

Supplementary information

Structure-based design of small imine reductase panels for target substrates

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Table S1. Hit rate in 20 sequences by IREDFisher and random selection. As a control, 20 sequences were randomly selected from the panels 1000 times and the average hit rate was calculated. The margin of error was calculated based on 95% confidence levels.

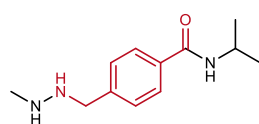
Reaction	Hit rate by IREDFisher (Conv. \geq 50%)	Hit rate by random selection (Conv. \geq 50%)	Improvement by IREDFisher ² (Conv. \geq 50%)	Hit rate by IREDFisher (Conv. \geq 2%)	Hit rate by random selection (Conv. \geq 2%)	Improvement by IREDFisher ² (Conv. \geq 2%)
1	20%	7% \pm 0.3%	1.90	30%	17% \pm 0.4%	0.82
2	15%	5% \pm 0.2%	2.33	55%	40% \pm 0.6%	0.39
3	25%	9% \pm 0.3%	1.66	70%	62% \pm 0.6%	0.14
4	5%	2% \pm 0.2%	1.27	75%	69% \pm 0.6%	0.08
5	0%	0% \pm 0.0%	NA	20%	6% \pm 0.3%	2.33
6	15%	6% \pm 0.3%	1.46	70%	66% \pm 0.6%	0.06
7	30%	21% \pm 0.5%	0.43	85%	65% \pm 0.6%	0.32
8	NA ¹	NA ¹	NA ¹	15%	11% \pm 0.4%	0.42
9	NA ¹	NA ¹	NA ¹	20%	12% \pm 0.4%	0.69
10	70%	36% \pm 0.5%	0.92	75%	57% \pm 0.5%	0.32
11	45%	25% \pm 0.5%	0.82	85%	66% \pm 0.5%	0.30
12	40%	23% \pm 0.4%	0.75	80%	68% \pm 0.5%	0.17
13	NA	NA ¹	NA ¹	35%	21% \pm 0.4%	0.70
14	45%	23% \pm 0.4%	0.96	90%	66% \pm 0.5%	0.37
15	NA	NA ¹	NA ¹	70%	36% \pm 0.5%	0.94
16	40%	18% \pm 0.4%	1.22	60%	34% \pm 0.5%	0.76
17	25%	11% \pm 0.3%	1.25	75%	62% \pm 0.5%	0.21
18	70%	54% \pm 0.6%	0.30	95%	91% \pm 0.3%	0.04
19	20%	14% \pm 0.4%	0.43	50%	46% \pm 0.6%	0.10
20	10%	8% \pm 0.3%	0.33	45%	40% \pm 0.6%	0.13
21	5%	3% \pm 0.2%	0.61	30%	37% \pm 0.6%	-0.19
22	60%	36% \pm 0.6%	0.66	80%	66% \pm 0.6%	0.22
23	15%	9% \pm 0.4%	0.60	60%	61% \pm 0.6%	-0.01
24	30%	20% \pm 0.5%	0.47	55%	61% \pm 0.6%	-0.09

¹ No hits with conversion over 50% were found in the overall screening.

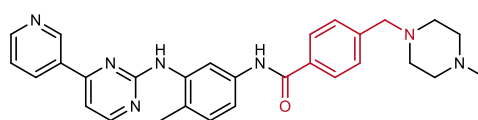
$$^2 \text{Improvement by IREDFisher} = \frac{\text{Hit rate by IREDFisher} - \text{Hit rate by random selection}}{\text{Hit rate by random selection}}$$

Table S2. Likelihood to retrieve the best hit by IREDFisher and Random selection. In most cases, IREDFisher was able to retrieve the best hit(s) from the whole panel. In some cases where the best hit did not appear, the second best hit was found. By comparison, random selection has a much lower chance of obtaining the best hit(s).

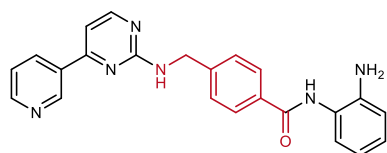
Reaction	Best hit	IREDFisher	Random selection
1	IR-10	Yes: Rank 10 th	22% ± 2.6%
2	IR-01	Yes: Rank 9 th	23% ± 2.6%
3	IR-01	Yes: Rank 10 th	22% ± 2.6%
4	IR-13	No, IR-10 Rank 11 th	23% ± 2.6%
5	IR-01	No, IR10 Rank 10 th	24% ± 2.6%
6	IR-01	Yes: Rank 9 th	25% ± 2.7%
7	IR-01	Yes: Rank 12 th	23% ± 2.6%
8	IR-01	Yes: Rank 16 th	25% ± 2.7%
9	IR01	No, IR-59 Rank 12 th	23% ± 2.6%
10	IR81	Yes: Rank 7 th	45% ± 3.1%
11	IR44	Yes: Rank 19 th	43% ± 3.1%
12	IR56	Yes: Rank 7 th	47% ± 3.1%
13	IR47	Yes: Rank 16 th	45% ± 3.1%
14	IR44	Yes: Rank 8 th	48% ± 3.1%
15	IR50	Yes: Rank 13 th	46% ± 3.1%
16	IR47	Yes: Rank 3 th	47% ± 3.1%
17	IR81	Yes: Rank 10 th	44% ± 3.1%
18	p-IR33	Yes: Rank 1 th	24% ± 2.6%
19	p-IR82	Yes: Rank 8 th	21% ± 2.5%
20	p-IR23	No; p-IR16 Rank 19 th	21% ± 2.5%
21	p-IR23	Yes: Rank 5 th	18% ± 2.4%
22	p- IR49	Yes: Rank 1 th	21% ± 2.5%
23	p-IR23	Yes: Rank 1 th	23% ± 2.6%
24	p-IR49	Yes: Rank 3 th	23% ± 2.6%



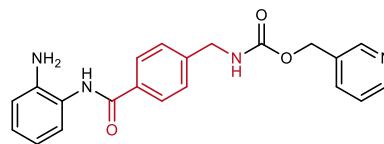
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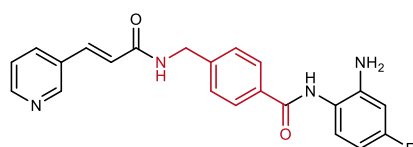
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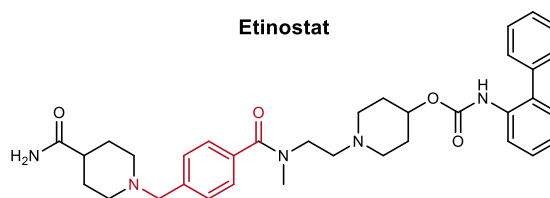
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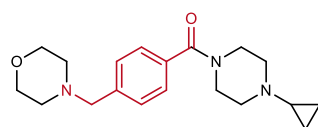
Etinostat



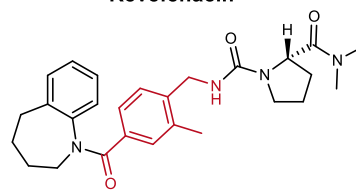
Tucidinostat



Revefenacin



Bavisant



Fedovapagon

Figure S1. Examples of pharmaceutically relevant 4-formylbenzoic acid benzaldehyde (highlighted in red) derivatives.

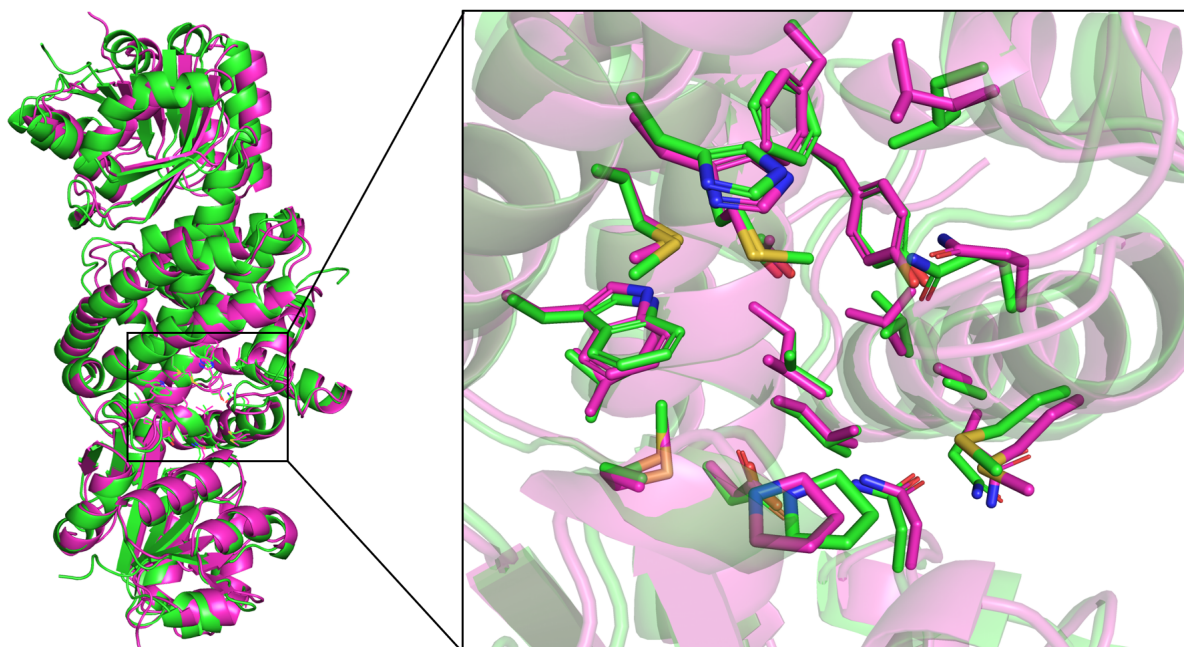


Figure S2. Structural comparison of an imine reductase predicted by homology modelling (in magenta) and AlphaFold (in green). Left panel, the overall structure. Right panel, a close-up view of amino acids in the substrate-binding site. Side chains in the active site were aligned, resulting in a root mean square deviation about 1.02 Å.

Analytical methods

GC-MS

Test reaction **18**, **26** and **27**. The mixture was analysed by GC-MS using the following conditions: hold at 40°C for 3 minutes, 40-300°C (30 °C / min) and 0.5 minutes for equilibration. Injector temperature 270 °C; inlet pressure 5.34 psi; detector temperature 300 °C; helium flow 2 ml/min; transfer line 320 °C, quadrupole 150 °C, ion source 230 °C; column Agilent HP-1MS (30 m × 0.32 mm × 0.25 µm).

HPLC and LC/MS

Test reaction **25** and **28**. The mixture was analysed by HPLC and corroborated by LC/MS using the following conditions: eluent MeCN/H₂O + 0.1% trifluoroacetic acid with a gradient method from 5% to 95% MeCN for 15 minutes. Flow 0.5 ml/min; column temperature 40 °C; injection volume 5 µl; UV detection at $\lambda = 210$ nm, column: Phenomenex Luna 3 µm C18(2) 100 Å.

Test reaction 18 scheme

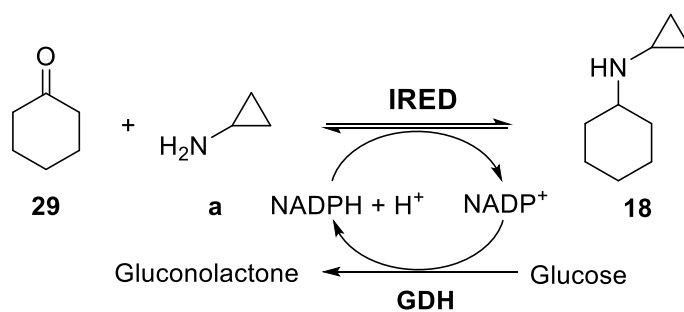
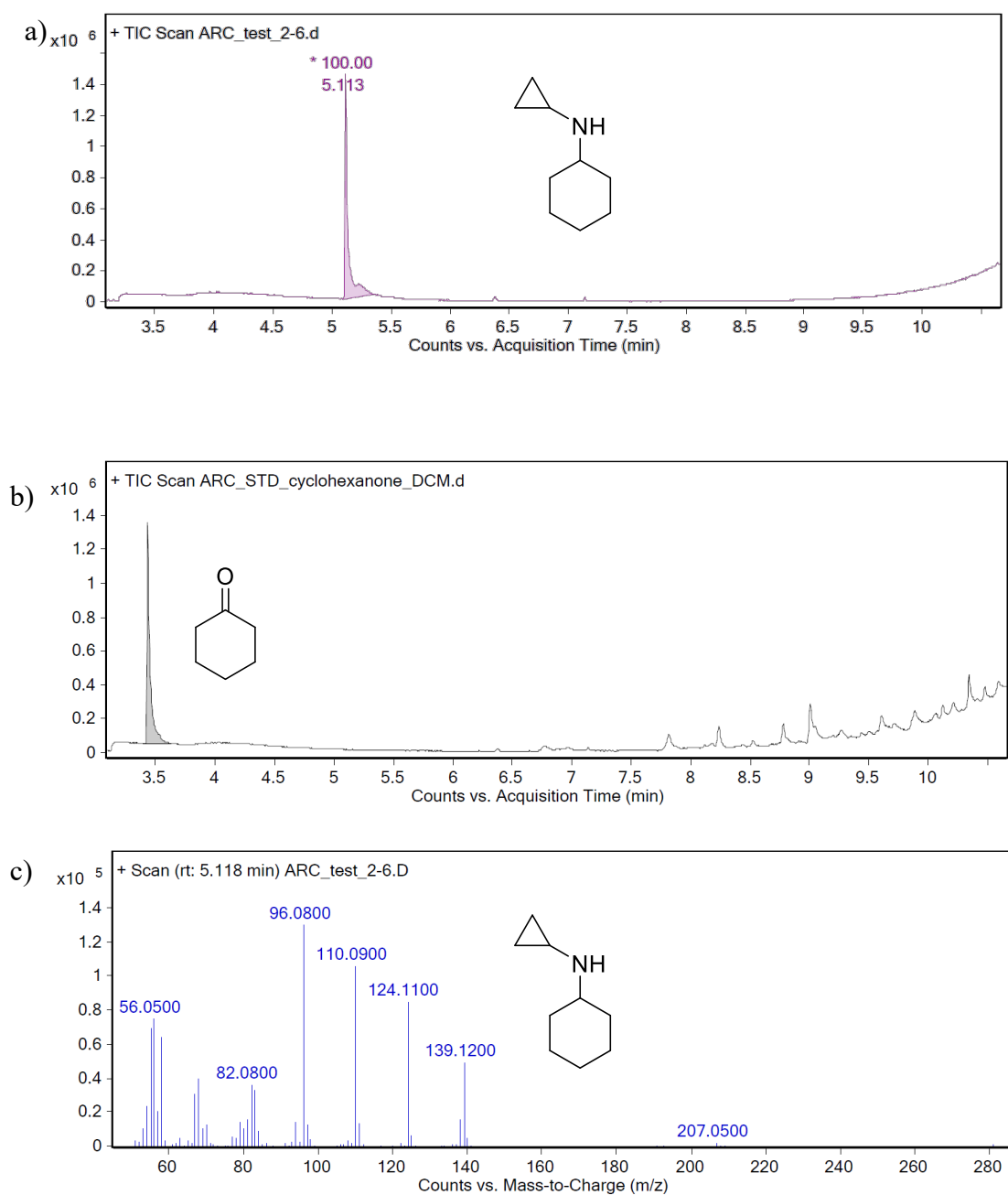


Figure S3. Scheme of reductive amination of cyclohexanone with cyclopropylamine for the synthesis of **18**. Cofactor regeneration system consists of GDH, NADP⁺ and glucose.

Test reaction 18 chromatograms



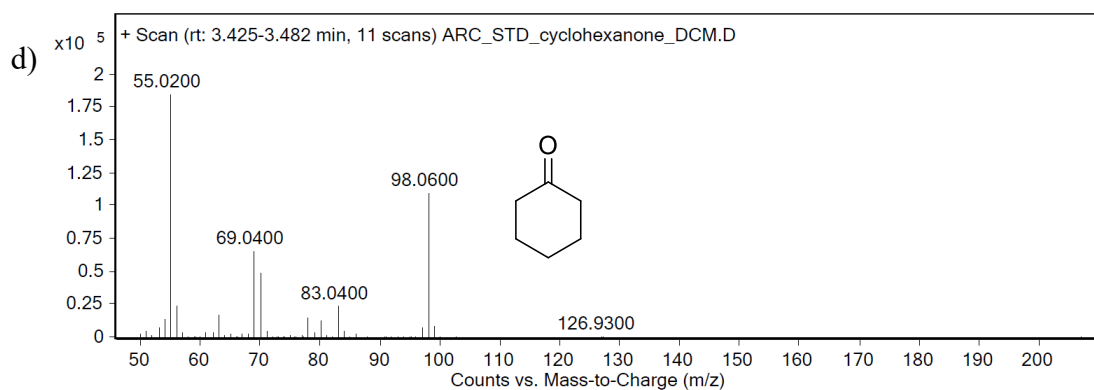


Figure S4. GC-MS spectra from screening for the synthesis of **18**. a) GC-MS chromatogram of cyclohexanone reductive amination with cyclopropylamine which exhibited >99% conversion. b) GC-MS chromatogram of cyclohexanone (**29**) standard. c) GC-MS spectra of peak at $R_t = 5.11$ min corresponding to GC-MS chromatogram a. d) GC-MS spectra of peak at $R_t = 3.45$ min corresponding to GC-MS chromatogram b.

Table S3. Retention times from GC-MS analysis for test reaction **18**.

Compound	Retention time (minutes)
<i>2-cyclohexanone (29)</i>	3.5
<i>N-cyclopropylcyclo hexanamine (18)</i>	5.1

Test reaction 26 scheme

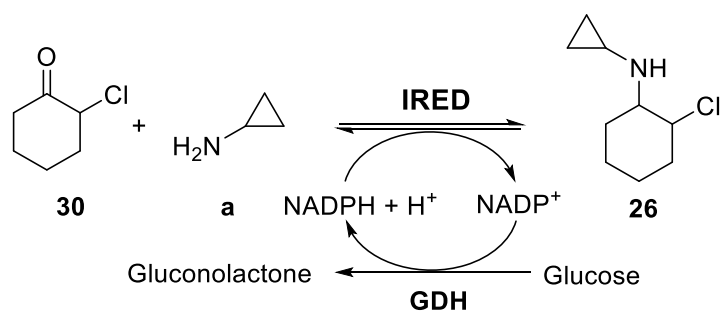
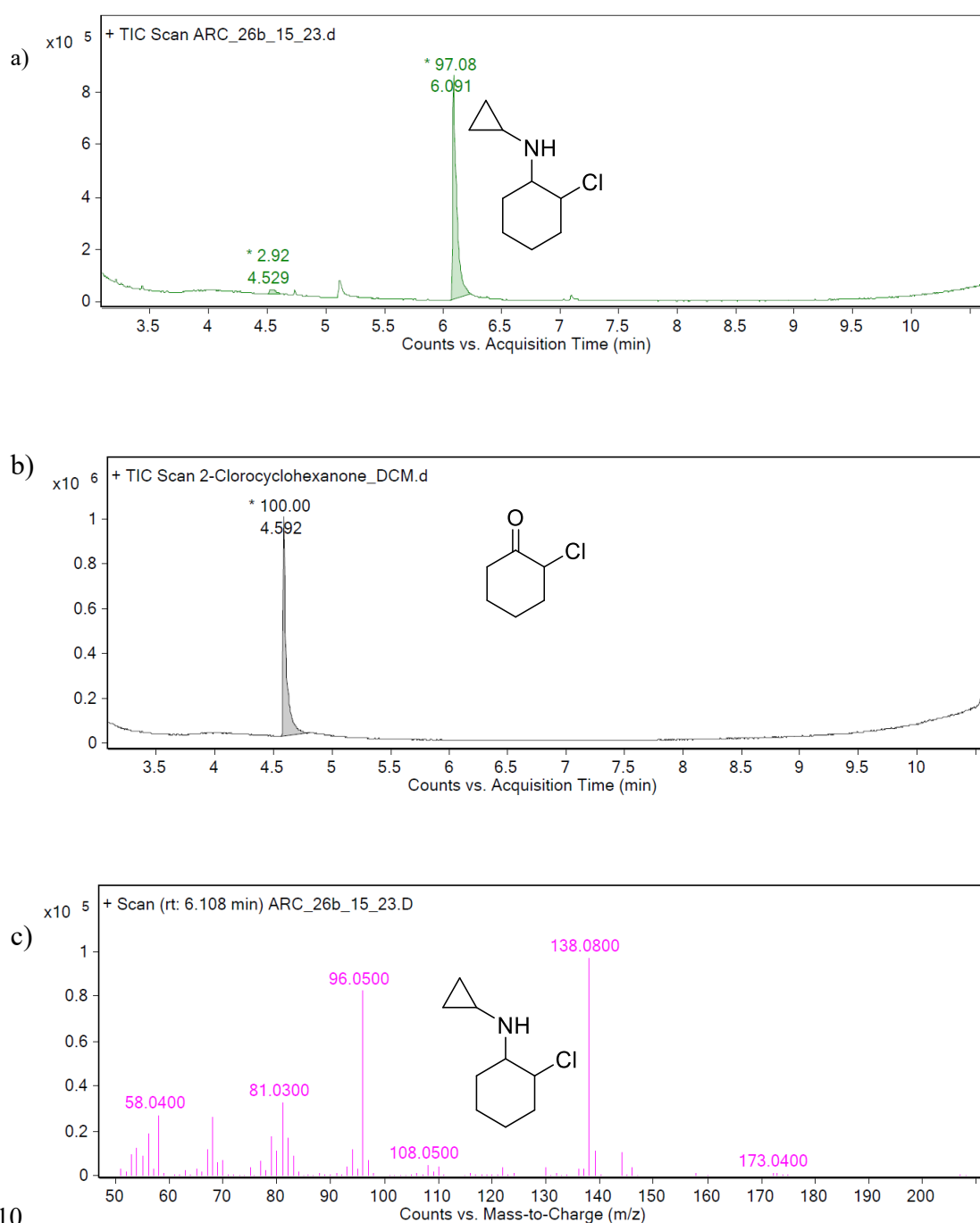


Figure S5. Scheme of reductive amination of 2-chlorocyclohexanone with cyclopropylamine for the synthesis of **26**. Cofactor regeneration system consists of GDH, NADP⁺ and glucose.

Test reaction 26 chromatograms



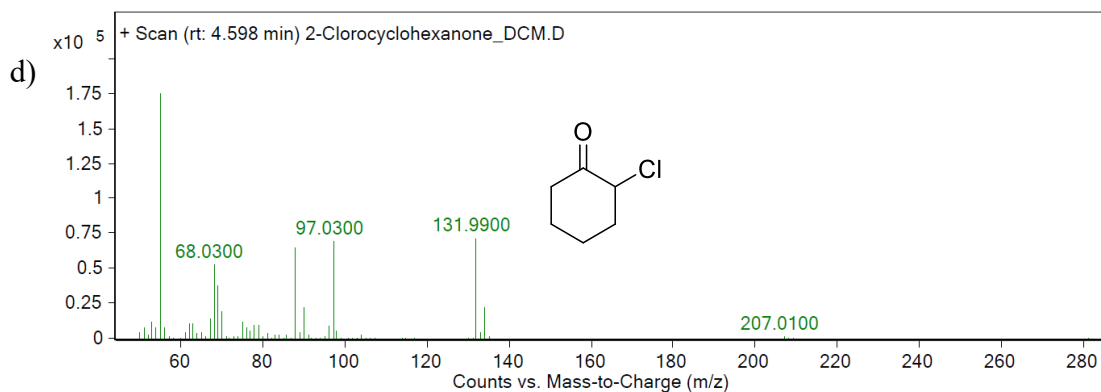


Figure S6. GC-MS spectra from screening for the synthesis of **26**. a) GC-MS chromatogram of 2-chlorocyclohexanone reductive amination with cyclopropylamine which exhibited 97% conversion. b) GC-MS chromatogram of cyclohexanone (**30**) standard. c) GC-MS spectra of peak at $R_t = 6.1$ min corresponding to GC-MS chromatogram a. d) GC-MS spectra of peak at $R_t = 4.6$ min corresponding to GC-MS chromatogram b.

Table S4. Retention times from GC-MS analysis for test reaction **26**.

Compound	Retention time (minutes)
<i>2-chloro-cyclohexanone (30)</i>	6.1
<i>2-chloro-N-cyclopropylcyclohexanamine (26)</i>	4.6

Test reaction 27 scheme

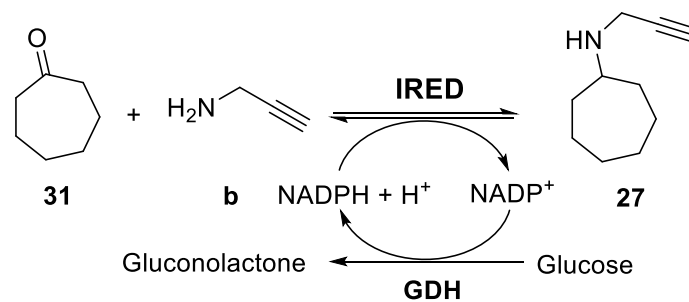
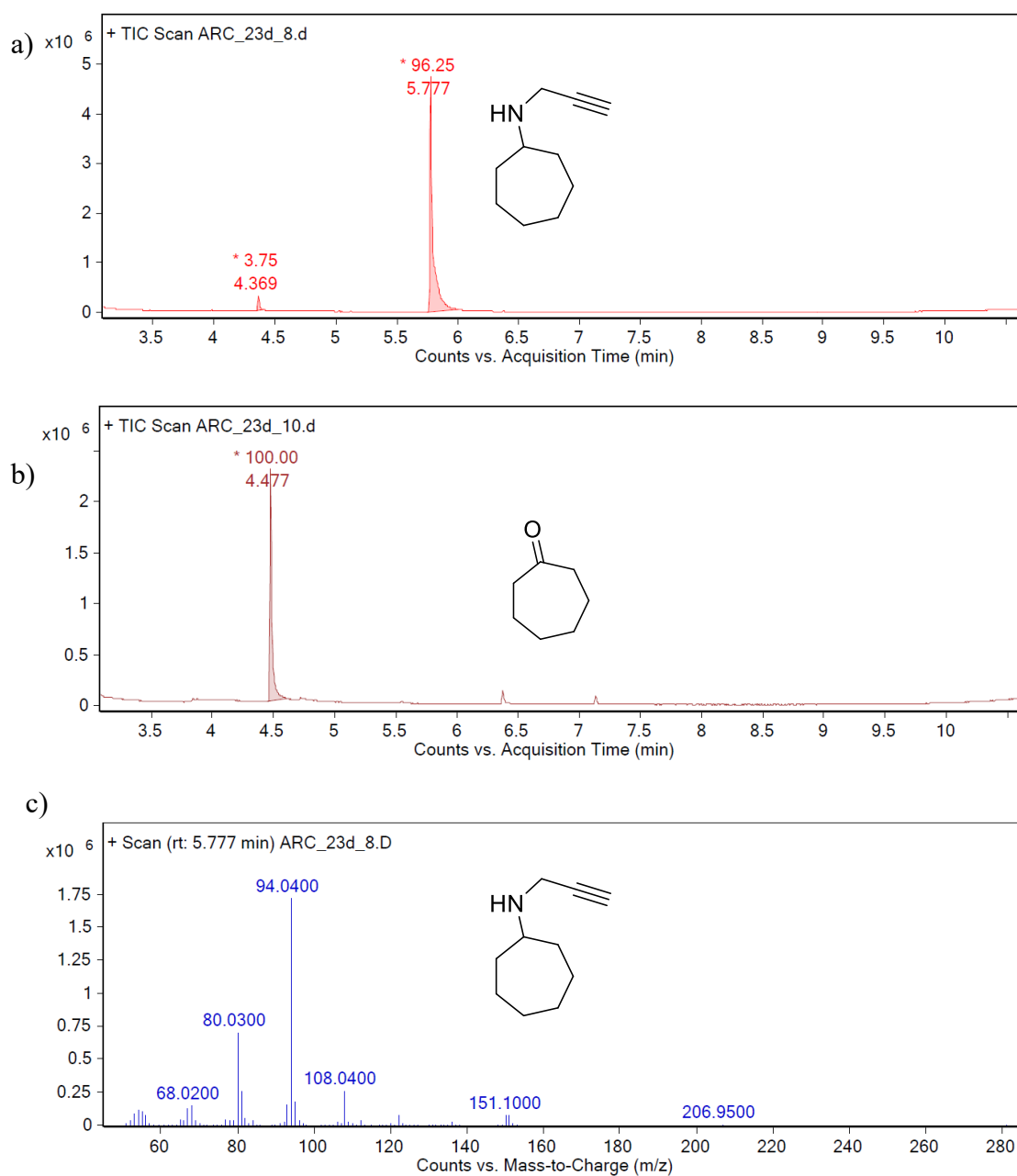


Figure S7. Scheme of reductive amination of cycloheptanone with propargylamine for the synthesis of **27**. Cofactor regeneration system consists of GDH, NADP^+ and glucose.
Test reaction 27 chromatograms



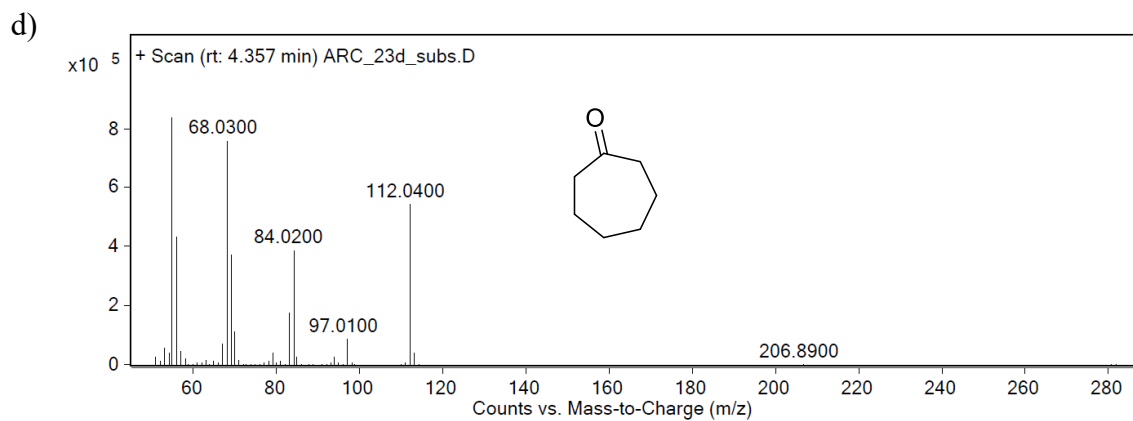


Figure S8. GC-MS spectra from screening for the synthesis of **27**. a) GC-MS chromatogram of cycloheptanone reductive amination with propargylamine which exhibited 96% conversion. b) GC-MS chromatogram of cyclohexanone (**31**) standard. c) GC-MS spectra of peak at $R_t = 5.8$ min corresponding to GC-MS chromatogram. d) GC-MS spectra of peak at $R_t = 4.4$ min corresponding to GC-MS chromatogram a.

Table S5. Retention times from GC-MS analysis for test reaction **27**.

Compound	Retention time (minutes)
<i>Cycloheptanone (31)</i>	5.8
<i>N-propargylcycloheptamine (27)</i>	4.4

Test reaction **25** scheme

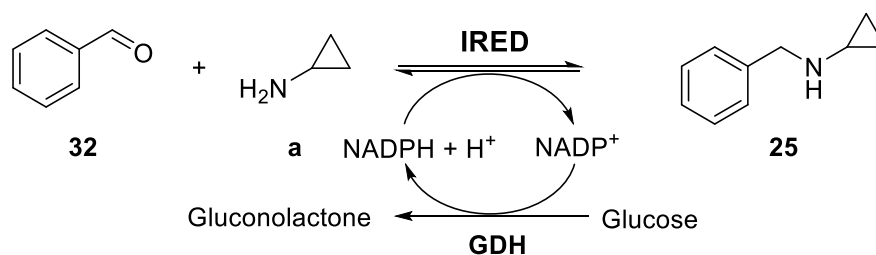


Figure S9. Scheme of reductive amination of benzaldehyde with cyclopropylamine for the synthesis of **25**. Cofactor regeneration system consists of GDH, NADP^+ and glucose.

Test reaction **25** chromatograms

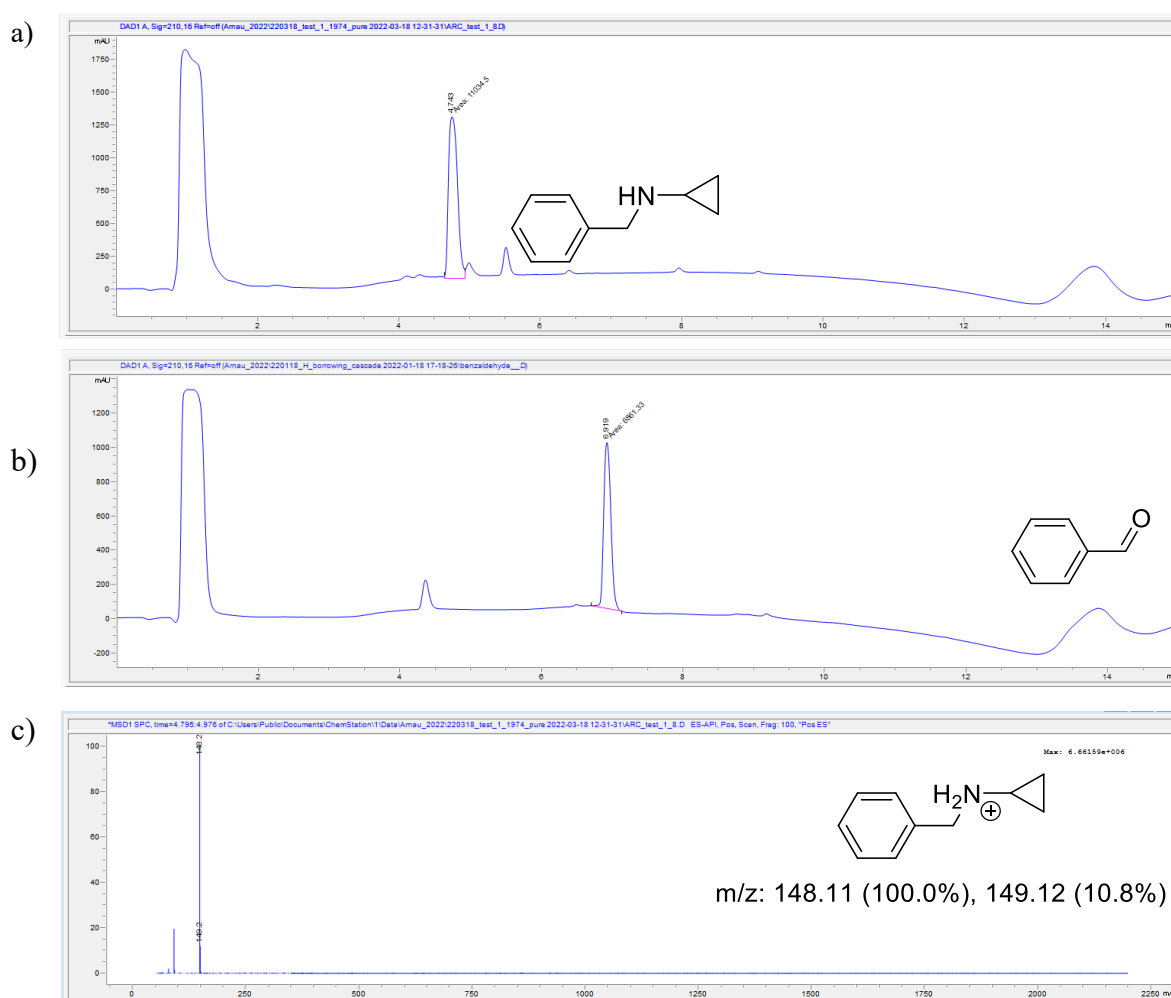


Figure S10. LC/MS chromatograms from screening for the synthesis of **25**. a) LC/MS chromatogram of benzaldehyde reductive amination with cyclopropylamine which exhibited 96% conversion. b) LC/MS chromatogram of benzaldehyde (**32**) standard c) LC/MS spectra of peak at $R_t = 4.7$ min corresponding to GC-MS chromatogram a.

Table S6. Retention times from HPLC analysis for test reaction **25**.

Compound	Retention time (minutes)
<i>Benzaldehyde (32)</i>	6.9
<i>N-benzylcyclopropylamine (25)</i>	4.7

Test reaction **28** scheme

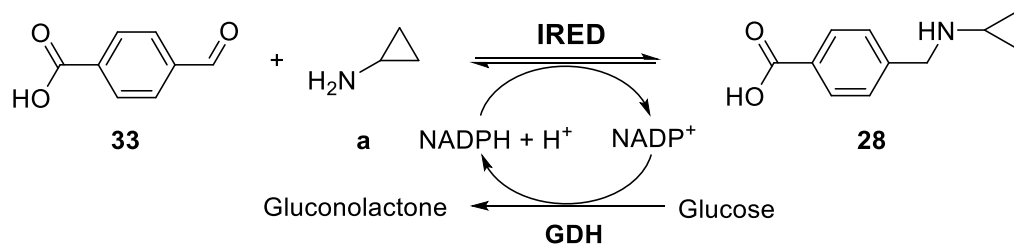


Figure S11. Scheme of reductive amination of 4-formylbenzoic acid with cyclopropylamine for synthesis of **28**. Cofactor regeneration system consists of GDH, NADP⁺ and glucose.

Test reaction **28** chromatograms

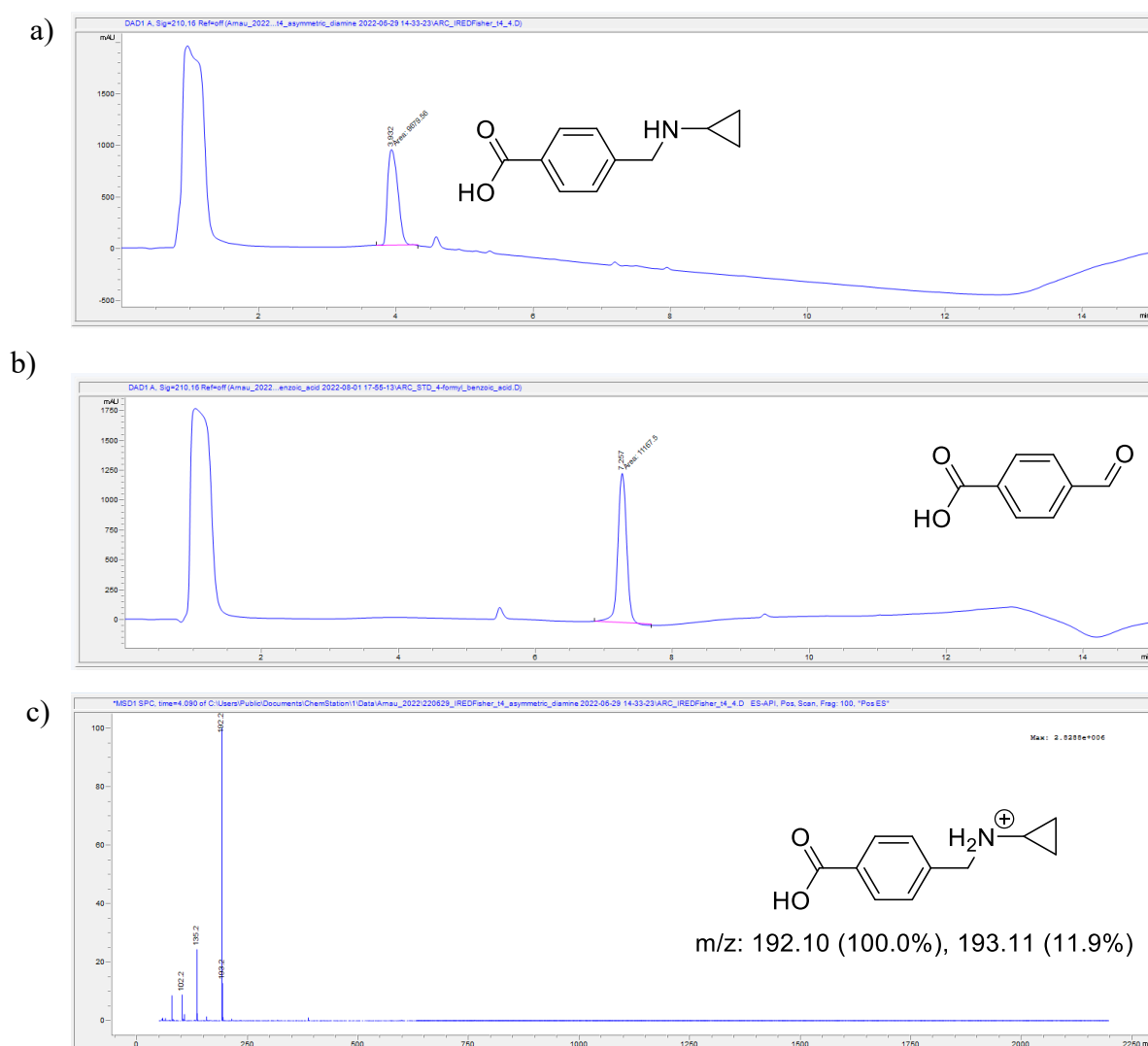


Figure S12. HPLC chromatograms from screening for the synthesis of **28**. a) LC/MS chromatogram of 4-formylbenzoic acid reductive amination with cyclopropylamine which exhibited 95% conversion. b) LC/MS chromatogram of 4-formylbenzoic acid (**33**) standard c) LC/MS spectra of peak at R_t = 3.9 min corresponding to GC-MS chromatogram a.

Table S7. Retention times from HPLC analysis for test reaction **28**.

Compound	Retention time (minutes)
<i>4-formylbenzoic acid (33)</i>	7.2
<i>4-((cyclopropylamino)methyl)benzoic acid (28)</i>	3.9

IRED Sequences

>yIRED1

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>yIRED16

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>yIRED17

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>yIRED19

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>yIRED20

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>yIRED21

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>yIRED26

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>yIRED27

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>yIRED31

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>yIRED32

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>yIRE38

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>yIRED42

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>yIRED44

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>yIRED46

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>yIRED47

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>yIRED49

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>yIRED51

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>yIRED53

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>yIRED54

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>yIRED55

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