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Full Paper

Solid Phase Synthesis of Sulfonimidamide Pseudopeptides and Library Generation

Praveen K. Chinthakindi,[a] Andrea Benediktsdottir,[a] Per I Arvidsson,[b, c] Yantao Chen,[d] and Anja Sandström*[a]

Abstract: Many synthetic routes have been explored to make small molecule sulfonimidamides (SIAs), however, its introduction into larger molecules such as oligopeptides has not been studied before. We herein demonstrate three alternative and complementary methods for synthesis of SIA based pseudopeptides, on solid phase, using both on and off-resin SIA-synthesis, *via* sulfonimidoyl chlorides from sulfonamides, in high conversion. Beside evaluation of various resins such as 2-CTC, Wang, and Rink amide-ChemMatrix, the possibilities to further *N*-functionalize and cyclize the SIA functionality on solid support are shown. The diastereomers of SIA containing pseudopeptides could in most cases be separated using normal reverse phase preparative HPLC. The solid phase SIA methodology has many advantages when it comes to handling and purification as compared to in solution, and will therefore enable exploration of the SIA group as isosteric substitutions and peptidomimetic building blocks in the development of drug-like pseudopeptides in many ways. Of particular note these approaches facilitate combinatorial library synthesis as demonstrated herein.

Introduction

Sulfur containing functional groups,[1] such as thioethers, sulfones, sulfonamides[2], etc., and their construction continue to play an important role in medicinal chemistry.[3] Synthesis and biochemical evaluation of compounds based on sulfonimidamides (SIAs) have gained much attention in recent time as judged by an increasing number of related publications,[4] patents[5] and reviews[6]. The sulfonimidamide functionality is an isostere to the sulfonamide, where one of the oxygens is replaced by an imine nitrogen (Figure 1). Thus, chirality is introduced and furthermore the extra "N" handle conveys either an additional hydrogen bond donor or the possibility of adding various substituents (i.e. =NH, =NR), which can tune physical-chemical and biological properties in different ways.[6b, 7] Moreover, single atom swapping has shown to improve biological activity in some cases.[8] All these aspects make the SIA group interesting from a drug discovery perspective, beside obvious applications within asymmetric synthesis.[9]

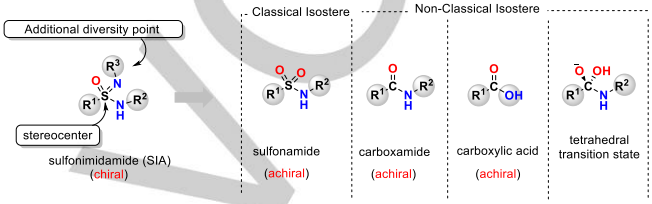

Figure 1. Comparison of sulfonimidamide and isosteres

We have previously studied synthetic routes to sulfonimidamides and acyl sulfonimidamides as well as their potential use as bioisosteres to sulfonamides and acyl sulfonamides, respectively, the latter being an isostere to carboxylic acids.[7b, 10] Moreover, the sulfonimidamide has similar geometry to a tetrahedral intermediate of amide bond hydrolysis. This similarity qualifies the SIA group as a transition state isostere (Figure 1), of potential use in inhibitors of hydrolases, such as proteases. Indeed, sulfonimidamide based carboxypeptidase inhibitors have been reported by Cathers and Schloss.[11]

Peptide drug discovery is going through a renaissance[12], partly driven by their potential use in new drug modalities addressing challenging undruggable targets like intracellular protein-protein interactions,[13] and for their use of various purposes in biomedicine, biotechnology, and bioengineering.[14] The transformation of peptides into peptidomimetics, mimicking the structure and conformation of physiologically active peptides, aims at improving stability against proteolysis, cell membrane permeability, and oral bioavailability.[4b] Approaches to make peptidomimetics include replacement of one or more of the metabolically unstable amide bonds of the backbone with amide bond isosteres such as sulfoximines[15] and sulfonamides;[16] incorporation of unnatural amino acids[17] is also a common practice (Figure 2).[18]

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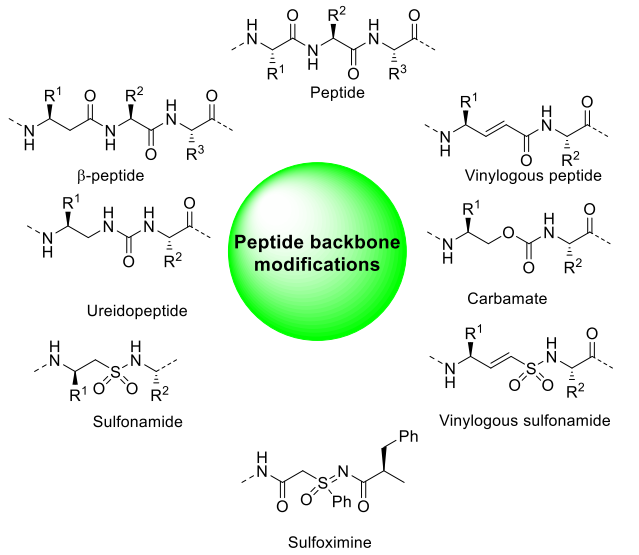
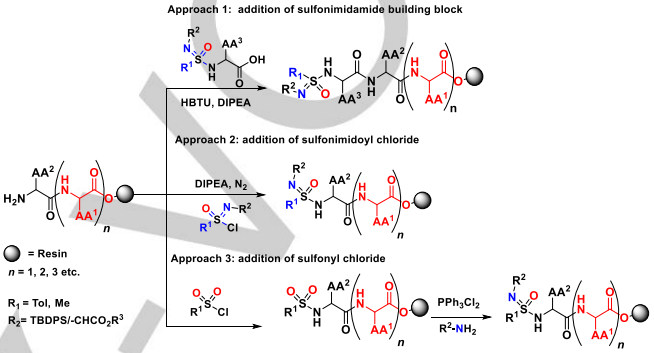


Figure 2. Peptide backbone modifications using amide bond isosteres.

Aiming at new peptide-based modalities, we felt encouraged to synthesize SIA-based pseudopeptides where the scissile peptide bond was replaced with chiral SIAs and to study the diastereomers forming when it comes to physicochemical properties, proteolytic stability, conformational preferences and target binding. Beside these obvious effects we saw great opportunities with the incorporation of the chiral SIA group to construct diverse high-quality compound libraries,[19] especially if the compounds could be synthesized on solid support, such as in solid phase peptide synthesis (SPPS).[20]

SPPS is considered as the best method for the manufacturing of peptide drugs.[21] In 1963 Bruce Merrifield invented the solid phase approach for peptide synthesis, and the methodology has thereafter been extensively used for oligonucleotide (DNA, RNA, PNA etc.), oligosaccharide, and combinatorial libraries synthesis.[22] In general, attaching the starting material covalently to an insoluble solid support has several advantages in comparison with solution phase synthesis. In solid phase synthesis reaction work-up is simplified because of excess reagents and soluble by-products can be removed by simple filtration using iterative resin washing steps. Also, it avoids chromatographic purification of each intermediates which increases the speed of the synthesis. However, this method normally requires final HPLC purification of the final peptide product. Overall, SPPS is a robust method for combinatorial chemistry applications and can easily be adjusted to automation.[23]

With the long term goal to combine the sulfonimidamide functionality and peptides we recently published a method to synthesize SIAs based amino acid building blocks using a one-pot method from *tert*-butyldiphenylsilyl (TBDPS)-protected sulfonamides as well as orthogonal protection strategies.[4b] In general, SIAs can be synthesized from non-commercially available sulfinamides (R^1SONHR^2),[24] sulfinylamines (RNSO),[25] sulfenamides (R^1SNHR^2),[26] thionyl tetrafluoride (SOF_4)[27] using multi step and often tedious and harsh conditions, as well as from commercially available sulfonamides ($R^1SO_2NHR^2$)[28]. Our method was optimized from a convenient one-pot method starting from TBS-protected sulfonamides, reported by Chen and Gibson.[28] Moreover, this is a cheap and metal free medicinal chemistry friendly method. In continuation to our previous work, we have now explored the possibility to combine the SIA synthesis with SPPS to make SIA-based pseudopeptides using alternative methods, which is herein presented.



Scheme 1: Solid phase synthesis approaches to SIA based pseudopeptides.

Results and discussion

Our investigation of the synthesis of SIA-based pseudopeptides on solid phase started by retrosynthetic analysis, which suggested three alternative methods according to Scheme 1. The first approach includes the synthesis of SIA-based amino acid building blocks[4b] (Scheme 1) followed by a standard peptide coupling on solid phase. Alternatively, instead of making SIA based amino acid building block in solution, we considered utilization of sulfonimidoyl chloride (SIC) solution made from a cocktail of TBDPS-protected sulfonamide and PPh_3Cl_2 directly on the *N*-terminal amine on the resin-bound peptide (approach 2, Scheme 1). Thirdly, a sulfonamide peptide could be made on solid phase using sulfonyl chloride, which could be further reacted with PPh_3Cl_2 to get SIC followed by addition of an amine (approach 3, Scheme 1).This would, however, require a solid phase reaction at low temperature (0 °C), which is not commonly explored and might be challenging to handle. All three approaches would be of use in library production since diversity is introduced in the last step, however approach 3 offers extra advantages since it introduces diversity on solid-phase in the three handles around chiral "S". Also, if the SIA synthesis is compatible with solid phase, advantages in purifications will be obvious. Moreover, the stoichiometry can be changed to support a higher yielding SIA synthesis by the allowing to use excess reagents that can be easily removed. Thus, the on-resin approaches 2 and 3 (Scheme 1) would be particularity advantageous in this regard.

Attachment of a SIA-based amino acid building blocks under SPPS conditions

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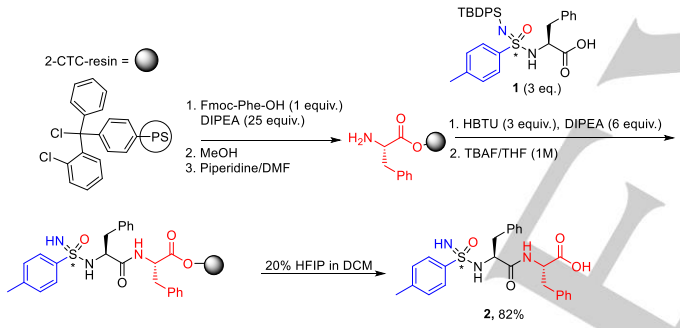
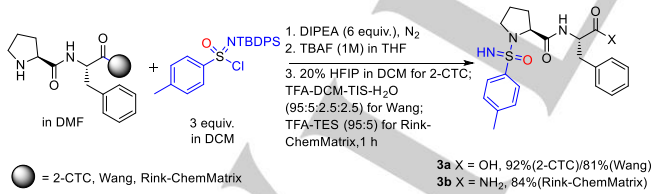
Initially, approach 1 (Scheme 1) was explored to see if the TBDPS-protected SIA based amino acids is compatible with SPPS (Scheme 2). Polystyrene 2-chlorotrityl chloride (2-CTC)-resin was swelled in DCM and Fmoc-Phe-OH was added and attached using by addition of diisopropylethylamine (DIPEA). Unreacted sites on the resin were capped through addition of MeOH, after which Fmoc was removed by 20% piperidine in DMF according to standard procedure. TBDPS-SIA-Phe-OH **1**[4b] was coupled with activation using HBTU and DIPEA. Mini-cleavage performed at this point using 20% hexafluoroisopropanol (HFIP) in DCM confirmed consumption of the starting material showing full conversion into the TBDPS-protected SIA-peptide. The TBDPS-group was successfully removed with 1M TBAF in THF, after which the product (SIA-Phe-Phe-OH, **2**) was released from the resin using 50% TFA in DCM. Analysis of the crude product after cleavage shows the SIA-Phe-Phe-OH product as a single major peak (according to LC-MS conversion at 254 nm), which after HPLC purification yielded **2** as a diastereomeric mixture (around 60:40) in 82% isolated yield. This proves that protected SIA-based amino acids could be used in SPPS and that the protection using TBDPS is advantageous since it allows a high yielding synthesis of SIA-based amino acids[4b] and since the protecting group can be removed selectively using TBAF also on resin.



Scheme 2. Approach 1. Attaching a SIA-based amino acid building block using SPPS conditions.



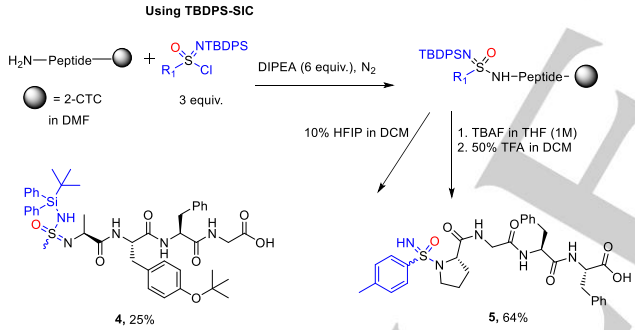
Scheme 3. Approach 2. On-resin SIA based pseudopeptides synthesis using addition of SIC.

On-resin SIA synthesis by addition of TBDPS protected SIC to a resin-bound peptide.

Next, the on-resin SIA synthesis using approach 2 (Scheme 1) was explored (Scheme 3). In this case we decided also to evaluate different resins, i.e. the 2-CTC, Wang and Rink-amide ChemMatrix resins. A model dipeptide (Pro-Phe) on each resin was chosen as starting material for the one pot sulfonimidamide chemistry. Thus, the synthesis started with the transformation of TBDPS-*p*-toluenesulfonamide into the SIC derivative at 0 °C degree in DCM instead of chloroform as previously described; this is partially due to resin swelling purpose and in order to avoid unwanted reaction with phosgene.[4b] Although, for SPPS convenience, we modified Chen's one pot protocol somewhat with respect to solvents and bases as will be described below.[4b] The SIC solution was thereafter transferred with a needle into the solid phase chamber containing the resin-bound dipeptide premixed with DIPEA in DMF under N_2-atmosphere and left shaking for 3-5 h. Thereafter, the resins were washed with DCM and DMF repeatedly. The TBDPS group was removed using 1M TBAF in THF. Finally, the sulfonimidamide based peptide was released from the ChemMatrix-Rink amide resin with TFA-TES (95:5), the Wang resin with TFA-TIS-H_2O (95:2.5:2.5) for 3 h, and from the 2-CTC resin with 50% TFA in DCM or 20% HFIP in DCM for 60 min (Scheme 3). Gratifyingly, according to analysis of the crude products by LC-MS the sulfonimidamide-dipeptides were formed as diastereomeric mixtures (**3a,** 60:40 and **3b,** 57:43) with full conversion on all three resins without any unreacted starting material left. It is also worth noting that, with this solid phase method, unreacted sulfonamide from the presynthesis of SIC, triphenyl phosphine oxide, and peptide coupling byproducts were easily washed off by the simple washing steps. Removing triphenyl phosphine oxide is normally relatively tedious in solution phase synthesis.[29] Moreover, it should be mentioned that conditions were initially screened and slightly modified for coupling of SIC to the *N*-terminal amine of the resin bound peptide. For example different bases (triethylamine, DIPEA) and different solvents (DCM, DMF) were evaluated. The usage of triethylamine in DCM in the solid-phase SIA synthesis at room temperature led to decomposition of SIC over the coupling to the amine residue of peptide. Thus, the reaction temperature of the peptide reactor was lowered from r.t. to 0-5^{0}C. Still, only modest conversion was achieved. We believe this could be due to sparingly soluble reaction mixture in DCM, which disfavored completion of the coupling. Therefore, the solvent was changed from DCM to DMF for better solubility of SIC, resulting in higher, although not satisfactory, partial (65-75%) conversion. After changing the base triethylamine to DIPEA almost full conversion was achieved. Also, various equivalents of the SIC mixture were evaluated showing that two equivalents were sufficient to get full conversion (for movie, see supporting information). Further, the synthesis of SIA peptide proved that the coupling of SIC to an amine on the solid support occurs similarly to in solution, without losing stereochemical integrity. Overall, in comparison with solution phase synthesis, the solid phase synthesis is robust with respect to conversion, purification and yields.

Having optimized conditions in hand, scope and limitations of the reaction (Scheme 3) were explored in the syntheses of other sequences and larger peptides (tetra) using either TBDPS protected toluene- and methane- SICs for the synthesis of *N*-terminal SIA peptides. We chose 2-CTC-resin for this study since this resin has many advantages related to handling and synthetic options.[30] For example, swelling, attachment of the first amino acid, and final cleavage from the 2-CTC resin are fast and performed under mild conditions. The coupling of TBDPS protected toluene-SIC to a resin bound Phe-Phe peptide

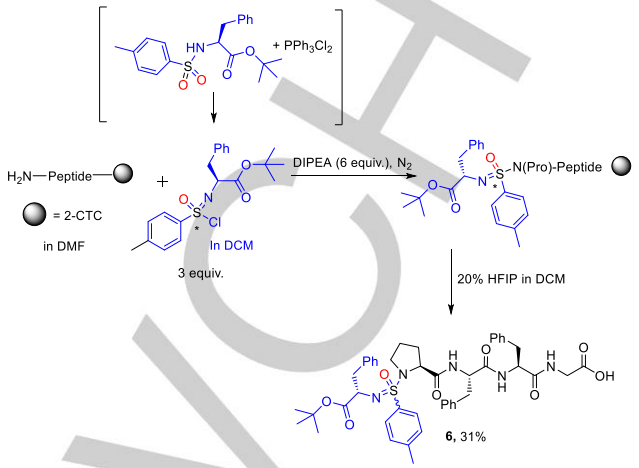
sequence was successful, giving compound **2** in an alternative way, as compared to in Scheme 2, although with less conversion in comparison to in the synthesis of proline based compound **3a** due to formation of side-products. It seems as e.g. a reactive iminophosphorane can form with the primary amine of phenylalanine under conditions with excess of the PPh_3Cl_2 solution. However, the product could easily be purified using HPLC giving a diastereomeric mixture of **2** in reasonable yields (70%). Next, we introduced SIA into two different tetrapeptides as outlined in Scheme 4, one with an *N*-terminal alanine and one with an *N*-terminal proline using either methane- or toluene-SIC, respectively. To our satisfaction, the reactions yielded desired diastereomeric products (**4**, dr 55:45 and **5,** dr 45:65) that easily could be isolated from each other using normal reversed-phase HPLC purification. Our gained experience indicate that sterically crowded or larger sequences may well give SIA-diastereomers, which can be easily separated from each other, which is greatly advantageous. Again, we observed a clean and full conversion reaction for the proline (secondary amine) sequence and a slightly lower yielding reaction for the alanine sequence (primary amine) due to side-product formation, although the latter can be easily purified using HPLC (Scheme 4). Furthermore, it was demonstrated that the peptides can be cleaved from the 2-CTC-resin with the TBDPS protection still intact as shown for the TBDPS-protected SIA peptide **4** using mild 10% HFIP in DCM for cleavage. These results demonstrate that SIA formation works equally well for longer peptides.



Scheme 4. Approach 2. On-resin SIA based (tetra) pseudopeptides synthesis using addition of SIC.

On-resin, protecting group free, SIA synthesis by addition of a functionalized SIC amino acid to a resin-bound peptide.

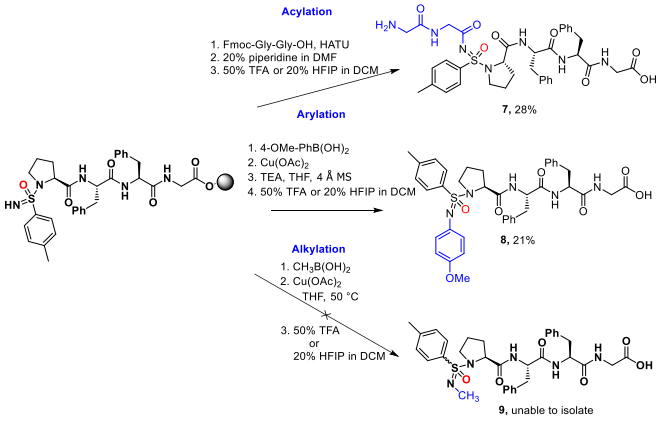
Our further investigations of the scope of the on-resin SIA-formation (approach 2) included the novel procedure to use a preformed amino acid based SIC-derivative, meaning formation of SIC from an amino acid toluene sulfonamide (instead of a TBDPS-protected toluene/methane sulfonamide), which gives us a bidirectional SIA-peptide with two C-terminal ends, as outlined in Scheme 5. To our satisfaction, the reaction worked with full conversion on a resin-bound tetrapeptide with an *N*-terminal proline, giving the peptidomimetic diastereomers (**6,** dr 60:40), which could be separated. Note that bidirectional peptides have in several cases shown useful in peptidomimetic protease inhibitor design, e.g. in simeprevir[31] and ACE-inhibitor[32]. Also, it mimics peptide urea analogs.[33]



Scheme 5. Approach 2. On-resin SIA based (tetra) pseudopeptides synthesis using addition of *functionalized SIC amino acid*.

Another way of making *N*-functionalized SIA-peptides is to functionalize the *N*-terminal imine of the SIA-group after its incorporation to peptides. We further investigated this possibility in acylation, arylation and alkylation reactions as outlined in Scheme 6.

On-resin N-Acylation of SIA-peptides: N-acylation is the easiest and most common method to grow a peptide, so we performed acylation at imine "N" of the sulfonimidamide using standard peptide coupling of Fmoc-Gly-Gly-OH with HBTU and DIPEA (Scheme 6). The reaction worked giving the acyl-SIA peptide **7**, although with 50% unreacted starting material left (Scheme 6). To improve the yield we opted double couplings, tried optimization by changing coupling reagents and reaction conditions (microwave, classical heating). Unfortunately, none of them improved the yields. This could be due to steric hindrance of proline groups flanked by SIA. Nevertheless, the product **7** could be isolated as diastereomers (28%, dr 86:14).

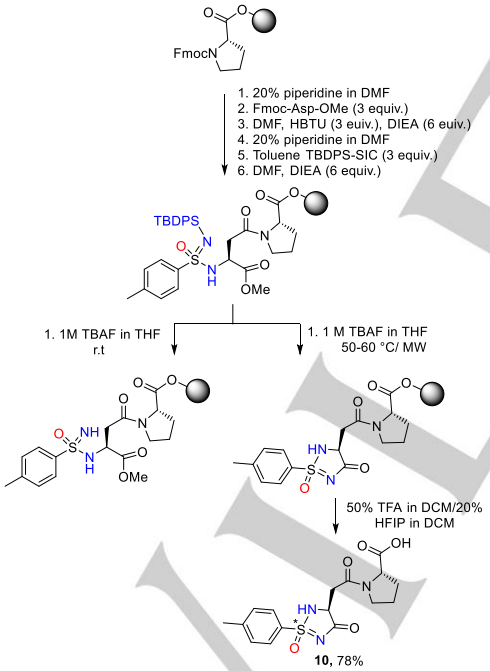


Scheme 6. On-resin *N*-functionalization of SIA based (tetra) pseudopeptides.

On-resin N-arylation of SIA-peptides: With the inspiration of *N*-arylation of sulfonamides[34] and amines[35] on solid support, we selectively carried out *N*-arylation of sulfonimidamide using Chan-Lam coupling conditions without affecting other functional groups like amides. To our delight, product **8** was successfully formed and isolated as pure diastereomers in moderate yields (21%, dr 72:28) (Scheme 6).

*On-resin N-alkylation of SIA-peptides***:** Various alkylation methods were tried with e.g. propargyl bromide and methyl iodide, however, only low conversion were observed. Alkylation with iodomethane worked with heating, however resulting in multiple methylations. Finally, using Chan-Lam conditions with methylboronic acid and copper acetate at 50 °C, the methylated SIA-peptides **9** was formed according to LC-MS, but unfortunately the compound could not be isolated during HPLC purification, possibly due to degradation (Scheme 6).

On-resin peptido five membered cyclic SIA formation (Scheme 7): A variant of the acylation reaction is intramolecular cyclization between the SIA-nitrogen and an amino acid carboxylic acid as we previously demonstrated in solution phase.[4b] Initially, we tried to synthesize the cyclic SIA-Asp-(Phe-Phe-OH), where the sidechain of Asp was coupled to the peptide, thus facilitating utilization of the α-carboxylic acid in formation of a five membered ring. This sequence was however accompanied by competing aspartimide[36] formation.

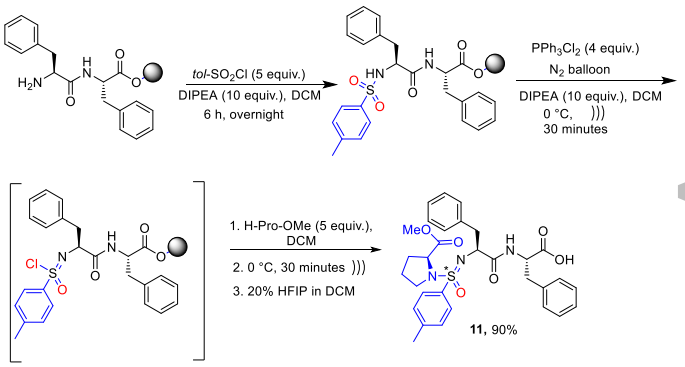


Scheme 7. On resin synthesis of 5-membered cyclic SIA based pseudopeptides.

In order to avoid the aspartimide formation we started our sequence with Fmoc-Pro-OH[37] and carried out Fmoc removal with 20% piperidine in DMF with 0.1 equiv. formic acid[36] instead of the standard 20% piperidine in DMF. Then, Fmoc-Asp-OMe was coupled followed by Fmoc deprotection. Thereafter, the SIC cocktail was added to the resin at 0-5 °C yielding the TBDPS-protected SIA peptide. Interestingly, at room temperature and in presence of TBAF, selective TBDPS deprotection took place (83% conversion as per LC-MS), while at 50-60 °C using conventional (95% crude yield as per LC-MS) or microwave heating (94% crude yield as per LC-MS), the simultaneous deprotection-cyclization was the predominated route (Scheme 7), yielding the desired product **10** (78%, dr 60:40). However, separation of the diastereomers was difficult using either preparative HPLC or SFC. We hypothesise that cyclic sulfonimidamide like **10**, i.e. having a shorter peptide sequence, might be difficult to separate. Possibly, increasing the peptide length might be helpful to separate the diastereomers. Based on our previous experience with solution-phase SIA synthesis, having bulkier functional groups around the sulfur atom is most often helpful for the separation of the diastereomers.

On-resin SIA synthesis, via on-resin SIC formation followed by addition of an amino acid.

Finally, we studied the possibility to make a peptide SIC-derivate on solid phase at 0 °C, followed by addition of an amino acid ester, as the amine, according to approach 3 (Scheme 1). To be able to get a simultaneous shaking and cooling in the solid phase reactor we considered using ultrasonication[38] in an ice bath. Thus, H-Phe-Phe-O-2-CTC-resin was synthesized using the standard procedure. An *N*-terminal sulfonamide was thereafter prepared by addition of toluene sulfonyl chloride at room temperature, as outlined in Scheme 8. After completion of the coupling, the resin was washed thoroughly and dried carefully. Thereafter, dry DCM was added to the resin under nitrogen atmosphere and the solid phase reactor cooled to 0 °C by adjusting the sonicator bath temperature with dry ice. Pre-dissolved PPh_3Cl_2 and triethylamine in DCM was then added dropwise to the solid phase reactor, which was further sonicated for 30 min at 0 °C forming the SIC. Subsequently, proline methyl ester and DIPEA were added and the reactor sonicated for another 30 min at 0 °C. Final cleavage using 20% HFIP in DCM successfully delivered the final products **11** as single products in an isolated yield of 90 %. The two diastereomers of **11** (dr 63:34) could be separated and isolated after standard HPLC (0.1% TFA-water-acetonitrile).



Scheme 8. Approach 3. On-resin SIC formation by followed SIA synthesis.

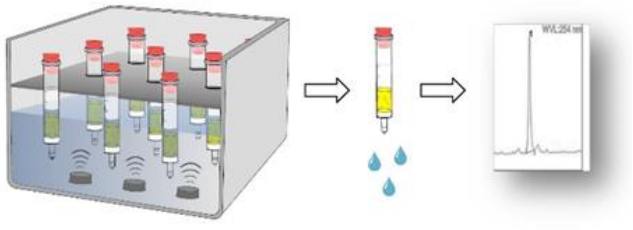


Figure 3. On-resin parallel combinatorial library synthesis.

The synthetic approach 3 was tried in a small parallel library format (for movie, see supporting information). Hence, cold SIC resin (as in Scheme 8) was dispensed into eight sealed syringes prepared with a filter and excess amount of different amine nucleophiles. The syringes were placed on a fitting plate to hold the syringes in the water bath and placed in a sonicator for 30 min at 0 °C (illustrated in Figure 3), followed by washing by filtration. Finally, a cleavage solution was added to each syringe, delivering desired products as major peaks according to LC-MS analysis at 254 nm (SerOEt 99% conversion, PhgOEt 100% conversion, ValOEt 100% conversion, AlaOEt 91% conversion, TyrOMe ~60% conversion, $MeNH_2$ ~96% conversion, aniline 99% conversion and propargylamine ~50% conversion). In case of the reaction with tyrosine methyl ester we observed several peaks in the LC-MS diagram. This could be due to presence of free phenol reacting with SIC on either the hydroxyl group of phenol or the activated aromatic ring. A similar situation was observed for serine methyl ester, where two major peaks with the same mass was observed, probably as a result of the free hydroxyl group reacting with SIC. These results suggest that protection of all other reactive functional groups in the starting material is needed in order to get good yields. In case of the propargyl amine, several other peaks were observed along with desired SIA with propargyl handle. This could be due to polymerization of the propargyl amine and chlorination of the triple bond with SIC under these conditions.

Conclusion

In summary, we have for the first time demonstrated three alternative methods for the introduction of the SIA group into a peptide backbone on solid phase using various resins. The first approach, using SIA-based amino acids as building blocks in SPPS, is suitable for construction of complex SIA based pseudopeptides with many reactive functional groups since the complex SIA synthesis is made beforehand in solution. The second approach where SIC is premade in solution and then added to a resin-bound peptide, is a faster option, since the SIA is made on solid-phase, which simply purification. This route works well for different peptides and with full conversion for secondary amines, such as proline at the *N*-terminal of the resin-bound peptide. Finally, the third approach is highly advantageous since the SIA-synthesis is made fully on solid-phase, making handling and purification easy. Moreover, this approach allows introduction of diversity at three handles around the chiral "S" atom in the last steps on solid-phase using different commercially available sulfonyl chlorides instead of sulfonamides. Taken together, the solid-phase SIA reactions are convenient and user friendly one-pot methods, with a wide scope that could be adapted to generate combinatorial libraries using automation. In this work we furthermore show the possibility to further functionalize and cyclize the SIA functionality on solid support, as well as to perform protection group free preparation of bidirectional SIA peptides. Overall, this study offers a critical basic foundation to flourish sulfonimidamide based pseudopeptides as a new modalities for various applications in medicinal chemistry and chemical biology, and constitute a valuable addition to peptide chemistry tool box.

Experimental Section

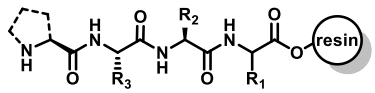
General Information:

Anhydrous solvents were collected from Innovative Technology (Model No. PS-MICRO) dry solvent system and other organic reagents in Sure-Seal™ bottles from various vendors and dried prior to use using standard drying procedures. Commercially available starting materials were used without purification unless otherwise stated. All amino acids are of L-configuration unless otherwise stated. N-(*tert*-butyl diphenyl silyl)-4-methyl benzenesulfonamide was prepared following previously described literature procedure.[4b] All solution phase reactions (sulfonimidoyl chloride solution preparation) were carried out in microwave vials (Biotage®) under nitrogen atmosphere. All solid phase reactions were carried out in manual solid phase syringe reactor (Biotage ISOLUTE® SPE reservoir) or on Biotage® Initiator+ Alstra™ Microwave Peptide Synthesizer. Thin layer chromatography was performed on pre-coated Silicagel 60 F_{254}, visualised by UV 254 nm. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance III (400 MHz) spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to the solvent residual peaks ($CDCl_3$ δH: 7.26 ppm, δC: 77.16 ppm; CD_3CN δH: 1.94 ppm, δC: 1.32, 118.26 ppm; CD_3OD δH: 3.31 ppm, δC: 49.00 ppm; DMSO-d_6 δH: 2.50 ppm, δC 39.52 ppm). Coupling constants (J) are reported in hertz (Hz). Splitting patterns are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br), or combination of these. Low-resolution mass spectra were recorded on Thermo Scientific Dionex UltiMate 3000 HPLC system with the MSQ™ Plus single quadrupol LCMS instrument. High-resolution mass spectra were recorded using a LC TOF (ES) ultra-spectrometer. Analytical HPLC/ESI-MS was performed using electrospray ionization (ESI) and a C18 column (50 × 3.0 mm, 2.6 µm particle size, 100 Å pore size) with CH_3CN/H_2O in 0.05 % aqueous HCOOH as mobile phase at a flow rate of 1.5 mL/min. LC analyses were run using a gradient of 5–100 % CH_3CN/H_2O in 0.05 % aqueous HCOOH as mobile phase at a flow rate of 1.5 mL/min for 2 min on a C18 column unless otherwise stated. Preparative RP-HPLC was performed on a system equipped with a Macherey–Nagel Nucleodur C18 HTec (21 mm × 125 mm, particle size 5 µm), with a H_2O/MeCN gradient with 0.1 % TFA as mobile phase at a flow rate of 10 mL/min for 20 min and with UV detection at 254/214 nm. IR was recorded on Agilent FTIR model Cary 630.

Note: PPh_3Cl_2 and sulfonimidoyl chloride (SIC) solutions are highly moisture sensitive thus trace amount of water presence seriously affects the outcome of the reaction conversion and yield.

General procedures:

Solid phase synthesis of peptide nucleophiles (Peptidyl resin):



All reactions were carried out in polypropylene plastic syringes fitted with polypropylene frits. Syntheses were performed manually or by automation. Amino acids were coupled using a 3-fold excess. 2-Cl-Trt (2-CTC) resin (1 g, 1.69 mmol/g) was activated using $SOCl_2$- DCM (1:9) (60 mL) overnight. ChemMatrix-rink amide, and Wang resin were used as received without any pre-activation. In case of 2-CTC, to load the first amino acid, Fmoc-L-AA−OH (1 equiv.) and DIEA (25 equiv.) were dissolved in DCM (3.5 mL) and shaken for 1 h. Then, MeOH (700 µL) was added and the mixture was shaken for 30 min to ensure full capping of the resin. The resin was washed with DMF (2 × 14 mL, 1 min), DCM (2 × 14 mL, 1 min), MeOH (2 × 14 mL, 1 min), DCM (2 × 14 mL, 1 min) and DMF (2 × 14 mL, 1 min). Fmoc removal was achieved with 20% piperidine in DMF (2 × 14 mL, 5 min) and the subsequent amino acids were added using the following coupling conditions: A solution of HBTU (3 equiv.), and DIEA (6 equiv.) in DMF, was added to the Fmoc-AA-OH (3 equiv.) and the amino acid was activated for 30 sec prior to addition to the resin. Fmoc-AA−OH/HBTU/DIEA (3:3:6) in DMF (3.5 mL) mixed for 30 min. Between the different steps, the resin was washed with DMF (2 × 35 mL), DCM (2 × 35 mL), and DMF (2 × 35 mL) for swelling. Every coupling was monitored by LC-MS after a mini-cleavage from the resin.

Note: The synthesis of peptide nucleophile was also carried out on Biotage® Initiator+ Alstra™ Microwave Peptide Synthesizer. Before coupling to sulfonimidoyl chloride (SIC), the peptide nucleophile was dried in a desiccator and purged with N2.

Synthesis of *tert*-butyl tosyl-L-phenylalaninate:

To a mixture of tert-butyl 2-amino-3-phenyl-propanoate hydrochloride (4.83 mmol) DMAP (0.525 mmol) and triethylamine (21 mmol.) in DCM (30 mL), at 0 ºC and under nitrogen atmosphere was added 4-methylbenzene-1-sulfonyl chloride (5.25 mmol) in small portions. The reaction was allowed to stir overnight at room temperature before being quenched by water (30 mL). The organic and aqueous layer were separated, and the aqueous layer extracted with 3 x 50 mL DCM. The organic layers were pooled and washed with brine solution and, dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to afford crude compound which was purified by column chromatography using 10-40% EtOAc in pentane as a mobile phase.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 – 7.64 (m, 2H), 7.26 – 7.21 (m, 5H), 7.16 – 7.11 (m, 2H), 5.05 (d, J = 9.2 Hz, 1H), 4.11-4.05 (m, 1H), 3.04 (dd, J = 13.6, 5.8 Hz, 1H), 2.99 (dd, J = 13.6, 6.1 Hz, 1H), 2.38 (s, 3H), 1.18 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.9, 143.6, 137.0, 135.4, 129.8, 129.7, 128.5, 127.4, 127.2, 82.8, 57.0, 39.8, 27.7, 21.6. IR (ATR): v = 3293, 2916, 1712, 1455, 1433, 1347, 1224, 1153, 922, 903 cm^{-1}. LCMS (ESI-TOF) m/z [M+CH_3CN+Na]+ calcd. for $C_{22}H_{28}N_2O_4NaS$: 439.1664, found 439.1667.

Synthesis of sulfonimidoyl chloride (SIC) from TBDPS-toluene sulfonamide:

To a stirred suspension of PPh_3Cl_2 (1.2 equiv, ca. 0.3M) in dry DCM under a N_2 atmosphere at room temperature, TEA (1.6 equiv.) was added dropwise. The reaction mixture was stirred for 20 min at room temperature where after it was cooled to -5 to 0 °C. A solution of the silyl protected sulfonamide (1 equiv.) in minimum amount of dry DCM was added, instantly resulting in a clear light-yellow coloured precipitate. The reaction mixture was stirred for ca. 20 min at -5 to 0 °C to get SIC.

The resulting reaction mixture was transferred with syringe to a solid phase reactor containing peptidyl-resin under nitrogen atmosphere.

Approach 1: **Attachment of a SIA-based amino acid building blocks[4b] under SPPS conditions**

Synthesis of (4-methylphenylsulfonimidoyl)-L-phenylalanyl-L-phenylalanine (diastereomeric mixture) (2):

The peptide was synthesized using standard Fmoc-SPPS from 2-CTC polystyrene (PS) resin (1 mmol/g) on a 0.5 mmol scale and the SIA amino acid building block (1) prepared in solution phase as described previously.[4b] 1 (834 mg, 1.5 mmol) was coupled using HBTU (568 mg, 1.5 mmol), DIPEA (521 µl, 3.0 mmol) in DMF under agitation at room temperature for 1 h. The title peptide (190 mg, 82%) was obtained as a diastereomeric mixture as a white fluffy powder after RP-HPLC purification and lyophilization. Gradient 0.1% TFA in acetonitrile and 0.1% TFA in water, with a linear gradient of 45% to 85% for 30 min at 10 ml min-1 and UV detection at 220 nm and 254 nm.

^{1}H NMR (400 MHz, $CDCl_3$) major isomer reported: δ 7.84 (br, 1H), 7.52 – 7.37 (m, 2H), 7.25 – 7.18 (m, 3H), 7.17 – 7.09 (m, 5H), 7.05 - 7.03 (m, 2H), 7.01 – 6.92 (m, 2H), 6.89 – 6.49 (br, 3H), 4.82 – 4.70 (m, 1H), 4.12-4.09 (m, 1H), 3.13-3.09 (m, 1H), 2.9-2.83 (m, 2H), 2.69 (dd, J = 13.9, 9.2 Hz, 1H), 2.37 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.0, 172.8, 136.4, 130.0, 129.6, 129.5, 128.8, 128.7, 128.6, 128.5, 127.5, 127.1, 127.1, 126.9, 58.6, 53.2, 39.0, 37.1, 21.7. IR (ATR): v = 1718, 1653, 1522, 1496, 1453, 1254, 1190, 1138, 1004, 810 cm^{-1}. HRMS (ESI-TOF) m/z [M+H]+ calcd. for $C_{25}H_{28}N_3O_4S$: 466.1801, found 466.1790.

Approach 2: On-resin SIA synthesis by addition of TBDPS protected SIC to a resin-bound peptide

The peptidyl-resin was dried under vacuum, bubbled with N_2 for 10 min and dry DMF was added for further N-capping with sulfonimidoyl chloride using DIPEA as a base under nitrogen atmosphere at 0 °C. The resulting solid phase reaction shook for 3-5 h on shaker. After completion of the reaction, the resin was washed with sufficient amount of DCM and DMF to wash off un-reacted starting materials (TBDPS-sulfonamide) and by-products (PPh_3O, DIPEA salt etc.). Thereafter, 1M TBAF in THF was added to remove TBDPS protecting group for 9 h to overnight. Finally, sulfonimidoyl peptidyl-resin was dried under vacuum and cleaved from the resin with the reagent cocktail: 20% HFIP in DCM (25 mL/1 g peptidyl-resin (or) 90% TFA, 5% DCM, 2.5% TIS and 2.5% H_2O) for 1 h and repeated with fresh cleavage cocktail to ensure full cleavage from the resin. The cleavage solution filtrates containing the peptide were collected and concentrated. The resulting crude peptide was purified on a semi preparative column to obtain the desired peptide. Recovered yields for the purified peptides were calculated based on the original resin loading (2-CTC resin 1mmol/g; Wang resin 0.67 mmol/g; ChemMatrix-Rink amide 0.4 mmol) and the amount of peptidyl-resin cleaved.

For demonstration of approach 2 - see movie in supporting information.

Synthesis of (4-methylphenylsulfonimidoyl)-L-prolyl-L-phenylalanine (diastereomeric mixture) (3a):

The peptide was synthesized using standard Fmoc-SPPS from 2-CTC resin (1 mmol/g) on a 0.5 mmol scale and SIC prepared and coupled according to the general procedure described above. The title peptide (190 mg, 92%) was obtained as a white fluffy powder after RP-HPLC purification and lyophilization. Gradient 0.1% TFA in acetonitrile and 0.1% TFA in water, with a linear gradient of 50% to 75% for 30 min at 10 ml min-1 and UV detection at 220 nm and 254 nm

Alternatively, the same procedure was applied on Wang resin (0.67 mmol/g) on a 0.33 mmol scale to get desired compound 3a (111 mg, 81%).

^{1}H NMR (400 MHz, $CDCl_3$) major isomer reported: δ 7.93-7.89 (m, 2H), 7.45 – 7.35 (m, 2H), 7.27 – 7.23 (m, 5H), 6.90-5.93 (br, 3H), 4.93 (dt, J = 9.7, 4.7 Hz, 1H), 4.09 (dd, J = 9.2, 3.1 Hz, 1H), 3.61-3.56 (m, 1H), 3.39 – 3.28 (m, 2H), 3.14-3.10 (m, 2H), 2.44 (s, 3H), 1.79-1.77 (m, 1H), 1.68-1.58 (m, 1H), 1.43 – 1.39 (m, 1H). ^{13}C NMR (101 MHz, CDCl3) δ 173.9, 171.5, 147.2, 136.7, 130.9, 129.7, 129.3, 128.8, 128.7, 127.1, 63.0, 53.1, 50.0, 36.9, 31.4, 24.1, 21.8. IR (ATR): v = 2965, 2877, 1727, 1654, 1595, 1526, 1455, 1259, 1177, 1131cm^{-1}. HRMS (ESI-TOF) m/z [M+H]+ calcd. for $C_{21}H_{26}N_3O_4S$: 416.1644, found 416.1632.

Alternatively, compound 2 was prepared using this approach 2 on 2-CTC resin (1 mmol/g) on a 0.5 mmol scale to get the desired product (145 mg, 70%).

Synthesis of N-((S)-1-amino-1-oxo-3-phenylpropan-2-yl)-1-(4-methylphenylsulfonimidoyl) pyrrolidine-2-carboxamide (3b):

The peptide was synthesized using standard Fmoc-SPPS from Chem-Matrix rink amide resin (0.4 mmol/g) on a 0.2 mmol scale and SIC prepared and coupled according to general procedure described above. The title peptide (68 mg, 84%) was obtained as a white fluffy powder after RP-HPLC purification and lyophilization. Gradient 0.1% TFA in acetonitrile and 0.1% TFA in water, with a linear gradient of 43% to 46% for 10 min at 10 ml min-1 and UV detection at 220 nm and 254 nm.

Isomer 1 (3b1): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.75 (m, 2H), 7.40-7.38 (m, 2H), 7.27-7.21 (m, 5H), 6.86-6.73 (m, 1H), 6.43-6.23 (br, 1H), 5.77-5.49 (br, 2H) 4.91-4.89 (m, 1H), 3.90-3.88 (m, 1H), 3.59-3.55 (m, 1H), 3.42-3.39 (m, 1H), 3.2-3.17 (m, 2H), 2.47 (s, 3H), 1.79-1.73 (m, 1H), 1.52-1.49 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.2, 171.9, 146.6, 137.0, 130.8, 129.4, 129.1, 128.8, 128.6, 127.2, 62.1, 53.4, 51.7, 36.7, 31.2, 24.5, 21.8. Isomer 2 (3b2): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80-7.74 (m, 2H), 7.38-7.34 (m, 2H), 7.32-7.28 (m, 2H), 7.25 (s, 1H), 7.24-7.20 (m, 2H), 6.97-6.93 (br, 1H), 4.92-4.85 (m, 1H), 4.21-4.18 (m, 3H), 4.02-3.98 (m, 1H), 3.42-3.40 (m, 2H), 3.08-3.02 (m, 2H), 2.45 (s, 3H), 1.78-1.73 (m, 1H), 1.68-1.63 (m, 1H), 1.53-1.46 (m, 1H), 1.39-1.31 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.7, 171.8, 146.4, 136.4, 130.7, 129.2, 129.1, 128.9, 128.6, 127.3, 63.3, 53.8, 49.5, 37.3, 31.4, 24.2, 21.8. IR (ATR): v = 3060, 2968, 1662, 1559, 1425, 1304, 1179, 1133, 1097, 814 cm^{-1}. HRMS (ESI-TOF) m/z [M+H]+ calcd. for $C_{21}H_{27}N_4O_3S$: 415.1804, found 415.1814.

Synthesis of ((2S)-3-(4-(tert-butoxy) phenyl)-2-((2S)-2-((((tert-butyldiphenylsilyl) amino) (methyl)(oxo)-l6-sulfanylidene)amino)propanamido)propanoyl)-L-phenylalanylglycine (4):

The peptide was synthesized using standard Fmoc-SPPS from 2-CTC resin (1 mmol/g) on a 0.5 mmol scale and SIC prepared and coupled according to the general procedure described above. The title peptide (103 mg, 25%) was obtained as a white fluffy powder after RP-HPLC purification and lyophilization. Gradient 0.05% formic acid in acetonitrile and 0.05% formic acid in water, with a linear gradient of 35% to 85% for 30 min at 10 ml min-1 and UV detection at 220 nm and 254 nm.

Isomer 1 (4a): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 – 7.66 (m, 4H), 7.64 – 7.27 (m, 6H), 7.25 – 7.12 (m, 3H), 7.11 – 7.05 (m, 2H), 6.96-6.94 (m, 2H), 6.81-6.78 (m, 2H), 4.86-4.81 (m, 1H), 4.68-4.64 (m, 1H), 4.10 (dd, J = 18.2, 5.6 Hz, 1H), 3.93-3.91 (m, 1H), 3.80 (dd, J = 18.2, 4.6 Hz, 1H), 3.17 (dd, J = 13.9, 5.6 Hz, 1H), 2.96 – 2.76 (m, 3H), 2.62 (s, 3H), 1.28 (s, 9H), 1.06 (s, 9H), 1.00 (d, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.9, 171.9, 171.3, 170.9, 154.6, 136.6, 135.6, 135.6, 134.9, 130.6, 129.9, 129.8, 129.5, 129.4, 128.5, 127.8, 127.8, 127.8, 127.0, 124.3, 78.7, 54.5, 54.1, 52.6, 46.3, 41.5, 38.3, 28.9, 27.1, 26.7, 19.8, 19.3. Isomer 2 (4b): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 – 7.70 (m, 4H), 7.49 – 7.32 (m, 4H), 7.28 – 7.25 (m, 6H), 7.20 – 7.13 (m, 1H), 6.84 – 6.78 (m, 2H), 6.68 – 6.66 (m, 2H), 6.58-6.56 (m, 1H), 4.85-4.79 (m, 1H), 4.25-4.22 (m, 2H), 3.81 – 3.72 (m, 2H), 3.46 (dd, J = 14.5, 4.4 Hz, 1H), 3.03-3.01 (m, 1H), 2.98 (s, 3H), 2.88 – 2.82 (m, 1H), 2.15 (dd, J = 14.5, 9.9 Hz, 1H), 1.31 (s, 9H), 1.11 (s, 9H), 0.78 (d, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.5, 172.5, 171.4, 171.2, 154.5, 137.6, 135.5, 135.4, 134.9, 130.9, 130.0, 129.8, 129.2, 129.1, 128.7, 128.3, 128.2, 127.8, 126.8, 124.5, 78.7, 56.3, 54.5, 53.7, 43.4, 41.8, 36.9, 28.9, 27.0, 26.7, 19.3, 18.7. IR (ATR): v = 2956, 1686, 1654, 1559, 1541, 1522, 1459, 1177, 1136, 924 cm^{-1}. HRMS (ESI-TOF) m/z [M+H]+ calcd. for $C_{44}H_{58}N_5O_7SSi$: 828.3826, found 828.3840.

Synthesis of (4-methylphenylsulfonimidoyl)-L-prolylglycyl-L-phenylalanyl-L-phenylalanine (5):

The peptide was synthesized using standard Fmoc-SPPS from 2-CTC resin (1 mmol/g) on a 1 mmol scale and SIC was prepared and coupled according to general procedure described above. The title peptide (396 mg, 64%) was obtained as a white fluffy powder as enriched isomers after RP-HPLC purification and lyophilization. Gradient 0.1% TFA in acetonitrile and 0.1% TFA in water, with a linear gradient of 37% to 85% for 30 min at 10 ml min-1 and UV detection at 220 nm and 254 nm

Isomer 1 (5a): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (br, 1H), 7.83 (d, J = 8.1 Hz, 2H), 7.53 (d, J = 7.2 Hz, 1H), 7.41 – 7.29 (m, 3H), 7.23 – 7.20 (m, 2H), 7.19-7.15 (m, 4H), 7.12 (d, J = 7.5 Hz, 2H), 6.70-5.86 (br, 4H), 4.74-4.66 (m, 1H), 4.56-4.49 (m, 1H), 4.12-4.06 (m,1H), 3.94-3.89 (m,1H), 3.80-3.71 (m, 1H), 3.56-3.46 (m, 1H), 3.26 (dd, J = 14.0, 5.1 Hz, 1H), 3.19-3.11 (m, 1H), 3.02-2.99 (m, 2H), 2.82 (dd, J = 14.2, 9.0 Hz, 1H), 2.45 (s, 3H), 2.01-1.95 (m, 1H), 1.84-1.82 (m, 2H), 1.56-1.53 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.3, 173.4, 171.9, 170.6, 151.5, 145.9, 136.9, 136.8, 131.2, 130.5, 129.4, 129.3, 128.7, 128.6, 128.5, 126.9, 62.4, 56.0, 54.2, 50.9, 43.4, 37.3, 37.2, 30.9, 24.9, 21.8. Isomer 2 (5b, major isomer reported): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.04 (br, 4H), 7.85-7.70 (m, 2H), 7.41-7.39 (m, 2H), 7.24 – 6.98 (m, 11H), 4.66-4.63 (m, 2H), 4.56-4.48 (m, 1H), 3.97-3.88 (m, 1H), 3.79-3.69 (m, 1H), 3.54-3.44 (m, 1H), 3.28 – 3.11 (m, 2H), 3.01 – 2.98 (m, 2H), 2.90-

2.81 (m, 1H), 2.46 (s, 3H), 2.10 – 1.89 (m, 3H), 1.79-1.70 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.8, 172.8, 172.2, 170.4, 147.3, 136.5, 136.1, 130.8, 130.3, 129.4, 129.3, 128.8, 128.6, 127.7, 127.2, 127.1, 70.64, 63.4, 49.8, 48.0, 37.8, 36.8, 29.9, 27.5, 24.8, 21.8. IR (ATR): v = 2868, 1779, 1751, 1636, 1528, 1444, 1420, 1340, 1205, 1120 cm^{-1}. HRMS (ESI-TOF) m/z [M+H]+ calcd. for $C_{32}H_{38}N_5O_6S$: 620.2543, found 620.2549.

Synthesis of (N-((S)-1-(tert-butoxy)-1-oxo-3-phenyl propan-2-yl) -4-methyl phenyl sulfonimidoyl) -L-prolyl-L-phenyl alanyl-L-phenyl alanyl glycine (6):

The peptide was synthesized using standard Fmoc-SPPS from 2-CTC resin (1 mmol/g) on a 1 mmol scale and SIC prepared and coupled according to the general procedure described above. The title peptide (255 mg, 31%) was obtained as a white fluffy powder after RP-HPLC purification and lyophilization. Gradient 0.1% TFA in acetonitrile and 0.1% TFA in water, with a linear gradient of 45% to 90% for 30 min at 10 ml min-1 and UV detection at 220 nm and 254 nm

Isomer 1 (6a): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.48 (br, 3H), 8.27-8.20 (m, 1H), 7.56-7.49 (m, 1H), 7.42 – 7.33 (m, 6H), 7.32-7.28 (m, 3H), 7.27 – 7.25 (m, 7H), 7.14 – 7.08 (m, 2H), 5.12 – 5.10 (m, 2H), 4.87-4.82 (m, 1H), 4.08 (dd, J = 18.7, 5.4 Hz, 1H), 3.91 – 3.74 (m, 2H), 3.68-3.60 (m, 1H), 3.32 – 3.06 (m, 7H), 2.51 (s, 3H), 2.35-2.22 (m, 1H), 2.03 – 2.0 (m, 2H), 1.83-1.72 (m, 1H), 1.62 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 171.0, 170.7, 170.2, 169.6, 161.3 (TFA), 160.9 (TFA), 147.2, 136.6, 136.1, 135.9, 130.7, 130.0, 129.7, 129.5, 129.5, 129.3, 128.8, 128.4, 128.3, 127.3, 127.1, 127.0, 116 (TFA), 114 (TFA), 83.3, 63.7, 59.5, 54.7, 53.8, 50.3, 41.8, 39.0, 38.9, 38.2, 31.6, 27.9, 24.7, 21.8. Isomer 2 (6b): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.55 (br, 3H), 8.01 - 7.98 (m, 2H), 7.41 – 7.27 (m, 7H), 7.20-7.17 (m, 7H), 7.11 – 7.07 (m, 2H), 7.0 – 6.98 (m, 2H), 4.88-4.81 (m, 1H), 4.69-4.61 (m, 1H), 4.39-4.28 (m, 1H), 4.20 – 4.13 (m, 1H), 3.99-3.89 (m, 2H), 3.26 (dd, J = 13.8, 6.4 Hz, 1H), 3.14-3.10 (m, 3H), 2.99-2.97 (m, 2H), 2.91-2.88 (m, 2H), 2.38 (s, 3H), 2.04-1.95 (m, 1H), 1.79-1.76 (m, 1H), 1.65 – 1.55 (m, 2H), 1.31 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 171.7, 171.6, 171.2, 170.3, 147.0, 136.6, 136.4, 136.2, 130.4, 129.8, 129.7, 129.5, 129.3, 129.1, 128.8, 128.6, 128.5, 127.2, 127.1, 127.0, 82.9, 63.9, 59.4, 55.2, 54.4, 49.4, 41.7, 39.7, 38.2, 37.7, 31.8, 27.7, 24.4, 21.7. IR (ATR): v = 3267, 2935, 1733, 1664, 1638, 1546, 1280, 1172, 1131, 1064 cm^{-1}. HRMS (ESI-TOF) m/z [M+H]+ calcd. for $C_{45}H_{54}N_5O_8S$: 824.3693, found 824.3704.

N-Acylation of imine “NH” of Sulfonimidoyl peptide:

Synthesis of (N-(glycylglycyl)-4-methylphenylsulfonimidoyl)-L-prolyl-L-phenylalanyl-L-phenylalanylglycine (7):

The peptide was synthesized using standard Fmoc-SPPS from 2-CTC resin (1 mmol/g) on a 0.5 mmol scale and SIC prepared and coupled according to the general procedure described above. Resin of attached toluene sulfonimidamide containing tetra-peptide (tol-SIA-Pro-Phe-Phe-Gly-2CTC) was made as per above mentioned by the SPPS protocol. Further, it was subjected to simple amide coupling using Fmoc-Gly-Gly-OH (5.0 equiv., 885 mg) as an acylating agent in presence of HBTU (5.0 equiv., 947 mg), DIPEA (10 equiv., 870 µL) in dry DMF (7 mL) at room temperature overnight, followed by a double coupling the next day. After completion of the reaction, the resin was washed thoroughly with DCM-DMF and the Fmoc group was deprotected using 20% piperidine in DMF. After completion of deprotection, the resin was washed thoroughly with DMF, DCM and dried under vacuum before cleavage of peptide with 20% HFIP in DCM. The title peptide (102 mg, 28%) was obtained as a white fluffy powder after RP-HPLC purification and lyophilization. Gradient 0.1% TFA in acetonitrile and 0.1% TFA in water, with a linear gradient of 25% to 75% for 30 min at 10 ml min-1 and UV detection at 220 nm and 254 nm.

Isomer 1 (7a): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.7-8.67 (m, 1H), 8.34 – 8.25 (m, 2H), 8.08 - 8.05 (m, 3H), 7.98-7.95 (m, 1H), 7.87 – 7.81 (m, 2H), 7.44 – 7.42 (m, 2H), 7.29 – 7.26 (m, 4H), 7.25-7.22 (m, 4H), 7.20 – 7.13 (m, 2H), 4.62-4.56 (m, 1H), 4.49 (ddd, J = 10.5, 8.4, 4.2 Hz, 1H), 4.28 (dd, J = 8.5, 2.8 Hz, 1H), 3.98 (dd, J = 17.88, 5.93 Hz, 1H), 3.88 (dd, J = 17.88, 5.66 Hz, 1H), 3.79 (d, J = 5.8 Hz, 2H), 3.64 - 3.60 (m, 2H), 3.10 – 2.99 (m, 4H), 2.83 – 2.75 (m, 2H), 2.41 (s, 3H), 1.67-1.62 (m, 1H), 1.59 – 1.45 (m, 2H), 1.21 – 1.14 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 176.2, 171.1, 171.0, 170.7, 170.4, 166.2, 144.5, 137.8, 137.5, 132.5, 130.0, 129.2, 129.1, 128.0, 128.0, 127.8, 126.3, 126.2, 60.9, 54.2, 53.7, 48.6, 45.7, 45.0, 40.6, 37.8, 37.2, 30.2, 23.6, 21.0. Isomer 2 (7b): ^{1}H NMR (400 MHz, CD_3CN) δ 8.42 (d, J = 8.5 Hz, 1H), 7.93 – 7.88 (m, 2H), 7.48-7.45 (m, 3H), 7.28-7.24 (m, 15H), 4.55 – 4.47 (m, 2H), 4.23 (dd, J = 9.0, 3.1 Hz, 1H), 3.99 - 3.90 (dd, J = 18.1, 6.5 Hz, 3H), 3.9 - 3.74 (dd, J = 18.1, 5.7 Hz, 2H), 3.27-3.24 (m, 3H), 3.17-3.12 (m, 1H), 2.95 – 2.89 (m, 3H), 2.44 (s, 3H), 1.74-1.69 (m, 1H), 1.64-1.60 (m, 1H), 1.49-1.45 (m, 1H), 1.24-1.19 (m, 1H). ^{13}C NMR (101 MHz, CD_3CN) δ 175.6, 172.8, 172.66, 172.3, 172.2, 172.1, 166.9, 147.2, 138.7, 138.6, 131.7, 131.3, 130.3, 130.2, 129.3, 129.3, 128.7, 127.5, 127.5, 63.0, 56.5, 55.7, 50.2, 45.1, 41.9, 37.9, 37.8, 31.4, 26.0, 24.8, 21.6. IR (ATR): v = 2958, 2916, 2849, 1718, 1653, 1522, 1457, 1377, 1202, 1181 cm^{-1}. HRMS (ESI-TOF) m/z [M+H]+ calcd. for $C_{36}H_{44}N_7O_8S$: 734.2972, found 734.2972.

N-Arylation of imine “NH” of Sulfonimidoyl peptide:

Synthesis of (N-(4-methoxyphenyl)-4-methylphenylsulfonimidoyl)-L-prolyl-L-phenylalanyl-L-phenylalanylglycine (8):

The peptide was synthesized using standard Fmoc-SPPS from 2-CTC resin (1 mmol/g) in a 1.0 mmol scale. Then SIC was prepared and coupled according to the general procedure described above.

Initially, sulfonimidamide resin (1 g, loading 1 mmol/g) was vacuum dried and swelled in dry THF (7 mL) and the following reagents were added in a sequential order: aryl boronic acid (5 equiv., 760 mg), anhydrous copper acetate (2.0 equiv., 362 mg), 4 Å powdered molecular sieves (100 mg), TEA (2.0 equiv., 300 µL) and dry THF (3 mL).[34] The heterogeneous mixture was mixed for 3 h. The resin was filtered off and washed with THF (×4), DCM (×4), followed by THF (×4) and charged with fresh reagents. The reaction mixture was again mixed for 3 h. The washing procedure was repeated, and the resin was charged again with fresh reagents and then shaken overnight. The washing procedure was repeated as above. The product was cleaved from the solid support by treatment with 50% TFA in DCM for 1 h. The cleavage solution was filtered off and washed with DCM. The combined filtrates were passed through celite and evaporated to dryness. The light green residue was re-dissolved in HPLC buffer and lyophilized. The resulting product was purified on a preparative column to obtain the desired peptide. The title peptide (153 mg, 21%) was obtained as a white fluffy powder after RP-HPLC purification and lyophilization. Gradient 0.1% TFA in acetonitrile

and 0.1% TFA in water, with a linear gradient of 45% to 95% for 25 min at 10 ml min-1 and UV detection at 220 nm and 254 nm.

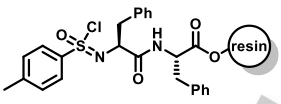
Isomer 1 (8a):^{1}H NMR (400 MHz, $CDCl_3$) δ 11.86 (br, 1H), 7.64 (d, J = 8.0 Hz, 2H), 7.25-7.19 (m, 13H), 7.08 – 7.01 (m, 2H), 6.97 (d, J = 8.7 Hz, 2H), 6.75 – 6.61 (m, 2H), 4.76-4.74 (m, 1H), 4.50-4.46 (m, 1H), 4.36 (dd, J = 9.2, 3.0 Hz, 1H), 4.21 (dd, J = 18.0, 6.1 Hz, 1H), 3.87 (dd, J = 17.7, 4.6 Hz, 1H), 3.64 (s, 3H), 3.18-3.12 (m, 4H), 2.97-2.89 (m, 1H), 2.32 (s, 3H), 2.24 (dd, J = 14.2, 10.6 Hz, 1H), 2.14 (dd, J = 14.3, 10.8 Hz, 1H), 1.77 – 1.64 (m, 1H), 1.62 – 1.51 (m, 1H), 1.14 – 1.05 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.9, 173.7, 171.7, 171.5, 155.6, 144.4, 137.7, 136.6, 136.4, 131.8, 130.3, 129.5, 129.4, 129.0, 128.9, 128.5, 127.2, 126.7, 125.1, 115.0, 63.9, 55.6, 54.8, 48.4, 45.8, 41.7, 36.9, 36.3, 30.2, 24.2, 21.6. Isomer 2 (8b): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (br, 3H), 7.88 (d, J = 8.1 Hz, 2H), 7.61 (d, J = 8.2 Hz, 1H), 7.38-7.34 (m, 3H), 7.25 – 7.16 (m, 3H), 7.11 – 7.06 (m, 3H), 7.01 – 6.92 (m, 3H), 6.66-6.62 (m, 4H), 5.10 – 4.96 (m, 1H), 4.44 – 4.32 (m, 1H), 4.26 (dd, J = 18.0, 6.3 Hz, 1H), 3.99 – 3.85 (m, 2H), 3.64 (s, 3H), 3.53 (dd, J = 15.2, 4.2 Hz, 1H), 3.29 – 3.17 (m, 2H), 3.10 – 2.99 (m, 2H), 2.39 (s, 3H), 2.27-2.20 (m, 1H), 1.78-1.70 (m, 1H), 1.40 – 1.27 (m, 2H), 1.12-1.02 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.0, 172.8, 172.4, 172.3, 156.5, 145.5, 136.7, 136.2, 134.8, 130.5, 129.6, 128.9, 128.8, 128.8, 128.7, 128.6, 127.2, 127.0, 125.5, 115.1, 62.6, 56.1, 55.6, 54.0, 50.1, 41.6, 36.7, 36.5, 30.6, 24.4, 21.7. IR (ATR): v = 2980, 1735, 1654, 1522, 1500, 1440, 1272, 1235, 1179, 1105 cm^{-1}. HRMS (ESI-TOF) m/z [M+H]+ calcd. for $C_{39}H_{44}N_5O_7S$: 726.2961, found 726.2973.

Synthesis of (2-((3S)-1-oxido-4-oxo-1-(p-tolyl)-3, 4-dihydro-2H-1l6,2,5-thiadiazol-3-yl) acetyl)-L-proline (10):

The peptide was synthesized using standard Fmoc-SPPS from 2-CTC resin (1 mmol/g) on a 0.5 mmol scale and SIC prepared and coupled according to the general procedure described above. Further, the resin attached SIA peptide was treated with 1.0 M TBAF in THF (10 mL) at 50 °C to get desired compound 10.[4b] The title peptide (141 mg, 78%) was obtained as a white fluffy powder after RP-HPLC purification and lyophilization. Gradient 0.1% TFA in acetonitrile and 0.1% TFA in water, with a linear gradient of 25% to 67% for 30 min at 10 ml min-1 and UV detection at 220 nm and 254 nm. Further, to separate the diastereomers, the HPLC purified fraction were subjected for SFC purification resulted in enriching of the isomers.

Isomer1 (10a, major isomer reported) : ^{1}H NMR (400 MHz, CD_3OD) δ 7.82 – 7.79 (m, 2H), 7.51 – 7.45 (m, 2H), 4.68-4.65 (m, 1H), 4.45 (dd, J = 8.6, 3.1 Hz, 1H), 3.68-3.55 (m, 2H), 3.36-3.33 (m, 1H), 3.24 – 3.12 (m, 1H), 2.80 (dd, J = 16.9, 10.4 Hz, 1H), 2.47 (s, 3H), 2.27-2.23 (m, 1H), 2.12 – 1.85 (m, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 181.4, 175.6, 170.5, 147.2, 136.6, 131.4, 128.8, 62.0, 60.3, 48.3, 40.3, 30.4, 25.5, 21.5. Isomer 2 (10b, major isomer reported): ^{1}H NMR (400 MHz, CD_3OD) δ 7.90-7.86 (m, 2H), 7.47 – 7.45 (m, 2H), 4.65 (dd, J = 9.4, 2.5 Hz, 1H), 4.41 (dd, J = 8.6, 3.2 Hz, 1H), 3.65-3.62 (m, 1H), 3.57 – 3.53 (m, 1H), 3.13 (dd, J = 17.0, 2.5 Hz, 1H), 2.80 (dd, J = 17.0, 9.5 Hz, 1H), 2.46 (s, 3H), 2.27-2.21 (m, 1H), 2.03-1.98 (m, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 181.9, 175.7, 170.2, 147.1, 136.3, 131.2, 129.4, 60.2, 59.9, 47.6, 38.5, 30.3, 25.6, 21.5. IR (ATR): v = 2143, 1716, 1636, 1453, 1405, 1261, 1190, 1142, 1097, 998 cm^{-1}. HRMS (ESI-TOF) m/z [M+H]+ calcd. for $C_{16}H_{20}N_3O_5S$: 366.1124, found 366.1128.

Approach 3: On-resin SIA synthesis, via on-resin SIC formation followed by addition of an amino acid (NH2-Phe-Phe-O-Resin) dipeptide nucleophile was synthesized as per above mentioned standard Fmoc-SPPS method on 0.5 mmol scale using 2-CTC resin. Next, toluene sulfonyl chloride (5.0 equiv., 476 mg) was coupled (N-capping) to the resin attached peptide in dry DCM, in presence of base and DIPEA (10 equiv., 900 µL). The resulting solid phase reaction shook for 6 h on shaker. Later, the resin was washed thoroughly with DCM, DMF and reaction was monitored by LC-MS after mini-cleavage. After successful completion of coupling, the resin was dried and purged with nitrogen gas. Dry DCM was added to the resin under nitrogen atmosphere and the solid phase reactor was cooled to 0 °C by adding dry ice to the sonicator bath.[38a] Pre-dissolved PPh_3Cl_2 (4.0 equiv., 600 mg), TEA (10 equiv., 850 µL) in dry DCM (6 mL) was added dropwise to solid phase reactor and further sonicated for 30 min at 0 °C to get SIC.



Later, proline methyl ester (5.0 equiv., 414 mg), DIPEA (10 equiv., 900 µL) in DCM (4 mL) was added and sonicated for 30 minutes. After completion of the reaction, the reactor was washed thoroughly with DMF and DCM. The resin attached peptide was cleaved from the resin using 20% HFIP in DCM (10 mL).

Synthesis of ((2S)-2-((((S)-2-(methoxycarbonyl) pyrrolidin-1-yl) (oxo)(p-tolyl)-l6-sulfaneylidene) amino)-3-phenylpropanoyl)-L-phenylalanine (11):

The title peptide (257 mg, 90%) was obtained as a white fluffy powder after RP-HPLC purification and lyophilization. Gradient 0.1% TFA in acetonitrile and 0.1% TFA in water, with a linear gradient of 60% to 65% for 15 min at 10 ml min-1 and UV detection at 220 nm and 254 nm.

Isomer 1 (11a): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (br, 1H), 7.45 – 7.28 (m, 2H), 7.25 – 7.07 (m, 10H), 6.97-6.95 (m, 2H), 6.57-6.32 (br, 1H), 5.0-4.83 (m, 1H), 4.36-4.28 (m, 1H), 4.26-4.11 (m, 1H), 3.62 (s, 3H), 3.31-3.28 (m, 2H), 3.06-2.97 (m, 4H), 2.48 (s, 3H), 1.84-1.82 (m, 2H)1.80-1.78 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.6, 174.9, 172.0, 146.0, 136.4, 130.7, 130.6, 130.3, 129.1, 129.1, 129.0, 128.9, 128.5, 127.7, 127.4, 63.3, 54.8, 53.6, 52.2, 49.5, 37.3, 31.3, 30.9, 24.2, 21.7. Isomer 2 (11b): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, J = 7.4 Hz, 2H), 7.51 (m, 1H), 7.37 – 7.28 (m, 6H), 7.23 (m, 1H), 7.14 – 7.06 (m, 3H), 7.06 – 7.00 (m, 2H), 4.85-4.79 (m, 1H), 4.40 (dd, J = 9.3, 3.4 Hz, 2H), 4.07-4.02 (m, 1H), 3.67 (s, 3H), 3.16 (dd, J = 13.3, 3.4 Hz, 1H), 3.10-3.06 (m, 1H), 2.80 (dd, J = 13.2, 9.3 Hz, 1H), 2.45 (s, 3H), 2.35-2.45 (m, 1H), 2.25-2.35 (m, 1H), 1.88-1.86 (m, 2H), 1.58-1.65 (m, 1H), 1.32-1.38 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.1, 172.9, 172.1, 145.7, 136.6, 135.8, 130.3, 130.2, 129.5, 128.8, 128.6, 128.4, 128.3, 127.1, 127.0, 61.6, 59.0, 53.1, 52.8, 49.3, 40.2, 37.4, 30.6, 24.6, 21.8. IR (ATR): v = 1735, 1623, 1522, 1453, 1436, 1271, 1190, 1149, 1008, 805 cm^{-1}. HRMS (ESI-TOF) m/z [M+H]+ calcd. for $C_{31}H_{36}N_3O_6S$: 578.2325, found 578.2328.

A combinatorial library synthesis was also demonstrated according to approach 3. On 0.5 mmol scale using 2-CTC resin.

For demonstration of approach 3 in library format - see movie and LC-MS chromatograms in supporting information.

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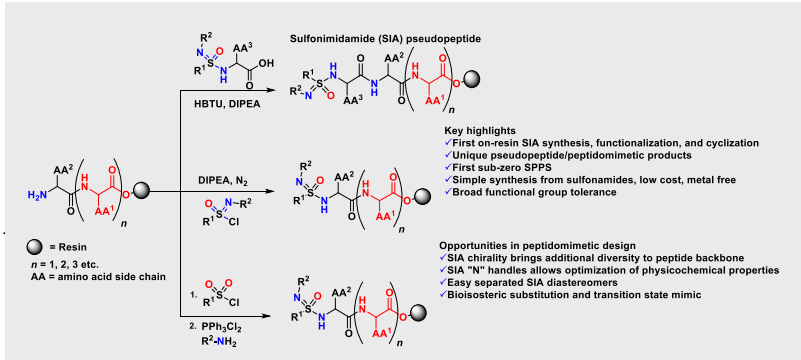

Sulfonimidamide Pseudopeptides

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Title: Solid Phase Synthesis of Sulfonimidamide Pseudopeptides and Library Generation

In this work the sulfonimidamide functionality has been combined with peptides forming sulfonimidamide pseudopeptides as potential new modalities in drug discovery. Three alternative synthetic methods to generate the pseudopeptides have been developed, all harmonized with classical solid-phase peptide synthesis (SPPS), including both on- and off-resin sulfonimidamide synthesis. The methods allow late stage modifications and parallel syntheses.

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