NMR-Solution Structures and Affinities for the Human Somatostatin G-Protein-Coupled Receptors $hsst_{1-5}$ of CF_3 Derivatives of *Sandostatin®* (Octreotide)

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The previously reported (*Helv. Chim. Acta* **2008**, *91*, 2035) derivatives of octreotide (**1**) with a (CF_3)-Trp substitution, *i.e.*, **3**, and with open-chain structures, *i.e.*, **2**, **4**, and **5**, have been tested for their affinities to $hsst_{1-5}$ receptors and subjected to a detailed NMR analysis. Their affinities vary from 15 nM to 5 μM, as compared to 0.6 nM to 0.8 μM for octreotide itself (*Table 1*). This decreased bioactivity may have had to be expected for the open-chain compounds **4** and **5**; possible reasons for this decrease in the case of CF_3 derivative of octreotide, **3**, are discussed. NMR Analysis (*Tables 2* and *3*) provides evidence for increased dynamics of all new derivatives **2**–**5**. The dynamics of the octreotide molecule **1** was analyzed by (natural-abundance) longitudinal ^{13}C-T_1-relaxation time measurements (*Table 4*), from which the conclusion is drawn that the backbone of the macrocycle is rather rigid on the time scale of this method.

We have recently reported the selective *S*-trifluoromethylations of cystein side chains in peptides [1], using the new electrophilic hypervalent iodine(III)-CF_3 reagents developed in one of our laboratories [2]. One of the substrates used in these investigations was the open-chain, reduced form **2** of octreotide (**1**; *Sandostatin®*). Besides the seven amide bonds in the backbone, this octapeptide derivative **2** contains two NH_2, three OH, and two SH groups on the side chains, and, in addition, it carries a highly nucleophilic 1*H*-indol-3-yl group in its Trp residue. Still, the trifluoromethylation can be carried out (in a protic medium MeOH/H_2O!) with preferred attack (> 80%) on the two S-atoms to give product **4**. Two other compounds **3** and **5** have also been isolated, in which the 1*H*-indole ring is trifluoromethylated at C(2). The isolation,

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[2]) The hitherto unpublished NMR investigation of the dynamic behavior of octreotide, described herein, was carried out in 1993 (in the course of Ph.D. thesis work by *T. B.*). Present address of *T. B.:* Fadacher 11, CH-8126 Zumikon.

[3]) Part of the Ph.D. thesis of *I. K.*, ETH-Zürich, ETH Dissertation No. 17990 (2008).

purification, and spectroscopic characterization of **2**–**5** are described in the Exper. Part of our previous paper [1].

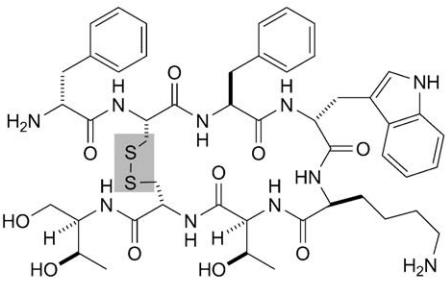
The cyclic disulfide octreotide [3] and its derivatives are used as diagnostic [4] and therapeutic [5] agents for the detection and treatment of certain types of cancers, which are characterized by overexpression of the somatostatin G-protein coupled receptor (GPCR) of the $hsst_2$ type on the surface of their cells, as compared to normal cells; these drugs are also used in endocrinological disorders such as acromegaly and further gastrointestinal indications. In the binding of somatostatin, octreotide, and analogs to the receptors $hsst_{1-5}$, a turn-type substructure, carrying the Trp and the Lys side chains, is thought to dock into receptor pockets[4]). To find out whether the new, open-chain trifluoromethylated octreotide derivatives **4** and **5** have conformational populatoins with the prerequisite turn structure, and whether a CF_3 group at C(2) of the 1*H*-indole ring of Trp in the trifluoromethylated octreotide **3** is tolerated, we have carried out affinity measurements (a biological test) and an NMR investigation (a spectroscopic, chemical test) of these compounds.

Affinities of Octreotide Derivatives **3**–**5** *for Somatostatin Receptors.* The three trifluoromethylated compounds **3**–**5** and, as a control, octreotide (**1**) itself were subjected to radioligand binding to establish their respective affinities for the recombinantly expressed somatostatin receptors $hsst_{1-5}$. The competition experiments with [^{125}I]LTTSRIF$_{28}$ from receptor proteins expressed in CCL-39 cell lines were carried out as described previously [6–9]. The results are listed in *Table 1.*

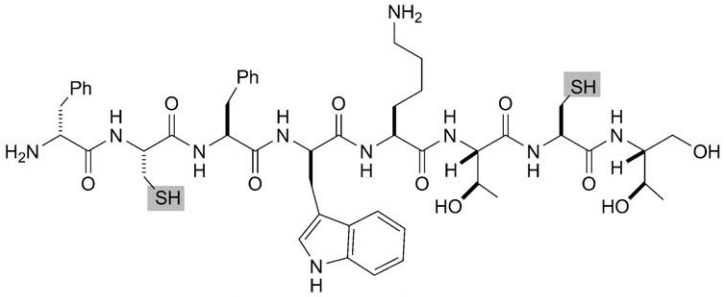
Table 1. *Affinities* (pK_d) *of the Octreotide Derivatives* **3**–**5** *as Compared to Octreotide* (**1**). Radioligand-binding assay (competition of the specific binding of [^{125}I]LTTSRIF$_{28}$) with the five known human somatostatin receptors ($hsst_{1-5}$) expressed in Chinese Hamster Lung Fibroblasts. *N*, independent determinations were used to calculate the pK_d values ($-\log M \pm SEM$), K_d values are also given.

Peptide		$hsst_1$	$hsst_2$	$hsst_3$	$hsst_4$	$hsst_5$
Octreotide (**1**)	pK_d	6.46 ± 0.04	9.21 ± 0.04	7.69 ± 0.04	6.08 ± 0.08	7.79 ± 0.04
	K_d	0.35 μM	0.61 nM	20.4 nM	0.83 μM	16.2 nM
	N	18	16	18	12	17
Assay control	pK_d	6.56 ± 0.04	9.13 ± 0.03	7.73 ± 0.26	6.06 ± 0.06	7.87 ± 0.07
	K_d	0.27 μM	0.74 nM	18.6 nM	0.87 μM	13.5 nM
	N	3	3	4	3	3
[F_3C-Trp]-Octreotide **3**	pK_d	6.32 ± 0.03	7.81 ± 0.10	6.29 ± 0.07	5.30 ± 0.07	6.46 ± 0.02
	K_d	0.48 μM	15.5 nM	0.51 μM	5.01 μM	0.35 μM
	N	3	4	4	3	3
4	pK_d	5.88 ± 0.09	7.11 ± 0.12	6.83 ± 0.14	6.34 ± 0.09	6.46 ± 0.05
	K_d	1.32 μM	77.6 nM	0.15 μM	0.46 μM	0.35 μM
	N	3	4	4	3	3
5	pK_d	6.37 ± 0.09	6.78 ± 0.07	6.54 ± 0.13	6.03 ± 0.02	5.84 ± 0.05
	K_d	0.43 μM	0.16 μM	0.29 μM	0.93 μM	1.44 μM
	N	3	3	4	3	3

[4]) For a recent discussion, see a survey of β-peptidic somatostatin mimics [6].



1



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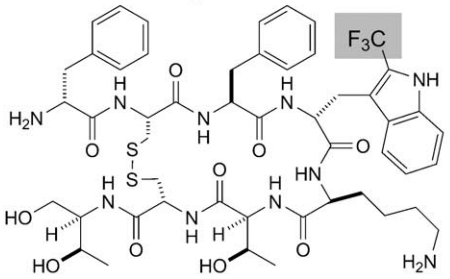
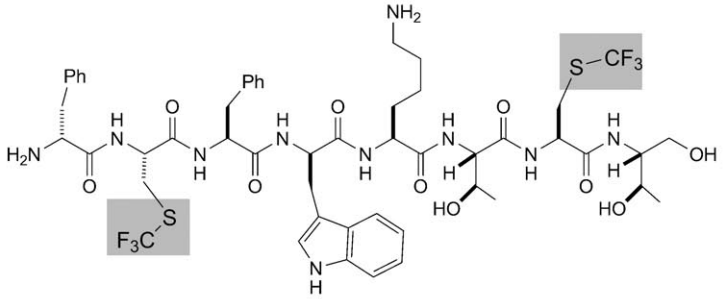


[F_3C-Trp]-Octreotide (**3**)



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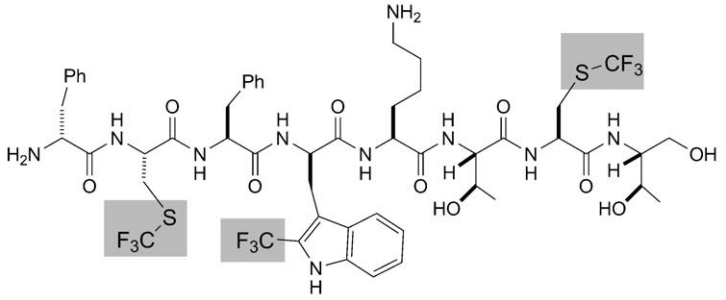

5

The binding patterns of octreotide (**1**; hsst $2 > 5 \approx 3 > 1 \approx 4$) and of the [$F_3$C-Trp]-octreotide (**3**; hsst $2 > 5 \approx 1 \approx 3 > 4$) are similar, albeit at a 5–25-fold lower level for the trifluoromethylated compound. Thus, the trifluoromethylation at C(2) of the 1*H*-indole ring in octreotide (**1**) leads to a substantial decrease in affinitiy, compatible with the view that the 1*H*-indol-3-yl substituent on the β-turn section provides a strong contribution to the binding of somatostatin, *Sandostatin*®, and their analogs to their cognate G protein-coupled receptors (GPCRs)[5]). The CF_3 group can be thought of exerting three different effects on the properties of the indole ring: *i*) there would be a steric hindrance introduced by the H/CF_3 replacement[6]), *ii*) the H-bond donating ability, *i.e.*, the acidity of the neighboring NH group in the indole ring is expected to be increased, *iii*) a possible π-interaction of the electron-rich indole ring in the receptor pocket should be weakened by the CF_3 group. Since the structures of the corresponding GPCRs (and their presumed ligand binding 'pockets') are unknown, it is not possible to state which effect or combination of effects is mainly responsible for the observed overall result (see also the increased dynamics of **3** *vs.* **1** derived from NMR analysis, below).

To our surprise, the two open-chain octreotide derivatives **4** and **5** have, with two exceptions, sub-micromolar affinities to all five receptors, with strongest binding of 78 and 160 nM, respectively, to $hsst_2$ (*Table 1*). Thus, the lack of the disulfide bond, *i.e.*, the open-chain structure, does not completely prevent binding. If the β-turn-structured D-Trp-Lys segment is, as commonly accepted, decisive for binding to their respective GPCRs, these molecules should have a turn structure or at least be capable of folding to a β-turn upon binding ('induced fit').

A CD-spectroscopic analysis [1] of the compounds **1**–**5** has provided intriguing differences between octreotide **1** and its CF_3 derivative **3** (a dramatic decrease of the intensity of the short-wavelength trough), and between the two open-chain compounds **2** and its tris(trifluoromethyl) derivative **5** (less strong *Cotton* effects of the latter). Furthermore, the CD spectra of the open-chain compounds have a similarity with those of α-helices, although this could be due to small populations of structures with intensive absorptions and large $\Delta\varepsilon$ (between right- and left-handed circularly polarized light) that are not 'seen' by other methods, *e.g.*, NMR. The decrease of the CD intensity, when going from octreotide to its CF_3 derivative, must be interpreted as a decrease of the contribution of that conformer which causes the negative *Cotton* effect at 202 nm, *i.e.*, the dynamics of the octreotide skeleton is likely to be changed by the CF_3 group.

NMR Analysis of the Octreotide Derivatives **1**–**5**. The conformations of the new compounds were further characterized by NMR spectroscopy, with the *caveat* that the NOE intensities are strongly biased towards short distances that may only transiently exist in a dynamic system. A list of the chemical shifts and of the coupling constants $J(\alpha,NH)$ of the five compounds is presented in *Table 2*, and the observed nuclear *Overhauser* effects are collected in *Table 3*. The data for octreotide **1** are taken from [12][13].

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[5]) In an investigation of γ-dipeptidic somatostatin mimics [10], however, major structural modifications on the Trp-indole ring led to derivatives with sub-micromolar hsst affinities.

[6]) The volume of a CF_3 hemisphere is *ca.* 43 Å^3 (*cf.* Scheme 7 in [11]).

Table 2. *^{1}H-NMR Chemical Shifts* [ppm] *and Scalar Coupling Constants* [Hz] *of the Compounds* **1** (in H_2O) *and* **2**–**5** (in CD_3OH). The values for **1** are taken from [12].

		1	**2**	**3**	**4**	**5**
D-Phe[1]	$C^\alpha H$	4.10	4.19	4.25	4.18	4.12
	$C^\beta H_2$	3.06, 3.16	3.11, 3.22	3.08, 3.26	3.07, 3.26	3.03, 3.22
Cys[2]	$J(\alpha,NH)$	7.6	7.8	8.7		
	NH	8.19	8.67	8.80	not obs.	not obs.
	$C^\alpha H$	4.67	4.72	5.19	4.87	4.84
	$C^\beta H_2$	2.75, 2.81	2.65, 2.65	2.93, 2.93	3.00, 3.27	3.02, 3.24
Phe[3]	$J(\alpha,NH)$	7.3	7.7	7.4	8.0	7.1
	NH	8.51	8.41	8.60	8.51	8.48
	$C^\alpha H$	4.67	4.62	4.63	4.62	4.60
	$C^\beta H_2$	2.93, 3.09	2.92, 2.97	2.93, 3.01	2.94, 2.97	2.95, 2.95
D-Trp[4]	$J(\alpha,NH)$	3.1	5.9	3.8	6.0	5.5
	NH	8.59	8.53	8.83	8.53	8.62
	$C^\alpha H$	4.25	4.35	4.05	4.42	4.38
	$C^\beta H_2$	2.84, 3.00	2.95, 3.14	2.88, 3.25	2.96, 3.18	3.04, 3.40
	C(2)H		7.05	n/a	7.04	n/a
	C(4)H		7.50	7.50	7.51	7.65
Lys[5]	$J(\alpha,NH)$	5.9	8.8	8.0	9.1	7.9
	NH	8.34	8.25	8.48	8.22	8.20
	$C^\alpha H$	3.81	4.14	3.85	4.23	4.12
	$C^\beta H_2$	1.28, 1.58	1.31, 1.73	1.20, 1.58	1.32, 1.76	1.28, 1.64
	$C^\gamma H_2$	0.35, 0.53	0.75	0.34	0.78	0.70
	$C^\delta H_2$	1.32	1.37	1.22	1.41	1.38
	$C^\varepsilon H_2$	2.70	2.66	2.58	2.68	2.70
Thr[6]	$J(\alpha,NH)$	7.8	9.0	9.1	9.1	8.1
	NH	8.01	8.12	8.18	8.11	8.10
	$C^\alpha H$	4.34	4.42	4.43	4.44	4.40
	$C^\beta H$	4.22	4.25	4.35	4.24	4.22
	$C^\gamma H_3$	1.26	1.22	1.24	1.24	1.23
Cys[7]	$J(\alpha,NH)$	7.7	8.0	8.8	8.0	7.9
	NH	7.92	8.34	8.36	8.66	8.64
	$C^\alpha H$	4.78	4.78	5.15	4.92	4.90
	$C^\beta H_2$	2.95, 3.19	2.87, 2.92	2.98, 3.15	3.28, 3.46	3.32, 3.48
Thr[8]-ol	$J(\alpha,NH)$	9.1	10.0	9.8	9.9	8.9
	NH	7.63	7.68	7.59	7.72	7.75
	$C^\alpha H$	3.87	3.82	3.80	3.85	4.20
	$C^\beta H$	4.02	4.07	4.12	4.06	4.02
	$C^\gamma H_3$	1.16	1.15	1.20	1.16	1.20
	CH_2		3.63, 3.65	3.64, 3.64	3.64, 3.67	3.62, 3.65

As can be seen from *Table 2*, the chemical shifts of H-atoms in analogous positions of all five compounds are similar and differ by less than 0.8 ppm, in half of the cases by less than 0.2 ppm, and in a third of the cases the deviations are less than 0.1 ppm. The largest differences to random coil chemical shifts are observed for the γ-CH_2 H-atoms of Lys[5], the signals of which are upfield shifted by 1.1 ppm in the cyclic derivatives **1** and

Table 3. *Observed NOEs in the NMR Spectra of Octreotide* (**1**) *and Its Derivatives* **2**–**4** (s, strong; m, medium; w, weak, vw, very weak). The upper distance bounds for *Sandostatin®* (**1**) are taken from [13] (data set B).

Atom 1		Atom 2		**1**	Upper bound [Å]	**2**	**3**	**4**
D-Phe[1]	$C^{\alpha}H$	Cys[2]	NH	s	2.6	s	s	
Cys[2]	NH	Phe[3]	NH			vw		
	$C^{\alpha}H$		NH	s	2.8	s	m	
	$C^{\alpha}H$	Cys[7]	$C^{\alpha}H$	w	4.0			
Phe[3]	NH	Trp[4]	NH	w	4.3	vw		overlap
	$C^{\alpha}H$		NH	s	2.6	s	s	s
	NH	Thr[6]	$C^{\beta}H$	vw	4.9			
	NH		NH				w	vw
	NH	Cys[7]	$C^{\alpha}H$	vw	4.6			
D-Trp[4]	NH	Lys[5]	NH			vw		vw
	$C^{\alpha}H$		NH	s	2.5	s	s	s
	C(4)H		$C^{\alpha}H$	w	4.9	w	w	vw
	HA	Thr[6]	NH	vw	4.6			
Lys[5]	NH	Thr[6]	NH	m	3.1	w	m	m
	$C^{\alpha}H$		NH	w		m	w	overlap
	$C^{\alpha}H$	Cys[7]	NH	vw	4.9			overlap
Thr[6]	NH	Cys[7]	NH	m	3.3	w	m	w
	$C^{\alpha}H$		NH	m	3.1	s	m	s
	$C^{\beta}H$		NH	w	4.7	m	w	m
	$C^{\alpha}H$	Thr[8]-ol	NH	vw	4.7			
	$C^{\gamma}H_3$	Cys[7]	NH			vw	vw	w
Cys[7]	NH	Thr[8]-ol	NH			w		w
	$C^{\alpha}H$	Thr[8]-ol	NH	m	3.0	m	m	

3, and by 0.7 ppm in the open-chain compounds **2**, **4**, and **5**. This effect, which is due to shielding by the aromatic π-system of the neighboring 1*H*-indol-3-yl group in the $\beta_{II'}$-turn-like structure **A** (*Fig.*) with an (*R*)- and an (*S*)-amino-acid residue, is present also in the open-chain compounds albeit to a lesser extent, presumably due to enhanced flexibility of the largely preserved turn structures. Small coupling constants $J(\alpha,NH)$ of 3.1 and 3.8 Hz are observed for D-Trp[4] in the cyclic compounds **1** and **3**, as is expected with a $\beta_{II'}$ turn (see *Fig.*); the values between 5.5 and 6.0 Hz in the open-chain counterparts **2**, **4**, and **5** also deviate from those expected in a random coil but indicate increased flexibility. The $J(\alpha,NH)$ values ≥ 8 Hz of Lys[5] – Thr[8]-ol in all compounds **2**–**5** indicate large torsion angles as is expected for the third residue of a type-II′ β turn (see π in *Fig.*) or in extended strands.

Inspection of *Table 3* shows that the NOE patterns[7]) are again similar in all derivatives, with the exception of a few long-range NOEs in the spectrum of octreotide (**1**), which are not observed for the other compounds, including the cyclic trifluoromethyl-octreotide **3**. As was discussed previously for octreotide [15], not all NOEs can

[7]) No NOE could be observed between the two CF_3 groups in the open-chain compound **4**, because the two signals are too close (42.69 and 42.74 ppm in the 376-MHz ^{19}F-NMR spectrum in DMSO).

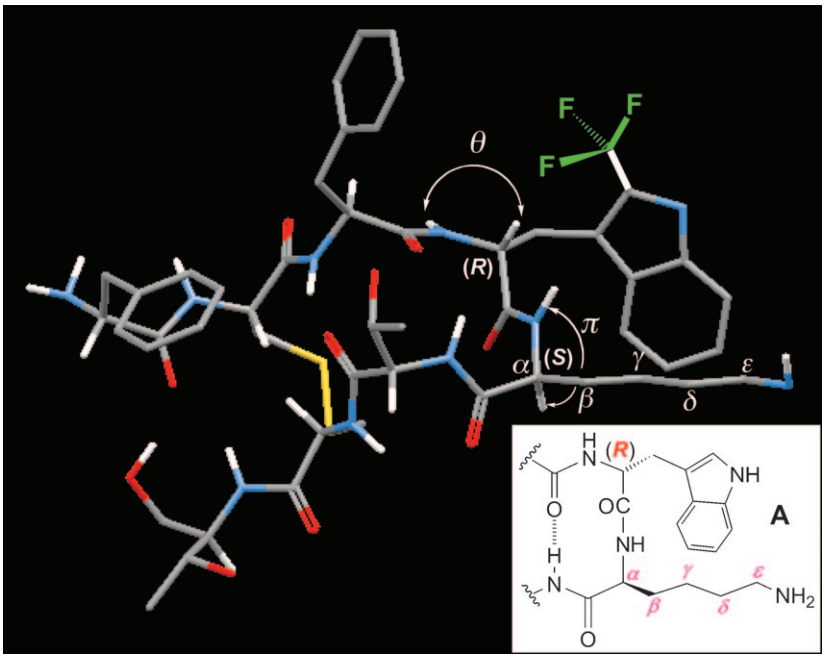

Figure. *Structural formula* **A** *of a typical* $\beta_{II'}$ *turn with an (*R*)- and an (*S*)-amino acid* (carrying the side chains of Trp and Lys) *and a structure of octreotide* (PDB code 1SOC, [14]) *with labeling of characteristic structural features and graphical attachment of a* CF_3 *group*. The structure of octreotide is derived from multiconformational NMR analyses, and the minimized *average β-sheet* structure with a – not quite ideal – β-turn (see the ten-membered 'H-bonded' ring) is chosen for the demonstration shown here; $\beta-\varepsilon$ are the CH_2 groups of the Lys side chain; θ ($+131°$) and π ($-138°$) are the H–N–C^α–H torsion angles in D-Trp^4 and Lys^5, respectively (values in an ideal $\beta_{II'}$ turn would be 120° and $-140°$, corresponding to ϕ-angles of 60° and $-80°$, respectively).

be satisfied simultaneously, which indicates the presence of conformational equilibria. This effect is noticably more pronounced in the new compounds, *e.g.*, the presence of medium or strong $C^\alpha H(i)-NH(i+1)$ NOEs as well as $NH(i)-NH(i+1)$ NOEs for the same residues. For this reason, it is not possible to determine unique three-dimensional structures from the data obtained for **2**–**5**. The data are insufficient for a determination of the conformational populations. However, the spectra suggest that the conformational space adopted by all compounds **2**–**5** is much more similar to, and includes, the conformations of octreotide (**1**) than it is to random coil. This was not necessarily to be expected for the open-chain compounds **2**, **4**, and **5** that lack the S–S bond present in the macrocycles **1** and **3**, but it is compatible with the observed biological activity. The reduced but still significant deviations from random-coil values for the coupling constant $J(\alpha,NH)$ of D-Trp^4 and the upfield shift of γ-CH_2 of Lys^5 in the latter two compounds provide semi-quantitative evidence for the increased flexibility of the linear over the cyclic compounds. The fact that trifluoromethylation of octreotide, *i.e.*, **1** → **3**, leads to a more flexible overall structure is surprising, but is in line with the

likewise unexpected substantial decrease of the negative *Cotton* effect observed in the CD spectrum.

An NMR analysis of the dynamics of octreotide **1** was carried out by analyzing the longitudinal ^{13}C relaxation behavior of a natural abundance sample in water at a magnetic field strength of 400 MHz and at a temperature of 300 K (for details, see *Exper. Part*). The T_1 relaxation times of the C-atoms are listed in *Table 4*. The rather homogeneous T_1 relaxation times of the backbone C^{α}-atoms of the amino acids residues integrated into the macrocycle indicate, in as far as the measurement of longitudinal relaxation at a single magnetic-field strength permits such interpretation [16], that the backbone of the macrocycle is rather rigid and undergoes an isotropic rotational tumbling motion with a correlation time of *ca.* 180 ps. It is, therefore, concluded that the exchange rate between conformers leading to NOEs that are not compatible with a single static structure is slower than the rotational correlation time, but faster than seconds as the chemical shifts of the conformers are not resolved.

Table 4. *^{13}C-NMR Chemical Shift Values,* nT_1 *Relaxation Times, and Assignments to the Individual ^{13}C-Atoms of Octreotide* (**1**) *at a Temperature of 300 K and at a Magnetic Field Strength of 400 MHz*. nT_1 Values are given with standard deviations, and values given in brackets indicate overlapping ^{13}C lines preventing reliable determination of nT_1 values. T_1 Values are multiplied by $n = 2$ or 3 for methylene or methyl C-atoms, respectively.

δ(C) [ppm]	T_1 [s]	C-Atom	δ(C) [ppm]	T_1 [s]	C-Atom
178.3	1.279 ± 0.143	D-Trp^4-C′	55.0	0.259 ± 0.020	Cys^7-α
177.9	1.700 ± 0.230	Lys^5-C′	69.46	0.305 ± 0.020	Thr^6-β
175.4	1.793 ± 0.249	Thr^6-C′	69.12	0.3611 ± 0.020	Thr^8-β
175.1	1.853 ± 0.271	Cys^7-C′	64.22	0.292 ± 0.016	Thr^8-C(1)
174.9	1.592 ± 0.206	Phe^3-C′	62.91	0.287 ± 0.021	Thr^6-α
172.6	1.544 ± 0.208	Cys^2-C′	59.44	0.387 ± 0.020	Thr^8-α
171.8	1.661 ± 0.271	D-Phe^1-C′	58.87	0.288 ± 0.021	D-Trp^4-α
139.3	1.958 ± 0.349	D-Trp^4-C(8)	58.07	0.290 ± 0.019	Phe^3-α
139.0	1.215 ± 0.124	Phe^3-γ	57.33	0.377 ± 0.023	D-Phe^1-α
136.6	1.615 ± 0.218	D-Phe^1-γ	57.62	0.288 ± 0.019	Lys^5-α
132.3	0.476 ± 0.011	D-Phe^1-δ	56.00	0.287 ± 0.021	Cys^2-α
132.2	0.350 ± 0.010	Phe^3-δ	44.90	0.314 ± 0.042	Cys^2-β
132.1	0.466 ± 0.011	D-Phe^1-ε	42.04	(0.840 ± 0.034)	Lys^5-ε
131.6	0.351 ± 0.010	Phe^3-ε	42.00	(0.840 ± 0.034)	Cys^7-β
131.0	0.387 ± 0.021	D-Phe^1-ζ	41.55	0.372 ± 0.036	Phe^3-β
130.2	0.291 ± 0.020	Phe^3-ζ	39.90	0.496 ± 0.034	D-Phe^1-β
129.6	2.024 ± 0.428	D-Trp^4-C(9)	32.35	0.428 ± 0.042	Lys^5-β
127.4	0.327 ± 0.024	D-Trp^4-C(2)	29.08	(0.746 ± 0.026)	Lys^5-δ
124.8	0.274 ± 0.021	D-Trp^4-C(6)	28.99	(0.746 ± 0.026)	D-Trp^4-β
122.2	0.295 ± 0.019	D-Trp^4-C(5)	24.48	0.594 ± 0.036	Lys^5-γ
121.2	0.252 ± 0.021	D-Trp^4-C(4)	22.16	1.446 ± 0.063	Thr^6-γ
114.8	0.271 ± 0.021	D-Trp^4-C(7)	21.96	1.71 ± 0.069	Thr^8-γ
111.1	2.144 ± 0.410	D-Trp^4-C(3)			

As concerns the side chains of the aromatic systems directly attached to the macrocyclic backbone (Phe^3 and D-Trp^4), one can see from the similar n^*T_1 values of the backbone C^{α}-atoms, the side-chain C^{β}-atoms, and the aromatic C^{ζ}- (Phe^3) and C(7)-

atoms (D-Trp[4]), which both are essentially unaffected by ring flips that, if there is motion at all around the $C^{\alpha}-C^{\beta}$ bond (χ_1), it must take place on a time scale which is not relaxation active. The ring flips about the $C^{\beta}-C^{\gamma}$ bond (χ_2) in the Phe[3] system, however, seem to be strongly relaxation-active, indicating fast flips with a correlation time in the sub-nanosecond range. On the other hand, relaxation times of aromatic C-systems in D-Trp[4] where the relaxation dominating C–H bonds have a large inclination angle with respect to the $C^{\beta}-C^{\gamma}$ bond (χ_2) are rather similar to the $n*T_1$ values of the backbone C-atoms, indicating that fast ring flips seem unlikely for the D-Trp[4] system.

The two amino acid residues D-Phe[1] and Thr[8]-ol not integrated into the macrocycle thus forming the terminal parts of the structure ('substituents' on the macrocycle) are more mobile than the macrocyclic backbone. In spite of their differring size and geometry, the dynamics of these two residues, as concerns the C^{α}-atoms, however, seems to take place on a similar time-scale.

In conclusion, the new octreotide derivatives **2**–**5** are all less well-ordered than octreotide (**1**) itself, in accordance with CD, NMR, and bioactivity evidence. This increased flexibility and dynamics was expected for the open-chain compounds **2**, **4**, and **5**. The cause for the unforeseen increased flexibility of the octreotide skeleton by introduction of a CF_3 group at C(2) of the Trp 1*H*-indole ring, as concluded from NMR and CD measurements of **3**, is not clear to us, at all. The possible reasons *i*–*iii*, proposed above, for the reduced bioactivity of the CF_3-octreotide, must be extended by the peculiar dynamics-increasing effect of the CF_3 group.

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Experimental Part

Binding Assays. Radioligand-binding assays were performed as described by *Siehler et al.* [8]. Briefly, 150 µl of membranes of CCL39 cells expressing recombinant human somatostatin receptors were incubated with 50 µl of [^{125}I]SRIF 28, in binding assay buffer containing $MgCl_2$ (5 mM) and the protease inhibitor bacitracin (5 µg/ml), and either 50 µl binding assay buffer (total binding) or with 50 µl of various concentrations of test compounds. Nonspecific binding was determined in the presence of 50 µl SRIF 28 (1 µM). After 1 h at r.t., the incubation was terminated by vacuum filtration through glass fibre filters pre-soaked in 0.3% (*w*/*v*) polyethyleneimine. The filters were rinsed twice with 5 ml of ice cold 10 mM *Tris*·HCl buffer, pH 7.4, and dried. Bound radioactivity was measured in a γ-counter (80% counting efficiency). Data were analyzed by nonlinear regression curve fitting. The data are expressed as mean pK_d ($-\log$ M) values of at least three independent determinations perfomed in triplicates.

NMR Measurements. Samples were prepared at 2-mM concentration in CD_3OH for resonance assignments, measurements of *J*-couplings, and NOEs. One-dimensional spectra, 2QF-COSY, TOCSY, and ROESY (spin lock time τ_m 200 ms) were recorded at 23° using standard parameters on a *Bruker DRX600* spectrometer at 600-MHz ^{1}H frequency.

^{13}C Chemical shifts of 20 mM octreotide (**1**) in aq. soln. (100 mM CD_3COOH adjusted to pH 5) were determined at natural abundance by recording gradient-enhanced HSQC and HMBC spectra at 600 MHz. Longitudinal relaxation times (T_1) are based on inversion-recovery measurements of the same sample on a 400-MHz spectrometer at 300 K. For the determination of the overall motional correlation time of the macrocycle, an isotropic rotational tumbling model was used for fitting the longitudinal relaxation times of the C^{α}-atoms embedded in the macrocyclic structure.

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