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Synthesis of Somatostatin Mimetics Based on 1-Deoxynojirimycin

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The synthesis of novel somatostatin mimetics from 1-deoxynojirimycin (DNJ) is described. The dipeptide mimetic, which respectively displayed the side chains of tryptophan and lysine at the nitrogen and O6 atoms of the iminosugar scaffold is a ligand ($K_i = 3.2\ \mu M$) for the human somatostatin receptor subtype 4 (hSSTR4) but has lower affinity ($K_i > 100\ \mu M$) for hSSTR5. A benzylated analogue of the Trp-Lys mimetic displays higher affinity for hSSTR5 ($K_i = 5\ \mu M$).

Introduction

Carbohydrate templates or scaffolds have found wide application in bioactive compound discovery.[1] A range of saccharide derivatives have been investigated such as (amino)sugars,[2] sugar amino acids[3] and disaccharides,[4] and the solid-phase syntheses of carbohydrate-based prospecting libraries have been of interest.[5] These multifunctional scaffolds have been applied to generate compounds of interest in peptidomimetic and glycomimetic research.[6,7]

Iminosugars, which are true structural analogues of pyranosides where the ring oxygen atom is replaced by a nitrogen atom, have not been widely investigated as scaffolds. This is presumably because they are not as readily available as pyranosides and their preparation is not trivial.[8,9] Potential advantages of investigating 1-deoxymannojirimycin (DMJ)[10] or 1-deoxynojirimycin (DNJ, **3**, Figure 1) or other iminosugars compared with pyranosides as scaffolds include the possibility that the protonated ring nitrogen atom could contribute a charged hydrogen bonding group to enhance interactions of a particular ligand with a receptor. Also pharmacophoric groups can be grafted to the ring nitrogen. Somatostatin (SST, **1**) is a tetradecapeptide that regulates, through binding to its receptors (SSTR), a number of processes including the release of growth hormone and other pituitary hormones.[11] The side chains of the Phe-Trp-Lys peptide fragment of **1** (residues *i*, $i+1$ and $i+2$ in Figure 1), which adopts a β-turn conformation, are important for the recognition of SSTRs, defining an important component of its pharmacophore. The low bioavailability and poor pharmacokinetics of somatostatin has led to the synthesis of both peptide and non-peptide mimetics of this hormone. Sandostatin[12] (D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol with a disulfide bridge) is a potent peptide-based mimetic of somatostatin that has found clinical application. Sandostatin differs from **1** as it contains D-tryptophan instead of the L-tryptophan residue but it can be postulated that the pharmacophoric side chains adopt a similar geometric arrangement in both sandostatin and **1**. The glucopyranoside **2**, for example, synthesised by Hirschmann, Nicolaou, and collaborators[13] is a mimetic of both **1** and sandostatin and had an IC_{50} value of 1.3 μM in a nonspecific SSTR binding assay. The C2, C1, and C6 substituents of **2** mimic the side chains of the pharmacophoric Phe-(D-)Trp-Lys fragment. Recently we synthesised a somatostatin mimetic **4**[14] based on DMJ,[15] which mimics the (D-)Trp-Lys dipeptide of **1**, and this ligand has a K_i value of 26 μM in a nonspecific SSTR binding assay. In a binding assay specific for SSTR4 and SSTR5, which are SSTR subtypes, the DMJ derivative **4** inhibited binding of the control ligand to SSTR4 by 46% at 1.0 μM, but did not show any binding to SSTR5 at the same concentration. We were interested to prepare novel DNJ-based analogues of both **2** and **4** and to evaluate their affinity for SSTRs. The synthesis and biological properties of two such peptidomimetics, **5** and **6**, are described herein. The latter derivative displays benzyl groups that could potentially mimic interactions of phenylalanine residues of **1** with its receptors, as is the case for the glucopyranoside **2**.

Results and Discussion

Molecular modelling

The spatial arrangement between the pharmacophoric indolylethyl and pentylamino side chains is clearly different when **2** is compared with **4**/**5**. This prompted a computational study as to whether these groups in **5** are presented in a close geometrical arrangement to that found in somatostatin or sandostatin. We chose to carry out the study using sandostatin as the 3D coordinates of its solution structure[16] are available at the protein data bank.[17] A model of **5** was built and was subjected to a Monte Carlo conformational searching protocol available in

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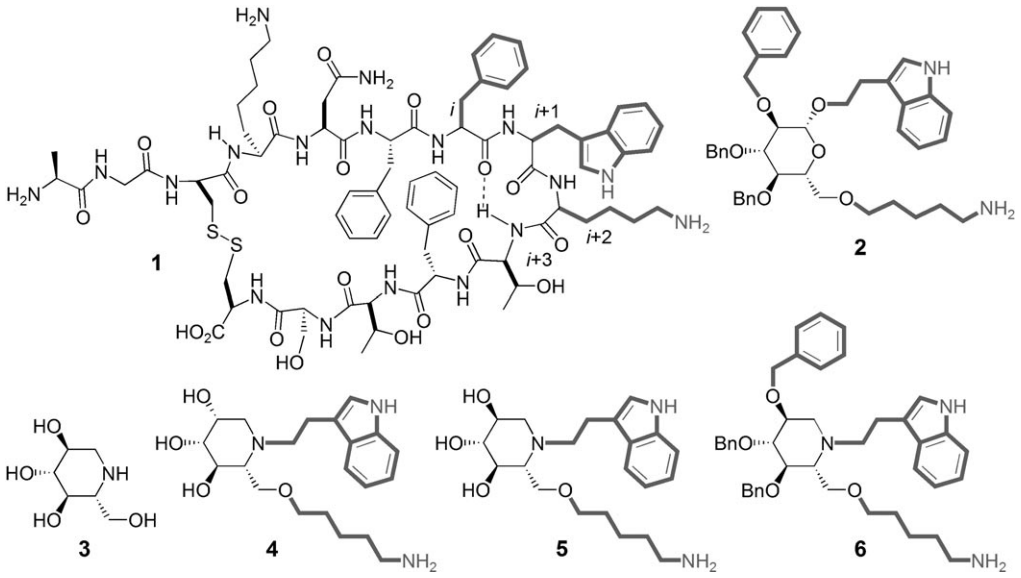


Figure 1. Structures of compounds **1**–**6**.

Macromodel 8.5[18] which accessed low energy isomers of **5**. Subsequently the lowest energy conformer of **5** was overlayed with that of the sandostatin solution structure (Figure 2). The

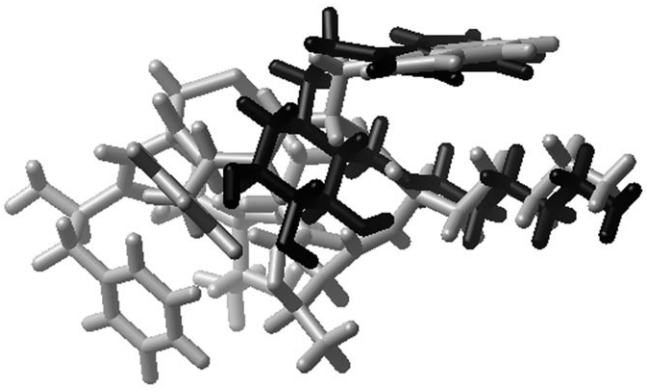
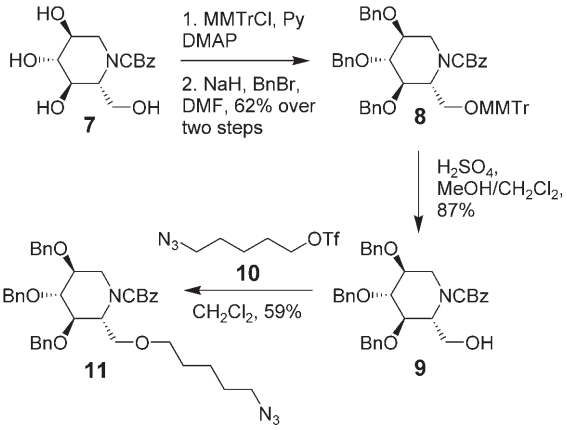


Figure 2. Overlay of sandostatin (grey) and a low-energy conformer of **5** (black).

indole and pentylamino residues of this conformer of **5** were found to adopt a similar geometrical arrangement to that found in sandostatin, indicating that **5** would bind to SSTRs that recognise the solution structure of sandostatin. Presumably **4** can adopt a similar arrangement between its pharmacophoric groups, explaining its biological activity.

Synthesis of somatostatin mimetics from DNJ

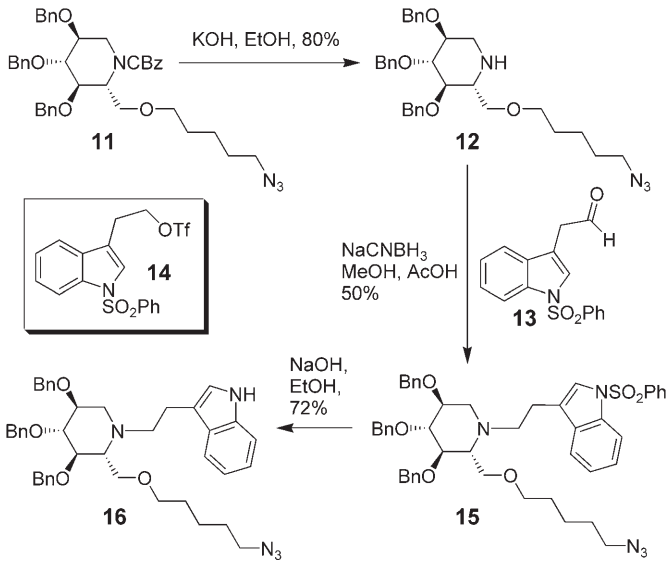
The synthesis of both **5** and **6** commenced from DNJ **3** which was first prepared as previously described[19] from L-sorbose and then **3** was converted into the CBz derivative **7**[20] as outlined previously. The reaction of **7** with monomethoxytrityl chloride in pyridine in the presence of DMAP followed by benzylation of the tritylated intermediate gave **8** (62% over two steps; see Scheme 1). The selective removal of the MMTr protecting group from **8** was achieved using sulfuric acid in a



Scheme 1. Synthesis of azide derivative **11**.

$MeOH/CH_2Cl_2$ mixture to give **9**. The next step, alkylation of the 6-OH group of **9**, needed to be carried out under non-basic conditions to preclude intramolecular carbamate formation, which has been a problem during the synthesis of **4**.[14] The azide derivative **10** was thus freshly prepared,[13] and the alkylation of **9** with **10** in dichloromethane gave **11** (59%); the experimental protocol involved the repeated evaporation of the more volatile triflate **10** from a solution of **9** at high vacuum.[21]

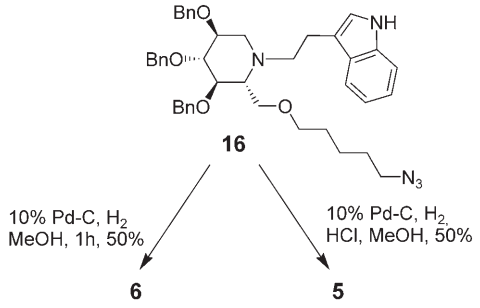
Next the CBz group was removed from **11** by heating in ethanolic potassium hydroxide to give **12** (80%) as shown in Scheme 2. The piperidine **12** was then converted into **15** by a reductive amination reaction using sodium cyanoborohydride and aldehyde **13** (50%).[10] Efforts to obtain **15** by the *N*-alkylation of **12** with the triflate **14** in the presence of NaH were not successful, despite this approach being successful during the



Scheme 2. Synthesis of indole derivative **16**.

preparation of **4**. The removal of the sulfonamide protecting group from the indole nitrogen atom was carried out under basic conditions to give **16**.

The reaction of **16** using 10% Pd-C in MeOH in the presence of H_2 for 1 h led to reduction of the azide group giving **6**; the use of the same conditions for 15 h in the presence of HCl led to reduction of the azide and the removal of the benzyl protecting groups and gave **5** (Scheme 3). The catalytic removal of the benzyl protecting groups in the presence of the DNJ nitrogen atom was only possible under these acidic conditions.



Scheme 3. Generation of compounds **5** and **6** from **16**.

Biological evaluation

The iminosugar derivatives **5** and **6** were evaluated in human SSTR4[22] and human SSTR5[23] binding assays in vitro and the results are summarised in Table 1. Somatostatin has a high affinity for these receptors ($K_i = 0.61–2.0$ nM). The dipeptide mimetic **5** was more potent ($K_i = 3.2$ μM) than **6** ($K_i = 4.4$ μM) as a ligand for the human SSTR4, despite **5** not presenting any benzyl groups that have the potential to mimic one or more of the phenylalanine residues of somatostatin. The DMJ derivative **4**, closely related to **5**, also showed a preference for the human SSTR4 previously, relative to SSTR5. Conversely, the dipeptide mimic **5** showed low binding to the human SSTR5 ($K_i > 100$ μM) whereas **6** was more potent ($K_i = 5$ μM) towards this receptor. The low affinity displayed by **4** and **5** when compared with ligand **6** for SSTR5 indicates that at least one of the benzyl groups of **6** makes an important binding interaction with SSTR5.

Table 1. Binding of peptidomimetics at human somatostatin receptors.

Compd	K_i [μM]	
	SST4	SST5
5	3.2	> 100
6	4.4	5.0

Summary and Conclusions

A synthetic route to somatostatin mimetics based on the DNJ scaffold has been developed which facilitates the synthesis of novel (D-)Trp-Lys mimetics. Grafting benzyl groups to the DNJ residue led to a small decrease in potency for SSTR4 and a significant enhancement in binding to SSTR5. This indicates that at least one benzyl group contributes an important binding interaction to SSTR5. The results could help in the design of selective inhibitors of both SSTR subtypes based on iminosugar scaffolds. The recent synthesis of differentially protected DNJ intermediates will aid in the regioselective introduction of pharmacophoric groups to the 2,3 and 4-OH groups of DNJ[24] with a view to optimising binding interactions of iminosugar-based peptidomimetics to SSTRs.

Experimental Section

General experimental conditions. NMR spectra were recorded with Varian 400 MHz or 500 MHz spectrometers in $CDCl_3$ at 25 °C unless otherwise stated. Chemical shifts are reported relative to internal Me_4Si in $CDCl_3$ ($\delta = 0.0$ ppm) or HOD for D_2O ($\delta = 4.79$ ppm) for ^1H and ($\delta = 77.16$ ppm) for ^{13}C. ^1H and ^{13}C NMR signals were assigned with the aid of COSY, DEPT-135, HSQC and HMBC. All coupling constants quoted arise from H,H coupling. Mass spectra were recorded on a Micromass LCT KC420 or Micromass Quattro. TLC was performed on aluminum sheets precoated with Silica Gel 60 (HF254, E. Merck) and spots visualised by UV and charring with 1:20 H_2SO_4/EtOH or ninhydrin. Flash column chromatography was generally employed and was carried out using Silica Gel 60 (0.040–0.630 mm, Merck) and employed a stepwise solvent polarity gradient correlated with the TLC mobility when gradient elution was used. Anhyd DMF and pyridine were used as purchased from Sigma–Aldrich. Dichloromethane, THF and MeOH were used as obtained from a Pure-Solv solvent purification system.

***N*-Benzyloxycarbonyl-1,5-dideoxy-1,5-imino-6-*O*-monomethoxytrityl-2,3,4-tri-*O*-benzyl-D-glucitol (8).** Compound **7**[20,24] (550 mg, 1.85 mmol) was dissolved in anhyd pyridine (4 mL) and DMAP (226 mg) was added. The mixture was cooled to 0 °C and MMTrCl (687 mg, 2.22 mmol) in anhyd pyridine (4 mL) was added dropwise. The reaction was then stirred at room temperature under N_2 for 12 h. The solvent was removed under diminished pressure and chromatography (EtOAc) of the residue gave the tritylated intermediate. To this intermediate (450 mg, 0.523 mmol) in dry DMF (25 mL) at 0 °C, was added slowly NaH (190 mg of a 60% disper-

sion in mineral oil, 3.1 mmol). The mixture was then stirred at 0 °C for 1 h and BnBr (0.57 mL, 3.1 mmol) was added dropwise and stirring was continued at 0 °C for 2 h and at room temperature for 15 h. Ice was added and the aqueous phase was extracted with EtOAc. The combined organic layers were dried (Na_2SO_4) and the solvent was removed under diminished pressure. Chromatography of the residue (cyclohexane/EtOAc, 95:5 and then 90:10) gave the title compound (515 mg, 62%) as a white solid; mp: 70 °C (dec); $[\alpha]_D^{20} = -4$ ($c = 0.1$, CH_2Cl_2); IR (NaCl): $\tilde{\nu} = 3031$, 2886, 1700, 1509, 1454, 1252, 1072, 734, 698 cm^{-1}; ^1H NMR (400 MHz): $\delta = 7.24$ (m, 32H, aromatic H), 6.75 (d, 2H, $^3J = 8.4$ Hz, aromatic H), 5.07 (br s, 2H), 4.73 (d, 1H, ^1J (H,H) = 11.0 Hz, benzyl CH), 4.58 (s, 2H), 4.47 (d, 2H, $^1J = 10.6$ Hz), 4.37 (br s, 1H), 4.06 (m, 1H, H-5), 4.03 (m, 1H, H-3), 3.72 (s, 5H, OCH_3, H-2,4), 3.41 ppm (d, 2H, $^3J = 14.4$ Hz, H-1); ^{13}C NMR (101 MHz): $\delta = 41.3$ (C1), 55.4 (OCH_3), 56.7 (C5), 62.0 (C6), 67.5 (CH_2), 70.7 (CH_2), 73.4 (CH_2), 74.2 (CH_2), 75.1 (C3), 78.7 (C2), 82.6 (C4), 113.4 (aromatic C), 126.0–144.0 (aromatic CH and C), 156.2 ($COCH_3$) 158.8 (C=O); ES-HRMS: found 862.3718 $[M+Na]^+$; $C_{55}H_{53}NO_7$ requires 862.3720.

***N*-Benzyloxycarbonyl-1,5-dideoxy-1,5-imino-2,3,4-tri-*O*-benzyl-D-glucitol (9).** The methoxytrityl derivative **8** (1.567 g, 1.87 mmol) was dissolved in MeOH/CH_2Cl_2 (2:1, 65 mL) and concentrated H_2SO_4 (30 μL, 0.4 mmol) was added slowly and the mixture was then stirred at room temperature for 12 h. Saturated $NaHCO_3$ was then added until the solution was neutral and the excess organic solvents were removed under vacuum. The aqueous layer was extracted with EtOAc (×2) and the combined organic layers were dried (Na_2SO_4) and the solvents removed under diminished pressure. Chromatography (EtOAc/cyclohexane; 30:70) of the residue gave the title compound **9** (900 mg, 87%) as a white solid; $[\alpha]_D^{20} = +3.7$ ($c = 1.85$, CH_2Cl_2); IR (NaCl): $\tilde{\nu} = 3446$, 3031, 2926, 2875, 1695, 1071 cm^{-1}; ^1H NMR (400 MHz): $\delta = 7.29$ (m, 20H, aromatic H), 5.11 (s, 2H, benzyl CH_2), 4.73 (d, $^1J = 11.5$ Hz, 1H, benzyl CH), 4.62–4.68 (overlapping signals, 2H, 2benzyl CH), 4.50 (d, $^1J = 12.4$ Hz, 1H, benzyl CH), 3.90 (br s, 1H, H-5), 3.86 (br s, 2H, H-6a,6b), 3.63–3.73 ppm (overlapping signals, 4H, H-1,2,3,4) ^{13}C NMR (101 MHz): $\delta = 156.5$ (C=O), 138.2 (2 s), 136.5 (each aromatic C), 128.0–129.0 (aromatic CH and C), 82.4 (C4), 77.7 (C2), 75.7 (C3), 73.7 (CH_2), 73.5 (CH_2), 71.52 (CH_2), 67.7 (CH_2), 61.7 (C6), 59.3 (C5), 43.1 ppm (C1); ES-HRMS: found 568.2690 $[M+H]^+$; $C_{35}H_{38}NO_6$ requires 568.2699.

6-*O*-(5′-Azidopentyl)-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-2,3,4-tri-*O*-benzyl-D-glucitol (11). To a stirred solution of 5-azido-1-pentanol[2] (18.2 mg, 0.14 mmol) and 2,6-di-*tert*-butyl-4-aminopyridine (28.2 mg, 0.14 mmol) in anhyd CH_2Cl_2 (1 mL) at 0 °C was added dropwise, triflic anhydride (23 μL, 0.14 mmol). The mixture was stirred at room temperature for 15 min, then poured into water (10 mL) and extracted with CH_2Cl_2 (×2). The organic layers were combined and dried ($MgSO_4$) and the solvent was removed under diminished pressure until a volume of ~20 mL remained. Some of this solution (6 mL) containing the triflate **11** was added to a flask containing **9** (20 mg, 35 μmol) and the reaction mixture was concentrated under diminished pressure and then placed under high vacuum for 2 h. This procedure was repeated (×3) and the final residue kept under high vacuum for 12 h. Chromatography (EtOAc/cyclohexane, 3:7) of the residue gave **11** as a colourless oil (15 mg, 59%); $[\alpha]_D^{20} = +4.0$ ($c = 0.1$, CH_2Cl_2); IR (NaCl): $\tilde{\nu} = 2921$, 2856, 2093, 1698, 1453, 1091 cm^{-1}; ^1H NMR (400 MHz): $\delta = 7.28$ (m, 20H, aromatic H), 5.15 (d, $^1J = 12.4$ Hz, 1H, benzyl CH), 5.10 (d, $^1J = 12.4$ Hz, 1H, benzyl CH), 4.74 (d, $^1J = 11.7$ Hz, 1H, benzyl CH), 4.66 (d, $^1J = 11.8$ Hz, 1H, benzyl CH), 4.63 (d, $^1J = 11.7$ Hz, 1H, benzyl CH), 4.60 (d, $^1J = 11.5$ Hz, 1H, benzyl CH), 4.59 (d, $^1J = 11.6$ Hz, 1H, benzyl CH), 4.47 (d, $^1J = 11.8$ Hz, 1H, benzyl CH), 4.19 (dd, $^3J = 9.2$, 4.4 Hz, 1H, H-5), 3.86 (t, $^3J = 6.1$ Hz, 1H, H-4), 3.74 (dd, $^3J = 6.3$, 4.5 Hz, 1H, H-3), 3.67 (dd, $^3J = 7.3$, 3.4 Hz, 1H, H-2), 3.56 (m, 2H, H-6a,6b), 3.32 (dd, $^1J = 14.4$ Hz, $^3J = 3.3$ Hz, 2H, H-1), 3.26 (m, 2H, CH_2CH_2O), 3.21 (t, $^3J = 6.9$ Hz, 2H, CH_2N_3), 1.55 (m, 2H, pentyl CH_2), 1.47 ppm (m, 2H, pentyl CH_2), 1.33 (m, 2H, pentyl CH_2); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 156.1$ (C=O), 138.5–136.9 (aromatic C), 128.7–127.8 (aromatic CH and C), 81.9 (C3), 78.2 (C2), 74.3 (C4), 73.2, 73.1, 71.0, 70.9, 69.2, 67.4 (each CH_2), 55.8 (C5), 51.5 (CH_2), 41.4 (C1), 29.9, 29.4, 28.9 ppm (each CH_2); ES-HRMS: found 679.3515 $[M+H]^+$; $C_{40}H_{47}N_4O_6$ requires 679.3496.

6-*O*-(5′-Azidopentyl)-1,5-dideoxy-1,5-imino-2,3,4-tri-*O*-benzyl-D-glucitol (12). The azide **11** (150 mg, 0.22 mmol) was dissolved in EtOH (25 mL) and KOH (25 mL of a 50% aqueous solution) was added. The reaction mixture was stirred whilst heating at reflux using an oil bath for 12 h. After cooling to room temperature, water (20 mL) was added. This was followed by extraction with CH_2Cl_2 (×4) and the combined organic layers were dried ($MgSO_4$), filtered and the solvent was removed under diminished pressure. Chromatography of the residue (EtOAc/cyclohexane, 10:90 to 40:60) gave **12** as a colourless oil (97 mg, 80%); $[\alpha]_D^{20} = +31$ ($c = 0.3$, CH_2Cl_2); IR (NaCl): $\tilde{\nu} = 3028$, 2922; 2863; 2095; 1100 cm^{-1}; ^1H NMR (400 MHz): $\delta = 7.29$ (m, 15H, aromatic H); 4.98 (d, 1H, $^1J = 10.9$ Hz, benzyl CH); 4.89 (d, 1H, $^1J = 11.0$ Hz, benzyl CH); 4.84 (d, 1H, $^1J = 10.9$ Hz, benzyl CH); 4.70 (d, 1H, $^1J = 11.7$ Hz, benzyl CH); 4.66 (d, 1H, $^1J = 11.7$ Hz, benzyl CH); 4.55 (d, 1H, $^1J = 11.0$ Hz, benzyl CH); 3.59 (dd, 1H, $^3J = 2.5$ Hz, $^1J = 9.0$ Hz, $CH_2CH(H)O$; 3.56 (t, $^3J = 9.2$ Hz, 1H, H-3); 3.51 (dd, $^3J = 10.0$, 4.8 Hz, 1H, H-2); 3.41 (m, 2H, H-6); 3.32 (overlapping signals, 2H, $CH_2CH(H)O$ and H-4); 3.24 (overlapping signals, 3H, CH_2N_3 and H-1a); 2.68 (ddd, 1H, $^3J = 2.6$ Hz, $^3J = 6.0$ Hz, $^3J = 9.5$ Hz, H-5); 2.51 (dd, 1H, $^3J = 10.4$ Hz, $^1J = 2.1$ Hz, H-1b); 1.84 (br s, 1H, NH); 1.60 (m, 2H, pentyl CH_2); 1.54 (m, 2H, pentyl CH_2); 1.41 ppm (m, 2H, pentyl CH_2); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 139.1$–139.8 (aromatic C), 128.6–127.8 (aromatic CH), 87.6 (C3), 80.9 (C2), 80.4 (C4), 75.9 75.4, 73.0, 71.2, 71.0 (each CH_2); 60.0 (C5), 51.6 (pentyl CH_2), 48.4 (C1), 29.4, 28.9, 23.7 ppm (each CH_2); ES-HRMS: found 545.3124 $[M+H]^+$; $C_{32}H_{41}N_4O_4$ requires 545.3128.

6-*O*-(5′-Azidopentyl)-1,5-dideoxy-1,5-imino-1-*N*-(2′-*N*′-phenylsulfonylindol-3-ylethyl)-2,3,4-tri-*O*-benzyl-D-glucitol (15). Sodium cyanoborohydride (26.7 mg, 0.42 mmol) was added to a solution of aldehyde **13**[10] (40 mg, 0.133 mmol) and the amine **12** (46 mg, 0.85 μmol) in MeOH (4 mL). Then acetic acid (0.6 mL) was added and the mixture was stirred at room temperature for 15 h under N_2. The solvent was removed under diminished pressure and the residue was partitioned between CH_2Cl_2 (30 mL) and aqueous Na_2CO_3 (pH 11). The aqueous layer was extracted with CH_2Cl_2 (30 mL, ×3) and the organic phases were combined, dried ($MgSO_4$) and the solvent was removed under diminished pressure. Chromatography (EtOAc/cyclohexane, 20:80, $R_f = 0.28$) of the residue gave **15** as a colourless oil (35 mg, 50%): $[\alpha]_D^{20} = -1.5$ ($c = 2$, CH_2Cl_2); IR (NaCl): $\tilde{\nu} = 2928$, 2861, 2095, 1448, 1370, 1175, 1095, 747, 698 cm^{-1}; ^1H NMR (400 MHz): $\delta = 8.00$ (d, $^3J = 8.3$ Hz, 1H, aromatic H), 7.85 (m, 1H, aromatic H), 7.22–7.50 (m, 23H, aromatic H), 4.97 (d, $^1J = 11.0$ Hz, 2H, 2 benzyl CH), 4.84 (d, $^1J = 11.0$ Hz, 1H, benzyl CH), 4.66 (d, $^1J = 11.6$ Hz, 1H, benzyl CH), 4.59 (d, $^1J = 11.5$ Hz, 2H, 2 benzyl CH), 3.65 (dd, $^1J = 10.8$, $^3J = 2.8$ Hz, 1H, H-6a), 3.59–3.63 (overlapping signals, 2H, H-2,6b), 3.59 (m, 1H, H-2), 3.53 (m, 2H, H-3,4), 3.38 (dt, $^1J = 9.5$ Hz, $^3J = 6.4$ Hz, 1H, $CH_2CH(H)O$), 3.30 (dt, $^1J = 9.6$ Hz, $^3J = 6.8$ Hz, 1H, $CH_2CH(H)O$), 3.13 (t, $^3J = 6.9$ Hz, 2H, CH_2N_3), 3.07–3.10 (overlapping signals, 2H, H-1 and $CH_2CH(H)O$) 2.89 (dt, $^1J = 13.1$, $^3J = 7.5$ Hz, 1H, $CH_2CH(H)N$), 2.78 (t, $^3J = 7.7$ Hz, 2H, CH_2CH_2N), 2.43 (dt, $^3J = 8.5$ and 2.1 Hz, 1H, H-5), 2.32 (t, $^1J =$

10.7 Hz, 1H, H-1b), 1.57 (m, 2H, CH_2), 1.50 (m, 2H, CH_2), 1.35 ppm (m, 2H, CH_2); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 137.9–122.0 (aromatic C and CH), 120.1 (N-CH=*C*), 118.3 (N-*C*H=C), 112.8 (aromatic CH), 86.2 (C3), 77.8 (C4), 77.5 (C2), 74.4, 74.3, 71.8 (each benzyl CH_2), 70.2 (CH_2CH_2O), 66.3 (C6), 63.2 (C5), 53.5 (C1), 50.7 (CH_2CH_2N), 50.2 (CH_2N_3), 28.1, 27.7, 22.5 (each pentyl CH_2), 19.4 ppm (CH_2CH_2N); ES-HRMS: found 828.3795 $[M+H]^+$; $C_{48}H_{54}N_5O_6S$ requires 828.3831. Some of the aldehyde **13** was recovered (18 mg, 40%).

6-*O*-(5′-Azidopentyl)-1,5-dideoxy-1,5-imino-1-*N*-(2′-(indol-3-yl)ethyl)-2,3,4-tri-*O*-benzyl-D-glucitol (16). The DNJ derivative **15** (25 mg, 30 μmol) was dissolved in EtOH (5 mL) and a solution of NaOH (0.85 mL of a 5 M solution) was added and the mixture was stirred whilst heating at reflux for 3 h. The EtOH was then removed under diminished pressure and water (20 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were then dried ($MgSO_4$), filtered and the solvent was removed under diminished pressure. Chromatography of the residue (EtOAc/cyclohexane, 20:80) gave **16** as a colourless oil (15 mg, 72%). $[\alpha]_D^{20}$ = +13.9 (*c* = 2, CH_2Cl_2); IR (NaCl): $\tilde{\nu}$ = 3427, 3031, 2920, 2862, 2095, 1454, 1099, 739, 698 cm^{-1}; ^{1}H NMR (400 MHz): δ = 7.96 (s, 1H, NH), 7.58–7.00 (m, 20H, aromatic H) 4.98 (d, ^1J = 11.1 Hz, 1H, benzyl CH), 4.97 (d, ^1J = 11.0 Hz, 1H, benzyl CH), 4.84 (d, ^1J = 11.1 Hz, 1H, benzyl CH), 4.70 (d, ^1J = 11.6 Hz, 1H, benzyl CH), 4.66 (d, ^1J = 11.6 Hz, 1H, benzyl CH), 4.60 (d, ^1J = 11.0 Hz, 1H, benzyl CH), 3.71 (broad signal, 2H, H-6a,6b), 3.68 (dd, ^3J = 10.1, 4.8 Hz, 1H, H-2), 3.61 (t, ^3J = 9.2 Hz, 1H, H-4), 3.53 (t, ^3J = 9.0 Hz, 1H, H-3), 3.41 (td, ^1J = 9.6, ^3J = 6.3 Hz, 1H, CH_2CH(H)O), 3.33 (td, ^1J = 9.6, ^3J = 6.8 Hz, 1H, CH_2CH(*H*)O), 3.24 (dd, ^1J = 11.1 Hz, 4.9 Hz, 1H, H-1a), 3.13 (t, ^3J = 6.9 Hz, 2H), 3.10 (m, 1H), 3.00 (m, 1H), 2.89 (dt, *J* = 10.0 Hz, 5.8 Hz, 2H), 2.47 (dt, ^3J = 9.3 and 2.0 Hz, 1H, H-5), 2.42 (t, ^1J = 10.7 Hz, 1H, H-1b), 1.57 (m, 2H), 1.51 (m, 2H), 1.35 ppm (m, 2H); ^{13}C NMR (101 MHz): δ = 139.0–111.1 (aromatic C and CH), 87.3, 78.9, 78.6, 75.4, 75.2, 72.8, 71.1, 66.9 (each CH_2), 64.0 (C5), 54.66 (C1), 52.8, 51.3, 29.1, 28.7, 23.5, 20.1 ppm (each CH_2); ES-HRMS: found 688.3882 $[M+H]^+$; $C_{42}H_{50}N_5O_4$ requires 688.3863.

6-*O*-(5′-Aminopentyl)-1,5-dideoxy-1,5-imino-1-*N*-(2′-(indol-3-yl)ethyl)-2,3,4-tri-*O*-benzyl-D-glucitol (6). The azide **16** (15 mg, 22 μmol) was dissolved in MeOH (10 mL) and 10% Pd-C (2 mg) was added and the mixture was stirred at room temperature under H_2 for 1 h. The mixture was then filtered through a short column of celite and the solvent removed under diminished pressure. Chromatography of the residue (CH_2Cl_2/MeOH/$NH_{3(aq)}$, 90:10:1) gave the title compound **6** as a yellow oil (7 mg, 50%); $[\alpha]_D^{20}$ = +40.1 (*c* = 2, CH_2Cl_2); IR (NaCl): $\tilde{\nu}$ = 3284, 3030, 2921, 2861, 1455, 1100, 739, 698 cm^{-1}; ^{1}H NMR (500 MHz, D_2O): δ = 8.54 (br s, 1H, NH), 7.57–7.02 (20H, aromatic H), 4.97 (d, ^1J = 11.0 Hz, 1H, benzyl CH), 4.96 (d, ^1J = 11.0 Hz, 1H, benzyl CH), 4.84 (d, ^1J = 11.0 Hz, 1H, benzyl CH), 4.72 (d, ^1J = 11.6 Hz, 1H, benzyl CH), 4.67 (d, ^1J = 11.6 Hz, 1H, benzyl CH), 4.60 (d, ^1J = 11.0 Hz, 1H, benzyl CH), 3.70 (dd, ^1J = 10.4, ^3J = 2.5 Hz, 1H, H-6a), 3.66 (overlapping signals, 2H, H-2,6b), 3.59 (t, ^3J = 9.20 Hz, 1H, H-4), 3.53 (t, ^3J = 9.0 Hz, 1H, H-3), 3.33 (m, 2H, CH_2CH_2O), 3.28 (dd, ^1J = 11.1, ^3J = 4.9 Hz, 1H, H-1a), 3.13 (dt, ^1J = 17.7 Hz, ^3J = 7.2 Hz, 1H, CH_2CH(H)N), 2.93 (m, 1H, CH_2CH(*H*)N), 2.88 (m, 2H, CH_2CH_2)N), 2.56 (br s, 2H, CH_2NH_2), 2.45 (d, ^3J = 9.3 Hz, 1H, H-5), 2.38 (t, ^1J = 10.8 Hz, 1H, H-1b), 1.49 (m, 2H, CH_2CH_2O), 1.41 (m, 2H, $CH_2CH_2NH_2$), 1.27 ppm (m, 2H, pentyl CH_2); ^{13}C NMR (101 MHz, D_2O): δ = 139.0–111.3 (aromatic CH and C), 87.33 (C3), 78.77 (C4), 78.55 (C2), 75.4, 75.3, 72.8, 71.1, 66.8 (each CH_2), 64.1 (C5), 54.4 (C1), 52.8, 40.8, 30.8, 28.9, 23.5, 20.2 ppm (each CH_2); ES-HRMS: found 662.3958 $[M+H]^+$; $C_{42}H_{52}N_3O_4$ requires 662.3978.

6-*O*-(5′-Aminopentyl)-1,5-dideoxy-1,5-imino-1-*N*-(2′-(indol-3-yl)ethyl)-D-glucitol (5). The azide **16** (70 mg, 0.1 mmol) was dissolved in MeOH (20 mL) and 10% Pd-C (7 mg) was added. The mixture was degassed by purging N_2 through it and a methanolic solution of HCl (4 mL of 1.2 M) was added. The mixture was stirred overnight at room temperature under H_2, then filtered through a short column of celite and the excess solvent was removed under diminished pressure. Chromatography using reverse phase silica (C18) using water as eluent gave the title compound **5** (20 mg, 50%) as a white solid; $[\alpha]_D^{20}$ = +36.2 (*c* 2, CH_2Cl_2); IR (NaCl): $\tilde{\nu}$ = 3332, 3310, 2941, 1618, 1458, 1106 cm^{-1}; ^{1}H NMR (500 MHz, D_2O): δ = 7.60 (d, ^3J = 7.9 Hz, 1H, aromatic H), 7.47 (d, ^3J = 8.2 Hz, 1H, aromatic H), 7.25 (s, 1H, C=C*H*NH), 7.21 (t, ^3J = 7.6 Hz, 1H, aromatic H), 7.13 (t, ^3J = 7.5 Hz, 1H, aromatic H), 3.80 (s, 2H, H-6a,6b), 3.77 (d, ^3J = 2.4 Hz, 1H, H-2), 3.73 (dd, ^1J = 12.6 Hz, ^3J = 4.0 Hz, 1H, H-1a), 3.60–3.66 (overlapping signals, 2H, CH_2CH(H)N and H-4), 3.47 (t, *J* = 9.3 Hz, 1H, H-3), 3.38 (overlapping signals, 3H, CH_2CH(H)N and CH_2CH_2O), 3.27 (overlapping signals, 2H, H-5 and C*H*(H)CH_2N), 3.19 (dd, *J* = 8.7, 5.8 Hz, 1H, CH(*H*)CH_2N), 3.10 (t, ^1J = 11.5 Hz, 1H, H-1b), 2.74 (t, ^3J = 7.7 Hz, 2H, CH_2NH_2), 1.47 (dt, ^1J = 15.5 Hz, ^3J = 7.7 Hz, 2H, pentyl CH_2), 1.39 (m, 2H, pentyl CH_2), 1.19 ppm (m, 2H, pentyl CH_2); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 136.3, 126.2 (each indole C), 124.0, 122.2, 119.5, 118.0, 112.1 (each indole CH), 108.4 (indole C), 75.8 (C3), 71.1 (CH_2CH_2O), 67.5 (C4), 66.0 (C2), 65.0 (C5), 62.5 (C6), 53.5 (CH_2), 53.0 (C1), 39.3, 27.9, 26.5, 22.2, 19.3 ppm (each CH_2); ES-HRMS: found 392.2538 $[M+H]^+$; $C_{21}H_{34}N_3O_4$: requires 392.2549.

Binding studies. The assays were carried out at Cerep.[25] Compounds were evaluated for their ability to inhibit [^{125}I]Tyr11-somatostatin-14 binding to human recombinant SSTR4[22] receptors (from CHO cells) and to human recombinant SSTR5[23] receptors (from CHO cells). The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) were determined by nonlinear regression analysis of competition curves using Hill equation curve fitting. The inhibition constants (K_i) were calculated from the Cheng Prusoff equation ($K_i = IC_{50}/(1+(L/K_D))$, where *L* = concentration of radioligand in the assay, and K_D = affinity of the radioligand for the receptor). Somatostatin-14, the reference compound, had a K_i value of 2.0 nM for SSTR4 and 0.61 nM for SSTR5 in these assays.

Molecular modelling. The Monte Carlo conformational searching protocol using the MCMM method available in Macromodel 8.5[18] was used to generate 10000 conformers of **5**. Each conformer was energy minimised (PRCG method up to 5000 iterations) using the OPLS-AA force field until convergence had been attained. The indole and pentyl amino atoms of lowest energy structural isomer obtained for **5** were superimposed with the same groups of the sandostatin solution structure[16] using the superimpose function in Macromodel 8.5 (Figure 2).

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