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(6) $HOs_3(CO)_{10}(CPh{=}CHPh)$: ir ($\nu_{CO}$, C_6H_{12}), 2102 m, 2062 s, 2050 m, 2024 s, 2005 m, 1994 m, 1982 w, 1955 vw; ^1H NMR ($CDCl_3$), τ 25.18 s (1), 2.6–3.3 m (10), 3.00 (1), position of vinylic hydrogen determined by difference from comparison with product from $D_2Os_3(CO)_{10}$; mass spectrum (field desorption), *m/e* 1036 (^{192}Os) plus fragment ions due to successive loss of ten carbonyls.

(7) $Os_3(CO)_{10}(C_2Ph_2)$: mp 118–119°; ir (ν_{CO}, C_6H_{12}), 2100 w, 2066 vs, 2047 s, 2028 s, 2011 s, 1996 m, 1982 sh, 1965 w; ^1H NMR ($CDCl_3$), τ 2.8–3.3 m; mass spectrum, *m/e* 1034 (^{192}Os) plus ion multiplets corresponding to loss of ten carbonyls.

(8) Johnson and co-workers have recently reported[9] that a metallocyclohexadienone complex, $Os_3(CO)_9((C_2HPh)_2CO)$ (A), derived from $H_2Os_3(CO)_{10}$ and excess phenylacetylene provided an osmiacyclopentadiene species after brief heating at 130°. A similar intermediate was proposed for the formation of 2. We have independently isolated complexes of type A from reactions of $H_2Os_3(CO)_{10}$ with terminal alkynes and have observed that they rearrange through intervening stages to analogues of **2**.[10] However, the temperatures required for this transformation are uniformly higher than for metallocyclopentadiene formation from $Os_3(CO)_{10}$(alkyne) complexes. Furthermore, the latter are not converted into type A species with excess alkyne nor has a complex related to A been observed for diphenylacetylene.

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(11) Two isomers are possible for **6** and **7** but only one was observed in each case. The NMR data obtained did not establish the configuration selected.

(12) Crystallographic data are: C_i^1–$P\bar{1}$; ρ_{calcd} = 2.653, ρ_{exptl} = 2.62 (2) g/cm^3; Z = 2 for $Os_3(CO)_{10}(C_2(C_6H_5)_2)$; a = 16.044 (3), b = 8.947 (3), c = 9.734 (3) Å; α = 113.99 (5)°, β = 87.39 (5)°, γ = 92.03 (5)°. The conventional R factor obtained from the full-matrix least-squares refinement of 2165 reflections measured on a Picker diffractometer is currently 0.082.

(13) It is interesting that the excess formal charge on Os(1) is not relieved by formation of semibridging carbonyls.[14] Nonbonding contacts for the trans (173°) carbonyls on Os(1) are not less than 2.88 Å.

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(15) A similar disposition of the diphenylacetylene moiety relative to the metal triangle was observed in the crystal structure of **3**.[3b] Therefore, it apparently does not result from specific steric interactions or the presence of a bridging metallocyclopentadiene group.

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Diphenyl Phosphorazidate (DPPA) and Diethyl Phosphorocyanidate (DEPC). Two New Reagents for Solid-Phase Peptide Synthesis and Their Application to the Synthesis of Porcine Motilin

Sir:

Solid-phase peptide synthesis ingeniously launched by Merrifield[1] has received great attention since it has now been automated and makes possible the very rapid preparation of peptides. Although many reagents for peptide bond formation are known,[2] *N,N'*-dicyclohexylcarbodiimide (DCCD) is the sole coupling reagent widely used for solid-phase synthesis.

Table I. Comparisons of the Reactivities of DCCD, DPPA, and DEPC in DMF and Methylene Chloride

coupling agent	Rate of coupling, %[a]		
	5 min	30 min	60 min
DCCD	12 (95)	20 (98.5)	32 (99)
DPPA[b]	40 (3)	72 (12)	80 (21)
DEPC[b]	98 (55)	98 (70.5)	99 (82)

[a] Rates of coupling of Boc-Ile·$^1/_2H_2O$ (1.5 equiv) and Gly-resin with 1.5 equiv of each coupling agent in dimethylformamide. Numbers in parentheses represent rates of coupling in methylene chloride. [b] Together with triethylamine (1.5 equiv).

We have previously disclosed that two *O,O'*-disubstituted phosphoropseudohalidates, diphenyl phosphorazidate ($N_3PO(OPh)_2$, DPPA)[3] and diethyl phosphorocyanidate ($NCPO(OEt)_2$, DEPC),[4] in combination with triethylamine are very efficient coupling reagents for racemization-free conventional (solution) peptide synthesis. We here report that these two reagents may also be useful for solid-phase peptide synthesis in both the stepwise and fragment condensation approaches and were successfully applied to the synthesis of porcine motilin, a gastrointestinal hormone exhibiting gastric motor stimulating activity.[5]

First, comparisons of DCCD with DPPA and DEPC were conducted by the coupling of Boc-Ile[6] with Gly-polystyrene-resin according to the general procedure described by Stewart and Young.[7] When DPPA and DEPC were the coupling reagents, they were added after the addition of Boc-Ile·$^1/_2H_2O$, followed by the addition of triethylamine. The reaction was followed by determining the unreacted amino group of the Gly-resin according to the Porath method.[8] When methylene chloride was used as the solvent, the reactivity of DCCD was higher than that of DPPA or DEPC. However, with dimethylformamide DPPA and DEPC were much more reactive than DCCD as shown in Table I.

The DPPA and DEPC methods were applied to the synthesis of Boc-Pro-Leu-Gly-NH_2, the amino acid sequence of which corresponds to the melanocyte release inhibiting hormone.[9] The synthesis[10] started from Boc-Gly-resin (1 equiv). Stepwise attachment of Boc-Leu (3 equiv) and Boc-Pro (3 equiv) using either DPPA or DEPC (3 equiv) in the presence of triethylamine (3 equiv) in dimethylformamide (each coupling reaction time was 2 hr, room temperature) gave Boc-Pro-Leu-Gly-resin. Treatment of the resin with ammonia in methanol afforded Boc-Pro-Leu-Gly-NH_2 in a 70% (DPPA) or a 76% (DEPC) yield. The sample of Boc-Pro-Leu-Gly-NH_2·$^1/_2H_2O$, mp 136–138°, $[\alpha]^{25}D$ −72° (c = 1.64, MeOH)[11] was identical with that similarly prepared from Gly-OMe by the solution method using DEPC.

To check racemization during fragment coupling on a solid support we adopted the Izumiya test[12] involving condensation of Boc-Gly-Ala with Leu-resin. Three equivalents of Boc-Gly-Ala and DPPA or DEPC were used together with triethylamine (3 × 0.95 equiv) in dimethylformamide. After the coupling reaction, the resin was treated with hydrogen bromide in trifluoroacetic acid to give Gly-Ala-Leu. The extent of racemization was determined with an amino acid analyzer. The coupling reaction proceeded almost quantitatively during 2 hr with little racemization: the extent of racemization = [D,L/(D,L + L,L)] × 100%; DPPA 2.5% at 20°, 2% at 0°; DEPC 1% at 20°, <0.5% at 0°. These values are considerably lower in the solid-phase method than are those for other coupling reagents.[13] Thus,

both DPPA and DEPC were proved useful for both the stepwise and fragment condensation approaches on a solid support.

Based on these satisfactory, preliminary experiments, we applied the method to the synthesis of porcine motilin, Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-Glu-Leu-Gln-Arg-Met-Gln-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln, which was recently synthesized by Yajima and his co-workers, using the conventional method.[14,15] As a strategy for the synthesis of motilin, its molecule was architecturally segmented into four subunits (see dotted lines in the above formula). Synthesis was carried out by a combination of the solution and solid-phase methods. The time for the coupling reactions on a solid support varied from 2 to 48 hr depending on the reactants. Z-Phe-Val-Pro ($\frac{1}{2}C_6H_6$ solvate), mp 99–102°, $[\alpha]^{20}D$ −49° (c = 0.74, DMF), was prepared from Pro-OMe by the stepwise addition of Z-Val and Z-Phe using either DPPA or DEPC, followed by alkaline treatment. This tripeptide derivative was condensed with Ile-Phe-Thr(Bzl)-Tyr(Bzl)-Gly-resin which had been prepared by the sequential incorporation of Boc-Tyr(Bzl), Boc-Thr(Bzl), Boc-Phe, and Boc-Ile into Gly-resin. The resultant resin was treated with methanol in the presence of triethylamine to give Z-Phe-Val-Pro-Ile-Phe-Thr(Bzl)-Tyr(Bzl)-Gly-OMe (monohydrate), mp 206–210°, $[\alpha]^{20}D$ −34.8° (c = 0.5, $CHCl_3$), in 48 and 68% yields using DPPA and DEPC, respectively, based on Boc-Gly-resin. Saponification of the methyl ester afforded Z-Phe-Val-Pro-Ile-Phe-Thr(Bzl)-Tyr(Bzl)-Gly-(hemihydrate), mp 198–201°, $[\alpha]^{20}D$ −23° (c = 0.6, $CHCl_3$). Boc-Leu-Gln-Arg(NO_2)-Met(monohydrate), $[\alpha]^{20}D$ −33° (c = 0.6, MeOH), was prepared from Met-OMe analogous to the preparation of Z-Phe-Val-Pro using DPPA or DEPC. The C-terminal nonapeptide resin, Gln-Glu(Bzl)-Lys(2-Cl-Z)-Glu(Bzl)-Arg(NO_2)-Asn-Lys(2-Cl-Z)-Gly-Gln-resin, prepared stepwise from Gln-resin, was successively condensed with Boc-Leu-Gln-Arg(NO_2)-Met, Boc-Glu(Bzl), and Z-Phe-Val-Pro-Ile-Phe-Thr(Bzl)-Tyr(Bzl)-Gly using DEPC in the presence of triethylamine in dimethylformamide to give the docosapeptide resin.

The resin was treated with hydrogen fluoride in the presence of anisole at −20° for 40 min, then at 0° for 40 min, followed by the usual treatment on a Dowex 1-X4 column (acetate form).[16] The deblocked peptide obtained was successively purified by column chromatography on SP-Sephadex C-25 (gradient elution with ammonium formate buffer), Sephadex G-25 (0.1 N acetic acid), QAE-Sephadex A-25 (gradient elution with ammonium formate buffer), and Biogel P4-P6 (4:1) (0.1 N acetic acid). This purified synthetic motilin appeared homogeneous in a variety of chromatographic systems[17] and gave excellent amino acid analyses,[18] after both acid hydrolysis and enzymic digestion (AP-M), the latter procedure showing the absence of racemization during synthesis.

The activity of synthetic motilin was determined using rabbit duodenum, jejunum, and colon contractile activity in vitro.[19] Synthetic motilin showed a potency similar to the natural one and the same biological activity pattern with other smooth muscle preparations of the alimentary tracts (rat, guinea pig, and rabbit).

The synthesis of motilin, as well as preliminary experiments including the Izumiya test, indicate that both DPPA and DEPC may be very efficient reagents for solid-phase peptide synthesis as well as for conventional synthesis.

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(18) Amino acid analyses after acid hydrolysis and enzymic digestion (values in parentheses): Lys 2.2 (1.8), Arg 2.1 (1.9), Asp 1.1 (0), Thr 0.9 (Asn + Thr + Gln 6.1), Glu 5.8 (3.3), Pro 1.0 (1.0), Gly 2.0 (1.9), Val 0.8 (1.2), Met 1.0 (1.0), Ile 0.9 (1.0), Leu 1.0 (1.0), Tyr 0.8 (0.8), Phe 1.8 (2.0).
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The Nuclear Overhauser Effect in ^{31}P Nuclear Magnetic Resonance

Sir:

The nuclear Overhauser effect (NOE) is the change in the NMR intensity of a nuclear spin upon saturation of a second spin interacting by a dipolar mechanism with the observed spin. The NOE is commonly observed in ^{13}C NMR, where carbons directly bound to hydrogens may display up to three times greater intensity due to dipolar interactions between the two nuclei. In this paper we report observation of a $^{31}P\{^1H\}$ NOE in a variety of phosphorus containing compounds. In our case saturation of all the protons in the sample can produce, under extreme narrowing conditions, a maximum increase in the intensity of the phosphorus resonance, or a nuclear Overhauser effect enhancement (NOEE), of 124%.[1] Failure to achieve the full enhancement may be due to competing relaxation mechanisms (other than the proton-^{31}P dipolar mechanism upon which the NOE depends) or to motions which are slower than required for the extreme narrowing limit. Quantitatively, the NOE enhancement in the ^{31}P, ^1H case in the extreme narrowing limit may be expressed as