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Modular Synthesis of the Pentacyclic Core of Batrachotoxin and Select Batrachotoxin Analogue Designs

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Abstract

Pentacyclic analogues of the potent voltage-gated sodium ion channel agonist batrachotoxin can be accessed through an intermediate furan by exploiting Diels-Alder cycloaddition reactions with ring-strained dienophiles. The use of 3-bromofuran as a 1,2-dianion equivalent, the application of carbamate reductive N-alkylation for homomorpholine ring assembly, and the demonstration of CsF as an effective reagent for generating benzyne, cyclohexyne, and related dienophiles underscore this work.

Introduction

Batrachotoxin (BTX, **1**), homobatrachotoxin, and batrachotoxinin A comprise a small family of complex steroidal alkaloids originally isolated in sparing amounts from the skin of Colombian poison dart frogs of the genus *Phyllobates*.[1,2] Batrachotoxin acts as a selective agonist of voltage-gated sodium channels (Na_Vs) and is among the most potent non-peptidic toxins known (LD_{50} in mice = 2 μg/kg).[3,4] BTX binding to Na_Vs results in a remarkably complex array of responses, including hyperpolarization of threshold activation, elimination of inactivation gating, and reduction of single channel conductance.[5] While there exist other small molecule modulators of Na_Vs, arguably none show activity that is as multifaceted as BTX. Severely limited quantities of BTX, however, frustrate any efforts to evaluate structure-function relationships and to utilize BTX or select analogues to interrogate mechanisms of ion selectivity and channel gating.[6,7] Such interests have motivated the synthetic studies described herein.

Batrachotoxin offers numerous intriguing challenges to the chemist interested in de novo synthesis.[8] The E-ring homomorpholine is unique among secondary metabolite structures, and the 9α-hydroxy 3β-hemiketal, C16–C17 unsaturation, and 20α pyrrole ester do not appear in other steroidal natural products. To date, only a single de novo route to (±)-batrachotoxinin A has been disclosed.[9,10] The impressiveness of this achievement notwithstanding, the length and linear nature of the synthesis does not provide a viable means to BTX analogues. Other reports describe preparations of A/B/C ring system variants; those that have been tested show modest to little effect as Na_V modulators.[11] To our knowledge, no reports of the preparation of the C/D/E skeleton have appeared, in spite of the fact that mouse lethality and electrophysiology data indicate that variations to the C, D, or E rings dramatically alter BTX activity.[12] Homology modelling and protein mutagenesis data suggest that BTX binds to the inner pore region of Na_V through primary contacts with the C/D/E ring unit.[13,14] With an interest in understanding how toxin binding alters Na_V function,

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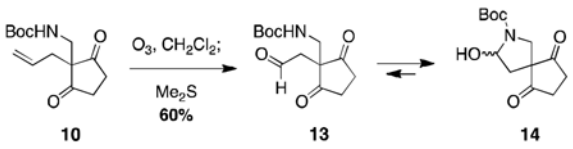
we have fixed on the preparation of molecular probes that conserve the C/D/E ring system of BTX and the pendant pyrrole ester. Our synthetic efforts have culminated in a synthesis of a furan-derived C/D/E ring intermediate, which is appropriately configured to enable access to both BTX and A/B ring BTX derivatives. The versatility of this furan structure is demonstrated below for the synthesis of the pentacyclic core of the natural product and related analogues.

Results and Discussion

Our retrosynthetic plan for preparing BTX is depicted in Figure 2. Of the strategies considered for assembling the A/B ring system from an advanced intermediate, a Diels-Alder sequence was selected using furan **3** as the diene component. The choice of this heterocycle as a B-ring surrogate offers flexibility in terms of dienophile selection and is expected to facilitate analogue production. Furan **3** is further deconstructed by unraveling the homomorpholine unit. Latent symmetry elements appear in the resulting tricyclic structure **4** that are revealed in ketone **5**. The preparation of **5** follows from dione **6** and a suitably derived furan. In this context, the furan is intended to serve as a 1,2-vicinal dianion equivalent.

Installation of the C13 quaternary carbon center is performed in the initial stages of the BTX synthesis through a three-step sequence from 1,3-cyclopentadione (Scheme 1). Mannich reaction with this diketone and a suitable imine or iminium electrophile are problematic, as the product readily reverts to starting materials under acidic or basic conditions.[15] The use of sulfone **8** as an imine precursor, however, enables formation of the N-Boc-protected Mannich product **9** in 86% yield.[16] Both sulfone **8** and the dione product **9** are easily prepared on multigram scales. Subsequent allylation of **9** under basic conditions does not induce retro-Mannich or β-elimination of the NHBoc group; instead, a 2:1 mixture of O- and C-allylated products is furnished. The choice of base has little influence on product selectivity; however, the mixture of isomers converges to the desired C-allylated product **10** upon heating in toluene (98%, two steps).

Ozonolysis of alkene **10** to reveal the tricarbonyl species **13**, while successful, gives exclusively hemi-aminal **14** (eq 1). All subsequent attempts to add nucleophiles to the masked aldehyde have been unfruitful. Efforts to protect the nitrogen center in **10** using various alkylating agents (e.g., MeI or Me_2SO_4) and bases (e.g., NaH or KHMDS) results in retro-Mannich reaction. Fortunately, the application of a recently developed protocol for *N*-methoxymethyl (MOM) protection of carbamates using paraformaldehyde and MeOH successfully affords **11** in 80% yield (Scheme 1).[17] Subsequent alkene cleavage with O_3 gives the desired aldehyde **12**, a critical component of our synthetic plan.



10 13 14

(1)

As originally conceived, sequential additional of a vicinal dianion nucleophile to **12** would make possible diastereoselective C-ring formation through a meso-breaking desymmetrization event (Figure 3). While several potential dianion surrogates were considered at this stage of planning, 3-bromofuran appeared optimal in the first-generation

design of our synthesis. Selective C2-lithiation of 3-bromofuran is possible using LiN^iPr_2; elimination to the heteroaryne does not occur at reduced temperature.[18] Addition of this anion to the aldehyde moiety in **12** was expected to furnish 2° alcohol **15** (R = H). Following alcohol protection, metal-halogen exchange would trigger cyclization to establish the *cis*-fused C/D ring junction. In theory, the intermediate furyl anion could add to either ketone group to generate diasteromeric products. A preferred transition structure in which the C11-alkoxy group is disposed in a pseudo-equatorial arrangement should favor the desired stereoisomer **16**.

Addition of 2-lithio-3-bromofuran to diketo-aldehyde **12** occurs exclusively at C11 to afford a 3:2 diastereomeric mixture of hemiacetals **18** rather than alcohol **17** (Scheme 2). Attempts to promote hemiacetal ring opening and 2° alcohol protection, however, did not prove successful; instead, use of MOMCl and amine base gives the unusual bis-acetal structures **19** in a slightly improved 2:1 diastereomeric ratio.[19] Although this intermediate was not conceived in our original retrosynthetic analysis, the value of the major diastereomer **19-endo** for generating the C/D ring system of BTX is evident. Treatment of **19** with *n*-BuLi at −78 °C results in selective lithium-halogen exchange, and intramolecular anion addition to the C14-ketone (BTX numbering) furnishes the bridged tetracycle **20**.[20] Access to **19-endo** thus enables C/D ring formation with perfect control of relative stereochemistry between the C11 and C14 centers.

Having established a preparative route to **20**, a three-step sequence makes possible elaboration of this structure to tricycle **22** (Scheme 3). High yielding, 3° alcohol allylation is effected using a biphasic reaction protocol with 50% aqueous NaOH and n-Bu_4NI as a phase-transfer agent.[21] Subsequent acetal hydrolysis reveals the C17 ketone, which is smoothly transformed to the corresponding vinyl triflate. Stille cross-coupling with (1-ethoxyvinyl)tributyltin followed by aqueous acid treatment affords methyl ketone **23** (81% yield over three steps). Notably, strong acid hydrolysis also cleaves the N-MOM group. Finally, diastereoselective 1,2-reduction of the C20 enone is achieved using i-Bu_2AlH as the hydride source. In this reaction, we speculate that the 2° carbamate may serve as a stereochemical controlling element to favor preferential addition to the *si*-face of the ketone. Exclusive formation of the β-configured C20-alcohol **24**, as required for completing the C/D/E ring synthesis, is consistent with the putative chelate addition model.[22]

Starting from alcohol **24**, homomorpholine ring synthesis is achieved through an efficient reductive amination sequence (Scheme 3). Despite the weakly nucleophilic nature of the carbamate moiety, condensation of this group with the intermediate aldehyde **25** is favorable under the action of acetic acid and $MgSO_4$. In situ iminium ion reduction occurs with $NaCNBH_3$ to afford the bridging 7-membered ring structure **26** in 74% yield. This is the first example of which we are aware for azepine synthesis involving reductive cyclization with an acyl-protected amine.[23,24] The success of this cyclization reaction is attributed to both the highly electrophilic character of the α-alkoxyaldehyde and the geometric constraints of the C/D ring fusion. Following a second metal hydride reaction, this one employing $LiAlH_4$ to reduce the N-Boc group, assembly of the fully functionalized C/D/E core **27** of the natural product is accomplished.

We have explored a variety of Diels-Alder reactions with furan **26** in an effort to complete the synthesis of BTX and to evaluate dienophile reactivity for BTX analogue design.[25] Cycloaddition reactions with electron-deficient alkynes showed encouraging preliminary results and revealed several important reactivity trends (Table 1, entries 1–3).[26] First, the substituted furan diene strongly biases regiochemical control in reactions with unsymmetrical alkyne dienophiles, as all three Diels-Alder adducts were formed as single constitutional isomers. The steric and electronic influence of C8–C9 substitution on the

furanyl unit favors bond formation between C6 and the most electrophilic site on the dienophile. Second, in all cases examined, approach of the dienophile occurs from the desired β-face of the furan, albeit not with the degree of selectivity anticipated from analysis of molecular models. The modest diastereomeric ratios may be reflective of thermodynamic energy differences between the oxo-norbornadiene products, as these alkyne-furan Diels-Alder reactions are reversible.[27]

The modest reactivity of furan as a diene for [4+2] cycloadditions and the proclivity of the oxo-bridged bicyclic products to undergo cycloreversion have foiled attempted reactions with several other common dienophiles.[28] We have, therefore, investigated reactions of **26** with highly strained dienophiles, which have been shown to cycloadd irreversibly with furan and simple furan derivatives. Benzyne, cyclohexyne, and cyclopropene derivatives react productively with **26** to give the expected Diels-Alder adducts (entries 4–6, Table 1).[29,30,31] Each of these structures comprises the pentacyclic framework of BTX. We have found dienophile generation to be most effective using CsF to promote elimination of a vicinally-substituted silyl-vinyl triflate or -vinyl halide, and reaction efficiency is excellent for two of the examples shown. With these [4+2] additions, however, facial selectivities across the furan diene are comparable to those obtained with alkyne dienophiles and only slightly in favor of the desired product.

While Diels-Alder cycloadditions with furan **26** have provided a collection of structurally unique analogues possessing the pentacyclic core of BTX, our inability to improve levels of diastereoinduction in these reactions has challenged us to reconsider elements of our synthetic strategy. To this end, we have investigated reactions of furan **21** with dienophiles, including cyclohexyne and a substituted benzyne (Figure 4). The three-dimensional topology of this tetracyclic structure effectively precludes approach of the dienophile from the α face of the furan, and the desired products **29** and **30** are furnished with >20:1 stereoselectivity. By contrast, treatment of **21** with a cyclohexenyl phenyliodonium salt, a known cyclohexyne precursor, gives none of **29**, further illustrating the utility of the CsF-promoted method for generating strained dienophiles.[32] Products such as **29** and **30** should facilitate access to BTX analogues with alternative D-ring and/or homomorpholine configurations than those highlighted in Table 1 and may allow for preparation of the natural product itself.

Conclusions

We have outlined a synthetic plan that makes available novel pentacyclic forms of BTX possessing the fully elaborated C/D/E ring core. Highlights of this work include the application of sulfone **8** for Mannich addition with cyclopentadione, the use of 3-bromofuran as a vicinal dianion equivalent, desymmetrization of dione **17** through bis-acetal formation, Li-Br exchange for Cring formation, and homomorpholine assembly through reductive alkylation. In addition, Diels-Alder reactions with furans **21** and **26** and ring-strained dienophiles have been examined for introduction of the A-ring moiety. Use of CsF to trigger benzyne, cyclohexyne, and cyclopropene formation from corresponding trimethylsilyl starting materials has proven to be a general and effective method, affording in most cases high yields of the desired cycloadducts. The evaluation of these unique toxin derivatives as modulators of voltage-gated sodium channels is currently underway in our laboratory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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19. The preference for isomer **19-endo**, in which the furan unit is located on the concave face of the bicyclic, may be the result of a stereoelectronic preference for O-lone pair → σ*(C11–C9) delocalization.
20. Lithium-halogen exchange is performed on a 2:1 **19-endo/exo** mixture to give 60% isolated yield of **20** (89% based on theoretical maximum) and 30% of the proto-debrominated exo product.
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24. The major byproduct of this reaction results from reduction of aldehyde **25** to a primary alcohol. Mesylation of this alcohol and subsequent intramolecular S_N2 displacement gives **26**, increasing the overall yield of homomorpholine ring closure to 84%. See supporting information for details.
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27. Re-subjecting the purified products to the reaction conditions results in cycloreversion to starting materials.
28. Other alkyne and alkene substrates tested include ethyl propiolate and 2-bromo-4-ethoxycyclohexa-1,3-dienecarbaldehyde. Use of high pressure conditions (> 6 kbar) with methyl maleic anhydride also resulted in no reaction.
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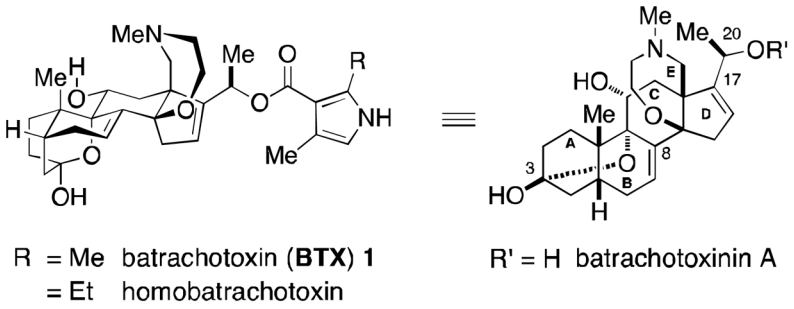


Fig. 1.
Structure of batrachotoxin and related compounds.

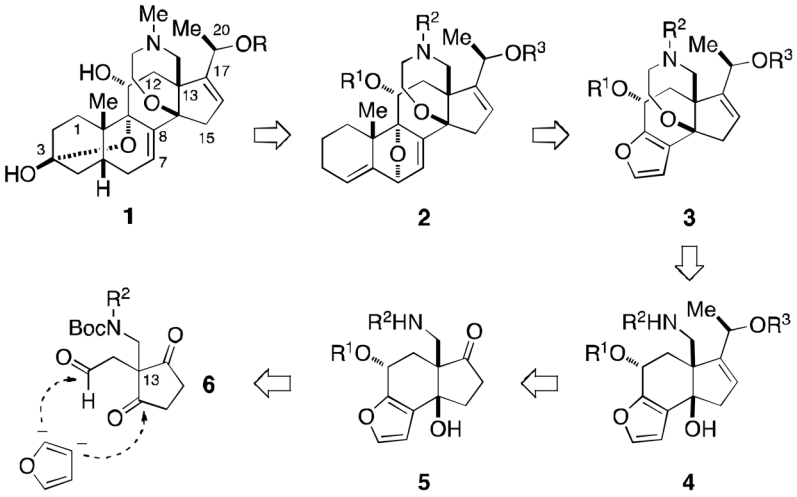


Fig. 2.
Retrosynthetic analysis of batrachotoxin **1**.

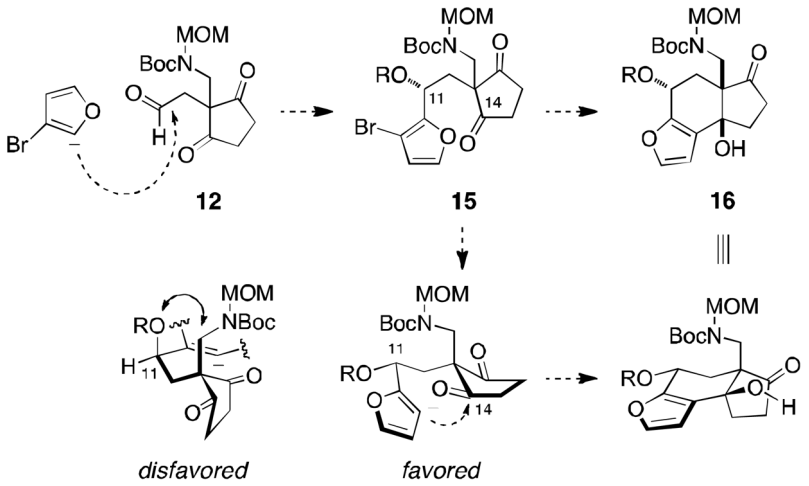


Fig. 3.
Predicted stereochemical outcome of furyl anion meso-breaking addition.

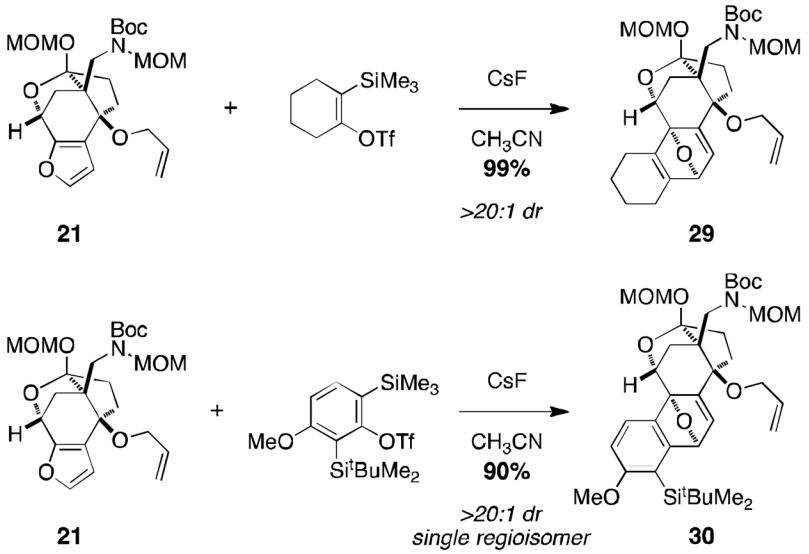
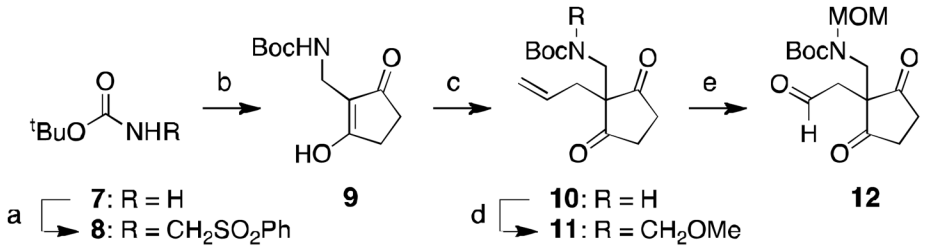


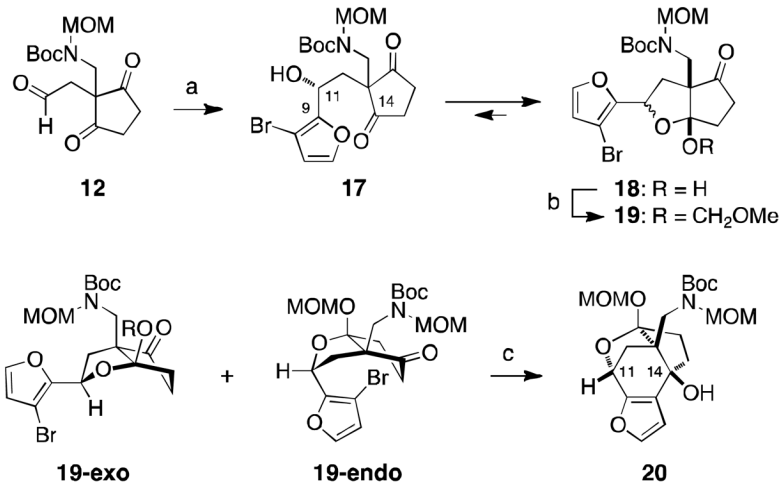
Fig. 4.

Highly selective cyclohexyne and benzyne Diels-Alder reactions [5] with furan **21**.



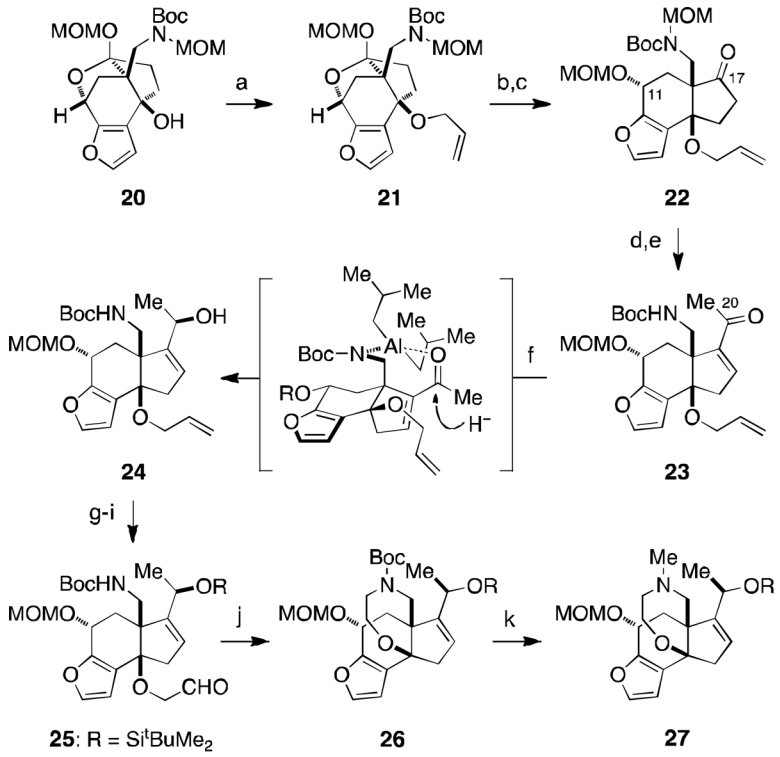
Scheme 1[a]

[a]Reagents and conditions: (a) $PhSO_2Na$, aq. $CH_2=O$, HCO_2H, MeOH, 78%; (b) 1,3-cyclopentadione, DBU, THF, 86%; (c) allyl bromide, K_2CO_3; toluene, 110 °C, (98%, 2 steps); (d) Me_3SiCl, paraformaldehyde, CH_2Cl_2, 0 °C; 9:1 MeOH/Et_3N, 5 °C, 80%; (e) O_3, CH_2Cl_2, −78 °C; Me_2S, 83%. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.



Scheme 2[a]

[a]Reagents and conditions: (a) 3-bromofuran, $LiN^{i}Pr_2$, THF, −78 °C, 3:2 dr, 89%; (b) MOMCl, *i*-$PrNEt_2$, 2:1 endo/exo, 80%; (c) *n*-BuLi, THF, −78 °C, 89% based on **19**-endo starting material.

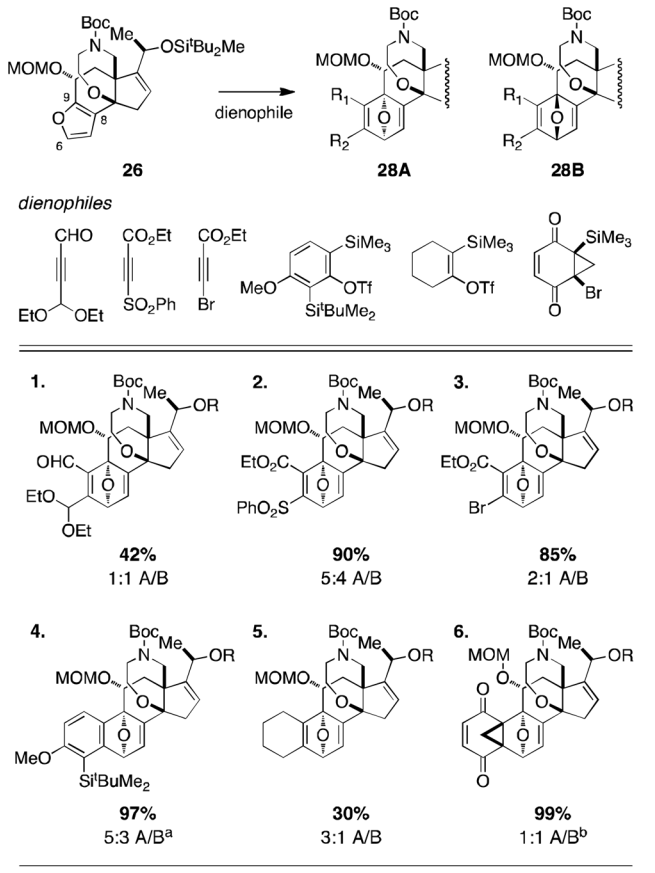


Scheme 3[a]

[a]Reagents and conditions: (a) allyl bromide, *n*-Bu_4NI, CH_2Cl_2, 50% aq. NaOH, 89%; (b) MeC(O)Cl, MeOH, 0 °C, 92%; (c) MOMCl, *i*-$PrNEt_2$, CH_2Cl_2, 40 °C, 92%; (d) $KN(SiMe_3)_2$, *N*-(5-chloro-2-pyridyl)triflimide, THF, −78 °C, 95%; (e) tributyl(1-ethoxyvinyl)tin, 10 mol% $Pd(PPh_3)_4$, CuCl, DMSO, 65 °C; 4N aq. HCl, EtOAc, 0 °C, 85%; (f) *i*-Bu_2AlH, toluene, −78 °C, 76%; (g) *t*-$BuMe_2SiOTf$, 2,6-lutidine, CH_2Cl_2, −78 °C, 99%; (h) 14 mol% OsO_4, NMO, H_2O/THF; (i) $Pb(OAc)_4$, CH_2Cl_2, 91% (over 2 steps); (j) $MgSO_4$, CH_3CO_2H, DCE; $NaBH_3CN$, 74%; (k) $LiAlH_4$, THF, 65 °C, 73%. NMO = *N*-methylmorpholine *N*-oxide.

Table 1

Late-stage Diels-Alder cycloaddition reactions with furan **26**.



[a] A regioisomeric Diels-Alder product having C6 *S,* C9 *R* stereochemistry is also obtained.

[b] The cyclopropane stereochemistry in B is inverted from that depicted (i.e., C5 *S*, C10 *R*). In addition, a third product having C5 S, C6 S, C9 *R*, C10 *R* stereochemistry is also obtained, see supporting information for details.