Graphical Abstract

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

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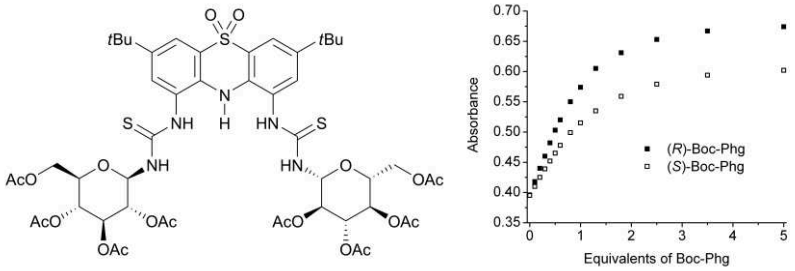
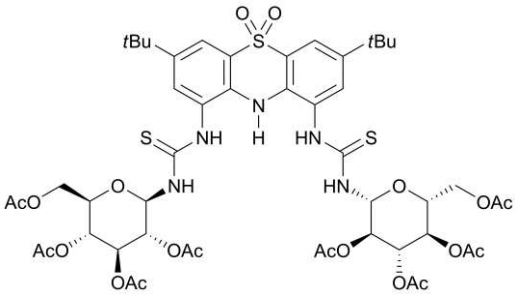
Attila Kormos, Ildikó Móczár, Dávid Pál, Péter Baranyai, József Kupai, Klára Tóth, Péter Huszthy



Stereochemistry Abstract

Attila Kormos, Ildikó Móczár, Dávid Pál, Péter Baranyai, József Kupai, Klára Tóth, Péter Huszthy



Ee > 97%
$[\alpha]_D^{28}$ = +75.3 (*c* 1.23, $CHCl_3$)
Source of chirality: D-glucose
Absolute configuration: 1*R*,2*R*,3*S*,4*R*,5*R*,1'*R*,2'*R*,3'*S*,4'*R*,5'*R*

$C_{50}H_{65}N_5O_{20}S_3$
N,*N*'-[(3,7-di-*tert*-butyl-5,5-dioxo-5,10-dihydro-5λ^6-phenothiazine-1,9-diyl)bis(azanediylcarbonothioyl)]bis(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine)

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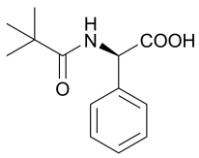

Ee > 97%
$[\alpha]_D^{25}$ = –155 (*c* 1.0, MeOH)
Source of chirality: (*R*)-phenylglycine
Absolute configuration: 2*R*

$C_{13}H_{17}NO_3$
(2*R*)-[(2,2-dimethylpropanoyl)amino](phenyl)acetic acid

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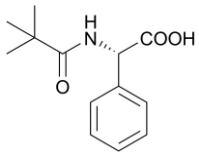

Ee > 97%
$[\alpha]_D^{25}$ = +157 (*c* 1.0, MeOH)
Source of chirality: (*S*)-phenylglycine
Absolute configuration: 2*S*

$C_{13}H_{17}NO_3$
(2*S*)-[(2,2-dimethylpropanoyl)amino](phenyl)acetic acid

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

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Abstract

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

Keywords: phenothiazine, neutral anion sensor, enantiomeric recognition, complexation, UV–vis spectroscopy

1. Introduction

Enantiomeric recognition as a special case 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 has great importance.

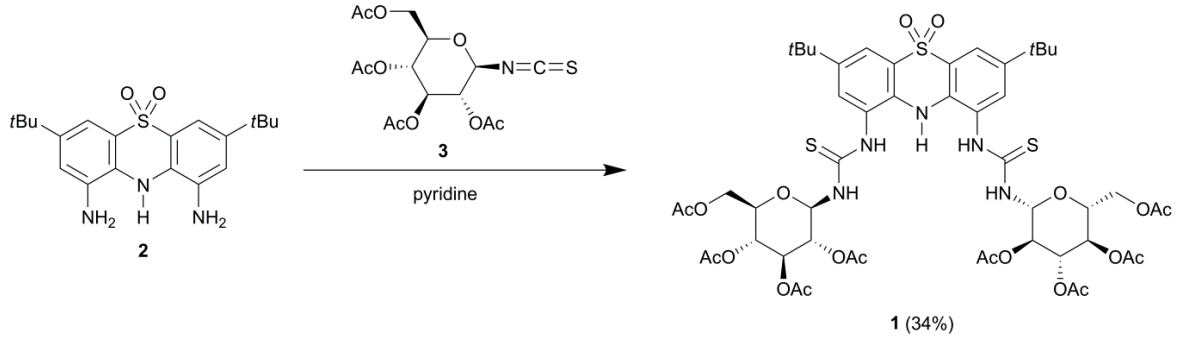
Carboxyl group is a particularly common functional group present in amino acids, enzymes, metabolic intermediates and several pharmaceutical molecules. Therefore, the synthesis and studies of sensor and selector molecules capable of discriminating the enantiomers of chiral carboxylic acids are of great 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 cheap sources of chirality and are used as asymmetric units in many host molecules,[5–7] there are only a few enantioselective anion sensors incorporating sugar units.[8–10] Korean researchers reported 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.

In this paper we report the synthesis of a novel 5,5-dioxophenothiazine bis(thiourea) containing tetra-*O*-acetyl-β-D-glucopyranosyl groups (**1**, Scheme 1) and the studies on its enantiomeric recognition ability toward the enantiomers of different optically active tetrabutylammonium carboxylates in acetonitrile.

2. Results and discussion

2.1. Synthesis

The synthesis of new optically active phenothiazine bis(thiourea) **1** was carried out as outlined 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**.



Scheme 1. Synthesis of receptor **1**.

2.2. Anion recognition studies

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

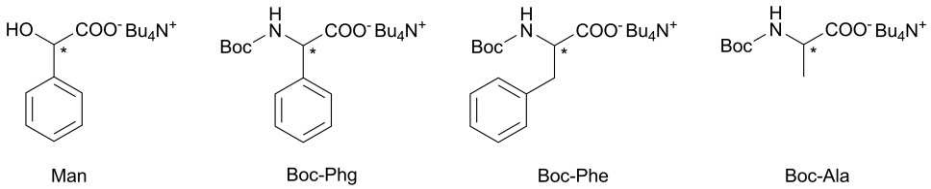


Figure 1. Optically active tetrabutylammonium carboxylates used in the enantiomeric recognition studies.

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).

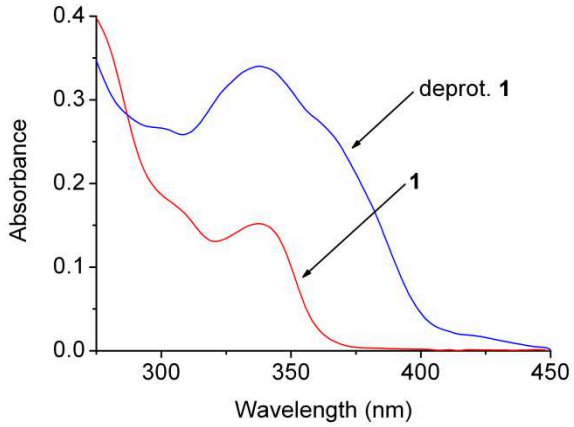


Figure 2. Absorption spectra of **1** (20 μM) and its deprotonated form in MeCN, optical path length: 1 cm.

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

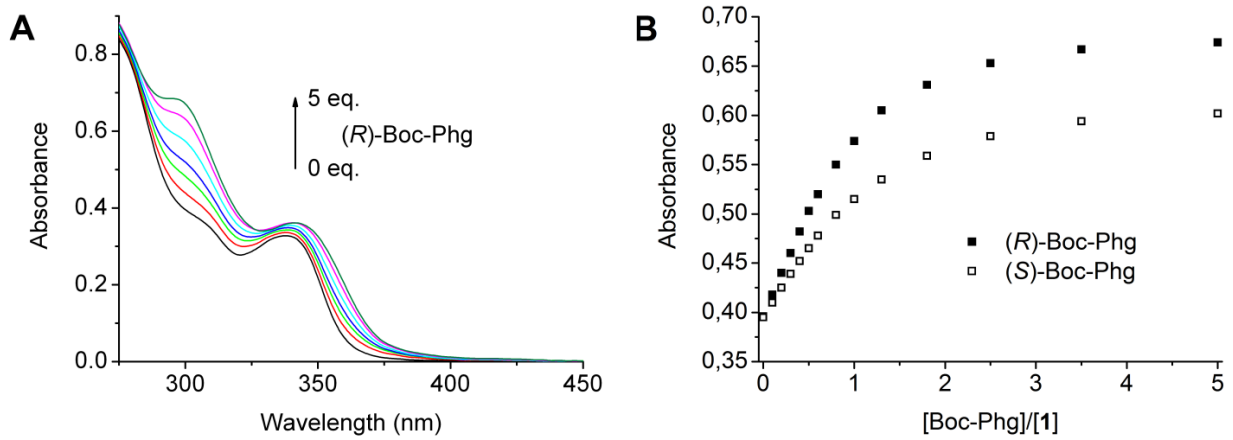


Figure 3. Absorption series of spectra upon titration of **1** (10 μM) with (*R*)-Boc-Phg (0–5 equiv) in MeCN, optical path length: 4 cm (A), and the titration curves (0–5 equiv) with (*R*)- and (*S*)-Boc-Phg at 300 nm (B).

Table 1

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

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

The results in Table 1 show that the groups at the stereogenic centres had reasonable effect on enantioselectivity. Moderate enantiomeric discrimination was observed in the cases of Boc-Phg and Man having an aromatic moiety (phenyl group) directly attached to their stereogenic centres. If the phenyl group is connected to the stereogenic centre by a methylene spacer (benzyl group in Boc-Phe), only poor enantiomeric recognition was experienced. Furthermore, there was no enantiomeric differentiation between the enantiomers of Boc-Ala, in which case an aliphatic group (methyl group) is the third substituent at the stereogenic centre. Receptor **1** formed relatively stable complexes with all 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 size of the protecting group on enantioselectivity was also examined in the case of Phg with the enantiomers of tetrabutylammonium salts of formyl-, acetyl- and pivaloyl-protected phenylglycine (Form-Phg, Ac-Phg and Piv-Phg, respectively, Fig. 4).

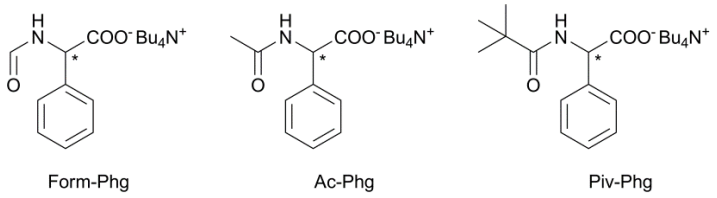


Figure 4. Tetrabutylammonium salts of protected phenylglycine derivatives used in the enantiomeric recognition studies.

With increasing bulkiness of the protecting group, the degree of enantiomeric differentation was increased (Table 2). Same enantioselectivity was observed in the cases of Piv-Phg and Boc-Phg having protecting groups of similar sizes. Bulkiness of the protecting group had no significant effect on stability of the complexes.

Table 2

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

	log *K*	Δ log *K*
(*R*)-Boc-Phg	5.43	0.22
(*S*)-Boc-Phg	5.21	
(*R*)-Form-Phg	5.16	0.05
(*S*)-Form-Phg	5.11	
(*R*)-Ac-Phg	5.45	0.18
(*S*)-Ac-Phg	5.27	
(*R*)-Piv-Phg	5.27	0.22
(*S*)-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 ^{1}H NMR spectroscopy (Fig. 5). Addition of 1 equiv of (*R*)-Boc-Phg or (*S*)-Boc-Phg caused broadening of the NH signals of receptor **1** 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 in the case of the formation of **1**–(*R*)-Boc-Phg complex, which also showed the enantiomeric differentiation ability of receptor **1**.

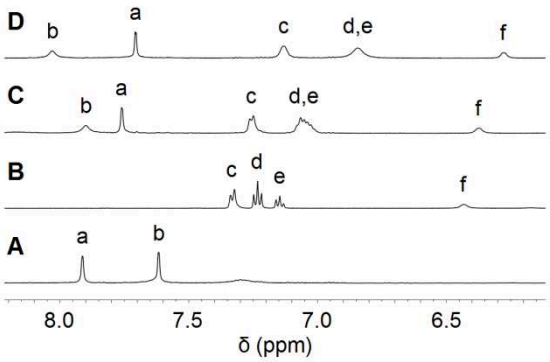


Figure 5. Partial ^{1}H NMR spectra of **1** (A), (*R*)-Boc-Phg (B, identical with the (*S*)-enantiomer), **1**–(*S*)-Boc-Phg complex (C) and **1**–(*R*)-Boc-Phg complex (D) in MeCN-d_3 (6 mM) at 500 MHz (a, b: aromatic protons of phenothiazine; c, d, e: aromatic protons of Boc-Phg; f: NH of Boc-Phg).

3. Conclusion

We studied the enantiomeric recognition properties of the newly synthesized 5,5-dioxophenothiazine bis(thiourea) containing tetra-*O*-acetyl-β-D-glucopyranosyl groups (**1**) toward the enantiomers of 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 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 F_{254} (Merck) plates were used for TLC. Silica gel 60 (70–230 mesh, Merck) was used for column chromatography. Ratios of 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. ^{1}H (500 MHz) NMR spectra were obtained on a Bruker DRX-500 Avance spectrometer. ^{1}H (300 MHz) and ^{13}C (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 method. Melting points were taken on a Boetius micro-melting point apparatus and were uncorrected.

UV–vis spectra were taken on a Unicam UV4-100 spectrophotometer. Quartz cuvettes with path length of 1 cm and 4 cm were used. Enantiomers of mandelic acid and Boc-protected amino acids were purchased from Sigma–Aldrich Corporation. Enantiomers of formyl-protected phenylglycine[14] and acetyl-protected phenylglycine[15] were prepared in our laboratory. Tetrabutylammonium salts of anions were prepared by adding 1 equiv of carboxylic acid to 1 equiv of Bu_4NOH dissolved in MeOH. After evaporating MeOH the salts were dried under reduced pressure over P_2O_5. 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 SPECFIT/32™ program.

4.2. *N*,*N*'-[(3,7-di-*tert*-butyl-5,5-dioxo-5,10-dihydro-5λ^6-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 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–CH_2Cl_2 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–CH_2Cl_2 1:20); $[\alpha]_D^{28}$ = +75.3,= +79.8,= +95.4,= +224 (*c* 1.23, $CHCl_3$); IR (KBr) ν_{max} 3407, 3346, 3245, 2964, 2873, 1755, 1608, 1531, 1494, 1368, 1288, 1237, 1142, 1097, 1037, 903 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) 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); ^{13}C NMR (75.5 MHz, $CDCl_3$) 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 $C_{50}H_{65}N_5O_{20}S_3$: 1151.3 Found (M–

H)$^-$: 1150.3; Anal. Calcd for $C_{50}H_{65}N_5O_{20}S_3$: 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}; ^{1}H 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); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 27.17, 38.00, 56.20, 127.67, 127.70, 137.58, 172.02, 177.12.

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