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Methyl-substituted *trans*-1,2-cyclohexanediamines as new ligands for oxaliplatin-type complexes

Ladislav Habala [a], Claudia Dworak [a], Alexey A. Nazarov [a,*], Christian G. Hartinger [a,†], Sergey A. Abramkin [a], Vladimir B. Arion [a], Wolfgang Lindner [b], Markus Galanski [a,*], Bernhard K. Keppler [a]

[a] *Institute of Inorganic Chemistry, University of Vienna, Waehringer Strasse 42, A-1090 Vienna, Austria*
[b] *Institute of Analytical Chemistry, University of Vienna, Waehringer Strasse 38, A-1090 Vienna, Austria*

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Abstract

Three different pathways for the synthesis of substituted *trans*-(±)-1,2-cyclohexanediamines as new ligands for oxaliplatin-type compounds are presented. The different synthetic routes lead (i) by the synthesis of the compound via *ortho*-bromination of a substituted cyclohexanone followed by reaction with hydroxylamine and reduction by hydrogen, (ii) by addition of azide to cyclohexene mediated by manganese(III) acetate and reduction by hydrogen, or (iii) by trans-dihydroxylation of cyclohexene, and subsequent conversion into the respective mesylate or tosylate, followed by substitution by azide, and reduction in the case of 4-methyl-*trans*-(±)-1,2-cyclohexanediamine to a preferentially equatorially, mainly axially, or exclusively equatorially or axially oriented 4-methyl group, respectively.

1. Introduction

Vicinal diamines are compounds of great importance in chemical and biological processes, e.g., as chiral auxiliaries, as metal ligands in catalytic asymmetric synthesis, or in the synthesis of the anticancer drug oxaliplatin.[1,2] Biotin (vitamin H),[3] peptidic antibiotics,[4,5] bleomycins,[6] antitumor agents,[7–9] or the anti-avian influenza drug Tamiflu[10] contain as an important structural element the diamine function.

Chirality is one of the main features of biology and many of the processes essential for life are stereospecific—therefore one out of two or more isomers works best in a particular physiological situation. The isolation and application of only one of the isomers were found to be advantageous for pharmaceutical purposes, when only one isomer is active or has advantageous pharmacological properties in contrast to the other, which causes adverse effects.[11]

(1*R*,2*R*)-1,2-Cyclohexanediamine [(1*R*,2*R*)-chxn] is the non-leaving group in the recently worldwide approved anticancer drug oxaliplatin [(*SP*-4-2)-{[(1*R*,2*R*)-1,2-cyclohexanediamine-κ^2N,N'][ethanedioato(2-)-$\kappa^2O1,O2$]platinum(II)}]. For oxaliplatin and its closest analogs with the (1*S*,2*S*)- and (1*R*,2*S*)-chxn ligands, the following order of activity for the platinum(II) compounds was found in studies on the structure–activity relationships in vitro and in vivo: (1*R*,2*R*)-chxn (trans-isomer)>(1*S*,2*S*)-chxn (trans-isomer)>(1*R*,2*S*)-chxn (cis-isomer).[12–19] The advantage of using the (1*R*,2*R*)-chxn isomer was found to be even more marked in cells with acquired resistance to cisplatin.[15–17,19] This enhancement of activity when compared to that of the cis-isomer could be explained by a favored binding of complexes with trans-oriented amino-groups to DNA. In complexes with *trans*-chxn ligands, the platinum center and the cyclohexane ring are almost

* Corresponding authors. Tel.: +43 1 4277 52609; fax: +43 1 4277 52680.
E-mail addresses: alex.nazarov@univie.ac.at (A.A. Nazarov), markus.galanski@univie.ac.at (M. Galanski).
† Current address: Laboratoire de Chimie Organométallique et Médicinale, Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland.

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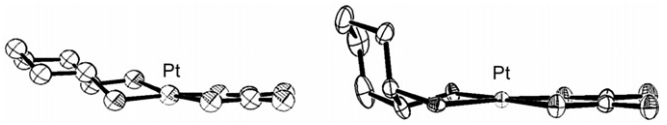

Figure 1. ORTEP plot of the molecular structures of oxaliplatin (left, adopted from Ref. 24) and the analogous complex with the *cis*-chxn ligand (right, adopted from Ref. 25) drawn at 50% probability level (ORTEP-3 for Windows; hydrogen atoms are omitted for clarity).

coplanar, while in the cis-isomer they are nearly perpendicular (Fig. 1) and lead to steric hindrance of DNA binding.[20–23]

The majority of oxaliplatin analogs was obtained by replacing the oxalato ligand by other carboxylic acids [26–30] and did not involve derivatization of the cyclohexane ring. The attachment of a substituent to the cyclohexane ring results in the formation of a third chiral center. This leads in turn to an additional pair of diastereomers for the cis- and trans-isomer. In order to simplify the separation work, there is need for a selective synthetic procedure to introduce an amine functionality to the cyclohexane ring and for a suitable method for the analysis of stereoisomeric mixtures.

In this study, three different synthetic pathways with different directing capabilities for methyl substituents are reported in order to obtain substituted *trans*-1,2-diaminocyclohexanes as ligands for new oxaliplatin-type coordination compounds. The ratio between the different isomers was determined by liquid chromatography with ultraviolet–visible (UV–vis) spectroscopy and electrospray ionization-mass spectrometry (ESI-MS) detection of selectively functionalized compounds, as well as by NMR spectroscopy.

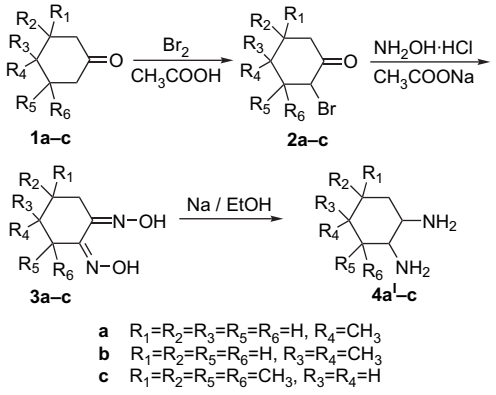

Figure 2. Synthesis of *trans*-(±)-1,2-cyclohexanediamines via pathway I.

2. Results and discussion

For the synthesis of mono-, di-, and tetramethyl-substituted 1,2-cyclohexanediamines **4a$^{I–III}$**, **4b**, and **4c**, respectively, with exclusively trans-oriented amino-groups three reaction pathways were evaluated. (i) The corresponding methyl-substituted cyclohexanones **1a–c** can be converted into the α-bromo-ketones followed by reaction with hydroxylamine and generation of oximes, which can be reduced by hydrogen (sodium/ethanol) to the diamines **4a^{I}**, **4b**, and **4c** (see Fig. 2). (ii) The substituted cyclohexenes are transformed via an addition reaction with sodium azide in the presence of manganese(III) μ_3-oxo acetate into the diazides, and then reduced to the diamines (see Fig. 3, **5**→**9a^{II}**→**4a^{II}**). (iii) The cyclohexenes are converted into the diazides via vicinal diols and the corresponding mesylates or tosylates as main products [see Fig. 3, **5**→**6**→**7** (**8**)→**9a^{III}**→**4a^{III}**].

2.1. Pathway I

Following pathway I the conversion of cyclohexanones **1a–c** into α-bromo-ketones was performed by reaction with bromine in aqueous acetic acid. The α-bromo-ketones were found to be of different stability: 2-bromo-4-methylcyclohexanone **2a** (slightly yellow liquid) is significantly more stable than the solid compounds 2-bromo-4,4-dimethylcyclohexanone **2b** and 2-bromo-3,3,5,5-tetramethylcyclohexanone **2c** (decomposition at room temperature occurs already in 1–2 h accompanied by changing color from white to orange).

In the next step, compounds **2a–c** were reacted with hydroxylamine to give the corresponding vicinal dioximes **3a–c**, which can be isolated from the reaction mixture by extraction with aromatic hydrocarbons. The trans-configured diamines **4a^{I}–c** were obtained by reduction of the dioximes **3a–c** by hydrogen (Na/EtOH). The diamines were isolated as sulfates (**4a^{I}**, **4b**), as L-mandelates (**4a^{I}**, **4b**), and as chlorides (**4c**). 4,4-Dimethyl-*trans*-(±)-1,2-cyclohexanediamine **4b** was isolated as both sulfate and L-mandelate but the latter was chosen for further studies because of the higher yield and purity of the product.

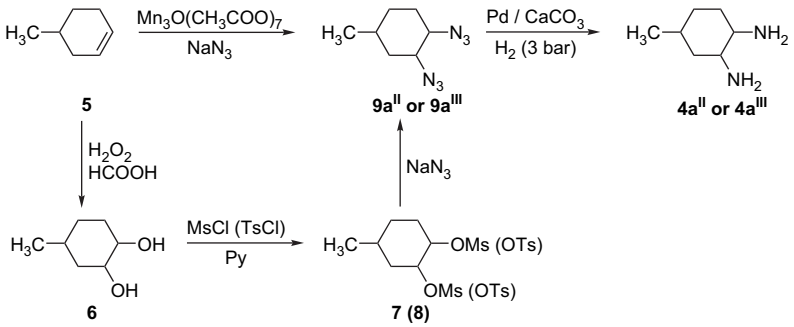

Figure 3. Synthetic pathways II (**5**→**9a^{II}**→**4a^{II}**) and III [**5**→**6**→**7** (**8**)→**9a^{III}**→**4a^{III}**] to obtain 4-methyl-*trans*-(±)-1,2-cyclohexanediamine.

All diamines synthesized by this method are racemic mixtures with exclusively trans-configured amino-groups. The amine **4a^{I}** was obtained as a diastereomeric mixture with the 4-methyl substituent being in equatorial or axial position with a ratio of ca. 90:10% (HPLC and NMR) in the case of the sulfate and of ca. 98:2% if the optically active L-mandelic acid was employed for isolation and the product was recrystallized from an ethanol/diethyl ether mixture. By applying this synthetic pathway, mainly the thermodynamically most stable form with the 4-methyl group in equatorial position was obtained.

2.2. Pathway II

In contrast to pathway I, the following synthetic procedure leads to 4-methyl-*trans*-(±)-1,2-cyclohexanediamine with the methyl substituent preferentially in axial position with a ratio of 88:12% according to HPLC and NMR analyses.

The synthesis started from 4-methylcyclohexene **5**, which was converted into the diazide **9a^{II}** by reaction with sodium azide in the presence of $Mn_3O(CH_3COO)_7$.[31,32] In the next step the azide was reduced with hydrogen at 3 bar in the presence of Pd to the diamine **4a^{II}**. The diastereomeric mixture of 4-methyl-*trans*-(±)-1,2-cyclohexanediamines was isolated as the respective diaminium sulfates. Addition of L-mandelic acid and recrystallization from an ethanol/diethyl ether mixture was applied for increasing the yield of the axial isomer. However, the isomeric ratio changed to 80:20%, and further decreased upon a second recrystallization step. The isomer with an axial methyl group with maximal purity (98%) was obtained from (4*S*)-4-methylcyclohexene, which was synthesized from commercially available (*S*)-(−)-citronellal (98%).[33]

2.3. Pathway III

Only a pair of enantiomers of the diamine **4a^{III}** with the 4-methyl group exclusively in equatorial position was obtained by modification of the synthetic pathway II: in a first step 4-methylcyclohexene **5** was trans-dihydroxylated with H_2O_2/HCOOH with formation of the vicinal diol **6**, which was isolated as the (1*S*,2*S*,4*R*)- and (1*R*,2*R*,4*S*)-enantiomers by recrystallization. Reaction with methanesulfonyl chloride or *p*-toluenesulfonyl chloride gave the mesyl- and tosyl-derivative **7** and **8**, respectively. An X-ray diffraction quality single crystal of 4-methyl-*O*,*O*′-di(*p*-toluenesulfonate)-*trans*-(±)-1,2-cyclohexanediol **8** was obtained by slow crystallization from a *n*-hexane/2-propanol mixture (9:1). Product **8** crystallized as a mixture of enantiomers in the monoclinic centrosymmetric space group C2/*c*. The result of the X-ray diffraction study is shown in Figure 4 and bond lengths and angles of **8** are given in Table S1 (Supplementary data). The cyclohexane ring adopts chair conformation with the two OTs-groups in axial and the methyl group in equatorial positions. The refinement of this structure revealed that **8** as a whole is statistically disordered over two resolvable positions with SOF as 0.5:0.5, which are related through a two-fold rotation axis.

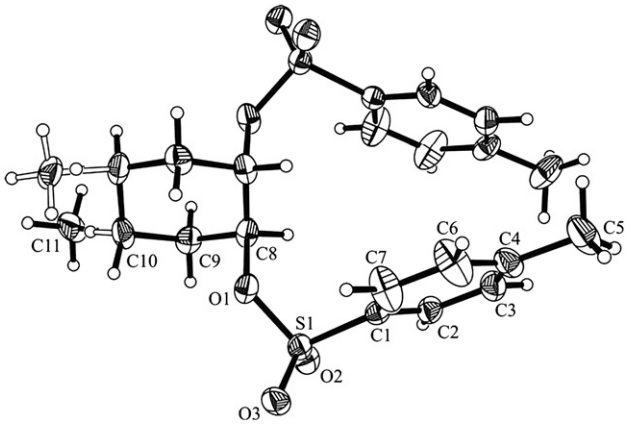

Figure 4. The structure of the tosylate **8** modeled with the ratio of SOF for C11 and C11′ of 0.5:0.5.

The mesylated or tosylated product was converted, by analogy to pathway II, into the diazide **9a^{III}** and then reduced to **4a^{III}**. The pure amine was isolated as in the case of **4a^{II}** as diaminium sulfate. This type of reaction via the tosylate or the mesylate leads to an inversion of the chiral centers in 1- and 2-position (in those mean products the methyl group is in an equatorial position and the OTs or OMs groups are axially oriented), while via pathway II no inversion step is part of the synthetic scheme. Therefore, in that case the mainly axially arranged product is obtained while by using the synthetic procedure described in pathway III the equatorially substituted product is isolated.

2.4. Synthesis of cis-configured 1,2-cyclohexanediamines

Only one way for the synthesis of cis-configured 1,2-cyclohexanediamines was reported in the literature so far. The 1,2-cyclohexanedicarboxylic acid anhydride was hydrolyzed in aqueous solution with sulfuric acid yielding the respective diacid, which was then converted into the diamine via an S_N2 reaction of the diazide intermediate. This pathway yields exclusively cis-configured diamines and was used for preparation of several representatives of this family of compounds (see Fig. 5 for the 4-methyl derivative).[34–36]

2.5. Characterization by NMR

For compounds **4a^{I}** and **4a^{II}** two sets of resonances were found in the ^{13}C NMR spectrum due to the stereochemistry of the methyl group in *trans*-(±)-1,2-cyclohexanediaminium sulfate at the C-4 atom, while applying pathway III leads to only one set of resonances. As a consequence of the equatorial position of the 4-methyl group, the ^{13}C resonances of C-1 and C-2 (both CH−NH_2 groups) at 52.4 and 52.5 ppm, respectively, were shifted similarly. However, in the case of an axially oriented 4-methyl group (minor isomer via pathway I and main product via pathway II) the chemical shifts for

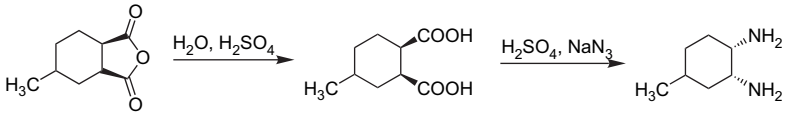

Figure 5. Synthetic pathway to 4-methyl-*cis*-(±)-1,2-cyclohexanediamine.

C-1 and C-2 (48.7 and 51.2 ppm) show a marked difference (see Fig. 6).

Contrary, in the case of 4-methyl-*cis*-(±)-1,2-cyclohexanediamine, the resonances for C-1 and C-2 of the equatorially- and axially-substituted derivatives were found between 48.7 and 51.1 ppm. Consequently, these resonances are well separated from those of the trans-analogs, allowing to distinguish between the cis- and trans-isomer.

Homonuclear decoupling experiments have been performed in order to receive further stereochemical information about the carbon atoms 1, 2, and 4. When equatorial protons H-3 and H-6 were irradiated, residual multiplets for protons H-1 and H-2 with two vicinal coupling constants of 11 Hz were found clearly confirming the axial position of both protons (trans-configuration at C-1 and C-2). Analogously, the configuration at C-4 was determined via irradiation in the methyl resonance.

For analogs **4b** and **4c**, both trans-configuration and equatorial arrangement of the amino functionality are indicated by the coupling constants of H-1 and H-2 signals (ca. 11 Hz), respectively.

2.6. HPLC analysis

In order to determine the ratio between the axially and equatorially substituted isomers, the synthesized diamines were functionalized with an UV active component (see Fig. 7) suitable for UV–vis detection after separation with HPLC.

N,*N*′-Bis(benzyloxycarbonyl)-4-methyl-*trans*-(±)-1,2-cyclohexanedicarbamates were synthesized from the respective diamines obtained via pathways I–III with the 4-methyl group

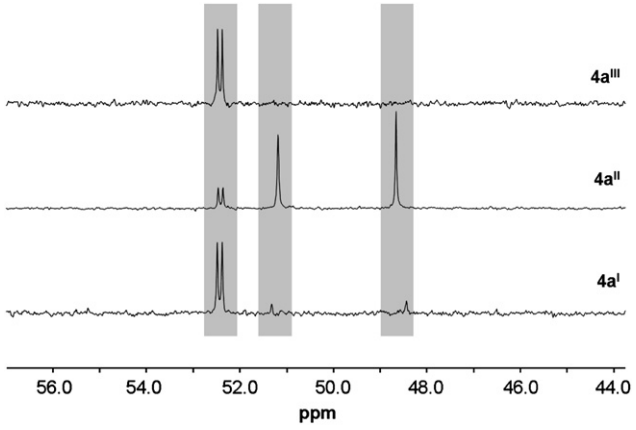

Figure 6. ^{13}C NMR spectra showing the region of the *C*H–NH_2 resonances of 4-methyl-*trans*-(±)-1,2-cyclohexanediamines **4a$^{I–III}$**; respectively.

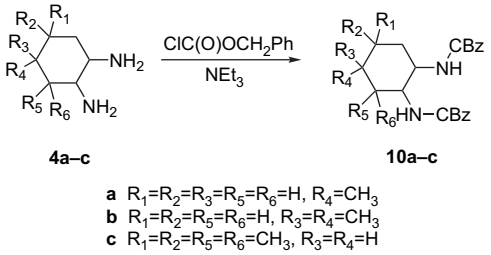

Figure 7. Synthesis of derivatized diamines equipped with an UV active group.

preferentially in equatorial (**4a^{I}**) and in axial (**4a^{II}**), or exclusively in equatorial position (**4a^{III}**). Prior to the reaction with benzyloxycarbonylchloride in the presence of triethylamine, the amines were recovered from their stable salts by dissolution in NaOH solution (5 M). Single crystals of **10a** and **10c** suitable for X-ray diffraction study were obtained by recrystallization from methanol (see Figs. 8 and 9, respectively; for crystallographic data see Table 1). Bond lengths and angles for **10a** and **10c** are given in Tables S3 and S5 (Supplementary data). The CBz-diamines **10a** and **10c** crystallize in the triclinic centrosymmetric space group *P*-1. The cyclohexane ring in compounds **10a** and **10c** has a chair conformation.

Determination of the ratios between the different isomers was done by HPLC on a Chiralcel OD column at 5 °C with an eluent mixture of *n*-hexane and 2-propanol (9:1) with a flow rate of 0.5–1.0 mL. Detection was done with an UV–vis-detector at 210 nm or by coupling to ESI-MS: after splitting the flow (1:100), the eluent was mixed online with methanol containing NaOH (0.5%, 0.1 M) in order to assist the spraying process (for experimental details see Table 2).

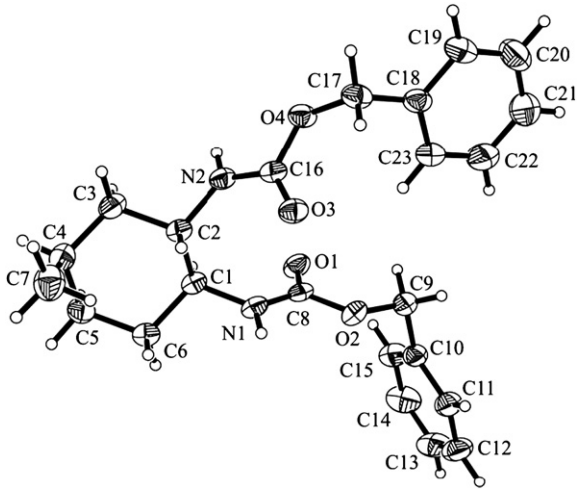

Figure 8. ORTEP view of **10a** with thermal ellipsoids drawn at the 50% probability level.

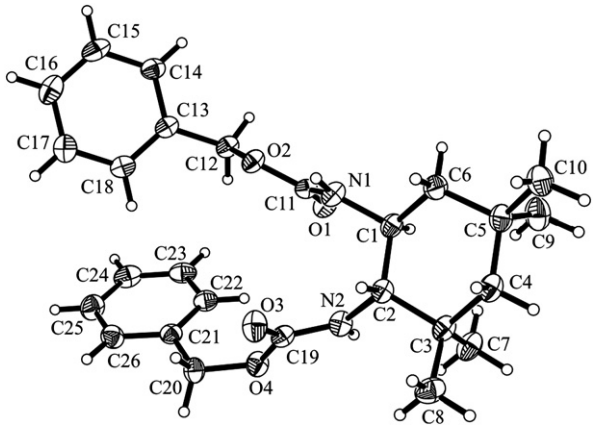

Figure 9. ORTEP view of **10c** with thermal ellipsoids drawn at the 50% probability level.

The HPLC–UV–vis chromatogram of **10a^{II}** (see Fig. 10, dashed line) contained two main signals at 24.5 and 38.1 min, which were assignable to the two enantiomers (peak area ca. 1:1). Additionally, a small peak was detected at 27.9 min. When performing the same HPLC experiment with **10a^{I}**, a converse result was obtained: the ratio between the two faster eluting peaks was reversed (see Fig. 10, solid line). When comparing the elution behavior with that of enantiomerically pure unsubstituted (1*R*,2*R*)- or (1*S*,2*S*)-CBz-chxn, it can be concluded that the fastest eluting species is the (1*S*,2*S*)-enantiomer. Taking into account the NMR data, it is suggested that the successfully separated peaks at about 25 and 28 min are the diastereomers with (1*S*,2*S*,4*R*)- and (1*S*,2*S*,4*S*)-configuration which are followed by the non-resolved (1*R*,2*R*,4*R*)- and (1*R*,2*R*,4*S*)-species (see Fig. 10, dashed line).

In the case of **10a^{III}** only two peaks were visible (see Fig. 10, inset). In order to prove that all assigned peaks in the HPLC–UV–vis chromatogram are isomeric forms of 4-methyl-1,2-cyclohexanediamine, a HPLC–ESI-MS method was developed allowing the identification of the species (for an extracted ion chromatogram [*m*/*z* 419.3±0.5] see Fig. 11). The measured extracted ion chromatogram of the $[M+Na]^+$ species (*m*/*z* 419.2) resembles accurately the result obtained by HPLC–UV–vis (compare Figs. 10 and 11) and the concluded facts based on NMR experiments.

In contrast to the 4-methyl derivatives, the 4,4-dimethyl derivative **10b** and the 3,3,5,5-tetramethyl compound **10c** were obtained only in a mixture of two enantiomers. The chromatogram shows in the case of **10b** only two peaks for the separated enantiomers. A HPLC study with *N*,*N*′-bis(benzyloxycarbonyl)-3,3,5,5-tetramethyl-*trans*-(±)-1,2-cyclohexanedicarbamate **10c** did not allow to separate the two enantiomers under the same conditions—lowering the flow rate to 0.1 mL/min gave a satisfying result accompanied by the shortcoming of very long analysis time (about 3–4 h for one run).

Table 2
Measurement parameters applied for the HPLC–ESI-MS study

Parameter	Settings
Eluent	*n*-hexane/2-propanol=9:1
Flow rate	0.5 mL/min
Column temperature	5 °C
Spray assistance	NaOH (0.5%, 0.1 M) in MeOH at 180 μL/h
Splitter	1:100
Accumulation time	3 ms
ICC Target	200.000
Averages	21 spectra
Scan range	*m*/*z* 50–1200
Trap Drive	23.6
Skim 1	29.0 V
Skim 2	10.0 V
HV capillary	4500 V
Dry temperature	300 °C
Dry gas	9 L/min
Nebulizer	10.0 psi

Table 1
Crystallographic data for **8**, **10a**, and **10c**

Compound	**8**	**10a**	**10c**
Chemical formula	$C_{21}H_{26}O_6S_2$	$C_{23}H_{28}N_2O_4$	$C_{26}H_{34}N_2O_4$
Formula weight	438.54	396.47	438.55
T/K	120	120	120
Space group	*C*2/*c*	*P*-1	*P*-1
a/Å	13.355(3)	9.110(2)	10.881(2)
b/Å	13.242(3)	10.658(2)	17.294(3)
c/Å	13.723(3)	11.818(2)	19.767(4)
α/°		115.16(3)	87.05(3)
β/°	115.30(3)	92.27(3)	76.85(3)
γ/°		92.72(3)	86.55(3)
V/Å^3	2194.1(8)	1035.2(3)	3612.8(12)
Z	4	2	6
μ_{calcd}/cm^{-1}	2.77	0.87	0.81
R_1[a]	0.0405	0.0507	0.0706
wR_2[b]	0.1169	0.1491	0.2003

[a] $R_1 = \sum ||F_o| - |F_c|| / \sum |F_o|$.
[b] $wR_2 = [\sum (w|F_o^2| - |F_c^2|)^2 / \sum w|F_o^2|^2]^{1/2}$.

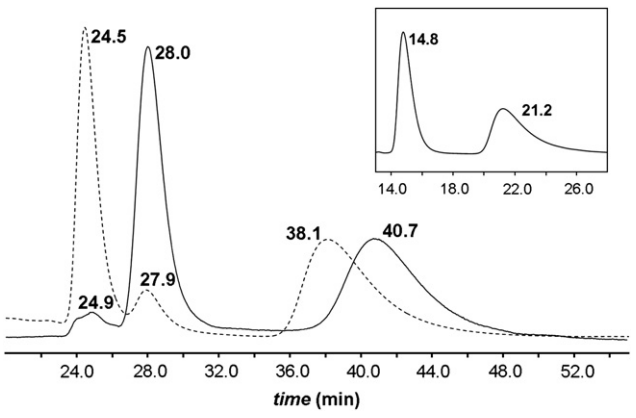

Figure 10. Chromatograms recorded for the separation of *N*,*N*′-bis(benzyloxycarbonyl)-4-methyl-*trans*-(±)-1,2-cyclohexanedicarbamates **10a^{I}** (4-methyl group mainly axial, solid line) and **10a^{II}** (4-methyl group mainly equatorial, dashed line) on a Chiracel OD column (0.5 mL/min, 5 °C). The inset shows the chromatogram of the isomer with exclusively equatorially oriented methyl-group **10a^{III}**.

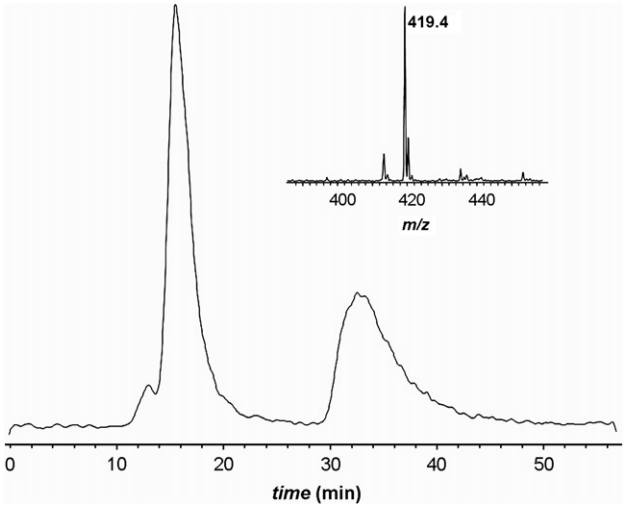

Figure 11. Extracted ion chromatogram (*m/z* 419.3±0.5) recorded for the separation of **10a**$^{\mathbf{I}}$ on a Chiracel OD column after mixing the eluent online with a solution of NaOH (0.5%, 0.1 M) in MeOH at a ratio of 1:0.6. In the inset the electrospray ionization mass spectrum at ca. 16 min (mass range *m/z* 380–460) is depicted.

3. Conclusions

A number of selectively trans-configured 1,2-cyclohexanediamines, bearing either 1, 2, or 4 methyl groups, were synthesized and characterized. The syntheses of the 4-Me-chxn derivatives were done applying different methods each with certain potential to yield compounds with the methyl group preferentially in axial, equatorial, or exclusively in equatorial position.

HPLC (after suitable derivatization of the amino-functionalities with CBz) with UV–vis and ESI-MS detection and NMR experiments allowed the determination of the ratio between axially and equatorially oriented methyl groups of 4-methyl-*trans*-(±)-1,2-cyclohexanediamine. Pathway I (via the vicinal hydroxime) yielded products with the methyl group oriented mainly equatorially, while the addition reaction of azide to cyclohexene derivatives mainly generates axially configured isomers (pathway II). The third synthetic method (via the diol and the mesylate or tosylate), including an inversion of the chiral center (S_N2 from tosylate or mesylate to the azide), yields exclusively the equatorially substituted compound **4a**$^{\mathbf{III}}$.

The diamines were structurally characterized on the basis of the CBz-protected compounds by X-ray diffraction analysis. Additionally, the molecular structure of a mean product of method III, i.e., the tosylate **8**, was determined in the solid state giving important information on the stereoselectivity of the reaction pathway.

4. Experimental section

4.1. General

All chemicals obtained from commercial suppliers were used as received and were of analytical grade. If necessary the reactions were carried out in dry solvents and under argon atmosphere. The NMR spectra were recorded on a Bruker Avance DPX 400 instrument (Ultrashield™ Magnet) at 400.13 MHz (^{1}H) and 100.63 MHz (^{13}C) at 25 °C in DMSO-d_6 or $CDCl_3$. Melting points were determined with a Büchi B-540 apparatus and are uncorrected. Electrospray ionization-mass spectra were recorded on a Bruker *esquire*$_{3000}$ in positive ion mode. The elemental analyses were performed by the Microanalytical Laboratoty of the University of Vienna with a Perkin–Elmer 2400 CHN Elemental Analyzer. Silica gel (Polygram® SIL G/UV$_{254}$) was used for thin layer chromatography. X-ray diffraction measurements were performed on a Nonius Kappa CCD diffractometer at 120 K. Single crystals were positioned at 30 mm from the detector, and 344, 297, and 502 frames were measured, each for 125, 50, and 170 s over 2°, 2°, and 1.5° scan width for **8**, **10a**, and **10c**, respectively. The data were processed using Denzo-SMN software.[37] Crystal data, data collection parameters, and structure refinement details are given in Table 1. The structures were solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters, all hydrogen atoms were inserted in calculated position and refined using a riding model. The structure of **8** was solved in the monoclinic space group *C*2/*c* with assumed disorder consisting in interchange of H atom and CH_3 group at the cyclohexane ring. Our attempt to solve the structure in the space group *Cc* resulted in a severe disorder for the whole molecule and unusual interatomic bond lengths and bond angles. The following computer programs were used: structure solution, SHELXS-97;[38] refinement, SHELXL-97;[39] molecular diagrams, ORTEP;[40] computer, and Pentium IV; scattering factors.[41]

HPLC analysis was carried out on a Dionex Summit® system equipped with a DAD controlled by the Dionex Chromeleon® 6.60 software. The sample concentration was 1.0 mg/mL with an injection volume of 5–15 μL introduced onto a Daicel Chiralcel OD column (250×4.6 mm, purchased from Chiral Technologies, Illkirch, France) with a guard column (50×4.6 mm) operated at 5 °C. The flow rate of the mobile phase (*n*-hexane/2-propanol=9:1) was 0.5 or 1.0 mL/min. For experimental setup of the HPLC–ESI-MS study see Table 2.

4.1.1. 4-Methyl-trans-(±)-1,2-cyclohexanediamine *4a*$^{\mathbf{I}}$

Bromine (23.8 mL, 0.46 mol) was added dropwise to a solution of 4-methylcyclohexanone **1a** (50.0 g, 0.45 mol) in a mixture of acetic acid (92 mL) and water (120 mL) at 35–40 °C. The reaction mixture was stirred for 1 h and then allowed to cool to room temperature. The crude 2-bromo-4-methylcyclohexanone was separated from the supernatant in a separator funnel and dried over Na_2CO_3. 2-Bromo-4-methylcyclohexanone (33.2 g, 0.17 mol) was added dropwise to a boiling solution of hydroxylamine hydrochloride (70.4 g, 1.0 mol) and sodium acetate (145.3 g, 1.1 mol) in a mixture of methanol (126 mL) and water (146 mL). The reaction mixture was refluxed for 1 h and, after cooling to room temperature, the methanol was removed almost completely, and the product was extracted with toluene (5×150 mL). The volume of the

combined organic fractions was reduced to 70 mL and the white or slightly pink oxime was slowly precipitated by addition of *n*-hexane (usually in 3–4 days). Sodium in small pieces (30 g, 1.3 mol) was added to a solution of 4-methylcyclohexane-1,2-dioxime (4 g, 25.6 mmol) in absolute ethanol (150 mL) and allowed to react completely. The product was isolated by water steam distillation (caution: be careful that the complete sodium has reacted) and treated with sulfuric acid (3 M) until pH 7 was reached. The solvent was removed in vacuum and the sulfate salt, consisting of two isomers, was obtained as a white solid.

Yield: 3.06 g (52%), mp>400 °C. Elemental analysis found: C, 37.27; H, 7.80; N, 12.17. Calcd for $C_7H_{16}N_2 \cdot H_2SO_4$: C, 37.15; H, 8.02; N, 12.38. MS (ESI^+) *m/z* 129.1 $[M+H]^+$. ^1H NMR in D_2O: δ=0.85 [d, 3H, CH_3 equatorial, ^3J=6.5 Hz], 0.90 [d, 3H, CH_3 axial, ^3J=7.0 Hz], 0.99 [m, 1H, H-5], 1.15 [m, 1H, H-3], 1.42–1.55 [m, 2H, H-4, H-6], 1.72 [m, 1H, H-5′], 1.99–2.10 [m, 2H, H-6′, H-3′], 3.23–3.39 [m, 2H, H-1, H-2]. ^{13}C NMR in D_2O: isomer with equatorial 4-methyl group (90%) δ=20.7 [CH_3], 29.5 [C-6], 30.2 [C-4], 31.5 [C-5], 37.6 [C-3], 52.4 [C-1], 52.5 [C-1]; isomer with axial 4-methyl group (10%) δ=17.9 [CH_3], 24.0 [C-6], 25.8 [C-4], 27.8 [C-5], 33.8 [C-3], 48.7 [C-1], 51.2 [C-2].

4.1.2. 4,4-Dimethyl-trans-(±)-1,2-cyclohexanediamine ***4b***

Bromine (7.8 mL, 0.15 mol) was added dropwise to a solution of 4,4-dimethylcyclohexanone **1b** (19.0 g, 0.15 mol) in a mixture of acetic acid (25 mL) and water (40 mL) at 50–55 °C. The reaction mixture was stirred for 1 h, cooled to 0 °C, and the crude product was allowed to crystallize from the mother liquid, which was decanted and the product was purified by recrystallization from hexane (at −50 °C) as white solid with a melting point of 60–62 °C being in accordance to the literature data.[42] A solution of 2-bromo-4,4-dimethylcyclohexanone **2b** (25.0 g, 0.12 mol) in methanol (50 mL) was added dropwise to a boiling solution of hydroxylamine hydrochloride (57.0 g, 0.82 mol) and sodium acetate (111.0 g, 0.82 mol) in a mixture of methanol (120 mL) and water (130 mL). The reaction mixture was refluxed for 2 h and, after allowing the solution to cool to room temperature, most of the solvent was removed and the product was extracted with toluene (5×150 mL). The volume of the combined organic fractions was reduced to 70 mL and the white or slightly pink oxime was precipitated by addition of *n*-hexane. Sodium in small pieces (15 g, 650 mol) was added to a solution of 4,4-dimethylcyclohexane-1,2-dioxime **3b** (2 g, 12 mmol) in absolute ethanol (150 mL). The mixture was allowed to react completely, and then it was cooled to room temperature and treated with concentrated hydrochloric acid (caution: be careful that the complete sodium has reacted) until pH 1 was reached. The white precipitate was filtered off and the solvent evaporated under reduced pressure. The brown oil was treated with 5 M NaOH solution and extracted with diethyl ether (3×50 mL). The combined ether fractions were evaporated, and the resulting oil was dissolved in water and neutralized with L-mandelic acid. The solvent was removed in vacuum, the semicrystalline compound was dissolved in 2-propanol, and the L-mandalate was finally obtained as a white solid upon addition of diethyl ether.

Yield: 1.31 g (25%), mp 196–197 °C. Elemental analysis found: C, 64.39; H, 7.70; N, 6.12. Calcd for $C_8H_{18}N_2 \cdot 2C_8H_8O_3$: C, 64.55; H, 7.67; N, 6.27. MS (ESI^+) *m/z* 143.1 $[M+H]^+$. ^1H NMR in D_2O: δ=0.82 [s, 3H, CH_3], 0.88 [s, 3H, CH_3], 1.22 [m, 1H, H-5], 1.31 [t, 1H, H-3, ^3J=13.0 Hz], 1.41 [m, 1H, H-5], 1.61 [m, 1H, H-6], 1.72 [dt, 1H, H-3′, ^3J=3.0 Hz, ^2J=13.0 Hz], 1.89 [m, 1H, H-6′], 3.16 [td, 1H, H-1, ^3J=5.0, 12.0 Hz], 3.41 [td, 1H, H-2, ^3J=4.5, 12.0 Hz], 4.87 [s, 2H, Ph(OH)CH], 7.31–7.25 [m, 10H, H-Ar]. ^{13}C NMR in D_2O: δ=23.3 [CH_3], 26.0 [C-6], 30.6 [CH_3], 31.1 [C-4], 35.7 [C-5], 41.9 [C-3], 50.0 [C-2], 52.9 [C-1], 75.3 [Ph(OH)CH], 127.4 [C-Ar], 128.6 [C-Ar], 129.1 [C-Ar], 140.8 [C-Ar], 179.8 [C(O)OH].

4.1.3. 3,3,5,5-Tetramethyl-trans-(±)-1,2-cyclohexanediamine ***4c***

Bromine (7.8 mL, 150 mmol) was added dropwise to a solution of 4,4-dimethylcyclohexanone **1c** (23.1 g, 150 mmol) in a mixture of acetic acid (25 mL) and water (10 mL) at 50–55 °C. The reaction mixture was stirred for 1 h, cooled to 0 °C, and the crude product was allowed to crystallize from the mother liquid, which was decanted. The product was purified by recrystallization from hexane (at −50 °C). A solution of 2-bromo-3,3,5,5-tetramethylcyclohexanone **2c** (27.8 g, 120 mmol) in methanol (50 mL) was added dropwise to a boiling solution of hydroxylamine hydrochloride (57 g, 0.82 mmol) and sodium acetate (111 g, 820 mmol) in a mixture of methanol (120 mL) and water (130 mL). The reaction mixture was refluxed for 2 h and, after cooling to room temperature, the methanol was removed and the product was extracted with toluene (5×150 mL). The volume of the combined organic fractions was reduced to 70 mL and the white or slightly pink oxime **3c** was precipitated by addition of *n*-hexane. Sodium in small pieces (15 g, 650 mmol) was added to a solution of 3,3,5,5-tetramethylcyclohexane-1,2-dioxime **3c** (2.4 g, 1.2 mmol) in absolute ethanol (150 mL) and allowed to react until no sodium was observable. The reaction mixture was cooled to room temperature and acidified with concentrated hydrochloric acid until pH 1 was reached (caution: be careful that the complete sodium has reacted). The white precipitate was filtered off and the solvent was evaporated under reduced pressure. The crude semicrystalline compound was dissolved in 2-propanol and the chloride was precipitated as a white solid by addition of diethyl ether.

Yield: 0.95 g (33%), mp 290–293 °C. Elemental analysis found: C, 49.57; H, 10.04; N, 11.75. Calcd for $C_{10}H_{22}N_2 \cdot 2HCl$: C, 49.38; H, 9.94; N, 11.51. MS (ESI^+) *m/z* 171.2 $[M+H]^+$. ^1H NMR in D_2O: δ=0.91 [s, 3H, CH_3], 1.01 [s, 3H, CH_3], 1.06 [s, 3H, CH_3], 1.07 [s, 3H, CH_3], 1.35–1.51 [m, 3H, H-4, H-4′, H-6], 1.87 [m, 1H, H-6′], 3.14 [d, 1H, H-2, ^3J=11.0 Hz], 3.68 [td, 1H, H-1, ^3J=3.5, 11.0 Hz]. ^{13}C NMR in D_2O: δ=21.4 [CH_3], 26.0 [CH_3], 30.2 [CH_3], 30.6 [C-5], 33.35 [CH_3], 35.21 [C-3], 42.87 [C-6], 48.79 [C-1], 51.30 [C-4], 61.27 [C-2].

4.1.4. 4-Methyl-trans-(±)-1,2-cyclohexanediol ***6***

4-Methylcyclohexene **5** (79.9 g, 0.83 mol) was added dropwise to a vigorously stirred mixture of hydrogen peroxide (30%, 160 mL, 1.56 mol) and formic acid (85%, 360 mL, 7.91 mol) at 40–45 °C. The reaction mixture was stirred for 1 h at 40 °C and for 3 h at room temperature. The solvents were removed in vacuum and NaOH (8 M, 200 mL) was added dropwise at 0 °C. Then it was stirred for 12 h at room temperature and water (100 mL) was added. The crude product was extracted from the reaction mixture with ethyl acetate (3×100 mL). The combined organic fractions were washed with water (2×100 mL), dried over Na_2SO_4, and the solvent was removed in vacuum to give a yellowish viscous liquid, which solidified overnight. The pure product was obtained after recrystallization from an ethanol/petroleum ether mixture (1:1).

Yield: 88.1 g (81%), mp 68–69 °C. Elemental analysis found: C, 64.63; H, 10.92. Calcd for $C_7H_{14}O_2$: C, 64.58; H, 10.84. MS (ESI^+) *m/z* 153.1 $[M+Na]^+$. ^1H NMR in $CDCl_3$: δ=1.00 [d, 3H, CH_3, ^3J=7.0 Hz], 1.40–1.64 [m, 4H, H-5, H-3, H-6, H-5′], 1.66–1.89 [m, 2H, H-3′, H-6′], 2.04 [m, 1H, H-4], 2.69 [br s, 1H, OH], 2.79 [s, 1H, OH], 3.43 [m, 1H, H-1], 3.67 [m, 1H, H-2]. ^{13}C NMR in $CDCl_3$: δ=19.5 [CH_3], 28.0 [C-4], 28.1 [C-6], 29.8 [C-5], 38.5 [C-3], 71.8 [C-2], 75.7 [C-1].

4.1.5. O,O′-Di(methanesulfonate)-4-methyl-trans-(±)-1,2-cyclohexanediol ***7***

Methanesulfonyl chloride (114.2 mL, 1.47 mol) was added dropwise to a solution of 4-methyl-*trans*-(±)-1,2-cyclohexanediol **6** (66.6 g, 0.51 mol) in dry pyridine (380 mL) at 0 °C. The reaction mixture was stirred for 2 h and then cold water (400 mL) was slowly added. The crude product was extracted with CH_2Cl_2 (3×200 mL), the combined organic fractions were washed with water (3×100 mL), dried over Na_2SO_4, and the solvent was removed in vacuum. The resulting viscous liquid was washed with petroleum ether to remove the pyridine. The pure product was obtained after recrystallization from an ethanol/*n*-hexane mixture (3:1).

Yield: 94.1 g (71%), mp 93–94 °C. Elemental analysis found: C, 37.73; H, 6.42. Calcd for $C_9H_{18}O_6S_2$: C, 37.74; H, 6.34. MS (ESI^+) *m/z* 309.1 $[M+Na]^+$. ^1H NMR in $CDCl_3$: δ=1.00 [d, 3H, CH_3, ^3J=6.5 Hz], 1.41 [m, 1H, H-5], 1.62 [m, 1H, H5′], 1.78 [m, 1H, H-3], 1.87–2.11 [m, 4H, H-3′, H-6, H-4, H-6′], 3.08 [s, 3H, CH_3], 3.09 [s, 3H, CH_3], 4.75 [m, 1H, H-1], 4.87 [m, 1H, H-2]. ^{13}C NMR in $CDCl_3$: δ=20.7 [CH_3], 26.4 [C-4], 26.8 [C-6], 28.2 [C-5], 35.8 [C-3], 38.9 [CH_3], 77.4 [C-2], 77.8 [C-1].

4.1.6. 4-Methyl-O,O′-di(p-toluenesulfonate)-trans-(±)-1,2-cyclohexanediol ***8***

Following the same procedure as described for **7**, compound **8** was obtained from 4-methyl-*trans*-(±)-1,2-cyclohexanediol (20.0 g, 0.15 mol), pyridine (140 mL), and *p*-toluenesulfonyl chloride (82.4 g, 0.43 mol).

Yield: 57.5 g (81%), mp 108–109 °C. Elemental analysis found: C, 57.39; H, 5.79. Calcd for $C_{21}H_{26}O_6S_2$: C, 57.51; H, 5.98. MS (ESI^+) *m/z* 461.1 $[M+Na]^+$. ^1H NMR in $CDCl_3$: δ=0.85 [d, 3H, CH_3, ^3J=7.0 Hz], 1.23 [m, 1H, H-5], 1.34–1.50 [m, 2H, H-3, H-5′], 1.54–1.67 [m, 2H, H-6, H-3′], 1.68–1.72 [m, 2H, H-4, H-6′], 2.50 [s, 6H, CH_3], 4.49 [m, 1H, H-2], 4.54 [m, 1H, H-1], 7.37–7.76 [m, 10H, H-Ar]. ^{13}C NMR in $CDCl_3$: δ=21.9 [CH_3], 22.1 [CH_3], 25.6 [C-4], 26.1 [C-6], 27.7 [C-5], 34.4 [C-3], 76.1 [C-2], 76.9 [C-1], 128.3 [C-Ar], 130.3 [C-Ar], 133.6 [C-Ar], 145.4 [C-Ar].

4.1.7. 4-Methyl-trans-(±)-1,2-cyclohexanediamine ***4a^{II}***

A solution of 4-methyl-1-cyclohexene **5** (20 g, 0.21 mol) in trifluoroacetic acid (240 mL) was added to a mixture of $Mn_3O(CH_3COO)_7$ (176 g, 0.62 mol) and NaN_3 (68 g, 1.04 mol) in acetonitrile (2400 mL) under N_2 atmosphere at −20 °C. The reaction mixture was stirred for 3 h at −19 to −21 °C and afterward $NaHSO_3$ (10%, 600 mL) was added. The product was extracted with petroleum ether (3×500 mL), washed with saturated Na_2CO_3 solution (2×200 mL) and brine (2×200 mL), and dried over Na_2SO_4. The solvent was removed in vacuum. A mixture of 4-methyl-*trans*-(±)-1,2-cyclohexanediazide **9a^{II}** (4.0 g, 22.2 mmol) and palladium on $CaCO_3$ (5% Pd, 1.6 g) in dry ethanol (50 mL) was stirred for 24 h under hydrogen atmosphere (3 bar). The catalyst was filtered off, the solvent was removed, and the product was precipitated by addition of sulfuric acid (3 M) until neutralization of the solution. Then the sulfate was filtered off, washed with diethyl ether (2×50 mL), and dried in vacuum.

Yield: 2.7 g (53%), mp 385–390 °C. Elemental analysis found: C, 37.31; H, 7.86; N, 12.25. Calcd for $C_7H_{16}N_2 \cdot H_2SO_4$: C, 37.15; H, 8.02; N, 12.38. MS (ESI^+) *m/z* 129.1 $[M+H]^+$. ^1H NMR in D_2O: δ=0.86 [d, 3H, CH_3 equatorial, ^3J=6.7 Hz], 0.88 [d, 3H, CH_3 axial, ^3J=7.0 Hz], 1.38 [m, 1H, H-5], 1.56 [m, 1H, H-5′], 1.62–1.82 [m, 3H, H-3, H-6, H-3′], 1.82–1.98 [m, 2H, H-6′, H-4], 3.37 [m, 1H, H-1], 3.54 [m, 1H, H-2]. ^{13}C NMR in D_2O: isomer with axial 4-methyl group (88%) δ=18.0 [CH_3], 24.0 [C-6], 25.8 [C-4], 27.8 [C-5], 33.8 [C-3], 48.7 [C-1], 51.2 [C-2]; isomer with equatorial 4-methyl group (12%) δ=20.7 [CH_3], 29.5 [C-6], 30.2 [C-4], 31.5 [C-5], 37.7 [C-3], 52.4 [C-1], 52.5 [C-2].

4.1.8. 4-Methyl-trans-(±)-1,2-cyclohexanediamine ***4a^{III}***

NaN_3 (14.0 g, 210 mol) in dry DMF (100 mL) was added to *O,O′*-di(methanesulfonate)-4-methyl-*trans*-(±)-1,2-cyclohexanediol **7** (15.1 g, 53 mmol) and the reaction mixture was kept for 48 h at 120 °C. After allowing the reaction mixture to cool to room temperature, water (300 mL) was added and the product was extracted with petroleum ether (3×100 mL). The combined organic fractions were washed with water (3×100 mL) and dried over Na_2SO_4. The crude diazide (4.3 g, yield: 46%) was obtained after removing the solvent in vacuum. A mixture of 4-methyl-*trans*-(±)-1,2-cyclohexanediazide **9a^{III}** (2.5 g, 14.3 mmol) and palladium on $CaCO_3$ (5% Pd, 500 mg) in dry ethanol (30 mL) was stirred for 24 h under hydrogen atmosphere (3 bar). The catalyst was filtered off, the solvent was removed, and the product was precipitated by addition of diluted sulfuric acid until pH 7 was reached. The sulfate was

filtered off, washed with diethyl ether (2×50 mL), and dried in vacuum.

Yield: 3.3 g (28%), mp>400 °C. Elemental analysis found: C, 37.28; H, 8.11; N, 12.54. Calcd for $C_7H_{16}N_2 \cdot H_2SO_4$: C, 37.15; H, 8.02; N, 12.38. MS (ESI^+) *m/z* 129.1 $[M+H]^+$. ^1H NMR in D_2O: δ=0.85 [d, 3H, CH_3, ^3J=6.5 Hz], 0.99 [m, 1H, H-5], 1.15 [m, 1H, H-3], 1.41–1.58 [m, 2H, H-4, H-6], 1.71 [m, 1H, H-5′], 1.98–2.10 [m, 2H, H-6′, H-3′], 3.20–3.38 [m, 2H, H-1, H-2]. ^{13}C NMR in D_2O: δ=20.7 [CH_3], 29.5 [C-6], 30.2 [C-4], 31.5 [C-5], 37.6 [C-3], 52.4 [C-1], 52.5 [C-2].

4.1.9. N,N′-Bis(benzyloxycarbonyl)-4-methyl-trans-(±)-1,2-cyclohexanedicarbamate ***10a^I***

4-Methyl-*trans*-(±)-1,2-cyclohexanediamine **4a^I** (0.30 g) was recovered from the respective diaminium sulfate by dissolution in aqueous NaOH solution (5 M, 15 mL) and extraction with dichloromethane (3×10 mL). Triethylamine (0.55 mL, 3.98 mmol) was added at 0–5 °C under Ar atmosphere to the combined CH_2Cl_2 fractions. The reaction mixture was treated with benzyloxycarbonylchloride (0.45 mL, 3.18 mmol) under vigorous stirring and kept at 0–5 °C for 15 min. Then it was allowed to warm to room temperature and stirred for another 3 h (TLC control, *n*-hexane/ethyl acetate=3:1). The reaction mixture was diluted with CH_2Cl_2 (5 mL), washed with brine (2×10 mL), and dried over Na_2SO_4. The solvent was removed to give a slightly brown crude product, which was purified by recrystallization from MeOH.

Yield: 0.24 g (47%), mp 154–155 °C. Elemental analysis found: C, 69.62; H, 7.04; N, 7.24. Calcd for $C_{23}H_{28}N_2O_4$: C, 69.66; H, 7.12; N, 7.07. MS (ESI^+) *m/z* 419.2 $[M+Na]^+$. ^1H NMR in $CDCl_3$: δ=0.94 [d, 3H, CH_3 equatorial, ^3J=6.5 Hz], 1.04 [d, 3H, CH_3 axial, ^3J=7.0 Hz], 0.93–1.08 [m, 2H, H-3, H-5], 1.32 [m, 1H, H-6], 1.54 [m, 1H, H-4], 1.72 [m, 1H, H-5′], 2.05–2.09 [m, 2H, H-3′, H-6′], 3.36–3.46 [m, 2H, H-2, H-1], 5.04–5.12 [m, 6H, OCH_2, NH], 7.34 [s, 10H, H-Ar]. ^{13}C NMR in $CDCl_3$: isomer with equatorial 4-methyl group (90%) δ=22.0 [CH_3], 31.9 [C-4], 32.6 [C-5], 33.7 [C-6], 41.6 [C-3], 55.5 [C-1], 55.7 [C-2], 67.0 [OCH_2], 128.3 [C-Ar], 128.4 [C-Ar], 128.9 [C-Ar], 137.0 [C-Ar], 157.2 [C(O)]; isomer with axial 4-methyl group (10%) δ=18.6 [CH_3], 27.6 [C-5], 27.7 [C-4], 30.3 [C-6], 38.3 [C-3], 50.9 [C-1, C-2], 67.1 [OCH_2], 128.3 [C-Ar], 128.4 [C-Ar], 128.9 [C-Ar], 137.0 [C-Ar], 157.1 [C(O)].

4.1.10. N,N′-Bis(benzyloxycarbonyl)-4-methyl-trans-(±)-1,2-cyclohexanedicarbamate ***10a^{II}***

Following the same procedure as described for **10a^I**, a diastereomeric mixture was obtained from 4-methyl-*trans*-(±)-1,2-cyclohexanediaminium sulfate (0.13 g), triethylamine (0.24 mL), and benzyloxycarbonylchloride (0.20 mL).

Yield: 74 mg (30%), mp 112–113 °C. Elemental analysis found: C, 69.57; H, 7.12; N, 7.09. Calcd for $C_{23}H_{28}N_2O_4$: C, 69.66; H, 7.12; N, 7.07. MS (ESI^+) *m/z* 419.2 $[M+Na]^+$. ^1H NMR in $CDCl_3$: δ=0.94 [d, 3H, CH_3 equatorial, ^3J=6.5 Hz], 1.04 [d, 3H, CH_3 axial, ^3J=7.0 Hz], 1.44–1.57 [m, 4H, H-3, H-3′, H-5, H-6], 1.79–1.90 [m, 2H, H-5′, H-6′], 2.05 [m, 1H, H-4], 3.41 [m, 1H, H-1], 3.69 [m, 1H, H-2], 4.96–5.18 [m, 6H, OCH_2, NH], 7.35 [s, 10H, H-Ar]. ^{13}C NMR in $CDCl_3$: isomer with axial 4-methyl group (88%) δ=18.6 [CH_3], 27.6 [C-5], 27.7 [C-4], 30.3 [C-6], 38.3 [C-3], 50.9 [C-2, C-1], 67.1 [OCH_2], 128.3 [C-Ar], 128.4 [C-Ar], 128.9 [C-Ar], 137.0 [C-Ar], 157.1 [C(O)]; isomer with equatorial 4-methyl group (12%) δ=22.1 [CH_3], 31.9 [C-4], 32.7 [C-5], 33.7 [C-6], 41.6 [C-3], 55.5 [C-2], 55.7 [C-1], 67.1 [OCH_2], 128.3 [C-Ar], 128.4 [C-Ar], 128.9 [C-Ar], 137.0 [C-Ar], 157.1 [C(O)].

4.1.11. 4-Methyl-N,N′-bis(benzyloxycarbonyl)-trans-(±)-1,2-cyclohexanedicarbamate ***10a^{III}***

Following the same procedure as described for **10a^I**, an enantiomeric mixture was obtained from 4-methyl-*trans*-(±)-1,2-cyclohexanediaminium sulfate (0.3 g), triethylamine (0.55 mL), and benzyloxycarbonylchloride (0.45 mL).

Yield: 0.16 g (31%), mp 175–176 °C. Elemental analysis found: C, 69.45; H, 7.18; N, 7.36. Calcd for $C_{23}H_{28}N_2O_4$: C, 69.66; H, 7.12; N, 7.07. MS (ESI^+) *m/z* 419.2 $[M+Na]^+$. ^1H NMR in $CDCl_3$: δ=0.94 [d, 3H, ^3J=6.5 Hz, CH_3], 0.93–1.08 [m, 2H, H-3, H-5], 1.32 [m, 1H, H-6], 1.54 [m, 1H, H-4], 1.72 [m, 1H, H-5′], 2.05–2.09 [m, 2H, H-3′, H-6′], 3.36–3.46 [m, 2H, H-2, H-1], 5.04–5.12 [m, 6H, OCH_2, NH], 7.34 [s, 10H, H-Ar]. ^{13}C NMR in $CDCl_3$: δ=22.0 [CH_3], 31.9 [C-4], 32.6 [C-5], 33.7 [C-6], 41.6 [C-3], 55.5 [C-1], 55.7 [C-2], 67.0 [OCH_2], 128.3 [C-Ar], 128.4 [C-Ar], 128.9 [C-Ar], 137.0 [C-Ar], 157.23 [C(O)].

4.1.12. N,N′-Bis(benzyloxycarbonyl)-4,4-dimethyl-trans-(±)-1,2-cyclohexanedicarbamate ***10b***

Following the same procedure as described for **10a^I**, compound **10b** was obtained from 4,4-dimethyl-*trans*-(±)-1,2-cyclohexanediaminium L-mandelate (0.52 g), triethylamine (0.60 mL), and benzyloxycarbonylchloride (0.49 mL).

Yield: 0.14 g (29%), mp 151–152 °C. Elemental analysis found: C, 69.93; H, 7.29; N, 6.86. Calcd for $C_{24}H_{30}N_2O_4$: C, 70.22; H, 7.36; N, 6.82. MS (ESI^+) *m/z* 433.2 $[M+Na]^+$. ^1H NMR in $CDCl_3$: δ=0.97 [s, 3H, CH_3], 1.01 [s, 3H, CH_3], 1.16 [m, 1H, H-3], 1.31 [m, 1H, H-5], 1.40–1.49 [m, 2H, H-5′, H-6], 1.77 [m, 1H, H-3′], 1.95 [m, 1H, H-6′], 3.33 [m, 1H, H-1], 3.62 [m, 1H, H-2], 4.96–5.17 [m, 6H, OCH_2, NH], 7.34 [s, 10H, H-Ar]. ^{13}C NMR in $CDCl_3$: δ=25.0 [CH_3], 29.1 [C-6], 31.9 [C-4], 32.7 [CH_3], 38.0 [C-5], 45.9 [C-3], 52.3 [C-1], 56.6 [C-2], 67.0 [OCH_2], 128.3 [C-Ar], 128.4 [C-Ar], 128.9 [C-Ar], 137.0 [C-Ar], 157.17 [C(O)].

4.1.13. N,N′-Bis(benzyloxycarbonyl)-3,3,5,5-tetramethyl-trans-(±)-1,2-cyclohexanedicarbamate ***10c***

Following the same procedure as described for **10a^I**, compound **10c** was obtained from 3,3,5,5-tetramethyl-*trans*-(±)-1,2-cyclohexanediaminium chloride (0.30 g), triethylamine (0.63 mL), and benzyloxycarbonylchloride (0.42 mL).

Yield 0.10 g (20%), mp 120–121 °C. Elemental analysis found: C, 70.94; H, 7.57; N, 6.38. Calcd for $C_{26}H_{34}N_2O_4$: C, 71.21; H, 7.81; N, 6.37. MS (ESI^+) *m/z* 461.3 $[M+Na]^+$. ^1H NMR in $CDCl_3$: δ=0.94 [s, 3H, CH_3], 0.97 [s, 3H, CH_3], 1.04 [s, 3H, CH_3], 1.10 [s, 3H, CH_3], 1.20–1.43 [m, 3H, H-

6, H-4, H-4′], 1.81 [m, 1H, H-6′], 3.28 [m, 1H, H-2], 3.80 [m, 1H, H-1], 4.88–4.98 [m, 3H, OCH_2, NH], 5.08–5.11 [m, 3H, OCH_2, NH], 7.34 [s, 10H, H-Ar]. ^{13}C NMR in $CDCl_3$: δ=22.5 [CH_3], 27.7 [CH_3], 31.9 [C-5], 32.0 [CH_3], 34.9 [CH_3], 36.4 [C-3], 46.7 [C-6], 49.8 [C-1], 53.1 [C-4], 63.9 [C-2], 67.1 [OCH_2], 128.2 [C-Ar], 128.2 [C-Ar], 128.9 [C-Ar], 137.0 [C-Ar], 157.2 [C(O)], 158.1 [C(O)].

Crystallographic data for the structural analysis has been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 663505 (**8**), 663504 (**10c**), 663503 (**10a**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.ac.uk).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.10.069.

References and notes

1. Lucet, D.; Le Gall, T.; Mioskowski, C. *Angew. Chem., Int. Ed.* **1998**, *37*, 2580–2627.
2. Kotti, S. R. S. S.; Timmons, C.; Li, G. *Chem. Biol. Drug Des.* **2006**, *67*, 101–114.
3. Marquet, A. *Pure Appl. Chem.* **1993**, *65*, 1249–1252.
4. Hettinger, T. P.; Craig, L. C. *Biochemistry* **1970**, *9*, 1224–1232.
5. Yoshioka, H.; Aoki, T.; Goko, H.; Nakatsu, K.; Noda, T.; Sakakibara, H.; Take, T.; Nagata, A.; Abe, J.; Wakamiya, T.; Shiba, T.; Kaneko, T. *Tetrahedron Lett.* **1971**, 2043–2046.
6. Umezawa, H.; Maeda, K.; Takeuchi, T.; Okami, Y. *J. Antibiot. (Tokyo), Ser. A* **1966**, *19*, 200–209.
7. West, C. E.; Waterworth, W. M.; Sunderland, P. A.; Bray, C. M. *Biochem. Soc. Trans.* **2004**, *32*, 964–966.
8. Marton, L. J.; Pegg, A. E. *Annu. Rev. Pharmacol. Toxicol.* **1995**, *35*, 55–91.
9. Galanski, M.; Jakupec, M. A.; Keppler, B. K. *Metal Compounds in Cancer Chemotherapy*; Perez, J. M., Fuertes, M. A., Alonso, C., Eds.; Research Signpost: Trivandrum, 2005; pp 155–185.
10. Karpf, M.; Trussardi, R. *J. Org. Chem.* **2001**, *66*, 2044–2051.
11. Andersson, T. *Clin. Pharmacokinet.* **2004**, *43*, 279–285.
12. Kidani, Y.; Noji, M.; Tsukagoshi, S.; Tashiro, T. *Gann* **1978**, *69*, 263–265.
13. Kidani, Y.; Noji, M.; Tashiro, T. *Gann* **1980**, *71*, 637–643.
14. Noji, M.; Okamoto, K.; Kidani, Y.; Tashiro, T. *J. Med. Chem.* **1981**, *24*, 508–515.
15. Jennerwein, M. M.; Eastman, A.; Khokhar, A. R. *Mutat. Res.* **1991**, *254*, 89–96.
16. Kido, Y.; Khokhar, A. R.; Al-Baker, S.; Siddik, Z. H. *Cancer Res.* **1993**, *53*, 4567–4572.
17. Kido, Y.; Khokhar, A. R.; Siddik, Z. H. *Anticancer Drugs* **1993**, *4*, 251–258.
18. Siddik, Z. H.; Al-Baker, S.; Thai, G.; Khokhar, A. R. *J. Cancer Res. Clin. Oncol.* **1994**, *120*, 409–414.
19. Pendyala, L.; Kidani, Y.; Perez, R.; Wilkes, J.; Bernacki, R. J.; Creaven, P. J. *Cancer Lett.* **1995**, *97*, 177–184.
20. Schmidt, W.; Chaney, S. G. *Cancer Res.* **1993**, *53*, 799–805.
21. Nehme, A.; Baskaran, R.; Nebel, S.; Fink, D.; Howell, S. B.; Wang, J. Y. J.; Christen, R. D. *Br. J. Cancer* **1999**, *79*, 1104–1110.
22. Jennerwein, M. M.; Eastman, A.; Khokhar, A. *Chem.-Biol. Interact.* **1989**, *70*, 39–49.
23. Boudny, V.; Vrana, O.; Gaucheron, F.; Kleinwachter, V.; Leng, M.; Brabec, V. *Nucleic Acids Res.* **1992**, *20*, 267–272.
24. Bruck, M. A.; Bau, R.; Noji, M.; Inagaki, K.; Kidani, Y. *Inorg. Chim. Acta* **1984**, *92*, 279–284.
25. Al-Allaf, T. A. K.; Rashan, L. J.; Steinborn, D.; Merzweiler, K.; Wagner, C. *Trans. Metal Chem.* **2003**, *28*, 717–721.
26. Furin, A.; Guiotto, A.; Baccichetti, F.; Pasut, G.; Deuschel, C.; Bertani, R.; Veronese, F. M. *Eur. J. Med. Chem.* **2003**, *38*, 739–749.
27. Top, S.; El Bachir, K.; Vessieres, A.; Leclercq, G.; Laios, I.; Ourevitch, M.; Deuschel, C.; McGlinchey, M. J.; Jaouen, G. *ChemBioChem* **2003**, *4*, 754–761.
28. Klenner, T.; Wingen, F.; Keppler, B. K.; Krempien, B.; Schmahl, D. *J. Cancer Res. Clin. Oncol.* **1990**, *116*, 341–350.
29. Galanski, M.; Slaby, S.; Jakupec, M. A.; Keppler, B. K. *J. Med. Chem.* **2003**, *46*, 4946–4951.
30. Rothenburger, C.; Galanski, M.; Arion, V. B.; Goerls, H.; Weigand, W.; Keppler, B. K. *Eur. J. Inorg. Chem.* **2006**, 3746–3752.
31. Snider, B. B.; Lin, H. *Synth. Commun.* **1998**, *28*, 1913–1922.
32. Fristad, W. E.; Brandvold, T. A.; Peterson, J. R.; Thompson, S. R. *J. Org. Chem.* **1985**, *50*, 3647–3649.
33. Nugent, W. A.; Feldman, J.; Calabrese, J. C. *J. Am. Chem. Soc.* **1995**, *117*, 8992–8998.
34. Galanski, M.; Yasemi, A.; Slaby, S.; Jakupec, M. A.; Arion, V. B.; Rausch, M.; Nazarov, A. A.; Keppler, B. K. *Eur. J. Med. Chem.* **2004**, *39*, 707–714.
35. Keppler, B. WO2003106469, 2003; *Chem. Abstr.* **2003**, *140*, 52202.
36. Saito, R.; Kidani, Y. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 1689–1694.
37. Otwinowski, Z.; Minor, W. *Methods in Enzymology: Macromolecular Crystallography, Part A*; Carter, C. W., Sweets, R. M., Eds.; Academic: New York, NY, 1997; pp 307–326.
38. Sheldrick, G. M. *SHELXS-97*; University Göttingen: Göttingen, Germany, 1997.
39. Sheldrick, G. M. *SHELXL-97*; University Göttingen: Göttingen, Germany, 1997.
40. Johnson, C.K. *OR TEP-II: A FORTRAN Thermal-Ellipsoid Plot Program for Crystal Structure Illustrations*; Oak Ridge National Laboratory Report ORNL-5138, 1976.
41. *International Tables for X-ray Crystallography*; Kluwer Academic: Dordrecht: The Netherlands, 1992; Vol. C.
42. Corey, E. J. *J. Am. Chem. Soc.* **1953**, *75*, 2301–2304.