Azapeptide Assembly Using Resonant Acoustic Mixing

William D. Lubell[1], Michael Quagliata[1], Justin Desjardins-Michaud[1], Nassim Maarouf-Mesli[1], Ida Boccino[1], Zahra Bigdeli[1] and Felix Polyak[1,2]

[1]*Département de Chimie, Université de Montréal, Complexe des Sciences, 1375 Avenue Thérèse-Lavoie-Roux, Montréal, Québec, H2V 0B3, Canada.*
[2]*Studio Bioscience Inc., 111 Rue Terry Fox, Verdun, Québec, H3E 1L4, Canada.*

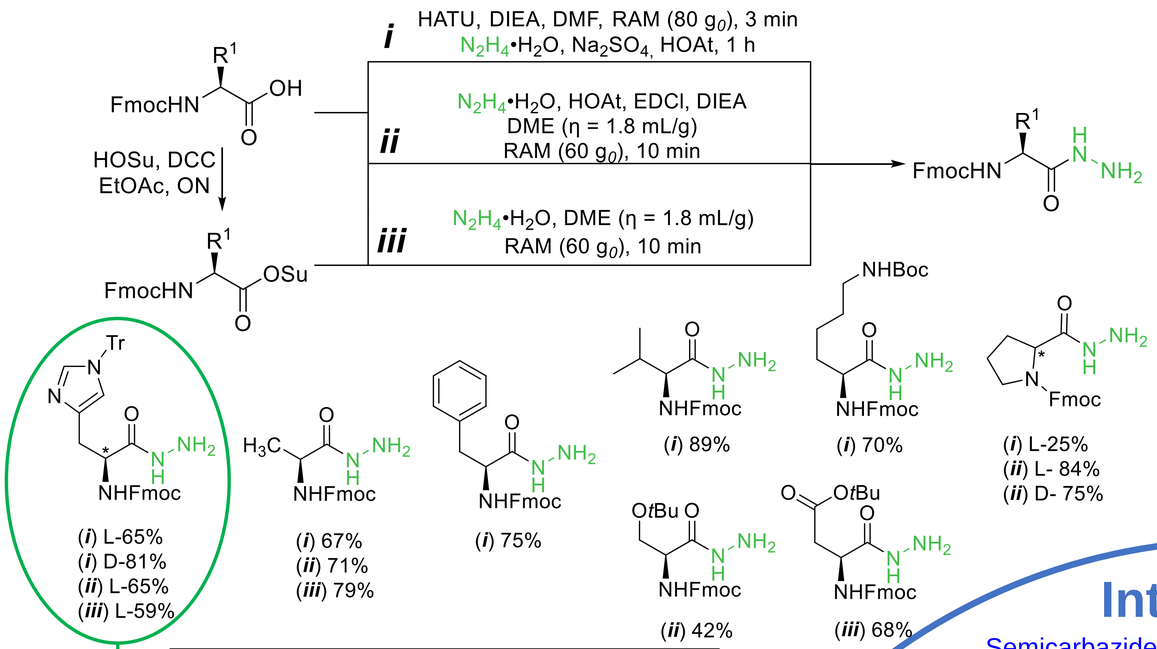
https://doi.org/10.17952/38EPS.2026.P132

Abstract

Azapeptide assembly amenable for Fmoc based peptide synthesis can be challenging because hydrazine differentiation requires selectivity. A greener solution-phase method is presented for making azapeptide by assembling *N*-(Fmoc)amino hydrazide and (Suoc)amino [*N*-succinyl carbamate] ester components at high concentration using resonant acoustic mixing (RAM). Hydrazinolysis was achieved without Fmoc cleavage, diacylation, and epimerization, eliminating chromatographic purification. Without hydantoin side products, (Suoc)amino acid analogs were effectively prepared and used in RAM-assisted semicarbazide synthesis. Aza-di-, tri-, and tetrapeptide building blocks were assembled using RAM with *N*-hydroxy-7-azabenzotriazole (HOAt) as catalyst and *N*-methylmorpholine (NMM) as solvent at high concentration. Aza-tripeptide building blocks proved useful in solid-phase synthesis of challenging bioactive targets. This sustainable, scalable approach minimizes hazardous reagents and solvents, facilitating azapeptides access for drug discovery.

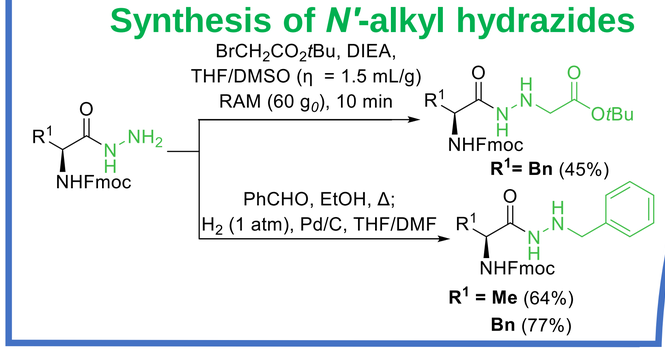
Synthesis of Fmoc-amino hydrazides

Hydrazine monohydrate was reported to cleave the Fmoc protecting group and liberate the amine in peptide synthesis [4]. The exothermic acylation of hydrazine hydrate using *N*-Fmoc-amino active esters was studied to minimize the generation of dibenzofulvene from Fmoc deprotection and diacyl-hydrazide byproduct [5]. Three strategies were successful employing *in-situ* generation of active ester (***i*** and ***ii***) and the direct acylation with succinimidyl esters (***iii***), all using RAM to expedite conversion.



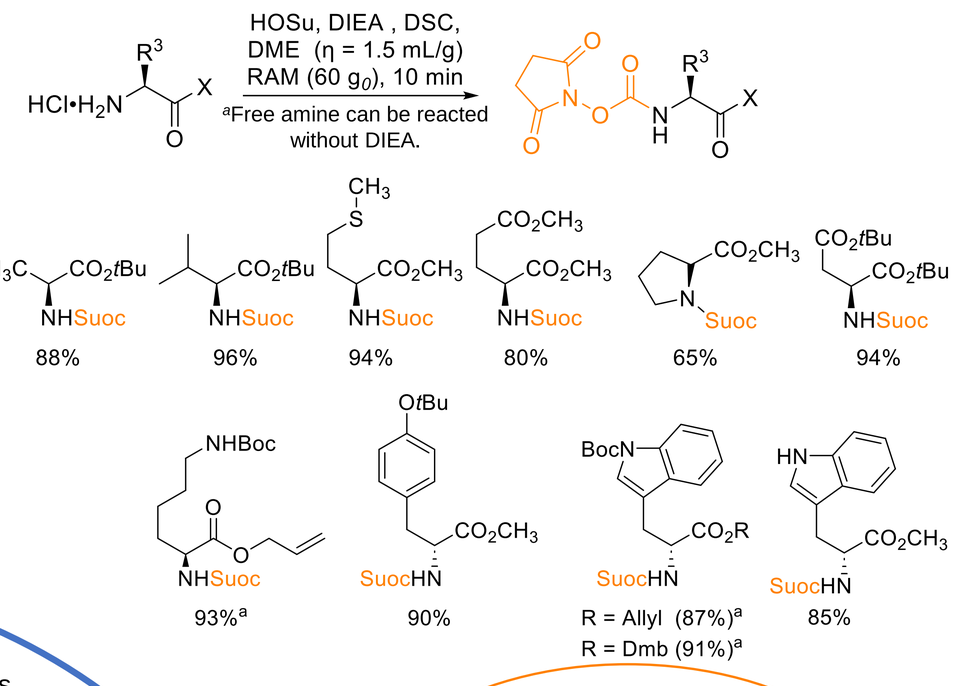
Condition	% diacyl-hydrazide	% dibenzofulvene	% D-epimer
i	<3	<2	9
ii	3	<2	<1
iii	<2	<2	<2

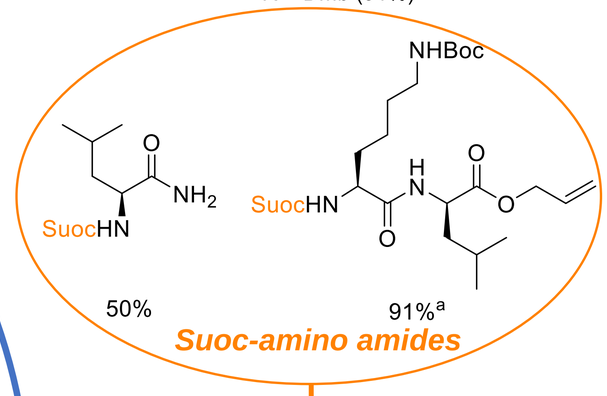
Synthesis of *N'*-alkyl hydrazides



Synthesis of Suoc-amino derivatives

Succinimidyl carbamates (Suoc) offer balanced stability and reactivity. They are readily prepared by RAM of amino ester/amide hydrochlorides with *N,N'*-disuccinimidyl carbonate (DSC), DIEA, and HOSu [6]. The HOSu (pKa 6.0) serves as buffer and reduces urea byproduct (0.4%).

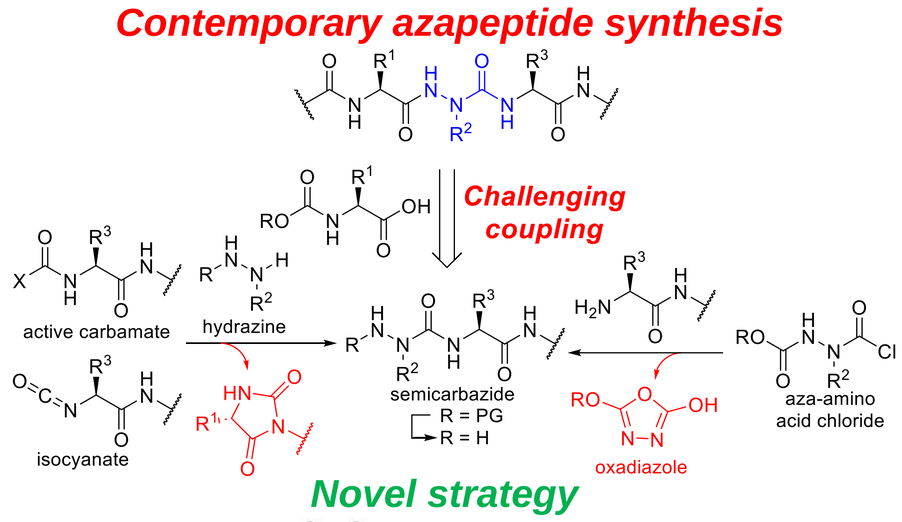




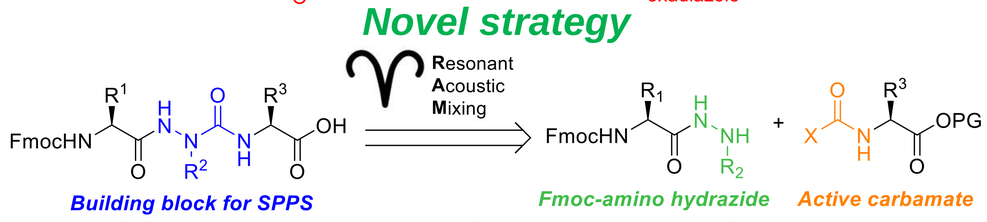
Introduction

Semicarbazides are amino amide surrogates. In azapeptides, semicarbazides can improve metabolic stability and enhance efficacy to achieve clinical success. The synthesis of semicarbazide peptidomimetics can be challenging [1].

Contemporary azapeptide synthesis

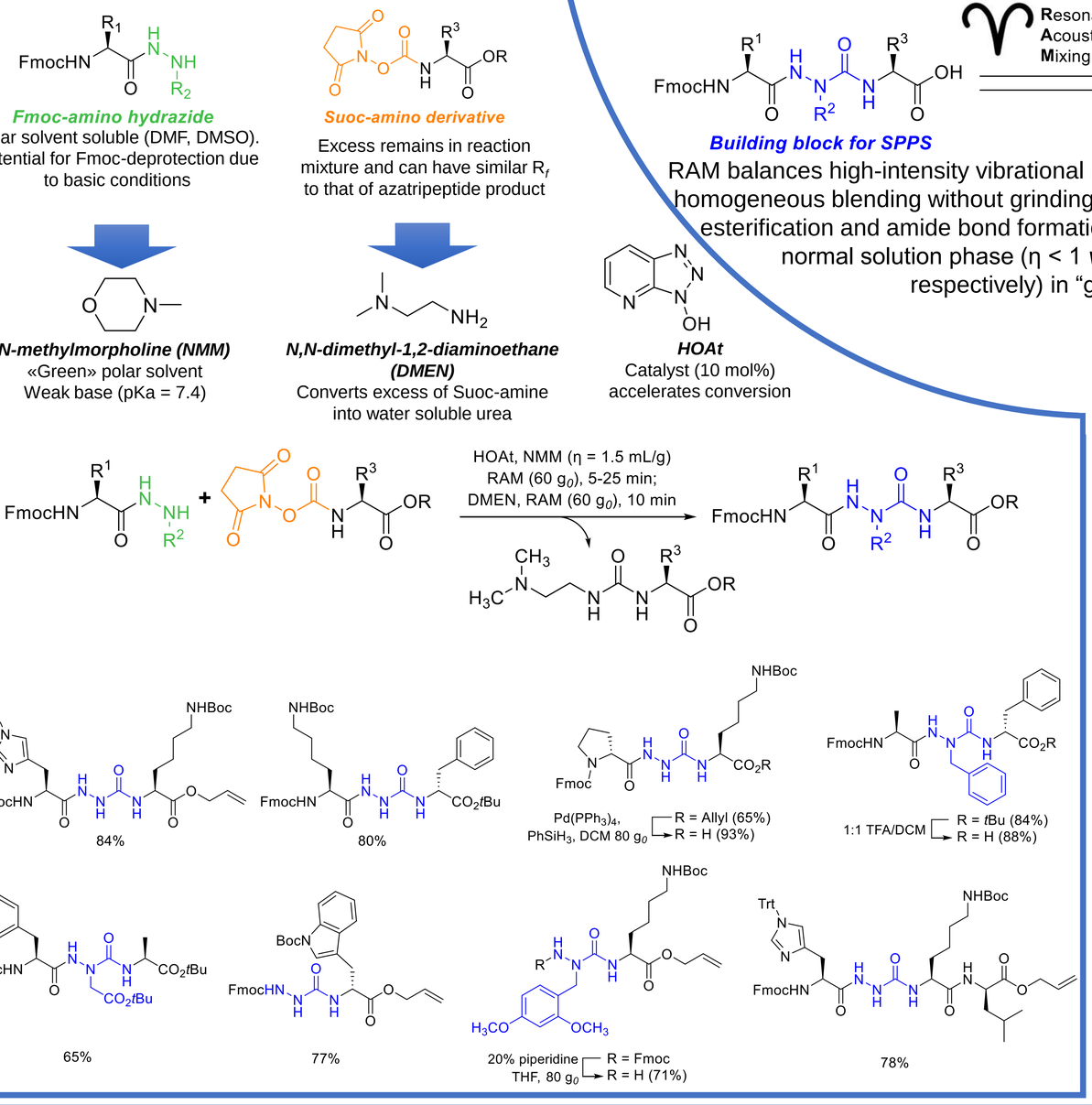


Novel strategy



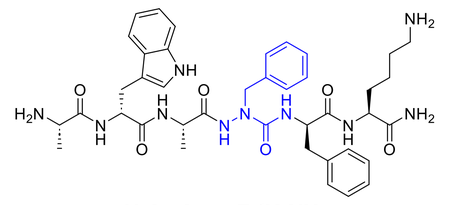
RAM balances high-intensity vibrational motion and acoustic frequency to expedite homogeneous blending without grinding. For peptide chemistry, RAM accelerated esterification and amide bond formation at higher concentration compared to normal solution phase (η < 1 *vs.* 10-20 mL/g of total reactant, respectively) in "green" solvent [2,3].

Synthesis of Fmoc protected azatripeptide



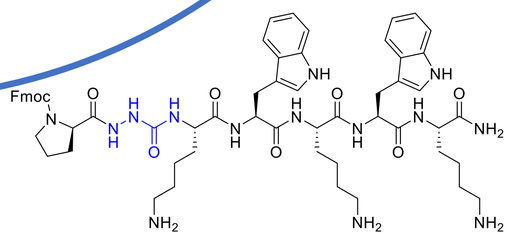
Building block incorporation into azapeptides

Biologically active peptide synthesis with aza-tripeptide building block was expedited by ultrasound-assisted SPPS (US-SPPS) [7].



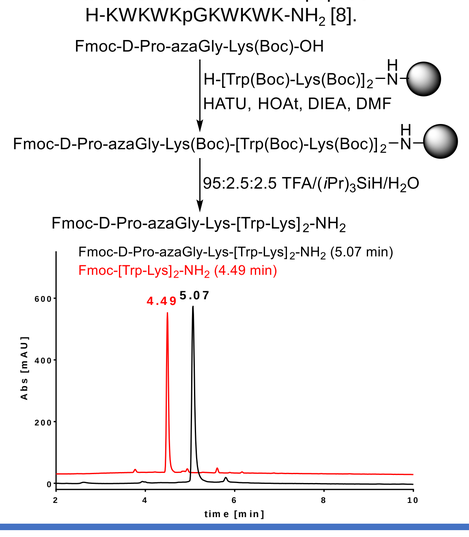
H-AwA-azaF-fK-NH$_2$

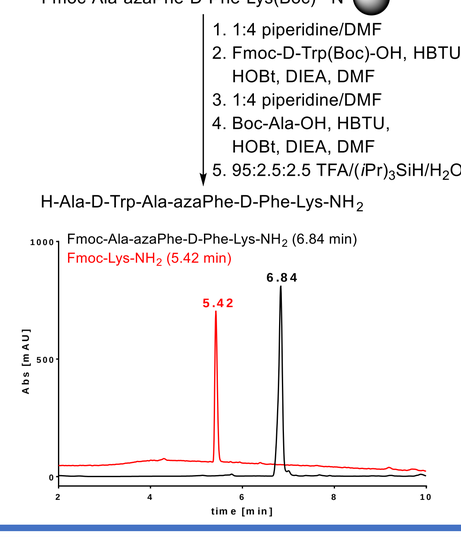
An aza-analogue of growth hormone releasing peptide-6 (GHRP-6) modulates the cluster of differentiation-36 receptor (CD36) exerting cardio protective activity against myocardial ischemia and reperfusion injury and dysfunction [9].



Fmoc-p-azaG-KWKWK-NH$_2$

Half sequence of aza-glycine variant of the antimicrobial peptide H-KWKWKpGKWKWK-NH$_2$ [8].





Conclusions

Azapeptides are promising therapeutic candidates. Their synthesis is challenging due to semicarbazide installation. An aza-tripeptide building block strategy has been developed and overcomes issues for scalable azapeptide synthesis by enabling efficient one-step coupling. Using RAM to prepare and couple *N*-(Fmoc)amino hydrazides with Suoc-amino esters, aza-tripeptide building blocks were effectively obtained and subsequently coupled onto peptide sequences linked to solid phase. Production methods for hydrazides, carbamates, ureas, and semicarbazides were all facilitated for the development of azapeptide therapeutics [10].

References

[1] C. Proulx *et* al., *Future Med. Chem.* **2011**, 3, 9, 1139–1164. https://doi.org/10.4155/fmc.11.74
[2] N. Maarouf-Mesli *et* al., *Green Chem*. **2026**, 28, 264−276. https://doi.org/10.1039/d5gc04619j
[3] N. Maarouf-Mesli *et* al., *J. Org. Chem.* **2026**, 91, 18, 6495–6501. https://doi.org/10.1021/acs.joc.6c00445
[4] V. Rodríguez *et* al., *Tetrahedron Lett*. **2019**, 60, 1, 48−51. https://doi.org/10.1016/j.tetlet.2018.11.054
[5] M. Quibell *et* al., *J. Chem. Soc. Perkin,* **1993**, 1, 22, 2843. https://doi.org/10.1039/P19930002843
[6] K. Takeda *et* al., *Tetrahedron Lett.*, **1983**, 24, 42, 4569-4572. https://doi.org/10.1016/S0040-4039(00)85958-4
[7] F. Merlino *et* al., *Org. Lett.* **2019**, 21, 16, 6378−6382. https://doi.org/10.1021/acs.orglett.9b02283
[8] L. Xu *et* al., *Amino Acids*, **2015**, 47, 11, 2385−2397. https://doi.org/10.1007/s00726-015-2029-7
[9] D. Huynh *et* al., *FASEB J.Off. Publ. Fed. Am. Soc. Exp. Biol.* **2018**, 32, 2, 807−818. https://doi.org/10.1096/fj.201700505R
[10] M. Quagliata *et* al., *Org. Lett*. **2026**. 28, 11, 3510-3516. https://doi.org/ 10.1021/acs.orglett.6c00458

Acknowledgements

For support of the Centre in Green Chemistry and Catalysis (CGCC, FRQNT 2020-RS4-265155-CCVC) and MQ Postdoctoral fellowship (https://doi.org/10.69777/370615)

For funding a Discovery Research Projects (RGPIN-2019-04079 and RGPIN-2025-04423)

For the project PJT-186296