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Asymmetric Synthesis of Phenylbis(glycines)

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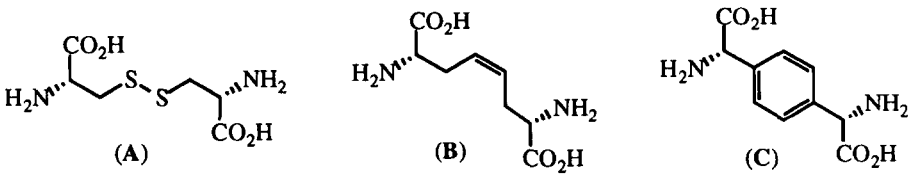
Abstract: (*S,S*) -α,α′-Diamino-1,4- and 1,3-benzenediacetic acids and *N*-Boc- and *N*-Fmoc-derivatives have been prepared by stereoselective syntheses. The chiral auxiliary (*S*)-4-benzyl-2-oxazolidinone formed bisimides with benzenediacetyl dichlorides The imides were lithiated at both α-sites to the carbonyl groups and azidated stereoselectively *alpha* to both carbonyl groups by trisyl azide to provide (*S,S*)-α,α′-diazidobenzenediacetic acid derivatives which were reduced to amines by Sn(II) chloride or by hydrogenolysis over Pd. Copyright © 1996 Elsevier Science Ltd

The disulfide linkage in the amino acid cystine is often involved in the formation of cyclic peptides and inter-linking peptide chains in physiologically essential (oligo)peptides and proteins. The disulfide linkage confers conformational constraint on the peptide or protein structure which may be essential for the spacing of the pharmacophoric region for its interaction with the receptor. When cystine (**A**, Scheme 1) exerts mainly a skeletal, structural function, isosteric structures may be envisaged to take its place. In the simplest case, the disulfide linkage may be replaced by an ethylene unit, and this may be variously substituted for subtle-tuning of conformational constraint in the amino acid and its peptides. Because of the medical implications which may arise from such interactions, we have initiated a program on the preparation of bridged amino acids as cystine substitutes.[1,2] In its simplest case, when the -CH_2S-SCH_2-bridge between the two glycine carbons in cystine is replaced by the all carbon -$(CH_2)_4$-bridge, the new bridge amino acid is (*S,S*)-2,7-diaminosuberic acid [(*S,S*)-2,7-diaminoctanedioic diacid].



Scheme 1

It has been known for some time that the (*S,S*)-2,7-diaminosuberic acid can replace cystine in essential cystine peptides such as oxytocin, calcitonin and somatostatin to furnish highly bioactive peptides.[3] These peptides will have a higher chemical stability than cystine peptides because of the absence of reducible disulfide linkages. Recently it has been found that cystine in the haemoregulatory peptide pGluGluAspCys(LysOH)S-SCys(LysOH)AspGlupGlu,[4] can be substituted with (*S,S*)-2,7-diaminosuberic acid to provide a peptide with an all-carbon bridge between the peptide chains which amounts to inter-connecting two glycine units in the

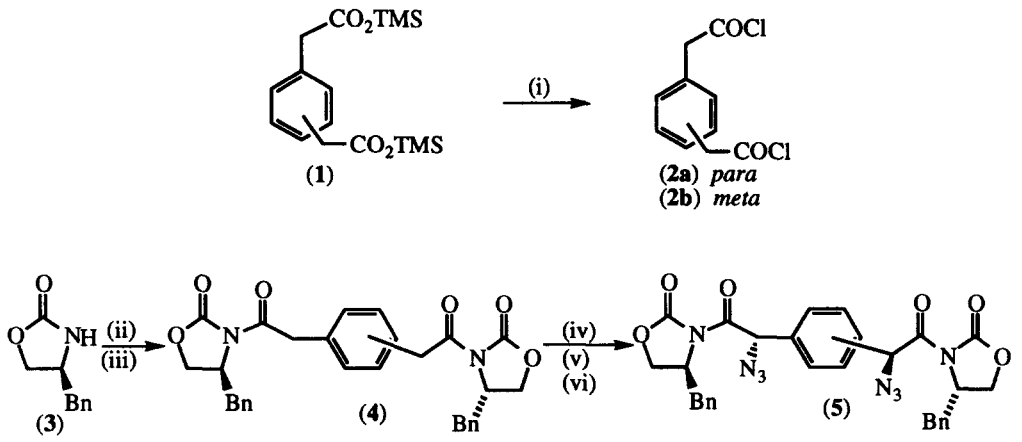
chains with a four-carbon bridge.[5] The new peptide is highly bioactive, stimulates colony formation of both human and murine CFU-GM *in vitro* and up-regulates murine myelopoeitic cells *in vivo*; it is of medical interest as a stimulator of the immune defence system in certain cases of pathological conditions. It is interesting to note that reduction of the disulfide linkage to furnish the corresponding cysteine derivative gives a peptide with opposite bioaction.[6] Unsaturation in the suberic acid decreases conformational freedom in the olefinic suberic acid and its peptide derivatives relative to suberic acid derivatives, an olefinic *cis*- or *trans*- bond takes the place of the disulfide linkage. Structure (**B**) exemplifies the *cis*-isomer and close analogy to a corresponding benzene derivative becomes obvious provided the bridge retains a four-carbon backbone between the α,α′-glycine units; the primary target molecule is therefore 1,4-benzenebis(glycine) (**C**) with increased constraint over that of the *cis* structure (**B**) because of the aromatic ring. A secondary target molecule was to be the sterically constrained 1,3-isomer which corresponds to a three-carbon bridge.

Several chiral auxiliaries are available for amino acid constructions.[7] We have used extensively the Schöllkopf "bislactim ether" chiral auxiliary in bridge forming reactions which involve alkylation of lithiated (2*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine. This excludes direct arylation unless the aryl group is strongly electrophilic as in metal complexes; reactions of chlorobenzenes-$Mn(CO)_3$ comlexes[8] or fluorobenzene-$Cr(CO)_3$ complexes have been reported.[9] Conversion of the chiral auxiliary to an electrophilic reagent can be used to effect arylation under Friedel-Crafts conditions provided the arene is strongly nucleophilic.[10] Analogous reactions can be effected with the Williams chiral oxazinone glycinate approach.[11] The most attractive approaches for the construction of arylglycines appeared to be offered by the Oppolzer sultam chiral auxiliary,[12] or the Evans chiral carboximide approach where the nitrogen is introduced as an electrophile on chiral phenylacetamide derivatives.[13] We chose the latter approach.

From the recent review on asymmetric synthesis of arylglycines,[14] it is apparent that stereoselective syntheses of arylbis(glycines) have not been reported so far. In the approach herein described two new chiral centers are created which require high stereoselectivity in the substitutions at both sites in order to obtain good diastereomeric excess (d.e.) for the overall reaction.

The 1,4- and 1,3-benzenediacetic acids for the *N*-acylation of the chiral auxiliary (*S*)-4-benzyl-2-oxazolidinone (**3**) were initially converted into trimethylsilyl esters by silylation with hexamethyldisilazane (HMDS) in the presence of trimethylsilyl chloride (TMS-Cl); the silyl esters react readily with oxalyl chloride at ambient temperature to form the diacid chlorides (**2**). The crude acid chlorides prepared by this procedure have a high degree of purity and were used for the acylation reaction without distillation to avoid partial decomposition of the somewhat labile acid chlorides. *N*-acylation of lithiated (*S*)-4-benzyl-2-oxazolidinone was run at -78 °C to form the diacetamides (**4**). The 1,3-isomer (**4b**) was recently reported to be formed in a yield of 27 % using acid chloride prepared directly from the acid.[15] Our approach with the acid chloride prepared from the TMS-ester (**1b**), gave 58 % of (**4b**).

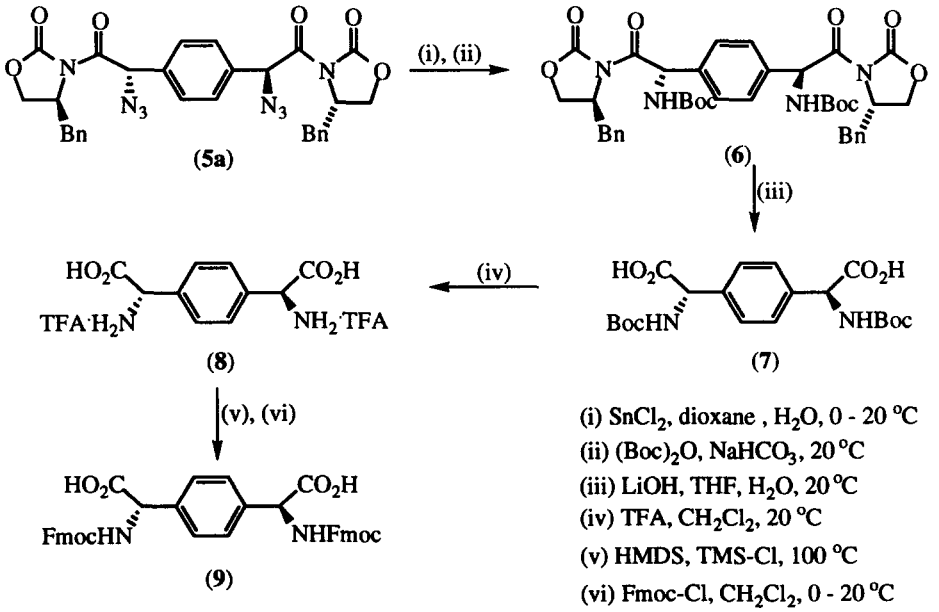
For the introduction of electrophilic nitrogen as 2,4,6-triisopropylbenzenesulfonyl azide (trisyl azide) on the α-carbons, the bisacetamides (**4**) were enolized by the addition of a slight excess of potassium hexamethyldisilazide (KHMDS) at -78 °C; enolization was effected at both α-carbons. The initially formed triazene in the substitution was converted to the azide by addition of acetic acid; potassium acetate is thereby formed and is the active reagent in this conversion.[16] The 1,4-isomer (**5a**) was isolated in a yield of 65 %, ratio for α,α′-diazido-isomers (**5a**) (*S,S*):(*R,S*) 4:1 which compare well to the data reported for azidation of phenylglycine (ratio 91:9) and substituted phenylacetic acids.[13] This may indicate that the stereoselectivity in



(i) $(COCl)_2$, CH_2Cl_2, DMF (cat), 0 - 20 °C
(ii) nBuLi, THF, -78 °C
(iii) (2), THF, -78 °C
(iv) KHMDS, THF, -78 °C
(v) $ArSO_2N_3$, THF, -78 °C
(vi) HOAc, -78 - 30 °C

Scheme 2

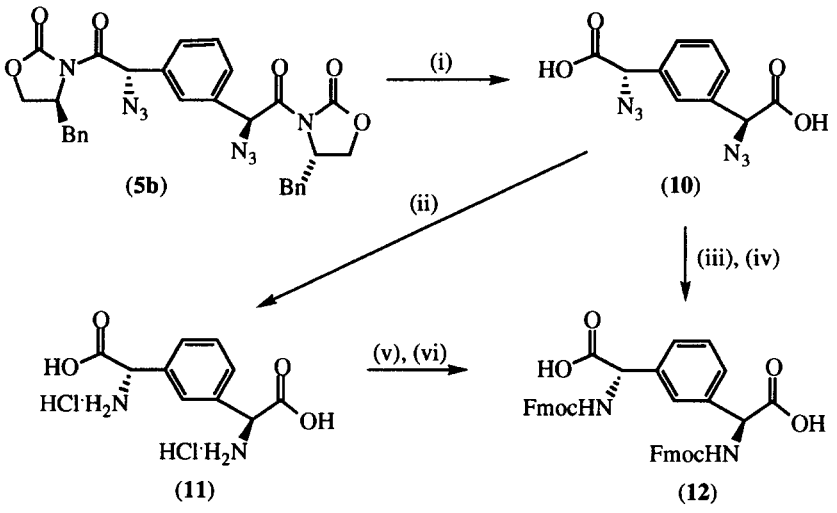
both steps in the formation of (**5a**) is similar. We did not succeed in separation of the (**5a**)-isomers, but isomer separation was readily effected by flash chromatography after reduction to amine and acylation to the *t*-butyloxycarbonyl (Boc) derivatives (**6**) (*vide infra*). In the formation of the 1,3-isomer (**5b**) the yield after recrystallization from acetonitrile was 73 %, d.e. > 95 %.



(i) $SnCl_2$, dioxane , H_2O, 0 - 20 °C
(ii) $(Boc)_2O$, $NaHCO_3$, 20 °C
(iii) LiOH, THF, H_2O, 20 °C
(iv) TFA, CH_2Cl_2, 20 °C
(v) HMDS, TMS-Cl, 100 °C
(vi) Fmoc-Cl, CH_2Cl_2, 0 - 20 °C

Scheme 3

The azido groups in the 1,4-isomer (**5a**) were reduced to amino groups by the reduction with tin(II) chloride in aqueous dioxane and the amino compound isolated and purified as the Boc-protected amine (**6**) by the reaction with *t*-butyloxycarbonyl anhydride; overall yield 56 %. Treatment of (**6**) with two equivalents of lithium hydroxide at ambient temperature cleaved the chiral auxiliary functions to furnish the Boc-protected 1,4-benzenebis(glycine) (**7**); yield > 95 %. The latter is a useful substrate in some of the protocols used in peptide coupling reactions. We have also deprotected (**7**) by treatment with trifluoroacetic acid (TFA) in dichloromethane at ambient temperature to furnish the amino acid (**8**); yield > 95 %. Diamino acids like (**8**) frequently possess low solubility in common organic solvents. For the conversion to the 7-fluoromethyloxycarbonyl (Fmoc) protected amino acid (**9**), the amino acid (**8**) was persilylated to its *N*-trimethylsilyl silyl ester by heating with HMDS in the presence of TMS-Cl. The persilylated intermediate is readily soluble in organic solvents; it was dissolved in dichloromethane and acylated by the treatment with Fmoc-Cl to form (**9**). The latter represents another mode of protection of an amino acid for peptide coupling reactions.



(i) LiOH, THF, H_2O, 0 °C
(ii) H_2/Pd/C (15 psi), EtOH, H_2O, HCl, 20 °C
(iii) $SnCl_2$, dioxane, H_2O, 0 - 20 °C
(iv) Fmoc-Cl, $NaHCO_3$, 0 - 20 °C
(v) HMDS, TMS-Cl, 100 °C
(vi) Fmoc-Cl, CH_2Cl_2, 0 - 20 °C

Scheme 4

In the 1,3-series the Fmoc-protected amino acid (**12**) was prepared by an alternative sequence. The chiral auxiliary in (**5b**) was first cleaved with lithium hydroxide; the hydrolysis proceeds under mild conditions. Hydrogenolysis of the diazido diacid (**10**) over palladium on charcoal in acidic aqeous ethanol gave the diamino diacid as hydrochloride (**11**). The latter was persilylated using HMDS and TMS-Cl, dissolved in cold dichloromethane and acylated by the reaction with Fmoc-Cl. In another variation of the procedure for the preparation of (**12**), the diazido diacid (**10**) was reduced with tin(II) chloride to the corresonding amine which was acylated by the reaction with Fmoc-Cl in aqueous sodium bicarbonate.

EXPERIMENTAL.

The mass spectra under electron impact conditions were recorded at 70 eV (EI). CH_4 was used for chemical ionization (CI). The spectra are presented as *m/z* (% rel. int.). The ^1H NMR spectra were recorded at 200 MHz or 300 MHz and the ^{13}C NMR spectra at 50 MHz or 75 MHz.

1,4-Benzenediacetyl dichloride (**2a**).[17] A mixture of 1,4-benzenediacetic acid (1.00 g, 5.15 mmol), hexamethyldisilazane (1.08 g, 10.30 mmol) and trimethylchlorosilane (3 drops) in dry 1,2-dichloroethane (30 ml) was refluxed overnight. The solution was evaporated at reduced pressure. The residual material (1.68 g, 4.97 mmol) was bis(trimethylsilyl) 1,4-benzenediacetate; yield (crude) 96 %. ^1H NMR ($CDCl_3$): δ 0.27 (s, 18H, TMS), 3.60 (s, 4H, CH_2), 7.23 (s, 4H, Ph). ^{13}C NMR ($CDCl_3$): δ -0.4 (TMS), 42.0 (CH_2), 129.3, 133.0 (Ph), 172.0 (CO).

The product thus obtained was dissolved in dry dichloromethane (10 ml) and DMF (2 drops), oxalyl chloride (1.39 g, 10.93 mmol) added dropwise to the solution at 0 °C, the solution stirred for 1 h at this temperature and for 1 h at ambient temperature before the solvent was removed at reduced pressure; yield (crude) 80 %. ^1H NMR ($CDCl_3$): δ 4.16 (s, 4H, CH_2), 7.30 (s, 4H, Ph). ^{13}C NMR ($CDCl_3$): δ 52.7 (CH_2), 129.8, 130.9 (Ph), 171.5 (CO).

1,3-Benzenediacetyl dichloride (**2b**).[17] Compound (**2b**) was prepared as above; intermediate bis(trimethylsilyl) 1,3-benzenediacetate; yield (crude) 92 %. ^1H NMR ($CDCl_3$): δ 0.26 (s, 18H, TMS), 3.60 (s, 4H, CH_2), 7.12-7.31 (m, 4H, Ph). ^{13}C NMR ($CDCl_3$): δ -0.3 (TMS), 42.8 (CH_2), 127.9, 128.5, 130.3, 134.5 (Ph), 171.9 (CO). Yield (crude) diacetyl chloride 97 %. ^1H NMR ($CDCl_3$): δ 4.16 (s, 4H, CH_2). 7.19-7.42 (m, 4H, Ph). ^{13}C NMR ($CDCl_3$): δ 52.7 (CH_2), 129.4, 129.6, 130.6, 132.0 (Ph), 171.7 (CO).

(*S,S*)-β,β′-Dioxo-β,β′-bis(4-benzyl-2-oxo-3-oxazolidinyl)-1,4-diethylbenzene (**4a**). 1.6 M nBuLi in hexane (9.68 ml, 15.485 mmol) was added dropwise with stirring to a solution of (*S*)-4-benzyl-2-oxazolidinone (2.744 g, 15.485 mmol) and triphenylmethane (indicator) (3 mg) in dry THF (20 ml) under argon at -78 °C until an orange colour persisted. The solution was kept between -65 °C and -78 °C during the addition. After recooling to -78 °C, 1,4-benzenediacetyl dichloride (1.896 g, 8.21 mmol) in dry THF (20 ml) was added dropwise. The mixture was warmed to 25 °C, treated with saturated aqueous sodium bicarbonate (30 ml) and stirred at 25 °C for 30 min. The mixture was extracted with ethyl acetate (3x). The organic extracts were combined, washed with 5 % aqueous sodium carbonate, washed with brine, dried ($MgSO_4$) and evaporated. The crude product was purified by flash chromatography eluting with hexane:ethyl acetate 1:2; yield 2.274 g (57 %) of a white glassy foam. Found: C, 70.08; H, 5.70. Calc. for $C_{30}H_{28}N_2O_6$: C, 70.29; H, 5.51. ^1H NMR ($CDCl_3$): δ 2.77 (dd, *J* 9.4, 13.3 Hz, 2H, CHHPh), 3.28 (dd, *J* 3.2, 13.3 Hz, 2H, CHHPh), 4.18-4.22 (m, 4H, CH_2O), 4.31 (s, 4H, CH_2CO), 4.64-4.72 (m, 2H, CHN), 7.35-7.13 (m, 14H, aromatics). ^{13}C NMR ($CDCl_3$): δ 37.7, 41.2, 55.2, 66.0, 126.9, 128.5, 129.0, 129.6, 132.1, 134.7, 152.9, 170.5. MS(EI): 512 (*M*, 40), 336 (14), 335 (60), 158 (100), 131 (43), 92 (25), 91 (23), 86 (19).

(*S,S*)-β,β′-Dioxo-β,β′-bis(4-benzyl-2-oxo-3-oxazolidinyl)-1,3-diethylbenzene (**4b**).[15] 1.6 M nBuLi in hexane (11.68 ml, 18.69 mmol) was added dropwise to a solution of (*S*)-4-benzyl-2-oxazolidinone (3.312 g, 18.69 mmol) and triphenylmethane (indicator) (3 mg) in dry THF (20 ml) under argon with stirring at -78 °C

until an orange colour persisted. The solution was kept between -65 °C and -78 °C during the addition. After recooling to -78 °C, 1,3-benzenediacetyl dichloride (2.29 g, 9.91 mmol) in dry THF (20 ml) was added dropwise. The mixture was warmed to 25 °C, treated with saturated aqueous sodium bicarbonate (30 ml) and stirred at 25 °C for 30 min. The mixture was extracted with ethyl acetate (3x). The organic extracts were combined, washed with 5 % aqueous sodium carbonate, washed with brine, dried ($MgSO_4$) and evaporated. The crude product was purified by flash chromatography on silica gel using hexane:ethyl acetate 1:1; yield (white solid) 2.789 g (58 %). M.p. 58-60 °C. ^1H NMR ($CDCl_3$): δ 2.75 (dd, *J* 9.5, 13.3 Hz, 2H, C<u>H</u>HPh), 3.28 (dd, *J* 3.3, 13.3 Hz, 2H, CH<u>H</u>Ph), 4.14-4.32 (m, 8H, CH_2O and CH_2CO), 4.63-4.71 (m, 2H, CHN), 7.11-7.35 (m, 14H, aromatics). ^{13}C NMR ($CDCl_3$): δ 37.7 (CH_2Ph), 41.5 (CH_2CO), 55.3 (CHN), 66.1 (CH_2O), 127.2, 128.7, 128.8, 129.3, 131.1, 133.7, 135.1 (aromatics), 153.3, 170.9 (CO). MS(EI): 512 (M, 85), 464 (49), 335 (94), 160 (89), 158 (100), 133 (53), 131 (61), 91(68).

<u>(*S,S,S,S*)-α,α′-Diazido-β,β′-bis(4-benzyl-2-oxo-3-oxazolidinyl)-β,β′-dioxo-1,4-diethylbenzene (**5a**).</u> A precooled (-78 °C) solution of (*S,S*)-β,β′-dioxo-β,β′-bis(4-benzyl-2-oxo-3-oxazolidinyl)-1,4-diethylbenzene (0.856 g, 1.67 mmol) in dry THF (16 ml) was added *via* a cannula to a solution of 0.5 M potassium bis(trimethylsilyl) amide (7.02 ml, 3.51 mmol) in toluene diluted with dry THF (18 ml) under argon at -78 °C. The resulting solution of the potassium enolate was kept at -78 °C for 30 min. A solution of precooled (-78 °C) 2,4,6-triisopropylbenzenesulfonyl azide (trisyl azide)[18] (1.14 g, 3.68 mmol) in dry THF (9 ml) was then added *via* a cannula. The solution was stirred for 10 min at -78 °C before the reaction was quenched by addition of acetic acid (0.922 g, 15.38 mmol). The slurry was immediately warmed to 30 °C on a water bath and stirred at this temperature for 1.5 h. The reaction slurry was subsequently partitioned between brine (200 ml) and ethyl acetate (250 ml), and the aqueous phase was washed twice with ethyl acetate (100 ml). The combined organic extracts were shaken with dilute aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated at reduced pressure. The crude product was purified by flash chromatography on silica gel eluting first with hexane:ethyl acetate 2:1, then with ethyl acetate. The product was purified further by flash chromatography eluting first with hexane:ethyl acetate 2:1, then with hexane:ethyl acetate 1:2; yield (pale yellow glassy foam) 646 mg (65 %), d.e. = 60 %. ^1H NMR (200 MHz, $CDCl_3$): δ 2.88 (dd, *J* 9.4, 13.3 Hz, 2H, CH<u>H</u>Ph), 3.41 (dd, *J* 3.2, 13.3 Hz, 2H, C<u>H</u>HPh), 4.17-4.20 (m, 4H, CH_2O), 4.64-4.73 (m, 2H, CHN), 6.13 (s, 2H, CHN_3), 7.21-7.42 (m, 10H, aromatics), 7.50 (s, 4H, aromatics). ^{13}C NMR (50 MHz, $CDCl_3$): δ 37.9, 55.8, 63.1, 66.6, 127.2, 128.7, 128.9, 129.0, 133.7, 134.1, 152.0, 168.2.

<u>(*S,S,S,S*)-α,α′-Diazido-β,β′-bis(4-benzyl-2-oxo-3-oxazolidinyl)-β,β′-dioxo-1,3-diethylbenzene (**5b**).</u> A precooled (-78 °C) solution of (*S,S*)-β,β′-dioxo-β,β′-bis(4-benzyl-2-oxo-3-oxazolidinyl)-1,3-diethylbenzene (1.94 g, 3.78 mmol) in dry THF (36 ml) was added *via* a cannula to a precooled solution of 0.5 M potassium bis(trimethylsilyl)amide (15.88 ml, 7.94 mmol) in toluene diluted with dry THF (40 ml) under argon at -78 °C. The solution was left at this temperature for 30 min, and a precooled solution (-78 °C) of 2,4,6-triisopropylbenzenesulfonyl azide (trisyl azide)[18] (2.571 g, 8.32 mmol) dissolved in dry THF (20 ml) added -78 °C at *via* a cannula. After stirring the solution for 10 min at -78 °C, the reaction was quenched by addition of acetic acid (2.087 g, 34.78 mmol). The slurry was immediately warmed to 30 °C on a water bath and stirred at this temperature for 2.5 h. The reaction slurry was then partitioned between brine (200 ml) and ethyl acetate

(250 ml), and the aqueous phase was washed twice with ethyl acetate (100 ml). The combined organic extracts were washed with dilute aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated at reduced pressure. The crude product was suspended and stirred in hexane:ethyl acetate 2:1, filtered and crystallized from acetonitrile; yield (white solid) 1.64 g (73 %), d.e. > 95 %. Found: C, 60.69; H, 4.43. Calc. for $C_{30}H_{26}N_8O_6$: C, 60.60; H, 4.41. $[\alpha]_D$ = +330.9° (c = 0.44, $CHCl_3$). M.p. 201-202 °C. ^{1}H NMR ($CDCl_3$): δ 2.85 (dd, *J* 9.7, 13.3 Hz, 2H, CHHPh), 3.41 (dd, *J* 3.2, 13.3 Hz, 2H, CHHPh), 4.12 (d, *J* 5.2 Hz, 4H, CH_2O), 4.62-4.71 (m, 2H, CHN), 5.99 (s, 2H, CHN_3), 7.20-7.54 (m, 14H, aromatics). ^{13}C NMR ($CDCl_3$): δ 37.7 (CH_2Ph), 55.7 (CHN), 64.0 (CHN_3), 66.7 (CH_2O), 126.5, 127.5, 129.1, 129.4, 130.6, 131.0, 133.2, 134.7 (aromatics), 152.3, 168.9 (CO).

(*S,S,S,S*)-α,α′-Bis(t-butoxycarbonylamino)-β,β′-bis(4-benzyl-2-oxo-3-oxazolidinyl)-β,β′-dioxo-1,4-diethylbenzene (6). A solution of (*S,S,S,S*)-α,α′-diazido-β,β′-bis(4-benzyl-2-oxo-3-oxazolidinyl)-β,β′-dioxo-1,4-diethylbenzene (100 mg, 0.168 mmol) in dioxane (3 ml) was added dropwise with stirring to a solution of $SnCl_2$ (192 mg, 1.01 mmol) in dioxane (6 ml) and water (3 ml) at 0 °C. The solution was subsequently stirred at ambient temperature overnight. $(Boc)_2O$ (367 mg, 1.68 mmol) and $NaHCO_3$ (141 mg, 1.68 mmol) were added, the solution stirred at ambient temperature for 1.5 h, acidified with aqueous potassium bisulfate, the mixture extracted with ethyl acetate, the organic layer shaken with aqueous sodium bicarbonate, washed with brine, dried (Na_2SO_4) and evaporated. The product was purified by flash chromatography on silica gel using hexane:ethyl acetate 2:1; yield (white solid): 70 mg (56 %), d.e. > 95 %. Found: C, 64.34; H, 6.28: Calc. for $C_{40}H_{46}N_4O_{10}$: C, 64.67; H, 6.24. M.p. 116 °C. ^{1}H NMR ($CDCl_3$): δ 1.43 (s, 18H, BOC), 2.84-2.91 (m, 2H, CHHPh), 3.33-3.40 (m, 2H, CHHPh), 4.06-4.17 (m, 4H, CH_2O), 4.59-4.63 (m, 2H, CHN), 5.50 (d, *J* 8Hz, 2H, CHNH), 6.56 (d, *J* 8Hz, 2H, NHCH), 7.21-7.40 (m, 14H, aromatics).

(*S,S*)-α,α′-Bis(t-butoxycarbonylamino)-1,4-benzenediacetic acid (7). $LiOH{\cdot}H_2O$ (65 mg, 1.55 mmol) in water (2 ml) was added dropwise to a solution of (*S,S,S,S*)-α,α′-bis(*t*-butoxycarbonylamino)-β,β′-bis(4-benzyl-2-oxo-3-oxazolidinyl)-β,β′-dioxo-1,4-diethylbenzene (288 mg, 0.388 mmol) in THF (6 ml) at 0 °C. The solution was stirred under argon for 45 min, 0.5 M sodium bicarbonate (7 ml) added and most of the THF evaporated. The solution was extracted (3x) with ethyl acetate and the aqueous phase was acidified (pH 3) with aqueous potassium bisulfate at 0 °C. The aqueous phase was extracted (3x) with ethyl acetate, dried (Na_2SO_4) and evaporated. The crude product was suspended and stirred in hexane:chloroform 95:5, filtered and dried; yield (white solid) 156 mg (95 %), d.e. > 95 %. Found: C, 57.01; H, 6.54. Calc. for $C_{20}H_{28}N_2O_8$: C, 56.60; H, 6.65. $[\alpha]_D$ = +174.8° (c = 0.55, EtOAc). M.p. 118-120 °C. ^{1}H NMR (DMF-d_7): δ 1.40 (s, 18H, Boc), 5.28 (d, *J* 8Hz, 2H, CH), 7.48-7.51 (m, 6H, aromatics and NH), 13.30 (br s, COOH). ^{13}C NMR (DMF-d_7): δ 28.3 (CH_3), 58.4 (CH), 79.0 (C), 128.4, 138.2 (aromatics), 156.0 (CO), 172.9 (CO).

(*S,S*)-α,α′-Diamino-1,4-benzenediacetic acid bis(trifluoroacetic acid) (8). TFA (3 ml) was added dropwise to a solution of (*S,S*)-α,α′-bis(*t*-butoxycarbonylamino)-1,4-benzenediacetic acid (155 mg, 0.366 mmol) in dry dichloromethane (3 ml) under argon at ambient temperature. The mixture was stirred for 1 h, the solvent evaporated and any residual TFA removed by azeotropic destillation with toluene (3x). The crude product was suspended and stirred in hexane, filtered and dried; yield (white solid) 157 mg (95 %), d.e. > 95 %. Found: C,

37.54; H 3.46. Calc. for $C_{14}H_{14}F_6N_2O_8$: C, 37.18; H, 3.12. $[\alpha]_D$ = +128° (c = 0.442, TFA). M.p. >230 °C (decomp.). ^{1}H NMR (CD_3OD / DMF-d_7 / TFA-d_1): δ 5.21 (s, 2H, CH), 7.65 (s, 4H, aromatics). ^{13}C NMR (CD_3OD / DMF-d_7 / TFA-d_1): 57.1 (CH), 130.3, 135.8 (aromatics), 170.3 (CO).

(*S,S*)-α,α′-Bis(9-fluorenylmethoxycarbonylamino)-1,4-benzenediacetic acid (**9**). A suspension of (*S,S*)-α,α′-diamino-1,4-benzenediacetic acid bis(trifluoroacetic acid) (110 mg, 0.243 mmol) in a mixture of hexamethyldisilazane (10 ml) and trimethylchlorosilane (1 ml) under argon was stirred and heated at 100 °C overnight. The resultant solution was evaporated to dryness at reduced pressure, the residual material dissolved in dry dichloromethane (5 ml), cooled in an ice/water bath and a solution of 9-fluorenylmethoxycarbonyl chloride (132 mg, 0.51 mmol) in dry dichloromethane (5 ml) added. The reaction mixture was stirred under argon at 0 °C for 1 h and overnight at ambient temperature. Dichloromethane was evaporated and the residue was dissolved in a mixture of THF (8 ml) and water (1 ml). The mixture was stirred for 30 min and then evaporated. The residue was taken up in ethyl acetate and washed (3x) with brine. The organic phase was dried ($MgSO_4$), filtered and evaporated. The crude product was suspended and stirred in hexane, filtered and purified by recrystallization from ethyl acetate:hexane; yield (white solid) 57 mg (35 %), d.e. > 95 %. M.p. 180-200 °C (decomp.). ^{1}H NMR (DMF-d_7): δ 4.28-4.38 (m, 6H, CH_2 and CH), 5.41 (d, *J* 8Hz, 2H, CH), 7.27-7.95 (m, 20H, aromatics), 8.26 (d, 8Hz, 2H, NH). ^{13}C NMR (DMF-d_7): δ 47.7 (CH), 58.9 (CH), 67.2 (CH_2), 120.7, 126.2, 127.8, 128.4, 128.6, 138.2, 141.8, 144.9 (aromatics), 156.8 (CO), 172.7 (CO).

(*S,S*)-α,α′-Diazido-1,3-benzenediacetic acid (**10**). LiOH·H_2O (189 mg, 4.51 mmol) in water (7 ml) was added dropwise to a solution of (*S,S,S,S*)-α,α′-Diazido-β,β′-bis(4-benzyl-2-oxo-3-oxazolidinyl)-β,β′-dioxo-1,3-diethylbenzene (670 mg, 1.12 mmol) in THF (20 ml) at 0 °C. The solution was stirred under argon at 0 °C for 1.5 h , 0.5 M sodium bicarbonate (24 ml) added, most of the THF evaporated, the solution extracted (3x) with ethyl acetate and the aqueous phase acidified (pH 3) with aqueous potassium bisulfate at 0 °C. The aqueous phase was extracted (3x) with ethyl acetate, dried (Na_2SO_4) and evaporated. The crude product was suspended and stirred in hexane:ethyl acetate 10:1, filtered and dried; yield (white solid) 292 mg (94 %), d.e. >95 %. Found: C. 43.75; H, 3.18. Calc. for $C_{10}H_8N_6O_4$: C, 43.48; H, 2.92. M.p. >120 °C (decomp.). ^{1}H NMR (DMF-d_7): δ 5.51 (s, 2H, CH), 7.46-7.56 (m, 4H, aromatics), 14.0 (br s, 2H, CO_2H). ^{13}C NMR (DMF-d_7): δ 65.7 (CH), 127.8, 129.0, 130.3, 137.0 (aromatics), 171.0 (CO).

(*S,S*)-α,α′-Diamino-1,3-benzenediacetic acid dihydrochloride (**11**). A solution of (*S,S*)-α,α′-diazido-1,3-benzenediacetic acid (185 mg, 0.67 mmol) in ethanol:HCl (1M) 8:1 (18 ml) was hydrogenated over Pd/C (10 %, 100 mg) under 15 psi H_2 at ambient temperature for 4 h, filtered through Celite, the Celite filter cake washed with several portions of the solvent, the combined solution and extracts evaporated at reduced pressure and any residual water removed by azeotropic destillation with benzene (3x). The product was suspended and stirred in ethyl acetate, filtered and dried; yield (white solid) 161 mg (81 %), d.e. < 95 %. Found: C, 41.21; H, 5.15. Calc. for $C_{10}H_{14}Cl_2N_2O_4$: C, 40.42; H, 4.75. M.p. 197-199 °C. ^{1}H NMR (D_2O / CD_3OD): δ 5.14 (s, 2H, CH), 7.57-7.62 (m, 4H, aromatics). ^{13}C NMR (D_2O / CD_3OD): δ 57.7 (CH), 129.0, 130.5, 131.8, 134.6 (aromatics), 172.0 (CO).

(*S,S*)-α,α′-Bis(9-fluorenylmethoxycarbonylamino)-1,3-benzenediacetic acid (**12**).

Method A: A suspension of (*S,S*)-α,α′-diamino-1,3-benzenediacetic acid dihydrochloride (71 mg, 0.239 mmol) in a mixture of hexamethyldisilazane (10 ml) and trimethylchlorosilane (1 ml) was stirred under argon at 100 °C overnight. The solution was then evaporated to dryness at reduced pressure, the residue dissolved in dry dichloromethane (5 ml), the solution cooled in an ice/water bath while a solution of 9-fluorenylmethoxycarbonyl chloride (186 mg, 0.717 mmol) in dry dichloromethane (5 ml) was added.The reaction mixture was stirred under argon at 0 °C for 1 h, for 3 h at ambient temperature, the solvent distilled off and the residue dissolved in a mixture of THF (8 ml) and water (1 ml). The mixture was stirred for 30 min at ambient temperature, evaporated at reduced pressure, the residue taken up in ethyl acetate, washed (3x) with brine, the organic phase dried ($MgSO_4$), filtered and evaporated. The crude product was suspended and stirred in hexane, filtered and purified by flash chromatography on silica gel using ethyl acetate:acetic acid 95:5; yield (pale yellow solid) 50 mg (32 %), d.e. > 95 %. M.p. 107-109 °C. ^{1}H NMR (DMF-d_7): δ 4.20-4.40 (m, 6H, CH_2 and CH), 5.37 (d, *J* 8Hz, 2H, CH), 7.28-7.89 (m, 20H, aromatics), 8.28 (d, *J* 8Hz, 2H, NH). ^{13}C NMR (DMF-d_7): δ 48.2 (CH), 60.0 (CH), 67.9 (CH_2), 120.9, 126.3, 127.9, 128.5, 139.3, 141.8, 144.9 (aromatics). 158.7 (CO), 173.0 (CO).

Method B: A solution of (*S,S*)-α,α′-diazido-1,3-benzenediacetic acid (140 mg, 0.507 mmol) in dioxane (5 ml) was added dropwise with stirring to a mixture of $SnCl_2$ (577 mg, 3.04 mmol) in dioxane (10 ml) and water (5 ml) under argon at 0 °C. The mixture was stirred at ambient temperature overnight, cooled to 0 °C and 1 M sodium bicarbonate (10 ml) added dropwise followed by a solution of 9-fluorenylmethoxycarbonyl chloride (1.312 g, 5.07 mmol) in dioxane (5 ml) The mixture was stirred at ambient temperature overnight, filtered, shaken with diethyl ether, the aqueous phase acidified with aqueous potassium bisulfate and extracted (3x) with ethyl acetate. The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The crude product was suspended and stirred in hexane, filtered and purified by flash chromatography on silica gel using ethyl acetate:acetic acid 95:5; yield 122 mg (36 %).

REFERENCES

1. (a) Undheim, K.; Kremminger, P., **PCT Int. Appl. WO 93 24,523;** *Chem.* Abstr. **1995**, *122*, 10682a; (b) Undheim, K.; Solbakken, M., **PCT Int. Appl. WO 93 24,522;** *Chem. Abstr.* **1994**, *121*, 281231e.
2. Fischer, P. M.; Solbakken, M.; Undheim, K., *Tetrahedron* **1994**, *50*, 2277-2288.
3. Nutt, R. F.; Strachan, R. G.; Veber, D. F.; Holly, F. W., *J. Org. Chem.* **1980**, *45*, 3078-3080 and references therein.
4. Laerum, O. D., Sletvold, O., Bjerknes, R., Eriksen, J. A., Johansen, J. H., Schanche, J. S, Tveteraas, T. and Paukovits, W R. *Exp. Hematol* **1988**, *16*, 274-280.
5. Alberts, D. P.; Agner, E.; Silvestri, J. S.; Kwon, C.;, Newlander, K.; King, A,; Pelus, L. M.; DeMarsh, P.; Frey, C.; Petteway, S.; Huffman, W.F.; Bhatnagar, P. K.; in *Thirteenth American Peptide Symposium*; Alberta, Canada, 1993; Poster P301.
6. Paukovits, W. R. and Laerum, O. D., *Z. Naturforsch.* **1982**, *37c*, 1297-1300
7. (a) R. O. Duthaler, *Tetrahedron* **50** (1994) 1539-1650; (b) R. M. Williams, *Synthesis of Optically Active Amino Acids,* Vol 7 of *Organic Chemistry Series*; J. E. Baldwin and P. D. Magnus, (Eds.), Pergamon, Oxford, 1989; (c) Ito, Y.; Sawamura, M.; Shirakawa, E.; Hayashizaki, K.; Hayashi, T., in *Symposia in-Print No 33*; Ed, O'Donnell, M., J., *Tetrahedron* **1988**, 5253-5262.
8. (a) Pearson, A. J.; Bruhn, P. R.; Gouzoules, F.; Lee, S.-H., *J. Chem. Soc. Chem. Commun.* **1989**, 659-661; (b) Pearson, A.J.; Lee, S.-H.; Gouzoles, F., *J. Chem. Soc. Perkin 1* **1990**, 2251-2254.
9. Chaari, M.; Jenhi, A.; Lavergne, J.-P.; Viallefont, P., *Tetrahedron* **1991**, *47*, 4619-4630.
10. Schöllkopf, U.; Gruttner, S.; Anderskewitz, R.; Egert, E., Dyrbusch, M., *Angew. Chem.* **1987**, *99*, 717-719.
11. Williams, R. M.; Hendrix, J. A., *J. Org. Chem.* **1990**, *55*, 3723-3729.
12. Oppolzer, W.; Tamura, O., *Tetrahedron Lett.* **1990**, *31*, 991-994
13. Evans, D. A.; Evrard, D. A.; Rychnovsky, S. D.; Früh, T.; Whittingham, W. G.; De Vries, K. M., *Tetrahedron Lett.*, **1992**, *33*, 1189-1192.
14. Williams, R. M.; Hendrix, J. A., *Chem. Rev.* **1992**, *92*, 889-917.
15. Trova, M. P.; Wang, Y., *Tetrahedron* **1993**, *49*, 4147-4158.
16. Evans, D. A.; Britton, T. C.; Ellman, J. A.; Dorow, R. L., *J. Am. Chem. Soc.* **1990**, *112*, 4011-4030.
17. Marve, C. S.; Kraiman, E. A., *J. Org. Chem.* **1953**, *18*, 707-714.
18. Harmon, R. E.; Wellman, G.; Gupta, S. K.; *J. Org. Chem.* **1973**, *38*, 11-16.

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