Supplementary information

IREDFisher: a structure-based web server for designing small screening panels with given substrates

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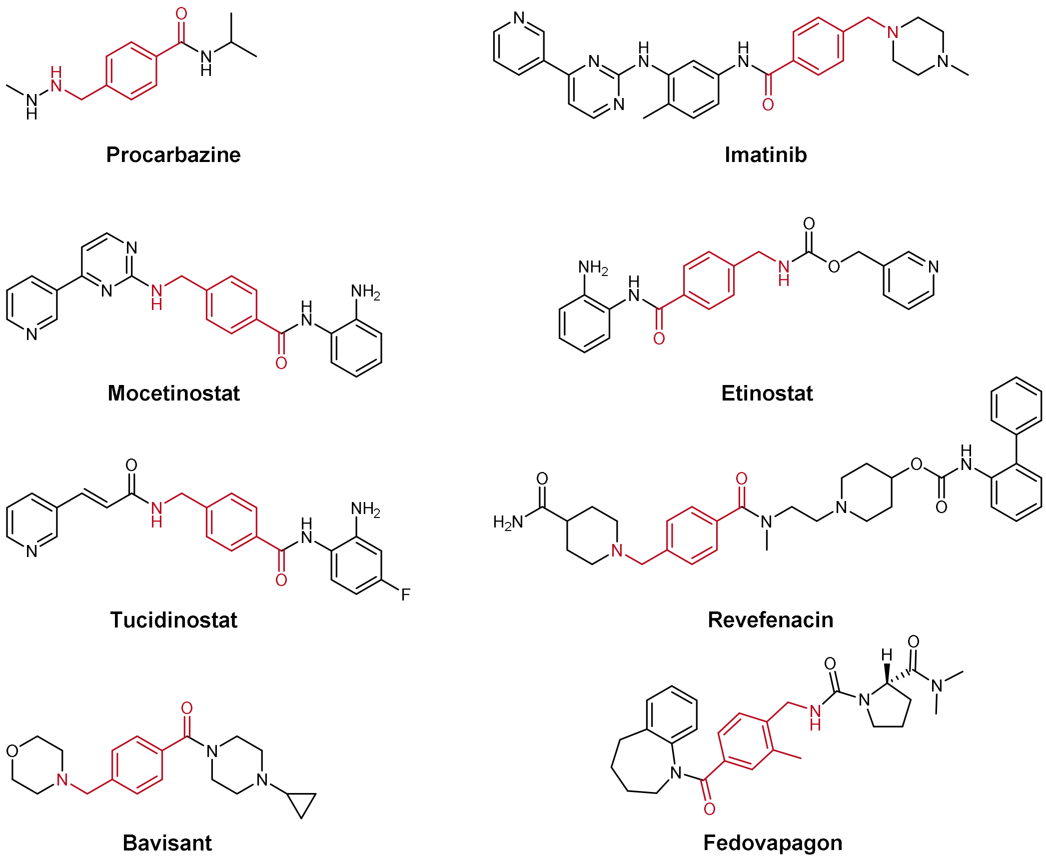
Table S1. Hits rate in 20 sequences by IREDFisher and random selection. In random selection, 20 sequences were draw from the panels for 1000 times and the average hit rate were calculated. The margin of error were calculated based on 95% confidence levels.

Reaction	Hit rate by IREDFisher (Conv. ≥50%)	Hit rate by random selection (Conv. ≥50%)	Improvement by IREDFisher (Conv. ≥50%)	Hit rate by IREDFisher (Conv. ≥2%)	Hit rate by random selection (Conv. ≥2%)	Improvement by IREDFisher (Conv. ≥2%)
1	20%	7% ± 0.3%	190%	30%	17% ± 0.4%	82%
2	15%	5% ± 0.2%	233%	55%	40% ± 0.6%	39%
3	25%	9% ± 0.3%	166%	70%	62% ± 0.6%	14%
4	5%	2% ± 0.2%	127%	75%	69% ± 0.6%	8%
5	0%	0% ± 0.0%	NA	20%	6% ± 0.3%	233%
6	15%	6% ± 0.3%	146%	70%	66% ± 0.6%	6%
7	30%	21% ± 0.5%	43%	85%	65% ± 0.6%	32%
8	NA[1]	NA[1]	NA[1]	15%	11% ± 0.4%	42%
9	NA[1]	NA[1]	NA[1]	20%	12% ± 0.4%	69%
10	70%	36% ± 0.5%	92%	75%	57% ± 0.5%	32%
11	45%	25% ± 0.5%	82%	85%	66% ± 0.5%	30%
12	40%	23% ± 0.4%	75%	80%	68% ± 0.5%	17%
13	NA	NA[1]	NA[1]	35%	21% ± 0.4%	70%
14	45%	23% ± 0.4%	96%	90%	66% ± 0.5%	37%
15	NA	NA[1]	NA[1]	70%	36% ± 0.5%	94%
16	40%	18% ± 0.4%	122%	60%	34% ± 0.5%	76%
17	25%	11% ± 0.3%	125%	75%	62% ± 0.5%	21%
18	70%	54% ± 0.6%	30%	95%	91% ± 0.3%	4%
19	20%	14% ± 0.4%	43%	50%	46% ± 0.6%	10%
20	10%	8% ± 0.3%	33%	45%	40% ± 0.6%	13%
21	5%	3% ± 0.2%	61%	30%	37% ± 0.6%	-19%
22	60%	36% ± 0.6%	66%	80%	66% ± 0.6%	22%
23	15%	9% ± 0.4%	60%	60%	61% ± 0.6%	-1%
24	30%	20% ± 0.5%	47%	55%	61% ± 0.6%	-9%

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

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 that the best hit did not appear, the second best hit were found. By comparison, random selection has much lower chance to obtain 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 10th	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 1**3**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%



Procarbazine

Imatinib

Mocetinostat

Etinostat

Tucidinostat

Revefenacin

Bavisant

Fedovapagon

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

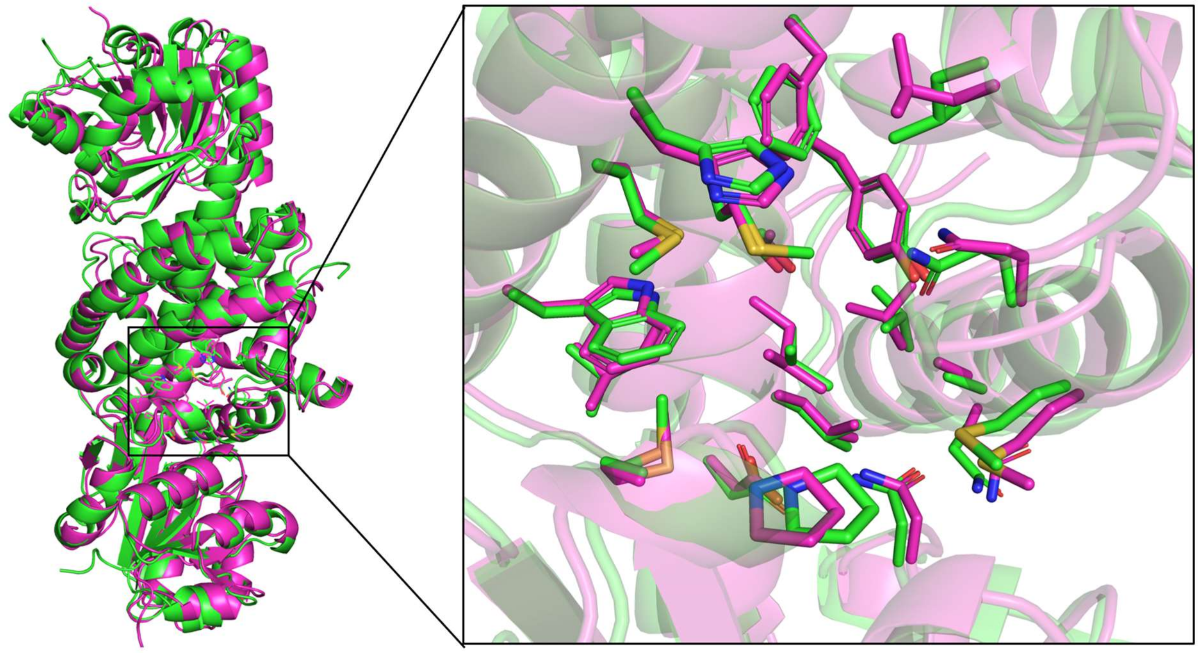


Figure S2. Structural comparison of a imine reductase predicted by homology modelling (in magenta) and AlphaFold (in green). (a) The overall structure (b) A close-up of amino acid in substrate-binding site. side chains in active site were aligned, resulting in an root mean square deviation about 1.02 Å.

Chromatographic Analysis

GC-MS method

Test reaction **18, 26** and **27:** The mixture was analysed by GC-MS on 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 methods

Test reaction **25** and **28**: The mixture was analysed by HPLC and corroborated by LC/MS on the following conditions: eluent MeCN/H_2O + 0.1% trifluoroacetic acid with a gradient method from 5% up 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.

GC/MS chromatograms

Test reaction **18** scheme

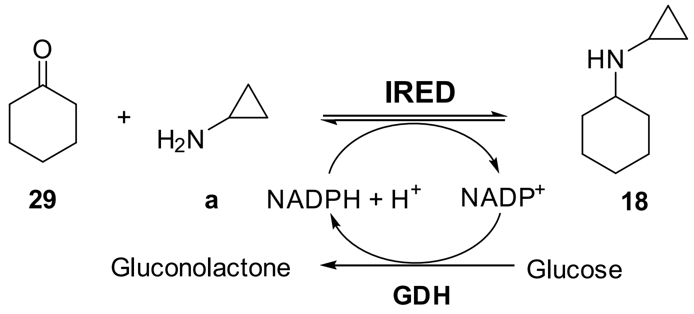
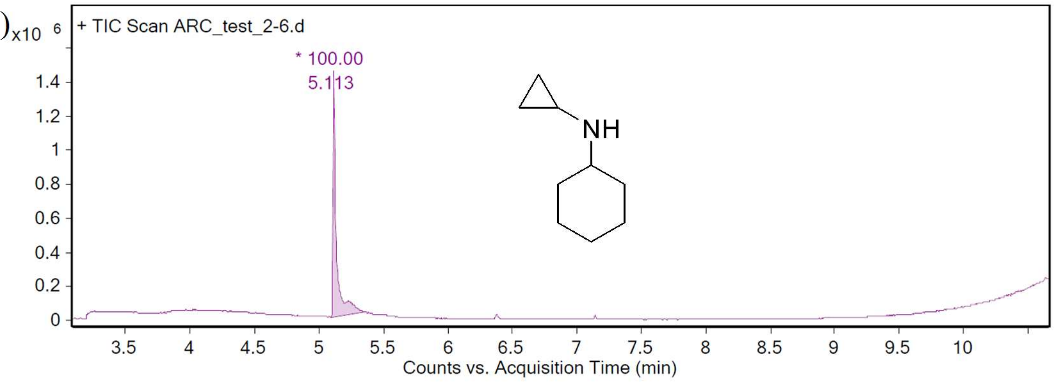


Figure S3. Scheme of reductive amination of cyclohexanone with cyclopropylamine for the synthesis of **18**. Cofactor regeneration system consist of GDH, $NADP^+$ and glucose.

Test reaction **18** chromatograms

a)



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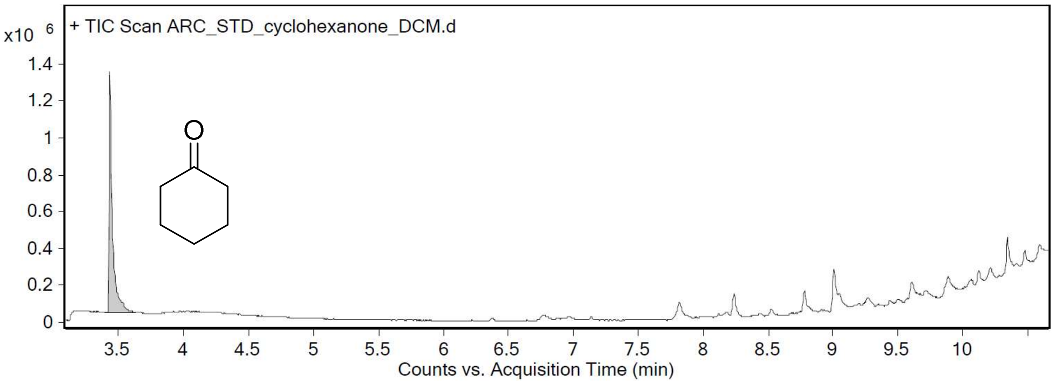

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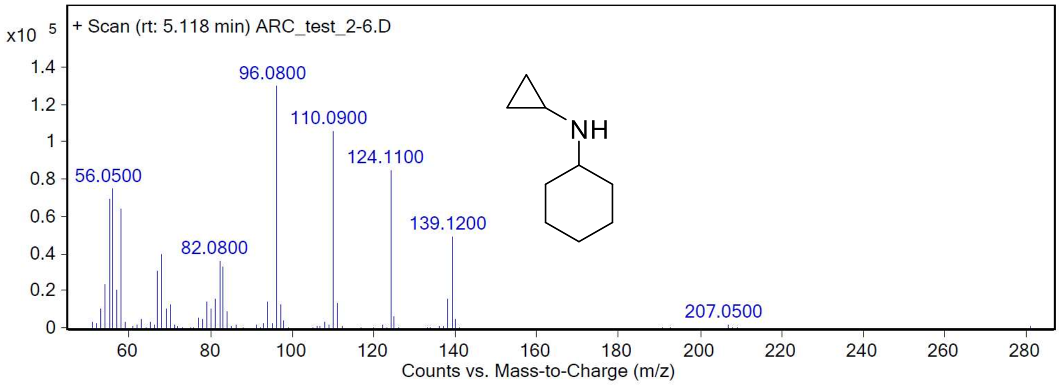


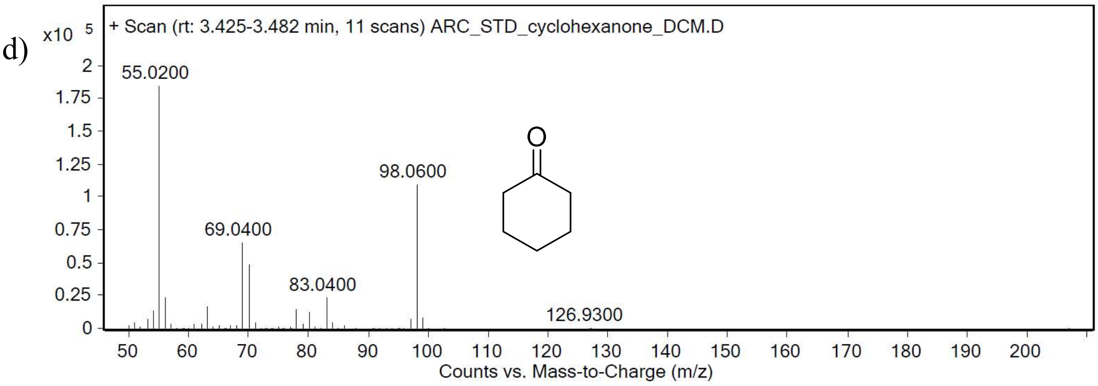


Figure S4. GC-MS spectra from screening for the synthesis of reaction **18**. a) GC-MS chromatogram of cyclohexanone reductive amination with cyclopropylamine which exhibited >99% conversion. b) GC-MS chromatogram of cyclohexanone (**29**) standard. c) GC-MS spectra of peak at R_t = 5.11 min corresponding 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)**
*2-cyclohexanone (**29**)*	3.5
*N-cyclopropylcyclo hexanamine (**18**)*	5.1

Test reaction **26** scheme

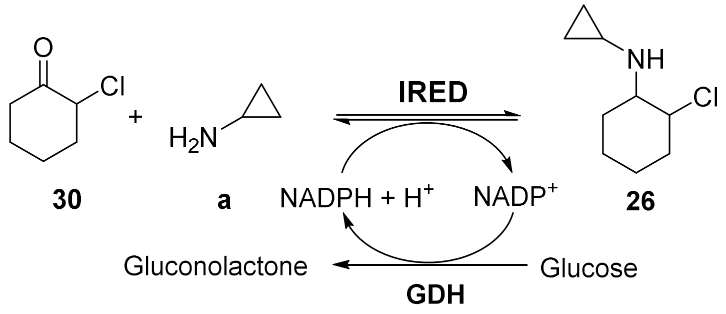
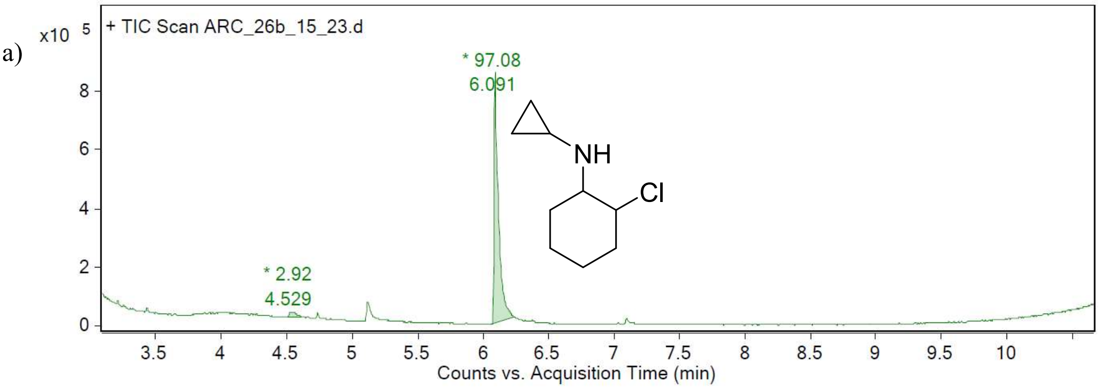


Figure S5. Scheme of reductive amination of 2-chlorocyclohexanone with cyclopropylamine for the synthesis of **26**. Cofactor regeneration system consist of GDH, NADP+ and glucose.

Test reaction **26** chromatograms



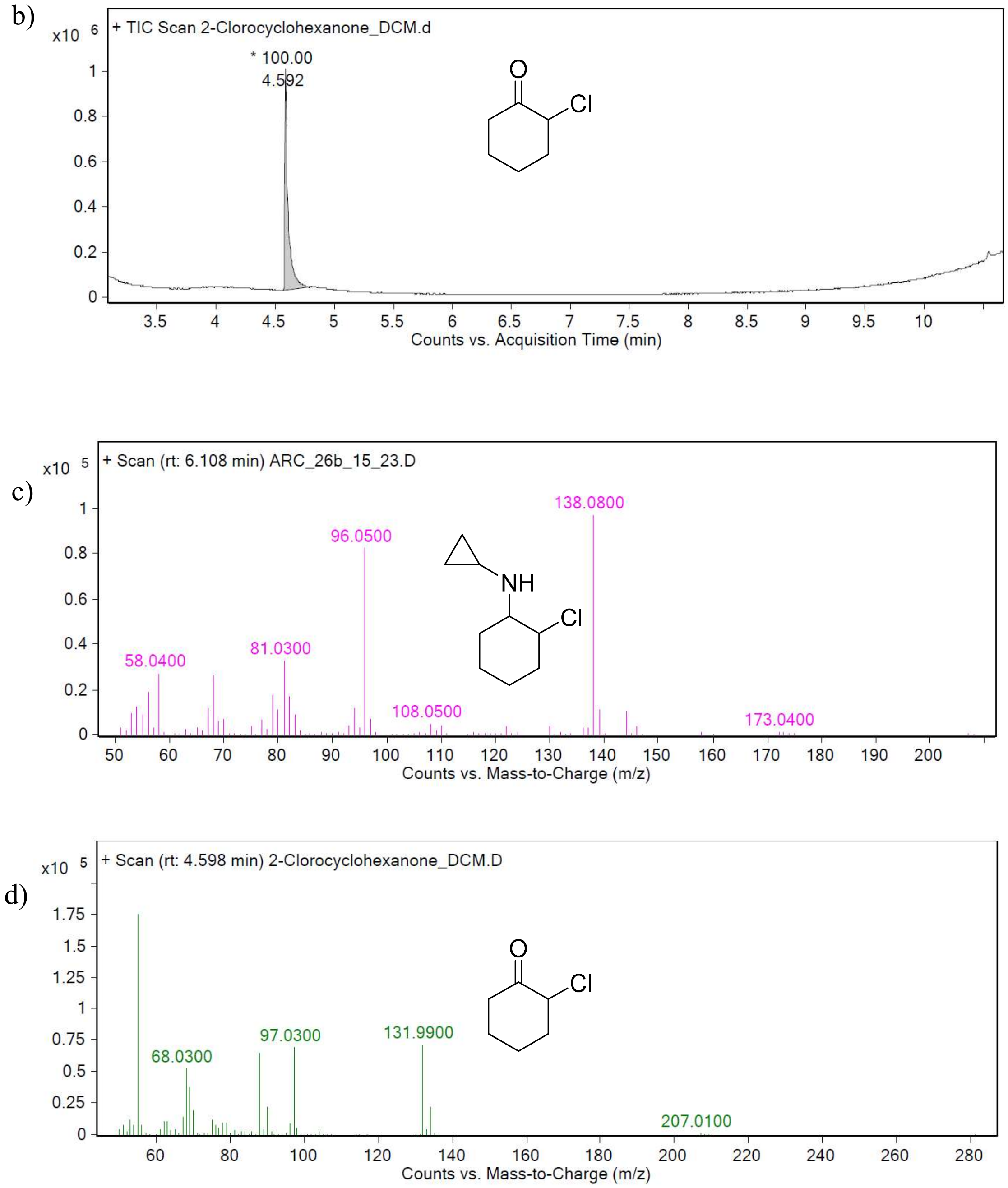


Figure S5. GC-MS spectra from screening for the synthesis of reaction **26**. a) GC-MS chromatogram of 2-chlorocyclohexanone reductive amination with cyclopropylamine which exhibited 97% conversion. b) GC-MS chromatogram of cyclohexanone (**30**) standard. c) GC-MS spectra of peak at R_t = 6.1 min corresponding 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)
*2-chloro-cyclohexanone (**30**)*	6.1
*2-chloro-N-cyclopropylcyclohexanamine (**26**)*	4.6

Test reaction **27** scheme

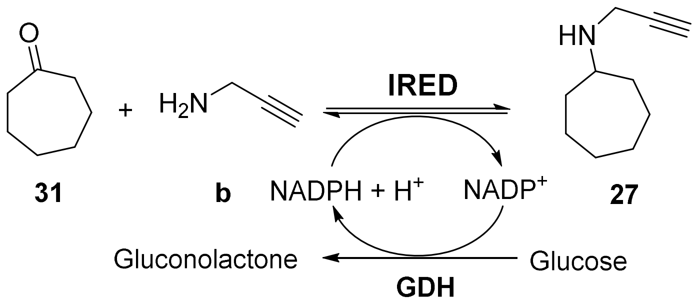
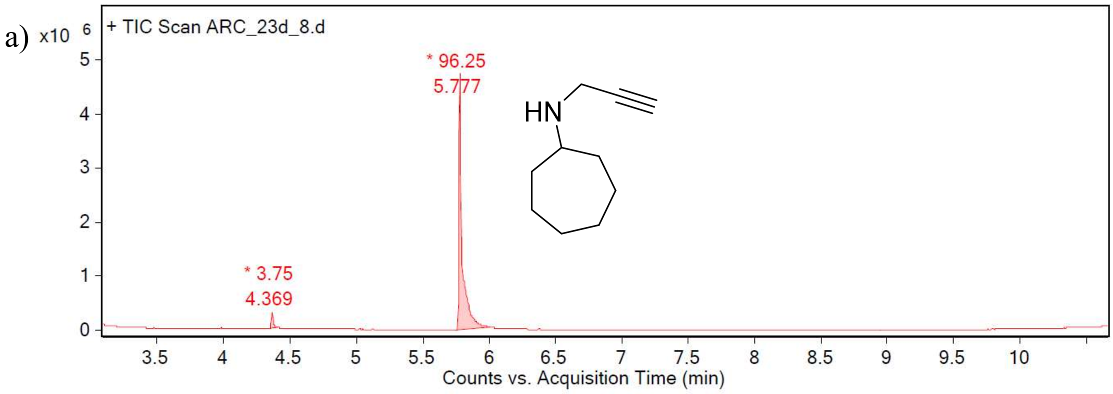
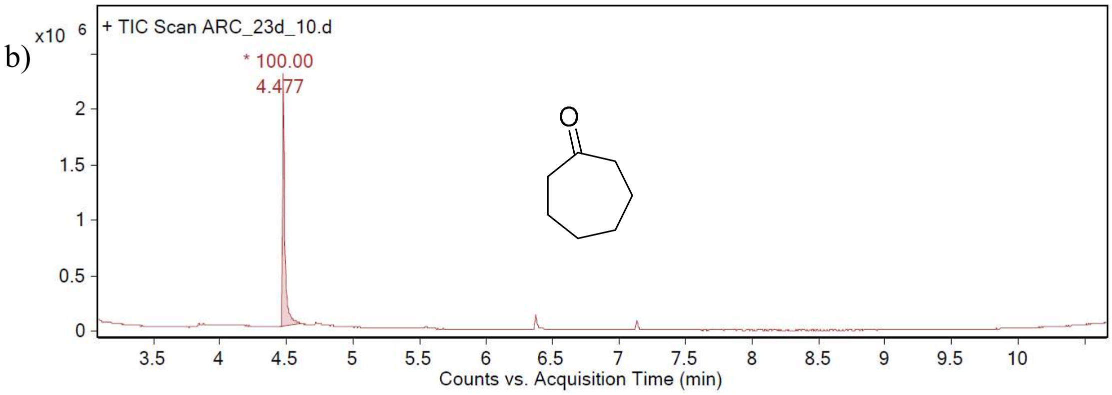


Figure S6. Scheme of reductive amination of cycloheptanone with propargylamine for the synthesis of **27**. Cofactor regeneration system consist of GDH, NADP+ and glucose.

Test reaction **27** chromatograms





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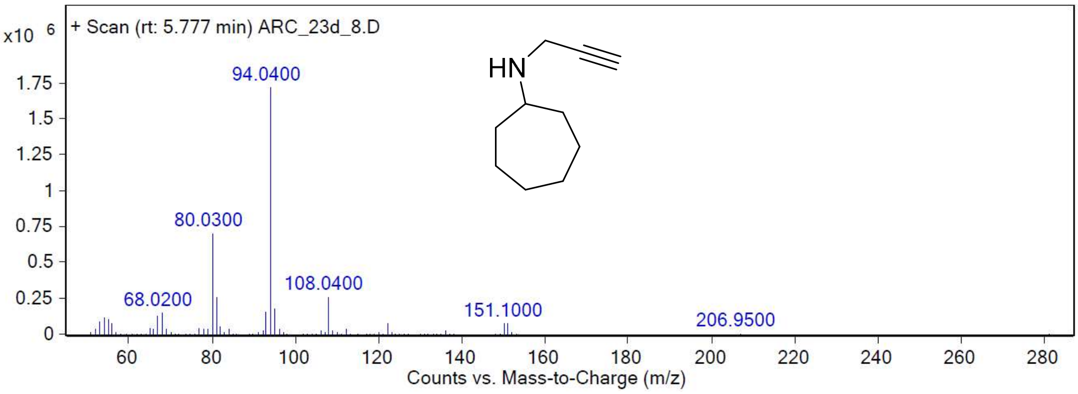

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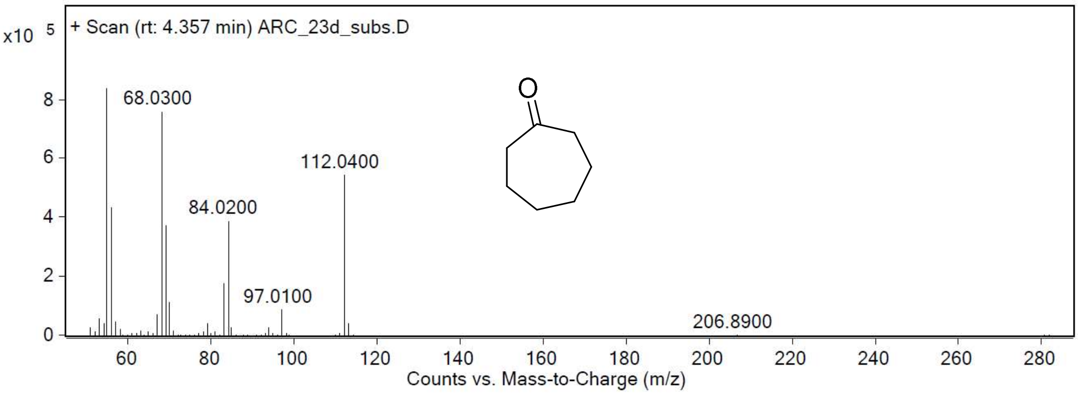


Figure S7. GC-MS spectra from screening for the synthesis of test reaction **27**. a) GC-MS chromatogram of cycloheptanone reductive amination with propargylamine which exhibited 96% conversion. b) GC-MS chromatogram of cyclohexanone (**31**) standard. c) GC-MS spectra of peak at R_t = 5.8 min corresponding 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)
Cycloheptanone (31)	5.8
N-propargylcycloheptamine (27)	4.4

HPLC and LC/MS chromatograms

Test reaction **25** scheme

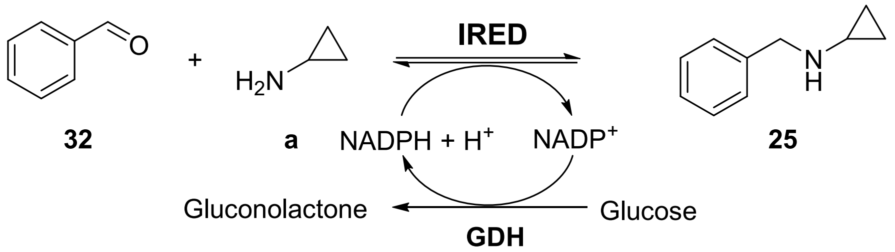
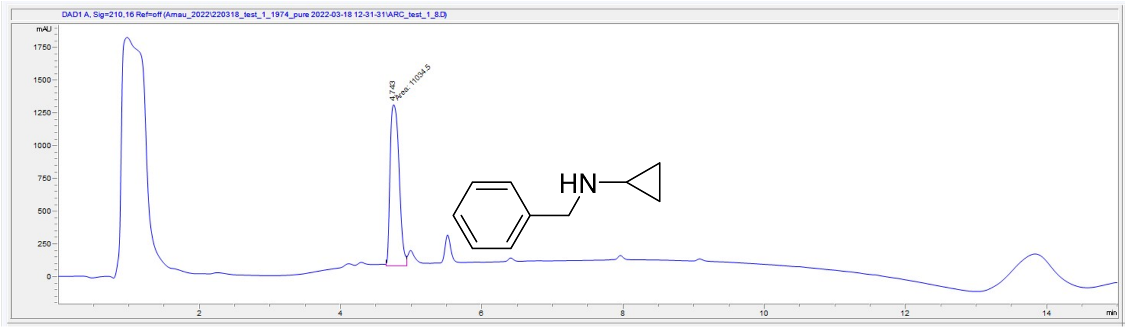


Figure S8. Scheme of reductive amination of benzaldehyde with cyclopropylamine for the synthesis of **25**. Cofactor regeneration system consist of GDH, NADP+ and glucose.

<u>Test reaction **25** chromatograms</u>

a)



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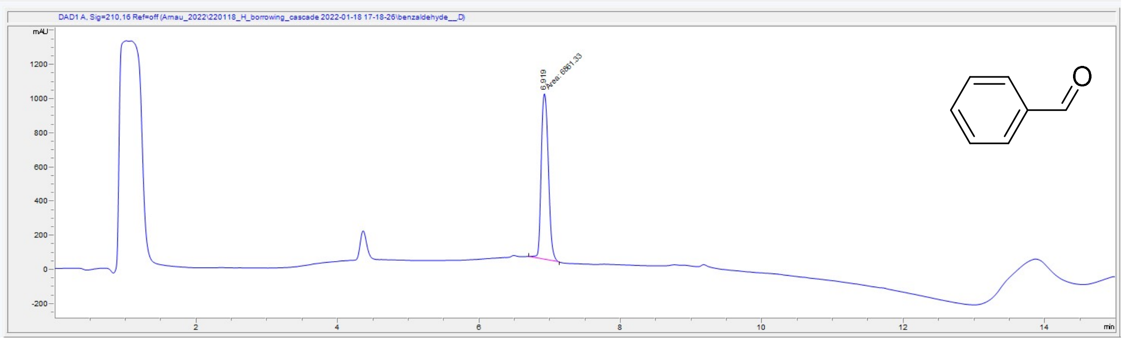

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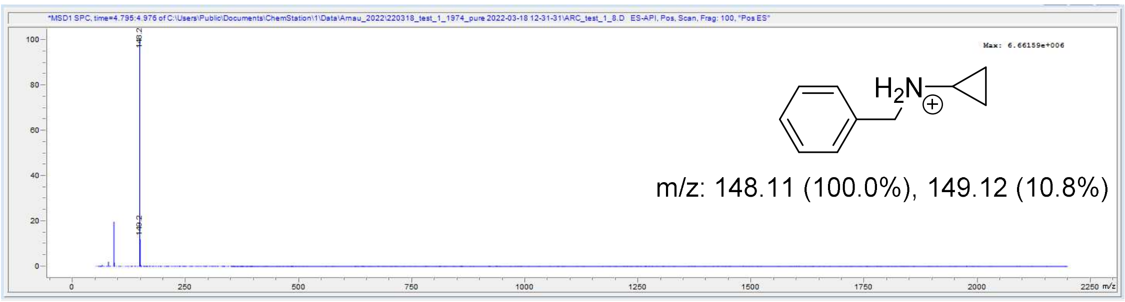


Figure S9. LC/MS chromatograms from screening for the synthesis of **25**. a) LC/MS chromatogram of benzaldehyde reductive amination with cyclopropylamine which exhibited 96% conversion. b) LC/MS chromatogram of benzaldehyde (**32**) standard c) LC/MS spectra of peak at $R_t = 4.7$ min corresponding to GC-MS chromatogram a.

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

Compound	Retention time (minutes)
*Benzaldehyde (**32**)*	6.9
*N-benzylcyclopropylamine (**25**)*	4.7

Test reaction **28** scheme

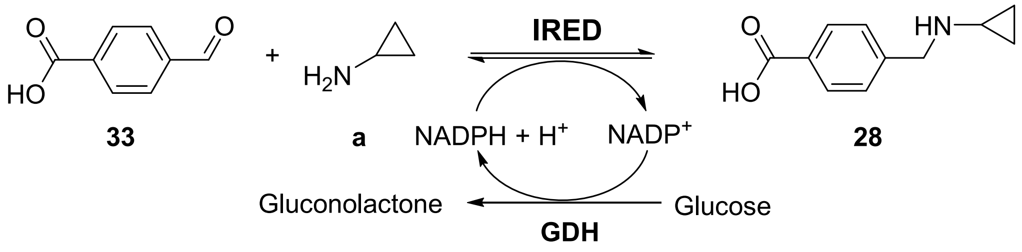
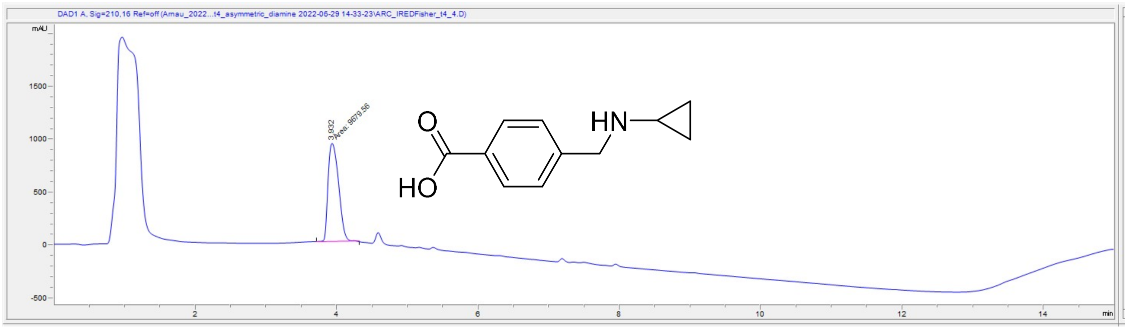


Figure S10. Scheme of reductive amination of 4-formylbenzoic acid with cyclopropylamine for synthesis of **28**. Cofactor regeneration system consist of GDH, NADP+ and glucose.

Test reaction **28** chromatograms

a)



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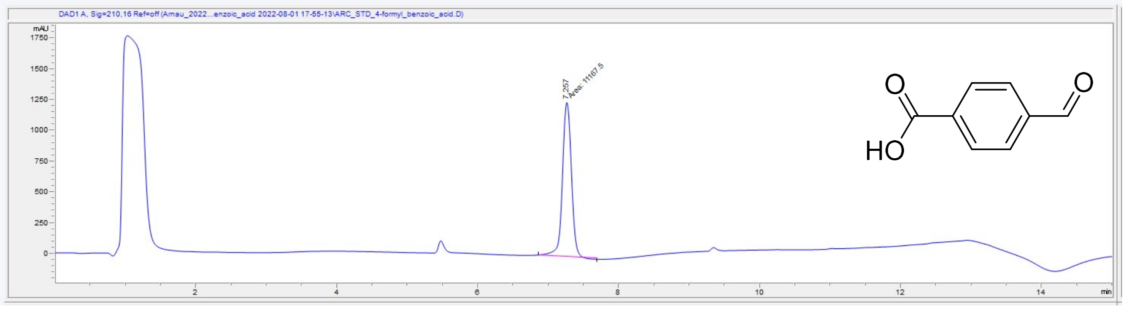

c)

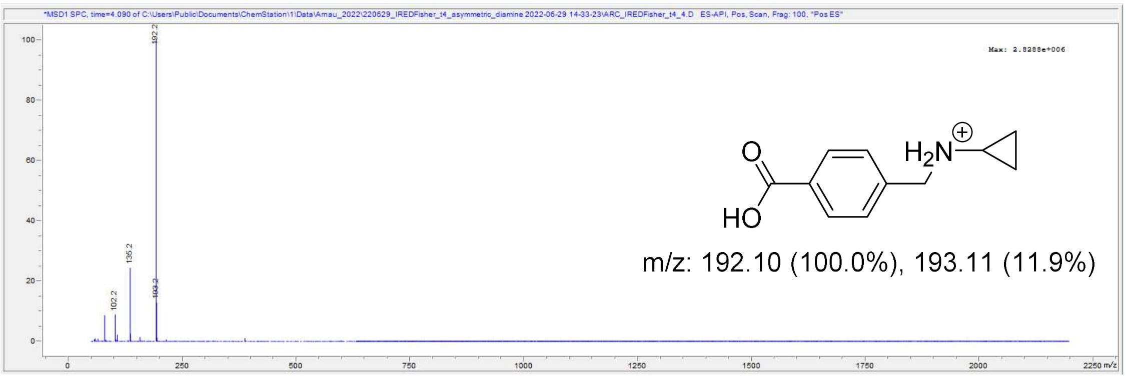

Figure S11. HPLC chromatograms from screening for the synthesis of compound **28**. a) LC/MS chromatogram of 4-formylbenzoic acid reductive amination with cyclopropylamine which exhibited 95% conversion. b) LC/MS chromatogram of 4-formylbenzoic acid (**33**) standard c) LC/MS spectra of peak at R_t = 3.9 min corresponding to GC-MS chromatogram a.

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

Compound	**Retention time (minutes)**
4-formylbenzoic acid (33)	7.2
4-((cyclopropylamino)methyl)benzoic acid (28)	3.9

Sequences

>yIRED1

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>yIRED12

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>yIRED16

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>yIRED17

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>yIRED23

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>yIRED35

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>yIRED37

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>yIRED58

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>yIRED59

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GEDHGLVALHEMAVMALMWSTLNGFLQGAALLGAAGVDAGAFVPYARQGAEAV
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>yIRED60

MKPGISVLGTGRMGSALVGAFLKQGYNVAVWNRTKSKCAPLAALGARVATTVRD
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QHELQYLDGAIMGTPNFIGEPGGTILYSGPGALFEKYKPVLLVLGGNSLHVGSDVGH
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KRIQTGRFAGDEKTLATVEAHHGALRHLIELCEEHGIHHAVPAAFGQLFQAALQAGH
AQDDFAVLNKFMK