Amino-acid 4-Methoxybenzyl Esters

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L-Amino-acid 4-methoxybenzyl ester hydrochlorides were synthesised by the interaction of 4-methoxybenzyl halides with amine or silver salts of the corresponding *N*-(2-nitrophenylthio)-L-amino-acids followed by removal of the *N*-protecting group. Direct esterification of the toluene-4-sulphonate of L-phenylalanine with 4-methoxybenzyl alcohol proceeded in rather low yield. Esters were cleaved with formic acid.

CARBOXY-PROTECTING groups cleaved by methods other than alkaline hydrolysis or catalytic hydrogenolysis are valuable in certain problems of peptide chemistry.[1] Among these are the 4-methoxybenzyl (anisyl) ester group,[2] and irradiation-sensitive ester groups.[3]

A number of *N*-protected amino-acid 4-methoxybenzyl esters have been reported. Esterification was performed either by *NN'*-dicyclohexylcarbodi-imide,[2,4] 4-methoxybenzyl chloride,[4] or *NN*-dimethylformamide dineopentyl acetal,[5] or by way of imidazole-promoted condensation of the corresponding 4-nitrophenyl esters with 4-methoxybenzyl alcohol.[6] However, a racemisation test conducted of the latter method demonstrated that the procedure can only be applied to glycine and to dipeptides bearing a glycine residue as *C*-terminal.[6] The preparation of *N*-unprotected amino-acid 4-methoxybenzyl esters has been confined so far to the β- and γ-derivatives, respectively, of L-aspartic and L-glutamic acids; they were synthesised by the interaction of 4-methoxybenzyl chloride with the sodium salt of the corresponding amino-acid copper complex.[7] We report here our studies on 4-methoxybenzyl ester hydrochlorides of optically pure *N*-unprotected mono-amino, mono-carboxylic acids, and sone experiments on the cleavage of this carboxy-protecting group.

The routes investigated for the ester synthesis involved either direct or indirect esterification. Direct esterification (possible in acid medium only) of the toluene-4-sulphonate of L-phenylalanine [8] with 4-methoxybenzyl alcohol was achieved by the standard method of azeotropic distillation. Although few steps were involved, the yield of the ester toluene-4-sulphonate was low. Moreover, a large excess of 4-methoxybenzyl alcohol was required. Even though most of the unchanged amino-acid salt was recovered, it was necessary to repeat the experiment in order to secure a substantial quantity of ester. Better yields were obtained by the indirect route, involving the interaction of 4-methoxybenzyl chloride [9] or bromide [10] with the silver (or amine) salts of *N*-(2-nitrophenylthio)amino acids [11] in chloroform. The ester hydrochlorides were obtained after the removal of the *N*-protecting group.[11] *N*-(2-Nitrophenylthio)amino-acid dicyclohexylammonium salts offer the advantage of being known compounds.[11] When *N*-(2-nitrophenylthio)-L-valine dicyclohexylammonium salt was treated with 4-methoxybenzyl bromide in chloroform suspension, the hydrochloride of the corresponding ester was obtained after removal of the amino-protecting group. Although the compound was obtained in lower yield than by using the corresponding silver salt, the procedure involves fewer steps.

$$2\text{-}NO_2\cdot C_6H_4S\cdot NH\cdot CHR\cdot CO_2^-(C_6H_{11})_2\overset{+}{N}H_2 \xrightarrow{4\text{-}MeO\cdot C_6H_4\cdot CH_2Br} 2\text{-}NO_2\cdot C_6H_4S\cdot NH\cdot CHR\cdot CO_2\cdot CH_2\cdot C_6H_4\cdot OMe\text{-}4$$

$$\xrightarrow[\text{ether}]{HCl} HCl,H_2N\cdot CHR\cdot CO_2\cdot CH_2\cdot C_6H_4\cdot OMe\text{-}4 \quad (I)$$

$$R = Pr^i$$

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The 4-methoxybenzyl ester group can be cleaved by brief action of cold trifluoroacetic acid;[2] under these conditions, benzyl ester groups remain intact.[2] Moreover, 4-methoxybenzyl esters of *N*-substituted carbamic acids are more sensitive to hydrogen halides than the corresponding benzyl esters.[12a,b,d] As in the case of diphenylmethyl esters,[1] the rate of cleavage of 4-methoxybenzyl esters by dilute solutions of hydrogen chloride in organic solvents is greatly influenced by the nature of the solvent itself. In ethyl acetate the cleavage takes place slowly, whereas in nitromethane (high dielectric constant) it is fast (*cf.* Table). It was not possible (at least for *N*-benzyloxycarbonyl-L-proline

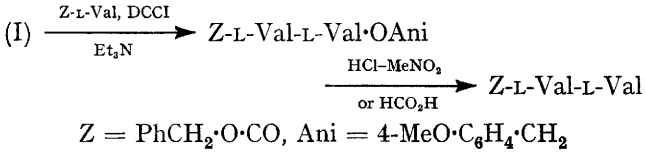
Removal of the ester functions from 4-methoxybenzyl (A) and diphenylmethyl (B) *N*-benzyloxycarbonyl-L-prolinate by hydrogen chloride

Compound	Solvent	*N*-Benzyloxycarbonyl-L-proline formed (%)
(A) [a]	Ethyl acetate	12 [b,c]
(B) (ref. 1)	Nitromethane	57 [b]
(A) [a]	Nitromethane	80 [b]

[a] *N*-Benzyloxycarbonyl-L-proline 4-methoxybenzyl ester was obtained as a viscous oil; it was prepared similarly (*cf.* Experimental section) to the L-alanine derivative; [b] cleavage by hydrogen chloride in the solvent indicated (0·2N; 3 equiv.); 30 min. at 30°. [c] The cleavage (%) had not increased after 3 hr.; a 90% recovery of the corresponding diphenylmethyl ester after similar treatment has been reported.[1]

esters under the cleavage procedures employed) to remove selectively by hydrogen chloride treatment either the 4-methoxybenzyl, the diphenylmethyl, or the t-butyl ester group in the presence of any one of the other two.* Similar results were obtained by use of formic acid, a reagent employed for the removal of groups such as 4-methoxybenzyloxycarbonyl [12c,d] and t-butoxycarbonyl.[14] 'Conventional' methods of cleavage, *e.g.* catalytic hydrogenolysis (used for diphenylmethyl esters [1]) or alkaline hydrolysis could perhaps remove selectively the 4-methoxybenzyl ester group in the presence of a t-butyl ester, since the former group is hydrogenolysed [12a] as well as saponified,[15] whereas t-butyl esters are difficult to saponify and are not hydrogenolysed.[16]

The ability of amino-acid 4-methoxybenzyl esters to couple with *N*-protected amino-acids was tested, as in analogous cases,[1] by the synthesis of the protected dipeptide *N*-benzyloxycarbonyl-L-valyl-L-valine 4-methoxybenzyl ester, by use of *NN'*-dicyclohexylcarbodi-imide (DCCI) [17] as condensing agent. The free dipeptide was obtained by catalytic hydrogenolysis of both protecting groups. The 4-methoxybenzyl ester group was selectively cleaved by treatment of the dipeptide ester with a dilute solution of hydrogen chloride in nitromethane for 3 hr. at 30°,† or with formic acid for 3 hr. at 24°. If the proper precautions were taken to avoid racemisation, the dipeptide chain could be lengthened at the carboxy-end. Alternatively, the same chain could be built up from the amino-end, provided that the 2-nitrophenylthio-group was used for amino-protection (this scheme was reported [6] after our work had been completed).



EXPERIMENTAL

4-Methoxybenzyl chloride was prepared by a modification of the instructions given in ref. 9*a*, *i.e.* by adding thionyl chloride to a benzene solution of 4-methoxybenzyl alcohol. Impure 4-methoxybenzyl chloride is reported to be unstable; [9c] however, the distilled product [9b] was kept in a deep-freeze for about a month without appreciable decomposition. 4-Methoxybenzyl bromide [10a] (purified by high-vacuum distillation) was more prone to decomposition under these conditions of storage (*cf.* ref. 10*b*).

For esterifications, cleavage of esters, and the coupling reaction, anhydrous reactants and dry solvents were used. Evaporations were carried out under reduced pressure at 35—40° (unless otherwise stated), after drying, if necessary, over anhydrous sodium sulphate. Light petroleum of b.p. 40—60° was used. M.p.s were taken in capillary tubes. Before analysis compounds were dried (P_2O_5) at 20—25° under high vacuum; microanalyses were performed by Dr. H. Mantzos, Analytical Laboratory of the Royal Hellenic Research Foundation. Evidence for the homogeneity of compounds was provided by t.l.c.[18] on Kieselgel G 16, or paper chromatography on Whatman No. 1 paper in n-butanol–acetic acid–water–pyridine; [19] spots were revealed by ninhydrin or iodine vapour.

N-Benzyloxycarbonyl-L-alanine 4-Methoxybenzyl Ester.—To a cold solution of *N*-benzyloxycarbonyl-L-alanine [20] (2·23 g., 0·01 mole) in chloroform (10 ml.), triethylamine (1·4 ml., 0·01 mole) and 4-methoxybenzyl bromide (2·01 g., 0·01 mole) were added. The mixture was left for 24 hr. in

* As reported,[13] *N*-benzyloxycarbonyl-L-phenylalanine diphenylmethyl ester was cleaved six times faster than the corresponding t-butyl ester by bubbling hydrogen chloride into a benzene solution of the ester at 0°.

† According to an observation of ours, the corresponding diphenylmethyl ester is cleaved faster than it has been reported; [1] further details on this matter will be included in a future publication from this Laboratory.

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the dark at room temperature, then chloroform was added and the solution was washed with water, aqueous potassium hydrogen carbonate, and water again, dried, and concentrated to dryness; light petroleum was added to the syrupy residue. Crystallisation was induced by cooling and scratching; the 4-*methoxybenzyl ester* (2 g., 59%) had m.p. 60—61° (from ethyl acetate–light petroleum), $[\alpha]_D^{18}$ −16·1° (*c* 5 in tetrahydrofuran) (Found: C, 66·9; H, 6·3; N, 3·9. $C_{19}H_{21}NO_5$ requires C, 66·45; H, 6·2; N, 4·1%).

L-*Alanine* 4-*Methoxybenzyl Ester Hydrochloride*.—The silver salt of *N*-(2-nitrophenylthio)-L-alanine [11] was prepared (84%) like silver *N*-(2-nitrophenylthio)-L-asparaginate [1] [from *N*-protected alanine (7·26 g., 0·03 mole) aqueous methanol (85% v/v; 45 ml.), diethylamine (3 ml., 0·03 mole), and silver nitrate (5·26 g., 0·031 mole) in water (5 ml.)]. To a suspension of the salt (8·55 g., 0·0245 mole) in chloroform (40 ml.), 4-methoxybenzyl bromide (4·44 g., 0·0221 mole) was added. The mixture was shaken in the dark for 20 hr. at 20—25°, silver bromide and unchanged starting material were filtered off (Celite) and the filtrate was concentrated to dryness. To a solution of the oily residue in ethyl acetate (5 ml.), a small amount of ether saturated with hydrogen chloride was added,[11] followed almost immediately by ether (*ca.* 180 ml.). Crystallisation was induced by cooling and scratching for a short time, then the crude ester salt was filtered off, washed repeatedly with ether, and suspended in water (70 ml.). Saturated aqueous sodium carbonate was added and the free ester liberated was taken up in ethyl acetate.* To the dried (K_2CO_3) organic layer, a small amount of ether containing hydrogen chloride was added; soon after, the mixture was diluted with more ether to yield crystalline L-*alanine* 4-*methoxybenzyl ester hydrochloride* (2·3 g., 42% †), m.p. 139—140° (from methanol–ether), $[\alpha]_D^{20}$ −7·8° (*c* 3 in $Me_2N{\cdot}CHO$) (Found: C, 53·8; H, 6·9; Cl, 14·7; N, 5·6. $C_{11}H_{15}NO_3$,HCl requires C, 53·8; H, 6·6; Cl, 14·4; N, 5·7%).

L-*Phenylalanine* 4-*Methoxybenzyl Ester Hydrochloride*.—(*a*) *N*-(2-Nitrophenylthio)-L-phenylalanine dicyclohexylammonium salt [11] was transformed into the silver salt (84%) as for the preparation of silver *N*-trityl-L-alaninate [1] [from the *N*-protected amino-acid salt (12·48 g., 0·025 mole), aqueous methanol (90% v/v; 260 ml.), and silver nitrate (4·58 g., 0·027 mole) in water (8 ml.)]. By the interaction of the silver salt (10·42 g., 0·0245 mole) with 4-methoxybenzyl bromide (4·44 g., 0·0221 mole) in chloroform (40 ml.) as described for the L-alanine derivative, *N*-(2-nitrophenylthio)-L-phenylalanine 4-methoxybenzyl ester was obtained as an oil. The crude *N*-protected amino-acid ester was treated with ether saturated with hydrogen chloride and then purified as before to yield L-*phenylalanine* 4-*methoxybenzyl ester hydrochloride* (4·2 g., 60%), m.p. 170—172° (froths) [from dioxan–methanol (2 : 1 v/v) by addition of ether], $[\alpha]_D^{23}$ +8·9° (*c* 2·9 in $Me_2N{\cdot}CHO$) (Found: C, 63·5; H, 6·4; Cl, 11·2; N, 4·25. $C_{17}H_{19}NO_3$,HCl requires C, 63·45; H, 6·3; Cl, 11·0; N, 4·35%).

(*b*) L-Phenylalanine toluene-4-sulphonate [8] (0·66 g., 0·002 mole) was dissolved in 4-methoxybenzyl alcohol ‡ (20 g., 0·145 mole) contained in the flask of a Soxhlet apparatus.[21] Carbon tetrachloride (120 ml.) was added and the clear solution obtained was refluxed for 6 hr. on a steam-bath with exclusion of atmospheric moisture ($CaCl_2$ tube); a drying cartridge (K_2CO_3) had been introduced into the extractor of the apparatus. The mixture was filtered and the crystalline precipitate was washed with carbon tetrachloride to yield starting material (0·35 g., 53%) contaminated with traces of the expected ester salt, m.p. and mixed m.p. with an authentic sample 168—169° (lit.,[8] 165—167°). The bulk of carbon tetrachloride was evaporated from the filtrate and a large amount of ether was added to the residue. The crystalline precipitate formed on cooling was filtered off to yield L-*phenylalanine* 4-*methoxybenzyl ester toluene*-4-*sulphonate* (0·3 g., 33%), m.p. 155—158° (decomp.) (from chloroform–ether) (Found: N, 3·25; S, 7·15. $C_{24}H_{27}NO_6S$ requires N, 3·1; S, 7·0%).

The compound was suspended in water, ether was added, and the free ester was liberated by shaking the mixture with aqueous sodium carbonate. Extraction with ether was repeated until no more salt was in suspension. The ether layer was dried (K_2CO_3) and the hydrochloride of the amino-acid 4-methoxybenzyl ester was obtained (75%) in the usual way; m.p. 170—172° (froths), $[\alpha]_D^{30}$ +9·6° (*c* 2·5 in $Me_2N{\cdot}CHO$) [from tetrahydrofuran–methanol (3 : 1 v/v) by addition of ether].

L-*Valine* 4-*Methoxybenzyl Ester Hydrochloride* (I).—*N*-(2-Nitrophenylthio)-L-valine dicyclohexylammonium salt [11] (4·51 g., 0·01 mole) was suspended in chloroform (25 ml.) and 4-methoxybenzyl bromide (2·01 g., 0·01 mole) was added. The mixture was shaken in the dark for 24 hr. at 20—25°, and the dicyclohexylammonium bromide formed filtered off along with unchanged starting material. The filtrate was concentrated to dryness, ethyl acetate was added, and the solution (after filtration) was repeatedly washed with water (until the aqueous layer became colourless) to remove dissolved starting material. It was then dried and concentrated to dryness. The oily residue was dissolved in ether (30 ml.) and the *N*-protecting group was removed as for the L-alanine derivative to yield, after the usual purification step, L-*valine* 4-*methoxybenzyl ester hydrochloride* (1·2 g., 44%), m.p. 114—114·5° (from ethyl acetate–ether), $[\alpha]_D^{30}$ −3·6° (*c* 3 in $Me_2N{\cdot}CHO$), $[\alpha]_D^{30}$ −10·0° (*c* 3 in tetrahydrofuran) (Found: C, 57·2; H, 7·3; Cl., 13·0; N, 4·7. $C_{13}H_{19}NO_3$,HCl requires C, 57·0; H, 7·4; Cl, 12·95; N, 5·1%).

The same compound was obtained (50%) by the interaction of silver *N*-(2-nitrophenylthio)-L-valinate [1] (9·24 g., 0·0245 mole) and 4-methoxybenzyl chloride (3·46 g., 0·0221 mole) as for the preparation of the L-alanine derivative by way of the corresponding bromide.

N-*Benzyloxycarbonyl*-L-*valyl*-L-*valine* 4-*Methoxybenzyl Ester* (II).—To a cold solution of L-valine 4-methoxybenzyl ester hydrochloride (I) (2·74 g., 0·01 mole) in chloroform (25 ml.) containing triethylamine (1·4 ml., 0·01 mole), *N*-benzyloxycarbonyl-L-valine [22] (2·51 g., 0·01 mole) and *NN'*-dicyclohexylcarbodi-imide [17] (2·06 g., 0·01 mole) were added. After 6 hr. at 25—30°, a few drops of aqueous acetic acid (50% v/v) were added and the insoluble precipi-

* Extraction with ethyl acetate was repeated if ester salt was still in suspension in the alkaline aqueous phase.

† Not substantially increased by using the bromide instead of the chloride.

‡ The commercial product was distilled in high vacuum under nitrogen. In subsequent runs, however, this step was omitted; instead, the required amount of 4-methoxybenzyl alcohol (Fluka 'purum') was dissolved in dry carbon tetrachloride and the solution was dried (Na_2SO_4) for *ca.* 20 hr. The mixture was filtered and the filtrate was used for the esterification step.

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tate of *NN'*-dicyclohexylurea (2·1 g., 93%) was filtered off. The filtrate was washed successively with water, dilute sulphuric acid, water, aqueous potassium hydrogen carbonate, and water again, dried, and evaporated to dryness. The crystalline residue was dissolved in warm ethyl acetate, the mixture was filtered, and ether was added to the filtrate to yield the N-*protected dipeptide ester* (3·3 g., 70%), 128·5—129·5° (from chloroform–ether), $[\alpha]_D^{22}$ −34·5° (*c* 1 in tetrahydrofuran) (Found: C, 66·7; H, 7·2; N, 5·8. $C_{26}H_{34}N_2O_6$ requires C, 66·4; H, 7·3; N, 5·95%).

Removal of the 4-Methoxybenzyl Ester Group.—(a) *By hydrogenolysis.* For the following examples, palladium black catalyst and methanol–tetrahydrofuran (3 : 1 v/v; 20 ml., plus 2—3 drops of water, each time) were employed.

(i) A solution of *N*-benzyloxycarbonyl-L-alanine 4-methoxybenzyl ester (0·68 g., 0·002 mole) was hydrogenated. After 3 hr., the free amino-acid formed and the catalyst were filtered off and washed with methanol then with water. The combined filtrates were evaporated to dryness and traces of water were removed from the residue by adding (twice) dry benzene and re-evaporating to dryness; after the addition of dry ether, the product was filtered off to yield L-alanine (0·17 g., 95%), $[\alpha]_D^{16}$ +14·5° (*c* 6·5 in N-HCl) {lit.,[23] $[\alpha]_D^{23}$ +14·4° (*c* 6·5 in N-HCl)}.

(ii) A solution of L-valine 4-methoxybenzyl ester hydrochloride (I) (0·54 g., 0·002 mole) was hydrogenated. After 45 min., the consumption of hydrogen ceased; the mixture was filtered, the filtrate was concentrated to dryness, and ether was added to the crystalline residue. The product was filtered off to yield L-valine hydrochloride (0·27 g., 90%), $[\alpha]_D^{24}$ +16·3° (*c* 5 in H_2O) {lit.,[1] $[\alpha]_D^{25}$ +16·3° (*c* 5 in H_2O)}.

(iii) A solution of *N*-benzyloxycarbonyl-L-valyl-L-valine 4-methoxybenzyl ester (II) (0·47 g., 0·001 mole) was hydrogenated. After 1·5 hr., the mixture (containing the precipitated free peptide) was diluted with methanol (10 ml.), water (15 ml.) was added, and the catalyst was filtered off. The clear filtrate was concentrated to dryness to yield L-valyl-L-valine (0·19 g., 90%), $[\alpha]_D^{25}$ +11° (*c* 2 in H_2O) {lit.,[24] $[\alpha]_D^{20}$ +10·8° (*c* 2 in H_2O)}.

(b) *By acidolysis.* (i) *In formic acid solution.* (1) *N*-Benzyloxycarbonyl-L-alanine 4-methoxybenzyl ester (0·34 g., 0·001 mole) was dissolved in formic acid (Fluka ' purum ', anhydrous; *ca.* 98%; 2 ml.). The clear solution obtained was turbid after 1 hr. at 22°. It was concentrated (at 30°) to dryness, the residue was dissolved in ethyl acetate, and the solution was extracted repeatedly with aqueous potassium hydrogen carbonate. The alkaline extracts were acidified with 5N-hydrochloric acid and concentrated to dryness. The residue was extracted many times with ethyl acetate, then the organic layer was washed with water to remove traces of the mineral acid, dried, and concentrated to dryness. The residue was triturated with light petroleum to yield *N*-benzyloxycarbonyl-L-alanine (0·18 g., 81%), m.p. 84—85°, (lit.,[20] 84°). When *N*-benzyloxycarbonyl-L-proline diphenylmethyl ester was similarly treated with formic acid for 15 min., or for 1 hr., the corresponding yields of cleaved product were 65 and 80%.

(2) *N*-Benzyloxycarbonyl-L-valyl-L-valine 4-methoxybenzyl ester (II) (0·47 g., 0·001 mole) was dissolved in formic acid as in (1). After 3 hr. at 24°, the turbid solution was worked up as before, with use, however, of chloroform instead of ethyl acetate. The *N*-protected dipeptide (0·3 g., 85%) had m.p. 138·5—139°, $[\alpha]_D^{20}$ −36·7° (*c* 3·2 in N-KOH) {lit.,[25] m.p. 139·5—140°, $[\alpha]_D^{20}$ −36·6° (*c* 3·2 in N-KOH)}.

(ii) *In 0·2N-hydrogen chloride solution in nitromethane.* *N*-Benzyloxycarbonyl-L-valyl-L-valine 4-methoxybenzyl ester (II) (0·24 g., 0·0005 mole) was suspended in nitromethane (6·3 ml.); most of the solid went into solution on gentle warming. As soon as the temperature of the mixture had dropped to 30°, 1·27N-hydrogen chloride solution (1·18 ml., 0·0015 mole) in nitromethane was added. The clear solution obtained was kept for 3 hr. at 30°, after which time the solvent was replaced with chloroform and the resulting solution was worked up as in (2) to yield *N*-benzyloxycarbonyl-L-valyl-L-valine (0·15 g., 86%), m.p. 138·5—139° (lit.,[25] 139·5—140°).

(c) *By alkaline hydrolysis.*—To a solution of *N*-benzyloxycarbonyl-L-alanine 4-methoxybenzyl ester (0·68 g., 0·002 mole) in dioxan (5 ml.), 4N-sodium hydroxide solution (0·53 ml., 0·0021 mole) and dioxan–water (2 : 1 v/v; 15 ml.) were added. After 15 min. at 20—25°, water (60 ml.) was added and the clear solution obtained was acidified with 5N-hydrochloric acid while cold. The mixture was extracted with ethyl acetate, then the organic layer was washed with water, dried, and concentrated to a small volume. Light petroleum was added to yield *N*-benzyloxycarbonyl-L-alanine (0·36 g., 81%), m.p. 84—85° (lit.,[20] 84°).

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