Supporting Information

Development of the late phase manufacturing process of ZPL 389: control of process impurities by enhanced process knowledge

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Analytical Methods

HPLC analytical method for compound **2a**

Instrument: Agilent 1290 UHPLC with high pressure mixing
Column: Acquity UPLC BEH C18, 100 mm x 3.0 mm x 1.7 μm, or equivalent column
Detection: UV 210 nm
Flow rate: 0.8 ml/min
Column temperature: 40°C
Mobile phase A: 0.1% H_3PO_4 in water
Mobile phase B: Methanol
Solvent 1: 100 mM Na_2CO_3 in water
Solvent 2: 2% (v/v) benzoyl chloride in acetonitrile

Gradient

Timen (min)	% B
0.0	5
14.0	90
17.0	90
17.1	5
20.0	5

Sample preparation (Derivatization): Dissolve 8-10 mg of sample in Sovent 1 in a 10-ml volumetric flask. Transfer 0.5 ml of this solution to an HPLC vial, add 0.5 ml Solvent 2. Shake well.

HPLC analytical method for compound **3**

Instrument: Agilent 1290 UHPLC with high pressure mixing
Column: Acquity UPLC BEH C18, 100 mm x 3.0 mm x 1.7 μm, or equivalent column
Detection: UV 230 nm
Flow rate: 0.8 ml/min
Column temperature: 40°C
Mobile phase A: 0.05% TFA in water/acetonitrile=95/5 (v/v)
Mobile phase B: 0.05% TFA in water/acetonitrile=5/95 (v/v)
Solvent: water/acetonitrile=50/50 (v/v)
Gradient

Timen (min)	% B
0.0	5
14.0	90
17.0	90
17.1	5
20.0	5

HPLC analytical method for compound **12**

Instrument: Agilent 1290 UHPLC with high pressure mixing
Column: Acquity UPLC BEH C18, 100 mm x 3.0 mm x 1.7 μm, or equivalent column
Detection: UV 210 nm
Flow rate: 0.8 ml/min
Column temperature: 40°C
Mobile phase A: 10 mM Na_2CO_3 in water/acetonitrile=95/5 (v/v)

Mobile phase B: 10 mM Na_2CO_3 in water/acetonitrile=15/85 (v/v)
Solvent: water/acetonitrile=10/90 (v/v)
Gradient

Timen (min)	% B
0.0	5
14.0	85
17.0	85
17.1	5
20.0	5

HPLC analytical method for compound **4**

Instrument: Agilent 1290 UHPLC with high pressure mixing
Column: Acquity UPLC BEH C18, 100 mm x 3.0 mm x 1.7 μm, or equivalent column
Detection: UV 210 nm
Flow rate: 0.8 ml/min
Column temperature: 40°C
Mobile phase A: 10 mM Na_2CO_3 in water/acetonitrile=95/5 (v/v)
Mobile phase B: 10 mM Na_2CO_3 in water/acetonitrile=15/85 (v/v)
Solvent: water/acetonitrile=10/90 (v/v)
Gradient

Time (min)	% B
0.0	5
14.0	85
17.0	85
17.1	5
20.0	5

HPLC analytical method for compound **5**

Instrument: Agilent 1290 UHPLC with high pressure mixing
Column: Acquity UPLC BEH C18, 100 mm x 3.0 mm x 1.7 μm, or equivalent column
Detection: UV 210 nm
Flow rate: 0.8 ml/min
Column temperature: 40°C
Mobile phase A: 10 mM Na_2CO_3 in water/acetonitrile=95/5 (v/v)
Mobile phase B: 10 mM Na_2CO_3 in water/acetonitrile=15/85 (v/v)
Solvent: water/acetonitrile=10/90 (v/v)
Gradient

Time (min)	% B
0.0	5
14.0	85
17.0	85
17.1	5
20.0	5

HPLC analytical method for compound **6**

Instrument: Agilent 1290 UHPLC with high pressure mixing
Column: Xbridge Phenyl, 150 mm x 3.0 mm x 3.5 μm, or equivalent column

Detection: UV 230 nm
Flow rate: 0.6 ml/min
Column temperature: 40°C
Mobile phase A: 0.05% TFA in water/methanol=95/5 (v/v)
Mobile phase B: 0.05% TFA in water/methanol=5/95 (v/v)
Solvent: water/methanol=50/50 (v/v)
Gradient

Timen (min)	% B
0.0	5
12.0	40
18.0	90
20.0	90
20.1	5
23.0	5

HPLC analytical method for compound **7**

Instrument: Agilent 1290 UHPLC with high pressure mixing
Column: Acquity UPLC BEH C18, 100 mm x 3.0 mm x 1.7 μm, or equivalent column
Detection: UV 230 nm
Flow rate: 0.8 ml/min
Column temperature: 50°C
Mobile phase A: 10 mM NH_4HCO_3 in water (pH=10.6)
Mobile phase B: methanol
Solvent: water/methanol=50/50 (v/v)
Gradient

Time (min)	% B
0.0	5
2.0	5
14.0	85
16.0	85
16.1	5
21.0	5

Screening experiments

Screening of conditions for the synthesis of **12**

The starting material **11** and the solvent were charged to a vial at room temperature, the base was added. 1.7 eq. DMS was charged in one portion and the mixture was stirred at room temperature and sampled after 2h.

Table S1. Screening of solvents and bases in the synthesis of **12**

Entry	Solvent	Base	HPLC (% A)		
			11	**12**	**Others**
1	Acetonitrile	NaO*t*Bu (2.0 eq)	<0.05	<0.05	100
2	DMF	NaO*t*Bu (2.0 eq)	0.90	54.85	44.25
3	MeTHF	NaO*t*Bu (2.0 eq)	1.94	43.63	54.43
4	THF	NaO*t*Bu (2.0 eq)	<0.05	23.41	76.59
5	Dioxane	NaO*t*Bu (2.0 eq)	<0.05	13.15	86.85
6	Sulfolane	NaO*t*Bu (2.0 eq)	<0.05	<0.05	100
7	Acetone	NaO*t*Bu (2.0 eq)	10.75	1.27	87.98
8	t-Butanol	NaO*t*Bu (2.0 eq)	45.56	2.46	51.98
9	n-Butyl acetate	NaO*t*Bu (2.0 eq)	39.71	<0.05	60.29
10 [1]	MeTHF	LiHMDS (2.0 eq)	7.4	30.30	62.27
11	DMF	KO*t*Bu (2.0 eq)	2.9	83.00	14.1
12	DMF	Na_2CO_3 (2.0 eq)	<0.05	<0.05	100
13	DMF	Na_2CO_3 (2.0 eq) [2]	<0.05	<0.05	100
14	DMF	K_2CO_3 (2.0 eq)	<0.05	<0.05	100
15	DMF	K_2CO_3 (2.0 eq) [2]	<0.05	<0.05	100
16	DMF	NaOH (2.0 eq)	<0.05	<0.05	100
17	DMF	DBU (2.0 eq)	20.27	<0.05	79.73

Note: [1] 5-gram scale, 1.9 eq. DMS, -5°C ;[2] 0.15 eq tetrabutylammonium sulfate added

Screening of conditions for the synthesis of **3**

The starting materials **1** and **2a** (1.1 eq) were charged to a vial and the solvent (10 v/w) and base (2.5 eq) were added. The vial was sealed and the mixture was heated with stirring to the temperature and for the time indicated in the table.

Table S2. Screening of reaction conditions for intermediate **3**

Entry	Solvent	Base	IT (°C)	Time (h)	IPC [% A (HPLC)]		
					3	1	Others
1	Acetonitrile/Water [1]	K_2CO_3	40	24	71.11	28.89	<0.05
2		EDIPA	40	24	84.89	15.11	<0.05
3		NMM	40	24	12.09	20.69	67.22
4	Methanol/Water [1]	K_2CO_3	40	24	89.24	9.15	1.61
5		EDIPA	40	24	78.14	17.30	4.56
6		NMM	40	24	11.29	75.79	12.92
7	Ethanol/Water [1]	K_2CO_3	40	24	57.63	28.09	14.28
8		EDIPA	40	24	75.95	15.02	9.03
9		NMM	40	24	8.70	60.18	31.12
10	1-Propanol/Water [1]	K_2CO_3	40	24	71.53	28.47	<0.05
11		EDIPA	40	24	72.87	24.70	2.43
12		NMM	40	24	10.90	72.85	16.25
13	2-Propanol/Water [1]	K_2CO_3	40	24	66.57	34.43	<0.05
14		EDIPA	40	24	67.22	32.78	<0.05
15		NMM	40	24	7.09	84.91	8.00
16	Acetone/Water [1]	K_2CO_3	40	24	68.39	27.42	4.19
17		EDIPA	40	24	69.93	23.73	6.34
18		NMM	40	24	10.05	47.02	42.93
19	MEK/Water [1]	K_2CO_3	40	24	68.21	31.79	<0.05
20		EDIPA	40	24	57.82	32.12	9.99
21		NMM	40	24	12.97	58.20	28.83
22	THF/Water [1]	K_2CO_3	40	24	73.40	26.60	<0.05
23		EDIPA	40	24	61.93	37.78	0.29
24		NMM	40	24	14.17	62.13	23.70
25	MeTHF/Water [1]	K_2CO_3	40	24	57.44	42.56	<0.05
26		EDIPA	40	24	37.40	62.40	0.20
27		NMM	40	24	13.12	82.67	4.21
28	2% aqueous TPGS	K_2CO_3	40	24	19.86	80.14	<0.05
29		EDIPA	40	24	37.27	32.34	30.39
30		NMM	40	24	3.76	88.37	7.87
31	2% aqueous TPGS/ THF (0.3 v/v)	K_2CO_3	40	24	25.12	74.88	<0.05
32		EDIPA	40	24	50.34	49.49	0.17
33		NMM	40	24	3.94	90.27	5.79
34	1-Propanol/Water [1]	K_2CO_3	90	24	98.46	0.96	0.58
35		EDIPA	90	24	98.71	0.87	0.42
36	1-Butanol/Water [1]	K_2CO_3	60	24	94.37	5.63	<0.05
37		NaOH 30%	60	24	74.02	<0.05	25.98
38		NaOH 30% [2]	60	24	85.08	0.28	14.64
39		NaOH 30% [3]	60	24	74.15	<0.05	25.85
40		K_2CO_3 [3]	90	90	71.94	15.32	12.74
41	Ethyl acetate	K_2CO_3	80	4	98.99	0.83	0.18

Notes: [1] Solvent/water=3:2 v/v; [2] Benzyl triethylammonium bromide added; [3] Tetrabutylammonium hydrogen sulfate added

Screening of conditions for the synthesis of **5** or **5'**

The starting materials **3** and either **4** or **41** were charged to a vial and the solvent and base (and the additive where applicable) were added. The vial was sealed and the mixture was heated with stirring to the temperature and for the time indicated in the table.

Compound **41** was used as surrogate of **4** due to the limited availability of **4** during development. Their reactivity was comparable in control experiments.

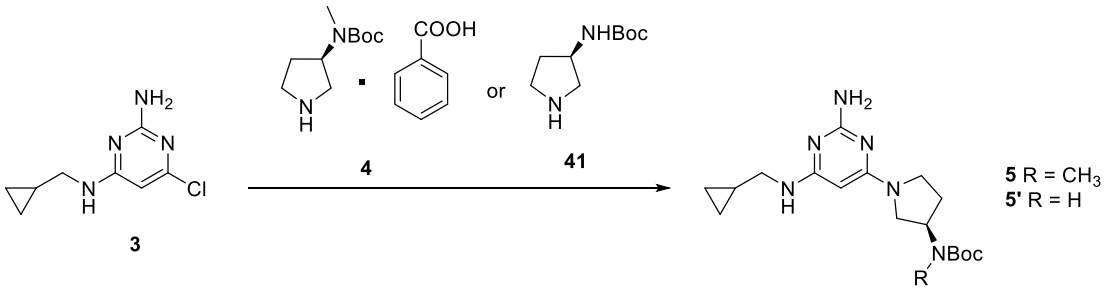

Table S3. Screening of conditions for the reaction of **3** with **4** or **41**

Entry	Solvent	Starting material (eq)	Base (eq) Additive (eq)	IT (°C)	Time (h)	HPLC (% A)		
						3	**5** or **5'**	**Others**
1	Sulfolane	**4** (1.15)	Na_2CO_3 (2.5)	130	17	23.42	75.20	1.38
2	Sulfolane	**4** (1.15)	-	130	17	6.23	70.60	23.17
3	Sulfolane	**4** (1.15)	Na_2CO_3 (2.5) $MgCl_2$ (0.2)	130	17	13.37	84.16	2.47
4	Sulfolane	**4** (1.15)	Tributyl amine (1.15)	130	17	5.29	89.30	5.42
5	Sulfolane	**4** (1.15)	Tributyl amine (1.15) $MgCl_2$ (0.2)	130	17	10.54	70.94	18.52
6	Sulfolane	**4** (1.15)	Tributyl amine (2.5)	130	24	7.13	83.89	8.98
7	Sulfolane	**4** (1.05)	Tributyl amine (2.5)	130	24	6.02	84.38	9.60
8	Sulfolane	**4** (1.05)	Tributyl amine (2.5)	140	19	13.46	79.15	7.39
9	NBP [1]	**4** (1.15)	Na_2CO_3 (2.5)	130	17	45.29	54.71	not integrated
10	NBP [1]	**4** (1.15)	-	130	17	9.62	82.35	8.03
11	NBP [1]	**4** (1.15)	Na_2CO_3 (2.5) $MgCl_2$ (0.2)	130	17	36.14	63.15	0.71
12	NBP [1]	**4** (1.15)	Tributyl amine (1.15)	130	17	12.13	85.14	2.73
13	NBP [1]	**4** (1.15)	Tributyl amine (1.15) $MgCl_2$ (0.2)	130	17	14.32	81.42	4.26
14	MeTHF [2]	**4** (1.20)	EDIPA (2.5)	80	39	17.03	82.97	<0.05
15	MeTHF [2]	**4** (1.20)	Na_2CO_3 (2.5)	80	39	54.00	46.00	<0.05
16	1-Butanol	**41** (1.05)	K_2CO_3 (2.5)	115	21	24.25	54.19	21.56
17	1-Butanol	**41** (1.05)	K_2CO_3 (2.5) CuI (5% mol)	115	21	29.45	52.37	18.18
18	PGMME [3]	**41** (1.15)	-	130	24	20.79	75.92	3.29
19	PGMME [3]	**41** (1.15)	K_2CO_3 (2.5)	130	32	34.38	50.6	15.02
20	PGMME [3]	**41** (1.15)	EDIPA (2.5)	130	32	35.55	52.2	12.25
21	Toluene	**41** (1.05)	K_2CO_3 (2.5)	110	24	44.12	51.45	4.43

Notes: [1] *N*-Butylpyrrolidone; [2] 2-Methyltetrahydrofuran; [3] Propylene glycol monomethylether

Screening of Lewis acids for the synthesis of **5** or **5'**

The starting materials **3** and either **4** or **41** were charged to a vial and the solvent (Propylene glycol monomethylether, PGMME) and additive(s) were added. The vial was sealed and the mixture was heated to 125°C and stirred for the time indicated in the table.

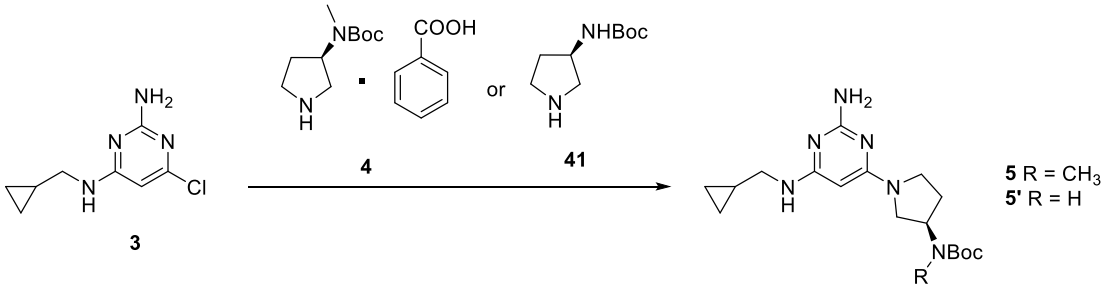

Table S4. Screening of Lewis acids in the reaction between **3** and **41**

Entry	Starting material	Additive (eq.)	Time (h)	HPLC (% A)		
				3	**5** or **5'**	**Others**
1	**41**	-	24	20.79	75.92	3.29
2	**41**	CuBr (0.2)	24	13.91	83.04	3.05
3	**41**	CuBr (0.2)/Phenantroline (0.4)	24	11.63	85.37	3.00
4	**41**	CuI (0.2)	24	14.07	82.68	3.25
5	**41**	CuI (0.2)/Phenantroline (0.4)	24	9.78	86.71	3.51
6	**41**	CuOTf (0.2)	24	13.77	82.72	3.51
7	**41**	CuOTf (0.2)/Phenantroline (0.4)	24	13.12	82.67	4.21
8	**41**	BF_3-THF (0.2)	24	7.38	89.3	3.32
9	**41**	$FeCl_3$ (0.2)	24	5.07	84.71	10.22
10	**41**	TMSCl (1.0) [1]	5	50.7	49.3	<0.10
11	**41**	TMSOTf (1.0) [1]	5	10.58	<0.10	89.42
12	**41**	$Sc(OTf)_2$ (0.2)	24	3.23	90.48	6.29
13	**41**	Cu_2Cl_2 (0.2)	24	17.15	76.97	5.88
14	**41**	$MgBr_2$ (0.2)	24	6.82	90.00	3.18
15	**41**	$MgCl_2$ (0.2)	24	4.57	87.71	7.72
16	**41**	CuBr (0.2)/K_2CO_3 (1.0)	24	29.86	55.66	14.48
17	**41**	$FeCl_3$ (0.2)/K_2CO_3 (1.0)	24	20.79	75.91	3.3
18	**41**	$MgCl_2$ (1.0)	11	46.95	51.67	1.38
19	**41**	BF_3-THF (0.1)	24	3.08	91.94	4.98
20	**41**	BF_3-THF (0.3)	24	3.4	93.71	2.89
21	**41**	$Zn(OAc)_2$ (0.2)	24	18.7	71.24	10.06
22	**41**	$Al(O\textit{i}Pr)_3$ (0.2)	24	6.84	90.98	2.18
23	**41**	$Ti(O\textit{i}Pr)_4$ (0.2)	24	3.52	92.9	3.58
24	**41**	$SnCl_4$ (0.2)	24	10.81	64.57	24.62
25	**41**	$Ce(OTf)_3$ (0.2)	24	16.23	78.98	4.79
26	**41**	$Mg(OAc)_2$* $4H_2O$ (0.2)	24	9.42	88.35	2.23
27	**41**	$CuBr_2$ (0.2)	24	23.04	68.55	8.41
28	**41**	$Cu(OTf)_2$ (0.2)	24	22.58	70.17	7.25
29	**41**	$Fe(OTf)_3$ (0.2)	24	18.1	77.29	4.61
30	**41**	$Mg(OEt)_2$ (3.0)	24	13.36	86.64	<0.05
33	**41**	$Mg(OTf)_2$ (3.0)	24	<0.05	<0.05	100
34	**4**	$B(C_6F_5)_3$ (0.05)	24	36.52	63.48	<0.05
35	**4**	$B(C_6F_5)_3$ (0.05) [2]	24	37.95	62.05	<0.05
36	**4**	$Mg(O\textit{t}Bu)_2$ (0.5)	24	16.50	82.06	1.44
37	**4**	$Mg(O\textit{t}Bu)_2$ (0.5) [2]	24	20.85	78.45	0.70

Notes: [1] Reaction in Toluene; [2] Reaction at 95°C