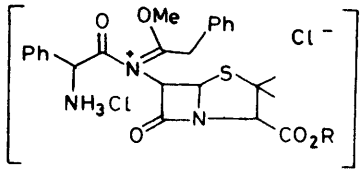
Transacylation of Cephalosporin; Isolation and Reactions of the Imidate Esters

By **Toshinori Saito,* Ken Nishihata,** and **Syunzo Fukatsu,** Central Research Laboratories, Meiji Seika Kaisha, Ltd., Morooka-cho, Kohoku-ku, Yokohama 222, Japan

The reaction of the imidate ester of methyl 7-acetamidoceph-3-em-4-carboxylate (2) and phenylacetyl chloride was followed by ^{13}C n.m.r. spectroscopy, which disclosed the intermediacy of the adduct of (2) with the acyl chloride. Transacylations of methyl 7-([1-^{13}C]acetamido)ceph-3-em-4-carboxylate (8) and methyl 7-{(±)-5-benzamido-5-methoxycarbonyl[1-^{13}C]valeramido}ceph-3-em-4-carboxylate (9) were also found to proceed through direct atack of the acyl chloride on the respective imidate ester.

CEPHALOSPORINS of pharmacological interest have been prepared by the exchange of the aminoadipoyl side-chain of cephalosporin C with other acyl groups. Methods of



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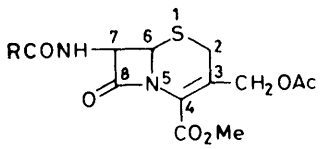
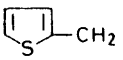
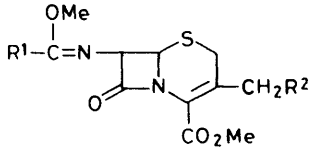
wide utility for these modifications are the acylation of 7-aminocephalosporanic acid (7-ACA), obtained *via* the corresponding imidate ester,[1] or alternatively the direct acylation of the imidate ester.[2] However, it has yet to be clarified whether for the latter procedure an acyl halide directly attacks the imidate ester or if methanolysis of the imidate ester[3] leading to 7-ACA precedes acylation. Isaka *et al.* studied the transacylation of natural penicillins and postulated the presence of the intermediate (1) from the i.r. and ^1H n.m.r. spectra of the reaction mixture.[4] We have recently succeeded in the isolation of cephalosporin imidate esters and now discuss the mechanism of transacylation by reference to n.m.r. studies on the ^{13}C-enriched compounds; there have previously been only a few reports on the isolation of the imidate esters in the cephamycin series.[5]

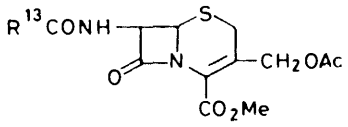
Reaction of methyl 7-acetamidoceph-3-em-4-carboxylate (2) with phosphorus pentachloride and *NN*-dimethylaniline in methylene chloride followed by the treatment of the imidoyl chloride with methanol gave methyl 7-(1-methoxyethylimino)ceph-3-em-4-carboxylate (5) in high yield. The imidate esters (6) and (7) were also obtained by a similar procedure. The imidate esters (5)—(7), though readily hydrolysed by atmospheric moisture, could be stored for about a month with adequate precautions. The structure of these imidate esters has been elucidated from their spectroscopic properties (^1H and ^{13}C n.m.r. data are collected in Table 1).



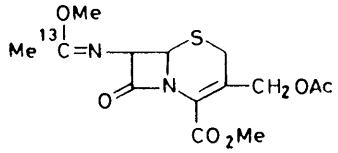
(2) R = Me
(3) R = $PhCH_2$
(4) R = 



(5) R^1 = Me, R^2 = OAc
(6) R^1 = Me, R^2 = H
(7) R^1 = Bu^n, R^2 = OAc



(8) R = Me
(9) R = $MeO_2CCH[CH_2]_3$ NHBz



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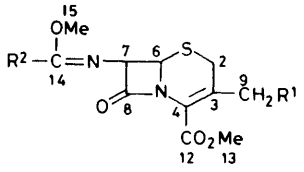
The imidate ester (5), when treated with phenylacetyl chloride in methylene chloride containing a trace amount

of methanol, afforded methyl 7-phenylacetamidoceph-3-em-4-carboxylate (3) in 82% yield. When this reaction was repeated in an n.m.r. tube for methyl 7-(1-methoxy-[1-^{13}C]ethylimino)ceph-3-em-4-carboxylate (10) (45% enriched), obtained from methyl 7-(2-thienylacetamido)-ceph-3-em-4-carboxylate (4) and [1-^{13}C]acetyl chloride, a singlet at δ 166.1 assignable to the labelled carbon of (10) slowly diminished in intensity and a new singlet began to develop at δ 180.7. After 5 h the former peak had disappeared, but the latter remained unchanged. However, the latter disappeared rapidly on addition of water or methanol, and a new signal attributable to methyl [1-^{13}C]acetate developed at δ 171.7. Alternatively, when phenyl[1-^{13}C]acetyl chloride (30% enriched) was used, the intensity of a singlet at δ 172.4 assignable to the enriched carbon of phenyl[1-^{13}C]acetyl chloride decreased and that of a singlet at δ 171.7 increased with the progress of the reaction. These results suggest that phenylacetyl chloride attacks the imidate carbon atom to form the intermediate (11) (the enriched imidate carbon signal shifted to δ 180.7), which is then decomposed by water or methanol to give (3) and methyl acetate.

TABLE 1

^{1}H and ^{13}C N.m.r. spectral data of imidate esters (5)—(7) [a]



R^1 = OCOMe, H

R^2 = Me, $CH_2CH_2CH_2Me$

	(5)	(6)	(7)
2-H	3.38 (2 H, ABq, *J* 18.8 Hz)	3.37 (2 H, ABq, *J* 18.8 Hz)	3.50 (2 H, ABq, *J* 18.8 Hz)
6-H	5.05 (1 H, d, *J* 5.0 Hz)	5.04 (1 H, d, *J* 5.0 Hz)	5.05 (1 H, d, *J* 5.0 Hz)
7-H	5.27 (1 H, d, *J* 5.0 Hz)	5.20 (1 H, d, *J* 5.0 Hz)	5.31 (1 H, d, *J* 5.0 Hz)
9-H	4.94 (2 H, ABq, *J* 12.5 Hz)	2.06 (3 H, s).	4.95 (2 H, ABq, *J* 12.5 Hz)
11-H	2.08 (3 H, s)		2.13 (3 H, s)
13-H	3.67 (3 H, s)	3.84 (3 H, s)	3.89 (3 H, s)
15-H	3.67 (3 H, s)	3.65 (3 H, s)	3.66 (3 H, s)
16-H	2.10 (3 H, s)	2.10 (3 H, s)	2.40 (2 H, t, *J* 7.0 Hz)
17,18-H			1.1—1.8 (4 H, m)
19-H			0.92 (3 H, t, *J* 7.0 Hz)

	(5)	(6)	(7)
C-2	26.4	30.3	26.6
C-3	125.6	131.0	125.0
C-4	125.1	122.0	125.5
C-6	67.8	67.3	67.2
C-7	58.8	58.2	58.9
C-8	162.1	162.9	162.1
C-9	63.1	20.3	63.3
C-10	170.8		171.0
C-11	20.9		21.0
C-12	165.8	165.6	165.8
C-13	53.2	52.6	53.1
C-14	166.1 [b]	166.0	168.9
C-15	53.4	53.3	53.2
C-16	16.9	17.0	30.2
C-17			28.1
C-18			22.6
C-19			13.9

[a] The assignments were made on the basis of the expected chemical shift behaviour and correlation with published data.[6] [b] Assigned on the basis of the values for ^{13}C-enriched compound (10).

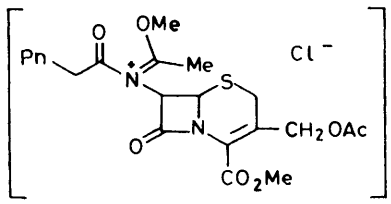
TABLE 2

Reactions of imidate esters (12a—c) with phenylacetyl chloride

Imidate ester	Yield (%) [a] (14)	Yield (%) [b] (14)		(15)
(12a)	89	92		
(12b)	88	64	(85) [c]	26
(12c)	82	77		

[a] In methylene chloride. [b] In chloroform. [c] The reaction mixture was treated with 6N-HCl to transform (15) into (14a).

The reactions of some relatively stable imidate esters [*viz.* ethyl *N*-phenylacetimidate (12a),[7] ethyl *N*-phenylpropionimidate (12b),[7] and ethyl *N*-(ethoxycarbonylmethyl)acetimidate (12c) [8]] were then studied; little is known on the behaviour of imidate esters towards acyl chlorides.[9] Treatment of (12a) and (12c), respectively, with phenylacetyl chloride in methylene chloride or chloroform, followed by addition of water into the reaction mixture, gave the amides (14a) and (14b) in good yield (Scheme 1). Similar treatment of (12b) in methylene chloride gave (14a) in good yield. However, the reaction of (12b) in chloroform gave (14a) and *N*-(1-ethoxyprop-1-enyl)benzylanilide (15) in 64 and 26%



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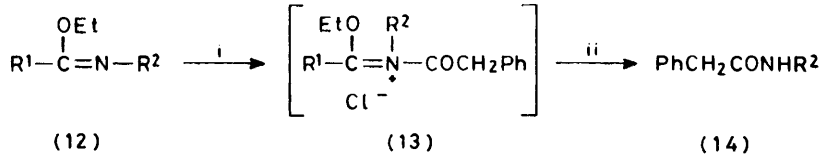


(12) (13) (14)

a; R^1 = Me, R^2 = Ph
b; R^1 = Et, R^2 = Ph
c; R^1 = Me, R^2 = CH_2CO_2Et

a; R^2 = Ph
b; R^2 = CH_2CO_2Et

SCHEME 1 *Reagents*: i, $PhCH_2COCl$; ii, H_2O

yield, respectively; the latter was then hydrolysed to (14a) by the action of hydrochloric acid. These results are summarized in Table 2. Formation of (15) would have proceeded through an intermediate (13b) (Scheme 2). ^{13}C N.m.r. spectroscopy shows the characteristic signal of the imidate carbon [δ 161.5 for (12a), 164.2 for (12b), and 164.9 for (12c)] to gradually diminish in intensity whilst a new signal associated with (13) appears at δ 174.8, 177.8, and 177.1, for (13a), (13b), and (13c), respectively, which then disappears on addition of water. The low-field shift observed for the imidate carbon signal can be explained in terms of the decrease in electron density at that carbon as a result of the formation of an acylimmonium ion. These results further support the foregoing suggestion that the transacylation proceeds through direct attack of acyl chloride on the imidate ester.

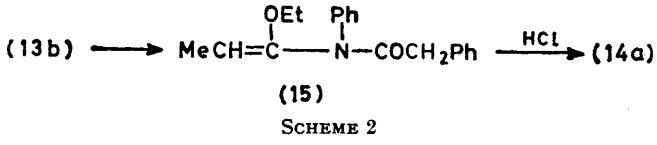
TABLE 3

Chemical shifts of the labelled carbons in each step of the transacylation reactions of (8) and (9)

		RC(=O)NH– *	RC(Cl)=N– *	RC(OMe)=N–HCl *	RC(OMe)=N– *	RC(=O)OMe *
(8)	R=Me	170.7	149.7	181.0	166.6	171.7
(9)	R=$MeO_2CCH[CH_2]_3$ NHCOPh	173.1 172.9 [a]	151.8	182.5	165.4	173.7

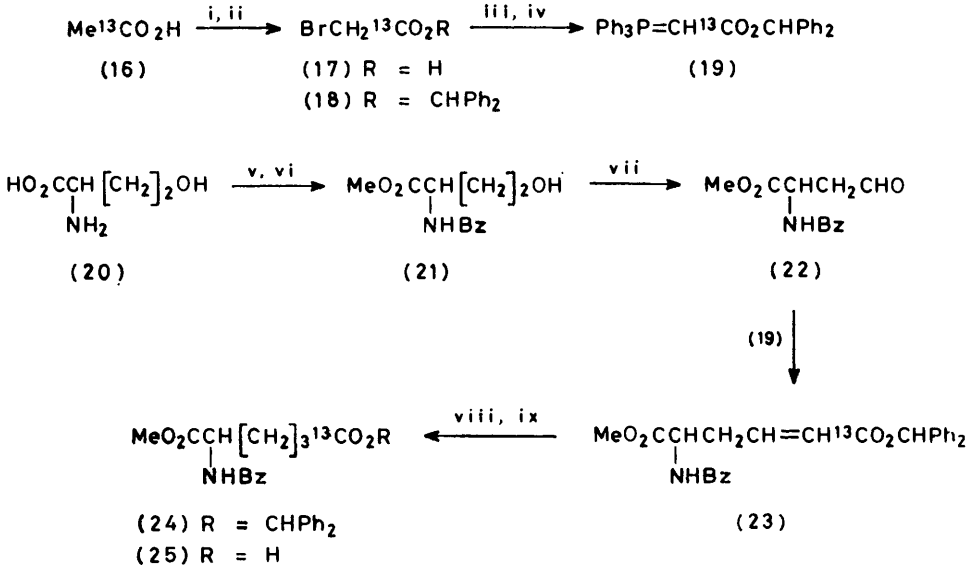
* ^{13}C-Labelled carbon.

[a] The splitting into two signals is due to diastereoisomers.



SCHEME 2

As the imidate ester of *N*-benzoylcephalosporin C dimethyl ester was too unstable to be isolated, its behaviour on transacylation was studied by ^{13}C n.m.r. spectroscopy and the result has been compared with that of the case of methyl 7-([1-^{13}C]acetamido)ceph-3-em-4-carboxylate (8). The methyl ester (8) and methyl 7-{(±)-5-benzamido-5-methoxycarbonyl[1-^{13}C]valeramido}ceph-3-em-4-carboxylate (9) * were treated successively with phosphorus pentachloride, methanol, phenylacetyl chloride, and water. Both the imidate ester hydrochlorides and imidate esters, derived from



SCHEME 3 *Reagents*: i, Br_2; ii, Ph_3CN_3; iii, Ph_3P; iv, NaOH; v, PhCOCl; vi, MeI; vii, DMSO–DCC, CF_3CO_2H–pyridine; viii, H_2/Pd–C; ix, CF_3CO_2H

(8) or (9), were stable in the reaction mixture as shown by the n.m.r. spectra (Table 3). New signals assignable to methyl acetate and methyl adipate appeared on addition of phenylacetyl chloride. The signals attributable to an intermediate such as (11) were not observed, presumably because this intermediate would be decomposed rapidly by methanol. Preparatively, (3) was

* The aminoadipoyl group of natural cephalosporin C has the (+)-configuration but we encountered no problems in using the (±)-mixture in the present work.

obtained from either (8) or (9) in 80 or 72% yields respectively.

The labelled compound (9) was synthesized as shown in Scheme 3. Bromo[1-^{13}C]acetic acid (17) {prepared [10] from [1-^{13}C]acetic acid (16) (90% enriched)} was converted into the diphenylmethyl ester (18) and then into the phosphorane (19). Methyl (±)-2-benzamido-4-hydroxybutyrate (21), obtained from (±)-homoserin (20) by standard methods, was oxidized with dicyclohexylcarbodi-imide in dimethyl sulphoxide to the aldehyde (22). Condensation of (19) and (22) gave diphenylmethyl (*E*)-(±)-5-benzamido-5-methoxycarbonyl[1-^{13}C]-pent-2-enoate (23) in 72% yield, from which the saturated free acid (25) was derived. Acylation of 7-ACA with (25) followed by methylation afforded (9).

We conclude from these results that the transacylation of cephalosporin proceeds through the intermediate (11).

EXPERIMENTAL

M.p.s were determined on a Yamato MP-21 melting-point apparatus. N.m.r. spectra were recorded on a Varian FT-80A spectrometer for solutions in deuteriochloroform with tetramethylsilane as internal standard (δ 0) unless otherwise stated. I.r. spectra were recorded on a Jasco IR-G instrument and mass spectra on a Hitachi M-80 double-beam spectrometer (70 eV). Chromatography was performed on Wako gel C-200. Methylene chloride, chloroform, and deuteriochloroform were dried over calcium chloride, methanol and dimethyl sulphoxide over 3A molecular sieves, and *NN*-dimethylaniline over sodium hydroxide. ^{13}C-Labelled compounds were 90% enriched unless otherwise stated.

Methyl 7-(1-Methoxyethylimino)ceph-3-em-4-carboxylate (5).*—To a suspension of methyl 7-acetamidoceph-3-em-4-carboxylate (2) (3.28 g) in methylene chloride (120 ml) cooled at −30 °C were added *NN*-dimethylaniline (4.24 g) and phosphorus pentachloride (5.20 g) and the mixture was stirred for 3 h. The temperature was lowered to −60 °C, dry methanol (10 ml) was added dropwise to the reaction mixture, and stirring was continued for 2 h. The mixture was poured into stirred aqueous sodium hydrogencarbonate at 0 °C with the pH being kept at 7—8.5 and the aqueous layer was extracted with methylene chloride. The extracts were washed twice with a 5% aqueous sodium hydrogencarbonate solution, dried (K_2CO_3), evaporated under reduced pressure, and the residual oil was dissolved in di-isopropyl ether (350 ml). The solution was treated with charcoal for 5 min and the filtrate was diluted with pentane (1.5 l) and allowed to stand overnight in a refrigerator to give needles (2.27 g, 66%), m.p. 110.5—112 °C; $\nu_{max.}$ (Nujol) 1 672 cm^{-1} (C=N); *m/e* 342 (M^+), 282, 255, 230, 210, and 170.

Methyl 7-(1-Methoxyethylimino)deacetoxyceph-3-em-4-carboxylate (6).*—This was prepared similarly in 80% yield. m.p. 56—58 °C; $\nu_{max.}$ (Nujol) 1 674 cm^{-1} (C=N); *m/e* 284 (M^+), 225, 172, and 152.

Methyl 7-(1-Methoxybutylimino)ceph-3-em-4-carboxylate (7).*—This was prepared similarly in 30% yield. m.p. 155.5—158 °C; $\nu_{max.}$ (Nujol) 1 668 cm^{-1} (C=N); *m/e* 384 (M^+), 325, 297, 236, 210, 172, and 155.

Reactions of the Imidate Esters with Phenylacetyl Chloride.—(*a*) A solution of the imidate ester (5) (1.0 g) and phenylacetyl chloride (0.6 g) in methylene chloride (20 ml) containing dry methanol (0.01 ml) was set aside at −30 °C. The reaction mixture was stirred for 5 h, washed with 5% aqueous sodium hydrogencarbonate, dried ($MgSO_4$), and evaporated under reduced pressure to dryness. Chromatography of the residue with chloroform afforded methyl 7-phenylacetamidoceph-3-em-4-carboxylate (3) (0.97 g, 80%), and methyl 7-acetamidoceph-3-em-4-carboxylate (2) (0.06 g, 6%), successively.

(*b*) To a cold (−20 °C) solution of the imidate ester (12b) (1.77 g) in chloroform (20 ml) was added phenylacetyl chloride (1.85 g). After standing for 20 h at that temperature, the reaction mixture was washed with 5% aqueous sodium hydrogencarbonate solution, dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography of the residue with benzene–ethyl acetate (20 : 1) afforded *N*-(1-ethoxyprop-1-enyl)benzylanilide (15) [(0.77 g, 26%). m.p. 44—47 °C; $\nu_{max.}$ (Nujol) 1 669 cm^{-1} (C=O) and 1 685 cm^{-1} (C=C); *m/e* 295 (M^+), 266, 211, 194, 177, 149, and 148; δ_H 1.26 (3 H, t, *J* 6.9 Hz, OCH_2Me), 1.47 (3 H, d, *J* 6.7 Hz, $=CHMe$), 3.76 (2 H, q, *J* 6.9 Hz, OCH_2), 3.76 (2 H, s, CH_2Ph), 4.58 (1 H, q, *J* 6.7 Hz, =CH), and 7.14—7.36 (10 H, m, ArH); δ_C 11.17 ($MeCH=$), 14.39 (OCH_2Me), 41.02 (CH_2Ph), 63.69 (OCH_2Me), 94.15 (=CHMe), 125.57, 126.35, 126.67, 128.31; 128.68; 129.77; 135.16, and 140.56 (aromatic), 150.17 (=CN), and 170.95 (C=O)], and phenylacetanilide (14a) [(1.36 g, 64%), m.p. 118 °C (lit.,[11] 117.5 °C)], successively.

(*c*) Similar treatment of the imidate ester (12c) (1.73 g) as described above followed by chromatography (benzene–ethyl acetate, 4 : 1) gave *N*-phenylacetylglycine ethyl ester (14b) (1.71 g, 77%), m.p. 82 °C (lit.,[12] 82 °C).

Transacylation Reaction.—To a solution of methyl 7-thienylacetamidoceph-3-em-4-carboxylate (4) (6.15 g) in methylene chloride (120 ml) cooled at −30 °C were added *NN*-dimethylaniline (6.36 g) and phosphorus pentachloride (7.8 g) and the mixture was stirred for 3 h. The temperature was lowered to −60 °C, methanol (12 ml) was added dropwise to the reaction mixture, and stirred continued at −40 °C for 2 h. *NN*-Dimethylaniline (17.9 g) and [1-^{13}C]acetyl chloride (1.0 g) were added successively to this solution at −30 °C, and stirring was continued at that temperature for 2 h. The reaction mixture was concentrated under reduced pressure and the residual oil was mixed with ethyl acetate (30 ml) and water (30 ml). After the pH was adjusted to 1.5 with 2N-HCl, the aqueous solution was extracted with ethyl acetate. The combined organic layers were washed five times with water, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was triturated with di-isopropyl ether to give *methyl 7-([1-^{13}C]acetamido)ceph-3-em-4-carboxylate* (8), which was recrystallized from methanol as prisms (3.06 g, 74%), m.p. 196—198 °C (decomp.) (Found: C, 47.79; H, 4.81; N, 8.44. $^{13}CC_{12}H_{16}N_2O_6S$ requires C, 47.69; H, 4.90; N, 8.51%); $\nu_{max.}$ (Nujol) 1 772, 1 741, 1 718, and 1 674 cm^{-1} (C=O); δ_H 2.06 (3 H, d, J_{C-H} 6.2 Hz, $Me^{13}CO$), 2.08 (3 H, s, OCOMe), 3.47 (2 H, ABq, *J* 18.0 Hz, 2-H_2), 3.86 (3 H, s, CO_2Me), 4.93 (1 H, d, *J* 5.1 Hz, 6-H), 4.96 (2 H, ABq, *J* 15.6 Hz, 3-CH_2), and 5.68 (1 H, q, *J* 8.6 Hz, 7-H); δ_C 20.97 ($OCOMe$), 23.00 (d, J_{C-C} 51.8 Hz, $Me^{13}CO$), 26.33 (C-2), 53.25 (CO_2Me), 57.21 (C-6), 58.86 (C-7), 63.12 (C-3, CH_2), 124.97 (C-3), 125.82 (C-4), 161.80 (CO_2Me), 165.25 (β-lactam), 170.68 (^{13}CO), and 170.92 (OCOMe).

Diphenylmethyl 2-Bromo[1-^{13}C]*acetate* (18).—To a solution

* This could be stored unchanged for *ca.* 1 month in a refrigerator (−20 °C) if moisture is excluded.

of 2-bromo[1-^{13}C]acetic acid (17) * (9.7 g) in chloroform (100 ml) was added a solution of diphenyldiazomethane (14.1 g) in chloroform (100 ml) at room temperature. The mixture was washed with 5% sodium hydrogencarbonate solution, dried, and evaporated under reduced pressure. Distillation gave a colourless *oil* (19.4 g, 91%), b.p. 157—160 °C at 2 Torr (Found: C, 59.4; H, 4.35. $^{13}CC_{14}H_{13}O_2Br$ requires C, 59.16; H, 4.28%); $\nu_{max.}$ (liquid film) 1 732 cm^{-1} (C=O); δ_H 3.79 (2 H, d, J_{C-H} 4.7 Hz), 6.88 (1 H, d, J_{C-H} 3.3 Hz), 7.28 (10 H, s); δ_C 25.81 (d, J_{C-C} 65.5 Hz, CH_2), 78.46 (CH), 127.03, 128.04, 128.46, and 139.39 (aromatic), and 165.84 (C=O).

Diphenylmethyl 2-Triphenylphosphoranylidene[1-^{13}C]*acetate* (19).—A solution of diphenylmethyl bromo[1-^{13}C]acetate (18) (18.3 g) and triphenylphosphine (16.5 g) in dry benzene (200 ml) was stirred for 4 h at room temperature in nitrogen. The phosphonium salt was filtered off, washed with benzene, and dried *in vacuo*, and then added to a mixture of benzene (300 ml) and water (150 ml) with stirring. The aqueous solution was basified to pH 9 with cold 1N-sodium hydroxide solution, separated from the organic layer after 30 min, and extracted with benzene. The combined organic layers were washed with water, dried ($MgSO_4$), and evaporated under reduced pressure to dryness. Recrystallization of the residue from ethanol gave the *phosphorane* as prisms (24.9 g, 86%), m.p. 195 °C (decomp.) (Found: C, 81.19; H, 5.84. $^{13}CC_{32}H_{27}O_2P$ requires C, 81.5; H, 5.6%); $\nu_{max.}$ (Nujol) 1 627 (C=O), 1 108 (Ph_3P), and 891 cm^{-1} (=CH); δ_H 3.02 (1 H, broad, =CH), 6.83 (1 H, d, J_{C-H} 3.6 Hz, C*H*Ph$_2$), 7.14—7.58 (15 H, m, Ph$_3$), and 7.17 (10 H, s, CH*Ph*$_2$); δ_C 30.81 (d, d, J_{C-P} 123.6 Hz, J_{C-C} 88.3 Hz, =CH), 75.48 (*C*HPh$_2$), 125.21, 125.79, 126.25, 126.86, 129.71, 129.85, 130.65, and 131.16 (aromatic), and 169.82 (d, $^2J_{C-P}$ 11.6 Hz, C=O).

Methyl (±)-2-*Benzamido*-4-*hydroxybutyrate* (21).—A solution of benzoyl chloride (56.2 g) in acetone (160 ml) was added dropwise into a mixture of (±)-homoserin (23.8 g) and potassium carbonate (82.9 g) in 50% aqueous acetone (240 ml) at −5 °C. After stirring for 30 min at 0 °C, precipitates were removed by filtration and the filtrate was concentrated under reduced pressure. The residual solution was washed with ethyl acetate and evaporated under reduced pressure to dryness. The residue was dissolved in *NN*-dimethylformamide (300 ml) containing methyl iodide (37.4 ml) and stirring was continued for 3 h at room temperature. To this solution was added water (1 l) containing acetic acid (3.5 ml) and the mixture was extracted five times with ethyl acetate. The combined extracts were washed with water, dried ($MgSO_4$), and evaporated under reduced pressure to leave a syrup, which was crystallized by allowing to stand overnight. The *amido-ester* (21) was washed with di-isopropyl ether and dried *in vacuo* (23.2 g, 49%). A sample, purified by chromatography with chloroform as eluant and recrystallized from water, had m.p. 89—91 °C (Found: C, 60.7; H, 6.4; N, 5.95. $C_{12}H_{15}NO_4$ requires C, 60.75; H, 6.37; N, 5.90%); $\nu_{max.}$ (Nujol) 1 726, 1 637, and 1 577 cm^{-1} (C=O); δ_H 1.65—2.44 (2 H, m, CHC*H*$_2$), 3.61—3.78 (2 H, m, C*H*$_2$OH), 3.77 (3 H, s, Me), 4.71—4.98 (1 H, m, CH), 7.38—7.46 (4 H, m, NH and Ph), and 7.74—7.86 (2 H, m, Ph); δ_C 35.11 (CH*C*H$_2$), 50.72 (CH), 52.61 (Me), 58.66 (CH$_2$OH), 127.19, 128.64, 131.98, and 133.42 (aromatic), 168.04 (*C*OPh), and 173.07 (*C*O$_2$Me).

Methyl (±)-3-*Formyl*-2-*benzamidopropionate* (22).—Methyl 2-benzamido-4-hydroxybutyrate (21) (11.9 g) was dissolved in a mixture of anhydrous dimethyl sulphoxide (75 ml) and benzene (75 ml) containing pyridine (4 ml) and trifluoroacetic acid (2 ml). Dicyclohexylcarbodi-imide (31 g) was added and the mixture was stirred overnight at room temperature. To the reaction mixture were added ethyl acetate (250 ml) and a solution of acetic acid (10 ml) in water (50 ml). After stirring for 1 h, dicyclohexylurea was filtered off and the filtrate was washed with 5% sodium hydrogencarbonate solution and water, dried (Na_2SO_4), and evaporated under reduced pressure to a syrup. Chromatography with benzene–ethyl acetate (2 : 1) gave compound (22) as a colourless oil (7.3 g, 62%), $\nu_{max.}$ (liquid film) 1 735, 1 724, 1 640, and 1 601 cm^{-1} (C=O); δ_H 3.18 (2 H, d, d, *J* 4.8, 0.4 Hz, CH$_2$), 3.75 (3 H, s, Me), 4.97 (1 H, t, d, *J* 4.8, 8.8 Hz, CH), 7.30—7.47 (4 H, m, NH and Ph), 7.73—7.82 (2 H, m, Ph), and 9.74 (1 H, d, *J* 0.4 Hz, CHO); δ_C 45.49 (CH$_2$), 47.90 (CH), 52.85 (Me), 128.62, 127.23, 131.97, and 133.51 (aromatic), 167.26 (*C*OPh), 171.34 (*C*O$_2$Me), and 199.64 (CHO).

Diphenylmethyl (E)-(±)-5-*Benzamido*-5-*methoxycarbonyl*-[1-^{13}C]*pent*-2-*enoate* (23).—A mixture of the labelled phosphorane (19) (24.4 g), the ester (22) (12.9 g), and benzene (200 ml) was heated under reflux for 2 h and evaporated under reduced pressure to leave a syrup, which was chromatographed with benzene–ethyl acetate (3 : 1) and then benzene–ethyl acetate (10 : 1). Compound (23) was recrystallized from ethanol as *prisms* (16.6 g, 75%), m.p. 108—109 °C (Found: C, 73.3; H, 5.65; N, 2.95. $^{13}CC_{26}H_{25}NO_5$ requires C, 73.17; H, 5.67; N, 3.15%); $\nu_{max.}$ (Nujol) 1 733, 1 638, and 1 600 cm^{-1} (C=O); δ_H 2.62—2.78 (2 H, m, CH$_2$), 3.71 (3 H, s, Me), 4.74—5.01 (1 H, m, NCH), 5.99 (1 H, d, d, $J_{CH=CH}$ 15.6 Hz, J_{C-H} 3.3 Hz, =CH^{13}C), 6.73—6.98 (1 H, m, CH=), 6.91 (1 H, s, C*H*Ph$_2$), 7.16—7.37 (4 H, m, NH and Ph), 7.28 (10 H, s, CH*Ph*$_2$), and 7.61—7.74 (2 H, m, Ph); δ_C 35.00 (CH$_2$), 51.76 (NCH), 52.73 (Me), 77.24 (*C*HPh$_2$), 124.96 (d, J_{C-C} 75.0 Hz, =*C*H^{13}C), 127.12, 127.92, 128.51, 128.66, 131.92, 132.05, 133.73, and 140.19 (aromatic), 143.03 (CH=), 164.73 (^{13}*C*O$_2$CHPh$_2$), 167.04 (*C*OPh), and 171.69 (*C*O$_2$Me).

(±)-5-*Benzamido*-5-*methoxycarbonyl*[1-^{13}C]*valeric Acid* (25).—The labelled ester (23) (10 g) was hydrogenated with a pressure of 3—4 kg cm^{-2} over 10% Pd–C (1 g) in ethanol (150 ml) at room temperature for 12 h. The catalyst was filtered off and the filtrate was evaporated under reduced pressure to dryness. The residue was dissolved in a mixture of 1,2-dichloroethane (60 ml) and anisole (20 ml). Trifluoroacetic acid (30 ml) was added to this solution maintained at 10—15 °C and stirring was continued at that temperature for 15 min. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in ethyl acetate–water (400 ml; 1 : 1). After the pH had been adjusted to 8.0 with aqueous sodium hydrogencarbonate, the organic layer was extracted with water. The combined aqueous layers were washed with ethyl acetate and then the pH was adjusted to 3.0 with 6N-HCl and further extracted with ethyl acetate. The combined organic layers were washed with water, dried ($MgSO_4$), evaporated under reduced pressure, and the residue was triturated with di-isopropyl ether. Compound (25) was recrystallized from ethyl acetate as *needles* (3.98 g, 63%), m.p. 103—105 C° (Found: C, 60.25; H, 6.05; N, 5.05.

* M.p. 46—48 °C (lit.,[10] m.p. 47—49 °C); δ_H 3.90 (2 H, d, J_{C-H} 4.7 Hz) and 11.45 (1 H, s); δ_C 25.04 (d, J_{C-C} 62.8 Hz, CH$_2$) and 173.42 (C=O).

$^{13}CC_{13}H_{17}NO_5$ requires C, 60.33; H, 6.12; N, 5.00%); $\nu_{max.}$ (Nujol) 1 730, 1 718, 1 692, and 1 544 cm^{-1} (C=O); δ_H ([$^2H_6$]DMSO) 1.55—1.90 (4 H, m, $CH_2CH_2CH_2{}^{13}C$), 2.15—2.37 (2 H, m, $CH_2{}^{13}C$), 3.65 (3 H, s, Me), 4.64—4.74 (1 H, m, CH), 7.46—7.58 (3 H, m, Ph), 7.82—7.94 (2 H, m, Ph), and 8.71 (1 H, d, J 7.2 Hz, NH); δ_C ([$^2H_6$]DMSO) 21.26 (d, $^2J_{C-C}$ 0.8 Hz, $CH_2CH_2{}^{13}C$), 29.91 ($CHCH_2$), 33.03 (d, J_{C-C} 55.0 Hz, $CH_2{}^{13}C$), 51.77 (CH), 52.42 (Me), 127.42, 128.19, 131.39, and 133.70 (aromatic), 166.58 (COPh), 172.69 (CO_2Me), and 174.06 (CO_2H).

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