Polymer-Bound Triarylphosphine–Iodine Complexes, Convenient Coupling Reagent Systems in Peptide Synthesis[1]

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N-protected α-amino acids are readily coupled with α-aminoacyl esters by polystyryl diphenylphosphine–iodine complex in very high yields and without detectable racemization. Protecting groups of general use for both amine and carboxyl functions, as well as the common side-chain-function protecting groups, are well tolerated under our experimental conditions. The workup is very easy since the only byproduct formed is a polymer-linked phosphine oxide that is simply filtered off to achieve the coupling product.

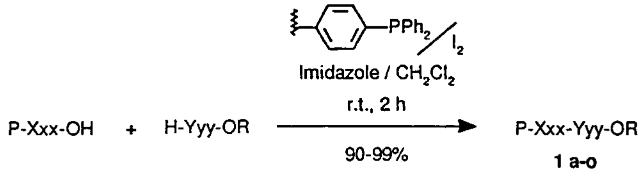
Electrophilic phosphorus-containing redox-condensation reagents from triphenylphosphine, such as $[Ph_3PE]^+X^-$, are nowadays of common use in organic synthesis. Some complex phosphonium salts, like BOP and BroP reagents,[2,3] also became very popular in peptide synthesis, mainly due to their ability to give very fast condensation reactions and minimize epimerization at the α-carbon of the reacting α-amino acids.

The action of such reagents consists of the conversion of the free carboxylic function of a *N*-protected α-amino acid into a highly reactive acyl-donor species which easily undergoes nucleophilic attack by the amine group of a second, *C*-protected, α-amino acid. However, their extensive utilization is plagued by several factors including their somewhat high cost, as well as the release of byproducts during the reaction (sometimes toxic[2,3] as in the case of hexamethylphosphoramide from BOP and BroP), which need time-consuming and circumstantial purification procedures to obtain pure peptides by solution methods.

Following our interest in the synthetic applications of triarylphosphine–halogen complexes,[4] we have now devised a new, very convenient procedure for the preparation of fully protected dipeptides by coupling *N*-protected α-amino acids and α-amino acid esters in the presence of polystyryl diphenylphosphine–iodine complex. The peptide bond formation, under our conditions, occurs in high yields, under mild conditions, and without detectable racemization of the reacting α-amino acids. The choice of polystyryl diphenylphosphine implies that the phosphine oxide, which is formed under our conditions as the only byproduct of the coupling reaction, is linked to a polymeric matrix and, thus, can be separated by simple filtration.

Our procedure involves treatment of a *N*-protected α-amino acid with freshly prepared polystyryl diphenylphosphine–iodine complex, in anhydrous dichloromethane solution, at room temperature and under inert atmosphere, for a few minutes to obtain the activated acyl-donor species. This is likely to be the *N*-protected α-aminoacyl iodide[5,6] which, by addition of a *C*-protected α-amino acid counterpart, is readily consumed to afford the expected fully protected dipeptide. Neutral conditions in the reaction medium are assured by the addition of excess imidazole which should act as a proton trap for the hydrogen iodide released in the reaction.[7]

This process is apparently effective (see Table) for α-amino acids carrying all the commonly used *N*-protecting groups, namely *N*-Fmoc (9-fluorenylmethoxycarbonyl), *N*-Boc (*tert*-butoxycarbonyl), and *N*-Cbz (benzyloxycarbonyl) α-amino acids. Various esters, like methyl, *tert*-butyl, benzyl, etc. are well tolerated, as well as the less common allylic and heptylic esters that have been rather recently introduced as protecting groups of the carboxylic function, since they can be easily removed under Pd(0) complex catalytic conditions[8] and enzymatic conditions,[9] respectively. All the common side-chain protecting groups, like tosyl (for arginine), trityl (for cysteine), *tert*-butyl (for glutamic acid), turned out to be quite unaffected by our reaction conditions.



1	P	Xxx	Yyy	R
a	Fmoc	L-Ala	L-Leu	All
b	Cbz	L-Met	L-Ala	Me
c	Fmoc	L-Cys(Trt)	L-Cys(Trt)	Me
d	Boc	L-Leu	L-Phe	Bu^t
e	Fmoc	L-Phe	Gly	Bu^t
f	Fmoc	D,L-Phe	Gly	Bu^t
g	Fmoc	L-Val	L-Val	All
h	Fmoc	L-Phe	L-Leu	Bn
i	Boc	L-Trp	L-Leu	Me
l	Fmoc	L-Glu(Bu^t)	L-Phe	All
m	Cbz	L-Ala	L-Phe	All
n	Boc	L-Arg(Ts)	L-Phe	Me
o	Cbz	L-Thr(OH)	L-Val	Hept

It is noteworthy that the trityl-protected cysteinyl SH groups (as in **1c**) do not undergo the reported[10] iodolysis process leading to disulfide bond formation. In addition, no byproducts could be detected when threonine, having a free chain OH (as in **1o**), was utilized under our experimental conditions.

The extent of epimerization at the α-carbon of the *N*-protected carboxylic moiety during the dipeptide formation was investigated by chiral column HPLC analysis

Table. Yields and Physical Properties of New Dipeptides **1a–o**

1[a]	Yield[b] (%)	mp[c] (°C) (Solvent)	$[\alpha]_D^{25}$[d] (c, Solvent)	^{1}H NMR[e] δ, J (Hz)
a	99	129–131 (CH_2Cl_2/hexane)	−19.90 (0.72, DMF)	0.90 (d, 3H, $J = 5.9$, CH_3-Leu), 0.92 (d, 3H, $J = 5.9$, CH_3-Leu), 1.40 (d, 3H, $J = 6.6$, CH_3-Ala), 1.56–1.70 (m, 3H, $CH_2\beta$ and CH-Leu), 4.15–4.30 (m, 2H, H9-Fmoc and Hα-Ala), 4.40 (d, 2H, $J = 6.9$, CH_2-Fmoc), 4.52–4.70 (m, 3H, $OC\mathit{H}_2CH{=}CH_2$ and Hα-Leu), 5.20–5.40 (m, 3H, $CH_2CH{=}C\mathit{H}_2$ and H-urethane), 5.90 (m, 1H, $CH_2C\mathit{H}{=}CH_2$), 6.24 (m, 1H, H-amide), 7.25–7.77 (m, 8H, H-Fmoc)
b	98	119–121 (EtOAc/hexane)	+1.14 (0.96, $CHCl_3$)	1.40 (d, 3H, $J = 7.1$, CH_3-Ala), 2.01 (m, 2H, $CH_2\beta$-Met), 2.10 (s, 3H, SCH_3), 2.60 (t, 2H, $J = 7.0$, $CH_2\gamma$-Met), 3.74 (s, 3H, OCH_3), 4.39 (m, 1H, Hα-Met), 4.56 (m, 1H, Hα-Ala), 5.11 (s, 2H, CH_2-Cbz), 5.52 (br s, 1H, H-urethane), 6.62 (br s, 1H, H-amide), 7.34 (s, 5H, H-Cbz)
c	95	110–111 (CH_2Cl_2/hexane)	−1.04 (1.00, $CHCl_3$)	2.53–2.69 (m, 4H, 2 × CH_2-Cys), 3.62 (s, 3H, OCH_3), 3.73 [dd, 1H, $J = 6.4$ Hα-Cys (*N*-protec.)], 4.18 (t, 1H, $J = 6.6$, H9-Fmoc), 4.25–4.48 [m, 3H, CH_2-Fmoc, and Hα-Cys (C-protec.)], 5.01 (br d, 1H, H-urethane), 6.33 (d, 1H, $J = 7.6$, H-amide), 7.10–7.78 (m, 38H, H-aromatic)
d	95	127–128 (EtOAc/hexane)	−23.65 (0.95, $CHCl_3$)	0.92 (2d, 6H, $J = 6.2$, 2 × CH_3-Leu), 1.40 (s, 9H, OBu^t), 1.45 (s, 9H, H-Boc), 1.52–1.75 (m, 3H, $CH_2\beta$ and CH-Leu), 3.12 (d, 2H, $J = 6.0$, CH_2-Phe), 4.10 (m, 1H, Hα-Leu), 4.72 (dd, 1H, $J = 6.0$ and $J = 8.2$, Hα-Phe), 4.85 (m, 1H, H-urethane), 6.53 (d, 1H, $J = 8.2$, H-amide), 7.18–7.35 (m, 5H, H-aromatic)
e	98	166–168 (CH_2Cl_2/hexane)	−29.12 (0.33, DMF)	1.44 (s, 9H, OBu^t), 3.08 (m, 2H, CH_2-Phe), 3.85 (dd, 2H, $J = 5.1$, CH_2-Gly), 4.17 (t, 1H, $J = 6.8$, H9-Fmoc), 4.28–4.46 (m, 3H, CH_2-Fmoc and Hα-Phe), 5.33 (m, 1H, H-urethane), 6.28 (m, 1H, H-amide), 7.13–7.78 (m, 13H, H-aromatic)
f	96	167–169 (CH_2Cl_2/hexane)		
g	99	140–142 (CH_2Cl_2/hexane)	−10.52 (2.33, DMF)	0.95 (2d, 12H, $J = 7.4$, 4 × CH_3-Val), 2.00–2.30 (m, 2H, 2 × CH-Val), 4.08 [m, 1H, Hα-Val (*N*-protec.)], 4.21 (t, 1H, $J = 6.9$, H9-Fmoc), 4.40 (m, 2H, CH_2-Fmoc), 4.51–4.70 [m, 3H, Hα-Val (C-protec.) and $C\mathit{H}_2CH{=}CH_2$], 5.33 (dd, 2H, $J_{trans} = 18.2$, $J_{cis} = 10.2$, $CH_2CH{=}C\mathit{H}_2$), 5.48 (d, 1H, $J = 8.5$, H-urethane), 5.90 (m, 1H, $CH_2C\mathit{H}{=}CH_2$), 6.45 (d, 1H, $J = 9.3$, H-amide), 7.20–7.78 (m, 8H, H-Fmoc)
h	95	153–155 (CH_2Cl_2/hexane)	−22.80 (0.82, DMF)	0.91 (t, 6H, $J = 6.1$, 2 × CH_3-Leu), 1.41–1.58 (m, 3H, $CH_2\beta$- and CH-Leu), 3.05 (m, 2H, CH_2-Phe), 4.18 (t, 1H, $J = 6.9$, H9-Fmoc), 4.25–4.50 (m, 3H, Hα-Phe and CH_2-Fmoc), 4.58 (m, 1H, Hα-Leu), 5.12 (s, 2H, CH_2-Ph), 5.29 (m, 1H, H-urethane), 6.10 (m, 1H, H-amide), 7.02–7.78 (m, 18H, H-aromatic)
i	94	59–61 (EtOAc/hexane)	−14.33 (1.05, $CHCl_3$)	0.83 (2d, 6H, $J = 6.1$, 2 × CH_3-Leu), 1.42 (s, 9H, H-Boc), 1.46–1.68 (m, 3H, $CH_2\beta$- and CH-Leu), 3.22 (m, 2H, $CH_2\beta$-Trp), 3.63 (s, 3H, OMe), 4.36–4.60 (m, 2H, Hα-Tpr and Hα-Leu), 5.15 (m, 1H, H-urethane), 6.15 (m, 1H, H-amide), 7.10–7.29 (m, 3H, H2-, H5-, and H6-Trp), 7.35 (d, 1H, $J = 7.7$, H7-Trp), 7.62 (d, 1H, $J = 9.6$, H4-Trp), 8.10 (br s, 1H, NH-Trp)
l	95	101–102 (CH_2Cl_2/hexane)	−13.42 (1.01, DMF)	1.44 (s, 9H, Bu^t), 1.93 (m, 2H, $CH_2\beta$-Glu), 2.35 (m, 2H, $CH_2\gamma$-Glu), 3.07 (dd, 1H, $J_{gem} = 15.2$ and $J_{vic} = 5.2$, CH_2–Phe), 3.18 (dd, 1H, $J_{gem} = 15.2$ and $J_{vic} = 5.2$, CH_2Phe), 4.12–4.28 (m, 2H, H9-Fmoc and Hα-Glu), 4.35 (d, 2H, $J = 8.0$, CH_2-Fmoc), 4.60 (d, 2H, $J = 6.2$, $C\mathit{H}_2CH{=}CH_2$), 4.87 (q, 1H, $J = 5.2$, Hα-Phe), 5.25 (dd, 2H, $J_{trans} = 17.5$, $J_{cis} = 9.2$, $CH_2CH{=}C\mathit{H}_2$), 5.65 (m, 1H, H-urethane), 5.85 (m, 1H, $CH_2C\mathit{H}{=}CH_2$), 6.80 (m, 1H, H-amide), 7.18–7.78 (m, 13H, H-aromatic)
m	94	103–104 (EtOAc/hexane)	−24.92 (1.05, DMF)	1.35 (d, 3H, $J = 7.0$, CH_3-Ala), 3.12 (m, 2H, CH_2-Phe), 4.18 (m, 1H, Hα-Ala), 4.62 (d, 2H, $J = 7.0$, $C\mathit{H}_2CH{=}CH_2$), 4.85 (m, 1H, Hα-Phe), 5.10 (s, 2H, CH_2-Cbz), 5.12–5.38 (m, 3H, H-urethane and $CH_2CH{=}C\mathit{H}_2$), 5.87 (m, 1H, $CH_2C\mathit{H}{=}CH_2$), 6.38 (m, 1H, H-amide), 7.05–7.40 (m, 10H, H-aromatic)
n	90	69–70 (EtOAc/hexane)	+10.35 (1.44, $CHCl_3$)	1.39 (s, 9H, H-Boc), 1.43–1.70 (m, 4H, $CH_2\beta$- and $CH_2\gamma$-Arg), 2.37 (s, 3H, CH_3-tosyl), 3.04 (m, 2H, CH_2-Phe), 3.22 (m, 2H, $CH_2\delta$-Arg), 3.66 (s, 3H, OCH_3), 4.10 (m, 1H, Hα-Arg), 4.75 (dd, 1H, $J = 3.0$ and 2.4, Hα-Phe), 5.37 (d, 1H, $J = 7.6$, H-urethane), 6.35 (br s, 3H, NH-Arg), 7.05–7.28 (m, 8H, H-amide, and H-Ar), 7.75 (d, 2H, $J = 8.1$, H_{ortho}-tosyl)
o	90	95–96 (EtOAc/hexane)	−33.21 (1.32, $CHCl_3$)	0.85–0.91 (m, 9H, 2 × CH_3-Val and CH_3-heptyl), 1.18 (d, 3H, $J = 6.5$, CH_3-Thr), 1.23–1.40 (m, 8H, CH_2-heptyl), 1.62 (m, 2H, $OCH_2C\mathit{H}_2C_5H_{11}$), 2.17 (m, 1H, CH-Val), 3.41 (br s, 1H, OH-Thr), 4.09–4.21 (m, 3H, Hα-Thr and $OC\mathit{H}_2C_6H_{13}$), 4.32 (m, 1H, CH-Thr), 4.48 (dd, 1H, $J = 4.8$ and 8.8, Hα-Val), 5.12 (2d, 2H, $J = 12.3$, CH_2-Cbz), 5.83 (d, 1H, $J = 7.5$, H-urethane), 7.00 (br d, 1H, $J = 8.8$, H-amide), 7.34 (m, 5H, H-Cbz)

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[a] Satisfactory microanalyses were obtained: C ± 0.27, H ± 0.33, N ± 0.32.

[b] Yields of pure crystalline product.

[c] Uncorrected, determined in capillary tubes.

[d] Rotations measured on a Perkin-Elmer 141 polarimeter (1.0 dm cell).

[e] Recorded on a Bruker WH270 spectrometer in $CDCl_3$ solutions (TMS i.s.).

of the protected dipeptide Fmoc-L-Phe-Gly-OBu^t (**1e**) in comparison with the specially prepared racemic dipeptide **1f**. Under our experimental conditions, no traces of Fmoc-D-Phe-Gly-OBu^t could be detected.

Polystyryl diphenylphosphine–iodine complex is an easy to prepare, handy, semicrystalline solid, reasonably stable at room temperature. Its utilization in peptide synthesis should find rather broad application if one considers the ready feasibility of the procedure (see experimental), simplified workup, no racemization and high yields even when α-amino acids like L-valine, well known[11] for their reluctance to give good coupling reactions, are used, as in the case of **1g**.

A future development of this coupling procedure might be the use of the soluble triphenylphosphine–iodine complex as a coupling reagent in solid-phase peptide synthesis and some work on this subject is already in progress in our laboratory.

N-protected α-amino acids were commercial chemicals. α-Amino acid alkyl esters were prepared from commercial α-amino acids according to standard literature methods.[12] Polystyryl diphenylphosphine (PDP) (Fluka, ≅ 3 mmol P/g) was dried under vacuum at 100 °C for 2 h before use. Dichloromethane was distilled from P_2O_5.

Synthesis of Fully Protected Dipeptides 1a–o; General Procedure:
To a magnetically stirred suspension of PDP (2.2 g) in anhydrous CH_2Cl_2 (20 mL) at r.t. was added dropwise I_2 (1.52 g, 6 mmol) dissolved in the same solvent (10 mL), stirring being gently continued until the solvent becomes colourless. The *N*-protected α-amino acid (3.0 mmol) and imidazole (7.5 mmol) dissolved in the same solvent (15 mL) were then added dropwise to the suspension. After 30 min stirring, the *C*-protected α-amino acid (3.2 mmol) dissolved in the minimum amount of anhyd. CH_2Cl_2 was added in one portion, the suspension being gently stirred for a further 2 h. Filtration of the polymeric phase on Celite and washing with small portions of CH_2Cl_2 gave a solution that was shaken with 5 N aq NaS_2O_3 (50 mL), then 0.1 N aq HCl (20 mL) and H_2O until neutral. Evaporation of the dried (Na_2SO_4) organic layer in vacuo afforded the crude fully protected dipeptide which was crystallized from a suitable solvent mixture (see Table).

HPLC Analysis of the Protected Dipeptides 1e and 1f:
A pure sample of **1f** mp 167–169 °C was dissolved in *i*-PrOH and 20 µl aliquots of the solution were injected into a HPLC system equipped with a Chiralcel OD (J. T. Baker) column using 92:8 hexane/*i*-PrOH mobile phase, flow rate 2 mL/min, and detection 263 nm. Under such conditions the retention times of L- and D-enantiomeric protected dipeptides **1f** were 15.5 min and 20.0 min, respectively. The protected dipeptide **1e** was analyzed under the same conditions and no traces of its D-enantiomer could be detected even operating at much higher sample concentrations.

All the other protected dipeptides, when analyzed by HPLC under suitable conditions, did not exhibit any peaks attributable to the co-occurrence of their diastereomers.

(1) Part V in the series *Polymer-Supported Phosphine-Halogen Complexes*. For part IV in the same series, see ref 4.
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(6) Some authors (cf. Landi, J.J.; Brinkman, H.R. *Synthesis* **1992**, 1093) reported somewhat hazy evidence of the formation of a polymer-linked anhydride species. Unfortunately, in our hands, experiments aimed to confirm this were quite unsuccessful.
(7) Imidazole was also suggested to play an active role when the triphenylphosphine–iodine reagent system is used to convert alcohols into iodides, although no experimental evidence of such a role was reported: cf. Garegg, P.J.; Samuelsson, B. *J. Chem. Soc., Perkin Trans. I* **1980**, 2866.
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