Esterification of Amino Acids and Dipeptides under Mild Conditions; Part I: via Phase Transfer Catalysis

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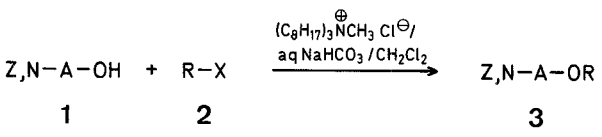
Many procedures for the esterification of carboxylic acids are available in the literature[1]. However, few are suitable for the fairly large scale esterification of *N*-protected amino acids and peptides while employing sufficiently mild conditions to be widely applicable to acid and/or base sensitive compounds.

0039-7881/79/1232-0957 $ 03.00

We wish to report a simple and convenient synthesis of esters of a wide variety of suitably protected amino acids and peptides via phase transfer catalysis[2]. The method is related to studies[3] on the transfer of amino acids and peptides from an aqueous into an apolar organic phase (dichloromethane) by means of a quaternary ammonium salt (Adogen, trioctylmethylammonium chloride). Under the reaction conditions (room temperature, vigorous stirring) the carboxylate anion is extracted into the organic phase where the alkylation takes place[4].



1 2 3

Z = *N*-protecting group
A = amino acid or dipeptide moiety
R X = see Table 1

The esters are readily isolated by extraction with dichloromethane and subsequent percolation on a silica gel column by eluting with hexane/ethyl acetate 8:2; by eluting with methanol, the ammonium salt may be recovered and recycled. No racemization takes place (<1% error), as verified for every L-amino acid and peptide reported, by simultaneously deprotecting the carboxy and the amino group by hydrogenolysis over 5% palladium on carbon and by comparing the $[\alpha]_D$ value of the newly recovered amino acid with that of the starting material. For this reason benzyloxycarbonyl (Z) was chosen as the *N*-protecting group together with the benzyl (Bzl) as the carboxy protecting group. The esterification is also effective with other protecting groups such as *t*-butyloxycarbonyl (Boc). Results are reported in Table 1; the spectroscopic properties of the esters are reported in Table 2.

The method is particularly convenient for acid sensitive amino acids such as tryptophan, but it also gives very good results with most amino acids. The process can be satisfactorily utilized in solid phase peptide synthesis (total esterification of Merrifield resins[8]). With diiodides, the reaction leads to the formation of monofunctionalized esters (**3q–w**), suitable for further bonding to polymers (carboxylic resins) through extended hydrocarbon chains, which place the amino acid moieties at varying distances from the matrix backbone (as for affinity chromatography[9]).

Z,N- and Boc,N-protected L-amino acids were obtained from Fluka (Switzerland). Peptides were synthesized and characterized as previously described[10]. Commercial Adogen-464 (trioctylmethylammonium chloride) obtained from Serva Chemie (Heidelberg) as a technical grade was purified as previously described[3b].

α,ω-Diiodoalkanes 2q-w:

α,ω-Diiodoalkanes are prepared from the corresponding dichloroalkanes (Fluka) by heating under reflux with aqueous potassium iodide in the presence of hexadecyltributylphosphonium bromide[11] for 4 h. The aqueous phase is separated from the organic phase, washed with ethyl ether (3 times), and the washings are added to the organic phase. By cooling at 5°, the phosphonium salt precipitates from the organic phase and may be recovered. The organic solvent is evaporated and the products are obtained in good yield by distillation and duly characterized.

Z,N- and Boc,N-Protected L-Amino Acid and Dipeptide Esters 3a-w; General Procedure:

To a solution of Z,N- or Boc,N-protected L-amino acid or dipeptide (1 mmol) in saturated aqueous sodium hydrogen carbonate so-

Table 1. Esterification of *N*-Protected L-Amino Acids and Dipeptides[a]

	Substrate 1	R—X 2	Molar ratios 2:1	Adogen:1	Product 3	Yield[b] [%]	m.p.	$[\alpha]_D^{25}$ (*c*, solvent)[c]	Molecular formula[d] or Lit. m.p.	M.S. *m/e* (M^+)
a	Z-Trp-OH	$C_6H_5CH_2$—Br	1.2	1	Z-Trp-OBzl	97[e]	103–104°	−5.8° (*c* 2, ethanol)	$C_{26}H_{24}N_2O_4$ (428.5)	428
b	Boc-Trp-OH	$C_6H_5CH_2$—Br	1.2	1	Boc-Trp-OBzl	93	143–144°	−2.0° (*c* 1, ethyl acetate)	Lit.[5] 140–142°	394
c	Z-Trp-OH	4-O_2N $C_6H_4CH_2$—Br	1.2	1	Z-Trp-O-p-NO_2Bzl	95[f]	130°	+9.8° (*c* 1, ethyl acetate)	$C_{26}H_{23}N_3O_6$ (473.5)	473
d	Z-Trp-OH	C_2H_5—J	3	1	Z-Trp-OC_2H_5	81[g]	84–85°	−6.7° (*c* 2, ethanol)	$C_{21}H_{22}N_2O_4$ (366.4)	366
e	Z-Phe-OH	$C_6H_5CH_2$—Br	1.2	1	Z-Phe-OBzl	98[e]	oil	−13.1° (*c* 2, ethanol)	$C_{24}H_{23}NO_4$ (389.4)	389
f	Z-Val-OH	$C_6H_5CH_2$ Br	1.2	1	Z-Val-OBzl	96[e]	oil	−24.3° (*c* 3, ethanol)	$C_{20}H_{23}NO_4$ (341.4)	341
g	Z-Ser-OH	$C_6H_5CH_2$ Br	1.2	1	Z-Ser-OBzl	81[g]	81–82°	−7.5° (*c* 3, ethanol)	$C_{18}H_{19}NO_5$ (329.3)	329
h	Z-Cys(Z)-OH	$C_6H_5CH_2$—Br	1.2	1	Z-Cys(Z)-OBzl	90[e]	52–53°	−37.9° (*c* 3, ethanol)	$C_{26}H_{25}NO_6S$ (479.5)	479
i	Z-Tyr(Z)-OH	$C_6H_5CH_2$ Br	1.2	1	Z-Tyr(Z)-OBzl	96[h]	95°	−11.0° (*c* 1, ethyl acetate)	$C_{32}H_{29}NO_7$ (539.6)	539

Table 1. Continued

	Substrate 1	R—X 2	Molar ratios 2:1	Adogen:1	Product 3	Yield[b] [%]	m.p.	$[\alpha]_D^{25}$ (c, solvent)[c]	Molecular formula[d] or Lit. m.p.	M.S. m/e (M^+)
j	Z-Asp-OH	$C_6H_5CH_2$—Br	1.2	1	Z-Asp-OBzl	45[e]	85°	+9.5° (c 1, ethyl acetate)	Lit.[6] 84–85	—
k	Z-Asp-OH	$C_6H_5CH_2$—Br	2	2	Z-Asp(Bzl)-OBzl	87[g]	65–66°	−11.8° (c 1, ethanol)	Lit.[7] 66.5°	447
l	Z-His-OH	$C_6H_5CH_2$—Br	2	1	Z-His(Bzl)-OBzl	93[e]	90–92°	+9.1° (c 1, ethanol)	$C_{28}H_{27}N_3O_4$ (469.5)	469
m	Z-Arg(di-Z)-OH	$C_6H_5CH_2$—Br	1.2	1	Z-Arg(di-Z)-OBzl	92[h]	103–104°	+10.4° (c 1, chloroform)	$C_{37}H_{38}N_4O_8$ (666.7)	—
n	Z-Phe-Gly-OH	$C_6H_5CH_2$—Br	1.2	1	Z-Phe-Gly-OBzl	90[g]	135°	−3.75° (c 1, acetone)	$C_{26}H_{26}N_2O_5$ (446.5)	446
o	Z-Phe-Phe-OH	$C_6H_5CH_2$—Br	1.2	1	Z-Phe-Phe-OBzl	70[g]	130°	−14.2° (c 1, acetone)	$C_{33}H_{32}N_2O_5$ (536.6)	536
p	Z-Trp-Gly-OH	$C_6H_5CH_2$—Br	1.2	1	Z-Trp-Gly-OBzl	70[g]	oil	+5.7° (c 1, ethyl acetate)	$C_{28}H_{27}N_3O_5$ (485.5)	485
q	Z-Trp-OH	J—$(CH_2)_3$—J	6	1.3	Z-Trp-O—$(CH_2)_3$—J	75[g]	68–70°	+6.1° (c 1, ethyl acetate)	$C_{22}H_{23}JN_2O_4$ (506.35)	506
r	Z-Trp-OH	J—$(CH_2)_5$—J	6	1.3	Z-Trp-O—$(CH_2)_5$—J	86[g]	wax	+8.3° (c 1, ethyl acetate)	$C_{24}H_{27}JN_2O_4$ (534.4)	534
s	Z-Trp-OH	J—$(CH_2)_6$—J	6	1.3	Z-Trp-O—$(CH_2)_6$—J	87[g]	wax	+7.5° (c 1, ethyl acetate)	$C_{25}H_{29}JN_2O_4$ (548.4)	548
t	Z-Trp-OH	J—$(CH_2)_8$—J	6	1.3	Z-Trp-O—$(CH_2)_8$—J	72[g]	63°	+7.2° (c 1, ethyl acetate)	$C_{27}H_{33}JN_2O_4$ (576.5)	576
u	Z-Trp-OH	J—$(CH_2)_{12}$—J	6	1.3	Z-Trp-O—$(CH_2)_{12}$—J	73[g]	oil	+4.9° (c 1, ethyl acetate)	$C_{31}H_{41}JN_2O_4$ (632.6)	632
v	Z-Trp-OH	J—$(CH_2)_2$—O—$(CH_2)_2$—J	10	2	Z-Trp-O—$(CH_2)_2$—O—$(CH_2)_2$—J	74[g]	60–62°	+6.7° (c 1, ethyl acetate)	$C_{23}H_{25}JN_2O_5$ (536.4)	536
w	Z-Trp-OH	1,4-di-JCH_2—C_6H_4	5	1	Z-Trp-O—$CH_2C_6H_4$—CH_2J-4	47[g]	oil[i]	—	$C_{27}H_{25}JN_2O_4$ (568.4)	—

[a] Z = benzyloxycarbonyl; Boc = *t*-butyloxycarbonyl; Bzl = benzyl.
[b] Yield of isolated product.
[c] Conc. = g/100 ml.
[d] All products gave satisfactory microanalyses (C ±0.30, H ±0.35, N ±0.29, J ±0.18).
[e] Reaction time 7 h.
[f] Reaction time 12 h.
[g] Reaction time 24 h.
[h] Reaction time 3 h.
[i] Unsatisfactory results due to rather fast decomposition (in the light).

Table 2. Spectral Properties of *N*-Protected L-Amino Acid and Dipeptide Esters **3**

Product	I.R. (KBr) ν [cm^{-1}]	^{1}H-N.M.R. (60 MHz, $CDCl_3$) δ [ppm]	U.V. (C_2H_5OH) λ [nm] (log ε)
3a	3400, 3350, 1740, 1690	3.25 (d, 2H, J=5 Hz); 4.70 (m, 1H); 5.03 (s, 4H); 5.30 (d, 1H, J=8 Hz); 6.64 (d, 1H, J=3 Hz); 6.80–7.50 (m, 14H); 8.05 (broad s, 1H)	269 (sh), 274 (3.77), 282 (3.80), 291 (3.74)
3b	3350, 1730, 1690	1.40 (s, 9H); 3.25 (d, 2H, J=5 Hz); 4.65 (m, 1H); 5.06 (s, 3H); 6.72 (d, 1H, J=3 Hz); 6.85–7.80 (m, 9H); 8.25 (broad s, 1H)	269 (sh), 274 (3.77), 281 (3.80), 290 (3.74)
3c	3420, 3370, 1725, 1705	3.25 (d, 2H, J=6 Hz); 4.65 (m, 1H); 5.03 (s, 4H); 6.68 (d, 1H, J=7 Hz); 6.80–8.20 (m, 14H); 10.2 (broad s, 1H)[a]	271 (3.68), 290 (3.50)
3d	3330, 1740, 1700	1.16 (t, 3H, J=7 Hz); 3.28 (d, 2H, J=5 Hz); 4.10 (q, 2H, J=7 Hz); 4.68 (m, 1H); 5.03 (s, 2H); 5.32 (d, 1H, J=8 Hz); 6.85 (d, 1H, J=2.5 Hz); 6.92–7.60 (m, 9H); 8.15 (broad s, 1H)	274 (3.77), 281 (3.80) 290 (3.72)
3e	3330, 1725	3.05 (d, 2H, J=5 Hz); 4.73 (m, 1H); 5.05 (s, 2H); 5.10 (s, 2H); 5.2 (d, 1H, J=8 Hz); 6.7–7.4 (m, 15H)	243 (2.34), 248 (2.48), 253 (2.62), 259 (2.73), 262 (2.61), 265 (2.65), 288 (2.44)
3f	3350, 1725	0.82 (d, 3H, J=5 Hz); 0.95 (d, 3H, J=6 Hz); 2.12 (m, 1H); 4.28 (m, 1H); 5.05 (s, 2H); 5.10 (s, 2H); 5.20 (d, 1H, J=8 Hz); 7.30 (s, 10H)	248 (2.39), 252 (2.52), 258 (2.63), 262 (2.52), 264 (2.55), 268 (2.36)
3g	3460, 3300, 1750	2.50 (t, 1H, J=5 Hz); 3.86 (m, 2H); 4.40 (m, 1H); 5.06 (s, 2H); 5.16 (s, 2H); 5.80 (d, 1H, J=8 Hz); 7.30 (s, 10H)	248 (2.37), 253 (2.51), 258 (2.63), 263 (2.51), 264 (2.55), 268 (2.35)
3h	3360, 1720	3.20–3.50 (m, 2H); 4.40–4.85 (m, 1H); 5.03 (s, 4H); 5.10 (s, 2H); 5.8 (d, 1H, J=8 Hz); 7.25 (s, 15H)	243 (2.60), 248 (2.64), 252 (2.74), 258 (2.84), 264 (2.76), 269 (2.56)
3i	3340, 1740, 1690	3.05 (d, 2H, J=6 Hz); 4.40–4.90 (m, 1H); 5.05 (s, 2H); 5.10 (s, 2H); 5.20 (d, 1H, J=8 Hz); 5.22 (s, 2H); 6.97 (s, 4H); 7.10–7.50 (m, 15H)	258 (2.88), 264 (3.01), 268 (2.97), 270 (2.99), 273 (2.83)
3j	3360, 1710	2.98 (m, 2H); 4.50–4.90 (m, 1H); 5.1 (s, 4H); 5.80–6.05 (m, 1H); 7.30 (s, 1H); 8.10 (s, 1H)	298 (2.77), 304 (2.78), 307 (2.73)
3k	3340, 1740, 1690	2.60–3.40 (m, 2H); 4.50–4.90 (m, 1H); 5.00 (s, 2H); 5.07 (s, 4H); 5.82 (d, 1H, J=8 Hz); 7.25 (s, 15H)	252 (2.68), 258 (2.80), 264 (2.73), 268 (2.53)
3l	3360, 1730, 1695	3.03 (d, 2H, J=5 Hz); 4.63 (m, 1H); 4.88 (s, 2H); 5.10 (s, 4H); 6.35 (s, 1H); 6.50 (d, 1H, J=8 Hz); 6.70–7.30 (m, 16H)	258 (2.73), 267 (2.64), 270 (2.66), 274 (2.52)
3m	3340, 3300, 1735, 1720, 1685, 1640	1.40–2.00 (m, 4H); 3.90 (m, 2H); 4.40 (m, 1H); 5.04 (s, 2H), 5.06 (s, 4H); 5.15 (s, 2H); 5.50 (d, 1H, J=8 Hz), 6.90–7.50 (m, 20H); 9.30 (broad s, 2H)	
3n	3310, 1750, 1700, 1660	3.07 (d, 2H, J=6 Hz); 3.93 (d, 2H, J=5 Hz); 4.50 (q, 1H, J=7 Hz); 5.03 (s, 2H); 5.14 (s, 2H); 5.56 (d, 1H, J=8 Hz); 6.65 (t, 1H, J=5 Hz); 7.20 (s, 5H); 7.29 (s, 5H); 7.34 (s, 5H)	253 (2.58), 259 (2.68), 262 (2.56), 265 (2.58), 268 (2.42)
3o	3300, 1730, 1695, 1646	2.85–3.20 (m, 4H); 4.20–5.20 (m, 2H); 5.02 (s, 2H); 5.05 (s, 2H), 5.45 (d, 1H, J=8 Hz); 6.30–7.90 (m, 21H)	248 (2.14), 252 (2.26), 258 (2.35), 265 (2.25), 268 (2.06)
3p	3400, 1710, 1665	3.18 (d, 2H, J=6 Hz); 3.83 (d, 2H, J=6 Hz); 4.25–4.75 (m, 1H); 5.02 (s, 4H); 5.68 (d, 1H, J=7 Hz); 6.30–7.90 (m, 16H); 8.37 (broad s, 1H)	277 (3.74), 283 (3.76), 292 (3.71)
3q	3350, 1730, 1710[b]	1.88 (q, 2H, J=6 Hz); 2.86 (t, 2H, J=6 Hz); 3.23 (d, 2H, J=6 Hz); 4.00 (t, 2H, J=6 Hz); 4.40–4.90 (m, 1H); 5.07 (s, 2H); 5.40 (d, 1H, J=8 Hz); 6.60–7.80 (m, 10H); 8.29 (broad s, 1H)	274 (3.79), 282 (3.81), 291 (3.75)
3r	3350, 1730, 1700[b]	1.00–2.00 (m, 6H); 3.07 (t, 2H, J=6 Hz); 3.27 (d, 2H, J=6 Hz); 3.97 (t, 2H, J=6 Hz); 4.40–4.90 (m, 1H); 5.07 (s, 2H); 5.35 (d, 1H, J=8 Hz); 6.70–7.60 (m, 10H); 8.26 (s, 1H)	275 (3.74), 282 (3.78), 291 (3.72)
3s	3350, 1725, 1700[b]	0.60–2.20 (m, 8H); 3.07 (t, 2H, J=6 Hz); 3.22 (d, 2H, J=6 Hz); 3.94 (t, 2H, J=6 Hz); 4.32–4.82 (m, 1H); 5.03 (s, 2H); 5.33 (d, 1H, J=8 Hz); 6.70–7.60 (m, 10H); 8.33 (broad s, 1H)	274 (3.79), 282 (3.82), 290 (3.75)
3t	3350, 1735, 1700	0.90–2.20 (m, 12H); 3.17 (t, 2H, J=7 Hz); 3.27 (d, 2H, J=6 Hz); 4.00 (t, 2H, J=6 Hz); 4.45–4.90 (m, 1H); 5.07 (s, 2H); 5.32 (d, 1H, J=8 Hz); 6.80–7.60 (m, 10H); 8.20 (s, 1H)	275 (3.79), 282 (3.81), 291 (3.75)
3u	3350, 1730, 1720	0.90–2.00 (m, 20H); 3.12 (t, 2H, J=6 Hz); 3.22 (d, 2H, J=5 Hz); 3.98 (t, 2H, J=6 Hz); 4.40–4.90 (m, 1H); 5.03 (s, 2H); 5.40 (d, 1H, J=8 Hz); 6.60–7.70 (m, 10H); 8.40 (broad s, 1H)	274 (3.77), 282 (3.79), 291 (3.73)
3v	3330, 1735, 1690[c]	2.90–3.80 (m, 8H); 4.07–4.32 (m, 2H); 4.40–4.95 (m, 1H); 5.06 (s, 2H); 5.36 (d, 1H, J=8 Hz); 6.80–7.70 (m, 10H); 8.22 (broad s, 1H)	275 (3.76), 282 (3.78), 291 (3.72)
3w	3400, 1730, 1690[b]	3.25 (d, 2H, J=6 Hz); 4.38 (s, 2H); 4.50–4.90 (m, 1H); 4.97 (s, 2H); 5.05 (s, 2H); 5.30 (d, 1H, J=8 Hz); 6.63 (s, 1H); 6.70–7.50 (m, 13H); 8.07 (s, 1H)	—[d]

[a] $CDCl_3$ + DMSO.
[b] Film.
[c] Nujol.
[d] Spectroscopic data were consistent with the analogous more stable Cl-derivative.

lution (1 ml), a mixture of trioctylmethylammonium chloride (Adogen-464; 0.404 g, 1 mmol) and organic halide **2** (1.2 mmol) in dichloromethane (1 ml) is added at room temperature. After the reaction is complete (3–24 h, see Table 1) the mixture is extracted twice with dichloromethane. The organic phase is washed with water, dried with sodium sulphate and concentrated to a small volume under vacuum at room temperature. The residue is purified by percolation on a silica gel column by eluting with hexane/ethyl acetate (8:2). Adogen may be recovered by eluting with methanol.

Deprotection of Z,N-Protected L-Amino Acid and Dipeptide Benzyl Esters by Hydrogenolysis; General Procedure:

A solution of the Z,N-L-amino acid-OBzl (2 mmol) (or -dipeptide-) in 95% ethanol (100 ml) in the presence of an equal amount of 5% palladium on carbon [in water (2.3 ml)] is stirred under a current of hydrogen for 1 h. The catalyst is subsequently removed by vacuum filtration on celite (2–3 cm thickness). The solution is evaporated to dryness and the amino acid (or dipeptide) recrystallized.

We gratefully acknowledge financial support by National Research Council (C.N.R.), Rome.

Received: July 17, 1979

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