New peptide-porphyrin conjugates targeting brain-residing viruses

Toni Todorovski[1,2], Giuseppina Guida[1], Patrizia Janković[2], Diogo Mendonça[3], Christine Cruz-Oliveira[3], Marco Cavaco[3], Daniela Kalafatović[2], Sira Defaus[1], Andrea Da Poian[4], Miguel Castanho[3], David Andreu[1]

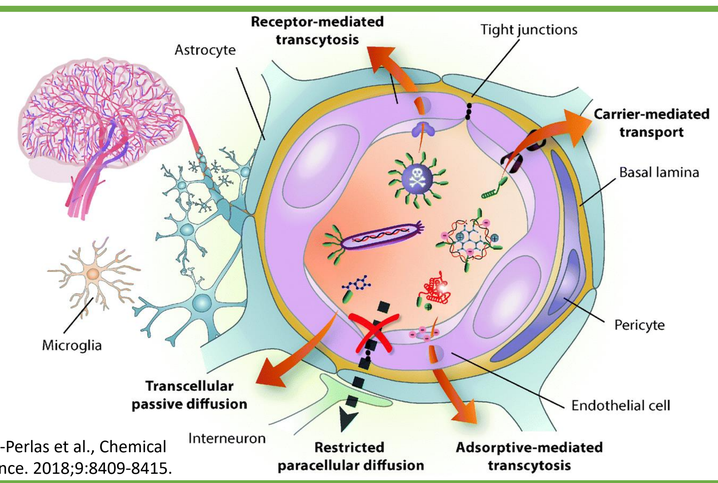
[1] Department of Medicine and Life Sciences (MELIS), Universitat Pompeu Fabra, Barcelona, Spain; [2] University of Rijeka, Faculty of Biotechnology and Drug Development, Rijeka, Croatia; [3] Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal; [4] Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Contact email: toni.todorovski@uniri.hr; toni.todorovski@upf.edu

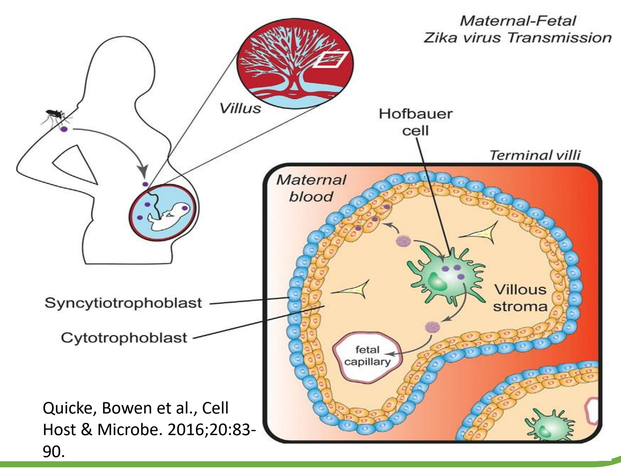
https://doi.org/10.17952/37EPS.2024.P1217

Introduction

Flaviviruses (Zika, dengue, West Nile, yellow fever, tick-borne encephalitis) are enveloped viruses with positive-sense RNA, able to cross the blood-brain barrier (BBB) and thus infect adult brains. The infection can affect the central nervous system (CNS) causing severe neurological complications such as encephalopathy, encephalitis, Guillain-Barré syndrome and microcephaly. There is no current drug to target brain-infecting viruses, and any such development faces the challenge of delivering the drug across the BBB to reach virus at its most sensitive site of action, the brain. Herein we present a comparative study of various on-resin conjugation strategies to generate linear and multimerized (juxtaposed or branched) peptide-porphyrin conjugates (PPCs) able to cross BBB with good translocation rates and high antiviral activity [1, 2].

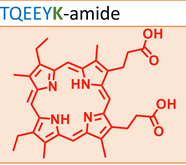
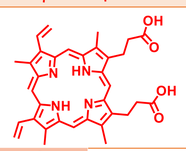
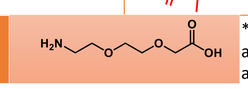


Diaz-Perlas et al., Chemical Science. 2018;9:8409-8415.

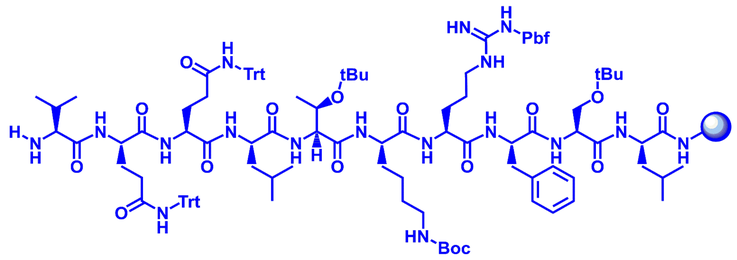


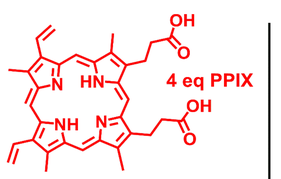
Quicke, Bowen et al., Cell Host & Microbe. 2016;20:83-90.

Conjugation chemistry

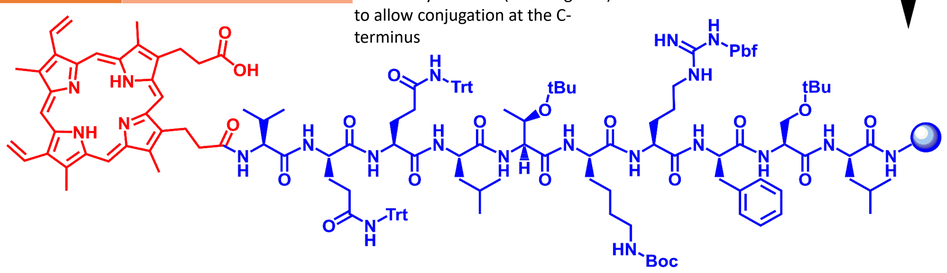
Entry number	Structure	M+H
P1	VQQLTKRFSL-amide	1218.7
P2*	VQQLTKRFSLK-amide	1346.8
P3	AGILKRW-amide	842.5
P4*	AGILKRWK-amide	970.6
P5	SGTQEEY-amide	812.3
P6*	SGTQEEYK-amide	940.4
Mesoporphyrin IX MPIX (MP)		
Protoporphyrin IX PPIX (PP)		
8-amino-3,6-dioxooctanoic acid (O2Oc)		

*P2, P4, P6 are versions of P1, P3 and P5, respectively, elongated with an extra Lys residue (in bold green) to allow conjugation at the C-terminus

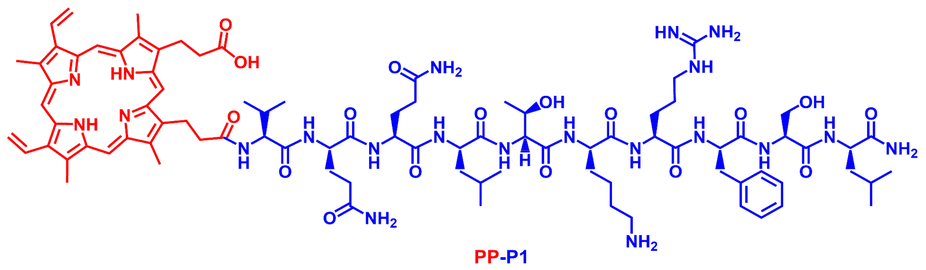




(1) 4eq Oxyma, 4eq DIC, RT, 3h+1h coupling
(2) Solvent mixture (DMF:DMSO:DCM) = 5:2:1
(3) 95% CF_3COOH / 2.5%TIS, 2.5% H_2O



3

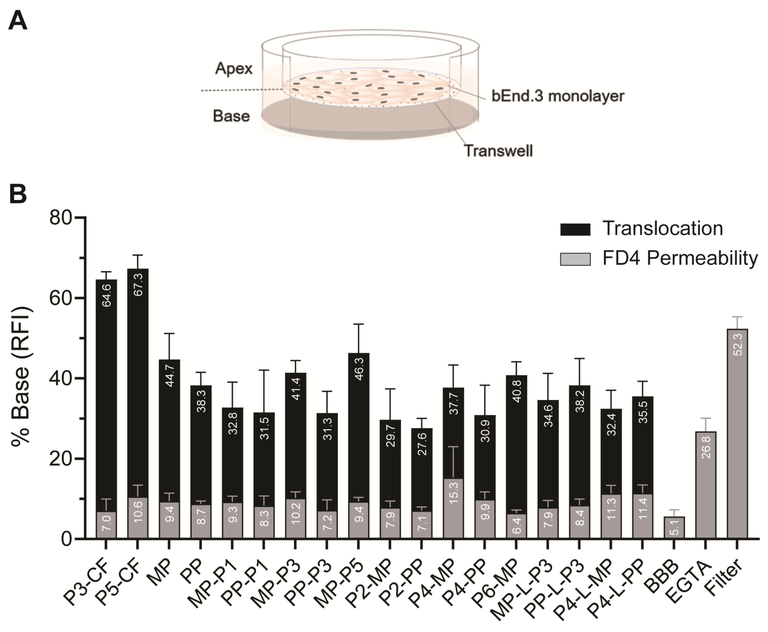


PP-P1

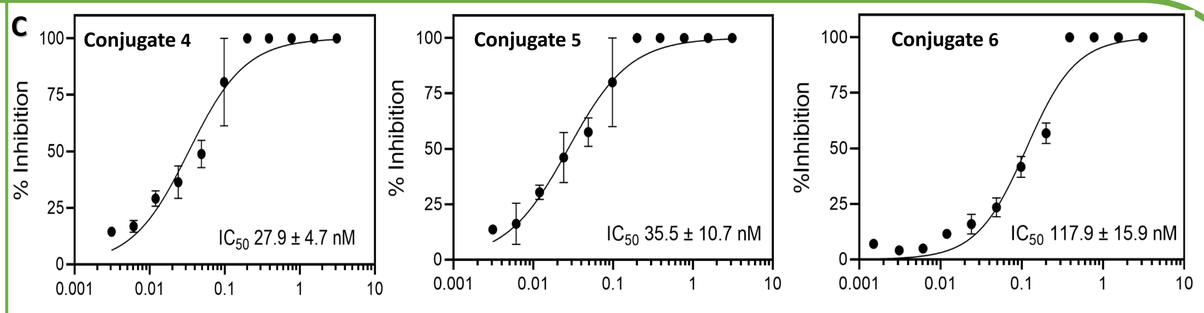
Porphyrin-peptide combinations	Activation method/Time (min)	Conversion (%)
PP-P1	PyBOP-DIPEA / 30	96.8
	HATU-DIPEA / 60	100
	TBTU-DIPEA / 30	97.6
	DIC-Oxyma / 180 + 60	**98.7**
MP-P3	PyBOP-DIPEA / 30	78.9
	HATU-DIPEA / 60	59.9
	TBTU-DIPEA / 30	98.6
	DIC-Oxyma-DIPEA / 180 + 60	**99.5**
PP-P3	TBTU-DIPEA / 30	90.1
	DIC-Oxyma / 180 + 60	**98.5**
MP-P1	TBTU-DIPEA / 30	67.3
	DIC-Oxyma-DIPEA / 180 + 60	**97.1**
P2-MP	**DIC-Oxyma-DIPEA / 180 + 60**	**99.6**
P2-PP	**DIC-Oxyma / 180 + 60**	**99.4**

The PPC production strategy involved six blood-brain barrier peptide shuttles (BBBpS), two porphyrins and one PEG-like linker (Table on the left side). Several on-resin conjugation chemistries and reaction times were tested (Table on the right side). The conjugation took place at *C*- (P2, P4 and P6) or *N*-terminal (P1, P3 and P5) part of the corresponding BBBpS. The scheme presented here show the synthesis of PP-P1 (conjugation at the peptide *N*-terminal part) using the optimized conditions. When conjugation was performed at the BBBpS *C*-terminal part, an additonal Lys with a orthogonally protected Mmt group was added at the peptide C-terminal end (Table on the left side, entries P2, P4 and P6). In these cases, first, the side-chain Mmt was selectively removed and afterwards the conjugation procedure is the same as showed in the scheme above. When the linker was used, coupling of the linker preceded the conjugation step.

BBB translocation rates and antiviral activity of some PPCs against Zika virus



In vitro PPC translocation. (**A**) Schematic representation of the BBB model transwell system. (**B**) *in vitro* BBB translocation measurements. PPC translocation values are depicted in black, while FD4 permeability values post-translocation are in grey (BBB—only cells, EGTA—tight junctions disruption control, Filter—no cells).



Conjugates 4, 5 and 6 were evaluated for Zika virus (ZIKV) inhibition *in vitro* using a plague assay **(C)**. The results showed that these PPCs are able to inhibit ZIKV *in vitro*, with IC_{50} values in the low nM range. These results are very promising, compared to IC_{50} values of the previously published PPCs [2] (Table on the right), and currently cytotoxicity and antiviral studies against other brain-residing viruses are undergoing.

IC50 (μM)	MP	PP	MP-P1	MP-P5	PP-P1	PP-P5	P2-MP	P6-MP	P2-PP	P6-PP
ZIKV	> 50	> 50	> 50	25.07 ± 0.05	**1.08 ± 0.14**	> 50	> 50	> 50	> 50	> 50

Conclusions

- We have developed successful on-resin synthesis of new PPCs by DIC/oxyma activation strategy as most suitable and effective approach.
- All conjugates were well characterized with purity > 90 % and most of them were able to successfully pass the BBB.
- We have identified several PPCs with high antiviral activity (some of them reaching nanomolar range) against ZIKV.
- **In any event, one may propose peptide-porphyrin conjugation as a promising strategy to tackle brain-resident viruses.**

References

1. Mendonça, D.A. et al. *Bioconjugate Chemistry* **(2021),** 32 (6), 1067-1077.
2. Todorovski, T. et al. *Pharmaceutics* **(2022),** *14*, 738.

Acknowledgement

This project has received funding from La Caixa Foundation (HR17_00409) and from the European Union´s Horizon 2020 research and innovation programme under grant agreement No 828774.