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The effect of substituents on the chiral solvating properties of (*S*)-1,6-dialkylpiperazine-2,5-diones

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ABSTRACT

The effect of substituents on the chiral solvating properties of 13 different (*S*)-1,6-dialkylpiperazine-2,5-diones (*S*)-**1a**–**m** and five (3*S*,6*S*)-1,3,6-trialkyl analogues (*S*,*S*)-**1n**–**r** was studied by NMR in $CDCl_3$ with methyl (*RS*)-*N*-benzoylleucinate (*RS*)-**2a** as the model analyte. Most diketopiperazines exhibited typical resolution, $\Delta\Delta\delta_{RS}^{-20} \sim 0.1$ ppm. Increased performance was observed with 6-CH_2R substituted compounds (*S*)-**1h**–**j**. The best resolution of the NH protons of (*R*)-**2a** and (*S*)-**2a**, $\Delta\Delta\delta_{RS}^{-20} = 0.227$ ppm, was obtained with (*S*)-1-isopropyl-6-(4-nitrobenzyl)piperazine-2,5-dione (*S*)-**1j**. An additional *syn*-oriented substituent at the C(3) position decreased the enantioselectivity. Association constants for the binding of (*S*)-**1j** to each enantiomer of (*RS*)-**2a** in $CDCl_3$ at −20 °C were determined by NMR titration.

1. Introduction

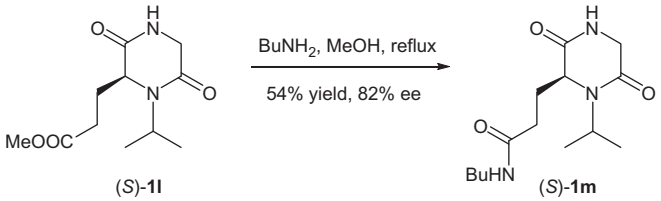
In addition to chromatographic methods (GC and HPLC), NMR spectroscopy represents a common and simple way for the determination of the enantiomeric excess and absolute configuration of chiral compounds. Various fluorinated α-hydroxymethylidene-camphor-based lanthanide shift reagents are nowadays commonly used as reagents for the determination of ee and absolute configuration by NMR.[1–3] However, a major disadvantage of chiral lanthanide shift reagents is the loss of resolution and broadening of signals, which may often prevent accurate ee determination. On the other hand, chiral solvation agents (CSA) that are not covalently attached to the enantiomers have also been successfully employed for the determination of enantiomer composition and absolute configuration. Examples of CSA include carboxylic acids and carboxamides,[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] Computational studies on chiral recognition and self-complexes of diketopiperazine derivatives have recently been reported.[15]

Within this context, we have recently reported the chiral solvating properties of (*S*)-1-benzyl-6-methylpiperazine-2,5-dione (*S*)-**1a**,[12] its 4-benzyl regioisomer, and its 1-pentafuorophenyl analogue.[13] Compound (*S*)-**1a** was recognised as being a suitable CSA for α-acylamino acids[12,13] and was used for determination of the enantiomeric excess of (*S*)-*tert*-butyl pyroglutamate[12] and tryprostatin B analogues.[14] By extension, it seemed reasonable to take a closer look at the structure–property relationship. To determine how the substituents affect chiral solvating activity, a series of novel enantiopure diketopiperazines (*S*)-**1b**–**r** were prepared[16] and their chiral solvating properties for α-acylamino acid esters then tested using methyl (*RS*)-*N*-benzoylleucinate (*RS*)-**2a** as a model analyte. Herein, we report the results of NMR experiments showing the effect of substituents on the chiral solvating properties of (*S*)-1,6-dialkylpiperazine-2,5-diones (*S*)-**1**.

2. Results and discussion

2.1. The synthesis of (*S*)-*N*-butyl-3-(1-isopropyl-2,5-dioxopiperazin-6-yl)propanamide (*S*)-1m

Following literature precedents, diketopiperazines (*S*)-**1a**,[17] (*S*)-**1b**–**l**, and (*S*,*S*)-**1n**–**r**[16] were prepared in three steps from commercially available amino acid esters. To add a representative with a C(6)-substituent that can act as a hydrogen bond donor, the 6-[(butylaminocarbonyl)ethyl] analogue (*S*)-**1m** was also prepared from the 6-[(methoxycarbonyl)ethyl] derivative (*S*)-**1l**. Thus, treatment of ester (*S*)-**1l** with excess butylamine in refluxing methanol gave the carboxamide (*S*)-**1m** in 54% yield and 82% ee (Scheme 1).



(*S*)-**1l** (*S*)-**1m**

Scheme 1.

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2.2. Discrimination of the enantiomers of (*RS*)-*N*-benzoylleucine methyl ester (*RS*)-2a with compounds (*S*)-1a–m and (*S,S*)-1n–r

In continuation of the preceding study,[13] (*RS*)-*N*-benzoylleucine methyl ester (*RS*)-**2a**[18] was chosen as the model analyte for testing the chiral solvating properties of compounds (*S*)-**1a**–**m** and (*S,S*)-**1n**–**r**. The ^{1}H NMR spectra of equimolar mixtures of (*S*)-**1** and (*RS*)-**2a** (**1**:**2a** = 1:1, *c* = 0.073 M) were taken in $CDCl_3$ at three different temperatures: 29, 0, and −20 °C. In accordance with our previous observations,[12–14] the signals (doublets) for the NH protons of compounds (*RS*)-**2a** and (*S*)-**1** were split, whilst all CH protons appeared as single sets of signals. Typically, well resolved doublets for the amide NH protons of each enantiomer of the analyte **2a** were obtained at −20 °C with the typical difference between the chemical shifts, $\Delta\Delta\delta_{RS}^{-20}$ being ∼0.1 ppm (Fig. 1, Tables 1 and 2).[†]

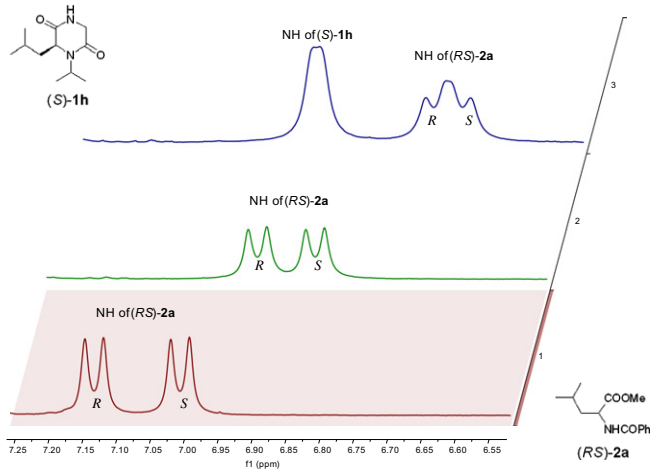


Figure 1. Signals for the NH protons in the partial ^{1}H NMR spectra of equimolar mixture of (*S*)-**1e** and (*RS*)-**2a** (*c* = 0.073 M) taken in $CDCl_3$ at 29, 0, and −20 °C.

2.3. Structure–property relationship

With some exceptions, these NMR measurements showed the relatively weak effect of most of the substituents on the performance of CSA (*S*)-**1**. In the 1-alkyl-6-methyl series (*S*)-**1a**–**e**, only the *N*-benzhydryl derivative (*S*)-**1d** was exceptionally effective ($\Delta\Delta\delta_{RS}^{-20}$ = 0.14 ppm), while the solvating properties of compounds (*S*)-**1a**–**c**,**e** were almost identical ($\Delta\Delta\delta_{RS}^{-20}$∼0.1 ppm). A similar efficacy of CSA (*S*)-**1e**–**g** ($\Delta\Delta\delta_{RS}^{-20}$∼0.1 ppm) showed that the bulk of the C(6)-substituent (Me, *i*-Pr, *t*-Bu) did not affect the resolution. On the other hand, compounds (*S*)-**1h**–**l** with a functionalised methylene group at the C(6) position exhibited moderate (6-isobutyl and 6-CH_2CH_2COOMe, $\Delta\Delta\delta_{RS}^{-20}$ = 0.124 and 0.138 ppm, respectively) to significantly improved chiral solvating properties (6-arylmethyl, $\Delta\Delta\delta_{RS}^{-20}$∼0.2 ppm). The best enantioselectivity, $\Delta\Delta\delta_{RS}^{-20}$ = 0.227 ppm, was obtained with (*S*)-1-isopropyl-6-(4-nitrobenzyl)piperazine-2,5-dione (*S*)-**1j**. It is noteworthy, that the electronic effect in the *para*-substituent in the 3-benzyl series (*S*)-**1i**–**k** was barely noticeable. On the other hand, compound (*S*)-**1l** with a hydrogen bond acceptor group (COOMe) showed increased efficacy ($\Delta\Delta\delta_{RS}^{-20}$ = 0.138 ppm), while the efficacy of its close analogue (*S*)-**1m** with the hydrogen bond donor group (CONHBu) was below average ($\Delta\Delta\delta_{RS}^{-20}$ = 0.062 ppm) (Table 1). The introduction of an additional *syn*-oriented substituent at the C(3)-position led to a decreased activity in comparison to the C(3)-unsubstituted analogues. In the 1,3,6-trialkylpiperazine-2,5-dione series (*S,S*)-**1n**–**r**, only compounds (*S,S*)-**1n** and (*S,S*)-**1q** were highly enantioselective, while the activities of (*S,S*)-**1o**,**p**,**r** were below average (Table 2). In conclusion, a typical resolution of enantiomers of (*RS*)-**2a** at −20 °C was ∼0.1 ppm; the best resolution was achieved with 6-benzyl substituted compounds (*S*)-**1i**–**k** ($\Delta\Delta\delta_{RS}^{-20}$∼0.2 ppm), while compounds (*S*)-**1d**,**h**,**l**,**n**,**q** also exhibited above average activities, $\Delta\Delta\delta_{RS}^{-20} \geqslant 0.12$ ppm (Fig. 2). However, the excellent efficacy of (*S*)-**1d**,**i**,**n**,**q** was counterbalanced by overlap between the aromatic protons and the NH protons (cf. Tables 1 and 2).

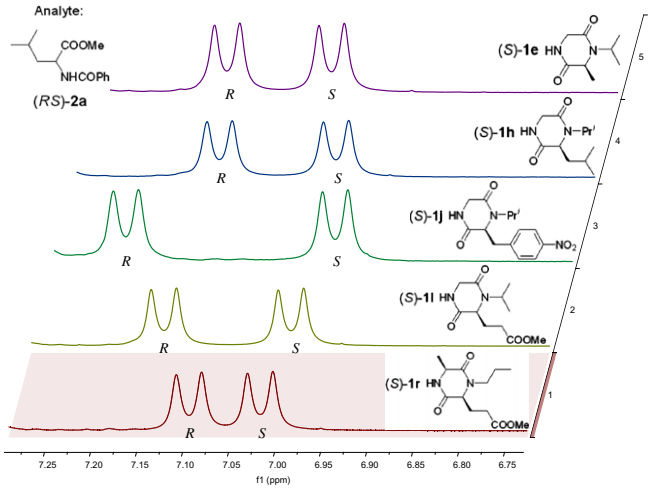


Figure 2. Signals for the NH protons of (*RS*)-**2a** in the partial ^{1}H NMR spectra of equimolar mixtures of (*S*)-**1e**,**h**,**j**,**l**,**q** and (*RS*)-**2a** (*c* = 0.073 M) taken in $CDCl_3$ at −20 °C.

These measurements showed the effect of *N*- and *C*-substituents of a CSA on the enantiodiscrimination of (*RS*)-**2a**, while influence of the α-substituent of the acylamino ester on the performance of (*S*)-**1a** has been determined previously.[13] Next, we took a closer look at the influence of the *N*-acyl group of **2** as well. Racemic *N*-acylvaline methyl esters (*RS*)-**2b**–**j** were prepared by acylation of (*RS*)-valine methyl ester hydrochloride (*RS*)-**3b**.[13,19] At −20 °C, the ^{1}H NMR spectra of equimolar mixtures of (*S*)-**1a** and (*RS*)-**2b**–**h** ($CDCl_3$, **1**:**2** = 1:1, *c* = 0.073 M) exhibited well resolved doublets for the NH protons corresponding to each of the enantiomer of racemic valine derivative (*RS*)-**2**. With the exception of (*RS*)-**2g**, the difference between the chemical shifts for the benzamide NH protons of (*R*)-**2** and (*S*)-**2** was sufficient in all other cases ($\Delta\Delta\delta_{RS}^{-20}$∼0.08 ppm). This led us to conclude that the performance of (*S*)-**1a** was not affected by the variation of the *N*-acyl group (Fig. 3, Table 3).

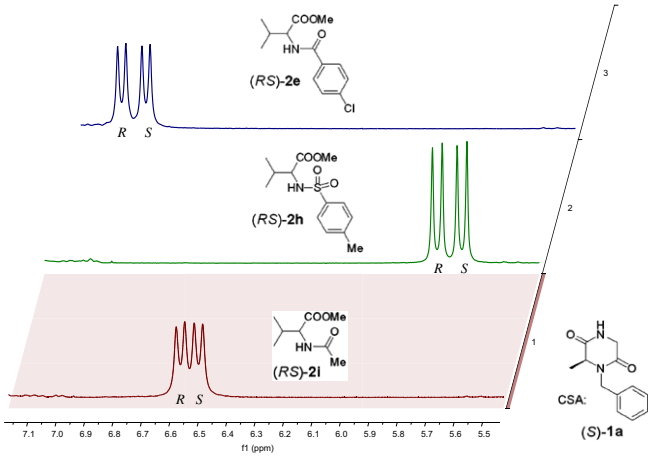


Figure 3. Signals for the NH protons of (*RS*)-**2e**,**h**,**i** in the partial ^{1}H NMR spectra of equimolar mixtures of (*S*)-**1a** and (*RS*)-**2e**,**h**,**i** (*c* = 0.073 M) taken in $CDCl_3$ at −20 °C.

[†] $\Delta\Delta\delta_{RS}T = |\delta_R T - \delta_S T|$, where δ_R^T and δ_S^T are chemical shifts of NH protons of (*RS*)-**2** in the presence of 1 equiv of (*S*)-**1** at a given temperature *T* (°C).

Table 1
Selected ^{1}H NMR data for equimolar mixtures (*S*)-**1a**–**m** and (*RS*)-**2a** (D,L-Bz-Leu-OMe)

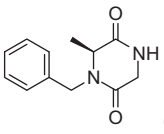
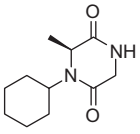
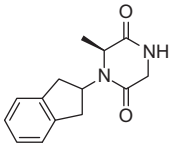
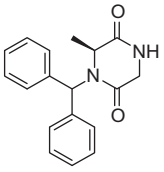
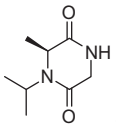
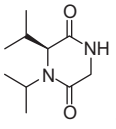
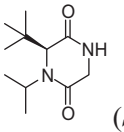
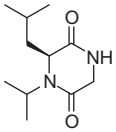
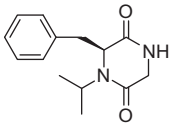
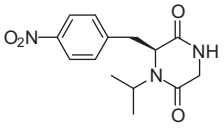
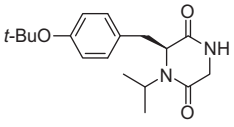
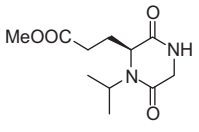
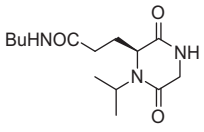
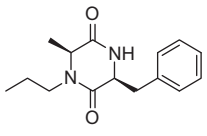
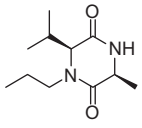
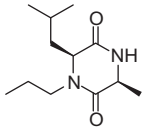
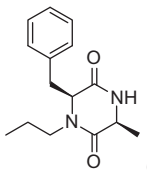
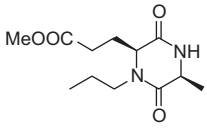
Compound **1**	*T* (°C)	Chemical shift of NH of (*RS*)-**2a** (ppm)			NH of **1**
		δ_R	δ_S	$\delta_R-\delta_S$	δ (ppm)
 (*S*)-**1a**	29	6.750	6.717	0.033	7.150
	0	6.948	6.875	0.073	7.704
	−20	7.129	7.025	0.104	8.088
 (*S*)-**1b**[a]	29	6.646	6.618	0.028	6.667
	0	6.801	6.738	0.063	7.246
	−20	6.956	6.862	0.094	7.684
 (*S*)-**1c**	29	6.737	6.707	0.030	7.050
	0	6.936	6.869	0.067	7.600
	−20	7.122	7.024	0.098	7.986
 (*S*)-**1d**	29	6.744	6.692	0.052	7.081
	0	6.948	6.848	0.100	7.671
	−20	7.15[b]	7.005	0.14[b]	8.129
 (*S*)-**1e**	29	6.756	6.717	0.039	7.057
	0	6.960	6.882	0.078	7.613
	−20	7.155	7.042	0.113	8.012
 (*S*)-**1f**	29	6.732	6.702	0.030	6.933
	0	6.934	6.865	0.069	7.5[b]
	−20	7.108	7.005	0.103	7.902
 (*S*)-**1g**	29	6.704	6.677	0.027	6.811
	0	6.890	6.828	0.062	7.379
	−20	7.076	6.980	0.096	7.85[b]
 (*S*)-**1h**	29	6.743	6.707	0.036	6.930
	0	6.952	6.867	0.085	7.52[b]
	−20	7.090	6.966	0.124	7.896
 (*S*)-**1i**	29	6.748	6.693	0.055	6.576
	0	6.948	6.822	0.126	6.974
	−20	7.19[b]	6.954	>0.2[b]	7.371
 (*S*)-**1j**	29	6.733	6.686	0.047	6.572
	0	6.965	6.841	0.123	6.081
	−20	7.216	6.989	0.227	~7.35[b]
 (*S*)-**1k**	29	6.734	6.689	0.045	6.481
	0	~7.0[b]	6.844	> 0.15[b]	6.991
	−20	7.195	6.990	0.205[b]	~7.4[b]

Table 1 (*continued*)

Compound **1**	T (°C)	Chemical shift of NH of (*RS*)-**2a** (ppm)			NH of **1**
		δ_R	δ_S	$\delta_R-\delta_S$	δ (ppm)
 (*S*)-**1l**	29	6.732	6.698	0.034	6.969
	0	6.935	6.852	0.083	7.51[b]
	−20	7.150	7.012	0.138	7.949
 (*S*)-**1m**	29	6.741	6.729	0.012	6.908
	0	6.925	6.880	0.045	7.400
	−20	7.067	7.005	0.062	7.702

[a] c = 0.0365 M.
[b] Overlapped by the signals of the aromatic protons.

Table 2
Selected ^{1}H NMR data for equimolar mixtures (*S*,*S*)-**1n–r** and (*RS*)-**2a** (**2a** = D,L-Bz-Leu-OMe)

Compound **1**	T (°C)	Chemical shift of NH of (*RS*)-**2a** (ppm)			NH of **1**
		δ_R	δ_S	$\delta_R-\delta_S$	δ (ppm)
 (*S*)-**1n**	29	6.760	6.712	0.052[b]	6.769
	0	7.012	6.908	0.104	7.362
	−20	7.27[a]	7.096	0.17[a]	7.790
 (*S*)-**1o**	29	6.734	6.734	0	6.991
	0	6.928	6.909	0.019	[a]
	−20	7.103	7.074	0.029	7.889
 (*S*)-**1p**	29	6.717	6.717	0	7.050
	0	6.903	6.909	0.026	7.600
	−20	7.071	7.029	0.042	7.986
 (*S*)-**1q**	29	6.707	6.707	0	6.790
	0	6.911	6.877	0.034	7.3[a]
	−20	7.123	7.04[b]	0.12[b]	7.695
 (*S*)-**1r**	29	6.731	6.709	0.022	7.073
	0	6.920	6.874	0.046	7.588
	−20	7.097	7.020	0.077	7.973

[a] Overlapped by the signals of the aromatic protons.
[b] Overlapped by the broad singlet of the N(1)–*H*.

2.4. Determination of binding constants, K_R and K_S

As shown previously, 1,6-disubstituted piperazine-2,5-diones (*S*)-**1** undergo association with various amino acid esters via C=*O*···*H*–N hydrogen bonded associates in $CDCl_3$ solution,[12–14] which is exhibited as a splitting (doubling) of signals in the NMR spectra (cf. Figs. 1–3). In addition to the previously determined binding constants, K_R = 45.4 M^{-1} and K_S = 38.6 M^{-1}, for the association of (*RS*)-**2a** with CSA (*S*)-**1a** ($\Delta\Delta\delta_{RS}^{-20}$∼0.104 ppm),[13] also K_R and K_S for association of the (*R*)- and the (*S*)-enantiomer of **2a** with the most effective CSA (*S*)-**1j** ($\Delta\Delta\delta_{RS}^{-20}$∼0.227 ppm) were determined by NMR titration. Thus, ^{1}H NMR spectra of mixtures of (*S*)-**1j** (c = 2.5→100 mM) and (*RS*)-**2a** (c = 5 mM) were taken in $CDCl_3$ at −20 °C to give the complexation-induced shifts, $\Delta\delta_R = \delta^0-\delta_R$ and $\Delta\delta_S = \delta^0-\delta_S$,[‡] for the NH protons of each enantiomer of a guest molecule, (*R*)-**2a** and (*S*)-**2a**, at different concentrations of a host molecule (*S*)-**1j** (Table 4). Benesi–Hildebrand treatment[20] of these data then furnished the complexation-induced

[‡] δ^0 Is chemical shift (ppm) of a NH proton of pure compound (*RS*)-**2a** (c = 5 mM) in $CDCl_3$ at −20 °C.

shifts at saturation binding, $\Delta\delta_{maxR} = -0.367$ ppm, $\Delta\delta_{maxS} = -0.254$ ppm, and the binding constants, $K_R = 40.0\ M^{-1}$ for the (*R*)-**2a** and $K_S = 27.6\ M^{-1}$ for the (*S*)-**2a** (Fig. 4, Table 4).

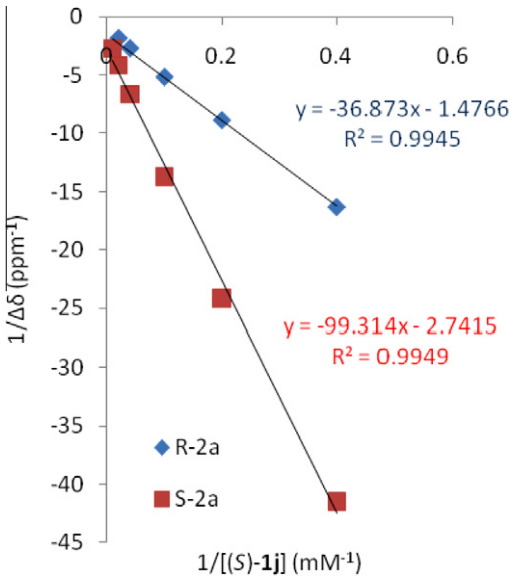


Figure 4. Benesi-Hildebrand data treatment for a mixture of (*RS*)-**2a** and (*S*)-**1j**. A double reciprocal plot of complexation-induced shifts for the NH proton of (*R*)-**2a** and (*S*)-**2a** ($1/\Delta\delta_R$ and $1/\Delta\delta_S$) as a function of 1/[(*S*)-**1j**] with slopes $1/K\Delta\delta_{maxR} = -36.87$ mM ppm^{-1} and $1/K\Delta\delta_{maxS} = -99.31$ mM ppm^{-1} and intercepts $1/\Delta\delta_{maxR} = -1.477$ ppm^{-1} and $1/\Delta\delta_{maxS} = -2.742$ ppm^{-1}, respectively.

2.5. Determination of the enantiomer composition by ^{1}H NMR

Although the applicability of CSA (*S*)-**1a** for the determination of the ee of α-acylamino acid derivatives has already been demonstrated,[12,14] we decided to provide additional examples. The first one chosen was determination of the ee of enantiomerically enriched (*S*)-**2a** with CSA (*S*)-**1l**. Known mixtures of enantiomers were weighed from the (*S*)- and (*RS*)-isomer of **2a**. For each known isomeric mixture, the enantiomer composition was then re-determined by ^{1}H NMR in $CDCl_3$ at −20 °C in the presence of equimolar amounts of (*S*)-**1l**. Enantiomeric excesses were established from the relative intensities of the signals of the NH group of (*R*)-**2a** and (*S*)-**2a**. The measured values were in good agreement with the actual enantiomer composition (Fig. 5).

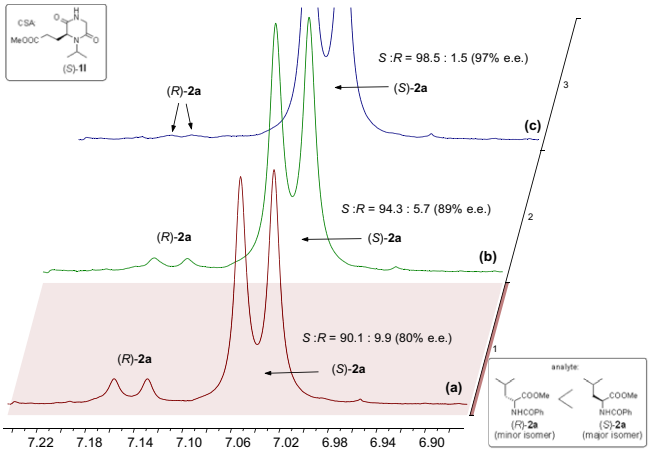


Figure 5. Signals for the NH protons in the partial ^{1}H NMR spectra ($CDCl_3$, −20 °C) of enantiomerically enriched and (*S*)-**2a** in the presence of (*S*)-**1l**. Actual (weighed) enantiomer composition: (a) (*R*)-**2a**: (*S*)-**2a** = 90.0:10.0 (80% ee); (b) (*R*)-**2a**: (*S*)-**2a** = 94.9:5.1 (90% ee); (c) (*R*)-**2a**: (*S*)-**2a** = 98.9:1.1 (98% ee).

The second example was the determination of the ee of diketopiperazine (*S*)-**1m** prepared by heating (*S*)-**1l** with excess butylamine in methanol. We assumed that such harsh conditions could lead to partial racemisation of (*S*)-**1m**. Indeed, the ^{1}H NMR spectrum of (*S*)-**1m** in the presence of a slight excess of (*S*)-**1l** in $CDCl_3$ at −20 °C revealed a splitting of signals for both NH protons of (*S*)-**1m**. An enantiomeric excess of 82% for (*S*)-**1m** was determined from the relative intensities of signals of NH groups of (*R*)-**1m** and (*S*)-**1m** (Fig. 6).

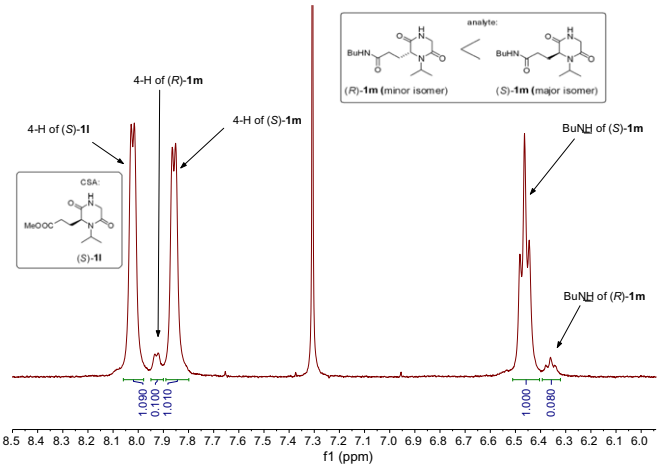


Figure 6. Signals for the NH protons in the partial ^{1}H NMR spectra ($CDCl_3$, −20 °C) of enantiomerically enriched and (*S*)-**1m** in the presence of a slight excess of (*S*)-**1l**. Right: determination of ee from the intensities of signals of BuNH protons, (*R*)-**1m**:(*S*)-**1m** = 92.6:7.4 (85% ee). Left: determination of ee from the intensities of signals of 4-NH protons, (*R*)-**1m**:(*S*)-**1m** = 91.0:9.0 (82% ee).

2.6. Correlation between the chemical shifts of the NH protons and the absolute configuration of 2a

Finally, a correlation between the chemical shifts of the NH protons and absolute configuration of *N*-benzoylleucine ester **2a** was tested next. The ^{1}H NMR spectrum of enantiomerically enriched leucine derivative (*S*)-**2a** was taken in $CDCl_3$ at −20 °C in the presence of CSA (*S*)-**1e**, (*S*)-**1h**, (*S*)-**1i**, and (*S*)-**1l** and the enantiomers of **2a** were identified by their relative intensities. In the presence of each CSA, the NH proton of (*R*)-**2a** appeared at a lower field than the NH proton of (*S*)-**2a** (Fig. 7). This clear correlation was in agreement with previous observations.[13] It is noteworthy that, in the presence of (*S*)-**1l**, the 4-NH proton of diketopiperazine (*R*)-**1m** also appeared at a lower field than that of the (*S*)-isomer (cf. Fig. 6).

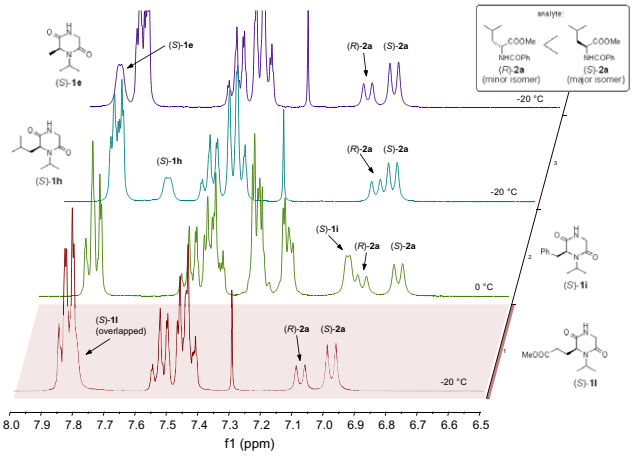
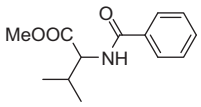
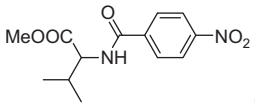
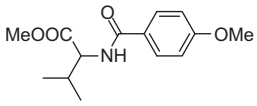
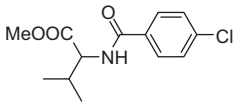
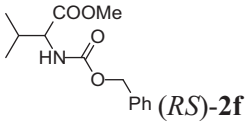
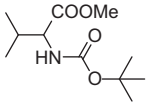
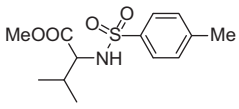
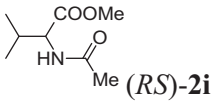
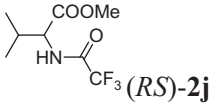


Figure 7. Signals for the NH protons in the partial ^{1}H NMR spectra ($CDCl_3$, 0 °C or −20 °C) of enantiomerically enriched (*S*)-**2a** in the presence of (*S*)-**1e**,**h**,**i**,**l** taken in $CDCl_3$ at 0 °C or −20 °C.

Table 3
Selected ^{1}H NMR data for equimolar mixtures (*S*)-**1a** and (*RS*)-**2b**–**j** (**2b**–**j** = D,L-Acyl-Val-OMe)

Compound (*RS*)-**2**	*T* (°C)	Chemical shift of NH of (*RS*)-**2a** (ppm)			NH of **1**
		δ_R	δ_S	$\delta_R-\delta_S$	δ (ppm)
 (*RS*)-**2b**[13]	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
 (*RS*)-**2c**	29	6.948	6.918	0.030	7.103
	0	7.156	7.087	0.069	7.689
	−20	[a]	[a]	[a]	8.096
 (*RS*)-**2d**	29	6.637	6.611	0.026	7.097
	0	6.763	6.712	0.051	7.698
	−20	6.874	6.793	0.081	8.125
 (*RS*)-**2e**	29	6.744	6.716	0.028	7.092
	0	6.901	6.845	0.056	7.680
	−20	7.037	6.952	0.085	8.089
 (*RS*)-**2f**	29	5.391	5.366	0.025	7.051
	0	5.517	5.471	0.046	7.615
	−20	5.626	5.554	0.072	8.020
 (*RS*)-**2g**	29	5.062	5.062	0	7.146
	0	5.133	5.133	0	7.730
	−20	5.194	5.179	0.015	8.159
 (*RS*)-**2h**	29	5.423	5.388	0.035	7.076
	0	5.632	5.569	0.063	7.633
	−29	5.800	5.713	0.087	8.011
 (*RS*)-**2i**	29	6.184	6.159	0.025	7.156
	0	6.393	6.354	0.039	7.730
	−20	6.576	6.513	0.063	8.122
 (*RS*)-**2j**	29	[b]	[b]	[b]	7.103
	0	[a]	7.174	>0.06[a]	7.685
	−20	7.501	[a]	>0.1[a]	8.092

[a] Overlapped by the signals of the aromatic protons.
[b] The signal for the N*H*COCF$_3$ group appeared as a broad signal (quazi singlet) partially overlapped by the signal (broad singlet) of the N(1)–*H*.

Table 4
^{1}H NMR titration data for mixtures (*S*)-**1j** (*c* = 2.5→100 mM) and (*RS*)-**2a** (*c* = 5 mM)[a,b]

[(*S*)-**1j**] (mM)	δ_R (ppm)	δ_S (ppm)	$\Delta\delta_R$ (ppm)	$\Delta\delta_S$ (ppm)
2.5	6.6687	6.6314	−0.0614	−0.0241
5	6.7207	6.6487	−0.1134	−0.0414
10	6.8027	6.6803	−0.1954	−0.073
25	6.9849	6.7576	−0.3776	−0.1503
50	7.1681	6.8461	−0.5608	−0.2388
100	7.3929	6.9732	−0.7856	−0.3659

[a] All spectra were taken in $CDCl_3$ at −20 °C.
[b] δ^0_{NH} of **2a** = 6.6073 ppm (*c* = 5 mM).

3. Conclusion

The present study showed the following effects of substituents on chiral solvating properties of (*S*)-1,6-dialkylpiperazine-2,5-diones (*S*)-**1** for racemic *N*-benzoylleucine ester (*RS*)-**2a**: (a) with the exception of a bulky benzyhydryl group, the effect of N(1)-substituents was negligible; (b) branching of the C(6)-substituent (Me→*i*-Pr→*t*-Bu) did not affect the enantioselectivity; (c) substituted methylene groups at the C(6) position (*i*-Bu, CH_2Ar, and CH_2CH_2COOMe) (significantly) increased the resolution of enantiomers; (d) a hydrogen bond acceptor group (CH_2CH_2COOMe) at C(6) increased the enantioselectivity, while a hydrogen bond donor group ($CH_2CH_2CONHBu$) at the same position decreased the enantioselectivity; and (e) an additional *syn*-oriented substituent at the C(3) position significantly decreased the enantioselectivity. A typical resolution of enantiomers of (*RS*)-**2a** in $CDCl_3$ solution at −20 °C was ~0.1 ppm, while the best result, $\Delta\Delta\delta_{RS}^{-20}$~0.227 ppm, was obtained with (*S*)-1-isopropyl-6-(4-nitrobenzyl)piperazine-2,5-dione (*S*)-**1j**. Additionally, the binding constants for the association of the (*R*)- and the (*S*)-enantiomer of **2a** with CSA (*S*)-**1j**, $K_R = 40.0^{-1}$ and $K_S = 27.6\ M^{-1}$, were determined by NMR titration, and showed a clear correlation between the chemical shift of the NH protons and the absolute configuration of **2a** with CSA (*S*)-**1e**,**h**,**i**,**l**. Two examples of the application of L-glutamic acid derived CSA (*S*)-**1l** for the determination of enantiomer composition were given. In conclusion, (*S*)-1,6-dialkylpiperazine-2,5-

diones (*S*)-**1** are easily available and effective non-lanthanide CSA's for the determination of the enantiomer composition of α-acylamino acid esters **2** by ^{1}H NMR with resolution, $\Delta\Delta\delta_{RS}^{-20} > 0.1$ ppm for the NH protons of each enantiomer of **2**. In terms of practical application, (possible) overlap between the aromatic protons of highly effective 1 (or 6)-benzyldiketopiperazines (*S*)-**1a**,**c**,**d**,**i**–**k** and the NH protons of (*RS*)-**2** can be a serious disadvantage. Therefore, 1,6-dialkylpiperazine-2,5-diones (*S*)-**1b**,**c**,**e**–**h**,**l** which had no aryl groups and somewhat lower resolution are considered as more suitable CSA for α-acylamino acid esters and analogous compounds.

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 spectrum on a Perkin-Elmer Spectrum BX FTIR spectrophotometer. Microanalysis was performed on a Perkin-Elmer CHN Analyzer 2400 II.

Dimethyl (*S*)-glutamate hydrochloride, (*RS*)-leucine, (*RS*)-valine, and 1-butylamine are commercially available (Sigma–Aldrich). (*S*)-1-Benzyl-6-methylpiperazine-2,5-dione (*S*)-**1a**,[17] diketopiperazines (*S*)-**1b**–**m** and (*S*,*S*)-**1n**–**r**,[16] (*RS*)-leucine methyl ester hydrochloride (*RS*)-**3a**, and (*RS*)-valine methyl ester hydrochloride (*RS*)-**3b** were prepared according to the literature procedures.[19] (*RS*)-*N*-Benzoylleucine methyl ester (*RS*)-**2a**, and (*RS*)-*N*-benzoylvaline methyl ester (*RS*)-**3b** were prepared from (*RS*)-**3a** and (*RS*)-**3b**, respectively, following the literature procedure for the preparation of the corresponding (*S*)-isomers, (*S*)-**2a** and (*S*)-**2b**.[18]

Sources 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; (ii) L-valine methyl ester hydrochloride (Fluka AG), product number 94670, puriss., ⩾99.0% (dried material AT), $[\alpha]_D^{20} = +23 \pm 1$ (*c* 2, MeOH), mp 165–170 °C (dec), ee not specified; (iii) L-*tert*-leucine methyl ester hydrochloride (Aldrich), product number 61891, puriss., ⩾99.0% (dried material AT), $[\alpha]_D^{20} = +17.5 \pm 1$ (*c* 1, MeOH), ee not specified; (iv) L-leucine methyl ester hydrochloride (Fluka AG), product number 61890 puriss., ⩾99.0% (dried material AT), $[\alpha]_D^{20} = +20 \pm 1$ (*c* 4.5, MeOH), ee ⩾98%; (v) L-phenylalanine methyl ester hydrochloride (Fluka AG), product number 78090 puriss., ⩾99.0% (dried material AT), $[\alpha]_D^{20} = +38 \pm 1.5$ (*c* 2, MeOH), mp 155–162 °C (dec), ee not specified; (vi) (*S*)-(+)-4-nitrophenylalanine methyl ester hydrochloride 97% (Aldrich), product number 658421, $[\alpha]_D^{20} = +7.0 - 13.0$ (*c* 1%, water), ee not specified; (vii) *O*-*tert*-butyl-L-tyrosine methyl ester hydrochloride, ⩾98.0%, product number 96627 (Aldrich), optical rotation and ee not specified; (viii) L-glutamic acid dimethyl ester hydrochloride (Aldrich), product number 49560 puriss., ⩾99.0% (dried material AT), $[\alpha]_D^{20} = 26 \pm 1$ (*c* 5, H_2O), ee not specified.

4.2. Synthesis of (*S*)-*N*-butyl-3-(1-isopropyl-2,5-dioxopiperazin-6-yl)propanamide (*S*)-1m

1-Butylamine (0.49 mL, 5 mmol) was added to a solution of ester (*S*)-**1l** (242 mg, 1 mmol) in methanol (5 mL). The mixture was refluxed for 20 h, after which volatile components were evaporated in vacuo. The semi-solid residue was dissolved in dichloromethane (25 mL) and the solution was washed with 1 M $NaHSO_4$ (3 × 10 mL) and brine (3 × 10 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and the filtrate was evaporated in vacuo to give (*S*)-**1m** as a viscous oil, which crystallized upon standing at rt for two days. Yield: 152 mg (54%) of white crystals; mp 70–73 °C; $[\alpha]_D^{27} = +94.6$ (*c* 0.5, CH_2Cl_2); (*R*)-**1m**: (*S*)-**1m** = 91:9 (82% ee). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.92 (3H, t, *J* = 7.0 Hz, $CH_3CH_2CH_2CH_2$); 1.24 and 1.30 (6H, 2d, 1:1, *J* = 6.9 Hz, $(CH_3)_2CH$); 1.32–1.43 (2H, m, $CH_3CH_2CH_2CH_2$); 1.44–1.57 (2H, m, $CH_3CH_2CH_2CH_2$); 1.88–2.05 and 2.22–2.40 (4H, 2 m, 1:3, CH_2CH_2COOMe); 3.32 (2H, m, $CH_3CH_2CH_2CH_2NH$); 3.80–3.91 (2H, m, 6-H and 3-Ha); 4.07 (1H, d, *J* = 17.0 Hz, 3-Hb); 4.46 (1H, septet, *J* = 6.8 Hz, $(CH_3)_2CH$); 6.50 (1H, br t, *J* = 5.5 Hz, N*H*Bu); 7.53 (1H, br d, *J* = 4.1 Hz, 4-N*H*). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.8, 20.0, 20.1, 20.7, 28.8, 31.7, 31.8, 39.4, 45.4, 47.5, 56.42, 164.8, 169.7, 171.4. *m*/*z* (ESI) = 282 (M^+−H). *m*/*z* (HRMS) Found: 282.1810 (M^+−H). $C_{14}H_{24}N_3O_3$ requires: *m*/*z* = 282.1818. (Found: C, 57.59; H, 9.13; N, 14.28. $C_{14}H_{25}N_3O_3{\cdot}½H_2O$ requires: C, 57.51; H, 8.96; N, 14.37.); ν_{max} (KBr) 3306, 2958, 2929, 1676 (C═O), 1640 (C═O), 1559, 1457, 1319, 1204, 1109, 668 cm^{-1}.

4.3. Synthesis of (*RS*)-*N*-benzoylleucine methyl ester (*RS*)-2a

This compound was prepared from (*RS*)-**3a** (0.908 g, 5 mmol) and benzoyl chloride (0.703 g, 580 μL, 5 mmol) following a literature procedure for the synthesis of (*S*)-**2a**.[18] Yield: 2.245 g (90%) of white solid; mp 96–98 °C. Spectroscopic data were in agreement with the literature data for the (*S*)-isomer.[18]

4.4. Synthesis of (*RS*)-*N*-benzoylvaline methyl ester (*RS*)-2b

Prepared from (*RS*)-**3b** (0.838 g, 5 mmol) and benzoyl chloride (0.703 g, 580 μL, 5 mmol) following the literature procedure for the synthesis of (*S*)-**2a**.[18] Yield: 1.972 g (84%) of white solid; mp 89–90 °C. Spectroscopic data were in agreement with the literature data for the (*S*)-isomer.[18]

4.5. General procedure for the synthesis of (*RS*)-*N*-acylvaline methyl esters (*RS*)-2c–j

A solution of acid chloride or anhydride (5 mmol) in anhydrous dichloromethane (2.5 mL) was added slowly to a stirred cold (0 °C) mixture of (*RS*)-valine methyl ester hydrochloride (*RS*)-**3b** (0.838 g, 5 mmol), dichloromethane (12.5 mL), and 4-methylmorpholine (1.38 mL, 12.5 mmol) and the reaction mixture was stirred at rt for 12 h. Water (12.5 mL) was added, and the mixture was stirred at rt for 5 min. after which the phases were separated. The organic phase was washed with 1 M aq $NaHSO_4$ (3 × 15 mL), satd aq $NaHCO_3$ (3 × 15 mL), and brine (3 × 15 mL), dried over anhydrous sodium sulfate, filtered, and the filtrate was evaporated in vacuo to give (*RS*)-**2c**–**j**.

4.5.1. (*RS*)-*N*-(4-Nitrobenzoyl)valine methyl ester (*RS*)-2c

Prepared from (*RS*)-**3b** and 4-nitrobenzoyl chloride (0.928 g, 5 mmol). Yield: 0.947 g (68%) of a yellowish solid; mp 83–86 °C (from EtOAc/hexanes). Spectroscopic data were in agreement with the literature data for the (*S*)-isomer.[20]

4.5.2. (*RS*)-*N*-(4-Methoxybenzoyl)valine methyl ester (*RS*)-2d

Prepared from (*RS*)-**3b** and 4-methoxybenzoyl chloride (0.853 g, 677 μL, 5 mmol). Yield: 0.171 g (13%) of a white solid; mp 88–90 °C (from EtOAc/hexanes). Spectroscopic data were in agreement with the literature data for the (*S*)-isomer.[21]

4.5.3. (*RS*)-*N*-(4-Chlorobenzoyl)valine methyl ester (*RS*)-2e

Prepared from (*RS*)-**3b** and 4-chlorobenzoyl chloride (0.875 g, 641 μL, 5 mmol). Yield: 0.747 g (55%) of a white solid; mp

105–106 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 and 1.01 (6H, 2d, 1:1, J = 6.8 Hz, $(CH_3)_2CH$); 2.28 (1H, doublet of septet, J = 5.2, 6.8 Hz, $(CH_3)_2CH$); 3.78 (3H, s, OCH_3); 4.77 (1H, dd, J = 4.9, 8.6 Hz, CHNH); 6.58 (1H, br d, J = 8.0 Hz, CHNH); 7.43 and 7.75 (4H, 2dt, 1:1, J = 8.7, 2.2 Hz, C_6H_4). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 18.2, 19.1, 31.8, 52.4, 57.7, 128.7, 129.0, 132.7, 138.2, 166.4, 172.8. m/z (ESI) = 270 (MH^+). m/z (HRMS) Found: 270.0900 (MH^+). $C_{13}H_{17}ClNO_3$ requires: m/z = 270.0897. (Found: C, 57.72; H, 5.77; N, 5.31. $C_{13}H_{16}ClNO_3$ requires: C, 57.89; H, 5.98; N, 5.19.); ν_{max} (KBr) 3330, 2973, 1719 (C=O), 1664 (C=O), 1597, 1529, 1488, 1446, 1373, 1321, 1244, 1218, 1174, 1162, 1091, 1015, 847, 764 cm^{-1}.

4.5.4. (*RS*)-*N*-(Benzyloxycarbonyl)valine methyl ester (*RS*)-2f

Prepared from (*RS*)-**3b** and benzyl chloroformate (0.898 g, 751 μL, 5 mmol). Yield: 0.820 g (62%) of a colourless oil. Physical and spectroscopic data were in agreement with the literature data for the (*RS*)-isomer[22a] and the (*S*)-isomer.[22b]

4.5.5. (*RS*)-*N*-(*tert*-Butoxycarbonyl)valine methyl ester (*RS*)-2g

Prepared from (*RS*)-**3b** and Boc-anhydride (1.091 g, 5 mmol). Yield: 0.990 g (86%) of a colourless oil. Spectroscopic data were in agreement with the literature data for the (*S*)-isomer.[23]

4.5.6. (*RS*)-*N*-Tosylvaline methyl ester (*RS*)-2h

Prepared from (*RS*)-**3b** and tosyl chloride (0.953 g, 5 mmol). Yield: 0.713 g (50%) of white solid; mp 97–99 °C (from CH_2Cl_2/*n*-hexane). Spectroscopic data were in agreement with the literature data for the (*S*)-isomer.[24]

4.5.7. (*RS*)-*N*-Acetylvaline methyl ester (*RS*)-2i

Prepared from (*RS*)-**3b** and acetyl chloride (0.393 g, 356 μL, 5 mmol). Yield: 0.579 g (67%) of white solid; mp 58–61 °C, lit[25a] mp 59–61 °C. Spectroscopic data were in agreement with the literature data for the (*RS*)-isomer[25a] and the (*S*)-isomer.[25b]

4.5.8. (*RS*)-*N*-Trifluoroacetylvaline methyl ester (*RS*)-2j

Prepared from (*RS*)-**3b** and trifluoroacetic anhydride (1.050 g, 695 μL, 5 mmol). Yield: 0.818 g (72%) of colourless oil. Spectroscopic data were in agreement with the literature data for the (*S*)-isomer.[26]

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