for 2 h. The coupling reaction was monitored by the ninhydrin test of Kaiser *et al.*[23]. The protected-peptide resin (2.23 g) was treated with anisole (3.35 mL) and hydrofluoric acid (22.3 mL) for 0.5 h at −20°, and for 0.3 h at 0°. The processing of the hydrogen fluoride-cleavage reaction was performed as described earlier[1], and lyophilization of the extract gave the crude glycopeptide (146 mg), which was purified by partition chromatography on Sephadex *G*-25 fine with solvent *B* to yield 67 mg. The fully acetylated glycopeptide was *O*-deacetylated with a saturated solution of ammonia in methanol as follows: the glycopeptide was introduced into a round-bottomed flask, sealed with a septum; dry benzene was added with a syringe, and the peptide was dried by vacuum distillation of benzene (2 × 5 mL) through a needle in the septum. Dry methanol (3 mL) and a saturated solution of ammonia in methanol (4.5 mL) were introduced through the septum at 0° and the mixture was kept for 3 h at room temperature. The solvent was evaporated (high vacuum, needle) at room temperature, and lyophilization of the residue in M acetic acid gave 53.5 mg of glycopeptide, which was further purified by partition chromatography (solvent *E*) to yield 35 mg of **6**: ^{1}H-n.m.r. (220 MHz, ^2H_2O): δ 4.59 (t, 1 H, *J* 6.5 Hz, H-3 of GlcNAc), 4.56 (d, 1 H, *J* 8.0 Hz, H-1 of GlcNAc), 4.16 (q, 1 H, *J* 7.5 Hz, CH-Ala),

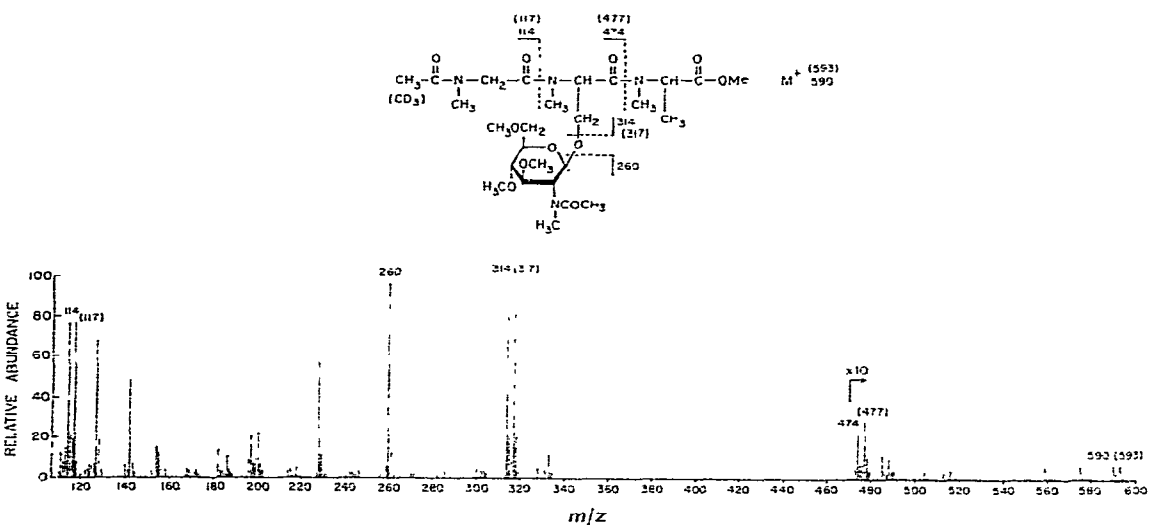


Fig. 1. Mass spectrum of **6** after *N*-acetylation and permethylation.

4.03 (q, 1 H, *J* 8.0, *J* 6.5 Hz, H-2 of GlcNAc), 3.98 (1 H, $J_{A,B}$ 15, *J* 2 Hz, H-6′ of GlcNAc), 3.88 (d, 2 H, *J* 2.5 Hz, CH_2-Gly), 3.70 (1 H, $J_{A,B}$ 15, *J* 5 Hz, H-6 of GlcNAc), 3.66 (q, 1 H, *J* 8.0, *J* 6.5 Hz, H-4 of GlcNAc), 3.56 (m, 1 H, C*H*-Ser), 3.52 (m, 1 H, H-5 of GlcNAc), 3.46 (m, 2 H, CH_2-Ser), 2.05 (s, 3 H, $COCH_3$), and 1.35 (d, 3 H, *J* 7.5 Hz CH_3-Ala); m.s., see Fig. 1.

Anal. Calc.: Ala, 1.0; Gly, 1.0; Ser, 1.0; GlcNAc, 1.0. Found: NH_3, 0.95; Ala, 1.03; Gly, 1.0; GlcNAc, 0.98.

L-*Alanyl*-L-*glycyl*-L-*cysteiyl*-L-*lysyl*-L-*asparaginyl*-L-*phenylalanyl*-L-*phenylalanyl*-L-*tryptophanyl*-L-*lysyl*-L-*threonyl*-L-*phenylalanyl*-L-*threonyl*-[3-O-(*2-acetamido-2-deoxy-β*-D-*glucopyranosyl*)-L-*seryl*]-L-*cysteine* (**7**). — The synthesis of *N*-(*tert*-butyloxycarbonyl)-*S*-(*p*-methoxybenzyl)-L-cysteinyl resin was performed as described for **6** to yield a content of 0.26 mmol of cysteine/g of resin. *N*-(*tert*-Butyloxycarbonyl)-L-alanine, *N*-(*tert*-butyloxycarbonyl)-L-glycine, *N*-(*tert*-butyloxycarbonyl)-*S*-(*p*-methoxybenzyl)-L-cysteine, 2-*N*-(*tert*-butyloxycarbonyl)-6-*N*-(2-chlorobenzyloxycarbonyl)-L-lysine, *N*-(*tert*-butyloxycarbonyl)-L-phenylalanine, 3-*O*-benzyl-*N*-(*tert*-butyloxycarbonyl)-L-threonine, and **5** were coupled to 5 g of resin, as previously described[1], except *N*-(*tert*-butyloxycarbonyl)-L-tryptophan, which was coupled in 1:9 (v/v) *N*,*N*-dimethylformamide–dichloromethane, and *N*-(*tert*-butyloxycarbonyl)-L-asparagine, which was coupled as its *p*-nitrophenyl ester with 1.5 equiv. of 1-hydroxybenzotriazole for 7 h. After cleavage as previously described[1], the peptide solution was diluted to 700 mL with degassed water and added dropwise to a ferricyanide solution, to form the disulfide bridge as described by Rivier *et al.*[20]. After oxidation, the peptide was chromatographed on both anion- and cation-exchange resins as previously described[20], and lyophilized. The residue (1.15 g) was purified by gel filtration twice on Sephadex G-25 fine to afford 347 mg of material, which was further purified by partition chromatography on Sephadex G-25 fine (*B*) to yield 135 mg. After *O*-deacetylation as described for **6**, the peptide (117.5 mg) was finally purified by partition chromatography (*B*) to give 76.5 mg of glycosomatostatin (**7**): t.l.c.: R_f 0.15 (*B*) (somatostatin, 0.17), 0.25 (*C*) (somatostatin, 0.27): $[\alpha]_D^{23}$ −36.2° (*c* 1, 1% acetic acid) {somatostatin, $[\alpha]_D^{23}$ −31.9° (*c* 1, 1% acetic acid)}; l.c. (iso., 18.4% acetonitrile in buffer *F*) 16.3 min, min. purity 97%, (somatostatin, iso., 23.2% acetonitrile in buffer *F*, 25.7 min, min. purity 98.5%).

Anal. Calc.: Ala, 1.0; Asp, 1.0; Cys, 2.0; Gly, 1.0; Lys, 2.8; Phe + GlcNAc, 4.0; Ser, 1.0; Thr, 2.0. Found: NH_3 12.5; Ala, 1.02; Asp, 0.80; Cys, 1.95; Gly, 1.00; Lys, 2.06; Phe + GlcNAc, 3.95; Ser, 1.00; Thr, 2.08.

ACKNOWLEDGMENTS

The authors thank Mr. R. Schroeder for the amino acid analyses, and Mrs. D. Maalouf, and Mrs. B. Gayer for secretarial assistance. The n.m.r. spectra (220 MHz) were recorded at the UCSD n.m.r.–m.s. research source facility supported by a grant (RR-00708) from the National Institutes of Health.

REFERENCES

1 S. LAVIELLE, N. LING, AND R. GUILLEMIN, *Carbohydr. Res.*, RJ-903.
2 S. LAVIELLE, N. LING, P. BRAZEAU, R. BENOIT, T. WASADA, D. HARRIS, R UNGER, AND R. GUILLEMIN, *Biochem. Biophys. Res. Commun.*, 91 (1979) 614–622.
3 P. BRAZEAU, W. VALE, R. BURGUS, N. LING, M. BUTCHER, J. RIVIER, AND R. GUILLEMIN, *Science*, 179 (1973) 77–79.
4 R. D. MARSHALL AND A. NEUBERGER, in A. GOTTSCHALK (Ed.), *Glycoproteins*, 2nd edn., Elsevier, Amsterdam. 1972, pp. 453–470.
5 H. G. GARG AND R. W. JEANLOZ, *Carbohydr. Res.*, 49 (1976) 482–488.
6 H. G. GARG AND R. W. JEANLOZ, *Carbohydr. Res.*, 52 (1976) 246–250 and references therein.
7 R. WERRIES AND E. BUDDECKE, *Hoppe-Seyler's Z. Physiol. Chem.*, 351 (1970) 1089–1099.
8 R. B. MERRIFIELD, *J. Am. Chem. Soc.*, 85 (1963) 2149–2154.
9 W. KOENIGS AND E. KNORR, *Ber.*, 34 (1901) 957–981.
10 B. HELFERICH AND K. F. WEDEMEYER, *Justus Liebigs Ann. Chem.*, 563 (1949) 139–145.
11 D. HORTON AND M. L. WOLFROM, *J. Org. Chem.*, 27 (1962) 1794–1800.
12 J. E. WALLACE AND L. L. SCHROEDER, *J. Chem. Soc., Perkin Trans.*, 2, (1977) 795–802.
13 N. LING, *Biochem. Biophys. Res. Commun.*, 74 (1977) 248–255.
14 M. W. MONAHAN AND C. GILON, *Biopolymers*, 12 (1973) 2513–2519.
15 W. KONIG AND R. GEIGER, *Chem. Ber.*, 103 (1970) 788–798.
16 S. SAKAKIBARA, Y. SHIMONISHI, M. OKADA, AND Y. KISHIDA, *Proc. Eur. Peptide Symp., 8th*, (1967) 44–49.
17 J. LENARD AND A. B. ROBINSON, *J. Am. Chem. Soc.*, 89 (1967) 181–182.
18 D. YAMASHIRO, *Nature (London)*, 201 (1964) 76–77.
19 N. LING, R. BURGUS, AND R. GUILLEMIN, *Proc. Natl. Acad Sci. U.S.A.*, 73 (1976) 3942–3947.
20 J. RIVIER, R. KAISER, AND B. GALYEAN, *Biopolymers*, 17 (1978) 1927–1938.
21 J. RIVIER, R. WOLBERS, AND R. BURGUS, *Proc. Am. Peptide Symp., 5th*, (1977) 52–55.
22 J. RIVIER AND R. BURGUS, *Chromatogr. Sci.*, 10 (1979) 147–161.
23 E. KAISER, R. COLESCOTT, C. BOSSINGER, AND P. COOK, *Anal. Biochem.*, 34 (1970) 595–598