Organic Process Research & Development **2000**, *4,* 259–263

Preparation of the Key Intermediate in a Novel Synthesis of ZD9063P: The Chemical Component of ADEPT, a Targeted Cytotoxic Therapy

David M. G. Martin* and Pawel S. Siedlecki

Process Research and Development Department, AstraZeneca, Silk Road Business Park, Charter Way, Macclesfield, Cheshire SK10 2NA, U.K.

Abstract:

A novel regioselective opening of the cyclic anhydride of a urethane derivative of glutamic acid using 4-(dimethylamino)-pyridine (DMAP) as the catalyst has greatly simplified the synthesis of the target compound, ZD9063P, 5-(*N*-[(*S*)-*N*-{*N*,*N*-bis(2-chloroethyl)amino}phenoxycarbonyl)-γ-glutamyl]amino)-isophthalic acid.

Introduction

The use of cytotoxic drugs is limited by their general toxicity and a highly desirable aim is the targeted delivery of the active cytotoxic agent. A novel approach under development is the dosing of the patient with an anti-body/enzyme complex which binds specifically at the site of the tumour. Subsequent treatment with a synthetic compound, the pro-drug, which bears a masked cytotoxic entity and has been specifically designed to be cleaved by the enzyme, delivers the cytotoxic agent, the drug, at the site of the tumour.

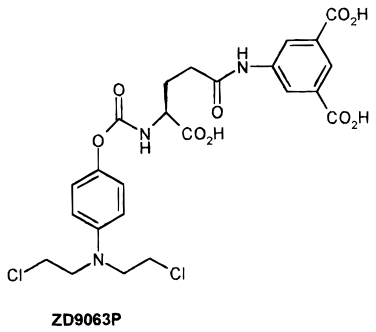
AstraZeneca has an **a**nti-body **d**irected **e**nzyme **p**ro-drug **t**herapy (**ADEPT**) program in progress, and this contribution describes improvements made in the synthesis of the pro-drug, ZD9063P (Figure 1).

Background

The original synthesis of ZD9063P involved the coupling of a differentially substituted derivative of (*S*)-glutamic acid **1** with the dibenzylester of 5-aminoisophthalic acid **2** to give the corresponding amide **3**.[1] Deprotection of the α-amino group and its subsequent activation with triphosgene gave **4**. This was condensed with **5** under phase transfer conditions to give **6** which was chlorinated to give **7**. This product, **7**, was hydrogenated and ZD9063P isolated as an amorphous solid (Scheme 1).

This synthetic approach was not ideal even though it was used to manufacture a kilogram of ZD9063P. The route involved the use of a capricious method for the construction of the key urethane linkage in **6**, and subsequent chlorination of the hydroxyl groups gave **7** which possessed physical properties offering little scope for purification via standard crystallisation techniques. Indeed, the product required chromatographic purification to remove impurities generated during formation of the urethane linkage.

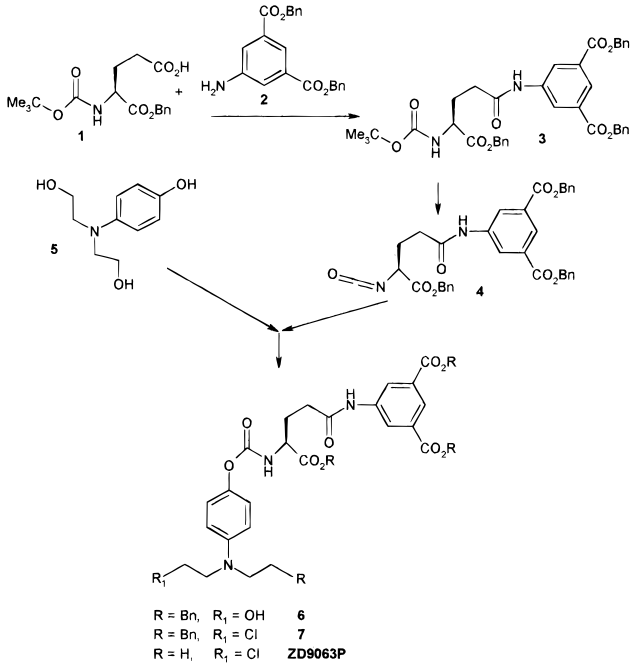
The incorporation of the dichloro analogue of **5** is not a practical option in avoiding the late-stage chlorination reaction as this compound, a nitrogen mustard, is the actual cytotoxic agent and would pose very significant containment problems if used on a manufacturing scale.



ZD9063P

Figure 1.

Scheme 1. **Initial route to ZD9063P**



Proposed Approach for a New Synthetic Route

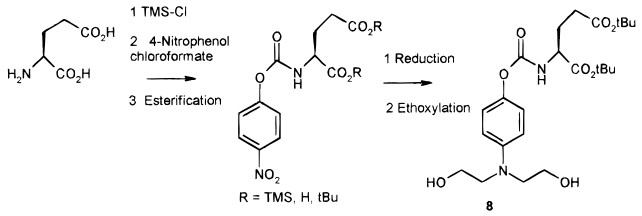
Significant quantities of a related molecule **8**, possessing the same basic aryl urethane derivative of (*S*)-glutamic acid as ZD9063P, were available and it seemed opportune to reinvestigate the choice of synthetic route to ZD9063P (Scheme 2).

The two key transformations required are the conversion of the hydroxyl groups to chloro groups and the regioselec-

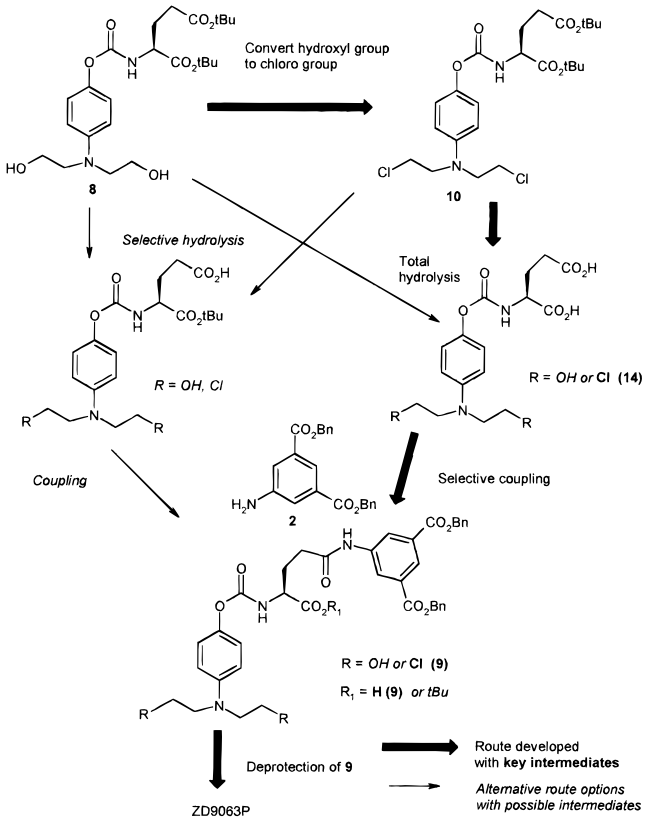
(1) Lee, S. A. Personal communication.

10.1021/op000016+ CCC: $19.00
Published on Web 05/23/2000

Scheme 2. **Original synthesis of the related derivative of (*S*)-glutamic acid**



Scheme 3. **Options for the key transformations**



tive coupling of the dibenzyl ester of 5-aminoisophthalic acid with the glutamic acid residue giving a compound **9** which can be converted to ZD9063P by catalytic hydrogenation. The key decision is the order in which the chlorination reaction and the introduction of the dibenzyl ester of 5-aminoisophthalic acid moiety are carried out (Scheme 3).

Initially, the partial hydrolyses of **8** and of its dichloro-analogue **10** were investigated to provide differentially protected derivatives of glutamic acid. Trifluoroacetic acid selectively cleaved the less hindered ester group, but it was not possible to obtain solution yields of the desired regioisomer of greater than about 50% because further de-esterification continually occurred.[2]

The physical characteristics of the chlorinated species as well as the desire to avoid the conversion of the hydroxyl group to the chloro group at a late stage in the synthesis suggested that a dichloro derivative should be chosen as the key starting material for coupling with the dibenzyl ester of 5-aminoisophthalic acid **2**. In addition, approaches based on the opening of the corresponding cyclic anhydride were considered to be more selective than activation of the acyclic system with, for example, a chloroformate ester.

Control of the regioselective opening of anhydrides of glutamic acid derivatives by methanol in the presence of triethylamine containing varying amounts of DMAP (4-dimethylaminopyridine) has been described. The ratio of regioisomers is reversed from about 1:7 to about 7:1 α/γ by the addition of DMAP.[3]

Regioselective opening of the anhydrides of *phthaloyl* derivatives of glutamic acid with amines has been described leading to the γ-isomer, and specific reference was made to the opening of the anhydrides of *urethane* derivatives of the anhydride of glutamic acid with ammonia leading to predominantly the α-isomer.[4] The regioselectivity of reaction with amines has also been controlled in urethane derivatives of glutamic and aspartic acid anhydrides by the choice of reaction solvent.[5−7] No examples in which DMAP affected the regioselectivity of nucleophilic attack by amines on derivatives of the cyclic anhydrides of glutamic acid have been found.

Model Studies

Derivatives of glutamic acid were used as mechanistic probes to investigate the reaction of anhydrides with the dibenzylester of 5-aminoisophthalic acid **2**.

CBZ-glutamic acid was converted by 1,3-dicyclohexylcarbodiimide to its cyclic anhydride **13** which was reacted with the *p*-toluenesulphonate salt of the dibenzylester of 5-aminoisophthalic acid **2** in the presence of an excess of a tertiary amine. Two products were seen in the reactions involving triethylamine and 4-methylmorpholine, whereas completely unexpectedly, a single product resulted when DMAP was used as the base. The compounds were isolated and shown to be **11** and **12**, the regioisomers from the opening of the anhydride ring system[8] (Scheme 4).

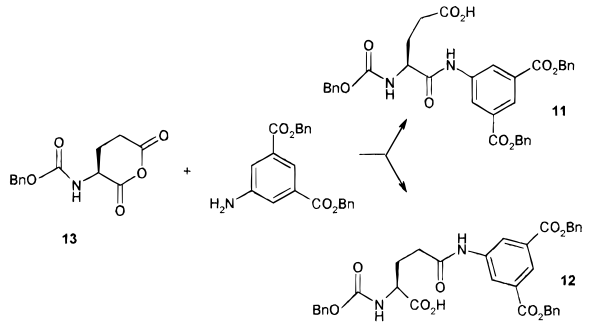
The single compound formed in the DMAP-catalysed reaction corresponded to the more polar regioisomer **12** which possesses the ZD9063P substitution pattern. This key observation means that the basic carbon skeleton of ZD9063P can be assembled without the need for an expensive, differentially protected, glutamic acid derivative.

Racemisation of the chiral centre of the glutamic acid residue is a possible problem with the base-catalysed opening of the anhydride ring. The proposed reaction was modelled

(2) The structure of the major regioisomer of the mixture was assigned by comparison of the chemical shifts in the NMR spectra of the protons adjacent to the carbonyl groups in the glutamic acid residue in the acid and salt forms of the product. Formation of the ammonium salt resulted in an upfield shift of approximately 0.2 ppm in the position of the methylene protons, thus showing them to be adjacent to a carboxylic acid group and not an ester function.

(3) Jouin, P.; Castro, B.; Zeggaf, C.; Pantaloni, A.; Senet, J. P.; Lecolier, S.; Sennyey, G. *Tetrahedron Lett*. **1987**, *28*, 1665.
(4) Sheehan, J. C.; Bolhofer, W. A. *J. Am. Chem. Soc.* **1950**, *72*, 2469.
(5) Cristea, I.; Mager, S.; Batiu, C.; Plé, G. *Rev. Roum. Chim*. **1994**, *39*(12), 1435.
(6) Huang, X.; Luo, X.; Roupioz, Y.; Keillor, J. W. *J. Org. Chem*. **1997**, *62*, 8821.
(7) Ksander, G. M.; Yuan, A. M.; Diefenbacher, C. G.; Stanton, J. L. *J. Med. Chem*. **1985**, *28*, 1606.
(8) The key feature of the structural assignment was a comparison of the NMR spectra with that of CBZ-glutamic acid. The chemical shift of the methine proton in the less polar compound was significantly different from the methine proton in CBZ-glutamic acid, whereas in the more polar compound the significant difference was in the methylene protons.

Scheme 4. **Initial experiment with CBZ-glutamic acid**



using the (−)-menthyloxycarbonyl derivative of (*R*)-glutamic acid because an enantiomeric analytical method for **9** was not initially available. The corresponding anhydride was reacted with the dibenzylester of 5-aminoisophthalic acid in the presence of various tertiary amines and the following results obtained[9,10], (Table 1).

The results demonstrate that a highly nucleophilic but weakly basic amine possesses the desired catalytic activity for the desired regioselective opening of the cyclic anhydride of a glutamic acid derivative by the dibenzyl ester of 5-aminoisophthalic acid **2**.

Development of the Ultimate Manufacturing Route

With the information from the modelling studies, the conversion of **8** to the desired cyclic anhydride was then reinvestigated with a high degree of confidence that the approach would ultimately lead to a practical manufacturing process for ZD9063P (Scheme 5).

The readily available starting material **8** reacts with methanesulphonyl chloride/*N*,*N*-diisopropylethylamine in methylene chloride solution to give an essentially quantitative yield of the corresponding dimesylate. The mesylate groups are displaced by the chloride ion present to give **10,** but the reaction is very slow even at reflux. An alternative solvent is not an attractive option as methylene chloride is the most suitable solvent for obtaining an initial solution of **8**, a necessary requisite for a high chemical conversion to the dimesylate. The solvent used must be compatible with the strongly acidic reaction conditions required for removal of the *tert*-butyl groups as the isolation of **10** as a crystalline solid is not easy and it is more convenient to proceed directly with the deprotection stage. In addition, both **10** and **14** contain the nitrogen mustard system, albeit in a less activated form, and handling of such intermediates would cause concern on a production scale.

The problem of the slow rate of the displacement reaction at reflux in methylene chloride solution (40 °C) was overcome, without introducing a change to a higher boiling solvent, by operating the reaction under pressure. Complete exchange occurs after 18 h at 75 °C, and the pressure generated in the system, about 2 BarG, is entirely consistent with operation in a standard production plant.

The de-esterification of **10** could be readily achieved by the extended reaction of trifluoroacetic acid, but a significant practical problem encountered was the complete removal of the excess trifluoroacetic acid prior to the activation step. A large excess of trifluoroacetic acid is required to displace the equilibrium set up between its *tert*-butyl ester and the corresponding *tert*-butyl ester of the substrate. Complete reaction is normally only achieved by multiple treatments removing volatile substances by distillation, a time-consuming procedure for large scale operation. The *tert*-butyl ester of methanesulphonic acid is unstable, and thus the corresponding equilibrium can be displaced by the formation of isobutylene which escapes from the system.[11] In this way, complete deprotection of **10** to **14** is achieved using only 0.75 mol equiv of methane sulphonic acid (18 h at 40 °C). Practical problems arose because the product precipitated as an oily solid in the presence of methane sulphonic acid. However, neutralisation of the added methane sulphonic acid with an equivalent of DMAP successfully overcame the problem giving a solution with a pH suitable for cyclisation to the anhydride with 1,3-dicyclohexylcarbodiimide.

The corresponding anhydride **15** reacts at −30 °C with the dibenzylester of 5-aminoisophthalic acid **2** in the presence of one equivalent of DMAP. The reaction is largely complete within 2 h.[12]

Although it is possible to isolate the free acid form of **9** from the reaction mixture after work-up by crystallisation of the evaporated residue from an ethyl acetate/cyclohexane solvent mixture, it was simpler to isolate the salt **16** formed with (*S*)-α-methylbenzylamine directly from a mixture of methylene chloride and acetonitrile. Formation of the salt gave considerable purification as well as an opportunity for any necessary subsequent enantiomeric enrichment.[13]

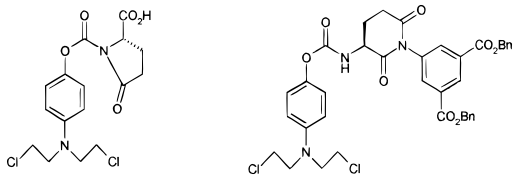
Currently the desired physical form of ZD9063P, a tri-acid, has not been defined, but the structure of **16** has been correlated with ZD9063P by conversion to the corresponding free acid followed by removal of the benzyl groups by catalytic hydrogenation. The resulting tri-acid was identical to an authentic sample of ZD9063P by ^{1}H and ^{13}C NMR and HPLC.[14]

(9) It was not possible to analyse (HPLC or NMR) the anhydride for the presence of the diastereomer to demonstrate the absence of racemisation during the 1,3-dicyclohexylcarbodiimide-mediated ring closure reaction.

(10) The anhydride of menthyloxycarbonyl-(*S*)-glutamic acid was subjected to a similar series of reactions. Comparison with the similar products from the enantiomeric acid allowed the four possible products to be assigned unambiguously on the HPLC trace.

(11) King, J. F.; du Manoir, J. R. *J. Am. Chem. Soc.* **1975**, *97*, 2566.

(12) Two minor by-products are observed.



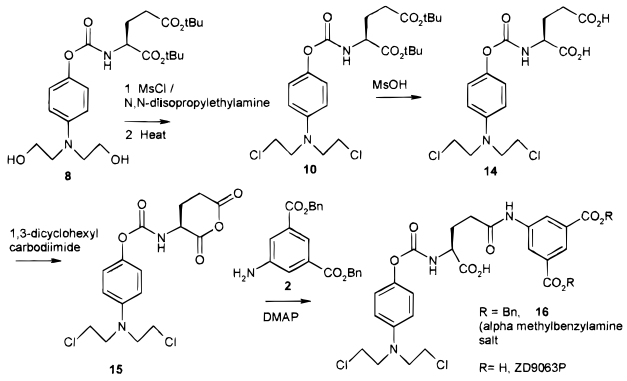
(13) A crystalline product was also obtained in about the same isolated yield with (*R*)-α-methylbenzylamine. It was noticed that the two diastereomeric salts did crystallise at significantly different rates from the same solvent mixture, but it is not known which salt would be more useful in further work on enhancing the purity of the derived ZD9063P.

(14) A sample of the regioisomeric dibenzyl ester was isolated by chromatography and its structure confirmed by NMR. Removal of the benzyl ester groups gave a compound very closely related to, but clearly different from, ZD9063P by HPLC and NMR analysis.

Table 1. **Results of opening anhydride 13 under various conditions (% yields)**

base	desired product	glutamic acid enantiomer of desired product	regioisomer	glutamic acid enantiomer of regioisomer
4-methylmorpholine	50	0	50	0
pyridine	50	0	50	0
triethylamine	25	25	25	25
no additional base	15	0	85	
4-pyrrolidinopyridine	82	16	2	0
DMAP (at + 20 °C)	88	8	4	0
DMAP (at − 30 °C)	96	2	2	0

Scheme 5. **Manufacturing route to ZD9063P**



Conclusions

In conclusion, we have developed a new, efficient, and convergent route to ZD9063P by converting a readily available starting material to a key intermediate suitable for both chemical and enantiomeric purification. The success of the synthetic approach arose from the use of a catalyst to obtain regiospecific opening of an anhydride ring, incorporation of a reaction at an elevated pressure to avoid a solvent exchange, and the development of an improved practical procedure for the deprotection of *tert*-butyl esters

Experimental Section

Reagents were purchased from standard suppliers.

NMR spectra were run at 270 MHz (proton) and at 67.7 MHz (carbon) in d_6-DMSO or d_6-DMSO/TFA solution and are reported in parts per million downfield from internal TMS. The signals assigned to TFA (159.0, q, $J = 60.9$ Hz, 115.3, q, $J = 440$ Hz) are omitted from the description of the ^{13}C spectrum for each compound.

HPLC analyses were conducted using a HiChrome RPB column, solvent system acetonitrile/water/TFA 640/360/1 (v/v/v), flow rate 1 or 2 mL/min and detection at 254λ

(*S*)-(α)-Methylbenzylamine Salt of the Dibenzyl Ester of 5-(*N*-[(*S*)-*N*-{*N*,*N*-Bis(2-chloroethyl)amino}phenoxycarbonyl)-γ-glutamyl]amino)isophthalic Acid (16). A solution of **8** (193 g, 0.40 mole) and *N*,*N*-diisopropylethylamine (124 g, 167 mL, 0.96 mole) in dichloromethane (1.5 L) was protected from atmospheric moisture and stirred at 0 °C. Methane sulphonyl chloride (101 g, 68 mL, 0.878 mol) was added at such a rate so that the reaction temperature remained between 0 and 5 °C. A wash of methylene chloride (200 mL) was added via the dropping funnel. Analysis by HPLC showed greater than 97% AN conversion to the corresponding dimesylate after 2h.[15] The solution was transferred to an autoclave, methylene chloride (460 mL) was added, and the solution was agitated and heated in a sealed vessel for 18 h (jacket temperature 75 °C, pressure generated 1.6 BarG). Analysis by HPLC showed a 97% AN conversion to **10**. A sample was isolated by chromatography for characterisation.

^{1}H NMR 7.95 (d, $J = 10$ Hz, 1H), 6.94, (d, $J = 10$ Hz, 2H), 6.72 (d, $J = 10$ Hz, 2H), 4.0−3.9, (m, 1H), 3.73 (s, 8H), 2.4−2.3 (m, 2H), 2.0−1.9 (m, 1H), 1.9−1.75 (m, 1H), 1.42 (s, 18H)

The cooled reaction mixture was washed with water (570 mL), aqueous citric acid solution (570 mL of 20% w/v), and water (570 mL). The final two separations emulsified slightly. The solution was passed through Whatman 1PS filter paper to remove extraneous water. Methanesulphonic acid (57.7 g, 38.9 mL, 0.60 mol) was added, the solution was stirred and distilled, and 1.0 L of distillate was collected. Methylene chloride (1.0 L) was added, the mixture was heated, and 1.0 L of distillate was collected. Further methylene chloride (0.7 L) was added, and the mixture was heated at reflux for 18 h to give an oily mixture. Analysis of the supernatant solution showed the absence of significant quantities of **10** and any intermediate mono esters. A solution of DMAP (70.8 g, 0.58 mol) in methylene chloride (200 mL) was added slowly. The oil dissolved to give a dark solution, and analysis by HPLC showed >95% AN conversion to the desired product, **14**, present as a salt with DMAP.

The methylene chloride solution of **14** was inerted by a stream of nitrogen, and a solution of 1,3-dicyclohexylcarbodiimide (88.3 g, 0.428 mol) in methylene chloride (220 mL) was added over about 1 h, maintaining a temperature of 5−10 °C. After a further hour, analysis by HPLC showed essentially complete conversion of **14** to the corresponding anhydride **15**. The solution was cooled to −50 °C, and a solution of DMAP (48.8 g, 0.40 mol in methylene chloride 200 mL) was added followed by a solution of the dibenzyl-ester of 5-aminoisophthalic acid (144 g, 0.40 mol) in methylene chloride (800 mL), the temperature being maintained throughout at −50 °C. Periodic analysis of the reaction mixture showed the reaction to be essentially complete after 3 h, and the mixture was allowed to reach ambient temperature overnight. Water (2.5 L) was added, and the mixture was stirred for 1 h and filtered to remove precipitated *N*,*N*^{1}-dicyclohexylurea, and the phases were separated. The organic phase was washed with aqueous citric acid (2 L of 10% w/v)

(15) AN-area normalised.

and water (2 L). The organic solution was filtered through Whatman 1PS filter paper to remove adventitious water and divided into two portions of 1.38 L each.

One portion was distilled to half of the volume at atmospheric pressure, diluted with acetonitrile (1.3 L), and stirred at 40 °C whilst a solution of (*S*)-α-methyl benzylamine (24.2 g, 0.20 mol) in acetonitrile (80 mL) was added. Crystallisation commenced quickly, and the slurry was allowed to cool to ambient temperature over 2−3 h. The product was filtered, washed with 2 × 100 mL methylene chloride/acetonitrile, 1/2 v/v, and dried at 30 °C.

The weight of product **16** was 134 g, 0.154 mol (76.9% yield from **8**).

Recrystallisation of the (α)-Methylbenzylamine Salt of the Dibenzyl Ester of 5-(*N*-[(*S*)-*N*-{*N*,*N*-Bis(2-chloroethyl)-amino}phenoxycarbonyl)-γ-glutamyl]amino)isophthalic Acid (16). The salt **16** (20.0 g, 22.9 mmol) was dissolved in acetonitrile (700 mL) at reflux and cooled to 20 °C. The crystalline product was filtered, washed with acetonitrile (100 mL), and dried at 50 °C. Yield 14.6 g, 16.7 mmol (73.0% yield).

^{1}H NMR 10.68 (s, 1H), 8.54 (s, 2H), 8.18 (s, 1H), 7.90 (d, J = 10 Hz, 1H), 7.52−7.23 (m, 15H), 6.90 (d, J = 10 Hz, 2H), 6.68 (d, J = 10 Hz, 2H), 5.38 (s, 4H), 4.28 (q, J = 10 Hz, 1H), 3.85−3.75 (m, 1H), 3.70 (s, 8H), 2.48−2.37 (m, 2H), 2.20−2.00 (m, 1H), 2.00−1.98 (m, 1H), 1.44 (d, J = 10 Hz, 3H)

^{13}C 173.8, 171.5, 165.1, 155.2, 144.1, 142.5, 140.6, 139.5, 136.2, 130.8, 129.0, 128.9, 128.8, 128.5, 128.4, 127.0, 124.5, 124.0, 122.9, 67.0, 53.8, 52.8, 50.5, 41.2, 33.1, 26.7, 20.8

Calcd for $C_{46}H_{48}N_4O_9Cl_2$: C, 63.37; H, 5.55; N, 6.43, Cl, 8.13. Found: C, 63.34; H, 5.52; N, 6.41; Cl, 8.13.

Quantitative analysis by ^{1}H NMR showed the strength to be 100% relative to an internal standard of maleic acid. Additionally, although quantitative analysis by HPLC showed that recrystallisation increased the strength of the product by only 1−2%, comparison of the ^{1}H spectra showed that low levels of structurally unrelated aliphatic components had been removed.

Dibenzyl Ester of 5-(*N*-[(*S*)-*N*-{*N*,*N*-Bis(2-chloroethyl)-amino}phenoxycarbonyl)-γ-glutamyl]amino)isophthalic Acid (9). The above salt **16** (5.0 g, 5.74 mmol) was partitioned between ethyl acetate (150 mL) and an aqueous solution of citric acid (100 mL of 20% w/v). The resulting organic phase was re-washed with an aqueous solution of citric acid (50 mL of 20% w/v) followed by water (3 × 50 mL). The organic phase was filtered through Whatman 1PS filter paper and the solvent removed under reduced pressure to give the desired product **9** as an amorphous foam. Yield 4.0 g, 5.34 mmol (93% yield).

^{1}H NMR 10.5, (s, 1H), 8.60, (s, 2H), 8.28 (s, 1H), 8.00 (d, J = 10 Hz, 1H), 7.5−7.3 (m, 10H), 6.94, (d, J = 10 Hz, 2H), 6.72 (J = 10 Hz, 2H), 5.38 (s, 4H), 4.2−4.1, (m, 1H), 3.73 (s, 8H), 2.68−2.50 (m, coincides with signal from d^6-DMSO) 2.62−2.50 (m, 2H), 2.32−2.15 (m, 1H), 2.1−1.9 (m, 1H)

^{13}C NMR 173.9, 171.8, 165.1, 155.5, 144.1, 142.5, 140.8, 136.1, 131.2, 128.8, 128.5, 128.2, 124.7, 124.4, 123.0, 112.6, 67.0, 53.8, 53.0, 41.6, 33.0, 27.0

Analysis showed the presence of less than 1% of the (*R*)-enantiomer.[16]

5-(*N*-[(*S*)-*N*-{*N*,*N*-Bis(2-chloroethyl)amino}phenoxy-carbonyl)-γ-glutamyl]amino)isophthalic Acid (ZD9063P). A sample of 10% palladium/carbon (200 mg) was slurry-washed with redistilled THF (20 mL) and transferred to a flask. A solution of **9** (0.30 g, 0.4 mmol) in redistilled THF (50 mL) was added. The slurry was hydrogenated at ambient temperature and pressure. After 18 h, when removal of the protecting groups was complete, the slurry was diluted with redistilled THF (10 mL) and the catalyst removed by filtration. The solvents were removed under vacuum to leave the product as an amorphous solid. Yield 0.18 g, 0.315 mmol (78.9% yield).

^{1}H NMR[17] 10.40, (s, 1H), 8.50, (s, 2H), 8.20, (s, 1H), 8.00 (d, J = 10 Hz, 1H), 7.00 (d, J = 10 Hz, 2H), 6.75 (d, J = 10 Hz, 2H), 4.23−4.08 (m, 1H), 3.73, (s, 8H), 2.65−2.52 (m) overlaps with the d_6-DMSO signal, 2.35, (m, 1H), 2.10−1.95, (m,1H)

^{13}C NMR 173.9, 171.2, 167.2, 155.3, 144.1, 142.6, 140.2, 132.2, 125.2, 124.2, 122.8, 113.0, 54.2, 53.3, 41.7, 33.2, 27.2

Acknowledgment

Simon D. Broady is thanked for helpful comments during the preparation of the manuscript.

Received for review February 16, 2000.

OP000016+

(16) Analysis by chiral HPLC using a CHI-DMB column, solvent system isohexane/2-propanol/TFA (75/25/0.1), flow 1 mL/min and detection at 254 λ. The authors are grateful to Miss Fiona J Bell for this result.

(17) Residual toluene and THF were detected in both spectra.