Articles

Nonpeptide Cholecystokinin-2 Receptor Agonists

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In the course of structural explorations around a series of potent CCK_2 receptor antagonists, it was noted that simple *N*-methylation of the indolic N−H in the parent molecule gave rise to behavior in vivo that was consistent with the compound acting as an agonist. Exploration in vitro confirmed this property, and it was shown that the agonist action could be blocked by the reference CCK_2 receptor antagonist, L-365,260. Further examples of this type of modification were explored, and a common theme with regard to agonist behavior was uncovered. Some molecular modeling is also presented in an attempt to throw light on the nature of the ligand receptor interactions that may be giving rise to the differing properties of these, apparently, structurally similar molecules.

Introduction

Agonists of peptide hormone receptors have, in general, been derived by minor structural modifications of the parent hormone.[1] In addition, a number of compounds, shown to be antagonists at peptide hormone receptors in particular tissues, have been shown to behave as agonists when examined in related systems. An example of this may be found in a series of peptoids, devised by Hughes et al.[2] as antagonists at cholecystokinin-2 (CCK_2) receptors, which were found to exhibit efficacy.[3] Peptides and close analogues usually suffer from a number of deficiencies which make their utility as drugs limited. Therefore it is surprising that there are relatively very few reports in the literature of nonpeptide-based small molecules that are agonists at peptide hormone receptors as these would make more credible drug candidates for any medical conditions requiring an enhancement of the endogenous effect of the peptide hormone. Kivlighn et al.[4] have described the discovery of L-162,313, a nonpeptide that mimics the actions of angiotensin II at the AT_2 receptor. This compound is a close analogue of a number of potent AT_2 antagonists such as L-158,809, which was derived from SAR studies on losartan.[5] More recently, a number of papers[6−8] from workers at Glaxo have disclosed a series of benzodiazepine-based CCK_1 receptor agonists and antagonists which also bear a close structural homology to each other. This area has recently been the subject of a review by Sugg.[9] Prompted by this literature we wish to report on our observations regarding the discovery of nonpeptide agonists at CCK_2 receptors. These compounds arose as part of a program in which the SAR of potent antagonists of these receptors were being explored.[10] The compounds differ from most other described CCK_1[11−15] and CCK_2 receptor agonists[16] which have been derived by a more direct consideration of the structure of tetragastrin, the minimum fully active fragment of the hormones, cholecystokinin and gastrin, at CCK_2 receptors.[17] In addition, we suggest, from a computer-generated molecular model, that steric rather than electrostatic fields around the molecules are an important determinant of the expression of efficacy in this receptor system.

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Scheme 1. Synthesis of Anhydrides **14**, **18**, and **20**
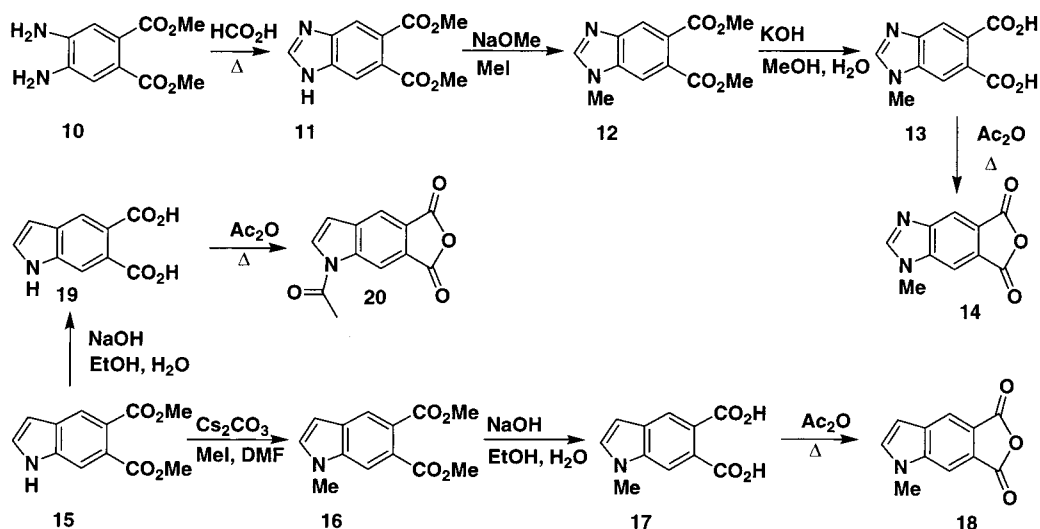
Scheme 2. Synthesis of Regioisomeric Mixtures **3**, **8**, and **9**
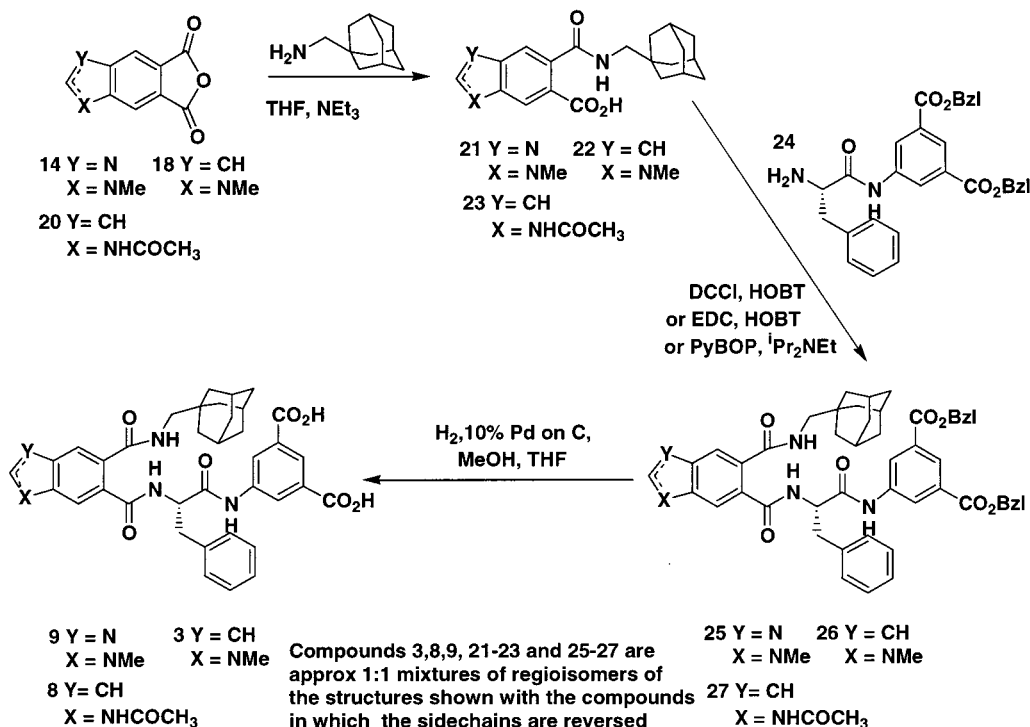

Chemistry

The anhydrides described for the first time in this paper were prepared according to the methodology outlined in Scheme 1. The *N*-methylbenzimidazole anhydride **14** was prepared from dimethyl 4,5-diaminophthalic acid **10** by treatment with hot formic acid to afford the benzimidazole derivative **11**, which was *N*-methylated using sodium methoxide and methyl iodide. The dimethyl ester **12** was hydrolyzed under basic conditions and the resulting diacid **13** converted to the target anhydride **14** using hot acetic anhydride. The modified indole anhydrides were both prepared from dimethyl indole-5,6-dicarboxylate **15**. *N*-Methylation of this intermediate attained using cesium carbonate and methyl iodide followed by hydrolysis and ring closure gave the requisite anhydride **18**, whereas hydrolysis to

the diacid **19** followed by ring closure in hot acetic anhydride gave rise to the *N*-acylated anhydride **20**.

Preparation of the mixtures of regioisomers from the anhydrides described above is shown in Scheme 2. In each case the anhydride was opened with 1-adamantanemethylamine in a mixture of triethylamine and THF to yield an approximately 1:1 mixture of regioisomeric amide acids. Treatment of the amide acids with compound **24** and a variety of coupling reagents gave rise to 1:1 mixtures of the benzyl ester-protected triamides. The target compounds were isolated as regioisomers following deprotection using hydrogen gas over a 10% palladium on charcoal catalyst in a solvent mixture of methanol and THF.

As the mixtures shown in Scheme 2 could not be separated at any stage by column chromatography, the regioisomerically pure indole derivatives required for

Scheme 3. Synthesis of Compounds **4–7**[a]
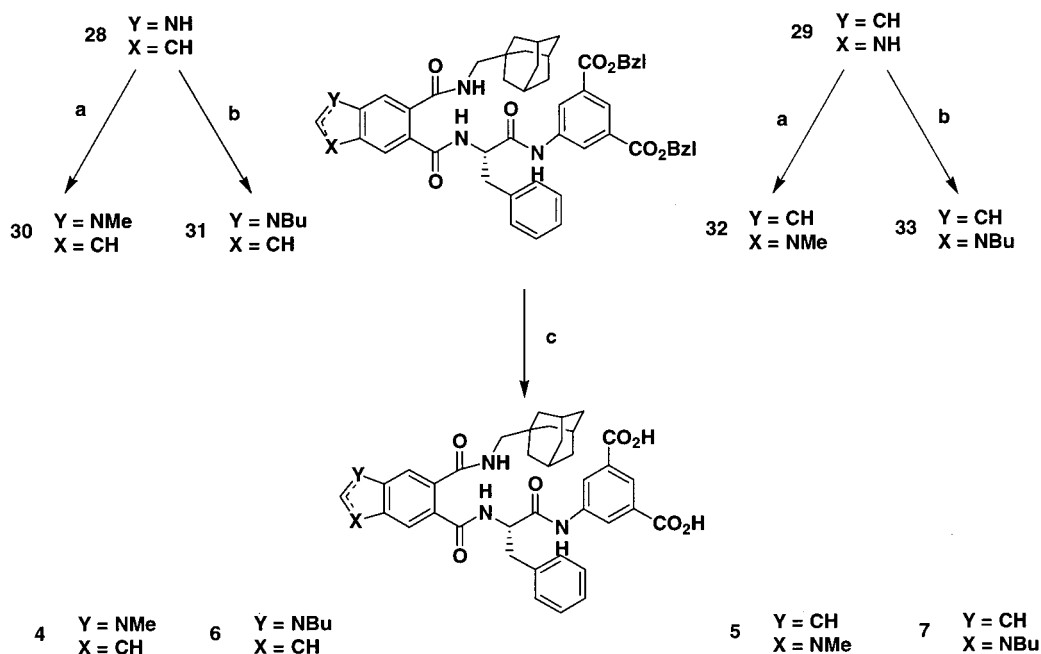
[a] (a) NaH, MeI, THF; (b) NaH, ^{n}BuI; (c) H$_2$, 10% Pd–C, MeOH/THF.

testing had to be prepared from separate intermediates that have already been described.[10] These compounds were prepared as shown in Scheme 3, initially by alkylation of compounds **28** and **29** using sodium hydride and the appropriate alkyl iodide, followed by hydrogenation using a catalyst of 10% palladium on charcoal in a solvent mixture of methanol and THF.

Results and Discussion

We have recently described a series of nonpeptide CCK$_2$ receptor antagonists based on a disubstituted indole framework.[10] These particular compounds, exemplified by **1** and **2**, had nanomolar affinities in the two principal CCK$_2$ receptor assays as shown in Table 1: namely the immature, isolated, lumen-perfused, pentagastrin-stimulated rat stomach[18] and a radioligand binding assay in mouse cortical membranes using [^{125}I]BH-CCK-8S as radiolabel.[19] Roberts et al.[20] and Harper et al.[19] suggest the existence of at least two CCK$_2$ receptor subtypes with apparently single and differing populations being present in these two particular tissues. From the data obtained for compound **1**, it may be suggested that it is slightly more selective for the single receptor site found in the rat stomach relative to the site found in the mouse cortex. In contrast, the isolated, lumen-perfused, pentagastrin-stimulated mouse stomach assay has been shown[20] to contain a mixed and variable population of at least two CCK$_2$ receptors, and this leads to complex behavior for selective antagonists. The affinity estimate obtained in the mouse stomach (pK_B 8.81) was similar to the value found in the cortex, consistent with the lower-affinity site being predominant in this particular experiment.

Due to the potential complexity in the behavior of compounds in the mouse stomach bioassay, compounds were not routinely assessed in this system. However, with respect to prototype drug discovery in the CCK$_2$ receptor field, the most important and useful property of this mouse stomach assay is that of all the in vitro

Table 1. Comparison of Receptor Activity Values for CCK$_2$ Ligands in Various Assays

no.[a]	Y	X	rat stomach[c]	mouse cortex[d]	mouse stomach[e]
1	NH	CH	9.90 ± 0.18	8.96 ± 0.24	no[f]
2	CH	NH	9.26 ± 0.28	7.87 ± 0.16	no[g]
3[b]	NMe	CH	9.80 ± 0.34	8.42 ± 0.11	yes
4	NMe	CH	8.99 ± 0.18	9.43 ± 0.18	yes
5	CH	NMe	8.57 ± 0.20	8.36 ± 0.02	yes
6	NBu	CH	7.35 ± 0.24	8.44 ± 0.30	yes
7	CH	NBu	6.86 ± 0.23	7.52 ± 0.21	yes
8	NCOCH$_3$	CH	8.72 ± 0.44	8.74 ± 0.09	yes
9[b]	NMe	N	8.95 ± 0.45	7.60 ± 0.03	yes

[a] Compounds were tested as the bis(*N*-methyl-D-glucamine) salts. [b] Compounds indicated were an approximately 50:50 mixture of regioisomers between the compound shown and that with X and Y reversed. [c] pK_B ± SEM values were estimated from single shifts of pentagastrin concentration–effect curves in the isolated, lumen-perfused immature rat stomach, calculated assuming an underlying Schild slope of unity and fitted using the Gaddum–Schild equation. [d] pK_B ± SEM competition with 20 pM [^{125}I]BH-CCK-8S for CCK$_2$ binding sites in mouse cortical homogenates from at least three separate experiments. [e] Observation of acid secretion due to compound alone in the isolated, lumen-perfused mouse stomach. [f] pK_B value of compound estimated from the lowest concentration which produced a significant shift of pentagastrin concentration–effect curves (8.81 ± 0.21) with assumptions and calculations as in footnote c. [g] pK_B value of compound estimated from the lowest concentration which produced a significant shift of pentagastrin concentration–effect curves (7.16 ± 0.43) with assumptions and calculations as in footnote c.

assays available, it is the most sensitive for detecting residual efficacy in molecules. This may be rationalized in terms of a number of tissue-dependent factors such as high receptor density or particularly efficient receptor G protein coupling.[21] Therefore, compounds such as PD-

134,308, which appear as antagonists in assays such as the isolated, immature, lumen-perfused rat stomach, can behave as low-efficacy agonists when examined in the analogous mouse stomach assay.[22] It should be noted that when PD-134,308 was examined in a chronic gastric fistula dog model in vivo, it behaved as a full agonist (data not shown) indicating that the relatively low level of agonism manifest in the mouse stomach was capable of substantial amplification. Thus examining selected compounds in the mouse stomach in order to minimize the chance of encountering agonist-type behavior in vivo is an important part of our screening strategy. Compounds **1** and **2** were found to be devoid of efficacy when examined in the mouse stomach assay, a finding that was further confirmed when compound **1** was examined in the chronic gastric fistula dog model.[10]

As part of the exploration of the salient features controlling the activity of compound **1**, it was decided to examine whether the hydrogen attached to the indole nitrogen atom had any direct interaction with the receptor via a hydrogen bond. For this reason, the indole *N*-methyl compound was specified and synthesized, initially as a mixture of regioisomers **3**. The affinity of this compound at CCK_2 receptors was similar to that of compound **1** in both the rat stomach assay and the mouse cortex radioligand binding assay. The first surprise with this compound came when it was examined in vivo in the chronic gastric fistula dog,[10] where it was ineffective at inhibiting pentagastrin-stimulated acid secretion at a dose of 1 μmol/kg by intravenous bolus (data not shown). This dose was 10-fold greater than that of compound **1**, which was able to cause a substantial inhibition in the same model. Intrigued by this result, we speculated that this apparent lack of antagonist activity might be because compound **3** was behaving as an agonist.

To address this possibility in vitro, we decided to examine the behavior of the compound in the isolated mouse stomach bioassay. In this assay, compound **3** was found to stimulate basal acid secretion, and the concentration—response curve obtained for the compound suggested that it was behaving as a partial agonist with a potency approximately equal to that of pentagastrin. We confirmed that this acid secretion was mediated by CCK_2 receptors by looking at whether the agonism could be blocked by the presence of a receptor antagonist and used L-365,260[23] for this experiment. It was found that inclusion of this antagonist shifted the dose response of compound **3**, and the pK_B obtained for the antagonist was approximately 7.0 (Figure 1 and Table 2). While this figure is rather lower than the commonly reported affinity of this compound at CCK_2 receptors (for example ref 23 quotes a pK_i of 8.7), both we[20] and others[24] have shown that in some tissues there are sites for which the compound has an affinity of this order. Given this low value, it was possible to infer that the agonism observed in this experiment was being mediated only through the CCK_2 site for which L-365, 260 has the lower affinity, and this is the one that is commonly found in the rat stomach. The dose—response curve obtained for pentagastrin in the same experiment is shown for comparative purposes. The agonism was confirmed in vivo in the chronic gastric fistula dog, as acid secretion could
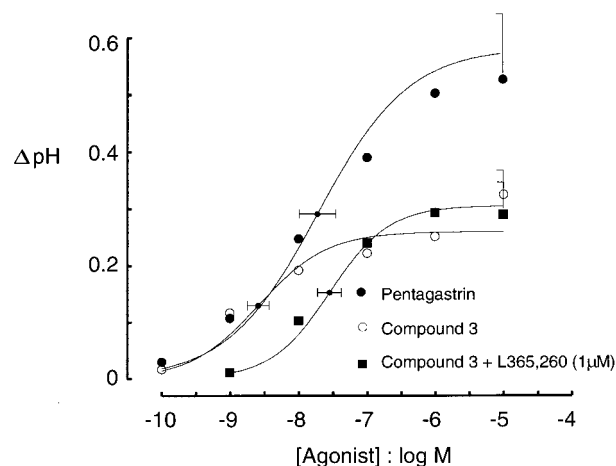

Figure 1. Agonist dose—response curves in isolated, lumen-perfused mouse stomach for compound **3** in the presence and absence of L-365,260 and pentagastrin ($n = 6$).

Table 2. Characterization of Agonist Response of Ligands in Isolated Mouse Stomach Bioassay

no.	agonist curve location[a]	affinity of L-365,260[b]	slope of Schild plot
3	8.40 ± 0.40^c	7.04 ± 0.35 (pK_B)[d]	ND^e
4	8.69 ± 0.15	7.02 ± 0.14 (pK_B)	1.02 ± 0.22
5	7.94 ± 0.17	7.90 ± 0.24 (pA_2)	0.39 ± 0.24
pentagastrin	7.73 ± 0.27	8.01 ± 0.30 (pA_2)	0.61 ± 0.15

[a] Mean pA_{50} based on the results of 7−10 different control concentration—response curves. [b] pK_B of L-365,260 obtained from Schild analysis with slope not significantly different from unity. A pA_2 was calculated when the Schild plot slope parameter was significantly different from unity and represents the shift of the agonist curve produced by the lowest concentration of the antagonist that produces a significant shift. [c] The standard errors associated with this data point are large due to the steepness of the dose—response curve. [d] pK_B of L-365,260 against agonism of the compound based on at least six individual data points at only a single concentration of the antagonist, calculated assuming an underlying Schild slope of unity and fitted using the Gaddum—Schild equation. [e] Schild analysis not performed.
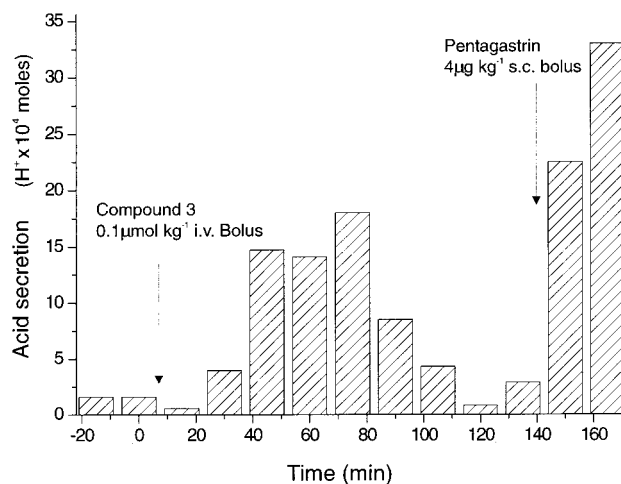

Figure 2. Typical acid secretory response due to the administration of an intravenous bolus dose of compound **3** and a subcutaneous bolus dose of pentagastrin to conscious, gastric fistula dogs. Each data block represents the total acid collected in a 15-min time period.

be seen when the compound was administered intravenously. Figure 2 shows the magnitude of this response relative to that induced by a 4 μg/kg subcutaneous bolus dose of pentagastrin.
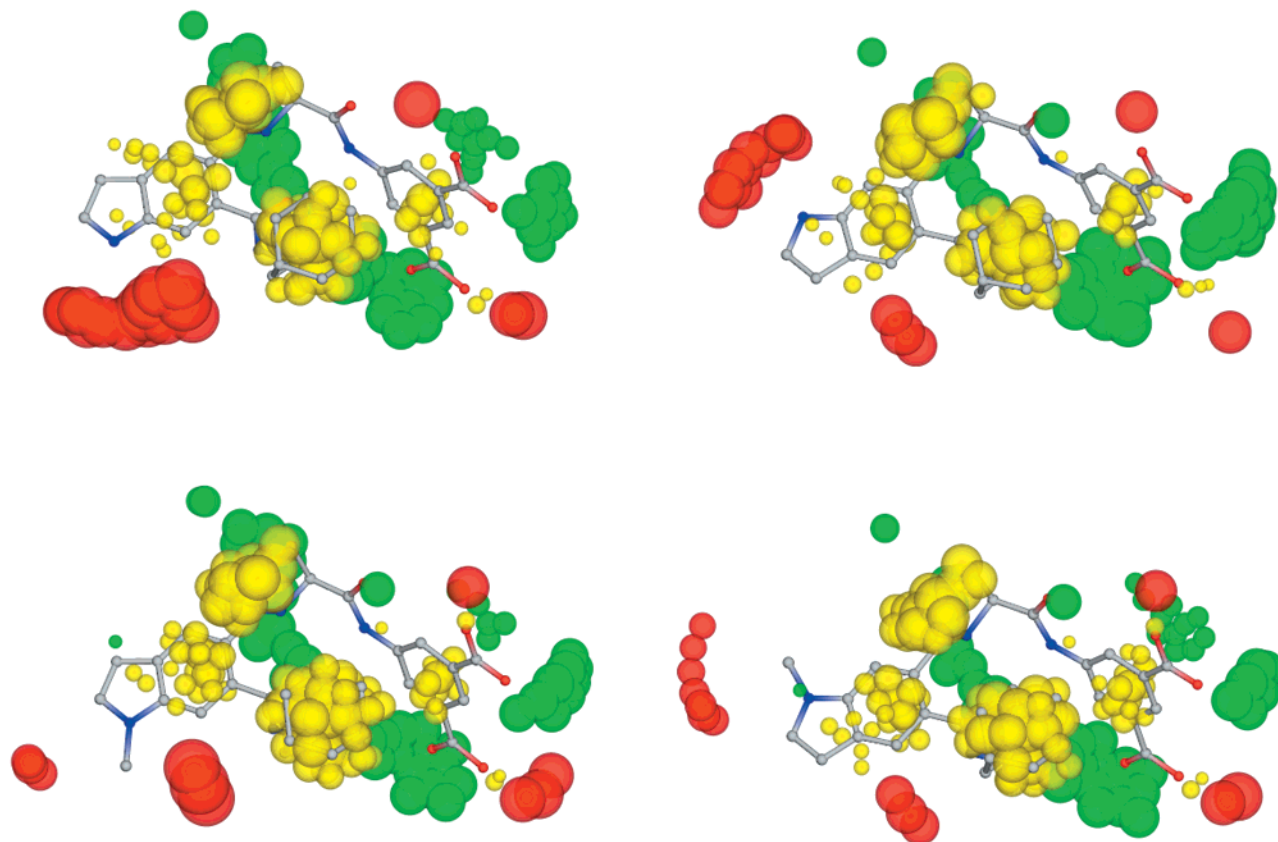
Figure 3. Typical low-energy fields of compounds **1** (top left), **2** (top right), **4** (bottom left), and **5** (bottom right) showing positive (red), negative (green), and steric (yellow) field points.

The mixture of regioisomers present in **3** were separated in order to see whether there was any difference in activity between them. As shown in Table 1, it was found that both the individual isomers **4** and **5** had broadly similar receptor affinities to their corresponding nonmethylated analogues and that they behaved as partial agonists in the mouse stomach assay, with compound **4**, the regioisomer corresponding to compound **1**, appearing slightly more potent. L-365,260 (30 nM−1 μM) behaved as an antagonist toward the acid secretion expressed by compound **4**, a finding that was consistent with this effect being CCK₂ receptor-mediated agonism. The Schild slope parameter was not significantly different from unity, indicating that the agonism produced by compound **4** was via a homogeneous receptor population for which L-365,260 expressed its lower affinity (Table 2, pK_B 7.02 ± 0.14). Interestingly, a similar experiment performed with compound **5** as the agonist had a flat Schild plot slope ($b = 0.39 ± 0.24$), and the pA_2 estimated from the lowest concentration of L-365,-260 which produced a significant shift of the agonist curve was approximately 7.90 suggesting that the acid secretion of this compound might be occurring through both the putative receptor sites present in this tissue. As discussed above, given the variable expression of the two receptors in the mouse stomach and relatively small amount of agonism detected, such effects are difficult to quantify accurately.

A number of further compounds were made in which the indolic N−H was substituted, and all those listed in Table 1 behaved qualitatively as partial agonists in the mouse stomach. The molecules made included the [n]butyl homologues of compounds **4** and **5**, the acetyl version of compound **3**, and an *N*-methylbenzimidazole derivative **9**. While maintaining respectable affinity at CCK₂ receptors, all these molecules, to a greater or lesser extent, caused an acid secretory effect in the mouse stomach. The amounts observed were generally small, so that it was not feasible to test whether the acid secretion was sensitive to L-365,260. However, given the structural similarities to compound **3** and the consistency of the observation of the phenomenon with this type of modification, we felt it was reasonable to conclude that the efficacy expressed by all these compounds was through CCK₂ receptors.

To try to rationalize the emergence of residual efficacy in the *N*-substituted indoles, the multiconformational composite molecular potential field method of Vinter and Trollope[25,26] was used to compare compounds **1**, **2**, **4**, and **5**. The first two compounds were antagonists[10] and the latter two were agonists when examined in the mouse stomach bioassay. Electrostatic and van der Waals field points[10] were calculated for all low-energy conformations of each molecule, and all possible combinations of these conformational sets were compared on the basis of these parameters. The results are illustrated in Figure 3, which shows typical low-energy representations of the four molecules, with the corresponding positive (red) and negative (green) electrostatic field points surrounding them. When represented in this way, while there are evident differences between molecules, we could see no consistent pattern of field points which distinguishes the antagonists from the agonists in the mouse stomach bioassay. Instead one must look to the steric differences around the indolic nitrogen atom for an explanation of the observations. Clearly, where

even modest bulk has been introduced, such as in compounds **4** and **5** with the presence of a methyl group, residual efficacy has become a property of these molecules. Intriguingly, substitution of both regioisomers gives rise to the same phenomenon, and there are two possible ways of rationalizing this finding. First, if one assumes that both compounds **4** and **5** bind to the receptor with the same relative orientation of the two side chains, then the methyl groups will vary in their position within the receptor binding pocket. This suggests that the feature of importance with regard to triggering efficacy at the receptor has to be equally accessible to methyl groups in both positions. To allow equivalent access, this putative residue on the receptor would probably have to be located in the plane perpendicular to that occupied by the bicyclic ring. The second possibility is that both *N*-substituted indoles are somewhat wider than their unsubstituted counterparts. Thus sterically, both would occupy a greater area of space and so may be impinging on residues in the receptor protein that might trigger efficacy.

It should be noted that apparently a similar steric requirement pertains when one considers the agonist properties of the CCK$_1$ ligands reported by Aquino,[6] in that the NH compound **34** is an antagonist, whereas the more sterically demanding, *N*-ethyl compound **35** behaves as an agonist. This suggests that the activation mechanism of the CCK$_1$ and CCK$_2$ receptors might share some common features.

34	R=H
35	R=Et
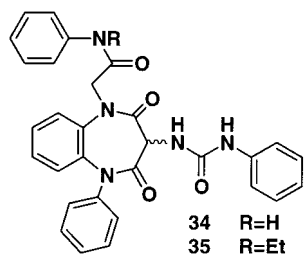
Our findings, and the other recent reports of nonpeptide agonists at peptide hormone receptors, also start to challenge a view that has been gaining credence about these classes of receptors: that peptide agonists and nonpeptide antagonists at the same receptor always bind at different sites on peptide hormone receptors. Certainly there is good evidence from point mutation studies that peptide hormones such as substance P bind to the extracellular domain of their GPCR receptors,[27,28] while smaller nonpeptide antagonists interact with residues in the transmembrane helices. However, there is physical evidence to suggest that this is not always the case: For example, in the CCK$_1$ receptor it is suggested that the hormone penetrates the transmembrane region.[29] The data described herein supports the more traditional view that agonist and antagonist binding sites on the protein can be congruent. Compounds **1** and **4** have broadly similar affinities in the rat stomach, whereas only compound **4** behaves as an agonist at the receptors in the mouse stomach. The nature of the receptor present in the two assays when our experiments were performed could not be distinguished on the basis of their affinity for L-365,260.[20] The simplest explanation of these data is that, given the similarities in the affinity and structure of the

molecules, both of these compounds bind at the same site on the unactivated CCK$_2$ receptor when they are behaving as antagonists as is the case in the rat stomach assay. However, compound **4**, which possesses residual efficacy, is able to induce, or stabilize, an activated form of the receptor, and in certain tissues, such as the mouse stomach, this is evident as agonism. Thus, unless compound **4** and other analogues possessing residual efficacy are able to migrate substantially within the receptor, or there are a multitude of pathways and binding positions possible from which to activate this receptor, a possibility that cannot be discounted based on a recent report from Blacker et al.,[30] it is likely that both molecules, the agonist and the antagonist, are sharing the same principal recognition site as the parent hormone. Thus, logically in this case, in contrast to the situation pertaining in the point mutation studies for substance P receptors referred to above, there is a substantial congruence between the site of interaction of the parent hormone with the receptor and the location of the binding pocket for the antagonist, compound **1**.

The contrast in behavior of the CCK$_2$ and substance P systems illustrates the dynamic nature of ligand–receptor interactions and indicates that there may be several possible sites from which an antagonist can exert an inhibitory influence on the actions of an agonist. When an agonist binds to the receptor it is able to induce conformational changes in that receptor, via a number of possible interactions, which lead to a cascade of events resulting in a signal. Antagonists, it would appear, fall into two distinct groups. There are those which compete directly for the agonist site but which either do not interact with or indeed, stabilize, the agonist 'trigger'. Alternatively, there are those which bind at other sites in the receptor protein either blocking conformational changes within the receptor that lead to signal transduction or promoting alternative conformational changes in a such a way as to distort the binding site for the agonist. This latter mechanism implies an allosteric interaction between the two sites.

In conclusion, during a program aimed at searching for CCK$_2$ receptor antagonists, we have found that a particular structural change gives rise to compounds that show agonist behavior in some of our assays. We have interpreted this observation in terms of subtle differences in the steric, rather than electrostatic, fields around the molecules. It is evident that seemingly minor changes in the structure of the ligand can lead to fundamental changes in the nature of the ligand–receptor interaction not only with respect to affinity but also in the expression of efficacy.

Experimental Section

General. NMR spectra were recorded on either a Nicolet GE300 or a Bruker DRX 300 machine. Elemental analyses were carried out at the London School of Pharmacy, and all compounds gave analytical results of ±0.4% of theoretical values. Flash column chromatography was performed using Merck Kieselgel 60 silica grade 9385.

Dimethyl Benzimidazole-5,6-dicarboxylate (11). Dimethyl 4,5-diaminophthalate (224 mg, 1.0 mmol), prepared in several standard steps from 4-nitrophthalic acid, was dissolved in 97% formic acid (1 mL) and stirred at a gentle reflux for 2 h. On cooling, the solution was carefully poured onto a saturated aqueous solution of sodium hydrogencarbonate (20

mL) and then extracted with dichloromethane (2 × 10 mL). The combined organic layers were dried (magnesium sulfate), filtered and evaporated resulting in a solid that was recrystallized from ethanol to leave the pale pink colored title compound (200 mg, 85%): ^{1}H NMR (CDCl₃) δ 8.2 (2H, s), 8.0 (2H, br s), 3.9 (6H, s).

Dimethyl 1-Methylbenzimidazole-5,6-dicarboxylate (12). Sodium (25 mg, 1.1 mmol) was dissolved in dry methanol (3 mL) under an atmosphere of dry nitrogen. Compound **11** (234 mg 1.0 mmol) was added and the solution stirred for 30 min. Methyl iodide (100 μL, 1.6 mmol) was added and the resulting solution stirred at room temperature overnight. The solution was then warmed briefly to reflux, cooled, evaporated and purified by column chromatography (silica, 95% dichloromethane−5% methanol) to leave the title compound (170 mg, 69%) as a white solid: ^{1}H NMR (CDCl₃) δ 8.2 (1H, s), 8.0 (1H, br s), 7.8 (1H, s), 3.94 (6H, s), 3.91 (3H, s).

1-Methylbenzimidazole-5,6-dicarboxylic Acid Anhydride (14). Step a. To compound **12** (2.27 g, 9.2 mmol) dissolved in 9:1 methanol:water (30 mL) was added potassium hydroxide (1.12 g, 20.0 mmol). The solution was stirred overnight at room temperature. The solution was evaporated and the residue taken up in a minimum volume of water and acidified with hydrochloric acid. The precipitated white solid was filtered, washed with water and dried in vacuo, to leave compound **13** (1.50 g) which was used without further purification.

Step b. The crude diacid was suspended in acetic anhydride (7 mL) and stirred and heated at a reflux for 2 h. The solution was cooled and diluted with diethyl ether, whereupon a yellow solid was precipitated, filtered, washed with diethyl ether and dried in vacuo. This left the title compound (0.95 g, 51%): ^{1}H NMR (DMSO-d₆) δ 8.7 (1H, s), 8.4 (1H, s), 8.3 (1H, s), 3.94 (6H, s), 4.0 (3H, s).

Dimethyl 1-Methylindole-5,6-dicarboxylate (16). Dimethyl indole-5,6-dicarboxylate[10] (**15**; 1.95 g, 8.3 mmol) was dissolved in dry dimethylformamide (10 mL); cesium carbonate (2.72 g, 8.3 mmol) and methyl iodide (2.6 mL, 42.0 mmol) were added. The reaction mixture was heated to a reflux for 15 min and then cooled and evaporated. The residue was taken up in ethyl acetate (50 mL) and washed successively with 2 M hydrochloric acid (25 mL), water (25 mL), saturated aqueous sodium hydrogencarbonate (25 mL) and brine (2 × 25 mL). The organic layer was dried (magnesium sulfate) filtered and evaporated resulting in a pale yellow gum. This was resuspended in dichloromethane and re-evaporated to leave the title compound as a beige solid (1.94 g, 94%): ^{1}H NMR (CDCl₃) δ 8.0 (1H, s), 7.7 (1H, s), 7.2 (1H, d), 6.6 (1H, d), 3.93 (3H, s), 3.91 (3H, s), 3.8 (3H, s).

1-Methylindole-5,6-dicarboxylic Acid (17). **16** (1.93 g, 7.8 mmol) was dissolved in 9:2 ethanol:water (20 mL) and sodium hydroxide (630 mg, 15.7 mmol) was added. The solution was stirred and heated to a reflux for 15 min. After cooling and evaporation to a volume of about 2 mL, 2 M hydrochloric acid was used to precipitate the title compound which was isolated by filtration, washed with water and dried in vacuo (1.69 g, 98%): ^{1}H NMR (DMSO-d₆) δ 12.6 (2H, Br s), 7.9 (1H, s), 7.7 (1H, s), 7.5 (1H, d), 6.6 (1H, d), 3.8 (3H, s).

1-Methylindole-5,6-dicarboxylic Acid Anhydride (18). **17** (1.69 g, 7.7 mmol) was suspended in acetic anhydride (14 mL) and stirred and heated at a reflux for 1 h. The solution was cooled and the yellow crystals were filtered, washed with diethyl ether and dried in vacuo. This left the title compound (1.42 g, 92%): ^{1}H NMR (DMSO-d₆) δ 8.3 (2H, br s), 7.8 (1H, br s), 6.8 (1H, br s), 4.0 (3H, br s).

Indole-5,6-dicarboxylic Acid (19). Dimethyl indole-5,6-dicarboxylate[10] (**15**; 3.05 g, 13.1 mmol) was dissolved in a mixture of ethanol (28 mL) and water (6 mL) and sodium hydroxide (1.31 g, 32.8 mmol) was introduced. The solution was stirred overnight at room temperature and concentrated hydrochloric acid was added so that a pH of 2 was achieved. The solvent was evaporated to give a white solid which was re-suspended in ethanol and evaporated followed by treatment with toluene and evaporation. The residue was dried in vacuo

and then extracted with hot acetone (5 × 20 mL). The combined acetone extracts were evaporated to leave the diacid as a yellow solid (2.07 g, 77%): ^{1}H NMR (DMSO-d₆) δ 11.6 (1H, s), 7.9 (1H, s), 7.7 (1H, s), 7.6 (1H, d), 6.6 (1H, d).

1-Acetylindole-5,6-dicarboxylic Acid Anhydride (20). **19** (1.01 g, 4.9 mmol) was dissolved in acetic anhydride (10 mL) and heated at reflux under an atmosphere of argon for 1h. On cooling a yellow, crystalline solid formed which was collected by filtration, washed in diethyl ether and dried in vacuo at 50 °C, to leave the title compound (504 mg, 44%): ^{1}H NMR (DMSO-d₆) δ 8.8 (1H, s), 8.33 (1H, s), 8.29 (1H, d), 7.0 (1H, d), 2.7 (3H, s).

1-Methyl-5(6)-[[(1-adamantylmethyl)amino]carbonyl]-benzimidazole-6(5)-carboxylic Acid (21). Compound **14** (950 mg, 4.7 mmol) was added to a stirred solution of 1-adamantylmethylamine (860 mg, 5.2 mmol) in dry THF (50 mL). The solution was stirred at room temperature for 1.5 h, whereupon the pale, yellow precipitate was isolated by filtration, washed with THF and dried in vacuo at 50 °C, to leave the title compound (1.65 g, 96%): ^{1}H NMR (DMSO-d₆) δ 9.5 (1H, bs), 8.3 (1H, s), 7.84 (1H, s), 7.75 (1H, 2 × s), 3.9 (3H, 2 × s), 2.9 (2H, m), 1.9 (3H, s), 1.73 (6H, q), 1.69 (6H, s). The spectrum being of regioisomers was more complex than would be expected for a single compound. Integration suggested a ca. 1:1 mixture.

1-Methyl-5(6)-[[(1-adamantylmethyl)amino]carbonyl]-indole-6(5)-carboxylic Acid (22). Compound **18** (1.42 g, 7.04 mmol) was added to a stirred solution of 1-adamantylmethylamine (1.16 g, 7.0 mmol) in dry THF (40 mL) and triethylamine (1.08 mL, 7.7 mmol). The solution was heated to reflux for 5 min and then stirred at room temperature for 2 h, whereupon 2 M hydrochloric acid was added and the precipitate that formed was isolated by filtration, washed with water and dried in vacuo, to leave the title compound (2.57 g, 99%): ^{1}H NMR (DMSO-d₆) δ 8.1−7.4 (3H, m), 6.5 (1H, m), 3.8 (3H, 2 × s), 2.9 (2H, m), 1.9 (3H, s), 1.6 (6H, q), 1.5 (6H, s). The spectrum being of regioisomers was more complex than would be expected for a single compound. Integration suggested a ca. 2:3 mixture, but the predominant regioisomer could not be identified.

1-Acetyl-5(6)-[[(1-adamantylmethyl)amino]carbonyl]-indole-6(5)-carboxylic Acid (23). **20** (578 mg, 2.5 mmol) was added to a stirred solution of 1-adamantylmethylamine (458 mg, 2.8 mmol) and DMAP (10 mg, catalytic) in dry THF (9 mL) and triethylamine (386 μL, 2.8 mmol). The solution was stirred at room temperature overnight, whereupon 2 M hydrochloric acid (20 mL) was added and the mainly aqueous solution was extracted with dichloromethane (3 × 20 mL). The organic layer was washed with brine, dried (magnesium sulfate) filtered and evaporated resulting in the title compound (1.03 g, 99%): ^{1}H NMR (DMSO-d₆) δ 8.7 and 8.4 (1H, 2 × s), 8.2 (1H, t), 8.0 (2H, m), 6.8 (1H, s), 2.9 (2H, m), 2.6 (3H, s), 1.9 (3H, s), 1.6 (6H, q), 1.5 (6H, s). The spectrum being of regioisomers was more complex than would be expected for a single compound. Integration suggested a ca. 1:1 mixture.

1-Methyl-5(6)-[[[1(*S*)-[[[3,5-bis(benzyloxycarbonyl)-phenyl]amino]carbonyl]-2-phenylethyl]amino]carbonyl]-6(5)-[[(1-adamantylmethyl)amino]carbonyl]-benzimidazole (25). **21** (367 mg, 1.0 mmol), 1(*S*)-[[[3,5-bis-(benzyloxycarbonyl)phenyl]amino]carbonyl]-2-phenylethylamine (**24**;[31] 510 mg, 1.0 mmol), hydroxybenzotriazole (135 mg, 1.0 mmol), EDC (192 mg, 1.0 mmol) and DMAP (5 mg, catalytic) were dissolved in dry DMF (3 mL) and stirred at room temperature overnight. The solution was poured into stirred water (30 mL) and the resulting buff precipitate was isolated by filtration, washed with water and dried in vacuo. Column chromatography (silica, 5% methanol−95% dichloromethane) left the title compound (250 mg, 29%): ^{1}H NMR (DMSO-d₆) δ 10.3 (1H, s), 8.8 (3H, m), 8.3 (3H, m), 7.9 (1H, 2 × s), 7.4−7.0 (16H, m), 5.4 (4H, s), 4.8 (1H, m), 3.9 (3H, 2 × s), 3.6−2.5 (4H, m), 1.8 (3H, s), 1.6−1.4 (12H, m). Integration suggested a ca. 1:1 mixture.

1-Methyl-5(6)-[[[1(*S*)-[[[3,5-bis(benzyloxycarbonyl)-phenyl]amino]carbonyl]-2-phenylethyl]amino]carbonyl]-

6(5)-[[[(1-adamantylmethyl)amino]carbonyl]indole (26). **22** (500 mg, 1.4 mmol) was dissolved in dry dichloromethane (40 mL) and PyBOP (710 mg, 1.4 mmol) and diisopropylethylamine (720 μL, 4.1 mmol) were added. After stirring at room temperature for 1 h, **24**[31] (694 mg, 1.4 mmol) was added and the mixture stirred overnight. The organic layer was washed successively with 5% potassium hydrogen sulfate (5 mL), saturated sodium hydrogen carbonate (5 mL) and saturated brine (5 mL). The organic layer was then dried, filtered and evaporated before being purified by column chromatography (silica, 20% ethyl acetate−80% dichloromethane) to leave the title compound (585 mg, 50%): ^{1}H NMR (DMSO-d_6) δ 10.3 (1H, s), 8.8 (3H, m), 8.4 (1H, m), 8.3 (1H, s), 7.9 and 7.8 (1H, 2 × s), 7.4 (16H, m), 7.1 and 6.8 (1H, 2 × s), 6.6 and 6.5 (1H, 2 × d), 5.4 (4H, s), 4.8 (1H, m), 3.8 (3H, m), 3.4 and 2.9 (4H, m), 1.8 (3H, s), 1.5 (6H, m), 1.4 (6H, s). Integration of key peaks suggested a ca. 1:1 mixture.

1-Acetyl-5(6)-[[[1(*S*)-[[[3,5-bis(benzyloxycarbonyl)phenyl]amino]carbonyl]-2-phenylethyl]amino]carbonyl]-6(5)-[[[(1-adamantylmethyl)amino]carbonyl]indole (27). **23** (1.18 g, 2.5 mmol), **24**[31] (958 mg, 1.9 mmol), 1-hydroxybenzotriazole (340 mg, 2.5 mmol) and DCCI (519 mg, 2.5 mmol) were dissolved in dry DMF (13 mL) and stirred at room temperature overnight. A precipitate was formed which was removed by filtration, the residue being washed with a small quantity of dichloromethane. Further dichloromethane (50 mL) was added to the filtrate and the solution washed successively with 2 M hydrochloric acid (10 mL), water (10 mL) and saturated sodium hydrogencarbonate (10 mL). After drying and filtering, the product was purified by column chromatography (silica, 20% ethyl acetate−80% dichloromethane) to leave the title compound (1.12 g, 67%): ^{1}H NMR (DMSO-d_6) δ 10.3 (1H, s), 9.9 and 9.0 (1H, 2 × d), 8.8 (2H, m), 8.6 (1H, s), 8.5 (1H, m), 8.3 (1H, d), 8.2 (1H, s), 8.0 and 7.9 (1H, 2 × m), 7.4 (15H, m), 6.9 and 6.8 (1H, 2 × d), 5.4 (4H, s), 4.7 (1H, m), 3.4 and 2.9 (4H, m), 2.7 (3H, 2 × s), 1.8 (3H, s), 1.6 (6H, m), 1.3 (6H, s). Integration suggested a ca. 1:1 mixture.

General Method for Hydrogenation. 1-Methyl-5(6)-[[[1(*S*)-[[(3,5-dicarboxyphenyl)amino]carbonyl]-2-phenylethyl]amino]carbonyl]-6(5)-[[[(1-adamantylmethyl)amino]carbonyl]indole (3). **26** (560 mg, 0.7 mmol) was dissolved in a 1:1 mixture of THF and methanol (30 mL) and a catalytic quantity of 10% palladium on charcoal was added. The suspension was stirred at room temperature under an atmosphere of hydrogen overnight, filtered through Celite and evaporated to leave the title compound (442 mg, 99%): ^{1}H NMR (DMSO-d_6) δ 13.2 (2H, br s), 10.2 (1H, 2 × s), 8.7 (3H, m), 8.4 (1H, m), 8.2 (1H, s), 7.9 and 7.8 (1H, 2 × s), 7.5 (1H, m), 7.4 (5H, m), 7.1 and 6.8 (1H, 2 × s), 6.6 and 6.5 (1H, 2 × d), 4.7 (1H, m), 3.8 and 3.7 (3H, 2 × s), 3.4 and 2.9 (4H, m), 1.8 (3H, s), 1.5 (6H, m), 1.4 (6H, s). Integration suggested a ca. 1:1 mixture; further characterized as the bis(*N*-methyl-D-glucamine) salt. Anal. ($C_{39}H_{40}N_4O_7 \cdot 2C_7H_{17}NO_5 \cdot 2.3H_2O$) C, H, N.

1-Acetyl-5(6)-[[[1(*S*)-[[(3,5-dicarboxyphenyl)amino]carbonyl]-2-phenylethyl]amino]carbonyl]-6(5)-[[[(1-adamantylmethyl)amino]carbonyl]indole (8). This was prepared from **27** using the general hydrogenation methodology described above for **3**: ^{1}H NMR (DMSO-d_6) δ 13.3 (2H, br s), 10.2 (1H, 2 × s), 9.0 and 8.9 (1H, 2 × d), 8.6 (3H, m), 8.14 (1H, s), 8.10 and 7.9 (1H, 2 × s), 8.0 (1H, s), 7.4 (5H, m), 6.9 and 6.8 (1H, 2 × d), 4.7 (1H, m), 3.4 and 2.9 (4H, m), 2.7 (3H, 2 × s), 1.8 (3H, s), 1.6 (6H, m), 1.3 (6H, s). Integration suggested a ca. 1:1 mixture; further characterized as the bis(*N*-methyl-D-glucamine) salt. Anal. ($C_{40}H_{40}N_4O_8 \cdot 2C_7H_{17}NO_5 \cdot 2.6H_2O$) C, H, N.

1-Methyl-5(6)-[[[1(*S*)-[[(3,5-dicarboxyphenyl)amino]carbonyl]-2-phenylethyl]amino]carbonyl]-6(5)-[[[(1-adamantylmethyl)amino]carbonyl]indole (9). This was prepared from **25** using the general hydrogenation methodology described above for **3**: ^{1}H NMR (DMSO-d_6) δ 13.0 (2H, br s), 10.2 (1H, s), 8.8 (1H, m), 8.7 (2H, s), 8.6 (2H, m), 8.2 (1H, s), 8.0 and 7.9 (1H, 2 × s), 7.4−7.0 (6H, m), 4.8 (1H, m), 3.9 (3H, 2 × s), 3.6−2.5 (4H, m), 1.8 (3H, s), 1.6−1.4 (12H, m).

Integration suggested a ca. 1:1 mixture; further characterized as the bis(*N*-methyl-D-glucamine) salt. Anal. ($C_{38}H_{39}N_5O_7 \cdot 2C_7H_{17}NO_5 \cdot 3.0H_2O$) C, H, N.

1-Methyl-5-[[[1(*S*)-[[[3,5-bis(benzyloxycarbonyl)phenyl]amino]carbonyl]-2-phenylethyl]amino]carbonyl]-6-[[[(1-adamantylmethyl)amino]carbonyl]indole (30). Compound **28**[10] (211 mg, 0.3 mmol) was dissolved in a mixture of THF (1 mL) and DMF (0.5 mL). The solution was stirred under an atmosphere of dry nitrogen and sodium hydride (15 mg, 0.3 mmol) was added. Hydrogen gas was evolved for about 5 min whereupon methyl iodide (40 μL, 0.6 mmol)) was added. The solution was stirred at room temperature for 1 h, diluted with brine (20 mL) and extracted with dichloromethane (20 mL). The organic layer was washed with brine (2 × 20 mL), dried (magnesium sulfate), filtered, evaporated and purified by column chromatography (silica, 85% dichloromethane−15% ethyl acetate) to leave the title compound (90 mg, 42%): ^{1}H NMR (DMSO-d_6) δ 10.3 (1H, s), 8.8 (3H, m), 8.4 (1H, m), 8.3 (1H, s), 7.8 (1H, s), 7.4 (16H, m), 7.1 (1H, s), 6.5 (1H, d), 5.4 (4H, s), 4.7 (1H, m), 3.8 (3H, s), 3.4 and 2.9 (4H, m), 1.8 (3H, s), 1.5 (6H, m), 1.4 (6H, s).

1-Methyl-5-[[[(1-adamantylmethyl)amino]carbonyl]-6-[[[1(*S*)-[[[3,5-bis(benzyloxycarbonyl)phenyl]amino]carbonyl]-2-phenylethyl]amino]carbonyl]indole (32). Compound **29**[10] was converted to the title compound using the same methodology as for the preparation of **30**: ^{1}H NMR (DMSO-d_6) δ 10.3 (1H, s), 8.8 (3H, m), 8.4 (1H, m), 8.3 (1H, s), 7.9 (1H, s), 7.4 (16H, m), 6.8 (1H, d), 6.6 (1H, d), 5.4 (4H, s), 4.7 (1H, m), 3.8 (3H, s), 3.4 and 2.9 (4H, m), 1.8 (3H, s), 1.5 (6H, m), 1.4 (6H, s).

1-Butyl-5-[[[1(*S*)-[[[3,5-bis(benzyloxycarbonyl)phenyl]amino]carbonyl]-2-phenylethyl]amino]carbonyl]-6-[[[(1-adamantylmethyl)amino]carbonyl]indole (31). **28**[10] was converted to the title compound using the same methodology as for the preparation of **30** except that an excess of butyl iodide was used as alkylating agent instead of methyl iodide: ^{1}H NMR (CDCl$_3$) δ 9.7 (1H, s), 8.8 (2H, s), 8.5 (1H, s), 7.4 (17H, m), 7.2 (1H, s), 6.5 (1H, d), 6.4 (1H, t), 6.3 (1H, t), 5.4 (4H, s), 5.1 (1H, m), 4.1 (2H, m), 3.3 (2H, m), 3.1 (2H, m), 1.9 (3H, s), 1.8 (2H, m), 1.5 (6H, m), 1.4 (6H, s), 1.3 (2H, m), 0.9 (3H, m).

1-Butyl-5-[[[(1-adamantylmethyl)amino]carbonyl]-6-[[[1(*S*)-[[[3,5-bis(benzyloxycarbonyl)phenyl]amino]carbonyl]-2-phenylethyl]amino]carbonyl]indole (33). Compound **29**[10] was converted to the title compound using the same methodology as for the preparation of **30** except that an excess of butyl iodide was used as alkylating agent instead of methyl iodide: ^{1}H NMR (CDCl$_3$) δ 10.0 (1H, s), 8.9 (2H, s), 8.5 (1H, s), 7.7 (1H, s), 7.4 (16H, m), 6.9 (1H, s), 6.5 (1H, d), 6.2 (2H, m), 5.4 (4H, s), 5.1 (1H, m), 4.1 (2H, m), 3.6 (1H, m), 3.3 (1H, m), 3.1 (2H, d), 1.9 (3H, s), 1.8 (2H, m), 1.5 (6H, m), 1.4 (6H, s), 1.3 (2H, m), 0.9 (3H, m).

1-Methyl-5-[[[1(*S*)-[[(3,5-dicarboxyphenyl)amino]carbonyl]-2-phenylethyl]amino]carbonyl]-6-[[[(1-adamantylmethyl)amino]carbonyl]indole (4). Compound **30** was converted to the title compound using the same methodology as for the preparation of **3**: ^{1}H NMR (DMSO-d_6) δ 13.2 (2H, br s), 10.2 (1H, s), 8.7 (3H, m), 8.4 (1H, m), 8.2 (1H, s), 7.8 (1H, s), 7.5 (1H, m), 7.4 (5H, m), 7.1 (1H, s), 6.5 (1H, d), 4.7 (1H, m), 3.7 (3H, s), 3.4 and 2.9 (4H, m), 1.8 (3H, s), 1.5 (6H, m), 1.4 (6H, s); further characterized as the bis(*N*-methyl-D-glucamine) salt. Anal. ($C_{39}H_{40}N_4O_7 \cdot 2C_7H_{17}NO_5$) C, H, N.

1-Methyl-5-[[[(1-adamantylmethyl)amino]carbonyl]-6-[[[1(*S*)-[[(3,5-dicarboxyphenyl)amino]carbonyl]-2-phenylethyl]amino]carbonyl]indole (5). Compound **32** was converted to the title compound using the same methodology as for the preparation of **3**: ^{1}H NMR (DMSO-d_6) δ 10.2 (1H, s), 8.7 (3H, m), 8.4 (1H, t), 8.2 (1H, s), 7.9 (1H, s), 7.5 (1H, d), 7.4 (5H, m), 6.8 (1H, s), 6.6 (1H, d), 4.7 (1H, m), 3.8 (3H, s), 3.4 and 2.9 (4H, m), 1.8 (3H, s), 1.5 (6H, m), 1.4 (6H, s); further characterized as the bis(*N*-methyl-D-glucamine) salt. Anal. ($C_{39}H_{40}N_4O_7 \cdot 2C_7H_{17}NO_5 \cdot 3H_2O$) C, H, N.

1-Butyl-5-[[[1(*S*)-[[(3,5-dicarboxyphenyl)amino]carbonyl]-2-phenylethyl]amino]carbonyl]-6-[[[(1-adamantylmethyl)amino]carbonyl]indole (6). Compound **31** was con-

verted to the title compound using the same methodology as for the preparation of **3**: ¹H NMR (DMSO-d_6) δ 13.2 (2H, br s), 10.2 (1H, s), 8.7 (3H, m), 8.4 (1H, t), 8.2 (1H, s), 7.8 (1H, s), 7.6 (1H, d), 7.4 (5H, m), 7.2 (1H, s), 6.5 (1H, d), 4.8 (1H, m), 4.2 (2H, m), 3.4 and 2.9 (4H, m), 1.8 (5H, m), 1.5 (6H, m), 1.4 (6H, s), 1.2 (2H, m), 0.9 (3H, m); further characterized as the bis(*N*-methyl-D-glucamine) salt. Anal. ($C_{42}H_{46}N_4O_7$·$2C_7H_{17}NO_5$·H_2O) C, H, N.

1-Butyl-5-[[(1-adamantylmethyl)amino]carbonyl]-6-[[[1(*S*)-[[(3,5-dicarboxyphenyl)amino]carbonyl]-2-phenylethyl]amino]carbonyl]indole (7). Compound **33** was converted to the title compound using the same methodology as for the preparation of **3**: ¹H NMR (DMSO-d_6) δ 13.3 (2H, br s), 10.2 (1H, s), 8.8 (3H, m), 8.4 (1H, t), 8.2 (1H, s), 7.9 (1H, s), 7.5 (1H, d), 7.4 (5H, m), 6.8 (1H, s), 6.6 (1H, d), 4.8 (1H, m), 4.2 (2H, m), 3.4 and 2.9 (4H, m), 1.8 (5H, m), 1.5 (6H, m), 1.4 (6H, s), 1.2 (2H, m), 0.9 (3H, m); further characterized as the bis(*N*-methyl-D-glucamine) salt. Anal. ($C_{42}H_{46}N_4O_7$·$2C_7H_{17}NO_5$·$2H_2O$) C, H, N.

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