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^{1}H NMR study on the intermolecular interactions of macrocyclic and single α-amino acids

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HIGHLIGHTS

- The ^{1}H NMR spectra of macrocyclic amino acid derivatives from L-tyrosine were analyzed.
- The formation of aggregates in solution is stabilized by π-stacking.
- Macrocyclic and simple amino acids are capable of retaining alcohol molecules.
- The alcohol molecule acts as a proton donor and the amino group acts as an acceptor.

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ABSTRACT

Through analysis of ^{1}H NMR spectra, evidence was found for intermolecular interactions between macrocyclic amino acid derivatives from L-tyrosine and their importance in the formation of aggregates in solution. It was also shown that both macrocyclic and simple amino acids are capable of retaining alcohol molecules through hydrogen bonding, where the alcohol molecule acts as a proton donor and the amino group acts as an acceptor.

1. Introduction

Cyclophanes are macrocyclic compounds that have two or more aromatic units linked by aliphatic bridges (spacers) in their structure [1]. The properties of cyclophanes are determined by their geometric and electronic features; aromatic rings act as structural units that provide rigidity to the molecule and contribute electron density, which enables the formation of complexes through non-covalent interactions such as π–π, π-cation, ion-dipole, Van der Waals, and hydrogen bonding. These compounds are capable of capturing ionic and neutral species [2,3]. The azacyclophane group, which is a prominent member of the cyclophane family, has one or more nitrogen atoms as spacers. These macrocyclic compounds have the combined features of high electron density from the aromatic rings and the acid–base properties of nitrogen heterocycles [2]. The use of amino acid residues in the synthesis of azacyclophanes is of interest because it enables the introduction of chiral centers into the molecules. Azacyclophanes from amino acid derivatives are usually composed of two amino acid residues and are therefore known as macrocyclic amino acids [4].

The synthesis of a new family of macrocyclic amino acid derivatives from tyrosine, formed by two units of 1,3-benzoxazine joined by two ethylene bridges (benzoxazinephanes **1**), was recently reported [5]. These macrocycles are the product of a double Mannich-type condensation of two units of L-tyrosine alkyl ester with four units of formaldehyde [5,6].

Acid treatment of benzoxazinephane **1** for short periods leads to selective hydrolysis of methylene groups in oxazine to form macrocyclic amino esters **2**, which are formed by two units of L-tyrosine alkyl ester joined by two methylene bridges [7]. When benzoxazinephane **1** derivatives from L-tyrosine methyl ester and L-tyrosine ethyl ester are subjected to acid treatment for a long time, they yield a macrocyclic amino acid formed by two L-tyrosine units joined by two methylene bridges **3** [8].

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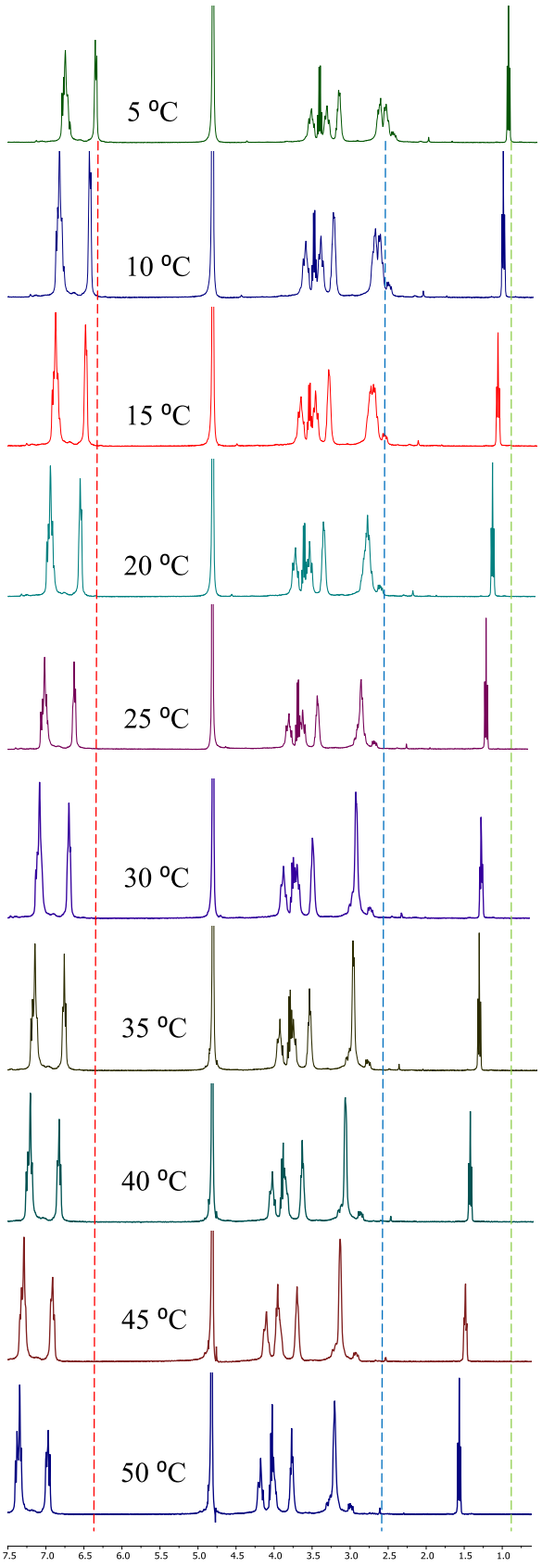

Fig. 1. Macrocyclic amino acid **4** ^{1}H NMR spectra at variable temperature.

Macrocyclic amino acids **2** and **3** have interesting structural features from a synthetic and supramolecular point of view: (a) they possess two stacked aromatic rings, (b) they exhibit a hydrophobic cavity, (c) the spacers contain hydrophilic substituents, and (d) they are molecules derived from an amino acid and are obtained under mild experimental conditions. Therefore, the products have a well-defined stereochemical meso structure. A previous study using theoretical calculations of compound **3** showed the existence of two intermolecular hydrogen bonds between the phenolic hydroxyls and amino groups by means of a six-membered cyclic structure. Spectroscopic studies showed the possible formation of aggregates in solution due to the amphiphilic nature of the molecule [8].

This paper studies the formation of aggregates of the macrocyclic amino acid **3** in solution by ^{1}H NMR analysis at different temperatures and describes new interactions between both macrocyclic and individual amino acids with alcohols.

2. Materials and methods

2.1. Materials

The reagents, L-amino acids (99.0%, Merck), and isopropyl alcohol (99.5%, J. T. Baker) were used as received. ^{1}H NMR spectra were acquired at 25 °C on a Bruker Avance 400 MHz spectrometer using TMS or the solvent residual signal as the reference for chemical shifts. Chemical shift values (δ) are reported in ppm, and coupling constants (J) are reported in Hz. Multiplicities are denoted as follows: s = singlet, d = doublet, t = triplet, dd = double doublet, and m = multiplet. The following commercial deuterated solvents were used: $CDCl_3$ (99.8%, Merck) and D_2O (99%, Aldrich). Mass spectra were recorded on a Shimadzu LC Time of Flight spectrometer using electrospray ionization (ESI). Azacyclophanes **1–3** were synthesized following the method reported [8].

2.2. Synthesis of compound *4*

Five milliliters of 10% NaOH was added to 0.500 g of macrocyclic amino acid **3**, and the resulting mixture was stirred until complete dissolution of compound **3**. Ethanol (25 mL) was added to the obtained solution. The precipitate formed was filtered and washed with ethanol and then dried in an oven at 120 °C for 24 h. The amount of product obtained was 0.53 g.

2.3. Synthesis of L-amino acid isopropyl esters

Concentrated sulfuric acid was added to a suspension of the respective L-amino acid (1 g, amino acid:sulfuric acid ratio 1:1.2) in isopropanol (10 mL). The resulting solution was refluxed

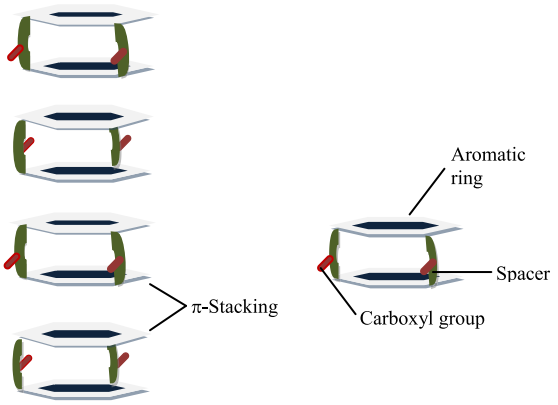

Fig. 2. π-Stacking of macrocyclic amino acids **3** and **4**.

for 12 h. After this time, the solution was cooled to 0 °C and neutralized with concentrated NH_4OH. The ammonium sulfate formed was filtered and washed with isopropanol (3 × 5 mL). The filtrate was concentrated under reduced pressure (50 mm Hg, 50 °C) to a third of its volume. The ester was extracted with chloroform (3 × 5 mL), the organic phase was dried with anhydrous sodium sulfate, and chloroform was removed under reduced pressure (50 mm Hg, room temperature).

L-Alanine isopropyl ester ($C_6H_{13}NO_2{\cdot}HOC_3H_7$) b.p.125–127 °C , yield 69%. ^{1}H NMR ($CDCl_3$) δ: 1.27 (d,6H, J = 6.8 Hz), 1.29 (d,6H, J = 6.4 Hz), 1.61 (d,3H, J = 7.2 Hz), 4.11 (q,1H, J = 7.2 Hz), 4.62 (heptet,1H, J = 6.4 Hz), 5.10 (heptet,1H, J = 6.4 Hz), ESI-MS: m/z 131.90 $[M + H]^+$.

L-phenylalanine isopropyl ester ($C_{12}H_{17}NO_2{\cdot}HOC_3H_7$) m.p. 81–84 °C, yield 53%. ^{1}H NMR ($CDCl_3$) δ: 1.08 (d,3H, J = 6.4 Hz), 1.16(d,3H, J = 6.0 Hz), 1.28 (d,6H, J = 6.0 Hz), 3.22 (dd,1H,J1,2 = 14 Hz, J1,3 = 8 Hz), 3.39 (dd,1H, J1,2 = 14 Hz, J1,3 = 5,2 Hz), 4.30 (dd,1H, J = 7.6 Hz and J = 5.6 Hz), 4.61 (heptet,1H, J = 6.4 Hz), 4.96 (heptet,1H, J = 6.4 Hz), 7.27 (br s,5H), ESI-MS: m/z 207.95 $[M + H]^+$.

Glycine isopropyl ester ($C_5H_{11}NO_2{\cdot}HOC_3H_7$) b.p. 118–120 °C, yield 71%. ^{1}H NMR ($CDCl_3$) δ: 1.26 (d,6H, J = 6 Hz), 1.27 (d,6H, J = 6 Hz), 3.87 (s,2H), 4.59 (heptet,1H, J = 6.4 Hz), 5.09 (heptet,1H, J = 6.4 Hz), ESI-MS: m/z 235.16 $[2 M + H]^+$.

***L-Valine isopropyl ester ($C_8H_{17}NO_2{\cdot}HOC_3H_7$) b.p. undetermined by decomposition, yield 43%. ^{1}H NMR ($CDCl_3$) δ: 1.09 (d,6H, J = 7.2 Hz), 1.30 (d,12H, J = 6.4 Hz), 2.37 (m,1H), 3.93 (d,1H, J = 4 Hz), 4.64 (heptet,1H, J = 6.4 Hz), 5.13 (heptet,1H, J = 6.4 Hz), ESI-MS: m/z 159.90 $[M + H]^+$.

L-isoleucine isopropyl ester ($C_9H_{18}NO_2{\cdot}HOC_3H_7$) b.p. 122–124 °C , yield 57%. ^{1}H NMR ($CDCl_3$) δ: 0.96 (t,3H, J = 7.2 Hz), 1.03 (d,3H, J = 6.8 Hz), 1.31 (d,12H, J = 6.4 Hz), 1.40 (m,1H), 1.53 (m,1H), 2.08 (m,1H), 4.00 (d,1H, J = 2.8 Hz), 4.63 (heptet,1H, J = 6.4 Hz), 5.12 (heptet,1H, J = 6 Hz), ESI-MS: m/z 173.96 $[M + H]^+$.

L-Serine isopropyl ester ($C_6H_{13}NO_3{\cdot}HOC_3H_7$) b.p. 134–135 °C, yield 35%. ^{1}H NMR ($CDCl_3$) δ: 1.29 (d,12H, J = 6.4 Hz), 4.01 (dd,1H,2 J = 12.4 Hz, 3 J = 5.2 Hz), 4.14 (d,1H, J = 12 Hz), 4.18 (br s,1H), 4.62 (heptet,1H, J = 6 Hz), 5,12 (heptet,1H, J = 6.4 Hz), ESI-MS: m/z 147.96 $[M + H]^+$.

3. Results and discussion

3.1. Formation of macrocyclic amino acid aggregates

To explore the possible formation of aggregates by macrocyclic amino acid **3** in aqueous solution, ^{1}H NMR spectra in D_2O at 25 °C and 50 °C were recorded. The spectra showed that all signals shifted approximately 0.4 ppm downfield with increasing temperature. No changes were observed in the number and multiplicity of the signals; these data show that the aggregates break down with increasing temperature, thereby exposing the different nuclei. However, this does not provide information about the type of interaction present.

To increase the amphiphilic character of compound **3** and gain information about the formation of aggregates, the synthesis of the corresponding sodium dicarboxylate **4** was performed. This product was obtained by dissolving compound **3** in NaOH solution (10%); the dicarboxylate was precipitated by addition of ethanol.

Azacyclophane dicarboxylate **4** was soluble in water and insoluble in organic solvents. The ESI-MS of **4** gave a molecular ion $[M]^+$ at 430.3 (m/z), consistent with a molecular formula of $C_{20}H_{20}N_2Na_2O_6$. The structure was determined by ^{1}H NMR; the spectroscopic data were also compared with the data reported for **2** and **3**. The ^{1}H NMR spectra presented the characteristic signals of 1,2,4-trisubstituted rings in the aromatic region. Diastereotopic hydrogens from both tyrosine units were observed as a multiplet at 2.75 ppm, which overlapped with two methylene groups in the molecule. The signals corresponding to the chiral methine were observed at 3.33 ppm. The signals of the N–CH_2–Ph groups were observed as overlapping doublets at 3.70 and 3.52 ppm because of the presence of two methylenes in the molecule.

In addition to the signals expected for compound **4**, two signals were observed in the aliphatic region, a triplet at 1.11 ppm and a quartet at 3.58 ppm. These two signals corresponded to ethanol molecules used in the synthesis and retained by interactions with azacyclophane, a behavior observed previously in benzoxazinephane-type molecules [6]. The integral ratio between the chiral carbon hydrogen and alcohol methyl was 1:0.7. Other synthetic

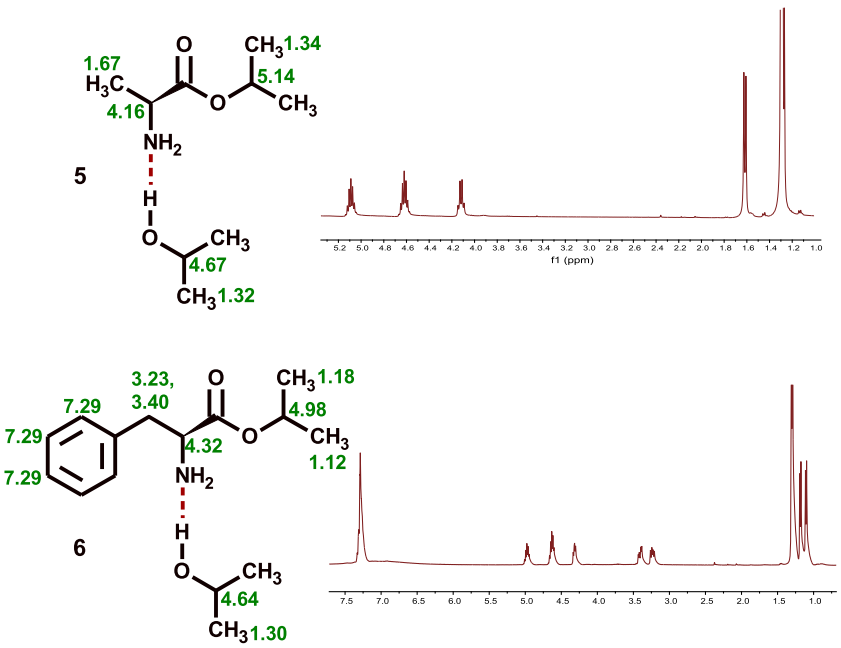

Fig. 3. ^{1}H NMR data for L-alanine isopropyl ester-isopropyl alcohol and L-phenylalanine isopropyl ester-isopropyl alcohol.

processes did not reproduce the cyclophane–ethanol ratio, thereby proving that this process only depended on adequate drying, which is usually performed at temperatures above 100 °C and for times longer than 12 h. Similar behavior was observed when methanol or isopropanol was used.

To confirm the formation of azacyclophane aggregates and to obtain information about the characteristics of the interaction with ethanol, ^{1}H NMR spectra in D_2O from 5 °C to 50 °C at intervals of 5 °C were recorded (Fig. 1).

Fig. 1 shows the same behavior observed for macrocycle **3**; all signals shifted downfield with increasing temperature. Differences close to 0.6 ppm were found in the spectra recorded between 0 °C and 50 °C. As in **3**, these shifts resulted from disaggregation with increasing temperature. Additionally, there was a change in the multiplicity of the signal corresponding to the diastereotopic hydrogens, seen as two multiplets at 2.55 and 2.63 ppm in the spectrum recorded at 0 °C that began to overlap with increasing temperature and form a singlet at 3.20 ppm at 50 °C. This phenomenon is evidence of increased conformational flexibility after the disaggregation process.

The change in chemical shift, which was of equal magnitude for all hydrogens present in the molecule, allows us to propose the formation of aggregates as symmetric arrays, most likely through aromatic ring stacking (Fig. 2). Dipole–dipole interactions between carboxyl groups in compound **3** and carboxylates in compound **4** should not be significant and are likely to break with the interaction with water molecules during the solution process. For this reason, no significant changes in chemical shift were observed between compounds **3** and **4**. Hydroxyl and amino groups were not involved in these interactions because they participate in the formation of intramolecular hydrogen bonds [8]. The structural features of compounds **3** and **4** allow us to propose that stacking is the most relevant chemical shift change induced by temperature (Fig. 2). Another possibility for Chemical shift changes is that the signal of HDO used as a reference depend on the temperature, this behavior is currently being investigated and will be reported in due course.

3.2. Interaction of macrocyclic and single amino acids with alcohols

An unexpected result was observed for the signals generated by the remaining ethanol. The spectrum at 0 °C showed a quartet at 3.42 ppm and a triplet at 0.95 ppm, signals that are at higher field than those usually generated by ethanol in water (3.65 and 1.17 ppm, respectively). The observed shifts showed a protective effect of the macrocyclic amino acid molecules on ethanol. This protective effect could be due to the inclusion of ethanol molecules in the macrocycle cavity and to alcohol retention in the aggregates. With increasing temperature, ethanol signals showed the same behavior as that of the macrocycle: chemical shifts at 4.03 and 1.57 ppm at 50 °C were observed. Deprotection of these hydrogens with increasing temperature led to the conclusion that retained alcohol molecules were not included within the cavity because inclusion of the alcohol inside the cavity would not allow large changes in chemical shift with increasing temperature. The change in chemical shift of ethanol's hydrogens downfield compared to the shift usually observed shows the existence of intermolecular interactions, most likely through a hydrogen-type bond between **4** and the alcohol. The increase in temperature induces a cyclophane disaggregation process and deprotection of all hydrogens present but does not induce the disruption of the cyclophane–ethanol interaction. For this reason, no signals with the expected shift for ethanol in water were observed.

The spectra of azacyclophane **4** at different temperatures showed the tendency of these compounds to form aggregates and the ability to retain alcohol molecules, which was previously observed in other azacyclophane derivatives from L-tyrosine.

The spectroscopic behavior of compound **4** and ethanol shows that the cyclophane cavity is not involved in the retention of ethanol molecules. If this is the case, then linear amino acid derivatives should also retain alcohol molecules. To confirm this hypothesis, some isopropyl esters soluble in aprotic solvents derived from amino acids were synthesized using drying and solvent-removal methods that do not require high temperatures, therefore preventing disruption of an amino–alcohol interaction.

Initially, the isopropyl ester was synthesized from L-alanine **5**. The ^{1}H NMR spectrum in $CDCl_3$ showed signals from L-alanine: chiral methine appeared as a quartet at 4.16 ppm and methyl as a doublet at 1.67 ppm. Signals from the isopropyl group were also observed: methine was a multiplet at 5.14 ppm and methyl was a doublet at 1.34 ppm. In addition to the signals expected for the L-alanine isopropyl ester molecule **5**, two more signals were evident: the first was a multiplet that appeared at 4.67 ppm. The multiplicity of this signal was in agreement with what is expected for the methine of an isopropyl group. Methyls of this isopropyl group appeared at 1.32 ppm, which overlaps with the signal for ester methyl groups. All signals appeared at 1.34 and 1.32 ppm for 12 hydrogen atoms. The number, integration, and multiplicity of these additional signals showed the presence of an additional isopropyl group. The downfield shift of these signals compared to what is expected for isopropanol in $CDCl_3$ can be explained by the formation of a hydrogen bond generated as a deprotection effect. This hydrogen bond must be formed between one isopropanol molecule serving as a proton donor and the amino group of the amino acid acting as an acceptor (Fig. 3). Similar behavior was observed for L-phenylalanine isopropyl ester **6**. Unlike L-alanine isopropyl ester **5**, L-phenylalanine isopropyl ester **6** gave two different signals for isopropyl group methyls (Fig. 3). The two signals in the ^{1}H NMR spectrum were due to the inability of the isopropyl group to rotate due to the U-shaped or "scorpion structure" previously observed in the L-tyrosine isopropyl ester [9].

The results obtained with esters **5** and **6** demonstrated a tendency of amino acid derivatives to interact with alcohols through

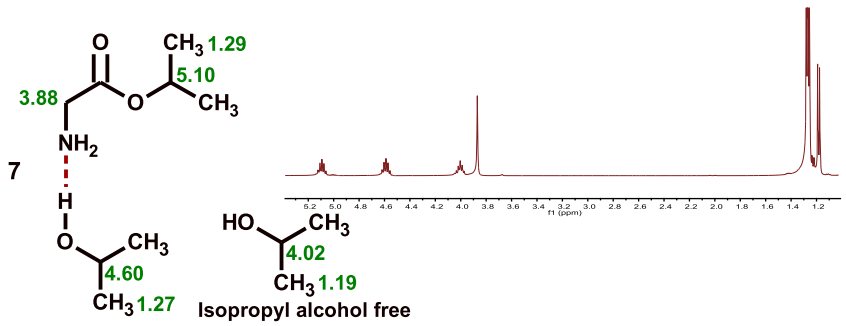

Fig. 4. ^{1}H NMR data of glycine isopropyl ester-isopropyl alcohol with an equivalent of isopropyl alcohol.

hydrogen bonds, as shown in Fig. 3. It was necessary to confirm this interaction and rule out the possibility of the presence of a free alcohol. To this end, glycine isopropyl ester **7** was synthesized using the same methodology employed to obtain **5** and **6** and the addition of one equivalent of isopropanol. The ^{1}H NMR spectrum showed the expected ester, interacting isopropanol molecules, and two additional signals for free isopropanol molecules (Fig. 4).

The experiment with glycine allowed us to demonstrate the interaction between alcohols and amino acid derivatives. Specifically, it allowed us to verify that the interaction occurs between one amino acid derivative molecule and one alcohol molecule. Esters derived from valine, isoleucine, serine, and threonine showed the same behavior, confirming the strong tendency of amino acid derivatives to interact with alcohols through hydrogen bonding.

4. Conclusions

Through analysis of ^{1}H NMR spectra, evidence was found for intermolecular interactions between macrocyclic amino acid derivatives from L-tyrosine and their importance in the formation of aggregates in solution. It was also shown that both macrocyclic and simple amino acids are capable of retaining alcohol molecules through hydrogen bonding, where the alcohol molecule acts as a proton donor and the amino group acts as an acceptor.

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