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An overview of chemical synthesis of antiviral peptides

*Rani Maharani** *and Muhamad Imam Muhajir*

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jawa Barat 45361, Indonesia

ABSTRACT

Antiviral peptides are a class of compounds with outstanding properties for the treatment of viral diseases. The sources of these peptides are varied, including plants and bacteria and they can also be chemically synthesized. As chemical synthesis is being increasingly applied, this review discusses the chemical synthesis of antiviral peptides. Initially, the antiviral peptides were grouped depending on their basic structures, including peptide and peptide conjugate groups, followed by a discussion of the synthetic approach. Two techniques commonly used to synthesize the peptides are solution-phase and solid-phase peptide synthesis, where the latter is mostly applied to make the peptides. The synthesis strategies, including the selection of the N-amino protecting group, coupling reagents, resin cleavage cocktails, resin, and other approaches, were also discussed.

***Corresponding author**:
Rani Maharani
r.maharani@unpad.ac.id

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1. INTRODUCTION

A viral disease is caused by an invasion of pathogenic viruses into an organism (Taylor et al., 2012). Viruses are tiny germs that can attach to and enter susceptible cells, and are comprised of a genetic substance, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), within a protein coating. They cause highly infectious illnesses, such as cold and flu, as well as critical diseases, such as human immunodeficiency virus (HIV), Ebola, and the recently-emerged COVID-19. The medicines used to treat viral diseases have come from natural products and many other sources (Lin et al., 2014). One class of compounds which also contributes in fighting viral disease is peptides.

Peptides have long been known as a class of compounds with attractive biological properties, such as anti-infection, neurotransmitters, hormones, growth factors, or ion channel ligands (Fosgerau and Hoffmann, 2015). Generally, peptides are safe, effective and well-tolerated by humans, hence have become targets in the design of novel pharmaceuticals. The structures of peptides, which are between small molecules and macromolecules like the biopharmaceuticals, are also beneficial because the production costs will be lower than those for biomolecules, and comparable to the costs of small molecules.

Structurally, antiviral peptides contain 3-116 residues, most of them are linear. These peptides have been isolated from higher plants, bacteria, oysters, spider venom, and other sources (Ussery et al., 1977; Andrei et al., 1994; Tripathi et al., 2006; Panya et al., 2019; Park et al., 2012; Zeng et al., 2008; Ji et al., 2019). In addition, antiviral peptides can be chemically synthesized (Table 1) via solid-phase synthesis, solution-phase synthesis, or a combination of both methods. Most synthetic methods involve the solid-phase option, which was introduced by Merrifield in the 1960s (Merrifield, 1963). The purification steps, which are characteristic of the solution-phase method, can be minimized in the solid-phase method since purification is only required in the last step, thus, the solid-phase method has become the method of choice for peptide synthesis.

This review focuses on the synthesis of peptides with good antiviral properties against several types of viruses that obtained from the literature for the period spanning 1995-2020. Much of the literature fails to detail the synthesis protocol, and some peptides are even purchased from custom peptide companies. However, the syntheses described in this review represent the synthesis of most antiviral peptides. The peptides discussed in the review are grouped into two categories, peptide and peptide conjugate, the latter involves a peptide conjugated with a non-peptidic compound.

2. CHEMICAL SYNTHESIS OF ANTIVIRAL PEPTIDES

Most effective antiviral peptides are varied in terms of structure, having linear or cyclic structures and some are conjugates with other compounds. Regarding synthesis, most antiviral peptides are synthesized using the solid-phase method but the solution-phase method, as well as a combination of the solid- and solution-phase methods, are also applied (Table 1).

Table 1. Synthetic methods of antiviral peptides

No.	Peptides	Sequence	Synthetic method	References
Peptide				
1	Thymosin α1	Ac-Ser-Asp-Ala-Ala-Val-Asp-Thr-Ser-Ser-Glu-Ile-Thr-Lys-Asp-Leu-Lys-Glu-Lys-Lys-Glu-Val-Val-Glu-Glu-Ala-Glu-Asn-OH	Solid method	(García-Ramos et al., 2009)
2	E1 GBV-C	Cyclo-Trp-Ile-Leu-Glu-Tyr-Leu-Trp-Lys-Val-Pro-Phe-Asp-Phe-Trp-Arg-Gln-Val-Ile	Solid method	(Gómara et al., 2016)
3	GBV-A NS5A-derived peptide	NH_2-Cys-Trp-Val-Arg-Leu-Gly-Arg-Tyr-Leu-Leu-Arg-Arg-Leu-Lys-Thr-Leu-Phe-Thr-OH	Combination of solid- and solution-method	(Liu et al., 2012)
4	BST2-TM-P1 peptide	NH_2-Lys-Arg-Ser-Lys-Leu-Leu-Leu-Gly-Ile-Gly-Ile-Leu-Val-Leu-Leu-Ile-Ile-Val-Ile-Leu-Gly-Val-Pro-Leu-Ile-Ile-Phe-Thr-Ile-Lys-Lys-Lys-Lys-Lys-Lys-Met-OH	Solid method	(Mi et al., 2014)
5	Bovine lactoferrin (bLf)	Cyclo-(Lys-His-Ser-Ser-Leu-Asp)-Cys-Val-Leu-Arg-Pro-OH	Solid method	(Scala et al., 2018)
6	C8 antiviral peptide	AcO-NH-Trp-Arg-Asp-Trp-Val-Gly-Trp-Ile-$CONH_2$	Solid method	(Scrima et al., 2014)
7	DP178	NH_2-Tyr-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-OH	Solid method	(Kliger et al., 2001)
8	Feglymicin	NH_2-(D-Hpg)-(D-Dpg)-(L-Val)-(D-Dpg)-(L-Hpg)-(D-Dpg)-(L-Hpg)-(D-Dpg)-(L-Val)-(D-Dpg)-(L-Hpg)-(L-Phe)-(L-Asp)-OH	Solution method	(Dettner et al., 2009)
9	Chimeric peptide	(NH_2-Ile-Tyr-Tyr-Asp-Tyr-Glu-Glu-Asp-Pro-Ala-Pro-Gly-Ser-Thr-Ala-OH)$_3$	Solid method	(Fontenot et al., 1996)
Peptide conjugate				
1	Anti-dengue peptide Hybrids	5-arylidenethiazolidine-2,4-dione-Arg-Lys-Phg-OH	Solid method	(Lima et al., 2015)
2	Ganciclovir (GCV)	Gly-Val-GCV diester	Solution method	(Patel et al., 2005)
3	Rhinovirus 3C protease inhibitor peptide	ethyl-(*S,E*)-4-(2*R*,5*S*)-2-(4-fluorobenzyl)-6-methyl-4-oxo-5-pyridazine-3-carboxamidoheptanamido-5-(*S*)-2-oxopyrrolidin-3-yl-pent-2-enoate	Solution method	(Tan et al., 2016)
4	Oligonuleotide containing KDEL	NH_2-R_1-Ala-Cys-Ala-Cys-Cys-Gly-Ala-Cys-Gly-Gly-Cys-Gly-R_2-S-CH_2-CO-Tyr-Lys-Asp-Glu-Leu-OH	Solid method	(Arar et al., 1995)
5	Biotinylated peptide	Biotin-Arg-Gln-Ile-Lys-Ile-Trp-Phe-Gln-Asn-Arg-Arg-Met-Lys-Trp-Lys-Lys-Gly-Ala-Gly-Ala-Leu-Gln-Leu-Pro-Pro-Leu-GluArg-Leu-Thr-Leu-Asp-OH Biotin-Arg-Gln-Ile-Lys-Ile-Trp-Phe-Gln-Asn-Arg-Arg-Met-Lys-Trp-Lys-Lys-Gly-Ala-Gly-Ala-Leu-Gln-Leu-Pro-Pro-Ala-Glu-Arg-Ala-Thr-Leu-Asp-OH	Solid method	(Flint et al., 2005)
6	Dendrimeric heparan sulphate-binding peptide	NH_2-Ala-Ser-Leu-Arg-Val-Arg-Ile-Lys-Lys-COOR	Combination of solid- and solution-method	(Donalisio et al., 2010)

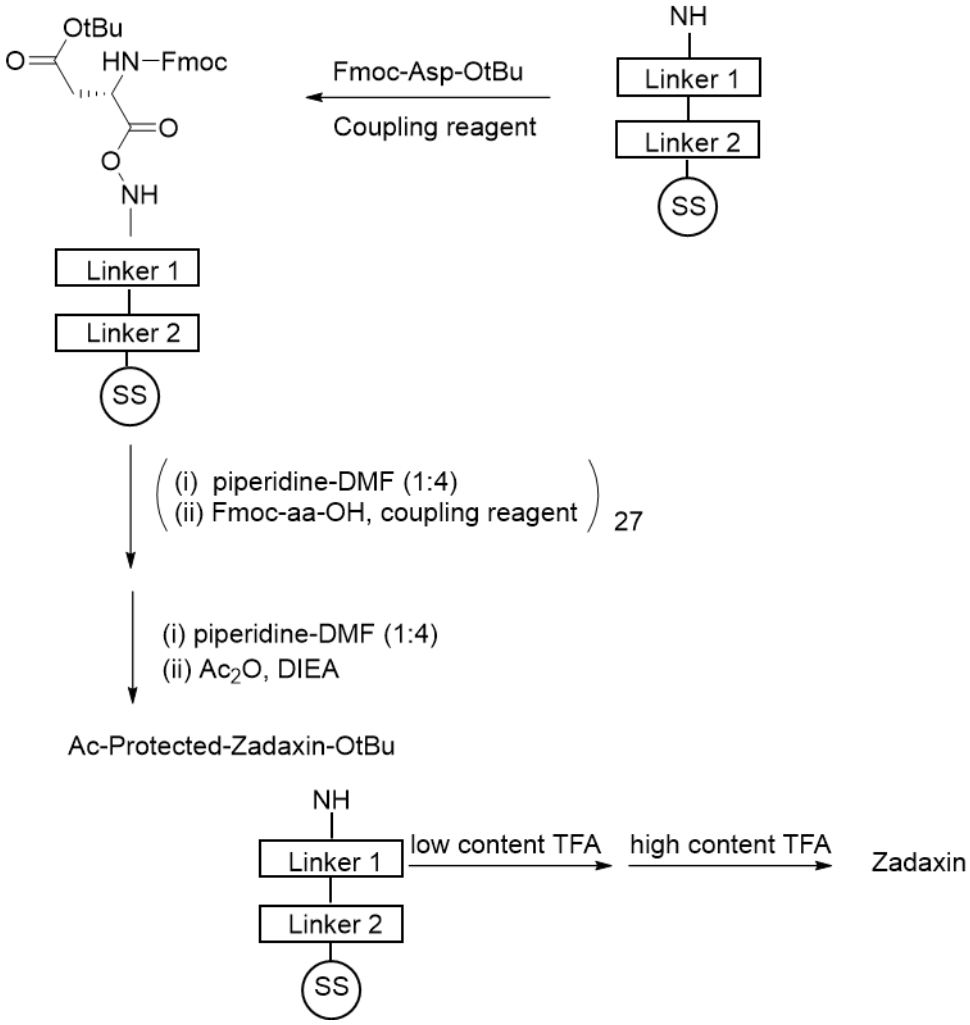
2.1 Peptides

2.1.1 Thymosin

Thymosin contains 28 amino acids (Table 1) and is used to treat chronic hepatitis C and D. At a dose of 1.6 mg twice weekly subcutaneous injections for 26 weeks, this peptide is effective in reducing viral replication in both HBeAg-positive and HBeAg-negative chronic hepatitis B patients (Li et al., 2010). However, the synthesis of this peptide is challenging due to its difficult sequence and tendency to form a β-sheet. Convergent solution-phase and solid-phase methods have been reported but their yields are low. García-Ramos et al. (2009) reported several attempts to synthesize thymosin using a solid-phase method (García-Ramos et al., 2009). The first attempt employing chlorotrityl chloride (CTC) resin (Figure 1) was unsuccessful due to its hydrophobic and bulky structure, together with the bulky structure of the first amino acid attached [Asn(triphenylmethyl; Trt)], leading to aggregation. The second attempt using fluorenylmethyloxycarbonyl (Fmoc) chemistry and a side-chain anchoring strategy into the Rink amide was successful (Scheme 1).

The side-chain anchoring method was applied trialling several amide-type resins, Rink-ChemMatrix, Rink-4-methylbenzhydrylamine hydrochloride (MBHA), Sieber, polyethylene glycol (PEG), and total PEG resins (Figure 1). Manual synthesis was performed first to test the method before synthesis on an automatic synthesizer. The total PEG and chemmatrix resin yielded 90% pure thymosin, hence a 100% PEG-based resin is suitable for large peptides.

O-(1*H*-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate (HCTU, Figure 2) was employed as a coupling reagent with ten equivalents used in the synthesis for 30-35 min coupling time. Hood et al. (2008) mentioned that the HCTU reagent was affordable and efficient for any fast Fmoc solid-phase synthesis. A side-chain protecting group is *t*-Bu, hence a simple scavenger such as water is required. The presence of Trt, Xan, or *S*-2,4,6-trimethoxybenzyl (Tmob) protecting group on Asn will require additional scavengers, such as triethylsilane. Acetylation is the final step using acetic anhydride in *N,N*-diisopropylethylamine (DIEA) and the peptide was cleaved with trifluoroacetic acid (TFA): H_2O (95:5).



Scheme 1. The side-chain anchoring method for thymosin α1 (zadaxin)
Note: SS is solid support.

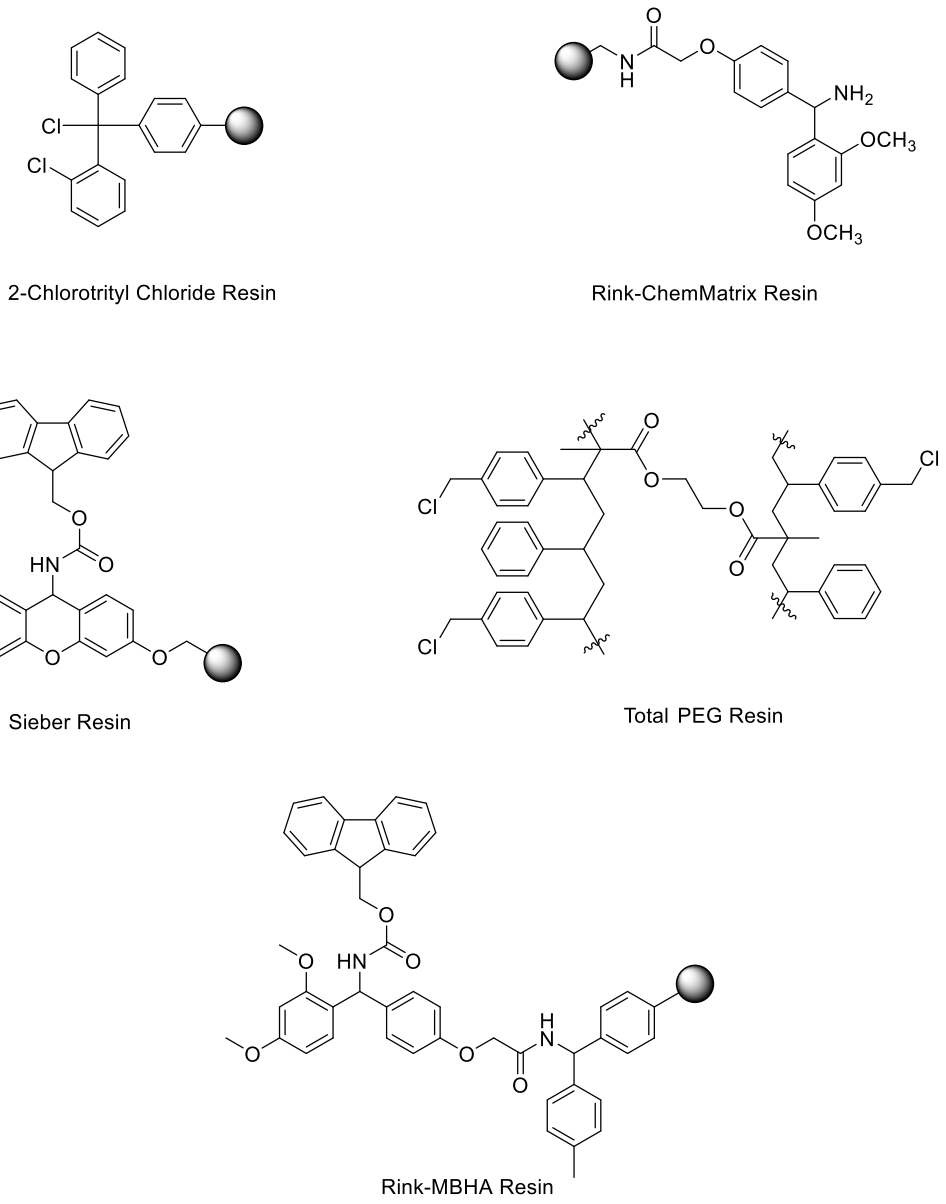


Figure 1. Structures of 2-chlorotrityl chloride, Rink-ChemMatrix, Rink-MBHA, Sieber, and total PEG resin

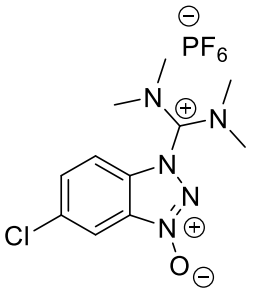


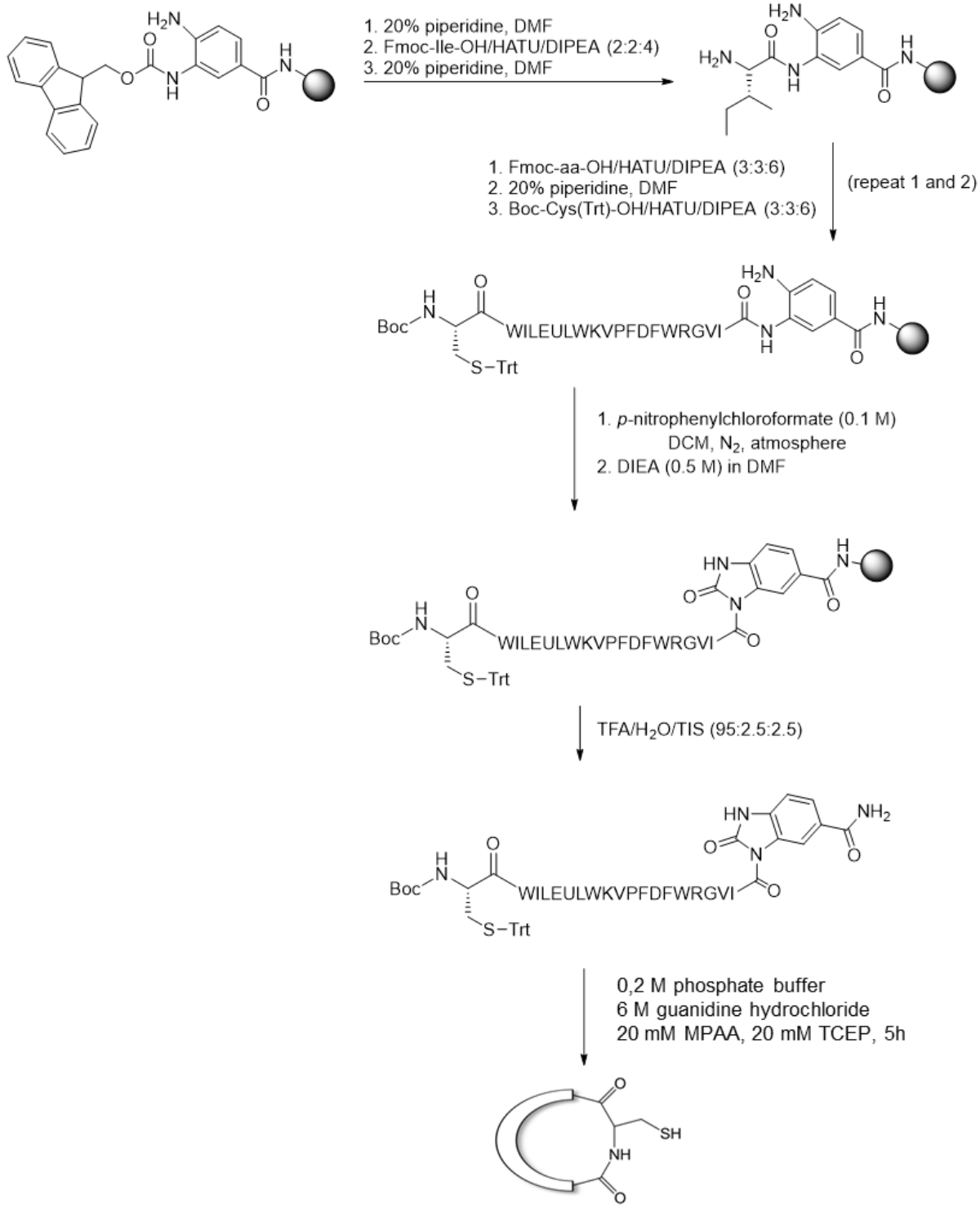
Figure 2. Structure of HCTU

2.1.2 E1P47 GBV-C

The synthesis of an anti-HIV peptide, E1P47 GBV-C, was described by Gómara et al. (2016) (Scheme 2). The peptide is a novel lead compound with an IC_{50} to HIV-1 NL4-3 of 7 ±0.3 µM. The report described that there is an interaction between the peptide and the N-terminal region of HIV-1 gp41 (the fusion peptide), which is crucial for antiviral activity and has a comparable mode of action to the inhibitor VIR576. It was concluded that E1P47 could interfere with HIV-1 entry.

The synthesis of this cyclic peptide involved a combination method with the 18-residue linear peptide prepared via semi-automated multiple solid-phase synthesis with a PEG-based resin, Dawson Dbz Novasyn TGR resin, and a diaminobenzoyl linker (Figure 3) applying the Fmoc strategy. Since there were two possible amino groups in the linker, a time limitation in the first amino acid loading was applied. *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate (HATU) reagent (Figure 4) was used in the amide bond formation to avoid racemization during the coupling (Carpino et al., 1994). Interestingly, imidazolinone (Nbz) was formed at the peptide C-terminus via the conversion of the diaminobenzoyl linker into Nbz by treatment with *p*-nitrophenylchloroformate in

dichloromethane (DCM) under nitrogen. The cyclization of the linear precursor was achieved in the solution-phase after the removal of the cysteine-side-chain and amino protecting groups. The native chemical ligation (NCL) strategy, which is based on an intramolecular transthioesterification reaction between a C-terminal thioester group and the N-terminal cysteinyl peptide, was applied to achieve a 20% yield of the final product.



Scheme 2. E1P47 synthesis
Note: MPAA is 4-Mercaptophenylacetic acid, TCEP is tris(2-carboxyethyl)phosphine.

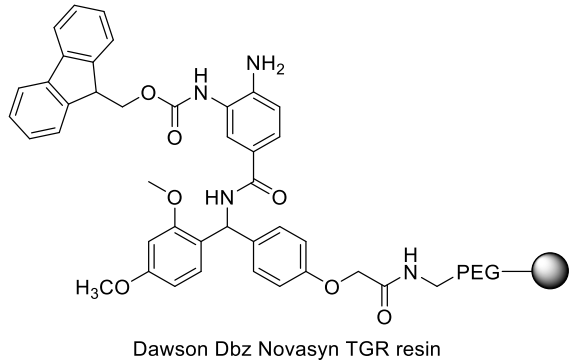


Figure 3. Structure of the Dawson Dbz Novasyn TGR resin with a diaminobenzoyl linker

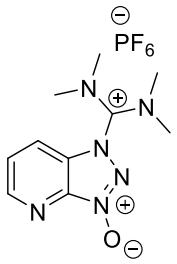


Figure 4. Structure of HATU

2.1.3 GBV-A NS5A-derived peptide

Liu et al. (2012) reported the Fmoc-based synthesis of an 18-residue peptide (NH_2-Cys-Trp-Val-Arg-Leu-Gly-Arg-Tyr-Leu-Leu-Arg-Arg-Leu-Lys-Thr-Leu-Phe-Thr-OH), which inhibited hepatitis C virus (HCV) with an IC_{50} of 1.9 µM towards H77 HCVpp. Wang resin (Figure 5) was employed in the synthesis (Sherrington, 1990) and treatment of the peptidyl resin by TFA/1,2-ethanedithiol (EDT)/ triisopropyl silane (TIS)/H_2O resulted in a cleaved peptide that was ready for purification to obtain the desired peptide. EDT and TIS were used to scavenge cations of the cleaved protecting group.

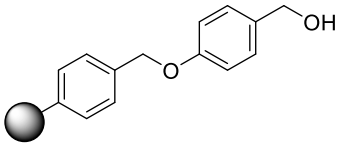


Figure 5. Structure of Wang resin

2.1.4 BST2-TM-P1 peptide

BST2-TM-P1 peptide is an antiviral peptide known to have anti-Herpes simplex virus (HSV) activity, with an IC_{50} of 10 µM. It has 37 amino acids (NH_2-Lys-Arg-Ser-Lys-Leu-Leu-Leu-Gly-Ile-Gly-Ile-Leu-Val-Leu-Leu-Ile-Ile-Val-Ile-Leu-Gly-Val-Pro-Leu-Ile-Ile-Phe-Thr-Ile-Lys-Lys-Lys-Lys-Lys-Lys-Met-OH) but exhibits no anti-HIV properties. The interaction between BST-2 TMD and Vpu TMD was disrupted by the peptide, which also blocked Vpu-mediated BST-2 downregulation, increased the BST-2 level at the cell surface, and extensively inhibited the replication of HIV-1 virion at a 10 µM concentration compared to the reference peptide, BST2-TM-P1-MT.

This peptide was synthesized on Fmoc-Rink Amide-MBHA resin using a standard Fmoc/tBu protocol (Mi et al., 2014). The *N,N′*-diisopropylcarbodiimide (DIPC)/ hydroxybenzo-triazole (HOBt) (Figure 6) coupling protocol was applied. DIPC is a carbodiimide reagent that is more applicable for solid-phase peptide synthesis than *N, N′*-dicyclohexylcarbodiimide (DCC) due to its solubility in DCM (Han and Kim, 2004). The additive HOBt is commonly used to minimize racemization during the coupling (Carpino, 1993). The crude peptide was precipitated in cold ether and dried in vacuo at 40°C to yield the final product.

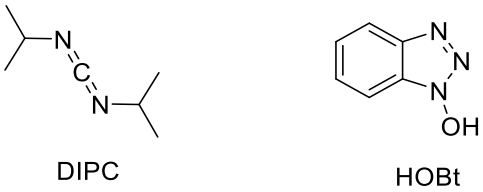


Figure 6. Structure of DIPC and HOBt

2.1.5 Bovine Lactoferrin (bLF)

An anti-influenza peptide with 11 amino acid residues in length was synthesized by Scala et al. (2018). This bLF has anti-influenza properties with an EC_{50} of 25 pM against H1N1, and can prevent virus hemagglutination and infection of all major virus subtypes, including H1N1 and H3N2, at low concentrations (pico-femtomolar).

Solid-phase synthesis with Fmoc chemistry on Fmoc-Rink-amide resin (100-200 mesh, 1% Divinylbenzene (DVB), 0.59 mmol/g) was applied to synthesize the peptide (Scheme 3). The amino acids Fmoc-Pro-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Leu-OH, Fmoc-Val-OH, Fmoc-Cys(Trt)-OH, Nα-Fmoc-Asp(2-PhiPr)-OH, Fmoc-Ala-OH, Fmoc-Leu-OH, Fmoc-Ser(tBu)-OH, Fmoc-His(Trt)-OH, and Fmoc-Lys(Mmt)-OH were used in the synthesis. A combination of *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uranium hexafluorophosphate (HBTU) (Figure 7) and HOBt reagents was employed in all coupling steps, including the on-resin cyclization step. Even though HBTU is less reactive than the aza derivative HATU, the reagent facilitates amide bond formation (Han and Kim, 2004). The synthesis of the lactam-bridged peptide, cyclo-(Lys-His-Ser-Ser-Leu-Asp)-Cys-Val-Leu-Arg-Pro-OH, was proven to be difficult, yielding undesired by-products such as aspartimide and α-piperidinyl derivatives according to LC-MS and NMR analyses. The most effective orthogonal strategy for the reduction of aspartimide formation during the synthesis is the use of (i) 2-PhiPr side-chain protection of aspartate, (ii) Trt side-chain protection of cysteine, and (iii) 4-methoxytrityl for lysine side-chain (yield: 30%). TFA/TIS /H_2O was used to release the peptide from the resin.

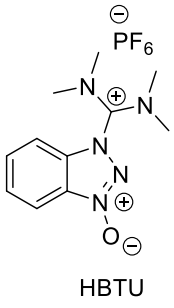
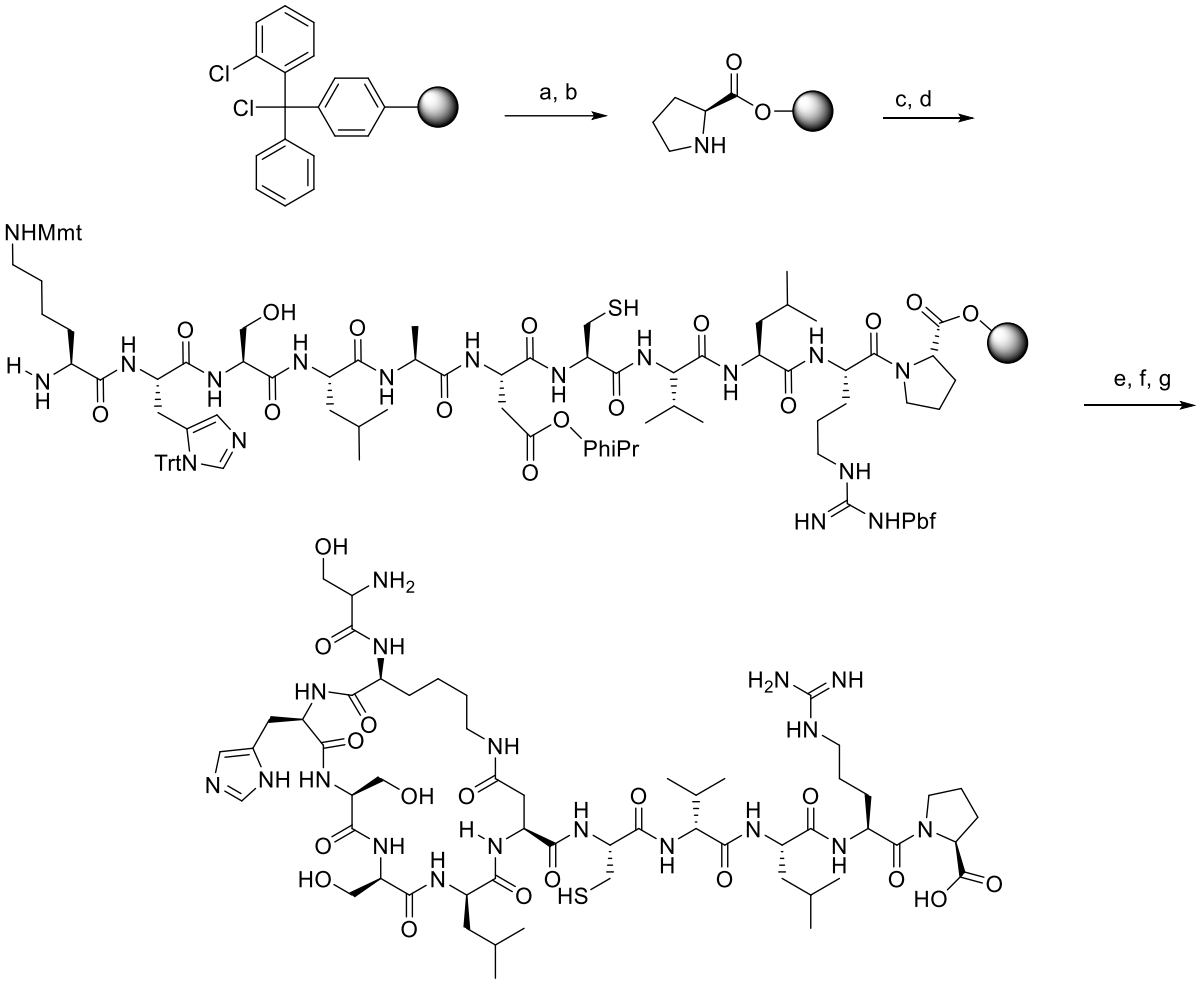


Figure 7. Structure of HBTU



Scheme 3. Synthesis of the lactam-bridged cyclic peptide. a. Fmoc-Pro-OH, DIEA. b. 25% piperidine, *N,N*-dimethylformamide (DMF). c. Fmoc-aa-OH/HBTU/HOBt/DIEA (3:3:3:6). d. 25% piperidine, DMF. e. 1% TFA/DCM. f. HBTU/HOBt/DIEA (3:3:6) for 2 h. g. TFA/TIS/H_2O (90:5:5) for 3h

2.1.6 C-8 peptide

An anti-HIV eight amino acid residue peptide (Figure 8) was synthesized using a manual conventional solid-phase strategy with Fmoc-based residues and Rink amide resin (Scrima et al., 2014). To improve peptide stability, the peptide was N-acetylated and C-amidated.

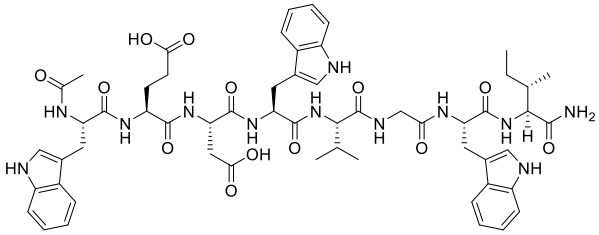


Figure 8. Structure of C8 antiviral peptide

2.1.7 DP178

The DP178 peptide was synthesized on 4-(hydroxymethyl) phenylacetamidomethyl-polystyrene (PAM) resin (Figure 9) using a *tert*-butyloxycarbonyl (Boc) strategy, with more acid-stable groups employed to protect the side chains (Merrifield method) (Kliger et al., 2001). The residue was then incorporated into the DCC/HOBt (Figure 10) coupling protocol and hydrofluoric acid (HF) was employed for the deprotection of Boc groups in the Nα. The peptide was detached from the resin using 12 N of hydrochloric acid (HCl)/phenol/HOAc (2: 1: 1) at 110°C for 24 h. The IC_{50} of DP178 towards phospholipid and solute redistributions mediated by the HIV-1 envelope glycoprotein was 43 and 335 nM, respectively.

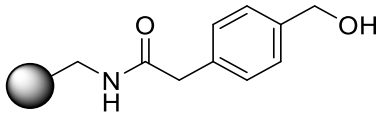


Figure 9. Structure of PAM resin

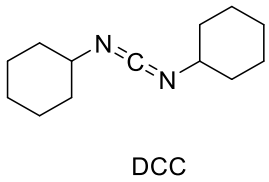


Figure 10. Structure of DCC

2.1.8 Feglymycin

Feglymycin has anti-HIV-1 properties with an IC_{50} of 1.9 μM. Some precursors of feglymycin also showed remarkable anti-HIV-1 activity. This 13-residue peptide was synthesized in the solution-phase via Boc chemistry (Dettner et al., 2009). The retrosynthetic analysis led to several fragments (Scheme 4) and since phenylglycine is the major residue of feglymycin, the initial step in the synthesis was to prepare this residue.

A four-step synthesis was involved in the preparation of the phenylglycine residue, starting from benzaldehyde (Scheme 5). The conversion involved a subsequent Wittig reaction, aminohydroxylation, and two oxidations before the phenylglycine (DPg) was produced. The synthesis was then followed by the preparation of a DPg-containing dipeptide (Scheme 6) that was used in the preparation of the hexapeptide unit (Scheme 7). The 3-(diethoxyphosphoryloxy) -1,2,3-benzotriazin-4(3*H*)-one (DEPBT, Figure 11) was used for the amide bond formation as it is an effective coupling agent for both solution- and solid-phase peptide synthesis (Ye et al., 2005). Carbodiimide-type reagent, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), combined with the additive 1-Hydroxy-7-azabenzotriazole (HOAt), was also involved in the formation of some peptidic bonds.

The synthesis of the heptapeptide unit was initiated by the synthesis of tetrapeptide and tripeptide fragments (Schemes 8 and 9), which were then conjugated to obtain the desired heptapeptide unit (Scheme 10).

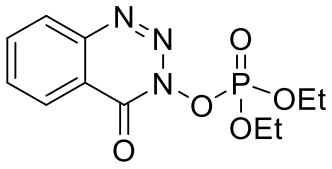
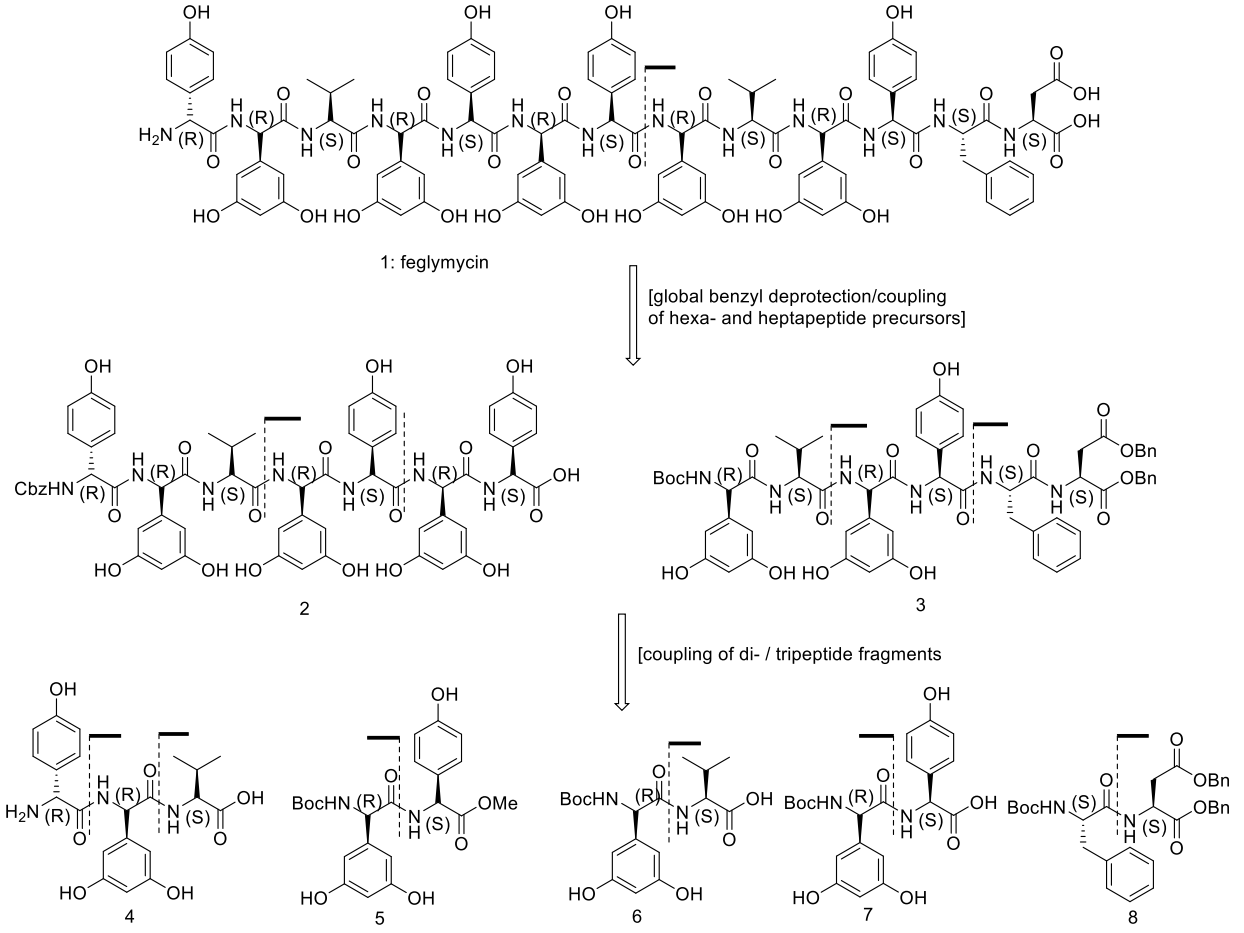
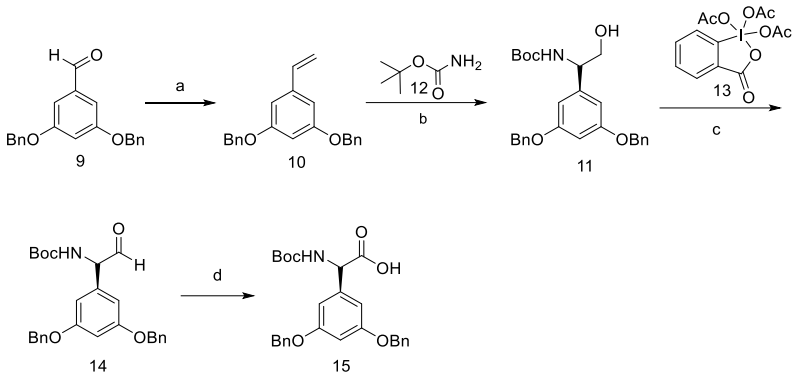


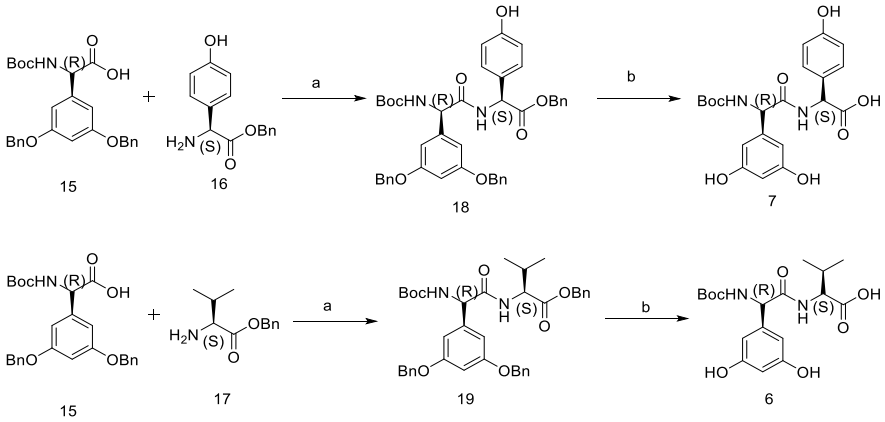
Figure 11. Structure of DEPBT



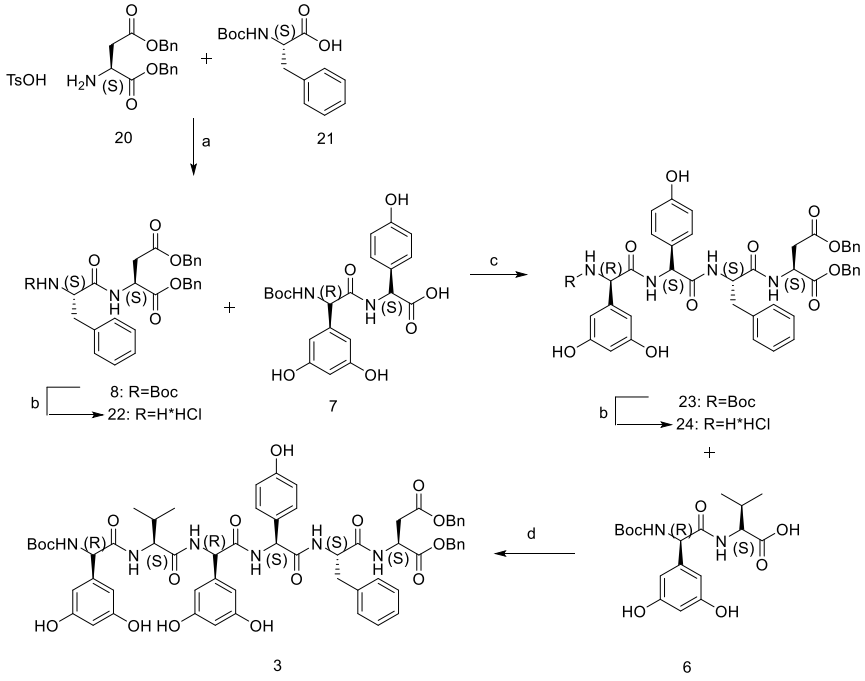
Scheme 4. Retrosynthesis of feglymycin



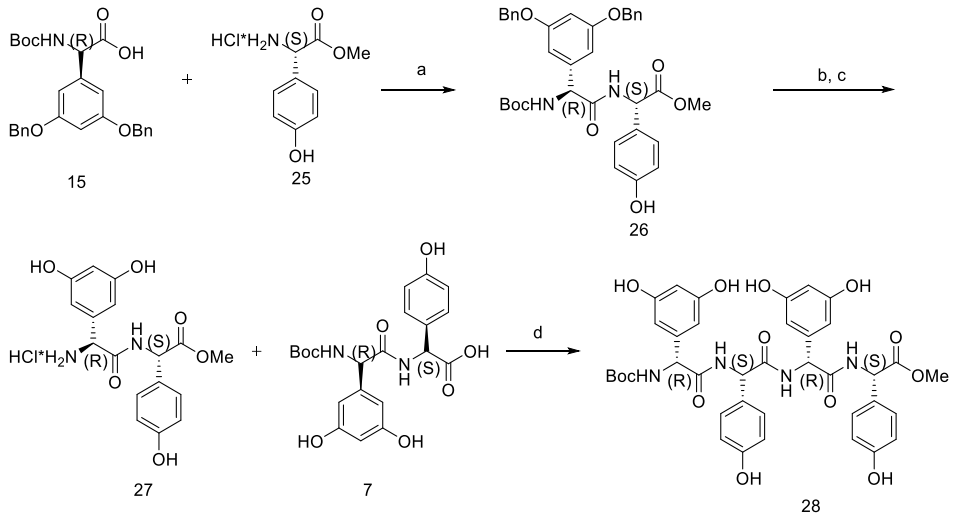
Scheme 5. Synthetic scheme of the preparation of phenylglycine (DPg): a. $[Ph_3PCH_3]Br$, n-BuLi, tetrahydrofuran (THF), -40°C ~ RT, 4.5 h, 91%; b. t-BuOCl, $K_2[OsO_2(OH)_4]$, $(DHQD)_2PHAL$,n-PrOH/H_2O (2:1), 0°C, 1 h, 52% 98% ee; c. 13, CH_2Cl_2, 0°C ~ RT, 2 h, quantitative; d. $NaClO_2$, 2-methyl-2-butene, H_2O, 25°C, 40 min, 97%



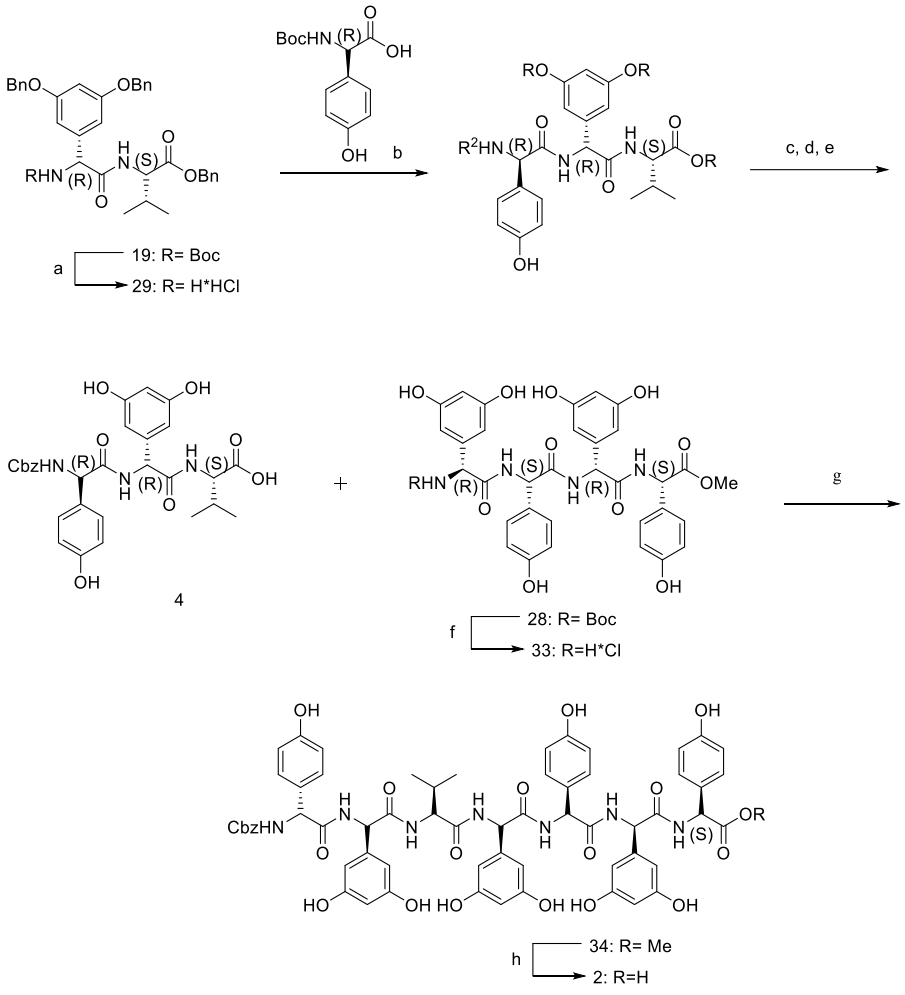
Scheme 6. Synthetic scheme of the preparation of DPg-contaning peptide: a. DEPBT, $NaHCO_3$, THF, 0°C ~ RT, 21 h, 80%; b. 10% Pd/C, H_2, THF, RT, 4 h



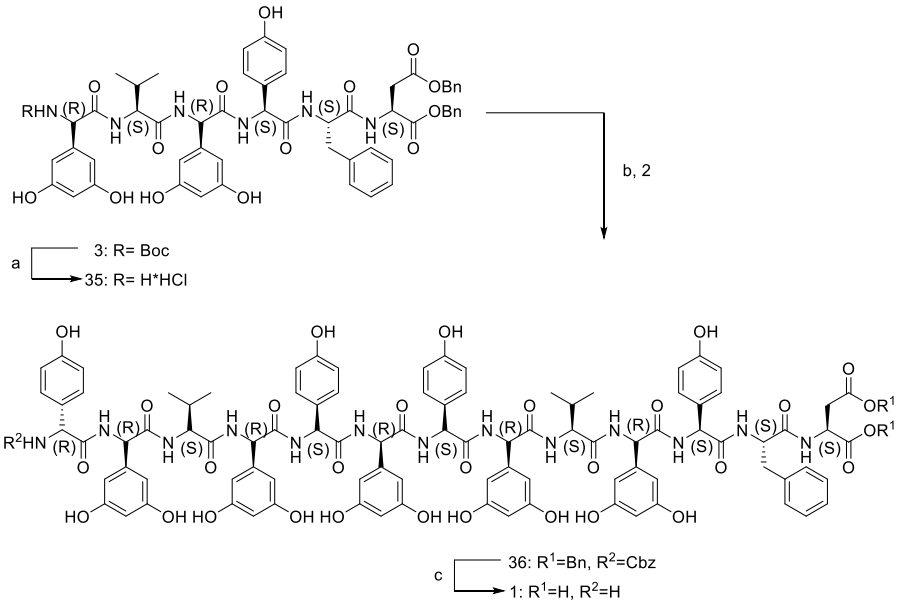
Scheme 7. Synthetic scheme of the preparation of hexapeptide unit: a. EDC, HOAt, $NaHCO_3$, DMF, 0°C ~ RT, 19 h, 77%; b. 4 N HCl/dioxane, 1 h, quantitative (for 22 and 24); c. DEPBT, $NaHCO_3$, THF, 0°C ~ RT, 21 h, 73%; d. DEPBT, $NaHCO_3$, THF, 0°C ~ RT, 21.5 h, 77%



Scheme 8. Synthetic scheme of tetrapeptide fragment: a. EDC, HOAt, $NaHCO_3$, DMF, 0°C ~ RT, 19 h, 77%; b. 10% Pd/C, H_2 THF, RT, 4 h; c. 4 N HCl/dioxane, 1.5 h, quantitative; d. DEPBT, $NaHCO_3$, DMF, 0°C ~ RT, 21.5 h, 54%



Scheme 9. Synthetic scheme of tripeptide fragment and conjugation of the tripeptide fragment into the tetrapeptide unit: 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, RT, 4 h, quantitative; d. 4 N HCl/dioxane, 55 min, quantitative; e. Benzyl chloroformate (CbzCl), $NaHCO_3$, H_2O/dioxane, RT, 1.5 h, 87%; f. 4 N HCl/dioxane, 55 min, quantitative; g. DEPBT, $NaHCO_3$, THF, 0°C ~ RT, 21 h, 52%; h. Tris(hydroxymethyl) aminomethane (TMTH), 1,2-dichloroethane, 85°C, 4 h, 84%



Scheme 10. Synthetic scheme of conjugation hexapeptide unit into the theptapeptide unit: 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, RT, 5.5 h, 89%

2.1.9 Chimeric C45 peptide

A proline-rich tandem repeat peptide MC60 (Figure 12) with 60 residues, $(NH_2$-Ile-Tyr-Tyr-Asp-Tyr-Glu-Glu-Asp-Pro-Ala-Pro-Gly-Ser-Thr-Ala-OH$)_3$, has anti-HSV-1 activity with an IC_{50} of 10 μM. This peptide was synthesized in a rapid multiple-peptide synthesizer with 0.1 mM rapid amide (2,3-dimethoxybenzhydrylamine) resin cartridges, employing 3-hydroxy-2,3-dihydro-4-oxo-benzotriazine (ODhbt) (Figure 13) and HOBt in the coupling reactions as they suppress racemization (Han and Kim, 2004). The peptide was cleaved from the resin by TFA-thioanisole-1,2-ethanedithiol-anisole (Fontenot et al., 1996).

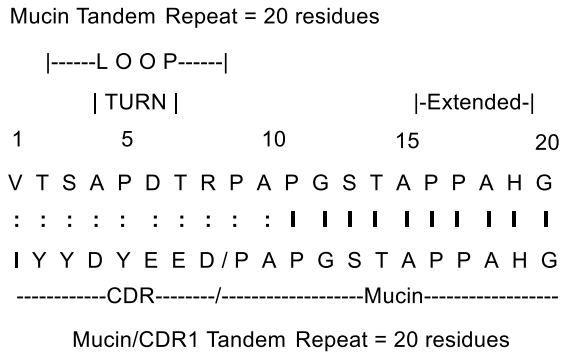


Figure 12. Design of one tandem repeat of MUC1-CDR1

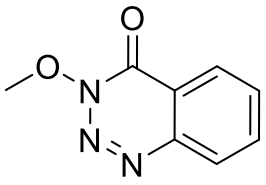


Figure 13. Structure of ODhbt

2.2 Conjugated peptides

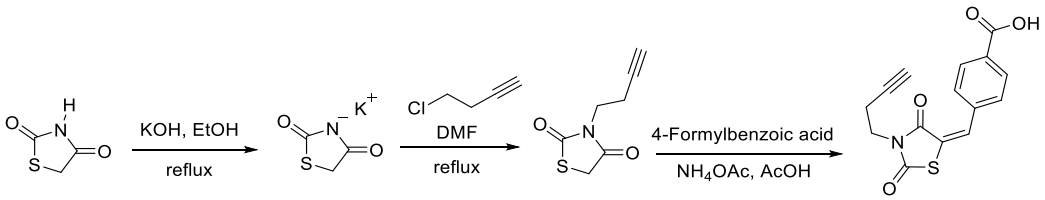
2.2.1 Conjugate of 5-arylidenethiazolidine-2,4-dione and NH_2-Arg-Lys-Phg-OH

An anti-dengue peptide containing 5-arylidenethiazolidine-2,4-dione moiety with anti-dengue virus activity (IC_{50} of 0.46 μM) and anti-dengue virus (WNF) activity (IC_{50} of 2.12 μM) was prepared on Rink amide resin using a Fmoc strategy (Lima et al., 2015). The synthesis was initiated by the preparation of 5-arylidenethiazolidine-2,4-dione from thiazolidine-2,4-dione (Scheme 11). Then, the peptide (NH_2-Arg-Lys-Phg-OH) was built on the resin, including attaching the 5-arylidenethiazolidine-2,4-dione to the resin in the last step (Scheme 12). HBTU was used to facilitate all amide bond formations and a cocktail of TFA/triisopropylsilane (TIPS)/water was employed to cleave the desired compound. The yield of the synthesis was 86%.

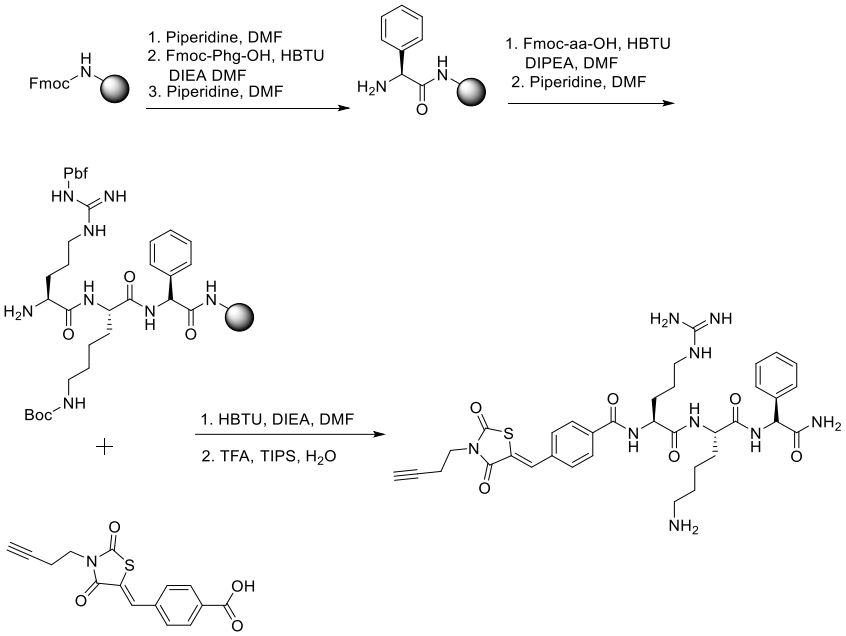
2.2.2 Ganciclovir (GCV)

Ganciclovir (GCV) (Figure 14) is a superior drug for the treatment of CMV retinitis. To enhance its efficacy, the GCV was conjugated as an ester with amino acids (Patel et al., 2005) (Scheme 13). The solution-phase synthesis was performed using Boc chemistry and Steglich esterification was applied to form an ester bond between GCV and amino acids, with 82% yield. In the DCC-based Steglich esterification, the by-product (dicyclohexylurea) was separated from the reaction mixture to make the purification easier (Neises and Steglich, 1978). The presence of 4-dimethylaminopyridine (DMAP) in the reaction accelerated the reaction and suppressed side reactions.

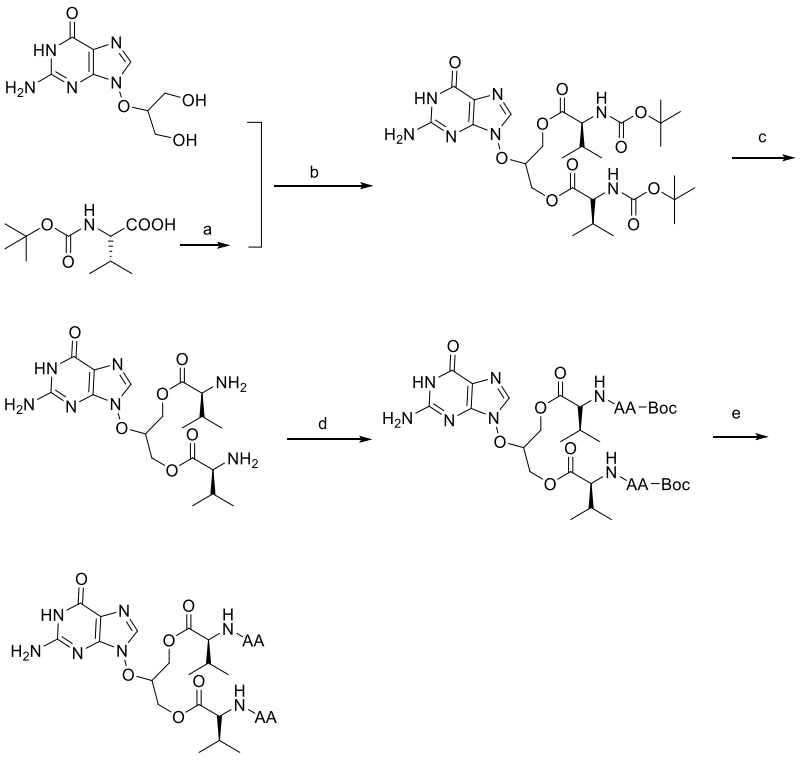
The peptide diester GCV and the ocular peptide transporter interact to a varying extent. An obvious enhancement of the intracellular drug concentration was accomplished due to the mutual effect of higher transcellular diffusion (lipophilicity) and major carrier-mediated transport by peptide transporters.



Scheme 11. Synthesis of 5-arylidenethiazolidine-2,4-dione



Scheme 12. Synthesis of 5-arylidenethiazolidine-2,4-dione-containing peptide



Scheme 13. Synthesis of Gly-Val-GCV diester: a. DCC, DMF, 0°C, 1h; b. DMAP, DMF, RT, 24 h; c. TFA, 0°C, 1 h; d. triethylamine (TEA), 15 min, Boc-AA-anhydride, DMAP, RT, 24 h; e. TFA, 0°C, 1 h

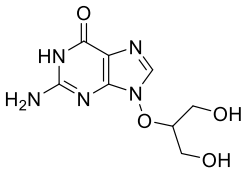
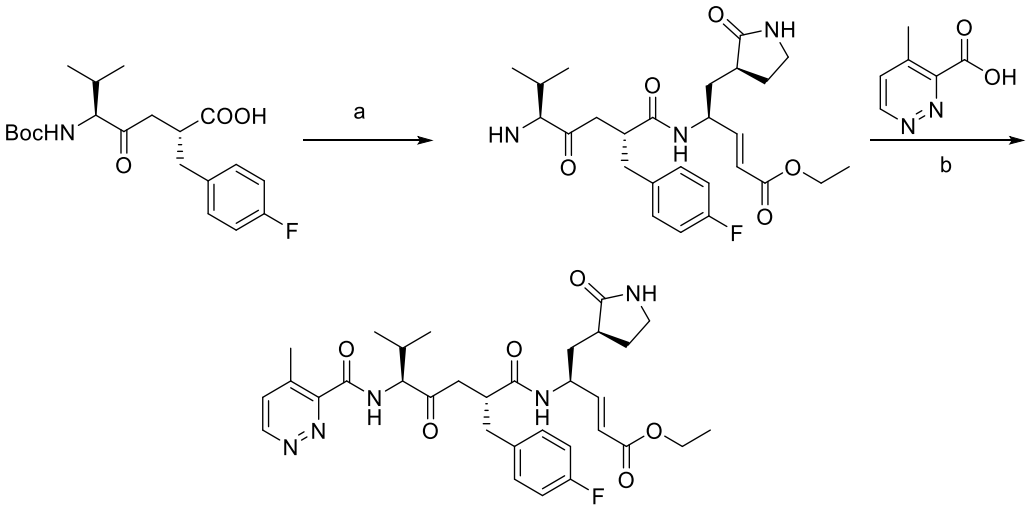


Figure 14. Structure of ganciclovir (GCV)

2.2.3 C-3 peptide

A rhinovirus 3C protease inhibitor tripeptide functions as an inhibitor of hand, foot and mouth disease (HFMD), as well as the EV71 cell virus, with an IC_{50} of 3.2 μM. The peptide NH_2-Val-Gly-Phe(F)-OH was conjugated with (*S,E*)-ethyl-4-amino-5-(*S*-2-oxopyrrolidin-3-yl)-pent-2-enoate to obtain the C-3 peptide (Tan et al., 2016). A solution-phase method with Boc chemistry was applied (Scheme 14) employing the, 6-chloro-benzotriazole-1-yloxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyClock) reagent (Figure 15) in the peptidic bond formation. PyClock is a highly reactive coupling agent (Subiros-Funosas et al., 2008). The Boc protecting group was removed with standard 95% TFA in DCM. The product was obtained in 50% overall yield.



Scheme 14. Synthetic scheme of C3 peptide: a. (1) (*S, E*)-ethyl-4-amino-5-(S-2-oxopyrrolidin-3-yl)pent-2-enoate, DIC, HOBt, DMF, 25°C, 16 h and (2) 95% TFA in CH_2Cl_2 (v/v), 25°C, 30 min; b. PyClock, DIEA, DMF, 25°C, 4 h

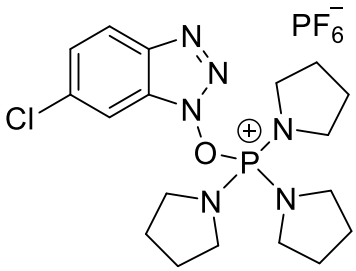


Figure 15. Structure of PyClock

2.2.4 KDEL-containing oligonucleotide

A peptide containing a KDEL (NH_2-Lys-Asp-Glu-Leu-OH) sequence was conjugated with a pentacosanucleotide by Arar et al. (1995) and was found to have anti-HIV properties, with an IC_{50} of 0.61 ± 0.03 μM. The peptide was synthesized via a solid-phase method on phenylacetamido resin (Figure 16) with Boc/ Benzyl (Bzl) chemistry by Merrifield. Carbodiimide (Figure 17) was employed as the coupling reagent and the bromo acetyl moiety was attached to the N-terminus of the peptide on the resin (Scheme 15). The peptide was cleaved using HF, then conjugated with the nucleotide. The oligonucleotide was synthesized in a disulfide derivative (Scheme 16) before it was conjugated to the peptide, resulting in a 100% yield of the antiviral peptide.

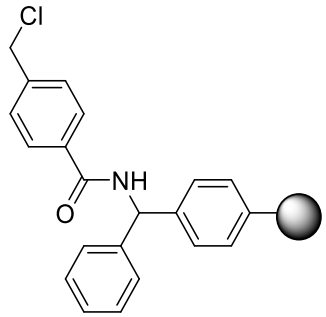


Figure 16. Structure of phenylacetomido resin

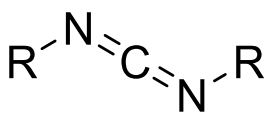
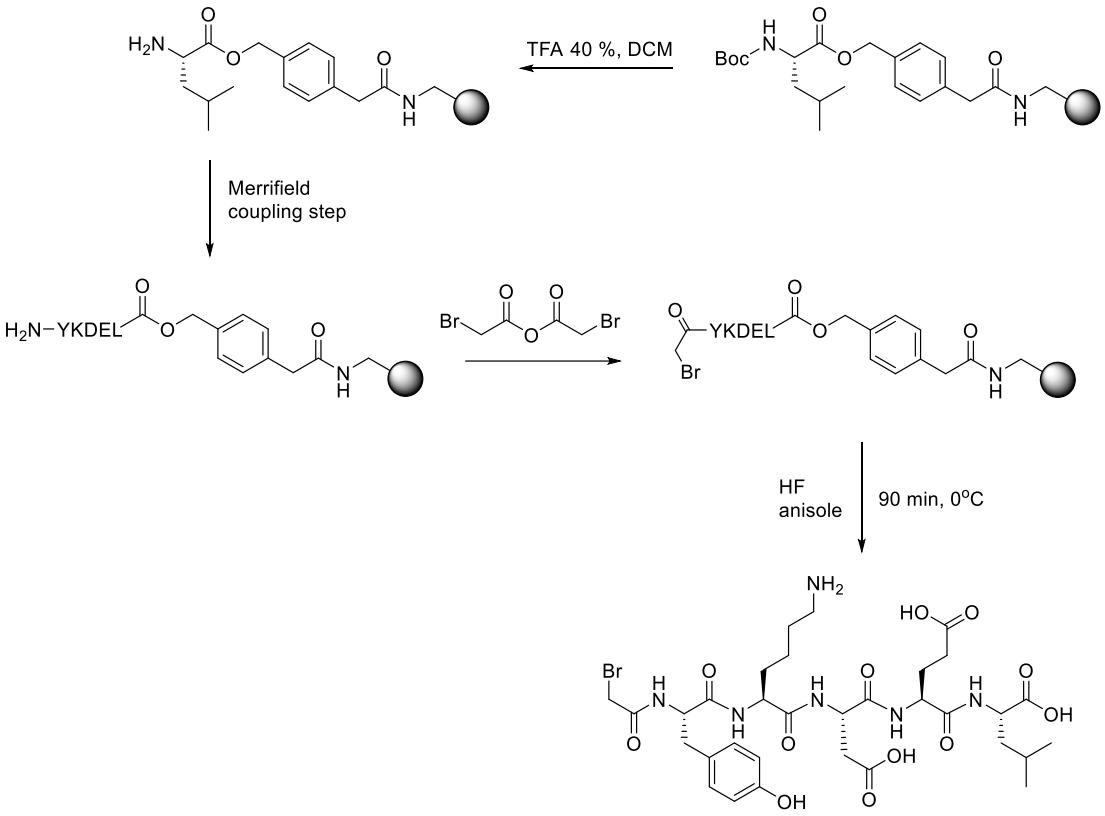
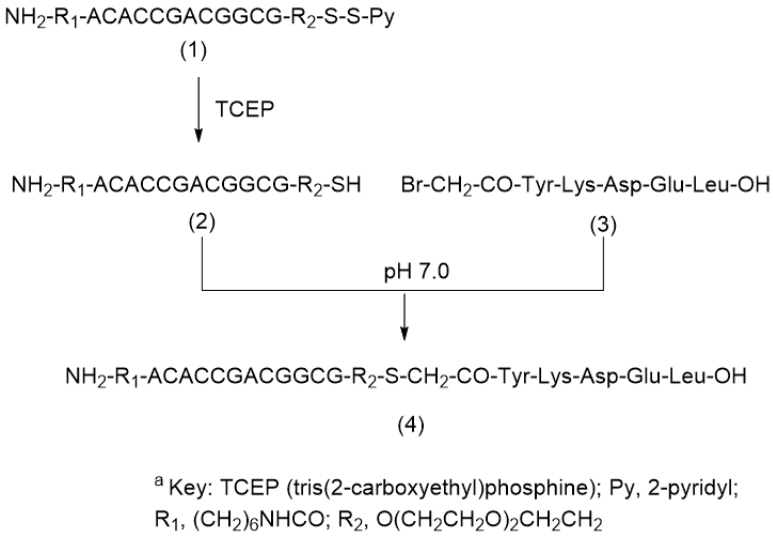


Figure 17. Structure of carbodiimide



Scheme 15. Synthetic of KDEL-containing peptide



Scheme 16. Conjugation of the KDEL-containing peptide and oligonucleotide

2.2.5 Biotinylated peptide

A 32-residue antiviral peptide was conjugated with biotin and this conjugate was synthesized via the solid-phase method by Flint et al. (2005) on a model 433A peptide synthesizer (Applied Biosystems) using Fmoc chemistry (Flint et al., 2005). The linear peptide was prepared in a peptide synthesizer with Fmoc chemistry. The peptidyl resin was removed from the instrument and reacted with biotin in a manual solid-phase synthesis to obtain the biotinylated peptide (Figure 18). The biotinylated peptide was released from the resin and deprotected using TFA.

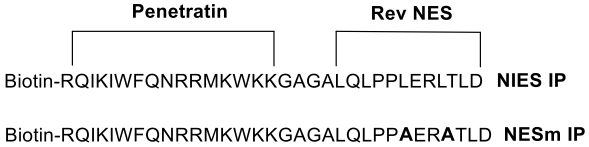


Figure 18. Sequences of the NES and NESm biotinylated peptides

2.2.6 Dendrimeric peptides

Tetrameric (Figure 19), dimeric and linear peptides with antiviral properties were synthesized by Donalisio et al. (2010) via a manual solid-phase method (Scheme 17). These peptides inhibited infection caused by human papillomaviruses HPV-16–SEAP PsV, with an IC_{50} of 2.8 μM. The peptide can bind to the capsids of the virus and also restrict the virus from entering the target cell. The biphasic binding of SB105-A10 to heparin was observed, where the first binding phase occurred at low concentrations and a relatively high affinity, leading to the higher potency of the peptide against HPV inhibition.

The synthesis of amide peptides was performed on a NovaPEG Rink amide resin using a Fmoc strategy (Scheme 17) and the acidic peptides were prepared on a preloaded Wang resin, with HBTU/HOBt employed in all coupling reactions. Protecting groups of the side chains involved 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl chloride (Pbf) for arginine, t-Bu for serine and Boc for lysine and tryptophan. The peptides were released from the resin using TFA/TIPS/water for complete cleavage.

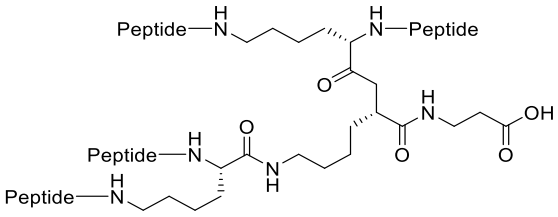
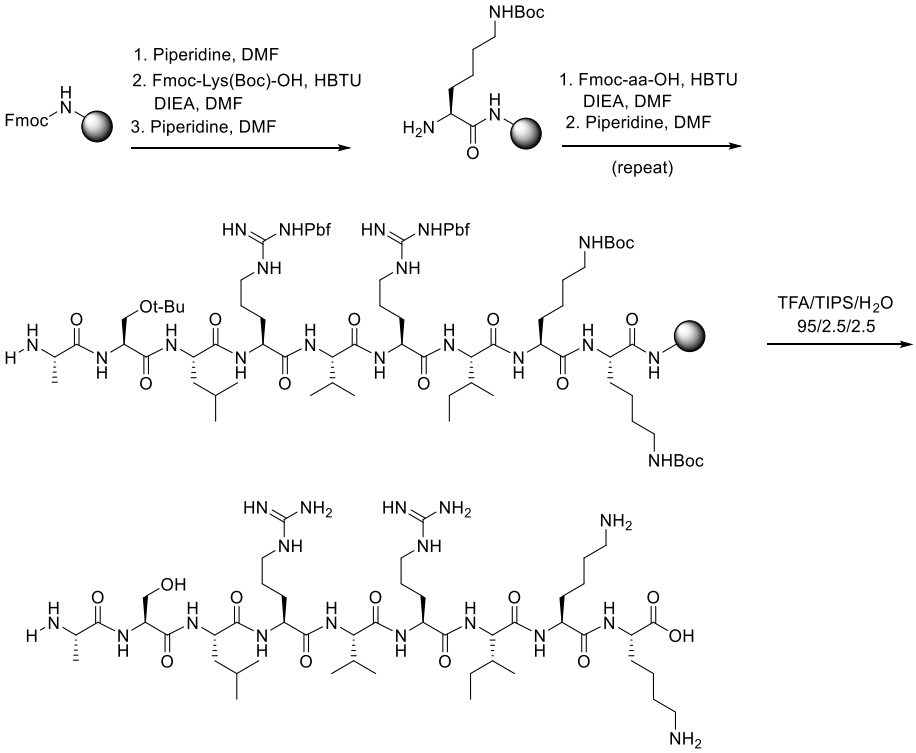


Figure 19. Structure of tetrameric peptides (SB105) A_{10}



Scheme 17. Synthesis of linear nonapeptide NH_2-ASLRVRIKK-OH

3. CONCLUSION

Antiviral peptides vary in terms of structure and include linear peptides and peptide conjugate. These peptides have good antiviral activity with different modes of action. Most antiviral linear peptides are synthesized via solid-phase peptide synthesis using a Fmoc strategy coupled with various types of resin, including total PEG-, PEG-, Wang-, and Rink amide-based resin. Typically, a long sequence peptide requires a non-hydrophobic and bulky resin. Boc chemistry can be coupled with a PAM resin for peptide synthesis. A solution-phase synthesis can also be applied but is more time-consuming due to the numerous purification steps required. Most solution-phase syntheses use Boc chemistry. When the peptide sequence contains unnatural residues, they must be prepared before elongation of the sequence. The amide bond formations can be facilitated by various coupling reagents, including HCTU, HATU, DIPC/HOBt, HBTU/HOAt, DCC/HOBt, DEPTBT, EDC/HOAt, and PyClock. Since most amino acids employed in the synthesis contain protecting groups on the side-chain, such as *t*-Bu, Pbf, Boc, Bn/Bzl, the strategy for the peptidic cleavage in the final step is of great importance and must be considered for solid-phase peptide synthesis (SPPS). Scavengers should be added to the cleavage cocktail, such as water, TIS, EDT, β-mercaptoethanol, or phenol. In the case of cyclic peptides, the linear peptide is frequently synthesized through the SPPS method, while cyclization is performed in the solution-phase.

Peptide conjugates with antiviral properties are generally a conjugation between a peptide and an organic compound that promotes antiviral properties. The organic compounds which are varied in terms of structure and the conjugation between the peptide and the organic compound can be achieved by several reactions, including amide bond formation with HBTU, PyClock, or carbodiimide coupling

agent and ester bond formation through Steglich esterification. The reactions can be performed in either the solution or solid-phase method. Most of solution-phase method involve Boc chemistry, while the solid-phase method involves either the Fmoc or Boc strategy.

The synthetic strategies described in this review give important information regarding the preparation of antiviral peptides, which can be used to inform more advanced synthetic investigations and may also aid in the design of more potent antiviral peptides as candidates for drug development.

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