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Discrimination of Enantiomers of Dipeptide Derivatives with Two Chiral Centers by Tetraaza Macrocyclic Chiral Solvating Agents Using ^1H NMR Spectroscopy

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Abstract

^1H NMR spectroscopy is often used to discriminate enantiomers of chiral analytes and determine their enantiomeric excess (ee) by various chiral auxiliaries. In reported research, these studies were mainly focused on chiral discrimination of chiral analytes with only one chiral center. However, many chiral compounds possessing two or more chiral centers are often found in natural products, chiral drugs, products of asymmetric synthesis and biological systems. Therefore, it is necessary to investigate their chiral discrimination by effective chiral auxiliaries using ^1H NMR spectroscopy. In this paper, a new class of tetraaza macrocyclic chiral solvating agents (TAMCSAs) with two amide (CONH), two amino (NH) and two phenolic hydroxyl (PhOH) groups has been designed and synthesized for chiral discrimination towards dipeptide derivatives with two chiral centers. These dipeptide derivatives are important chiral species because some of them are used as clinical drugs and special dietary supplements for treatment of human diseases, such as *L*-alanyl-*L*-glutamine and aspartame. The results show that these TAMCSAs have excellent chiral discriminating properties and offer multiple detection possibilities pertaining to ^1H NMR signals of diagnostic split protons. The nonequivalent chemical shifts (up to 0.486 ppm) of various types of protons of these dipeptide derivatives were evaluated with the assistance of well-resolved ^1H NMR signals in most cases. In addition, enantiomeric excesses (ee) of the dipeptide derivatives with different optical compositions have been calculated based on integration of well-separated proton signals. At the same time, the possible chiral discriminating behaviors have been discussed by means of Job plots, ESI mass spectra and a proposed theoretical model of ($\pm$)-**G1** with TAMCSA **1c**. Additionally, the association constants of enantiomers of ($\pm$)-**G5** with TAMCSA **1a** were calculated by employing the nonlinear curve-fitting method.

Graphical Abstract

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[†]Electronic Supplementary Information (ESI) available: synthesis and characterization of TAMCSAs **1a-1c** and enantiomers of dipeptide derivatives with two chiral centers **G1-8**, experimental detail and original NMR spectra of chiral discrimination. See DOI: 10.1039/x0xx00000x

Enantiomers of dipeptide derivatives with two chiral centers were effectively discriminated by their split ^1H NMR signals of various types of protons in the presence of a new class of tetraaza macrocyclic chiral solvating agents (TAMCSAs) **1a-1c** with multiple amide, amino and phenolic hydroxyl groups by ^1H NMR spectroscopy.

Introduction

In the field of chiral molecular recognition, NMR spectroscopy has been extensively utilized as one of the most effective techniques towards discriminating enantiomers of various chiral analytes by virtue of chiral auxiliaries,¹ such as chiral derivatizing agents (CDAs),² chiral lanthanide shift reagents (CLSR),³ chiral shift reagents (CSRs)⁴ and chiral solvating agents (CSAs).⁵ Compared with chiral chromatography and X-ray crystallography, the more prominent advantages of NMR spectroscopy are its attributes as a fast, accurate and convenient method.⁶ In reported papers, NMR spectroscopy was mainly focused on enantiodifferentiation of chiral analytes with only one chiral center by chiral auxiliaries.⁷ However, studies on enantiomeric discrimination of chiral compounds with two or more chiral centers were rarely reported in the past.⁸ Nevertheless, these chiral compounds often exist in natural products, chiral drugs, products of asymmetric synthesis and biological systems.⁹ Therefore, it is necessary to intensively develop more effective chiral auxiliaries for their chiral discrimination by ^1H NMR spectroscopy.¹⁰ Herein, dipeptide derivatives were selected as one of the most representative species with two stereo configurations. Generally, dipeptides or their derivatives contain two identical or different chiral amino acid residues with one or two stereocenters. Thus, these dipeptides or their derivatives are likely composed of one, two, three and even four stereoisomers (*D,D*; *L,L*; *D,L*; *L,D*) under the same amino acid sequence, due to the reasons noted above. Of course, they are also achiral compounds. However, different stereoisomers have different biological and pharmacological activities, and even result in the opposite physiological effect.¹¹ Therefore, analysis and determination of the optical compositions of these dipeptides or their derivatives, especially used as clinical drugs and special dietary supplements (such as *L*-alanyl-*L*-glutamine¹² and aspartame¹³), are of important significance for the treatment of human diseases. Furthermore, this work addresses a challenging and fundamental problem, due to the very similar chemical structures and more complicated stereochemistries involved. In this paper, we report methodology towards achieving highly effective chiral discrimination of dipeptide derivatives with two chiral centers, in the presence of a new class of tetraaza macrocyclic chiral solvating agents (TAMCSAs) **1a-1c**, by employing ^1H NMR spectroscopy.

Results and discussion

Design and synthesis of TAMCSAs **1a-1c** for chiral discrimination

TAMCSAs **1a-1c** have been designed and synthesized from *D*-($-$)- α -phenylglycine and (1*S*, 2*S*)-(+)-1,2-diaminocyclohexane for chiral discrimination of dipeptide derivatives with two chiral centers by ^1H NMR spectroscopy.¹⁴ The detailed procedure of synthesis of TAMCSAs **1a-1c** is available in the supporting information. Their chemical structures are shown in Figure 1.

As shown in Figure 1, the most outstanding features of TAMCSAs **1a-1c** are the presence of two amide, two amino and two phenolic hydroxyl groups as potential intermolecular interaction sites. Other notable features include an overall C_2 -symmetry and a 12-membered cavity as part of the framework.

The newly generated chiral centers of TAMCSAs **1a-1c** in the reductive coupling reaction are assigned to possess *S*, *S* absolute configurations based on their X-ray crystallographic analysis. Their X-ray crystal structures are shown in Figure 2.

To investigate the chiral discriminating ability of TAMCSAs **1a-1c** towards dipeptide derivatives with two chiral centers by ^1H NMR spectroscopy, the following stereoisomers of dipeptide derivatives as guests were derived from the corresponding *D*- and *L*-amino acids (Figure 3).

Their chemical structures are characterized by ^1H NMR, ^{13}C NMR, IR and HRMS methods. However, some protons cannot be clearly assigned on this NMR evidence alone, due to close chemical shift values, almost identical coupling constants and similar peak shapes. Therefore, ^1H - ^1H COSY and ^1H - ^{13}C HSQC spectra were measured and the assignments of the disputed protons were properly determined on the basis of their correlated signals.

As a typical example, the assignments of some related protons of **G4-1** were clarified by ^1H - ^1H COSY and ^1H - ^{13}C HSQC. In the ^1H NMR spectrum of **G4-1**, signals of the protons of PhCH_2 and BnCH groups of phenylalanine residue of **G4-1** are easily determined according to their chemical shift values, coupling constants and split peak shapes. However, the protons with chemical shift values at 4.93 and 5.40 ppm cannot be properly assigned owing to their same coupling constants and peak shapes (*d*, $J = 6.96$ Hz). Nevertheless, based on the correlation of their ^1H - ^1H COSY and ^1H - ^{13}C HSQC features, they can be very clearly assigned to be PhCH (5.40 ppm) of the phenylglycine residue and NH proton (4.93 ppm) of the TsNH group of *N*-*Ts*-phenylalanine residue. All relevant ^1H - ^1H COSY and ^1H - ^{13}C HSQC spectra are available in the supporting information.

To evaluate chiral discriminating properties of TAMCSAs **1a-1c**, first the ^1H NMR spectrum of ($\pm$)-**G1** (**G1-1** (○) and **G1-2** (●)) was measured in the presence of TAMCSA **1b**. The result shows that the ^1H NMR signals of the corresponding protons for the PhCH_2 , PhCH_2O , TsNH , CONH and ArH groups of ($\pm$)-**G1** were split and their nonequivalent chemical shifts (δ) were found to be 0.013, 0.020, 0.012, 0.013, 0.151, 0.081 and 0.020 ppm (Figure 4).

Among them, δ of the *NH* proton for the Ts*NH* group was demonstrated to provide the maximum value (up to 0.151 ppm). Two protons belonging to PhCH₂ or PhCH₂O groups, with a subtle difference in stereochemistry, are also differentiated. The aforementioned results suggest that strong hydrogen bonding interactions may exist in diastereomeric complexes of TAMCSA **1a** with (±)-**G1**. Consequently, under the same conditions, ¹H NMR spectra of **G1-1** and **G1-2** were also measured, respectively. Assignment of the two enantiomers of (±)-**G1** was easily determined by comparing their chemical shift values, coupling constants and peak shapes. These preliminary results suggest that TAMCSA **1b** has excellent binding properties towards chiral discrimination of (±)-**G1**.

To obtain better baseline resolution for chiral discrimination, the following chiral discriminating conditions were optimized. Firstly, ¹H NMR spectra of (±)-**G1** with TAMCSA **1b** were performed in different deuterated solvents such as CDCl₃, CDCl₃ containing 10% C₆D₆, CD₃COCD₃, CD₃OD and DMSO-*d*₆, respectively. The changes of δ of the selected protons without any interfering signals are summarized in Table 1.

As shown in Table 1, upon enhancement of solvent polarity, the intermolecular interactions between enantiomers of (±)-**G1** and TAMCSA **1b** were weakened and even disappeared. The results suggest that CDCl₃ is more suitable for chiral discrimination by ¹H NMR spectroscopy.

Secondly, samples of (±)-**G1** with different concentrations were prepared in the presence of TAMCSA **1b** in CDCl₃, and their ¹H NMR spectra were measured. Overlaid ¹H NMR spectra featuring the *NH* proton of the CONH group are shown in Figure 5.

As the concentration gradually increase, the δ values of the *NH* proton of the CONH group exhibit a gradually increasing trend from 0.021 (2 mM) to 0.166 ppm (40 mM). Based on general requirements for concentration in ¹H NMR spectroscopy, and common solubilities of hosts and guests, a concentration of 10 mM was used for chiral discrimination. It should be clarified that both a double peak at 6.17 ppm and another double peak between 6.40 and 6.58 ppm at 40 mM derive from ¹H NMR signals of *NH* protons of the Ts*NH* group of a pair of enantiomers of (±)-**G1**. It should be clarified that both a double peak at 6.17 ppm and another double peak between 6.40 and 6.58 ppm at 40 mM derive from ¹H NMR signals of *NH* protons of the Ts*NH* group of a pair of enantiomers of (±)-**G1**.

Lastly, upon increasing the molar ratio of TAMCSA **1b**, the δ values of the *NH* protons of the CONH group of (±)-**G1** were showing a gradually increasing trend. The largest change of δ was found to be 0.038 ppm upon varying the molar ratios of TAMCSA **1b** over (±)-**G1** from 1:1.5 to 1:1 (Figure 6). Based on these results, the 1:1 molar ratio of (±)-**G1** with TAMCSAs **1b** was used for chiral discrimination.

Upon completion of the aforementioned optimization study, and in order to obtain further evidence to demonstrate the chiral discriminating ability of TAMCSAs **1a-1c**, ¹H NMR spectra were measured for all (±)-**G1-G8** samples prepared in the presence of TAMCSAs **1a-1c**, with the exception of (±)-**G1** that was prepared only in the presence of TAMCSAs **1b**. The ¹H NMR spectra show that signals of various types of key protons belonging to (±)-

G1-G8 exhibit splitting. Furthermore, TAMCSAs **1a-1c** provide multiple detection capabilities for chiral discrimination in most cases. Among them, δ value of the *NH* proton (Ts*NH* group) of ($\pm$)-**G8** is found to exhibit a maximum (0.486 ppm) in the presence of TAMCSA **1a**. In particular, ^1H NMR signals due to eight types of protons of ($\pm$)-**G5** can be differentiated in the presence of TAMCSAs **1a-1c**. ^1H NMR spectra of the corresponding protons of ($\pm$)-**G5** in the presence of TAMCSA **1a** are shown in different colors (Figure 7).

As shown in Figure 7, the δ values of protons belonging to CH_3CH , PhCH_3 , PhCH_2CH , CH_3CH , PhCH_2O , Ts*NH*, *CONH* and *ArH* groups of the ($\pm$)-**G5** enantiomers are found to be 0.024, 0.025, 0.054, 0.054, 0.006, 0.293, 0.136 and 0.025 ppm in sequence. In particular, two aromatic hydrogens (*ArH*, pink) of the benzene ring (Ts group) were discriminated as well. This indicates that π - π stacking interactions may operate in the diastereoisomeric complexes of ($\pm$)-**G5** with TAMCSA **1a**.

To further test the intermolecular non-covalent interactions, the association constants of complexes of **G5-1** and **G5-2** with TAMCSA **1a** were calculated by the nonlinear least-squares methods, respectively.¹⁵ The detailed results are summarized in Table 2.

Following measurements of the enantiomeric discrimination patterns noted above, ^1H NMR spectra of each enantiomer of **G1-G8** with TAMCSAs **1a-1c** were obtained under the same conditions. The assignments of enantiomers of ($\pm$)-**G1-G8** were determined by comparing their chemical shift values, coupling constants and peak shapes. As an example, the partial ^1H NMR spectra and nonequivalent chemical shifts of the *NH* proton belonging to the Ts*NH* group of ($\pm$)-**G1-G8** are demonstrated in Table 3, with the exception of ($\pm$)-**G3** in the presence of TAMCSA **1a** and ($\pm$)-**G6** in the presence of TAMCSAs **1b** and **1c**, since the ^1H NMR signal of the corresponding *NH* proton (Ts*NH* group) exhibit interference by signals from other protons or are not discriminated (Table 3). Nonequivalent chemical shifts of other discriminated protons of ($\pm$)-**G1-G8** are summarized in Table 4. Their partial ^1H NMR spectra are available in the supporting information.

As shown in Table 4, an unprecedented number of different types of protons from the same chiral analyte can be discriminated by TAMCSAs **1a-1c** using ^1H NMR spectroscopy. These results show that TAMCSAs **1a-1c** are highly effective chiral solvating agents with respect to the dipeptide derivatives ($\pm$)-**G1-G8**.

Now that TAMCSAs **1a-1c** have been established to demonstrate excellent chiral discriminating abilities, their practical applicability was tested in determining enantiomeric excess (*ee*) of dipeptide derivatives with different optical compositions. Samples containing **G1-1** with 85%, 65%, 45%, 25%, 0%, -25%, -45%, -65% and -85% *ee* were prepared in the presence of TAMCSA **1c** and then their ^1H NMR spectra were recorded. The enantiomeric excesses (*ee*) of all samples were accurately obtained based on the integration of ^1H NMR signals of the *NH* protons (Ts*NH* group) of enantiomers **G1-1** and **G1-2** (Figure 8).

Additionally, in order to further investigate chiral discriminating behavior, Job plots of ($\pm$)-**G1** were performed in the presence of TAMCSA **1c**. The Job plots of ($\pm$)-**G1** exhibited a

maximum value ($X^* \delta = 0.042$ ppm) at a molar fraction of $X = 0.5$, which suggests that a diastereoisomer complex with 1:1 stoichiometry was established between ($\pm$)-**G1** and TAMCSA **1c** (Figure 9).

To verify the results noted above, the stoichiometric interaction of ($\pm$)-**G1** with TAMCSA **1c** was evaluated by electrospray ionization (ESI) mass spectroscopy. The partial ESI mass spectrum of TAMCSA **1c** and ($\pm$)-**G1** exhibit formation of a 1:1 complex [**1c**+**G1**+H]⁺ (m/z 1289.3025). Partial and expanded ESI mass spectra derived from the same spectrum are shown in Figure 10. The full ESI mass spectrum of ($\pm$)-**G1** and TAMCSA **1c** is available in the supporting information.

Finally, to better understand the chiral discriminating behavior between ($\pm$)-**G1** and TAMCSA **1c**, theoretical calculations were carried out using an AM1 model by Gaussian 03 program.¹⁶ The proposed model suggests that a pair of diastereoisomer complexes of ($\pm$)-**G1** with TAMCSA **1c** are formed with 1:1 stoichiometry. The proposed model exhibits the distances of hydrogen bonds (S=O...HNCO and NHCO...HNSO₂) of **G1-1** and **G1-2** with TAMCSA **1c** as 1.91 and 2.02, 2.05 and 1.97 Å, respectively. The models separated from the following proposed binding model are available in the supporting information. The proposed binding model is shown in Figure 11.

Conclusions

In summary, we have developed a new class of tetraaza macrocyclic chiral solvating agents (TAMCSAs) **1a-1c** with two amide, two amino and two phenolic hydroxyl groups, featuring C₂-symmetry and a 12-membered cavity as a framework for chiral discrimination. All enantiomers of the dipeptide derivatives with two chiral centers ($\pm$)-**G1-G8** were successfully discriminated by the corresponding ¹H NMR signals of various types of protons in the presence of TAMCSAs **1a-1c**. Enantiomeric excesses (*ee*) of **G1** with different optical compositions were calculated based on the integration of ¹H NMR signals of *NH* protons of the Ts*NH* group of enantiomers **G1-1** and **G1-2** in the presence of TAMCSA **1c**. The results have proved that the unique structural features of TAMCSAs **1a-1c** are enormously helpful for promoting chiral discrimination. The chiral discriminating behavior has been evaluated by means of Job plots, ESI mass spectra and a theoretically proposed model of ($\pm$)-**G1** with TAMCSA **1c**. In addition, the association constants of enantiomers of ($\pm$)-**G5** with TAMCSA **1a** were calculated by the nonlinear curve-fitting method. In conclusion, TAMCSAs **1a-1c** have exhibited strong binding properties, better baseline resolution and multiple detection windows for chiral discrimination. These results demonstrate that TAMCSAs **1a-1c** are especially suitable for chiral discrimination of these dipeptide derivatives by ¹H NMR spectroscopy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TAMCSA **1a**: R = H
TAMCSA **1b**: R = Cl
TAMCSA **1c**: R = Br

Figure 1.
Structures of TAMCSAs **1a-1c**.

Figure 2.
X-ray crystal structures of TAMCSAs **1a**·2CH₃COCH₃ (a), **1b** (b) and **1c**·CDCl₃ (c).

G1-1-G8-2

G1-1: <i>N</i> -Ts- <i>D</i> -Phg- <i>D</i> -PheOBn	G5-1: <i>N</i> -Ts- <i>D</i> -Ala- <i>D</i> -PheOBn
G1-2: <i>N</i> -Ts- <i>L</i> -Phg- <i>L</i> -PheOBn	G5-2: <i>N</i> -Ts- <i>L</i> -Ala- <i>L</i> -PheOBn
G2-1: <i>N</i> -Ts- <i>D</i> -Phg- <i>L</i> -PheOBn	G6-1: <i>N</i> -Ts- <i>D</i> -Val- <i>D</i> -PheOBn
G2-2: <i>N</i> -Ts- <i>L</i> -Phg- <i>D</i> -PheOBn	G6-2: <i>N</i> -Ts- <i>L</i> -Val- <i>L</i> -PheOBn
G3-1: <i>N</i> -Ts- <i>D</i> -Phe- <i>D</i> -PhgOBn	G7-1: <i>N</i> -Ts- <i>D</i> -Glu- <i>D</i> -PheOBn
G3-2: <i>N</i> -Ts- <i>L</i> -Phe- <i>L</i> -PhgOBn	G7-2: <i>N</i> -Ts- <i>L</i> -Glu- <i>L</i> -PheOBn
G4-1: <i>N</i> -Ts- <i>D</i> -Phe- <i>L</i> -PhgOBn	G8-1: <i>N</i> -Ts- <i>D</i> -Phg- <i>D</i> -AlaOBn
G4-2: <i>N</i> -Ts- <i>L</i> -Phe- <i>D</i> -PhgOBn	G8-2: <i>N</i> -Ts- <i>L</i> -Phg- <i>L</i> -AlaOBn

Figure 3.
Structures of dipeptide derivatives with two chiral centers **G1-1** – **G8-2**.

Figure 4. Overlaid ^1H NMR spectra (400 MHz) of $(\pm)\text{-G1}$ in the presence of TAMCSA **1b** (TAMCSA **1b**/ $(\pm)\text{-G1}$ = 1:1, 10 mM) in CDCl_3 , free $(\pm)\text{-G1}$ and free TAMCSA **1b** from top to bottom.

Figure 5. Overlaid ^1H NMR spectra of NH proton of CONH group of $(\pm)\text{-G1}$ (**G1-1** (○), **G1-2** (●)) with different concentrations in the presence of TAMCSA **1b**. The mole ratio of TAMCSA **1b** and $(\pm)\text{-G1}$ is 1:1, unchangeable.

Figure 6.

Overlaid ¹H NMR spectra of *NH* proton of CONH group of (±)-**G1** (**G1-1** (○), **G1-2** (●)) with various molar ratios in the presence of TAMCSA **1b**. The concentration of (±)-**G1** is 10 mM in CDCl₃, unchangeable.

Figure 7. Overlaid ^1H NMR spectra (400 MHz) of $(\pm)\text{-G5}$ in the presence of TAMCSA **1a** (TAMCSA **1a**/ $(\pm)\text{-G5}$ = 1:1, 10 mM), free $(\pm)\text{-G5}$ and free TAMCSA **1a** from top to bottom.

Figure 8.

Determination of enantiomeric excesses of **G1**, $ee\% = [(\text{G1-1}) - (\text{G1-2})] \%$, **G1-1** and **G1-2** stand for two enantiomers of $(\pm)$ -**G1**. (a) The overlaid ^1H NMR spectra of NH proton of TsNH group of **G1-1** (left) and **G1-2** (right) in the presence of equal amounts TAMCSA **1c** (5 mM) in CDCl_3 at room temperature. (b) Linear correlation between the theoretical (Y) and observed (X) $ee\%$ values.

Figure 9.

Job plots for complex of (±)-**G1** and TAMCSA **1c**. δ stands for chemical shift change of *NH* proton of Ts*NH* group of **G1-1** and **G1-2** in the presence of TAMCSA **1c**. X stands for the molar fraction of (±)-**G1**, ($X = [(\pm)\text{-G1}] / [(\pm)\text{-G1}] + [1c]$).

Figure 10.

Partial and expanded ESI mass spectra of (a) $[G1+Na]^+$: calcd for $C_{31}H_{30}N_2NaO_5S$ $[M+Na]^+$ 565.1773, found 565.1765; (b) $[1c+H]^+$: calcd for $C_{36}H_{37}Br_2N_4O_4$ $[M+H]^+$ 747.1176, found 747.1177; (c) $[1c+G1+H]^+$: calcd for $C_{67}H_{67}Br_2N_6O_9S$ $[M+H]^+$ 1289.3051, found 1289.3025.

Figure 11.
Proposed model for the intermolecular interactions of **G1-1** and **G1-2** with TAMCSA **1c**.

Table 1

Nonequivalent chemical shifts (δ , ppm) of protons of PhCH_2O , TsNH , CONH and ArH of ($\pm$)-**G1** in the presence of TAMCSA **1b** in different deuterated solvents by ^1H NMR spectroscopy (400 MHz) at room temperature.^a

Solvent	PhCH_2O	TsNH	CONH	ArH
CDCl_3	0.012/0.013	0.151	0.081	0.020
$\text{CDCl}_3/\text{C}_6\text{D}_6$ (10%)	0.003/0.004	0.060	0.035	0.007
$\text{CDCl}_3/\text{CD}_3\text{COCD}_3$ (10%)	0.000/0.000	0.038	0.018	0.000
$\text{CDCl}_3/\text{CD}_3\text{OD}$ (10%)	0.000/0.000	-	-	0.000
$\text{CDCl}_3/\text{DMSO-d}_6$ (10%)	0.000/0.000	-	-	0.000

^a Concentrations of ($\pm$)-**G1** and TAMCSA **1b** are 10 mM (1:1).

Table 2Association constants of **G5-1** and **G5-2** with TAMCSA **1a**.

TAMCSA	Guest	K_a^a (M ⁻¹)	– G (KJ/mol)
1a	G5-1	$(1.05 \pm 0.45) \times 10^3$	17.04 ± 1.14
1a	G5-2	$(1.06 \pm 0.45) \times 10^3$	17.07 ± 1.14

^a K_a values were calculated by the nonlinear curve-fitting method.

Table 3

Nonequivalent chemical shifts (δ , ppm) and partial ^1H NMR spectra of *NH* proton of Ts*NH* group of ($\pm$)-**G1-G8** in the presence of TAMCSAs **1a-1c** (except ($\pm$)-**G3** with TAMCSA **1a** and ($\pm$)-**G6** with TAMCSAs **1b** and **1c**) by ^1H NMR spectroscopy at room temperature, respectively (400 MHz)^a.

TAMCSA / Guest $\Delta\Delta\delta$	Spectra ^{b,d}	Spectra ^{c,d}	TAMCSA / Guest $\Delta\Delta\delta$	Spectra ^{b,d}	Spectra ^{c,d}
1a / ($\pm$)- G1 /0.154			1a / ($\pm$)- G5 /0.293		
1b / ($\pm$)- G1 /0.151			1b / ($\pm$)- G5 /0.133		
1c / ($\pm$)- G1 /0.160			1c / ($\pm$)- G5 /0.132		
1a / ($\pm$)- G2 /0.142			1a / ($\pm$)- G6 /0.048		
1b / ($\pm$)- G2 /0.143			1a / ($\pm$)- G7 /0.141		
1c / ($\pm$)- G2 /0.140			1b / ($\pm$)- G7 /0.104		
1b / ($\pm$)- G3 /0.060			1c / ($\pm$)- G7 /0.105		
1c / ($\pm$)- G3 /0.059			1a / ($\pm$)- G8 /0.486		
1a / ($\pm$)- G4 /0.199			1b / ($\pm$)- G8 /0.425		
1b / ($\pm$)- G4 /0.243			1c / ($\pm$)- G8 /0.432		
1c / ($\pm$)- G4 /0.242					

Table 4

Nonequivalent chemical shifts (δ , ppm) of corresponding protons of ($\pm$)-**G1-G8** (except *NH* protons of Ts*NH* group) in the presence of TAMCSAs **1a-1c** obtained by ^1H NMR spectroscopy at room temperature, (400 MHz)^a.

TAMCSA/Guest	Proton	δ (ppm)	TAMCSA/Guest	Proton	δ (ppm)
1a/(\pm)-G1	PhCH ₂	0.021	1b/(\pm)-G5	ArH	0.025
		0.032		CH ₃	0.014
	PhCH ₂ O	0.011		PhCH ₃	0.015
		0.012		PhCH ₂	0.029
	CONH	0.066		CH ₃ CH	0.048
	ArH	0.021		PhCH ₂ O	0.007
1b/(\pm)-G1	PhCH ₂	0.013	1c/(\pm)-G5		0.009
		0.020		CONH	0.075
	PhCH ₂ O	0.012		ArH	0.019
		0.013		CH ₃	0.014
	CONH	0.081		PhCH ₃	0.014
	ArH	0.020		PhCH ₂	0.029
1c/(\pm)-G1	PhCH ₂	0.014	1a/(\pm)-G6	CH ₃ CH	0.049
		0.022		PhCH ₂ O	0.008
	PhCH ₂ O	0.013			0.010
		0.013		CONH	0.072
	CONH	0.089		ArH	0.017
	ArH	0.021		(CH ₃) ₂ CH	0.011
1a/(\pm)-G2	PhCH ₂	0.013	1b/(\pm)-G6		0.009
		0.013		PhCH ₂	0.025
	PhCH ₂ O	0.004			0.014
		0.004		(CH ₃) ₂ CHCH	0.057
	CONH	0.034		PhCH ₂ O	0.010
	ArH	0.008		CONH	0.089
1b/(\pm)-G2	PhCH ₂	0.008	1c/(\pm)-G2	ArH	0.021
		0.007		(CH ₃) ₂ CH	0.007
	CONH	0.035			0.005
	ArH	0.007		PhCH ₂	0.015
1c/(\pm)-G2	PhCH ₂	0.007	1a/(\pm)-G3	(CH ₃) ₂ CHCH	0.048
		0.008		PhCH ₂ O	0.010
	CONH	0.073		CONH	0.081
	ArH	0.006		ArH	0.017
1a/(\pm)-G3	CH ₃	0.009	1c/(\pm)-G6	(CH ₃) ₂ CH	0.007
	PhCH ₂	0.009			0.005

TAMCSA/Guest	Proton	δ (ppm)	TAMCSA/Guest	Proton	δ (ppm)
1b/(±)-G3		0.014	1a/(±)-G7	PhCH ₂	0.014
	PhCH ₂ O	0.004		(CH ₃) ₂ CHCH	0.043
	PhCH	0.008		PhCH ₂ O	0.008
	ArH	0.006		CONH	0.074
	CH ₃	0.011		ArH	0.015
	PhCH ₂	0.015		PhCH ₃	0.022
		0.017		PhCH ₂	0.017
	PhCH ₂ O	0.007			0.035
	PhCH	0.003		PhCH ₂ O	0.008
	ArH	0.006			0.008
1c/(±)-G3	CH ₃	0.011	1b/(±)-G7	ArH	0.005
	PhCH ₂	0.015		PhCH ₃	0.012
		0.016		PhCH ₂ O	0.007
	PhCH ₂ O	0.006		PhCH ₃	0.012
	PhCH	0.004		PhCH ₂ O	0.007
1a/(±)-G4	ArH	0.007	1a/(±)-G8	CH ₃	0.100
	PhCH ₂ O	0.052		PhCH	0.060
		0.071		PhCH ₂ O	0.011
	PhCH	0.047		CONH	0.250
	PhCH ₂ O	0.005		ArH	0.055
1b/(±)-G4		0.029	1b/(±)-G8	CH ₃	0.077
	PhCH	0.031		PhCH	0.058
	PhCH ₂ O	0.006		PhCH ₂ O	0.009
		0.029		CONH	0.247
	PhCH	0.031		ArH	0.052
1a/(±)-G5	CH ₃	0.024	1c/(±)-G8	CH ₃	0.078
	PhCH ₃	0.025		PhCH	0.056
	PhCH ₂	0.054		PhCH ₂ O	0.009
	CH ₃ CH	0.054		CONH	0.253
	PhCH ₂ O	0.006		ArH	0.052
	CONH	0.136			

^a Nonequivalent chemical shifts (δ , ppm) of (±)-**G1-G8** (10×10^{-3} M) in the presence of TAMCSAs **1a-1c**, respectively, H: G = 1:1.