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Synthesis of Peptide Hydrazides: Hydrazone Resin *vs.* Hydrazine 2CT Resin

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Introduction

Peptide hydrazides are widely applied as precursors of peptide thioesters, valuable building blocks for the synthesis of proteins by native chemical ligation. Conversion of hydrazides into azides or isocyanates makes them useful intermediates for the conjugation with different nucleophiles. Finally, they can be applied for the selective modification of cargo or carrier molecules using hydrazone ligation technique (Fig. 1).

Solid-phase synthesis of peptide hydrazides commonly can be achieved by hydrazinolysis of peptidyl-Wang resin or using standard cleavage/deprotection protocols and hydrazine 2CT resin[1,2,3]. Both methods possess their own limitations depending on peptide sequence and protecting group combination. Previously, we described the synthesis of a new pyruvoyl hydrazone resin (Fig. 2) and examples of its practical application[4]. Herein, we report a comparative investigation of different synthetic protocols in parallel synthesis of peptide hydrazides using hydrazine 2CT and hydrazone resin.

Results and Discussions

In this study, we demonstrated that in the case of the hydrazone resin, all synthetic procedures from the attachment of the linker group to the cleavage of the final product can be performed by using only "recommended" and "usable" organic solvents (Fig. 3; Table 1). Moreover, the resin permits to fulfil selective Mtt group removal and possesses complete stability to acidic additives such as 6-Cl-HOBt and OxymaPure during peptide chain elongation (Fig. 4; Table 2).

In contrast, synthesis of the Fmoc-hydrazine 2CT resin in "usable" and green solvents proceeds with moderate efficiency (Fig. 5). Attempts of Mtt group removal with 4% TFA solution in DCM or toluene resulted in complete peptide release from the solid support (Table 2).

At the same time, HPLC analysis of different peptide hydrazides synthesized using hydrazine 2CT and hydrazone resin demonstrated comparable purity of the crude products (Fig. 6).

Investigation of the cleavage conditions revealed that apart from TFA, the peptide can be efficiently released from the polymeric support with simultaneous removal of ^{t}Bu based protecting groups using 5% HCl (aq) in acetone. While this protocol demands subsequent peptide deprotection in the case of other protecting groups, it benefits of significantly reduced TFA consumption.

It should be mentioned that cleavage and deprotection of peptide hydrazides using TFA can be accompanied with the side reaction of hydrazide trifluoroacetylation. In accordance with the literature data, extent of trifluoroacetylation can be reduced by the additional amount of water (8%) in the cleavage cocktail[5]. However, in the case of peptide with C-terminal glycine we still observed about 10% of the corresponding side product. Subsequent experiments demonstrated that its formation can be minimized by the addition of 10% of HCl (aq) to the cleavage cocktail (Fig. 7; Table 3).

To prove the practical utility of the suggested resin, we synthesized a set of model compounds including, but not limited to SV40 T antigen nuclear localization signal, *cyclo*-RGDfK(TPP), and an analog of Szeto-Schiller peptides (Table 4). Parallel synthesis of several peptides using hydrazine 2CT and hydrazone resin demonstrated comparable weight gain, cleavage yield, and crude peptide purity in both cases. Taking into account the improved stability in acidic conditions, the possibility of selective Mtt group removal and peptide cleavage using green solvent, hydrazone resin can be considered as a useful alternative for peptide hydrazides synthesis.

Conclusions

- Synthesis of peptide hydrazides on hydrazine 2CT and hydrazone resin resulted in comparable weight gain, cleavage yield, and crude peptide purity.
- Hydrazone resin permits to fulfil selective Mtt group removal and possesses complete stability to acidic additives during peptide chain elongation.
- Side reaction of hydrazide trifluoroacetylation can be minimized by the addition of 10% HCl (aq) to the cleavage cocktail.

Fig. 3. Efficiency of HP resin loading with the first amino acid.
Coupling conditions: 3 eqv of Fmoc-Ala-OH; DIC/Oxyma; 20 h.
Solvents classification: – banned; – usable; – green

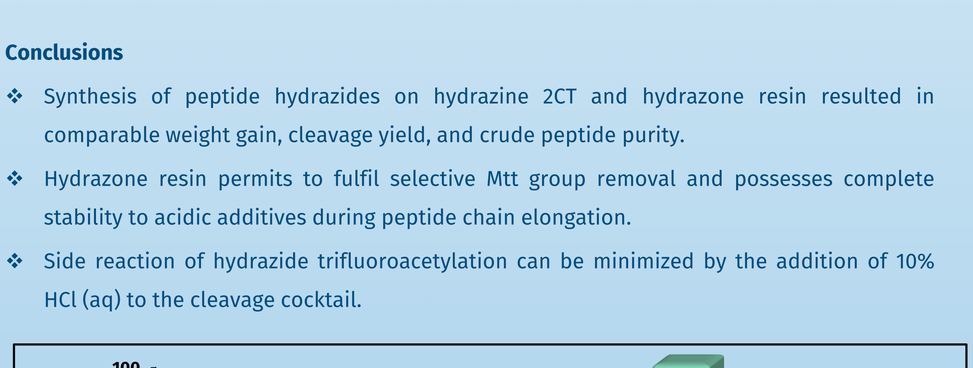

Fig. 5. Efficiency of 2CTC resin loading with Fmoc-NH-NH_2.
Solvents classification: – banned; – usable; – green

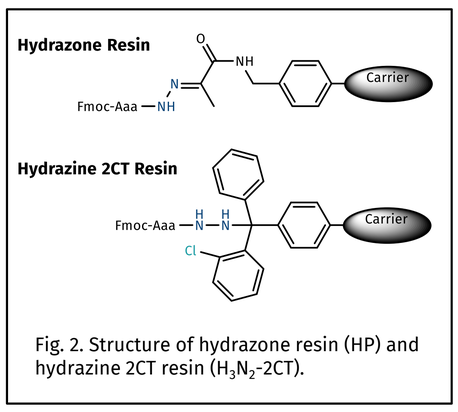

Fig. 2. Structure of hydrazone resin (HP) and hydrazine 2CT resin (H_3N_2-2CT).

Fig. 1. Applications of peptide hydrazides

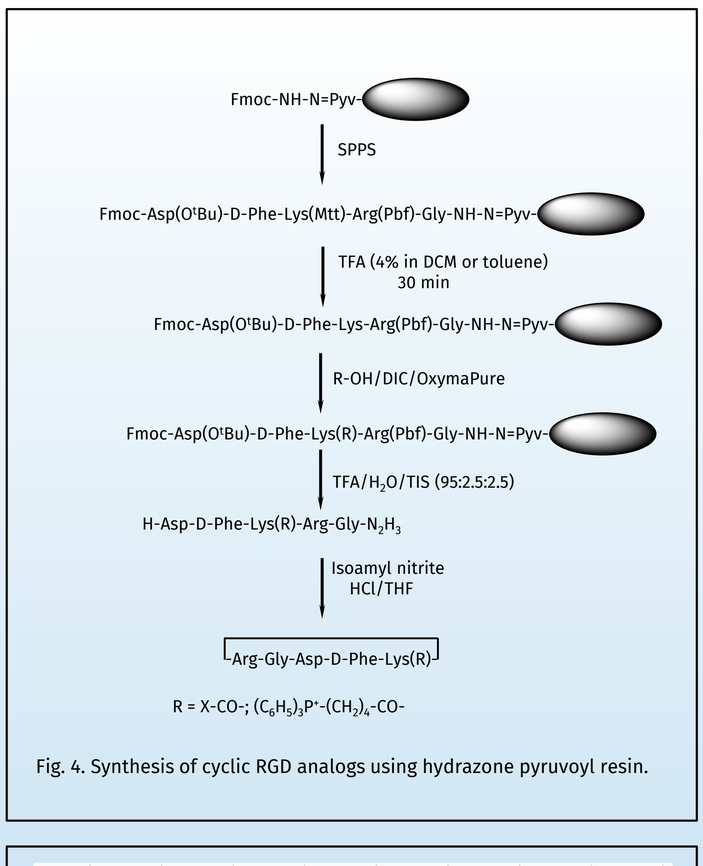

Fig. 4. Synthesis of cyclic RGD analogs using hydrazone pyruvoyl resin.

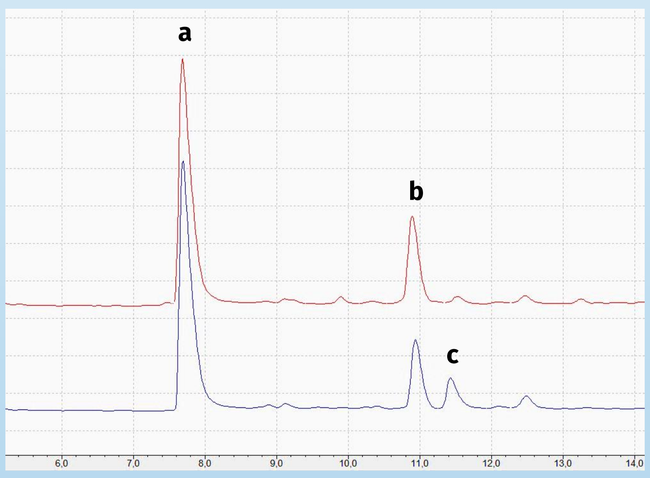

Fig. 6. HPLC analysis of crude product. Synthesis on H_3N_2-2CT resin (red) and HP resin (blue). Cleavage conditions: TFA/H_2O/TIS (95:2.5:2.5).
a - PKKKRKVG hydrazide;
b - Trifluoroacetyl hydrazide;
c - Peptide with free carboxyl group.

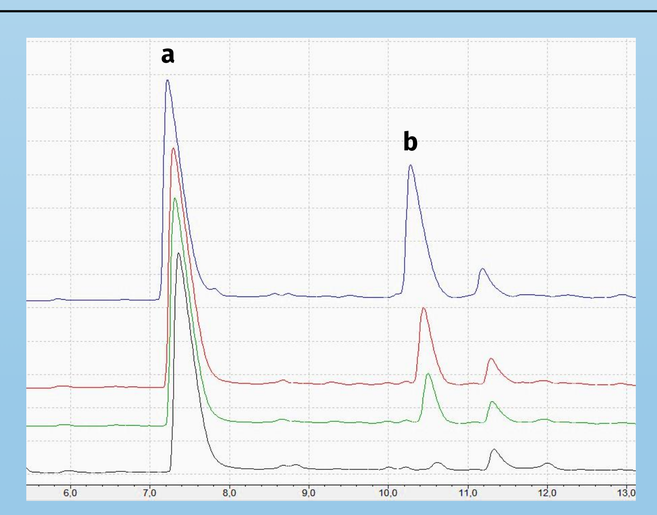

Fig. 7. Suppression of hydrazide trifluoroacetylation using different cleavage cocktails. TFA/DODT/TIS 95:2.5:2.5 (blue); TFA/H_2O/TIS 95:2.5:2.5 (red); TFA/H_2O/TIS 89.5:8:2.5 (green); TFA/HCl(aq)/TIS 87.5:10:2.5 (black)
a - PKKKRKVG hydrazide;
b - Trifluoroacetyl hydrazide.

Table 1. Synthesis of peptide hydrazides in "greener" solvent mixtures.

Entry	Hydrazone resin	H_3N_2-2CT resin
Attachment of the linker group	DMSO/EtOAc	EtOAc/AcN
Resin loading with the first amino acid residue	BuOAc	DMSO/EtOAc
Coupling and Fmoc removal	DMSO/EtOAc	DMSO/EtOAc
Cleavage/deprotection	HCl(aq)/Acetone; TFA/H_2O	HCl(aq)/Acetone; TFA/H_2O

Table 2. On-resin removal of Mtt group from DfKRG precursor.

Solvent	Polymer capacity (mmol/g)			
	HP resin		H_3N_2-2CT resin	
	A	B	A	B
DCM	0.28	0.35*	0.46	0.00
Toluene	0.28	0.35*	0.46	0.03

A – Before the treatment with 4% TFA for 30 min.
B – After the treatment.
*The loading value was recalculated due to Mtt group removal.

Table 3. Trifluoroacetylation of PKKKRKVG hydrazide in the course of peptide deprotection.

Cleavage cocktail	Desired product (%)	Trifluoroacetylation (%)
TFA/DODT/TIS 95:2.5:2.5	53.6	27.6
TFA/H_2O/TIS 95:2.5:2.5	70.4	14.8
TFA/H_2O/TIS 89.5:8:2.5*	75.4	10.6
TFA/HCl(aq)/TIS 92.5:5:2.5	76.1	9.3
TFA/HCl(aq)/TIS 87.5:10:2.5	80.8	2.3

*Recommended in the literature for the suppression of trifluoroacetylation[5].

Table 4. Synthesis of peptide hydrazides on hydrazone resin.

Entry	[M+H]$^+$ Calc.	[M+H]$^+$ Found	Yield* (%)
PKKKRKVG	953.66	953.67	55
TKPR	514.33	514.33	42
FrFK	610.37	610.36	47
RADA	445.24	–**	35
RADARADA	858.44	858.44**	41
cyclo-RGDfK(TPP)	948.45	948.48	28

* Yield of purified hydrazides and peptide obtained by azide cyclization.
TPP – triphenylphosphonium moiety.
** Peptides were prepared from the common precursor.

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