Pergamon

Tetrahedron Vol. 50, No. 40, pp. 11781-11792, 1994

0040-4020/94 $7.00+0.00

0040-4020(94)00729-2

New Synthetic Probes of the Iron Transport System of *Paracoccus denitrificans*

George M.Buckley, Gerald Pattenden*, and Donald A. Whiting

Department of Chemistry, The University, Nottingham, NG7 2RD

Abstract A range of analogues of parabactin A **4** and agrobactin A **5** have been synthesised. All these comounds were able to chelate iron, and to promote the growth of iron-starved *Paracoccus denitrificans* to varying degrees. A degree of flexibilty in the siderophore receptor system is thus demonstrated, which may be exploitable for the transport of new siderophore - antibiotic conjugates.

Many vital metallo-enzymes rely on the unique chemistry of iron for their function,[1] *e.g.* the cytochromes, ferredoxins, haemoglobin, nitrogenase, and RNA reductase. The supply of iron raises a number of special problems for organisms, at different levels. Thus iron III has a very low solubility at pH 7, making acquisition from the environment and internal transport and storage problematic, while free ferric iron is a promoter of both oxygen radical chemistry and of microbial growth, with potential toxicity consequences to higher organisms.

Microorganisms and plants have evolved the apparatus for the biosynthesis of a structurally diverse range of very specific iron chelators, named siderophores.[2] These compounds are excreted into the organism's habitat to sequester iron III; the metal complex is actively transported through the cell membrane *via* specific protein channels. Release of iron by various mechanisms then follows. Synthesis of both the siderophores and the receptor proteins is initiated by conditions of iron deficiency.

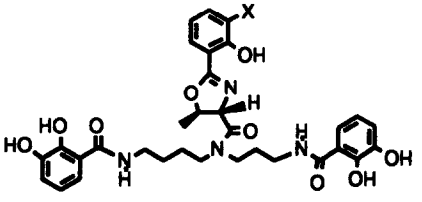
The membrane channels for siderophores are, as with other specific transport systems, open to misuse by toxins.[3] A classic case is that of the natural antibiotic albomycin,[4,5] which has been used clinically, in which the trihydroxamate siderophore ferrioxamine is linked to a thioribosylpyrimidine. The ferric complex of albomycin is ferried across the cell membrane, when the nucleoside analogue considered to act as the toxophore is released. This example of natural drug transport has prompted a number of attempts to link catecholate or hydroxamate siderophores or related compounds, to antibiotics such as sulphonamides or β-lactams, with some success in enhancing antibiotic activity.[6,7,8,9]

One of the prerequisites of such work is a knowledge of the receptor flexibility and the variations in siderophore structures which permit transport. Notable 'receptor mapping' work in this area includes studies on enterobactin [10a-d] and ferrichrome[10e] In both cases analogues were developed with microbial growth promoting abilities comparable to those of the natural siderophores.

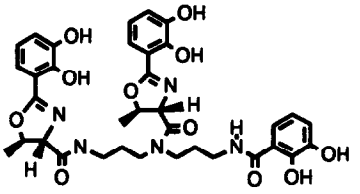
Our interests in this area focussed on the iron accumulation system of *Paracoccus denitrificans*, which synthesises the triscatechol siderophore parabactin **1**,[11] constructed on a spermidine backbone. Related natural products are agrobactin **2**,[12](*Agrobacterium tumefaciens*) and vibriobactin **3**,[13] (*Vibrio choleare*). Parabactin A **4** and agrobactin A **5**, with uncyclized threonine residues, are also known.[12] *Paracoccus denitrificans* has been considered to be able to deploy both high and low affinity iron - protein receptors.[14] The former is used by ferriparabactin, while the complexes of parabactin A **4** and the

enantiomer of parabactin are bound by the second uptake system.

We set out to investigate an aspect of the relationship between structure and iron transporting ability in *Paracoccus* by new parabactin and agrobactin analogues. Our initial aim was to identify any sites of structural tolerance which might be suitable for the attachment of antibiotic residues. Some encouraging flexibility has been reported[14] for this microorganism, in that both (+)- and (-)-parabactin and both (+)- and (-)-parabactin A will mediate iron supply albeit to differing extents. We considered that an antibiotic based on a modified siderophore would not need to be a highly efficient iron transporter to be an effective bactericide.



1; X=H
2; X=OH



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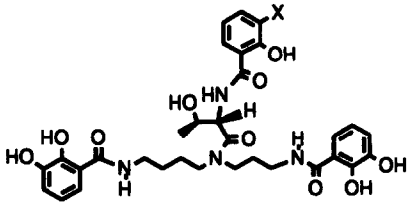
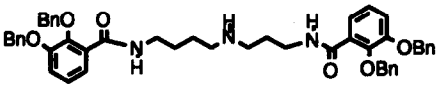
The first requirement of this study was a viable synthetic route to parabactin **1** which would act as a model protocol for the projected target compounds and which would provide material for biological tests. Parabactin has been prepared previously by two methods[15] and some parabactin analogues have been reported.[16] We opted to employ the short and efficient route of Bergeron and coworkers[15a] with the modification of using *O*- benzyl protection throughout instead of *O*- methyl, for ease of deblocking. Thus bis (2,3-dibenzyloxybenzoyl) spermidine **6** was first prepared by the methods of Nagao and Fujita.[15b]



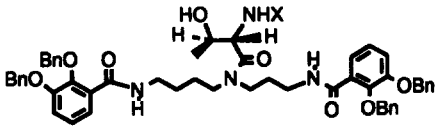
4; X=H
5; X=OH



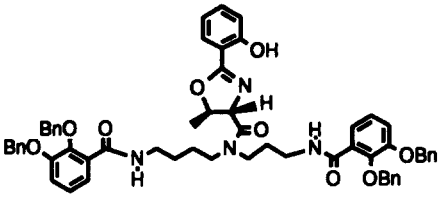
6

This was condensed with the N-hydroxysuccinimide ester of BOC-L-threonine in DMF over 48 h to give the peptide **7**. Removal of the t-butoxycarbonyl group with formic acid next provided **8** which was condensed with ethyl 2-hydroxybenzimidate to form the *trans* oxazoline **9**. Finally debenzylation was carried out by transfer hydrogenolysis using cyclohexene over palladium hydroxide on charcoal, to yield

parabactin 1, which showed spectroscopic properties esentially identical to those reported for the natural and synthetic product.[15]



7;X=BOC
8;X=H



9

We chose for our specific targets two sets of compounds, *viz* **12** and **13**, analogues of the siderophores parabactin A **4** and agrobactin A **5**. Structural variations in the pendant amino acids in these series did not appear, from studies of models, to exert a major effect on iron binding, and thus seemed to offer the best chance of introducing a bulky substituent into a catecholamide of these types without major disruption of the biological function. Fig.1 illustrates this point, showing the preferred conformation of the iron complex of the *tris* catechol **13b**; R^2=H, minimised (in vacuum) using the Spartan v.3.1 program (Wavefunction Inc., 18401 Von Karman, Suite 370, Irvine, California 92715) and the SYBYL forcefield.

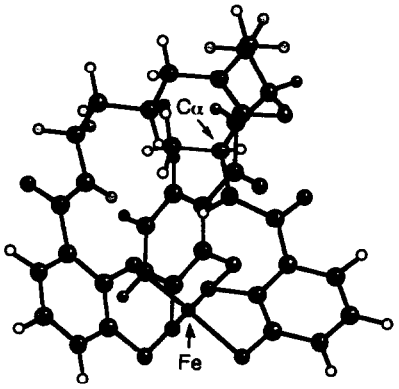


Fig.1

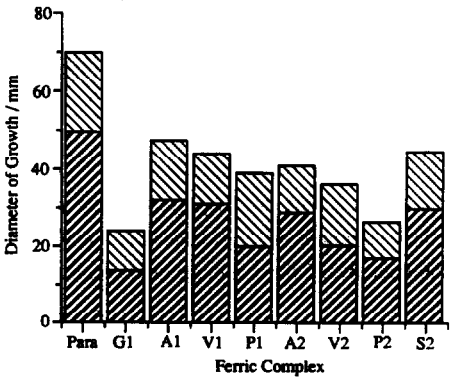
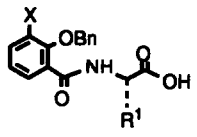


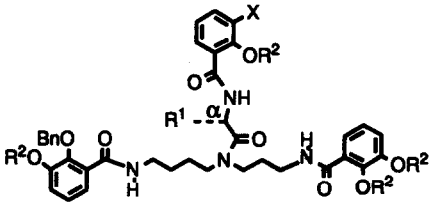
Fig.2

The required compounds **12** and **13** were made by similar methods to those described for parabactin itself. Thus the appropriate amino acid methyl esters were first aroylated with 2-benzyloxybenzoyl chloride or 2,3- bis(benzyloxy)benzoyl chloride and the product esters were then saponified with aqueous sodium hydroxide to provide the acids **10** and **11**. Coupling of these acids with bis-(2,3-dibenzyloxybenzoyl) spermidine **6** next afforded the desired perbenzylated products. Debenzylation as previously then yielded the target compounds in the two series **12 a-d, R^2=H** (compounds G1, A1, V1, and P1 respectively) and **13 a-e, R^2=H** (compounds G2, A2, V2, P2, and

S2 respectively). These compounds were finally converted into their ferric complexes using ferric acetoacetonate, after the method of Bergeron and Weimer,[14b] and the chelates were purified by reverse phase chromatography on a C_{18} column.



10; X=H
11; X=OBn



12; X=H **a; R^1=H b; R^1=Me**
13; X=OR^2 **c; R^1=$CHMe_2$ d; R^1=CH_2Ph**
e; R^1=CH_2OH

The abilities of the ferric complexes of the products **12** and **13** to act as siderophores were evaluated by a simple qualitative test involving reversal of iron starvation. In this assay agar plates were prepared containing deferrated ethylenediamine-di (*o*-hydroxyphenylacetic acid) and inoculated with *P. denitrificans* by the spread plate method. Ethylenediamine-di (*o*-hydroxyphenylacetic acid) chelates any available iron in the medium and cannot itself act as a siderophore, thus preventing bacterial growth by iron limitation. The prospective siderophores were added at known concentrations to a well cut into the cente of the agar, and the plate was incubated at 37°C. The ability of addenda to mediate iron supply is shown by the appearance of circular growth zones. A concentration gradient is set up from the centre of the plate outwards, and the diameter of the growth zone reflects the efficiency of a given iron chelate as a siderophore. The test is not quantitative but does indicate relative activities in a qualitative fashion suitable for the purpose in hand.

The results for eight compounds (G1, A1, V1, P1, A2, V2, P2, S2; compound G2 proved too insoluble for testing) are displayed in Figs .2, 3, and 4. Fig. 2 shows the bacterial growth zone diameters for the compounds at 15nM concentration after 24h and 48h. Figs. 3 and 4 show the variations in growth stimulation over 24h for a range of concentrations.

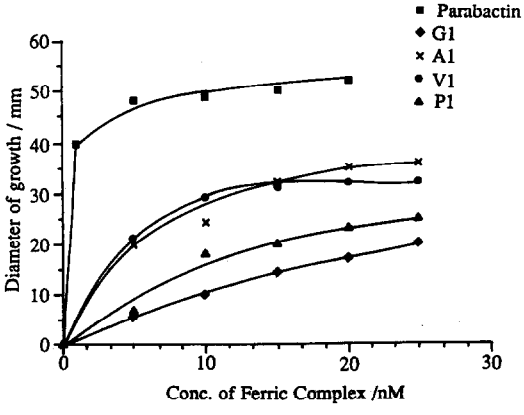


Fig.3

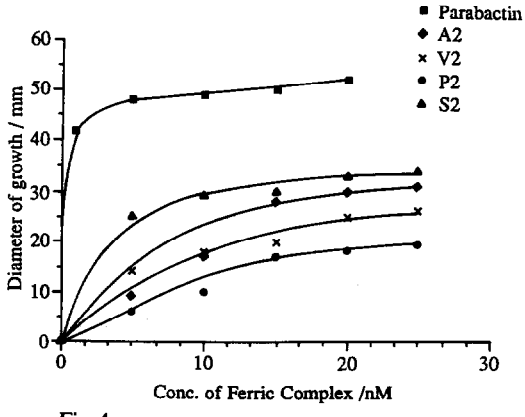


Fig.4

It can be seen that all the compounds tested are able to act as siderophores to *P. denitrificans* with efficiencies varying over a relatively narrow range; not surprisingly, none are as good as natural parabactin. With incubation for 24h at 15nM the best synthetic compounds were A1, V1, S2, and A2, with approx. 60% growth stimulation zone diameters compared to natural parabactin while the worst compound was G1 (28%). Differentials were more apparent at 5nm, with S2, A1 and V1 in the best set (40-50%) and P1, P2 around 10%. It thus appears that structural variations on parabactin A and agrobactin A of the type described here are possible, with a fair degree of retention of growth stimulating capability. An element of flexibility in the ferrisiderophore - receptor complex is thus apparent, encouraging further investigation into exploitation of the iron transport channel for 'smuggling' of antibiotics as siderophore conjugates.

EXPERIMENTAL

General Details. All melting points were recorded on a Köfler hot-plate apparatus and are uncorrected. ^{1}HNMR and ^{13}CNMR chemical shifts were recorded relative to an internal TMS standard. Multiplicities in ^{13}CNMR were obtained using a DEPT sequence. All reactions were monitored to completion by TLC, and NMR was used to indicate successful outcome. However full interpretation of NMR spectra was complicated by the presence of rotamers leading to doubling of peaks; complete resolution was thus very difficult and in most cases with n carbons rather less than 2n lines could be reliably discerned in ^{13}CNMR. This situation has been observed previously with parabactin. Data are listed as observed for characterisation. Mass spectra were recorded using electron ionisation (EI) or fast atom bombardment (FAB) tecniques. Protected and activated amino acids were prepared by standard methods.

L-N^4-[N-(tert-Butoxycarbonyl)threonyl]-N^1,N^8-bis-(2,3-dibenzyloxybenzoyl)spermidine (7).
A solution of L-*N*-hydroxysuccinimido-*N*-(*tert*-butoxy-carbonyl)threonate (0.05g, 0.16 mmol.) in dimethylformamide (10cm^3) was added to a solution of *N,N-bis*-(2,3-dibenzyloxybenzoyl)spermidine (**6**) (0.11g, 0.14 mmol.) in dimethylformamide (10cm^3) at 0°C. The solution was stirred under nitrogen at room temperature for 48 hours and the solvent evaporated *in vacuo*. The residue was re-dissolved in dichloromethane (20cm^3), washed with cold aqueous hydrochloric acid (3% w/v)(10cm^3) and cold water, dried, filtered and the filtrate evaporated *in vacuo*. The residue was chromatographed on a silica gel column with 5% methanol / dichloromethane as eluent to yield *L-N^4-[N-(tert-butoxy-carbonyl)threonyl]-N^1,N^8-bis-(2,3-di-benzyloxybenzoyl)spermidine* as a hydroscopic colourless gum (0.09g, 65%):
(Found: C, 71.0%; H, 6.5%; N, 5.6%; [M+H]=980. Required for $C_{58}H_{66}N_4O_{10}$: C, 71.1%; H, 6.8%; N, 5.7%; M=979); ν_{max} ($CHCl_3$)/cm^{-1} 3670, 3610 (O-H), 3440 (N-H), 3020, 2980, 2900 (C-H), 1700 (C=O), 1640 (C=O); δ_H [400MHz FT](CDCl_3) [two rotamers] 7.90-8.10 (m, 3H, N*H*CO), 7.60-7.75 (m, 2H, Ar*H*), 7.27-7.47 (m, 20H, Ar*H*), 7.11-7.14 (m, 4H, Ar*H*), 5.37 (d, *J*=9 Hz, 1H, α-C*H*), 5.15 (s, 2H, PhCH_2O-), 5.14 (s, 2H, PhCH_2O-), 5.10 (s, 2H, PhCH_2O-), 5.09 (s, 2H, PhCH_2O-), 4.10-4.30 (m, 1H, O*H*), 3.95 (m, 1H, β-C*H*), 3.10-3.40 (m, 8H, CH_2N-), 1.43-1.75 (m, 6H, CH_2-), 1.40 (s, 9H, (CH_3)C-), 1.10 and 1.12 (each d, 3H, *J*=6Hz, CH_3-); m/e (FAB) 980 $[M+H]^+$ (0.16%), 979 $[M^+]$ (1.15%), 879 $[980\text{-BOC}]^+$ (7.2%), 779 $[980\text{-(BOC-Threonine)}]^+$ (1.55%).

L-N^4-Threonyl-N^1,N^8-bis-(2,3-dibenzyloxy-benzoyl)spermidine (8).
L-N^4-[*N*-(*tert*-Butoxycarbonyl)threonyl]-N^1,N^8-*bis*-(2,3-dibenzyloxybenzoyl)spermidine (0.2g, 0.2mmol.) was dissolved in formic acid (20cm^3) and the solution stirred for 3 hours at room temperature under nitrogen. The solvent was then partially removed *in vacuo* and the residue made basic using cold aqueous sodium carbonate (30%w/v). The product was extracted with dichloromethane (30cm^3). The

organic layer was washed with cold water (2x30) and dried. This was filtered and the filtrate evaporated *in vacuo*. The residue was chromatographed on a silica gel column with 10% methanol / dichloromethane as eluent to yield *L-N^4-threonyl-N^1,N^8-bis-(2,3-dibenzyloxybenzoyl)spermidine* as a clear gum (0.1g, 56%): (Found [M+H]= 880. Required for $C_{53}H_{58}N_4O_8$ M= 879); ν_{max} ($CHCl_3$)/cm^{-1} 3650 (O-H), 3460 (N-H), 3010, 2960, 2900 (C-H), 1640 (C=O); δ_H [80MHz FT]($CDCl_3$) [rotamers]7.88-8.14 (m, 2H, N*H*CO), 7.59-7.82 (m, 2H, Ar*H*), 7.23-7.52 (m, 20H, Ar*H*), 7.05-7.15 (m, 4H, Ar*H*), 5.35-5.40 (m, 1H, α-C*H*), 5.20 (s, 4H, PhCH_2O-), 5.15 (s, 2H, PhCH_2O-), 5.10 (s, 2H, PhCH_2O-), 4.05-4.32 (m, 3H, NH_2 and O*H*), 4.00 (m, 1H, β-C*H*), 3.06-3.50 (m, 8H, CH_2N-), 1.43-1.95 (m, 6H, CH_2-), 1.10 and 1.12 (d each (rotamers), 3H, *J*=6Hz, CH_3-); m/e (FAB) 880 $[M+H]^+$ (0.26%), 879$[M^+]$ (1.06%), 789 $[880-C_7H_7]^+$ (0.37%), 778 [880-Threonine]$^+$ (0.2%).

L-N-[3-(2,3-Dibenzyloxybenzamido)propyl]-N-[4-(2,3-dibenzyloxybenzamido)butyl]-2-(2-hydroxyphenyl)-trans-5-methyloxazoline-4-carboxamide (9).

A solution of L-N^4-threonyl-N^1,N^8-*bis*-(2,3-dibenzyloxybenzoyl)spermidine (0.6g, 0.68mmol.) and ethyl 2-hydroxybenzimidate (0.15g, 0.75mmol.) in dry methanol (20cm^3) was stirred at reflux for 24 hours under nitrogen. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel with 2% methanol / dichloromethane as eluent to yield *L-N-[3-(2,3-di-benzyloxybenzamido)propyl]-N-[4-(2,3-di-benzyloxybenzamido)butyl]-2-(2-hydroxyphenyl)-trans-5-methyl-oxazoline-4-carboxamide* as a colourless oil (90%); (Found: C, 73.2%; H, 5.9%; N, 5.5%; (M+H)= 982. Required for $C_{60}H_{60}N_4O_9$: C, 73.5%; H, 6.2%; N, 5.7%; M=981); ν_{max} ($CHCl_3$)/cm^{-1} 3380 (O-H), 3070 , 3010, 2940, 2880 (C-H), 1640, 1575, 1530, 1490; δ_H [400MHz FT]($CDCl_3$) [Mixture of rotamers] 11.64 (s, 1H, O*H*), 7.92-8.10 (m, 2H, N*H*CO-), 7.61-7.72 (m, 2H, Ar*H*), 7.22-7.51 (m, 22H, Ar*H*), 7.09-7.20 (m, 4H, Ar*H*), 6.85-6.95 (m, 2H,), 5.35-5.42 (m, 1H, α-C*H*), 5.13 (s, 2H, PhCH_2O-), 5.12 (s, 2H, PhCH_2O-), 5.10 (s, 2H, PhCH_2O-), 5.09 (s, 2H, PhCH_2O-), 4.51 and 4.46 (d each (rotamers), *J*=6 Hz, 1H, β-C*H*), 3.13-3.35 (m, 8H, CH_2-N), 1.23-1.71 (m, 6H partially obscured, CH_2-), 1.43 and 1.40 (d each (rotamers), 3H, *J*=6 Hz, CH_3-); m/e (FAB) 982 $[M+H]^+$ (3.42%), 981 $[M]^+$ (4.75%), 203 (1.82%), 91 $[C_7H_7]^+$(100%).

L-N-[3-(2,3-Dihydroxybenzamido)propyl]-N-[4-(2,3-dihydroxybenzamido)butyl]-2-(2-hydroxyphenyl)-trans-5-methyloxazoline-4-carboxamide. (Parabactin) (1)

Moistened 10% palladium (II) hydroxide on charcoal (0.09g) was added to a solution of L-*N*-[3-(2,3-dibenzyloxybenzamido)-propyl]-*N*-[4-(2,3-dibenzyl-oxybenzamido)butyl]-2-(2-hydroxyphenyl)-*trans*-5-methyloxazoline-4-carboxamide. (0.09g, 0.09mmol) in ethanol (3cm^3) and cyclohexene (7cm^3). The solution was stirred at reflux for 3 hours under nitrogen and then the solvent was evaporated *in vacuo*. The residue was chromatographed on silica gel with 10% methanol / dichloromethane as eluent. Finally, the product was recrystallised from ethyl acetate to yield *L-N-[3-(2,3-dihydroxybenzamido)propyl]-N-[4-(2,3-dihydroxy-benzamido)butyl]-2-(2-hydroxyphenyl)-trans-5-methyl-oxazoline-4-carboxamide* as a white solid (0.05g, 0.08mmol.; 88%), m.p. 115-118°C. (lit.[12] m.p. 114-117°C); (Found: M= 620. Required for $C_{32}H_{36}N_4O_9$, M=620.66); ν_{max} (KBr)/cm^{-1} 3348 (O-H), 2940, 2880 (C-H), 1623 (C=N), 1600, 1545, (C=O); δ_H [400MHz FT](4:1 $CDCl_3$ / CD_3OD) [Mixture of rotamers] 7.70 and 7.68 (d each (rotamers), 1H, *J*=7.5 Hz, Ar*H*), 7.41 and 7.39 (dd each (rotamers), 1H, *J*=7.5, 7.5 Hz, Ar*H*), 7.08-7.16 (m, 2H, Ar*H*), 6.90-7.00 (m, 4H, Ar*H*), 6.64-6.77 (m, 2H, Ar*H*), 5.41-5.49 (m, 1H, α-C*H*), 4.67 and 4.61 (d each (rotamers), 1H, *J*=6Hz, β-C*H*), 3.30-3.68 (m, 8H, CH_2-N), 1.60-1.85 (m, 6H, CH_2-), 1.55 and 1.46 (d each (rotamers), 3H, *J*=6Hz, CH_3-); δ_C [100MHz FT]($CDCl_3$) 170.49, 170.06, 169.45, 166.48, 159.36, 149.20, 149.13, 145.91, 145.72, 134.09, 128.70, 119.36, 118.64, 118.16, 117.87, 116.68, 116.61, 116.27, 114.23, 113.97, 110.61, 77.98, 72.55, 47.47, 42.63, 39.09, 35.78, 27.24, 26.55, 26.47, 20.22; m/e (FAB) 621 $[M+H]^+$ (5.04%), 620 $[M]^+$ (1.49%), 417 (1.11%), 203 (2.57%).

General procedure for coupling protected aminoacids to N,N-bis-(2,3-dibenzyloxybenzoyl)-spermidine.- A solution of diphenylphosphoryl azide (0.173g, 0.63mmol.) in dimethylformamide (5cm^3) was added to a solution of the aminoacid derivative (0.63mmol.) and *N,N-bis*-(2,3-dibenzyloxybenzoyl)-spermidine (0.5g, 0.63mmol.) in dimethylformamide (10cm^3) stirring at 0°C. Triethylamine (0.1cm^3, 0.72mmol.) and dimethylaminopyridine (7mg) were then added and the solution stirred at room temperature under nitrogen for 2 days. The solvent was removed *in vacuo* and the residue dissolved in dichloromethane (20cm^3). The organic solution was washed with aqueous hydrochloric acid (2M) (10cm^3), saturated aqueous sodium hydrogen carbonate (10cm^3), water (10cm^3) and brine (5cm^3). The organic solution was then dried, filtered and the filtrate evaporated *in vacuo*. The residue was chromatographed on silica gel with 1:1 ethyl acetate / hexane as eluent to yield the desired products.

In this way the following were prepared:-

N-[3-(2,3-dibenzyl-oxybenzoylamino)propyl]-N-[4-(2,3-dibenzyloxybenzoyl-amino)-butyl]-2-benzyloxybenzoylglycylamide (**12a, R^2=Bn**) as a colourless oil (82%); (Found: C, 74.5%; H, 6.3%; N, 5.1%; [(M+Na]=1068. Required for $C_{65}H_{64}N_4O_9$: C, 74.7%; H, 6.2%; N, 5.4%; M=1045); ν_{max} ($CHCl_3$)/cm^{-1} 3380 (N-H), 2992, 2935, 2866 (C-H), 1651 (C=O), 1600, 1456; δ_H [250MHz FT]($CDCl_3$) [Mixture of rotamers] 8.15-8.19 (dd, 1H, *J*=8 and 2Hz Ar*H*), 7.97-8.07 (m, 2H, N*H*CO–), 7.62-7.74 (m, 2H, Ar*H*), 6.91-7.49 (m, 33H, Ar*H* and N*H*CO-), 5.04-5.36 (m, 10H, PhCH_2O-), 4.18 and 4.14 (d each, *J*=4 Hz, 2H, α-CH_2-), 2.99-3.30 (m, 8H, CH_2-N), 1.26-1.61 (m, 6H, CH_2-); m/e (FAB) 1068 [M+Na]$^+$ (1.67%), 778 (0.7%), 268 (2.42%), 91 [C_7H_7]$^+$ (100%).

N-[3-(2,3-Dibenzyloxybenzoylamino)propyl]-N-[4-(2,3-dibenzyloxybenzoylamino)butyl]-2-benzyloxybenzoylalanylamide (**12b R^2=Bn**) as a colourless gum; (Found: C, 74.5%; H, 6.3%; N, 5.1%; [M+Na]=1082. Required for $C_{66}H_{66}N_4O_9$: C, 74.8%; H, 6.3%; N, 5.3%; M=1058); ν_{max} ($CHCl_3$)/cm^{-1} 3378 (N-H), 2992, 2940, 2865 (C-H), 1651 (C=O), 1600, 1497, 1575 ; δ_H [80MHz FT]($CDCl_3$) [Mixture of rotamers] 8.48-8.70 (d, 1H, *J*=5.5 Hz, N*H*CO-), 7.80-8.30 (m, 2H, N*H*CO–), 6.85-7.80 (m, 35H, Ar*H*), 5.07 (s, 10H, PhCH_2O-), 4.70-4.90 (m, partially obscured, 1H, α-C*H*), 2.80-3.55 (m, 8H, CH_2-N), 0.90-1.80 (m, 9H, CH_2- and CH_3-); m/e (FAB) 1082 [M+Na]$^+$ (1.12%), 1059 [M+H]$^+$(0.31%), 778 (2.31%), 282 (10%), 91 [C_7H_7]$^+$ (100%).

N-[3-(2,3-Dibenzyloxybenzoylamino)propyl]-N-[4-(2,3-dibenzyloxybenzoylamino)butyl]-2-benzyloxybenzoyl-L-valylamide (**12c. R^2=Bn**) as a colourless gum (78%); (Found: C, 74.9%; H, 6.3%; N, 5.0%; [M+Na]=1110. Required for $C_{68}H_{70}N_4O_9$: C, 75.1%; H, 6.5%; N, 5.2%; M=1087); ν_{max} ($CHCl_3$)/cm^{-1} 3380 (N-H), 2942, 2869 (C-H), 1651 (C=O), 1600, 1496, 1575; δ_H [80MHz FT]($CDCl_3$) [Mixture of rotamers] 8.53-8.63 (m, 1H, N*H*CO-), 6.96-8.20 (m, 37H, N*H*CO- and Ar*H*), 5.08 (s, 6H, PhCH_2O-), 5.04 (s, 4H, PhCH_2O-), 4.80-4.95 (m partially obscured, 1H, α-C*H*), 2.90-3.50 (m, 9H, b-C*H* and CH_2-N), 1.10-1.70 (m, 6H, CH_2-), 0.64-0.95 (m (rotamers), 6H, CH_3-); m/e (FAB) 1125 [M+K-H]$^+$(1.8%), 1110 [M+Na]$^+$ (1%), 1109 [M+Na-H]$^+$(0.34%), 778 (2.44%), 310 (2.04%), 91 [C_7H_7]$^+$ (100%).

N-[3-(2,3-Dibenzyloxybenzoylamino)propyl]-N-[4-(2,3-dibenzyl-oxybenzoylamino)butyl]-2-benzyloxybenzoyl-phenylalanylamide (**12d, R^2=Bn**) as a colourless gum (99%); (Found: C, 74.4%; H, 6.3%; N, 5.0; [M+H]=1136. Required for $C_{72}H_{70}N_4O_9.H_2O$: C, 75.0%; H, 6.3%; N, 4.9%; M=1135); ν_{max} ($CHCl_3$)/cm^{-1} 3389 (N-H), 2942 (C-H), 1644 (C=O), 1600, 1575, 1106 (C-O); δ_H [80MHz FT]($CDCl_3$) [Mixture of rotamers] 7.58-8.55 (m, 3H, N*H*CO-), 6.80-7.50 (m, 40H, Ar*H*), 5.10 (s, 6H, PhCH_2O-), 5.05 (s, 4H, PhCH_2O-), 4.80-5.00 (m, 1H, α-C*H*), 2.71-3.33 (m, 10H, CH_2-N and PhCH_2-), 0.81-1.50 (m, 6H, CH_2-); m/e (FAB) 1136 [M+H]$^+$(0.1%), 776 (0.5%), 358

(1.0%), 91 $[C_7H_7]^+$ (100%).

N-[4-(2,3-Dibenzyloxybenzoyl-amino)butyl]-2,3-dibenzyloxybenzoylglycylamide[16b] (**13a, R^2=Bn**) as a colourless gum (84%); (Found: M=1151. Calculated for $C_{72}H_{70}N_4O_{10}$: M=1151); ν_{max} ($CHCl_3$)/cm^{-1} 3375 (N-H), 2928, 2855 (C-H), 1651 (C=O), 1576, 1498, 1576 (N-H), 1311, 1098; δ_H [250MHz FT]($CDCl_3$) 8.55-8.65 (t, 1H, *J*=2Hz, N*H*CO-), 7.97-8.07 (m, 2H, N*H*CO-), 7.55-7.70 (m, 3H, Ar*H*), 7.03-7.49 (m, 36H, Ar*H*), 4.95-5.12 (m, 12H, PhCH_2O-), 3.92-4.03 (d, 2H, *J*=5Hz, α-CH_2-), 2.94-3.23 (m, 8H, CH_2-N), 1.26-1.51 (m, 6H, CH_2-); m/e (FAB) 1151 $[M]^+$ (45%), 1061 $[(M+H)-C_7H_7]^+$(7%), 778 (64%), 374 (53%), 92 $[(C_7H_7)+H]^+$ (91%).

N-[3-(2,3-Dibenzyloxybenzoylamino)propyl]-N-[4-(2,3-dibenzyl-oxybenzoylamino)-butyl]-2,3-dibenzyloxybenzoyl-L-alanylamide (**13b, R^2=Bn**) as a colourless gum (72%); (Found: C, 75.0%; H, 6.2%; N, 4.8%; M =1165. Required for $C_{73}H_{72}N_4O_{10}$: C, 75.2%; H, 6.2%; N, 4.8%; M=1165); ν_{max} ($CHCl_3$)/cm^{-1} 3375 (N-H), 2992, 2935, 2866 (C-H), 1652 (C=O), 1575, 1496, 1575; δ_H [80MHz FT]($CDCl_3$) [Mixture of rotamers] 8.52-8.60 (m, 1H, N*H*CO-), 7.10-8.33 (m, 41H, Ar*H* and N*H*CO–), 5.10 (s, 12H, PhCH_2O-), 4.80-5.00 (m, partially obscured, 1H, α-C*H*), 2.86-3.31 (m, 8H, CH_2-N), 0.98-1.68 (m, 9H, CH_2- and CH_3-); m/e (FAB) 1165 $[M]^+$ (0.15%), 778 (4.72%), 388 (2.26%), 91 $[C_7H_7]^+$ (100%).

N-[3-(2,3-Dibenzyloxybenzoylamino)propyl]-N-[4-(2,3-dibenzyl-oxybenzoylamino)-butyl]-2,3-dibenzyloxybenzoyl-valylamide (**13c, R^2=Bn**) as a colourless gum (72%); (Found: C, 75.2%; H, 6.5%; N, 4.5%; M=1193. Required for $C_{75}H_{76}N_4O_{10}$: C, 75.5%; H, 6.4%; N, 4.7%; M=1193); ν_{max} ($CHCl_3$)/cm^{-1} 3355 (N-H), 2987, 2930, 2886 (C-H), 1649 (C=O), 1595, 1500, 1575; δ_H [80MHz FT]($CDCl_3$) [Mixture of rotamers] 8.40-872 (m, 1H, N*H*CO-), 7.75-8.15 (m, 2H, N*H*CO-), 6.85-7.70 (m, 39H, Ar*H*), 5.10 (s, 6H, PhCH_2O-), 5.08 (s, 6H, PhCH_2O-), 4.75-4.95 (m, 1H, α-C<u>H</u>), 2.80-3.40 (m, 8H, CH_2-N), 1.98-2.05 (m, 1H, β-C*H*), 1.05-1.75 (m, 6H, CH_2-), 0.65-0.95 (m (rotamers), 6H, *J*=7Hz, CH_3-); m/e (FAB) 1193 $[M]^+$ (0.1%), 776 (0.3%), 416 (0.5%), 91 $[C_7H_7]^+$ (100%).

L-N-[3-(2,3-Dibenzyloxy-benzoylamino)propyl]-N-[4-(2,3-dibenzyl-oxybenzoylamino)butyl]-2,3-dibenzyloxybenzoyl-phenylalanylamide (**13d, R^2=Bn**) as a colourless gum (88%); (Found: C, 74.4%; H, 6.2%; N, 4.5%; (M+Na)=1264. Required for $C_{79}H_{76}N_4O_{10}.2H_2O$: C, 74.3%; H, 6.3%; N, 4.4%; M=1241); ν_{max} ($CHCl_3$)/cm^{-1} 3380 (N-H), 2946 (C-H), 1651 (C=O), 1575, 1490, 1575; δ_H [80MHz FT]($CDCl_3$) [Mixture of rotamers] 9.60 (d, 1H, *J*=7Hz, N*H*CO-), 7.8-8.05 (m, 2H, N*H*CO-), 6.82-7.75 (m, 41H, Ar*H*), 5.11 (m partially obscured, 1H, α-C*H*), 5.08 and 5.06 (s, 6H each, PhCH_2O-), 3.10-3.38 (m, 10H, CH_2-N), 2.89-3.08 (m, (rotamers), 2H, PhCH_2-), 0.87-1.63 (m, 6H, CH_2-); m/e (FAB) 1264 [M+Na] (0.28%), 778 (8.03%), 468 (1.17%), 91 $[C_7H_7]^+$ (100%).

N-[3-(2,3-Dibenzyloxybenzoylamino)propyl]-N-[4-(2,3-dibenzyl-oxybenzoylamino)butyl]-2,3-dibenzyloxybenzoyl-L-serylamide (**13e, R^2=Bn**)as a colourless gum (89%); (Found: C, 74.4%; H, 6.3%; N, 5.1%; [M+Na]=1204. Required for $C_{31}H_{36}N_4O_{11}$: C, 74.2%; H, 6.1%; N, 4.7%; M=1181).(Found: M+Na=1204. Required for $C_{73}H_{72}N_4O_{11}$: M=1181); ν_{max} ($CHCl_3$)/cm^{-1} 3455 (O-H), 1645 (C=O), 1601, 1495, 1568 (N-H); δ_H [80MHz FT]($CDCl_3$) [Mixture of rotamers] 8.65-8.75 (t, 1H, *J*=2Hz, N*H*CO-), 7.90-8.15 (m, 2H, N*H*CO-), 6.90-7.85 (m, 39H, Ar*H*), 5.00-5.15 (m, 12H, PhCH_2O-), 4.50-4.55 (m, 1H, α-C*H*), 3.91-4.15 (broad s, 1H, O*H*), 3.65-3.80 (m, (rotamers), 2H,

CH_2-O*H*), 2.80-3.40 (m, 8H, CH_2-N), 1.10-1.60 (m, 6H, CH_2-); m/e (FAB) 1204 $[M+Na]^+$ (0.15%), 777 (0.5%), 405 (0.4%), 91 $[C_7H_7]^+$ (100%).

General procedure for debenzylation of catecholamides (**12, R^2=Bn and 13, R^2=Bn**)
Moistened 10% palladium (II) hydroxide on charcoal (8mg) was added to a solution of the perbenzyl ether(0.08g, 0.077mmol.) in ethanol ($5cm^3$) and cyclohexene ($2cm^3$). The solution was stirred at reflux for 3 hours under nitrogen and the solvent was evaporated *in vacuo*.The residue was chromatographed on silica gel with 10% methanol / dichloromethane as eluent. The product was recrystallised from ethyl acetate to yield the free phenolic product. In this way the following were prepared:-

3-(2,3-Hydroxybenzoylamino)propyl]-N-[4-(2,3-dihydroxy-benzoylamino)butyl]-2-hydroxybenzoyl-glycylamide[17] (**12a, R^2=H**) as a white solid (47%), m.p. 98-100°C; (Found: C, 59.0%; H, 5.9%; N, 9.1%; M+H=595. Required for $C_{30}H_{34}N_4O_9.H_2O$: C, 58.8%; H, 5.9%; N, 9.1%; M=594); ν_{max} (KBr)/cm^{-1} 3378 (O-H, N-H), 2924 (C-H), 1634 (C=O), 1596, 1490, 1543; δ_H [400MHz FT](d_4 Methanol) 7.81-7.84 (m, 1H, Ar*H*), 7.34-7.39 (m, 1H, Ar*H*), 7.18-7.23 (m, 2H, Ar*H*), 6.87-6.93 (m, 4H, Ar*H*), 6.65-6.93 (m, 2H, Ar*H*), 4.29 (s, 2H, α-CH_2-), 3.32-3.50 (m, 8H, CH_2-N), 1.60-2.02 (m, 6H, CH_2-); δ_C [100MHz FT](d_4Methanol) 171.72, 171.59, 171.52, 170.69, 170.49, 170.21 (*C*), 160.67, 150.36, 150.25, 147.35 (*C*), 134.86, 129.85, 120.26, 119.62, 118.79, 118.68, 118.61, 118.29 (*C*H) 117.25, 116.82, 116.69 (*C*) 48.02, 46.99, 46.10, 44.83, 42.31, 42.26, 40.00, 39.84, 38.02, 37.85, 29.55, 27.71, 26.91, 25.96 (CH_2); m/e (FAB) 595 $[M+H]^+$ (7%), 418 (9%), 91 $[C_7H_7]^+$ (100%).

3-(2,3-Dihydroxybenzoylamino)-propyl]-N-[4-(2,3-dihydroxy-benzoylamino)butyl]-2-hydroxybenzoylalanylamide (**12b R^2=H**) as a white solid (82%), m.p. 103-105°C; (Found: C, 60.4%; H, 6.2%; N, 9.0%; (M+Na) = 632. Required for $C_{31}H_{36}N_4O_9$: C, 61.2%; H, 6.0%; N, 9.2%; M= 609); ν_{max} (KBr)/cm^{-1} 3374 (O-H, N-H), 2938 (C-H), 1636 (C=O), 1595, 1489, 1543; δ_H [400MHz FT](d_4 Methanol) [Mixture of rotamers] 7.83-7.87 (m, 1H, Ar*H*), 7.33-7.38 (m, 1H, Ar*H*), 7.17-7.23 (m, 2H, Ar*H*), 6.86-6.93 (m, 4H, Ar*H*), 6.64-6.71 (m, 2H, Ar*H*), 4.99-5.05 (q, 1H, *J*=7Hz, α-C*H*), 3.30-3.61 (m, 8H, CH_2-N), 1.60-2.15 (m, 6H, CH_2-), 1.43 and 1.40 (d each (rotamers), 3H, *J*=7Hz, CH_3); δ_C [100MHz FT](d_4Methanol) 175.21, 174.73, 171.87, 171.70, 171.59, 169.75 (*C*), 160.48, 150.35, 147.36 (*C*), 134.87, 129.94, 120.33, 119.64, 118.72, 118.65, 118.60, 118.21 (*C*H), 117.25, 116.76, 116.75 (*C*), 47.25 (*C*H), 47.01, 46.80, 44.91, 40.06, 39.74, 38.05, 37.70, 29.90, 28.49, 27.69, 27.61, 27.24, 25.94 (CH_2), 18.42, 18.26 (CH_3); m/e (FAB) 632 $[M+Na]^+$ (5.02%), 418 (10.18%), 192 (6.47%).

3-(2,3-Dihydroxybenzoylamino)propyl]-N-[4-(2,3-dihydroxy-benzoylamino)butyl]-2-hydroxybenzoyl-L-valylamide (**12c, R^2=H**) as a white solid (69%), m.p. 96-98°C; (Found: C, 60.5%; H, 6.7%; N, 9.6%; (M+Na) =660. Required for $C_{33}H_{40}N_4O_9.H_2O$: C, 60.5%; H, 6.5%; N, 8.6%; M=637); ν_{max} (KBr)/cm^{-1} 3366 (O-H, N-H), 2963, 2872 (C-H), 1638 (C=O), 1596, 1490, 1544; δ_H [400MHz FT](d_4Methanol) [Mixture of rotamers] 7.71-7.90 (m, 1H, Ar*H*), 7.17-7.38 (m, 3H, Ar*H*), 6.81-6.93 (m, 4H, Ar*H*), 6.65-6.73 (m, 2H, Ar*H*), 4.65-4.95 (m, 1H, partially obscured, α-C*H*), 3.24-3.66 (m, 8H, CH_2-N), 2.02-2.25 (m, 1H, β-C*H*), 1.62-2.01 (m, 6H, CH_2-), 0.91-1.00 (m (rotamers), 6H, CH_3-); δ_C [100MHz FT](d_4Methanol) 172.9, 172.6, 170.80, 169.0 (*C*), 159.8, 158.7, 158.3, 149.58, 146.58 (*C*), 133.98, 129.85, 128.62, 119.80, 119.48, 118.83, 117.70, 1117.54, 117.25 (*C*H), 115.89, 116.0 (*C*), 64.32 (*C*H), 46.83, 46.28, 44.71, 44.23, 39.11, 37.07, 36.95, 32.94, 28.47, 27.73, 27.03, 26.83, 25.82, 25.22, (CH_2), 18.84, 18.55 (CH_3); m/e (FAB) 675 $[M+K]^+$ (3.47%), 660 $[M+Na]^+$(3.84%), 418 (5.44%), 220 (2.87%).

3-(2,3-Hydroxy-benzoylamino)propyl]-N-[4-(2,3-dihydroxybenzoylamino)butyl]-2-hydroxy-benzoyl-L-phenylalanylamide as a white solid (**12d, R^2=H**) (83%), m.p. 98-100°C; (Found: C, 64.4%; H, 6.3%; N, 7.9%; (M+Na) =707. Required for $C_{37}H_{40}N_4O_9$: C, 64.9%; H, 5.9%; N, 8.2%; M=684); ν_{max} (KBr)/cm^{-1} 3361 (O-H, N-H), 2934 (C-H), 1638 (C=O), 1600, 1490, 1542; δ_H [400MHz FT](d$_4$Methanol) [Mixture of rotamers] 7.82-7.89 (m, 1H, Ar*H*), 7.32-7.38 (m, 1H, Ar*H*), 7.08-7.28 (m, 7H, Ar*H*), 6.84-6.96 (m, 4H, Ar*H*), 6.64-6.74 (m, 2H, Ar*H*), 5.20-5.28 (m (rotamers), 1H, α-C*H*), 3.00-3.55 (m, 10H, PhCH_2- and CH_2-N), 1.52-1.97 (m, 6H, CH_2-); δ_C [100MHz FT](d$_4$Methanol) 171.62, 170.09, 167.69, 162.35, 156.59, 151.26, 147.83, 146.33 (*C*), 132.54, 132.02, 129.46, 128.73, 128.22, 127.96, 118.79, 118.15, 117.99, 117.53 , 117.27, 117.06 (*C*H), 116.58 (*C*), 52.35 (*C*H), 47.22, 45.64, 40.01, 38.47, 37.31, 27.46, 27.05, 26.60 (*C*H$_2$); m/e (FAB) 708 [M+H+Na]$^+$ (5.2%), 707 [M+Na]$^+$(12%), 418 (26%), 268 (10%).

N-[4-(2,3-Dihydroxybenzoylamino)butyl]-2,3-dihydroxybenzoylglycylamide[16a] (**13a, R^2=H**) as a white solid (75%), m.p. 101-102°C; (Found: (M+H)=611. Required for $C_{30}H_{34}N_4O_{10}$: M=610); ν_{max} (KBr)/cm^{-1} 3384 (O-H, N-H), 2927 (C-H), 1637 (C=O), 1589, 1487, 1545; δ_H [400MHz FT](d$_4$Methanol) 7.26-7.29 (m, 1H, Ar*H*), 7.18-7.22 (m, 2H, Ar*H*), 6.89-6.95 (m, 3H, Ar*H*), 6.65-6.75 (m, 3H, Ar*H*), 4.28 (d, 2H, *J*=6Hz, CH_2-C=O), 3.32-3.51 (m, 8H, CH_2-N), 1.59-1.88 (m, 6H, CH_2-); δ_C [100MHz FT](d$_4$Methanol) 171.44, 171,62, 171.27, 170.72, 170.23 (*C*), 150.36, 150.26, 149.99, 147.36 (*C*), 119.84, 119.77, 119.30, 118.77, 118.69, 118.63 (*C*H), 116.9, 116.74 (*C*), 48.04, 47.01, 46.12, 44.85, 42.23, 42.16, 40.00, 39.84, 39.03, 37.87, 29.59, 28.49, 27.72, 26.93, 25.98 (*C*H$_2$); m/e (FAB) 612 [M+2H]$^+$ (13.8%), 611 [M+H]$^+$ (5.3%), 610 [M]$^+$(1.2%), 417 (1.3%), 193 (11.5%).

3-(2,3-Hydroxybenzoylamino)propyl]-N-[4-(2,3-dihydroxybenzoylamino)butyl]-2,3-dihydroxy-benzoyl-L-alanylamide (**13b, R^2=H**) as a white solid (67%), m.p. 108-110°C; (Found: C, 58.0%; H, 6.1%; N, 8.6%; (M+H)=625. Required for $C_{31}H_{36}N_4O_{10}.H_2O$: C, 57.9%; H, 6.0%; N, 8.7%; M=624); ν_{max} (KBr)/cm^{-1} 3374 (O-H , N-H), 2936 (C-H), 1637 (C=O), 1595, 1490, 1545; δ_H [400MHz FT] (d$_4$Methanol) [Mixture of rotamers]7.17-7.34 (m, 4H, Ar*H*), 6.87-6.94 (m, 2H, Ar*H*), 6.64-6.73 (m, 3H, Ar*H*), 4.97-5.03 (d, 1H, *J*=7Hz, α-C*H*), 3.25-3.62 (m, 8H, CH_2-N), 1.59-1.98 (m, 6H, CH_2-), 1.42 and 1.39 (d each (rotamers), 3H, *J*=7Hz, CH_3-); δ_C [100MHz FT](d$_4$Methanol) 175.20, 174.73, 171.71, 171.60, 170.50 (*C*), 150.36, 149.84, 147.39, 147.23 (*C*) 130.26, 124.46, 121.35, 119.75, 119.63, 118.63 (*C*H), 116.95, 116.72 (*C*), 47.27 (CH) 47.03, 46.79, 44.91, 40.06, 39.73, 38.05, 37.71, 29.91, 28.51, 27.72, 27.64, 27.23, 25.95 (*C*H$_2$), 18.27, 18.12 (*C*H$_3$); m/e (FAB) 647 [M+Na]$^+$ (9.8%), 625 [M+H]$^+$ (3.3%), 418 (8.7%), 207 (10.8%).

3-*(2,3-Dihydroxybenzoylamino)-propyl]-N-[4-(2,3-dihydroxybenzoylamino)butyl]-2,3-dihydroxybenzoyl-L-valylamide* (**13c, R^2=H**) as a white solid (0.08g, 73%), m.p. 91-93°C; (Found: (M+Na)=675. Required for $C_{33}H_{40}N_4O_{10}$: M=652); ν_{max} (KBr)/cm^{-1} 3372 (O-H, N-H), 2962,2871 (C-H), 1638 (C=O), 1596, 1489, 1543; d_H [400MHz FT](d$_4$Methanol) [Mixture of rotamers] 7.31-7.36 (m, 1H, Ar*H*), 7.15-7.29 (m, 3H, Ar*H*), 6.90-7.09 (m, 3H, Ar*H*), 6.61-6.77 (m, 2H, Ar*H*), 4.45-4.59 (m, 1H, a-C*H*), 3.21-3.67 (m, 8H, CH_2-N), 2.11-2.25 (m (rotamers), 1H, b-C*H*), 1.44-2.04 (m, 6H, CH_2-), 1.00 and 0.99 (d each (rotamers), 6H, *J*=7Hz, CH_3-); δ_C [100MHz FT](d$_4$Methanol) 173.7, 173.5, 171.59, 170.00, 150.39, 147.37, 147.18, 149.1 (*C*), 121.65, 120.66, 120.16, 120.05, 119.96, 119.51, 119.43, 118.67 (*C*H), 116.72, 111.74 (*C*),59.97, 55.69 (*C*H), 47.11, 45.06, 40.02, 39.65, 37.91, 37.73,(*C*H$_2$), 32.62, 32.11 (*C*H), 30.19, 28.54, 27.55, 27.58, 26.88 (*C*H$_2$), 18.64, 19.89 (*C*H$_3$); m/e (FAB) 714 [M+K+Na]$^+$ (1.8%), 676 [M+Na+H]$^+$(1.0%), 418 (4.8%), 236 (3.5%).

3-(2,3-Hydroxy-benzoylamino)propyl]-N-[4-(2,3-dihydroxybenzoylamino)butyl]-2,3-dihydroxybenzoyl-L-phenylalanylamide (**13d, R^2=H**) as a white solid (0.15g, 83%), m.p. 95-97°C; (Found: C, 62.2%; H, 6.0%; N, 7.7%; (M+Na)=723. Required for $C_{37}H_{40}N_4O_{10}.H_2O$: C, 61.8%; H, 5.9%; N, 7.8%; M=700); ν_{max} (KBr)/cm^{-1} 3360 (O-H, N-H), 2934 (C-H), 1635 (C=O), 1596, 1490, 1540; δ_H [400MHz FT](d$_6$DMSO) [Mixture of rotamers] 8.98 (s, 1H, broad, O*H*), 8.72 (s, 3H, broad, O*H*), 7.92-7.94 (d, 1H, *J*=7Hz, N*H*CO-), 7.07-7.38 (m, 10H, Ar*H* and N*H*CO), 6.86-6.99 (m, 4H, Ar*H*), 6.63-7.69 (m, 2H, Ar*H*), 5.11 (m (rotamers), 1H, a-C*H*), 3.15-3.72 (m, 8H, CH_2-N), 2.89-3.08 (m (rotamers), 2H, PhCH_2-), 1.32-1.97 (m, 6H, CH_2-); δ_C [100MHz FT](d$_6$DMSO) 170.58, 170.31, 169.90, 169.66, 169.59, 167.18, 167.08, 158.67, 149.68, 146.15, 137.25, 137.08 (*C*), 133.48, 129.71, 129.44, 129.13, 129.09, 128.97, 128.77, 128.54, 128.15, 128.07, 126.47, 126.41, 125.77, 124.22, 121.99, 119.86, 119.81, 118.99, 118.65, 117.75, 117.70, 117.04 (*C*H), 50.63, 50.73 (CH), 46.79, 45.03, 43.40, 41.86, 38.61, 37.91, 36.61, 28.45, 27.13, 26.21, 26.05, 24.56 (*C*H$_2$); m/e (FAB) 723 [M+Na]$^+$ (0.2%), 418 (16.7%), 282 (7.07%).

3-(2,3-Hydroxybenzoylamino)propyl]-N-[4-(2,3-dihydroxy-benzoylamino)butyl]-2,3-dihydroxybenzoyl-L-seryl-amide (**13e, R^2=H**) as a white solid (83%), m.p. 128-130°C; (Found: C, 58.0%; H, 6.1%; N, 8.6%; [(M+H)-central portion]=418. Required for $C_{31}H_{36}N_4O_{11}$: C, 58.1%; H, 5.7%; N, 8.8%; M=640); ν_{max} (KBr)/cm^{-1} 3379 (O-H, N-H), 2938 (C-H), 1638 (C=O), 1589, 1488, 1546; δ_H [400MHz FT](d$_4$Methanol) [Mixture of rotamers] 7.13-7.14 (d, 2H, *J*=7Hz, Ar*H*), 6.83-6.85 (d, 2H, *J*=7Hz, Ar*H*), 6.61-6.32 (d, 2H, *J*=7Hz, Ar*H*), 4.01 (d, *J*=7 Hz, 1H, α-C*H*), 3.10-3.34 (m, 8H, CH_2-N), 2.50-2.52 (m (rotamers), 2H, CH_2-O), 1.48-1.77 (m, 6H, CH_2-); δ_C [100MHz FT](d$_4$Methanol) 171.49, 171.45, 150.42, 150.19, 149.51, 147.25 (*C*), 120.35, 120.13, 119.84, 119.76, 119.64, 119.90, 118.76, 118.70, 118.45, 116.73, 116.51 (*C*H), 61.53 (*C*H$_2$), 50.77 (CH), 47.53, 46.57, 40.06, 39.87, 37.84, 27.84, 27.73, 27.59 (*C*H$_2$); m/e (FAB) 418 (2.5%), 223 (1.1%), 91 [C_7H_7]$^+$ (9.4%).

ACKNOWLEDGEMENTS

We thank Zeneca Agrochemicals for financial support and Dr J.M. Clough (Zeneca Agrochemicals) for his valuable interest. We also thank Dr P Williams (Department of Pharmaceutical Sciences, University of Nottingham), for his advice and cooperation in setting up growth promotion measurements with *Paracoccus denitrificans*.

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(*Received in UK* 3 *August* 1994; *accepted* 19 *August* 1994)