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# Synthesis and Conformational Studies of N-Glycosylated Analogues of the HIV-1 Principal Neutralizing Determinant[†]

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ABSTRACT: The principal neutralizing determinant (PND) of HIV-1 is found in the $V_3$ loop of the envelope glycoprotein. Antibodies elicited by peptides from this region, containing the GlyProGlyArgAlaPhe (GPGRAF) sequence, were able to neutralize diverse HIV-1 isolates [Javaherian et al. (1990) *Science 250*, 1590–1593]. The GPGR tetrapeptide was predicted to adopt a type II β-turn conformation. Earlier, we showed that glycosylation of synthetic T cell epitopic peptides at natural glycosylation sites stabilized β-turns [Ötvös et al. (1991) *Int. J. Pept. Protein Res. 38*, 467–482]. To evaluate the secondary structure modifying effect of the introduction of an N-glycosylated asparagine residue and to find a correlation between conformation and a possible PND potential, a series of glycopeptide derivatives, N(sugar)GPGRAFY-$NH_2$ (**4a–f**), have been prepared, together with the parent peptides GPGRAFY-$NH_2$ (**2**) and NGPGRAFY-$NH_2$ (**3**), by solid-phase peptide synthesis [sugars: (a) β-D-glucopyranosyl (Glc); (b) β-D-galactopyranosyl (Gal); (c) Glc-β(1→4)-Glc; (d) 2-acetamido-2-deoxy-β-D-glucopyranosyl (GlcNAc); (e) 2-acetamido-2-deoxy-β-D-galactopyranosyl (GalNAc); (f) GlcNAc-β(1→4)-GlcNAc; sugars are attached through a β(1→$N^\beta$) linkage to asparagine (N).] Peptides **2–4** were characterized by amino acid analysis, reversed-phase HPLC, and fast atom bombardment mass spectrometry. Circular dichroism (CD) and Fourier-transform infrared (FT-IR) spectroscopic studies were performed in trifluoroethanol (TFE) and water ($D_2O$ was used in FT-IR experiments). Nonglycosylated peptides showed significantly different CD spectra in aqueous and TFE solution. Moreover, a continuous spectral change was observed for all the peptides investigated when going from water to TFE. The chiral contribution of the aromatic side chains and acetamido sugars was also estimated. On the basis of CD and FT-IR evidence, the introduction of an N-glycosylated Asn residue does not destroy but rather stabilizes the suggested type II β-turn conformation of the PND peptide.

The principal neutralizing determinant (PND)[1] of HIV-1 is found in a disulfide-bridged loop ($V_3$) of the variable region of the envelope glycoprotein gp120 (LaRosa et al., 1990). Antibodies elicited to the amino acid backbone of PND peptides, containing the GlyProGlyArgAlaPhe (GPGRAF) sequence, were able to neutralize diverse HIV-1 isolates (Javaherian et al., 1990). This sequence was predicted to adopt a type II β-turn conformation encompassing the GPGR tetrapeptide (LaRosa et al., 1990). Since the PND sequence is surrounded by hypervariable regions at both sides, this type II β-turn structure is believed to be sensitive to environmental conditions.

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[1] Abbreviations: PND, principal neutralizing determinant; CD, circular dichroism; TFE, trifluoroethanol; FT-IR, Fourier-transform infrared; Glc, β-D-glucose; GlcNAc, 2-acetamido-2-deoxy-β-D-glucopyranose.

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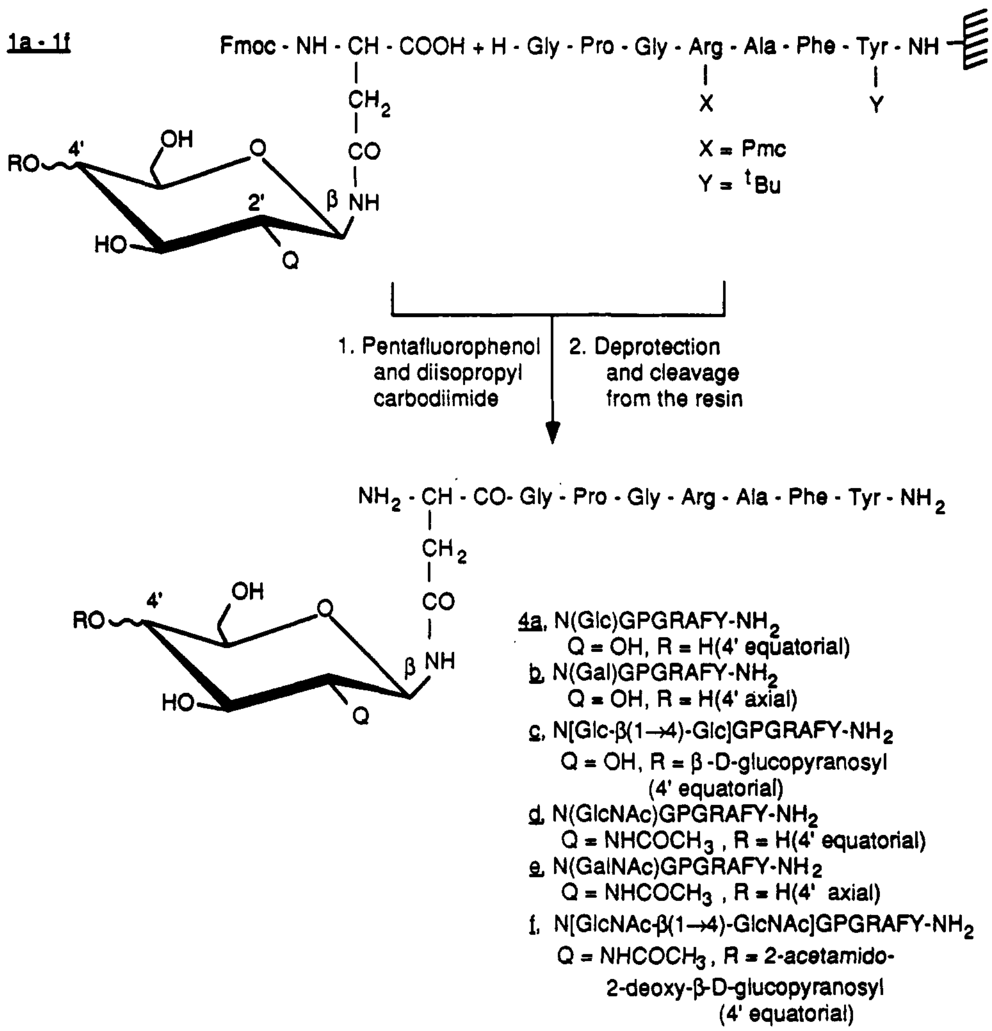

FIGURE 1: Scheme of synthesis of glycopeptides of PND.

The common feature of the major oligosaccharide antennae of *N*-glycoproteins is a mannotriosido-di-*N*-acetylchitobiose core linked to asparagine through a $\beta(1\rightarrow N^{\beta})$ glycosidic bond (Kobata, 1984).

Solid-phase synthesis is the method of choice to produce peptides suitable for screening epitopic regions of viral proteins. It was demonstrated earlier that $N^{\alpha}$-Fmoc-Asn[GlcNAc]-OH and $N^{\alpha}$-Fmoc-Asn[GlcNAc-$\beta$(1→4)-GlcNAc]-OH, with free sugar hydroxyls, can be used in glycopeptide synthesis (Ötvös et al., 1989, 1990). Recently, we described the preparation of $N^{\beta}$-glycosides of $N^{\alpha}$-Fmoc-asparagine containing a variety of simple mono- and disaccharides (Ürge et al., 1991). These reagents (**1a–f**, Figure 1) allow synthesizing model glycopeptides designed to study the influence of the presence of the acetamido group(s) and the configuration (gluco, galacto, etc.) of the first and second monosaccharide residues on epitopic recognition.

Since the N-glycosylation of the HIV-1 envelope protein gp120 could play a role in the virulence of the virus by blockage of T cell epitopic presentation, we decided to synthesize a series of *N*-glycopeptide analogues, N(sugar)-GPGRAFY-$NH_2$ (**4**), of the PND peptide GPGRAF in order to investigate the effect of glycosylation on the conformation of PND. Loosening or stabilization of the conformation of PND may result in altered immunological reactions or breaking tolerance among the diverse isolates. In many PND sequences, phenylalanine (F) is followed by tyrosine (Y), and the residue preceding the first glycine (G) appears not to have any immunological importance based on immunological studies. Earlier we demonstrated that incorporation of mono- and disaccharides into T cell epitopic peptides at natural glycosylation sites broke the $\alpha$-helical structure of the parent peptides and resulted in the formation of repeating type I $\beta$-turns or a $3_{10}$-helix (Ötvös et al., 1991). We expected that addition of sugars at artificial positions would also stabilize the type II turn of the PND sequence in a sugar structure-directed manner.

This paper reports the synthesis (Figure 1) and chemical and conformational characterization of peptides GPGRAFY-$NH_2$ (**2**) and NGPGRAFY-$NH_2$ (3) and *N*-glycopeptides N(sugar)GPGRAFY-$NH_2$ (**4**).

## MATERIALS AND METHODS

*Peptide Synthesis, Purification, and Characterization.* Peptides (consisting of L-amino acids) were made on a SAM 2 automated synthesizer. Standard Fmoc-synthetic protocol was used for peptide chain assembly (Atherton et al., 1978) on a modified methylbenzhydrylamine resin (Bachem, Philadelphia, PA). Fmoc-Asn(sugar)-OH derivatives (**1a–f**) (Ürge et al., 1991) were used for carbohydrate incorporation (Figure 1). A trifluoroacetic acid–thioanisole solution (95:5 v/v) was used to detach peptides from the solid support. Peptides were isolated and purified by reversed-phase high-performance liquid chromatography using a Beckman ODS $C_{18}$ column and a 1.33%/min linear gradient of acetonitrile in 0.1% aqueous trifluoroacetic acid (starting at 6.7 min). After this purification, peptides **2–4** are present in the form of trifluoroacetate salts (see FT–IR studies). The structure of the peptides was

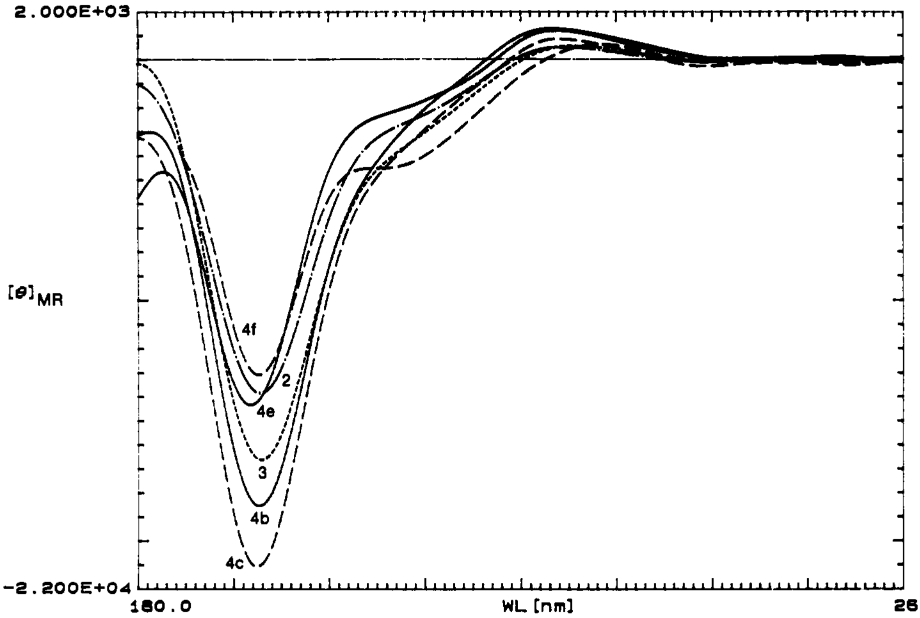

FIGURE 2: Circular dichroism spectra in water of GPGRAFY-$NH_2$ (**2**) (—·—), NGPGRAFY-$NH_2$ (**3**) (···), and N(sugar)GPGRAFY-$NH_2$ derivatives **4b** and **4e** (—) and **4c** and **4f** (‐‐); $c$ = 0.5 mg/mL. **4d** shows practically the same CD spectrum as **4e** (—).

Table I: Characterization of PND Peptides and Their N-Glycosylated Analogues

| no. | abbreviated name | RP-HPLC, retention time (min) | fast atom bombardment MS |
|---|---|---|---|
| **2** | GPGRAFY-$NH_2$ | 24.96 | M + Na, 790 |
| **3** | NGPGRAFY-$NH_2$ | 24.87 | M + Na, 902 |
| **4a** | N(Glc)GPGRAFY-$NH_2$ | 24.26 | M + Na, 1065 |
| **4b** | N(Gal)GPGRAFY-$NH_2$ | 24.27 | M + H, 1043 |
| **4c** | N[Glc-β(1→4)-Glc]-GPGRAFY-$NH_2$ | 24.29 | M, 1204 |
| **4d** | N[GlcNAc]GPGRAFY-$NH_2$ | 24.67 | M, 1083;<br>M + Na, 1105 |
| **4e** | N[GalNAc]GPGRAFY-$NH_2$ | 24.52 | M, 1083 |
| **4f** | N[GlcNAc-β(1→4)-GlcNAc]-GPGRAFY-$NH_2$ | 24.10 | M, 1286 |

verified by fast atom bombardment mass spectroscopy on a VG Analytical ZAB-E instrument at the Department of Chemistry of the University of Pennsylvania (Philadelphia, PA). Data are found in Table I. The structures were verified by amino acid analyses and were within 5% of the expected values.

*Circular Dichroism* (*CD*) *Measurements.* CD spectra were taken on a Jasco J720 circular dichrograph at room temperature in a 0.2-mm path-length cell. Double-distilled water and nuclear magnetic resonance spectroscopy grade trifluoroethanol (TFE) (Aldrich, Milwaukee, WI) were used as solvents. The peptide concentration was 0.5 mg/mL as determined by amino acid analysis. The mean residue ellipticity ($[\theta]_{MR}$) is expressed in deg $cm^2$ $dmol^{-1}$ by using a mean residue weight calculated from the molecular weight by dividing with the number of the amino acid residues in the peptide or glycopeptide.

*FT-IR Spectroscopy.* Infrared spectra were recorded on a Digilab FTS-60 instrument at room temperature. Measurements were performed in ~0.5 mg/mL solutions (100 μL) of $D_2O$ or TFE. For each spectrum, 512 interferograms were coadded and Fourier-transformed to give a resolution of 2 $cm^{-1}$. Overlapping infrared bands were resolved using Fourier self-deconvolution procedures (Mantsch et al., 1988).

*Enzyme-Linked Immunoadsorbent Assay.* Binding of 0.5-μg amounts of the synthetic peptides and glycopeptides was tested with 1:50 and 1:500 dilutions of HIV-seropositive sera samples on Immunolon 2 plates. A 1:2000 dilution of goat anti-human immunoglobulin horseradish peroxidase conjugate was used as a secondary antibody. Color development was made with 3,3′,5,5′-tetramethylbenzidine dihydrochloride and was measured at 450 nm.

## RESULTS

*CD Spectroscopic Studies.* CD spectra of peptides **2** and **3** and glycopeptides **4a–f** were measured in TFE, water, and TFE–water mixtures. In water the spectra of **2–4** show a strong negative band between 190 and 195 nm (Figure 2) as a sign of the predominance of nonperiodic (unordered) conformer populations (Woody, 1985). The decreased amplitude of the negative band in the spectra of glycopeptides **4d–f** with 2- and 2′-acetamido groups can be attributed to the positive spectral contribution above 190 nm of oligosaccharides containing an acetamido group in position 2 (Figure 3). The spectra of **2–4** are markedly changed when going from water to TFE. In TFE they show a broad negative band and a positive one below 200 nm (Figure 4). The negative band is composed of two constituents, an intense band between 210 and 220 nm and a second band or shoulder near 230 nm (Figure 4). The magnitude of the positive band definitely depends on the structure of the molecule. The highest-intensity positive bands are found in the spectra of 2-acetamido sugar derivatives **4d–f**. Peptide NGPGRAFY-$NH_2$ (**3**) and glycopeptides with a galactosyl (**4b**) or cellobiosyl residue (**4c**) show positive bands of comparable and intermediate magnitude. The positive band of the peptide without sugar is, however, red-shifted and somewhat broader. The lowest-intensity positive CD is observed in the spectrum of the glucosyl derivative **4a**; excluding GPGRAFY-$NH_2$ (**2**), the positive band of its spectrum has negligible intensity.

The spectra of nonglycosylated peptides **2** and **3** and glycopeptides **4a–f** show a continuous spectral transition between the two extremes measured in water and TFE as shown for the cellobiose-containing peptide (**4c**) in Figure 5. This suggests that, in TFE–water mixtures, the conformational equilibria of all peptides contain two major populations of conformers.

The chiral contribution of the aromatic side chains of Phe and Tyr and that of the sugar residue in glycopeptides **4a–f** were estimated by measuring the CD spectra of *N*-acetylphenylalanyltyrosinamide (Ac-FY-$NH_2$, **5**), 1-acetamido-1-

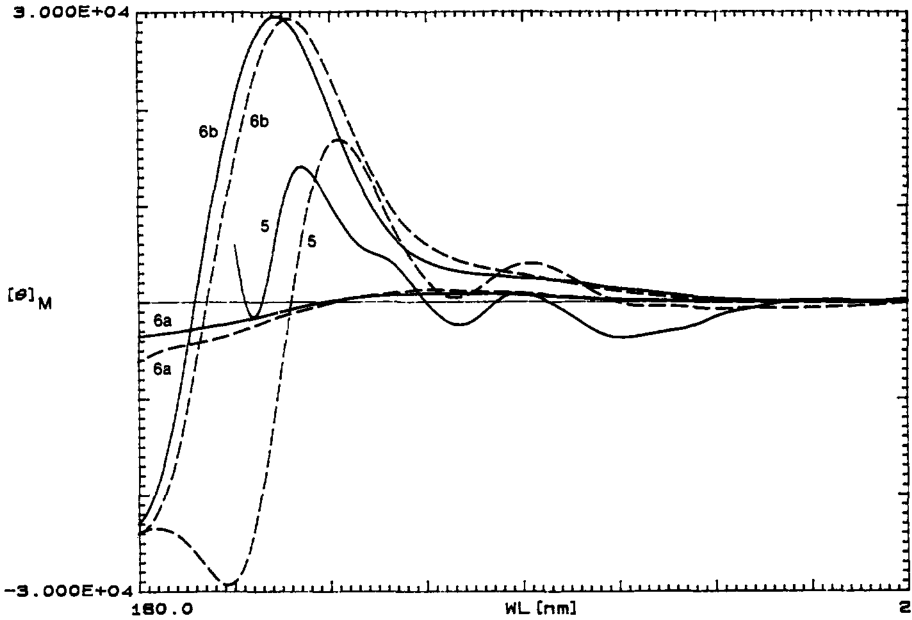

FIGURE 3: Circular dichroism spectra of Ac-FY-$NH_2$ (**5**), 1-acetamido-1-deoxy-β-D-galactopyranose (**6a**) and 1,2-diacetamido-1,2-dideoxy-β-D-glucopyranose (**6b**) in TFE (—) and water (--); $c = 0.1$–$0.5$ mg/mL. Ellipticities are given on a molar basis ($\theta_M$).

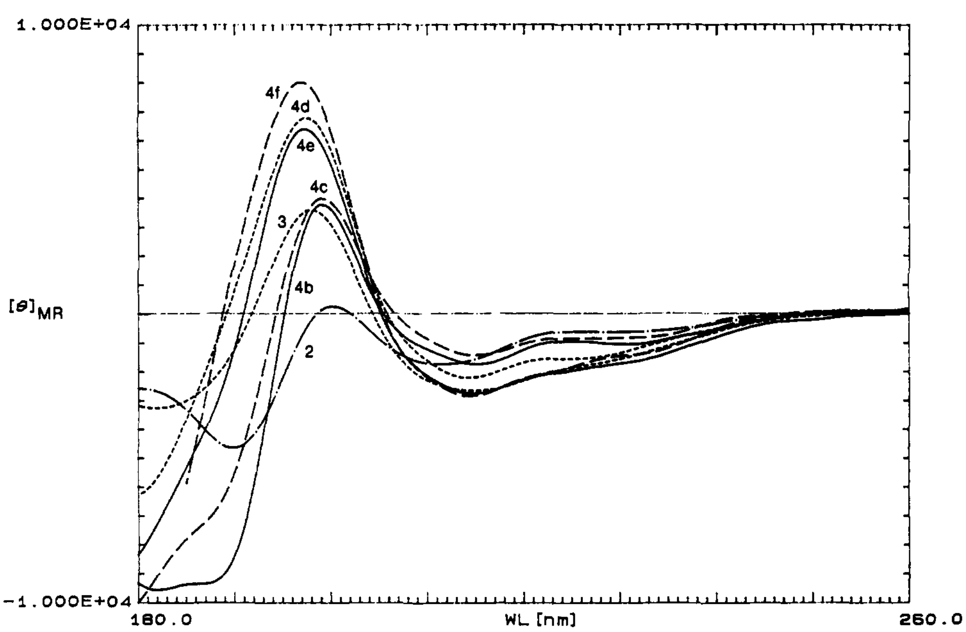

FIGURE 4: Circular dichroism spectra in TFE of GPGRAFY-$NH_2$ (**2**) (—·—), NGPGRAFY-$NH_2$ (**3**) (···), and N(sugar)GPGRAFY-$NH_2$ derivatives **4b** and **4e** (—), **4d** (···), and **4c** and **4f** (--); $c = 0.5$ mg/mL.

deoxy-β-D-galactopyranose (**6a**), and 1,2-diacetamido-1,2-dideoxy-β-D-glucopyranose (**6b**) (Figure 3). Subtracting the CD spectrum of **5** from those of **2–4** results, at the first approximation of the CD of peptides and glycopeptides terminated by alanine. (This procedure neglects the CD effect of the interaction of aromatic side chains with the remaining part of the molecule.) As shown in Figure 6, the spectrum of N[GlcNAc]GPGRAFY-$NH_2$ (**4d**), obtained after the aromatic correction, features bands which compare in position and magnitude of the negative band with that measured for the full-length peptide in TFE.

The CD spectrum of the diacetamido sugar **6b** is marked by a positive band near 195 nm. It has almost the same position and intensity in TFE and water (Figure 3). Substracting the spectrum of the diacetamido sugar **6b** in TFE from those of 2- and 2′-acetamido derivatives **4d–f** in TFE gives rise to spectra with a red-shifted positive band of reduced intensity (Figure 6). The same correction of the spectra measured in water leads to an intensity increase of the negative band. (Again, corrections by the spectrum of sugars neglect any CD contribution which might arise from the interaction of the sugar with the backbone and side-chain groups of the peptide.)

1-Acetamido-1-deoxy-β-D-glucopyranose and 1-acetamido-1-deoxy-β-D-galactopyranose have low-intensity CD spectra in both TFE and water (Figure 3). Correcting the spectra of glycopeptides without acetamido groups **4a–c** by that of 1-acetamido sugars has practically no effect on the band positions and intensities.

Most notably, the CD spectra of 1-acetamido and 1,2-diacetamido sugars do not show a significant solvent dependence (Figure 3). Consequently, the transition between the spectra measured in water and TFE is due to a marked change of the backbone conformation. On the basis of the increased intensity of the positive band in the spectra of peptides **3** and **4**, the elongation of peptide **2** with Asn or N-glycosylated Asn ap-

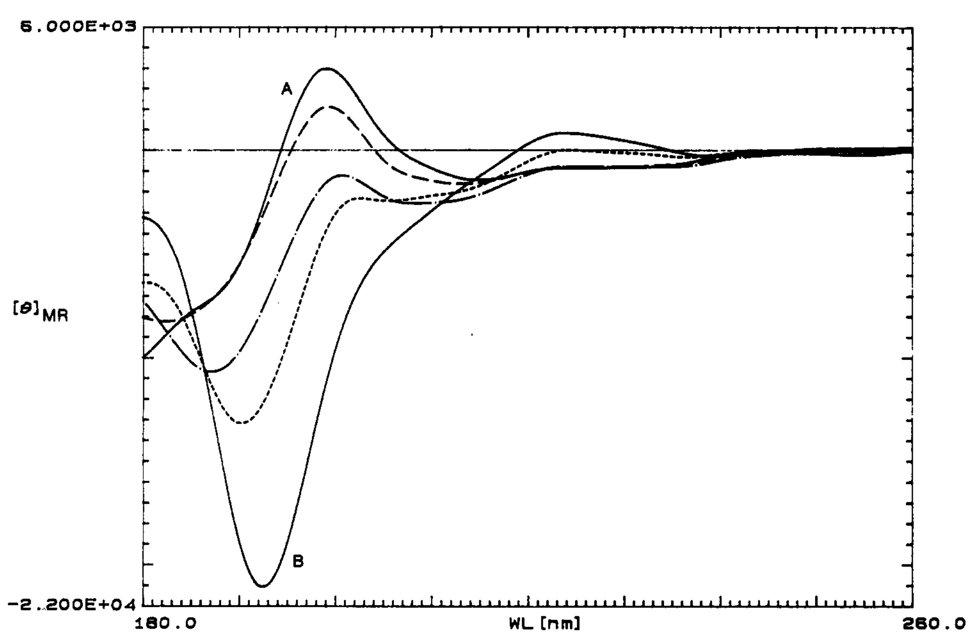

FIGURE 5: Circular dichroism spectra of N[Glc-β(1→4)-Glc]GPGRAFY-NH$_2$ (**4c**) in TFE (—, A), 75% TFE–water (– –), 50% TFE–water (–•–), 25% TFE-water (···), and water (—, B); $c$ = 0.5 mg/mL.

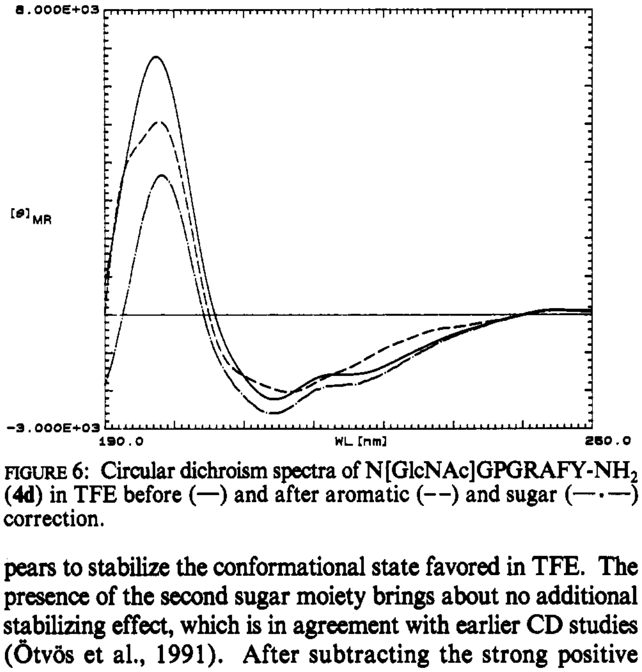

FIGURE 6: Circular dichroism spectra of N[GlcNAc]GPGRAFY-NH$_2$ (**4d**) in TFE before (—) and after aromatic (– –) and sugar (—•—) correction.

pears to stabilize the conformational state favored in TFE. The presence of the second sugar moiety brings about no additional stabilizing effect, which is in agreement with earlier CD studies (Ötvös et al., 1991). After subtracting the strong positive spectral contribution of the 1,2-diacetamido sugar, the spectra of peptides with the acetamido group(s), **4d–f**, compare with the corrected spectra of glycopeptides **4a–c** having an OH group in position 2 (Figure 6).

*FT-IR Spectroscopic Studies.* Infrared spectra of the peptides **2**, **3**, and **4a–f** were measured both in D$_2$O and in TFE solution. Figure 7A illustrates the spectrum of the heptapeptide **2** in water (D$_2$O). Since the peptides were eluted with a solvent mixture containing trifluoroacetic acid, all peptides contain the counterion trifluoroacetate. After subtracting the band at 1673 cm$^{-1}$, due to the antisymmetric COO$^-$ stretching vibration of trifluoroacetate, the spectra in D$_2$O are dominated by a strong band at 1646 ± 2 cm$^{-1}$ (Figure 7B). This band can be assigned to a peptide backbone without any ordered secondary structure (Surewicz & Mantsch, 1988). The weak bands near 1612 and 1587 cm$^{-1}$ are due to the arginine side chain, while the band at 1516 cm$^{-1}$ and a second band at 1610 cm$^{-1}$ arise from the tyrosine and phenylalanine side chains.

In TFE, the FT-IR spectra resemble each other but differ

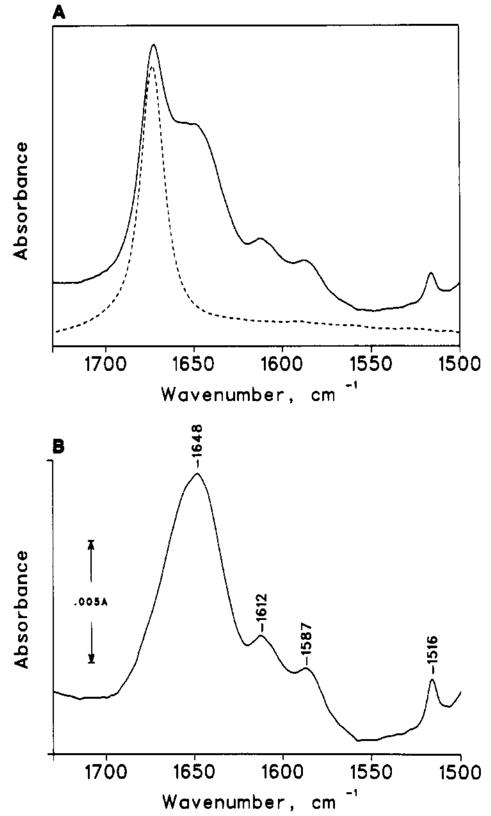

FIGURE 7: (A) Infrared spectra as D$_2$O solutions of the heptapeptide GPGRAFY-NH$_2$ (**2**) (solid trace) and of sodium trifluoroacetate (broken trace). (B) Infrared spectrum of the peptide in (A) after subtraction of the spectrum of trifluoroacetate. The same spectrum was obtained after exchange of the counterion trifluoroacetate with bicarbonate on an Amberlite IR-45 minicolumn.

significantly from those measured in D$_2$O. The trifluoroacetic acid-subtracted and resolution-enhanced spectra all have a major band at 1661 cm$^{-1}$ and two other bands at 1679 and 1636 cm$^{-1}$ (Figure 8). Again, the weaker bands between 1618 and 1580 cm$^{-1}$ and the narrow band at 1518 cm$^{-1}$ are due to side-chain vibrations (arginine, tyrosine, and phenylalanine). The 1661-cm$^{-1}$ band can be assigned to weakly H-bonded (solvated) amide carbonyls and the bands at 1679 and 1636

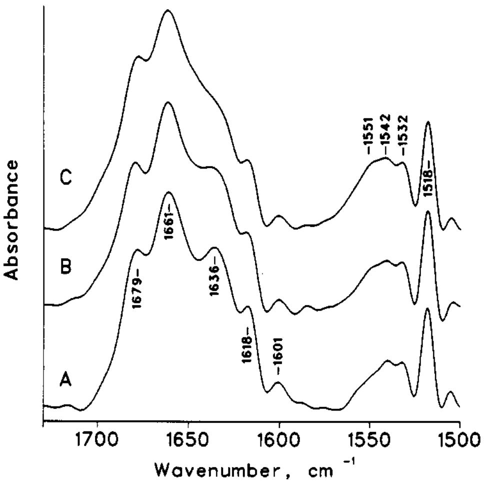

FIGURE 8: Infrared spectra as solutions in TFE of **2** (A), **4a** (B), and **4d** (C) after band narrowing by Fourier self-deconvolution, using a bandwidth of 15 $cm^{-1}$ and a resolution enhancement factor of 2.

$cm^{-1}$ to turns. The 1636-$cm^{-1}$ band is located in the region of $\beta$-pleated sheets, which suggests the formation of a strong intramolecular H-bond. From the force field calculations of Krimm et al. (1986), a type II $\beta$-turn is expected to be higher in energy than the type I or III $\beta$-turns. Thus, a band below 1640 $cm^{-1}$ may be indicative of the occurrence of type II $\beta$-turn conformation. On the basis of recent infrared studies on bridged cyclic models with well-characterized $\beta$-turns (Mantsch et al., unpublished results), a band at 1640 ± 3 $cm^{-1}$ can be assigned to the acceptor amide carbonyl of both type I and type II $\beta$-turns. Accordingly, infrared spectroscopy reflects only the presence of a strongly H-bonded $\beta$-turn, not its type (geometry). The strong band near 1660 $cm^{-1}$ suggests that the majority of amide carbonyls is exposed to the solvent and takes part in weak H-bondings. The ratio of band intensities of **2** and **3** is similar to that in glycopeptides **4a–f**, which may indicate a similar population of conformers with turn structures in the TFE solution of these peptides. As shown in Figure 8, the amide I bands appear in the same positions; however, in the glycopeptides **4a** and **4d**, the bands (particularly the band at 1636 $cm^{-1}$ assigned to $\beta$-turns) become broader.

The broad band contour with peaks at 1532, 1542, and 1551 $cm^{-1}$ represents the amide II band (primarily an amide NH bending vibration). In $D_2O$, this band is shifted below 1500 $cm^{-1}$ due to the NH to ND exchange. The observation of three amide II bands and three amide I bands in TFE (whereby the 1532-$cm^{-1}$ band can be correlated with the 1637-$cm^{-1}$ band, the 1542-$cm^{-1}$ band with the 1661-$cm^{-1}$ band, and the 1551-$cm^{-1}$ band with the 1679-$cm^{-1}$ band) reflects the presence of three different types of amide bonds.

*Enzyme-Linked Immunoadsorbent Assay.* Neither the synthetic peptides (**2**, **3**) nor the glycopeptides (**4**) were able to cross-react with the serum of an HIV-infected patient. The same serum recognized the entire V3 loop and its 23–24 amino acid long N-terminal and C-terminal fragments. This recognition was altered when the V3 loop was glycosylated at its natural glycosylation sites. This suggests—in accordance with expectations—that although the GPGRAF sequence is the dominant recognition site for the V3-directed antibodies, the hexapeptide alone is too short to be recognized without the flanking sequences.

## DISCUSSION

Recently, we reported a general method to incorporate the starting carbohydrate structure of natural glycosylation sites into T cell epitopic peptides (Ötvös et al., 1990) and also the protocol to prepare reagents for glycopeptide synthesis from almost any reducing sugar (Urge et al., 1991). The structures of various sugars show a wide diversity range, and these carbohydrates seem to be good candidates for de novo design of new peptide variants.

Solid-phase synthesis is the method of choice to generate large numbers of peptide analogues to screen epitopic regions (Barany et al., 1987). The major concerns in synthesis of glycopeptides are the proposed acid lability of the glycosidic bond and reduced coupling efficiency of glycosylated asparagine residues due to steric hindrance (Ötvös et al., 1989). Earlier, we found that the sugars already on the resin do not interfere with the coupling efficiency of the consecutive amino acids. For this reason, no particular problems could be expected if we had continued the building of the peptide chain further to the N-terminus of the V3 loop. Generally, incorporation of asparagine with disaccharides was less effective than that with the appropriate monosaccharides, but all six crude products were well within the easy purification range. Reversed-phase chromatography appears to be a suitable tool for purification of the glycopeptides. All glycopeptides could be eluted from the column earlier than the nonglycosylated parent molecules, and the decrease in retention times was only dependent on the number of added carbohydrate moieties (Table I).

The CD spectra of the PND peptides (**2**, **3**) and glycopeptides (**4**) in TFE with a negative band near 220 nm and a positive band below 200 nm resemble the class B CD spectrum, which is indicative of $\beta$-turns (Woody, 1974).

On the basis of a theoretical study on the CD contribution of $\beta$-turns, Woody predicted that the CD spectra of standard type I ($\phi_{i+1} = -60°$, $\psi_{i+1} = -30°$, $\phi_{i+2} = -90°$, $\psi_{i+2} = 0°$) and type II $\beta$-turns ($\phi_{i+1} = -60°$, $\psi_{i+1} = 120°$, $\phi_{i+2} = 80°$, $\psi_{i+2} = 0°$), which were previously described by Venkatachalam (1968), should resemble those of $\beta$-sheets but with red-shifted maxima (class B spectrum: a negative band between 220 and 230 nm, a stronger positive band above 200 nm, and a second negative band near 190 nm) (Woody, 1974). Comparative CD spectroscopic and conformational studies on models of $\beta$-turns have shown that type I $\beta$-turns have an $\alpha$-helix-like (class C) CD curve rather than class B (Gierasch et al., 1981; Bandekar et al., 1982; Hollósi et al., 1987). The correlation between a type I $\beta$-turn and a helix-like (class C) CD spectrum is also supported by more recent theoretical (Sathyanarayana & Applequist, 1986) and X-ray crystallographic studies (Perczel et al., 1991) on models of $\beta$-turns. Thus, CD spectroscopy allows distinguishing between type I and type II $\beta$-turns, but only if their population in the equilibrium in solution is high enough.

Assuming an additivity of the chiral contribution of the peptide backbone, the sugar residue, and the aromatic side chains, the CD spectra measured in TFE were corrected by subtracting the spectrum of Ac-FY-$NH_2$ and/or that of 1,2-diacetamido-1,2-dideoxy-$\beta$-D-glycopyranose. 1-Acetamido-1-deoxy sugars have no significant chiral contribution in the 180–260-nm spectral range (Figure 3). After the aromatic correction, the class B character of the spectra of NGPGRAFY-$NH_2$ (**3**) and its N-glycosylated derivatives **4** was still preserved (Figure 6). The aromatic residues are present in both the PND peptides (**2**, **3**) and their glycosylated derivatives (**4**). The preserved class B character of the corrected CD spectra gives support to the idea that in octapeptide **3** and especially glycopeptides **4** the type II $\beta$-turn conformational state is highly populated in TFE solution. On the basis of CD data, the amount of this conformer is less in the TFE solution of heptapeptide **2**. As revealed by the CD spectra of glycopeptides **4d–f**, corrected by the chiral contribution of the sugar, 2-acetamido-2-deoxy sugars do not appear to have an extra turn-stabilizing effect (Figure 6).

Generally, results of FT-IR spectroscopic studies are in agreement with those of CD measurements. The predominant amide I band appearing at 1646 ± 2 $cm^{-1}$ suggests an unordered peptide backbone in $D_2O$ solution, while the band at 1636 $cm^{-1}$ in TFE can be correlated with the strongly H-bonded carbonyl of a $\beta$-turn, likely type II. However, the relative amount of the $\beta$-turn conformation cannot be rigorously determined by means of CD and FT-IR spectroscopy.

Our comparative CD and FT-IR spectroscopic studies, indicating the high population of a type II $\beta$-turn conformer in TFE solution of **3** and **4**, are in agreement with X-ray crystallography-based data on proteins (Chou & Fasman, 1979) which show that the dipeptide PG has a high frequency of occurrence in the central ($i$+1 and $i$+2) position of a type II $\beta$-turn. Similarly, phase-sensitive 2D NMR studies on proline-containing pentapeptides, representing one of the immunodominant regions of influenza virus hemagglutinin, have shown that a substantial population of the type II $\beta$-turn conformer is present even in aqueous solution (Dyson et al., 1988). The CD spectrum of one of the peptides, YPGDV, was found to show a positive band near 206 nm and a minimum near 190 nm, which are also indicative of the presence of a $\beta$-turn. The high population of the $\beta$-turn conformer in an aqueous solution of YPGDV was attributed to a turn-stabilizing ionic interaction between the oppositely charged $NH_3^+$ (N-terminal) and $COO^-$ (Asp) groups. However, in the case of PND peptides, there is no possibility for ionic interactions, and in aqueous solution the H-bonding(s) between the backbone and side-chain functional groups (amide, guanidinium, and hydroxyl) is (are) not effective enough to fix the folded conformation of the peptide. The peptides alone were not recognized by the V3-directed antibodies because of their shortness. The structural changes due to glycosylation, however, would result in glycopeptides with diverse $\beta$-turn structures if attempts to synthesize longer peptides (20–25 amino acid residues) were made. Provided the conformation of the entire V3 loop is regulated (at least partly) by the conformation of the PND sequence at the tip of the loop, the wide range of the conformational variants of the longer glycopeptides will be recognized by a similarly wide range of structure-dependent antibodies, originating from different HIV isolates.

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