ORGANIC PROCESS RESEARCH & DEVELOPMENT
OPR&D



Full Paper

# Continuous-flow Synthesis of Cationic Lipid SST-01 via Safe and Scalable Aerobic Oxidation and Reductive Amination

Katsuaki Fujiwara, Haruro Ishitani, and Shu Kobayashi

*Org. Process Res. Dev.*, **Just Accepted Manuscript** • DOI: 10.1021/acs.oprd.0c00084 • Publication Date (Web): 22 Jul 2020



is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

# Continuous-flow Synthesis of Cationic Lipid SST-01 via Safe and Scalable Aerobic Oxidation and Reductive Amination


## Authors

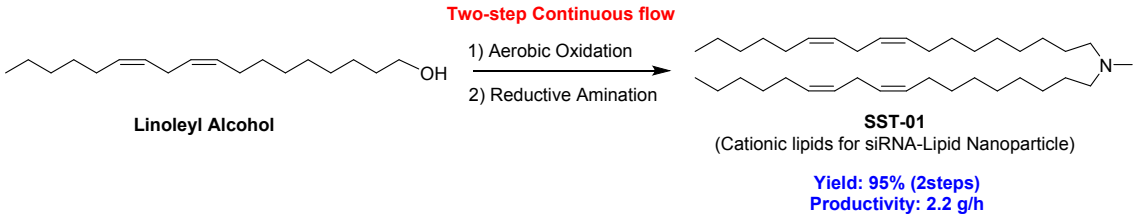
Katsuaki Fujiwara,*[†‡] Haruro Ishitani,[§] and Shū Kobayashi*[‡§]

### Affiliation

[†]Fuji Research Park, Research Functions Unit, R&D Division, Kyowa Kirin, Co., Ltd., 1188, Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8731, Japan

[‡]Department of Chemistry, School of Science, and [§]Green & Sustainable Chemistry Social Cooperation Laboratory, Graduate School of Science, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan


## Abstract Graphic



**Abstract**

The synthesis of a cationic lipid **SST-01**, a key component of Kyowa Kirin's siRNA-lipid nanoparticle (LNP), through a two-step homogeneous continuous-flow process is described. Safe and scalable aerobic oxidation with a catalytic $Cu^{I}$/TEMPO system and a diluted oxygen (5% $O_2$ in $N_2$) was utilized in the first step to provide a lipid aldehyde. Subsequent reductive amination of the first step eluent with methylamine using tetramethylammonium triacetoxyborohydride provided the target **SST-01** with an overall yield of 95%.

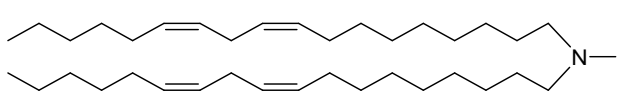
**Keywords**

Continuous-flow chemistry, Catalytic oxidation, Reductive amination, Cationic lipid, Lipid nanoparticle

## INTRODUCTION

Continuous-flow synthesis[1] is a technology that has attracted a great deal of attention from industry as well as academia, because it provides safe, compact, efficient and environmentally benign chemical manufacturing processes, including those for manufacturing fine and specialty chemicals like drugs.[2–5] In comparison to conventional batch methods, continuous-flow methods offer large benefits to pharmaceutical industries, such as accurate quality control, efficient inventory management, easy automation, and faster scale-up applications;[6–8] these allow compounds from the laboratory to be brought to markets faster and cheaper.

Lipid nanoparticle (LNP) is one of the most successful lipid-mediated delivery vehicle systems for short interfering RNA (siRNA) and is now considered a reliable therapeutic modality after the U.S. Food and Drug Administration (FDA) approved Alnylam Pharmaceuticals' PATISIRAN[9]. LNPs are approximately 100 nm in size and consist of siRNA, neutral lipids, PEG-lipids, and cationic lipids. Cationic lipids, key components of siRNA-LNP, are protonated in acidic endosomes, increase the electric charge on the surface of nanoparticles, and induce the migration of siRNA into cytosol. Medicinal chemists have shown that sophisticated structural modification of cationic lipids influences the in vivo performance of siRNA-LNPs. In a previous study[10, 11] at Kyowa Kirin Co., Ltd., we conducted a medicinal chemistry campaign and found that cationic lipid **SST-01** (**1**; Figure 1), which possesses a relatively simple chemical structure of two long alkyl chains connected to a nitrogen atom, exerts prominent siRNA delivery activity, especially for solid tumors.

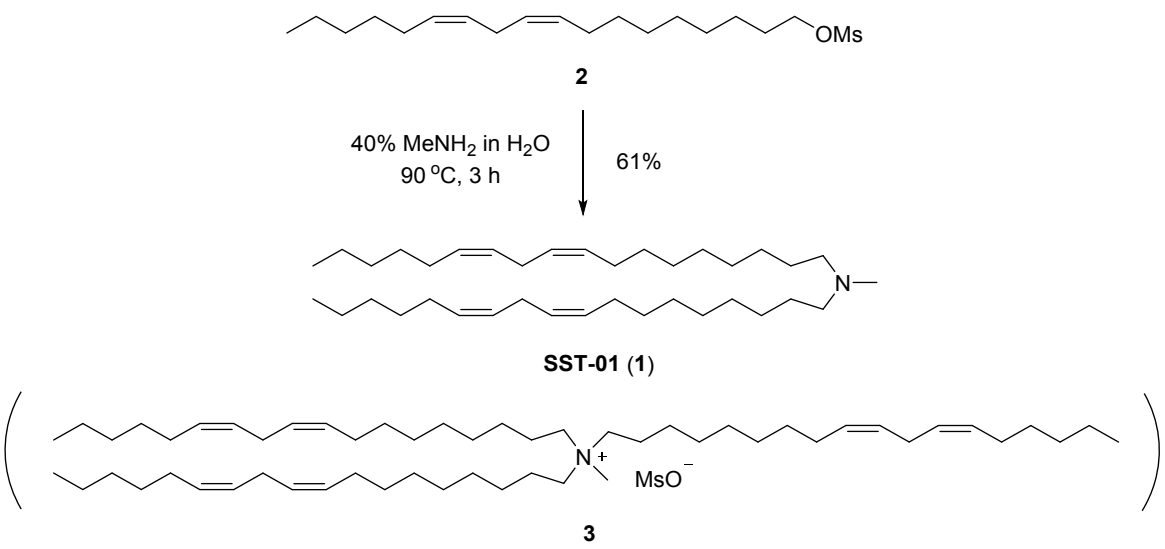



**SST-01** (**1**)

**Figure 1.** Structure of cationic lipid **SST-01** (**1**)

We have been conducting a batch process to supply **SST-01** (**1**) for use in a preclinical study (Scheme 1).[12] However, the manufacturing cost of the present batch method, which consists of dialkylation with lipid mesylate **2**, was inadmissibly high due to the use of expensive raw material **2**. In addition, the formation of a byproduct, the corresponding quaternary ammonium salt (**3**), decreased the yield of the desired **1**. In order to reduce the manufacturing cost, the present batch method with conventional *N*-alkylation should be improved. We therefore predict that the utilization of continuous-flow methods with a novel synthetic route using inexpensive raw material would achieve such an improvement. In this paper, we report the details of our improvement in preparing **1** with sequential continuous-flow reactions.

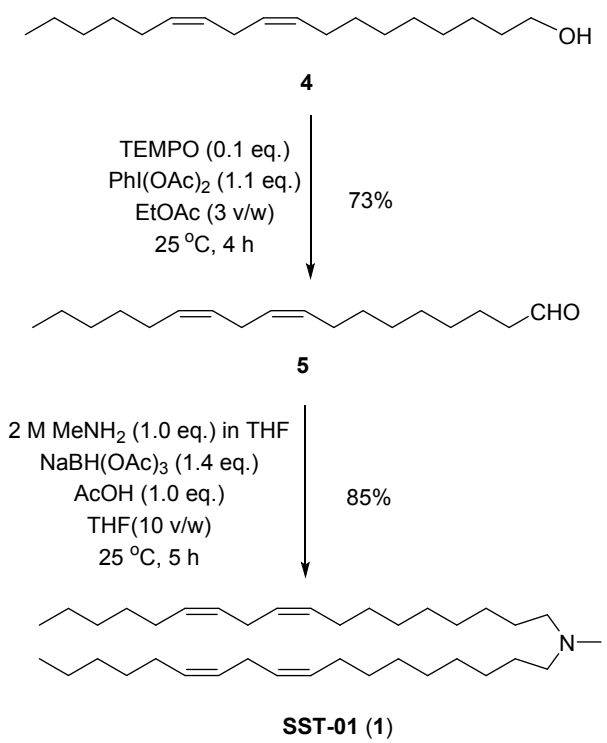
**Scheme 1.** Structure of cationic lipid **SST-01** (**1**) and the first-generation synthetic route in batch




## RESULTS AND DISCUSSION

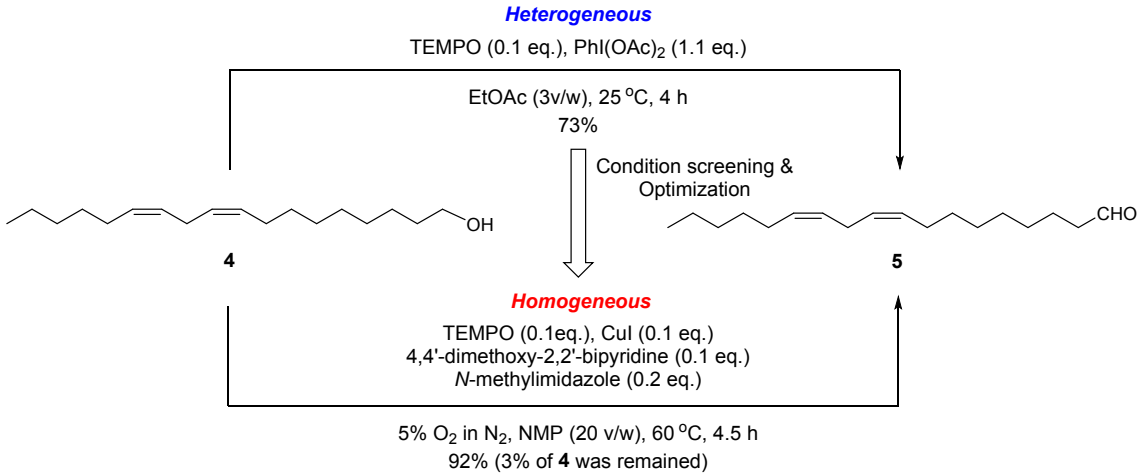
Prior to investigations into continuous-flow methods, we conducted comprehensive research on the synthesis of **1** and envisioned a new two-step protocol starting with less-expensive linoleyl alcohol (**4**). For the new protocol, oxidation of an alcohol and reductive double amination would provide target **1** without the formation of an undesired quaternary ammonium salt. However, preliminary studies revealed that a known process shown in Scheme 2 gave the corresponding aldehyde in relatively low yield, and a co-oxidant, $PhI(OAc)_2$, was hard to dissolve into the reaction medium. In addition, a reductant in the amination step, $NaBH(OAc)_3$, has no solubility in THF, producing a heterogeneous system. We expected that these drawbacks would account for troubles in continuous-flow; thus, we re-investigated both steps individually.

**Scheme 2.** New synthetic route of **SST-01** (**1**)




Since the oxidation of primary alcohols to aldehydes using the flow method has been reported [13-17], some of these conditions were examined in the batch method (Table S1). As a result, it was found that aerobic oxidation conditions with a $Cu^{I}$/TEMPO catalyst system developed by Stahl et al.[18] gave the desired aldehyde in a satisfactory 81% isolated yield (Table S1, entry 6). Although this Stahl oxidation was already applied into a homogeneous flow reaction,[19] it was necessary to adjust the original conditions with our substrates to maintain the reduced cost. Several conditions, such as solvents, concentrations of substrates, reaction temperature, oxygen amounts, and Cu sources[20] and ligands[21] were widely screened (Table S2-S6), and it was found that the combination of CuI and 4,4'-dimethoxy-2,2'-bipyridine showed sufficient activity in NMP at 60°C. We also examined an oxygen concentration by considering the limiting oxygen concentration (LOC) of NMP (7.6% at 200°C, 20 bar)[22] for the safety aspect in continuous-flow conditions, and decided to use 5% oxygen diluted with nitrogen instead of air. Under these optimized conditions, a complete homogeneous system could be realized and a 92% yield of the desired aldehyde was obtained (Scheme 3).

**Scheme 3.** Optimized conditions of the oxidation step in batch




To apply the abovementioned optimized conditions to a homogeneous continuous-flow system, the flow set-up shown in Figure 2 was assembled. An NMP solution of substrate **4** (Solution A in Reservoir A) was flowed using Pump A, and an NMP solution of a catalyst system (Solution B in Reservoir B), consisting of CuI, the ligand, TEMPO, and *N*-methylimidazole (NMI), was fed using Pump B at the same flow rate of Solution A. These streams were mixed at a T-shaped mixer (M1), and then 5% oxygen in nitrogen that was regulated with a mass flow controller (MFC) at another T-shaped mixer (M2) was combined. Finally, the whole mixture was fed into a 30 m SUS tube reactor (i.d. 1.0 mm). The tube reactor was heated in an aluminum heating block and a back pressure regulator (BPR) was attached at the outlet.

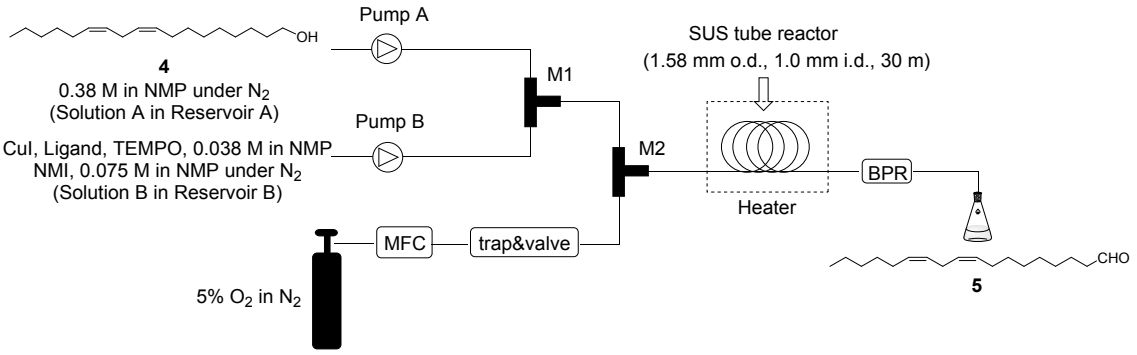
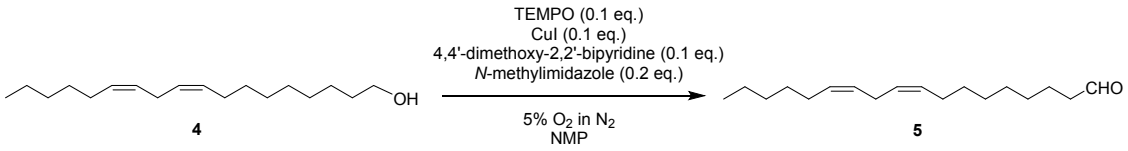



**Figure 2.** Set-up of the continuous-flow oxidation step.

The results of representative optimization studies using this flow system are shown in Table 1. High yield and high selectivity were obtained under slow feed rate conditions (0.04 mL/min, entry 1). In addition, the reaction time was shown to be markedly shorter than with the batch method (Table 1, entry 1 vs. Scheme 3). The flow regime was an annular mist flow. We considered a tube reactor in flow had a larger interfacial area to volume ratio than that of a stirred tank reactor in batch, and the

reaction solution and diluted oxygen gas were sufficiently mixed. We assumed this was why the reaction time was significantly shortened. On the other hand, the stability of the catalyst was maintained for up to 21 hours, which was monitored in Reservoir A (Table S7, entry 3). A decrease in yield at 143 hours raised the suspicion of catalyst deterioration; however, the yield recovered to 92% yield (HPLC area) when the reaction temperature was elevated to 80°C (Table S7, entries 4 and 5).

**Table 1.** Optimization of reaction conditions of the oxidation step in flow




| Entry | Flow rate (mL/min) | | Pressure (bar) | Temp. (°C) | Oxygen (eq.) | Residence time (min) [b] | HPLC area (%) | |
|---|---|---|---|---|---|---|---|---|
| | Solution A, B [a] | 5% $O_2$ in $N_2$ | | | | | **4** | **5** |
| 1 | 0.040, 0.040 | 15 | 0 | 60 | 1.8 | 1.6 | 2 | 97 |
| 2 | 0.10, 0.10 | 15 | 0 | 60 | 0.7 | 1.6 | 35 | 64 |
| 3 | 0.10, 0.10 | 38 | 0 | 60 | 1.8 | 0.6 | 14 | 85 |
| 4 | 0.10, 0.10 | 15 | 5 | 60 | 4.3 | 1.6 | 6 | 91 |
| 5 | 0.10, 0.10 | 20 | 5 | 60 | 5.7 | 1.2 | 6 | 92 |
| 6 | 0.10, 0.10 | 15 | 5 | 70 | 4.2 | 1.6 | 1 | 98 |
| 7 | 0.20, 0.20 | 15 | 5 | 70 | 2.1 | 1.5 | 20 | 79 |
| 8 | 0.20, 0.20 | 15 | 5 | 80 | 2.0 | 1.5 | 19 | 80 |
| 9 | 0.20, 0.20 | 30 | 5 | 80 | 4.0 | 0.8 | 2 | 96 |
| 10 | 0.40, 0.40 | 50 | 8 | 80 | 5.1 | 0.5 | 8 | 91 |
| 11 | 0.40, 0.40 | 50 | 8 | 90 | 4.9 | 0.5 | 5 | 94 |
| 12 | 0.40, 0.40 | 50 | 8 | 100 | 4.8 | 0.5 | 3 | 95 |

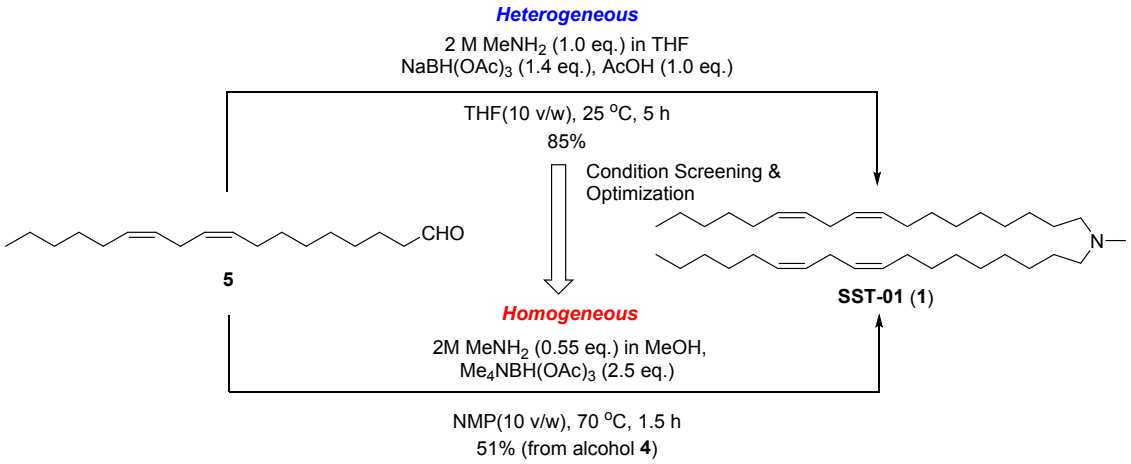
[a]Solution A: **4** in NMP (0.38 M). Solution B: CuI, Ligand, TEMPO in NMP (0.038 M), NMI in NMP (0.075 M).
[b]The residence time was calculated based on the assumption that the total flow rate was the sum of the flow rate of the gas and liquid components.

To improve productivity, the flow rates of Solutions A and B were increased from 0.04 mL/min to 0.40 mL/min in a stepwise manner. The decreased conversion of substrate **4** was essentially observed by increasing the rate; however, this could be overcome by increasing the oxygen feed rate, back pressure, and reaction temperature (entries 2-9). Final optimization at 0.40 mL/min was started with an oxygen feed rate of 50 mL/min, 8 bar back pressure, and a reaction temperature of 80°C, and a 91% yield (HPLC area) was obtained with the co-existence of 8% substrate **4.** Elevating the reaction temperature to 100°C improved the conversion of the desired **5** and 95% yield (HPLC area)

was obtained (entry 12). Furthermore, continuous operation for 20 hours under optimized conditions produced 40 g of the target aldehyde (**5**) (95% yield). Under these conditions, the flow regime was a slug flow (Figure S2). In comparison to the previously reported continuous-flow $Cu^{I}$/TEMPO oxidation of aliphatic alcohol, the usage of inexpensive CuI, low pressure of oxygen gas, and a short residence time were successfully achieved.

Next, a preliminary investigation of the reductive amination process was conducted in batch. To avoid the use of an insoluble metal hydride reductant, such as $NaBH(OAc)_3$, screening of other conditions was investigated (Table S8-S12). Sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al) and catalytic hydrogenation conditions were both invalid; however, switching the cation of the acetoxyborohydride from $Na^+$ to $Me_4N^+$ made borohydride species soluble and a reasonable yield of the desired **SST-01** (**1**) was attained (Table S12, entry 2). Under these conditions, the one-pot two-step reaction was confirmed to be possible using a crude reaction mixture from the first oxidation step (Scheme 4).

**Scheme 4.** Optimized conditions of the reductive amination step in batch




To apply the above system in a flow process, other set-ups consisting of the following apparatuses were assembled (Figure 3)**.** A 0.19 M NMP solution of aldehyde **5** (Solution C) and an unpurified crude mixture from the oxidation step were flowed from Reservoir C using Pump C, and this stream was mixed with a MeOH solution of methylamine (Solution D) fed from Pump D at a T-shaped mixer (M3). The combined substrate solutions were then forgathered with a NMP solution of $Me_4NBH(OAc)_3$ from Pump E (Solution E) at a T-shaped mixer (M4), and were carried into a 20 m SUS tube reactor (i.d. 1.0 mm), which was heated by an aluminum heating block.

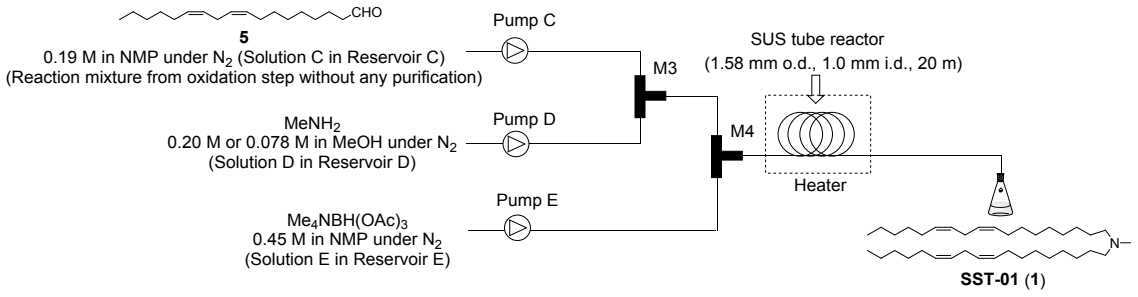
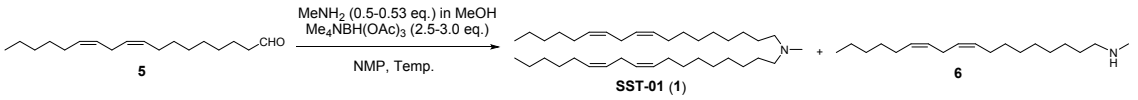



**Figure 3.** Set-up of the continuous-flow reductive amination step.

The conditions optimized in batch were first applied to the flow reaction. The conversion of aldehyde **5** was not full and a moderate 61% yield of the dialkylated amine **1** was obtained along with 6% of monoalkylated amine **6** (Table 2, entry 1). In this case, precipitation in the resulting solution could be found, possibly due to the evaporation of MeOH in the reactor. We assumed that the precipitation was a derivative of $Me_4NBH(OAc)_3$ since MeOH was able to dissolve the reductant. To increase the relative MeOH amount and suppress the evaporation of MeOH, we diluted the solution D to 0.078 M and the reaction temperature was reduced to 25°C. These modifications were effective for homogenization of the reaction medium, and a better yield of the desired **1** was obtained when the reaction was performed at 40°C (entries 2 and 3). Increasing the flow rate of Solution C did not affect the conversion or yield; thus, productivity of 0.15 mmol/min could be achieved under the conditions shown in entry 5. The stability of the present flow system was also checked under the conditions of entry 4, and no clogging and no change in the outcome could be confirmed during the 11-hour operation.

**Table 2.** Optimization of the reaction condition of the reductive amination step in flow

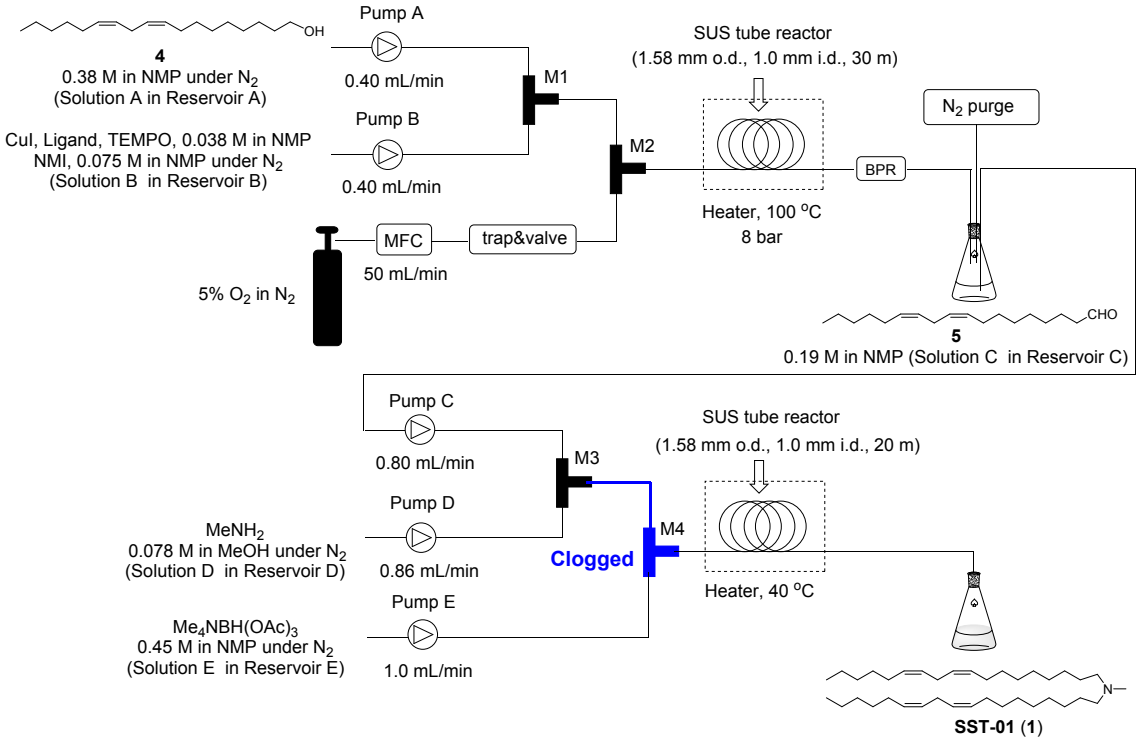



| Entry | Flow rate (mL/min) | | | Temp. (°C) | Residence Time (min) | HPLC area (%) | | | Two Steps Yield (%) [g] |
|---|---|---|---|---|---|---|---|---|---|
| | Solution C [a] | Solution D [b] | Solution E [c] | | | **5** | **SST-01** | **6** | |
| 1 | 0.20 | 0.10 [d] | 0.21 [f] | 70 | 31 | 19 | 61 | 6 | |
| 2 | 0.20 | 0.24 | 0.21 [f] | 25 | 24 | 12 | 64 | 8 | |
| 3 | 0.15 | 0.19 [e] | 0.19 | 40 | 30 | 0 | 82 | 7 | 70 |
| 4 | 0.30 | 0.38 [e] | 0.38 | 40 | 15 | 0 | 83 | 8 | 71 |
| 5 | 0.80 | 0.96 | 1.00 | 40 | 6 | 2 | 81 | 7 | 75 |

[a] **5** in NMP (0.19 M), reaction mixture from the oxidation step without any purification. [b] $MeNH_2$ in MeOH (0.078 M, 0.5 eq.). [c] $Me_4NBH(OAc)_3$ in NMP (0.45 M, 3.0 eq.). [d] $MeNH_2$ in MeOH (0.20 M, 0.5 eq.). [e] $MeNH_2$ in MeOH (0.078 M, 0.53 eq.). [f] $Me_4NBH(OAc)_3$ in NMP (0.45 M, 2.5 eq.). [g] Yield from alcohol **4**. Yield based on HPLC assay

using biphenyl as an internal standard.

Using a combined flow set-up, as illustrated in Figure 4, we next tried to connect the homogeneous $Cu^{I}$/TEMPO-catalyzed oxidation with the reductive amination. To avoid any contamination of oxygen into the reduction step in the first step, a gas-liquid separation flask (Reservoir C) was placed between the two units, and Reservoir C was purged by nitrogen gas to keep the oxygen concentration at ≤5% (considering LOC of NMP).




The equivalent of reagents for **4**: CuI (0.1 eq.), Ligand (0.1 eq.), TEMPO (0.1 eq.), NMI (0.2 eq.), methylamine (0.45 eq.), $Me_4NBH(OAc)_3$ (3.0 eq.)

**Figure 4.** Set-up of the continuous-flow oxidation and reductive amination steps.

The two-step flow reaction proceeded smoothly for 30 minutes; however, clogging occurred at the T-shaped mixer (M4) on the inlet part of the second reduction reactor due to the deposition of a reddish-brown solid. We suspected that the solid was a derivative of Cu species due to its very low solubility in any solvent. Although one potential problem of this system was compatibility of copper species supplied in the oxidation step for the following reductive amination step, the previous single reductive amination investigation under flow conditions proved that there was no deposition from the copper-included substrate solution (Solution C) in the reductive amination step.

To clarify the causes of the generation of the insoluble solid, we carefully checked the precipitation process among Solutions C, D, and E, especially for a solid growth process from a seed. i) Methanol and an insoluble solid seed were added to Solution C, resulting in the immediate generation of a

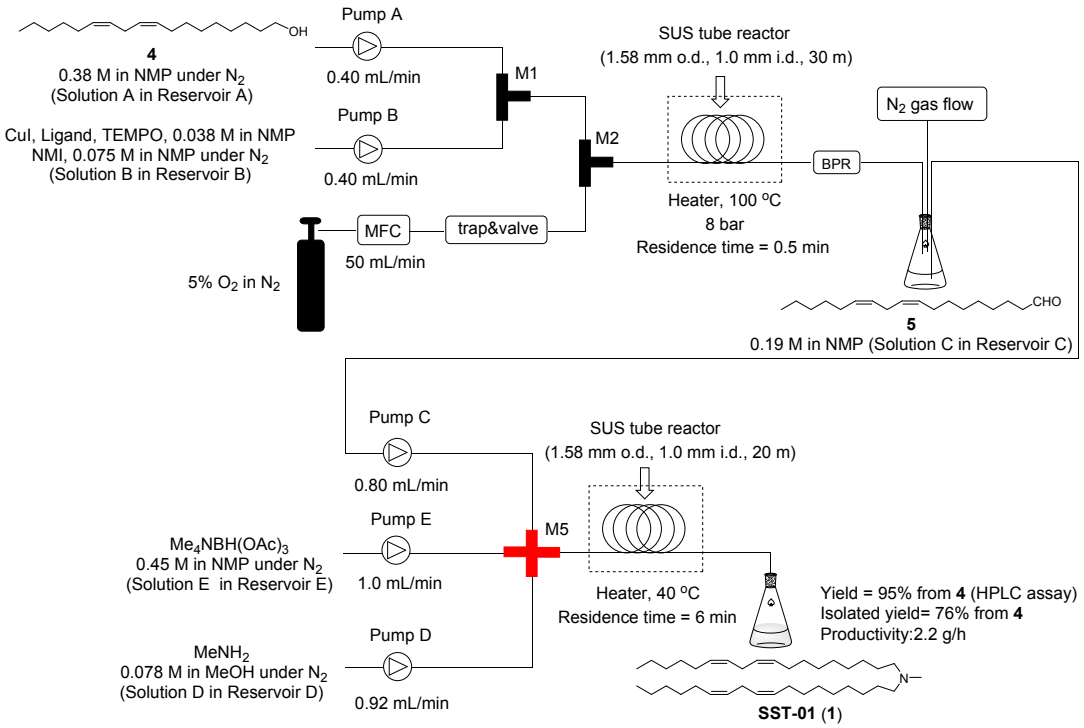
considerable amount of solid (Table 3, entry 1). ii) A methanol solution of methylamine and insoluble solid seed were added to Solution C, resulting in the immediate generation of a considerable amount of solid (entry 2). iii) A methanol solution of methylamine and a NMP solution of $Me_4NBH(OAc)_3$ were added to Solution C at the same time in the presence of the solid seed, resulting no further growth of the solid (entry 3). These careful observations suggested that the solid growth occurred in the intersection of Solutions C and methanol. We assume that methanol generated the solid by working as an anti-solvent. Given the results of entry 3, solution E was considered important for dissolving the solid, and we suspected that acidic sources from $Me_4NBH(OAc)_3$ would be effective for dissolving the solid.

**Table 3.** Investigation of the causes of the generation of the insoluble solid

| Entry | Condition | Insoluble solid |
|---|---|---|
| 1 | Solution C[a] +MeOH + Seed of the insoluble solid | Yes |
| 2 | Solution C[a] +$MeNH_2$ in MeOH + Seed of the insoluble solid | Yes |
| 3 | Solution C[a] +$MeNH_2$ in MeOH + $Me_4NBH(OAc)_3$ in NMP + Seed of the insoluble solid | No |

[a] **5** in NMP (0.19 M), reaction mixture from oxidation step without any purification.

Therefore, we changed the method to mix them all together using a cross-shaped mixer (M5, Figure 5). As a result, no insoluble solid was observed, and continuous operation without clogging became possible. The overall yield of **SST-01** (**1**) from the oxidation step was determined to be 95% (HPLC assay) from samples collected between 4.5 and 5 hours, and the target compound was obtained with an isolated yield of 76%. Productivity of 2.2 g/h was achieved.




The equivalent of reagents for **4**: CuI (0.1 eq.), Ligand (0.1 eq.), TEMPO (0.1 eq.), NMI (0.2 eq.), methylamine (0.48 eq.), $Me_4NBH(OAc)_3$ (3.0 eq.)

**Figure 5.** Modified set-up of the continuous-flow oxidation and reductive amination steps

Finally, the comparison of the batch and flow methods examined in the present report is summarized in Table 4. Compared to the original batch method (entry 1), the final optimized flow method (entry 4) showed a reduction in the raw material cost, shortened reaction time and improved total yield.

**Table 4.** The comparison with the synthetic route and method of **SST-01** (**1**)

| Entry | Synthetic route and method of **SST-01** (**1**) | Cost of Starting Material | Number of Steps | Total Reaction Time (h) | Total Isolated Yield (%) |
|---|---|---|---|---|---|
| 1 | Shown in Scheme 1, Batch method | High | 1 | 3 | 61 |
| 2 | Shown in Scheme 2, Batch method | Low | 2 | 9 | 62 |
| 3 | Shown in Scheme 3 and 4, Batch method | Low | 2 (1) [a] | 6 | 51 |
| 4 | Shown in Figure 5, Flow method | Low | 2 (1) [a] | 0.1 [b] | 76 |

[a]The reaction mixture from the oxidation step without any purification was directly used for the reductive amination step. [b] The residence time of the oxidation step was calculated based on the assumption that the total flow rate was the sum of the flow rate of gas and liquid components.

## CONCLUSION

A two-step continuous-flow process for the cationic lipid **SST-01** (**1**), which is an important component of Kyowa Kirin's siRNA-lipid nanoparticle (LNP), was developed. Safe, scalable catalytic oxidation with 5% oxygen and subsequent homogeneous reductive amination were utilized in this process. To maintain homogeneity during the two-step operation, the solvents, reagents, and mixing order were finely optimized and the target lipid amine **SST-01** (**1**) could be synthesized in 95% yield (HPLC assay, 76% isolated yield) and with 2.2 g/h productivity from inexpensive linoleyl alcohol (**4**).

## EXPERIMENTAL SECTION

**General Information.** All reagents and solvents were procured from commercial sources and used as received. Amino silica gel (CHROMATOREX NH-DM1020) was purchased from Fuji Silysia Chemical Ltd. $^{1}H$ and $^{13}C$ NMR spectra were recorded with JEOL ECA-500 spectrometers in $CDCl_3$ (7.24 and 77.0 ppm). HPLC analyses were performed on a Hitachi Chromaster ® system. HPLC analyses were performed with the following conditions: Agilent ZORBAX SB-CN (5 μm, 4.6 × 150 mm); column temperature, 60°C; flow rate, 1.0 mL/min; photodiode array detection (206 nm), and mobile phase gradient, A/B=60/40 (0-10 min), A/B=0/100 (30 min), A/B=0/100 (45 min), mobile phase A, 2-PrOH/10 mM aq.$NH_4OAc$=15/85; mobile phase B, 2-PrOH/10 mM aq.$NH_4OAc$=85/15. The HPLC yield was calculated by an HPLC assay using biphenyl as an internal standard and with purified standard compounds. The HPLC area % was calculated without reagent and solvent peaks. High-resolution mass spectrometry was carried out with a Synapt G2 HDMS (Waters). As apparatuses for the flow systems, an HPLC pump (SHIMADZU LC 20AD x 2), plunger pump (FLOM, Intelligent Pump Model UI-22-110 x 1, FLOM, Dual Pump KP-22 x 2) were used. Aluminum block heaters SynFlex (EYELA, RCH-1000, BBS-230RB, Aluminum beads) were used for flow reactions. PTFE tubes (1.0 mm i.d., 1.58 mm o.d.) were used to connect the pumps with the SUS tube reactor (1.0 mm i.d., 1.58 mm o.d., 20 m or 30 m). 5 % $O_2$ in $N_2$ gas was regulated by a mass-flow controller (HORIBA STEC, SEC-E40MK3, 10-50mL/min, 10 bar) connected with control unit (HORIBA STEC, CU-2130). An SUS cross (GL Sciences, U-430, Cat. No. 6010-72358) and PCTFE fitting 3 neck joint (GL Sciences, Cat. No. 6010-47210) were used as a mixer. The pressure was regulated by a back pressure regulator (DFC, FC-BPV-500) and monitored at the HPLC pump display.

### Preparation of starting material and reagent solution

***Continuous-flow oxidation***: Linoleyl alcohol (**4**) (20.0 g, 75.1 mmol) was added to a 200 mL volumetric flask and dissolved in NMP (0.375 M). Then, the solution was transferred to a recovery flask (Reservoir A) and purged with $N_2$ (Solution A). CuI (3.57 g, 18.8 mmol), 4,4'-dimethoxy-2, 2'-bipyridine (4.06 g, 18.8 mmol), TEMPO (2.93 g, 18.8 mmol) and NMI (2.99 mL, 37.5 mmol)

were mixed in a 500 mL volumetric flask and dissolved in NMP (Cu concentration: 0.0375 M). Then, the solution was transferred to a recovery flask (Reservoir B) and purged with $N_2$ (Solution B). Solution A (0.400 mL/min) and Solution B (0.400 mL/min) were flowed by HPLC pumps (SHIMADZU LC 20AD x 2) and mixed in a T-shape mixer (M1, GL Sciences, PCTFE fitting 3 neck joint). The resulting stream and 5% $O_2$ diluted by $N_2$ (50 mL/min) were introduced into an SUS tube reactor (1.0 mm i.d., 1.58 mm o.d., 30 m) through a T-shape mixer (M2, GL Sciences, PCTFE fitting 3 neck joint). The tube reactor was heated to 100°C and the pressure of the reactor was adjusted to 8 bar by a back pressure regulator (DFC, FC-BPV-500). The resulting solution (Solution C) was collected in a flask (Reservoir C) purging by $N_2$. To analyze the resulting Solution C, an appropriate volume of Solution C was picked up and analyzed by HPLC.

***Two-step continuous-flow*:** 9.8 M $MeNH_2$ in methanol (4.00 mL, 39.2 mmol) was added to a 500 mL volumetric flask and diluted with methanol to 0.0784 M. Then, the solution was transferred to a recovery flask (Reservoir D) and purged with $N_2$ (Solution D). $Me_4NBH(OAc)_3$ (59.3 g, 225 mmol) was added to a 500 mL volumetric flask and dissolved in NMP (0.450 M). Then, the solution was transferred to a recovery flask (Reservoir E) and purged with $N_2$ (Solution E). Solution C was pumped out from Reservoir C by a plunger pump (FLOM Intelligent Pump, UI-22-110) and combined with streams of Solutions D and E, fed from plunger pumps, in a cross-shaped mixer (M5, GL Sciences, SUS cross). The combined reaction solution was introduced into a SUS tube reactor (1.0 mm i.d., 1.58 mm o.d., 20 m) heated to 40°C. The outlet solution was collected for 35 min (94.9 mL) in a recovery flask and analyzed to determine the yields by an HPLC assay using biphenyl as an internal standard (HPLC yield of **SST-01**=94.5% from **4**). For the isolation of the product, the recovered reaction mixture (94.9 mL) was diluted with heptane (200 mL), washed successively with 1 M aq. NaOH solution (100 mL) and saturated aq. NaCl solution (200 mL), and dried over anhydrous magnesium sulfate. The resultant was concentrated under reduced pressure, and was purified by amino silica gel column chromatography (heptane only) to give **SST-01** (**1**) (1.02 g, isolated yield=75.9 %, HPLC area =93.6%).

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: . Optimization studies for the oxidation and reductive amination in batch; Evaluation of Stability of catalyst solution in flow; Characterization.

## AUTHOR INFORMATION

Corresponding Author

*E-mail: katsuaki.fujiwara.wj@kyowakirin.com

*E-mail: shu_kobayashi@chem.s.u-tokyo.ac.jp.

ORCID

Shū Kobayashi: 0000-0002-8235-4368

Notes

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We thank Koji Hagihara, Masashi Taga for giving us helpful advice on this research subject. We thank for Takayuki Atago for developing the original batch method. We thank Dr. Hideyuki Onodera, Akira Ogawa, and Dr. Takeshi Kuboyama for supporting the editing of this manuscript.

**ABBREVIATIONS**

TEMPO, 2,2,6,6-tetramethylpiperidine 1-oxyl;

NMP, *N*-methylpyrrolidone; NMI, *N*-Methylimidazole

**REFERENCES**

(1) Plutschack, M. B.; Gilmore, K.; Seeberger, P. H. The Hitchhiker's Guide to Flow Chemistry. *Chem. Rev.* **2017**, *117*, 11796–11893.

(2) Porta, R.; Benaglia, M.; Puglisi, A. Flow Chemistry : Recent Developments in the Synthesis of Pharmaceutical Products. *Org. Process Res. Dev.* **2016**, *20*, 2–25.

(3) Souza, R. O. M. A. De; Watts, P. Flow Processing as a Tool for API Production in Developing Economies. *J. Flow Chem.* **2017**, *7*, 146–150.

(4) Baumann, M.; Baxendale, I. R. The Synthesis of Active Pharmaceutical Ingredients (APIs) Using Continuous Flow Chemistry. *Beilstein J. Org. Chem* **2015**, *11*, 1194–1219.

(5) Gutmann, B.; Cantillo, D.; Kappe, C. O. Continuous-Flow Technology — A Tool for the Safe Manufacturing of Active Pharmaceutical Ingredients Angewandte. *Angew.Chem. Int. Ed* **2015**, *54*, 6688–6728.

(6) Lummiss, J. A. M.; Morse, P. D.; Beingessner, R. L.; Jamison, T. F. Towards More Efficient , Greener Syntheses through Flow Chemistry. *Chem. Rec* **2017**, No. 17, 667–680.

(7) Poechlauer, P.; Colberg, J.; Fisher, E.; Jansen, M.; Johnson, M. D.; Koenig, S. G.; Lawler, M.; Laporte, T.; Manley, J.; Martin, B.; et al. Pharmaceutical Roundtable Study Demonstrates the Value of Continuous Manufacturing in the Design of Greener Processes. *Org. Process Res. Dev.* **2013**, *17*, 1472–1478.

(8) Morse, P. D.; Beingessner, R. L.; Jamison, T. F. Enhanced Reaction Efficiency in Continuous Flow. *Isr.J.Chem.* **2017**, *57*, 218–227.

(9) Adams, D.; Gonzalez-Duarte, A.; O'Riordan, W. D.; Yang, C. C.; Ueda, M.; Kristen, A. V.; Tournev, I.; Schmidt, H. H.; Coelho, T.; Berk, J. L.; et al. Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. *N. Engl. J. Med.* **2018**, *379* (1), 11–21

(10) Kuboyama, T.; Yagi, K.; Naoi, T.; Era, T.; Yagi, N.; Nakasato, Y.; Yabuuchi, H.; Takahashi, S.; Shinohara, F.; Iwai, H.; et al. Simplifying the Chemical Structure of Cationic Lipids for SiRNA-Lipid Nanoparticles. *ACS Med. Chem. Lett.* **2019**, *10*, 749–753.

(11) Sasayama, Y.; Hasegawa, M.; Taguchi, E.; Kubota, K.; Kuboyama, T. In Vivo Activation of PEGylated Long Circulating Lipid Nanoparticle to Achieve Efficient SiRNA Delivery and Target Gene Knock down in Solid Tumors. *J. Control. Release* **2019**, *311–312*, 245–256.

(12) Atago, T. Method for producing aliphatic tertiary amine compound. Patent WO2018074568A1, 2018.

(13) Gemoets, H. P. L.; Su, Y.; Shang, M.; Hessel, V.; Luque, R.; Noël, T. Liquid Phase Oxidation Chemistry in Continuous-Flow Microreactors. *Chem. Soc. Rev.* **2016**, *45* (1), 83–117.

(14) Sharma, Y.; Moolya, S.; Joshi, R. A.; Kulkarni, A. A. Continuous Flow Telescopic Oxidation of Alcohols: Via Generation of Chlorine and Hypochlorite. *React. Chem. Eng.* **2017**, *2* (3), 304–308.

(15) Baxendale, I. R.; Deeley, J.; Griffiths-Jones, C. M.; Ley, S. V.; Saaby, S.; Tranmer, G. K. A Flow Process for the Multi-Step Synthesis of the Alkaloid Natural Product Oxomaritidine: A New Paradigm for Molecular Assembly. *Chem. Commun.* **2006**, No. 24, 2566–2568.

(16) Van Der Linden, J. J. M.; Hilberink, P. W.; Kronenburg, C. M. P.; Kemperman, G. J. Investigation of the Moffatt-Swern Oxidation in a Continuous Flow Microreactor System. *Org. Process Res. Dev*. **2008**, *12* (5), 911–920.

(17) Kobayashi, S.; Miyamura, H.; Akiyama, R.; Ishida, T. Highly Active, Immobilized Ruthenium Catalysts for Oxidation of Alcohols to Aldehydes and Ketones. Preparation and Use in Both Batch and Flow Systems. *J. Am. Chem. Soc.* **2005**, *127* (25), 9251–9254.

(18) Hoover, J. M.; Stahl, S. S. Highly Practical Copper (I)/ TEMPO Catalyst System for Chemoselective Aerobic Oxidation of Primary Alcohols. *J. Am. Chem. Soc.* **2011**, *133*, 16901–16910.

(19) Greene, J. F.; Hoover, J. M.; Mannel, D. S.; Root, T. W.; Stahl, S. S.; Mizuno, N.; Yamaguchi, K. Continuous-Flow Aerobic Oxidation of Primary Alcohols with a Copper(I)/TEMPO Catalyst. *Org. Process Res. Dev.* **2013**, *17*, 1247–1251.

(20) Steves, J. E.; Preger, Y.; Martinelli, J. R.; Welch, C. J.; Root, T. W.; Hawkins, J. M.; Stahl, S. S. Process Development of CuI/ABNO/NMI-Catalyzed Aerobic Alcohol Oxidation. *Org. Process Res. Dev*. **2015**, *19* (11), 1548–1553.

(21) Steves, J. E.; Stahl, S. S. Copper(I)/ABNO-Catalyzed Aerobic Alcohol Oxidation: Alleviating Steric and Electronic Constraints of Cu/TEMPO Catalyst Systems. *J. Am. Chem. Soc*. **2013**, *135* (42), 15742–15745.

(22) Osterberg, P. M.; Niemeier, K.; Welch, C. J.; Hawkins, J. M.; Martinelli, J. R.; Johnson, T. E.; Root, T. W.; Stahl, S. S. Experimental Limiting Oxygen Concentrations for Nine Organic Solvents at Temperatures and Pressures Relevant to Aerobic Oxidations in the Pharmaceutical Industry. *Org. Process Res. Dev.* **2015**, *19*, 1537–1543.