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Chapter 5

A General Synthetic Method Towards Uridylylated Picornavirus VPg Proteins

Introduction

*Picornaviridae* are single stranded, positive sense RNA viruses sub-classified in the genera: *Enterovirus, Rhinovirus, Hepatovirus, Parechovirus, Kobuvirus, Erbovirus, Cardiovirus, Aphtovirus* and *Teschovirus*. Well-known examples of notorious human pathogens from this family of viruses are Rhinovirus, Hepatitis A virus and Poliovirus, which cause the common cold, acute liver infection and inflammation of the central neural system, respectively. Other members of this family of viruses are known agricultural pathogens, such as Foot-and-mouth-disease virus which causes high fever and blisters in the mouth and on the feet of cloven-hoofed animals. Encephalomyocarditis virus and Theiler’s virus, which cause inflammation of the heart muscle and brain in rodents, are other examples.

All these viruses share the same genomic structure: a 5’ non-translated region (NTR) with an internal ribosome entry site and specific cloverleaf structure, an open reading frame coding for all viral capsid and replication proteins, a 3’ NTR and a 3’ poly(A)-tail. In addition, the most intriguing feature is that picornaviruses carry a small VPg-protein (Viral Protein genome-linked) at the 5’-end of their genome whereas in other species the 5’-end of the RNA is commonly protected with a cap structure such as the modified nucleotide 7-methyl guanylate, which is attached via an 5’ - 5’ triphosphate linkage. The VPg protein is a 22-24 amino acid sequence that is highly conserved in all picornaviruses, and plays a crucial role in viral replication.

Upon infection by picornaviruses the viral genome is transcribed by the host ribosome generating viral replication proteins, amongst which the protein VPg. The positive sense genome is copied using its own replication proteins into a negative sense RNA strand, which acts as template for the formation of 40 to 70 copies of progeny positive sense RNA.
more (+) strand RNA is formed than (-) strand RNA the replication is called asymmetric, indicating multiple replication mechanisms must be involved.

Since both strands are 5’ capped with the VPg protein, it was long believed that uridylylated VPg was involved as primer in both (+) and (-) strand synthesis. However, it is now proposed that this uridylylated VPg only acts as an essential primer in the synthesis of progeny (+) strand RNA, using the 3’- poly(A)-tail of the genome as template. The viral 3D polymerase is responsible for uridylylating VPg using a functional domain in the viral genome called the cis-replication element (CRE) as template. In contrast, (-) strand RNA synthesis is now believed to be primed by the non-uridylylated VPg-protein. These findings, suggesting a different primer for (-) and (+) strand formation, seem more in line with the asymmetric replication found for picornaviruses. The exact mode of action in this replication process, however, remains unclear.

Outbreaks of picornavirus related diseases are rapid due to their high virulence and ease of communicability. The consequences can be severe, as demonstrated by the outbreak of Foot-and-mouth-disease virus (FMDV) among United Kingdom farm animals in 2001. Understanding the role of the RNA linked VPg protein in viral replication in more detail might give insight in the development of new vaccines and drugs against diseases caused by these viruses. Synthetically made VPgpU and VPgpUpU proved to be useful tools in the elucidation of Poliovirus replication.

Figure 1. Coxsackie A24, Coxsackie B3W, Human Entero Virus 71, consensus Human Entero Virus and Poliovirus 1 VPg(pU)’s
Previous reports describe the synthesis of Poliovirus VPgpU using a block condensation strategy\textsuperscript{20} and the online SPPS synthesis of Poliovirus, Coxsackie B3 and Cowpea mosaic virus VPgpU’s.\textsuperscript{21} As an extension of the latter procedure this chapter describes the solid phase synthesis of the VPg proteins as well as the VPgpU nucleoproteins from two additional coxsackie strands (CX A24 and CX B3W), human enterovirus 71 (HEV71), Poliovirus (PV1) and a consensus sequence for human enterovirus (conHEV)\textsuperscript{22} (see Figure 1). It was envisaged that the application of a pre-uridylylated tyrosine building block would result in a universal solid phase synthesis strategy for all Tyr-O\textsuperscript{4} linked VPgpU’s.

Results and Discussion

Guided by an earlier synthesis, uridylylated tyrosine building block 11 was selected for the solid phase synthesis of the target VPgpU’s (see Scheme 1).\textsuperscript{20} A modified route of synthesis to 11 was undertaken that started with the protection of commercially available Fmoc-Tyr(tBu)-OH 6 as its allyl ester. When cesium carbonate and allyl bromide were used to introduce the allyl ester, the Fmoc group in 6 was found to be cleaved. To avoid this unwanted side-reaction silver carbonate was used as a base and 7 was obtained in quantitative yield. Subsequent removal of the t-butyl ether using TFA gave phenol 8. Phosphitylation of 8 with uridine-5’-phosphoramidite 9 under the agency of 4,5-dicyanoimidazole and subsequent oxidation of the intermediate phosphate using t-BuOOH yielded fully protected tyrosine 10. Liberation of the acid functionality by palladium catalyzed removal of the allyl protecting group gave target tyrosine derivative 11. In this reaction tributyltin hydride acts as a nucleophile, a process reported to proceed very efficient in allyl and Alloc cleavage in amino acid and peptide derivatives.\textsuperscript{23}

![Scheme 1](image)

Reagents and conditions: i. Ag\textsubscript{2}CO\textsubscript{3}, DMF, r.t., 15 min; then All-Br, 2.5 h, r.t., (quantitative), ii. 70% TFA in DCM, 30 min, r.t., (quantitative), iii. 4,5-dicyanoimidazole, MeCN, 30 min, r.t., iv. t-BuOOH, 45 min, rt, (90% based on 8), v. Pd(PPh\textsubscript{3})\textsubscript{4}, Bu\textsubscript{3}SnH, AcOH, THF/DCM, 1h, r.t., (62%).
The solid phase synthesis of VPgpU’s 1b – 5b started with immobilization of the first glutamic acid residue via its side chain acid moiety to Rink amide resin (Tentagel RAM). This was achieved by HATU mediated coupling of Fmoc-Glu-OtBu to the resin. During the final acidic cleavage of the VPgpU from the resin a carboxamide was generated, converting the initially attached glutamic acid into the desired C-terminal glutamine residue (see Scheme 2).

Scheme 2. SPPS for Coxsackie A24 VPg 1a and VPgpU 1b

Preparation of Coxsackie A24 VPgpU 1b is elaborated by removal of the Fmoc protection and repetitive elongation of the resin bound glutamine using standard automated Fmoc solid phase peptide synthesis and HATU as coupling reagent, to give the immobilized 19-mer peptide 12. The immobilized peptide 12 was elongated performing a manual double coupling protocol using Fmoc-Tyr(pU)-OH 11 and BOP/HOBt as activating agents. Subsequent piperidine treatment liberated the terminal amine, and concomitantly cleaved the cyanoethyl group resulting in the formation of a phosphodiester. The presence of the phosphodiester gave no problems in the subsequent attachment of the last two N-terminal amino acids, as no side reactions were detected. Final removal of the Fmoc group followed by treatment with a mixture of 95% TFA, 2.5 % TIS and 2.5% H2O, to effectuate removal of acid labile protective groups and release from the solid support yielded crude partially protected VPgpU. An alkaline treatment with NH4OH in dioxane to remove 2’-O and 3’-O acetyl groups from the ribose
residue yielded the completely deprotected crude nucleopeptide, which was purified using RP-HPLC to afford homogeneous Coxsackie A24 VPgpU nucleoprotein 1b. Elongation of resin bound peptide 12 with Fmoc-Tyr(tBu)-OH, Fmoc-Ala-OH and Fmoc-Gly-OH gave protected VPg peptide 13a. A final Fmoc deprotection step and acid treatment followed by RP-HPLC purification afforded Coxsackie A24 VPg protein 1a.

In a similar fashion, using uridilylated tyrosine 11 or commercially available Fmoc-Tyr(tBu)-OH the preparation of nucleoproteins 2b – 5b and proteins 2a – 5a, respectively, proceeded uneventfully in a yield between 3 and 35%.24

**Conclusion**

In this chapter suitably protected pre-uridylylated Fmoc tyrosine building block 11 is synthesized. The general applicability of a sequential solid phase based strategy is demonstrated by the successful synthesis of five picornavirus VPg proteins 1a – 5a and five uridylylated VPg proteins 1b – 5b.
Experimental

General: All chemicals were used as received. MeCN and THF were stored over activated 4Å molecular sieves at least 24 hours prior to use. DCM was distilled over CaH₂ prior to use. Solvents were removed by rotary evaporation under reduced pressure at a temperature not exceeding 40°C. TLC analysis, silica gel column chromatography, NMR, LCMS, HPLC and HRMS techniques used are described in Chapter 2.

Procedure for solid phase peptide synthesis: The peptides were prepared on an ABI-433A (Applied Biosystems, division of Perkin-Elmer) automated peptide synthesizer using the Fmoc based peptide synthesis protocol. The peptides were prepared on 50 μmol scale starting from Tentagel S RAM resin (with the exception of Coxsackie A24 VPg and VPgpU; 100 μmol). The first amino acid (Fmoc-Glu-OtBu) was coupled to the resin via the side-chain and converted in a Gln residue in course of the final cleavage off the resin. The consecutive steps performed in each cycle were:
- Deprotection of the Fmoc-group with 20 % piperidine in NMP for 5 x 1 min.
- Coupling of the appropriate amino acid applying a five-fold excess. Generally the amino acid (0.25 mmol) was dissolved in NMP (0.5 mL) and subsequently 0.25 mmol of HATU (0.25 M in DMF/NMP, 1/1, v/v) and 0.32 mmol of DiPEA (1.0 M in NMP) were added. The resulting solution was transferred to the reaction vessel, which was shaken for 1 hour.
- The unreacted amine functions were capped using 0.5 M acetic anhydride, 0.125 M DiPEA and 0.015 M HOBt in NMP. The resulting suspension was shaken for 1 min.

Double couplings were conducted on β-branched amino acids and on Fmoc-Asn(Trt)-OH and Fmoc-Arg(Pbf)-OH.

The 19-mer peptide containing resin was split in two equal portions of which one portion was elongated on the automated peptide synthesizer using a double coupling protocol for the last three AA’s to obtain VPg peptides 1a – 5a. In the synthesis of the VPgpU’s 1b – 5b the last three amino acids, Fmoc-Tyr(pU)-OH, Fmoc-Ala-OH and Fmoc-Gly-OH, were attached manually using a double coupling protocol. The consecutive steps performed in each cycle were:
- Deprotection of the Fmoc-group with 2 % DBU in DMF for 5 x 1 min.
- Coupling of the appropriate amino acid applying a five-fold excess. Generally the amino acid (0.25 mmol) was dissolved in NMP (0.5 mL) and subsequently 0.25 mmol of BOP/HOBt (0.5 M BOP/ 0.5 M HOBt in DMF/NMP, 1/1, v/v) and 0.32 mmol of DiPEA (1.25 M in NMP) were added. The resulting solution was transferred to the reaction vessel, which was shaken for 1 hour.
- The unreacted amine functions were capped using 0.5 M acetic anhydride, 0.125 M DiPEA and 0.015 M HOBt in NMP. The resulting suspension was shaken for 1 min.

After final Fmoc deprotection, the peptide was cleaved off the resin using TFA/TIS/H₂O (95/5/5, v/v/v, 10 mL) by shaking for 4 hours. After filtration into cold Et₂O (35 mL) the precipitate was collected and coevaporated with toluene (2 x 5 mL) and then treated with 25% aq. ammonia (2.5 mL) in 1,4-dioxane (2.5 mL). After stirring for 4 hours concentration of the reaction mixture afforded the crude title compound. RP-HPLC purification was conducted on a Gilson automated HPLC supplied with a semi-preparative Phenomenex Gemini C₁₈ column (250 x 10.00 mm, 5 μ, flow: 4 mL/min). The applied eluent system was A: 0.5 % TFA in H₂O, B: MeCN and detection at 250 nm.
A General Synthetic Method Towards Uridylylated Picornavirus VPg Proteins

\[ N^\alpha - \text{Fmoc-(O-tet-butyl)-tyrosine allyl ester (7)} \]

To a solution of \( N^\alpha - \text{Fmoc-(O-tet-butyl)-tyrosine} \) (6) (2.3 g, 5.0 mmol) in DMF (25 mL) at 0 °C was added Ag\(_2\)CO\(_3\) (2.7 g, 9.8 mmol) under exclusion of light. The reaction mixture was warmed to room temperature and stirred for 15 min. prior to addition of allylbromide (2.0 mL, 23 mmol). After 2.5 hours TLC analysis (70/29/1 EtOAc: PE: AcOH) indicated complete consumption of the starting material. The reaction mixture was filtered over celite and the filtrate was diluted in 100 mL EtOAc. The organic phase was subsequently washed with 10% KHSO\(_4\) (2 x 25 mL) and water (2 x 30 mL), dried (MgSO\(_4\)) and concentrated. The residue was applied to a silica gel column and eluted with a gradient of EtOAc in PE (0/100 – 40/60, v/v) to yield the title compound quantitatively. 1H-NMR (400 MHz, CDCl\(_3\)) δ 7.75 (d, \( J = 7.5 \) Hz, 2H, Arom. Fmoc), 7.61 - 7.52 (m, 2H, Arom. Fmoc), 7.38 (t, \( J = 7.4 \) Hz, 2H, Arom. Fmoc), 7.30 (t, \( J = 7.3 \) Hz, 2H, Arom. Fmoc), 7.11 - 6.95 (m, 2H, Arom. Tyr.), 6.89 (d, \( J = 7.7 \) Hz, 2H, Arom. Tyr.), 5.84 (ddd, \( J = 21.2, 10.2, 5.0 \) Hz, 1H, CH Allyl), 5.36 (d, \( J = 8.2 \) Hz, 1H, NH), 5.33 - 5.15 (m, 3H, CH\(_2\) Allyl), 4.74 - 4.49 (m, 3H, CH\(_2\) Allyl, CH\(_\alpha\)), 4.47 - 4.29 (m, 2H, CH\(_2\) Fmoc), 4.19 (t, \( J = 6.8 \) Hz, 1H, CH Fmoc), 3.19 - 2.96 (m, 2H, CH\(_2\) Fmoc), 1.31 (s, 9H, CH\(_2\) tBu). 13C-NMR (101 MHz, CDCl\(_3\)) δ 171.3 (CO\(_\alpha\)), 155.6, 154.5 (CO Fmoc, Cq Tyr.), 143.9, 143.8, 141.3 (Cq Arom. Fmoc), 131.5 (CH Allyl), 130.5 (CH\(_\alpha\)), 129.87 (Arom. Tyr.), 129.82, 127.76, 127.1, 125.2, 125.1, 124.2, (Arom. Fmoc), 120.0 (CH\(_2\) Allyl), 119.1 (Arom. Tyr.) 78.4 (Cq tBu), 67.0 (CH\(_2\) Fmoc), 66.1 (CH\(_2\) Allyl), 54.9 (CH\(_\alpha\)), 47.2 (CH Fmoc), 37.7 (CH\(_2\) β), 28.9 (CH\(_2\) tBu). IR: 2980, 1718, 1506, 1236, 1161, 757, 740.\( \alpha_D \) (CHCl\(_3\)) = + 14.6°. HRMS: [C\(_{31}\)H\(_{33}\)NO\(_5\) + H]\(^+\); calcd. 500.2432, found 500.2431.

\[ N^\alpha - \text{Fmoc-tyrosine-allyl ester (8)} \]

Allyl ester (7) was dissolved in 30 mL TFA/DCM (7:3, v/v) and stirred for 30 min. at room temperature. Toluene (50 mL) was added and the solvents were removed. The residue was applied to a silica gel column and eluted with a gradient of EtOAc in toluene (0/100 – 10/90, v/v) to yield the title compound quantitatively. 1H-NMR (400 MHz, CDCl\(_3\)) δ 7.73 (d, \( J = 7.5 \) Hz, 2H, Arom. Fmoc), 7.58 – 7.47 (m, 2H, Arom. Fmoc), 7.37 (t, \( J = 7.4 \) Hz, 2H, Arom. Fmoc), 7.28 (t, \( J = 7.3 \) Hz, 2H, Arom. Fmoc), 6.92 (d, \( J = 8.0 \) Hz, 2H, Arom. Tyr.), 6.70 (d, \( J = 8.0 \) Hz, 2H, Arom. Tyr.), 6.36 (s, 1H, OH), 5.90 - 5.81 (m, 1H, CH Allyl), 5.40 (d, \( J = 8.3 \) Hz, 1H, NH), 5.36 - 5.19 (m, 2H, CH\(_2\) Allyl), 4.77 - 4.49 (m, 3H, CH\(_2\) Allyl, CH\(_\alpha\)), 4.42 - 4.32 (m, 2H, CH\(_2\) Fmoc), 4.17 (t, \( J = 6.8 \) Hz, 1H, CH Fmoc), 3.09 - 2.97 (m, 1H, CH\(_2\) β). 13C-NMR (101 MHz, CDCl\(_3\)) δ 171.7 (CO\(_\alpha\)). 156.0, 155.3 (CO Fmoc, Cq Tyr.), 143.9, 143.7, 141.3 (Cq Arom. Fmoc), 131.5 (CH Allyl), 130.5 (CH\(_\alpha\)), 127.8 (Arom. Tyr.), 127.2, 125.2, 125.15, 120.1 (Arom. Fmoc), 119.4 (CH\(_2\) Allyl), 115.6 (Arom. Tyr.), 67.2 (CH\(_2\) Fmoc), 66.3 (CH\(_2\) Allyl), 55.1 (CH\(_\alpha\)), 47.2 (CH Fmoc), 37.5 (CH\(_2\) β). IR: 3317, 1689, 1516, 1450, 1214, 758, 739. \( \alpha_D \) (CHCl\(_3\)) = + 14.6°. HRMS: [C\(_2\)H\(_3\)N\(_3\)O\(_5\) + H]\(^+\); calcd. 444.1806, found 444.1804.
2-cyanoethyl-(Nα-Fmoc-tyrosin-4-yl-allyl ester)-(2', 3'-di-O-acetyluridyl-5'-yl) phosphate (10)

Phosphoramidite 9 was prepared by reacting 2', 3'-di-O-acetyluridine (0.98 g, 3 mmol), coevaporated with MeCN (2 x 7.5 mL) in DCM (15 mL), containing TEA (1.4 mL, 10 mmol) with 2-cyanoethoxy-N,N-diisopropylaminochlorophosphine (0.75 mL, 3.75 mmol) under an argon atmosphere. The reaction mixture was stirred for 45 min at room temperature. After $^{31}$P NMR showed complete consumption of the aminochlorophosphine and formation of phosphoramidite $^9$ (161 MHz, CDCl$_3$; δ $^{150.49}$, $^{148.68}$), 30 mL DCM was added and the reaction mixture was washed with water (20 mL) and 5% NaHCO$_3$ (10 mL). The organic phase was dried (MgSO$_4$) and concentrated. Approximately 0.6 mmol $^9$ was added to a solution of $N^\alpha$-Fmoc-tyrosine-allyl ester (8) (0.21 g, 0.47 mmol) in MeCN (10 mL). 4,5-Dicyanoimidazole (0.15 g, 0.6 mmol) was added and the reaction mixture was stirred for 30 min until $^{31}$P NMR showed complete consumption of the phosphoramidite and formation of the phosphite (161 MHz, CDCl$_3$; δ $^{135.12}$, $^{134.97}$). A solution of tBuOOH (5.5 M in nonane) was added to the reaction mixture until after 45 minutes $^{31}$P NMR showed complete consumption of the phosphate and formation of the phosphotriester (161 MHz, CDCl$_3$; δ $^{-6.48}$, $^{-6.83}$). The reaction mixture was diluted in DCM and washed with 5% Na$_2$S$_2$O$_3$ and water. The organic phase was dried with MgSO$_4$ and concentrated. The residue was applied to a silica gel column and eluted with a gradient of MeOH in DCM (0/100 – 2/98, v/v), to yield the title compound as a mixture of epimers at P as a white foam in 90 % (0.37 g, 0.42 mmol) based on 8. 1H-NMR (400 MHz, CDCl$_3$); δ $^{9.78}$ (d, J = 10.0 Hz, 1H, NH U), $^{7.76}$ (ap. d, J = 7.5 Hz, 2H, Arom. Fmoc), $^{7.62}$ - $^{7.53}$ (m, 2H, Arom. Fmoc), $^{7.45}$ - $^{7.35}$ (m, 3H, Arom. Fmoc, H6), $^{7.34}$ - $^{7.26}$ (m, 2H, Arom. Fmoc), $^{7.20}$ - $^{7.04}$ (m, 4H, Arom. Tyr), $^{6.13}$ - $^{6.00}$ (m, 1H, H1'), $^{5.96}$ - $^{5.82}$ (m, 1H, CH Allyl), $^{5.79}$ - $^{5.58}$ (m, 2H, H5, NH α), $^{5.45}$ - $^{5.37}$ (m, 1H, H3'), $^{5.37}$ - $^{5.12}$ (m, 3H, H2', CH2 Allyl), $^{4.71}$- $^{4.25}$ (m, 10H, 4', 5', CH2 Allyl, CH2 Fmoc, CH α), $^{4.20}$ (t, J = 7.0 Hz, 1H, CH Fmoc), $^{3.21}$ - $^{3.01}$ (m, 2H, OAc’s), $^{2.86}$ - $^{2.61}$ (m, 2H, CH2 CNEO), $^{2.18}$ - $^{2.02}$ (m, 6H, OAc’s). $^{13}$C-NMR (101 MHz, CDCl$_3$); δ $^{171.1}$, $^{171.0}$ (CO α), $^{169.83}$, $^{169.80}$, $^{169.73}$, $^{169.71}$ (CO Ac), $^{163.2}$, $^{163.1}$ (C4), $^{155.7}$ (CO Fmoc), $^{150.5}$ (C2), $^{149.03}$, $^{148.96}$ (C4 Tyr.), $^{143.84}$, $^{143.75}$, $^{141.29}$ (Cq Fmoc), $^{139.9}$, $^{139.7}$ (C6), $^{133.8}$ (Cq Tyr.), $^{131.4}$, $^{131.12}$ (Arom. Tyr.), $^{127.76}$, $^{127.09}$, $^{125.16}$, $^{125.11}$ (Aro. Fmoc), $^{119.3}$ (Arom. Fmoc), $^{117.9}$ (CH2 Allyl), $^{117.87}$, $^{116.44}$ (CN), $^{103.5}$ (C5), $^{87.3}$, $^{87.0}$ (C1’), $^{80.59}$, $^{80.54}$, $^{80.52}$, $^{80.47}$ (C4’), $^{72.5}$ (C3’), $^{69.83}$, $^{69.82}$ (C2’), $^{67.36}$, $^{67.30}$, $^{67.24}$, $^{66.92}$, $^{66.88}$, $^{66.21}$, $^{66.17}$ (CH2 Allyl, CH2 CNEO, CH2 Fmoc), $^{63.18}$, $^{63.08}$ (C5’), $^{54.8}$ (CH α), $^{47.1}$ (CH Fmoc), $^{37.3}$ (CH2 β), $^{20.56}$, $^{20.54}$, $^{20.4}$ (CH3 Ac), $^{19.7}$, $^{19.6}$ (CH2 CNEO). $^{31}$P-NMR (81 MHz, CDCl$_3$); δ $^{-6.48}$, $^{-6.83}$. IR: 3067, 1694, 1506, 1452, 1378, 1211, 1037, 961. LCMS (10 - 90 % B in 15 min), Rt = 8.91. ESI-MS; m/z 887.40 [M + H$^+$]. HRMS: [C$_{43}$H$_{43}$N$_4$O$_{15}$P + H]$^+$; calcd. 887.2535, found 887.2542.
2-cyanoethyl-(N\textsuperscript{α}-Fmoc-tyrosin-4-yl)-(2',3'-di-O-acetyluridyl-5'-yl) phosphate (11)

To a stirred solution of 10 (2.15 g, 2.4 mmol) coevaporated with MeCN (7.5 mL x 2) in 1:1 THF/DCM (10 mL, v/v), containing AcOH (0.6 mL, 11 mmol), was added Bu\textsubscript{3}SnH (1.3 mL, 4.9 mmol) and Pd(PPh\textsubscript{3})\textsubscript{4} (115 mg, 0.1 mmol) under an argon atmosphere. After one hour TLC analysis (10% MeOH/DCM) revealed complete consumption of the starting material. The reaction mixture was concentrated and purified using a silica gel column with a gradient of MeOH in DCM (0/100 – 4/96), to yield the title compound as a mixture of epimers as white foam in 62% (1.26 g, 1.49 mmol).  

1H-NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 9.99 (s, 1H), 7.73 – 7.13 (m, 13H), 6.00 (dd, \(J = 15.1, 5.3\) Hz, 1H), 5.94 – 5.80 (m, 1H), 5.61 (dd, \(J = 56.2, 8.0\) Hz, 1H), 5.49 – 5.36 (m, 1H), 5.35 – 5.27 (m, 1H), 4.68 – 4.62 (m, 1H), 4.51 – 4.37 (m, 3H), 4.37 – 4.23 (m, 4H), 4.21 – 4.08 (m, 1H), 3.26 – 3.00 (m, 2H), 2.82 – 2.55 (m, 2H), 2.19 – 1.95 (m, 6H).  

13C-NMR (100 MHz, CDCl\textsubscript{3}); \(\delta\) 173.4 (CO\textalpha), 169.77, 169.75, 169.7, 169.6 (CO Acetyl), 164.2, 164.0 (Ur. 4), 155.7 (CO Fmoc), 150.24, 150.17 (Ur. 6), 148.83, 148.77 (Cq Tyr.), 143.8, 143.7, 141.2 (Cq Fmoc), 134.0 (Cq Tyr.), 131.2, 131.1 (Arom. Tyr.), 127.6, 127.0, 125.1 (Arom. Fmoc), 119.9, 119.7 (Arom. Fmoc, Arom. Tyr.), 116.5 (CN), 103.0, 102.9 (Ur. 5), 87.7, 87.3 (C'1), 80.5, 80.4 (C'4), 72.6, 72.5 (C'2'), 69.7, 69.6 (C'3'), 67.3, 67.1 (CH2 Fmoc), 66.8 (C'5'), 63.2, 63.1 (CH2 CNEO), 54.6 (C\textalpha), 47.1 (CH Fmoc), 37.0 (CB), 20.40, 20.37, 20.3 (CH3 OAc), 19.53, 19.45 (CH2 CNEO).  

31P-NMR (162 MHz, CDCl\textsubscript{3}); \(\delta\) -6.59, -6.95. ESI-MS: \(m/z = 847.40 \text{ [M+H]}^+\). LCMS (10 – 90 B in 15 min.), Rt = 7.75.  

HRMS: \([C_{40}H_{39}N_4O_{15}P + H]^+; \text{calcd.} 847.2222, \text{found} 847.2229.\)

Coxsackie A24 Virus VPg, (H-Gly-Ala-Tyr-Thr-Gly-Pro-Asn-Lys-Lys-Pro-Ser-Val-Pro-Thr-Val-Arg-Thr-Ala-Lys-Val-Gln-OH), (1a)

HPLC gradient: 10 - 60 % B in 3 CV. The appropriate fractions were lyophilized, yielding 22.47 mg, 7.79 \mu mol (16 % based on original loading of resin) of the nucleopeptide as TFA salt. LCMS (0 – 50 % B in 15 min.), Rt = 6.54 min. ESI-MS: \(m/z = 771.47 \text{ [M + 2H]}^+\), 1157.13 \text{ [M + 3H]}^+. HRMS \([C_{103}H_{174}N_{30}O_{30} + 2H]^+; \text{calcd.} 1156.6578, \text{found} 1156.6577, [C_{103}H_{174}N_{30}O_{30} + 3H]^+; \text{calcd.} 771.4407, \text{found} 771.4325, \text{found} 787.8325 \text{, found} 787.8323, [C_{103}H_{174}N_{30}O_{30} + 5H]^+; \text{calcd.} 463.2675, \text{found} 463.2674. \)  

1H NMR (600 MHz, D\textsubscript{2}O) \(\delta\) 7.17 (d, \(J = 8.5\) Hz, 2H), 6.83 (d, \(J = 8.6\) Hz, 2H), 4.70 - 4.61 (m, 2H), 4.58 (ap. dd, \(J = 8.7, 5.5\) Hz, 1H), 4.54 - 4.46 (m, 4H), 4.45 - 4.40 (m, 2H), 4.39 - 4.28 (m, 7H), 4.28 - 4.19 (m, 3H), 4.19 - 4.10 (m, 3H), 3.92 - 3.80 (m, 8H), 3.77 - 3.70 (m, 1H), 3.70 - 3.61 (m, 2H), 3.22 (ap. t, \(J = 7.0\) Hz, 2H), 2.73 (m, 2H), 2.20 - 1.42 (m, 41H), 1.40 (ap. d, \(J = 7.2\) Hz, 5H), 1.13 (ap. d, \(J = 7.2\) Hz, 3H), 1.23 (ap. dd, \(J = 8.3, 6.5\) Hz, 7H), 1.17 (ap. d, \(J = 6.4\) Hz, 3H), 1.00 (ap. d, \(J = 6.7\) Hz, 4H), 0.97 - 0.89 (m, 19H).  

Coxsackie A24 Virus VPgpU, (H-Gly-Ala-Tyr(pU)-Thr-Gly-Pro-Asn-Lys-Lys-Pro-Ser-Val-Pro-Thr-Val-Arg-Thr-Ala-Lys-Val-Gln-OH), (1b)

HPLC gradient: 10 - 60 % B in 3 CV. The appropriate fractions were lyophilized, yielding 54.72 mg, 17.19 \mu mol (34 % based on original loading of resin) of the nucleopeptide as TFA salt. LCMS (0 – 50 % B in 15 min.), Rt = 6.32 min. ESI-MS: \(m/z = 1310.13 \text{ [M + 2H]}^+\), 873.73 \text{ [M + 3H]}^+. HRMS \([C_{112}H_{185}N_{32}O_{38}P + 2H]^+; \text{calcd.} 1309.6705, \text{found} 1309.6705, [C_{112}H_{185}N_{32}O_{38}P + 3H]^+; \text{calcd.} 873.4499, \text{found} 873.4499, [C_{112}H_{185}N_{32}O_{38}P + 4H]^+; \text{calcd.} 524.4725, \text{found} 524.4721. \)  

31P-NMR (162 MHz, D\textsubscript{2}O); \(\delta\) - 4.28. 1H NMR (600 MHz, D\textsubscript{2}O) \(\delta\) 7.69 (d, \(J = 8.1\) Hz, 1H), 7.19 (d, \(J = 8.5\) Hz, 2H), 7.12 (d, \(J = 8.3\) Hz, 2H), 5.94 (ap. d, \(J = 4.6\) Hz, 1H), 5.68 (ap. d, \(J = 8.1\) Hz, 2H), 4.68 - 4.62 (m, 1H), 4.51 - 4.37 (m, 3H), 4.37 - 4.23 (m, 4H), 4.21 - 4.08 (m, 1H), 3.26 - 3.00 (m, 2H), 2.82 - 2.55 (m, 2H), 2.19 – 1.95 (m, 6H).
Hz, 2H), 7.11 (d, 2H), 3.97 - 3.79 (m, 9H), 3.76 - 3.69 (m, 1H), 3.69 - 3.62 (m, 2H), 3.21 (ap. t, J = 7.0 Hz, 2H), 3.12 (ap. dd, J = 13.9, 6.9 Hz, 1H), 3.01 (ap. dd, J = 17.6, 7.9 Hz, 7H), 2.89 - 2.72 (m, 2H), 2.37 (ap. t, J = 7.7 Hz, 4H), 2.35 - 1.42 (m, 42H), 1.40 (ap. d, J = 7.2 Hz, 7H), 1.31 (ap. d, J = 7.2 Hz, 3H), 1.22 (ap. dd, J = 8.1, 6.6 Hz, 6H), 1.19 (ap. d, J = 6.4 Hz, 3H), 0.99 (ap. d, J = 6.7 Hz, 4H), 1.04 - 0.79 (m, 24H).

Coxsackie B3 Virus Woodruff strain VPg, (H-Gly-Ala-Tyr-Thr-Thyr-Gly-Ala-Pro-Asn-Gln-Lys-Pro-Lys-Val-Pro-Thr-Leu-Arg-Gln-Ala-Lys-Val-Gln-OH), (2a)

HPLC gradient: 10 - 30 % B in 3 CV. The appropriate fractions were lyophilized, yielding 11.47 mg, 3.87 μmol (16 % based on original loading of resin) of the nucleopeptide as TFA salt. LCMS (0 - 50 % B in 15 min.), Rt = 6.54 min. ESI-MS: m/z 1198.20 [M+2H]^{2+}, 799.13 [M+3H]^{3+}. HRMS [C_{107}H_{180}N_{32}O_{30} + 2H]^{2+}; calcd. 1197.6844, found 1197.6846, [C_{107}H_{180}N_{32}O_{30} + 3H]^{3+}; calcd. 798.7920, found 798.7920, [C_{107}H_{180}N_{32}O_{30} + 4H]^{4+}; calcd.599.3458, found 599.3457, [C_{107}H_{180}N_{32}O_{30} + 5H]^{5+}; calcd. 479.6781, found 479.6780. \(^{1}H\) NMR (600 MHz, D_{2}O) δ 7.16 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 8.5 Hz, 2H), 4.67 (ap. t, J = 8.0 Hz, 1H), 4.65 - 4.60 (m, 1H), 4.57 (ap. dd, J = 9.1, 5.1 Hz, 1H), 4.47 (ap.dd, J = 16.1, 7.8 Hz, 3H), 4.42 (ap.dd, J = 7.9, 6.6 Hz, 2H), 4.40 - 4.24 (m, 11H), 4.24 - 4.19 (m, 1H), 4.13 (ap.d, J = 7.9 Hz, 1H), 3.99 - 3.81 (m, 7H), 3.77 - 3.68 (m, 2H), 3.68 - 3.59 (m, 1H), 3.21 (ap.t, J = 7.0 Hz, 2H), 3.10 - 2.97 (m, 8H), 2.85 - 2.77 (m, 2H), 2.43 - 2.23 (m, 9H), 2.20 - 1.41 (m, 46H), 1.39 (ap.d, J = 7.2 Hz, 5H), 1.33 (ap.d, J = 7.2 Hz, 3H), 1.24 (ap.d, J = 6.4 Hz, 3H), 1.17 (ap.d, J = 6.4 Hz, 4H), 1.00 (ap.d, J = 6.8 Hz, 3H), 1.01 - 0.92 (m, 18H), 0.88 (ap. t, J = 6.4 Hz, 6H).

Coxsackie B3 Virus Woodruff strain VPgpU, (H-Gly-Ala-Tyr(pU)-Thr-Thyr-Gly-Ala-Pro-Asn-Gln-Lys-Pro-Lys-Val-Pro-Thr-Leu-Arg-Gln-Ala-Lys-Val-Gln-OH), (2b)

HPLC gradient: 15 - 25 % B in 3 CV. The appropriate fractions were lyophilized, yielding 2.76 mg, 0.84 μmol (3 % based on original loading of resin) of the nucleopeptide as TFA salt. LCMS (0 - 50 % B in 15 min.), Rt = 6.39 min. ESI-MS: m/z 1351.13 [M+2H]^{2+}, 900.93 [M+3H]^{3+}. HRMS [C_{116}H_{191}N_{34}O_{38}P + 2H]^{2+}; calcd. 1350.6970, found 1350.6971, [C_{116}H_{191}N_{34}O_{38}P + 3H]^{3+}; calcd. 900.8004, found 900.8004. \(^{31}P\)NMR (162 MHz, D_{2}O) δ = 4.27. \(^{1}H\) NMR (600 MHz, D_{2}O) δ 7.69 (d, J = 8.1 Hz, 1H), 7.19 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.2 Hz, 2H), 5.94 (d, J = 4.6 Hz, 1H), 5.68 (d, J = 8.1 Hz, 1H), 4.70 (ap. t, J = 7.6 Hz, 1H), 4.64 - 4.55 (m, 2H), 4.47 (ap. dd, J = 14.8, 7.8 Hz, 3H), 4.44 - 4.40 (m, 2H), 4.40 - 4.26 (m, 13H), 4.23 - 4.14 (m, 4H), 4.13 (ap. d, J = 7.9 Hz, 1H), 3.99 - 3.78 (m, 7H), 3.78 - 3.67 (m, 2H), 3.67 - 3.60 (m, 1H), 3.21 (ap. t, J = 7.0 Hz, 2H), 3.12 (ap. dd, J = 13.9, 6.9 Hz, 1H), 3.01 (ap. dd, J = 16.6, 7.6 Hz, 7H), 2.89 - 2.73 (m, 2H), 2.47 - 2.23 (m, 10H), 2.21 - 1.41 (m, 47H), 1.39 (ap.d, J = 7.2 Hz, 5H), 1.31 (ap.d, J = 7.2 Hz, 3H), 1.24 (ap. d, J = 6.4 Hz, 3H), 1.19 (ap. d, J = 6.5 Hz, 4H), 1.00 (ap. d, J = 6.7 Hz, 3H), 0.98 - 0.92 (m, 14H), 0.88 (ap. dd, J = 13.2, 6.1 Hz, 7H).

Human Entero Virus 71 VPg, (H-Gly-Ala-Tyr-Ser-Gly-Ala-Pro-Lys-Gln-Val-Leu-Lys-Pro-Pro-Leu-Arg-Thr-Ala-Ala-Val-Gln-OH), (3a)

HPLC gradient: 10 - 30 % B in 3 CV. The appropriate fractions were lyophilized, yielding 10.89 mg, 3.98 μmol (16 % based on original loading of resin) of the nucleopeptide as TFA salt. LCMS (0 - 50 % B in 15 min.), Rt = 6.36 min. ESI-MS: m/z 1143.13 [M+2H]^{2+}, 762.40 [M+3H]^{3+}. HRMS [C_{102}H_{174}N_{30}O_{29} + 2H]^{2+}; calcd. 1142.6604, found 1142.6608, [C_{102}H_{174}N_{30}O_{29} + 3H]^{3+}; calcd. 762.1093, found 762.1093. \(^{1}H\) NMR (600 MHz, D_{2}O) δ 7.16 (d, J = 8.5 Hz, 2H), 6.84 (d, J = 8.5 Hz, 2H), 4.67 - 4.56 (m, 3H), 4.49 - 4.29 (m, 13H), 4.27 - 4.23 (m 3H), 4.21 - 4.13 (m, 2H), 4.09 (ap. d, J = 8.3 Hz, 1H), 3.90 (ap. d, J = 5.0 Hz, 2H), 3.87 - 3.81 (m, 5H), 3.78 (ap. dd, J = 11.6, 5.2 Hz, 1H), 3.65 (ap. dd, J = 17.0, 7.0 Hz, 2H), 3.23 (ap. t, J = 6.9 Hz, 2H), 3.13 - 2.91 (m, 8V), 2.42 - 2.26 (m, 6H), 2.23 - 1.45 (m, 40H), 1.43 (ap. d, J = 7.2 Hz...
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Hz, 5H), 1.37 (ap. dd, J = 13.1, 7.1 Hz, 7H), 1.33 (ap. d, J = 7.2 Hz, 3H), 1.22 (ap. dd, J = 6.3, 4.8 Hz, 6H), 1.05 - 0.91 (m, 19H), 0.89 (ap. dd, J = 11.0, 6.1 Hz, 7H).

Human Enterovirus 71 VPgpU, (H-Gly-Ala-Tyr(pU)-Ser-Gly-Ala-Pro-Lys-Val-Lys-Lys-Pro-Ala-Leu-Arg-Thr-Ala-Thr-Val-Gln-OH), (3b)

HPLC gradient: 10 - 30 % B in 3 CV. The appropriate fractions were lyophilized, yielding 2.35 mg, 0.67 μmol (3 % based on original loading of resin) of the nucleopeptide as TFA salt. LCMS (0 – 50 % B in 15 min.), Rt = 6.24 min. ESI-MS: m/z 1296.07 [M+2H] 2+, 864.47 [M+3H] 3+. HRMS [C_{111}H_{185}N_{32}O_{37}P + 2H]^{2+}; calcd. 864.1178, found 864.1173.

1H-NMR (600 MHz, D2O) δ 7.69 (d, J = 8.1 Hz, 1H), 7.19 (d, J = 8.6 Hz, 2H), 7.12 (d, J = 8.3 Hz, 2H), 5.94 (d, J = 8.1 Hz, 1H), 4.65 (ap. t, J = 7.6 Hz, 1H), 4.60 (ap. dt, J = 9.6, 6.1 Hz, 2H), 4.45 - 4.22 (m, 18H), 4.22 - 4.12 (m, 4H), 4.09 (ap. d, J = 8.2 Hz, 1H), 3.99 - 3.76 (m, 5H), 3.68 - 3.62 (m, 2H), 3.23 (ap. t, J = 6.3 Hz, 7H), 1.01 - 0.90 (m, 19H), 0.88 (ap. dd, J = 11.1, 6.1 Hz, 6H).

Consensus Human Enterovirus VPg, (H-Gly-Ala-Tyr-Thr-Gly-Leu-Pro-Asn-Gln-Lys-Pro-Lys-Val-Pro-Thr-Ile-Arg-Thr-Ala-Lys-Val-Gln-OH), (4a)

HPLC gradient: 10 - 35 % B in 3 CV. The appropriate fractions were lyophilized, yielding 11.25 mg, 3.83 μmol (15 % based on original loading of resin) of the nucleopeptide as TFA salt. LCMS (0 – 50 % B in 15 min.), Rt = 6.56 min. ESI-MS: m/z 1184.07 [M+2H] 2+, 790.33 [M+3H] 3+. HRMS [C_{106}H_{179}N_{31}O_{30} + 2H]^{2+}; calcd. 1184.1789, found 1184.1789, [C_{106}H_{179}N_{31}O_{30} + 3H]^{3+}; calcd. 789.7887, found 789.7887.

1H-NMR (600 MHz, D2O) δ 7.16 (d, J = 8.6 Hz, 1H), 6.83 (d, J = 8.6 Hz, 1H), 4.67 (t, J = 8.0 Hz, 1H), 4.65 - 4.59 (m, 1H), 4.57 (dd, J = 9.1, 5.2 Hz, 1H), 4.53 - 4.39 (m, 3H), 4.38 - 4.27 (m, 5H), 4.25 - 4.18 (m, 2H), 4.18 - 4.11 (m, 1H), 3.95 - 3.80 (m, 3H), 3.80 - 3.58 (m, 2H), 3.21 (ap. t, J = 7.0 Hz, 1H), 3.09 - 2.96 (m, 4H), 2.89 - 2.75 (m, 1H), 2.35 (ap. t, J = 7.8 Hz, 3H), 2.33 - 2.24 (m, 1H), 2.20 - 1.95 (m, 7H), 1.40 (ap. d, J = 7.2 Hz, 2H), 1.33 (ap. d, J = 7.2 Hz, 2H), 1.22 (ap. t, J = 5.9 Hz, 3H), 1.17 (ap. d, J = 6.4 Hz, 2H), 1.00 (ap. d, J = 6.8 Hz, 2H), 0.98 - 0.93 (m, 8H), 0.91 (ap. d, J = 6.8 Hz, 2H), 0.87 (ap. t, J = 7.4 Hz, 2H).

Consensus Human Enterovirus VPgpU, (H-Gly-Ala-Tyr(pU)-Thr-Gly-Leu-Pro-Asn-Gln-Lys-Pro-Lys-Pro-Thr-Ile-Arg-Thr-Ala-Lys-Val-Gln-OH), (4b)

HPLC gradient: 10 - 30 % B in 3 CV. The appropriate fractions were lyophilized, yielding 8.2 mg, 2.43 μmol (10 % based on original loading of resin) of the nucleopeptide as TFA salt. LCMS (0 – 50 % B in 15 min.), Rt = 6.44 min. ESI-MS: m/z 1338.13 [M+2H]^{2+}, 892.13 [M+3H]^{3+}. HRMS [C_{115}H_{190}N_{33}O_{38}P + 2H]^{2+}; calcd. 1337.1916, found 1337.1926, [C_{115}H_{190}N_{33}O_{38}P + 3H]^{3+}; calcd. 891.7974, found 891.7974, [C_{115}H_{190}N_{33}O_{38}P + 4H]^{4+}; calcd. 669.0998, found 669.0998. 31P-NMR (162 MHz, D2O) δ = 4.27. 1H-NMR (600 MHz, D2O) δ 7.69 (d, J = 8.1 Hz, 1H), 7.19 (d, J = 8.3 Hz, 2H), 7.13 (d, J = 8.5 Hz, 2H), 7.12 (d, J = 8.3 Hz, 2H), 5.94 (d, J = 4.6 Hz, 1H), 5.68 (d, J = 8.1 Hz, 1H), 4.74 - 4.68 (m, 1H), 4.67 - 4.56 (m, 2H), 4.51 - 4.39 (m, 5H), 4.39 - 4.26 (m, 12H), 4.26 - 4.16 (m, 5H), 4.16 - 4.10 (m, 2H), 3.93 (s, 2H), 3.92 - 3.77 (m, 5H), 3.76 - 3.70 (m, 1H), 3.69 - 3.59 (m, 2H), 3.21 (ap. t, J = 7.0 Hz, 2H), 3.12 (ap. dd, J = 14.0, 6.8 Hz, 1H), 3.01 (ap. dd, J = 15.8, 7.9 Hz, 7H), 2.89 - 2.75 (m, 2H), 2.44 - 2.24 (m, 7H), 2.19 - 1.41 (m, 44H), 1.40 (ap. d, J = 7.2 Hz, 4H), 1.31 (ap. d, J = 7.2 Hz, 2H), 1.22 (ap. t, J = 6.0 Hz, 6H), 1.19 (ap. d, J = 6.4 Hz, 4H), 1.00 (ap. d, J = 6.8 Hz, 3H), 0.98 - 0.89 (m, 18H), 0.87 (ap. t, J = 7.4 Hz, 4H).
Poliovirus VPg, (H-Gly-Ala-Tyr-Gly-Leu-Pro-Asn-Lys-Lys-Pro-Asn-Val-Pro-Thr-Ile-Arg-Thr-Ala-Lys-Val-Gln-OH), (5a)

HPLC gradient: 10 - 30 % B in 3 CV. The appropriate fractions were lyophilized, yielding 48.57 mg, 17.29 μmol (35 % based on original loading of resin) of the nucleopeptide as TFA salt. LCMS (0 – 50 % B in 15 min.), Rt = 6.48 min. ESI-MS: m/z 1178.13 [M+2H]2+, 785.47 [M+3H]3+. HRMS [C105H177N31O30 + 2H]2+; calcd. 1177.1711, found 1177.1690, [C105H177N31O30 + 3H]3+; calcd. 785.1165, found 785.1156, [C105H177N31O30 + 4H]4+; calcd. 589.0892, found 589.0900, [C105H177N31O30 + 5H]5+; calcd. 471.4728, found 471.4736. 1H NMR (600 MHz, D2O) δ 7.16 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 8.5 Hz, 2H), 4.74 - 4.66 (m, 2H), 4.58 (ap. dd, J = 8.8, 5.3 Hz, 1H), 4.52 - 4.45 (m, 2H), 4.40 - 4.27 (m, 8H), 4.26 - 4.19 (m, 3H), 4.19 - 4.10 (m, 2H), 3.95 - 3.78 (m, 7H), 3.78 - 3.70 (m, 1H), 3.70 - 3.61 (m, 1H), 3.61 (m, 2H), 3.21 (ap. t, J = 7.0 Hz, 2H), 3.14 - 2.94 (m, 8H), 2.92 - 2.68 (m, 4H), 2.40 - 2.23 (m, 5H), 2.20 - 1.43 (m, 40H), 1.40 (ap. d, J = 7.2 Hz, 7H), 1.33 (ap. d, J = 7.2 Hz, 3H), 1.27 - 1.19 (m, 7H), 1.17 (ap. d, J = 6.4 Hz, 3H), 1.00 - 0.89 (m, 21H), 0.87 (ap. t, J = 7.4 Hz, 3H).

Poliovirus VPgpU, (H-Gly-Ala-Tyr(pU)-Gly-Leu-Pro-Asn-Lys-Lys-Pro-Asn-Val-Pro-Thr-Ile-Arg-Thr-Ala-Lys-Val-Gln-OH), (5b)

HPLC gradient: 10 - 50 % B in 3 CV. The appropriate fractions were lyophilized, yielding 13.29 mg, 4.26 μmol (17 % based on original loading of resin) of the nucleopeptide as TFA salt. LCMS (0 – 50 % B in 15 min.), Rt = 6.38 min. ESI-MS: m/z 1330.07 [M+2H]2+, 887.40 [M+3H]3+. HRMS [C114H189N33O38P + 2H]2+; calcd. 1330.1838, found 1330.1826, [C114H189N33O38P + 3H]3+; calcd. 887.1255, found 887.1248, [C114H189N33O38P + 4H]4+; calcd. 665.5955, found 665.5960, [C114H189N33O38P + 5H]5+; calcd. 532.6778, found 532.6768. 31P-NMR (162 MHz, D2O); δ – 4.28. 1H NMR (600 MHz, D2O) δ 7.69 (d, J = 8.1 Hz, 1H), 7.19 (d, J = 8.5 Hz, 2H), 7.12 (d, J = 8.3 Hz, 2H), 5.94 (d, J = 4.6 Hz, 1H), 5.68 (d, J = 8.1 Hz, 1H), 4.73 - 4.66 (m, 1H), 4.66 - 4.61 (m, 2H), 4.60 (ap. dd, J = 8.8, 5.3 Hz, 1H), 4.52 - 4.39 (m, 5H), 4.39 - 4.26 (m, 12H), 4.25 - 4.09 (m, 6H), 3.98 - 3.80 (m, 7H), 3.77 - 3.69 (m, 1H), 3.69 - 3.63 (m, 2H), 3.21 (ap. t, J = 7.1 Hz, 2H), 3.12 (ap. dd, J = 13.9, 6.9 Hz, 1H), 3.05 - 2.93 (m, 7H), 2.89 - 2.69 (m, 4H), 2.37 (ap. t, J = 7.6 Hz, 4H), 2.36 - 2.24 (m, 3H), 2.24 - 2.16 (m, 1H), 2.14 - 1.97 (m, 9H), 1.97 - 1.43 (m, 29H), 1.40 (ap. d, J = 7.2 Hz, 4H), 1.31 (ap. d, J = 7.2 Hz, 3H), 1.27 - 1.20 (m, 7H), 1.19 (ap. d, J = 6.4 Hz, 3H), 1.01 - 0.95 (m, 12H), 0.92 (ap. dd, J = 12.2, 6.6 Hz, 9H), 0.87 (ap. t, J = 7.4 Hz, 4H).
Notes and References


22. The consensus HEV VPg was designed using software that used 33 aligned HEV-VPg’s to assign the conserved physicochemical properties and on the basis of these findings model a consensus sequence.


24 Preliminary results indicate that all synthetically prepared VPg’s, including the consensus HEV protein, were recognized as substrate by viral 3D polymerases based on their ability to be uridylylated. Poliovirus and coxsackie A virus polymerases showed a preference for their own VPg with respect to other viral strand VPg’s, but the consensus VPg was found to be uridylylated as well or even better as the wild type VPg.