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PHOSPHORUS IN ORGANIC SYNTHESIS—XI[1a]

AMINO ACIDS AND PEPTIDES—XXI[1b]

REACTION OF DIETHYL PHOSPHOROCYANIDATE(DEPC) WITH CARBOXYLIC ACIDS. A NEW SYNTHESIS OF CARBOXYLIC ESTERS AND AMIDES[2]

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Abstract—Diethyl phosphorocyanidate(DEPC) reacts with carboxylic acids in the presence of triethylamine leading to transient formation of acyl cyanides, but in presence of alcohols or amines, carboxylic esters or amides are produced. DEPC is especially effective for the synthesis of amides and peptides, and showed a satisfactory result on the Young racemization test.

Diethyl phosphorocyanidate(DEPC) was first synthesized by Saunders *et al.*[3] as a probable chemical weapon during World War II. Later Takamizawa and *et al.*[4] slightly modified the Saunders' method, and Chinese workers[5] reported another synthesis.

We have already disclosed that diphenyl phosphorazidate(DPPA)[6] is a quite useful synthetic reagent for peptide synthesis[7,8] and the Curtius reaction.[1a,7,9] DEPC, which is a dialkyl phosphoropseudohalide, was considered to be a compound related to DPPA, and our attention was focussed on the synthetic utility of DEPC which has not been described.

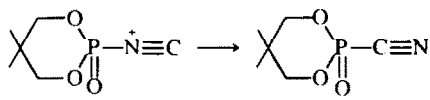
$X\text{–}PO(OR)_2$ DEPC: X = CN, R = Et
DPPA: X = N_3, R = Ph

Synthesis, purification, and properties of DEPC

DEPC is easily synthesized by the Arbuzov reaction of triethylphosphite with cyanogen bromide according to the method of Takamizawa *et al.*[4] DEPC, freshly prepared and distilled, has, however, two medium peaks in the nitrile region on the IR spectrum. Holmstedt and Larsson[10] have already recognized this phenomenon, and they assigned the absorption band at 2200 cm^{-1} to the C≡N stretching vibrations and the band at 2085 cm^{-1} either due to the isocyanide ($\overset{+}{N}\equiv\overset{-}{C}$) group obtained as a by-product in the synthesis or to hydrogen cyanide formed by a partial hydrolysis of DEPC.

When a colorless sample of DEPC showing two peaks in the nitrile region was allowed to stand at room temperature, it colored and the peak intensity at the lower wavenumber decreased. After several weeks, this peak completely disappeared. Redistillation afforded a pure colorless DEPC containing a single peak at 2200 cm^{-1}. Heating a crude DEPC below 100° caused no change on its IR spectrum. Raising the heating temperature to 120–140° increased the 2200 cm^{-1} band with decreasing to the 2085 cm^{-1} band, and cooling to room temperature resulted in the same relative intensity in both bands as that before heating. Polymerization or decomposition of DEPC seemed to occur above 180°. These facts suggest the band at the lower wavenumber is due to the isocyanide.[11]

During the preparation of this manuscript, Polish workers[12] have published an interesting paper on the synthesis of a dialkyl phosphoroisocyanidate. They prepared the isocyanodioxaphosphorinan **A** which readily isomerized to the cyano compound **B**:



A **B**

The structures of **A** and **B** have been well supported by spectral data (IR: ν_{NC} 2080 cm^{-1} and ν_{CN} 2210 cm^{-1}; ^{31}P-NMR(C_6H_6): +34.5 (> P(O)NC) and +28.5 ppm (> P(O)CN)). Furthermore they have claimed that the Arbuzov reaction of 2 - methoxy - 5,5 - dimethyl - 1,3,2 - dioxaphosphorinan with cyanogen bromide afforded a mixture of the 2-cyano compound **B** and the corresponding 2-bromo compound (^{31}P-NMR(C_6H_6): +14.7 ppm) in a 57:43 ratio.

The method for the preparation of DEPC might allow the formation of diethyl phosphorobromidate to some extent. However, diethyl phosphorobromidate prepared according to a literature[13] was quite a labile compound, especially to heat and showed no peak between 2400 and 1900 cm^{-1}. Thus DEPC freshly prepared and distilled may not contain any bromide. This was further confirmed by the ^{31}P-NMR spectral study. Freshly prepared DEPC showing two peaks at 2200 and 2085 cm^{-1} has two signals at +21.8 and +27.0 ppm in an integral ratio 10:1 corresponding to > P(O)CN and > P(O)NC structures, respectively. Purified DEPC, however, has a signal at 21.8 ppm only. Thus, the reported procedure[4] for the synthesis of DEPC was proved to give a mixture of DEPC and the corresponding isocyanide, which afforded pure DEPC upon standing followed by distillation.

Although attempted purification by the fractional distillation of a mixture of the cyanide and the isocyanide on a spinning band column failed, the isocyanide was considered to form a complex with some transition metal halides.[11] Preliminary experiments using IR spectral technique revealed that the order of the complexing ability of various metal chlorides with the isocyanide

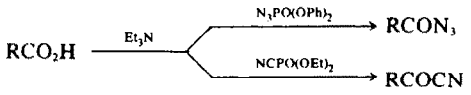
isomer was as followed: $CoCl_2$, $SnCl_2 > FeCl_2$, $CuCl_2$, $HgCl_2$, $AlCl_3$, CuCl, $FeCl_3$, $NiCl_2 > CdCl_2$, $SrCl_2$, $PbCl_2$, $MnCl_2$, $BaCl_2$, $MgCl_2$, HgCl. Thus, anhydrous cobaltous chloride was added to a sample of crude DEPC, followed by dilution with diethyl ether, to give hygroscopic, dark green precipitates containing a broad peak around 2100 cm^{-1} due to the isocyanide function. The filtrate on distillation gave pure DEPC showing a single peak at 2200 cm^{-1}. This work-up is quite rapid as the purification of crude DEPC, but further experiments will be required to obtain pure DEPC in satisfactory yield.

In view of the statement[14] that 95% of DEPC was hydrolysed at pH 7.2 during 1 h, investigation was made about the stability of DEPC toward acid and base. Treatment of DEPC in a mixture of ethyl acetate and benzene with 5% aqueous hydrochloric acid gave DEPC with 64% recovery. Further washing the DEPC solution with saturated aqueous sodium bicarbonate afforded DEPC with the recovery of 53%. This suggests that DEPC may be removed to some extent by acid and base washings when an excess of DEPC is used for the reaction.

Unless otherwise stated, pure DEPC containing a single peak at 2200 cm^{-1} was used for the reactions hereafter.

Reaction of carboxylic acids with DEPC

As the reaction of carboxylic acids with DPPA in the presence of triethylamine affords acyl azides,[6-9] DEPC was expected to give acyl cyanides[15] under similar reaction conditions:



Since ester and amide formations via acyl cyanides prepared by the reaction of alkylidene phosphoranes with nitrosonium ion was reported from our laboratories,[16] we had much interest in providing an improved method for the synthesis of acyl cyanides. Accordingly, benzoic acid was allowed to react with DEPC in the presence of triethylamine. Instead of the expected benzoyl cyanide, the corresponding dimer **1**[17] was obtained in 51% yield. As benzoyl cyanide is subject to dimerization by basic

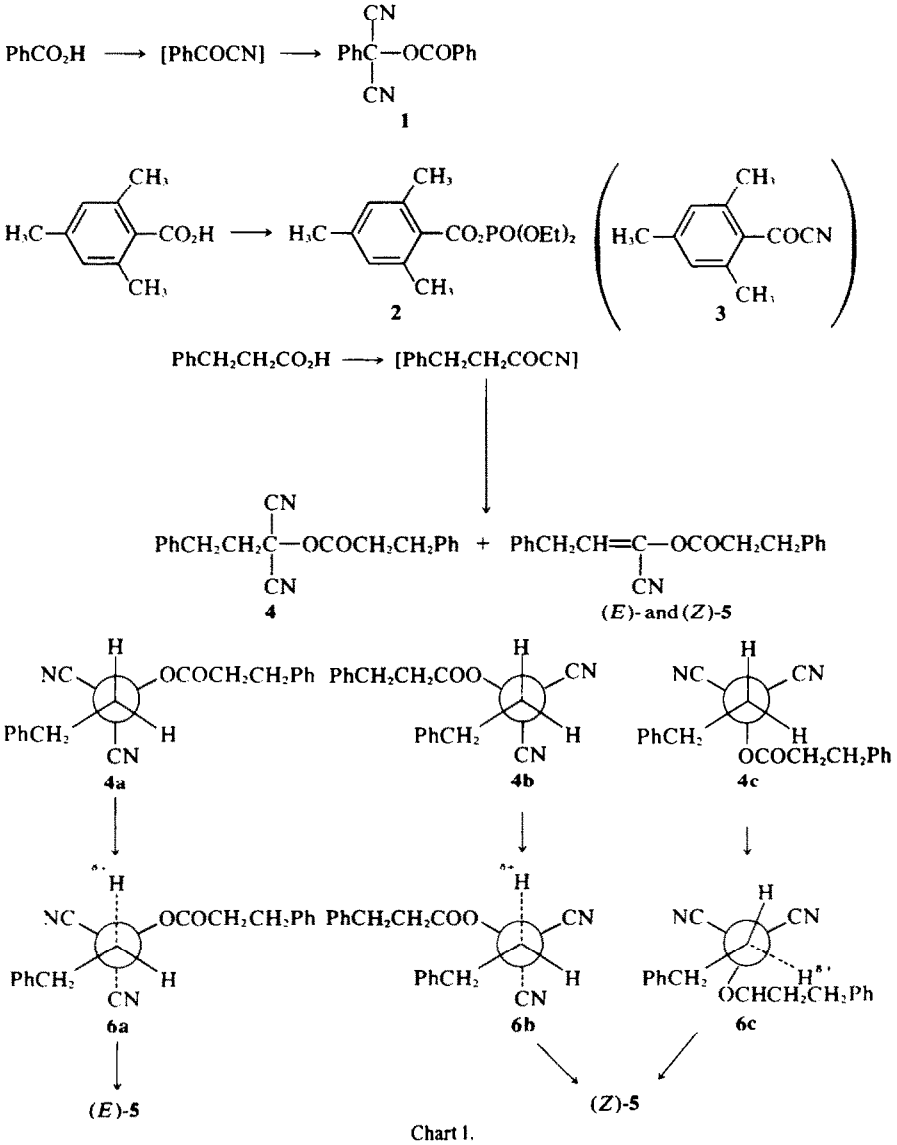


Chart 1.

catalysis,[15,17,18] the formation of the dimer **1** reveals the transient formation of benzoyl cyanide. To prevent the dimerization of acyl cyanides, 2,4,6-trimethylbenzoic acid expected to have a steric hindrance was chosen and treated with DEPC in the presence of triethylamine. Surprisingly, however, the stable acyl phosphate **2** was obtained in good yield in place of the expected acyl cyanide **3**.

Reaction of 3-phenylpropionic acid with DEPC in the presence of triethylamine also afforded the dimer **4** of 3-phenylpropionyl cyanide in 19% yield. Furthermore, two compounds having the same structural formula were isolated. Their spectral and analytical data shown in Experimental revealed that they are geometrical isomers, (*E*)- and (*Z*)-**5**, being produced by the elimination of hydrogen cyanide from the dimer **4**. The respective yields of (*E*)- and (*Z*)-**5** were 18 and 5.5%. Both (*E*)- and (*Z*)-**5** were also obtained in 40 and 23% yields, respectively, by the reaction of 3-phenylpropionyl bromide with cuprous cyanide. These two compounds afforded methyl 3-phenylpropionate with methanolic potassium hydroxide or triethylamine in good yields. The configurational assignment of (*E*)- and (*Z*)-**5** was not possible by their spectroscopic studies. However, tentative assignment was made by the mechanistic considerations. If the elimination of hydrogen cyanide from the dimer **4** proceeds via E2 mechanism, three conformations **4a–c** depicted as the Newman projection will be possible, among which **4a** will be the most stable because of the fewer non-bonded interactions. In the transition states **6a–c**, the least skew interaction will be expected in **6a** derived from **4a**. Thus **4a** will undergo E2 elimination to give the predominant isomer (*E*)-**5**. The other less stable rotamers **4b** and **4c** will similarly afford the minor isomer (*Z*)-**5** via **6b** and **6c**, as shown in Chart 1.

The above various reactions of carboxylic acids with DEPC in the presence of triethylamine will be summarized as Chart 2. The carboxylate anion will attack the phosphorus atom of DEPC to give the pentacovalent phosphorus compound **7** and the mixed anhydride **8**, both being in equilibrium. The reaction will stop at the stage of **8** in the case of sterically hindered 2,4,6 - trimethylbenzoic acid. The S_Ni type rearrangement of the cyano group in **7** or S_N2 type reaction of **8** with the cyanide ion will afford the acyl cyanide **9**, which is dimerized to give **10**. The processes are quite similar to the reaction of carboxylate anions with DPPA.[8]

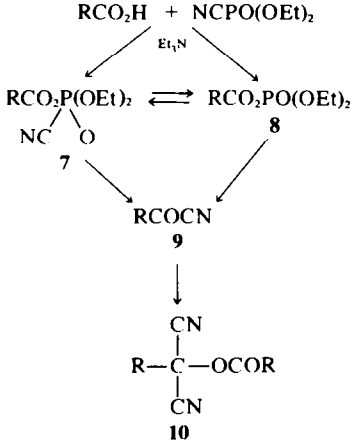


Chart 2.

Esterification of carboxylic acids

As the above experiments revealed that reactive acyl cyanides or acyl phosphates will be formed by the reaction of carboxylate anions with DEPC, the reaction was carried out in the presence of a suitable nucleophile.[15,16] First, reaction of benzoic acid or 3-phenylpripionic acid with DEPC in the presence of alcohols was investigated. The results were summarized in Table 1, showing that both carboxylic acids underwent esterification. The yields were not satisfactory in each case, but the advantage of the esterification using DEPC may be that the reaction proceeds under comparatively mild conditions (− 15° ~ room temperature, almost neutral solution).

Table 1. Esterification of carboxylic acids

$$RCO_2H + R'OH \xrightarrow[Et_3N]{NCPO(OEt)_2} RCO_2R'$$

Expt.	Ester (yield, %)
(a)	$PhCO_2Me$ (35)
(b)	$PhCO_2Et$ (56)
(c)	$PhCO_2$-iso-Pro (47)
(d)	$Ph(CH_2)_2CO_2Me$ (52)
(e)	$Ph(CH_2)_2CO_2$-iso-Pro (28)

A new synthesis of amides[19]

In general, an amine is a much superior nucleophile to an alcohol. DPPA was already revealed as a convenient reagent for the synthesis of amides, especially for the racemization-free peptide synthesis.[6–8] These facts led us to investigate the possibility of DEPC as a reagent for the amide synthesis from carboxylic acids and amines.

Benzoic acid was allowed to react with cyclohexylamine and DEPC in the presence of triethylamine. The amide bond formation smoothly occurred to give N-cyclohexylbenzamide in quite a high yield. The presence of triethylamine is indispensable because N-cyclohexylbenzamide was obtained in lower yield when the above reaction was conducted without triethylamine. Interestingly, N,N'-dicyclohexylcarbodiimide, a well-known coupling reagent, was completely uneffective in the above case.

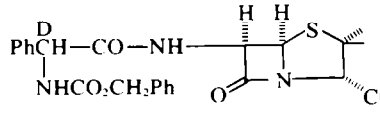
As shown in Table 2, the amide synthesis from a carboxylic acid and an amine, both including aromatic and aliphatic ones, was conveniently achieved by the combination of DEPC and triethylamine in dimethylformamide. It is noteworthy that the method could be effectively applied to the synthesis of derivatives of important antibiotics, ampicillin and cephalexin.[20] The reaction of 2,4,6 - trimethylbenzoic acid with tert-butylamine sluggishly proceeded to give N - tert - butyl - 2,4,6 - trimethylbenzamide in only 9% yield, while the condensation of 2,4,6 - trimethylbenzoic acid with n-butylamine afforded the corresponding N - n - butyl amide in 43% yield. This is not unexpected because of their severe steric hindrance. However, the mixed anhydride **2** from 2,4,6 - trimethylbenzoic acid and DEPC reacted with tert-butylamine to furnish N - tert - butyl - 2,4,6 - trimethylbenzamide in 35% yield.

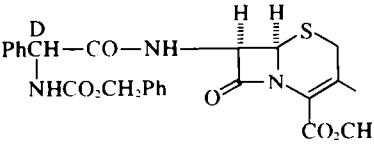
Finally, our attention was focused on the utility of DEPC for racemization-free peptide synthesis. The supersensitive Young test[21] involving the condensation of benzoyl-L-leucine with glycine ethyl ester was adopted to check the possibility. The results summarized in Table 3 show that DEPC may be efficiently used for the

Table 2. Preparation of amides

$$RCO_2H + R'NHR'' \xrightarrow[Et_3N]{NCPO(OEt)_2} RCONR'R''$$

Expt.	Amide (yield, %)
(f) (i)	PhCONH-cyclohexyl (97)
(f) (ii)	PhCONH-cyclohexyl (70)
(f) (iii)	PhCONH-cyclohexyl (0)
(g)	PhCONH-n-Bu (94)
(h)	$PhCONEt_2$ (91)
(i)	(3-CH_3)-$PhCONEt_2$ (86)
(j)	$PhCH_2CONHPh$ (83)
(k)	$CH_3(CH_2)_4CONHPh$ (84)
(l)	$CH_3(CH_2)_4CONHCH_2Ph$ (85)
(m)	(2,4,6-TriCH_3)-PhCONH-n-Bu(43)
(n) (i)	(2,4,6-TriCH_3)-PhCONH-t-Bu (9)
(n) (ii)	(2,4,6-TriCH_3)-PhCONH-t-Bu (35)

(o)  (71)

(p)  (100)

racemization-free peptide synthesis. The reaction proceeds quite rapidly, and 1 h (reaction at 0° for 0.5 h and then at 20° for 0.5 h) will be sufficient to couple benzoyl-L-leucine with glycine ethyl ester. No or little "chloride effect"[22,23] was observed in the DEPC method, because glycine ethyl ester hydrochloride together with an equivalent of triethylamine can be used without apparent racemization. This as well as the suitability of highly polar dimethylformamide is quite similar to the case of the DPPA method.[6-8] We also examined the coupling ability of dimethyl phosphorocyanidate in this case. In contrast to DEPC, the dimethyl analogue was not so effective for the Young test on the material yield.

Application of the DEPC method to the peptide synthesis and the other synthetic utility of DEPC are now actively under way.†‡

EXPERIMENTAL

Unless otherwise stated, mps were measured on a hot stage apparatus and uncorrected; IR spectra were measured in either Nujol mulls or KBr tablets for crystals and in liquid films for oils; ^1H NMR spectra (60 or 100 MHz) were measured in $CDCl_3$ or CCl_4, and chemical shifts (δ) are given in ppm relative to internal TMS; ^{31}P NMR spectra (24.3 MHz) were measured without solvent, and chemical shifts are reported in ppm upfield from external 85% H_3PO_4; mass spectra were measured at 70 eV. Silica gel (Wakogel C-200) was used for column chromatography. The organic solns were dried over either Na_2SO_4 or $MgSO_4$ before vacuum evaporation.

Diethyl phosphorocyanidate (DEPC). Prepared from triethylphosphite and cyanogen bromide according to the literature.[4] Distillation at 104–105° (19 mm Hg) (lit.[4] 103–104° (20 mm Hg)) gave a colorless oil, IR 2200, 2085 cm^{-1}, ^1H NMR 1.4 (6H, triplet, J = 7 Hz, 2 × CH_3), 4.3 (4H, multiplet, 2 × CH_2), ^{31}P NMR +21.8 (P–C≡N), +27.0 (P–N≡C). After standing at room temp. for several weeks, it colored and the peak at 2085 cm^{-1} disappeared. Redistillation at 94–95° (14 mm Hg) afforded pure DEPC in 70% yield as a colorless oil, IR 2200, 1305, 1165, 1030, 800, 760 cm^{-1}. ^1H NMR 1.4 (6H, triplet, J = 7 Hz, 2 × CH_3), 4.3 (4H, multiplet, 2 × CH_2), ^{31}P NMR +21.8 (P–C≡N).

Attempted purification of crude DEPC *by the metal complex formation.* To a crude sample of DEPC (5 g) showing two peaks at 2200 and 2085 cm^{-1} on its IR spectrum was added anhydrous cobaltous chloride (1 g). The mixture became hot and gelatinous. After 0.5 h, the mixture became a blue green solution. Dry diethyl ether (30 ml) was added to the solution after 1 h. The resultant precipitates showing a broad peak around 2100 cm^{-1} were filtered. The filtrate was evaporated and distilled at 81–82° (6 mm Hg) to give pure DEPC (1.45 g, 29% recovery) exhibiting a single peak at 2200 cm^{-1}.

Stability of DEPC *toward acid and base.* A solution of DEPC (0.551 g) in a mixture of AcOEt (20 ml) and C_6H_6 (10 ml) was successively washed with 5% HCl aq (2 × 10 ml), H_2O (10 ml), and sat NaCl aq (2 × 10 ml). Drying followed by evaporation afforded 0.35 g (64% recovery) of DEPC.

A solution of DEPC after washing as above was further washed with sat $NaHCO_3$ aq (2 × 10 ml), H_2O (10 ml), and sat NaCl aq. Drying followed by evaporation afforded 0.29 g (53% recovery) of DEPC.

Dimethyl phosphorocyanidate. Prepared by the action of trimethylphosphite with cyanogen bromide and purified analogously as the preparation of DEPC, 50% yield, b.p. 65–66°

Table 3. The Young test

$$\text{Bz-L-Leu-OH} + \text{H-Gly-OEt} \cdot \text{HCl} \xrightarrow[Et_3N]{NCPO(OEt)_2} \text{Bz-Leu-Gly-OEt}$$

Expt.	Reaction temp (°C)	Reaction time(h)	Yield (%)	m.p.[a] (°C)	$[\alpha]_D$ in EtOH	L-Isomer[b] (%)
(q)	0; 20	0.5; 0.5	83	160–161	−32.3	95
(r)	0; 20	0.5; 1	87	158–160	−32.1	94
(s)	0; 20	0.5; 4	86	158.5–160	−32.7	96
(t)[c]	0; 20	0.5; 4	53	158–160	−32.3	95

[a] Lit. values: L-isomer, 156.5–157°; racemate, 146°.

[b] Calculated by the equation: observed $[\alpha]_D \times 100/-34°$ (optical rotation of pure Bz-L-Leu-Gly-OEt).

[c] Using dimethyl phosphorocyanidate.

†The utility of DEPC for the direct preparation of thiol esters from carboxylic acids and thiols (S. Yamada, Y. Yokoyama and T. Shioiri, *J. Org. Chem.* **39**, 3302 (1974)) as well as for solid-phase peptide synthesis (S. Yamada, N. Ikota, T. Shioiri and S. Tachibana, *J. Am. Chem. Soc.* **97**, 7174 (1975)) was communicated in preliminary form.

‡Recently W.-Y. Chen and R. K. Olsen (*J. Org. Chem.* **40**, 350 (1975)) have reported that studies of selected coupling methods for attachment of amino acid derivatives to *cis*- and *trans* - 4 - aminocyclohexanecarboxylic acids have shown the DEPC method to be a much more satisfactory coupling method than the carbodi-imide, p-nitrophenyl active ester, and symmetrical anhydride methods.

(4 mm Hg) (lit.[5] 80° (9 mm Hg)), IR 2200, 1300, 1175, 1030, 850, 785 cm^{-1}, NMR 3.9 (6H, doublet, J = 13 Hz, $2 \times CH_3$).

Reaction of carboxylic acids with DEPC

Reaction of benzoic acid with DEPC *in the presence of triethylamine.* To a mixture of benzoic acid (1.22 g, 10 mM) and DEPC (1.63 g, 10 mM) in THF (10 ml) was added triethylamine (1.01 g, 10 mM) in THF (5 ml) during 1 h with ice-cooling and stirring. After the addition, the mixture was stirred at 0° for 10 min, then at room temp. for 5 min. Evaporation at 20° afforded a yellow oil which was purified over silica gel (60 g) with n-hexane and AcOEt (20:1) to give a pale yellow solid (0.91 g). Recrystallization from n-hexane and AcOEt afforded the dimer (**1**, 0.67 g, 51%) as colorless needles, mp 98–99° (lit.[18] 94–95°), IR 2270, 2260, 1745, 1605 cm^{-1}. (Found: C, 73.25; H, 3.79; N, 10.69. $C_{16}H_{10}O_2N_2$ requires: C, 73.27; H, 3.84; N, 10.68%).

Reaction of 2,4,6-*trimethylbenzoic acid with* DEPC *in the presence of triethylamine.* To a stirred mixture of 2,4,6-trimethylbenzoic acid (0.656 g, 4 mM) and DEPC (0.716 g, 4.4 mM) in DMF (10 ml) was added triethylamine (0.424 g, 4.4 mM) with ice-cooling. The mixture was stirred at 0° for 15 min, and at room temperature for 4 h. After dilution with AcOEt (100 ml) and C_6H_6 (50 ml), the mixture was successively washed with 5% HCl aq (2×40 ml), H_2O (40 ml), sat NaCl aq (2×40 ml), sat $NaHCO_3$ aq (2×40 ml), H_2O (40 ml), and sat NaCl aq (2×40 ml). Drying followed by evaporation gave the mixed anhydride (**2**, 1.064 g, 88.7%) as an orange yellow liquid. IR 1770, 1610, 1300, 1160, 1030 cm^{-1}, NMR 1.40 (6H, triplet, J = 7 Hz, $2 \times CH_3CH_2$), 2.30 (3H, singlet, 4-CH_3), 2.36 (6H, singlet, 2,6-di-CH_3), 4.3 (4H, quintet, $2 \times CH_2CH_3$), 6.78 (2H, singlet, 3,5-di-H). (Found: C, 55.34; H, 7.08. $C_{14}H_{21}O_5P$ requires: C, 56.00; H, 7.00%).

3-Phenylpropionyl bromide. A mixture of 3-phenylpropionic acid (9.0 g, 0.06 M) and phosphorus tribromide (16.2 g, 0.06 M) was heated at 100° for 1 h. C_6H_6 was added to the mixture, and the C_6H_6 solution was decanted. Evaporation of the C_6H_6 followed by distillation at 125–128° (6 mm Hg) gave 3-phenylpropionyl bromide (8.7 g, 68%) as a colorless oil, IR 1810, 750, 700 cm^{-1}.

Reaction of 3-phenylpropionyl bromide with cuprous cyanide. A mixture of 3-phenylpropionyl bromide (8.5 g, 0.04 M) and cuprous cyanide (8.0 g, 0.09 M) in toluene (25 ml) containing a little amount of P_2O_5 was refluxed for 18 h. The resultant precipitates were filtered and washed with C_6H_6. The combined filtrates were evaporated to give a dark brown oil (5.9 g), which was fractionated by a silica gel (250 g) column chromatography using C_6H_6 and n-hexane (1:1). The first fraction to be eluted was (*Z*)-**5** (1.33 g, 23%) as a pale yellow oil, b.p. 190° (0.06 mm Hg), IR 2320, 1775 cm^{-1}, NMR 2.5–3.0 (4H, multiplet, $(CH_2)_2Ph$), 3.53 (2H, doublet, J = 8 Hz, CH_2CH), 6.00 (1H, triplet, J = 8 Hz, CH), 7.1 (10H, singlet, $2 \times C_6H_5$). Mass *m/e* 291 (M^+), 133, 105 (base peak), 91. (Found: C, 78.56; H, 5.98; N, 5.00. $C_{19}H_{17}O_2N$ requires: C, 78.33; H, 5.88; N, 4.81%). The second fraction to be eluted was (*E*)-**5** (2.3 g, 40%) as a pale yellow oil, b.p. 188° (0.04 mm Hg); IR 2320, 1775 cm^{-1}; NMR 2.5–3.0 (4H, multiplet, $(CH_2)_2Ph$), 3.16 (2H, doublet, J = 8 Hz, CH_2CH), 5.97 (1H, triplet, J = 8 Hz, CH), 7.1 (10H, singlet, $2 \times C_6H_5$); Mass *m/e* 291 (M^+), 133, 105, 91 (base peak). (Found: C, 78.52; H, 5.93; N, 4.90. $C_{19}H_{17}O_2N$ requires: C, 78.33, H, 5.88; N, 4.81%).

Reaction of 3-phenylpropionic acid with DEPC *in the presence of triethylamine.* To a stirred mixture of 3-phenylpropionic acid (0.75 g, 5 mM) and DEPC (0.83 g, 5 mM) in THF (5 ml) was added triethylamine (0.50 g, 5 mM) in THF (5 ml) during 0.5 h with ice-cooling. The mixture was stirred at 0° for 10 min, and then at 25° for 5 min. Evaporation at 25° gave a pale yellow oil which was chromatographed over silica gel (60 g) with n-hexane and AcOEt (10:1) to give a yellow oil (0.39 g), which was again subjected to a silica gel (30 g) column chromatography with n-hexane and C_6H_6 (4:1).

The first fraction (0.16 g) was a 1:3 mixture of (*Z*)-**5** and **4**, the structures and ratio of which were determined by its NMR spectral analysis. The yield of (*Z*)-**5** was 5.5%. A similar experiment to the above in a larger scale afforded pure **4** as colorless prisms (n-hexane), m.p. 65–70°, IR 2250, 1765 cm^{-1}, NMR 2.4–3.1 (8H, multiplet, $4 \times CH_2$), 7.2 (10H, multiplet, $2 \times C_6H_5$), Mass *m/e* 318 (M^+), 168, 107, 105, 91 (base peak), 77. (Found: C, 75.17; H, 5.76; N, 8.93. $C_{20}H_{18}O_2N_2$ requires: C, 75.45; H, 5.70; N, 8.80%). The second fraction (0.07 g) was a 3:4 mixture of **4** and (*E*)-**5**, the structures and ratio of which were determined by its NMR spectral analysis. The total yield of **4** was 19%. The third fraction (0.09 g) was (*E*)-**5**, the total yield of which was 18%.

Methyl 3-phenylpropionate from **5**. (*i*) To a mixture of (*E*)-**5** (0.20 g, 0.69 mM) in MeOH (2 ml) was added 1% KOH–MeOH (0.4 ml). The mixture was stirred at room temperature overnight, and evaporated. The residue was dissolved in C_6H_6, and the C_6H_6 soln was successively washed with 10% HCl aq, H_2O, sat $NaHCO_3$ aq, and sat NaCl aq. Drying followed by evaporation gave methyl 3-phenylpropionate (0.19 g, 84%) as a colorless oil, b.p. 74–76° (3 mm Hg) (lit.[24] 118–120° (21 mm Hg)), IR 1750, 1610, 750, 700 cm^{-1}, NMR 2.5 and 2.9 (4H, two sets of multiplet, $2 \times CH_2$), 3.56 (3H, singlet, CH_3), 7.1 (5H, singlet, C_6H_5).

The same treatment of (*Z*)-**5** with methanolic potassium hydroxide as above afforded methyl 3-phenylpropionate.

(ii) A mixture of (*Z*)-**5** (0.56 g, 1.9 mM) and triethylamine (0.19 g, 1.9 mM) in MeOH (4 ml) was stirred at room temp. overnight, and evaporated to give methyl 3-phenylpropionate (0.61 g, 98%). The same treatment of (*E*)-**5** with triethylamine in methanol as above afforded methyl 3-phenylpropionate.

Esterification of carboxylic acids

General procedure. To a stirred mixture of a carboxylic acid (5 mM), DEPC (5 mM), and an alcohol (10 mM) in DMF (5 ml) was added triethylamine (5 mM) in DMF (5 ml) at −10 ~ −15°, and the mixture was stirred at room temp. overnight. Benzene (100 ml) was added to the mixture, and the benzene solution was successively washed with 10% HCl aq, H_2O, sat $NaHCO_3$ aq, and sat NaCl aq. Drying followed by evaporation gave an oil which was purified by a silica gel column chromatography with n-hexane and AcOEt (9:1). The yields are given in Table 1.

(a) *Methyl benzoate.* Prepared as above using 5 mM of MeOH; a colorless oil, IR 1730, 1280, 710 cm^{-1}, NMR 3.82 (3H, singlet, CH_3), 7.3 (3H, multiplet, 3,4,5-tri-H), 7.9 (2H, multiplet, 2,6-di-H).

(b) *Ethyl benzoate.* The reaction was carried out at 0° for 0.5 h and then at 30° for 1 h using 5.5 mM of EtOH; a colorless liquid, b.p. 64° (3 mm Hg) (lit.[25] 94° (14 mm Hg)), IR 1725, 1290, 720 cm^{-1}, NMR 1.40 (3H, triplet, J = 7 Hz, CH_3), 4.25 (2H, quartet, J = 7 Hz, CH_2), 7.4 (3H, multiplet, 3,4,5-tri-H), 8.0 (2H, multiplet, 2,6-di-H).

(c) *Isopropyl benzoate.* Prepared as in the general procedure; a colorless oil, b.p. 102° (24 mm Hg) (lit.[26] 106.5–107.5° (21 mm Hg)), IR 1720, 1270, 1110, 700 cm^{-1}, NMR 1.35 (6H, doublet, J = 7 Hz, $2 \times CH_3$), 5.2 (1H, quintet, J = 7 Hz, CH), 7.4 (3H, multiplet, 3,4,5-tri-H), 8.0 (2H, multiplet, 2,6-di-H).

(d) *Methyl 3-phenylpropionate.* Prepared analogously and identified with a sample obtained from **5**.

(e) *Isopropyl 3-phenylpropionate.* Obtained as a colorless oil, IR 1740, 1380, 1170, 740, 700 cm^{-1}, NMR 1.10 (6H, doublet, J = 8 Hz, $2 \times CH_3$), 2.6 (4H, multiplet, $2 \times CH_2$), 4.85 (1H, quintet, J = 8 Hz, CH), 7.05 (5H, singlet, C_6H_5).

A new synthesis of amides

General procedure. To a stirred mixture of a carboxylic acid (4 mM), an amine (4.4 mM), and DEPC (4.4 mM) in DMF (10 ml) was added triethylamine (4.2 mM) at 0° during 10 min. The mixture was stirred at 0° for 0.5 h, and then at room temp. for 1 h. The mixture was diluted with AcOEt (100 ml) and C_6H_6 (50 ml), and successively washed with 5% HCl aq, H_2O, sat $NaHCO_3$ aq, and sat NaCl aq. Drying followed by evaporation gave a crude amide, which was purified by distillation, recrystallization or column chromatography. The yields were given in Table 2.

(f) N-*Cyclohexylbenzamide.* (i) The reaction was carried out as above on half the scale. Purification was made by Al_2O_3 column chromatography using benzene: colorless needles (EtOH), m.p. 151–152° (lit.[27] 149°), IR 3200, 1630, 1540, 700 cm^{-1}, NMR 1.5 (10H, multiplet, C_5H_{10}), 3.9 (1H, multiplet, CH), 6.0 (1H, broad singlet, NH), 7.4 (H, multiplet, 3,4,5-tri-H), 7.7 (2H, multiplet, 2,6-di-H). (Found: C, 76.56; H, 8.54; N, 6.87. $C_{13}H_{17}ON$ requires: C, 76.84; H, 7.88; N, 6.90%).

(ii) The reaction was carried out as above without triethylamine, and the reaction time was extended to 24 h.

(iii) A mixture of benzoic acid (0.488 g, 4 mM), cyclohexylamine

(0.436 g, 4.4 mM) and N,N'-dicyclohexylcarbodiimide (0.824 g, 4 mM) in DMF (15 ml) was stirred at room temp. overnight. The resultant precipitates were filtered, and proved to be cyclohexylamine salt of benzoic acid (0.581 g, 66%) by comparisons with the authentic sample prepared from cyclohexylamine and benzoic acid.

The filtrate was treated as in the general work-up, during which N,N'-dicyclohexylurea was filtered. Evaporation gave an orange liquid which has no spot of N-cyclohexylbenzamide on its thin layer chromatogram.

(g) N-*n-Butylbenzamide.* The reaction was carried out in a half scale as in the general procedure. The crude product was purified by distillation at 112° (0.2 mm Hg) (lit.[28] m.p. 28°) to give a colorless oil, which was identified with the authentic sample of N-n-butylbenzamide.[8]

(h) N,N-*Diethylbenzamide.* The reaction was carried out in a half scale as in the general procedure. Purification was made by distillation at 111° (2 mm Hg) (lit.[29] 150–151° (15 mm Hg)) to give a colorless oil, IR 1630, 740, 710 cm^{-1}, NMR 1.18 (6H, triplet, J = 7 Hz, 2 × CH_3), 3.41 (4H, quartet, J = 7 Hz, 2 × CH_2), 7.34 (5H, singlet, C_6H_5).

(i) N,N-*Diethyl*-3-*methylbenzamide.* Prepared as in the general procedure; a colorless oil, b.p. 126–127° (2.5 mm Hg) (lit.[30] 160° (19 mm Hg)), IR 1630, 800, 750 cm^{-1}, NMR 1.13 (6H, triplet, J = 7 Hz, 2 × CH_3), 2.34 (3H, singlet, 3-CH_3), 3.30 (4H, quartet, J = 7 Hz, 2 × CH_2), 7.0 (4H, multiplet, C_6H_4).

(j) *Phenylacetanilide.* Prepared as in the general procedure; colorless needles (EtOH), m.p. 118–119° (lit.[31] 117°), IR 3260, 1650, 1570, 705 cm^{-1}, NMR 1.70 (1H, singlet, NH), 3.70 (2H, singlet, CH_2), 7.3 (10H, multiplet, 2 × C_6H_5). (Found: C, 79.63; H, 6.23; N, 6.84. $C_{14}H_{13}ON$ requires: C, 79.59; H, 6.20; N, 6.63%).

(k) *Hexananilide.* Prepared as in the general procedure; colorless needles (EtOH), m.p. 97.5–98° (lit.[32] 95°), IR 3300, 1675, 1605, 1550, 755 cm^{-1}, NMR 0.90 (3H, triplet, J = 6 Hz, CH_3), 1.3 and 1.7 (6H, multiplet, 3 × CH_2), 2.33 (2H, triplet, J = 7 Hz, CH_2CO), 5.7 (1H, broad singlet, NH), 7.3 (5H, multiplet, C_6H_5). (Found: C, 75.38; H, 8.98; N, 7.30. $C_{12}H_{17}ON$ requires: C, 75.38; H, 8.90; N, 7.33%).

(l) N-*Benzylhexanamide.* Prepared as in the general procedure; colorless plates (EtOH aq), m.p. 52–52.5°, IR 3280, 1630, 1555, 730, 700 cm^{-1}, NMR 0.84 (3H, triplet, J = 7 Hz, CH_3), 1.2 and 1.5 (6H, multiplet, 3 × CH_2), 2.03 (2H, triplet, J = 7 Hz, CH_2CO), 4.09 (2H, doublet, J = 7 Hz, CH_2N), 6.9 (1H, broad singlet, NH), 7.10 (5H, singlet, C_6H_5). (Found: C, 76.32; H, 9.45; N, 6.57. $C_{13}H_{19}ON$ requires: C, 76.05; H, 9.33; N, 6.82%).

(m) N - *n* - *Butyl* - 2,4,6 - *trimethylbenzamide.* The reaction was carried out as in the general procedure, but the reaction time was extended to 20 h. The crude product was purified by silica gel column chromatography using AcOEt–n-hexane (1 : 4) followed by recrystallization from n-hexane to give colorless needles, m.p. 85–87° (lit.[28] 80–81°), IR 3240, 1650, 1550, 860, 730 cm^{-1}, NMR 0.95 (3H, triplet, J = 7 Hz, CH_3CH_2), 1.45 (4H, multiplet, 2 × CH_2), 2.22 (9H, singlet, 2,4,6-tri-CH_3), 3.4 (2H, multiplet, CH_2N), 5.7 (1H, broad singlet, NH), 6.76 (2H, singlet, 3,5-di-H). (Found: C, 76.46; H, 9.47; N, 6.68. $C_{14}H_{21}ON$ requires: C, 76.66; H, 9.65; N, 6.39%).

(n) N - *tert* - *Butyl* - 2,4,6 - *trimethylbenzamide.* (i) The reaction was carried out as in the general procedure using 16 mM of tert-butylamine, and the reaction time was extended to 70 h. The resultant precipitates were filtered, and recrystallized from C_6H_6–$CHCl_3$–n-hexane to give tert-butylammonium diethyl phosphate (60%) as colorless needles, m.p. 145–148°, IR 2720, 2620, 2530, 2200, 1630, 1550, 1205, 1040, 950 cm^{-1}, NMR 1.23 (6H, triplet, J = 7 Hz, 2 × CH_3CH_2), 1.36 (9H, singlet, tert-butyl), 3.67 (1H, broad singlet, NH_2).

The filtrate was worked up as usual, and the crude product was purified by silica gel column chromatography with n-hexane–AcOEt (5 : 1) followed by recrystallization from MeOH to give N - tert - butyl - 2,4,6 - trimethylbenzamide as colorless crystals, m.p. 144–146°, IR 3240, 1640, 1560, 845 cm^{-1}, NMR 1.42 (9H, singlet, tert-butyl), 2.24 (9H, singlet, 2,4,6-tri-CH_3), 5.4 (1H, broad singlet, NH), 6.72 (2H, singlet, 3,5-di-H), (Found: C, 76.39; H, 9.63; N, 6.51. $C_{14}H_{21}ON$ requires: C, 76.66; H, 9.65; N, 6.31%).

(ii) To the mixed anhydride (2, 0.30 g, 1 mM) in DMF (5 ml) was added tert-butylamine (0.146 g, 2 mM) in DMF (5 ml) at 0°. The mixture was stirred at 0° for 0.5 h, and at room temp. for 24 h, followed by intermittent addition of tert-butylamine (0.180 g, 2.5 mM) in DMF (8 ml) during 48 h. The resultant precipitates of tert-butylammonium diethyl phosphate (0.16 g, 70.5%) were filtered, and the filtrate was worked up as in (n) (i) to give N - tert - butyl - 2,4,6 - trimethylbenzamide (0.077 g, 35%).

(o) *Phenacyl* 6 - (N - *benzyloxycarbonyl* - D - α - *phenylglycylamido*) - *penicillanate.* To a stirred mixture of hydrochloride of phenacyl 6-aminopenicillanate (0.371 g, 1 mM) and N-benzyloxycarbonyl - D - α - phenylglycine (0.314 g, 1.1 mM) in DMF (5 ml) was added DEPC (0.179 g, 1.1 mM) in DMF (5 ml) at 0°, followed by the addition of triethylamine (0.212 g, 2.1 mM) in DMF (5 ml). The mixture was stirred at 0° for 0.5 h, and then at room temp. for 24 h. After successive addition of N - benzyl - oxycarbonyl - D - α - phenylglycine (0.114 g, mM), DEPC (0.065 g, 0.4 mM), and triethylamine (0.040 g, 0.4 mM), the mixture was stirred at room temp. for 1.5 h. The mixture was diluted with C_6H_6 (30 ml) and AcOEt (60 ml) and washed successively with 0.001 N HCl aq (2 × 20 ml), H_2O (20 ml), sat NaCl aq (2 × 20 ml), 1.5% $NaHCO_3$ aq (2 × 20 ml), H_2O (20 ml), and sat NaCl aq (2 × 20 ml). Drying followed by evaporation afforded a yellow oil which was passed over silica gel with n-hexane–AcOEt (6 : 7) to give a pale yellow oil (0.426 g, 71%) which was solidified by tritulation with n-hexane. Recrystallization from AcOEt–petroleum ether afforded colorless prisms, m.p. 172–173°, $[\alpha]_D^{20}$ + 97.06° (c = 0.5, $CHCl_3$), IR 3300, 1790, 1765, 1740, 1715, 1660, 1550 cm^{-1}, NMR 1.57 (3H, singlet, 2α-CH_3), 1.58 (3H, singlet, 2β-CH_3), 4.46 (1H, singlet, 3-H), 5.03 (2H, singlet, $CH_2C_6H_5$), 7.3–7.8 (15H, multiplet, 3 × C_6H_5). (Found: C, 63.46; H, 5.15; N, 7.26. $C_{32}H_{31}O_7N_3S$ requires: C, 63.89; H, 5.16; N, 6.99%).

(p) *Phenacyl* 7 - (N - *benzyloxycarbonyl* - D - α - *phenylglycylamido*) - 3 - *methyl* - 3 - *cephem* - 4 - *carboxylate.* The reaction was carried out as in the case of the penicillin derivative using phenacyl 7 - amino - 3 - methyl - 3 - cephem - 4 - carboxylate (0.331 g, 1 mM), N - benzyloxycarbonyl - D - α - phenylglycine (0.314 g, 1.1 mM), DEPC (0.179 g, 1.1 mM), triethylamine (0.112 g, 1.1 mM), and DMF (15 ml). After stirring at room temp. 6 h, N - Benzyloxycarbonyl - D - α - phenylglycine (0.114 g, 0.4 mM), DEPC (0.065 g, 0.4 mM), and triethylamine (0.040 g, 0.4 mM) was added to the mixture, which was stirred at room temp. for 18 h. Dilution of the reaction mixture with AcOEt (100 ml) and C_6H_6 (50 ml), washing as in (o), followed by evaporation afforded a pale yellowish powder (0.618 g), which was dissolved in AcOEt (100 ml) and reprecipitated by the addition of n-hexane (250 ml) to give a pale yellowish powder (0.60 g, 100%), m.p. 214–218°, IR 3260, 1775, 1730, 1690, 1655, 1530 cm^{-1}, NMR in DMSO-d_6, 2.08 (3H, singlet, 3-CH_3), 5.02 (2H, singlet, $CH_2C_6H_5$), 7.3–7.9 (15 H, multiplet, 3 × C_6H_5)). (Found: C, 64.22, H, 5.27; N, 6.51. $C_{32}H_{29}O_7N_3S$ requires: C, 64.09; H, 4.87; N, 7.01%).

The Young test

General procedure. To a stirred mixture of benzoyl-L-leucine (0.47 g, 2 mM) and glycine ethyl ester hydrochloride (0.31 g, 2.2 mM) in DMF (5 ml) was added DEPC (0.36 g, 2.2 mM) in DMF (5 ml) at 0°, followed by the addition of triethylamine (0.424 g, 4.2 mM) in DMF (5 ml) during 5–10 min. The mixture was stirred at 0° and then at room temp. (20°). Reaction time is indicated in the Table 3 together with reaction temp. The reaction mixture was diluted with C_6H_6 (50 ml) and AcOEt (100 ml), and successively washed with 5% HCl aq (2 × 20 ml), H_2O (20 ml), sat NaCl aq (2 × 20 ml), sat $NaHCO_3$ aq (2 × 20 ml), H_2O (20 ml), and sat NaCl aq (2 × 20 ml). Drying followed by evaporation gave colorless crystals, which were subjected to silica gel column chromatography. Elution with $CHCl_3$–AcOEt (10 : 1) gave colorless crystals of benzoyl-leucylglycine ethyl ester, which was identified with the authentic sample.[8] The crystals were weighed, and the m.ps and optical rotations ($[\alpha]_D^{20-23}$ in EtOH (C = 2–3)) were determined. The results were summarized in Table 3. The Young test using dimethyl phosphorocyanidate was conducted analogously as above.

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