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Inverse-electron-demand Diels–Alder approach to densely substituted bicyclo[3.2.2]nonanes: Discovery of an autophagy regulator

Jiulong Li[a,b], Pengxin Ren[a,b], Lin Wang[c], Yuting Zhang[c], Peng Yang[a,*], Weiwei He[c,*], Ang Li[b,*]

[a] College of Chemistry & Henan Institute of Advanced Technology, Zhengzhou University, Zhengzhou 450001, China
[b] State Key Laboratory of Chemical Biology, Shanghai Institute of Organic Chemistry, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai 200032, China
[c] Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China

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ABSTRACT

Natural products bearing a bicyclo[3.2.2]nonane motif pose a considerable challenge to chemical synthesis. We developed a europium-promoted inverse-electron-demand Diels–Alder reaction of benzo[2,3]tropone derivatives with electron-rich olefins, which offers an expeditious approach to densely substituted bicyclo[3.2.2]nonanes. This method enabled the concise synthesis of a tetracyclic amine, subsequently identified as a downstream suppressor of autophagy.

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The three-dimensional structural motifs of natural products [1] are increasingly recognized for their significance in chemical biology [2,3] and drug discovery [4]. Polycyclic natural products containing a bicyclo[3.2.2]nonane motif (highlighted in red in Fig. 1) [5–7], such as goupiolone B [5], phleghenrine A [6], and acremoxanthone A [7] (**1**–**3**, Fig. 1), present a formidable challenge to chemical synthesis. A variety of methods have been developed to construct such a structural motif in natural products and related compounds [8–17]. However, many of them involve multistep sequences [8–13]. Strategically, the intermolecular Diels–Alder cycloaddition of tropone derivatives and olefins (highlighted in blue in Fig. 1) offers a desirable approach for assembling bicyclo[3.2.2]nonanes [15–17]. Benzo[2,3]tropones [18] would be advantageous substrates for the [4 + 2] cycloaddition [19,20] because they are not prone to undesired [6 + 4] or [8 + 2] cycloaddition [21,22] compared to standard tropones. However, most reported examples are indeed formal [4 + 2] reactions [5,23–25] proceeding *via* enolate intermediates, which limit the applicability [5,24] and sometimes result in unsatisfactory efficiency [25]. To our knowledge, the inverse-electron-demand Diels–Alder (IEDDA) reaction [26,27] involving benzo[2,3]tropones remains largely unexplored. Following our continued interest in the Diels–Alder cycloaddition for complex molecule synthesis [28–36], we initiated an investigation into the IEDDA reaction of benzo[2,3]tropone-type dienes with electron-rich dienophiles and explored its application in the synthesis of polycyclic natural products and biologically active molecules, particularly autophagy regulators [37,38] and cell cycle inhibitors [39,40]. Herein, we report the assembly of densely substituted bicyclo[3.2.2]nonanes *via* such a cycloaddition reaction, which leads to the discovery of an autophagy suppressor.

We first explored the cycloaddition of 6-methoxycarbonylbenzo[2,3]tropone (**4**) and cyclopentadiene (**5**) (Table 1). Notably, Ghosh and co-workers demonstrated that benzo[2,3]tropone acted as a dienophile in a normal-electron-demand Diels–Alder (NEDDA) reaction with **5** [41]. Introducing the methoxycarbonyl group would not only provide a functional handle for further derivatization of the product(s), but also enhance the dienic reactivity of benzo[2,3]tropone in the IEDDA reaction. To our delight, thermal treatment of **4** and **5** at 80 °C afforded the desired cycloadducts (**6a** and **6b**) in a *ca.* 18:1 ratio (entry 1), with a strong preference for the *endo* product. The position of the C=C bond within the cyclopentene ring of each product was determined by X-ray crystallographic analysis (Table 1). The excellent *endo* selectivity may be attributable to secondary orbital interactions [42]. Despite complete suppression of the NEDDA reaction (Table 1), a substantial portion of **4** was recovered, which

* Corresponding authors.
E-mail addresses: yangpeng_imb@zzu.edu.cn (P. Yang), heweiwei@ecust.edu.cn (W. He), ali@sioc.ac.cn (A. Li).

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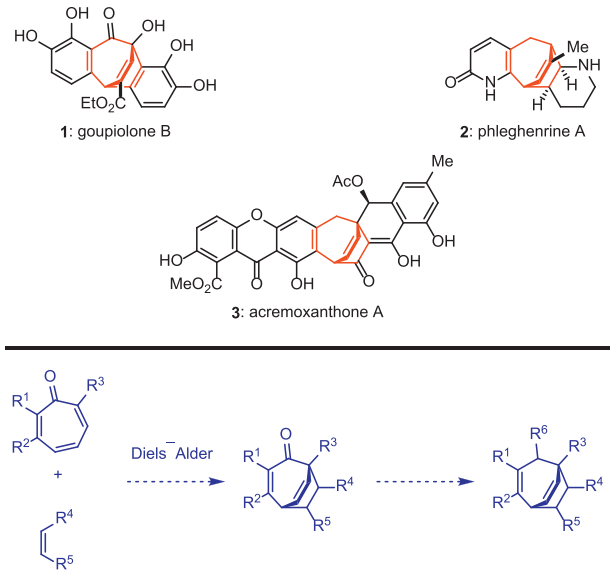
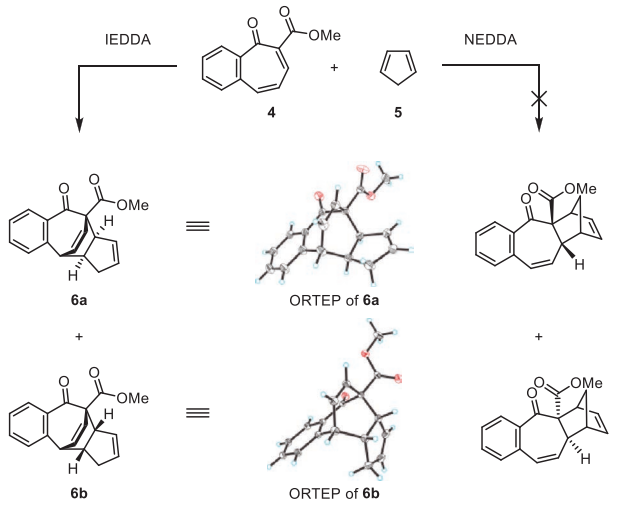


Fig. 1. Selected natural products containing a bicyclo[3.2.2]nonane motif and an envisioned IEDDA approach to such a structural motif.

Table 1
Investigation of the conditions for the IEDDA reaction.



Entry	Conditions [a]	6a + 6b	6a:6b[b]
1	Toluene, 80 °C	29%	18:1
2[c]	$BF_3{\cdot}OEt_2$, CH_2Cl_2, −20 °C	70%	9:1
3[c]	Me_2AlCl, CH_2Cl_2, 0 °C	66%	8:1
4[c]	$Bi(OTf)_3$, CH_2Cl_2, 22 °C	79%	5:1
5[c]	$Eu(OTf)_3$, CH_2Cl_2, 22 °C	81%	17:1
6[c]	$Eu(fod)_3$, CH_2Cl_2, 22 °C	92%	>20:1
7[c]	$Eu(fod)_3$, THF, 22 °C	32%	>20:1

[a] Reactions were performed with substrates **4** (1.0 equiv.) and **5** (3.5 equiv.) at the indicated temperature for 6 h.
[b] Determined by ^1H NMR spectroscopic analysis.
[c] 10 mol% Lewis acid.

resulted in reduced efficiency of the IEDDA reaction (entry 1). Thus, we turned to Lewis acids to lower the LUMO energy of **4**. As shown in Table 1 (entries 2–4), $BF_3{\cdot}OEt_2$, Me_2AlCl, and $Bi(OTf)_3$ significantly increased the dienic reactivity of **4** but concomitantly compromised the *endo*/*exo* selectivity. Our experience with lanthanide-mediated Diels–Alder cycloaddition [29,32,36] prompted us to examine europium-based promoters for this reaction. $Eu(OTf)_3$ improved both efficiency and selectivity (entry 5), while $Eu(fod)_3$ rendered the optimal results (92% combined yield, *endo*:*exo* > 20:1) (entry 6). Switching the solvent from CH_2Cl_2 to THF markedly reduced the yield of the desired products (entry 7).

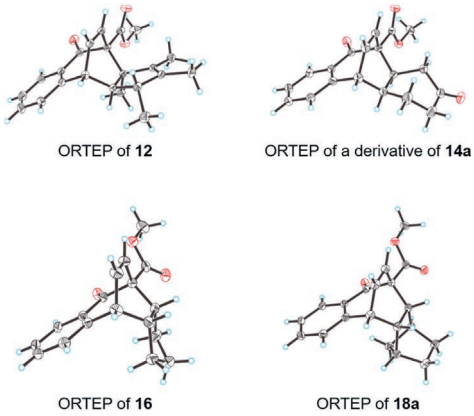


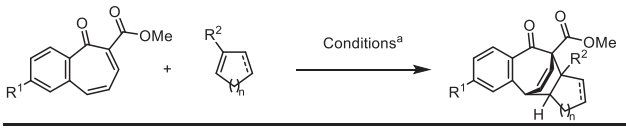
Fig. 2. ORTEP representations of compounds **12**, **16**, **18a**, and a derivative of **14a**.

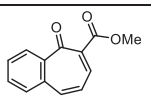
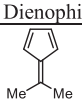
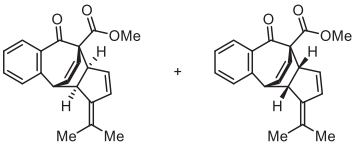
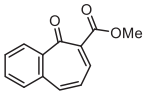
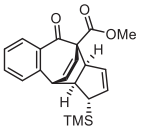
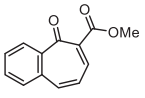
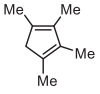
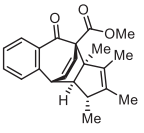
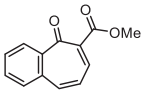
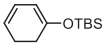
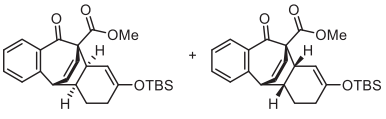
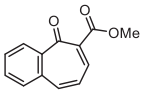
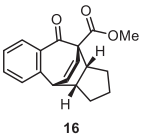
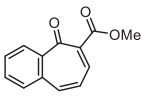
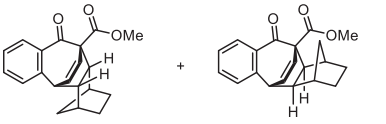
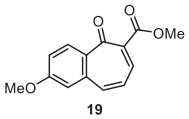
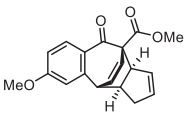
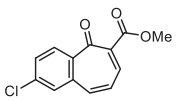
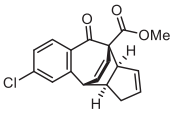
Having established the optimal conditions, we investigated the substrate scope of the IEDDA reaction (Table 2). As shown in entries 1–4, the cycloaddition of **4** with various cyclic dienes demonstrated high *endo* selectivity. 6,6-Dimethylfulvene (**7**) performed well in this reaction; cycloadducts **8a** and **8b** (*ca.* 10:1) were isolated in 82% combined yield (entry 1). Despite steric hindrance, TMS-cyclopentadiene (**9**) reacted smoothly with **4** to afford compound **10** as the sole detectable diastereomer in 67% yield (entry 2). Retention of the TMS group in **10** supported a concerted rather than a stepwise mechanism. 1,2,3,4-Tetramethyl-1,3-cyclopentadiene (**11**) could equilibrate *in situ* to its positional isomer through a [1,5]-H shift [32,43], which subsequently underwent cycloaddition to form **12**, a remarkably crowded tetracyclic compound, in 44% yield (entry 3). The IEDDA reaction between 1-siloxycyclohexadiene **13** and **4** afforded **14a** and **14b** (*ca.* 6:1) in 90% combined yield (entry 4). Due to the lower reactivity of mono-alkenes, the stronger promoter $Bi(OTf)_3$ was required for their cycloaddition with **4** (entries 5 and 6). For cyclopentene (**15**), the *exo* product (**16**) was obtained as the single detectable diastereomer in 66% yield (entry 5). For norbornene (**17**), a mixture of the *exo* and *endo* products (**18a** and **18b**; *ca.* 2:1) was isolated in 68% yield (entry 6). Notably, two analogs of **4** (**19** and **20**), bearing different substituents on the benzene ring, underwent smooth cycloaddition with **5** to give *endo* products **21** and **22**, respectively, as single diastereoisomers (entries 7 and 8). The structures of **12**, **16**, **18a**, and a derivative of **14a** were confirmed by X-ray crystallographic analysis (Fig. 2).

We further derivatized the obtained bicyclo[3.2.2]nonane-containing compounds to identify biologically active candidates. A representative example is illustrated in Scheme 1. Selective epoxidation of **6a** with *m*-CPBA in the presence of $NaHCO_3$ afforded compound **23** in 72% yield. Acidic hydrolysis of the epoxide, followed by oxidative cleavage of the resulting diol with aqueous $NaIO_4$, provided bis-aldehyde **24**, which underwent double reductive amination [$BnNH_2$, $NaBH_3CN$, HOAc] to furnish tetracyclic amine **25** in 74% overall yield.

In light of our sustained interest in modulating autophagy with small molecules [37,38], we evaluated the effect of the above compounds on autophagic flux. The autophagy marker microtubule-associated protein light chain 3B (LC3B) [44,45] was analyzed in human lung carcinoma cells (A549) by immunoblotting. As shown in Fig. 3A, compound **25** induced a dose-dependent increase in the levels of LC3B-II, the phosphatidylethanolamine-conjugated form of LC3B, which pointed to this compound as a potential regulator

Table 2
IEDDA cycloaddition of benzo[2,3]tropone derivatives with electron-rich olefins.

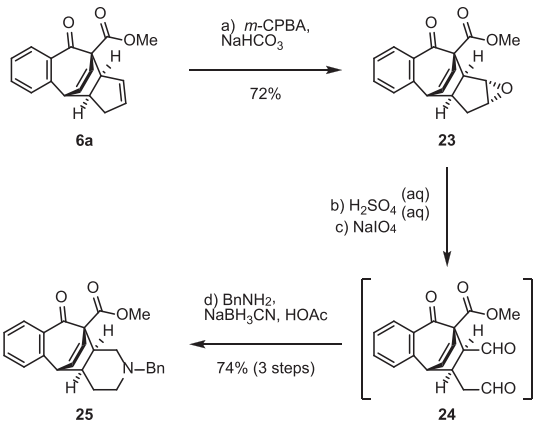


Entry	Diene	Dienophile	Cycloadduct	Yield (%) (ratio[b])
1	 **4**	 **7**	 **8a** + **8b**	82 (*ca.* 10:1)
2	 **4**	 **9**	 **10**	67
3	 **4**	 **11**	 **12**	44
4	 **4**	 **13**	 **14a** + **14b**	90 (*ca.* 6:1)
5[c]	 **4**	 **15**	 **16**	66
6[c]	 **4**	 **17**	 **18a** + **18b**	68 (*ca.* 2:1)
7	 **19**	 **5**	 **21**	79
8	 **20**	 **5**	 **22**	83

[a] Reactions were performed with dienes (1.0 equiv.) and dienophiles (3.5 equiv.) in the presence of 10 mol% $Eu(fod)_3$ at 22 °C for 6 h.
[b] Determined by ^1H NMR spectroscopic analysis.
[c] $Bi(OTf)_3$ was used in place of $Eu(fod)_3$.

of autophagy. To investigate whether compound **25** stimulates autophagy upstream or inhibits it downstream, we compared LC3B-II levels in A549 cells treated with **25** alone *versus* those co-treated with **25** and the autophagy inducer rapamycin (RAPA) [44,45]. The co-treatment significantly increased LC3B-II levels (Fig. 3B), which suggested a suppressive role for this compound in downstream autophagy. Autophagic flux was further assessed by using mCherry–eGFP–LC3B [46], a tandem fluorescent protein-tagged LC3 reporter. In A549 cells expressing this reporter, the fluorescence pattern of LC3B-positive puncta revealed that treatment with **25** led to an accumulation of autophagosomes and a reduction in autolysosomes (Fig. 3C), which indicated that this compound inhibits autophagy at a downstream stage. These findings highlight this compound as a promising chemical scaffold for developing autophagy modulators [47].

In conclusion, we developed a facile IEDDA approach to densely substituted bicyclo[3.2.2]nonanes, which enabled the discovery of an autophagy regulator. Exploration of the synthesis of bicyclo[3.2.2]nonane-containing natural products *via* this method is currently underway in our laboratories.



Scheme 1. Synthesis of a tertiary amine bearing a bicyclo[3.2.2]nonane motif.

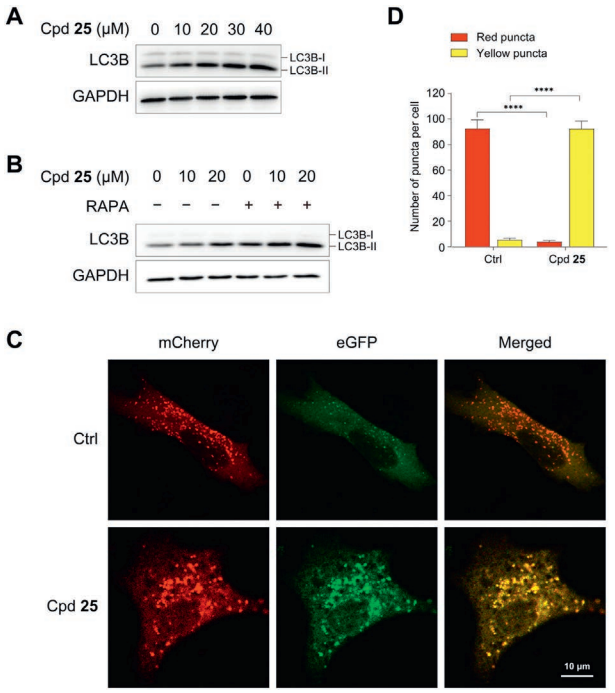


Fig. 3. Compound **25** suppresses downstream autophagy in A549 cells. (A) Immunoblot analysis of LC3B in A549 cells treated with **25** at the indicated concentrations for 12 h. (B) Comparison of LC3B levels in A549 cells treated with **25** alone at the indicated concentrations for 12 h *versus* those co-treated with **25** at the indicated concentrations and RAPA (200 nmol/L) for 12 h. μM = μmol/L. (C) Microscopy analysis of fluorescent puncta in A549 cells expressing mCherry–eGFP–LC3B. Cells were treated with **25** (20 μmol/L) for 12 h. (D) Quantitative analysis of the number of yellow puncta and red puncta per cell in the above experiment. Data are presented as mean ± SEM. $^{****}P < 0.0001$ ($n = 25$, two-tailed Student's *t*-test). DMSO served as the vehicle control for the compounds.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Jiulong Li: Writing – original draft, Investigation. **Pengxin Ren:** Investigation. **Lin Wang:** Investigation. **Yuting Zhang:** Investigation. **Peng Yang:** Writing – original draft, Supervision. **Weiwei He:** Writing – original draft, Supervision. **Ang Li:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2025.111381.

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