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Synthesis of a dysidiolide-inspired compound library and discovery of acetylcholinesterase inhibitors based on protein structure similarity clustering (PSSC)

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Abstract

Biologically relevant compound collections are a major prerequisite for efficient protein ligand development and ultimately for drug discovery. We herein describe the development of a compound collection inspired by the decalin core motif of two natural products, dysidiolide **1** and sulfiricin **2**, both inhibitors of the Cdc25A phosphatase. Several keto-functionalized decalinols were synthesized in solution, immobilized on Merrifield resin equipped with a dihydropyranyl linker, and then subjected to aldol condensation reactions with different aldehydes leading to exocyclic *E*-configured olefins. Further diversity-increasing transformations on the solid support included Sonogashira, Suzuki, and Heck reactions, Cu-catalyzed conjugate addition and Grignard reactions, alkylation reactions in the α-position to a ketone, Wittig reactions, and reductive animations. In total, 483 compounds were synthesized.

Cdc25A and AChE exhibit structural similarity in their ligand-sensing cores and were thus grouped into a protein structure similarity cluster (PSSC). A screen for AChE inhibition of a subset of 162 compounds yielded three micromolar inhibitors of AChE with IC_{50} values <20 μM.

Keywords: Solid-phase synthesis; Protein structure similarity clustering (PSSC); Acetylcholinesterase; Biology-oriented synthesis

1. Introduction

The efficient identification of small molecules that modulate protein function in vitro and in vivo is at the heart of chemical biology and medicinal chemistry research, and the development of new therapies and diagnostics for disease.

Key to the discovery of novel modulators of protein function is the identification of appropriate starting points in chemical structure space for compound library development, which could serve as 'leitmotifs' for the synthesis of biologically relevant compound collections. To gain access to novel chemical structures in library formats, several new strategies for library design that focus for instance on natural product-guided,[1] protein structure- and natural product-guided,[2–4] and biology-oriented[5–16] or diversity-oriented[17] synthesis have emerged.

Natural products (NPs) can be regarded as evolutionarily selected ligands for the ligand-sensing cores of proteins. They emerge via biosynthesis by proteins and often fulfill various biological functions through interaction with multiple proteins.[4,18] Their underlying structures define structural prerequisites for binding to proteins and biological activity. While the entire biologically relevant chemical space may be larger than the structural space occupied by natural products, their structural scaffolds represent the biologically relevant and prevalidated fractions of chemical structure space explored by nature so far. Consequently, compound collections designed to mimic the structures and properties of NP classes will have greater biological relevance than the libraries obtained on the basis of chemical feasibility alone,[1,19–21] and it is expected that NP guided compound library development[1,4] will persist

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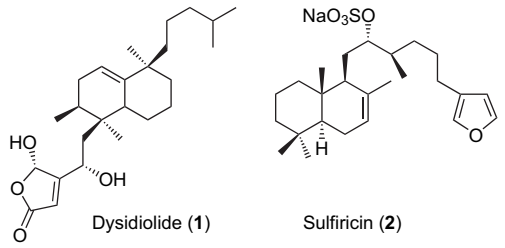


Figure 1. The natural products dysidiolide **1** and sulfiricin **2** served as structural guiding principles for the synthesis of a decalin scaffold-based compound library.

as a guiding principle for the identification of small molecules for chemical biology and medicinal chemistry research.[18]

Both dysidiolide **1** and sulfiricin **2** (Fig. 1) are natural products known to inhibit Cdc25A (IC_{50} values of 9.4 and 7.8 μM, respectively).[22,23] A systematic study with sulfiricin, varying the scaffold of the compound, revealed that with analogs bearing benzimidazole, benzothiazole or naphthalene moieties instead of the decalin moiety, phosphatase-inhibiting activity completely disappeared. Thus, we hypothesized that the decalin moiety may represent a 'privileged'[24] core structure, which conveys biological relevance to compound collections derived thereof. We thus headed for the synthesis of a compound collection on the solid support using the decalin moiety as 'leitmotif', which we describe herein in full experimental detail. We also demonstrate how the application of protein structure similarity clustering (PSSC) led to the identification of novel acetylcholinesterase inhibitors.

2. Results and discussion

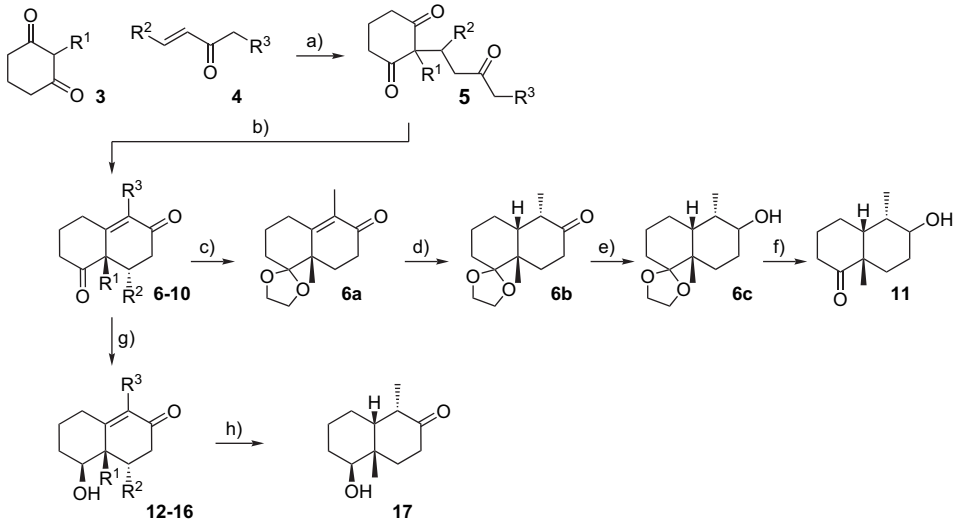
2.1. Synthesis of decalin building blocks in solution

Differently functionalized decalin derivatives were synthesized in solution as building blocks for further derivatization on the solid support as shown in Scheme 1.

Unsaturated decalinols **12**–**16** were synthesized employing the enantioselective Robinson annulation[25–27] as the key C–C bond forming step (Scheme 1). In addition, intermediate **6** was further derivatized. The carbonyl functionality was protected as 1,3-dioxolane (**6a**) followed by hydrogenation of the double bond leading to ketal **6b**. Treatment with sodium methoxide and reduction of the carbonyl group yielded alcohol **6c**. After cleavage of the 1,3-dioxolane, alcohol **11** was obtained. A further decalinol building block (**17**) was synthesized starting from alcohol **12**, which was achieved by catalytic hydrogenation and subsequent treatment with sodium methoxide.

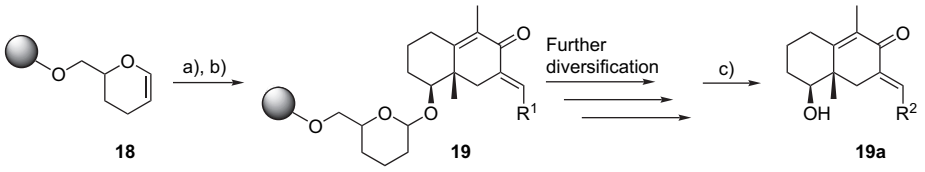
2.2. Immobilization on solid support and first diversification

As shown in Scheme 2 for decalinol **12** as a representative example, the decalin-derived alcohols were immobilized on Merrifield resin equipped with a dihydropyranyl linker **18**.[28]



Compound	6	7	8	9	10
	12	13	14	15	16
R^1 =	Me	Me	Me	Benzyl	Allyl
R^2 =	H	Me	H	H	Me
R^3 =	Me	H	H	Me	H

Scheme 1. Solution phase synthesis of decalin-based building blocks. (a) 1.0 equiv 1,3-dione, 1.5 equiv enone, water, rt, 7 days; (b) 0.66 equiv D-CSA, 0.97 equiv L-phenylalanine, DMF, rt to 70 °C in 6 days; (c) 2 ml/mM 2-ethyl-2-methyl-1,3-dioxolane/ethylene glycol (cat.), *p*-toluenesulfonic acid (cat.), rt, 2 days, 90%; (d) 5 mol % Pd/C, H_2 (12 bar), 65 °C, 3 days, then 1.3 equiv NaOMe in MeOH, reflux, 2 h, 82%; (e) 1.01 equiv DIBAH, Et_2O, −78 °C to rt, 1 h, then rt, 15 min, 89%; (f) 2.5 equiv PPTS, acetone/water (1:1), 70 °C, 2 h, 82%; (g) 0.3 equiv $NaBH_4$, EtOH, rt, yields over three steps: 43–49%; (h) 5 mol % Pd/C, H_2 (1 bar), rt, 1 day, then 1.3 equiv NaOMe in MeOH, reflux, 2 h, 62%.



Scheme 2. Attachment of the scaffolds to Merrifield resin and functionalization by means of aldol condensation. (a) 1.0 equiv resin, 0.5 equiv *p*-TsOH, 5.0 equiv alcohol (**12**–**17**), CH_2Cl_2, rt, 1 day, cleaning: 3×CH_2Cl_2, 3×CH_2Cl_2/MeOH=1:1 (v/v), 3×MeOH, 3×CH_2Cl_2/MeOH=1:1 (v/v), 3×CH_2Cl_2 (dry), drying in vacuum, loading level: 1.06 mmol g^{-1}; (b) 0.95 equiv LDA, 1.1 equiv aldehyde, 0 °C to rt, 2 h; (c) 5% TFA in CH_2Cl_2, rt, 10 min (three times).

The immobilized ketones were then subjected to aldol condensation reactions with different aldehydes leading to exocyclic *E*-configured olefins, e.g., **19**. After further derivatization (see next chapter), the synthesized products (**19a**) were cleaved from the solid support using 5% trifluoroacetic acid in DCM.

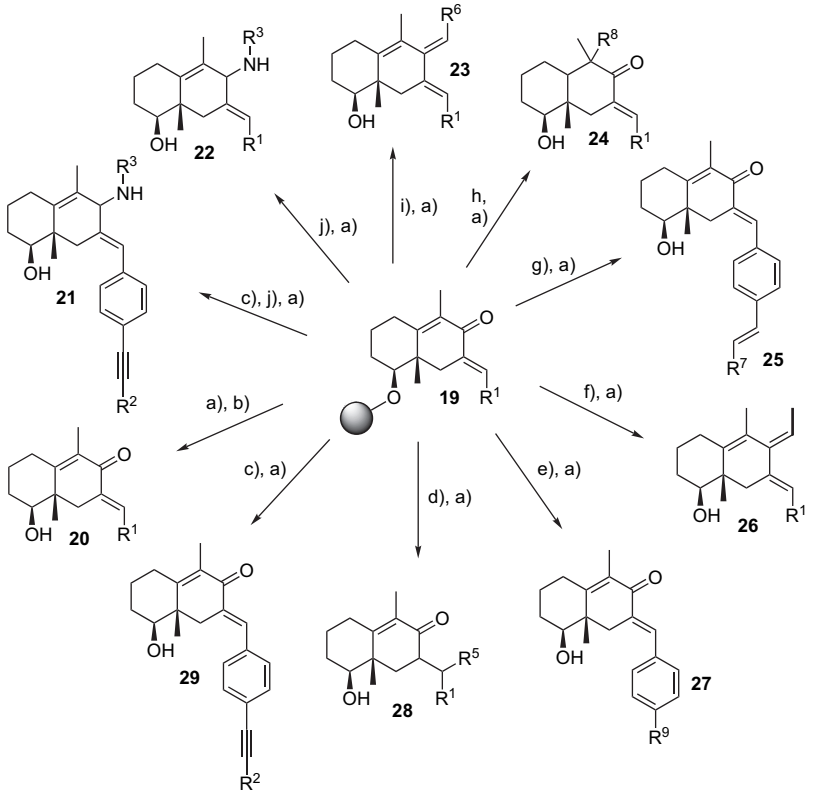
2.3. Diversification of aldol condensation products on solid support

The immobilized aldol condensation products were then subjected to a variety of different transformations to increase the diversity of the library. As shown in Scheme 3 for one representative scaffold (compound **19**), these reactions included Sonogashira, Suzuki, and Heck reactions, Cu-catalyzed conjugate addition and Grignard reactions, alkylation reactions in α-position to a ketone, Wittig reactions, and reductive animations.

After release from the solid support by treatment with trifluoroacetic acid the desired compounds were obtained in purities of 23–98% and purified to homogeneity by means of preparative HPLC. In total, 483 compounds were obtained in multimilligram amounts. Typical overall yields were 40–60% after the 3–5 step reaction sequences on the polymeric carrier using the tea bag method in combination with radio frequency coding to increase efficiency.

2.4. PSSC-guided discovery of acetylcholinesterase inhibitors

Based on the protein structure similarity clustering (PSSC) concept developed by us,[4] Cdc25A, the target protein of the two guiding compounds of the library described here, dysidiolide **1** and sulfiricin **2**, was found to exhibit significant structural similarity with the ligand-sensing core of



Scheme 3. (a) 10% TFA in CH_2Cl_2, rt, 10 min (three times); (b) 5 equiv TBAF, THF, rt, overnight; (c) 1.0 equiv CuI, 0.5 equiv $Pd(PPh_3)_4$, 20 equiv DIPEA, 15 equiv alkyne, DMF, 90 °C, overnight; (d) 4.5 equiv EtMgBr, 0.35 equiv CuBr, 14 equiv DMPU, THF, −20 °C to 0 °C, 3 h; (e) 2.0 equiv Na_2CO_3, 0.5 equiv $Pd(PPh_3)_4$, 2.0 equiv boronic acid, THF, 60 °C, overnight; (f) 5.5 equiv EtMgBr, THF, −20 °C to rt, 3 h; (g) 5.0 equiv alkene, 0.2 equiv $Pd(OAc)_2$, 2.0 equiv PPh_3, 1.5 equiv TMEDA, DMF, 70 °C overnight; (h) 5.0 equiv KH, THF, rt, 30 min then add 8 equiv primary bromide, rt to 50 °C, 3 h; (i) 10 equiv triphenylphosphonium bromide, 8 equiv BuLi, toluene, 100 °C, overnight; (j) 1.6 equiv $TiCl_4$, 3.8 equiv amine, toluene, 90 °C, overnight, then 1.5 equiv $NaBH_3CN$, THF, rt, overnight.

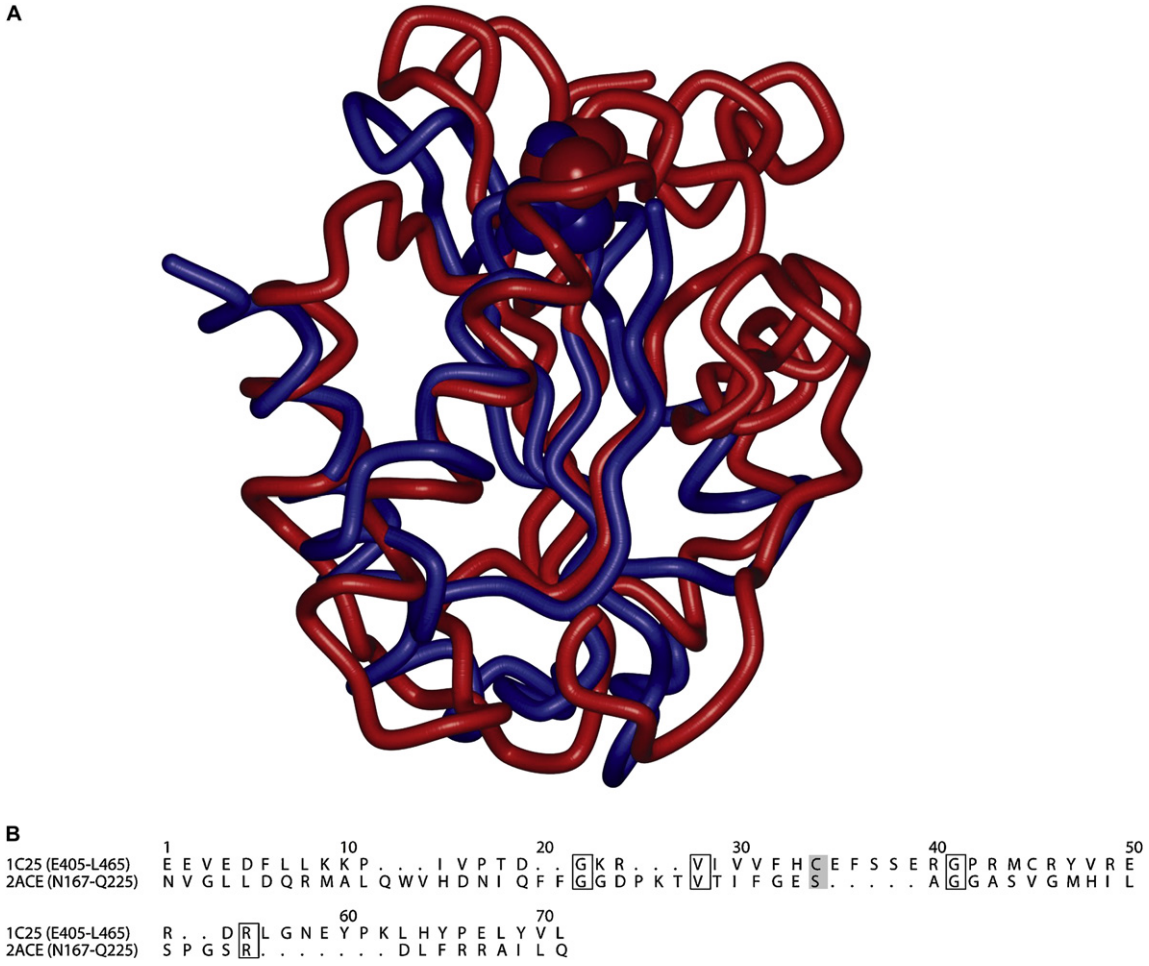


Figure 2. The two catalytic cores of Cdc25A and AChE exhibit significant structural similarity. (A) Superimposed catalytic cores of AChE (blue) and Cdc25A (red). The ligand-sensing cores of both enzymes were aligned with a rms deviation of 2.74 Å at an alignment length of 49 residues. The sequence identities amount to 8.2%. Also shown, in Corey–Pauling–Koltun (CPK) representation, are the catalytic residues, Ser-200 (AChE) and Cys-430 (Cdc25A) that are collocated in space. (B) Structure-based sequence alignment of the two superimposed catalytic cores. The catalytic key residues (Cys-430 and Ser-200) are correlated and gray-shaded.

acetylcholinesterase (Fig. 2). The catalytic residues, Ser-200 (for AChE) and Cys-430 (for Cdc25A), of both enzymes share the same location in space. This structural similarity also translates well into sequence similarity between the two catalytic cores where the key catalytic residues are correlated.

In the light of this structural similarity, a subset of this compound collection (in total 162 compounds) was subjected to biochemical investigation for possible inhibition of AChE screen. Compounds displaying IC_{50} values <20 μM were considered as hits. Three compounds were qualified as hits in the AChE with IC_{50} values ranging from 3.7 to 15.9 μM (see Table 1). Thus, the hit rate in the AChE inhibition screen amounts to ~2%, which is an acceptable value for an initial screen aimed exclusively at identifying hit classes. None of the identified compounds and the compound class in general have been described as AChE inhibitors before.

3. Conclusion

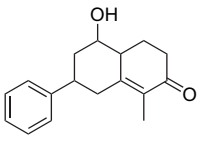
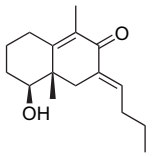
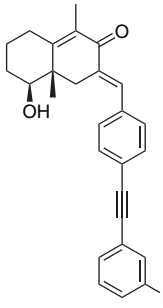
In conclusion, we have synthesized a natural product-inspired compound collection based on the decalin scaffold by means of a combination of both solution phase and solid phase methodologies. Employing the PSSC concept we have proven that the synthesized compounds are valuable and biologically relevant starting points in chemical space for the development of protein inhibitors for medicinal chemistry and chemical biology research.

4. Experimental section

4.1. General

Unless otherwise noted, chemicals were obtained from Aldrich, Acros or Fluka and were used without further purification. Regular Merrifield resin (1.7 mmol g^{-1}, 1% DVB, 100–200 mesh) was purchased from Novabiochem. All solvents were distilled by standard procedures. All reactions were performed under argon with freshly distilled and dried solvents. Analytical chromatography was performed using Merck silica gel 60 F_{254} aluminum sheets. Flash chromatography was performed using Merck silica gel 60. ^{1}H and ^{13}C NMR data were recorded on a Bruker DRX 500 or Bruker DRX 400 spectrometer at room

Table 1
Identified AChE inhibitors

Compound	Structure	IC_{50} [μm]
30		3.7±0.4
31		5.3±1.1
32		15.9±6.7

temperature. NMR spectra were calibrated to the solvent signals of $CDCl_3$ (7.26 ppm and 77.00 ppm) and the following abbreviations are used to indicate signal multiplicities: s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), sext (sextet), sept (septet), br (broad), ap (apparent). GC–MS (EI) analysis was performed on a Hewlett–Packard 5890 series II gas chromatograph connected to a Hewlett–Packard 5973 series mass spectrometer; column: H&W 19091σ-102 HP-5MS, capillary: 0.2 μm, 25 m×0.2 mm nominal. LC–MS was performed on a Hewlett–Packard 1100 series connected to a Finnigan LCQ ESI-spectrometer. High resolution mass spectra (HRMS) were measured on a Finnigan MAT 8200 spectrometer. The optical rotation was determined with a Perkin–Elmer polarimeter 241.

4.2. Synthesis of decalin-based building blocks

4.2.1. 2-Methyl-2-(3-oxopentyl)cyclohexane-1,3-dione, **5** (R^1=Me, R^2=H, R^3=Me)

Pent-1-en-3-one **4** (R^2=H, R^3=Me) (50.0 ml, 512.4 mmol) was added to a solution of 2-methylcyclohexane-1,3-dione **3** (R^1=Me) (37.5 g, 297.5 mmol) in water (75 ml). EtOH (2.5 ml) was added to improve the solubility. The resulting suspension was stirred for 7 days at room temperature (after 6 days the solid dissolved entirely). Toluene (300 ml) was added to the reaction mixture and the aqueous phase extracted with DCM (3×300 ml). The combined organic phases were washed with brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the product purified by flash chromatography to provide the product as a colorless oil (53.7 g, 86%). R_f value: 0.16 (cyclohexane/EtOAc 3:1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.02 (t, J=7.4 Hz, 3H), 1.24 (s, 3H), 1.98–2.10 (m, 2H), 2.2–2.58 (m, 10H). GC–MS, *m/z* (rel int. %): 210 (49) [M^+], 192 (18), 181 (21), 153 (15), 139 (82), 111 (100), 97 (45), 69 (43), 57 (64).

4.2.2. (*S*)-3,4,8,8a-Tetrahydro-5,8a-dimethylnaphthalene-1,6(2*H*,7*H*)-dione, **6**

L-Phenylalanine (37.0 g, 224 mmol) and D-camphorsulfonic acid (27.0 g, 166 mmol) were added to a solution of 2-methyl-2-(3-oxopentyl)cyclohexane-1,3-dione **5** (49.0 g, 233 mmol) in DMF (400 ml). The solution was stirred overnight at room temperature, and then the temperature was elevated by 10 °C every 24 h until 70 °C is reached. The reaction was cooled to 0 °C and satd $NaHCO_3$ (600 ml) was added. After stirring for 30 min, the aqueous phase was extracted with Et_2O (3×400 ml). The combined organic phases were washed with brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the product purified by flash chromatography to yield a white solid (32.0 g, 71%). Melting point: 46 °C. R_f value: 0.24 (cyclohexane/EtOAc 3:1, v/v). $[\alpha]_D^{20}$ +136 (*c* 1.00, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41 (s, 3H), 1.80 (s, 3H), 2.02–2.21 (m, 4H), 2.38–2.59 (m, 4H), 2.81–2.94 (m, 2H). GC–MS, *m/z* (rel int. %): 192 (34) [M^+], 177 (38), 149 (57), 136 (100), 121 (41), 107 (74), 93 (82), 79 (49), 55 (31), 39 (23).

4.2.3. (4a*S*,5*S*)-4,4a,5,6,7,8-Hexahydro-5-hydroxy-1,4a-dimethylnaphthalene-2(3*H*)-one, **12**

Sodium borohydride (1.68 g, 44.4 mmol) in EtOH (70 ml) was added over 3 h to a solution of (*S*)-3,4,8,8a-tetrahydro-5,8a-dimethylnaphthalene-1,6(2*H*,7*H*)-dione **6** (32.0 g, 166.7 mmol) in distilled EtOH (250 ml). After an additional 30 min of stirring, acetic acid was added until gas evolution has stopped. The solution was then coevaporated in vacuo with toluene (50 ml). The residue was redissolved in chloroform (500 ml), washed twice with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude mixture is purified by flash chromatography to yield the product as a colorless oil (25.2 g, 78%). R_f value: 0.12 (cyclohexane/EtOAc 3:1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.11 (s, 3H), 1.21–1.35 (m, 1H), 1.69 (s, 3H), 1.58–1.92 (m, 4H), 1.94–2.10 (m, 2H), 2.30–2.39 (m, 2H), 2.55–2.65 (m, 1H), 3.34 (dd, J=4.5, 7.2 Hz, 1H), 3.60 (br s, 1H). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ 11.5, 16.0, 23.1, 27.3, 30.1, 33.6, 42.2, 78.3, 129.9, 161.8, 199.6. GC–MS, *m/z* (rel int. %): 194 (81) [M^+], 179 (7), 151 (12), 138 (100), 123 (41), 110 (34), 91 (27), 77 (19), 67 (9), 55 (11) 41 (11).

4.2.4. (1*S*,4a*S*,5*S*,8a*R*)-Octahydro-5-hydroxy-1,4a-dimethylnaphthalene-2(1*H*)-one, **17**

A solution of (4a*S*,5*S*)-4,4a,5,6,7,8-hexahydro-5-hydroxy-1,4a-dimethylnaphthalen-2(3*H*)-one **12** (18.0 g, 92.8 mmol) in pyridine (100 ml) and Pd/C (0.4 g) was stirred vigorously overnight under a H_2 atmosphere. DCM (250 ml) was added and the suspension was filtered over Celite. The filtrate was then washed with satd NH_4Cl solution followed by brine and evaporated under reduced pressure. The crude mixture was redissolved in MeOH (150 ml) and heated for 2 h at reflux with a 5.4 M solution of sodium methoxide (23.0 ml, 124 mmol).

After cooling to room temperature, DCM and water (300 ml each) were added and the phases separated. The aqueous phase was extracted three times with DCM. The combined organic phases were washed with satd NH_4Cl, then brine and dried over $MgSO_4$. The solution was evaporated under reduced pressure and the crude product was purified by flash chromatography to afford the product as a colorless oil (11.2 g, 62%). R_f value: 0.15 (cyclohexane/EtOAc 3:1, v/v). $[\alpha]_D^{20}$ −98 (*c* 1.00, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.96 (d, J=5.9 Hz, 3H), 1.07 (s, 3H), 1.17–1.85 (m, 9H), 2.19–2.55 (m, 4H), 3.22 (dd, J=4.5, 7.0 Hz, 1H). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ 10.3, 11.7, 24.3, 25.1, 30.0, 34.0, 37.7, 39.2, 44.8, 50.8, 79.0, 213.0. GC–MS, *m/z* (rel int. %): 196 (26) [M^+], 181 (21), 163 (17), 145 (10), 135 (51), 111 (100), 107 (32), 93 (12), 79 (9), 67 (11), 55 (10), 41 (9). HRMS (FAB) calcd for $C_{12}H_{20}O_2$: 196.1463, found: 197.1558 [M+H]$^+$.

4.2.5. (8S,8aS)-3,4,8,8a-Tetrahydro-8,8a-dimethylnaphthalene-1,6(2H,7H)-dione, ***7***

trans-Pent-3-en-2-one **4** (R^2=Me, R^3=H) (7.4 g, 88.2 mmol) was added to a solution of 2-methylcyclohexane-1,3-dione **3** (R^1=Me) (10.0 g, 80.1 mmol) in water (75 ml). EtOH (1.5 ml) was added to improve the solubility. The resulting suspension was stirred for 7 days at room temperature (after 6 days the solid dissolved entirely). Toluene (200 ml) was added to the reaction mixture and the aqueous phase was extracted with DCM (3×200 ml). The combined organic phases were washed with brine and dried over $MgSO_4$. The crude was evaporated under reduced pressure. DMF (300 ml) was added along with L-phenylalanine (18.5 g, 112 mmol) and D-camphorsulfonic acid (13.5 g, 83.2 mmol). The solution was stirred overnight at room temperature, and then the temperature was elevated by 10 °C every 24 h until 70 °C was reached. The reaction was cooled to 0 °C and satd $NaHCO_3$ (300 ml) was added. After stirring for 30 min, the aqueous phase was extracted with Et_2O (3×250 ml). The combined organic phases were washed with brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the product purified by flash chromatography to yield a white solid (9.49 g, 61%). Melting point: 58 °C. R_f value: 0.22 (cyclohexane/EtOAc 3:1, v/v). $[\alpha]_D^{20}$ +72 (*c* 1.00, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.78 (d, J=6.8 Hz, 3H), 1.36 (s, 3H), 1.52–1.65 (m, 1H), 1.94–2.03 (m, 1H), 2.09–2.15 (m, 2H), 2.31–2.38 (m, 1H), 2.43–2.67 (m, 4H), 5.85 (s, 1H). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ 16.8, 20.8, 26.2, 32.0, 35.8, 39.0, 41.5, 54.6, 124.8, 164.2, 197.9, 211.8. GC–MS, *m/z* (rel int. %): 192 (15) [M^+], 174 (17), 150 (22), 135 (100), 122 (11), 107 (13), 94 (16), 79 (16), 55 (13), 39 (11). HRMS (FAB) calcd for $C_{12}H_{16}O_2$: 192.1150, found: 193.1221 [M+H]$^+$.

4.2.6. (4S,4aS,5S)-4,4a,5,6,7,8-Hexahydro-5-hydroxy-4,4a-dimethylnaphthalene-2(3H)-one, ***13***

Sodium borohydride (776 mg, 20.5 mmol) in EtOH (30 ml) was added over 3 h to a solution of (8*S*,8a*S*)-3,4,8,8a-tetrahydro-8,8a-dimethylnaphthalene-1,6(2*H*,7*H*)-dione **7** (15.9 g, 82.8 mmol) in distilled EtOH (150 ml). After an additional 30 min of stirring, acetic acid was added until gas evolution had stopped. The solution was then coevaporated in vacuo with toluene (20 ml). The residue was redissolved in chloroform (300 ml), washed twice with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude mixture was purified by flash chromatography to yield the product as a colorless oil (12.7 g, 79%). R_f value: 0.12 (cyclohexane/EtOAc 3:1, v/v). $[\alpha]_D^{20}$ +88 (*c* 1.00, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.79 (d, J=7.0 Hz, 3H), 1.26 (s, 3H), 1.20–1.40 (m, 1H), 1.40–1.59 (m, 1H), 1.98–2.04 (m, 2H), 2.11–2.18 (m, 1H), 2.43–2.67 (m, 6H), 6.07 (s, 1H). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ 17.6, 18.6, 26.0, 31.6, 36.3, 37.9, 42.3, 47.6, 82.8, 127.8, 177.1, 197.9.

4.2.7. (S)-3,4,8,8a-Tetrahydro-8a-methylnaphthalene-1,6(2H,7H)-dione, ***8***

But-3-en-2-one **4** (R^2=H, R^3=H) (10.5 g, 150 mmol) in MeOH (60 ml) was added to a solution of 2-methylcyclohexane-1,3-dione **3** (R^1=Me) (12.6 g, 100 mmol) and benzyltrimethylammonium hydroxide (Triton B) (2.5 M in MeOH, 4.4 ml, 11.0 mmol) in MeOH (60 ml). The suspension was stirred for 5 h at 60 °C and subsequently overnight at room temperature. The solvent was then evaporated under reduced pressure and the product purified by flash chromatography. The product was dissolved in DMF (300 ml) and combined with L-phenylalanine (23.3 g, 141 mmol) and D-camphorsulfonic acid (17.0 g, 105 mmol). The solution was stirred overnight at room temperature, and then the temperature was elevated by 10 °C every 24 h until 70 °C was reached. The reaction was cooled to 0 °C and satd $NaHCO_3$ (300 ml) was added. After stirring for 30 min, the aqueous phase was extracted with Et_2O (3×250 ml). The combined organic phases were washed with brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the product purified by flash chromatography to yield a colorless oil (12.7 g, 60%). R_f value: 0.27 (cyclohexane/EtOAc 3:1, v/v). $[\alpha]_D^{20}$ +103 (*c* 1.09, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 1.42 (s, 3H), 1.61–1.78 (m, 1H), 2.03–2.20 (m, 3H), 2.36–2.53 (m, 4H), 2.66–2.78 (m, 2H), 5.84 (s, 1H). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ 23.0, 23.4, 29.7, 31.8, 33.7, 37.7, 50.6, 125.7, 165.6, 198.0, 210.7. GC–MS, *m/z* (rel int. %): 178 (29) [M^+], 160 (49), 150 (16), 136 (41), 121 (100), 108 (76), 93 (83), 79 (91), 55 (34), 39 (27). HRMS (FAB) calcd for $C_{11}H_{14}O_2$: 178.0994, found: 179.1085 [M+H]$^+$.

4.2.8. (4aR,5R)-4,4a,5,6,7,8-Hexahydro-5-hydroxy-4a-methylnaphthalene-2(3H)-one, ***14***

Sodium borohydride (946 mg, 25.0 mmol) in EtOH (30 ml) was added over 3 h to a solution of (*S*)-3,4,8,8a-tetrahydro-8a-methylnaphthalene-1,6(2*H*,7*H*)-dione **8** (17.0 g, 95.5 mmol) in distilled EtOH (150 ml). After an additional 30 min of stirring, acetic acid was added until gas evolution had stopped. The solution was then coevaporated in vacuo with toluene (20 ml). The residue was redissolved in chloroform (300 ml), washed twice with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude mixture was purified by flash chromatography to yield the product as a colorless oil (13.9 g, 81%). R_f value: 0.12 (cyclohexane/EtOAc 3:1, v/v).

$[\alpha]_D^{20}$ +78 (*c* 1.00, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 1.17 (s, 3H), 1.22–1.53 (m, 6H), 1.80–2.78 (m, 5H), 3.31 (t, *J*=6.4 Hz, 1H), 5.71 (s, 1H). ^{13}C NMR (125.6 MHz, $CDCl_3$): 14.3, 15.5, 21.2, 27.4, 30.3, 32.3, 33.8, 41.7, 60.6, 125.4, 171.6, 200.2. GC–MS, *m/z* (rel int. %): 180 (44) [M^+], 162 (10), 152 (12), 137 (16), 124 (100), 109 (69), 91 (31), 79 (35), 67 (23), 55 (28), 43 (35). HRMS (FAB) calcd for $C_{11}H_{16}O_2$: 180.1150, found: 181.1217 $[M+H]^+$.

4.2.9. 2-Benzyl-2-(3-oxopentyl)cyclohexane-1,3-dione, 5

2-Benzylcyclohexane-1,3-dione **3** (R^1=benzyl) (20.2 g, 100 mmol) in MeOH (60 ml) was added to a solution of pent-1-en-3-one **4** (R^2=H, R^3=Me) (12.6 g, 150 mmol) and benzyltrimethylammonium hydroxide (Triton B) (2.5 M in MeOH, 4.4 ml, 11.0 mmol) in MeOH (60 ml). The suspension was stirred for 5 h at 60 °C and subsequently overnight at room temperature. The solvent was then evaporated under reduced pressure and toluene (400 ml) was added. The phases were separated and the aqueous phase extracted three times each with DCM (300 ml). The combined organic phases were washed with brine and then dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the product purified by flash chromatography to yield a colorless oil (22.9 g, 80%). R_f value: 0.21 (cyclohexane/EtOAc 3:1, v/v). ^1H NMR (400 MHz, $CDCl_3$): δ 0.99 (t, *J*=7.0 Hz, 3H), 1.93–2.03 (m, 2H), 2.11–2.64 (m, 10H), 3.66 (s, 2H), 7.17–7.37 (m, 5H). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ 7.9, 16.1, 21.4, 35.9, 36.9 (2C), 37.1, 38.5, 70.7, 127.2, 128.1 (2C), 128.7 (2C), 135.2, 200.0, 210.0, 210.4. GC–MS, *m/z* (rel int. %): 286 (32) [M^+], 258 (12), 202 (13), 187 (31), 173 (56), 158 (42), 115 (27), 91 (100), 57 (21), 42 (10). HRMS (FAB) calcd for $C_{18}H_{22}O_3$: 286.1569, found: 287.1655 $[M+H]^+$. The ^1H NMR data are in accordance with the literature.[29]

4.2.10. (R)-8a-Benzyl-3,4,8,8a-tetrahydro-5-methylnaphthalene-1,6(2H,7H)-dione, 9

L-Phenylalanine (11.1 g, 66.9 mmol) and D-camphorsulfonic acid (8.1 g, 49.8 mmol) were added to a solution of 2-benzyl-2-(3-oxopentyl)cyclohexane-1,3-dione **5** (20.0 g, 69.9 mmol) in DMF (400 ml). The solution was stirred overnight at room temperature, and then the temperature was elevated by 10 °C every 24 h until 70 °C was reached. The reaction was cooled to 0 °C and satd $NaHCO_3$ (500 ml) was added. After stirring for 30 min, the aqueous phase was extracted with Et_2O (3×300 ml). The combined organic phases were washed with brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the product purified by flash chromatography to yield a white solid (13.3 g, 71%). Melting point: 67 °C. R_f value: 0.12 (cyclohexane/EtOAc 3:1, v/v). $[\alpha]_D^{20}$ +78 (*c* 1.00, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 1.65–1.75 (m, 1H), 1.86 (s, 3H), 1.87–1.91 (m, 1H), 1.91–2.02 (m, 2H), 2.07–2.12 (m, 1H), 2.36–2.52 (m, 5H), 2.60–2.71 (m, 1H), 2.83–2.91 (m, 1H), 6.97–7.03 (m, 1H), 7.24–7.30 (m, 2H), 7.37–7.42 (m, 2H). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ 11.8, 21.5, 27.1, 29.3, 37.0, 38.9, 43.2, 56.1, 127.2, 128.7, 129.8 (4C), 141.2, 157.9, 197.7, 211.1. GC–MS, *m/z* (rel int. %): 268 (17) [M^+], 176 (9), 141 (8), 115 (11), 91 (100), 77 (12), 65 (12). HRMS (FAB) calcd for $C_{18}H_{20}O_2$: 268.1463, found: 269.1530 $[M+H]^+$.

4.2.11. (4aR,5S)-4a-Benzyl-4,4a,5,6,7,8-hexahydro-5-hydroxy-1-methylnaphthalene-2(3H)-one, 15

Sodium borohydride (473 mg, 12.5 mmol) in EtOH (20 ml) was added over 3 h to a solution of (*R*)-8a-benzyl-3,4,8,8a-tetrahydro-5-methylnaphthalene-1,6(2*H*,7*H*)-dione **9** (12.8 g, 47.8 mmol) in distilled EtOH (100 ml). After an additional 30 min of stirring, acetic acid was added until gas evolution had stopped. The solution was then coevaporated in vacuo with toluene (20 ml). The residue was redissolved in chloroform (300 ml), washed twice with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude mixture was purified by flash chromatography to yield the product as a colorless oil (13.9 g, 81%). R_f value: 0.10 (cyclohexane/EtOAc 3:1, v/v). $[\alpha]_D^{20}$ +72 (*c* 1.01, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 1.23–1.57 (m, 6H), 1.82 (s, 3H), 1.90–2.09 (m, 2H), 2.26–2.51 (m, 2H), 2.74–2.87 (m, 1H), 2.95–3.02 (m, 1H), 3.20–3.26 (m, 1H), 7.04–7.12 (m, 1H), 716–7.20 (m, 2H), 7.35–7.40 (m, 2H). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ 11.7, 23.8, 27.7, 30.9, 31.1, 33.7, 36.7, 46.8, 80.1, 126.9, 129.9 (4C), 132.7, 138.6, 157.6, 199.2. GC–MS, *m/z* (rel int. %): 270 (41) [M^+], 178 (35), 161 (100), 137 (16), 105 (22), 91 (76), 79 (25), 67 (13), 55 (18), 43 (25). HRMS (FAB) calcd for $C_{18}H_{22}O_2$: 270.1620, found: 271.1712 $[M+H]^+$.

4.2.12. (8S,8aS)-8a-Allyl-3,4,8,8a-tetrahydro-8-methylnaphthalene-1,6(2H,7H)-dione, 10

Pent-3-en-2-one **4** (R^2=Me, R^3=H) (8.2 g, 97.2 mmol) was added to a solution of 2-allylcyclohexane-1,3-dione **3** (R^1=allyl) (13.4 g, 88.1 mmol) in water (75 ml). EtOH (1.5 ml) was added to improve the solubility. The resulting suspension was stirred for 7 days at room temperature (after 6 days the solid dissolved entirely). Toluene (200 ml) was added to the reaction mixture and the aqueous phase extracted with DCM (3×200 ml). The combined organic phases were washed with brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. DMF (400 ml) was added along with L-phenylalanine (24.8 g, 150 mmol) and D-camphorsulfonic acid (18.1 g, 111 mmol). The solution was stirred overnight at room temperature, and then the temperature was elevated by 10 °C every 24 h until 70 °C was reached. The reaction was cooled to 0 °C and satd $NaHCO_3$ (400 ml) was added. After stirring 30 min, the aqueous phase was extracted with Et_2O (3×300 ml). The combined organic phases were washed with brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the product purified by flash chromatography to yield a white solid (10.4 g, 54%). Melting point: 52 °C. R_f value: 0.37 (cyclohexane/EtOAc 3:1, v/v). $[\alpha]_D^{20}$ +54 (*c* 1.00, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.84 (d, *J*=7.6 Hz, 3H), 1.54–1.70 (m, 1H), 1.91–2.74 (m, 11H), 5.01–5.13 (m, 2H), 5.58–5.71 (m, 1H). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ 17.0, 21.1, 33.2, 33.7, 40.3, 41.2, 43.8, 58.6, 119.3, 125.3, 132.7, 163.8, 197.9, 210.2. GC–MS, *m/z* (rel int. %): 218 (52) [M^+], 203 (31), 190 (10), 175 (24), 161 (22), 149 (100), 135 (26), 119

(14), 105 (41), 91 (49), 77 (36), 55 (27), 41 (23). HRMS (FAB) calcd for $C_{14}H_{18}O_2$: 218.1307, found: 219.1391 $[M+H]^+$.

4.2.13. (4S,4aS,5S)-4a-Allyl-4,4a,5,6,7,8-hexahydro-5-hydroxy-4-methylnaphthalene-2(3H)-one, ***16***

Sodium borohydride (48 mg, 12.8 mmol) in EtOH (20 ml) was added over 3 h to a 0 °C cooled solution of (*S*)-8a-benzyl-3,4,8,8a-tetrahydro-5-methylnaphthalene-1,6(2*H*,7*H*)-dione **10** (9.9 g, 45.5 mmol) in distilled EtOH (100 ml). After an additional 30 min of stirring, acetic acid was added until gas evolution had stopped. The solution was then coevaporated in vacuo with toluene (20 ml). The residue was redissolved in chloroform (300 ml), washed twice with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude mixture was purified by flash chromatography to yield the product as a colorless oil (13.9 g, 81%). R_f value: 0.14 (cyclohexane/EtOAc 3:1, v/v). $[\alpha]_D^{20}$ +66 (*c* 1.03, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (d, *J*=8.0 Hz, 3H), 1.28–2.74 (m, 11H), 3.21–3.26 (m, 1H), 5.04–5.17 (m, 2H), 5.58–5.70 (m, 2H). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ 16.9, 23.6, 28.5, 32.6, 35.4, 36.1, 44.7, 51.8, 79.5, 115.9, 123.8, 139.1, 170.0, 199.1. GC–MS, *m/z* (rel int. %): 220 (13) $[M^+]$, 202 (11), 179 (22), 161 (39), 137 (61), 119 (100), 105 (29), 91 (31), 77 (28), 65 (15), 55 (14), 41 (23). HRMS (FAB) calcd for $C_{14}H_{20}O_2$: 220.1463, found: 221.1551 $[M+H]^+$.

4.2.14. (S)-4,4a,5,6,7,8-Hexahydro-5-(1,3-dioxolan)-1,4a-dimethylnaphthalene-2(3H)-one, ***6a***

2-Ethyl-2-methyl-1,3-dioxolan (100 ml) and catalytic amounts of ethylene glycol and *p*-toluenesulfonic acid were added to a solution of (*S*)-3,4,8,8a-tetrahydro-5,8a-dimethylnaphthalene-1,6(2*H*,7*H*)-dione **6** (10.5 g, 53.8 mmol) in DCM (100 ml). The solution was stirred for 2 days at room temperature, then triethylamine (4 ml) and DCM (400 ml) were added. The organic phase was washed with brine and then dried over $MgSO_4$. The solution was evaporated under reduced pressure and the crude product purified by flash chromatography to yield **6a** (11.0 g, 90%) as a colorless oil. R_f value: 0.33 (cyclohexane/EtOAc 3:1, v/v). $[\alpha]_D^{20}$ +125 (*c* 1.39, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.34 (s, 3H), 1.57–1.72 (m, 5H), 1.76 (d, *J*=1.2 Hz, 3H), 2.02–2.25 (m, 2H), 2.32–2.50 (m, 2H), 2.67–2.76 (m, 1H), 3.87–4.00 (m, 4H). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ 11.5, 20.9, 21.4, 26.4, 26.5, 29.7, 33.7, 45.3, 65.0, 65.3, 112.8, 130.1, 156.0, 198.5. GC–MS, *m/z* (rel int. %): 236 (21) $[M^+]$, 180 (12), 149 (21), 121 (17), 107 (15), 99 (100), 91 (34), 77 (25), 55 (18), 41 (12). HRMS (FAB) calcd for $C_{14}H_{20}O_3$: 236.1412, found: 237.1477 $[M+H]^+$.

4.2.15. (1S,4aS)-Octahydro-5-(1,3-dioxolan)-1,4a-dimethylnaphthalene-2(1H)-one, ***6b***

Pd/C (0.2 g) was added to a solution of (*S*)-4,4a,5,6,7,8-hexahydro-5-(1,3-dioxolan)-1,4a-dimethylnaphthalene-2(3*H*)-one **6a** (2.79 g, 11.8 mmol) in pyridine (50 ml). The suspension was stirred vigorously for 3 days under 12 bar H_2 atmosphere at 65 °C. DCM (250 ml) was then added and the suspension filtered over Celite. The filtrate was washed with satd NH_4Cl, then with brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the crude product was dissolved in MeOH (20 ml). A 5.4 M solution of sodium methoxide in MeOH (2.3 ml, 12.4 mmol) was added and the solution was heated for 2 h at reflux. After the reaction had cooled to room temperature, DCM and water (200 ml each) were added. The phases were separated and the aqueous phase backwashed twice with DCM. The combined organic extracts were washed with satd NH_4Cl and brine, and dried over $MgSO_4$. The crude organic extracts were evaporated under reduced pressure and the product purified by flash chromatography to yield a colorless oil (2.31 g, 82%). R_f value: 0.31 (cyclohexane/EtOAc 3:1, v/v). $[\alpha]_D^{20}$ +83 (*c* 1.09, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.94 (d, *J*=6.6 Hz, 3H), 1.25 (s, 3H), 1.32–1.83 (m, 8H), 1.90–2.00 (m, 1H), 2.07–2.29 (m, 2H), 2.37–2.48 (m, 1H), 3.83–4.00 (m, 4H). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ 12.4, 17.5, 22.6, 22.8, 25.9, 29.8, 38.2, 43.1, 44.1, 49.8, 65.4 (2C), 112.6, 213.4. GC–MS, *m/z* (rel int. %): 238 (17) $[M^+]$, 209 (31), 138 (11), 112 (74), 99 (100), 86 (78), 67 (14), 55 (21), 41 (16). HRMS (FAB) calcd for $C_{14}H_{22}O_3$: 238.1569, found: 239.1659 $[M+H]^+$.

4.2.16. (1S,4aS)-Decahydro-5-(1,3-dioxolan)-1,4a-dimethylnaphthalene-2-ol, ***6c***

A 1 M solution of diisobutylaluminum hydride (14.5 ml, 14.5 mmol) was added at −78 °C to a solution of (1*S*,4a*S*)-octahydro-5-(1,3-dioxolan)-1,4a-dimethylnaphthalene-2(1*H*)-one **6b** (3.41 g, 14.3 mmol) in distilled Et_2O (20 ml). The solution was stirred at −78 °C for 1 h, then left to warm up to room temperature. The solution was stirred at room temperature for 15 min before being cooled to −78 °C for quenching with a saturated solution of NH_4Cl. After warming to room temperature, the phases were separated and the aqueous phase backwashed twice with Et_2O. The combined organic extracts were washed with brine and dried over $MgSO_4$. The crude extract was evaporated under reduced pressure and the product was purified by flash chromatography to yield a colorless oil (3.05 g, 89%). R_f value: 0.33 (cyclohexane/EtOAc 3:1, v/v). $[\alpha]_D^{20}$ +113 (*c* 1.39, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92–1.00 (m, 3H), 1.12 (s, 3H), 1.24–1.83 (m, 12H), 2.31 (br s, 1H), 3.16 (m, 1H), 3.83–4.00 (m, 4H). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ 12.7, 16.8, 20.9, 22.8, 23.6, 29.8, 32.0, 44.2, 44.3, 44.8, 65.4 (2C), 80.0, 112.8. GC–MS, *m/z* (rel int. %): 240 (11) $[M^+]$, 222 (13), 195 (10), 178 (12), 160 (16), 145 (15), 125 (26), 113 (22), 99 (100), 86 (71), 67 (10), 55 (18), 41 (12). HRMS (FAB) calcd for $C_{14}H_{24}O_3$: 240.1725, found: 241.1817 $[M+H]^+$.

4.2.17. (5S,8aS)-Octahydro-6-hydroxy-5,8a-dimethylnaphthalene-1(2H)-one, ***11***

2-Hydroxy-pyridinium *p*-toluenesulfonate (15.0 g, 56.1 mmol) was added to (1*S*,4a*S*)-decahydro-5-(1,3-dioxolan)-1,4a-dimethylnaphthalene-2-ol **6c** (5.30 g, 22.1 mmol) in a 1:1 solution of acetone and water (100 ml). The mixture was stirred for 2 h at 70 °C. After cooling to room temperature, satd $NaHCO_3$ (400 ml) was added and the phases

separated. The aqueous phase was backwashed three times with DCM (500 ml each). The combined organic phases were washed with brine and dried over $MgSO_4$. The crude mixture was purified by flash chromatography to yield a colorless oil (3.51 g, 82%). R_f value: 0.33 (cyclohexane/EtOAc 3:1, v/v). $[\alpha]_D^{20}$ +125 (*c* 1.39, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.97 (d, J=6.4 Hz, 3H), 1.27 (s, 3H), 1.38–2.05 (m, 10H), 2.12–2.31 (m, 2H), 3.10–3.17 (m, 1H). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ 11.0, 21.4, 23.1, 27.2, 28.7, 35.6, 39.0, 45.2, 45.4, 55.2, 79.5, 214.3. GC–MS, *m*/*z* (rel int. %): 196 (32) [M^+], 178 (81), 163 (31), 135 (100), 111 (75), 107 (71), 95 (62), 81 (66), 67 (69), 55 (73), 41 (78). HRMS (FAB) calcd for $C_{12}H_{20}O_2$: 196.1463, found: 196.1481 [$M]^+$.

4.3. General procedures for solid-supported synthesis

4.3.1. General procedure for resin loading (general procedure 1)

THP-functionalized resin (1.0 equiv) was preswelled for 15 min in DCM (15 ml/g). The alcohol (5 equiv) to be coupled was then added along with *p*-toluenesulfonic acid monohydrate (0.5 equiv) and the suspension shaken overnight. The resin was filtered and washed three times each with DCM, DCM/MeOH=1:1 (v/v), MeOH, DCM/MeOH=1:1 (v/v), and DCM, and then dried in vacuo.

Loading determination procedure: resin was preswelled for 15 min in DCM, then a 10% solution of trifluoroacetic acid in DCM (5 ml) was added and shaken for 10 min. The solution was then filtered and coevaporated with toluene (3 ml). The amount and quality of the cleaved alcohol determines the loading of the resin.

4.3.2. General procedure for the solid phase aldol reaction (general procedure 2)

n-BuLi in hexane (9 equiv) was added to a solution of diisopropyl amine (10 equiv) in THF (1 ml/mmol) at −78 °C. After the solution warmed to room temperature, it was then added to the resin (1 equiv), which was washed twice and preswelled in THF (15 ml). The mixture was shaken at room temperature for 30 min then cooled to 0 °C. The aldehyde (12 equiv) was then added and the mixture shaken for a further 30 min at 0 °C, then 2 h at room temperature. After filtration of the solvent, the resin was washed three times each with THF, DCM/MeOH=1:1 (v/v), MeOH, DCM/MeOH=1:1 (v/v), and DCM, and then vacuum dried.

4.3.3. General procedure for the cleavage (general procedure 3)

The dry resin (30 mg) was preswelled in DCM (2 ml) for 15 min. After filtration of the solvent, a 10% TFA solution in DCM (3 ml) was added and the mixture shaken for 10 min. The solution was filtered and the resin washed twice with the TFA solution. Toluene (3 ml) was added to the combined filtrates and the solution evaporated under reduced pressure. The crude product was dissolved in ca. 100 µl acetonitrile and purified by HPLC.

4.3.4. General procedure for the Sonogashira reaction on solid support (general procedure 4)

The resin (1 equiv) was combined under an argon atmosphere with CuI (1.0 equiv), $Pd(PPh_3)_4$ (0.5 equiv), DMF (15 ml/g), diisopropylethyl amine (20 equiv), and the terminal alkyne (15 equiv). The mixture was shaken overnight at 90 °C. The solution was filtered and the resin washed three times with THF/H_2O=2:1, DCM/MeOH=1:1 (v/v), MeOH, DCM/MeOH=1:1 (v/v), and DCM, and then dried under reduced pressure.

4.3.5. General procedure for the reductive amination on solid support (general procedure 5)

Dry resin (1 equiv) was combined with toluene (5 ml/g resin), titanium(IV) chloride (1.6 equiv) in toluene, and the amine (3.8 equiv). The mixture was shaken overnight at 60 °C. The solution was then filtered and the resin washed three times with toluene, DCM/MeOH=1:1 (v/v), MeOH, DCM/MeOH=1:1 (v/v), and DCM, and then dried in vacuo. The resin was then suspended in THF (3 ml/g resin) with sodium cyanoborohydride (1.5 equiv) and shaken overnight at room temperature. The solution was then filtered and resin washed three times with THF, DCM/MeOH=1:1 (v/v), MeOH, DCM/MeOH=1:1 (v/v), and DCM, and then dried in vacuo.

4.3.6. General procedure for the amination of aryl iodides on solid support (general procedure 6)

Amine (3.0 equiv) was added to the resin (1 equiv) and combined under an argon atmosphere with CsOAc (3.6 equiv), CuI (1.5 equiv), and DMF (8 ml/g resin). The reaction mixture was heated to 90 °C for 18 h (deep blue solution). The solution was then filtered, and the resin washed three times with toluene, DCM/MeOH=1:1 (v/v), MeOH, DCM/MeOH=1:1 (v/v), and DCM, and then dried in vacuo.

4.3.7. General procedure for the alkylation on solid support (general procedure 7)

The polymer-bound ketone (1.0 equiv) was washed twice and preswelled in dry THF (2 ml/g resin). Potassium hydride (5.0 equiv) in THF (3 ml/mmol) was then added and the reaction shaken for 30 min at room temperature. The primary bromide (8.0 equiv) was then added and the reaction shaken for 1 h at room temperature and 2 h at 50 °C. After draining the solvent, the resin was washed three times each with THF, DCM/THF=1:1 (v/v), MeOH, DCM/MeOH=1:1 (v/v), and DCM, and then vacuum dried.

4.3.8. General procedure for the Wittig reaction on solid support (general procedure 8)

The polymer-bound ketone (1.0 equiv) was suspended in toluene (2 ml/g resin) and shaken for 15 min. Triphenylphosphonium bromide (10 equiv) and *n*-BuLi (8 equiv) in toluene were then added and the reaction shaken for 15 min at room temperature then overnight at 100 °C. The solvent was then drained, and the resin washed three times with toluene, DCM/toluene=1:1 (v/v), DCM/MeOH=1:1 (v/v), MeOH, DCM/MeOH=1:1 (v/v), and DCM, and then dried in vacuo.

4.3.9. General procedure for the cleavage of the silyl protecting group on solid phase (general procedure 9)

The polymer-bound silyl protected alcohol was preswelled in THF (2 ml/g resin) and shaken for 15 min. Tetrabutylammonium fluoride trihydrate was then added and the mixture shaken overnight at room temperature. The solvent was drained and the resin washed three times with THF, DCM/THF=1:1 (v/v), DCM/MeOH=1:1 (v/v), MeOH, DCM/MeOH=1:1 (v/v), and DCM, and then dried in vacuo.

4.3.10. General procedure for the Heck reaction on solid support (general procedure 10)

The resin (1.0 equiv) was preswelled for 15 min in DMF (10 ml/g). Olefin (5.0 equiv), $Pd(OAc)_2$ (0.1 equiv), PPh_3 (2.0 equiv), and tetramethylethylene diamine (1.0 equiv) were added and the suspension heated to 70 °C and shaken overnight. The solution was filtered away and the resin washed three times with THF/H_2O=2:1, DCM/MeOH=1:1 (v/v), MeOH, DCM/MeOH=1:1 (v/v), and DCM, and then dried in vacuo.

4.3.11. General procedure for the Suzuki reaction on solid support (general procedure 11)

The resin (1.0 equiv) was preswelled in THF (10 ml/g). Na_2CO_3 (0.5 equiv), $Pd(PPh_3)_4$ (0.5 equiv), and boronic acid (2.0 equiv) were then added and the suspension heated to 60 °C and shaken overnight. The solution was filtered, the resin washed with THF/H_2O=2:1, DCM/MeOH=1:1 (v/v), MeOH, DCM/MeOH=1:1 (v/v), and DCM, and then dried in vacuo.

4.4. Inhibition of acetylcholinesterase (AChE)

4.4.1. Materials

Acetylcholinesterase (AChE, type III, electric eel, solution, 200 units, Sigma–Aldrich), acetylthiocholine iodide (ATC, Sigma–Aldrich), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB, Sigma–Aldrich), $Na_2HPO_4 \cdot 2H_2O$ (Mallinckrodt Baker), $NaH_2PO_4 \cdot H_2O$ (Mallinckrodt), DMSO (Serva Electrophoresis GmbH), Triton X-100 (Serva Electrophoresis GmbH), acetonitrile (HPLC-Grad, LGC Promochem GmbH), 96-well microtiter plates (Falcon, Becton Dickinson GmbH).

4.4.2. Assay

The inhibitory activity was measured according to the spectrophotometric method of Ellman et al.,[30,31] which was adapted to microtiter plate format. ATC was used as the substrate of the enzymatic reaction and DTNB for the measurement of AChE activity. Microtiter plates were washed with 100 mM sodium phosphate buffer pH 7.2 prior to each assay. In this procedure, 1 µl inhibitor solution was added to 20 µl of a diluted enzyme solution (0.25 Units/ml) in 100 mM sodium phosphate buffer pH 7.2 and preincubated for 15 min at room temperature. Inhibitors were dissolved in acetonitrile/DMSO=1:1 (v/v) to keep the final DMSO concentration in the assay low (DMSO itself is a competitive inhibitor of AChE![32]). After incubation, 79 µl of a solution containing 0.253 mM ATC and 0.380 mM DTNB in 100 mM sodium phosphate buffer pH 8.0 were added to give a final assay volume of 100 µl and end concentrations of 200 µM ATC[33] and 300 µM DTNB.[34] Similarly, control experiments in the presence of 0.01% Triton X-100 were performed.[35] The reaction rate was calculated from the absorption difference between 30 and 90 s reaction time and was determined to be 0.059±0.003 ΔA/min in the absence of inhibitor.[34] After screening the whole library at 20 µM inhibitor concentration, IC_{50} values of the most promising candidates (best percent inhibition) were determined. Different concentrations of each test compound were assayed and the percent inhibition due to the presence of test compound was calculated. IC_{50} values were determined from log concentration versus inhibition curves by using the IC_{50} fit function that is implemented in the program GRAFIT version 5.0.4 (Erithacus, Surrey, UK).

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Supplementary data

Full analytical characterization of the whole library and results of the AChE assay are provided. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.02.106.

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