Design of Selective Peptidomimetic Agonists for the Human Orphan Receptor BRS-3[#]

Dirk Weber,[†] Claudia Berger,[‡] Peter Eickelmann,[‡,§] Jochen Antel,[‡] and Horst Kessler*[,†]

Institut für Organische Chemie und Biochemie, Technische Universität München, Lichtenbergstrasse 4, 85747 Garching, Germany, and Solvay Pharmaceuticals GmbH, Hans-Böckler-Allee 20, 30173 Hannover, Germany

Received November 11, 2002

New tool substances may help to unravel the physiological role of the human orphan receptor BRS-3 and its possible use as a drug target for the treatment of obesity and cancer. In continuation of our work on BRS-3, the solid- and solution-phase synthesis of a library of low molecular weight peptidomimetic agonists based on the recently developed short peptide agonist **4** is described. Functional potencies of the compounds were determined measuring calcium mobilization in a fluorometric imaging plate reader (FLIPR) assay. Focusing on the N-terminus, the D-Phe-Gln moiety of **4** was modified in a combinatorial SAR-oriented medicinal chemistry approach. With the incorporation of N-arylated glycine and alanine building blocks azaglycine, piperazine, or piperidine and the synthesis of semicarbazides and semicarbazones, a number of highly potent and selective compounds with a reduced number of peptide bonds were obtained, which also should have enhanced metabolic stability.

Introduction

G-protein-coupled receptors (GPCRs) represent the largest and most important class of all drug targets.[1] Currently, worldwide more than 50% of all drugs are GPCR-based and their annual sales exceeded 30 billion U.S. dollars in 2001.[2] Owing to this proven track of being excellent drug targets, it is commonly assumed that orphan GPCRs, which emerged from genomic research, will offer a similar aptitude in the future.[3,4] About 1−3% of our genome encode approximately 1000 GPCRs, a number that was until recently predicted to be much larger.[5] Excluding sensory receptors, there are presently only about 150 orphan GPCRs.[4] Although the orphan receptor strategy and reverse pharmacology approach[6] have led to the identification of many natural ligands in recent years, the proportion of available orphan receptor sequences that have been "deorphanized" in this manner remains relatively small. New approaches such as the constitutively activating receptor technology (CART) are gaining increasing attention,[7] also because it is now a generally accepted concept that various ligands can bind to different binding sites to have different effects on GPCRs.[7−9] The identification of a natural ligand is an important step in the target validation of orphan GPCRs;[10] however, in the case of the orphan receptor bombesin receptor subtype 3 (BRS-3), a naturally occurring high-affinity ligand is still unknown. The BRS-3 receptor was assigned to the bombesin (Bn) receptor familiy because of its high sequence homology with the two mammalian bombesin receptors neuromedin B receptor (NMB-R) and gastrin-releasing peptide receptor (GRP-R).[11,12] Data obtained from the knock-out mouse model implicated BRS-3 with the regulation of energy balance, body weight, and blood pressure because mice lacking functional BRS-3 developed mild obesity, hypertension, and diabetes.[13] Second, it has been observed that the BRS-3 receptor is expressed on several human carcinoma cell lines.[11,14] In general, bombesin-like peptides (BLPs) are involved in the growth regulation of various cancers, especially small-cell lung cancer (SCLC) cell lines.[15] Expression of BRS-3 in mammals, as determined by distribution of BRS-3 mRNA, is species-dependent and limited[16,17] compared to the widely expressed NMB-R and GRP-R.[18] Very recently, BRS-3 was connected with the treatment of neurological disorders.[19] Despite the development of the high-affinity ligand [D-Tyr^6,β-Ala^{11},Phe^{13},Nle^{14}]Bn(6−14) (**1**) and its analogues,[20] which allowed receptor pharmacology studies,[20,21] more tool substances are needed to find out about the possible usefulness of BRS-3 as a drug target. Recently, we and others[22,23] performed structure−activity relationship studies of **1** mainly by utilizing its ability to mobilize intracellular calcium in BRS-3 transfected CHOGα-16 cells in a FLIPR assay. With the obtained information, we applied a strategy similar to the "peptoid" approach,[24] which was used to design peptidomimetic antagonists for several GPCRs[25−27] and subsequent C-terminal optimization to develop the selective short peptide agonist **4** (Table 1 and Figure 1) for the human BRS-3 receptor.[22] In continuation of this work, we describe the solid- and solution-phase synthesis of a library of low molecular weight peptidomimetic BRS-3 agonists based on the short peptide agonist **4**, which should have enhanced metabolic stability. Besides the incorporation of peptoid building blocks,[28−32] the synthesized BRS-3 agonists include a number of azapeptides,[33] a class of compounds that was successfully developed into drugs,[33−35] semicarbazides, semicarbazones, and compounds containing a piperazine/piperidine scaffold (Figure 1).

[#] Dedicated to Prof. George M. Sheldrick on the occasion of his 60th birthday.

* To whom correspondence should be addressed. Phone: ++49+89/289-13300. Fax: ++49+89/289-13210. E-mail: kessler@ch.tum.de.

[†] Technische Universität München.

[‡] Solvay Pharmaceuticals GmbH.

[§] Present address: Boehringer Ingelheim Pharma KG, Biberach an der Riss, Germany.

10.1021/jm0210921 CCC: $25.00
Published on Web 04/15/2003

Table 1. Functional Potencies from 4 to 30 Independent Concentration−Response Curves of [D-Phe^6,*β*-Ala^{11},Phe^{13},Nle^{14}]Bn(6−14) (**1**), Endogenous Ligands NMB (**2**) and GRP (**3**), and the Synthetic Agonist **4** in CHO Cells Transfected with the Human Bombesin Receptors NMB-R, GRP-R, and BRS-3[a]

		EC_{50} [nM]		
compd	peptide	NMB-R	GRP-R	BRS-3
1	[D-Phe^6,*β*-Ala^{11},Phe^{13},Nle^{14}]Bn(6−14)	310 (260−380)	84 (69−100)	151 (134−170)
2	NMB	120 (91−150)	41 (36−47)	4500 (4100−4900)
3	GRP	3200 (2400−4400)	34 (24−49)	6400 (6100−6700)
4	fQw-1-(2-phenylethyl)amide	inactive	inactive	710 (590−860)

[a] For further experimental details see Table 2 or Supporting Information.

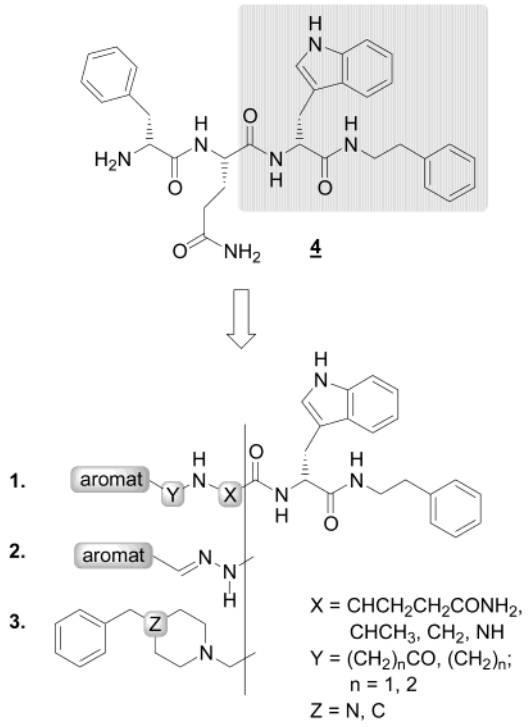


Figure 1. Development of peptidomimetic BRS-3 agonists from lead structure **4**. The marked region of structure **4** shows the previously optimized part of the molecule, which was left unchanged in this study.

Chemistry

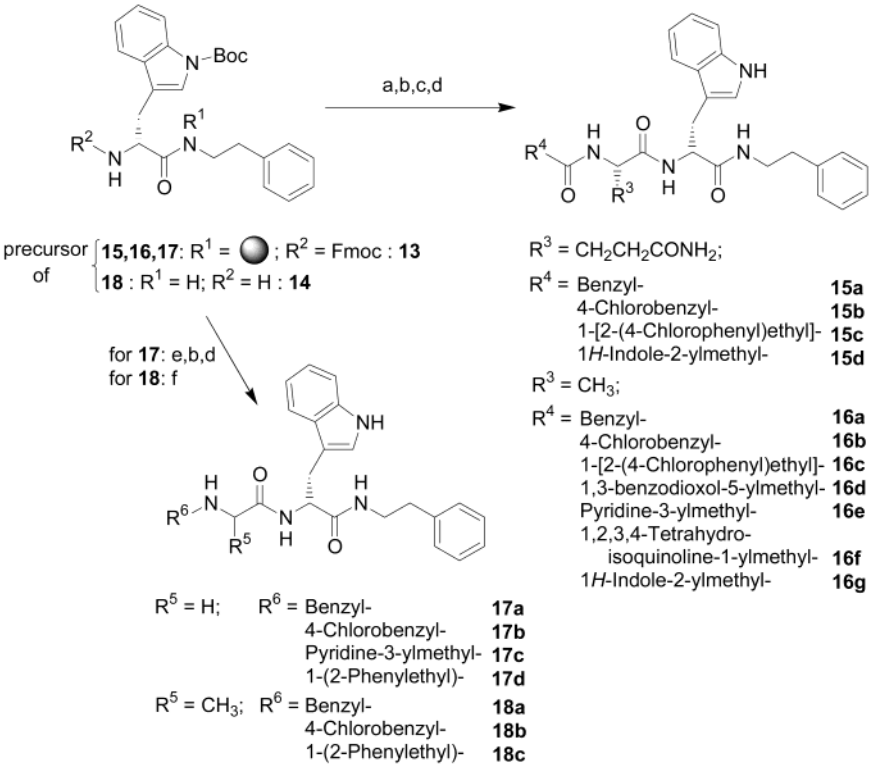
General Strategy. Recently, we described the design of the short peptide agonist H-D-Phe-Gln-D-Trp-1-(2-phenylethyl)amide (**4**).[22] Previous results suggested that the side chain of the Gln residue is not essential for functional activity; however, all three aromatic moieties are essential.[22] Furthermore, a stereochemical change of the N-terminal D-Phe did not affect functional potency.[22] Consequently, our work was directed toward the N-terminal part of the molecule while leaving the C-terminal part D-Trp-1-(2-phenylethyl)amide, which already has been optimized,[22] unchanged. We tried to modify the residues D-Phe-Gln step by step, to elaborate the structural features of this part of the agonist required for biological activity, to reduce it to a minimum fragment, and moreover to replace it with proteolytically more stable surrogates.

Removal of N-Terminal NH_2 and Side Chain of the Gln (Compounds 15a−d, 16a−g). First, to remove the N-terminal chirality and increase lipophilicity, peptides with removed N-terminal amino function (**15a**−**d**) were prepared (Scheme 1).[36] For synthesis, FMPE resin was reductively aminated and loaded with Fmoc-D-Trp-OH to yield **13** in a procedure similar to the one previously described.[37] Chain extension including the coupling of the N-terminal building blocks **7a**−**c**,**g**[38−41] was carried out using standard Fmoc protocols[42] with TBTU/HOBt/DIEA activation.[43] Peptides with an additional substitution of Gln by Ala (**16a**−**g**) were prepared similarly (Scheme 1).

Insertion of N-Substituted Glycine/Alanine (Compounds 17a−d and 18a−c). In the second step, the D-Phe-Gln unit was replaced with N-substituted glycines and alanines. All Fmoc-protected peptoid monomers, except **11c**, were synthesized in two steps. First, 2-(arylalkylamino)acetic acid ethyl esters (**8a**,**b**,**d**) and 2-(arylalkylamino)propionic acid ethyl esters (**10a**−**c**) were prepared in a modified, previously described procedure.[44] Then, the esters were hydrolyzed with aqueous NaOH and Fmoc was introduced without intermediate purification to yield building blocks **11a**,**b**,**d** and also **12a**−**c**, which were obtained as racemic mixtures. 2-{Pyridine-3-ylmethyl[(9*H*-fluorene-9-ylmethoxy)carbonyl]amino}acetic acid (**11c**) was synthesized in three steps[45] via the *tert*-butyl ester **8c**, which was Fmoc-protected to give **9**. Final cleavage of the *tert*-butyl group was carried out with TFA/TIPS (10:1) (v/v). Assembly of the peptoid−peptide hybrids **17a**−**d** was carried out on solid support[36] using TBTU/HOBt/DIEA activation[43] (Scheme 1). For synthesis of compounds **18a**−**c**, Fmoc-protected peptoid monomers **12a-c** were coupled to **14** in solution with HATU/HOAt/collidine activation[46] (Scheme 1). Cleavage of the Boc-protecting group from D-Trp turned out to be incomplete after treatment with TFA/TIPS (10:1) (v/v) at 0 °C. ESI mass spectral data indicated that the carbaminic acid was stable under these highly acidolytic water-free conditions.[47] Destruction of the carbaminic acid was achieved by treatment with DMSO/H_2O/HOAc (8:1:1) (v/v). Compounds **18a**−**c** were tested as racemic mixtures.

Synthesis of Azapeptides 21a−e. In the next step, Gln was replaced by azaglycine. Numerous routes have been described for the synthesis of azapeptides in solution,[33] but besides the synthesis of azatides,[48] only a few were reported for the preparation on solid support.[49−51] Other methods suffer from major drawbacks such as hydantoin formation and a slow reaction rate[50] or give rise to considerable amounts of byproducts such as the in situ activation of Fmoc-hydrazine with triphosgene.[51,52a] To overcome these drawbacks, we incorporated azaglycine in the solid-phase synthesis of azapeptides **21a**−**e** using an excess of freshly prepared 5-(9*H*-fluoren-9-ylmethoxy)-1,3,4-oxadiazol-2(3*H*)-one (**19**).[52] Therefore, **13** was Fmoc-deprotected and reacted with **19**, which was obtained from the activation of Fmoc-hydrazine with an excess of a solution of phosgene

Scheme 1[a]



[a] Reagents: (a) (only **13**, 20% piperidine/NMP), Fmoc-Gln(Trt)-OH or Fmoc-Ala-OH, TBTU/HOBt/DIEA, NMP; (b) 20% piperidine/NMP; (c) carboxylic acid building blocks **7a**−**g** (compounds **7a**−**e** were purchased from commercial sources), TBTU/HOBt/DIEA, NMP; (d) TFA/TIPS/H_2O (18:1:1); (e) {arylalkyl-[9*H*-fluoren-9-ylmethoxy)carbonyl]amino}acetic acid (**11a**−**d**), TBTU/HOBt/DIEA, NMP; (f) (1) 2-{arylalkyl-[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}propionic acid (**12a**−**c**), HATU/HOAt/collidine, DMF, (2) TFA/CH_2Cl_2/TIPS (10:10:1), (3) DMSO/HOAc/H_2O (8:1:1), (4) 20% piperidine/DMF (65−87%).

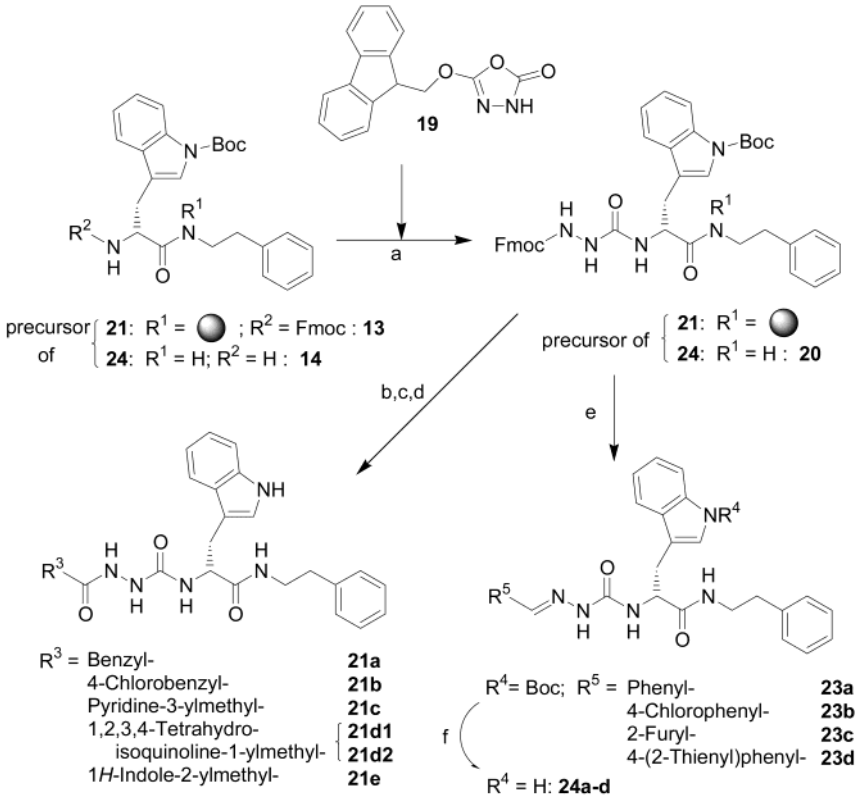
in toluene as previously described [52] (Scheme 2). Diastereomers **21d1** and **21d2** were separated by HPLC.

Synthesis of Semicarbazones 24a−d. Analogues of the azapeptides **21** lacking an N-terminal peptide bond and a C−N double bond were prepared in solution (Scheme 2). Acylation of amine **14** with freshly prepared oxadiazolone **19** in DMF at room temperature resulted in aza compound **20**, which was then Fmoc-deprotected and without intermediate purification reacted with aldehydes **22a**−**d** in THF at room temperature. Imine bond formation was slow probably because of residual basicity; however, since the desired compounds **23a**−**d** were obtained in acceptable yields, optimization of this process was not pursued. Aldehydes **22a**−**c** were purchased from commercial sources, whereas 4-(2-thienyl)-benzaldehyde (**22d**) was synthesized from 4-bromobenzaldehyde via Suzuki coupling.[53] Final Boc deprotection under first strong and then mild acidic conditions gave semicarbazones **24a**−**d**.

Synthesis of Semicarbazides 28a−c. The synthesis of analogues of azapeptides **21** lacking an N-terminal peptide bond afforded the preparation of suitably protected azaamino acid constituents (Scheme 3). Therefore, Boc-hydrazine was reacted to the Boc-protected hydrazones **25a**−**c** with the appropriate aldehydes in THF.[54] Contrary to previous reports, no conversion was observed when reduction of **25a** was attempted through hydrogenation over 5% Pd/C in THF as a solvent at room temperature,[54] both at atmospheric pressure or at 50 atm. However, reduction with sodium cyanoborohydride[55] afforded the desired Boc-protected arylalkylhydrazines **26a**−**c** in good yields. It was further planned to convert these building blocks into acylating agents selectively at the unsubstituted nitrogen atom of the hydrazine after Boc deprotection in the following steps. Because the site of acylation is dependent on the nature of the substituted hydrazine and the activation reagent and therefore was difficult to predict,[48,52a,54] Fmoc was introduced at the arylalkylated nitrogen to give building blocks **27a**−**c**. Preliminary attempts to cleave the Boc protecting group were carried out by careful treatment with TFA/CH_2Cl_2/TIPS/H_2O (4:14:1:1) (v/v) at 0 °C and showed approximately 70% deprotection after 1 h as monitored by TLC and HPLC−MS. However, we observed unexpected degradation of the dried deprotected species at room temperature within 24 h. Surprisingly, attempts to remove the Boc with 4 N HCl in dioxane as a solvent at room temperature[35a] failed because of insufficiently low reaction rates. Therefore, it was decided to cleave the Boc group of **27a**−**c** under more drastic conditions than aforementioned with TFA/CH_2Cl_2/TIPS (20:20:1) (v/v). Without further workup, activated carbazic acid esters were immediately generated using bis(pentafluorophenyl)carbonate and without isolation instantly reacted with the amine **14**.[48,56] This method worked very well and was found to be superior to the use of a solution of phosgene in toluene,[52a] which seems to require more subtle adjustment of the reaction conditions. Direct cleavage of Boc and Fmoc without intermediate purification finally furnished the desired semicarbazides **28a**−**c** (Scheme 3).

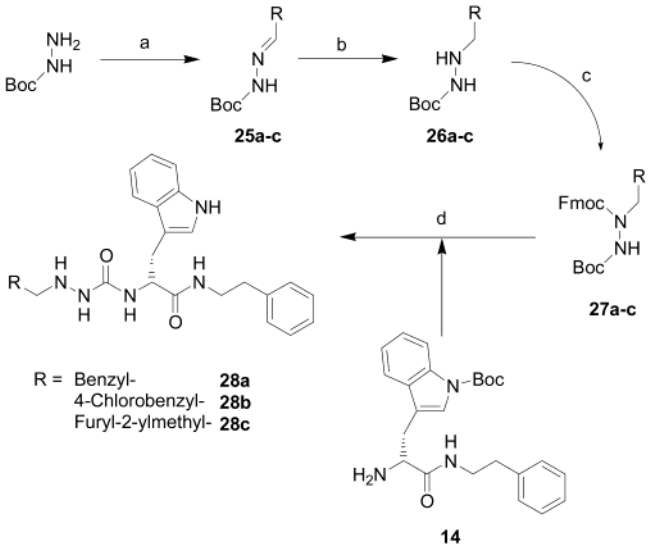
Scaffolds Containing Piperidine/Piperazine (Compounds 35 and 36). Building blocks (4-benzylpiperazin-1-yl)acetic acid (**31**) and its piperidine ana-

Scheme 2[a]



[a] Reagents: (a) 5-(9*H*-fluoren-9-ylmethoxy)-1,3,4-oxadiazol-2(3*H*)-one (**19**), CH_2Cl_2 (68%, for R^1 = H); (b) 20% piperidine/NMP; (c) carboxylic acid building blocks **7a,b,e**−**g** (compounds **7a,b,e** were purchased from commercial sources), TBTU/ HOBt/DIEA, NMP; (d) TFA, TIPS, H_2O (18:1:1); (e) (1) 20% piperidine/DMF, (2) arylaldehydes **22a**−**d** (compounds **22a**−**c** were purchased from commercial sources), THF (47−83%); (f) (1) TFA/CH_2Cl_2/TIPS (30:30:1), (2) DMSO/HOAc/H_2O (8:1:1) (56−63%).

Scheme 3[a]



[a] Reagents: (a) arylaldehydes **22a**−**c** (compounds were purchased from commercial sources), THF (82−96%); (b) (1) $NaCNBH_3$, THF, AcOH, (2) $NaHCO_3$, (3) 1 N NaOH, MeOH (71−93%); (c) Fmoc-Cl, $NaHCO_3$, dioxane (72−98%); (d) (1) TFA/CH_2Cl_2/TIPS (20:20:1), (2) *tert*-butyl-3-{(2*R*)-2-amino-2-[1-(2-phenylethyl)carbamoyl]ethyl}-1*H*-1-indolecarboxylate (**14**), bis(pentafluorophenyl)-carbonate, DMAP, CH_2Cl_2, (3) TFA/TIPS (40:1), CH_2Cl_2, (4) 20% piperidine/DMF (9−21%).

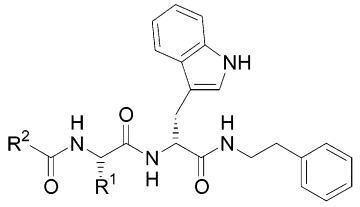
logue **32** were prepared in two steps similar to that described for **8a**−**d** followed by saponification. After coupling to amine **14** using TBTU/HOBt/DIEA activation, Boc deprotection carried out as described above for the synthesis of **18a**−**c** yielded compounds **35** and **36**.

Results and Discussion

The synthesized library is derived from the structure of the recently published BRS-3 agonist **4**, which was obtained from the C-terminal optimization of the lead structure H-D-Phe-Gln-D-Trp-Phe-NH_2.[22] Functional activity of the compounds was assessed in a FLIPR assay. Therefore, their ability to increase the level of intracellular calcium in CHO cells transfected with the human bombesin receptors NMB-R, GRP-R, and BRS-3 was measured and referenced to the peptide agonist [D-Phe^6,β-Ala^{11},Phe^{13},Nle^{14}]Bn(6−14) (**1**) for BRS-3, endogenous ligands NMB (**2**) for NMB-R, and GRP (**3**) for GRP-R. Further experimental details of this assay have been published earlier[22] and are given in the Supporting Information.

One of the most reliable methods in medicinal chemistry to improve in vitro activity is to incorporate properly positioned lipophilic groups or, as could be suggested for our case, to remove "unnecessary" hydrophilic groups. Although C-terminal optimization of the aforementioned lead structure, which resulted in the removal of the amide function, was sufficient to increase functional potency about 15-fold to generate agonist **4** with an EC_{50} of 710 nM,[22] we hoped that further improvement of functional activity could be achieved by exploiting the N-terminal SAR of this molecule. On the other hand, we were aiming at oral availability, which requires solubility. Therefore, regarding Lipinski's "rule of five",[57] lipophilicity, which can be estimated and

Table 2. Functional Potencies from 4 to 11 Independent Concentration−Response Curves of Modified Analogues of **4** with N-Terminally Deleted Amino Function (**15a**−**d**)[a]



			BRS-3		
compd	R^1	R^2	EC_{50} [nM]	E_{max}	cLogP
15a	$(CH_2)_2CONH_2$	benzyl-	1200 (680−2100)	38 ± 0.8	1.81
15b	$(CH_2)_2CONH_2$	4-chlorobenzyl-	1400 (990−2000)	62 ± 3.8	2.52
15c	$(CH_2)_2CONH_2$	1-[2-(4-chlorophenyl)ethyl]-	230 (170−320)	68 ± 5.9	2.91
15d	$(CH_2)_2CONH_2$	1*H*-indole-2-ylmethyl-	57 (26−120)	82 ± 4.7	1.8
16a	CH_3	benzyl-	2.1 (1.2−3.9)	71 ± 4.7	3.62
16b	CH_3	4-chlorobenzyl-	140 (75−270)	75 ± 8.5	4.33
16c	CH_3	1-[2-(4-chlorophenyl)ethyl]-	6400 (5800−7200)	30 ± 2.2	4.72
16d	CH_3	1,3-benzodioxol-5-ylmethyl-	7.8 (2.8−22)	75 ± 7.0	3.18
16e	CH_3	pyridine-3-ylmethyl-	32 (23−44)	74 ± 1.6	2.12
16f	CH_3	1,2,3,4-tetrahydroiso- quinoline-1-ylmethyl-	310 (250−390)	69 ± 8.1	3.83
16g	CH_3	1*H*-indole-2-ylmethyl-	79 (50−120)	69 ± 1.2	3.61

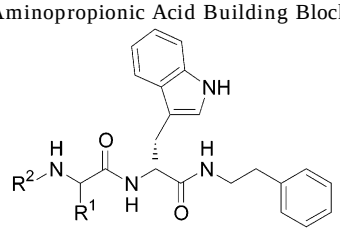
[a] Additionally, in compounds **16a**−**g**, Gln was replaced by Ala. Functional potencies of the peptides were determined in a previously described FLIPR assay[22] (see also Supporting Information) and are given as EC_{50} ± SEM.[63] E_{max} represents the Ca^{2+} signal at 16 μM in percent of maximal response of **1**. All compounds tested were inactive on the NMB-R and GRP-R except for **15a**, with EC_{50} = 15.0 μM (13.9−16.3) on NMB-R and EC_{50} = 19.1 μM (15.5−23.5) on GRP-R, and for **15b**, with EC_{50} = 18.5 μM (16.6−20.7) on NMB-R and EC_{50} = 12.2 μM (11.1−13.3) on GRP-R. For further experimental details, see Supporting Information.

expressed by the *n*-octanol/water partition coefficient cLogP value, should not exceed the magnitude of five. First, we deleted the N-terminal aminofunction of the agonist **4** (Scheme 1, Figure 1). As a result of this, the cLogP value increased from 1.3 to 1.81 (Table 2), compared to the augmentation of 0.1 of H-D-Phe-Gln-D-Trp-Phe-NH_2 to 1.3 of agonist **4**. As shown in Table 2 (compounds **15a**−**d**), the effect of this increase of lipophilicity on functional potency at BRS-3 is low except for compound **15d,** with an increase of about 12-fold. Compounds **15a** and **15b** showed functional potencies on the NMR-R and GRP-R in the micromolar range.

In the second step, a series of compounds, where in addition to the deleted amino function Gln was replaced by Ala and the N-terminal aromatic moiety was differently substituted or replaced by heteroaromatic moieties, were investigated (Table 2). Although the synthetic effort to get from lead structure H-D-Phe-Gln-D-Trp-Phe-NH_2 to compounds **16** was relatively small, the impact on improvement of functional potency was enormous. With compounds **16a** and **16d**, potencies in the nanomolar range were obtained. Furthermore, all compounds showed excellent selectivity for BRS-3. As seen in the preceding series, for compounds **15a**−**d**, indole-2-yl/3-pyridyl was clearly favored as the N-terminal aromatic moiety over 4-chlorophenyl. On the other hand, these alterations probably do not represent much progress concerning proteolytic stability, since these compounds still comprised three peptide bonds. Therefore, efforts were directed toward a reduction of the peptidic character of the compounds.

In compounds **17a**−**d** the N-terminal peptide bond was removed by incorporation of N-substituted glycine, and in compounds **18a**−**c** it was removed by N-substituted alanine (Table 3). Thus, it is assumed that these modifications introduce a greater flexibility to the backbone accompanied by enhanced metabolic stability.[28−31] Deletion of the carbonylfunction caused cLogP values to slightly increase by about 0.2−0.7.

Table 3. Functional Potencies from 7 to 11 Independent Concentration−Response Curves of Modified Analogues of **4** Containing Peptoid Monomer Building Blocks (**17a**−**d**) and N-Arylated 2-Aminopropionic Acid Building Blocks (**18a**−**c**)[a]



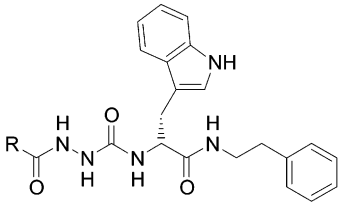
			BRS-3		
compd	R^1	R^2	EC_{50} [nM]	E_{max}	cLogP
17a	H	benzyl-	21 (14−31)	87 ± 6.4	3.86
17b	H	4-chlorobenzyl-	2.9 (1.2−6.9)	81 ± 1.7	4.58
17c	H	pyridine-3-ylmethyl-	21 (12−36)	82 ± 8.4	2.37
17d	H	1-(2-phenylethyl)-	17 (8.9−31)	92 ± 16.0	4.08
18a	CH_3	benzyl-	46 (34−64)	86 ± 12.8	4.17
18b	CH_3	4-chlorobenzyl-	4.0 (2.3−7.3)	83 ± 12.5	4.89
18c	CH_3	1-(2-phenylethyl)-	25 (19−34)	82 ± 9.6	4.39

[a] All compounds tested were inactive on the NMB-R and GRP-R. For further experimental details, see Table 2 or Supporting Information. E_{max} represents the Ca^{2+} signal at 16 μM in percent of maximal response of **1**.

Here, in contrast to the preceding series, the 4-chloro-substituted compounds **17b** and **18b** showed the highest functional potency with EC_{50} values around 3−4 nM. The explanation for this shift of high functional potency could probably be that a high lipophilicity is favorable but only at the correct distance from the tryptophan. This distance is obviously too large for compounds **15** and **16**. Moreover, a basic functionality located more closely to the tryptophan seems to enhance functional activity. The overall series of compounds **17** and **18** demonstrated that the carbonyl function or the planarity caused by the peptide bond was not contributing to the binding mode of the compound to the receptor and therefore could be omitted.

Moreover, we attempted to exchange $C^{\alpha}H$ by N^{α}, which leads to a loss of one chiral center and to a

Table 4. Functional Potencies of Azapeptides **21a**−**e** from 7 to 14 Independent Concentration−Response Curves[a]



compd	R	EC_{50} [nM]	E_{max}	cLogP
21a	benzyl-	3.1 (2.1−4.6)	86 ± 9.5	3.61
21b	4-chlorobenzyl-	0.19 (0.06−0.58)	89 ± 13.5	4.33
21c	pyridine-3-ylmethyl-	1.4 (0.7−2.5)	102 ± 26.1	2.12
21d1	1,2,3,4-tetrahydroiso-	9.3 (7.1−12.3)	95 ± 15.0	3.83
21d2	quinoline-1-ylmethyl-	19 (15−25)	91 ± 15.1	3.83
21e	1*H*-indole-2-ylmethyl-	2.2 (0.8−5.9)	84 ± 4.3	3.6

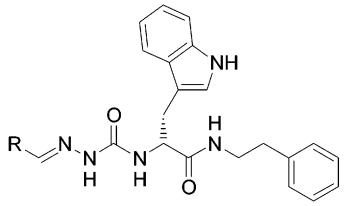
[a] All compounds tested were inactive on the NMB-R and GRP-R. For further experimental details, see Table 2 or Supporting Information. E_{max} represents the Ca^{2+} signal at 16 μM in percent of maximal response of **1**.

conformational change.[58] Nevertheless, there are many examples where amino acids have been successfully replaced by their corresponding azaamino acids with retention of biological activity accompanied by an increase of metabolic stability.[35,49,52] Ab initio calculations showed that diacylhydrazine has relatively easy access to different conformations; however, its flexibility was substantially constrained by N-methylation.[58a] It is generally believed that a critical amount of rigidity is a prerequisite for high activity and selectivity.[59] On the other hand, scaffolds bearing azaglycine as their core and structurally dominating unit demonstrated possession of these features.[52] Obviously the same proves to be true in this case. All azapeptides show excellent activities and selectivities, especially compound **21b** with an EC_{50} value in the subnanomolar range (Table 4). Although the distance to the lipophilic 4-chloro-substituted moiety seems to be larger than in compounds **17** and **18** with the removed peptide bond, it is feasible that the azaglycine induces a bentlike structure. Therefore, the actual length of the compound in its bioactive conformation could resemble that of the peptoid−peptide hybrids.

In the next step, we investigated the necessity of the N-terminal peptide bond in the aza compounds. Functional potencies of the semicarbazides **28a**−**c** were lower than those of the corresponding azapeptides **21a**−**e** but, as expected, were still excellent (Table 6). As for the peptoid−peptide hybrids, removal of the carbonyl group caused the cLogP values to increase about 0.3 order of magnitude, and the 4-chloro-substituted compound **28b** showed the highest activity. Insertion of a C−N double bond (semicarbazones **24a**−**d**, Table 5) caused another increase of the cLogP value of about 0.3. This increase was tolerated for 2-furyl as the aromatic moiety but not for too lipophilic residues. In the latter, the drop of functional activity correlated with increasing cLogP value, and because of the high activity of **24c**, it probably was not conformationally related. Taken together, the results confirm those obtained from the peptoid−peptide hybrids **17** and **18**, namely, that the N-terminal peptide bond is not involved in receptor activation.

Finally compounds with incorporated piperazine (**35**) and piperidine (**36**) have been tested (Table 7). Here,

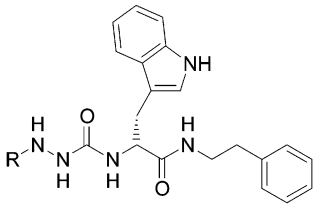
Table 5. Functional Potencies of Semicarbazones **24a**−**d** from 6 Independent Concentration−Response Curves[a]



		BRS-3		
compd	R	EC_{50} [nM]	E_{max}	cLogP
24a	phenyl-	240 (110−490)	89 ± 0.4	4.21
24b	4-chlorophenyl-	2700 (1500−5200)	78 ± 0.1	4.92
24c	2-furyl-	1.5 (0.7−3.2)	94 ± 2.6	3.38
24d	4-(2-thienyl)phenyl-	7900 (3800−16200)	58 ± 7.3	5.98

[a] All compounds tested were inactive on the NMB−R and GRP−R. For further experimental details see Table 2 or Supporting Information. E_{max} represents the Ca^{2+} signal at 16 μM in percent of maximal response of **1**.

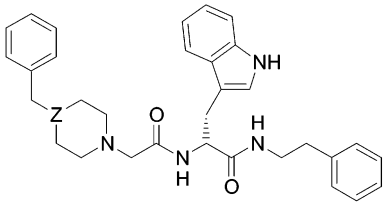
Table 6. Functional Potencies of Semicarbazides **28a**−**c** from 6 Independent Concentration−Response Curves[a]



		BRS-3		
compd	R	EC_{50} [nM]	E_{max}	cLogP
28a	benzyl-	15 (8.5−25)	81 ± 0.4	3.9
28b	4-chlorobenzyl-	6.0 (2.3−16)	83 ± 5.3	4.62
28c	furyl-2-ylmethyl-	24 (18−33)	96 ± 14.3	3.08

[a] All compounds tested were inactive on the NMB-R and GRP-R. For further experimental details, see Table 2 or Supporting Information. E_{max} represents the Ca^{2+} signal at 16 μM in percent of maximal response of **1**.

Table 7. Functional Potencies of Compounds **35** and **36** from 6 Independent Concentration−Response Curves[a]



		BRS-3		
compd	Z	EC_{50} [nM]	E_{max}	cLogP
35	N	70 (57−85)	90 ± 1.2	4.98
36	C	30 (4.9−190)	84 ± 5.3	5.85

[a] Compounds tested were inactive on the NMB-R and GRP-R. For further experimental details, see Table 2 or Supporting Information. E_{max} represents the Ca^{2+} signal at 16 μM in percent of maximal response of **1**.

high activities and selectivities were obtained too; however, the cLogP value clearly exceeded the upper limit of 5.

Additional FLIPR experiments were carried out to assess the selectivity of the new agonists to the BRS-3 receptor over the NMB and the GRP receptors. The ability of compounds **15**−**18**, **21**, **24**, **28**, **35**, and **36** to inhibit receptor activation of the agonists NMB at NMB-R and GRP at GRP-R was investigated.

As a reference, the known antagonists [D-Phe^{12}]Bn, [D-Phe^{6},Leu^{13},*p*-chloro-Phe^{14}]Bn(6−14), and [D-Phe^{6},Leu-NHEt^{13},desMet^{14}]Bn(6−14) were used.[60−62] First, a solution of the new agonists and reference antagonists at eight different concentrations were added to CHO cells transfected with the NMB or GRP receptor followed by a solution of the agonist, and calcium mobilization was permanently measured. In all measurements, no reduction of the calcium emission induced by the presence of the aforementioned compounds could be observed. Thus, we conclude that the investigated compounds are not antagonists of the NMB-R and GRP-R.

At present, we are unable to explain the selectivity of the compounds for the BRS-3 receptor.

Conclusions

In summary, we have performed detailed N-terminal structure−activity studies on the known BRS-3 agonist H-D-Phe-Gln-D-Trp-1-(2-phenylethyl)amide (**4**). On the basis of previous results, which suggested that the side chain of Gln is not essential for functional potency, we developed a library of peptidomimetics with a conserved C-terminal D-Trp-1-(2-phenylethyl)amide moiety and structural variations on the H-D-Phe-Gln unit. It was demonstrated that the N-terminal increase of lipophilicity by simple deletion of the amino function combined with removal of the Gln side chain furnished selective BRS-3 agonists in the nanomolar range. Furthermore, substitution of the H-D-Phe-Gln unit by peptoid monomers showed that the N-terminal peptide bond is not required for receptor activation. Gln can further be replaced by azaglycine, leading to compounds with subnanomolar activities (**21b**: EC_{50} = 0.19 nM). For azapeptides, too, a removal of the N-terminal peptide bond is possible, as realized in the semicarbazides **28a**−**c**. However, as shown by the semicarbazones **24a**−**d**, a too lipophilic N-terminus does not seem to be tolerated. The finding that piperidine or piperazine can also be incorporated suggests that a large number of different spacers are able to mimic the function of the former Gln at this position. From the analysis of the substitution and distance pattern for the different series of compounds, it seems very likely that a lipophilicity placed at a certain distance from the tryptophan combined with a basic functionality located between them is favorable for functional potency. A further FLIPR experiment in which the ability of the described peptidomimetics to inhibit activation of the NMB-R and GRP-R by their natural ligands was investigated, showed that these compounds are not antagonists of the NMB-R and GRP-R. This work therefore describes the development of selective tool substances for BRS-3 with improved pharmacokinetic properties, which may prove to be helpful in the understanding of the physiological role of this orphan receptor.

Experimental Section

General. Fmoc-protected amino acids and 2-(4-formyl-3-methoxyphenoxy)ethylpolystyrene (FMPE) resin was purchased from Novabiochem (Darmstadt, Germany). [D-Phe^{6},*β*-Ala^{11},Phe^{13},Nle^{14}]Bn(6−14) (**1**) was purchased from Polypeptide Laboratories (Wolfenbüttel, Germany). NMB (**2**), GRP (**3**), [D-Phe^{12}]Bn, [D-Phe^{6},Leu^{13},*p*-chloro-Phe^{14}]Bn(6−14), and [D-Phe^{6},Leu-NHEt,^{13}desMet^{14}]Bn(6−14) were obtained from Bachem (Heidelberg, Germany), HATU and HOAt from Perseptive Biosystems (Hamburg, Germany), and TBTU and HOBt from Quantum Appligene (Heidelberg, Germany). TFA was obtained from Solvay (Hannover, Germany). Bis(pentafluorophenyl)carbonate was purchased from Senn Chemicals (Dielsdorf, Switzerland) and 3-(4-chlorophenyl)propionic acid from CPS Chemie + Service (Düren, Germany). All other chemicals were purchased from Aldrich (Deisenhofen, Germany), Merck (Darmstadt, Germany), Lancaster (Mühlheim, Germany), or Fluka (Seelze, Germany). Solid-phase synthesis was carried out in PE syringes (2, 5, or 20 mL) from Becton-Dickinson (Fraga, Spain) or Braun (Melsungen, Germany) equipped with PE frits from Roland Vetter Laborbedarf (Ammerbuch, Germany). Air- or moisture-sensitive reactions were carried out in dry glassware and under argon (99.996%) atmosphere. There was no attempt to optimize yields. Thin-layer chromatography was performed on TLC aluminum sheets covered with silica gel 60 F_{254} from Merck (Darmstadt). For flash chromatography, silica gel 60 (230−400 mesh ASTM, Korngrösse 0.040−0.063 mm) from Merck (Darmstadt) was used. Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, DMSO-d_6, or ACN-d_3 as solvent on Bruker AC250 and DMX500 instruments at 300 K. TMS or the solvent peak was used as internal reference. Chemical shifts (δ) are reported in parts per million (ppm), and coupling constants (J values) are given in Hertz (Hz). Purification of compounds **15**−**18**, **21**, **23d**, **24**, **28**, **35**, and **36**, analyses, and analytical purity determination were carried out on RP-HPLC instruments from Amersham Pharmacia Biotech or Beckman (System Gold), equipped with Omicron YMC columns (preparative, ODS-A C_{18}, 250 mm × 30 mm, 10 μm, flow rate of 25 mL/min; semipreparative, ODS-A C_{18}, 250 mm × 20 mm, 5 μm or 10 μm, flow rate of 8 mL/min; analytical, ODS-A C_{18}, 250 mm × 4.6 mm, 5 μm, flow rate of 1 mL/min) or a Macherey-Nagel column (preparative, Nucleosil C_{18}, 250 mm × 40 mm, 7 μm, flow rate of 25 mL/min). Compounds were eluted with linear gradients (30 min) of acetonitrile in water and 0.1% (v/v) trifluoroacetic acid, detected at 220 nm, and lyophilized after purification. ESI mass spectra were obtained on a LCQ Finnigan mass spectrometer, and GC mass spectra were obtained on a Finnigan MAT 8200 instrument. High-resolution mass spectra were recorded on a Finnigan spectrometer using the electrospray ionization time-of-flight (ESI-TOF) technique. cLogP values were calculated using Sybyl 6.8 from Tripos Inc.

Synthesis of *N*1-(2-Phenylethyl)-(2*R*)-2-[(9*H*-fluoren-9-ylmethoxy)carboxamido]-3-[1-(*tert*-butoxycarbonyl)-3-indolyl]propanamide on FMPE Resin (13).[6] FMPE resin (2.378 g, theoretical 0.5 mmol/g, 1.19 mmol) was preswollen in DCE (24 mL) for 10 min. Then TMOF (12 mL), 1-(2-phenylethyl)amine (11.89 mmol), and $NaBH(OAc)_3$ (2.52 g, 11.89 mmol) were added and the mixture was submitted to an ultrasonicator for 10 min and then shaken overnight at room temperature. After the mixture was washed with CH_2Cl_2 (3 × 20 mL) and NMP (3 × 20 mL), Fmoc-D-Trp-OH (2.38 mmol, 2 equiv) was coupled to the resin using [HATU (2 equiv)]/[HOAt (2 equiv)]/[collidine (20 equiv)] activation in NMP (20 mL) for 5 h. Washing with NMP (3 × 20 mL) was followed by a double-coupling step overnight. Finally the resin was washed with NMP (3 × 20 mL) and CH_2Cl_2 (3 × 20 mL) and dried thoroughly in vacuo to give resin-bound *N*1-(2-phenylethyl)-(2*R*)-2-[(9*H*-fluoren-9-ylmethoxy)carboxamido]-3-[1-(*tert*-butoxycarbonyl)-3-indolyl]propanamide (**13**).

***N*1-{(1*R*)-2-(1*H*-3-Indolyl)-1-[1-(2-phenylethyl)carbamoyl]ethyl}-(2*S*)-2-(benzylcarboxamido)pentanediamide (15a).** Resin-bound **13** (124 mg, 0.315 mmol/g, 0.039 mmol) was treated two times with 20% piperidine (v/v) in NMP (5 mL) for 15 min and washed after each time with NMP (5 × 5 mL). Then Fmoc-Gln(Trt)-OH (48 mg, 0.078 mmol) dissolved in NMP (2 mL) was coupled two times to the resin for 45 min using [TBTU (2 equiv)]/[HOBt (2 equiv)]/[DIEA (5.7 equiv)] activation. Coupling steps were followed by a washing step with NMP (5 × 5 mL). After repeated Fmoc removal and coupling of **7a** (10.6 mg, 0.078 mmol), the resin was washed

with CH_2Cl_2 (3 × 5 mL) for 30 min and the compound was deprotected and cleaved from the resin with [TFA (18 equiv)]/[TIPS (1 equiv)]/[H_2O (1 equiv)] (3 × 2 mL, 30 min each). The combined filtrates were reduced in vacuo. RP-HPLC purification yielded 11.1 mg of **15a**: colorless powder; mp 226−227 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.77 (s, 1H), 8.30 (d, 1H, J = 7.1 Hz), 8.18 (d, 1H, J = 8.3 Hz), 7.95 (t, 1H, J = 5.0 Hz), 7.55 (d, 1H, J = 8.0 Hz), 7.31 (d, 1H, J = 8.1 Hz), 7.23−7.27 (m, 6H), 7.16−7.19 (m, 2H), 7.11 (d, 2H, J = 7.5 Hz), 7.07 (s, 1H), 7.05 (t, 1H, J = 7.7 Hz), 6.97 (t, 1H, J = 7.2 Hz), 6.72 (bs, 2H), 4.39−4.44 (m, 1H), 4.15−4.21 (m, 1H), 3.47 (d, 2H, J = 6.0 Hz), 3.16−3.21 (m, 2H), 3.11 (dd, 1H, J = 4.9/14.5 Hz), 2.86 (dd, 1H, J = 8.9/14.7 Hz), 2.57 (t, 2H, J = 8.0 Hz), 1.92−2.00 (m, 1H), 1.85−1.92 (m, 1H), 1.69−1.77 (m, 1H), 1.60−1.69 (m, 1H); MS (ESI) m/z 554.2 [M + H]$^+$.

***N*1-{(1*R*)-2-(1*H*-3-Indolyl)-1-[1-(2-phenylethyl)carbamoyl]ethyl}-(2*S*)-2-[(4-chlorobenzyl)carboxamido]pentanediamide (15b).** Preparation as described for **15a**, using 128 mg of **13** (0.357 mmol/g, 0.046 mmol) and **7b** (15.6 mg, 0.091 mmol), yielded 6.3 mg of **15b**: colorless powder; mp 219−220 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.77 (s, 1H), 8.33 (d, 1H, J = 7.5 Hz), 8.19 (d, 1H, J = 9.0 Hz), 7.94 (t, 1H, J = 5.0 Hz), 7.55 (d, 1H, J = 7.5 Hz), 7.29−7.32 (m, 3H), 7.23−7.26 (m, 4H), 7.17 (t, 1H, J = 7.3 Hz), 7.11 (d, 2H, J = 7.6 Hz), 7.08 (s, 1H), 7.05 (t, 1H, J = 7.7 Hz), 6.97 (t, 1H, J = 8.0 Hz), 6.73 (bs, 2H), 4.39−4.44 (m, 1H), 4.16−4.20 (m, 1H), 3.47 (d, 2H, J = 6.0 Hz), 3.16−3.21 (m, 2H), 3.10 (dd, 1H, J = 5.0/14.3 Hz), 2.86 (dd, 1H, J = 8.5/14.8 Hz), 2.56 (t, 2H, J = 7.0 Hz), 1.92−1.99 (m, 1H), 1.84−1.92 (m, 1H), 1.68−1.76 (m, 1H), 1.60−1.68 (m, 1H); MS (ESI) m/z 588.2 [M + H]$^+$.

***N*1-{(1*R*)-2-(1*H*-3-Indolyl)-1-[1-(2-phenylethyl)carbamoyl]ethyl}-(2*S*)-2-[1-(2-(4-chlorophenyl)ethyl)carboxamido]pentanediamide (15c).** Preparation as described for **15a**, using 100 mg of **13** (0.354 mmol/g, 0.035 mmol) and **7c** (13.1 mg, 0.07 mmol), yielded 16.8 mg of **15c**: colorless powder; mp 213-215 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (s, 1H), 8.14 (d, 1H, J = 8.2 Hz), 8.08 (d, 1H, J = 7.4 Hz), 7.99 (t, 1H, J = 5.8 Hz), 7.55 (d, 1H, J = 8.5 Hz), 7.28−7.32 (m, 3H), 7.26 (t, 2H, J = 7.4 Hz), 7.21 (d, 2H, J = 8.0 Hz), 7.18 (t, 1H, J = 7.3 Hz), 7.15 (d, 2H, J = 7.6 Hz), 7.08 (s, 1H), 7.05 (t, 1H, J = 7.7 Hz), 6.97 (t, 1H, J = 7.7 Hz), 6.72 (bs, 2H), 4.39−4.45 (m, 1H), 4.15−4.21 (m, 1H), 3.18−3.29 (m, 2H), 3.11 (dd, 1H, J = 4.7/14.4 Hz), 2.88 (dd, 1H, J = 8.8/14.8 Hz), 2.79 (t, 2H, J = 7.7 Hz), 2.64 (t, 2H, J = 7.5 Hz), 2.34−2.46 (m, 2H), 1.82−1.98 (m, 2H), 1.65−1.74 (m, 1H), 1.55−1.65 (m, 1H); MS (ESI) m/z 602.2 [M + H]$^+$.

***N*1-{(1*R*)-2-(1*H*-3-Indolyl)-1-[1-(2-phenylethyl)carbamoyl]ethyl}-(2*S*)-2-[(1*H*-2-indolylmethyl)carboxamido]pentanediamide (15d).** Preparation as described for **15a**, using 100 mg of **13** (0.354 mmol/g, 0.035 mmol) and **7g** (12.3 mg, 0.07 mmol), yielded 6.6 mg of **15d**: colorless powder; mp 195−201 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.86 (s, 1H), 10.76 (s, 1H), 8.24 (d, 1H, J = 7.5 Hz), 8.21 (d, 1H, J = 8.2 Hz), 7.96 (t, 1H, J = 5.6 Hz), 7.55 (d, 1H, J = 7.8 Hz), 7.39 (d, 1H, J = 7.8 Hz), 7.29 (t, 2H, J = 8.2 Hz), 7.22 (t, 2H, J = 7.4 Hz), 7.14 (t, 1H, J = 7.4 Hz), 7.06−7.08 (m, 3H), 7.04 (t, 1H, J = 8.0 Hz), 6.95−6.99 (m, 2H), 6.90 (t, 1H, J = 7.4 Hz), 6.72 (bs, 2H), 6.20 (s, 1H), 4.41−4.45 (m, 1H), 4.23−4.27 (m, 1H), 3.63 (d, 2H, J = 3.4 Hz), 3.13−3.24 (m, 2H), 3.09 (dd, 1H, J = 5.4/14.5 Hz), 2.86 (dd, 1H, J = 8.7/14.5 Hz), 2.56 (t, 2H, J = 7.6 Hz), 1.87−2.01 (m, 2H), 1.71−1.78 (m, 1H), 1.61−1.69 (m, 1H); MS (ESI) m/z 593.2 (100) [M + H]$^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[(1*S*)-1-(benzylcarboxamido)ethyl]carboxamido}-3-(1*H*-3-indolyl)propanamide (16a).** Preparation as described for **15a**, using 100 mg of **13** (0.354 mmol/g, 0.035 mmol) and Fmoc-Ala-OH (22 mg, 0.07 mmol), yielded 12.6 mg of **16a**: colorless powder; mp 205−207 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (s, 1H), 8.24 (d, 1H, J = 6.8 Hz), 8.18 (d, 1H, J = 8.4 Hz), 8.00 (t, 1H, J = 5.5 Hz), 7.57 (d, 1H, J = 7.9 Hz), 7.31 (d, 1H, J = 8.1 Hz), 7.23−7.27 (m, 6H), 7.16−7.19 (m, 2H), 7.14 (d, 2H, J = 7.5 Hz), 7.06 (s, 1H), 7.05 (t, 1H, J = 7.7 Hz), 6.97 (t, 1H, J = 7.3 Hz), 4.38−4.43 (m, 1H), 4.21−4.25 (m, 1H), 3.45 (s, 2H), 3.19−3.24 (m, 2H), 3.11 (dd, 1H, J = 4.6/14.7 Hz), 2.84 (dd, 1H, J = 9.6/14.6 Hz), 2.61 (t, 2H, J = 7.6 Hz), 1.01 (d, 3H, J = 7.0 Hz); MS (ESI) m/z 497.2 (80) [M + H]$^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[(1*S*)-1-((4-chlorobenzyl)carboxamido)ethyl]carboxamido}-3-(1*H*-3-indolyl)propanamide (16b).** Preparation as described for **16a**, using **7b** (12.1 mg, 0.070 mmol), yielded 10.4 mg of **16b**: colorless powder; mp 198−200 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (s, 1H), 8.28 (d, 1H, J = 6.8 Hz), 8.18 (d, 1H, J = 8.4 Hz), 7.99 (t, 1H, J = 5.5 Hz), 7.57 (d, 1H, J = 7.9 Hz), 7.29−7.32 (m, 3H), 7.24−7.27 (m, 4H), 7.18 (t, 1H, J = 7.3 Hz), 7.13 (d, 2H, J = 7.5 Hz), 7.07 (s, 1H), 7.05 (t, 1H, J = 7.7 Hz), 6.97 (t, 1H, J = 7.4 Hz), 4.37−4.44 (m, 1H), 4.20−4.26 (m, 1H), 3.45 (s, 2H), 3.16−3.26 (m, 2H), 3.11 (dd, 1H, J = 4.5/14.7 Hz), 2.84 (dd, 1H, J = 9.6/14.6 Hz), 2.60 (t, 2H, J = 7.6 Hz), 1.00 (d, 3H, J = 7.0 Hz); MS (ESI) m/z 531.1 [M + H]$^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[(1*S*)-1-(1-(2-(4-chlorophenyl)ethyl)carboxamido)ethyl]carboxamido}-3-(1*H*-3-indolyl)propanamide (16c).** Preparation as described for **16a**, using **7c** (13.1 mg, 0.07 mmol), yielded 10.2 mg of **16c**: colorless powder; mp 214−217 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (s, 1H), 8.14 (d, 1H, J = 8.5 Hz), 8.02−8.04 (m, 2H), 7.57 (d, 1H, J = 7.9 Hz), 7.24−7.32 (m, 5H), 7.17−7.21 (m, 5H), 7.07 (s, 1H), 7.05 (t, 1H, J = 7.5 Hz), 6.97 (t, 1H, J = 7.4 Hz), 4.39−4.43 (m, 1H), 4.20−4.26 (m, 1H), 3.22−3.29 (m, 2H), 3.11 (dd, 1H, J = 4.5/14.6 Hz), 2.86 (dd, 1H, J = 9.5/14.6 Hz), 2.78 (t, 2H, J = 7.7 Hz), 2.68 (t, 2H, J = 7.5 Hz), 2.36−2.40 (m, 2H), 0.97 (d, 3H, J = 7.0 Hz); MS (ESI) m/z 545.1 [M + H]$^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[(1*S*)-1-((1,3-benzodioxol-5-ylmethyl)carboxamido)ethyl]carboxamido}-3-(1*H*-3-indolyl)propanamide (16d).** Preparation as described for **16a**, using **7d** (12.8 mg, 0.07 mmol), yielded 12.1 mg of **16d**: colorless powder; mp 211 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (s, 1H), 8.17−8.19 (m, 2H), 7.99 (t, 1H, J = 5.5 Hz), 7.57 (d, 1H, J = 7.9 Hz), 7.31 (d, 1H, J = 8.1 Hz), 7.26 (t, 2H, J = 7.5 Hz), 7.18 (t, 1H, J = 7.3 Hz), 7.13 (d, 2H, J = 7.5 Hz), 7.07 (s, 1H), 7.05 (t, 1H, J = 7.7 Hz), 6.97 (t, 1H, J = 7.4 Hz), 6.81 (s, 1H), 6.78 (d, 1H, J = 7.9 Hz), 6.68 (d, 1H, J = 8.0 Hz), 5.91 (s, 2H), 4.38−4.43 (m, 1H), 4.19−4.24 (m, 1H), 3.35 (s, 2H), 3.18−3.27 (m, 2H), 3.11 (dd, 1H, J = 4.5/14.6 Hz), 2.83 (dd, 1H, J = 9.7/14.6 Hz), 2.61 (t, 2H, J = 7.6 Hz), 0.99 (d, 3H, J = 7.0 Hz); MS (ESI) m/z 541.2 [M + H]$^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[(1*S*)-1-((3-pyridyl)methylcarboxamido)ethyl]carboxamido}-3-(1*H*-3-indolyl)propanamide (16e).** Preparation as described for **16a**, using **7e** (12.3 mg, 0.07 mmol), yielded 13.2 mg of **16e**: colorless powder; mp 95−97 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (s, 1H), 8.57−8.59 (m, 2H), 8.40 (d, 1H, J = 7.0 Hz), 8.20 (d, 1H, J = 8.4 Hz), 7.97−8.01 (m, 2H), 7.57 (d, 2H, J = 7.8 Hz), 7.31 (d, 1H, J = 8.1 Hz), 7.26 (t, 2H, J = 7.5 Hz), 7.18 (t, 1H, J = 7.2 Hz), 7.13 (d, 2H, J = 7.5 Hz), 7.07 (s, 1H), 7.05 (t, 1H, J = 7.7 Hz), 6.97 (t, 1H, J = 7.4 Hz), 4.04−4.45 (m, 1H), 4.23−4.29 (m, 1H), 3.61 (s, 2H), 3.18−3.28 (m, 2H), 3.09 (dd, 1H, J = 4.7/14.6 Hz), 2.84 (dd, 1H, J = 9.6/14.6 Hz), 2.60 (t, 2H, J = 7.5 Hz), 1.01 (d, 3H, J = 7.1 Hz); MS (ESI) m/z 498.2 [M + H]$^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[(1*S*)-1-((1,2,3,4-tetrahydro-1-isoquinolinyl)methylcarboxamido)ethyl]carboxamido}-3-(1*H*-3-indolyl)propanamide (16f).** Preparation as described for **16a**, using **7f** (29 mg, 0.07 mmol), yielded 4.7 mg of **16f**: colorless powder; cis/trans isomeric ratio of 1:1.08; mp 110−113 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (s, 1H), 8.80 and 9.21 (bs, 1H), 8.39 and 8.45 (d, 1H, J = 7.5 Hz), 8.21 and 8.23 (d, 1H, J = 8.5 Hz), 8.10 (s, 1H), 7.60 (d, 1H, J = 7.8 Hz), 7.31 (d, 1H, J = 8.1 Hz), 7.15−7.28 (m, 9H), 7.08 (s, 1H), 7.05 (t, 1H, J = 7.6 Hz), 6.97 (t, 1H, J = 7.5 Hz), 4.77−4.82 (m, 1H), 4.46−4.52 (m, 1H), 4.33−4.44 (m, 1H), 3.40−3.48 (m, 1H), 3.27−3.36 (m, 2H), 3.17−3.24 (m, 1H), 2.81−3.08 (m, 6H), 2.64 (t, 2H, J = 7.5 Hz), 0.94 and 1.02 (d, 3H, J = 7.0 Hz); MS (ESI) m/z 552.3 [M + H]$^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[(1*S*)-1-((1*H*-2-indolyl)methylcarboxamido)ethyl]carboxamido}-3-(1*H*-3-indolyl)propanamide (16g).** Preparation as described for **16a**, using **7g** (12.3 mg, 0.07 mmol), yielded 5.8 mg of **16g**: colorless powder;

mp 188−193 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.86 (s, 1H), 10.76 (s, 1H), 8.20 (d, 1H, J = 8.5 Hz), 8.17 (d, 1H, J = 7.1 Hz), 8.01 (t, 1H, J = 5.5 Hz), 7.56 (d, 1H, J = 8.1 Hz), 7.38 (d, 1H, J = 7.8 Hz), 7.29 (t, 2H, J = 9.1 Hz), 7.22 (t, 2H, J = 7.4 Hz), 7.15 (t, 1H, J = 7.4 Hz), 7.11 (d, 2H, J = 7.6 Hz), 7.06 (s, 1H), 7.04 (t, 1H, J = 7.6 Hz), 6.94−6.99 (m, 2H), 6.90 (t, 1H, J = 7.0 Hz), 6.19 (s, 1H), 4.41−4.45 (m, 1H), 4.26−4.31 (m, 1H), 3.60 (d, 2H, J = 3.5 Hz), 3.15−3.29 (m, 2H), 3.08 (dd, 1H, J = 4.8/14.6 Hz), 2.84 (dd, 1H, J = 9.5/14.4 Hz), 2.61 (t, 2H, J = 7.6 Hz), 1.00 and 1.01 (s, 3H); MS (ESI) m/z 536.2 [M + H]$^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[(benzyl)amino]methylcarboxamido}-3-(1*H*-3-indolyl)propanamide (17a).** Preparation as described for **15a**, using 100 mg of **13** (0.354 mmol/g, 0.035 mmol) and **11a** (27 mg, 0.07 mmol), yielded 9.8 mg of **17a**: colorless powder; mp 107−109 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.82 (s, 1H), 9.11 (bs, 1H), 8.69 (d, 1H, J = 8.1 Hz), 8.24 (m, 1H), 7.62 (d, 1H, J = 7.9 Hz), 7.38−7.43 (m, 5H), 7.32 (d, 1H, J = 8.1 Hz), 7.26 (t, 2H, J = 7.4 Hz), 7.18 (t, 1H, J = 7.2 Hz), 7.16 (d, 2H, J = 7.4 Hz), 7.10 (s, 1H), 7.06 (t, 1H, J = 7.4 Hz), 6.98 (t, 1H, J = 7.5 Hz), 4.57 (q, 1H, J = 5.7 Hz), 4.02 (m, 2H), 3.63−3.66 (m, 1H), 3.53−3.56 (m, 1H), 3.27−3.34 (m, 1H), 3.20−3.26 (m, 1H), 3.04 (dd, 1H, J = 5.1/14.5 Hz), 2.86 (dd, 1H, J = 9.0/14.5 Hz), 2.64 (t, 2H, J = 7.5 Hz); MS (ESI) m/z 455.2 [M + H]$^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[(4-chlorobenzyl)amino]methylcarboxamido}-3-(1*H*-3-indolyl)propanamide (17b).** Preparation as described for **17a**, using **11b** (29.8 mg, 0.07 mmol), yielded 11 mg of **17b**: colorless powder; mp 138−140 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.82 (s, 1H), 9.13 (bs, 1H), 8.68 (d, 1H, J = 8.2 Hz), 8.25 (m, 1H), 7.61 (d, 1H, J = 7.9 Hz), 7.49 (d, 2H, J = 8.2 Hz), 7.41 (d, 2H, J = 8.1 Hz), 7.32 (d, 1H, J = 8.1 Hz), 7.26 (t, 2H, J = 7.4 Hz), 7.18 (t, 1H, J = 7.3 Hz), 7.16 (d, 2H, J = 7.5 Hz), 7.10 (s, 1H), 7.06 (t, 1H, J = 7.3 Hz), 6.97 (t, 1H, J = 7.5 Hz), 4.57 (q, 1H, J = 5.8 Hz), 4.03 (m, 2H), 3.62−3.67 (m, 1H), 3.51−3.56 (m, 1H), 3.26−3.34 (m, 1H), 3.19−3.26 (m, 1H), 3.04 (dd, 1H, J = 5.1/14.5 Hz), 2.86 (dd, 1H, J = 9.0/14.5 Hz), 2.64 (t, 2H, J = 7.5 Hz); MS (ESI) m/z 489.2 [M + H]$^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[(3-pyridyl)methylamino]methylcarboxamido}-3-(1*H*-3-indolyl)propanamide (17c).** Preparation as described for **17a**, using the TFA salt of **11c** (35.4 mg, 0.07 mmol), yielded 6.6 mg of **17c**: colorless powder; mp 89−93 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.82 (s, 1H), 9.20 (bs, 1H), 8.71 (d, 1H, J = 8.0 Hz), 8.59−8.62 (m, 2H), 8.26 (t, 1H, J = 5.5 Hz), 7.83 (d, 1H, J = 8.0 Hz), 7.62 (d, 1H, J = 8.0 Hz), 7.46 (dd, 1H, J = 4.7/7.9 Hz), 7.32 (d, 1H, J = 8.0 Hz), 7.26 (d, 2H, J = 7.4 Hz), 7.18 (t, 1H, J = 7.2 Hz), 7.16 (d, 2H, J = 7.5 Hz), 7.11 (s, 1H), 7.06 (t, 1H, J = 7.0 Hz), 6.98 (t, 1H, J = 7.0 Hz), 4.57 (q, 1H, J = 6.0 Hz), 4.09 (m, 2H), 3.69−3.75 (m, 1H), 3.57−3.64 (m, 1H), 3.27−3.35 (m, 1H), 3.19−3.27 (m, 1H), 3.05 (dd, 1H, J = 5.2/14.9 Hz), 2.87 (dd, 1H, J = 8.8/14.6 Hz), 2.64 (t, 2H, J = 7.1 Hz); MS (ESI) m/z 456.2 [M + H]$^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[1-(2-phenylethyl)amino]methylcarboxamido}-3-(1*H*-3-indolyl)propanamide (17d).** Preparation as described for **17a**, using **11d** (28.3 mg, 0.07 mmol), yielded 2.7 mg of **17d**: colorless powder; mp 197−199 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.82 (s, 1H), 8.79 (bs, 1H), 8.74 (d, 1H, J = 8.5 Hz), 8.26 (t, 1H, J = 5.5 Hz), 7.62 (d, 1H, J = 8.0 Hz), 7.31−7.34 (m, 3H), 7.25−7.28 (m, 3H), 7.19−7.21 (m, 3H), 7.16 (d, 2H, J = 7.6 Hz), 7.12 (s, 1H), 7.05 (t, 1H, J = 7.5 Hz), 6.98 (t, 1H, J = 7.5 Hz), 4.58 (q, 1H, J = 5.5 Hz), 3.73−3.79 (m, 1H), 3.62−3.67 (m, 1H), 3.29−3.34 (m, 1H), 3.20−3.25 (m, 1H), 3.04−3.08 (m, 3H), 2.84−2.92 (m, 3H), 2.64 (t, 2H, J = 7.4 Hz); MS (ESI) m/z 469.2 [M + H]$^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[1-(benzylamino)ethyl]carboxamido}-3-(1*H*-3-indolyl)propanamide (18a).** Compound **14** (0.30 g, 0.736 mmol) and **12a** (0.295 g, 0.735 mmol) were coupled in DMF (7 mL) at room temperature within 1 h using [HATU (1.5 equiv)]/[HOAt (1.5 equiv)]/[collidine (15 equiv)] activation. The solvent was removed in vacuo, the residue was chromatographed (ethyl acetate/hexane, 1:1) and subsequently dissolved in CH_2Cl_2 (2.5 mL), and triisopropylsilane (0.25 mL) was added. After the mixture was cooled to 0 °C, TFA (2.5 mL) was added dropwise over 5 min and the solution was stirred for 1 h. Then the solvent was removed in vacuo and the residue was treated with a mixture of DMSO (20 mL), water (2.5 mL), and acetic acid (2.5 mL) and stirred at room temperature overnight. Evaporation to dryness was followed by treatment with 20% (v/v) piperidine in DMF (10 mL) and stirring for 30 min at room temperature. Finally the solvent was removed in vacuo, and chromatography (ethyl acetate) yielded 0.25 g (73%) of **18a**: colorless powder; the ratio of the two isomers was determined from the NMR integrals of the CH_3 group as 1:1.52; mp 100−105 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.83 and 10.85 (s, 1H), 9.1 (m, 1H), 8.74−8.78 (m, 1H), 8.27 and 8.34 (t, 1H, J = 5.6 Hz), 7.63 and 7.68 (d, 1H, J = 7.8 Hz), 7.17−7.21 and 7.24−7.33 and 7.33−7.37 and 7.40−7.43 (m, 11H), 7.09 and 7.14 (s, 1H), 6.96−7.07 (m, 2H), 4.62−4.68 (m, 1H), 3.98−4.01 (m, 1H), 3.76−3.78 (m, 1H), 3.61−3.67 and 3.41−3.43 (m, 1H), 3.34−3.39 (m, 1H), 3.26−3.30 (m, 1H), 3.05−3.09 (m, 1H), 2.84−2.93 (m, 1H), 2.67−2.72 (m, 2H), 1.11 and 1.34 (d, 3H, J = 6.9 Hz); MS (ESI) m/z 469.2 [M + H]$^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[1-((4-chlorobenzyl)amino)ethyl]carboxamido}-3-(1*H*-3-indolyl)propanamide (18b).** Preparation as described for **18a**, using **12b** (0.32 g, 0.734 mmol), yielded 320 mg (87%) of **18b**: colorless powder; the ratio of the two isomers was determined from the NMR integrals of the CH_3 group as 1:1.24; mp 107−110 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.85 and 10.83 (s, 1H), 9.2 (m, 1H), 8.72 and 8.77 (d, 1H, J = 8.5 Hz), 8.28 and 8.34 (t, 1H, J = 5.5 Hz), 7.68 and 7.63 (d, 1H, J = 7.7 Hz), 7.17−7.20 and 7.24−7.33 and 7.40−7.50 (m, 10H), 7.09 and 7.15 (s, 1H), 6.96−7.07 (m, 2H), 4.61−4.69 (m, 1H), 3.98−4.02 (m, 1H), 3.75 (m, 1H), 3.60−3.67 and 3.39−3.43 (m, 1H), 3.34−3.38 (m, 1H), 3.24−3.29 (m, 1H), 3.05−3.08 (m, 1H), 2.85−2.92 (m, 1H), 2.67−2.71 (m, 2H), 1.33 and 1.10 (d, 3H, J = 6.9 Hz); MS (ESI) m/z 503.2 [M + H]$^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[1-(1-(2-phenylethyl)amino)ethyl]carboxamido}-3-(1*H*-3-indolyl)propanamide (18c).** Preparation as described for **18a**, using **12c** (0.305 g, 0.737 mmol), yielded 230 mg (65%) of **18c**: colorless powder; the ratio of the two isomers was determined from the NMR integrals of the CH_3 group as 1:1.59; mp 89−91 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.83 and 10.79 (s, 1H), 8.88 (m, 1H), 8.77 (m, 1H), 8.24 and 8.32 (t, 1H, J = 5.4 Hz), 7.63 (d, 1H, J = 7.8 Hz), 7.15−7.18 and 7.21−7.28 and 7.29−7.33 (m, 11H), 7.04−7.12 (m, 2H), 6.94−7.00 (m, 1H), 4.59−4.64 (m, 1H), 3.82−3.86 (m, 1H), 3.29−3.35 (m, 1H), 3.22−3.30 (m, 1H), 2.75−3.09 (m, 6H), 2.66 (t, 2H, J = 7.2 Hz), 1.35 and 1.12 (d, 3H, J = 6.9 Hz); MS (ESI) m/z 483.3 [M + H]$^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[*N*′-(benzoyl)hydrazino]carboxamido}-3-(1*H*-3-indolyl)propanamide (21a).** Resin-bound **13** (100 mg, 0.354 mmol/g, 0.035 mmol) was treated two times with 20% piperidine (v/v) in NMP (5 mL) for 15 min, washed after each time with NMP (5 × 5 mL), finally washed with dry CH_2Cl_2 (5 × 5 mL), and subsequently left in dry CH_2Cl_2 (5 mL) for 30 min. Then a solution of **19** (30.5 mg, 0.108 mmol) in dry CH_2Cl_2 (1 mL) was added and the resin was shaken for 90 min at room temperature and washed with CH_2Cl_2 (5 × 5 mL) and NMP (5 × 5 mL). After repeated Fmoc cleavage, **7a** (9.5 mg, 0.07 mmol) was coupled two times to the resin for 45 min using [TBTU (2 equiv)]/[HOBt (2 equiv)]/[DIEA (5.7 equiv)] activation. Coupling steps were followed by a washing step with NMP (5 × 5 mL). Finally the resin was washed with CH_2Cl_2 (3 × 5 mL) for 30 min and the compound was deprotected and cleaved from the resin with [TFA (18 equiv)]/[TIPS (1 equiv)]/[H_2O (1 equiv)] (3 × 2 mL, 30 min each). The combined filtrates were reduced in vacuo. RP-HPLC purification yielded 2.6 mg of **21a**: colorless powder; mp 109−114 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.79 (s, 1H), 9.80 (s, 1H), 7.95−8.00 (m, 2H), 7.51 (d, 1H, J = 7.9 Hz), 7.30 (d, 1H, J = 8.2 Hz), 7.23−7.28 (m, 6H), 7.19−7.22 (m, 1H), 7.16 (t, 1H, J = 7.1 Hz), 7.13 (d, 2H, J = 7.5 Hz), 7.02−7.04 (m, 2H), 6.95 (t, 1H, J = 7.5 Hz), 6.36 (d, 1H, J = 8.0 Hz), 4.36 (q, 1H, J = 6.6 Hz), 3.41 (s, 2H), 3.14−3.29 (m, 2H),

3.01 (dd, 1H, J = 5.7/14.6 Hz), 2.91 (dd, 1H, J = 7.5/14.5 Hz), 2.58 (t, 2H, J = 7.1 Hz); MS (ESI) m/z 484.3 $[M + H]^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[*N*′-(4-chlorobenzoyl)-hydrazino]carboxamido}-3-(1*H*-3-indolyl)propanamide (21b).** Preparation as described for **21a**, using **7b** (12 mg, 0.07 mmol), yielded 3.0 mg of **21b**: colorless powder; mp 195/196 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.79 (s, 1H), 9.81 (s, 1H), 8.00 (s, 1H), 7.94 (bs, 1H), 7.51 (d, 1H, J = 7.9 Hz), 7.23−7.34 (m, 7H), 7.17 (t, 1H, J = 7.3 Hz), 7.13 (d, 2H, J = 7.5 Hz), 7.02−7.05 (m, 2H), 6.95 (t, 1H, J = 7.5 Hz), 6.38 (d, 1H, J = 8.1 Hz), 4.36 (q, 1H, J = 6.7 Hz), 3.42 (s, 2H), 3.16−3.24 (m, 2H), 3.01 (dd, 1H, J = 5.6/14.9 Hz), 2.91 (dd, 1H, J = 7.5/14.5 Hz), 2.58 (t, 2H, J = 7.5 Hz); MS (ESI) m/z 518.2 $[M + H]^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[*N*′-(2-(3-pyridyl)ethanoyl)-hydrazino]carboxamido}-3-(1*H*-3-indolyl)propanamide (21c).** Preparation as described for **21a**, using **7e** (12 mg, 0.07 mmol), yielded 4.5 mg of **21c**: colorless powder; mp 116−120 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.79 (s, 1H), 9.89 (s, 1H), 8.63 (s, 1H), 8.61 (d, 1H, J = 5.0 Hz), 8.02−8.04 (m, 2H), 7.96 (bs, 1H), 7.63 (dd, 1H, J = 5.8/7.3 Hz), 7.51 (d, 1H, J = 7.9 Hz), 7.30 (d, 1H, J = 8.2 Hz), 7.25 (t, 2H, J = 7.6 Hz), 7.17 (t, 1H, J = 7.4 Hz), 7.13 (d, 2H, J = 7.5 Hz), 7.02−7.04 (m, 2H), 6.95 (t, 1H, J = 8.0 Hz), 6.44 (d, 1H, J = 8.1 Hz), 4.32−4.36 (m, 1H), 3.59 (s, 2H), 3.22−3.26 (m, 1H), 3.15−3.19 (m, 1H), 3.01 (dd, 1H, J = 5.6/14.4 Hz), 2.91 (dd, 1H, J = 7.4/14.6 Hz), 2.58 (t, 2H, J = 7.5 Hz); MS (ESI) m/z 485.3 $[M + H]^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[*N*′-(2-(1,2,3,4-tetrahydro-1-isoquinolinyl)ethanoyl)hydrazino]carboxamido}-3-(1*H*-3-indolyl)propanamide (21d).** Prepared as described for **21a**, using **7f** (29 mg, 0.07 mmol). The two isomers were separated by RP-HPLC and obtained as colorless powders. **21d1**: 1.6 mg; mp 105−110 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.79 (s, 1H), 9.94 (s, 1H), 8.75 and 9.25 (bs, 1H), 8.12 (s, 1H), 8.04 (t, 1H, J = 5.5 Hz), 7.54 (d, 1H, J = 7.5 Hz), 7.31 (d, 1H, J = 8.0 Hz), 7.17−7.26 (m, 7H), 7.14 (d, 2H, J = 7.4 Hz), 7.05 (s, 1H), 7.04 (t, 1H, J = 7.7 Hz), 6.96 (t, 1H, J = 7.0 Hz), 6.49 (d, 1H, J = 8.0 Hz), 4.75−4.84 (m, 1H), 4.35−4.39 (m, 1H), 3.31−3.48 (m, 2H), 3.24−3.28 (m, 1H), 3.16−3.20 (m, 1H), 2.87−3.01 (m, 6H), 2.59 (t, 2H, J = 7.5 Hz); MS (ESI) m/z 539.3 $[M + H]^+$. **21d2**: 0.9 mg; mp 120−123 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.79 (s, 1H), 9.95 (s, 1H), 8.78 and 9.26 (bs, 1H), 8.12 (s, 1H), 8.03 (t, 1H, J = 5.8 Hz), 7.54 (d, 1H, J = 7.7 Hz), 7.31 (d, 1H, J = 8.1 Hz), 7.17−7.26 (m, 7H), 7.14 (d, 2H, J = 7.3 Hz), 7.03−7.05 (m, 2H), 6.95 (t, 1H, J = 7.0 Hz), 6.49 (d, 1H, J = 8.2 Hz), 4.80−4.86 (m, 1H), 4.37 (q, 1H, J = 3.6 Hz), 3.31−3.48 (m, 2H), 3.22−3.28 (m, 1H), 3.15−3.20 (m, 1H), 2.87−3.05 (m, 6H), 2.59 (t, 2H, J = 7.5 Hz); MS (ESI) m/z 539.3 $[M + H]^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[*N*′-(2-(1*H*-2-indolyl)ethanoyl)hydrazino]carboxamido}-3-(1*H*-3-indolyl)propanamide (21e).** Preparation as described for **21a**, using **7g** (12.3 mg, 0.07 mmol), yielded 5.7 mg of **21e**: colorless powder; mp 109−115 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.92 (s, 1H), 10.79 (s, 1H), 9.80 (s, 1H), 8.04 (s, 1H), 7.97 (bs, 1H), 7.52 (d, 1H, J = 7.9 Hz), 7.40 (d, 1H, J = 7.7 Hz), 7.30 (d, 2H, J = 8.0 Hz), 7.24 (t, 2H, J = 7.4 Hz), 7.16 (t, 1H, J = 7.3 Hz), 7.12 (d, 2H, J = 7.5 Hz), 6.90−7.05 (m, 5H), 6.45 (d, 1H, J = 8.0 Hz), 6.23 (s, 1H), 4.36−4.40 (m, 1H), 3.60 (s, 2H), 3.15−3.26 (m, 2H), 3.02 (dd, 1H, J = 5.7/14.5 Hz), 2.91 (dd, 1H, J = 7.4/14.6 Hz), 2.58 (t, 2H, J = 7.4 Hz); MS (ESI) m/z 523.2 $[M + H]^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[*N*′-(phenylmethylene)-hydrazino]carboxamido}-3-(1*H*-3-indolyl)propanamide (24a).** Compound **23a** (120 mg, 0.217 mmol) was dissolved in CH_2Cl_2 (3 mL), triisopropylsilane (0.1 mL) was added, and the solution was cooled to 0 °C. Then TFA (3 mL) was added dropwise over 5 min and the solution was stirred for 30 min. The solvent was removed in vacuo and the residue was treated with DMSO (8 mL), water (1 mL), and acetic acid (1 mL) and stirred overnight at room temperature. Then the solution was evaporated to dryness. RP-HPLC purification yielded 62 mg (63%) of **24a**: colorless powder; mp 183 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.85 (s, 1H), 10.43 (s, 1H), 8.06 (t, 1H, J = 5.5 Hz), 7.81 (s, 1H), 7.60 (d, 1H, J = 7.9 Hz), 7.54 (d, 2H, J = 7.4 Hz), 7.36−7.41 (m, 3H), 7.32 (d, 1H, J = 8.1 Hz), 7.25 (t, 2H, J = 7.5 Hz), 7.14−7.19 (m, 3H), 7.11 (s, 1H), 7.05 (t, 1H, J = 7.4 Hz), 6.94 (t, 1H, J = 7.5 Hz), 6.72 (d, 1H, J = 8.0 Hz), 4.44 (q, 1H, J = 7.4 Hz), 3.27−3.35 (m, 1H), 3.19−3.27 (m, 1H), 3.11 (d, 2H, J = 6.4 Hz), 2.66 (t, 2H, J = 7.4 Hz); MS (ESI) m/z 454.2 $[M + H]^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[*N*′-(4-chlorophenylmethylene)hydrazino]carboxamido}-3-(1*H*-3-indolyl)propanamide (24b).** Preparation from **23b** (120 mg, 0.204 mmol) as described for **24a** yielded 56 mg (56%) of **24b**: colorless powder; mp 173−175 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.85 (s, 1H), 10.51 (s, 1H), 8.05 (t, 1H, J = 5.5 Hz), 7.79 (s, 1H), 7.59 (d, 1H, J = 8.0 Hz), 7.56 (d, 2H, J = 8.4 Hz), 7.46 (d, 2H, J = 8.3 Hz), 7.33 (d, 1H, J = 8.1 Hz), 7.25 (t, 2H, J = 7.5 Hz), 7.15−7.18 (m, 3H), 7.11 (s, 1H), 7.05 (t, 1H, J = 7.4 Hz), 6.94 (t, 1H, J = 7.5 Hz), 6.73 (d, 1H, J = 8.0 Hz), 4.43 (q, 1H, J = 7.5 Hz), 3.27−3.35 (m, 1H), 3.19−3.27 (m, 1H), 3.11 (d, 2H, J = 6.2 Hz), 2.66 (t, 2H J = 7.5 Hz); MS (ESI) m/z 488.2 $[M + H]^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[*N*′-(furan-2-ylmethylene)-hydrazino]carboxamido}-3-(1*H*-3-indolyl)propanamide (24c).** Preparation from **23c** (100 mg, 0.184 mmol) as described for **24a** yielded 46 mg (57%) of **24c**: colorless powder; mp 100/101 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.82 (s, 1H), 10.41 (s, 1H), 8.09 (t, 1H, J = 5.5 Hz), 7.76 (s, 1H), 7.73 (s, 1H), 7.55 (d, 1H, J = 7.9 Hz), 7.30 (d, 1H, J = 8.1 Hz), 7.25 (t, 2H, J = 7.4 Hz), 7.14−7.17 (m, 3H), 7.07 (s, 1H), 7.03 (t, 1H, J = 7.4 Hz), 6.93 (t, 1H, J = 7.5 Hz), 6.74 (d, 1H, J = 3.2 Hz), 6.58−6.60 (m, 2H), 4.45 (q, 1H, J = 6.8 Hz), 3.24−3.31 (m, 1H), 3.17−3.24 (m, 1H), 3.02−3.10 (m, 2H), 2.62 (t, 2H, J = 7.4 Hz); MS (ESI) m/z 444.2 $[M + H]^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[*N*′-(4-(2-thienyl)phenylmethylene)hydrazino]carboxamido}-3-(1*H*-3-indolyl)-propanamide (24d).** Preparation from **23d** (40 mg, 0.063 mmol) as described for **24a** yielded 25 mg (74%) of **24d**: colorless powder; mp 219−221 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.87 (s, 1H), 10.48 (s, 1H), 8.06 (t, 1H, J = 5.5 Hz), 7.81 (s, 1H), 7.69 (d, 2H, J = 8.2 Hz), 7.57−7.61 (m, 5H), 7.33 (d, 1H, J = 8.1 Hz), 7.25 (t, 2H, J = 7.5 Hz), 7.16−7.18 (m, 4H), 7.13 (s, 1H), 7.06 (t, 1H, J = 7.7 Hz), 6.95 (t, 1H, J = 7.5 Hz), 6.74 (d, 1H, J = 7.9 Hz), 4.43 (q, 1H, J = 7.7 Hz), 3.27−3.34 (m, 1H), 3.20−3.27 (m, 1H), 3.12 (d, 2H, J = 6.4 Hz), 2.66 (t, 2H, J = 7.4 Hz); MS (ESI) m/z 536.1 $[M + H]^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[*N*′-(benzyl)hydrazino]carboxamido}-3-(1*H*-3-indolyl)propanamide (28a).** Compound **27a** (0.24 g, 0.54 mmol) was dissolved in CH_2Cl_2 (2 mL), triisopropylsilane (0.1 mL) was added, and the solution was cooled to 0 °C. Then TFA (2 mL) was added dropwise over 5 min and the solution was stirred for 30 min. The solvent was removed in vacuo, and the residue was redissolved in a solution of dry CH_2Cl_2 (8 mL) and DMAP (66 mg, 0.54 mmol). This solution was added dropwise and under stirring to a solution of bis(pentafluorophenyl)carbonate (213 mg, 0.54 mmol) in dry CH_2Cl_2 (20 mL) over a period of 20 min. Upon completion of the addition, a solution of **14** (0.22 g, 0.54 mmol), DMAP (66 mg, 0.54 mmol), and dry CH_2Cl_2 (8 mL) was added. The resulting mixture was stirred for 30 min at room temperature. Then the solvent was removed in vacuo, the residue was redissolved in CH_2Cl_2 (4 mL) and triisopropylsilane (0.1 mL), and the solution was cooled to 0 °C. TFA (4 mL) was added dropwise over 5 min, and the solution was stirred for 30 min. The solution was evaporated to dryness and then treated with a solution of 20% (v/v) piperidine in DMF (10 mL) for 30 min at room temperature. Finally the solvent was removed in vacuo and RP-HPLC purification yielded 34.2 mg (14%) of **28a**: colorless powder; mp 81−84 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.56 (d, 1H, J = 7.9 Hz), 7.34 (d, 1H, J = 8.1 Hz), 7.16−7.29 (m, 9H), 7.11 (t, 1H, J = 7.1 Hz), 6.94−6.95 (m, 3H), 6.88 (bs, 1H), 5.99 (bs, 1H), 4.43 (q, 1H, J = 6.5 Hz), 3.91 (bs, 2H), 3.34−3.42 (m, 1H), 3.16−3.24 (m, 1H), 3.14 (dd, 1H, J = 5.5/14.0 Hz), 3.07 (dd, 1H, J = 7.4/14.3 Hz), 2.45−2.59 (m, 2H); MS (ESI) m/z 456.1 $[M + H]^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[*N*′-(4-chlorobenzyl)hydrazino]carboxamido}-3-(1*H*-3-indolyl)propanamide (28b).** Preparation from **27b** (0.30 g, 0.63 mmol) and **14** (0.26 g, 0.63

mmol) as described for **28a** yielded 26.8 mg (9%) of **28b**: colorless powder; mp 75−80 °C; ^{1}H NMR (500 MHz, ACN-d_3) δ 9.74 (bs, 1H), 7.91 (d, 1H, J = 7.7 Hz), 7.98 (d, 1H, J = 8.1 Hz), 7.74−7.83 (m, 5H), 7.71 (t, 1H, J = 7.4 Hz), 7.66−7.69 (m, 4H), 7.62 (t, 1H, J = 7.5 Hz), 7.58 (s, 1H), 6.99 (bs, 1H), 6.89 (bs, 1H), 4.87 (q, 1H, J = 6.9 Hz), 4.34 (bs, 2H), 3.87−3.94 (m, 1H), 3.76−3.82 (m, 1H), 3.66 (d, 2H, J = 6.2 Hz), 3.17 (t, 2H, J = 7.3 Hz); MS (ESI) m/z 490.1 $[M + H]^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[*N*′-(2-furylmethyl)hydrazino]carboxamido}-3-(1*H*-3-indolyl)propanamide (28c).** Preparation from **27c** (0.30 g, 0.69 mmol) and **14** (0.28 g, 0.69 mmol) as described for **28a** yielded 65.1 mg (21%) of **28c**: colorless powder; mp 59−62 °C; ^{1}H NMR (500 MHz, ACN-d_3) δ 9.72 (s, 1H), 8.14 (d, 1H, J = 7.9 Hz), 7.97 (d, 1H, J = 8.3 Hz), 7.95 (s, 1H), 7.82 (t, 2H, J = 7.5 Hz), 7.76 (t, 1H, J = 7.2 Hz), 7.70 (t, 1H, J = 7.4 Hz), 7.67 (d, 2H, J = 7.5 Hz), 7.62 (t, 1H, J = 7.6 Hz), 7.60 (s, 1H), 7.05 (bs, 2H), 6.89 (s, 1H), 6.80 (s, 1H), 4.89 (q, 1H, J = 6.8 Hz), 4.41 (bs, 2H), 3.82−3.92 (m, 2H), 3.68 (dd, 1H, J = 5.9/14.5 Hz), 3.64 (dd, 1H, J = 7.7/14.0 Hz), 3.17 (t, 2H, J = 7.0 Hz); MS (ESI) m/z 446.2 $[M + H]^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[(4-benzylpiperazino)methyl]carboxamido}-3-(1*H*-3-indolyl)propanamide (35).** Compound **33** (170 mg, 0.273 mmol) was dissolved in CH_2Cl_2 (3 mL), and triisopropylsilane (0.1 mL) was added. After the mixture was cooled to 0 °C, TFA (3 mL) was added dropwise over 5 min and the solution was stirred for 1 h. Then the solvent was removed in vacuo and the residue was treated with DMSO (8 mL), water (1 mL), and acetic acid (1 mL). The mixture was stirred overnight at room temperature. Evaporation to dryness and RP-HPLC purification yielded 97 mg (68%) of **35**: colorless powder; mp 67−70 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.60 (d, 1H, J = 7.9 Hz), 7.55 (d, 1H, J = 7.5 Hz), 7.43−7.49 (m, 3H), 7.34−7.37 (m, 3H), 7.23−7.27 (m, 2H), 7.19 (t, 2H, J = 7.2 Hz), 7.13 (t, 1H, J = 7.5 Hz), 7.05 (d, 2H, J = 7.4 Hz), 6.96 (s, 1H), 6.26 (s, 1H), 4.68 (q, 1H, J = 7.5 Hz), 4.03 (dd, 2H, J = 12.9/23.8 Hz), 3.48−3.55 (m, 1H), 3.38−3.45 (m, 1H), 3.23 (dd, 1H, J = 7.4/14.8 Hz), 3.14 (dd, 1H, J = 7.2/14.8 Hz), 3.08 (m, 2H), 2.70 (t, 2H, J = 6.9 Hz), 2.61−2.75 (m, 8H); MS (ESI) m/z 524.4 $[M + H]^+$.

***N*1-(2-Phenylethyl)-(2*R*)-2-{[(4-benzylpiperidino)methyl]carboxamido}-3-(1*H*-3-indolyl)propanamide (36).** Preparation from **34** (180 mg, 0.289 mmol) as described for **35** yielded 109 mg (72%) of **36**: colorless powder; mp 73−75 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.84 (s, 1H), 8.81 (d, 1H, J = 8.3 Hz), 8.25 (t, 1H, J = 5.2 Hz), 7.61 (d, 1H, J = 7.7 Hz), 7.14−7.31 (m, 11H), 7.09 (s, 1H), 7.02 (t, 1H, J = 7.3 Hz), 6.96 (t, 1H, J = 6.9 Hz), 4.60 (q, 1H, J = 6.9 Hz), 3.81 (d, 1H, J = 15.2 Hz), 3.67 (d, 1H, J = 13.1 Hz), 3.21−3.34 (m, 3H), 3.06 (dd, 1H, J = 4.9/14.4 Hz), 2.97−2.93 (m, 3H), 2.59−2.67 (m, 3H), 2.46−2.48 (m, 2H), 1.57−1.70 (m, 3H), 1.32−1.46 (m, 2H); MS (ESI) m/z 523.3 $[M + H]^+$.

Acknowledgment. The authors thank B. Cordes, Mr. Krause (Technische Universität München), and H. Wölk (Solvay Pharmaceuticals) for their professional assistance in ESI-MS and GC−MS and in molecular and cell biology. We thank Mr. Reineke and Dr. Mühlebach (Solvay Pharmaceuticals) for their excellent technical assistance in aquiring HRMS data. The authors express their gratitude to James Battey, Ph.D. (NIDCD, NIH), who kindly provided the receptor cDNA to Solvay Pharmaceuticals (Hannover, Germany). Financial support by the Fonds der Chemischen Industrie is gratefully acknowlegded.

Appendix

Abbreviations. BLP, bombesin-like peptide; Bn, bombesin; Boc, *tert*-butyloxycarbonyl; BRS-3, bombesin receptor subtype 3; $[Ca^{2+}]_i$, intracellular calcium; CART, constitutively activating receptor technology; CHO, Chinese hamster ovary; DCE, dichloroethane; DIEA, diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EC, effect concentration; ESI, electrospray ionization; FLIPR, fluorometric imaging plate reader; Fmoc, 9-fluorenylmethoxycarbonyl; FMPE, 2-(4-formyl-3-methoxyphenoxy)ethyl; GPCR, G-protein-coupled receptor; GRP, gastrin-releasing peptide; GRP-R, gastrin-releasing peptide receptor; HATU, 2-(1*H*-9-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOAc, acetic acid; HOAt, *N*-hydroxy-9-azabenzotriazole; HOBt, *N*-hydroxybenzotriazole; mRNA, messenger ribonucleic acid; MS, mass spectrometry; NMB, neuromedin B; NMB-R, neuromedin B receptor; NMP, *N*-methylpyrrolidinone; PE, polyethylene; propylamide is *n*-propylamide; RP-HPLC, reverse-phase high-pressure performance chromatography; SAR, structure−activity relationship; SCLC, small-cell lung cancer; TBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; THF, tetrahydrofuran, TIPS, triisopropylsilane; TLC, thin-layer chromatography; TMS, trimethylsilane; TMOF, trimethyl orthoformate; TOF, time of flight.

Supporting Information Available: Detailed descriptions of molecular cloning, transfection, cell culture, the FLIPR assay, and the FLIPR antagonist experiment, synthesis procedures of compounds **7f**, **7g**, **8**−**12**, **14**, **20**, **22d**, **23**, **25**−**27**, **29**−**34**, and tables of analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Drews, J. Drug Discovery: A Historical Perspective. *Science* **2000**, *287* (5460), 1960−1963.
(2) Source: IMS Health, Fairfield, CT (2001).
(3) (a) Stadel, J. M.; Wilson, S.; Bergsma, D. J. Orphan G protein-coupled receptors: a neglected opportunity for pioneer drug discovery. *Trends Pharmacol. Sci.* **1997**, *18*, 430−437. (b) Wilson, S.; Bergsma, D. J.; Chambers, J. K.; Muir, A. I.; Fantom, K. G. M.; Ellis, C.; Murdock, P. R.; Herrity, N. C.; Stadel, J. M. Orphan G-protein-coupled receptors: the next generation of drug targets? *Br. J. Pharmacol.* **1998**, *125* (7), 1387−1392.
(4) Dowell, S. J. Understanding GPCRs—from orphan receptors to novel drugs. *Drug Discovery Today* **2001**, *6* (17), 884−886.
(5) (a) International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* **2001**, *409*, 860−921. (b) Venter, J. C.; et al. The sequence of the human genome. *Science* **2001**, *291*, 1304−1351.
(6) (a) Libert, F.; Vassart, G.; Parmentier, M. Current developments in G-protein coupled receptors. *Curr. Opin. Cell Biol.* **1991**, *8*, 218−223. (b) Civelli, O. Functional genomics: the search for novel neurotransmitters and neuropeptides. *FEBS Lett.* **1998**, *430*, 55−58. (c) Civelli, O.; Reinscheid, R.; Nothacker, H.-P. Orphan receptors, novel neuropeptides and reverse pharmaceutical research. *Brain Res.* **1999**, *848*, 63−65. (d) Civelli, O.; Saito, Y.; Lin, S.; Nothacker, H.-P.; Renscheid, R.; Wang, Z. The orphan receptor strategy and the discovery of novel neuropeptides. *Trends Neurosci.* **2001**, *24*, 230−237.
(7) For a review, see the following. Chalmers, D. T.; Behan, D. P. The use of constitutively active GPCRs in drug discovery and functional genomics. *Nat. Rev. Drug Discovery* **2002**, *1*, 103−110.
(8) Gether, U.; Kobilka, B. K. G-protein coupled receptors. II. Mechanism of agonist activation. *J. Biol. Chem.* **1998**, *273*, 17979−17982.
(9) Schwartz, T. W.; Rosenkilde, M. M. Is there a "lock" for all agonist "keys" in 7TM receptors? *Trends Pharmacol. Sci.* **1996**, *17*, 213-216.
(10) Wess, G.; Urmann, M.; Sickenberger, B. Medicinal Chemistry: Challenges and Opportunities. *Angew. Chem., Int. Ed.* **2001**, *40*, 3341−3350.
(11) Fathi, Z.; Corjay, M. H.; Shapira, H.; Wada, E.; Benya, R.; Jensen, R.; Viallet, J.; Sausville, E. A.; Battey, J. F. BRS-3: A Novel Bombesin Receptor Subtype Selectively Expressed in Testis and Lung Carcinoma Cells. *J. Biol. Chem.* **1993**, *268*, 5979−5984.
(12) Gorbulev, V.; Akhundova, A.; Büchner, H.; Fahrenholz, F. Molecular cloning of a new bombesin receptor subtype expressed in uterus during pregnancy. *Eur. J. Biochem.* **1992**, *208*, 405−410.

(13) (a) Okhi-Hamazaki, H.; Watase, K.; Yamamoto, K.; Ogura, H.; Yamano, M.; Yamada, K.; Maeno, H.; Imaki, J.; Kikuyama, S.; Wada, E.; Wada, K. Mice lacking bombesin receptor subtype-3 develop metabolic defects and obesity. *Nature* **1997**, *390*, 165−169. (b) Yamada, K.; Wada, E.; Imaki, J.; Ohki-Hamazaki, H.; Wada, K. Hyperresponsiveness to Palatable and Aversive Taste Stimuli in Genetically Obese (Bombesin Receptor Subtype-3-Deficient) Mice. *Physiol. Behav.* **1999**, *66*, 863−867.

(14) (a) DeMichele, M. A.; Davis, A. L.; Hunt, J. D.; Landreneau, R. J.; Siegfried, J. M. Expression of mRNA for three bombesin receptor subtypes in human bronchial epithelial cells. *Am. J. Respir. Cell Mol. Biol.* **1994**, *11*, 66−74. (b) Toi-Scott, M.; Jones, C. L.; Kane, M. A. Clinical correlates of bombesin-like peptide receptor subtype expression in human lung cancer cells. *Lung Cancer* **1996**, *15*, 341−354. (c) Kiaris, H.; Schally, A. V.; Nagy, A.; Sun, B.; Armatis, P.; Szepeshazi, K. Targeted cytotoxic analogue of bombesin/gastrin-releasing peptide inhibits the growth of H-69 human small-cell lung carcinoma in nude mice. *Br. J. Cancer* **1999**, *81*, 966−971. (d) Sun, B.; Schally, A. V.; Halmos, G. The presence of receptors for bombesin/GRP and mRNA for the three receptor subtypes in human ovarian epithelial cancers. *Regul. Pept.* **2000**, *90*, 77−84.

(15) (a) Cuttitta, F.; Carney, N.; Mulshine, J.; Moody, T. W.; Fedorko, J.; Fischler, A.; Minna, J. D. Bombesin-like peptides can function as autocrine growth factors in human small-cell lung cancer. *Nature* **1985**, *316*, 823−826. (b) Carney, D. N.; Cuttitta, F.; Moody, T. W.; Minna, J. D. Selective stimulation of small cell lung cancer clonal growth by bombesin and gastrin-releasing peptide. *Cancer Res.* **1987**, *47*, 821−825.

(16) (a) Gorbulev, V.; Akhundova, A.; Grzeschick, K. H.; Fahrenholz, F. Organization and chromosomal localization of the gene for the human bombesin receptor subtype expressed in pregnant uterus. *FEBS Lett.* **1994**, *340*, 260−264. (b) Okhi-Hamazaki, H.; Wada, E.; Matsui, K.; Wada, K. Cloning and expression of the neuromedin B receptor and the third subtype of bombesin receptor genes in the mouse. *Brain Res.* **1997**, *762*, 165−172. (c) Whitley, J. C.; Moore, C.; Giraud, A. S.; Shulkes, A. Molecular cloning, genomic organization and selective expression of bombesin receptor subtype 3 in the sheep hypothalamus and pituitary. *J. Mol. Endocrinol.* **1999**, *23*, 107−116.

(17) Liu, J.; Lao, Z. J.; Zhang, J.; Schaeffer, M. T.; Jiang, M. M.; Guan, X.-M.; Van der Ploeg, L. H. T.; Fong, T. M. Molecular Basis of the Pharmacological Difference between Rat and Human Bombesin Receptor Subtype-3 (BRS-3). *Biochemistry* **2002**, *41*, 8954−8960.

(18) (a) Vigna, S. R.; Giraud, A. S.; Soll, A. H.; Walsh, J. H.; Mantyn, P. W. Bombesin Receptors on Gastrin Cells. *Ann. N. Y. Acad. Sci.* **1988**, *547*, 131−137. (b) Battey, J.; Wada, E. Two distinct receptor subtypes for mammalian bombesin-like peptides. *Trends Neurosci.* **1991**, *14*, 524−528. (c) Battey, J. F.; Way, J. M.; Corjay, M. H.; Shapira, H.; Kusano, K.; Harkins, R.; Wu, J.; Slattery, T.; Mann, E.; Feldman, R. Molecular cloning of the bombesin/gastrin-releasing peptide receptor from Swiss 3T3 cells. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 395−399. (d) Wada, E.; Way, J.; Shapira, H.; Kusano, K.; Lebacq-Verheyden, A.; Coy, D. H.; Jensen, R. T.; Battey, J. F. cDNA cloning, characterization, and brain region-specific expression of a neuromedin-B-preferring bombesin receptor. *Neuron* **1991**, *6*, 421−430. (e) Vigne, P.; Feolde, E.; Van Renterghem, C.; Breittmayer, J. P.; Frelin, C. Properties and functions of a neuromedin-B-preferring bombesin receptor in brain microvascular endothelial cells. *Eur. J. Biochem.* **1995**, *233*, 414−418.

(19) Smart, D.; Strijbos, P. Bombesin receptor subtype 3 polynucleotides, polypeptides and ligands for use in treating neurological disorders. PTC Int. Appl. WO 0168120, 2001; 31 pp.

(20) (a) Mantey, S. A.; Weber, H. C.; Sainz, E.; Akeson, M.; Ryan, R. R.; Pradhan, T. K.; Searles, R. P.; Spindel, E. R.; Battey, J. F.; Coy, D. H.; Jensen, R. T. Discovery of a High Affinity Radioligand for the Human Orphan Receptor, Bombesin Receptor Subtype 3, Which Demonstrates That It Has a Unique Pharmacology Compared with Other Mammalian Bombesin Receptors. *J. Biol. Chem.* **1997**, *272*, 26062−26071. (b) Pradhan, T. K.; Katsuno, T.; Taylor, J. E.; Kim, S. H.; Ryan, R. R.; Mantey, S. A.; Donohue, P. J.; Weber, H. C.; Sainz, E.; Battey, J. F.; Coy, D. H.; Jensen, R. T. Identification of a unique ligand which has high affinity for all four bombesin receptor subtypes. *Eur. J. Pharmacol.* **1998**, *343*, 275−287. (c) Mantey, S. A.; Coy, D. H.; Pradhan, T. K.; Igarashi, H.; Rizo, I. M.; Shen, L.; Hou, W.; Hocart, S.; Jensen, R. T. Rational Design of a Peptide Agonist That Interacts Selectively with the Orphan Receptor, Bombesin Receptor Subtype 3. *J. Biol. Chem.* **2001**, *276*, 9219−9229.

(21) Ryan, R. R.; Weber, H. C.; Hou, W.; Sainz, E.; Mantey, S. A.; Battey, J. F.; Coy, D. H.; Jensen, R. T. Ability of Various Bombesin Receptor Agonists and Antagonists To Alter Intracellular Signaling of the Human Orphan Receptor BRS-3. *J. Biol. Chem.* **1998**, *273*, 13613−13624.

(22) Weber, D.; Berger, C.; Heinrich, T.; Eickelmann, P.; Antel, J.; Kessler, H. Systematic Optimization of a Lead-Structure Identities for a Selective Short Peptide Agonist for the Human Orphan Receptor BRS-3. *J. Pept. Sci.* **2002**, *8*, 461−475.

(23) Darker, J. G.; Brough, S. J.; Heath, J.; Smart, D. Discovery of Potent and Selective Peptide Agonists at the GRP-Preferring Bombesin Receptor (BB_2). *J. Pept. Sci.* **2001**, *7*, 598−605.

(24) (a) Horwell, D. C.; Howson, W.; Rees, D. C. "Peptoid" Design. *Drug Des. Discovery* **1994**, *12*, 63−75. (b) Horwell, D. C. The "peptoid" approach to the design of non-peptide, small molecule agonists and antagonists of neuropeptides. *Trends Biotechnol.* **1995**, *13*, 132−134. (c) Horwell, D. C. Design of non-peptide agonists and antagonists at neuropeptide receptors starting from the chemical structure of neuropeptides. *Lett. Pept. Sci.* **1998**, *5*, 115−116.

(25) (a) Horwell, D. C.; Hughes, J.; Hunter, J. C.; Pritchard, M. C.; Richardson, R. S.; Roberts, E.; Woodruff, G. N. Rationally Designed "Dipeptoid" Analogues of CCK. α-Methyltryptophan Derivatives as Highly Selective and Highly Active Gastrin and CCK-B Antagonists with Potent Anxiolytic Properties. *J. Med. Chem.* **1991**, *34*, 404−414. (b) Boden, P. R.; Higginbottom, M.; Hill, D. R.; Horwell, D. C.; Hughes, J.; Rees, D. C.; Roberts, E.; Singh, L.; Suman-Chauhan, N.; Woodruff, G. N. Cholecystokinin Dipeptoid Antagonists: Design, Synthesis and Anxiolytic Profile of Some Novel CCK-A and CCK-B Selective and "Mixed" CCK-A/CCK-B Antagonists. *J. Med. Chem.* **1993**, *36*, 552−565. (c) Singh, L.; Field, M. J.; Hill, D. R.; Horwell, D. C.; McKnight, A. T.; Roberts, E.; Tang, K. W.; Woodruff, G. N. Peptoid CCK Receptor Antagonists: Pharmacological Evaluation of CCKA, CCKB and Mixed CCKA/B Receptor Antagonists. *Eur. J. Pharmacol.* **1995**, *286*, 185−191. (d) Trivedi, B. K.; Padia, J. K.; Holmes, A.; Rose, S.; Wright, D. S.; Hinton, J. P.; Pritchard, M. C.; Eden, J. M.; Kneen, C.; Webdale, L.; Suman-Chauhan, N.; Boden, P.; Singh, L.; Field, M. J.; Hill, D. Second Generation "Peptoid" CCK-B Receptor Antagonists: Identification and Development of *N*-(Adamantyloxycarbonyl)-α-methyl-(*R*)-tryptophan Derivative (CI-1015) with an Improved Pharmacokinetic Profile. *J. Med. Chem.* **1998**, *41*, 38−45.

(26) (a) Boyle, S.; Guard, S.; Hodgson, J.; Horwell, D. C.; Howson, W.; Hughes, J.; McKnight, A. T.; Martin, K.; Pritchard, M. C.; Watling, K. J. Rational Design of High Affinity Tachykinin NK_2 Receptor Antagonists. *Bioorg. Med. Chem.* **1994**, *2*, 101−113. (b) Boden, P.; Eden, J. M.; Hodgson, J.; Horwell, D. C.; Hughes, J.; McKnight, A. T.; Lewthwaite, R. A.; Pritchard, M. C.; Raphy, J.; Meecham, K.; Ratcliffe, G. S.; Suman-Chauhan, N.; Woodruff, G. N. Use of Dipeptide Chemical Library in the Development of Non-Peptide Tachykinin NK_3 Receptor Selective Antagonists. *J. Med. Chem.* **1996**, *39*, 1664−1675. (c) Maggi, C. A.; Patacchini, R.; Giuliani, S.; Giachetti, A. In Vivo and In Vitro Pharmacology of SR 48968, A Non-Peptide Tachykinin NK_2 Receptor Antagonist. *Eur. J. Pharmacol.* **1993**, *234*, 83−90. (d) Boden, P.; Eden, J. M.; Hodgson, J.; Horwell, D. C.; Pritchard, M. C.; Raphy, J.; Suman-Chauhan, N. The Development of a Novel Series of Non-Peptide Tachykinin NK_3 Receptor Selective Antagonists. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1773−1778.

(27) (a) Eden, J. M.; Hall, M. D.; Higginbottom, M.; Horwell, D. C.; Howson, W.; Hughes, J.; Jordan, R. E.; Lewthwaite, R. A.; Martin, K.; McKnight, A. T.; O'Toole, J. C.; Pinnock, R. D.; Pritchard, M. C.; Suman-Chauhan, N.; Williams, S. C. PD 165929—The First High Affinity Non-Peptide Neuromedin-B (NMB) Receptor Selective Antagonist. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2617−2622. (b) Ashwood, V.; Brownhill, V.; Higginbottom, M.; Horwell, D. C.; Hughes, J.; Lewthwaite, R. A.; McKnight, A. T.; Pinnock, R. D.; Pritchard, M. C.; Suman-Chauhan, N.; Webb, C.; Williams, S. C. PD 176252—The First High Affinity Non-Peptide Gastrin-Releasing Peptide (BB_2) Receptor Antagonist. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2589−2594.

(28) Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C. K.; Spellmeyer, D. C.; Tan, R.; Frankel, A. D.; Santi, D. V.; Bartlett, P. A. Peptoids: A modular approach to drug discovery. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367−9371.

(29) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. Efficient Method for the Preparation of Peptoids [Oligo(N-substituted glycines)] by Submonomer Solid-Phase Synthesis. *J. Am. Chem. Soc.* **1992**, *114*, 10646−10647.

(30) Kessler, H. Peptoids: key compounds in a new path to pharmaceutically active substances. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 543−544.

(31) Miller, S. M.; Simon, R. J.; Ng, S.; Zuckermann, R. N.; Kerr, J. M.; Moos, W. H. Proteolytic studies of homologous peptide and N-substituted glycine peptoid oligomers. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2657−2662.

(32) (a) Zuckermann, R. N.; Martin, E. J.; Spellmeyer, D. C.; Stauber, G. B.; Shoemaker, K. R.; Kerr, J. M.; Figliozzi, G. M.; Goff, D. A.; Siani, M. A.; Simon, R. J.; Banville, S. C.; Brown, E. G.; Wang, L.; Richter, L. S.; Moos, W. H. Discovery of Nanomolar

Ligands for 7-Transmembrane G-Protein-Coupled Receptors from a Diverse *N*-(Substituted)glycine Peptoid Library. *J. Med. Chem.* **1994**, *37*, 2678−2685. (b) Hamy, F.; Felder, E. R.; Heizmann, G.; Lazdins, J.; Aboul-ela, F.; Varani, G.; Karns, J.; Klimkait, T. An inhibitor of the TAT/TAR RNA interaction that effectively suppresses HIV-1-replication. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 3548−3553.

(33) For a review, see the following. Gante, J. Azapeptides. *Synthesis* **1989**, *6*, 405−413.

(34) Gante, J. Peptidomimetics-tailored enzyme inhibitors. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699−1720.

(35) (a) Bold, G.; Fässler, A.; Capraro, H.-G.; Cozens, R.; Klimkait, T.; Lazdins, J.; Mestan, J.; Poncioni, B.; Rösel, J.; Stover, D.; Tintelnot-Blomley, M.; Acemoglu, F.; Beck, W.; Boss, E.; Eschbach, M.; Hürlimann, T.; Masso, E.; Roussel, S.; Ucci-Stoll, K.; Wyss, D.; Lang, M. New Aza-Dipeptide Analogues as Potent and Orally Absorbed HIV-1 Protease Inhibitors: Candidates for Clinical Development. *J. Med. Chem.* **1998**, *41*, 3387−3401. (b) Witherell, G. Novartis/Bristol-Myers Squibb. *Curr. Opin. Invest. Drugs* **2001**, *2*, 340−347.

(36) Floersheimer, A.; Riniker, B. Solid-phase synthesis of peptides with the highly acid-sensitive HMPB linker. *Proc. Eur. Pept. Symp., 21th, 1990* **1991**, 131.

(37) Dörner, B.; White, P. Preparation of carboxy-modified peptide fragments using alkoxybenzaldehyde resins. *Proc. Eur. Pept. Symp., 25th, 1998*, **1999**, 90.

(38) Phenylacetic acid (**7a**), 4-chlorophenylacetic acid (**7b**), 3-(4-chlorophenyl)propionic acid (**7c**), 2-(1,3-benzodioxol-5-yl)acetic acid (**7d**), and 3-pyridylacetic acid (**7e**) were obtained from commercial sources. 2-(1,2,3,4-Tetrahydro-1-isoquinolinyl)acetic acid was prepared in three steps according to literature procedures.[39] Fmoc protection was carried out in a modified procedure of the one previously described[40] and yielded **7f** as racemic mixture. (1*H*-Indol-2-yl)acetic acid ethyl ester was prepared in two steps according to literature procedures.[41] Hydrolysis of the ester with aqueous NaOH yielded **7g**.

(39) (a) Gretler, R.; Askitoglu, E.; Kühne, H.; Hesse, M. Die massenspektrometrische retro-Diels−Alder-Reaktion: 1,2,3,4-Tetrahydroisochinolin und 1,2,3,4-Tetrahydronaphthalin (Tetralin) (The mass spectrometry of retro-Diels−Alder reaction: 1,2,3,4-tetrahydroisoquinoline and 1,2,3,4-tetrahydronaphthalene (Tetralin). *Helv. Chim. Acta* **1978**, *61* (5), 1730−1755. (b) Pelletier, J. C.; Cava, M. P. The Reaction of 3,4-Dihydroisoquinolines with Malonic Acid and Its Derivatives. *Synthesis* **1987**, *5*, 474−477.

(40) Carpino, L. A.; Han, G. Y. The 9-Fluorenylmethoxycarbonyl Amino-Protecting Group. *J. Org. Chem.* **1972**, *37*, 3404−3409.

(41) (a) Mai, A.; Artico, M.; Sbardella, G.; Massa, S.; Novellino, E.; Greco, G.; Loi, A. G.; Tramontano, E.; Marongiu, M. E.; La Colla, P. 5-Alkyl-2-(alkylthio)-6-(2,6-dihalophenylmethyl)-3,4-dihydropyrimidin-4(3*H*)-ones: Novel Potent and Selective Dihydroalkoxy-benzyl-oxopyrimidine Derivatives. *J. Med. Chem.* **1999**, *42*, 619−627. (b) Moody, C. J.; Rahimtoola, F. Diels−Alder Reactivity of Pyrano[4,3-*b*]indol-3-ones, Indole 2,3-Quinodimethane Analogues. *J. Chem. Soc., Perkin Trans. 1* **1990**, 673−679.

(42) (a) Fields, G. B.; Noble, R. L. Solid-phase peptide synthesis utilizing 9-fluorenylmethoxycarbonyl amino acids. *Int. J. Pept. Protein Res.* **1990**, *35*, 161−214. (b) Chan, C.; White, D. *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*; Oxford University Press: Oxford, 2000.

(43) (a) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788−798. (b) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 2024−2033.

(44) (a) Speziale, A. J.; Jaworski, E. G. *N*-Substituted Glycinate and Alaninate Esters. *J. Org. Chem.* **1960**, *25*, 728−732. (b) Stewart, H. C. A Surface Active Sydnone. *Austr. J. Chem.* **1961**, *14*, 654−656.

(45) Dechantsreiter, M. Doctoral Thesis, Technical University of Munich, Germany, 1998.

(46) (a) Dourtoglou, V.; Gross, B.; Lambropoulou, V.; Zioudrou, C. *O*-Benzotriazolyl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate as coupling reagent for the synthesis of peptides of biological interest. *Synthesis* **1984**, *7*, 572−574. (b) Carpino, L. A.; 1-Hydroxy-7-azabenzotriazole. An Efficient Peptide Coupling Additive. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.

(47) White, P. Fmoc-Trp(Boc)-OH: A new derivative for the synthesis of peptides containing tryptophan. *Pept.: Chem., Struct. Biol., Proc. Am. Pept. Symp., 12th* **1992**, 537−538.

(48) Han, H.; Janda, K. D. Azatides: Solution and Liquid Phase Synthesis of a New Peptidomimetic. *J. Am. Chem. Soc.* **1996**, *118* (11), 2539−2544.

(49) (a) Wermuth, J. Doctoral Thesis, Technical University of Munich, Germany, 1996. (b) Schmitt, J. S. Doctoral Thesis, Technical University of Munich, Germany, 1998.

(50) (a) Quibell, M.; Turnell, W. G.; Johnson, T. Synthesis of Azapeptides by the Fmoc/*tert*-Butyl/Polyamide Technique. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2843−2849. (b) Liley, M.; Johnson, T. Solid phase synthesis of azapeptides utilizing reversible amide bond protection to prevent hydantoin formation. *Tetrahedron Lett.* **2000**, *41*, 3983−3985.

(51) (a) André, F.; Marraud, M.; Tsouloufis, T.; Tzartos, S. J.; Boussard, G. Triphosgene: an efficient carbonylating agent for liquid and solid-phase aza-peptide synthesis. Application to the synthesis of two aza-analogues of the AchR MIR decapeptide. *J. Pept. Sci.* **1997**, *3*, 429−441. (b) Frochot, C.; Vanderesse, R.; Driou, A.; Linden, G.; Marraud, M.; Cung, M. T. A solid-phase synthesis of three aza-, iminoaza- and reduced aza-peptides from the same precursor. *Lett. Pept. Sci.* **1997**, *4*, 219−225.

(52) (a) Gibson, C.; Goodman, S. L.; Hahn, D.; Hölzemann, G.; Kessler, H. Novel Solid-Phase Synthesis of Azapeptides and Azapeptoides via Fmoc-Strategy and Its Application in the Synthesis of RGD-Mimetics. *J. Org. Chem.* **1999**, *64*, 7388−7394. (b) Gibson, C.; Sulyok, G. A. G.; Hahn, D.; Goodman, S. L.; Hölzemann, G.; Kessler, H. Nonpeptidic $\alpha v\beta 3$ Integrin Antagonist Libraries: On-Bead Screening and Mass Spectrometric Identification without Tagging. *Angew. Chem., Int. Ed.* **2001**, *40*, 165−169. (c) Sulyok, G. A. G.; Gibson, C.; Goodman, S. L.; Hölzemann, G.; Wiesner, M.; Kessler, H. Solid-Phase Synthesis of a Nonpeptide RGD Mimic Library: New Selective $\alpha v\beta 3$ Integrin Antagonists. *J. Med. Chem.* **2001**, *44*, 1938−1950.

(53) Wright, S. W.; Hageman, D. L.; McClure, L. D. Fluoride-Mediated Boronic Acid Coupling Reactions. *J. Org. Chem.* **1994**, *59*, 6095−6097.

(54) Dutta, A. S.; Morley, J. S. Polypeptides. Part XIII. Preparation of α-Aza-amino-acid (Carbazic Acid) Derivatives and Intermediates for the Preparation of α-Aza-peptides. *J. Chem. Soc., Perkin Trans. 1* **1975**, *17*, 1712−1720.

(55) Calabretta, R.; Giordano, C.; Gallina, C.; Morea, V.; Consalvi, V.; Scandurra, R. Peptidyl and azapeptidyl methylketones as substrate analog inhibitors of papain and cathepsin B. *Eur. J. Med. Chem.* **1995**, *30* (12), 931−941.

(56) Hansen, T. K. Synthesis of azapeptides from hindered amines leading to novel growth hormone secretagogues. *Tetrahedron Lett.* **1999**, *40* (51), 9119−9120.

(57) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **1997**, *23*, 3−25.

(58) (a) Reynolds, C. H.; Hormann, R. E. Theoretical study of the structure and rotational flexibility of diacylhydrazines: Implications for the structure of nonsteroidal ecdysone agonists and azapeptides. *J. Am. Chem. Soc.* **1996**, *118*, 9395−9401. (b) André, F.; Boussard, G.; Bayeul, D.; Didierjean, C.; Aubry, A.; Marraud, M. Aza-peptides. II. X-Ray structures of aza-alanine and aza-asparagine containing peptides. *J. Pept. Res.* **1997**, *49*, 556−562. (c) André, F.; Vicherat, A.; Boussard, G.; Aubry, A.; Marraud, M. Aza-peptides. III. Experimental structural analysis of aza-alanine and aza-asparagine-containing peptides. *J. Pept. Res.* **1997**, *50*, 372−381. (d) Lee, H.-J.; Anh, I.-A.; Ro, S.; Choi, K.-H.; Choi, Y.-S.; Lee, K.-B. Role of azaamino acid residue in β-turn formation and stability in designed peptide. *J. Pept. Res.* **2000**, *56*, 35−46.

(59) Haubner, R.; Finsinger, D.; Kessler, H. Stereoisomeric peptide libraries and peptidomimetics for designing selective inhibitors of the $\alpha v\beta 3$ integrin for a new cancer therapy. *Angew. Chem., Int. Ed.* **1997**, *3*, 11374−11389.

(60) Heinz-Erian, P.; Coy, D. H.; Tamura, M.; Jones, S. W.; Gardner, J.; Jensen, R. T. [D-Phe^{12}]bombesin analogs: a new class of bombesin receptor antagonists. *Am. J. Physiol.* **1987**, *252*, G439−G442.

(61) Thomas, F.; Arvelo, F.; Antoine, E.; Jacrot, M.; Poupon, M. F. Antitumoral activity of bombesin analogues on small cell lung cancer xenografts: relationship with bombesin receptor expression. *Cancer Res.* **1992**, *52*, 4872−4877.

(62) Coy, D. H.; Taylor, J. E.; Jiang, N. Y.; Kim, S. H.; Wang, L. H.; Huang, S. C.; Moreau, J. P.; Jensen, R. T. Short-chain bombesin receptor antagonists with IC50s for cellular secretion and growth approaching the picomolar region. *Pept.: Chem., Struct. Biol., Proc. Am. Pept. Symp., 11th* **1990**, 65−67.

(63) EC_{50} values were calculated using Graphpad Prism (version 3.00, Graphpad Software) and were obtained as $pEC_{50} \pm$ SEM. For clarity of presentation, they are presented as $EC_{50} \pm$ SEM. Values exceeding 10 nM have been approximated to the full number, values exceeding 100 nM to the full 10, and values larger than 1000 nM to the full 100.

JM0210921