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BRIEF ARTICLE

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Chiral Indolo[3,2-*f*][3]benzazecine-Type Dopamine Receptor Antagonists: Synthesis and Activity of Racemic and Enantiopure Derivatives

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Supporting Information

ABSTRACT: Racemic and enantiopure 8-substituted derivatives of the lead dopamine receptor antagonist LE 300 (**1**) were prepared, and their affinities for the dopamine receptors (D_1–D_5) were tested. The separate enantiomers showed significantly different affinities; the (8*S*)-methyl and (8*R*)-hyroxymethyl derivatives where the substituents point below the reference plane of the indolo[3,2-*f*][3]benzazecine scaffold were markedly more active than their enantiomeric counterparts. The racemic 8-carboxy derivative was shown to be selective for the D_5-receptor, even against D_1.

INTRODUCTION

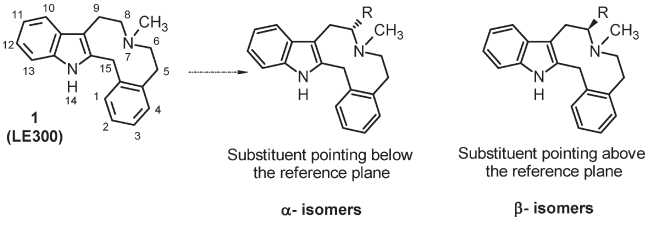
Dysfunctions of the dopaminergic system have been linked with several neurological and psychiatric disorders, mainly Parkinson's disease,[1] schizophrenia,[2,3] depression,[4] attention-deficit hyperactivity disorder,[5] and alcohol dependence.[6]

Partially hydrogenated indolo[3,2-*f*][3]benzazecines[7,8] constitute a structurally novel class of dopamine receptor antagonists distinguished by their nanomolar to subnanomolar affinities for all dopamine receptors and a general preference for the receptors of the D_1 family. Since the discovery of the lead compound 7-methyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]benzazecine (**1**, LE300),[8] considerable work has been accomplished in developing the SAR of this class of compounds.[7,9,10] This revealed that the heterocyclic backbone scaffold is optimally made up of two aromatic rings (indole and benzene, or two benzene rings) surrounding a central hydrogenated azecine moiety. Both aromatic rings should be separated by a methylene group. Further studies showed that small substituents at the indole nitrogen are well tolerated or may even result in increased affinities.[11]

The aim of the present work was to investigate the first indolo[3,2-*f*][3]benzazecine derivatives substituted at a C-atom of the azecine ring next to the central nitrogen. Since this type of substitution results in the formation of a chiral center, we wanted to investigate not only the racemic but also the enantiopure derivatives (Chart 1).

Our primary target compounds were indolobenzazecine derivatives substituted at position 8 with a small residue such as methyl, hydroxymethyl, and carboxyl groups. The assignment of the configuration of these substituents differs from the hydroxymethyl and carboxy to the methyl derivatives. This is due to the change in the priorities of the groups involved at the chiral center.

Chart 1. Substitution of the Lead Indolobenzazecine Derivative 1 at Position 8



According the Cahn–Ingold–Prelog priority rules, a hydroxymethyl or a carboxyl group has a different priority than a methyl group. To allow better presentation of the results, those enantiomers where the substituents point below the reference plane will be defined as α-isomers. Those bearing substituents pointing above the reference plane will be defined as β-isomers (Chart 1).

CHEMISTRY

To obtain the *R* and *S* enantiomers (α and β isomers, respectively) of the target 8-methylated benzindoloazecines ((+)-**5** and (−)-**5**), racemic α-methyltryptamine (**2**) was first converted to the quinolizine derivative **3** by reaction with 2-(2-bromoethyl)benzaldehyde following a previously established procedure for the synthesis of analogous quinolizines.[12] Subsequent quaternization with methyl iodide and reduction of the obtained quaternary salts under Birch conditions (Na, liq NH_3) yielded the

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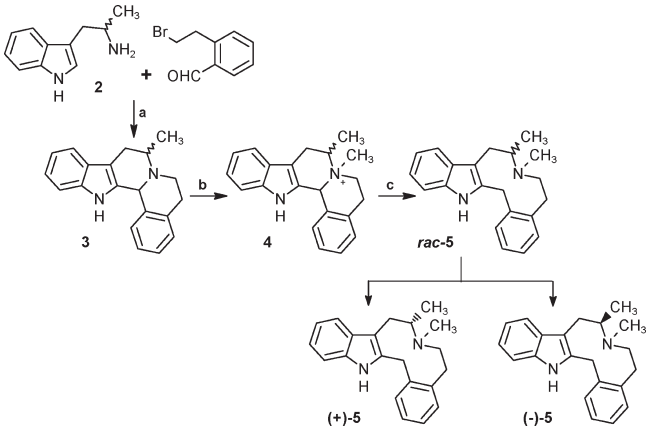
target azecine *rac*-**5** (Scheme 1). Resolution of the racemate to obtain the enantiomers (+)-**5** and (−)-**5** was achieved by means of preparative chiral HPLC on a cellulose-based chiral stationary phase. Attempts to crystallize the enantiopure derivatives to assign the absolute configuration by X-ray were not successful. To determine the configurations of these separated enantiomers, an alternative synthetic route was adopted (Scheme 3), where we started from an enantiopure quinolizine derivative. This will be described in more detail below.

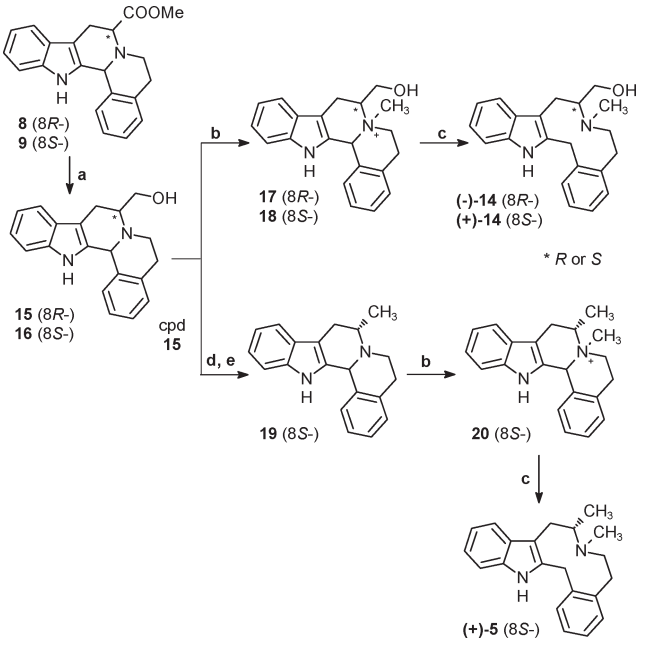
The quinolizine ester derivatives **8** and **9**, obtained from the enantiomerically pure methyl esters of D- and L-tryptophan (**6** and **7**), served as starting materials for the preparation of further 8-substituted indolobenzazecine derivatives. Conversion of the quinolizine esters **8** and **9** into the corresponding azecines, however, failed at the last step, namely, the ring cleavage of the quaternary salts **10** and **11** under Birch conditions. As an alternative route, the ester function of the quaternary salts **10** and **11** was first hydrolyzed, and the resulting carboxylic acid derivatives **12** were reduced under Birch conditions, yielding the amino acid azecine derivatives *rac*-**13**. These were in turn reduced using lithium aluminum hydride to give the hydroxymethyl derivatives *rac*-**14** (Scheme 2). However, investigations of the enantiomeric purity of the hydroxymethyl derivatives *rac*-**14** by means of chiral HPLC (enantiomeric excess (ee) 0.12% and 0.52%, respectively) and measurement of their optical rotation revealed that these derivatives were obtained in a racemized form, despite the fact that we started from enantiopure compounds. It can be presumed that this racemization took place by deprotonation/reprotonation due to the basic conditions used for the hydrolysis of the ester function of the quaternary salts **10** and **11** to the corresponding carboxylic acid derivative **12**, despite the mild conditions applied (1 N sodium hydroxide, 0 °C for 2 h).

Scheme 1. Synthesis of the 8-Methylbenzinodoloazecine Derivative 5 and Its Enantiomeric Separation[a]



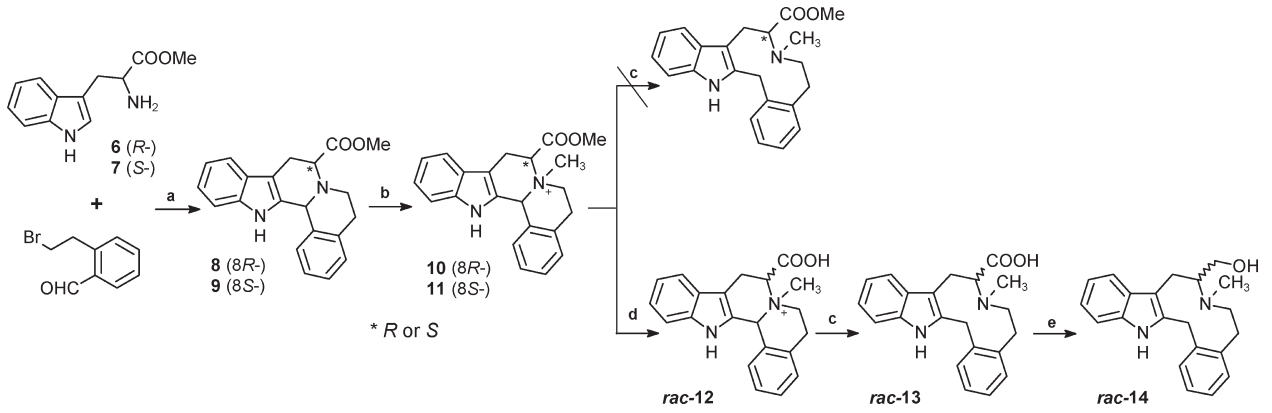
[a] Reagents and conditions: (a) TFA, dioxane, reflux, 6 h; (b) MeI, acetone, 24 h; (c) Na^0, liq NH_3, −40 °C, 10 min.

Scheme 3. Synthetic Route B: 8-Hydroxymethylquinolizine as starting Material for Enantiopure 8-Hydroxymethyl- and 8-Methylindolobenzazecines[a]



[a] Reagents and conditions: (a) $LiAlH_4$, dry THF, room temp, 90 min; (b) MeI, acetone, 24 h; (c) Na^0, liq NH_3, −40 °C, 10 min; (d) MsCl, TEA, DCM, 12 h, room temp; (e) $LiAlH_4$, dry THF, reflux, 2 h.

Scheme 2. Synthetic Route A for the Preparation of Benzinodoloazecines Derived from D- and L-Tryptophan[a]



[a] Reagents and conditions: (a) TFA, dioxane, reflux, 3 h; (b) MeI, acetone, 35 °C, 24 h; (c) Na^0, liq NH_3, −40 °C, 10 min; (d) 1 N NaOH, MeOH, 0 °C, 2 h; (e) $LiAlH_4$, dry THF, reflux, 5 h.

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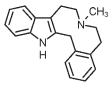
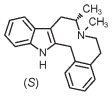
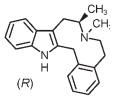
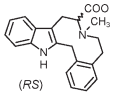
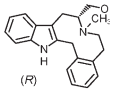
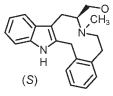
Table 1. Affinities (K_i, nM) for Human D_1−D_5 Receptors, Determined by Radioligand Binding Experiments[a]

Compounds		K_i, (nM) D_1	D_2	D_3	D_4	D_5
1*		1.9 ± 0.9	44.5 ±15.8	25.9	108 ± 39	7.5 ± 0.3
(+)-5	 α-isomer	6.1 ± 2.2	85.3 ± 15.2	304 ± 1	64.1 ± 19.3	4.4 ± 2.5
(-)-5	 β-isomer	640 ± 119	>10000	>10000	>10000	389 ± 0.8
***rac*-13**		>10000	>10000	>10000	>10000	657 ± 163
(-)-14	 α-isomer	10.3 ± 1.4	268 ± 56	681 ± 68	408 ± 31	11.4 ± 2.3
(+)-14	 β-isomer	114 ± 9	2923 ± 442	4676 ± 1593	4861 ± 803	91.4 ± 8.5

[a] K_i values are the mean of two to three experiments, performed in triplicate (±SEM). The asterisk (*) indicates values taken from ref 10. CHO cell lines were used for $D_{4.4}$. HEK cell lines were used for D_1, D_{2L}, D_3, and D_5.

Accordingly, the hydrolysis step was circumvented, and the ester function of the quinolizines **8** and **9** was first reduced using lithium aluminum hydride. The resulting hydroxymethylquinolizines **15** and **16** were subsequently quaternized, and the quaternary salts **17** and **18** were finally reduced with sodium in liquid ammonia to give the target azecines (−)-**14** and (+)-**14** (Scheme 3). The enantiopurity of the obtained azecines was ascertained by measuring their specific rotation ($[\alpha]^{rt}_{496}$ of −158.4° and +161°, respectively) and by chiral HPLC. These findings substantiate our earlier speculations that in the previously adopted synthetic procedure racemization took place at a step prior to the synthesis of the hydroxymethyl derivative *rac*-**14** and that it most probably occurred during hydrolysis. It could also be concluded that the amino acid derivative *rac*-**13** is not enantiomerically pure and that it racemized to the same extent as the hydroxymethyl derivative *rac*-**14**.

In order to identify the configuration of the aforementioned separated *S* and *R* enantiomers of 8-methylindolobenzazecine ((+)-**5** and (−)-**5**), Scheme 1), we adopted an alternative route for the synthesis of the (8*S*)-enantiomer (+)-**5** (α-isomer) starting from an enantiomerically pure precursory substance, namely, the (8*R*)-hydroxymethylquinolizine **15** (α-isomer). This was first converted to the corresponding mesylate, which was directly reduced with lithium aluminum hydride to give the methylquinolizine **19**. Reduction with sodium in liquid ammonia yielded the corresponding (8*S*)-azecine derivative (+)-**5** (α- isomer) (Scheme 3). Comparison of the respective specific rotation of the herein obtained azecine with the previously obtained ones (Scheme 1, (+)-**5** and (−)-**5**)) enabled us to assign the respective configuration.

PHARMACOLOGY

All the enantiopure target compounds ((+)-**5**, (−)-**5**, (+)-**14**, and (−)-**14**) and the racemized amino acid derivative *rac*-**13** were screened for their affinities for the human cloned dopamine receptor subtypes D_1, D_{2L}, D_3, $D_{4.4}$, and D_5. These receptors were stably expressed in HEK293 or CHO cells. [^{3}H]SCH 23390 and [^{3}H]spiperone were used as radioligands at the D_1-like and D_2-like receptors, respectively. K_i values are given in nanomolar units (Table 1). A detailed protocol has been elsewhere described.[15] Additionally, the compounds were tested in an intracellular Ca^{2+} assay to determine their functionality (agonist or antagonist) at the D_1 and D_2 receptors. HEK293 cells stably expressing the respective D-receptor were loaded with a fluorescent dye, and after preincubation with rising concentrations of the test compound, an agonist (SKF 38393 for D_1 and quinpirole for D_2) was added and the Ca^{2+}-induced fluorescence was measured with a microplate reader. The ability of the test compound to suppress the agonist-induced Ca^{2+} influx is an indication of antagonistic or inverse agonistic properties at the receptor.[13]

RESULTS AND DISCUSSION

Indolobenzazecine derivatives substituted at position 8 with three different residues (methyl, hydroxymethyl, and carboxylic acid) were prepared. The 8-methyl and 8-hydroxymethyl derivatives were obtained as the separated α and β isomers ((+)-**5**, (−)-**5**, (−)-**14**, and (+)-**14**, respectively), while the amino acid derivative could only be obtained in a racemized form (*rac*-**13**).

All tested compounds showed antagonistic properties at the investigated dopamine receptors in the functional Ca^{2+} assay.

Compared with many other indolobenzazecine derivatives, the racemized amino acid derivative *rac*-**13** showed a pronounced decrease in the affinities for all dopamine receptors. Interestingly however, *rac*-**13** displayed practically no affinities for all dopamine receptors (K_i > 10 000 nM) except for the D_5 receptor, where it displayed submicromolar K_i (~657 nM).

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Hitherto, compounds with a significant selectivity for D_5 over D_1 have not been achieved, with the exception of some other azecine-type ligands,[14,15] which renders the compound highly attractive as a potential D_5-selective pharmacological tool. Separation of the enantiomers of compound *rac*-**13** could not be accomplished, but it would be of great value, as one of the enantiomers would presumably show higher affinities or more pronounced selectivity.

The separated α and β enantiomers of 8-methylbenzindoloazecine derivatives ((+)-**5** and (−)-**5**) displayed a high discrepancy in their affinities. The α-enantiomer (+)-**5** was almost as active as the lead indolobenzazecine **1**. The corresponding β-isomer (−)-**5** exhibited at least 100-fold reduction in affinities for all dopamine receptors, showing only micromolar affinities for D_1-like receptors (D_1 and D_5), and was practically devoid of affinities for D_2-like receptors (D_2, D_3, and D_4).

A similar but less pronounced difference was observed between the α and β enantiomers of 8-hydroxymethylindolobenzazecine ((−)-**14** and (+)-**14**). The α-enantiomer (−)-**14** was also more active than the β-enantiomer (+)-**14**. However, only a 10-fold difference in affinities between both enantiomers was found. While the α-isomer of 8-hydroxymethylindolobenzazecine was relatively less active than its 8-methylated counterpart, the β-isomer of the hydroxymethyl derivative was more active than the methylated one.

EXPERIMENTAL SECTION

General Methods. Melting points are uncorrected and were measured in open capillary tubes using a Gallenkamp melting point apparatus. ^{1}H and ^{13}C NMR spectral data were obtained from a Bruker Advance 250 spectrometer (250 MHz) and Advance 400 spectrometer (400 MHz). TLC was performed on silica gel F254 plates (Merck). MS data were determined by GC/MS using a Hewlett-Packard GCD-Plus (G1800C) apparatus (HP-5MS column, J&W Scientific). Purities of the compounds were determined by elemental analysis, performed on a Heraeus Vario EL apparatus, or by HPLC. All values for C, H, and N were found to be within ±0.4. All compounds showed >95% purity. The HPLC system for the preparative resolution of (+)-**5** and (−)-**5** consisted of a Labomatic-HD-200 pump and UV/vis filter−photometer (Knauer, Berlin) detector (220 nm). Separation was performed on a Chiralcel OD column [cellulose tris(3,5-dimethylphenylcarbamate)] (50 cm × 5 cm) with a cooling mantle. Analytical separation of *rac*-**5** and analysis of the separated (+)-**5** and (−)-**5** was performed on HPLC system consisting of a system controller SCL10-Avp, autoinjector SIL-10A, 50 μL sample loop, UV detector SPD-10A, two pumps LC-10A (Shimadzu, Duisburg), and a Chiralcel OD column (10 μm, 250 mm × 4.6 mm) (Daicel Chemical Industries, Tokyo). The ee values were calculated from peak areas. Chiral HPLC analyses of *rac*-**14**, (+)-**14**, and (−)-**14** were performed on HPLC instrument (Water, U.S.) equipped with a pump (model PU-980), a UV/vis detector (model UV-975), and injection valve with 20 μL sample loop. The chiral stationary phase (CSP) used in this separation was the macrolide-type antibiotic teicoplanin, known as Teicoplanin T (150 mm × 4.6 mm i.d.) purchased from Advanced Separation Technologies (Whippany, NJ, U.S.) in the polar-organic separation mode.

General Procedure for the Preparation of the Quinolizine Derivatives 3, 8, and 9 Starting from the Respective Amines. A solution of the respective arylethylamine (α-methyltryptamine, methyl D- or L-tryptophanate) (1 mmol), 2-(2-bromoethyl)benzaldehyde (1.2 mmol), and trifluoroacetic acid (1 mmol) in dioxane was refluxed under nitrogen for 3 (tryptophanate esters) to 6 h (methyltryptamine). The solvent was then evaporated under reduced pressure, the residue basified with 2 N NaOH. The resulting quinolizine base was extracted with dichloromethane and recrystallized or purified chromatographically.

(8*RS*,14b*RS*)-8-Methyl-5,6,8,9,14,14b-hexahydroindolo-[2′,3′:3,4]pyrido[2,1-*a*]isoquinoline (3). Crystallization from isopropanol/hexane yielded pale yellow needle-shaped crystals. Yield 70%. Mp: 75−77 °C (for analytical data, see Supporting Information).

Methyl (8*R*)-5,6,8,9,14,14b-Hexahydroindolo[2′,3′:3,4]pyrido-[2,1-*a*]isoquinoline-8-carboxylate (8). **8** was purified by column chromatography, dichloromethane/methanol (100:1). Yield 55%. Mp 84−87 °C (for analytical data, see Supporting Information).

Methyl (8*S*)-5,6,8,9,14,14b-Hexahydroindolo[2′,3′:3,4]pyrido-[2,1-*a*]isoquinoline-8-carboxylate (9). **9** was purified by column chromatography, dichloromethane/methanol (100:1). Yield 59%. Mp 84−86 °C (for analytical data, see Supporting Information).

General Procedure for the Ring-Opening. Ammonia was condensed in a three-necked 100 mL flask, which was equipped with a balloon and a stopper and cooled in a liquid nitrogen bath. After filling $^3/_4$ of the flask's volume, the cooling bath was removed and ammonia was allowed to liquefy. The respective quaternary salts were then added to the stirred liquid ammonia. This was followed by gradual addition of small pieces of sodium metal until the blue color remained for 10 min. A few drops of saturated ammonium chloride solution were added to terminate the reaction, and the mixture was stirred under nitrogen until the ammonia completely evaporated. An amount of 10 mL of water was added to the residue, and the mixture was then extracted with 30 mL of diethyl ether. The organic phase was dried over Na_2SO_4, and the solvent was removed under reduced pressure.

(8*RS*)-7,8-Dimethyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]benzazecine (*rac*-5) and Its Separated *R* and *S* Enantiomers (+)-5 and (−)-5. Creamy white solid. Yield 89%. Mp 82−84 °C (for analytical data, see Supporting Information).

The enantiomers were separated using a preparative Chiracel OD column (cellulose tris(3,5-dimethylphenylcarbamate): eluent, hexane/ethanol 9:1; flow rate, 194 mL/min; temp, 25 °C; UV detector λ = 220 nm. The isolation was accomplished by 1 mL injection of a solution of *rac*-**5** (75 mg in 1 mL of *n*-hexane/ethanol 1:1). Enantiomeric purity was assessed by analytical chiral HPLC (column, Chiralcel OD; eluent *n*-hexane/ethanol 9:1; flow rate, 1.3 mL/min; UV detector λ = 220 nm), where the first eluted enantiomer ((+)-**5**) showed 99.4% ee (retention time, 11.8 min) and the second eluted compound ((−)-**5**) showed 98.5% ee (retention time, 22.8 min). Specific rotation of (+)-**5** was $[\alpha]^{rt}_{546}$ 132° (*c* 1, $CHCl_3$), and for (−)-**5** was $[\alpha]^{rt}_{546}$ −128° (*c* 1, $CHCl_3$).

Alternatively, (8*S*)-7,8-dimethyl-6,7,8,9,14,15-hexahydro-5*H*-indolo-[3,2-*f*][3]benzazecine ((+)-**5**) was prepared starting from **20** (Scheme 3). HPLC retention time: 11.64 min (column, Chiralcel OD; eluent *n*-hexane/ethanol 9:1; flow rate, 1.3 mL/min; UV detector λ 220 nm). $[\alpha]^{rt}_{546}$ 136° (*c* 1, $CHCl_3$). This revealed that the first eluted enantiomer (compound (+)-**5**) bears an *S* configuration at position 8.

7-Methyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]-benzazecine-8-carboxylic Acid (*rac*-13). The same procedure for ring-opening was applied. After evaporation, water was added and the obtained solution was carefully treated with 1 N HCl until the target amino acid precipitated as a creamy white solid (pH 6), which was filtered off and dried. White solid. Yield 60−67%. Compound chars without melting at 220 °C (for analytical data, see Supporting Information).

[(8*R*)-7-Methyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*]-[3]benzazecin-8-yl]methanol ((−)-14). (−)-**14** was purified by column chromatography, dichloromethane/methanol (11:1). Yellowish white solid. Yield 66.5%. Mp 83−85 °C (for analytical data, see Supporting Information).

[(8*S*)-7-Methyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*]-[3]benzazecin-8-yl]methanol ((+)-14). (+)-**14** was purified by column chromatography, dichloromethane/methanol (11:1). Yellowish white solid. Yield 62.6%. Mp 83−85 °C (for analytical data, see Supporting Information).

General Procedure for the Reduction of the Ester Function. To a suspension of lithium aluminum hydride (2 mmol) in dry THF was added a solution of the respective ester (1 mmol) in dry THF, and the mixture was stirred at room temperature for 90 min (heated under reflux for 3 h for compound *rac*-**14**). Excess lithium aluminum hydride was carefully quenched with methanol. The mixture was filtered off, and the filtrate was evaporated under reduced pressure.

[(8*RS*)-7-Methyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*]-[3]benzazecin-8-yl]methanol (*rac*-14). *rac*-**14** was purified by column chromatography, dichloromethane/methanol (11:1). Creamy white solid. Yield 42%. Mp 100−103 °C (for analytical data, see Supporting Information).

[(8*R*)-5,6,8,9,14,14b-Hexahydroindolo[2′,3′:3,4]pyrido[2,1-*a*]-isoquinolin-8-yl]methanol (15). **15** was crystallized from acetone/hexane, giving yellowish crystals. Yield 82.6%. Mp 198−200 °C (for analytical data, see Supporting Information).

[(8*S*)-5,6,8,9,14,14b-Hexahydroindolo[2′,3′:3,4]pyrido[2,1-*a*]-isoquinolin-8-yl]methanol (16). 16 was scrystallized from acetone/hexane, giving yellowish crystals. Yield 79.3%. Mp 197−199 °C (for analytical data, see Supporting Information).

8-Carboxy-7-methyl-5,6,8,9,14,14b-hexahydroindolo[2′,3′:3,4]-pyrido[2,1-*a*]isoquinolin-7-ium Iodide (12). To an ice cold-suspension of **10** or **11** (4.4 mmol) in 80 mL of methanol was slowly added 27 mL of 1 N NaOH. The mixture was stirred at 0 °C for 2 h and then acidified with 1 N HCl. Methanol was removed under reduced pressure, and the remaining aqueous suspension was cooled in an ice bath for 30 min. The obtained pale yellow precipitate was filtered off and dried. Yield 71−77%. Compound chars without melting at 235 °C (for analytical data, see Supporting Information).

(8*S*,14b*RS*)-8-Methyl-5,6,8,9,14,14b-hexahydroindolo-[2′,3′:3,4]pyrido[2,1-*a*]isoquinoline (19). To an ice-cold solution of **15** (1.7 mmol) and triethylamine (2 mmol) in 30 mL of dichloromethane was slowly added mesyl chloride (2.5 mmol). The mixture was then stirred for 12 h, allowing it to reach room temperature. The mixture was then washed with 100 mL of 0.1 N HCl, then with 100 mL of dilute K_2CO_3 solution, and finally with 100 mL of brine. The organic phase was evaporated under reduced pressure leaving a yellow solid, which was used without further purification.

To a suspension of 100 mg of lithium aluminum hydride was added a solution of the crude mesylate derivative in 20 mL of dry THF. The mixture was heated under reflux for 2 h. Excess lithium aluminum hydride was carefully quenched with a saturated solution of potassium sodium tartarate. The mixture was filtered off and the filtrate was evaporated under reduced pressure, leaving a creamy white solid. Crystallization from isopropanol/hexane yielded a pale yellow compound. Yield 88%. Mp: 75−77 °C.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures and physical and spectral data for some target and intermediary compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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