



Synthesis and enantiomeric recognition studies of a novel 5,5-dioxophenothiazine-1,9 bis(thiourea) containing glucopyranosyl groups

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ARTICLE INFO

Article history:

Received 7 November 2012

Accepted 29 November 2012

ABSTRACT

A novel optically active 5,5-dioxophenothiazine-1,9 bis(thiourea) containing glucopyranosyl groups was synthesized and its enantiomeric recognition properties were examined towards the enantiomers of tetrabutylammonium salts of chiral α -hydroxy and *N*-protected α -amino acids using UV–vis spectroscopy.

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1. Introduction

Enantiomeric recognition, a special type of molecular recognition, is a ubiquitous and vital phenomenon in Nature. Examples of its action include the metabolism of single enantiomeric forms of amino acids and sugars in biosynthetic pathways. Since the individual enantiomers of a biologically active compound may have different pharmacological and toxicological properties, the determination of the enantiomeric composition of organic compounds is of great importance.

The carboxyl group is a particularly common functional group present in amino acids, enzymes, metabolic intermediates and several pharmaceutical molecules. Therefore, the synthesis and studies of sensor and selector molecules capable of discriminating between the enantiomers of chiral carboxylic acids are of great interest. The enantiomers of chiral carboxylic acids can be differentiated not only in their neutral forms, but also as their carboxylates by enantioselective anion sensors.^{1–4} The most frequently used chiral building blocks in anion sensors are amino acids, BINOL and steroid units.^{1–4} Although many monosaccharides are inexpensive sources of chirality and are used as asymmetric units in many host molecules,^{5–7} there are only a few enantioselective anion sensors that incorporate sugar units.^{8–10} Yoon et al have reported on the synthesis and enantiomeric recognition studies of anthracene- and azophenol-based bis(thiourea) type receptors containing tetra-*O*-acetyl- β -D-glucopyranosyl groups.^{8,9} They found that the latter sensor molecules showed the highest enantioselectivity for the enantiomers of the tetrabutylammonium salt of *tert*-butoxycarbonyl-protected alanine.

Herein we report the synthesis of a novel 5,5-dioxophenothiazine bis(thiourea) containing tetra-*O*-acetyl- β -D-glucopyranosyl

groups (scheme 1) and studies on its enantiomeric recognition ability towards the enantiomers of different optically active tetrabutylammonium carboxylates in acetonitrile.

2. Results and discussion

2.1. Synthesis

The synthesis of the new optically active phenothiazine bis(thiourea) **1** was carried out as shown in Scheme 1. Diamine **2**¹¹ was reacted with tetra-*O*-acetyl- β -D-glucopyranosyl isothiocyanate **3**¹² in pyridine to give bis(thiourea) derivative **1**.

2.2. Anion recognition studies

The enantiomeric recognition ability of receptor **1** was studied in acetonitrile towards the enantiomers of the tetrabutylammonium salts of mandelic acid (Man), *tert*-butoxycarbonyl-protected phenylglycine (Boc-Phe), *tert*-butoxycarbonyl-protected phenylalanine (Boc-Phe) and *tert*-butoxycarbonyl-protected alanine (Boc-Ala) (Fig. 1) using UV–vis spectroscopy.

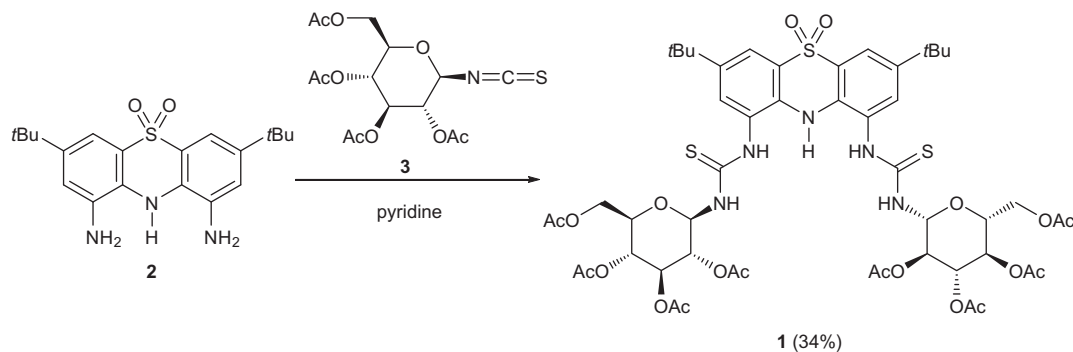
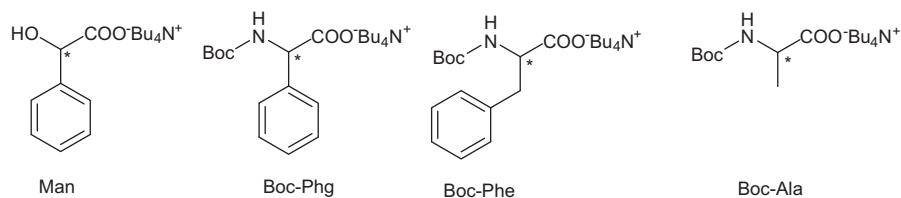
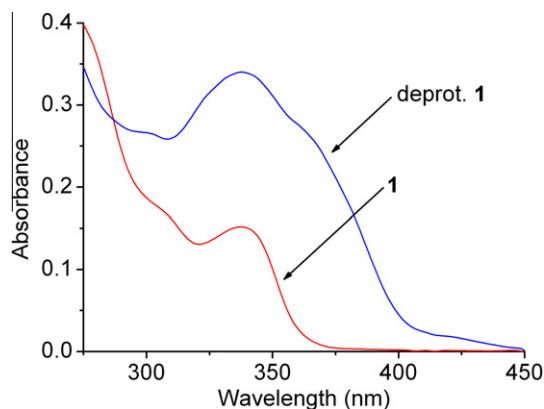
Since basic anions often cause the deprotonation of neutral anion sensors, we recorded the spectrum of the deprotonated form of receptor **1** using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a strong base (Fig. 2).

Upon the addition of carboxylate anions only complex formation was observed (Fig. 3) and all of the titration series of spectra could be fitted satisfactorily assuming the formation of a 1:1 complex (Table 1).

The results in Table 1 show that the groups at the stereogenic centres had a reasonable effect on the enantioselectivity. Moderate enantiomeric discrimination was observed in the cases of Boc-Phe and Man, which have an aromatic moiety (phenyl group) directly attached to their stereogenic centres. If the phenyl group is

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**Scheme 1.** Synthesis of receptor **1**.**Figure 1.** Optically active tetrabutylammonium carboxylates used in the enantiomeric recognition studies.**Figure 2.** Absorption spectra of **1** (20 μ M) and its deprotonated form in MeCN, optical path length: 1 cm.

connected to the stereogenic centre by a methylene spacer (the benzyl group in Boc-Phe), poor enantiomeric recognition was experienced. Furthermore, there was no enantiomeric differentiation between the enantiomers of Boc-Ala, in which compound an aliphatic group (methyl group) is the third substituent at the

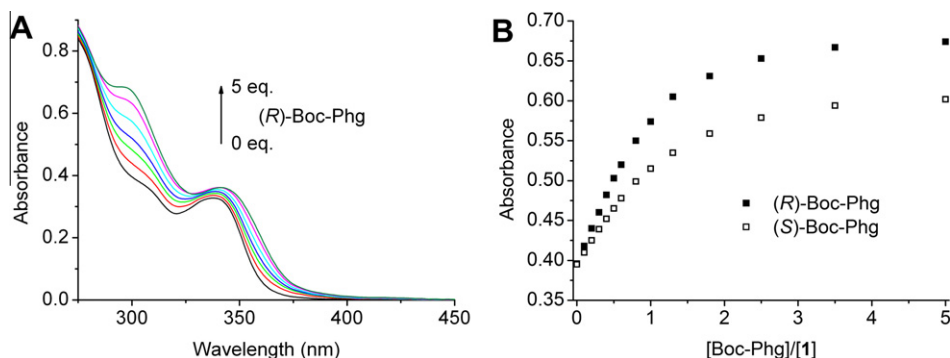
Table 1

Stability constants of complexes of **1** with the enantiomers of optically active tetrabutylammonium carboxylates and the degrees of enantiomeric differentiation

	log <i>K</i>	Δ log <i>K</i>
(<i>R</i>)-Man	4.92	0.19
(<i>S</i>)-Man	4.73	
(<i>R</i>)-Boc-Phg	5.43	0.22
(<i>S</i>)-Boc-Phg	5.21	
(<i>R</i>)-Boc-Phe	5.88	0.09
(<i>S</i>)-Boc-Phe	5.79	
(<i>R</i>)-Boc-Ala	5.94	0.04
(<i>S</i>)-Boc-Ala	5.90	

stereogenic centre. Receptor **1** formed relatively stable complexes with all of the optically active tetrabutylammonium carboxylates studied, but the complex stability constants were slightly lower in the cases when a phenyl group was directly attached to the stereogenic centre (Boc-Phg and Man).

The effect of the size of the protecting group on the enantioselectivity was also examined in the case of Phg with the enantiomers of the tetrabutylammonium salts of formyl-, acetyl- and pivaloyl-protected phenylglycine (Form-Phg, Ac-Phg and Piv-Phg, respectively, Fig. 4).

**Figure 3.** Absorption series of spectra upon titration of **1** (10 μ M) with (*R*)-Boc-Phg (0–5 equiv) in MeCN, optical path length: 4 cm (A), and the titration curves (0–5 equiv) with (*R*)- and (*S*)-Boc-Phg at 300 nm (B).

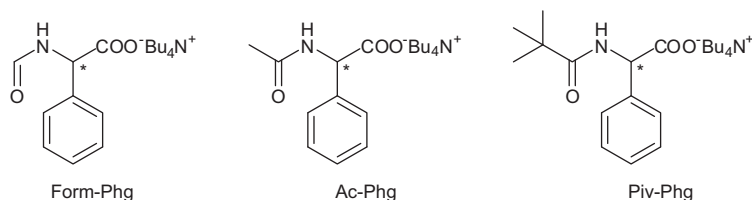


Figure 4. Tetrabutylammonium salts of the protected phenylglycine derivatives used in the enantiomeric recognition studies.

Increasing the bulk of the protecting group, increased the degree of enantiomeric differentiation (Table 2). The same enantioselectivity was observed in the cases of Piv-Phg and Boc-Phg, which have protecting groups of similar sizes. The bulk of the protecting group had no significant effect on the stability of the complexes.

Table 2

The effect of the protecting groups of the Phg derivatives on the enantiomeric recognition by **1**

	log <i>K</i>	Δlog <i>K</i>
(<i>R</i>)-Boc-Phg	5.43	0.22
(<i>S</i>)-Boc-Phg	5.21	
(<i>R</i>)-Form-Phg	5.16	0.05
(<i>S</i>)-Form-Phg	5.11	
(<i>R</i>)-Ac-Phg	5.45	0.18
(<i>S</i>)-Ac-Phg	5.27	
(<i>R</i>)-Piv-Phg	5.27	0.22
(<i>S</i>)-Piv-Phg	5.05	

In the case of Boc-Phg, we also examined the enantiomeric recognition properties of receptor **1** in acetonitrile-*d*₃ using ¹H NMR spectroscopy (Fig. 5). The addition of 1 equiv of (*R*)-Boc-Phg or (*S*)-Boc-Phg broadened the NH signals of receptor **1**, thus indicating multiple hydrogen bonds in the complexes. The signals of the NH and the aromatic protons of Boc-Phg and one of the phenothiazine protons shifted upfield, while the other phenothiazine proton shifted downfield upon complexation. The changes of the chemical shifts were larger upon formation of the **1**–(*R*)-Boc-Phg complex, which also showed the enantiomeric differentiation ability of receptor **1**.

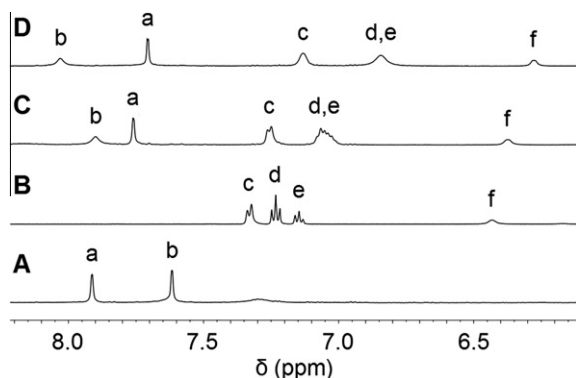


Figure 5. Partial ¹H NMR spectra of **1** (A), (*R*)-Boc-Phg [B, identical to the (*S*)-enantiomer], **1**–(*S*)-Boc-Phg complex (C) and **1**–(*R*)-Boc-Phg complex (D) in MeCN-*d*₃ (6 mM) at 500 MHz (a, b: aromatic protons of phenothiazine; c, d, e: aromatic protons of Boc-Phg; f: NH of Boc-Phg).

3. Conclusion

We have studied the enantiomeric recognition properties of the newly synthesized 5,5-dioxophenothiazine bis(thiourea) containing tetra-*O*-acetyl-β-*D*-glucopyranosyl groups **1** towards the enantiomers of the tetrabutylammonium salts of optically active α-

hydroxy and *N*-protected α-amino acids. We have shown that the type of the third group (methyl, benzyl or phenyl) at the stereogenic centre had a considerable effect on the enantioselectivity. The size of the protecting group (formyl, acetyl, pivaloyl or *tert*-butoxycarbonyl) of the phenylglycine also influenced the enantiomeric discrimination.

4. Experimental

4.1. General

Starting materials were purchased from Sigma–Aldrich Corporation unless otherwise noted. Silica gel 60 F₂₅₄ (Merck) plates were used for TLC. Silica gel 60 (70–230 mesh, Merck) was used for column chromatography. Ratios of the solvents for the eluents are given in volumes (mL/mL). Solvents were dried and purified according to well established methods.¹³ Evaporations were carried out under reduced pressure unless otherwise stated.

IR spectra were recorded on a Bruker Alpha-T FT-IR spectrometer. ¹H (500 MHz) NMR spectra were obtained on a Bruker DRX-500 Avance spectrometer. ¹H (300 MHz) and ¹³C (75 MHz) NMR spectra were obtained on a Bruker 300 Avance spectrometer. Elemental analysis was performed in the Microanalytical Laboratory of the Department of Organic Chemistry, Institute for Chemistry, L. Eötvös University, Budapest, Hungary. The mass spectrum was recorded on an Agilent-1200 Quadrupole LC/MS instrument using ESI methods. Melting points were taken on a Boetius micro-melting point apparatus and are uncorrected.

UV–vis spectra were taken on a Unicam UV4-100 spectrophotometer. Quartz cuvettes with path lengths of 1 cm and 4 cm were used. The enantiomers of mandelic acid and Boc-protected amino acids were purchased from Sigma–Aldrich Corporation. The enantiomers of formyl-protected phenylglycine¹⁴ and acetyl-protected phenylglycine¹⁵ were prepared in our laboratory. Tetrabutylammonium salts of anions were prepared by adding 1 equiv of carboxylic acid to 1 equiv of Bu₄NOH dissolved in MeOH. After evaporating the MeOH, the salts were dried under reduced pressure over P₂O₅. The concentrations of the solutions of receptor **1** during the titrations were 20 μM for Man, 10 μM for Boc-Phg, Form-Phg, Ac-Phg and Piv-Phg, 5 μM for Boc-Phe and 4 μM for Boc-Ala. The concentrations of the titrant solutions of chiral carboxylates were 1 mM. Stability constants of the complexes were determined by global nonlinear regression analysis using SPEC-FIT/32™ program.

4.2. *N,N'*-[(3,7-Di-*tert*-butyl-5,5-dioxo-5,10-dihydro-5λ⁶-phenothiazine-1,9-diyl)bis(azanediylcarbonothioyl)] bis(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosylamine) **1**

To a solution of isothiocyanate **3** (117 mg, 0.30 mmol) in pyridine (3 mL) was added a solution of diamine **2** (53 mg, 0.142 mmol) in pyridine (2 mL) under Ar at rt. The mixture was then stirred at rt for 1 h. After the reaction was completed, the solution was poured into a water–ice mixture, and the pH was adjusted to 1 using concentrated aqueous HCl solution. The

precipitate was filtered off, and the crude product was purified by column chromatography on silica gel using 1:20 MeOH–CH₂Cl₂ as an eluent to give receptor **1** (56 mg, 34%) as pale yellow crystals. Mp: 152–155 °C; R_f: 0.70 (silica gel TLC, MeOH–CH₂Cl₂ 1:20); $[\alpha]_D^{28} = +75.3$, $[\alpha]_{578}^{28} = +79.8$, $[\alpha]_{546}^{28} = +95.4$, $[\alpha]_{436}^{28} = +224$ (c 1.23, CHCl₃); IR (KBr) ν_{\max} 3407, 3346, 3245, 2964, 2873, 1755, 1608, 1531, 1494, 1368, 1288, 1237, 1142, 1097, 1037, 903 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 1.28 (s, 18H), 1.82 (s, 6H), 2.00 (s, 6H), 2.03 (s, 6H), 2.08 (s, 6H), 3.63–3.86 (m, 4H), 5.14 (t, *J* = 9 Hz, 2H), 5.25–5.30 (m, 4H), 5.40 (t, *J* = 11 Hz, 2H), 5.56 (t, *J* = 9 Hz, 2H), 7.26 (br s, 1H, NH), 7.28 (br s, 2H, NH), 7.40 (d, *J* = 2 Hz, 2H), 7.93 (d, *J* = 2 Hz, 2H), 9.50 (br s, 2H, NH); ¹³C NMR (75.5 MHz, CDCl₃) δ 19.47, 19.68, 19.84, 20.94, 30.19, 33.89, 61.25, 68.39, 70.14, 73.80, 74.88, 82.28, 117.88, 121.17, 124.26, 129.62, 131.67, 144.35, 168.12, 169.38, 169.49, 173.98, 184.47; MS calcd for C₅₀H₆₅N₅O₂₀S₃: 1151.3 Found (M–H)⁺: 1150.3; Anal. Calcd for C₅₀H₆₅N₅O₂₀S₃: C 52.12, H 5.69, N 6.08, S 8.35, found: C 51.92, H 5.99, N 5.87, S 8.13.

4.3. (2*R*)- and (2*S*)-[(2,2-Dimethylpropanoyl)amino] (phenyl) acetic acid

To a solution of (*R*)- or (*S*)-phenylglycine (1.512 g, 10 mmol) in 4% aqueous NaOH (30 mL) was added pivaloyl chloride (1.568 g, 13 mmol), and the resulting mixture was stirred at rt overnight. The solution was acidified to pH 1 using concentrated aqueous HCl solution, and the precipitate was filtered off. The crude product was recrystallized from water to give the pure acids [799 mg, 34% for the (*R*)-enantiomer and 901 mg, 38% for the (*S*)-enantiomer] as white crystals. (*R*)-enantiomer, mp: 134–137 °C; $[\alpha]_D^{25} = -155$ (c 1.0, MeOH). (*S*)-enantiomer, mp: 135–138 °C; $[\alpha]_D^{25} = +157$ (c 1.0, MeOH); IR (KBr) ν_{\max} 3422, 3300–2400, 1733, 1633, 1587, 1516, 1456, 1416, 1365, 1315, 1298, 1253, 1200, 1183, 1162, 1072,

1030, 979, 900, 862, 724, 701, 665, 636, 582, 533, 491 cm⁻¹; ¹H NMR (500 MHz, DMSO-*d*₆) δ 1.14 (s, 9H), 5.38 (d, *J* = 10 Hz, 1H), 7.30 (t, *J* = 7 Hz, 1H), 7.35 (t, *J* = 7 Hz, 2H), 7.40 (d, *J* = 7 Hz, 2H), 7.88 (d, *J* = 7 Hz, 1H, NH) 12.82 (br s, 1H); ¹³C NMR (75.5 MHz, DMSO-*d*₆) δ 27.17, 38.00, 56.20, 127.67, 127.70, 137.58, 172.02, 177.12.

Acknowledgements

Financial support of the Hungarian Scientific Research Fund (OTKA No. K 81127 and PD 104618) is gratefully acknowledged. The authors express their appreciation to Dr. József Nagy for helpful discussions.

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