Supporting Information

Imines that React with Phenols in Water over a Wide pH Range

Maki Minakawa, Hai-Ming Guo, Fujie Tanaka*

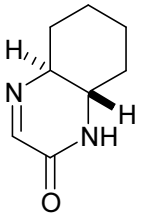
Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

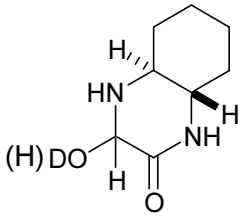
ftanaka@scripps.edu

General: For thin layer chromatography (TLC), silica gel plates with fluorescent indicator were used. Flash column chromatography was performed using silica gel 32-63 mesh, 60 Å. For ^{1}H NMR spectra, proton chemical shifts (δ) are given in ppm relative to tetramethylsilane (0.00 ppm) in $CDCl_3$ or 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (0.00 ppm) in D_2O. Multiplicities are indicated by s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). For ^{13}C NMR spectra, carbon chemical shifts were internally referenced to the deuterated solvent signal of $CDCl_3$ (77.00 ppm).

1. Synthesis of imines 1 and 2

trans-4a,5,6,7,8,8a-Hexahydro-1*H*-quinoxalin-2-one (1)

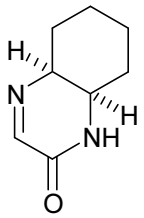




hydrated form in D_2O

To a solution of *trans*-1,2-diaminocyclohexane (228 mg, 2.00 mmol) in 2-PrOH (3.0 mL), a solution of ethyl glyoxylate polymer form (45-50% in toluene, 0.21 mL, 1.00 mmol) in 2-PrOH (3.0 mL) was added dropwise over 15 min at room temperature (23 ºC). The mixture was stirred for 2 h at the same temperature, concentrated under reduced pressure, and purified by flash column chromatography (AcOEt) to afford **1** (151 mg, 99%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.28-1.37 (m, 1H), 1.39-1.49 (m, 3H), 1.80-1.84 (m, 1H), 1.88-1.92 (m, 1H), 1.94-1.98 (m, 1H), 2.35-2.41 (m, 1H), 3.06-3.12 (m, 1H, C*H*N=CH), 3.17 (dt, *J* = 3.9 Hz, 11.6 Hz, 1H, C*H*NHC=O), 7.07 (brs, 1H, N*H*), 7.72 (t, *J* = 2.8 Hz, 1H, N=C*H*CO). ^{1}H NMR (500 MHz, D_2O): δ1.24-1.36 (m, 1H), 1.38-1.48 (m, 3H), 1.77-1.80 (m, 1H), 1.83-1.90 (m, 1H), 1.96-1.99 (m, 1H), 2.32-2.32 (m, 1H), 3.18-3.29 (m, 2H), 7.66 (d, *J* = 2.5 Hz, 1H, N=C*H*CO); hydrated form 2.83 (m, C*H*NHCH(OD)CO), 3.09 (m, C*H*NHCO), 4.82 (s, NHC*H*(OD)C=O, major isomer), 4.93 (s NHC*H*(OD)C=O, minor isomer). ^{13}C NMR (125 MHz, $CDCl_3$): δ 23.6, 25.2, 31.0, 31.5, 54.1, 63.0, 156.3, 158.0. HRMS: calcd for $C_8H_{13}N_2O$ (MH^+) 153.1022, found 153.1019. Synthesis of **1** in other solvents; H_2O (99%), toluene (99%), DMF (99%), DMSO (99%), NMP (99%), AcOEt (81%), THF (80%), $CHCl_3$ (78%), and CH_2Cl_2 (62%).

cis-4a,5,6,7,8,8a-Hexahydro-1*H*-quinoxalin-2-one (2)



To a solution of *cis*-1,2-diaminocyclohexane (228 mg, 2.00 mmol) in 2-PrOH (3.0 mL), a solution of ethyl glyoxylate polymer form (45-50% in toluene, 0.21 mL, 1.00 mmol) in 2-PrOH (3.0 mL) was added dropwise over 15 min at room temperature (23 ºC). The mixture was stirred for 17 h at the same temperature, concentrated under reduced pressure,

and purified by flash column chromatography (AcOEt) to afford **2** (114 mg, 75%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.37-1.45 (m, 1H), 1.46-1.55 (m, 3H), 1.62-1.68 (m, 1H), 1.70-1.77 (m, 2H), 1.86-1.92 (m, 1H), 3.70 (m, 1H, C*H*N=CH), 3.85-3.87 (m, 1H, C*H*NCHC=O), 5.79 (brs, 1H, N*H*), 7.75 (t, J = 2.3 Hz, 1H, N=C*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.7, 22.8, 28.2, 28.8, 48.5, 57.7, 155.9, 157.8. HRMS: calcd for $C_8H_{13}N_2O$ (MH^+) 153.1022, found 153.1026.

2. Reactions of imines 1 and 2 with 4-methylphenol

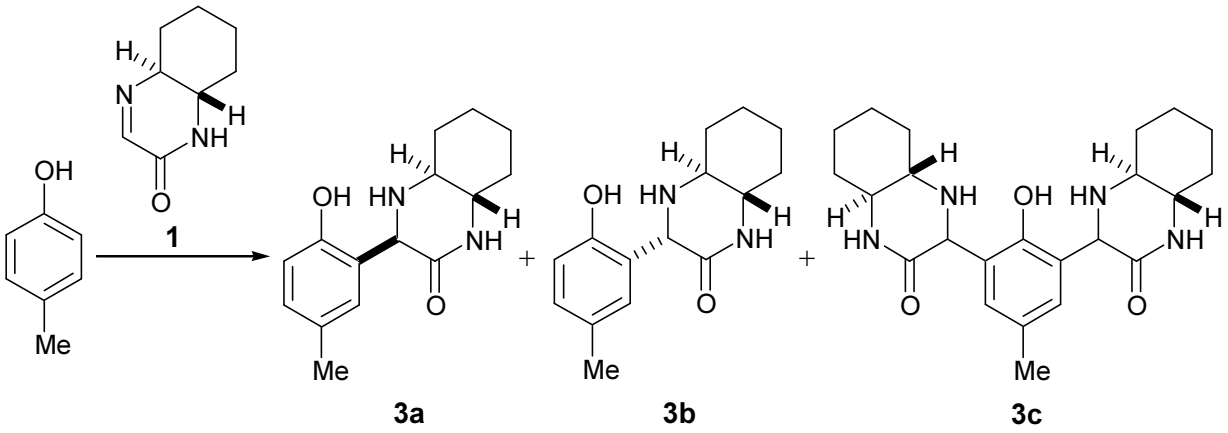
2-1. Reactions of imine 1 with 4-methylphenol (Table 1)

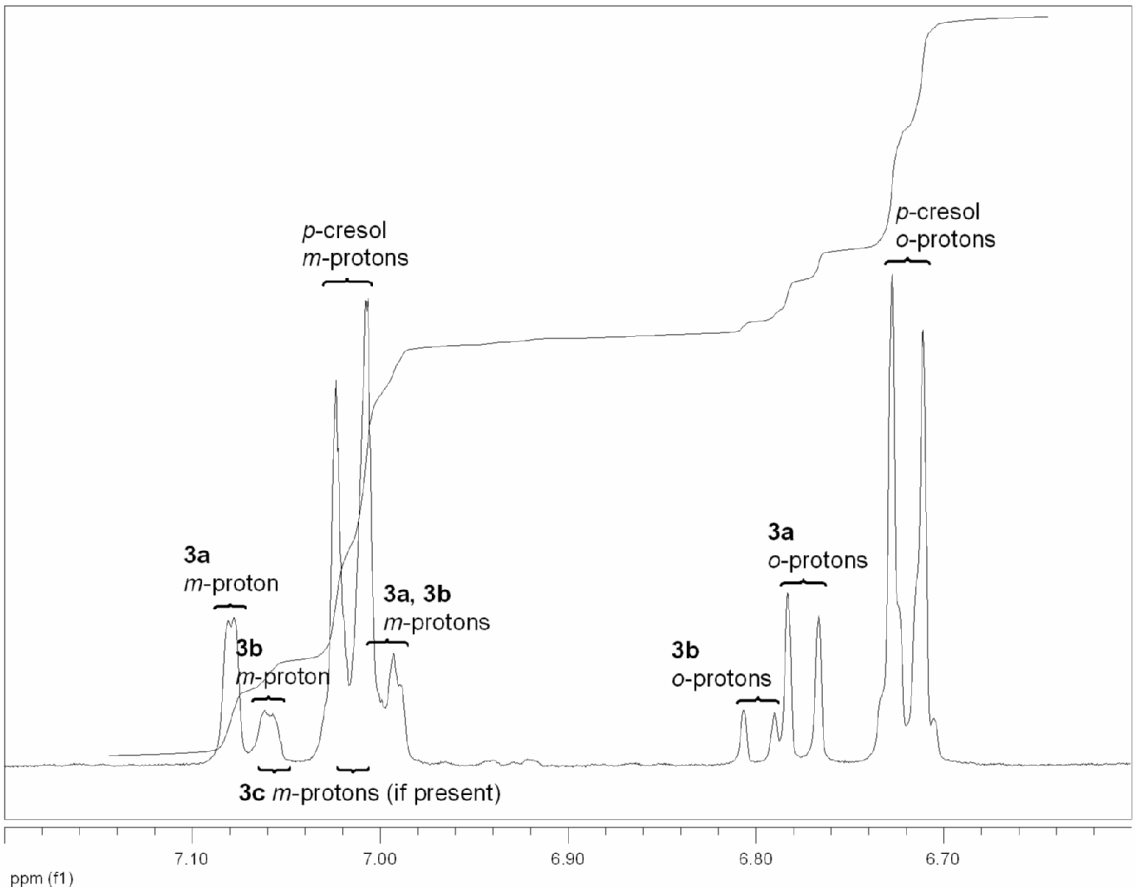
General procedure for Table 1, entries 1-15 and 20. A 100 mM solution of imine **1** in 200 mM sodium phosphate buffer or indicated buffer or aqueous component was prepared immediately before starting reaction. To the 100 mM solution of **1** (950 μL), a 500 mM solution of 4-methylphenol in DMSO (50 μL) was added at rt (23 ºC) and the mixture was stirred at the same temperature for 24 h. After pH of the reaction mixture was adjusted to pH 7~8 with 200 mM Na_2HPO_4 or with 200 mM NaH_2PO_4, the mixture was extracted with CH_2Cl_2. Organic layers were combined, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude mixture was dissolved in $CDCl_3$ and was analyzed by ^{1}H NMR to determine the yield. The ratio of **3a** and **3b** to *p*-cresol was determined by the comparison of the areas of the *o*-proton signals. Comparison of the areas of the *o*-proton signals and areas of *m*-proton signals led to determination of the ratio of **3c** to **3a** and **3b** and to *p*-cresol. The ratios were then converted to the yields.

General procedure for Table 1, entries 16-19. A 100 mM solution of imine **1** in 200 mM sodium phosphate buffer (prepared with D_2O) was prepared immediately before starting reaction. The 100 mM solution of **1** (0.5 mL) was added to a 20 mM solution of 4-methylphenol in D_2O (0.5 mL) in a glass vial at rt (23 ºC) and the mixture was shaken for 24 h at 37 ºC. To analyze ^{1}H NMR, approximately 0.5 mL of the reaction mixture was transferred to a NMR tube. ^{1}H NMR was recorded at 25 ºC to determine the yield. Proton chemical shifts in the ^{1}H NMR spectra of compounds depend on pH. In most cases, signals of *o*-protons (to phenolic OH) of mono-addition products **3a** and **3b** did not overlap with signals of *o*-protons of starting material phenol (4-methylphenol or tyrosine phenol). In these cases, the ratio of mono-addition products to starting material phenol was determined by comparison of the areas of the *o*-proton signals. Comparison of the areas of the *o*-proton signals and areas of *m*-proton signals led to determination of the ratio of

di-addition product **3c** to mono-addition products and/or to starting material phenol. The ratios were then converted to yields of the products. When signals of *o*-protons of mono-addition products overlapped with signals of *o*-protons of starting material phenol, areas of *o*-proton signals and areas of *m*-proton signals were compared to determine percentage of modified *o*-positions of starting material phenol.

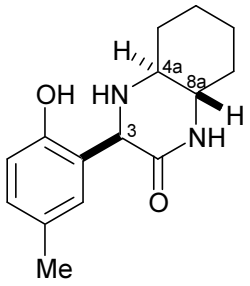
^{1}H NMR of the extracted mixture of the reaction (Table 1, entry 10)



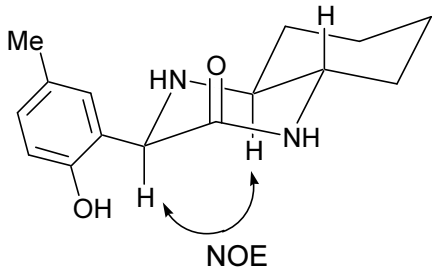


2-2. Synthesis of standards of Mannich products 3a, 3b, and 3c

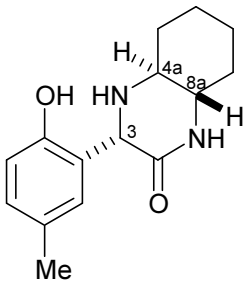
(3*R**,4a*S**,8a*S**)-3-(2-Hydroxy-5-methylphenyl)octahydroquinoxalin-2(1*H*)-one (3a)



To a solution of imine **1** (152 mg, 1.00 mmol) in 200 mM sodium phosphate, pH 8.0 (9.50 mL), a solution of 4-methylphenol (130 mg, 1.20 mmol) in DMSO (0.50 mL) was added and the mixture was stirred for 14 h at 23 ºC. The reaction mixture was diluted with water and extracted with CH_2Cl_2. Organic layers were combined, washed with brine, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography (AcOEt) to afford **3a** (84.3 mg, 32%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$-CD_3OD): δ 1.30-1.47 (m, 4H), 1.81-1.86 (m, 2H), 1.89-1.92 (m, 2H), 2.25 (s, 3H, C*H*$_3$), 2.65 (ddd, *J* = 3.5 Hz, 9.5 Hz, 11.0 Hz, 1H, C*H*NHCHAr), 3.31-3.26 (m, 1H, C*H*NHC=O), 4.59 (s, 1H, C*H*Ar), 6.71 (d, *J* = 8.0 Hz, 1H, Ar*H*), 6.98 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H, Ar*H*), 7.04 (d, *J* = 2.0 Hz, 1H, Ar*H*). ^{1}H NMR (500 MHz, D_2O-CD_3OD): δ 6.83 (d, *J* = 8.0 Hz, 1H, Ar*H*), 7.10 (s, 1H, Ar*H*), 7.13 (d, *J* = 8.0 Hz, 1H, Ar*H*). ^{13}C NMR (125 MHz, $CDCl_3$-CD_3OD): δ 19.8, 23.4, 24.2, 30.0, 30.7, 57.2, 57.7, 62.3, 115.9, 123.1, 128.5, 129.6, 131.0, 152.7, 170.9. HRMS: calcd for $C_{15}H_{21}N_2O_2$ (MH^+) 261.1597, found 261.1606. The relative stereochemistry of **3a** was determined by ROESY analysis.

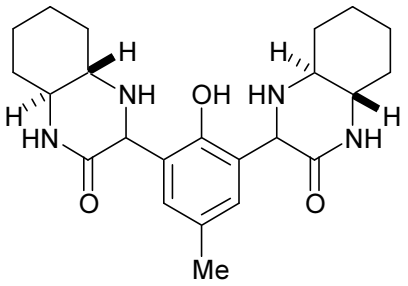


(3*S,4a*S**,8a*S**)-3-(2-Hydroxy-5-methylphenyl)octahydroquinoxalin-2(1*H*)-one (3b)**



A mixture of imine **1** (245 mg, 1.61 mmol), 4-methylphenol (130 mg, 1.20 mmol), $MgSO_4$ (291 mg, 2.42 mmol), and TFA (117 μL, 1.61 mmol) in CH_2Cl_2 (5.0 mL) was stirred for 5 h at 23 ºC. The reaction mixture was added to saturated $NaHCO_3$ aqueous solution and extracted with CH_2Cl_2. Organic layers were combined, washed with brine, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography (AcOEt) to afford **3b** (221 mg, 53%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.18-1.36 (m, 4H), 1.75 (m 1H), 1.79-1.86 (m, 2H), 1.96 (dd, J = 2.8, 12.6 Hz, 1H), 2.26 (s, 3H, CH_3), 2.64 (ddd, J = 3.5 Hz, 9.5 Hz, 11.5 Hz, 1H, C*H*NHCHAr), 3.08 (dt, J = 4.0 Hz, 10.5 Hz, 1H, C*H*NHC=O), 4.96 (s, 1H, C*H*Ar), 6.76 (brs, 1H), 6.80 (d, J = 8.0 Hz, 1H, Ar*H*), 7.00 (dd, J = 2.0 Hz, 8.0 Hz, 1H, Ar*H*), 7.06 (s, 1H, Ar*H*) 10.8 (br, 1H, O*H*). ^{1}H NMR (500 MHz, D_2O): δ 6.91 (d, J = 8.5 Hz, 1H, Ar*H*), 6.96 (d, J = 2.0 Hz, 1H, Ar*H*), 7.13 (dd, J = 2.5 Hz, 8.0 Hz, 1H, Ar*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.8, 23.7, 24.8, 30.6, 31.6, 53.4, 58.9, 59.4, 117.5, 122.1, 127.3, 128.7, 129.7, 154.7, 170.6. HRMS: calcd for $C_{15}H_{21}N_2O_2$ (MH^+) 261.1597, found 261.1595.

Di-addition product (3c)



To a solution of imine **1** (172 mg, 1.13 mmol) in 200 mM NaH_2PO_4 aqueous, pH 5 (11.0 mL), a solution of 4-methylphenol (29.7 mg, 0.275 mmol) in 200 mM NaH_2PO_4 aqueous, pH 5 (11.0 mL) was added and the mixture was shaken for 65 h at 37 ºC. The reaction mixture was diluted with water and extracted with CH_2Cl_2. Organic layers were combined, washed with brine, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography (CH_2Cl_2:AcOEt:MeOH=1:1:0.1) to afford **3c** (34.7 mg, 31%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.24-1.39 (m, 8H), 1.76-1.85 (m

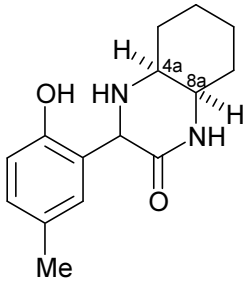
8H), 2.25 (s, 3H, C*H*$_3$), 2.55-2.64 (m, 2H, C*H*NHCHAr), 3.15-3.21 (m, 2H, C*H*NHC=O), 4.54 (s, 1H, C*H*Ar), 4.75 (s, 1H, C*H*Ar), 6.23 (s, 1H), 6.33 (s, 1H), 7.02 (s, Ar*H*), 7.07 (s, 1H, Ar*H*). ^{1}H NMR (500 MHz, D_2O): δ 1.31-1.42 (m, 8H), 1.79-1.95 (m 8H), 2.27 (s, 3H, C*H*$_3$), 2.70-2.74 (m, 2H, C*H*NHCHAr), 3.28-3.33 (m, 2H, C*H*NHC=O), 4.64 (s, 1H, C*H*Ar), 4.66 (s, 1H, C*H*Ar), 7.07 (s, 2H, Ar*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.5, 20.6, 23.8, 24.6, 24.7, 30.50, 30.53, 31.3, 31.4, 57.8, 58.2, 58.4, 61.4, 62.9, 124.7, 125.2, 128.3, 128.7, 130.5, 131.6, 151.7, 170.6, 170.7. HRMS: calcd for $C_{23}H_{33}N_4O_3$ (MH^+) 413.2547, found 413.2544. Compound **3c** characterized here was the main diastereomer of the di-addition product. Other diastereomers were minor or were not formed.

2-3. Reactions of cyclic imine 2 with 4-methylphenol

Reactions in Table 2 were performed using imine **2** by the procedures for the reactions of Table 1.

2-4. Synthesis of standards of Mannich product 4

(4a*S,8a*R**)-3-(2-Hydroxy-5-methylphenyl)octahydroquinoxalin-2(1*H*)-one (4)**



To a solution of imine **2** (152 mg, 1.00 mmol) in 200 mM sodium phosphate, pH 8.0 (9.50 mL), a solution of 4-methylphenol (130 mg, 1.20 mmol) in DMSO (0.50 mL) was added and the mixture was stirred for 24 h at 23 ºC. The reaction mixture was diluted with water and extracted with CH_2Cl_2. Organic layers were combined, washed with brine, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography (AcOEt) to afford **4** (90.7 mg, 35%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.33-1.39 (m, 1H), 1.40-1.46 (m, 1H), 1.55-1.59 (m, 3H), 1.63-1.69 (m, 1H), 1.72-1.78 (m, 1H), 1.88-1.95 (m, 1H), 2.25 (s, 3H, C*H*$_3$), 3.26 (dt, *J* = 7.5 Hz, 3.5 Hz, 1H, C*H*NHCHAr), 3.64 (br, d, *J* = 3.5, 1H, C*H*NHC=O), 4.81 (s, 1H, C*H*Ar), 6.57 (brs, 1H), 6.77 (d, *J* = 8.0 Hz, 1H, Ar*H*), 6.99 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H, Ar*H*), 7.06 (d, *J* = 2.0 Hz, 1H, Ar*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.6, 21.3, 22.0, 27.4, 30.5, 48.6, 51.8, 58.1, 117.3, 122.1, 128.7, 129.0, 129.8, 154.5, 169.9. HRMS: calcd for $C_{15}H_{21}N_2O_2$

(MH^+) 261.1597, found 261.1594.

3. Stability analyses of compounds 3a, 3b, and 4

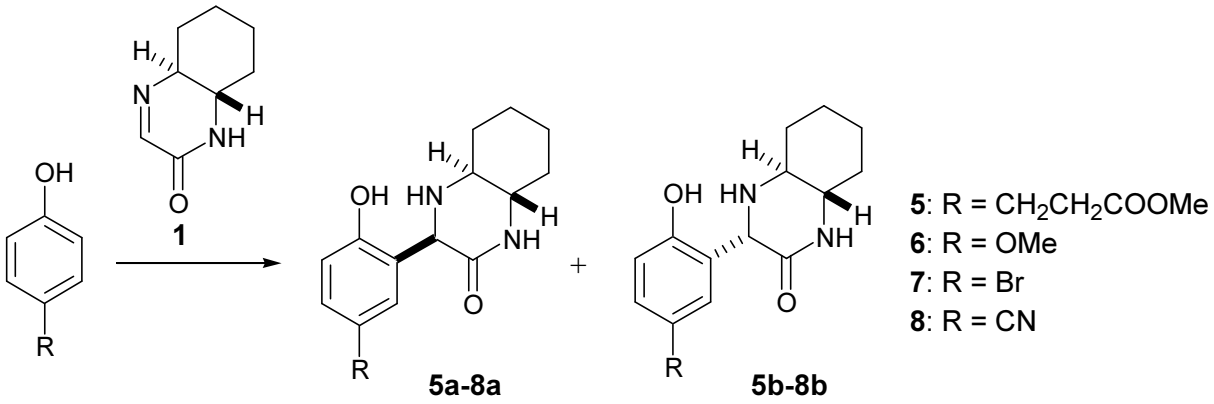
A solution of compound (indicated concentration) in 5% DMSO/indicated aqueous buffer (or aqueous component) was stirred at 23 ºC for 24 h. After pH of the mixture was adjusted to pH 7 with 200 mM Na_2HPO_4 or with 200 mM NaH_2PO_4, the mixture was extracted with CH_2Cl_2. The crude mixture was analyzed by ^1H NMR ($CDCl_3$).

Table S1. Stability experiments in 5% DMSO/aqueous buffer.

entry	compound (concentration)	buffer (or aqueous component)	results
1	**3a** (2.50 mM)	1% TFA, pH 1	recovered[a]
2	**3a** (2.38 mM)	10% citric acid, pH 3	recovered[a]
3	**3a** (2.57 mM)	200 mM NaH_2PO_4, pH 5	recovered[a]
4	**3a** (0.98 mM)	200 mM sodium phosphate, pH 8.0	recovered[a]
5	**3b**[b] (3.19 mM)	1% TFA, pH 1	recovered[a]
6	**3b**[b] (3.07 mM)	10% citric acid, pH 3	recovered[a]
7	**3b**[b] (3.34 mM)	200 mM NaH_2PO_4, pH 5	**3a**:**3b** = 3:97
8	**3b**[b] (3.96 mM)	200 mM sodium phosphate, pH 8.0	**3a**:**3b** = 6:94
9	**4** (2.96 mM)	1% TFA, pH 1	recovered[a]
10	**4** (2.80 mM)	10% citric acid, pH 3	recovered[a]
11	**4** (2.96 mM)	200 mM NaH_2PO_4, pH 5	recovered[a]
12	**4** (2.80 mM)	200 mM sodium phosphate, pH 8.0	recovered[a]

[a] Corresponding starting material was recovered quantitatively. No other compounds, including 4-methylphenol, were detected. [b] **3b**:**3a** = 1:>99

4. Reactions of imine 1 with phenol derivatives



Method A. Reactions were performed by the procedures for the reactions of Table 1, entries 1-15. Imine, 95 mM; phenol, 25 mM; 5% DMSO/200 mM sodium phosphate buffer; 24 h.

Method B. Reactions of **1** with phenols were performed in DMSO/200 mM sodium phosphate, pH 8.0 and the major diastereomer was isolated. See synthesis of **5a**, **6a**, **7a**, and **8a** described below.

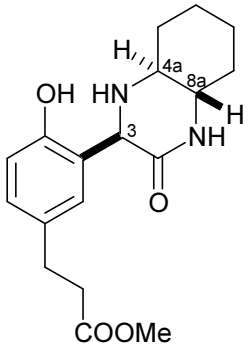
Table S2. Reaction of imine **1** with phenols

entry	method	5% DMSO/200 mM Na phosphate	product	yield (%)	ratio (**a**:**b**)
1	**A**	pH 6.0	**5**	30[a]	1.7 : 1
2	**A**	pH 7.5	**5**	41[a]	3.1 : 1
3	**A**	pH 8.0	**5**	41[a]	3.3 : 1
4	**B**	pH 8.0	**5a**	32[b]	-[c]
5	**A**	pH 7.5	**6**	41[a]	6.9 : 1
6	**B**	pH 8.0	**6a**	40[b]	-[c]
7	**A**	pH 7.5	**7**	14[a]	2.8 : 1
8	**B**	pH 8.0	**7a**	23[b]	-[c]
9	**A**	pH 7.5	**8**	2[a]	4.6 : 1
10	**B**	pH 8.0	**8a**	4[b]	-[c]

[a] Conversion yield determined by ^{1}H NMR. [b] Isolated yield. [c] The minor diastereomer was not isolated and the ratio was not determined.

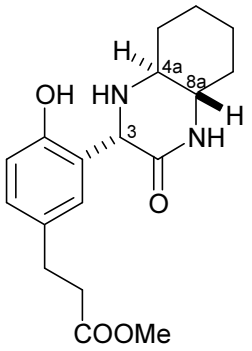
Synthesis of standards of Mannich products 5a-8a

3-{4-Hydroxy-3-[(3*R,4a*S**,8a*S**)-2-oxodecahydroquinoxalin-3-yl]phenyl}propionic acid methyl ester (5a)**



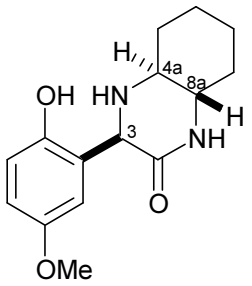
To a solution of imine **1** (152 mg, 1.00 mmol) in 200 mM sodium phosphate, pH 8.0 (9.50 mL), a solution of methyl 3-(4-hydroxylphenyl)propionate (216 mg, 1.20 mmol) in DMSO (0.50 mL) was added and the mixture was stirred for 24 h at 23 ºC. The reaction mixture was diluted with water and extracted with CH_2Cl_2. Organic layers were combined, washed with brine, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography (AcOEt) to afford **5a** (60.7 mg, 32%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30-1.48 (m, 4H), 1.81-1.85 (m, 4H), 2.60 (dd, J = 6.5 Hz, 8.6 Hz, 2H, $CH_2CH_2COOCH_3$), 2.68-2.72 (m, 1H, C*H*NHCHAr), 2.83-2.92 (m, 2H, $CH_2CH_2COOCH_3$), 3.24-3.28 (m, 1H, C*H*NHC=O), 3.66 (s, 3H, CH_3), 4.73 (s, 1H, C*H*Ar), 6.02 (brs, 1H), 6.80 (d, J = 8.0 Hz, 1H, Ar*H*), 7.02 (dd, J = 2.0 Hz, 8.0 Hz, 1H, Ar*H*), 7.11 (d, J = 2.0 Hz, 1H, Ar*H*), 10.0 (br, 1H). ^{13}C NMR (125 MHz, $CDCl_3$-CD_3OD): δ 23.3, 24.1, 29.6, 29.8, 30.6, 35.4, 51.0, 57.1, 57.5, 62.1, 115.9, 123.5, 128.7, 130.4, 131.1, 153.3, 170.8, 173.7. HRMS: calcd for $C_{18}H_{25}N_2O_4$ (MH^+) 333.1809, found 333.1815.

3-{4-Hydroxy-3-[(3*S,4a*S**,8a*S**)-2-oxodecahydroquinoxalin-3-yl]phenyl}propionic acid methyl ester (5b)**



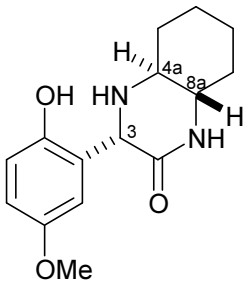
A mixture of imine **1** (457 mg, 3.00 mmol), methyl 3-(4-hydroxylphenyl)propionate (649 mg, 3.60 mmol), $MgSO_4$ (542 mg, 4.50 mmol), and TFA (223 μL, 3.00 mmol) in CH_2Cl_2 (10.0 mL) was stirred for 12 h at 23 ºC. The reaction mixture was added to saturated $NaHCO_3$ aqueous solution and extracted with CH_2Cl_2. Organic layers were combined, washed with brine, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography (AcOEt:CH_2Cl_2 = 1:1) to afford **5b** (665 mg, 67%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.20-1.40 (m, 4H), 1.75-1.77 (m, 1H), 1.80-1.85 (m, 2H), 1.97-1.99 (m, 1H), 2.57-2.60 (m, 2H, $CH_2CH_2COOCH_3$), 2.61-2.65 (m, 1H, C*H*NHCHAr), 2.87 (t, *J* = 8.0 Hz, 2H, $CH_2CH_2COOCH_3$), 3.08-3.13 (m, 1H, C*H*NHC=O), 3.67 (s, 3H, CH_3), 4.96 (s, 1H, C*H*Ar), 6.82 (d, *J* = 8.0 Hz, 1H, Ar*H*), 6.85 (brs, 1H), 7.02 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H, Ar*H*), 7.11 (d, *J* = 2.0 Hz, 1H, Ar*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 23.7, 24.8, 30.4, 30.5, 31.5, 36.0, 51.5, 53.5, 58.8, 59.3, 117.8, 122.4, 126.7, 128.9, 131.5, 155.4, 170.7, 173.5. HRMS: calcd for $C_{18}H_{25}N_2O_4$ (MH^+) 333.1809, found 333.1810.

(3*R,4a*S**,8a*S**)-3-(2-Hydroxy-5-methoxyphenyl)octahydroquinoxalin-2(1*H*)-one (6a)**



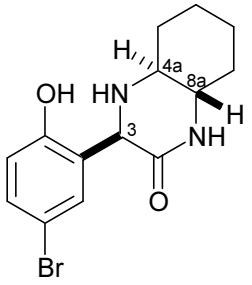
To a solution of imine **1** (152 mg, 1.00 mmol) in 200 mM sodium phosphate, pH 8.0 (9.50 mL), a solution of 4-methoxyphenol (149 mg, 1.20 mmol) in DMSO (0.50 mL) was added and the mixture was stirred for 5 h at 23 ºC. The reaction mixture was diluted with water and extracted with CH_2Cl_2. Organic layers were combined, washed with brine, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography (AcOEt) to afford **6a** (111.8 mg, 40%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$-CD_3OD): δ 1.26-1.46 (m, 4H), 1.81-1.90 (m, 4H), 2.64-2.69 (m, 1H, C*H*NHCHAr), 3.21-3.26 (m, 1H, C*H*NHC=O), 3.76 (s, 3H, OCH_3), 4.64 (s, 1H, C*H*Ar), 6.74-6.79 (m, 2H, Ar*H*), 6.86 (d, *J* = 2.5 Hz, 1H, Ar*H*). ^{13}C NMR (125 MHz, $CDCl_3$-CD_3OD): δ 23.5, 24.2, 30.1, 30.7, 55.6, 57.1, 57.9, 62.4, 114.7, 115.9, 117.2, 124.2, 149.2, 152.7, 170.4. HRMS: calcd for $C_{15}H_{21}N_2O_3$ (MH^+) 277.1547, found 277.1554.

(3*S,4a*S**,8a*S**)-3-(2-Hydroxy-5-methoxyphenyl)octahydroquinoxalin-2(1*H*)-one (6b)**



A mixture of imine **1** (231 mg, 1.52 mmol), 4-methoxyphenol (226 mg 1.82 mmol), $MgSO_4$ (274 mg, 2.28mmol), and TFA (113 μL, 1.52 mmol) in CH_2Cl_2 (10.0 mL) was stirred for 15 h at 23 ºC. The reaction mixture was added to saturated $NaHCO_3$ aqueous solution and extracted with CH_2Cl_2. Organic layers were combined, washed with brine, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography (AcOEt:CH_2Cl_2 = 1:1) to afford **6b** (57.5 mg, 14%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.31-1.48 (m, 4H), 1.81-1.86 (m, 4H), 2.22 (br, 1H, N*H*CHAr), 2.69-2.73 (m, 1H, C*H*NHCHAr), 3.24-3.29 (m, 1H, C*H*NHC=O), 3.76 (s, 3H, OCH_3), 4.76 (s, 1H, C*H*Ar), 5.93 (brs, 1H, N*H*C=O), 6.77 (dd, J = 3.0 Hz, 8.8 Hz, 1H, Ar*H*), 6.82 (d, J = 8.8 Hz, 1H, Ar*H*), 7.00 (d, J = 3.0 Hz, 1H, Ar*H*) 9.66 (br, 1H, O*H*). ^{13}C NMR (125 MHz, $CDCl_3$-CD_3OD): 23.5, 24.2, 29.8, 30.1, 30.8, 55.6, 57.1, 58.0, 114.7, 115.8, 117.5, 124.1, 149.3, 152.7, 170.2. HRMS: calcd for $C_{15}H_{21}N_2O_3$ (MH^+) 277.1547, found 277.1549.

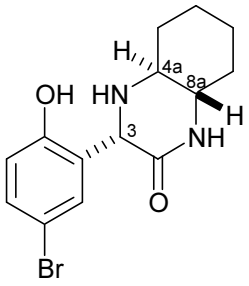
(3*R,4a*S**,8a*S**)-3-(2-Hydroxy-5-bromophenyl)octahydroquinoxalin-2(1*H*)-one (7a)**



To a solution of imine **1** (152 mg, 1.00 mmol) in 200 mM sodium phosphate, pH 8.0 (9.50 mL), a solution of 4-bromophenol (208 mg, 1.20 mmol) in DMSO (0.50 mL) was added and the mixture was stirred for 6 h at 23 ºC. The reaction mixture was diluted with water and extracted with CH_2Cl_2. Organic layers were combined, washed with brine, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography (AcOEt) to afford **7a** (75.0 mg, 23%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$-CD_3OD): δ 1.31-1.44 (m, 4H), 1.81-1.90 (m, 4H), 2.67 (dt, J = 3.8 Hz, 10.8 Hz, 1H, C*H*NHCHAr), 3.21-3.26 (m, 1H, C*H*NHC=O), 4.66 (s, 1H, C*H*Ar), 6.74 (d, J =

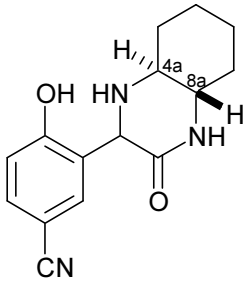
8.6 Hz, 1H, Ar*H*), 7.27 (dd, *J* = 2.5 Hz, 8.6 Hz, 1H, Ar*H*), 7.40 (d, *J* = 2.5 Hz, 1H, Ar*H*). ^{13}C NMR (125 MHz, $CDCl_3$-CD_3OD): δ 23.5, 24.2, 30.1, 30.7, 57.1, 57.9, 61.8, 111.4, 118.5, 125.6, 132.0, 132.8, 154.8, 170.0. HRMS: calcd for $C_{14}H_{18}BrN_2O_2$ (MH^+) 325.0546, found 325.0545.

(3*S,4a*S**,8a*S**)-3-(2-Hydroxy-5-bromophenyl)octahydroquinoxalin-2(1*H*)-one (7b)**



A mixture of imine **1** (231 mg, 1.52 mmol), 4-bromophenol (315 mg 1.82 mmol), $MgSO_4$ (274 mg, 2.28mmol), and TFA (110 μL, 1.52 mmol) in CH_2Cl_2 (5.0 mL) was stirred for 24 h at 23 ºC. The reaction mixture was added to saturated $NaHCO_3$ aqueous solution and extracted with CH_2Cl_2. Organic layers were combined, washed with brine, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography (AcOEt:CH_2Cl_2 = 1:1) to afford **7b** (107 mg, 22%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$-CD_3OD): δ 1.23-1.39 (m, 4H), 1.78-1.81 (m, 3H), 1.90-1.92 (m, 1H), 2.47-2.52 (m, 1H, C*H*NHCHAr), 3.11-3.15 (m, 1H, C*H*NHC=O), 4.93 (s, 1H, C*H*Ar), 6.75 (d, *J* = 8.5 Hz, 1H, Ar*H*), 7.27 (dd, *J* = 2.0 Hz, 8.5 Hz, 1H, Ar*H*), 7.30 (d, *J* = 2.0 Hz, 1H, Ar*H*). ^{13}C NMR (125 MHz, $CDCl_3$-CD_3OD): δ 23.3, 24.2, 29.8, 30.6, 52.3, 57.6, 57.7, 110.8, 117.7, 125.5, 130.1, 131.4, 155.0, 169.9. HRMS: calcd for $C_{14}H_{18}BrN_2O_2$ (MH^+) 325.0546, found 325.0551.

(4a*S,8a*S**)-3-(2-Hydroxy-5-cyanophenyl)octahydroquinoxalin-2(1*H*)-one (8a)**



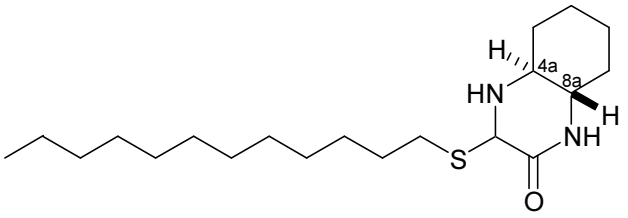
To a solution of imine **1** (152 mg, 1.00 mmol) in 200 mM sodium phosphate, pH 8.0 (9.50 mL), a solution of 4-cyanophenol (143 mg, 1.20 mmol) in DMSO (0.50 mL) was added and the mixture was stirred for 72 h at 23 ºC. The reaction mixture was diluted with water and extracted with CH_2Cl_2. Organic layers were combined, washed with brine, dried over

Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography (AcOEt: CH_2Cl_2 = 1:1) to afford **8** (10.4 mg, 4%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$-CD_3OD): δ 1.27-1.45 (m, 4H), 1.82-1.93 (m, 4H), 2.66-2.71 (m, 1H, C*H*NHCHAr), 3.22-3.26 (m, 1H, C*H*NHC=O), 4.71 (s, 1H, C*H*Ar), 6.90 (d, J = 8.5 Hz, 1H, Ar*H*), 7.49 (dd, J = 2.1 Hz, 8.5 Hz, 1H, Ar*H*), 7.61 (d, J = 2.1 Hz, 1H, Ar*H*). ^{13}C NMR (125 MHz, $CDCl_3$-CD_3OD): δ 23.4, 24.1, 30.0, 30.6, 57.0, 57.6, 61.4, 101.9, 117.3, 119.1, 124.8, 133.4, 134.8, 160.1, 169.5. HRMS: calcd for $C_{15}H_{18}N_3O_2$ (MH^+) 272.1393, found 272.1392.

5. Reactions of imine 1 with potential nucleophiles

A 100 mM solution of imine **1** in 200 mM sodium phosphate buffer, pH 7.5 was prepared immediately before starting reaction. To the 100 mM solution of **1** (950 μL), a 500 mM solution of 4-methylphenol in DMSO (50 μL) was added at rt (23 ºC) and the mixture was stirred at the same temperature for 24 h. The reaction mixture was diluted with water and the mixture was extracted with CH_2Cl_2. Organic layers were combined, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude mixture was dissolved in $CDCl_3$ and analyzed by ^{1}H NMR. Potential nucleophiles tested: imidazole, indole, 3-methylindole, *n*-butylamine, 2-propanol, *p*-anisidine, and 1-dodecanethiol. For reactions with imidazole, reaction using 125 mM (final concentration) of imidazole was also tested. No addition reactions proceeded except reactions with indole and with 1-dodecanethiol. For reactions with indole and with 1-dodecanethiol, addition products were isolated. See below.

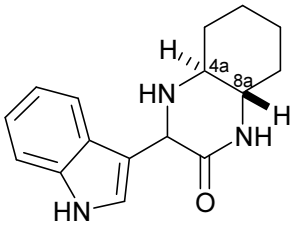
(4a*S,8a*S**)-3-(Dodecylthio)octahydroqunoxalin-2(1*H*)-one (9)**



To a solution of imine **1** (152 mg, 1.00 mmol) in 200 mM sodium phosphate, pH 8.0 (9.50 mL), a solution of 1-dodecanethiol (243 mg, 1.20 mmol) in DMSO (0.50 mL) was added and the mixture was stirred for 12 h at 23 ºC. The reaction mixture was diluted with water and extracted with CH_2Cl_2. Organic layers were combined, washed with brine, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography (AcOEt) to afford **9** (250 mg, 71%) as a colorless solid. ^{1}H NMR (500

MHz, $CDCl_3$): δ 0.88 (t, J = 7.0 Hz, 3H, CH_3), 1.20-1.35 (m, 18H, $CH_3(CH_2)_9CH_2CH_2S$), 1.35-1.46 (m, 4H), 1.61-1.67 (m, 2H), 1.73-1.79 (m, 4H), 1.84 (br, 1H, N*H*), 2.74-2.83 (m, 2H, CH_2S), 2.89-2.94 (m, 1H, C*H*NHCHAr), 3.03-3.08 (m, 1H, C*H*NHC=O), 4.74 (s, 1H, NHC*H*S), 5.69 (brs, 1H, N*H*C=O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 22.7, 23.8, 24.3, 29.0, 29.2, 29.3, 29.51, 29.58, 29.62, 29.64, 29.8, 30.3, 31.0, 31.9, 32.8, 53.6, 57.9, 64.6, 169.0. HRMS: calcd for $C_{20}H_{39}N_2OS$ (MH^+) 355.2777, found 355.2777.

(4a*S,8a*S**)-3-(1*H*-Indol-3-yl)octahydroqunoxalin-2(1*H*)-one (10)**



To a solution of imine **1** (29.1 mg, 0.191 mmol) in 200 mM sodium phosphate, pH 7.0 (9.50 mL), a solution of indole (33.6 mg, 0.287 mmol) in DMSO (0.50 mL) was added and the mixture was stirred for 24 h at 23 ºC. The reaction mixture was diluted with water and extracted with CH_2Cl_2. Organic layers were combined, washed with brine, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography (CH_2Cl_2:MeOH = 10:1) to afford **10** (44.7 mg, 87%, dr = 1:1) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.23-1.87 (m, 8H), 2.65 (m, 1Hx1/2H, C*H*NHCHAr), 2.72 (m, 1Hx1/2, C*H*NHCHAr), 3.10-3.15 (m, 1Hx1/2, C*H*NHC=O), 3.26-3.31 (m, 1Hx1/2, C*H*NHC=O), 4.93 (s, 1Hx1/2, NHC*H*C=O), 5.14 (s, 1Hx1/2, NHC*H*C=O), 6.21 (brs, 1Hx1/2, N*H*C=O), 6.26 (brs, 1Hx1/2, N*H*C=O), 6.97 (s, 1Hx1/2, C=C*H*NH), 7.11 (s, 1Hx1/2, C=C*H*NH), 7.09-7.20 (m, 2H, Ar*H*), 7.31 (m, 1Hx1/2, Ar*H*), 7.34 (m, 1Hx1/2, Ar*H*), 7.68 (d, J = 7.9 Hz, 1Hx1/2, Ar*H*), 7.72 (d, J = 7.9 Hz, 1Hx1/2, Ar*H*), 8.46 (brs, 1Hx1/2, C=CHN*H*), 8.53 (brs, 1Hx1/2, C=CHN*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 23.6, 24.3, 24.6, 30.2, 30.3, 31.1, 31.1, 31.2, 52.6, 57.2, 57.8, 57.9, 58.2, 111.5, 111.5, 118.1, 118.6, 119.3, 119.5, 121.8, 121.9, 123.3, 123.5, 124.4, 125.9, 136.3, 171.6. HRMS: calcd for $C_{16}H_{20}N_3O$ (MH^+) 270.1601, found 270.1603.

6. Reactions of imine 1 and 11 with peptides and proteins

Reactions in Table 3 were performed by the procedures for the reactions of Table 1, entries 16-18.

Addition products of Gly-Tyr to imine **1**: Mono-addition product HRMS: calcd for $C_{19}H_{27}N_4O_5$ (MH^+) 391.1976, found 391.2085. Di-addition product HRMS: calcd for $C_{27}H_{39}N_6O_6$ (MH^+) 543.2925, found 543.2917.

Addition products of Ala-Tyr-Ala to imine **1**: Mono-addition product HRMS: calcd for $C_{23}H_{34}N_5O_6$ (MH^+) 476.2503, found 476.2524. Di-addition product HRMS: calcd for $C_{31}H_{46}N_7O_7$ (MH^+) 628.3463, found 628.3471.

Addition products of Gly-Tyr to imine **11**: Mono-addition product HRMS: calcd for $C_{21}H_{29}N_4O_7$ (MH^+) 449.2031, found 449.2043. Di-addition product HRMS: calcd for $C_{31}H_{42}N_6O_{10}$ (MH^+) 659.3035, found 659.3046.

Addition products of Ala-Tyr-Ala to imine **11**: Mono-addition product HRMS: calcd for $C_{25}H_{36}N_5O_8$ (MH^+) 534.2558, found 534.2563. Di-addition product HRMS: calcd for $C_{35}H_{50}N_7O_{11}$ (MH^+) 744.3563, found 744.3560.

Reactions of imine 1 with 24-mer peptide YKLLKELLAKLKWLLRKLLGPTSL

Condition 1. [Imine **1**] 25 mM and [peptide] 3.75 mM in 150 mM sodium phosphate buffer, pH 7.0 at 37 ºC for 24 h. Peptides were purified by C18 Spin Column. Addition product of the peptide to imine **1**: ESI-MS: (+2 charged) 1495.5. Unmodified peptide: ESI-MS: (+2 charged) 1419.4.

Condition 2. [Imine **1**] 6.2 mM and [peptide] 99 μM in 74 mM Tris buffer, pH 8.0 at 23 ºC for 24 h. Peptides were purified by C18 Spin Column. Addition product of the peptide to imine **1**: ESI-MS: (+2 charged) 1497.9. Unmodified peptide: ESI-MS: (+2 charged) 1419.4.

Condition 3. [Imine **1**] 6.2 mM and [peptide] 99 μM in 19 mM HEPES buffer, pH 7.5 at 23 ºC for 24 h. HRMS analysis was performed without purification. Addition product of the peptide to imine **1**: HRMS: calcd for $C_{138}H_{240}N_{33}O_{36}$ x 1/2 ($[M+2H]^{2+}$ x 1/2) (+2

charged) 1495.9023, found 1494.9311.

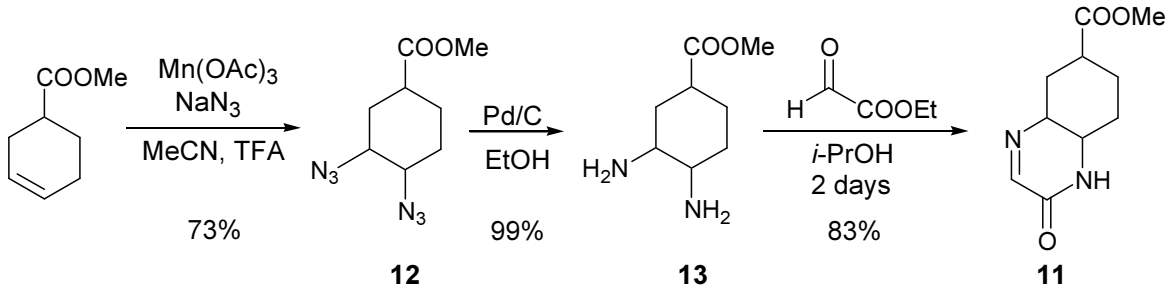
Reactions of imine 1 with lysozyme (from chicken egg white)

Condition 1. [Imine **1**] 50 mM and [lysozyme] 250 μM in 100 mM sodium phosphate buffer, pH 7.0 at 37 ºC for 48 h. Proteins were purified by C18 Spin Column. Addition product: ESI-MS: 14458. Unmodified protein: ESI-MS: 14306.

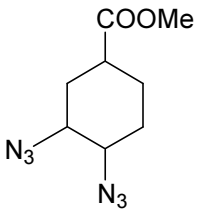
Condition 2. [Imine **1**] 50 mM and [lysozyme] 50 μM in 100 mM sodium phosphate buffer, pH 7.0 at 37 ºC for 48 h. Proteins were purified by C18 Spin Column. Addition product: ESI-MS: 14458. Unmodified protein: ESI-MS: 14306.

Myoglobin, which does not have an accessible tyrosine on the folded surface,[1] was not modified with **1** under the conditions used for the reactions of **1** with lysozyme.

7. Synthesis of 11



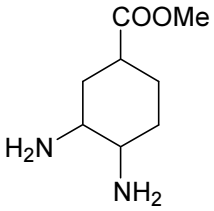
3,4-Diazidocyclohexanecarboxylic acid methyl ester (12)



To a mixture of methyl 3-cyclohexene-1-carboxylate (981 mg, 7.00 mmol), Mn $(OAc)_3 \cdot 2H_2O$ (5.63 g, 21.0 mmol), and NaN_3 (2.28 g, 35.0 mmol) in CH_3CN (70.0 mL), TFA (7.00 mL) was added at -20 ºC under Ar.[2] The mixture was stirred for 5 h at the same temperature. The reaction was added to saturated $NaHSO_3$ aqueous solution, and extracted with CH_2Cl_2. Organic layers were combined, washed with saturated Na_2CO_3 aqueous solution and with brine, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography (hexane:AcOEt = 10:1) to afford **12**

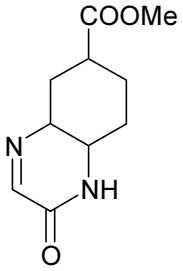
(diastereomer mixture, 1.15 g, 73%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ (major isomer) 1.54-1.68 (m, 3H), 1.92-1.98 (m, 1H), 2.00-2.06 (m, 1H), 2.27-2.32 (m, 1H), 2.72-2.76 (m, 1H), 3.38 (m, 1H), 3.61 (m, 1H), 3.71 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.4, 24.8, 26.2, 26.6, 29.7, 30.1, 32.9, 36.7, 37.8, 40.9, 52.0, 60.1, 60.6, 61.0, 62.2, 63.7, 63.9, 174.2. HRMS: calcd for $C_8H_{12}N_6O_2Na$ (MNa^+) 247.0914, found 247.0921.

3,4-Diaminocyclohexanecarboxylic acid methyl ester (13)



A mixture of compound **12** (1.55 g, 6.91 mmol) and Pd/C (200 mg) in EtOH (100 mL) was stirred under H_2 for 20 h at rt. The reaction mixture was filtrated and concentrated under reduced pressure to afford **13** (1.19 g, 99%). ^{1}H NMR (500 MHz, $CDCl_3$): δ (major isomer) 1.25-1.37 (m, 2H), 1.50-1.58 (m, 1H), 1.76-1.81 (m, 1H), 2.11-2.16 (m, 1H), 2.24-2.33 (m, 2H), 2.50 (m, 1H), 2.72-2.76 (m, 1H), 3.69 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (major isomer) 26.2, 31.7, 35.3, 39.3, 51.7, 54.0, 56.8, 175.1. HRMS: calcd for $C_8H_{17}N_2O_2$ (MH^+) 173.1284, found 173.1282.

Compound 11



To a solution of compound **13** (1.19 g, 6.90 mmol) in 2-PrOH (10.0 mL), a solution of ethyl glyoxylate polymer form (45-50% in toluene, 0.73 mL, 3.46 mmol) in 2-PrOH (10.0 mL) was added dropwise over 15 min at rt. The mixture was stirred for 2 days at the same temperature, concentrated under reduced pressure, and purified by flash column chromatography (CH_2Cl_2:MeOH = 20:1) to afford **11** (602 mg, 83%) as a pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.51-1.74 (m, 4H), 2.25-2.39 (m, 2H), 2.79-2.94 (m, 1H), 3.07-3.21 (m, 1H), 3.34-3.45 (m, 1H), 3.72 (s, 3H, OC*H*$_3$), 6.49 (br, 1H, N*H*), 7.73 (m, 1H, N=C*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.9, 26.7, 27.8, 28.2, 31.8, 32.2, 38.0, 39.1,

50.8, 52.0, 52.1, 53.9, 59.6, 62.7, 156.5, 156.7, 174.1, 174.4. HRMS: calcd for $C_{10}H_{15}N_2O_3$ (MH^+) 211.1077, found 211.1077.

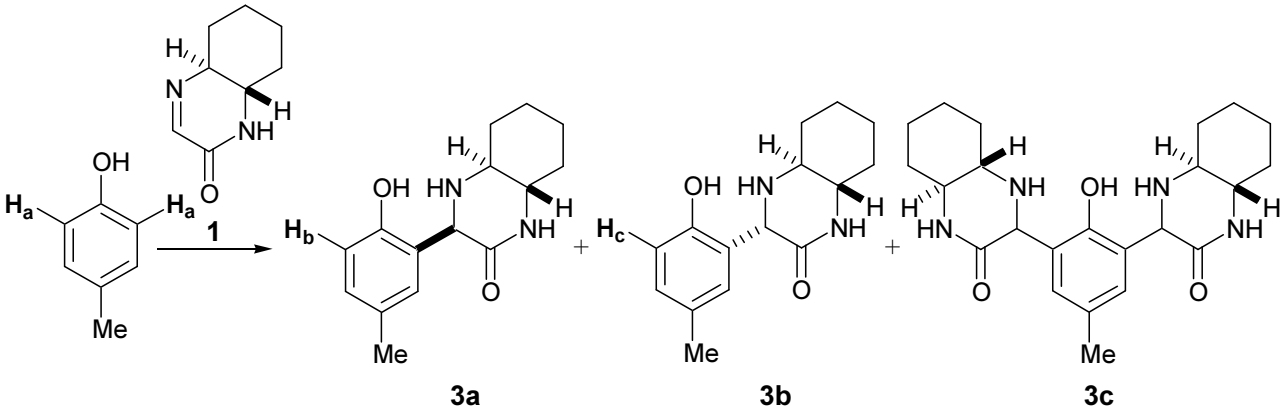
References

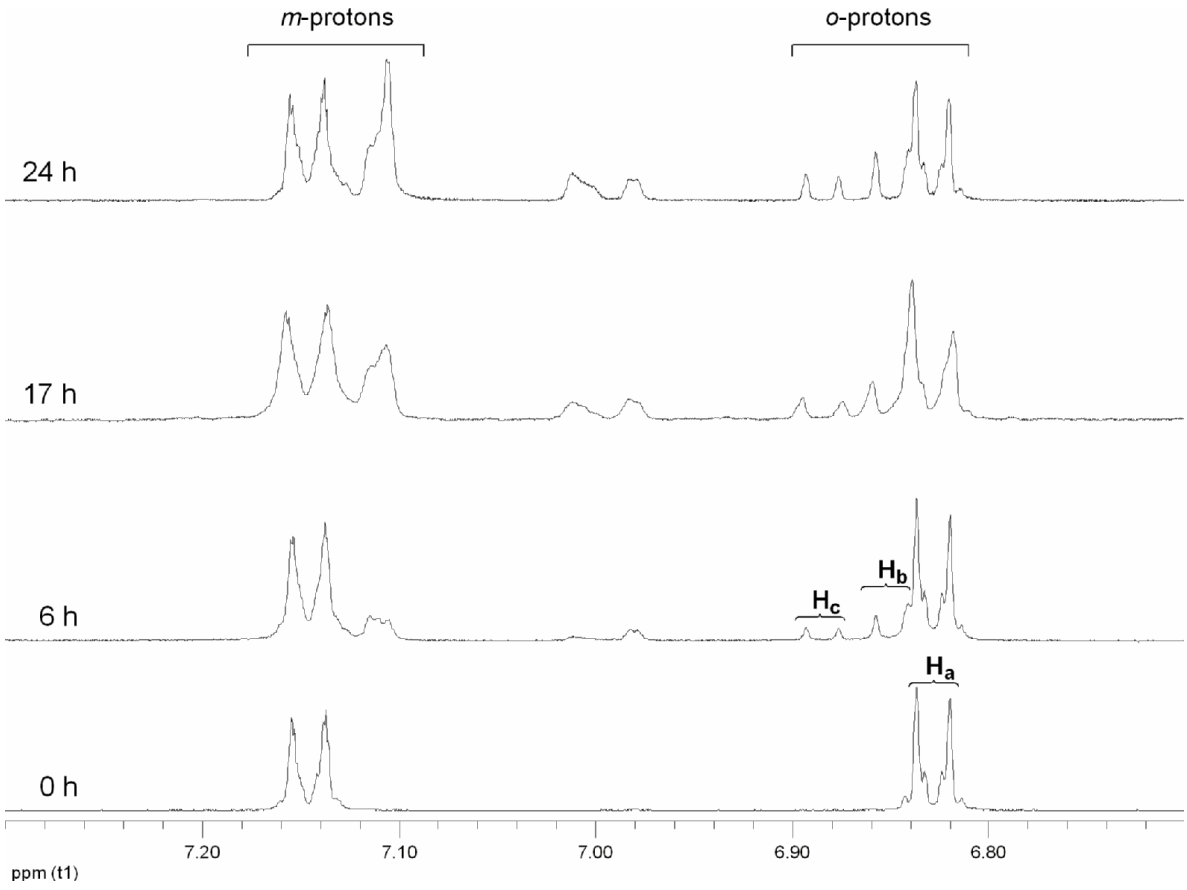
1. (a) Joshi, N. S.; Whitaker, L. R.; Francis, M. B. *J. Am. Chem. Soc.* **2006**, *128*, 1080. (b) Ly, T.; Julian, R. R. *J. Am. Chem. Soc.* **2008**, *130*, 351.
2. (a) Snider, B. B.; Lin, H. *Synth. Commun.* **1998**, *28*, 1913. (b) Fristad. W. F.; Brandvold, T. A.; Peterson, J. R.; Thompson, S. R. *J. Org. Chem.* **1985**, *50*, 3647.

8. ^{1}H NMR spectra of reaction mixtures

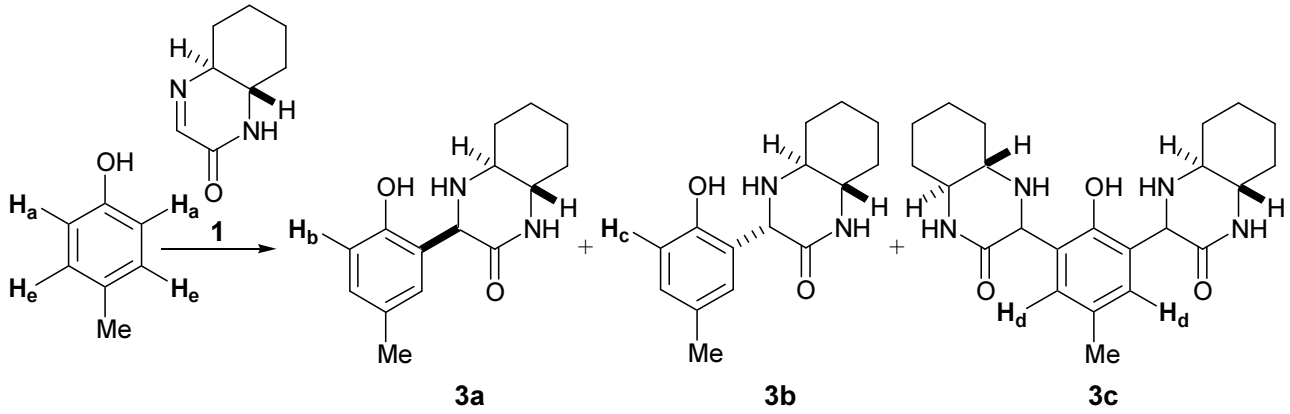
See also section 2-1.

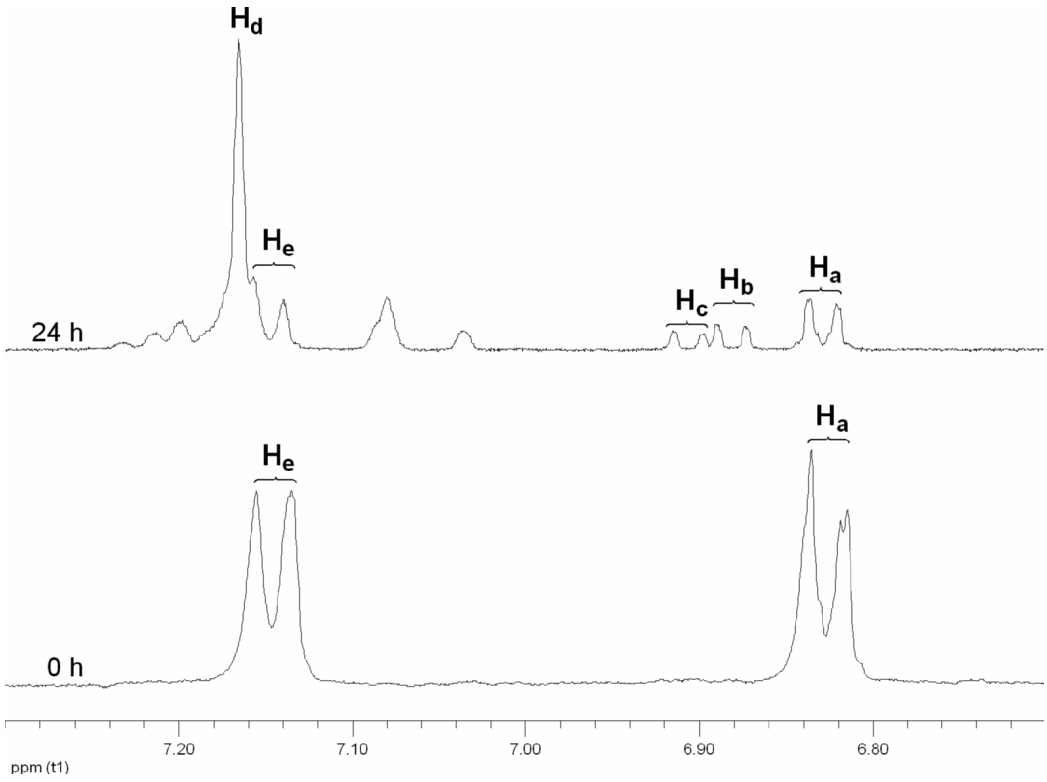
^{1}H NMR spectra of the reaction of Table 1, entry 18 (pH 7)



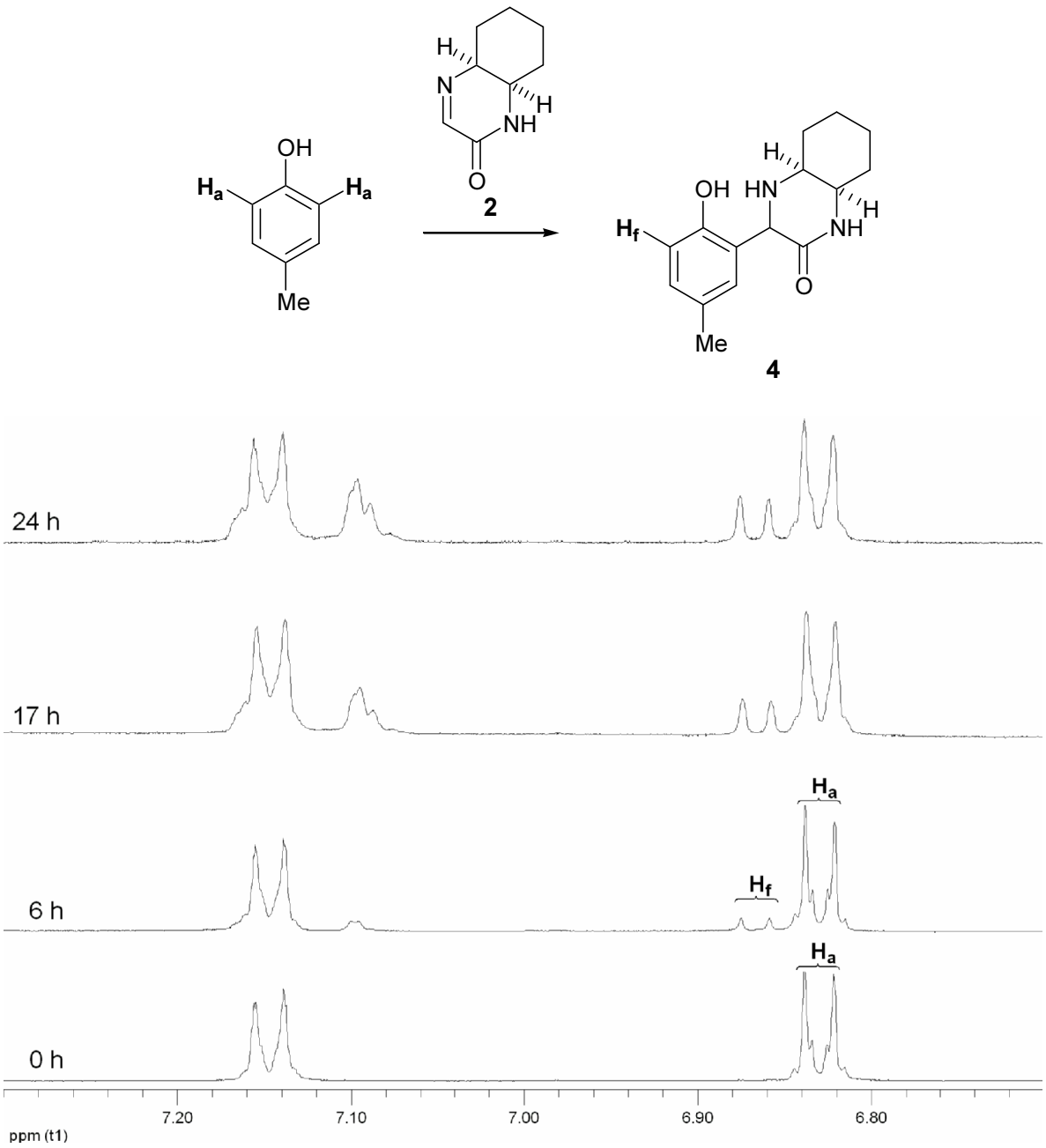


^{1}H NMR spectra of the reaction of Table 1, entry 16 (pH 5)





^{1}H NMR spectra of the reaction of Table 2, entry 7 (pH 7)



^{1}H NMR spectra of the reaction of Table 3, entry 2 (pH 7)

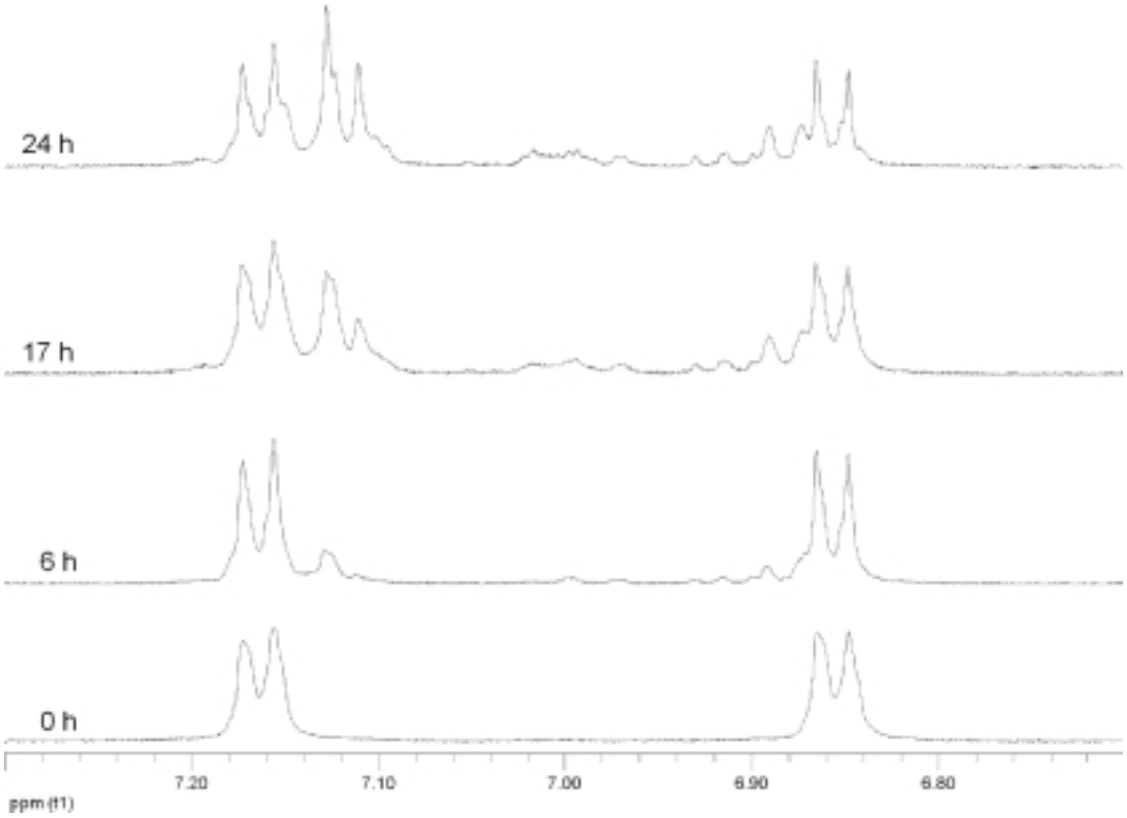


Table S1. Chemical shifts of aryl protons of compounds reported in Table 3.[a]

entry of Table 3	*ortho* protons of the phenolic OH			*meta* protons of the phenolic OH		
	sm[b]	products[c]	range[d]	sm[b]	products[c]	range[d]
entry 1	6.83 (d)	6.89 (d), 6.90 (d), 6.91 (d), 6.92 (d)	6.80-6.93	7.14 (d)	7.13 (s), 7.15 (s)	7.10-7.23
entry 2	6.86 (d)	6.88 (d), 6.90 (d), 6.92 (d)	6.81-6.94	7.16 (d)	7.12 (d), 7.13 (s), 7.15 (s)	7.07-7.21
entry 3	6.85 (d)	6.90 (d), 6.91 (d)	6.80-6.92	7.16 (d)		7.15-7.20
entry 4	6.87 (d)	6.89 (d), 6.90 (d), 6.92 (d), 6.93 (d)	6.83-7.05	7.18 (d)		7.10-7.21
entry 5	6.86 (d)	6.88 (d), 6.90 (d), 6.92 (d)	6.80-6.93	7.17 (d)		7.07-7.20
entry 6	6.87 (d)		6.83-6.94	7.18 (d)		7.10-7.25
entry 7	6.87 (d)		6.83-6.94	7.18 (d)		7.10-7.25

[a] Selected chemical shifts that were assigned. For doublet (d), J = 8.1~8.5 Hz. [b] Unmodified peptide. [c] Diastereomers of mono- and di-addition products. [d] Areas of the signals of these ranges were used for the determination of percentage of modified *ortho* positions of the phenolic OH.

As described in the main text, reactions of imine **1** with *p*-cresol afforded product diastereomers. For the reactions of peptides, the number of possible diastereomers of the mono-addition is four, because the peptides are enantiomerically pure and imine **1** is racemic. In addition, di-addition products form. When the reaction mixtures in Table 3 were analyzed by HPLC with mass analysis, multiple peaks corresponding to the diastereomers of the products were observed. Some peaks overlapped under all HPLC conditions tested. Thus, HPLC with mass analysis did not provide simple estimations of the yields.