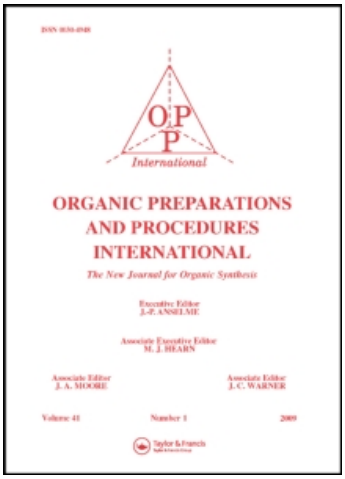

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SYNTHESIS OF *cis* AND *trans*-4-AMINOCYCLOHEXYL-D-ALANINE DERIVATIVES AND DETERMINATION OF THEIR STEREOCHEMISTRY

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SYNTHESIS OF *cis* AND *trans*-4-AMINOCYCLOHEXYL-D-ALANINE DERIVATIVES AND DETERMINATION OF THEIR STEREOCHEMISTRY

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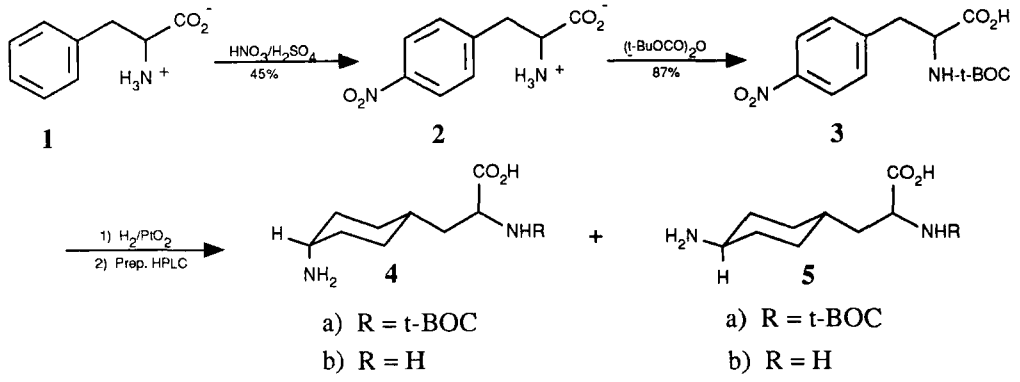
Over the past decade, several potent GnRH antagonists, exemplified by [N-Ac-D-2-Nal[1], D-*p*-ClPhe[2], D-3-Pal[3] , D-Arg[6], Trp[7], D-Ala[10]]-GnRH have been developed.[1,2] These effectively cause 100% inhibition of ovulation in rats at doses in the nanogram range.[2] Unfortunately, the strongly basic amino acids D-Arg[6] and Arg[8] have been implicated in the therapeutically unacceptable side-effects of histamine release and anaphylactoid reactions.[3] Recent structural modifications of the above antagonist by Folkers and coworkers[4] have led to a series of new antagonists, which cause negligible histamine release while maintaining almost equipotent antiovulatory activity (AOA) in the rat. These results were achieved in part by substituting weakly basic acylated D-Lys[6] derivatives, notably D-Niclys[6], for the strongly basic D-Arg[6] as in [N-Ac-D-2-Nal[1], D-*p*-ClPhe[2], D-3-Pal[3], Niclys[5], D-Niclys[6], I Lys[8], D-Ala[10]]-GnRH. In order to investigate further on the structural modification of GnRH antagonists, without untoward side-effects, some novel conformationally constrained derivatives of lysine, such as *cis*- and *trans*-isomers of 4-aminocyclohexyl-D-alanine, for substitution at position 6 of GnRH antagonists were needed. Their synthesis and stereochemistry are

presented.

D-Phenylalanine (**1**) was nitrated with a mixture of concentrated sulfuric and nitric acids to give the 4-nitrophenyl-D-alanine.[5] Treatment of the *p*-nitro derivative (**2**) with di-*tert*-butyldicarbonate in *tert*-butyl alcohol and water afforded the N^{α}-*t*-BOC protected amino acid (**3**) in good yield. Catalytic hydrogenation of the nitro derivative (**3**) in the presence of platinum oxide gave a mixture of *cis*- and *trans*-aminocyclohexyl derivatives (**4a**) and (**5a**).



The *cis* and *trans* isomeric mixture was separated into its pure components by preparative high performance liquid chromatography (HPLC). Other methods of purification such as flash chromatography and dry column chromatography were investigated, but preparative liquid chromatography proved to be the most efficient method in terms of both time and product recovery. We found that the *cis* and *trans* mixture could be separated by thin layer chromatography (t.l.c.) on silica using a solvent mixture of chloroform-methanol-water (60:30:5, visualization by ninhydrin) and this also proved to be the solvent of choice for HPLC. For preparative chromatography, the *cis* and *trans* mixtures were dissolved in the eluting solvent at a concentration of 2 g/10 ml and 20 ml were chromatographed at a time and several fractions (150-300 ml) were collected. Each fraction was checked by t.l.c. and like fractions were combined and concentrated *in vacuo*. The optical purity of these amino acid isomers was determined by a method described by Kinoshita *et al.*[7] The D-glucopyranosyliso-thiocyanate (GITC) derivatives were prepared and checked by analytical HPLC. The *cis*

isomer (**4a**) was found to be 100% optically pure whereas the *trans* isomer (**5a**) was found to have 97.5 % optical purity.

The stereochemistry of *cis* and *trans* compounds (**4a**) and (**5a**) was assigned based on the following considerations. It has been observed by us as well as others,[8] that in a given *cis/trans* mixture of 4-alkyl substituted cyclohexanecarboxylic acid esters, the *cis* isomer will be the least polar isomer under normal phase chromatography such as t.l.c., HPLC, g.l.c., etc.. In addition, the *trans* isomer, as the free acid, will be the higher melting of the two isomers. In the preparative HPLC separation of the isomers, the more mobile fractions were enriched in the *cis* isomer (**4a**), with the latter fractions being enriched in the *trans* isomer (**5a**). As expected, the pure *trans* compounds (**5a** and **5b**) had higher melting points (mp. 251-252° dec.) and (mp. 235-237°) respectively, whereas the *cis* compounds (**4a** and **4b**) melted at slightly lower temperature (mp. 234-237° dec.) and (mp. 227-228°) respectively.

The unequivocal assignment of the stereochemistry of compounds **4** and **5** was made by the use of ^{1}H nmr (300 M Hz) studies. The ^{1}H nmr spectral data of the *cis* and *trans* isomers of the free amino acids and their *t*-BOC derivatives was presented in the Table below.

TABLE. ^{1}H NMR Data Of Compounds 4 and 5[a]

Compound	C-4 proton (d)	Coupling Constant (J,Hz)	Half-band Width (Hz)
D - 5a	3.126 (t)	13.50	27.00
D - 4a	3.352 (br s)	-----	14.16
D - 5b	2.296 (t)	10.00	22.23
D - 4b	2.662 (br s)	-----	15.26

a) Solvent: Deuterated water, HOD resonance set to 4.80.

It is reasonable to assume that **4a** and **5a** exist in the normal chair conformation at ambient temperature. With the 4-amino group in an equatorial position for the *trans* isomer (**5a**), the C-4 proton lies in an axial position and its resonance will occur slightly upfield from the corresponding equatorial proton of the *cis* isomer (**4a**). Also, the axial C-4 proton in the *trans* isomer (**5a**) will exhibit distinct axial-axial coupling in the range of 8-14 Hz. This was indeed what was observed in the present case. For the *trans* isomer (**5a**), the C-4 axial proton was centered at 3.126 ppm as a broadened triplet (J = 13.5 Hz),

while the equatorial C-4 proton of the *cis* isomer was located slightly downfield at 3.352 ppm as a broadened singlet. Furthermore, it was established that axial-axial couplings give peaks with larger peak-width at half height.[9] For the *trans* isomer (**5a**) this value was approximately 27 Hz, while the *cis* isomer gave a value of 14 Hz. Similarly for the free amino acids the C-4 axial proton in the *trans* isomer (**5b**) was centered at 2.296 ppm as a broad triplet (J = 10 Hz), whereas the equatorial C-4 proton in the *cis* isomer was at 2.662. The peak width at half height for the *trans* isomer (**5b**) was larger (22 Hz) compared to the *cis* isomer (**4b**) (15 Hz).

EXPERIMENTAL SECTION

Most chemicals and solvents were analytical grade and used without further purification. D-Phenylalanine was purchased from Aldrich Chemical Company, Milwaukee, WI. Di-*tert*-butyldicarbonate was obtained from Fluka Chemical Corp., Happauge, NY. Platinum (IV) oxide was purchased from Englehard Corp., Newark, NJ. Acetobromo-α-D-glucose was obtained from Sigma Chemical Co., St. Louis, MO. Purity and identity of new compounds were established by normal spectral (nmr) and analytical (t.l.c., optical rotation) techniques. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and were uncorrected. Proton nmr spectra were obtained with Varian EM-390 (90 MHz) and GE-300 (300 MHz) spectrometers. Optical rotations were measured with a Rudolph Research Autopol II polarimeter in a 1 dm cell. Optical and isomeric purities of final compounds were established by derivatization with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) followed by analytical chromatography on Waters Associates equipment employing a reverse phase C_{18} column (Whatman Partisil ODS-3, 5μ 25cm x 4.6mm). Preparative high pressure liquid chromatography was accomplished on a Waters Prep500 liquid chromatograph using one silica PrepPak cartridge.

4-Nitrophenyl-D-alanine (**2**).- Sulfuric acid (conc., 120 ml) was added slowly to nitric acid (conc., 120 ml) with stirring. The reaction vessel was kept in an ice water bath to keep the mixture at ~20-25°. D-Phenylalanine (**1**, 100 g, 0.60 mol) was added as a solid portionwise over a period of 15 min. The mixture was kept in an ice bath to maintain the temperature below 55°. Upon completion of the addition, the mixture was heated to 55° for 45 min. The solution was then poured into rapidly stirred ice water (4 L) and conc. ammonium hydroxide (~390 ml) was added until the solution became turbid and 4-nitro-D-phenylalanine (**2**) precipitated out. The mixture was cooled overnight in the refrigerator, and the solid was collected. The solid was then transferred to a 2 liter Erlenmeyer flask and dissolved in boiling water (~1400 ml). The aqueous solution was cooled in the refrigerator (4°) and thereupon 4-nitrophenyl-D-alanine (**2**) crystallized from H_2O as an off white solid (56.88 g, 45% yield). mp. 252-253°, lit.[6] mp. 238-240°.

Anal. Calcd for $C_9H_{10}N_2O_4 \cdot 1H_2O$: C, 47.37; H. 5.30
Found: C, 47.07; H, 5.32

$[\alpha]_D^{25}$ = -7.9° (c 2.15 in 1N HCl, lit.[6] -8.9° (c 2.41 in 1N HCl). ^{1}H NMR: (90 MHz, CF_3CO_2H) δ 3.40-4.03(m, βH, 2H), 4.60-5.07(m, αH, 1H), 7.37-7.83(m, NH_3), 7.71 and 8.37 (2d, J = 8 Hz, aromatic H).

N^{α}-*t*-BOC-4-Nitrophenyl-D-alanine (3).- 4-Nitrophenyl-D-alanine (**2**, 118.3 g, 0.56 mol) was suspended in *tert*-butyl alcohol (473 ml) and water (178 ml) and the pH was adjusted to 8.5 with a few drops of 4 N sodium hydroxide. Di-*tert*-butyldicarbonate (147 g, 0.67 mol) was added portionwise to the suspension over a period of 2 h. Sodium hydroxide (4N) was added as needed to maintain pH 8.5 during addition of the carbonate and for the 2 h following. The reaction mixture was stirred overnight at room temperature, then diluted with water (500 ml). The mixture was extracted with hexane (3x) to remove excess di-*tert*-butyldicarbonate. The hexane extracts were washed with water (1x), and the aqueous layers were combined and acidified to pH 2 with conc. hydrochloric acid. This caused the N^{α}-*t*-BOC derivative (**3**) to precipitate as an oil. The material was extracted with ethyl acetate (3x) and the extracts were washed with water (1x) and brine (2x), then combined, dried (Na_2SO_4), filtered and concentrated *in vacuo*. For crystallization, the residue was dissolved in ethyl acetate (~400 ml), treated with charcoal, and filtered. Hexane (700 ml) was added, together with a few drops of ethyl acetate to prevent the amino acid derivative (**3**) from oiling out while cooling. Cooling of the ethyl acetate:hexane solution in the refrigerator effected crystallization. After collection of the solid (133.25 g, mp. 110-115°), a second crop of the N^{α}-*t*-BOC derivative (**3**) was obtained in a similar manner (18.27 g, mp. 108-110°). An analytical sample was prepared by recrystallization from ethyl acetate:hexanes. Combined yield = 86.8%).

Anal. Calcd for $C_{14}H_{18}N_2O_6$: C, 54.19; H, 5.85
Found: C, 54.00; H, 5.79

Crop 1:$[\alpha]_D^{23}$ = -8.30° (c 1.21 in methanol); Crop 2: $[\alpha]_D^{23}$ = -7.4° (c 1.08 in methanol). ^{1}H NMR: (90 MHz, $CDCl_3$): δ 1.39(s, *t*-Bu), 2.93-3.53(m, βCH_2), 4.33-4.87(m, αCH), 5.07-5.37(m, NH), 7.43 and 8.22 (2d, J = 8 Hz, aromatic H).

N^{α}-*t*-BOC-*cis* and *trans*-4-Aminocyclohexyl-D-alanine (**4a**) and (**5a**).- N^{α}-*t*-BOC-4-Nitrophenyl-D-alanine (**3**, 10 g, 0.032 mol) was dissolved in a mixture of absolute ethanol (120 ml), water (6 ml) and acetic acid (6 ml). Platinum oxide (0.6 g) was added and the mixture was hydrogenated at 40 psi for 5 h. The catalyst was removed from the mixture by filtration under nitrogen and the filtrate was concentrated *in vacuo* to give the 4-aminocyclohexyl amino acids **4a** and **5a**, (12.3 g) as a *cis* and *trans* mixture. Nmr of the crude material showed the absence of aromatic protons. Chromatographic separation (Si, $CHCl_3$:MeOH:H_2O, 60:30:5) of the two isomers was seen by t.l.c. analysis. The *cis* and *trans* isomers were separated by preparative liquid chromatography on a Waters Prep/500 Chromatograph using one silica PrepPak column and chloroform:methanol:water (60:30:5) as the eluting solvent. A total of 160 g of the *cis:trans* mixture was purified, and as much as 4 g of the mixture in 20 ml of eluting solvent could be processed at one time. Several fractions were collected with each run and all were checked by t.l.c.. Like fractions were combined and concentrated *in vacuo*. The GITC derivatives were prepared and compared by analytical HPLC (Whatman 5μ ODS-3 column, MeOH:KH_2PO_4 buffer, 0.05 M, pH 3). The more mobile fractions contained predominantly *cis* isomer (**4a**) contaminated with 11% *trans* isomer (**5a**). The slower running fraction contained the *trans* isomer (**5a**) 18.87 g, (25.5%) with ~2.5% *cis* isomer. This fraction was not purified any further. The *cis* isomer (**4a**, 25 g) was repurified by HPLC to give 17.02 g, (23%) of pure *cis* (**4a**). A total of 51.6 g of the *cis:trans* mixture (~50:50) was recovered.

trans isomer (**5a**), mp. 251-252° (dec.).

Anal. Calcd for $C_{14}H_{26}N_2O_4$: C, 54.19; H, 5.85
Found: C, 54.00; H, 5.79

$[\alpha]_D^{26}$ = +10.6° (c 2.46 in 25% acetic acid). ^1H NMR: (90 MHz, D_2O/CD_3OD) δ 1.00-2.17(m, 11H), 1.33(s, *t*-Bu, 9H), 3.10(m, 1H), 3.96 (m, 1H).

cis isomer (**4a**), mp. 234-237° (dec.).

Anal. Calcd for $C_{14}H_{26}N_2O_4 \cdot 1/2\ H_2O$: C, 56.93; H, 9.21
Found: C, 57.33; H, 8.94

$[\alpha]_D^{26}$ = +10.6° (c 2.07 in 25% acetic acid). ^1H NMR: (90 MHz, D_2O) δ 1.17-1.97(m, 11H), 1.44(s, *t*-Bu, 9H), 3.38(m, 1H).

It is interesting to note that the optical rotations of both isomers are identical.

cis and *trans*-4-Aminocyclohexyl-D-alanine (4b) and (5b). - N^{α}-*t*-BOC-*cis*-4-Aminocyclohexyl-D-alanine (**4a**) or N^{α}-*t*-BOC-*trans*-4-aminocyclohexyl-D-alanine (**5a**) (100 mg) was stirred for 1 hr at room temperature in trifluoroacetic acid (2 ml). The solvent was removed *in vacuo* and the residue was taken up in absolute ethanol and evaporated to remove the last traces of trifluoroacetic acid. The resulting material was dissolved in a minimum amount of ethanol and trituration with ether gave a solid. The solid was dissolved in aqueous hydrochloric acid (6 N) and the solution was loaded onto an ion exchange column. (Dowex 50 X 8-200 strongly acidic cation). The column was eluted with water until the eluent was free of chloride ions ($AgNO_3$ test). The column was then eluted with ammonium hydroxide (2·0N) and the ninhydrin positive fractions were combined and evaporated *in vacuo* to afford the amino acid.

trans isomer (**5b**, 60 mg recovered, 92%), mp. 235-237°.

Anal. Calcd for $C_9H_{18}N_2O_2 \cdot 2/3\ H_2O$: C, 54.52; H, 9.83
Found: C, 54.53; H, 9.71

$[\alpha]_D^{25}$ = -9.16° (c 1.20 in 25% acetic acid). ^1H NMR: (90 MHz, D_2O) δ 1.00-2.17 (m, 11H), 2.296(*t*, J = 10 Hz, 1-H), 2.95(br. t, 1H).

cis isomer (**4b**, 45 mg recovered, 75%), mp. 227-228°.

Anal. Calcd for $C_9H_{18}N_2O_2 \cdot 1/3\ H_2O$: C, 56.23; H, 9.79
Found: C, 56.33; H, 9.76

$[\alpha]_D^{25}$ = -9.25° (c 0.54 in 25% acetic acid). ^1H NMR: (90 MHz, D_2O) δ 1.00-2.17(m, 11-H), 2.662(br. s., 1-H), 2.96(br. t, 1H).

Once again it is interesting to note that the optical rotations for both isomers are nearly identical, but have become negative.

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