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(*S*)-*N*-Benzyl-3(6)-methylpiperazine-2,5-diones as chiral solvating agents for *N*-acylamino acid esters

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ABSTRACT

Three closely related diketopiperazines, (*S*)-1-benzyl-6-methylpiperazine-2,5-dione (*S*)-**1a**, (*S*)-1-benzyl-3-methylpiperazine-2,5-dione (*S*)-**1b**, and (*S*)-6-methyl-1-(pentafluorobenzyl)piperazine-2,5-dione (*S*)-**1c**, were prepared and screened as potential chiral solvating agents in NMR spectroscopy. The ^{1}H NMR spectra of 13 racemic α-amino acid derivatives (*RS*)-**5a**–**5m** were taken in $CDCl_3$ in the presence of equimolar amounts of enantiopure diketopiperazines (*S*)-**1a**–**1c** at 29 °C, 0 °C, and −20 °C. Compound (*S*)-**1a** exhibited the strongest chiral solvating properties for racemic α-amino acid derivatives (*RS*)-**5a**–**5m** and was recognized as a suitable CSA for the determination of their enantiomer composition. Weaker interactions of diketopiperazines (*S*)-**1b** and (*S*)-**1c** with compounds (*RS*)-**5a**–**5m** indicate that the position and properties of substituents play an important role in the binding affinity of diketopiperazines **1** towards amino acid derivatives **5**. Association constants for binding of (*S*)-**1a** to each enantiomer of the leucine derivative (*RS*)-**5d** in $CDCl_3$ at −20 °C were also determined by NMR titration.

1. Introduction

NMR spectroscopy represents one of the most common ways for determining the enantiomeric excess and absolute configuration of chiral compounds. In addition to various fluorinated α-hydroxymethylidenecamphor-based chiral lanthanide shift reagents, which are nowadays commonly used reagents for determination of ee and absolute configuration,[1–3] chiral solvation agents (CSA) that are not covalently attached to the enantiomers have also been successfully employed for the determination of enantiomeric purity and absolute configuration. Examples of CSA are carboxylic acids and amides,[4] 1,1′-(anthracene-9,10-diyl)bis(2,2,2-trifluoroethanol),[5] cyclodextrins,[6] crown ethers,[7] calixarenes,[8] binaphthyls,[9] α-amino acid derivatives,[10] porphyrins,[11] and diketopiperazines.[12–14]

Within this context, we have recently reported chiral solvating properties of (*S*)-1-benzyl-6-methylpiperazine-2,5-dione (*S*)-**1a**,[12] which was used as a CSA for the determination of the enantiomeric purity of *tert*-butyl pyroglutamate[12] and tryprostatin B analogues.[13,14] In an extension, (*S*)-1-benzyl-6-methylpiperazine-2,5-dione (*S*)-**1a**, its regioisomer (*S*)-1-benzyl-3-methylpiperazine-2,5-dione (*S*)-**1b**,[15] and its analogue (*S*)-6-methyl-1-(pentafluorobenzyl)piperazine-2,5-dione (*S*)-**1c** were prepared and tested as CSA for a set of 13 racemic N-acylated α-amino acid esters **5**. Herein, we report the results of this study, which showed a significant effect of the N-substituents on chiral solvating properties of closely related diketopiperazines (*S*)-**1a**–**c**.

2. Results and discussion

First, we decided to take a closer look at the interactions of regioisomeric diketopiperazines (*S*)-**1a** and (*S*)-**1b** with a series of 12 N-benzoylated α-amino acid methyl esters (*RS*)-**5a**–**5l** in $CDCl_3$ solution. Both (*S*)-1-Benzyl-6-methylpiperazine-2,5-dione (*S*)-**1a**[16–19] and (*S*)-1-benzyl-3-methylpiperazine-2,5-dione (*S*)-**1b**[15] were prepared from (*S*)-alanine methyl ester hydrochloride (*S*)-**2** following literature procedures. Similarly, the model amino acid derivatives (*RS*)-**5a**–**5l** were prepared in two steps according to known general protocols[20,21] from the corresponding racemic α-amino acids: D,L-alanine (*RS*)-**6a**, D,L-valine (*RS*)-**6b**, D,L-norvaline (*RS*)-**6c**, D,L-leucine (*RS*)-**6d**, D,L-β-phenylalanine (*RS*)-**6e**, D,L-α-phenylglycine (*RS*)-**6f**, D,L-methionine (*RS*)-**6g**, D,L-aspartic acid (*RS*)-**6h**, D,L-glutamic acid (*RS*)-**6i**, D,L-serine (*RS*)-**6j**, D,L-histidine (*RS*)-**6k**, and D,L-tryptophan (*RS*)-**6l**. Thus, compounds **6a**–**l** were first esterified with thionyl chloride in methanol[20] and the crude esters **7a**–**l** were subsequently benzoylated (Scheme 1).[21]

Once the racemic amino acid derivatives (*RS*)-**5a**–**l** were prepared, the ^{1}H NMR spectra of equimolar mixtures of (*S*)-**1a** and (*RS*)-**5a**–**l** (**1**:**5** = 1:1, *c* = 0.073 M) were taken in $CDCl_3$ at three different temperatures: 29 °C, 0 °C, and −20 °C. In these spectra, only the signals (doublets) for the NH protons of compounds (*RS*)-**5** were split, while all CH protons and the NH of (*S*)-**1a** appeared as single sets of signals. This observation was in agreement with

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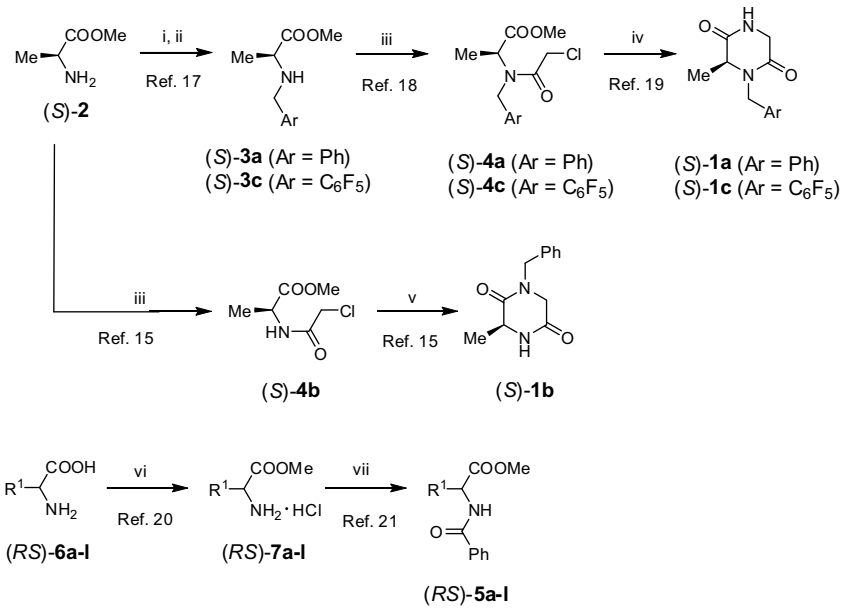


Scheme 1. Reagents and conditions: (i) ArCHO (Ar = phenyl or pentafluorophenyl), $HC(OMe)_3$, MeOH, rt; (ii) $NaCNBH_3$, rt; (iii) $ClCH_2COCl$, CH_2Cl_2, Et_3N, 0 °C; (iv) NH_3, MeOH, rt; (v) $BnNH_2$ (excess), MeOH, rt; (vi) $SOCl_2$, MeOH, 0–20 °C; (vii) PhCOCl, H_2O, CH_2Cl_2, Et_3N, rt.

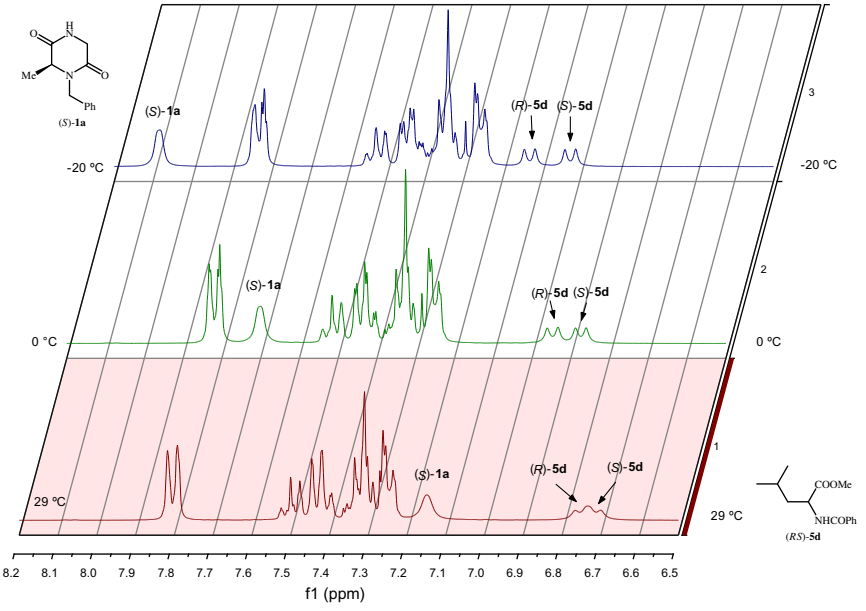
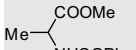
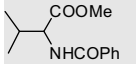
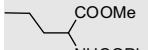
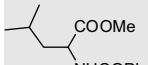
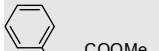
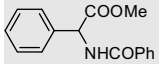
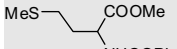
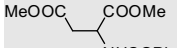
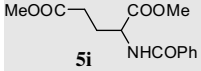
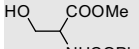
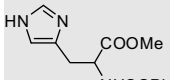
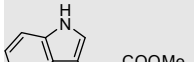


Figure 1. Signals for the NH protons in partial ^1H NMR spectra of equimolar mixture of (*S*)-**1a** and (*RS*)-**5d** (c = 0.073 M) taken in $CDCl_3$ at 29 °C, 0 °C, and −20 °C.

almost exclusive NH splitting in closely related examples, described previously.[12–14] Thus, splitting of NH doublets was observed in mixtures of (*S*)-**1a** and (*RS*)-**5a**–**e**,**g**,**i**,**l**; however, it could not be established for mixtures (*S*)-**1a** and (*RS*)-**5f**,**h**,**j** due to an overlap with the signals of the aromatic protons. The only compound which exhibited a single doublet for the NH proton even at −20 °C was the histidine ester (*RS*)-**5k**. Typically, the ^1H NMR spectra at 29 °C exhibited broad triplets for the NH proton of compounds (*RS*)-**5a**–**e**,**g**,**i**,**l** as a consequence of two partially resolved doublets. Resolution increased with decreasing temperature and broad triplets observed at 29 °C evolved into well-resolved doublets at −20 °C corresponding to each of the enantiomers of racemic amino acid derivatives (*RS*)-**5a**–**e**,**g**,**i**,**l**. The best resolution was observed with leucine derivative (*RS*)-**5d** (Fig. 1). Typically, the difference between the chemical shifts, $\delta_R - \delta_S$,[†] for the benzamide NH protons of (*R*)-**5** and (*S*)-**5** increased from ∼0.02 ppm (29 °C) to ∼0.05 ppm (0 °C) and ∼0.08 ppm (−20 °C). Along with decreasing temperature, the δ chemical shift of the amidic protons increased, that is, doublets for NH of (*R*)- and (*S*)-**5a**–**l** and a singlet for NH of (*S*)-**1a** were shifted toward higher δ values (Fig. 1, Table 1). Finally, the ^1H NMR spectrum of enantiomerically enriched (*S*)-**5d** was taken in $CDCl_3$ at −20 °C in the presence of (*S*)-**1a**, and the enantiomers of **5d** were identified by relative intensities. In the presence of (*S*)-**1a**, the NH proton of the (*R*)-enantiomer of **5d** appeared at a

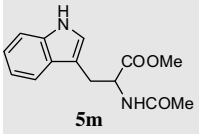
[†] δ_R and δ_S are chemical shifts of NH protons of (*RS*)-**5** in the presence of 1 equiv of (*S*)-**1**.

Table 1
Selected ^{1}H NMR data for equimolar mixtures (*S*)-**1a** and (*RS*)-**5a–l**[a]

Compound **5**	*T* (°C)	Chemical shift of NH of (*R*)- and (*S*)-**5a–l** (ppm)			NH of **1a** δ (ppm)
		δ_R	δ_S	$\delta_R - \delta_S$	
 **5a**	25[b]	6.859[b]	6.836[b]	0.023[b]	7.005[b]
	−15[b]	7.111[b]	7.050[b]	0.061[b]	7.948[b]
	−40[b]	7.282[b]	7.190[b]	0.092[b]	8.403[b]
 **5b**	29	6.750	6.728	0.022	7.060
	0	6.847	6.799	0.048	7.628
	−20	6.957	6.880	0.078	8.049
 **5c**	29	6.804	6.781	0.023	~7.2[c]
	0	6.962	6.910	0.052	7.757
	−20	7.098	7.021	0.077	8.129
 **5d**	29	6.750	6.717	0.033	7.150
	0	6.948	6.875	0.073	7.704
	−20	7.129	7.025	0.104	8.088
 **5e**	29	6.698	6.677	0.021	7.068
	0	6.783	6.823	0.060	7.645
	−20	6.867	6.926	0.059	8.059
 **5f**	29	[c]	[c]	[c]	7.041
	0	[c]	[c]	[c]	7.606
	−20	[c]	[c]	[c]	8.034
 **5g**	29	7.112	7.092	0.020	7.162
	0	[c]	[c]	[c]	7.704
	−20	[c]	[c]	[c]	8.100
 **5h**	29	[c]	[c]	[c]	7.171
	0	[c]	[c]	[c]	7.735
	−20	[c]	[c]	[c]	8.134
 **5i**	29	7.144	7.128	0.016	7.114
	0	[c]	[c]	[c]	7.655
	−20	[c]	[c]	[c]	8.047
 **5j**	29	[c]	[c]	[c]	7.037
	0	[c]	[c]	[c]	7.557
	−20	[c]	[c]	[c]	7.925
 **5k**	29	8.206[d]	8.206[d]	0[d]	6.860
	0	8.288[d]	8.288[d]	0[d]	7.631
	−20	8.549[d]	8.549[d]	0[d]	7.897[c]
 **5l**	29	6.782	6.760	0.022	~7.01[b]
	0	6.879	6.853	0.026	~7.57[b]
	−20	6.958	6.927	0.031	7.990

(*continued on next page*)

Table 1 (*continued*)

Compound **5**	T (°C)	Chemical shift of NH of (*R*)- and (*S*)-**5a–l** (ppm)			NH of **1a** δ (ppm)
		δ_R	δ_S	$\delta_R - \delta_S$	
 **5m**	29	6.009	6.089	0.020	6.702
	0	6.248	6.219	0.029	[c]
	−20	6.369	6.327	0.041	7.654

[a] Unless otherwise specified, all spectra were taken in $CDCl_3$ at 29 °C, 0 °C, and −20 °C with a 300 MHz spectrometer.
[b] Spectrum was taken with a 600 MHz spectrometer. For details see Ref. 12.
[c] Overlapped by the signals for aromatic protons.
[d] The signal for the benzamide NH of (*RS*)-**5** was not resolved in the presence of (*S*)-**1a**.

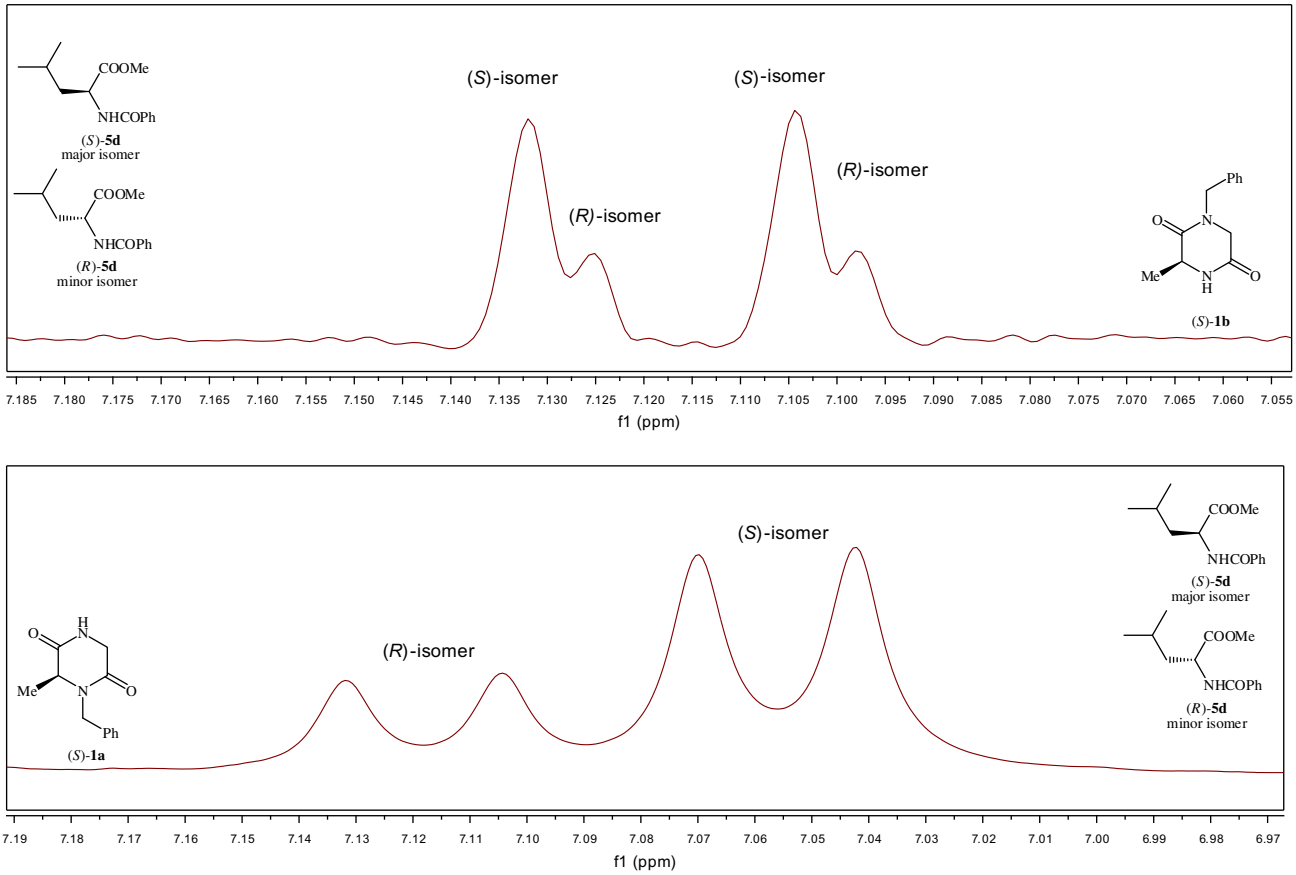


Figure 2. Signals for the NH protons in partial ^{1}H NMR spectra ($CDCl_3$, −20 °C) of enantiomerically enriched (*S*)-**5d** in the presence of (*S*)-**1a** and (*S*)-**1b**.

higher chemical shift (7.118 ppm) than the NH proton of (*S*)-**5d** (7.056 ppm, Fig. 2).

Next, we investigated the chiral solvating properties of (*S*)-1-benzyl-3-methylpiperazine-2,5-dione (*S*)-**1b**, a regioisomer of (*S*)-**1a**. Again, the ^{1}H NMR spectra of equimolar mixtures (*S*)-**1b** and (*RS*)-**5a–l** (**1**:**5** = 1:1, c = 0.073 M) were taken in $CDCl_3$ at 29 °C, 0 °C, and −20 °C. Somewhat to our surprise, compound (*S*)-**1b** turned out to be a less effective CSA than its regioisomer (*S*)-**1a**. Thus, no signal splitting was observed at 29 °C, while at 0 °C, just a slight splitting was observed for 'the most active' leucine derivative (*RS*)-**5d**. At −20 °C, however, the spectra of compounds (*RS*)-**5c–5e**,**j**,**l** exhibited two sets of signals for the NH protons. A typical difference between the chemical shifts at −20 °C, $\delta_S - \delta_R \sim 0.01$ ppm, was two times as low than in the presence of (*S*)-**1a** at 29 °C ($\delta_R - \delta_S \sim 0.02$ ppm, cf. Table 1). The best result in the (*S*)-**1b** series was also obtained with the leucine derivative (*RS*)-**5d** at −20 °C ($\delta_S - \delta_R$ = 0.021 ppm), while splitting could not be established for mixtures (*S*)-**1b** and (*RS*)-**5f–i**,**k** due to an overlap with the aromatic protons (Fig. 3, Table 2). The ^{1}H NMR spectrum of enantiomerically enriched leucine derivative (*S*)-**5d** was also taken in $CDCl_3$ at −20 °C in the presence of (*S*)-**1b** in order to identify the enantiomers of (*RS*)-**5d** by the relative intensity of the signals. In the presence of (*S*)-**1b**, the δ chemical shift of the NH proton of (*S*)-**5d** was higher than the chemical shift of the NH proton of (*R*)-**5d** (Fig. 2).

On the basis of these data we concluded that (*S*)-**1a** is a more effective CSA for *N*-acylamino acid esters **5** than its regioisomer (*S*)-**1b**. However, a minor disadvantage of (*S*)-**1a** was, that the sig-

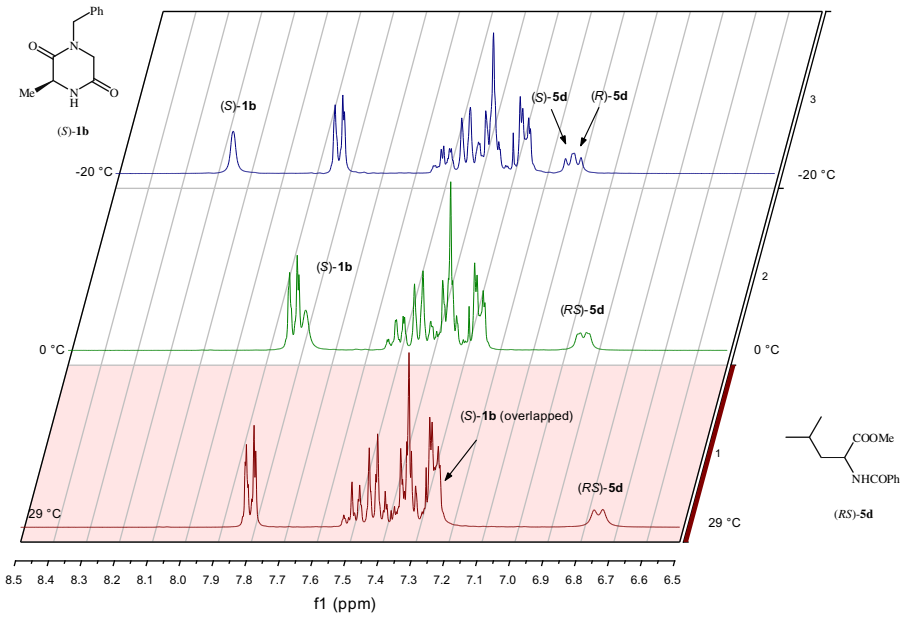


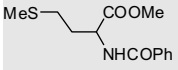
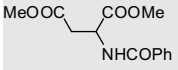
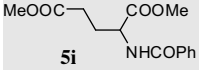
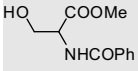
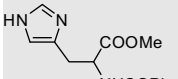
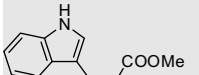
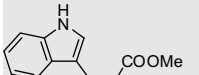
Figure 3. Signals for the NH protons in partial ^{1}H NMR spectrum of equimolar mixture of (*S*)-**1b** and (*RS*)-**5d** in $CDCl_3$ at 29 °C, 0 °C, and −20 °C.

Table 2
Selected ^{1}H NMR data for equimolar mixtures (*S*)-**1b** and (*RS*)-**5a–l**[a]

Compound **5**	*T* (°C)	Chemical shift of NH of (*RS*)-**5a–m** (ppm)			NH of **1b** (ppm)
		δ_R	δ_S	$\delta_S - \delta_R$	
Me, COOMe, NHCOPh **5a**	29	6.895[b]	6.895[b]	0[b]	[c]
	0	7.047[b]	7.047[b]	0[b]	7.769
	−20	7.179[b]	7.179[b]	0[b]	8.135
COOMe, NHCOPh **5b**	29	6.741[b]	6.741[b]	0[b]	[c]
	0	6.865[b]	6.865[b]	0[b]	7.784
	−20	6.976[b]	6.976[b]	0[b]	8.166
COOMe, NHCOPh **5c**	29	6.808[b]	6.808[b]	0[b]	[c]
	0	6.963[b]	6.963[b]	0[b]	~7.84[b]
	−20	7.093	7.103	0.010	8.196
COOMe, NHCOPh **5d**	29	6.747[b]	6.747[b]	0[b]	[c]
	0	6.933	6.941	0.008	7.780
	−20	7.101	7.122	0.021	8.144
COOMe, NHCOPh **5e**	29	6.715[b]	6.715[b]	0[b]	[c]
	0	6.846[b]	6.846[b]	0[b]	7.789
	−20	6.950	6.955	0.005	8.167
COOMe, NHCOPh **5f**	29	[c]	[c]	[c]	[c]
	0	[c]	[c]	[c]	7.773
	−20	[c]	[c]	[c]	8.159

(*continued on next page*)

Table 2 (*continued*)

Compound **5**	T (°C)	Chemical shift of NH of (*RS*)-**5a–m** (ppm)			NH of **1b** (ppm)
		δ_R	δ_S	$\delta_S - \delta_R$	
 **5g**	29	7.086[b]	7.086[b]	0[b]	[b]
	0	[c]	[c]	[c]	7.776
	−20	[c]	[c]	[c]	8.150
 **5h**	29	7.176[b]	7.176[b]	[c]	[c]
	0	[c]	[c]	[c]	7.727
	−20	[c]	[c]	[c]	8.125
 **5i**	29	7.154[b]	7.154[b]	0[b]	[c]
	0	[c]	[c]	[c]	7.790
	−20	[c]	[c]	[c]	8.154
 **5j**	29	[c]	[c]	[c]	7.133
	0	[c]	[c]	[c]	7.621
	−20	7.600	7.625	0.025	7.965
 **5k**	29	6.904[b]	6.904[b]	0[b]	[c]
	0	[c]	[c]	[c]	[c]
	−20	[c]	[c]	[c]	7.952
 **5l**	29	6.790[b]	6.790[b]	0[b]	[c]
	0	6.872[b]	6.872[b]	0[b]	7.628
	−20	6.939	6.947	0.008	8.008
 **5m**	29	6.099	6.119	0.020	6.826
	0	6.236	6.265	0.029	[c]
	−20	6.350	6.398	0.048	7.736

[a] All spectra were taken in $CDCl_3$ at 29 °C, 0 °C, and −20 °C.
[b] The signal for the benzamide NH of (*RS*)-**5** was not resolved in the presence of (*S*)-**1b**.
[c] Overlapped by the signals for aromatic protons.

nals of the NH proton of the N-benzoylated amino acids **5** were frequently overlapped by the signals of the aromatic protons, especially in the case of more functionalized amino acids, such as histidine, serine, aspartic acid, glutamic acid, and methionine (cf. Tables 1 and 2). In order to circumvent this inconvenience, we prepared (*S*)-1-(pentafluorobenzyl)-6-methylpiperazine-2,5-dione (*S*)-**1c** as a pentafluoro analogue of (*S*)-**1a**, which does not exhibit aromatic signals in ^{1}H NMR. Compound (*S*)-**1c** was prepared from (*S*)-alanine methyl ester (*S*)-**2** and pentafluorobenzaldehyde following a three-step literature procedure for the preparation of its non-fluorinated analogue (*S*)-**1a** (cf. Scheme 1).[16–19] Then, ^{1}H NMR spectra of the valine ester (*RS*)-**5b** and the leucine ester (*RS*)-**5d** were taken in $CDCl_3$ in the presence of (*S*)-**1c** (**1c**:**5b**,**d** = 1:1, c = 0.073 M) at 29 °C, 0 °C, and −20 °C. To our disappointment, no splitting of the NH signals or any other signals was observed in these spectra (Fig. 4).

The above experiments showed that N-substituents in diketopiperazine **1** play an important role in the efficacy of diketopiperazines **1a–c** as CSA in NMR spectroscopy. Since we were interested in the influence of the *N*-acyl group as well, ^{1}H NMR spectra of *N*-acetyltryptophan methyl ester (*RS*)-**5m**[20j,21g] were taken in the presence of equimolar amounts of (*S*)-**1a** and (*S*)-**1b** (**1**:**5** = 1:1, c = 0.073 M) in $CDCl_3$ under standard conditions, that is, at 29 °C, 0 °C, and −20 °C. With (*RS*)-**5m**, both diketopiperazines, (*S*)-**1a** and (*S*)-**1b**, acted as reasonably effective CSA with a typical difference in chemical shifts $\delta_R - \delta_S$ for the acetamide NH protons of (*RS*)-**5m** increasing from ∼0.02 ppm (29 °C) to ∼0.03 ppm (0 °C) and ∼0.05 ppm (−20 °C). The signal of the indole's NH proton also appeared as two singlets in the presence of (*S*)-**1a** at 0 °C and −20 °C and in the presence of (*S*)-**1b** at −20 °C (Figs. 5 and 6, Tables 1 and 2).

As shown previously, diketopiperazine (*S*)-**1a** undergoes association with various amino acid esters via C=*O*···*H*–N hydrogen bonded associates in $CDCl_3$ solution.[12–14] Within this context, the binding mode between (*RS*)-*N*-benzoylalanine methyl ester (*RS*)-**5a** and CSA (*S*)-**1a** in $CDCl_3$ solution has been determined by ^{1}H NMR, ^{13}C NMR, and ROESY spectroscopy.[12] Accordingly, the binding mode of diketopiperazines (*S*)-**1a–c** with N-benzoylated amino acid esters (*RS*)-**5a-m** in $CDCl_3$ solution should be the same. Interactions between diketopiperazines (*S*)-**1a–c** and N-acylated amino acid esters **5a–m** should result in a reversible association, that is, in the reversible formation of the corresponding diastereomeric associates (*R*,*S*)-**8**, (*S*,*S*)-**8**, (*R*,*S*)-**9**, and (*S*,*S*)-**9** (Fig. 7), which is exhibited as the splitting (doubling) of signals in the NMR spectra

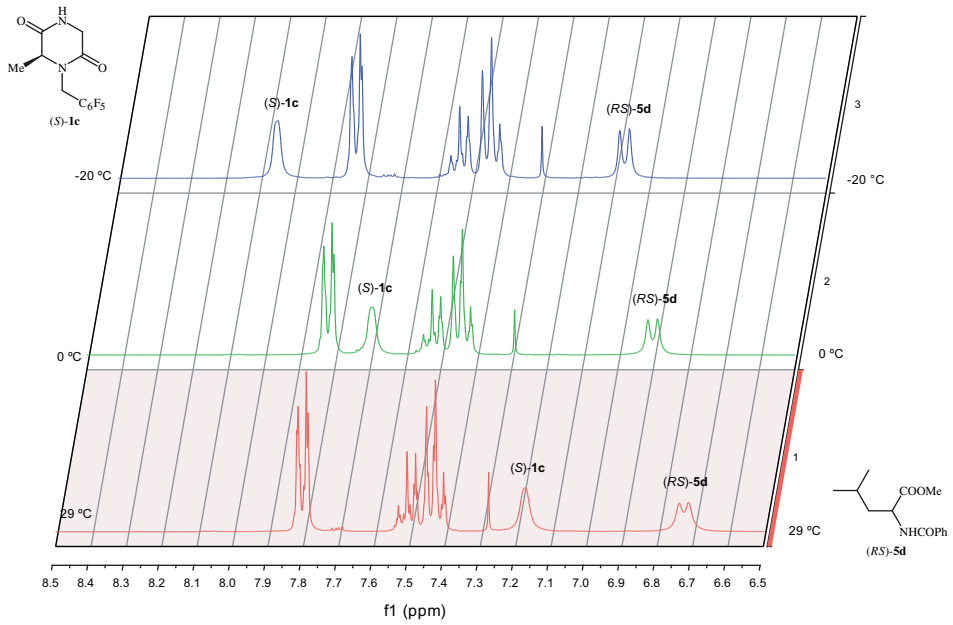


Figure 4. Signals for the NH protons in partial ^{1}H NMR spectrum of equimolar mixture of (*S*)-**1c** and (*RS*)-**5d** in $CDCl_3$ at 29 °C, 0 °C, and −20 °C.

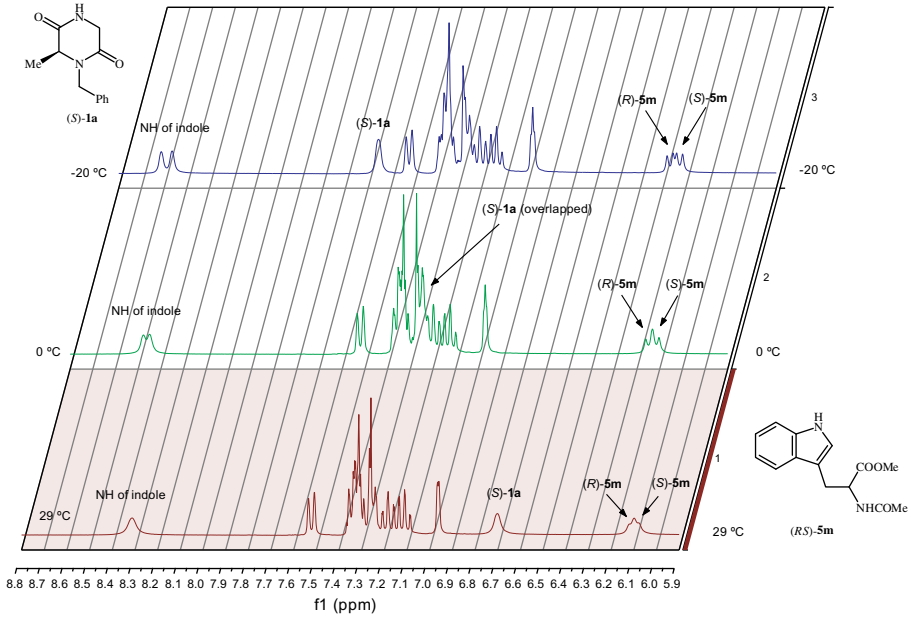


Figure 5. Signals for the NH protons in partial ^{1}H NMR spectrum of equimolar mixture of (*S*)-**1a** and (*RS*)-**5m** in $CDCl_3$ at 29 °C, 0 °C, and −20 °C.

(cf. Figs. 1–6, Tables 1 and 2). On the basis of these NMR data, it can be concluded that (*S*)-**1a** is the most effective CSA among the investigated diketopiperazines (*S*)-**1a–c**. The different efficacies of (*S*)-**1a** and (*S*)-**1b** can be explained by the distance between the N*H* proton of (*RS*)-**5** and the stereogenic center of (*S*)-**1** in the corresponding associates **8** and **9**. Namely, the distance d_{1a} between the N*H* hydrogen atom of (*RS*)-**5** and the chiral center *C*(6) of (*S*)-**1a** in associates **8** is shorter than the distance d_{1b} between N*H* of (*RS*)-**5** and C(3) of (*S*)-**1b** ($d_{1a} < d_{1b}$, Fig. 7). Consequently, (*S*)-**1a** is a more effective CSA than (*S*)-**1b**. The distances, $d_{1a} \sim 0.45$ nm and $d_{1b} \sim 0.55$ nm, could also be estimated on the basis of the X-ray structure of the enamino analogue of (*S*)-**1a** comprising two C=*O*···*H*–N hydrogen bonded molecules in a crystal cell.[12] Unfortunately, we do not have an explanation for the lack of chiral solvating properties of (*S*)-**1c**, which might be due to electronic properties of the pentafluorobenzyl group. In contrast to the N-substituents in diketopiperazines (*S*)-**1a–c**, the α-C-substituents of the acylamino acid esters **5** did not significantly influence the efficiency of **1a–c** as CSA in $CDCl_3$ solution. For example, the splitting of the NH signals observed for intermolecular cross-interactions of (*S*)-**1a** with α-amino acid esters (*RS*)-**5a–e,g,l,m** was pretty much the same for the whole range of amino acid derivatives (*RS*)-**5a–e,g,l,m**. Exceptions were compounds (*RS*)-**5h,j,k**, where the signals for the NH protons appeared at higher chemical

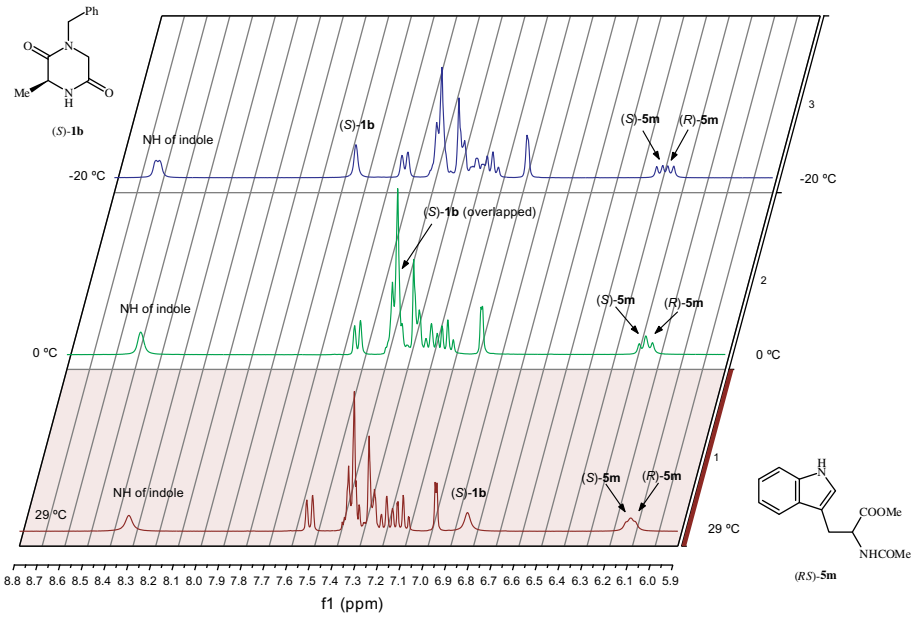


Figure 6. Signals for the NH protons in partial ^{1}H NMR spectrum of equimolar mixture of (*S*)-**1b** and (*RS*)-**5m** in $CDCl_3$ at 29 °C, 0 °C, and −20 °C.

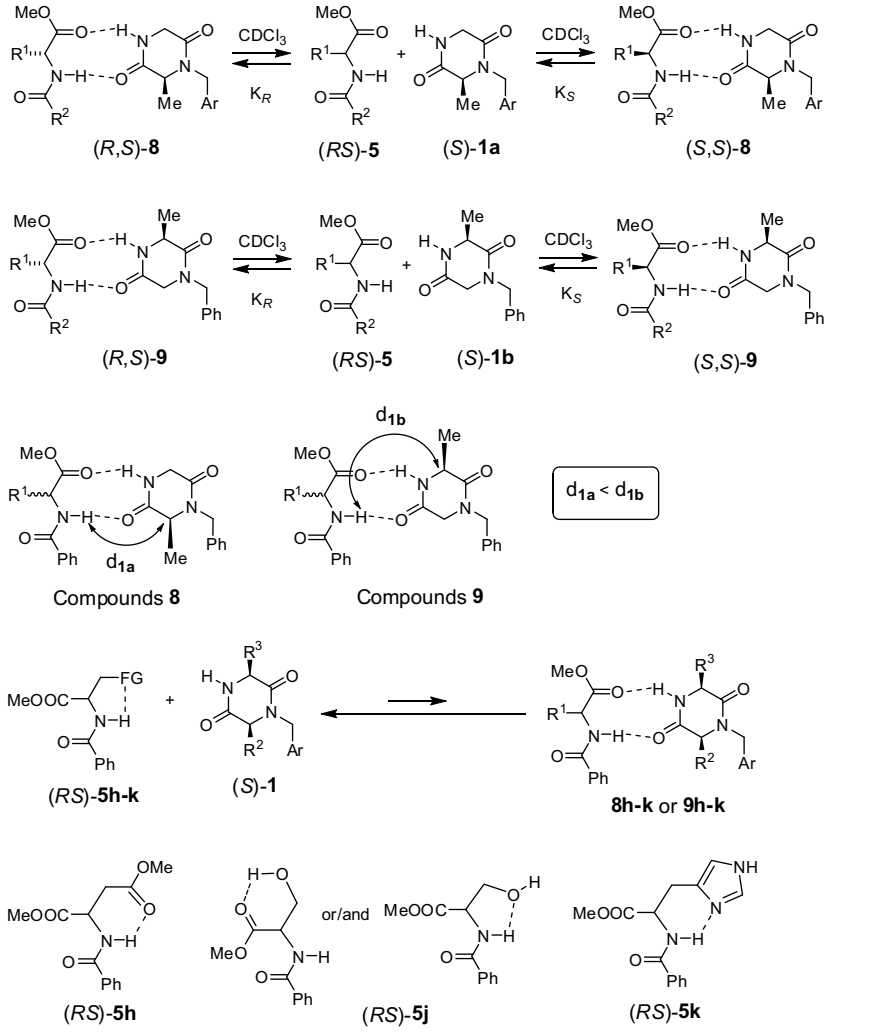


Figure 7.

Table 3
^{1}H NMR titration data for mixtures (*S*)-**1a** (c = 2.5→100 mM) and (*RS*)-**5d** (c = 5 mM)[a,b]

[(*S*)-**1a**] (mM)	δ_R (ppm)	δ_S (ppm)	$\Delta\delta_R$ (ppm)	$\Delta\delta_S$ (ppm)
2.5	6.625	6.610	−0.038	−0.023
5	6.649	6.625	−0.062	−0.038
10	6.687	6.650	−0.100	−0.063
25	6.783	6.714	−0.196	−0.127
50	6.886	6.787	−0.299	−0.200
100	6.972	6.854	−0.385	−0.267

[a] All spectra were taken in $CDCl_3$ at −20 °C.
[b] δ^0_{NH} of **5d** = 6.587 ppm (c = 5 mM).

shift (>7.0 ppm, cf. Table 1). Deshielding of the N*H* protons in compounds (*RS*)-**5h**,**j**,**k** with functionalized α-substituents can be explained by the preferential formation of intramolecular hydrogen bonds, either between the NH group and a hydrogen bond acceptor functional group at the β-position in the case of compounds (*RS*)-**5h**,**j**,**k** or between the ester group and the hydroxy group in the case of the serine derivative (*RS*)-**5j** (Fig. 7).

Finally, the binding constants for association of the (*R*)- and the (*S*)-enantiomer of *N*-benzoyl-D,L-leucine methyl ester (*RS*)-**5d** with CSA (*S*)-**1a**, K_R and K_S, were determined. First, ^{1}H NMR spectra of mixtures of (*S*)-**1a** (c = 2.5→100 mM) and (*RS*)-**5d** (c = 5 mM) were taken in $CDCl_3$ at −20 °C. This NMR titration gave the complexation-induced shifts, $\Delta\delta_R = \delta^0 - \delta_R$ and $\Delta\delta_S = \delta^0 - \delta_R$,[‡] for the NH protons of each enantiomer of a guest molecule, (*R*)-**5d** and (*S*)-**5d**, at different concentrations of a host molecule (*S*)-**1a** (Table 3). Benesi–Hildebrand treatment[22] of these data then furnished the complexation-induced shifts at saturation binding, $\Delta\delta_{maxR} = -0.362$ ppm, $\Delta\delta_{maxS} = -0.254$ ppm, and the binding constants, K_R = 45.4 M^{-1} for the (*R*)-**5d** and K_S = 38.6 M^{-1} for the (*S*)-**5d** (Fig. 8).

3. Conclusion

The present study on the utilization of (*S*)-1-benzyl-6-methylpiperazine-2,5-dione (*S*)-**1a**, its regioisomer (*S*)-1-benzyl-3-methylpiperazine-2,5-dione (*S*)-**1b**, and its close analogue (*S*)-1-(pentafluorobenzyl)-6-methylpiperazine-2,5-dione (*S*)-**1c** as CSA for racemic acylamino acid esters (*RS*)-**5a–m** in $CDCl_3$ solution showed that the position and properties of substituents strongly influence the efficiency of diketopiperazines (*S*)-**1a–c** as a CSA. With respect to this, compound (*S*)-**1a** is a superior CSA for racemic N-benzoylated amino acid esters (*RS*)-**5a–m**. In addition, the association constants, K_R = 45.4 M^{-1} and K_S = 38.6 M^{-1}, for the formation of the diastereomeric complexes (*R*,*S*)-**8d** and (*S*,*S*)-**8d** between (*S*)-**1a** and both enantiomers of the leucine ester (*R*)-**5d** and (*S*)-**5d** in $CDCl_3$ were determined. In conclusion, (*S*)-**1a** is an easily available and effective CSA, which can be employed for a simple determination of ee of α-acylamino acid esters **5** and analogous chiral amides by ^{1}H NMR.[12–14] Exceptions are esters of β-functionalized *N*-acylamino acids, such as aspartic acid, serine, and histidine, where intermolecular association with CSA (*S*)-**1a** is diminished by competitive intramolecular hydrogen bonding. An important advantage of (*S*)-**1a** over the lanthanide-based chiral shift reagents is retained resolution of ^{1}H NMR spectra of the investigated compounds due to absence of a paramagnetic lanthanide nuclei. Since the substituents exhibit a significant impact on the enantioselectivity of diketopiperazine type CSA, a further study on the preparation and utilization of novel diketopiperazine-based CSA and their testing against various types of chiral substrates is currently in progress.

[‡] δ^0 is chemical shift (ppm) of a NH proton of pure compound (*RS*)-**5d** (c = 5 mM) in $CDCl_3$ at −20 °C.

4. Experimental

4.1. General methods

Melting points were determined on a Kofler micro hot stage. The NMR spectra were obtained on a Bruker Avance DPX 300 at 300 MHz for ^{1}H and 75.5 MHz for ^{13}C nucleus, using $CDCl_3$ as solvent with TMS as the internal standard. Optical rotations were measured on a Perkin-Elmer 241MC Polarimeter. Mass spectra were recorded on AutoSpecQ and Q-Tof Premier spectrometers and IR spectra on a Perkin-Elmer Spectrum BX FTIR spectrophotometer. Microanalyses were performed on a Perkin-Elmer CHN Analyzer 2400 II.

(*S*)-Alanine methyl ester hydrochloride (*S*)-**2** and (*RS*)-amino acids **6a–l** are commercially available (Sigma-Aldrich). (*S*)-1-Benzyl-6-methylpiperazine-2,5-dione (*S*)-**1a**,[17–19] (*S*)-1-benzyl-3-methylpiperazine-2,5-dione (*S*)-**1b**,[15] and N-benzoylated amino acid methyl esters (*RS*)-**5a**,[20a] (*RS*)-**5b**,[20b,21a] (*RS*)-**5c**,**d**,[20c,21a] (*RS*)-**5e**,[20d,21a] (*RS*)-**5f**,[20e,21b] (*RS*)-**5g**,[20f,21a] (*RS*)-**5h**,**i**,[20g,21c] (*RS*)-**5j**,[20h,21d] (*RS*)-**5k**,[20i,21e] (*RS*)-**5l**,[20j,21f] and (*RS*)-**5m**,[20j,21g] were prepared according to the literature procedures.

Source of chirality: (i) L-alanine methyl ester hydrochloride (Fluka AG), product number 05200, puriss, ⩾99.0% (dried material AT), $[\alpha]_D^{20} = +7.5 \pm 0.5$ (c 2, MeOH), mp 107–110 °C, ee not specified.

4.2. Synthesis of (*S*)-1-(pentafluorobenzyl)-6-methylpiperazine-1,4-dione (*S*)-1c

This compound was prepared from (*S*)-alanine methyl ester hydrochloride (*S*)-**2** and pentafluorobenzaldehyde following the literature procedure for the preparation of its analogue (*S*)-**1a**[12,16] via reductive benzylation[17] (**2**→**3c**), chloroacetylation[18] (**3**→**4c**), and cyclization with ammonia[19] (**3**→**1c**).

4.2.1. Methyl (*S*)-2-(pentafluorobenzylamino)propanoate (*S*)-3c

This compound was prepared following a slightly modified literature procedure for the preparation of its benzyl analogue (*S*)-**3a**.[17] Compound (*S*)-**2** (1.40 g, 10 mmol), trimethyl orthoformate (20 mL), anhydrous methanol (7.5 mL), and triethylamine (1.4 mL, 10 mmol) were added to a 250 mL flask, the flask was flushed with argon, and closed with a septum. Then, pentafluorobenzaldehyde (1.54 mL, 12.5 mmol) was added via syringe and the mixture was stirred at room temperature for 1 h. Next, a solution of $NaBH_3CN$ (3.14 g, 50 mmol) in a mixture of trimethyl orthoformate (20 mL) and anhydrous methanol (5 mL) was added via syringe and stirring at room temperature was continued for 2 h. The reaction mixture was cooled to 0 °C, the septum was removed, after which the mixture was carefully acidified with 2 M hydrochloric acid (20 mL), and extracted with dichloromethane (2 × 25 mL). The organic phases were combined and extracted with 3.8 M hydrochloric acid (2 × 50 mL). The aqueous phases were combined, cooled to 0 °C, and made slightly alkaline (pH ∼ 8) with 50% aq NaOH, and the product was extracted with dichloromethane (4 × 40 mL). The organic phases were combined, dried over anhydrous Na_2SO_4, filtered, and the filtrate was evaporated in vacuo to give (*S*)-**3c**, which was used in the next step without purification. Yield: 2.574 g (91%) of a yellowish oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.32 (3H, d, J = 7.2 Hz, CH_3); 3.37 (1H, q, J = 7.2 Hz, CH); 3.72 (3H, s, OMe); 3.84 (1H, d, J = 13.2 Hz, 1H of CH_2Ar); 3.94 (1H, d, J = 13.2 Hz, 1H of CH_2Ar). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.0, 29.3, 38.8, 51.9, 55.9, 175.4. m/z

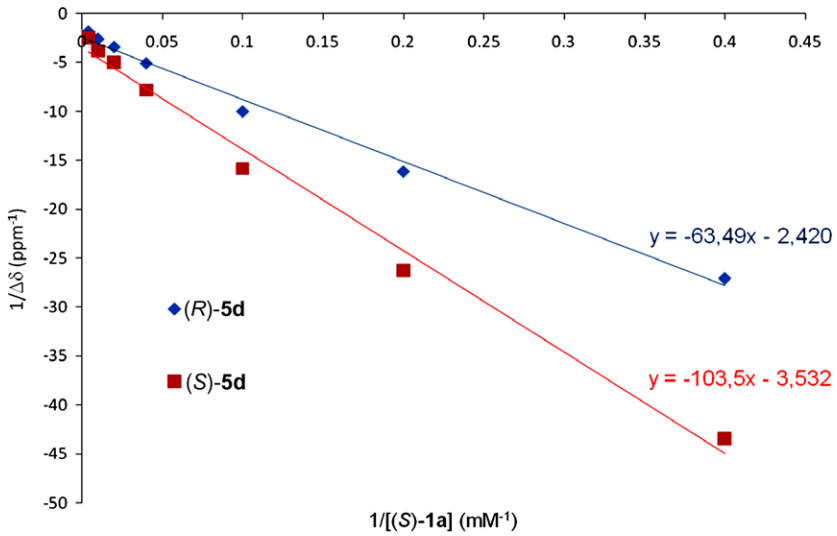


Figure 8. Benesi–Hildebrand data treatment for a mixture of (*R*)-**5d** and (*S*)-**1a**. A double reciprocal plot of complexation-induced shifts for the NH proton of (*R*)-**5d** and (*S*)-**5d** ($1/\Delta\delta_R$ and $1/\Delta\delta_S$) as a function of 1/[(*S*)-**1a**] with slopes $1/K\Delta\delta_{maxR} = -63.5$ mM ppm^{-1} and $1/K\Delta\delta_{maxS} = -103.5$ mM ppm^{-1} and intercepts $1/\Delta\delta_{maxR} = -2.420$ ppm^{-1} and $1/\Delta\delta_{maxS} = -3.532$ ppm^{-1}, respectively.

(ESI) = 284 (MH^+). *m*/*z* (HRMS) Found: 284.0710 (MH^+). $C_{11}H_{10}F_5NO_2$ requires: *m*/*z* = 284.0720 (MH^+). ν_{max} (NaCl) 3457, 3341, 2957, 1739 (C=O), 1656 (C=O), 1520, 1505, 1452, 1203, 1150, 1028, 937, 722 cm^{-1}.

4.2.2. Methyl (*S*)-2-[2-chloro-*N*-(pentafluorobenzyl)-acetylamino]propanoate (*S*)-4b

This compound was prepared following a slightly modified literature procedure for the preparation of its benzyl analogue (*S*)-**4a**.[18] Chloroacetyl chloride (0.8 mL, 10 mmol) was added slowly to a stirred cold solution (0 °C) of the crude (*S*)-**3c** (2.8 g, 10 mmol) and triethylamine (1.75 mL, 12.5 mmol) in dichloromethane (12 mL). The reaction mixture was allowed to warm to room temperature and stirred at room temperature for 1 h. Hydrochloric acid (3.8 M, 3 mL) was then added and the mixture was extracted with dichloromethane (3 × 12 mL). The organic phases were combined, dried over anhydrous Na_2SO_4, filtered, after which the filtrate was evaporated in vacuo, and the residue was purified by FC (EtOAc–hexanes, 1:4). Fractions containing the product were combined and evaporated in vacuo to give (*S*)-**4c**, which was used in the next step without purification. Yield: 1.908 g (53%) of a yellowish oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 and 1.59 (3H, 2d, 65:35, *J* = 6.6, 5.4 Hz, C*H*$_3$CH); 3.63 and 3.71 (3H, 2s, 65:35, OMe); 3.91 (1H, q, *J* = 6.6 Hz, C*H*CH$_3$); 4.20–4.37 (2H, m, CH$_2$); 4.60–4.85 (2H, m, CH$_2$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.9, 29.3, 40.7, 41.1 (B), 52.4, 52.8, 65.1, 166.2, 167.4, 170.6, 170.9. *m*/*z* (ESI) = 360 (MH^+). *m*/*z* (EI-HRMS) Found: 360.042587 (MH^+). $C_{13}H_{10}F_5NO_3$ requires: *m*/*z* = 360.042587 (MH^+). ν_{max} (NaCl) 3463, 2995, 2955, 1747 (C=O), 1666 (C=O), 1523, 1504, 1438, 1223, 1123, 1027, 946, 799 cm^{-1}.

4.2.3. (*S*)-1-(Pentafluorobenzyl)-6-methylpiperazine-1,4-dione (*S*)-1c

This compound was prepared following a slightly modified literature procedure for the preparation of its benzyl analogue (*S*)-**1a**.[19] Ammonia was bubbled through a cold (0 °C) solution of the crude (*S*)-**4c** (3.59 g, 10 mmol) in anhydrous methanol (12 mL) for 10 min and the reaction mixture was stirred at room temperature for 27 h. The precipitate was collected by filtration and washed with cold (0 °C) aqueous ethanol (50%, 5 mL) to give (*S*)-**1c**. Yield: 1.324 g (43%) of white crystals; mp 186–188 °C (from EtOH–H_2O); $[\alpha]_D^{28} = +4.2$ (*c* 0.15, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.55 (3H, d, *J* = 7.2 Hz, CH$_3$); 3.86 (1H, q, *J* = 7.2 Hz, 6–H); 3.99 (1H, dd, *J* = 17.7, 3.6 Hz, 1H of C*H*$_2$NH); 4.08 (1H, d, *J* = 17.7 Hz, 1H of C*H*$_2$NH); 4.09 (1 H, d, *J* = 14.7 Hz, 1H of C*H*$_2$Ar); 5.40 (1H, d, *J* = 14.7 Hz, 1H of C*H*$_2$Ar); 6.36 (1H, s, NH). *m*/*z* (EI) = 308 (M^+). *m*/*z* (HRMS) Found: 308.058419 (M^+). $C_{12}H_9F_5N_2O_2$ requires: *m*/*z* = 308.059520. (Found: C, 46.74; H, 2.88; N, 9.04. $C_{12}H_9F_5N_2O_2$ requires: C, 46.76; H, 2.94; N, 9.09.); ν_{max} (KBr) 3212, 3078, 2984, 1687 (C=O), 1667 (C=O), 1522, 1509, 1441, 1327, 1302, 1132, 1020, 947, 849, 783 cm^{-1}.

Acknowledgments

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