Supporting information
for
Catalyst discovery using a ‘tethering’ strategy

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1. Synthesis and characterization of compounds 3A, 3B, and 3I.

A synthetic scheme was used that allows a very facile access to compounds 3 via the common intermediate 5.

![Chemical structures](image)

**Scheme SI-1.** (a) CH$_3$I, 60°C, CH$_3$CN, (b) 1. HCl, MeOH. 2. HOBT, EDC, R-COOH, CH$_3$CN, (c) acetic anhydride, CH$_3$CN.

**Compound 5.** Compound 4$^1$ (1.1g, 4.61mmol) was dissolved in CH$_3$CN and the resulting clear solution was transferred in a pyrex bottle with a screw cap. After addition of methyl iodide (3.5 ml, 56.2 mmol) the solution was stirred at 60 °C for 5h, after which the solvent was evaporated under reduced pressure. The resulting solid was crystallized (EtOAc) to give 5 as a white solid (541mg, 47% yield).

$^1$H-NMR (250 MHz, CD$_3$CN) δ (ppm): 10.34-9.94 (m, 2H), 7.09 (dd, $J = 8.18$, 3.42 Hz, 1H), 6.95 (t, $J = 7.50$, 7.50 Hz, 1H), 4.67 (s, 2H), 3.20 (s, 3H), 1.36 (s, 9H).

$^{13}$C-NMR (62.5 MHz, CD$_3$CN) δ (ppm): 157.25, 133.78, 132.92, 121.22, 116.50, 85.26, 60.87, 46.47, 27.98, 25.48.

**ESI-MS(+)** MeOH+0.1%HCOOH: [M+H]$^+$ 253m/z

**HPLC** (Jupiter Proteo 4u 250x4.60x4u, flow=0.8ml/min, 0-15min 10-90% ACN+0.1%TFA, 15-25min 90%ACN+0.1%TFA, λ=226nm): 18.8 min

**General procedure for the synthesis of compounds 6**

Compound 5 (300 mg, 1.19mmol) was dissolved in 25ml of a 1.75 M solution of HCl in MeOH. After one night the solvent was evaporated, which yielded BOC-deprotected 5 in quantitative yield, which was used as such.

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$^1$H-NMR (250 MHz, CD$_3$CN) δ (ppm): 7.70-7.06 (m, 2H), 6.95-6.82 (m, 2H), 4.30 (s, 2H), 2.84 (s, 3H)

$^{13}$C-NMR (62.5 MHz, CD$_3$CN) δ (ppm): 157.71, 133.62, 132.53, 120.93, 117.73, 116.40, 60.32, 44.72

ESI-MS(+) MeOH+0.1%HCOOH: [M+H]$^+$ 153 m/z

The commercial carboxylic acid (1.5 eq) was dissolved in anhydrous CH$_3$CN and HOBT (2eq) was added. After stirring the resulting suspension for 20 minutes in an ice bath, EDC (2 eq) was added. Stirring was continued for 15 minutes at 0 °C and at room temperature for 15 minutes. After renewed cooling to 0 °C, a solution of the BOC-deprotected 5 (1 eq) and Et$_3$N (6eq) in CH$_3$CN was added dropwise. The reaction mixture was stirred at 0 °C for 4h and one night at room temperature. Subsequently, the solvent was removed under reduced pressure and the residue was purified by silica gel chromatography.

Compound 6A
$^1$H-NMR (250 MHz, CD$_3$CN) δ (ppm): 7.37-7.15 (m, 6H), 7.12-7.01 (m, 1H), 6.84-6.73 (m, 2H), 3.96 (s, 2H), 3.44 (s, 2H), 2.55 (s, 3H)

$^{13}$C-NMR (62.5 MHz, CD$_3$CN) δ (ppm): 170.47, 158.15, 135.90, 131.04, 130.69, 130.05, 129.46, 127.83, 121.80, 120.20, 117.15, 62.39, 43.15, 41.29

ESI-MS(+) MeOH+0.1%HCOOH: [M+H]$^+$ 271 m/z, [M+Na]$^+$ 293 m/z,

HPLC (Jupiter Proteo 4u 250x4.60x4u, flow=0.8 ml/min, 0-15min 10-90% ACN+0.1%TFA, 15-25min 90%ACN+0.1%TFA, λ=280nm): 16.11 min

Compound 6B
$^1$H-NMR first isomer (50%) (250 MHz, CD$_3$OD) δ (ppm): 7.38-7.03 (m, 2H), 6.99-6.68 (m, 2H), 4.12 (s, 2H), 4.11 (s, 2H), 3.16 (s, 9H), 2.71 (s, 3H)

$^1$H-NMR second isomer (50%) (250 MHz, CD$_3$OD) δ (ppm): 7.38-7.03 (m, 2H), 6.99-6.68 (m, 2H), 4.12 (s, 2H), 4.11 (s, 2H), 3.04 (s, 9H), 2.61 (s, 3H)

$^{13}$C-NMR first isomer (50%) (75 MHz, CD$_3$OD) δ (ppm): 167.74, 157.87, 133.95, 130.91, 123.92, 120.72, 116.50, 64.85, 60.71, 54.86, 45.94

$^{13}$C-NMR first isomer (50%) (75 MHz, CD$_3$OD) δ (ppm): 163.19, 157.57, 131.79, 130.58, 122.84, 117.11, 116.50, 63.93, 59.92, 54.50, 44.21

ESI-MS(+) MeOH+0.1%HCOOH: [M]$^+$ 252 m/z

Compound 6I
$^1$H-NMR (250 MHz, CD$_3$OD) δ (ppm): 7.42-7.29 (m, 2H), 7.27-7.17 (m, 2H), 7.16-7.05 (m, 2H), 7.01-6.93 (m, 1H), 6.85-6.72 (m, 2H), 3.82 (s, 2H), 2.56 (m, 3H)

$^{13}$C-NMR (62.5 MHz, CD$_3$OD) δ (ppm): 158.67, 157.57, 140.23, 133.08, 130.54, 129.80, 124.34, 123.88, 120.65, 120.53, 116.42, 61.54, 44.92

ESI-MS(+) MeOH+0.1%HCOOH: [M+H]$^+$ 272 m/z, [M+Na]$^+$ 294 m/z

General procedure for the synthesis of compounds 3

Compound 6 was dissolved in anhydrous CH$_3$CN and acetic anhydride (2eq) was added. The mixture was stirred for 8h, after which the solvent was evaporated under reduced pressure. The final compounds 3A and 3B were purified by preparative HPLC. Compound 3C was purified by recrystallization from ethylacetate.
Compound 3A
ESI-MS(+) MeOH+0.1%HCOOH: [M+H]^+ 313 m/z, [M+Na]^+ 335 m/z, [M+K]^+ 351 m/z,
HPLC (Agilent Eclipse XDB-C18, 5µm 150x4.60mm, flow=0.8ml/min, 0-15min 10-90% ACN+0.1%TFA, 15-25min 90%ACN+0.1%TFA, λ=280nm): 11.83 min

Compound 3B
ESI-MS(+) MeOH+0.1%HCOOH: [M]^+ 294 m/z
HPLC (Jupiter Proteo 4u 250x4.60x4u, flow=0.8ml/min, 0-15min 10-90% ACN, 15-25min 90%ACN, λ=280nm): 21.4 min.

Compound 3I
^1H-NMR (250 MHz, CDCl₃) δ (ppm): 7.95 (s, 1H), 7.44-6.91 (m, 9H), 5.34 (s, 1H), 3.75 (s, 2H), 2.63 (s, 3H), 2.33 (s, 3H)
^13C-NMR (62.5 MHz, CDCl₃) δ (ppm): 169.90, 155.38, 149.74, 138.45 131.86, 129.67, 128.69, 128.05, 126.39, 122.83, 122.74, 119.15, 59.57, 46.54, 30.94
ESI-MS(+) MeOH+0.1%HCOOH: [M+Na]^+ 336 m/z
2. HSQC spectra of scaffolds 1 and 2 with hydrazide libraries A-I

HSQC spectra were recorded at a Bruker Avance 300. Samples were prepared by adding either scaffold 1 or 2 (5 mM) to a mixture of hydrazides A-I (7.5 mM each) in MeOD and subsequent equilibration at 50 ºC. Fingerprint parts of the resulting spectra are shown in Figures SI-1 and SI-2 for scaffolds 1 and 2, respectively. All hydrazones could be identified by comparison with the $^1$H and $^{13}$C spectra of the pure hydrazones. In some cases, the total amount of hydrazone was calculated based on correction of a single isomer peak with the known isomer distribution. The resulting concentrations (relative to the concentration of hydrazone 1A or 2A, respectively) are shown in Figure SI-3. To illustrate the excellent correlation, also the relative concentrations for the competition experiments of each separate hydrazine against hydrazide A are given. Division of the numbers for each separate hydrazone gives the amplification numbers shown in Figure 1 of the main text.

**Figure SI-1.** Fingerprint part of the HSQC spectrum of the library 2A-2I in MeOD (300 MHz, 50 ºC).
Figure SI-2. Fingerprint part of the HSQC spectrum of the library 1A-1I in MeOD (300 MHz, 50 °C).

Figure SI-3. Relative concentrations of each hydrazone (a) 1B-1I and (b) 2B-2I with respect to hydrazone 1A or 2A, respectively, obtained from the HSQC-spectra ( ) or direct competition experiments ( ).
3. Fingerprint parts of the $^1$H NMR spectra used for determination of the amplification.

Hydrazone formation between scaffolds 1 and 2 and hydrazides A and B is extensively discussed (including all experimental details) in a previous communication (Chem.Commun. 2007, 1340-1342). In Figure SI-4 fingerprint parts of the $^1$H NMR spectra are shown of the competition experiments between hydrazide A and either one of the library members C-I using either scaffold 1 (left) or scaffold 2 (right). The spectrum of hydrazones 1A and 1B is shown as a reference (Figure SI-4, a, peaks are highlighted in all spectra). In cases where assignment of signals was not entirely clear due to overlap, the spectrum of the pure hydrazone was used for unambiguous assignment. Some hydrazones are present as two isomers due to $E,Z$-isomerism around the NH-C(O) bond based on related studies in the literature (F.V. Bagrov, T.V. Vasil’eva Russ. J. Org. Chem. 2002, 38, 1309-1313.). Hydrazone ratios were determined by integrating the corresponding signals (occasionally using deconvolution) and are reported in Table SI-1.

Table SI-1. Amplification numbers obtained from the NMR competition experiments.[a]

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<th>Scaffold 1</th>
<th>Scaffold 2</th>
<th>Amplif.</th>
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<td>2X (%) 2A (%) K$_{2,ref}$</td>
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<tr>
<td>B</td>
<td>67 33 2.1</td>
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<tr>
<td>C</td>
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<td>72 28 2.6</td>
<td>1.3</td>
</tr>
<tr>
<td>D</td>
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<td>52 48 1.1</td>
<td>1.1</td>
</tr>
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<td>50 50 1.0</td>
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<tr>
<td>I</td>
<td>88 12 7.3</td>
<td>85 15 5.5</td>
<td>1.3</td>
</tr>
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</table>

(a) All spectra obtained in CD$_3$OD (300 MHz). (b) From a previous communication (Chem.Commun. 2007, 1340-1342).
Figure SI-4. Fingerprint parts of the $^1$H NMR spectra (300 MHz) obtained for the competition experiments between hydrazide A and one of the hydrazides C-I (b-h,●) using either scaffold 1 (left) or 2 (right). As a reference the spectra of hydrazones 1A (a, left) and 2A (a, right) are shown. All spectra were obtained after equilibrating a mixture of 1 or 2 (5mM) and 5 equivalents of both hydrazide B and the other hydrazide (B-I) in CD$_3$OD at 50 °C. Equilibration was continued until no further changes were detected in the $^1$H NMR spectra.
3. Plot of log $k_{\text{obs}}$ against pH for the hydrolysis of compounds 3A and 3B.

Figure SI-5. Plot of log $k_{\text{obs}}$ as a function of pH for the hydrolysis of compounds 3A (□) and 3B (■) at different pH. Conditions [3] = 0.6 mM, H$_2$O:CH$_3$CN = 50:50, 45 °C. The pH was buffered using 60 mM of CAPS (pH 11 and 10), CHES (pH 9), EPPS (pH 8) or HEPES (pH 7). The pH refers to the aqueous component. Kinetics were followed by measuring the increase in absorbance at 280 nm and fitted to a first order exponential.