(+)-9-Benzyloxy-α-Dihydrotetrabenazine as an Important Intermediate for the VMAT2 Imaging Agents: Absolute Configuration and Chiral Recognition

CHUNYI LIU, ZHENGPING CHEN,* XIAOMIN LI, JIE TANG, AND XIAOFENG QIN

Key Laboratory of Nuclear Medicine, Ministry of Health, Jiangsu Key Laboratory of Molecular Nuclear Medicine, Jiangsu Institute of Nuclear Medicine, Wuxi, Jiangsu, China, 214063

ABSTRACT This article reports, for the first time, on the absolute configuration of (+)-9-benzyloxy-α-dihydrotetrabenazine (**8**), as determined from the perspective of X-ray crystallography. Compound **8** was prepared by a six-step reaction using 3-benzyloxy-4-methoxybenzaldehyde (**1**) as a starting material. The X-ray crystal diffraction structure of two compounds, racemic 9-benzyloxy-tetrabenazine (**5**) and the diastereomeric salt of compound **8**, is also described for the first time in this article. The X-ray results and the chiral HPLC helped elucidate that compound **8** has an absolute configuration as 2*R*,3*R*,11b*R*. The crystal structure of racemic compound **5** contains two symmetry-independent molecules in the unit cell. Interestingly, while they are structural isomers, they are enantiomers, too, i.e., in solution, because they are not mirror images of each other in the crystal lattice. In order to elucidate the intermolecular interaction mechanism of the diastereomeric salt of compound **8**, its crystal packing was investigated with regard to the weak interactions, such as salt bridge, OH…O and CH…O hydrogen bonds, and intermolecular CH…π interaction. The results showed that the carbonyl-assisted salt bridges and the OH…O hydrogen bonds formed polar columns in the crystal structure of the diastereomeric salt of compound **8**, resembling butterflies with open wings as viewed along the *c*-axis. These polar columns were extended to three-dimensional network by intermolecular CH…O hydrogen bonds and intermolecular CH…π interactions. *Chirality 25:215–223, 2013*.

KEY WORDS: VMAT2; dihydrotetrabenazine; chiral recognition; absolute configuration

INTRODUCTION

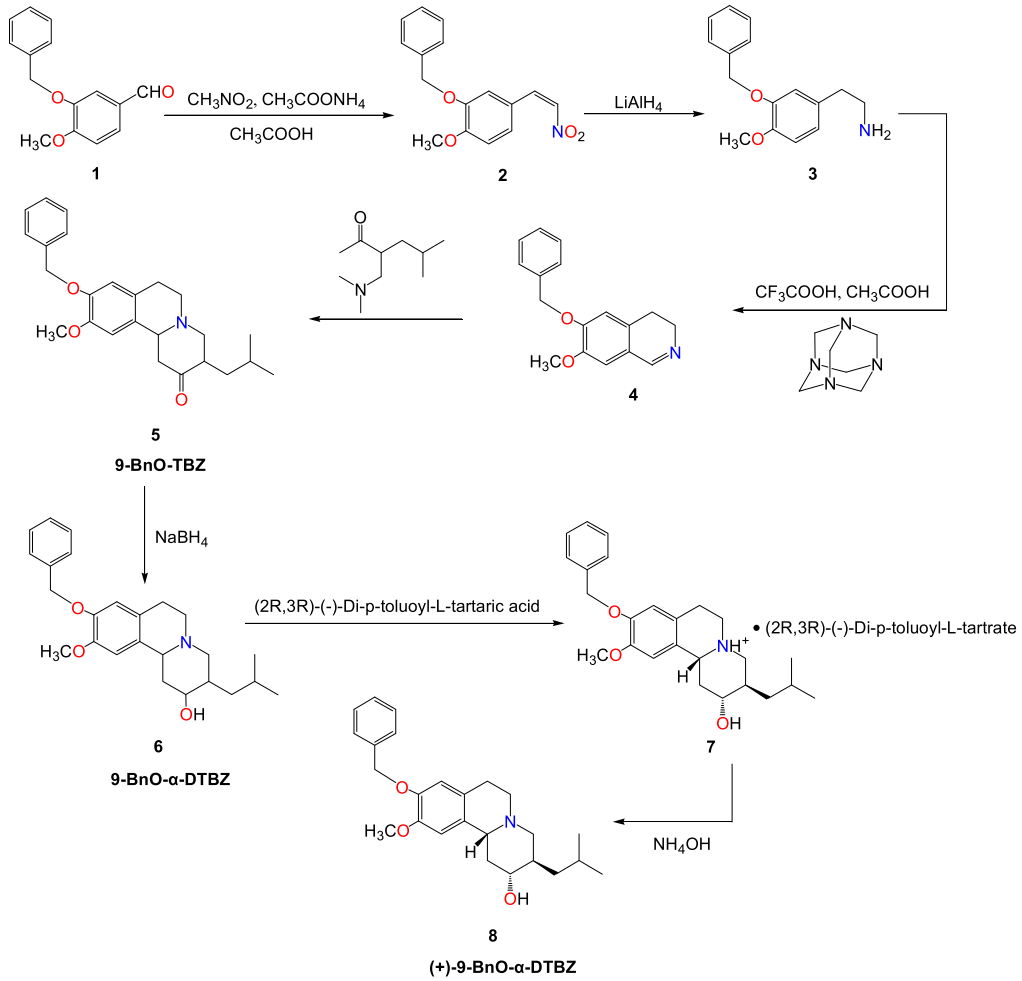
The vesicular monoamine transporter (VMAT) is a transport protein integrated into the membrane of intracellular vesicles of presynaptic neurons. It acts to transport monoamines into the synaptic vesicles. The VMAT exists in two pharmacologically distinct forms: VMAT1 and VMAT2. In humans, VMAT1 is primarily found in the adrenal tissue and VMAT2 is expressed nearly exclusively in the brain. Therefore, imaging VMAT2 in the brain with positron emission tomography (PET) and related imaging agents provides a measurement reflecting the integrity of monoaminergic neurons, and is used to monitor or diagnose neurodegenerative disorders such as Parkinson's, Alzheimer's, Tourette's, and Huntington's disease.[1]

Recently, (+)-9-desmethyl-α-dihydrotetrabenazine (DTBZ) has been labeled with carbon-11 and fluorine-18 radioisotopes and used for in vitro and in vivo studies of VMAT2 in both the animal and human brain. For example, ^{11}C-(+)-α-DTBZ[2–5] and ^{18}F-(+)-FP-α-DTBZ[6–8] have been prepared as PET imaging agents targeting VMAT2. Two preparation methods of (+)-9-desmethyl-α-dihydrotetrabenazine were found in the literature.[8–10] One method was selective O-demethylation of (±)-DTBZ, followed by enantiomeric separation by chiral high-performance liquid chromatography (HPLC). This method was not suitable for large-scale preparation of (+)-9-desmethyl-α-dihydrotetrabenazine. On the contrary, deprotection of the benzyl group of (+)-9-benzyloxy-α-dihydrotetrabenazine (**8**) via hydrogenolysis gave the precursor for the VMAT2 imaging agents, (+)-9-desmethyl-α-dihydrotetrabenazine, in a good yield. Therefore, compound **8** is an important intermediate for the VMAT2 imaging agents and it is necessary to confirm its absolute configuration. Boldt and colleagues first reported the synthesis of this compound[9] in 2008. Until now, however, its absolute configuration has not been determined. We report here, for the first time, the X-ray crystal structure analysis of the diastereomeric salt of compound **8** (namely, compound **7**), which allows the assignment of the absolute configuration of compound **8**. In the synthesis of the title compound, in order to know the chiral configuration of intermediate compounds, the single crystal of racemic 9-benzyloxy-tetrabenazine (**5**) was also determined by X-ray diffraction in this article. In addition, the total synthesis of compound **8** used by our group is shown in Scheme 1.

9-Benzyloxy-dihydrotetrabenazine has three chiral carbon atoms. Therefore, in theory, 9-benzyloxy-dihydrotetrabenazine has eight isomers, as shown in Figure 1. By means of X-ray diffraction, we used the most effective way to confirm that compound **8** has 2*R*,3*R*,11b*R* stereochemistry. X-ray diffraction for determination of the absolute configuration of

Additional Supporting Information may be found in the online version of this article.

Contract grant sponsor: National Natural Science Foundation of China; Contract grant number: 30970844.
Contract grant sponsor: Outstanding Professionals Foundation of Jiangsu Health Bureau; Contract grant number: RC2011096.
Contract grant sponsor: Natural Science Foundation of Jiangsu Province; Contract grant number: BK2010155.
Contract grant sponsor: Youth Research Foundation of Jiangsu Institute of Nuclear Medicine; Contract grant number: QN201109.
*Correspondence to: Zhengping Chen, 20 Qianrong Road, Wuxi 214063, P.R. China. E-mail: chenzhengping@hotmail.com
Received for publication 12 July 2012; Accepted 21 September 2012
DOI: 10.1002/chir.22126
Published online in Wiley Online Library (wileyonlinelibrary.com).



Scheme 1. Synthesis of (+)-9-benzyloxy-α-dihydrotetrabenazine.

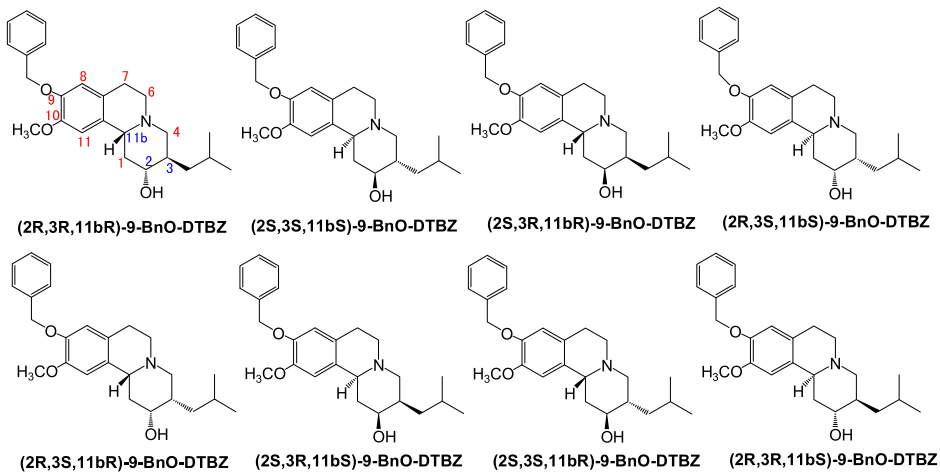


Fig. 1. Structures of eight 9-benzyloxy-dihydrotetrabenazine isomers.

molecules includes direct and indirect methods. Our test sample contains only C, H, N, and O elements and no heavy elements that may help to directly determine absolute configuration, so we adopted the indirect method. The absolute configuration of compound **8** was established using a reference molecule ((2*R*,3*R*)-(−)-di-p-toluoyl-L-tartaric acid). Furthermore, as X-ray diffraction analysis of the sample was conducted on a single crystal, the result was not universal. The chiral

HPLC analysis was applied to racemic 9-benzyloxy-α-dihydrotetrabenazine (**6**) and compound **8**.

Compound **7** was formed by compound **8** and (2*R*,3*R*)-(−)-di-p-toluoyl-L-tartaric acid. The (2*R*,3*R*)-(−)-di-p-toluoyl-L-tartrate anion and the (+)-9-benzyloxy-α-dihydrotetrabenazine cation acted as a proton acceptor and donor, respectively. The crystal structure of compound **7** was investigated for weak interactions, such as salt bridge, NH...O and CH...O hydrogen bonds, and intermolecular CH...π interactions. The identification of these weak intermolecular interactions in the crystal would be useful in understanding the chiral recognition mechanism of the parent structure of compound **8** for the amino acids in the VMAT2.

EXPERIMENTAL

Materials and Physical Measurements

All solvents and reagents were of analytical grade and were used without further purification. 6-Benzyloxy-7-methoxy-3,4-dihydroisoquinoline (**4**) was synthesized according to literature methods.[11–13] 3-Dimethylaminomethylheptan-2-one (**12**) was synthesized by our own methods. Melting points were determined by using a Yanagimoto micro melting point apparatus. MS spectra were recorded with a Waters Platform ZMD 4000 mass spectrometer. Proton NMR spectra were obtained on a Bruker Avance III 400MHz Digital NMR Spectrometer in $CDCl_3$. A Bruker CCD APEX2 diffractometer was used for the X-ray structure study.

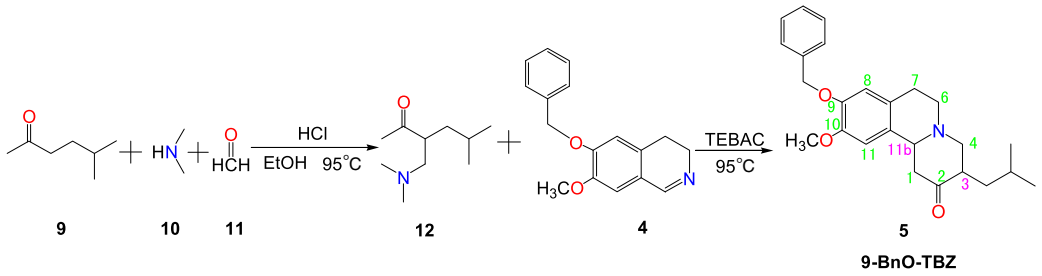
Synthesis of racemic 9-benzyloxy-tetrabenazine (5). 3-Dimethylaminomethylheptan-2-one (**12**) (8.00 g, 46.71 mmol), 6-benzyloxy-7-methoxy-3,4-dihydroisoquinoline (**4**) (9.05 g, 33.85 mmol), and triethylbenzylammonium chloride (2.40 g, 10.54 mmol) were dissolved in water (100 ml). The mixture was stirred and heated under reflux at 95°C for 6 h. The residue was extracted thoroughly with ethyl ether. Organic extracts were combined and concentrated in vacuum. The crude product was purified by crystallization with methanol, giving racemic compound **5** (4.20 g, 10.67 mmol) as colorless crystals. Yield: 32%. mp: 133–135°C. MS *m/z*: 394 $[M+H]^+$; 416 $[M+Na]^+$. ^{1}H NMR($CDCl_3$, 400 MHz), δ: 7.46–7.30 (m, 5H), 6.67 (s, 1H), 6.60 (s, 1H), 5.14 (s, 2H), 3.86 (s, 3H), 3.53 (d, *J* = 10.92 Hz, 1H), 3.32–3.28 (m, 1H), 3.14–3.06 (m, 2H), 2.95–2.91 (m, 1H), 2.77–2.53 (m, 4H), 2.37 (t, *J* = 11.51 Hz, 1H), 1.86–1.79 (m, 1H), 1.72–1.65 (m, 1H), 1.09–1.02 (m, 1H), 0.94 (d, *J* = 4.77 Hz, 3H), 0.92 (d, *J* = 4.77 Hz, 3H).

Synthesis of racemic 9-benzyloxy-α-dihydrotetrabenazine (6). Racemic compound **6** (1.82 g, 4.60 mmol) was prepared from racemic compound **5** (3.62 g, 9.20 mmol) and sodium borohydride (3.50 g, 92 mmol). The reaction time was 12 h. See Ref. 9 for details. Yield: 50%. mp: 178–180°C. MS *m/z*: 396 $[M+H]^+$; 378 $[M-OH]^+$. ^{1}H NMR($CDCl_3$, 400 MHz), δ: 7.43–7.29 (m, 5H), 6.70 (s, 1H), 6.61 (s, 1H), 5.11 (s, 2H), 3.85 (s, 3H), 3.43–3.35 (m, 1H), 3.13–2.94 (m, 4H), 2.61–2.55 (m, 2H), 2.46–2.39 (td, *J* = 10.79, 3.48 Hz, 1H), 1.96 (t, *J* = 11.36 Hz, 1H), 1.76–1.65 (m, 2H), 1.58–1.44 (m, 3H), 1.09–1.03 (m, 1H), 0.94 (d, *J* = 6.49 Hz, 3H), 0.91 (d, *J* = 6.45 Hz, 3H).

Synthesis of (+)-9-benzyloxy-α-dihydrotetrabenazine (8). Compound **8** (0.13 g, 0.33 mmol) was prepared from racemic compound **6** (0.63 g, 1.59 mmol) and (2*R*,3*R*)-(−)-di-p-toluoyl-L-tartaric acid (0.61 g, 1.58 mmol). See Ref. 9 for details. Yield: 21%. mp: 139–141°C. $[\alpha]_D^{20}$ = +58.3° (*c* = 1, chloroform); MS *m/z*: 396.3 $[M+H]^+$; 378.3 $[M-OH]^+$. ^{1}H NMR($CDCl_3$, 400 MHz), δ: 7.43–7.26 (m, 5H), 6.70 (s, 1H), 6.61 (s, 1H), 5.11 (s, 2H), 3.85 (s, 3H), 3.42–3.35 (m, 1H), 3.13–2.94 (m, 4H), 2.61–2.55 (m, 2H), 2.46–2.39 (td, *J* = 10.85, 3.54 Hz, 1H), 1.96 (t, *J* = 11.35 Hz, 1H), 1.73–1.44 (m, 5H), 1.09–1.02 (m, 1H), 0.94 (d, *J* = 6.49 Hz, 3H), 0.91 (d, *J* = 6.45 Hz, 3H).

X-ray Crystallography

The X-ray diffraction data for the crystals of compound **5** and compound **7** were collected on a Bruker APEX2 diffractometer equipped with graphite-monochromatic Mo-Kα radiation (λ = 0.71073 Å). The



Scheme 2. Synthesis of racemic 9-benzyloxy-tetrabenazine.

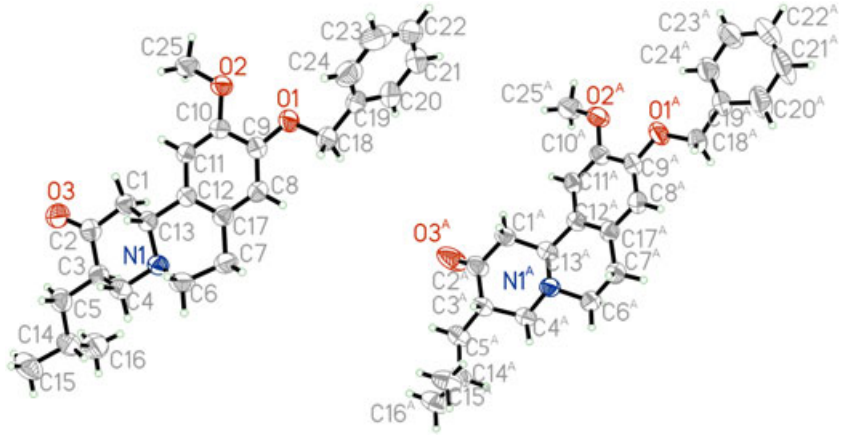


Fig. 2. The molecular structure of racemic compound **5**; 30% probability ellipsoids are shown.

TABLE 1. Crystallographic data and structure refinement details for racemic compound 5 and compound 7

Compound	racemic compound **5**	compound **7**
CCDC deposit number	857040	857041
Molecular formula	$C_{25}H_{31}NO_3$	$C_{35}H_{44}NO_8$
Formula weight	393.51	606.71
Temperature(K)	296(2)	298(2)
Wavelength(Å)	0.71073	0.71073
Crystal system	Triclinic	Orthorhombic
Space group	*P*-1	$P2_12_12$
a(Å)	10.6228(10)	14.6804(18)
b(Å)	14.8996(14)	26.824(3)
c(Å)	15.7681(15)	8.7820(10)
α(°)	105.996(2)	90.00
β(°)	107.034(2)	90.00
γ(°)	100.230(2)	90.00
V(Å^3)	2201.4(4)	3458.2(7)
Z	4	4
$D_{ca.}$(g · cm^{-3})	1.187	1.165
F(000)	848	1300
Absorption coeff. (mm^{-1})	0.077	0.082
Θ range(°)	1.44 -25.00	1.52 -25.10
Index ranges	$-12 \le h \le 12$; $-17 \le k \le 17$; $-18 \le l \le 18$	$-17 \le h \le 17$; $-27 \le k \le 32$; $-10 \le l \le 10$
Reflections collected	12314	19360
Independent reflections	7676 [R_{int} = 0.0215]	6169 [R_{int} = 0.0398]
Observed reflections	5786	5145
Data/restraints/parameters	7676/0/529	6169/12/412
Goodness-of-fit on F^2	1.058	1.082
R, wR indices[I > 2σ(I)]	0.0553, 0.1632	0.0692, 0.2062
R, wR indices(all data)	0.0726, 0.1904	0.0815, 0.2188
Largest diff. peak and hole(e · Å^{-3})	0.241, -0.228	0.496, -0.237

structure was solved by direct and difference Fourier map methods with SHELXS-97.[14] Non-hydrogen atoms were refined by full-matrix least-squares techniques on F^2 with anisotropic thermal parameters, using SHELXL-97.[15] All H atoms were allowed to ride on their parent atoms at distances of 0.93 Å (C-H aromatic), 0.96 Å (C-H methyl), 0.97 Å (C-H methylene), and 0.98 Å (C-H tert-methyl) with U_{iso}(H) values of 1.2–1.5 times U_{eq} of the parent atoms.*

Chromatographic Analysis of Compound 6 and Compound 8

The analysis of compound **6** and compound **8** was investigated on a chiral HPLC column (Phenomenex Chirex (S)-Val and (R)-NEA; 250 × 4.6 mm) using isocratic 95% A/5% B at 1.0 ml/min with ultraviolet detection at 280 nm with solvent A being hexane/1,2-dichloroethane (2:1) and solvent B being 0.1% trifluoroacetic acid (TFA) ethanol solution.

*Crystallographic data for the structures of racemic compound **5** and compound **7** reported in this article have been deposited in the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 857040 and 857041.These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by e-mailing data_request@ccdc.cam.ac.uk, or by contacting the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223−336033.

RESULTS AND DISCUSSION

Synthesis

In this article, on the basis of previous work and our experimental study, we report the total synthesis of (+)-9-benzyloxy-α-dihydrotetrabenazine (**8**) by using a commercial compound as starting material in moderate conditions.

Synthesis of 6-benzyloxy-7-methoxy-3,4-dihydroisoquinoline (**4**) was performed as previously reported.[11–13] The process was as follows. A mixture of 3-benzyloxy-4-methoxybenzaldehyde (**1**), nitromethane, ammonium acetate, and acetic acid was heated under reflux, which gave 3-benzyloxy-4-methoxy-β-nitrostyrene (**2**). Reduction of compound **2** via lithium aluminum hydride generated 3-benzyloxy-4-methoxy phenylethylamine (**3**). Under the presence of acetic acid and trifluoroacetic acid, compound **3** and hexamethylenetetramine yielded compound **4**.

Compound **5** was first synthesized by our own concise means as shown in Scheme 2. 3-Dimethyl-aminomethylheptan-2-one (**12**) was prepared using the three-component one-pot Mannich reaction. Utilizing concentrated hydrochloric acid and ethanol as a catalyst system, the Mannich reaction of 5-methyl-2-hexanone (**9**), dimethylamine (**10**), and formaldehyde solution (**11**) occurred directly. This three-component one-pot synthesis strategy makes the preparation of the Mannich base easier and more convenient, and it could have potential applications. To synthesize compound **5**, a mixed solution of 3-dimethyl-aminomethylheptan-2-one (**12**), compound **4**, and triethylbenzylammonium chloride was stirred and heated under reflux at 95 °C for 6 h. This reaction occurred in aqueous medium. Our preparation method was simple, mild, and met the requirements of green chemistry.

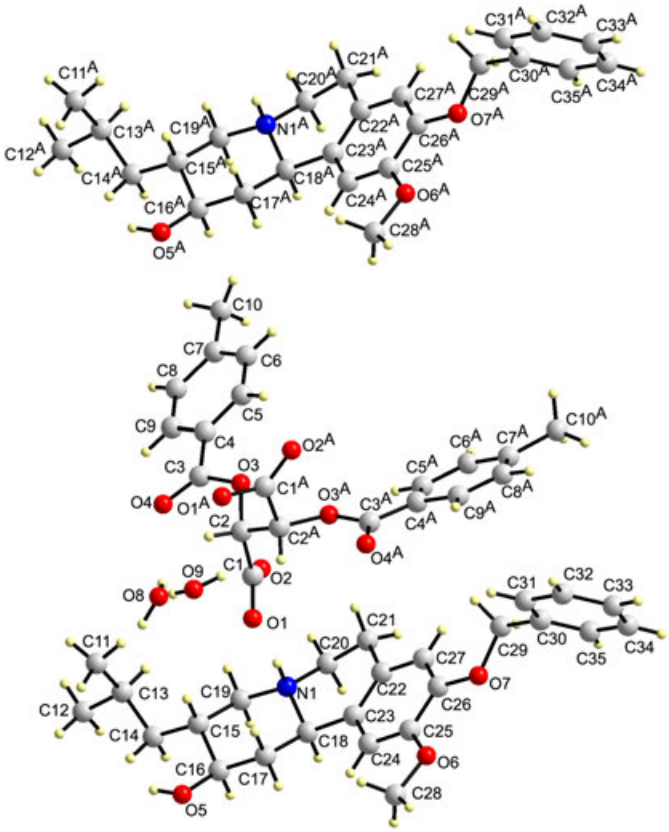


Fig. 3. The molecular structure of compound **7**.

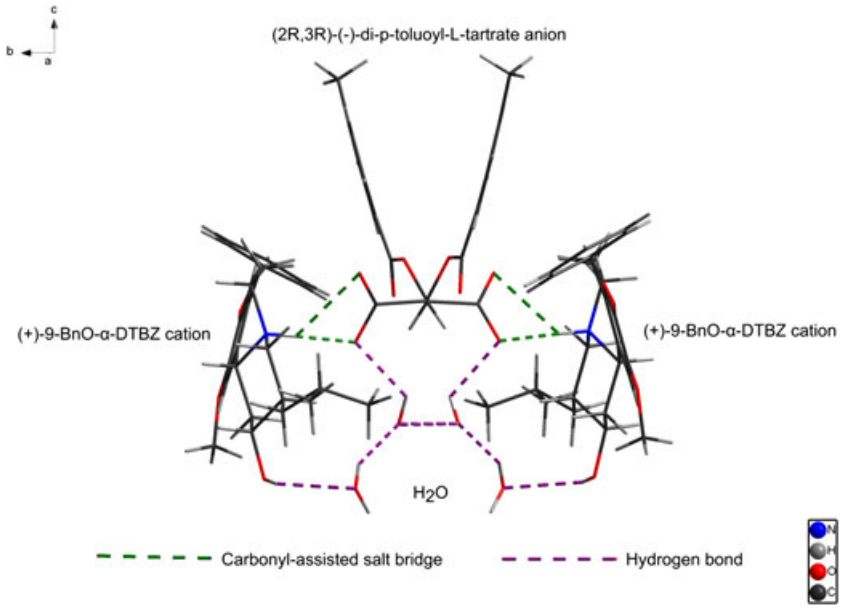
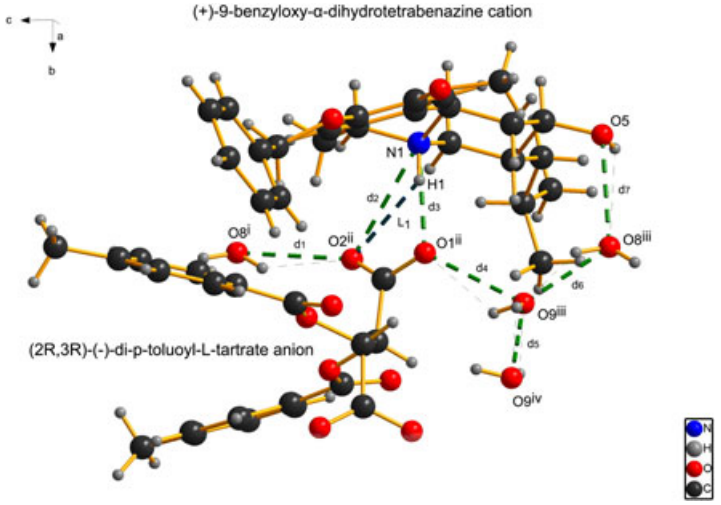


Fig. 4. Chiral recognitions in compound **7**.

TABLE 2. Hydrogen bond parameters of compound 7



D—H. . .A	D—A (Å)	D—H. . .A (°)
$O8^{i}$—H. . .$O2^{ii}$	2.68 (1) [d_1]	125.97 (54)
N1—H1. . .$O2^{ii}$	3.17 (1) [d_2]	121.35 (17)
N1—H1. . .$O1^{ii}$	2.65 (1) [d_3]	173.80 (18)
$O9^{iii}$—H. . .$O1^{ii}$	2.63 (1) [d_4]	118.47 (85)
$O9^{iii}$—H. . .$O9^{iv}$	2.28 (1) [d_5]	117.80 (100)
$O8^{iii}$—H. . .$O9^{iii}$	2.45 (1) [d_6]	143.93 (69)
O5—H. . .$O8^{iii}$	2.73 (1) [d_7]	80.35 (31)
Interatomic distance (Å)		
$O2^{ii}$. . .H1 [=L_1]	2.60 (1)	

The van der Waals radii (Å): H, 1.20; O, 1.52; N, 1.55.
Symmetry operators: i) 2-x,1-y,z; ii) 2-x,2-y,z; iii) 2-x,1-y,-1+z; iv) x,1+y,-1+z.

Compounds **6** and **8** were also synthesized through procedures described in the literature[9] and the synthesis steps were achieved through slight optimization of some reaction conditions. Compound **5** was reduced with sodium borohydride in ethanol to give compound **6**. (2*R*,3*R*)-(−)-Di-p-toluoyl-L-tartaric acid was utilized to separate the isomers of compound **6**, and then the title compound was obtained.

Crystal Structure of Compound 5

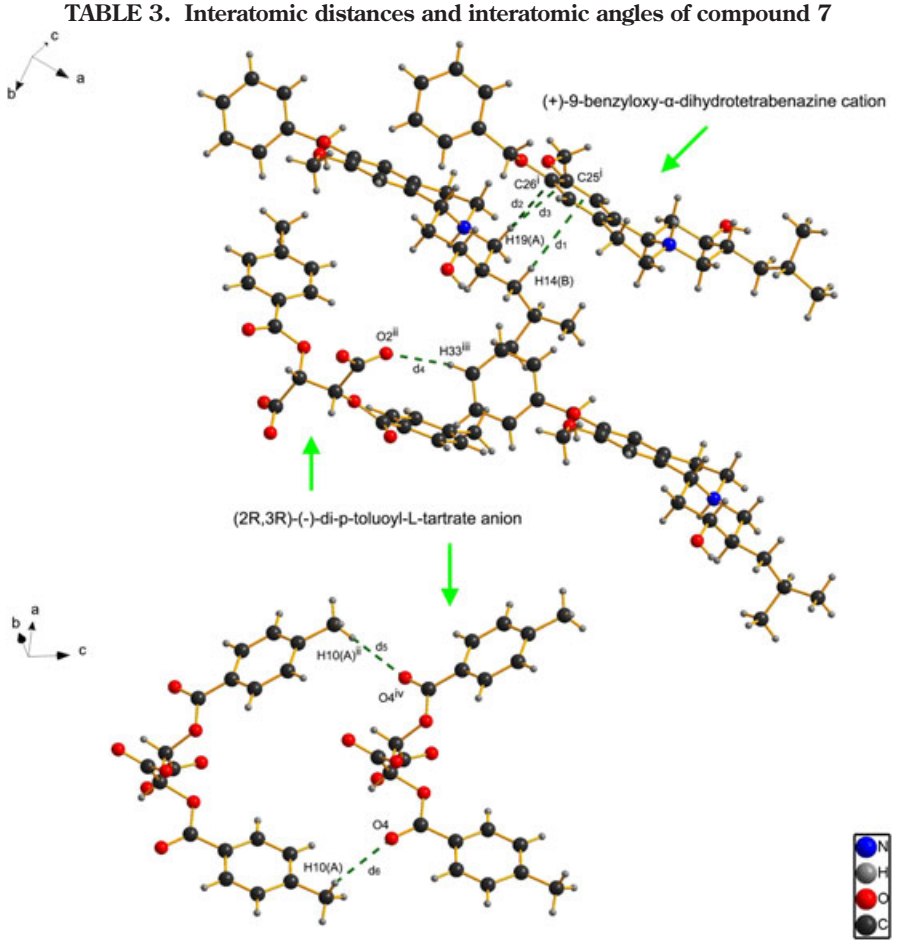
The single crystal of racemic compound **5** was grown from ethanol solution by slow evaporation at room temperature; it was a colorless prism. The molecular ellipsoid diagram of racemic compound **5** is shown in Figure 2 and the crystal data and refinement details are listed in Table 1. Racemic compound **5** crystallized in the triclinic system, space group $P\bar{1}$ with two sets of molecules in a unit cell.

The X-ray crystal diffraction structure of racemic compound **5** showed that it consists of (*R*,*R*) and (*S*,*S*) isomers. In the two isomers, the dihedral angles between the phenyl ring and isoquinoline aromatic ring were 69.35(8)° and 3.25(9)°, respectively. There were four rings in every isomer: two aromatic rings and two six-membered heterocyclic rings. They did not share a common plane. The aromatic rings were planar in conformation, and the other two heterocyclic rings were chair conformations. The two oxygen atoms of the benzyloxy and methoxy groups were all in the isoquinoline aromatic ring plane. Additional detailed single crystal X-ray investigations of compound **5** are shown in supporting information.

Crystal Structure and Chiral Recognition of Compound 7

(2*R*,3*R*)-(−)-Di-p-toluoyl-L-tartaric acid was used to resolve the isomers of compound **6**, and then compound **7** ($[\alpha]_D^{20}$ = −15.8° (*c* = 1, methanol); mp: 143–145°C) was obtained. The single crystal of compound **7** was grown from ethanol solution by slow evaporation at room temperature; it was also a colorless prism. The molecular structure of compound **7** is

TABLE 3. Interatomic distances and interatomic angles of compound 7



Interatomic distance (Å)					
H14(B)…π	(=d_1)	3.24 (1)	$O2^{ii}$…$H33^{iii}$	(=d_4)	2.46 (1)
H19(A)…$C26^{i}$	(=d_2)	2.86 (1)	$H10(A)^{ii}$…$O4^{iv}$	(=d_5)	2.67 (1)
H19(A)…$C25^{i}$	(=d_3)	2.75 (1)	H10(A)…O4	(=d_6)	2.67 (1)
Interatomic angle (°)					
$C33^{iii}$—$H33^{iii}$…$O2^{ii}$		161.06 (47)	$C10^{ii}$—$H10(A)^{ii}$…$O4^{iv}$		126.37 (34)
C10—H10(A)…O4		126.37 (34)			

The van der Waals radii (Å): C, 1.70; H, 1.20; O, 1.52; the half-thickness of aromatic ring, 1.77.
Symmetry operators: i) 0.5 + x,1.5-y,2-z; ii) 2-x,2-y,z; iii) 1 + x,y,-1 + z; iv) 2-x,2-y,1 + z.

shown in Figure 3. One molecule of compound **7** consisted of one molecule of (2*R*,3*R*)-(−)-di-p-toluoyl-L-tartaric acid, two molecules of compound **8**, and two molecules of water. The crystal data and refinement details are listed in Table 1. Compound **7** crystallized in the orthorhombic system, space group $P2_12_12$, with three sets of molecules in a unit cell.

Compound **7** does not contain a heavy atom. The data on its crystal were collected on a Bruker APEX2 diffractometer equipped with graphite-monochromatic Mo-Kα radiation (λ = 0.71073 Å). Therefore, the Flack parameter was not significant for absolute configuration of compound **7**. However, the configuration of (2*R*,3*R*)-(−)-di-p-toluoyl-L-tartaric acid is known. The absolute configuration of compound **8** was established using this reference molecule. This indirect method showed that compound **8** has the absolute configuration of *R*,*R*,*R*.

In 1967, Corey introduced the concept of synthon in the field of organic synthesis and Desiraju modified it later.[16] This concept is also suitable for all kinds of supramolecular systems. It is the common spatial arrangements of intermolecular interaction in crystal. In compound **7**, the ammonium-carboxylate ion pair was formed by a supramolecular synthon and some OH...O hydrogen bond interactions as shown in Figure 4. The supramolecular synthon consisted of a carbonyl-assisted salt bridge. Table 2 shows the hydrogen bond parameters of compound **7**. The interatomic distance between the ammonium nitrogen atom N1 and the carboxylate oxygen atom $O1^{ii}$ was 2.65(1) Å and the angle of N1—H1...$O1^{ii}$ was 173.80(18)°, suggesting that N1—H1...$O1^{ii}$ was a salt bridge interaction. In addition, the sum of O...H van der Waals radii was 2.72 Å and the oxygen atom $O2^{ii}$ and the neighboring hydrogen atom H1 were at a distance of 2.60(1) Å. This indicated that N1—H1...$O2^{ii}$ was a weak hydrogen bond. The multi-point recognition between ammonium and carboxylate ion was considered to enhance the robustness of this synthon.[16]

Sakai and colleagues proposed the concept of a space filler. They believed that the diastereomeric salt method was the most suitable way to separate the enantiomers, when the chiral resolving agent and the racemic substrate had the same molecular length, namely the same number of bonds between the most distant carbon atom and the carboxy or amino groups.[17] Obviously, the molecular lengths of the (2*R*,3*R*)-(−)-di-p-toluoyl-L-tartrate anion and the (+)-9-benzyloxy-α-dihydrotetrabenazine cation were not identical. According to this theory, compound **7** should have an external solvent molecule as space filler, such as water, acetone, or ethanol. This was consistent with the actual situation. The water molecules were connected tightly with the ammonium cation and the carboxylate anion by OH...O hydrogen bond interactions in the crystal structure.

In the (+)-9-benzyloxy-α-dihydrotetrabenazine cation, the phenyl ring and the isoquinoline aromatic ring were not coplanar and their dihedral angle was 76.33(15)°. The dihedral angle between the two phenyl rings in the (2*R*,3*R*)-(−)-di-p-toluoyl-L-tartrate anion was 29.86(15)°. Some short distances were also observed among the various molecules in compound **7**. Table 3 shows the details. The interatomic distance between the hydrogen atom H14(B) and the isoquinoline aromatic ring was 3.24(1) Å, and the hydrogen atom H14(B) almost faced the center of the adjacent isoquinoline aromatic ring. In addition, the carbon atom $C25^{i}$ and the neighboring hydrogen atom H19(A) were at a distance of 2.75(1) Å, which was shorter than the sum of the C...H van der Waals radii. This phenomenon indicated that there was a weak intermolecular CH...π interaction between the hydrogen atom H14(B) and the isoquinoline aromatic ring. The

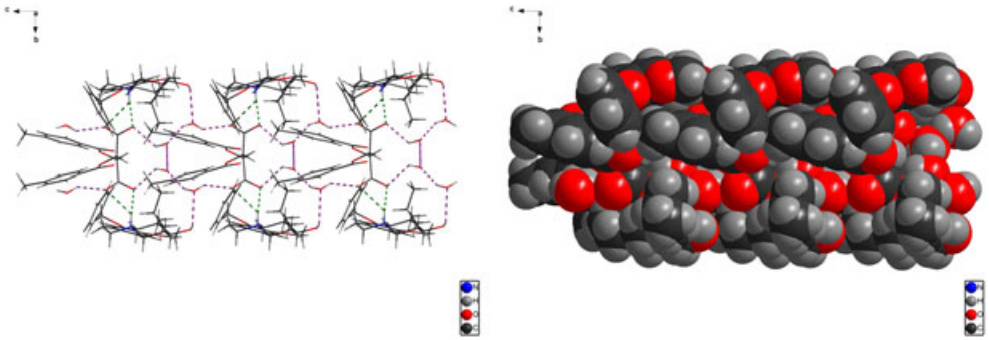


Fig. 5. One-dimensional supramolecular chain of compound **7** as viewed along the the *a*-axis.

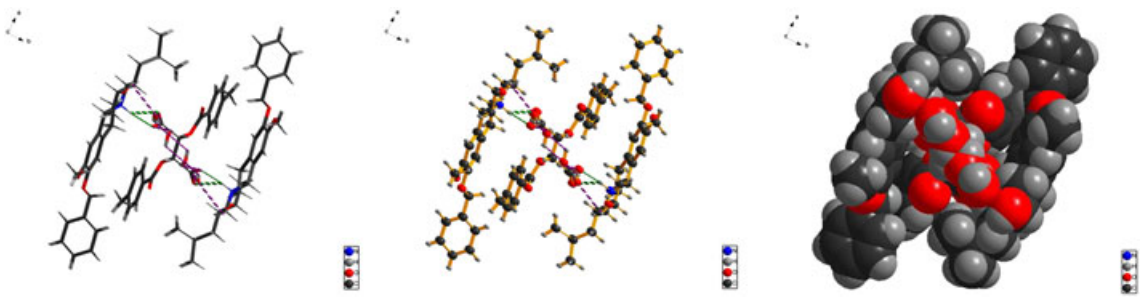


Fig. 6. One-dimensional supramolecular chain of compound **7** as viewed along the the *c*-axis.

distances between H33[iii] and O2[ii] and between H10(A) and O4 were 2.46(1) Å and 2.67(1) Å, respectively, which suggested contribution of weak intermolecular CH...O hydrogen bond interactions.

Compound **7** displayed a polar column through the carbonyl-assisted salt bridges and the OH...O hydrogen bonds as shown in Figure 5. This polar column skeleton played a solid supportive role in the whole crystal structure. The polar column was a one-dimensional supramolecular chain. Figure 6 shows that it resembles a butterfly with open wings as viewed along the *c*-axis. Owing to the weak intermolecular CH...O hydrogen bond interactions and the weak intermolecular CH...π interactions, one-dimensional polar columns extended to a three-dimensional network structure along the *a*-axis and *b*-axis, respectively. This three-dimensional network structure is shown in Figure 7. As mentioned above, the multiple interactions helped stabilize the crystal structure of compound **7**.

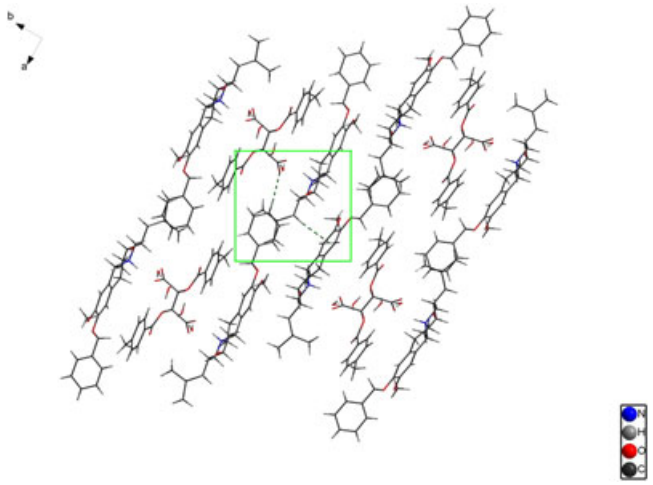


Fig. 7. Crystal packing of compound **7** as viewed along the the *c*-axis.

Configuration of Compound 5, Compound 6, and Compound 8

As reported by Yao[18] and Yu,[19] the *cis*-isomer of compound **5** was thermodynamically unstable. Under the synthesis conditions in this work, only the enantiomers of compound **5** [(*R*,*R*) and (*S*,*S*) isomers] were formed. Our earlier work on using ^{1}H NMR to determine the composition of tetrabenazine (TBZ) could be used to confirm this point.[20] If compound **5** consisted of diastereomers, in the ^{1}H NMR spectrum of compound **5**, the two aromatic ring proton resonances at C-8 and C-11 (the numbered compound **5** shown in Scheme 2) would split into multiple peaks, rather than two singlet peaks. The two aromatic ring proton resonances in compound **5** were two singlet peaks. Therefore, compound **5** consisted of (*R*,*R*) and (*S*,*S*) enantiomers or (*R*,*S*) and (*S*,*R*)

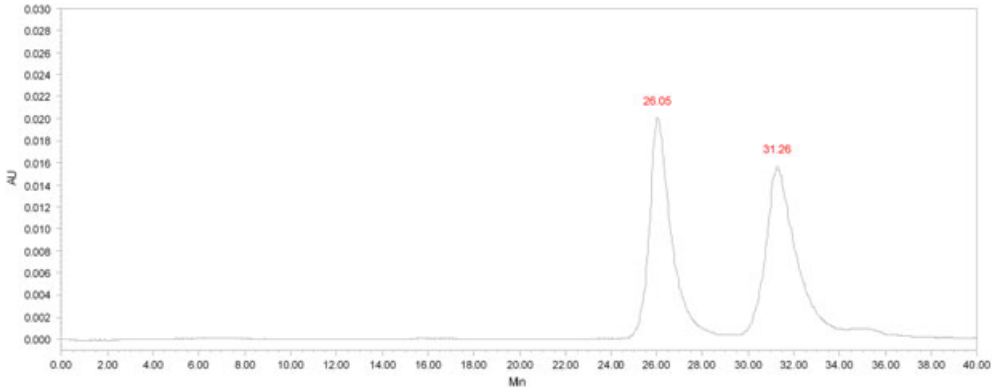


Fig. 8. The chiral HPLC graph of racemic compound **6**.

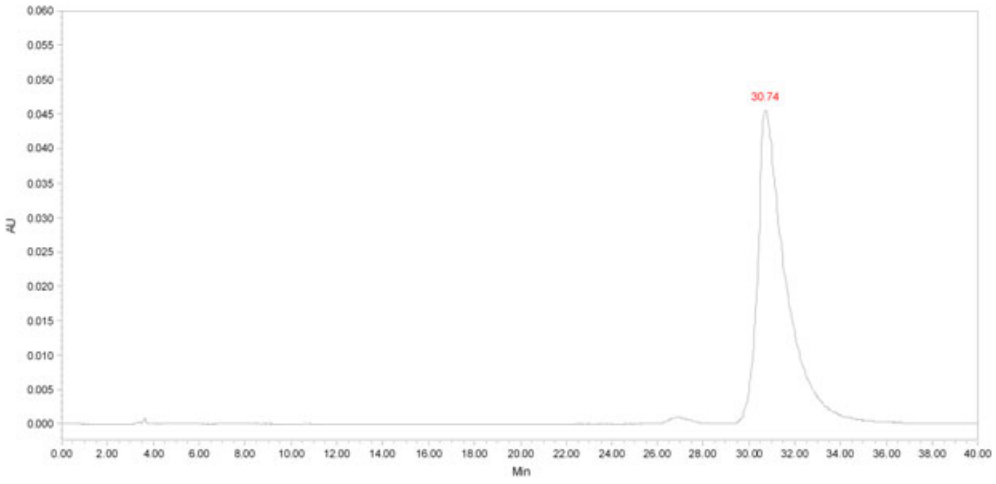


Fig. 9. The chiral HPLC graph of compound **8**.

enantiomers. Combined with the X-ray crystal diffraction structure of racemic compound **5**, we could infer that compound **5** consists of (*R*,*R*) and (*S*,*S*) enantiomers.

Racemic compound **6** was synthesized from racemic compound **5** and a new chiral carbon generated. Therefore, compound **6** may contain a maximum of four isomers: α configuration [(*R*,*R*,*R*) isomer and (*S*,*S*,*S*) isomer] and β configuration [(*R*,*R*,*S*) isomer and (*S*,*S*,*R*) isomer]. The β configuration isomers could be removed by recrystallization.[9] For this reason, racemic compound **6** obtained by this method was α configuration; in other words, it consists of two isomers. This was exactly in line with the results of the chiral HPLC of racemic compound **6** as shown in Figure 8.

Compound **8** was obtained by the separation of racemic compound **6**. The X-ray results showed that compound **8** has the absolute configuration of a (*R*,*R*,*R*) isomer. However, given the X-ray diffraction analysis of the sample only in a single crystal, the result was not universal. The chiral HPLC was also investigated for compound **8**. There was only one peak in the HPLC graph as shown in Figure 9. As discussed above, compound **8** is a single configuration compound and it has the absolute configuration of *R*,*R*,*R*.

CONCLUSIONS

(+)-9-benzyloxy-α-dihydrotetrabenazine (**8**) was synthesized by a six-step reaction. The crystal organizations of racemic compound **5** and compound **7** were established first. X-ray crystal diffraction and chiral HPLC indicated that compound **8** consists of only one isomer and has the absolute configuration of 2*R*,3*R*,11b*R*. Furthermore, two symmetry-independent molecules were found in the crystal of racemic compound **5**. In compound **7**, the (2*R*,3*R*)-(−)-di-p-toluoyl-L-tartrate anion and the (+)-9-benzyloxy-α-dihydrotetrabenazine cation formed a close ion pair through a carbonyl-assisted salt bridge and the OH. . .O hydrogen bond interactions. In addition, these two interactions also created polar columns in the crystal. The polar column was a one-dimensional supramolecular chain and resembled a beautiful butterfly with open wings as viewed along the *c*-axis. Therefore, the carbonyl-assisted salt bridge and the OH. . .O hydrogen bond interactions play a key role in the chiral recognition of compound **7**. These facts clarified the chiral recognition mechanism of compound **7** and they should be helpful in investigating the chiral recognition mechanism of the parent structure of compound **8** for the amino acids in the VMAT2.

ACKNOWLEDGMENTS

We are grateful for the financial support provided by the National Natural Science Foundation of China (30970844) and the Outstanding Professionals Foundation of Jiangsu Health Bureau (RC2011096). This work was also additionally supported in part by the Natural Science Foundation of Jiangsu Province (BK2010155) and the Youth Research Foundation of Jiangsu Institute of Nuclear Medicine (QN201109).

LITERATURE CITED

1. Kilbourn MR, Hockley B, Lee L, Hou C, Goswami R, Ponde DE, Kung MP, Kung HF. Pharmacokinetics of [^{18}F]fluoroalkyl derivatives of dihydrotetrabenazine in rat and monkey brain. Nucl Med Biol 2007;34:233–237.
2. Simpson NR, Souza F, Witkowski P, Maffei A, Raffo A, Herron A, Kilbourn M, Jurewicz A, Herold K, Liu E, Hardy MA, Heertum RV, Harris PE. Visualizing pancreatic beta-cell mass with [^{11}C]DTBZ. Nucl Med Biol 2006;33:855–864.
3. Jewett DM, Kilbourn MR, Lee LC. A simple synthesis of [^{11}C]dihydrotetrabenazine (DTBZ). Nucl Med Biol 1997;24:197–199.
4. Kilbourn MR, Butch ER, Desmond T, Sherman P, Harris PE, Frey KA. Dopamine depletion increases in vivo [^{11}C]dihydrotetrabenazine ([^{11}C] DTBZ) binding in rat striatum. Nucl Med Biol 2010;37:3–8.
5. Koeppe RA, Gilman S, Junck L, Wernette K, Frey KA. Differentiating Alzheimer's disease from dementia with Lewy bodies and Parkinson's disease with (+)-[^{11}C]dihydrotetrabenazine positron emission tomography. Alzheimers Dement 2008;4:S67–S76.
6. Kung MP, Hou C, Goswami R, Ponde DE, Kilbourn MR, Kung HF. Characterization of optically resolved 9-fluoropropyl-dihydrotetrabenazine as a potential PET imaging agent targeting vesicular monoamine transporters. Nucl Med Biol 2007;34:239–246.
7. Goswami R, Ponde DE, Kung MP, Hou C, Kilbourn MR, Kung HF. Fluoroalkyl derivatives of dihydrotetrabenazine as positron emission tomography imaging agents targeting vesicular monoamine transporters. Nucl Med Biol 2006;33:685–694.
8. Zheng PG, Lieberman BP, Choi SR, Plöessl K, Kung HF. Synthesis and biological evaluation of 3-alkyl-dihydrotetrabenazine derivatives as vesicular monoamine transporter-2 (VMAT2) ligands. Bioorg Med Chem Lett 2011;21:3435–3438.
9. Boldt KG, Brine GA, Rehder K. Synthesis of (+)-9-O-desmethyl-dihydrotetrabenazine, precursor for the high affinity VMAT2 imaging pet radioligand [^{11}C]-(+)-dihydrotetrabenazine. Org Prep Proced Int 2008;40:379–384.
10. Kilbourn MR, Lee LC, Heeg MJ, Jewett DM. Absolute configuration of (+)-α-dihydrotetrabenazine, an active metabolite of tetrabenazine. Chirality 1997;9:59–62.
11. Schmiechen R, Mulzer J, Zuhse R. Enantioselective synthesis of the antidepressant rolipram by Michael addition to a nitroolefin. Angew Chem Int Ed Engl 1992;31:870–872.
12. Yonan PK, Grove M. 1-Aryl-3,4-dihydro-2(1H)-isoquinoline carbonyl chlorides. US Pat 1977;4001244.
13. Roche FH, Aktiengesellschaft C. Novel tetrahydro-isoquinoline derivatives and a process for the manufacture thereof. GB Pat 1964;950159.
14. Sheldrick GM. SHELXS-97, program for crystal structure solution. Göttingen, Germany: University of Göttingen; 2008.
15. Sheldrick GM. SHELXL-97, program for crystal structure solution. Göttingen, Germany: University of Göttingen; 2008.
16. Desiraju GR. Supramolecular synthons in crystal engineering—a new organic synthesis DOI:10.1002/anie.199523111 . Angew Chem Int Ed Engl 1995;34:2311–2327.
17. Ichikawa A, Ono H, Echigo T, Mikata Y. Crystal structures and chiral recognition of the diastereomeric salts prepared from 2-methoxy-2-(1-naphthyl)propanoic acid. CrystEngComm 2011;13:4536–4548.
18. Yao Z, Wei X, Wu X, Katz JL, Kopajtic T, Greig NH, Sun H. Preparation and evaluation of tetrabenazine enantiomers and all eight stereoisomers of dihydrotetrabenazine as VMAT2 inhibitors. Eur J Med Chem 2011;46:1841–1848.
19. Yu QS, Luo WM, Deschamps J, Holloway HW, Kopajtic T, Katz JL, Brossi A, Greig NH. Preparation and characterization of tetrabenazine enantiomers against vesicular monoamine transporter 2. ACS Med Chem Lett 2010;1:105–109.
20. Liu CY, Chen ZP, Li XM, Tang J. A concise synthesis of tetrabenazine and its crystal structure. Mol Cryst Liq Cryst 2012;557:39–49.