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# Feglymycin, a Novel Inhibitor of the Replication of the Human Immunodeficiency Virus

## Fermentation, Isolation and Structure Elucidation

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The novel peptide feglymycin has been isolated from cultures of *Streptomyces* sp. DSM 11171 by solid phase extraction, size exclusion chromatography and repeated reversed-phase chromatography. The molecular weight was found to be 1900.90 g/mol and the molecular formula is $C_{95}H_{97}N_{13}O_{30}$. Feglymycin contains 13 amino acids of which four are 3-hydroxyphenylglycine and five are 3,5-dihydroxyphenylglycine residues. The structure of the linear peptide has been determined by $^{1}H$ and $^{13}C$ NMR spectroscopy. The sequence was confirmed by the observed mass spectroscopic fragmentation pattern. As well as having weak antibacterial activity, feglymycin inhibits the replication of the human immunodeficiency virus (HIV) *in vitro*.

It is still not possible to cure diseases caused by infection with the human immunodeficiency virus (HIV)[1]. Many synthetic compounds have been developed and are in use in the treatment of acquired immunodeficiency syndrome (AIDS), but due to the increasing resistance of HIV new drugs constantly have to be developed[1]. Several peptide inhibitors of virus replication have been found in microbial cultures, including RPI-856 A, B, C and D[2], siamycin I and II[3], Mer-N5075A[4], RP71955[5] (identical to aborycin[6]), chloropeptins I and II[7], complestatin[8], and FR901724[9]. These compounds either inhibit the retroviral protease or the binding of the HIV envelope glycoprotein gp120 to the CD4 molecule on the surface of the target human cell.

In this communication we report on the novel peptide feglymycin isolated from *Streptomyces* sp. DSM 11171 which strongly inhibits the syncytium formation typical of HIV infections[9] in cell cultures. The fermentation of *Streptomyces* sp. DSM 11171 and the isolation and structure elucidation of feglymycin are described.

## Materials and Methods

### Fermentation

To prepare the spore suspension, 100 ml of nutrient solution [20 g of malt extract, 2 g of yeast extract, 10 g of glucose, 0.5 g of $(NH_4)_2HPO_4$ in 1 liter of tap water, pH before sterilization: 6.0] in a 500 ml sterile Erlenmeyer flask was inoculated with the strain *Streptomyces* sp. DSM 11171 and incubated on a rotating shaker for 72 hours at 25°C and 140 rpm. 120 ml of culture fluid was then uniformly distributed in a sterile 500 ml Erlenmeyer flask containing a nutrient medium of oatmeal infusion (2.0 g/liter) to which 15 g of agar/liter had been added for solidification, and decanted. The cultures were incubated at 25°C for 10 to 14 days. The resulting spores

in the flask were rinsed with 500 ml of deionized water containing one drop of Triton X100 (Serva), immediately reused or stored at −22°C in 50% glycerol or at −140°C in 10% dimethyl sulfoxide.

Shake cultures were prepared in sterile 500 ml Erlenmeyer flasks containing 100 ml of the nutrient solution described above, which were inoculated with 0.2 ml of spore suspension and incubated in the dark at 25°C on a shaker operating at 140 rpm. The maximum production of feglymycin was achieved after about 72 hours. A 72 hour old immersion culture (incubation quantity about 5%) was used to inoculate 10 liter fermenters.

The 10 liter fermenters (Biostat V, B. Braun Melsungen, Germany) were operated under the following conditions: nutrient medium: 2% oat flakes, 0.25% trace element solution. Trace elements: $CaCl_2 \cdot 2H_2O$ 0.3%, Fe(III) citrate 0.1%, $MnSO_4$ 0.02%, $ZnCl_2$ 0.01%, $CuSO_4 \cdot 5H_2O$ 0.002%, sodium tetraborate 0.02% and sodium molybdate 0.001% in water. The incubation time was 48 hours, temperature 28°C, stirrer speed 200 rpm, and the air flow rate was 5 liters/minute.

## Isolation

27 liters of culture broth was centrifuged and the cell material (~1.1 liter) was extracted by stirring twice with 2.2 liter volumes of methanol. The combined extracts were concentrated *in vacuo* and dried, and the dry matter was treated with diethyl ether. The degreased residue (40 g) was dissolved in water and applied to a 3 liter column packed with the adsorption resin MCI Gel CHP20P (11.3 cm × 30 cm). Elution was carried out with a solvent gradient from 5 to 50% isopropanol in water and the column eluent was checked by HPLC. The feglymycin-containing fractions were collected, concentrated *in vacuo*, and freeze-dried (3.2 g). Three gram were subjected to column (10 cm × 50 cm) chromatography on Fractogel TSK HW-40 (E. Merck, Darmstadt) and developed with 50% acetonitrile in 10 mM sodium phosphate buffer, pH 7.0. The active fractions were combined and concentrated under reduced pressure. The concentrate was applied to a Nucleosil $12C_{18}$AB column (Macherey-Nagel, Düren, Germany) (32 mm × 250 mm) and was separated using a gradient from one liter 5% acetonitrile to one liter of 30% acetonitrile, each in 10 mM potassium phosphate buffer, pH 7. The final purification and desalination was carried out on Nucleosil $12C_{18}$AB, eluting with 25% acetonitrile in 0.05% trifluoracetic acid to give a white powder (18 mg).

Feglymycin could be detected during HPLC by monitoring at 210 nm. A LiChroCART 250-4 Superspher 100 RP-18 e (E. Merck) column was used and the mobile phase was 25% acetonitrile in 0.1% trifluoracetic acid. Feglymycin had a retention time of 12.9 minutes at a flow rate of 1.0 ml/minute.

## NMR Measurements and Sample Preparation

NMR samples were prepared by dissolving either 7 mg or 15 mg of feglymycin in 0.5 ml dimethylsulfoxide-$d_6$. Wilmad NMR tubes (grade 507) with a diameter of 5 mm were used.

All spectra were recorded on a BRUKER ARX500 spectrometer operating at 500.13 MHz and 125.76 MHz for $^1H$ and $^{13}C$, respectively. A 5 mm inverse broadband probe was used. The sample temperature was set at 300 K. All experiments were performed in phase-sensitive mode using standard pulse sequences employing time proportional phase incrementation (TPPI) for quadrature detection in $F_1$[10]. For the homonuclear experiments (double quantum filtered [DQF]-COSY[11,12], $^1H$-$^1H$ total correlation spectroscopy (TOCSY)[13], NOESY[14,15]) the spectral width was set to 14 ppm in both dimensions, $F_1$ and $F_2$. In DQF-COSY and TOCSY experiments 512 increments in $t_1$ were recorded with 2048 complex data points in $t_2$. 32 transients were averaged for each $t_1$ value. In the TOCSY experiments an MLEV17 spin lock sequence was applied for 70 ms at a $B_1$ field strength of 10 kHz[16]. Delays corresponding to the 90° pulse width were inserted as specified by GRIESINGER *et al.*[17]. NOESY spectra were recorded with 1024 increments in $t_1$ and 2048 complex data points in $t_2$. 16 transients were averaged and mixing times of 50, 100, and 200 ms were used.

To record the heteronuclear single quantum correlation (HSQC) spectra[18] 512 increments with either 16 or 48 scans each and 2048 complex data points in $t_2$ were collected using sweep widths of 14 ppm in the proton dimension and 150 ppm in the carbon dimension. A bilinear rotation decoupling (BIRD) pulse was applied to suppress magnetization of protons bound to $^{12}C$. The heteronuclear multiple bond correlation (HMBC) spectra[19] were acquired with a sweep width of 14 ppm in the proton dimension and 200 ppm in the carbon dimension. 1024 increments in $t_1$ and 2048 complex data points in $t_2$ were recorded. The delay for the evolution of long-range correlations was set to correspond to $^nJ_{CH}$ coupling constant values of 7 and 10 Hz. Up to 48 scans per increment were collected.

Prior to Fourier transformation all time domain data were subjected to apodization using adjusted sine and

squared sine bell window functions. The software packages XWINNMR and AURELIA supplied by Bruker, Rheinstetten were used for data processing.

Mass spectrometry

The mass spectra were recorded on an ion trap mass spectrometer (LCQ, Finnigan) equipped with an electrospray ionization source. The sample solution was introduced using a syringe pump.

The mass spectrometer was operated in the positive mode, with electrospray ionization (ESI). The heated capillary was kept at 180°C, and an 11 V potential was applied to it. The conversion dynode was set at 15 kV, the electron multiplier at 0.8 kV and the spray voltage at 5.0 kV.

The sheath gas was nitrogen at a pressure of 80 psi. No auxillary gas was used. To carry out MS/MS analysis, a 3 u wide window was used for isolation of the precursor. A relative excitation energy of 28% was applied to dissociate the precursor ion. The daughter ions were unit resolved across the scan range. Three microscans were acquired over the mass range of 265~2000 with a target threshold of $1 \times 10^6$ for automatic gain control. High resolution peaks were recorded with a zoom scan over a 10 u window.

The mobile phase was 50 : 50 acetonitrile : water (0.01 M $NH_4OAc$, pH 4.5) with a flow rate of 1 $\mu$l/minute. The peptide was dissolved in the mobile phase to give a concentration of 0.5 mg/ml.

The software package Navigator version 1.1 (Finnigan) was used for instrument control and data processing.

Syncytium Formation Inhibition Assay

Stock solutions of the compounds investigated were prepared by dissolving 16.7 $\mu$g/ml DMSO.

Virus: Infectious HTLV-$III_{MN}$ (HIV-$1_{MN}$) was obtained from the supernatant of infected H9 cell cultures and stored at −80°C. H9 cell cultures were prepared as recommended by the American Type Culture Collection (ATCC).

Test of antiviral activity: H9 cells were incubated with HIV-$1_{MN}$ ($2 \sim 4 \times 10^5$ thymus-derived lymphocyte infectious units/$10^6$ cells) for 30 minutes. The virus-containing supernatant was removed and the infected cells were distributed over 24-well trays. The test compounds were added immediately, using a fivefold dilution series (1, 0.2, 0.04 *etc.*). The final cell concentration in each well was $2.5 \times 10^5$ cells/ml. The trays were kept in a $CO_2$ incubator (5% $CO_2$, 37°C) for 72 hours. The extent of virus replication was determined by microscopic observation of the syncytia formed. In the case of reduced formation the amount of HIV in the corresponding sample was determined using a commercially available p24 antigen test kit.

p24 antigen test: Vironostatika HIV-1 Antigen Micro ELISA System, ORGANON TEKNIKA, Eppelheim (Germany). The test was carried out in accordance with the manufacturer's instructions. All samples were diluted by a factor of ten before testing.

*In Vitro* Antibacterial Susceptibility Testing

A doubling dilution series of feglymycin was prepared in sterile physiological saline and incorporated into Mueller Hinton agar (Difco) resulting in final compound concentrations ranging between 0.002 $\mu$g/ml and 128 $\mu$g/ml. Fresh overnight cultures of the test strains were used as inocula. The surface of the agar plates was inoculated by using a multipoint inoculator (Denley) which delivered $5 \times 10^4$ colony forming units (cfu) per spot. The minimum inhibitory concentrations (MIC) were taken as the lowest compound concentrations that suppressed visible growth after 24 hours incubation at 37°C. A single colony or a fine, barely visible haze of growth was disregarded.

## Results and Discussion

Isolation and Physico-chemical Properties

The active compound feglymycin was isolated from the mycelium of *Streptomyces* sp. DSM 11171 by extraction with methanol and enriched by adsorption chromatography on MCI GEL CHP20P. Further purification was achieved by size exclusion chromatography and repeated preparative HPLC on a reversed-phase column at pH 7.0 and 2.5, giving feglymycin of 99% purity. The colorless compound is soluble in methanol, water, acetonitrile and DMSO. Its physico-chemical properties are summarized in Table 1. Feglymycin contains the elements C, H, O, and N, as demonstrated by elemental analysis. The IR spectrum showed absorption bands at 1641 and 1514 $cm^{-1}$, suggesting the presence of peptide bonds. The amino acids L-Val (2), L-Phe (1), and L-Asp (1) were identified as components of feglymycin after hydrolysis in boiling hydrochloric acid at 105°C for 18 hours.

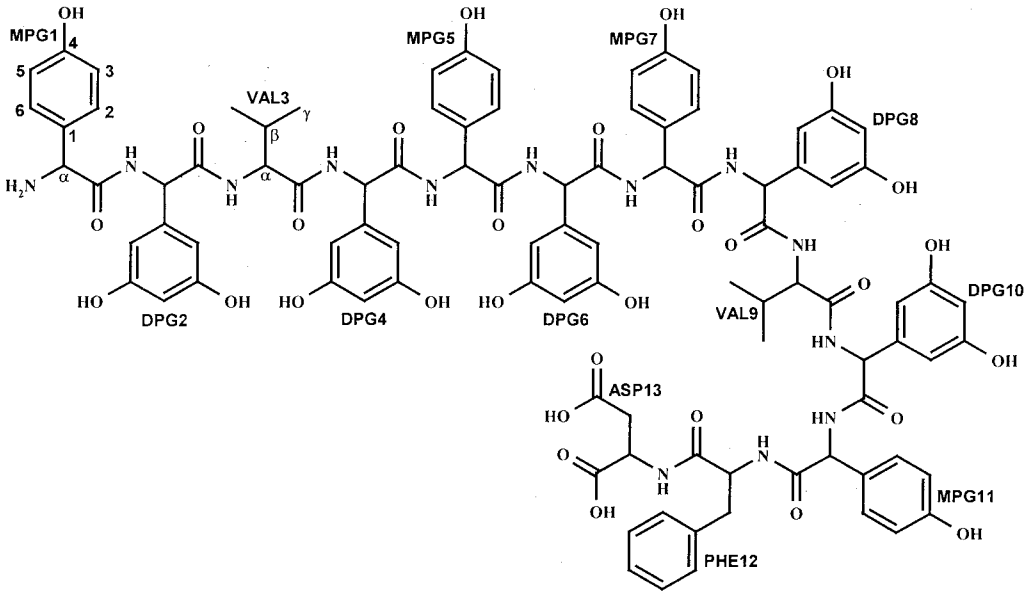
Table 1. Physico-chemical properties of feglymycin.

| | Feglymycin |
|---|---|
| Appearance | White powder |
| $[\alpha]_D^{21}$ | $-106^\circ$ ($c$ 0.78, $CH_3OH$) |
| Molecular formula | $C_{95}H_{97}N_{13}O_{30}$ |
| Molecular weight | 1900.90 |
| ESI-MS ($m/z$) | 1900.6 $[M+H]^+$ |
| UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$) | 280 (14,500) |
| UV $\lambda_{max}^{water-0.1\,N\,HCl}$ | 278 (10,000) |
| UV $\lambda_{max}^{water-0.1\,N\,NaOH}$ | 296 (18,400) |
| IR $\nu_{max}\,cm^{-1}$ (KBr) | 3288, 1641, 1514, 1340, 1155, 1006, 843 |
| Solubility | |
| Soluble | Water, MeOH, acetonitrile, DMSO |
| HPLC retention time | 5.1 minutes[a], 10.9 minutes[b] |

[a] Column: LiChroCart 250-4, Superspher 100 RP-18 e (E. Merck), eluent: 20% acetonitrile in 10 mM sodium phosphate buffer, pH 7.0, flow rate at 1.0 ml/minute.

[b] Column: LiChroCart 250-4, Superspher 100 RP-18 e (E. Merck), eluent: 25% acetonitrile in 0.1% trifluoracetic acid, flow rate at 1 ml/minute.

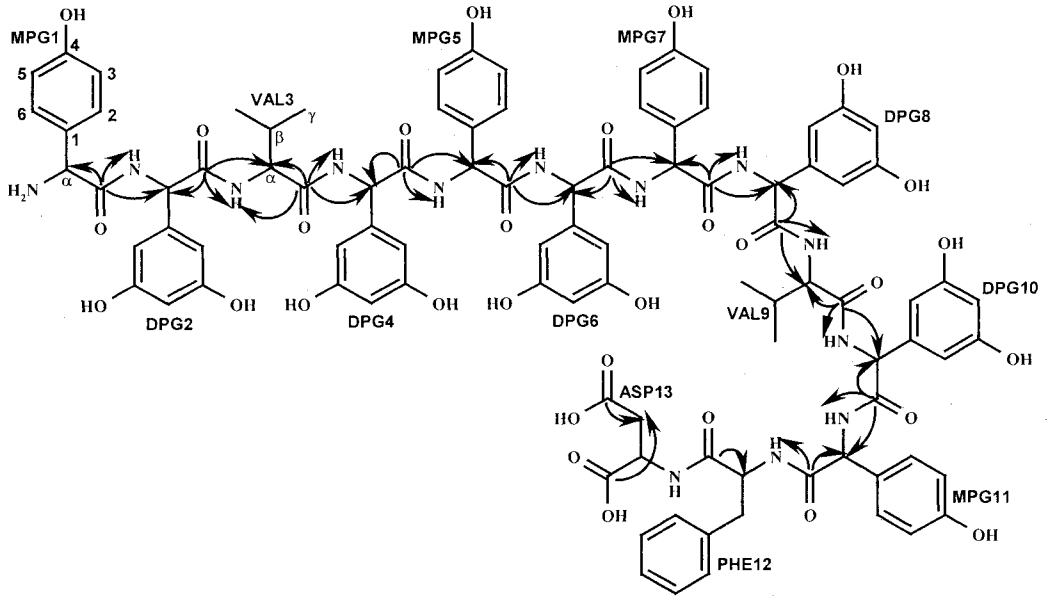
Fig. 1. Chemical structure of feglymycin.




## Structure Elucidation

The structure of feglymycin (Fig. 1) was determined by NMR spectroscopy using $^1H$, DQF-COSY, TOCSY, NOESY, $^1H$-$^{13}C$ HSQC and $^1H$-$^{13}C$ HMBC experiments. The results of MS-MS investigations confirmed the amino acid sequence (Fig. 1). Feglymycin is a linear peptide which contains an unusually large proportion of the rare amino acids 4-monohydroxyphenylglycine (MPG) (4 of 13 residues) and 3,5-dihydroxyphenylglycine (DPG) (5 of 13 residues). The peptide also contains two valine residues, one phenylalanine and one aspartate residue.

Fig. 2. Sequential $^{1}H$-$^{13}C$ long-range correlations observed in the HMBC spectrum.




Arrows originating at the carbon atoms and pointing towards the correlated protons mark the correlations detected. For reasons of clarity, only those connectivities relevant to the sequence assignment are shown.

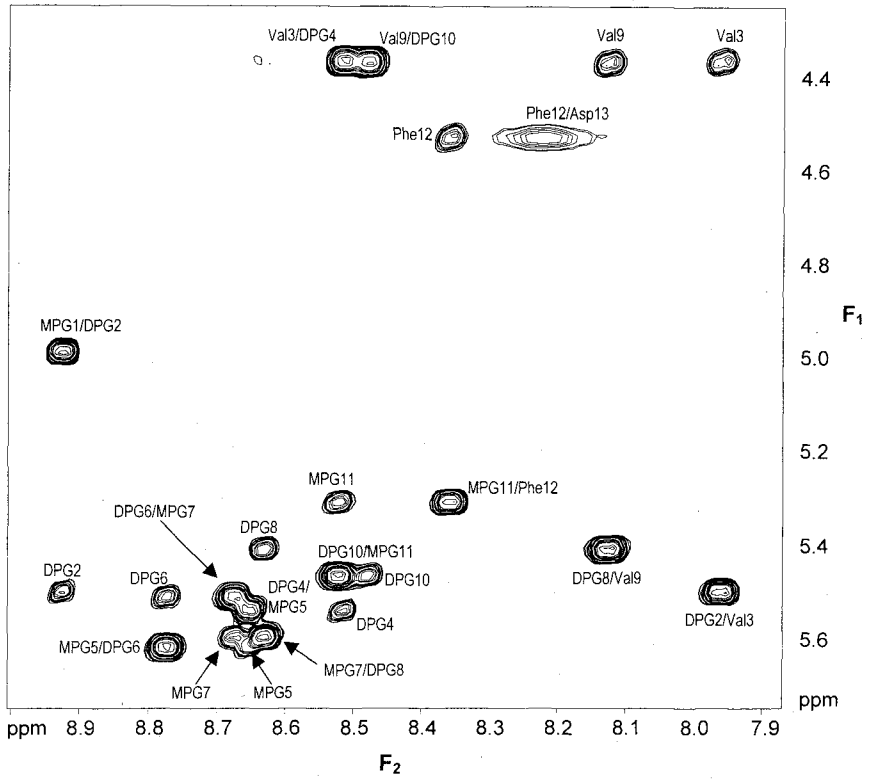
## NMR Spectroscopy

The proton spin systems of feglymycin were identified from the homonuclear scalar correlated 2D spectra (DQF-COSY, TOCSY). Both valine residues could be recognized by the characteristic cross peak pattern of their side chains. The aspartate resonances and the aliphatic spin system of Phe12 could be assigned in the same way. In the case of the AX spin systems (peptide backbone) of the monohydroxyphenylglycine (MPG) and dihydroxyphenylglycine (DPG) residues cross peaks have been observed connecting the amide proton with the $H^{\alpha}$ proton. Due to exchange broadening of the *N*-terminal amine proton resonances this connectivity could not be detected for the monohydroxyphenylglycine in position 1 (MPG1). Nevertheless, the $\alpha$-proton resonance of this residue was later identified as a broad singlet at 4.98 ppm. The aromatic spin systems of the monohydroxyphenylglycine residues showed the typical AA'XX' pattern while the aromatic protons of the dihydroxyphenylglycine residues gave two singlet signals with an intensity ratio 2:1 (H2/6:H4). An obviously small $^{4}J_{HH}$ coupling constant meant that cross peaks between these two resonances could be detected in the TOCSY experiment. The hydroxyl proton resonances have been assigned to the appropriate MPG or DPG residue using both NOE and $^{1}H$-$^{13}C$ long range correlations. The $^{13}C$ resonance assignments of feglymycin were achieved by analysis of the $^{1}H$-$^{13}C$ heteronuclear correlation spectra (HSQC, HMBC). The remaining AMM'XX' spin system could easily be assigned to the aromatic side chain of phenylalanine.

The aromatic side chains of the MPG and DPG residues have been related to the corresponding NH-$C^{\alpha}(H)$ fragment by both NOE effects between the $C^{\alpha}$ proton and the aromatic protons H2/6 and $^{1}H$-$^{13}C$ long range correlations between the $C^{\alpha}$ proton and the aromatic carbon atoms in position 1 ($^{2}J_{CH}$) and 2/6 ($^{3}J_{CH}$). The aromatic side chain of the phenylalanine residue has been related to the $C^{\beta}$ position in the same way.

The partial amino acid structures were linked by analysis of NOE- and $^{1}H$-$^{13}C$ long range correlations, yielding the amino acid sequence (Fig. 2). The sequential correlations observed in the HMBC spectrum are indicated by arrows in Fig. 2. An almost complete $^{1}H$-$^{13}C$ connectivity network was obtained. The sequential assignment of amino acid residues by NOE correlations relies on the fact that in proteins or peptides

Fig. 3. Part of a NOESY spectrum (200 ms mixing time) of feglymycin displaying the NH-αH region.




Besides the intraresidual NOEs connecting the amide and α protons within a particular amino acid residue, all sequential $d\alpha_N$ NOE correlations are observed connecting consecutive amino acid residues.

there are always one or two distances between backbone or $C^\beta$ rotons of adjacent amino acids which are no longer than 0.36 nm[20]. The distances of interest are $d_{NN}(i, i+1)$, $d_{\alpha N}(i, i+1)$ and $d_{\beta N}(i, i+1)$ which are defined from the *N* to the *C* terminus and connect the two amide protons, the α-proton and the amide proton, and the β-proton and the amide proton of consecutive amino acids respectively[21]. In the case of feglymycin all possible $d\alpha_N$ (Fig.3) and $d_{\beta N}$ NOE correlations were observed. In addition, $d_{NN}$ NOE effects have been observed for the stretches DPG2 to DPG6, DPG8 to DPG10, and MPG11 to Phe12. The sequential assignment is further substantiated by NOE correlations between the side chains of adjacent amino acids. The assigned proton and carbon resonances are summarized in Table 2.

## Mass Spectrometry

Feglymycin gives a strong protonated molecular ion at *m/z* 1900.6 and a smaller doubly protonated ion at *m/z* 950.9 (see insert Fig. 3). From this, it was possible to deduce a monoisotopic molecular weight of 1899.6, indicating an odd number of nitrogens.

Despite its lower intensity, the doubly charged ion was chosen as the precursor mass for sequence confirmation because of the larger scan range at lower masses, as the start mass of the ion trap scan is limited to about 30% of the parent mass.

The MS/MS spectrum is dominated by B - and Y″-type fragments, comprising the *N*-terminus or the *C*-terminus respectively. The sum of the masses of the B- and corresponding Y″-fragments add up to the doubly protonated molecular mass, the corresponding fragments can therefore easily be assigned (Table 3).

Except for the $B_1$- and the $Y_1''$-fragment (due to the mass range limitation) all B- and Y″-fragments in the spectrum were identified (see Fig. 4).

Table 2. $^{1}H$ and $^{13}C$ NMR data for feglymycin at 300 K in DMSO-$d_6$.

| Residue | Position | $^{1}H$ [ppm] | $^{13}C$ [ppm] | Residue | Position | $^{1}H$ [ppm] | $^{13}C$ [ppm] |
|---|---|---|---|---|---|---|---|
| MPG 1 | $NH_2$ | n.d. | — | DPG8 | NH | 8.63 | — |
| | αH | 4.98 | 54.6 | | αH | 5.40 | 55.9 |
| | 1 | — | 123.9 | | 1 | — | 140.7 |
| | 2, 6 | 7.34 | 129.2 | | 2, 6 | 6.20 | 105.1 |
| | 3, 5 | 6.81 | 115.3 | | 3, 5 | — | 157.6 |
| | 4 | — | 157.9 | | 4 | 6.05 | 101.3 |
| | OH | 9.73 | — | | OH | 9.09 | — |
| | CO | | 166.6 | | CO | — | 169.0 |
| DPG2 | NH | 8.93 | — | Val9 | NH | 8.13 | — |
| | αH | 5.49 | 56.2 | | αH | 4.36 | 56.6 |
| | 1 | — | 140.4 | | βH | 1.88 | 31.2 |
| | 2, 6 | 6.37 | 105.5 | | γH | 0.61 | 17.4 |
| | 3, 5 | — | 157.7 | | γH | 0.59 | 18.9 |
| | 4 | 6.13 | 101.6 | | CO | — | 170.0 |
| | OH | 9.22 | — | DPG10 | NH | 8.48 | — |
| | CO | — | 168.6 | | αH | 5.46 | 55.5 |
| Val3 | NH | 7.96 | — | | 1 | — | 140.6 |
| | αH | 4.36 | 56.8 | | 2, 6 | 6.29 | 105.4 |
| | βH | 1.88 | 31.2 | | 3, 5 | — | 157.7 |
| | γH | 0.55 | 18.9 | | 4 | 6.08 | 101.4 |
| | γH | 0.52 | 17.0 | | OH | 9.15 | — |
| | CO | — | 169.8 | | CO | — | 168.6 |
| DPG4 | NH | 8.52 | — | MPG11 | NH | 8.52 | — |
| | αH | 5.53 | 55.5 | | αH | 5.30 | 55.2 |
| | 1 | — | 140.7 | | 1 | — | 128.1 |
| | 2, 6 | 6.25 | 105.2 | | 2, 6 | 6.93 | 128.0 |
| | 3, 5 | — | 157.6 | | 3, 5 | 6.57 | 114.5 |
| | 4 | 6.07 | 101.3 | | 4 | — | 156.2 |
| | OH | 9.14 | — | | OH | 9.32 | — |
| | CO | — | 168.6 | | CO | — | 169.2 |
| MPG5 | NH | 8.65 | — | Phe12 | NH | 8.36 | — |
| | αH | 5.61 | 54.7 | | αH | 4.52 | 53.5 |
| | 1 | — | 128.3 | | βH | 3.04/2.81 | 37.3 |
| | 2, 6 | 7.07 | 127.8 | | 1 | — | 137.2 |
| | 3, 5 | 6.55 | 114.5 | | 2, 6 | 7.24 | 129.1 |
| | 4 | — | 156.2 | | 3, 5 | 7.22 | 127.8 |
| | OH | 9.28 | — | | 4 | 7.16 | 126.0 |
| | CO | — | 169.2 | | CO | — | 170.0 |
| DPG6 | NH | 8.77 | — | Asp13 | NH | 8.22 | — |
| | αH | 5.50 | 55.5 | | αH | 4.43 | 48.4 |
| | 1 | — | 140.2 | | βH | 2.58/2.49 | 35.9 |
| | 2, 6 | 6.17 | 105.2 | | COO*H*§ | 12.45 | — |
| | 3, 5 | — | 157.6 | | COO*H*§ | 12.70 | — |
| | 4 | 6.05 | 101.3 | | *C*OOH§ | — | 171.3 |
| | OH | 9.09 | — | | *C*OOH§ | — | 171.9 |
| | CO | — | 168.6 | | | | |
| MPG7 | NH | 8.68 | — | | | | |
| | αH | 5.59 | 54.8 | | | | |
| | 1 | — | 128.3 | | | | |
| | 2, 6 | 7.05 | 127.8 | | | | |
| | 3, 5 | 6.58 | 114.5 | | | | |
| | 4 | — | 156.2 | | | | |
| | OH | 9.30 | — | | | | |
| | CO | — | 169.1 | | | | |

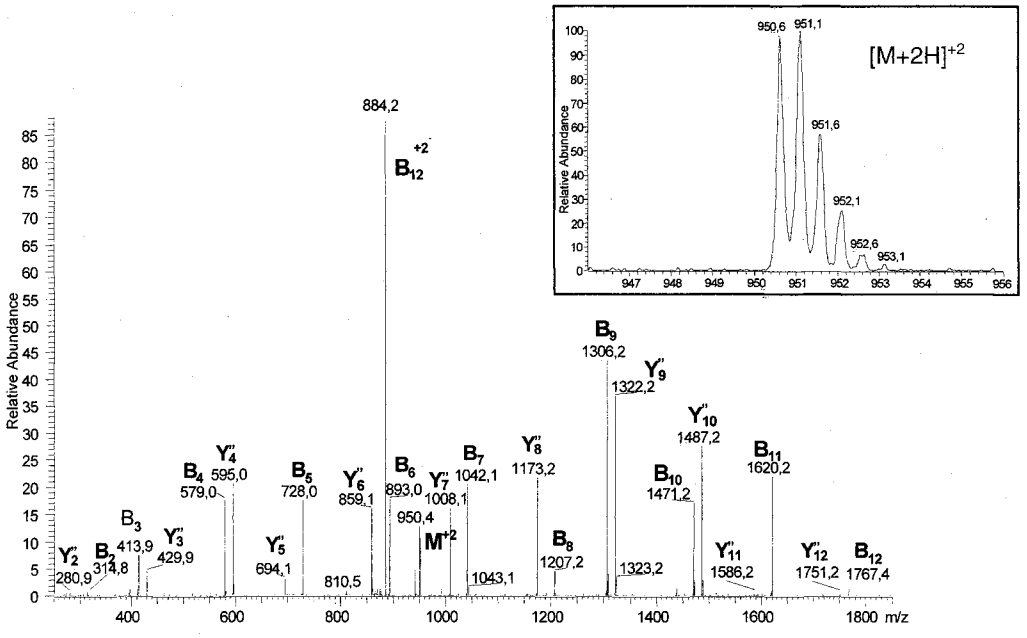
Table 3. Expected B- and Y″-fragments of feglymycin.

| | | | |
|---|---|---|---|
| $B_1$ | 150 | $Y''_1$ | 134 |
| $B_2$ | 315 | $Y''_2$ | 281 |
| $B_3$ | 414 | $Y''_3$ | 430 |
| $B_4$ | 579 | $Y''_4$ | 595 |
| $B_5$ | 728 | $Y''_5$ | 694 |
| $B_6$ | 893 | $Y''_6$ | 859 |
| $B_7$ | 1042 | $Y''_7$ | 1008 |
| $B_8$ | 1207 | $Y''_8$ | 1173 |
| $B_9$ | 1306 | $Y''_9$ | 1322 |
| $B_{10}$ | 1471 | $Y''_{10}$ | 1487 |
| $B_{11}$ | 1620 | $Y''_{11}$ | 1586 |
| $B_{12}$ | 1767 | $Y''_{12}$ | 1751 |

Table 4. Antibacterial activity of feglymycin.

| Test organism | MIC ($\mu$g/ml) |
|---|---|
| *Staphylococcus aureus* SG 511 | 64 |
| *Staphylococcus aureus* 285 | 64 |
| *Staphylococcus aureus* 503 | 32 |
| *Staphylococcus aureus* FH 1982 | 64 |
| *Staphylococcus aureus* 701E | 64 |
| *Staphylococcus aureus* 9 Tüb | 64 |
| *Staphylococcus aureus* 8236 | 64 |
| *Staphylococcus epidermidis* ZH 2c | 64 |
| *Staphylococcus epidermidis* 763 | 64 |
| *Staphylococcus epidermidis* 799 | 64 |
| *Streptococcus pyogenes* 308A | 32 |
| *Streptococcus pyogenes* 77A | 32 |
| *Escherichia coli* 078 | >128 |
| *Pseudomonas aeruginosa* 9027 | >128 |

Fig. 4. MS/MS spectrum of the doubly protonated molecular ion of feglymycin at $m/z$ 950.9.




The insert shows a zoom scan of this ion with an isotope spacing of 0.5 u, indicative for the doubly charged character of the ion.

## Biological Activities

Feglymycin significantly inhibits the formation of HIV syncytia *in vitro*. An $IC_{50}$ of $\sim 5\,\mu$M was estimated visually by microscopic observations. An $IC_{50}$ concentration of 3.6 $\mu$M was found in the p24 antigen test. No cytotoxic effects were observed. Feglymycin also showed weak antibiotic activity against Gram-positive bacteria (Table 4).

The molecular mechanism of the antiviral activity has not yet been investigated.

Feglymycin is an unusual peptide containing a high

proportion of the rare 4-hydroxy- and 3,5-hydroxy-substituted phenylglycine residues. Interestingly, among the virostatic substances mentioned earlier in the text the replication inhibitors RPI-856 also contain 3,5-dihydroxyphenylglycine while the inhibitor chloropeptin contains both 4-hydroxy- and 3,5-hydroxyphenylglycine moieties. While RPI-856A, B, C, and D are inhibitors of the HIV-1 protease, chloropeptin inhibits the gp120-CD4 binding process.

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