Natural Products Synthesis

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Total Synthesis of the Antiviral Peptide Antibiotic Feglymycin**

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Dedicated to Professor Rudolf Wiechert on the occasion of his 80th birthday and to Professor Helmut Schwarz on the occasion of his 65th birthday

Feglymycin (**1**), a naturally occurring peptide isolated from *Streptomyces* sp. DSM 11171, strongly inhibits the formation of HIV syncytia in vitro and was reported to show weak antibacterial activity against Gram-positive bacteria.[1] The molecular structure of **1** was first determined by mass spectrometry and NMR spectroscopy in the mid-1990s and was proven in 2005 by Sheldrick and co-workers by X-ray crystallography.[2] The unusual primary structure of **1** consists of an alternating sequence of mostly aromatic *S*- and *R*-configured amino acid residues. Remarkably, the X-ray crystal structure shows the formation of a double-stranded antiparallel β-helical dimer, which is stabilized by a network of intermolecular hydrogen bonds between phenolic OH groups (Figure 1). These structural features are strongly reminiscent of those of membrane-spanning peptides, such as gramicidin.[3] With a high proportion of unusual amino acid residues, such as 4-hydroxyphenylglycine (Hpg) and 3,5-dihydroxyphenylglycine (Dpg), feglymycin belongs to a family of natural products with interesting pharmacological properties. Other members include the glycopeptide antibiotic vancomycin,[4] the antiviral compound complestatin,[5] and the antimicrobial agent ramoplanin.[6] Owing to its unique chemical structure and biological activity, **1** is an auspicious new natural product whose mechanisms of antibacterial and antiviral activity have not yet been investigated.

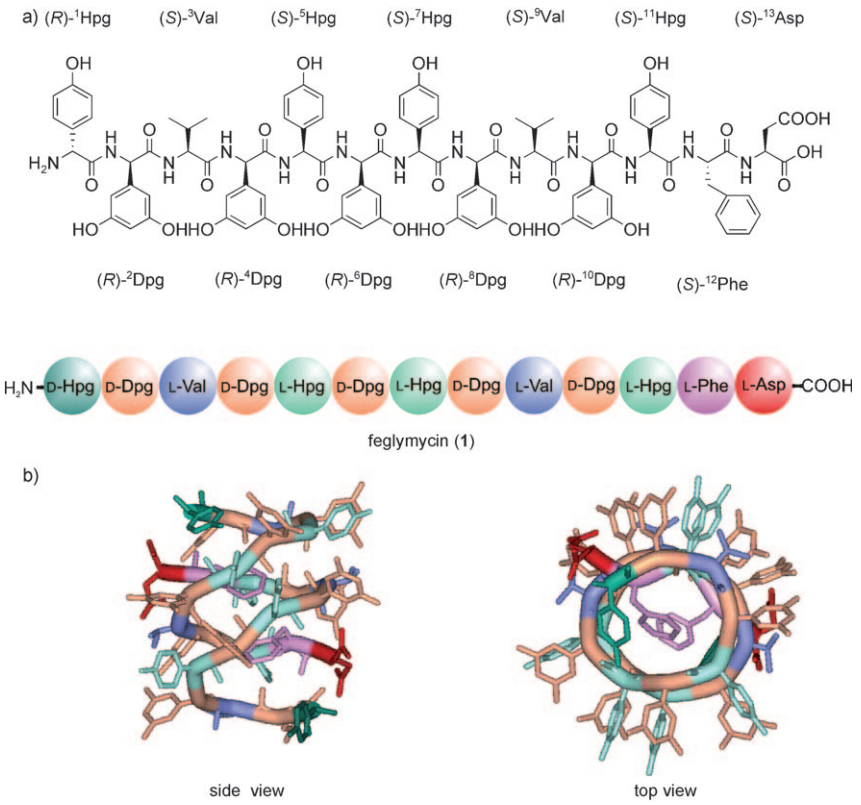

Figure 1. a) Primary structure of feglymycin (**1**); b) X-ray crystal structure of a double-stranded antiparallel β-helical dimer of feglymycin.[2]

Herein we report a first highly convergent total synthesis of the 13 amino acid peptide feglymycin (**1**) and its enantiomer **1′** by fragment condensation. We also describe structure–activity-relationship (SAR) studies with intermediate synthetic peptides, the results of which shed light on structural features relevant to the molecular mode of action of feglymycin.

The two main challenges in the total synthesis of **1** were the establishment of a racemization-free coupling protocol for Hpg and Dpg, and the development of an adequate protecting-group strategy, which should prevent the epimerization of sensitive amino acid building blocks and guarantee the

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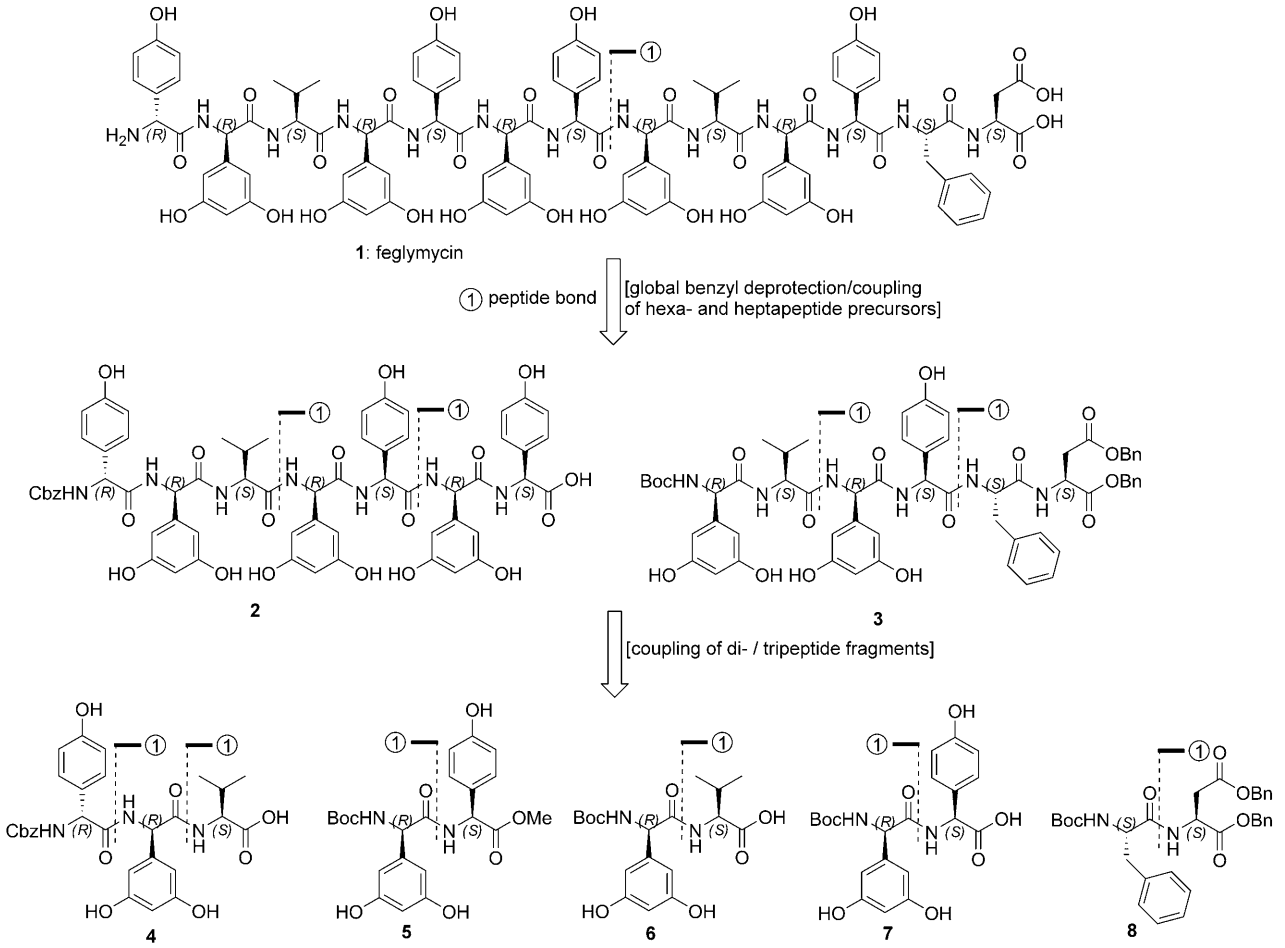
solubility of synthetic intermediates. Moreover, the amino acids Hpg and Dpg are themselves difficult to prepare in the desired protected enantiomerically pure form and do not tolerate standard coupling procedures in peptide synthesis or basic conditions because of their ease of epimerization.[7] In particular, the coupling of the highly racemization prone amino acid Dpg in most cases led to the formation of large amounts of the diastereomeric product as a result of epimerization at the C^{α} position. Especially in more advanced stages of feglymycin synthesis, diastereomeric mixtures were not separable by standard chromatographic procedures (data not shown). Therefore, an iterative coupling of single amino acid building blocks was not possible.

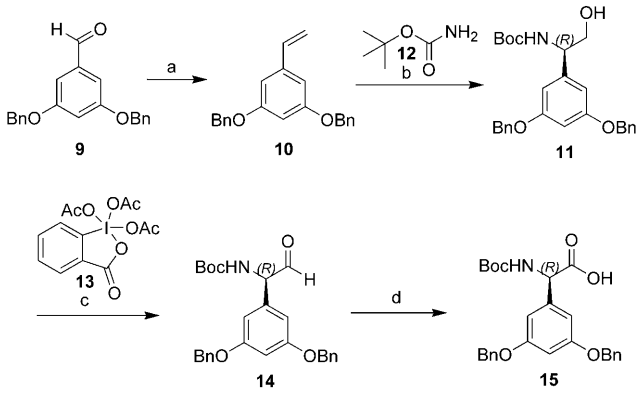
With our retrosynthetic strategy, we aimed to avoid the activation of Dpg by dividing the parent structure of **1** into suitable di-, tri-, hexa-, or heptapeptide fragments. Thus, peptide-coupling steps only required the activation of Val, Hpg, or Phe (Scheme 1), and the hepta- and hexamer precursors **2** and **3**, obtained from trimer **4** and dimers **5**–**8**, could be coupled to furnish **1**. We settled on the general strategy depicted in Scheme 1 because peptide fragments with fully protected side chains, at the latest from the level of a hexapeptide, such as **3**, turned out to be nearly insoluble in all solvents, including dimethyl sulfoxide (DMSO) and *N*,*N*-dimethylformamide (DMF). The *tert*-butoxycarbonyl (Boc) group was chosen as the temporary N-terminal protecting group during chain extensions because it could be cleaved under epimerization-free reaction conditions in short reaction times and almost quantitative yield. Nearly all peptide-coupling reactions were carried out with 3-(diethyloxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT) and $NaHCO_3$ in THF or DMF.[8] The use of alternative coupling reagents led to significantly lower conversion as well as substantial epimerization of hydroxyphenylglycine residues.

Starting from 3,5-dibenzoxybenzaldehyde (**9**), a Wittig reaction with methyltriphenylphosphonium bromide[9] (Scheme 2) led to 3,5-dibenzoxystyrene (**10**). A subsequent Sharpless asymmetric aminohydroxylation with *tert*-butyl carbamate (**12**) and the alkaloid ligand $(DHQD)_2PHAL$[10] resulted in the formation of the Boc-protected α-amino-alcohol **11** on a multigram scale (for the enantiomer **11′**, the ligand $(DHQ)_2PHAL$ was used). Subsequent oxidation with Dess–Martin periodinane (**13**) afforded aldehyde **14** in quantitative yield. Because of its low stability, the aldehyde was converted immediately with $NaClO_2$ into the key amino acid (*R*)-*N*-Boc-3,5-dibenzoxyphenylglycine (**15**).

With **15** in hand, the next task was to prepare the C-terminal hexamer building block **3**. The required dipeptide fragments **7** and **6** were obtained by the condensation of **15** with either (*S*)-4-hydroxyphenylglycine benzyl ester hydro-

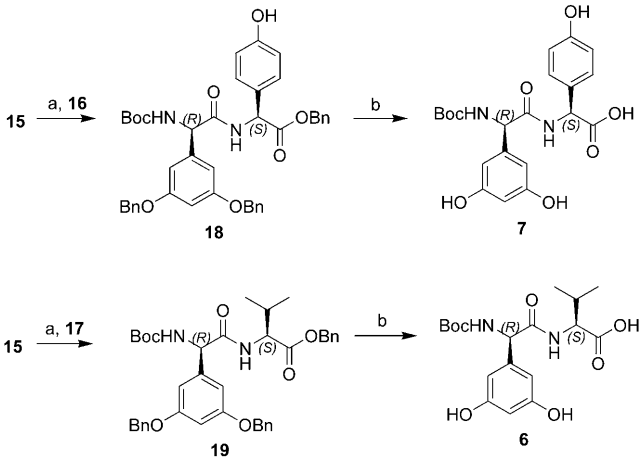


Scheme 1. Retrosynthetic analysis of feglymycin (**1**), with coupling of fragments by condensation (①). Bn = benzyl, Cbz = benzyloxycarbonyl, Boc = *tert*-butyloxycarbonyl.



Scheme 2. Synthesis of the key amino acid (*R*)-*N*-Boc-3,5-dibenzoxyphenylglycine (**15**): a) $[Ph_3PCH_3]Br$, *n*BuLi, THF, −40 °C→RT, 4.5 h, 91 %; b) *t*BuOCl, $K_2[OsO_2(OH)_4]$, $(DHQD)_2PHAL$, *n*PrOH/H_2O (2:1), 0 °C, 1 h, 52 %, 98 % *ee*; c) CH_2Cl_2, 0 °C→RT, 2 h, quantitative; d) $NaClO_2$, 2-methyl-2-butene, H_2O, 25 °C, 40 min, 97 %.

chloride (**16**)[11] or (*S*)-valine benzyl ester hydrochloride (**17**) in the presence of DEPBT and $NaHCO_3$ in THF[12] to first yield the fully protected dipeptides **18** and **19** (Scheme 3).

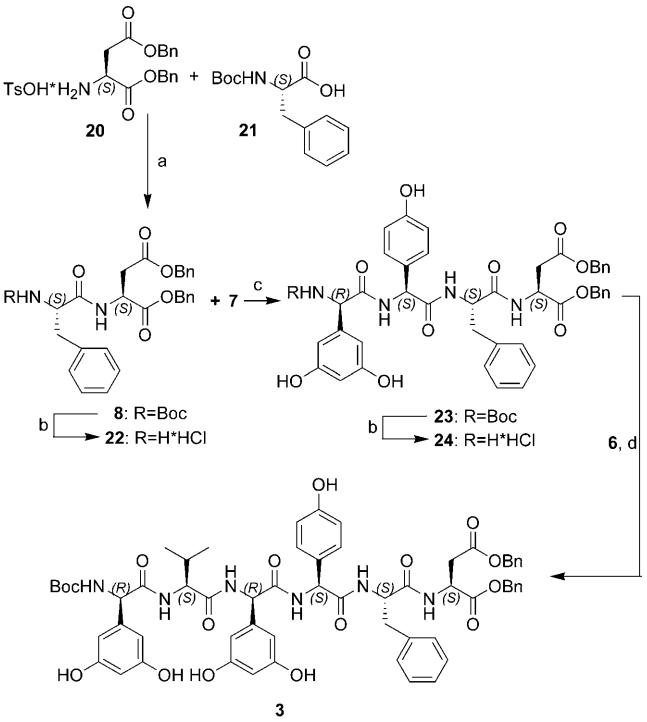


Scheme 3. Construction of the Dpg-containing dipeptide fragments **6** and **7**: a) DEPBT, $NaHCO_3$, THF, 0 °C→RT, 21 h, 80 % (98 % for **19**); b) 10 % Pd/C, H_2, THF, room temperature, 4 h, quantitative (for **6** and **7**).

Thin-layer chromatography in both cases indicated a minute amount of the undesired diastereomer, which could not be isolated from the mixture in pure form, but which was perfectly separated from the desired product. The subsequent removal of the three benzylic protecting groups by hydrogenolysis with 10 % Pd/C in THF led to **7** and **6**, respectively.

As mentioned above, the synthesis of side-chain-protected hexapeptide derivatives led to severe solubility problems. Therefore, methyl esters were introduced as C-terminal protecting groups of (*S*)-aspartic acid to minimize the increase in hydrophobicity. Unfortunately, the final cleavage of these methyl ester groups from the readily prepared 13-mer peptide led to the decomposition of the substrate. As an alternative, a mild final C-terminal deprotection of benzyl esters by hydrogenolysis was chosen. Fortunately, the introduction of the benzyl ester groups did not cause solubility problems during the subsequent coupling sequence.

The coupling of (*S*)-aspartic acid dibenzyl ester *p*-toluenesulfonate (**20**) and (*S*)-*N*-Boc-phenylalanine (**21**) with EDC/HOAt/$NaHCO_3$ in DMF[13] provided the dipeptide **8** in 77 % yield with no detectable racemization (Scheme 4).



Scheme 4. Synthesis of the C-terminal hexapeptide **3**: a) EDC, HOAt, $NaHCO_3$, DMF, 0 °C→RT, 19 h, 77 %; b) 4 N HCl/dioxane, 1 h, quantitative (for **22** and **24**); c) DEPBT, $NaHCO_3$, THF, 0 °C→RT, 21 h, 73 %; d) DEPBT, $NaHCO_3$, THF, 0 °C→RT, 21.5 h, 77 %. DMF = *N*,*N*-dimethylformamide, EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, HOAt = 1-hydroxy-7-azabenzotriazole.

Removal of the Boc group with 4 N HCl/dioxane and subsequent coupling of the hydrochloride **22** with dimer **7** (DEPBT/$NaHCO_3$) afforded the tetrapeptide **23**, which could be purified readily by flash chromatography (silica; $CHCl_3$/MeOH 9:0.5).

The C-terminal hexapeptide **3** was obtained subsequently by linking dipeptide **6** with the tetrapeptide hydrochloride **24** under the same reaction conditions. However, the workup and isolation of the product by flash chromatography (silica; $CHCl_3$/MeOH 9:0.5) were significantly more difficult than in case of the tetramer. These increasing difficulties in purification ruled out a convenient peptide assembly by the iterative coupling of dipeptides **7** and **6** and the tripeptide **4**. Hence, the construction of an N-terminal heptapeptide fragment **2** and one final coupling of **2** and **3** to yield the 13-mer peptide became indispensable.

During the elaboration of our final synthetic strategy based on fragment condensation, we orignially prepared a

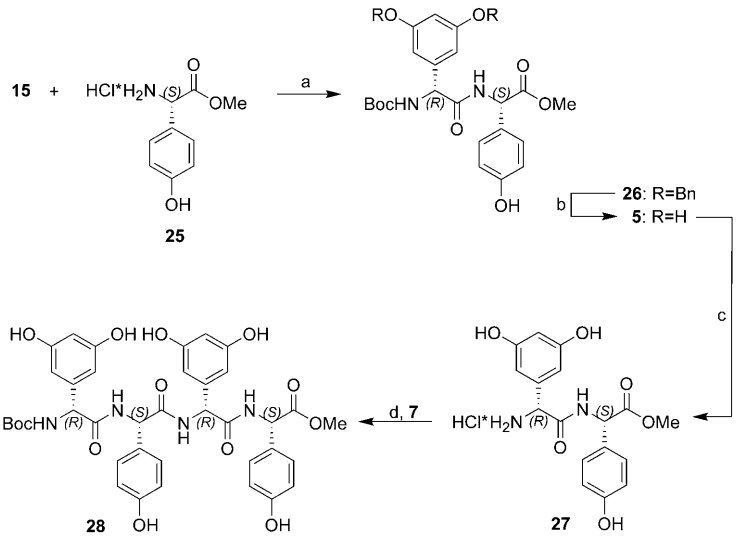
heptapeptide analogue of **2** with an N-terminal Boc protecting group on (*R*)-[1]Hpg. Cleavage of the Boc group of this derivative proceeded cleanly in only 10 min with 4 N HCl in dioxane or trifluoroacetic acid in CH_2Cl_2. However, the exposure of the C-terminally protected 13-mer to the same cleavage conditions resulted in almost complete decomposition of the substrate. Since the thermal cleavage of the Boc group also did not occur reproducibly ($T > 100\,°C$),[14] a benzyloxycarbonyl (Cbz) group was introduced on (*R*)-[1]Hpg. This approach enabled simultaneous N- and C-terminal deprotection of three benzylic groups from the 13-mer peptide by hydrogenolysis under mild conditions.

A methyl ester was used for the interim C-terminal protection of the required N-terminal heptamer **2** at (*S*)-[7]Hpg. This protecting group is commonly removed under strong basic conditions with LiOH or NaOH. Owing to the ease of epimerization of Dpg, a mild procedure was required. Cleavage of the methyl ester was possible under slightly basic conditions with trimethyltin hydroxide (TMTH) in 1,2-dichloroethane at 85 °C.[15]

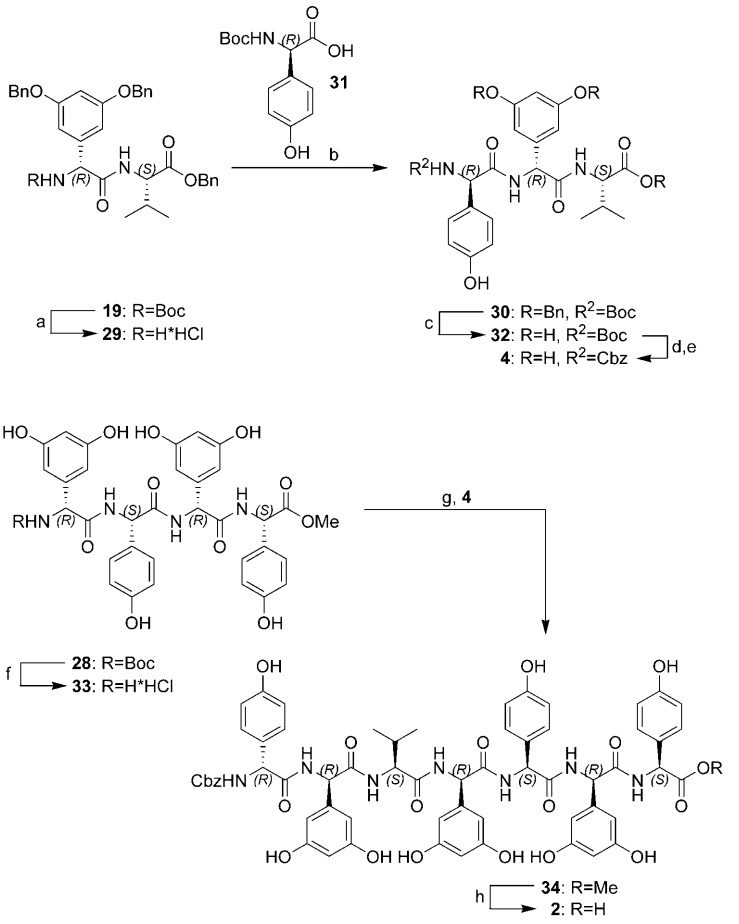
The N-terminal heptapeptide **2** was assembled as follows: (*S*)-4-Hydroxyphenylglycine methyl ester hydrochloride (**25**)[16] was coupled with **15** in the presence of DEPBT and $NaHCO_3$ to give dipeptide **26** (Scheme 5). Cleavage of the benzyl ether groups (10% Pd/C, H_2) then yielded **5** quantitatively and thus improved the solubility of all subsequent peptide intermediates. Following the removal of the Boc group with 4 N HCl in dioxane to give hydrochloride **27**, DEPBT-mediated coupling with **7** furnished tetrapeptide **28**. Multiple attempts to optimize the moderate yield of 54% in this step by varying the reaction conditions failed. Only the use of reagent systems such as EDC/HOAt led to slightly better conversion; however, undesired diastereomer formation was also observed under these conditions.

Removal of the Boc group from dipeptide **19** (4 N HCl/dioxane) afforded the hydrochloride **29**, the coupling of which with (*R*)-*N*-Boc-4-hydroxyphenylglycine (**31**)[17] led to the protected tripeptide **30** (Scheme 6). Hydrogenolysis of **30** (10% Pd/C, H_2) resulted in the formation of the trimer building block **32**. Since the cleavage of an N-terminal Boc group in the 13-mer peptide resulted in decomposition of the substrate, as mentioned above, the Boc group of tripeptide **32** was replaced with a Cbz group to give tripeptide **4**.

Standard DEPBT-mediated coupling of **4** with hydrochloride **33** furnished heptamer **34**. This compound could be purified by flash chromatography (CH_2Cl_2/MeOH 9:2) without difficulty and was converted into acid **2** with TMTH (20 equiv) in 1,2-dichloroethane at 85 °C within 4 h.[15a] This reaction proceeded without detectable epimerization with 84% conversion; a complicated chromatographic purification of **2** was not necessary.



Scheme 5. Synthesis of the tetrapeptide **28** as a precursor to the N-terminal heptapeptide **2**: a) DEPBT, $NaHCO_3$, DMF, 0 °C→RT, 23 h, 78%; b) 10% Pd/C, H_2, THF, room temperature, 4 h, quantitative; c) 4 N HCl/dioxane, 1.5 h, quantitative; d) **7**, DEPBT, $NaHCO_3$, DMF, 0 °C→RT, 21.5 h, 54%.



Scheme 6. Synthesis of tripeptide **4** and the heptapeptide unit **2**: a) 4 N HCl/dioxane, 55 min, quantitative; b) DEPBT, $NaHCO_3$, THF, 0 °C→RT, 19.5 h, 79%; c) 10% Pd/C, H_2, THF, temperature, 4 h, quantitative; d) 4 N HCl/dioxane, 55 min; e) CbzCl, $NaHCO_3$, H_2O/dioxane, room temperature, 1.5 h, 87% over two steps; f) 4 N HCl/dioxane, 1 h, quantitative; g) DEPBT, $NaHCO_3$, THF, 0 °C→RT, 21 h, 52%; h) TMTH, 1,2-dichloroethane, 85 °C, 4 h, 84%.

All that now remained in the synthesis of the target compound feglymycin (**1**) was the coupling of the C-terminal hexamer and N-terminal heptamer fragments, followed by the cleavage of all protecting groups of the resulting 13-mer peptide. Removal of the Boc group in **3** led to hydrochloride **35**, which was coupled to acid **2** with DEPBT/$NaHCO_3$ in DMF (Scheme 7). The 13-mer product **36** was obtained after 24 h at 0 °C and an additional 24 h at room temperature with 42% conversion. Changes in these reaction parameters resulted in lower yields, in particular when the reaction time at 0 °C was shortened. On the other hand, extension of the coupling time, either at 0 °C or room temperature, led only to a significant increase in the formation of by-products. The separation of protected feglymycin **36** after this step posed a challenge, because conventional chromatographic purification techniques typically resulted in a substantial loss of material. Only size-exclusion chromatography (sephadex LH-20, MeOH) afforded almost pure 13-mer **36**, the global deprotection of which by hydrogenolysis (10% Pd/C, H_2, MeOH) gave **1**.

Enantiomer **1′** was also prepared by the synthetic route described herein for **1**. Synthetic **1** (and **1′**) exhibited identical physical properties (R_f, HPLC, ^{1}H NMR, MS) to those of natural feglymycin.[1,18]

For the investigation of biological activity, the natural product and a selection of synthetic intermediates were chosen for antiviral testing (see the Supporting Information for complete data). The anti-HIV-1 activity of the compounds was evaluated in the human MT-4 cell line.[19] Compounds **1** and **2** (also enantiomers **1′** and **2′**) and heptapeptide **34** had activities between 1.9 and 8.9 μg mL^{-1} and showed no cytotoxicity at 100 μg mL^{-1}. The other compounds, including **34′**, had no significant anti-HIV-1 activity, as their IC_{50} value was too close to their toxic concentration (Table 1). The IC_{50} value of the bicyclam fusion inhibitor AMD3100,[20] a CXCR4 coreceptor antagonist, is shown for reference.

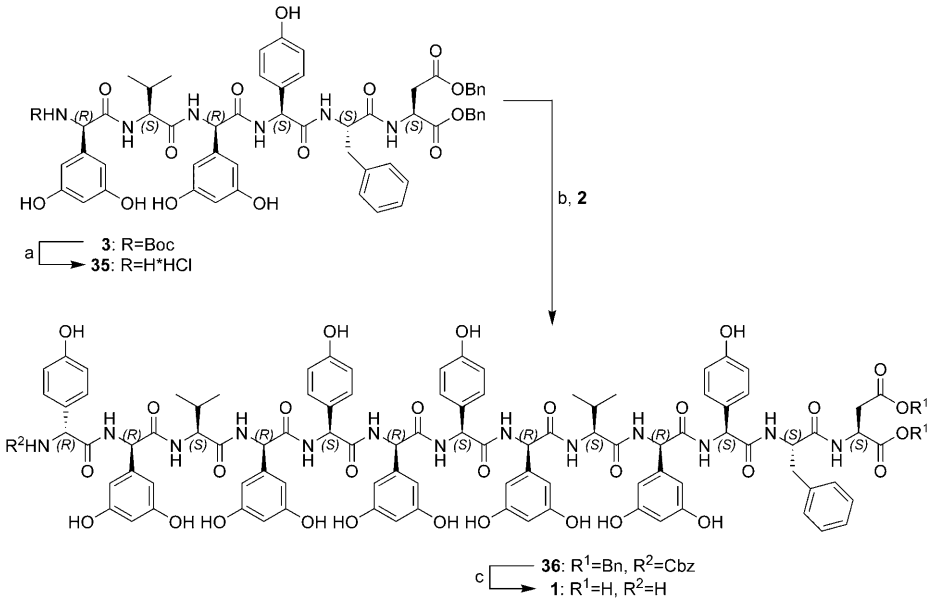
Table 1: Anti-HIV-1 activity of feglymycin and derivatives in MT-4 cells.

Compound	IC_{50}[a] [μg mL^{-1}]	CC_{50}[b] [μg mL^{-1}]	Enantiomer	IC_{50} [μg mL^{-1}]	CC_{50} [μg mL^{-1}]
1	1.9	>100	**1′**	7.9	>100
2	8.9	>100	**2′**	8.3	>100
3	>100	>100	**3′**	>100	>100
26	>4	12.4	**26′**	>13	13.2
34	7.8	>100	**34′**	7.7	57.1
AMD3100	0.0037	>10.0			

[a] IC_{50}: 50% inhibitory concentration, or drug concentration required to inhibit the virus-induced cytopathic effect (CPE) of HIV-1 NL4.3 in human MT-4 cells by 50%. [b] CC_{50}: 50% cytotoxic concentration, or drug concentration required to inhibit the cell growth of MT-4 cells by 50%.

The IC_{50} value of feglymycin (1.0 μM) is comparable to that of the nucleoside analogue reverse transcriptase inhibitor (NARTI) zalcitabine (0.95 μM),[21] which was investigated in previous studies under similar conditions.[19] Besides **1**, the N-terminal heptapeptides **2** and **34** show remarkable activity, whereas the C-terminal hexamer **3** appears to be ineffective. As a small molecule, dipeptide **26** also shows interesting anti-HIV-1 activity; however, it displays cytotoxic effects. A comparison of the IC_{50} values (μM) shows that feglymycin is at least four times more active than all other peptide derivatives tested. The IC_{50} values of the enantiomeric compounds **1′**, **2′**, and **34′** are comparable to those of the natural derivatives, which indicates that the absolute configuration of these substances seems to be of minor importance for their biological activity. In conclusion, it may be assumed that the potential pharmacophore is located in the N-terminal region. Antibacterial tests (see the Supporting Information) showed exceptional activity of synthetic **1** and a sample of natural **1** against *Staphylococcus aureus* (MIC = 1-4 μg mL^{-1}), contrary to the previous results of Vértesy and coworkers.[1]

In summary, we have described a convergent and stereoselective synthesis of the highly acid labile antiviral 13-mer peptide feglymycin (**1**) and its enantiomer **1′** by the DEPBT-mediated condensation of repeating fragments. The approach enables fast access to new potentially interesting derivatives without significant changes to the reaction protocols. Future investigations will involve more detailed studies of biological activity to shed light on the molecular mode of action of feglymycin.



Scheme 7. Completion of the total synthesis of feglymycin (**1**): a) 4 N HCl/dioxane, 55 min, quantitative; b) DEPBT, $NaHCO_3$, DMF, 0 °C→RT, 48 h, 42%; c) 10% Pd/C, H_2, methanol, room temperature, 5.5 h, 89%.

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