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# CHIRAL AMINOACID CONTAINING ACYCLIC LIGANDS - I. SYNTHESES AND CONFORMATIONS

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Abstract — Synthesis and spectral properties of optically active acyclic ligands, containing two (S)-phenylalanine residues (3a-f) are described. The synthesis is achieved by two different routes. Conformational studies in different solvents are performed by dilution and temperature-dependent experiments of $^{1}H$ and $^{13}C$ NMR spectroscopy.

Chiral recognition both in the solid state and in solution has long been pursued, but only recently has it been subjected to systematic study.[1]

The construction of chiral molecular receptors is greatly facilitated if suitable chiral synthons are available, which may be used as building blocks. A number of crown-type compounds have been synthesized which utilized either natural (carbohydrate,[2] tartaric acid,[3] D-ψ-ephedrine,[4] aminoacid[5]) or synthetic (cyclohexane-1,2-diol,[6] binaphthol,[7] etc.) chiral synthons incorporated in polyethyleneglycolic macrocycles.

In contrast, only a few open chain ligands which possess centers of chirality have been studied,[8] besides Vögtle's coronands[9] non-chiral, though able to wrap around a cation in a helical chiral fashion.

Now, we think that this type of compounds deserves further attention specially since, on account of their higher flexibility, which should allow conformational changes in the binding and releasing processes of metal ions, they should be able to act as better carriers through biological type membranes.

As models for biotic acyclic ionophores such as nigericin,[10] we report here the design and the synthesis of open chain ligands containing aminoacids as source of chirality, joined *via* amide bonds by oligoethyleneoxide chains. We report also the major conformational characteristics of the ligands in different solvents, as inferred by temperature and dilution experiments of $^{1}H$ and $^{13}C$ NMR spectroscopy.

*Design.* For this first study, (S)-phenylalanine was chosen as aminoacid in order to meet the lipophilic requirements of carrier-type structures. The two aminoacid residues are joined *via* amide bonds through oligoethyleneoxide bridges of different length, geometry and potential binding sites (3c-f). Also the $CH_2$ and S containing analogues (3a,b) were synthesized for comparison purposes (see Scheme 1).

A point of particular interest in these molecules is the presence of two terminal carboxyl groups, which could eventually enforce in apolar solvents pseudo-cyclic conformations *via* hydrogen bonding, or undergo deprotonation/protonation processes, very desirable in transport experiments.

*Syntheses.* The synthesis of ligands (3a-f) was performed by two routes. Both utilized bridging bisacid chlorides (a-f), obtained from the oligoethyleneoxide glycols by oxidation with nitric acid to the diacids and subsequent reaction with oxalyl chloride.[11]

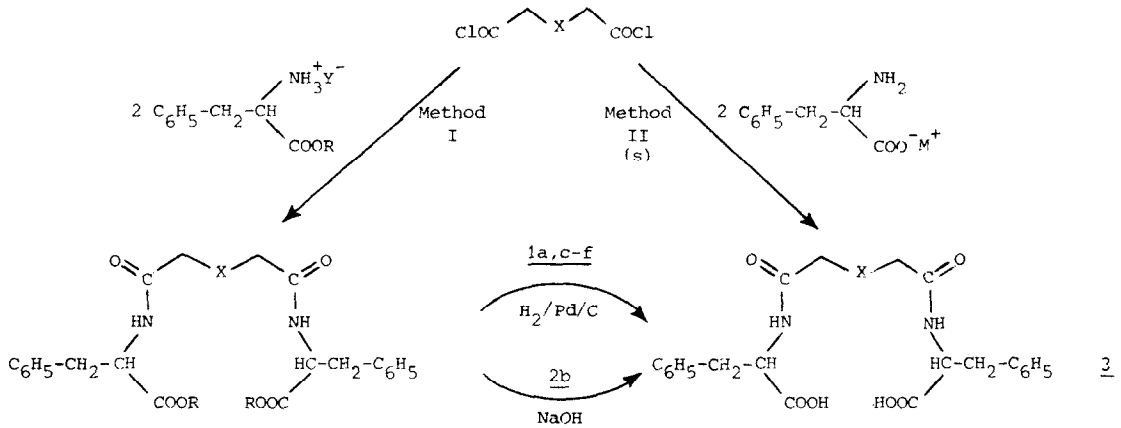
*I Method.* The first method involves the traditional protection of the aminoacid carboxyl groups either as benzyl or ethyl esters and subsequent condensation with the bisacid chloride in a molar ratio 2 : 1 in toluene, in the presence of triethylamine (see Scheme 1). Yields are strongly dependent on the purity of the bridging bisacid chloride and average 70%. Benzyl and ethyl esters were purified by preparative TLC and recrystallized. Their physical and spectral properties are reported in Table 1.

Catalytic hydrogenolysis (Pd/C in MeOH) of benzyl esters (1a, c-f) led to the diacid

(3a, c-f) in quantitative yields. Ligand (3b) was prepared from the ethyl ester hydrochloride (2b) by mild alkaline hydrolysis (0.1 N NaOH in EtOH at 0°).

able to compete with the amino group as nucleophile for the acid chloride.§

Although, at the present, the overall yields are slightly in favour of the former, the latter method is much more convenient, being much simpler, easier, extremely rapid, and thus more amenable for large scale preparations.




1 R = Bz $Y^- = C_6H_5SO_3^-$
2 R = Et $Y^- = Cl^-$

a X = $CH_2$
b X = S
c X = O
d X = O O
e X = O O O
f X = O O O O

$M^+ = Na^+, K^+, nBu_4N^+$
s = DMF, toluene, methylene chloride, acetonitrile, dioxane

Scheme 1

*Method II.* This method is a one-step synthesis starting from the aminoacid by titrating it stoichiometrically with a base, such as sodium, potassium or tetrabutylammonium hydroxide and by reacting it with the bisacid chloride in a molar ratio 2 : 1 (see Scheme 1). The organic base offers the advantage that its salts are more soluble in organic solvents. In order to overcome the heterogeneity of the reaction, several solvents were used: DMF in connection with the alkali salts and toluene, methylene chloride, acetonitrile and dioxane for the organic salts. Both the rate and the overall yields of the reaction are deeply affected by the cation and solvent effect: thus, with sodium and potassium salts in DMF, after 24 h, yields are in the order of 35-40%, while for the organic salts yields increase with the polarity of the solvent up to 60% in dioxane and acetonitrile, after 1 h. The yields reported concern isolated products after purification by HPLC (reverse phase) and, whenever possible, crystallization.

Apparently, the reaction reaches an optimum when the carboxylate is sufficiently protected against a nucleophilic attack from the $NH_2$ of the aminoacid and, however, is not

There is not substantial difference in the optical activity of the ligands prepared by the two routes, so that there is no evidence of carboxylate induced racemization under the present conditions.

Physical and analytical data of the acid ligands (3a-f) are reported in Table 2.

*Conformational studies.* Significant $^1H$ NMR data of the title compounds (3a-f) in $CDCl_3$ and in MeOD are tabulated in Table 3. Although all molecules are essentially symmetric, spectra are quite complex in $CDCl_3$: the $CH_2$ resonance of the $COCH_2O$ group always appears as a broad† singlet near 3.8-4.0 ppm. For compounds 3d-f a

§Actually, we cannot exclude the eventual formation of a mixed anhydride, by nucleophilic attack by the carboxylate at the acid chloride, and further reaction.

†The peak broadening observed only in $CDCl_3$ suggests a certain rigidity existing in the molecule skeleton, probably due to the formation of pseudo-cyclic structures.

Table 1. Physical and spectral data of benzyl (1) and ethyl (2) diesters

| Diesters | m.p. °C | $[\alpha]_D^{25}$ [a] | $M^+$ [b] m/e | IR [c] $\nu_{max}$ $cm^{-1}$ | NMR [d] δ ppm |
|---|---|---|---|---|---|
| 1a | 140-141 | -6.6 | 606 | 3350,1730,1660, 1520 | 2.05 (6H,m,$(CH_2)_3$); 2.8-3.1 (4H,m,$ArCH_2CH$); 4.7-5.0 (2H,m,CH); 5.15 (4H,s,$OCH_2Ar$); 7.0-7.5 (20H,m,Ar); |
| 1c | 78-79 | -3.0 | 608 | 3150,1730,1640 | 3.1-3.2 (4H,d,$CH_2Ar$); 3.90 (4H,s,$CH_2CO$); 4.7-5.5 (2H,m,CH); 5.15 (4H,s,$OCH_2Ar$); 6.8-7.3 (20H,m,Ar) |
| 1d | oil | -9.9 | 652 | 3300,1730,1670, 1530 [e] | 3.0-3.2 (4H,d,$CH_2Ar$); 3.45 (4H,s,$CH_2O$); 3.85 (4H,s,$CH_2CO$); 4.7-5.0 (2H,m,CH); 5.10 (4H,s,$OCH_2Ar$); 6.9-7.3 (20H,m,Ar) |
| 1e | oil | -0.75 | 696 | 3200,1740,1660, 1520 [e] | 3.10 (4H,d,$CH_2Ar$); 3.5-3.8 (8H,m,$CH_2O$); 3.98 (2H,d,$CH_2O$); 4.30 (2H,d,$CH_2CO$); 4.90 (2H,m,CH); 5.10 (4H,s,$OCH_2Ar$); 7.10 (10H,s,Ar); 7.20 (10H,s,Ar) |
| 1f | oil | -0.9 | 740 | 3400,3350,1740, 1680,1520 [e] | 3.0-3.3 (4H,d,$CH_2Ar$); 3.45 (12H,m,$CH_2O$); 3.88 (4H,s,$CH_2CO$); 4.6-5.0 (2H,m,CH); 5.10 (4H,s,$OCH_2Ar$); 7.15 (10H,s,Ar); 7.30 (10H,s,Ar) |
| 2b | 110 | +79.0 | 500 | 3300,1740,1670, 1530 | 1.27 (6H,t,$CH_3$); 2.6-3.4 (8H,m,$CH_2S$ + $CH_2Ar$); 4.20 (4H,q,$CH_2$); 4.85 (2H,m,CH); 7.0-7.7 (10H,m,Ar); 7.55 (2H,d,NH) |
| 2c | 122-123 | +63.5 | 484 | 3080,1750,1640, 1550 | 1.27 (6H,t,$CH_3$); 3.17 (4H,d,$CH_2Ar$); 3.97 (4H, s,$CH_2O$); 4.20 (4H,q, $CH_2$); 4.90 (2H,m,CH); 6.90 (2H,d,NH); 7.0-7.3 (10H,m, Ar) |
| 2d | wax | +18.9 | 528 | 3200,1740,1675, 1540 [e] | 1.23 (6H,t,$CH_3$); 3.16 (4H,m,$CH_2Ar$); 3.50 (4H,broad s,$CH_2O$); 3.92 (4H,s,$CH_2CO$); 4.18 (4H,q,$CH_2$); 4.92 (2H,m, CH); 7.22 (10H,m,Ar) |

[a] (c 1, EtOH). [b] Elemental analysis (C, H, N) within 0.3%. [c] (KBr) unless stated otherwise. [d] ($CDCl_3$). [e] (NaCl) film.

Table 2. Physical and spectral data of diacids

| Diacids[a] | m.p. °C | $[\alpha]_D^{25}$[b] | IR[c] $\nu_{max}$ $cm^{-1}$ | UV[d] $\lambda_{max}$ (log ε) nm |
|---|---|---|---|---|
| 3a | 70-73 | +14.2 | 3300,1720,1630, 1540 | 242 (2.23), 248 (2.32), 253 (2.43), 259 (2.51), 264 (2.39), 268 (2.22) |
| 3b | 80-82 | +46.2 | 3300,1725,1640, 1530 | 252 (2.77), 258 (2.72), 264 (2.62), 269 (2.52) |
| 3c | 140-142 | +26.8 | 3300,1740,1650, 1530 | 247 (2.24), 253 (2.36), 258 (2.44), 264 (2.34), 267 (2.15) |
| 3d | 52-55 | +37.1 | 3300,1740,1650, 1560 | 247 (2.34), 252 (2.45), 258 (2.52), 264 (2.43), 266 (2.27) |
| 3e | wax | +34.5 | 3300,1740,1660, 1520[e] | 247 (2.37), 252 (2.50), 258 (2.58), 264 (2.46), 269 (2.19) |
| 3f | wax | +32.8 | 3400,3200,1740, 1680,1630,1535[e] | 249 (2.41), 253 (2.50), 258 (2.57), 264 (2.45), 268 (2.27) |

[a]Satisfactory C, H, N analyses were obtained. The molecular ion (MS) is present only in traces. [b](c 1, EtOH). [c](KBr) unless stated otherwise. [d](EtOH). [e](NaCl).

Table 3. Significant $^1H$ NMR data in $CD_3OD$ and $CDCl_3$ of diacids (δ ppm)[a]

| Diacids | $OCH_2CH_2O$ | $XCH_2CO$ | $H_A$ | $H_B$ | $H_M$ | NH |
|---|---|---|---|---|---|---|
| 3a | | 2.10 | 2.85 | 3.20 | 4.71 | |
| | | | $J_{AM}$= 8.8 | $J_{BM}$= 5.1 | $J_{AB}$= 13.8 | |
| 3b | | 3.30 | 2.80 | 3.30 | 4.75 | |
| | | | $J_{AM}$= 8.0 | $J_{BM}$= 5.0 | $J_{AB}$= 13.8 | |
| 3c | | 3.81 (4.00) | 2.95 (3.20) | 3.35 (3.20) | 4.70 (4.80) | (7.50) |
| | | | $J_{AM}$= 8.5 | $J_{BM}$= 5.5 | $J_{AB}$= 13.5 | $J_{NH,CH}$ = 8.0 |
| 3d | 3.48 (3.31) | 3.86 (3.76) | 2.95(3.13) | 3.35 (3.13) | 4.70 (4.80) | (7.43) |
| | | | $J_{AM}$= 8.5 | $J_{BM}$= 5.5 | $J_{AB}$= 13.6 | $J_{NH,CH}$ = 8.0 |
| 3e | 3.54 (3.43) | 3.91 (3.86) | 2.95 (3.13) | 3.35 (3.13) | 4.70 (4.80) | (7.43) |
| | | | $J_{AM}$= 8.3 | $J_{BM}$= 5.5 | $J_{AB}$= 13.5 | $J_{NH,CH}$ = 8.0 |
| 3f | 3.55 (3.51) | 3.86 (3.95) | 3.00 (3.16) | 3.32 (3.16) | 4.70 (4.80) | (7.40) |
| | | | $J_{AM}$= 8.1 | $J_{BM}$= 5.0 | $J_{AB}$= 13.8 | $J_{NH,CH}$ = 7.8 |

[a]Coupling constants are in Hz. The COOH band is always broad and dependent on concentration. All values in brackets are referred to $CDCl_3$ solutions. The center of the unresolved multiplet due to the various $CH_2$'s is reported.

singlet near 3.5 ppm due to the $OCH_2CH_2O$ group is also present. The remaining protons of the phenylalanine residues consist of an ABMX system ($CH_AH_B$-$CH_M$-NH) with signals in the range 2.9-3.5 ppm for $CH_2$ and near 4.7 and 7.4 ppm for CH and NH respectively. No variation of the coupling constants is observed along the series 3a-f.

Spectra in $CD_3OD$ exhibit some modifications of the chemical shifts, in particular a downfield shift of the $COCH_2O$ and $OCH_2CH_2O$ signals, as expected for oxygens involved in H bonding with a protic solvent.[12] Indeed, these compounds may assume preferred conformations in different solvents, being able to form either intra- or intermolecular H bonding between the acidic protons (NH and COOH) and the acceptor atoms present in the molecule (ethereal and carbonyl oxygens) or with the solvent.

H bonding both for NH and COOH was studied by variable temperature experiments in $CDCl_3$ and DMSO-$d_6$. Whereas the resonance of the amide proton is essentially temperature-independent in $CDCl_3$, it undergoes a marked variation in DMSO-$d_6$ (see Fig. 1).

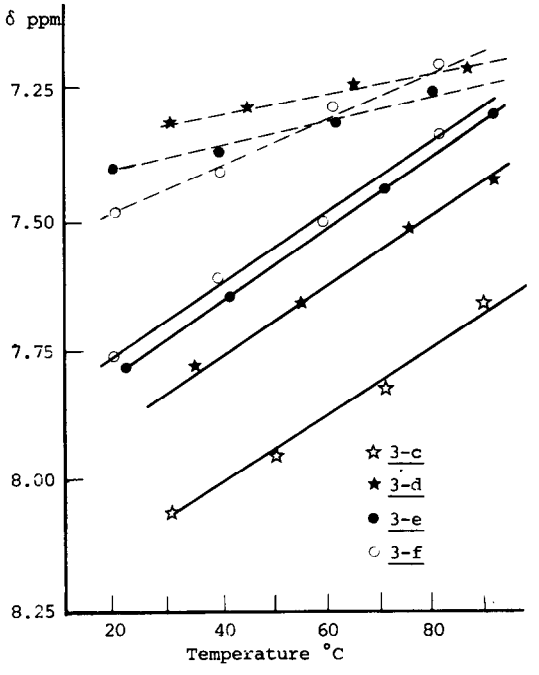
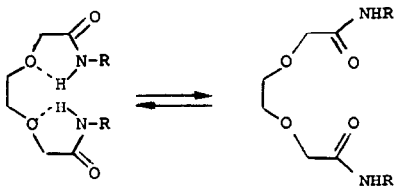



Fig. 1. Temperature dependence of the amide proton chemical shift of ligands in DMSO (-) and $CDCl_3$ (--)

On the ground of these experiments, it is possible to conclude that several conformations exist in solution and that in an apolar medium as chloroform the most probable is the one with the NH's directed inwards away from the solvent to form strong intramolecular H bonds with the ethereal oxygens (I), whereas in H bonding solvents (DMSO, MeOH), NH's are directed outwards and preferably interact with the solvent (II) (Scheme 2).




I II

Scheme 2

The contribution of conformation I in $CDCl_3$ is dependent also on the size of the oxa-ethylenic bridge, becoming less important for larger sized ligands. Infact, as shown in Table 4, the magnitude of the NH shift with the temperature increases with the size of the bridge, whereas

Table 4. Temperature dependence of the CONH chemical shift

| Ligand | $\Delta\delta/\Delta T$ (ppm/degree $10^3$)[a] DMSO | $CDCl_3$ |
|---|---|---|
| 3-c | 5.4 | not detected |
| 3-d | 5.4 | 1.0 |
| 3-e | 5.0 | 2.3 |
| 3-f | 5.0 | 3.3 |

[a]Measured in the range +30° to + 90°C.

it remains nearly constant in DMSO, approaching the value assignable to weakly bonded NH's.[13]

Further support to conformation I in chloroform is obtained by solvent titration experiments by $^1H$ and $^{13}C$ NMR. Accordingly, the NH resonance moves very slightly upfield on adding TFE up to 100% (v/v),[14] whereas the amide carbonyl signal is strongly affected and shows a large downfield shift (1.9 ppm).

The carboxyl groups in chloroform are, as

expected, extensively H-bonded. On varying the temperature (from 30° to 60°C) their signals undergo a large upfield variation (0.5 ppm), in agreement with intermolecular H-bond breaking.

Significant upfield shift of the carboxyl signals in $^1H$ NMR are also observed, whereas all other signals remain practically unchanged.

The IR spectra in $CHCl_3$ do not show any variation over a concentration range from $1 \cdot 10^{-1}$ M to $5 \cdot 10^{-3}$ M, so that it is possible to conclude that extensive intermolecular association exists even at high dilution.

## EXPERIMENTAL

M.ps were taken on a Büchi apparatus and are uncorrected. $[\alpha]_D^{25}$ were measured on a Rudolph Research Polarimeter III, at 589 nm by using a thermostated cell at 25°C. $^1H$ NMR spectra were recorded on a Varian EM 360 spectrometer at 60 MHz, using TMS as internal standard. $^{13}C$ NMR spectra were recorded on a Varian XL 100 spectrometer (Fourier transform). Mass spectra were taken on a Varian Mat CH-5 mass spectrometer. IR spectra were recorded on Perkin Elmer 298 and 283 B spectrophotometers. UV spectra were recorded on a Jasco Uvidec 505 spectrophotometer in EtOH (95%). TLC was carried out on Merck silica gel $PF_{254}$. HPLC was performed on an ALC-244 Waters chromatograph equipped with a UV detector ($\lambda_{max}$ 254 nm) by using a μ-Bondapack (30 x 0.8 cm) column and eluting with MeOH:$H_2O$ = 65:35 (2-2.5 ml/min).

*Bisacid chloride* (a-f). Polyethylene glycols were oxidized to the corresponding diacids with nitric acid, then (20 mmol) were reacted with oxalyl chloride (60 mmol) in dry benzene (40 ml) in the presence of few drops of pyridine.[11] The reaction mixture was stirred for 24 h at room temperature, then filtered and evaporated twice under vacuum in the presence of benzene and the bisacid chloride obtained was immediately used.

*Method I*

*Synthesis of N,N'-pentanoyl-(3a), 3-oxa-pentanoyl-* (3c) *and poly-oxa-alkanoyl-(S,S)-diphenylalanine* (3d-f). To (S)-phenylalanine benzyl ester benzene sulphonate (20 mmol) and bisacid chloride (a, c-f) (10 mmol) in dry toluene cooled at 0°C anhydrous $Et_3N$ was added dropwise. The mixture was stirred for 15 h at room temperature, evaporated under vacuum at 40°C, diluted with water, acidified with 1N HCl to pH = 2 and extracted with ethyl acetate. The solvent was then removed and the esters (1a, c-f) were recovered as solids and subsequently crystallized from hexane-acetate or purified by column chromatography on silica gel, using ethyl acetate as eluent. Upon hydrogenolysis of 1a,c-f in MeOH in the presence of Pd/C for 3h, diacids (3a, c-f) were obtained in quantitative yields.

*Synthesis of ethyl diesters* (2b-d). The reaction was performed as above described using (S)-phenylalanine ethyl ester hydrochloride.

*Synthesis of N,N'-3-thia-pentanoyl-(S,S)-diphenylalanine.* (3b) was obtained by alkaline hydrolysis (0.1 N NaOH in EtOH) of the corresponding ethyl ester (2b) at 0°C.

*Method II*

*Synthesis of* (3a-f). (S)-phenylalanine (20 mmol) was titrated with a base (NaOH, KOH or $n\text{-}Bu_4NOH$, 20 mmol) and dried under vacuum for at least 1H. The salt was then mixed with the solvent (DMF for the alkali salts and toluene, dioxane, acetonitrile and methylene chloride for the organic salts) (dry, 50 ml) under nitrogen. Bisacid chloride (10 mmol in 10 ml of solvent) was added dropwise over 1 h. The mixture was allowed to react (for 24 h the alkali salts and for 1h the organic salts) at room temperature, then it was washed four times with acidic water and extracted with chloroform. The organic phase was dried over $Na_2SO_4$ and purified by HPLC. The yields were as follows: from (S)-phe-$O^-Na^+$ in DMF 35%; from (S)-phe-$O^-K^+$ in DMF 40%; from (S)-phe-$O^-nBu_4N^+$ in toluene 25%, in methylene chloride 35%, in acetonitrile and dioxane 60%.

*NMR Experiments*

NMR measurements of the $^1H$ and $^{13}C$ chemical shifts of ligands (3a-f) were performed on 0.1 M solutions. Variable temperature experiments were carried out in the range 20-90°C, using standard precalibrated samples of ethylene glycol.

Dilution and titration solvent experiments were performed on 0.1 M solutions, by adding $CDCl_3$ or TFE up to 0.01 M and 100% respectively.

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