Rapid Catalyst Identification for the Synthesis of the Pyrimidinone Core of HIV Integrase Inhibitors**

Ana Bellomo, Nihan Celebi-Olcum, Xiaodong Bu, Nelo Rivera, Rebecca T. Ruck, Christopher J. Welch, Kendall N. Houk,* and Spencer D. Dreher*

Microscale multiparallel chemistry platform technologies enable modern synthetic chemists to rapidly apply known potential solutions for a given synthetic transformation to new, high-complexity synthetic problems. This paradigm is typically most successful for solving problems where a rich history of relevant literature precedents and a thorough mechanistic understanding of the target reaction already exist. For example, a Suzuki reaction platform employing an array of known effective phosphine ligands, reaction solvents, and inorganic bases can be screened to uncover optimal substrate-specific reaction conditions with a high probability of success. However, many synthetic problems arise for which rationally designed platforms are not available, and cannot be designed owing to a lack of precedent or understanding. Nevertheless, microscale high-throughput experimentation (HTE) tools can be used to maximize the opportunity to serendipitously improve reaction performance, and several recent, high-profile reports have highlighted some initial progress in this area.[1–8]

Herein, we describe a broad-based, microscale additive-screening platform that was designed to minimize complexity and cost/time bottlenecks in the discovery of new ways to improve the performance for a wide variety of reactions. By using this approach, a single chemist was able to set up and analyze 475 different reaction conditions in a single day to identify new catalysts for the preparation of high-value pyrimidinone heterocycles, which are at the core of HIV Integrase inhibitors.[9,10] Follow-up investigations of identified conditions combined with quantum mechanical calculations

led to the proposal of a single-electron transfer (SET) activation mechanism for this catalysis, thus illustrating how the pursuit of serendipitous solutions can sometimes lead to improved understanding.

We envisioned creating a 96-well plate additives platform containing 95 different, highly practical pre-dosed compounds which might activate a reaction through a variety of different mechanistic pathways; there was one empty well for a comparative control reaction. The additives that were chosen are displayed in Figure S1 of the Supporting Information. Ideally, this reaction improvement engine would be inexpensive and simple to use, while providing results that would be a robust predictor of scale-up performance. To minimize reagent cost, we utilized very small 250 μL HPLC-vial inserts (microvials)[11] as reaction vials, thus permitting reaction screening at approximately 20 μL of solvent (ca. 1 mg substrate per reaction). This is about 4–5 times smaller than the 1 mL vials that are the current HTE standard in industry and academic laboratories, and in our opinion represents the present lower limit that still enables robust chemistry development. The platform setup simply requires weighing out the substrates, reagents, and solvent into a single vial, then distributing this mixture to the additives plate using a pipettor, a process that only takes a few minutes to complete. Finally, after reaction incubation, we envisioned using MISER chromatography[12,13] for sample analysis, as this method enables an entire 96-well plate to be evaluated in about 1 hour, and produces a convenient graphical output that facilitates hit identification.

We were interested in applying this platform to the synthesis of 2-substituted-5-hydroxy-6-oxo-1,6-dihydropyrimidine-4-carboxylic acid (pyrimidinone) derivatives of type **2** (Scheme 1), an important class of compounds with well-documented biological activity.[9,10,14–16] Currently, the most effective approach for the synthesis of pyrimidinones employs a two-step process which involves a Michael addition of an *N*-hydroxy amidine to an acetylynic diester with a subsequent

---

[*] Dr. A. Bellomo
Department of Chemistry, University of Pennsylvania
Philadelphia, PA 19104-6323 (USA)

Dr. N. Celebi-Olcum, Prof. Dr. K. N. Houk
Department of Chemistry and Biochemistry, University of California
Los Angeles, CA 90095 (USA)
E-mail: houk@chem.ucla.edu

Dr. X. Bu, Dr. N. Rivera, Dr. R. T. Ruck, Dr. C. J. Welch,
Dr. S. D. Dreher
Department of Process Chemistry, Merck Research Laboratories
P.O. Box 2000, Rahway, NJ 07065 (USA)
E-mail: spencer_dreher@merck.com

Supporting information for this article (general synthetic procedures, ^{1}H and ^{13}C NMR and HPLC data for compounds listed in all tables, and MISER data) is available on the WWW under http://dx.doi.org/10.1002/anie.201201720.
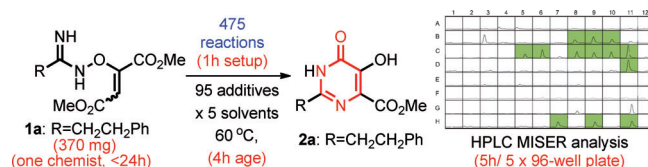
Scheme 1. Catalyst screening for the synthesis of pyrimidinone **2a** from Michael adduct **1a** using the additive platform. The pyrimidone core is highlighted in red. A MISER chromatogram for the 96 reactions run in 1,4-dioxane reveals which additives resulted in significant product formation (highlighted in green).

thermal dissociation/recombination event to afford the key pyrimidinone scaffold, typically in 15–50 % overall yield.[15–20] This thermal rearrangement requires elevated temperatures (140 °C) to convert both *E* and *Z* isomers into product, thus leading to significant decomposition and low to moderate yields. To date, there has been no report of using catalysis to improve the yields of these reactions, or to decrease the reaction temperatures.

To use the additives-screening platform for this project we first established, through temperature screening, that the highest temperature at which no pyrimidinone product **2a** was formed thermally from **1a** in a variety of solvents was 60 °C. The additive screening was carried out by dosing the substrate **1a** (2.5 µmol) and internal standard (0.25 µmol biphenyl) in 20 µL of five different solvents (*o*-xylene, 1,4-dioxane, methanol, NMP and DMF) to five of the 250 µL additives plates containing 20–50 % of the pre-dosed additives. Each reaction was then heated for 4 hours at 60 °C. Upon completion, the reactions were diluted with MeOH and the five plates were subjected to HPLC MISER analysis (see the Supporting Information).[12,13] Thus, with this platform and workflow in hand, 475 different reactions were evaluated with just 370 mg of material in a single day.

Figure S1 in the Supporting Information shows the MISER chromatograms for the 96 reactions run in 1,4-dioxane. The reactions giving product are easily identified by the presence of a semiquantitative peak at the relevant mass. As expected, the control well (A01), which contains no additive, showed no product formation. Rewardingly, product **2a** was formed in significant quantities with a number of practical catalysts (Cu, Fe, Ni) and also several ligands. The assay yields (AY) of the successful leads were then determined by conventional quantitative HPLC analysis, with the best leads tabulated in Table 1.

The best catalyst lead, dibromo(1,10-phenanthroline)/Cu^{II} (**3**; 50 mol %), from the initial screening in 1,4-dioxane afforded 74 % AY of the desired pyrimidinone **2a** (under noncatalyzed conditions at 140 °C, 50 % AY was obtained for product **2a**). We decided to further optimize this lead using multiparallel screening in 250 µL vials (Table 2). First, we found that the loading of **3** could be reduced from 50 mol % to 5 mol % in 1,4-dioxane without impacting the reaction yield (entries 1 and 2). Subsequently, we found that the dichloro analogue **4** performed identically to **3** (entry 3), which had limited commercial availability. We next evaluated several environmentally friendly solvents as alternatives to 1,4-dioxane (entries 4–8), and found that 2-methyltetrahydrofuran (2-MeTHF) and cyclopentylmethyl ether (CPME) were effective green replacements, with 2-MeTHF affording **2a** in 84 % AY (entry 8). We next evaluated the effects of temperature and loading with catalyst **4** on the reaction performance (entries 9–13). Reactions at 60 °C, 40 °C, and room temperature, at 5 mol % and 1 mol % catalyst loadings showed that the best reaction conditions in terms of yield, loading, and time were using 5 mol % of catalyst for 4 hours at 40 °C, thus giving 90 % HPLC AY (entry 10). Remarkably, overnight reactions at room temperature (entry 12) afforded complete conversion, and hence we had identified a practical catalyst that was able to reduce the reaction temperature by 120 °C.
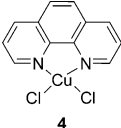
Table 1: Best leads obtained using the additive plate in 1,4-dioxane.[a]

Well	Additive	2a AY [%]
B08	FeCl$_2$	32
B09	FeCl$_3$	35
B10	[Fe(acac)$_2$]	51
C05	[NiCl$_2$(PPh$_3$)$_2$]	12
C06	CuCl	48
C07	CuCl$_2$	20
C08	CuBr	44
C09	CuI	29
C10	[Cu(acac)$_2$]	36
C11	dibromo-(1,10-phenanthroline)/Cu^{II}	74
D11	Cu powder < 75 micron	34
H07	dppf	2
H09	XPhos	8
H11	phenanthroline	19

[a] Wells B03, G08 and G11 indicated mass hits by MISER analysis, but did not contain the desired product by follow-up HPLC analysis. acac = acetylacetonate, dppf = 1,1′-bis(diphenylphosphanyl)ferrocene, XPhos = 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl.

Table 2: Reaction optimization for substrate **1a** using the most active additive on 2.5 µmol scale.
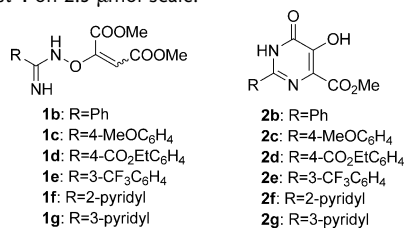
Entry	Catalyst, loading	T [°C]	Solvent	AY [%] 2
1	**3**, 50 mol %	60	1,4-dioxane	74
2	**3**, 5 mol %	60	1,4-dioxane	77
3	**4**, 5 mol %	60	1,4-dioxane	75
4	**4**, 5 mol %	60	CPME	79
5	**4**, 5 mol %	60	THF	56
6	**4**, 5 mol %	60	MeOH	40
7	**4**, 5 mol %	60	NMP[a]	42
8	**4**, 5 mol %	60	2-MeTHF	84
9	**4**, 1 mol %	60	2-MeTHF	74
10	**4**, 5 mol %	40	2-MeTHF	90
11	**4**, 1 mol %	40	2-MeTHF	78
12	**4**, 5 mol %	RT	2-MeTHF	84
13	**4**, 1 mol %	RT	2-MeTHF	70

[a] NMP = *N*-methylpyrrolidone.

The substrate scope for pyrimidinone formation was next explored with a series of electronically diverse and pyridine-containing Michael reaction adducts (**1b–g**), each prepared by the method described by Culbertson (Table 3).[20] The optimized procedure using catalyst **4** produced compounds **2b** and **2c** in excellent yields (entries 1 and 2). This catalyst also promoted the reaction with the remaining substrates **1d–g** at 60 °C, however the yields were not as high (entries 3–8).

To improve the yields of **2d–g**, we next evaluated solvent, temperature, and catalyst loading effects on these substrates

Table 3: Best reaction conditions for the synthesis of compounds **2b–g** using catalyst **4** on 2.5 µmol scale.

1b: R=Ph
1c: R=4-MeOC$_6$H$_4$
1d: R=4-CO$_2$EtC$_6$H$_4$
1e: R=3-CF$_3$C$_6$H$_4$
1f: R=2-pyridyl
1g: R=3-pyridyl

2b: R=Ph
2c: R=4-MeOC$_6$H$_4$
2d: R=4-CO$_2$EtC$_6$H$_4$
2e: R=3-CF$_3$C$_6$H$_4$
2f: R=2-pyridyl
2g: R=3-pyridyl

| Entry	Substrate	Catalyst loading	T [°C]	Solvent	2 AY [%]
1	**1b**	5 mol%	60	2-MeTHF	94
2	**1c**	5 mol%	60	2-MeTHF	88
3	**1d**	5 mol%	60	2-MeTHF	51
4	**1e**	5 mol%	60	2-MeTHF	16
5	**1e**	5 mol%	40	DCE	71
6	**1f**	5 mol%	60	2-MeTHF	17
7	**1g**	5 mol%	60	2-MeTHF	51
8	**1g**	50 mol%	60	2-MeTHF	75

and found that the yield of compound **2e** could be improved to 71% by using 5 mol% of **4** in 1,2-dichloroethane (DCE) at 40°C (entry 5). Similarly, the yield of compound **2g** was improved to 75% when we increased the loading of catalyst **4** back to 50 mol% (entry 8). However, we were unsuccessful in improving the yields for compounds **2d** and **2f** in this way.[21]

Also present in the initial screen of additives, were several ligands (dppf, XPhos, and phenanthroline), which gave some conversion of **1a** into **2a.** Follow-up experiments revealed that these ligands gave product slowly and did not turnover as catalysts. We hoped, however that we might be able to identify a ligand that could act as an effective catalyst, so we employed a pre-dosed microscale ligand-screening approach (again in 250 µL vials) with which we could rapidly evaluate 112 sterically and electronically diverse, mono- and bidentate achiral phosphine ligands. The phosphine screen was conducted for 4 hours at 60°C in total reaction volume of 20 µL of 1,4-dioxane containing 2.5 µmol of **1a** and 5 mol% of ligand per reaction. In this way a single ligand, 1,1′-(ferrocenediyl)phenylphosphine (**5**) was identified to have significant catalytic activity. This ligand gave excellent conversion and 78% HPLC AY of **2a** at a 5 mol% catalyst loading (see the Supporting Information).

We then applied this ligand to the synthesis of compounds **2a–g** while also evaluating seven solvents, and found that each substrate had a different solvent that was optimal (see the Supporting Information). Most importantly, we were able to identify a new catalyst that afforded **2f** in a very high, 88% AY (Scheme 2).

With identification of two different, novel catalytic systems, using **4** and **5**, for the synthesis of pyrimidinones we were interested in determining how these small-scale screening results would translate to a 0.4 mmol (160×) scale. Indeed, the small-scale assay yields proved to be highly predictive of scale-up performance, as demonstrated in Table 4.

With these successful synthetic results identified through serendipity, we were interested in determining if we could
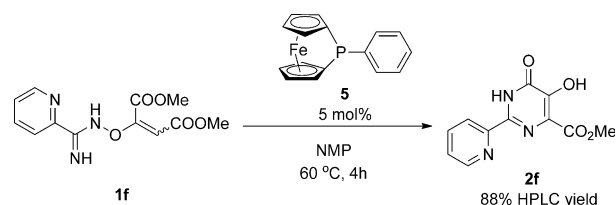
Scheme 2. High-yielding reaction conditions for the synthesis of compound **2f** using catalyst **5**.

Table 4: Reaction conditions for substrates **1a–g** using the two catalysts discovered by comparing yields obtained in microscale screening and scale-up results.

| | Conditions A[a] | | Conditions B[b] | |
	Microvials AY [%]	Scale-up AY [%]	Microvials AY [%]	Scale-up AY [%]
2a	90	94 (85)	quant.	90 (88)
2b	94	92 (83)	quant.	99 (94)
2c	88	83 (80)	94	74 (74)
2d	51	55 (50)	49	34 (30)
2e	71	71 (73)	41	44 (40)
2f	17	25	88	quant. (91)[c]
2g	75	77 (75)[c]	46	43

[a] Conditions A: **4** (5 mol%), 2-MeTHF, 60°C; **2a**: 24 h, 40°C; **2b**: 48 h; **2c**: 24 h; **2d**: 24 h; **2e**: 24 h, DCE, 40°C; **2f**: 48 h (56% conv.); **2g**: 24 h, 50 mol% additive. [b] Conditions B: **5** (5 mol%), 60°C; **2a**: 2-MeTHF, 24 h; **2b**: DME, 24 h; **2c**: NMP, 48 h (74% conv.); **2d**: NMP, 48 h (55% conv.); **2e**: NMP, 24 h (95% conv.); **2f**: NMP, 6 h; **2g**: NMP, 48 h (87% conv.). Numbers within parentheses correspond to yields of isolated products. Control reactions with no catalyst were run at 60°C and 40°C and no product formation was observed. [c] **2f** and **2g** could not be purified in good yield by silica gel chromatography because streaking of the products on the column and therefore the yields of isolated products shown here correspond to their characterization as benzoates **6f** and **6g** (see the Supporting Information). Compound **2g** was always obtained with variable amounts of impurities.

establish a mechanism for the reaction. It was previously proposed and supported through density functional calculations, that the thermal synthesis of the pyrimidinones involves a direct N−O cleavage to form a polar radical pair (PRP) with a substantial preference for recombination.[22] With our new catalysts, we surmised that the Cu and Fe may be working to reduce the energy of the transition state through a SET reduction mechanism. Quantum mechanical calculations[23] at the B3LYP/6-31G(d) level showed that indeed the dissociation and recombination of the radical anion should be much more facile than that of the neutral species (Figure 1).

While copper has a well-documented legacy as a SET catalyst,[24] we were interested in determining whether the ligand **5** also proceeded through SET activation or whether it proceeded through a new mechanism. Experimentally, we determined that ferrocene itself is not a catalyst in this reaction, and also that other ferrocenyl ligands (dppf, dippf, dtbpf) gave product but were significantly less reactive than **5** (see the Supporting Information). The calculated oxidation potential of the catalyst **5** (E^0_{calc} Fc(PPh)$^+$/Fc(PPh) = −0.62 V in acetonitrile vs. Fc$^+$/Fc) suggests that **5** may be a better reducing agent than ferrocene itself, thus supporting its role as a SET catalyst. We intend to study the specific catalytic
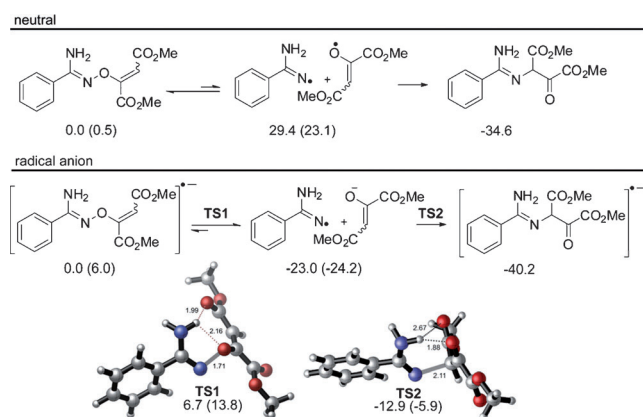
Figure 1. Energy profile for SET catalysis mechanism. B3LYP/6-31G(d) free energies calibrated with G3(MP2)B3 (ΔG, kcal mol^{-1}) for the *Z* and *E* isomers (values for *E* isomers in parenthesis) in the gas phase.

activity in more detail and to pursue other potential applications.

Herein, we demonstrated how a pre-dosed, microscale additives platform can enable a single chemist to evaluate 475 discrete reactions in a single day, thus providing a convenient and powerful "improvement engine" for organic synthesis. Employing this platform and subsequent microscale optimization techniques rapidly led to the identification of robust substrate-specific procedures for a range of biologically important pyrimidinones which are at the core of HIV Integrase inhibitors. We envision that similar, generally useful platform tools will soon become more widely available, thus dramatically impacting chemistry development and enabling increased access to chemical diversity and lower-cost synthesis. Most importantly, we believe that such platforms will lead to the discovery of new and potentially useful chemical reactivity and reaction mechanisms.

[1] I. W. Davies, C. J. Welch, *Science* **2009**, *325*, 701.
[2] S. D. Dreher, P. G. Dormer, D. L. Sandrock, G. A. Molander, *J. Am. Chem. Soc.* **2008**, *130*, 9257.
[3] G. A. Molander, S. L. J. Trice, S. D. Dreher, *J. Am. Chem. Soc.* **2010**, *132*, 17701.
[4] D. W. Robbins, J. F. Hartwig, *Science* **2011**, *333*, 1423.
[5] A. McNally, C. K. Prier, D. W. C. MacMillan, *Science* **2011**, *334*, 1114.
[6] M. T. Reetz, K. M. Kühling, A. Deege, H. Hinrichs, D. Belder, *Angew. Chem.* **2000**, *112*, 4049; *Angew. Chem. Int. Ed.* **2000**, *39*, 3891.
[7] J. Kobayashi, Y. Mori, K. Okamoto, R. Akiyama, M. Ueno, T. Kitamori, S. Kobayashi, *Science* **2004**, *304*, 1305.
[8] O. Trapp, S. K. Weber, S. Bauch, W. Hofstadt, *Angew. Chem.* **2007**, *119*, 7447; *Angew. Chem. Int. Ed.* **2007**, *46*, 7307.
[9] G. R. Humphrey, P. J. Pye, Y.-L. Zhong, R. Angelaud, D. B. Askin, P. E. Maligres, D. E. Mancheno, R. A. Miller, R. A. Reamer, S. A. Weissman, *Org. Process Res. Dev.* **2011**, *15*, 73.
[10] V. Summa, A. Petrocchi, F. Bonelli, B. Crescenzi, M. Donghi, M. Ferrara, F. Fiore, C. Gardelli, O. Gonzalez Paz, D. J. Hazuda et al., *J. Med. Chem.* **2008**, *51*, 5843.
[11] Previous experiments run in our lab using 250 µL vials for a variety of academic and drug development projects showed excellent scalability. S. Goble, S. Berritt, S. D. Dreher, unpublished results.
[12] C. J. Welch, X. Gong, W. Schaefer, E. C. Pratt, T. Brkovic, Z. Prizada, J. F. Cuff, B. Kosjek, *Tetrahedron: Asymmetry* **2010**, *21*, 1674.
[13] *High-throughput Analysis in the Pharmaceutical Industry* (Ed.: P. G. Wang), CRC Press, **2009**.
[14] D. E. O'Brien, L. T. Weinstock, R. H. Springer, C. C. Cheng, *J. Heterocycl. Chem.* **1967**, *4*, 49.
[15] V. Summa, A. Petrocchi, V. G. Matassa, M. Taliani, R. Laufer, R. D. Francessco, S. Altmura, P. Pace, *J. Med. Chem.* **2004**, *47*, 5336.
[16] E. Wagner, L. Becan, E. Nowakowska, *Bioorg. Med. Chem. Lett.* **2004**, *12*, 265.
[17] S. D. Dreher, N. Ikemoto, V. Gresham, J. Liu, P. G. Dormer, J. Balsells, D. Mathre, T. J. Novak, J. D. I. Armstrong, *Tetrahedron Lett.* **2004**, *45*, 6023.
[18] V. A. Boyd, J. Mason, P. Hanumesh, J. Price, C. J. Russell, T. R. Webb, *J. Comb. Chem.* **2009**, *11*, 1100.
[19] Y.-L. Zhong, H. Zhou, D. R. Gauthier, J. Askin, *Tetrahedron Lett.* **2006**, *47*, 1315.
[20] T. P. Culbertson, *J. Heterocycl. Chem.* **1979**, *16*, 1423.
[21] In the case of compound **2d** the moderate HPLC AY obtained was due in part to the formation of an imidazole by-product which was tentatively identified by LC-MS. Generation of this type of by-product was also described in Ref. [9] of this paper. This by-product was also observed for compound **2e**. To attempt to find high-yielding reaction conditions for the synthesis of pyrimidinones **2d** and **2e** we went back to our original 96-additive plate and we evaluated the corresponding *Z/E* adducts using 2-MeTHF as the solvent. No more active additives other than **4** were found for both substrates.
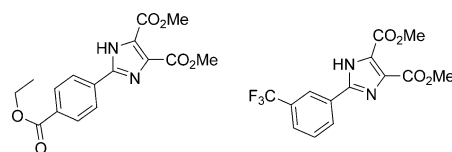
[22] P. J. Pye, Y.-L. Zhong, G. O. Jones, R. A. Reamer, K. N. Houk, D. Askin, *Angew. Chem.* **2008**, *120*, 4202; *Angew. Chem. Int. Ed.* **2008**, *47*, 4134.
[23] Gaussian 09 (Revision A.02), M. J. Frisch, et al.; see the Supporting Information.
[24] A. E. King, L. M. Huffman, A. Casitas, M. Costas, X. Ribas, S. S. Stahl, *J. Am. Chem. Soc.* **2010**, *132*, 12068, and references therein.