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Maleimide-based metal-free ligation with dienes: a comparative study

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A brief literature survey reveals that metal-free ligation such as the maleimide-based cycloaddition with electron-rich (hetero)diene is a widespread tool for the assembly of (bio)molecular systems with applications in biotechnology, materials science, polymers and bio-organic chemistry. Despite their everyday use, only scattered data about their kinetics as well as the stabilities of corresponding products under physiological conditions, are accessible. These key parameters are yet, of paramount importance to ensure the rapid and effective preparation of stable compounds. Herein is reported a systematic study regarding the different classes of dienes used in chemoselective ligation, including their accessibility and stability, as well as comparative kinetic experiments and products stability assays. We took advantage of these data to develop a double labeling strategy from the combined use of cyclopentadiene and oxazole dienes.

Introduction

Maleimide is indisputably a privileged scaffold for the design of biomolecular systems and tailor-made materials that are finding widespread utility in biotechnology, polymer and materials science.¹⁻⁴ The constant interest in maleimide derivatives is partly due to their remarkable reactivity, chemoselectivity, stability, and to the fact that a wide range of maleimide-activated labels are now commercially available (Figure 1). Moreover, maleimide-based chemical reactions proceed under metal-free and physiological conditions suitable for biomolecules modification.

On the one hand, maleimide readily undergoes Michael addition with thiols or biothiols and prominent examples in this context include the design of marketed antibody-drug conjugate (ADCs), like Kadcyra and Adcetris used for cancer treatment.⁵ On the other hand, this cyclic electron-poor system is a particularly convenient dienophile partner for Diels-Alder (D-A) cycloaddition reactions with diene-modified (bio)molecules, which have been exploited for the site-specific derivatization of peptides and proteins,⁶⁻⁸ monoclonal antibodies,^{9,10} oligonucleotides,¹¹⁻¹⁴ polysaccharides, as well as for their immobilization on surface.^{15,16} In this context, recent examples include the preparation of carbohydrate-based affinity adsorbents by Fort and coworkers through a furan/maleimide strategy for immobilizing oligosaccharides on solid matrices.¹⁶ The resulting glycoconjugates were used for the purification of lectins and the capture of anti-blood-group

A and -B antibodies. In addition, Read de Alaniz and Christie reported the production of an antibody-drug conjugate by reacting the maleimide-based drug-linker AZ1508 with a cyclopentadiene-based non canonical lysine residue genetically incorporated into the human IgG1 antibody.¹⁷ Finally, Overkleeft and coworkers demonstrated the applicability of the maleimide-based D-A strategy for monitoring the activity of endogenously expressed proteases in cellular extracts.¹⁸

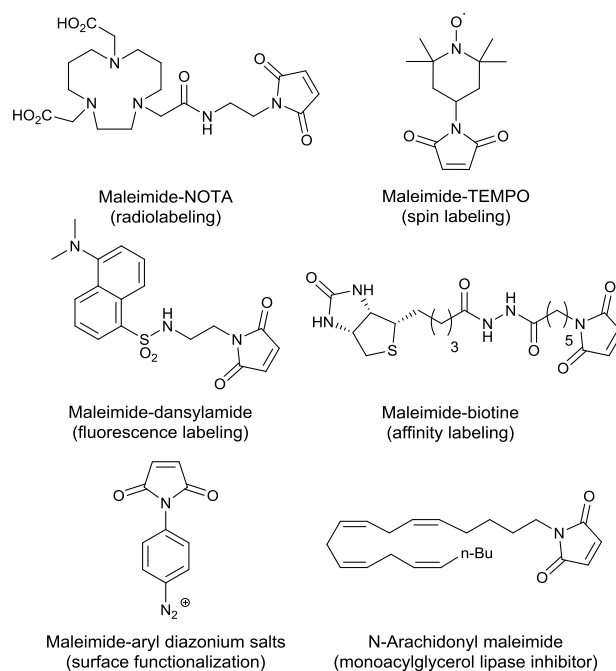


Figure 1. Representative examples of commercial maleimide-activated labels.

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We have recently reviewed the current state-of-the-art in the use of maleimides for bioconjugate reactions,⁴ and noticed, however, that only a few scattered comparative studies between the different dienes were reported. Thus selecting the appropriate D-A partner according to the targeted applications is not straightforward, although important theoretical studies have recently been reported on the prediction of rate constants¹⁹ and factors governing D-A reactions.^{20,21} To address this gap, we report, herein, a systematic comparative study of maleimide-based D-A strategies in terms of dienes accessibility and stability, reaction rates, as well as cycloadducts isolation and stability. Large differences in reaction rate of D-A suggest that dienes could be used concomitantly in sequential labeling strategies, which should open a new route for the design of multiple conjugate systems.

Results and discussions

Diene synthesis/accessibility

Dienes of different natures (linear, cyclic, polycyclic, heterocyclic), which were already reported to react specifically with maleimides in chemoselective ligation, were investigated in this study. Position of the substituent on the diene moiety was selected according to their previous or potential use in ligation experiments (Figure 2).

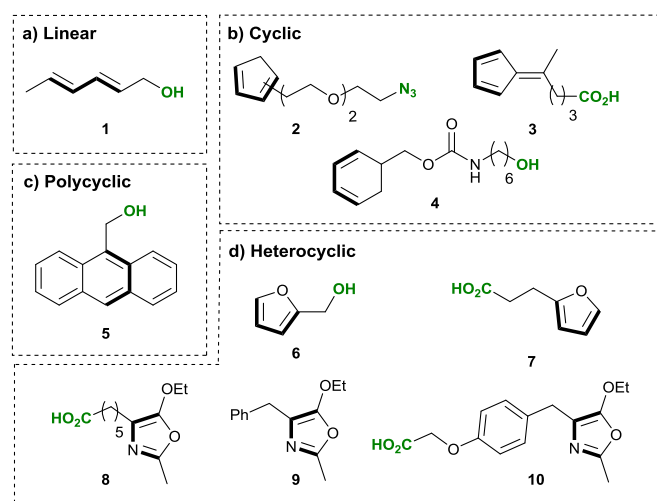
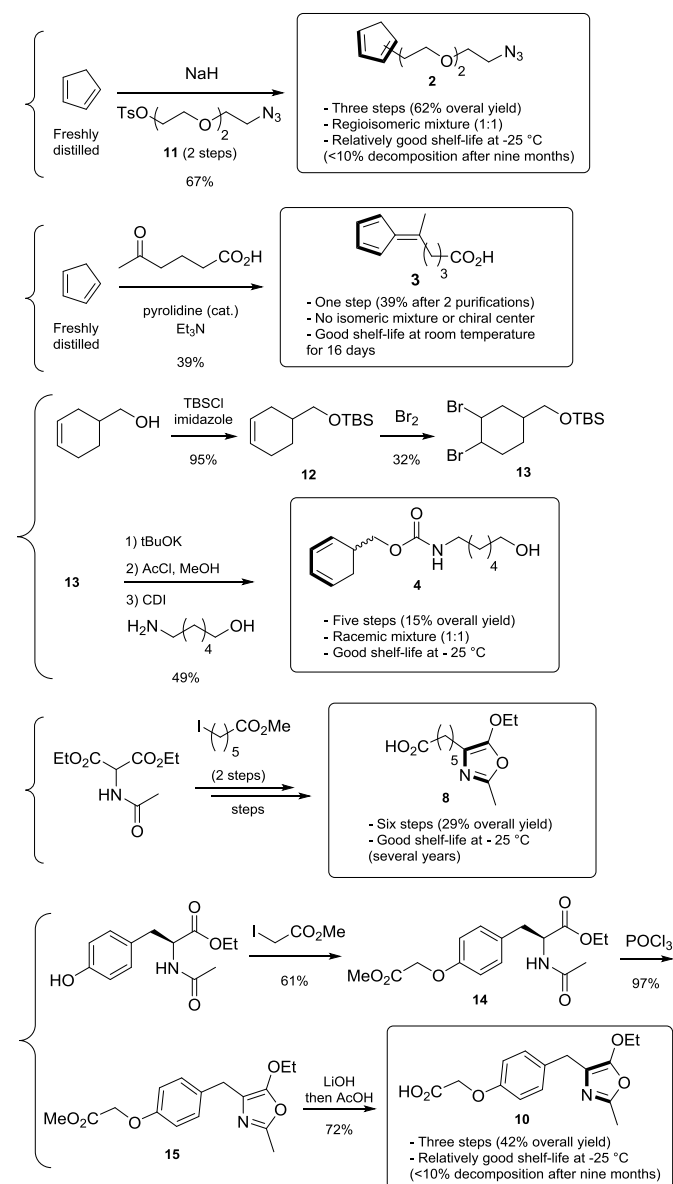


Figure 2. Examples of functionalized dienes used in chemoselective ligations with maleimides.

Dienes **1**^{6,7,11,18,22}, and **5-7**^{11,23} are commercially available, while **2**,^{15,24} **3**,²⁵ **4**^{12,14} and **8-9**⁸ were prepared according to known procedures. In addition, we also report here a novel tyrosine-based oxazole derivative **10** readily obtained from commercially available tyrosine ethyl ester. First, the cyclopentadiene derivative **2** was synthesized in a total of three steps through freshly distilled cyclopentadiene, and

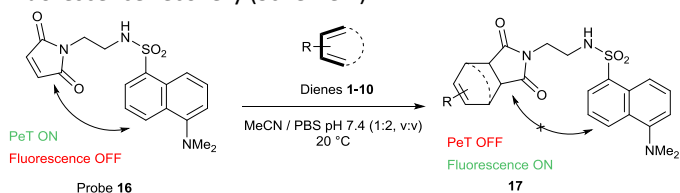
tosylate **11**,²⁶ which was obtained in two steps from commercially available 2-[2-(2-chloroethoxy)ethoxy]ethanol (Scheme 1). Monosubstituted cyclopentadiene **2** was isolated as a mixture of two inseparable position isomers, 1- and 2-cyclopentadiene (ratio \approx 1:1), resulting from a well-established fast [1,5]-hydride shift.²⁷ Additionally, a fast dimerization of **2** was observed after 36 h at room temperature (Figures S34-S35). Nevertheless, it was relatively stable when stored at -25 °C, as less than 10% dimerization was observed after 9 months of storage. It is also worth noting that Read de Alaniz and Christie observed that cyclopentadiene-modified lysine was stable over the 11-day antibody expression process.¹⁷ Despite a modest isolated yield of 39 %, fulvene **3** requires only one synthetic step from commercially available starting materials.²⁵ Furthermore, no isomeric mixture nor chiral centre is present in this structure. In contrast to cyclopentadiene **2**, fulvene **3** was fully stable for more than 16 days at room temperature (Figure S36). Next, the racemic cyclohexadiene **4** was prepared from commercial cyclohex-3-en-1-ylmethanol in a total of 5 synthetic steps and 15% overall yield (Scheme 1).^{12,14} This cyclic diene was, however, isolated along with 6 % of its aromatized analog, benzyl carbamate (Figure S37), as an inseparable mixture, whatever the purification method used (normal-phase flash chromatography, RP-HPLC or recrystallization in water). These past years, our team reported the preparation of 5-ethoxyoxazole dienes from commercial α -hydroxy- or α -amino esters for the total synthesis of natural compounds,²⁷⁻³⁰ and for bioconjugation purposes.^{8,31,32} Importantly, the azaphthalimide linkers generated from corresponding 5-ethoxyoxazoles revealed fluorescence behaviors, especially in aqueous media (Figure S2). In the context of modification of peptides, 5-ethoxyoxazole **8** bearing a carboxylic acid function was prepared on the gram scale in a total of six steps and 29% overall yield.⁸ Interestingly, this compound proved stable for several years at -25 °C. We postulated that protonation of the nitrogen atom of the oxazole ring by the carboxylic acid moiety preserved **8** against oxidative degradation, such as the formation of the N-oxide derivative which could be observed by HRMS analysis in a sample of degraded **9** (Figure S39). Thus, to support this hypothesis and to find a reliable procedure for long-term storage of 5-alkoxyoxazoles, the model compound **9** containing 1 equiv. of acetic acid was also stored at -25 °C for nine months. After that time, only 16% of degradation was observed, while at the same time the model oxazole **9** contained more than 80% of oxidation product (Figure S38). As a result, a new bioconjugatable analogue **10** of compound **9**, bearing a carboxylic acid, was prepared from commercially available ethyl N-acetyl-L-tyrosinate in only three steps and 42% overall yield. As expected, this diene proved stable since less than 10% of oxidation was observed after nine months of storage at -25 °C (Figure S40).



Scheme 1. Syntheses of conjugatable non-commercially available dienes **2-4** and 5-ethoxyoxazoles **8** and **10**.

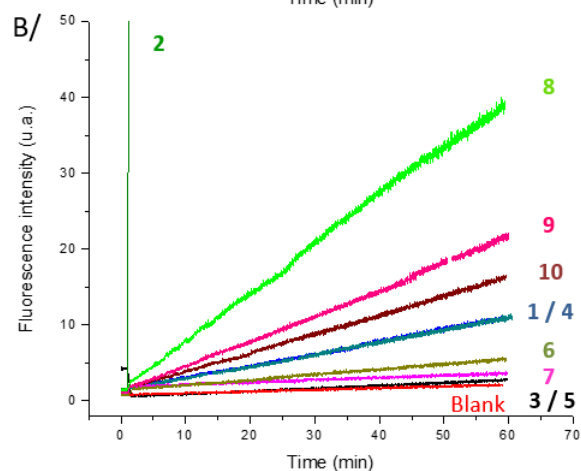
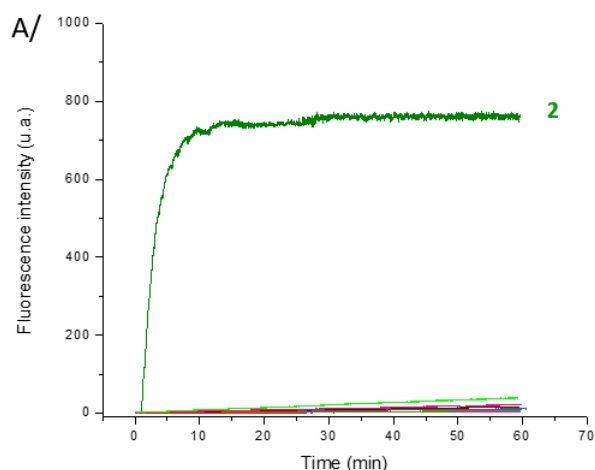
Comparative kinetics study in D-A reactions of dienes with maleimide: towards double labeling strategies

With dienes **1-10** in hand, their ability to react with maleimides was investigated through the use of a maleimide-based probe **16** whose fluorescence is quenched by a photoinduced electron-transfer (PeT) process, which occurs between the maleimide and the dansyl dye.^{34,35} Then, upon reaction with dienes, the maleimide scaffold is transformed into the corresponding cycloadduct, which leads to a full dansyl fluorescence recovery (Scheme 2).



Scheme 2. D-A cycloaddition reactions between dienes **1-10** and probe **16** for kinetic comparisons study.

The comparative efficiency of dienes for cycloaddition with maleimides was thus easily assayed by determining the time course of fluorescence intensity enhancement over a 60-minute time scale. Results are summarized in Figure 3. Dienes from each class of compounds were also subject to RP-HPLC analysis at $t = 10$ minutes for cyclopentadiene **2**, and $t = 60$ minutes for other dienes to confirm fluorescence recovery assays (Table 1). The cyclopentadiene derivative **2** exhibited by far the highest rate of fluorescence enhancement. After 10 min of reaction, maleimide **16** was entirely consumed, which was also confirmed by RP-HPLC analysis (Figure S10). A calibration curve that correlates the fluorescence intensity to the cycloadduct concentration enabled us to determine the second order kinetic rate constant $k_2 = 6.50 \pm 0.02 \text{ M}^{-1} \cdot \text{s}^{-1}$ in MeCN/PBS pH 7.4 (1:2, v:v) at $T = 20 \text{ }^\circ\text{C}$ (Figures S19-S21). While results obtained by fluorescence experiments matched well with the ones determined by RP-HPLC, the cycloaddition with fulvene and anthracene are the exception. In fact, no fluorescence intensity change could be detected after 60 minutes while cycloadducts formation was observed by RP-HPLC. This result from the fact that fulvene and anthracene absorb the incident UV light, which is confirmed by their absorption profiles that exhibit a band at 330 nm, preventing



thus the excitation of the dansyl dye (Figure S2).

Figure 3. A) Time-dependent fluorescence intensity increase of dansyl-based probe **16** (1 mM, 1 equiv.) in the presence of dienes **1-10** (5 mM, 5 equiv.) in MeCN/PBS pH 7.4 (1:2, v:v) recorded at λ_{em} 560 nm upon excitation at λ_{ex} 330 nm. B) Enlarged view of Figure 3A).

Of note their corresponding cycloadducts do not absorb at 330 nm (Figures S5-S8). Moderate to low conversions were observed with the other dienes. A conversion of 39 % was reached after 60 minutes of reaction with fulvene **3**, the second fastest diene. Then, 5-ethoxyoxazoles and anthracene have shown to react more slowly, with only 17, 13 and 11% of conversion for compounds **8**, **5** and **9**, respectively, after 60 minutes. Finally, cyclohexadiene **4**, *trans-trans*-2,4-hexadiene **1**, and furans **6-7** gave even lower kinetics, with less than 5% conversion after 60 minutes. The same trend was observed with a lower probe concentration (50 μ M, Figure S3). Following these results, k_2 was determined for several of these dienes. Due to their apparent slow kinetics, these values were determined by RP-HPLC analysis from calibration curves (Figures S22-S25), and results are summarized in Table 1.

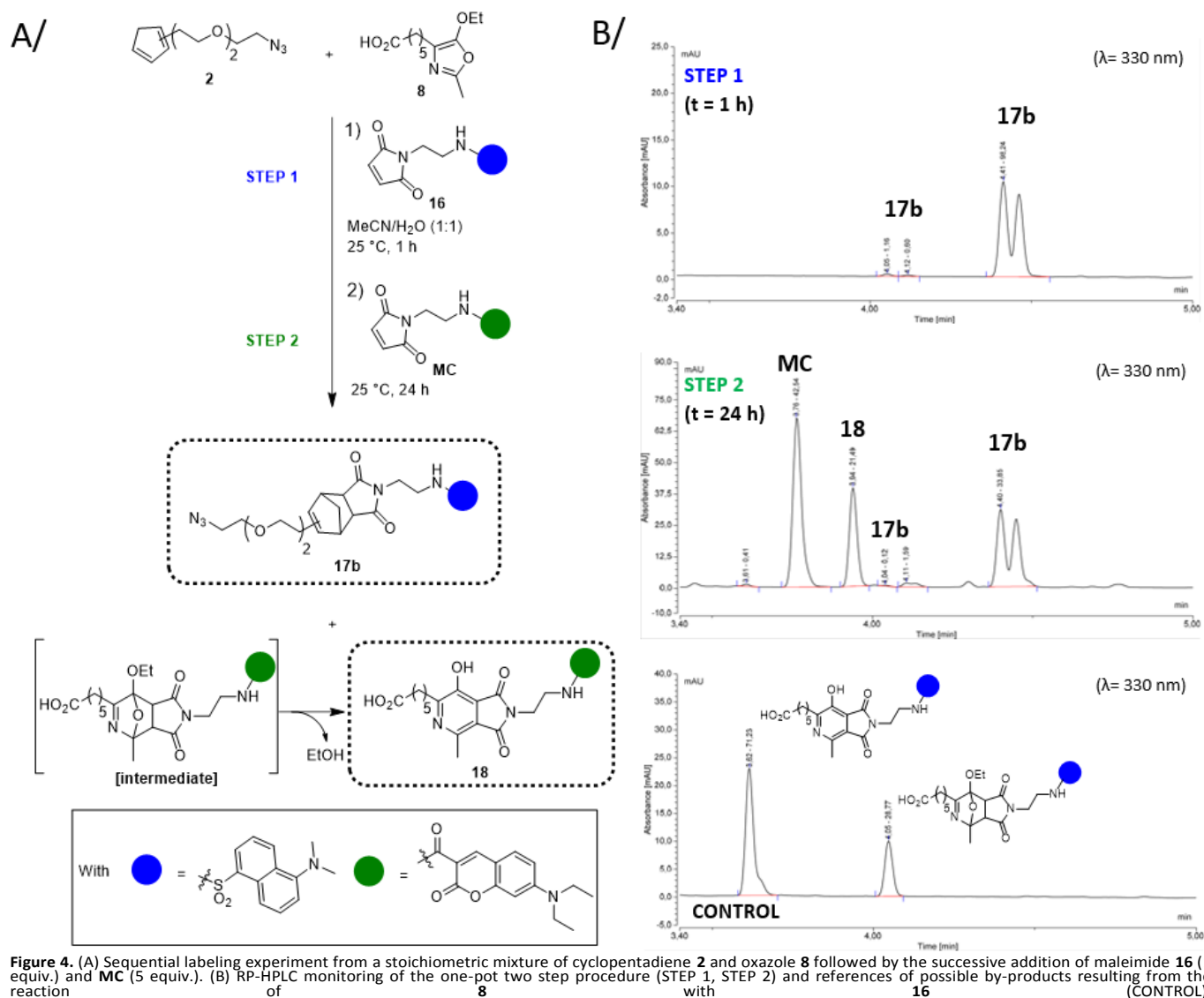
Table 1 Kinetic Data for the D-A Reaction in aqueous media.^a

Diene	% conv. (min)	k_2 ($M^{-1}.s^{-1}$)
1	1 (60)	$(8.9 \pm 0.7) \cdot 10^{-4}$
2	>99 (10)	6.50 ± 0.02
3	39 (60)	0.15 ± 0.01
4	4 (60)	n.d. ^b
5	13 (60)	n.d.
6	<1 (60)	$(8.9 \pm 0.1) \cdot 10^{-4}$
7	<1 (60)	n.d.
8	17 (60)	$(1.2 \pm 0.4) \cdot 10^{-2}$
9	11 (60)	$(5.1 \pm 0.3) \cdot 10^{-3}$
10	n.d.	n.d.

^a Reaction in MeCN/PBS pH 7.4 (1:2, v:v) at 20 °C monitored by RP-HPLC ($\lambda = 330$ nm), except for diene **2** where fluorescence spectroscopy was used. ^b n.d.: not determined.

The large difference in reaction rates observed between the cyclopentadiene and other dienes, such as 5-ethoxyoxazoles, prompted us to investigate their combined use in the prospect, for example, of developing one-pot double labeling strategies potentially useful for biological applications, or for the design of (hetero)multifunctional cross-linking reagents.^{3,36-39} Such an approach generally relies on the use of mutually orthogonal chemoselective ligations,⁴⁰ or the catalytic activation of one of the two ligations when they share a common chemical function such as copper-catalyzed alkyne azide cycloadditions (CuAAC) used in the combination of strain-promoted alkyne azide cycloaddition (SPAAC).⁴¹ In the present study, since one of the maleimide-based reagent was common to both chemical ligations, we questioned whether the selectivity for one of the two dienes could be controlled by their large kinetic difference. To verify this hypothesis, a stoichiometric mixture of the two sufficiently kinetically different dienes, namely cyclopentadiene **2**/oxazole **8** (1 equiv. each), was treated with maleimide **16** (1 equiv.) at 25 °C and stirred for 1 h (STEP 1, Figure 4A), before adding the coumarin-based maleimide **MC**⁴² (5 equiv.) (STEP 2). This one-pot 2 steps protocol was followed by RP-HPLC analysis. Figure 4B shows relevant enlarged views of RP-HPLC chromatograms recorded at 330 nm (λ_{abs} of dansyl) in order to simplify chromatograms and focus on the selectivity outcome of the first step. Full-view data also determined at 254 nm are also available in the ESI. The first step led exclusively to cycloadduct **17b**, observed as a mixture of 4 distinguishable isomers. Indeed, the formation of the competitive cycloadduct or azaphthalimide resulting from the reaction of the oxazole **8** with maleimide **16** was not observed (STEP 1 versus CONTROL, Figure 4B). Then, the addition of the second maleimide (**MC**) to the reaction mixture provided mostly the corresponding azaphthalimide **18** after 24 h (STEP 2). The aromatization of the oxabicyclic intermediate under “neutral conditions”, we previously observed in acidic conditions,⁸ and ending up in an irreversible rearomatization reaction process, is presumably promoted by the presence of an internal proton source thanks to the carboxylic acid function, combined with a long reaction time.

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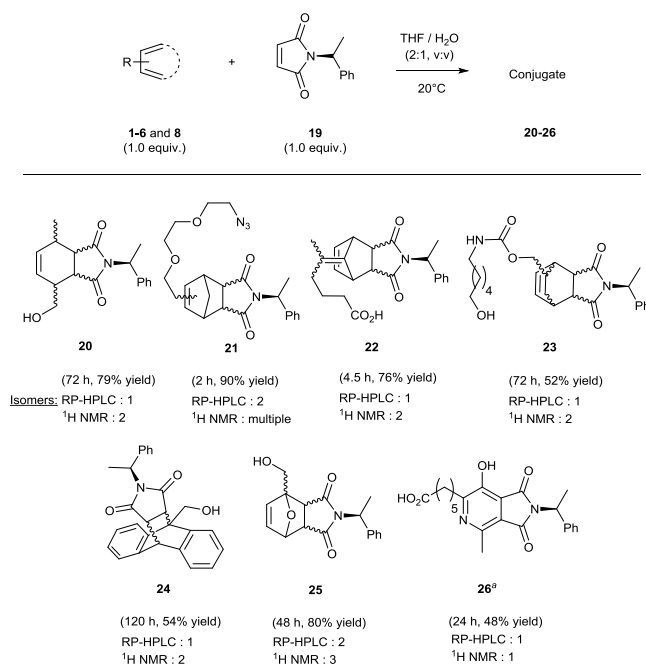


This experiment clearly illustrates that the kinetics difference between the D-A reactions could be exploited to achieve sequential bioconjugate reactions and to offset the need for chemoselectivity between the reactive functions. Moreover, a perfect chemical compatibility between **2** and **8** was observed in our study, which made very convenient this one-pot procedure.

Isolation and stability of conjugates, evaluation of isomer amounts.

Next, an enantiomerically pure maleimide partner **19**, namely (R)-1-(1-phenylethyl)-1H-pyrrole-2,5-dione, was selected as dienophile partner to study first how its chirality could impact HPLC analyses of isolated cycloadducts in terms of number of isomers, while avoiding issues related to the short half-life of chiral biomolecules for the stability study in blood plasma. In fact, the formation of uncontrolled stereogenic centers during the cycloaddition process combined to those already present in biomolecules, may lead to the formation of multiple isomers. This may complicate the reaction monitoring, as well as the bioconjugate isolation and characterization in particular with low-molecular weight conjugates (a few kDa).^{5,13,43}

Besides, isomeric products may display significant difference in biological, or physicochemical properties.⁴⁴ Accordingly, products **20-26** were prepared in moderate to good yields from corresponding dienes **1-6** and **8** in a solution of THF/water mixture, whereas one equivalent of trifluoroacetic acid was also added to the reaction mixture for compound **8** to furnish the corresponding the fluorescent azaphthalimide **26** (Scheme 3). Multiple isomers were observed by ¹H NMR analysis for all cycloadducts **20-25**, with the exception of azaphthalimide **26** whose stereogenic centers were suppressed by the aromatization step. Moreover, a mixture of two isomers was observed by RP-HPLC only for cycloadducts **21** and **25** generated from the cyclopentadiene and furan derivatives, respectively. This set of experiments clearly illustrates the advantage of the oxazole- based bioconjugate reaction for low molecular weight bioconjugates, since, provided the occurring further aromatization process, this is the only reaction yielding only one isomer (either regio or stereoisomer). Consequently, this is the only diene avoiding the formation of heterogeneous conjugates likely to have different properties.

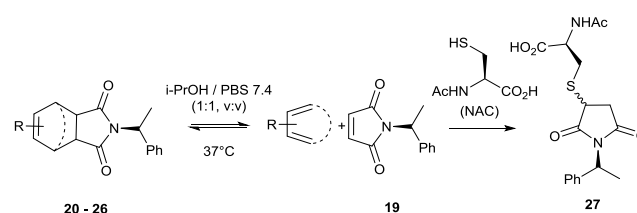


Scheme 3. Conjugates **20-26** obtained from Diels-Alder reactions of dienes **1-6** and **8** with maleimide **19**, and experimentally identified isomers. ^a Prepared in the presence of TFA (1 equiv.).

The stability of the conjugate adduct is important to ensure that the two ligated units will not prematurely dissociate in biological media. In fact, the reversible character of the D-A reaction is closely associated with the nature of the diene-dienophile pair. Moreover, imide-containing cycloadducts may also be subject to hydrolysis under physiological conditions, as reported for example for some thiosuccinimides resulting from the addition of biothiols to maleimides.^{4,45-48} Accordingly, the reversibility, and more generally the stability of imide-based cycloadducts obtained from different classes of dienes was

next investigated. It should be stressed that the oxazole strategy leads directly to the aromatized azaphthalimide scaffold under acidic conditions (the oxabicyclic intermediate being generally unstable during chromatographic purification, but prone to aromatization), and therefore could not be subjected to a retro-D-A process. The reversible behaviour of cycloadducts **20-25** was investigated at 37 °C, in a iPrOH/PBS pH 7.4 (1:1) solution containing 10 equiv. of N-acetylcysteine (NAC) as a fast maleimide-trapping agent (Figure S49).⁴⁹ Formation of the thiol Michael adduct **27**, resulting from thio-Michael addition reaction between NAC and **19**, will be observed only if rD-A reactions occurred (Scheme 4). Thioether **27** was synthesized, characterized and used as reference for this study. This compound was found to be fully stable after 24 h in iPrOH/PBS pH 7.4 (1:1), 37 °C. Stability experiments revealed that all cycloadducts were stable for 24 h under physiological conditions, except oxabicyclic **25**, for which thiol Michael adduct was observed in noticeable amount (30%, Figure S55) which was consistent with recent studies.⁵⁰ Of note, the azaphthalimide **26** was stable under these reaction conditions.

Finally, the chemical and enzymatic stability of conjugates were investigated by incubating compounds **20-26** in human blood plasma (HBP) at 37 °C for 24 h.⁵¹ HBP is composed of proteins (8%), organic compounds (1%), and mineral salts (1%). After 24 h incubation, HPLC-MS analyses showed that most of cycloadducts appeared to be stable, except compounds **20** and **26** for which monohydrolysis of the corresponding imide was observed in about 35 and 4%, respectively, along with presumably epimerization for **20** and traces of N-oxidation for **26**. A plausible explanation for this is that bridged ring systems in **21-25** provide additional steric hindrances in the vicinity of the imide function protecting this imide against hydrolysis, compared to simply fused ring scaffolds **20** and **26**. Of note, degradation products of **20** and **26** maintain nevertheless a covalent linkage between the two conjugation partners through the remaining amide bond.



Scheme 4. rD-A study in the presence of an excess of NAC as fast maleimide-trapping agent.

Conclusions

Herein is reported a comparative study of a collection of (hetero)diene systems involved in maleimide-based cycloaddition reactions that had proved useful applications for chemoselective ligation. It was observed that non-commercially available dienes were stable for several months at -25 °C or days at room temperature, and that 5-

alkoxyoxazoles required stabilization by an internal or external source of protons to protect them from long-term air N-oxidation. From this observation, it resulted the preparation of a novel and storable conjugable tyrosine-based oxazole, obtained within three steps and a good overall yield. In addition, while cyclopentadiene proved particularly unstable, a recent study reported *in situ* deprotection of cyclopentadienes to overcome their important lack of stability.⁵²

With regard to the kinetic study, it was established that cyclopentadiene conjugate reacts with maleimides between one and three orders of magnitude faster than the other diene families. The second order kinetic rate constants were found to be between 10^{-4} and $10 \text{ M}^{-1}\cdot\text{s}^{-1}$, comparatively, the Staudinger ligation has a rate constant of $10^{-3} \text{ M}^{-1}\cdot\text{s}^{-1}$.⁵³ Dienes may be ranked in descending order in terms of their reactivity as follows: cyclopentadiene, fulvene, 5-ethoxyoxazoles family and anthracene, cyclic and linear dienes and finally furan based derivatives. Such difference was leveraged in a proof-of-concept for sequential labeling strategy. The chemical compatibility observed between the cyclopentadiene and 5-ethoxyoxazole could further be exploited in the design of novel heterobifunctional cross-linking reagents with useful applications in bioconjugation and materials science.

The formation of multiple isomeric products was observed with all dienes, except for oxazole whose aromatization step leads to the formation of a single product, with unique fluorescent properties.

In addition, stability experiments revealed that only furan cycloadduct underwent cleavage through a rD-A process leading to the dissociation of the reaction partners under physiological conditions, whereas unbridged fused systems were mainly subject to monohydrolysis in HBP.

To conclude, since none of the dienes combines all the appropriate aforementioned properties (*i.e.* starting material commercially available and indefinitely stable at room temperature, ligation rate faster than $1 \text{ M}^{-1}\cdot\text{s}^{-1}$, single isomeric product, etc...), we hope this article will be a useful guide for the reader in choosing the most convenient diene according his targeted application and the (bio)molecular system which will be involved, in particular with regard to the accessibility/availability of dienes, reaction rates, products isolation and stability or fluorescence properties.

Conflicts of interest

There are no conflicts to declare.

Experimental

General

All chemicals were used as received from commercial sources without further purification, except cyclopentadiene which was freshly distilled prior to use. Solvents, unless otherwise stated, were purchased in reagent grade or HPLC grade and used as received. PBS (pH 7.4, 0.1 M) and aq. mobile phases

for HPLC were prepared with water that was purified by means of a MilliQ system (purified to 18.2 M Ω cm). For Diels-Alder reaction in aqueous media, MilliQ H₂O was used in the solvents system. Human blood plasma (HBP) was furnished by the Centre Hospitalier Universitaire de Rouen and was collected under medical conditions and supervisions from unknown donors. (CAUTION! HBP is a biological substance that must be handled with care by trained personnel. Vials, needles, pipettes, etc. must be considered and treated as biological wastes). All reactions were monitored by thin layer chromatography (TLC) and/or RP-HPLC. TLC were carried out on Merck DC Kieselgel 60 F-254 aluminum sheets. Visualization of spots was performed under a UV lamp at $\lambda = 254$ or 365 nm, and/or staining with a KMnO₄ solution/K₂CO₃ + 5% NaOH, developed with heat. Flash column chromatography purifications were performed manually on silica gel (40–63 μM) under pressurized air flow.

Instruments and methods

System A: RP-HPLC analyses were performed with a Thermo Scientific Ultimate[®] 3000 RS instrument, equipped with a diode array detector (DAD-3000RS) and temperature of the column compartment was fixed at 25 °C. A Thermo Fisher Hypersyl GOLD[®] column (1.9 μm , 2.1 \times 50 mm) was used with a binary solvent system composed of MeCN and 0.1% aq. formic acid (aq. FA, pH 2) as eluents (linear gradient from 5 to 100% MeCN over 6 min; 100% MeCN for 1.5 min; linear gradient from 100 to 5% MeCN over 1.5 min; 5% MeCN for 2 min) at a flow rate of 0.600 mL/min. **System B:** RP-HPLC analyses were performed with a Thermo Scientific Ultimate[®] 3000 RS instrument, equipped with a diode array detector (DAD-3000RS) and temperature of the column compartment was fixed at 25 °C. An Agilent SB-C18[®] column (1.8 μm , 2.1 \times 50 mm), equipped with a Phenomenex AJO-9000[®] C18 pre-column, was used with a binary solvent system composed of MeCN and 0.1% aq. formic acid (aq. FA, pH 2) as eluents (linear gradient from 5 to 100% MeCN over 6 min; 100% MeCN for 1.5 min; linear gradient from 100 to 5% MeCN over 1.5 min; 5% MeCN for 2 min) at a flow rate of 0.600 mL/min. **System C:** RP-HPLC-MS analysis were performed on a Agilent 1200 Series[®] and Bruker HCT ultra[®] instruments, equipped with a Thermo Fisher Accucore[®] C18 column (2.6 μm , 2.1 \times 150 mm). Temperature was maintained to 22 °C. Injection volume was 4 μL . UV detector (set at 254 nm) was coupled with a low-resolution MS detector (ESI/ion trap). Solvent system was composed of MeCN and 0.1% aq. formic acid (aq. FA, pH 2) as eluents (5% MeCN for 1 min, linear gradient from 5 to 100% MeCN over 14 min; 100% MeCN for 3 min; linear gradient from 100 to 5% MeCN over 1 min; 5% MeCN for 11 min) at a flow rate of 0.300 mL/min. **System D:** Semi-preparative RP-HPLC were performed with a Interchim PuriFlash[®] 4250 instrument equipped with a UV-visible detector, and a Hypersil Gold[®] column (5 μm , 250 \times 20 mm) with MeCN and 0.1% aq. FA as eluents (5% MeCN for 5 min, 5–100% MeCN for 48 min) at a flow rate of 20 mL/min.

High Resolution Mass spectrometry (HRMS) was performed using a Waters Micromass LCT Premier XE[®] equipped with an orthogonal acceleration time-of-flight (oa-TOF) and an electrospray source in positive or negative mode.

¹H, ¹³C spectra were recorded on 300 MHz Bruker FT-NMR machine operating at ambient probe temperature. The solvent resonance was used as the internal standard for ¹H-NMR (chloroform-d at 7.26 ppm ; acetonitrile-d₃ at 1.94 ppm ; acetone-d₆ at 2.05 ppm ; methanol-d₄ at 3.31 ppm ; DMSO-d₆ at 2.50) and ¹³C-NMR (chloroform-d at 77.0 ppm ; acetonitrile-d₃ at 1.3 ppm ; acetone-d₆ at 29.8 ppm ; methanol-d₄ at 49.0 ppm ; DMSO-d₆ at 39.5). Chemical shift (δ) were quoted in parts per million (ppm). Coupling constants (*J*) were quoted in Hertz (Hz). The following abbreviations were used to give the multiplicity of the NMR signals: s: singlet, bs: broad singlet, d: doublet, t: triplet, dd: doublet of doublet...

Fluorescence spectroscopic studies (emission/excitation spectra and time course) were performed on a Varian Cary Eclipse[®] spectrophotometer using a quartz fluorescence cell (Hellma, 104F-QS, 10 × 4 mm, pathlength 10 mm, chamber volume 3.5 mL or 1.4 mL). Excitation/emission spectra were recorded at 20 °C (330/560 nm, excitation and emission filters: auto, excitation and emission slit: 5 nm or 10 nm). UV-Vis spectroscopy was performed on a Agilent Cary 60 UV-Vis[®] at 20 °C.

For conjugates stabilities in HBP, centrifugations were performed with a VWR MicroStar 12[®] at 11.000 rotations/min for 30 min.

Synthesis

Diene synthesis

Synthesis of the cyclopentadiene (2)

Tosylate-azide (11). To a solution of 2-(2-(2-azidoethoxy)ethoxy)ethanol⁵⁴ (2.5 g, 14.3 mmol, 2 equiv.) in dry acetonitrile (20 mL), were successively added Et₃N (8.47 mL, 62.7 mmol, 4.4 equiv.) and TsCl (1.36 g, 7.1 mmol, 1.0 equiv.). The reaction mixture was stirred at room temperature for 2 h. After completion, a solution of saturated aq. NH₄OH (50 mL) was poured onto the reaction mixture, followed by brine (25 mL). The aqueous layer was extracted with EtOAc (3 × 50 mL). Combined organic layers were washed with brine (50 mL), dried over MgSO₄, and evaporated under vacuum. The crude product was purified by flash-column chromatography (silica gel, cyclohexane/EtOAc as gradient from 100:0 to 90:10, v/v) to provide the product (2.32 g, 7.03 mmol, 99 % yield) as a yellow oil. The following ¹H NMR analysis was in accordance with literature data.²⁵ ¹H NMR (300 MHz, CDCl₃): δ 7.82 – 7.76 (m, 2H), 7.37 – 7.30 (m, 2H), 4.19 – 4.13 (m, 2H), 3.73 – 3.66 (m, 2H), 3.66 – 3.61 (m, 2H), 3.60 (s, 4H), 3.40 – 3.33 (m, 2H), 2.44 (s, 3H).

Cyclopentadiene (2). To a solution of freshly distilled cyclopentadiene (380 μ L, 4.56 mmol, 3 equiv.) in dry THF (5 mL) cooled at 0 °C, under argon atmosphere, was added sodium hydride (60% w/w in mineral oil, 182 mg, 4.56 mmol, 3 equiv.)

in one portion into the reaction mixture, which became rapidly dark red. After 15 min, the homogenous mixture was cooled at -78 °C, and a solution of 2-(2-(2-azidoethoxy)ethoxy)ethyl 4-methylbenzenesulfonate **11** (500 mg, 1.52 mmol, 1 equiv.) in dry THF (5 mL) was introduced dropwise to the mixture. After stirring at -78 °C for 1 h, the reaction mixture was allowed to warm to 0 °C over 2 h. After completion, the mixture was filtered on a Celite[®] plug and the precipitate was washed four times with EtOAc and DCM. The filtrate was concentrated under vacuum to give a brown solid, which was purified by flash-column chromatography (silica gel, cyclohexane/EtOAc as gradient from 100:0 to 90:10, v/v) to afford 1-(2-(2-azidoethoxy)ethoxy)ethylcyclopenta-1,3-diene **2** (227 mg, 0.95 mmol, 67 % yield) as a light yellow oil. IR (neat): 2924, 2865, 2099, 1745, 1709, 1442, 1346, 1284, 1110, 928, 811. Two inseparable isomers were observed by NMR in a 1:1 ratio and are reported below: ¹H NMR (300 MHz, CDCl₃): δ 6.44 (ddq, *J* = 12.0, 3.1, 1.5 Hz, 3H), 6.27 (ddd, *J* = 5.3, 2.7, 1.4 Hz, 1H), 6.24 – 6.19 (m, 1H), 6.11 – 6.05 (m, 1H), 3.71 – 3.59 (m, 16H), 3.38 (t, *J* = 5.0 Hz, 4H), 2.94 (dtd, *J* = 4.2, 2.9, 1.5 Hz, 4H), 2.94 (dtd, *J* = 4.2, 2.9, 1.5 Hz, 4H), 2.75 – 2.63 (m, 4H), 1.66 (s, 1H), 1.34 – 1.18 (m, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 146.0, 143.6, 134.7, 133.8, 132.4, 131.1, 127.7, 127.4, 77.5, 77.1, 76.7, 71.3, 70.8, 70.7, 70.3, 70.2, 70.1, 50.7, 43.8, 41.4, 31.0, 30.3. HRMS (ESI+) Calculated for C₁₁H₂₁N₄O₂ [M + H]⁺: 241.1665; found: 241.1664. HPLC System A (λ = 254 nm) t_R = 4.24 min.

Synthesis of the cyclohexadiene (3).

tert-Butyl(cyclohex-3-en-1-ylmethoxy)dimethylsilane (12).

To a solution of cyclohex-3-en-1-ylmethanol (2.00 g, 17.8 mmol, 1 equiv.) in dry DMF (8 mL) under argon atmosphere, was added imidazole (2.43 g, 35.7 mmol, 2 equiv.). The reaction mixture was cooled to 0 °C and then, *tert*-butyldimethylsilyl chloride (3.23 g, 21.4 mmol, 1.2 equiv.) was added. After 5 min, the reaction was allowed to warm to room temperature for and was stirred for 1 h. After completion, the reaction was quenched with water (80 mL) and the resulting mixture was extracted with Et₂O (3 × 40 mL). Combined organic layer was washed with water (50 mL) and brine (50 mL) and was dried over MgSO₄, and evaporated under vacuum. The crude product was purified by flash-column chromatography (silica gel, cyclohexane) to provide the title compound (3.65 g, 16.1 mmol, 95 % yield) as a colorless liquid. The following ¹H NMR analysis was in accordance with literature data.¹⁴ ¹H NMR (300 MHz, CDCl₃): δ 5.67 (d, *J* = 2.2 Hz, 2H), 3.48 (d, *J* = 1.9 Hz, 1H), 3.49 (d, *J* = 1.9 Hz, 1H), 2.12 – 2.00 (m, 3H), 1.85 – 1.63 (m, 3H), 1.34 – 1.15 (m, 1H), 0.90 (s, 9H), 0.04 (s, 6H).

tert-Butyl((3,4-dibromocyclohexyl)methoxy)dimethylsilane (13).

To a solution of *tert*-butyl(cyclohex-3-en-1-ylmethoxy)dimethylsilane **11** (500 mg, 2.2 mmol, 1 equiv.) in dry DCM (5 mL), cooled to 0 °C under argon atmosphere, was added NaHCO₃ (185 mg, 2.2 mmol, 1.0 equiv.). A solution of bromine (0.12 mL, 2.42 mmol, 1.2 equiv.) in DCM (3 mL) was added dropwise at 0 °C. The reaction mixture was stirred at 0

°C for 2 h after the addition of bromine solution. Then, reaction was quenched with a mixture of sat. aq. Na₂S₂O₃ and sat. aq. Na₂CO₃ (15 mL, 1:1, v/v). The resulting mixture was extracted with EtOAc (3 × 10 mL). Combined organic layer dried over MgSO₄, and evaporated under vacuum. The crude product was purified by flash-column chromatography (silica gel, cyclohexane/EtOAc as gradient from 100:0 to 95:5, v/v) to provide the title compound (270 mg, 0.70 mmol, 32 % yield) as a light yellow oil. This procedure was applied several times in order to get sufficient amount of product. The following ¹H NMR analysis was in accordance with literature data.¹⁴ ¹H (300 MHz, CDCl₃): δ 4.73 – 4.64 (m, 2H), 3.48 (d, *J* = 5.7 Hz, 2H), 2.54 – 2.40 (m, 1H), 2.26 – 2.14 (m, 1H), 2.14 – 2.02 (m, 1H), 2.02 – 1.90 (m, 2H), 1.67 – 1.54 (m, 2H), 0.90 (s, 9H), 0.05 (s, 6H).

Cyclohexadiene (4). To a solution of tert-butyl((3,4-dibromocyclohexyl)methoxy)dimethylsilane **13** (685 mg, 1.77 mmol, 1 equiv.) in dry THF (25 mL), at room temperature and under argon atmosphere, was added Aliquat 336 (0.02 mL, 0.035 mmol, 0.02 equiv.). *t*BuOK (436 mg, 3.89 mmol, 2.2 equiv.) was rapidly added in one portion to the mixture, which was stirred for 3 h. After, completion, the reaction was quenched with water (5 mL) and sat. aq. NH₄Cl (20 mL). The resulting mixture was extracted with EtOAc (2 × 30 mL). Combined organic layer dried over MgSO₄, and evaporated under vacuum. The crude product was partially purified by a rapid flash-column chromatography (silica gel, cyclohexane) and a light yellow oil was obtained, which was directly engaged in the next step. To a solution of tert-butyl(cyclohexa-2,4-dien-1-ylmethoxy)dimethylsilane (≈ 1.77 mmol, 1 equiv.) in dry methanol (10 mL), at 0 °C and under argon atmosphere, was added acetyl chloride (0.02 mL, 0.27 mmol, 0.15 equiv.). After 30 min, the solvent was evaporated under vacuum. The crude product was partially purified by a rapid flash-column chromatography (silica gel, cyclohexane/EtOAc, 8:2, v/v) and a light yellow oil was obtained, which was in part directly engaged in the next step. To a solution of cyclohexa-2,4-dien-1-ylmethanol (255 mg, 2.32 mmol, 1.0 equiv.) in dry MeCN (8 mL) under argon atmosphere and room temperature, was added di(1H-imidazol-1-yl)methanone (451 mg, 2.78 mmol, 1.2 equiv.). The reaction was stirred for 1 h at room temperature. After that time, 6-aminohexan-1-ol (326 mg, 2.78 mmol, 1.2 equiv.) was added and the reaction was stirred overnight. After completion, the solvent was removed by vacuum. The crude mixture was dissolved in DCM (20 mL) and was washed with H₂O (2 × 20 mL), then brine (20 mL). The organic layer was dried over MgSO₄ and solvents were removed by vacuum. The crude product was purified by flash-column chromatography (silica gel, cyclohexane/EtOAc as gradient from 100:0 to 60:40, v/v) to provide the racemic product **4** (56 mg, 0.22 mmol, 49 % yield) as a white solid. **NB:** Aromatized analog was observed by ¹H NMR (6 %) but was inseparable from the title compound by flash-column chromatography, RP-HPLC (Systems A and B) or recrystallization in water. The following ¹H NMR analysis was in accordance with literature data.¹⁴ ¹H NMR (300 MHz, acetone-d₆): δ 6.19 (bs, 1H), 6.00 – 5.60 (m, 4H), 4.01 – 3.85 (m, 2H), 3.58 – 3.47 (m, 2H), 3.11 (dd, *J* = 13.0, 6.9 Hz, 2H), 2.68 – 2.48

(m, 1H), 2.30 – 2.16 (m, 2H), 1.60 – 1.25 (m, 8H). HPLC System A (λ = 254 nm) *t*_R = 3.65 min.

Synthesis of oxazoles

Oxazole (9). To a solution of (S)-ethyl 2-acetamido-3-phenylpropanoate (1.00 g, 4.25 mmol, 1 equiv.) in dry toluene (10 mL), was added POCl₃ (0.61 mL, 6.38 mmol, 1.5 equiv.). The mixture was heated at 80 °C for 3 h. After completion, the reaction mixture was cooled to room temperature and poured onto a saturated solution of aq. NaHCO₃ (100 mL) cooled to 0 °C. The aqueous layer was extracted with EtOAc (2 × 100 mL). The organic layers were combined and washed with brine (50 mL), dried over MgSO₄, and evaporated under vacuum. The crude product was purified by flash-column chromatography (silica gel, cyclohexane/EtOAc 70:30, v/v) to furnish the corresponding product **8** (691 mg, 3.18 mmol, 75 % yield) as a yellow oil. The following ¹H NMR analysis was in accordance with literature data.²⁷ ¹H NMR (300 MHz, CDCl₃): δ 7.32 – 7.13 (m, 5H), 4.07 (q, *J* = 7.1 Hz, 2H), 3.71 (s, 2H), 2.30 (s, 3H), 1.31 (t, *J* = 7.1 Hz, 3H).

Tyrosine ethyl ester (14). To a solution of ethyl acetyl-L-tyrosinate (1.454 g, 6.2 mmol, 1 equiv.) in dry DMF (7.2 mL), was added K₂CO₃ (1.5 g, 12.4 mmol, 2 equiv.). The resulting reaction mixture was stirred at room temperature for 30 min, then methyl iodide (1.17 mL, 12.4 mmol, 2 equiv.) was added. The reaction mixture was stirred at room temperature for 12 h. The solution was diluted with EtOAc (30 mL) and the resulting solution was washed with water (5 × 50 mL), dried over MgSO₄, and evaporated under vacuum. The crude product was purified by flash-column chromatography (silica gel, cyclohexane/EtOAc 50:50, v/v) to provide the title compound (1.095 g, 3.39 mmol, 61 % yield) as a white amorphous powder. mp 56 °C. IR (neat): 3312, 1736, 1635, 1514, 1374, 1294, 1225, 1198, 1138, 1077, 1006, 862. ¹H (300 MHz, CDCl₃): δ 7.02 (d, *J* = 8.7 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 2H), 5.94 (d, *J* = 7.5 Hz, 1H), 4.81 (dt, *J* = 7.8, 5.8 Hz, 1H), 4.60 (s, 2H), 4.16 (tt, *J* = 7.2, 3.7 Hz, 2H), 3.80 (s, 3H), 3.06 (dd, *J* = 5.7, 1.8 Hz, 2H), 1.98 (s, 3H), 1.24 (t, *J* = 7.2 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 171.68, 169.73, 169.27, 156.78, 130.32, 129.23, 114.54, 65.19, 61.29, 53.24, 52.09, 36.90, 22.86, 14.00. HRMS (ESI+) Calculated for C₁₆H₂₂NO₆ [M + H]⁺: 324.1447; found: 324.1459.

Oxazole Methyl ester (15). To a solution of ethyl (S)-2-acetamido-3-(4-(2-methoxy-2-oxoethoxy)phenyl)propanoate **13** (500 g, 1.54 mmol, 1 equiv.) in dry toluene (5 mL), was added POCl₃ (187 mL, 2.01 mmol, 1.3 equiv.). The resulting reaction mixture was stirred at 80 °C for 4.5 h. After completion, the solution was added dropwise to a solution of saturated aq. NaHCO₃ (50 mL), and the resulting mixture was extracted with EtOAc (3 × 25 mL). The organic layers were combined, dried over MgSO₄, and evaporated under vacuum. The crude product was purified by flash-column chromatography (silica gel, cyclohexane/EtOAc 50:50, v/v) to furnish the title compound (454.2 mg, 1.49 mmol, 97% yield) as a pale yellow oil. IR (neat): 2993, 2955, 1760, 1669, 1612, 1586, 1510, 1438, 1375, 1348, 1269, 1204, 1176, 1085, 1022,

953, 909. ^1H (300 MHz, CDCl_3): δ 7.17 (d, J = 8.3 Hz, 2H), 6.87 – 6.78 (d, J = 8.3 Hz, 2H), 4.59 (d, J = 0.9 Hz, 2H), 4.07 (qd, J = 7.1, 1.2 Hz, 2H), 3.79 (d, J = 1.4 Hz, 3H), 3.63 (s, 2H), 2.29 (d, J = 1.1 Hz, 3H), 1.31 (td, J = 7.1, 1.2 Hz, 3H). ^{13}C NMR (75 MHz, CDCl_3): δ 169.61, 156.38, 153.98, 152.55, 132.97, 129.71, 115.96, 114.74, 70.47, 65.59, 52.28, 30.16, 15.11, 14.40. HRMS (ESI+) Calculated for $\text{C}_{16}\text{H}_{20}\text{NO}_5$ [M + H] $^+$: 306.1341; found: 306.1341.

Oxazole (10). To a solution of methyl 2-(4-((5-ethoxy-2-methyloxazol-4-yl)methyl)phenoxy)acetate **14** (200 mg, 0.644 mmol, 1 equiv.) in THF (6 mL), were successively added deionized water (0.91 mL) and LiOH (31 mg, 1.31 mmol, 2 equiv.). The resulting reaction mixture was stirred at 70 °C for 3 h. After completion, the reaction mixture was cooled to room temperature and glacial AcOH was added (150 mL, 2.62 mmol, 4 equiv.). The reaction mixture was stirred at room temperature for 1 h. The solution was diluted with deionized water (2 mL) and extracted with EtOAc (3 \times 20 mL), and the combined organic layers dried over MgSO_4 , and evaporated under vacuum. The crude product was co-evaporated several times with toluene (5 \times 8 mL) to obtain the title compound (137 mg, 0.47 mmol, 72 % yield) as a colorless oil. IR (neat): 2927, 2507, 1739, 1673, 1612, 1583, 1510, 1440, 1375, 1250, 1207, 1177, 1112, 1078, 1013, 976, 909. ^1H (300 MHz, CDCl_3): δ 11.43 (s, 1H), 7.13 (d, J = 8.7 Hz, 2H), 6.85 – 6.73 (d, J = 8.7 Hz, 2H), 4.53 (s, 2H), 4.09 (q, J = 7.1 Hz, 2H), 3.65 (s, 2H), 2.33 (s, 3H), 1.31 (t, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, CDCl_3): δ 171.94, 156.59, 153.94, 153.64, 132.15, 129.69, 115.42, 114.69, 70.76, 65.47, 29.32, 15.06, 13.85. HRMS (ESI+) Calculated for $\text{C}_{15}\text{H}_{16}\text{NO}_5$ [M + H] $^+$: 290.1028; found: 290.1033.

Maleimide synthesis

Maleimide-dansylamide (16). To a solution of 1-(2-aminoethyl)-pyrrole-2,5-dione hydrochloride (113 mg, 0.81 mmol, 2.2 equiv.) and dansyl chloride (100 mg, 0.37 mmol, 1.0 equiv.) in DMF (6 mL) was added DIEA (142 μL , 0.82 mmol, 2.2 equiv.). The reaction mixture was stirred at room temperature for 2 h. After completion, the reaction was poured onto water (60 mL) and the aqueous layer was extracted with DCM (3 \times 20 mL). Combined organic layers were dried over MgSO_4 and solvents were removed by vacuum. The crude product was purified by flash-column chromatography (silica gel, DCM/EtOAc 90:10, v/v) to provide the title compound (97 mg, 0.26 mmol, 70 % yield) as a pale yellow solid. The following ^1H NMR analysis was in accordance with literature data.⁷ ^1H NMR (300 MHz, CDCl_3): δ 8.51 (d, J = 8.6 Hz, 1H), 8.24 – 8.15 (m, 2H), 7.53 (ddd, J = 10.1, 8.6, 7.5 Hz, 2H), 7.17 (dd, J = 7.6, 0.6 Hz, 1H), 6.34 (s, 2H), 5.07 (t, J = 6.1 Hz, 1H), 3.53 (t, J = 5.7 Hz, 2H), 3.16 (dd, J = 11.5, 6.0 Hz, 2H), 2.88 (s, 6H). HPLC System A (λ = 254 nm) t_{R} = 3.40 min.

Chiral maleimide (19). To a solution of (*R*)-1-phenylethanol (500 mg, 4.09 mmol, 1.0 equiv.) in dry THF (15 mL, 0.41 M) and under argon atmosphere, cooled to 0 °C, were successively added PPh_3 (1.180 mg, 4.5 mmol, 1.1 equiv.) and diisopropyl azodicarboxylate (0.88 mL, 4.5 mmol, 1.2 equiv.). The reaction was stirred for 15 min at 0 °C, then 1H-pyrrole-2,5-dione (597

mg, 6.14 mmol, 1.5 equiv.) was introduced into the mixture, which was stirred for 30 min at 0 °C, and overnight at room temperature. After completion, the solvent was removed by vacuum. The crude product was purified by flash-column chromatography (silica gel, cyclohexane/EtOAc 90:10, v/v) to furnish the title compound (278 mg, 1.38 mmol, 34 % yield) as a yellow oil. The following ^1H NMR analysis was in accordance with literature data.⁵⁵ ^1H NMR (300 MHz, CDCl_3): δ 7.43 – 7.37 (m, 2H), 7.34 – 7.21 (m, 3H), 6.61 (s, 2H), 5.34 (q, J = 7.4 Hz, 1H), 1.81 (d, J = 7.4 Hz, 3H). HPLC System A (λ = 254 nm) t_{R} = 3.76 min.

Preparation of cycloadducts

Cycloadduct linear diene-dansylamide (17a). To a solution of (2E,4E)-hexa-2,4-dien-1-ol **1** (10.6 mg, 0.108 mmol, 2.0 equiv.) in a solution of THF/ H_2O (0.5 mL, 2:1 v/v) was added a solution of **16** (20 mg, 0.054 mmol, 1.0 equiv.) in THF/ H_2O (0.5 mL, 2:1 v/v). The mixture was stirred at room temperature for 2 days. After completion, the reaction mixture was diluted with water (10 mL) and the aqueous layer extracted with EtOAc (3 \times 10 mL). The organic layers were combined, dried over MgSO_4 , and solvents were removed under vacuum. The crude product was purified by flash-column chromatography (silica gel, DCM/EtOAc as gradient from 100:0 to 90:10, 80:20 and 50:50 v/v) to provide the title compound (22 mg, 0.047 mmol, 86 % yield) as a pale yellow oil. IR (neat): 3451, 3284, 2926, 2851, 1767, 1684, 1588, 1573, 1403, 1327, 1141, 975, 945, 789.

^1H NMR (300 MHz, MeOD): δ 8.56 (d, J = 8.5 Hz, 1H), 8.26 (d, J = 8.7 Hz, 1H), 8.14 (dd, J = 7.3, 1.2 Hz, 1H), 7.63 – 7.52 (m, 2H), 7.29 (d, J = 7.6 Hz, 1H), 5.69 (dt, J = 9.0, 3.0 Hz, 1H), 5.61 (dt, J = 9.2, 2.9 Hz, 1H), 3.97 (dd, J = 11.1, 6.9 Hz, 1H), 3.86 (dd, J = 11.0, 8.1 Hz, 1H), 3.39 (t, J = 6.1 Hz, 2H), 2.97 (t, J = 6.2 Hz, 2H), 2.95 – 2.90 (m, 1H), 2.88 (s, 6H), 2.73 (dd, J = 8.4, 7.0 Hz, 1H), 2.41–2.30 (m, 2H), 1.34 (d, J = 7.4 Hz, 3H), 1.31 – 1.24 (m, 1H). ^{13}C NMR (75 MHz, MeOD): δ 179.6, 179.2, 153.2, 136.8, 135.8, 131.2, 130.8, 130.3, 129.2, 124.4, 120.6, 116.5, 63.4, 46.2, 45.8, 43.8, 40.8, 40.1, 38.9, 32.2, 17.1. HRMS (ESI+) Calculated for $\text{C}_{24}\text{H}_{30}\text{N}_3\text{O}_5\text{S}$ [M+H] $^+$: 472.1906; found: 472.1895. HPLC System B (λ = 330 nm) t_{R} = 3.84 min.

Cycloadduct cyclopentadiene-dansylamide (17b). To a solution of 1-(2-(2-(2-azidoethoxy)ethoxy)ethyl)cyclopenta-1,3-diene and 2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)cyclopenta-1,3-diene **2** (47 mg, 0.21 mmol, 5.0 equiv.) in a solution of MeCN/ H_2O (1 mL, 1:2 v/v) was added compound **16** (15 mg, 0.042 mmol, 1.0 equiv.). The mixture was stirred at room temperature for 3 h. After completion, the reaction mixture was diluted with water (10 mL) and the aqueous layer extracted with EtOAc (2 \times 10 mL). Combined organic layers were dried over MgSO_4 , and solvents were removed under vacuum. The crude product was purified by flash-column chromatography (silica gel, DCM/EtOAc as gradient from 100:0 to 90:10, and 80:20, v/v) to obtain the product (22 mg, 0.037 mmol, 92 % yield) as a dark yellow foam. At least 2 stereoisomers were observed by NMR in a ratio of 0.83:1.00. IR (neat): 3276, 2925, 2868, 2100, 1769, 1692, 1588, 1574, 1396, 1329, 1143, 909, 790, 727. ^1H NMR (300 MHz, CDCl_3): δ 8.52 (d, J = 8.6 Hz, 2H), 8.25 (d, J = 8.7 Hz, 2H), 8.20 (ddd, J = 7.3, 3.4, 1.2 Hz, 2H), 7.59 (dd, J = 8.5, 7.7 Hz, 1.9H), 7.55 – 7.46 (m, 2.1H), 7.17 (d, J = 7.6 Hz, 2H), 5.93 (dd, J = 5.7, 3.0 Hz,

0.8H), 5.80 (d, $J = 5.6$ Hz, 0.8H), 5.64–5.50 (m, 1H), 5.38–5.30 (t, $J = 5.6$ Hz, 1H), 5.11 (d, $J = 5.6$ Hz, 0.9H), 3.70–3.57 (m, 11H), 3.57–3.42 (m, 8H), 3.41–3.33 (m, 8H), 3.25–3.18 (m, 2H), 3.16–3.11 (m, 1H), 3.09–2.96 (m, 5H), 2.94 (dd, $J = 2.8$, 1.5 Hz, 2H), 2.87 (s, 11H), 2.36–2.02 (m, 6H), 1.74–1.64 (m, 2.7H), 1.41 (t, $J = 9.4$ Hz, 3H), 1.29–1.24 (m, 1.8H), 1.21 (t, $J = 7.0$ Hz, 1.8H), 1.14 (d, $J = 6.4$ Hz, 1H), 0.92–0.77 (m, 2.9H). ^{13}C NMR (75 MHz, CDCl_3): δ 178.3, 177.8, 177.7, 177.4, 152.0, 152.0, 146.9, 137.8, 134.5, 134.4, 134.2, 130.4, 130.4, 129.9, 129.9, 129.8, 129.4, 129.4, 128.4, 128.4, 126.3, 123.3, 119.0, 118.9, 115.2, 70.7, 70.5, 70.1, 70.0, 70.0, 69.9, 69.0, 68.7, 56.7, 55.7, 52.1, 50.7, 50.7, 48.9, 48.1, 47.3, 46.9, 45.7, 45.4, 45.2, 44.7, 41.9, 41.7, 37.5, 37.2, 31.1, 30.6, 29.7. HRMS (ESI+) Calculated for $\text{C}_{29}\text{H}_{36}\text{N}_6\text{O}_6\text{S}$ $[\text{M}+\text{H}]^+$: 597.2495; found: 597.2493. HPLC System A ($\lambda = 254$ nm) $t_{\text{R}} = 4.56$ min, 4.61 min.

Cycloadduct fulvene-dansylamide (17c). To a solution of 5-(cyclopenta-2,4-dien-1-ylidene)hexanoic acid **3** (10 mg, 0.058 mmol, 1.0 equiv.) in a solution of THF/ H_2O (0.7 mL, 2:1 v/v) was added a solution of **16** (24 mg, 0.064 mmol, 1.1 equiv.) in THF/ H_2O (0.5 mL, 2:1 v/v). The mixture was stirred at room temperature for 3 h. After completion, the reaction mixture was diluted with water (10 mL) and the aqueous layer extracted with EtOAc (3×10 mL). The organic layers were combined, dried over MgSO_4 , and solvents were removed under vacuum. The crude product was purified by flash-column chromatography (silica gel, DCM/EtOAc/MeOH as gradient from 100:0:0 to 90:10:0, 80:20:0, 70:30:0 and 50:40:10 v/v) to provide the title compound (29 mg, 0.053 mmol, 91 % yield) as a pale yellow oil. IR (neat): 3268, 2927, 2858, 2794, 2253, 1770, 1689, 1589, 1575, 1397, 1324, 1143, 909, 728. ^1H NMR (300 MHz, CDCl_3): δ 8.52 (d, $J = 8.5$ Hz, 1H), 8.30–8.13 (m, 2H), 7.65–7.45 (m, 2H), 7.19 (d, $J = 7.5$ Hz, 1H), 6.12–6.01 (m, 2H), 5.22 (bs, 1H), 3.76–3.63 (m, 2H), 3.40 (overlapped triplet, $J = 5.7$ Hz, 2H), 3.03 (dd, $J = 11.4$, 5.8 Hz, 2H), 2.98–2.89 (m, 1H), 2.85 (s, 6H, overlapped with multiplet at 2.84 ppm), 2.84 (m, 1H, overlapped with singlet at 2.85 ppm), 2.29 (overlapped triplet, $J = 7.2$ Hz, 2H), 1.97 (t, $J = 7.3$ Hz, 2H), 1.70 (dt, $J = 7.2$, 7.2 Hz, 2H), 1.54 (s, 3H). ^{13}C NMR (75 MHz, CDCl_3): δ 178.4, 177.4, 177.3, 151.9, 148.7, 134.8, 134.7, 134.4, 130.4, 129.8, 129.4, 128.5, 123.3, 119.0, 118.96, 115.3, 113.9, 45.4, 45.1, 44.8, 44.4, 44.2, 41.6, 37.5, 32.9, 32.6, 22.6, 16.9. HRMS (ESI-) Calculated for $\text{C}_{29}\text{H}_{34}\text{N}_3\text{O}_6\text{S}$ $[\text{M}+\text{H}]^+$: 552.2168; found: 552.2166. HPLC System B ($\lambda = 330$ nm) $t_{\text{R}} = 4.16$ min, 4.25 min.

Cycloadduct furan-dansylamide (17d). To a solution of furan-2-ylmethanol **6** (25 mg, 0.27 mmol, 5.0 equiv.) in a solution of THF/ H_2O (1.0 mL, 2:1 v/v) was added a solution of **16** (20 mg, 0.054 mmol, 1.0 equiv.) in THF/ H_2O (0.5 mL, 2:1 v/v). The mixture was stirred at room temperature for 3 days. After completion, the reaction mixture was diluted with water (10 mL) and the aqueous layer extracted with EtOAc (3×15 mL). The organic layers were combined, dried over MgSO_4 , and solvents were removed under vacuum. The crude product was purified by flash-column chromatography (silica gel, DCM/EtOAc as gradient from 100:0 to 90:10, 80:20, 70:30, 60:40 and 50:50 v/v) to provide the title compound (21 mg, 0.045 mmol, 82 % yield) as a pale yellow oil. IR (neat): 3483, 3290, 2921, 2828, 1692, 1398, 1323, 1141, 789. ^1H NMR (300 MHz, MeOD): δ 8.61–8.51 (m, 1.4H), 8.27 (d, $J = 8.7$ Hz, 1H), 8.26 (d, $J = 8.7$ Hz, 0.35H), 8.15 (dd, $J = 7.4$, 1.0 Hz, 1.34H), 7.64–7.51 (m, 2.75H), 7.29 (d, $J = 7.3$ Hz, 1H), 7.27 (d, $J = 7.7$ Hz, 0.36H), 6.52–6.46 (m, 0.67H), 6.35 (dd, $J = 5.8$, 1.6 Hz, 1H), 6.22 (d, $J = 5.8$ Hz, 1H), 5.14 (dd, $J = 5.5$, 1.6 Hz, 1H), 5.03 (d, $J = 1.0$ Hz, 0.34H), 4.86 (s, 8H), 4.15–3.95 (m, 2.48H), 3.80 (d, $J = 12.8$ Hz, 0.33H), 3.45 (t, $J = 6.5$ Hz, 0.84H), 3.35 (s, 2.77H), 3.25 (dd, $J = 7.6$, 5.5 Hz, 1.35H), 3.06 (d, $J = 7.8$ Hz, 1H), 3.05 (d, $J = 6.1$ Hz, 0.66H), 2.96 (t, $J = 6.1$ Hz, 2.25H), 2.88 (s, 6H), 2.86 (s, 2H), 2.60 (d, $J = 6.4$ Hz, 0.34H), 2.52 (d, $J = 6.5$ Hz, 0.34H). ^{13}C NMR (75 MHz, MeOD): δ 178.0, 176.9, 176.8, 176.6, 153.3, 138.8, 138.0, 136.7, 136.6, 136.2, 131.3, 131.26, 130.8, 130.77, 130.4, 130.35, 129.2, 124.4, 120.6, 116.6, 93.5, 92.9, 82.1, 80.4, 61.5, 60.8, 51.5, 46.7, 45.8, 40.9, 40.7, 39.4, 38.9. HRMS (ESI+) Calculated for $\text{C}_{23}\text{H}_{26}\text{N}_3\text{O}_6\text{S}$ $[\text{M}+\text{H}]^+$: 472.1542; found: 472.1541. HPLC System B ($\lambda = 330$ nm) $t_{\text{R}} = 3.19$ min, 3.49 min.

Azaphthalimide - coumarin (18). To a solution of 6-(5-ethoxy-2-methylxazol-4-yl)hexanoic acid **7** (9.4 mg, 0.039 mmol, 1.0 equiv.) in a solution of THF/ H_2O (0.5 mL, 2:1 v/v) were added 7-(diethylamino)-N-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-2-oxo-2H-chromene-3-carboxamide **MC**⁴² (15 mg, 0.039 mmol, 1.0 equiv.) and TFA (3 μL , 0.043 mmol, 1.1 equiv.). The mixture was stirred at room temperature for 26 h. After completion, and solvents were removed under vacuum. The crude product was purified by RP-HPLC (System D) and fractions were collected and lyophilized to provide the title compound (12 mg, 0.019 mmol, 49 % yield) as a yellow powder. IR (neat): 3330, 2976, 2927, 2858, 1708, 1617, 1581, 1514, 1402, 1352, 1227, 1189, 1134, 910, 795. ^1H NMR (300 MHz, DMSO- d_6): δ 11.95 (bs, 1H), 8.74 (t, $J = 6.1$ Hz, 1H), 8.57 (s, 1H), 7.65 (d, $J = 9.0$ Hz, 1H), 6.79 (dd, $J = 8.9$, 2.3 Hz, 1H), 6.60 (d, $J = 2.0$ Hz, 1H), 3.76–3.65 (m, 2H), 3.58–3.50 (m, 2H), 3.48 (q, $J = 7.0$ Hz, 4H), 2.81 (t, $J = 7.6$ Hz, 2H), 2.58 (s, 3H), 2.20 (t, $J = 7.3$ Hz, 3H), 1.69–1.57 (m, 2H), 1.56–1.46 (m, 2H), 1.42–1.31 (m, 2H), 1.14 (t, $J = 7.0$ Hz, 6H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 174.5, 168.0, 162.8, 161.3, 157.2, 152.4, 147.6, 131.5, 120.4, 110.1, 109.4, 107.6, 95.9, 44.3, 37.6, 37.4, 33.6, 32.1, 28.4, 27.3, 24.3, 19.6, 12.3. HRMS (ESI+) Calculated for $\text{C}_{30}\text{H}_{35}\text{N}_4\text{O}_8$ $[\text{M}+\text{H}]^+$: 579.2455; found: 579.2453. HPLC System A ($\lambda = 254$ nm) $t_{\text{R}} = 3.96$ min.

Cycloadduct linear diene-chiral maleimide (20). To a solution of (2E,4E)-hexa-2,4-dien-1-ol **1** (25 mg, 0.25 mmol, 1.0 equiv.) in a solution of THF/ H_2O (1 mL, 2:1 v/v) was added a solution of **19** (50 mg, 0.25 mmol, 1.0 equiv.) in THF/ H_2O (1 mL, 2:1 v/v). The mixture was stirred at room temperature for 3 days. After completion, the reaction mixture was diluted with water (10 mL) and the aqueous layer extracted with EtOAc (3×15 mL). The organic layers were combined, dried over MgSO_4 , and solvents were removed under vacuum. The crude product was purified by flash-column chromatography (silica gel, cyclohexane/EtOAc as gradient from 100:0 to 90:10, 80:20 and 70:30 v/v) to provide the title compound (59 mg, 0.20 mmol, 79 % yield) as a pale yellow oil. Two stereoisomers were observed by NMR in a ratio of 1:1. IR (neat): 3437, 3090, 3065, 3034, 2969, 2934, 2878, 2847, 1764, 1687, 1452, 1363, 1225, 1189, 912, 727, 694. ^1H NMR (300 MHz, CDCl_3): δ 7.37–7.19 (m, 10H), 5.82–5.54 (m, 4H), 5.35 (q, $J = 7.2$ Hz, 1H), 5.35 (q, $J = 7.2$ Hz, 1H), 4.05–3.85 (m, 3H), 3.76 (t, $J = 10.5$ Hz, 1H), 3.28 (dd, $J = 15.9$, 7.4 Hz, 1H), 3.16 (bs, 1H), 3.00 (ddd, $J = 8.6$, 6.2, 5.0 Hz, 2H), 2.62–2.49 (m, 2H), 2.49–2.32 (m, 2H), 1.72 (d, $J = 7.3$ Hz, 3H), 1.71 (d, $J = 7.3$ Hz, 3H), 1.48 (d, $J = 7.4$ Hz, 3H), 1.43 (d, $J = 7.4$ Hz, 3H). ^{13}C NMR (75 MHz, CDCl_3): δ 178.8, 178.7, 177.3, 177.2, 139.2, 139.2, 135.2, 135.2, 128.4, 128.3, 128.2, 128.2, 127.6, 127.5, 127.1, 127.1, 62.7, 62.7, 49.8, 44.7, 44.6, 42.6, 42.4, 38.2, 31.2, 16.7, 16.7, 16.5, 16.4. HRMS (ESI+)

Calculated for $C_{18}H_{22}NO_3$ $[M+H]^+$: 300.1600 ; found : 300.1609. HPLC System A ($\lambda = 254$ nm) $t_R = 3.88$ min.

Cycloadduct cyclopentadiene-chiral maleimide (21). To a solution of 1-(2-(2-(2-azidoethoxy)ethoxy)ethyl)cyclopenta-1,3-diene and 2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)cyclopenta-1,3-diene **2** (39 mg, 0.17 mmol, 1.0 equiv.) in a solution of THF/H₂O (1 mL, 2:1 v/v) was added a solution of **19** (50 mg, 0.25 mmol, 1.0 equiv.) in THF/H₂O (1 mL, 2:1 v/v). The mixture was stirred at room temperature for 2 h. After completion, the reaction mixture was diluted with water (10 mL) and the aqueous layer extracted with EtOAc (2 × 15 mL). Combined organic layers were dried over MgSO₄, and solvents were removed under vacuum. The crude product was purified by flash-column chromatography (silica gel, cyclohexane/EtOAc as gradient from 100:0 to 90:10, 80:20 and 70:30, v/v) to provide the title compound (65 mg, 0.15 mmol, 90 % yield) as a white foam. At least 4 stereoisomers were observed by NMR in a ratio 1.00:1.00:1.70:0.95. IR (neat): 2941, 2968, 2100, 1694, 1453, 1354, 1188, 1113, 913, 728, 698. ¹H NMR (300 MHz, CDCl₃): δ 7.43 – 7.34 (m, 10H), 7.33 – 7.26 (m, 9H), 7.25 – 7.18 (m, 6H), 5.98 (dd, $J = 5.6, 2.9$ Hz, 1H), 5.89 – 5.81 (m, 2H), 5.70 (d, $J = 5.0$ Hz, 1H), 5.60 – 5.55 (m, 1.7H), 5.50 (s, 1H), 5.23 (dd, $J = 14.5, 7.6$ Hz, 4.4H), 3.69 – 3.56 (m, 29.5H), 3.56 – 3.47 (m, 7.4H), 3.41 – 3.33 (m, 11.4H), 3.31 – 3.16 (m, 18.9H), 3.01 (dd, $J = 7.3, 6.1$ Hz, 2.2H), 2.42 – 2.26 (m, 2.5H), 2.26 – 2.10 (m, 4.3H), 1.97 – 1.83 (m, 3.8H), 1.72 – 1.64 (m, 20.5H), 1.49 – 1.42 (m, 5.2H). ¹³C NMR (75 MHz, CDCl₃): δ 177.8, 177.7, 177.4, 177.4, 177.2, 177.2, 177.1, 177.1, 146.8, 146.7, 139.9, 139.6, 139.5, 137.8, 137.7, 134.1, 134.0, 128.2, 128.1, 128.0, 127.8, 127.6, 127.6, 127.5, 127.5, 126.5, 126.5, 70.6, 70.6, 70.0, 69.9, 69.9, 69.9, 69.0, 68.9, 68.7, 57.0, 56.9, 55.5, 55.5, 52.2, 50.6, 50.1, 49.6, 49.6, 49.5, 48.3, 47.9, 47.8, 46.9, 46.8, 46.6, 46.6, 45.5, 45.3, 45.3, 45.0, 45.0, 31.1, 31.1, 30.8, 30.4, 16.8, 16.3, 16.2, 16.0. HRMS (ESI+) Calculated for $C_{23}H_{29}N_4O_4$ $[M+H]^+$: 425.2189 ; found : 425.2180. HPLC System A ($\lambda = 254$ nm) $t_R = 4.55$ min, 4.71 min.

Cycloadduct fulvene-chiral maleimide (22). To a solution of 5-(cyclopenta-2,4-dien-1-ylidene)hexanoic acid **3** (45 mg, 0.25 mmol, 1.0 equiv.) in a solution of THF/H₂O (0.75 mL, 2:1 v/v) was added a solution of **19** (30 mg, 0.15 mmol, 1.0 equiv.) in THF/H₂O (0.5 mL, 2:1 v/v). The mixture was stirred at room temperature for 3 days. After completion, the reaction mixture was diluted with water (10 mL) and the aqueous layer extracted with EtOAc (3 × 15 mL). The organic layers were combined, dried over MgSO₄, and solvents were removed under vacuum. The crude product was purified twice by flash-column chromatography (silica gel, cyclohexane/EtOAc as gradient from 100:0 to 90:10, 80:20, 70:30 and 60:40 v/v) to furnish the title compound (72 mg, 0.19 mmol, 76 % yield) as a colorless oil. Two stereoisomers were observed by NMR in a ratio of 1:1. IR (neat): 2939, 1766, 1693, 1378, 1354, 1219, 1188, 911, 835, 768, 729, 698. ¹H NMR (300 MHz, CDCl₃): δ 8.67 (bs, 1H), 7.40 – 7.33 (m, 2H), 7.33 – 7.23 (m, 3H), 6.17 – 6.09 (m, 1H), 6.02 – 5.94 (m, 1H), 5.26 (q, $J = 7.3$ Hz, 1H), 3.83 – 3.69 (m, 2H), 3.17 – 3.05 (m, 2H), 2.24 (overlapped triplet, $J = 7.3$ Hz, 2H), 1.97 (overlapped triplet, $J = 7.3$ Hz, 2H), 1.69 (d, $J =$

7.3 Hz, 3H, overlapped with signal at 1.68 ppm), 1.68 (dt, $J = 7.3, 7.3$ Hz, 2H, overlapped with signal at 1.69 ppm), 1.55 (overlapped singlet, 3H). ¹³C NMR (75 MHz, CDCl₃): δ 179.1, 177.0, 176.9, 148.7, 139.4, 134.6, 134.6, 134.5, 128.1, 127.6, 113.6, 50.0, 44.76, 44.72, 44.70, 44.66, 44.52, 44.46, 44.41, 44.35, 32.94, 32.60, 22.6, 16.9, 16.4. HRMS (ESI-) Calculated for $C_{23}H_{24}NO_4$ $[M-H]^-$: 378.1705 ; found : 378.1703. HPLC System B ($\lambda = 254$ nm) $t_R = 4.36$ min.

Cycloadduct cyclohexadiene-chiral maleimide (23). To a solution of cyclohexa-2,4-dien-1-ylmethyl (6-hydroxyhexyl)carbamate **4** (38 mg, 0.15 mmol, 1.0 equiv.) in a solution of THF/H₂O (1 mL, 2:1 v/v) was added a solution of **19** (30 mg, 0.15 mmol, 1.0 equiv.) in THF/H₂O (1 mL, 2:1 v/v). The mixture was stirred at room temperature for 3 days. After completion, the reaction mixture was diluted with water (10 mL) and the aqueous layer extracted with EtOAc (3 × 15 mL). The organic layers were combined, dried over MgSO₄, and solvents were removed under vacuum. The crude product was purified twice by flash-column chromatography (silica gel, cyclohexane/EtOAc as gradient from 100:0 to 90:10, 80:20, 70:30 and 60:40 v/v) to furnish the title compound (48 mg, 0.11 mmol, 70 % yield) as a white foam. Two stereoisomers were observed by NMR in a ratio of 1:1. IR (neat): 3358, 2931, 2860, 1687, 1528, 1453, 1361, 1234, 1190, 1056, 1024, 698. ¹H NMR (300 MHz, CDCl₃): δ 7.39 – 7.30 (m, 5H), 7.30 – 7.27 (m, 3H), 7.25 – 7.19 (m, 2H), 6.20 – 5.88 (m, 4H), 5.30 (q, $J = 7.3$ Hz, 2H), 4.64 (bs, 2H), 3.79 (dd, $J = 10.8, 5.8$ Hz, 2H), 3.64 (t, $J = 6.5$ Hz, 2H), 3.64 (t, $J = 6.5$ Hz, 2H), 3.59 – 3.47 (m, 3H), 3.25 – 3.03 (m, 9H), 2.86 – 2.67 (m, 4H), 2.18 – 1.96 (m, 2H), 1.94 – 1.74 (m, 1H), 1.68 (d, $J = 7.3$ Hz, 6H), 1.61 – 1.44 (m, 10H), 1.44 – 1.27 (m, 10H), 1.21 (t, $J = 7.0$ Hz, 3H), 1.14 (d, $J = 6.6$ Hz, 3H), 0.98 – 0.70 (m, 4H). ¹³C NMR (75 MHz, CDCl₃): δ 178.4, 178.4, 178.21, 156.4, 139.4, 133.0, 132.8, 130.1, 129.9, 128.1, 127.5, 127.4, 66.9, 62.6, 49.9, 43.8, 43.8, 43.4, 43.3, 40.8, 35.9, 33.5, 32.5, 32.0, 29.9, 27.8, 27.8, 26.3, 25.3, 16.4, 16.4. HRMS (ESI+) Calculated for $C_{26}H_{35}N_2O_5$ $[M+H]^+$: 455.2546 ; found : 455.2538. HPLC System A ($\lambda = 254$ nm) $t_R = 4.09$ min.

Cycloadduct anthracene-chiral maleimide (24). To a solution of anthracen-9-ylmethanol **5** (52 mg, 0.25 mmol, 1.0 equiv.) in a solution of THF/H₂O (1 mL, 2:1 v/v) was added a solution of **19** (50 mg, 0.25 mmol, 1.0 equiv.) in THF/H₂O (1 mL, 2:1 v/v). The mixture was stirred at room temperature for 120 h. After completion, the reaction mixture was diluted with water (10 mL) and the aqueous layer extracted with EtOAc (2 × 15 mL). The organic layers were combined, dried over MgSO₄, and solvents were removed under vacuum. The crude product was purified by flash-column chromatography (silica gel, cyclohexane/EtOAc as gradient from 100:0 to 50:50, v/v) to provide the title compound (56 mg, 0.14 mmol, 54 % yield) as a colorless solid. Two stereoisomers were observed by NMR in a ratio of 0.8:1. IR (neat): 3448, 3065, 3031, 2969, 2941, 1688, 1457, 1359, 1221, 1195, 907, 728, 696. ¹H NMR (300 MHz, CDCl₃): δ 7.61 (dd, $J = 5.2, 3.6$ Hz, 1.7H), 7.40 – 7.30 (m, 2.7H), 7.29 – 7.26 (m, 1.1H), 7.26- 7.22 (m, 2.4H), 7.21 – 7.09 (m, 13.4H), 6.99 – 6.91 (m, 2H), 6.90 – 6.82 (m, 1.6H), 5.13 (dt, $J = 12.8, 6.7$ Hz, 1.3H), 5.07 – 4.84 (m, 4.2H), 4.76 (d, $J = 3.3$ Hz,

0.8H), 4.73 (d, $J = 2.9$ Hz, 0.9H), 3.35 – 3.20 (m, 3.6H), 2.70 (t, $J = 6.3$ Hz, 1.1H), 2.67 (t, $J = 6.3$ Hz, 1H), 1.31 (d, $J = 7.3$ Hz, 2.4H), 1.24 (d, $J = 6.2$ Hz, 3H). ^{13}C NMR (75 MHz, CDCl_3): δ 176.7, 176.6, 176.5, 176.4, 142.2, 142.2, 142.2, 139.3, 139.3, 139.0, 138.5, 138.5, 128.2, 127.3, 127.2, 126.9, 126.8, 126.8, 126.7, 126.5, 126.4, 125.2, 123.9, 123.9, 123.2, 123.2, 122.3, 60.2, 60.1, 49.6, 49.5, 49.3, 49.3, 47.4, 47.4, 45.6, 45.5, 45.5, 15.84, 15.7. HRMS (ESI+) Calculated for $\text{C}_{27}\text{H}_{24}\text{NO}_4$ $[\text{M}+\text{H}]^+$: 410.1756; found: 410.1757. HPLC System A ($\lambda = 254$ nm) $t_{\text{R}} = 4.67$ min.

Cycloadduct furan-chiral maleimide (25). To a solution of furan-2-ylmethanol **6** (24.5 mg, 0.25 mmol, 1.0 equiv.) in a solution of THF/ H_2O (1 mL, 2:1, v/v) was added a solution of **19** (50 mg, 0.25 mmol, 1.0 equiv.) in THF/ H_2O (1 mL, 2:1, v/v). The mixture was stirred at room temperature for 48 h. After completion, the reaction mixture was diluted with water (10 mL) and the aqueous layer extracted with EtOAc (2×15 mL). The organic layers were combined and dried over MgSO_4 , and solvents were removed under vacuum. The crude product was purified by flash-column chromatography (silica gel, cyclohexane/EtOAc as gradient from 100:0 to 70:30 and finally 50:50, v/v) to furnish the title compound (60 mg, 0.20 mmol, 80 % yield) as a colorless oil. Three stereoisomers were observed by NMR in a ratio of 0.7:1:1. IR (neat): 3449, 2925, 2857, 1691, 1378, 1356, 1219, 1188, 1025, 983, 969, 730, 697. ^1H NMR (300 MHz, CDCl_3): δ 7.41 – 7.27 (m, 13.2H), 6.60 (dd, $J = 5.7, 1.5$ Hz, 0.7H), 6.55 – 6.50 (m, 0.7H), 6.28 (dd, $J = 5.8, 1.7$ Hz, 1H), 6.20 (d, $J = 5.8$ Hz, 1H), 6.12 (dd, $J = 5.8, 1.7$ Hz, 1H), 6.02 (d, $J = 5.8$ Hz, 0.9H), 5.39 (q, $J = 7.3$ Hz, 0.9H), 5.30 – 5.15 (m, 4.8H), 4.29 – 4.06 (m, 5.4H), 4.03 – 3.83 (m, 1.4H), 3.58 (dd, $J = 7.8, 5.4$ Hz, 1.9H), 3.32 (dd, $J = 7.8, 1.7$ Hz, 2H), 2.95 (dd, $J = 6.6, 1.0$ Hz, 0.8H), 2.92 – 2.89 (m, 0.8H), 2.76 (dt, $J = 28.2, 7$ Hz, 1H), 2.12 – 2.02 (m, 2H), 1.80 (d, $J = 7.4$ Hz, 2H), 1.70 (d, $J = 7.6$ Hz, 5.7H). ^{13}C NMR (75 MHz, CDCl_3): δ 175.9, 175.8, 175.7, 175.7, 175.1, 175.0, 174.6, 170.6, 167.7, 142.4, 140.2, 139.0, 138.9, 138.9, 138.8, 138.4, 138.3, 136.9, 136.8, 135.3, 135.2, 134.6, 134.5, 133.9, 132.4, 130.8, 128.4, 128.4, 128.31, 128.1, 128.1, 127.8, 127.6, 127.6, 127.5, 127.1, 127.0, 126.9, 110.3, 107.6, 92.2, 92.2, 91.5, 91.4, 81.0, 80.7, 79.6, 79.6, 68.1, 67.9, 61.3, 61.3, 60.7, 60.5, 57.3, 50.3, 50.1, 50.1, 49.6, 49.6, 47.7, 47.7, 47.5, 47.47, 45.6, 45.5, 38.6, 30.3, 29.6, 28.8, 25.5, 23.7, 22.9, 17.6, 16.4, 15.9, 15.9, 14.0, 10.9. HRMS (ESI+) Calculated for $\text{C}_{17}\text{H}_{18}\text{NO}_4$ $[\text{M}+\text{H}]^+$: 300.1236; found: 300.1240. HPLC System A ($\lambda = 254$ nm) $t_{\text{R}} = 3.20$ min, 3.25 min.

Azaphthalimide (26). To a solution of 6-(5-ethoxy-2-methyloxazol-4-yl)hexanoic acid **8** (60 mg, 0.25 mmol, 1.0 equiv.) in a solution of THF/ H_2O (1 mL, 2:1 v/v) were added a solution of **19** (50 mg, 0.25 mmol, 1.0 equiv.) in THF/ H_2O (1 mL, 2:1 v/v) and TFA (19 μL , 0.25 mmol, 1.0 equiv.). The mixture was stirred at room temperature for 24 h. After completion, the reaction mixture was diluted with water (10 mL) and the aqueous layer extracted with EtOAc (3×15 mL). The organic layers were combined, dried over MgSO_4 , and solvents were removed under vacuum. The crude product was purified by RP-HPLC (System D) and fractions were collected and lyophilized to furnish the title compound (61 mg, 0.12 mmol, 48 % yield) as a yellow powder. One stereoisomer was

observed by NMR. IR (neat): 3375, 2941, 2865, 1699, 1393, 1335, 1197, 1146, 1061, 759, 698. ^1H NMR (300 MHz, $\text{CD}_3\text{CN} + 5\%$ methanol- d_4): δ 7.47 – 7.23 (m, 5H), 5.49 (q, $J = 7.4$ Hz, 1H), 2.89 (t, $J = 7.6$ Hz, 2H), 2.66 (s, 3H), 2.27 (t, $J = 7.4$ Hz, 2H), 1.85 (d, $J = 7.3$ Hz, 3H), 1.72 (dd, $J = 15.1, 7.7$ Hz, 2H), 1.66 – 1.54 (m, 2H), 1.47 – 1.34 (m, 2H). ^{13}C NMR (75 MHz, $\text{CD}_3\text{CN} + 5\%$ methanol- d_4): δ 176.2, 168.8, 160.4, 146.8, 146.5, 141.7, 129.6, 128.7, 128.1, 122.8, 122.1, 118.6, 50.5, 34.5, 32.7, 29.7, 28.7, 25.6, 19.5, 17.9. HRMS (ESI-) Calculated for $\text{C}_{22}\text{H}_{23}\text{N}_2\text{O}_5$ $[\text{M}-\text{H}]^-$: 395.1607; found: 395.1617. HPLC System A ($\lambda = 254$ nm) $t_{\text{R}} = 4.22$ min.

Azaphthalimide model (AM). To a solution of 4-benzyl-5-ethoxy-2-methyloxazole **9** (54 mg, 0.25 mmol, 1.0 equiv.) in a solution of THF/ H_2O (1 mL, 2:1 v/v) were successively added a solution of **19** (50 mg, 0.25 mmol, 1.0 equiv.) in THF/ H_2O (1 mL, 2:1 v/v) and TFA (20 μL , 0.25 mmol, 1.0 equiv.). The mixture was stirred at room temperature for 24 h. After completion, the reaction mixture was diluted with water (10 mL) and the aqueous layer extracted with EtOAc (2×15 mL). The organic layers were combined, dried over MgSO_4 , and solvents were removed under vacuum. The crude product was purified by flash-column chromatography (silica gel, cyclohexane/EtOAc as gradient from 100:0 to 95:5) to yield the title compound (70 mg, 0.19 mmol, 75 % yield) as a yellow oil. One stereoisomer was observed by NMR. IR (neat): 3430, 3088, 3064, 3029, 2979, 2933, 1764, 1695, 1602, 1395, 1362, 1328, 1146, 953, 906, 726, 695. ^1H NMR (300 MHz, CDCl_3): δ 7.50 – 7.41 (m, 3H), 7.38 – 7.14 (m, 7H), 5.48 (q, $J = 7.3$ Hz, 1H), 4.24 (s, 2H), 2.74 (s, 3H), 1.89 (d, $J = 7.3$ Hz, 3H). ^{13}C NMR (75 MHz, CDCl_3): δ 169.0, 167.6, 156.9, 147.5, 145.3, 139.6, 137.6, 129.1, 128.5, 128.4, 127.9, 127.3, 126.5, 120.4, 119.7, 49.7, 38.8, 20.1, 17.3. HRMS (ESI+) Calculated for $\text{C}_{20}\text{H}_{21}\text{N}_2\text{O}_3$ $[\text{M}+\text{H}]^+$: 373.1552; found: 373.1550. HPLC System A ($\lambda = 254$ nm) $t_{\text{R}} = 5.09$ min.

Preparation of Michael adducts

Thio Michael adduct (27). To a solution of **19** (25 mg, 0.12 mmol, 1.0 equiv.) in a solution of MeCN/PBS pH 7.4 (1 mL, 1:1, v/v) was added N-acetylcysteine (23 mg, 0.14 mmol, 1.2 equiv.). The mixture was stirred at room temperature for 2 h. After completion, the reaction mixture was diluted with water (10 mL) and the aqueous layer extracted with EtOAc (2×15 mL). The organic layers were combined, dried over MgSO_4 , and solvents were removed under vacuum. The crude product was purified twice by RP-HPLC (System D) and fractions were collected and lyophilized to furnish the title compound (20 mg, 0.055 mmol, 46 % yield) as a lyophilized white powder. Two stereoisomers were observed by NMR in a ratio of 1:1. IR (neat) 3342, 3276, 2985, 2941, 2106, 1695, 1538, 1362, 1219, 1183, 765, 698. ^1H NMR (300 MHz, methanol- d_4): δ 7.42 – 7.20 (m, 10H), 5.37 (q, $J = 7.3$ Hz, 2H), 4.71 (dd, $J = 9.1, 4.4$ Hz, 1H), 4.64 (t, $J = 6.3$ Hz, 1H), 3.94 (t, $J = 3.6$ Hz, 1H), 3.91 (t, $J = 3.6$ Hz, 1H), 3.51 (dd, $J = 13.9, 4.4$ Hz, 1H), 3.24 – 3.17 (m, 2H), 3.17 – 3.10 (m, 2H), 2.91 (dd, $J = 13.9, 9.2$ Hz, 1H), 2.50 (dd, $J = 18.6, 3.9$ Hz, 1H), 2.38 (dd, $J = 18.6, 3.6$ Hz, 1H), 2.00 (s, 3H), 1.99 (s, 3H), 1.79 (d, $J = 0.8$ Hz, 3H), 1.77 (d, $J = 0.8$ Hz, 3H). ^{13}C NMR (75 MHz, methanol- d_4): δ 178.5, 178.3, 176.6, 176.5, 173.4, 173.4, 173.3, 141.0, 141.0, 129.4, 129.4, 128.6, 128.2, 128.1, 53.7, 52.9, 51.6, 51.5, 41.3, 39.9, 37.1, 36.6, 34.3, 34.1, 22.4,

16.9, 16.8 . HRMS (ESI+) Calculated for $C_{17}H_{21}N_2O_5S$ $[M+H]^+$: 365.1171 ; found : 365.1165. HPLC System A ($\lambda = 254$ nm) $t_R = 3.23$ min.

Conflicts of interest

There are no conflicts to declare.

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