Arginine building blocks with increased lipophilicity – synthesis and application

UMO-2019/35/D/NZ7/00174

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INTRODUCTION

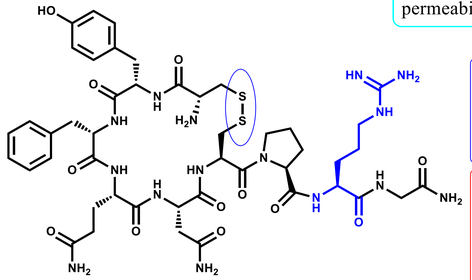
PEPTIDES
crucial role in all living organisms [1]

application → - hormones, antibodies, neurotransmitters [1]
- development of some diseases [2]
- functional materials [3]
- pharmaceuticals [4]

shortcomings → - inability to cross intestinal epithelial barrier
- limited stability toward proteolysis [4][5]

solution → **lipophilic prodrug charge masking (LPCM)** [6] → positive charged group is masked by lipophilic alkyl residues, the compound with increased lipophilicity and better membrane permeability

Arginine vasopressin (AVP)



Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2

Function:
- **antidiuretic [8]**
- **increases the blood pressure in septic shock**
- **maintains cardiovascular homeostasis [8a,8c]**

Shortcomings:
- **short biological half-life**
- **lack of specificity for receptors [9]**
- **may cause hyponatremia and decreasing of cardic output**
- **increases bilirubin level in blood [10]**

AIM

- preparation of arginine (Arg) building blocks with masked guanidine group
- SPPS of AVP analogues with increased lipophilicity

Table 1. Optimization of reaction conditions for step 1.

Entry	m (guanidine hydrochloride)/g	base/eq.	solvent/mL	t/°C	chloroformate/2.2 eq.	η/%
1	0.25	TEA/4	ACN/H_2O/3/1	0→rt	butyl	13
2	0.10[a]	TEA/4	ACN/H_2O/1.2/1.2	0→rt	butyl	-
3	**0.10**	**TEA/4**	**DMF/2**	**0→rt**	**butyl**	**76**
4	0.10	DIPEA/4	ACN/H_2O/1.2/1.2	0→rt	butyl	44
5	0.10	1M NaOH	ACN/H_2O/1.2/1.2	0→rt	butyl	-
6	0.10	TEA/4	ACN/H_2O/1.2/1.2	40	butyl	-
7	0.10	NaH/4	ACN/H_2O/1.2/1.2	0→rt	butyl	70
8	**1.00**	**TEA/4**	**DMF/20**	**0→rt**	**butyl**	**84**

[a] DMAP was added in the reaction.

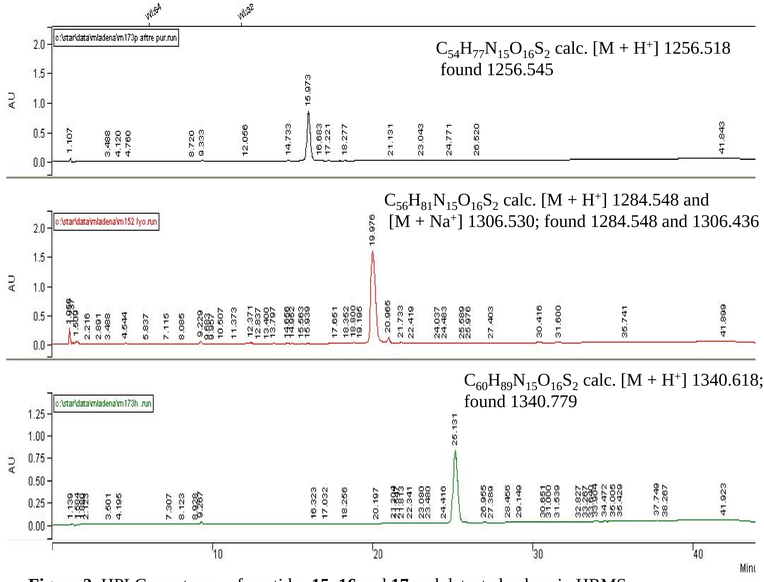
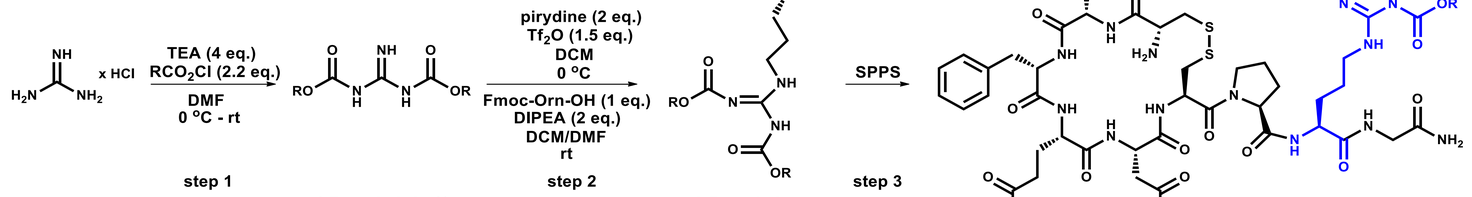


Figure 2. HPLC spectrums of peptides **15**, **16** and **17** and detected values in HRMS.

SYNTHESIS

Synthesis of modified arginine started with optimization of reaction conditions (Table 1) for preparation of guanidynylating reagents **1** – **7** (Figure 1) [11]. All reactions were conducted using butyl chloroformate and the best results were obtained using triethylamine (TEA) as base in dimethylformamide (DMF) on 0 °C (entry 3, Table 1). Reaction was performed on gram-scale, and the product was isolated in 84 % (entry 8, Table 1). Reactions with other chlororformates (ethyl-, propyl-, hexyl-, octyl-, decyl- and dodecyl-) were performed under the same reaction conditions on the gram-scale and the products **1** – **7** were isolated in 35 – 94 %.



1 R = ethyl; 35 %
2 R = propyl; 57 %
3 R = butyl; 84 %
4 R = hexyl; 67 %
5 R = octyl; 70 %
6 R = decyl; 53 %
7 R = dodecyl; 94 %

8 R = ethyl; -
9 R = propyl; 12 %
10 R = butyl; 5 %
11 R = hexyl; 14 %
12 R = octyl; -
13 R = decyl; -
14 R = dodecyl; -

15 R = propyl; 23 %
16 R = butyl; 22 %
17 R = hexyl; 30 %

Figure 1. Synthesis of Arg with masked guanidine group and its application in the SPPS of AVP analogues.

In the next step, compounds **1** – **7** were reacted with ornithine. The reaction was performed simultaneously in two flasks. In the first flask reacted compound **1** – **7**, respectively, with trifluoroacetic anhydride (Tf_2O) in dichloromethane (DCM) with addition of pirydine on 0 °C. In the second flask, reacted Fmoc protected ornithine with diisopropylamide (DIPEA) in DCM/DMF on rt. After 20 minutes of stirring, two parts were connected. Arginine derivatives with masked guanidine group **9** – **11** were isolated in modest yield of 5 – 14 %.

Last part was synthesis of AVP analogues, applying SPPS. Peptides **15** – **17** were obtained in moderate yield of 22 – 30 %. The main difference between peptides is in the group (ethyl-, propyl-, hexyl-) with which the Arg guanidine moiety is masked. The structure of all compounds were confirmed by NMR (1D and 2D), HRMS and HPLC.

CONCLUSION

- guanidynylating reagents **1** – **7** were isolated in moderate to excellent yield of 35 – 94 %, respectively
- only three compounds **9** – **11**, with masked Arg guanidine group were isolated in modest yield of 5 – 14 %
- three AVP analogues **15** – **17** were prepared and isolated in moderate yield of 22 – 30 %
- further research including computional studies is planned as well as the biological testing of prepared peptides

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