for bradykinin. The difference in pD_2 values compared to concurrent control reflected the percentage recovery of agonist response.

Guinea Pig Ileum in Vitro pA_2 Measurement. Male Dunkin Hartley guinea pigs (350–450 g) were killed by cervical dislocation, and the ilea removed. Segments of ileum 2.5 cm in length were prepared and mounted under 2 g of resting tension in 4-mL tissue baths containing Tyrode's solution at 37 °C and bubbled with O_2/CO_2 (95%/5%). Concentration–effect curves were constructed for bradykinin in the absence and presence of the antagonist (preincubated for 5 min). Antagonist potency and recovery from antagonism were calculated as described above.

Rationally Designed "Dipeptoid" Analogues of CCK. A Free–Wilson/Fujita–Ban Analysis of Some α-Methyltryptophan Derivatives as CCK-B Antagonists

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A Free–Wilson/Fujita–Ban (FW/FB) analysis is reported on 36 "dipeptoid" antagonists of the CCK-B receptor. This series of compounds includes [*R*-(*R**,*R**)]-4-[[2-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]amino]propyl]amino]-1-phenylethyl]amino]-4-oxobutanoic acid (CI-988, 1, Figure 1), the first rationally designed non-peptide antagonist of a neuropeptide receptor. The analysis treats the compounds in three parts: the N-terminus, variants on the tryptophan moiety, and the C-terminus. A highly significant correlation was found ($n = 36$, $r^2 = 0.97$, $s = 0.22$, $F = 57$, $p = 2 \times 10^{-8}$), suggesting that these three domains of these compounds contribute to binding affinity independently of each other, and are therefore additive in their effects on receptor affinity. The relative free-energies of binding of the individual substituents are calculated from the coefficients of the regression equation. The substitution of D-α-methyltryptophan for L-tryptophan increases the free-energy of binding by 3.5 kcal mol^{-1}. This increase in binding energy is explained by a 300-fold difference in conformational entropy between the methylated and desmethyl analogues.

Introduction

The synthesis of potent, highly selective non-peptide antagonists for the central cholecystokinin (CCK-B) receptor has been described previously.[1-5] The strategy involved the independent optimization of the N and C terminal structure–activity relationships (SAR) of compound 2[3] in Figure 1. Such a strategy assumes that the binding energies of the N and C terminus groups are additive when the ligand binds to the receptor. This is thought to be a reasonable approach with these semirigid molecules.

This paper describes a justification of this assumption using Free–Wilson/Fujita–Ban (FW/FB) analysis[6,7] for the binding of these "dipeptoids" to the CCK-B receptor. This quantitative structure–activity relationship (QSAR) method assumes that the individual substituents act independently of each other, and that they act in an additive fashion. Thus any deviation from this assumption should be evident in the differences between predicted and actual binding constants (K_i). The coefficients of the regression equation gained from this analysis are directly proportional to the free-energy change (ΔG) when replacing one group with another.[8] Therefore, in addition to testing the additivity hypothesis for this series a quantitative measure of the binding contribution of each group is also gained. The relative binding of the substituents are interpreted in terms of the additivity concept of the binding constants of the relevant functional groups put forward previously by Andrews et al.[9]

The choice of substituents (and hence compounds) used in any QSAR analysis is of considerable importance.[10] In

(1) Horwell, D. C.; Beeby, A.; Hughes, J. Synthesis and binding affinities of analogues of Cholecystokinin-(30-33) as probes for central nervous system Cholecystokinin receptors. *J. Med. Chem.* **1987**, *30*, 729–732.

(2) Birchmore, B.; Boden, P. R.; Hewson, G.; Higginbottom, M.; Horwell, D. C.; Ho, Y. P.; Hughes, J.; Hunter, J. C.; Richardson, R. S. α-Methyl tryptophanylphenylalanines and their arylamine "dipeptoid" analogues of the tetrapeptide cholecystokinin (30-33). *Eur. J. Med. Chem.* **1990**, *25*, 53–60.

(3) 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 CCK-B antagonists with potent anxiolitic properties. *J. Med. Chem.* **1991**, *34*, 404–414.

(4) Eden, J. M.; Higginbottom, M.; Hill, D. R.; Horwell, D. C.; Hunter, J. C.; Martin, K.; Pritchard, M. C.; Richardson, R. S.; Roberts, E. Rationally designed "dipeptoid" analogues of CCK. C-Terminal structure–activity relationships of α-Methyltryptophan derivatives. *J. Med. Chem.*, submitted for publication, October 1991.

(5) Eden, J. M.; Higginbottom, M.; Hill, D. R.; Horwell, D. C.; Hunter, J. C.; Martin, K.; Pritchard, M. C.; Richardson, R. S.; Roberts, E. Rationally designed "dipeptoid" analogues of CCK. N-Terminal structure–activity relationships of α-Methyltryptophan derivatives. *J. Med. Chem.*, submitted for publication, October 1991.

(6) Free, S. M.; Wilson, J. W. A mathematical contribution to structure–activity studies. *J. Med. Chem.* **1964**, *7*, 395–399.

(7) Fujita, T.; Ban, T. Structure–activity relations, 3. Structure–activity study of phenethylamines as substrates of biosynthetic enzymes of sympathetic transmitters. *J. Med. Chem.* **1971**, *14*, 148–152.

(8) Kubinyi, H. The Free–Wilson method and its relationship to the extrathermodynamic approach. In *Comprehensive Medicinal Chemistry*; Hansch, C., Ed.; Permagon Press: New York, 1990; Vol. 4, 589–643.

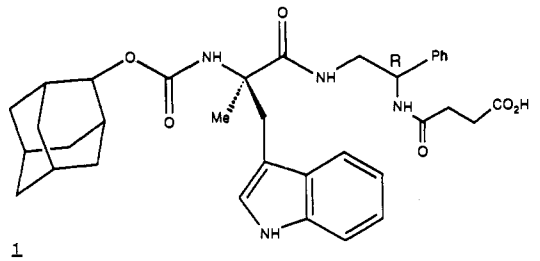
(9) Andrews, P. R.; Craik, D. J.; Martin, J. L. Functional group contributions to drug–receptor interactions. *J. Med. Chem.* **1984**, *27*, 1648–1657.

0022-2623/92/1835-1572$03.00/0

Table I. Designation and Structures of the Dataset of Compounds 1–36[a]

compd	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	binding affinities: CCK-B observed pK_i	residual[b] pK_i
1	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	8.84	0.08
2	0	0.5	0	0.5	0	1	0	0	1	0	0	0	0	0	0	0	7.34	0.18
3	0	0.5	0	0.5	1	0	0	0	1	0	0	0	0	0	0	0	6.87	0.06
4	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	8.22	0.03
5	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	7.67	−0.23
6	0	0.5	0	0.5	0	0	0	1	1	0	0	0	0	0	0	0	6.90	−0.15
7	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	7.69	−0.03
8	0	0.5	0	0.5	0	0	1	0	1	0	0	0	0	0	0	0	6.72	0.03
9	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	6.09	0.10
10	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	5.68	−0.03
11	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	7.82	−0.02
12	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	8.69	0.05
13	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	6.52	0.10
14	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	7.21	−0.07
15	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	7.81	−0.26
16	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	6.01	−0.27
17	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	6.45	−0.11
18	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	8.43	0.02
19	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	7.91	−0.05
20	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	6.78	−0.05
21	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	6.40	0.23
22	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	6.52	0.07
23	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	9.15	−0.07
24	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	8.66	−0.28
25	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	9.29	0.24
26	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	8.41	−0.30
27	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	7.09	0.34
28	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	8.05	0.21
29	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	8.94	0.07
30	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	8.40	0.09
31	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	9.83	0.39
32	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	6.61	0.02
33	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	8.05	−0.02
34	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	7.90	−0.06
35	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	6.86	−0.39
36	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	7.03	0.07

[a] The contents of columns A to P indicate the presence (1), or absence (0), of the substituents indicated in Figure 2. The presence of two values of 0.5 in a single row indicates that this compound is racemic at the tryptophan center. [b] The residual pK_i is defined as (observed pK_i) − (fitted pK_i).



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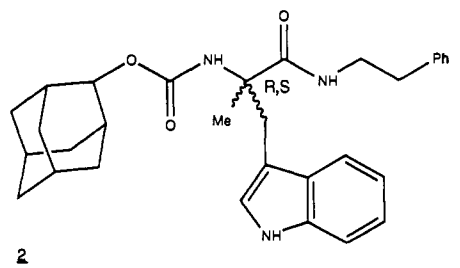

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Figure 1.

this dataset each substituent occurs at least twice, and there are no linear dependencies such as A and B always occurring together. The activities of the 36 compounds cover a large range (pK_i 5.66 to 9.83) which is distributed evenly about their average. All compounds reported thus far with the substituents of interest are used in this analysis.

Table II. Substituent Constants and Statistical Data from the FW/FB Analysis[a]

subst	ΔG^b kcal mol^{-1}	fitted coeff[c]	SD	signif level
A	0.40	0.285	0.151	0.072[d]
B	3.48	2.477	0.137	<0.0001
D	1.41	1.000	0.164	<0.0001
E	−0.49	−0.348	0.111	0.004
G	−0.66	−0.467	0.177	0.015
H	−0.16	−0.113	0.111	0.318[d]
J	0.41	0.290	0.139	0.048
K	0.57	0.408	0.162	0.019
L	1.86	1.322	0.196	<0.0001
M	1.21	0.860	0.137	<0.0001
N	1.63	1.157	0.161	<0.0001
O	2.17	1.545	0.158	<0.0001
P	0.24	0.172	0.187	0.367[d]

[a] $n = 36$, $r^2 = 0.97$, $s = 0.22$, $F = 56.8$, $p = 2 \times 10^{-8}$. [b] $\Delta G = 1.41 \times$ fitted coefficient. [c] The fitted coefficients for C, F, and I, are all zero. [d] The fitted coefficient is not significantly different from zero at the 95% confidence level.

Results and Discussion

Figure 2 gives three lists: a list of tryptophan substituents, a list of N-terminus substituents, and a list of C-terminus substituents. Each compound consists of three

(10) Hudson, D. R.; Bass, G. E.; Purcell, W. P. Quantitative structure–activity models. Conditions for application and statistical interpretation. *J. Med. Chem.* **1970**, *13*, 1184–1189.

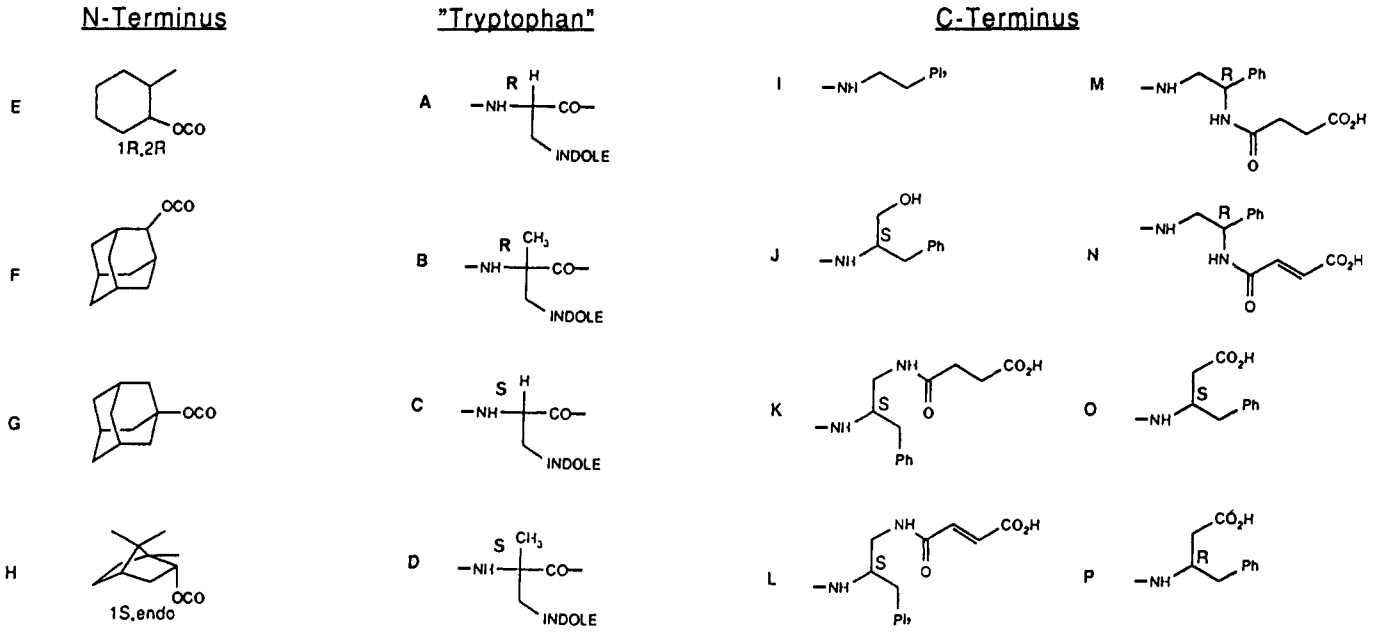

Figure 2. Key to the substituents A to P in Table I.

substituents, one from each list. The complete list of compounds together with their respective binding affinities is given in Table I.

The multiple linear regression was performed on the elements of Table I after the removal of columns C, F, and I with the statistical package RS/1.[11] The compound represented by these columns becomes the fixed point to which the affinities of new compounds and substituents are to be compared. It has a calculated $pK_i = 5.42$ which is found at the intercept of the regression equation. All columns are assumed to be linearly independent and are all used in the analysis. This results in the highly significant correlation detailed in Table II. The coefficients from the regression analysis (column 2 of Table II) may be used to calculate the affinity of any compound that is made up from the substituents in Figure 2. For example the affinity of compound 31 is 5.42 + B + F + O (Table I) or 5.42 + 2.477 + 0 + 1.545 (Table II), and the calculated $pK_i = 9.442$ and the experimental $pK_i = 9.83$. The difference between these figures (0.39) being the residual reported in Table I. Since FW/FB analysis assumes that substituents bind independently, if there is any interaction between them, the method will produce a poor correlation with large discrepancies between fitted and experimental activities for certain substituents. This is evidently not the case for this dataset (Table I). Hence, the application of the additivity hypothesis to this series is justified.

A feature of FW/FB analysis is that the coefficients of the regression equation are directly proportional to ΔG when affinities are represented as the logarithms of equilibrium constants (pK_i). We can therefore calculate the ΔG for substituting 1-[(adamantyloxy)carbonyl] (1-adoc) for 2-[(adamantyloxy)carbonyl] (2-adoc), and compare this with the ΔG involved for various ligand–receptor interactions and that ΔG expected on the basis of the analysis of Andrews et al.[9] The ΔG associated with substituting 1-adoc for 2-adoc is −0.66 kcal mol^{-1} (Tables II and V). The difference in ΔG cannot be explained as a consequence of lipophilicity since this parameter remains unchanged. The intrinsic binding energy data (Table III) suggests that the binding energy for the addition of one

(11) RS/1 (Release 4) Supplied by BBN software of 10 Fawcett Street, Cambridge, MA 02238.

Table III. Intrinsic Binding Energies[9]

functional group	modal value, kcal mol^{-1}	range, kcal mol^{-1}
DOF[a]	−0.7	−(0.7–1.0)
C(sp^2)	0.7	0.6–0.8
C(sp^3)	0.8	0.1–1.0
N+	11.5	10.4–15.0
N	1.2	0.8–1.8
CO_2^-	8.2	7.3–10.3
OPO_3^{2-}	10.0	7.7–10.6
OH	2.5	2.5–4.0
C=O	3.4	3.2–4.0
O, S	1.1	0.7–2.0
halogen	1.3	0.7–2.0

[a] DOF = degrees of freedom (rotatable bonds).

Table IV. Typical Bond Strengths of Drug–Receptor Interactions[12]

bond type	binding energy, kcal mol^{-1}
van der Waals	−(0.5–1.0)
hydrophobic	−1[a]
charge transfer	−(1–7)
hydrogen bond	−(1–7)
dipole–dipole	−(1–7)
ionic	−5
reinforced ionic	−10

[a] Per methylene group.

methylene group is −0.7 kcal mol^{-1}. The interpretation of these results is that 2-adoc provides a better "fit" than 1-adoc to the receptor. This is consistent with one extra methylene group in the 2-adoc derivatives being in contact with the receptor binding site than in the corresponding 1-adoc derivatives. This observation is in accordance with the data of Farmer[12] (Table IV). For the other similarly bulky N-terminal substitution *endo*-borneol (H), the ΔG is not significantly different from 2-adoc at the 95% confidence level, and presumably has a similar area of contact with the receptor as 2-adoc. It is interesting to note that the 2-methylcyclohexyl derivatives (E) have binding en-

(12) Farmer, P. S. Bridging the gap between bioactive peptides and non-peptides: some perspectives in drug design. In *Drug Design*, Ariens, E. J., Ed.; Academic Press: New York, 1980; Vol. 10, 134.

ergies only 0.49 kcal mol^{-1} less than 2-adoc despite having three methylene groups less. This suggests that at least two of the methylene groups of the 2-adoc are not involved in binding.

The free energy change on replacement of the natural L-tryptophan (C) by D-α-methyltryptophan (B) is 3.5 kcal mol^{-1} (Table II). This is a significant increase in affinity. There are several possible interpretations of this observation. Firstly, the methyl group itself might be involved in lipophilic interaction with the receptor. However, this is not likely, as the data in Tables III and IV show that the maximum hydrophobic binding available to one methyl group is 1 kcal mol^{-1}. Secondly, the methylated compounds may achieve an "active" conformer that is not a low-energy conformer of the desmethyl compounds. Thirdly, the increase in affinity could reflect a decrease in the methylated analogues conformational entropy. Since the receptor may be presumed to recognize only one conformer there is a conformational entropy penalty involved in binding, i.e. the receptor imposes order on the ligand and this costs free-energy. If the flexibility of the ligand is reduced, however, this penalty will become smaller. Since the second explanation is perhaps more difficult to demonstrate, as it would require the precise active conformer to be found, an attempt has been made to quantify the conformational entropy change between the methylated and nonmethylated species.

The change in entropy is given by $\Delta S = k \ln (n_{me}/n_H)$.[13] In this case we may identify n with the number of accessible conformers. Using the conformational search tool developed by Marshall[14,15] these numbers were evaluated for the compounds in Figure 3:

$$n_H = 6\,385\,722$$

$$n_{Me} = 48\,380$$

Using these figures the conformational ΔS may be calculated:

$$\Delta S = R \ln (6\,385\,722/48\,380)$$

$$= 40.6 \text{ J K}^{-1} \text{ mol}^{-1}$$

The free energy change associated with this is

$$\Delta G = \Delta H - T\Delta S$$

$$= -T\Delta S$$

$$= 12.6 \text{ kJ mol}^{-1}$$

$$= 3.1 \text{ kcal mol}^{-1}$$

where $\Delta H = 0$ and $T = 310$ K. This figure is not significantly at variance with the difference in binding energy observed between the methylated and desmethyl analogues of D-tryptophan. This "order of magnitude" calculation shows that the loss of conformational entropy is sufficient to account for the increased binding affinity. This effect may be thought of as an increase in the concentration of the active conformer by n_H/n_{Me}, resulting in a decrease in the inhibition constant by n_H/n_{Me}.

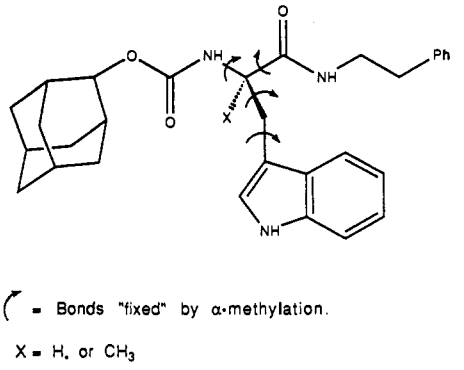

Figure 3.

Table V. ΔG for the Groups A–P Calculated from the Functional Group Substituent Constants of Table III

substituent	ΔG FW/FB, kcal mol^{-1}	average,[9] kcal mol^{-1}
A	0.40	0
B	3.48	0.8
D	1.41	0.8
E	−0.49	−2.4
G	−0.66	0
H	−0.16	0
J	0.41	2.6
K	0.57	11.7
L	1.86	12.2
M	1.21	11.6
N	1.63	12.1
O	2.17	7.5
P	0.24	7.5

The extra affinity observed with the α-methylated analogues may also be seen as the loss of four degrees of freedom. From Table III this would provide 3–4 kcal mol^{-1} of binding energy. The bonds which are restricted in their degree of freedom may be identified as those shown in Figure 3, which is supported by the conformational search results.

Finally the changes in binding energies associated with C-terminus modifications may also be considered. Table V compares those energies found from the FW/FB analysis with those expected from the average values given by Andrews et al.[9] for these functional groups; again, these are changes relative to the substituents C, F, and I. This comparison shows that in contrast to the rest of the molecule, changes in binding energy at the C-terminus are smaller than one might expect. This implies that the additional binding of the C-terminal acids relative to the phenethylamide (I) is inefficient, and in particular is difficult to rationalize in terms of the potential to form a salt bridge with the receptor (−5 or −10 kcal mol^{-1}, Table IV). The binding of this acid group is probably by a weaker interaction such as a dipole–dipole, or induced dipole–dipole interaction (1–7 kcal mol^{-1}, see Table IV).

Conclusions

This work shows that for the "dipeptoids" the additivity hypothesis leading to the development of CI-988 is valid.[3] This is a consequence of the flexibility of these compounds. The profound effect of α-methylation on binding affinity is ascribed to the reduction of conformational entropy between the tryptophan and the corresponding α-methyl tryptophan derivatives. There is clearly a balance here between the relative merits of rigid and flexible molecules. A flexible molecule has more chance of finding a good fit with a receptor but achieves this at the cost of a large conformational entropy. A rigid molecule has little conformational entropy but is unlikely to fit the receptor

(13) McClelland, B. J. *Statistical Thermodynamics.* Chapman and Hall: New York, 1973; 33.

(14) Marshall, G. R.; Van Opdenbosch, N.; Font, J. Systematic search of conformational space: use and visualization. Proceeding of the 2nd SCI-RSC Medicinal Chemistry Symposium, Cambridge, U.K., 12–14 Sept, 1983, pp 96–105 (*Spec. Publ.-R. Soc. Chem.*).

(15) SYBYL 5.3 supplied by Tripos Associates of South Hanley Road, Suite 303, St. Louis, MO 63144.

Table VI. Physical and Chemical Data of Novel Compounds

compd	mp, °C	found, %			calculated, %			molecular formula
		C	H	N	C	H	N	
7	147–155	72.25	7.40	7.95	72.56	7.42	7.93	$C_{33}H_{39}N_3O_4$
9	90–92	71.56	7.19	8.12	72.21	7.23	8.14	$C_{31}H_{37}N_3O_4$
10	136–140	72.07	7.28	8.13	72.21	7.23	8.14	$C_{31}H_{37}N_3O_4$
11	124–131	70.31	7.53	8.53	70.21	7.62	8.47	$C_{29}H_{37}N_3O_4 \cdot 0.25H_2O$
15	84–86	71.85	7.72	7.83	71.80	7.80	7.85	$C_{32}H_{41}N_3O_4 \cdot 0.2H_2O$
16	84–88	66.01	6.81	8.55	66.00	6.84	9.06	$C_{34}H_{40}N_4O_6 \cdot H_2O$
17	108–113	65.93	6.60	9.11	66.00	6.84	9.06	$C_{34}H_{40}N_4O_6 \cdot H_2O$
20	106–110	67.91	7.00	8.77	67.78	7.11	8.78	$C_{36}H_{44}N_4O_6 \cdot 0.5H_2O$
21	103–107	66.02	6.86	9.02	65.78	7.15	9.03	$C_{34}H_{42}N_4O_6 \cdot H_2O$
22	102–107	66.15	7.01	9.02	65.78	7.14	9.03	$C_{34}H_{42}N_4O_6 \cdot H_2O$
27	110–115	67.19	7.06	8.29	76.06	6.88	8.69	$C_{34}H_{40}N_4O_6 \cdot 0.5C_4H_8O_2$
28	76–80	70.56	7.57	8.49	70.85	7.59	8.55	$C_{29}H_{37}N_3O_4$
32	102–107	70.80	7.02	7.59	71.07	7.05	7.53	$C_{33}H_{39}N_3O_5$
33	108–112	70.61	7.00	7.61	70.62	7.08	7.49	$C_{33}H_{39}N_3O_5 \cdot 0.2H_2O$
34	102–106	70.90	7.04	7.48	71.07	7.05	7.53	$C_{33}H_{39}N_3O_5$
35	85–90	69.79	7.26	7.43	69.54	6.93	7.60	$C_{32}H_{37}N_3O_5 \cdot 0.5H_2O$
36	70–75	69.95	7.37	6.86	69.48	7.03	7.15	$C_{32}H_{37}N_3O_5 \cdot 0.5C_4H_8O_2$

optimally. The semirigid α-methyl tryptophan derivatives achieve a balance between these two extremes.

Experimental Section

Binding Assays. CCK-B receptor binding assays[3] were performed on male mouse cerebral cortex. Tissue homogenized in ice-cold Tris-HCl (pH = 7.4) (50 mL of a 50 mM solution) was centrifuged at 20000g. The pellet was washed and resuspended in SAB (pH = 7.4) to a tissue concentration of 2.0 mg of original wet weight per mL of buffer.

For each of the binding assays, aliquots of tissue (400 μL) were incubated at 21 °C for 120 min with 35 pM [^{125}I]Bolton Hunter CCK-26-33 in the absence and presence of a range of the test compound in a final volume of 500 μL. Nonspecific binding was estimated, 1 μM CCK-8S. After each incubation, the assay was terminated by filtration onto Whatman GF/B filters, and the radioactivity was measured with a Packard Series 5000 γ-counter. Binding affinities of all compounds are the geometric mean of three independent measurements of K_i, the result being expressed as the logarithm pK_i.

Molecular Modeling. The conformational searches were performed with the SEARCH[14] algorithm in SYBYL[15] (version 5.3). The systematic search option was used throughout. This method finds all low-energy conformers available to a molecule using a set of discrete bond rotation increments. The criteria for low-energy conformers is that of no van der Waals (VDW) contacts. For both molecules the default parameters for VDW contact were used.

The structures of both molecules were built within SYBYL. The molecules were then subject to energy minimization using the Tripos force field[16] with MAXIMIN II.[17] The same bond increments were used for both searches: single bonds (sp^2–sp^3, or sp^3–sp^3) over 360° in 30° increments. Amide bonds as cis (0°) or trans (180°). The bond from the tryptophan α-C to X (Figure 2) was not rotated when X = Me.

Chemistry. Melting points were determined with a Mettler FP800 or a Reichart Thermovar hot stage apparatus and are uncorrected. Proton NMR spectra were recorded on a Bruker AM300 spectrometer; chemical shifts were recorded in parts per million (ppm) downfield from tetramethylsilane. IR spectra were recorded with the compound (neat) on a sodium chloride disk and a Perkin-Elmer 1750 Fourier transform spectrophotometer. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. Mass spectra were recorded with a Finnigan 4500 or a ZAB-E VG Analytical mass spectrometer. Elemental analysis were determined by CHN Analysis Limited, Leicester, U.K.

All novel compounds were prepared using methods analogous to those given in ref 3 (see also refs 2, 4, and 5). We report here only the analytical data, and the binding affinities at the CCK-B receptor, of these new compounds in Tables VI and I, respectively.

Carbamic acid, [2-[[1-(hydroxymethyl)-2-phenylethyl]amino]-1-(1*H*-indol-3-ylmethyl)-1-methyl-2-oxoethyl]-, tricyclo[3.3.1.1^{3,7}]dec-1-yl ester, [*R*-(*R,*S**)]- (7):** $[\alpha]^{20}_D$ = +23.4° (c = 1, MeOH); IR (film) 3500–3200, 2912, 2854, 1674, 1650, 1520, 1250, 1075, 740 cm^{-1}; NMR ($CDCl_3$) δ 1.33 (3 H, s), 1.68 (6 H, s), 2.12 (6 H, s), 2.20 (3 H, s), 2.81 (1 H, d, *J* = 4.5 Hz), 2.84 (1 H, d, *J* = 4.5 Hz), 2.85–2.95 (1 H, m), 3.32 (1 H, d, *J* = 14.7 Hz), 3.46 (1 H, d, *J* = 14.7 Hz), 3.44–3.51 (1 H, m), 3.75–3.85 (1 H, m), 4.20–4.30 (1 H, m), 4.82 (1 H, s), 6.18 (1 H, d, *J* = 8.2 Hz), 6.95–7.31 (8 H, m), 7.38 (1 H, d, *J* = 8 Hz), 7.61 (1 H, d, *J* = 7.7 Hz), 8.15 (1 H, s); MS *m/e* (EI) 130 (100), 135 (146), 152 (11), 334 (11), 400 (10), 514 (3), 529 (M^+, 0.2).

Carbamic acid, [2-[[1-(hydroxymethyl)-2-phenylethyl]amino]-1-(1*H*-indol-3-ylmethyl)-2-oxoethyl]-, tricyclo[3.3.1.1^{3,7}]dec-2-yl ester, [*R*-(*R,*S**)]- (9):** NMR ($CDCl_3$) δ 1.40–1.60 (2 H, m), 1.65–2.10 (12 H, m), 2.60 (2 H, m), 2.70–3.00 (1 H, br), 3.10–3.60 (4 H, m), 4.05 (1 H, m), 4.45 (1 H, m), 4.80 (1 H, s), 5.30–5.60 (1 H, br s), 6.00–6.20 (1 H, br s), 6.85–7.25 (8 H, m), 7.35 (1 H, d, *J* = 8 Hz), 7.65 (1 H, d, *J* = 8 Hz), 8.10–8.30 (1 H, br s).

Carbamic acid, [2-[[1-(hydroxymethyl)-2-phenylethyl]amino]-1-(1*H*-indol-3-ylmethyl)-2-oxoethyl]-, tricyclo[3.3.1.1^{3,7}]dec-2-yl ester, [*S*-(*R,*R**)]- (10):** NMR ($CDCl_3$) δ 1.40–2.00 (14 H, m), 2.10 (1 H, br s), 2.60 (2 H, m), 3.10 (1 H, m), 3.30 (3 H, m), 4.00 (1 H, m), 4.43 (1 H, m), 4.82 (1 H, s), 5.20–5.50 (1 H, br s), 5.70–5.90 (1 H, br s), 7.00 (3 H, m), 7.10–7.25 (5 H, m), 7.36 (1 H, d, *J* = 8 Hz), 7.66 (1 H, d, *J* = 8 Hz), 8.21 (1 H, s).

Carbamic acid, [2-[[1-(hydroxymethyl)-2-phenylethyl]amino]-1-(1*H*-indol-3-ylmethyl)-1-methyl-2-oxoethyl]-, 2-methylcyclohexyl ester, [1(±)-[1α[*R(*S**)],2β]]- (11):** IR (film) 3500–3200, 2932, 2858, 1690, 1660, 1497, 1255, 1074, 739 cm^{-1}; NMR ($CDCl_3$) δ 0.90 and 0.91 (each 1.5 H, each d, *J* = 6.5 and 6.3 respect.), 1.00–1.75 (8 H, m), 1.36 and 1.39 (each 1.5 H, each s), 1.92–2.00 (1 H, m), 2.70–2.80 (3 H, m), 3.28–3.48 (3 H, m), 3.65–3.80 (1 H, m), 4.10–4.38 (2 H, m), 4.94 and 4.99 (each 0.5 H, each s), 6.11 and 6.14 (each 0.5 H, each d, *J* = 7.7 and 7.8 Hz respect.), 6.91 (1 H, s), 7.05–7.30 (7 H, m), 7.35 (1 H, d, *J* = 8 Hz), 7.57 (1 H, d, *J* = 7.8 Hz), 8.08 (1 H, s); MS *m/e* (FAB) 492 (M + H, 100), 352 (43).

Carbamic acid, [2-[[1-(hydroxymethyl)-2-phenylethyl]amino]-1-(1*H*-indol-3-ylmethyl)-1-methyl-2-oxoethyl]-, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, [1*R*-[1α,2β[*S(*R**)],4α]]- (15):** $[\alpha]^{20}_D$ = +27.2° (c = 1, MeOH); IR (film) 3500–3200, 2955, 1694, 1650, 1498, 1456, 1256, 1074, 741 cm^{-1}; NMR ($CDCl_3$) δ 0.84 (3 H, s), 0.87 (3 H, s), 0.91 (3 H, s), 0.90–1.00 (1 H, s), 1.15–1.30 (2 H, m), 1.37 (3 H, s), 1.65–1.85 (3 H, m), 2.30–2.45 (1 H, m), 2.75–2.85 (3 H, m), 3.33 (1 H, d, *J* = 14.7 Hz), 3.44 (1 H, d, *J* = 14.7 Hz), 3.35–3.50 (1 H, m), 3.70–3.80 (1 H, m),

(16) Clarke, M.; Cramer, R. D., III.; Van Opdenbosch, N. Validation of the Tripos 5.2 force field. *J. Comput. Chem.* **1989**, *10*, 982–1012.

(17) Labanowski, J.; Motoc, I.; Naylor, C. B.; Mayer, D.; Dammkoehler, R. A. Three-dimensional quantitative structure–activity relationships. 2. Conformational mimickry and topological similarity of flexible molecules. *Quant. Struct.–Act. Relat.* **1986**, *5*, 138–152.

4.15–4.25 (1 H, m), 4.80–4.85 (1 H, m), 4.99 (1 H, s), 6.12 (1 H, d, J = 8.0 Hz), 6.91 (1 H, d, J = 2.4 Hz), 7.05–7.30 (7 H, m), 7.35 (1 H, d, J = 8.0 Hz), 7.56 (1 H, d, J = 7.8 Hz), 8.07 (1 H, s); MS m/e (FAB) 532 (M + H, 100), 402 (30), 352 (99), 334 (40), 266 (42), 221 (16).

Butanoic acid, 4-[[2-[[3-(1*H*-indol-3-yl)-1-oxo-2-[[(tricyclo[3.3.1.1.^{3,7}]dec-2-yloxy)carbonyl]amino]propyl]amino]-1-phenylethyl]amino]-4-oxo-, [*S*-(*R,*S**)]- (16)**: $[\alpha]^{20}_D$ = −35.4° (c = 0.5, MeOH); IR (film) 3155, 2908, 1794, 1713, 1667 cm^{-1}; NMR (d_6-DMSO) 330 K δ 1.46–1.95 (14 H, m), 2.40–2.45 (4 H, m), 2.85 (1 H, dd, J = 14.6, 8.9 Hz), 3.03 (1 H, dd, J = 14.6, 4.5 Hz), 3.25–3.35 (1 H, m), 3.45–3.55 (1 H, m), 4.20–4.30 (1 H, m), 4.57 (1 H, s), 6.65 (1 H, br s), 6.97 (1 H, t, J = 6.9 Hz), 7.00–7.10 (1 H, m), 7.20–7.35 (7 H, m), 7.56 (1 H, d, J = 7.8 Hz), 7.82 (1 H, m), 8.05 (1 H, d, J = 8.0 Hz), 10.60 (1 H, s), 11.9 (1 H, br s); MS m/e (FAB) 601 (M + H).

Butanoic acid, 4-[[2-[[3-(1*H*-indol-3-yl)-1-oxo-2-[[(tricyclo[3.3.1.1.^{3,7}]dec-2-yloxy)carbonyl]amino]propyl]amino]-1-phenylethyl]amino]-4-oxo-, [*R*-(*R,*R**)]- (17)**: $[\alpha]^{20}_D$ = −13.4° (c = 0.5, MeOH); IR (film) 2958, 2901, 1795 cm^{-1}; NMR (d_6-DMSO) δ 1.40–2.10 (14 H, m), 2.30–2.50 (4 H, m), 2.75–2.90 (1 H, m), 2.97 (1 H, dd, J = 14.5, 4.3 Hz), 3.30 (2 H, m), 4.15–4.25 (1 H, m), 4.50–4.60 (1 H, s), 4.90–5.00 (1 H, m), 6.90–7.30 (10 H, m), 7.56 (1 H, d, J = 7.6 Hz), 7.95–8.10 (1 H, m), 8.26 (1 H, d, J = 8.3 Hz), 10.75 (1 H, s), 11.2 (1 H, br s); MS m/e (FAB) 601.7 (M + H).

Butanoic acid, 4-[[2-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1.^{3,7}]dec-2-yloxy)carbonyl]amino]propyl]amino]-3-phenylpropyl]amino]-4-oxo-, [*S*-(*R,*R**)]- (20)**: $[\alpha]^{20}_D$ = −1.7° (c = 0.64, MeOH); IR (film) 1700, 1657 cm^{-1}; NMR (CD_3OD) δ 1.33 (3 H, s), 1.56 (2 H, m), 1.70–2.20 (2 H, m), 2.35.–2.80 (6 H, m), 3.00–3.10 (1 H, m), 3.13 (1 H, d, J = 14 Hz), 3.30–3.50 (2 H, m), 4.10–4.20 (1 H, m), 4.60–4.90 (1 H, m), 6.81 (1 H, s), 6.90–7.30 (8 H, m), 7.30 (1 H, d, J = 8 Hz), 7.51 (1 H, d, J = 8 Hz).

Butanoic acid, 4-[[2-[[3-(1*H*-indol-3-yl)-1-oxo-2-[[[(1,7,7-trimethylbicyclo[2.2.1]hept-2-yl)oxy]carbonyl]amino]propyl]amino]-1-phenylethyl]amino]-4-oxo-, [1*S*-[1α,2β-[*R(*S**)],4α]]- (21)**: $[\alpha]^{20}_D$ = −48.9° (c = 1.0, MeOH); IR 2957, 1795, 1723, 1668 cm^{-1}; NMR (d_6-DMSO) δ 0.55–0.65 (1 H, m), 0.81 (3 H, s), 0.86 (6 H, s), 1.05–1.30 (2 H, m), 1.55–1.80 (2 H, m), 1.90–2.00 (1 H, m), 2.05–2.20 (1 H, m), 2.50–2.60 (4 H, m), 2.70–3.00 (2 H, m), 3.20–3.30 (1 H, m), 3.45–3.55 (1 H, m), 4.10–4.25 (1 H, m), 4.55–4.70 (1 H, br), 4.95–5.05 (1 H, m), 6.55 and 6.95–7.20 (4 H, m), 7.20–7.40 (6 H, m), 7.62 (1 H, d, J = 7.7 Hz), 8.10–8.20 (1 H, m), 8.27 (1 H, d, J = 8.4 Hz), 10.80 (1 H, s); MS m/e (FAB) 603 (M + H).

Butanoic acid, 4-[[2-[[3-(1*H*-indol-3-yl)-1-oxo-2-[[[(1,7,7-trimethylbicyclo[2.2.1]hept-2-yl)oxy]carbonyl]amino]propyl]amino]-1-phenylethyl]amino]-4-oxo-, [1*S*-[1α,2β-[*S(*S**)],4α]]- (22)**: $[\alpha]^{20}_D$ = −25.7° (c = 1.0, MeOH); IR (film) 3055, 2987, 1710, 1669 cm^{-1}; NMR (d_6-DMSO) 330 K δ 0.67 (3 H, s), 0.82 (3 H, s), 0.75–1.00 (1 H, m), 1.00–1.30 (2 H, m), 1.55–1.70 (2 H, m), 1.80–1.95 (1 H, m), 2.10–2.25 (1 H, m), 2.40 (4 H, m), 2.86 (1 H, dd, J = 14.7, 8.9 Hz), 3.02 (1 H, dd, J = 14.7, 4.7 Hz), 3.37 (2 H, t, J = 6.2 Hz), 4.15–4.25 (1 H, m), 4.66 (1 H, d, J = 9.1 Hz), 4.90–5.05 (1 H, m), 6.68 (1 H, s), 6.95 (1 H, t, J = 7.6 Hz), 7.00–7.10 (2 H, m), 7.20–7.35 (6 H, m), 7.52 (1 H, d, J = 7.7 Hz), 7.71 (1 H, br), 8.04 (1 H, d, J = 8.2 Hz), 10.6 (1 H, s), 11.0 (1 H, br s); MS m/e (FAB) 603.5 (M + H).

2-Butenoic acid, 4-[[2-[[3-(1*H*-indol-3-yl)-1-oxo-2-[[[(1,7,7-trimethylbicyclo[2.2.1]hept-2-yl)oxy]carbonyl]amino]propyl]amino]-1-phenylethyl]amino]-4-oxo-, [1*S*-[1α,2β[*S[*S**(*E*)]],4α]]- (27)**: $[\alpha]^{20}_D$ = −45.4° (c = 1.0, MeOH); IR (film) 3375, 2906, 1704, 1656, 1516; NMR (d_6-DMSO) 340 K δ 0.68 (3 H, s), 0.83 (3 H, s), 0.84 (3 H, s), 0.80–0.90 (1 H, m), 1.10–1.20 (2 H, m), 1.60–1.75 (2 H, m), 1.80–1.90 (2 H, m), 2.10–2.20 (1 H, m), 2.90 (1 H, m), 3.00–3.10 (1 H, m), 3.40–3.50 (2 H, m), 4.20–4.30 (1 H, m), 4.65–4.70 (1 H, m), 5.05 (1 H, m), 6.55 (1 H, d, J = 15.5 Hz), 6.30–6.65 (1 H, br s), 6.90–7.10 (4 H, m), 7.20–7.35 (6 H, m), 7.52 (1 H, d, J = 7.7 Hz), 7.72 (1 H, m), 8.63 (1 H, d, J = 8.1 Hz), 10.55 (1 H, s); MS m/e (FAB) 601 (M + H).

Carbamic acid, [2-[[1-(hydroxymethyl)-2-phenylethyl]amino]-1-(1*H*-indol-3-ylmethyl)-1-methyl-2-oxoethyl]-, 2-methylcyclohexyl ester, [1*R*-[1α[*R(*S**)],2β]]- (28)**: $[\alpha]^{20}_D$ = −3.3° (c = 0.52, MeOH); IR (film) 3500–3200, 2932, 2859, 1690, 1663 cm^{-1}; NMR ($CDCl_3$) δ 0.89 (3 H, d, J = 6.5 Hz), 1.00–1.80 (8 H, m), 1.35 (3 H, m), 1.90–2.00 (1 H, m), 2.70–2.85 (2 H, m), 2.90–3.00 (1 H, br s), 3.29 (1 H, d, J = 14.7 Hz), 3.43 (1 H, d, J = 14.7 Hz), 3.50–3.60 (1 H, m), 3.65–3.75 (1 H, m), 4.15–4.25 (1 H, m), 4.32 (1 H, dt, J = 10, 4 Hz), 5.00 (1 H, s), 6.14 (1 H, d, J = 8 Hz), 6.90 (1 H, d, J = 2.3 Hz), 7.05–7.25 (7 H, m), 7.34 (1 H, d, J = 8.0 Hz), 7.55 (1 H, d, J = 7.8 Hz), 8.32 (1 H, s); MS m/e (FAB) 492 (M + H, 100), 352 (39), 362 (27), 334 (25).

Benzenebutanoic acid, β-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]amino]propyl]amino]-, [*S*-(*R,*S**)]- (32)**: $[\alpha]^{24}_D$ = −11.6 (c = 1, MeOH); IR (film) 3500–3200, 2907, 2856, 1708, 1657 cm^{-1}; NMR ($CDCl_3$) δ 1.49 (4 H, s), 1.54 (1 H, s), 1.70–2.05 (12 H, m), 2.20–3.00 (1 H, br s), 2.40–2.50 (2 H, m), 2.72 (1 H, dd, J = 13.6, 8.0 Hz), 2.84 (1 H, dd, J = 13.6 Hz, 6.5 Hz), 3.24 (1 H, d, J = 14.7 Hz), 3.44 (1 H, d, J = 14.7 Hz), 4.40–4.50 (1 H, m), 4.81 (1 H, s), 5.30–5.35 (1 H, br s), 6.84 (1 H, d, J = 7.8 Hz), 6.93 (1 H, s), 7.04–7.28 (7 H, m), 7.31 (1 H, d, J = 8.0 Hz), 7.56 (1 H, d, 7.7 Hz), 8.34 (1 H, s); MS m/e (FAB) 558 (M + H, 50), 428 (10), 400 (10), 362 (12), 323 (12), 217 (100).

Benzenebutanoic acid, β-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1.^{3,7}]dec-2-yloxy)carbonyl]amino]propyl]amino]-, [*R*-(*R,*R**)]- (33)**: $[\alpha]^{21}_D$ = +36° (c = 1, MeOH); IR (film) 3450–3200, 2908, 2854, 1711, 1659 cm^{-1}; NMR ($CDCl_3$) δ 1.00–3.00 (1 H, br), 1.50 (1 H, s), 1.53 (4 H, s), 1.70–2.00 (12 H, m), 2.65–2.85 (2 H, m), 3.22 (1 H, d, J = 14.7 Hz), 3.40 (1 H, d, J = 14.7 Hz), 4.35–4.45 (1 H, m), 4.80 (1 H, s), 5.41 (1 H, s), 6.70–6.80 (1 H, m), 6.91 (1 H, s), 7.05–7.27 (7 H, m), 7.33 (1 H, d, J = 8.1 Hz), 7.56 (1 H, d, J = 7.9 Hz), 8.28 (1 H, s); MS m/e (FAB) 558 (M + H, 22), 514 (12), 445 (27), 444 (100), 428 (25), 418 (72), 401 (13), 380 (25), 307 (33).

Benzenebutanoic acid, β-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]amino]propyl]amino]-, [*S*-(*R,*R**)]- (34)**: $[\alpha]^{24}_D$ = −36.6° (c = 1, MeOH); IR (film) 3500–3200, 2919, 2859, 1712, 1658 cm^{-1}; NMR ($CDCl_3$) δ 1.50 (1 H, s), 1.53 (4 H, s), 1.70–2.00 (12 H, m), 2.27 (1 H, dd, J = 16.2, 5.1 Hz), 2.00–3.00 (1 H, br s), 2.36 (1 H, dd, J = 16.2, 5.5 Hz), 2.71 (1 H, dd, J = 13.7, 7.7 Hz), 2.81 (1 H, dd, J = 13.7, 6.3 Hz), 3.22 (1 H, d, J = 14.6 Hz), 3.40 (1 H, d, J = 14.6 Hz), 4.35–4.45 (1 H, m), 4.80 (1 H, s), 5.43 (1 H, s), 6.76 (1 H, d, J = 8.2 Hz), 6.91 (1 H, d, J = 2.3 Hz), 7.05–7.25 (7 H, m), 7.32 (1 H, d, J = 8 Hz), 7.56 (1 H, d, J = 7.7 Hz), 8.33 (1 H, s); MS m/e (FAB) 558 (M + H, 100), 428 (42), 400 (63), 362 (40), 307 (29), 249 (24).

Benzenebutanoic acid, β-[[3-(1*H*-indol-3-yl)-1-oxo-2-[[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]amino]propyl]amino]-, [*R*-(*R,*S**)]- (35)**: $[\alpha]^{20}_D$ = −35.1° (c = 1.0, MeOH); IR 3409, 3155, 2918, 1712, 1666 cm^{-1}; NMR (d_6-DMSO) 330 K δ 1.50 (2 H, d, J = 11.4 Hz), 1.65–2.00 (12 H, m), 2.39 (2 H, d, J = 6.5 Hz), 2.70–3.10 (4 H, m), 4.20–4.40 (2 H, m), 4.62 (1 H, s), 6.52 (1 H, br s), 6.95–7.10 (3 H, m), 7.20–7.40 (5 H, m), 7.59 (1 H, d, J = 7.8 Hz), 7.68 (1 H, d, J = 8.0 Hz), 10.60 (1 H, s); MS m/e (FAB) 544.2 (M + H).

Benzenebutanoic acid, β-[[3-(1*H*-indol-3-yl)-1-oxo-2-[[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]amino]propyl]amino]-, [*S*-(*R,*R**)]- (36)**: $[\alpha]^{20}_D$ = 0.30° (c = 1.0, MeOH); IR (film) 3404, 2855, 1706, 1660, 1525; NMR (d_6-DMSO) δ 1.35–2.00 (14 H, m), 2.30–2.40 (2 H, m), 2.60–2.90 (4 H, m), 4.10–4.20 (1 H, m), 4.20–4.30 (1 H, m), 4.55 (1 H, m), 6.80–7.10 (4 H, m), 7.10–7.30 (6 H, m), 7.50–7.60 (1 H, m), 7.98 (1 H, d, J = 8.1 Hz), 10.70 (1 H, s), 12.30 (1 H, br s); MS m/e (FAB) 544.5 (M + H).