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Biocatalytic Organic Synthesis of Optically Pure (*S*)-Scoulerine and Berbine and Benzylisoquinoline Alkaloids

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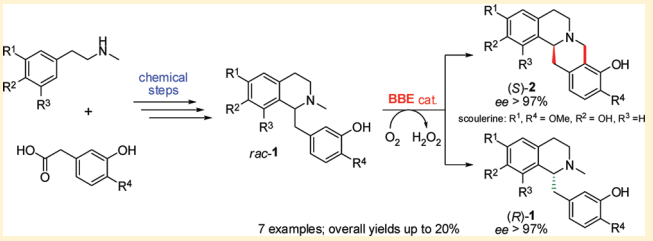
Supporting Information

ABSTRACT: A chemoenzymatic approach for the asymmetric total synthesis of the title compounds is described that employs an enantioselective oxidative C−C bond formation catalyzed by berberine bridge enzyme (BBE) in the asymmetric key step. This unique reaction yielded enantiomerically pure (*R*)-benzylisoquinoline derivatives and (*S*)-berbines such as the natural product (*S*)-scoulerine, a sedative and muscle relaxing agent. The racemic substrates *rac*-**1** required for the biotransformation were prepared in 4−8 linear steps using either a Bischler−Napieralski cyclization or a C1−Cα alkylation approach. The chemoenzymatic synthesis was applied to the preparation of fourteen enantiomerically pure alkaloids, including the natural products (*S*)-scoulerine and (*R*)-reticuline, and gave overall yields of up to 20% over 5−9 linear steps.



INTRODUCTION

Benzylisoquinolines and berbines[1] are two closely related classes of alkaloids encompassing more than 100 known structures. Both alkaloid families are associated with a broad range of biological activities: Many 1-benzyl-1,2,3,4-tetrahydroisoquinolines act as antispasmodic or hypotensive and some, such as norcoclaurine, coclaurine, and *N*-methylcoclaurine, possess anti-HIV activity *in vitro*.[2] Berbines show diverse biological activities such as analgesic, sedative, hypnotic, or anti-inflammatory effects,[3] and the non-natural derivative *l*-chloroscoulerine is currently investigated as a novel treatment of schizophrenia.[4] In addition, tetrahydroisoquinolines have recently been employed as chiral ligands for metal-catalyzed transfer-hydrogenation.[5]

Because of their biological significance, benzylisoquinolines and berbines have been targets for organic synthesis for a long time, and their asymmetric synthesis has been achieved by many different strategies.[6,7] However, a large number of steps and harsh reaction conditions are often required, resulting in limited overall yields and ee values. Furthermore, among the published procedures only few catalytic processes are found, with metal-catalyzed asymmetric hydrogenation,[8] intramolecular allylic amination or amidation,[9] and various metal- or organocatalyzed asymmetric alkylation reactions[10] representing the most notable exceptions. Despite the impressive progress in these areas, enantiomerically pure (ee > 99%) substances are rarely obtained. On the other hand, optically pure benzylisoquinoline[11] and berbine alkaloids are produced by a number of plants belonging mainly to the Berberidaceae and Papaveraceae families. However, isolation of the natural products is cumbersome, and biotransformations using plant cell cultures[12] afford minute amounts only. The production of benzylisoquinolines and related alkaloids from the morphine and sanguinarine pathways using recombinant enzymes in *Escherichia coli* and *Saccharomyces cerevisiae* has recently been reported,[13] but conversions were rather low (<15%) in these fermentative processes and product isolation was not reported. In addition, this approach is limited to a small number of target molecules and is therefore not as flexible as chemical and biocatalytic synthetic methods.

Biocatalytic steps in organic synthesis have already proven to be an efficient, highly stereoselective and flexible option in the preparation of many target compounds.[14] Of special interest are C−C bond-forming enzymes to set up the carbon framework of the organic molecules.[15] Berberine bridge enzyme (BBE) represents an outstanding biocatalyst enabling an aerobic oxidative C−C bond formation transforming benzylisoquinolines to berbines. BBE catalyzes the first committed step in the benzophenanthridine, protoberberine, and protopine biosynthesis pathways[16] in plants as it converts (*S*)-reticuline to (*S*)-scoulerine by intramolecular C−C coupling, forming the so-called "berberine bridge" (Scheme 1).

This transformation takes place via oxidative C−H activation of the substrate's *N*-methyl group at the expense of molecular oxygen, a reaction unparalleled in organic synthesis.[17] BBE from *Eschscholzia californica* (California poppy) has been heterologously expressed in *Pichia pastoris*, and its X-ray crystal structure and molecular mechanism have been solved.[17,18]

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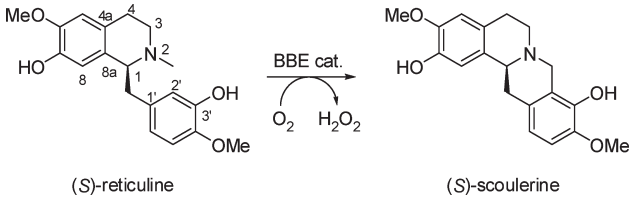
Scheme 1. C−C Bond Formation Leading to (*S*)-Scoulerine Catalyzed by Berberine Bridge Enzyme (BBE)



Recently, it has been shown that BBE accepts also non-natural substrates, whereby it transforms exclusively the (*S*)-enantiomer of racemic benzylisoquinolines to optically pure (*S*)-berbines. Since this reaction represents a highly enantioselective kinetic resolution, it provides access to the remaining optically pure (*R*)-substrates as well.[19]

In the present paper, we demonstrate the broad applicability of the enzyme to establish a novel synthetic route to optically pure (*S*)-berbines and (*R*)-benzylisoquinolines, including the first asymmetric total synthesis of naturally occurring (*S*)-scoulerine.

RESULTS AND DISCUSSION

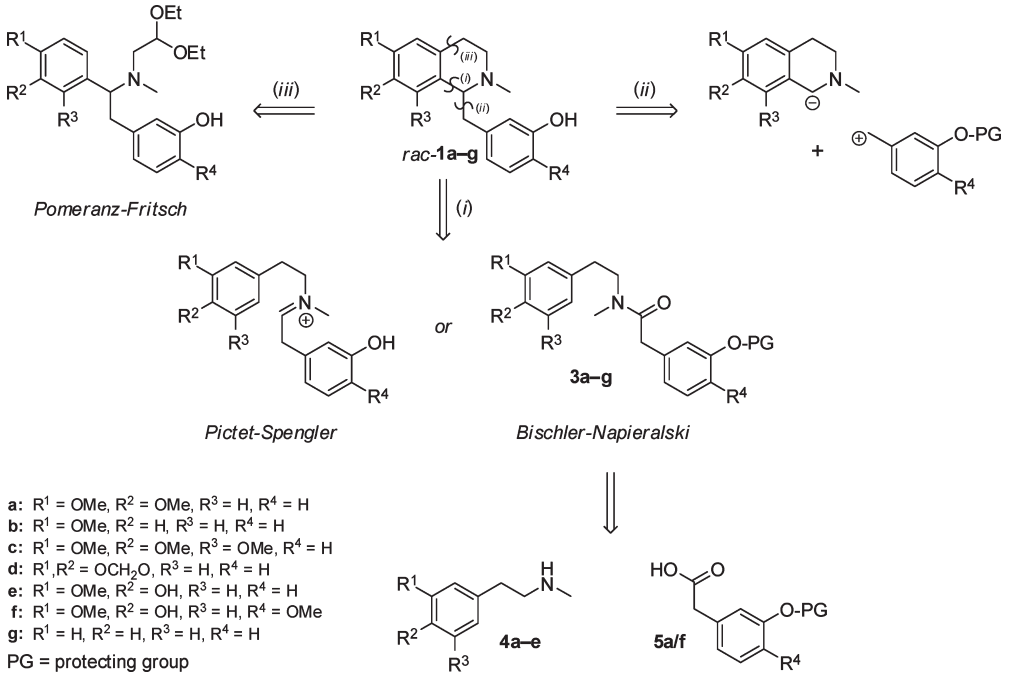
The synthesis of racemic 1-substituted tetrahydroisoquinolines **1** usually relies on one out of three different strategies: (i) formation of the C1−C8a bond of the isoquinoline core employing either the Pictet−Spengler[20] or the Bischler−Napieralski[21] cyclization, (ii) alkylation at position C1 of the isoquinoline via nucleophilic or electrophilic activation,[22] and (iii) formation of the C4−C4a bond by a Pomeranz−Fritsch reaction (Scheme 2).[23] The first two approaches are particularly appealing since the target molecule is disconnected at central bonds leading to simple starting materials. We focused first on the Bischler−Napieralski cyclization of amides **3a**−**g**, since it offers a broad scope and mild reaction conditions. Therefore, the *N*-methylphenethylamines **4a**−**g** and phenylacetic acid derivatives **5a** and **5f** were needed for synthesis of the amides **3a**−**g** used as educts in the cyclization reaction.

Although *N*-methyl-(3,4-dimethoxyphenyl)ethylamine (*N*-methylhomoveratrylamine) **4a** as well as *N*-methylphenethylamine **4g** were commercially available, all other phenethylamines had to be synthesized. Compounds **4b**−**d** were prepared from the corresponding phenylacetic acid derivatives **6b**−**d** via conversion into the *N*-methylamides **7b**−**d** followed by reduction (Scheme 3). The latter transformation was first attempted employing $LiAlH_4$ as reducing agent; however, only incomplete conversion was achieved even with a 3-fold excess of $LiAlH_4$ and prolonged reaction time under reflux heating (48 h). Fortunately, borane proved to be more efficient: the reduction of **7b** with $BH_3 \cdot THF$ led to full conversion as judged by TLC and GC−MS, giving **4b** in 72% isolated yield.

Compound **4e** was obtained starting from cheap and readily available vanillin **8**.[24] Benzylation followed by Henry-reaction with nitromethane and $LiAlH_4$-reduction afforded the primary amine derivative **9** in 47% overall yield (Scheme 3). In a first trial, the amine **9** was reacted with acid chloride **5a** to give the corresponding amide. *N*-Methylation of this compound was attempted following a published procedure,[25] but unfortunately alkylation occurred not only at the nitrogen but also on the α-carbon of the amide, giving an undesired dimethylated product in 72% yield. In a second trial, cyclization of the secondary amide formed from **9** and **5a** led to the desired tetrahydroisoquinoline; however, *N*-methylation employing methyl iodide in the presence of sodium hydride and triethylamine[26] did not lead to any conversion. Finally, the third attempt was successful: the monomethylation of **9** was performed prior to amide formation via conversion into a carbamate and $LiAlH_4$ reduction, giving the desired *N*-methylphenethylamine derivative **4e** in 66% yield.

For the preparation of the phenylacetic acid building blocks two different approaches were investigated. Compound **5a** was obtained from 3-hydroxyphenylacetic acid by selective monobenzylation

Scheme 2. Strategies for the Construction of 1-Substituted 1,2,3,4-Tetrahydroisoquinolines



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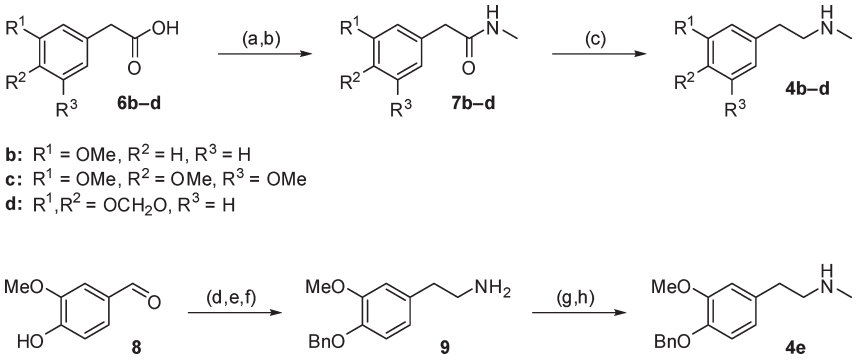
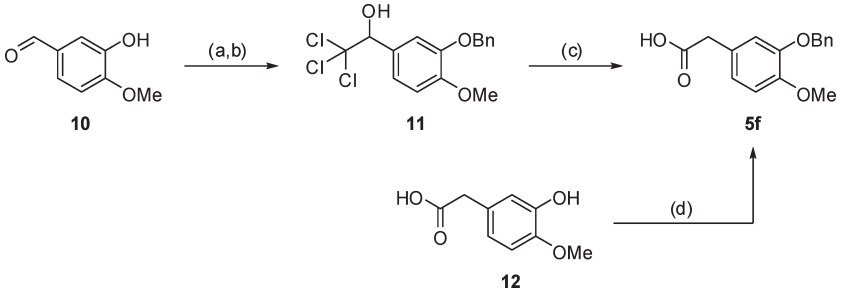
Scheme 3. Synthesis of Phenethylamine Derivatives 4b−e[a]



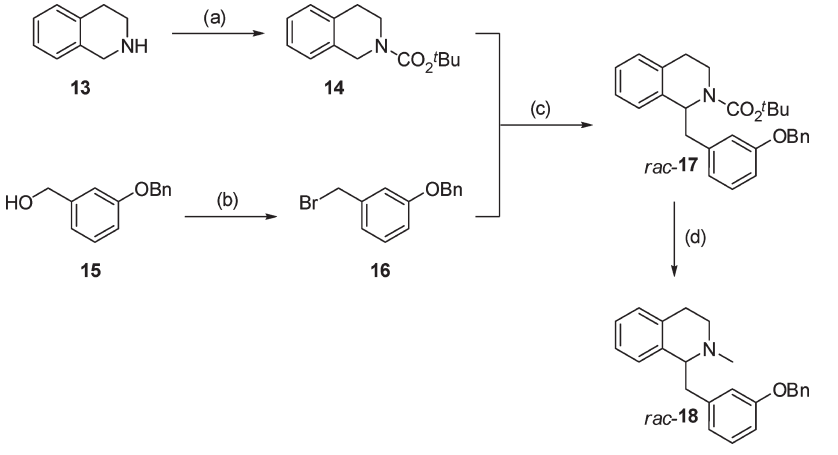
[a] Reagents and conditions: (a) $(COCl)_2$ (1.2 equiv), DMF cat., toluene, room temperature, 2 h, quant. (b) $MeNH_2$, aq NaOH, CH_2Cl_2, 0 °C to room temperature, 16 h, 78−95%. (c) $BH_3 \cdot THF$ (5 equiv), THF, reflux, 16 h, 58−78%. (d) BnBr (1.0 equiv), K_2CO_3, argon, room temperature, 20 h, 89%. (e) $MeNO_2$ (3.2 equiv), NH_4OAc, HOAc, reflux, 5 h, 68%. (f) $LiAlH_4$ (5 equiv), THF, reflux, 20 h, 77%. (g) $ClCO_2Et$ (1.2 equiv), Et_3N, CH_2Cl_2, 0 °C to room temperature, 3 h, 99%. (h) $LiAlH_4$ (5 equiv), THF, 0 °C to reflux, 4 h, 67%.

Scheme 4. Synthesis of Phenylacetic Acid Derivative 5f[a]



[a] Reagents and conditions: (a) BnBr (1.0 equiv), K_2CO_3 (1.1 equiv), EtOH, argon, room temperature, 20 h, 91%. (b) $CHCl_3$ (3.6 equiv), KOH (1.3 equiv), DMF, argon, −10 °C, 2.5 h, 97%. (c) $(PhSe)_2$ (1.05 equiv), $NaBH_4$ (2.1 equiv), NaOH (6.0 equiv), ethanol, room temperature, 30 min, 40 °C, 18 h, 31%. (d) BnBr (1.1 equiv), KOH, NaI cat., EtOH, 100 °C, 16 h, 67%.

Scheme 5. Synthesis of 1-(3-Benzyloxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline 18[a]



[a] Reagents and conditions: (a) Boc_2O (1.02 equiv), CH_2Cl_2, room temperature, 2 h, quant. (b) CBr_4 (1.05 equiv), PPh_3 (1.04 equiv), CH_2Cl_2, 0 °C to room temperature, 3 h, 94%. (c) *t*-BuLi (1.05 equiv), TMEDA (1.05 equiv), THF, −78 to −50 °C, 4 h, 51%. (d) $LiAlH_4$ (5 equiv), THF, 0 °C to reflux, 16 h, 74%.

of the dianion.[27] 3-Benzyloxy-4-methoxyphenylacetic acid **5f** was also obtained by this method, requiring the commercially accessible acid **12** as starting material. Alternatively, **5f** was synthesized from isovanillin **10** in a three-step sequence involving one-carbon homologation via the α-trichloromethylcarbinol **11** (Scheme 4).[28]

With the required building blocks (**4** and **5**) in hand, amide coupling was performed. The carboxylic acids **5** were converted

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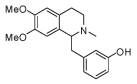
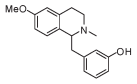
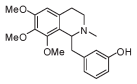
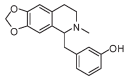
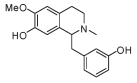
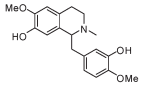
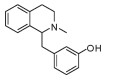
into the corresponding acyl chlorides using oxalyl dichloride in toluene and connected with the amines under Schotten–Baumann conditions. Amides **3a**–**g** were obtained in yields ranging from 63% to 97%. The best results were generally obtained when the acyl chloride was applied in slight excess.

Next, the Bischler–Napieralski cyclization of these amides was investigated to obtain the corresponding racemic tetrahydroisoquinolines **1**. A broad range of reagents, employed in a wide variety of solvents, has been reported to effect this transformation.[21a]

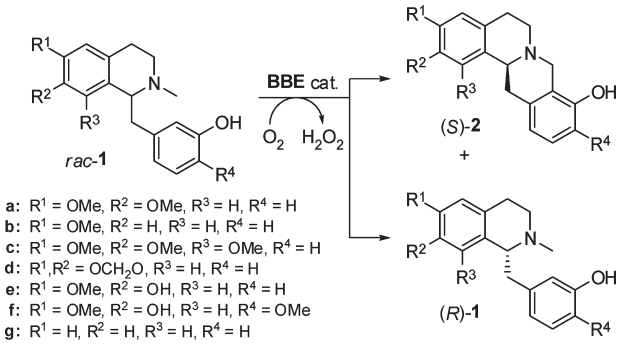
Table 1. Overall Yields of Chemical Route to Racemic Tetrahydroisoquinolines *rac*-1a–g

Product	Structure	Steps (linear)	Overall Yield [%]
rac-**1a**		5 (5)	40
rac-**1b**		8 (6)	42
rac-**1c**		8 (6)	28
rac-**1d**		8 (6)	43
rac-**1e**		10 (8)	21
rac-**1f**		10 (8)	16
rac-**1g**		5 (4)	33

Cyclization of **3a** employing PCl_5 in chloroform at room temperature followed by $NaBH_4$-reduction afforded the desired tetrahydroisoquinoline, albeit only in 13% yield. The best results were obtained using phosphorus oxychloride in refluxing acetonitrile, followed by $NaBH_4$-reduction in methanol. This sequence gave the tetrahydroisoquinolines in yields of 85–97%. Only the cyclization of **3g** failed under these conditions, most likely owing to the lack of electron-donating substituents on the aromatic ring of the original amine building block. Although the Bischler–Napieralski reaction of nonactivated arenes is described in literature,[21a,29] no conversion was achieved in our case even under the most forcing reaction conditions employed (P_2O_5 in tetralin at 206 °C). Consequently, we had to change our strategy for the synthesis of **1g**. Alkylation of a C1-lithiated tetrahydroisoquinoline derivative appeared promising and lithiation of tetrahydroisoquinoline carbamates employing *t*-BuLi has previously been described.[22a] Carbamate **14** and 3-benzyloxybenzyl bromide **16** were prepared and reacted following the published procedure to give the desired C–C coupling product in 29% yield (Scheme 5). By slightly changing the reaction conditions, i.e., higher temperature during the alkylation stage (see Experimental Section), this value could be improved to 51%, which approaches the reported yields obtained with less hindered nucleophiles.[22a] The alkylated carbamate was converted into the *N*-methyltetrahydroisoquinoline by $LiAlH_4$ reduction. This represents an improvement on the original report, where this transformation was achieved in a two-step deprotection/reductive amination sequence. In our case the carbamate moiety serves a triple purpose: it protects the nitrogen atom, directs the lithiation, and serves as precursor of the *N*-methyl group.

The synthesis of racemic tetrahydroisoquinolines **1a**–**g** (summarized in Table 1) was completed by hydrogenolytic cleavage of the benzyl ether protective groups, which proceeded quantitatively and generally gave the target compounds without the need for chromatographic purification. For instance, racemic

Table 2. Yields of BBE-Catalyzed Oxidative Kinetic Resolution via C–C Bond Formation



entry	substrate	*c* [%][a]	yield (*S*)-**2** [%][b]	ee (*S*)-**2** [%][c]	yield (*R*)-**1** [%][b]	ee (*R*)-**1** [%][c]	*E*[d]
1	*rac*-**1a**[e]	50	42	>97	50	>97	>200
2	*rac*-**1b**[e]	50	36	>97	36	>97	>200
3	*rac*-**1c**[e]	50	39	>97	47	>97	>200
4	*rac*-**1d**[e]	50	31	>97	46	>97	>200
5	*rac*-**1e**	50	22	>97	49	>97	>200
6	*rac*-**1f**	50	47	>97	37	>97	>200
7	*rac*-**1g**	50	46	>97	49	>97	>200

[a] Determined by HPLC on an achiral stationary phase. [b] Isolated yield (maximum theoretical yield = 50%). [c] Determined by HPLC on a chiral stationary phase. [d] Determined from the ee of substrate and product. [e] Kinetic resolution from ref 19.

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reticuline *rac*-**1f**, as the most complex structure, was obtained with 16% overall yield, while the most efficient synthesis in terms of yield was achieved for *rac*-**1d** and *rac*-**1b** with 43% and 42% isolated overall yield, respectively.

Finally, the racemic tetrahydroisoquinolines *rac*-**1a**−**g** were subjected to enantioselective oxidative ring closure catalyzed by BBE, leading to the untouched optically pure (*R*)-substrates and the optically pure (*S*)-berbine products **2a**−**g** via kinetic resolution (Table 2). The reaction was performed employing 1 g/L BBE, 5 g/L catalase, and 20 g/L substrate in a toluene/buffer (70:30) biphasic mixture.[19] Under these conditions, substrate solubility is not an issue. Maximum conversion (50%) was achieved within 24 h in all cases, and the enantiomerically pure products (ee > 97%, HPLC) were obtained in good to excellent yields (Table 2). For instance, the kinetic resolution of racemic reticuline *rac*-**1f** yielded optically pure (*R*)-reticuline (*R*)-**1f** and optically pure (*S*)-scoulerine (*S*)-**2f** in 37% and 47% isolated yield.

CONCLUSION

The combination of chemical synthesis of racemic 1-benzyl-1,2,3,4-tetrahydroisoquinolines with biocatalytic enantioselective intramolecular oxidative C−C coupling by BBE provided a new and efficient synthetic route to enantiomerically pure benzylisoquinoline and berbine alkaloids. The racemic substrates for BBE were prepared by two different pathways: either via Bischler−Napieralski cyclization or by alkylation of Boc-protected tetrahydroisoquinoline. BBE-catalyzed kinetic resolution proceeded with excellent enantioselectivity (E > 200), affording optically pure products in all cases. The overall chemoenzymatic synthesis resulted in yields of up to 20% for the benzylisoquinolines and 17% for the berbines, which represents a competitive alternative to the conventional asymmetric syntheses of these compounds.[21d,e,24,30] In particular, this novel synthetic route enabled the first asymmetric total synthesis of naturally occurring (*S*)-scoulerine, a sedative and muscle-relaxing agent,[3b,31] yielding 230 mg (7.4%) of the enantiomerically pure alkaloid over 9 linear steps.

EXPERIMENTAL SECTION

Synthesis of Amides 7b−d. A literature procedure[32] was adapted for our purpose: A solution of phenylacetic acid derivative **6b**−**d** (20.0 mmol), oxalyl chloride (2.89 g, 22.8 mmol) and one drop of DMF in dry toluene (50 mL) was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure to give the acyl chloride (quant), which was used without further purification. A solution of the crude acyl chloride (20.0 mmol) in CH_2Cl_2 (40 mL) was cooled to 0 °C on an ice bath. A solution of amino methane (40% in H_2O; 4.11 g, 52.9 mmol) in 2 M aqueous NaOH (20 mL) was added dropwise over 1 h. The ice bath was removed and stirring was continued overnight. The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with 2 N HCl solution (100 mL), saturated $NaHCO_3$ solution (100 mL), and water (100 mL) and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure yielded the amides **7b**−**d**, which were used in the following transformation without further purification.

3-Methoxyphenyl-*N*-methylacetamide (7b). Yield: 3.40 g (95%) as a pale yellowish solid. Mp: 41−44 °C. TLC (petroleum ether/EtOAc = 1/1): R_f = 0.22. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19,33]

***N*-Methyl-3,4,5-trimethoxyphenylacetamide (7c).** Yield: 3.74 g (78%) as a pale yellowish solid. Mp: 87−89 °C (lit.[34] 90.5−91.5 °C). TLC (petroleum ether/EtOAc = 1/1): R_f = 0.12. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19]

(3,4-Methylenedioxy)phenyl-*N*-methylacetamide (7d). Yield: 3.68 g (95%) as a pale yellowish solid. Mp: 100−101 °C (lit.[35] 99−101 °C). TLC (petroleum ether/EtOAc = 1/1): R_f = 0.20. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19,32]

Reduction of Amides 7b−d Giving Amines 4b−d. A literature procedure[32] was adapted for our purpose: BH_3·THF (1.0 M in THF; 100 mL, 100 mmol) was added to a solution of amide **7b**−**d** (17.4−20.0 mmol) in anhydrous THF (100 mL) and the mixture was gently refluxed for 18 h under an argon atmosphere. The solution was allowed to cool to room temperature, and 6 N HCl solution (20 mL) was cautiously added. After stirring for 30 min, the resulting solution was concentrated under reduced pressure, basified by addition of 2 M NaOH solution (100 mL), and saturated with NaCl. The product was extracted into EtOAc (3 × 30 mL), and the combined organic phases were washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to give the crude product as a yellowish liquid. Flash chromatography (silica; CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1) afforded the pure amine **4b**−**d**.

***N*-Methyl-3-methoxyphenethylamine (4b).** Yield: 2.23 g (72%) as a pale yellowish liquid. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.21. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19,33]

***N*-Methyl-3,4,5-trimethoxyphenethylamine (4c).** Yield: 3.54 g (78%) as a pale yellowish liquid, which crystallized upon standing to a pale yellowish solid. Mp: 175−177 °C (lit.[34] 178 °C). TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.37. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19]

***N*-Methyl-(3,4-methylenedioxy)phenethylamine (4d).** Yield: 1.81 g (58%) as a pale yellowish liquid. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.20. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19,35]

4-Benzyloxy-3-methoxybenzaldehyde[36]. K_2CO_3 (20.1 g, 0.146 mol) and benzyl bromide (22.5 g, 0.132 mol) were added to a solution of vanillin **8** (20.0 g, 0.131 mol) in ethanol (120 mL). The mixture was stirred for 20 h at room temperature under argon atmosphere. The solution was filtered through Celite and washed with CH_2Cl_2 (3 × 100 mL), and the solvent was evaporated under reduced pressure. The residue was taken up in CH_2Cl_2 (200 mL), washed with 5% NaOH solution (100 mL) and dried over K_2CO_3. Evaporation of the solvent under reduced pressure yielded 30.5 g of a yellow solid. Recrystallization from ethanol gave 4-benzyloxy-3-methoxybenzaldehyde (28.1 g, 89%) as a white solid. Mp: 61−63 °C (lit.[36] 61−62 °C). TLC (petroleum ether/EtOAc = 3/1): R_f = 0.62. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[36]

4-Benzyloxy-3-methoxy-β-nitrostyrene[37]. A solution of 4-benzyloxy-3-methoxy-benzaldehyde (22.7 g, 0.094 mol), nitromethane (18.4 g, 0.301 mol), and NH_4OAc (18.4 g, 0.239 mol) in AcOH (220 mL) was refluxed for 5 h. The mixture was poured into ice−water (300 mL), followed by addition of CH_2Cl_2 (150 mL) to dissolve the formed precipitate. The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were washed with water (100 mL), half-saturated Na_2CO_3 solution (50 mL), and brine (50 mL), dried over Na_2SO_4, and evaporated under reduced pressure to give 21.4 g of a brown solid. Recrystallization from ethanol gave 4-benzyloxy-3-methoxy-β-nitrostyrene (18.1 g, 68%) as a yellow solid. Mp: 119−121 °C (lit.[36] 124−125 °C). TLC (petroleum ether/EtOAc = 3/1): R_f = 0.47. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[36]

4-Benzyloxy-3-methoxyphenethylamine (9)[36]. To a suspension of $LiAlH_4$ (8.05 g, 212 mmol) in dry THF (120 mL) under argon was added dropwise a solution of 4-benzyloxy-3-methoxy-β-nitrostyrene (12.0 g, 42.0 mmol) in dry THF (80 mL) over 1 h. The reaction

mixture was refluxed for 16 h, then diluted with THF (100 mL), and cooled to 0 °C on an ice bath. To the vigorously stirred mixture were added water (8 mL), 15% NaOH solution (8 mL), and water (24 mL), the ice bath was removed, and stirring was continued for 1 h at room temperature. The resulting suspension was filtered through Celite, washed with THF, and evaporated under reduced pressure. The residue was dissolved in 10% HCl solution (20 mL) and washed with ether; afterward the aqueous layer was made basic and extracted with ether (3 × 50 mL). The combined organic phases were washed with water (20 mL) and brine (20 mL), dried over K_2CO_3, and evaporated under reduced pressure to give 8.28 g (77%) of 4-benzyloxy-3-methoxyphenethylamine as a yellowish liquid that crystallized upon standing to a yellowish solid. Mp: 63−65 °C (lit.[38] 59−61 °C). TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.27. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[36]

Ethyl 4-Benzyloxy-3-methoxyphenethylcarbamate. A literature procedure[39] was adapted for our purpose: To a solution of 4-benzyloxy-3-methoxyphenethylamine **9** (4.00 g, 15.5 mmol) in dichloromethane (120 mL) were added triethylamine (1.75 g, 17.3 mmol) and ethyl chloroformate (2.01 g, 18.4 mmol), and the mixture was stirred for 3 h at room temperature. Water (100 mL) was added, the phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure to give 5.06 g (99%) of ethyl 4-benzyloxy-3-methoxyphenethylcarbamate as a yellow liquid that crystallized upon standing to a yellowish solid. Mp: 80−81 °C. TLC (petroleum ether/EtOAc = 3/1): R_f = 0.23. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.24 (3H, t, J = 7.1 Hz, OCH_2CH_3), 2.75 (2H, t, J = 7.1 Hz, Ar-CH_2CH_2-N), 3.41 (2H, dt, J_1 = 6.6 Hz, J_2 = 6.5 Hz, Ar-CH_2CH_2-N), 3.89 (3H, s, OCH_3), 4.12 (2H, q, J = 7.2 Hz, OCH_2CH_3), 4.71 (1H, br s, NH), 5.15 (2H, s, $PhCH_2O$), 6.66−6.86 (3H, m, Ar), 7.31−7.47 (5H, m, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.7, 30.3, 35.8, 42.2, 56.0, 60.7, 71.1, 112.5, 114.3, 120.7, 127.3, 127.8, 128.5, 132.0, 137.3, 146.8, 149.7, 156.6. MS (EI, 70 eV): m/z = 329 (M^+, 13), 240 (27), 137 (59), 91 (100).

4-Benzyloxy-3-methoxy-*N*-methylphenethylamine (4e). A literature procedure[39] was adapted for our purpose: A solution of 4-benzyloxy-3-methoxyphenethylcarbamate (7.43 g, 22.6 mmol) in anhydrous THF (160 mL) under argon atmosphere was cooled to 0 °C on an ice bath. $LiAlH_4$ (4.33 g, 114 mmol) was added in portions to the stirred solution; afterward the ice bath was removed and the mixture was refluxed for 4 h. The suspension was diluted with THF (50 mL) and cooled to 0 °C on an ice bath. To the vigorously stirred mixture were added water (4.3 mL), 15% NaOH solution (4.3 mL) and water (12.9 mL), the ice bath was removed, and stirring was continued for 1 h at room temperature. The resulting suspension was filtered through Celite, washed with THF, dried over Na_2SO_4, and evaporated under reduced pressure to give 6.44 g of a brownish liquid. Flash chromatography (silica; CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1) afforded 4-benzyloxy-3-methoxy-*N*-methylphenethylamine (4.16 g, 67%) as an orange liquid. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.22. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.73 (1H, br s, NH), 2.44 (3H, s, NCH_3), 2.73−2.86 (4H, m, CH_2-CH_2-N), 3.90 (3H, s, OCH_3), 5.14 (2H, s, $PhCH_2O$), 6.68−6.84 (3H, m, Ar), 7.28−7.46 (5H, m, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 35.8, 36.4, 53.3, 56.0, 71.2, 112.6, 114.3, 120.6, 127.3, 127.8, 128.5, 133.2, 137.4, 146.6, 149.6. MS (EI, 70 eV): m/z = 271 (M^+, <1), 228 (46), 137 (18), 137 (18) 91 (58), 44 (100).

3-Benzyloxyphenylacetic Acid (5a). A literature procedure[40] was adapted for our purpose: A solution of 3-hydroxyphenylacetic acid (6.02 g, 39.6 mmol), KOH (6.0 g, 107 mmol), and NaI (0.2 g, 1.4 mmol) in ethanol (200 mL) was heated to 90 °C. Benzyl bromide (8.01 g, 46.8 mmol) was added, whereupon the mixture was refluxed at 100 °C for 16 h. The resulting suspension was concentrated to 70 mL and poured into water (200 mL) to give a slightly brownish solution from which the product was precipitated by addition of conc hydrochloric acid. The precipitate was filtered and recrystallized from H_2O/AcOH (1/1, 70 mL) to yield 6.61 g (69%) of 3-benzyloxyphenylacetic acid as a white solid. Mp =124−125 °C (lit.[41] 119 °C). TLC (EtOAc): R_f = 0.60. The ^{1}H NMR data are in accordance with literature.[41] ^{13}C NMR ($CDCl_3$, 75 MHz): δ 41.1, 70.0, 113.7, 116.0, 122.0, 127.6, 128.0, 128.6, 129.7, 134.7, 136.9, 159.0, 177.6. MS (EI, 70 eV): m/z = 242 (M^+, 9), 91 (100), 65 (10).

3-Benzyloxy-4-methoxyphenylacetic Acid (5f). *Method A.* A literature procedure[40] was adapted for our purpose: A solution of 3-hydroxy-4-methoxyphenylacetic acid (2.00 g, 11.0 mmol), KOH (1.73 g, 30 mmol), and NaI (0.06 g, 0.4 mmol) in ethanol (60 mL) was heated to 90 °C. Benzyl bromide (2.59 g, 16.5 mmol) was added, whereupon the mixture was refluxed at 100 °C for 16 h. The resulting suspension was poured into water (110 mL) to give a brownish solution from which the product was precipitated by addition of conc hydrochloric acid. The precipitate was filtered and recrystallized from H_2O/AcOH (1/1, 35 mL) to yield 1.99 g (67%) of 3-benzyloxy-4-methoxyphenylacetic acid as an off-white solid. Mp =117−118 °C (lit.[42] 124−125 °C). TLC (petroleum ether/EtOAc = 3/1 + 1 drop of AcOH): R_f = 0.63. The ^{1}H NMR data are in accordance with literature.[41] ^{13}C NMR ($CDCl_3$, 75 MHz): δ 40.5, 56.1, 71.1, 111.9, 115.3, 122.2, 125.6, 127.5, 127.9, 128.5, 137.0, 148.2, 149.1, 177.7.

Method B. A literature procedure[28,43] was adapted for our purpose: To a stirred solution of isovanillin **10** (20.0 g, 0.131 mol) in ethanol (120 mL) were added K_2CO_3 (20.1 g, 0.145 mol) and benzyl bromide (22.5 g, 0.131 mol). The mixture was stirred for 20 h at room temperature under argon atmosphere. The solution was filtered through Celite and washed with CH_2Cl_2 (3 × 100 mL), and the solvent was evaporated under reduced pressure. The residue was taken up in CH_2Cl_2 (200 mL), washed with 5% NaOH solution (100 mL), and dried over K_2CO_3. Evaporation of the solvent under reduced pressure yielded 31.3 g of a yellow solid. Recrystallization from ethanol gave 3-benzyloxy-4-methoxybenzaldehyde (29.2 g, 91%) as a white solid. Mp: 62−63 °C (lit.[24] 61−62 °C). TLC (petroleum ether/EtOAc = 3/1): R_f = 0.29. The ^{1}H and ^{13}C NMR data are in accordance with literature.[44] MS (EI, 70 eV): m/z = 242 (M^+, 13), 91 (100), 65 (9).

A solution of 3-benzyloxy-4-methoxybenzaldehyde (29.0 g, 0.120 mol) and chloroform (35 mL) in DMF (120 mL) under argon atmosphere was cooled to −10 °C on an ice/NaCl bath. A solution of KOH (8.88 g, 0.158 mol) in methanol (30 mL) was added dropwise over 30 min and the resulting mixture was stirred for 2 h at −10 °C. The reaction was quenched with 1 N hydrochloric acid (270 mL) and stirred for an additional 30 min at −10 °C. Afterward, the mixture was allowed to warm to room temperature, toluene (100 mL) was added, and the phases were separated. The aqueous phase was extracted with toluene (2 × 100 mL), and the combined organic phases were washed with water (30 mL) and brine (30 mL) and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure gave 42.9 g (97%) of 1-(3-benzyloxy-4-methoxyphenyl)-2,2,2-trichloroethanol **11** as a yellowish solid, which was used in the next step without further purification. TLC (petroleum ether/EtOAc = 3/1): R_f = 0.53. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.71 (1H, br s, OH), 3.92 (3H, s, OCH_3), 5.09 (1H, s, CH−OH), 5.21 (2H, s, $PhCH_2O$), 6.89 (1H, d, J = 8.7 Hz, Ar), 7.14−7.21 (2H, m, Ar), 7.26−7.46 (5H, m, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 55.9, 71.0, 84.1, 103.4, 110.6, 115.1, 122.6, 127.3, 127.4, 127.9, 128.6, 137.0, 147.1, 150.5. MS (EI, 70 eV): m/z = 360 (M^+, 1), 243 (36), 91 (100).

Diphenyl diselenide (36.9 g, 0.118 mol) was dissolved in deoxygenated ethanol (300 mL; purged with argon for 1 h). $NaBH_4$ (9.0 g, 0.238 mol) was added in portions over 30 min, upon which the previously orange solution turned colorless. The resulting mixture was stirred for 30 min at room temperature before addition of **11** (40.8 g, 0.113 mol) followed by NaOH (27.1 g, 0.678 mol). The reaction was then stirred for 18 h at 40 °C. The solvent was evaporated under reduced

pressure, and the solid residue was dissolved in water (200 mL). The pH of the solution was adjusted to 1.0 by addition of conc hydrochloric acid, and the product was extracted into EtOAc (5 × 100 mL). The combined organic phases were dried over Na_2SO_4, and the solvent was evaporated under reduced pressure to give an orange solid, which was recrystallized from petroleum ether/acetone to afford **5f** (11.0 g, 31%) as an off-white solid. The spectroscopic and chromatographic data are identical to those of **5f** obtained by method A.

Synthesis of Amides 3a−g. A literature procedure[45] was adapted for our purpose: A solution of phenylacetic acid derivative **5a** or **5f** (10.5−12.0 mmol), oxalyl chloride (1.95 g, 15.4 mmol), and one drop of DMF in dry toluene (40 mL) was stirred at room temperature under argon for 1 h. The solvent was evaporated under reduced pressure to give the acyl chloride (quant), which was used without further purification.

The amine **4a−g** (10.0−13.5 mmol) was dissolved in $CHCl_3$ (30 mL). A 3% NaOH solution (150 mL) was added, and the mixture was cooled to 0 °C on an ice bath. A solution of the crude phenylacetyl chloride derivative (10.6−12.8 mmol) in chloroform (20 mL) was added dropwise over 1 h to the vigorously stirred mixture. The ice bath was removed, and stirring was continued for 16 h at room temperature. The phases were separated, and the aqueous phase was extracted with $CHCl_3$ (50 mL). The combined organic phases were washed with dilute HCl solution (100 mL) and then water (100 mL) and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure yielded the crude amide **3a−g**, which was purified by flash chromatography (silica; petroleum ether/EtOAc = 1/1). The product is obtained as a mixture of rotamers, to which NMR signals are assigned based on the peak intensities as well as the DEPT, COSY, and HSQC spectra.

2-(3-Benzyloxyphenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide (3a). Yield: 2.97 g (64%) as an off-white solid. Ratio *trans*/*cis* = 1.15/1. Mp: 97−98 °C. TLC (petroleum ether/EtOAc = 1/1): R_f = 0.55. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19] HRMS: calcd for $C_{26}H_{29}NO_4$ 419.2097; found 419.2099.

2-(3-Benzyloxyphenyl)-*N*-(3-methoxyphenethyl)-*N*-methylacetamide (3b). Yield: 2.79 g (68%) as a pale yellowish liquid. Ratio *trans*/*cis* = 1.05/1. TLC (petroleum ether/EtOAc = 1/1): R_f = 0.37. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19] HRMS: calcd for $C_{25}H_{27}NO_3$ 389.1991; found 389.1990.

2-(3-Benzyloxyphenyl)-*N*-methyl-*N*-(3,4,5-trimethoxyphenethyl)acetamide (3c). Yield: 3.63 g (63%) as a pale yellowish liquid. Ratio *trans*/*cis* = 1.05/1. TLC (petroleum ether/EtOAc = 1/1): R_f = 0.18. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19] HRMS: calcd for $C_{27}H_{31}NO_5$ 449.2202; found 449.2224.

2-(3-Benzyloxyphenyl)-*N*-(3,4-methylenedioxy)phenethyl-*N*-methylacetamide (3d). Yield: 3.88 g (97%) as a pale yellowish liquid. Ratio *trans*/*cis* = 1.07/1. TLC (petroleum ether/EtOAc = 1/1): R_f = 0.37. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19] HRMS: calcd for $C_{25}H_{25}NO_4$ 403.1783; found 403.1796.

2-(3-Benzyloxyphenyl)-*N*-(4-benzyloxy-3-methoxyphenethyl)-*N*-methylacetamide (3e). Yield: 5.19 g (78%) as a pale yellowish liquid. Ratio *trans*/*cis* = 1.11/1. TLC (petroleum ether/EtOAc = 1/1): R_f = 0.26. MS (EI, 70 eV): m/z = 495 (M^+, 5), 240 (48), 197 (5), 149 (12), 91 (100). HRMS: calcd for $C_{32}H_{33}NO_4$ 495.2410; found 495.2440. *trans*-**3e**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.76 (2H, t, J = 7.5 Hz, CH_2-CH$_2$-N), 2.82 (3H, s, N-CH$_3$), 3.56 (2H, t, J = 7.5 Hz, CH$_2$-CH_2-N), 3.65 (2H, s, CH$_2$-CO), 3.81 (3H, s, OCH$_3$), 5.04 (2H, s, Ph-C H_2-O), 5.10 (2H, s, Ph-C H_2-O), 6.61−6.64 (1H, m, Ar), 6.74−6.90 (5H, m, Ar), 7.18−7.40 (11H, m, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 33.3 (CH$_2$), 36.6 (CH$_3$), 41.4 (CH$_2$), 50.3 (CH$_2$), 56.0 (CH$_3$), 69.9 (CH$_2$), 71.1 (CH$_2$), 112.6 (CH), 113.2 (CH), 114.3 (CH), 115.3 (CH), 120.7 (CH), 121.4 (CH), 127.3 (CH), 127.5 (CH), 127.8 (CH), 128.0 (CH), 128.5 (CH), 128.6 (CH), 129.7 (CH), 132.4 (C), 136.5 (C), 137.0 (C), 137.2 (C), 146.7 (C), 149.7 (C), 159.1 (C), 170.6 (C). *cis*-**3e**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.60 (2H, t, J = 7.2 Hz, CH_2-CH$_2$-N), 2.95 (3H, s, N-CH$_3$), 3.41 (2H, s, CH$_2$-CO), 3.42 (2H, t, J = 7.1 Hz, CH$_2$-C H_2-N), 3.84 (3H, s, OCH$_3$), 5.02 (2H, s, Ph-C H_2-O), 5.10 (2H, s, Ph-C H_2-O), 6.53−6.90 (2H, m, Ar), 6.74−6.90 (4H, m, Ar), 7.18−7.40 (11H, m, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 33.6 (CH$_3$), 34.3 (CH$_2$), 40.8 (CH$_2$), 52.2 (CH$_2$), 56.1 (CH$_3$), 69.9 (CH$_2$), 71.1 (CH$_2$), 112.5 (CH), 113.2 (CH), 114.5 (CH), 115.2 (CH), 120.7 (CH), 121.3 (CH), 127.3 (CH), 127.5 (CH), 127.9 (CH), 128.0 (CH), 128.5 (CH), 128.6 (CH), 129.7 (CH), 131.4 (C), 136.9 (C), 137.0 (C), 137.1 (C), 147.0 (C), 149.9 (C), 159.1 (C), 170.8 (C).

2-(3-Benzyloxy-4-methoxyphenyl)-*N*-(4-benzyloxy-3-methoxyphenethyl)-*N*-methylacetamide (3f). Yield: 2.33 g (76%) as an off-white solid. Ratio *trans*/*cis* = 1.14/1. Mp: 126−127 °C. TLC (petroleum ether/EtOAc = 1/1): R_f = 0.64. MS (EI, 70 eV): m/z = 525 (M^+, 3), 240 (22), 149 (11), 105 (14), 91 (100). HRMS: calcd for $C_{33}H_{35}NO_5$: 525.2515; found 525.2523. *trans*-**3f** ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.74 (2H, t, J = 7.5 Hz, CH_2-CH$_2$-N), 2.79 (3H, s, N-CH$_3$), 3.54 (2H, t, J = 7.7 Hz, CH$_2$-CH_2-N), 3.59 (2H, s, CH$_2$-CO), 3.84 (3H, m, OCH$_3$), 3.88 (3H, s, OCH$_3$), 5.13 (2H, s, Ph-C H_2-O), 5.16 (2H, s, Ph-C H_2-O), 6.53−6.86 (6H, m, Ar), 7.28−7.47 (10H, m, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 33.3 (CH$_2$), 36.5 (CH$_3$), 40.8 (CH$_2$), 50.2 (CH$_2$), 56.0 (CH$_3$), 70.9 (CH$_2$), 71.1 (CH$_2$), 112.0 (CH), 112.6 (CH), 114.2 (CH), 114.7 (CH), 120.7 (CH), 121.5 (CH), 127.3 (CH), 127.4 (CH), 127.8 (CH), 127.9 (CH), 128.5 (CH), 132.4 (C), 137.1 (C), 146.6 (C), 148.2 (C), 149.6 (C), 170.9 (C). *cis*-**3f**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.58 (2H, t, J = 7.2 Hz, CH_2-CH$_2$-N), 2.94 (3H, s, N-CH$_3$), 3.35 (2H, s, CH$_2$-CO), 3.39 (2H, t, J = 6.9 Hz, CH$_2$-CH_2-N), 3.87 (3H, s, OCH$_3$), 3.88 (3H, s, OCH$_3$), 5.13 (2H, s, Ph-CH_2-O × 2), 6.53−6.90 (2H, m, Ar), 6.74−6.90 (4H, m, Ar), 7.18−7.40 (11H, m, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 33.6 (CH$_3$), 34.2 (CH$_2$), 40.8 (CH$_2$), 52.0 (CH$_2$), 56.0 (CH$_3$), 70.8 (CH$_2$), 71.1 (CH$_2$), 111.9 (CH), 112.6 (CH), 114.5 (CH), 114.5 (CH), 120.7 (CH), 121.3 (CH), 127.3 (CH), 127.4 (CH), 127.7 (CH), 127.8 (CH), 128.5 (CH), 131.4 (CH), 137.1 (CH), 137.3 (CH), 147.0 (CH), 148.6 (CH), 149.8 (CH), 171.2 (CH).

2-(3-Benzyloxyphenyl)-*N*-phenethyl-*N*-methylacetamide (3g). Yield: 2.30 g (63%) as a pale yellowish liquid. Ratio *trans*/*cis* = 1.05/1. TLC (petroleum ether/EtOAc = 1/1): R_f = 0.51. *trans*-**3g**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.79−2.84 (5H, s + t overlap, N-CH$_3$ + CH_2-CH$_2$-N), 3.57 (2H, t, J = 7.5 Hz, CH$_2$-CH_2-N), 3.63 (2H, s, CH$_2$-CO), 5.03 (2H, s, Ph-C H_2-O), 6.73−6.88 (3H, m, Ar), 7.05−7.07 (1H, m, Ar), 7.14−7.42 (10H, m, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 33.7 (CH$_2$), 36.6 (CH$_3$), 41.4 (CH$_2$), 50.2 (CH$_2$), 69.9 (CH$_2$), 113.2 (CH), 115.3 (CH), 121.5 (CH), 126.3 (CH), 127.5 (CH), 128.0 (CH), 128.6 (CH), 128.8 (CH), 128.9 (CH), 129.7 (CH), 136.6 (C), 137.0 (C), 139.1 (C), 159.1 (C), 170.8 (C). *cis*-**3g**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.66 (2H, t, J = 7.3 Hz, CH_2-CH$_2$-N), 2.95 (3H, s, N-CH$_3$), 3.40 (2H, s, CH$_2$-CO), 3.44 (1H, t, J = 7.3 Hz, CH$_2$-CH_2-N), 5.01 (2H, s, Ph-CH_2-O), 6.73−6.88 (3H, m, Ar), 7.05−7.07 (1H, m, Ar), 7.14−7.42 (10H, m, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 33.6 (CH$_3$), 34.7 (CH$_2$), 40.8 (CH$_2$), 52.0 (CH$_2$), 69.9 (CH$_2$), 113.3 (CH), 115.2 (CH), 121.4 (CH), 126.8 (CH), 127.5 (CH), 128.0 (CH), 128.5 (CH), 128.6 (CH), 128.9 (CH), 129.7 (CH), 136.9 (C), 137.0 (C), 138.3 (C), 159.1 (C), 170.7 (C).

Bischler−Napieralski Cyclization of Amides 3a−f. A literature procedure[46] was adapted for our purpose: A solution of amide **3a−f** (7.0 mmol) and $POCl_3$ (21.0 mmol) in dry acetonitrile (60 mL) was refluxed for 3 h under argon atmosphere. The solvent and excess $POCl_3$ were evaporated under reduced pressure, and the residue was dissolved in dry methanol (50 mL), flushed with argon, and cooled to −5 °C on an ice/NaCl bath. $NaBH_4$ (50.0 mmol) was added in portions to the stirred mixture. The ice bath was then removed, and stirring was continued for 16 h at room temperature. The solvent was evaporated, and the residue was treated with half-saturated Na_2CO_3 solution (60 mL). The product was extracted with CH_2Cl_2 (3 × 30 mL) and the combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure to give the crude tetrahydroisoquinoline, which was purified by flash chromatography (silica; CH_2Cl_2/MeOH/NH_3(aq) = 96/3/1).

1-(3-Benzyloxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline. Yield: 2.78 g (94%) as a yellowish liquid. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.75. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19] HRMS: calcd for $C_{26}H_{28}NO_3$ [$(M - H)^+$] 402.2069; found 402.2071.

1-(3-Benzyloxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline. Yield: 2.43 g (94%) as a pale yellowish liquid. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.61. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19] HRMS: calcd for $C_{25}H_{26}NO_2$ [$(M - H)^+$] 372.1964; found 372.1974.

1-(3-Benzyloxybenzyl)-2-methyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline. Yield: 2.89 g (85%) as a pale yellowish viscous liquid. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.75. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19] HRMS: calcd for $C_{27}H_{30}NO_4$ [$(M - H)^+$] 432.2175; found 432.2194.

1-(3-Benzyloxybenzyl)-6,7-methylenedioxy-2-methyl-1,2,3,4-tetrahydroisoquinoline. Yield: 3.59 g (97%) as a pale yellowish liquid. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.76. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19] HRMS: calcd for $C_{25}H_{24}NO_3$ [$(M - H)^+$] 386.1756; found 386.1740.

1-(3-Benzyloxybenzyl)-7-benzyloxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline. Yield: 4.36 g (88%) as a pale yellowish liquid. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.28. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.56 (3H, s, NCH_3), 2.58−2.90 (4H, m, CH_2), 3.10−3.20 (2H, m, CH_2), 3.68 (1H, dd, J_1 = 6.9 H, J_2 = 5.7 Hz, CH), 3.86 (3H, s, OCH_3), 4.76 (1H, d, J = 12.3 Hz, $PhCH_2O$), 4.86 (1H, d, J = 12.0 Hz, $PhCH_2O$), 5.01 (2H, s, Ph-CH_2O), 6.13 (1H, s, Ar), 6.60 (1H, s, Ar), 6.69−6.88 (3H, m, Ar), 7.20 (1H, t, J = 8.0 Hz, Ar), 7.28−7.45 (10H, m, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.7, 41.1, 42.7, 47.0, 55.9, 64.6, 69.9, 70.8, 111.7, 112.3, 113.7, 116.4, 122.6, 126.5, 127.3, 127.5, 127.7, 127.9, 128.4, 128.6, 129.1, 129.4, 137.1, 137.1, 141.9, 145.6, 147.9, 158.7. MS (EI, 70 eV): m/z = 478 [$(M - H)^+$, <1], 282 (100), 191 (30), 162 (18), 91 (37). HRMS: calcd for $C_{32}H_{32}NO_3$ [$(M - H)^+$] 478.2382; found 478.2391.

1-(3-Benzyloxy-4-methoxybenzyl)-7-benzyloxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline. Yield: 1.98 g (97%) as a yellowish liquid. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.56. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.47 (3H, s, NCH_3), 2.50−2.58 (1H, m, CH_2), 2.66−2.83 (3H, m, CH_2), 2.97−3.12 (2H, m, CH_2), 3.55 (1H, dd, J_1 = 6.9 H, J_2 = 5.2 Hz, CH), 3.85 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 4.80 (1H, d, J = 12.3 Hz, $PhCH_2O$), 4.87 (1H, d, J = 12.3 Hz, $PhCH_2O$), 5.07 (2H, s, Ph-CH_2O), 6.10 (1H, s, Ar), 6.55−6.57 (2H, m, Ar), 6.64 (1H, d, J = 1.9 Hz, Ar), 6.77 (1H, d, J = 8.2 Hz, Ar), 7.26−7.38 (8H, m, Ar), 7.42−7.45 (2H, m, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.8, 40.5, 42.7, 47.2, 55.9, 56.0, 64.6, 70.8, 70.9, 111.4, 111.6, 113.7, 115.7, 122.5, 126.8, 127.2, 127.3, 127.7, 127.7, 128.4, 128.5, 129.3, 132.4, 137.3, 145.6, 147.7, 147.8, 148.0. MS (EI, 70 eV): m/z = 507 [$(M - 2H)^+$, <1], 282 (100), 191 (25), 162 (13), 91 (21). HRMS: calcd for $C_{33}H_{33}NO_4$ [$(M-2H)^+$] 507.2410; found 507.2435.

***tert*-Butyl 3,4-Dihydro-2(1*H*)-isoquinolinecarboxylate (14)[22a].** A solution of di-*tert*-butyl dicarbonate (11.11 g, 50.9 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a solution of 1,2,3,4-tetrahydroisoquinoline **13** (6.66 g, 50.0 mmol) in CH_2Cl_2 (30 mL). After stirring at room temperature for 2 h, the solvent was evaporated under reduced pressure to give 11.74 g (100%) of **14** as an orange liquid. TLC (petroleum ether/EtOAc = 3/1): R_f = 0.62. The ^{1}H NMR data are in accordance with literature.[22a] ^{13}C NMR ($CDCl_3$, 75 MHz): δ 28.5, 29.0, 40.7, 45.9, 85.2, 126.2, 126.3, 128.7, 134.8, 154.9. MS (EI, 70 eV): m/z = 218 [$(M - CH_3)^+$, <1), 176 (100), 160 (24), 142 (9), 132 (70), 117 (13), 104 (52), 77 (13), 57 (78), 41 (22).

3-Benzyloxybenzyl bromide (16). A literature procedure[47] was adapted for our purpose: A solution of 3-benzyloxybenzyl alcohol **15** (8.01 g, 37.4 mmol) and tetrabromomethane (13.1 g, 39.4 mmol) in CH_2Cl_2 (60 mL) was cooled to 0 °C on an ice/NaCl bath. Triphenylphosphine (10.22 g, 39.0 mmol) was added in portions to the stirred mixture, the cooling bath was removed, and the solution was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure, and the liquid residue was poured into well-stirred petroleum ether (100 mL), resulting in the formation of a white precipitate. The solid was removed by filtration and washed with petroleum ether (3 × 50 mL), and the filtrate was evaporated under reduced pressure to give 18.4 g of an orange liquid. Flash chromatography (silica; petroleum ether → petroleum ether/EtOAc = 9/1) afforded **16** (9.75 g, 94%) as a white crystalline solid. Mp: 54−55 °C (lit.[48] 37−39 °C). TLC (petroleum ether/EtOAc = 3/1): R_f = 0.76. The ^{1}H and ^{13}C NMR data are in accordance with literature.[48] MS (EI, 70 eV): m/z = 276 (M^+, 8), 197 (15), 91 (100).

***tert*-Butyl 1-(3-Benzyloxybenzyl)-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate (17).** A literature procedure[22a] was adapted for our purpose: A solution of *tert*-butyl 3,4-dihydro-2(1*H*)-carboxylate **14** (2.33 g, 10.0 mmol) and tetramethylethylene-diamine (1.22 g, 10.5 mmol) in anhydrous THF under argon atmosphere was cooled to −78 °C. *tert*-Butyl lithium solution (1.7 M in pentane; 6.2 mL, 10.5 mmol) was added dropwise over 30 min, resulting in a deep red solution, which was stirred at −78 °C for 30 min. A solution of 3-benzyloxybenzyl bromide (**5**; 2.77 g, 10.0 mmol) in anhydrous THF (10 mL) was added dropwise over 30 min. The mixture was then stirred for 3 h, during which time the temperature was allowed to rise to −50 °C. The resulting yellow suspension was quenched with saturated NH_4Cl solution (10 mL). Water (30 mL) was added, the phases were separated, and the aqueous phase was extracted with *tert*-butyl methyl ether (2 × 20 mL). The combined organic phases were dried over Na_2SO_4, and the solvent was evaporated under reduced pressure to give 5.52 g of an orange liquid. Flash chromatography (silica; petroleum ether → petroleum ether/EtOAc = 95/5) afforded **17** (2.21 g, 51%) as a colorless liquid. TLC (petroleum ether/EtOAc = 3/1): R_f = 0.59. MS (EI, 70 eV): m/z = 429 (M^+, <1), 232 (16), 176 (57), 132 (100), 91 (38). HRMS: calcd for $C_{28}H_{31}NO_3$ (M^+) 429.2304; found 429.2333. The product is obtained as a mixture of rotamers (ratio *cis*/*trans* = 2/1), to which NMR signals are assigned based on the peak intensities as well as the DEPT, COSY, and HSQC spectra. *cis*-**17**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.25 (9H, s, CH_3), 2.62−3.07 (4H, m, CH_2), 3.22−3.31 (1H, m, CH_2CH_2N), 4.19 (1H, ddd, J_1 = 13.1 Hz, J_2 = 5.6 Hz, J_3 = 3.5 Hz, CH_2CH_2N), 5.01 (2H, s, $PhCH_2O$), 5.22 (1H, dd, J_1 = 8.2 Hz, J_2 = 5.6 Hz, CH), 6.68−6.92 (3H, m, Ar), 7.03−7.19 (5H, m, Ar), 7.32−7.49 (5H, m, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 28.1 (CH_3), 28.5 (CH_2), 37.0 (CH_2), 43.0 (CH_2), 56.7 (CH), 69.9 (CH_2), 79.6 (C), 112.7 (CH), 116.3 (CH), 122.4 (CH), 125.9 (CH), 126.7 (CH), 127.3 (CH), 127.5 (CH), 127.9 (CH), 128.6 (CH), 129.1 (CH), 129.3 (CH), 134.8 (C), 137.0 (C), 137.1 (C), 140.2 (C), 154.4 (C), 158.8 (C). *trans*-**17**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.42 (9H, s, CH_3), 2.62−3.07 (4H, m, CH_2), 3.22−3.31 (1H, m, CH_2CH_2N), 3.78 (1H, dt, J_1 = 11.3 Hz, J_2 = 5.2 Hz, CH_2CH_2N), 4.96 (2H, s, $PhCH_2O$), 5.38 (1H, t, J = 6.7 Hz, CH), 6.68−6.92 (3H, m, Ar), 7.03−7.19 (5H, m, Ar), 7.32−7.49 (5H, m, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 28.4 (CH_2), 28.6 (CH_3), 39.4 (CH_2), 42.7 (CH_2), 55.5 (CH), 69.8 (CH_2), 79.5 (C), 113.0 (CH), 116.0 (CH), 122.5 (CH), 125.9 (CH), 126.6 (CH), 127.3 (CH), 127.5 (CH), 127.9 (CH), 128.4 (CH), 129.0 (CH), 129.3 (CH), 134.6 (C), 137.0 (C), 137.2 (C), 139.8 (C), 154.7 (C), 158.6 (C).

1-(3-Benzyloxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline (18). A solution of *tert*-butyl 1-(3-benzyloxybenzyl)-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate **17** (3.55 g, 8.26 mmol) in anhydrous THF (160 mL) under argon atmosphere was cooled to 0 °C on an ice bath. $LiAlH_4$ (1.60 g, 42.2 mmol) was added in portions to the stirred solution; afterward the ice bath was removed and the mixture was refluxed for 16 h. The suspension was diluted with THF (50 mL) and cooled to 0 °C on an ice bath. Water (1.6 mL), 15% NaOH solution (1.6 mL), and again water (4.8 mL) were added to the vigorously stirred

mixture, the ice bath was removed, and stirring was continued for 1 h at room temperature. The resulting suspension was filtered through Celite, washed with THF, dried over Na_2SO_4, and evaporated under reduced pressure to give 2.89 g of a yellow liquid. Flash chromatography (silica; CH_2Cl_2/MeOH/NH_3(aq) = 98/1/1) afforded **18** (2.09 g, 74%) as a yellowish liquid. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.56. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.54 (3H, s, NCH_3), 2.67−2.83 (2H, m, CH_2), 2.88−2.98 (2H, m, CH_2), 3.14−3.27 (2H, m, CH_2), 3.87 (1H, t, J = 6.2 Hz, CH), 5.04 (2H, s, $PhCH_2O$), 6.78−6.89 (4H, m, Ar), 7.06−7.24 (4H, m, Ar), 7.36−7.46 (5H, m, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 26.1, 41.5, 42.9, 47.1, 65.0, 69.9, 112.4, 116.2, 122.4, 125.4, 126.0, 127.6, 127.9, 128.0, 128.6, 128.8, 129.0, 134.4, 137.3, 137.9, 141.7, 158.6. MS (EI, 70 eV): m/z = 342 [$(M - H)^+$, <1], 146 (100), 131 (6), 91 (10). HRMS: calcd for $C_{24}H_{24}NO$ [$(M - H)^+$] 342.1858; found 342.1851.

Hydrogenolytic Deprotection Affording Tetrahydroisoquinolines 1a−g. A literature procedure[24] was adapted for our purpose: A mixture of benzyl-protected tetrahydroisoquinoline (5.75−9.16 mmol), Pd 10% on activated charcoal (0.20−0.30 g), acetic acid (12.5−20.0 mmol), and dry methanol (50 mL) was stirred under H_2 atmosphere (balloon) for 16 h. The mixture was filtered through Celite, washed with methanol (100 mL), and evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (30 mL) and washed with half-saturated $NaHCO_3$ solution (40 mL). The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure to afford pure **1a**−**g**.

6,7-Dimethoxy-1-(3-hydroxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline (1a). Yield: 2.07 g (98%) as an off-white solid foam. Mp: 127−128 °C (lit.[49] 135 °C). TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.53. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19,50] HRMS: calcd for $C_{19}H_{22}NO_3$ [$(M - H)^+$] 312.1600; found 312.1589.

1-(3-Hydroxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (1b). Yield: 1.53 g (94%) as an off-white solid foam. Mp =113−116 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.51. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19] HRMS: calcd for $C_{18}H_{20}NO_2$ [$(M - H)^+$] 282.1494; found 282.1499.

1-(3-Hydroxybenzyl)-2-methyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline (1c). Yield: 1.87 g (84%) as a highly viscous yellowish liquid. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.51. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19] HRMS: calcd for $C_{20}H_{24}NO_4$ [$(M - H)^+$]: 342.1705; found 342.1727.

1-(3-Hydroxybenzyl)-6,7-methylenedioxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (1d). Yield: 2.19 g (81%) as a white solid foam. Mp: 143−145 °C (lit.[49] 145 °C). TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.45. The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19] HRMS: calcd for $C_{18}H_{18}NO_3$ [$(M - H)^+$] 296.1287; found 296.1297.

1-(3-Hydroxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (1e). Yield: 2.11 g (98%) as an off-white solid foam. Mp: 103−106 °C (lit.[49] 111−113 °C). TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.25. The NMR data are in accordance with literature.[50] MS (EI, 70 eV): m/z = 298 [$(M - H)^+$, <1), 192 (100), 177 (19), 148 (5). MS (EI, 70 eV): m/z = 298 [$(M - H)^+$, <1), 192 (100), 177 (19), 148 (5). HRMS: calcd for $C_{18}H_{20}NO_3$ [$(M - H)^+$] 298.1443; found 298.1450.

Reticuline (1f). Yield: 0.90 g (70%) as an off-white solid foam. Mp: 83−84 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.29. The NMR data are in accordance with literature.[24] MS (EI, 70 eV): m/z = 328 [$(M - H)^+$, <1], 192 (100), 177 (21). HRMS: calcd for $C_{19}H_{22}NO_4$ [$(M - H)^+$] 328.1549; found 328.1571.

1-(3-Hydroxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline (1g). Yield: 1.25 g (94%) as an off-white solid foam. Mp: 129−130 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.47. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.50 (3H, s, NCH_3), 2.73−3.05 (4H, m, CH_2), 3.17 (1H, dd, J_1 = 13.8 Hz, J_2 = 6.0 Hz, CH_2), 3.28−3.37 (1H, m, CH_2), 3.92 (1H, t, J = 6.5 Hz, CH), 6.60−6.63 (3H, m, Ar), 6.76 (1H, d, J = 7.8 Hz, Ar), 7.02−7.15 (4H, m, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.5 (CH_2), 41.7 (CH_2), 41.9 (CH_3), 46.0 (CH_2), 64.9 (CH), 113.9 (CH), 116.6 (CH), 121.1 (CH), 125.6 (CH), 126.4 (CH), 128.1 (CH), 128.9 (CH), 129.4 (CH), 133.2 (C), 136.8 (C), 140.9 (C), 156.7 (C). MS (EI, 70 eV): m/z = 252 [$(M - H)^+$, <1], 146 (100), 131 (7). HRMS: calcd for $C_{17}H_{18}NO$ [$(M - H)^+$] 252.1388; found 252.1403.

BBE-Catalyzed Kinetic Resolution of 1a−g[19]. Substrate **1a**−**g** (500 mg, 1.5−2.0 mmol) was dissolved in toluene (17.5 mL) and buffer (7.5 mL, 10 mM Tris-HCl, pH 9.0, 10 mM $MgCl_2$) containing BBE (1.5 mL enzyme solution, final concentration = 1 g/L = 0.017 mM) and crude catalase (125 mg, final concentration 5 g/L). The mixture was shaken in a light-shielded round-bottom flask (50 mL) at 200 rpm and 40 °C for 24 h. The reaction was stopped by phase separation, followed by extraction of the aqueous phase with ethyl acetate (3 × 10 mL). The combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure to give the crude product. Flash chromatography (silica; **a**−**f**, CH_2Cl_2/MeOH/NH_3(aq) = 96/3/1; **g**, CH_2Cl_2/MeOH/NH_3(aq) = 98/1/1) afforded pure (*S*)-**2a**−**g** and (*R*)-**1a**−**g**.

(*S*)-2,3-Dimethoxy-9-hydroxyberbine (*S*)-2a. Yield: 207 mg (42%) as an off-white solid foam. Mp: 90−95 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.78. $[\alpha]^{20}_D$ = −273.4 ($CHCl_3$, c = 1.0); lit.[50] (*R*) +176 (MeOH, c = 0.34). The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19,50] HRMS: calcd for $C_{19}H_{21}NO_3$ 311.1521; found 311.1519.

(*R*)-6,7-Dimethoxy-1-(3-hydroxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline (*R*)-1a. Yield: 249 mg (50%) as an off-white solid foam. Mp: 151−153 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.53. $[\alpha]^{20}_D$ = −109.4 ($CHCl_3$, c = 1.0). The ^{1}H and ^{13}C NMR as well as MS data are in agreement with those obtained for the racemic compound. HRMS: calcd for $C_{19}H_{22}NO_3$ [$(M - H)^+$] 312.1600; found 312.1591. The NMR data are in accordance with literature.[50]

(*S*)-9-Hydroxy-3-methoxyberbine (*S*)-2b. Yield: 177 mg (36%) as an off-white solid foam. Mp: 192−195 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.56. $[\alpha]^{20}_D$ = −280.6 ($CHCl_3$, c = 0.5). The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19] HRMS: calcd for $C_{18}H_{19}NO_2$ 281.1416; found 281.1415.

(*R*)-1-(3-Hydroxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*R*)-1b. Yield: 181 mg (36%) as a highly viscous yellowish liquid. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.47. $[\alpha]^{20}_D$ = −76.3 ($CHCl_3$, c = 0.63). The ^{1}H and ^{13}C NMR as well as MS data are in agreement with those obtained for the racemic compound. HRMS: calcd for $C_{18}H_{20}NO_2$ [$(M - H)^+$] 282.1494; found 282.1504.

(*S*)-9-Hydroxy-1,2,3-trimethoxyberbine (*S*)-2c. Yield: 194 mg (39%) as an off-white solid foam. Mp: 85−89 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.60. $[\alpha]^{20}_D$ = −226.5 ($CHCl_3$, c = 0.57). The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19] HRMS: calcd for $C_{20}H_{23}NO_4$ 341.1627; found 341.1623.

(*R*)-1-(3-Hydroxybenzyl)-2-methyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline (*R*)-1c. Yield: 237 mg (47%) as highly viscous yellowish liquid. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.33. $[\alpha]^{20}_D$ = −75.4 ($CHCl_3$, c = 0.75). The ^{1}H and ^{13}C NMR as well as MS data are in agreement with those obtained for the racemic compound. HRMS: calcd for $C_{20}H_{24}NO_4$ [$(M - H)^+$] 342.1705; found 342.1703.

(*S*)-9-Hydroxy-2,3-methylenedioxyberbine (*S*)-2d. Yield: 155 mg (31%) as an off-white solid foam. Mp: 177−180 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.50. $[\alpha]^{20}_D$ = −342.5 ($CHCl_3$, c = 0.63). The ^{1}H and ^{13}C NMR as well as MS data are in accordance with literature.[19] HRMS: calcd for $C_{18}H_{17}NO_3$ 295.1208; found 295.1209.

(*R*)-1-(3-Hydroxybenzyl)-6,7-methylenedioxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*R*)-1d. Yield: 231 mg (46%) as an off-white solid foam. Mp: 165−167 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.38. $[\alpha]^{20}_D$ = −83.2 ($CHCl_3$, c = 0.31). The ^{1}H and ^{13}C NMR as well as MS data are in agreement with those obtained for the racemic compound. HRMS: calcd for $C_{18}H_{18}NO_3$ $[(M - H)^+]$ 296.1287; found 296.1303.

(*S*)-2,9-Dihydroxy-3-methoxyberbine (*S*)-2e. Yield: 129 mg (22%) as an off-white solid foam. Mp: 135 °C (decomp.). TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.63. $[\alpha]^{20}_D$ = −281.5 ($CHCl_3$, c = 0.28); lit.[50] −129° ($CHCl_3$, c = 0.3). The ^{1}H and ^{13}C NMR data are in accordance with literature.[50] MS (EI, 70 eV): m/z = 297 (M^+, 100), 296 (92), 282 (15), 178 (60), 176 (82), 163 (16), 149 (19), 120 (24), 86 (52). HRMS: calcd for $C_{18}H_{19}NO_3$ 297.1365; found 297.1373.

(*R*)-1-(3-Hydroxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*R*)-1e. Yield: 247 mg (49%) as an off-white solid foam. Mp: 89−90 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.39. $[\alpha]^{20}_D$ = −29.1 ($CHCl_3$, c = 0.36); lit.[50] (*S*): +43 (MeOH, c = 0.5). The ^{1}H and ^{13}C NMR as well as MS data are in agreement with those obtained for the racemic compound. HRMS: calcd for $C_{18}H_{20}NO_3$ $[(M - H)^+]$ 298.1443; found 298.1453. The NMR data are in accordance with literature.[50]

(*S*)-Scoulerine (*S*)-2f. Yield: 232 mg (47%) as an off-white solid foam. Mp: 194−195 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.49. $[\alpha]^{20}_D$ = −248.3 ($CHCl_3$, c = 0.27); lit.[51] −315 (MeOH, c = 0.11). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.63−2.70 (2H, m, CH_2), 2.83 (1H, dd, J_1 = 15.9 Hz, J_2 = 11.4 Hz, CH_2), 3.11−3.28 (3H, m, CH_2), 3.49−3.57 (2H, m, N-CH_2-Ar + CH), 3.87 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.25 (1H, d, J = 15.6 Hz, N-CH_2-Ar), 6.61 (1H, s, Ar), 6.68 (1H, d, J = 8.3 Hz, Ar), 6.75 (1H, d, J = 8.3 Hz, Ar), 6.84 (1H, s, Ar). ^{13}C NMR ($CHCl_3$, 75 MHz): δ 29.2 (CH_2), 36.3 (CH_2), 51.6 (CH_2), 53.5 (CH_2), 55.9 (CH_2), 56.2 (CH_3), 59.2 (CH), 109.0 (CH), 110.6 (CH), 111.4 (CH), 119.3 (CH), 121.2 (C), 126.1 (C), 128.2 (C), 130.6 (C), 141.5 (C), 143.9 (C), 144.0 (C), 145.1 (C). MS (EI, 70 eV): m/z = 327 (M^+, 55), 310 (8), 178 (100), 176 (32), 163 (13), 150 (48), 135 (27), 107 (16). HRMS: calcd for $C_{19}H_{21}NO_4$ 327.1471; found 327.1490.

(*R*)-Reticuline (*R*)-1f. Yield: 182 mg (37%) as an off-white solid foam. Mp: 74−75 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.29. $[\alpha]^{20}_D$ = −64.6 ($CHCl_3$, c = 0.26); lit.[52] −55 (EtOH, c = 0.44). The ^{1}H and ^{13}C NMR as well as MS data are in agreement with those obtained for the racemic compound. HRMS: calcd for $C_{19}H_{22}NO_4$ $[(M - H)^+]$ 328.1549; found 328.1600. The NMR data are in accordance with literature.[24]

(*S*)-9-Hydroxyberbine (*S*)-2g. Yield: 230 mg (46%) as an off-white solid foam. Mp: 103−104 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.58. $[\alpha]^{20}_D$ = −328.8 ($CHCl_3$, c = 1.0). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.55−2.71 (2H, m, CH_2), 2.86 (1H, dd, J_1 = 16.3 Hz, J_2 = 11.5 Hz, CH_2), 3.10−3.20 (2H, m, CH_2), 3.26 (1H, dd, J_1 = 16.5 Hz, J_2 = 3.7 Hz, CH_2), 3.36 (1H, d, J = 15.6 Hz, N-CH_2-Ar), 3.62 (1H, dd, J_1 = 11.2 Hz, J_2 = 3.3 Hz, CH), 4.09 (1H, d, J = 15.6 Hz, N-CH_2-Ar), 6.21 (1H, d, J = 7.9 Hz, Ar), 6.58 (1H, d, J = 7.6 Hz, Ar), 6.78 (1H, t, J = 7.8 Hz, Ar), 7.04−7.21 (3H, m, Ar). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 29.1 (CH_2), 36.2 (CH_2), 51.3 (CH_2), 53.6 (CH_2), 59.4 (CH), 112.5 (CH), 120.5 (CH), 121.8 (C), 125.5 (CH), 126.1 (CH), 126.3 (CH), 126.8 (CH), 128.9 (CH), 134.4 (C), 135.9 (C), 137.5 (C), 152.4 (C). MS (EI, 70 eV): m/z = 251 (M^+, 70), 132 (100), 130 (50), 130 (32), 91 (27). HRMS: calcd for $C_{17}H_{17}NO$ 251.1310; found 251.1308.

(*R*)-1-(3-Hydroxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline (*R*)-1g. Yield: 247 mg (49%) as an off-white solid foam. Mp: 110−111 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.45. $[\alpha]^{20}_D$ = −58.4 ($CHCl_3$, c = 1.0). The ^{1}H and ^{13}C NMR as well as MS data are in agreement with those obtained for the racemic compound. HRMS: calcd for $C_{17}H_{17}NO$ $[(M - 2H)^+]$: 251.1310; found 251.1338.

Determination of Absolute Configuration. Absolute configurations of benzylisoquinolines **1a**−**g** and berbines **2a**−**g** were assigned based on optical rotation, circular dichroism, and HPLC elution order analogies as previously described.[19]

Preparation of Racemic Reference Samples for Chiral HPLC Analysis. Racemic samples of berbines **2a**−**g** for use as HPLC reference were prepared as previously described.[19]

Enzyme Expression and Purification. BBE expression was carried out in a 7 L glass fermenter according to the following protocol:

Preparation of Inoculum. Overnight cultures (ONCs) of *Pichia pastoris* colonies containing the BBE expression plasmid [pPICZα-BBE-ER] were grown in 50 mL of YPD medium containing 100 μg/mL zeocin (in 300 mL Erlenmeyer flasks) at 30 °C and 150 rpm for 20 h. The ONC was used to inoculate 300 mL of YPD medium (in 2 L baffled Erlenmeyer flasks) to an initial OD_{600} of 1.0. The cultures were grown to an OD_{600} of 10−15 at 30 °C and 150 rpm.

Preparation of Fermenter. Feeding flasks and tubing for base, antifoam, glycerol, and methanol addition as well as inoculum flasks were autoclaved. The fermenter was equipped with a calibrated pH electrode, a pO_2 electrode, and a sampling nozzle, filled with fermentation basal salts medium (3.5 L, see below), sterilized, and cooled to 30 °C. Trace salts solution (15 mL, for composition see below) and antifoam (Struktol J650, 1:10 dilution; 100 mL) were added, and the pH was adjusted to 5.0 by addition of 25% aqueous ammonia. The pO_2 electrode was calibrated using N_2 and air saturation for adjusting the 0% and 100% values, respectively.

Inoculation and Glycerol Batch Phase. The fermenter was inoculated with the shaking flask culture (300 mL, the initial OD_{600} in the fermenter should be 1.0), and the batch was stirred overnight with automatic control of pO_2 (≥30%), pH (pH 5.0) and temperature (30 °C). The next morning, the culture had consumed all glycerol present in the medium (as indicated by a sharp rise in the pO_2 value).

Glycerol Fed-Batch Phase. A glycerol feed (50% w/v; containing 12 mL/L of trace salts solution) was started with an initial feed rate of 15 g/h, causing the pO_2 to drop. After about 5 min, when the pO_2 had reached again a value above 30%, the feed rate was raised continuously to 30 g/h over 30 min. Three hours later, the feed rate was raised continuously to 45 g/h over 30 min. This feed rate was maintained overnight. The next morning, 1000 g of 50% glycerol had been added in total. A sample was taken and analyzed for wet cell weight (WCW). A WCW of 280 g/L was reached at the end of the glycerol batch-phase.

Methanol Fed-Batch Phase (Induction Phase). After approximately 1000 g of glycerol had been added, methanol adaptation was started by pumping 5 g of methanol feed (HPLC grade, methanol containing 12 mL/L of trace salts solution) into the fermenter. Subsequently, the glycerol feed was continuously reduced to 15 g/h over 1 h 59 min and finally reduced to zero within 1 min. During these 2 h of decrease of glycerol feed, the pH value was raised to 6.0 by addition of base. This pH adjustment is crucial for protein expression. As soon as the glycerol feed had been stopped, the methanol feed was started with an initial feed rate of 3 g/h. During the next 12 h, the feed rate was slowly raised to 9 g/h in several steps. This rate was maintained until the end of fermentation. Samples were taken every 24 h and analyzed for WCW and BBE activity. The activity rose over time, while the WCW stayed constant. After addition of 750 g of methanol in total (96 h of induction), pH and pO_2 control were disabled, and $(NH_4)_2SO_4$ was added to the batch to a final concentration of 1 M. The culture was aliquoted into centrifuge beakers (1 L), and the cells were pelleted by centrifugation (4000 rpm,

30 min, 4 °C). The supernatant was subjected to protein purification (see below).

Protein Purification. Hydrophobic Interaction Chromatography (HIC). The fermentation supernatant was loaded onto a Phenyl Sepharose column (XK50/20, Phenyl Sepharose 6 High Sub) equilibrated with HIC start buffer (50 mM $K_2HPO_4 \cdot 3H_2O$, 1 M $(NH_4)_2SO_4$, pH 7.5; filtered and degassed) with maximum flow rate and the column was washed with HIC start buffer until absorption (280, 375, 450 nm) and conductivity readings were constant. BBE was eluted using a gradient of HIC start buffer against 20% aqueous ethanol (100% start buffer to 50% in 25 min; 50% to 20% in 60 min; 20% to 0% start buffer in 60 min) and a flow rate of 6 mL/min. Fractions of 12 mL were collected, activity assays were performed and the active fractions were pooled and concentrated using the Centriprep centrifugal filtration system.

Gel Filtration (GF). The pooled and concentrated fractions from HIC were loaded onto a Superdex 200 column (XK16/100, Superdex 200, prep grade) equilibrated with GF buffer (100 mM Tris-HCl, 150 mM NaCl, pH 8.0; filtered and degassed) using a 3 mL sample loop. BBE was eluted with GF buffer at a flow rate of 1 mL/min. Fractions of 3 mL were collected, activity assays were performed and the active fractions were pooled and concentrated as above. The protein solution was flash frozen by dripping into liquid nitrogen.

Activity Assay. A mixture of *rac*-reticuline solution (4 μL, 10 mM in reaction buffer/DMSO = 9/1), 4 μL BBE solution (protein purification fraction, fermentation supernatant, etc.), and BBE assay buffer (17 μL, 100 mM Tris-HCl, pH 9.0) were incubated at 37 °C for 10 min. The reaction was analyzed by TLC (silica; CH_2Cl_2/MeOH/NH_4OH = 90/9/1; visualization by UV irradiation).

Media and Feed Solutions. YPD Medium (for 1 L). Bacto yeast extract (10 g) and Bacto peptone (20 g) were dissolved and autoclaved in 900 mL of H_2O; glucose (20 g) was dissolved and autoclaved in 100 mL of H_2O. The two solutions were mixed after autoclaving.

Basal Salts Medium (according to Hartner & Winkler; 3.5 L). A total of 0.6 g $CaSO_4 \cdot 2H_2O$, 8.1 g $MgSO_4 \cdot 7H_2O$, 10 g K_2SO_4, 7 g KOH, 0.77 g NaCl, 112.8 g glycerol, 44.6 mL phosphoric acid, and water (bidest.) ad 3500 mL.

Trace Salts Solution (200 mL). Dissolve 40 mg biotin, 16 mg NaI, 40 mg $Na_2MoO_4 \cdot 2H_2O$, 4 mg H_3BO_3, and 146 mg $CoCl_2 \cdot 6H_2O$ in 100 mL H_2O (bidest). Additionally, 1.2 g $CuSO_4 \cdot 5H_2O$, 590 mg $MnCl_2 \cdot 4H_2O$, 4 g $ZnCl_2$, 13 g $FeSO_4 \cdot 7H_2O$ and 1 mL H_2SO_4 (conc) are dissolved in 100 mL H_2O (bidest.). The two solutions were mixed, filter-sterilized, and stored at 4 °C.

Glycerol Feed (1.5 L). Mix 750 g glycerol, water (bidest.) ad 1500 mL; autoclave, add 18 mL trace salts solution.

Methanol Feed (1.5 L). Mix 1.5 L HPLC grade MeOH and 18 mL trace salts solution.

Base (400 mL). Ammonium hydroxide 25% solution.

Fermenter Settings. Temperature. Setpoint: 30 °C, Mode: AUTO

Stirrer. Mode: CASC, Min: 25% (= 500 rpm), Max: 75% (= 1500 rpm), Ramp: 20%/sec

pH. Setpoint: 5.00, Mode: Auto, Pump: ---/BASE

pO_2. Setpoint: 30%, Mode: AUTO, Casc: STIRR AIRFL, Parameter: HTime: 1 min, Dead: 0.5%, Stirr Min: 25%, Stirr Max: 75%, Airfl Min: 25%, Airfl Max: 100%

Foam. Mode: AUTO, Pump: AFOAM, Cycle: 0:10 m:s

Airflow. Mode: CASC, Min: 25% (= 2.5 L/min), Max: 100% (= 10 L/min)

■ ASSOCIATED CONTENT

Supporting Information. General experimental information; analytical methods; ^1H and ^{13}C NMR spectra, MS spectra, and HRMS results of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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