Efficient Synthesis of Differentially Protected (*S*,*S*)-2,7-Diaminooctanedioic Acid, the Dicarba Analogue of Cystine

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Convenient preparative syntheses of differentially protected forms of (*S*,*S*)-2,7-diaminooctanedioic acid (**2**), suitable for application in peptide chemistry, are described. The key compound, the di-Cbz-protected phenacyl monoester **7a** (Cbz = [(benzyloxy)carbonyl]), was obtained by means of *Schöllkopf* bis-lactim ether methodology and optimized monoesterification procedures. Selective amino deprotection at the non-esterified amino-acid function of **7a** by dichloromethyl-methyl-ether-induced '*N*-carboxyanhydride' formation, and hydrolysis permitted access to the Boc/Cbz- and Fmoc/Cbz-protected monophenacyl esters **11a** and **11b**, as well as to the fully orthogonally protected Fmoc/Boc monophenacyl ester **12** (Boc = (*tert*-butoxy)carbonyl, Fmoc = (9*H*-fluoren-9-ylmethoxy)carbonyl).

Introduction. – Disulfide bonding through cystine residues is a main feature of peptide and protein architecture. For many applications of polypeptides it is desirable to replace cystine **1** with its non-reducible isosteric dicarba analogue, *i.e.*, (*S*,*S*)-2,7-diaminooctanedioic acid (= L,L-2,7-diaminosuberic acid; **2**) [1]. For symmetrical polypeptides containing such a cystine replacement, this does not present any particular synthetic problem as *N*-protected derivatives of **2** are available: mixtures of stereoisomers of **2** can be obtained by *Gabriel* synthesis from octanedioic acid [2], and a number of related synthetic routes are known [3]. Separation of the stereoisomers has been shown to be possible but is tedious [4]. For this reason, asymmetric-synthesis methods have been developed. Electrochemical dimerization *via Kolbe* synthesis from glutamic-acid derivatives [5] has been used preparatively for this purpose [6]. Furthermore, asymmetric-synthesis methods using various chiral auxiliaries have been used for the preparation of optically pure derivatives of certain diamino dicarboxylic acids [7]. The results we present here for the synthesis of derivatives of diaminooctanedioic acid **2** are based on the use of one such method, *viz*. the *Schöllkopf* bis-lactim ether technique [8].

For most applications where a cystine residue in a polypeptide is to be replaced with the isoster **2**, however, the resulting synthetic bridged intermediates will be unsymmetrical, and differentially protected derivatives of **2** are required for unambiguous and selective synthesis. Originally, α-(*tert*-butyl) N^{α}-Boc-$N^{\alpha'}$-Cbz-L,L-2,7-diaminosuberate[3]) (= 8-(*tert*-butyl) 1-hydrogen (*S*,*S*)-2-{[(benzyloxy)carbonyl]amino}-7-{[(*tert*-

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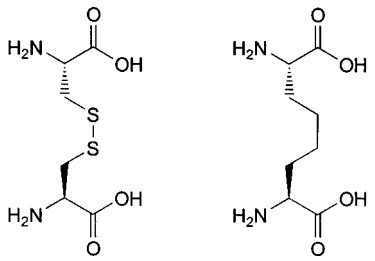
[3]) Cbz = (Benzyloxy)carbonyl, Boc = (*tert*-butoxy)carbonyl, Fmoc = (9*H*-fluoren-9-ylmethoxy)carbonyl, Pac = phenacyl = 2-oxo-2-phenylethyl.



1 **2**

butoxy)carbonyl]amino}octanedioate) was obtained by mixed *Kolbe* electrolytic decarboxylative dimerization of appropriate glutamic-acid derivatives [9], and this approach has recently been extended to the synthesis of orthogonally protected diamino dicarboxylic acids containing up to four different protecting groups [10]. The disadvantage of electrolytic dimerization methods lies in the fact that statistically controlled product mixtures are formed from which the desired derivatives have to be isolated.

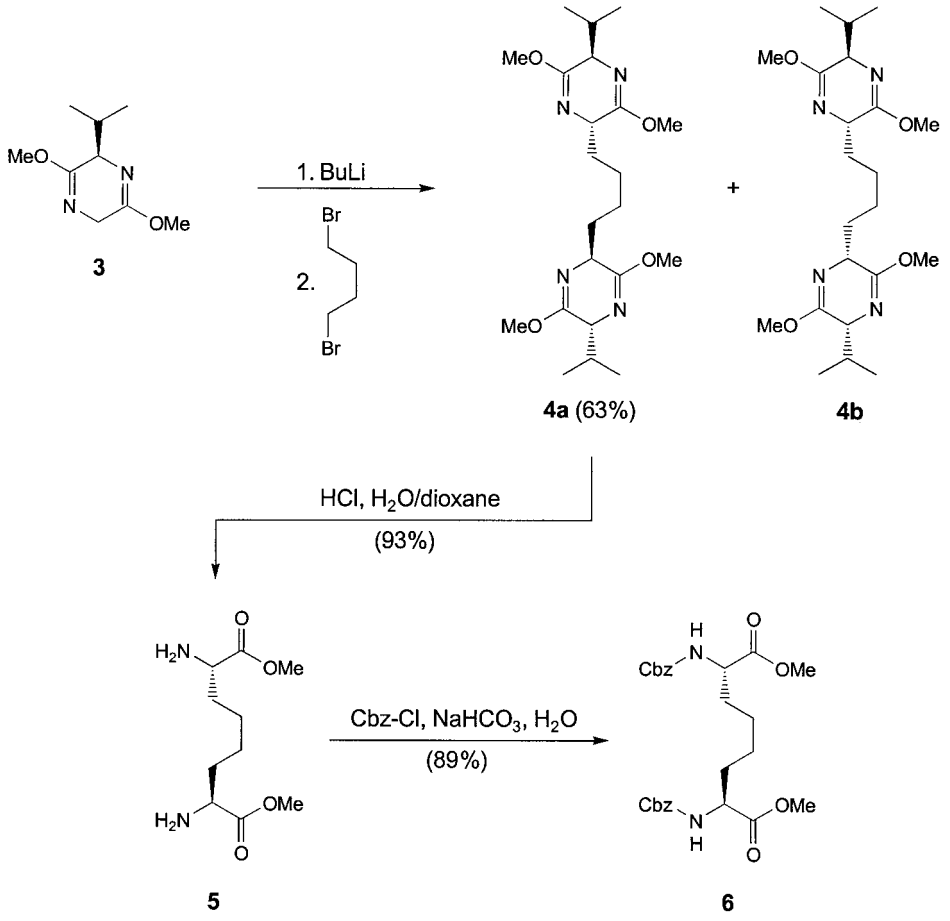
Differentially protected derivatives of diaminoheptanedioic acid (= diaminopimelic acid) have also been synthesized *via* connection and elaboration of two different chiral auxiliaries [11]; a similar approach was adopted for the preparation of differentially protected 2,9-diaminodecanedioic acid [12]. Furthermore, it was shown in principle that diaminooctanedioic-acid derivatives with two different amino-protecting groups are accessible using the chiral auxiliary Boc-BMI of *Seebach* and coworkers [13]. Starting from diaminoheptanedioic acid, selective introduction of protecting groups with the aid of dimeric copper chelates has also been shown to be feasible [14]. The most recent approach towards differentially protected diaminodicarbocylic acids uses *Grubb*'s ring closure olefin metathesis reactions on allylglycine-derived templates [15].

The method we have developed for the differential protection of **2** is based on work by *Arendt et al.* [16] who showed that using racemic di-Cbz-protected[3]) 4-nitrobenzyl hydrogen 2,6-diaminopimelate (*i.e.*, 4-nitrobenzyl hydrogen 2,6-bis{(benzyloxy)carbonyl]amino}heptanedioate), it was possible to address selectively the α-amino-acid function containing the free carboxylic acid rather than the ester function by formation of an '*N*-carboxyanhydride' (NCA). Using this approach, we show that enantiomerically pure derivatives of **2**, in orthogonally protected forms directly applicable to peptide synthesis, can be obtained.

Results and Discussion. – The bis-lactim ether **3** (*Scheme 1*), synthesized from cyclo(D-Val-Gly) [17], was lithiated and alkylated with 0.5 mol-equiv. of 1,4-dibromobutane to afford a mixture of diastereoisomers **4a** and **4b** in a ratio of $\geq 4:1$. The desired stereoisomer **4a** was obtained in enantiomerically pure form after column chromatography (silica gel) and recrystallization. Controlled hydrolysis of the imino-ether functions in **4a** provided the diester **5** after removal of valine methyl ester by high-vacuum distillation. Rather than hydrolysis of the methyl esters prior to the introduction of the *N*-Cbz groups as is usually the practice, we chose to reverse the

process. This is advantageous due to the problems attendant on N-protection of free 2,7-diaminooctanedioic acid [18]. Thus, the di-Cbz-protected diester **6** was obtained in high yield from **5**.

Scheme 1



Monoesterification of diaminodicarboxylic acids is most easily effected using methods relying on alkylation of carboxylates, since the alkylating agent can be employed in stoichiometric amounts. Thus, 4-nitrobenzyl esters have been used for this purpose [14]. We chose the phenacyl (Pac[3]) ester [19], which can be introduced similarly and has the additional advantage of being orthogonal with respect to both the Cbz and Boc[3]) groups in terms of deprotection methods. Thus, it is possible to remove the Pac ester from amino-acid derivatives as well as from peptides, *e.g.*, with the aid of Zn in AcOH or with Bu_4NF [20]. Furthermore, its acid stability is such that both the Boc and Cbz groups can be removed acidolytically without interference with the Pac ester.

In our early experiments, we achieved monoesterification by treatment of di-Cbz-protected diaminooctanedioic acid (after acid hydrolysis of the methyl esters in **6**; *Scheme 2*) with 1 mol-equiv. of Pac-Br in DMF in the presence of Et_3N. With this procedure, monoester **7a** and diester **7b** were formed in a ratio of 2:1 at best, and the chemical yields were low. We subsequently found that much better yields were achieved if **6** was hydrolysed carefully using LiOH in H_2O/dioxane, followed directly by treatment of the hydrolysate with Pac-Br in the same solvent mixture. Under appropriate concentration conditions, the monoester **7a** precipitated immediately upon formation, and excess Pac-Br could be used to drive the reaction nearly to completion, thus, only trace amount of diester **7b** were observed.

Conversion of Cbz-amino acids to NCAs is usually achieved by preparation of the corresponding acyl chlorides with PCl_5 [14] or $SOCl_2$ [21], followed by heating of the reaction mixtures. Using these strategies, we were able to detect the formation of what is likely to be the acyl chloride **8** by TLC, as well as the NCA **9**[4]). Unfortunately, the transformations were not smooth, and several unidentified by-products were observed. Thus, we took recourse to a method of NCA formation from Cbz-protected amino acids using the reagent dichloromethyl methyl ether [22]. Analyses of reaction mixtures obtained with this reagent showed clean and rapid formation of **9**, and no additional solvent was necessary. Hydrolysis of the NCA **9** with aqueous AcOH to give **10**, followed by introduction of a Boc or Fmoc[3]) protecting group, without purification of the hydrolysed intermediate, finally afforded the triprotected target compounds **11a** and **11b**, respectively, in excellent yield and purity.

In terms of the envisaged use of our triprotected target compounds in peptide syntheses, the combination of protecting groups in **11a** and **11b** is not fully satisfactory due to the fact that hydrogenolytic deblocking of the Cbz group is compatible with neither the Pac nor the Fmoc [23] groups. We have demonstrated the compatibility of the Fmoc and Pac groups [24] and thus, in principle, the three protecting groups in **11b** are fully orthogonal if the Cbz group is removed acidolytically rather than hydrogenolytically. However, a further element of orthogonality is desirable with respect to the amino-acid side-chain protecting groups present in prospective peptides incorporating derivatives of **2**. These protecting groups are commonly of the benzyl type, and the choice of Cbz as one of the amino-blocking groups in **2** is, therefore, not optimal. Compound **12**, on the other hand, is fully orthogonal when permanent benzyl-type peptide protecting groups are to be used. This derivative was readily prepared by removal of the Cbz groups from **11b** with strong acid, followed by introduction of a Boc group in the usual manner.

The absence of racemization during LiOH saponification of the methyl diester **6** and the following esterification step was verified by removal of the ester functions in the diester **7b** and comparison of the resulting product with authentic [18] (*S*,*S*)-2,7-bis{[(benzyloxy)carbonyl]amino}octanedioic acid (^{13}C-NMR and optical rotation). Similarly, chiral integrity during the transformation of monoester **7a** to amino acid **10** *via* NCA **9** was established by comparison of authentic material with the product obtained after reprotection of **10** by Cbz and Pac deprotection.

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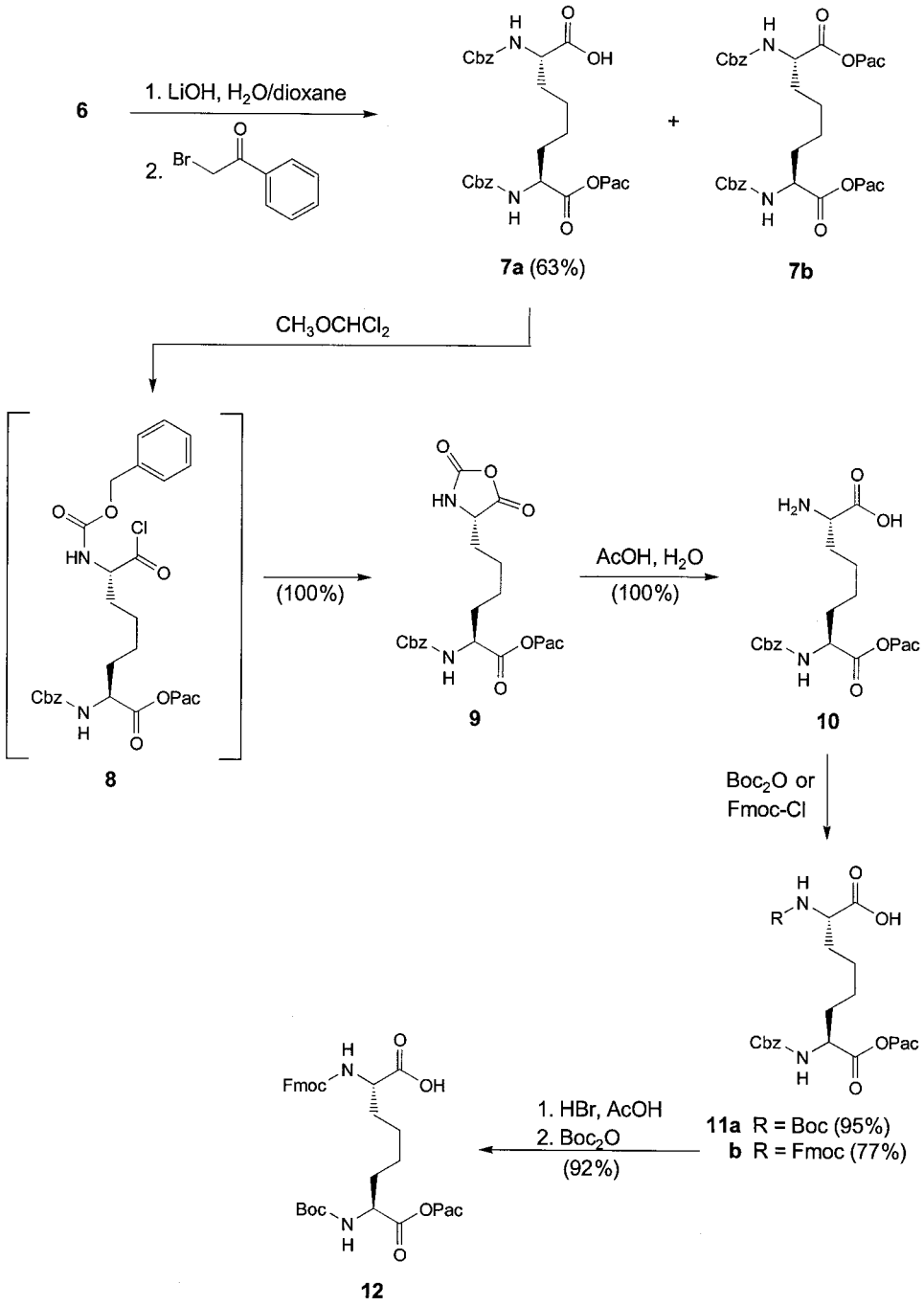
4) The R_f value upon TLC (AcOEt/hexane/AcOH 50:45:5) was 0.57 for the methyl ester derived from acyl chloride **8** (after quenching with MeOH), 0.47 for NCA **9**, and 0.42 for the starting material **7a**.

Scheme 2


6
1. LiOH, H2O/dioxane
2. Br
7a (63%)
7b
CH3OCHCl2
8
(100%)
9
AcOH, H2O
(100%)
10
Boc2O or
Fmoc-Cl
11a R = Boc (95%)
b R = Fmoc (77%)
1. HBr, AcOH
2. Boc2O
(92%)
12

Experimental Part

General. Reagents and solvents were of the highest commercial grades available and were used as supplied, except THF, which was freshly distilled from sodium diphenylketyl. Column chromatography [25]: *Merck* silica gel *60*, 230–400 mesh. TLC: *Merck* silica gel *60* F_{254} anal. plates; detection by UV light or by spraying with 2% ninhydrin soln. or 5% phosphomolybdic acid in 95% EtOH and heating. Optical rotations: *Perkin-Elmer-141* polarimeter, $d = 10$ cm, c in g/100 ml. NMR Spectra: *Varian Unity 300*; δ values in ppm rel. to $SiMe_4$, coupling constants J in Hz. MS: performed at the Laboratory for Mass Spectrometry, Chemical Institute, University of Oslo, Norway (EI-MS) and by *M-Scan Ltd.*, Ascot, Berkshire, England (FAB-MS). Elemental analyses were carried out by *Ilse Beetz Mikroanalytisches Laboratorium*, Kronach, Germany.

*2,2′-(Butane-1,4-diyl)bis[(2*S,5R*)-2,5′-dihydro-3,6-dimethoxy-5-isopropylpyrazine]* (**4a**). A soln. of 1.60M BuLi in hexane (26.0 ml, 41.6 mmol) was injected into a soln. of bis-lactim ether **3** (7.0 g, 37.8 mmol) and 1,3-dimethylimidazolidin-2-one (8.3 ml, 75.6 mmol) in THF (375 ml) at $-78°$ while stirring. Stirring was continued for 15 min to complete the formation of the aza-enolate. A soln. of 1,4-dibromobutane (2.3 ml, 4.1 g, 18.9 mmol) in THF (75 ml) was then added slowly, and stirring was continued for 12 h at $-78°$ to 0°. After addition of phosphate buffer (pH 7), the mixture was allowed to warm up to r.t. and evaporated and the residue partitioned between H_2O (200 ml) and Et_2O (300 ml). The aq. layer was extracted with Et_2O (2 × 100 ml), the combined extract washed with brine, dried ($MgSO_4$), and evaporated, and excess of 1,4-dibromobutane removed by bulb-to-bulb distillation (50°/0.2 Torr). The crude alkylation product (diastereoisomer ratio **4a**/**4b** 4:1; d.e. 90% by capillary GLC) was chromatographed (silica gel (850 g), Et_2O/hexane 1:20; TLC; R_f 0.28). Pure (> 99% d.e.) **4a** (5.03 g, 63%) was obtained after recrystallization from MeOH or MeCN. Colourless solid. M.p. 84°. IR (CH_2Cl_2): 1690*s* (C=N). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.67, 1.04 (2*d*, $J = 7.7$, 2 Me_2CH); 1.14–1.34 (*m*, $CH_2(CH_2)_2CH_2$); 1.61–1.84 (*m*, $CH_2(CH_2)_2CH_2$); 2.27 (*dsept.*, $J = 7.7$, 3.6, 2 MeC*H*); 3.67, 3.68 (2*s*, 4 MeO); 3.91 (*dd*, $J = 4.0$, 3.6, 2 H, H–C(5)); 4.00 (*ddd*, $J = 4.3$, 4.0, 2 H, H–C(2)). ^{13}C-NMR (75.4 MHz, $CDCl_3$): 16.54, 19.07 (Me_2CH); 24.47, 34.12 (($CH_2)_4$); 28.60 (C(2)); 31.63 (Me_2CH); 52.26, 52.29 (MeO); 55.44 (C(2)); 60.97 (C(5)); 163.40, 163.89 (C=N). HR-EI-MS: 422.2893 ($C_{22}H_{38}N_4O_4$, M^+; calc. 422.2893). Anal. calc. for $C_{22}H_{38}N_4O_4$ (422.56): C 62.53, H 9.06, N 13.26; found: C 62.51, H 9.08, N 13.28.

*(*S,S*)-2,7-Diaminooctanedioic Acid Dimethyl Ester* (**5**). To a stirred soln. of **4a** (6.0 g, 14.2 mmol) in MeCN (40 ml), 0.5M aq. HCl (170.4 ml, 85.2 mmol) was added dropwise. Stirring was continued overnight at r.t. The mixture was extracted with Et_2O (50 ml), the aq. phase adjusted to pH 11 with NH_4OH soln. and extracted with $CHCl_3$ (3 × 50 ml), and the $CHCl_3$ phase dried ($MgSO_4$) and evaporated. The valine methyl ester was removed from the residue by bulb-to-bulb distillation (35°/0.05 Torr) to afford **5** (3.07 g, 93%) which was used in the next step without further purification. Colourless solid. IR (CH_2Cl_2): 1732*s* (C=O). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.23–1.41 (*m*, 2 NH_2); 1.42–1.59 (*m*, $CH_2(CH_2)_2CH_2$); 1.60–1.71 (*m*, $CH_2(CH_2)_2CH_2$); 3.26–3.46 (*m*, 2 CH); 3.65 (*s*, 2 MeO). ^{13}C-NMR (75.4 MHz, $CDCl_3$): 25.19, 34.51 (($CH_2)_4$); 51.81; 51.71 (MeO); 54.11 (CH); 176.34 (C=O).

*(*S,S*)-2,7-Bis[[(benzyloxy)carbonyl]amino}octanedioic Acid Dimethyl Ester* (**6**). Amino ester **5** (3.0 g, 12.9 mmol) was dissolved in dioxane (30 ml) and the soln. cooled (0°). Benzyl carbonochloridate Cbz-Cl; (6.4 ml, 45.2 mmol) and 1M aq. $NaHCO_3$ (45.2 ml, 45.2 mmol) were added simultaneously and gradually with stirring. After the addition, the mixture was further stirred for 3 h at 0° and for 5 h at r.t. Dioxane was removed by evaporation and the residue extracted with $CHCl_3$ (3 × 30 ml). The combined extract was washed with brine, dried ($MgSO_4$), and evaporated and the residue chromatographed (silica gel (170 g), hexane/AcOEt 2:1, TLC: R_f 0.25): **6** (5.8 g, 89%). Colourless solid. M.p. 88°. $[\alpha]_D^{20} = +14.2$ ($c = 1.0$, $CHCl_3$). IR (CH_2Cl_2): 1743*s*, 1718*s*, 1700*s* (C=O). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.21–1.46 (*m*, $CH_2(CH_2)_2CH_2$); 1.55–1.62, 1.63–1.89 (2*m*, $CH_2(CH_2)_2CH_2$); 3.73 (*s*, 2 MeO); 4.35 (*ddd*, $J = 4$, 2 CH); 5.10 (*s*, 2 PhCH_2); 5.31 (*d*, $J = 8.7$, 2 NHC=O); 7.33 (*s*, 2 *Ph*CH_2). ^{13}C-NMR (75.4 MHz, $CDCl_3$): 24.71, 32.45 (($CH_2)_4$); 52.37 (MeO); 53.68 (CH); 67.02 (PhCH_2); 128.12, 128.19, 128.52, 136.23 (*Ph*CH_2); 155.82 (NHC=O); 172.81 (C=O). HR-EI-MS: 500.2199 ($C_{26}H_{32}N_2O_8$, M^+; calc. 500.2199). Anal. calc. for $C_{26}H_{32}N_2O_8$ (500.54): C 62.39, H 6.44, N 5.60; found: C 62.38, H 6.42, N 5.65.

*(*S,S*)-2,7-Bis[[(benzyloxy)carbonyl]amino}octanoic Acid 2-Oxo-2-phenylethyl Ester* (**7a**) *and (*S,S*)-2,7-Bis[[(benzyloxy)carbonyl]amino}octanoic Acid Bis(2-oxo-2-phenylethyl) Ester* (**7b**). To a soln. of **6** (2.5 g, 5.0 mmol) in dioxane (25 ml) and H_2O (12.5 ml), 1M aq. LiOH (10 ml, 10 mmol) was added. The mixture was stirred overnight at r.t. When methyl ester hydrolysis was complete (TLC), 2-bromo-1-phenylethan-1-one (= phenacyl bromide, Pac-Br; 1.1 g, 5.5 mmol) in dioxane (1 ml) was added. Onset of precipitation could normally be observed soon after addition was complete. If this was not the case, H_2O (1–5 ml) was added until the mixture became turbid. Stirring was continued for 2 d, when more Pac-Br (0.22 g, 1.10 mmol) was added.

After a further 2 d stirring, the solvents were evaporated, and the residue was chromatographed (200 g of silica gel). The by-product **7b** was eluted with $CHCl_3$ and **7a** (1.86 g, 63%) with hexane/AcOEt 1:2 containing 2% AcOH.

Data of **7a**: Amorphous powder. $[\alpha]_D^{20} = -3.4$ ($c = 1.0$, DMF). IR (CH_2Cl_2): 1747*s*, 1714*s* (C=O). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.28–1.58 (*m*, $CH_2(CH_2)_2CH_2$); 1.60–1.92, 1.93–2.12 (2*m*, $CH_2(CH_2)_2)CH_2$); 4.31–4.43, 4.48–4.61 (2*m*, 2 CH); 5.10, 5.12 (2*s*, 2 PhCH_2); 5.27, 5.50 (2*dd*, $J = 18.3$, 2.3, CH_2COPh); 5.36 (*d*, $J = 16.3$, NHC=O); 5.41 (*d*, $J = 17.0$, NHC=O); 7.34 (*s*, 2 *Ph*CH_2); 7.47 (*ddd*, $J = 15.0$, 6.6, 1.3, 2 H, CH_2CO*Ph*); 7.56–7.65 (*m*, 1 H, CH_2CO*Ph*); 7.88 (*dd*, $J = 8.3$, 1.3, 2 H, CH_2CO*Ph*). ^{13}C-NMR (75.4 MHz, $CDCl_3$): 24.63, 24.69, 32.38, 32.39 ($(CH_2)_4$); 52.31, 53.73 (CH); 66.42, 66.97 (Ph*C*H_2, *C*H_2COPh); 127.71, 128.08, 128.14, 128.48, 128.85, 128.89, 133.92, 133.98, 136.98 (*Ph*CH_2, CH_2CO*Ph*); 155.82 (NHC=O); 171.88, 171.96, 172.86 (C=O); 191.36 (COOH). HR-FAB-MS: 591.2348 ($C_{32}H_{35}N_2O_9$, $[M+H]^+$; calc. 591.2343). Anal. calc. for $C_{32}H_{34}N_2O_9$ (590.62): C 65.07, H 5.80, N 4.74; found: C 64.97, H 5.85, N 4.88.

Data of **7b**: Colourless solid. M.p. 161–164°. $[\alpha]_D^{20} = +14.3$ ($c = 1.0$, $CHCl_3$). IR (CH_2Cl_2): 1753*s*, 1698*s* (C=O). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.48–1.59 (*m*, $CH_2(CH_2)_2CH_2$); 1.78–1.93, 1.98–2.12 (2*m*, $CH_2(CH_2)_2$-CH_2); 4.51–4.61 (*m*, 2 CH); 5.12 (2*s*, 2 PhCH_2); 5.27, 5.50 (2*d*, $J = 18.3$, 2 CH_2COPh); 5.43 (*d*, $J = 9.3$, 2 NHC=O); 7.34 (*s*, 2 *Ph*CH_2); 7.46 (*dd*, $J = 8.3$, 4 H, CH_2CO*Ph*); 7.60 (*dd*, $J = 8.3$, 2 H, CH_2CO*Ph*); 7.88 (*d*, $J = 8.3$, 4 H, CH_2CO*Ph*). ^{13}C-NMR (75.4 MHz, $CDCl_3$): 24.72, 32.34 ($(CH_2)_4$); 53.80 (CH); 66.46, 67.01 (Ph*C*H_2, *C*H_2COPh); 127.77, 128.10, 128.13, 128.51, 128.89, 133.97, 134.04, 136.30 (*Ph*CH_2); 155.42 (NHC=O); 171.98 (C=O); 191.44 (COOH). HR-FAB-MS: 709.2753 ($C_{41}H_{41}N_2O_{10}$, $[M+H]^+$; calc. 709.2761). Anal. calc. for $C_{40}H_{40}N_2O_{10}$ (708.75): C 67.78, H 5.69, N 3.95; found: C 67.88, H 5.74, N 3.98.

Phenacyl Ester Deprotection of **7b**. A sample of **7b** (0.10 g, 0.14 mmol) in AcOH (25 ml) was treated with Zn powder (0.18 g, 2.8 mmol). The suspension was stirred at 60° for 2 h. More Zn (0.18 g, 2.8 mmol) was added and stirring continued at r.t. for 4 h. The mixture was filtered through a pad of *Celite* and the filtrate evaporated. The residue was partitioned between H_2O (10 ml) and CH_2Cl_2 (25 ml). The aq. phase was extracted twice more with CH_2Cl_2 (10 ml), the combined extract washed with brine, dried ($MgSO_4$), and evaporated, and the reside crystallized from MeCN/Et_2O: *(*S,S*)-2,7-bis{[(benzyloxy)carbonyl]amino}octanedioic acid* (62 mg, 94%). $[\alpha]_D^{20} = -10.5 \pm 0.5$ ($c = 1.0$, DMF); [18]: $[\alpha]_D = -10.8$ ($c = 1$, DMF); [6]: $[\alpha]_D = -9.8$ ($c = 5$, DMF). ^{13}C-NMR: in accordance with [6].

*(*S,S*)-7-Amino-2-{[(benzyloxy)carbonyl]amino}octanedioic Acid 1-(2-Oxo-2-phenylethyl) Ester 7-(*N*-Carboxyanhydride)* (=(α*S*,4*S*)-α-{[(*Benzyloxy)carbonyl]amino}-2,5-dioxooxazolidine-4-hexanoic Acid 2-Oxo-2-phenylethyl Ester*; **9**). A soln. of **7a** (1.50 g, 2.54 mmol) in dichloromethyl methyl ether (20.0 g, 174 mmol) was stirred at 60° for 30 min and then for 2 h at r.t. The mixture was evaporated: **9** (1.23 g, 100%) which was not purified (semistable compound). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.38–1.62 (*m*, $CH_2(CH_2)_2CH_2$); 1.64–1.92, 1.94–2.09 (2*m*, $CH_2(CH_2)_2CH_2$); 3.93–4.05, 4.48–4.59 (2*m*, 2 CH); 5.06, 5.15 (2*d*, $J = 13.7$, PhCH_2); 5.29, 5.50 (2*d*, $J = 18.3$, CH_2COPh); 5.67, 7.15 (2*s*, 2 NHC=O); 7.33 (*s*, *Ph*CH_2); 7.48 (*dd*, $J = 8.3$, 2 H, CH_2CO*Ph*); 7.61 (*dd*, $J = 8.3$, 1 H, CH_2CO*Ph*); 7.87 (*d*, $J = 8.3$, 2 H, CH_2CO*Ph*). ^{13}C-NMR (75.4 MHz, $CDCl_3$): 23.44, 23.68, 31.36, 32.34 ($(CH_2)_4$); 53.05, 56.78 (CH); 66.66, 67.20 (Ph*C*H_2, *C*H_2COPh); 127.82, 128.04, 128.09, 128.60, 128.93, 129.05, 133.75, 134.31, 136.36 (*Ph*CH_2, CH_2CO*Ph*); 152.35, 156.34 (NHC=O); 170.37, 171.88, 191.86 (C=O).

*(*S,S*)-2-Amino-7-{[(benzyloxy)carbonyl]amino}octanedioic Acid 8-(2-Oxo-2-phenylethyl) Ester* (**10**). A soln. of **9** (1.20 g, 2.49 mmol) in AcOH/H_2O 3:1 (50 ml) was stirred at r.t. for 12 h. After evaporation, the residue was suspended in $CHCl_3$, evaporated again, and dried: acetate salt of **10** (1.29 g, 100%) which was not purified. IR (CH_2Cl_2): 1740*s* (C=O). ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.22–1.52 (*m*, $CH_2(CH_2)_2CH_2$); 1.62–1.89 (*m*, $CH_2(CH_2)_2CH_2$, NH_2); 3.55–3.68, 4.10–4.23 (2*m*, 2 CH); 5.03 (*s*, 2 PhCH_2); 5.46, 5.58 (2*d*, $J = 18.3$, CH_2COPh); 7.36 (*s*, *Ph*CH_2); 7.54 (*dd*, $J = 8.3$, 2 H, CH_2CO*Ph*); 7.67 (*dd*, $J = 8.3$, 1 H, CH_2CO*Ph*); 7.82 (*d*, $J = 8.7$, NHC=O); 7.95 (*d*, $J = 8.3$, 2 H, CH_2CO*Ph*). ^{13}C-NMR (75.4 MHz, (D_6)DMSO): 24.72, 25.61, 30.63, 31.21 ($(CH_2)_4$); 53.25, 54.34 (CH); 66.05, 67.25 (Ph*C*H_2, *C*H_2COPh); 128.23, 128.30, 128.34, 128.52, 128.87, 129.43, 134.51, 137.41 (*Ph*CH_2, CH_2CO*Ph*); 155.70 (NHC=O); 172.57 (C=O); 193.09 (COOH).

*(*S,S*)-2-{[(Benzyloxy)carbonyl]amino}-7-{[(*tert*-butoxy)carbonyl]amino}octanedioic Acid 1-(2-Oxo-2-phenylethyl) Ester* (**11a**). To a cooled (0°) soln. of **10** (0.40 g, 0.77 mmol) in CH_2Cl_2 (30 ml), Boc_2O (0.34 g, 1.55 mmol) and Et_3N (0.32 ml, 2.32 mmol) were added gradually with stirring. The mixture was stirred for further 3 h at 0° and 5 h at r.t. After evaporation, the residue was suspended in H_2O and extracted with $CHCl_3$ (3×30 ml), the combined extract washed with brine, dried ($MgSO_4$), and evaporated, and the crude product chromatographed (silica gel (170 g), hexane/AcOEt 5:1; TLC; R_f 0.28): **11a** (0.41 g, 95%). Amorphous powder. $[\alpha]_D^{20} = -2.8$ ($c = 1.0$, DMF). IR (CH_2Cl_2): 1748*s*, 1694*s* (C=O). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.42 (*s*, *t*-Bu); 1.59–1.81 (*m*, $CH_2(CH_2)_2CH_2$); 1.85–2.38 (*m*, $CH_2(CH_2)_2CH_2$); 4.37–4.46, 4.48–4.58 (2*m*, 2 CH);

5.11 (*s*, PhCH_2); 5.21 (*s*, NHC=O); 5.28, 5.48 (2*d*, *J* = 18.3, CH_2COPh); 5.62 (*s*, NHC=O); 7.34 (*s*, *Ph*CH_2); 7.47 (*dd*, *J* = 8.3, 2 H, CH_2CO*Ph*); 7.60 (*dd*, *J* = 8.3, 1 H, CH_2CO*Ph*); 7.88 (*d*, *J* = 8.3, 2 H, CH_2CO*Ph*). ^{13}C-NMR (75.4 MHz, $CDCl_3$): 24.68, 24.86, 32.22 ((CH_2)$_4$); 28.26 (*Me*$_3$); 53.74 (CH); 66.46, 67.04 (Ph*C*H_2, *C*H_2COPh); 79.96 (Me_3C); 127.74, 128.08, 128.12, 128.47, 128.87, 133.94, 133.98, 136.16 (*Ph*CH_2, CH_2CO*Ph*); 156.06 (NHC=O); 172.03 (C=O); 191.53 (COOH). HR-FAB-MS: 557.2459 ($C_{29}H_{37}N_2O_9^+$, $[M+H]^+$; calc. 557.2499). Anal. calc. for $C_{29}H_{36}N_2O_9$ (556.60): C 62.58, H 6.52, N 5.03; found: C 62.49, H 6.54, N 5.00.

*(*S,S*)-2-{[(Benzyloxy)carbonyl]amino}-7-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}octanedioic Acid 1-(2-Oxo-2-phenylethyl) Ester* (**11b**). To a cooled (0°) soln. of **10** (0.75 g, 1.45 mmol) in dioxane (20 ml), Fmoc-Cl (0.75 g, 2.90 mmol) and 1M $NaHCO_3$ (5.8 ml, 5.8 mmol) were added gradually with stirring. The mixture was stirred for further 3 h at 0° and for 9 h at r.t. The reaction was quenched by adding AcOH (2 ml), and the solvents were evaporated. The residue was suspended in H_2O and extracted with $CHCl_3$ (3 × 30 ml), the combined extract washed with brine, dried ($MgSO_4$), and evaporated and the crude product chromatographed (silica gel (170 g), hexane/AcOEt 2 : 1; TLC: R_f 0.31): **11b** (0.76 g, 77%). Amorphous powder. $[\alpha]_D^{20} = -4.2$ (*c* = 1.0, DMF). IR (CH_2Cl_2): 1746*s*, 1693*s* (C=O). ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.25–1.34 (*m*, $CH_2(CH_2)_2CH_2$); 1.52–1.78 (*m*, CH_2(CH_2)$_2$CH_2); 3.84–3.92 (*m*, CH); 4.10–4.28 (*m*, 3 H, CH, $C_{13}H_9CH_2$); 5.03 (*s*, PhCH_2); 5.42, 5.59 (2*d*, *J* = 18.7, CH_2COPh); 7.23–7.41 (*m*, 10 H, NHCO, *Ph*CH_2, CH_2CO*Ph*, $C_{13}H_9CH_2$); 7.50–7.58, 7.61–7.73 (2*m*, 6 H, CH_2CO*Ph*, $C_{13}H_9CH_2$); 7.81 (*d*, *J* = 7.6, NHC=O); 7.86, 7.95 (2*d*, *J* = 8.3, 4 H, CH_2CO*Ph*, $C_{13}H_9CH_2$). ^{13}C-NMR (75.4 MHz, (D_6)DMSO): 24.79, 32.11 ((CH_2)$_4$); 52.36, 53.74 (CH); 66.49, 66.75, 67.08 (Ph*C*H_2, *C*H_2COPh, $C_{13}H_9CH_2$); 120.02, 124.92, 127.08, 127.56, 127.67, 127.92, 128.10, 128.15, 128.50, 128.87, 128.99 (*Ph*CH_2, CH_2CO*Ph*, $C_{13}H_9CH_2$); 133.92, 134.01 (CH_2CO*Ph*); 136.20 (*Ph*CH_2); 141.25, 143.95 ($C_{13}H_9CH_2$); 156.24 (NHC=O); 171.33, 172.19 (C=O); 191.69 (COOH). HR-FAB-MS: 679.2658 ($C_{39}H_{39}N_2O_9^+$, $[M+H]^+$; calc. 679.2656). Anal. calc. for $C_{39}H_{38}N_2O_9$ (678.73): C 69.01, H 5.64, N 4.13; found: C 69.10, H 5.59, N 4.02.

*(*S,S*)-2-{[(*tert*-Butoxy)carbonyl]amino}-7-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}octanedioic Acid 1-(2-Oxo-2-phenylethyl) Ester* (**12**). A soln. of **11b** (0.70 g, 1.03 mmol) in 47% HBr/AcOH (7 ml) at 0° was stirred for 2 h at r.t. After evaporation, the residue was suspended in $CHCl_3$ and dried under high vacuum for 24 h: HBr salt (0.64 g, 99%) of Cbz-deprotected **11b** which was not purified. IR (CH_2Cl_2): 1747*s*, 1703*s* (C=O). ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.24–1.82 (*m*, $CH_2(CH_2)_2CH_2$); 1.89 (*s*, NH_3^+); 3.90–3.98 (*m*, CH); 4.18–4.32 (*m*, CH, $C_{13}H_9CH_2$); 5.63, 5.73 (2*d*, *J* = 19.0, CH_2COPh); 7.30 (*dd*, *J* = 8.6, 2 H, $C_{13}H_9CH_2$); 7.40 (*dd*, *J* = 8.3, 2 H, $C_{13}H_9CH_2$); 7.56 (*dd*, *J* = 8.6, 3 H, CH_2CO*Ph*, $C_{13}H_9CH_2$); 7.65 (*d*, *J* = 9.0, NHC=O); 7.70 (*dd*, *J* = 8.6, 3 H, CH_2CO*Ph*, $C_{13}H_9CH_2$); 7.88, 7.97 (2*d*, *J* = 8.3, 4 H, CH_2CO*Ph*, $C_{13}H_9CH_2$); 8.43 (*s*, COOH). ^{13}C-NMR (75.4 MHz, (D_6)DMSO): 24.18, 25.64, 30.57, 30.95 ((CH_2)$_4$); 52.32, 58.14 (CH); 66.10, 68.13 (*C*H_2COPh, $C_{13}H_9CH_2$); 120.63, 125.77, 127.58, 128.16, 128.40, 128.82, 129.18 (CH_2CO*Ph*, $C_{13}H_9CH_2$); 134.04, 134.75 (CH_2CO*Ph*); 141.22, 144.28 ($C_{13}H_9CH_2$); 156.06, 169.88 (C=O); 192.47 (COOH).

An aliquot of this material (0.50 g, 0.80 mmol) in CH_2Cl_2 (20 ml) was cooled (0°) and treated gradually with Boc_2O (0.53 g, 2.40 mmol) and Et_3N (0.39 ml, 2.80 mmol). The mixture was stirred for 3 h at 0° and 5 h at r.t. After evaporation, the residue was suspended in H_2O and extracted with $CHCl_3$ (3 × 30 ml), the combined extract washed with brine, dried ($MgSO_4$), and evaporated, and the crude product chromatographed (silica gel (170 g), hexane/AcOEt 1 : 1 (2% AcOH); TLC: R_f 0.30): **12** (475 mg, 92%). Amorphous powder. $[\alpha]_D^{20} = -1.5$ (*c* = 1.0, DMF). IR (CH_2Cl_2): 1749*s*, 1703*s* (C=O). ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.17–1.39 (*m*, $CH_2(CH_2)_2CH_2$); 1.35 (*s*, 9 H, Me_3C); 1.44–1.83 (*m*, CH_2(CH_2)$_2$CH_2); 3.66–3.84, 3.98–4.11 (2*m*, 2 CH); 4.13–4.32 (*m*, 2 H, $C_{13}H_9CH_2$); 5.41, 5.56 (2*d*, *J* = 19.0, CH_2COPh); 7.02 (*d*, *J* = 7.3, NHC=O); 7.30 (*dd*, *J* = 8.6, 4 H, CH_2CO*Ph*, $C_{13}H_9CH_2$); 7.39 (*dd*, *J* = 8.3, 2 H, CH_2CO*Ph*, $C_{13}H_9CH_2$); 7.52 (*dd*, *J* = 8.6, 2 H, CH_2CO*Ph*, $C_{13}H_9CH_2$); 7.62 (*d*, *J* = 7.3, NHC=O); 7.69 (*dd*, *J* = 8.6, 2 H, CH_2CO*Ph*, $C_{13}H_9CH_2$); 7.86, 7.94 (2*d*, *J* = 8.3, 4 H, CH_2CO*Ph*, $C_{13}H_9CH_2$). ^{13}C-NMR (75.4 MHz, (D_6)DMSO): 25.58, 26.01, 31.45, 32.33 ((CH_2)$_4$); 28.70 (Me_3C); 54.06, 55.12 (CH); 65.84, 67.08 ($C_{13}H_9CH_2$, *C*H_2COPh); 78.68 (Me_3C); 120.52, 121.87, 125.77, 127.56, 128.08, 128.40, 129.39, 134.37 ($C_{13}H_9CH_2$, CH_2CO*Ph*); 137.93, 139.92 ($C_{13}H_9CH_2$); 143.08, 156.06, 172.86 (C=O); 193.11 (COOH). HR-FAB-MS: 645.2811 ($C_{36}H_{41}N_2O_9$, $[M+H]^+$; calc. 645.2812). Anal. calc. for $C_{36}H_{40}N_2O_9$ (644.71): C 67.07, H 6.25, N 4.35; found: C 67.34, H 6.17, N 4.19.

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Received July 27, 1998