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Continuous flow macrocyclization at high concentrations: synthesis of macrocyclic lipids†

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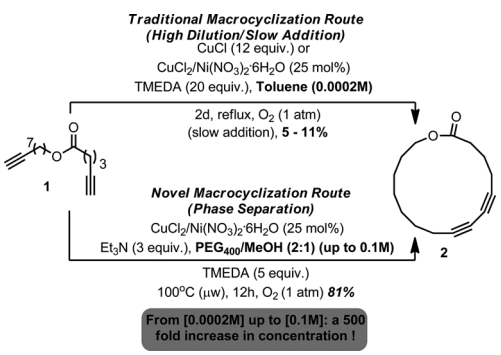
A phase separation/continuous flow macrocyclization protocol eliminates the need for high-dilution conditions and can be used to prepare gram quantities of biologically relevant macrocyclic lipid structures. The method presents several green advantages towards macrocycle synthesis: (1) the prevention of unwanted oligomers and waste, (2) a reduction in the large quantities of toxic, volatile organic solvents and (3) the use of PEG as an environmentally benign reaction media. Macrocycles could be synthesized in high yields (up to 99%) in short reaction times (1.5 h) and on gram scales without the need to alter the reaction conditions.

Continuous flow strategies for green chemical synthesis have impacted every area of organic synthesis, from academic to industrial research.[1] Flow chemistry represents a powerful technology whose advantages include precise control of reaction time, temperature, concentration and stoichiometry. Consequently, many of the principles of green chemistry are fulfilled such as reduced energy requirements, minimized exposure to hazardous chemicals or intermediates and reduced amounts of unwanted by-products and waste. Given these advantages, flow chemistry is an ideal technique to use in tackling the challenges associated with macrocycle synthesis. Macrocycles are important structural motifs found in compounds with applications in pharmaceutical, agrochemical, cosmetic and material sciences.[2] Their synthesis is challenging, often requiring significant amounts of solvent to control concentration effects and prevent the formation of undesirable oligomers. When the dilution requirements and inconvenient set-up are considered, macrocyclization reactions in general would benefit from improved protocols employing flow techniques.

Macrocyclization *via* continuous flow presents several challenges, including: (1) the need to accelerate the normally slow cyclization reactions to improve their efficiency and most importantly, (2) the need to prevent oligomerization, as these by-products have low solubilities and could block the flow reactor. Recently, James and co-workers have reported the first flow-macrocyclization reaction that involved the synthesis of constrained macrocycles *via* alkyne–azide cycloaddition chemistry.[3] The reaction rates of the macrocyclizations reported were accelerated through judicious choice of ligands and the exploitation of copper tubing as the flow reactor. The efficient heat transfer that can be achieved in flow would seem to provide a solution to the challenge of macrocyclization efficiency, however the challenge of reaction dilution is often more daunting.[4]

Consequently, our group has recently reported a general, efficient and green macrocyclization protocol *via* oxidative Glaser–Hay coupling at high concentrations through the use of a "phase separation" strategy (Scheme 1).[5] The concentration effects of the macrocyclization reaction were controlled through the aggregation properties of poly(ethylene)glycol$_{400}$ (PEG$_{400}$). The "phase separation" technique allowed for significantly higher concentrations (150–500×), a reduction of the amounts of toxic organic solvents that are eventually converted to waste, and replacement of the majority of the volatile organic solvents by the environmentally benign PEG. Given the advantages presented by the "phase separation" strategy, we sought to apply it to the development of a continuous flow process to synthesize important medicinally relevant classes of



Scheme 1 Advantages of the "phase separation" strategy in macrocyclization.

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macrocycles. Consequently, herein we report on the development of efficient continuous flow macrocyclization for the preparation of macrocyclic lipids that mimic those found in Archaeal membranes.[6]

The investigations into a continuous flow/macrocyclization protocol began by transposing the previously optimized reaction conditions for the Glaser–Hay coupling utilizing microwave heating.[7] The preliminary studies utilized the formation of macrolactone **4**, as it provides a valid comparison for the continuous flow protocol *versus* other existing macrocyclization strategies (Table 1). TMEDA was chosen as the ligand as its bidentate nature provides increased solubility and stability of the resulting transition metal complexes at high temperatures.[5] Intermolecular Glaser–Hay couplings have been previously studied in continuous flow settings, where semi-permeable Teflon AF-2400 membranes[8] were used to increase contact with oxygen gas. In the present study, the solutions for macrocyclization were only sparged with O_2 for 5 min before injection into the flow apparatus. First, we investigated the effect of the temperature on the yields of the desired 21-membered macrolactone **4** (Table 1, entries 1→4). When the reaction was performed at the same temperature as in the microwave heated reaction (120 °C), **4** was formed in higher yield using a flow strategy (85% *vs.* 81%, entry 3). Lower temperatures resulted in lower conversions and isolated yields. Increasing the temperature (140 °C) resulted in a decrease of selectivity (macrocyclization *vs.* oligomerization) as the yield of **4** decreased (72%) and oligomers could be observed. Changing the ratio of PEG_{400}/MeOH (Table 1, entries 4→6) did not result in significant changes in isolated yield, although larger ratios of PEG result in better solubility of the organic substrates. Higher ratios of PEG_{400}/MeOH were not explored as previous work has indicated that catalyst inhibition becomes problematic at higher ratios.[5]

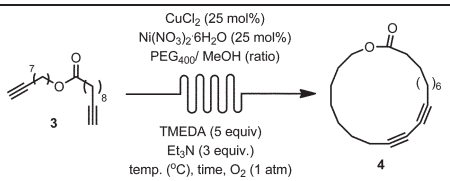
The influence of reaction time and flow/rate were also investigated (Table 1, entries 7→9). Due to the viscosity of PEG_{400}, high flow rates were found to cause irregularities in pressure control. It was found that by using lower flow rates, pressure control was easily maintained and helped to provide more reproducible results. Gratifyingly, it was found that flow rates of 0.22 mL min^{-1} and a residence time of 1.5 h afforded a nearly quantitative yield (97%) of the macrocyclic product **4**. The high yield of macrocycle **4** achieved at relatively high concentration ([0.03 M]) denotes the high efficiencies that are possible when combining the two green chemistry techniques of phase separation and continuous flow synthesis. The calculated E_{mac} (a recently proposed meter for grading the efficiency of macrocyclizations)[9] of ~7.4 for the macrocyclization (**3**→**4**) is high and similar to those obtained in James' previously reported continuous flow synthesis of constrained macrocycles *via* alkyne–azide cycloadditions.

In an effort to evaluate the generality of the optimized continuous flow conditions, the macrocyclization of three other diynes was performed (Table 2, entries 2→4). In each case, the results of the cyclization using the continuous flow conditions were compared with the isolated yields obtained utilizing the previously developed microwave heating protocol. First, the isolated yield of the 21-membered macrolactone **4** obtained *via* continuous flow (97%) was found to be much better than that obtained *via* microwave heating (81%). Given the excellent yield obtained for **4**, the macrocyclization to afford **4** was scaled to 1 mmol and a 93% yield was obtained.

The macrocyclization of other diynes was then investigated. When the cyclization to form the 20-membered macrocyclic diester **5** was investigated, a 72% yield of the product was obtained utilizing continuous flow. A much lower yield (47%) of **5** was obtained using the microwave heating protocol. The preparation of macrocyclic ether **6** was initially problematic, as its corresponding diyne precursor was partially insoluble in the PEG_{400}/MeOH (1 : 1) mixture. However, as previous experiments demonstrated very little difference in yield between PEG_{400}/MeOH (2 : 1) and (1 : 1) mixtures, the cyclization to form **6** was conducted using the higher ratio of PEG_{400}/MeOH (2 : 1). The macrocycle **6** was once again obtained in higher yields (71%) when using the continuous flow strategy. However, it was noted that when the synthesis of a smaller and significantly more strained macrocycle **2** was investigated, the trend reversed. The 16-membered macrocycle **2** was obtained in higher yield using microwave heating (75%) *versus* the continuous flow (58%).

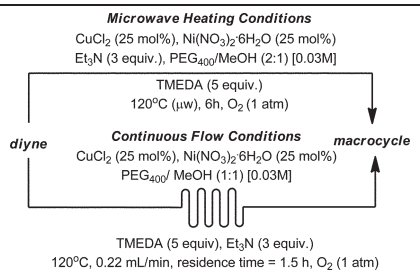
Encouraged by the preliminary optimization of a continuous flow-macrocyclization protocol employing a "phase separation" strategy, the optimal conditions were also explored with a macrocyclic lipid precursor **7** which bears a protected glycerol motif (Table 3). The macrocyclic Archaeal lipids are typically composed of polyprenyl chains with glycerol or polyol head groups.[6] Within the structure of the lipids, macrocyclization can occur through the alkyl chains of the same head group or between two lipids, producing dimers. As such, 36- or 72-membered rings can be formed.[10] Recently, much attention has been given to macrocyclic lipids for potential anti-cancer activities or in the design of novel vehicles for liposomal drug delivery.[11] When the benzyl-protected glycerol derivative **7** was

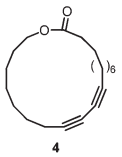
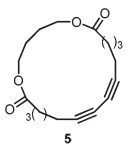
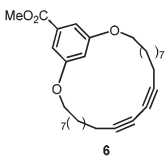
Table 1 Optimization of reaction conditions for macrocyclic Glaser–Hay coupling of **3**



Entry	Temp. (°C)	PEG_{400}/ MeOH (ratio)	Flow rate (mL min^{-1}); residence time (h)	Yield (%)
1	80	2 : 1	5; 3	74
2	100	2 : 1	5; 3	73
3	120	2 : 1	2225; 3	85
4	140	2 : 1	5; 3	72
5	120	1 : 1	5; 3	86
6	120	1 : 2	5; 3	82
7	120	1 : 1	5; 1.5	96
8	120	1 : 1	1; 1.5	91
9	120	1 : 1	0.22; 1.5	97

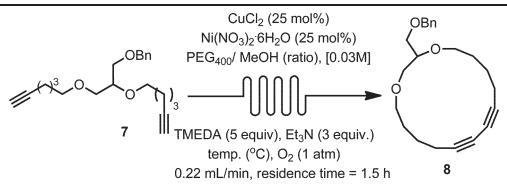
Table 2 Macrocyclic lactones and ethers synthesized *via* continuous flow-macrocyclization using "phase separation"



Entry	Product	Yield: flow (%)	Yield: microwave (%)
1	 **4**	97[a]	81
2	 **2**	58	75
3	 **5**	72	47
4	 **6**	71[b]	65

[a] On a 1 mmol scale, **4** was isolated in 93% yield. [b] The corresponding diyne precursor of **7** was insoluble in PEG_{400}/MeOH mixtures. As such, a PEG_{400}/MEOH (2 : 1) ratio was used.

Table 3 Optimization of reactions conditions for the synthesis of a protected macrocyclic lipid **8**



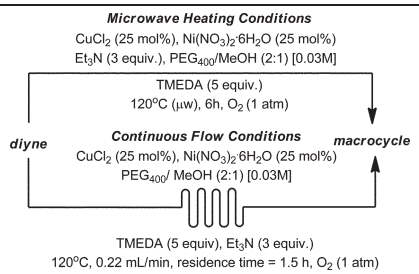
Entry	Temp. (°C)	PEG_{400}/MeOH (ratio)	Yield (%)
1	120	1 : 1	—[a]
2	120	2 : 1	71
3	100	2 : 1	66
4	140	2 : 1	63

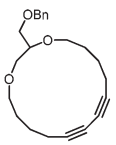
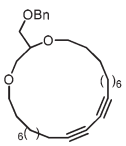
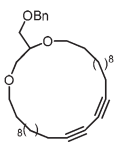
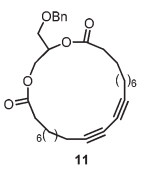
[a] Diyne **7** precipitates from the solution when a PEG_{400}/MeOH (1 : 1) ratio is used.

subjected to the optimized conditions from Table 2, it was found that **7** was poorly soluble in the 1 : 1 PEG_{400}/MeOH solvent mixture (Table 3, entry 1). However, when the ratio of PEG_{400}/MeOH was increased (2 : 1), the 16-membered macrocyclic lipid **9** was isolated in 71% yield. Variations in the reaction temperature followed the same trends that were observed for the synthesis of macrolactone **4** (Table 3). Lower temperatures resulted in lower conversions and isolated yields, although excellent selectivity was observed and the remaining mass balance was recovered starting diyne **7** (Table 3, entry 3). Increasing the reaction temperature once again lowered selectivity, resulting in the formation of oligomers and decreased isolated yields (63%).

With optimized continuous flow conditions in hand for the synthesis of macrocyclic lipids, the macrocyclization of 3 other substrates were performed (Table 4). The isolated yield of the 16-membered macrolipid **8** was found to be slightly higher

Table 4 Macrocyclic lipids synthesized *via* continuous flow-macrocyclization using "phase separation"



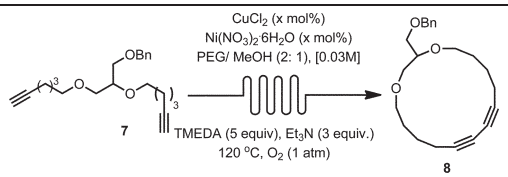
Entry	Product	Yield: flow (%)	Yield: microwave (%)
1	 **8**	71[a]	65
2	 **9**	78	62
3	 **10**	99	92
4	 **11**	45	42

[a] When cyclization was performed on 3.8 mmol scale, the macrocycle was obtained in 66% yield.

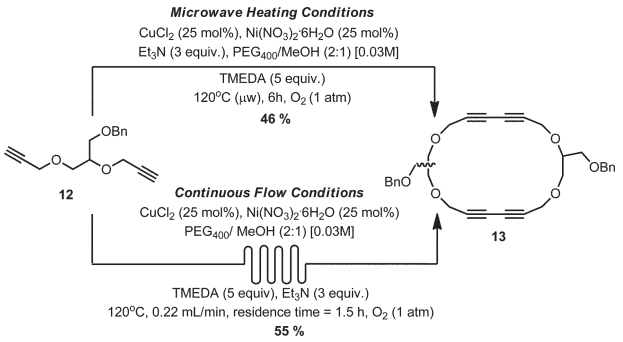
under continuous flow conditions (71%) compared to cyclization under microwave heating (65%) (Table 4, entry 1). As one of the advantages of continuous flow methods is the facile scale-up, the synthesis of **8** was repeated on ~4 mmol scale. The yield of the 16-membered macrolipid **8** did not change significantly (66% isolated yield on ~4 mmol scale). Similar macrolipids based upon a glycerol head group were then prepared having different ring sizes. When the 26-membered macrocycle **9** was prepared *via* continuous flow, the isolated yield of 78% was again higher than what was obtained *via* microwave heating (62%). Similarly, the 30-membered macrocycle **10** was isolated in 99% yield after synthesis *via* continuous flow (92% isolated yield was obtained using microwave heating). As many macrocyclic lipids can be prepared with ester linkages, the macrocycle **11** was prepared by continuous flow synthesis and was isolated in 45% yield. In all of the macrocyclizations, the products were obtained in higher yield when using continuous flow *versus* microwave heating. In general, the yields of the larger 21-, 26- and 30-membered macrocyclic lipids were higher than smaller macrocycles, perhaps due to the ring strain caused by the incorporation of a linear 1,3-diyne within the cyclic motif.

In an effort to further improve the continuous flow process, the use of other PEG solvents were investigated with the goal of reducing the catalyst loadings (Table 5).[5] When the diyne **7** had been previously cyclised under optimum conditions using 25 mol% of catalysts and PEG_{400} as a co-solvent, the yield of the corresponding lipid macrocycle **8** was 71%. When the solvent PPG_{425} was used as a substitute for PEG_{400} at identical catalyst loadings a similar yield was observed (Table 5, entry 2). However, when the catalyst loading was dropped to 10 mol%, continuous flow conditions could be developed so that the desired macrocycle **8** could be isolated in 92% yield. These preliminary results demonstrate that the catalyst loadings could be lowered and the yields increased through judicious choice of the PEG co-solvent in the continuous flow conditions. Further reductions in the catalyst loading to 5 mol% also provided good yields of macrocycle **8** (78%) and excellent selectivity for macrocyclization was observed, as the remaining mass balance was recovered diyne **7**. Longer reaction times may be necessary for effective use of reduced catalyst loadings.

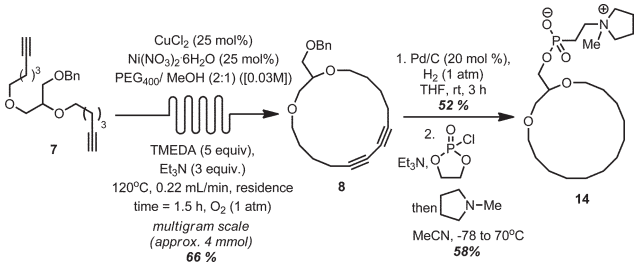
Table 5 Reduction of catalyst loadings when using PPG_{425} for the synthesis of a protected macrocyclic lipid **8**



Entry	Mol%	PEG	Flow rate (mL min^{-1}); residence time (h)	Yield (%)
1	25	PEG_{400}	0.22; 1.5	71
2	25	PPG_{425}	0.22; 1.5	65
3	10	PPG_{425}	0.11; 3	92
4	5	PPG_{425}	0.11; 3	78



Scheme 2 Synthesis of a dimeric macrocyclic lipid **13** as a mixture of head-to-tail and head-to-head isomers.



Scheme 3 Conversion of Bn-protected macrocycle **8** into novel macrocyclic phosphonate containing lipid **14**.

As dimeric macrocyclic lipids are found in Nature,[6] the macrocyclization of the diyne **12** was investigated under the optimized reaction conditions (Scheme 2). Acyclic diyne **12** cannot undergo intramolecular cyclization due to ring strain but selectively forms the dimer **13** in 55% yield *via* continuous flow (46% using microwave heating).

To demonstrate the utility of the novel macrocycles prepared in Table 5 as lipids, the synthesis of the 16-membered macrocyclic diyne **8** was performed on a multigram scale (~4 mmol) and similar yields were obtained even when the reaction was scaled by a factor of 30× (Scheme 3). Upon isolation, macrocyclic lipid **8** was functionalized with a polar phosphonate group. A global hydrogenation (Pd/C, H_2 (1 atm)) of macrocycle **8** results in cleavage of the Bn protecting group and exhaustive hydrogenation of the 1,3-diyne to afford the corresponding saturated macrocycle in 52% yield. Subsequent phosphonation provided the saturated macrocyclic lipid **14** in 58% yield.

Conclusions

The above studies demonstrate that a continuous flow-macrocyclization protocol utilizing "phase separation" eliminates the need for extremely high-dilution conditions and can be used to prepare meaningful quantities of biologically relevant macrocyclic lipid structures. The combination of the "phase

separation" strategy with a continuous flow synthesis presents several green advantages towards macrocycle synthesis: (1) the precise control of reaction time and temperature prevent the formation of unwanted oligomers and waste, (2) the high concentrations reduce the large quantities of solvents that would have to be disposed of in environmentally damaging processes and (3) the use of PEG as a solvent represents a non-volatile and non-toxic alternative to traditional organic solvents. Macrocycles could be synthesized in high yields (up to 99%), in short reaction times (1.5 h) and on large scales without the need to alter the reactions conditions. Preliminary results demonstrate that reduced catalyst loadings may be possible when using modified PEG co-solvents. The demonstration of the viability of the phase separation/continuous flow synthetic strategy should find applicability in the synthesis of other macrocycles in academia and industry.

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