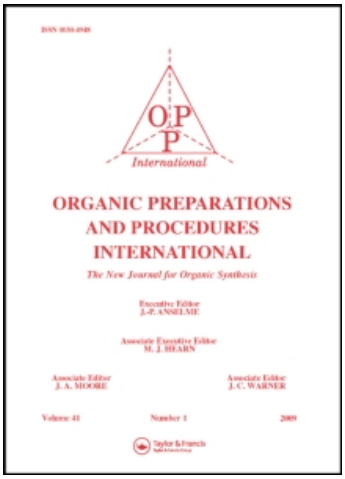

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THE PREPARATION OF N^{α}-t-BOC-2,3-DIAMINOPROPIONIC ACID (DPR) AND OF N^{α}-t-BOC-2,4-DIAMINOBUTYRIC ACID (DBR) DERIVATIVES SUITABLE FOR SOLID PHASE PEPTIDE SYNTHESIS

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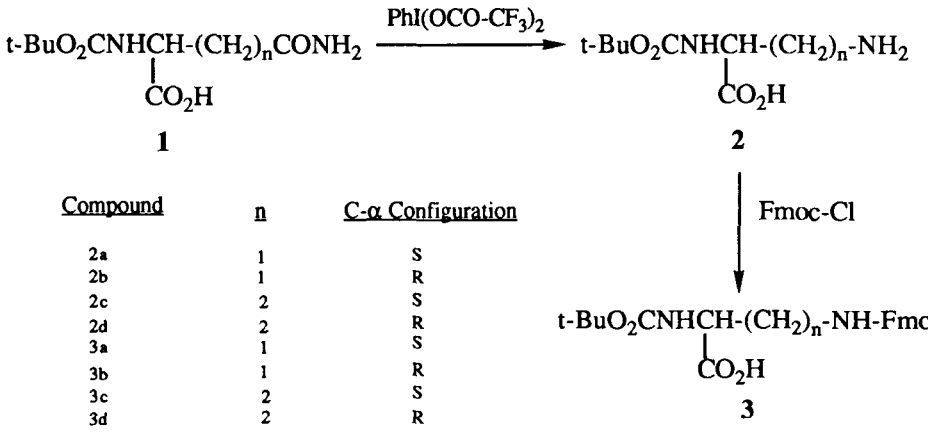
Cyclic peptides have become increasingly important recently due to the fact that conformational restriction is induced by cyclizing a peptide. This restriction may reduce the number of conformers which are available to the peptide, e.g. from an almost infinite number for a linear peptide to a few well-defined conformations in favorable cases. The reduction of possible conformations which a peptide can adopt is essential for eliciting information regarding the bioactive conformation, antagonist design, and development of potent agonists. Additionally, cyclic peptides are resistant to degradation, and therefore may have prolonged biological activity.

The possible methods of cyclizing peptides can be divided into four categories: (a) C-terminus to N-terminus; (b) C-terminus to side-chain; (c) N-terminus to side-chain; (d) side-chain to side-chain. Of the four possibilities for peptide cyclization, three require that an amino acid possess a reactive functional group in its side-chain - generally an amine, an alcohol, or a carboxylic acid. This manuscript describes the preparation of 2,3-diaminopropionic acid (Dpr)[1] and 2,4-diaminobutyric acid (Dbr)[2] and their corresponding protected derivatives which are suitable for cyclization using either a solid phase or solution phase synthetic methodology. The side-chain β-amino or γ-amino group was protected as the Fmoc

derivative to enable side-chain to side-chain cyclization by our recently reported strategy.[3]

N^{α}-t-Boc-2,3-diaminopropionic acid (Dpr) and N^{α}-t-Boc-2,4-diaminobutyric acid (Dbr) were synthesized by Hofmann rearrangement of Boc-asparagine or Boc-glutamine, respectively.



Compound	n	C-α Configuration
2a	1	S
2b	1	R
2c	2	S
2d	2	R
3a	1	S
3b	1	R
3c	2	S
3d	2	R

Experimental details are provided for the preparation of four new derivatives: N^{α}-t-Boc-(L)-Dpr-OH (2a), N^{α}-t-Boc-(L)-Dpr(Fmoc)-OH (3a), N^{α}-t-Boc-(L)-Dbr-OH (2c), and N^{α}-t-Boc-(L)-Dbr-(Fmoc)-OH (3c). In addition, the corresponding D-enantiomers (2b, 3b, 2d and 3d) have also been prepared. Except for 3b, these compounds were difficult to crystallize and retained varying amounts of water. However, spectral data fully support their structure.

The Fmoc protecting group was introduced for side-chain amine protection using a standard procedure employing the chloroformate in aqueous dioxane.[5] We have used the products, N^{α}-t-Boc-N^{β}-Fmoc-(L)-Dpr-OH (3a) and N^{α}-t-Boc-N^{γ}-Fmoc-(L)-Dbr-OH (3c) for the solid-phase synthesis of peptide hormone analogs. It is anticipated that the ready availability of both enantiomers of Dpr and Dbr, in protected form, will be generally useful for

side-chain amino group cyclization, cross-linking, or other synthetic purposes.

EXPERIMENTAL SECTION

Melting points were determined using open capillary tubes and are uncorrected. [Bis(trifluoroacetoxyiodo]benzene was purchased from Aldrich Chemical Co. and used without further purification. Dimethylformamide (Fisher) and pyridine (Fisher) were Spectroquality. The protected amino acids were purchased from Bachem, Inc.(Torrance, CA.). All other solvents and reagents were reagent grade or better. Low resolution (60 MHz) ^{1}H NMR were recorded on a Varian T-60 instrument. High resolution ^{1}H NMR were obtained at 250 MHz on a Bruker instrument. Mass spectra were recorded in the fast atom bombardment (FAB) mode. Detailed preparation of four compounds is provided: N^{α}-t-Boc-(L)-Dpr-OH (2a), N^{α}-t-Boc-N^{β}-Fmoc-(L)-Dpr-OH (3a), N^{α}-t-Boc-(L)-Dbr-OH (2c) and N^{α}-t-Boc-N^{γ}-Fmoc-(L)-Dbr-OH (3c). The preparation of the enantiomers was carried out using the same experimental procedures.

N^{α}-t-Boc-(L)-2,3-diaminopropionic Acid (2a).- A mixture of 40 mL of aqueous DMF (1/1, v/v) and [bis(trifluoroacetoxy)iodo]benzene (3.2 g, 7.4 mmol)[4] was placed into a 100 mL round bottom flask equipped with a magnetic stirrer. The mixture was stirred at 20° until the yellow solid was dissolved (3-4 minutes) and then N^{α}-t-Boc-(L)-asparagine (1.3 g, 5.6 mmol) was added in one portion as a solid. The reaction mixture was stirred for 15 minutes and pyridine (0.8 mL, 786 mg, 10.4 mmol) was added to the clear yellow solution and stirring was continued for 3 hrs. The solvents were evaporated *in vacuo* and the residue was dissolved in 50 mL of water. The aqueous solution was extracted with diethyl ether (4 x 25 mL) to remove neutral by-products. The aqueous layer was evaporated *in vacuo* at R.T. and the residue was evaporated with absolute ethanol to remove residual water. The N^{α}-t-Boc-(L)-Dpr-OH was recrystallized from ethanol/diethyl ether to yield 900 mg (79%) of yellow crystals, mp. 205-205.5°; Kaiser test positive; $[\alpha]_{D}^{25}$ = +16.12° (c, 0.98; MeOH); mass spectrum: $[M + H]^{+}$, m/e 205.

Anal. Calcd. for $C_8H_{16}N_2O_4 \cdot H_2O$: C, 43.22; H, 8.16; N, 12.61

Found: C, 43.95; H, 7.18; N, 12.48

N^{α}-t-Boc-(D)-Dpr (2b).- The (D) enantiomer was prepared using the procedure described above for 2a. Yield: 51%, mp. 196-196.5°; Kaiser test positive; $[\alpha]_D^{25}$ -18.74° (c, 1.2; MeOH).

Anal. Calcd. for $C_8H_{16}N_2O_4 \cdot 1/2\ H_2O$: C, 45.13; H, 8.05; N, 13.16

Found: C, 44.84; H, 7.46; N, 12.55

N^{α}-t-Boc-N^{β}-Fmoc-(L)-2,3-diaminopropionic Acid (3a).- N^{α}-t-Boc-(L)-2,3-diaminopropionic acid (6.3 g, 30.9 mmol) (2a) was dissolved in 10% Na_2CO_3 (62 mL) in a 250 mL round bottom flask equipped with a magnetic stirrer and a pressure equalizing addition funnel. The solution was cooled with stirring in an ice bath, and dioxane (35 mL) was added. 9-Fluorenylmethyl chloroformate (8.0 g, 31.1 mmol) was placed in the addition funnel, followed by 46 mL of dioxane. The funnel was agitated briefly to dissolve the chloroformate. The chloroformate solution was added dropwise over 30 minutes to the amino acid solution. The ice bath was removed after one hr, and the reaction was allowed to warm to room temperature, stirred for 2 hrs, and poured over ice/water (800 mL). The aqueous dioxane was extracted with diethyl ether (2 x 250 mL). The aqueous phase was cooled in an ice bath, acidified to pH 2-3 with 1N sulfuric acid, and extracted rapidly at 0° with ethyl acetate (2 x 300 mL). Ice was added to the separatory funnel for each extraction to maintain approximately 0°. The combined ethyl acetate extracts were washed with water (3 x 150 ml), dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was dried *in vacuo* overnight to obtain the N^{α}-t-Boc-N^{β}-Fmoc-(L)-Dpr-OH as white, foamy solid (3.7 g, 28%); Kaiser test negative; mp. sublimes at 75-100°; ^1H NMR (60 MHz, $CDCl_3$): δ 7.7-7.1 (m, 9H, Fmoc aromatics (8), -NH-), 6.0 (m, 1H, -NH-), 4.4-3.5 (m, 6H, α-CH-, β-CH_2-, -O-CH_2-CH-), 1.4 (s, 9H, $(CH_3)_3C$-); $[\alpha]_D^{25}$ = +0.20° (c, 1.0; $CHCl_3$).

Anal. Calcd. for $C_{23}H_{26}N_2O_6 \cdot 1.5\ H_2O$: C, 60.94; H, 6.01; N, 6.18

Found: C, 60.46; H, 5.76; N, 6.02

N^{α}-t-Boc-N^{β}-Fmoc-(D)-Dpr (3b).- The (D) enantiomer was prepared using the procedure described above for 3a. Yield: 38%; Kaiser test negative; mp. sublimes at 75-95°; $[\alpha]_D^{25} = -0.60°$ (c, 1.0; $CHCl_3$).

Anal. Calcd. for $C_{23}H_{26}N_2O_6 \cdot 1/2\ H_2O$: C, 63.46; H, 6.25; N, 6.44

Found: C, 63.01; H, 6.52; N, 6.57

N^{α}-t-Boc-(L)-2,4-diaminobutyric Acid (2c).- A mixture of 50 mL of aqueous DMF and [bis(trifluoroacetoxy)iodo]benzene (4.0 g, 9.2 mmol) was placed into a 100 mL round bottom flask equipped with a magnetic stirrer. The mixture was stirred at 20° until the solid was dissolved. N^{α}-t-Boc-(L)-glutamine (1.7 g, 6.9 mmol) was added in one portion as a solid. The reaction mixture was stirred for 15 minutes at 20°, and pyridine (0.9 mL, 870 mg, 11.4 mmoL) was added. The reaction mixture was stirred at 20° overnight (22 hours). The solvents were evaporated *in vacuo* at R.T. and the residue was dissolved in water (50 mL). The aqueous solution was extracted with diethyl ether (4 x 25 mL) and the aqueous layer was evaporated *in vacuo*. Attempts to recrystallize the N^{α}-t-Boc-(L)-Dbr-OH were unsuccessful. The N^{α}-t-Boc-(L)-Dbr was obtained as a clear, colorless oil (1.4g, 93%) which was used directly for conversion to the N^{γ}-Fmoc derivative (3c); Kaiser test positive; $[\alpha]_D^{25} = +7.34°$ (c, 1.1; MeOH); ^1H NMR (60 MHz, $CDCl_3$): δ 4.2-4.0 (m, 1H, α-CH-), 2.8-2.6 (m, 2H, γ-CH_2-), 1.5-1.4 (m, 2H, β-CH_2-), 1.4 (s, 9H, $(CH_3)_3C$-. Mass spectrum: $[M + H]^+$; m/e 219.

Anal. Calcd. for $C_9H_{18}N_2O_4$: C, 49.52; H, 8.33; N, 12.84

Found: C, 49.62; H, 7.54; N, 12.55

N^{α}-t-Boc-(D)-Dbr (2d).- The (D) enantiomer was prepared using the procedure described above for 2c. Yield: 34.8%; Kaiser ninhydrin test positive; mp. 197-198.0°; $[\alpha]_D^{25} = -11.3°$ (c, 0.8; MeOH); Mass spectrum: $[M + H]^+$; m/e 219.

Anal. Calcd. for $C_9H_{18}N_2O_4 \cdot H_2O$: C, 45.74; H, 8.53; N, 11.85

Found: C, 46.27; H, 7.76; N, 11.72

N^{α}-t-Boc-N^{γ}-Fmoc-(L)-2,4-diaminobutyric Acid (3c).- N^{α}-t-Boc-(L)-2,4-diaminobutyric acid (1.4 g, 5.7 mmol) (2c) was dissolved in 10% Na_2CO_3 (20 mL) in a 100 mL round bottom flask equipped with a magnetic stirrer. The solution was cooled in an ice bath with stirring, and dioxane (10 mL) was added. From a pressure equalizing addition funnel, 9-fluorenylmethyl chloroformate (1.85g, 7.2 mmol) in 20 mL of dioxane was added to the amino acid solution dropwise over 45 minutes. The reaction was stirred at 0° for one hour after the addition of the chloroformate was completed. The ice bath was removed and the reaction was allowed to warm to R.T., stirred overnight (ca. 14 hours), and poured into ice water (300 mL). The aqueous dioxane was extracted with diethyl ether (2 x 250 mL). The aqueous phase was cooled to 0°, acidified to pH 2-3 with 1N sulfuric acid, and extracted with ethyl acetate (2 x 150 mL). Ice was added for each extraction to keep the temperature at 0°. The combined ethyl acetate extracts were washed with brine (3 x 75 mL), dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The N^{α}-t-Boc-N^{γ}-Fmoc-(L)-Dbr was obtained as white crystals after the residue was dried under high vacuum overnight; 1.4 g (35%); Kaiser test negative; mp. sublimes at 85-95°; $[\alpha]_D^{25} = -3.63°$ (c, 1.0; $CHCl_3$). ^1H NMR (60 MHz, $CDCl_3$): δ 7.9-7.1 (m, 9H, Fmoc aromatics (8), -NH-), 4.3-4.2 (m, 3H, -O-CH_2-CH-), 4.1-3.9 (m, 1H, α-CH-), 3.2-2.9 (m, 2H, γ-CH_2-), 2.0-1.6 (m, 2H, β-CH_2-), 1.4 (s, 9H, Boc).

Anal. Calcd. for $C_{24}H_{28}N_2O_6 \cdot 1/4\ H_2O$: C, 64.78; H, 6.45; N, 6.29

Found: C, 65.19; H, 7.03; N, 6.44

Boc-N^{α}-t-Boc-N^{γ}-Fmoc-(D)-Dbr (3d).- The (D) enantiomer was prepared using the procedure described above for 3c. Yield: 38%; Kaiser test negative; mp. sublimes at 85-100°; $[\alpha]_D^{25} = +2.22°$ (c, 0.9; $CHCl_3$).

Anal. Calcd. for $C_{24}H_{28}N_2O_6 \cdot 1/4\ H_2O$: C, 64.78; H, 6.45; N, 6.29

Found: C, 64.87; H, 6.43; N, 6.16

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