AN EFFICIENT BIS-THIOUREA CSA FOR THE ENANTIODISCRIMINATION OF AMINO ACID DERIVATIVES BY NMR SPECTROSCOPY

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INRODUCTION

Different response of biological systems depending on the divergent pharmacological activity exhibited by enantiomers of an active ingredient has urged the improvement of methods[1] for monitoring and quantifying stereoisomers.[2]

On this issue, NMR spectroscopy makes available reliable and accurate tools based on the use of chiral auxiliaries, aimed to transfer the enantiomers in a diastereoisomeric environment. Among chiral auxiliaries for NMR spectroscopy, chiral solvating agents (CSA) are greatly advantageous on the practical point of view, being simply mixed into NMR tube to the chiral compound under investigation, before NMR observation.[3-5] Diastereoisomeric solvates are formed in solution, which are intrinsically anisochronous and, in principle, can be differentiated and quantified in the NMR spectra (Figure 1).

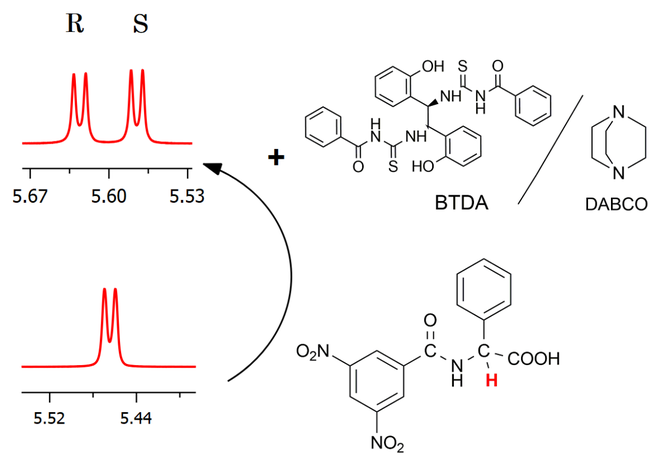


Figure 1. ^{1}H NMR enantiodiscrimination of amino acid derivative in the presence of DABCO and **BTDA** (30 mM, 1:1:1).

AIM OF THE WORK

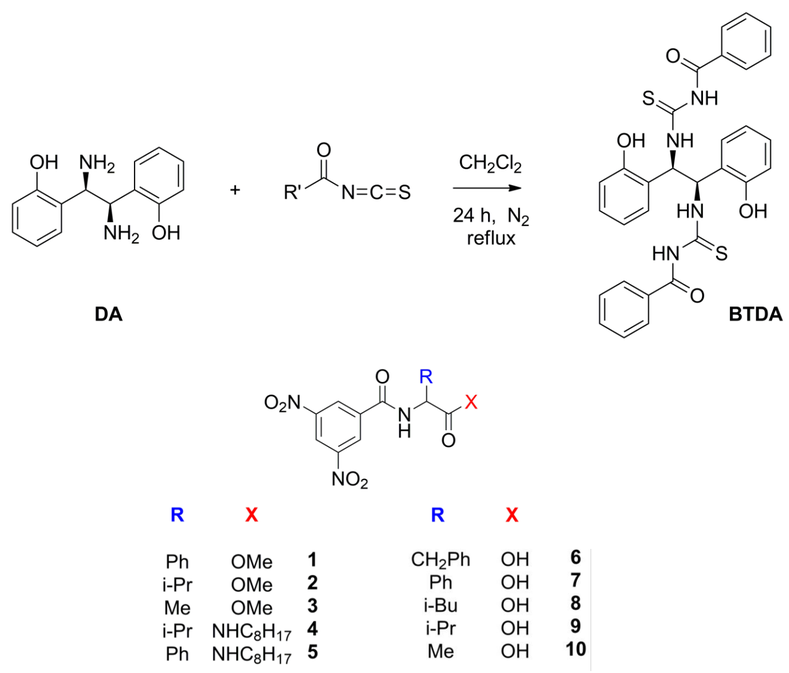
Ability of the new bis-thiourea CSA **BTDA** (Figures 1,2) to differentiate enantiomers of *N*-(3,5)-dinitrobenzoyl α-amino acid derivatives (Figures 1,2), in ternary mixtures containing DABCO, was investigated by NMR (Figure 1).[6]

Chiral discrimination mechanism was carefully investigated by NMR, through the determination of the complexation stoichiometries, association constants and stereochemistry of the diastereomeric solvates.

RESULTS

SYNTHESIS

The reaction of diamine **DA** (Figure 2) with 2 equivalents of benzoyl isothiocyanate proceeded selectively and quantitively at the amino groups, leading to the thiourea derivative **BTDA** (Figure 2).



R	X		R	X	
Ph	OMe	1	CH_2Ph	OH	6
i-Pr	OMe	2	Ph	OH	7
Me	OMe	3	i-Bu	OH	8
i-Pr	NHC_8H_{17}	4	i-Pr	OH	9
Ph	NHC_8H_{17}	5	Me	OH	10

Figure 2. Synthesis of **BTDA** and structures of amino acid derivatives **1**-**10**.

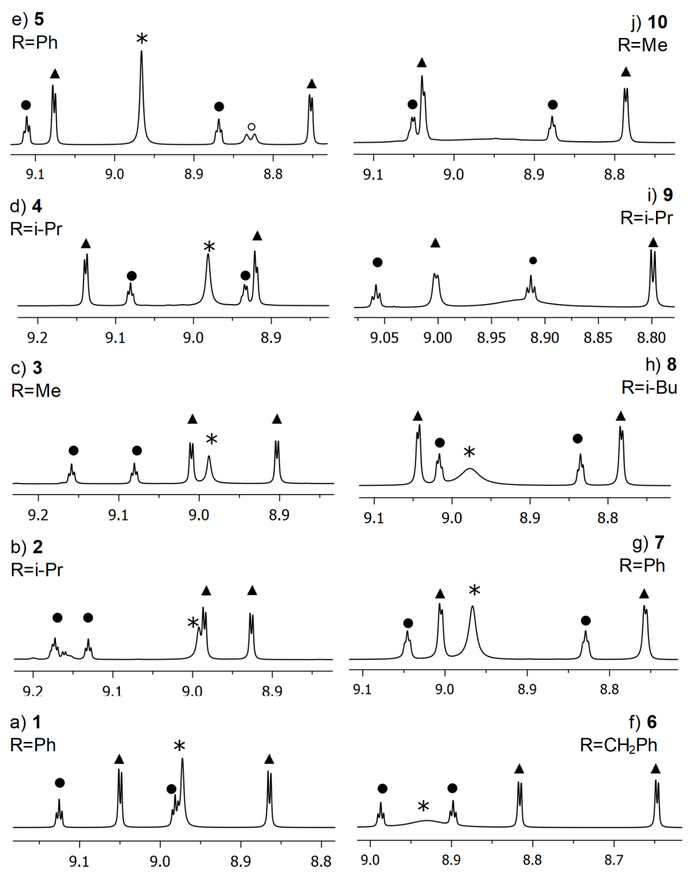


Figure 3. ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C) spectral regions corresponding to ortho (▲) and para (●) DNB protons of 1:1 mixture **BTDA** (30 mM)/racemic: a) **1**, b) **2**, c) **3**, d) **4**, e) **5**, and of 1:1:1 mixture **BTDA** (30 mM)/DABCO/racemic: f) **6**, g) **7**, h) **8**, i) **9**, j) **10**. CSA resonances (*) and NH proton of **5** (○)

NMR ENANTIODISCRIMINATION EXPERIMENTS

^{1}H enantiodiscrimination experiments were carried out by adding one equivalent of **BTDA** and DABCO to the $CDCl_3$ solution of the selected amino acid derivative (Figure 3 and Table 1). Remarkably higher nonequivalences ($\Delta\Delta\delta=|\Delta\delta_R-\Delta\delta_S|$) were detected respect to those obtained in the presence of previously reported monomer chiral auxiliary **TMA** (Figure 4).[7]

Table 1. ^{1}H-NMR (600 MHz, $CDCl_3$, 25 °C) nonequivalences (ΔΔδ, ppm) of **1**-**5** (30 mM) in the presence of **BTDA** (1:1) and of **6**-**10** (30 mM) in the presence of **BTDA**/DABCO (1:1:1).

	pDNB[a]	oDNB[b]	NH	CH[c]		pDNB[a]	oDNB[b]	NH	CH[c]
1	0.141	0.186	0.210	0.043	**6**	0.089	0.169	0.042	0.050
2	0.041	0.060	0.021	0.008	**7**	0.216	0.247	0.173	0.049
3	0.078	0.106	0.009	0.005	**8**	0.180	0.260	0.053	0.080
4	0.147	0.219	0.047	0.034	**9**	0.145	0.202	0.045	0.096
5	0.243	0.324	0.113	0.028	**10**	0.174	0.253	0.074	0.081

[a]Para and [b]ortho protons of DNB moiety. [c]Methine proton of chiral centre.

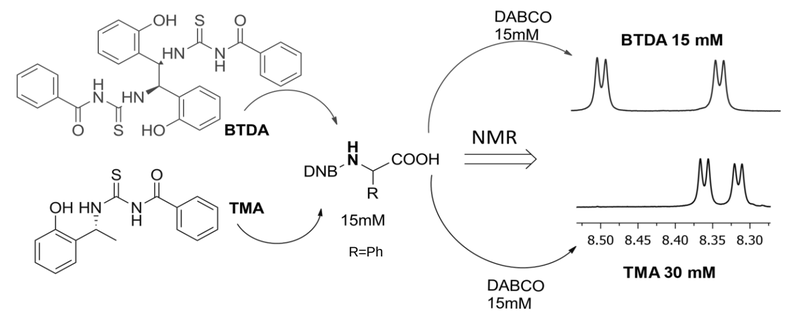


Figure 4. Comparison of nonequivalences of *NH* proton of amino acid derivatives in the presence of DABCO and **BTDA** or **TMA.**

ENANTIODIFFERENTIATION MECHANISM

The conformation of CSA and its diastereoisomeric solvates formed with the two enantiomers of **7** was defined by 1D and 2D ROE experiments. **BTDA** assumes a **cleft conformation** and by means of an extended hydrogen bonding network interacts with enantiomeric substrates, which bisect the major grooves of CSA structure (Figure 5). CSA/substrate interaction is mediated by DABCO, which produces relevant dipolar interactions both with amino acid protons and aromatic moieties of the CSA, suggesting that the base lies between all of these groups (Figure 6).

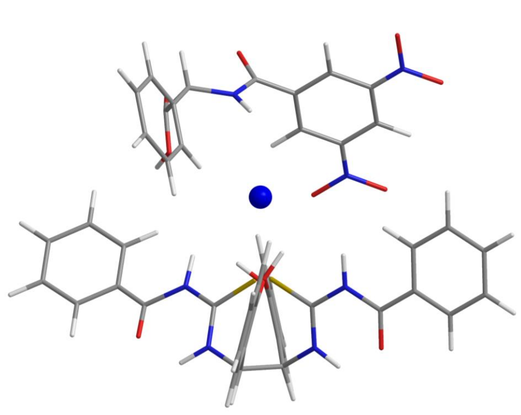


Figure 5. 3D representation of (*R*)-**7**/**BTDA**/DABCO according to NMR data.

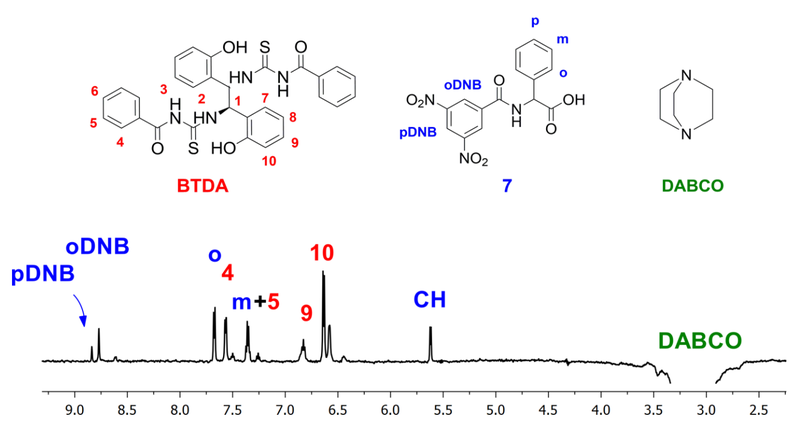


Figure 6. 1D-ROESY experiments (600 MHz, 30 mM, $CDCl_3$, 25 °C) with selective perturbation of DABCO protons in **7**/**BTDA**/DABCO mixtures (1:1:1).

A 1 to 1 complexation stoichiometry was found on the basis of Job's method (Figure 7) and the association constants, calculated by dilution data, were 297±13 M^{-1} and 54±3 M^{-1} for (*R*)- and (*S*)-**7**/**BTDA**/DABCO complexes, respectively (Figure 8) .

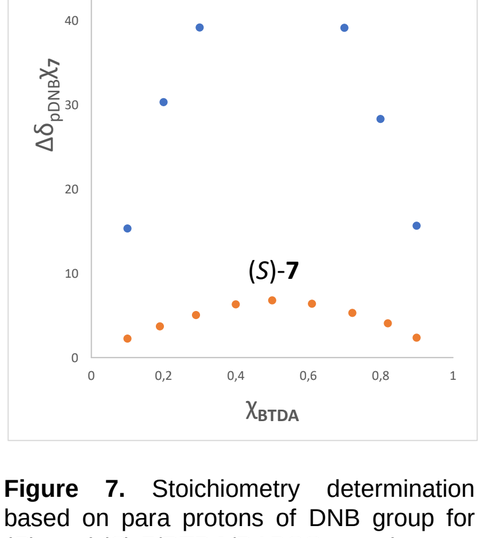


Figure 7. Stoichiometry determination based on para protons of DNB group for (*R*)- and (*S*)-**7**/**BTDA**/DABCO complexes.

$$C_0 = \frac{(\delta_{obs} - \delta_f)(\delta_b - \delta_f)}{K(\delta_b - \delta_{obs})^2}$$

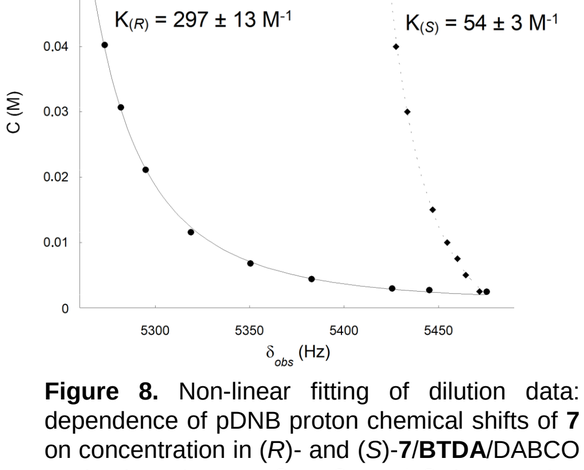


Figure 8. Non-linear fitting of dilution data: dependence of pDNB proton chemical shifts of **7** on concentration in (*R*)- and (*S*)-**7**/**BTDA**/DABCO equimolar mixtures. δ_{obs}, δ_b and δ_f in equation are the observed chemical shift, and the chemical shift in the bound and free state, respectively.

CONCLUSION

Dimeric thiourea **BTDA** represents a new tweezer-like artificial receptor with a remarkable enantiodiscriminating ability towards *N*-3,5-dinitrobenzoyl derivatives of amino acids. Electron-rich aromatic moieties of CSA originate attractive interactions with the electron-poor 3,5-dinitrophenyl group of the substrates. DABCO plays an active role in the enantiodiscrimination processes by acting as a bridge in the interaction between the carboxylate of the amino acid and with the acidic hydroxyl of the phenolic moiety.

REFERENCES: [1] T. H. Webb, C. S. Wilcox, Chem. Soc. Rev. 22, 383–395 (1993); [2] J. D. Morrison, Asymmetric Synthesis, 1, (1983); [3] F. Balzano, G. Uccello-Barretta, F. Aiello, In Chiral Analysis: Advances in Spectroscopy, Chromatography and Emerging Methods; Elsevier. Ch. 9, 367–427 (2018); [4] G. Uccello-Barretta, F. Balzano, Top. Curr. Chem. 341, 69–131 (2013); [5] T. J. Wenzel, Differentiation of Chiral Compounds Using NMR Spectroscopy; Wiley & Sons (2018); [6] A. Recchimurzo, C. Micheletti, G. Uccello-Barretta, F. Balzano, J. Org. Chem. 86, 7381-7389 (2021); [7] A. Recchimurzo, C. Micheletti, G. Uccello-Barretta, F. Balzano, J. Org. Chem. 85, 5342–5350 (2020).

REF [6]