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Synthesis of Rhenium-Centric Reverse Turn Mimics

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Abstract: Molecular scaffolds have been shown to facilitate and stabilise secondary structural turn elements, with a central core-arranging functionality in a defined three-dimensional orientation. In a peptide-based molecular imaging probe, this approach is of particular value as it would essentially "hide" a metal radioisotope within the ligand framework, making the labelling element a critical component of the receptor-bound structure. Starting from a 1,2-diaminoethane loaded 2-chlorotrityl resin, a versatile set of triamine ligand systems were synthesised by using solid-phase Fmoc-based peptide chemistry. The resultant resin-bound peptides then underwent amide reduction by treatment with borane-THF at 65 °C. This provided complete conversion to the corresponding polyamine entities in high purity for the majority of the amino acids utilised. The triamines were then coordinated on solid support by using $[NEt_4]_2[Re(CO)_3(Br)_3]$ followed by resin cleavage and HPLC purification, to give the desired rhenium coordinated species. We have shown that amino acid sequences can be assembled, reduced and coordinated on-resin, resulting in a versatile set of metal–ligand constructs. These studies could be expanded to generate libraries of turn-based peptidomimetics containing Re/Tc^{I} organometallic scaffolds, with the intention of developing an improved approach for finding new diagnostic and therapeutic radiopharmaceutical entities.

Keywords: molecular diversity · peptidomimetics · radiopharmaceuticals · rhenium · solid-phase synthesis

Introduction

Proteins are the primary means of cellular communication in most biological systems and with such a vast array of amino acid sequence assemblies available, they have the potential to provide an abundance of distinct signalling messages. The majority of this cellular communication occurs through the interaction of protein messengers with cell surface receptors, relying on a defined spatial orientation of amino acid functionality. However, the development of peptides as drug candidates has proven difficult due to poor bioavailability, rapid enzymatic degradation and limited shelf stability.[1] Although a number of clinically relevant peptides have been identified as drug targets,[2] it is exceedingly likely that future lead compounds will emerge from research directed at identifying non-peptidic structures. Therefore, there is a need in drug development to generate an efficient method of conveying the three-dimensional information of peptide pharmacophores into non-peptidic drug candidates.

Reverse turns are common motifs found in the inherent organisation of numerous biomolecules, encompassing a wide range of structures with a well-defined three-dimensional arrangement.[3] Recognition of turn elements generally only involves an interaction between the amino acid side chains and the receptor. This insinuates that the peptide backbone is not a required constituent, and could be replaced by a scaffold, displaying functionality in the correct orientation for ligand–receptor interaction to take place.

One area of research in which scaffolds have been extensively studied is in the development of G protein-coupled receptor (GPCR) targeting analogues.[4,5] GPCRs play an important role in a variety of diseases from cancer and diabetes, to inflammatory and respiratory disorders,[6] and are therefore of great interest in drug development. They have become the most heavily investigated drug targets in the pharmaceutical industry. In fact, around 40 % of all prescription drugs currently on the market act by targeting GPCRs either directly or indirectly.[7,8] Upon cross-target analysis it has also been revealed that many GPCR ligands have common structural binding motifs.[9,10] These so-called "privileged structures" have demonstrated that a single molecular framework can be utilised to create libraries of compounds targeting a diverse set of GPCRs through structural modification of the functionality extending from a central core.

Metal-based scaffolds as secondary structure mimics: A metal-based molecular scaffold could also act as a privileged structure, organising the presentation of ligands in a defined

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three-dimensional orientation at a targeted binding site. Whereas carbon is limited to linear, trigonal planar and tetrahedral geometries, transition metals have higher structural diversity enabling a further exploration of chemical space.

This concept of a metal-based scaffold has proven to be effective at generating high affinity ligands. Katzenellenbogen's group, revealed that a cyclopentadienyl rhenium(I) tricarbonyl scaffold, with phenol functionality radiating from the central core, could yield compounds with nanomolar affinity for the human estrogen receptor.[11] Meggers has shown that the rigid backbone conformation of ruthenium metal complexes, functionalised with various ligand sets, produces structures similar to that of a peptide reverse turn, providing potent inhibitors for a number of protein kinases.[12] The literature also supports the use of a ferrocenyl moiety to induce the formation of a peptide turn,[13] in which the ferrocene derivatives can be coupled to subsequent amino acids and peptides to give the corresponding β-sheet-like bioconjugates.

Using a metal-based scaffold we can move away from the traditional peptide-based molecular imaging probe and progress toward that of a privileged structure, in which the radiometal acts as a skeleton displaying the ligand framework in a desired orientation. These proposed metal-centric peptide mimics, would result in the formation of an integrated radiopharmaceutical, one in which the labelling element forms a critical component of the receptor–ligand structure (Figure 1). This is contrary to the current, most widely utilised pendant approach in developing a receptor binding radiopharmaceutical, where the radioisotope is attached to the peptide via a side chain lysine, or through functionalization of the carboxy or amine terminus of the ligand.[14,15]

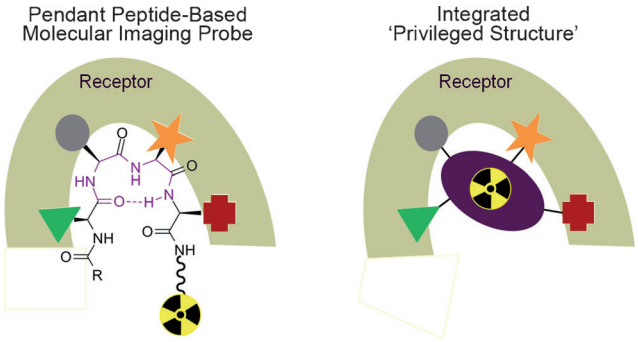


Figure 1. A pendant versus integrated approach to designing radiopharmaceuticals. The pendant design places the radiolabel at a location removed from the biologically active site. In contrast, the integrated privileged structure incorporates the radiolabel directly into the framework of the receptor ligand.

An integrated secondary structural mimic would allow for a significant decrease in the molecular weight of the ligand, improved in vivo behaviour, and the ability to perform structure–activity studies by simply modifying the amino acid side chain functionality extending from the central core. With this design, prior to metal coordination, the free ligand will display low binding affinity toward the related biological receptor. However, once the biomolecule is coordinated to the Re/Tc tricarbonyl core, it will be locked in the desired turn orientation, resulting in a high-affinity ligand; this means that metal incorporation is required in order for biological activity to be present.

This project aims to design a synthetic approach towards generating a diverse set of non-peptidic metal-based turn mimics. We have accomplished this through the preparation of rhenium-based scaffolds functionalised with various triamine coordination spheres. These metal constructs can have application in a wide variety of biological ligands that contain a reverse-turn, providing a new approach for researchers who are involved in the radiolabelling of peptide-like molecules.

Results and Discussion

The synthetic approach utilised focuses on the preparation of a triamine chelation core, comprised of three variable positions allowing for chemical and spatial diversity (Figure 2).

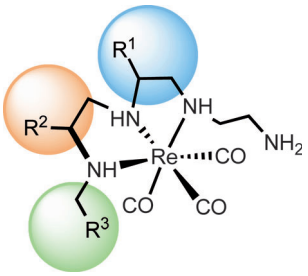


Figure 2. Three areas of spatial diversity (R^1, R^2 and R^3) present in a rhenium-based scaffold containing a tridentate {NNN} chelation sphere.

In this design, R^1 and R^2 can consist of any D, L or unnatural amino acid residue, while R^3 can be any acid or anhydride-containing compound (such as hydrocinnamic acid, acetic anhydride or 4-methylvaleric acid). Simply modifying the functionality present in these locations will allow for the potential to create a diverse collection of chemical entities. For example, if 40 different compounds were utilised in each of the three variable sites, a library consisting of approximately 64 000 compounds would be generated.

The advantage of this synthetic approach is that the central core is composed of a transition metal complex. Organic compounds containing sp^2 and sp^3 carbon atoms have limited variability in their three-dimensional orientation. The use of a metal complex enables further exploration of chemical space due to the existence of a variety of available geometries and coordination numbers.[16] These metal complexes will have a unique structural distinction from regular organic constructs, which would then allow for an increase in the number of chemical entities within a library that are able to bind to known biological targets.

The crystal structure of a simple, unsubstituted rhenium triamine has previously been reported.[17] From these data it can be estimated that the distance between N^1 and N^3 in the structure of a rhenium diethylenetriamine complex is approximately 3.5 Å (Figure 3). It has also been stated that the

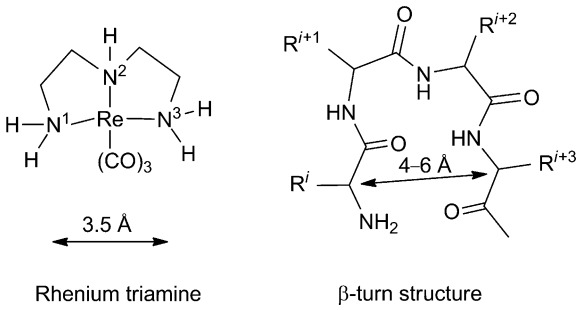


Figure 3. Comparison of a simple rhenium triamine with the distance between N^1 and N^3 estimated at 3.5 Å, and a β-turn structure with a measured distance of 4–6 Å between α-carbon i and α-carbon $i+3$.

complex fits in a sphere of 7 Å in diameter.[18] This small size correlates well with the dimensions of a β-turn structure. The distance between the α-carbon of residue i and the α-carbon of residue $i+3$ has been determined experimentally to be between 4–6 Å in various naturally occurring reverse turn structures.[19] Therefore, due to their small compact structure these rhenium diethylenetriamine metal constructs are of an appropriate scale to have application in the targeting of receptors that bind biologically active molecules in a turn conformation.

In order to further validate the use of rhenium/technetium-containing tridentate chelation systems as turn mimics, we have postulated that the skeleton of a Re/Tc complex consisting of a tridentate {NNN} ligand system, not only is of the appropriate size but also has a spatial orientation similar to that of a naturally occurring reverse turn peptide.[20] X-ray crystallography data (.cif) of a {NNN} tricoordinate $Re(CO)_3$ complex[18] was fit with the conformation of a published NMR structure for Met-enkephalin,[21] a peptide known to exist in a β-turn conformation (Figure 4, shown in cyan, by using the modelling software Sybyl 7.3, Tripos, St. Louis).

The three nitrogen atoms of the rhenium chelator were lined up with the amide backbone atoms of the turn region of enkephalin. Although enkephalin has substantial flexibility, the folded forms of the peptide overlay well with the {NNN} tridentate chelation system. When these two structures are superimposed, good atom overlap is produced, with a calculated three-atom fit of 0.48 Å root mean square (RMS) shown in blue. This demonstrates that tridentate rhenium/technetium(I) tricarbonyl complexes are able to exist in the proper orientation to mimic peptide-based turns.

C-terminal carboxylic acid; general approach: The first step in generating a library of rhenium-containing turn mimics

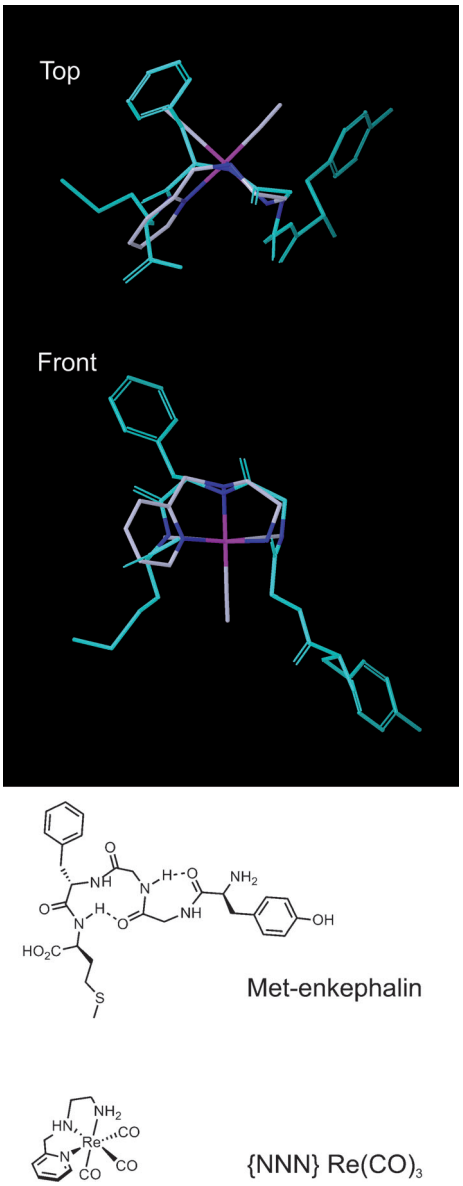


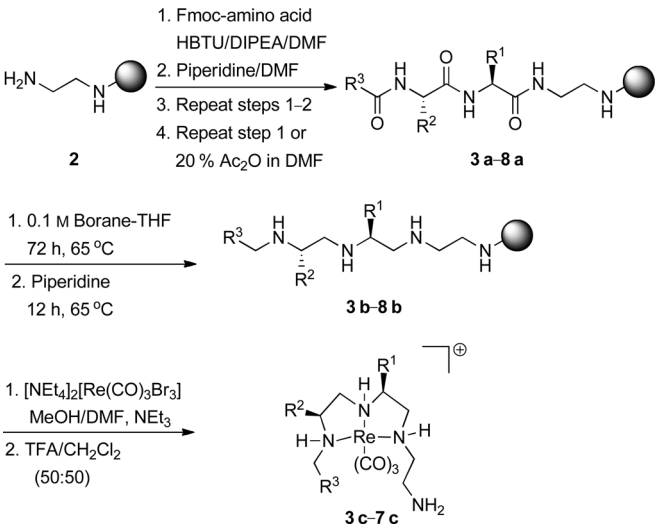
Figure 4. Modelling of NMR spectroscopy data for Met-enkephalin (cyan)[21] and X-ray crystallography data of a tridentate {NNN} $Re(CO)_3$ chelation system[18] gives a three-atom fit of 0.48 Å RMS (blue).

involves the development of a chelation sphere that promotes chemical diversity. This can be accomplished through the use of amino acid building blocks. However, a peptide backbone consisting entirely of amide functionality is undesirable as it will not readily coordinate to the Re/Tc^I tricarbonyl core. One way to overcome this obstacle is to assemble the desired peptide sequence and then reduce the backbone amides to create a polyamine species, preserving the functionality present in the amino acid side chains. Acyclic triamine moieties are known to form highly stable, hydrophilic cationic complexes upon coordination to rhenium/technetium.[18] When evaluated in vivo, these metal compounds display rapid clearance from blood and organs. This indicates that triamines are biologically relevant alternatives

to more common ligands, such as histidine or iminodiacetic acid, which form neutral metal complexes with Re^{I} upon coordination.

The research groups of Hall and Houghten have widely reported on the synthesis of polyamines through a borane induced amide reduction while on a solid-phase polymer support.[22–26] The initial approach that we attempted involved the synthesis of a tetrapeptide loaded onto a chlorotrityl resin through the C-terminal carboxylic acid (Scheme 1). Once the linear peptide sequence was assembled the resin was treated with borane-tetrahydrofuran (BH_3-THF) to reduce the backbone amide functionality. The resultant polyamine was then treated with $[NEt_4]_2[Re(CO)_3Br_3]$ while still attached to the solid support, followed by trifluoroacetic acid (TFA) cleavage. This synthetic route was designed to produce the unnatural amino acid **1**, containing a free C-terminal carboxylic acid and a protected N terminus. This would allow for easy incorporation into larger peptide structures where it could act as a turn inducing element. However, after numerous attempts and modifications this method was abandoned as upon analysis of the filtrate it was determined that the peptide was being cleaved from the solid support during the on-resin reduction step. There is support in the literature for a carboxylic triphenylmethyl ester being stable to borane reduction conditions, as according to Greene, a trityl protected acid is expected to be inert to diborane at 0 °C.[27] However, under our conditions of heating at 65 °C, it was observed that the ester attachment was cleaved, resulting in no observable amount of the desired product being salvaged.

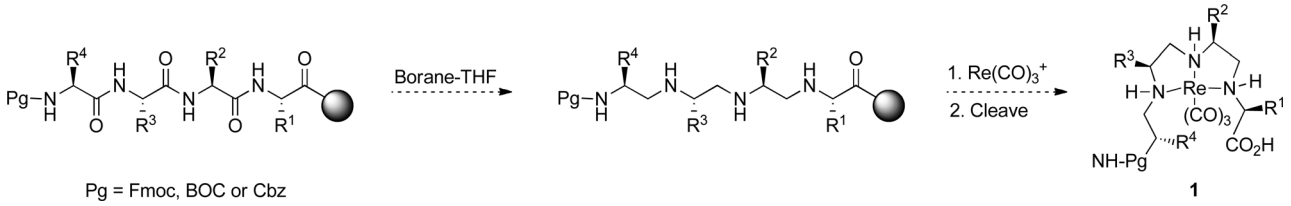
C-terminal diaminoethane; general approach: A new approach was then developed, in which a diamino moiety was loaded onto the trityl resin as the C-terminal attachment.[22,23] The technique of having an amine appended to the trityl resin proved more successful than that of a C-terminal carboxylic acid. Starting with 1,2-diaminoethane preloaded onto a 2-chlorotrityl resin **2**, the respective amino acid residues were constructed onto the solid support by using standard 9-fluorenylmethoxycarbonyl (Fmoc)-based solid-phase peptide chemistry (Scheme 2). In this design the N-terminal amino acid was substituted with a more structurally simple modified acid or anhydride moiety, thus limiting the potential complexity caused by having an N-terminal amine protecting group. The first two amino acids were assembled onto the resin, followed by a third coupling reaction or acetylation giving variable functionality in R^1, R^2 and R^3.



Scheme 2. General synthetic route towards the generation of rhenium-centric reverse turn peptidomimetics, with the amino acid side chain protecting groups removed in the final rhenium coordinated products **3c**–**7c** by TFA treatment.

The resultant resin-bound peptides **3a**–**8a** then underwent a 72 h amide reduction through reaction with a 0.1 M BH_3-THF solution at 65 °C. After treatment with borane, complete conversion to the polyamine entities was achieved in high purity for the majority of the amino acids utilised. However, the generated secondary amines were found to form very robust BH_3-amine adducts, the cleavage of which requires an extensive work-up. A mild oxidative method was first attempted that involved the addition of iodine to the resin in a THF buffered solvent mixture containing acetic acid and diisopropylethylamine.[22] Iodine is used to cleave the borane–amine complexation and the buffered solution traps the formed hydroiodic acid. This prevents the solution from becoming too acidic, which would result in the peptide being prematurely released from the solid support. Although this approach produced the desired triamine product, a low purity was consistently observed by HPLC analysis.

An alternative work-up procedure, involving the addition of piperidine at 65 °C, was utilised for ensuing attempts.[26] The potential for racemisation to occur during both reduction and basic work-up steps has been examined previously and it was reported that this methodology does not affect the stereochemistry present at the α-carbon of the amino acid residues.[26] Upon treatment with piperidine at 65 °C for 12 h, the desired diethylenetriamine species **3b**–**8b** were ob-



Scheme 1. Initial attempt at synthesising a rhenium-containing unnatural amino acid by on-resin amide reduction methodology.

tained in high purity. The triamines were then coordinated on solid support with $[NEt_4]_2[Re(CO)_3(Br)_3]$ to give the resultant resin-bound metal complexes. In a final step, the metal complexes were treated with TFA to afford cleavage from the resin and removal of all side chain protecting groups. Due to the presence of a primary amine at the C terminus of the peptide, the rhenium coordination must take place on-resin as opposed to via solution-phase methodologies. This amine would act as a fourth chelation site, competing with the tridentate coordination sphere created by the amide reduction, resulting in a complicated mixture of products. Treatment of the polyamine with rhenium results in the formation of isomers of the desired product due to the presence of secondary amines.[28] The crude material was purified by preparative HPLC, giving the desired rhenium coordinated species **3c**–**7c**, which were further analysed by ESI-MS as well as by NMR spectroscopy. Reaction progress and purity of each synthetic step was monitored by taking a small portion of the resin, treating it with TFA to afford cleavage from the solid support, followed by LC-MS analysis.

Rhenium complexes: In an attempt to test the limits of this synthetic approach, amino acid residues containing an array of side chain functionality and protecting groups were incorporated into the various rhenium complexes. Borane is a mild reducing agent that should reduce the amide functionality present in the peptide backbone, leaving the side chain protected functionality in its current state. It has been previously reported that *t*Bu-protected carboxylic acid containing amino acids, such as aspartic acid and glutamic acid, will undergo reduction to the corresponding *t*Bu-protected ethers under borane reduction conditions.[22,26] However, these residues were still employed because we wanted to ensure that rhenium coordination was unaffected. Boc-protected histidine has been reported to not be able to withstand the borane reduction protocol[22] and therefore has been left out of this investigation.

Using this synthetic approach, complete reduction to the desired triamine species was achieved for the majority of the amino acids utilised (Table 1); HPLC purity and ESI-MS data for seven different systems are shown in Table 2. However, we did encounter some synthetic challenges along the way. Glutamine is often a problematic amino acid when located near the N terminus of the peptide chain as it undergoes cyclisation, forming the corresponding pyroglutamate lactam.[29,30] This is a very common post-translational enzymatic modification found in many proteins and natural peptides. However, this cyclisation will also occur spontaneously under both acidic and basic conditions.

In this instance, even though glutamine was not positioned near the N terminus of peptide **5**, once cleaved from the resin, the residue is in very close proximity to the C-terminal amine functionality. Since resin cleavage occurs under acid conditions, the amide bond connecting glutamine to the diamine linker could react with the side chain of the glutamine residue forming a six-membered ring. Alternatively, the C-terminal primary amine may react with the amide side chain of the glutamine residue, resulting in the formation of a nine-membered ring. Even though both of these options seem unfavourable, the HPLC-MS data of the linear

Table 1. Summary of amino acid compatibility. Amino acid building blocks utilised in positions 1, 2 and 3, which remain intact upon BH_3-THF treatment.

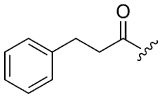
Position 1	Position 2	Position 3
Phe	Ala	Ac = 
Ser	Leu	Hc = 
Thr	Lys Tyr Val	

Table 2. HPLC purity and exact mass found for the linear peptide, reduced sequence and rhenium complex after TFA micro-cleavage.

Compound	R^1	R^2	R^3	HPLC purity [%]	M_W calcd[a]	M_W found
3a	$CH_2C_6H_5$	CH_3	CH_3	95	321.2	321.3 $[M+H]^+$
3b	$CH_2C_6H_5$	CH_3	CH_3	65[b]	279.3	279.3 $[M+H]^+$
3b	$CH_2C_6H_5$	CH_3	CH_3	96	279.3	279.3 $[M+H]^+$
3c	$CH_2C_6H_5$	CH_3	CH_3	92[c]	549.2	549.2 $[M]^+$
4a	CH_2OtBu	$CH_2C_6H_4OtBu$	$(CH_2)_2C_6H_5$	90	443.2	443.3 $[M+H]^+$
4b	CH_2OtBu	$CH_2C_6H_4OtBu$	$(CH_2)_2C_6H_5$	89	401.3	401.4 $[M+H]^+$
4c	CH_2OH	$CH_2C_6H_4OH$	$(CH_2)_2C_6H_5$	98[c]	671.2	671.3 $[M]^+$
5a	$(CH_2)_2CONH(Trt)$	$CH(CH_3)_2$	CH_3	96[d]	330.2	330.3 $[M+H]^+$
5b	$(CH_2)_3NH(Trt)$	$CH(CH_3)_2$	CH_3	96[e]	274.3	274.3 $[M+H]^+$
5c	$(CH_2)_3NH_2$	$CH(CH_3)_2$	CH_3	99[c]	544.2	544.2 $[M]^+$
6a	$CH(OtBu)CH_3$	$CH_2CH(CH_3)_2$	$(CH_2)_2C_6H_5$	96	407.3	407.3 $[M+H]^+$
6b	$CH(OtBu)CH_3$	$CH_2CH(CH_3)_2$	$(CH_2)_2C_6H_5$	92	365.3	365.4 $[M+H]^+$
6c	$CH(OH)CH_3$	$CH_2CH(CH_3)_2$	$(CH_2)_2C_6H_5$	90[c]	635.3	635.3 $[M]^+$
7a	$CH_2CO(OtBu)$	$(CH_2)_2CO(OtBu)$	$(CH_2)_2C_6H_5$	99	437.2	437.1 $[M+H]^+$
7b	$(CH_2)_2OtBu$	$(CH_2)_3C(OtBu)$	$(CH_2)_2C_6H_5$	98	367.3	367.3 $[M+H]^+$
7c	$(CH_2)_2OH$	$(CH_2)_3COH$	$(CH_2)_2C_6H_5$	99[c]	637.2	637.2 $[M]^+$
8a	$CH_2C_8H_5NBoc$	$(CH_2)_4NHBoc$	CH_3	98	417.3	417.3 $[M+H]^+$
8b	$CH_2C_8H_5NBoc$	$(CH_2)_4NHBoc$	CH_3	–	374.3	–

[a] All protecting groups were removed during TFA micro-cleavage. [b] Mild work-up conditions were attempted with this peptide sequence (I_2 buffered solution as reported by Hall),[22] resulting in a decreased purity. [c] Determined after HPLC purification. [d] Combined value from desired product and cyclised glutamine residue. [e] Purity determined by HPLC-MS mass TIC chromatogram due to the absence of UV active functionality.

peptide sequence **5a** show two different products, one with the desired mass and a second peak with a mass loss equivalent to that of ammonia.

The purity reported in Table 1 for compound **5a** is a combined integration of what we believe to be a cyclised lactam and the desired linear peptide sequence, as cyclisation is apparently inevitable during these highly acidic conditions. It is, however, important to note that this modification only occurs during the micro-cleavage analysis step and that the linear peptide sequence, which remains on the solid support, is still of the correct orientation. Therefore, this outcome does not affect the subsequent reduction and metal coordination reaction steps. A second structural alteration, which occurred to the Gln(Trt) residue, was complete conversion of the amide functionality to the corresponding primary amine during the borane reduction step. This reduced amide side chain results in the formation of an ornithine amino acid, which could still be desirable in a library synthesis. Since this modified product **5b** was obtained in high purity, the reaction to form the rhenium coordinated species was still attempted. Also, as previously reported, we observed a reduction of the glutamic acid and aspartic acid side chains in **7** to the corresponding primary alcohol functionality.

The Boc-protected tryptophan residue in **8** proved to be most problematic during the reaction, as it appeared to decompose or polymerise upon BH_3-THF treatment. Houghten also reported that aggregates or polymerised products were observed when using tryptophan residues;[26] however, Hall witnessed that the Trp(Boc) residue remained intact throughout the synthesis of their polyamines.[23] Due to these contrasting results, both the piperidine, as well as the mild oxidative iodine work-ups were attempted with compound **8b**. Still, no discernable amount of the desired triamine was obtained; therefore, rhenium coordination was not attempted with this system. Also, since the HPLC-MS chromatograms displayed multiple peaks, none of which matched the molecular weight of the desired product, it was difficult to determine if the Lys(Boc) had endured the borane reductions conditions. However, since the structure of lysine and ornithine are very similar, with lysine only containing one additional methylene group in the side chain, for future attempts it is believed that lysine would be able to withstand the reaction conditions as well. Apart from these limitations associated with tryptophan and amino acid residues containing acid/amide side chain functionality, there remains ample diversity available with this protocol through the use of other D, L and unnatural amino acids.

On-resin complexation and analysis: The resultant triamine species **3b–7b** were coordinated on-resin by using the rhenium(I) reagent $[NEt_4]_2[Re(CO)_3(Br)_3]$, which was synthesised according to a procedure published by Alberto.[31] The complexation reaction was stirred for 4 h at 65 °C, upon which time the peptide was analysed by HPLC-MS. One disadvantage of using a secondary amine-based coordination sphere is that diastereomers can form upon metal complexation.[28] However, in this particular instance where the end goal is creating a library of metal constructs, the formation of diastereomers may in fact be beneficial in that it increases the likelihood of attaining the desired conformation for biological affinity, even though only one of the isomers may be active. Since there are three secondary amines in the coordination sphere, there is the potential to produce eight different rhenium-containing products. However, only a maximum of four isomers were ever seen upon cleavage from the solid support for **3c–7c**, and the major isomer was isolated for characterisation by preparative HPLC in high purity.

After HPLC purification, **3c–7c** were analysed by ^1H and ^1H-^1H gCOSY NMR spectroscopy. Upon coordination to rhenium, the methylene groups in the diethylenetriamine backbone become diastereotopic in nature, due to the locked conformation enforced by the metal. Also upon coordination, the NH protons no longer exchange with the solvent, and therefore show correlations to the aliphatic region of the spectrum, enabling full characterisation of the complexes. These purified rhenium triamine species were also analysed by HPLC-MS, with the effectiveness of this technique in generating rhenium-centric peptide mimic structures illustrated in Figure 5 and Figure 6.

The analytical HPLC, as well as ESI mass spectral data for compound **4** through each of the three reaction steps are illustrated in Figure 5. This peptide was synthesised to contain two *tert*-butyl protecting groups, adding further complexity due to the presence of an additional functionality. The top panel of this Figure shows the crude HPLC UV chromatogram of the linear peptide sequence **4a**, and displays a peak corresponding to the desired species in 90% purity. The identity of the compound was verified by ESI mass spectrometry with the desired $[M+H]^+$ exact mass presented in the inset on the right-hand side. Panel B displays the crude HPLC trace of the peptide following on-resin BH_3-THF reduction and subsequent piperidine work-up conditions, giving compound **4b** in 89% purity. The corresponding mass spectrum confirms the identity of the triamine species, as the retention time of these two compounds does not vary that greatly by HPLC. The bottom panel shows an analytical trace of the rhenium complex **4c** after preparative HPLC purification, with the corresponding mass spectrum verifying the presence of rhenium through observation of the characteristic isotopic signature.

Confirmation of purity and reaction progression throughout the three synthetic steps is also shown for compound **6** (Figure 6). With this example, however, there is a visible difference in the crude HPLC retention time for the linear peptide sequence **6a** and that of the reduced peptide **6b**. This clear separation in retention time confirms that the on-resin reduction does in fact go to completion as there is no visible starting material remaining after reacting for 72 h at 65 °C. This validates the borane-THF on-resin reduction approach as a viable technique to generate libraries of metal constructs. In a library system, reaction progress is not always easily monitored; therefore, this on-resin reduction and subsequent coordination approach, which we have de-

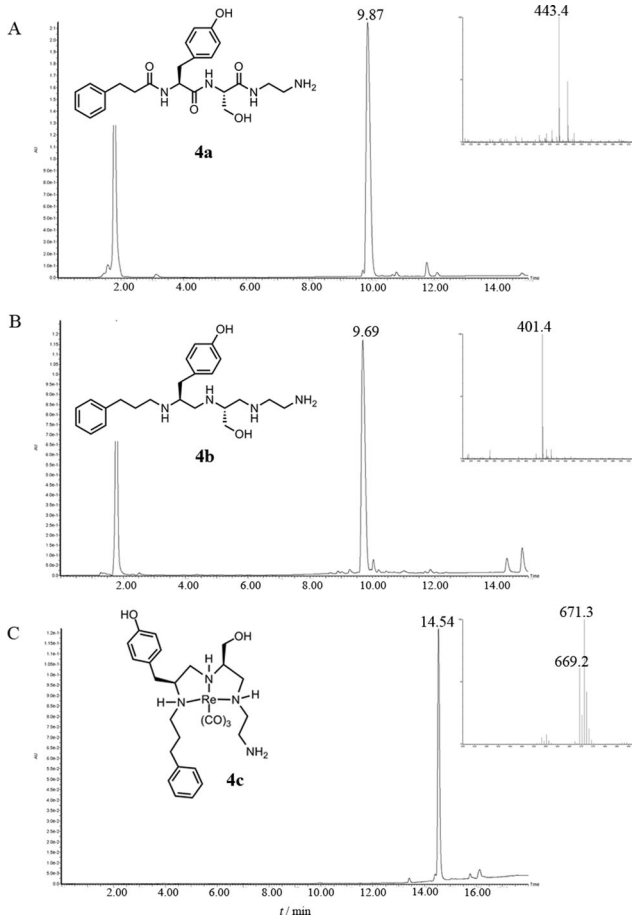


Figure 5. Analytical HPLC and ESI-MS (inset) traces for the peptide derivative **4**. A) The linear peptide **4a**. B) The reduced triamine **4b**, following treatment with borane-THF and piperidine work-up. C) The rhenium coordinated triamine **4c** after HPLC purification.

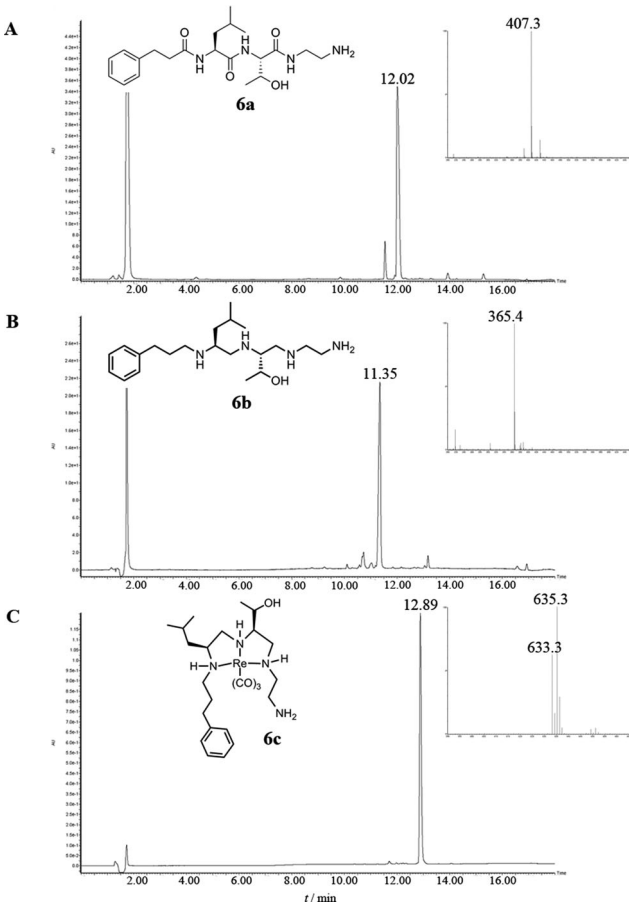


Figure 6. Analytical HPLC and ESI-MS traces for the peptide derivative **6**. A) The linear peptide **6a**. B) The peptide **6b**, following treatment with borane-THF and piperidine work-up. C) The rhenium coordinated triamine **6c** after HPLC purification.

veloped, will provide the desired rhenium diethylenetriamine species in high purity. Such a reliable approach would be especially advantageous in a one-bead one-compound methodology in which identity of peptide sequences and verification of purity is rarely performed prior to biological screening.[32]

Conclusion

In conclusion, it has been demonstrated that amino acid sequences can be assembled, reduced and coordinated on-resin, resulting in a versatile set of rhenium-ligand constructs for use as reverse-turn mimics. Due to the existence of a variety of available geometries and coordination numbers, these metal complexes will have a unique structural distinction from regular organic molecules, potentially allowing for an increase in the number of chemical entities displaying affinity towards various biological targets. Through the use of simple D, L and unnatural amino acid residues, this synthetic approach will allow for the generation of a diverse set of turn-based peptidomimetics containing a metal scaffold, and aide in the discovery of new drug-like diagnostic and therapeutic radiopharmaceutical entities.

Experimental Section

General procedures and materials: All chemicals were purchased from commercial sources and used without further purification unless otherwise noted. Analytical HPLC was performed by using a Waters Atlantis T3 C18 column 4.6×150 mm, 5 μm or a Waters Sunfire C18 column 4.6×150 mm, 5 μm. Preparative HPLC was performed by using a Waters Atlantis T3 Prep C18 OBD column 19×150 mm, 5 μm or a Waters Sunfire Prep C18 OBD column 19×150 mm, 5 μm. A gradient system was used consisting of: CH_3CN+0.1% TFA (solvent A) or MeOH (solvent B)+0.1% TFA and H_2O+0.1% TFA (solvent C) and the absorbance was detected by using a Waters 2998 photodiode array detector. Electrospray ionisation mass spectra were obtained by using a Micromass Quatro Micro LCT mass spectrometer. NMR spectroscopy data were obtained on the Varian Inova 600 MHz in $[D_4]$MeOH with the chemical shifts referenced to solvent signals ($[D_4]$MeOH, ^{1}H 3.31 ppm) relative to TMS. For compounds containing rhenium, both ^{185}Re and ^{187}Re were observed in the ESI-MS and HRMS chromatogram in the correct isotopic ratio; however, only the more abundant ^{187}Re molecular weight is reported in this section.

General peptide synthesis: Fully protected resin-bound peptides were synthesised by standard Fmoc solid-phase peptide chemistry by using manual peptide synthesis methods. 2-Chlorotrityl resin preloaded with 1,2-diaminoethane (1.0 mmol, loading 0.9 mmol g^{-1}) was utilised as the solid support. All *N*-Fmoc amino acids were employed. Fmoc removal was achieved by treatment with piperidine (20%) in DMF for 5 and 20 min with consecutive DMF and CH_2Cl_2 washes after each addition. For all Fmoc amino acid coupling, the resin was treated twice with Fmoc amino acids (3 equiv), HBTU (3 equiv) and DIPEA (6 equiv) in DMF (20 mL) for 30 min to 2 h. Once the dipeptide was synthesised, following Fmoc removal, the resin was treated twice with a solution of either: 1) acetic anhydride (10%) in DMF for 15 min to afford N-terminal acetylation, or 2) a third coupling reaction consisting of 3-phenylpropanoic acid (3 equiv), HBTU (3 equiv) and DIPEA (6 equiv) in DMF for 30 min to 2 h. Once the linear sequence was assembled a micro-cleavage was performed where a very small portion of the peptide was deprotected and removed from the trityl resin by treatment with a 50:50 mixture of TFA/CH_2Cl_2 for 3 h to determine the reaction progression and purity through HPLC-MS analysis.

General amide reduction procedure: The resin containing the linear peptide sequence was placed in an oven-dried round-bottom flask under argon. A solution of borane-THF was added (0.1 M, 10 equiv per amide) to the flask and the reaction mixture was stirred at 65 °C for 72 h. Over the course of the reaction, the resin changed from a pale yellow grainy texture to a fluffy white consistency. At this time, the entire reaction mixture was poured back into a glass peptide vessel to remove the borane-THF solution. The resin was then thoroughly washed with THF (×3) and MeOH (×3).

Oxidative work-up procedure A (compounds 3 and 7): A buffered THF solution (7:2:1, THF/AcOH/DIPEA) was added to a peptide vessel containing the reduced diamine species attached to the trityl support in a volume of 10 mL per every gram of resin utilised. This was followed by the addition of iodine (5 equiv per amine) as a concentrated THF solution. The vessel was placed on an orbital for 4 h, after which the resin was rinsed extensively with THF (×3), 1:3 NEt_3/DMF (×3), MeOH (×3) and CH_2Cl_2 (×3). At this point, a micro-cleavage was performed to check the purity of the reduced peptides by HPLC-MS.

Oxidative work-up procedure B (compounds 4, 5, 6 and 7): After rinsing, the resin was then placed into a round-bottom flask, followed by the addition of piperidine (30 mL). The reaction mixture was subsequently heated to 65 °C and stirred for 12 h. The heterogeneous solution was then poured back into a peptide vessel, where it was drained and the resin was rinsed with THF (×3), MeOH (×3), CH_2Cl_2 (×3) and DMF (×3). Again, a micro-cleavage was performed by treatment with 50:50, TFA/CH_2Cl_2 for 3 h to determine reaction progression and purity by HPLC-MS analysis.

General rhenium coordination on-resin procedure: The resin containing the reduced peptide was placed in a round-bottom flask with methanol (6 mL) and DMF (6 mL), followed by the addition of NEt_3 (209 μL, 1.5 equiv). The $[NEt_4]_2[Re(CO)_3(Br)_3]$ (1.1 equiv) was dissolved in a 50:50 solvent ratio of MeOH/DMF (8 mL) and then added to the reaction mixture. The heterogeneous solution was stirred at 65 °C for 4 h, at which time the contents of the flask were poured into a peptide vessel where the resin was rinsed extensively with MeOH (×3) and DMF (×3). A micro-cleavage was performed where a small portion of peptide was deprotected and cleaved from the resin by treatment with 50:50, TFA/CH_2Cl_2 for 3 h to determine reaction progression and purity by HPLC-MS analysis.

$[Re(3b)(CO)_3]^+[CF_3CO_2]^-$ (3c): Purification was carried out by preparative HPLC (linear gradient of 15 to 60% solvent A in C) and the purity of the isolated yellow oil was determined to be 92% by analytical HPLC (linear gradient 20 to 40% solvent A in C, $t_R = 5.55$ min). Yield: 15 mg (3%); ^{1}H NMR (600 MHz, CD_3OD): $\delta = 7.34$–7.25 (m, 5H, C*H*, ar), 6.45 (m, 1H, N*H*), 5.13 (m, 1H, N*H*), 4.90 (m, 1H, N*H*), 3.56 (m, 1H, C*H*), 3.40 (m, 2H), 3.29 (m, 2H), 3.20–3.05 (m, 5H), 2.84 (m, 2H), 2.65 (m, 2H), 1.36 (t, 3H, CH_3), 1.24 ppm (d, 3H, CH_3); HRMS (ESI): *m*/*z* calcd for $C_{19}H_{30}N_4O_3{}^{187}Re$: 549.1876; found: 549.1896 $[M]^+$.

$[Re(4b)(CO)_3]^+[CF_3CO_2]^-$ (4c): Purification was carried out by preparative HPLC (linear gradient of 40 to 80% solvent B in C) and the purity of the isolated pale yellow powder was determined to be 90% by analytical HPLC (linear gradient 40 to 80% solvent B in C, $t_R = 7.87$ min). Yield: 50 mg (8%); ^{1}H NMR (600 MHz, CD_3OD): $\delta = 7.32$–7.23 (m, 4H, C*H*, ar), 7.20 (m, 1H, C*H*, ar), 6.99 (m, 2H, C*H*, ar), 6.71 (m, 2H, C*H*, ar), 6.35 (m, 1H, N*H*), 5.21 (m, 1H, N*H*), 5.12 (m, 1H, N*H*), 3.67–3.53 (m, 3H), 3.50 (m, 1H, C*H*), 3.41 (m, 1H, C*H*), 3.33 (m, 1H, C*H*), 3.27–3.13 (m, 4H), 3.04 (dd, $^3J_{H-H} = 3.9$, 13.9 Hz, 1H, C*H*), 2.90 (m, 2H), 2.82–2.63 (m, 4H), 2.51 (dd, $^3J_{H-H} = 9.2$, 13.9 Hz, 1H, C*H*), 2.25 (m, 1H, C*H*), 2.00 ppm (m, 1H, C*H*); HRMS (ESI): *m*/*z* calcd for $C_{26}H_{36}N_4O_5{}^{187}Re$: 671.2243; found: 671.2260 $[M]^+$.

$[Re(5b)(CO)_3]^+[CF_3CO_2]^-$ (5c): Purification was carried out by preparative HPLC (linear gradient of 20 to 60% solvent B in C) and the purity of the isolated white powder was determined to be 99% by analytical HPLC (linear gradient 20 to 60% solvent B in C, $t_R = 7.14$ min). Yield: 75 mg (13%); ^{1}H NMR (600 MHz, CD_3OD): $\delta = 6.42$ (m, 1H, N*H*), 5.00 (m, 1H, N*H*), 4.77 (m, 1H, N*H*), 3.66 (m, 2H), 3.44 (m, 2H), 3.32 (m, 1H, C*H*), 3.25 (m, 1H, C*H*), 3.18 (m, 1H, C*H*), 3.07–2.92 (m, 5H), 2.82 (ddd, $^3J_{H-H} = 3.9$, 13.9, 13.9 Hz, 1H, C*H*), 2.52 (m, 1H, C*H*), 2.21 (m, 1H, C*H*), 1.88 (m, 1H, C*H*), 1.76 (dq, $^3J_{H-H} = 7.5$, 7.5 Hz, 2H, CH_2), 1.65 (m, 1H, C*H*), 1.38 (t, 3H, CH_3), 1.09 (d, 3H, CH_3), 1.00 ppm (d, 3H, CH_3); HRMS (ESI): *m*/*z* calcd for $C_{17}H_{35}N_5O_3{}^{187}Re$: 544.2298; found: 544.2212 $[M]^+$.

$[Re(6b)(CO)_3]^+[CF_3CO_2]^-$ (6c): Purification was carried out by preparative HPLC (linear gradient of 50 to 90% solvent B in C) and the purity of the isolated white powder was determined to be 98% by analytical HPLC (linear gradient 50 to 90% solvent B in C, $t_R = 6.80$ min). Yield: 65 mg (10%); ^{1}H NMR (600 MHz, CD_3OD): $\delta = 7.30$–7.18 (m, 5H, C*H*, ar), 6.39 (m, 1H, N*H*), 5.18 (m, 1H, N*H*), 5.00 (m, 1H, N*H*), 3.88 (dq, $^3J_{H-H} = 6.2$, 6.2 Hz, 1H, C*H*), 3.55 (m, 2H), 3.43 (m, 2H), 3.29 (m, 2H), 3.05 (ddd, $^3J_{H-H} = 5.4$, 12.0, 12.0 Hz, 1H, C*H*), 2.92–2.65 (m, 7H), 2.28 (m, 1H, C*H*), 1.98 (m, 1H, C*H*), 1.46 (m, 2H), 1.28 (m, 4H, C*H* and CH_3), 0.89 (d, $^3J_{H-H} = 6.2$ Hz, 3H, CH_3), 0.85 ppm (d, $^3J_{H-H} = 6.2$ Hz, 3H, CH_3); HRMS (ESI): *m*/*z* calcd for $C_{24}H_{40}N_4O_4{}^{187}Re$: 635.2607; found: 635.2640 $[M]^+$.

$[Re(7b)(CO)_3]^+[CF_3CO_2]^-$ (7c): Purification was carried out by preparative HPLC (linear gradient of 30 to 60% solvent B in C) and the purity of the isolated white powder was determined to be 99% by analytical HPLC (linear gradient 10 to 80% solvent A in C, $t_R = 10.6$ min). Yield: 49 mg (8%); ^{1}H NMR (600 MHz, CD_3OD): $\delta = 7.31$–7.19 (m, 5H, C*H*, ar), 6.39 (m, 1H, N*H*), 5.24 (m, 1H, N*H*), 5.05 (m, 1H, N*H*), 3.72 (m, 2H), 3.64 (m, 1H, C*H*), 3.58 (m, 2H), 3.51 (m, 1H, C*H*), 3.44 (m, 2H), 3.35 (m, 1H, C*H*), 3.26 (m, 1H, C*H*), 3.14 (m, 2H), 3.00 (m, 1H, C*H*), 2.82 (m, 1H, C*H*), 2.72 (m, 4H), 2.24 (m, 1H, C*H*), 2.01 (m, 1H, C*H*), 1.91 (m, 3H), 1.56 (m, 1H, C*H*), 1.51 ppm (m, 2H); HRMS (ESI): *m*/*z* calcd for $C_{23}H_{38}N_4O_5{}^{187}Re$: 635.2400; found: 635.2372 $[M]^+$.

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[1] R. Haag, F. Kratz, *Angew. Chem.* **2006**, *118*, 1218–1237; *Angew. Chem. Int. Ed.* **2006**, *45*, 1198–1215.
[2] V. Marx, *Chem. Eng. News* **2005**, *83*, 17–24.
[3] J. S. Richardson, *Adv. Protein Chem.* **1981**, *34*, 167–339.
[4] J. D. A. Tyndall, B. Pfeiffer, G. Abbenante, D. P. Fairlie, *Chem. Rev.* **2005**, *105*, 793–826.
[5] R. Jones, *Curr. Opin. Pharmacol.* **2003**, *3*, 530–543.
[6] S. Lee, J. Xie, X. Chen, *Biochemistry* **2010**, *49*, 1364–1376.
[7] D. Filmore, *Mod. Drug Discovery* **2004**, *7*, 24–28.

[8] J. P. Overington, B. Al-Lazikani, A. L. Hopkins, *Nat. Rev. Drug Discovery* **2006**, *5*, 993–996.
[9] B. E. Evans, K. E. Rittle, M. G. Bock, R. M. DiPardo, R. M. Freidinger, W. L. Whitter, G. F. Lundell, D. F. Veber, P. S. Anderson, *J. Med. Chem.* **1988**, *31*, 2235–2246.
[10] T. Klabunde, G. Hessler, *ChemBioChem* **2002**, *3*, 928–944.
[11] E. S. Mull, V. J. Sattigeri, A. L. Rodriguez, J. A. Katzenellenbogen, *Bioorg. Med. Chem.* **2002**, *10*, 1381–1398.
[12] H. Bregman, P. J. Carroll, E. J. Meggers, *J. Am. Chem. Soc.* **2006**, *128*, 877–884.
[13] L. Barisić, M. Cakić, K. A. Mahmoud, Y.-n. Liu, H.-B. Kraatz, H. Pritzkow, S. I. Kirin, N. Metzler-Nolte, V. Rapić, *Chem. Eur. J.* **2006**, *12*, 4965–4980.
[14] J. C. Reubi, H. R. Maecke, *J. Nucl. Med.* **2008**, *49*, 1735–1738.
[15] S. M. Okarvi, *Med. Res. Rev.* **2004**, *24*, 357–397.
[16] E. J. Meggers, *Curr. Opin. Chem. Biol.* **2007**, *11*, 287–292.
[17] S. Mundwiler, L. Candreia, P. Häfliger, K. Ortner, R. Alberto, *Bioconjugate Chem.* **2004**, *15*, 195–202.
[18] P. Häfliger, S. Mundwiler, K. Ortner, B. Spingler, R. Alberto, G. B. Andócs, L. Balogh, K. Bodo, *Synth. React. Inorg. Met.-Org. Nano-Met. Chem.* **2005**, *35*, 27–34.
[19] M. Hata, G. R. Marshall, *J. Comput. Aided Mol. Des.* **2006**, *20*, 321–331.
[20] J. L. Hickey, E. J. Simpson, L. G. Luyt, *J. Labelled Compd. Radiopharm.* **2009**, *52*, S71.
[21] I. Marcotte, F. Separovic, M. Auger, S. M. Gagne, *Biophys. J.* **2004**, *86*, 1587–1600.
[22] D. G. Hall, C. Laplante, S. Manku, J. Nagendran, *J. Org. Chem.* **1999**, *64*, 698–699.
[23] S. Manku, C. Laplante, D. Kopac, T. Chan, D. G. Hall, *J. Org. Chem.* **2001**, *66*, 874–885.
[24] A. Nefzi, M. A. Giulianotti, R. A. Houghten, *Tetrahedron* **2000**, *56*, 3319–3326.
[25] A. Nefzi, J. M. Ostresh, J. Yu, R. A. Houghten, *J. Org. Chem.* **2004**, *69*, 3603–3609.
[26] A. Nefzi, J. M. Ostresh, M. Giulianotti, R. A. Houghten, *J. Comb. Chem.* **1999**, *1*, 195–198.
[27] T. W. Greene, P. G. M. Wuts, *Protective Groups in Organic Synthesis* 3rd ed., Wiley, New York, **1999**.
[28] E. J. Simpson, J. L. Hickey, D. Breadner, L. G. Luyt, *Dalton Trans.* **2012**, *41*, 2950–2958.
[29] K. Richter, E. Kawashima, R. Egger, G. Kreil, *EMBO J.* **1984**, *3*, 617–621.
[30] H. C. Brown, Y. M. Choi, S. Narasimhan, *J. Org. Chem.* **1982**, *47*, 3153–3163.
[31] R. Alberto, A. Egli, U. Abram, C. K. Hegetschweiler, V. Gramlich, P. A. Schubiger, *J. Chem. Soc. Dalton Trans.* **1994**, 2815–2820.
[32] K. S. Lam, S. E. Salmon, E. M. Hersh, V. J. Hruby, W. M. Kazmierski, R. J. Knapp, *Nature* **1991**, *354*, 82–84.

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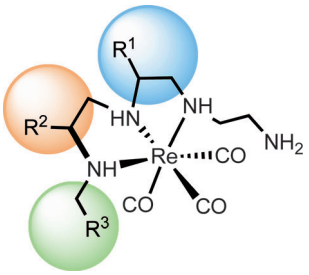
Peptidomimetics



Redefining chemical space: Molecular scaffolds have been shown to facilitate and stabilise 2° structural turn elements, with a central core arranging functionality in a defined 3-dimensional orientation. By utilising a $Re(CO)_3^+$ centre (see figure), we have demonstrated that amino acid sequences can be assembled, reduced and coordinated on-resin, resulting in a diverse set of turn-based peptidomimetics.