Benzotriazole-assisted Synthesis of α-Acylaminonitriles and a Conceptually Novel Method for Peptide Elongation

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A general method for the synthesis of α-acylamino nitriles is reported. Initially, the adducts resulting from Mannich-type condensation of benzotriazole with an aldehyde and an amide are prepared. These undergo elimination of benzotriazole with cyanide to give α-arylamino nitriles in high yields. Subsequent hydrolysis of the nitrile function to the di-amides, followed by a repetition of the cyanoalkylation sequence, constitutes a novel method for the elongation of peptides.

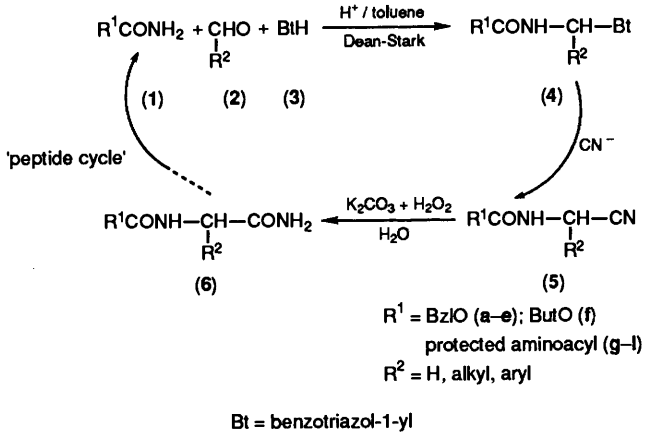
Reports on α-acylamino nitriles are relatively rare in the literature. Apart from a few reported biological effects,[1,2] such compounds have been mainly prepared as intermediates for further transformations of the cyano group to various heterocycles, such as thiazoles[3] or tetrazoles[4] or to 'unusual' derivatives such as thiazoline-peptide[5] or iminopeptide[6] derivatives. Apart from the cyclic Reissert compounds, the syntheses of α-acylamino nitriles are usually accomplished (i) by acylations of Strecker-product amino nitriles (often called the 'Delepine' method),[1,2,7,8] or (ii) by dehydration of α-acylamino carboxamides (usually by tosyl chloride in pyridine).[3,5,6]

Direct Strecker-analogue preparations of α-acylamino nitriles have been classified in the literature as amidoalkylations of HCN.[9] The AB + C (A = amide, B = oxo component, C = HCN) and the A + BC type reactions have both been reported, but only for a few cases. Thus, (i) phthalimidomethyl derivatives of type $PhtNCH_2X$ (X = Br[10] or $^+NM_3$[11]), (ii) 2-methoxy-1-acyl-1,4-benzoxazines,[12] and (iii) *N*-(1,2,2-trichloroethyl)benzamides,[13] each react with CN^- to give α-amido nitriles, with those from route (iii) suffering simultaneous HCl elimination. *N*-(Benzyloxycarbonyl)trifluoroalanine nitrile was prepared from benzyl (1-chloro-2,2,2-trifluoroethyl)carbamate by an analogous reaction.[14] Reactions of α-X-lactams (X = leaving group), such as 2-hydroxy- or 2-acyloxy-pyrrolidin-5-ones,[15] and 4-ethoxy-1,2,3,4-tetrahydroquinazoline-2-thiones,[16] with CN^- have also been reported. Reactions of amides with cyanohydrins (A + BC type) require a high temperature and give good yields only with formamide.[17] Apart from these peripheral examples, no general method for the amidoalkylation of HCN has been published previously.

We now report such a general method. The Mannich adducts (**4**) (Scheme 1), derived from benzotriazole (**3**), an aldehyde (**2**), and an amide (**1**), react smoothly with CN^- to give the corresponding α-acylamino nitriles (**5**) in high yield. Furthermore, hydrolysis of the nitrile function to amide, followed by repetition of the cyanoalkylation on the new amide group, results in a cyclic sequence (Scheme 1), which enables the stepwise synthesis of peptides.

Earlier we reported on the preparation of benzotriazole adducts (**4**) with either carboxamides[18] (aliphatic or aromatic), carbonates or protected amino acid amides[19] as the amide component. The use of glyoxylic acid, or its ester, as the aldehyde component resulted in carboxy-functionalised derivatives.[20]

Mannich adducts, resulting from an amide and secondary amine, were reported[21] to react with CN^- to yield α-amino nitriles by displacement of the amide component.[22] By contrast,



Scheme 1. Preparation of α-acylamino nitriles, and the 'peptide-cycle'.

in the adducts (**4**) the benzotriazolyl entity is a better leaving group than the amido group, and thus the reactions lead exclusively to α-acylamino nitriles (**5**). In our initial attempts, the displacement reaction was carried out with KCN in methanol, but side-products were always observed resulting from the attack of the MeO^- anion on both the central carbon atom [to give $R^1CONHCH(R^2)OMe$] and the urethane carbonyl group (trans-esterification). In a tetrahydrofuran and water two-phase system, the reaction is clean, but very slow; using either Bu_4NOH or $C_{16}H_{33}NMe_3Cl$ as a phase-transfer catalyst increases the reaction rate (see Experimental part, Method A). In dimethyl sulphoxide (Method B), the transformation is much faster, and the reactions are usually completed within a few hours. In Method A the product is isolated by extraction, whereas in Method B it can often be isolated simply by precipitation with water. In both cases the benzotriazole side-product is removed in the alkaline aqueous phase as its potassium salt and can be recovered by acidification. The isolated products always give clean NMR spectra (Tables 2–4), and often correct C, H, N analyses, without further purification (Table 1). While some of the protected amino acid nitriles (**5a,c–e**) have been previously obtained by other methods, compounds (**5b,f**) and all of the protected peptide nitriles prepared are novel derivatives.

The stereoselectivity of the formation of the new chiral centre in dipeptide nitriles (**5h–l**) was also investigated. No dominant stereoselectivity was observed in the condensation step; adducts (**4**) with chiral R^1 (*i.e.* protected aminoacyl) were always obtained as diastereoisomeric mixtures. For (**4h**) and (**4j**) diastereo adducts were separated by crystallisation. To

Table 1. α-Acylamino nitriles (**5a–f**) and protected peptide nitriles (**5g–l**)[a] [$R^1CONHCH(R^2)CN$].

Compound	St[b]	R^1[c]	R^2[c]	Method	Rect. time[d]	Yield[e] (%)	R_f[f] (TLC)	Cryst. solvent[g]	M.p.[h] (°C)	Lit. m.p. (°C)	Analysis[h] (Calc./found) C(%)	H(%)	N(%)
(5a)	A	BzlO	H	A	2 w	79	0.5/B	Pr^i_2O	58	62[8]	63.15/63.3	5.3/5.2	14.7/14.9
				B	36 h	62							
(5b)	A	BzlO	Pr	A	1 w	90	0.7/A		oil		67.2/67.0	6.9/7.0	12.1/12.1
(5c)	A	BzlO	Pr^i	A	1 w	90	0.7/A		oil	oil[4]	67.2/67.1	6.9/7.0	12.1/11.9
(5d)	A	BzlO	Bu^i	A	1 w	86	0.7/A	hexane	59–61	53–54[4]	68.3/68.3	7.4/7.4	11.4/11.4
(5e)	A	BzlO	Ph	A	2 d	79	0.8/A	Pr^i_2O	103–105	114[8]	72.2/72.1	5.3/5.4	10.5/10.1
(5f)	A	tBoc	Pr^i	B	16 h	90	0.7/C		76–78		60.6/60.7	9.2/9.4	14.1/14.2
(5g)	A	BzGly	Pr^i	B	16 h	73	0.4/B		118–121		64.6/64.7	6.6/6.6	16.2/16.1
(5h)	D	Z-Phe	Pr^i	B	10 h	95	0.8/A		147–149		69.6/69.6	6.6/6.7	11.1/11.0
(5i)	C	Z-L-Val	Bu^i	B	16 h	85	0.6/C		120–132		66.1/66.4	7.9/8.1	12.2/12.1
(5j)	B, D[i]	Z-Phg	Bu^i	B	16 h	87	0.4/C	Pr^i_2O	94–97		69.6/69.6	6.6/6.6	11.1/11.0
(5k)	*j*	Z-Ileu	Pr^i	B	16 h	86	0.5/C	EtOAc + hexane	108–122		66.1/66.2	7.9/8.0	12.2/12.2
(5l)	C	Z-L Phe-L Val	Bu^i	B	16 h	94	0.6/A	EtOH	164–168		68.3/68.5	7.4/7.5	11.4/11.4

[a] The monochiral products are racemates; those with more chiral centre are ~1:1 diastereoisomeric mixtures. [b] Stereocomposition of the starting material (**4**): A = racemate; B = ~1:1 diastereo-mixture, racemate; C = 1:1 diastereo-mixture, single enantiomer; D = diastereo-homogeneous, racemate. [c] For abbreviations see Scheme 2. [d] w = week, d = day, h = hour. [e] Yield given for crude, TLC-pure products. [f] Commercial (Kodak) silica gel plates, A: hexane–acetic acid–chloroform = 1:1:8; B: benzene–methanol = 10:1; C: diethyl ether–hexane = 4:1. [g] If not given, m.p. and correct analysis were obtained for the crude product. [h] M.p. and analysis obtained for the same (crude or crystallized) sample. [i] Both reactions gave the same diastereo-mixture product. [j] C_α(Ileu):C_α(Val) = ~5:1; C_α(Ileu):C_β(Ileu) = ~1:1.

Table 2. ^1H and ^{13}C NMR spectra[a] of α-acylamino nitriles (**5a–f**) [$R^1CONHCH(R^2)CN$].

Compound	^1H NMR signals R^1	NH[b]	α-CH	R^2	^{13}C NMR signals R^1	CONH	α-CH	R^2	CN
(5a)	7.3 s, 5 H 5.09 s, 2 H	5.88 br s	3.96 br s[c]	3.96 br s[c]	135.0, 128.5, 128.4, 128.3 67.6	155.8	29.3		116.4
(5b)	7.34 s, 5 H 5.12 s, 2 H	5.4 br	4.6 br	0.96 t, 3 H 1.76 m, 2 H 1.5 m, 2 H	135.1, 128.5, 128.4, 128.2 67.6	155.9	42.5	35.1, 18.5, 13.1	118.6
(5c)	7.33 s, 5 H 5.13 s, 2 H	5.52 br d	4.48 br t	2.1–1.95 m, 1 H 1.1–1.0 2 × d, 6 H	135.5, 128.5, 128.3, 128.1 67.6	155.4	48.9	31.6, 18.4, 17.8	117.7
(5d)	7.35 s, 5 H 5.13 s, 2 H	5.3 d	4.64 q	1.9–1.75 m, 1 H 1.75–1.55 m, 2 H 1.1–0.85 m, 6 H	135.6, 128.5, 128.4, 128.2 67.6	155.3	41.8	41.4, 24.6, 22.0 21.7	118.8
(5e)	7.5–7.2 m, 5 H[b] 5.13 s, 2 H	5.7 m[b]	5.7 m[b]	7.5–7.2 m, 5 H[b]	135.4, 128.5, 128.4, 128.2 67.8	155.3	46.4	133.0, 129.5, 129.2, 126.8	117.4
(5f)	1.48 s, 9 H	5.2 br	4.5 br t	2.04 q, 1 H 1.07 d, 3 H 1.12 d, 3 H	28.1[d]	154.5	48.4	31.7, 18.4, 17.9	118.0

[a] δ ppm, in $CDCl_3$ + TMS. [b] $J_{NH,CH}$ = 9 Hz. [c] Overlapping signals. [d] The signal of the quaternary carbon was not seen.

investigate the stereoselectivity of the Bt/CN displacement step, both single diastereoisomers were treated with KCN to give products (**5h**) and (**5j**) in near quantitative yield. Analysis by ^1H and ^{13}C NMR of (**5h**) and (**5j**) showed duplication of most of the signals indicating that diastereoisomeric mixtures had been formed. A good separation of the peptide NH signals for (**5h**), and also the protected NH signals for (**5j**), suggest a 1:1 ratio and indicate that no stereoselectivity had occurred in either reaction. Apart from the NMR analysis, the nitrile (**5h**) was also converted into the corresponding amide (**6h**), which was compared by NMR, m.p. and TLC with an independently prepared, stereo-L,L-Z-Phe-Val-NH_2 sample.[23] Each method clearly showed that (**6h**) is an approximately 1:1 mixture of the two diastereoisomers (see Experimental section). Because of the lack of retention of the stereo-purity, all the other adducts were subjected to the KCN reaction as crude, diastereoisomeric mixtures, giving nitriles (**5i–l**), and consequently their hydrolysis product amides (**6h,j**) and (**6l**), as ~1:1 diastereoisomeric mixtures (see NMR data in Tables 3 and 4). Recrystallisation of the nitrile (**5l**) resulted in a 2:1 mixture, whereas for the amide (**6j**) a complete isolation of both diastereoisomers was achieved. No separation of the diastereoisomers could be obtained by recrystallisation of (**5j**) and (**5k**).

Completion of the 'peptide-cycle' (Scheme 1) required a high-yielding method for the hydrolysis of the nitrile function. Although Japanese authors reported[22] difficulties in the selective hydrolysis of α-acylamino nitriles, we found that use of the Radziszewski method,[24] particularly in DMSO as a solvent,[25] effects these transformations in selective reactions to afford high yields of pure products (see Experimental section).

Application of the new cyclic elongation sequence is demonstrated by the syntheses of a dipeptide and of a tripeptide amide (Scheme 2). Benzyloxycarbonyl-phenylglycyl-leucine amide (Z-Phg-Leu-NH_2), and benzyloxycarbonyl-phenylalanyl-valyl-leucine amide (Z-Phe-Val-Leu-NH_2) have each been prepared by two repeated cycles, starting from benzyl

Table 3. ^{1}H NMR spectra[a] of protected peptide nitriles (**5g–l**) [$R^1CONHCH(R^2)CONHCH(R^3)CN$].

Compound	R^1	$(R^1CO)NH$	$(CHCO)NH$	$CH(R^2)$	$CH(R^3)$	R^2	R^3
(**5g**)	7.5–7.1 m, 5 H	8.26 d	7.87 t	4.15 b[b]	4.57 t	4.15[b]	1.9 q, 1 H; 1.2–0.7 m, 6 H
(**5h**)	7.4–7.0 m, 5 H[b]	6.1 br s	8.1 and 7.9 2 × b	4.7–4.4 b[b]	4.7–4.4 b[b]	7.4–7.0 m, 5 H[b] 3.0 b, 2 H	2.0–1.7 m, 1 H 1.0–0.7 2 × d, 6 H
(**5i**)	7.32 s, 5 H 5.1 s, 2 H	5.8 2 × d	7.54 d	4.15–4.0 2 × t	4.86 q	2.3–1.9 m, 1 H 1.1–0.8 m, 6 H[b]	1.9–1.5 m, 3 H 1.1–0.8 m, 6 H[b]
(**5j**)	7.3 br s, 5 H[b] 5.05 s, 2 H	6.4 and 6.35 2 × d	7.9 br	5.47 and 5.43 2 × d	4.85–4.7 br	7.3 br s, 5 H[b]	1.6–1.35 m, 2.5 H 1.75–1.6 m, 0.5 H 0.85 d, 3 H 0.78 and 0.74 2 × d, 3 H
(**5k**)	7.34 s, 5 H 5.12 s, 2 H	5.7 2 × d	7.5 2 × d	4.4–4.1 m	4.8–4.7 br t	1.92–1.7 br, 1 H 1.65–1.35 br, 1 H[c] 1.3–0.8 m, 7 H[b]	2.1–1.92 b, 1 H 1.3–0.8 m, 6 H[b]
(**5l**)	7.4–7.1 m, 5 H[b] 5.14–5.3 m, 2 H	5.89 d 5.68 d	7.65–7.5 2 × d	4.7–4.5 2 × bq	4.95–4.75 m	7.4–7.1 m, 5 H[b] 3.2–1.95 m, 2 H	1.85–1.5 m, 3 H 1.05–0.75 m, 6 H[b]

[a] δ ppm, in $CDCl_3$ + TMS. [b] Overlapping signals. [c] The other half of the γ-CH_2 signal is overlapped with the CH_3 multiplet (1.3–0.8 ppm). [d] Signals of the middle amino acid unit [$NHCH(Pr^i)CO$]: 6.83 (2/3 H, d, NH; 1/3 H overlapping with Ar signals); 4.4–4.25 (1 H, m, N-CH); 2.25–2.05 (1 H, m, Pr^iCH); 1.05–0.75 (6 H, br m, Pr^iMe).

Table 4. ^{13}C NMR spectra[a,b] of protected peptide nitriles (**5g–m**) [$R^1CONHCH(R^2)CONHCH(R^3)CN$].

Compound	R^1	$(R^1)CO$	$(CH)CO$	$CH(R^2)$	$CH(R^3)$	R^2	R^3	CN
(**5g**)	132.9, 131.8, 128.4, 127.1	169.4[c]	168.3[c]	43.3	46.8		31.1, 18.4, 17.9	117.6
(**5h**)	135.9, 128.6, 128.4, 127.1,[c] 67.2,	156.1	170.9	56.1	46.5	129.2, 128.2, 127.7, 127.6,[c]	31.4, 18.4, 17.7	117.3
	67.1	155.7	171.0		46.4	38.7[c]	31.3, 17.6	117.2
(**5i**)	135.9, 128.5, 128.2, 127.8, 67.2,	156.7	171.3	60.2	41.5	31.3, 19.1, 18.2	38.9, 24.8, 22.15, 21.9	118.7
	67.1		171.2		41.2	31.0, 19.0, 17.9	38.8, 24.75, 21.8, 21.7	118.6
(**5j**)	135.7, 129.0, 128.3, 127.7,[c] 67.2	156.1	169.8	58.2	41.0	137.4, 129.0, 128.5, 126.85[c]	39.2, 24.5, 22.0, 21.6	118.8
		156.0		58.0	40.9	136.8, 128.9, 128.4, 126.8	38.9, 24.4, 21.7, 21.5	118.4
(**5k**)	136.0, 128.5, 128.2, 127.7, 67.2,	156.5	171.5	59.4	46.5	37.4, 24.7, 15.5, 11.2, 24.6,	31.5, 18.5, 17.9	117.6
	67.1		171.3	59.2	46.4	15.3, 11.0		117.5
(**5l**)[d]	136.1, 129.2–127.0[e] 6.75, 67.0	157.0	170.7	56.7	41.6	135.7, 129.2–127.0,[e] 39.0	38.1, 24.9, 22.2, 21.9	118.9
			170.4	56.3	41.3	38.8	38.0, 24.8, 22.0, 21.7	118.6

[a] δ ppm, in $CDCl_3$ + TMS. [b] Whenever separated, signals of the diastereoisomers are listed in separate lines. [c] Interchangeable signals. [d] Signals of the middle amino acid unit ($NHCH(Pr^i)CO$): 171.7 and 171.5 (CO); 58.5 and 58.3 (CH_α), 30.8 and 30.2, 19.2 and 19.0, 18.3 and 17.6 (Pr^i). [e] Complex.

carbamate and from Z-Phe-NH_2, respectively. The chemical yields of the cycles are high (the average overall yield of a 3-step cycle is 65–70%), but the stereoselectivity at the new chiral centres is poor (see above). Earlier Lipshutz and co-workers reported[26] stereoselective transformation of a dipeptide amide from a 1:1 to a 6.5:1 diastereo-mixture *via* ring closure to trifluoroacetamido-oxazole derivatives. Application of this, or a similar technique, may allow the preparation of diastereo-pure peptide amides from our products. We are planning to continue our work in this direction.

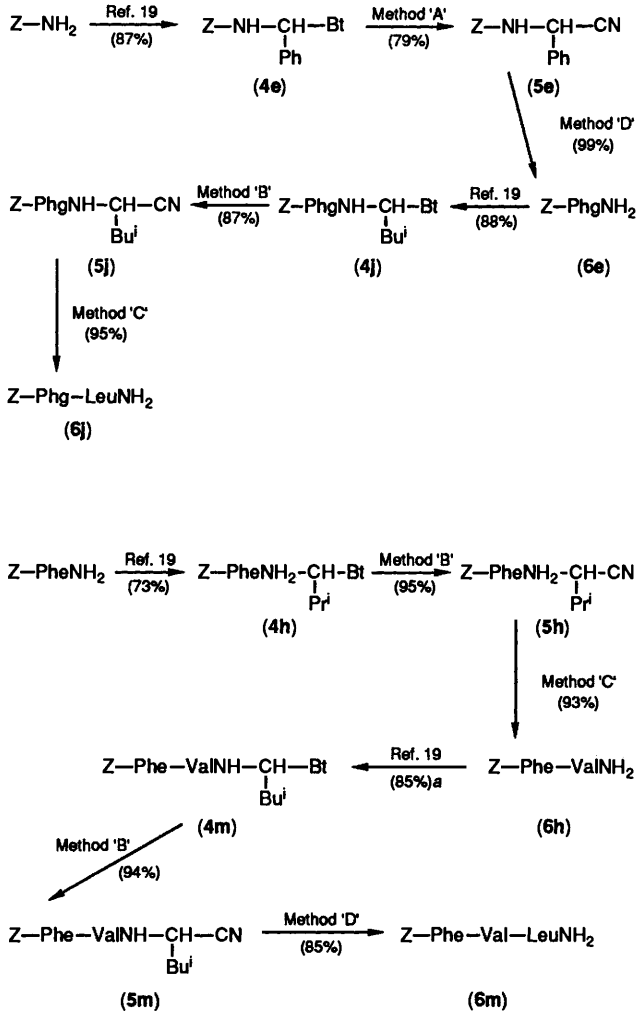
Experimental

M.p.s were determined on a hot stage microscope and are uncorrected. NMR spectra were recorded on a Varian EM-360 (60 MHz), on a Varian XL-200 (200 or 50 MHz) and on a Varian VXR-300 (300 or 75 MHz) instrument as solutions in deuteriochloroform ($CDCl_3$) using TMS (δ = 0.0 ppm) for proton, and the solvent signal (δ = 77.0 ppm) for carbon spectra as reference. Elemental analyses were performed in this department on a Carlo Erba-1106 instrument under the supervision of Dr. D. Powell.

1-(α-*Acylaminoalkyl*)*benzotriazoles* (**4a–e**) *and* (**4g–k**) were prepared according to the literature.[19]

t-*Butyl* [N-(1-*Benzotriazolyl*-2-*methylpropyl*)*carbamate* (**4f**).—t-Butyl carbamate (1.17 g, 10 mmol), benzotriazole (1.8 g, 15 mmol), isobutyraldehyde (1.08 g, 15 mmol), and toluene-*p*-sulphonic acid monohydrate (0.1 g, as catalyst) in toluene (30 ml) were refluxed in a Dean-Stark apparatus for 1 h, then stirred at 20 °C overnight. Ethyl acetate (30 ml) was added, and the mixture was washed with aqueous K_2CO_3 (1 mol l^{-1}; 2 × 10 ml), followed by water (2 × 10 ml). The organic solution was dried ($MgSO_4$), evaporated, and the product was isolated from ether (20 ml) as a white solid (1.2 g, 43%). Evaporation of the mother liquor and treatment of the residue with Pr^i_2O (10 ml) yielded a second fraction of the product 0.46 g, 15%). In solution (NMR) both fractions showed a ~2:1 ratio of the benzotriazol-1-yl, and -2-yl isomers; these isomers are probably present also in the solid state, resulting in the wide melting point range. TLC: R_f = 0.6 (ether–hexanes 4:1, silica gel); m.p. 145–154 °C; $\delta_H(CDCl_3)$ 8.1–7.3 (4 H, m, Bt), 6.3–5.8 (2 H, m, CHN + NH), 2.9–2.4 (1 H, m, Pr^i-CH), 1.4 (9 H, s, Bu^t), 1.2 (2 H, d), 1.12 (1 H, d), and 0.75 (3 H, d) Pr^i-Me) signals; $\delta_C(CDCl_3)$ 155.0 (CO), 126.3, 124.0, 118.3, 109.9 (benzotriazol-1-yl), 127.5, 119.7 (benzotriazol-2-yl), 69.9 (N–C–N), 33.3, 18.6, 18.3 (major isomer Pr^i signals), 34.1, 18.9, 18.1 (minor isomer Pr^i signals), 28.1 (Bu^t) (quaternary signals were not observed) (Found: C, 61.6; H, 7.7; N, 19.2. $C_{15}H_{22}N_4O_2$ requires C, 62.0; H, 7.6; N, 19.3%).

1-[1-(N-*Benzyloxycarbonyl*-*phenylalanyl*-*valylamino*)-3-*methylbutyl*]*benzotriazole* (**4l**).—*N*-Benzyloxycarbonyl-L-phenylalanyl-L-valine amide[23] (1.19 g, 3 mmol), benzotriazole



Legend: Z = benzyloxycarbonyl; Bt = benzotriazol-1-yl; Phg = C-phenylglycine; Leu = leucine; Phe = phenylalanine; Val = valine. *a* started with stereo-pure L,L (**6h**); adduct (**4m**) (oil) was used for the next step without purification.

Scheme 2. Benzotriazole-assisted peptide elongations. [a] Z-Phg-LeuNH$_2$. [b] Z-Phe-Val-LeuNH$_2$.

(0.56 g, 4.5 mmol), isovaleraldehyde (0.4 g, 4.5 mmol) and toluene-*p*-sulphonic acid monohydrate (0.05 g, as catalyst) in toluene (20 ml) were refluxed in a Dean-Stark apparatus for 2 h. Additional benzotriazole (0.3 g) and isobutyraldehyde (0.25 g) were added, and the reflux was continued for 3 h. The solution was extracted with K_2CO_3 solution (1 mol l^{-1}; 3 × 5 ml) and water (2 × 5 ml), dried ($MgSO_4$), and evaporated to yield a solid foam (1.5 g, 85%). TLC: R_f = 0.6 (silica gel; hexane–AcOH–$CHCl_3$, 1:1:8). The product gave complex NMR spectra and was used in a crude state for further transformation.

α-Acylamino nitriles (**5**). *Method A*.—The adduct (**4**) (10 mmol) was dissolved [or suspended for (**5b**)] in tetrahydrofuran (25 ml). Aqueous KCN solution (1 mol^{-1}; 12 ml) and Bu_4NOH (*c* = 40% in H_2O, 5 drops) were added, and the mixture was vigorously stirred at 20 °C. The reaction was followed by TLC. After completion (see Table 1) ether [or ethyl acetate for (**5e**)] was added, the aqueous layer removed, and the organic phase washed with aqueous K_2CO_3 (1 mol l^{-1}; 2 × 10 ml) and water (2 × 10 ml). After drying ($MgSO_4$), the solvent was evaporated to give the crude acylamino nitriles. Further purifications are given in Table 1.

Method B. The adduct (**4**) (10 mmol) and powdered KCN (0.7 g, 10.8 mmol) were stirred in dimethyl sulphoxide (10 ml) at 20 °C. When the reaction was complete as determined by TLC, water (20 ml) was added. Products (**5f–i**) and (**5k**) were filtered off, and washed with water. Products (**5a,j**) and (**5l**) were extracted with ether (**5a,j**) or ethyl acetate (**5l**), washed with aqueous K_2CO_3 (1 mol l^{-1}; 2 × 10 ml) and water (2 × 10 ml). After drying ($MgSO_4$), the solvent was evaporated to give the crude acylaminonitriles. Further purifications are given in Table 1.

Hydrolysis of α-Acylamino Nitriles to Amides: Method C. N-*Benzyloxycarbonyl*-D,L-*phenylglycine Amide* (**6e**) *and* N-*Benzyloxycarbonyl*-L-*phenylalanyl*-L-*valyl*-D,L-*leucine Amide* (**6l**). The nitrile (**5e**) (0.8 g, 3 mmol) or (**5l**), (0.2 g, 0.4 mmol) was stirred in dimethyl sulphoxide (3 ml). Aqueous K_2CO_3 [0.2 g in H_2O (0.3 ml)] and 30% H_2O_2 (0.5 ml, dropwise) were added at 20 °C, while cooling in ice–water. After being stirred for 10 min, (**5e**), or overnight, (**5l**), water (9 ml) was added to give a white solid. This was filtered off and washed with water to give the amide (**6e**) (0.84 g, 99%), or (**6l**) (0.17 g, 85%), respectively.

N-*Benzyloxycarbonyl*-D,L-*phenylglycine amide* (**6e**). M.p. 150–152 °C, TLC: R_f 0.2 (silica gel, benzene–methanol 10:1), identical with the authentic sample; δ_H([$^2H_6$]-DMSO) 7.83 (1 H, d, NH), 7.5–7.25 (10 H, m, 2 × Ph), 7.54 (1 H, br s) and 7.19 (1 H, br s) (NH_2), and 5.06 (2 H, s, CH_2); δ_C([$^2H_6$]-DMSO) 171.8 ($CONH_2$), 155.6 (Z-CO), 138.8, 137.0, 128.3, 128.2, 127.8, 127.7, 127.5, 127.2 (2 × Ph), 65.6 (CH_2), and 58.1 (CH) (Found: C, 67.5; H, 5.7; N, 9.85. $C_{16}H_{16}N_2O_3$ requires C, 67.6; H, 5.7; N, 9.85%).

N-*Benzyloxycarbonyl*-L-*phenylalanyl*-L-*valyl*-D,L-*leucine amide* (**6l**). (Recrystallised from acetic acid–water 7:3; ~1:1 mixture of two diastereoisomers exhibiting duplicated signals in their NMR spectra): m.p. 228–233 °C; TLC: R_f 0.2 (silica gel, benzene–methanol 10:1); δ_H([$^2H_6$]-DMSO) 8.18 (0.5 H, d) and 8.05–7.9 (1.5 H, m) (2 × NH), 7.6–7.5 (1 H, s × br s) and 7.05–6.95 (1 H, 2 × br s) (NH_2), 4.95 (2 H, s, CH_2-Z), 4.4–4.15 (3 H, m, 3 × α-CH), 3.05–2.9 br d and 3.8–3.6 br t (2 H, CH_2-Phe), 2.1–1.9 (1 H, m, CH-Pr^i), 1.7–1.55 (1 H, m, CH-Bu^i), 1.55–1.4 (2 H, m, CH_2-Bu^i), 0.95–0.75 (12 H, br, 6 × Me); δ_C([^2H]-DMSO) 174.2 and 173.9 ($CONH_2$), 170.7 and 170.5 (CO-Phe), 155.8 (CO-Z), 138.1 and 138.0, 129.2, 127.6, 126.2 (Ph-Phe), 137.0, 128.3, 128.0, 127.4 (Ph-Z), 65.2 (CH_2-Z), 58.1 and 57.7 (CH-Val), 56.0 (CH-Phe), 50.8 and 50.7 (CH-Leu), 37.2 (CH_2-Phe), 41.0 and 40.6, 24.2, 23.3 and 23.0, 21.6 and 21.1 (Bu^i), 30.6, 19.2 and 19.1, 18.1 (Pr^i) (Found: C, 65.7; H, 7.6; N, 10.8. $C_{28}H_{38}N_4O_5$ requires C, 65.9; H, 7.5; N, 11.0%).

Method D. Benzyloxycarbonyl-phenylalanyl-valine amide (**6h**) *and Benzyloxycarbonyl-α-phenylglycyl-leucine amide* (**6j**). The nitrile (**5h**) or (**5j**) (0.5 g, 1.3 mmol) was stirred in a mixture of acetone (15 ml or 5 ml, respectively), K_2CO_3 (1 mol l^{-1}; 2 ml) and 30% H_2O_2 (2 ml) at 20 °C for 24 h. Water (10 ml) was added, and the acetone was removed under reduced pressure. The formed white solid was filtered off and washed with water and ether to give the amide (**6h**) (0.49 g, 93%) or (**6j**) (0.4 g, 76%), respectively. Recrystallisation of the amide (**6j**) from ethanol led to isolation of a single diastereoisomer (A); addition of ether to the mother liquor gave the other isomer (B) in 80% d.e. purity (calculated from the Me(Pr^i) ^{1}H signals).

Benzyloxycarbonyl-phenylalanyl-valine amide (**6h**). M.p. 200–205 ° (lit., m.p. L,L-isomer[23]: 241–242 °C; L,D isomer[27]: 125–127 °C); TLC: R_F 0.15 and 0.2 (silica gel, benzene–methanol 10:1); L,L isomer: R_F 0.15) (Found: C, 66.5; H, 6.9; N, 10.5. $C_{22}H_{27}N_3O_4$ requires C, 66.5; H, 6.8; N, 10.6%); δ_H([$^2H_6$]-DMSO + $CDCl_3$) 8.15 (2 H, s, NH_2), 7.4–7.1 (10 H, m, 2 × Ph), 4.95 (2 H, s, Z-CH_2), 4.5–4.1 (2 H, 2 × m, 2 × NCHCO), 3.1–2.7 (2 H, 2 × m, Phe-CH_2), 2.05 (1 H, m, Pr^i-CH), 1.0–0.7 (6 H, m, Pr^i-Me).

The Pr^i-Me multiplet consisted of a triplet (*i.e.* 2 × d, overlapping) at 0.9 ppm and a doublet at 0.8 ppm, which upon decoupling of the Pr^i-CH gave a doublet and a singlet, respectively. The two signals belong to the two diastereoisomers, and can serve as an analytical tool for their quantitative estimation. (See also the spectrum of the L,L isomer.) ^1H NMR of the L,L isomer $\delta_H([^2H_6]$-DMSO + $CDCl_3)$ 7.7 (1 H, d) and 7.4, (1 H, d) (2 × H, d) (2 × NH), 7.25 (10 H, br s, 2 × Ph), 4.95 (2 H, s, Z-CH_2), 4.4 (1 H, m, Phe-CH), 4.23 (1 H, t, Val-CH), 3.2–2.7 (2 H, 2 × m, Phe-CH_2), 2.05 (1 H, m, Pr^i-CH), 0.9 (6 H, t, Pr^i-Me); $\delta_C([^2H_6]$-DMSO + $CDCl_3)$ 172.7, 171.2 (CONH and $CONH_2$), 155.5 (OCO), 137.7, 137.5, 129.0, 128.9, 128.0, 127.7, 127.4, 127.3, 127.2, 126.0 (Ph signals), 65.3 and 65.2 (Z-CH_2), 57.1 (Val-CH), 56.1 and 56.0 (Phe-CH), 37.5 and 37.2 (Phe-CH_2), 30.6 and 30.0 (Pr^i-CH), 19.1 and 19.0, 17.6 and 17.4 (Pr^i-Me); ^{13}C NMR of the L,L isomer $\delta_C([^2H_6]$-DMSO + $CDCl_3)$ 172.7, 171.2 (CONH and $CONH_2$); 155.5 (OCO); 137.7, 136.5, 129.0, 128.0, 127.8, 127.6, 127.5, 126.0 (Ph signals), 65.3 (Z-CH_2), 57.1 (Val-CH), 56.0 (Phe-CH), 37.5 (Phe-CH_2), 30.6 (Pr^i-CH), 19.1 and 17.6 (Pr^i-Me).

Benzyloxycarbonyl-α-phenylglycyl-leucine amide (**6j**), *A diastereoisomer*. M.p. 230–233 °C; TLC: R_F 0.4 (silica gel; hexane–AcOH–$CHCl_3$, 1:1:8) (Found: C, 66.5; H, 6.9; N, 10.5. $C_{22}H_{27}N_3O_4$ requires C, 66.5; H, 6.85; N, 10.6%); $\delta_H([^2H_6]$-DMSO) 8.27 (1 H, d, Phg-NH), 7.95 (1 H, d, Leu-NH), 7.5–7.2 (11 H, m, 2 × Ph × 1/2 NH_2), 7.0 (1 H, br s, 1/2 NH_2), 5.35 (1 H, d, Phg-CH), 5.07 (2 H, s, Z-CH_2), 4.29 (1 H, q, Leu-CH), 1.67–1.52 (1 H, m, Bu^i-CH), 1.52–1.36 (2 H, br t, Bu^i-CH_2), 0.88 and 0.83 (6 H, 2 × d, 2 × Me); $\delta_C([^2H_6]$-DMSO) 173.7 ($CONH_2$), 169.5 (Phg-CO), 155.7 (Z-CO), 138.5, 136.9, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6, 127.5, 127.3 (2 × Ph), 65.6 (Z-CH_2), 58.3 (Phg-CH), 50.9 (Leu-CH), and 41.0, 24.1, 22.8, 21.6 (Bu^i).

B diastereoisomer (major signals given). M.p. 187–195 °C; TLC: R_F 0.35 (silica gel; hexane–AcOH–$CHCl_3$, 1:1:8); $\delta_H([^2H_6]$-DMSO) 8.33 (1 H, d, Phg-NH), 7.90 (1 H, d, Leu-NH), 7.5–7.2 (11 H, m, 2 × Ph + 1/2 NH_2), 6.9 (1 H, br s, 1/2 NH_2), 5.32 (1 H, d, Phg-CH), 5.07 (2 H, s, Z-CH_2), 4.21 (1 H, q, Leu-CH), 1.52–1.36 (2 H, br t, Bu^i-CH_2), 1.4–1.25 (1 H, m, Bu^i-CH), and 0.76 and 0.67 (6 H, 2 × d, 2 × Me). $\delta_C([^2H_6]$-DMSO) 174.0 ($CONH_2$), 169.7 (Phg-CO), 155.6 (Z-CO), 138.3, 136.6, 128.1, 128.0, 127.9, 127.55, 127.5, 127.3, 127.05, 127.0 (2 × Ph), 65.6 (Z-CH_2), 58.1 (Phg-CH), 50.9 (Leu-CH), and 40.6, 24.0, 22.8, 21.0 (Bu^i).

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