Rationally Designed "Dipeptoid" Analogues of CCK. α-Methyltryptophan Derivatives as Highly Selective and Orally Active Gastrin and CCK-B Antagonists with Potent Anxiolytic Properties

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This paper describes the synthesis and structure–activity relationships (SAR) leading to the first rational design of "dipeptoid" analogues of the neuropeptide cholecystokinin (CCK). Compounds [*R*-(*R**,*S**)]-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-2-butenoic acid (**18**), [*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-oxy)carbonyl]amino]propyl]amino]-1-phenylethyl]amino]-4-oxo-2-butenoic acid (**27**), and [*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 (**29d**) have CCK-B binding affinities of IC_{50} = 0.8, 0.7, and 1.7 nM with a CCK-A/CCK-B ratio of 550, 1100, and 2500, respectively. Compound **27** is well-absorbed and is equiactive by the subcutaneous (sc) and intravenous (iv) routes of administration in the Ghosh and Schild test in rats in inhibiting pentagastrin stimulated gastric acid secretion with ED_{50} = 0.07 (0.01–0.34) μmol/kg. Compound **29d** is anxiolytic in mice in the black–white test box over the range 0.0001–30 mg/kg sc, comparable in activity to diazepam over the range 0.125–1 mg/kg ip), and also active in this test when dosed *orally* over a wide range from 0.0001 to 10 mg/kg.

We have previously reported on the rational design of α-methyl-(*R*)-tryptophan "dipeptoids" with micromolar affinity for the central cholecystokinin (CCK-B) receptor, by systematic investigation of central CCK-B receptor binding of fragments of CCK-26–33.[1-3] We now report the development of our scheme resulting in the discovery of the selective and potent CCK-B dipeptoids given in Tables II and III. These are the first rationally designed non-peptide ligands for neuropeptide receptors (CCK-B and gastrin) which have oral activity, central nervous system activity, low molecular weight, nanomolar affinity, and ca. 1000-fold selectivity over CCK-A and a wide selection of other receptors. The key to the design of these compounds has been the independent optimization of the N- and C-terminal structure–activity relationships (SAR) required for high CCK-B binding affinity and then combining the optimal substitution patterns. This was rationalized as feasible with these semirigid molecules to produce the "super-ligand" compounds in Tables II and III.

Synthesis

Intermediates and all compounds in Tables I–III were prepared according to Schemes I–IV.

Scheme I shows the syntheses of the versatile intermediates **6** and **8** used in later schemes. Mitsunobu reaction of Fmoc-(*S*)-phenylalaninol (**5**) with hydrazoic acid provided an efficient, one-pot route to the protected amino azide which was deprotected with aqueous lithium hydroxide in dioxane. This method for removing the Fmoc group was favored over the traditional method (piperidine/DMF), due to the ease with which the product was isolated.

The regiospecifically protected diamine **8** was prepared from the corresponding N-protected phenylglycinol **7** by tosylation (TsCl, Et_3N, CH_2Cl_2) followed by displacement with azide (NaN_3, DMF, 80 °C) and subsequent hydrogenation of the azide. Use of the Lindlar catalyst did not remove the Cbz protecting group.

Table I. Physical and Chemical Data of Compounds and Intermediates

no.	molecular formula	mp, °C	anal.	method of purifn[a]
5a	$C_{24}H_{23}NO_3$	147	C,H,N	A
5b	$C_{24}H_{23}NO_3$	146	C,H,N	A
6b	$C_9H_{12}N_2$	*b*	*c*	B
7a	$C_{16}H_{17}NO_3$	102–103	C,H,N	A
7b	$C_{16}H_{17}NO_3$	88–90	C,H,N	A
10a	$C_{23}H_{28}N_2O_4 \cdot 0.5C_4H_8O_2$	105–110	C,H,N	A
10b	$C_{23}H_{28}N_2O_4 \cdot 0.25C_4H_8O_2$	100–105	C,H,N	A
10c				A
11a	$C_{31}H_{37}N_3O_3 \cdot 0.25H_2O$	90–94	C,H,N	C
11b	$C_{31}H_{37}N_3O_3 \cdot 0.4H_2O$	73–77	C,H,N	C
11c	$C_{31}H_{37}N_3O_3$	84–86	C,H,N	C
12	$C_{35}H_{41}N_3O_7$	94–100	C,H,N	C
13	$C_{32}H_{39}N_3O_4$	134–140	C,H,N	A
14	$C_{36}H_{43}N_3O_7$	66–69	C,H,N	C
15e	$C_{32}H_{38}N_6O_3$	77–78	C,H,N	C
16e	$C_{32}H_{40}N_4O_3 \cdot C_4H_8O_2$	134–139	C,H,N	A
17	$C_{37}H_{44}N_4O_6 \cdot H_2O$	161–166	C,H,N	C
18	$C_{36}H_{42}N_4O_6$	166–170	C,H,N	C
19	$C_{36}H_{48}N_4O_6 \cdot 0.9H_2O$	85–94	C,H,N	B
20	$C_{37}H_{46}N_4O_6 \cdot 0.75H_2O$	110–121	C,H,N	C
21d	$C_{36}H_{44}N_4O_6 \cdot 0.5H_2O$	104–106	C,H,N	A
21e	$C_{36}H_{44}N_4O_6$	110–114	C,H,N	A
21f	$C_{36}H_{44}N_4O_6$	101–105	C,H,N	A
21g	$C_{36}H_{44}N_4O_6 \cdot 0.5H_2O$	106–110	C,H,N	A
22	$C_{36}H_{44}N_4O_6 \cdot 0.6H_2O$	84–92	C,H,N	B
23	$C_{35}H_{42}N_4O_6 \cdot 1.5H_2O$	121–134	C,H,N	B
24d	$C_{39}H_{44}N_4O_5 \cdot 0.5H_2O$	68–73	C,H,N	D
25d	$C_{31}H_{38}N_4O_3 \cdot 0.75H_2O$	91–94	C,H,N	E
26	$C_{36}H_{42}N_4O_6 \cdot 0.5CHCl_3$	109–112	C,H,N	D
27	$C_{35}H_{40}N_4O_6 \cdot 0.6H_2O$	145–150	C,H,N	C
28	$C_{36}H_{44}N_4O_6 \cdot 0.9C_2H_4O_2$	94–98	C,H,N	B[d]
29d	$C_{35}H_{42}N_4O_6 \cdot 0.5H_2O$	142–146	C,H,N	F
29e	$C_{35}H_{42}N_4O_6 \cdot 0.75C_4H_8O_2$	117–121	C,H,N	F
29f	$C_{35}H_{42}N_4O_6 \cdot 0.75C_4H_8O_2$	117–121	C,H,N	F
29g	$C_{35}H_{42}N_4O_6 \cdot 1.5H_2O$	127–130	C,H,N	F
30	$C_{35}H_{42}N_4O_6$	85–90	C,H,N	D
31	$C_{34}H_{40}N_4O_6 \cdot 0.25H_2O$	122–127	C,H,N	C

[a] Chromatography solvent systems: A, recrystallized from EtOAc or EtOAc/*n*-hexane; B, 2–5% MeOH/CH_2Cl_2; C, 75% MeOH/H_2O (reverse-phase chromatography); D, 50–75% EtOAc/*n*-hexane; E, not purified; F, triturated from MeOH. [b] Compounds was obtained as a viscous oil and elemental analysis and melting point were not measured. [c] *m/e* 177.1440 (MH^+ requires 177.1440). [d] Chromatography eluant contained 0.5% AcOH.

(1) Horwell, D. C.; Beeby, A.; Clark, C. R.; Hughes, J. *J. Med. Chem.* **1987**, *30*, 729.

(2) (a) Horwell, D. C. *Topics in Medicinal Chemistry, 4th SCI-RSC Medicinal Chemistry Symposium*; Leeming, P. R., Ed.; Royal Society of Chemistry Special Publication No. 65; Royal Society of Chemistry: Letchworth, UK, 1988; p 62. (b) Carty, R. P.; Chen, J.; Lubowsky, J.; Avitable, M.; Shah, D.; Scheraga, H. A.; Murphy, R. B. *Proc. Natl. Acad. Sci.* **1987**, *84*, 4821. (c) Chuong, P. P. V. *Horm. Recept. Proc. Int. Symp.*; Horm. *Recept. Dig. Tract Physiol., 2nd.*, **1979**, 33.

(3) Birchmore, B.; Boden, P. R.; Hewson, G.; Higginbottom, M.; Horwell, D. C.; Ho, Y. P.; Hughes, J.; Hunter, J. C.; Richardson, R. S. *Eur. J. Med. Chem.* **1990**, *25*, 53.

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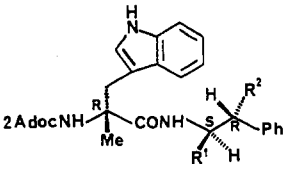
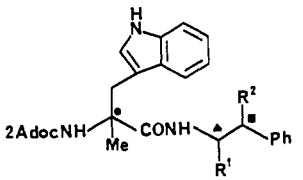
Table II. CCK Receptor Binding Affinites[a]



no.	R^1	R^2	IC_{50}, nM CCK-B	IC_{50}, nM CCK-A	A/B ratio
12[b]	H	$OCO(CH_2)_2CO_2H$	8.7 (5.5–12)	810 (790–820)	93
13	CH_2OH	H	6.3 (4.2–8.9)	780 (690–850)	120
14	$CH_2OCO(CH_2)_2CO_2H$	H	3.4 (2.5–5.8)	740 (690–790)	220
18	$CH_2NHCOCH{=}CHCO_2H$	H	0.8 (0.4–1.2)	440 (430–440)	550
20	$CH_2NHCO(CH_2)_3CO_2H$	H	4.6 (4.0–6.0)	1100 (1000–1400)	250
21e	$CH_2NHCO(CH_2)_2CO_2H$	H	4.2 (2.9–6.3)	950 (740–1100)	230
23	$CH_2NHCOCH_2CO_2H$	H	2.6 (1.8–3.5)	500 (390–600)	190
27	H	$NHCOCH{=}CHCO_2H$	0.7 (0.5–1.0)	790 (680–1000)	1100
28	H	$NHCO(CH_2)_3CO_2H$	14 (12–17)	1300 (250–3000)	93
29d	H	$NHCO(CH_2)_2CO_2H$	1.7 (1.3–2.7)	4300 (1200–8500)	2500
31	H	$NHCOCH_2CO_2H$	0.8 (0.5–1.0)	870 (620–1500)	1100
1, devazepide (MK329)			31 (18–43)	0.1 (0.03–0.2)	0.0032
2, L-365,260			5.1 (4.6–5.4)	230 (170–380)	45
3, CCK-8S			0.3 (0.2–0.3)	0.1 (0.08–0.2)	0.33
4, pentagastrin			0.8 (0.5–0.9)	600 (500–660)	750

[a] IC_{50} represents the concentration (nM) producing half-maximal inhibition of specific binding of [^{125}I]Bolton Hunter CCK-8 to CCK receptors in the mouse cerebral cortex (CCK-B) or the rat pancreas (CCK-A). The values given are the geometric mean and the range from at least three separate experiments. [b] A mixture of two diastereoisomers (*R,S* and *R,R*).

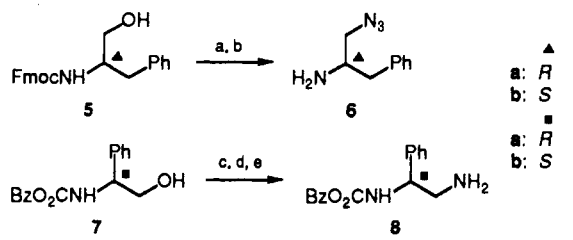
Table III. CCK Receptor Binding Affinities for the Stereoisomers of Compounds 11, 21, and 29[a]



compd	●	▲	■	R^1	R^2	IC_{50}, nM CCK-B	IC_{50}, nM CCK-A	A/B ratio
11a	*R*			H	H	32 (16–47)	650 (510–760)	20
11b	*S*			H	H	330 (190–530)	620 (430–760)	1.9
11c	*RS*			H	H	48 (34–59)	380 (250–560)	8.0
21d	*R*	*R*		$CH_2NHCO(CH_2)_2CO_2H$	H	23 (15–31)	850 (800–1000)	37
21e	*R*	*S*		$CH_2NHCO(CH_2)_2CO_2H$	H	4.2 (2.9–6.3)	950 (740–1100)	230
21f	*S*	*R*		$CH_2NHCO(CH_2)_2CO_2H$	H	170 (160–180)	580 (430–890)	3.4
21g	*S*	*S*		$CH_2NHCO(CH_2)_2CO_2H$	H	180 (150–210)	>1000	>56
29d	*R*		*R*	H	$NHCO(CH_2)_2CO_2H$	1.7 (1.3–2.7)	4300 (1200–8500)	2500
29e	*R*		*S*	H	$NHCO(CH_2)_2CO_2H$	43 (34–50)	3100 (2200–4600)	72
29f	*S*		*R*	H	$NHCO(CH_2)_2CO_2H$	63 (44–79)	18000 (2500–72000)	290
29g	*S*		*S*	H	$NHCO(CH_2)_2CO_2H$	160 (120–190)	2500 (1200–4400)	16

[a] Binding affinities defined in footnote *a*, Table II.

Scheme I[a]



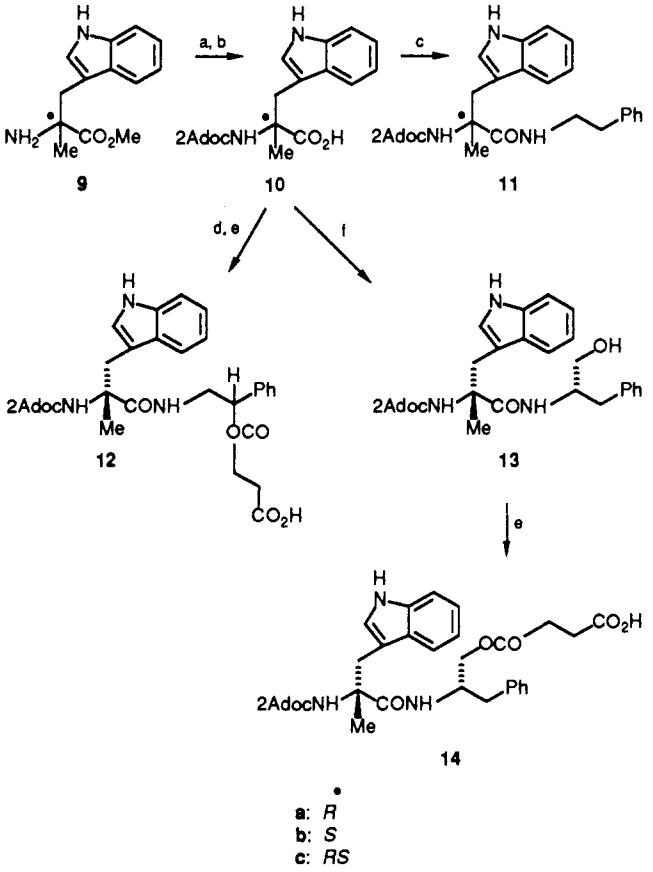
[a] (a) HN_3, PPh_3, $iPrO_2CN{=}NCO_2iPr$, THF; (b) LiOH, aqueous 1,4-dioxane; (c) *p*-TsCl, Et_3N, CH_2Cl_2; (d) NaN_3, DMF, Δ; (e) H_2, Lindlar catalyst, EtOAc.

N^{α}-2-Adoc-α-methyltryptophan (**10**), prepared from methyl ester **9**, was converted into phenethylamide **11** by coupling the acid with phenethylamine (DCC, PFP, EtOAc) (Scheme II). The racemate **11c** as well as the two enantiomers **11a** (*R*) and **11b** (*S*) were prepared from the racemic or appropriately resolved α-methyltryptophan methyl ester.[3]

N^{α}-2-Adoc-α-methyl-(*R*)-tryptophan (**10a**) was converted into the corresponding hydroxy amides on coupling to (*RS*)-2-amino-1-phenylethanol or (*S*)-phenylalaninol, respectively [DCC, HOBt(PFP), EtOAc]. Acylation of the free hydroxyl groups (succinic anhydride, DMAP, EtOAc) yielded the corresponding hydrogen succinate half-esters **12** and **14** in good yield (Scheme II).

Representative α-substituted phenethylamides were prepared from intermediate **16** (Scheme III). Coupling **10c** to **6a** (DCC, PFP, EtOAc) gave azide **15**, which was converted directly to the amine **16** by hydrogenation (H_2, 10% Pd/C, EtOH). Acylation of the amine with (i) monomethyl fumarate (DCC, PFP), (ii) methyl-4-(chloroformyl)butyrate (Et_3N, THF), (iii) succinic anhydride, or (iv) methyl (chloroformyl)acetate yielded the correspond-

Scheme II[a]



[a] (a) 2-AdocCl, Et_3N, THF; (b) LiOH, aqueous THF; (c) **10c**, DCC, PFP, $PhCH_2CH_2NH_2$, EtOAc; (d) **10a**, DCC, HOBt, (*RS*)-2-amino-1-phenylethanol; (e) succinic anhydride, DMAP, EtOAc; (f) **10a**, DCC, PFP, (*S*)-phenylalaninol, EtOAc.

ing methyl esters **17** and **19**, acid **21**, and ester **22** directly; each of the esters was saponified under mild conditions (0.1 N aqueous LiOH/THF) and gave the corresponding acids **18**, **20**, and **23**.

The synthesis of representative β-substituted phenethylamides is illustrated in Scheme IV. **10a** was coupled to **8a** with DCC/HOBt to give benzyl urethane **24** from which the free β-amine **25** was obtained by hydrogenolysis (10% Pd/C, EtOH). Acylation of the amine with methyl (chloroformyl)acetate (THF, Et_3N) yielded methyl ester **30**, saponification (0.1 M LiOH, THF) of which gave the free malonamidic acid **31** while acylation of **25** with glutaric anhydride in ethyl acetate solution led directly to glutaramidic acid **28**. Succinyl analogue **29** was similarly prepared. The four diastereomers of **21** (**21d–g**) and **29** (**29d–g**) were prepared by procedures similar to those in Schemes III and IV, respectively, but starting with the corresponding (*R*)- and (*S*)-α-methyltryptophans (**10a,b**) and the corresponding (*R*)- and (*S*)-phenylalaninols and 2-amino-1-phenylethanols.

Results and Discussion

1. Development of the N-Terminal SAR. We have previously shown that the N-terminal carbamate alkyl moiety requires a cycloalkyl or bulky substituent rather than a straight chain or aromatic hydrocarbon to achieve micromolar CCK-B receptor affinity.[3]

Our attention became focused on bulky, fused C-10 cyclic systems, because the [(1-adamantyloxy)carbonyl]-α-methyl-(*RS*)-tryptophan phenethylamide was shown to be best among C-4 to C-12 cyclic and branched hydrocarbons, with K_i = 5 μM.[3] The 2-adamantyl group was subsequently shown to be superior to the 1-adamantyl group. Furthermore, the [(2-adamantyloxy)carbonyl]-α-methyl-(*R*)-tryptophan-configured derivative **11a** showed a 10-fold higher affinity than the corresponding α-methyl-(*S*)-tryptophan-configured analogue **11b** (Table III).

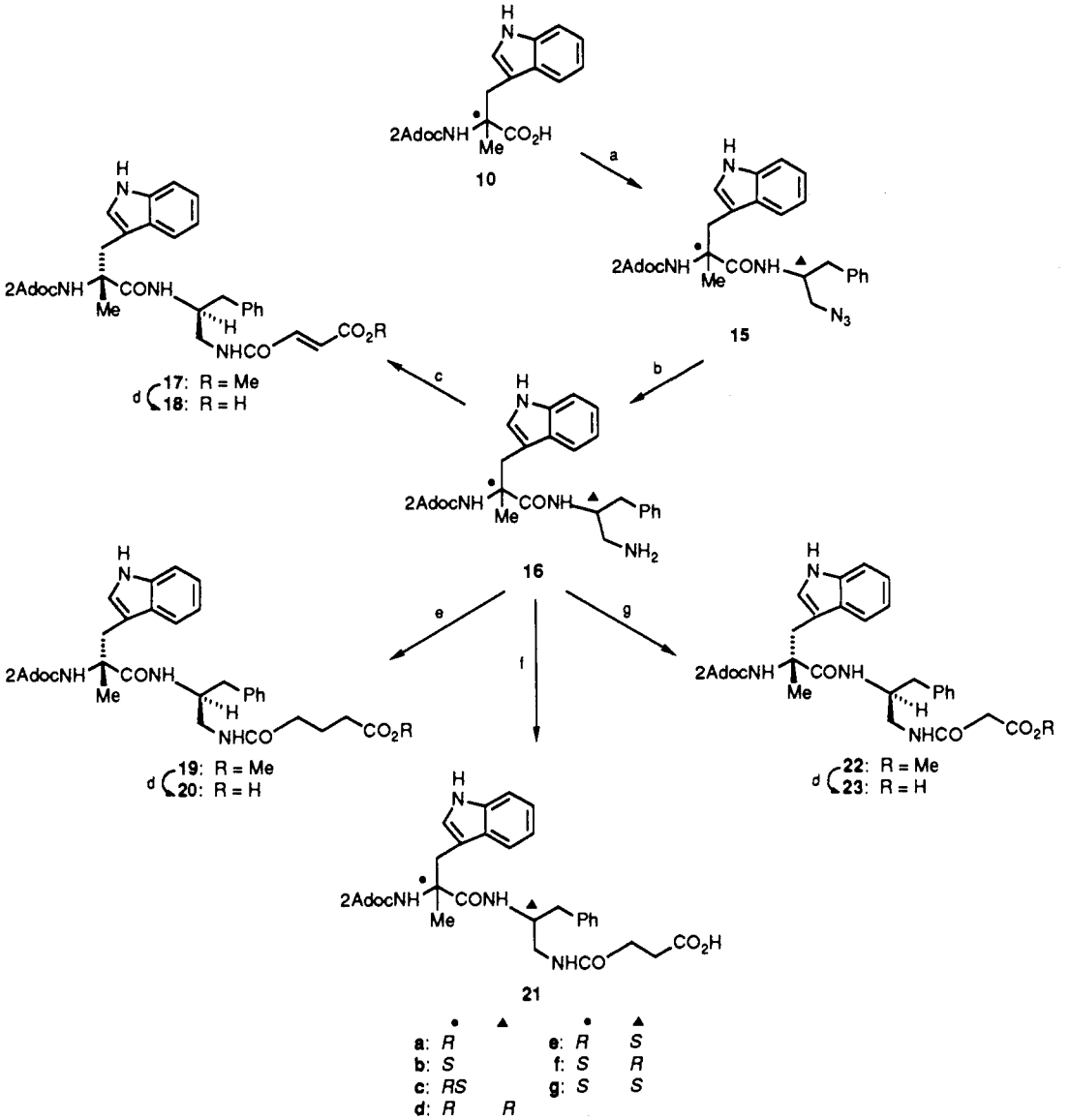
2. Development of C-Terminal SAR. Having established that the optimal N-terminal group is [(2-adamantyloxy)carbonyl]-α-methyl-(*R*)-tryptophan, the SAR of the C-terminus was investigated while the N-terminal group (2-adamantyloxy)carbonyl (2-Adoc) was kept. We were confident that the SAR would most likely be additive to produce the required "super ligand" because these molecules are only semirigid and hence allow the side chains at the C-terminus to explore and find their optimal energy minima at the CCK-B receptor independent of the N-terminal. This indeed proved to be the case.

We have previously shown that the C-terminal could tolerate many changes to the α-phenylalanine side chain, such as (*S*)-Phe-NH_2, (*S*)-Phe-O-H, and (*S*)-Phe-piperidide, with no loss in binding affinity.[3] Furthermore, we have also shown that in the peptide series, the full tetrapeptide structure Trp-Met-Asp-Phe-NH_2 (CCK-30–33) is necessary for nanomolar affinity.[1] These data show, therefore, that the Trp and Phe residues are needed to impart micromolar affinity, but molecular recognition information in the Met and Asp residues enhances affinity a further 1000-fold. Published computer graphics energy minimization of CCK-30–33 (nanomolar affinity) indicates a preferred folded structure.[2b] This structure brings the Trp and Phe residues within 5–8 Å, in agreement with ORD data.[2c] Dreiding model comparison of this folded structure of CCK-30–33 with overlap of the Trp and Phe moieties of Boc-α-methyl-(*R*)-tryptophan (*S*)-phenylalaninamide (micromolar affinity) allowed us to conclude that the Asp residue of CCK-30–33 is readily available to serve as an accessory binding group, in which the terminal COOH group is free to explore a large volume of space.[2] Hence we decided to explore the SAR of mobile chains on the C-terminus which terminate in the COOH group. This accessory binding group concept has precedent in being successfully applied to enhance the potency of certain enzyme inhibitors. For example, the inhibition of dihydrofolate reductase by trimethoprim,[4] and penicillopepsin by pepstatin analogues[5] has been increased 80–100-fold by introduction of a chain into these inhibitors that terminates in a COOH and NH_2 group, respectively. In these enzyme cases, X-ray of the binary complexes show that these groups are able to form salt bridges with complementary amine (arginine) and acid (aspartic acid) groups, respectively, in the enzyme–protein framework.

Hence, the compounds with C-terminal chains terminating in COOH, compounds **14**, **18**, **20**, **21e**, and **23**, were prepared and all had greatly improved affinity in the nanomolar range (IC_{50} = 3.4, 0.8, 4.6, 4.2, and 2.6 nM, respectively, Table II) compared with the corresponding phenylethylamide derivative **11a** (IC_{50} = 32 nM, Table III). Having established that this strategy indeed worked, examination of the Newman projection about the (α,β)-C–C–Ph bond revealed that the orientation in space of the α-substituted (*S*)-phenethylamide derivatives, e.g. **21e**, could be mimicked by the similar *R*-configured side chain in the β-position. This would lead to derivatives that had the *R* configuration at both chiral centers, and the C-

(4) Kuyper, L. F.; Roth, B.; Baccanari, D. P.; Ferone, R.; Beddell, C. R.; Champness, J. N. *J. Med. Chem.* **1982**, *25*, 1120.
(5) Salituro, F. G.; Agarwal, N.; Hofmann, T.; Rich, D. H. *J. Med. Chem.* **1987**, *30*, 286.

Scheme III[a]



[a] (a) 6, DCC, PFP, EtOAc; (b) 10% Pd/C, H_2, EtOH; (c) $MeO_2CCH{=}CHCO_2H$, DCC, PFP, EtOAc; (d) 0.1 M LiOH, THF; (e) $MeO_2C(CH_2)_3COCl$, Et_3N, THF; (f) succinic anhydride, EtOAc; (g) MeO_2CCH_2COCl, Et_3N, THF.

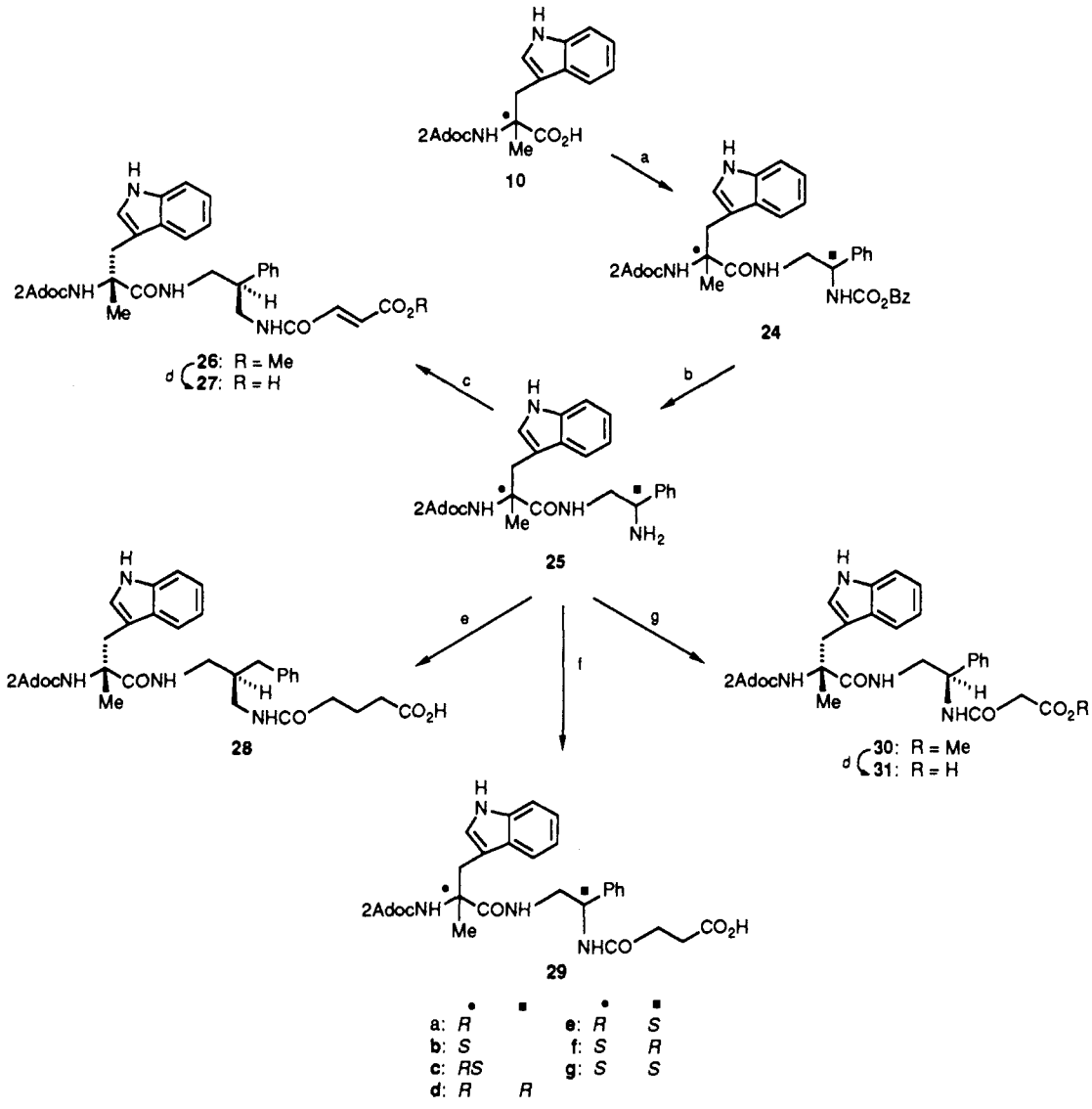
terminal would no longer be expected to be a good substrate for carboxypeptidases as the chain is fused from the β- rather than the "natural" amino acid α-position. Compound **29d** was prepared to test this hypothesis. This compound could be synthesized from (*R*)-α-phenylglycinol which has the same *through bond* separation of the terminal COOH group as **21e** from the α-methyltryptophan residue. This compound **29d** along with the fumaryl analogue **27** retained nanomolar binding affinity, IC_{50} = 1.7 and 0.7 nM, respectively. Even the shorter chain acid **31** has an IC_{50} of 0.8 nM. These dipeptoids all contain the major structural elements of CCK-4 distributed in such a way that they show nanomolar binding affinity to the CCK-B receptor comparable to those of the CCK agonists **3** (CCK-26–33) (CCK-8 sulphated) and **4** (pentagastrin) (Table II). All four diastereomers of **21** (**21d–g**) and **29** (**29d–g**) were prepared for comparison of their binding affinities (Table III). It is concluded that in the α-phenylethylamide series **21d–g** that the *R,S* configuration is optimal, and in the β-phenylethylamide series **29d–g** the *R,R* configuration is optimal for CCK-B affinity and selectivity over CCK-A. Table II also gives comparative binding data for the CCK-A-selective antagonist devazepide (**1**) and the CCK-B-selective antagonist L-365,260 (**2**).

Biological Activity

We consider our strategy to design peptoids from neuropeptides to have been vindicated.[1-3] Having obtained these compounds with nanomolar CCK-B affinities and selectivity over the CCK-A binding of >1000:1 for compounds **27**, **29d**, and **31**, we examined their pharmacological properties.

Compounds **27** and **29d**, for example, are potent, highly selective gastrin antagonists, as assayed by their intravenous (iv) and subcutaneous (sc) administration against maximal stimulation of gastric acid secretion by pentagastrin in the Parsons-modified Ghosh and Schild test in rats. Thus, gastric acid secretion stimulated by a continuous infusion of pentagastrin (1 μg/kg per min) was blocked by an iv infusion of 0.5 μmol/kg of compound **29d** (Figure 1). Compound **27** was *equiactive* in blocking acid secretion in this test by both the iv and sc routes of administration with ED_{50} = 0.07 (0.01–0.34) μmol/kg. Hence this compound appears to be well-absorbed by the subcutaneous route of administration and is more potent than

Scheme IV[a]



[a] (a) DCC, HOBt, 8, EtOAc; (b) Pearlman's catalyst, H_2, EtOH; (c) $MeO_2CCH{=}CHCO_2H$, DCC, HOBt, EtOAc; (d) 0.1 M LiOH, THF; (e) glutaric anhydride, EtOAc; (f) succinic anhydride, EtOAc; (g) MeO_2CCH_2COCl, Et_3N, EtOAc.

ranitidine [ED_{50} = 0.19 (0.11–0.36) μmol/kg] in this test (Figure 2).

The recently described benzodiazepine-derived CCK-B/gastrin antagonist **2** (L-365,260) also appears to inhibit gastric acid secretion in mice.[6]

Compound **29d** was chosen for examination of CCK-B behavioral effects on the central nervous system (CNS). The overall lipophilicity of these compounds is high, i.e. C – log, P for **13** and **29d** = 5.4 and 5.0, respectively, and is comparable with known CNS-active drugs that are well-absorbed orally and cross the blood–brain barrier by passive diffusion, e.g. chlorpromazine and imipramine have C – log P = 5.28 and 4.62, respectively. Hence, as CCK-B receptors are found in the brain[6,7] which appear to have comparable affinity for pentagastrin to the functional gastrin receptors in gut,[6] we rationalized that these ligands were ideal probes to explore the functional role of CCK-B receptors in the brain. The highly water soluble *N*-methyl-D-glucamine salts of these carboxylic acids were used for this purpose. CCK is known to coexist with GABA in some cortical interneutrons,[8] and agents that modify GABA may have utility as anxiolytic agents.[9]

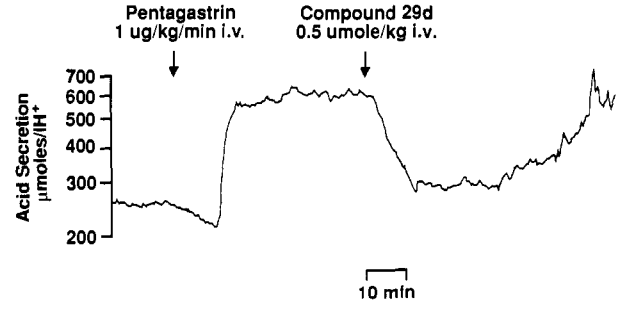


Figure 1. Reversal of pentagastrin-stimulated acid secretion by compound **29d**.

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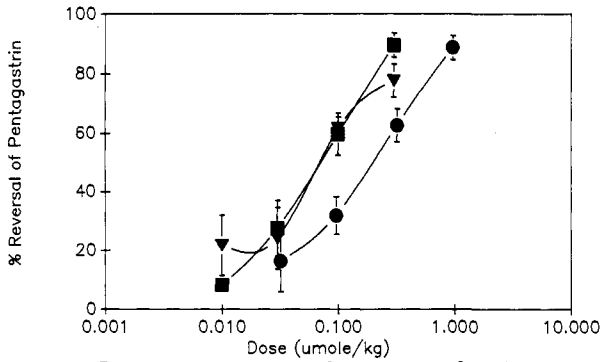

Figure 2. Dose–response curves for compound **27** (sc, ▼; iv, ■) and ranitidine (sc, ●). $n = 4$.

Therefore, among other CNS activities, we elected to study the potential of these CCK antagonist ligands as anxiolytic agents. Further support for a role of CCK in anxiety is the recent observation that CCK-30–33 (a selective CCK-B agonist) precipitates anxiogenesis in man, the effect of which appeared to be blocked by the clinically effective anxiolytic lorazepam.[10] Furthermore, anxiolytic activity of the CCK-A-selective antagonist devazepide in mice in the black–white test box has recently been reported.[11]

Compound **29d** was investigated in the black–white test box, which has shown to be a sensitive test for anxiolytics in which diazepam, buspirone, and the 5-HT_3 antagonists such as GR 38032F (ondansetron) and ICS 205,930 are active.[12]

The results obtained with **29d** are shown in Figures 3 and 4. Control mice, not treated with **29d** but subjected to the aversive stimulus, showed a high level of rearing and line-crossing activity in the black compartment compared to the white compartment (Figure 3). Treatment with **29d** (0.0001–30 mg/kg sc) reversed this response and the mice now showed higher rearing and line-crossing activity in the white compartment than in the black compartment. These results suggest that **29d** possesses anxiolytic activity in this test. Figure 4 shows that **29d** is also effective as an anxiolytic agent in this test over a wide dose range (0.0001–10 mg/kg) by the *oral* route of administration. Figure 5 shows the comparable effects with the reference anxiolytic diazepam in this test over the dose range 0.125–1 mg/kg ip. The potential anxiolytic activity of these compounds in three other tests, including the elevated X-maze, will be reported elsewhere.[13]

Conclusion

Non-peptide and pseudo-peptide ligands of both A and B subtypes of CCK receptors that have been described during the past 5 years include cyclic and linear analogues of CCK-26–33,[14] cyclic CCK-B antagonist analogues of CCK-26–33,[15] small molecule antagonists with improved potency over benzotript (A-65186[16]) and proglumide (lorglumide[17]), as well as the CCK-A and -B selective antagonist benzodiazepine derivatives devazepide and L-365,260, which were designed starting from the chemical lead of the benzodiazepine ring containing fungal metabolite asperlicin.[6,18] These data have been recently reviewed.[18]

The molecules described in this paper (Tables II and III) represent the *first* rationally designed non-peptide ligands for neuropeptide receptors. Our scheme as described above has utilized receptor binding affinity as the guide to use medicinal chemistry rationale in starting with the full neuropeptide structure (CCK-26–33) and logically arrive at the highly selective CCK-B/gastrin "dipeptoid" antagonists given in Tables II and III which are orally active and have potent antigastrin and central anxiolytic actions.

Experimental Section

Biological Assays. 1. CCK Receptor Binding Assay. CCK-A receptor binding assays were performed on male rat pancreas. Tissue (250 mg) homogenized in ice-cold Tris-HCl (pH 7.4) (50 mL of a 50 mM solution) was centrifuged at 20000*g*. The pellet was washed and resuspended in a standard assay buffer (SAB) comprising 10 mM Hepes (pH 7.2 at 21 °C), 130 mM NaCl, 5 mM $MgCl_2$, 4.7 mM KCl, 1 mM 1,2-bis(2-aminoethoxy)ethane, and 0.25 mg/mL bacitracin at a tissue concentration of 0.5 mg of original wet weight per mL of buffer.

CCK-B receptor binding assays 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 20000*g*. The pellet was washed and resuspended in SAB 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 concentrations 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 measured with a Packard series 5000 γ-counter. **2.** Blockade of pentagastrin-stimulated gastric acid secretion was performed by the Parsons modification[19] of the Ghosh and Schild test.[20] **3.** The black–white test box procedure for anxiolytic activity in mice was performed by the method of Costall.[12]

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 Finnegan 4500 or a ZAB-E VG Analytical. Elemental analyses were determined by CHN Analysis Limited, Leicester, UK. Normal phase silica gel used for chromatography was Kieselgel-60 (230–400 mesh); reverse-phase silica gel used was Lichroprep RP-18 (230–400 mesh); both were supplied by E. Merck, A.G., Darmstadt, Germany. Anhydrous solvents were dried over 4-Å molecular sieves prior to use. The filter aid used throughout was Celite, purchased from the Aldrich Chemical Co. Ltd., Gillingham, England. 2-Adamantanol, (*RS*)-2-amino-1-phenylethanol, and (*S*)-phenylalaninol were purchased from the Aldrich Chemical Co. Ltd., Gillingham, England. α-Methyltryptophan was purchased from Bachem, A.G., Bubendorf, Switzerland, and was resolved by

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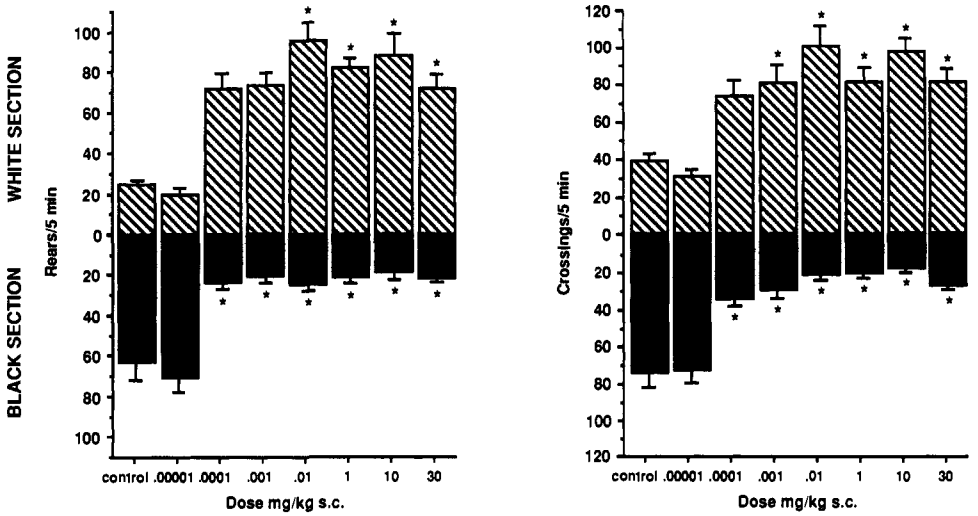

Figure 3. Anxiolytic action of compound **29d** assessed in the mouse black–white test box. $n = 5$–10. $^*P < 0.001$ (anxiolytic action). Pretreatment time was 40 min.

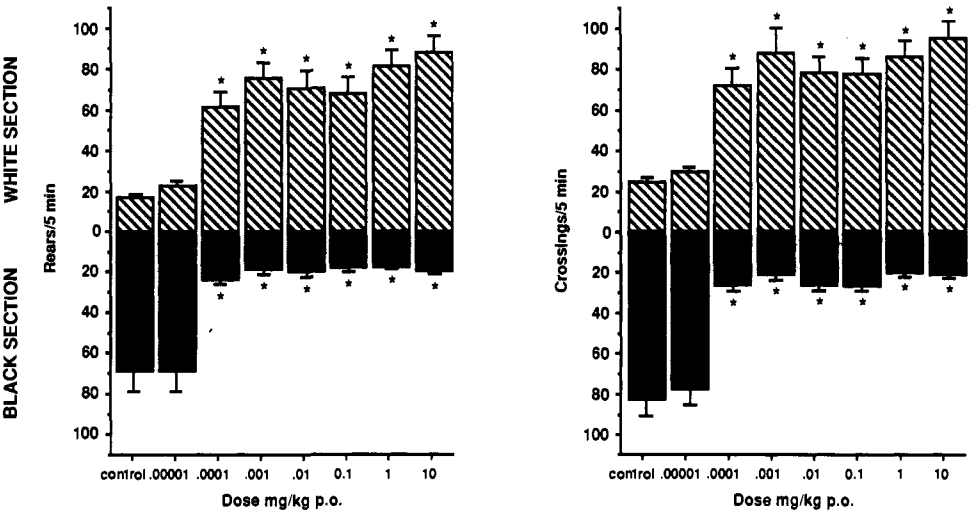

Figure 4. Anxiolytic activity of orally administered compound **29d** assessed in the mouse black–white test box. $n = 5$. $^*P < 0.001$ (anxiolytic action). Pretreatment time was 40 min.

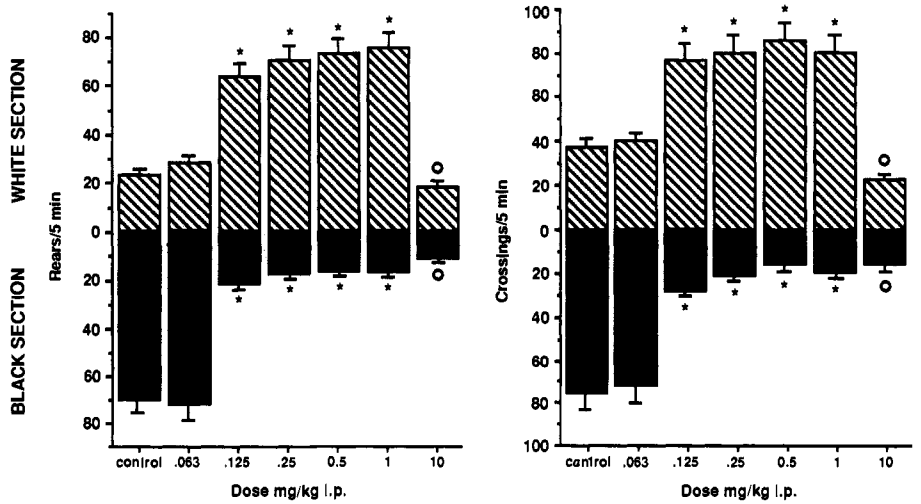

Figure 5. The effect of diazepam in the mouse black–white test box. $n = 5$. $^*P < 0.001$–0.001. SEMs are for original data. o indicates sedation.

chymotrypsin digestion of the methyl ester.[3]

9*H*-Fluoren-9-ylmethyl (*S*)-[1-(Hydroxymethyl)-2-phenylethyl]carbamate (5b). A solution of (*S*)-(−)-phenylalaninol (10 g, 66 mmol) in 2:1 H_2O/1,4-dioxane (750 mL) was treated dropwise with a solution of 9-fluorenylmethyl chloroformate (17.1 g, 66.1 mmol) in 1,4-dioxane (250 mL) and stirred for 18 h at room temperature. The solvent was evaporated in vacuo and the residue suspended between H_2O (400 mL), 1 *M* citric acid solution (100 mL), and EtOAc (500 mL). Both phases were filtered simultaneously, and the white precipitate was washed with H_2O (200 mL) and cold ether (200 mL) to give 10.7 g of product **5b**. The organic phases were combined, the solvents were

removed in vacuo, and the residue was crystallized from ether to give a further 11.8 g of product. An analytically pure sample was obtained by recrystallization from EtOAc: total yield 22.5 g, 91.5% $[\alpha]^{20}_D$ = −40.8° (c = 0.5, MeOH); IR (film) 1689 cm^{-1}; NMR (DMSO-d_6) δ 2.61 (1 H, dd, J = 13.5 and 9 Hz), 2.86 (1 H, dd, J = 13.5 and 5 Hz), 3.30–3.45 (2 H, m), 3.60–3.70 (1 H, m), 4.10–4.30 (3, H, m), 3.75 (1 H, t, J = 5.5 Hz), 7.10–7.50 (10 H, m), 7.60–7.70 (2 H, m), 7.88 (2 H, d, J = 7 Hz).

9*H*-Fluoren-9-ylmethyl (*R*)-[1-(Hydroxymethyl)-2-phenethyl]carbamate (5a). The method was as for **5b** except using (*R*)-(+)-phenylalaninol: yield 22.15 g, 90%. Data was as for **5b** except $[\alpha]^{20}_D$ +41.8° (c = 0.5, MeOH).

(*S*)-α-(Azidomethyl)-2-phenylethanamine (6b). To a solution of **5b** (3.73 g, 10.0 mmol) in anhydrous THF (30 mL) was added hydrazoic acid in toluene (20 mL of a 0.6 M solution, 12 mmol) followed by a solution of triphenylphosphine (2.62 g, 10.0 mmol) in anhydrous THF (20 mL). A solution of diisopropyl azodicarboxylate (2.22 g, 11.0 mmol) in anhydrous THF (20 mL) was added dropwise with stirring at room temperature. After 1 h the solvents were distilled in vacuo, and the residue was chromatographed over silica gel using 20% EtOAc in *n*-hexane as eluant, giving a white solid (3.18 g, 80%); mp 72–73 °C; IR (film) 2101 and 1698 cm^{-1}; NMR ($CDCl_3$) δ 2.85 (2 H, m), 3.30–3.50 (2 H, m), 4.00 (1 H, br s), 4.19 (1 H, t, J = 7 Hz), 4.39 (1 H, br s), 4.85 (1 H, br d), 7.15–7.55 (11 H, m), 7.76 (2 H, d, J = 9 Hz). This solid (1.0 g, 2.5 mmol) in 1:1 H_2O/1,4-dioxane (20 mL) was treated with $LiOH·H_2O$ (105 mg, 2.5 mmol) and the mixture left for 18 h at room temperature. The solvents were removed in vacuo and the residue was suspended between H_2O (20 mL) and EtOAc (30 mL); both phases were filtered simultaneously, and the aqueous phase was extracted with EtOAc (3 × 30 mL). The combined organic phases were dried over $MgSO_4$, and the solvent was removed in vacuo. The residue was purified by chromatography to give amine **6b** as a syrup (377 mg, 86%): $[\alpha]^{20}_D$ = +14.0° (c = 1.75, $CHCl_3$); IR (film) 2130 cm^{-1}; NMR ($CDCl_3$) δ 1.4 (2 H, br s), 2.59 (1 H, dd, J = 13.5 and 8 Hz), 2.78 (1 H, dd, J = 13.5 and 5 Hz), 3.15–3.25 (2 H, m), 3.35–3.40 (1 H, m), 7.10–7.40 (5 H, m).

(*R*)-α-(Azidomethyl)-2-phenylethanamine (6a). The method was exactly as for **6b** except using 9*H*-fluoren-9-ylmethyl (*R*)-[1-(hydroxymethyl)-2-phenylethyl]carbamate (**5a**): yield 7.0 g, 63% over two steps; $[\alpha]^{20}_D$ = −14.7 ° (c = 1, $CHCl_3$).

***N*-[(Benzyloxy)carbonyl]-(*R*)-β-amino-2-phenylethanol (7a).** A mixture of (*R*)-phenylglycinol (4.00 g, 29.2 mmol) and benzyl chloroformate (5.47 g, 33.4 mmol) in anhydrous THF (50 mL) at 0 °C was treated with a solution of triethylamine (3.24 g, 32.1 mmol) in THF (10 mL) over a 5-min period. This mixture was stirred a further 18 h at room temperature then filtered and concentrated in vacuo to give a pale brown solid (7.4 g) which was recrystallized from EtOAc/*n*-hexane to yield urethane **7a** as white needles (6.9 g, 87%): $[\alpha]^{20}_D$ = −35.1° (c = 1, MeOH); IR (film) 3325, 1687, and 1542 cm^{-1}; NMR ($CDCl_3$) δ 2.27 (1 H, br s), 3.87 (2 H, br s), 4.83 (1 H, br s), 5.06 (1 H, d, J = 12 Hz), 5.12 (1 H, d, J = 12 Hz), 5.53 (1 H, br s), 7.25–7.40 (10 H, m).

***N*-[(Benzyloxy)carbonyl]-(*S*)-β-amino-2-phenylethanol (7b).** The method was as for **7a** except using (*S*)-phenylglycinol: yield 3.28 g, 83%. Data was as for **7b** except $[\alpha]^{20}_D$ = +36.2° (c = 1, MeOH).

***N*β-[(Benzyloxy)carbonyl]-(*R*)-β-amino-2-phenylethanamine (8a).** A solution of **7a** (6.44 g, 23.8 mmol) in anhydrous CH_2Cl_2 (50 mL) was treated with triethylamine (2.88 g, 28.5 mmol), followed by a solution of *p*-toluenesulfonyl chloride (5.43 g, 28.5 mmol) in CH_2Cl_2 (20 mL). After stirring for 18 h at room temperature, the reaction mixture was washed with 1 M citric acid solution (2 × 50 mL), the organic phase dried over $MgSO_4$ and filtered, and the solvent evaporated in vacuo to give a crude, pale yellow solid (8.49 g): mp 103–105.5 °C (EtOAc/*n*-hexane); IR (film) 3410, 1703, 1361, and 1190 cm^{-1}; NMR ($CDCl_3$) δ 2.42 (3 H, s), 4.25 (2 H, m), 4.98 (1 H, br s), 5.07 (2 H, s), 5.35 (1 H, br s), 7.20–7.40 (12 H, m), 7.65 (2 H, d, J = 8 Hz). This crude solid (7.57 g) was dissolved in anhydrous DMF (100 mL), treated with sodium azide (1.21 g, 18.6 mmol), then warmed to 80 °C for 3 h, cooled, and poured into ice water (200 mL). This mixture was extracted with Et_2O (2 × 200 mL), and the combined organic phases were washed with H_2O (200 mL), dried over $MgSO_4$, and evaporated in vacuo to yield a yellow oil (4.95 g): IR (film) 3300, 2103, and 1697 cm^{-1}; NMR ($CDCl_3$) δ 3.66 (2 H, m), 4.95 (1 H, m), 5.09 (1 H, d, J = 11 Hz), 5.12 (1 H, d, J = 11 Hz), 5.31 (1 H, m), 7.25–7.45 (10 H, m). This crude oil (5 g) in EtOAc (100 mL) was treated with Lindlar catalyst (2 g, 40% w/w) and placed under an atmosphere of hydrogen at 45 psi at 30 °C for 6 h then filtered through filter aid to give a solution of the desired amine **8a** which was used immediately, assuming a quantitative yield; IR (film) 3300, 1703 cm^{-1}.

α-Methyl-*N*-[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]-(*R*)-tryptophan (10a). To a stirred solution of 2-adamantanol (912 mg, 5.99 mmol) in anhydrous CH_2Cl_2 (15 mL) was added bis(trichloromethyl) carbonate (653 mg, 2.20 mmol) and pyridine (474 mg, 5.99 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 h. The solvent was removed in vacuo at 30 °C and the residue dissolved EtOAc (30 mL) and stirred for 10 min. The precipitate was filtered off and the solvent removed in vacuo at 30 °C to give an oil which solidified upon standing (1.29 g, 100%): IR (film) 1778 cm^{-1}; NMR ($CDCl_3$) δ 1.55–1.65 (2 H, m), 1.70–1.80 (4 H, m), 1.85–1.95 (4 H, m), 2.00–2.10 (2 H, m), 2.15–2.20 (2 H, m), 5.02 (1 H, d, J = 3 Hz). To a stirred solution of this solid (965 mg, 4.5 mmol) in anhydrous THF (10 mL) was added a solution of α-methyl-(*R*)-tryptophan methyl ester **9a** (928 mg, 4.00 mmol) in anhydrous THF (20 mL) followed by a solution of triethylamine (808 mg, 7.98 mmol) in anhydrous THF (20 mL) dropwise. After 15 min, the reaction mixture was filtered, the solvent removed in vacuo, and the residue column chromatographed using 2% MeOH in CH_2Cl_2 as eluant to yield a syrup (1.42 g, 89%): IR (film) 1740–1695 cm^{-1}; NMR ($CDCl_3$) δ 1.50–1.60 (2 H, m), 1.67 (3 H, s), 1.70–2.10 (12 H, m), 3.38 (1 H, d, J = 14.5 Hz), 3.50–3.60 (1 H, br s), 3.68 (3, H, s), 4.86 (1, H, br s), 5.28 (1 H, br s), 6.93 (1 H, d, J = 2 Hz), 7.04–7.10 (2 H, m), 7.33 (1 H, d, J = 8 Hz), 7.54 (1 H, d, J = 8 Hz), 8.18 (1 H, br s). To a stirred solution of this syrup (1.36 g, 3.31 mmol) in H_2O/1,4-dioxane (20 mL) was added $LiOH·H_2O$ (210 mg, 5.00 mmol) and the mixture stirred at room temperature for 18 h. After removal of the solvent in vacuo, the residue was chromatographed to yield acid **10a** (953 mg, 90%) as a white solid, crystallized from EtOAc/*n*-hexane: $[\alpha]^{20}_D$ = +15.9° (c = 1, MeOH); IR (film) 1689 cm^{-1}; NMR ($CDCl_3/D_2O$) δ 1.3–2.2 (14 H, m), 1.70 (3 H, s), 3.26 (1. H, d, J = 13.5 Hz), 3.63 (1 H, d, J = 13.5 Hz), 4.77 (1 H, br s), 6.85–7.60 (5 H, m).

α-Methyl-*N*-[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]-(*S*)-tryptophan (10b). The methods were exactly as for **10a**: yield 10.8 g, 88% from α-methyl-(*S*)-tryptophan; $[\alpha]^{20}_D$ = −17.0° (c = 1, MeOH).

α-Methyl-*N*-[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]-(*RS*)-tryptophan (10c). The methods were exactly as for **10a**: yield 2.4 g, 61% from α-methyl-(*RS*)-tryptophan.

Tricyclo[3.3.1.1^{3,7}]dec-2-yl (*R*)-[1-(1*H*-Indol-3-ylmethyl)-1-methyl-2-oxo-2-[(2-phenylethyl)amino]ethyl]carbamate (11a). A solution of **10a** (390 mg, 0.982 mmol) and pentafluorophenol (184 mg, 1.00 mmol) in anhydrous EtOAc (10 mL) at 0 °C was treated with a solution of *N*,*N*′-dicyclohexylcarbodiimide (216 mg, 1.05 mmol) in EtOAc (5 mL). This mixture was stirred at 0 °C for 18 h then filtered. A solution of phenethylamine (145 mg, 1.20 mmol) was added to the filtrate and left for a further 48 h. The mixture was then washed with 1 M HCl (50 mL), H_2O (50 mL), 1 M NaOH (50 mL), and H_2O (50 mL). The organic phase was dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was chromatographed to give the product as a white foam (415 mg, 83%): $[\alpha]^{20}_D$ = +20.8° (c = 0.42, MeOH); IR (film) 3324, 1701, and 1656 cm^{-1}; NMR ($CDCl_3$) δ 1.50–2.0 (17 H, m), 2.67 (2 H, t, J = 7 Hz), 3.26 (1 H, d, J = 15 Hz), 3.40–3.50 (3 H, m), 4.80 (1 H, br s), 5.25 (1 H, br s), 6.18 (1 H, br s), 6.95 (1 H, d, J = 2 Hz), 7.10–7.30 (7 H, m), 7.35 (1 H, d, J = 8 Hz), 7.58 (1 H, d, J = 8 Hz), 8.08 (1 H, br s); MS *m/e* (FAB) 500 (100).

Tricyclo[3.3.1.1^{3,7}]dec-2-yl (*S*)-[1-(1*H*-Indol-3-ylmethyl)-1-methyl-2-oxo-1-[(1-phenylethyl)amino]ethyl]carbamate (11b). The method was as for **11a** except using **10b**: yield 234 mg, 49%; $[\alpha]^{20}_D$ = −19.1° (c = 1.1, MeOH).

Tricyclo[3.3.1.1^{3,7}]dec-2-yl (*RS*)-[(1*H*-Indol-3-ylmethyl)-1-methyl-2-oxo-2-[(2-phenylethyl)amino]ethyl]carbamate (11c). The method was as for **11a** except using **10c**: yield 385 mg, 77%.

Mono[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] Butanedioate (Trp center is *R*, other center is *RS*) (12). A solution of **10a** (60 mg, 0.15 mmol) in EtOAc (7 mL) was treated with *N,N'*-dicyclohexylcarbodiimide (34 mg, 0.17 mmol) and 1-hydroxybenzotriazole hydrate (25 mg, 0.16 mmol). After stirring for 2 h at room temperature (*RS*)-2-amino-1-phenylethanol (21 mg, 0.15 mmol) in EtOAc (2 mL) was added and the mixture stirred for a further 2 h. The suspension was filtered and the filtrate concentrated in vacuo. The residue was chromatographed over alumina using 75% EtOAc in *n*-hexane as eluant to give a slightly impure mixture of two diastereoisomers (58 mg): IR (film) 3338, 1690, and 1622 cm^{-1}; NMR (inter alia) ($CDCl_3$) δ 1.50–2.05 (17 H, m) 3.15–3.55 (4 H, m), 3.75 (1 H, m), 4.85 (1 H, m), 5.10 (0.5 H, s), 5.20 (0.5 H, s), 6.55 (1 H, m), 7.00–7.40 (9 H, m), 7.60 (1. H, d, J = 9 Hz), 8.16 (0.5 H, s), 8.18 (0.5 H, s). This impure mixture (58 mg) as a solution in EtOAc (10 mL) was refluxed with succinic anhydride (13 mg, 0.13 mmol) and 4-(dimethylamino)pyridine (27 mg, 0.22 mmol) for 24 h. The reaction mixture was washed with 1 M citric acid solution (10 mL) and the organic phase dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was chromatographed to give **12** as an amorphous white solid and a mixture of two diastereoisomers (21 mg, 22% overall from **10a**): IR (film) 3352, 1722, and 1665 cm^{-1}; NMR ($CDCl_3$) δ 1.50–2.10 (17 H, m), 2.55–2.75 (4 H, m), 3.20–3.55 (3 H, m), 3.85–4.00 (1 H, m), 4.88 (0.5 H, s), 4.94 (0.5 H, s), 5.48 (0.5 H, s), 5.58–5.68 (1 H, m), 5.83 (0.5 H, d, J = 7 Hz), 6.65–6.75 (1 H, m), 6.96 (0.5 H, d, J = 2 Hz), 7.07–7.40 (8.5 H, m), 7.58 (0.5 H, d, J = 8 Hz), 7.62 (0.5 H, d, J = 8 Hz), 8.15 (0.5 H, s), 8.22 (0.5 H, s).

Tricyclo[3.3.1.1^{3,7}]dec-2-yl [*R*-(*R,*S**)]-[2-[[1-(Hydroxymethyl)-2-phenylethyl]amino]-1-(1*H*-indol-3-ylmethyl)-1-methyl-2-oxoethyl]carbamate (13).** The method was as for **11a** except using (*S*)-phenylalaninol: yield 5.3 g, 79%; $[\alpha]^{20}_D$ = +11.6° (c = 1.02, MeOH); IR (film) 1695 and 1659 cm^{-1}; NMR ($CDCl_3$) δ 1.39 (3 H, s), 1.50–2.00 (14 H, m), 2.75–2.85 (3 H, m), 3.32 (1 H, d, J = 15 Hz), 3.40–3.50 (2 H, m), 3.70–3.80 (1 H, m), 4.15–4.25 (1 H, m), 4.82 (1 H, br s), 5.01 (1 H, s), 6.19 (1 H, d, J = 8 Hz), 6.91 (1 H, d, J = 2.5 Hz), 7.05–7.25 (7 H, m), 7.35 (1 H, d, J = 8 Hz), 7.58 (1 H, d, J = 8 Hz), 8.10 (1 H, br s).

Mono[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] [*R*-(*R,*S**)]Butanedioate (14).** A solution of **13** (5.0 g, 9.5 mmol) in EtOAc (50 mL) was treated with succinic anhydride (1.04 g, 10.4 mmol) and 4-(dimethylamino)pyridine (1.38 g, 11.4 mmol) and refluxed for 24 h. The reaction mixture was washed with 1 M citric acid solution (50 mL), the organic phase dried over $MgSO_4$ and filtered, and the filtrate concentrated in vacuo. The residue was chromatographed to give **14** as a white, amorphous solid (5.83 g, 98%): $[\alpha]^{20}_D$ = +14.1° (c = 1.01, MeOH); IR (film) 1718 and 1660 cm^{-1}; NMR ($CDCl_3$) δ 1.54 (5 H, m), 1.70–2.00 (12 H, m), 2.62 (4 H, s), 2.70 (1 H, dd, J = 7 and 13 Hz), 2.80 (1 H, dd, J = 7 and 14 Hz), 3.25 (1 H, d, J = 15 Hz), 3.45 (1 H, d, J = 15 Hz), 3.90 (2 H, m), 4.35 (1 H, m), 4.88 (1 H, br s), 5.55 (1 H, br s), 6.61 (1 H, d, J = 8 Hz), 7.00 (1 H, br s), 7.05–7.33 (8 H, m), 7.34 (1 H, d, J = 8 Hz), 7.60 (1 H, d, J = Hz), 8.25 (1 H, br s).

Tricyclo[3.3.1.1^{3,7}]dec-2-yl [*R*-(*R,*S**)]-[2-[[1-(Azidomethyl)-2-phenylethyl]amino]-1-(1*H*-indol-3-ylmethyl)-1-methyl-2-oxoethyl]carbamate (15e).** The method was as for **11a** except using amine **6b**: yield 12.7 g, 92%; $[\alpha]^{20}_D$ = +17.0° (c = 0.2, MeOH); IR (film) 3339, 2102, 1699, and 1666 cm^{-1}; NMR ($CDCl_3$) δ 1.47 (3 H, s), 1.52–2.02 (14 H, m), 2.68 (1 H, dd, J = 8 and 14 Hz), 2.74 (1 H, dd, J = 6.5 and 14 Hz), 3.23 (2 H, m), 3.26 (1 H, d, J = 15 Hz), 3.51 (1 H, d, J = 15 Hz), 4.28 (1 H, m), 4.84 (1 H, br s), 5.10 (1 H, s), 6.43 (1 H, d, J = 8 Hz), 6.94 (1 H, d, J = 2.5 Hz), 7.06–7.33 (7 H, m), 7.34 (1 H, d, J = 8 Hz), 7.58 (1 H, d, J = 8 Hz), 8.19 (1 H, s).

Tricyclo[3.3.1.1^{3,7}]dec-2-yl [*R*-(*R,*S**)]-[2-[[1-(Aminomethyl)-2-phenylethyl]amino]-1-(1*H*-indol-3-ylmethyl)-1-methyl-2-oxoethyl]carbamate (16e).** A solution of azide **15e** (2.77 g, 4.99 mmol) in 2% AcOH in absolute EtOH (100 mL) was treated with 10% Pd/C (270 mg, 10% w/w) and put under an atmosphere of hydrogen at pressure of 50 psi at 30 °C with agitation. Once hydrogen uptake had ceased, the mixture was filtered through filter aid and concentrated in vacuo to a foam which was taken up in EtOAc (100 mL) and washed with saturated $NaHCO_3$ solution (100 mL) then H_2O (100 mL). The organic phase was dried over $MgSO_4$ and evaporated in vacuo to a white solid which was recrystallized from EtOAc (2.5 g, 95%): $[\alpha]^{20}_D$ = +27.8° (c = 0.5, MeOH); IR (film) 3306, 1696, and 1659 cm^{-1}; NMR ($CDCl_3$) δ 1.38 (3 H, s), 1.3–2.1 (16 H, m), 2.51 (1 H, dd, J = 7 and 13 Hz), 2.6–2.8 (3 H, m), 3.30 (1 H, d, J = 15 Hz), 3.47 (1 H, d, J = 15 Hz), 4.15 (1 H, m), 4.80 (1 H, br s), 5.03 (1 H, br s), 6.29 (1 H, d, J = 9 Hz), 6.89 (1 H, d, J = 2 Hz), 7.05–7.25 (7 H, m), 7.32 (1 H, d, J = 8 Hz), 7.58 (1 H, d, J = 8 Hz), 8.10 (1 H, br s).

Tricyclo[3.3.1.1^{3,7}]dec-2-yl [*R*-[*R,*S**(*E*)]]-3-(1*H*-Indol-3-ylmethyl)-3-methyl-4,9,12-trioxo-6-(phenylmethyl)-13-oxa-2,5,8-triazatetradec-10-enoate (17).** A suspension of monomethyl fumarate (200 mg, 1.54 mmol) in EtOAc (20 mL) was treated with pentafluorophenol (340 mg, 1.85 mmol) and *N,N'*-dicyclohexylcarbodiimide (349 mg, 1.69 mmol) and allowed to stir for 3 h. After this time the suspension was filtered and the filtrate treated with amine **16e** (816 mg, 1.54 mmol) and left stirring for 18 h at room temperature. The reaction mixture was then filtered, and filtrate evaporated in vacuo, and the residue chromatographed to give ester **17** as an amorphous, white solid (867 mg, 88%): $[\alpha]^{20}_D$ = +13.3° (c = 1.04, MeOH); IR (film) 1728, 1700, and 1666 cm^{-1}; NMR ($CDCl_3$) δ 1.34 (3 H, s), 1.50–1.60 (2 H, m), 1.70–2.10 (12 H, m), 2.73 (2 H, d, J = 7 Hz), 3.10–3.25 (1 H, m), 3.28 (1 H, d, J = 15 Hz), 3.38 (1 H, d, J = 15 Hz), 3.70–3.80 (1 H, m), 3.75 (3 H, s), 4.25–4.35 (1 H, m), 4.80 (1 H, s), 5.00 (1 H, s), 6.12 (1 H, d, J = 8 Hz), 6.80 (1 H, d, J = 16 Hz), 6.92 (1 H, d, J = 16 Hz), 6.93 (1 H, d, J = 2 Hz), 7.05–7.30 (8 H, m), 7.35 (1 H, d, J = 8 Hz), 7.57 (1 H, d, J = 8 Hz), 8.21 (1 H, s).

[*R*-(*R,*S**)]-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-2-butenoic Acid (18).** Methyl ester **17** (867 mg, 1.35 mmol) as a solution in THF (35 mL) at 0 °C was treated dropwise with aqueous LiOH solution (13.5 mL of a 0.1 M solution, 1.35 mmol). The resultant mixture was stirred at 0 °C for 4.5 h, allowed to warm to room temperature, and acidified with 1 M citric acid solution. The mixture was concentrated to one-third of its original volume and the residue extracted with EtOAc (75 mL) and washed with H_2O (75 mL). The organic phase was dried over $MgSO_4$, filtered, and evaporated in vacuo. The residue then purified by chromatography to give **18** as an amorphous, white solid (611 mg, 72%): $[\alpha]^{20}_D$ = +105.2° (c = 1.07, MeOH); IR (film) 3341, 1706, and 1665 cm^{-1}; δ NMR ($CDCl_3$) 1.38 (3 H, s), 1.45–1.55 (2 H, m), 1.70–2.10 (12 H, m), 2.00 (CO_2H and H_2O), 2.60–2.80 (2 H, m), 3.10–3.20 (1 H, br m), 2.22 (1 H, d, J = 12 Hz), 3.34 (1 H, d, J = 14.5 Hz), 3.50–3.60 (1 H, br m), 4.20–4.35 (1 H, br m), 4.78 (1 H, s), 5.23 (1 H, s), 6.35–6.45 (1 H, br m), 6.75 (1 H, d, J = 15.5 Hz), 6.89 (1 H, d, J = 15.5 Hz), 6.90 (1 H, d, J = 2 Hz), 7.00–7.30 (8 H, m), 7.31 (1 H, d, J = 8 Hz), 7.54 (1 H, d, J = 8 Hz), 8.54 (1 H, s).

Tricyclo[3.3.1.1^{3,7}]dec-2-yl [*R*-(*R,*S**)]-3-(1*H*-Indol-3-ylmethyl)-3-methyl-4,9,13-trioxo-6-(phenylmethyl)-14-oxa-2,5,8-triazapentadecanoate (19).** Methyl 4-(chloroformyl)-butyrate (222 mg, 1.35 mmol) in anhydrous EtOAc (5 mL) was added dropwise to a solution of amine **16e** (322 mg, 0.61 mmol) and triethylamine (134 mg, 1.33 mmol) in EtOAc (50 mL). This reaction mixture was stirred at room temperature for 4 h then filtered and the filtrate concentrated in vacuo. The residue was chromatographed to give **19** as a white, amorphous solid (275 mg, 69%): $[\alpha]^{20}_D$ = +50.9° (c = 0.11, EtOH); IR (film) 1723, 1695, and 1657 cm^{-1}; NMR ($CDCl_3$) δ 1.35 (3 H, s), 1.51–2.00 (16 H, m), 2.19 (2 H, t, J = 7 Hz), 2.35 (2 H, t, J = 7 Hz), 2.60 (1 H, dd, J = 7 and 14 Hz), 2.70 (1 H, dd, J = 7 and 14 Hz), 2.95–3.04 (1 H, m), 3.34 (2 H, s), 3.63–3.58 (4 H, m), 4.25 (1 H, m), 4.80 (1 H, br s), 5.17 (1 H, s), 6.26 (1 H, br d, 7 Hz), 6.51 (1 H, br), 6.92–7.26 (8 H, m), 7.34 (1 H, d, J = 8 Hz), 7.57 (1 H, d, J = 8 Hz), 8.48 (1 H, br s); MS m/e (CI) 657 (100).

[*R*-(*R,*S**)]-5-[[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]-5-oxopentanoic Acid (20).** The method was as for **18** except ester **19** was used: yield 127 mg, 65%; $[\alpha]^{20}_D$ = +45.5° (c = 0.14, EtOH); IR (film) 1696 and 1652 cm^{-1}; NMR ($CDCl_3$) δ 1.36 (3 H, s), 1.51–1.99 (16 H, m), 2.20 (2 H, t, J = 9 Hz), 2.35 (2 H, t, J = 7 Hz, 2.56–2.69 (2 H, m), 2.94 (1 H, m), 3.26 (1 H, d, J = 15 Hz), 3.30 (1 H, d, J = 15 Hz), 3.55

(1 H, br), 4.26 (1 H, br), 4.78 (1 H, br s), 5.30 (1 H, br), 6.32 (1 H, br), 6.68 (1 H, br), 6.93–7.23 (8 H, m), 7.33 (1 H, d, J = 8 Hz), 7.55 (1 H, d, J = 8 Hz), 8.67 (1 H, br s); MS m/e (CI) 643 (5), 115 (100).

[*R*-(*R,*S**)]-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-oxobutanoic Acid (21e).** The method was as for 14 except using amine **16e** at room temperature in the absence of 4-(dimethylamino)pyridine: yield 1.25 g, 66%; $[\alpha]^{20}_D$ = +52.9° (c = 1, MeOH); IR (film) 3306, 1695, and 1651 cm^{-1}; NMR ($CDCl_3$) δ 1.34–1.97 (17 H, m), 2.38 (2 H, m), 2.55 (2 H, m), 2.62 (2 H, m), 2.98 (1 H, m), 3.27 (2 H, m), 3.45 (1 H, m), 4.20 (1 H, m), 4.77 (1 H, s), 5.43 (1 H, br s), 6.05 (1 H, br), 6.43 (1 H, br s), 6.85–7.55 (10 H, m), 8.91 (1 H, s).

[*S*-(*R,*S**)]-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-oxobutanoic Acid (21f).** The method was exactly as for **21e** except using **10b** and **5a**. Data was as for **21e** except $[\alpha]^{20}_D$ = −54.1° (c = 0.56, MeOH).

[*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]-3-phenylpropyl]amino]-4-oxobutanoic Acid (21d).** The method was exactly as for **21e** except using **5a**: $[\alpha]^{20}_D$ = +1.80° (c = 0.64, MeOH); IR (film) 1700 and 1657 cm^{-1}; NMR ($CDCl_3$) δ 1.33 (3 H, s), 1.56 (2 H, m), 1.70–2.20 (12 H, m), 2.35–2.80 (6 H, m), 3.00–3.01 (1 H, m), 3.13 (1 H, d, J = 14 Hz), 3.30–3.38 (1 H, m), 3.38–3.50 (1 H, m), 4.10–4.20 (1 H, m), 4.60–4.90 (1 H, m), 6.81 (1 H, s, NH), 6.90–7.30 (8 H, m), 7.30 (1 H, d, J = 8 Hz), 7.51 (1 H, d, J = 8 Hz).

[*S*-(*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]-3-phenylpropyl]amino]-4-oxobutanoic Acid (21g).** The method was exactly as for **21e** except using **10b**. Data was as for **21d** except $[\alpha]^{20}_D$ = −1.71° (c = 0.64, MeOH).

Tricyclo[3.3.1.1^{3,7}]dec-2-yl [*R*-(*R,*S**)]-3-(1*H*-Indol-3-ylmethyl)-3-methyl-4,9,11-trioxo-6-(phenylmethyl)-12-oxa-2,5,8-triazatridecanoate (22).** The method was as for **19** except using methyl (chloroformyl)acetate: yield 101 mg, 45%; $[\alpha]^{20}_D$ = +32.1° (c = 0.01, EtOH); IR (film) 1742, 1690, and 1663 cm^{-1}; NMR ($CDCl_3$) δ 1.40 (3 H, s), 1.51–2.00 (14 H, m), 2.58 (1 H, dd, J = 7 and 14 Hz), 2.72 (1 H, dd, J = 7 and 14 Hz), 3.12–3.21 (1 H, m), 3.27 (2 H, s), 3.34 (2 H, s), 3.50–3.52 (1 H, m), 3.71 (3 H, s), 4.24 (1 H, br m), 4.79 (1 H, br s), 5.20 (1 H, s), 6.41 (1 H, br), 6.92 (1 H, d, J = 2 Hz), 7.03–7.25 (8 Hz, m), 7.34 (1 H, d, J = 8 Hz), 7.58 (1 H, d, J = 8 Hz), 8.30 (1 H, br); MS m/e (FAB) 629 (56), 251 (100).

[*R*-(*R,*S**)]-3-[[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]-3-oxopropanoic Acid (23).** The method was as for **18** except using ester **22**: yield 150 mg, 68%; $[\alpha]^{20}_D$ = +25.4° (c = 0.19, EtOH); IR (film) 1725, 1693, and 1659 cm^{-1}; NMR ($CDCl_3$) δ 1.32 (3 H, s), 1.54–1.99 (14 H, m), 2.66 (2 H, d, J = 7 Hz), 2.96 (1 H, m), 3.25 (2 H, s), 3.29 (2 H, s), 3.66 (1 H, br), 4.26 (1 H, br), 4.78 (1 H, br s), 5.14 (1 H, br), 6.05 (1 H, br), 6.92–7.25 (9 H, m), 7.35 (1 H, d, J = 8 Hz), 7.54 (1 H, d, J = 8 Hz), 8.38 (1 H, br); MS m/e (FAB) 615 (71), 237 (100).

Tricyclo[3.3.1.1^{3,7}]dec-2-yl [*R*-(*R,*R**)]-3-(1*H*-Indol-3-ylmethyl)-3-methyl-4,9-dioxo-7,11-diphenyl-10-oxa-2,5,8-triazaundecanoate (24d).** A solution of acid **10a** (4.60 g, 11.6 mmol) in EtOAc (30 mL) was treated with 1-hydroxybenzotriazole hydrate (1.96 g, 12.8 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (2.87 g, 13.9 mmol) and stirred at room temperature for 2 h before amine **8a** (4.56 g, 16.9 mmol) in EtOAc (10 mL) was added. After stirring for a further 18 h, the mixture was filtered, concentrated in vacuo, and purified by silica gel chromatography to give urethane **24d** as a white solid (6.17 g, 56%): $[\alpha]^{20}_D$ = +8.9° (c = 1, MeOH); IR (film) 3350, 1700, and 1662 cm^{-1}; NMR ($CDCl_3$) δ 1.54 (5 H, br), 1.60–1.95 (14 H, m), 3.23 (1 H, d, J = 14 Hz), 3.35 (1 H, m), 3.43 (1 H, d, J = 14 Hz), 3.72 (1 H, m), 4.79 (2 H, br s), 5.07 (2 H, s), 5.13 (1 H, s), 5.90 (1 H, br s), 6.43 (1 H, br s), 6.93 (1 H, s), 7.10–7.40 (13 H, m), 7.55 (1 H, d, J = 8 Hz), 7.95 (1 H, s).

Tricyclo[3.3.1.1^{3,7}]dec-2-yl [*R*-(*R,*R**)]-[2-[(2-Amino-2-phenylethyl)amino]-1-(1*H*-indol-3-ylmethyl)-1-methyl-2-oxoethyl]carbamate (25d).** A solution of the benzyl urethane **24d** (6.17 g, 8.94 mmol) in absolute EtOH (50 mL) was treated with Pearlman's catalyst (620 mg, 10% w/w). The mixture was put under an atmosphere of hydrogen at 45 psi for 18 h at 25 °C, filtered, and concentrated in vacuo to yield amine **25d** as a white foam, pure enough to be used directly in the next step (4.44 g, 89%): $[\alpha]^{20}_D$ = +10.3° (c = 1, MeOH); IR (film) 3340, 1701, and 1658 cm^{-1}; NMR ($CDCl_3$) δ 1.54 (5 H, br s), 1.70–2.05 (14 H, m), 3.15 (1 H, ddd, J = 6, 8, and 14 Hz), 3.31 (1 H, d, J = 15 Hz), 3.54 (1 H, d, J = 15 Hz), 3.55 (1 H, m), 3.97 (1 H, m), 4.82 (1 H, s), 5.15 (1 H, s), 6.49 (1 H, br s), 6.96 (1 H, d, J = 2 Hz), 7.10–7.40 (8 H, m), 7.59 (1 H, d, J = 8 Hz), 8.19 (1 H, s).

Tricyclo[3.3.1.1^{3,7}]dec-2-yl [*R*-(*R,*R**)]-3-(1*H*-Indol-3-ylmethyl)-3-methyl-4,9,12-trioxo-7-phenyl-13-oxa-2,5,8-triazatetradec-10-enoate (26).** A solution of monomethyl fumarate (470 mg, 3.62 mmol) in EtOAc (30 mL) was treated with 1-hydroxybenzotriazole hydrate (550 mg, 3.62 mmol) in EtOAc (10 mL) followed by *N*,*N*′-dicyclohexylcarbodiimide (740 mg, 3.60 mmol) in EtOAc (10 mL). This suspension was stirred for 2 h and filtered and the filtrate added to amine **25d** (2.0 g, 3.6 mmol) in EtOAc (20 mL) and left for 18 h at room temperature. The reaction mixture was then filtered and evaporated in vacuo and the residue chromatographed to give **26** as a pale yellow foam (1.93 g, 81%): $[\alpha]^{20}_D$ = +12.9° (c = 0.62, Me_2CO); IR (film) 3400–3300, 1720, and 1667 cm^{-1}; NMR ($CDCl_3$) δ 1.42 (3 H, s), 1.54 (2 H, m), 1.70–2.05 (12 H, m), 3.34 (1 H, d, J = 14 Hz), 3.42 (1 H, m), 3.50 (1 H, d, J = 14 Hz), 3.79 (3 H, s), 4.05 (1 H, m), 4.84 (1 H, br s), 5.03 (1 H, s), 5.20 (1 H, m), 6.35 (1 H, m), 6.82 (1 H, d, J = 15 Hz), 6.95–7.35 (10 H, m), 7.57 (2 H, d, J = 8 Hz), 8.30 (1 H, s).

[*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-oxy)carbonyl]amino]propyl]amino]-1-phenylethyl]amino]-4-oxo-2-butenoic Acid (27).** The method was as for **18** except using ester **26**: yield 324 mg, 46%; $[\alpha]^{20}_D$ = +13.7° (c = 0.24, $CHCl_3$); IR (film) 3300, 1706, and 1667 cm^{-1}; NMR (DMSO-d_6) δ 1.18 (3 H, s), 1.47 (2 H, m), 1.65–2.00 (12 H, m), 3.30–3.50 (4 H, + H_2O), 4.66 (1 H, br s), 5.06 (1 H, m), 6.52 (1 H, d, J = 15 Hz), 6.77 (1 H, br s), 6.90–7.10 (4 H, m), 7.20–7.35 (6 H, m), 7.44 (1 H, d, J = 8 Hz), 7.82 (1 H, t, J = 6 Hz), 8.78 (1 H, br s), 10.85 (1 H, s).

[*R*-(*R,*R**)]-5-[[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]-5-oxopentanoic Acid (28).** The method was as for **21e** except using glutaric anhydride and amine **25d**: yield 84 mg, 70%; $[\alpha]^{20}_D$ = −11.9° (c = 0.02, $CHCl_3$); IR (film) 3320, 1708, and 1658 cm^{-1}; NMR (DMSO-d_6) δ 1.20 (3 H, s), 1.25 (1 H, s), 1.42–1.57 (2 H, m), 1.68–2.03 (11 H, m), 2.17–2.27 (6 H, m), 3.12–3.50 (4 H + H_2O, m), 4.70 (1 H, br s), 4.99 (1 H, m), 6.73 (1 H, br s), 6.91 (1 H, s), 6.95 (1 H, s), 7.03 (1 H, t, J = 7 Hz), 7.22–7.37 (6 H, m), 7.44 (1 H, d, J = 8 Hz), 7.78 (1 H, t, J = 5.5 Hz), 8.10 (1 H, m), 10.88 (1 H, s).

[*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 (29d).** The method was exactly as for **14** except using **25** at room temperature in the absence of 4-(dimethylamino)pyridine: yield 190 mg, 80%; $[\alpha]^{20}_D$ = −8.8° (c = 0.22, Me_2CO); IR (film 3306, 1713, and 1670 cm^{-1}; NMR (DMSO-d_6) δ 1.20 (3 H, s), 1.49 (2 H, br s), 1.65–1.85 (8 H, m), 1.95 (4 H, m), 2.39 (4 H, br s), 3.40 (4 H + H_2O), 4.69 (1 H, br s), 4.96 (1 H, m), 6.70 (1 H, s), 6.90 (2 H, s), 7.01 (1 H, t, J = 7 Hz), 7.22 (1 H, m), 7.31 (5 H, br s), 7.44 (1 H, d, J = 7 Hz), 7.78 (1 H, br s), 8.30 (1 H, s), 10.85 (1 H, s).

[*S*-(*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 (29g).** The method was exactly as for **29d** except using **7b** and **10b**. Data was as for **29d** except $[\alpha]^{20}_D$ = +8.9° (c = 0.2, Me_2CO).

[*R*-(*R,*S**)]-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 (29e).** The method was exactly as for **29d** except using **7b**: $[\alpha]^{20}_D$ = +65.1° (c = 0.21, Me_2CO); IR (film) 3323 and 1659 cm^{-1}; NMR (DMSO-d_6) 1.21 (3 H, s), 1.49 (2 H, br s), 1.72–1.83 (8 H, m), 1.95 (4 H, m), 2.40 (4 H, br s), 3.30–3.50 (4 H + H_2O), 4.72 (1 H, br s), 4.96 (1 H, m), 6.93 (2 H, br s), 7.04 (1 H, t, J = 7.5 Hz), 7.22 (1 H, m), 7.32 (3 H, br s), 7.45 (1 H, d, J = 8 Hz), 7.78 (1 H, br s), 8.26 (1 H, d, J = 11 Hz), 10.86 (1 H, s).

[*S*-(*R,*S**)]-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 (29f).** The

method was exactly as for **29d** except using acid **10b**. Data was as for **29e** except $[\alpha]^{20}_D = -63.4°$ (c = 0.21, Me_2CO).

Tricyclo[3.3.1.1^{3,7}]dec-2-yl [*R*-(*R,*R**)]-3-(1*H*-Indol-3-ylmethyl)-3-methyl-4,9,11-trioxo-7-phenyl-12-oxa-2,5,8-triazatridecanoate (30).** The method was exactly as for **22** except using methyl (chloroformyl)acetate and amine **25d**: yield 43 mg, 43%; $[\alpha]^{20}_D = -11.5°$ (c = 0.27, MeOH); IR film 1740, 1700, and 1660 cm^{-1}; NMR ($CDCl_3$) δ 1.49 (3 H, s), 1.52–1.60 (3 H + H_2O, m), 1.73–2.01 (11 H, m), 3.30–3.46 (5 H, m), 3.73 (3 H, s), 3.89 (1 H, m), 4.84 (1 H, br s), 5.13 (2 H, br s), 6.48 (1 H, m), 6.98 (1 H, d, J = 2 Hz), 7.07–7.37 (8 H, m), 7.57 (1 H, d, J = 8 Hz), 7.68 (1 H, d, J = 8 Hz), 8.23 (1 H, s).

[*R*-(*R,*R**)]-3-[[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]-3-oxopropanoic Acid (31).** The method was as for 18 except using ester **30**: yield 81 mg, 69%; $[\alpha]^{20}_D = -8.4°$ (c = 0.17, $CHCl_3$); IR (film) 3310, 1707, and 1663 cm^{-1}; NMR (DMSO-d_6) δ 1.23 (3 H, br s), 1.48–1.62 (2 H, m), 1.72–2.07 (12 H, m), 3.14–3.55 (6 H + H_2O, m), 4.72 (1 H, br s), 5.02 (1 H, s), 6.72 (1 H, br s), 6.92–6.97 (2 H, m), 7.02 (1 H, t, J = 7 Hz), 7.24–7.36 (6 H, m), 7.81 (1 H, m), 8.96 (1 H, m), 10.91 (1 H, br s).

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Registry No. **5a**, 130406-30-3; **5b**, 129397-83-7; **6a**, 130406-33-6; **6b**, 130406-34-7; **6b** (*N*-Fmoc derivative), 130406-32-5; **7a**, 120666-53-7; **7a** (*O*-tosylate derivative), 130406-37-0; **7b**, 130406-31-4; **8a**, 130406-35-8; **8b**, 130406-36-9; **9a**, 96551-27-8; **9a** (*N*-2Adoc derivative), 130406-39-2; **10a**, 130406-40-5; **10b**, 130406-41-6; **10c**, 130406-42-7; **11a**, 130406-43-8; **11b**, 130406-44-9; **11c**, 130466-73-8; (*R*,*R*)-**12**, 130406-48-3; (*R*,*R*)-**12** (free alcohol), 130406-45-0; (*R*,*S*)-**12**, 130406-49-4; (*R*,*S*)-**12** (free alcohol), 130406-46-1; **13**, 130406-47-2; **14**, 130406-50-7; **15e**, 130406-51-8; **16e**, 130406-52-9; **17**, 130406-53-0; **18**, 130406-56-3; **19**, 130406-54-1; **20**, 130406-57-4; **21d**, 130406-58-5; **21e**, 130406-59-6; **21f**, 130406-60-9; **21g**, 130406-61-0; **22**, 130406-55-2; **23**, 130406-62-1; **24d**, 130406-63-2; **25d**, 130406-64-3; **26**, 130406-65-4; **27**, 130406-67-6; **28** (free base), 130406-68-7; **28**·HOAc, 130406-73-4; **29d**, 130332-27-3; **29e**, 130406-69-8; **29f**, 130406-70-1; **29g**, 130406-71-2; **30**, 130406-66-5; **31**, 130406-72-3; (*R*)-(+)-$H_2NCH(CH_2Ph)CH_2OH$, 5267-64-1; (*S*)-(−)-$H_2NCH(CH_2Ph)CH_2OH$, 3182-95-4; (*R*)-$H_2NCHPhCH_2OH$, 56613-80-0; (*S*)-$H_2NCHPhCH_2OH$, 20989-17-7; (*R*)-$BzO_2CNHCHPhCH_2N_3$, 130406-38-1; $PhCH_2CH_2NH_2$, 64-04-0; (*RS*)-$H_2NCH_2CHPhOH$, 1936-63-6; (*E*)-MeO_2CCH=$CHCOOH$, 2756-87-8; $MeO_2C(CH_2)_3COCl$, 1501-26-4; MeO_2CCH_2COCl, 37517-81-0; α-methyl-(*S*)-tryptophan, 16709-25-4; α-methyl-(*RS*)-tryptophan, 153-91-3; 2-adamantanol, 700-57-2; succinic anhydride, 108-30-5; glutaric anhydride, 108-55-4.

Preparation, Characterization, and Anticancer Activity of a Series of *cis*-$PtCl_2$ Complexes Linked to Anthraquinone Intercalators

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A new series of complexes of the type *cis*-PtL_2X_2 [where L is a monodentate AQ–$Y(CH_2)_nNH_2$ and L_2 is a bidentate AQ–$Y(CH_2)_nNH(CH_2)_2NH_2$; AQ = anthraquinone, X = Cl, I, Y = NH, O] in which anthraquinone intercalators are tethered to the *cis*-$PtCl_2$ unit via an (aminoalkyl)amino, (oxyalkyl)amino, or polyethylene glycol (aminoethyl)amino linker chains was prepared and screened in vitro against P388 leukemia. In vivo toxicity studies were carried out on selected complexes. All complexes were characterized by means of elemental analysis, ^{195}Pt NMR spectroscopy, and FTIR. The 1:1 Pt–intercalator complexes displayed much higher in vitro cytotoxic activities than the 1:2 Pt–intercalator complexes. The dichloride complexes were consistently more active than their diiodide counterparts. Among the 1:1 Pt–intercalator complexes those with the shorter linker chains (n = 2, 3) exhibited the highest cytotoxic activities. Three compounds, [[2-[[2-(anthraquinon-1-ylamino)ethyl]amino]ethyl]amine-*N*,*N*']dichloroplatinum(II), [[2-[[3-(anthraquinon-1-ylamino)propyl]amino]ethyl]amine-*N*,*N*']dichloroplatinum(II), and [[2-[[3-(anthraquinon-1-yloxy)propyl]amino]ethyl]amine-*N*,*N*']dichloroplatinum(II), were as active in vitro as cisplatin (ED_{50} = 2–4 × 10^{-7} M) while on a molar basis their acute in vivo toxicity was significantly lower than that of cisplatin. In vivo screening against P388 leukemia indicated that these complexes have activity comparable to cisplatin.

Introduction

cis-Diamminedichloroplatinum(II) (*cis*-DDP) is a clinically effective widely used anticancer agent which has been studied extensively.[1,2] The drug is believed to effect cytotoxicity by covalently modifying the DNA, its putative biological target, and arresting DNA replication.[3-5] The major adduct of *cis*-DDP with DNA, both in vitro and in vivo, is the covalent chelate formed between $Pt(NH_3)_2^{2+}$ and the d(GpG) fragment.[6-8] The detailed geometry as well as the metrical parameters of this adduct have been recently described in a single crystal X-ray diffraction study.[9,10] Despite its clinical success, *cis*-DDP suffers from

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