15. Preparation of Boc-Amino-Acid or Peptide Aldehydes *via* Reduction of Corresponding Phenyl Esters

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A new alternative method for the preparation of amino-acid and peptide aldehydes based on reduction of corresponding phenyl esters with lithium tri(*tert*-butoxy)aluminium hydride is described.

Introduction. – In a framework of synthesis of enzyme inhibitors, we studied new synthetic routes to N^2-protected amino-aldehydes and peptide aldehydes.

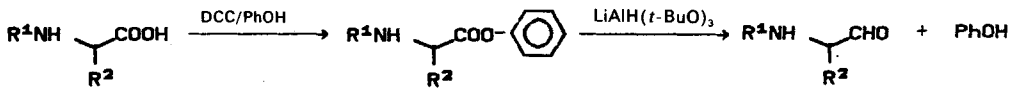
Known methods for the preparation of N^2-protected amino-aldehydes are the reduction of corresponding methyl esters with diisobutyl aluminium hydride ($AlH(i\text{-}Bu)_2$) [1], reduction of *Wienreb*'s amide with $LiAlH_4$ [2], oxidation of *N*-protected amino alcohols by the *Pfitzer-Moffat* method [3], electrochemical reduction of N^2-Boc-amino acids [4] (Boc = (*tert*-butoxy)carbonyl), triethylsilane/Pd-mediated reductive cleavage of Fmoc-amino acid benzylthio esters [5] (Fmoc = (9*H*-fluoren-9-yl)methoxycarbonyl), or, recently, reduction of mixed anhydrides with pivalic or diphenylacetic acids generated *in situ* [6].

The pivalic-anhydride method gave sufficient yields of relatively pure N^2-Boc-amino-aldehydes with low racemisation but failed in the preparation of N^2-Boc-N^7-nitroarginal, where a cyclic δ-lactam was obtained instead [6]. This lactam was readily reduced to N^2-Boc-N^7-nitroargininal by $LiAlH_4$ [7], but this aldehyde was more conveniently prepared by $AlH(i\text{-}Bu)_2$ reduction of the methyl ester [8] or by the *Fehrentz-Castro* method [9].

Reduction of phenyl esters of aliphatic acids with lithium tri(*tert*-butoxy)aluminium hydride ($LiAlH(t\text{-}BuO)_3$) leads to corresponding aldehydes in high yields [10], but this method was not exploited up to now, because usually these phenyl esters are prepared from acyl chlorides which are themselves easily reduced to aldehydes by several methods [11–13].

Results and Discussion. – Phenyl esters **2** of N^2-Boc-amino acids and peptides **1** can be readily prepared in high yields by the DCC (dicyclohexylcarbodiimide) method [14]. This fact prompted us to study the reduction of these esters to the corresponding aldehydes **3** by $LiAlH(t\text{-}BuO)_3$ (see *Scheme 1*). We found the method efficient for the synthesis of some N^2-Boc-amino-aldehydes, including N^2-Boc-N^7-nitroargininal **3g**, N^2-Boc-N^7-Z-argininal **3h**, N^2-Boc-N^7,$N^{7'}$di-Z-argininal **3i**, and leupeptin precursor acetyl-leucyl-leucyl-N^δ,N^ωdi-Z-argininal **3j**. Some aldehydes **3** will be described in our forthcoming communication [6]; the ^{1}H-NMR data of the others were conforming with the proposed structures (see *Exper. Part*).

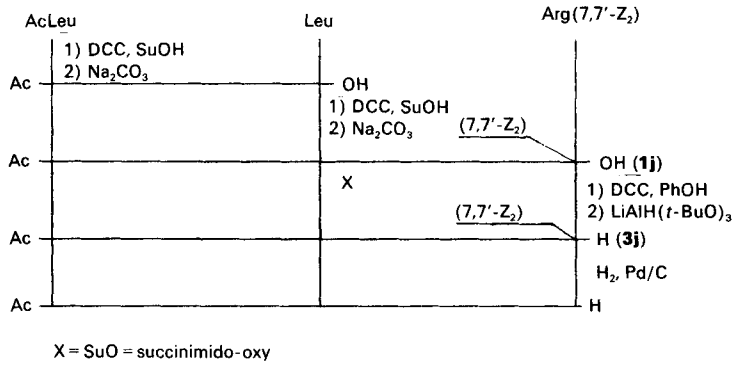
Scheme 1



1 2

3a R^1 = Boc, R^2 = Me
b R^1 = Boc, R^2 = Me_2CH
c R^1 = Boc, R^2 = Me_2CHCH_2
d R^1 = Boc, R^2 = $MeSCH_2CH_2$
e R^1 = Boc, R^2 = $PhCH_2$
f R^1 = Boc, R^2 = (1*H*-indol-3-yl)methyl
g R^1 = Boc, R^2 = NO_2–NHC(=NH)NH$(CH_2)_3$
h R^1 = Boc, R^2 = Z–NHC(=NH)NH$(CH_2)_3$
i R^1 = Boc, R^2 = Z–NHC(=NZ)NH$(CH_2)_3$
j R^1 = Ac-Leu-Leu, R^2 = Z–NHC(=NZ)NH$(CH_2)_3$

Scheme 2



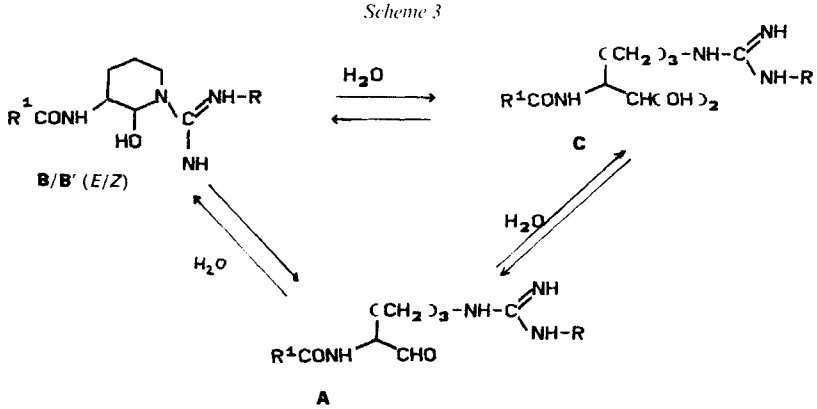
X = SuO = succinimido-oxy

The synthesis of leupeptin, a peptide aldehyde, was performed *via* **1j** and **3j** according to *Scheme 2*.

In the literature, the selective hydrogenolytic removal of a NO_2 group from a guanidino function in the presence of an aldehyde [9] and semicarbazone [8] group is described. But according to our and other's experiences [7] [17], neither an aldehyde nor a semicarbazone function is able to survive to prolonged hydrogenolysis which is required for total removal of the NO_2 group from the guanidino moiety: In our hands, N^2-Boc-N^7-nitroarginal **3g** yielded N^2-Boc-argininol, and N^2-Boc-$N^{7'}$-nitroargininal semicarbazone gave a mixture of ninhydrin-positive products. However, reductive cleavage of a single (**3h**) or both Z-groups (**3i**) during 50 min gave N^2-Boc-argininal, and similarly acetyl-leucyl-leucyl-N^7,$N^{7'}$-di(Z)-argininal **3j** gave leupeptin.

It is known that N^7- or $N^{7'}$-substituted argininals can adopt several forms in aqueous solution, some as a mixture of (*E*)- and (*Z*)-isomers, which complicates their NMR spectra [3] [16]. In the case of N^2-Boc-N^7-nitroargininal **3g** and *N*-Boc-argininal, the free-aldehyde form **A**, the internal hemi-aminoacetal (with N(6)), as an (*E*/*Z*)-mixture **B** and **B'**, as well as the hydrated form **C** could be identified by ^{13}C-NMR spectroscopy (see *Scheme 3*). In dry (D_6)DMSO, **3g** shows the ^{13}C-NMR signals of the expected three forms **A**, **B**, and **B'**, the gem-diol form **C** being present only in very minor amounts (*Table 1*).

Scheme 3



After addition of H_2O, the signals of the aldehyde form **A** decrease (*C*HO at 202.75 ppm), and a new one appears at 91.432 ppm, attributed to the *C*H(OH)$_2$ group of **C**. Other peaks were assigned as shown in *Table 1*. N^2-Boc-Argininal in (D$_6$)DMSO/H_2O exhibits essentially the signals of the forms **B**, **B′**, and **C**, the peak of the free aldehyde group of **A** being considerably less important than in the case of **3g** (*Table 2*).

Table 1. *^{13}C-NMR Shifts* [ppm] *of* N^2-*Boc*-N^7-*Nitroargininal* **3g**

	A	B	B′	C
CH(OH)$_2$	–	–	–	80.937
O–CH–N	–	77.237	76.867	–
CH–N	51.421	51.340	50.724	51.526
CH_2	25.604	25.401	25.631	25.358
CH_2	23.128	23.728	23.336	23.198
CH_2	40.415	40.445	41.746	39.478
guanidino (C(N)$_3$)	158.326	157.378	158.018	157.041
OCONH	161.378	160.157	160.157	160.056
Me_3CO	80.937	80.937	80.937	80.937
Me_3CO	29.434	29.139	19.147	29.158
CHO	202.746	–	–	–

Table 2. *^{13}C-NMR Shifts* [ppm] *of* N^2-*Boc-Argininal*[a])

	B	B′	C
CH(OH)$_2$	–	–	90.326
O–CH–N	80.406	80.406	–
CH–N	54.807	54.754	56.314
CH_2 (3)	26.521	26.521	26.165
CH_2 (4)	23.278	23.362	22.746
CH_2 (5)	40.935	41.983	41.732
guanidino (C(N)$_3$)	155.647	155.647	155.806
OCONH	178.388	178.388	174.325
Me_3CO	80.858	80.858	80.858
Me_3CO	28.773	28.773	28.688

[a]) The CHO group of the **A** form is visible at 203.120 ppm.

Experimental Part

General. All chemicals were purchased from *Merck*, Boc-amino acids from *Fluka*, and dansyl-amino acids as standards from *Sigma*. TLC: *Silufol UV 254*; detection by UV light (254 nm), sat. (dinitrophenyl)hydrazine in 2N HCl, *Sakaguchi* reagent, or chlorine/toluidine reagent; HPLC: column *Tessek C-18*, 25 × 0.5 cm. M.p.: *Boetius* apparatus; uncorrected. Optical rotations: *Polamat A* (*Karl Zeiss-Jena*); in MeOH, $c = 1$. NMR: *Varian 200*; *d* in ppm rel. to Me_4Si, *J* in Hz. Amino-acid analyses were performed using the dansyl-chloride method after hydrolysis with 9N HCl in a sealed tube at 120° during 22 h. Elemental analyses: *Carlo Erba 1106*.

N^2-*Boc-Amino-Acid or Peptide Aldehydes* **3**: *General Procedure.* To a soln. of N^2-Boc-amino acid or peptide **1** (0.01 mol) in dry THF/MeCN 1:1 (20 ml), freshly distilled phenol (0.94 g, 0.01 mol) was added and the mixture cooled under stirring to −10°. Then, DCC (2.06 g, 0.01 mol) was added followed by 4-(dimethylamino)pyridine (50 mg). The mixture was stirred at r.t. overnight. Dicyclohexylurea was filtered and washed with 2 small portions of dry THF. The filtrate was transferred to a three-necked flask and cooled to 0°. The soln. of $LiAlH(t\text{-}BuO)_3$ (2.6 g, 0.011 mol) in dry THF (25 ml) was dropwise added under N_2 and at 0°. After the addition, the mixture was stirred for 1 h at 0°, poured into 20% citric acid (30 ml), and extracted with AcOEt (4 ×). The org. phase was dried (Na_2SO_4) and evaporated to a small volume. Then, $(i\text{-}Pr)_2O$ (25 ml) was added and the precipitate or oil separated and dissolved in AcOEt (10 ml). Addition of $(i\text{-}Pr)_2O$ separated the aldehyde **3** as an amorphous powder or oil. Aldehydes **3g** and **3h** were purified by flash chromatography (silica gel, column 5 × 40 cm, Et_2O/hexane 2:9, then Et_2O/MeOH 10:1). The (dinitrophenyl)hydrazin-positive fraction was evaporated.

N^2-*[(*tert-*Butoxy)carbonyl]*-L-*alaninal* (**3a**): Yield 76%. $[\alpha]_D = -25.52$. ^{1}H-NMR: 9.58 (*s*, 1 H); 5.5 (*s*, NH); 4.2 (*q*, $J = 8.72$, 1 H); 3.7 (*q*, $J = 7.28$, 1 H); 1.45 (*s*, 9 H); 1.25 (*s*, 3 H).

N^2-*[(*tert-*Butoxy)carbonyl]*-L-*valinal* (**3b**): Yield 79%. $[\alpha]_D = -11.6$. ^{1}H-NMR: 9.6 (*s*, 1 H); 5.33 (*s*, 1 H, NH); 4.23 (*dd*, $J = 8.56$, 1 H); 2.29 (*m*, 1 H); 1.46 (*s*, 9 H); 0.95 (2*d*, 6 H).

N^2-*[(*tert-*Butoxy)carbonyl]*-L-*leucinal* (**3c**): Yield 85%. $[\alpha]_D = -30.62$. ^{1}H-NMR: 9.55 (*s*, 1 H); 5.45 (*d*, 1 H, NH); 4.2 (*m*, 1 H); 1.9–1.45 (*m*, 2 H); 1.45 (*m*, 9 H); 0.95 (*d*, $J = 9.72$, 6 H).

N^2-*[(*tert-*Butoxy)carbonyl]*-L-*methioninal* (**3d**): Yield 82%. $[\alpha]_D = -21.30$. ^{1}H-NMR: 9.6 (*s*, 1 H); 5.78 (*d*, 1 H); 4.3 (*m*, 1 H); 3.2 (*m*, 2 H); 2.55 (*m*, 2 H); 2.1 (*s*, 3 H); 1.5 (*s*, 9 H).

N^2-*[(*tert-*Butoxy)carbonyl]*-L-*phenylalaninal* (**3e**): Yield 78%. $[\alpha]_D = -36.03$. ^{1}H-NMR: 9.4 (*s*, 1 H); 7.25 (*m*, 5 H); 5.1 (*s*, NH); 4.2 (*m*, 1 H); 3.65 (*q*, $J = 9.36$, 1 H); 3.6 (*d*, $J = 9.32$, 2 H); 1.45 (*s*, 9 H).

N^2-*[(*tert-*Butoxy)carbonyl]*-L-*tryptophanal* (**3f**): Yield 81%. $[\alpha]_D = -8.50$. ^{1}H-NMR: 9.7 (*s*, 1 H); 7.5–7.6 (*d*, 1 H); 6.8–7.4 (*m*, 4 H); 8.3 (*s*, NH(ind)); 5.3 (*s*, 1 H); 3.6–3.8 (*m*, 1 H); 1.5–1.6 (*d*, $J = 8.54$, 2 H); 1.3–1.45 (*s*, 9 H).

N^2-*[(*tert-*Butoxy)carbonyl]*-N^7-*nitro*-L-*argininal* (**3g**): Yield 74%. $[\alpha]_D = -4.6$. R_f (AcOEt/EtOH 20:1) 0.26–0.36. ^{13}C-NMR: see *Table 1*.

N^7-*[(Benzyloxy)carbonyl*-N^2-*[(*tert-*butoxy)carbonyl]*-L-*argininal* (**3h**): Yield 74%. $[\alpha]_D = -2.52$. ^{1}H-NMR ($CDCl_3$): 1.43 (*s*, 9 H, Me_3C); 1.56–1.76 (*m*, 1 H, $CH_2(4)$); 3.11–3.23 (*m*, 1 H, CHN); 3.36–3.57 (*m*, 2 H, $CH_2(5)$); 5.33 (*s*, 2 H, CH_2O); 5.55–5.68 (*m*, 1 H, OCHN); 6.78–6.66 (*m*, 5 arom. H); 9.55 (*s*, 0.15 H, CHO). ^{13}C-NMR ($CDCl_3$): 23.783, 24.576, 25.555 (CH_2(Arg)); 28.279, 28.290, 28.333 (*Me*$_3$C); 44.468, 44.936, 45.178 ($CH_2(5)$); 53.538, 53.768, 53.996 (CHN); 65.563, 66.661 (OCHN); 127.654–128.924 (arom. CH); 134.876, 134.902 (arom. C); 155.376 ($C(N)_3$); 159.376, 159.578, 159.936, 160.378, 162.572 (OCONH, CONH); 200.115 (CHO).

N^7,$N^{7'}$-*Bis[(benzyloxy)carbonyl]*-N^2-*[(*tert-*butoxy)carbonyl]*-L-*argininal* (**3i**): Yield (after precipitation from $(i\text{-}Pr)_2O$): 71%. $[\alpha]_D = -2.52$. R_f (AcOEt/EtOH 20:1) 0.83. ^{1}H-NMR ($CDCl_3$): 1.41 (*s*, 9 H, Me_3C); 1.80–1.88 (*m*, 2 H, $CH_2(4)$); 3.19–3.27 (*t*, $J = 8.76$, 2 H, $CH_2(5)$); 3.57–3.61 (*m*, 2 H, $CH_2(3)$); 3.91–4.00 (*m*, 1 H, CHN); 5.30 (*s*, 2 H, CH_2O); 5.33 (*s*, 2 H, CH_2O); 7.10–7.51 (*m*, 10 arom. H); 9.55 (*s*, 1 H, CHO). ^{13}C-NMR ($CDCl_3$): 25.329 ($CH_2(4)$); 28.388 (*Me*$_3$C); 31.361 ($CH_2(3)$); 43.935 ($CH_2(5)$); 56.700 (CHN); 66.654 (CH_2O); 66.956 (CH_2O); 126.811–127.223 (C_p); 127.468–127.559 (C_m); 127.793 (C_o); 140.961 (C_{ipso}); 141.688 (C_{ipso}); 155.632 (OCONH); 156.721 (OCONH); 156.902 (OCONH); 160.421 ($C(N)_3$); 200.220 (CHO).

N^2-*Acetyl*-L-*leucyl*-L-*leucine.* A soln. of N^2-acetyl-L-leucine (3.68 g, 0.02 mol) and *N*-hydroxysuccinimide (1.82 g, 0.02 mol) in dry MeCN (50 ml) was cooled under stirring to −20°. Then, DCC (4.12 g, 0.02 mol) was added and the mixture stirred for 6 h at r.t. (drying tube ($CaCl_2$)). Dicyclohexylurea was filtered and washed with a little amount of MeCN. The filtrate was added to a stirred soln. of L-leucine (2.84 g, 0.02 mol) and Na_2CO_3 (2.12 g, 0.02 mol) in H_2O (50 ml). The mixture was stirred overnight and then concentrated to half of its volume. H_2O (150 ml) was added and the pH adapted to 2.5–2.6. Precipitated product was filtered and recrystallized from H_2O/EtOH: 5.56 g (90%). M.p. 191–192° ([9]: 190–191°). $[\alpha]_D = -33.7$ ([9]: −51.74 ($c = 2.495$)). Amino-acid analysis: Leu (1.00). Anal. calc. for $C_{14}H_{22}N_2O_4$ (352.21): C 58.74, H 9.15, N 9.79; found: C 58.71, H 9.05, N 9.71.

N^2-*Acetyl*-L-*leucyl*-L-*leucyl*-N^7,$N^{7'}$-*bis[(benzyloxy)carbonyl]*-L-*arginine* (**1j**). To a stirred soln. of Ac-Leu-Leu-OH (3.08 g, 0.01 mol) and *N*-hydroxysuccinimide (0.91 g, 0.01 mol) in dry MeCN (40 ml) which was cooled to

−20° was added DCC (2.06 g, 0.01 mol) under stirring. The mixture was stirred overnight at r.t. Dicyclohexylurea was filtered and washed with two little portions of MeCN and the filtrate added to a stirred mixture of N^7,$N^{7'}$-bis-[(benzyloxy)carbonyl]-L-arginine (4.42 g, 0.01 mol) and Na_2CO_3 (1.06 g) in H_2O (30 ml). The mixture was stirred for 6 h at r.t. and diluted with H_2O (50 ml) and the pH adapted to 2.5–2.6. The precipitated resin was extracted with AcOEt (3 ×), the org. layer dried (Na_2SO_4) and concentrated to *ca.* 10 ml, $(i\text{-Pr})_2O$ (10 ml) added, and the mixture left in the freezer overnight (−20°). The white precipitate was filtered, washed with $(i\text{-Pr})_2O$, and stored. The filtrate was concentrated to *ca.* 5 ml and $(i\text{-Pr})_2O$ (5 ml) added. Standing overnight in the freezer gave another crop of **1j**. Yield: 3.62 g (62%). $[\alpha]_D = -8.51$. R_f (AcOEt) 0.52. M.p. 160–165°. Amino-acid analysis: Leu (2.01), Arg (0.98). Anal. calc. for $C_{36}H_{50}N_6O_9$ (710.09): C 59.23, H 7.05, N 11.85; found: C 59.39, H 7.00, N 11.63.

Hydrogenolysis of **3g**. A soln. of **3g** (1 g) in MeOH/AcOH 7:3 (10 ml) was hydrogenated under 1 atm over 10% Pd/C (100 mg) for 11.6 h. The catalyst was removed by filtration, and the filtrate evaporated: 0.71 g of N^2-*[(tert-butoxycarbonyl]-*L*-argininal*. Oil. TLC: *Sakaguchi*-positive and (dinitrophenyl)hydrazin-negative. $[\alpha]_D = +9.2$. ^{13}C-NMR (MeOH): 24.325 (CH_2(4)); 28.645 (Me_3C); 34.673 (CH_2(3)); 41.760 (CH_2(5)); 51.587 (CHN); 59.721 (CH_2OH); 80.376 (Me_3C); 155.543 (C(N)$_3$); 156.076 (OCONH).

N^2-*[(tert-Butoxy)carbonyl]-*L*-argininal Acetate*. A soln. of **3h** (0.9 g) was hydrogenated for 50 min and worked up as above: yellow foam (0.52 g). TLC: only 1 spot, R_f (BuOH/AcOH/H_2O 4:1:1) 0.21; *Sakaguchi*- and (dinitrophenyl)hydrazine-positive. $[\alpha]_D = +22.3$. ^{13}C-NMR: *Table 2*. Anal. calc. for $C_{13}H_{26}N_4O_5$ (318.06): C 49.05, H 8.17, N 17.61; found: C 48.67, H 8.74, N 17.36.

Leupeptin Hydrochloride. According to the *General Procedure*, N^2-*acetyl-*L*-leucyl-*L*-leucyl-*N^7,$N^{7'}$-*bis[(benzyloxy)carbonyl]-*L*-argininal* (**3j**) was prepared from **1j** and deprotected without any special purification: A soln. of **3j** (0.78 g) in MeOH/AcOH 10:3 (13 ml) was hydrogenated over 10% Pd/C under normal pressure for 90 min. The catalyst was filtered and washed with MeOH. 2N HCl (2 ml) was added to the filtrate, and it was evaporated. The residue was dissolved in MeOH (3 ml), applied to a column of *Fractogel TSK HW-40(F)* (100 × 1.5 cm), and eluted with MeOH (20 ml/h, 5-ml portions). *Sakaguchi*- and (dinitrophenyl)hydrazine-positive fractions were evaporated to constant weight: white amorphous powder (0.24 g). $[\alpha]_D = -59.37$ ([9]: −54.7). Anal. calc. for $C_{20}H_{38}N_6O_4Cl$ (488.5): C 52.12, H 7.41, N 18.24, Cl 7.49; found: C 52.37, H 7.80, N 18.59, Cl 7.88.

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