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Study of conditions for streamlined assembly of a model bacteriochlorophyll from two dihydrodipyrrin halves

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Knoevenagel condensation followed by double-ring closure (Nazarov cyclization, electrophilic aromatic substitution, and elimination of methanol) and optional zinc insertion afford bacteriochlorophyll model compounds.

Abstract

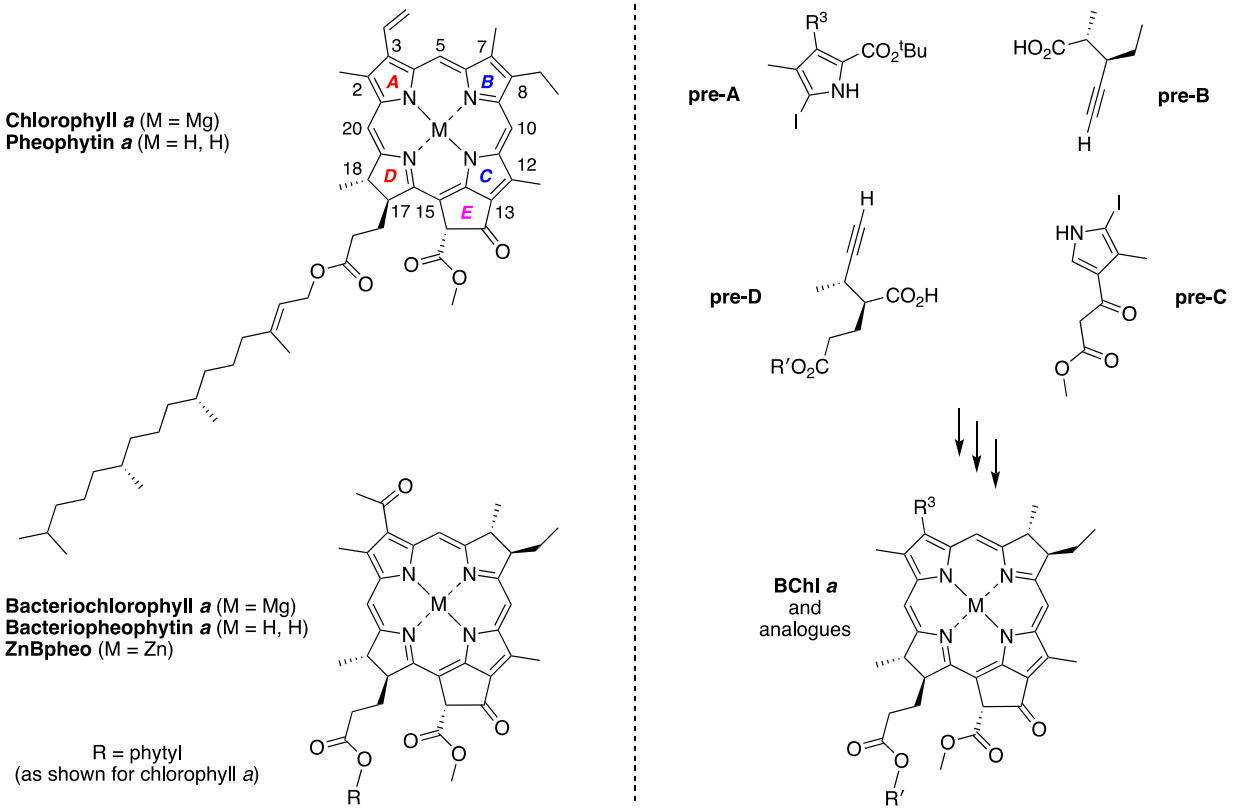
A long-term goal is to gain synthetic access to native photosynthetic bacteriochlorophylls. A recently developed route entails Knoevenagel condensation of an AD dihydrodipyrrin (**I**, bearing a carboxaldehyde attached to pyrroline ring D) and a BC dihydrodipyrrin (**II**, bearing a β-ketoester attached to pyrrole ring C) to form the Z/E-enone. Acid-mediated double-ring closure of the E-enone **III-*E*** (Nazarov cyclization, electrophilic aromatic substitution, and elimination of methanol) affords the bacteriochlorophyll skeleton **BC-1** containing the isocyclic ring (E), a *trans*-dialkyl group in ring D, and a gem-dimethyl group in ring B. Prior work established the synthesis and the integrity of the resulting *trans*-dialkyl groups and bacteriochlorin chromophore. The counterpart report here concerns an in-depth study of conditions for the double-ring closure: catalyst/solvent surveys; grid search including time courses of **III-*E*** versus acid concentrations emphasizing *equimolar*, *inverse molar*, and *variable acid* lines of inquiry; and chlorin byproduct quantitation. Key findings are that (1) the double-ring closure can be carried out in 4 h ($t_{1/2}$ ~40 min) instead of 20 h, affording ~1/5th the chlorin byproduct (0.16%) while maintaining the yield of **BC-1** (up to 77%); (2) the Z/E-enones of **III** have comparable reactivity; (3) sub-stoichiometric quantities of acid are ineffective; (4) the Knoevenagel condensation (40 mM, room temperature, piperidine/acetic acid in acetonitrile) and the acid-mediated double-ring closure (0.20 mM, 80 °C, $Yb(OTf)_3$ in acetonitrile) can be carried out in a two-step process; and (5) zinc insertion to form **ZnBC-1** is straightforward. Together, the results enable streamlined conversion of dihydrodipyrrin reactants to the bacteriochlorophyll model compounds.

Introduction

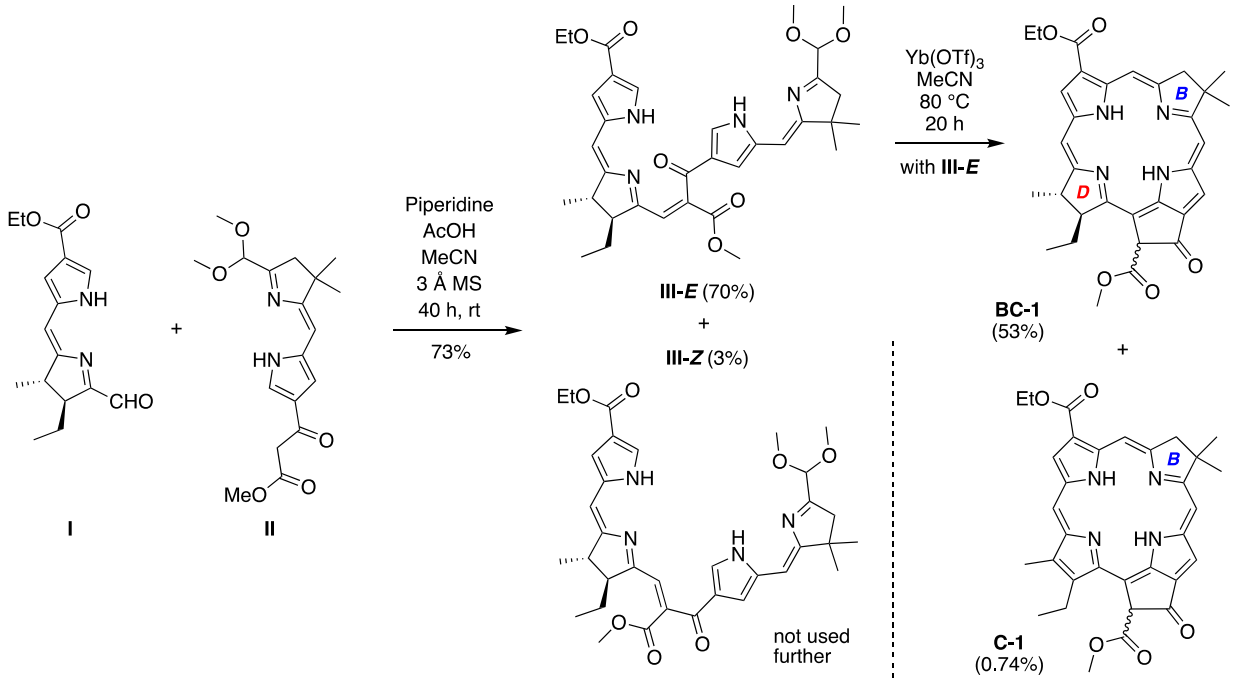
Chlorophyll *a* and bacteriochlorophyll *a* are the predominant members of the native photosynthetic tetrapyrrole macrocycles (Scheme 1, left).[1] *De novo* synthetic efforts toward (bacterio)chlorophylls apparently have been little pursued.[2] A key challenge is to install the *trans*-dialkyl substituents in each pyrroline ring: rings B and D of bacteriochlorophylls, ring D of chlorophylls. We recently outlined a conceptual path toward the native macrocycles that installs the stereodefined substituents at a very early stage of the synthesis (Scheme 1, right), and have begun developing the requisite methodology to realize the path.[3] The advantage of an early installation of stereodefined substituents is the ability to rely on well-established methodology for asymmetric synthesis. A companion challenge is to carry the stereodefined, *trans*-dialkyl group(s) through multiple transformations without epimerization or dehydrogenation. Epimerization would obviously cause loss of stereochemistry, which while unattractive, might be suffered through depending on the scientific objective. Dehydrogenation, on the other hand, would cause intolerable loss of the target chromophore, providing a porphyrin or chlorin instead of a bacteriochlorin, or a porphyrin instead of a chlorin.[3]

The feasibility of the strategy toward native bacteriochlorophylls was explored very recently by synthesis of a model bacteriochlorophyll compound. The model bacteriochlorophyll is a free base ligand and contains *trans*-dialkyl substituents in ring D but a gem-dimethyl group in ring B (Scheme 2).[3] The inclusion of stereodefined substituents in a single pyrroline ring enabled sharp focus on precursor stability and stereochemical outcome. The synthesis relies on joining two dihydrodipyrrins, an AD half and a BC half. The AD half (**I**) bears a carboxaldehyde whereas the BC half (**II**) bears β-ketoester and 1,1-dimethoxymethyl substituents. Knoevenagel condensation of the respective halves yields Z and E-enones, which were separated by chromatography. The more abundant, E-enone (**III-*E***), was carried forward in a process that

entails Nazarov cyclization (forming the isocyclic ring, ring E), electrophilic aromatic substitution (S_EAr, forming the macrocycle), and elimination of methanol (giving the aromatic system). Examination of the crude reaction mixture by fluorescence spectroscopy prior to purification showed the presence of a small amount (0.74%) of chlorin byproduct (putative **C-1**, by dehydrogenation of ring D) accompanying bacteriochlorin **BC-1** (53% yield) as assessed by independent synthesis of chlorin **C-1**.[3] Formation of chlorins while handling bacteriochlorins is a well-known phenomenon that was first identified more than 50 years ago.[4] The product **BC-1** (devoid of chlorin impurity) was isolated as a 7:1 mixture of epimers owing to the configuration at the 13^2-position (site of attachment of the methoxycarbonyl group). The presence of such diastereomers is well established for chlorophylls but much less so for bacteriochlorophylls.[5-9] The dominant epimer has the *trans-trans* configuration proceeding along the 17-18-13^2 positions.



Scheme 1. Native hydroporphyrins (left) and proposed route to bacteriochlorophyll *a* and analogues (right).



Scheme 2. Synthesis of model bacteriochlorophyll **BC-1**.[3]

In the synthesis of **BC-1**, the Knoevenagel reaction was carried out with 38 mM and 48 mM of **I** and **II**, respectively, for 40 h at room temperature, whereas the double-ring closure of ***III-E*** to give **BC-1** was carried out in 200-fold more dilute solution (0.19 mM) at 80 °C for 20 h. The conditions resemble those employed for the synthesis of bacteriochlorophyll model compounds wherein both pyrroline rings contain a gem-dimethyl group.[10] While the quantity of chlorin **C-1** was relatively tiny (1:71 relative to **BC-1**), given the desire to use bacteriochlorophyll model compounds in diverse photochemical studies where spectroscopic purity is at a premium, reaction conditions that further diminish the quantity of chlorin byproduct are desired. Short reaction durations (including exposure to elevated temperature and various reagents) and limited handling are generally regarded as desirable to maintain integrity of the bacteriochlorin chromophore. The vulnerable site on the macrocycle to adventitious dehydrogenation is the *trans*-dialkyl group (in ring D), whereas the gem-dimethyl group (in ring B) lacks such susceptibility;[11] indeed, **BC-1** was

designed as a test case where any occurrence of dehydrogenation was localized to a single site to enable scrutiny and quantitation as needed for evaluation of the synthetic route.

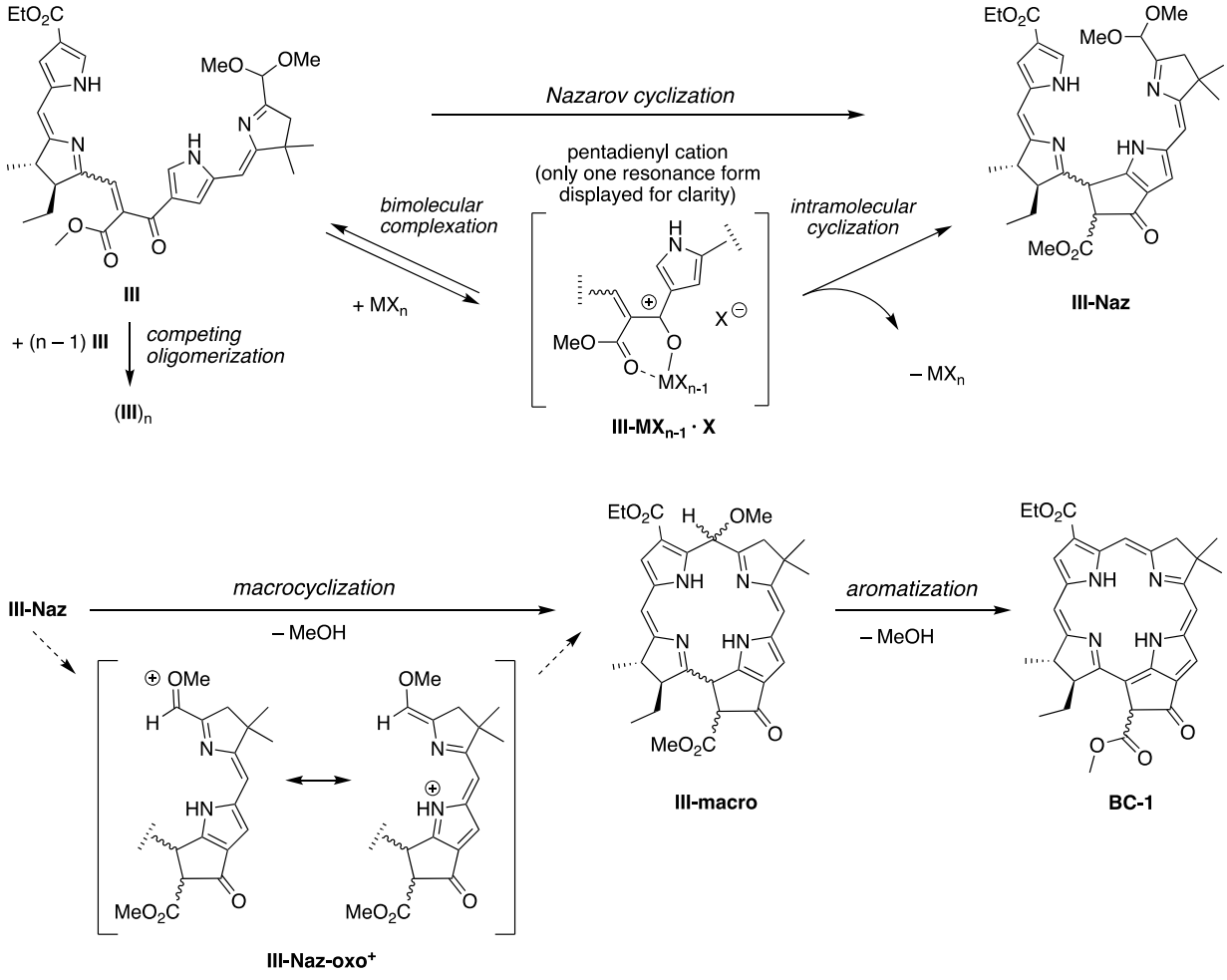
The prior work focused on the fidelity of the formation of the *trans*-dialkyl-substituted dihydrodipyrrin (**I**) and conversion to the corresponding bacteriochlorin chromophore with intact stereochemical integrity of the ring-D substituents. The counterpart studies reported herein concern an in-depth examination of reaction conditions. In particular, we sought to address a handful of questions: (1) Are there suitable catalysis conditions for the double-ring closure that enable reactions at lower temperature and/or shorter reaction time while affording a cleaner product (e.g., diminished quantity of chlorin byproduct)? (2) Does the small amount of dehydrogenation observed (giving the chlorin in the bacteriochlorin crude product) originate at some point along the path to **I**, where detection is difficult, or in the double-ring closure of **III**? (3) For preparations at increased scale, are there conditions that enable use of concentrations of the enone **III** that are >0.19 mM? (4) Do the E and Z enones react similarly, thereby obviating chromatographic separation? (5) Can the conversion of **I** and **II** to form the bacteriochlorin – presently relying on purification of the intermediate enone isomers – be conducted in a sequential process without purification? (6) Does formation of the zinc chelate alter the ratio of epimers that arise from the configuration of the 13^2-carbomethoxy group? In this paper, we report studies that address the aforementioned questions. The results enable a streamlined conversion of **I** and **II** to form **BC-1** in shorter time and greater purity, support metalation to give the zinc chelate thereof, **ZnBC-1**, and should establish a foundation for syntheses of the native macrocycles.

Results and Discussion

I. Reconnaissance

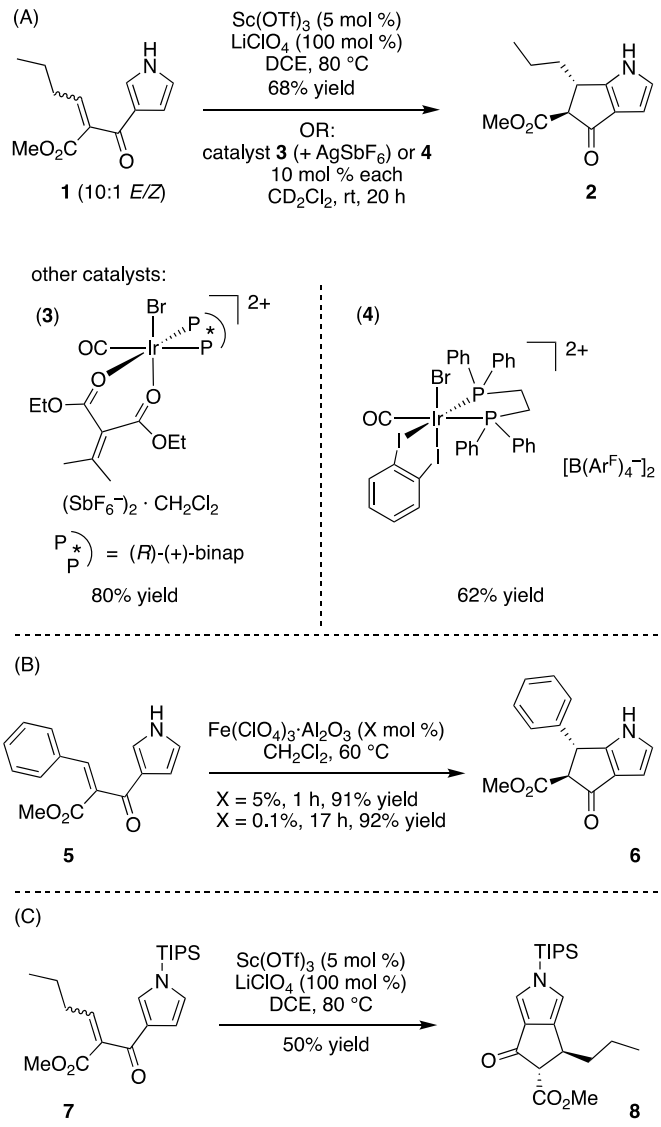
What reaction conditions should be used for the conversion of the enone **III** to form the bacteriochlorin? The conversion of enone **III-*E*** to bacteriochlorin **BC-1** entails S_EAr to form the

macrocycle, Nazarov cyclization to form the isocyclic ring, and elimination of methanol to create the aromatic π system. The acid catalysis conditions chosen for this process must suffice for all three reactions; poor catalysis in any one of the three reactions would crimp the yield of the bacteriochlorin product (**BC-1**). The order of the three reactions is not known, but if methanol elimination is the third step, as would seem likely, then the only unknown is the order of the Nazarov cyclization and S_EAr process. The three steps are shown in Scheme 3, with the Nazarov cyclization preceding the S_EAr process. In this section, the reactions are discussed to highlight considerations that impinge on the choice of catalysts and conditions for the overall transformation.



Scheme 3. Possible steps in the double-ring closure of **III** to give **BC-1**.

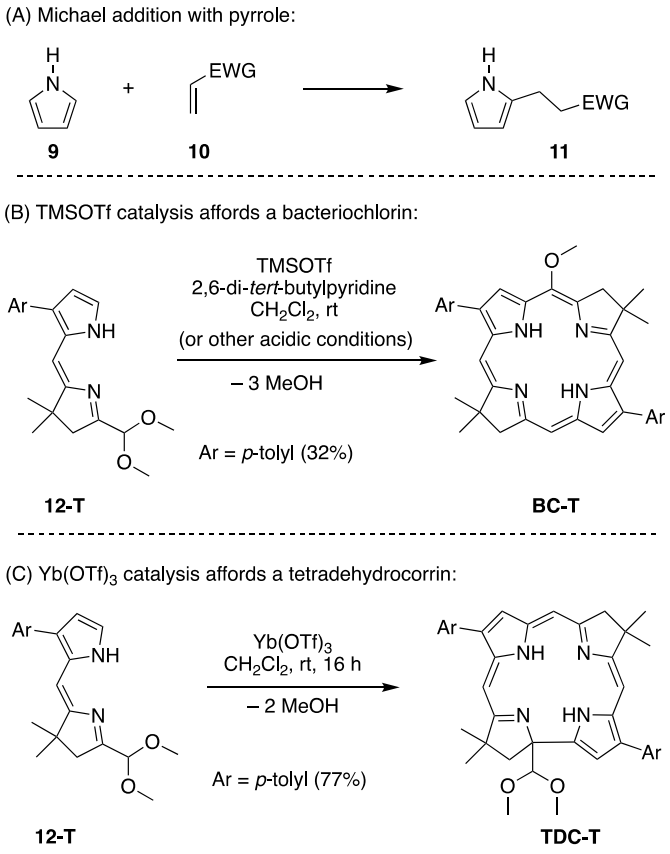
The Nazarov cyclization finds growing use in heterocyclic chemistry[12-14] including with pyrroles, but only rarely with pyrroles is the propenoyl group at the 3-position[3,10,15-18] (versus the more popular 2-position). The Nazarov cyclization proceeds via formation of a pentadienyl cation, which is achieved by complexation of the β-ketoester with a Lewis acid, typically a rare earth. The reaction conditions generally employ a high concentration (e.g., 0.1–0.3 M) of the substrate and a catalytic quantity of acid.[15,17,19] The known examples with simple pyrroles are shown in Scheme 4. First, Frontier and coworkers carried out the Nazarov cyclization of **1** (0.3 M) in the presence of $Sc(OTf)_3$ (15 mM) to obtain the Nazarov product **2** in 68% yield (panel A).[15] The reaction of **1** (0.13 M) also was carried out in CD_2Cl_2 at room temperature in the presence of iridium catalyst **3** (13 mM) and $AgSbF_6$ (13 mM) for 20 h to afford **2** in 80% isolated yield.[17] Identical reaction in the presence of iridium catalyst **4** alone afforded **2** in 62% yield. Second, Itoh and coworkers carried out the reaction of **5** (0.5 M) with the catalyst $Fe(ClO_4)_3 \cdot Al_2O_3$ in quantities of 5 or 0.1 mol% (25 or 0.5 mM) and obtained **6** in isolated yields of 91 or 92%, respectively (panel B).[16] And third, a pyrrole bearing a sterically encumbered substituent at the nitrogen atom (**7**) underwent Nazarov cyclization to give the 3,4-annulated product **8** (panel C),[15] which illustrates that reaction can occur at both the 2- and 4-positions of a pyrrole.



Scheme 4. Nazarov reactions with 3-substituted pyrroles.

Here, high concentrations of **III** are not feasible due to limited solubility as well as possible competing intermolecular reactions. The general phenomenon of competition between cyclization (intramolecular) and addition (intermolecular) is well known.[20] There are at least two plausible types of intermolecular reactions with **III** under acid-catalysis conditions to yield oligomers denoted by $\mathbf{(III)_n}$ in Scheme 3: acetal + pyrrole condensation, and enone + pyrrole (Michael) addition. (1) The reaction of the pyrrole of one molecule of **III** with the dimethyl acetal of a second

molecule of **III** is simply the intermolecular equivalent of the macrocyclization shown in Scheme 3. (2) A perhaps less obvious potential side reaction is Michael addition. The Michael addition of pyrrole (**9**) with an activated alkene (**10**) (Scheme 5, A), first reported as early as the 1950s,[21] proceeds under neutral or acid-catalyzed conditions[22] to give alkylpyrroles (e.g., **11**). The reaction encompasses aryl-substituted enones.[23] For pyrrole–enone **III**, there are six possible nucleophilic sites, including four unsubstituted pyrrole carbons and two pyrrole nitrogens.



Scheme 5. Diverse reactions potentially germane to the double-ring closure of **III** (*vide supra*).

One logical solution is to first carry out the Nazarov cyclization under the best Nazarov conditions, then carry out the macrocyclization under the best macrocyclization conditions (or *vice versa* if the order is reversed); however, the conditions to date for the Nazarov cyclization (lanthanoids in CH_3CN, 80 °C[3]) are thought to be more demanding than for the macrocyclization,

given that analogous macrocyclizations to form bacteriochlorins (e.g., **BC-T**) have been carried out by self-condensation of a dihydrodipyrrin-acetal (e.g., **12-T**) in the presence of an acid such as TMSOTf under proton-scavenging conditions (e.g., use of 2,6-di-*tert*-butylpyridine, DTBP) in CH_2Cl_2 at room temperature (Scheme 5, B).[24] Evidence supportive of resonance-stabilized oxocarbenium ions analogous to **III-Naz-oxo**$^+$ has been obtained by studies of diverse substrates.[25] In this case, the Nazarov and macrocyclization processes would occur sequentially or simultaneously; thus, the conversion of **III** to **BC-1** is carried out in dilute solution. To accomplish the Nazarov cyclization in dilute solution, however, may require an excess quantity of acid given the concentration-dependent binding of acid with **III** to form the pentadienyl cation.

One further issue centers on the choice of acid for the Nazarov and macrocyclization processes. The Nazarov reaction of a β-ketoester-substituted pyrrole typically relies on an oxophilic catalyst such as $Sc(OTf)_3$ or $Yb(OTf)_3$. The reaction of a dihydrodipyrrin-acetal (**12-T**) in the presence of $Yb(OTf)_3$, however, inexplicably affords the tetradehydrocorrin (e.g., **TDC-T**) instead of the desired bacteriochlorin (Scheme 5, C).[26] In short, the acid catalysts for the reaction of a dihydrodipyrrin-acetal to give a specific macrocycle are not universally fungible.

II. Reaction Refinement

The prior conversion of **III-*E*** to **BC-1** was carried out in acetonitrile wherein the concentrations of **III-*E*** and the acid $Yb(OTf)_3$ were 0.19 and 1.9 mM, respectively.[3] The conditions arose from a limited survey of the condensation of a gem-dimethyl-substituted analogue of enone **III-*E***.[10] Here, we carried out a broader survey in an effort to find conditions that afford improvement in any of a number of metrics – higher yield, lesser chlorin byproduct, faster reaction, and/or higher concentration.

Acids, Solvents and Temperature. Reactions of **III-*E*** were carried out at the microscale level in various solvents containing acid catalysts. The reactions were monitored by absorption spectroscopy with measurement of the intensity of the long-wavelength absorption band of **BC-1** (λ_{abs} = 749 nm, ε = 72,100 $M^{-1}cm^{-1}$).[3] In each case, the concentration of **III-*E*** was 0.20 mM and the concentration of acid was 2.0 mM unless noted otherwise.

The previous reaction of **III-*E*** to form **BC-1** was carried out at a nominal temperature of 80 °C, which was measured external to the reaction vessel.[3] Subsequent assessment of the same reaction apparatus indicates the internal temperature (of the reaction medium) was likely ~50 °C. Here, all stated temperatures refer to that of the internal reaction medium assessed by placement of a thermometer into the reaction mixture. The reactions at 80 °C (internal temperature) were achieved with an oil bath set at substantially higher temperature.

First, the reaction was examined with $Yb(OTf)_3$ in solvents of polarity varying from toluene (ε = 2.38) to acetonitrile (ε = 36.6) at 80 °C. The results are shown in Table 1 (see Figure S1 for spectra). The yields ranged from 0–78% (entries 1–7). While the highest yield was obtained in acetonitrile (entry 7), other polar solvents such as *n*-propanol and nitromethane gave no observable product (entries 5 and 6). The reaction with $Yb(OTf)_3$ in solvents at other temperatures gave no better results: in acetonitrile at room temperature or 50 °C, the yield was 8 or 36% (entries 8 and 9), whereas reaction at 110 °C in toluene, chlorobenzene or butyronitrile gave yields of 41–60% (entries 10–12).

Table 1. Conditions for the conversion of **III-*E*** to **BC-1** with $Yb(OTf)_3$ catalysis.[a]

Entry	Solvent	Temp. (°C)	Yield (%)[b]
1	Toluene	80	38
2	Pentafluorobenzene	80	37
3	Diglyme	80	-[c]

4	1,2-Dichloroethane	80	46
5	*n*-Propanol	80	-[c]
6	Nitromethane	80	-[c]
7	Acetonitrile	80	78
8	Acetonitrile	rt	8
9	Acetonitrile	50	36
10	Toluene	110	41
11	Chlorobenzene	110	54
12	Butyronitrile	110	60

[a]Each reaction was carried out with **III-*E*** (0.20 mM) and $Yb(OTf)_3$ (2.0 mM) at 0.1 μmol scale for 20 h. [b]Determined by absorption spectroscopy in toluene at room temperature. [c]No absorption in the region >700 nm was detected.

Second, the reaction in acetonitrile at 80 °C was screened with acids other than $Yb(OTf)_3$. The acids (with citations to prior use in tetrapyrrole reactions) include the following: $Sc(OTf)_3$,[27,28] $Ga(OTf)_3$,[29] $Sn(OTf)_2$,[29] $Sm(OTf)_3$,[29] $Gd(OTf)_3$, $Er(OTf)_3$,[24] $Hf(OTf)_4 \cdot H_2O$,[24,29] $Bi(OTf)_3$,[24,29] and $InCl_3$.[29,30] Triflates were chosen given the enhanced catalysis, via electrostatic activation, of the lanthanide triflates toward β-dicarbonyl compounds.[31] The results are shown in Table 2. Among the non-lanthanide triflates [$Sc(OTf)_3$, $Ga(OTf)_3$, $Sn(OTf)_2$, $Hf(OTf)_4 \cdot H_2O$ and $Bi(OTf)_3$; entries 1–3, 8, 9], only $Sc(OTf)_3$, which was employed previously in Nazarov cyclization of heteroaromatic compounds,[15] gave **BC-1** in significant yield (27%). On the other hand, the lanthanide triflates [$Sm(OTf)_3$, $Gd(OTf)_3$, and $Er(OTf)_3$; entries 4–6] gave yields ranging from 47–53%.

Table 2. Other acids for the conversion of **III-*E*** to **BC-1** in acetonitrile at 80 °C.[a]

Entry	Acid	Yield of **BC-1** (%)[b]
1	$Sc(OTf)_3$	27
2	$Ga(OTf)_3$	trace[c]

3	$Sn(OTf)_2$	-[d]
4	$Sm(OTf)_3$	47
5	$Gd(OTf)_3$	48
6	$Er(OTf)_3$	53
7	$Hf(OTf)_4 \cdot H_2O$	trace[c]
8	$Bi(OTf)_3$	trace[c]
9	$InCl_3$	13[g]
10	TMSOTf / DTBP (2.0 mM / 2.6 mM)	0
11	$Yb(OTf)_3$ / DTBP (2.0 mM / 2.6 mM)	0[e]
12	$Yb(OTf)_3$ / TMSOTf / DTBP (0.2 mM / 2.0 mM / 2.6 mM)	43%[f]

[a]Each reaction was carried out with **III-*E*** (0.20 mM) in acetonitrile at 80 °C with an acid (2.0 mM) at 0.1 μmol scale for 20 h unless noted otherwise. [b]Determined by absorption spectroscopy (in acetonitrile for entries 1–9; in toluene for entries 10–12). [c]A characteristic band was observed but the yield was <5%. [d]An absorption was observed at 792 nm. [e]An absorption was observed at 764 nm. [f]16 h reaction. [g]In addition to absorption at 749 nm, an absorption at 778 nm (putative indium complex of **BC-1**) was also observed.

Third, reactions were carried out to examine catalysts employed in the self-condensation of a dihydrodipyrrin-acetal (e.g., **12-T**) leading to a bacteriochlorin, which include TMSOTf + DTBP.[24] Such conditions are derived from reactions of silyl enol ethers and acetals[32] and also from cleavage of acetals.[33] The base DTBP selectively binds Brønsted acids.[34] While bacteriochlorin formation from dihydrodipyrrin-acetal self-condensation is carried out at room temperature in CH_2Cl_2 (or more polar solvents),[24] and is believed to proceed through a resonance-stabilized oxocarbenium ion (or similar species)[25] as shown in Scheme 3 (bottom panel), the reactions here were examined in acetonitrile at 80 °C. The reactions were examined using TMSOTf + DTBP alone and in conjunction with $Yb(OTf)_3$. Thus, reaction with TMSOTf + DTBP alone gave no **BC-1** (Table 2, entry 10), nor did $Yb(OTf)_3$ and DTBP (entry 11). On the other

hand, the inclusion of TMSOTf + DTBP with only 0.20 mM $Yb(OTf)_3$ gave **BC-1** in 43% yield (entry 12), to be compared with 78% from the reaction with 2.0 mM $Yb(OTf)_3$ with no additives (Table 1, entry 7). The combination of $Yb(OTf)_3$ and TMSOTf has been employed previously in catalysis of imino–ene reactions[35] and crossed aldol condensations.[36] In summary, the highest yield was obtained in acetonitrile (80 °C) containing $Yb(OTf)_3$, which is (1) the most oxophilic among all lanthanides,[37,38] (2) a widely used catalyst in organic chemistry,[39-43] and (3) the acid chosen previously[10] from a more limited survey for formation of the bacteriochlorophyll skeleton.

Grid search. A more systematic study of the role of concentration of $Yb(OTf)_3$ and **III-*E*** was carried out. The reactions were carried out in an anaerobic glove box with reaction volumes ranging from 0.28–7.0 mL. Samples were collected periodically and examined by absorption spectroscopy to determine the yield of **BC-1** and any chlorin byproduct as a function of time. The time courses are described below; we first focus on the yields of **BC-1** at the reaction endpoint (21 h unless noted otherwise) as shown in Figure 1. Several features were examined.

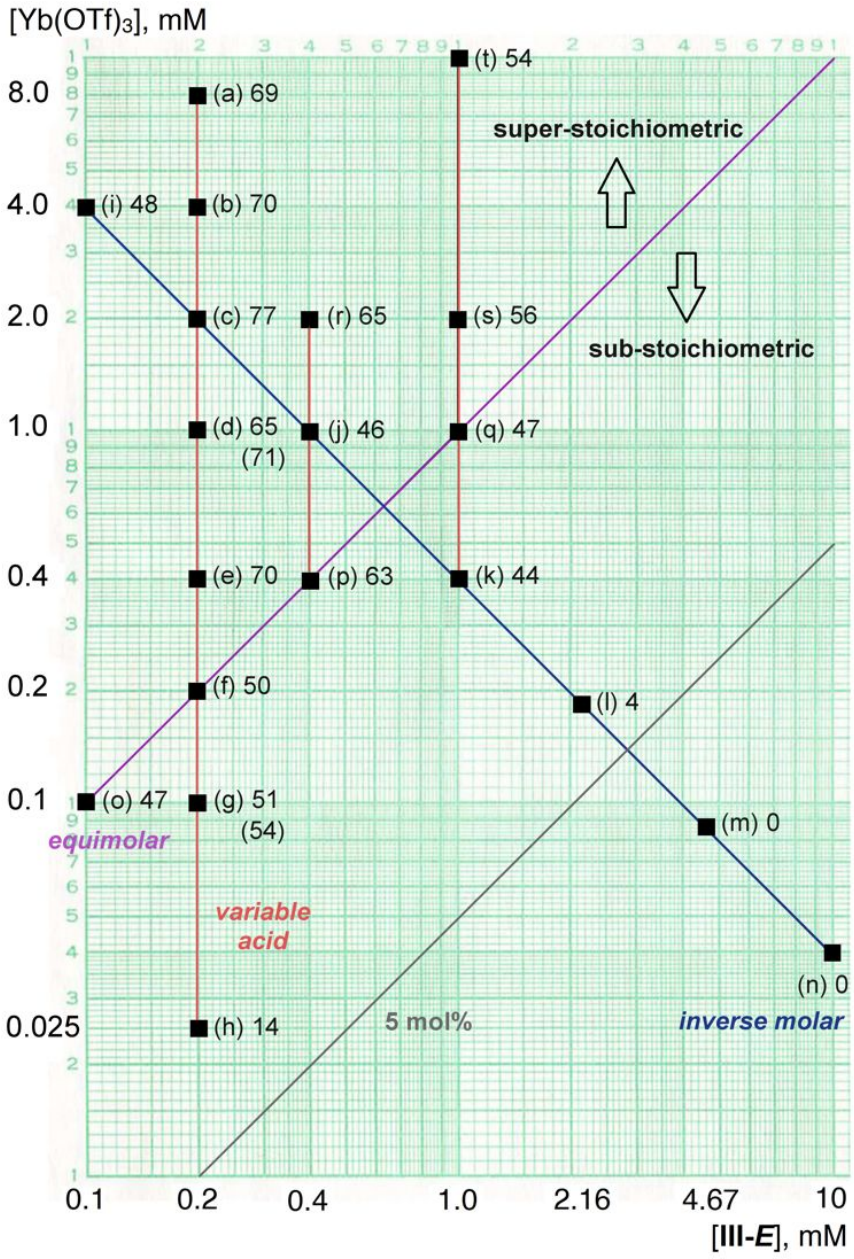


Figure 1. Percent yields of **BC-1** at 21 h upon variation in the quantity of $Yb(OTf)_3$ ("*variable acid*" line), inverse variation in the quantity of $Yb(OTf)_3$ and **III-*E*** ("*inverse molar*" line), and proportional variation in the quantity of $Yb(OTf)_3$ and **III-*E*** ("*equimolar*" line). All reactions were in acetonitrile at 80 °C.

(1) Fixed substrate with variable acid concentration: Reactions were carried out with **III-*E*** at 0.20 mM with concentrations of $Yb(OTf)_3$ from 8.0 mM to 0.025 mM (a 320-fold range). Eight experiments were carried out, defining the *"variable acid"* line (points a–h). Proceeding

from 40 to 0.125 equivalents of $Yb(OTf)_3$ (8.0 to 0.025 mM) to **III-*E***, the yield of **BC-1** went from 69% through the maximum of 77% to 14%. All of the reactions with 2–40 equivalents of $Yb(OTf)_3$ to **III-*E*** gave good yields (65–77%) whereas the reaction with 1 (or 0.5) equivalent gave 50% (or 51%), and 0.125 equivalents gave only 14% yield of **BC-1**. Two other *"variable acid"* lines (points p, j, r; k, q, s, t) are shown using **III-*E*** at higher concentrations (0.40, 1.0 mM), but a smaller range of acid catalyst concentration was examined, chiefly at greater than stoichiometric amounts. The yields ranged from 54–65%.

(2) Constant product of acid and substrate concentrations: The quantities of $Yb(OTf)_3$ and **III-*E*** were varied inversely so that the product of the two concentrations was fixed. Thus, the concentration of $Yb(OTf)_3$ was decreased from 4.0 to 0.040 mM while the concentration of **III-*E*** was increased from 0.10 to 10. mM. Seven experiments were carried out, defining the *"inverse molar"* line (points i, c, j-n). Proceeding from the lowest to highest concentration of **III-*E***, the yield went from 48% through a maximum of 77% to 0%. While the product of the concentrations of the substrate and acid ([**III-*E***]·[$Yb(OTf)_3$]) remained constant, the number of equivalents of $Yb(OTf)_3$ to **III-*E*** varied over the range of 40 to 0.004. The highest yield (77%) of **BC-1** was obtained with 2 mM $Yb(OTf)_3$ and 0.2 mM **III-*E***. Reaction with 2.5 or 0.40 equivalents of $Yb(OTf)_3$ to **III-*E*** gave similar yields (46, 44%) whereas 0.085 equivalents gave 4% yield, and fewer equivalents gave no detected yield. Thus, the use of diminished catalyst concentration commensurate with increased substrate concentration was of no utility below 10 equivalents of $Yb(OTf)_3$ for **III-*E*** at 0.20 mM.

(3) Acid and substrate concentrations in lockstep: The quantities of $Yb(OTf)_3$ and **III-*E*** were set equal across the concentration range of 0.10 mM to 1.0 mM. Four experiments were carried out, defining the *"equimolar"* line (points o, f, p, q). The yields of **BC-1** were little affected, giving 47% at the bookends (0.10 mM, 1.0 mM) and a maximum of 63% at 0.40 mM.

In general, the reactions with super-stoichiometric quantities (2–20-fold) of $Yb(OTf)_3$ versus **III-*E*** gave good yields of **BC-1**. The inverse molar concentration line employed the product $[Yb(OTf)_3] \cdot [\textbf{III-}\boldsymbol{E}] = 0.4\ mM^2$. The thinking was that as the concentration of **III-*E*** is increased, $Yb(OTf)_3$ could be diluted without altering the fraction of bound **III-*E*** · catalyst, or the propensity to form such a complex, which is essential for reaction. We note that the Nazarov reaction of pyrrole **1** → **2** using 5 mol% catalyst had a value for [acid]·[pyrrole] = 4500 mM^2,[15] and **5** → **6** with 0.1 mol% catalyst had 250 mM^2.[16] The 5 mol% line for $Yb(OTf)_3$ catalysis of the reaction of **III-*E*** is shown in Figure 1. The high values of the term [acid]·[pyrrole] for the reactions of **1** and **5** must be at the source of the ability to use a sub-stoichiometric (i.e., "catalytic") quantity of acid versus the equimolar or super-stoichiometric quantity required for the much more dilute reaction of **III-*E***.

Time course. Each of the 20 experiments shown in Figure 1 was monitored over time. The time courses were quite revealing – previously all reactions were allowed to proceed for 21 h.[3] To our surprise, almost all of the reactions were essentially complete in ~4 h or less, the only exceptions originating with the few reactions at <0.4 equivalents of $Yb(OTf)_3$ versus **III-*E***. The time at half-maximal yield ($t_{1/2}$) for each experiment ranged from ~30–300 minutes. The time courses for the acid-variation study (points a–h) are shown in Figure 2A. Additional time courses are shown in Figure S2, and the yields at 4 h for all 20 reactions in the grid are displayed in Figure S3. The same general trends observed at 21 h are apparent at 4 h although there are slight differences in yields. All of the yields (21 h) and the $t_{1/2}$ values for **BC-1** are summarized in Table 3. Again, the highest yield was observed with $Yb(OTf)_3$ (2.0 mM) and **III-*E*** (0.20 mM), which exhibited $t_{1/2}$ at 43 min (point "c").

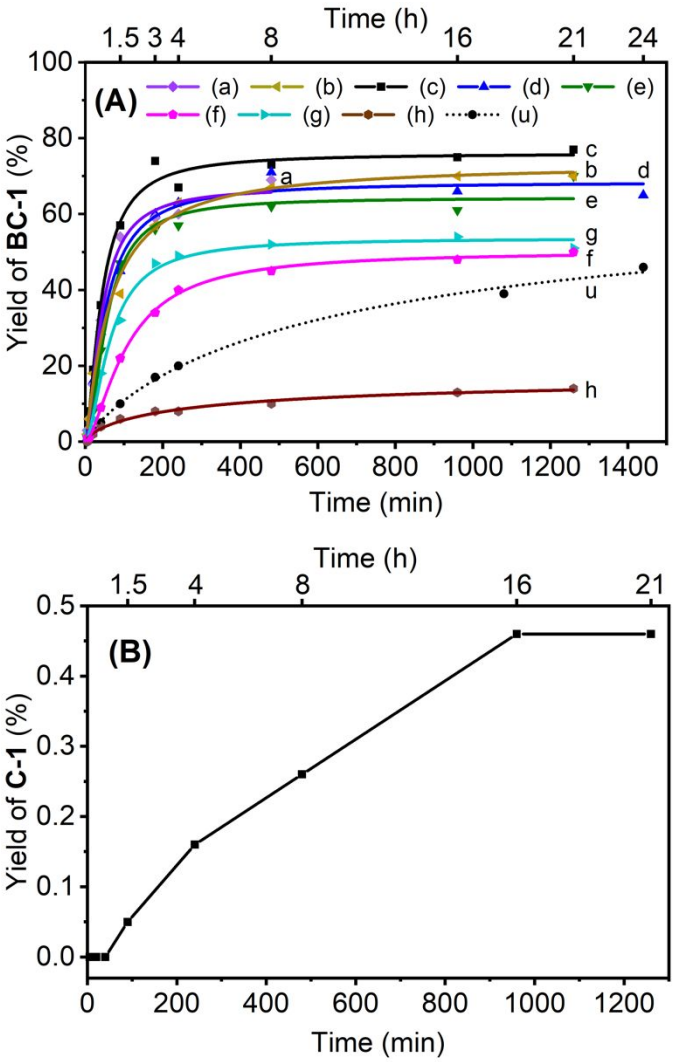


Figure 2. (A) Time course for the yield of **BC-1** with various concentration of $Yb(OTf)_3$ and constant concentration of **III-*E*** in acetonitrile at 80 °C (solid line) or 50 °C (dotted line). (B) Time course for the yield of chlorin **C-1** upon reaction in acetonitrile containing 2.0 mM $Yb(OTf)_3$ (10 equiv) and 0.20 mM **III-*E*** at 80 °C. Note the different scales on the Y-axes for **BC-1** (0–100%) and **C-1** (0–0.5%).

Table 3. Yields of **BC-1** and **C-1** with various concentrations of $Yb(OTf)_3$ and **III-*E***.

Entry	[$Yb(OTf)_3$] mM	[**III-*E***] mM	equiv	**BC-1** Yield (%)[a]	$t_{1/2}$ (min)	**C-1** Yield (%)[b]
a	8.0	0.20	40	69 (69)[c]	43	0.61
b	4.0	0.20	20	70 (70)	62	<0.05[d]
c	2.0	0.20	10	77 (77)	43	0.16
d	1.0	0.20	5	65 (71)[e]	51	0.09
e	0.40	0.20	2	70 (70)	55	0.27
f	0.20	0.20	1	50 (50)	106	0.24
g	0.10	0.20	0.5	51 (54)	63	2.01
h	0.025	0.20	0.125	14 (14)	292	<0.05[d]
i	4.0	0.10	40	48 (48)	67	0.23
j	1.0	0.40	2.5	46 (46)	71	0.10
k	0.40	1.0	0.4	44 (44)	56	0.22
l	0.185	2.16	0.086	4 (4)	–[f]	–
m	0.086	4.67	0.018	0 (0)	–	–
n	0.040	10.	0.004	0 (0)	–	–
o	0.10	0.10	1	47 (47)	143	0.43
p	0.40	0.40	1	63 (63)	91	0.21
q	1.0	1.0	1	47 (47)	55	0.18
r	2.0	0.40	5	65 (65)	36	0.18
s	2.0	1.0	2	56 (56)	43	0.16
t	10.	1.0	10	54 (54)	31	0.27
u[g]	2.0	0.20	10	46 (46)	645	<0.05

[a]Yields were recorded at the end of the reaction course; values in parentheses are the highest yields obtained at any point during the reaction course. [b]Yields are at 4 h of reaction. [c]Reaction ended at 8 h. [d]Below the limits of detection. [e]Reaction ended at 24 h. [f]Not determined. [g]Reaction at 50 °C, ended at 24 h.

The yield of chlorin byproduct was examined in all reaction samples taken at 4 h. The assay relies on fluorescence emission spectroscopy with excitation at 406 nm, a wavelength at which the chlorin (**C-1**) absorbs strongly but the bacteriochlorin (**BC-1**) absorbs weakly.[3] Integration was carried out for the emission over the range 675–715 nm after subtraction of the baseline from a blank sample. The limit of detection of **C-1** alone is 1 nM in the fluorescence cuvette, which for a reaction at 0.20 mM **III-*E*** corresponds to a yield of ~0.05%, as shown in Figure S4. This lower limit may not be reliably achieved if other pigments arise that emit in this region. Previously, the chlorin byproduct was only assessed at the end of the reaction,[3] in which case there were two limiting interpretations concerning the source of dehydrogenation: (1) there pre-existed a small amount of dehydrogenated species (i.e., the dipyrrin analogue of the AD-dihydrodipyrrin unit) in the sample of precursor **I** or **III**, which upon macrocycle formation would directly form the chlorin; or (2) during the course of formation and handling of the bacteriochlorin, some dehydrogenation occurred to form the chlorin.

The time course for formation of chlorin is shown in Figure 2 panel B. Inspection of the time course shows no detectable chlorin at the earliest reaction times. Such results are consistent with the chlorin originating during or after macrocycle formation, not from trace dehydrogenated impurities in any precursors. The yields of chlorin **C-1** at 4 h of reaction were generally <0.2%, although some higher values were observed. The 20-point grid displaying chlorin yields is provided in Figure S5. The yield of **C-1** for the reaction under optimal conditions [2.0 mM $Yb(OTf)_3$ and 0.20 mM **III-*E***] was 0.16% after 4 h and 0.46% after 21 h, to be compared with 68% and 78%, respectively, of **BC-1**. The amount of chlorin as a percentage of the bacteriochlorin ([**C-1**]/[**BC-1**]) is thus 0.24% and 0.60%, respectively. The prior synthesis of **BC-1** (11.5 mg, 53% yield, 21 h) under the same reaction conditions gave **C-1** in 0.74% (absolute) and 1.4% (fractional amount) yield.[3] The ability to carry out the reaction in 1/5 – 1/10th the time (2–4 h

instead of 21 h) affords the bacteriochlorin in good yield with ~1/5th the amount of chlorin byproduct.

Reaction temperature. One reaction was carried out at 50 °C in acetonitrile with concentrations of $Yb(OTf)_3$ (2.0 mM) and **III-*E*** (0.20 mM) found optimal for the reactions at 80 °C. The time course is shown in Figure 2 panel A (trace "u"). The yield of **BC-1** grew in far more slowly than the reaction at 80 °C ($t_{1/2}$ = 645 versus 43 min) and was on a slow, increasing trajectory when the reaction was discontinued (24 h), affording a yield of 46%. Further characterization of the interplay of temperature and other reaction conditions was beyond the scope of the present study.

III. Streamlined Reaction Conditions

Our objective was to carry out the Knoevenagel reaction of dihydrodipyrrins **I** + **II** to form enones **III-*E/Z***, and carry the mixture of the two isomers on to the bacteriochlorin **BC-1**. The following studies were carried out in this regard.

(1) Comparison of the reactivity of **III-*E*** and **III-*Z***. The Knoevenagel reaction[44-46] previously afforded **III-*E*** and **III-*Z*** in isolated yields of 70 and 3%, respectively.[3] The limited quantity of **III-*Z*** precluded extensive experiments concerning formation of **BC-1**. The *E* and *Z* isomers of related enones are known to interconvert under the conditions of the Nazarov reaction.[15,19,47-50] Here, the reactions of **III-*Z*** and **III-*E*** were carried out in parallel under identical conditions (0.20 mM each with 2.0 mM $Yb(OTf)_3$ in acetonitrile at 80 °C). Enone **III-*Z*** gave **BC-1** in 70% yield and $t_{1/2}$ = 38 min versus 77% and 43 min for **III-*E***. The comparable values for the two isomers imply that (i) the enones must isomerize during the course of the Nazarov reaction; and (ii) the mixture of the two isomers can be carried forward to the bacteriochlorin-forming reaction without separation, which previously was quite tedious.

(3) Examination of a two-step procedure. The Knoevenagel reaction previously was carried out with **I** and **II** (to form **III**) at 38 and 48 mM, respectively, whereas the double-ring cyclization of **III** (to form **BC-1**) was carried out at 0.19 mM.[3] To use the crude reaction mixture of **III** without any workup would require dilution by ~200-fold and addition of $Yb(OTf)_3$. The Knoevenagel reaction employs piperidine/acetic acid (15 mM/15 mM) in acetonitrile and 3Å molecular sieves (MS). A trial reaction of **III-*E*** in the presence of diluted piperidine/acetic acid solution (0.08 mM / 0.08 mM in acetonitrile) at 80 °C for 21 h afforded **BC-1** in 69% ($t_{1/2}$ = 56 min) or 64% yield ($t_{1/2}$ = 41 min) with or without use of 3Å molecular sieves, respectively. The results indicate the crude mixture from the Knoevenagel reaction can be employed without workup in the subsequent bacteriochlorin-forming reaction.

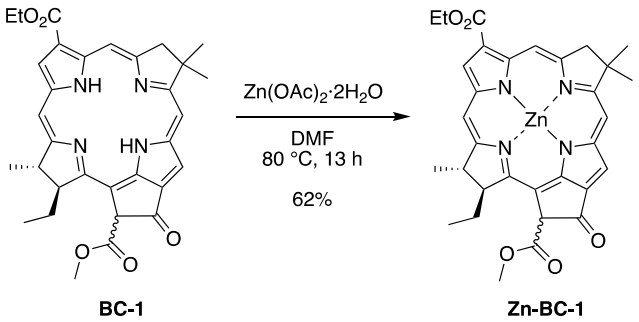
(3) Streamlined synthesis. The Knoevenagel condensation of **I** and **II** (40 mM each) was carried out in acetonitrile containing piperidine/acetic acid (15 mM/15 mM) at room temperature for 40 h. Filtration to remove 3Å molecular sieves, addition of $Yb(OTf)_3$ (10 equiv) along with supplemental acetonitrile to reach 0.20 mM, and subsequent stirring at 80 °C for 4 h gave the crude product. Purification by column chromatography (Figure S6) gave **BC-1** in 22% yield. ^{1}H NMR analysis in $CDCl_3$ indicated that the ratio of 13^2-epimers was 88:12 for **BC-1** formed from the reaction of **I** and **II**, identical with that observed previously upon reaction of **III-*E***.[3]

IV. Zincation of BC-1

Zinc has long been employed as a surrogate for magnesium in fundamental studies pertaining to the native bacteriochlorophylls given the greater chemical stability of zinc versus magnesium chelates, the lesser difficulty of metalation of bacteriochlorins with zinc versus magnesium,[51-55] yet the otherwise rather similar physicochemical properties of the two metal chelates.[56,57] Indeed, an aerobic anoxygenic photosynthetic bacterium of the genus *Acidophilium*

has been found to employ zinc bacteriochlorophylls (rather than the typical magnesium) in both antenna and reaction center complexes.[58] The physiological rationale stems from the greater stability of the chelate of zinc versus magnesium in an acidic milieu, given that optimum growth occurs at pH 3.5.[59] Zinc bacteriochlorophylls also have been incorporated deliberately in photosynthetic reaction centers via use of magnesium chelatase mutants.[60]

Treatment of a small sample of bacteriochlorin **BC-1** with $Zn(OAc)_2 \cdot 2H_2O$ in $MeOH/CH_2Cl_2$ at room temperature did not afford metalation, but reaction in DMF at 80 °C gave a high degree of conversion (Scheme 6). The product was isolated in 62% yield by chromatography on powdered sugar, a well-known adsorbent in chlorophyll chemistry.[61] The absorption and fluorescence spectra (in toluene) of the free base **BC-1** and the zinc chelate **ZnBC-1** are shown in Figure 3. The long-wavelength absorption band of **ZnBC-1** appears at 766 nm, a bathochromic shift of 17 nm versus the free base **BC-1**. The spectrum of **ZnBC-1** is characteristic of metallobacteriochlorophylls[56,57] and is quite similar to that of the zinc(II) chelate of bacteriopheophytin *a* (**ZnBPheo**)[62] as summarized in Table 4. The fluorescence quantum yield (Φ_f) value of **ZnBC-1** is 0.19 (determined by comparison with a known standard), versus that of 0.11[62] for the zinc chelate of bacteriochlorophyll *a* (**ZnBPheo**). ^{1}H NMR analysis in $CDCl_3$ indicated that the ratio of 13^2-epimers was 81:19 for **ZnBC-1**, almost identical with that for **BC-1**.



Scheme 6. Zincation of **BC-1**.

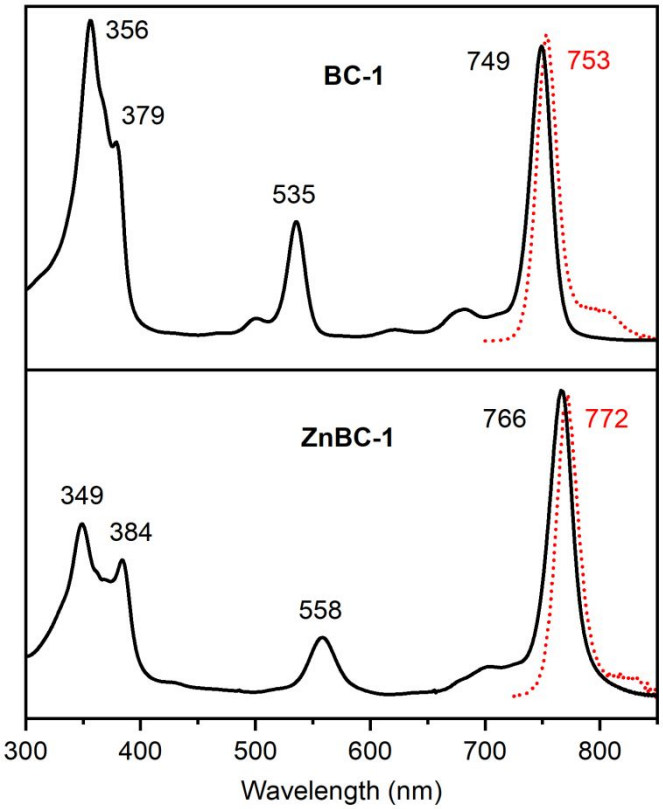


Figure 3. Absorption and fluorescence spectra in toluene at room temperature of the free base **BC-1** and the zinc chelate **ZnBC-1**.

Table 4. Spectral properties of zinc(II) bacteriochlorophylls[a]

Parameter	**ZnBPheo**[b]	**ZnBC-1**
λ_{abs} (B), nm	355, 393	349, 384
λ_{abs} (Q_y), nm	773	766
Q_y (abs) fwhm, nm	35	25
I_{Qy}/I_B	1.40	1.74
λ_{em}	790	772
Stokes shift, nm	17	6
Φ_f	0.11	0.19

[a]All data are from samples in toluene at room temperature. [b]Data from Musewald *et al.*[62]

Outlook

The present work concerns the development of methodology for the synthesis of model bacteriochlorophyll compounds, and as such constitutes a step toward *de novo* syntheses of the native macrocycles. The key findings are as follows.

(1) Refined conditions have been identified for the double-ring closure of **III-*E*** that enable reaction in 4 h (versus 20 h previously), a 77% yield of **BC-1**, and <0.16% chlorin byproduct (versus 0.74% previously). The reaction also can be carried out at higher reactant concentration (1.0 mM versus 0.19 mM previously) while affording a 56% yield of **BC-1**.

(2) Reasonable variation is tolerable of concentrations of **III** and $Yb(OTf)_3$, although the yields of **BC-1** fall off with significant sub-stoichiometric concentrations of the acid. The typical use of 5 mol% of acid in Nazarov reactions of small molecules does not afford any reaction given the already low concentration of **III** (0.20 mM).

(3) The enones **III-*E*** and **III-*Z*** react comparably, enabling conversion to **BC-1** without chromatographic separation.

(4) The insertion of zinc does not significantly alter the epimeric composition at the 13^2-position of the bacteriochlorin.

(5) A streamlined route from two dihydrodipyrrin halves to the bacteriochlorophyll model compound requires only the intervention of filtration and then dilution of the Knoevenagel enone with CH_3CN, treatment with $Yb(OTf)_3$, and heating.

(6) Refinement of a fluorescence assay allows the chlorin byproduct to be determined with limits of detection of 0.05%. Application of improved reaction conditions affords a chlorin content in the crude reaction mixture that is $1/5^{th}$ of that observed previously (0.74%).

(7) Relatively little is known concerning the rate of epimerization of bacteriochlorophylls and analogues compared with that for chlorophylls.[5-9] The nascence of a family of bacteriochlorophyll model compounds will enable a fundamental study in this regard.

(8) Many systematic studies of the interplay of parameters that affect reactions employ factorial design studies, including full factorials and partial factorial designs, which almost always entail survey of a regular grid. We have focused on a subset of points concerning the concentrations of **III-*E*** and $Yb(OTf)_3$ that define selected lines of investigation – *variable acid concentration*, *equimolar concentrations*, and *inverse molar concentrations*. Together with placement of the exploratory lines so as to span regions of sub- and super-stoichiometric acid concentrations, the approach has delineated key features germane to reaction implementation without the expansive experimentation often characteristic of factorial design studies.

The conversion of the enone **III** to the bacteriochlorophyll skeleton **BC-1** has hallmarks of a covalent self-assembly process. Development of the principles of covalent self-assembly as a means of rapid and effective construction of molecular architectures[63] was prompted by results from 35 years ago concerning formation and derivatization of tetrapyrrole macrocycles, bringing the present work full circle. The use of $Yb(OTf)_3$ in acetonitrile for the double-ring closure of **III-*E*** to form **BC-1** can be rationalized on the basis of (1) oxophilicity in binding to the β-dicarbonyl motif, thereby triggering the Nazarov cyclization,[31,37,38] and (2) facilitated cleavage of the acetal moiety, thereby forming an oxocarbenium ion or similar reactive entity.[64] Yet the reaction of a dihydrodipyrrin-acetal (e.g., **12-T**) that in the presence of TMSOTf and DTBP affords a bacteriochlorin (e.g., **BC-T**, Scheme 5)[24] generates instead the tetradehydrocorrin in the presence of $Yb(OTf)_3$ (Scheme 5).[26] Here, the pre-organization provided by the enone **III-*E*** apparently precludes formation of the tetradehydrocorrin (**TDC-T**). In summary, the questions posed at the

outset of this work have largely been answered. A next objective is to apply the conditions with dihydrodipyrrin reactants where each half contains the *trans*-dialkyl and other substituents appropriate for the synthesis of native bacteriochlorophylls.

Experimental Section

General methods

All temperatures reported herein refer to the internal contents of the reaction vessel. Acetonitrile employed in all reactions was HPLC grade.

Grid search protocol

A stock solution of **III-*E*** was prepared by dissolving a known amount (e.g., 24 mg) in anhydrous CH_2Cl_2 (e.g., 14 mL). A sample was aliquoted (e.g., 250 μL) into a conical reaction vial, which was then placed in a warm reaction block (near 40 °C) to evaporate the CH_2Cl_2 over the course of ~30 min. Then, the appropriate quantity of $Yb(OTf)_3$ was added, either directly as a weighed solid or from a stock solution in acetonitrile. The reaction vial was then transferred to the glove box, which contained an atmosphere of argon. Degassed acetonitrile inside the glove box was added to give the volume for the desired concentrations. The final volumes generally ranged from 0.70 – 7.0 mL, although the reactions with >1.0 mM concentrations of **III-*E*** were carried out in a volume of ~0.28 mL. In most cases, the quantity of **III-*E*** in each reaction vial was 0.43 mg (0.70 μmol), although larger amounts were necessarily employed to explore the higher concentration ranges while keeping the reaction volume at least ~0.28 mL.

The reaction vials were composed of glass and were chosen to accommodate the various volumes; conical vials were used for all reaction volumes ≤ 3.5 mL whereas 20-mL flat-bottom vials were used for the reactions at 7.0 mL. The conical vials contained a conical, Teflon-coated magnetic stir bar, whereas the flat-bottom vials contained a rod-shaped, Teflon-coated stir bar. Upon filling, each reaction vial was sealed with a screw cap containing a septum. The reaction vial was then placed on an aluminum heating block that was situated on a heater-stirrer for microscale reactions. The temperature of the heater was set to give 80 °C for the solvent inside the reaction vial. The reactions were allowed to proceed with stirring.

For time-course monitoring, a reaction vial was removed from the heating block, uncapped, and an aliquot was removed. The aliquot was placed into a 3.5-mL vial preloaded with the

appropriate quantity of toluene, which was determined by weighing for high accuracy (e.g, 1.61 g, 1.85 mL). The resulting vial containing the diluted aliquot in toluene was capped and removed from the glove box for analysis by absorption spectroscopy. For the assays wherein the beginning volume of acetonitrile was less than 3.5 mL, the aliquot volume taken for absorption spectroscopy was also proportionally adjusted (e.g., a 50 μL aliquot was taken for each absorption measurement when a 700 μL volume of acetonitrile was used at the outset). The volume of toluene added to the aliquot was also modified accordingly to keep the volume of the resulting solution constant at 2.1 mL prior to the absorption spectroscopic measurement.

The yield of **BC-1** was determined according to equation 1.

$$\text{Yield of } \mathbf{BC-1}\ (\%) = \frac{A_{749} \times 0.0021\ (\text{L}) \times 10^{-6}\ (\mu\text{mol/mol}) \times 14}{72{,}100\ (\text{M}^{-1}\text{cm}^{-1}) \times 1\ (\text{cm}) \times 0.70\ (\mu\text{mol})} \times 100 \qquad \text{(eq 1)}$$

Here, A_{749} is the absorbance of **BC-1** in solution at 749 nm; 72,100 $M^{-1}cm^{-1}$ is the molar absorption coefficient of **BC-1**;[3] 1 (cm) is the cuvette pathlength; 0.0021 L is the volume of the sample upon dilution in toluene for absorption spectroscopic measurement; 10^{-6} is the conversion factor between mol and μmol; 14 is the ratio of the aliquot volume to the reaction mixture volume; and 0.70 (μmol) is the total quantity of **BC-1** in the reaction. The yields were corrected for small but systematic solvent loss, which was determined to be a total of 4.9% over the course of 21 h with collection of 10 samples.

Fluorescence quantum yield determination

The fluorescence quantum yield of **ZnBC-1** was determined by comparison with that of the standard 2,12-di-*p*-tolyl-8,8,18,18-tetramethylbacteriochlorin (Φ_f = 0.18 in toluene).[65] The spectra were corrected for instrument sensitivity as a function of wavelength, and the integrated intensity was ratioed for sample absorption at the wavelength of excitation. The instrument parameters included the following: excitation and emission slit widths = 0.375 mm (1.5 nm bandpass); photomultiplier tube (Hamamatsu R928P) voltage = 1000; and integration time = 2 nm/s.

Fluorescence assay for chlorin content in reaction mixtures

A sample of **C-1** (1.9 mg, 3.44 μmol) was dissolved in toluene (10 mL) to afford a stock solution, from which 580 μL (corresponding to 0.2 μmol of **C-1**) was removed and further diluted in toluene to a final volume of 25 mL. The resulting **C-1** solution (8.0 μM) was then used to prepare five dilute **C-1** solutions (with concentration from 1–20 nM) for use in the fluorescence

assay. Each **C-1** solution was examined by fluorescence spectroscopy (λ_{exc} = 406 nm). Each fluorescence spectrum was corrected first for instrument sensitivity as a function of wavelength and second by subtraction of the spectrum obtained from a solvent blank. The resulting spectrum was then integrated over the range 675–715 nm. The value upon integration from each of the five samples gave a linear regression plot over concentration (Figure S4). The instrument parameters were identical with those for the Φ_f measurements.

Samples taken from time-course reactions after the absorption spectroscopic assay (to determine the yield of **BC-1**) were diluted 12-fold in toluene prior to fluorescence spectroscopic examination. As with the calibrants, each spectrum was corrected first for instrument sensitivity as a function of wavelength and second by subtraction of the spectrum obtained from a solvent blank. From the linear regression plot, the quantity of **C-1** in each sample was readily determined, affording the absolute yield of **C-1** in the reaction samples.

Streamlined synthesis of BC-1

(*17S,18S*)-3-Carboethoxy-13^2-carbomethoxy-17-ethyl-8,8,18-trimethyl-13^1-oxobacteriophorbine (BC-1). A mixture of samples of **I** (12 mg, 40 μmol), **II** (14 mg, 40 μmol), and dried molecular sieves powder (3 Å, 14 mg) was treated with a solution of piperidine/acetic acid in acetonitrile (15 mM/15 mM, 1.1 mL, 16 μmol/16 μmol). The reaction mixture was stirred at room temperature for 40 h, upon which the resulting mixture was filtered through a Pasteur pipette containing a plug of Celite. The filtrate was diluted in acetonitrile to 200 mL, then $Yb(OTf)_3$ (248 mg, 400 μmol) was added. The reaction mixture was then stirred at 80 °C for 4 h under argon in a glove box. Upon completion, the reaction mixture was filtered through a Celite pad, concentrated, and chromatographed [silica, hexanes/ethyl acetate 2:1] to afford the title compound (4.80 mg, 22%). ^{1}H NMR (500 MHz, $CDCl_3$) δ –0.68 (br, 1H), 0.66 (br, 1H), 0.99 (t, J = 7.4 Hz, 3H), 1.66 (t, J = 7.1 Hz, 3H), 1.73 (d, J = 7.3 Hz, 3H), 1.86 (s, 3H), 1.90 (s, 3H), 1.96–2.05 (m, 1H), 2.26–2.31 (m, 1H), 3.84 (s, 3H), 3.88–3.91 (m, 1H), 4.24–4.31 (m, 3H), 4.70–4.75 (m, 2H), 6.08 (s, 1H), 8.40 (s, 1H), 8.43 (s, 1H), 8.49 (s, 1H), 9.14 (d, J = 2.0 Hz, 1H), 9.49 (s, 1H); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 10.6, 12.3 (unknown), 14.6, 17.7 (unknown), 22.7, 27.5, 29.7 (unknown), 30.9, 31.0, 44.4, 49.3, 52.8, 52.9, 53.1, 61.3, 64.2, 98.8, 100.1, 100.5, 108.2, 109.0, 126.5, 129.7, 131.1, 137.6, 137.7, 140.9, 149.5, 159.3, 164.6, 165.1, 169.4, 171.0, 171.2, 188.8; HRMS (ESI-TOF) *m/z*: $[M + H]^+$ calcd for $C_{32}H_{35}N_4O_5$ 555.2602; found 555.2591. λ_{abs} = 749 nm; λ_{em} (λ_{ex} 535 nm) = 753 nm (toluene).

Zincation of BC-1

Zn(II) (*17S,18S*)-3-Carboethoxy-13^2-carbomethoxy-17-ethyl-8,8,18-trimethyl-13^1-oxobacteriophorbine (ZnBC-1). A solution of **BC-1** (4.8 mg, 8.7 μmol) and $Zn(OAc)_2 \cdot 2H_2O$ (57.1 mg, 260 μmol) in DMF (2.2 mL) was stirred at 80 °C for 13 h, followed by dilution in CH_2Cl_2. The organic solution was then washed with saturated aqueous $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The mixture was chromatographed on powdered sugar (Figure S6) with elution by hexanes to afford a deep blue residue (3.29 mg, 62%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.97 (t, J = 6.6 Hz, 3H), 1.61 (t, J = 7.0 Hz, 3H), 1.73 (d, J = 7.1 Hz, 3H), 1.87 (s, 3H), 1.89 (s, 3H), 1.95–2.00 (m, 1H), 2.19–2.26 (m, 1H), 3.83–3.87 (m, 4H), 4.21–4.28 (m, 3H), 4.63 (br, 2H), 5.96 (s, 1H), 8.29 (s, 1H), 8.40 (s, 1H), 8.47 (s, 1H), 9.10 (s, 1H), 9.38 (s, 1H); HRMS (ESI-TOF) *m/z*: $[M]^+$ Calcd for $C_{32}H_{32}N_4O_5Zn$ 616.1659; Found 616.1653. The absorption and fluorescence features are listed in Table 4.

Associated content

Electronic supplementary information. Additional data from exploratory studies of reaction conditions including time courses and grid searches; chlorin assay calibration data; and ^{1}H NMR and $^{13}C\{^1H\}$ NMR data for new compounds.

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Conflict of interest

The authors declare the following competing financial interest(s): J.S.L. is a cofounder of NIRvana Sciences, which has licensed aspects of technology antecedent to that described herein.

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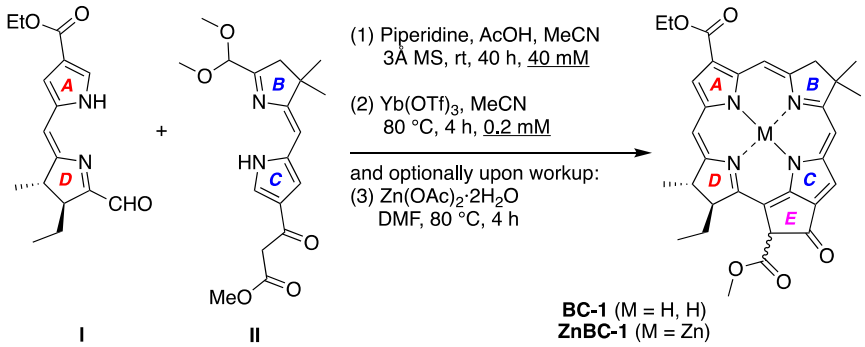
TOC graphic



I II

BC-1 (M = H, H)
ZnBC-1 (M = Zn)