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Synthesis of *Aza*-Rocaglates *via* ESIPT-Mediated (3+2) Photocycloaddition

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Abstract

Synthesis of *aza*-rocaglates, nitrogen-containing analogues of the rocaglate natural products, is reported. The route features ESIPT-mediated (3+2) photocycloaddition of 1-alkyl-2-aryl-3-hydroxyquinolinones with the dipolarophile methyl cinnamate. A continuous photoflow reactor was utilized for photocycloadditions. An array of compounds bearing the hexahydrocyclopenta[b]indole core structure was synthesized and evaluated in translation inhibition assays.

Graphical abstract

Aza-rocaglates were prepared from 3-hydroxyquinolinones and methyl cinnamate using ESIPT (3+2) photocycloaddition in a continuous photoflow reactor. A collection of compounds

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containing hexahydrocyclopenta[*b*]indole core was synthesized using this method and evaluated in translation inhibition assays.

Keywords

Rocaglate; Photocycloaddition; ESIPT; Flow Chemistry; Heterocycle

Flavaglines are a family of natural products isolated from plants of the genus *Aglaia* which feature the cyclopenta[*b*]-benzofuran core structure.[1] After the first isolation of rocaglamide (**1**, Figure 1) by King and coworkers in 1982,[2] more than 100 natural flavaglines (rocaglates) have been isolated, characterized, and tested for insecticidal, anti-inflammatory, and anticancer activities.[3] Due to their intriguing structural complexity and biological activities, a number of synthetic chemists have undertaken syntheses of natural flavaglines as well as targeted derivatives with improved or novel biological properties.[4]

However, with the exception of the carbocyclic analogue **4** for which biological data was not reported,[5] such studies have been limited to variations of the cyclopenta[*b*]benzofuran core.[4] Previously, we reported a biomimetic approach towards rocaglates using excited state intramolecular proton transfer (ESIPT)-mediated (3+2) photocycloaddition of 3-hydroxyflavones and dipolarophiles.[6] Later, we successfully extended the methodology to employ 1,2-dimethyl-3-hydroxyquinolinone (DMQ, **6**) to access various cycloadducts.[7] For example, photocycloaddition of DMQ (**6**) with methyl cinnamate **7** provided bridged ketone cycloadduct **8** (Scheme 1).

In the current study, we considered syntheses of nitrogen-containing rocaglate analogues employing ESIPT photocycloaddition of 2-aryl-3-hydroxyquinolinones such as **9** wherein it was thought that π-π stacking interactions between the 2-aryl substituent of substrate **9** and the phenyl group of methyl cinnamate **7** may favor the desired cycloaddition geometry (Scheme 2). *Aza*-aglain cycloadduct **10** may further undergo α-ketol (acyloin) rearrangement to **11** which may be followed by diastereoselective reduction to access *aza*-rocaglates such as **5** bearing the hexahydrocyclopenta[*b*]-indole scaffold. Herein, we outline synthetic and mechanistic studies regarding the *aza*-rocaglates as well as translation inhibition studies of the natural product variants.

We first performed pilot studies with 2-aryl-3-hydroxyquinolinone substrate **12**.[8b] Under photoirradiation of **12** with dipolarophile **7** (λ>330 nm), we did not observe satisfactory conversion to the corresponding cycloadduct **13** (Scheme 3). As our previous studies were performed using a *N*-methyl-3-hydroxyquinolinone substrate (*cf.* Scheme 1), 1-methyl-2-aryl-3-hydroxyquinolinone (*N*-Me-3-HQ, **15**) was chosen as a modified substrate for ESIPT photocycloaddition. The synthesis of derivatives related to **15** has been previously reported.[8] However, using substrate **14** and polyphosphoric acid (PPA) as solvent,[8b] a lengthy workup and purification was required and only 34% of the desired product **15** was observed. In order to obtain practical amounts of **15**, development of a new method for the synthesis of *N*-alkyl-3-hydroxyquinolinones was required. After evaluation of reaction conditions, we found that treatment of phenacyl anthranilate **14** with NaH (1.0 equiv.) in

THF led to the production of **15** in 83% yield (Scheme 4A). A proposed mechanism (Scheme 4B) may proceed through formation of hemiaminal **14a** followed by rearrangement to alkoxide **14b**. 3,1-Benzoxazin-4-ones related to **14b** have previously been reported from treatment of phenacyl anthranilates with acetic acid.[8c] Further reaction of **14b** to iminium **14c** followed by isomerization should generate the intramolecular hydrogen bond-stabilized enolate **14d** (Scheme 4C).[9] A DFT model of **14d** (Scheme 4C) shows a well-defined arrangement for further transformations.[10] Cyclization of **14d** to benzoxazepine **14e** followed by rearrangement to zwitterion **14f**, elimination, and acidic workup provides 3-hydroxyquinolinone **15**. Similar benzoxazepine structures have been reported previously in the literature by treatment of phenacyl anthranilates with phosphoryl chloride.[11] To the best of our knowledge, the synthesis of 1-alkyl-3-hydroxyquinolinones from phenacyl anthranilates under basic conditions has not been previously described.

When substrate **15** was subjected to photoirradiation (λ>330 nm) in the presence of methyl cinnamate, cycloadducts were indeed generated. After condition optimization, we found that using trifluorotoluene and trifluoroethanol as co-solvents, *aza*-aglain derivatives **16** and **17** could be isolated in 40% yield (5:1 ratio). However, 48 h was needed to obtain the desired products in which case substantial decomposition was observed. Taking advantage of a recently developed continuous photoflow reactor,[12] we found that the reaction was complete in 9 h (46% yield, 89% yield b.r.s.m., **16:17**= 5:1). In addition, significantly less decomposition was observed in comparison to batch reactions which facilitated product purification. Reduction of the minor isomer **17** using $LiAlH_4$ provided an unstable diol which was acylated to afford the *bis-para*-bromobenzoate **18**. The structure and relative stereochemistry of **18** was confirmed using HMBC and NOESY experiments.[10] Using sodium methoxide-mediated α-ketol rearrangement, followed by diastereoselective reduction, *aza*-rocaglate **20** could be obtained in 77% yield (2 steps) from cycloadduct **16**. The structure of **20** was unambiguously confirmed by X-ray crystal structure analysis of the derived bromobenzoate **21** (Figure 2).[13] Interestingly, inspection of the X-ray structure reveals that the *N*-methyl moiety is coplanar to the adjacent aryl group indicating a non-pyramidalized nitrogen.[14] This is likely due to steric interaction of *N*-methyl moiety with the two nearby aryl substituents which appears to prevent nitrogen pyramidalization.

In order to provide a plausible explanation for the differential reactivity of 3-hydroxyquinolinones **12** and **15**, we measured their UV-Vis absorption spectra.[15] We found that in CH_2Cl_2:MeOH (2:1), both substrates possess an absorption band at approximately 370 nm which can be attributed to charge transfer excitation. More interestingly, we observed an additional absorption (310 nm) for substrate **12** (Figure 3). Therefore, we hypothesized that in solution, **12** may also be represented by an aromatic, dihydroxyquinoline tautomeric form which may diminish its photoreactivity.[16] In order to support this assertion, we synthesized 3,4-dimethoxyquinoline **22** by treatment of **12** with TMS diazomethane (Scheme 6) for evaluation of its UV absorption properties.[17] An absorption shoulder at 320 nm was also observed for compound **22** which provides information on the existence of tautomeric form **12a** in solution.

We next targeted the synthesis of more highly functionalized *aza*-rocaglates. Starting with *N*-Me-Br-3-HQ **23** as substrate, obtained from commercially available materials in four steps,[10] photocycloaddition with methyl cinnamate **7** in the continuous flow reactor led to the production of cycloadduct **24** in 43% yield and excellent selectivity.[18] Ketol rearrangement, followed by diastereoselective reduction, provided *aza*-rocaglate **26** in good yield. (Scheme 7) Compound **26** was then further transformed to the corresponding hydroxamate **27**[19] by saponification and coupling.

As part of our study, we also targeted the synthesis of the *N*-H-containing *aza*-rocaglate **5**. After an evaluation of nitrogen protecting groups for synthesis of 3-hydroxyquinolinone substrates, only alkyl protecting groups on the nitrogen could enable access to photocycloadducts (*cf.* Scheme 4). Therefore, an allyl protecting group was chosen to synthesize *N*-allyl-3-HQ **28** using the previously described method. Upon photoirradiation of substrate **28** in the continuous photoflow reactor, cycloadduct **29** could be obtained from *N*-allyl-3-HQ **28** in 43% yield (Scheme 8). Ketol rearrangement of **29** led to the production of **30** which was subjected to hydroxyl-directed reduction to afford *N*-allyl-*aza*-rocaglate **31**. After a thorough evaluation of the allyl deprotection conditions, we found that using Pd (0) / 1,4-*bis*(diphenylphosphino)-butane complex and Meldrum's acid as nucleophile and proton source,[20] *N*-H-*aza*-rocaglate **5** could be obtained in 45% yield. Other *N*-deallylation conditions reported in the literature failed to provide the desired product.[21] In order to demonstrate the possibility for further functionalization of the *N*-allyl moiety, **31** was converted into acrylate **32** using olefin cross metathesis.[22]

Rocaglates have been shown to behave as potent inhibitors of translation by interfering with the activity of eukaryotic initiation factor (eIF) 4A, an RNA helicase necessary for cap-dependent protein synthesis.[23] We therefore assessed the biological activities of compounds **5**, **26**, **27**, **31**, and **32** *in vitro* in a translation assay programmed with the bicistronic mRNA, FF/HCV/Ren (Figure 4A). In this system firefly luciferase (FF) production is eIF4A-dependent whereas Renilla luciferase (Ren) is not.[24] Silvestrol (*not shown*) and rocaglates **CR-1-31B**[19a] and **SDS-1-021**-(−)[3e] potently inhibited FF production (5–10 fold) at 5 μM. The only *aza*-rocaglate to affect FF production was the *N*-methyl derivative **26**, showing a ~30% reduction in FF production at 20 μM (Figure 4A). To assess activity towards cellular protein synthesis, HeLa cells were incubated with silvestrol, **CR-1-31B**, or **26** for 1 h and metabolic protein synthesis quantitated (Figure 4B). Whereas 100 nM silvestrol or **CR-1-31B** completely blocked protein synthesis, compound **26** showed modest activity at only at 50 μM (~20% inhibition).

Consistent with these findings, we also found no evidence for translation inhibition over a 24 h period at concentrations up to 10 μM using a whole cell assay based on constitutive expression of the rapidly turned-over reporter protein firefly luciferase (Figure 5A). Likewise the compounds had minimal cytotoxic activity in a standard 3-day growth assay over the same concentration range (Figure 5B). Together, these results indicate that *aza*-rocaglates do not possess the same inhibitory potency towards protein synthesis in comparison to related rocaglates such as RHT.[19c]

In summary, we have employed ESIPT photocycloaddition methodology to synthesize *aza*-rocaglates. Our studies have uncovered differential photocycloaddition reactivities between *N*-H- and *N*-Me-substituted 2-aryl-3-hydroxyquinolinone substrates. A novel method to access 1-alkyl-2-aryl-3-hydroxyquinolinones was also developed for (3+2) photocycloaddition. Use of a continuous photoflow reactor facilitated synthesis of *aza*-rocaglates with both *N*-alkyl and *N*-H substitution. Initial protein synthesis and translation inhibition data indicates that *aza*-rocaglates do not possess activity in comparison to related rocaglates which provides further information on the SAR of the natural product scaffold.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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13. CCDC 1457719 (**21**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.
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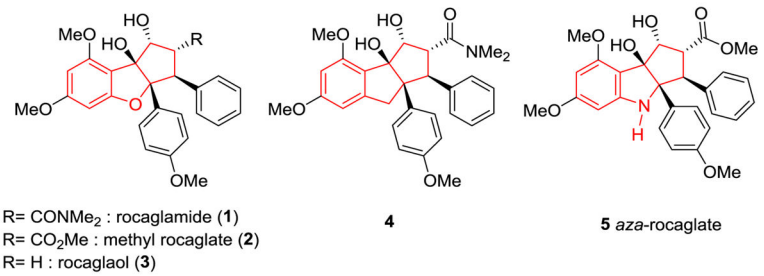


R= $CONMe_2$: rocaglamide (**1**)
R= CO_2Me : methyl rocaglate (**2**)
R= H : rocaglaol (**3**)

4

5 *aza*-rocaglate

Figure 1.
Rocaglate and Related Natural Products, a Carbocyclic Analogue, and *Aza*-Rocaglate Structures.

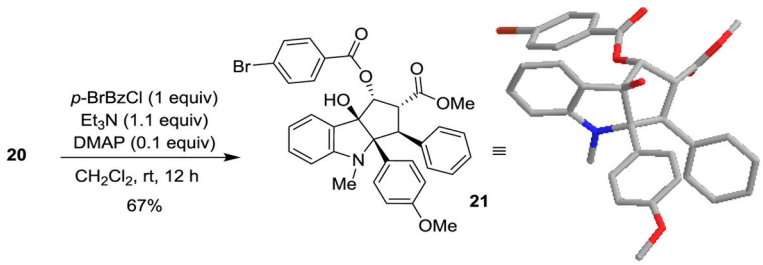


Figure 2.
Synthesis and X-ray Crystal Structure of *Aza*-Rocaglate **21**.

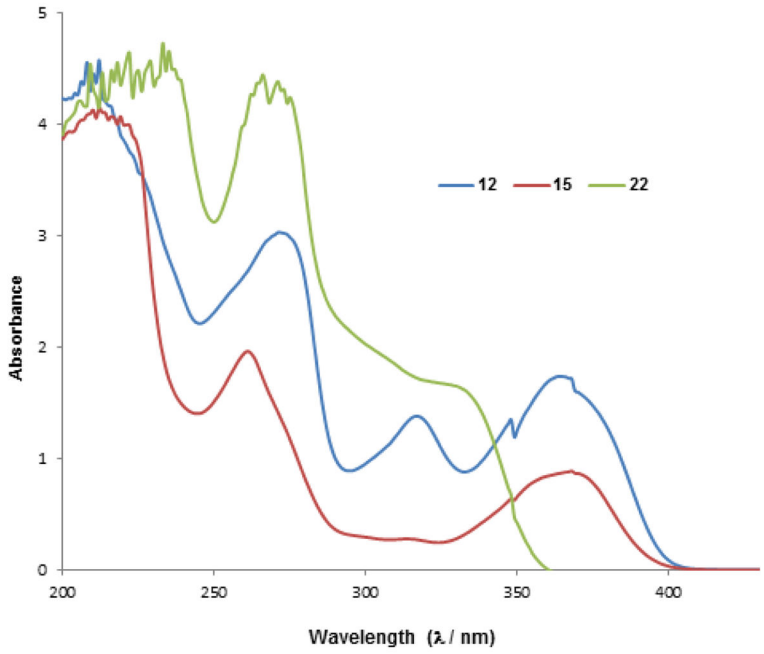


Figure 3.
UV Absorption Spectra of 3-Hydroxyquinolinones **12** and **15** in Comparison to Dimethoxyquinoline **22**.

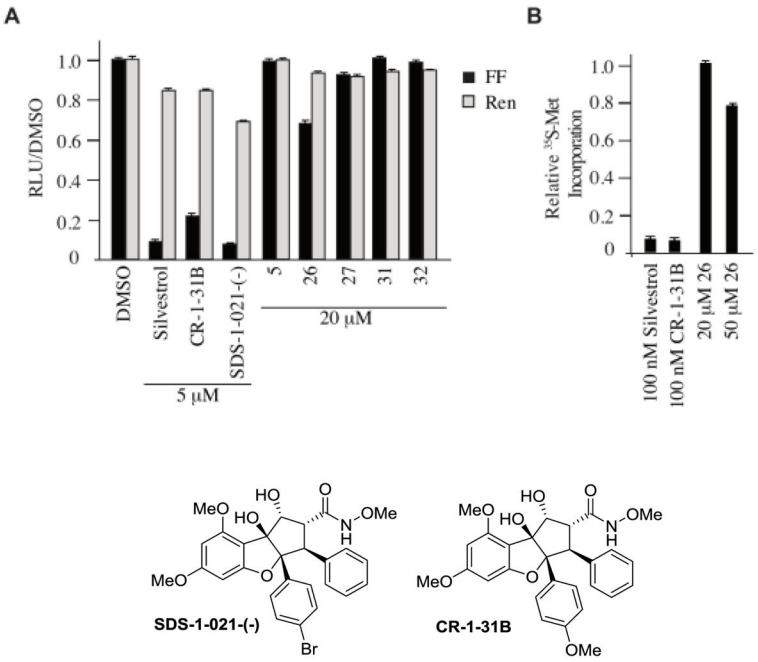


Figure 4.

A. Effect of *aza*-rocaglates on *in vitro* translation of FF/HCV/Ren. **B.** Assessing translation inhibition activity of **26** on HeLa cells.

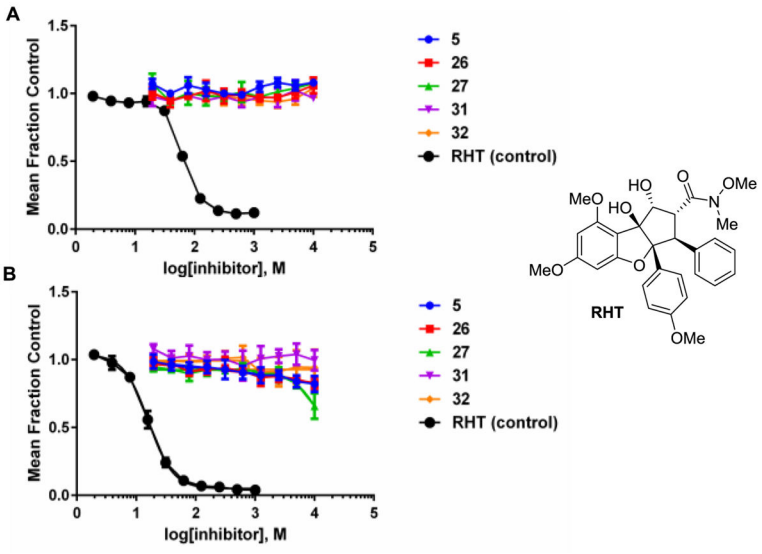
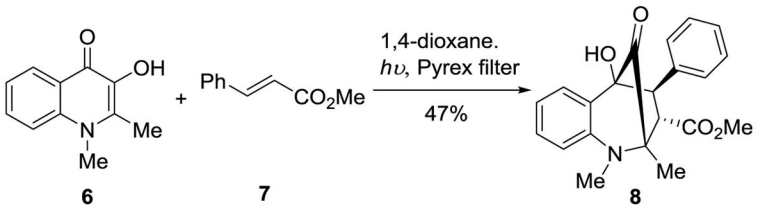


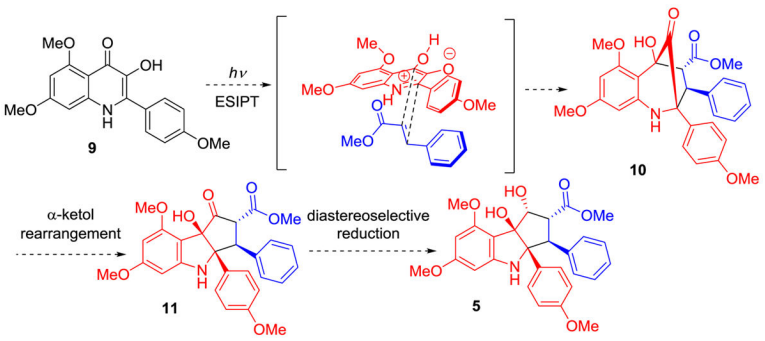
Figure 5.

A. Translation inhibition (10 μM) in whole cells based on constitutive expression of firefly luciferase. **B**. 3-day growth assay (Human 293T cancer cells) over the same concentration.

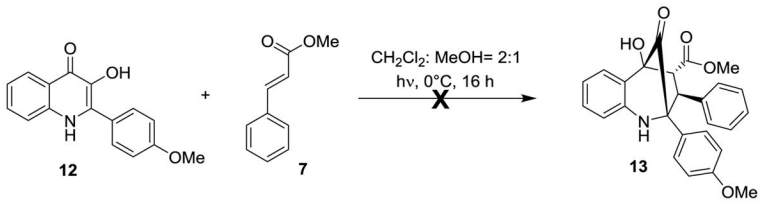


Scheme 1.

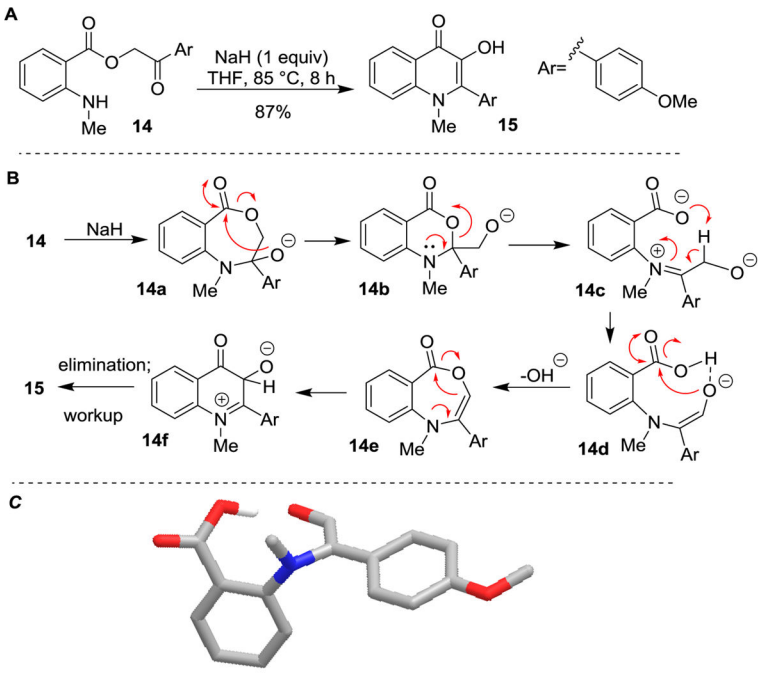
Previous Studies on Photocycloadditions Using 3-Hydroxyquinolinones (3-HQ's).



Scheme 2.
Planned Synthesis of *Aza*-Rocaglates.

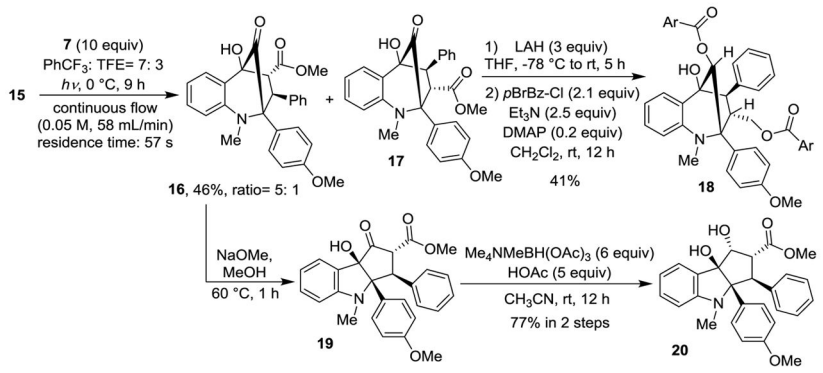


Scheme 3.
Attempted (3+2) Photocycloaddition of **12**.



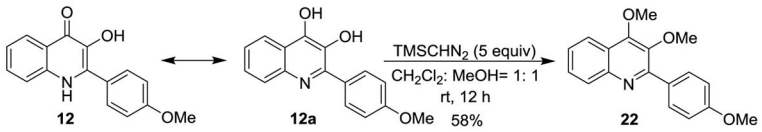
Scheme 4.

A Synthesis of *N*-Me-3-HQ **15; B.** Proposed Mechanism; **C.** DFT Model of Intermediate **14d** (B3LYP_6–31G**++).

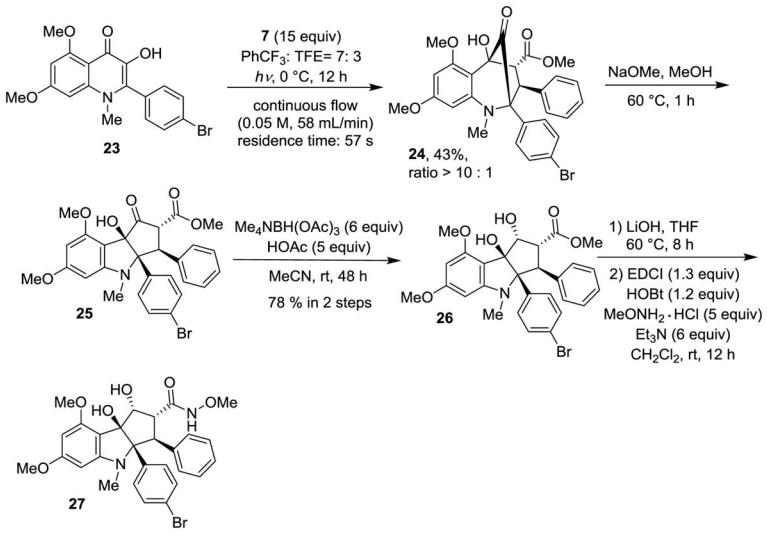


Scheme 5.

(3+2) Photocycloaddition and Prototype Synthesis of an *Aza*-Rocaglate.

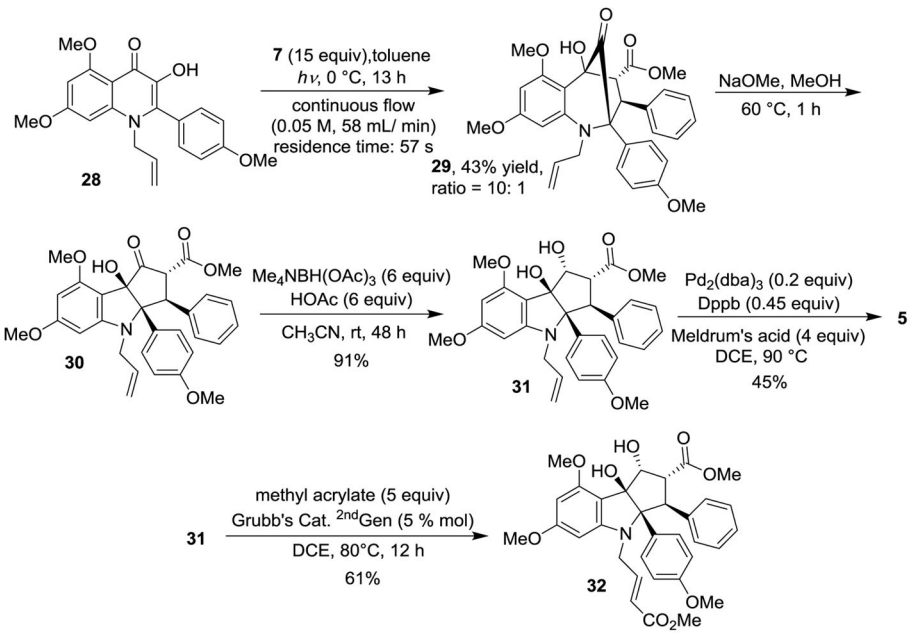


Scheme 6.
Methylation of an *N*-H-3-hydroxyquinolinone.



Scheme 7.

Synthesis of an Advanced *N*-methyl-*aza*-rocaglate **27**.



Scheme 8.
Synthesis of *N*-H-*aza*-rocaglate **5**.