Amino-acids and Peptides. Part I. Esterification of the Carboxy-Group of Penicillins and Cephalosporins by Hydrazone Oxidation

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A new and efficient process for esterifying *N*-blocked amino-acids and peptides with readily removed protecting groups is described. Illustrations are given with particular reference to *S*-oxides of penam acids. The new process comprises oxidation of a hydrazone in an organic or aqueous organic solvent in the presence of the *N*-blocked amino-acid or peptide, and with a trace of iodine as catalyst.

We describe here a simple and efficient process for esterifying the carboxy-function of *N*-blocked amino-acids. The process is illustrated by particular reference to the preparation of aralkyl esters of penam *S*-oxides (I). The β-lactam ring of penicillins and cephalosporins is sensitive to hydrolysis, and the choice of temporary protecting groups is limited to those which can be removed without concomitant ring cleavage. Several groups satisfying this requirement have been the subject of patents, *e.g.* 2,2,2-trichloroethyl,[1] *p*-nitrobenzyl,[2] and trimethylsilyl.[3] Another, the diphenylmethyl group, has been extensively employed in the laboratory for the synthesis of simple peptides [4] and in transformations of penicillins and

[1] (*a*) R. B. Woodward, *Science*, 1966, **153**, 487; (*b*) R. B. Woodward, B.P. 1 155 016.

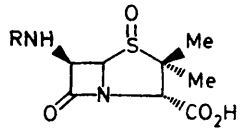
[2] (*a*) Beecham Research Ltd., S. Afr. P. 8 894/1967; (*b*) Eli Lilly Corp., Belg. P. 745 845; (*c*) R. and L. Research Ltd., U.S. P. 3 579 506.

[3] (*a*) H. W. D. Weissenburger and M. G. van der Hoeven, *Rec. Trav. chim.*, 1970, **89**, 1081; (*b*) Kon. Nederlandsche Gist., S. Afr. P. 2 927/1967.

[4] (*a*) M. Bethell, D. B. Bigley, and G. W. Kenner, *Chem. and Ind.*, 1963, 653; (*b*) R. G. Hiskey and J. B. Adams, *J. Amer. Chem. Soc.*, 1965, **87**, 3969; (*c*) A. A. Aboderin, G. R. Delpierre, and J. S. Fruton, *ibid.*, 5468; (*d*) G. C. Stelakatos, A. Paganou, and L. Zervas, *J. Chem. Soc.* (*C*), 1966, 1191.

cephalosporins.[5] This group has found little favour outside the laboratory however, owing to the hazard and expense involved in the preparation of its precursor, diphenyldiazomethane.

Our early experiments were directed at the introduc-



a ; R = $PhCH_2 \cdot CO$
b ; R = $PhO \cdot CH_2 \cdot CO$

(I)

tion of the diphenylmethyl group. By analogy with the oxidation of hydrazine to di-imide,[6] we reasoned that the penam *S*-oxide (I) might be esterified by using reactive intermediates arising from the oxidation of benzophenone hydrazone to benzophenone. The species (IIa–c) could conceivably be produced in such oxidations and any of these might be trapped by a protic acid to yield a diphenylmethyl ester. Horner and Fernekess [7] have

$Ph_2C{=}N{\cdot}NH{\cdot}OH$ (IIa) $Ph_2CH{\cdot}N{=}N{\cdot}OH$ (IIb) Ph_2CN_2 (IIc)

observed the formation of esters and ethers following oxidation of benzophenone hydrazone by peracetic acid in the presence of such substrates as acetic acid, benzoic acid, phenol, and ethanol. They considered that 'nascent diphenyldiazomethane' was the reactive species. The yields obtained were moderate, except when the substrate was present in great excess. The fact that commercial peracetic acid contains *ca.* 39% w/w free acetic acid, itself esterifiable, could account for this feature. Nonetheless, in one example quoted, competition between approximately equimolar quantities of phenol and acetic acid was resolved in favour of the weaker acid. Diphenylmethyl phenyl ether was isolated in 62.5% yield; 25% of the benzophenone hydrazone was converted into benzophenone azine. Apparently, under the conditions employed, acetic acid did not compete very strongly for the esterifying agent.

As a guide for further work, we treated (1*S*,3*S*,5*R*,6*R*)-2,2-dimethyl-6-phenylacetamidopenam-3-carboxylic acid 1-oxide (Ia) with 1.1 mol. equiv. of diphenyldiazomethane in the presence of 3.6 mol. equiv. of acetic acid. A 91.2% yield of the penam diphenylmethyl ester was obtained, indicating that if diphenyldiazomethane were being produced in benzophenone hydrazone oxidations, acetic acid would probably not compete effectively with (Ia) for esterification.

The penam *S*-oxide was suspended in dichloromethane containing 1.1—1.5 mol. equiv. of benzophenone hydrazone. Addition of peracetic acid between 0 and 10 °C produced fairly good yields of the diphenylmethyl ester. The best results were obtained by using *ca.* 8% excess of peracetic acid with respect to the hydrazone.

[5] (*a*) B. Fechtig, H. Peter, H. Bickel, and E. Vischer, *Helv. Chim. Acta*, 1968, **51**, 1108; (*b*) See also 'Cephalosporins and Penicillins, Chemistry and Biology,' ed. E. H. Flynn, Academic Press, New York and London, 1972.

The penam (Ia) was prepared by oxidation of penicillin G with sodium periodate or peracetic acid. The product obtained by using sodium periodate performed consistently better in the esterification than that obtained with peracetic acid. It also produced a transient colouration when treated with peracetic acid. This led us to suspect the presence of traces of iodine compounds in the substrate. When catalytic quantities of iodine were added in the esterifications of the substrate (Ia) obtained by oxidation with peracetic acid, the yield of ester was greatly enhanced. For example, addition of peracetic acid (1.4 mol) to a solution of benzophenone hydrazone (1.3 mol), (Ia) (1.0 mol), and iodine (0.002 mol) in chloroform gave an almost quantitative yield of the penam diphenylmethyl ester. In simple organic systems the iodine was conveniently added as such but in aqueous organic systems it could, with equal effect, be added in the form of compounds such as potassium iodide. The optimum level of iodine was about 0.0015 mol. equiv. with respect to the hydrazone; levels significantly greater or less were associated with lower yields.

A wide range of organic solvents proved suitable for the process, *e.g.* hydrocarbons, chlorinated hydrocarbons, ethers, esters, alcohols, ketones, amides, and nitriles. Mixtures of these with each other and with water were also effective. For the esterification of (Ia), the yields increased according to solvent in the following sequence: formamide < methanol < butanol ~ tetrahydrofuran < acetonitrile < acetone ~ ethyl acetate < chloroform ~ dichloromethane ~ 1,2-dichloroethane. The position of acetone in this sequence is worth noting, as ketones react rapidly with hydrazones to form azines.

Other oxidising agents such as periodic acid, ozone, and manganese dioxide have been employed in the esterification process. Peracids proved to be generally more efficient and more convenient.

Simple carboxylic acids, *N*-protected amino-acids, and penicillin and cephalosporin *S*-oxides have all been esterified by the method now described. Free amino-acids could be esterified in aqueous alcohols in the presence of strong acids such as hydrochloric acid. The reaction under these conditions was however usually incomplete; satisfactory efficiencies depended on recovery of unchanged substrate acid. Sulphur-containing acids, such as penicillins and cephalosporins were generally more sensitive to oxidation than were hydrazones; consequently such acids were not esterified without predominant oxidation of the sulphur atom. Thus, esterification of (6*R*)-6-phenoxyacetamidopenicillanic acid (penicillin V) gave almost entirely the ester of the *S*-oxide (Ib). Even when esterification of penicillin V was attempted at low temperature with 1.0 mol. equiv. of peracetic acid and 1.4 mol. equiv. of hydrazone, the *S*-oxide ester was the predominant product.

Several hydrazones have been employed for the esteri-

[6] E. J. Corey, W. L. Mock, and D. J. Pasto, (*a*) *Tetrahedron Letters*, 1961, 347; (*b*) *J. Amer. Chem. Soc.*, 1961, **83**, 2957; (*c*) S. Hünig, H. R. Müller, and W. Thier, *Tetrahedron Letters*, 1961, 353.

[7] L. Horner and H. Fernekess, *Chem. Ber.*, 1961, **94**, 712.

fications by the present method. Results are given in the Table.* 2-Furylmethyl esters have not previously been reported. Relatively stable hydrazones, *e.g.* benzophenone hydrazone, gave good yields of the corresponding esters when treated with peracetic acid in the presence of the substrate acid. Small amounts of azine and parent ketone were the principal by-products. More labile hydrazones, *e.g.* furfuraldehyde hydrazone, were better used by adding them to a mixture of the substrate acid with peracetic acid. Furthermore, in such instances, lower reaction temperatures and greater proportions of reagents to substrate were advantageous. Thus the best yields of (Ia) 2-furylmethyl ester were obtained when 2.5 mol. equiv. of furfuraldehyde hydrazone and 2.75 mol. equiv. of peracetic acid were employed at temperatures below −15 °C. In this context the results obtained with the hydrazones of *p*-anisaldehyde, phenyl 2-thienyl ketone, and fluorenone were probably less than optimum (Table). When, in this scheme, the substrate acid and oxidising agent are pre-mixed, it is important to recognise that penicillins are oxidised by peracetic acid to penicillaminic acid and that this reaction can be violent above 25 °C.

Esterification of carboxylic acids *via* hydrazone oxidation *

Substrate acid	Solvent [a]	Hydrazone [b]		Molar proportions AcO_3H	Molar proportions I_2 ($\times 10^{-3}$)	Reaction sequence [c]	Reaction temp. (°C)	Cryst. solvent	Yield [d] (%)	M.p. (°C)	$[\alpha]_D$(°)
(Ia)	A	Benzophenone	(1.38)	1.42	1.97	P	−7 to −5	PriOH	98	146	+192
	B	2-Methylbenzophenone	(1.30)	1.42	1.88	P	0	PriOH	97	147.5—148.5	
	B	3-Methylbenzophenone	(1.32)	1.42	1.88	P	−4 to −7	PriOH	97	68—70	
	A	Furfuraldehyde	(2.5)	2.75	3.62	H	−23 to −16	PriOH	91	150	+211
	B	Cyclohexanone	(2.5)	2.75	3.62	H	−23 to −16	EtOH	38	135 [e]	+188
	B	Fluorenone	(1.1)	1.33	2.04	H	17 to 25	†	74	140 [f]	+138
	A	Phenyl 2-thienyl ketone	(1.3)	1.42	1.88	P	−2 to −8	PriOH	62	125—126	
	E	*p*-Anisaldehyde	(1.1)	1.18	0.8	H	15 to 20	BuⁿOH	47	149	
	D	Acetophenone	(1.1)	1.1		H	15 to 20	MeOH	79	174—5	+227
(Ib)	B	Benzophenone	(1.38)	1.42	1.97	P	0 to 5	PriOH	95	149	+162
	F	Furfuraldehyde	(2.3)	2.52	3.35	H	+5		78	155	+175
Cephalothin *S*-oxide	F	Benzophenone	(1.38)	1.44	2.48	P	10		91	188	+34.3
(−)-D-PhCH(NH_3+Cl^-)·CO_2H	C	Benzophenone	(1.38)	1.42	1.88	H	0 to 5		74	190—191 (decomp.)	
(−)-D-PhCH(CO_2H)·NH·CO_3·CH_2·CCl_3	B	Benzophenone	(1.3)	1.42	1.88	P	−2 to +7	Me_2CO	91	178 [g]	−37

[a] A, 1,2-dichloroethane; B, chloroform; C, ethanol; D, tetrahydrofuran; E, dichloromethane; F, acetone. [b] Figures in parentheses indicate molar proportions with respect to substrate acid. [c] H, hydrazone; P, peracid; each, where indicated, added to a mixture of the other two reactants. [d] Based on starting material consumed. [e] Recrystallised from ethanol. [f] Recrystallised from ether with petroleum. [g] Recrystallised from acetone.

* Analytical data are available as Supplementary Publication No. SUP 21465 (2 pp). For details of Supplementary Publications see Notice to Authors No. 7, *J.C.S. Perkin I*, 1974, Index issue. † Ethereal solution added to light petroleum.

The diphenylmethyl ester of (Ia) was isolated in two polymorphic forms differing in their solid-phase i.r. spectra (Nujol) and in their m.p.s.

An attractive feature of the use of diphenylmethyl esters for the protection of carboxy-groups is the ease with which diphenylmethanol, produced on hydrolysis of the ester, can be recycled to benzophenone hydrazone.[8]

We have undertaken no work to define the role played by iodine in our esterification process. Speculation concerning the possible intermediacy of the diazohydroxide (IIb) and diphenyldiazomethane (IIc) led to studies to be reported later.

EXPERIMENTAL

M.p.s were determined with a Townson and Mercer instrument. N.m.r. spectra were obtained with a Varian A60, and i.r. spectra with a Perkin-Elmer 257 instrument.

Diphenylmethyl (1S,3S,5R,6R)-2,2-*Dimethyl*-6-*phenylacetamidopenam*-3-*carboxylate* 1-*Oxide*.—The acid (Ia) (35 g, 100 mmol), benzophenone hydrazone (27 g, 138 mmol), and iodine (1% w/v solution in chloroform; 5.0 ml, 0.197 mmol) in 1,2-dichloroethane (90 ml) were stirred and cooled to −5 °C. Peracetic acid (38% w/w solution in acetic acid; 25 ml, 142 mmol) was added during 45 min at −7 to −5 °C, and rinsed through with 1,2-dichloroethane (10 ml). After stirring for a further 30 min the mixture was washed with water (2 × 200 ml), aqueous sodium hydrogen carbonate (3.75 g in 150 ml), and water (150 ml), the aqueous washings being re-extracted sequentially with 1,2-dichloroethane (50 ml). The bulked organic liquors were evaporated under reduced pressure and the gum so obtained was crystallised from propan-2-ol (260 ml). The crystalline slurry was chilled to −5 °C and the crystals filtered off, washed with chilled (−5°) propan-2-ol (50 ml), and dried *in vacuo* at 40°, to give the *diphenylmethyl ester* (50.7 g, 98.2%), m.p. 146°, $[\alpha]_D$ + 192° (1% in $CHCl_3$), $\nu_{max.}$ (Nujol) 3 380 (amide NH), 1 795 (β-lactam C=O), and 1 677 and 1 500 cm^{-1} (amide I and II), τ (60 MHz; $CDCl_3$) 9.14 and 8.37 (each 3H, s, CMe_2), 6.45 (2H, s, CH_2·CO), 5.28 (1H, s, 3-H), 5.09 (1H, d, *J* 4.5 Hz, 5-H), 4.00 (1H, dd, *J* 10 and 4.5 Hz, 6-H), 3.04 (1H, s, CO_2·CH), 2.73 (5 H, s, *Ph*CH_2CO), and 2.69 and 2.67 (each 5 H, s, Ph_2C).

In other experiments a less stable crystalline form was obtained, m.p. 127—128°. Solution spectra were unchanged but $\nu_{max.}$ (Nujol) for the β-lactam carbonyl was shifted to 1 815 cm^{-1} and three new, sharp bands appeared at 1 413, 1 350, and 1 330 cm^{-1}.

2-Furylmethyl (1S,3S,5R,6R)-2,2-*Dimethyl*-6-*phenylacetamidopenam*-3-*carboxylate* 1-*Oxide*.—The acid (Ia) (700.8 g, 2.00 mol), peracetic acid (37.2% w/w solution in acetic acid; 990 ml, 5.495 mol), and iodine (1% w/v solution in 1,2-dichloroethane; 184 ml, 7.245 mmol) in 1,2-dichloroethane (1 l), were stirred and cooled to −20 °C. Furfuraldehyde hydrazone (550 g, 4.995 mol) in 1,2-dichloroethane (600 ml) was added during 90 min at −23 to −16 °C and rinsed through with 1,2-dichloroethane (400 ml). After stirring for a further 30 min the mixture was washed with water (2 × 4 l), aqueous sodium hydrogen carbonate (75 g in 3 l), and water (3 l), the aqueous washings being re-extracted sequentially with 1,2-dichloroethane (2 × 500 ml). The bulked organic liquors were evaporated under reduced pressure and the residue dissolved in propan-2-ol (2 l) at 50 °C. The solution was cooled and the crystals

* Dr. E. M. Wilson, of this Department, suggested the preparation of these esters. They are readily hydrolysed by strong acids.

[8] S. M. Rivkin, (*a*) Russ. P. 38 634; (*b*) *J. Appl. Chem.* (*U.S.S.R.*), 1938, **11**, 83 (*Chem. Abs.*, 1938, **32**, 4566).

harvested at −5 °C, washed with chilled (−5 °C) propan-2-ol (1 l), and dried *in vacuo* at 40 °C, to give the *2-furylmethyl ester* (783.8 g, 91.1%), m.p. 150°, $[\alpha]_D$ +211° (1% in $CHCl_3$), $\nu_{max.}$ (Nujol) 3 392 (amide NH), 1 785 and 1 780 (β-lactam C=O), 1 758 and 1 738 (ester C=O), 1 680 and 1 512 (amide I and II), and 1 012 cm^{-1} (sulphoxide), τ [60 MHz; $(CD_3)_2SO$] 8.92 and 8.48 (each 3H, s, CMe_2), 6.39 (2H, s, CH_2·CO), 5.52 (1H, s, 3-H), 4.85 and 4.61 (2H, ABq, *J* 13 Hz, CO_2·CH_2), 4.54 (1H, d, *J* 5 Hz, 5-H), 4.16 (1H, dd, *J* 9 and 5 Hz, 6-H), 3.51 (1H, dd, *J* 3.5 and 2 Hz, furyl 4-H), 3.38 (1H, dd, *J* 3.5 and 1 Hz, furyl 3-H), 2.69 (5H, s, aromatic), 2.28 (1H, dd, *J* 2 and 1 Hz, furyl 5-H), and 2.08 (1H, d, *J* 9 Hz, NH).

Diphenylmethyl (−)-D-2-*Phenyl*-N-(2,2,2-*trichloroethoxycarbonyl*)*phenylglycinate*.— (−)-D-2-Phenyl-*N*-(2,2,2-trichloroethoxycarbonyl)glycine (16.33 g, 50 mmol), benzophenone hydrazone (13.0 g, 65 mmol), and iodine (1% w/v solution in chloroform; 2.4 ml, 0.094 mmol), were stirred in chloroform (100 ml) and cooled to −2 °C. Peracetic acid (38% w/w solution in acetic acid; 12.5 ml, 71 mmol) was added during 7 min, with the temperature kept below 7 °C. The mixture was then stirred for 50 min without cooling (temperature rose to 19 °C) and filtered, and the solid was washed with ether (80 ml) and dried to give the *diphenylmethyl ester* (18.8 g), m.p. 177—178°. The ether washing from the first crop was evaporated under reduced pressure and the residue dissolved in chloroform (50 ml) and added to the filtered reaction mixture. The combined chloroform solutions were washed with water (2 × 100 ml), aqueous sodium hydrogen carbonate (5 g in 200 ml), and water (150 ml), the aqueous washings being sequentially re-extracted with chloroform (50 ml). The bulked chloroform liquors were dried ($MgSO_4$) and evaporated under reduced pressure, and the residue was triturated with ether to yield a second crop of the ester (3.6 g, total 91%), m.p. 175—177°.

The ester was recrystallised from acetone to give needles, m.p. 178—179°, homogeneous on t.l.c. [silica; 2:1 C_6H_6–EtOAc; detection by aq. 1% $KMnO_4$ or u.v. (λ 245 nm)], $[\alpha]_D$ −37° (0.8% in $CHCl_3$), $\nu_{max.}$ (Nujol) 3 392 (amide NH), 1 742 (ester C=O), and 1 724 and 1 530 (amide I and II), $\nu_{max.}$ ($CHBr_3$) 3 452 (amide NH), 1 735 (ester C=O), and 1 735 and 1 500 cm^{-1} (amide I and II), τ [60 MHz; $(CD_3)_2SO$] 5.15 (2H, s, Cl_3C·CH_2·O), 4.48 (1H, d, *J* 8 Hz, 2-H), 3.16 (1H, s, CO_2·CH), 2.80 (5H, s, Ph), 2.62 (10H, s, Ph_2C), and 1.13 (1H, d, *J* 8 Hz, NH).

Diphenylmethyl (−)-D-2-*Phenylglycinate Hydrochloride*.— (−)-D-2-Phenylglycine (15.1 g, 100 mmol) was stirred with ethanol (200 ml) and concentrated hydrochloric acid (8.62 ml, 100 mmol) was added at ambient temperature. Almost complete dissolution occurred, followed by formation of a thick suspension of phenylglycine hydrochloride. Peracetic acid (36% w/w solution in acetic acid; 26.5 ml, 142 mmol) and iodine (1% w/v solution in 1,2-dichloroethane; 4.8 ml, 0.189 mmol) were added and the mixture was cooled to *ca.* 2 °C. Almost complete dissolution occurred.

Benzophenone hydrazone (27 g, 138 mmol) was added in portions during 67 min at 2—4 °C, and the mixture was then stirred until evolution of gas ceased (22 min) and for a further 1 h. The mixture was filtered, the solid was washed with ethanol, the filtrate and washing were combined and evaporated under reduced pressure (≤36 °C). The residue was dispersed in 2N-hydrochloric acid (200 ml) and the slurry was filtered. The solid was washed with water and ether, and dried *in vacuo* at 40 °C, to give the *diphenylmethyl ester* (12.09 g), m.p. 190—191° (decomp.), $\nu_{max.}$ (Nujol) 2 673 and 2 598 (NH_3^+), 1 732 and 1 219 (ester C=O), 1 580, 1 551, 730, and 682 cm^{-1} (aromatic), τ [60 MHz; $(CD_3)_2SO$] 4.53 (1H, s, 2-H), 3.08 (1H, s, CO_2·CH), 2.6 (5H, s, Ph), 2.2—3.0 (10H, Ph_2C), and 0.7br (NH_3^+ and H_2O).

Diphenylmethyl (6R,7R)-3-*Acetoxymethyl*-7-[2-(2-*thienyl*)-*acetamido*]*ceph*-3-*em*-4-*carboxylate* 1-*Oxide* (*Cephalothin* S-*Oxide*).—Benzophenone hydrazone (6.55 g, 33.4 mmol), the cephem-4-carboxylic acid *S*-oxide (10.0 g, 24.2 mmol), and iodine (0.06 mmol) were suspended in acetone (110 ml) at 10 °C. Peracetic acid (37% w/w; 6.15 ml, 34.8 mmol) was added dropwise over 30 min with stirring at 10 °C. The mixture was stirred for an additional 30 min, the temperature being allowed to rise to 23 °C. The filtered solution (140 ml) was treated with warm water (140 ml) at 35 °C and the precipitated solid was cooled and stirred at 0 °C for 30 min. The solid was filtered off and washed with a little cold 50% v/v acetone–water to yield the *diphenylmethyl ester* (12.8 g, 91.3%), m.p. 188°, $[\alpha]_D$ +34.3°. A recrystallised sample, m.p. 201—203° (from acetone–water), showed $\nu_{max.}$ (Nujol) 3 290 (amide NH), 1 795 (β-lactam), 1 748 (ester C=O), 1 722 (acetate), 1 664 and 1 531 (amide I and II), and 1 040 cm^{-1} (sulphoxide), $[\alpha]_D$ +37.1° (1% in $CHCl_3$).

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