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Ligand Design for Somatostatin Receptor Isoforms 4 and 5

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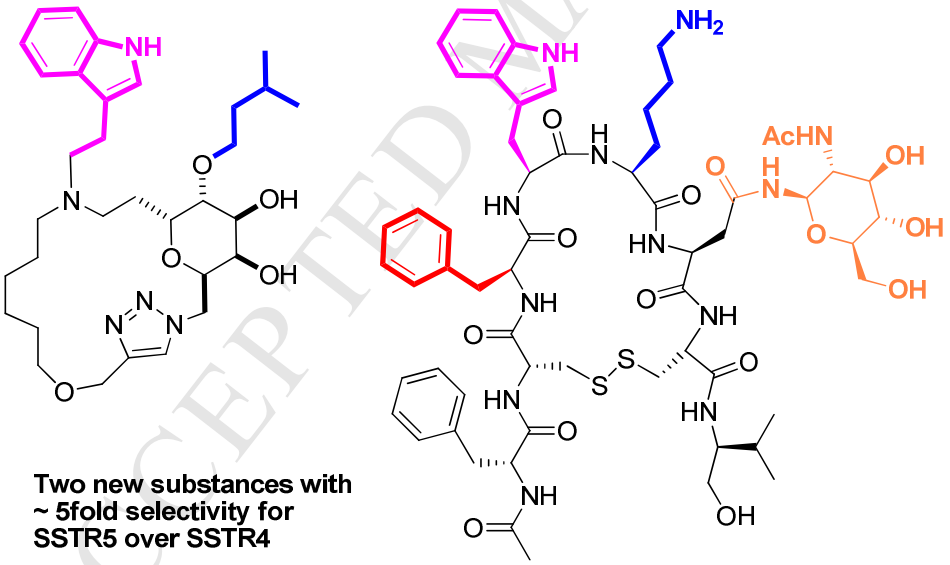
Highlights

- Small to large sized peptidomimetics were synthesized
- They all contain Phe, Trp, Lys sidechains or similar bioisosteres
- An iminosugar derivative showed 5-fold or greater selectivity for SSTR-4 over SSTR-5
- A new glycopeptide presenting GlcNAc and new macrocyclic derivative showed ~5-6 fold selectivity for SSTR-5
- Homology models of SSTR4 and SSTR-5 were constructed, evaluated and are made available

Graphical Abstract



Abstract

The somatostatin receptor (SSTR) isoforms, SSTR-4 and SSTR-5 are targets in numerous disorders and diseases. Although there has been some success in achieving selective isoform inhibition, structure-based drug design and development in this area has faced a challenge, mainly attributed to the lack of availability of SSTR-4 and SSTR-5 crystal structures. Previous structure activity relationship (SAR) studies have included work on non-peptide peptidomimetics or β-turn peptidal peptidomimetics where side chains of lysine, tryptophan, and phenylalanine

(i.e. functional epitopes) are presented on a scaffold or molecular framework. However, there could be more structural information that would help design ligands selective for one or more of these isoforms. Here, we include synthesis of new mimetics and include their evaluation as ligands for SSTR-4 and SSTR-5. Inhibitors based on small to larger sized scaffolds (ManNAc, iminosugars, Eannaphane macrocycles, acyclic and cyclised peptide structures) are compared. These scaffolds have been grafted with side chains of lysine, tryptophan, and phenylalanine or similar bioisosteres/pharmacophoric groups. A new macrocycle as well as an iminosugar derivative show 5-fold or greater selectivity for SSTR-4 over SSTR-5. A new glycopeptide presenting GlcNAc showed ~6 fold selectivity for SSTR-5, which contrasted with the non-glycosylated peptide. A number of non-peptide dual inhibitors (K_i values of 0.58 μM to 5 μM) were also identified. Conceivable molecular interactions of these inhibitors were studied with newly constructed homology models of SSTR-4 and SSTR-5 isoforms.

Keywords: Somatostatinergic system, Diverse Scaffolds, Synthesis, Ligand Based Design, Somatostatin Receptor 4, Somatostatin Receptor 5

Introduction

The human somatostatin receptors (SSTRs) belong to the G-protein coupled receptors (GPCRs) and have 5 isoforms (SSTR-1 to 5), which closely resemble each other in structural homology and functional efficacy [1-4]. Their high structural resemblance is also linked to their synchronised roles in numerous cellular homeostases and in several disorders, based on their tissue specific isoform localisation. Additional interest has been placed in SSTR-4 and SSTR-5 in recent years. Agonism of SSTR-4 is believed to be relevant in Alzheimer disease [5], influencing memory strategies in the human brain [6]. Targeting SSTR-4 selectively also represents a promise for non-opioid pain control, the latter successfully shown by clinically studied agent J2156, which is a potent selective inhibitor of this target [7-9]. SSTR-4 is also believed to have a role in the migration of hepatic oval cells [10]. On the other hand, SSTR-5 has found roles in proliferation in pancreatic cancer [11], neuroendocrine tumours [12] and glucose homeostasis [13]. Therefore, the identification of compounds which have preferential selectivity or differential binding for these receptors is important and is tied in with the identification of agonists or antagonists and there are a number of implications [14-16]. These receptors have not been crystallised to date and this hinders structure-based drug design for them. However, ligand-based design strategies, have established some key important features that a ligand should possess, to recognise these receptors. Accordingly, studies on the binding of somatostatins (SRIFs), especially its tetradecapeptide form (SRIF-14, see figure 1) and its *N*-terminally extended peptide form (SRIF-28) with SSTRs, have indicated that tryptophan-8 (trp8) and lysine-9 (lys9) residues in these structures are recognised by all isomeric forms of SSTR, while phenylalanine-6 (phe6) is highlighted as being specifically important for SSTR-4 activation [17-22].

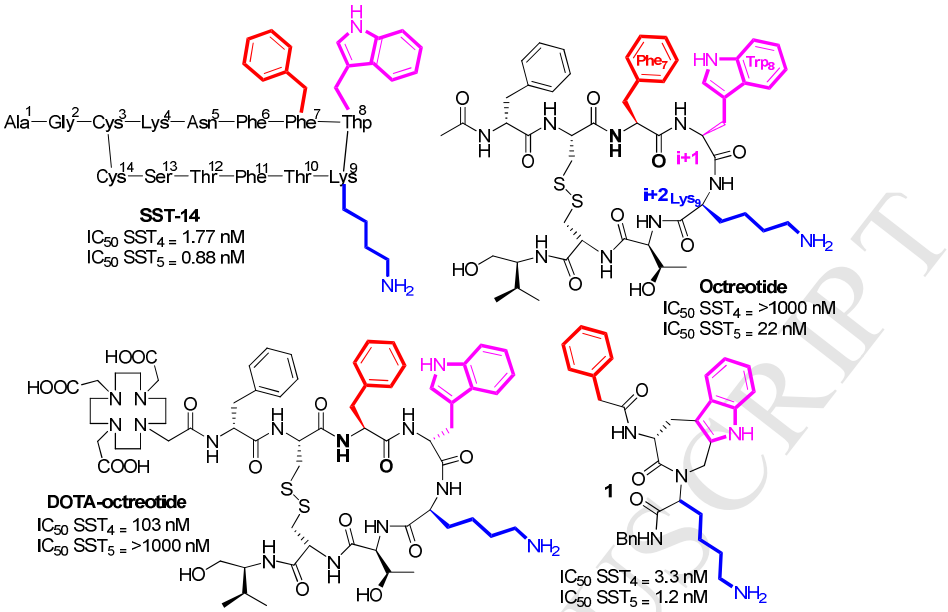


Figure 1 Structures of SST-14 and selected peptidomimetics with high binding affinities for SSTRs. Important pharmacophoric groups are shown in blue, purple and red.

To date, various non-peptide, and peptide analogues of somatostatin, with pharmacophoric groups have been synthesized and evaluated against SSTR-4 and SSTR-5, and their activity ranges from *n*M to *m*M; some of these molecules are shown in Figure 1. It is unclear why these molecules [23-29], have varying degree of affinities. In this research, a set of peptidomimetics based on pyranose, iminosugar (multi-hydroxylated piperidine), macrocycle and peptidyl scaffolds were synthesised. To each scaffold was grafted pharmacophoric groups which are identical or bioisosteric to those found in amino acid sidechains (Figure 2 and Figure 3). Two of these molecules have show preferential selectivity for SSTR-4 over SSTR5. Docking to respective homology models of the proteins is included as part of this work.

Results and Discussion

Compounds designed to target SSTRs. Syntheses of some compounds used in this study has been reported earlier (see Figure 2) and these include the iminosugars [30-33], benzomacrolactones [34] and pyranoside **3** originally designed by Hirschmann and co-workers [35]. New compounds based on the pyranose ManNAc, the Eannaphane macrocycle, as well as acyclic/cyclic peptidyl scaffolds are shown in Figure 3. The basic design concept involved using the functional groups, inherent in the scaffolds, to graft pharmacophoric groups, and thus defining the points of attachment for amino acid side chains or their bioisosteres. For the pyranoses or iminosugars and benzomacrolactones, like **9** and **10**, these were inspired by natural product ring systems found in nature. Whereas **13-15** are not natural products, to the best of our knowledge, their core scaffolds can be considered to be 'natural product like', in that they are chiral macrocycles and have functional groups found in natural products. The pharmacophoric groups were placed at

distances from each other on the scaffolds approximating to those in SST-14. Thus there are 3-5 bonds between atoms to where the pharmacophoric groups are attached. In addition, peptidyl mimetics **16-18** are included, which are similar in structure to octreotide.

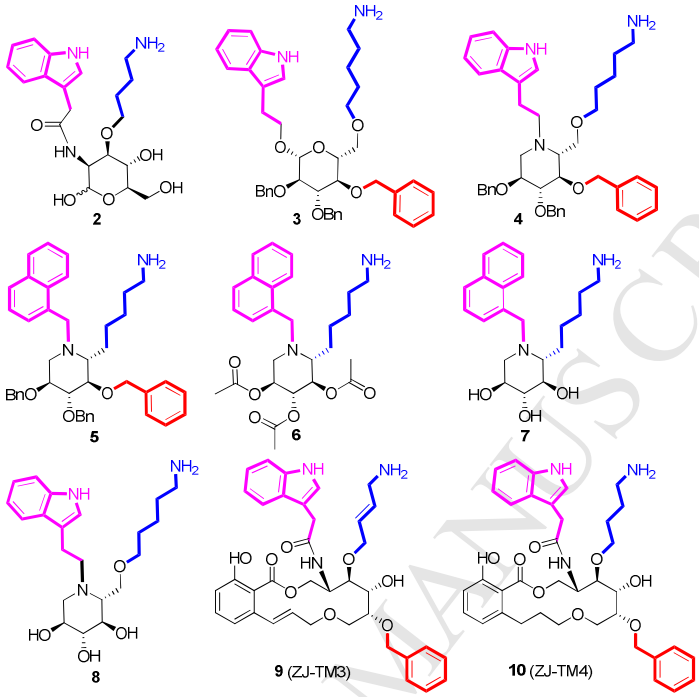


Figure 2 Structures of compounds previously synthesised *in-house* and Hirschman's pyranoside **3**. The colour codes are used to display the relevant amino acid side chain or their bioisosteres corresponding to those found to be important in somatostatin ligands.

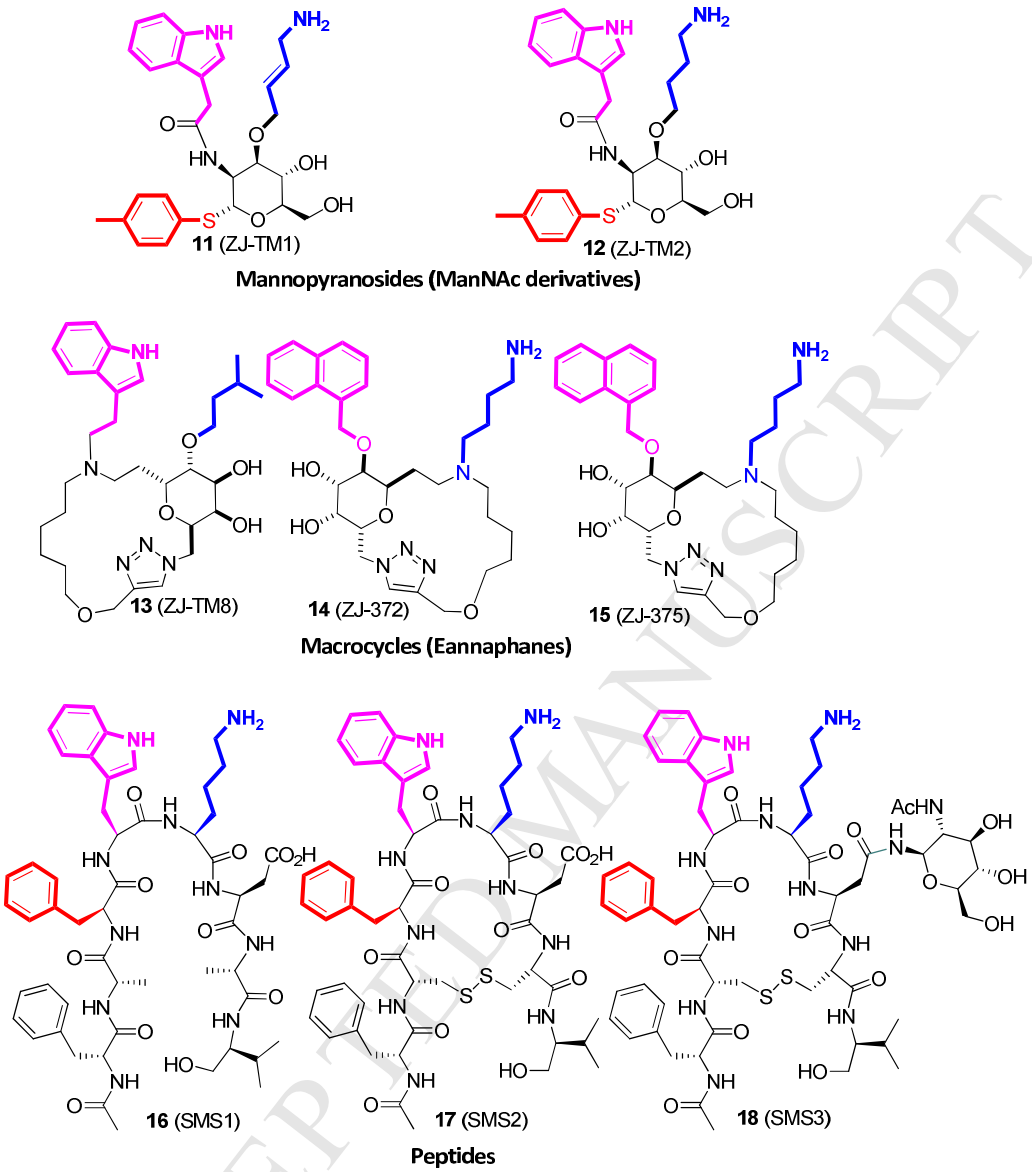
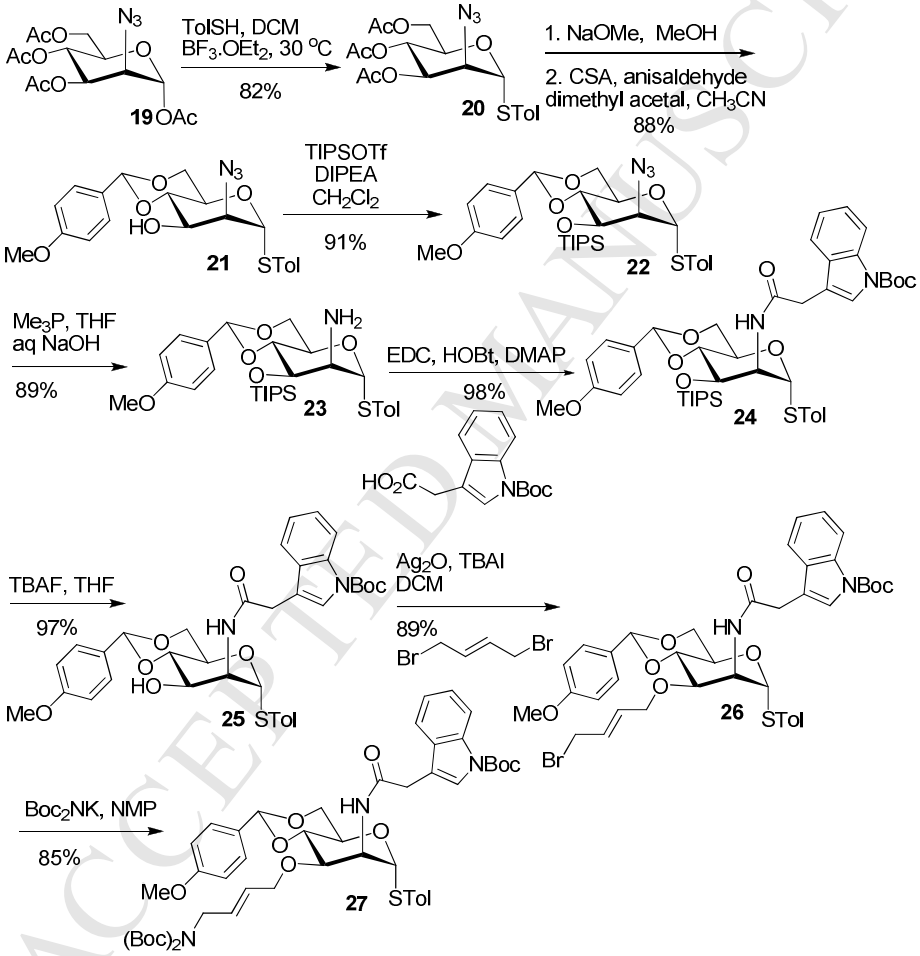


Figure 3 Structures of various new potential somatostatin mimetics included herein. The colour codes are used to display the relevant amino acid side chains or their bioisosteres corresponding to those found important in somatostatin ligands

*Synthesis of somatostatin mimetics based on 2-deoxy-2-acetamido-*D*-mannopyranose*

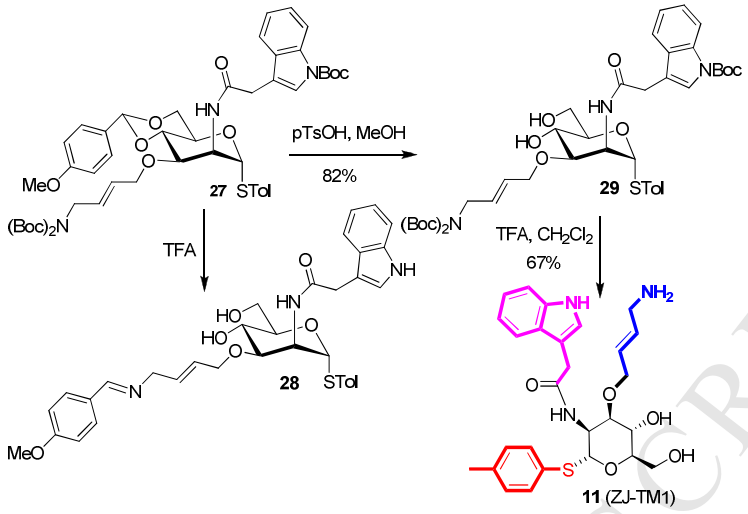
The earlier work of Hirshmann and co-workers on synthesis of glucopyranose-based somatostatin ligands encouraged us to synthesize new 2-deoxy-2-acetamido-D-mannopyranose (ManNAc) based mimetics (Fig. 3), where the amine of the 2-deoxy-2-aminomannopyranose could be used for grafting an isostere of the trp sidechain. In addition the C-3 alcohol was used as the point of attachment for the lys sidechain. The spacing between the trp isostere and lysine side chain is equal to that found in the benzomacrolactone **10**. In addition the STol group can potentially mimic the phe side chain. The synthesis of the ManNAc derivatives was initiated by Lewis acid promoted glycosidation reaction of **19** [36] to give the α-thioglycoside **20**.

Subsequent de-*O*-acetylation and acetalisation gave **21**. Next a TIPS group was introduced at the C-3 OH group to give **22**, and its subsequent reduction using the Staudinger reaction gave amine **23**, needed for the grafting. In the latter reaction the utilisation of Me_3P was found to be more efficient than the use of Bu_3P. The coupling of **23** with a protected indole acetic acid derivative gave **24**. The TIPS group was removed from **24** to give **25**. Monoalkylation reactions of **25** were investigated and the use of (*E*)-1,4-dibromobut-2-ene in the presence of silver oxide and tetra-*N*-butyl ammonium iodide (TBAI) gave the desired product **26** in 89% yield; these reagents and conditions were found superior to the use of NaH in DMF (no product obtained) or the use of NaH in the presence of TBAI in dichloromethane (50%). Next, we found that attempts to carry out a Gabriel reaction with PhthK were unsuccessful and led to decomposition. However, the use of Boc_2NK gave the useful intermediate **27** (Scheme 1) [34].



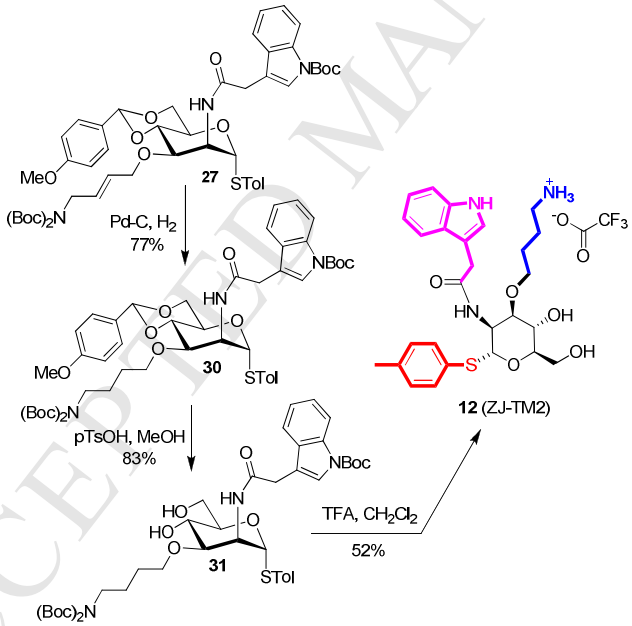
Scheme 1 Synthesis of intermediate **27**

The attempted TFA induced removal in one pot of the benzylidene group and Boc groups from **27**, resulted in the formation of the Schiff base **28**. In contrast, the step by step removal of the benzylidene group giving **29** followed by Boc removal using TFA afforded the desired peptidomimetic **11** (Scheme 2).



Scheme 2 Synthesis of **11**

The alkene of **27** was converted to **30** using catalytic hydrogenation and then the stepwise removal of protecting groups gave the ManNAc derivative **12** via **31** (Scheme 3).

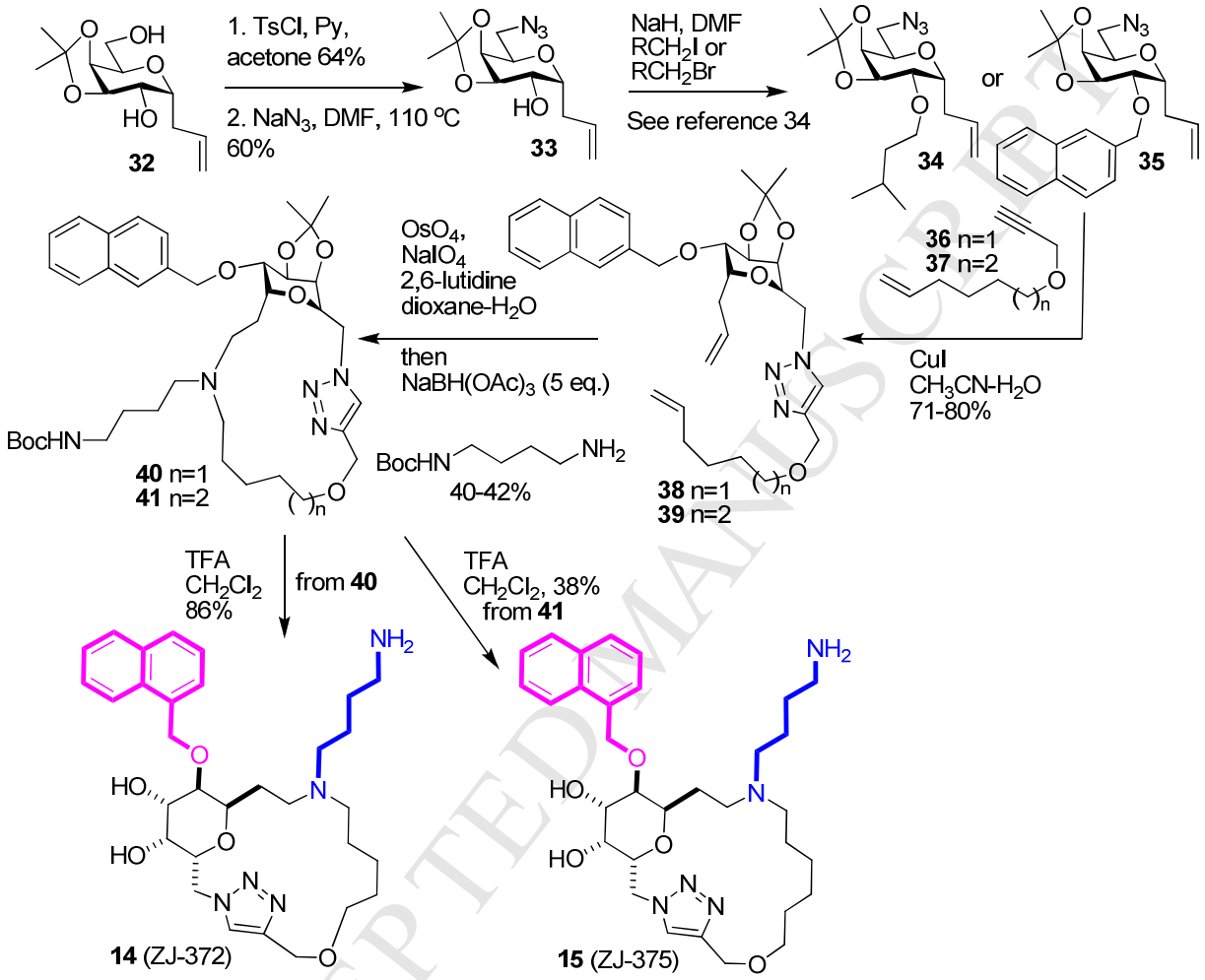


Scheme 3 Synthesis of **12**

Synthesis of somatostatin mimetics based on macrocyclic scaffolds with an embedded monosaccharide

The synthesis of natural product like macrocyclic compounds was carried out via **33**, **36** and **37** which were prepared as previously described [37]. Alkylation of the galactose 2-OH group of **33** gave **34/35**. Next the Cu(I) catalyzed azide-alkyne cycloaddition of **35** with **36/37**, gave 1,4-triazoles **38**/**39** [38, 39]. Sequential oxidative cleavage of the alkenes of **38/39**, gave dialdehydes, which then after double reductive amination cyclisation [40] gave **40/41**.

Subsequent removal of the protecting groups, gave **14** and **15** as shown in Scheme 4. Analogous procedures involving **34**, and **36** with tryptamine gave **13** (see supporting information for details).

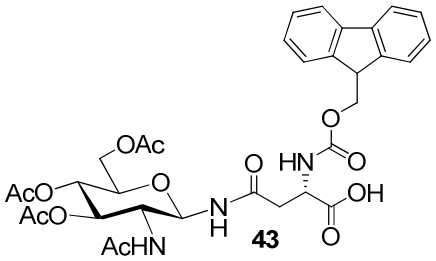


Scheme 4 Synthesis of **14** & **15**

Somatostatin mimetics based on peptides.

Our investigation also explored peptides, which potentially had the *β*-turn as found in somatostatin, along with the incorporation of the key residue sequence Phe-Trp-Lys. Kelly and co-workers had outlined the stabilization of reverse *β*-turns with the inclusion of a glycosylated asparagine (*i*) two or three places after a phenylalanine residue (*i+2* and *i+3*) through a carbohydrate-*π* interaction [41, 42]. For this reason, we synthesized a glycosylated peptididomimetic **18** (SMS3) with this feature. The glycosylated **18** and other related peptidyl mimetics were prepared from aspartate derivative **43** [43], prepared according to the previously

reported procedures in high yield [44-46]. Solid phase peptide synthesis based on the rink amide resin, was then used to give **16-18 (**SMS1-3**)** (see supplementary information) [47] after deacetylation of the acetylated glycopeptide and subsequent purification using by high-performance liquid chromatography.



Scheme 5 Structure of **43**

Binding affinities, molecular modelling and structure activity relationship

The binding affinity assays were performed against two somatostatin receptor isoforms (SSTR-4 and SSTR-5) and inhibitory constants (K_i) determined and these are shown in Table 1. Most of molecules based on the different scaffolds displayed similar affinities, in most cases in the low micromolar range, for both isoforms with a limited number of exceptions where > fivefold selectivity differences were observed. The macrocycle **13** showed preferential selectivity for SSTR-5 over SSTR-4 while the iminosugar **8** had greater selective for SSTR-4 compared to SSTR-5. The iminosugar **7** and the glycosylated peptide **18** were the only agents which showed moderate preferential selectivity for SSTR-5 (Table 1). Iminosugar **7** differs from analogues **5** and **6**, which have similar affinities for both isoforms, in that **7** contains free hydroxyl groups. Peptide **18** differs only from **17** due to the presence of the GlcNAc residue linked via the asparagine and **17** displayed similar activity for both isoforms.

Table 1 Binding affinities (K_i) of peptidomimetics for SSTRs: **SSTR-4** [48]**:** Binding studies were carried out at Cerep (www.cerep.fr). According to Cerep's procedures, they were performed with cell membranes from transiently transfected COS-1 cells as described [49]. 10 µg of membrane protein was incubated in 10 mM Hepes (pH 7.5), 5 mM. $MgCl_2$, bacitracin (20 jig/ml), 0.5% bovine serum albumin, and ^{125}I-labeled [Tyr^{11}]-somatostatin-14 (30,000 cpm) with various concentrations of unlabeled somatostatin 14 (1 µg) and compounds of interest for 2 h at room temperature. Later, scintillation counting method was used for detection; **SSTR-5** [50]: The SSTR-5 gene was cloned into pCMV6c expression vector [51] and transfected into COS1 cells. 20 µg of membrane protein preparation was incubated in 500 µL of Na^+-free binding buffer (10 mM HEPES, 1% bovine serum albumi, 5mM $MgCl_2$, 1mg/ml bacitracin, pH 7.45) containing approximately 10 pM of [^{125}I-Tyr^{11}]-somatostain-14 alone or with somatostatin-14 and compounds of interest at various concentrations for 2 hrs at room temperature. Later, scintillation counting was used for detection. (detailed experimental data are provided in Supplementary Information).

Somatostatin mimetic	**Scaffold type**	$K_{i,\ SSTR\text{-}4}$ (μM)	$K_{i,\ SSTR\text{-}5}$ (μM)
2	Pyranose	>100	>100
3 [35]	Pyranose	1.1	not available
4	Iminosugar	4.4 ± 0.89	5.0 ± 0.66
5	Iminosugar	1.9 ± 0.37	1.3 ± 0.08
6	Iminosugar	5.4 ± 0.60	5.1 ± 0.91
7	Iminosugar	>100	23 ± 1.39
8	Iminosugar	3.2 ±0.57	>100
9	Macrocycle	0.58 ± 0.23	1.1 ± 0.25
10	Macrocycle	1.9 ± 0.41	3.2 ± 0.71
11	Pyranose	2.1 ± 0.26	3.9 ± 0.23
12	Pyranose	6.8 ± 1.02	12 ± 1.35
13	Macrocycle	21 ± 1.47	4.1 ± 0.88
14	Macrocycle	>100	>100
15	Macrocycle	>100	>100
16	Peptide	>100	>100
17	Peptide	7.2 ± 0.73	10 ± 1.12
18	Peptide	20 ± 1.77	3.4 ± 0.52

In order, to hypothesise how the selectivities of these ligands might be influenced by scaffold and pharmacophoric groups, homology models of SSTR-4 and SSTR-5 were developed and utilised in docking. For the homology modelling, template-based modelling was implemented, which was based on accessed templates obtained from a BLASTp search. A selection was made based on complementarity with respect to the sequences of SSTR-4 and SSTR-5 [52, 53]. A protein sequence of the human delta opioid 7-transmembrane receptor (PDB: 4N6H [54],

resolution = 1.80 Å, 48% sequence identity, 44.01% similarity, 72% coverage) was selected for SSTR-4 and a sequence of the nociceptin-orphanin FQ peptide receptor (PDB: 5DHH [55], resolution 3.0 *Å*, 41.76% sequence identity, 40.08% seq. similarity, 70% coverage) was selected for SSTR-5. These were retrieved from the Research Collaboratory for Structural Bioinformatics - Protein Data Bank (RCSB-PDB).

The built homology models were evaluated for structural consistency in a qualitative manner. Firstly, the RMSD value was compared between the homology model and the respective template. These were within acceptable limits (0.887 *Å* for SSTR-4 and 0.609 *Å* for SSTR-5, see *Supplementary Information*). Next Ramachandran plots were evaluated to investigate the geometry of amino acid residues in the homology models. For SSTR-4 93.4% of the amino acids were in the most favoured geometries and for SSTR-5 this was 96% see *Supplementary Information*). The ERRAT plots, which are for the determination of errors in model building indicate a high degree of confidence (83.45% for SSTR4, 91.20% for SSTR-5). The *z*-score plots were obtained by the protein structure analysis tool (ProSA), as it evaluated the overall model quality; a score of -3.87 was obtained for SSTR-4 as compared with -4.51 for the template PDB file used (4N6H). A score of -3.08 was obtained for SSTR-5 as compared with a score of -3.23 for the template PDB used (5DHH) [56]. These results provided assurance of a reasonable structural quality of the constructed homology models.

Table 2 Comparative analysis of key residues within active site domain of individual isoforms with the template amino acids.

Template	**SSTR4**	**SSTR5**	**Template**	**SSTR4**	**SSTR5**	**Template**	**SSTR4**	**SSTR5**
Gln107	Val67	Leu96	*Tyr131*	*Gly91*	*Gly120*	Val279	*Phe239*	*Phe264*
Asp110	Ser70	Gln99	*Met134*	*Met94*	*Gln123*	Gln280	*Tyr240*	*Phe265*
Ile111	Ala71	Asn100	*Phe135*	*Phe95*	*Phe124*	Val283	*Gln243*	*Asn268*
Trp116	Trp76	Trp105	*Cys200*	*Cys162*	*Cys186*	Leu301	Asn257	Tyr286
Val126	Val86	Val115	Ile219	Thr179	Thr205	Arg302	*His258*	*Phe287*
Ile127	*Leu87*	*Met116*	Ser223	Gly183	Gly209	Thr305	Leu261	Val290
Asp130	*Asp90*	*Asp119*	Trp276	Trp236	Trp261	Tyr309	*Tyr265*	*Tyr294*

Underlined amino acids are highly conserved through the GPCR family; Italics denote the key residues involved in antagonist/agonist binding interactions

To identify the active cavity on the surface of both proteins, the Molecular Operating Environment active site detection tool (MOE 2015.1001) was employed [57, 58]. Nehrung *et al* had performed mutation based studies on Asp90 (SSTR-4) and Asp119 (SSTR-5) in transmembrane domain 3 (TM3) of both SSTR-4/5 isoforms, and this showed that an ionic interaction with the positively charged lysine amine group in the side chain is important for the

binding of endogenous somatostatin (SRIF-14) [59, 60]. However, Kontoyianni *et al.* suggested two possible ligand binding modes, indicating one which makes a *H*-bond with Gln243 while the other mode involves interaction with Asp90 with the latter also indicating that heteroaryl moieties could engage in π-stacking within the hydrophobic domain constituted by Phe175, Phe239, Trp171 and Tyr240 in the SSTR4 receptor [61]. Ozenberger and Hadcock reported a single site tyrosine substitution for Phe265 in the region of transmembrane domain 6 (TM6) of SSTR-5 and this resulted in altered ligand binding selectivity and loss of the binding preference of SSTR-5 for SRIF-28 over SRIF-14 [62]. To the best of our knowledge, at the time of writing of this manuscript, no more structural data information was available for these proteins and their interactions with their ligands. This led us to compare the active sites in the homology models with that of the template protein (human delta opioid receptor) used for generating the SSTR-4 homology model in the form where it was co-crystallised with its ligand. The template is also a GPCR protein, which would help to evaluate whether amino acids in the active site could be important for the binding of somatostatins and their mimetics (see **Table 2**). The binding cavity was found to be relatively large, which is commensurate with its requirement to bind SST-14. Smaller non-peptidic ligands could thus occupy different parts of this cavity. To explore these possibilities, docking was investigated.

Firstly, a comparison of glucopyranoside **3** in SSTR-4 with peptidomimetics based on the iminosugar scaffold was made. The iminosugar derivative **4** retained potency to an extent, compared to **3**, but had low isoform selectivity ($K_{i,\ \text{SSTR-4/ SSTR-5}}$ = 1.13, see Table 1). In the docking, the interaction of **4** with receptor site was found to be highly influenced by π-stacking interactions of its own aromatic rings (Fig. 4) as well as with residues in the receptor. These were observed in both isoforms and may explain lack of selectivity observed.

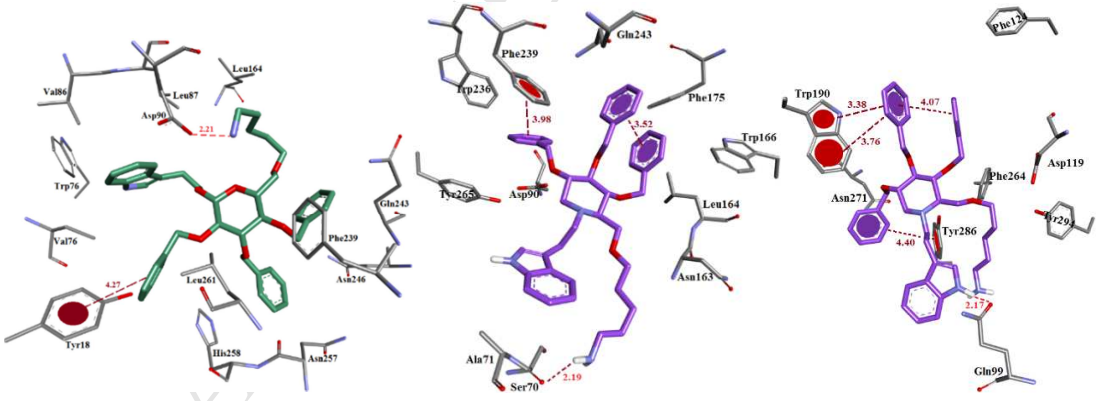
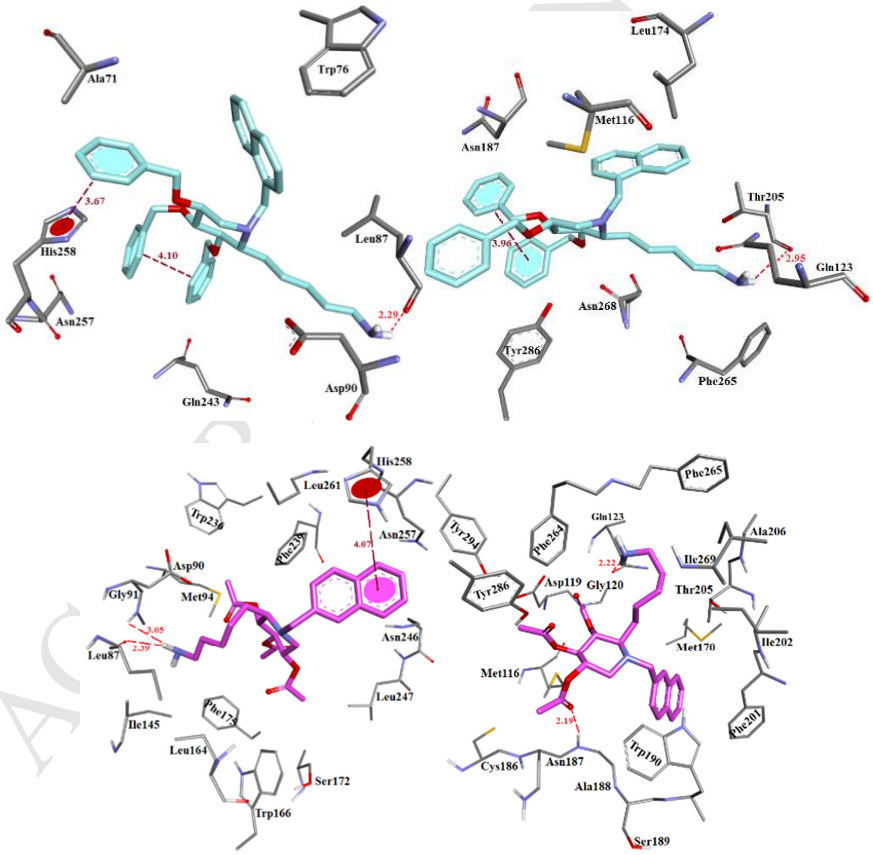


Fig. 4 Compound **3** (green) exhibits a distinct binding mode, *via* interacting with Asp90 (2.21 *Å*) and aromatic π-π interaction with Tyr18. Iminosugar **4** (purple) lacks selectivity, displaying π-π interactions in SSTR-4 (3.52 *Å*) and in SSTR-5 (4.07 *Å*). 2D ligand interaction diagrams are in addition provided in the supplementary information.

A similar loss of preferential isoform selectivity was also observed ($K_{i,\ \text{SSTR-4: SSTR-5}}$ = 1.46, see Table 1) when comparing **4** with **5**. The similar π-stacking interactions of benzyl group aromatic rings for both isoforms are again believed to be the major influence in there being little

selectivity (see, Fig. 5), although there was a slight variation in the docked binding mode for **5**, when compared to **4**. The acetylated analogue **6**, containing acetates rather than aromatic residues, showed low selectivity ($K_{i,\ \text{SSTR-4: SSTR-5}}$ = 1.05) to **4** and **5**, which may be due to various interactions in the orthosteric binding sites of both these proteins [63-65].

In contrast, the trihydroxylated **7** displayed selectivity towards SSTR-5 albeit with low potency (shown in Fig. 5). Molecular modelling of **7** showed that a *H*-bond donor interaction with one of the hydroxyl groups of the iminosugar core with the backbone of Asn268 of the receptor and there was an interaction of the naphthyl group with Phe201; these may explain the selectivity seen for **7** for SSTR-5. The modelled structure of **8** (more selective for SSTR-4) shows that its amino group has a shared interaction with the backbone of Asp90-Gly91 (2.82 *Å*) residues of SSTR-4 while its indole ring engages in *H*-bond donor interactions with the amide group of the Gln243 (2.74 *Å*) side chain and π-π interactions with Phe239 (3.89 *Å*). These indicate that the presence of the indole group is helpful in stabilising/adopting the bound structure of **8** to SSTR-4; these interactions are not possible for SSTR5 and could account for the selectivity shown for **8**.



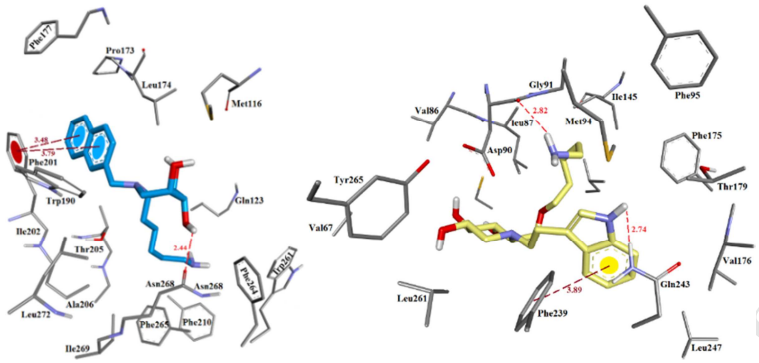


Fig. 5 Iminosugars shown in a docked interactive mode. Tri-O-benzylated **5** (Cyan) with SSTR-4 (π-π interactions with His258 (3.67 *Å*) & *H*-bond donor interaction with Leu87 (2.29 *Å*)) and with SSTR-5 (*H*-bond donor interaction with Thr205 (2.95 *Å*)); Compound **6** (pink) with SSTR-4 (*H*-bond donor interactions with the backbone of Gly91(3.05 *Å*) and Leu87 (2.39 *Å*), π-π interaction with His258 (4.07 *Å*)) and with SSTR-5 (*H*-bonding donor-acceptor interactions with Ala188 (2.19 *Å*) & Gln123 (2.32 *Å*)); Interaction of compound **7** (blue) with SSTR-5 (naphthyl functionality has π-π interaction with Phe201 (3.48 and 3.79 *Å*) and polar carbohydrate head has *H*-bond donor interaction with Asn268 (2.44 *Å*)); Compound **8** (yellow) with SSTR-4 (*H*-bond donor interactions with Asp90-Gly91 and Gln243). 2D ligand interaction diagrams are in addition provided in the supplementary information.

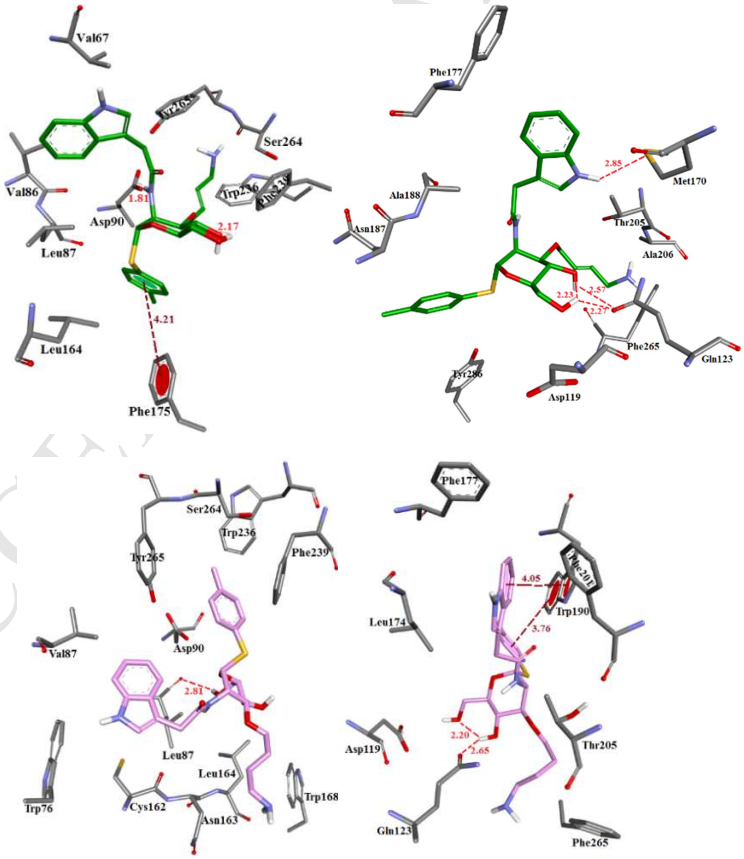
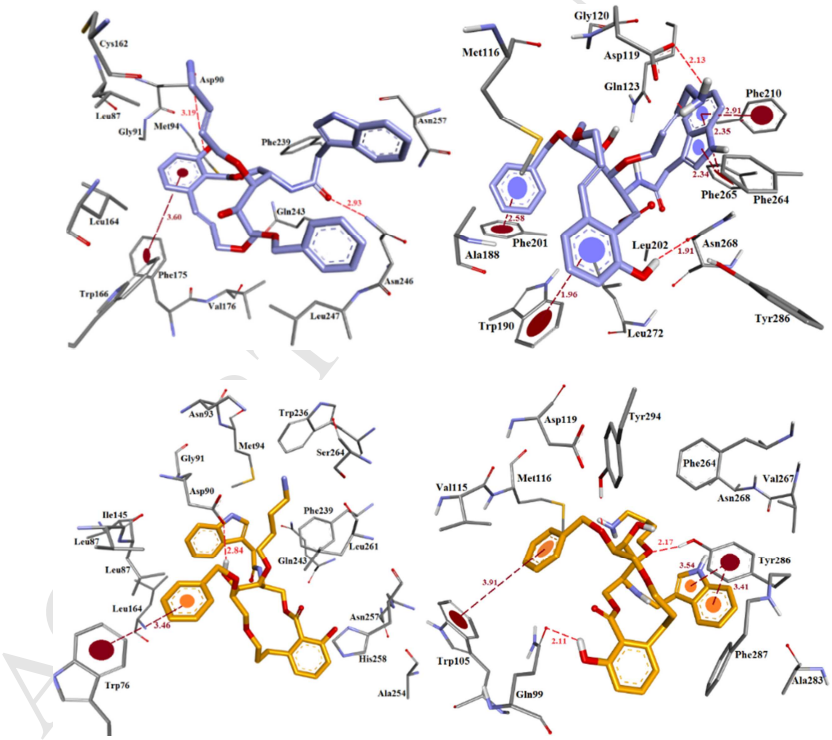


Fig. 6 Docked mode of ManNAc derivatives: Compound **11** (green) with SSTR-4 (*H*-bond donor with Asp90 (1.81 *Å*) & π-π interaction with Phe175 (4.21 *Å*)) and with SSTR-5 (*H*-bond donor interaction with Met170 (2.85 *Å*),

Gln123 (2.27 & 2.57 *Å*); Compound **12** (pink) with SSTR4 (*H*-bond donor interaction with Leu87 (2.81 *Å*) and *para*-OMe-phenyl ring inserted into the hydrophobic cavity, constituted by the Tyr265, Trp236 and Phe239), and with SSTR-5 (π-π interaction with Trp190 (3.76 *Å* & 4.05 *Å*) and *H*-bond with Gln123 (2.65 *Å*) respectively). 2D ligand interaction diagrams are in addition provided in the supplementary information.

The binding modes of ManNAc derivatives, **11** and **12** (Fig. 6) showed interactions with both receptors consistent with relatively low selectivity ($K_{i,\ \text{SSTR-4: SSTR-5}} \approx 1.8$, see Table 1). The SSTR isoform's active site cavities are large and this may contribute to poor selectivity observed for these pyranose derivatives [66].

The benzomacrolactone based mimetics **9** and **10** (Fig. 7) which although having improved affinities compared to iminosugar and pyranose-based scaffolds showed low selectivities for the SSTR isoforms and this was also consistent with interactions observed in both binding sites. Changing the structure of the macrocyclic scaffold to that found in **14** and **15** showed a complete loss of affinity to both isoforms of these receptors, despite these scaffolds presenting naphthyl and alkyl amine groups found in **5-7**. On the other hand if the tryptophan side chain is incorporated onto this type of macrocycle, even without the apparently required lysine residue as in **13** then affinity is restored to a degree [67, 68]. Five-fold isoform selectivity was observed for **13** for SSTR-5 (see Fig. 7) and this is proposed to arise due a π-π interaction with its Tyr-294.



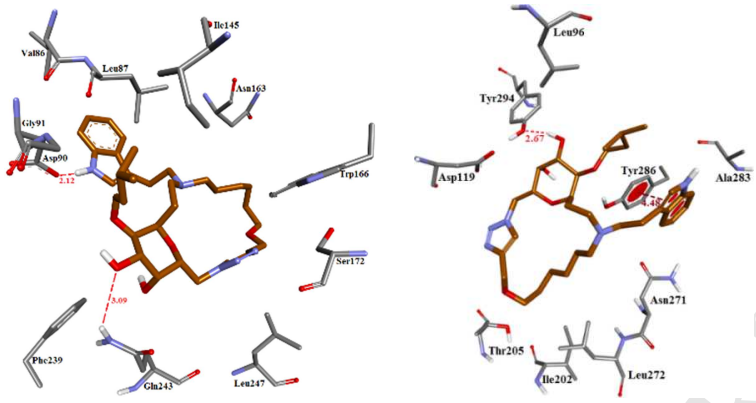


Fig. 7 Interactive mode of macrocycles**:** (a) **9** (blue) with SSTR-4. The phenol moiety has *π-π* interaction with Phe175 (3.60 *Å*), the butenylamine shows *H*-bond donation to the COOH group of Asp90 (3.19 *Å*) and the amide carbonyl of the indole side chain has a *H*-bond acceptor interaction with Asn246 (2.93 *Å*)). For SSTR-5 the phenol moiety has a *π-π* interaction with Trp190 (1.96 *Å*), a *H*-bond acceptor interaction with the amide group of Asn268 (1.91 *Å*), a *π-π* interaction of the benzyl and indole groups with Phe201 (2.58 *Å*) and Phe265 (2.35 *Å* & 2.34 *Å*) respectively, and there are additional *H*-bond donor interactions of the butenylamine with the COOH group of Asp119 side chain (2.13 *Å*)). (b) **10** (orange) with SSTR4: a benzene residue was parallel to Trp76 in a π-sacking manner (3.46 *Å*) and a *H*-bond donor interaction was observed for the free hydroxyl group with Asp90. This compound with SSTR-5 showed that the benzene residue has a T-shaped *π-π* interaction [69] with Trp105 (3.91 *Å*) whereas the indole displayed a sandwich type *π-π* interaction [69] with Tyr286 (3.54 *Å* and 3.41 *Å*), whereas the phenolic (OH) and macrocycle *oxygen atom* shows *H*-bond donor and acceptor interactions with Gln99 (2.11 *Å*) and phenol of Tyr286 (2.17 *Å*) respectively); (c) **13** (brown) with SSTR-4 utilised *H*-bond donor/acceptor interactions with Asp90 (2.12 *Å*) and Gln123 (3.09 *Å*) respectively, while it shows a *H*-bond donor interaction with Tyr294 (2.67 *Å*) and *π-π* interaction with Tyr286 (4.48 *Å*) of SSTR-5. 2D ligand interaction diagrams are in addition provided in the supplementary information.

Next, we compared the larger peptide ligands (**16-18**). Molecular modelling showed three features. Firstly **16** occupied a greater space in the receptor than the other ligands shown so far [70]. Constraining the peptide into a cyclic structure giving **17** reduced the overall size of the cavity occupied by the peptide and also reduced conformational flexibility leading to improved binding [71]. The presence of the GlcNAc residue in **18** led to an increase in selectivity for SSTR-5 [72]. Interestingly, the GlcNAc unit of **18** showed intramolecular *H*-bond interactions in both the binding poses to both SSTR-4 and SSTR-5 isoforms, which led **18** to adopt a different conformation to **17**. For SSTR-5, the indole of **18** was proximal to Phe201 (shown in Fig. 13) and this interaction was not observed for the various non-peptide-based structures described above. This indicates the potential utilisation of an alternative site which could be explored in drug design (Fig. 8).

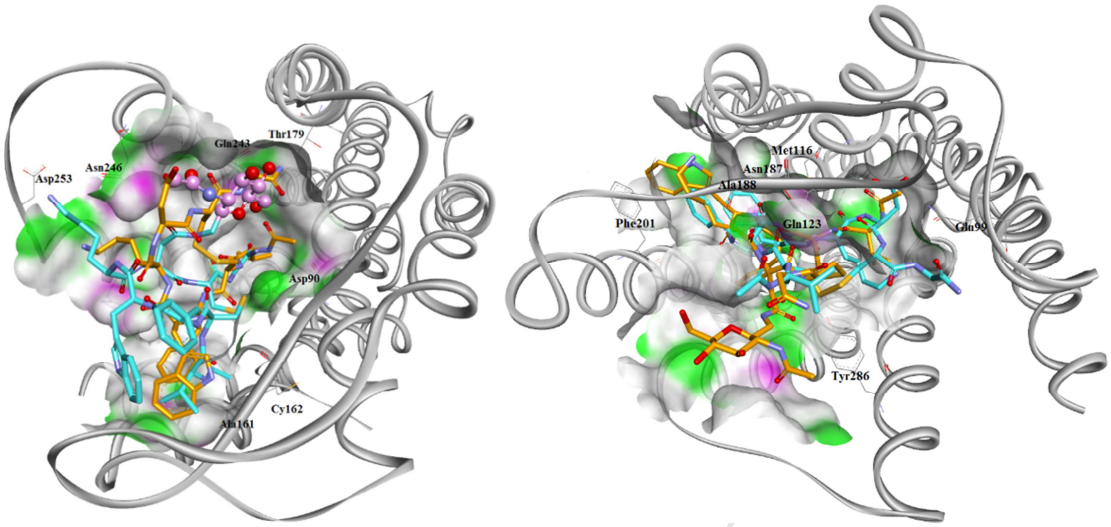


Fig. 8 Peptides **17** (cyan) and **18** (orange) are docked to SSTR-4 (left) and SSTR-5 (right). In both cases, we found number of peptide backbone intermolecular *H*-bond interactions. The amino acid functional epitopes (Lys, Trp, Phe) along with the glycosylated amino acid (ball and stick representation in the first structure on left, SSTR-4) behaviour with proteins, was primarily considered. As observed for SSTR-4, **18** and **17** spanned the wide binding cavities and adopted unrelated binding modes. The **35.ii** peptide backbone had *H*-bond acceptor/donor interactions with Asp90 (2.22 *Å* & 2.40 *Å*), Thr179 (2.75 *Å*), Gln243 (2.29 *Å*) and its Trp indole group displayed a *H*-bond donor interaction with the backbone of Cys162 (2.80 *Å*). In the case of **18,** the peptide backbone also showed *H*-bond acceptor/donor interactions with Asp90 (3.02 *Å*) as well as with Cys162 (2.65 *Å*), Asn246 (2.54 *Å*), His258 (2.18 *Å*). The Lys sidechain of SMS3 had *H*-bond acceptor interactions with the backbone of Asp253 (2.47 *Å*). However, in case of SSTR-5, there was a similar binding pattern for both **17/18**, as their peptide backbone interacted with the same amino acids of the cavity. The **17** peptide backbone interacts through *H*-bond acceptor/donor interactions with Ala188 (2.16 *Å*), Asn187 (2.99 *Å*), Tyr286 (2.80 *Å*), Gln99 (2.34 *Å*) & Met116 (2.72 *Å*). On the other hand, the **18** peptide backbone interacts through *H*-bond acceptor/donor interactions with Gln123 (2.54 *Å*), Ala188(2.76 *Å*), Tyr286 (2.31 *Å*). The indole ring of **18** had π-stacking interactions with Phe201 (3.82 *Å* and 4.14 *Å*) while the GlcNAc residue had *H*-bond acceptor interactions with Asn187 (2.80 *Å*). 2D ligand interaction diagrams are provided in the supplementary information.

Conclusions and Future Perspectives

Various scaffold or scaffold-based ligand design for somatostatin receptors, including iminosugars, ManNAc, (glyco)peptides and macrocycles inspired by natural products have been explored as ligands for somatostatin receptors. Their binding affinities for somatostatin receptors were determined and hypotheses for their modes of interaction with these receptors generated using molecular modelling. This has included construction of homology models for SSTR-4 and SSTR-5, which disclosed some key interactions with certain amino acid residues which, from the docking experiments, was ligand dependent. The study showed that various pharmacophoric groups could interact with these key amino acid residues in a wide ranging manner and this was due to the large active site cavity. Many of these key amino acids are evolutionarily conserved and only a few have been found to be mutated, and these are comprehensively listed in **Table 2**. Because of the size of this cavity, smaller sized ligands, especially those based on the (imino)sugars and the macrocycles, could adopt various different binding poses [73]. Some

observations were also made in the case of the docked larger peptidyl somatostatin mimetics. The peptide backbone of **17** bonded to SSTR-4 more often through H-bond donor interactions which was very different to its proposed binding to SSTR-5. Study of the glycosylated **18** (shown in Table 3), indicated the possibility of two binding modes to SSTR-4 (indicated in Table 3) [61].

Moreover, it has been postulated previously that the interaction of ligands with His258 of SSTR-4 could lead to agonist activity. This interaction was seen in the docking of the iminosugars **5** and **6**, and glycosylated **18** [61]. However, we have not received bioassay support as of yet for this for these compounds and this could form the basis for the design of new agonists.

In general, favourable interactions are predicted when the hydroxyl groups on various scaffolds were not protected allowing these hydrophilic groups interact with polar surface areas of the receptors. This was seen computationally in case of compounds **7**, **8**, **11-13** and **18** [74, 75].

This study has contributed to identification of new inhibitors and provides a basis for the design and synthesis of ligands for SSTR-4 and SSTR-5 as well for generating hypotheses regarding their modes of binding. The homology models provided can be useful for performing any further structure-based ligand design for these receptors in lieu of crystal structures. More generally, ligand-based design has potential to identify new pharmacophoric groups together with new scaffolds that can identify new chemical entities that target large active sites in proteins.

Supplementary Information

Experimental details concerning synthesis and NMR spectra of final compounds. Somatostatin receptor affinity *K*i plots and measurements, Homology modelling data and related qualitative plots and figures. 2D Ligand interaction diagrams.

Conflicts of interest

The authors declare no competing financial interest.

Author Contributions

AN generated the homology models and did the docking, interpreted the models and contributed to drafting and refining the manuscript. JZ synthesised new compounds **11-15** and drafted experimental data. SS synthesised new peptides **16-18** and drafted experimental data. PVM planned the work with the other authors and contributed to drafting and finalising the manuscript.

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