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Unlocking the Potential of Retro-Inverso (RI) Peptides as Future Drug Candidates

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Abstract

Background With the rising demand for peptide-based drugs, enhancing their stability against proteolytic degradation has become a critical challenge. Strategies to improve peptide stability include cyclization, substitution of L-amino acids with D-amino acids, incorporation of β-amino acids, and various formulation techniques. An innovative approach involves modifying the peptide backbone by reversing the amide bond direction and inverting the stereochemistry of amino acids in the same segment. This approach results in the formation of retro-inverso peptides, which offer increased stability, permeability, and cellular uptake.

Purpose The aim of this review is to provide a comprehensive analysis of retro-inverso peptides, focusing on their concept, synthesis, and applications as potential therapeutic agents, drug delivery systems, and in aesthetic applications.

Methods The review explores the theoretical underpinnings of retro-inverso peptide design and its application to both linear and cyclic peptides. The synthesis strategies of retro-inverso peptides are discussed in detail, along with their formulation and practical utility in various biomedical fields.

Results Retro-inverso peptides show promise in enhancing peptide stability and improving biological properties such as permeability and cellular uptake. Their unique structure offers advantages in drug development and potential as therapeutic agents or drug carriers.

Conclusion Retro-inverso peptides represent a valuable strategy for overcoming the limitations of conventional peptides, especially regarding stability and bioavailability. This review highlights their potential in therapeutic development and other applications, reinforcing the importance of continued research and innovation in peptide chemistry.

Keywords Peptides · Retro-inverso · Aesthetic · Drug discovery · Peptide synthesis

Introduction

In 2023, global pharmaceutical revenues reached a total of $1.6 trillion (Mikulic 2024). Within this expansive market, the UK's pharmaceutical sector, is expected to reach $31.3 billion in 2024, representing a substantial and influential segment, accounting for about 2.6% of the global pharmaceutical market (Statista Team 2024). The three largest and fastest-growing categories of new biotherapeutic products in development are peptides, monoclonal antibodies, and oligonucleotides (Torre and Albericio et al. 2024). Peptide-based drugs now make up nearly one-tenth of all drugs and represent an increasing proportion of new medicines (Al Shaer et al. 2023). Pharmaceutical manufacturing is crucial for the future of medicine and is a strong industrial player in the UK. Medicinal chemistry has been instrumental in delivering peptides with diverse structures and applications. Interestingly, therapeutic peptides are considered a complement to, and in many cases more favourable than, small molecules and biologics. They offer a tolerable safety profile and can address unmet medical challenges.

Various strategies are employed to enhance the proteolytic stability of peptides, ensuring their efficacy as therapeutic agents. Cyclisation is a prevalent chemical modification employed to enhance the proteolytic stability (White and Yudin 2011; Pelay-Gimeno et al. 2013; Mourão et al. 2020),

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incorporating non-proteinogenic amino acids (Werner et al. 2016; Wei et al. 2014), *N*-methylation (Werner et al. 2016; Chatterjee et al. 2013), and substitution with β-amino acids (Cheloha et al. 2016). However, these approaches could lead to a potential loss of the peptide's biological activity (Al Musaimi et al. 2022). An important class of peptides introduced by Dr Goodman in 1970, known as retro-inverso (RI) peptides offer an alternative approach to enhancing peptide stability. It involves inverting the stereochemistry of amino acid residues and reversing the amide bond in peptides, thereby maintaining the side chain orientation while switching the positions of the carbonyl and amide groups. This strategy was initially tested on the aspartame sweetener (MacDonald et al. 1980). RI peptides hold significant promise for extending the in vivo lifetime of biologically active peptides, addressing one of the major challenges in peptide delivery (Pallai et al. 1983). This innovative approach has since been applied to various biologically active peptides, finding utility across diverse fields including immunology, diagnostics, and drug discovery (Chorev 2005). However, it's important to acknowledge the numerous unsuccessful attempts where these analogues have lost their biological activity.

In this review, the concept of RI is reviewed in detail, including its adoption for both linear and cyclic peptides, various synthetic approaches, and its potential applications in therapeutics and other fields.

Linear Peptides

The RI concept can yield a variety of structural conformations depending on the number of modified amide bonds and the specific amino acid residues involved (Pallai et al. 1983). These modifications alter the peptide's backbone structure, while maintaining the spatial distribution of side chains. This alteration makes the molecules less susceptible to enzymatic cleavage at the reversed bonds (Pallai et al. 1983). The RI approach is particularly intriguing because it offers versatile analogues with various conformational effects, which is advantageous for peptides that are susceptible to cleavage at multiple sites. When a single amide bond is reversed, it is referred to as a pairwise modification. This transformation renders a pseudopeptide that includes a gem diamino alkyl and a 2-substituted malonyl residues (alkyl = R_3, Fig. 1A). Reversing an additional adjacent amide bond will result in a pseudopeptide where a new 2-substituted malonyl residue replaces the original one (alkyl = R_4, Fig. 1B). Reversing multiple amide bonds, along with the incorporation of some D-amino acids, is known as extended modification.

It is important to note that pairwise modification within an α-helical array disrupts the hydrogen-bonding network. This disruption is further exacerbated by repulsive interactions between adjacent carbonyl groups and adjacent NH groups (Gomez et al. 1989). In contrast, the extended modification enables the construction of fully hydrogen-bonded parallel and antiparallel β-sheets (Gomez et al. 1989).

Extending the RI concept to every amino acid in a linear peptide is known as retro-enantio or retro-all-D peptides. This process involves switching the end-groups, resulting in a different arrangement of side chains compared to the parent peptide (Fig. 2). Various research groups have endeavored to address this issue by attempting to restore the original topological arrangement of each terminal end.

End-Group Problem

The end-groups play a crucial role in such modified peptides, where various RI peptides have been found to be completely devoid of biological activity. As discussed by Goodman and Chorev (1979), failure to address the end-group issue has resulted in the loss of activity for several biologically active peptides. Examples includes the RI modification of bradykinin (Vogler and Lanz 1966), α-MSH-(5–9)-pentapeptide (Chung and Li 1967), and RI tuftsin (Dutta et al. 1976). The rationale for not addressing the end-group problem in bradykinin is because both termini contain the same amino acid (Arg). However, the loss of activity may be attributed to the crucial role of the carboxyl functionality. The retro-isomer of bradykinin did not exhibit kinin activity, possibly due to

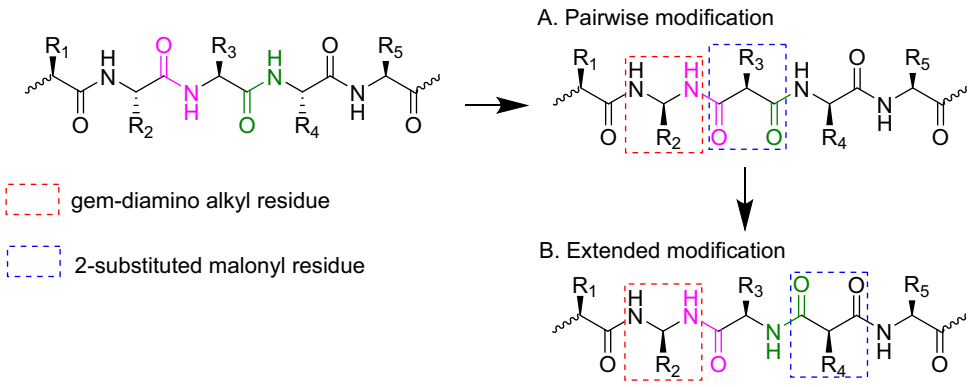


Fig. 1 Structural transformations: **A** Pairwise modification (one amide bond is reversed only). **B** Extended modification (multiple amide bonds are reversed and the chirality of amino acids involved is inverted)

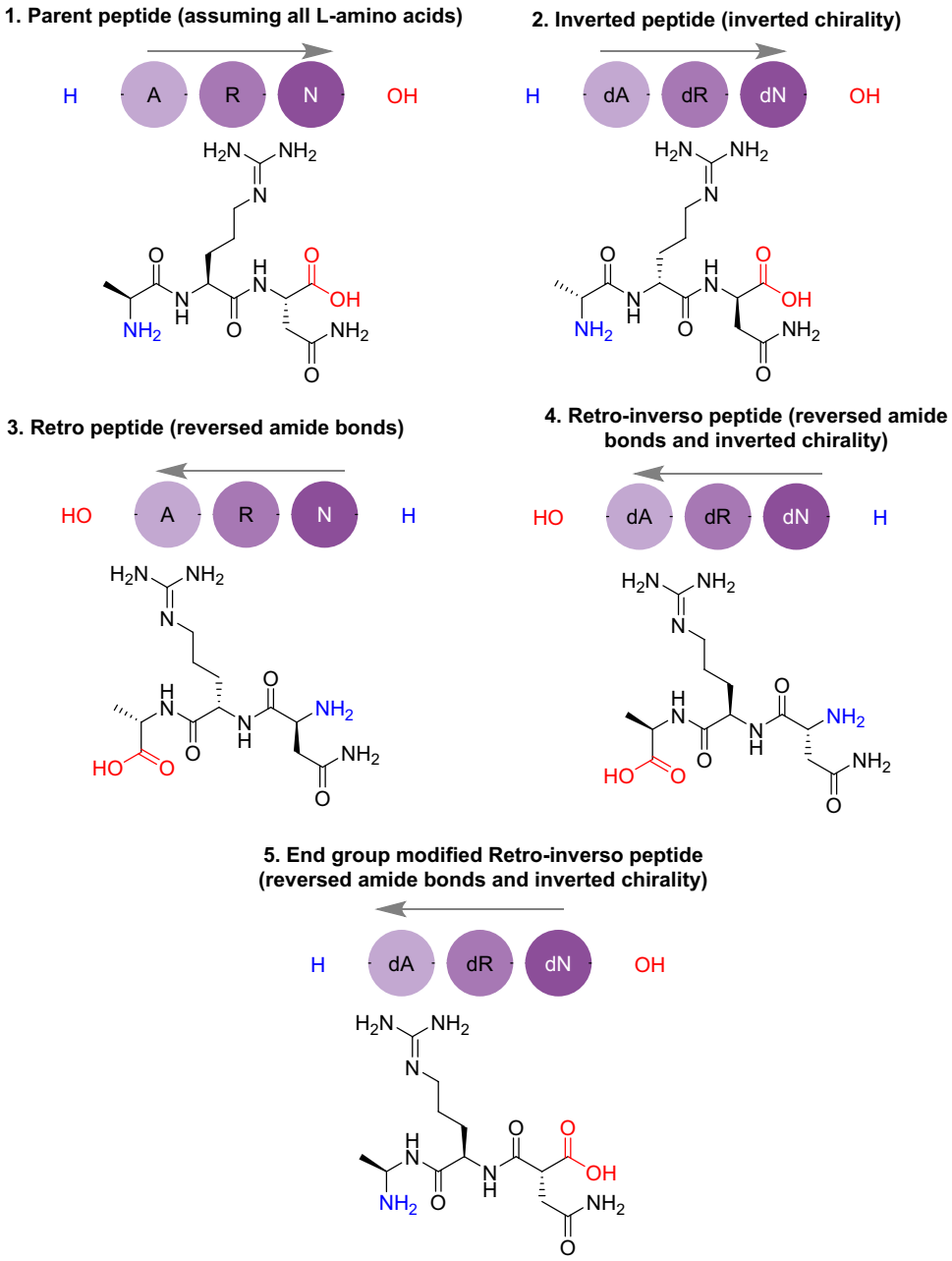


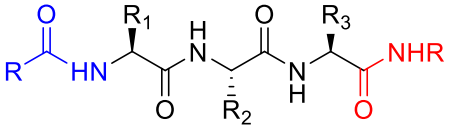
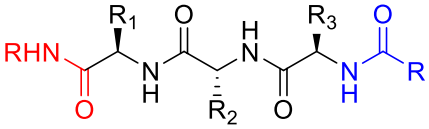
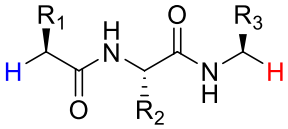
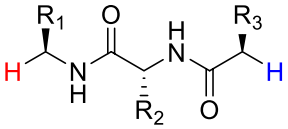
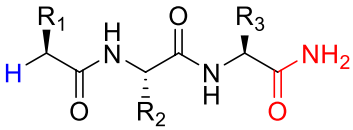
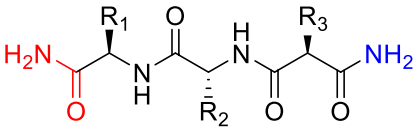
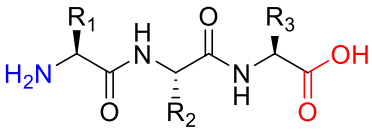
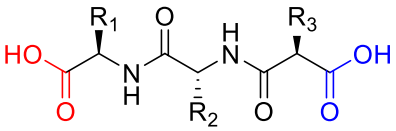
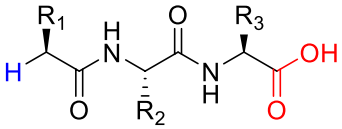
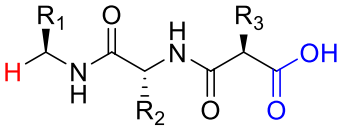
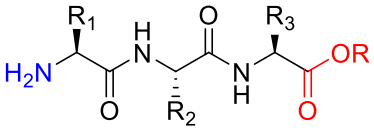
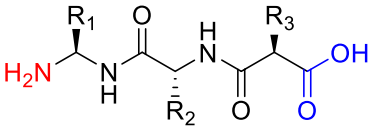
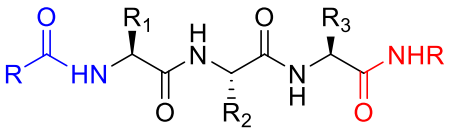
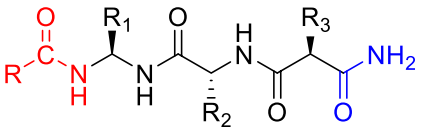
Fig. 2 Structural transformations of peptide. Arbitrary amino acids (A, R, N) selected for demonstration. Grey arrow indicates the direction of amide bond

the presence of Proline in its sequence. When the end-groups are free, they hinder the realisation of the RI concept. Therefore, to solve this issue, the end-groups should be protected (blocked), removed, or modified to achieve closer complementarity with the parent peptide and resolve this problem (Table 1). Protecting and/or eliminating the end-groups are shown in (#1 and 2, Table 1), whereas modifying them is shown in (#3–7, Table 1).

Shemyakin and colleagues suggested blocking the free end-groups (structure 4, Fig. 2) with appropriate blocking groups to ensure identical topochemical circumstances as the parent peptide (#1, Table 1) (Shemyakin et al. 1969). They proved the activity of Ac-LY-$NHCH_3$ and its RI analogue Ac-yl-$NHCH_3$ in terms of pepsin inhibition ability (Shemyakin et al. 1969). Somatostatin is a cyclic peptide stabilised by a disulfide bridge. Since the ring closure does not involve the peptide backbone, it can be considered akin to a linear peptide regarding the end-group problem. Activity towards growth hormone was observed in a modified RI analogue of somatostatin, where the end-groups were eliminated (#2, Table 1). On the other hand, the activity was absent in the case of the modified end-group RI (Immer et al. 1976).

Although scenarios (#1 and 2, Table 1) help minimise the influence of the end-groups by appropriately blocking and/or eliminating them, they also have adverse effects by altering

Table 1 Scenarios to solve the end-group problem

No	Modification	*N*-terminal	*C*-terminal	*N*-terminal	*C*-terminal
1	Blocking both termini	RCONH–	–CONHR′	RNHCO–	–NHCOR′
					
2	Eliminating both termini (Desamino and descarboxy)	H–	–H	H–	–H
					
3	Desamino plus maloamyl residue	H–	–$CONH_2$	H–	–$CONH_2$
					
4	Malonyl residue	H_2N–	–COOH	HCOO–	–COOH
					
5	Desamino plus malonyl residue	H–	–COOH	H–	–COOH
					
6	Gem-diaminoalkyl and malonyl ester residue	H_2N–	–COOR	H_2N–	–COOR
					
7	*N*-acetylated-gem-diaminoalkyl and maloamyl residue	RCONH–	–$CONH_2$	RCONH–	–$CONH_2$
					

the chemical environment around the end-groups compared to that of the parent peptide. To overcome this challenge, Goodman and colleagues proposed preserving the proximity of the end-groups and instead converting them into another motif that resembles the structure of the parent peptide. Their solution involves converting the *C*-terminal into a *C*α-alkyl malonyl moiety and the *N*-terminal into an α,α-dialkyl moiety using Curtius rearrangement (structure 5, Fig. 2) (Chorev 2005). Interestingly, structure 5 in Fig. 2 closely resembles the parent peptide structure. It involves reversing the amide bonds, inverting the chirality of all amino acids, and modifying the end-groups to have the same orientation with respect to the side chains as in the parent structure. This modification was first proposed by Rudinger (Momany et al. 1981). Morely followed modification #3 from Table 1 and synthesised end-group modified RI peptide, D-2-benzylmalonamyl-D-Asp-D-Met-tryptamide, however, it did not exhibit biological activity (Dutta et al. 1976). RI angiotensin analogues were tested following modifications #3 and 4 from Table 1 (Goodman and Chorev 1979). The authors recognised the challenge posed by proline-containing peptides. Proline's structure includes a pyrrolidine ring that needs to engage with the backbone nitrogen to form a ring. In RI analogues, the nitrogen and carbonyl positions are reversed,

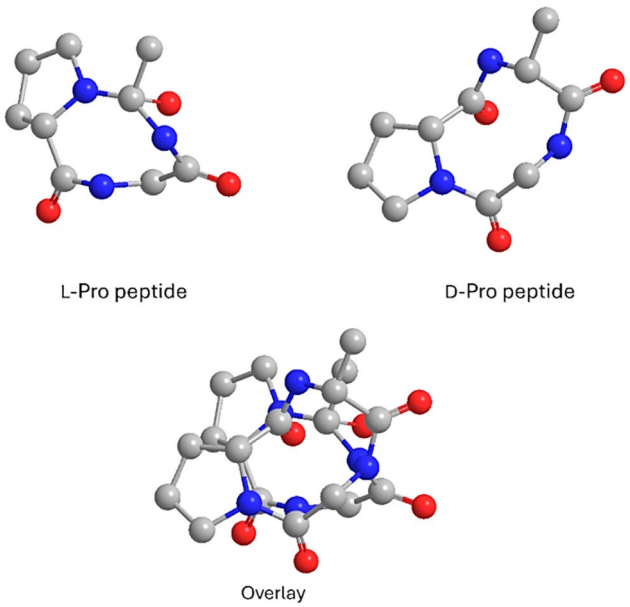


Fig. 3 3-D representation of APG cyclic peptide and its RI showing the offset problem due to proline residue

preventing the pyrrolidine ring from fulfilling this requirement (Fig. 3) (Goodman and Chorev 1979).

Such challenges can significantly affect the interaction and engagement of the peptide with its therapeutic receptor. Therefore, maintaining the biological activity of new analogues is crucial. In addressing this issue, the authors replaced the proline residue with β-alanine in some analogues. Interestingly, this single modification successfully restored bioactivity (Goissis et al. 1976). Moreover, the presence of a secondary chiral centre, such as in Ile or Thr, can lead to improper three-dimensional positioning of the side chains (Brugidou et al. 1995). Therefore, in some cases, Ile can be substituted with Val, as studies have shown that such a change does not compromise the peptide's internalisation capacity (Derossi et al. 1994). On the other hand, Awahara and colleagues demonstrated that substituting Ile residues in the human T-cell leukaemia virus (HTLV)-1 protease substrate with D-allo-Ile significantly lowered the protease's affinity and increased its inhibitory activity (Awahara et al. 2022).

Not to underestimate the significance of the carbonyl and amine backbone, the case of Aspartame being 200 times sweeter than sucrose highlights the importance of the free α-amino group, the methoxycarbonyl end-groups, and the carboxymethylene and benzyl side chains (Chorev 2005). Therefore, even the end-group modified RI Aspartame was tasteless, which, as Goodman highlighted, is attributable to the steric, electronic, and directional properties of the amide bond (MacDonald et al. 1980). On the other hand, while the end-group modified RI thiorphan retains its capacity to form a crucial hydrogen bond between the carbonyl of the amide bond in thiorphan and the complementary acceptor in the enkephalinase active site, this was not the case with the acceptor in the active site of the ACE (Roques et al. 1983). Also, scandium-44 radiolabeled-^{D}A7R conjugate has lost affinity towards neuropilin-1 (NRP-1) because the crucial *C*-terminal Arg with a free COOH, necessary for receptor interaction, has been moved to the *N*-terminal with an amine group instead (Masłowska et al. 2023).

Cyclic Peptides

Due to the absence of termini in head-to-tail cyclised peptide structures, there are no issues with end-groups. Cyclic peptides that incorporate the RI concept exhibit different stereochemical structures (Goodman and Chorev 1979). In terms of enantiomeric differences, there are two types: enantiomers and cycloenantiomers. Enantiomers have reversed amide bonds, and their reflection in a plane results in a new distribution of chiral centres. Cycloenantiomers, on the other hand, also have reversed amide bonds but maintain the same distribution of chiral centres upon reflection in a plane (Goodman and Chorev 1979). Cyclic peptides can be classified as cyclodiastereoisomers, where the in-plane reflection results in a distribution of chiral centres and reversed amide bonds that are not superimposable. This class arises due to the lack of a C_2 axis of symmetry in enantiomers (Goodman and Chorev 1979).

It is important to note that introducing different amino acid residues in cyclic structures can result in structural isomers (cycloisomers A and B). These isomers do not have a symmetrical distribution of their amino acids, which becomes evident when starting from a specific amino acid and proceeding in different directions of the amide bond (Fig. 4) (Goodman and Chorev 1979). The asymmetrical distribution of these cycloisomers is apparent when moving

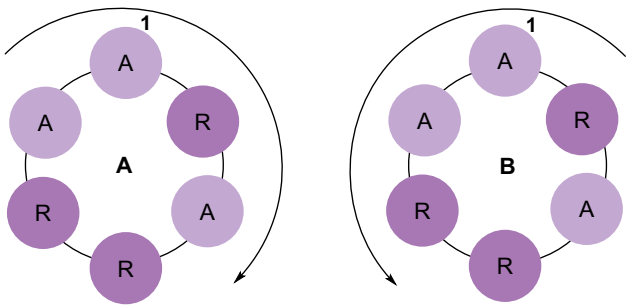


Fig. 4 Structural isomers of cyclic peptide (cycloisomers). Arbitrary amino acids (Arg and Ala) are for demonstration purposes. Arrows show the direction of amide bond

clockwise (ARARRA) or counterclockwise (AARRAR) from amino acid (1) (Goodman and Chorev 1979).

Heterocyclic peptides exhibit a wide range of structural transformations (Goodman and Chorev 1979). Reversing the amide bonds in peptide A results in retro-isomer B; however, it is not a cycloisomer because it does not have the same sequence as the parent peptide. Peptide C, the mirror image of peptide B, is the RI (enantiomer) of peptide A (Fig. 5).

However, topochemical equivalence does not always adversely affect the final biological activity of peptides. The RI [D-Tyr^6] antamanid has maintained its antioxidant activity with respect to the parent analogue, [Tyr^6] antamanid (Wieland et al. 1972). Nevertheless, the authors consider that topochemical investigation as a powerful tool for structure-activity relationship (SAR) studies (Wieland et al. 1972).

Synthesis of Gem-Diamino Alkyl Derivatives

The azide precursor is obtained from the free acid by coupling with NaN3 or from an acyl hydrazide. The hydrazide can be converted to an azide using various methods. An appropriate amino acid serves as a precursor to the isocyanate. In the Curtius rearrangement, the carboxylic acid is converted to an isocyanate via an acyl azide intermediate (Fig. 6). Alternative routes to these compounds include

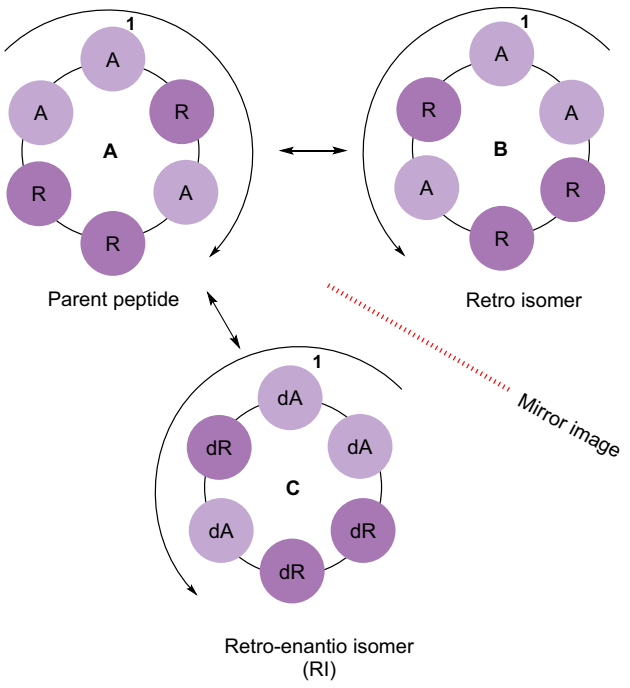


Fig. 5 Schematic representation of possible isomerisation of heterocyclic peptides. Arbitrary amino acids (Arg and Ala) are for demonstration purposes. Arrows show the direction of amide bond

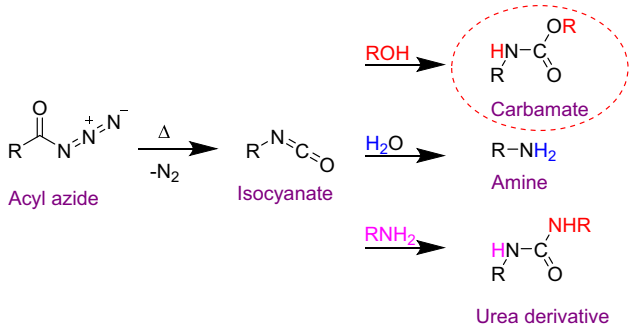


Fig. 6 Schematic representation of carbamate production from acyl azide

the Curtius, Hofmann, and Schmidt rearrangements. The Curtius route is labour-intensive and requires an additional deprotection step to prepare the target molecule, whereas the Hofmann and Schmidt rearrangements have limited applicability for peptides due to their harsh reaction conditions (Pallai and Goodman 1982).

To deliver the important partially modified RI (PMRI) peptides, monoacyl2-alkyl gem-diamines is required (Pallai and Goodman 1982). Several approaches can be pursued for their synthesis. Introducing gem-diaminoalkyl and 2-alkylmalonyl residues enables the alteration of specific peptide bonds' direction without impacting the end-groups (Pallai et al. 1983). The synthesis of optically pure monoacyl 2-alkyl gem-diamines directly from their peptide amide precursors is achieved using [bis(trifluoroacetoxy)iodo]benzene (TIB). It was introduced by Loudon et al., where the amide is converted into an amine using I,I-bis(trifluoroacetoxy) iodobenzene under mild conditions and without the need to trap the isocyanate intermediate (Fig. 7) (Radhakrishna et al. 1979).

Later on, the Goodman group found that the conversion of dipeptide amides using this reagent proceeds without racemisation and with appreciable yield (Pallai and Goodman 1982). However, not all derivatives of gem-diaminoalkyl are stable to this reagent, as exemplified by Z-Phe-NH_2 or Boc-NH_2, where hydrolysis could occur (Pallai and Goodman 1982). These observations were consistent with those

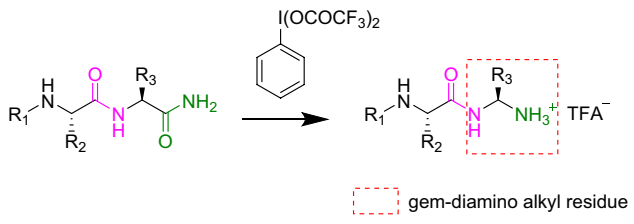


Fig. 7 Schematic representation of the conversion of amide into non-chiral alkyl amine

obtained by Loudon (Loudon et al. 1981). Moreover, due to the oxidative nature of this reagent, it cannot be used with oxidatively sensitive amino acids, where side reactions are likely to occur. For instance, incubating Boc-Tyr(Bzl)-OH with TIB results in oxidative cleavage of the side chain protecting group after 3 h. In contrast, *t*Bu protecting groups have been shown to be stable against this reagent. Since the coupling reaction of gem-diaminoalkyl-containing fragments can be slow, malonate is considered an alternative in such cases (Pallai et al. 1983).

So, the Hofmann rearrangement of N-carbamate protected amino acids is not straightforward under solution conditions. This is due to the instability of the generated gem-diamine in solution. Another tactic to avoid this problem is to carry out the reaction using urethane or acyl-protected dipeptide amides. Fehrentz and co-workers suggested an alternative approach using solid-phase settings (Cantel et al. 2003). They anchored the amino acid to the resin via its amine functionality (Fig. 8), allowing for various chemistries at the carboxylic acid group. They incorporated amino acids with an amide terminal instead of an acid. Then, the Hofmann rearrangement was carried out accordingly to convert it into an amine functionality, enabling the synthesis of various molecules thereafter.

Applications

The RI modification of peptides results in having distinct structural conformation while maintaining the same three-dimensional orientation of the side chains (Chorev 2005). RI peptides incorporate D-amino acids, maintaining the same side chain topology but with reversed amide bonds. Experimental and theoretical studies have demonstrated that this modification significantly impacts the way the peptide interacts with biological targets and can either enhance or reduce its activity (Shemyakin et al. 1969). While a noticeable enhancement in the stability of RI peptides was achieved by substituting L-amino acids with D-amino acids, various studies have shown that this modification compromises biological activity. This confirms that the backbone plays a crucial role in the engagement process with the biological target as well (MacDonald et al. 1980; Chorev 2005; Roques et al. 1983; Masłowska et al. 2023).

Notably, reversing the peptide sequence increases helicity, with the RI peptide exhibiting a 40% helicity compared to the 20% helicity of the parent P53 peptide (Atzori et al. 2013). This enhanced helicity is attributed to a higher fraction of hydrogen bonding, which plays a crucial role in the peptide's biological activity. Specifically, these structural changes impact the peptide's interaction with cell membranes, its penetration capabilities, and cellular uptake, thereby influencing its overall biological function (Atzori et al. 2013). Moreover, reversing the sequence leads to a distinct peptide surface behaviour and alters its interaction with lipids compared to a molecule with the same structure (Atzori et al. 2013).

RI peptides demonstrate enhanced proteolytic resistance compared to their parent analogues. Studies have shown that these derivatives can induce higher and longer-lasting levels of antibodies than conventional peptides. Moreover, the affinity of immunoglobulin G1 (IgG1) isotype antibodies for the reversed peptide is significantly greater than for the parent peptide of identical structure (Guichard et al. 1996). Modifying the side chain is crucial for receptor interactions. However, there is increasing interest in modifying the main chain due to numerous studies demonstrating its impact on conformational stabilisation, intermolecular interactions, and enzymatic cleavage processes. PMRI shows promise in extending the lifetime of biologically active peptides. Importantly, in PMRI, the termini are not swapped as they are when reversing the entire sequence (Pallai et al. 1983). This approach is also favoured by the inventor of the concept, Goodman. In Goodman's research group, they demonstrated that the PMRI analogue of Met-Enkephalin exhibits higher activity in vitro compared to the native peptide (Chorev 2005).

Verdini and colleagues demonstrated that the PMRI analogue of the tuftsin peptide reproduces the anti-inflammatory activity of the parent molecule and is more potent than the parent peptide in stimulating the production of antibody-secreting cells (Verdini et al. 1991). Its enhanced in vivo activity could be ascribed to its resistance to peptidases (Verdini et al. 1991). Modifying indolicidin results in a peptide that is equipotent to the parent peptide in its antimicrobial activity against *Salmonella typhimurium* (Nagpal et al. 2002). Interestingly, RI of Antp-derived peptide resulted in 3.4-fold higher intracellular concentration compared

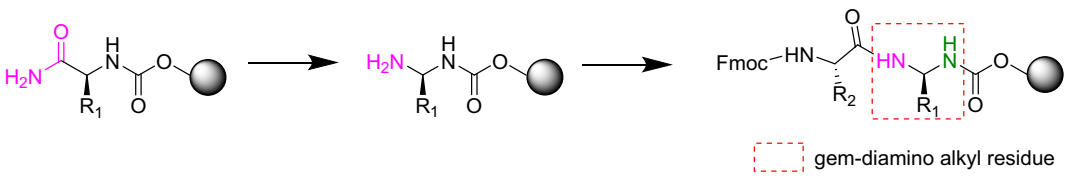


Fig. 8 Schematic representation of Hofmann rearrangement of *N*-carbamate protected amino acid on resin

to the parent peptide (Brugidou et al. 1995). Most importantly, retro inversion of peptides with potent T-cell epitopes results in loss of their ability to bind to MHC II molecules, rendering them poor immunogens. In conclusion, RI offers good penetration, high resistance against enzymatic degradation and lack of immunogenicity and antigenicity (Hervé et al. 1997). In the internalisation process, the motor region is considered the main component, while other regions play diverse roles in enhancing the internalisation process (Aldrian-Herrada et al. 1998; Gallazzi et al. 2003). Various studies have demonstrated the plasma stability of RI peptides and their ability to internalise into cells (Aldrian-Herrada et al. 1998; Gallazzi et al. 2003). Cao and co-workers have designed RI and inverso peptide isomers that can bind to and inhibit α7 nAChR with an IC_{50} of 0.35 μM, where better stability in the plasma was noticed in the case of D-peptide (Cao et al. 2024).

A fascinating study exploited RI to block the autoimmune response between HLA-DQ8 positive APCs and CD4+ T-cells. The idea is that RI can occupy the same pocket in the HLA groove, preventing the binding of pathogenic peptides and thereby stopping the destruction of β cells (Lombardi et al. 2020). Similarly, another study has employed a similar strategy, utilising an RI peptide with high affinity for the HLA-DRβ1-Arg74 peptide binding pocket to block thyroid antigen binding. This approach has potential applications in autoimmune thyroid diseases (AITD) (Li et al. 2023).

In cell-penetrating peptides (CPPs), modified peptides are effectively internalised into cells. Moreover, some of these peptides were found to be more stable in insulin-secreting cell lines (Snyder et al. 2004). Hybrid peptides derived from HIV-TAT and p53, along with their RI forms, were also shown to be effectively internalised. The hybrid HIV-TAT peptide remained stable in the insulin-secreting cell line (βTC-3) for 2 weeks and provided complete protection against IL-1β-induced apoptosis during this period. This suggests its potential as a therapeutic agent for protecting β-cells from autoimmune destruction associated with type 2 diabetes (Snyder et al. 2004; Borsello et al. 2003). RI analogue of the hybrid peptide p53[361–382]-Gly-TAT-HIV(Taylor et al. 2000; Wang et al. 2018; Dong et al. 2023; Liu et al. 2015; Carver et al. 1997; Zhang et al. 2022; Muller et al. 1998; Errante et al. 2024; Pandey et al. 2020; Suzuki et al. 2024; Cushman et al. 1977) efficiently activates the p53 tumour suppressor pathway, offering promising treatments for various preclinical cancer models (Snyder et al. 2004).

Prosaptide peptides derived from the 14-amino acid region of prosaposin [326–339], where neurotrophic activity is localised, have been investigated (Taylor et al. 2000). Two RI analogues of prosaptide-derived undecapeptides (D4 and D5) have shown biological activity, the ability to cross the blood-brain barrier (BBB), and stability in vivo (Taylor et al. 2000). Wei et al., demonstrated that angiopep, a 19-mer peptide, and its RI analogue exhibit high uptake capacity via lipoprotein-related protein 1 (LRP-1), which is expressed on brain capillary endothelial cells (Wei et al. 2014). Moreover, the RI analogue was observed to be more stable in rat blood compared to the parent analogue, with 85% degradation of the parent analogue occurring over 2 h (Wei et al. 2014). So, RI peptides show significant potential for the advancement of treatments for central neurodegenerative diseases. RI peptides have demonstrated better resistance against proteolytic degradation than the parent analogue, attributed to the presence of D-amino acids (Brugidou et al. 1995). A 16-mer peptide from antennapedia is known to be susceptible to degradation due to the presence of dibasic sites, namely Lys-Lys and Arg-Arg, which are well recognised by neuronal proteases. Its RI peptidomimetic analogue has shown complete stability against these endoproteases, with eight times more internalisation than the parent analogue (Brugidou et al. 1995).

RI peptide showed promising performance in the tumour tissue penetration. C-end rule peptide (CendR) is a heptapeptide (RGERPPR) that is able to enhance the permeability of the tumour blood vessels and tumour tissue through binding to neuropilin-1 (NRP-1). Wang et al., investigated the RI analogue of this peptide and obtained higher binding affinity with NRP-1 and more cellular uptake, and improved cell penetration in comparison to the parent peptide (Wang et al. 2018). In 2023, Dong and co-workers investigated the same CendR peptide as well as its inverso and RI analogues (Dong et al. 2023). They modified the three peptides on the outer surface of human H chain ferritin and loaded paclitaxel (PTX) into each ferritin inner cavity (Dong et al. 2023). The inverso modified analogue displayed a stronger cellular uptake as well as penetration efficiency, thus, it is holds promise in providing effective dual-targeting platform for treatment of cancers (Dong et al. 2023). iRGD is a cyclic nonapeptide (CRGDKGPDC), the Arg, Gly, Asp amino acids motif has high binding affinity towards αvβ3 and αvβ5 integrins abundant in tumour vasculatures (Liu et al. 2015). iRGD can specifically bind integrins and NRP-1 that are overexpressed in on various tumours (Liu et al. 2015). The cleaved form of iRGD binds to NRP-1 and prompts the NRP-1 dependent endocytosis hence enhancing the tumour penetration (Liu et al. 2015). RI based on RGD peptides coupled with CPP offer enhanced tumour targeting efficiency similar to RDG motif alone (Liu et al. 2015). RI of RGD-containing peptide showed the same antigenic activity with respect to the parent peptide (Carver et al. 1997). Although both analogues have different backbone conformation, they adopt the same structure, quasi-planer, which implies similar topologies with a flexibility around the Arg (Carver et al. 1997). RI follicle-stimulating hormone (FSH) peptide has been engineered for the delivery of pyruvate dehydrogenase

kinase 2 (PDK2) shRNA to target chemo-resistant ovarian cancer (Zhang et al. 2022). This approach resulted in higher PDK2 expression, which subsequently inhibited proliferation and migration and promoted apoptosis in both cisplatin-sensitive and cisplatin-resistant ovarian cancer cells (Zhang et al. 2022).

RI peptides have also potential in synthetic peptide vaccines (Muller et al. 1998). A PNA antisense targeting AUG translation initiation was linked to a RI peptide. This bioconjugate was rapidly internalised by cultured cerebral cortex neurons within minutes e (Aldrian-Herrada et al. 1998). The PNA alone can also enter the cell, albeit at a slower rate (Aldrian-Herrada et al. 1998).

RI found applications in cosmeceutical as well aiming at enhancing the resistance against dermal enzymes. Rovero and coworkers have designed a RI tetrapeptide (Ac-KVVN-NH_2), a sequence that was devised from a serine protease inhibitor (serpin A1) (Errante et al. 2024). Higher in vitro activity was observed with the designed RI peptide with respect to the parent one. Its high resistance against human proteases is ascribed to their inability to recognise such rationally modified peptides.

From another perspective, RI peptides can not only mimic the biological activity of their parent analogues but also interact with pre-formed aggregates of the parent peptide. A study conducted by Pandey and colleagues demonstrated that an RI of amylin, a 37-mer peptide used to treat type 2 diabetes, can inhibit the formation of amylin fibrils, which are often formed in diabetic conditions for unknown reasons (Pandey et al. 2020). Moreover, RI peptides can be utilised to investigate the role of hydrogen bonds in supramolecular structures and aggregates (Pandey et al. 2020).

Sometimes, the biological activity could be devoid by adopting RI and does not always reproduce the bioactive conformation. For instance, RI of a bicyclic hexapeptide showed no cytotoxicity towards certain cancer cell lines (Suzuki et al. 2024). According to the crystal structure, the three Tyr residues reside in the same vicinity, while structural differences in the peptide backbone and in the CH_3 of the Ala residue were observed with respect to the parent peptide. The authors believes that the loss of the activity ($IC_{50} > 150$ μM) might be attributed to those differences, which could have hampered the interaction with the ribosome of eukaryotic cells (Suzuki et al. 2024).

Table 2 summarises various additional peptides and their modified analogues, along with the observed effects of their RI counterparts.

Various RI peptides have paved the way for clinical studies. uPAR-FPR1 analogues represent promising leads for developing anti-metastasis therapeutics (#13, Table 2) (Carriero et al. 2017). NR58-3.14.3 with its nano-molar effective dose (ED50), it could serve as a useful broad-specificity chemokine inhibitor in vivo (#8, Table 2). However, the authors emphasised that further research will be needed to determine if molecules like NR58-3.14.3 have clinically relevant anti-inflammatory properties in humans (Reckless et al. 2001). Interestingly, NR58-3.14.4 shares the same sequence with NR58-3.14.3, with an additional D-Ala linked to the side chain of Asp, but it showed no activity as a chemokine inhibitor (Reckless et al. 2001). GnRH RI peptides have important therapeutic implications for patients with GnRH receptor-activating autoantibodies (AAb), providing a basis for their use in clinical applications, including diagnostic testing and therapeutic interventions (#14, Table 2) (Li et al. 2022). The all-D and RI forms of HHC36 (#17, Table 2) reduce the effective concentrations of DOX and D-peptides, particularly all-D HHC36, showing potential for combination treatments in in vivo models. The authors are considering these studies in their laboratory (Behzadi et al. 2022). MID (#19, Table 2) shows promise for expressing a long-lasting muscle-building effect in skeletal muscles (Takayama et al. 2022). The cyclic-inverso AHSG 1e30 peptide (#21, Table 2) holds potential for future applications in osteoarthritis (Akker et al. 2023). In addition, ^{68} Ga-labelled TMTP1 (NVVRQ) (#27, Table 2) is promising for the early detection of highly metastatic primary hepatocellular carcinoma (Wang et al. 2024). These advancements are likely to find applications in the expanding field of Peptide Receptor Radionuclide Therapy (PRRT) (Bergsma et al. 2012; Al Musaimi 2024).

According to the examples in Table 2, we can conclude that RI peptides hold promise in various fields, often demonstrating similar or even better binding affinities with potential receptors. However, in some cases, the RI strategy led to a loss of affinity and consequently a reduction or even a complete loss of biological activity. Furthermore, some studies have reported inconsistent results when using RI in different applications. For instance, DA7R exhibited promising outcomes in terms of affinity towards VEGFR2 and NRP-1 when coupled to a liposome system (#15, Table 2) but lost its affinity towards NRP-1 when conjugated to scandium-44 (#16, Table 2). In conclusion, RI can be effective in some applications but not in others, and this variability applies to common peptides as well, not just RI peptides. For further readings about the applications of RI in cancer, immunology, neurodegenerative, and antimicrobials, readers are encouraged to refer to the detailed review by Doti et al. (Doti et al. 2021).

Conclusion

Since their inception, RI peptides have evolved through several developmental stages, demonstrating their utility across various peptide applications, which are increasingly important and in demand. The RI strategy offers the

Table 2 Example of selected peptides and their RI analogues

#	Peptide	Modification	Outcome	Explanation	References
1	Thiorphan	End-group modified RI	A. retained 50% inhibiting potency of enkephalinase compared to parent thiorphan, and B. > 1600-fold less potent in inhibiting ACE	A. Ability to form H-bonds between the amide carbonyl and the enkephalinase receptor B. Inability to form H-bonds with the ACE receptor	Cushman et al. 1977)
2	L-H-Phe-Gly-OH	End-group modified RI	Inhibited enkephalinase with potency similar to the natural dipeptide	Ability to form H-bonds between the amide carbonyl and the enkephalinase receptor	Roques et al. 1983)
3	IRGERA	RI	The affinity of the generated antibodies against the modified analogue is 7–75-fold higher than their affinity toward the parent hexapeptide	The side chains of each essential residue have identical orientations	Benkirane et al. 1995)
4	Immunodominant loop of foot-and-mouth disease virus (FMDV)	End-group modified RI	Greater and longer-lasting antibody titers than parent peptide, 20-fold greater half-life than parent peptide	Due to its high stability conferred by D-amino acids	Briand et al. 1997)
5	C reactive protein (CRP)	RI	Increases the expression and activation of inducible nitric oxide synthase (NOS)	Due to high stability conferred by D-amino acids	Arcoleo et al. 1997)
6	Pseudopeptide [$Gly^{58}\Psi$(NH–CO) Leu^{59}]M(58–66)	PMRI	More effective than the wild-type sequence in stimulating $CD8^{+}$CTL, and more resistant to proteolysis than the parent peptide	Nonbonded energy calculation of amino acid 59 is intermediate for this PMRI, and the PMRI-HLA-A2 complex is more stable than with parent peptide	Ostankovitch et al. 1998)
7	Arg(N^{ω}-NO_2)-Lys-NH_2	RI	Potent and selective inhibitor of the neuronal NOS	Due to the binding of the nitroarginine residue to the same binding site. D-Arg at the *C*-terminus might flip 180° to assume an L-nitroarginine-like configuration at the *N*-terminus for binding	Huang et al. 1999)
8	NR58-3.14.3	RI	100–1000-fold more potent than the parent peptide in inhibiting leukocyte migration	NR	Reckless et al. 2001)
9*	S12-RI	RI	Failed to cross-react with anti-B13 antibodies in the sera from T	RI analogue tended to form an opposite right hand α-helix compared to parent analogue	Iwai et al. 2001)
10	Indolicidin		Equipotent antimicrobial potency	The hydrophobic side chains maintained the same spatial arrangement, and the charge-bearing Arg side chains exhibited the same flexibility	Nagpal et al. 2002)

Table 2 (continued)

#	Peptide	Modification	Outcome	Explanation	References
11	111In-[PTD-4]-Gly-Lys(N^{ε}-DOTA)	RI	RI showed superior plasma stability, active in mediating internalisation of anti-bcl-2 PNA, and nuclear accumulation	Due to high stability conferred by D-amino acids	Gallazzi et al. 2003)
12	Human gonadotropin-releasing hormone (GnRH)	RI	Improved immunogen and does not require conjugation to an immunogenic carrier protein	RI is not recognised due to D-amino acids	Fromme et al. 2003)
13	uPAR-FPR1	RI	More stable in vitro, and are effective and well tolerated in vivo	Molecular dynamics proved that the RI analogue adopts similar turn structure	Carriero et al. 2017)
14	GnRH receptor-activating autoantibodies	RI	Significantly inhibited polycystic ovary syndrome (PCOS) serum IgG-induced GnRH receptor activation, and high proteolytic stability	Due to same side chain topology of the parent analogue	Li et al. 2022)
15	^{D}A7R and ^{D}A7R-modified liposome	RI	Enhanced the tumour targeting efficiency of liposomes compared to ^{L}A7R, and higher serum stability	Similar binding affinity to its receptors in vitro (VEGFR2 and NRP-1), and higher tumour accumulation	Ying et al. 2016)
*16	Scandium-44 radiolabeled-^{D}A7R	RI	Lack in the affinity towards Neuropilin-1 (NRP-1)	Lack of affinity for b1 subdomain of the NRP-1 binding pocket due to important *C*-terminal Arg with free COOH being moved to the *N*-terminal with amine group instead	Masłowska et al. 2023; Parrasia et al. 2023)
17	HHC36	All D and RI	Higher antibacterial activity, and higher tryptic stability	They are able to self-assemble like the parent analogue and get access to the hydrophobic membrane interior	Behzadi et al. 2022)
18	^{99m}Tc-PEG 6-RD-PDP2	RI	Better tumour imaging intensity, and higher in vivo stability	Higher PD-L2-binding specificity both in vitro, and in vivo	Luo et al. 2022)
19	Myostatin inhibitory D-peptide (MID)	RI	Superior in vivo efficacy and stability compared to MIPE-1686	improved physicochemical properties	Takayama et al. 2022)
20	(ri)-r(P)ApoBS^{Pro}	RI	Increased activity against Gram-negative rather than Gram-positive bacterial strains compared to its parent analogue, lower toxic effects toward human dermal fibroblasts (HDFs), and higher stability in serum proteases	The substitution of L-amino acids with D-amino acids, enhanced its stability without affecting the antimicrobial activity or toxicity	Cesaro et al. 2022a; Cesaro et al. 2022b)
21	cyclic-inverso AHSG 1e30 peptide	RI	Significant improvement in histopathological osteoarthritis score and animal mobility, and lower serum levels of IFNγ	The substitution of L-amino acids with D-amino acids, enhanced its stability without affecting the efficient inhibition of precipitation	Akker et al. 2023)

Table 2 (continued)

#	Peptide	Modification	Outcome	Explanation	References
22	FOXM1 (1-138aa)	RI	Improved stability and cell inhibitory activity compared to the parent peptide	Due to enhanced stability conferred by D-amino acids, with similar side chains orientation	Cheng et al. 2023)
23	[^{177}Lu]DOTA-L-A9	Inverso and RI	Higher secondary structure stabilisation of the RI than the inverso, RI showed better pharmacokinetic and significantly higher tumour uptake than the inverso and the original peptide, both also showed improved in vivo metabolic stability over the original peptide	Improved conformational stability	Kumar Sharma et al. 2023)
24	PD00	RI	RI showed high affinity for CD81, higher metabolic stability, and higher half-life	Molecular docking showed that the binding site of PD00 is generally conserved	Ma et al. 2023)
25	FOXO4	RI	RI analogue selectively induced p53 nuclear exclusion and eliminated senescent cells by competing with FOXO4 for the binding of p53	RI analogue may function by competing with FOXO4 for the binding of p53	Liu et al. 2023; Born et al. 2023)
26*	bicyclic hexapeptide RA-VII	RI	No cytotoxic activity of the RI analogue	The analogue exhibited distinct structural characteristics compared to the parent peptide	Suzuki et al. 2024)
27	^{68} Ga-labelled TMTP1 (NVVRQ)	RI	Exceptional in vivo stability, suitable physicochemical properties, and remarkable diagnostic capabilities	Due to higher lipophilicity of the RI which implies increased propensity for crossing biomembranes	Wang et al. 2024)

CRP C reactive protein, *FMDV* foot-and-mouth disease virus, *GnRH* human gonadotropin-releasing hormone, *MCP-1* human monocyte chemoattractant protein-1, *NR* not reported, *PCOS* polycystic ovary syndrome, *VEGFR2* vascular endothelial growth factor 2

*These analogues lost their functionalities

possibility to enhance peptide potency by increasing their binding potential with receptors, activating or deactivating them (Lombardi et al. 2020; Li et al. 2023; Cushman et al. 1977; Arcoleo et al. 1997; Fromme et al. 2003; Ying et al. 2016; Ma et al. 2023). RI peptides demonstrated greater resistance to enzymatic degradation, including enzymes involved in dermal degradation, leading to subsequently prolonged half-lives. Interestingly, RI strategies often lead to peptides that do not bind effectively to MHC II, making them poor immunogens and eliciting less immunogenic responses. Indeed, some studies demonstrate the potential of RI analogues to interact with the parent peptide and inhibit its aggregation formation process that arise from certain pathological diseases.

Although RI help in boosting the stability of peptides, they could also compromise their biological activity, therefore, proper evaluation studies have to be considered to establish the optimum modifications that render an active analogue (Li et al. 2010).

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Declarations

Conflict of interest The author declares no conflict of interest.

Ethical Approval Not applicable.

References

Al Musaimi O (2024) Peptide Therapeutics: unveiling the potential against cancer—a journey through 1989. Cancers. https://doi.org/10.3390/cancers16051032

Al Musaimi O, Lombardi L, Williams DR, Albericio F (2022) Strategies for improving peptide stability and delivery. Pharmaceuticals 15(10):1283. https://doi.org/10.3390/ph15101283

Al Shaer D, Al Musaimi O, Albericio F, de la Torre BG (2023) FDA TIDES (peptides and oligonucleotides) harvest. Pharmaceuticals 2024:17. https://doi.org/10.3390/ph17020243

Aldrian-Herrada G, Méry J, Brugidou J, Desarménien MG, Orcel H, Boissin-Agasse L, Rabié A (1998) A peptide nucleic acid (PNA) is more rapidly internalized in cultured neurons when coupled to a retro-inverso delivery peptide. The antisense activity depresses the target mRNA and protein in magnocellular oxytocin neurons. Nucleic Acids Res Spec Publ 26(21):4910–4916. https://doi.org/10.1093/nar/26.21.4910

Arcoleo F, Milano S, D'Agostino P, Misiano G, Cappelletti S, Gromo G, Marcucci F, Leoni F, Cillari E (1997) Effect of partially modified retro-inverso analogues derived from C-reactive protein on the induction of nitric oxide synthesis in peritoneal macrophages. Br J Pharmacol 120(7):1383–1389. https://doi.org/10.1038/sj.bjp.0701050

Atzori A, Baker AE, Chiu M, Bryce RA, Bonnet P (2013) Effect of sequence and stereochemistry reversal on p53 peptide mimicry. PLoS ONE 8(7):e68723. https://doi.org/10.1371/journal.pone.0068723

Awahara C, Oku D, Furuta S, Kobayashi K, Teruya K, Akaji K, Hattori Y (2022) The Effects of side-chain configurations of a retro–inverso-type inhibitor on the human T-Cell leukemia virus (HTLV)-1 protease. Molecules. https://doi.org/10.3390/molecules27051646

Behzadi M, Eghtedardoost M, Bagheri M (2022) Endocytosis involved d-oligopeptide of tryptophan and arginine displays ordered nanostructures and cancer cell stereoselective toxicity by autophagy. ACS Appl Mater Interfaces 14(13):14928–14943. https://doi.org/10.1021/acsami.1c23846

Benkirane N, Guichard G, van Regenmortel MHV, Briand JP, Muller S (1995) Cross-reactivity of antibodies to retro-inverso peptidomimetics with the parent protein histone H3 and chromatin core particle. J Biol Chem 270:11921–11926

Bergsma H, van Vliet EI, Teunissen JJM, Kam BLR, de Herder WW, Peeters RP, Krenning EP, Kwekkeboom DJ (2012) Peptide receptor radionuclide therapy (PRRT) for GEP-NETs. Best Pract Res Clin Gastroenterol 26(6):867–881. https://doi.org/10.1016/j.bpg.2013.01.004

Born E, Lipskaia L, Breau M, Houssaini A, Beaulieu D, Marcos E, Pierre R, Do Cruzeiro M, Lefevre M, Derumeaux G et al (2023) Eliminating senescent cells can promote pulmonary hypertension development and progression. Circulation 147(8):650–666. https://doi.org/10.1161/circulationaha.122.058794

Borsello T, Clarke PG, Hirt L, Vercelli A, Repici M, Schorderet DF, Bogousslavsky J, Bonny C (2003) A peptide inhibitor of c-Jun N-terminal kinase protects against excitotoxicity and cerebral ischemia. Nat Med 9(9):1180–1186. https://doi.org/10.1038/nm911

Briand J-P, Benkirane N, Guichard G, Newman JFE, Van Regenmortel MHV, Brown F, Muller S (1997) A retro-inverso peptide corresponding to the GH loop of foot-and-mouth disease virus elicits high levels of long-lasting protective neutralizing antibodies. PNAS 94(23):12545–12550. https://doi.org/10.1073/pnas.94.23.12545

Brugidou J, Legrand C, Mery J, Rabie A (1995) The retro-inverso form of a homeobox-derived short peptide is rapidly internalized by cultured neurons: a new basis for an efficient intracellular delivery system. Biochem Biophys Res Commun 214(2):685–693. https://doi.org/10.1006/bbrc.1995.2340

Cantel S, Boeglin D, Rolland M, Martinez J, Fehrentz J-A (2003) Synthesis of gem-diamino derivatives on solid support. Tetrahedron Lett 44(25):4797–4799. https://doi.org/10.1016/S0040-4039(03)00925-0

Cao X, Liu T, Wang T, Wang X, Xu Z, Zhou L, Tian C, Sun DD (2024) Novo screening and mirror image isomerization of

linear peptides targeting α7 nicotinic acetylcholine receptor. ACS Chem Biol 19(3):592–598. https://doi.org/10.1021/acschembio.3c00674

Carriero MV, Bifulco K, Ingangi V, Costantini S, Botti G, Ragone C, Minopoli M, Motti ML, Rea D, Scognamiglio G et al (2017) Retro-inverso urokinase receptor antagonists for the treatment of metastatic sarcomas. Sci Rep 7(1):1312. https://doi.org/10.1038/s41598-017-01425-9

Carver JA, Esposito G, Viglino P, Fogolari F, Guichard G, Briand JP, Van Regenmortel MH, Brown F, Mascagni P (1997) Structural comparison between retro-inverso and parent peptides: molecular basis for the biological activity of a retro-inverso analogue of the immunodominant fragment of VP1 coat protein from foot-and-mouth disease virus. Biopolymers 41(5):569–590. https://doi.org/10.1002/(sici)1097-0282(19970415)41:5%3c569::Aid-bip8%3e3.0.Co;2-k

Cesaro A, Torres MDT, Gaglione R, Dell'Olmo E, Di Girolamo R, Bosso A, Pizzo E, Haagsman HP, Veldhuizen EJA, de la Fuente-Nunez C et al (2022a) Synthetic antibiotic derived from sequences encrypted in a protein from human plasma. ACS Nano 16(2):1880–1895. https://doi.org/10.1021/acsnano.1c04496

Cesaro A, Gaglione R, Chino M, De Luca M, Di Girolamo R, Lombardi A, Filosa R, Arciello A (2022b) Novel retro-inverso peptide antibiotic efficiently released by a responsive hydrogel-based system. Biomedicines. https://doi.org/10.3390/biomedicines10061301

Chatterjee J, Rechenmacher F, Kessler H (2013) N-methylation of peptides and proteins: an important element for modulating biological functions. Angew Chem Int Ed Engl 52(1):254–269. https://doi.org/10.1002/anie.201205674

Cheloha RW, Watanabe T, Dean T, Gellman SH, Gardella TJ (2016) Backbone modification of a parathyroid hormone receptor-1 antagonist/inverse agonist. ACS Chem Biol 11(10):2752–2762. https://doi.org/10.1021/acschembio.6b00404

Cheng H, Yuan J, Pei C, Ouyang M, Bu H, Chen Y, Huang X, Zhang Z, Yu L, Tan Y (2023) The development of an anti-cancer peptide M1–21 targeting transcription factor FOXM1. Cell Biosci 13(1):114. https://doi.org/10.1186/s13578-023-01059-7

Chorev M (2005) The partial retro–inverso modification: a road traveled together. Biopolym (Pept Sci) 80(2–3):67–84. https://doi.org/10.1002/bip.20219

Chung D, Li CH (1967) The synthesis of D-tryptophanyl-D-arginyl-D-phenylalanyl-D-histidyl-D-glutamic acid and its effect on melanotropic activity. Biochim Biophys Acta 136(3):570–571. https://doi.org/10.1016/0304-4165(67)90018-9

Cushman DW, Cheung HS, Sabo EF, Ondetti MA (1977) Design of potent competitive inhibitors of angiotensin-converting enzyme. Carboxyalkanoyl and mercaptoalkanoyl amino acids. Biochemistry 16(25):5484–5491. https://doi.org/10.1021/bi00644a014

de la Torre BG, Albericio F (2024) The pharmaceutical industry in 2023: an analysis of FDA drug approvals from the perspective of molecules. Molecules. https://doi.org/10.3390/molecules29030585

Derossi D, Joliot AH, Chassaing G, Prochiantz A (1994) The third helix of the antennapedia homeodomain translocates through biological membranes. J Biol Chem 269(14):10444–10450

Dong Y, Yang K, Xu Z, Li X, Wang F, Zhang Y (2023) Effective delivery of paclitaxel-loaded ferritin via inverso CendR peptide for enhanced cancer therapy. Mol Pharm 20(2):942–952. https://doi.org/10.1021/acs.molpharmaceut.2c00616

Doti N, Mardirossian M, Sandomenico A, Ruvo M, Caporale A (2021) Recent applications of retro-inverso peptides. Int J Mol Sci 22(16):8677. https://doi.org/10.3390/ijms22168677

Dutta AS, Furr BJA, Giles MB, Morley JS (1976) Synthesis and biological activity of α-azapeptides: α-aza-analogues of luteinizing hormone releasing hormone. Clin Endocrinol 5(s1):s291–s298. https://doi.org/10.1111/j.1365-2265.1976.tb03837.x

Errante F, Pallecchi M, Bartolucci G, Frediani E, Margheri F, Giovannelli L, Papini AM, Rovero P (2024) Retro-inverso collagen modulator peptide derived from serpin A1 with enhanced stability and activity in vitro. J Med Chem 67(6):5053–5063. https://doi.org/10.1021/acs.jmedchem.4c00137

Fromme B, Eftekhari P, Van Regenmortel M, Hoebeke J, Katz A, Millar R (2003) A novel retro-inverso gonadotropin-releasing hormone (GnRH) immunogen elicits antibodies that neutralize the activity of native GnRH. Endocrinol 144(7):3262–3269. https://doi.org/10.1210/en.2002-221135

Gallazzi F, Wang Y, Jia F, Shenoy N, Landon LA, Hannink M, Lever SZ, Lewis MR (2003) Synthesis of radiometal-labeled and fluorescent cell-permeating peptide−PNA conjugates for targeting the bcl-2 proto-oncogene. Bioconjugate Chem 14(6):1083–1095. https://doi.org/10.1021/bc034084n

Goissis G, Nouailhetas VLA, Paiva ACM (1976) Synthesis of biologically active retroenantiomers of angiotensin peptides. J Med Chem 19(11):1287–1290. https://doi.org/10.1021/jm00233a006

Gomez EJ, Vitoux B, Marraud M, Sakarellos C, Masdouri LE, Aubry A (1989) Conformational perturbations in retro-analogs of the tBuCO-Ala-Gly-NHiPr dipeptide crystal structure of the retro-dipeptide with a reversed Ala-Gly amide bond. J Pept Protein Res 34(6):480–486. https://doi.org/10.1111/j.1399-3011.1989.tb01397.x

Goodman M, Chorev M (1979) On the concept of linear modified retropeptide structures. Acc Chem Res 12(1):1–7. https://doi.org/10.1021/ar50133a001

Guichard G, Connan F, Graff R, Ostankovitch M, Muller S, Guillet JG, Choppin J, Briand JP (1996) Partially modified retro-inverso pseudopeptides as non-natural ligands for the human class I histocompatibility molecule HLA-A2. J Med Chem 39(10):2030–2039. https://doi.org/10.1021/jm9509511

Hervé M, Maillére B, Mourier G, Texier C, Leroy S, Ménez A (1997) On the immunogenic properties of retro-inverso peptides. Total retro-inversion of T-Cell epitopes causes a loss of binding to MHC II molecules. Mol Immunol 34(2):157–163. https://doi.org/10.1016/S0161-5890(97)00004-7

Huang H, Martasek P, Roman LJ, Masters BSS, Silverman RB (1999) Nω-nitroarginine-containing dipeptide amides. Potent and highly selective inhibitors of neuronal nitric oxide synthase. J Med Chem 42(16):3147–3153. https://doi.org/10.1021/jm990111c

Immer HU, Abraham NA, Nelson VR, Robinson WT, Sestanj K (1976) Peptides 1976: proceedings of the fourteenth European peptide symposium. Universite de Bruxelles, Belgium

Iwai LK, Duranti MA, Abel LCJ, Juliano MA, Kalil J, Juliano L, Cunha-Neto E (2001) Retro-inverso peptide analogues of *Trypanosoma cruzi* B13 protein epitopes fail to be recognized by human sera and peripheral blood mononuclear cells. Peptides 22(6):853–860. https://doi.org/10.1016/S0196-9781(01)00409-0

Kumar Sharma A, Sharma R, Dev Sarma H, Mukherjee A, Das T, Satpati D (2023) Design, synthesis and evaluation of 177Lu-labeled inverso and retro-inverso A9 peptide variants targeting HER2-overexpression. Bioorg Chem 140:106761. https://doi.org/10.1016/j.bioorg.2023.106761

Li C, Pazgier M, Li J, Li C, Liu M, Zou G, Li Z, Chen J, Tarasov SG, Lu W-Y et al (2010) Limitations of peptide retro-inverso isomerization in molecular Mimicry. J Biol Chem 285(25):19572–19581. https://doi.org/10.1074/jbc.M110.116814

Li H, Guo Y, Deng J, Gali H, Weedin EA, Burks HR, Craig LB, Yu X (2022) GnRH receptor-activating autoantibodies in polycystic ovary syndrome: identification of functional epitopes and development of epitope mimetic inhibitors. Endocrine 75(3):959–963. https://doi.org/10.1007/s12020-021-02944-2

Li CW, Osman R, Menconi F, Hou H, Schechter C, Kozhakhmetova A, Tomer Y (2023) Effective inhibition of thyroid antigen presentation using retro-inverso peptides in experimental autoimmune thyroiditis: a pathway toward immune therapies of thyroid autoimmunity. Thyroid 33(4):492–500. https://doi.org/10.1089/thy.2022.0511

Liu Y, Mei L, Yu Q, Zhang Q, Gao H, Zhang Z, He Q (2015) Integrin αvβ3 targeting activity study of different retro-inverso sequences of RGD and their potentiality in the designing of tumor targeting peptides. Amino Acids 47(12):2533–2539. https://doi.org/10.1007/s00726-015-2043-9

Liu Y, Hou Q, Wang R, Liu Y, Cheng Z (2023) FOXO4-D-retro-inverso targets extracellular matrix production in fibroblasts and ameliorates bleomycin-induced pulmonary fibrosis in mice. Naunyn-Schmiedeberg's Arch Pharmacol 396(10):2393–2403. https://doi.org/10.1007/s00210-023-02452-2

Lombardi A, Concepcion E, Hou H, Arib H, Mezei M, Osman R, Tomer Y (2020) Retro-inverso D-peptides as a novel targeted immunotherapy for type 1 diabetes. J Autoimmun 115:102543. https://doi.org/10.1016/j.jaut.2020.102543

Loudon GM, Almond M, Jacob JN (1981) Mechanism of hydrolysis of N-(1-aminoalkyl)amides. J Am Chem Soc 103(15):4508–4515. https://doi.org/10.1021/ja00405a036

Luo Q, Zhang Y, Wang Z, Sun Y, Shi L, Yu Y, Shi J, Hu Z, Wang F (2022) A novel peptide-based probe 99mTc-PEG6-RD-PDP2 for the molecular imaging of tumor PD-L2 expression. Chin Chem Lett 33(7):3497–3501. https://doi.org/10.1016/j.cclet.2022.02.068

Ma S, Yang K, Li Z, Li L, Feng Y, Wang X, Wang J, Zhu Z, Wang Z, Wang J et al (2023) A retro-inverso modified peptide alleviated ovalbumin-induced asthma model by affecting glycerophospholipid and purine metabolism of immune cells. Pulm Pharmacol Ther 78:102185. https://doi.org/10.1016/j.pupt.2022.102185

MacDonald SA, Willson CG, Chorev M, Vernacchia FS, Goodman M (1980) Peptide sweeteners. 3. Effect of modifying the peptide bond on the sweet taste of L-aspartyl-L-phenylalanine methyl ester and its analogs. J Med Chem 23(4):413–420. https://doi.org/10.1021/jm00178a012

Masłowska K, Redkiewicz P, Halik PK, Witkowska E, Tymecka D, Walczak R, Choiński J, Misicka A, Gniazdowska E (2023) Scandium-44 radiolabeled peptide and peptidomimetic conjugates targeting neuropilin-1 co-receptor as potential tools for cancer diagnosis and anti-angiogenic therapy. Biomedicines. https://doi.org/10.3390/biomedicines11020564

Mikulic M (2024) Revenue of the worldwide pharmaceutical market from 2001 to 2023. https://www.statista.com/statistics/263102/pharmaceutical-market-worldwide-revenue-since-2001/#:~:text=The%20global%20pharmaceutical%20market%20has,billion%20dollars%20compared%20to%202022

Momany FA (1981) Conformational analysis and polypeptide drug design. In: Metzger RM (ed) Crystal cohesion and conformational energies. Springer, Berlin, pp 41–79

Mourão CBF, Brand GD, Fernandes JPC, Prates MV, Bloch C Jr, Barbosa J, Freitas SM, Restano-Cassulini R, Possani LD, Schwartz EF (2020) Head-to-tail cyclization after interaction with trypsin: a scorpion venom peptide that resembles plant cyclotides. J Med Chem 63(17):9500–9511. https://doi.org/10.1021/acs.jmedchem.0c00686

Muller S, Benkirane N, Guichard G, Regenmortel MHVV, Brown F (1998) The potential of retro-inverso peptides as synthetic vaccines. Expert Opin Investig Drugs 7(9):1429–1438. https://doi.org/10.1517/13543784.7.9.1429

Nagpal S, Kaur KJ, Jain D, Salunke DM (2002) Plasticity in structure and interactions is critical for the action of indolicidin, an antibacterial peptide of innate immune origin. Protein Sci 11(9):2158–2167. https://doi.org/10.1110/ps.0211602

Ostankovitch M, Guichard G, Connan F, Muller S, Chaboissier A, Hoebeke J, Choppin J, Briand J-P, Guillet J-G (1998) A partially modified retro-inverso pseudopeptide modulates the cytokine profile of CTL specific for an influenza virus epitope1. J Immun 161(1):200–208. https://doi.org/10.4049/jimmunol.161.1.200

Pallai P, Goodman M (1982) Preparation of optically pure monoacyl 2-alkyl gem-diamines from peptide amides. Chem Commun 5:280–281. https://doi.org/10.1039/C39820000280

Pallai PV, Richman S, Struthers RS, Goodman M (1983) Approaches to the synthesis of retro-inverso peptides. Int J Pept Protein Res 21(1):84–92. https://doi.org/10.1111/j.1399-3011.1983.tb03081.x

Pandey P, Nguyen N, Hansmann UHE (2020) d-Retro inverso amylin and the stability of amylin fibrils. J Chem Theory Comput 16(8):5358–5368. https://doi.org/10.1021/acs.jctc.0c00523

Parrasia S, Rossa A, Roncaglia N, Mattarei A, Honisch C, Szabò I, Ruzza P, Biasutto L (2023) DA7R: a 7-letter zip code to target PDAC. Pharmaceutics. https://doi.org/10.3390/pharmaceutics15051508

Pelay-Gimeno M, Tulla-Puche J, Albericio F (2013) Head-to-side-chain cyclodepsipeptides of marine origin. Mar Drugs 11:1693–1717. https://doi.org/10.3390/md11051693

Radhakrishna AS, Parham ME, Riggs RM, Loudon GM (1979) New method for direct conversion of amides to amines. J Org Chem 44(10):1746–1747. https://doi.org/10.1021/jo01324a048

Reckless J, Tatalick LM, Grainger DJ (2001) The pan-chemokine inhibitor NR58-3.14.3 abolishes tumour necrosis factor-alpha accumulation and leucocyte recruitment induced by lipopolysaccharide in vivo. Immunology 103(2):244–254. https://doi.org/10.1046/j.1365-2567.2001.01228.x

Roques BP, Lucas-Soroca E, Chaillet P, Costentin J, Fournié-Zaluski MC (1983) Complete differentiation between enkephalinase and angiotensin-converting enzyme inhibition by retro-thiorphan. PNAS 80(11):3178–3182. https://doi.org/10.1073/pnas.80.11.3178

Shemyakin MM, Ovchinnikov YA, Ivanov VT (1969) Topochemical investigations on peptide systems. Angew Chem Int Ed Engl 8(7):492–499. https://doi.org/10.1002/anie.196904921

Snyder EL, Meade BR, Saenz CC, Dowdy SF (2004) Treatment of Terminal peritoneal carcinomatosis by a transducible p53-activating peptide. PLOS Biol 2(2):e36. https://doi.org/10.1371/journal.pbio.0020036

Statista Team (2024) Pharmaceuticals - United Kingdom. https://www.statista.com/outlook/hmo/pharmaceuticals/united-kingdom

Suzuki R, Masuda R, Yamamoto H, Fukaya H, Hasuda T, Hitotsuyanagi Y (2024) Synthesis and cytotoxicity evaluation of retro-inverso analogue of antitumor bicyclic hexapeptide RA-VII. Tetrahedron Lett 134:154839. https://doi.org/10.1016/j.tetlet.2023.154839

Takayama K, Hitachi K, Okamoto H, Saitoh M, Odagiri M, Ohfusa R, Shimada T, Taguchi A, Taniguchi A, Tsuchida K et al (2022) Development of myostatin inhibitory d-peptides to enhance the potency, increasing skeletal muscle mass in mice. ACS Med Chem Lett 13(3):492–498. https://doi.org/10.1021/acsmedchemlett.1c00705

Taylor EM, Otero DA, Banks WA, O'Brien JS (2000) Retro-inverso prosaptide peptides retain bioactivity, are stable in vivo, and are blood-brain barrier permeable. J Pharmacol Exp Ther 295(1):190–194

van den Akker GGH, Steijns JSJJ, Stassen RHMJ, Wasilewski GB, Peeters LCW, Wijnands KAP, Schurgers LJ, Caron MMJ, van Rhijn LW, Welting TJM (2023) Development of a cyclic-inverso AHSG/fetuin a-based peptide for inhibition of calcification in osteoarthritis. Osteoarthr Cartil 31(6):727–740. https://doi.org/10.1016/j.joca.2022.11.007

Verdini AS, Silvestri S, Becherucci C, Longobardi MG, Parente L, Peppoloni S, Perretti M, Pileri P, Pinori M (1991) Immunostimulation by a partially modified retro-inverso-tuftsin analog containing Thr1.Sum.psi.[NHCO](R, S)Lys2 modification. J Med Chem 34:3372–7339. https://doi.org/10.1021/JM00116A005

Vogler K, Lanz P (1966) Synthesis of all-D-isomers in the bradykinin series. In: Erdös EG, Back N, Sicuteri F, Wilde AF (eds) Hypotensive Peptides. Springer, Berlin

Wang R, Shen Q, Li X, Xie C, Lu W, Wang S, Wang J, Wang D, Liu M (2018) Efficacy of inverso isomer of CendR peptide on tumor tissue penetration. Acta Pharm Sin B 8(5):825–832. https://doi.org/10.1016/j.apsb.2018.06.006

Wang Y, Sun Y, Zeng X, Zhuang R, Huang J, Zhang X, Guo Z, Li Y (2024) 68Ga-labeled TMTP1 modified with d-amino acid for positron emission tomography diagnosis of highly metastatic hepatocellular carcinoma. J Med Chem 67(3):2165–2175. https://doi.org/10.1021/acs.jmedchem.3c02090

Wei X, Zhan C, Chen X, Hou J, Xie C, Lu W (2014) Retro-inverso isomer of angiopep-2: a stable d-peptide ligand inspires brain-targeted drug delivery. Mol Pharm 11(10):3261–3268. https://doi.org/10.1021/mp500086e

Werner HM, Cabalteja CC, Horne WS (2016) Peptide backbone composition and protease susceptibility: impact of modification type, position, and tandem substitution. ChemBioChem 17(8):712–718. https://doi.org/10.1002/cbic.201500312

White CJ, Yudin AK (2011) Contemporary strategies for peptide macrocyclization. Nat Chem 3(7):509–524. https://doi.org/10.1038/nchem.1062

Wieland T, Penke B, Birr C (1972) Antamanide. XVI. All-D-retro-antamanide and D-tyr 6 -all-D-retro-antamanide. Justus Liebigs Ann Chem 759:71–75

Ying M, Shen Q, Liu Y, Yan Z, Wei X, Zhan C, Gao J, Xie C, Yao B, Lu W (2016) Stabilized heptapeptide A7R for enhanced multifunctional liposome-based tumor-targeted drug delivery. ACS Appl Mater Interfaces 8(21):13232–13241. https://doi.org/10.1021/acsami.6b01300

Zhang M, Du M, Qi X, Wang Y, Li G, Xu C, Zhang X (2022) Retro-inversion follicle-stimulating hormone peptide-modified nanoparticles for delivery of PDK2 shRNA against chemoresistant ovarian cancer by switching glycolysis to oxidative phosphorylation. Cancer Nanotechnol 13(1):23. https://doi.org/10.1186/s12645-022-00129-8