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A palladium-catalyzed synthetic approach to new Huperzine A analogues modified at the pyridone ring

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Abstract—The synthesis of two new Huperzine A analogues is reported. Both products present an amino substituted benzo-fused system in place of the pyridone ring of the natural alkaloid. The synthetic strategy to the two analogues is based on three different key palladium-catalyzed steps, namely a carbonylation reaction, an epoxide isomerization and a bicycloannulation reaction.

1. Introduction

Alzheimer's disease (AD) is a chronic and progressive degenerative neurological disorder characterized by dementia and behavioral symptoms. Because the cognitive symptoms of AD result in part from an impaired cholinergic transmission in the central nervous system (CNS), the inhibition of acetylcholinesterase (AChE), the key brain enzyme that degrades acetylcholine (ACh), is a useful therapeutic approach for the palliative treatment of AD, at least at the onset of the disease.[1] Huperzine A (**1**, HA, Fig. 1),[2] a Lycopodium alkaloid, is a potent, reversible AChE inhibitor with excellent penetration into the CNS and a remarkable in vivo half-life.[3] In addition to its activity as an AChE inhibitor, recent findings suggest that HA decreases neuronal cell death caused by glutamate particularly in primary cultures derived from the hippocampus and cerebellum of the embryonic rat.[4] This dual pharmacological action suggests that HA may be a unique and important drug for the treatment of AD patients, since it may serve both to alleviate reduced ACh levels in the brain and to decrease neuronal cell death. In the past, several HA analogues were developed by our research group and potent AChE inhibitors were identified.[5] To add further insights into the structure–activity relationships of this class of AChE inhibitors we investigated the synthesis of new analogues of the natural product.

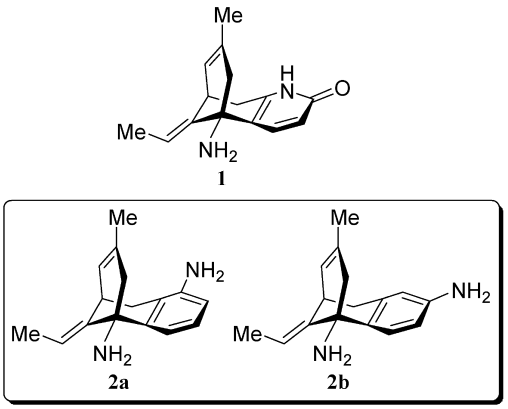

Figure 1. Structures of Huperzine A (**1**), 1-amino and 2-amino analogues (**2a** and **2b**).

The X-ray structure of HA as a complex with *Torpedo Californica* AChE shows that in the active site gorge the pyridone ring of the natural alkaloid forms one hydrogen bond with Tyr130 and another hydrogen bond with Glu199 via a water bridge (W619, Fig. 2).[6] Based on these data we decided to investigate the effect on affinity of replacing the pyridone ring of the natural alkaloid by an amino substituted benzo-fused system. The amino groups are placed respectively in position 1 or 2 (**2a** and **2b**, Fig. 1), and the synthesis of the new analogues is discussed.

2. Results and discussion

The synthesis of **2a**,**b** was performed as described in Scheme 1. The esters **5a**,**b** were obtained starting from the

Keywords: palladium; carbonylation; epoxide isomerization; bicycloannulation reaction.

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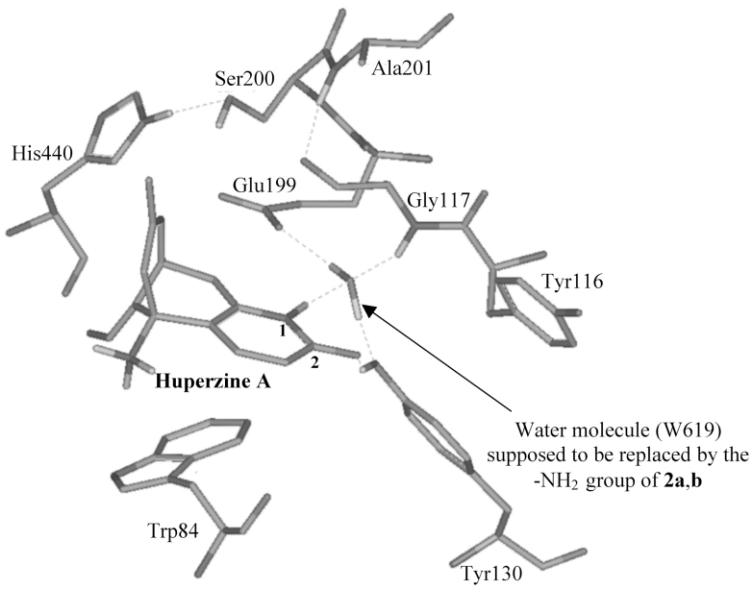
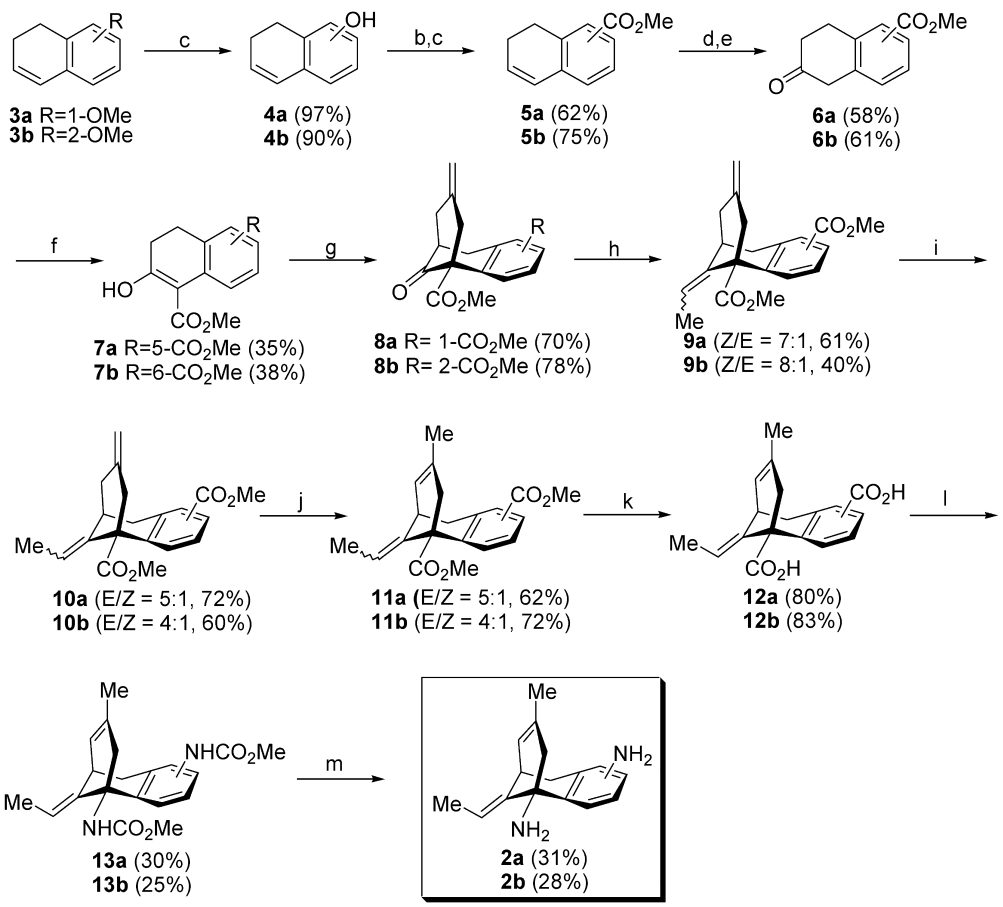


Figure 2. The hydrogen bond network formed between the pyridone ring of Huperzine A and its surrounding amino acid residues in the X-ray determined structure of AChE, and the supposed W619 replacement by the amino analogues **2a**,**b**. Hydrogens, except those involved in hydrogen bonds and those of the protonated amino group of the natural alkaloid, are omitted for clarity.

previously described compounds **3a** and **3b**.[7,8] Accordingly, the ethers **3a**,**b** were demethylated using sodium ethanethiolate in *N*,*N*-DMF at 120°C to give the phenols **4a**,**b** in good yield. After the hydroxyl groups of **4a**,**b** were triflated using triflic anhydride in pyridine at room temperature, the crude products were immediately converted into the corresponding carboxylic esters **5a**,**b** by a carbonylation reaction using palladium(II) acetate/dppf as a catalyst, in the



Scheme 1. *Reagents and conditions*: (a) NaSEt, DMF; (b) Tf_2O, pyr; (c) $Pd(OAc)_2$, dppf, CO, Et_3N, MeOH, DMSO; (d) *m*-CPBA, CH_2Cl_2; (e) $Pd(OAc)_2$, PPh_3, C_6H_6; (f) LDA, HMPA, $NCCO_2Me$; (g) $Pd(OAc)_2$, PPh_3, DBU, 2-methylene-1,3-propanediol diacetate, dioxane; (h) $EtPPh_3Br$, *n*-BuLi, THF; (i) AIBN, thiophenol, MeC_6H_5; (j) TfOH, dioxane; (k) NaOH 20%, THF/MeOH; (l) (i) $(PhO)_2P(O)N_3$, Et_3N, toluene, (ii) MeOH; (m) (i) Me_3SiI, $CHCl_3$; (ii) MeOH.

presence of methanol and carbon monoxide.[9] The synthetic strategy herein described to the intermediate **5b**,[10] as well as to compound **4a**,**b**[11,12] led to the desired molecules with much higher yields than those reported in literature, since, in our case, there was no need to separate complex isomeric mixtures. In the subsequent steps of the synthesis, the β-tetralones **6a**,**b** were obtained from olefins **5a**,**b** through an epoxidation/isomerization sequence: after double bond epoxidation with *m*-CPBA, the epoxides were regioselectively isomerized to carbonyl compounds **6a**,**b** using a catalytic amount of tetrakis(triphenylphosphine)palladium(0) prepared in situ.[13] To our knowledge this is the first example of a palladium-catalyzed isomerization reaction applied to cyclic aryl-substituted epoxides and proved more convenient than the classical isomerization protocols based on the use of Lewis acid catalysts. In fact this palladium-catalyzed isomerization reaction provided **6a**,**b** in almost 60% yield, higher than that obtained by using ZnI_2 or BF_3 etherate. The unstable β-tetralones **6a**,**b** were rapidly converted into lithium enolates and were subsequently exposed to methyl cyanoformate[14] to obtain the β-ketoesters **7a**,**b**. At this stage the remaining three-carbon bridge was introduced through a palladium-catalyzed bicycloannulation protocol.[15] Accordingly, the compounds **7a**,**b** were treated with 2-methylene-1,3-propanediol diacetate, DBU and a catalytic amount of palladium(II) acetate/triphenylphosphine to provide the annulation products **8a**,**b**.

The standard Wittig olefination of tricyclic compounds **8a**,**b** with $EtPPh_3Br$ and *n*-BuLi, gave the *Z*-olefins **9a**,**b** as the major isomers (for **9a** *Z*/*E*=8:1, for **9b** *Z*/*E*=5:1) which were isomerized with thiophenol and AIBN to the *E*-olefins **10a**,**b** (for **10a** *E*/*Z*=5:1, for **10b** *E*/*Z*=4:1).[16] Next, a second isomerization step was conducted in order to selectively obtain the products with an endocyclic double bond at C7,8 (**11a**,**b**). By treating **10a**,**b** with triflic acid in dioxane at 95°C in a sealed tube,[17] **11a**,**b** were obtained as the sole C7,8 unsaturated isomers. Saponification of both ester groups of **11a**,**b** using 20% sodium hydroxide in tetrahydrofuran/methanol 2:1 furnished the *E*-acids **12a**,**b** as single geometric isomers (the *Z*-isomers failed to undergo hydrolysis under these conditions, since more hindered). Curtius rearrangement of both carboxyl groups of **12a**,**b** with diphenyl azidophosphate and triethylamine, followed by methanolysis of the resulting isocyanates provided the dicarbamates **13a**,**b**.[18] Finally trimethylsilyl iodide promoted deprotection of **13a**,**b**, in refluxing chloroform, gave the desired compounds **2a**,**b**. Lower yields were obtained performing the deprotection step by using lithium *n*-propylmercaptide in HMPA at 100°C.

The biological activity of the HA analogues **2a**,**b** was evaluated as previously described,[19] and preliminary data indicated that both **2a** and **2b** ($Ki_{FBSAChE}$ of >1.1 and 0.8 μM respectively) showed an AChE inhibition potency lower than that of the natural alkaloid ($Ki_{FBSAChE}$ of 0.31 nM). This fact may possibly be due to the inability of the novel analogues to displace the tightly bound water molecule 619, establishing a direct interaction with the γ carboxylate group of Glu199 (**2a**), or (**2b**) to the impossibility to establish a possible hydrogen bond with the hydroxyl group of Tyr130, that could stabilize the AChE/inhibitor complex.

3. Conclusions

In conclusion, we presented herein a practical route to the HA analogues **2a**,**b** based on three different palladium-catalyzed functional group transformations. Exploiting the versatility of the synthetic approach described, this strategy could be useful to obtain further HA derivatives modified at the pyridone ring.

4. Experimental

4.1. General

Melting points were determined using an Electrothermal 8103 apparatus and are uncorrected. IR spectra were taken with Perkin–Elmer 398 and FT 1600 spectrophotometers. ^1H NMR spectra were recorded on a Bruker 200 MHz spectrometer with TMS as internal standard; the values of chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hertz (Hz). All reactions were carried out in an argon atmosphere. Mass spectra (EI) were recorded using a VG 70-250S spectrometer. Elemental analyses were performed on a Perkin–Elmer 240°C elemental analyzer and the results were within ±0.3% of the theoretical values, unless otherwise noted. Yields refer to purified products and are not optimized.

4.1.1. 7,8-Dihydro-1-hydroxynaphthalene (4a). A stirred solution of **3a** (8.8 g, 55.0 mmol) and sodium ethanethiolate (10.0 g, 120 mmol) in of anhydrous *N*,*N*-dimethylformamide (100 mL) was heated to 120°C overnight. After cooling to room temperature the mixture was poured into crushed ice (100 g) and the aqueous phase was extracted with ethyl acetate (200 mL). The extracts were dried (Na_2SO_4) and the solvent was removed in vacuo. The residue was chromatographed eluting with ethyl acetate/hexane 1:7 to obtain the title compound **4a** (7.8 g, 97%) as white solid. Spectroscopic data are consistent with those reported in the literature.[11]

4.1.2. 7,8-Dihydro-2-hydroxynaphthalene (4b).[12] The title compound was prepared from **3b** in a manner similar to that described for **4a**. Flash-chromatography of the crude product (ethyl acetate/hexane 1:7) afforded pure **4b** (730 mg, 90%) as a colorless viscous oil. ^1H NMR ($CDCl_3$) δ 6.89 (d, *J*=8.8 Hz, 1H), 6.64–6.59 (m, 2H), 6.40 (d, *J*=9.7 Hz, 1H), 5.93–5.84 (m, 1H), 4.84 (s, 1H), 2.74 (t, *J*=8.7 Hz, 2H), 2.32–2.22 (m, 2H). IR ($CHCl_3$) ν_{max} 3480–3060, 1620, 1510 cm^{-1}. Anal. calcd for $C_{10}H_{10}O$: C, 82.16; H, 6.89. Found: C, 82.12; H, 6.74.

4.1.3. 7,8-Dihydronaphthalene-1-carboxylic acid methyl ester (5a). Trifluoromethanesulfonic anhydride (5.9 mL, 35.4 mmol) was added dropwise to a solution of **4a** (4.4 g, 30.0 mmol) in anhydrous pyridine (40 mL) cooled to 0°C. The reaction mixture was stirred at room temperature overnight and then was poured into crushed ice (20 g). The aqueous phase was extracted with ethyl acetate (100 mL) and the extracts were washed with 4N hydrochloric acid (40 mL), dried (Na_2SO_4) and evaporated. The crude product was dissolved in anhydrous dimethyl sulfoxide (40 mL) and the solution was added to a warm mixture of palladium(II)

acetate (11.4 mg, 0.051 mmol), 1,1′-bis(diphenylphosphino)-ferrocene (56.5 mg, 0.102 mmol), triethylamine (0.75 mL, 5.2 mmol) and methanol (1.4 mL, 34 mmol) in anhydrous dimethylsulfoxide (11 mL), under a carbon monoxide atmosphere. The reaction mixture was stirred at 60°C for 8 h. After cooling to room temperature, the mixture was poured into brine (100 mL) and the aqueous phase was extracted with ethyl acetate (100 mL). The organic extracts were dried (Na_2SO_4) and the solvent was removed in vacuo. The residue was chromatographed eluting with ethyl acetate and hexane 1:9 to obtain the title compound **5a** (3.5 g, 62%) as a colorless viscous oil. ^{1}H NMR ($CDCl_3$) δ 7.65 (dd, J=2.1, 6.8 Hz, 1H), 7.18–7.11 (m, 2H), 6.45 (d, J=9.9 Hz, 1H), 6.13–6.04 (m, 1H), 3.88 (s, 3H), 3.16 (t, J=8.3 Hz, 2H), 2.34–2.23 (m, 2H). m/z (EI) 188 (M^+), 173, 157, 143, 129 (100%), 115, 102. IR ($CHCl_3$) ν_{max} 3021, 1709 cm^{-1}. Anal. calcd for $C_{12}H_{12}O_2$: C, 76.57; H, 6.43. Found: C, 76.30; H, 6.31.

4.1.4. 7,8-Dihydronaphthalene-2-carboxylic acid methyl ester (5b). The title compound was prepared from **4b** in a manner similar to that described for **5a**. Flash-chromatography of the crude product (ethyl acetate/hexane 1:7) afforded pure **5b** (470 mg, 75%) as a colorless solid. Spectroscopic data are consistent with those reported in the literature.[10]

4.1.5. 5,6,7,8-Tetrahydro-6-oxonaphthalene-1-carboxylic acid methyl ester (6a). To a solution of **5a** (3.5 g, 18.6 mmol) in anhydrous dichloromethane (85 mL), cooled in an ice-water bath, *m*-chloroperoxybenzoic acid (4.6 g, 20.5 mmol, 77%) was added in small portions, and the mixture was stirred at 0–5°C overnight. The organic phase was washed with sodium hydrogen carbonate solution (100 mL, sat. aq.) and dried (Na_2SO_4). After removing the solvent the crude epoxide was dissolved in anhydrous benzene (15 mL) and the solution was added to a mixture of anhydrous triphenylphosphine (0.72 g, 2.7 mmol) and palladium(II) acetate (0.20 g, 0.9 mmol) in degassed benzene (35 mL). The reaction mixture was heated under reflux for 5 h, and after cooling to room temperature, the solvent was evaporated. The residue was rapidly filtered over silica gel with ethyl acetate and hexane 1:1 and the crude product **6a** (yellow oil, 2.4 g, 58%) was immediately used in the next reaction. ^{1}H NMR ($CDCl_3$) δ 7.72–7.66 (m, 1H), 7.20–7.16 (m, 2H), 3.83 (s, 3H), 3.52 (s, 2H), 3.41 (t, J=6.6 Hz, 2H), 2.41 (t, J=6.6 Hz, 2H). IR ($CHCl_3$) ν_{max} 1726, 1708 cm^{-1}. Anal. calcd for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92. Found: C, 70.76; H, 5.89. Due to the instability of the title compound, **6a** was not further characterized.

4.1.6. 5,6,7,8-Tetrahydro-6-oxonaphthalene-2-carboxylic acid methyl ester (6b). The title compound **6b** was obtained as a pale yellow oil (150 mg, 61%) starting from **5b** in a manner similar to that described for **6a**. ^{1}H NMR ($CDCl_3$) δ 7.88–7.83 (m, 1H), 7.24–7.13 (m, 2H), 3.88 (s, 3H), 3.60 (s, 2H), 3.08 (t, J=6.7 Hz, 2H), 2.53 (t, J=6.7 Hz, 2H). IR ($CHCl_3$) ν_{max} 1718, 1707 cm^{-1}. Anal. calcd for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92. Found: C, 70.38; H, 5.96. Due to the instability of the title compound, **6b** was not further characterized.

4.1.7. 1,2,3,4-Tetrahydro-2-oxonaphthalene-1,5-dicarboxylic acid dimethyl ester (7a). To a solution of diisopropylamine (330 μL, 2.5 mmol) in anhydrous tetrahydrofuran (5.5 mL), cooled to-20°C, *n*-butyllithium (1.20 mL, 1.6 M solution in hexane) was added. After 10 min the solution was cooled to −78°C and a solution of ketone **6a** (400 mg, 2.1 mmol) in anhydrous tetrahydrofuran (2.0 mL) was added. The mixture was warmed to 0°C and stirred for 1 h, before cooling again to −78°C, hexamethylphosphoramide (340 μL, 2.1 mmol) and methyl cyanoformate (190 μL, 2.5 mmol) were added. After 10 min water (1.0 mL) was added and the solvent was evaporated. The aqueous phase was extracted with ethyl acetate (5 mL) and the extracts dried (Na_2SO_4) and evaporated. The residue was purified by flash-chromatography eluting with ethyl acetate/hexane 1:2 to yield the title compound **7a** (195 mg, 35%) as a pale yellow solid. Mp (hexane)=73–74°C. ^{1}H NMR ($CDCl_3$) δ 13.23 (s, 1H), 7.79 (d, J=7.9 Hz, 1H), 7.60 (d, J=7.9 Hz, 1H), 7.28–7.20 (m, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.18 (t, J=7.5 Hz, 2H), 2.47 (t, J=7.5 Hz, 2H). m/z (EI) 262 (M^+), 247, 203 (100%), 172, 156. IR ($CHCl_3$) ν_{max} 1709, 1645 cm^{-1}. Anal. calcd for $C_{14}H_{14}O_5$: C, 64.12; H, 5.38. Found: C, 64.39; H, 5.40. The title compound was used in the next step without further characterization.

4.1.8. 1,2,3,4-Tetrahydro-2-oxonaphthalene-1,6-dicarboxylic acid dimethyl ester (7b). The title compound was prepared from **6b** in a manner similar to that described for **7a**. Flash-chromatography of the crude product (ethyl acetate/hexane 1:2) afforded pure **7b** (170 mg, 38%) as a pale yellow solid. Mp (hexane)=77–78°C. ^{1}H NMR ($CDCl_3$) δ 13.49 (s, 1H), 7.81–7.70 (m, 3H), 3.91 (s, 3H), 3.88 (s, 3H), 2.84 (t, J=7.5 Hz, 2H), 2.54 (t, J=7.5 Hz, 2H). m/z (EI) 262 (M^+), 247, 203 (100%), 172, 156. IR ($CHCl_3$) ν_{max} 1710, 1641 cm^{-1}. Anal. calcd for $C_{14}H_{14}O_5$: C, 64.12; H, 5.38. Found: C, 63.94; H, 5.48. The title compound was used in the next step without further characterization.

4.1.9. (±)-7,8,9,10-Tetrahydro-7-methylene-11-oxo-5,9-methanobenzocyclooctene-1,5(6*H*)-dicarboxylic acid dimethyl ester (8a). Anhydrous triphenylphosphine (15.2 mg, 0.058 mmol) was added to a suspension of palladium(II) acetate (6.5 mg, 0.029 mmol) in anhydrous and degassed dioxane (3.0 mL). After 10 min a solution of 2-methylene-1,3-propanediol diacetate (90 μL, 0.57 mmol), **7a** (150 mg, 0.57 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (120 μL, 0.80 mmol) in dioxane (1.0 mL) was added. The mixture was heated under reflux and after 30 min DBU (60 μL, 0.40 mmol) was added. After 2 h the reaction mixture was cooled to room temperature and the solvent was evaporated. The residue was chromatographed over silica gel using a mixture of ethyl acetate and hexane 1:2 as eluent to obtain the title compound **8a** (126 mg, 70%) as a white solid. Mp=119–120°C (hexane). ^{1}H NMR ($CDCl_3$) δ 7.83 (d, J=7.9 Hz, 1H), 7.29–7.21 (m, 1H), 6.90 (d, J=7.8 Hz, 1H), 4.70 (d, J=1.5 Hz, 1H), 4.38 (d, J=1.5 Hz, 1H), 3.85 (s, 3H), 3.77 (s, 3H), 3.65–3.61 (m, 2H), 3.15 (d, J=14.0 Hz, 1H), 2.96–2.90 (m, 1H), 2.80–2.46 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 208.9, 171.6, 167.5, 139.6, 139.1, 136.0, 131.1, 130.2, 128.6, 127.1, 115.7, 64.1, 52.6, 52.0, 49.0, 45.4, 44.0, 37.2. m/z (EI) 314 (M^+), 282, 267, 255, 237, 223, 195 (100%), 165, 152, 127. IR ($CHCl_3$) ν_{max} 3016, 1765, 1750, 1714 cm^{-1}. Anal. calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.69; H, 5.92.

4.1.10. (±)-7,8,9,10-Tetrahydro-7-methylene-11-oxo-5,9-methanobenzocyclooctene-2,5(6*H*)-dicarboxylic acid dimethyl ester (8b). The title compound was prepared from **7b** in a manner similar to that described for **8a**. Flash-chromatography of the crude product (ethyl acetate/hexane 1:4) afforded pure **8b** (156 mg, 78%) as a white solid. Mp (hexane)=138–139°C. ^{1}H NMR ($CDCl_3$) δ 7.85–7.79 (m, 2H), 6.84 (d, J=8.1 Hz, 1H), 4.73 (s, 1H), 4.39 (s, 1H), 3.89 (s, 3H), 3.81 (s, 3H), 3.51 (dd, J=17.4, 6.5 Hz, 1H), 3.21–3.10 (m, 2H), 2.94–2.52 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 208.4, 171.2, 166.6, 142.4, 138.9, 134.2, 129.3, 128.9, 127.9, 127.0, 115.9, 63.8, 52.6, 52.1, 48.6, 45.5, 43.8, 37.6. *m/z* (EI) 314 (M^+), 282, 267, 255, 226 (100%), 207, 195, 167, 141, 128. IR ($CHCl_3$) ν_{max} 3018, 1768, 1752, 1719, 1684 cm^{-1}. Anal. calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.91; H, 5.84.

4.1.11. (11*Z*)-(±)-11-Ethylidene-7,8,9,10-tetrahydro-7-methylene-5,9-methanobenzocyclooctene-1,5(6*H*)-dicarboxylic acid dimethyl ester (9a). To a suspension of ethyltriphenylphosphonium bromide (304 mg, 0.82 mmol) in anhydrous tetrahydrofuran (3.0 mL), cooled to 0°C, *n*-butyllithium (430 μL, 1.6 M solution in hexane) was slowly added. The resulting orange solution was stirred at room temperature for 1 h, then was cooled to 0°C and a solution of **8a** (55.0 mg, 0.17 mmol) in tetrahydrofuran (2.0 mL) was added over 30 min. After stirring at room temperature for 2 h, the reaction was quenched with ammonium chloride solution (2.0 mL, sat. aq), the solvent was evaporated and the aqueous phase was extracted with ethyl acetate (3.0 mL). The extracts were dried (Na_2SO_4) and the solvent was removed in vacuo. The residue was chromatographed with ethyl acetate/hexane 1:4 to obtain **9a** (35 mg, 61%) as a colorless amorphous solid consisting of a 7:1 mixture of *Z*- and *E*-olefins. *Z*-olefin: ^{1}H NMR ($CDCl_3$) δ 7.71 (d, J=8.0 Hz, 1H), 7.19–7.12 (m, 1H), 6.89 (d, J=7.8 Hz, 1H), 5.58 (q, J=7.5 Hz, 1H), 4.48 (d, J=1.7 Hz, 1H), 4.19 (d, J=1.7 Hz, 1H), 3.84 (s, 3H), 3.73 (s, 3H), 3.44 (dd, J=6.5, 18.4 Hz, 1H), 3.12 (d, J=18.4 Hz, 1H), 2.87–2.77 (m, 2H), 2.57–2.23 (m, 3H), 1.54 (d, J=7.5 Hz, 3H). *m/z* (EI) 326 (M^+), 311, 295, 267 (100%), 251, 235, 219, 207, 179, 165, 152. IR ($CHCl_3$) ν_{max} 3009, 1760, 1711 cm^{-1}. Anal. calcd for $C_{20}H_{22}O_4$: C, 73.60; H, 6.79. Found: C, 73.71; H, 6.61.

4.1.12. (11*Z*)-(±)-11-Ethylidene-7,8,9,10-tetrahydro-7-methylene-5,9-methanobenzocyclooctene-2,5(6*H*)-dicarboxylic acid dimethyl ester (9b). The title compound was prepared from **8b** in a manner similar to that described for **9a**. Flash-chromatography of the crude product (ethyl acetate/hexane 1:2) afforded **9b** (1.2 g, 40%) as a colorless amorphous solid consisting of a 8:1 mixture of *Z*- and *E*-olefins. *Z*-olefin. ^{1}H NMR ($CDCl_3$) δ 7.76–7.71 (m, 2H), 6.79 (d, J=8.3 Hz, 1H), 5.60 (q, J=7.3 Hz, 1H), 4.48 (d, J=1.6 Hz, 1H), 4.19 (d, J=1.6 Hz, 1H), 3.87 (s, 3H), 3.75 (s, 3H), 3.26 (dd, J=17.1, 6.4 Hz, 1H), 2.88–2.73 (m, 3H), 2.56–2.27 (m, 3H), 1.56 (d, J=7.3 Hz, 3H). *m/z* (EI) 326 (M^+), 311, 295, 267 (100%), 251, 235, 219, 207, 179, 165, 152. IR ($CHCl_3$) ν_{max} 3010, 1886, 1763, 1716 cm^{-1}. Anal. calcd for $C_{20}H_{22}O_4$: C, 73.60; H, 6.79. Found: C, 73.78; H, 6.74.

4.1.13. (11*E*)-(±)-11-Ethylidene-7,8,9,10-tetrahydro-7-methylene-5,9-methanobenzocyclooctene-1,5(6*H*)-dicarboxylic acid dimethyl ester (10a). A mixture of **9a** (111 mg, 0.34 mmol, *Z*/*E*=7:1), thiophenol (52 μL, 0.51 mmol) and α,α′-azoisobutyronitrile (41.2 mg, 0.25 mmol) in anhydrous toluene (1.0 mL) was heated to 85°C for 21 h. After cooling to room temperature the solvent was removed and the residue was dissolved in dichloromethane (2.0 mL). The organic phase was washed with brine (3 mL), dried (Na_2SO_4) and evaporated. The residue was purified by flash-chromatography eluting with ethyl acetate/hexane 1:3 to obtain **10a** (80 mg, 72%) as a colorless amorphous solid consisting of a 5:1 mixture of *E*- and *Z*-olefins. A small quantity of *E*-olefin was obtained as a single isomer by crystallization from hexane. *E*-olefin. Mp (hexane)=106–107°C. ^{1}H NMR ($CDCl_3$) δ 7.74 (d, J=7.9 Hz, 1H), 7.20–7.12 (m, 1H), 6.90 (d, J=7.9 Hz, 1H), 5.19 (q, J=6.6 Hz, 1H), 4.54 (s, 1H), 4.22 (s, 1H), 3.85 (s, 3H), 3.78 (s, 3H), 3.43–3.33 (m, 2H), 3.18 (dd, J=3.5, 20.6 Hz, 1H), 2.88 (d, J=12.8 Hz, 1H), 2.47 (d, J=12.8 Hz, 1H), 2.43–2.33 (m, 2H), 1.72 (d, J=6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 175.4, 168.0, 142.5, 140.5, 138.6, 137.8, 130.9, 129.2, 128.7, 125.7, 115.5, 112.5, 58.3, 52.0, 51.8, 49.3, 42.9, 35.8, 31.7, 12.7. *m/z* (EI) 326 (M^+), 311, 295, 271 (100%), 251, 235, 207, 179, 165, 152. IR ($CHCl_3$) ν_{max} 3018, 1752, 1710, 750 cm^{-1}. Anal. calcd for $C_{20}H_{22}O_4$: C, 73.60; H, 6.79. Found: C, 73.31; H, 6.78.

4.1.14. (11*E*)-(±)-11-Ethylidene-7,8,9,10-tetrahydro-7-methylene-5,9-methanobenzocyclooctene-2,5(6*H*)-dicarboxylic acid dimethyl ester (10b). The title compound was prepared from **9b** in a manner similar to that described for **10a**. Flash-chromatography of the crude product (ethyl acetate/hexane 1:3) afforded **10b** (934 mg, 60%) as a colorless amorphous solid consisting of a 4:1 mixture of *E*- and *Z*-olefins. *E*-olefin. ^{1}H NMR ($CDCl_3$) δ 7.79–7.71 (m, 2H), 6.80 (d, J=8.0 Hz, 1H), 5.21 (q, J=6.6 Hz, 1H), 4.53 (d, J=1.5 Hz, 1H), 4.28 (d, J=1.5 Hz, 1H), 3.87 (s, 3H), 3.80 (s, 3H), 3.36–3.04 (m, 2H), 2.91–2.73 (m, 2H), 2.55–2.29 (m, 3H), 1.72 (d, J=6.6 Hz, 3H). *m/z* (EI) 326 (M^+), 267, 149, 207 (100%), 193, 167, 149, 103. IR ($CHCl_3$) ν_{max} 3020, 1758, 1716, 763 cm^{-1}. Anal. calcd for $C_{20}H_{22}O_4$: C, 73.60; H, 6.79. Found: C, 73.66; H, 6.76.

4.1.15. (11*E*)-(±)-11-Ethylidene-9,10-dihydro-7-methyl-5,9-methanobenzocyclooctene-1,5(6*H*)-dicarboxylic acid dimethyl ester (11a). A solution of **10a** (250 mg, 0.77 mmol, *E*/*Z*=5:1) and trifluoromethanesulfonic acid (10 μL, 0.12 mmol) in anhydrous dioxane (4.0 mL) was heated in a sealed tube to 95°C overnight. After cooling to room temperature the solvent was removed and the residue was dissolved in dichloromethane (5 mL). The organic phase was washed with solution of sodium hydrogen carbonate solution (3 mL, sat. aq.), dried (Na_2SO_4) and the solvent was removed. The crude product was purified by flash-chromatography with ethyl acetate/hexane 1:2 to yield **11a** (156 mg, 62%) as a colorless amorphous solid consisting of a 5:1 mixture of *E*-and *Z*-olefins. *E*-olefin. ^{1}H NMR ($CDCl_3$) δ 7.70 (d, J=8.4 Hz, 1H), 7.17–7.09 (m, 1H), 7.01 (d, J=7.8 Hz, 1H), 5.28–5.25 (m, 1H), 5.00 (q, J=6.7 Hz, 1H), 3.79 (s, 3H), 3.65 (s, 3H), 3.61–3.51 (m, 1H), 3.22–3.20 (m, 2H), 3.04 (d, J=16.9 Hz, 1H), 2.21 (d, J=16.9 Hz, 1H), 1.64 (d, J=6.7 Hz, 3H), 1.45 (s, 3H). *m/z* (EI) 326 (M^+), 311, 295, 271 (100%), 251, 235, 207, 192, 165, 152. IR ($CHCl_3$) ν_{max} 3020, 1753, 1710 cm^{-1}. Anal. calcd for $C_{20}H_{22}O_4$: C, 73.60; H, 6.79. Found: C, 73.77; H, 6.79.

4.1.16. (11*E*)-(±)-11-Ethylidene-9,10-dihydro-7-methyl-5,9-methanobenzocyclooctene-2,5(6*H*)-dicarboxylic acid dimethyl ester (11b). The title compound was prepared from **10b** in a manner similar to that described for **11a**. Flash-chromatography of the crude product (ethyl acetate/hexane 1:3) afforded **11b** (872 mg, 72%) as a colorless amorphous solid consisting of a 4:1 mixture of *E*- and *Z*-olefins. *E*-olefin. ^{1}H NMR ($CDCl_3$) δ 7.77–7.72 (m, 2H), 6.93 (d, J=8.0 Hz, 1H), 5.30–5.28 (m, 1H), 5.03 (q, J=6.7 Hz, 1H), 3.82 (s, 3H), 3.69 (s, 3H), 3.61–3.56 (m, 1H), 3.10–3.02 (m, 2H), 2.83 (d, J=16.5 Hz, 1H), 2.20 (d, J=16.5 Hz, 1H), 1.66 (d, J=6.7 Hz, 3H), 1.47 (s, 3H). *m/z* (EI) 326 (M^+), 311, 295, 271 (100%), 251, 235, 207, 192, 165, 152. IR ($CHCl_3$) ν_{max} 3015, 1756, 1713 cm^{-1}. Anal. calcd for $C_{20}H_{22}O_4$: C, 73.60; H, 6.79. Found: C, 73.39; H, 6.92.

4.1.17. (11*E*)-(±)-11-Ethylidene-9,10-dihydro-7-methyl-5,9-methanobenzocyclooctene-1,5(6*H*) dicarboxylic acid (12a). The olefin **11a** (136 mg, 0.42 mmol, *E*/*Z*=5:1) was dissolved in a 2:1 mixture of methanol and tetrahydrofuran (1.0 mL) and a sodium hydroxide solution (0.66 mL, 20% aq.) was added. The mixture was heated to 80°C for 48 h and then concentrated. The aqueous phase was extracted with ethyl acetate, acidified (pH 4) with 1N hydrochloric acid and finally extracted with ethyl acetate (10 mL). The organic extracts were dried (Na_2SO_4) and the solvent was removed. The crude product was purified by crystallization (dichloromethane/hexane) to obtain the diacid **12a** (100 mg, 80%) as a colorless crystalline solid. Mp (dichloromethane/hexane)=262–263°C. ^{1}H NMR (acetone-d_6) δ 7.78–7.74 (m, 1H), 7.25–7.22 (m, 2H), 5.35–5.21 (m, 2H), 3.64–3.61 (m, 1H), 3.33 (dd, J=2.3, 17.8 Hz, 1H), 3.10 (dd, J=5.1, 17.8 Hz, 1H), 2.98 (d, J=17.0 Hz, 1H), 2.24 (d, J=17.0 Hz, 1H), 1.68 (d, J=6.8 Hz, 3H), 1.47 (s, 3H). ^{13}C NMR (acetone-d_6) δ 175.8, 169.0, 143.4, 138.4, 137.7, 133.7, 132.4, 131.6, 130.0, 126.6, 125.4, 114.1, 56.8, 47.6, 36.7, 33.6, 22.8, 12.7. *m/z* (EI) 298 (M^+), 253 (100%). IR (nujol) ν_{max} 1702, 1698 cm^{-1}. Anal. calcd for $C_{18}H_{18}O_4$: C, 72.47; H, 6.08. Found: C, 72.66; H, 5.94.

4.1.18. (11*E*)-(±)-11-Ethylidene-9,10-dihydro-7-methyl-5,9-methanobenzocyclooctene-2,5(6*H*) dicarboxylic acid (12b). The title compound was prepared from **11b** in a manner similar to that described for **12a**. Crystallization of the crude product (dichloromethane/hexane) afforded pure **12b** (150 mg, 83%) as a colorless crystalline solid. Mp (dichloromethane/hexane)=265–266°C. ^{1}H NMR (acetone-d_6) δ 11.0 (bs, 1H), 7.79–7.71 (m, 2H), 7.14 (d, J=8.3 Hz, 1H), 5.36–5.24 (m, 2H) 3.68–3.66 (m, 1H), 3.03–2.86 (m, 3H), 2.21 (d, J=17.2 Hz, 1H), 1.68 (d, J=6.3 Hz, 3H), 1.47 (s, 3H). ^{13}C NMR (acetone-d_6) δ 174.9, 171.1, 148.4, 147.0, 138.4, 136.4, 133.8, 131.3, 128.6, 127.9, 125.0, 114.8, 56.6, 47.2, 37.5, 33.7, 22.8, 12.7. *m/z* (EI) 298 (M^+), 253 (100%). IR (nujol) ν_{max} 1700, 1694 cm^{-1}. Anal. calcd for $C_{18}H_{18}O_4$: C, 72.47; H, 6.08. Found: C, 72.74; H, 5.99.

4.1.19. (11*E*)-(±)-(11-Ethylidene-9,10-dihydro-5(6*H*)-methoxycarbonylamino-7-methyl-5,9-methanobenzocycloocten-1-yl)-carbamic acid methyl ester (13a). A mixture of **12a** (47.0 mg, 0.16 mmol), diphenyl azidophosphate (68.0 μL, 0.32 mmol) and triethylamine (44.0 μL, 0.32 mmol) in anhydrous toluene (2.0 mL) was heated to 85°C for 5 h. After cooling to room temperature, the solvent was evaporated, the residue was dissolved in anhydrous methanol (6.0 mL) and the solution was heated under reflux for 20 h. After cooling to room temperature, the solvent was evaporated and the residue was chromatographed with a mixture of ethyl acetate and chloroform 1:4 to obtain **13a** (17.0 mg, 30%) as a white solid. Mp (hexane)=92–93°C. ^{1}H NMR ($CDCl_3$) δ 7.61–7.59 (m, 1H), 7.22–7.14 (m, 2H), 6.25 (s, 1H), 5.41–5.34 (m, 2H), 5.02 (s, 1H), 3.75 (s, 3H), 3.70–3.68 (m, 1H), 3.61 (s, 3H), 2.83–2.62 (m, 3H), 2.31 (d, J=15.5 Hz, 1H), 1.69 (d, J=6.8 Hz, 3H), 1.49 (s, 3H). ^{13}C NMR ($CDCl_3$) 155.3, 154.9, 138.6, 137.9, 135.1, 134.8, 132.4, 130.2, 126.9, 120.9, 120.2, 111.8, 58.9, 52.3, 51.9, 49.2, 36.7, 33.5, 22.5, 12.5. *m/z* (EI) 356 (M^+), 324, 297, 281 (100%), 266, 249, 234, 221, 206, 167, 149. IR ($CHCl_3$) ν_{max} 3340, 1725, 1713 cm^{-1}. Anal. calcd for $C_{20}H_{24}N_2O_4$: C, 67.40; H, 6.79; N, 7.86. Found: C, 67.66; H, 6.91; N, 7.83.

4.1.20. (11*E*)-(±)-(11-Ethylidene-9,10-dihydro-5(6*H*)-methoxycarbonylamino-7-methyl-5,9-methanobenzocycloocten-2-yl)-carbamic acid methyl ester (13b). The title compound was prepared from **12b** in a manner similar to that described for **13a**. Flash-chromatography of the crude product (ethyl acetate/chloroform 1:4) afforded pure **13b** (28.0 mg, 25%) as a colorless solid. Mp (hexane)=80–81°C. ^{1}H NMR ($CDCl_3$) δ 7.30 (d, J=8.4 Hz, 1H), 7.13–7.05 (m, 2H), 6.54 (s, 1H), 5.38–5.28 (m, 2H), 5.01 (s, 1H), 3.73 (s, 3H), 3.60–3.59 (m, 4H), 3.03 (dd, J=4.0, 15.9 Hz, 1H), 2.77–2.69 (m, 2H), 2.23 (d, J=15.6 Hz, 1H), 1.68 (d, J=6.8 Hz, 3H), 1.48 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 154.8, 154.0, 138.0, 137.3, 136.3, 136.2, 132.4, 125.2, 124.9, 118.8, 117.2, 111.4, 59.4, 52.2, 51.8, 49.3, 37.1, 34.3, 22.6, 12.5. *m/z* (EI) 356 (M^+), 324, 297, 281 (100%), 266, 249, 234, 221, 206, 167, 149. IR ($CHCl_3$) ν_{max} 3343, 1725, 1718 cm^{-1}. Anal. calcd for $C_{20}H_{24}N_2O_4$: C, 67.40; H, 6.79; N, 7.86. Found: C, 67.14; H, 6.71; N, 7.75.

4.1.21. (11*E*)-(±)-11-Ethylidene-9,10-dihydro-7-methyl-5,9-methanobenzocycloocten-1,5(6*H*)-diamine (2a). Iodo trimethylsilane (8.8 μL, 0.062 mmol) was added to a stirred solution of **13a** (11.0 mg, 0.031 mmol) in anhydrous chloroform (2.0 mL). The reaction mixture was heated under reflux for 6 h, then the solvent was evaporated and the residue was dissolved in anhydrous methanol (3.0 mL). The mixture was heated under reflux for 18 h, cooled to room temperature and the solvent was evaporated. The residue was purified by flash-chromatography with ethyl acetate and methanol 5:1 to obtain the title compound **2a** (2.3 mg, 31%)as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.24 (d, J=5.4 Hz, 1H), 7.07–6.99 (m, 1H), 6.53 (d, J=7.9 Hz, 1H), 5.53 (q, J=6.7 Hz, 1H), 5.40–5.37 (m, 1H), 3.69–3.71 (m, 1H), 2.61–2.59 (m, 2H), 2.20–2.29 (m, 2H), 1.71 (d, J=6.7 Hz, 3H), 1.51 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 138.6, 138.0, 137.9, 132.0, 131.1, 128.2, 124.4, 118.6, 116.5, 109.1, 51.7, 49.7, 36.8, 33.2, 22.5, 12.6. *m/z* (EI) 240 (M^+), 167, 149, 137, 123, 111, 97, 69 (100%). IR ($CHCl_3$) ν_{max} 3425–3340 cm^{-1}. Anal. calcd for $C_{16}H_{20}N_2$: C, 79.96; H, 8.39; N, 11.66. Found: C, 79.72; H, 8.49; N, 11.78.

4.1.22. (11*E*)-(±)-11-Ethylidene-9,10-dihydro-7-methyl-5,9-methanobenzocycloocten-2,5(6*H*)-diamine (2b). The title compound was prepared from **13b** in a manner similar

to that described for **2a**. Flash-chromatography of the crude product (ethyl acetate/methanol 5:1) afforded pure **2b** (7.3 mg, 28%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.55 (d, J=8.3 Hz, 1H), 6.53 (dd, J=2.1, 8.1 Hz, 1H), 6.33 (d, J=2.1 Hz, 1H), 5.48 (q, J=6.7 Hz, 1H), 5.38–5.36 (m, 1H), 3.61–3.58 (m, 1H), 2.94 (dd, J=5.1, 16.1 Hz, 1H), 2.69 (d, J=16.1 Hz, 1H), 2.24–2.22 (m, 2H), 1.69 (d, J=6.7 Hz, 3H), 1.51 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 138.0, 138.3, 137.3, 136.3, 135.8, 130.2, 123.5, 123.0, 115.3, 109.9, 52.4, 49.3, 37.1, 33.2, 22.4, 12.5. m/z (EI) 240 (M^+, 100%), 225, 208, 185, 145, 115, 91. IR ($CHCl_3$) ν_{max} 3420–3340 cm^{-1}. Anal. calcd for $C_{16}H_{20}N_2$: C, 79.96; H, 8.39; N, 11.66. Found: C, 80.02; H, 8.44; N, 11.65.

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