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Conformational Investigations on Glycosylated Asparagine-Oligopeptides of Increasing Chain Length

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Abstract: Stepwise solution syntheses of the homo-oligomers Boc-$(Asn)_n$-$NHCH_3$ ($n = 1–5$; $\mathbf{I}_{1-5}$), Boc-$\{[GlcNAc(Ac)_3\beta]Asn\}_n$-$NHCH_3$ ($n = 1–8$; $\mathbf{II}_{1-8}$), and Boc-$[(GlcNAc\beta)Asn]_n$-$NHCH_3$ ($n = 1–8$; $\mathbf{III}_{1-8}$) are described. Members of the series **III** were obtained by deacetylation of the corresponding members of the series **II**. The conformational preferences of the *N*-protected homo-peptides of the three series were investigated by spectroscopic techniques. ^{1}H-NMR measurements were carried out in various solvents; the CD spectra were recorded in water, aqueous SDS and TFE. The poor solubility of the oligomers of the three series prevented FT-IR measurements in solution. NMR and IR measurements indicate the existence of unordered structures containing some γ-turns in the carbohydrate-free oligomers and the presence of β-turns in the glycosylated oligopeptides, whether acetylated or not. The CD spectra do not indicate the presence of organized structures. The sugar moieties apparently do not have a structure-inducing effect on the asparagine homo-oligomer main chain.

Keywords: circular dichroism; FT-IR absorption; glycopeptides; ^{1}H nuclear magnetic resonance; oligopeptides; peptide conformation

INTRODUCTION

We already described [1] the synthesis and the conformational preferences of the three homo-oligomer series Z-$(Thr)_n$-$NHCH_3$ ($n = 1–4$), Z-$\{[Gal(Ac)_4\beta]Thr\}_n$-$NHCH_3$ ($n = 1–5$) and Z-$[(Gal\beta)Thr]_n$-$NHCH_3$ ($n = 1–5$). Conformational investigations indicated the existence of unordered structures in the carbohydrate-free oligomers and of more or less extended organized structures in the glycosylated derivatives. The sugar moieties apparently has a structure-inducing effect on the peptide chain. For a further investigation on the conformational properties of glycosylated polymers the following homo-oligomers have been prepared by stepwise synthesis in solution: Boc-$(Asn)_n$-$NHCH_3$ ($n = 1–5$; $\mathbf{I}_{1-5}$), Boc-$\{[GlcNAc(Ac)_3\beta]Asn\}_n$-$NHCH_3$ ($n = 1–8$; $\mathbf{II}_{1-8}$), and Boc-$[(GlcNAc\beta)Asn]_n$-$NHCH_3$ ($n = 1–8$; $\mathbf{III}_{1-8}$). Members of the series **III** were obtained by deacetylation of the corresponding members of the series **II**. The conformational preferences of the *N*-protected homo-peptides of the three series were investigated by spectroscopic techniques. Proton NMR measurements were carried out in various solvents; CD spectra were recorded in water, aqueous SDS and TFE. The poor solubility of the oligomers of the three series prevented FT-IR measurements in solution.

Abbreviations: Abbreviations listed in the guide published in *J. Peptide Sci.* 1999; **5**: 465–471 are used without explanation. Other abbreviations: AcOEt, ethyl acetate; EDC, *N*-(3-dimethylaminopropyl),*N*′-ethylcarbodiimide; Gal, D-Galactopyranosyl; $Gal(Ac)_4$, 2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl; GlcNAc, 2-acetamido-2-deoxy-D-glucopyranosyl; $GlcNAc(Ac)_3$, 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyl; HFIP, 1,1,1,3,3,3-hexafluoro-propan-2-ol; TDM, *N*,*N*,*N*′,*N*′-tetramethyl-4,4-diamino-diphenylmethane.

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MATERIALS AND METHODS

EDC, HOBt, BOP, $(Boc)_2O$, DMSO-d_6 (99.96% d_6) and 2-acetamido-2-deoxy-D-glucopyranose were obtained from Fluka. 2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosylamine [2], 1-*N*-acetyl-2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosylamine [3] and 1-*N*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosylamine [3] were synthesized according to the literature. $HOBt{\cdot}NH_2CH_3$ was prepared by the procedure already described [4] for the preparation of the corresponding salt of *N*-hydroxysuccinimide. All other chemicals were of the best grade commercially available. Melting points were taken on a Buchi 150 apparatus in an open capillary, and are not corrected. Optical rotations were determined with a Perkin Elmer 241 polarimeter. Ascending TLC was performed on Merck F254 silica plates using the following solvent systems: E1: butan-1-ol/acetic acid/water (3 : 1 : 1 by vol); E2: ethyl acetate/butan-1-ol/acetic acid/water (5 : 3 : 1 : 1 by vol); E4: chloroform/methanol/acetic acid (9/0.8/0.2 by vol). Amino acid derivatives and peptides were visualized by one or more of the following procedures: ninhydrin, TDM reagent [5], UV light, or 10% H_2SO_4 in methanol (for the trityl derivatives). Sugar and sugar derivative-containing products were located by spraying the plates with 10% H_2SO_4 in ethanol, followed by heating for 10 min at 100° C. Low pressure liquid chromatography was performed on a Buchi 688 Chromatographic Pump (Silica Gel F 60, 230 × 49 mm column, flow rate 48 ml/min. Eluant: ethyl acetate/methylene chloride/acetic acid 70 : 30 : 1 by vol) connected with a Buchi UV/Vis Filter-Photometer Detector (254 nm) and a Knauer recorder. Analytical HPLC separations were performed on an Aquapore RP-300 column (220 × 4.6 mm, 7 μm, Brownlee Labs., flow rate 1.5 ml/min), on a Delta-Pack C-18 column (300 × 3.9 mm, 15 μm, flow rate 1.5 ml/min) or on a Vydac Diphenyl C-18 column (250 × 4.6 mm, 10 μm, flow rate 1.5 ml/min), using a Perkin Elmer series 3B liquid chromatograph equipped with a LC-90 UV detector and LC-100 integrator. Semipreparative HPLC separations (Delta Pack C-18, 30 × 1.9 cm, 15 μm, column, flow rate 15 ml/min, or Vydac Diphenyl C-18, 25 × 2.2 cm, 10 μm, column, flow rate 15 ml/min) were performed on a Shimadzu series LC-6A chromatograph equipped with two independent model. LC-8A pump units, an SPD-6A detector, and a C-R6A integrator. Eluant A (0.1% TFA in 90% aqueous acetonitrile) and B (0.1% aqueous TFA) were used for preparing binary gradients. See the text for elution conditions. Solvents were dried and freshly distilled, and evaporations were carried out under reduced pressure, at 40°–50 °C, using a rotary evaporator. Yields are based on the weights of vacuum-dried products. Sodium sulphate or magnesium sulphate were used for drying purposes.

Mass Spectra

Molecular weight determinations were made by MALDI-TOF MS carried out on a Bruker Reflex TOF MS instrument operating at nominal accelerating potential of +20 KV (matrix α-cyano-4-hydroxycinnamic acid).

Infrared Absorption

Solid-state IR absorption spectra (KBr disk technique) were recorded using a Perkin Elmer Model 1720 FT-IR spectrophotometer (Norwalk, Connecticut, USA) connected with a PC IBM PS/2 Model 50 Z. Elaboration of the spectra by baseline subtraction and second derivative formation was achieved using the Spectra Calc. program (Galactic, Salem, USA).

Proton Nuclear Magnetic Resonance

^{1}H-NMR spectra were recorded using a Bruker AM-X-300 spectrometer, operating at a frequency of 300.13 MHz for the ^{1}H nucleus. The chemical shifts were measured in δ (ppm) and referenced to the solvent. Different solvents (DMSO-d_6, TFE/H_2O (75 : 25 v/v), D_2O and H_2O/D_2O (90 : 10 v/v) were used and the spectra were acquired at different temperatures (278 and 298 K) and at different pH values (8.9, 6.7 and also 3.5 for peptides of series **I**). Sample concentrations were in the range 8–10 mg/ml.

Circular Dichroism

CD measurements were performed, in the solvents indicated, at 298 K, over the 250–185 nm region, using a Jasco-715 spectropolarimeter connected to a PC IBM PS/2 Model 40 SIC for the spectra elaboration (J700 Windows program). The number of accumulated scans ranged from 6 to 8, depending on the intensity of the CD signal. Sample concentrations were about 1 mM and the optical pathlength was 0.02 cm. Samples of the different peptides were dissolved in the minimum amount of water (peptides $\mathbf{I}_{1-5}$ and $\mathbf{III}_{1-8}$) or TFE

(peptides $\mathbf{II}_{1-8}$). Peptide concentrations were determined by quantitative amino acid analysis. Appropriate volumes of the aqueous solutions were diluted with water or aqueous 30 mM SDS up to the desired peptide concentration. Increasing amounts of TFE were added to the aqueous solutions. The solvent composition still securing complete sample solubility was 75 : 25 v/v TFE-water for peptides $\mathbf{I}_{1-5}$, and 5 : 95 v/v TFE-water for glycopeptides $\mathbf{III}_{1-8}$. The TFE solutions of the glycopeptides of series **II** were either diluted with the same solvent or water, or aqueous 30 mM SDS, added to a final 95% (v/v) of water.

The contribution of 1-*N*-acetyl-2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosylamine and 1-*N*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosylamine to the optical activity of the different homo-peptides was subtracted from the CD spectra of the oligomer [6]. The spectra reported are original computer-drawn CD curves; $[\theta]_R$ represents the residue molar ellipticity (deg $cm^2 dmol^{-1}$).

Peptide Synthesis

The homo-oligomers of the series **I** and **II** were prepared by stepwise synthesis in solution starting, respectively, from H-Asn(Trt)-$NHCH_3$ and H-[GlcNAc(Ac)$_3\beta$]Asn-$NHCH_3$, both obtained by acid treatment of the corresponding N^{α}-Boc derivatives which were synthesized by reacting Boc-Asn(Trt)-OH or Boc-[GlcNAc(Ac)$_3\beta$]Asn-OH with HOBt·NH_2CH_3 in the presence of EDC as the coupling agent. During the synthesis, the amino group of the growing peptide chain was deprotected by treatment either with 2N HCl (series **I**) or 95% TFA (series **II**). Activation of the carboxyl component was achieved by the EDC-HOBt procedure (homo-oligomers $\mathbf{I}_{1-5}$) or the BOP procedure (homo-oligomers $\mathbf{II}_{1-8}$). Treatment of peptides $\mathbf{I}_{1-5}$ with 95% aqueous TFA removed both the Trt and the Boc groups. Reprotection of the α-amino function was achieved with $(Boc)_2O$. Deacetylation of the carbohydrate moieties of the homo-oligomers $\mathbf{II}_{1-8}$ by treatment with hydrazine in DMF yielded the corresponding glycopeptides $\mathbf{III}_{1-8}$. All peptides synthesized, which were practically homogeneous by TLC and analytical HPLC in the conditions indicated, were characterized by optical rotation and IR spectroscopy and, in some cases, by ^{1}H-NMR and molecular weight determination.

Boc-Asn(Trt)-$NHCH_3$

EDC (6.53 g, 34.1 mmol) and HOBt·NH_2CH_3 (5.90 g, 35.5 mmol) were added to an ice-cold solution of Boc-Asn(Trt)-OH (12.43 g, 30.4 mmol) [7] in DMF (60 ml). After 20 min stirring at 0 °C and a further 3 h at room temperature, the reaction mixture was evaporated to dryness and the oily residue taken up with AcOEt (350 ml). The resulting solution was washed with aqueous 5% $NaHCO_3$ (3 × 100 ml) and saturated aqueous NaCl (3 × 100 ml), dried, evaporated to dryness and the residue was triturated with light petroleum. The yield was 14.26 g (96%); single spot by TLC in E4; m.p. 190 °C; $[\alpha]_D^{25°}$ −17° (*c* 1.3, methanol); homogeneous by analytical HPLC (Aquapore column; elution: isocratic 20% A for 2 min, linear gradient 20%–70% A in 20 min, 70%–90% A in 5 min). ^{1}H-NMR data are shown in Table 1.

Boc-(Asn(Trt))$_2$-$NHCH_3$

2 N HCl (200 ml) was added to a suspension of Boc-Asn(Trt)-$NHCH_3$ (13.39 g, 28.2 mmol) in isopropanol (260 ml). The mixture was heated to 50 °C and the reaction was monitored by TLC in E4. After 4 h the solvent was removed and the residue [H-Asn(Trt)-$NHCH_3$ hydrochloride] was triturated with ether and dried *in vacuo* over KOH pellets. The yield was 9.85 g (82.4%), single spot by TLC in E4; m.p. 142 °C dec.; $[\alpha]_D^{25°}$ +7° (*c* 0.85, methanol).

Boc-Asn(Trt)-OH (7.51 g, 15.8 mmol), H-Asn(Trt)-$NHCH_3$ hydrochloride (6.72 g, 15.8 mmol) and HOBt (3.01 g, 22.3 mmol) were dissolved in DMF (60 ml). The solution was cooled to 0 °C, DIPEA (2.71 ml, 15.8 mmol) and EDC (3.27 g, 17.1 mmol) were added and, after 2.5 h stirring, the reaction mixture was evaporated to dryness. The oily residue was taken up with 5% aqueous $NaHCO_3$, stirred for 60 min and the resulting solid compound was collected by filtration and precipitated from methanol with water. The yield was 12.5 g (94%); single spot by TLC in E4; m.p. 217 °C; $[\alpha]_D^{25°}$ −11.7° (*c* 0.75, methanol). For the ^{1}H-NMR data see Table 1.

Boc-(Asn(Trt))$_3$-$NHCH_3$

2N HCl (120 ml) was added to a suspension of Boc-[Asn(Trt)]$_2$-$NHCH_3$ (14.2 g, 16.8 mmol) in isopropanol (120 ml). The mixture was heated to 60 °C and AcOEt (400 ml) was slowly added to give a clear solution. The deblocking reaction was monitored by TLC (E4) and after 3 h the organic solvents were removed *in vacuo*. The resulting oil was extracted with AcOEt (3 × 200 ml), the combined organic layers were washed with water (3 × 200 ml) and saturated aqueous NaCl (3 × 200 ml), dried and concentrated to small volume.

Table 1 ^{1}H-NMR Chemical Shifts of Boc-[Asn(Trt)]$_n$-NHCH$_3$ (n = 1–5) in DMSO at 298 K

n	Boc	NHα ($J_{NH\text{-}H\alpha}$)	NHγ	CHα	CHβ	N<u>H</u>-CH$_3$ (J_{NH-CH_3})	NH-C<u>H</u>$_3$	Trt
1	1.38	7.13 (7.94)	8.54	4.18	2.52	7.71 (4.46)	2.56	7.22
2	1.36	6.98 (7.44), 8.17 (8.15)	8.65, 8.68	4.20, 4.45	2.64 (4Hβ)	7.49 (4.73)	2.36	7.21
3	1.38	7.06 (8.11), 8.08 (7.94), 8.01 (6.96)	8.51,8.55, 8.79	4.44 (3Hα)	2.70 (6Hβ)	7.47 (4.63)	2.25	7.21
4	1.39	7.01 (8.27), 8.08 (3NHα)	8.50,8.55, 8.64, 8.76	4.40 (2Hα), 4.60 (2Hα),	2.87–2.55 (8Hβ)	7.45 (4.58)	2.24	7.20
5	1.39	6.99, 8.02 (4NHα)	8.69–8.46 (5Hγ)	4.69–4.33 (5Hα)	2.78–2.62 (10 Hβ)	7.43	2.24	7.19

Addition of light petroleum gave a precipitate {H-[Asn(Trt)]$_2$-NHCH$_3$hydrochloride} which was collected and dried. The yield was 12.2 g (93%); single spot by TLC in E4; m.p. 175°–179 °C; $[\alpha]_D^{25°}$ + 2.3° (*c* 0.84, methanol). DIPEA (2.6 ml, 15.3 mmol) and EDC (3.52 g, 18.4 mmol) were added to an ice-cold DMF solution (70 ml) of Boc-Asn(Trt)-OH (7.28 g, 15.3 mmol), H-[Asn(Trt)]$_2$-NHCH$_3$ hydrochloride (11.95 g, 15.3 mmol) and HOBt (2.61 g, 19.3 mmol). After stirring for 20 min at 0 °C and a further 5 h at room temperature, the reaction mixture was evaporated to dryness. The residue was taken up with CH_2Cl_2 (400 ml), washed with 0.1M $KHSO_4$ (3 × 100 ml), 0.5N Na_2CO_3 (3 × 100 ml), water (3 × 100 ml), saturated aqueous NaCl, and dried. The solvent was removed and the residue was precipitated from CH_2Cl_2 with ether. The yield was 15.2 g (83%); single spot by TLC in E4; m.p. 186°–187 °C; $[\alpha]_D^{25°}$ −6.6° (*c* 1.06, methanol); homogeneous by analytical HPLC (Aquapore column, elution: isocratic 65% A for 2 min, linear gradient 65%–100% A in 20 min). For the ^{1}H-NMR data see Table 1.

Boc-(Asn(Trt))$_4$-NHCH$_3$

Boc-[Asn(Trt)]$_3$-NHCH$_3$ (12.49 g, 10.4 mmol) was N^α-deprotected by treatment with 2N HCl (80 ml) as described for Boc[Asn(Trt)$_3$]-NHCH$_3$, and the resulting H-[Asn(Trt)]$_3$-NHCH$_3$ hydrochloride (10.66 g, 9.4 mmol, 90%) was dissolved in DMF (50 ml). Boc-Asn(Trt)-OH (4.46 g, 9.4 mmol), HOBt (1.69 g, 12.5 mmol), DIPEA (1.6 ml, 9.4 mmol) and EDC (2.18 g, 11.4 mmol) were added and the reaction mixture was worked up as described for the preparation of Boc-[Asn(Trt)]$_3$-NHCH$_3$. Final crystallization from AcOEt gave 12.6 g (86%) of the title compound. Single spot by TLC in E4; m.p. 203°–206 °C; $[\alpha]_D^{25°}$ −6.9° (*c* 0.86, methanol); homogeneous by analytical HPLC (column and elution conditions as described for Boc-[Asn(Trt)]$_3$-NHCH$_3$). For the ^{1}H-NMR see Table 1.

Boc-(Asn(Trt))$_5$-NHCH$_3$

Boc-[Asn(Trt)]$_4$-NHCH$_3$ (8.46 g, 5.4 mmol) was treated with 2N HCl (50 ml) as described above and the resulting [Asn(Trt)]$_4$-NHCH$_3$ hydrochloride (7.36 g, 4.93 mmol, 91%) was dissolved in DMF (35 ml). Boc-Asn(Trt)-OH (2.37 g, 4.99 mmol), HOBt (0.89 g, 6.59 mmol), DIPEA (0.845 ml, 4.93 mmol) and EDC (1.2 g, 6.26 mmol) were added and the reaction mixture was worked up as described for the preparation of Boc-[Asn(Trt)]$_3$-NHCH$_3$. Yield 8.22 g (87%). An aliquot of the crude product (7.9 g) was purified by low pressure liquid chromatography. The final yield was 4.5 g (48%); single spot by TLC in E4; m.p. 167°–169 °C; $[\alpha]_D^{25°}$ −7.5° (*c* 0.95, methanol); homogeneous by analytical HPLC (column and elution conditions as described for Boc-[Asn(Trt)]$_3$-NHCH$_3$). For the ^{1}H-NMR data see Table 1.

Boc-Asn-NHCH$_3$ (I_1)

Boc-Asn(Trt)-NHCH$_3$ (1.15 g, 2.36 mmol) was dissolved in 95% aqueous TFA (30 ml). After stirring at room temperature for 17 h, the solvent was removed and the residue taken up with ether (200 ml), collected by filtration, washed with ether and dried. The product (0.58 g, 95%, single spot by TLC in E1) was dissolved in water (4 ml) containing $Na_2CO_3 \cdot 10H_2O$ (0.68 mg). The solution was cooled to 0° and $(Boc)_2O$ (0.8 g, 3.66 mmol) in dioxane (4 ml) was added. After

5 h the mixture was concentrated to small volume, the precipitate was collected by filtration, thoroughly washed with water and dried. The yield was 0.54 g (98%); single spot by TLC in E1; m.p.130°–133 °C dec.; $[\alpha]_D^{25°}$ +23.9° (*c* 0.86, HFIP); homogeneous by analytical HPLC (Aquapore column, elution: isocratic 1% A for 2 min, linear gradient 1%–30% A in 20 min and 30%–90% A in 5 min; Delta-Pack C-18 column, elution: isocratic 5% A for 2 min, linear gradient 5%–30% A in 20 min and 30%–90% A in 5 min). For the ^{1}H-NMR and IR data see Tables 2 and 3, respectively.

Boc-(Asn)$_2$-NHCH$_3$ (**I**$_2$)

Boc-[Asn(Trt)]$_2$-NHCH$_3$ (1.73 g, 2.05 mmol) was dissolved in 95% aqueous TFA (40 ml) and worked up as described for **I**$_1$. The dipeptide trifluoroacetate was precipitated from methanol with ether. The product (0.54 g, 73%, single spot by TLC in E1) was dissolved in water (3 ml) containing $Na_2CO_3 \cdot 10H_2O$ (0.4 g), cooled to 0° and $(Boc)_2O$ (0.73 g, 3.3 mmol) in dioxane (3 ml) was added. After 26 h at room temperature the precipitate was collected by filtration, thoroughly washed with 0.1 M $KHSO_4$ and water, dried and triturated with ether and methanol. The yield was 0.43 g (84%); single spot by TLC in E1; m.p. 220°–222 °C dec.; $[\alpha]_D^{25°}$ +13.0° (*c* 0.86, HFIP); homogeneous by analytical HPLC (elution conditions as described for **I**$_1$). For the ^{1}H-NMR and IR data see Tables 2 and 3, respectively.

Boc-(Asn)$_3$-NHCH$_3$ (**I**$_3$)

The title compound was prepared from Boc-[Asn(Trt)]$_3$-NHCH$_3$ (2.53 g, 2.1 mmol) as described for **I**$_2$. The yield was 0.73 g (75%); single spot by TLC in E1; m.p. 222°–223 °C dec.; $[\alpha]_D^{25°}$ −6.8° (*c* 0.83, HFIP); homogeneous by analytical HPLC (column and elution conditions as described for **I**$_1$). For the ^{1}H-NMR and IR data see Tables 2 and 3, respectively.

Table 2 ^{1}H-NMR Chemical Shifts of Boc-(Asn)$_n$-NHCH$_3$ (n = 1–5) in DMSO at 298 K

n	Boc	NHα ($J_{NH-H\alpha}$)	NHγ	CHα	CHβ	N<u>H</u>-CH$_3$ (J_{NH-CH_3})	NH-C<u>H</u>$_3$
1	1.36	6.82 (8.44)	7.24, 6.82	4.17	2.36	7.66 (4.58)	2.52
2	1.37	7.00, 8.09, (7.42)	7.39, 7.00, 7.31, 6.84	4.16, 4.43	2.43–2.30 (4Hβ)	7.63 (4.35)	2.54
3	1.37	6.97, 8.09 (2NHα)	7.34, 6.93, 7.47, 7.02, 7.23, 6.81	4.25–4.42 (2Hα)	2.45 (6Hβ)	7.62 (4.63)	2.55
4	1.36	7.00, 8.18, (6.82), 8.13 (6.89), 8.00 (7.55)	7.50–7.20 (4Hγ), 7.00, 6.87, 7.23, 6. 82	4.25, 4.5–4.3 (3Hα)	2.56–2.49 (8Hβ)	7.45 (4.58)	2.50
5	1.36	7.00, 8.18–8.10 (2NHα) 8.04 (7.18), 7.95 (7.71)	7.41–7.36 (4Hγ), 7.24, 6.82, 6.97–6.90 (4Hγ)	4.26, 4.5–4.4 (4Hα)	2.57–2.49 (10 Hβ)	7.51 (4.38)	2.50

Table 3 IR Frequencies (cm^{-1}) of Boc-(Asn)$_n$-NHCH$_3$ (**I**$_{1-5}$) in the Solid State (KBr pellets)

	Amide A						Amide I					Amide II	
I$_1$		3390m	3338s	3328m		3196w	1693s	1684m	1658s	1650m	1620w	1559w	1529m
I$_2$	3402m	3383w		3225s	3293s	3281s 3206m	1691s		1669s	1649w	1624w	1546w	1535m
I$_3$	3414sh	3393m		3326s	3289s	3280s 3210m	1690s		1671s	1650s	1624w	1547w	1531s
I$_4$	3399s	3381m		3324s	3287s	3272s 3200s	1691s	1675m	1662s	1646m	1620w	1546m	1530s
I$_5$		3395s		3328m	3282s	3201s	1692m	1672sh	1656s			1543w	1530w

w, weak; m, medium; s, strong; sh, shoulder.

Boc-(Asn)$_4$-NHCH$_3$ (I$_4$)

The title compound was prepared from Boc-[Asn(Trt)]$_4$-NHCH$_3$ (3.8 g, 2.44 mmol) as described for **I**$_2$. The yield was 1.0 g (70%); single spot by TLC in E1; m.p. 222°–224 °C dec.; $[\alpha]_D^{25°}$ −21.9° (*c* 0.70, HFIP); homogeneous by analytical HPLC (column and elution conditions as described for **I**$_1$). For the ^{1}H-NMR and IR data see Tables 2 and 3, respectively.

Boc-(Asn)$_5$-NHCH$_3$ (I$_5$)

The title compound was prepared from Boc-[Asn(Trt)]$_5$-NHCH$_3$ (2.19 g, 1.14 mmol) as described for **I**$_2$. The crude product (0.59 g) was purified by semipreparative HPLC (Delta-Pack C-18 column, elution: isocratic 3% A for 3 min, linear gradient 3%–17% A in 15 min and 17%–90% A in 5 min). The yield was 0.48 g (62%); single spot by TLC in E1; m.p. 266°–268 °C dec.; $[\alpha]_D^{25°}$ −21.9° (*c* 0.70, HFIP); homogeneous by analytical HPLC (column and elution conditions as described for **I**$_1$). For the ^{1}H-NMR and IR data see Tables 2 and 3, respectively.

Boc-(GlcNAc(Ac)$_3\beta$)Asn-NHCH$_3$ (II$_1$)

EDC (1.43 g, 7.48 mmol) was added to an ice-cold solution of Boc-[GlcNAc(Ac)$_3\beta$]Asn-OH (3.0 g, 5.34 mmol) [8] and HOBt·NH$_2$CH$_3$ (1.24 g, 7.48 mmol) in DMF (20 ml). After 20 min at 0° and further 60 min at room temperature the mixture was diluted with water (50 ml) and the resulting precipitate was collected by filtration, washed with water and dried. The yield was 2.4 g (78%); single spot by TLC in E4; m.p. 231°–232 °C; $[\alpha]_D^{25°}$ +11.2° (*c* 1.08, methanol), $[\alpha]_D^{25°}$ = +23.2° (*c* 0.75, TFE); homogeneous by analytical HPLC (Aquapore column, elution: isocratic 15% A for 2 min, linear gradient 15%–50% A in 30 min and 50%–90% A in 5 min). MALDI-TOF [M + Na] 597.3 (597.58), [M + K] 612.4 (612.23). For the IR data see Table 4.

Boc-{(GlcNAc(Ac)$_3\beta$)Asn}$_2$-NHCH$_3$ (II$_2$)

Boc-[GlcNAc(Ac)$_3\beta$]Asn-NHCH$_3$ (**II**$_1$) (2.45 g, 4.26 mmol) was dissolved in TFA (7 ml) and excess ether was added after 20 min. The resulting precipitate was collected by filtration and added to a DMF solution (100 ml) of Boc-[GlcNAc(Ac)$_3\beta$]Asn-OH (2.24 g, 3.99 mmol). DIPEA (1.72 ml, 9.97 mmol) and BOP (1.76 g, 3.99 mmol) were added to the reaction mixture and, after 2 h stirring, the solution was diluted with excess AcOEt. The resulting precipitate was collected by centrifugation, thoroughly washed with AcOEt and dried. The yield was 3.95 g (97%); single spot by TLC in E2; m.p. 248°–250 °C; $[\alpha]_D^{25°}$ +26.2° (*c* 1.08, TFE); homogeneous by analytical HPLC (column and elution conditions as described for **II**$_1$). MALDI-TOF [M + Na] 1040.4 (1041), [M + K] 1057 (1057). For the IR data see Table 4.

Boc-{(GlcNAc(Ac)$_3\beta$)Asn}$_3$-NHCH$_3$ (II$_3$)

The title compound was prepared from Boc-[GlcNAc(Ac)$_3\beta$]Asn-OH (1.75 g, 3.12 mmol) and **II**$_2$ (3.24 g, 3.12 mmol) as described for **II**$_2$. The isolated tripeptide (4.4 g, 96%) showed the presence of a few minor impurities by analytical HPLC (column and elution conditions as described for **II**$_1$). An aliquot (300 mg) of the crude product was triturated several times with methanol, collected and dried. The yield was 250 mg (83%); single spot by TLC in E2; m.p. 244°–245 °C dec.; $[\alpha]_D^{25°}$ +31.2° (*c* 0.91, TFE); homogeneous by analytical HPLC. MALDI-TOF [M + Na] 1484.0 (1484.41), [M + K] 1498.9 (1499.41). For the IR data see Table 4.

Boc-{(GlcNAc(Ac)$_3\beta$)Asn}$_4$-NHCH$_3$ (II$_4$)

The title compound was prepared from Boc-[GlcNAc(Ac)$_3\beta$]Asn-OH (1.37 g, 2.44 mmol) and **II**$_3$ (3.6 g, 2.44 mmol) as described for **II**$_2$. The reaction time was 3 h. The isolated product (4.6 g, 98%) showed the presence of minor impurities by analytical HPLC (column and elution conditions as described for **II**$_1$). An aliquot (150 mg) of the crude product was purified by semipreparative HPLC (Vydac-Diphenyl C-18 column, elution: isocratic 30% A for 2 min, linear gradient 30%–35% A in 5 min, 35%–40% A in 12 min and 40%–90% A in 3 min). The yield was 100 mg (67%); single spot by TLC in E1 and E2; $[\alpha]_D^{25°}$ +31.1° (*c* 0.95, TFE); MALDI-TOF [M + Na] 1928.0 (1927.82), [M + K] 1943.4 (1943.82). For the IR data see Table 4.

Boc-{(GlcNAc(Ac)$_3\beta$)Asn}$_5$-NHCH$_3$ (II$_5$)

The title compound was prepared from Boc-[GlcNAc(Ac)$_3\beta$]Asn-OH (1.04 g, 1.85 mmol) and **II**$_4$ (3.55 g, 1.85 mmol) as described for **II**$_2$. The reaction time was 3 h. A portion (250 mg) of the crude product (4.2 g, 97%), was purified by semipreparative HPLC (column and elution conditions as described for **II**$_4$). The yield was 160 mg (64%); single peak

Table 4 IR Frequencies (cm^{-1}) in the Solid State (KBr pellets) of 1-*N*-acetyl-2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosylamine (GlcN**), Boc-{[GlcNAc(Ac)$_3$ β]Asn}$_n$-NHCH$_3$ (**II**$_{1-8}$) and poly-[GlcNAc(Ac)$_3\beta$]Asn$_n$-OH (M.W. $\approx$ 8500) [14] (Poly **II**)

	Amide A							
GlcN**			3339s	3318s	3308s			3212w
II$_1$			3339s	3319s				
II$_2$	3474w	3375ww		3325s		3299m	3284sh	3220w
II$_3$	3485w	3375ww			3311s	3299sh	3286sh	3215w
II$_4$	3455w		3331s		3310m		3286m	3213w
II$_5$	3474w	3375w		3321s	3310sh		3287s	3220m
II$_6$	3480w	3374m	3332m		3314m		3284m	3216m
II$_7$	3476w	3376m			3311m		3278m	3214m
II$_8$	3485w		3347m		3314m		3278m	3218m
Poly **II**	3460m		3357m				3276m	3210m

	Amide I							Amide II	
GlcN**		1748s	1738s			1664m		1553w	1540m
II$_1$		1748s		1694s	1680ww	1663s	1640ww	1554w	1525 m
II$_2$	1756s	1746s	1728m	1696s	1680sh	1664s	1652m	1556ww	1533 m
II$_3$	1755s	1747s	1728m	1694m	1674m	1663s	1640m	1555ww	1536 m
II$_4$	1755s	1750s	1728m	1693w	1682s	1664s	1645m	1544m	1533ww
II$_5$	1755sh	1749s	1728w	1697m	1682m	1664s	1645m	1543m	
II$_6$	1756s	1745sh	1728w	1693w	1680sh	1668m	1645sh	1544b	
II$_7$	1757s	1745sh	1728w	1692w	1680sh	1667m	1645sh	1542b	
II$_8$	1756	1745sh	1728w	1692w	1680sh	1669m	1645sh	1542b	
Poly **II**	1755s	1745sh	1728m	1690sh		1667m		1544b	

ww, very weak; w, weak; m, medium; s, strong; sh, shoulder; b, broad.

by analytical HPLC (Aquapore column, elution: isocratic 30% A for 2 min, linear gradient 30%–70% A in 30 min and 70%–90% A in 5 min); $[\alpha]_D^{25^\circ}$ +30.1° (*c* 1.0, TFE); MALDI-TOF [M + Na] 2370.2 (2371.23), [M + K] 2385.7 (2387.21). For the IR data see Table 4.

Boc-{(GlcNAc(Ac)$_3\beta$)Asn}$_6$-NHCH$_3$ (II$_6$)

The title compound was prepared from Boc-[GlcNAc(Ac)$_3\beta$]Asn-OH (0.67 g, 1.18 mmol) and **II**$_5$ (2.8 g, 1.18 mmol) as described for **II**$_2$. The reaction time was 20 h. A portion (270 mg) of the crude product (2.9 g, 88%) was purified as described for **II**$_5$. The yield was 135 mg (50%); single peak by analytical HPLC (column and elution conditions as for **II**$_5$); $[\alpha]_D^{25^\circ}$ +47.4° (*c* 0.85 TFE); MALDI-TOF [M + Na] 2813.1 (2814.64). For the IR data see Table 4.

Boc-{(GlcNAc(Ac)$_3\beta$)Asn}$_7$-NHCH$_3$ (II$_7$)

The title compound was prepared from Boc-[GlcNAc(Ac)$_3\beta$]Asn-OH (0.39 g, 0.7 mmol) and **II**$_6$ (2.0 g, 0.7 mmol) as described for **II**$_2$. The reaction time was 20 h. An aliquot (200 mg) of the crude product (1.85 g, 82%) was purified as described for **II**$_5$. Yield 85 mg (43%); single peak by analytical HPLC (column and elution conditions as for **II**$_5$); $[\alpha]_D^{25^\circ}$ +38.3° (*c* 0.75 TFE); MALDI-TOF [M + Na] 3256.5 (3258.05). For the IR data see Table 4.

Boc-{(GlcNAc(Ac)$_3\beta$)Asn}$_8$-NHCH$_3$ (II$_8$)

The title compound was prepared from Boc-[GlcNAc(Ac)$_3\beta$]Asn-OH (0.12 g, 0.21 mmol) and **II**$_7$ (0.7 g, 0.21 mmol) as described for **II**$_2$. The reaction time was 20 h. An aliquot (200 mg) of the crude product (0.6 g, 76%) was purified by semipreparative HPLC as described for **II**$_5$. The yield was 80 mg (40%); single peak by analytical HPLC (column and elution conditions as for **II**$_5$); $[\alpha]_D^{25^\circ}$ +34.2° (*c* 0.75 TFE); $[\alpha]_D^{25^\circ}$ +2.4° (*c* 0.76 DMF); MALDI-TOF [M + Na] 3698.9 (3701.46). For the IR data see Table 4.

Deacetylation Procedure

Deacetylation of the oligomers $\mathbf{II}_{1-8}$ by treatment with hydrazine hydrate yielded the corresponding glycopeptides $\mathbf{III}_{1-8}$. Glycopeptides $\mathbf{III}_{1-6}$ were homogeneous by analytical HPLC (Delta-Pack C-18 column, elution conditions: isocratic 5% A for 2 min, linear gradient 5%–12% A in 15 min, 12%–90% A in 5 min). The oligomers $\mathbf{III}_7$ and $\mathbf{III}_8$ were further purified by semipreparative HPLC [Delta Pack-C18 column, elution conditions: isocratic 5% A for 2 min, linear gradient 5%–12% A in 15 min ($\mathbf{III}_7$) and isocratic 6% A for 2 min, linear gradient 6%–8% A in 5 min ($\mathbf{III}_8$)]. IR data in the 1400–1700 cm^{-1} range are shown in Table 5. Yields and analytical data are shown in Table 6. As an example, the deacetylation of Boc-[Glc–Ac(Ac)$_3\beta$]Asn-$NHCH_3$ ($\mathbf{II}_1$) is described in detail: Boc-[GlcNAc(Ac)$_3\beta$]Asn-$NHCH_3$ (0.3 g, 0.52 mmol) was suspended in DMF (10 ml) and NH_2-NH_2·H_2O (1.17 ml, 23.4 mmol) was added. After 4 h stirring excess ethanol was added and the resulting precipitate was collected by filtration, washed with ethanol and ether, dissolved in water and precipitated with ethanol. The yield was 190 mg (81%). The analytical data are shown in Table 6.

RESULTS AND DISCUSSION

Infrared Absorption

As pointed out above, the poor solubility of the oligomers of the three series prevented FT-IR absorption measurements in solution, and their conformational preferences were examined only in the solid state (KBr pellets). The infrared absorption frequencies of the oligomers $\mathbf{I}_{1-5}$ in the

Table 5 IR Frequencies (cm^{-1}) in the Solid State (KBr pellets) of 1-*N*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosylamine (GlcN*), Boc-[(GlcNAcβ)Asn]$_n$-$NHCH_3$ ($\mathbf{III}_{1-8}$) and poly-[(GlcNAcβ)Asn]$_n$-OH (M.W. ≈6000) [14] (Poly **III**)

	Amide I			Amide II		
GlcN*		1674s	1659m	1556w	1543m	
$\mathbf{III}_1$	1690s	1666s	1656s	1556w	1545m	1526m
$\mathbf{III}_2$	1697s	1664s	1648s	1556sh	1541s	1526m
$\mathbf{III}_3$	1698m	1664sh	1656s	1556m	1540s	1525m
$\mathbf{III}_4$	1690sh	1664sh	1656s	1554sh	1544s	
$\mathbf{III}_5$	1690sh	1667sh	1657s	1556sh	1545s	
$\mathbf{III}_6$	1690sh	1670sh	1657s	1556sh	1544s	
$\mathbf{III}_7$	1690sh	1682m	1656s	1555sh	1545s	
$\mathbf{III}_8$	1690sh	1682m	1657s	1556sh	1545s	
Poly **III**	1690sh	1682m	1660s	1550sh		

w, weak; m, medium; s, strong; sh, shoulder; b, broad.

Table 6 Yields and Analytical Data for the Oligomers of Series **III**

Oligomer		Reaction time	Yield (%)	$[\alpha]_D^{25°}$	c (5% CH_3ACOH)
Boc-(GlcNAcβ)Asn-$NHCH_3$	$\mathbf{III}_1$	4 h	81	+20.6°	1.16
Boc-[(GlcNAcβ)Asn]$_2$-$NHCH_3$	$\mathbf{III}_2$	8 h	73	+18.7°	0.76
Boc-[(GlcNAcβ)Asn]$_3$-$NHCH_3$	$\mathbf{III}_3$	8 h	58	+14.6°	0.81
Boc-[(GlcNAcβ)Asn]$_4$-$NHCH_3$	$\mathbf{III}_4$	8 h	86	+15.2°	0.91
Boc-[(GlcNAcβ)Asn]$_5$-$NHCH_3$	$\mathbf{III}_5$	8 h	93	+13.3°	1.02
Boc-[(GlcNAcβ)Asn]$_6$-$NHCH_3$	$\mathbf{III}_6$	10 h	92	+13.2°	0.81
Boc-[(GlcNAcβ)Asn]$_7$-$NHCH_3$	$\mathbf{III}_7$	10 h	12	+11.8°	0.50
Boc-[(GlcNAcβ)Asn]$_8$-$NHCH_3$	$\mathbf{III}_8$	10 h	32	+11.6°	0.64

3500–3200 cm^{-1} region (N-H stretching mode) and in the 1800–1500 cm^{-1} region (C=O stretching mode) are shown in Table 3.

In the 3500–3200 cm^{-1} region, the spectrum of $\mathbf{I}_1$ exhibits bands at 3390, 3338 and 3228 cm^{-1} and a weak band at about 3200 cm^{-1}. In peptides $\mathbf{I}_2$, $\mathbf{I}_3$ and $\mathbf{I}_4$ the band at 3338 disappears and two absorptions are visible between 3290 and 3280 cm^{-1}. In $\mathbf{I}_5$ the two bands overlap in an absorption centred at about 3280 cm^{-1}. The intensity of the band at 3200 cm^{-1} gradually increases on increasing the main chain length. The **FT-IR** absorption spectra suggest that structures stabilized by five-atom H-bonded rings (C_5) [9, 10] and γ-turns (C_7) [11] predominate in the oligomers $\mathbf{I}_{1-4}$.

The low-frequency bands suggest the presence of H-bonded β-turns [12] in the oligomers $\mathbf{I}_4$ and $\mathbf{I}_5$. The presence of intramolecularly H-bonded structures increases with the length of the peptide chain. It was not possible to establish if the polypeptide backbone or the side chain amide functions are involved in the turn formation.

No peptide shows bands which can be assigned to non-H-bonded NH groups. In the 1800–1600 cm^{-1} region, the band at about 1690 cm^{-1} which appears in the spectra of all oligomers may be ascribed to the urethane C=O group being involved in intramolecular hydrogen bonding. The other bands are consistent with the type of secondary structure suggested by the N-H stretching bands, but the bands at about 1650 cm^{-1} suggest the presence of unordered structures [13].

The infrared absorption frequencies of the oligomers $\mathbf{II}_{1-8}$ in the 3500–3200 cm^{-1} and 1800–1500 cm^{-1} regions are shown in Table 4. For comparison, the absorption frequencies determined for 1-*N*-acetyl-2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosylamine (GlcN**) and for the poly-{[GlcNAc(Ac)$_3\beta$]Asn} (Poly **II**, M.W. $\approx$ 8500) [14] are also reported. In the N-H stretching region, both $\mathbf{II}_1$ and the peracetylated monosaccharide (GlcN**) show bands ascribable to γ-turn (C_7) [11] conformations. C_7 H-bonded structures may involve the urethane C=O group and the *C*-terminal NH in the monomer [15], and the acetamido functions in the peracetylated monosaccharide molecule.

The absorption bands between 3375 and 3280 cm^{-1} in the oligomers with a longer main chain are also consistent with the existence of structures stabilized by $4 \rightarrow 1$ H-bonds [16, 17] such as type I β-turns [12] or 3_{10} helices [18]. The intensity of the bands ascribable to aggregation processes ($\approx$3215 cm^{-1}) and to non-H-bonded NH groups ($\approx$3475 cm^{-1}) increases from $\mathbf{II}_2$ to $\mathbf{II}_8$.

The different C=O groups give rise to three main different bands in the 1800–1600 cm^{-1} region. The ester C=O groups are non bonded and show absorption maxima at 1757–1728 cm^{-1}. The urethane C=O group gives a band at about 1695 cm^{-1}, whose intensity decreases on increasing the main chain length. Possibly this C=O group is involved as an H-bond acceptor in cyclic structures (7 or 10 member rings) [10, 17].

The amide I band of the peptides of the series **II** appears at 1664 cm^{-1}, and overlaps the band of the acetamido function of the sugar. In peptides $\mathbf{II}_3$, $\mathbf{II}_4$ and $\mathbf{II}_5$ two new bands are identified at about 1680 and 1645 cm^{-1} and the amide II bands appear at about 1555 and 1533 cm^{-1}. Similar absorption frequencies have been ascribed to 3_{10} helical structures and type I/III β-turns [18]. From $\mathbf{II}_6$ to $\mathbf{II}_8$ the intensity of the bands at 1680 and 1645 cm^{-1} decreases, the amide I band shifts to 1667–1669 cm^{-1} and the large amide II band appears at 1542–1544 cm^{-1}. The spectra are very similar to that of the polymer, suggesting that, starting from $\mathbf{II}_6$, a different secondary structure is formed. The IR spectra of the deacetylated derivatives $\mathbf{III}_{1-8}$ and of the poly-[(GlcNAcβ)Asn] (Poly **III**, M.W. $\approx$ 6000) [14] in the 3500–3200 cm^{-1} region are characterized by intense, broad absorption bands that cannot be resolved by the second derivative. In the 1800–1600 cm^{-1} region (Table 5) the spectrum of the 2-acetamido-1-*N*-acetyl-2-deoxy-β-D-glucopyranosylamine suggests the involvement of the C=O groups as weak H-bond acceptors in the stabilization of possible C_7 glyco turns [19].

In this region, the spectra of the homo-oligomers $\mathbf{III}_{1-3}$ exhibit intense, sharp bands, including the urethane band at 1690 cm^{-1}, which become broad with the main chain elongation.

The amide I bands at 1664 and 1655 cm^{-1} may be assigned either to weakly H-bonded C=O groups, or to unordered structures as suggested also by the amide II bands at 1555 and 1545 cm^{-1} [20]. The spectra of the high molecular weight oligomers and that of the polymer are quite similar.

Circular Dichroism

The far ultraviolet CD spectra of the oligomers of the series **I** in water, 30 mM SDS and aqueous 75% TFE are shown in Figure 1. The spectra of the peptides $\mathbf{I}_{2-4}$ are quite similar and independent of the environment. They are characterized by

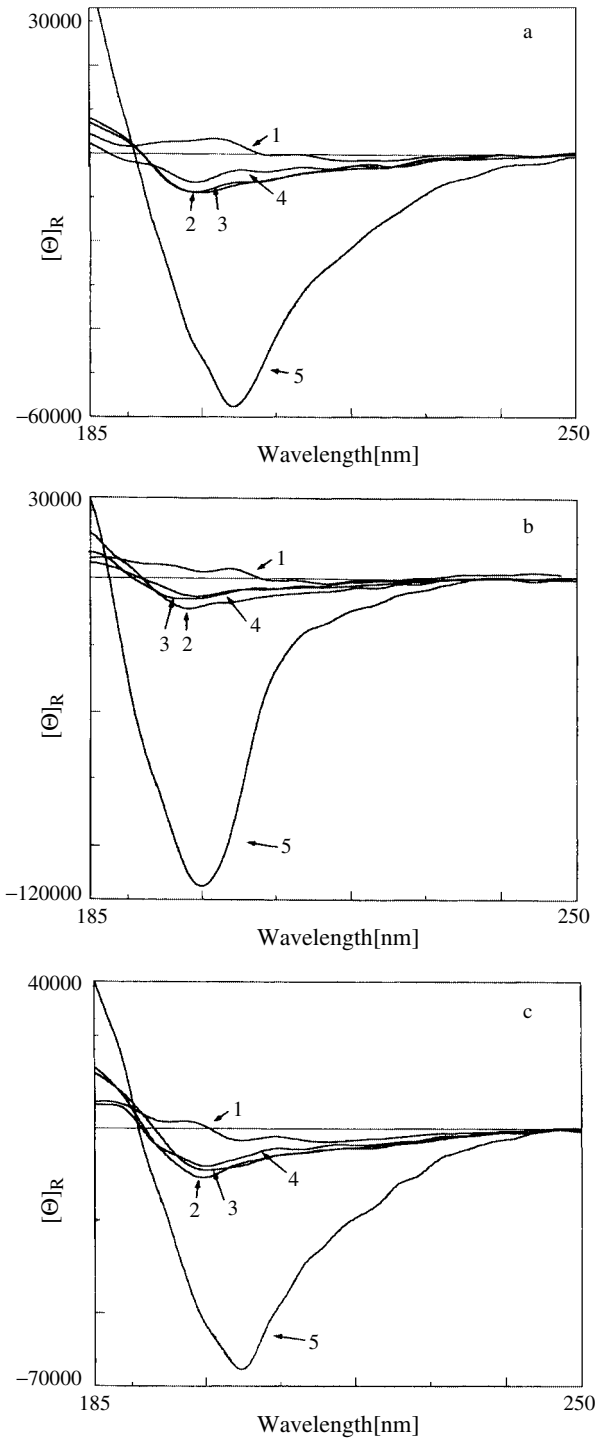

Figure 1 CD spectra of Boc-$(Asn)_n$-$NHCH_3$ ($n = 1–5$), series **I**, at 298 K. Peptide concentration ≈1 mM in: (a) water; (b) aqueous 30 mM SDS, (c) TFE-water (75:25 v/v).

a wide negative band at 197–200 nm and a shoulder at ≈220 nm, indicating the predominance of an essentially unordered conformer population. However, the widening of the band and the shoulder at ≈220 nm may suggest the presence of conformers with a more or less ordered structure. The CD spectra of $\mathbf{I}_5$ in water and aqueous TFE (Figure 1a and 1c) show a positive band below 185 nm, and a very strong negative band at 205 nm with a shoulder at ≈220 nm. These spectra resemble a class C spectrum [21] which, according to the definition proposed by Woody [22], is indicative of type I/III β-turn structures. The strong increase of the band intensity may arise from a decrease of conformational mobility probably through side chain-backbone interactions [23]. In 30 mM SDS solution (Figure 1b), the spectrum of $\mathbf{I}_5$ is blue-shifted relative to the spectra in water and in TFE, and shows a very strong negative dichroism at 200 nm. The spectrum resembles that of a random coil structure [24] but the negative ellipticity of the $\pi–\pi^*$ band is significantly greater than that generally expected for disordered conformations. The unusual pattern may be resulting from an intermolecular association of random type in the hydrophobic environment, with formation of rigid structure [12]. The oligomers $\mathbf{I}_{2-4}$, which on the basis of the solid state IR spectra exhibit a certain amount of γ turn conformation, according to the CD spectra show significant backbone flexibility in all the conditions examined. Comparison of the IR and CD data suggests that $\mathbf{I}_5$ exhibits a similar conformation both in the solid state and in aqueous solutions.

The CD spectra of the peracetylated glycopeptides (series **II**) in water-TFE (95:5 v/v) (Figure 2a) and in aqueous 30 mM SDS-TFE (95:5 v/v) (Figure 2b) are rather similar. The CD curves of $\mathbf{II}_1$ are characterized by a broad minimum at 210–220 nm, a maximum at about 200 nm and another minimum at 190 nm. The shapes, but not the amplitudes, of the spectra are similar to a type II β turn CD curve [22]. The monomer $\mathbf{II}_1$ probably adopts a structure involving interactions between the α-NH and the C=O of the acetamido function of the sugar moiety and between the urethane C=O and the NH of the asparagine side chain. NMR studies on model glycopeptides [25] showed the presence of this type of hydrogen bond when the backbone orientation is close to a type II β turn. In the same solvents, the oligomers $\mathbf{II}_{2-8}$ show a positive band under 185 nm, and a broad negative band between 195 and 230 nm. The intensities of the negative bands are essentially independent of the peptide chain length. Comparison of the experimental curves with

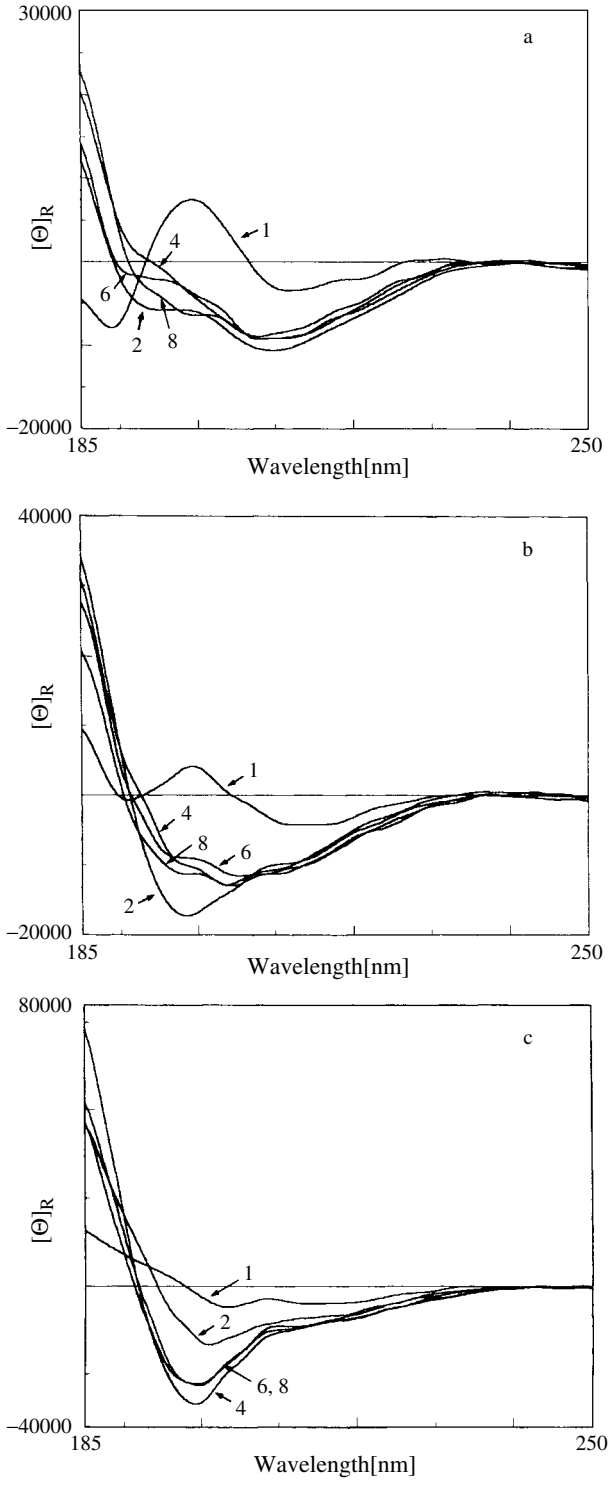

Figure 2 CD spectra of Boc-{[GlcNAc(Ac)$_3\beta$]Asn}$_n$-NHCH$_3$ (n = 1,2,4,6,8), series **II**, at 298 K. Peptide concentration ≈1 mM in: (a) water-TFE (95:5 v/v); (b) aqueous 30 mM SDS-TFE (95:5 v/v); (c) TFE.

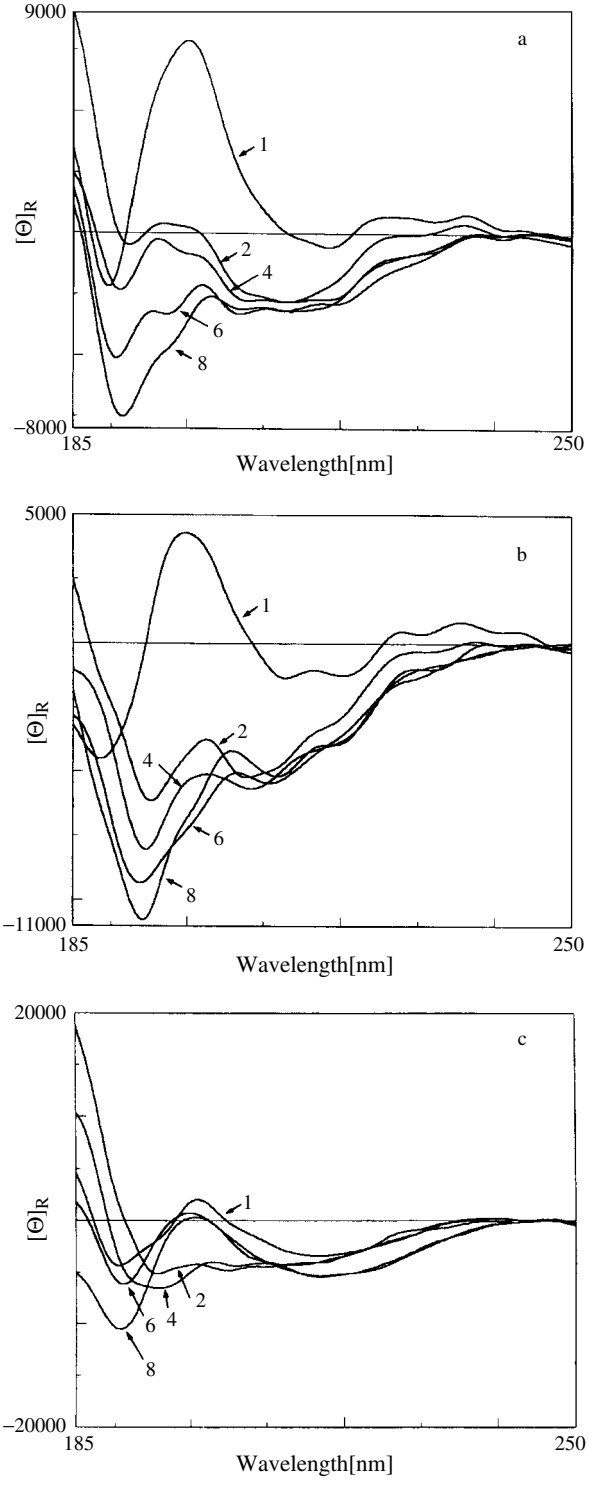

Figure 3 CD spectra of Boc-[(GlcNAcβ)Asn]$_n$-NHCH$_3$ (n = 1,2,4,6,8), series **III**, at 298 K. Peptide concentration ≈1 mM in: (a) water; (b) aqueous 30 mM SDS; (c) TFE-water (95:5 v/v).

the CD spectra calculated for different mixtures of type I β turns, type II β turns, and random conformations [23, 26] suggests mixture of β turn type I prevailing and random conformers. The CD spectra of $\mathbf{II}_{1-8}$ in TFE (Figure 2c) show a positive absorption below 185 nm, a negative band shifting from 205 nm to 198 nm on increasing the main chain length, and a large shoulder at about 215 nm. The dichroic absorption becomes more negative on going from $\mathbf{II}_1$ to $\mathbf{II}_4$ and exhibits a constant value for the higher oligomers. This feature could indicate the presence of more ordered structures starting from $\mathbf{II}_5$. The spectra of the oligomers $\mathbf{II}_{5-8}$ (spectrum of $\mathbf{II}_5$ not shown) are similar to class C spectra [21] correlated with prevailing type I β turns and random conformations.

The spectra of the deacetylated glycopeptides of the series **III** in water and in 30 mM SDS (Figures 3a and 3b) are substantially similar. In particular, the spectra of $\mathbf{III}_1$ in water (Figure 3a) in 30 mM SDS (Figure 3b) and in aqueous 95% TFE (Figure 3c) are quite similar. The CD spectra of $\mathbf{III}_{2-8}$ in water (Figure 3a) exhibit a positive ellipticity below 185 nm, a negative band at 192 nm, and a pronounced negative shoulder at approximately 215 nm. The intensity of the negative band increases regularly with the main chain elongation, whereas the shoulder intensity remains practically constant. The steady variation of the dichroic bands of the homopeptides $\mathbf{III}_{2-8}$ underlines the absence of conformational transitions. The spectra of $\mathbf{III}_{3-8}$ in SDS solution (Figure 3b) show only negative ellipticity in the 185–250 nm range. The $\pi-\pi^*$ bands, shifted to 195 nm, and the shoulders at about 210 nm, are more intense than the corresponding signals in water. The spectra, particularly those in SDS containing solution, are indicative of essentially unordered conformations, even if the widening of the shoulders might indicate the presence of ordered structures. The spectra of the oligomers $\mathbf{III}_{2-4}$ in TFE, are characterized by a positive band below 185 nm and a broad negative absorption in the $\pi-\pi^*$ and n–π^* regions, probably indicating the existence of a mixture of various reverse-turn conformers. Completely different CD curves are recorded for the oligomers $\mathbf{III}_{5-8}$ (spectrum of $\mathbf{III}_5$ not shown). The spectra show a negative band at 220 nm, a slightly positive band at 200 nm, and another negative band at 190 nm. Although the bands do not present typical intensities, the spectra are similar to class B spectra [22], which is indicative of type II β structures. In the structure stabilizing solvent (TFE), starting from $\mathbf{III}_5$, the deacetylated glycopeptides undergo a conformational variation due to the formation of a certain amount of type II β population besides unordered conformations.

Proton Nuclear Magnetic Resonance

^{1}H-NMR spectroscopy in DMSO-d_6 was used for characterization of the Boc-[Asn(Trt)]$_n$-NHCH$_3$ (n = 1–5) oligomers (Table 1) and the corresponding detritylated derivative (Table 2). An attempt to investigate the conformational features of the synthetic oligomers by ^{1}H-NMR performed at 300 MHz, in different solvents and at different temperatures, gave unsatisfactory results.

The protons of the peptide chain, specifically NH and Hα, experience very similar chemical shifts in all the three series of oligomers and are not significant enough to give conformational information. The spectra performed in H_2O for the series **I** and **III**, showed the NH proton of the first unit around 7.2 ppm and the others between 8.4 and 8.7 ppm. Likewise, Hα of the *N*- and *C*-terminal asparagine residue resonate at 4.4 and 4.6 ppm, respectively. The Hα protons of the other asparagine residues resonate all together at 4.5 ppm for the series **I** and at 4.7 ppm for the series **III**. The spectra of the oligomers of the series **II** were determined in TFE/H_2O and showed a similar trend. In the glycosylated series, the analysis of the NMR spectra in the typical region of the sugar rings (the anomeric protons resonate around 5.0 ppm, while the other protons are concentrated between 3.5 and 3.8) allowed us to obtain, but only for the dimer, the coupling constant values relative to protons from H-1′ to H-4′, which indicate a glucopyranosidic structure for the two glucose units and the β configuration for the glycosidic bonds ($J_{1'2'}$ = 10.0 Hz, $J_{2'3'}$ = 10.0 Hz, $J_{3'4'}$ = 8.5 Hz).

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