Toward True αvβ8 Selectivity: Cyclopeptide Ligands that Discriminate Homologous Integrins

C. Savian[1], M. Spirelli[1], A. Sartori[1], P. Delcanale[2], C. Viappiani[2], M. Civera[3], F. Vasile[3], L. Belvisi[3], F. Zanardi[1] & L. Battistini[1]

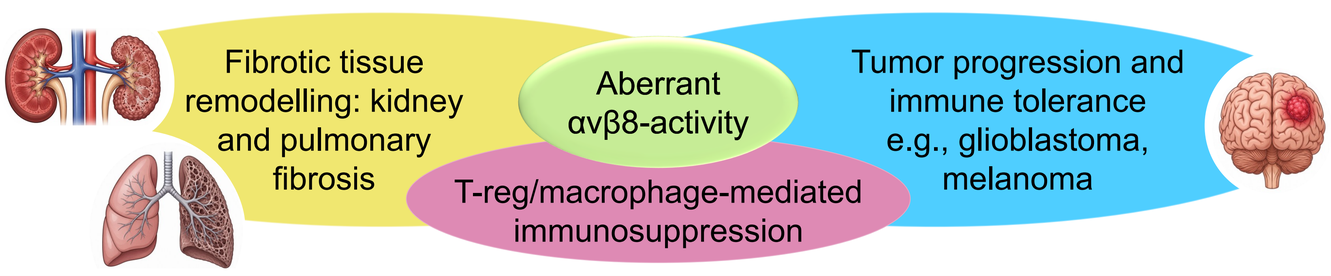
[1]*Department of Food and Drug, University of Parma, Parma, Italy*; [2]*Department of Mathematical, Physical and Computer Sciences, University of Parma, Parma, Italy;* [3]*Department of Chemistry, University of Milan, Milan, Italy*.

Email: chiara.savian@unipr.it **https://doi.org/10.17952/38EPS.2026.P139**

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αvβ8 Integrin: latent TGF-β activator

Together with αvβ6, αvβ8 integrin is a key activator of TGF-β, regulating its bioavailability and downstream signalling, and thereby influencing its pathophysiological roles.[1,2]



Challenge: αvβ8 and αvβ6 homology

The high structural homology between integrins αvβ8 and αvβ6 represents a major challenge for the development of truly αvβ8-selective ligands. Both integrins bind the RGDLXX(L/I) motif in TGF-β1/β3 with nanomolar affinity. To date, no high-affinity, αvβ8-selective small molecules have been reported, except for miniproteins described in 2023.[3]



Integrin heterodimers αv (green), β6 (blue), and β8 (violet) with RGDLXX(L/I) (yellow). Image adapted from ref. 3.

Dual c-AmpRGD ligands: starting point

The affinity of the Amp-RGD cyclopeptides was assessed by competitive solid-phase binding assays using immobilized LAP (pro-TGF-β) and soluble αvβ8 or αvβ6 integrins, revealing dual integrin-binding activity.

Structure	IC_{50} (nM) for αvβ8	IC_{50} (nM) for αvβ6
c(Amp[BuN_3]LRGDL)	**2.28** ± 0.12	4.52 ± 1.02
c(Amp[$HexN_3$]LRGDL)	**1.09** ± 0.05	30.3 ± 5.1
c(Amp[$HexN_3$]FRGDL)	**1.29** ± 0.03	43.6 ± 4.9
c(Amp[$HexN_3$]RLD)	**> 10000**	**> 10000**

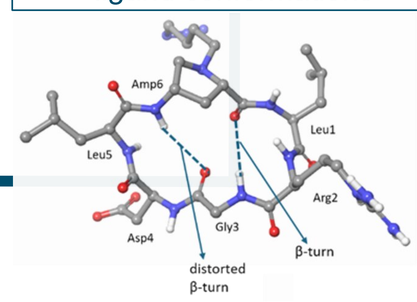
NMR and computational studies

Molecular dynamic simulation (500 ns, 320K) → 5000 frames → Filters from experimental studies (NMR, X-ray structure)
- d(Amp C=O - Gly3 NH) ≤ 4.5 Å
- d(Leu1/Phe1 NH – Arg2 NH) ≤ 3 Å
- d(Cβ-Arg2 and Cβ-Asp4) > 8.25 Å

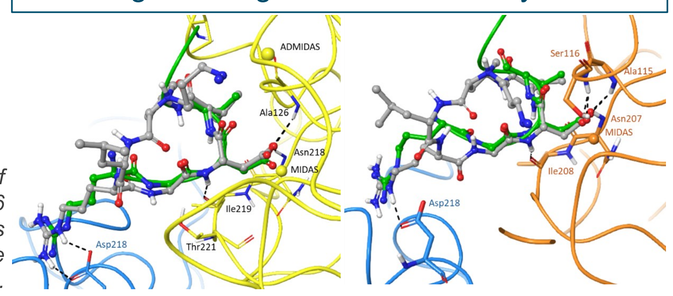
→ MD-NMR-derived ligand conformations

A molecular dynamic-driven docking protocol was implemented to identify the crucial structural determinants and bioactive conformations of dual c-AmpRGD ligands.

Glide SP peptide docking in αvβ8 and αvβ6 → Binding mode selection → Redocking of one selected pose for each ligand → Ligand integrin interaction analysis



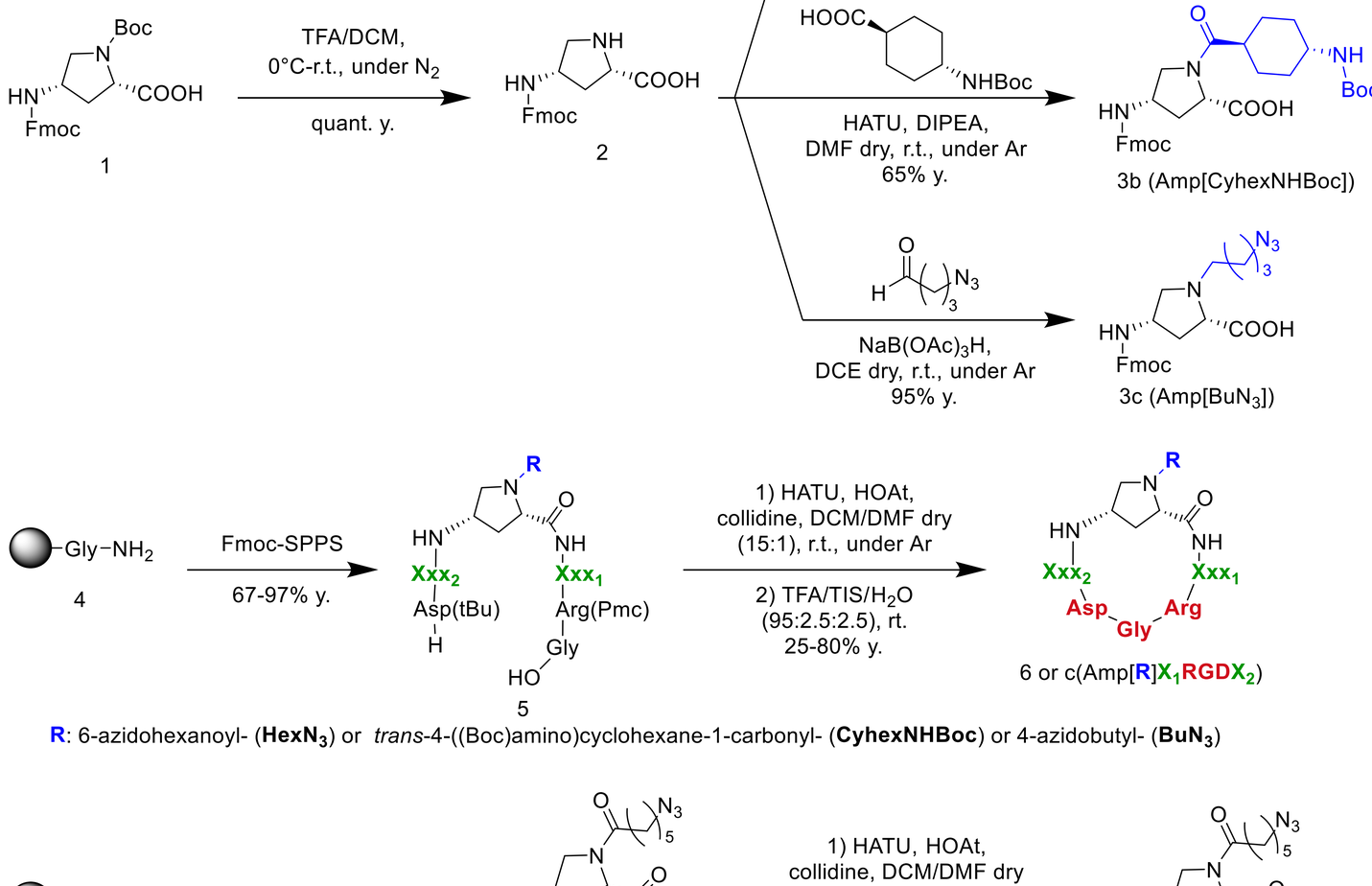
Representative MD-NMR-derived conformation of c(Amp[$HexN_3$]LRGDL).



Docking best poses of c(Amp[$HexN_3$]LRGDL) (grey) in αvβ6 (left) and αvβ8 (right) integrins overlaid to the X-ray structure of the TGF-β3/ β1 (green).

Synthesis of FITC-probe and cAmpRGD ligands

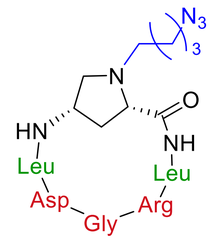
The Amp-RGD cyclopeptides[4] and a FITC-labelled decapeptide probe[5] have been synthesized via standard solid- and solution-phase procedures, purified by flash chromatography or preparative HPLC, and characterized using LC/MS and NMR.



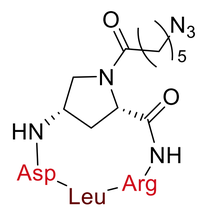
Fmoc-SPPS: (*i) Coupling*: Fmoc-Arg(Pmc)-OH, **Fmoc-$Xxx_{1/2}$-OH** (**$Xxx_{1/2}$**: **none**, **Phe**, **Leu**), **3a** or **3b** or **3c**, Fmoc-Asp(tBu)-OH, Fmoc-Lys(Boc)-OH; HATU, HOAt, collidine, DMF, r.t. *(ii) Fmoc-deprotection*: piperidine, DMF, r.t. *(iii) Resin cleavage*: AcOH, TFE, DCM, r.t.

Fluorescence anisotropy displacement binding assays

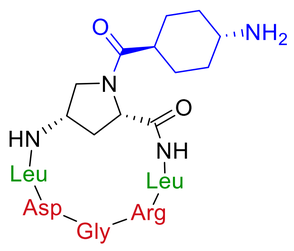
The FITC-labelled probe and an in-house focused library of cyclopeptides with known αvβ6/αvβ8 affinity were used to establish a rapid and reliable cell-free displacement binding assay based on fluorescence anisotropy.



c(Amp[BuN_3]LRGDL)
αvβ6/αvβ8 dual affinity

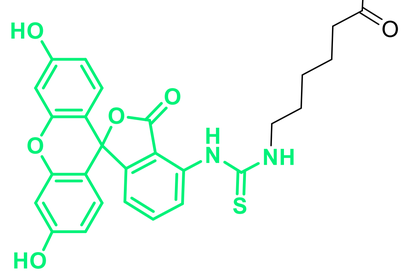


c(Amp[$HexN_3$]RLD)
negative control



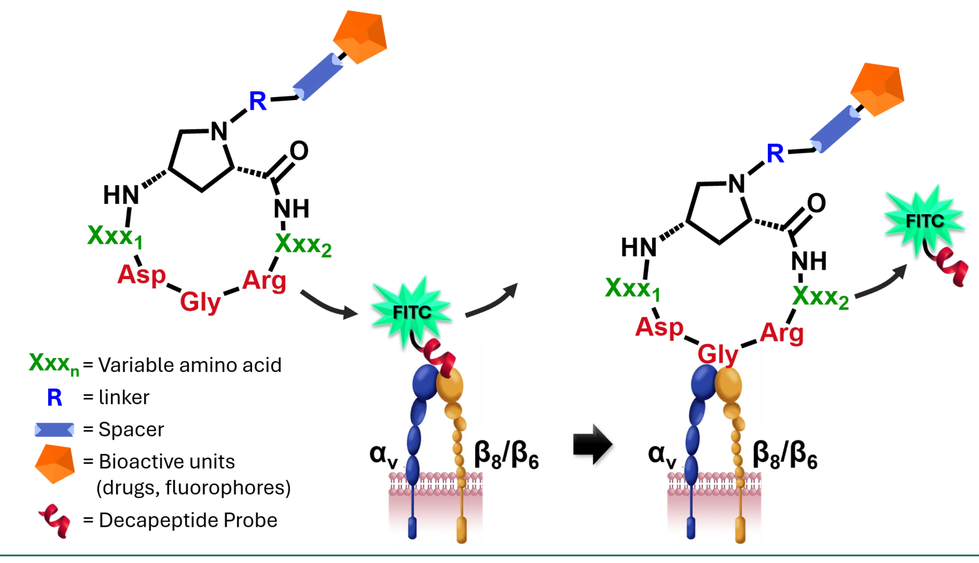
c(Amp[$CyhexNH_2$]LRGDL)

HO—Lys-Lys-Leu-Arg-Gly-Leu-Asp-Gly-Arg-Gly-NH



FITC-probe

The setup of the biophysical binding assay has enabled the affinity evaluation of the newly synthesized Amp-RGD cyclopeptides (*experiment ongoing*).



Conclusions and future perspectives

Once the affinity and selectivity of the newly developed ligands have been established, the most promising ones will be optimized to improve their binding profiles and subsequently functionalized with fluorescent or therapeutic payload through suitable linker/spacer moieties.

By integrating structure-guided design, chemical synthesis, and biophysical screening, this approach aims to generate αvβ8-selective Amp-cyclopeptides as innovative platforms for αvβ8-targeted biomedical and pharmacological applications.

References

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