Supplemental Data

Rational Design of Broad-Spectrum Antibacterial Activity based on a Clinically Relevant Enoyl-ACP Reductase Inhibitor*

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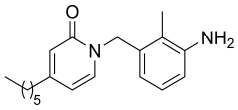
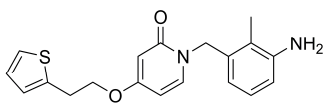
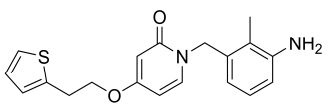
Supplemental Tables

TABLE S1. **Data Collection and Refinement Statistics (*S. aureus* FabI).**

	PT173	**CG400549-I**	**CG400549-II**
Inhibitor			
Data collection			
Cell dimensions			
a, b, c (Å)	61.5, 109.2, 289.4	61.9, 108.5, 296.8	77.0, 113.1, 117.7
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Resolution[1] (Å)	60.3-3.10 (3.27-3.10)	47.6-1.95 (2.06-1.95)	45.6-2.20 (2.32-2.20)
Observed reflections	146,382 (20,325)	2,435,819 (313,695)	257,814 (36,976)
Unique reflections	36,439 (5,219)	146,661 (21,138)	52,885 (7,580)
Completeness (%)	100.0 (100.0)	100.0 (100.0)	100.0 (99.9)
Average redundancy	4.0 (3.9)	16.6 (14.8)	4.9 (4.9)
R_{merge}[2] (%)	10.4 (48.8)	16.1 (134.8)	13.0 (46.6)
R_{pim}[3] (%)	6.0 (28.2)	4.0 (36.1)	6.6 (23.5)
$\langle I / \sigma(I) \rangle$	9.4 (2.7)	15.6 (2.4)	8.7 (3.2)
Monomers per AU	8	8	4
Refinement			
Resolution (Å)	60.1-3.10	47.6-1.95	45.6-2.20
R_{cryst}[4] (%)	20.7	16.8	20.5
R_{free} (%)	25.8	23.1	27.7
Number of atoms	16,154	17,795	8,665
rmsd bond lengths (Å)	0.006	0.011	0.014
rmsd bond angles (°)	1.05	1.63	1.55
Average B-factor (Å^2)	81.8	30.1	27.1
Ramachandran-plot[5]			
Favored (%)	95.2	96.8	95.9
Allowed (%)	4.4	3.1	4.0
Outliers (%)	0.4	0.1	0.1
Maximum likelihood based estimated coordinate error (Å)	0.27	0.13	0.22
PDB code	4CUZ	4CV1	4CV0

[1]Values in parenthesis refer to the highest resolution shell

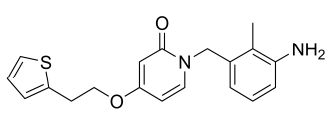
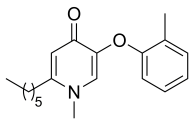
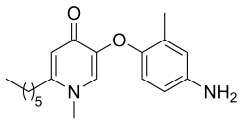
[2] $R_{merge} = \sum_{hkl}\sum_{i}\left|I_i - \langle I \rangle\right| \Big/ \sum_{hkl}\sum_{i} I_i$

[3] $R_{pim} = \sum_{hkl}\left[1/(N-1)\right]^{1/2}\sum_{i}\left|I_i - \langle I \rangle\right| \Big/ \sum_{hkl}\sum_{i} I_i$ (1)

[4] $R_{cryst} = \sum_{hkl}\left|F_{obs} - F_{calc}\right| \Big/ \sum_{hkl} F_{obs}$

[5]According to Molprobity, version 3.19 (2)

TABLE S2. **Data Collection and Refinement Statistics (*E. coli* and *B. pseudomallei* FabI).**

	ecFabI-NADH-CG400549	**ecFabI-NADH-PT166**	**bpFabI-NAD$^+$-PT155**
Inhibitor			
Data collection			
Cell dimensions			
a, b, c (Å)	80.1, 80.1, 320.2	79.7, 79.7, 330.2	74.3, 76.0, 89.4
α, β, γ (°)	90, 90, 120	90, 90, 120	90, 90, 90
Space group	$P6_122$	$P6_122$	I222
Resolution[1] (Å)	47.1-1.80 (1.90-1.80)	47.7-1.95 (2.06-1.95)	38.0-1.84 (1.94-1.84)
Observed reflections	818,706 (97,180)	306,907 (46,321)	108,611 (14,641)
Unique reflections	56,987 (7,945)	46,243 (6,503)	22,291 (3,219)
Completeness (%)	98.7 (96.6)	99.5 (98.6)	100.0 (100.0)
Average redundancy	14.4 (12.2)	6.6 (7.1)	4.9 (4.5)
R_{merge}[2] (%)	12.5 (109.7)	9.6 (64.7)	6.9 (26.1)
R_{pim}[3] (%)	3.2 (30.4)	4.0 (25.6)	3.5 (13.7)
$\langle I / \sigma(I) \rangle$	12.6 (2.3)	12.6 (2.7)	14.6 (5.0)
Monomers per AU	2	2	1
Refinement			
Resolution (Å)	69.4-1.80	69.0-1.95	38.0-1.84
R_{cryst}[4] (%)	17.5	17.6	13.7
R_{free} (%)	20.7	21.2	16.2
Number of atoms	4,057	3,943	2,367
rmsd bond lengths (Å)	0.012	0.015	0.008
rmsd bond angles (°)	1.60	1.77	1.14
Average B-factor (Å^2)	33.0	37.9	13.8
Ramachandran-plot[5]			
Favored (%)	97.4	97.4	97.3
Allowed (%)	2.6	2.6	2.7
Outliers (%)	0.0	0.0	0.0
Maximum likelihood based estimated coordinate error (Å)	0.08	0.09	0.18
PDB code	4CV2	4CV3	4BKU

[1]Values in parenthesis refer to the highest resolution shell

[2] $R_{merge} = \sum_{hkl}\sum_{i}\left|I_i - \langle I \rangle\right| \Big/ \sum_{hkl}\sum_{i} I_i$

[3] $R_{pim} = \sum_{hkl}\left[1/(N-1)\right]^{1/2}\sum_{i}\left|I_i - \langle I \rangle\right| \Big/ \sum_{hkl}\sum_{i} I_i$ (1)

[4] $R_{cryst} = \sum_{hkl}\left|F_{obs} - F_{calc}\right| \Big/ \sum_{hkl} F_{obs}$

[5]According to Molprobity, version 3.19 (2)

TABLE S3. **EcFabI kinetic parameters for the mechanistic model in Figure 2B.**

Parameter	Estimate	Rationale
K_S	4.25 mM for cro-CoA	Approximated based on a previously reported K_m value (3).
K_{NADH}	7.8 μM	Based on a previously reported K_d value (3).
K_{NAD}	1.8 mM	Based on a previously reported K_d value (3).
$k_{on,S}$	$6 \cdot 10^8$ M^{-1} min^{-1}	This arbitrary estimate is within an order of magnitude of the diffusion-limited rate constant, which represents the theoretical ceiling. The value will only affect the kinetic system if it is low enough to be rate limiting (approx. $6 \cdot 10^5$ M^{-1} min^{-1}).
$k_{on,NADH}$	$9 \cdot 10^7$ M^{-1} min^{-1}	This value approximates the $K_{m,NADPH}^{app}$ previously obtained with 500 μM cro-CoA (3).
$k_{off,\ NAD}$	$8 \cdot 10^3$ min^{-1}	We noticed that the dissociation rate of PT52 from saFabI was similar to ecFabI. Thus, we made the assumption that the K_i is identical for the two FabI homologues. Based on this assumption, we determined the value of $k_{off,\ NAD}$ that best replicates the experimental progress curves. Consistently, the ratio of $k_{off,\ NAD}$ for ecFabI to $k_{off,\ NADP}$ for saFabI is calculated to be very similar to the ratio of $k_{off,\ NADH}$ for ecFabI to $k_{off,\ NADPH}$ for saFabI (4).
k_{cat}	600 min^{-1} for cro-coA	Based on a previously reported k_{cat} value (3).

Supplemental Figures

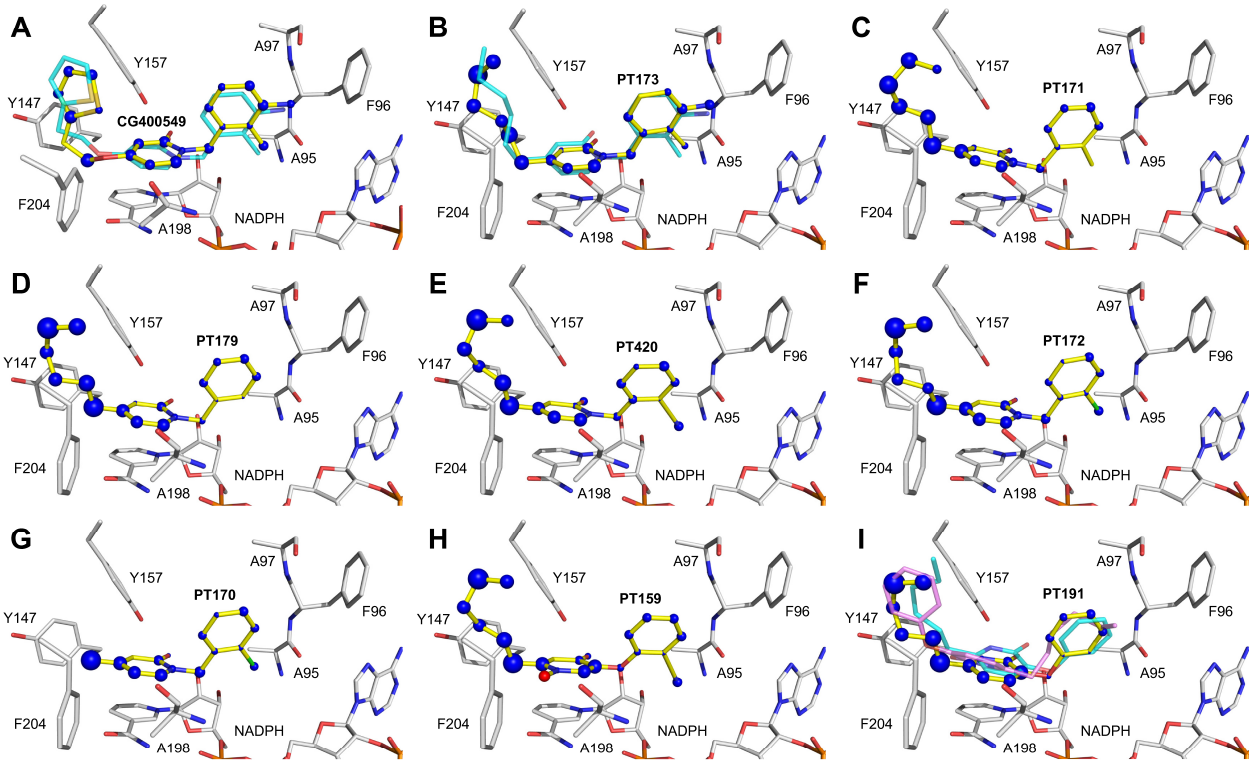


FIGURE S1. **Putative binding modes of pyridone inhibitors bound to saFabI.** All pyridone inhibitors investigated in this study (Table 1) were docked into the saFabI binding pocket as described in the Experimental Procedures section. The radii of the blue (red) spheres represent the value of the favorable (unfavorable) per-atom score, as determined by DrugScore^X. Selected residues of the saFabI binding pocket (CG400549-I structure; according to the score, the more 'open' subunit C was selected for the bulky CG400549 compound, whereas subunit A was preferred for the other inhibitors) are depicted as gray sticks along with the putative inhibitor binding mode (yellow sticks). A, Comparison of the generated CG400549 docking pose (yellow stick model) with the experimentally observed binding mode (cyan stick model). The best-ranked docking pose differs only slightly from the CG400549 binding geometry observed in the crystal structure (rmsd = 0.71 Å). B, Comparison of the generated PT173 docking pose (yellow) with the experimental binding geometry (cyan). The top-ranked docking pose is very similar to the PT173 binding geometry observed in the crystal structure (rmsd = 0.83 Å). C, Putative binding mode of PT171 (best-ranked pose). D, Putative binding mode of PT179. The six highest-ranked docking poses were excluded due to the lack of the central hydrogen bonding network between the inhibitor, Tyr157 and NADPH. E, Putative binding mode of PT420 (best-ranked pose). F, Putative binding mode of PT172 (top-ranked pose). G, Putative binding geometry of PT170. The best-ranked docking pose was excluded via visual inspection due to the absence of a hydrogen bond with Tyr157 and the cofactor. H, Putative binding mode of PT159 (best-ranked pose). I, Docking results for PT191. The FlexX docking procedure exclusively generated "unexpected" binding poses (the best-ranked pose is shown as pink stick model) without the central hydrogen

bonding network. We attribute this to the free NH group and the low affinity of this particular C-substituted 2-pyridone (Table 1). However, docking of the tautomeric hydroxypyridine form of PT191, which likely exists in equilibrium with the pyridone form (5), resulted in the "expected" binding mode (cyan sticks; best-ranked docking pose was observed with subunit C of CG400549-I). Interestingly, the most favorable score was achieved when mutating PT179 in its putative binding mode (panel D) to PT191 (yellow sticks; subunit A of CG400549-I) (DrugScore of -261,110 compared to -246,554 and -225,496 for the best-ranked docking poses with the pyridone and hydroxypyridine forms of PT191, respectively).

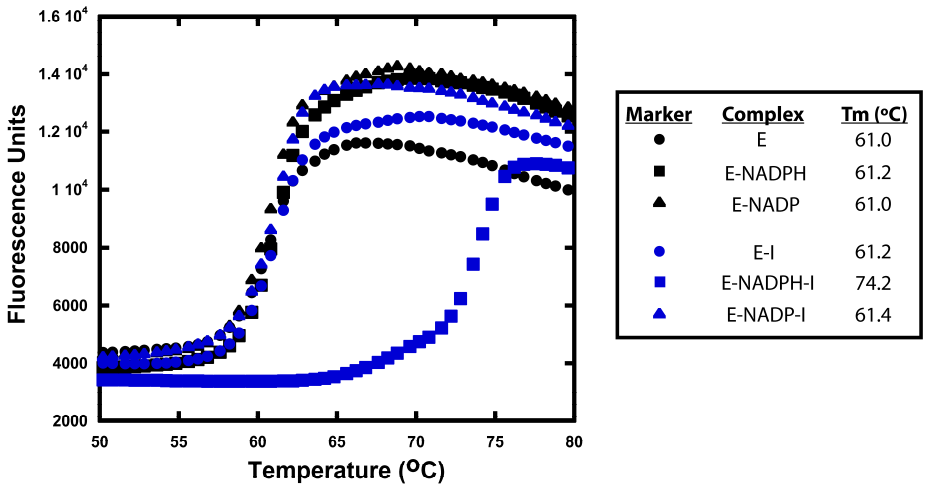
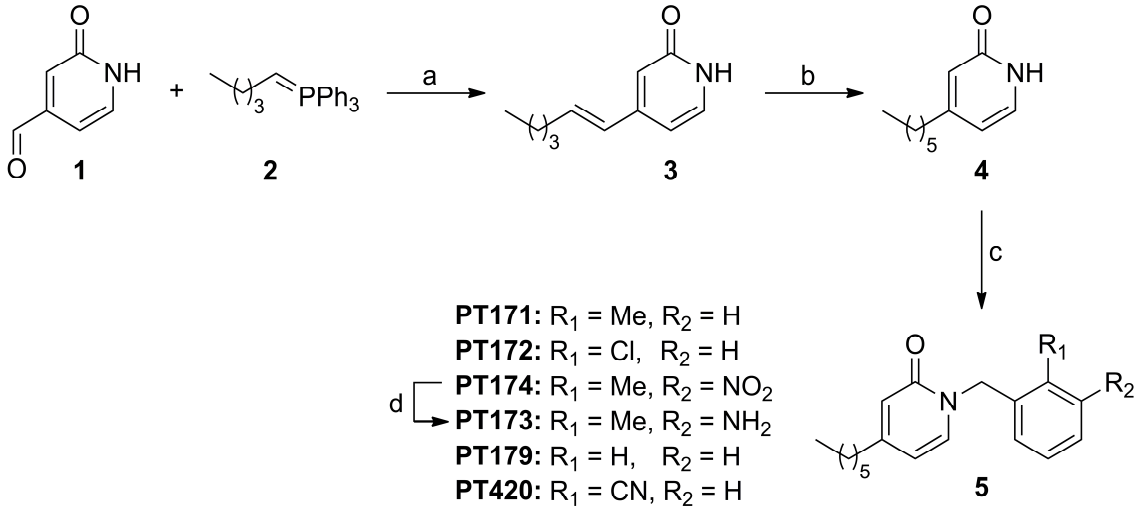


FIGURE S2. **Cofactor preference of 4-pyridones.** Thermal shift analyses of saFabI bound to NADPH, $NADP^+$ and/or inhibitor (PT166). The measurement variability is approximately ±0.2 °C.

Supplemental Experimental Procedures

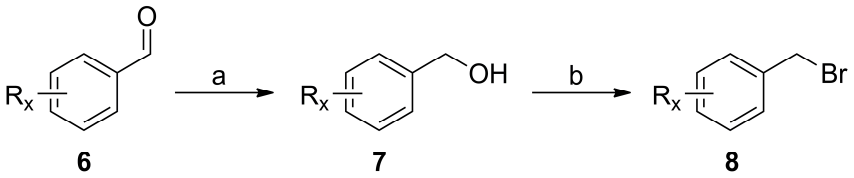
Synthesis of N-substituted 2-pyridones

The synthesis of the N-substituted 2-pyridones is shown in Scheme S1. The first step was a Wittig reaction between aldehyde **1** and n-pentyl triphenyl phosphine bromide **2** in the presence of the base n-BuLi at -78 ºC. This was followed by catalytic reduction of **3** to yield the intermediate **4.** Compound **4** was then treated with different benzylbromides **8** in the presence of K_2CO_3 at 80 ^oC for 5 h leading to the formation of the final compounds **5**.



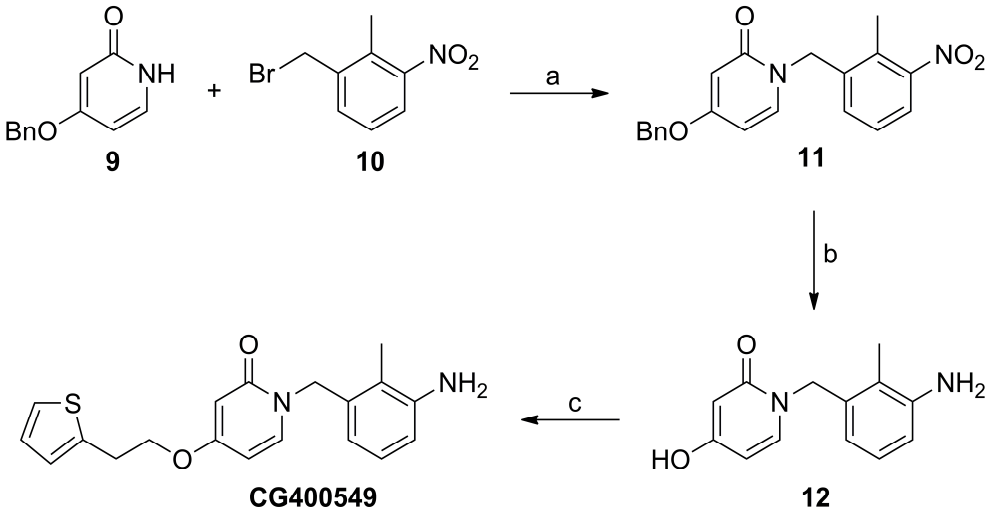
SCHEME S1. **Synthesis of N-substituted 2-pyridone inhibitors.** Reagents and conditions: a) n-BuLi, THF, -78 ºC; b) H_2, Pd/C, MeOH, RT; c) K_2CO_3, CH_3CN, 80 ºC, R_x-$PhCH_2Br$ (**8**); d) Zn, NH_4Cl, MeOH:H_2O = 5:1, 65 °C.

If the benzylbromide (**8**) for the final reaction was commercially unavailable, it was synthesized as shown in Scheme S2. The corresponding aldehyde **6** was reduced using $NaBH_4$ to form the primary alcohol **7**. This alcohol then undergoes substitution reactions in the presence of PBr_3 to form the bromo-compound **8**. Both of these reactions are complete and do not require further purification.



SCHEME S2. **Synthesis of the substituted benzylbromides.** Reagents and conditions: a) $NaBH_4$, MeOH, RT; b) PBr_3, Et_2O, RT.

We also synthesized the 2-pyridone CG400549 as shown in Scheme S3. Commercially available **9** undergoes a substitution reaction with the corresponding bromide **10** forming compound **11**. Catalytic reduction of **11** leads to debenzylation and concomitant reduction of the nitro to an amine group forming compound **12**. Compound **12** then undergoes a substitution reaction with the electrophile 2-(2-bromoethyl)-thiophene in the presence of Cs_2CO_3 and NaI forming the final product.



SCHEME S3. **Synthesis of compound 3.** Reagents and conditions: a) K_2CO_3, CH_3CN, 80 ºC; b) H_2, Pd/C, MeOH, RT; c) 2-(2-bromoethyl)-thiophene, Cs_2CO_3, NaI, DMF, RT.

4-((*E*)-hex-1-enyl)pyridin-2(1H)-one (**3**)

Reaction a (Scheme S1) was used to convert **1** and **2** to the title product. Under nitrogen atmosphere, n-BuLi (8.9 mmol) was added drop wise to n-pentyl triphenyl phosphine bromide **2** (10.2 mmol) dissolved in dry THF at -78 ºC. The color of the solution changed to orange and the reaction was stirred for 30 minutes. Subsequently, 2-hydroxypyridine-4-carbaldehyde **1** (4.1 mmol) was dissolved in dry DMSO and added drop wise to the orange solution. The reaction was kept overnight. After completion, the reaction was quenched with NH_4Cl. THF was evaporated and the work up was performed using CH_2Cl_2. Product **3** was purified via flash chromatography (70 w/v% ethyl acetate in hexane). After the removal of the solvent, the product was obtained as a light yellow liquid. Yield 88%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (t, *J* = 6.5 Hz, 3H), 1.31-1.48 (m, 4H), 2.30-2.35 (m, 2H), 5.82-5.87 (m, 1H), 6.18 (d, *J* = 11.5 Hz, 1H), 6.23 (d, *J* = 6 Hz, 1H), 6.47 (s, 1H), 7.34 (d, *J* = 6 Hz, 1H) ppm; ESI-MS (*m/z*): 178.0 $[M+H]^+$.

4-hexylpyridin-2(1H)-one (**4**)

Reaction b (Scheme S1) was used to convert **3** to the title product. Compound **3** (1 mmol) was dissolved in methanol and supplemented by 5 w/v% Pd/C and 10 v/v% HCl (1.7 ml). Then H_2 gas was passed into the round bottom flask. The reaction was completed within 4 h. Methanol was evaporated and the work up was performed with EtOAc. Product **4** was obtained as a white solid. Yield 89%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (t, *J* = 7 Hz, 3H), 1.27-1.32 (m, 6H), 1.57-1.64 (m, 2H), 2.48 (t, *J* = 8 Hz, 2H), 6.15 (d, *J* = 6 Hz, 1H), 6.39 (s, 1H), 7.27 (d, *J* = 6 Hz, 1H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 14.0, 22.5, 28.7, 29.2, 31.6, 35.7, 108.6, 118.0, 113.6, 158.1, 165.6 ppm; ESI-MS (*m/z*): 180.1 $[M+H]^+$.

For the following chemical conversions, reaction c (Scheme S1) was used to generate the final products (**5**). In general, intermediate **4** (0.3 mmol) and the appropriate benzylbromide **8** (0.4 mmol) were dissolved in acetonitrile and supplemented with K_2CO_3 (8.23 mmol). The reaction was heated to 80 °C under reflux for 6 h. After completion, the reaction mixture was filtered and the solvent was evaporated. Work up was performed using CH_2Cl_2. The organic layer was dried over $MgSO_4$ and the product isolated via flash chromatography. If the benzylbromide **8** for this reaction was not commercially available, it was synthesized via the following 2-step procedure: The appropriate benzaldehyde **6** (4 mmol) was dissolved in methanol and cooled to 0 ºC. Then, $NaBH_4$ (5.2 mmol) was added in small portions. The appearance of the solution changed from yellow to colorless. The reaction was completed within 15 minutes and was subsequently quenched with NH_4Cl. Methanol was evaporated under reduced pressure and the reaction was worked up using ethyl acetate. Without further purification, the resulting alcohol **7** (3.4 mmol) was dissolved in dry diethyl ether under argon atmosphere. Subsequently, PBr_3 (5.1 mmol) was added drop wise. The reaction was completed within 1 h and was quenched by adding water at 0 ºC. The reaction was quantitative and thus the product **8** was used without further purification.

1-(2-chlorobenzyl)-4-methylpyridin-2(1H)-one (**PT170**)
2-hydroxy-4-methyl-pyridine (1 mmol) and 2-chloro-benzylbromide (1.2 mmol) were dissolved in acetonitrile and the same procedure was followed as for reaction c (Scheme S1). The product obtained was a yellow liquid. Yield 87%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.18 (s, 3H), 5.23 (s, 2H), 6.00-6.03 (dd, *J* = 7.2 Hz, *J* = 1.8 Hz, 1H), 6.43 (br s, 1H), 7.22-7.26 (m, 4H), 7.26-7.39 (m, 1H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 22.1, 51.4, 107.8, 118.2, 126.7, 128.2, 128.7, 129.2, 132.3, 134.5, 136.4, 153.5, 161.3 ppm; ESI-MS (*m/z*): 234.7 $[M+H]^+$.

1-(2-methylbenzyl)-4-hexylpyridin-2(1H)-one (**PT171**)
Reaction c (Scheme S1) was used to convert **4** and 2-methyl-benzylbromide to the title product. The product obtained was a brown liquid. Yield 82%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.94 (t, *J* = 6.6 Hz, 3H), δ 1.31-1.40 (m, 6H), δ 1.57-1.65 (m, 2H), δ 2.32 (s, 3H), δ 2.48 (t, *J* = 7.5 Hz, 2H), δ 5.16 (s, 2H), δ 6.03-6.06 (dd, *J* = 6.9 Hz, *J* = 1.8 Hz, 1H), δ 6.49 (d, *J* = 1.2 Hz, 1H), δ 7.00 (d, *J* = 6.9 Hz, 1H), δ 7.09 (d, *J* = 6.9 Hz, 1H), δ 7.21-7.33 (m, 3H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 13.6, 14.2, 22.1, 28.3, 28.6, 31.1, 34.8, 48.6, 107.5, 118.1, 126.8, 128.7, 129.1, 129.3, 132.8, 133.6, 136.2, 155.3, 162.3 ppm; ESI-MS (*m/z*): 284.1 $[M+H]^+$.

1-(2-chlorobenzyl)-4-hexylpyridin-2(1H)-one (**PT172**)
Reaction c (Scheme S1) was used to convert **4** and 2-chloro-benzylbromide to the title product. The product obtained was a light yellow solid. Yield 88%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.92 (t, *J* = 10 Hz, 3H), 1.32-1.38 (m, 6H), 2.48 (t, *J* = 8 Hz, 2H), 5.26 (s, 2H), 6.06 (d, *J* = 7 Hz, 1H), 6.45 (s, 1H), 7.20-7.29 (m, 4H), 7.42 (m, 2H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 13.9, 22.8, 27.1, 29.1, 32.5, 35.4, 49.5, 108.2, 117.8, 127.8, 128.9, 129.5, 130.1, 133.4, 134.2, 135.5, 155.6, 161.5 ppm; ESI-MS (*m/z*): 304.8 $[M+H]^+$.

1-(2-methyl-3-nitrobenzyl)-4-hexylpyridin-2(1H)-one (**PT174**)
Reaction c (Scheme S1) was used to convert **4** and 3-nitro-2-methyl-benzylbromide to the title product. The product obtained was a brown liquid. Yield 89%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, *J* = 6.9 Hz, 3H), 1.23-1.32 (m, 6H), 1.51-1.53 (m, 2H), 2.41 (s, 3H), 2.45 (t, *J* = 7.2 Hz, 2H), 5.17 (s, 2H), 6.06 (dd, *J* = 1.8, 7.2 Hz, 1H), 7.02 (d, *J* = 7 Hz, 1H), 7.17 (d, *J* = 7.5 Hz, 1H), 7.27 (m, 1H), 7.69 (dd, *J* = 1, 8 Hz, 1H) ppm; ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.9, 14.4, 22.3,

28.6, 28.9, 31.4, 35.1, 49.2, 108.4, 118.4, 123.3, 126.6, 131.4, 135.7, 137.2, 151.3, 155.9, 162.4 ppm; ESI-MS (*m/z*): 329.1 $[M+H]^+$.

1-(3-amino-2-methylbenzyl)-4-hexylpyridin-2(1H)-one (**PT173**)
For the subsequent reduction of the **PT174** nitro group (reaction d, Scheme S1), Zn (1.3 mmol), NH_4Cl (1.9 mmol), and **PT174** (0.13 mmol) were dissolved in a 1:5 water-methanol mixture. The reaction was heated to 65 ºC for 30 minutes. The solvent was removed under reduced pressure and the product was purified using flash chromatography. The product obtained was a light yellow solid. Yield 90%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, *J* = 8 Hz, 3H), 1.24-1.28 (m, 8H), 2.01 (s, 3H), 2.40 (t, *J* = 7.8 Hz, 2H), 3.7 (br s, 2H), 5.10 (s, 2H), 5.93-5.95 (dd, *J* = 7.2 Hz, 8.7 Hz, 1H), 6.41 (br s, 1H), 6.59 (d, *J* = 3 Hz, 1H), 6.71 (d, *J* = 8 Hz, 2H), 6.94 (d, *J* = 6 Hz, 1H), 7.03 (t, *J* = 6 Hz, 1H) ppm; ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.9, 22.5, 28.7, 29.0 31.5, 35.2, 49.1, 108.0, 118.6, 127.2, 129.1, 129.5, 130.0, 133.4, 134.0, 136.4, 155.7, 162.8 ppm; ESI-MS (*m/z*): 299.1 $[M+H]^+$.

1-benzyl-4-hexylpyridin-2(1H)-one (**PT179**)
Reaction c (Scheme S1) was used to convert **4** and benzylbromide to the title product. The product obtained was a yellow liquid. Yield 90%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.84 (t, *J* = 7 Hz, 3H), 1.26-1.32 (m, 6H), 1.5-1.56 (m, 2H), 2.38 (t, *J* = 5 Hz, 2H), 5.08 (s, 2H), 5.97 (d, *J* = 7 Hz), 6.39 (s, 1H), 7.11 (d, *J* = 7 Hz, 1H), 7.24-7.32 (m, 5H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 13.9, 22.5, 22.7, 29.0, 31.5, 35.2, 51.4, 108.0, 118.7, 127.8, 128.1, 128.8, 136.1, 136.6, 155.5, 162.8 ppm; ESI-MS (*m/z*): 270.2 $[M+H]^+$.

2-((4-hexyl-2-oxopyridin-1(2H)-yl)methyl)benzonitrile (**PT420**)
Reaction c (Scheme S1) was used to convert **4** and 2-cyano-benzylbromide to the title product. The product obtained was a dark yellow liquid. Yield 92%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, *J* = 7 Hz, 3H), 1.25-1.34 (m, 6H), 1.54-1.63 (m, 2H), 2.43 (t, *J* = 8 Hz, 2H), 5.31 (s, 2H), 6.07 (d, *J* = 7.5 Hz, 2H), 6.42 (s, 1H), 7.34 (d, *J* = 7.5 Hz, 1H), 7.38-7.41 (m, 2H), 7.53-7.55 (m, 1H), 7.66 (d, *J* = 8 Hz, 1H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 13.9, 22.4, 28.7, 28.9, 31.5, 35.2, 49.9, 108.4, 111.6, 117.6, 118.7, 128.3, 129.7, 132.7, 133.3, 136.6, 140.1, 156.2, 162.6 ppm; ESI-MS (*m/z*): 295.3 $[M+H]^+$.

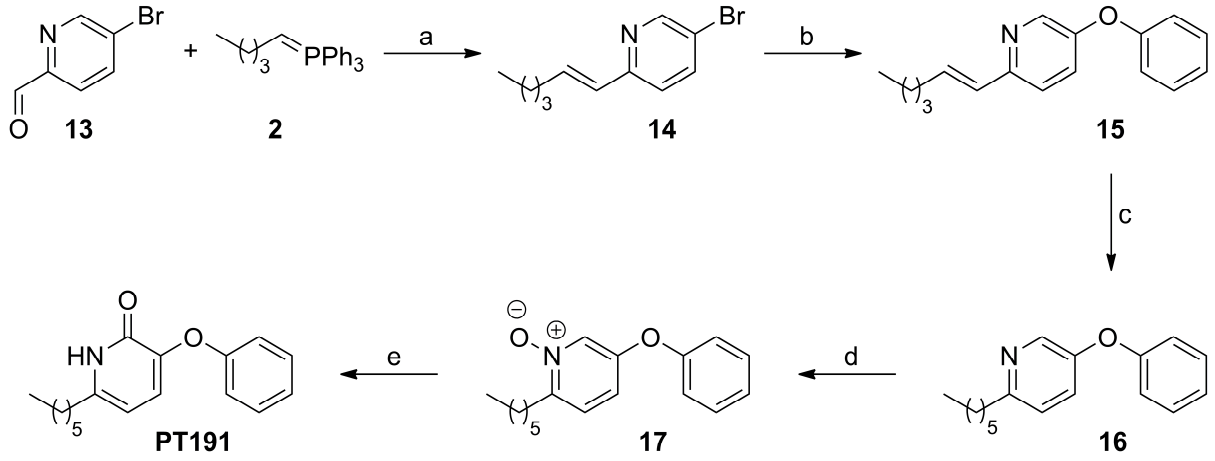
1-(2-methyl-3-nitrobenzyl)-4-(benzyloxy)pyridin-2(1H)-one (**11**)
Reaction a (Scheme S3) was used to convert the starting material 4-(benzyloxy)pyridin-2(1H)-one **9** and 3-nitro-2-methyl-benzylbromide **10** to the title product (procedure as described for synthesis of **5**). The product obtained was a yellow liquid. Yield 84%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.26 (s, 3H), 4.87 (s, 2H), 4.99 (s, 2H), 5.87-5.91 (m, 2H), 6.89 (d, *J* = 7.5 Hz, 1H), 7.03 (d, *J* = 7.5 Hz, 1H), 7.14-7.17 (m, 3H), 7.22-7.25 (m, 3H), 7.56 (d, *J* = 8 Hz) ppm; ^{13}C NMR (500 MHz, $CDCl_3$): δ 14.4, 48.9, 70.2, 98.2, 101.9, 123.4, 126.7, 127.7, 128.5, 128.6, 130.3, 131.2, 134.8, 136.6, 137.2, 151.2, 163.7, 167.1 ppm; ESI-MS: ESI-MS (*m/z*): 351.0 $[M+H]^+$.

1-(3-amino-2-methylbenzyl)-4-hydroxypyridin-2(1H)-one (**12**)
The title product was obtained by catalytic hydrogenation of compound **11** (procedure as described for synthesis of **4**; reaction b, Scheme S3). ESI-MS (*m/z*): 231.1 $[M+H]^+$.

1-(3-amino-2-methylbenzyl)-4-(2-(thiophen-2-yl)ethoxy)pyridin-2(1H)-one (**CG400549**)
The title product was obtained using reaction c (Scheme S3). Compound **12** (1 mmol), 2-(2-bromoethyl)-thiophene (1.2 mmol), Cs_2CO_3 (1.2 mmol) and NaI (0.1 mmol) were dissolved in DMF at room temperature. The reaction was stirred for 12 h. After completion of the reaction, work up was performed using diethyl ether. The product was purified using flash chromatography. Yield 80%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.00 (s, 3H), 3.28 (t, *J* = 6.5 Hz, 3H), 3.61-3.79 (br s, 2H), 4.13 (t, *J* = 6.5 Hz, 3H), 5.04 (s, 2H), 5.84 (dd, *J* = 3 Hz, 8 Hz, 1H), 5.93 (d, *J* = 2.5 Hz, 1H), 6.56 (d, *J* = 7 Hz, 1H), 6.68 (d, *J* = 8 Hz, 1H), 6.87-6.99 (m, 2H), 7.16 (dd, *J* = 1 Hz, 5.5 Hz, 1H) ppm; ^{13}C NMR (500 MHz, $CDCl_3$): δ 12.3, 29.6, 49.1, 68.3, 97.5, 100.9, 115.5, 120.3, 120.9, 124.1, 125.6, 126.7, 126.8, 134.6, 136.2, 139.5, 145.4, 163.1, 166.7 ppm; ESI-MS (*m/z*): 341.1 $[M+H]^+$.

Synthesis of C-substituted 2-pyridones

The synthesis of the C-substituted 2-pyridone **PT191** is shown in Scheme S4**.** Commercially available **13** reacts with n-pentyl triphenyl phosphine bromide **2** under Wittig reaction conditions to form compound **14**. This compound is then coupled with phenol to form compound **15**. The side chain of compound **15** is reduced by catalytic hydrogenation forming compound **16**. Compound **16** is then oxidized in the presence of MCPBA leading to the formation of compound **17** that finally undergoes a rearrangement in the presence of Ac_2O followed by HCl treatment forming compound **PT191**.



SCHEME S4. **Synthesis of the 2-pyridone PT191 with a bridging oxygen.** Reagents and conditions: a) n-BuLi, THF, -78 ºC; b) PhOH, picolinic acid, CuI, K_3PO_4, DMSO, 90 ºC; c) H_2, Pd/C, MeOH, RT; d) MCPBA, $CHCl_3$, RT; e) (i) Ac_2O, reflux; (ii) HCl, 90 ºC.

6-hexyl-3-phenoxypyridin-2(1H)-one (16)

Reaction a (Scheme S4) was used to convert **13** and **2** to **14**. Under nitrogen atmosphere, n-BuLi (8.9 mmol) was added drop wise to n-pentyl triphenyl phosphine bromide **2** (10.2 mmol) dissolved in dry THF at -78 ºC. The color of the solution changed to orange and the reaction was stirred for 30 minutes. Subsequently, 5-bromo-pyridine-2-carbaldehyde **13** (4.1 mmol) was dissolved in dry DMSO and added drop wise to the orange solution. The reaction was kept overnight. After completion, the reaction was quenched with NH_4Cl. THF was evaporated and the work up was performed using CH_2Cl_2. Product **14** was purified via flash chromatography and coupled with phenol (reaction b, Scheme S4). For this, compound **14** (1 mmol), CuI (0.1 mmol), picolinic acid (0.2 mmol), phenol (1.2 mmol) and K_3PO_4 (2 mmol) were dissolved in dry DMSO under argon atmosphere. The reaction mixture was heated to 90 ºC for 24 h. After completion, the reaction mixture was cooled to room temperature. After the work up with diethyl ether, **15** was purified using flash chromatography. Subsequently, **15** was catalytically hydrogenated in the presence of 5 w/v% Pd/C and 10 v/v% HCl (0.5 ml) to obtain the final product **16** as a dark yellow liquid (procedure as described for synthesis of **4**; reaction c, Scheme S4). Yield 85%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (t, J = 7 Hz, 3H), 1.27-1.39 (m, 6H), 1.70-1.76 (m, 2H), 2.78 (t, J = 8 Hz, 2H), 7.01 (d, J = 8 Hz, 1H), 7.12 (d, J = 8.5 Hz, 1H), 7.22-7.24 (m, 2H), 7.35 (m, 2H),

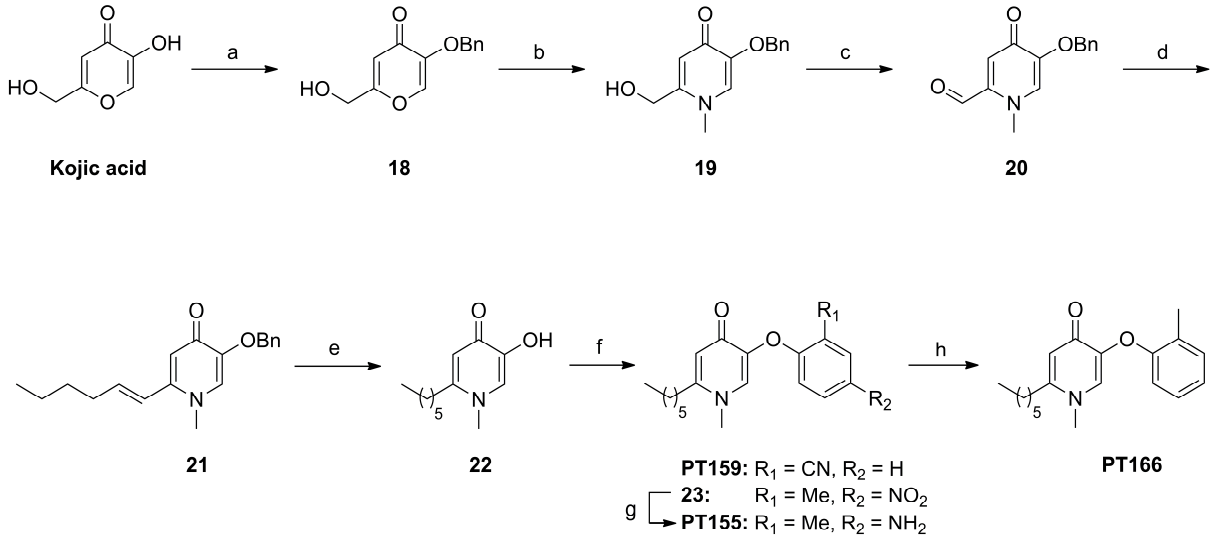
8.36 (d, J = 2.5 Hz, 1H) ppm; ^{13}C NMR (500 MHz, $CDCl_3$): δ 14.0, 22.6, 29.0, 29.9, 31.7, 37.6, 118.4, 122.9, 123.5, 126.4, 129.8, 140.8, 151.5, 157.0, 157.4 ppm; ESI-MS (*m/z*): 256.2 $[M+H]^+$.

6-hexyl-3-phenoxypyridin-N-oxide-2(1H)-one (**17**)
Reaction d (Scheme S4) was used to oxidize compound **16** to the title product. Compound **16** (1 mmol) was added into a solution containing *m*-chloroperbenzoic acid (2.5 mmol) in $CHCl_3$. The reaction was stirred overnight at room temperature. Subsequently, the reaction mixture was washed with KI (10 w/v%), $Na_2S_2O_7$ (20 w/v%), NaOH (10 w/v%) and H_2O. The organic layer was collected and the solvent was removed under reduced pressure. Purification via flash chromatography led to the product obtained as a dark yellow liquid. Yield 50%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J = 7.5 Hz, 3H), 1.24-1.43 (m, 6H), 1.64-1.74 (m, 2H), 2.86 (t, J = 8.1 Hz, 2H), 6.92 (d, J = 7 Hz, 1H), 7.15-7.20 (m, 3H), 7.34-7.41 (m, 2H), 7.45 (d, J = 7 Hz, 1H), 8.02 (d, J = 2.1 Hz, 1H) ppm; ESI-MS (*m/z*): 272.4 $[M+H]^+$.

6-hexyl-3-phenoxypyridin-2(1H)-one (**PT191**)
Reaction e (Scheme S4) was used to convert compound **17** to the title product. Compound **17** (0.5 mmol) was added to 1.5 ml of acetic anhydride. The reaction mixture was refluxed for 7 h. Subsequently, the solvent was removed under vacuum and a dark oily liquid was obtained that was treated with water. This aqueous mixture was heated to 100 °C for 6 h. After cooling to room temperature, conc. HCl (1.5 ml) was added. This mixture was heated to 90 °C for 24 h. The work up was performed using CH_2Cl_2 and was washed with saturated Na_2CO_3 and brine. The organic layer was then dried over Na_2SO_4. Purification via flash chromatography led to a yellow liquid. Yield 30%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, J = 7 Hz, 3H), 1.23-1.29 (m, 6H), 1.55-1.60 (m, 2H), 2.47 (t, J = 7.5 Hz, 2H), 5.92 (d, J = 7.5 Hz, 1H), 6.96 (d, J = 7.5 Hz, 1H), 7.01-7.02 (m, 2H), 7.03-7.05 (m, 2H), 7.31-7.32 (m, 2H) ppm; ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.9, 22.5, 28.4, 28.6, 31.4, 32.6, 103.7, 117.7, 122.9, 127.3, 129.5, 143.8, 144.9, 157.0, 160.4 ppm; ESI-MS (*m/z*): 272.1 $[M+H]^+$.

Synthesis of 4-pyridones

4-Pyridones were prepared as described in Scheme S5. Kojic acid was benzylated to give compound **18**, which was converted into 4-pyridone using a replacement reaction in a mild condition. Oxidation of **19** was attempted with pyridinium chlorochromate (PCC), Parikh–Doering reaction (SO_3•Py), and MnO_2, but only MnO_2 yielded the desired compound **20**. A Wittig reaction with pentenyl ylide then led to compound **21**, which was hydrogenated to compound **22** by removing the benzyl group and reducing the double bond simultaneously. In this reaction, 3 equivalents of HCl are necessary to prevent the tertiary amine from coordinating to the palladium charcoal. Once compound **22** was obtained, **PT159** and **23** were synthesized with the corresponding phenyl fluoride. Compound **PT155** was obtained from **23** using a Clemmensen reduction, and the following deamination with *t*-$BuNO_2$ (6) yielded compound **PT166**.



SCHEME S5. **Synthesis of PT159 and PT166.** Reagents and conditions: a) BnBr, KOH (5 w/v%), MeOH, H_2O, reflux, 1 h, 86%; b) $MeNH_2$, MeOH, RT, 12 h, 74%; c) MnO_2, $CHCl_3$, reflux, 16 h, 83% ; d) *n*-$C_5H_9PPh_3$, *n*-BuLi, toluene, CH_2Cl_2, RT, 4 h, 88%; e) HCl, H_2, Pd/C, EtOH, 6 h, 52%; f) K_2CO_3, DMF, 18-crown-6, 110°C, 3 h; g) Zn, NH_4Cl, MeOH, reflux, 1 h, 79%; h) *t*-$BuNO_2$, DMF, 65°C, 1 h, 62%.

5-(Benzyloxy)-2-(hydroxymethyl)-4H-pyran-4-one (18)
An aqueous solution of KOH (5 w/v%, 250 ml) was added to a solution of kojic acid (14.2 g, 0.1 mol) and benzyl bromide (13.1 ml, 18.8 g, 0.11 mol) in MeOH (250 ml). The mixture was stirred and heated to 95 °C to reflux for approximately 1 h. After TLC (10 v/v% MeOH/CH_2Cl_2) showed completion of the reaction, the mixture was poured into 500 ml iced water and neutralized with 10 v/v% HCl. The solution was extracted with EtOAc (300 ml, 3X), and the organic layers were combined and evaporated to obtain the crude product. Purification by flash chromatography (MeOH/CH_2Cl_2 8 v/v%) led to the pure compound **18** as white crystals. Yield 86%; ^{1}H NMR (400 MHz, CD_3OD): δ 8.17 (s, 1H), 7.45 (d, *J* = 7.2 Hz, 2H), 7.41-7.31 (m, 3H), 6.52 (s, 1H), 5.03 (s, 2H), 4.42 (s, 2H) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ 175.60,169.23, 146.89, 141.74,

135.86, 128.21, 128.03, 127.73, 110.67, 71.15, 59.64 ppm; ESI-MS (*m/z*): calculated for $C_{13}H_{12}O_4$ $[M+H]^+$ 233.1; found, 233.1.

5-(Benzyloxy)-2-(hydroxymethyl)-1-methylpyridin-4(1H)-one (19)
A solution of methyl amine (33 v/v%, 40 ml) in EtOH was added into a solution of **18** (4.6g, 20 mmol) in MeOH (200 ml). The mixture was stirred overnight in a round bottom flask that was sealed with a rubber septum. The product precipitated as a white solid. When TLC (10 v/v% MeOH/CH_2Cl_2) showed completion of the reaction, the reaction was concentrated to approximately 50 ml and cooled in an ice bath. Product **19** was obtained by filtration of the cold mixture. Yield 74%; white crystal; ^{1}H NMR (400 MHz, DMSO-d6): δ 7.57 (s, 1H), 7.42-7.32 (m, 5H), 6.23 (s, 1H), 5.55 (s, 1H), 4.99 (s, 2H), 4.37 (s, 2H), 3.58 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d6): δ 172.07, 149.18, 147.45, 137.70, 129.71, 128.78, 128.44, 128.30, 115.52, 71.05, 59.73, 39.69 ppm.

5-(Benzyloxy)-1-methyl-4-oxo-1,4-dihydropyridine-2-carbaldehyde (20)
A solution of **19** (2.5 g, 10 mmol) and activated MnO_2 (3.4 g, 40 mmol) in $CHCl_3$ (150 ml) was stirred and heated to 80 °C to reflux overnight. When TLC (10 v/v% MeOH/CH_2Cl_2) showed completion of the reaction, the mixture was cooled to RT and filtered through Celite 545 to remove the MnO_2. Purification by flash chromatography (MeOH/CH_2Cl_2 8 v/v%) gave the pure compound **20** as light yellow crystals. Yield 83%; ^{1}H NMR (300 MHz, CD_3OD): δ 9.75 (s, 1H), 7.57 (s, 1H), 7.46 (d, *J* = 7.2 Hz, 2H), 7.38-7.31 (m, 3H), 6.77 (s, 1H), 5.08 (s, 2H), 3.80 (s, 3H) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ 172.45, 147.86, 147.75, 136.37, 129.07, 128.12, 127.80, 127.78, 113.73, 93.33, 70.98, 40.46 ppm; ESI-MS (*m/z*): calculated for $C_{14}H_{13}NO_3$ $[M+H]^+$ 244.1; found, 244.1.

(*E*)-5-(benzyloxy)-2-(hex-1-en-1-yl)-1-methylpyridin-4(1*H*)-one (21)
A solution of *n*-BuLi (2.0 M in cyclohexane, 3.0 ml, 6.0 mmol) was added dropwise into a solution of *n*-pentyltriphenylphosphonium bromide (2.1 g, 5.5 mmol) in toluene (100 ml). After 30 min, the solution became dark red, and a solution of **20** (1.2 g, 5.0 mmol) in CH_2Cl_2 (100 ml) was added dropwise into the flask. The reaction was stirred at RT for approximately 1 h. When TLC (MeOH/CH_2Cl_2 5 v/v%) showed completion of the reaction, the reaction was quenched with HCl (10 v/v%, 10 ml) and washed with water (200 ml) and CH_2Cl_2 (100 ml). The aqueous layer was then washed with CH_2Cl_2 (50 ml, 2X), and the organic layers were combined and evaporated to yield the crude product. Purification by flash chromatography (MeOH/CH_2Cl_2 4 v/v%) led to the pure compound **21** as a white crystal. Yield 88%.

2-Hexyl-5-hydroxy-1-methylpyridin-4(1*H*)-one (22)
A solution of **21** (594.8 mg, 2.0 mmol), HCl (10 v/v%, 2.2 ml, 6.0 mmol), and palladium charcoal (27.5 mg) in EtOH (100 ml) was sealed under vacuum and flushed with hydrogen for at least three times. The reaction mixture was stirred at RT under hydrogen gas for approximately 6 h. When TLC (MeOH/CH_2Cl_2 5 v/v%) showed completion of the reaction, the mixture was filtered through Celite 545 and concentrated yielding the crude product. Purification by flash chromatography (MeOH/CH_2Cl_2 4 v/v%) led to the pure compound **22** as a pale solid. Yield 52%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.13 (s, 1H), 6.33 (s, 1H), 5.30 (s, br, 1H), 3.61 (s, 3H), 2.53 (t, *J* = 7.2 Hz, 2H), 1.60 (m, 2H), 1.41-1.24 (m, 6H), 0.89 (t, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.71, 151.87, 149.61, 124.73, 115.12, 43. 38, 35.07, 34.13, 31.47, 30.82, 25.16, 16.66 ppm; ESI-MS (*m/z*): calculated for $C_{12}H_{19}NO_2$ $[M+H]^+$ 210.1; found, 210.1.

2-Hexyl-1-methyl-5-(2-methyl-4-nitrophenoxy)pyridin-4(1H)-one (23)
Compound **22** (209.3 mg, 1.0 mmol), 2-fluoro-5-nitrotoluene (155.1 mg, 1.0 mmol), K_2CO_3 (345.5 mg, 2.5 mmol), and 18-crown-6 (10.5 mg) were sealed under vacuum and flushed with nitrogen gas for three times, after which DMF (50 ml) was added to dissolve the solid. The reaction mixture was heated to 110 °C and stirred for approximately 3 h. When TLC ($MeOH/CH_2Cl_2$ 5 v/v%) showed completion of the reaction, the reaction was cooled to RT and washed with water (100 ml) and CH_2Cl_2 (100 ml) three times. The organic layers were combined and evaporated to yield the crude product. Purification by flash chromatography ($MeOH/CH_2Cl_2$ 3 v/v%) led to the pure compound **23** as a yellow oil. Yield 83%.

5-(4-Amino-2-methylphenoxy)-2-hexyl-1-methylpyridin-4(1H)-one (PT155)
A solution of **23** (344.4 mg, 1.0 mmol), activated Zn powder (400 mg), and NH_4Cl (300 mg) in MeOH (30 ml) was heated to 70 °C and refluxed for 1 h. When TLC ($MeOH/CH_2Cl_2$ 5 v/v%) showed completion of the reaction, the mixture was filtered and evaporated to yield the crude product. Purification by flash chromatography ($MeOH/CH_2Cl_2$ 3 v/v%) led to the pure compound **PT155** as a yellow oil. Yield 79%.

2-((6-Hexyl-1-methyl-4-oxo-1,4-dihydropyridin-3-yl)oxy)benzonitrile (PT159)
Compound **22** (209.3 mg, 1.0 mmol), 2-fluorobenzonitrile (121.1 mg, 1.0 mmol), K_2CO_3 (345.5 mg, 2.5 mmol), and 18-crown-6 (10.5 mg) were sealed under vacuum and flushed with nitrogen gas three times, after which DMF (50 ml) was added into the flask to dissolve the solid. The reaction mixture was heated to 110 °C and stirred for approximately 3 h. When TLC ($MeOH/CH_2Cl_2$ 5 v/v%) showed completion of the reaction, the reaction was cooled to RT and washed with water (100 ml) and CH_2Cl_2 (100 ml) three times. The organic layers were combined and evaporated to yield the crude product. Purification by flash chromatography ($MeOH/CH_2Cl_2$ 3 v/v%) led to the pure compound **PT159** as a yellow oil. Yield 86%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.57-7.52 (m, 2H), 7.40 (td, J = 10.8, 2.0 Hz, 1H), 7.02 (td, J = 10.4, 1.2 Hz, 1h), 6.78 (d, J = 11.2 Hz, 1H), 6.38 (s, 1H), 3.64 (s, 1H), 2.54 (t, J = 10.4 Hz, 2h), 1.67-1.57 (m, 2H), 1.45-1.28 (m, 6H), 0.92-0.87 (m, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.80, 159.13, 151.30, 142.20, 135.27, 134.01, 133.24, 122.16, 118.86, 116.32, 115.08, 101.74, 40.75, 31.96, 31.40, 28.75, 27.78, 22.42, 13.92 ppm; ESI-MS (*m/z*): calculated for $C_{19}H_{22}N_2O_2$ $[M+H]^+$ 311.2; found, 311.1.

2-Hexyl-1-methyl-5-(o-tolyloxy)pyridin-4(1H)-one (PT166)
A solution of *t*-$BuNO_2$ (118.7 mg, 0.137 ml, 1.15 mmol) in DMF (30 ml) was heated to 65 °C under nitrogen, and a solution of **PT155** (314.4 mg, 1.0 mmol) in CH_2Cl_2 (20 ml) was added dropwise into the flask. After stirring at 65 °C for 30 min, the reaction became dark red. When TLC ($MeOH/CH_2Cl_2$ 5 v/v%) showed completion of the reaction, the mixture was cooled to RT and washed with water (100 ml) and CH_2Cl_2 (100 ml). The organic layer was dried over $MgSO_4$ and evaporated to yield the crude product. Purification by flash chromatography ($MeOH/CH_2Cl_2$ 3 v/v%) led to the pure compound **PT166** as a red oil. Yield 62%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21 (d, J = 5.8 Hz, 1H), 7.14 (dd, J = 13.4, 6.4 Hz, 2H), 7.03 (d, J = 5.8 Hz, 1H), 6.80 (d, J = 6.4 Hz, 1H), 6.66 (s, 1H), 3.65 (s, 3H), 2.59 (t, J = 6.2 Hz, 2H), 2.28 (s, 3H), 1.65-1.62 (m, 2H), 1.45-1.33 (m, 6H), 0.92 (t, J = 5.2 Hz, 3H) ppm; ESI-MS (*m/z*): calculated for $C_{19}H_{25}NO_2$ $[M+H]^+$ 300.2; found, 300.2.

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