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Chemoselective sulfonyl fluoride exchange (SuFEx)-induced macrocyclization of tyrosine-containing peptides in aqueous media

Peptide macrocycles have emerged as a powerful class of therapeutics capable of targeting challenging protein–protein interactions with high affinity and selectivity. This work introduces a mild, aqueous method for chemoselective peptide macrocyclization using sulfur fluoride exchange (SuFEx) chemistry. The approach selectively targets tyrosine residues to form sulfonate-tyrosine ester macrocyclic peptides (*STEMtides*) without metal catalysts or organic cosolvents. The method exhibits exceptional sequence-length tolerance, side-chain compatibility, and yields, enabling efficient cyclization of therapeutically relevant peptides such as leuprorelin, β-MSH, liraglutide, and RGD analogs.

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Chemoselective sulfonyl fluoride exchange (SuFEx)-induced macrocyclization of tyrosine-containing peptides in aqueous media

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Cyclic peptides generally exhibit enhanced metabolic stability, cell permeability, and binding affinity to biological targets compared to linear peptide sequences, making them attractive scaffolds for therapeutic development. Due to the chemical heterogeneity of peptides, the development of new, chemoselective methods for peptide macrocyclization remains a significant challenge. Here, we report a tyrosine-selective strategy for the synthesis of Sulfonate-Tyrosine Ester Macrocyclic peptides (STEMtides) in aqueous buffer under mild conditions. This method leverages sulfur fluoride exchange (SuFEx) chemistry to engage the phenolic side chain of tyrosine residues with sulfonyl fluorides, achieving efficient and chemoselective cyclization without the need for additional reagents. The approach is highly tolerant of native side chain functionality and enables access to cyclic peptides ranging from 2 to 13 residues in length. To demonstrate the scope and translational potential of the method, STEMtide analogs of several clinically relevant peptides, including leuprorelin, β-MSH, liraglutide, and cilengitide, a cyclic RGD peptidomimetic, were successfully synthesized in high yield using the SuFEx-mediated strategy. RGD STEMtide analogs exhibited low toxicity to MCF-7 cells, as well as potent inhibition of cell adhesion comparable to cilengitide itself, highlighting the therapeutic potential of this new class of peptide macrocycles.

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Introduction

Peptides possess large surface areas and can closely mimic structural features of protein surfaces, making them ideal candidates to modulate protein function and disrupt protein–protein interactions.[1,2] Despite this potential, the clinical utility of peptides is often limited by poor proteolytic stability, low membrane permeability, and rapid *in vivo* clearance.[3] Cyclization of linear peptides offers a powerful strategy to mitigate these poor pharmacological properties. Constraining a peptide's conformation through macrocyclization can improve resistance to enzymatic degradation, increase binding affinity and specificity, and reduce entropic penalties for target engagement.[4–9] Owing to these advantages, over 40 cyclic peptide drugs have gained FDA approval in the past two decades, accounting for two-thirds of all new peptide therapeutics during that time.[10]

The development of general, selective, and operationally simple methods for peptide cyclization remains a formidable challenge. Peptide macrocyclization must overcome both entropic and enthalpic barriers. Long linear sequences suffer from conformational flexibility that entropically disfavors intramolecular reaction, while short sequences risk increased ring strain.[11] Intermolecular reactions often compete with desired macrocyclization, leading to undesired oligomerization. The inherent chemical heterogeneity of peptides, rich in functional group diversity, further complicates selective transformation. Additionally, peptides intended for therapeutic applications are often soluble in water but not organic solvents, preventing the use of harsh or water sensitive reagents. For these reasons, few orthogonal methods exist for chemoselective peptide macrocyclization, limiting the chemical diversity of macrocyclic peptides.

Common orthogonal strategies for peptide cyclization include lactamization, azide–alkyne cycloaddition, olefin ring-closing metathesis, and thiol-based nucleophilic substitution (Fig. 1a). These methods have proven powerful in many contexts. However, the increasing complexity of next-generation peptide therapeutics demands a broader repertoire of selective and mutually compatible cyclization strategies. A compelling example is Merck compound 44, a tricyclic peptide inhibitor of PCSK9 developed as a potential treatment for hypercholesterolemia (Fig. 1b).[12,13] The synthesis of this molecule required the sequential application of four distinct peptide cyclization strategies. As peptide therapeutics continue to evolve toward more structurally complex and polycyclic architectures, the development of new, chemoselective macrocyclization methods will be critical to fully realize their therapeutic potential.

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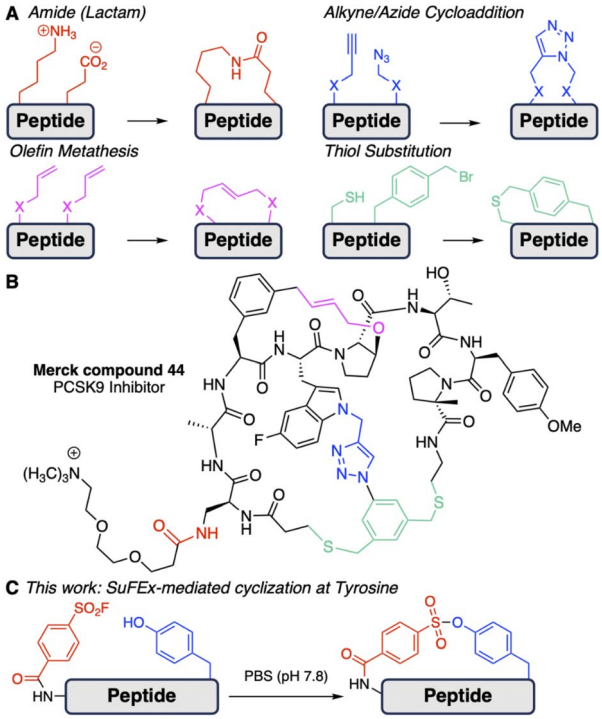

Fig. 1 (A) Common orthogonal strategies for peptide macrocyclization. (B) Merck compound 44, a tricyclic peptide inhibitor of PCSK9, which requires sequential application of the four orthogonal strategies. (C) Our orthogonal SuFEx-mediated method for peptide cyclization at tyrosine.

While many cyclization strategies have focused on highly nucleophilic residues, such as cysteine and lysine,[4–6] strategies for selective cyclization at tyrosine remain comparatively underdeveloped. Notable tyrosine-mediated methods include a 1,3,5-triazine-based bis-tyrosine cyclization,[14] a urazole-derived triazolinedione strategy,[15] and an enzymatic tyrosinase biocatalytic approach.[16] These methods, however, often exhibit limited functional group tolerance or reduced yields in the presence of reactive side chains such as cysteine, lysine, or arginine. Advancements in tyrosine-selective cyclization strategies would expand the ability to construct conformationally constrained and chemically diverse therapeutic peptides.

Sulfur fluoride exchange (SuFEx) chemistry offers a promising approach for tyrosine-selective peptide cyclization. Introduced as a next-generation click reaction by Sharpless and coworkers, SuFEx reactions proceed with high efficiency under aqueous, aerobic, and biologically compatible conditions.[17] Sulfonyl fluorides, key reagents in SuFEx reactions, exhibit significant resistance to hydrolysis and reduction, yet remain reactive toward nucleophiles under mild conditions.[18] This unique reactivity has enabled widespread use in chemical biology, drug discovery, and materials science.[19–28]

Here, we report a SuFEx-mediated peptide cyclization strategy that leverages the mild electrophilicity of sulfonyl fluorides to chemoselectively target the phenolic side chain of tyrosine. This method enables the efficient formation of Sulfonate-Tyrosine Ester Macrocyclic peptides (STEMtides) across a broad range of sequence lengths and peptide scaffolds, with excellent chemoselectivity, functional group tolerance, and operational simplicity. Our approach offers a robust and orthogonal platform for the synthesis of a new class of conformationally constrained peptide therapeutics.

Results and discussion

Optimization of reaction conditions

We initiated the development of a SuFEx-mediated peptide macrocyclization strategy using 4FSB-GAGY (**1a**), a model tetrapeptide bearing an N-terminal 4-(fluorosulfonyl)benzoyl (4FSB) cap and a C-terminal tyrosine residue (Fig. 2a). A range of conditions were evaluated for their ability to promote intramolecular cyclization of the linear precursor. Initial screens employing base additives, such as TEA, DIEA and NaOH, in mixed aqueous/organic media yielded rapid conversion (<5 min) but low to modest conversion yields (0–40%) of the desired sulfonate-linked macrocycle **2a** (Fig. S1). These conditions also resulted in substantial formation of side products, such as hydrolysis of the sulfonyl fluoride.

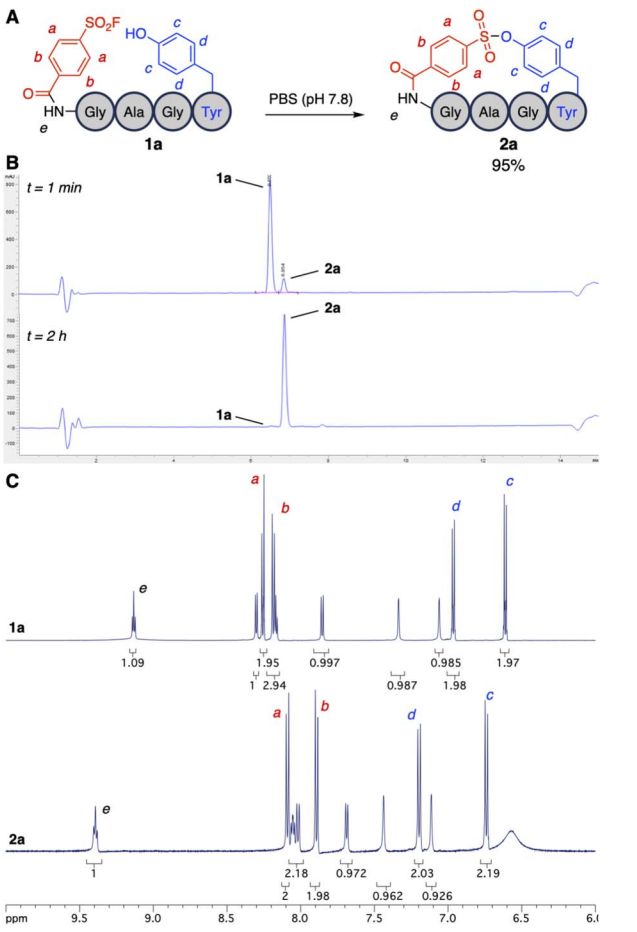

Fig. 2 (A) SuFex-mediated peptide cyclization of **1a** in mildly basic buffer. (B) Crude HPLC chromatograms highlight efficient conversion of SuFEx-mediated cyclization with minimal side products. (C) ^{1}H-NMR spectra of linear **1a** and cyclized **2a** peptides.

Recognizing the relatively low pK_a of the tyrosine phenol, we hypothesized that selective activation under mildly basic aqueous conditions could favor intramolecular reaction of the phenolic nucleophile while suppressing undesired hydrolysis. Buffer systems across a pH range of 6.5–8.0 were evaluated. Optimal conditions were identified as phosphate-buffered saline (PBS) at pH 7.8, under which the linear peptide underwent clean and efficient macrocyclization upon simple stirring at ambient temperature for 2 hours, affording **2a** in 95% isolated yield. Reaction progress, monitored by analytical HPLC, confirmed full conversion of the linear peptide and minimal byproduct formation (Fig. 2b). Both linear and cyclic peptides

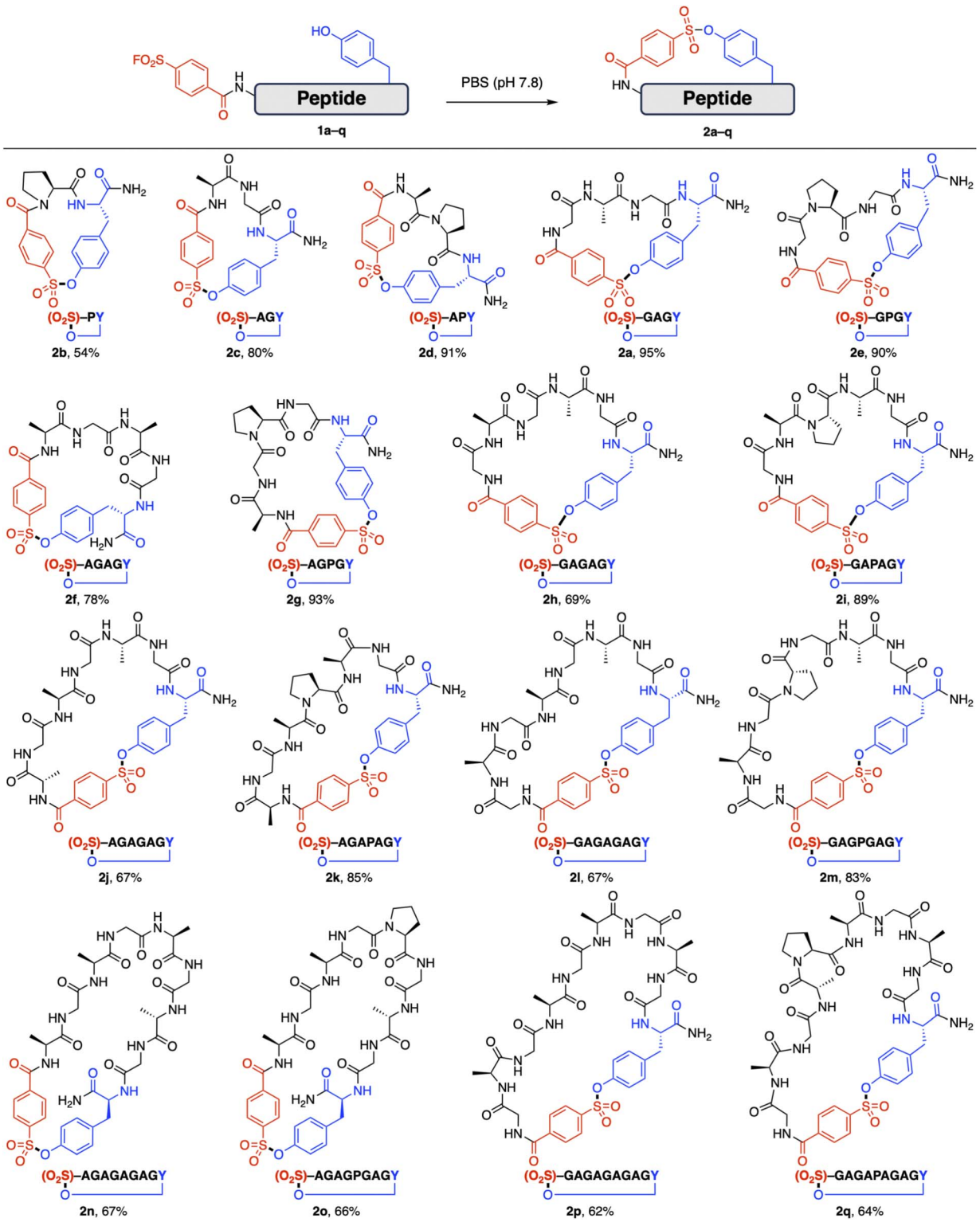

Fig. 3 Effect of sequence length on SuFEx-mediated peptide cyclization. Yields shown are isolated yields following purification by RP-HPLC.

were characterized by ^{1}H-NMR spectroscopy, verifying successful formation of the sulfonate-linked macrocycle (Fig. 2c).

Effect of sequence length on peptide cyclization

The propensity of a peptide to undergo cyclization is highly dependent on the number of residues separating the reactive functional groups.[11] When the intervening sequence is too short, limited conformational flexibility and backbone rigidity can prevent proper alignment of the reactive groups, often resulting in substantial ring strain due to unfavorable bond and torsional angles. Conversely, an excessive number of residues increases conformational freedom, making it less likely for the peptide to adopt the specific geometry required for cyclization without incurring significant entropic penalties.

To investigate the influence of sequence length on the efficiency of SuFEx-mediated cyclization, a series of linear peptides containing 2–10 residues was synthesized, each featuring a C-terminal tyrosine and N-terminal 4FSB cap. Cyclization was initiated by dissolving the peptides in mildly basic aqueous buffer (PBS, pH 7.8). All sequences (**1a–q**) underwent efficient macrocyclization to generate STEMtide analogs **2a–q** (Fig. 3). A plot of isolated yields as a function of sequence length revealed an optimal cyclization range of 3–5 residues, with yields gradually declining for shorter or longer sequences (Fig. S6). Notably, the presence of proline generally resulted in enhanced cyclization efficiency, an effect most pronounced in peptides 5–8 residues in length (Fig. S7). The cyclic side chain of proline restricts its φ dihedral angle, introducing a conformational kink in the peptide backbone that could potentially decrease the entropic cost of cyclization. In shorter sequences, proline can exacerbate ring strain due its rigidity. For example, the two-residue peptide **2b**, exhibited the slowest conversion and lowest isolated yield (54%), likely due to ring strain imposed by the compact macrocyclic structure. This hypothesis was supported by X-ray crystallographic analysis, which revealed significant distortion of aromatic bond angles, with both the aryl sulfonate and tyrosine side chain deviating up to 10° from planarity (Fig. S8).

Chemoselectivity of peptide cyclization at tyrosine

To evaluate the reactivity of alternative nucleophilic side chains under these conditions, a series of 4FSB-GAGX peptides (X = K, H, S, T, or C) was synthesized and incubated in mildly basic aqueous buffer (PBS, pH 7.8). No cyclization was observed for the histidine, serine, threonine, or cysteine analogs, indicating that these nucleophiles are unreactive toward SuFEx-mediated cyclization

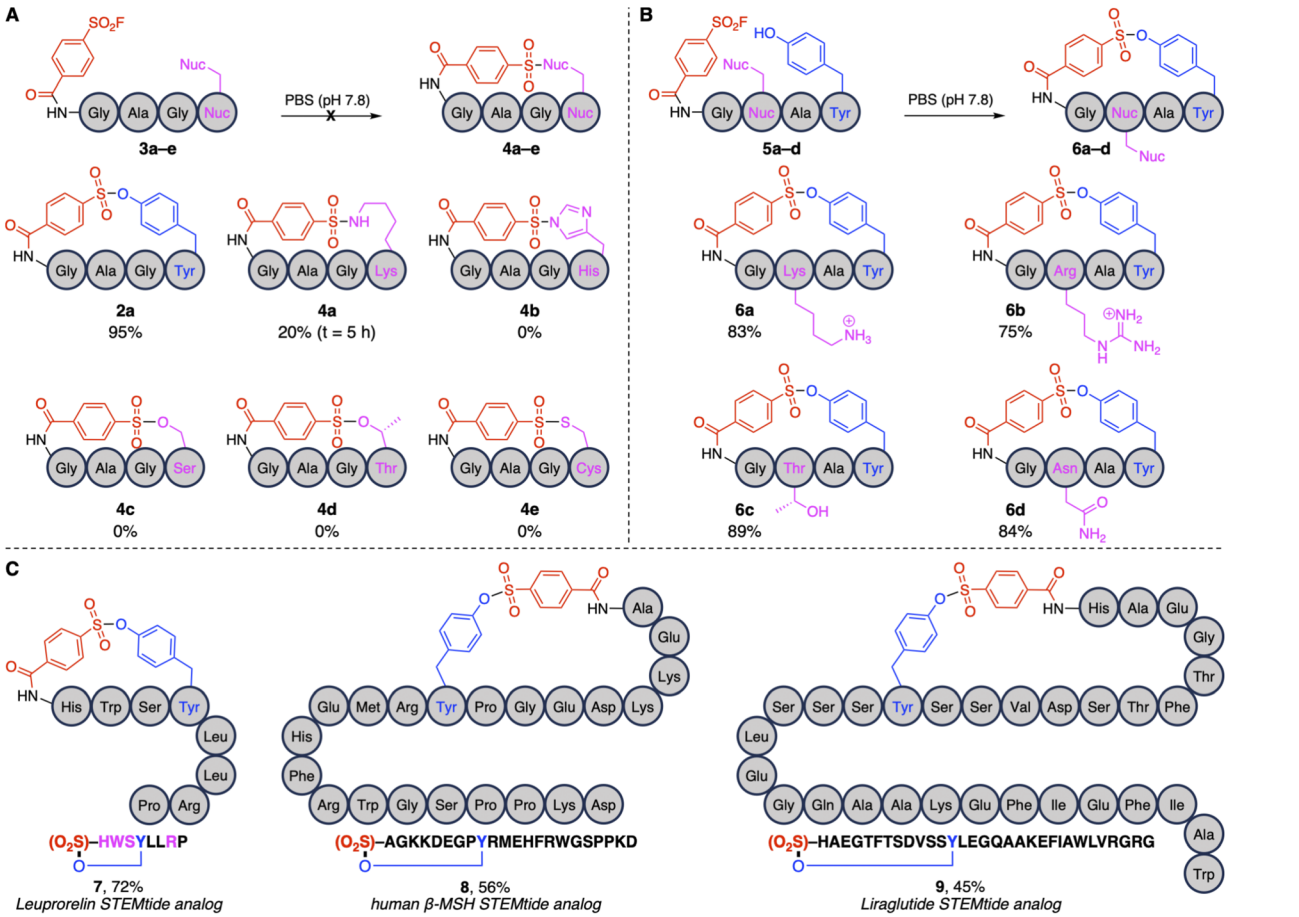

Fig. 4 (A) SuFEx-mediated peptide cyclization is effective with tyrosine residues but ineffective with other nucleophilic amino acid residues. (B) SuFEx-mediated peptide cyclization occurs chemoselectively at tyrosine. (C) Synthesis of cyclic STEMtide analogs of clinically relevant peptides.

under the tested conditions (Fig. 4a). The lysine-containing peptide underwent slow conversion, yielding the corresponding sulfonamide-linked macrocycle **4a** in 20% isolated yield after 5 hours. The stark reduction in both rate and yield for cyclization at lysine, combined with the lack of reactivity at histidine, serine, threonine, and cysteine, highlight the chemoselective potential for tyrosine-mediated cyclization.

The chemoselectivity of the SuFEx-mediated cyclization method for tyrosine was further explored in a series of 4FSB-GXGY (X = K, T, R, or N) peptides. Each analog featured a C-terminal tyrosine residue and a residue with a potentially reactive side chain (*i.e.*, primary amine, aliphatic alcohol, guanidinium, primary amide). Incubation of these linear peptides in mildly basic aqueous buffer (PBS, pH 7.8) resulted in tyrosine-selective cyclization to **6a–d** with isolated yields of 75–89% (Fig. 4b). HPLC chromatograms of the crude reaction mixtures show clean conversion of each linear peptide to the corresponding cyclic STEMtide with few other new peaks visible, underscoring the chemoselectivity of the method. For peptide **6a**, which possesses both tyrosine and lysine residues, NMR experiments were conducted to provide additional evidence of chemoselective Tyr cyclization (Fig. S9).

Application to clinically relevant peptides

Thus far, we have employed model peptide sequences to systematically demonstrate the broad sequence-length tolerance and tyrosine selectivity of our SuFEx-mediated cyclization strategy. To further validate the method and assess its practical utility, we next applied it to the cyclization of clinically relevant bioactive peptides: leuprorelin, β-melanocyte stimulating hormone (β-MSH), and liraglutide (Fig. 4c). Leuprorelin (leuprolide) is a nonapeptide analog of gonadotropin-releasing hormone (GnRH) that functions as a partial agonist of the GnRH receptor.[29–31] Although it initially stimulates testosterone and estrogen secretion, sustained activation leads to suppression of these hormones, enabling its clinical use in prostate cancer therapy and gender-affirming therapy. β-MSH is a 22-residue neuropeptide secreted by melanocytes and keratinocytes in response to ultraviolet exposure. It activates melanocortin receptors (notably MC4R) to promote melanogenesis, while also regulating energy balance and food intake.[32–34] Liraglutide, a 31-residue synthetic analog of glucagon-like peptide-1 (GLP-1), acts as a GLP-1 receptor agonist to stimulate insulin secretion and suppress appetite, and is widely used for the treatment of type 2 diabetes and obesity.[35–37] Each of these

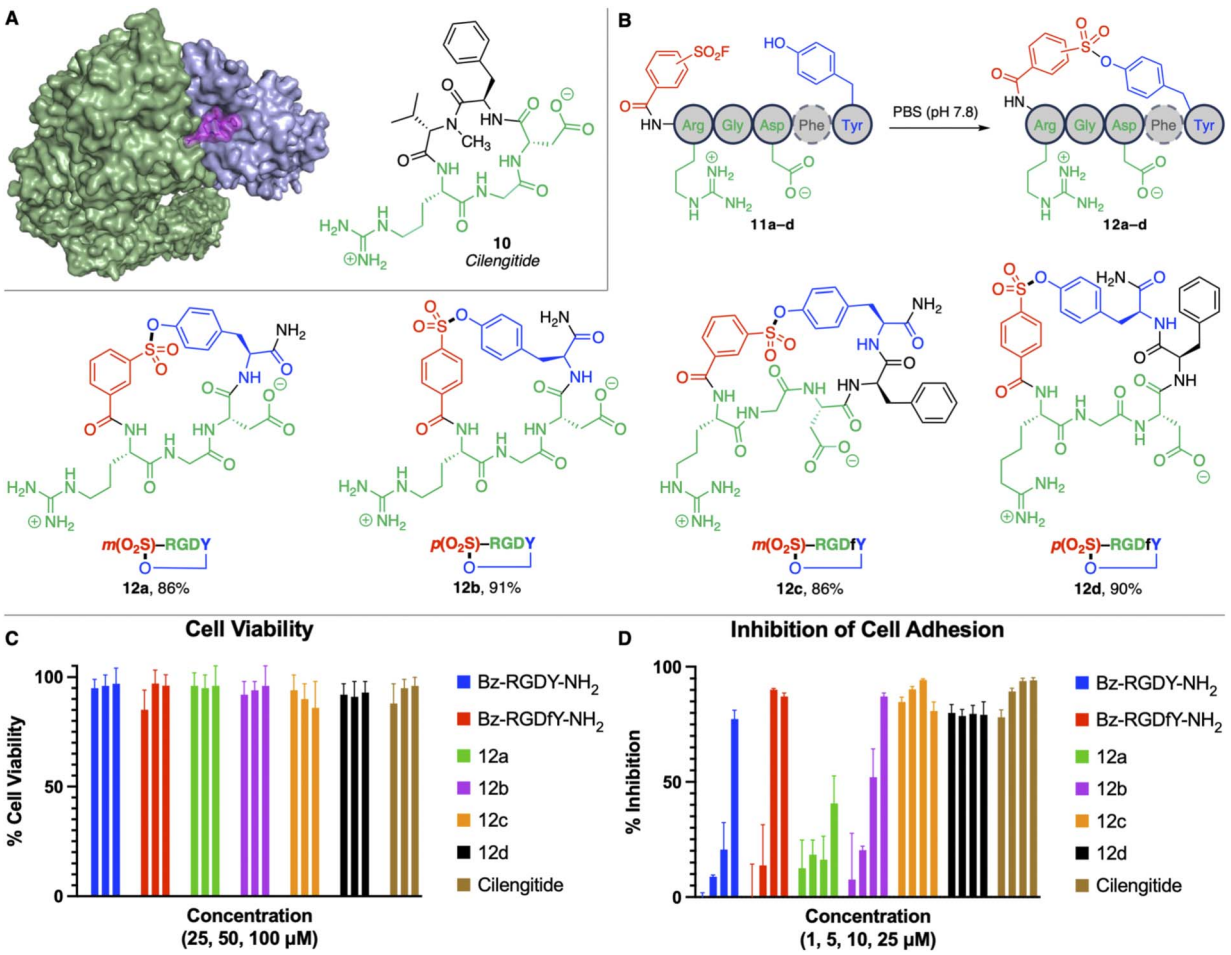

Fig. 5 (A) Cryo-EM structure of cyclic RGD peptide cilengitide (magenta) bound to integrin $\alpha_v\beta_3$ (green/blue, PDB 8XER). (B) Synthesis of RGD STEMtide analogs. (C) MCF-7 cell viability in the presence of linear and STEMtide RGD analogs. (D) Inhibition of cell adhesion in MCF-7 cells by linear and STEMtide RGD analogs.

peptides contains a single tyrosine residue along with multiple other potentially nucleophilic and reactive side chains, making them ideal candidates for assessing the chemoselectivity and generality of SuFEx-mediated macrocyclization in the context of complex peptide sequences.

Linear analogs of leuprorelin, β-MSH, and liraglutide were synthesized with an N-terminal 4FSB group to enable SuFEx-mediated macrocyclization. For leuprorelin, the 4FSB cap replaced the native N-terminal pyroglutamate. Upon incubation in PBS buffer (pH 7.8), all three peptides underwent efficient cyclization to yield the corresponding STEMtide analogs leuprorelin **7** (72% yield), β-MSH **8** (56% yield), and liraglutide **9** (45% yield). In each case, cyclization occurred exclusively at the tyrosine residue, with no evidence of reaction at other potentially nucleophilic side chains, underscoring the high chemoselectivity of the method. The number of residues separating the 4FSB cap and the tyrosine side chain varied substantially, including four residues in leuprorelin, nine residues in β-MSH, and thirteen residues in liraglutide, demonstrating the broad sequence-length tolerance of the SuFEx-based macrocyclization strategy.

Bioactivity of RGD STEMtide analogs

Cell-surface integrins mediate adhesion to the extracellular matrix through recognition of a conserved Arg-Gly-Asp (RGD) motif.[38] The $\alpha_v\beta_3$ isoform plays a central role in the regulation of cellular processes, including cell migration, angiogenesis, wound healing, and inflammatory responses.[38] Notably, integrin $\alpha_v\beta_3$ is often overexpressed in tumor cells and has been implicated in cancer progression and metastasis, making it an attractive target for anticancer therapeutics.[39] Cilengitide **10**, a synthetic cyclic RGD-containing peptide (Fig. 5a), has been clinically investigated as an $\alpha_v\beta_3$ inhibitor to disrupt integrin-mediated adhesion and induce apoptosis in cancer cells.[40–45]

To explore the utility of SuFEx-mediated macrocyclization for generating bioactive molecules, we synthesized a small set of cyclic RGD-containing STEMtide analogs as potential integrin antagonists (Fig. 5b). The library consisted of four- and five-residue peptides featuring the minimal RGD motif, with variants either retaining or omitting the D-phenylalanine residue found in cilengitide. To probe the impact of linker geometry on conformation and potential isoform selectivity, cyclization was performed using either *meta*- or *para*-substituted sulfonyl fluorides. Upon incubation in PBS (pH 7.8), linear precursors underwent efficient cyclization to the corresponding RGD-STEMtide analogs (**12a–d**) in 86–91% yield, with no major side products detected, highlighting the robustness of the SuFEx-mediated approach. Linear peptides bearing N-terminal benzoyl caps (Bz-RGDY-NH_2 and Bz-RGDfY-NH_2) were also synthesized as controls to examine the impact of cyclization on inhibitory activity.

The RGD STEMtide analogs, as well as linear and cilengitide controls, were evaluated for cytotoxicity and cell adhesion inhibition in human MCF-7 breast cancer cells. No significant cytotoxicity was observed at concentrations up to 100 μM following 48-hour exposure (Fig. 5c), indicating the analogs are well-tolerated.

To assess their functional activity, RGD STEMtide analogs, as well as linear and cilengitide controls, were tested for inhibition of cell adhesion at 1, 5, 10, and 25 μM concentrations (Fig. 5d). All peptides demonstrated dose-dependent inhibition, with STEMtide analogs generally exhibiting more effective inhibition relative to their linear counterparts (*e.g.*, **12b** *vs.* Bz-RGDY-NH_2 or **12c,d** *vs.* Bz-RGDfY-NH_2). Both linear and cyclic peptides possessing an additional D-phenylalanine residue (*e.g.*, Bz-RGDfY-NH_2, **12c**, **12d**, cilengitide) demonstrated enhanced inhibition compared to peptides lacking this residue (*e.g.*, Bz-RGDY-NH_2, **12a**, **12b**). Of particular significance, STEMtides **12c** and **12d** exhibited similar inhibitory activity to that of cilengitide, a clinical candidate being explored as a potential anticancer therapeutic. Notably, the extent of inhibition among the STEMtide analogs varied across the series, indicating that small structural differences result in distinct activity profiles. These findings highlight how subtle modifications to peptide sequence or macrocyclic constraints can influence conformation and, consequently, biological activity.

Enhanced stability to hydrolytic and enzymatic degradation

Given the potential susceptibility of sulfonate ester linkages to hydrolysis, the hydrolytic stability of STEMtide analog **2a** was evaluated under acidic (pH 4) and basic (pH 9) conditions at elevated temperatures (40 °C and 65 °C). At pH 4 (40 °C or 65 °C) and pH 9 (40 °C), no significant degradation was observed over

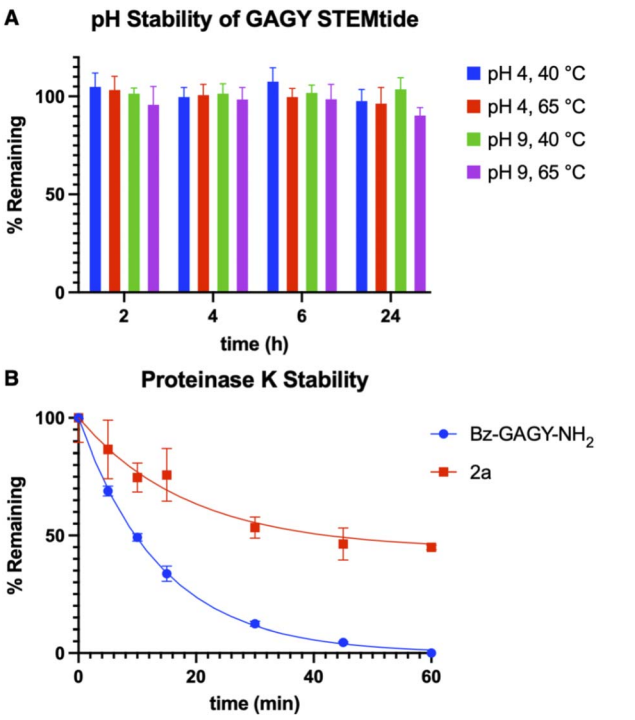

Fig. 6 Stability of cyclic STEMtides to hydrolytic and enzymatic degradation. (A) STEMtide **2a** incubated in acidic (pH 4) or basic (pH 9) buffer at 40 °C or 65 °C demonstrates stability to hydrolysis. (B) STEMtide analog **2a** exhibits enhanced resistance to proteolytic degradation by proteinase K compared to linear peptide control Bz-GAGY-NH_2.

24 h (Fig. 6a). Even under harsher basic conditions (pH 9, 65 °C), 90% (±3%) of the peptide remained intact after 24 h, demonstrating the excellent chemical stability of the sulfonate ester linkage to both acidic and basic conditions.

A primary motivation of peptide cyclization is the enhanced resistance to proteolytic degradation that limits the therapeutic utility of linear peptide sequences. To assess the effect of SuFEx-mediated peptide cyclization on proteolytic susceptibility, STEMtide **2a** and linear peptide control Bz-GAGY-NH_2 were incubated with proteinase K, a promiscuous serine protease. Although these assays may not accurately model the biological environment, they have been used previously as general tools to evaluate non-specific proteolysis and to detect amide bonds prone to enzymatic cleavage.[46–49] The linear peptide underwent rapid degradation with a half-life of 10 minutes (Fig. 6b). The cyclic STEMtide analog exhibited enhanced resistance to proteolysis, with over 45% of the peptide remaining after 60 minutes. These results demonstrate that SuFEx-mediated cyclization enhances proteolytic stability, supporting its utility in the development of metabolically stable peptide therapeutics.

Conclusions

In summary, we have developed a robust and chemoselective method for peptide macrocyclization based on SuFEx chemistry that selectively targets tyrosine residues under mild, aqueous conditions. This tyrosine-directed strategy enables the efficient synthesis of Sulfonate-Tyrosine Ester Macrocyclic peptides (STEMtides) across a broad range of sequence lengths and structural motifs, with high tolerance for diverse amino acid side chains. The method proceeds without the need for metal catalysts or organic cosolvents and exhibits exclusive reactivity toward tyrosine over other nucleophilic residues. The generality and practical utility of the approach were demonstrated through the successful cyclization of clinically relevant peptides, including leuprorelin, β-MSH, liraglutide, and cyclic RGD analogs derived from cilengitide. RGD STEMtides retained favorable bioactivity profiles, including potent dose-dependent inhibition of cell adhesion with minimal cytotoxicity. Together, these findings establish SuFEx-mediated tyrosine cyclization as a powerful platform for the streamlined synthesis of conformationally constrained peptide therapeutics. We are currently pursuing the design, synthesis, and evaluation of STEMtides as chemical tools to study and potential therapeutics to modulate biological processes.

Author contributions

H. S. and V. K. O conceived the project, H. S. and H. H. V. carried out the experimental work. V. K. O. supervised the work. V. K. O. wrote the manuscript. All authors contributed to editing the manuscript.

Conflicts of interest

There are no conflicts to declare.

Data availability

CCDC 2472077 contains the supplementary crystallographic data for this paper.[50]

Experimental procedures, characterization data, and X-ray data can be found in the supplementary information (SI). Supplementary information is available. See DOI: https://doi.org/10.1039/d5sc06993a.

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References

1 T. A. Cardote and A. Ciulli, Cyclic and Macrocyclic Peptides as Chemical Tools To Recognise Protein Surfaces and Probe Protein-Protein Interactions, *ChemMedChem*, 2016, **11**, 787–794, DOI: 10.1002/cmdc.201500450.
2 G. J. B. Philippe, D. J. Craik and S. T. Henriques, Converting peptides into drugs targeting intracellular protein–protein interactions, *Drug Discovery Today*, 2021, **26**, 1521–1531.
3 K. Fosgerau and T. Hoffmann, Peptide therapeutics: current status and future directions, *Drug Discovery Today*, 2015, **20**, 122–128.
4 C. J. White and A. K. Yudin, Contemporary strategies for peptide macrocyclization, *Nat. Chem.*, 2011, **3**, 509–524, DOI: 10.1038/nchem.1062.
5 Y. H. Lau, P. de Andrade, Y. Wu and D. R. Spring, Peptide stapling techniques based on different macrocyclisation chemistries, *Chem. Soc. Rev.*, 2015, **44**, 91–102, DOI: 10.1039/c4cs00246f.
6 C. Bechtler and C. Lamers, Macrocyclization strategies for cyclic peptides and peptidomimetics, *RSC Med. Chem.*, 2021, **12**, 1325–1351, DOI: 10.1039/d1md00083g.
7 M. A. Abdalla and L. J. McGaw, Natural Cyclic Peptides as an Attractive Modality for Therapeutics: A Mini Review, *Molecules*, 2018, **23**, 2080, DOI: 10.3390/molecules23082080.
8 L. Reguera and D. G. Rivera, Multicomponent Reaction Toolbox for Peptide Macrocyclization and Stapling, *Chem. Rev.*, 2019, **119**, 9836–9860, DOI: 10.1021/acs.chemrev.8b00744.
9 X. Ji, A. L. Nielsen and C. Heinis, Cyclic Peptides for Drug Development, *Angew Chem. Int. Ed. Engl.*, 2024, **63**, e202308251, DOI: 10.1002/anie.202308251.
10 L. Wang, N. Wang, W. Zhang, X. Cheng, Z. Yan, G. Shao, X. Wang, R. Wang and C. Fu, Therapeutic peptides: current applications and future directions, *Signal Transduct. Targeted Ther.*, 2022, **7**, 48, DOI: 10.1038/s41392-022-00904-4.

11 A. K. Yudin, Macrocycles: lessons from the distant past, recent developments, and future directions, *Chem. Sci.*, 2015, **6**, 30–49, DOI: 10.1039/c4sc03089c.
12 C. Alleyne, R. P. Amin, B. Bhatt, E. Bianchi, J. C. Blain, N. Boyer, D. Branca, M. W. Embrey, S. N. Ha, K. Jette, D. G. Johns, A. D. Kerekes, K. A. Koeplinger, D. LaPlaca, N. Li, B. Murphy, P. Orth, A. Ricardo, S. Salowe, K. Seyb, A. Shahripour, J. R. Stringer, Y. Sun, R. Tracy, C. Wu, Y. Xiong, H. Youm, H. J. Zokian and T. J. Tucker, Series of Novel and Highly Potent Cyclic Peptide PCSK9 Inhibitors Derived from an mRNA Display Screen and Optimized via Structure-Based Design, *J. Med. Chem.*, 2020, **63**, 13796–13824, DOI: 10.1021/acs.jmedchem.0c01084.
13 T. J. Tucker, M. W. Embrey, C. Alleyne, R. P. Amin, A. Bass, B. Bhatt, E. Bianchi, D. Branca, T. Bueters, N. Buist, S. N. Ha, M. Hafey, H. He, J. Higgins, D. G. Johns, A. D. Kerekes, K. A. Koeplinger, J. T. Kuethe, N. Li, B. Murphy, P. Orth, S. Salowe, A. Shahripour, R. Tracy, W. Wang, C. Wu, Y. Xiong, H. J. Zokian, H. B. Wood and A. Walji, A Series of Novel, Highly Potent, and Orally Bioavailable Next-Generation Tricyclic Peptide PCSK9 Inhibitors, *J. Med. Chem.*, 2021, **64**, 16770–16800, DOI: 10.1021/acs.jmedchem.1c01599.
14 Y. Zhang, R. Yin, H. Jiang, C. Wang, X. Wang, D. Wang, K. Zhang, R. Yu, X. Li and T. Jiang, Peptide Stapling through Site-Directed Conjugation of Triazine Moieties to the Tyrosine Residues of a Peptide, *Org. Lett.*, 2023, **25**, 2248–2252, DOI: 10.1021/acs.orglett.3c00499.
15 E. D. Keyes, M. C. Mifflin, M. J. Austin, B. J. Alvey, L. H. Lovely, A. Smith, T. E. Rose, B. A. Buck-Koehntop, J. Motwani and A. G. Roberts, Chemoselective, Oxidation-Induced Macrocyclization of Tyrosine-Containing Peptides, *J. Am. Chem. Soc.*, 2023, **145**, 10071–10081, DOI: 10.1021/jacs.3c00210.
16 M. C. Fleming, M. M. Bowler, R. Park, K. I. Popov and A. A. Bowers, Tyrosinase-Catalyzed Peptide Macrocyclization for mRNA Display, *J. Am. Chem. Soc.*, 2023, **145**, 10445–10450, DOI: 10.1021/jacs.2c12629.
17 J. Dong, L. Krasnova, M. G. Finn and K. B. Sharpless, Sulfur(VI) fluoride exchange (SuFEx): another good reaction for click chemistry, *Angew Chem. Int. Ed. Engl.*, 2014, **53**, 9430–9448, DOI: 10.1002/anie.201309399.
18 A. Narayanan and L. H. Jones, Sulfonyl fluorides as privileged warheads in chemical biology, *Chem. Sci.*, 2015, **6**, 2650–2659, DOI: 10.1039/c5sc00408j.
19 Y. You, H. S. Kim, I. H. Bae, S. G. Lee, M. H. Jee, G. Keum, S. K. Jang and B. M. Kim, New potent biaryl sulfate-based hepatitis C virus inhibitors, *Eur. J. Med. Chem.*, 2017, **125**, 87–100, DOI: 10.1016/j.ejmech.2016.09.031.
20 Q. Zhao, X. Ouyang, X. Wan, K. S. Gajiwala, J. C. Kath, L. H. Jones, A. L. Burlingame and J. Taunton, Broad-Spectrum Kinase Profiling in Live Cells with Lysine-Targeted Sulfonyl Fluoride Probes, *J. Am. Chem. Soc.*, 2017, **139**, 680–685, DOI: 10.1021/jacs.6b08536.
21 B. Gao, L. Zhang, Q. Zheng, F. Zhou, L. M. Klivansky, J. Lu, Y. Liu, J. Dong, P. Wu and K. B. Sharpless, Bifluoride-catalysed sulfur(VI) fluoride exchange reaction for the synthesis of polysulfates and polysulfonates, *Nat. Chem.*, 2017, **9**, 1083–1088, DOI: 10.1038/nchem.2796.
22 Z. Liu, J. Li, S. Li, G. Li, K. B. Sharpless and P. Wu, SuFEx Click Chemistry Enabled Late-Stage Drug Functionalization, *J. Am. Chem. Soc.*, 2018, **140**, 2919–2925, DOI: 10.1021/jacs.7b12788.
23 N. Wang, B. Yang, C. Fu, H. Zhu, F. Zheng, T. Kobayashi, J. Liu, S. Li, C. Ma, P. G. Wang, Q. Wang and L. Wang, Genetically Encoding Fluorosulfate-l-tyrosine To React with Lysine, Histidine, and Tyrosine via SuFEx in Proteins in Vivo, *J. Am. Chem. Soc.*, 2018, **140**, 4995–4999, DOI: 10.1021/jacs.8b01087.
24 D. E. Mortenson, G. J. Brighty, L. Plate, G. Bare, W. Chen, S. Li, H. Wang, B. F. Cravatt, S. Forli, E. T. Powers, K. B. Sharpless, I. A. Wilson and J. W. Kelly, "Inverse Drug Discovery" Strategy To Identify Proteins That Are Targeted by Latent Electrophiles As Exemplified by Aryl Fluorosulfates, *J. Am. Chem. Soc.*, 2018, **140**, 200–210, DOI: 10.1021/jacs.7b08366.
25 A. S. Barrow, C. J. Smedley, Q. Zheng, S. Li, J. Dong and J. E. Moses, The growing applications of SuFEx click chemistry, *Chem. Soc. Rev.*, 2019, **48**, 4731–4758, DOI: 10.1039/c8cs00960k.
26 P. Martín-Gago and C. A. Olsen, Arylfluorosulfate-Based Electrophiles for Covalent Protein Labeling: A New Addition to the Arsenal, *Angew Chem. Int. Ed. Engl.*, 2019, **58**, 957–966, DOI: 10.1002/anie.201806037.
27 B. Y. Li, L. Voets, R. Van Lommel, F. Hoppenbrouwers, M. Alonso, S. H. L. Verhelst, W. M. De Borggraeve and J. Demaerel, SuFEx-enabled, chemoselective synthesis of triflates, triflamides and triflimidates, *Chem. Sci.*, 2022, **13**, 2270–2279, DOI: 10.1039/d1sc06267k.
28 B. Yang, H. Wu, P. D. Schnier, Y. Liu, J. Liu, N. Wang, W. F. DeGrado and L. Wang, Proximity-enhanced SuFEx chemical cross-linker for specific and multitargeting cross-linking mass spectrometry, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, 11162–11167, DOI: 10.1073/pnas.1813574115.
29 G. L. Plosker and R. N. Brogden, Leuprorelin: a review of its pharmacology and therapeutic use in prostatic cancer, endometriosis and other sex hormone-related disorders, *Drugs*, 1994, **48**, 930–967.
30 F. Haviv, T. D. Fitzpatrick, R. E. Swenson, C. J. Nichols, N. A. Mort, E. N. Bush, G. Diaz, G. Bammert and A. Nguyen, Effect of N-methyl substitution of the peptide bonds in luteinizing hormone-releasing hormone agonists, *J. Med. Chem.*, 1993, **36**, 363–369.
31 Z. Abouelfadel and E. D. Crawford, Leuprorelin depot injection: patient considerations in the management of prostatic cancer, *Therapeut. Clin. Risk Manag.*, 2008, **4**, 513–526.
32 R. D. Cone, D. Lu, S. Koppula, D. I. Vage, H. Klungland, B. Boston, W. Chen, D. N. Orth, C. Pouton and R. A. Kesterson, The melanocortin receptors: agonists, antagonists, and the hormonal control of pigmentation, *Recent Progress in Hormone Research*, 1996, 51, 287–317.
33 Y. S. Lee, B. G. Challis, D. A. Thompson, G. S. H. Yeo, J. M. Keogh, M. E. Madonna, V. Wraight, M. Sims, V. Vatin

and D. A. Meyre, POMC variant implicates β-melanocyte-stimulating hormone in the control of human energy balance, *Cell Metab.*, 2006, **3**, 135–140.

34 H. Biebermann, T. R. Castañeda, F. van Landeghem, A. von Deimling, F. Escher, G. Brabant, J. Hebebrand, A. Hinney, M. H. Tschöp and A. Grüters, A role for β-melanocyte-stimulating hormone in human body-weight regulation, *Cell Metab.*, 2006, **3**, 141–146.

35 S. P. Marso, G. H. Daniels, K. Brown-Frandsen, P. Kristensen, J. F. E. Mann, M. A. Nauck, S. E. Nissen, S. Pocock, N. R. Poulter and L. S. Ravn, Liraglutide and cardiovascular outcomes in type 2 diabetes, *N. Engl. J. Med.*, 2016, **375**, 311–322.

36 L. B. Knudsen and J. Lau, The discovery and development of liraglutide and semaglutide, *Front. Endocrinol.*, 2019, **10**, 155.

37 M. J. Davies, R. Bergenstal, B. Bode, R. F. Kushner, A. Lewin, T. V. Skjøth, A. H. Andreasen, C. B. Jensen, R. A. DeFronzo and NN8022-1922 Study Group, Efficacy of liraglutide for weight loss among patients with type 2 diabetes: the SCALE diabetes randomized clinical trial, *JAMA*, 2015, **314**, 687–699.

38 E. Ruoslahti and M. D. Pierschbacher, New perspectives in cell adhesion: RGD and integrins, *Science*, 1987, **238**, 491–497.

39 C. J. Avraamides, B. Garmy-Susini and J. A. Varner, Integrins in Angiogenesis and Lymphangiogenesis, *Nat. Rev. Cancer*, 2008, **8**, 604–617.

40 M. A. Dechantsreiter, E. Planker, B. Mätha, E. Lohof, A. Jonczyk, S. L. Goodman and H. Kessler, N-Methylated Cyclic RGD Peptides as Highly Active and Selective αVβ3 Integrin Antagonists, *J. Med. Chem.*, 1999, **42**, 3033–3040.

41 C. Mas-Moruno, F. Rechenmacher and H. Kessler, Cilengitide: The First Anti-Angiogenic Small Molecule Drug Candidate. Design, Synthesis and Clinical Evaluation, *Anti-Cancer Agents Med. Chem.*, 2010, **10**, 753–768.

42 D. A. Reardon, B. Neyns, M. Weller, J. C. Tonn, L. B. Nabors and R. Stupp, Cilengitide: An RGD Pentapeptide αvβ3 and αvβ5 Integrin Inhibitor in Development for Glioblastoma and Other Malignancies, *Future Oncol.*, 2011, **7**, 339–354.

43 G. D. Maurer, I. Tritschler, B. Adams, G. Tabatabai, W. Wick, R. Stupp and M. Weller, Cilengitide Modulates Attachment and Viability of Human Glioma Cells, but Not Sensitivity to Irradiation or Temozolomide in Vitro, *Neuro Oncol.*, 2009, **11**, 747–756.

44 F. Rechenmacher, S. Neubauer, J. Polleux, C. Mas-Moruno, M. De Simone, E. A. Cavalcanti-Adam, J. P. Spatz, R. Fässler and H. Kessler, Functionalizing αvβ3- or α5β1-Selective Integrin Antagonists for Surface Coating: A Method to Discriminate Integrin Subtypes in Vitro, *Angew. Chem., Int. Ed.*, 2013, **52**, 1572–1575.

45 A. C. Conibear, S. Chaousis, T. Durek, K. Johan Rosengren, D. J. Craik and C. I. Schroeder, Approaches to the Stabilization of Bioactive Epitopes by Grafting and Peptide Cyclization, *Biopolymers*, 2016, **106**, 89–100.

46 H. S. Haase, K. J. Peterson-Kaufman, S. K. Lan Levengood, J. W. Checco, W. L. Murphy and S. H. Gellman, Extending foldamer design beyond α-helix mimicry: α/β-peptide inhibitors of vascular endothelial growth factor signaling, *J. Am. Chem. Soc.*, 2012, **134**, 7652–7655.

47 W. S. Horne, L. M. Johnson, T. J. Ketas, P. J. Klasse, M. Lu, J. P. Moore and S. H. Gellman, Structural and biological mimicry of protein surface recognition by α/β-peptide foldamers, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 14751–14756.

48 L. M. Johnson, D. E. Mortenson, H. G. Yun, W. S. Horne, T. J. Ketas, M. Lu, J. P. Moore and S. H. Gellman, Enhancement of α-helix mimicry by an α/β-peptide foldamer via incorporation of a dense ionic side-chain array, *J. Am. Chem. Soc.*, 2012, **134**, 7317–7320.

49 V. K. Outlaw, R. W. Cheloha, E. M. Jurgens, F. T. Bovier, Y. Zhu, D. F. Kreitler, O. Harder, S. Niewiesk, M. Porotto, S. H. Gellman and A. Moscona, Engineering Protease-Resistant Peptides to Inhibit Human Parainfluenza Viral Respiratory Infection, *J. Am. Chem. Soc.*, 2021, **143**, 5958–5966, DOI: **10.1021/jacs.1c01565**.

50 CCDC 2472077: Experimental Crystal Structure Determination, 2025, DOI: **10.5517/ccdc.csd.cc2nzdfh**.