Pergamon

Tetrahedron Vol. 51, No. 36, pp. 10013-10024, 1995

0040-4020/95 $9.50+0.00

0040-4020(95)00573-0

Photolabile Benzoin and Furoin Esters of a Biologically Active Peptide

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Abstract: Benzoin and furoin esters of *N*-carbobenzyloxyglycylphenylalanine were prepared and photolyzed under a variety of conditions. The photochemistry of peptide-derived benzoin esters is more efficient than that of furoin esters and is appropriate for the photolytic initiation of biochemical processes. Methodology to assess the enantiomeric purity of the resulting free peptide was developed and used to monitor the protection-deprotection chemistry. Synthesis based on alkylation of the cesium salt of the peptide proved more effective than DCCI-mediated chemistry on stereochemical grounds.

INTRODUCTION

Photochemical methods for the initiation of biochemical processes are of continuing interest. For example physiologists have long used photolabile precursors to nucleoside phosphates, metal ions, and protons in studies on single cells.[2,3] Our primary concern is the development of photochemistry to underpin the new methodology of time-resolved X-ray diffraction of proteins.[4] This technique may provide detailed information about the dynamics of enzyme-catalysed processes but the information available from these experiments is dependent on the synchronisation of such processes;[5,6] co-crystallising an enzyme and photoprotected substrate provides a means of simultaneous release of substrate throughout the crystal, triggered by a high-energy pulse of light. Scheidig *et al*[7] have exploited this methodology to observe details of the hydrolysis of guanosine 5'-triphosphate (GTP) to guanosine 5'-diphosphate (GDP) by the H-ras p21 protein.

These, and similar,[8,9] studies have utilised the photolysis of 2-nitrobenzyl, and related, esters.[10] Unfortunately this methodology suffers from serious draw backs. The principal side-products of the photolysis reactions are the electrophilic nitroso carbonyl compounds, 2-nitrosobenzaldehyde from the photolysis of 2-nitrobenzyl esters and 2-nitrosoacetophenone from the photolysis of α-methyl-2-nitrobenzyl esters.[11] These can become especially serious in time-resolved X-ray experiments which require high substrate concentrations and where the conditions often preclude the use of pH changes (the use of low pH to suppress the reactivity of lysine residues) or scavengers (e.g. thiols). In studies on glycogen phosphorylase *b* using the photolabile phosphate precursor α-methyl-*ortho*-nitrobenzylphosphate, the 2-nitrosoacetophenone produced resulted in modifications to the enzyme which were visible in the electron density maps[12] indicating the limitations of this methodology.

The use of benzoin esters[13,14] might circumvent these limitations. Benzoin esters may photolyse via two possible pathways (figure 1). The relative amounts of α-cleavage and acid release are influenced by, amongst other things, leaving group ability and solvent polarity.[15] When the leaving group is the conjugate base of a strong acid photolysis to give the inert side product, 2-phenylbenzofuran, **1**, predominates.

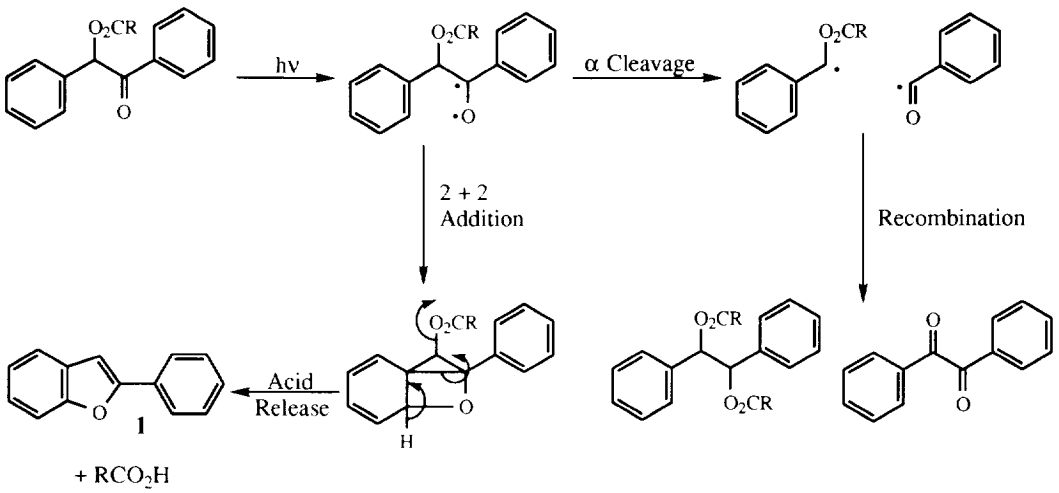
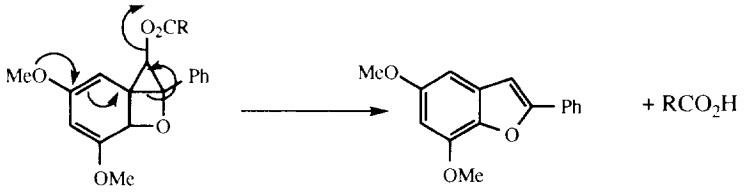


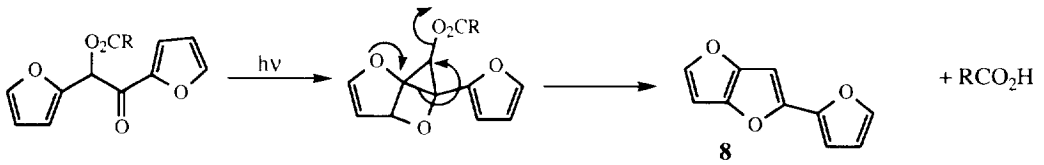
Figure 1 - photolysis pathways of benzoin esters.

The benzofuran is expected to be inert towards proteins. The kinetics of these photochemical processes are known to be favourable for time-resolved experiments.[16,17] Consequently, the unmasking of carboxylate or phosphate[18] groups by photolysis of benzoin esters might be used as the trigger in a kinetic X-ray experiment. Sheehan[13] noted that the photolysis of 3',5'-dimethoxybenzoinyl acetate to liberate acetic acid and 2-phenyl-5,7-dimethoxybenzofuran, **2**, is more facile than that of benzoin acetate. He suggested that in this case electron donation by the methoxy substituents facilitated collapse of a 'hausane' intermediate, **3**.



3 **2**

We decided to investigate the potential of benzoin and furoin esters as protecting groups for the carboxyl terminus of peptides. Furoin esters were perceived as an alternative means of introducing alkoxy groups at appropriate sites of the protecting group, thereby enhancing photolability.



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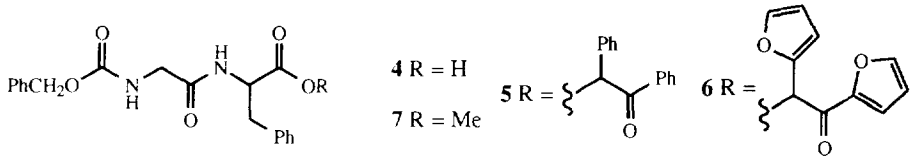
Protection of peptides was chosen as a goal as it relates to a wide variety of biochemical processes. Hence we sought a mild and racemisation-free method of introducing benzoin groups directly into biologically interesting peptides. We chose esters of *N*-carbobenzyloxyglycylphenylalanine (Z-Gly-PheOH, **4**), a substrate of carboxypeptidase A (CPA), as our target. Carboxypeptidase A (CPA) is a zinc dependent metalloenzyme which catalyses the hydrolysis of the carboxyl-terminal amide bond in a polypeptide chain.[19] Binding and

catalysis are dependent upon the peptide possessing a terminal carboxylate function; if this is masked, hydrolysis does not occur. We conceived that release of Z-Gly-PheOH by photolysis of the corresponding benzoin and furoin esters could provide a biologically-compatible means of initiating the enzyme-mediated reaction.

RESULTS AND DISCUSSION

We initially investigated dicyclohexylcarbodiimide (DCCI)-mediated coupling as a means of generating the desired peptide esters. In the absence of catalyst no esterification was observed when Z-Gly-PheOH, **4**, was mixed with benzoin. Inclusion of one equivalent of 4-*N*,*N*-dimethylaminopyridine (DMAP), a known catalyst for related reactions,[20] resulted in quantitative esterification in 48 hours. Chromatography (to remove dicyclohexyl urea) followed by crystallisation gave the desired product **5** as a 2 to 1 mixture of diastereomers. Synthesis of the analogous furoin ester, **6**, was accomplished by the dicyclohexylcarbodiimide (DCCI)-mediated coupling of Z-Gly-PheOH and furoin, catalysed by DMAP.



Photolysis studies were undertaken on the two esters under deoxygenated conditions. The benzoin ester was comfortably soluble in acetonitrile, methanol and 30% aqueous methanol at the concentrations of around 0.1 mM used for the photolyses. The progress of the photolysis was monitored by U.V. spectroscopy. In all three solvent systems photolytic deprotection was smooth and quantitative, with 5 mg of ester being deprotected in around 45 minutes. Formation of 2-phenylbenzofuran was verified by comparison with an authentic sample.[21] The U.V. spectra are shown in figure 2. The decay of the ester (λ_{max} *ca.* 250 nm) is evident, as is the build-up of benzofuran (λ_{max} *ca.* 300 nm). Two isosbestic points are clearly evident. The spectra in methanol show a build up of 2-phenylbenzofuran as the photolysis proceeds, whilst those in acetonitrile show that the concentration of 2-phenylbenzofuran rises to a maximum after around 10 minutes and then decays. The ^{1}H nmr spectra of the photolysis products correlated with the UV spectra demonstrating the clean formation of Z-Gly-PheOH, along with 2-phenylbenzofuran.

Flash-photolysis studies were conducted using a xenon flash-lamp, recording the spectra with a diode-array spectrophotometer.[22] The initial spectrum is subtracted from the current spectrum and the data are represented in the form of a stacked plot. Photolysis in methanol (figure 3a) shows steady build-up of 2-phenylbenzofuran, **1**, (λ_{max} *ca.* 300 nm), echoing the results of the bulk photolysis. The spectra from the flash-photolysis in acetonitrile show the benzofuran, **1**, concentration rising to a maximum after around 10 flashes and then slowly decaying with further flashes (figure 3b).

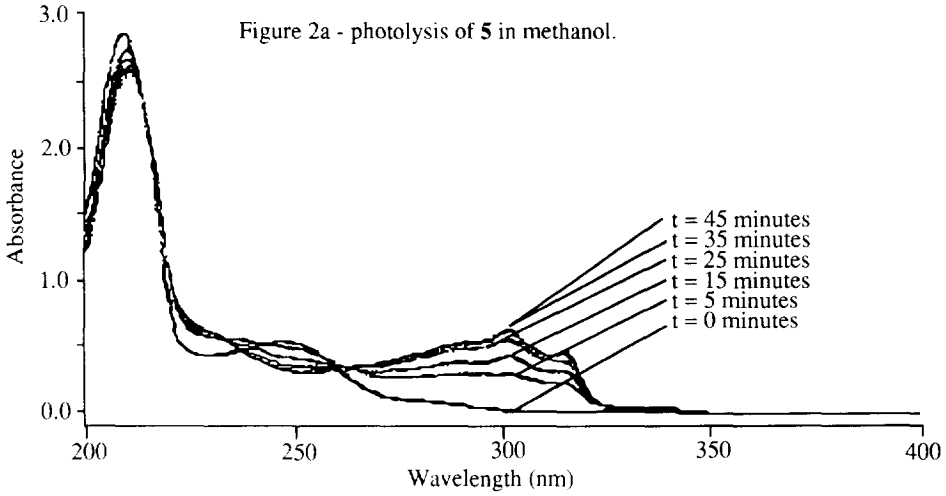


Figure 2a - photolysis of **5** in methanol.

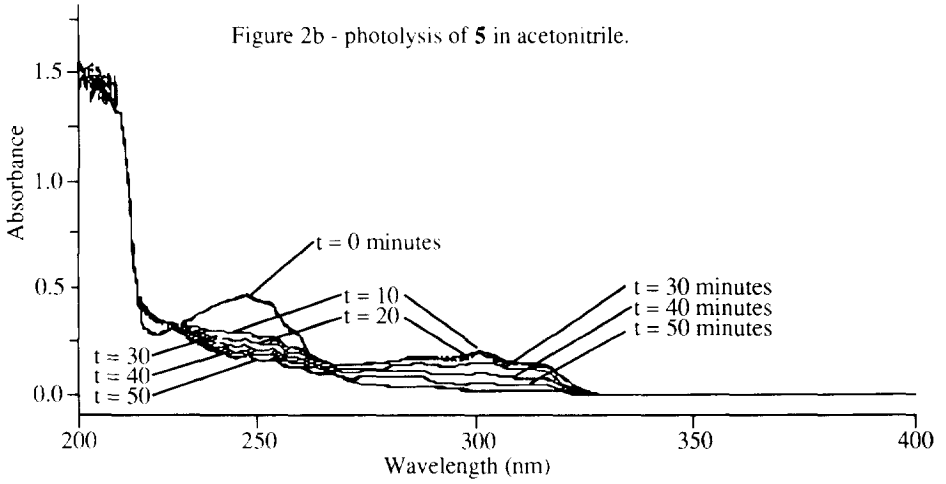


Figure 2b - photolysis of **5** in acetonitrile.

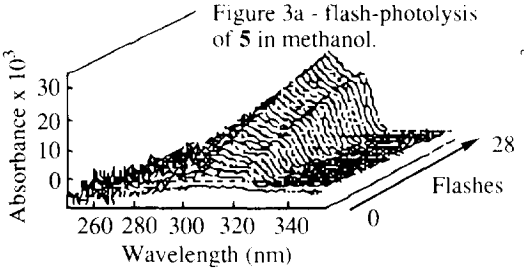
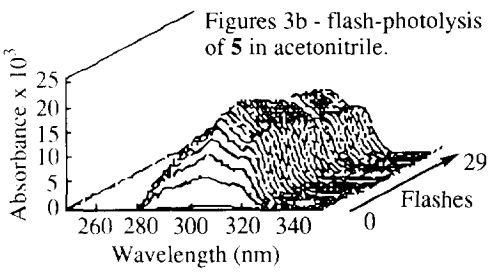


Figure 3a - flash-photolysis of **5** in methanol.



Figures 3b - flash-photolysis of **5** in acetonitrile.

The photolysis of the furoin ester, **6**, was not so encouraging. Deprotection of a 0.1 mM solution of ester in methanol took around 10 hours. Photolysis was monitored by U.V. spectroscopy and the spectra are shown in figure 4.

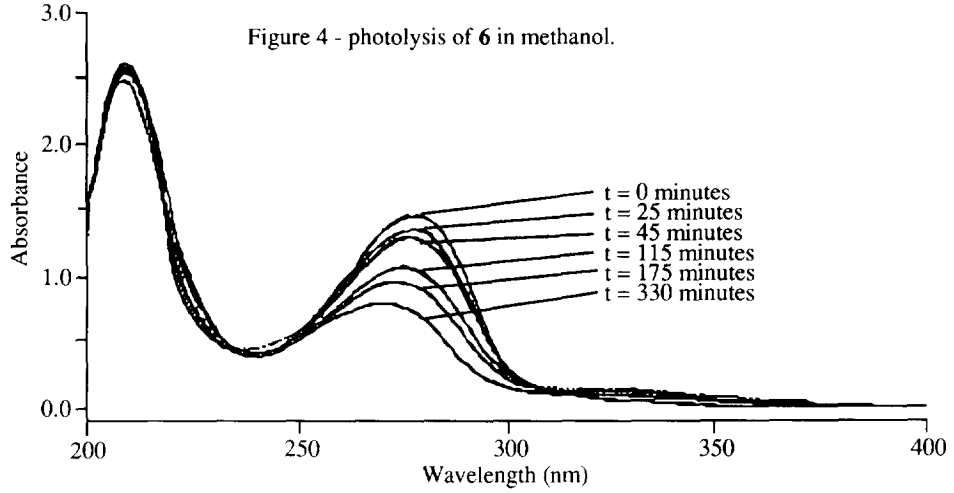


Figure 4 - photolysis of **6** in methanol.

Smooth decay of the ester peak (λ_{max} *ca.* 280 nm) is evident, as is build-up and subsequent decay of a side product (λ_{max} *ca.* 325 nm) postulated to be 2-furylfuranofuran, **8**. During the course of the photolysis a fast-running spot was observed by tlc, consistent with furanofuran formation, although this did not accumulate in concentrations sufficient to allow for isolation. As the U.V. spectra show, the concentration of this side product was always small and its decomposition, possibly by photolysis, appeared to be facile.

The photolysis studies indicated that benzoin, rather than furoin, esters of peptides show the most promise as photolabile protecting groups for this peptide and so the synthesis of these esters was investigated further. In particular, maintenance of the stereochemical integrity at the centre α- to the carboxyl function is a demanding and important constraint on protection-deprotection protocols. We set out to measure the extent of epimerisation at the Phe centre of benzoinyl *N*-carbobenzyloxyglycylphenylalanate, **5**, in order to minimise this problem.

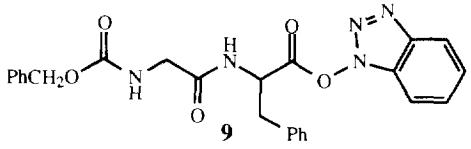
The mixture of esters, **5**, resulting from DCCI-DMAP coupling of benzoin exhibited a specific optical rotation of 0°, suggesting complete loss of stereochemical integrity at the phenylalanyl chiral centre. We developed an nmr-based method to determine the stereochemical integrity of the phenylalanyl centre of products resulting from synthetic manipulations. We chose to photolytically deprotect the esters and use ^{1}H nmr spectroscopy in conjunction with the chiral solvating agent *(R)*-2,2,2-trifluoro-1-(9-anthryl)ethanol[23] to measure the enantiomeric excess (*e.e.*) of the deprotected peptide. These experiments were unsuccessful on the free peptide. Masking the carboxyl terminus by treatment with diazomethane provided the methyl ester, **7**, which was soluble in deuterobenzene, a solvent which does not interfere with the coordination between peptide and solvating agent. Addition of five molar equivalents of *(R)*-2,2,2-trifluoro-1-(9-anthryl)ethanol to the benzene solution split the resonance due to the Phe α-proton into two with baseline resolution of the signals.

As anticipated, the liberated Z-Gly-PheOH was completely racemic. To provide evidence that racemisation was occurring during the DCCI-mediated coupling (rather than during *e.e.* determination), the experiment was repeated using *(S)*-Z-Gly-PheOH and *(S)*-benzoin. Coupling without racemisation of the phenylalanyl chiral centre would lead to a single diastereomer, the *S,S*-isomer, of the product ester **2** (it was thought unlikely that benzoin would racemise under the conditions of the coupling). In the event, an equimolar mixture of diastereomers was observed (as judged by ^{1}H nmr spectroscopy), in accordance with the DCCI-

mediated coupling being responsible for the loss of stereochemical integrity (presumably via enolisation of an intermediate azlactone).[24]

1-Hydroxybenzotriazole (HOBt) has long been added to DCCI-mediated peptide coupling reactions to suppress racemisation.[25] It is believed to act by intercepting the highly activated intermediates produced in the coupling before azlactone formation occurs; the resultant ester is less-highly activated and therefore less prone to side-reactions. Z-Gly-PheOH was completely consumed on treatment with DCCI, benzoin and one equivalent of HOBt for 1 hour. ^{1}H Nmr spectroscopy revealed the presence of two peptide-derived products in the ratio 3:1, neither of which was the benzoin ester. These compounds, which could not be satisfactorily purified, were tentatively identified as the benzotriazolyl ester **9** of Z-Gly-PheOH and the symmetrical anhydride[26] (Z-Gly-Phe)$_2$O. In particular, there was a pair of doublets in the ratio 3:1 at δ_H 3.81 and δ_H 3.75 for the glycine -CH_2-, as well as a doublet of triplets at δ_H 4.94 and δ_H 4.97 and a multiplet centred around δ_H 5.62. Further tentative evidence for the existence of **9** was provided by observation of a protonated molecular ion at m/z 473 and the molecular ion plus ammonium at m/z 491 in the CI$^+$ mass spectrum of the crude mixture. Although the mass spectrum showed the presence of high mass fragments possibly resulting from the symmetrical anhydride (Z-Gly-Phe)$_2$O the molecular ion was not detected, even with the FAB technique. On prolonged standing this mixture slowly hydrolysed back to Z-Gly-PheOH.

O-Benzotriazolyl-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HBTU, **10**)[27] has been shown to yield racemisation-free peptide couplings with some of the most challenging substrates. Unfortunately HBTU-mediated esterification also gave the benzotriazolyl ester **9** and none of the desired ester **5**. It appears that benzoin was insufficiently nucleophilic to displace the benzotriazolyloxy group from **9**.



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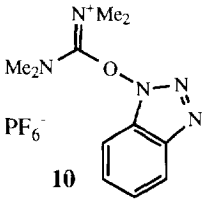

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These experiments underscore the poor nucleophilicity of benzoin. It did not react with several activated peptide-coupling intermediates; coupling was only observed with highly reactive acylating agents. In attempting to couple benzoin to Z-Gly-PheOH we must balance two conflicting factors: achieving a sufficient degree of activation whilst avoiding racemisation. It may be that such a balance is unattainable for benzoin/peptide couplings.

Thus we decided to try an alternative strategy based on alkylation of the peptide carboxylate as the key step. This approach generally precludes racemisation α- to the carboxylate. Two benzoin derivatives were evaluated as electrophiles for this alkylation chemistry: diazobenzil (PhC(N_2)COPh) and desyl bromide (PhCHBrCOPh). Displacement of a leaving group, by S_N2 chemistry, is expected to be facile for a benzylic centre α- to a ketone.

Diazoalkanes react with carboxylic acids to form esters *via* proton transfer, to form a diazonium carboxylate ion pair which undergoes nucleophilic substitution at the diazo carbon. Preliminary studies indicated that diazobenzil was unreactive towards simple carboxylic acids in dry dichloromethane or ether.

However, addition of a few drops of boron trifluoride etherate effected rapid and quantitative ester formation in these solvents. For our experiments on Z-Gly-PheOH we once again used acetone as the solvent. In this solvent only partial esterification was observed in the presence of boron trifluoride etherate; in the absence of Lewis acid smooth transformation to **5** occurred using a five-fold excess of the diazoketone to drive the reaction to completion. Purification of **5** was complicated because of the requirement for excess diazobenzil. Chromatography afforded **5** (as a 1 to 1 mixture of diastereomers) in 42% yield. Following our standard photolysis/esterification protocol the *e.e.* of the deprotected peptide was found to be 40%. This result was disappointing and unexpected.

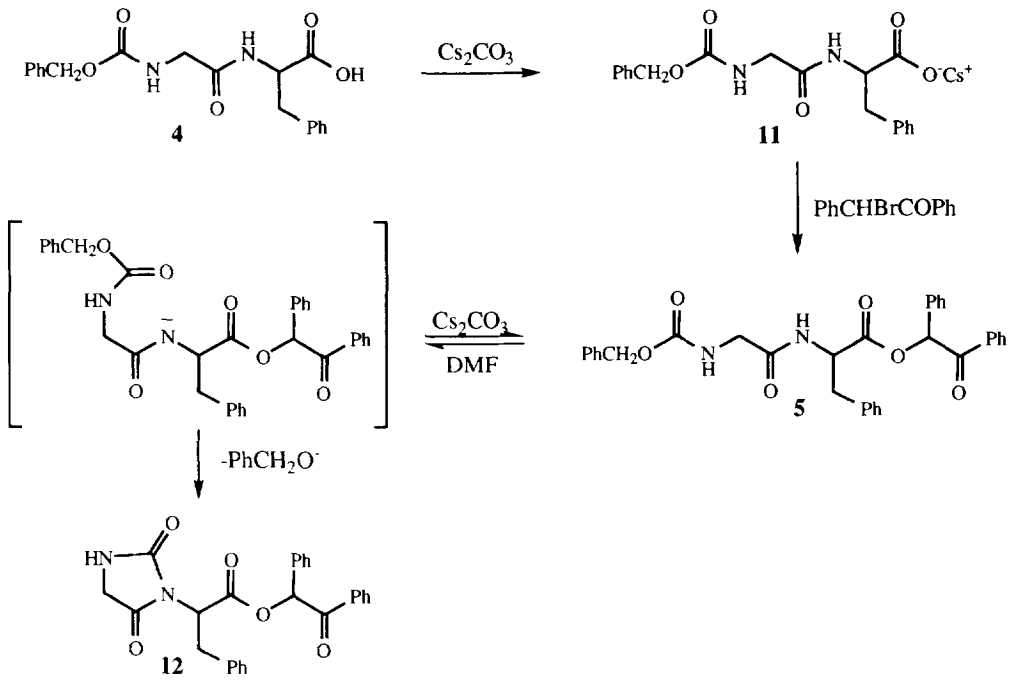
We sought an alternative alkylation procedure which might furnish the ester more effectively. Givens[28] has employed desyl bromide in the synthesis of phosphate esters of benzoin. The esterification of cesium carboxylates by alkyl halides in polar aprotic solvents such as dimethylformamide (DMF) is a facile reaction; Wang *et al.* have applied this chemistry to the synthesis of peptide esters.[29] We hoped that the reaction of desyl bromide with the cesium salt of Z-Gly-PheOH would provide a more satisfactory route to **2**.

The cesium salt **11** of Z-Gly-PheOH was prepared by stirring **4** with cesium carbonate in a methanol/water solution. Reaction of the salt with desyl bromide in DMF was complete within 30 minutes. However, in addition to **5** (a 1 to 1 mixture of diastereomers), a further product, tentatively identified as hydantoin **12**, was isolated. A similar mixture of **5** and **12** was generated when the experiment was repeated in dimethyl sulphoxide (DMSO) and when an analogous 'one pot' reaction (stirring Z-Gly-PheOH, cesium carbonate and desyl bromide) was undertaken in DMF. The side product, **12**, did not result from the reaction work up. These results were disappointing; Wang *et al* have made several different esters of a range of Z-protected peptides without any reported side-reactions.

Most examples of the esterification of carboxylate salts have been in polar aprotic solvents such as DMF, DMSO and hexamethylphosphoramide (HMPA). Reports of esterification in less polar solvents such as acetone are rare.[30] Nevertheless, since Z-Gly-PheOH shows good solubility in acetone and desyl bromide is highly reactive, we decided to attempt the esterification in acetone to evaluate solvent dependency of the side-reaction.

Stirring Z-Gly-PheOH with one equivalent of desyl bromide and half an equivalent of cesium carbonate in acetone for 16 hours cleanly gave the two diastereomers of **5** without a trace of the side-product which had plagued the reaction in DMF. Following evaporation of the solvent the product was obtained as an oil which crystallised on prolonged standing, allowing us to dispense with chromatographic purification.

It would appear that in the polar aprotic solvents DMF and DMSO, cesium carbonate forms a small but significant amount of amide anion which is highly nucleophilic in these conditions and reacts intramolecularly to form hydantoin **12**. This deprotonation/cyclisation chemistry is suppressed in the less polar solvent acetone. Esterification is slower in the less polar solvent but now proceeds without competitive side products. The *e.e.* of the phenylalanyl chiral centre in **5** was determined to be in excess of 90% using our standard protocol.



The 'one pot' cesium carbonate-mediated reaction of peptide **5** with desyl bromide in acetone was therefore found to be the method of choice for formation of the corresponding diastereomeric benzoin esters **2** in high yield without significant racemisation of the derivatised amino acid residue.

In conclusion, peptides can be derivatised as benzoin esters and deprotected by continuous or flash photolysis. Furoin esters are less appropriate for this purpose. The methodology developed is appropriate for the protection and photodeprotection of a range of peptides and should be widely applicable to time-resolved biophysical experiments.

ACKNOWLEDGEMENTS

We are grateful to the SERC for funding and to Drs J. Hajdu, J. H. Jones and Professor P. Bailey for helpful discussions.

EXPERIMENTAL

All solvents were distilled before use. Petrol refers to petroleum ether, b.p. 40-60 °C. Acetone was distilled from 4Å molecular sieves and used immediately. Flash column chromatography was performed using Merck Kieselgel 60, 230-400 mesh. Thin layer chromatography was performed on Merck Kieselgel DC-Alufolien 60 F_{254} aluminium-backed plates with visualisation by U.V. light. Melting points were determined on a Gallenkamp hot stage apparatus and are uncorrected. Infra-red spectra were recorded on a Perkin-Elmer 1750 fourier transform instrument. Only selected absorbances are recorded. Microanalysis was performed in the Dyson Perrins Laboratory by Mrs V. Lamburn. ^{1}H Nmr spectra were recorded on Varian Gemini 200, Bruker AM250 and Bruker AM500 instruments at 200, 250 and 500 MHz respectively and are referenced to

residual solvent peak. *J*-Values are given in Hz. ^{13}C Nmr spectra were recorded on a Bruker AM500 instrument at 125 MHz and are referenced to residual solvent peak. Mass spectra were recorded on V.G. Micromass ZAB1F and V.G. Micromass 20-250 instruments. Only the major peaks are reported, with intensities quoted as percentages of the base peak. U.V. spectra were recorded on Perkin-Elmer 555 and Cary 3 U.V.-visible spectrophotometers. Optical rotations were measured on a Perkin-Elmer 241 polarimeter.

Photolysis was performed using a Hanovia medium-pressure mercury lamp with a pyrex jacket. A solution of the ester of approximately 0.1 mM concentration in a pyrex reaction vessel was deoxygenated by passing a nitrogen stream through it for 20 minutes. The solution was maintained under a nitrogen atmosphere and magnetically stirred throughout the photolysis. Flash photolysis was performed using a xenon flash lamp, recording the spectra with an Oriel Instaspec diode array spectrophotometer. A drop of the solution to be photolysed was mounted in a 1 mm quartz capillary tube and the ends sealed with wax. The tube was mounted in the beamlines of the spectrophotometer and flash lamp, these two being arranged perpendicular to one another.

Benzoinyl N-*Carbobenzyloxyglycylphenylalanate, 5,* via *DCCI-DMAP Coupling.*

To a stirred solution of *N*-carbobenzyloxyglycylphenylalanine, **4**, (500 mg, 1.4 mmol) in acetone (5 ml) was added DCCI (640 mg, 3.1 mmol). This was followed by DMAP (170 mg, 1.5 mmol) and benzoin (295 mg, 1.4 mmol). The mixture was stirred under nitrogen for 48 hours. The precipitate of dicyclohexyl urea was filtered off and the solvent evaporated. The resulting oil was purified by flash column chromatography (ethyl acetate:petrol, 3:2) to afford a white solid which was recrystallised from xylene to yield the *title compound* as a 2 to 1 mixture of diastereomers (480 mg, 63%) m.p. 152-154 °C (Found: C, 71.77; H, 5.52; N, 5.07. $C_{33}H_{30}N_2O_6$ requires C, 71.98; H, 5.49; N, 5.09%); $[\alpha]^{20}_D$ 0 (c 1 in acetone); ν_{max} ($CHCl_3$)/cm^{-1} 3419w (NH), 1732, 1697; λ_{max} (MeOH)/nm 210 (ε/dm^3 mol^{-1} cm^{-1} 27500), 248 (6000); δ_H (500 MHz; CD_3CN) major diastereomer: 3.03 and 3.15 (2H, ABX, J_{AB} 14, J_{AX} 5.5, J_{BX} 8, Phe -CH_2-), 3.68 (2H, d, *J* 6, Gly -CH_2-), 4.86 and 4.87 (1H, dt, *J* 8, Phe α-H), 5.07 (2H, s, Z -CH_2-), 5.89 (1H, br, ex D_2O, -NH-), 6.87 (1H, br, ex D_2O, -NH-), 6.93 (1H, s, -OC*H*(Ph)COPh), 7.1-7.6 (18H, m, Ar), 7.97 (2H, d, *J* 7, Ar); minor diastereomer 3.07 and 3.37 (2H, ABX, J_{AB} 14, J_{AX} 5.2, J_{BX} 8.6, Phe -CH_2-), 3.65 (2H, d, *J* 6, Gly -CH_2-), 4.79 and 4.80 (1H, dt, *J* 8, Phe α-H), 5.07 (2H, s, Z -CH_2-), 5.89 (1H, br, ex D_2O, -NH-), 6.87 (1H, br, ex D_2O, -NH-), 6.97 (1H, s, -OC*H*(Ph)COPh), 7.1-7.6 (18H, m, Ar), 7.99 (2H, d, *J* 7, Ar); δ_C (125 MHz; CD_3CN) mixture of diastereomers 38.0, 38.1, 54.2, 67.3, 79.0, 127.7, 127.8, 128.7, 128.9, 129.4, 129.6, 129.9, 130.0, 130.3, 130.4, 134.3, 134.8, 135.4, 137.5, 137.8, 138.0, 157.5, 170.1, 170.3, 171.6, 171.8, 194.5, 194.7, 207.4; *m/z* ($CI^+(NH_3)$) 568 (MNH_4^+, <1%), 551 (1, MH^+), 357 (30), 266 (55), 249 (100), 197 (38), 148 (53), 108 (28), 91 (65, $C_7H_7^+$).

Furoinyl N-*Carbobenzyloxyglycylphenylalanate, 6*

To a stirred solution of *N*-carbobenzyloxyglycylphenylalanine, **4**, (100 mg, 0.28 mmol) in acetone (1 ml) was added DCCI (128 mg, 0.62 mmol). This was followed by DMAP (34 mg, 0.3 mmol) and furoin (54 mg, 0.28 mmol). After stirring overnight under nitrogen the dicyclohexyl urea was filtered off and the solvent evaporated to afford a pale yellow oil. This was purified by flash column chromatography (ethyl acetate:petrol, 3:2) and the resulting oil crystallised from dichloromethane-petrol to yield the *title compound* as a 3 to 1 mixture of diastereomers (114 mg, 77%) m.p. 140-141 °C; Found: C, 65.91; H 5.12; N 5.30. $C_{29}H_{26}N_2O_8$ requires C, 65.65; H, 4.94; N, 5.28); $[\alpha]^{20}_D$ 0 ° (c 1 in acetone); ν_{max} (KBr)/cm^{-1} 3339 (NH), 1758, 1719,

1687, 1675; λ_{max} (MeOH)/nm 211 (ε/dm^3 mol^{-1} cm^{-1} 26900), 279 (14800); δ_H (500 MHz; $CDCl_3$) major diastereomer: 3.2-3.4 (2H, m, Phe -CH_2-), 3.8 and 3.9 (2H, m, Gly -CH_2-), 5.07 and 5.08 (1H, dt, *J* 6, Phe α-H), 5.12 (1H, s, Z -CH_2-), 5.35 (1H, br, ex D_2O, -NH-), 6.43 (1H, m, Ar), 6.45 (1H, br, ex D_2O, -NH-), 6.54 (2H, m, Ar), 6.77 (1H, s, -OC*H*(Ar)COAr), 7.0-7.4 (11H, m, Ar), 7.49 (1H, d, *J* 2, Ar), 7.58 (1H, d, *J* 1.5, Ar); minor diastereomer: 3.2-3.4 (2H, m, Phe -CH_2-), 3.8 and 3.9 (2H, m, Gly -CH_2-), 4.99 and 5.00 (1H, dt, *J* 6, Phe α-H), 5.11 (1H, s, Z -CH_2-), 5.35 (1H, br, ex D_2O, -NH-), 6.41 (1H, m, Ar), 6.45 (1H, br, ex D_2O, -NH-), 6.54 (2H, m, Ar), 6.81 (1H, s, -OC*H*(Ar)COAr), 7.0-7.4 (11H, m, Ar), 7.47 (1H, d, *J* 2, Ar), 7.60 (1H, d, *J* 1.5, Ar); δ_C (125 MHz; DMSO) major diastereomer 37.0, 43.3, 53.5 (Phe α-C), 65.6, 70.9, 111.4, 112.6, 113.0, 121.0, 126.8, 127.2, 127.8, 127.9, 128.4, 128.5, 129.3, 136.8, 137.2, 145.1, 146.3, 149.1, 156.5, 169.3, 170.6, 178.7 (4 x C=O); minor diastereomer 36.6, 43.3, 53.2 (Phe α-C), 65.6, 70.9, 111.5, 112.8, 113.0, 121.0, 126.8, 127.2, 127.8, 127.9, 128.4, 128.5, 129.3, 136.8, 137.1, 145.2, 146.3, 149.1, 156.5, 169.6, 171.0, 178.8 (4 x C=O); *m/z* ($CI^+(NH_3)$) 548 (MNH_4^+, 12%), 531 (47, MH^+), 374 (6), 357 (24), 339 (44), 192 (40), 177 (100), 91 (26, $C_7H_7^+$).

Methyl N-*Carbobenzyloxyglycylphenylalanate* , **7**

A solution of benzoinyl *N*-carbobenzyloxyglycylphenylalanate , **5**, (5 mg, 0.01 mmol) in the chosen solvent was photolysed as above until none of the ester remained by tlc (ethyl acetate:petrol, 3:2). The solvent was evaporated and the residue taken up in acetone (1 ml) and treated with an excess of an etherial solution of diazomethane (1 ml, *ca.* 0.15 mmol). After ten minutes a drop of glacial acetic acid was added to quench any remaining diazomethane, the solvent removed by evaporation and the residue subjected to flash column chromatography (ethyl acetate:petrol, 3:2) to furnish the *title compound* as a colourless oil (2 mg, 62%) δ_H (200 MHz; C_6D_6) 2.82 and 2.98 (2H, ABX, J_{AB} 14, J_{AX} 6, J_{BX} 6, Phe -CH_2-), 3.16 (3H, s, -CH_3), 3.40 (2H, m, Gly -CH_2-), 4.40 (1H, s, br, ex D_2O, -NH-), 4.87 and 4.91 (1H, dt, *J* 6, Phe α-H), 4.97 (2H, s, Z -CH_2-), 6.40 (1H, s, br, ex D_2O, -NH-), 6.9-7.2 (10H, m, 2 x Ph); *m/z* ($CI^+(NH_3)$) 371 (MH^+, 50%), 263 (100), 162 (52), 108 (23), 91 (51, $C_7H_7^+$).

Determination of Enantiomeric Purity of Methyl N-*Carbobenzyloxyglycylphenylalanate, 7, by* ^{1}H *NMR Spectroscopy*

To a solution of methyl *N*-carbobenzyloxyglycylphenylalanate, **7**, (1 mg, 0.003 mmol) in C_6D_6 (0.4 ml) was added *(R)*-2,2,2-trifluoro-1-(9-anthryl)ethanol (7 mg, 0.015 mmol). Δδ for the resonances due to the Phe α-H was approximately 0.05 ppm (500 MHz), with the signals for (*R*)-**7** resonating upfield to those for (*S*)-**7**.

Benzoinyl N-*Carbobenzyloxyglycylphenylalanate, 5,* via *Diazobenzil.*

Diazobenzil[31] (1.38 g, 6.2 mmol) was added to a stirred solution of *N*-carbobenzyloxyglycylphenylalanine, **4**, (1 g, 2.8 mmol) in acetone (10 ml). After stirring overnight the solvent was evaporated to give an orange oil. Flash column chromatography (ethyl acetate:petrol, 3:2) afforded a 1 to 1 mixture of diastereomers of the *title compound* as a yellow oil (640 mg, 42%) which was crystallised from xylene, m.p. 146-148 °C; $[\alpha]^{20}_D$ -1.14 ° (c 1 in acetone). Other data as above.

Cesium N-Carbobenzyloxyglycylphenylalanate, 11.

To a stirred solution of *N*-carbobenzyloxyglycylphenylalanine, **4**, (500 mg, 1.4 mmol) in methanol (30 ml) and water (10 ml) was added dropwise a solution of cesium carbonate (326 mg, 1 mmol) in water (5 ml) until the solution was neutral to universal indicator. The methanol was removed by evaporation and the residue

freeze dried to afford a quantitative yield (685 mg) of the *title compound* as a white solid which was used without further purification, m.p. 178-180 °C; $[\alpha]^{20}_D$ +33.6 (c 1 in methanol); δ_H (250 MHz; DMSO) 2.91 and 3.02 (2H, ABX, J_{AB} 13, J_{AX} 5, J_{BX} 5, Phe -CH_2-), 3.48 and 3.59 (2H, ABX, J_{AB} 17, J_{AX} 6, J_{BX} 6, Gly -CH_2), 3.87 and 3.90 (1H, dt, *J* 5, Phe α-H), 5.02 (2H, s, Z -CH_2-), 7.0-7.4 (11H, m, Ar, -NH-), 7.58 (1H, br, -NH-); *m/z* (FAB) 621 (MCs^+, 20%), 133 (100, Cs^+).

Reaction of Cesium N-*Carbobenzyloxyglycylphenylalanate with Desyl Bromide in DMF.*

Cesium *N*-carbobenzyloxyglycylphenylalanate, **11**, (50 mg, 0.1 mmol) was dissolved with gentle warming in DMF (3 ml). To this solution was added desyl bromide (55 mg, 0.2 mmol) and the mixture stirred in a stoppered flask protected from the light. After 30 minutes the DMF solution was partitioned between water (100 ml) and ethyl acetate (3 x 75 ml). The organic layer was washed with brine (2 x 100 ml), dried with magnesium sulphate and evaporated to afford an oil which was subjected to flash column chromatography, eluting with ethyl acetate:petrol, 3:2. In addition to unreacted desyl bromide two fractions were isolated. The first fraction eluted was obtained as an oil (23 mg, 30%) and was confirmed as a 1 to 1 mixture of diastereomers of *benzoinyl* N-*carbobenzyloxyglycylphenylalanate, 5*, from its ^1H nmr spectrum. The second fraction was also obtained as an oil (20 mg) and was tentatively identified as *hydantoin 12*; δ_H (250 MHz; CD_3CN) 3.3-3.5 (2H, m), 3.6-3.9 (2H, m), 5.0-5.1 (1H, m), 6.0 (1H, br, ex D_2O, -NH-), 7.02 and 7.05 (1H, 2 x s, PhC*H*(OCOR)-), 7.2-7.6 (13H, m), 8.0 (2H, m); *m/z* ($CI^+(NH_3)$) 460 (MNH_4^+, 62%), 443 (100, MH^+), 231(38, MH^+-benzoin), 195 (31), 105 (46, $C_7H_5O^+$), 91 (5, $C_7H_7^+$).

Benzoinyl N-*Carbobenzyloxyglycylphenylalanate, 5,* via *Cesium Carbonate in Acetone.*

Desyl bromide (385 mg, 1.4 mmol) and cesium carbonate (228 mg, 1.4 mmol) were added to a stirred solution of *N*-carbobenzyloxyglycylphenylalanine, **4**, (500 mg, 1.4 mmol) in acetone (20 ml). The flask was stoppered and the solution stirred overnight. After filtering the solvent was evaporated to afford a quantitative yield (770 mg) of a 1 to 1 mixture of diastereomers of the *title compound* as an oil, $[\alpha]^{20}_D$-3.53 (c 1.5 in acetone). Other data as above.

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(*Received in UK* 18 *May* 1995; *revised* 11 *July* 1995; *accepted* 14 *July* 1995)