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Critical modifications of the ISO-1 scaffold improve its potent inhibition of macrophage migration inhibitory factor (MIF) tautomerase activity

Kai Fan Cheng[a] and Yousef Al-Abed[a,b,*]

[a]*Department of Medicinal Chemistry, The Feinstein Institute for Medical Research, North Shore-LIJ Health System, 350 Community Drive, Manhasset, NY 11030, USA*
[b]*New York School of Medicine, New York, NY, USA*

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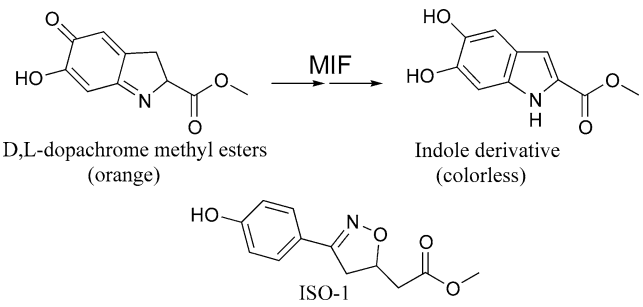
Abstract—Based on the scaffold of (*S*,*R*)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1), an inhibitor of the proinflammatory cytokine MIF, two critical modifications and chiral resolution have significantly improved the potency of the inhibition. Compound (***R***)-**17** is 20-fold more potent than ISO-1 and inhibits MIF tautomerase activity with an IC_{50} of 550 nM.

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine, critically involved in the pathogenesis of inflammatory disorders.[1,2] Our recent studies have clearly defined MIF as a critical factor in the pathophysiology of sepsis.[3] We showed that abolition of MIF activity during sepsis by antibodies or ISO-1 improves cardio-circulatory efficiency and prevents the lethality associated with sepsis.[3,4] We designed our specific inhibitor ISO-1 to fit into the hydrophobic active site of MIF, an interaction confirmed by the crystal structure of the MIF complex with ISO-1 (Scheme 1).[5] Administration of ISO-1 in a clinically relevant model of sepsis confers moderate protection (80% vs 40% control). These results identify ISO-1 as the first small molecule inhibitor of MIF proinflammatory activities with therapeutic implications and indicate the potential of the MIF active site as a novel target for therapeutic interventions in human sepsis. To improve the potency of ISO-1, herein, we explored the SAR of ISO-1.

Previously, we have identified critical functional groups within the ISO-1 scaffold as evidenced by the loss of its MIF inhibitory effect upon methylation of the *para*-hydroxyl functional group, oxidation of 4,5-dihydroisoxazole to isoxazole or reduction of methyl ester to alcohol.[5] Herein, we discovered that mono-fluorination onto the ortho position of the phenolic group of ISO-1 improved the inhibition of MIF activity up to 41% (Scheme 5). Hence, we investigated the alkyl tail of ISO-1 with various ester and amide analogues. The new synthetic route provides the precursor (ISO-1-acid) in large scale (Scheme 2).[9] Esterification and amide formation between the ISO-1-acid and alcohol (or amine) were accomplished using a standard DCC coupling protocol (DCC, DMAP or HOBt) (Scheme 3).[9]

As summarized in Schemes 3 and 4, we found that the ester analogues are more potent inhibitors than the amide counterpart (e.g., compounds **14** (IC_{50} = 2.5 μM) vs **6** (IC_{50} = 24 μM) and **3** (IC_{50} = 8.5 μM) vs



Scheme 1. MIF tautomerizes dopachrome methyl esters and the structure of MIF inhibitor dubbed 'ISO-1.'

Keywords: MIF; ISO-1; Tautomerase activity; Enzyme.
* Corresponding author. Tel.: +1 516 562 3406; fax: +1 516 562 1022; e-mail: yalabed@nshs.edu

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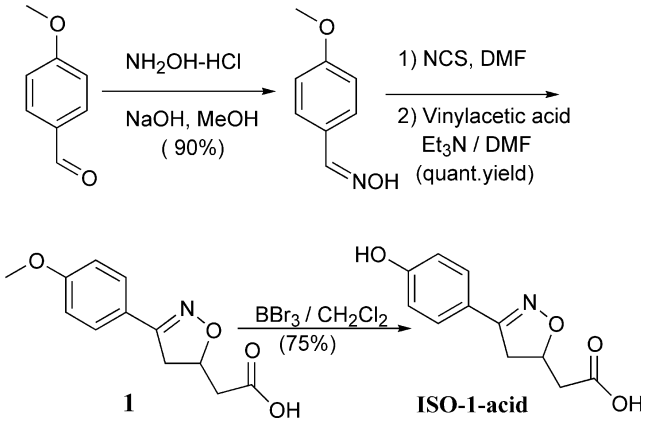
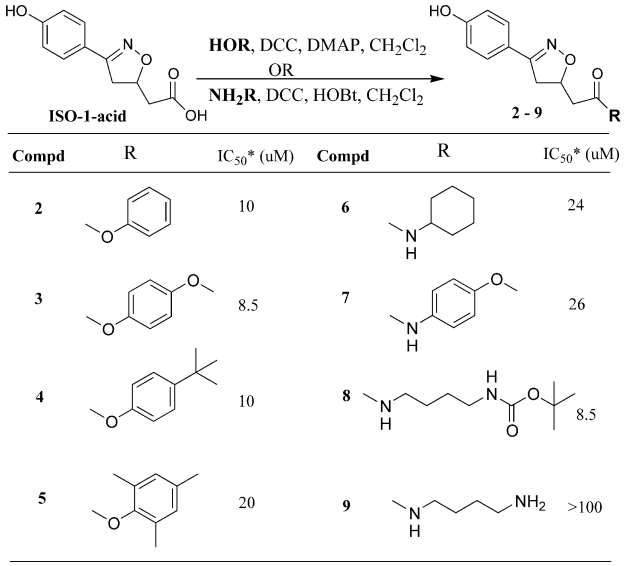


Scheme 2. Synthesis of the ISO-1-acid.



Compd	R	IC_{50}* (uM)	Compd	R	IC_{50}* (uM)
2		10	6		24
3		8.5	7		26
4		10	8		8.5
5		20	9		>100

*From ref. [8]

Scheme 3. Synthesis of compounds **2**–**9**.

7(IC_{50} = 26 μM)). We further investigated the influence of the bulkiness of the ester group on the potency of inhibiting MIF activity. The esterification process of ISO-1-acid was accomplished with TMSCl using the following alcohols: ethanol, **10**; 1-propanol, **11**; 2-propanol, **12**; 1-butanol, **13**; cyclohexanol, **14**; cyclohexylmethanol, **15**; and neopentylalcohol, **16** (Scheme 4).[9] As shown in Scheme 4, the most bulky alcohol (compound **16**, neopentyl ester analogue) shows a superior inhibition activity (IC_{50} = 1.5 μM) which is ten times more potent than ISO-1.

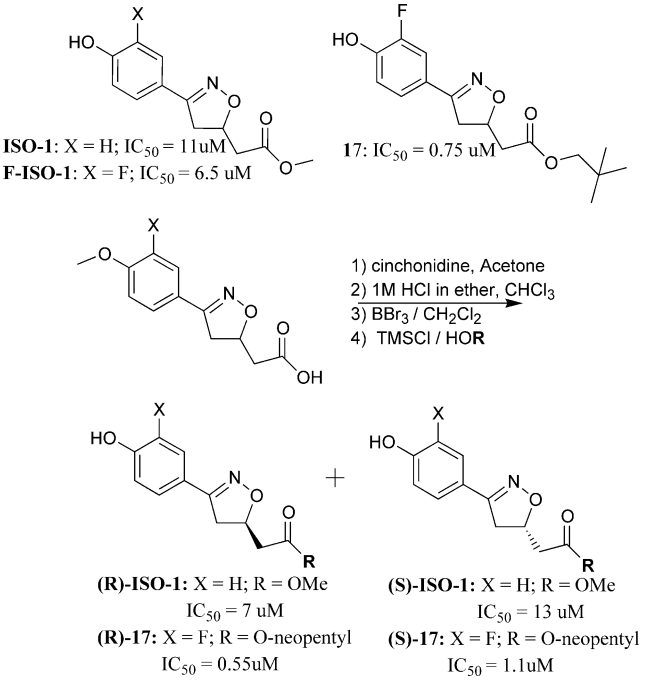
The crystal structure analysis of MIF/ISO-1 complex predicted that the (*R*)-isomer of ISO-1 binds in the active site, suggesting that the (*R*)-isomer would bind with higher affinity to the MIF active site. This is evident from the hydrogen bond formation between the side-chain nitrogen of Lys-32 and both the carbonyl oxygen of the carboxymethyl group and the oxygen of the isoxazoline ring.[6] Previously, we have shown the importance of the absolute configuration of the amino acid

Compd	R	IC_{50}* (uM)	Compd	R	IC_{50}* (uM)
ISO-1		11	13		3
10		12	14		2.5
11		4	15		5
12		6	16		1.5

*From ref. [8]

Scheme 4. Synthesis of compounds **10**–**16**.

Schiff bases in the inhibitory effect of MIF.[5] Hence, we needed to resolve the optically active ISO-1 and determine the inhibitory activity for each isomer. Chiral resolution was accomplished as previously described through fractional crystallization of the diastereomeric cinchonidine salts of methylated ISO-1-acid (Scheme 5).[7,9] The (*S*)-configuration salts were found to be less soluble and were crystallized out from the chiral mixture. The (*S*)- and (*R*)-configuration salts were then acidified by 1 N HCl, demethylated with BBr_3, and esterified with TMSCl in methanol to yield (*S*)-ISO-1 (90% ee) and (*R*)-ISO-1 (90% ee). Both isomers were tested for activity in the MIF dopachrome tautomerase



Scheme 5. Synthesis of stereoisomers of **ISO-1** and compound **17**.

assay. (*R*)-ISO-1 inhibited MIF tautomerase activity with an IC_{50} of 7 μM, but (*S*)-ISO-1 was 50% less active with an IC_{50} of 13 μM (Scheme 5).

In order to obtain the most potent, specific, small molecule, MIF inhibitor, three critical steps need to be integrated into the ISO-1 scaffold: (1) mono-fluorination onto the ortho position of the phenolic group of ISO-1; (2) a neopentyl ester functional group replacing the methyl ester of ISO-1; (3) chiral resolution to obtain an (*R*)-isomer. Compound **17** emerged after all as a potent inhibitor of MIF activity with an IC_{50} of 750 nM. After the classical chiral resolution, (***R***)-**17** inhibited MIF tautomerase activity with an IC_{50} of 550 nM, while the (***S***)-**17** was 50% less active with an IC_{50} of 1.1 μM (Scheme 5). Compound (***R***)-**17** is 20 times more potent than the parent compound ISO-1.

After two critical modifications on ISO-1 scaffold, we have improved the inhibition of MIF tautomerase activity to a nanomolar concentration (550 nM), which is 20 times potent than ISO-1. The in vivo and in vitro studies of the new inhibitor are currently under investigation.

References and notes

1. Calandra, T.; Roger, T. *Nat. Rev. Immunol.* **2003**, *3*, 791.
2. Riedemann, N. C.; Guo, R. F.; Ward, P. A. *Nat. Med.* **2003**, *9*, 517.
3. Al-Abed, Y.; Dabideen, D.; Aljabari, B.; Valster, A.; Messmer, D.; Ochani, M.; Tanovic, M.; Ochani, K.; Bacher, M.; Nicoletti, F.; Metz, C.; Pavlov, V. A.; Miller, E. J.; Tracey, K. J. *J. Biol. Chem.* **2005**, *280*, 36541.
4. Lin, X.; Sakuragi, S.; Metz, C.; Ojamaa, K.; Skopicki, H.; Wang, P.; Al-Abed, Y.; Miller, E. J. *Shock* **2005**, *24*, 556.
5. Lubetsky, J. B.; Dios, A.; Han, J.; Aljabari, B.; Ruzsicska, B.; Mitchell, R.; Lolis, E.; Al-Abed, Y. *J. Biol. Chem.* **2002**, *277*, 24976.
6. Dios, A.; Mitchell, R. A.; Aljabari, B.; Lubetsky, J.; O'Connor, K.; Liao, H.; Senter, P. D.; Manogue, K. R.; Lolis, E.; Metz, C.; Bucala, R.; Callaway, D. J. E.; Al-Abed, Y. *J. Med. Chem.* **2002**, *45*, 2410.
7. Wityak, J.; Sielecki, T. M.; Pinto, D. J.; Emmett, G.; Sze, J. Y.; Liu, J.; Tobin, A. E.; Wang, S.; Jiang, B.; Ma, P.; Mousa, S. A.; Wexler, R. U.; Olson, R. E. *J. Med. Chem.* **1997**, *40*, 50.
8. MIF tautomerase activity was measured by UV–vis recording spectrophotometry (SHIMADZU, UV1600U). A fresh stock solution of L-dopachrome methyl ester was prepared at 2.4 mM through oxidation of L-3,4-dihydroxyphenylalanine methyl ester with sodiumperiodate. 1 μL of MIF solution (800–900 ng/mL) and 1 μL of a DMSO solution with various concentrations of the enzymatic inhibitor were added into a plastic cuvette (10 mm, 1.5 mL) containing 0.7 mL assay buffer (50 mM potassium phosphate, pH 7.2). Then L-dopachrome methyl ester solution (0.3 mL) was added to the assay buffer mixture. Activity was determined at room temperature and the spectrometric measurements were made at $\lambda = 475$ nm for 20 seconds by monitoring the rate of decolorization of L-dopachrome methyl ester in comparison to a standard solution.
9. All solvents were HPLC-grade from Fisher Scientific. Silica gel (Selecto Scientific, 32–63 μm average particle size) was used for flash column chromatography (FCC). Aluminum-backed Silica Gel 60 with a 254 nm fluorescent indicator TLC plates were used. Spots on TLC plates were visualized under a short wavelength UV lamp or stained with I_2 vapor. NMR spectra were performed on a Jeol Eclipse 270 spectrometer at 270 MHz for ^1H NMR spectra and 67.5 MHz for the ^{13}C NMR spectra. Coupling constants are reported in Hertz (Hz), and chemical shifts are reported in parts per million (ppm) relative to deuterated solvent peak. The coupling constants (*J*) are measured in Hertz (Hz) and assigned as s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). Low-resolution mass spectra were acquired using Thermofinnigan LCQ DecaXPplus quadrupole ion trap MS with negative-ion or positive-ion mode. *Preparation of 4-methoxybenzaldehyde oxime*. To a solution of 4-methoxybenzaldehyde (5.0 g, 36.8 mmol) in methanol (300 mL) were added hydroxylamine hydrochloride (7.6 g, 110.4 mmol) and 2 N NaOH (37 mL, 73.6 mmol). The mixture was stirred at room temperature for 6 h. The mixture was neutralized to pH 4 by using 1 N HCl. Excess methanol was removed in vacuo to precipitate out the oxime. The precipitations were filtered and washed with water. The product was dried under vacuum and yielded a white solid (4.9 g, 88%): ^1H NMR (270 MHz, acetone-d_6) δ 8.07 (s, 1H), 7.55 (d, $J = 8.2$ Hz, 2H), 6.95 (d, $J = 8.2$ Hz, 2H), 3.82 (s, 3H). *Preparation of ISO-1-acid*. To a solution of 4-methoxybenzaldoxime (4 g, 26 mmol) in anhydrous DMF (500 mL) was added NCS (5.2 g, 39 mmol). The reaction mixture was stirred for 5 h at rt affording the chloro oxime. To this solution, vinylacetic acid (6.6 mL, 78 mmol) was added, followed by the dropwise addition of triethylamine (5.5 mL, 39 mmol) in DMF (50 mL). The reaction mixture was stirred under N_2 at rt for 48 h. The solvent was removed in vacuo and the residue was taken up in EtOAc. The EtOAc solution was washed with 0.5 N HCl, water, and brine, and dried with anhydrous $MgSO_4$. The final solution was concentrated in vacuo and dried under vacuum pump to afford **1** in quantitative yield. A solution of **1** (40–50 mM in dry dichloromethane) was treated with an excess (8–10 equiv) of boron tribromide (1 M solution in dichloromethane, Aldrich cat No.: 211222) at 0 °C under N_2. The reaction mixture was allowed to reach room temperature over 5–6 h and then quenched with aqueous saturated $NaHCO_3$ (caution: BBr_3 reacts violently with water). The mixture was stirred for 1/2 h and then diluted with water and CH_2Cl_2. The organic layer was separated from aqueous and discarded. The aqueous portion was neutralized with 1N HCl to pH 4 and extracted with EtOAc. The combined EtOAc solution was washed with brine and dried with anhydrous $MgSO_4$ to afford ISO-1-acid as a pale yellow powder in good yield (75%). ^1H NMR (300 MHz, acetone-d_6) δ 10.65 (br, 1H), 8.75 (s, 1H), 7.52 (d, $J = 8.7$ Hz, 2H), 6.85 (d, $J = 8.7$ Hz, 2H), 5.01 (m, 1H), 3.50 (m, 1H), 3.05 (m, 1H), 2.68 (m, 2H); ESI-MS *m/z* 220 (M^-). *General DCC coupling procedure for formation of esters or amides*. A solution of ISO-1-acid (100 mM in dry dichloromethane) was treated with 1.1 equiv DCC, 0.2 equiv DMAP, and 1.5 equiv alcohols (or 0.2 equiv HOBt and 1.5 equiv amines). The mixture was stirred for 8 h at rt. The formed white precipitate was filtered off and washed with CH_2Cl_2 and the filtrate was evaporated to dryness. The residue was purified on silica gel (hexane/EtOAc/MeOH 4:3:1) to give the esters or amides as a white solid. Compound **2** (65% yield): ^1H NMR (300 MHz, acetone-d_6) δ 8.75 (br, 1H), 7.54 (d, $J = 8.7$ Hz, 2H), 7.38 (m, 2H), 7.22 (m, 1H), 7.11 (m, 2H), 6.86 (d, $J = 8.7$ Hz, 2H), 5.10 (m, 1H), 3.54 (m, 1H), 3.27 (m, 1H), 2.96 (m, 2H); ESI-MS *m/z* 296 (M^-). Compound **3** (60% yield): ^1H NMR (300 MHz, acetone-d_6) δ 8.78 (s, 1H), 7.52 (d, $J = 8.7$ Hz, 2H), 7.02 (d, $J = 8.7$ Hz, 2H), 6.90 (d, $J = 8.7$ Hz, 2H), 6.86 (d, $J = 8.7$ Hz, 2H), 5.10

(m, 1H), 3.76 (s, 3H), 3.53 (m, 1H), 3.25 (m, 1H), 2.76 (m, 2H); ESI-MS *m*/*z* 326 (M^-). Compound **4** (40% yield): ^{1}H NMR (300 MHz, acetone-d_6) δ 8.75 (br, 1H), 7.55 (d, J = 8.7 Hz, 2H), 7.39 (d, J = 8.7 Hz, 2H), 7.03 (d, J = 8.7 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 5.11 (m, 1H), 3.55 (m, 1H), 3.23 (m, 1H), 2.95 (m, 2H), 1.30 (s, 9H); ESI-MS *m*/*z* 352 (M^-). Compound **5** (30% yield): ^{1}H NMR (300 MHz, acetone-d_6) δ 8.75 (s, 1H), 7.72 (d, J = 8.7 Hz, 2H), 7.17 (d, J = 8.7 Hz, 2H), 6.86 (s, 2H), 5.10 (m, 1H), 3.55 (m, 1H), 3.25 (m, 1H), 2.96 (m, 2H), 2.05 (s, 9H); ESI-MS *m*/*z* 338 (M^-). Compound **6** (80% yield): ^{1}H NMR (300 MHz, acetone-d_6) δ 8.79 (s, 1H), 7.52 (d, J = 8.7 Hz, 2H), 7.00 (br, 1H), 6.85 (d, J = 8.7 Hz, 2H), 4.94 (m, 1H), 3.64 (m, 1H), 3.42 (m, 1H), 3.10 (m, 1H), 2.52 (m, 1H), 2.37 (m, 1H) 1.90–1.00 (m, 10H); ESI-MS *m*/*z* 301 (M^-). Compound **7** (88% yield): ^{1}H NMR (300 MHz, acetone-d_6) δ 9.10 (br,1H), 8.80 (br, 1H), 7.53 (m, 4H), 6.84 (m, 4H), 5.06 (m, 1H), 3.72 (s, 3H), 3.49 (m, 1H), 3.17 (m, 1H), 2.73 (m, 1H), 2.60 (m, 1H); ESI-MS *m*/*z* 325 (M^-). Compound **8** (95% yield): ^{1}H NMR (270 MHz, acetone-d_6) δ 7.52 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 6.07 (br, 1H), 5.02 (m, 1H), 3.46 (m, 1H), 3.13 (m, 5H), 2.56 (m, 2H), 1.51 (m, 4H), 1.38 (s, 9H); ESI-MS *m*/*z* 414 ($M+Na^+$). Compound **9** (90% yield): ^{1}H NMR (270 MHz, acetone-d_6) δ 8.63 (br, 1H), 7.52 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 5.04 (m, 1H), 3.84 (m, 2H), 3.28 (m, 3H), 2.58 (m, 3H), 1.86 (m, 2H), 1.65 (m, 2H); ESI-MS *m*/*z* 292 (M^+). General TMSCl esterification procedure. To a solution of ISO-1-acid (50 mg. 0.23 mmol) in a 3 mL alcohol (ethanol, **10**; 1-propanol, **11**; 2-propanol, **12**; 1-butanol, **13**; cyclohexanol, **14**; cyclohexylmethanol, **15**; and neopentylalcohol, **16**) was added 0.1 mL TMSCl. The mixture was stirred for 2 hrs at rt. (for **14**, **15** and **16**: 3 h at 50 °C). The mixture was evaporated to dryness and the residue was subjected to purification on silica gel (hexane/EtOAc 4:3) to afford a white solid or pale yellow oil in quantitative yield. Compound **10**: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.74 (s, 1H), 7.52 (d, J = 8.7 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 4.97 (m, 1H), 4.10 (q, 2H), 3.51 (m, 1H), 3.12 (m, 1H), 2.66 (m, 2H) 1.19 (t, 3H); ESI-MS *m*/*z* 248 (M^-). Compound **11**: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.75 (s, 1H), 7.51 (d, J = 8.7 Hz, 2H), 6.86 (d, J = 8.7 Hz, 2H), 4.98 (m, 1H), 4.01 (t, 2H), 3.51 (m, 1H), 3.15 (m, 1H), 2.66 (m, 2H) 1.60 (m, 2H), 0.89 (t, 3H); ESI-MS *m*/*z* 262 (M^-). Compound **12**: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.74 (s, 1H), 7.51 (d, J = 8.7 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 4.97 (m, 2H), 4.72 (m, 1H), 3.51 (m, 1H), 3.12 (m, 1H), 2.63 (m, 2H) 1.18 (d, j = 6.3 Hz, 6H); ESI-MS *m*/*z* 262 (M^-). Compound **13**: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.78 (s, 1H), 7.52 (d, J = 8.7 Hz, 2H), 6.86 (d, J = 8.7 Hz, 2H), 4.99 (m, 1H), 4.05 (t, 2H), 3.51 (m, 1H), 3.12 (m, 1H), 2.68 (m, 2H) 1.10–1.60 (m, 4H), 0.88 (t, 3H); ESI-MS *m*/*z* 276 (M^-). Compound **14**: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.84 (br, 1H), 7.52 (d, J = 8.7 Hz, 2H), 6.86 (d, J = 8.7 Hz, 2H), 4.98 (m, 1H), 4.72 (m, 1H),3.51 (m, 1H), 3.15 (m, 1H), 2.66 (m, 2H) 1.90–1.20 (m, 10H); ESI-MS *m*/*z* 302 (M^-). Compound **15**: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.78 (s, 1H), 7.55 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 5.02 (m, 1H), 3.90 (d, J = 6.7 Hz, 2H), 3.51 (m, 1H), 3.15 (m, 1H), 2.72 (m, 2H) 1.80–0.90 (m, 11H); ESI-MS *m*/*z* 302 (M^-). Compound **16**: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.79 (s, 1H), 7.54 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 5.05 (m, 1H), 3.82 (m, 2H), 3.52 (m, 1H), 3.18 (m, 1H), 2.75 (m, 2H) 0.96 (s, 9H); ESI-MS *m*/*z* 292 (M^+). Classical resolution of acid 1 via crystallization of the cinchonidine salts. (***R***,***S***)**-1** (1.3 g, 5.5 mmol) was dissolved in hot acetone (25 mL), cinchonidine (1.61 g, 5.5 mmol) was added, and the solution was cooled to rt and allowed to stand at −20 °C overnight. The resulting white solid was filtered to give (***S***)**-1** salts. The filtrate was concentrated in vacuo to afford (***R***)**-1** salts. To a solution of (***R***)**-1** salts or (***S***)**-1** salts (200 mg, 0.4 mmol) in chloroform (3 mL) was added 1 N HCl in ether (3 mL, 3 mmol). The resulting white precipitate was filtered off and the filtrate was concentrated in vacuo to give (***R***)**-1** or (***S***)**-1** in quantitative yield.