Supporting Information

Solid-Phase Synthesis of β-Amino Ketones Via DNA-Compatible Organocatalytic Mannich Reactions

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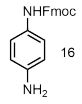
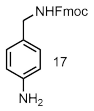
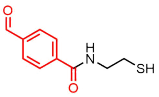
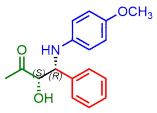
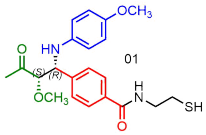
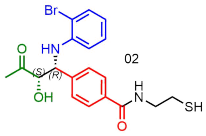
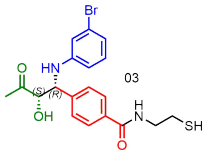
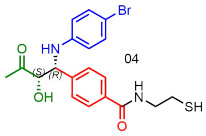
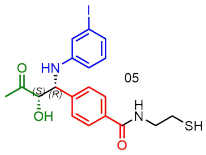
Supp. Table 1: Table of all chemicals used, excluding solvents and reagents for DNA damage assay

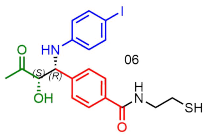
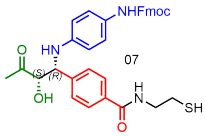
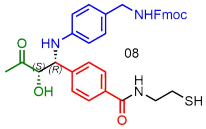
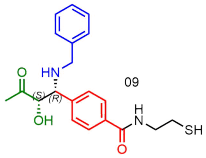
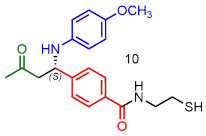
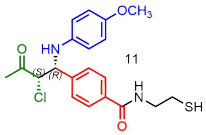
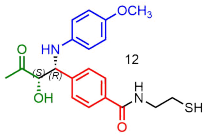
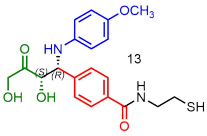
Name	Other name	CAS	Source
4-methoxyaniline	p-anisidine	104-94-9	Sigma-Aldrich
1-hydroxypropan-2-one	hydroxyacetone	116-09-6	Acros Organics
(R)-proline	(D)-proline	344-25-2	Alfa Aesar
(S)-proline	(L)-proline	147-85-3	Fisher Scientific
benzaldehyde		100-52-7	Opko Chemical
3-bromoaniline	m-bromoaniline	591-19-5	Sigma-Aldrich
4-iodoaniline	p-iodoaniline	540-37-4	Sigma-Aldrich
benzene-1,4-diamine	p-phenylenediamine	106-50-3	Alfa Aesar
4-aminobenzylamine	4-(Aminomethyl)aniline	4403-71-8	Sigma-Aldrich
benzylamine	1-Phenylmethanamine	100-46-9	Sigma-Aldrich
propan-2-one	acetone	67-64-1	Acros Organics
1-chloropropan-2-one	chloroacetone	78-95-5	Acros Organics
1-methoxypropan-2-one	methoxyacetone	5878-19-3	Matrix Scientific
1,3-dihydroxypropan-2-one	dihydroxyacetone	96-26-4	Ark Pharm
cyclohexanone		108-94-1	Acros Organics
1-azidopropan-2-one	azidoacetone	4504-27-2	Keitou Shu
TentaGel S NH_2 resin			Rapp Polymere GmbH
Bromoacetic acid	BAA	79-08-3	Sigma-Aldrich
N,N-diisopropylcarbodiimide	DIC	693-13-0	Fluka Analytical
2,2'-dithiobis(ethylamine)	Cystamine dihydrochloride	51-85-4	Acros Organics
N-ethyl-N-(propan-2-yl)propan-2-amine	N,N-diisopropylethylamine, DIPEA	7087-68-5	Sigma-Aldrich
4-formyl benzoic acid	4-Carboxybenzaldehyde	619-66-9	Oakwood Chemicals
ethyl-2-cyano-2-hydroxyiminoacetate	oxyma	3849-21-6	Oakwood Chemicals
3,3,3-phosphanetriyltripropanoic acid HCl	Tcep-HCl	51805-45-9	Acros Organics
9-Fluorenylmethoxycarbonyl chloride	Fmoc-chloride	28920-43-6	Oakwood Chemicals
9-Fluorenylmethyl N-succinimidyl carbonate	Fmoc-OSu	82911-69-1	Chem Impex
sodium hydrogen carbonate	sodium bicarbonate	144-55-8	Amresco
magnesium sulfate		7487-88-9	Fisher Scientific
ammonium sulfate		7757-82-6	Fluka Analytical

Supp. Table 2: Table of all products investigated and their high resolution mass spectrometry

Structure	Formula	Molecular mass	Predicted MH^+	Recorded MH^+
	$C_{21}H_{18}N_2O_2$	330.387	331.14465	331.14429
	$C_{22}H_{20}N_2O_2$	344.414	345.16030	345.15891
	$C_{10}H_{11}NO_2S$	209.263	210.05887	210.05835
	$C_7H_{19}NO_3$	285.343	286.14432	286.14395
	$C_{21}H_{26}N_2O_4S$	402.509	403.16915	403.16768
	$C_{19}H_{21}BrN_2O_3S$	437.352	437.05345 439.05140	437.05313 439.05099
	$C_{19}H_{21}BrN_2O_3S$	437.352	437.05345 439.05140	437.05356 439.05127
	$C_{19}H_{21}BrN_2O_3S$	437.352	437.05345 439.05140	437.05310 439.05069
	$C_{19}H_{21}IN_2O_3S$	484.352	485.03958	485.03835

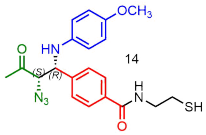
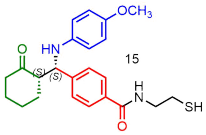
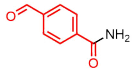
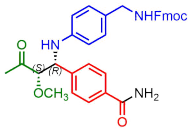
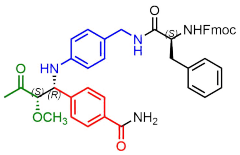
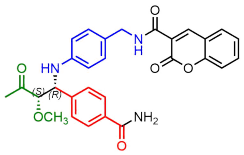
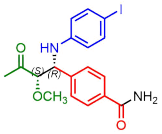
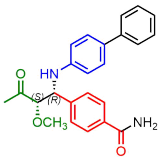
Some products received a designated number next to their structure for quick reference.

Supp. Table 3: Table of all products investigated and their high resolution mass spectrometry (cont.)

Structure	Formula	Molecular mass	Predicted MH^+	Recorded MH^+
	$C_{19}H_{21}IN_2O_3S$	484.352	485.03958	485.03949
	$C_{34}H_{33}N_3O_5S$	595.714	596.22192	596.22223
	$C_{35}H_{35}N_3O_5S$	609.741	610.23757	610.23761
	$C_{20}H_{24}N_2O_3S$	372.483	373.15859	nd
	$C_{20}H_{24}N_2O_3S$	372.483	373.15859	373.15732
	$C_{20}H_{23}ClN_2O_3S$	406.925	407.11962 409.11667	407.11921 409.11600
	$C_{20}H_{24}N_2O_4S$	388.482	389.15350	389.15359
	$C_{19}H_{21}BrN_2O_3S$	404.481	405.14842	405.14764

Some products received a designated number next to their structure for quick reference.

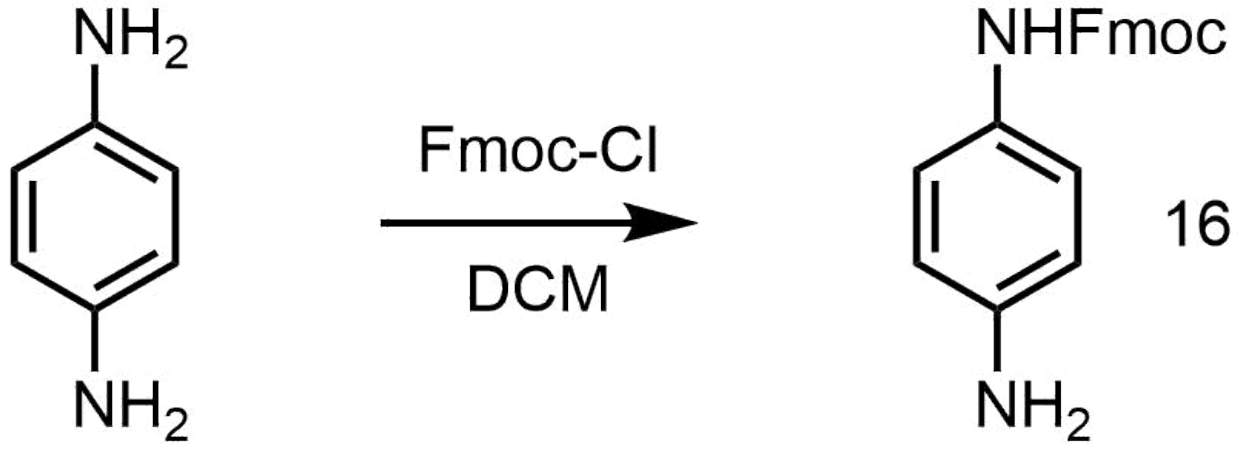
Supp. Table 4: Table of all products investigated and their high resolution mass spectrometry (cont.)

Structure	Formula	Molecular mass	Predicted MH^+	Recorded MH^+
	$C_{20}H_{23}N_5O_3S$	413.496	414.15999	nd
	$C_{23}H_{28}N_2O_3S$	412.548	413.18989	nd
	$C_8H_7NO_2$	149.149	150.05550	150.05502
	$C_{34}H_{33}N_3O_5$	563.654	564.24985	564.24841
	$C_{43}H_{42}N_4O_6$	710.831	711.31826	711.31677
	$C_{29}H_{27}N_3O_6$	513.550	514.19781	514.19769
	$C_{18}H_{19}IN_2O_3$	438.265	439.05186	439.05048
	$C_{24}H_{24}N_2O_3$	388.467	389.18652	389.18623

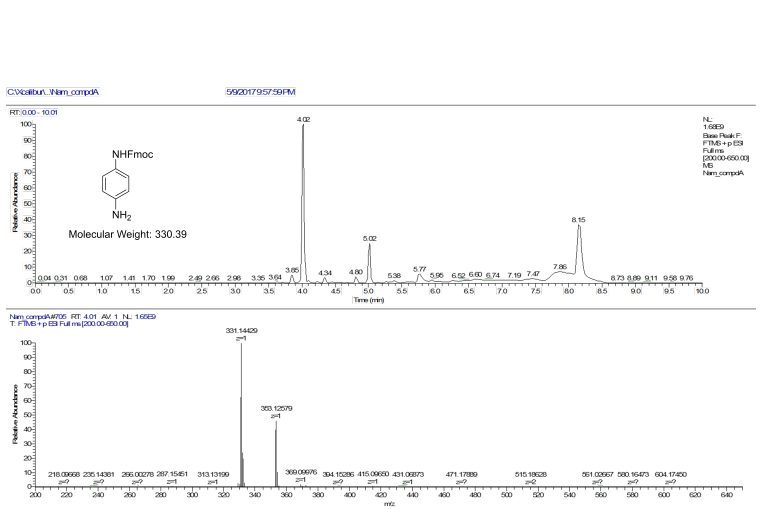
Some products received a designated number next to their structure for quick reference.

Synthesis of two aniline components for Mannich reaction

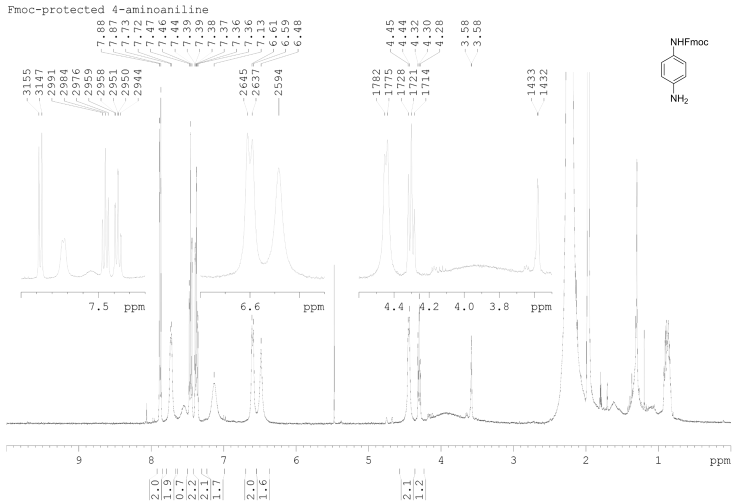
Synthesis of mono-Fmoc-protected benzene-1,4-diamine. Procedure adapted from Lee[1] and Gawande.[2] CAS of expected product: 205688-13-7. Following Scheme 1, to a 250 mL round bottom flask containing 23.1 mL solution of 0.4M benzene-1,4-diamine (1.02 g, 9.25 mmol, 1 eq.) in DCM (23.1 mL), 23.1 mL solution of 0.38M Fmoc-Cl (2.27 g, 8.77 mmol, 0.95 eq.) in DCM (23.1 mL) is added slowly, drop-wise, under vigorous stirring. Solution quickly turns into white slush. After an hour upon adding all Fmoc-chloride solution, the white solid is collected, washed with 100 mL saturated $NaHCO_3$, washed three times with water, then washed three times with cold DCM, dried on filter, ground, and collected. Product is white solid (2.634 g, 7.97 mmol, 0.91 yield). **Solubility**: only appreciably soluble in hot DMF and DMSO; quickly dissove/decompose in solution 20% v/v piperidine in DMF. **Melting**: decompose to black solid at 182-186 ℃. **HMRS**: calculated for MH^+ 331.14465; found 331.14429. **^{1}H-NMR** (400 MHz, d3-acetonitrile): 4.301 (t, 1H, J = 6.74 Hz), 4.444 (d, 2H, J = 6.16 Hz), 6.484 (s br, 2H), 6.600 (d, 2H, J = 7.64 Hz), 7.132 (s br, 2H), 7.374 (dt, 2H, J = 0.88 Hz, J = 7.44 Hz), 7.456 (t, 2H, J = 7.32), 7.726 (d br, 2H, J = 5.77), 7.875 (d, 2H, J = 7.57).



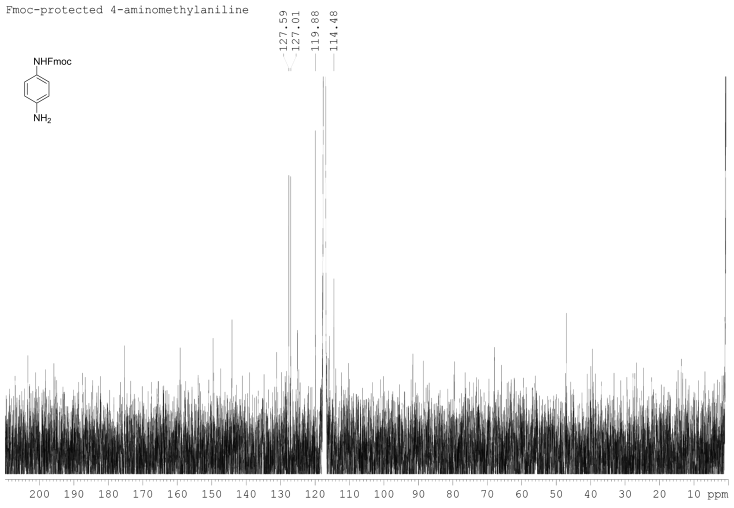
Supp. Scheme 1: Synthesis of mono-Fmoc-protected benzene-1,4-diamine



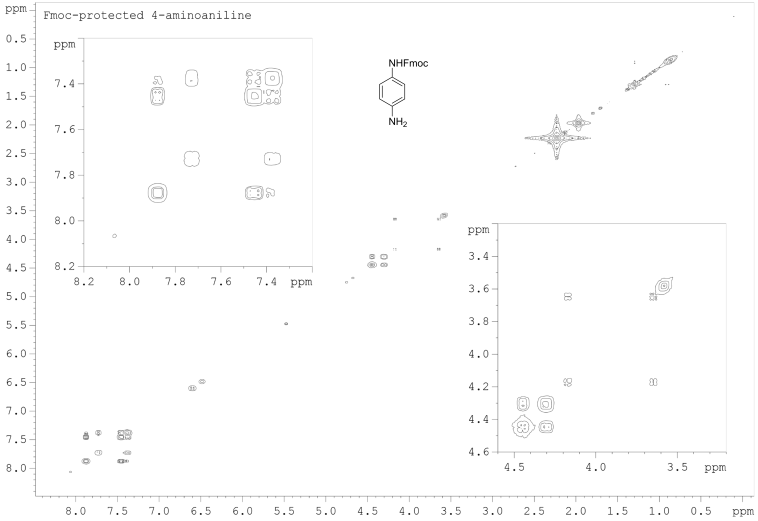
(a) HRMS



(b) HNMR



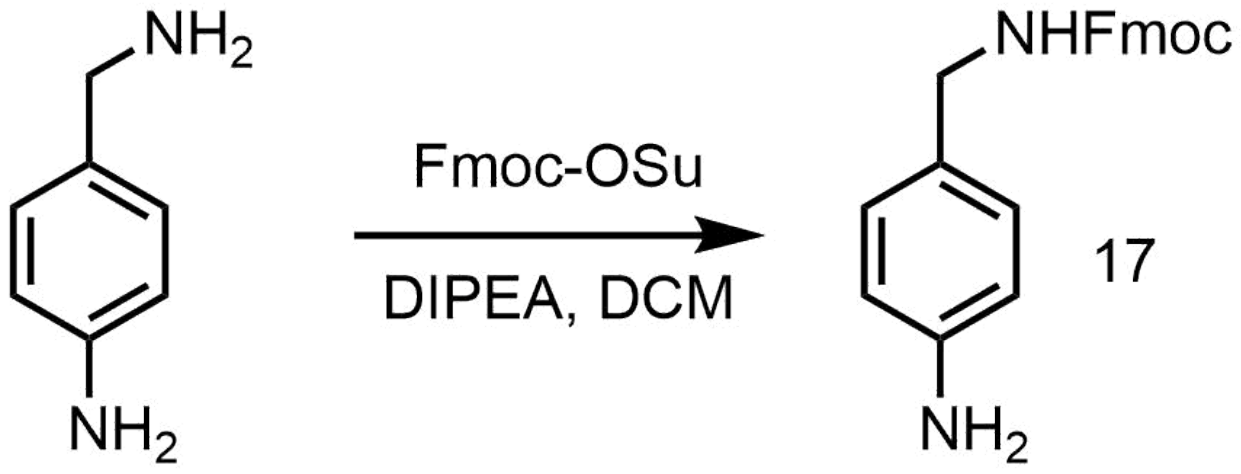
(c) CNMR



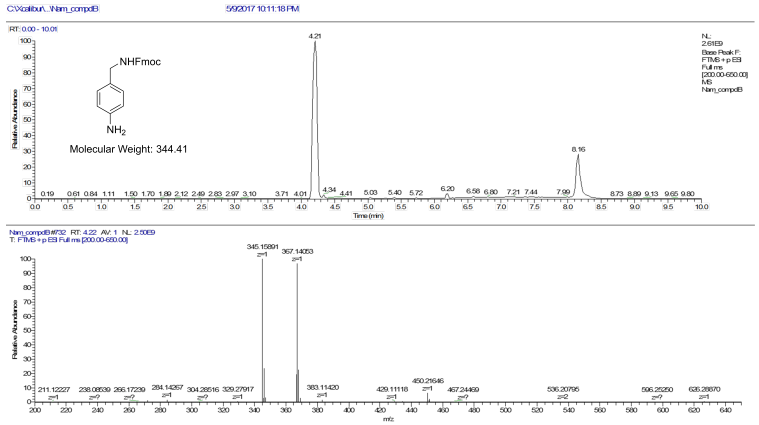
(d) COSY

Supp. Figure 1: Spectra of Fmoc-protected 4-aminoaniline

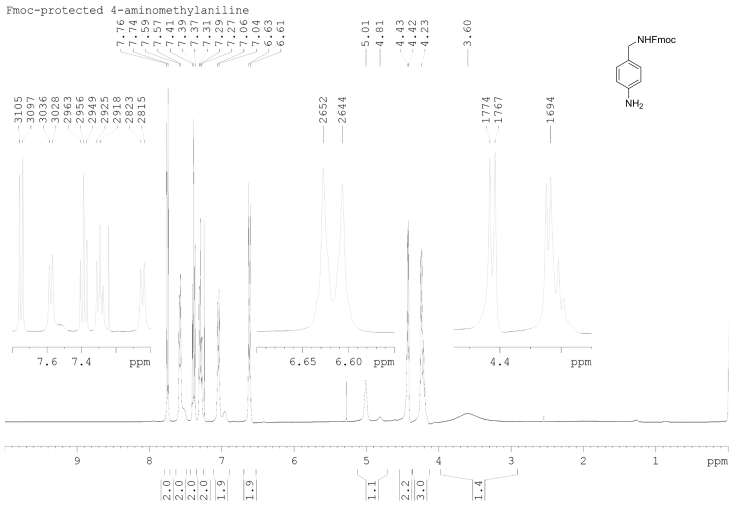
Synthesis of Fmoc-protected 4-aminomethylaniline. Procedure adapted from Montalvao.[3] CAS of expected product: 159790-81-5. Following Scheme 2, to a 250 mL round bottom flask containing 71 mL solution of 0.3M 4-aminobenzylamine (2.41 mL, 18.30 mmol, 1 eq.) and 0.285M DIPEA (2.72 mL) in DCM (71 mL), 71 mL solution of 0.285M Fmoc-OSu (7.10 g, 21.05 mmol, 0.95 eq.) in DCM (71 mL) is added slowly, drop-wise, under vigorous stirring. Solution turns into white slush. After an hour upon adding all Fmoc-OSu solution, the solution mixture is vaporized at low pressure, yielding a mixture of white solid and yellow solid. Product is re-crystalized with DCM: sufficient DCM is added to dissolve all solid; then, the mixture is chilled to −70 ℃using dry ice/acetone bath; the re-crystalized white solid is filtered, dried, ground, and collected. Product is creamy white solid (5.6743 g, 16.48 mmol, 0.78 yield - most solid is lost through mechanical transfer). **Melting**: 126.6-127.2 ℃. **HMRS**: calculated for MH^+ 345.16030; found 345.15891. **^{1}H-NMR** (400 MHz, $CDCl_3$): 4.211 (t, 1H, J = 6.96 Hz), 4.241 (s, 2H), 4.424 (d, 2H, J = 7.18 Hz), 5.008 (s br, 1H), 6.617 (d, 2H, J = 8.34 Hz), 7.046 (d, 2H, J = 8.10 Hz), 7.293 (t, 2H, J = 7.30 Hz), 7.387 (t, 2H, J = 7.34 Hz), 7.577 (d, 2H, J = 7.40 Hz), 7.750 (d, 2H, J = 7.56 Hz). **^{13}C-NMR** (400 MHz, $CDCl_3$): 44.758, 47.317, 66.603, 115.230, 119.989, 125.079, 127.062, 127.678, 128.974, 141.338, 143.999, 145.884, 156.369.



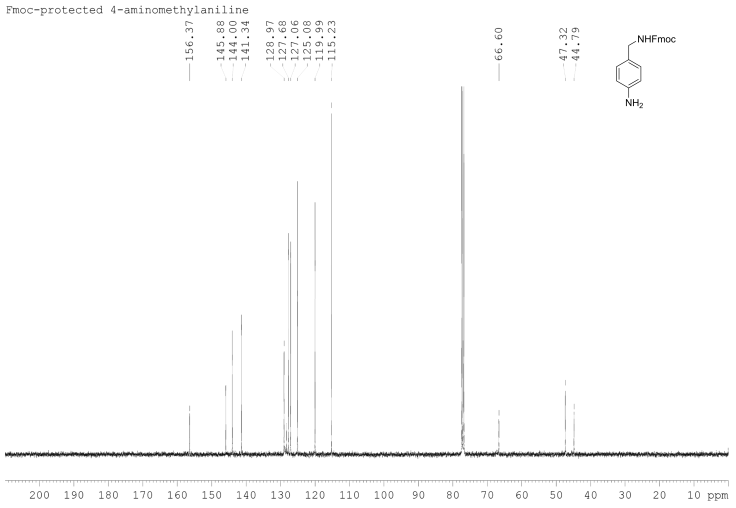
Supp. Scheme 2: Synthesis of Fmoc-protected 4-aminomethylaniline



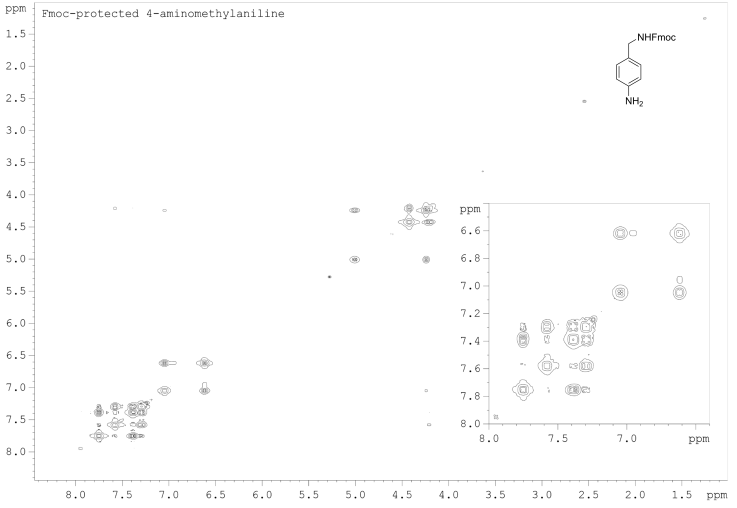
(a) HRMS



(b) HNMR



(c) CNMR



(d) COSY

Supp. Figure 2: Spectra of Fmoc-protected 4-aminomethylaniline

Resin formation and cleavage

Resin washing and re-equilibration/re-suspension. For washing, sufficient amount of solvent is added to resin, so that resin completely immersed in solvent (500 µL for 50 mg resin). Resin and solvent mixture is pulsed with vortex, sonicated to separate resin chink if necessary, and drained. For re-equilibration/re-suspension, the process is similar: solvent is introduced to resin, and the mixture is pulsed and sonicated.

General procedure for coupling amine linker onto NH_2 resin. First, NH_2 resin is incubated with solution of 1M BAA, 1M DIC in DMF for 5 minutes at 37℃. After draining BAA solution and washing, resin is incubated with solution of 1M amine linker in DMF for 1 hour at 37℃. Resin is drained and washed with DMF afterwards. All reagents are used in excess, 30 eq. compared to resin loading. Note: because of cystamine hydrochloride solubility, 2/1 v/v MeOH/DIEPA solvent mixture is used for coupling cystamine instead of DMF, with additional resin washing steps.

General procedure for coupling carboxylic acid linker onto NH_2 resin. Solution of 0.2M carboxylic acid linker, 0.2M oxyma, 0.2M DIC in DMF is incubated off resin for 5 minutes at room temperature (4 eq. each compared to resin loading). The resin is then incubated in the solution for 1 hour at room temperature. Resin is drained and washed with DMF afterwards

General procedure for disulfide bond cleavage using Tcep-HCl. Resin is incubated in solution of 0.2M tcep-HCl (4 eq.), 0.8M $NaHCO_3$ (16 eq.) in water for 1.5 hours, at room temperature. Cleavage study using LC-MS suggests that at 3 hours on-wards the amount of substance cleaved from resin remains unchanged, and at 1.5 hours the amount cleaved is about 94% of the amount obtained at 3 hours. Note on making cleavage solution: corresponding amount of tcep is added into 0.8M $NaHCO_3$ solution in open vessel to evacuate CO_2.

General procedure for methionine cleavage using cyanogen bromide. Resin is cleaved with solution of 25mg CNBr per 1 mL in 0.2M HCl, or solution of 25mg CNBr per 1 mL in 5/4/1 v/v/v ACN/AcOH/H_2O 5/4/1, at 20 eq. CNBr comparing to resin loading, for 1.5 hours at room temperature

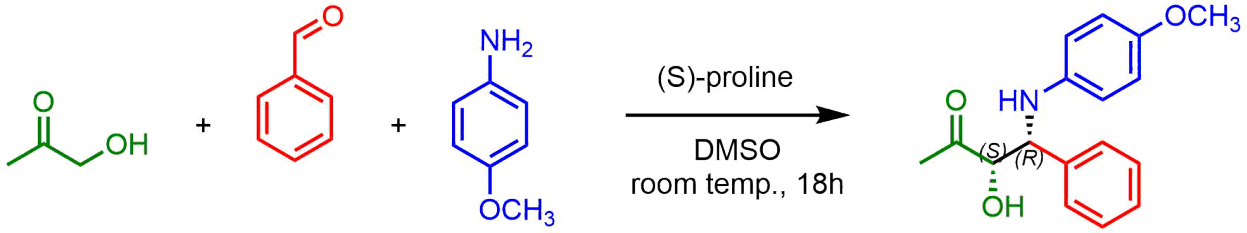
General procedure for RAM cleavage using trifluoroacetic acid. Resin is cleaved with solution of 95/2.5/2.5 v/v/v TFA/H_2O/TIPS, 500 µL for 50 mg resin, for 30 minutes at room temperature (TIPS: triisopropylsilane). After TFA is dried under a running stream of Ar, the product is redissoved in appropriate solvent.

Note: after DCM washing, and especially when equilibrating in DMSO, the resin displaying aldehyde moiety turns dark green from the regular white yellowish color of TentaGel resin.

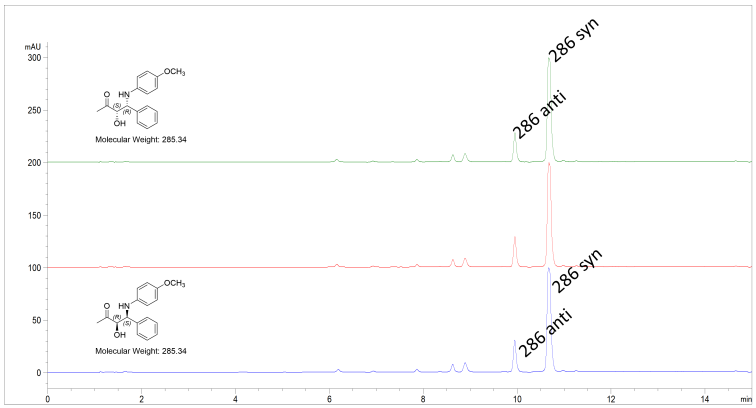
Mannich reaction

Mannich reaction in solution phase. Procedure adapted from List.[4] CAS of expected product: 402741-05-3. Following Scheme 3, solution of 0.4M benzaldehyde (0.1 mL, 0.9 mmol, 1 eq.) and 0.44M 4-methoxyaniline (133 mg, 1.1 mmol, 1.1 eq.) in DMSO (2.057 mL) is prepared in small RBF and left to react for 5 min at room tempterature. Solution of 0.1M (S)-proline (28 mg, 0.245 mmol, 0.25 eq.) and 4M 1-hydroxypropan-2-one (686 µL, 8.74 mmol, 10 eq.) in DMSO (2.057 mL) is prepared and sonicated for 5 minutes. Then, the sonicated/suspended ketone/proline solution is added to the small RBF. The RBF is flushed with argon, sealed. The reaction is left to proceed for 18 hours, under gentle stirring, at room temperature. Afterwards, the reaction is work-up with one 5 mL saturated $(NH_4)_2SO_4$ wash, extracted with two 15 mL Hex/EtOAc 2/3 v/v, dried with $MgSO_4$, and purified by column chromatography, silica gel, Hex/EtOAc 3/1 v/v. Product is yellowish oil (0.2521 g, 0.89 yield). **HMRS**: calculated for MH^+ 286.14432; found 286.14395. **Stereoselectivity**: dr 18:82 by LC-MS, assuming equal molar attenuation coefficient of diastereoisomers; ee 0.86 by chiral LC. **^{1}H-NMR** (400 MHz, $CDCl_3$): 2.229 (s, 3H), 3.608 (s, 3H), 4.262 (br s, 2H),

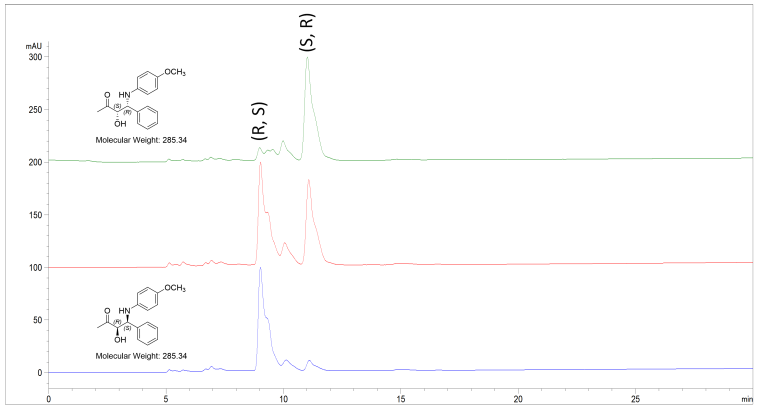
4.324 (d, 1H, J = 2.64 Hz), 4.854 (d, 1H, J = 2.6 Hz), 6.461 (d, 2H, J = 8.96 Hz), 6.631 (d, 2H, J = 8.96 Hz), 7.181-7.342 (m, 5H). **^{13}C-NMR** (400 MHz, $CDCl_3$): 25.39, 55.54, 59.24, 80.78, 114.78, 115.18, 127.47, 128.58, 139.60, 140.33, 152.34.



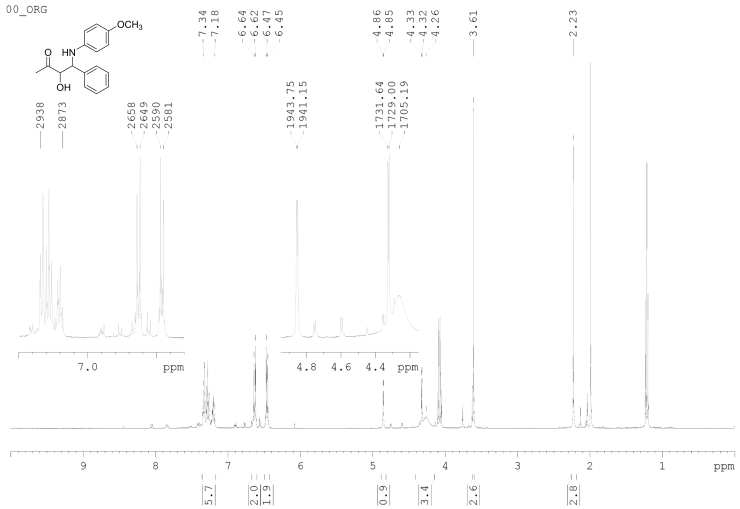
Supp. Scheme 3: Scheme of Mannich reaction with benzaldehyde, 4-methoxyaniline, and 1-hydroxypropan-2-one, catalyzed by proline



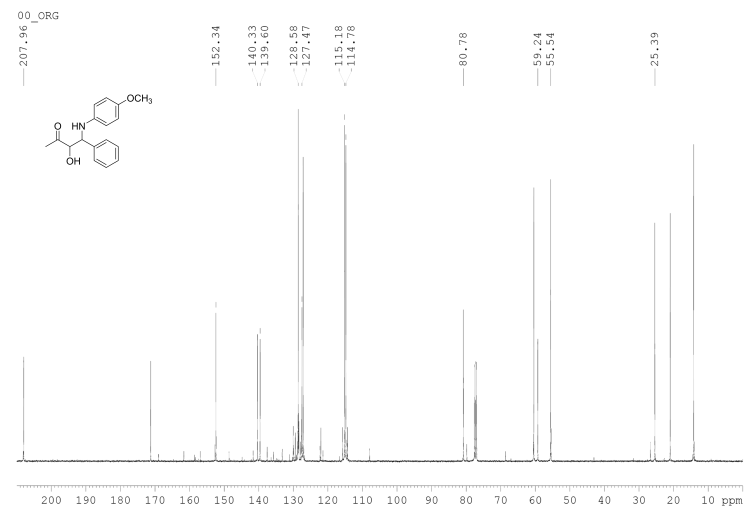
(a) LCMS



(b) Chiral LC



(c) HNMR

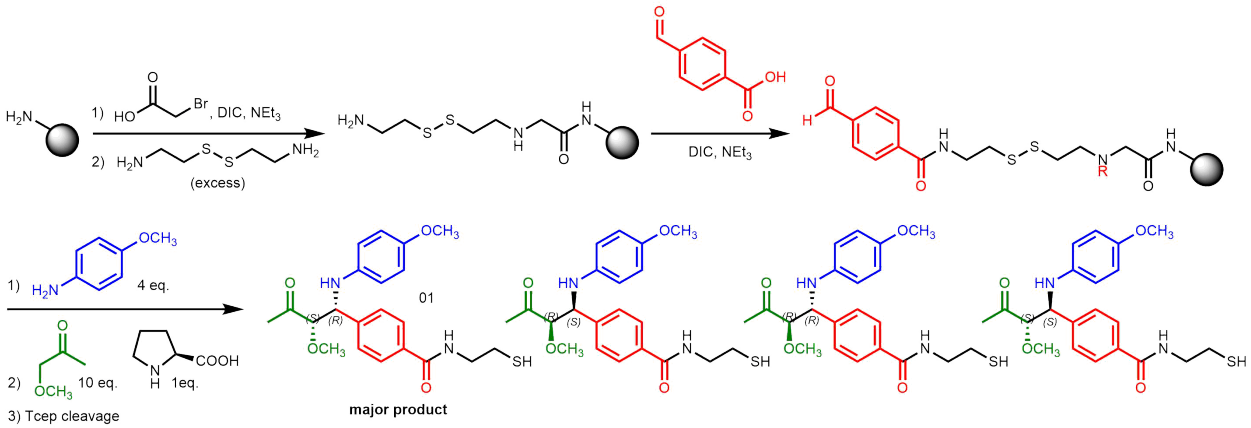


(d) CNMR

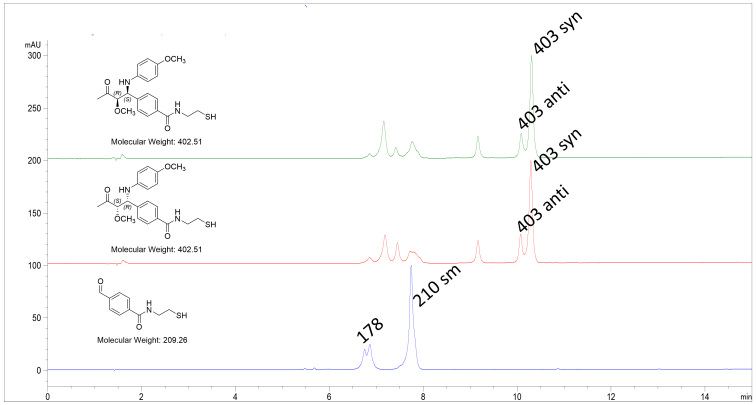
Supp. Figure 3: Spectra of Mannich reaction with benzaldehyde, 4-methoxyaniline, and 1-hydroxypropan-2-one, catalyzed by proline. **a) LC-MS:** the LC traces show (upwards order) Mannich reaction catalyzed by (S)-proline, coinjection of products from reactions catalyzed by (S)- and (R)-proline, and Mannich reaction catalyzed by (R)-proline. The number adjacent to each LC peak indicate the major molecular ion associated. The structures are known major products, and the notations syn/anti are assigned to each LC peaks.[4] **b) chiral LC:** the LC traces show (upwards order) Mannich reaction catalyzed by (S)-proline, coinjection of products from reactions catalyzed by (S)- and (R)-proline, and Mannich reaction catalyzed by (R)-proline. The notations (S, R), (R, S) are assigned to known major products following X-ray crystallography.[4]

Mannich reaction on solid support. Procedure adapted from List.[4] CAS of expected product: n/a. Following the last part of Supp Scheme 4. Resin displaying aldehyde moiety is washed three times with DMSO and re-equilibrating in DMSO in a reaction vessel. Solution 145 µL 0.4M 4-methoxyaniline (7.14 mg, 4 eq.) in DMSO (145 µL); and 145 µL solution of 1M 1-methoxypropan-2-one (13.5 µL, 10 eq.) with 0.1M (S/L)-proline catalyst (1.67 mg, 1 eq.) in DMSO (145 µL) are prepared. Resin is first incubated with solution of 4-methoxyaniline for 5 minutes, after a couple of second quick mixing by vortex resulting in a slight change of color. The ketone-proline solution is sonicated for 5 minutes, also resulting in a slight change of color. Then, the sonicated/suspension ketone-proline solution is introduced onto resin without draining the 4-methoxyaniline solution. After flushing the reaction vessel with Ar, sealing the vessel, and quick mixing by vortex, the resin is incubated in the mixture for 18 hours at room temperature, under gentle shaking. Afterwards, the reaction mixture is drained from resin, and resin is washed three times with DMSO, three times with DMF, three times with DCM, three times with water, and cleaved by incubated with 290 µL solution of 0.2M tris(2-carboxyethyl)phosphine hydrochloride (tcep–HCl) (16.63 mg, 4 eq.) and 0.8M $NaHCO_3$ (19.49 mg, 16 eq.) in water (290 µL), pH 6-7, for 1.5 hour at room temperature, under gentle shaking. The resin and resulting solution after cleavage are separated, and each extracted five times with 500 µL DCM. All extracted fractions are combined, dried with $MgSO_4$, filtered into vial, flushed with Ar, sealed, and stored at 4℃. Product is a yellowish oil (0.76 yield, comparing to cleaving only the starting material - resin displaying aldehyde moiety). Product quickly decomposes out of solution. **HMRS**: calculated for MH^+ 403.16915; found 403.16768. **Stereoselectivity**: dr 21:79 by LC-MS, assuming equal molar attenuation coefficient of diastereoisomers; ee 81% by chiral LC; the specific conformation syn/(S, R) is assigned by comparing retention times/order of peaks to those of Mannich reaction in solvent phase. The syn/anti diastereoisomer is separated by HPLC. For major-syn diastereoisomer, retention time 25.3-25.9 min. **^{1}H-NMR** (400 MHz, $CDCl_3$): 1.39 (t, 1H, J = 8.6 Hz), 2.20 (s, 3H), 2.68 (q, 2H, J = 7.2 Hz), 3.30 (s, 3H), 3.48 (q, 2H, J = 6.3

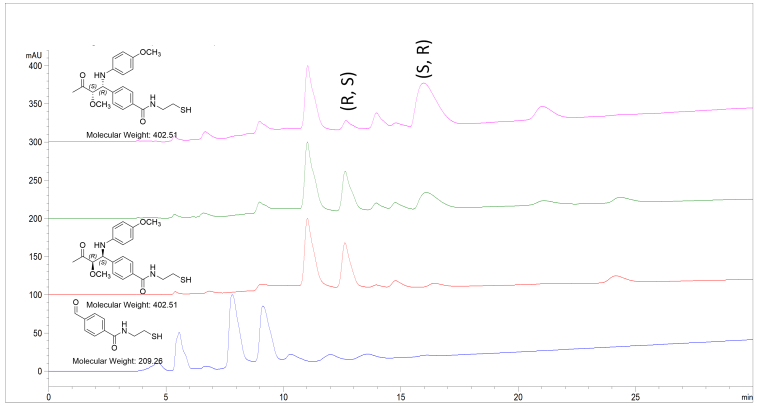
Hz), 3.67 (s, 3H), 3.81 (d, 1H, J = 2.8 Hz), 4.80 (d, 1H, J = 3.2 Hz), 6.46 (d, 2H, J = 9.2 Hz), 6.66 (d, 2H, J = 9.2 Hz), 7.44 (d, 2H, J = 8.0 Hz), 7.76 (d, 2H, J = 8.4 Hz). For minor anti-diastereoisomer, retention time 23.5-23.9 min. **^{1}H-NMR** (400 MHz, $CDCl_3$): 1.38 (t, 1H, J = 8.4 Hz), 1.89 (s, 3H), 2.69 (q, 2H, J = 7.1 Hz), 3.39 (s, 3H), 3.48 (q, 2H, J = 6.2 Hz), 3.69 (s, 3H), 3.90 (d, 1H, J = 5.5 Hz), 4.66 (d, 1H, J = 5.7 Hz), 6.52 (d, 2H, J = 9.0 Hz), 6.68 (d, 2H, J = 9.0 Hz), 7.42 (d, 2H, J = 8.4 Hz), 7.72 (d, 2H, J = 8.4 Hz). ^{1}H-NMR spectra of the syn diastereoisomerr is consistent with reported spectra of similar product from solvent phase Mannich reaction, having $\frac{2.8+3.2}{2}$ = 3.0 Hz vicinal coupling constant at chiral centers, instead of $\frac{5.5+5.7}{2}$ = 5.6 Hz for the anti diastereoisomer.



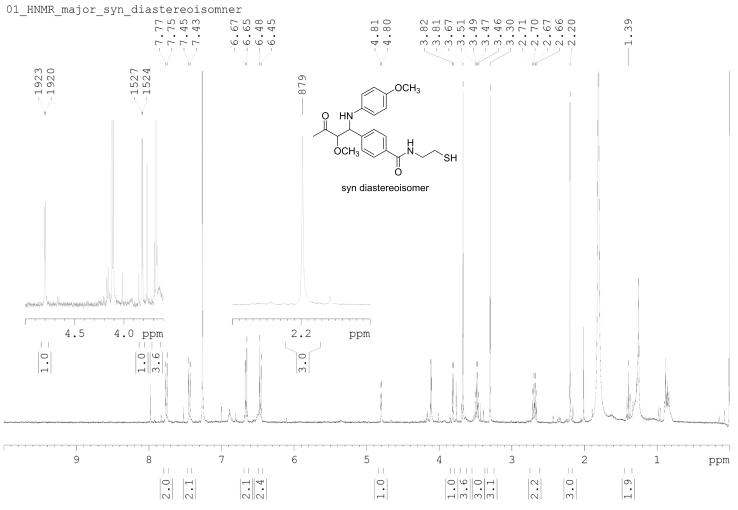
Supp. Scheme 4: Mannich reaction on TentaGel microsphere, with disulfide-containing linker for cleavage using TCEP. Note that the addition of 4-formyl benzoic acid and subsequent Mannich couplings likely occurs on the secondary amine in the linker as well. Since this material is never released from the bead and analyzed, it is abbreviated as -R



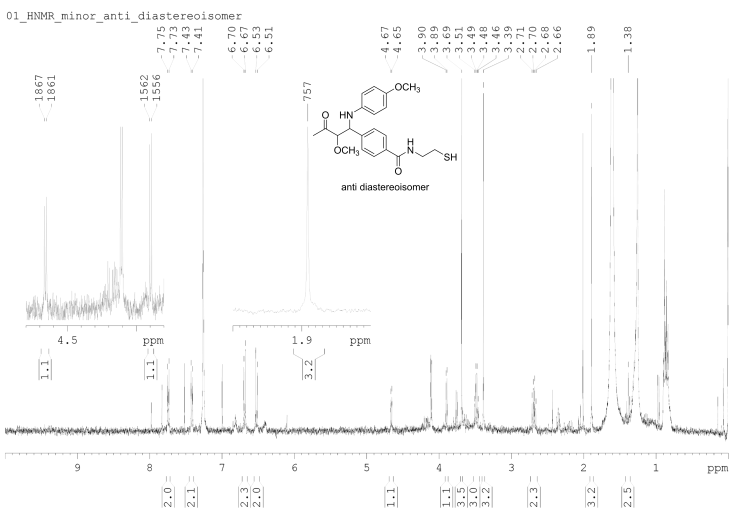
(a) LC-MS



(b) chiral LC

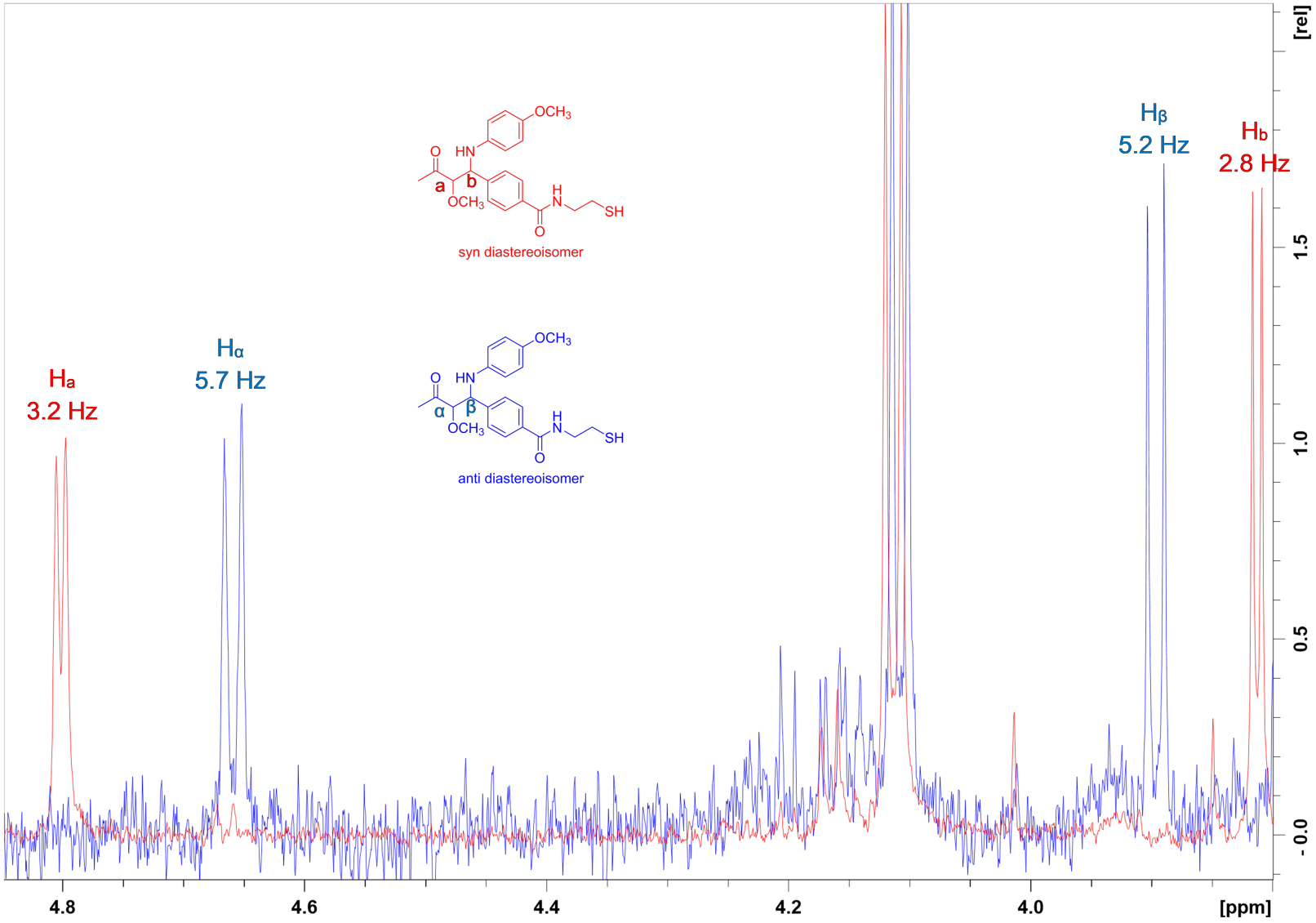


(c) ^{1}H-NMR of major syn diastereoisomer



(d) ^{1}H-NMR of minor anti diastereoisomer

Supp. Figure 4: Spectra of Mannich reaction with immobilized aldehyde, 4-methoxyaniline, and 1-methoxypropan-2-one, catalyzed by proline. **a) LC-MS:** the LC traces show (upwards order) starting material, Mannich reaction catalyzed by (S)-proline, and Mannich reaction catalyzed by (R)-proline. The number adjacent to each LC peak indicate the major molecular ion associated. The structures are expected products, and the notations syn/anti are assigned to each LC peaks following ^{1}H-NMR analysis and similar order of elution to the one of solution phase reaction. **b) chiral LC:** the LC traces show starting material, Mannich reaction catalyzed by (R)-proline, coinjection of products from reactions catalyzed by (S)- and (R)-proline, and Mannich reaction catalyzed by (S)-proline. The notations (S, R), (R, S) are assigned to products by identifying/comparing major peaks among the spectra and by similar order of elution to the one of solution phase reaction. **c) ^{1}H-NMR:** Integration to 3 hydrogens near 2 ppm confirms regioselectivity at carbon adjacent to methoxy group of 1-methoxypropan-2-one. Coupling constants from vicinal hydrogens (3.8-4.0 ppm and 4.6-4.9 ppm) adjacent to chiral centers of products indicate their conformation: 3.0 Hz for syn- and 5.6 Hz for anti-diastereoisomer.



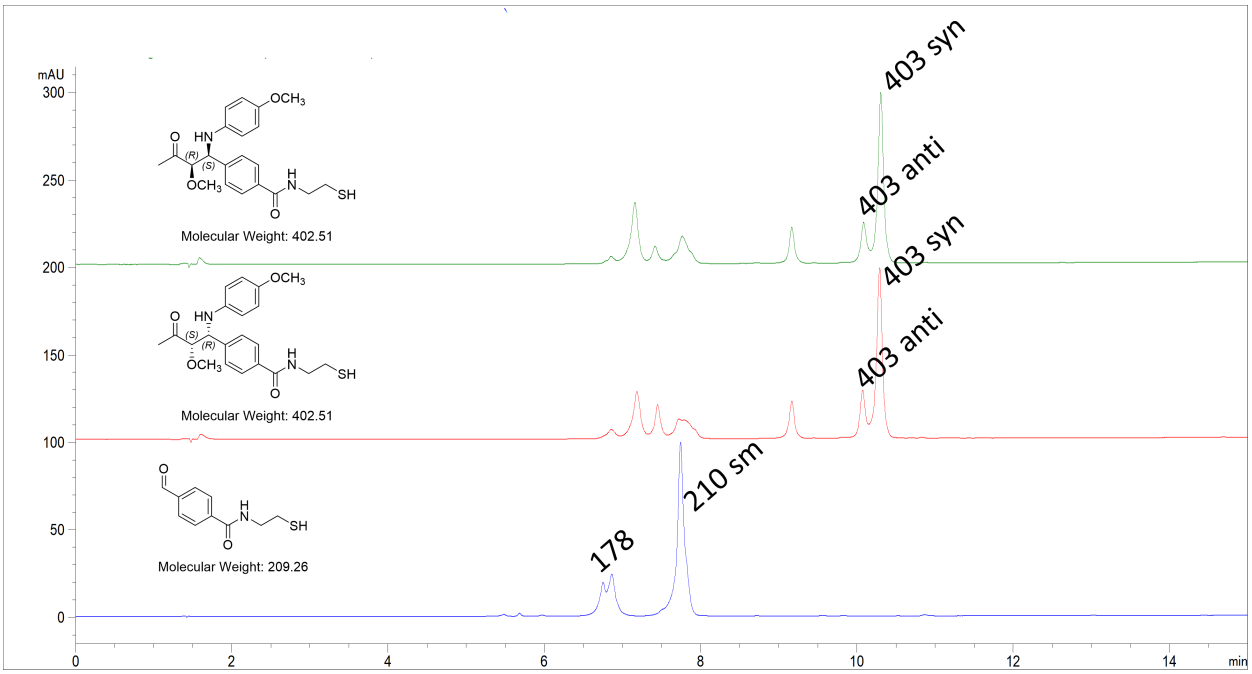
Supp. Figure 5: Spectra of Mannich reaction with immobilized aldehyde, 4-methoxyaniline, and 1-methoxypropan-2-one, catalyzed by proline. ^{1}H-NMR: Coupling constants from vicinal hydrogens (3.8-4.0 ppm and 4.6-4.9 ppm) adjacent to chiral centers of products indicate their conformation: 3.0 Hz for syn diastereoisomer, and 5.6 Hz for anti diastereoisomer.

LC-MS and chiral-LC data of crude Mannich products

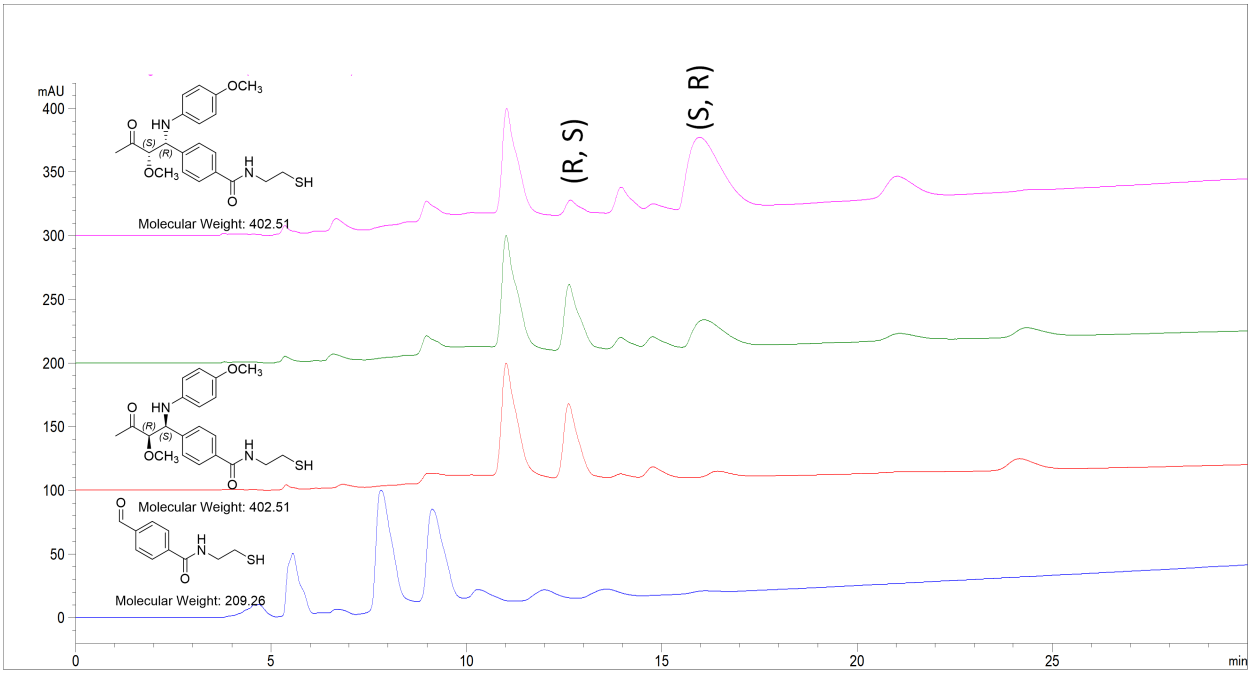
LCMS spectra and Chiral LC spectra of all crude Mannich products are provided.

Please refer to Supp. Tables 2, 3, and 4 for designated numbers.

Please refer to Supp. Figures 3 or 4 for analogous descriptions.

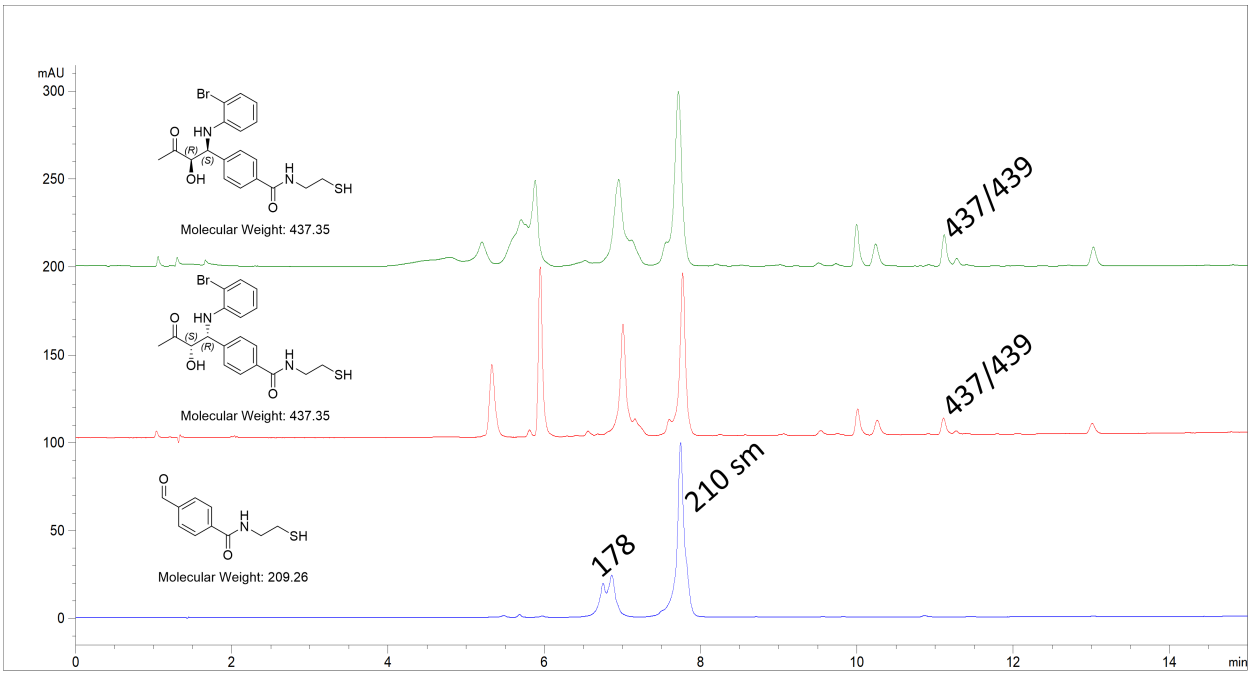


(a) LC-MS

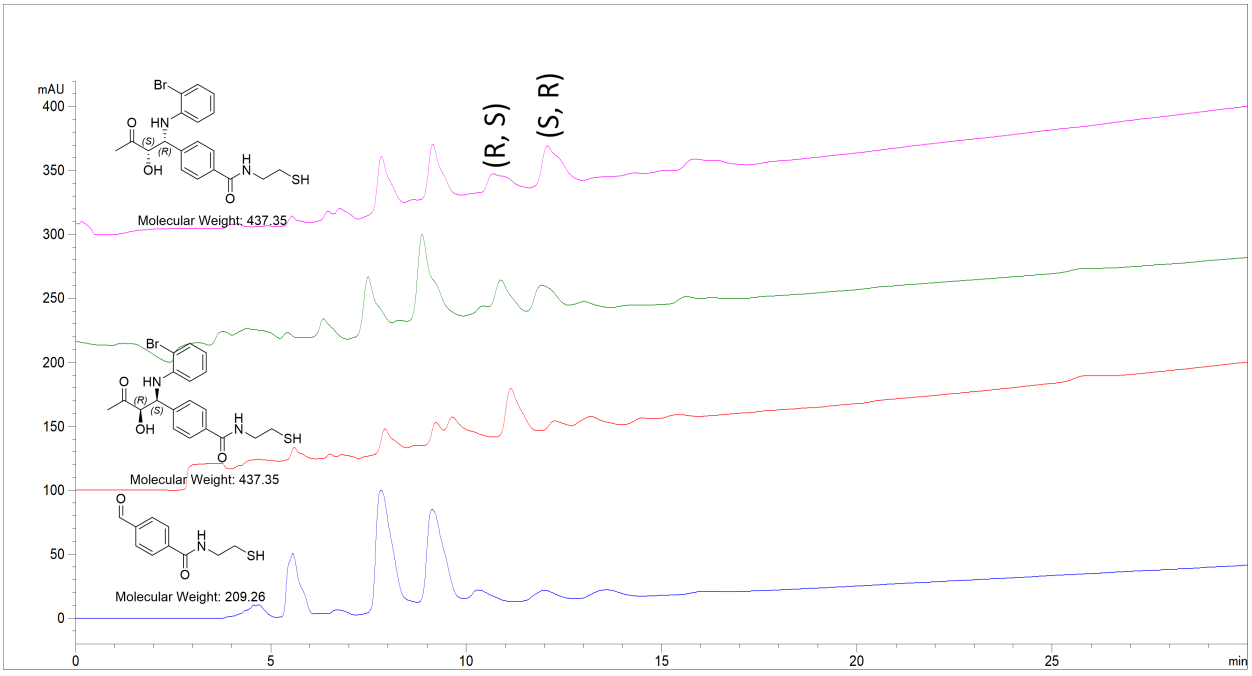


(b) chiral LC

Supp. Figure 6: LCMS and Chiral LC spectra of Mannich product 01

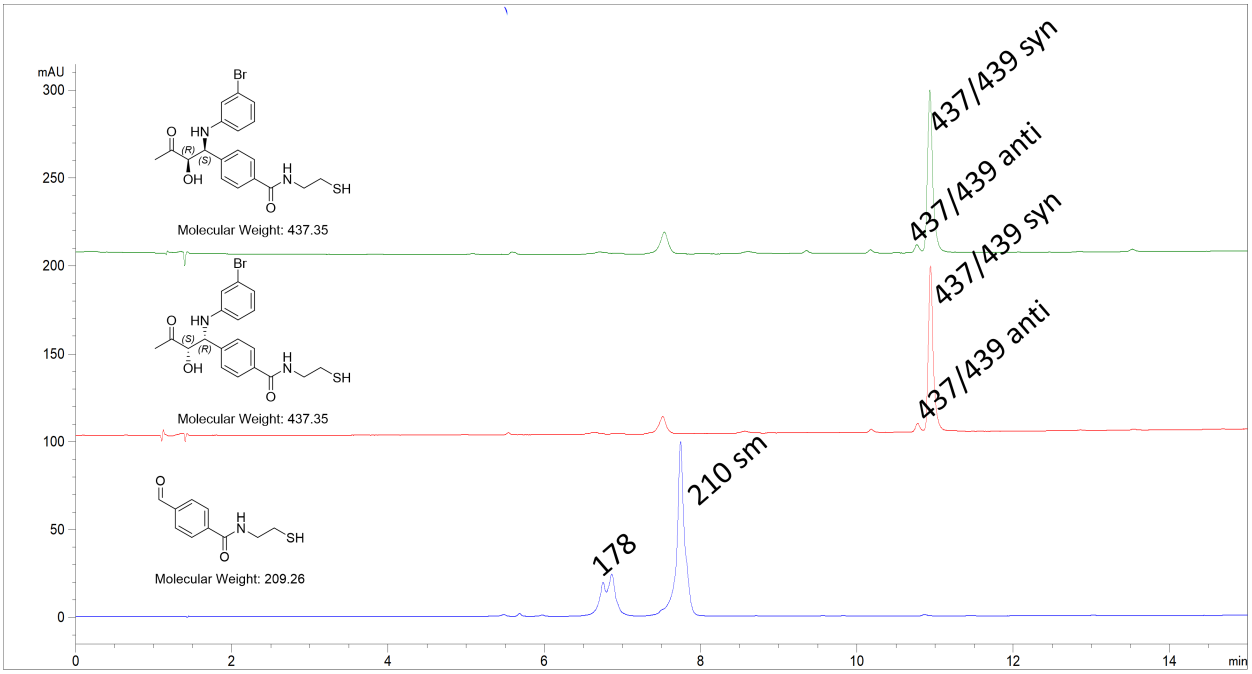


(a) LC-MS

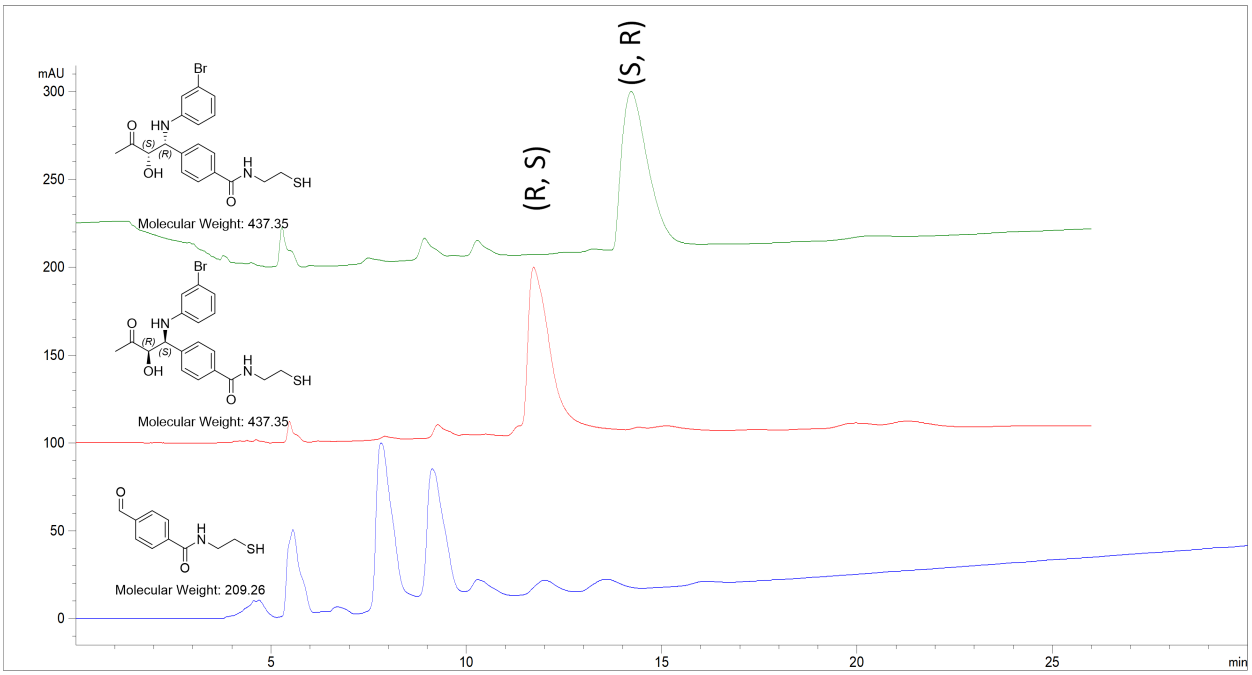


(b) chiral LC

Supp. Figure 7: LCMS and Chiral LC spectra of Mannich product 02

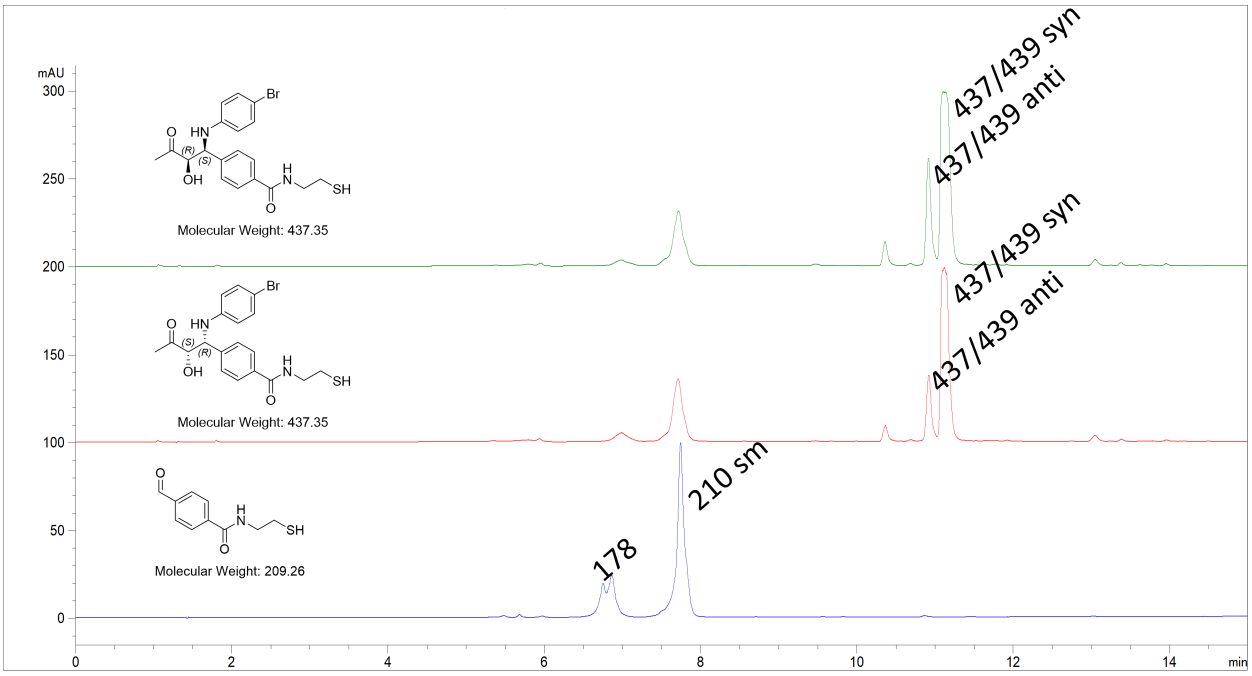


(a) LC-MS

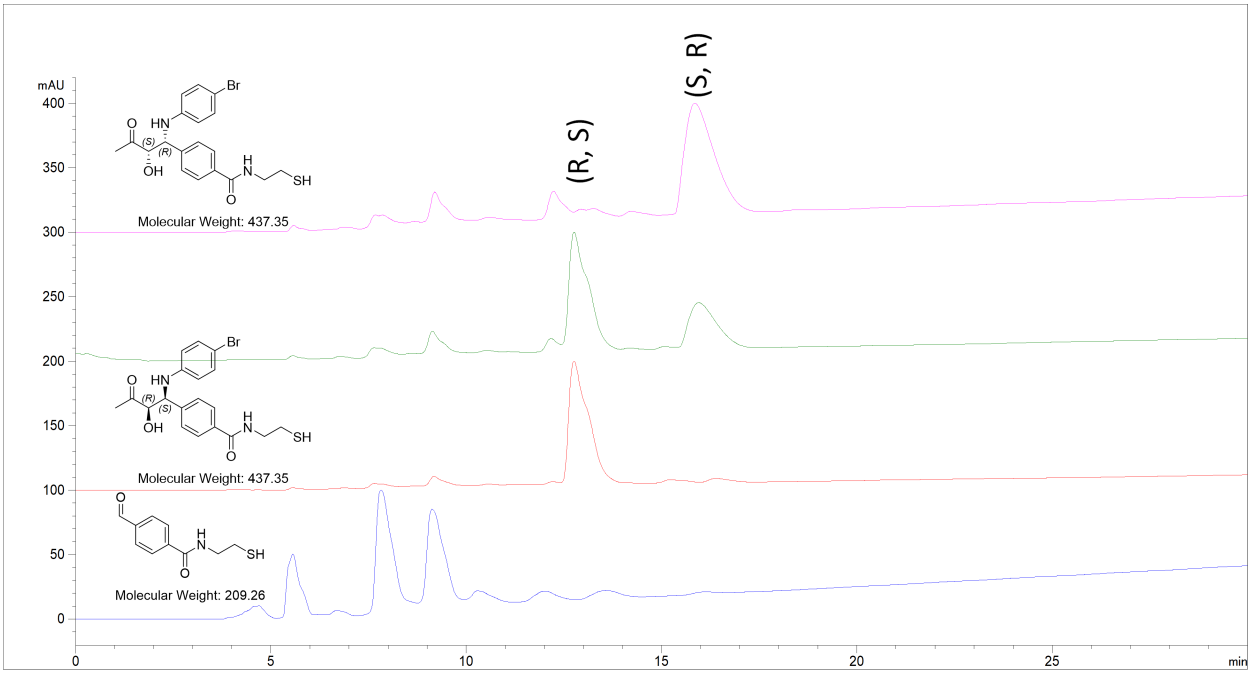


(b) chiral LC

Supp. Figure 8: LCMS and Chiral LC spectra of Mannich product 03

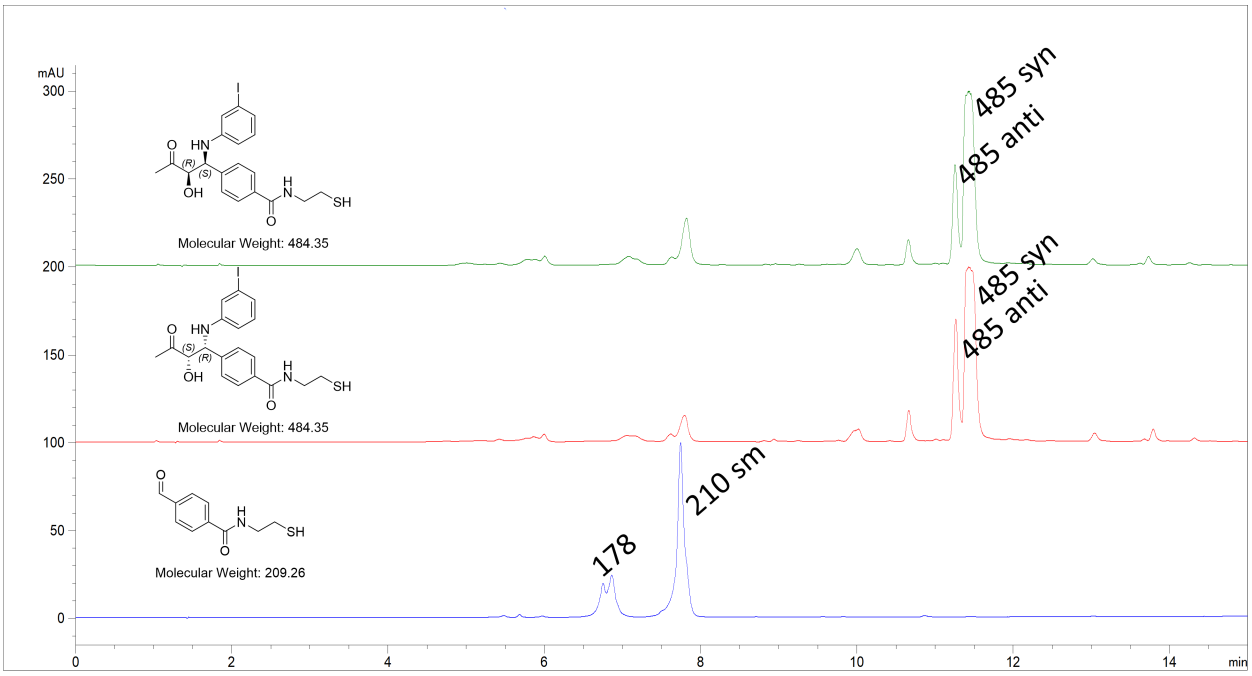


(a) LC-MS

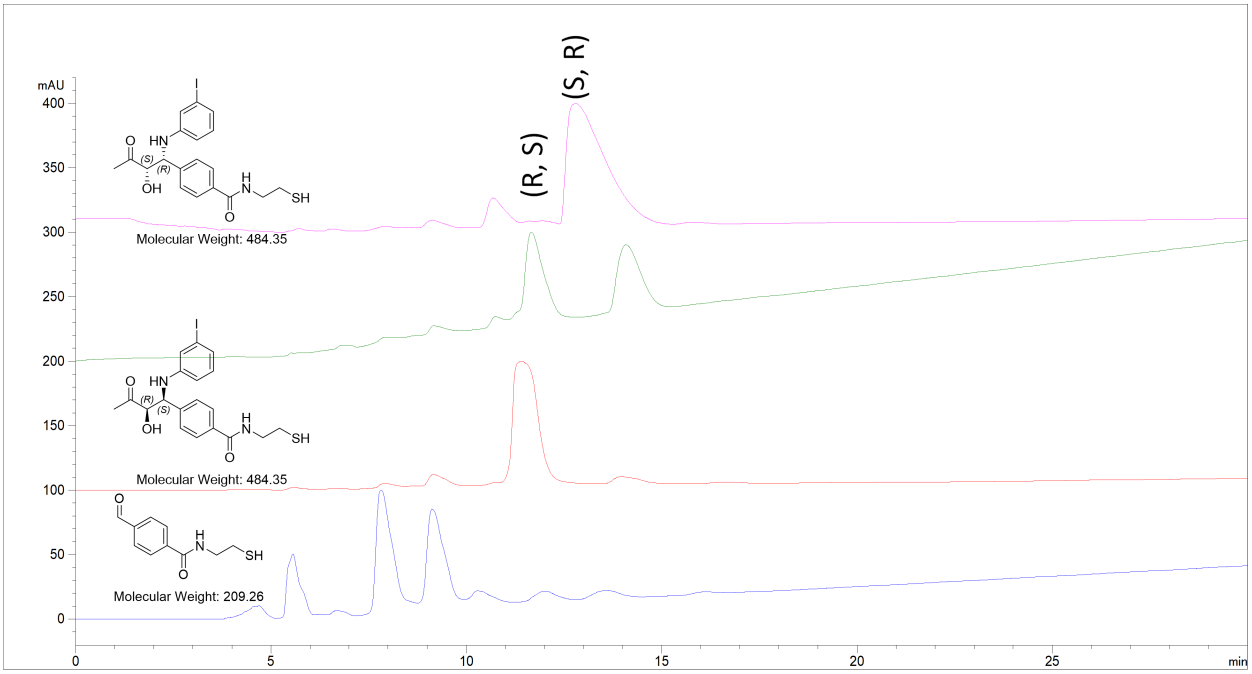


(b) chiral LC

Supp. Figure 9: LCMS and Chiral LC spectra of Mannich product 04

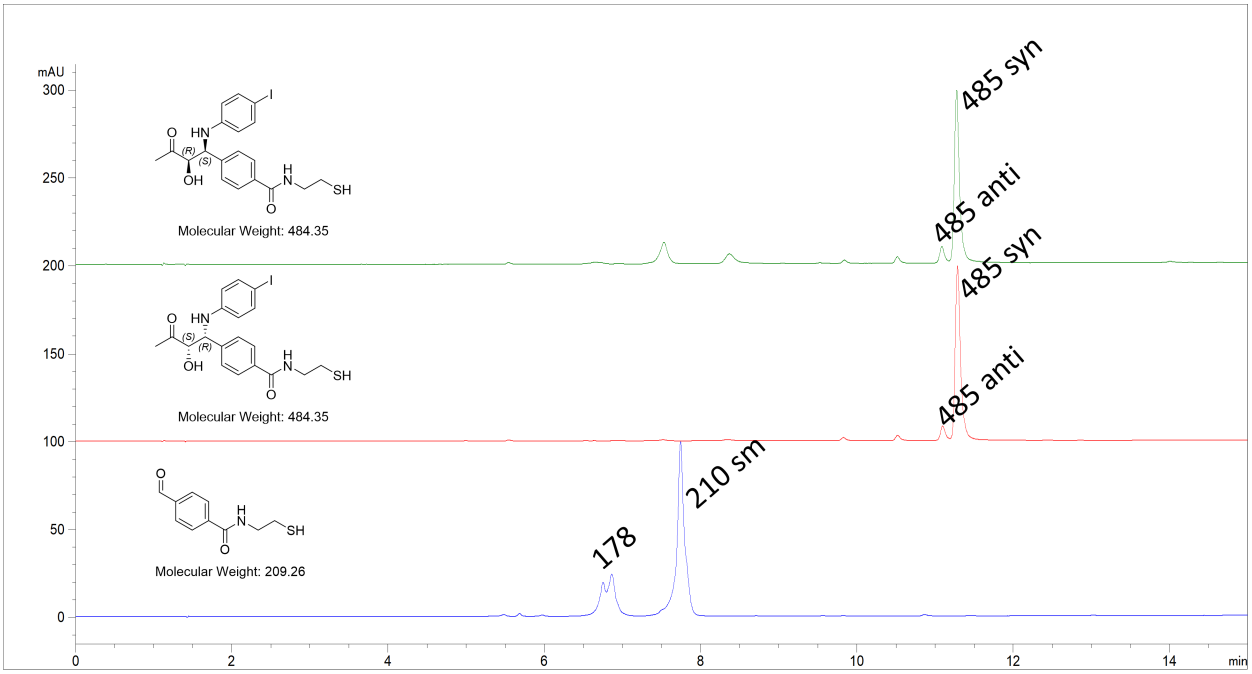


(a) LC-MS

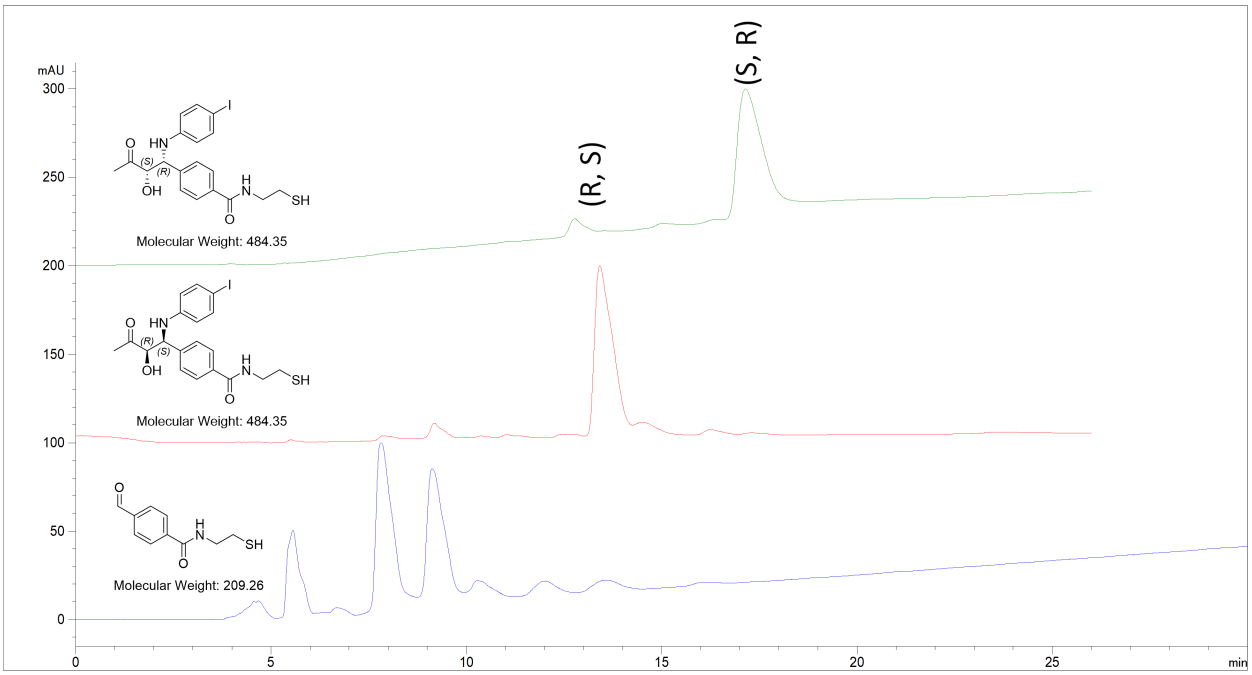


(b) chiral LC

Supp. Figure 10: LCMS and Chiral LC spectra of Mannich product 05

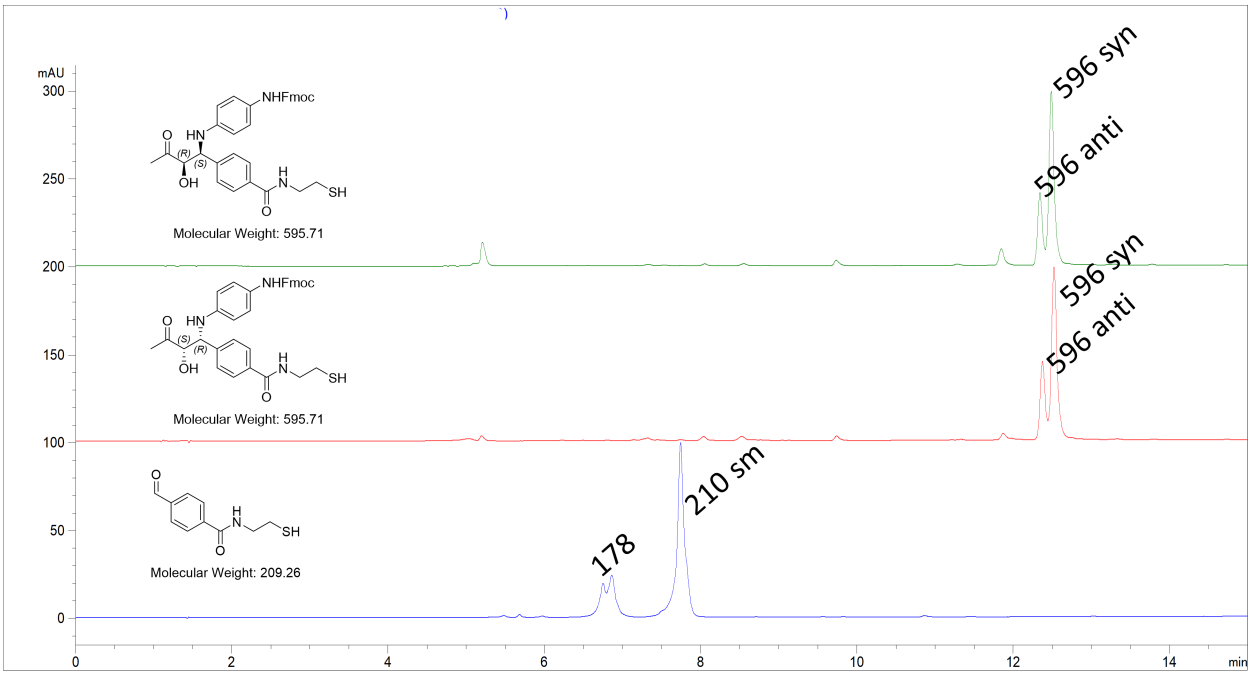


(a) LC-MS

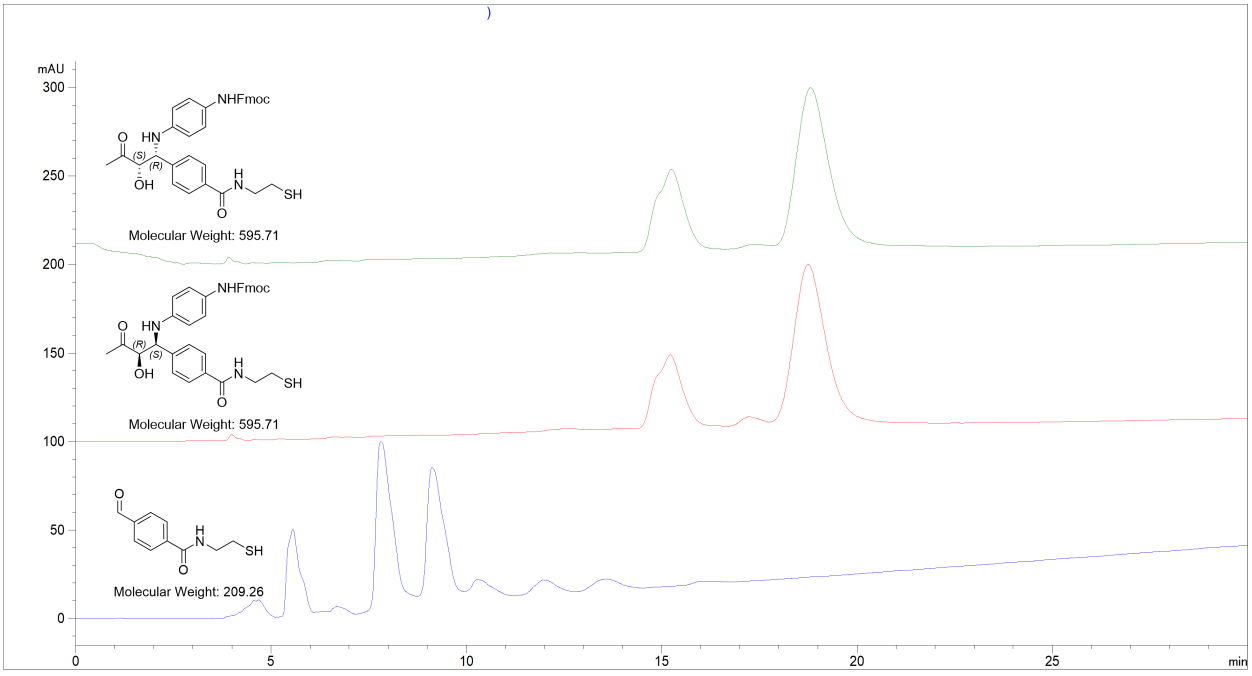


(b) chiral LC

Supp. Figure 11: LCMS and Chiral LC spectra of Mannich product 06

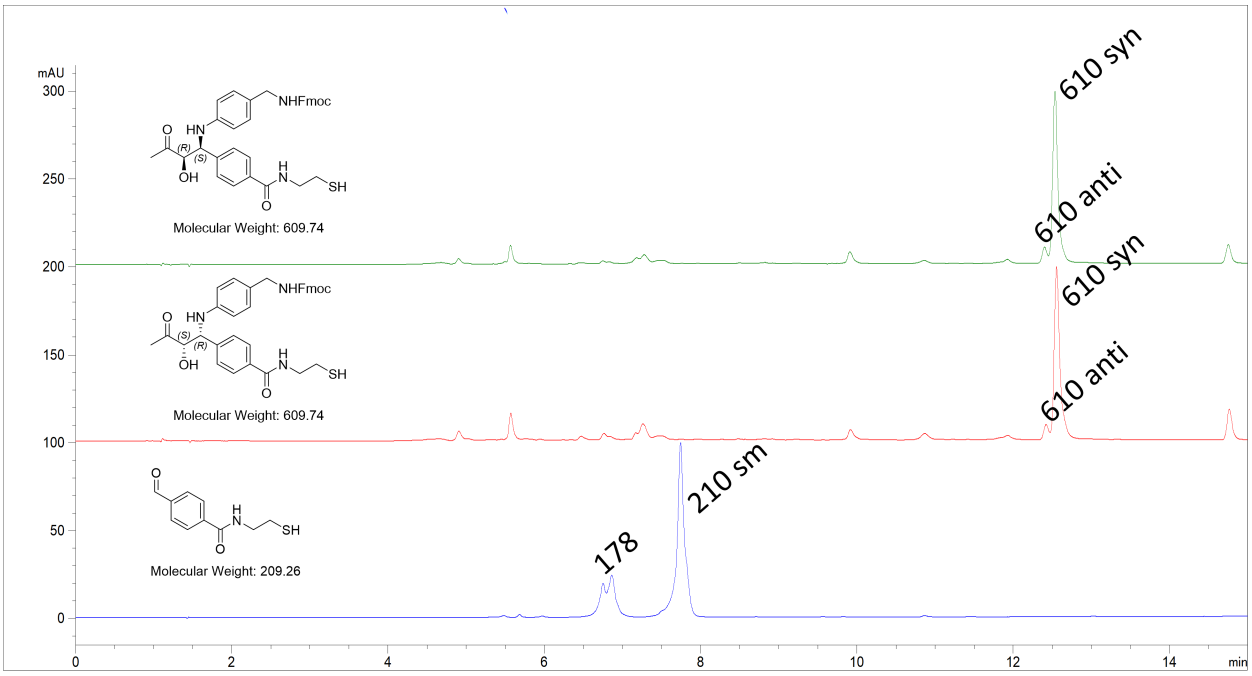


(a) LC-MS

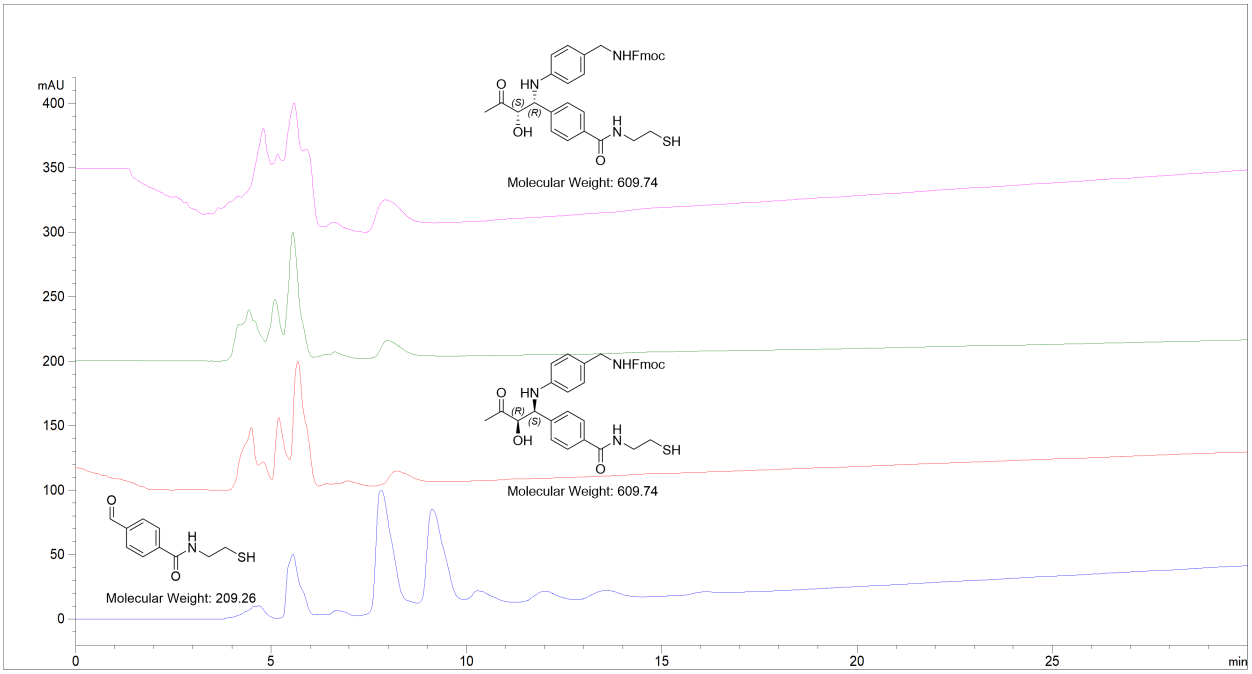


(b) chiral LC

Supp. Figure 12: LCMS and Chiral LC spectra of Mannich product 07

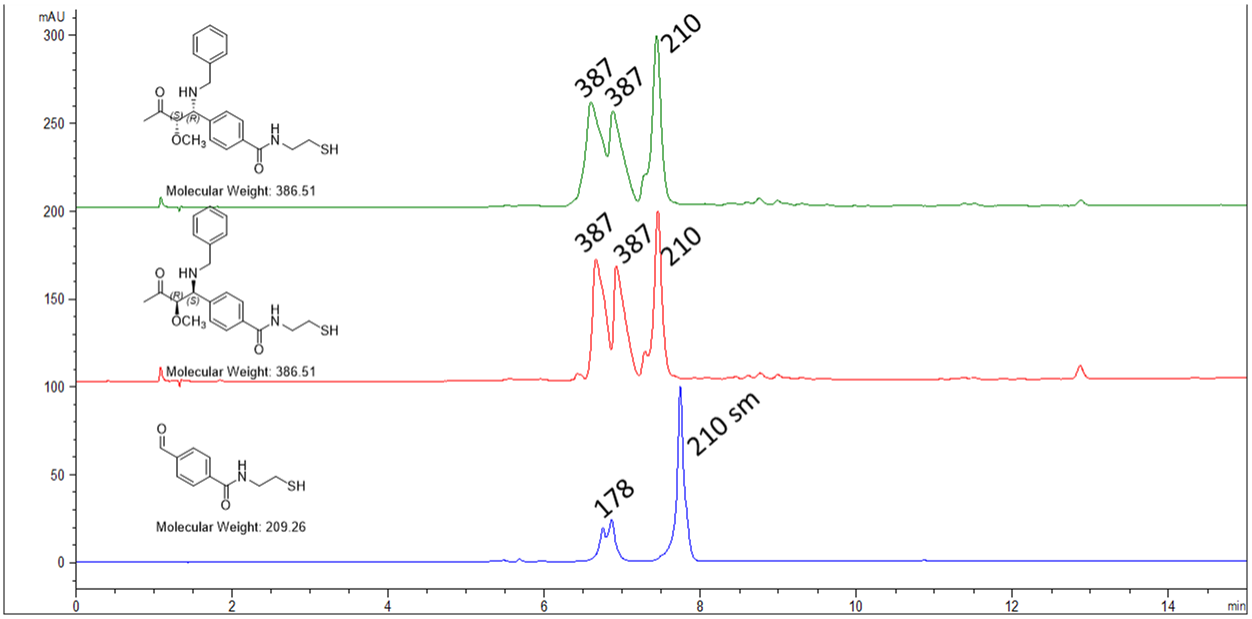


(a) LC-MS

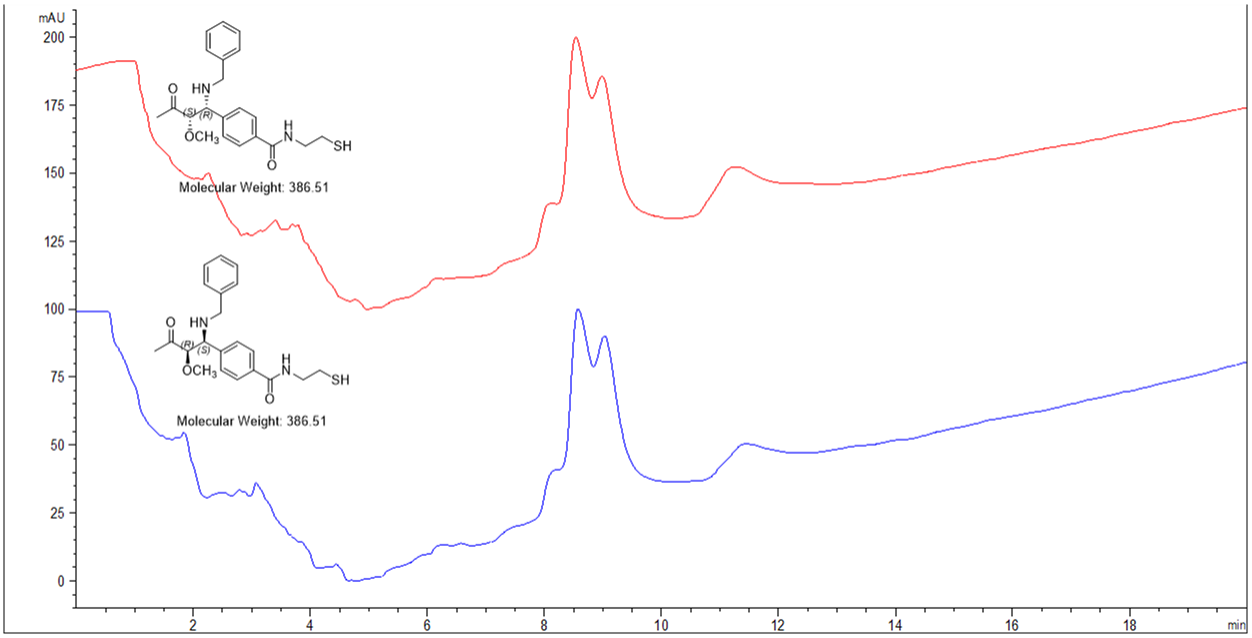


(b) chiral LC

Supp. Figure 13: LCMS and Chiral LC spectra of Mannich product 08

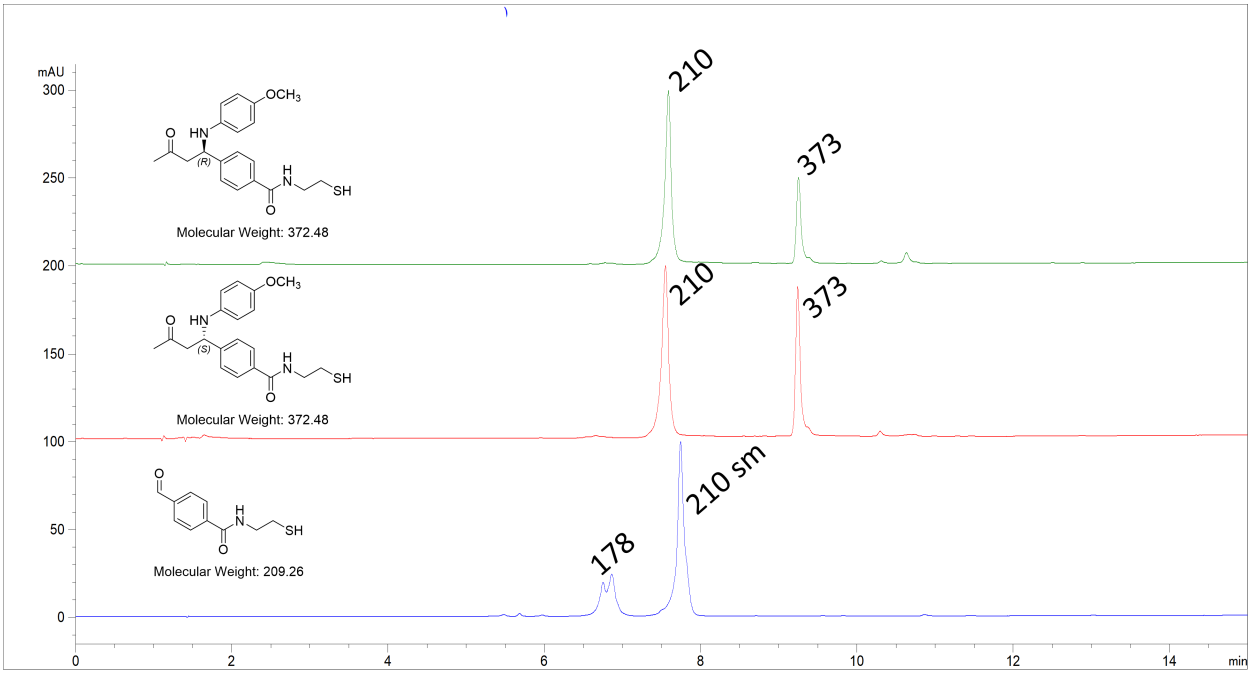


(a) LC-MS

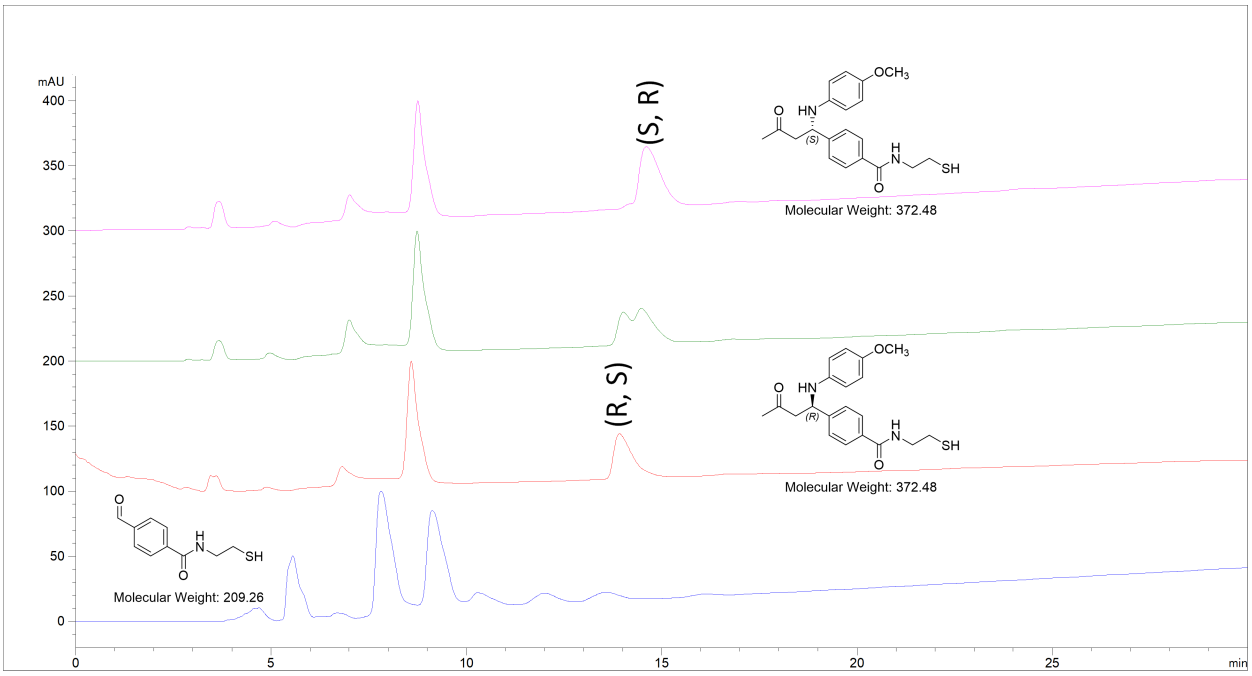


(b) chiral LC

Supp. Figure 14: LCMS and Chiral LC spectra of Mannich product 09

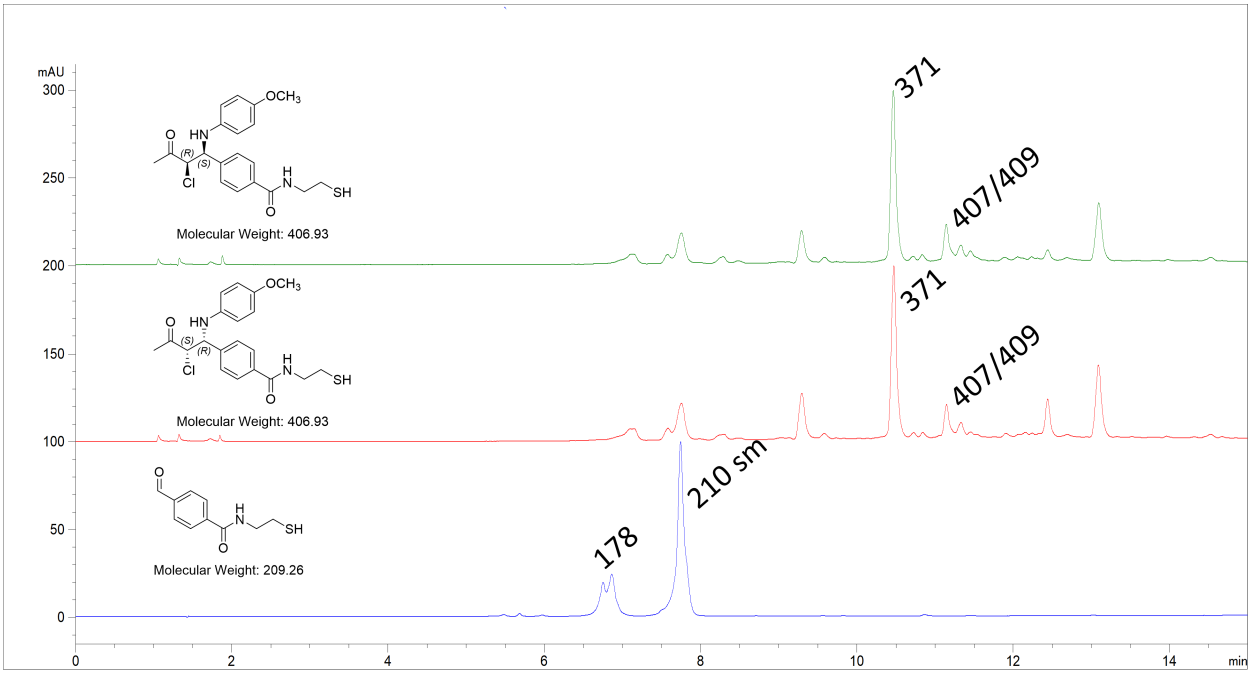


(a) LC-MS

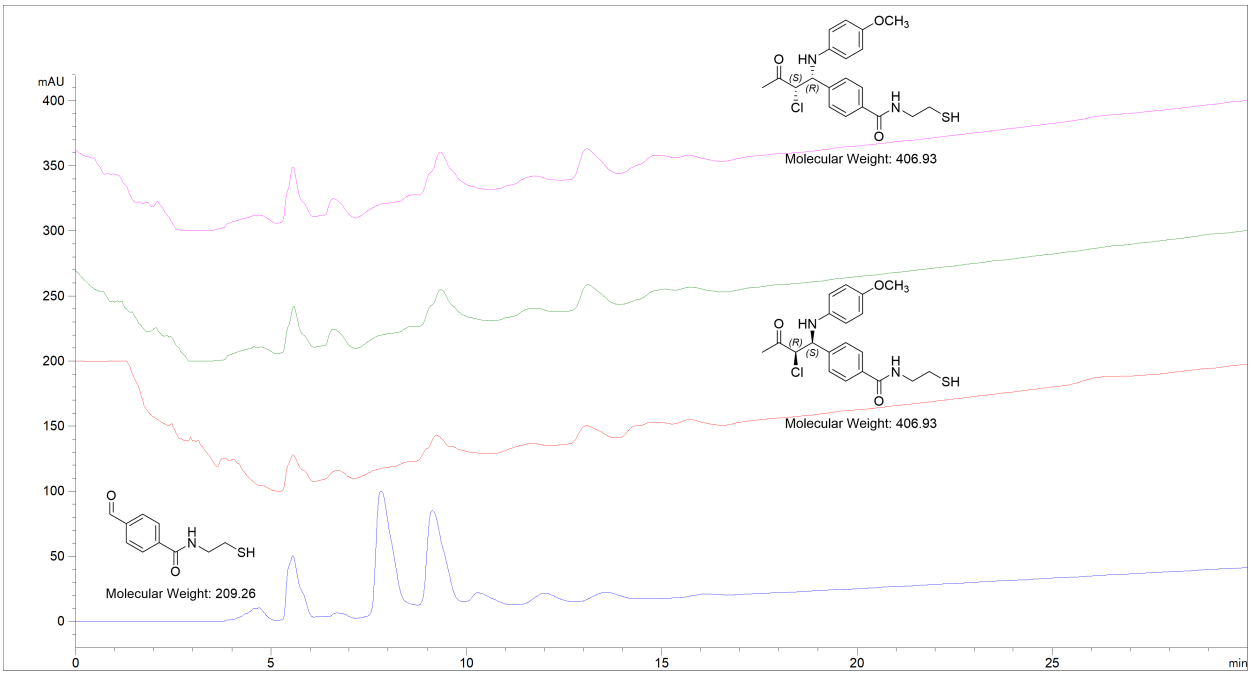


(b) chiral LC

Supp. Figure 15: LCMS and Chiral LC spectra of Mannich product 10

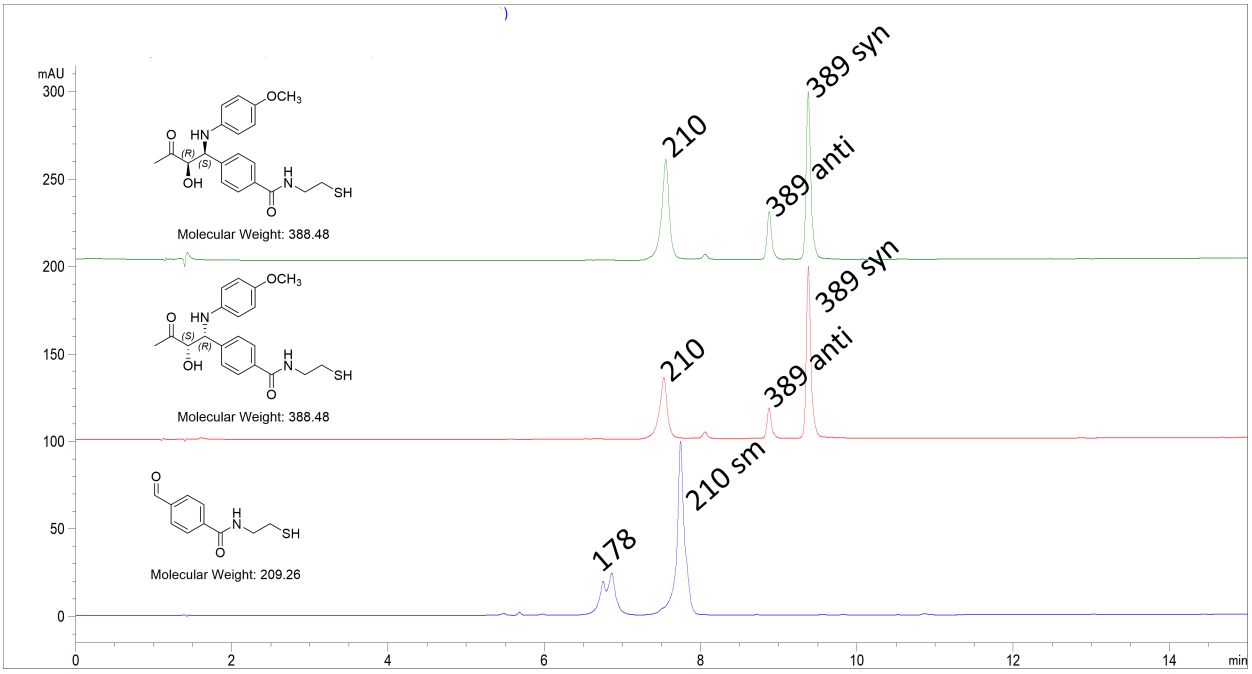


(a) LC-MS

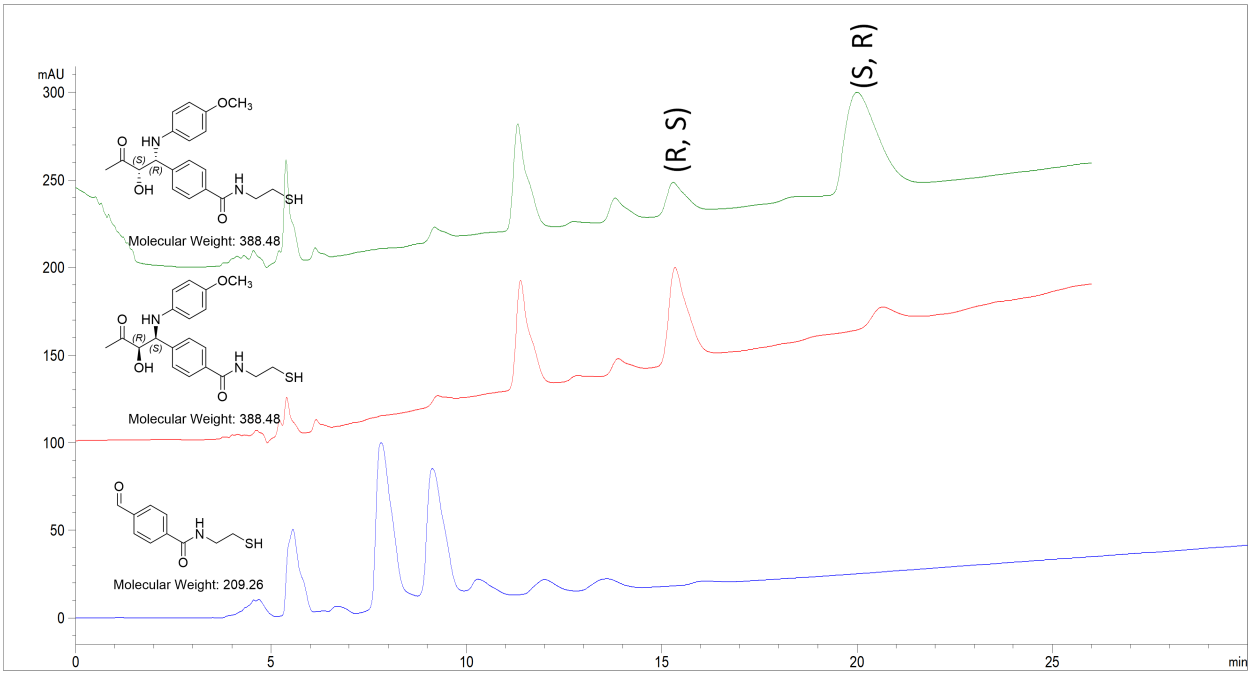


(b) chiral LC

Supp. Figure 16: LCMS and Chiral LC spectra of Mannich product 11

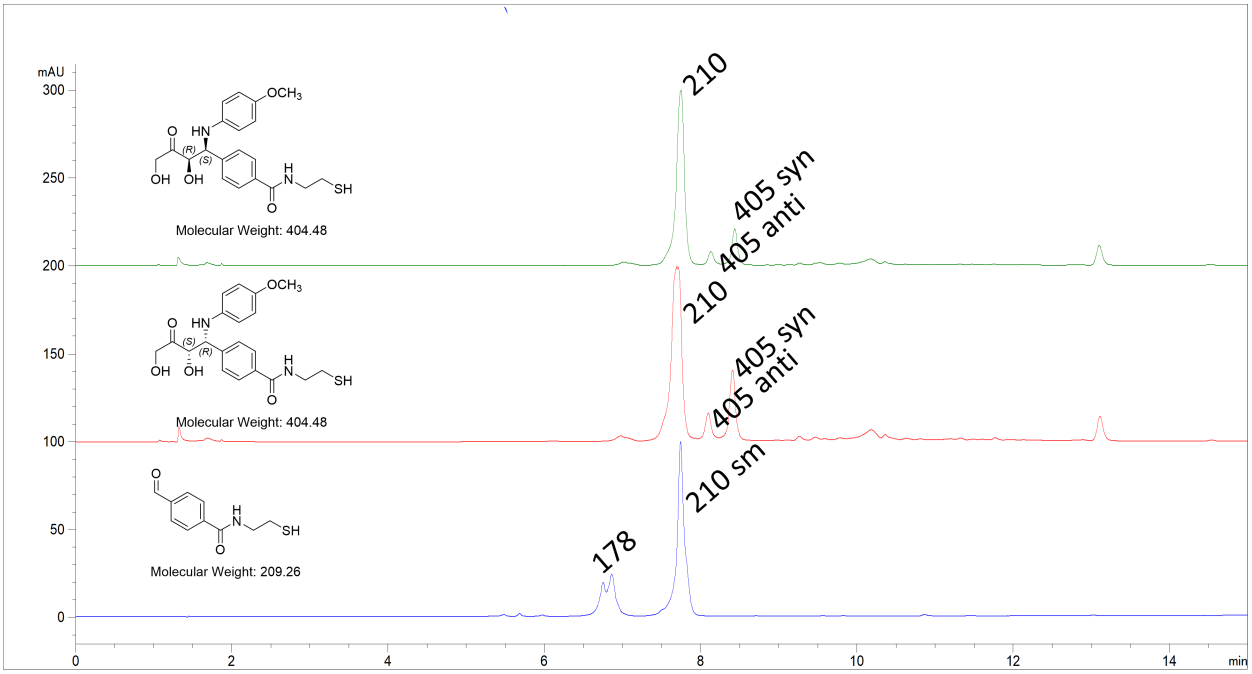


(a) LC-MS

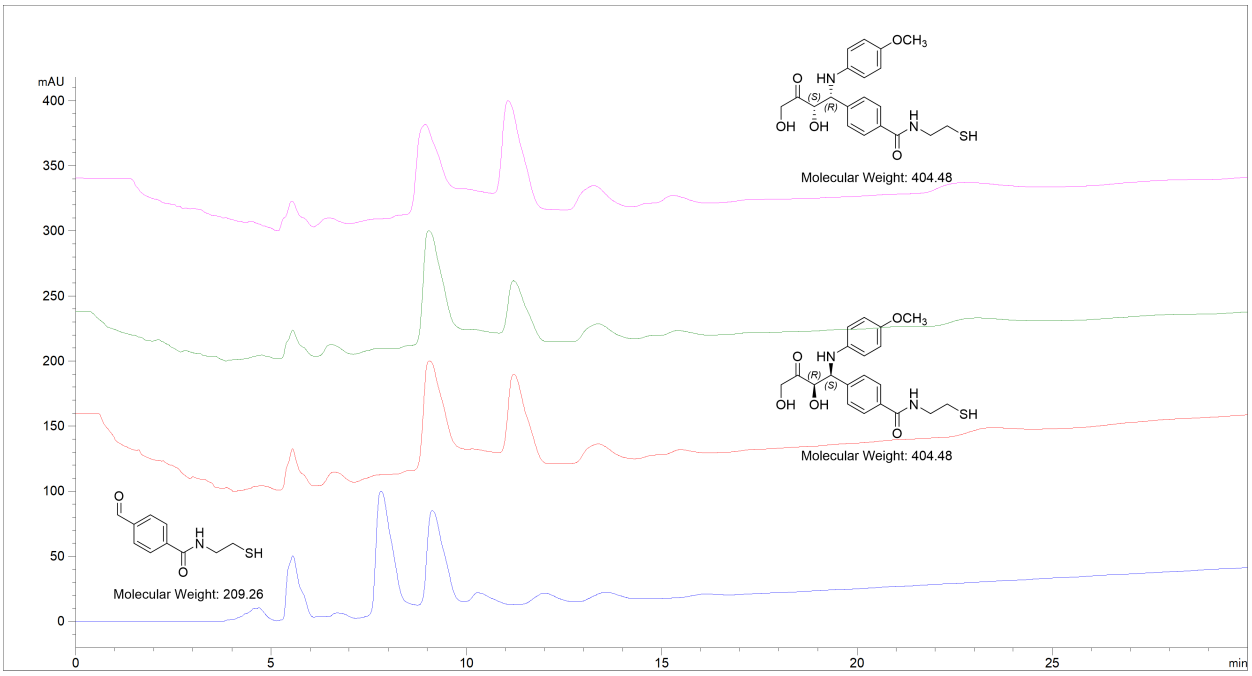


(b) chiral LC

Supp. Figure 17: LCMS and Chiral LC spectra of Mannich product 12

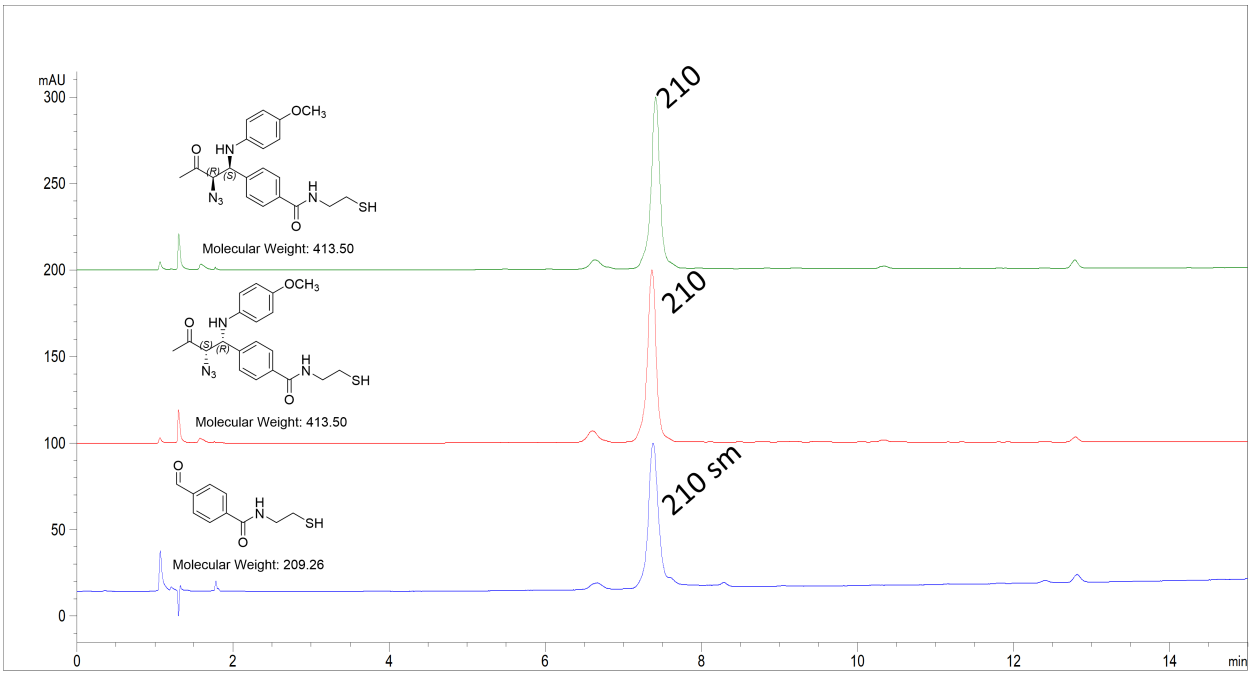


(a) LC-MS

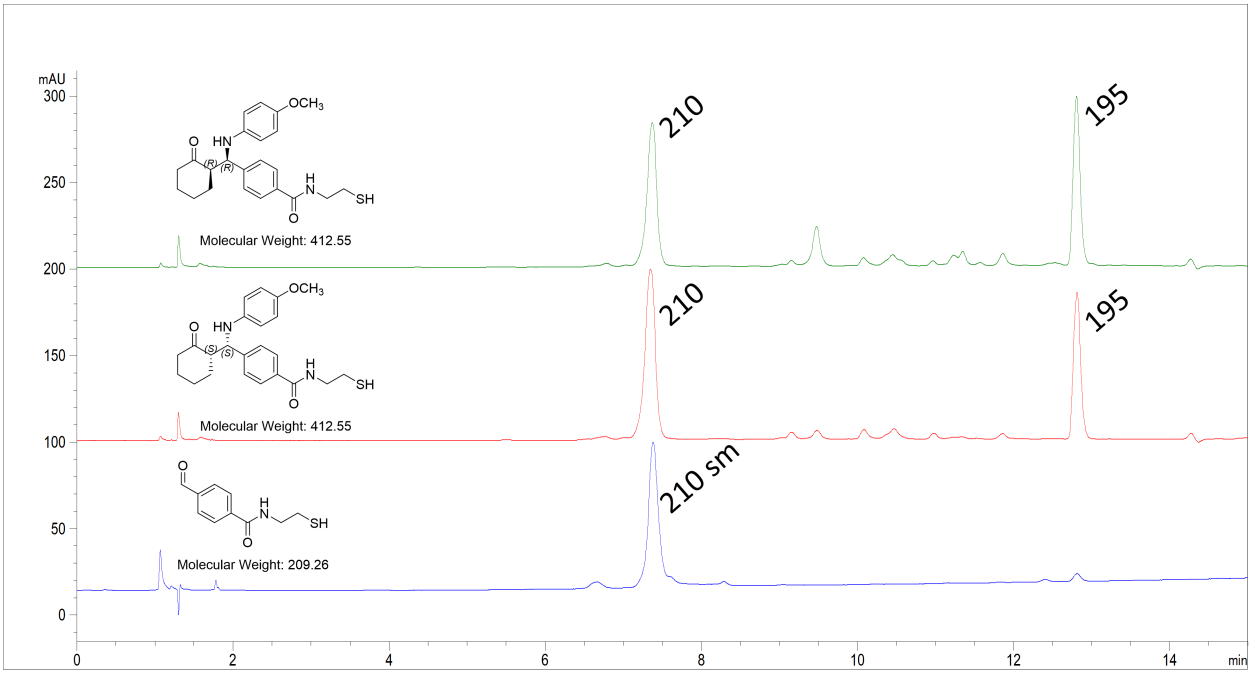


(b) chiral LC

Supp. Figure 18: LCMS and Chiral LC spectra of Mannich product 13

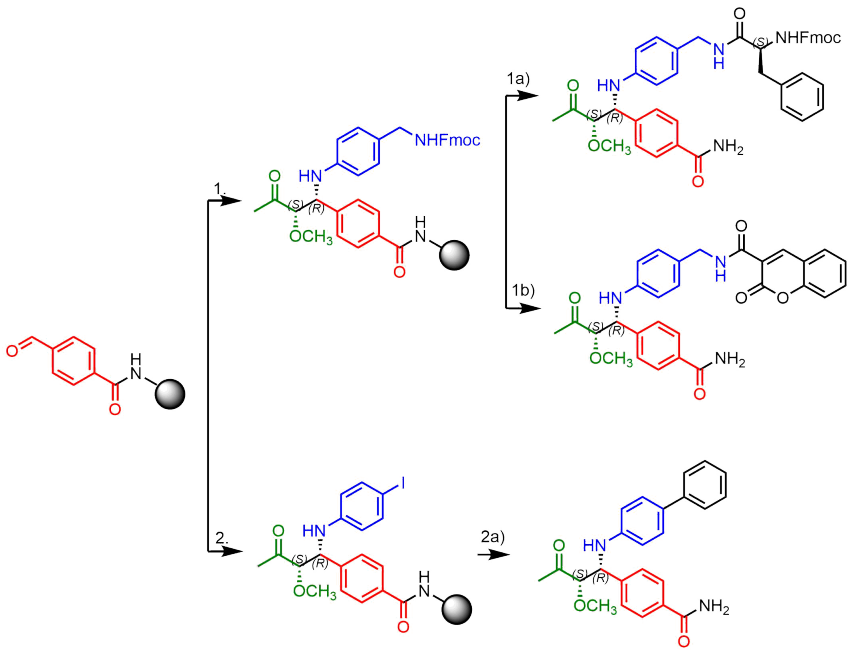


Supp. Figure 19: LC-MS spectra of Mannich product 14



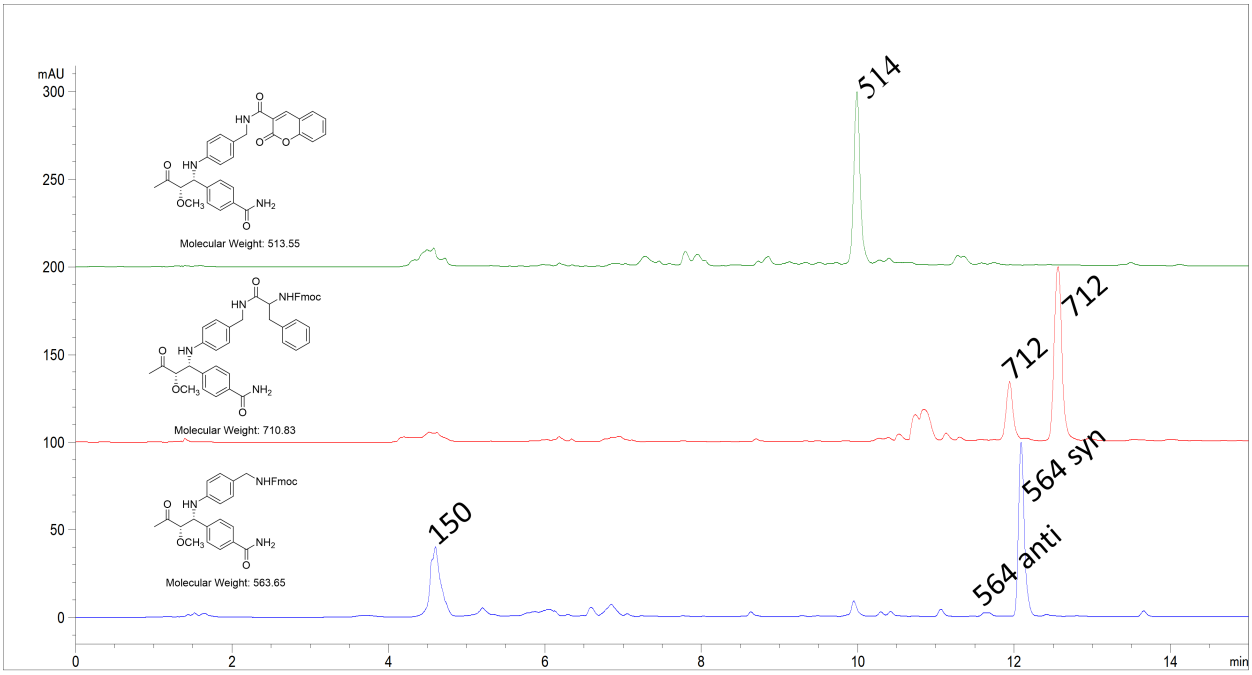
Supp. Figure 20: LC-MS spectra of Mannich product 15

Extension from Mannich products

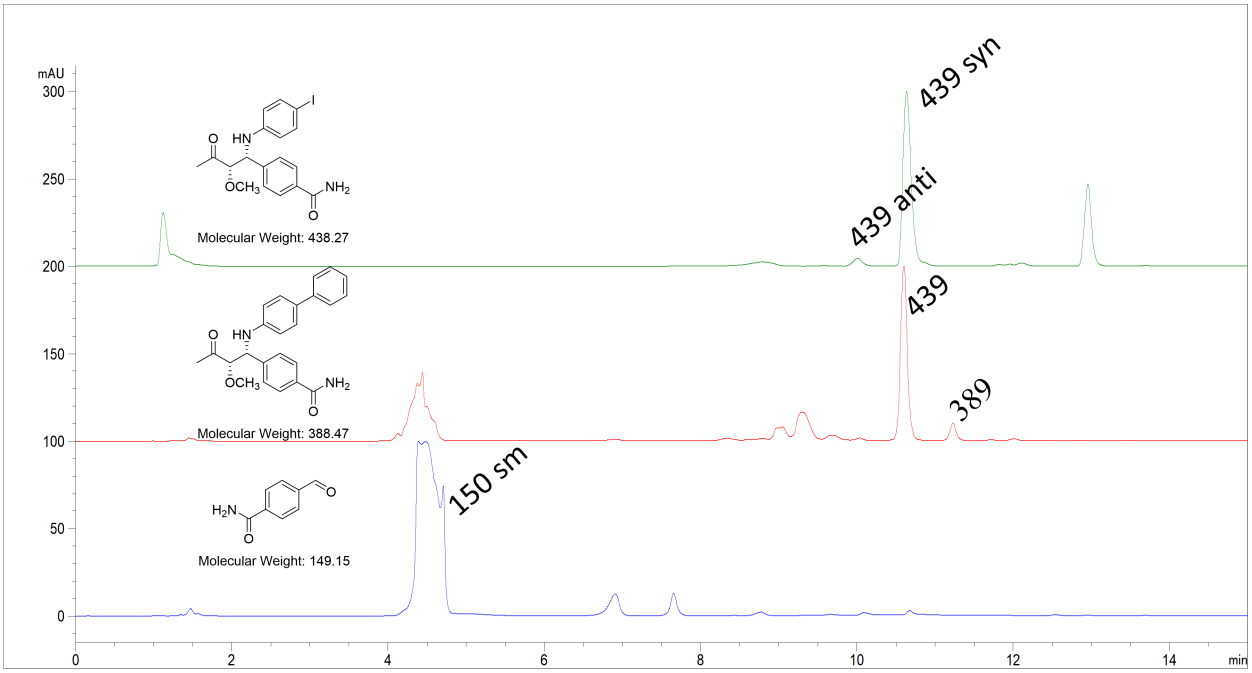


Supp. Scheme 5: Extension strategy for Mannich products: Oxyma coupling on Fmoc-protected 4-aminomethyl anilines, and Suzuki coupling on 4-iodoaniline.

Further elaboration of the Mannich products were attempted, following SUpp. Scheme 5. From Table 1, the product derived from Fmoc-protected 4-aminomethyl anilines was subjected to common peptide/peptoid solid phase synthesis with Oxyma coupling and Fmoc-deprotection, Supp. Figure 21a. Alternatively, the products from 3- or 4-halogenated anilines participated in Suzuki coupling Supp. Figure 21b.



(a) From Oxyma coupling



(b) From Suzuki coupling

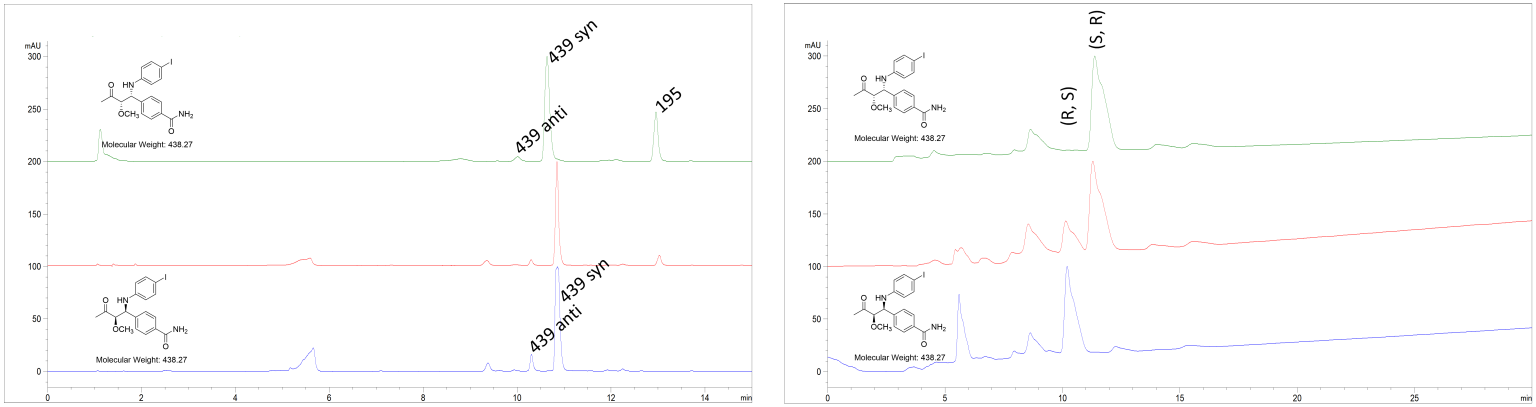
Supp. Figure 21: LC-MS spectra of chain extension on Mannich product. **a) Oxyma:** the LC traces show (upwards order) starting material, product from Oxyma coupling with Fmoc-protected (S)-Proline, and product from Oxyma coupling with coumarin-3-carboxylic acid. **b) Suzuki:** the LC traces show immobilized aldehyde on RAM resin, starting material, and product from Suzuki coupling with phenylboronic acid. In both spectra, the number adjacent to each LC peak indicate the major molecular ion associated.

Expanded reaction conditions and scope

The Mannich reaction reported can be scaled down to 1mg resin on microfilter plate, giving similar yield. The reaction does not exhibit plate effect – the position of the wells, whether on the border or in the interior of the plate, does not influence the reaction.

TentaGel solid support displaying different aldehyde moieties are tried: solid support incorporated with 3-formylbenzoic acid, 1,8-Naphthalaldehydic acid, 5-Formyl-2-furancarboxylic acid are used, in addition to the original 4-formylbenzoic acid. Mannich reaction is observed with all the resin above except for the one with 1,8-Naphthalaldehydic acid component. Various ketones/aldehydes (replacing ketone with aldehyde) and anilines are employed to screen the extended reaction scope: most aniline gives good conversion, while only ketones with adjacent hydroxy or methoxy group react efficiently. Other resin with different cleavage conditions are also tested: the product of Mannich reaction is stable upon disulfide cleaved by TCEP, methionine cleavage by CNBr, and RAM cleavage by TFA, Supp. Figure 22.

The product of the Mannich reaction disintegrates in aqueous solution after a few days. It is expected that stability would decrease further in acidic or basic condition. Attempts to directly determine the specific configuration through Mosher ester[5,6] are fruitless, likely because of the inaccessibility of the secondary alcohols.



(a) LCMS

(b) Chiral LC

Supp. Figure 22: Mannich product is stable, preserving both dr and ee upon cleavage by TFA. For both spectra, the LC traces show (upwards order) Mannich reaction catalyzed by (R)-proline, coinjection of products from reactions catalyzed by (S)- and (R)-proline, and Mannich reaction catalyzed by (S)-proline. **a) LC-MS:** The number adjacent to each LC peak indicate the major molecular ion associated. The structures are expected products, and the notations syn/anti are assigned to each LC peaks following similar order of elution. **b) chiral LC:** The notations (S, R), (R, S) are assigned to products by identifying/comparing major peaks among the spectra and by similar order of elution.

DNA damage assay

Supp. Table 5: Table of all reagents used for DNA damage assay, excluding solvents

Name	Description	Source
Dynabeads M-270 Carboxylic acid	magnetic bead	Life Technologies
propagylamine	2450-71-7	Acros Organics
HOAt	39968-33-7	AK Scientific
N_3–HDNA		K. Pels
BTPWB	buffer	
BTPBB	buffer	
BTPBBE	buffer	
forward primer MLM014		K. Pels & V. Dang
reverse primer MLM005		K. Pels & V. Dang
fluorescent probe BMP259	FAM probe	K. Pels & V. Dang
Taq polymerase		New England Biolabs
PCR buffer		P. Dickson

Components.

1. DNA sequence on sensor bead and soluble DNA: ligation product of MLM003, MLM004, MLM005, MLM006
2. MLM003: 5'-/5Phos/GCC GCC CAG TCC TGC TCG CTT CGC TAC ATG GAC AAA GAG CCG ACG ACG ACT TCC CCG CGG TCT AAA CCT CAA -3'
3. MLM004: 5'-/5Phos/AGG CTT GAG GTT TAG ACC GCG GGG AAG TCG TCG TCG GCT CTT TGT CCA TGT AGC GAA GCG AGC AGG ACT GGG CGG CGG -3'
4. MLM006: 5'-/5Phos/GCC TCG GTA TCA GGG ATA TGC TCA GTG -3'
5. Bis-Tris propane wash buffer BTPWB: 50 mM NaCl, 0.04% Tween 20, 10 mM Bis-Tris, pH 7.6.
6. Bis-Tris propane breaking buffer BTPBB: 100 mM NaCl, 10 mM EDTA, 1% SDS, 1% Tween 20, 10 mM Bis-Tris, pH 7.6.

7. Bis-Tris propane breaking buffer with EDTA BTPBBE: 10 mM NaCl, 1% SDS, 1% Tween-20, 10 mM EDTA, 10 mM Bis-Tris, pH 7.6

8. copper-catalyzed azide-alkyne cycloaddition reaction buffer CuAAC: 1.8 mM $CuSO_4$, 2.2 mM TBTA, 10 mM ascorbic acid, 1% Tween 20, 11.1 µM N3-HDNA, 40% DMSO, 1M TEAA, pH 7

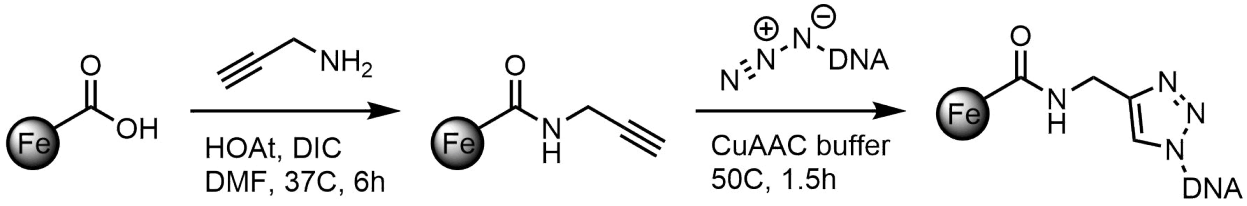
9. Forwards primer MLM005: 5-CAC TGA GCA TAT CCC TGA TAC CG-3

10. Reverse primer MLM014: 5-CCT GCT CGC TTC GCT ACA TGG ACA AAG-3.

11. Fluorescent probe BMP259: /56-FAM/CC GAC GAC G/ZEN/AC TTC CCC GCG/ 3IABkFQ/3'.

12. 10X PCR buffer: 2 mM dATP, 2 mM dGTP, 2 mM dCTP, 2 mM dTTP (generally, 2mM dNTPs), 15 mM MgCl2, 500 mM KCl, 100 mM Tris, pH 8.3.

Preparing magnetic sensor beads Magnetic sensor beads are prepared following Malone and Paegel reported procedure.[7]

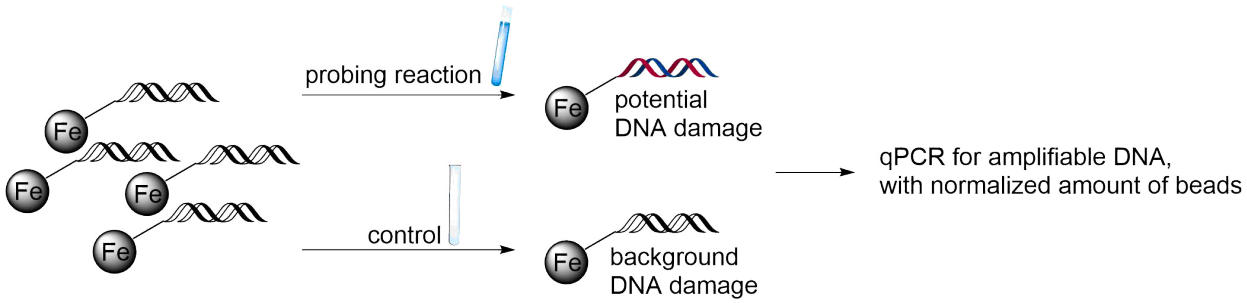


Supp. Scheme 6: Preparing magnetic probe beads displaying DNA sequence

First, following the initial part of Supp. Scheme 6, carboxylic acid-functionalized magnetic resin (Dynabeads M-270 Carboxylic acid) is washed three times with DMF and suspended in DMF. To the resin, 50 µL solution of 2M propargylamine in DMF, 50 µL solution of 2.8M DIC in DMF, and 100 µL solution of 1M HOAt in DMF are added. The resin is incubated with rotation for five hours, at 37°C. After the resin is washed six times with DMF, three times with DCM, three times with solution of 1% Tween 20 in HPLC grade water, it is then incubated in solution of 1% Tween 20 for one hour, at 55 °C, re-suspended in 1% Tween 20,

and stored at 4 °C.

Second, following the later part of Supp. Scheme 6, N-propargyl-functionalized magnetic resin is re-suspended in 27 µL copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction buffer, containing 1.8 mM $CuSO_4$, 2.2 mM TBTA, 10 mM ascorbic acid, 1% Tween 20, 11.1 µM N3-HDNA, 40% DMSO, 1M TEAA, pH 7. The resin is incubated in buffer for 1.5 h, at 50 °C. Resin is then washed three times with BTPBBE, re-suspended and incubated in BTPBBE for 18h at 50 °C.



Supp. Scheme 7: DNA damage assay, developed by Malone and Paegel[7]

Probing Mannich reaction. Following Supp. Scheme 7, to each reaction vessel, 5 µL of stock solution, concentration of about 20000 beads per µL in BTPWB, is distributed. After the course of the reaction, using Magna-Sep Magnetic Particle Separator, sensor beads are separated, washed three times with 100 µL BTPBB, incubated for 16h at room temperature in 500 µL BTPBB under gentle shaking, washed three times with 100 µL BTPBB, washed three times with 100 µL DI water, washed three times with 100 µL BTBWB, and re-suspended in 100 µL BTPWB.

qPCR. The amount of amplifiable DNA on sensor beads are analyzed by qPCR: the 20 µL solution undergoing qPCR analysis consists of 1x PCR buffer, 0.5 µM forwards primer, 0.5 µM reverse primer, 0.35 µM fluorescent probe, 0.05 U/µL Taq polymerase, 300 sensor beads per µL, and BTBWB. In practice, a stock solution is made from mixing 10x PCR buffer ($n \times 2$ µL), 20 µM forwards primer ($n \times 0.50$ µL), 20 µM reverse primer ($n \times 0.50$ µL), 100 µM fluorescent probe ($n \times 0.07$ µL), 5 U/µL Taq polymerase ($n \times 0.2$ µL), and then 3.27

µL stock solution is distributed into each reading well, followed by sufficient sensor bead solution containing 6000 beads, and sufficient BTBWB to dilute to 20 µL. The thermal cycle settting is 95°C, 20s; [65.8 °C, 20s; 68 °C, 15s] × 28 cycles, on C1000 Touch Thermal Cycler with fluorescence monitoring (channel 1, CFX96 Real-Time System).

Analysis method. The amount of quantifiable DNA on sensor beads that observed the reaction is compared to the amount of DNA on naive sensor beads, with normalized bead count and confirming the instrument detection range with soluble DNA control. For Mannich reaction, the fraction of remaining amplifiable DNA is 0.87 ± 0.05 (Table 6 and Supp Figure 23).

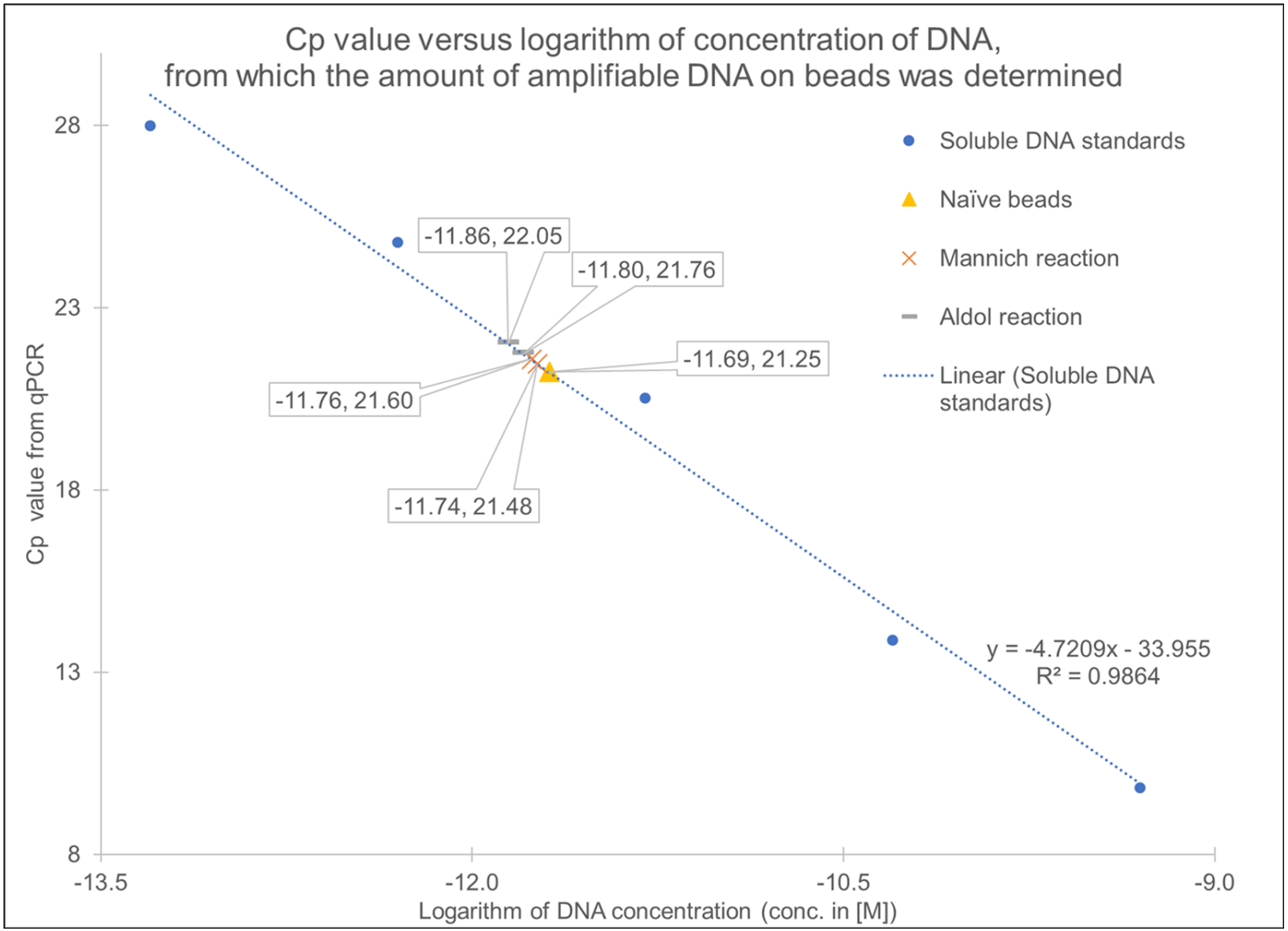
Instruments. Magna-Sep Magnetic Particle Separator (Invitrogen Life Science Technologies); C1000 Touch Thermal Cycler (Bio-Rad); CFX96 Real-Time System (Bio-Rad).

Note: the concentration of solution of sensor beads, i.e. the amount of beads per µL, is quantified by counting with a hemacytometer.

Supp. Table 6: Table of analyzed DNA for DNA damage assay

Sample	Description	Cq measurements			Mean Cq	Known Conc. (M)	Inferred Conc. (M)	fraction
S6	soluble DNA	9.67	10.03	9.80	9.83	5×10^{-10}		
S5		13.98	13.79	13.84	13.87	5×10^{-11}		
S4		19.94	21.53	20.07	20.51	5×10^{-12}		
S3		24.46	24.85	25.02	24.78	5×10^{-13}		
S2		27.73	28.60	27.63	27.98	5×10^{-14}		
S1		n/a	n/a	n/a	n/a	5×10^{-15}		
S0	BTPWB	13.98	13.79	13.84	13.87	5×10^{-11}		
Naive sensor beads		21.47	21.05	21.24	21.25		2.05×10^{-12}	1.0000
Mannich beads, trial 1		21.67	21.53	21.60	21.60		1.73×10^{-12}	0.8466
Mannich beads, trial 2		21.41	21.64	21.37	21.48		1.84×10^{-12}	0.8984
Aldol beads, trial 1		22.27	21.84	22.04	22.05		1.39×10^{-12}	0.6817
Aldol beads, trial 2		21.58	21.93	21.79	21.76		1.60×10^{-12}	0.7818

pool measurements: 0.87 ± 0.04 amplifiable DNA after probing Mannich reaction.
pool measurements: 0.73 ± 0.07 amplifiable DNA after probing Aldol reaction.



Supp. Figure 23: Cp value versus logarithm of concentration of DNA, from which the amount of amplifiable DNA on beads was determined. After probing Mannich reaction, 87% DNA on beads remains amplifiable, comparing to naive control beads'.

LC-MS instrument

Agilent 1100/1200 system: binary pump, autosampler, column thermostat, diode array detector, mass spectrometer detector. Column: Agilent ZOR3AX S8-C18 3.5 µm 4.6x100; temperature: 25℃; flow rate 1.000 mL/min; max pressure 400 bar; run time 0-15 min. Solvent: A 95% H_2O 5% ACN 0.1% formic acid; B 5% H_2O 95% ACN 0.1% formic acid. Gradient:

Time	% A	% B	Flow (mL/min)
0.00	100.00	0.00	1.000
1.00	100.00	0.00	1.000
1.50	94.33	5.67	1.000
13.50	16.67	83.33	1.000
14.00	0.00	100.00	1.000
15.00	0.00	100.00	1.000

HPLC instrument

Waters 1525 HPLC. Column: Sunfire Prep C18 OBD 5 µm 19x250 mm; temperature: not controlled; flow rate 1.000 mL/min; max pressure 400 bar; run time 0-50 min. Solvent: A H_2O 0.1% trifluoroacetic acid; B ACN 0.1% trifluoroacetic acid. Gradient:

Time	% A	% B	Flow (mL/min)
0.00	100.00	0.00	10.00
4.00	100.00	0.00	10.00
5.00	90.00	10.00	10.00
35.00	20.00	80.00	10.00
36.00	0.00	100.00	10.00
40.00	0.00	100.00	10.00
42.00	100.00	0.00	10.00
46.00	100.00	0.00	10.00
50.00	0.00	100.00	0.00

Chiral LC instrument

Agilent 1100 system: quartenary pump, autosampler, column thermostat, diode array detector. Column: Chiralpak AD 10 µm 4.6x250; temperature: not controlled; flow rate 0.750 mL/min; max pressure 300 bar; run time 0-30 min. Solvent: A isopropanol 0.1% trifluoroacetic acid; B hexane. Sample prepared in 50/50 v/v IPA/Hex. Gradient:

Time	% A	% B	Flow (mL/min)
0.00	20.00	80.00	0.750
25.00	80.00	20.00	0.750
30.00	80.00	20.00	0.750

HRMS instrument

ThermoScientific Dionex UltiMate 300 system: RS pump, column compartment, RS autosampler, with ThermoScientific Q Exactive hybrid quadrupole-Orbitrap mass spectrometer. Column: Pheromenex C0D-4725-AN Kinetex 2.6 µm EVO C16 100 ÅLC column

100x2.1; temperature: 25℃; flow rate 0.300 mL/min; max pressure 400 bar; run time 0-10 min. Solvent: A 0.1% formic acid in H_2O; B 0.1% formic acid in ACN. Gradient:

Time	% A	% B	Flow (mL/min)
0.00	95.00	5.00	0.300
0.50	95.00	5.00	0.300
5.00	5.00	95.00	0.300
7.00	5.00	95.00	0.300
7.10	95.00	5.00	0.300
10.00	95.00	5.00	0.300

NMR instrument

Bruker AMX-400 instrument

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