Conformational Analyses of Somatostatin-Related Cyclic Hexapeptides Containing Peptoid Residues

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We report the conformational analysis by ^{1}H NMR in DMSO and computer simulations involving distance geometry and molecular dynamics simulations of a series of peptoid analogues of the cyclic hexapeptide c[Phe^{11}-Pro^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}] (**1**). The proline residue in compound **1** is replaced with the peptoid residues *N*-benzylglycine (Nphe) (compound **2**), *N*-(*S*)-α-methylbenzylglycine [(*S*)-*β*-MeNphe] (compound **3**), and *N*-(*R*)-α-methylbenzylglycine [(*R*)-*β*-MeNphe] (compound **4**). The peptoid analogues **2** and **4** exhibit potent binding activities to the hsst2 receptor, while the binding affinities to the hsst5 and to the hsst3 receptors are reduced compared to that of the parent compound **1**. Compound **3** shows reduced binding activities to the hsst2, hsst3, and hsst5 receptors compared to compound **1**. The results of in vivo assays indicate that these compounds inhibit the growth hormone release but do not affect the insulin release. These peptoid-containing analogues show two sets of NMR signals corresponding to cis and trans conformations of the peptide bond between Phe^{11} and Nxaa^6. We demonstrate that the backbone conformation and the orientation of the relevant side chains of compound **1** are maintained in the cis isomers of the peptoid analogues which adopt a type VI *β*-turn centered around residues 11 and 6 and a type II′ *β*-turn with D-Trp in the *i*+1 position. The enhanced selectivity of the peptoid-containing analogues compared to compound **1** and the results of the conformational analysis suggest that the presence of a conformationally constrained hydrophobic group in position 6 in complementary topology to the Phe^{11} side chain enhances selective binding to the hsst2 receptor.

Introduction

Since the discovery of the highly potent somatostatin analogue c[Phe^{11}-Pro^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}] (**1**) by Veber and co-workers[1,2] (the numbering refers to the location of the residues in native somatostatin[3−5]), numerous cyclic hexapeptides related to somatostatin have been synthesized and their conformations in solution have been studied.[6−10] It has been demonstrated that **1** and most of the related compounds share common structural motifs such as a type II′ *β*-turn with D-Trp in the *i*+1 position and a type VI *β*-turn in the so-called bridging region Xaa^{11}-Xbb^6 characterized by a cis peptide bond or mimicked by a disulfide or lanthionine bridge as in Sandostatin analogues.[11,12]

It has been postulated that the tetrapeptide sequence Phe^7-D-Trp^8-Lys^9-Thr^{10} is the biologically active portion, interacting with the receptor, while the Xaa^{11}-Xbb^6 sequence is important for maintaining the proper orientation of the tetrapeptide portion. Conformational studies on **1** have also shown that the molecule adopts two backbone conformations which are both consistent with all NMR data: a "flat" conformation and a conformation which is "folded" about Phe^7 and Thr^{10}.[13]

However, active hexapeptide analogues of somatostatin with a trans peptide bond in the bridging region have been reported as well,[5] and studies of active molecules incorporating changes in the bridging region have suggested that this region might play a role in the binding and does not simply function as a bracket to hold the 7−10 region in the appropriate position.[14]

The conformational analysis of a series of α- and *β*-methylated analogues of **1** revealed valuable information regarding the "bioactive" conformation of the side chains and of the backbone by restricting the conformational flexibility of these analogues compared to the parent compound **1**.[15,16] It was deduced from these studies that the "folded" and not the "flat" conformation might be the "bioactive" conformation.

In this paper we report the conformational analysis of a series of analogues of **1** containing novel peptoid[17,18] residues. The conformations of the cyclic hexapeptide analogues c[Phe^{11}-Nphe^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}] (**2**), c[Phe^{11}-(*S*)-*β*-MeNphe^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}] (**3**), and c[Phe^{11}-(*R*)-*β*-MeNphe^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}] (**4**) were studied by ^{1}H NMR in DMSO-d_6 and by computer simulations. The peptoid analogues **2** and especially **4** show increased hsst2 selectivity compared to compound **1**. In particular, the binding activities to the hsst3 and hsst5 receptors are weaker for these analogues compared to compound **1**, while their binding to the hsst2 receptor is in the same range as that measured for compound **1**. Compound **3**, on the other hand, shows weaker binding to hsst2 and hsst5 receptors compared to compound **1**. The syntheses of these analogues and a detailed discussion of the binding to the five recombinant somatostatin receptors and of their in vivo bioactivity is reported in the accompanying paper.[19]

Results

NMR Studies. The complete assignments of the proton resonances are given in Table 1S of the Supporting Information. The NOEs observed in the ROESY

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Table 1. Summary of the Observed Backbone NOEs for c[Phe[11]-Nphe[6]-Phe[7]-D-Trp[8]-Lys[9]-Thr[10]] (**2**), c[Phe[11]-(*S*)-*β*-MeNphe[6]-Phe[7]-D-Trp[8]-Lys[9]-Thr[10]] (**3**), and c[Phe[11]-(*R*)-*β*-MeNphe[6]-Phe[7]-D-Trp[8]-Lys[9]-Thr[10]] (**4**)[a]

	2		**3**		**4**	
	cis	trans	cis	trans	cis	trans
Phe[11]NH-Phe[11]H[a]	m	m	m	m	m	s
Phe[11]NH-ThrH[a]	s	s	s	m	*b*	s
Phe[11]H[a]-Nxaa[6]H[a]	s		s		s	
Phe[11]H[a]-Nxaa[6]H[b]		s		s		s
Phe[7]NH-Phe[7]H[a]	m	m	m	s	s	s
Phe[7]NH-Nxaa[6]H[a]	w	m	w	w	w	m
Trp NH-Phe[7]H[a]	s	s	s	m	s	s
Trp NH-TrpH[a]	w	m	w	s	s	m
LysNH-LysH[a]	m	m	m	s	m	s
LysNH-TrpH[a]	s	s	s	*b*	s	s
ThrNH-ThrH[a]	m	s	m	m	s	s
ThrNH-LysH[a]	m	w	w	m	m	m
LysNH-ThrNH	s	m	m	m	s	s
TrpNH-Phe[7]NH	w					w

[a] The NOEs corresponding to distances ≤ 2.5 Å are classified as strong (s); those corresponding to distances > 2.5 and ≤ 3.5 Å are classified as medium (m); the NOEs corresponding to distances > 3.5 and ≤ 4.5 Å are classified as weak (w). [b] Measurement of intensity difficult due to overlap.

Table 2. $J_{NH-C^{\alpha}H}$ Coupling Constants (Hz) and Calculated ϕ Angles (deg)[a]

	2		**3**		**4**	
	cis	trans	cis	trans	cis	trans
Phe[11]	5.2 Hz	3.6 Hz	5.8 Hz	4.6 Hz	4.3 Hz	7.1 Hz
	96	108	91	101	103	79
	24	12	29	19	17	41
	−166	−176	−162	−170	−172	−154
	−74	−64	−78	−70	−68	−86
Phe[7]	6.7 Hz	5.4 Hz[b]	4.9 Hz	6.8 Hz	7.4 Hz	6.9 Hz[b]
	84	95	98	83	76	82
	36	25	22	37	44	38
	−157	−165	−168	−156	−152	−156
	−83	−75	−72	−84	−88	−84
D-Trp	6.1 Hz	7.1 Hz[b]	7.3 Hz	7.0 Hz[b]	4.9 Hz	7.4 Hz
	−31	−41	−43	−39	−22	−44
	−89	−79	−77	−81	−98	−76
	80	86	87	85	72	88
	160	154	153	155	168	152
Lys	7.5 Hz	8.8 Hz	7.3 Hz	9.1 Hz	4. 5 Hz	7.4 Hz
	75	−141	77	−139	102	76
	45	−99	43	−101	18	44
	−152		−153		−171	−152
	−88		−87		−69	−88
Thr	7.1 Hz[b]	8.9 Hz	7.3 Hz	9.9 Hz	9.5 Hz	7.5 Hz
	79	−140	76	−125	−134	74
	41	−100	43	−115	−106	46
	−154		−153			−152
	−86		−87			−88

[a] Values were calculated using $J_{NH-C^{\alpha}H} = A \cos^2 |\phi \pm 60°| - B \cos |\phi \pm 60°| + C$, where (+) is for a D-configuration, (−) is for a L-configuration, and the coefficients are those proposed by Bystrov et al. for a chiral residue.[20] [b] From DQF-COSY.

experiment were assigned as strong, medium, and weak relative to each other according to their intensities, and the relevant backbone NOEs are shown in Table 1. A complete table with all NOEs observed is given in Table 2S. The $J_{NHC^{\alpha}H}$ coupling constants were used to calculate the ϕ angles,[20,21] and the results are given in Table 2. The temperature coefficients of the NH protons are discussed in the appropriate sections, and are given in Table 3S of the Supporting Information. The β-protons were stereospecifically assigned,[22] and the $J_{C^{\alpha}H-C^{\beta}H}$ coupling constants were used to calculate the side-chain populations. For the calculation of aliphatic amino acids, Pachler's equations[23] were used, while Cung's equations[24] were used for aromatic residues. The results are discussed in the appropriate sections and are given in the Supporting Information (Table 4S).

Table 3. Backbone Torsion Angles (deg) for Cis and Trans Isomers of the Peptoid Analogues of Compound **1**: c[Phe[11]-Nxaa[6]-Phe[7]-D-Trp[8]-Lys[9]-Thr[10]]

structure		Phe[11]	Nxaa[6]	Phe[7]	Trp	Lys	Thr
Compound **2**, c[Phe[11]-Nphe[6]-Phe[7]-D-Trp[8]-Lys[9]-Thr[10]]							
cis1	ϕ	−57	−83	−84	62	−69	−86
	ψ	128	−2	72	−119	−31	66
	ω	15	−177	−178	180	174	173
	χ_1	−174	−85	57	−176	−62	−58
cis2	ϕ	−90	−117	−165	61	−90	−147
	ψ	123	64	140	−126	20	178
	ω	−2	−177	173	180	177	169
	χ_1	−173	−87	69	177	−55	−164
cis3	ϕ	99	119	−88	62	−86	−88
	ω	−99	−92	81	−120	−78	66
	ω	12	178	−173	179	−176	−179
	χ_1	−69	99	−178	−58	−62	−57
trans1	ϕ	−63	−69	−154	101	−72	−114
	ψ	−29	−12	67	−125	168	168
	ω	177	−180	−174	−174	−172	−172
	χ_1	−52	−59	50	173	−176	−176
trans2	ϕ	−56	−74	−173	64	−74	−114
	ψ	−48	−12	130	−118	−15	168
	ω	178	−175	166	−178	176	−168
	χ_1	−57	−97	55	175	−65	164
Compound **3**, c[Phe[11]-(*S*)-*β*-MeNphe[6]-Phe[7]-D-Trp[8]-Lys[9]-Thr[10]]							
cis1	ϕ	−53	−81	−85	68	−66	−89
	ψ	126	−7	66	−107	−25	67
	ω	19	−178	172	−179	−175	−176
	χ_1	−174	−152	−56	178	−60	−175
cis2	ϕ	−57	−109	−167	84	−79	−138
	ψ	136	31	132	−126	−10	162
	ω	2	178	170	179	−177	173
	χ_1	−178	−147	−178	175	−62	77
trans1	ϕ	−54	100	−161	68	−83	−152
	ψ	122	−35	132	−151	53	153
	ω	174	176	179	174	174	176
	χ_1	−60	−141	−173	−177	−63	59
trans2	ϕ	72	84	−90	55	−116	−143
	ψ	78	−65	138	−131	36	80
	ω	179	−179	−179	178	−178	176
	χ_1	−55	−151	−63	−175	−62	59
Compound **4**, c[Phe[11]-(*R*)-*β*-MeNphe[6]-Phe[7]-D-Trp[8]-Lys[9]-Thr[10]]							
cis1	ϕ	−60	−93	−85	59	−72	−86
	ψ	135	13	84	−136	−13	73
	ω	8	179	−171	178	−169	−179
	χ_1	−60	−72	−176	−57	−63	−51
cis2	ϕ	−171	−81	−158	85	−80	−149
	ψ	128	−4	166	−119	−20	174
	ω	5	179	177	−173	179	−179
	χ_1	−59	−73	−169	179	−60	−176
trans1	ϕ	70	84	−80	61	−67	−87
	ψ	91	−58	73	−129	−34	59
	ω	−179	−176	−173	−177	−172	172
	χ_1	−172	76	−59	−176	−63	−177
trans2	ϕ	40	84	−84	75	−71	−154
	ψ	68	−54	73	−103	−27	159
	ω	174	−170	166	−174	171	178
	χ_1	−59	126	−172	174	−69	−171

Each of the four peptoid analogues shows two sets of NMR signals corresponding to cis and trans conformations of the Phe[11]-Nxaa[6] peptide bond. The ROESY experiment was used to differentiate between cis and trans conformations based upon strong cross-peaks between Phe[11]H$^{\alpha}$ and either Xaa[6]H$^{\alpha}$ for the cis isomers or Xaa[6]H$^{\beta}$ for the trans isomers. The ratios of cis to

trans isomers as determined by integration are 1:0.6 for compound **2**, 1:0.7 for compound **3**, and 1:1.6 for compound **4**. The presence of both, cis and trans isomers, in the peptoid analogues is different from compound **1** which shows exclusively cis orientation of the peptide bond between residues 11 and 6. In the analogues with β-methylated peptoid residues (compounds **3** and **4**) cis−trans isomerization does not occur on the NMR time scale. Contrary to that, the observation of several exchange cross-peaks between the two conformations in the ROESY experiment of the Nphe analogue **2** proves that cis-trans isomerization occurs during the mixing time of the ROESY experiment. Spectral overlap and low concentration of the minor conformations lead to a lack of experimental data especially for the minor conformation.

The NMR data of compounds **2**−**4** are very similar and suggest that the backbone conformations of these three compounds are not considerably different. The cis conformations of compounds **2**−**4** exhibit very similar NOE patterns, and data such as a medium NOE between ThrNH and LysNH, a strong sequential NOE between TrpH$^{\alpha}$ and LysNH, and low temperature coefficients of the ThrNH protons (0.6 −ppb/K for compound **2**, 0.4 −ppb/K for compound **3**, and 0.3 −ppb/K for compound **4**) suggest that the cis isomers of all three compounds adopt a type II′ β-turn with D-Trp in the i+1 position. There are considerable differences in the temperature coefficients of the Phe^{7}NH protons which are 3.0 −ppb/K for compound **2**, 2.4 −ppb/K for compound **3**, and 0.8 −ppb/K for compound **4**. The cis peptide bond between Phe^{11} and the peptoid residue suggests the presence of a type VI β-turn in this region. However, the differences in the temperature coefficients of the Phe^{7}NH indicate that the stability of this turn is different among these three compounds. The type VI β-turn seems to be considerably more stable in compound **4** compared to compounds **2** and **3**.

The calculation of the side chain populations in the cis isomers of compounds **2**−**4** indicates a relatively large degree of flexibility except for the Lys side chain which shows in all three compounds a strong preference for the g− orientation. There is an upfield shift of the Lys γ-protons (compared to the chemical shift in a disordered structure) observable for the cis isomers of all three compounds which indicates close spatial proximity with the aromatic side chain of D-Trp.[25] It is worthwile noting that the D-Trp side chain in compound **4** shows a preference for the trans rotamer, while this side chain has an almost equal distribution of all three possible rotamers in compounds **2** and **3**. The side chain of Phe^7 in compound **3** has a preference for g+, while compounds **2** and **4** adopt all three rotamers for this side chain. Finally, the Phe^{11} side chain of compound **4** prefers a g− orientation. In compound **3** this side chain does not show a preferred rotamer. It is important to note that the side chains in the parent compound **1** are also quite flexible.

The NMR data of the trans isomers of compounds **2**−**4** are also very similar compared to each other and suggest that the type II′ β-turn is maintained in the trans conformations. This is supported by medium NOEs between LysNH and ThrNH, strong sequential NOEs between D-Trp and Lys, and low temperature

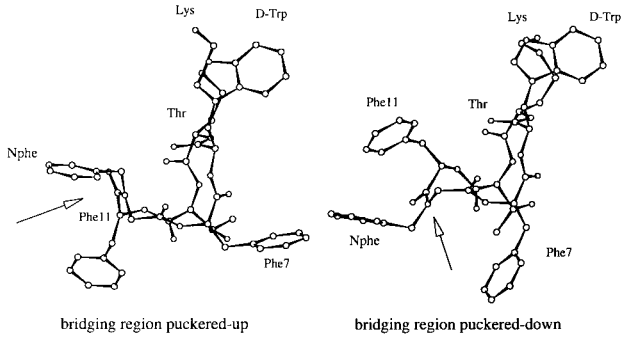

Figure 1. Structures of the "puckered-up" and "puckered-down" conformations of the cis isomer of c[Phe^{11}-Nphe^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}] (**2**).

coefficients of the ThrNH protons (0.0 −ppb/K for compound **2**, 1.8 −ppb/K for compound **3**, and 2.1 −ppb/K for compound **4**). The temperature coefficients of Phe^{7}NH proton are in all three compounds very low (0.0 −ppb/K in compound **2**, 0.1 −ppb/K in compound **3**, and 0.5 −ppb/K in compound **4**) and indicate that these NH protons are involved in a second hydrogen bond. As far as the nature of this second turn is concerned, difficulties in the evaluation by ^{1}H NMR arise from the N-benzylated structure of the peptoid residues.

The side-chain populations in the trans isomers are also very similar among compounds **2**−**4**. The Phe^7 side chain prefers a g+ orientation in all three compounds, and the Lys side chain adopts g− as seen for the cis isomers. The upfield shift of the Lys γ-protons (compared to the chemical shift in a disordered structure) indicating close spatial proximity between the Lys and D-Trp side chain is also observable in the trans isomers.

Computer Simulations. The conformational search for the cis isomers resulted in three conformational families for compound **2** and two conformational families each for compounds **3** and **4** which are consistent with the NMR data (Table 3, see also Figures 1S−3S of the Supporting Information). The conformations cis1 and cis2 observed for each compound are very similar to those described for compound **1**, cis1 representing a "folded" structure with a C^7 conformation about Phe^7 and Thr^{10} and cis2 representing a "flat" conformation. For compound **2** a second "folded" structure (cis3) was found, in which the bridging region around residues 11 and 6 can be described as "puckered-up" compared to conformer cis1 (Figure 1).

In all these structures the molecules adopt type II′ β-turns with D-Trp in the i+1 position. The presence of the cis peptide bonds between residues 11 and 6 leads to type VI β-turns spanning residues Phe^{11} and the peptoid residues. To obtain information about the accessible side-chain orientations in the peptoid residues, we carried out grid searches to determine the minimum-energy orientations of the torsion angle χ^1 (C^{11}=O−N^6−Cβ^6−Cγ^6) of the peptoid side chains in the model compounds acetyl-Nphe-NHMe, acetyl-(*R*)-β-MeNphe-NHMe, and acetyl-(*S*)-β-MeNphe-NHMe for both cis and trans orientations of the amide bond between the acetyl and peptoid residue. This search demonstrated that the peptoid side chain especially in the β-methylated peptoids shows little flexibility and adopts only two minimum-energy conformations. The

transition between the two minimum-energy orientations of the peptoid side chain in the β-methylated peptoids demands considerably more energy than for the unsubstituted Nphe compound. The results of these grid searches for the different compounds are discussed below, and a summary is given in the Supporting Information (Table 5S). For the cis isomer of compound **2** the peptoid side chain adopts only two conformations throughout all structures consistent with the NMR data characterized by the side chain torsion angle χ^1 (C^{11}=O−N^6−Cβ^6−Cγ^6) which adopts values of −82° to −95° or +98°. These values are in very good agreement with those obtained for the model compounds Ac-Nphe-NHMe which showed two minimum-energy conformations characterized by $\chi^1 = -97°$ or +90°. For the β-methylated peptoid analogues, only one side-chain orientation of the peptoid side chain was observed for each compound, −148° to −160° for compound **3** and −66° to −75° for compound **4**. In the model compound acetyl-(*S*)-β-MeNphe-NHMe with a cis orientation of the amide bond between the acetyl and (*S*)-β-MeNphe residues, two minimum-energy orientations of $\chi^1 = +64°$ and −100° were found. For the Ac-(*R*)-β-MeNPhe-NHMe values of χ^1 (C^{11}=O−N^6−Cβ^6−Cγ^6) = −79° and +126° were observed.

Computer simulations of the trans isomers resulted in two conformational families for each compound consistent with the experimental data (Table 3). All conformations adopt a type II′ β-turn spanning residues D-Trp and Lys. There are, however, differences in the conformations about residues 11 and 6. Structures trans1 and trans2 found for compound **2** and structure trans1 observed for compound **3** show a slightly distorted type II β-turn centered at Phe^{11} and the peptoid residues. Conformations trans2 of compound **3** and structures trans1 and trans2 of compound **4** adopt γ-turns about the peptoid residues. As mentioned, the evaluation of the conformation about the peptoid residue by NMR is difficult due to the lack of indicative NOEs involving the N-benzylated residue. Studies of the model compounds Ac-Phe^{11}-$Nxaa^6$-Phe^7-NHMe, however, suggest that the distances between the Phe^{11}NH proton and the β-protons of the peptoid residue in structures containing γ-turns about $Nxaa^6$ should be short enough to observe an NOE between these protons. In a type II β-turn, on the other hand, the distances between these protons are longer than 4 Å. In both conformations the Phe^7HN and the β-protons of the peptoid residues are close enough to lead to an observable cross-peak. The observation of weak NOEs between the Phe^7NH and the β-protons of the peptoid residues and the absence of NOEs between the Phe^{11}-NH and the β-protons of the peptoid residues in compounds **2** and **3** favor a type II β-turn spanning residues Phe^{11} and $Nxaa^6$ over structures containing γ-turns about $Nxaa^6$. For compound **4** a medium NOE between the Phe^{11}NH and the β-proton of the peptoid residue was observed which favors the γ-turn structures over the conformations containing β-turns about residues 11 and 6.

The side chain of the peptoid residues in the trans isomers of compound **2** shows more flexibility than that in the cis isomer. This is in agreement with the low activation barrier between the two minimum-energy conformations determined for this side chain in the trans orientation of the bond between the acetyl and Nphe residue of the model compound Ac-Nphe-NHMe. In the structures obtained from modeling, the peptoid residue adopts orientations characterized by χ^1 (C^{11}=O−N^6−Cβ^6−Cγ^6) values of −57°, −97°, −115°, −122°, and −139°. The conformational search for compound **3** suggests that the side chain orientation of the peptoid residue is restricted to values of χ^1 (C^{11}=O−N^6−Cβ^6−Cγ^6) around −150° in all structures consistent with the experimental data. This is in agreement with the results obtained for the model compound acetyl-(*S*)-β-MeNphe-NHMe with a trans orientation of the amide bond. For this model compound two minimum-energy conformations were found which are characterized by the torsional angle χ^1 (C^{11}=O−N^6−Cβ^6−Cγ^6) = −136° and +54°. In compound **4**, the peptoid residue seems to be restricted to values from 75° to 130° which is in good agreement with the torsional angles found for the model compound acetyl-(*R*)-β-MeNphe-NHMe with a trans orientation of the amide bond which adopted minimum-energy orientations of −61° and +83° to +120°.

Conclusions

We have shown that the cis isomers which we believe to be the bioactive isomers of compounds **2**−**4** adopt backbone conformations similar to those reported for the parent compound **1**. The side chains except for the Lys and the peptoid side chain are not particularly constrained. Conformations which are "folded" about Phe^7 and Thr^{10} and "flat" conformations were found. Both types of conformations contain a type II′ β-turn with D-Trp in the i+1 position and a type VI β-turn with the cis peptide bond between residues 11 and 6 in the bridging region. In addition to those two conformations the Nphe analogue **2** shows more flexibility in the bridging region than compounds **3** and **4** and adopts a second "folded" structure in which the bridging region can be described as "puckered-up" compared to the other "folded" conformation (Figure 1). Only the "puckered-down" conformation was observed for compounds **3** and **4**.

The side-chain orientation of the peptoid side chain can be described by the torsion angle χ^1 (C^{11}−N6−$C^6\beta$−$C^6\gamma$) which adopts values around −70° for compound **4** and around −150° for compound **3**. The spatial arrangement of the peptoid side chain results in a considerably closer proximity of the side chains of Phe^{11} and the peptoid residue in the cis isomer of compound **4** compared to compound **3**. The resulting steric effects provide a possible explanation for the different populations of cis and trans isomers in compounds **3** and **4** which is reflected in the relative energies of the cis and trans conformations in compounds **3** and **4**, respectively (see Figures 1S−3S in the Supporting Information). The unsubstituted peptoid side chain of compound **2** has considerably more flexibility and can adopt several different orientations.

Figure 2 shows the superimposed minimum-energy structures of the "flat" and "folded" conformational families of the cis isomers of compounds **2**−**4**. The superimposition demonstrates that compound **2**, although it is more flexible than compounds **3** and **4**, can

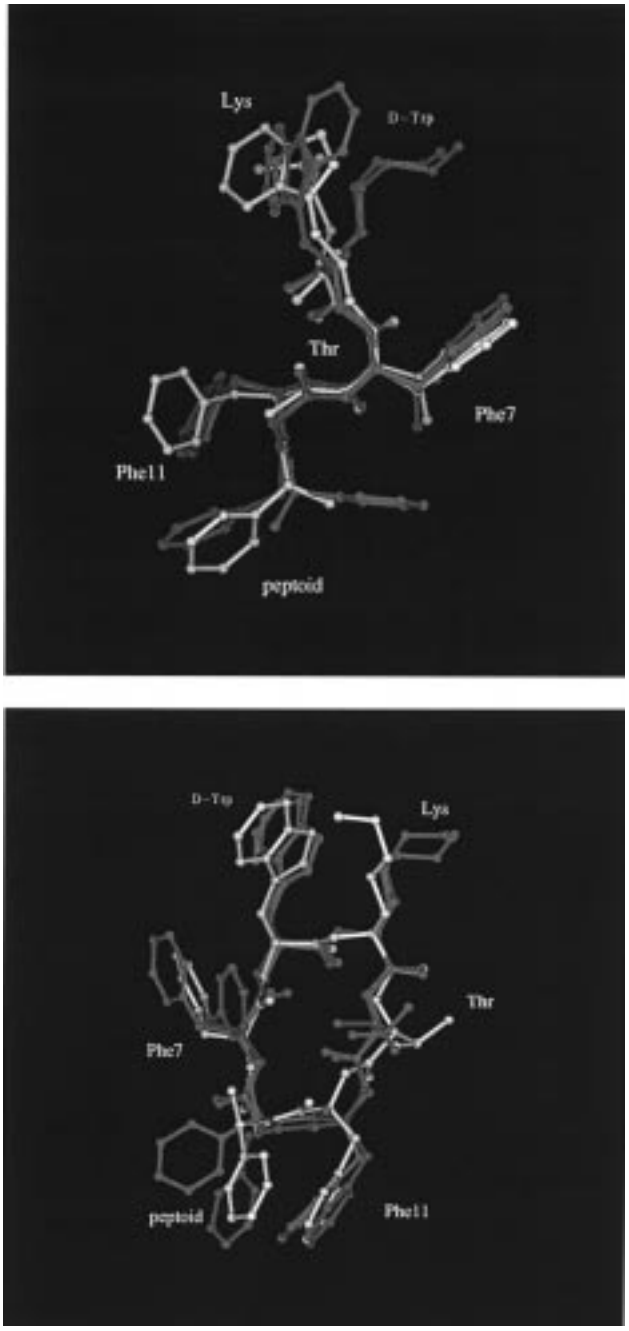

Figure 2. Superimposed structures of the "folded" (top) and "flat" (bottom) conformations of the cis isomers of c[Phe^{11}-Nphe^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}] (**2**) (green), c[Phe^{11}-(*S*)-β-MeNphe^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}] (**3**) (red), and c[Phe^{11}-(*R*)-β-MeNphe^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}] (**4**) (white).

access conformations very similar to those found for compound **4**. These conformations are characterized by a hydrophobic stacking of the peptoid and Phe^{11} side chains which leads to an almost parallel orientation of these two side chains. In compound **3**, on the other hand, the peptoid side chain points away from the Phe^{11} side chain and is oriented toward the Phe^{7} side chain. This is also reflected in the different populations of Phe^{7} and Phe^{11} side chains found experimentally. In compound **3**, the Phe^{7} side chain has a preferred orientation (g+), while this side chain in compounds **2** and **4** shows equal population of all three rotamers. In compound **4**, on the other hand, Phe^{11} has a preferred side-chain orientation (g−), while this side chain has no conformational preference in compound **3**. This is consistent with the close proximity of the peptoid side chain and the Phe^{11} side chain in compound **4** and the proximity of the peptoid and Phe^{7} side chains in compound **3**.

The results of the free molecular dynamics simulations at 300 K of compound **4** demonstrate that the type II′ β-turn is stable during the entire simulation and the areas of the largest flexibility within this molecule are ϕ(Phe^{7}), ψ(Phe^{7}), and ψ[(*R*)-βMeNphe^{6}). The ϕ(Thr^{10}) and ψ(Thr^{10}) show some flexibility but are much more rigid than those of Phe^{7} (Figure 4S of the Supporting Information). The backbone torsions of Phe^{7} and Thr^{10} are of special interest since the "folded" conformation is characterized by two hydrogen bonds about Phe^{7} (formed by TrpHN−(*R*)-βMeNpheC═O) and Thr (formed by Phe^{11}HN−LysC═O). These hydrogen bonds are not present in the "flat" conformation. The torsional angles of the Phe^{7} and Thr^{10} residues are significantly different in the "folded" compared to the "flat" conformations. The "folded" conformation adopts ϕ angles of −80° to −90° and ψ angles around 65° for both residues, whereas the "flat" conformation is characterized by ϕ angles of −130° to −160° and ψ angles of 130° to 180° for Phe^{7} and Thr^{10}. The hydrogen bond between TrpHN and (*R*)-βMeNpheC═O is not very strong, and that part of the molecule "folds" and "unfolds" numerous times during the molecular dynamics simulation. Contrary to that, the hydrogen bond about Thr (Phe^{11}HN−LysC═O) is very stable. As a consequence of the "folding" and "unfolding", the torsional angles ϕ(Phe^{7}), ψ(Phe^{7}), and ψ[(*R*)-βMeNphe^{6}) undergo significant changes, while changes in the Thr^{10} torsional angles are considerably smaller. The enhanced conformational stability of the hydrogen bond about Thr^{10} is consistent with a stable hydrophobic stacking of the peptoid aromatic side chain and the Phe^{11} side chain, which leads to a more rigid conformation in the bridging region especially around Phe^{11}. As a consequence, the hydrogen bond about Thr which involves the Phe^{11}HN is considerably more stable than that about Phe^{7}. The backbone torsional angles of Thr show much fewer changes than those of Phe^{7}, and the hydrogen bond (Phe^{11}HN−LysC═O) is maintained during the entire simulation.

Since the backbone conformation and the side chain orientations of the relevant side chains of compound **1** are maintained in the cis isomers of the peptoid analogues **2**−**4,** the changes in the bioactivity such as weaker binding affinities to the hsst3 and hsst5 receptors compared to those of compound **1** suggest that the presence of an additional hydrophobic group in position 6 in close spatial proximity of the Phe^{11} side chain might be responsible for the enhancement of selectivity to the hsst2 receptor. This leads us to propose a model according to which an aromatic peptoid side chain in an parallel arrangement with the Phe^{11} side chain such as seen in compounds **2** and **4** can be tolerated by the hsst2 receptor but leads to reduced binding activities to the hsst3 and hsst5 receptors. The orientation of the peptoid side chain in compound **3** toward the Phe^{7} side chain on the other hand leads to a topology which seems to be less favorable for both the hsst2 and hsst5 receptors and results in a lower binding potency to the hsst2 receptor for this compound compared to those of compounds **2** and **4**.

The enhanced flexibility of compound **2** compared to compound **4** results in a slightly lower binding affinity to the hsst2 receptor. The in vivo tests indicate that the peptoid analogues inhibit the release of growth hormone but seem to have no detectable effect on the release of the inhibition of insulin.[19]

Experimental Section

^{1}H NMR Measurements. The ^{1}H NMR spectra were recorded on a Bruker AMX 500 spectrometer operating at 500 MHz. Temperatures were maintained at given values within ±0.1 °C. All experiments were carried out in DMSO-d_6 with the solvent peak (2.49 ppm) as internal standard. The peak assignments were made using TOCSY,[26−28] DQF-COSY,[29−31] and ROESY[32] experiments. The ROESY experiment was used for the sequential assignments.[33] The TOCSY experiments employed the MLEV-17 spin-locking sequence suggested by Bax and Davis[26] with a spin-locking field of 10 kHz and a mixing time of 75 ms. The ROESY experiments were carried out using mixing times of 100 and 200 ms with a spin−locking field of 2.5 kHz. All two-dimensional spectra were obtained using 4K data points in the f_2 domain and 400 points in the f_1 domain for the TOCSY and ROESY experiment and 512 data points in the f_1 domain for the DQF-COSY. The time proportional phase increment was used. Applying zero-filling procedures resulted in a final matrix of 2K × 2K data points. Multiplication with a 30° shifted sine bell function was used for the TOCSY and DQF-COSY; multiplication with a 90° shifted sine bell function was applied for the ROESY to enhance the spectra. The ROESY cross-peaks were calibrated against the distance between the indole HN and H2 protons of D-Trp and against the geminal protons of the peptoid residue. An error of ±0.5 Å was estimated, and the upper and lower distances were set to the measured distance of ±0.5 Å.

Computer Simulation. All calculations were performed on an Iris 4D-340 computer (Silicon Graphics). The distance geometry program DGEOM[35] was used to generate structures consistent with the distance constraints derived from the NOEs. Temperature coefficient of NH protons indicating hydrogen bonds and ϕ angles calculated from J_{NH-H^α} were used to filter out structures that did not meet the experimental data. An error of ±30° was tolerated for the ϕ angles calculated from J_{NH-H^α} at this stage of refinement. In the case of the hydrogen bond-based selection, structures were retained in which the NH protons with an absolute value of the temperature coefficient of <2 ppb/K donate at least one hydrogen bond fulfilling the loose threshold of 3.0 Å and 110° for the NH proton-acceptor distance and for the angle defined by the three atoms N−H−oxygen of the acceptor carbonyl. Structures which did not fulfill these requirements were discarded. The remaining structures were subjected to molecular dynamics. Energy minimization and molecular dynamics computation were carried out in vacuo using the DISCOVER program[36] with the CFF91 force field. To approximate the solvatation, a distance-dependent dielectric constant was used. To search the accessible space more thoroughly, the distance geometry structures which were consistent with the experimental data were subjected to 10 ps of molecular dynamics at 1000 K with a step size of 1 fs. At intervals of 1 ps, conformations were extracted and energy-minimized by steepest descent until the maximum derivative was less than 1. Starting from each of the minimized structures, 10 ps of molecular dynamics was performed at 300 K. At regular intervals of 1 ps structures were extracted. These structures were subjected to unrestrained minimization using the VA09A algorithm until the maximum derivative was less than 0.001 kcal/mol. Using this procedure, 100 structures were created starting from each of the remaining distance geometry structures. The structures which were consistent with the temperature coefficients, calculated ϕ angles, and NOEs derived from the ROESY experiment were subjected to cluster analysis using a range of ±30° of the backbone torsional angles. Deviations less than 20° were tolerated for the ϕ angles estimated from the J_{NH-H^α} at this stage of the refinement. The other conformations were discarded. The low-energy conformation of each conformational family was subjected to free molecular dynamics at 300 K.

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Supporting Information Available: Tables of proton assignments, NOEs, temperature coefficients, and coupling constants and figures of minimum-energy structures as mentioned in the text (12 pages). Ordering information is given on any current masthead page.

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