Synthesis and Platelet Aggregation Inhibiting Activity of 1'-Carboxyl Modified Hydantoin Prostaglandin Analogues

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A series of hydantoin prostaglandin analogues, in which the 1'-carboxy group was replaced by tetrazole, amine, alcohol, amide, cyanamide, or sulphonamide functionalities, was prepared and evaluated for platelet aggregation inhibiting activity. The 2'-decarboxy-2'-(tetrazol-5-yl) analogue (**30**) proved to have interesting activity, being approximately equipotent (*ca.* $17 \times PGE_1$) to BW245C (**1**). Activity was often lost when the carboxy group was replaced by neutral or basic moieties. Substitution of the carboxy terminus by other groups of similar acidity gave rise to reduced activity. These results suggest that the platelet receptor which mediates these effects possesses a cationic site requiring binding of an anionic group at the agonist 1'-position. However, additional factors are clearly involved in determining agonist potency.

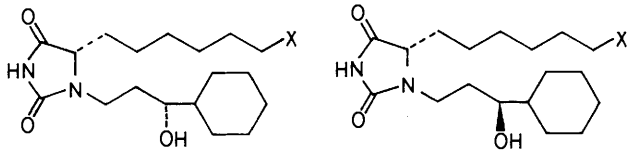
The hydantoin prostaglandin (PG) analogue, BW245C(**1**) is a potent inhibitor of human platelet aggregation which displays pronounced vasodilation.[1] The effects of (**1**) in healthy human volunteers have been investigated in the clinic.[2] Preliminary results[3] suggest that a more selective agent that retains the platelet anti-aggregatory activity of BW245C but which has reduced cardiovascular effects would have greater therapeutic potential. In particular, such an agent should give rise to a reduced incidence of tachycardia, facial flushing, and headache.

The search for an analogue of BW245C possessing better platelet selectivity would be facilitated by a knowledge of the PG receptor sub types that mediate the platelet and cardiovascular effects of (**1**). Initial studies[4–8] have led to the hypothesis that these effects are mediated by PGD_2 receptors. Literature reports of structure-activity studies on the platelet anti-aggregatory activity of PGD_2 analogues are limited.[8,9] We now report, as part of our detailed studies of hydantoin PGD_2 mimics, the synthesis and evaluation of the platelet effects of BW245C analogues (**8**)—(**15**), (**18**)—(**26**), and (**30**), in which the 1'-carboxy group is replaced by other polar, acidic, or basic groups.

Synthesis of Hydantoin Prostaglandin Analogues.— Racemic BW245C(**1**) and its epimer BW246C(**2**) were available in quantity in these laboratories and proved to be useful starting materials for most of the new analogues.† The mixed anhydrides (**3**—**7**) were of particular value for carboxy coupling reactions, being of more general utility than reactive intermediates derived from dicyclohexylcarbodi-imide (DCC). However, the phenacyl ester (**8**) was obtained in good yield by coupling (**1**) with *p*-hydroxyacetophenone in the presence of DCC.

Mixed anhydrides (**3**,**4**), obtained by the reaction of (**1**,**2**) with ethyl chloroformate and triethylamine, were reduced by sodium borohydride to give the alcohols (**9**,**10**). H.p.l.c. separation of the diastereoisomeric mixture gave pure (**9**). The amides (**11**,**13**) and the hydroxamic acid (**15**) were also obtained *via* anhydride (**3**) by the reaction with ammonium chloride–triethylamine, morpholine, and hydroxylamine hydrochloride–triethylamine respectively.

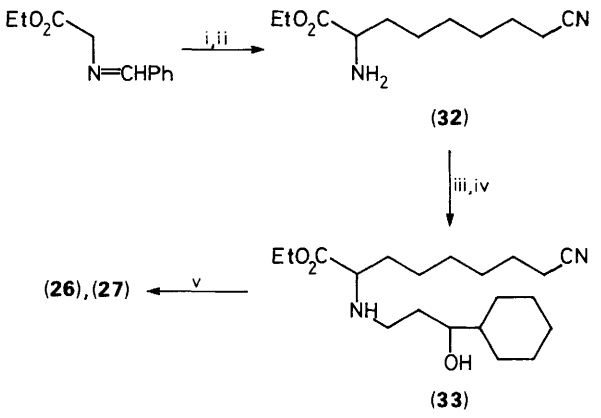
Attempts to prepare the sulphonamide (**18**) by DCC coupling of (**1**) with methanesulphonamide proved unsuccessful, the unwanted acylurea (**16**) being obtained instead as the sole product. In this case, the initial adduct (**17**) presumably undergoes *O*-*N* acyl migration faster than attack by the relatively poorly nucleophilic sulphonamide.

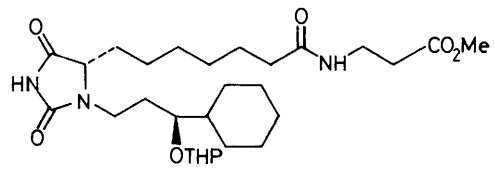


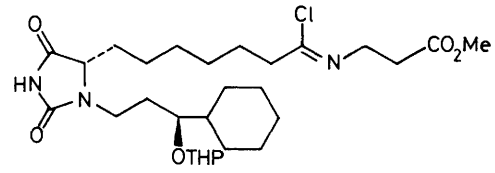
(**1**) BW245C	X = CO_2H	(**2**) BW246C
(**3**)	X = $CO{\cdot}O{\cdot}CO_2Et$	(**4**)
(**5**)	X = $CO{\cdot}O{\cdot}CO_2Me$	(**6**)
(**7**)	X = $CO{\cdot}O{\cdot}CO_2CH_2CHMe_2$	
(**8**)	X = $CO{\cdot}OC_6H_4$-*p*Ac	
(**9**)	X = CH_2OH	(**10**)
(**11**)	X = $CO{\cdot}NH_2$	(**12**)
(**13**)	X = morpholinocarbonyl	(**14**)
(**15**)	X = CO·NHOH	
(**16**)	X = $CO{\cdot}N(C_6H_{11})CO{\cdot}NHC_6H_{11}$	
(**17**)	X = $CO{\cdot}O{\cdot}C{=}NC_6H_{11}{\cdot}NHC_6H_{11}$	
(**18**)	X = $CO{\cdot}NHSO_2Me$	(**19**)
(**20**)	X = $CO{\cdot}NHSO_2C_6H_{13}$	(**21**)
(**22**)	X = CO·NHCN	
(**23**)	X = *N*-tetrazol-5-ylcarbamoyl	(**24**)
(**25**)	X = NH_2	
(**26**)	X = CN	(**27**)
	X = $CO{\cdot}NH(CH_2)_2CO_2Me$	(**28**)
	X = 1-(2-methoxycarbonylethyl)-tetrazol-5-yl	(**29**)
(**30**)	X = tetrazol-5-yl	(**31**)

Reaction of the anhydride (**3**) with methanesulphonamide at 100 °C in the absence of solvent, however, gave the desired sulphonamide (**18**) along with some of its epimer (**19**). A modification of this method led to sulphonamides (**20**,**21**), these being obtained by reaction of (**7**) with the sodium salt of hexanesulphonamide at room temperature. In a similar manner, the cyanamide (**22**) was obtained by treating the anhydride (**5**) with sodium cyanamide. The (tetrazol-5-yl)amide (**23**) was obtained by condensation of (**1**) with 5-aminotetrazole at 80 °C in the presence of 1,1'-carbonyldi-1*H*-imidazole. Hofmann

† All analogues are racemic; relative stereochemistry is shown.







Scheme. *Reagents:* i, $LiNPr^i_2$, $Br(CH_2)_6CN$; ii, 2MHCl; iii, CH_2=CH·CO·C_6H_{11}; iv, $NaBH_4$; v, KCNO,HCl then heat.

reaction of the amide (**11**) using [bis(trifluoroacetoxy)]-iodobenzene[10] at room temperature gave the amine (**25**).

The tetrazole (**30**) proved to be the least accessible of the desired analogues. The first approach to (**30**) involved treatment of the nitrile (**26**) [prepared by modification of the original route [11] to BW245C (Scheme)] with sodium azide–ammonium chloride in DMF at 140 °C for 60h, but gave only trace amounts of the required tetrazole. To circumvent this problem, an alternative route exploiting the relative ease of synthesis [12] of 1,5-disubstituted tetrazoles was employed. Thus, reaction of the anhydride (**4**) with methyl 3-aminopropionate gave the amide (**28**), which was converted smoothly into the tetrahydropyranyl ether (**34**) by reaction with dihydropyran and toulene-*p*-sulphonic acid. The imidoyl chloride (**35**) was obtained from (**34**) by treatment with phosgene–pyridine. Reaction of (**35**) with trimethylsilylazide, followed by an acidolytic work up to remove the THP group, gave the disubstituted tetrazole (**29**). Deprotection of the tetrazole ring of (**29**) with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) or sodium methoxide gave the desired monosubstituted tetrazole (**30**) together with the epimer (**31**), the epimerisation occurring concomitantly with the retro-Michael-type reaction. This deprotection step proved, in our hands, difficult to reproduce and invariably gave low yields. Since this route did not provide a practical means of obtaining gram quantities of (**30**) we reinvestigated methods for elaborating the nitrile (**26**) to (**30**) in the light of the reported [13] conversion of RCN into RCN_4 (R is arachidonyl) using tributyltin azide. The previously described nitriles (**26**,**27**) were more conveniently prepared in quantity by dehydration of the amides (**11**) and (**12**) with trifluoroacetic anhydride. As hoped, the desired tetrazoles (**30**) and (**31**) were obtained by reaction of the nitriles (**26**) and (**27**) with tributyltin azide in dioxane at 160 °C in an autoclave. This procedure gave a 45% isolated yield of the products and proved easily reproducible, being adequate for the provision of sufficient material for detailed pharmacological evaluation.

In several cases, the more polar diastereoisomer could be epimerized at C-5 of the hydantoin ring with dilute aqueous sodium hydroxide or DBN. Separation of the resulting diastereoisomeric mixtures then furnished more of the less polar epimer for pharmacological evaluation. For this reason, and in some cases to aid characterisation, the pharmacologically less interesting more polar isomers were often isolated and purified and are described in the Experimental section.

Pharmacology.—The ability of the 1′-carboxy modified analogues to inhibit ADP-induced platelet aggregation was measured *in vitro* with human platelet rich plasma (PRP). These analogues were also tested for their blood pressure lowering effects in normotensive anaesthetized rats. Results are given in the Table and details of the assays in the Experimental section.

Structure-activity Relationships.—In comparison with major efforts to delineate the structure-activity relationships (s.a.r.s) of the prostanoid ring and the ω-chain, the effect on pharmacological activity of modifying the 1-carboxy group of prostanoids has received relatively little attention. No systematic study of the effects of modification of the 1-carboxy group of prostaglandins in the 'D' or 'E' series on platelet anti-aggregatory activity has yet been reported. This lack of s.a.r. information meant that no guidelines were available to assist a well defined study of hydantoin PGD_2 mimics. Our selection of 1′-substituents is such that they have allowed us to vary chosen physicochemical parameters (charge, steric bulk, lipophilicity) in a controlled manner.

The activity of the phenacyl ester (**8**) is fully developed on incubation in human PRP and is presumably due to (**1**), formed by the hydrolysis of (**8**) by esterases. Thus (**8**) could be a potentially useful pro-drug for BW245C.

The alcohol (**9**), the amide (**11**), and the nitrile (**26**) are devoid of activity. These results suggest that uncharged polar groups at the 1′-position are not compatible with potent agonism at the platelet receptor. The inactivity of the amide (**11**) is not surprising in view of the lack of agonism displayed by PGD_2 carboxamide.[7] The only analogue possessing a totally unionised C-1′ group which displayed even modest activity (0.8 times that of PGE_1) was the morpholine amide (**13**). This modest activity may be due to partial hydrolysis of (**13**) to (**1**) under the assay conditions. However, other factors such as lipophilic binding or hydrogen bond donor capacity may be responsible for the significant difference in agonist activity between (**13**) and (**11**).

Hydroxamic acid (**15**) proved to be much less potent (0.4 times that of PGE_1) than (**1**). From its estimated pK_a, (**15**) would not be expected to be significantly ionized at physiological pH. In contrast, the acid (**1**) would be fully ionized at pH 7.4. If agonist activity is associated with a carboxylate-like C1′-anion the 30-fold lower activity of hydroxamic acid (**15**) is probably due primarily to differences in anion formation. Also if the platelet receptor possessed a Zn^{2+} or Fe^{3+} metalloprotein site, then both the hydroxamic acid (**15**) and the anion of the acid (**1**) might be expected to be good ligands at this site. It seems reasonable to speculate from the modest activity of hydroxamic acid (**15**), however, that the platelet receptor does not possess a Zn^{2+} or Fe^{3+} ion site, which would require tight binding of a chelating group (at the agonist 1′-position), for the expression of agonism.

Table. Pharmacological activities of BW245C analogues

Compd.	Inhibn. of ADP-induced human platelet aggregn. IC_{50}, nM[a] (no. of experiments)	Relative Potency (PGE_1 = 1)	Blood Pressure Lowering Activity in Rat: Relative Potency[b] (PGI_2 = 1)	pK_a[c]
(1)	4.0 ± 0.4(10)	14	0.12 (*n* = 4)	5.0[d]
(2)	195 ± 29(3)	0.3	0.002	5.0[d]
(8)	3.1 ± 0.9(3)	15	0.08	
(9)	I(2)		I	
(11)	I(2)		I	
(13)	78 ± 25(2)	0.8	0.01	
(15)	180 ± 35(2)	0.4		9.0[e]
(18)	200 ± 50(2)	0.3	0.005	5.2[f]
(20)	I(2)		I	
(22)	100 ± 20(2)	0.6	0.009	4.2[d,g]
(23)	I(2)			4.5[h]
(25)	I(2)			
(26)	I(2)			
(30)	3.0 ± 0.4(6)	17	0.007 (*n* = 4)	5.1[d]

[a] IC_{50}, concentration required to reduce the aggregation to 50% of its control amplitude; I = inactive, IC_{50} > 500nM; values given are mean ± s.e.m. for (*n*) experiments. Potencies relative to PGE_1 are approximate. BW245C is approximately 8 and 0.2 times as potent as PGD_2 and PGI_2 respectively. [b] Values given are relative to prostacyclin (PGI_2) in the same animal; I = inactive, relative potency <0.001 the potency of PGI_2; number of experiments (*n*) is 2 unless stated otherwise. Prostacyclin (0.25μg kg^{-1} i.v.) caused a fall in blood pressure of 42 ± 5 mmHg (*n* = 4); BW245C (5.0μg kg^{-1} i.v.) reduced the b.p. by 35 ± 3 mmHg (*n* = 4), and tetrazole (**30**) (7.5μg kg^{-1} i.v.) led to a b.p. reduction of 4 ± 1.0 mmHg (*n* = 4). [c] Relates to equilibrium between un-ionised analogue and its anion (proton loss from 1′-substituent). [d] Experimentally determined by potentiometric titration in water–ethanol (98:2) at 25 °C. p*k*2 (hydantoin) was 9.3. [e] Value for MeCO·NHOH in water taken from ref. 24. [f] Value for $Ph_3{}^+P(CH_2)_3CO{\cdot}NHSO_2MeBr^-$ taken from ref 25. [g] The reported pK_a of *N*-cyanoacetamide is 4.[26] [h] Experimentally determined for (**24**) by spectrophotometric method in water–ethanol (98:2) at 25 °C. pK_2 (hydantoin) was 9.20. Analogue (**24**) was found to be more soluble than its epimer (**23**) under these conditions.

The human platelet PGE_1/prostacyclin receptor has been isolated and purified.[14] Investigations of ligand-receptor interactions showed [14] that the presence of Mg^{2+} was needed for optimal binding of PGE_1 to the purified receptor. Studies to characterise the structure of the PGD_2 receptor of human platelet membranes, however, have not yet been reported. Whether the PGD_2 receptor also has a requirement for Mg^{2+} or whether the present speculation is correct await the outcome of such studies.

The sulphonamides (**18**) and (**20**), the cyanamide (**22**), and the tetrazolylamide (**23**) are all less active than (**1**) despite being essentially fully ionised at pH 7.4. The reason(s) for the low potency of sulphonamide (**18**) remain obscure. A further large decrease in activity is observed when the size of the alkyl group on the sulphonamide moiety is increased *c.f.* (**20**). The inactivity of the tetrazolylamide (**23**) may be a consequence of its low solubility in the assay medium. The amine (**25**) is also inactive. Thus it would appear that an ammonium cation cannot mimic a 1′-carboxylate anion in eliciting an agonist response. The tetrazole (**30**) was approximately equipotent with BW245C in its ability to inhibit human platelet aggregation. This result is perhaps not too surprising given the similarity[15,16] of the carboxylate and tetrazol-5-yl moieties with respect of pK_a and lipophilicity.* The tetrazol-5-yl group is thus the best bioisostere for the carboxy group found to date for this series of hydantoin PGD_2 mimics. It will be of interest to see if the corresponding tetrazole analogue of PGD_2 has a similar activity towards the platelet as PGD_2 itself.

In summary, it would appear that an acidic group is preferred in the agonist 1′-position. The factors responsible for the differences in potency between carboxy and other acidic groups remain unclear, there being no simple correlation of agonism with lipophilicity, steric bulk, or electronic factors. These results suggest that the platelet receptor (probably the PGD_2 receptor) which mediates these effects possesses a cationic site requiring binding of an anionic group at the agonist 1′-position. The nature of this cationic site is at present not known.

Initial results of a broader pharmacological evaluation of the tetrazole (**30**) suggest it has higher platelet selectivity as an agonist than BW245C. Thus (**30**) is less effective than BW245C as a vasodilator in some species; in the anaesthetized rat, for example, (**30**) is 17 times less potent than (**1**) as an hypotensive agent. If this reduced hypotensive effect is also expressed in man, the tetrazole (**30**) will be a more selective probe than BW245C for investigating the clinical utility of platelet aggregation inhibition. PG Analogues of this isosteric type are thus worthy of wider study.

* The log *P* values for compounds (**1**) and (**30**) are 2.56 and 2.20 respectively at pH 1.1, where *P* is the octanol–water partition coefficient.

Experimental

M.p.s were taken in open capillary tubes and are uncorrected. ^1H N.m.r spectra were obtained on Bruker HFX90, AM-200, or WM-360 spectrometers. E.i. mass spectra were obtained on an A.E.I. MS 902 spectrometer, interfaced to a VG MULTISPEC data system at 70 eV. Fast atom bombardment (f.a.b.) mass spectra were obtained from a Kratos MS 50 mass spectrometer equipped with an RF magnet. The instrument was calibrated to 2,000 a.m.u. with caesium iodide. The samples were admixed with thioglycerol on the probe tip, introduced *via* the vacuum lock to the source on the mass spectrometer, and bombarded with fast Xe atoms. Thin layer chromatography (t.l.c.) was performed on Merck silica gel 60 F_{254}, gravity column chromatography with Merck silica gel (60—120 mesh), and flash chromatography with Merck silica gel (230—400 mesh). High performance liquid chromatography (h.p.l.c.) was conducted on an instrument employing Bio-sil silica (20—44 u). Separation of the hydantoin diastereoisomers was achieved with dichloromethane–methanol–acetic acid mixtures (*e.g.* 93:5:2) as eluant. Hydantoin analogues obtained as viscous oils often contained a little occluded solvent and attempts to obtain analytical samples or accurate mass measurements by e.i. mass spectroscopy were usually unsuccessful. In all cases except one, however, one of the diastereoisomers of each analogue proved to be crystalline and satisfactory microanalytical data could be obtained. Since each pair of diastereoisomers could be interconverted by base this protocol allowed full characterisation of each analogue. Organic solutions of BW245C should be handled with care since absorption on skin may cause facial flushing.

(±)-5-[6-(4-*Acetylphenoxycarbonyl*)*hexyl*]-1-(3-*cyclohexyl*-3-*hydroxypropyl*)*hydantoin* (**8**).—To a stirred solution of the acid (**1**) (770 mg, 2.09 mmol) in dichloromethane (15 ml) was added *p*-hydroxyacetophenone (272 mg, 2.0 mmol) and then dicyclohexylcarbodi-imide (432 mg, 2.10 mmol). The reaction was stirred at room temperature for 16 h, filtered, diluted with dichloromethane, and then washed twice with aqueous sodium hydrogen carbonate and then with water. The organic extract was dried (Na_2SO_4) and the solvent removed under reduced pressure to give a pale yellow viscous oil (1.1 g). Purification of this material by h.p.l.c. and crystallization from ether gave (**8**)

(450 mg, 45%), m.p. 74—76 °C (Found: C, 66.8; H, 7.95; N, 5.62. $C_{27}H_{38}N_2O_6$ requires C, 66.6; H, 7.87; N, 5.76%); δ_H (360 MHz; $CDCl_3$) 0.85—2.0 (23 H, m, aliphatic H), 2.55 (2 H, t, *J* 7 Hz CH_2CO), 2.58 (3 H, s, COMe), 2.80 (1 H, br s, OH, exchangeable), 3.08 (1 H, m, NC*H*H), 3.90 (1 H, m, NCH*H*) 3.26 (1 H, m, C*H*OH), 4.03 (1 H, br t, *J* 4 Hz, CHN), 7.15 (2 H, d, *J* 8 Hz, ArH), 8.0 (2 H, d, *J* 8 Hz, ArH), and 8.90 (1 H, br s, NH, exchangeable).

(±)-1-(3-*Cyclohexyl*-3-*hydroxypropyl*)-5-(7-*hydroxyheptyl*)-*hydantoin* (**9**) *and* (**10**).—A solution of ethyl chloroformate (108.5 mg, 1.0 mmol) in dry tetrahydrofuran (0.5 ml) was added to a mixture of the acid epimers (**1** and **2**) (368 mg, 1.0 mmol), triethylamine (101 mg, 1.0 mmol), and tetrahydrofuran (3 ml) at −5 °C, over 20 min. The reaction mixture was stirred at −5 °C for 15 min, filtered to remove triethylamine hydrochloride, and the filtrate added dropwise over 30 min to a solution of sodium borohydride (100 mg, 2.5 mmol, 95%) in water (1 ml), cooled in ice to maintain the temperature at *ca.* 15 °C. After addition was complete, the solution was stirred at room temperature for 2h, diluted with water, and then acidified with 2M hydrochloric acid. The resulting aqueous suspension was extracted with ether, and the combined organic extracts were washed with aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$), and evaporated under reduced pressure to yield a colourless viscous oil (330 mg). The diastereoisomers were separated by h.p.l.c. yielding (**9**) (90 mg,51%) as a colourless solid, m.p. 73—77 °C (Found: C, 64.2; H, 9.52; N, 7.70. $C_{19}H_{34}N_2O_4$ requires C, 64.4; H, 9.67; N, 7.90%); R_F 0.63 ($CHCl_3$–MeOH–HOAc, 90:5:5); δ_H (90 MHz; $CDCl_3$) 0.8—2.0 (25 H, m, aliphatic H), 2.8—3.4 (4 H, m, NC*H*H + CH_2O*H* + CHOH, 2 H exchangeable), 3.68 (2 H, t, *J* 7 Hz, CH_2OH), 3.95 (1 H, m, NCH*H*), 4.02 (1 H, t, *J* 4 Hz, CHN) and 8.70 (1 H, br s, NH, exchangeable). Compound (**10**) (60 mg, 34%) was obtained as a syrup, R_F 0.60 ($CHCl_3$–MeOH–HOAc, 90:5:5); δ_H(90 MHz; $CDCl_3$) 0.8—2.0 (25 H, m, aliphatic H), 2.50 (2 H, br s, OH, 2 H exchangeable), 3.2—3.7 (5 H, m, C*H*OH + CH_2OH + NCH_2), 4.04 (1 Ht, *J* 4Hz, CHN), and 9.10 (1 H, br s, NH, exchangeable).

(±)-5-(6-*Carbamoylhexyl*)-1-(3-*cyclohexyl*-3-*hydroxypropyl*)*hydantoin* (**11**) *and* (**12**).—A solution of the anhydride (**3**) in tetrahydrofuran (5 ml) was prepared as above from the acid (**1**) (736 mg, 2.0 mmol), triethylamine (202 mg, 2.0 mmol), and ethyl chloroformate (220 mg, 2.0 mmol). A solution of ammonium chloride (320 mg, 6 mmol) in water (1 ml) and tetrahydrofuran (1.5 ml), which had been neutralised with triethylamine (606 mg, 6.0 mmol), was added quickly to the anhydride solution and the resulting mixture was stirred for 2 h, the temperature being allowed to rise to 20 °C over this period. The volatiles were removed under reduced pressure, and the residue was diluted with water and acidified with 2M hydrochloric acid. The liberated oil was extracted with ethyl acetate (3 ×), and washed with aqueous sodium hydrogen carbonate and water, dried (Na_2SO_4) and evaporated under reduced pressure. The residue crystallized from ether to give (**11**) (0.50 g, 68%), m.p. 132—134 °C (Found: C, 61.9; H, 9.21; N, 11.4. $C_{19}H_{33}N_3O_4$ requires C, 62.1; H, 9.05; N, 11.4%); *m/z* 367 (M^+); R_F 0.36 ($CHCl_3$–MeOH–HOAc, (90:5:5). The acid epimers (**1**) and (**2**) were converted in a similar manner into the amides (**11**) and (**12**) (R_F 0.36, 0.32 respectively in $CHCl_3$–MeOH–HOAc, 90:5:5) which were used without further purification for the preparation of the nitriles (**26**) and (**27**).

(±)-1-(3-*Cyclohexyl*-3-*hydroxypropyl*)-5-(6-*morpholinocarbonylhexyl*)*hydantoin* (**13**) *and* (**14**).—A solution of morpholine (73 mg, 0.83 mmol) in dichloromethane (2 ml) was added at 0 °C to a solution of the anhydride (**3**) in dichloromethane (15 ml), prepared as above from acid (**1**) (300 mg, 0.81 mmol), triethylamine (83 mg, 0.82 mmol), and ethyl chloroformate (89 mg, 0.82 mmol). After overnight storage, the reaction mixture was acidified with 2M hydrochloric acid and washed with water. The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure and the residue (300 mg) purified by preparative t.l.c. to yield (**13**) as a colourless gum (190 mg, 53%), R_F 0.63 ($CHCl_3$–MeOH–HOAc, 90:5:5); *m/z* 438 (M^+ + 1, f.a.b.); δ_H (90 MHz; $CDCl_3$) 0.9—2.1 (23 H, m, aliphatic H), 2.31 (2 H, t, *J* 7 Hz, CH_2CO), 2.90—3.90 (12 H, m, 3 × NCH_2 + 2 × CH_2O + CHOH, 1 H exchangeable), 4.01 (1 H, t, *J* 4 Hz, CHN), and 7.0 (1 H, br s, NH, exchangeable). In a similar manner, the anhydride (**4**), obtained from the acid (**2**), gave a 45% yield of (**14**), m.p. 105—107 °C (Found: C, 63.0; H, 9.05; N, 9.30. $C_{23}H_{39}N_3O_5$ requires C, 63.1; H, 8.98; N, 9.60%); R_F 0.60, ($CHCl_3$–MeOH–HOAc, 90:5:5); δ_H(90 MHz; $CDCl_3$) 0.9—2.1 (23 H, m, aliphatic H), 2.29 (2 H, t, *J* 7 Hz, CH_2CO), 2.70 (1 H, v. br s, OH, exchangeable), 3.30—3.80 (11 H, m, 3 × NCH_2 + 2 × CH_2O + C*H*OH), 4.04 (1 H, t, *J* 4 Hz, CHN), and 8.3 (1 H, v. br s, NH, exchangeable).

(±)-1-(3-*Cyclohexyl*-3-*hydroxypropyl*)-5-[(6-N-*hydroxy carbamoyl*)*hexyl*]*hydantoin* (**15**).—A solution of the anhydride (**3**) in dichloromethane (20 ml) was prepared from the acid (**1**) (500 mg, 1.36 mmol), triethylamine (138 mg, 1.36 mmol), and ethyl chloroformate (148 mg, 1.36 mmol). Hydroxylamine hydrochloride (188 mg, 2.71 mmol) and then triethylamine (274 mg, 2.71 mmol) were added to this at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 24 h. It was then evaporated under reduced pressure, and the residue treated with 1M hydrochloric acid (25 ml). The mixture was extracted (3 ×) with ethyl acetate and the combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give a colourless gum (485 mg) which was purified by flash chromatography, eluting $CHCl_3$–MeOH–HOAc (90:5:5), to give (**15**) (210 mg, 41%) as a colourless solid, no sharp m.p. (decomp.) (Found: C, 56.7; H, 8.30; N, 9.32. $C_{19}H_{33}N_3O_5$·1.0 HOAc requires C, 56.9; H, 8.41; N, 9.47%); *m/z* 384 (M^+ + 1, f.a.b.); δ_H {360 MHz; [$(CD_3)_2SO$]} 0.88—1.80 (23 H, m, aliphatic H), 1.92 (2 H, t, *J* 7Hz,CH_2CO), 3.03 (1 H, m, NC*H*H), 3.15 (1 H, m, NCHH_2), 3.50 (1 H, m, C*H*OH), 4.04 (1 H, t, *J* 4 Hz, CHN), and 4.4 (1 H, v.br.s, exchangeable).

(±)-1-(3-*Cyclohexyl*-3-*hydroxypropyl*)-5-[6-(N,N′-*dicyclohexylallophanoyl*)*hexyl*]*hydantoin* (**16**).—To a solution of the acid (**1**) (368 mg, 1.0 mmol) and methanesulphonamide (100 mg, 1.05 mmol) in dry tetrahydrofuran (8 ml) was added dicyclohexylcarbodi-imide (216 mg, 1.05 mmol). The reaction mixture was stirred overnight, filtered, and concentrated under reduced pressure. The residual gum was stirred with 0.5M aqueous sodium hydroxide until no more dissolution occurred, and then the solution was filtered through charcoal, and the filtrate acidified with 2M hydrochloric acid. The resulting solid (190 mg) was filtered off and recrystallized twice from aqueous methanol to yield (**16**) (0.12 g, 21%), m.p. 162—164 °C (Found: C, 66.7; H, 9.48; N, 9.60. $C_{32}H_{54}N_4O_5$ requires C, 66.9; H, 9.47; N, 9.75%); *m/z* 575 (M^+ + 1, f.a.b.).

(±)-1-(3-*Cyclohexyl*-3-*hydroxypropyl*)-5-[6-(N-*methanesulphonylcarbamoyl*)*hexyl*]*hydantoin* (**18**) *and* (**19**).—Methanesulphonamide (320 mg, 3.36 mmol) in dichloromethane (2 ml) was added to a solution of anhydride (**3**) in dichloromethane (30 ml), prepared from the acid (**1**) (1.23 g, 3.34 mmol), triethylamine (340 mg, 3.36 mmol), and ethyl chloroformate (370 mg, 3.41 mmol) and the mixture stirred at room temperature for 1 h (t.l.c. indicated the absence of any reaction). The mixture was then evaporated under reduced pressure and the residual viscous paste heated on a steam-bath for 4 h. After cooling, the reaction mixture was dissolved in dichloromethane

(40 ml), and washed with 0.1M hydrochloric acid and then with water. The organic extract was dried ($MgSO_4$) and the solvent removed under reduced pressure to yield a pale yellow gum (1.4 g). T.l.c. indicated the presence of a diastereoisomeric mixture and separation by h.p.l.c. gave (**18**) (0.23 g, 16%) as a colourless wax; δ_H (90 MHz, $CDCl_3$) 0.9—2.1 (23 H, m, aliphatic H), 2.33 (2 H, t, *J* 7 Hz, CH_2CO), 3.28 (3 H, s, Me), and 3.0—4.2 (5 H, m, NCH_2 + CHOH + CHN); *m/z* 445 (M^+); R_F, 0.44 ($CHCl_3$–MeOH–HOAc, 90:5:5); and (**19**), (0.20 g, 14%), m.p. 102—104 °C (Found: C, 54.1; H, 8.21; N, 9.22. $C_{20}H_{35}N_3O_6S$ requires C, 53.9; H, 7.92; N, 9.43%); R_F 0.41 ($CHCl_3$–MeOH–HOAc, 90:5:5); δ_H (90 MHz, $CDCl_3$) 0.9—2.1 (23 H, m, aliphatic H), 2.33 (2 H, t, *J* 7 Hz, CH_2CO), 3.28 (3 H, s, Me), 3.20—3.90 (4 H, m, NCH_2 + CHOH), and 4.17 (1 H, t, *J* 4 Hz, CHN).

(±)-1-(3-*Cyclohexyl*-3-*hydroxypropyl*)-5-[6-N-(*hexanesulphonylcarbamoyl*)*hexyl*]*hydantoin* (**20**) *and* (**21**).—Hexanesulphonamide[17,18] 1.65 g, 10 mmol) was added to a freshly prepared solution of sodium (210 mg, 9.2 mmol) in methanol (12 ml), after which the solvent was removed under reduced pressure; the last traces of methanol were removed from the residue by azeotroping with benzene. The resulting sodium hexanesulphonamide was added at 0 °C to a solution in dimethylformamide (8 ml) of anhydride (**7**), prepared from acid (**1**) (736 mg, 2.0 mmol), triethylamine (225 mg, 2.11 mmol), and isobutyl chloroformate (290 mg, 2.12 mmol). Hexamethylphosphoramide (2 ml) was then added and the reaction mixture stirred at room temperature for 16 h. After this it was diluted with water, acidified with 2M hydrochloric acid, and extracted with ethyl acetate (3 ×). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give a viscous oil; t.l.c. analysis indicated the presence of a diastereoisomeric product mixture and some unchanged hexanesulphonamide. The latter was removed by stirring the crude product with aqueous sodium hydrogen carbonate and washing the soluble fraction with ether. Acidification of the aqueous solution and work-up as before gave a viscous oil (1.2 g). Separation of the diastereoisomers by h.p.l.c. yielded (**20**) (390 mg, 38%), m.p. 90—92 °C (Found: C, 58.2; H, 8.6; N, 8.05. $C_{25}H_{45}N_3O_6S$ requires C, 58.2; H, 8.80; N, 8.15%); R_F 0.64 ($CHCl_3$–MeOH–HOAc, 90:5:5) and (**21**) (310 mg, 28%) m.p. 59—62 °C; *m/z* 516 (M^+ + 1, *f.a.b.*); R_F 0.58 ($CHCl_3$–MeOH–HOAc, 90:5:5).

(±)-5-[6-(N-*Cyanocarbamoyl*)*hexyl*]-1-(3-*cyclohexyl*-3-*hydroxypropyl*)*hydantoin* (**22**).—Sodium cyanamide (0.30 g, 4.7 mmol) and hexamethylphosphoramide (2 ml) were added at 0 °C to a solution in dimethylformamide (10 ml) of the anhydride (**3**), prepared from acid (**1**) (1.0 g, 2.72 mmol), triethylamine (0.28 g, 2.77 mmol), and ethyl chloroformate (0.30 g, 2.76 mmol). The reaction mixture was stirred overnight and then poured into 0.5M hydrochloric acid. The mixture was extracted with ethyl acetate, and the combined extracts were washed with water, dried ($MgSO_4$) and evaporated under reduced pressure to give the crude product, which was purified by h.p.l.c. to give (**22**) (0.50 g, 47%) as a hygroscopic colourless solid, m.p. 45—48 °C (decomp.) (Found: C, 50.1; H, 8.52; N, 11.3. $C_{20}H_{32}N_4O_4 \cdot 5H_2O$ requires C, 49.8; H, 8.77; N, 11.6%); ν_{max} (KBr) 2 165, 1 769, and 1 715 cm^{-1}; δ_H (360 MHz; $[(CD_3)_2SO]$) 0.9—1.8 (23 H, m, aliphatic H), 1.91 (2 H, t, *J* 7 Hz, CH_2CO), 3.11 (2 H, m, NC*H*H + C*H*OH), 3.50 (1 H, m, NCH*H*), 4.07 (1 H, br t, *J* 4 Hz, CHN), 4.3 (1 H, v. br s, NH, exchangeable), and 0.6 (1 H, v. br s, NH, exchangeable); R_F 0.3 ($CHCl_3$–MeOH, 85:15).

(±)-1-(3-*Cyclohexyl*-3-*hydroxypropyl*)-5-[6-(N-*tetrazol*-5-*ylcarbamoyl*)*hexyl*]*hydantoin* (**23**) *and* (**24**)—1,1′-Carbonyl di-1*H*-imidazole (0.95 g, 5.86 mmol) was added to a solution of the acid (**2**) (2.0 g, 5.43 mmol) in dry dimethylformamide (40 ml) under dry nitrogen and the mixture was heated at 95 °C for 4 h. Anhydrous 5-aminotetrazole (0.50 g, 5.87 mmol), obtained from the commercially available monohydrate by heating at 85 °C under high vacuum, was then added and heating continued at 95 °C for a further 2 h. After the mixture had cooled to room temperature, it was evaporated under reduced pressure and the residue treated with 0.5M hydrochloric acid (50 ml). The mixture was extracted with ethyl acetate (4 ×) and the combined extracts were dried ($MgSO_4$), and evaporated under reduced pressure to yield an oil (1.2 g). This was purified by h.p.l.c. and crystallized from ethyl acetate to give (**24**) (610 mg, 26%), m.p. 120—122 °C (Found: C, 54.8; H, 7.75; N, 22.3. $C_{20}H_{33}N_7O_4$ requires C, 55.2; H, 7.63; N22.5%); δ_H (90 MHz, $[(CD_3)_2SO]$) 0.9—2.0 (23 H, m, aliphatic H), 2.40 (2 H, t, *J* 7 Hz, CH_2CO), 3.34 (3 H, m, NCH_2 + C*H*OH), 3.94 (1 H, t, *J* 4 Hz, CHN), 9.7 (1 H, br s, exchangeable), and 11.8 (1 H, br s, exchangeable); R_F 0.15 ($CHCl_3$–MeOH–HOAc, 90:5:5).

An analogous procedure starting from the acid (**1**) gave (**23**) (20%), m.p. 204—207 °C (Found: C, 55.4; H, 7.70; N, 22.3. $C_{20}H_{33}N_7O_4$ requires, C, 55.2; H, 7.63; N, 22.5%); *m/z* 436 (M^+ + 1, f.a.b.); δ_H (90 MHz; $[(CD_3)_2SO]$) 0.9—2.0 (23 H, m, aliphatic H), 2.35 (2 H, t, *J* 7 Hz, CH_2CO), 2.8—3.8 (4 H, m, NCH_2 + CHOH, 1 H exchangeable), and 4.04 (1 H, t, *J* 4 Hz, CHN); R_F 0.2 ($CHCl_3$–MeOH–HOAc, 90:5:5).

(±)-5-(6-*Aminohexyl*)-1-(3-*cyclohexyl*-3-*hydroxypropyl*)-*hydantoin Hydrochloride* (**25**).—[Bis(trifluoroacetoxy)]iodobenzene (3.58 g, 8.32 mmol) was added to a solution of the amide (**11**) (2.33 g, 6.34 mmol) in acetonitrile–water (50 ml, 1:1) and the reaction mixture was stirred at room temperature for 5 h. It was then evaporated under reduced pressure and 2M hydrochloric acid (64 ml) added to the residue; and the volatiles were then again removed under reduced pressure. The residue was dissolved in water (60 ml) and ethanol (14 ml), treated with sodium naphthalene-2-sulphonate (1.6 g), and warmed until dissolution occurred. As the mixture cooled, crystals formed which were filtered off and recrystallized from propan-2-ol–ethyl acetate to give the naphthalenesulphonate salt of the title compound (1.72 g), m.p. 117–122 °C (Found: C, 61.2; H, 7.55; N, 7.6. $C_{28}H_{41}N_3O_6S$ requires C, 61.4; H, 7.55; N, 7.67%). This salt was dissolved in ethanol–water (1:1) and passed through a column of Amberlite resin IRA 400 (Cl^-) to yield (**25**) hydrochloride (1.0 g, 42%) as a colourless glass, δ_H(360 MHz; $[(CD_3)_2SO]$) 0.90—1.84 (25 H, m, aliphatic H), 2.73 (2 H, t, *J* 7 Hz, C*H*$_2$NH$_2$·HCl), 2.94—3.20 (2 H, m, NC*H*H + C*H*OH), 3.4—3.64 (1 H, m, NCH*H*), and 4.08 (1 H, t, *J*4 Hz, CHN); R_F 0.36 [$CHCl_3$–MeOH–NH_3 (0.88), 20:5:1].

(±)-5-(6-*Cyanohexyl*)-1-(3-*cyclohexyl*-3-*hydroxypropyl*)-*hydantoin* (**26**) *and* (**27**).—(*A*) *From ethyl* 2-*amino*-8-*cyanooctanoate* (**32**). A solution of lithium di-isopropylamide in dry tetrahydrofuran (1 l) was prepared from di-isopropylamine (20.2 g, 0.20 mol) and butyl-lithium in hexane (1.65 M; 121 ml). Hexamethylphosphoramide (300 ml) was added and the stirred solution cooled to −78 °C. A solution of *N*-benzylidene glycine ethyl ester[19] (42 g, 0.22 mol) in a little dry tetrahydrofuran was added slowly and the mixture stirred at −78 °C for 30 min. 1-Bromo-6-cyanohexane[20,21] (38.0 g 0.20 mol) in a little dry tetrahydrofuran was then added and the resulting solution was allowed to warm to room temperature and then stirred for 18 h. Most of the solvent was removed under reduced pressure and the residue was diluted with ether and washed with aqueous ammonium chloride. The organic extract was dried and evaporated under reduced pressure and the residual yellow oil suspended in 1M hydrochloric acid (2 l) with stirring for 1 h. The resulting suspension was thoroughly extracted with ether, the separated aqueous phase made alkaline (pH 11—12) with solid

sodium carbonate, and the mixture extracted with chloroform. The extract was dried and the solvent removed under reduced pressure to give (**32**) (22 g, 52%) as a pale yellow oil, b.p. 119 °C 0.012 mmHg); δ_H (90 MHz; $CDCl_3$) 1.26 (3 H, t, *J* 7 Hz, Me), 1.70 (2 H, br s, exchangeable, NH_2), 1.85 (10 H, m, $5 \times CH_2$), 2.40 (2 H, t, *J* 7 Hz, CH_2CN), 3.42 (1 H, m, CHN), and 4.15 (2 H, q, *J* 7 Hz, OCH_2); $\nu_{max.}$(film) 2 250 cm^{-1}.

Ethyl 2-amino-8-cyano-octanoate (**32**) (8.51 g, 0.04 mol) and cyclohexyl vinyl ketone[11] (5.52 g, 0.04 mol) were mixed at 0 °C and set aside at room temperature overnight to give ethyl 2-(3-cyclohexyl-3-oxopropylamino)-8-cyano-octanoate as an oil. A stirred solution of this ketone (13.5 g) in ethanol (140 ml) was treated dropwise at 0 °C with sodium borohydride (0.75 g, 0.02 mol) in ethanol (70 ml), kept at room temperature for 3.5 h, and then concentrated at 40 °C under reduced pressure. Water was added, the mixture was brought to pH 6 by addition of dilute hydrochloric acid and the product was extracted into ether. The extract was washed with water, dried ($MgSO_4$), and evaporated to give (**33**) (12 g) as an oil consisting of two diastereoisomers, R_F 0.40 and 0.45 (SiO_2; $CHCl_3$–MeOH, 50:1) and minor impurities which could be removed by chromatography (SiO_2; $CHCl_3$–MeOH, 50:1).

To the foregoing crude amino-alcohol (4 g, 0.01 mol), dissolved in ethanol (20 ml) and 2M hydrochloric acid (10 ml), a solution of potassium cyanate (1.62 g, 0.02 mol) in water (5 ml) was added gradually with cooling and stirring, and the solution was left at room temperature overnight. Most of the alcohol was evaporated, water was added, and the oil was extracted into ether. Evaporation of the washed and dried ethereal solution left an oil which was heated on the steam-bath for 5 h, to give the crude nitrile as a yellow oily mixture (4.1 g) of diastereoisomers, R_F 0.5 and 0.65 (SiO_2; $CHCl_3$–MeOH, 9:1), containing some impurities. This material was stirred with 0.5M aqueous sodium hydroxide (50 ml) for 15 min. The insoluble non-acidic material was removed by washing with ether and the clear alkaline solution was acidified with 2M hydrochloric acid; the liberated hydantoin was extracted to give the crude nitrile, as a pale yellow viscous oil (3.25 g). The individual diastereoisomers were obtained by h.p.l.c.: (**26**) (1.3 g. 37%), m.p. 97–99 °C (Found: C, 65.5; H, 8.72; N, 12.2. $C_{19}H_{31}N_3O_3$ requires C, 65.3; H, 8.94; N, 12.0%). (**27**) (1.0 g, 28%), viscous oil; $\nu_{max.}$(film) 2 250 cm^{-1}; *m/z* 349 (M^+).

(*B*) *From the amides* (**11**) *and* (**12**). Dry pyridine (10 ml) was added to a solution of the amides (**11**) and (**12**) (6.80 g. 0.02 mol) in dry tetrahydrofuran (40 ml). The mixture was cooled to 0 °C and trifluoroacetic anhydride (5 ml, 0.04 mol) was added dropwise over 15 min. The reaction mixture was stirred for a further hour at room temperature and then poured onto ice (150 g). The mixture was extracted with chloroform (2 ×) and the combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The crude residue was extracted with aqueous sodium hydroxide as described above, and was further purified by flash chromatography eluting with chloroform–methanol (97:3) to give the nitrile epimers (**26**) and (**27**) (3.9 g, 60%).

(±)-1-(3-*Cyclohexyl*-3-*hydroxypropyl*)-5-{6-[N-(2-*methoxycarbonylethyl*)*carbamoyl*]*hexyl*}*hydantoin* (**28**).—A solution of the acid (**2**) (2.45 g, 6.6 mmol) and triethylamine (0.672 g, 6.65 mmol) in dichloromethane (35 ml) was stirred at 0 °C and treated dropwise with ethyl chloroformate (0.735 g, 6.8 mmol) in dichloromethane (7 ml). After 1 h at 0 °C, a freshly made solution of β-alanine methyl ester hydrochloride (0.932 g, 6.7 mmol) and triethylamine (0.672 g, 6.7 mmol) in dichloromethane (15 ml) was added, and the resulting solution stirred overnight at room temperature. The solution was washed with water, dried ($MgSO_4$), and evaporated under reduced pressure. The residual gum was purified by column chromatography (chloroform–methanol, 9:1) to give the amide (**28**) (3.0 g 99%) as a colourless glass, δ_H (90 MHz; $CDCl_3$) 0.9—2.0 (23 H, m, aliphatic H), 2.56 (4 H, br t, *J* 7 Hz, 2 × CH_2CO), 3.2—3.8 (5 H, m, $NHCH_2$ + NCH_2 + C*H*OH), 3.74 (3 H, Me), 4.13 (1 H, m, CHN), and 9.48 (1 H, br s, exchangeable); *m/z* 453 (M^+).

(±)-1-(3-*Cyclohexyl*-3-*hydroxypropyl*)-5-{6-[1-(2-*methoxycarbonylethyl*)*tetrazol*-5-*yl*]*hexyl*}*hydantoin* (**29**).—The hydantoin amide (**28**) (0.908 g, 2.0 mmol) in dichloromethane (8 ml) was treated with dihydropyran (0.7 ml, 7.7 mmol) and then toluene-*p*-sulphonic acid (20 mg) and the mixture stirred at room temperature for 2 h. The solvent was removed under reduced pressure and the residual gum purified by column chromatography to give the desired intermediate THP ether (**34**) as a colourless glass (1.18 g, 87%). This was dissolved in dichloromethane (7 ml) and dry pyridine (0.25 ml). The stirred solution was cooled to 0 °C and a solution of phosgene in toluene (12.5% w/w, 2.1 ml, 2.41 mmol) diluted with dichloromethane (4.5 ml) was added dropwise. The solution was stirred at room temperature for 3 h and then a solution of trimethylsilyl azide (0.265 g, 2.3 mmol) in dichloromethane (4.5 ml) was added dropwise. After 18 h at room temperature, methanol (2.0 ml) was added and the solution was stirred for a further hour and then washed with water. The organic phase was separated, dried ($MgSO_4$), and evaporated under reduced pressure. The residual gum was dissolved in tetrahydrofuran (23 ml) and water (18 ml), and concentrated hydrochloric acid (1.1 ml) was added. After 3 h at room temperature, water was added, the solution taken to pH 6—7 by addition of solid sodium hydrogen carbonate, and the resulting mixture was extracted with chloroform. The extract was dried ($MgSO_4$) and evaporated under reduced pressure and the residue was purified by column chromatography to give the desired *tetrazole ester* (**29**) as a colourless glass (0.71 g, 67%), δ_H (90 MHz; $CDCl_3$) 0.9—2.0 (23 H, m, aliphatic H), 2.8—3.2 (4 H, m, CH_2CO + CH_2CN_4), 3.2—3.7 (3 H, m, NCH_2 + C*H*OH), 3.76 (3 H, s, Me), 4.12 (1 H, t, *J* 5 Hz, CHN), and 4.74 (2 H, t, *J* 7 Hz, CH_2N_4C); $\nu_{max.}$(film) 1 765 sh, 1 710, and 1 517w (tetrazole) cm^{-1}.

(±)-1-(3-*Cyclohexyl*-3-*hydroxypropyl*)-5-[6-(*tetrazol*-5-*yl*)*hexyl*]*hydantoin* (**30**) *and* (**31**).—*Method A*. The ester (**29**) (0.71 g) was dissolved in chloroform (50 ml), 1,5-diazabicyclo[4.3.0]non-5-ene (0.55 g) was added, and the solution was refluxed for 18 h. The cooled solution was washed with 0.1M hydrochloric acid, dried ($MgSO_4$), and evaporated under reduced pressure to give a pale yellow gum (*ca.* 0.7 g). This was purified by h.p.l.c. to give the less polar diastereoisomer (**30**) (32 mg, 5.5%) as a white crystalline solid, m.p. 140—141 °C (from chloroform–ether) (Found: C, 58.3; H, 8.00; N, 21.2. $C_{19}H_{32}N_6O_3$ requires C, 58.1; H, 8.22; N, 21.4%); $\nu_{max.}$(KBr) 1 760 (weak), 1 705, 1 580 (weak), and 1 565 (weak) cm^{-1}; *m/z* 392 (M^+); and (**31**) (64 mg, 11%) as a colourless glass, δ_H (90 MHz; $CDCl_3$) 0.85—2.0 (23 H, m, aliphatic H), 2.90 (2 H, t, *J* 7 Hz, CH_2CN_4), 3.2—3.6 (3 H, m, NCH_2 + C*H*OH), and 4.04 (1 H, t, *J* 4Hz, CHN); *m/z* 392 (M^+).

Method B. The nitriles (**26**) and (**27**) (3.90 g, 11 mmol) and tributyltin azide[22] (4.07 g, 12 mmol), were stirred in dioxane (12 ml) until they had dissolved, and the solution was then heated in a small autoclave at 160 °C for 30 h. The reaction mixture was diluted with dioxane (40 ml) and then stirred with concentrated hydrochloric acid (5 ml) for 2 h. Saturated aqueous sodium hydrogen carbonate was then added until the mixture was at pH 10 and it was then washed twice with ether–hexane (1:1). The aqueous phase was acidified to pH 1 with 2M hydrochloric acid and extracted twice with ethyl acetate. The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give a viscous oil (3.2 g). The major components of this material were identical with those produced above and the tetrazole

epimers [R_F 0.69, 0.73 in CH_2Cl_2–MeOH–Et_2O (13:10:2)] were separated by h.p.l.c. as before to give (**30**) (1.0 g, 45%) and (**31**) (0.85 g, 38%).

Inhibition of Platelet Aggregation in Vitro.—Human blood was freshly collected into siliconized (Siloclad: Clay Adams) plastic (Sterilin Ltd.) vessels containing trisodium citrate (3.15%; 0.1 volume with 0.9 volume blood) and centrifuged (200 g for 15 min) at room temperature. The platelet-rich plasma (PRP) was withdrawn into plastic containers and kept at room temperature. Inhibition of platelet aggregation was determined by a Born-type aggregometer as described previously[23] by incubating aliquots (0.5 ml) of the PRP for 1 min at 37 °C with or without the prostaglandin analogue prior to addition of sufficient adenosine diphosphate (ADP) to just cause a non-reversing control aggregation. Dose-inhibition curves were constructed for each compound and the IC_{50} (conc. causing 50% inhibition) was calculated as that required to reduce the aggregation to 50% of its control amplitude. Prostacyclin was used as a standard for each batch of PRP. Comparison of the IC_{50} values of analogues with those of prostacyclin then allowed a potency ratio to be calculated; *e.g.* BW245C/PGI_2 is 0.2. The potencies of analogues relative to PGE_1, were estimated from potency ratios of PGI_2/PGE_1 observed previously[5,23] in human PRP.

Cardiovascular Actions in Rats.—Anaesthesia was induced in male Wistar rats (250—300 g body weight) with sodium pentobarbitone (30 mg kg^{-1}, i.v.) and supplemented (3 mg kg^{-1}) when required. Arterial pressure was recorded from a cannulated femoral artery, the resting values being in the range of 100—140 mmHg. Rectal temperature was maintained at 37 °C by thermistor-controlled radiant heat. Each compound was injected into a femoral vein in a volume of 0.25 ml and flushed in with 0.2 ml of saline (0.9% w/v. Dose-response relationships for the fall in mean systemic arterial blood pressure (b.p.) with the prostaglandin analogues were constructed and compared to those obtained with prostacyclin (PGI_2) in the same animal, and a potency ratio calculated.

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