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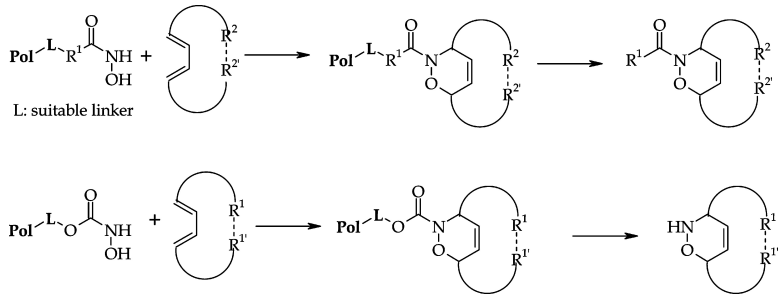

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Solid-Supported Nitroso Hetero Diels–Alder Reactions. 1. Acylnitroso Dienophiles: Scope and Limitations

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Polymer-supported acylnitroso dienophiles were prepared and used in hetero Diels–Alder (HDA) reactions with a variety of dienes. The transient acylnitroso dienophiles were prepared in situ from immobilized hydroxamates, which were attached to solid supports via several linkers each cleavable by different cleavage reagents, and served for the synthesis of both N-unsubstituted and N-derivatized HDA adducts. Model compounds were used to (i) optimize reaction conditions for solid-supported HDA reactions, (ii) evaluate the outcome of the reactions with various dienes, (iii) compare relative reactivities of dienes, and (iv) assess the stability of HDA adducts toward cleavage conditions typically used in solid-phase syntheses. Cleaved products were submitted to biological assays, and the results are reported. The accompanying paper, focused on complementary arylnitroso HDA reactions, includes a comparison of both HDA reactions.

Introduction

The appeal of acyl- and arylnitroso hetero-Diels–Alder (HDA) reactions[1–5] for the creation of novel structural and functional diversity in chemical space consists of the formation of two new carbon−heteroatom bonds, while simultaneously introducing a rigid element, the dihydro[1,2]-oxazine ring, that dramatically changes the original spatial arrangement of the incoming diene. These attributes, coupled with the immense diversity of accessible dienes, particularly those that originate from natural products,[6] make HDA reactions attractive for the generation of new molecular libraries. In addition to the fact that HDA adducts are attractive compounds per se,[7] synthetic HDA cycloadducts represent a rich pool of intermediates amenable to further transformations. Among HDA-specific transformations, that is, those concerning the oxazine ring modification, ring opening by cleavage of the component C═C, N−O, and C−O bonds attracted attention.

Oxidative cleavage of the olefin yielded novel diacids.[8,9] Reductive cleavage of the N−O bond gave 1,4-aminoalcohols.[10,11] Scission of the C−O bond yielded N-substituted hydroxamates. Lewis acid, including iron(III),[12,13] copper(II),[13] and palladium(0),[12] mediated ring opening in the presence of an alcohol provided access to cycloalkene-derived hydroxamates. Copper-catalyzed reactions of cyclopentadiene HDA adducts with Grignard reagents[14] and palladium(0)/indium iodide-mediated allylic additions to aldehydes and ketones[15] led to controlled formation of new C−C bonds.

Nitroso HDA reactions have become valuable and often key transformations in total syntheses of natural products such as the alkaloids narciclasin,[16] azimine, and carpaine,[17] as well as numerous biologically active compounds,[14,18–21] including generation of novel *N*(4)-hydroxy-1,4-benzodiazepines.[22]

There are numerous reports describing Diels–Alder reactions on solid-phase supports (reviewed, for example, in ref 23). The polymer-supported hetero-Diels–Alder reaction has become a valuable transformation for synthesis of heterocyclic compounds including synthesis of dihydropyrans,[24–26] di- and tetrahydropyridines,[27–29] di- and tetrahydropyridazines,[30–32] pyridazines,[33] fused pyrrolidine rings,[34,35] and complex fused ring systems.[34–41] Although acyl- and arylnitroso HDA reactions have frequently been used in solution for the synthesis of variety of targets, the acylnitroso HDA reaction has not been widely used in solid-support chemistry.[42,43]

In this and the accompanying paper, we describe results of our efforts to adopt and use solid-supported acyl- and arylnitroso dienophiles in HDA reactions with dienes as counterparts in solution. Because of the large variety of dienes, including complex natural products available as substrates, we have chosen to attach nitroso dienophiles to solid supports and subject them to reactions with various dienes in solution rather than developing specific routes for immobilization of individual dienes. From the chemical point of view, the immobilization of nitroso dienophiles represents the more challenging task because the more-reactive species is immobilized on the solid support. Although both acyl- and arylnitroso HDA reactions yield dihydro[1,2]oxazine derivatives, the syntheses, properties, and reactivity of acyl- and arylnitroso dienophiles differ substantially and therefore

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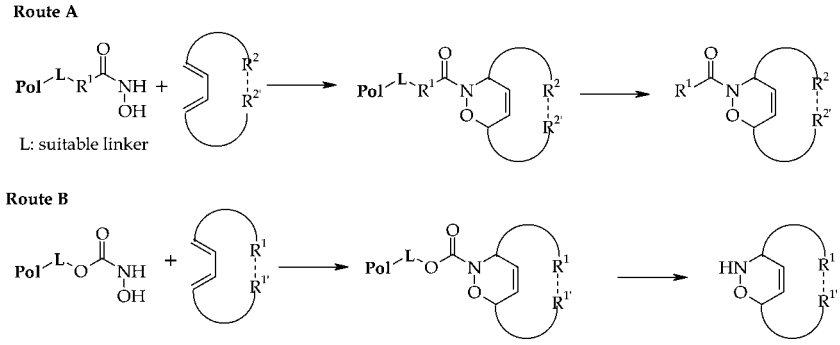
Scheme 1. Two Strategies for Polymer-Supported Transformations of Diverse Dienes via HDA Reactions



they will be dealt with independently in two consecutive contributions.[44]

Results and Discussion

In this, our first paper dedicated to polymer-supported HDA reactions, we focus on transformations of dienes by solid-phase-supported acylnitroso species. When compared to the HDA reaction with arylnitroso dienophiles, the acylnitroso HDA reaction is more challenging, but offers larger variety for chemical modifications of targeted dienes. To perform the HDA transformation on a range of structurally unrelated dienes, we immobilized the acylnitroso dienophile precursor hydroxamates. We considered two different synthetic scenarios to access the dihydro[1,2]oxazine products (Scheme 1). Route A facilitated introduction of an additional diversity element at the nitrogen atom of the dihydro[1,2]oxazine moiety. Route B was designed to provide products in a traceless manner, that is, with no additional functional group attached to the transformed diene at the point of the acylnitroso HDA reaction. This latter route was designed to be carried out on linkers that serve to "catch" the HDA product for subsequent combinatorial transformations and "release" the transformed diene. Both routes also enable further transformations of peripheral positions of the diene (in a combinatorial fashion while still attached to the resin) to provide a structurally diverse set of derivatives.

Unlike most arylnitroso compounds that are stable enough to be isolated, acylnitroso species are unstable and must be prepared in situ for the subsequent cycloaddition reaction. Typically, hydroxamates are oxidized to acylnitroso derivatives in the presence of a diene to yield a HDA adduct. Thus, this reaction route precludes the use of dienes that do not tolerate the presence of an oxidant. This problem was overcome by creating a HDA adduct that undergoes retro-HDA reaction in the presence of an oxidation sensitive diene.[45] For example, transient acylnitroso species can be trapped by 9,10-dimethylanthracene (DMA). The DMA−HDA adduct is not thermally stable and, upon gentle heating, undergoes retro-HDA reaction, liberating the acylnitroso species, that, in the presence of a diene, forms a HDA adduct with the new diene.[45] We focused a part of our effort to adopt the retro HDA reaction to solid phase. An alternative procedure for generation of acylnitroso species in the absence of an oxidant, applied also to solid phase, uses photolysis of 1,2,4-oxadiazole-4-oxides.[43]

Because the acylnitroso HDA reaction has not been systematically studied on a solid support,[42] we focused on the development of reaction conditions for polymer-supported acylnitroso HDA reactions including access to hydroxamates, oxidation to acylnitroso dienophiles in the presence of a diene, and retro-HDA reactions. In addition, numerous HDA adducts are unstable in strongly acidic medium, typically used for cleavage of target compounds from solid support. This limitation-required selection of linkers allows mild conditions for the release of acid-sensitive HDA adducts from the resin.

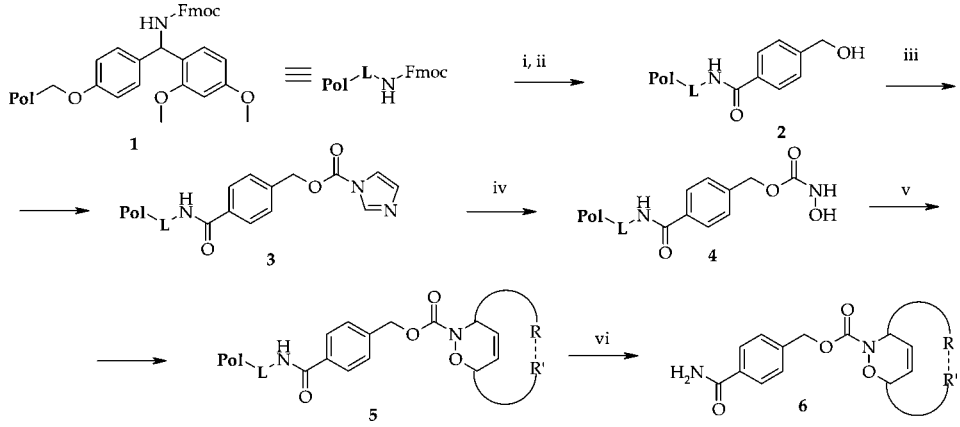
Feasibility Study. To study the feasibility of the polymer-supported acylnitroso HDA reaction, we synthesized several model polymer-supported hydroxamate substrates that addressed syntheses of both N-substituted and N-unsubstituted HDA adducts, different cleavage conditions, and reaction conditions for dienes not compatible with an oxidant.

N-Substituted Dihydro[1,2]oxazine: Rink Linker. First, we describe syntheses of N-substituted HDA adducts. We used a resin-bound Rink amide linker acylated with 4-hydroxymethylbenzoic acid to introduce the hydroxamate.

Briefly, the Fmoc group of commercially available Rink resin (**1**) was cleaved by piperidine, and the liberated amino group was acylated with 4-hydroxymethylbenzoic acid (HMBA). The resulting polymer-supported alcohol, **2**, was reacted with carbonyldiimidazole (CDI) to form activated resin **3**, followed by reaction with hydroxylamine hydrochloride in pyridine. The resulting resin-bound hydroxamate, **4**, was oxidized with tetrabutylammonium periodate in the presence of dienes to generate polymer-supported products, **5**, which, upon treatment with 50% trifluoroacetic acid (TFA) released crude products, **6** (Scheme 2). Subsequent analyses provided information on the effectiveness of the multistep solid-phase process.

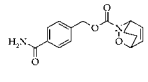
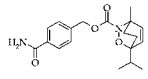
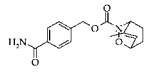
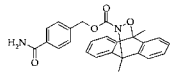
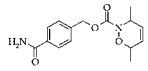
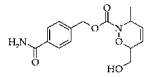
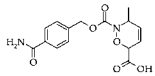
While the HDA adduct with 2,4-hexadiene, 2,4-hexadienol, and sorbic acid provided expected products **6e**, **6h**, and **6i** cleanly (Table 1), the LCMS analysis of the cleaved sample from the reaction with 1,3-cyclohexadiene revealed a complex reaction mixture that contained five components in double-digit percent yields, including the expected product **6a**. Milder cleavage conditions using 10% TFA for 30 min cleanly generated the expected cleavage product, indicating instability of the product **6a** in acidic media (however, the yield was lower because of incomplete cleavage from the Rink linker). Cyclohexadienes with alkyl substituents (α-terpinene and 1,3,5,5-tetramethylcyclohexadiene) did not provide any product upon cleavage with 10% TFA. LCMS analysis revealed the presence of the starting hydroxamate, indicating either incomplete oxidation or possible novel

Scheme 2. Synthesis of N-Substituted HDA Adducts Using an Acid Cleavable Linker[a]



[a] **L** stands for the Rink linker. Reagents and conditions: (i) 20% piperidine, DMF, RT, 30 min; (ii) HMBA, DIC, HOBt, RT, 16 h; (iii) CDI, pyridine, DCM, 1 h, RT; (iv) $NH_2OH \cdot HCl$, pyridine, RT, 2 h; (v) 0.1 M nBu_4NIO_4, diene, DCM, RT, 1 h; (vi) 50% TFA, DCM 30 min.

Table 1. HDA Adducts from Rink Resin-Derived Dienophiles[a]

Product	Diene	Structure	$[M+H]^+$	Isomers	Yield
6a	1,3-cyclohexadiene (CHD)		289.2	-	39%
6b	α-terpinene		not detected		
6c	1,3,5,5-tetramethyl-CHD		not detected		
6d	9,10-dimethylanthracene		cf. the text		
6e	2,4-hexadiene		291.2	-	73%
6h	trans,trans-2,4-hexadien-1-ol		307.5	74:26	53%
6i	sorbic acid		321.5	93:7	56%

[a] Electrospray ionization (ESI) mass spectrometry provided the expected $[M+H]^+$ signal. The yields and relative ratios of regioisomers were estimated from ^1H NMR spectra.

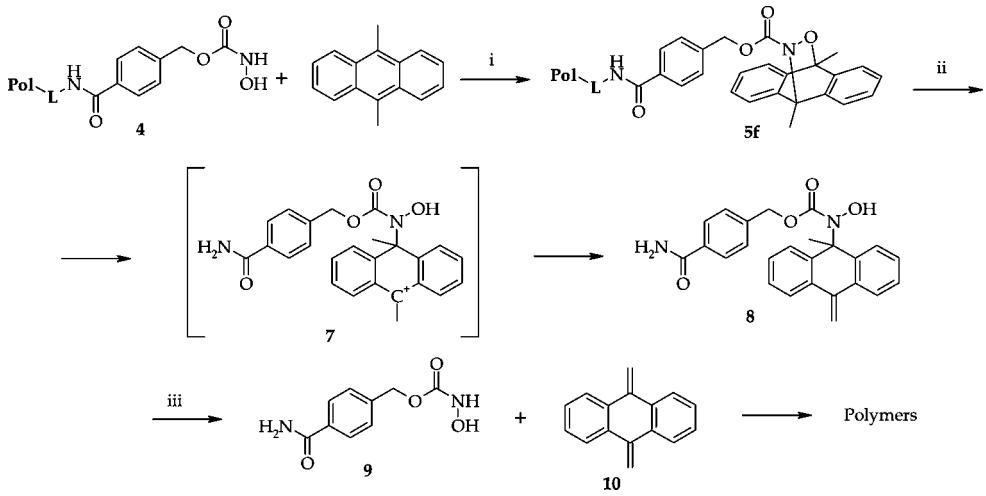
chemistry of the expected cycloadduct under the conditions used to cleave the product from the resin. The application of acid instability for preparatively useful post-HDA modifications will be described in a dedicated paper.

The structures of the regioisomers were assigned using 2D NMR spectra and are described for the entire set of acyl and arylnitroso HDA adducts in the accompanying paper.[44] In general, the major regioisomer of each set contained the bulkier group attached to the C6 carbon of the dihydro[1,2] oxazine ring.

The products obtained from TFA cleavage after the HDA reaction with DMA was more complex than anticipated. The LCMS analysis of a sample cleaved by 50% TFA in dichloromethane (DCM) revealed the presence of the starting hydroxamate with no traces of the expected product. Considering the previous results with electron rich dienes, we suspected that the HDA adduct **5d** was formed, but it was not stable toward the conditions of the cleavage because the *O*-(9,10-dimethyl-9,10-dihydro-anthracen-9-yl) group can be cleaved by 50% TFA in 30 min, typical conditions for cleavage of amides from the Rink linker. Thus, we carried out the cleavage under milder conditions, 10% TFA for 15 min. The HPLC traces of a cleaved sample was complex and, in addition to starting material, indicated the presence of a compound that provided a MS signal corresponding to the mass of the expected product. However, the ^1H NMR spectrum of the HPLC-purified compound contained a signal for only one methyl group and two methylene signals. In addition, the NMR spectrum contained a resonance signal of the OH proton of the hydroxamate (δ 10.0). The *O*-(9,10-dimethyl-9,10-dihydro-anthracen-9-yl) group was cleaved, and the intermediate carbocation, **7**, lost a proton to generate the corresponding alkene, **8**. However, alkene **8** also was not stable to the acidic conditions and reacted further to form the starting hydroxamate **9** and 9,10-dimethylene-9,10-dihydro-anthracene **10**, which polymerized (Scheme 3). Thus, recovery of the initial hydroxamate did not indicate that the initial oxidation to generate the corresponding acylnitroso moiety had failed but that subsequent acidic cleavage conditions induced additional chemistry of the initial diene and regeneration of the starting hydroxamate! These interesting results encouraged further studies, especially related to alternative conditions for release of acid-labile acylnitroso cycloadducts from solid supports.

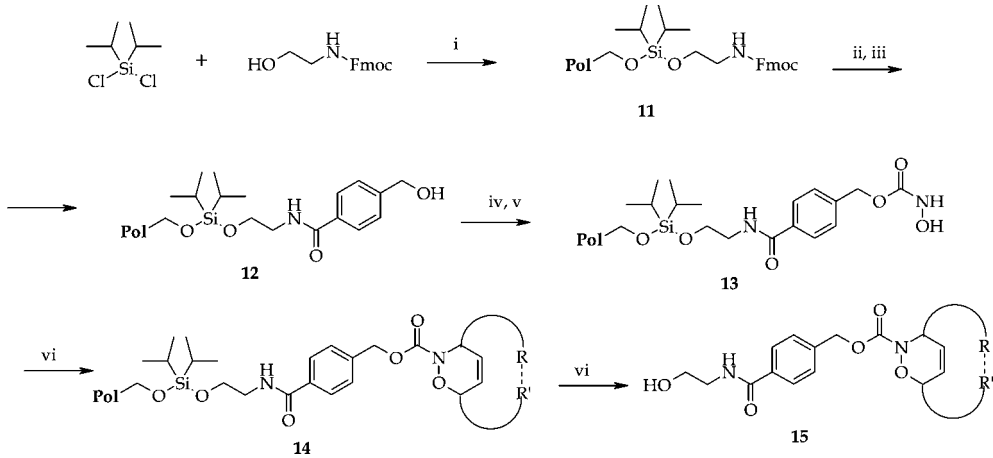
While these model experiments demonstrated the feasibility of polymer-supported acylnitroso HDA reactions and that the conditions employed are compatible with syntheses of HDA adducts that tolerate a TFA-containing cleavage cocktail, the instability of resin-bound intermediates toward TFA prompted us to prepare polymer-supported acylnitroso dienophiles on linkers that allow cleavage by mild reagents. To avoid the use of acidic cleavage cocktails, we chose to explore two types of linkers, a silyloxy-based linker cleavable by tetra-*n*-butylammonium fluoride (TBAF) and an electron-rich benzyl ether Wang-derived linker cleavable by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).[46,47] In the process of developing new linker technology, we also

Scheme 3. Cleavage of the DMA Adduct by TFA[a]



[a] Reagents and conditions: (i) 0.1 M nBu$_4$NIO$_4$, DCM, RT, 1 h; (ii) 10% TFA, DCM, 15 min; (iii) 50% TFA, DCM 30 min.

Scheme 4. Synthesis of the HDA Adducts on a TBAF Cleavable Linker[a]



[a] Reagents and conditions: (i) imidazole, NMP, RT, 1 h, then Wang resin, RT, 1 h; (ii) 20% piperidine, DMF, RT, 30 min; (iii) HMBA, DIC, HOBt, RT, 1 h; (iv) CDI, pyridine, DCM, 1 h, RT; (v) $NH_2OH \cdot HCl$, pyridine, RT, 2 h; (vi) diene, 0.1 M nBu$_4$NIO$_4$, DCM, RT, 1 h; (vii) 0.1M TBAF, THF, RT, 30 min.

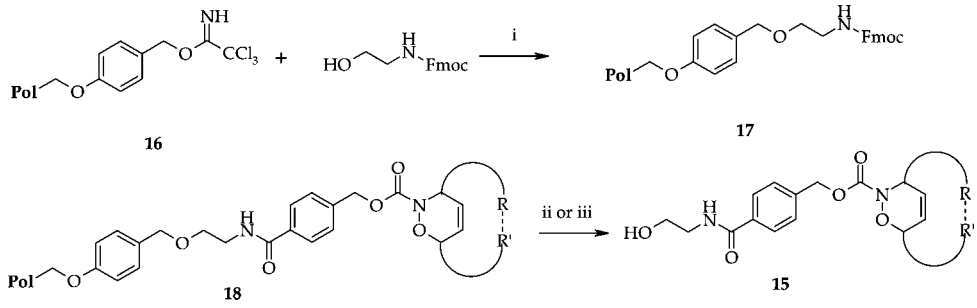
intended to design polymer-supported dienophiles for HDA reactions suitable for combinatorial syntheses.

N-Substituted Dihydro[1,2]oxazine: Si Linker. Of the numerous silyl linkers reported in the literature,[48,49] we selected the diisopropylsilyl linker,[50] which has been successfully used for the solid-phase syntheses of polyketides.[51] The diisopropylsilyl linker[50] facilitated incorporation of amino alcohols, practical building blocks for future combinatorial syntheses. Thus, *N*-Fmoc-ethanolamine was reacted with diisopropyldichlorosilane and the formed monosilyl ether was added to standard hydroxymethyl resin to provide resin **11**. The Fmoc group was removed by piperidine, followed by acylation with HMBA. The resulting polymer-supported alcohol, **12**, was reacted with CDI and subsequently with hydroxylamine hydrochloride to provide the required intermediate *N*-hydroxy carbamate, **13**, for subsequent HDA reactions. Thus, oxidation to the nitroso species was carried out by treatment with nBu$_4$NIO$_4$ in the presence of dienes. The cycloaddition products, **15**, were cleaved from the newly derivatized solid support **14** by TBAF and analyzed. Because the silyl linker is acid-sensitive, the target HDA adducts **15** were also released by separate treatment with TFA to evaluate the acid stability of products (Scheme 4). The results were compared with those using different linkers and discussed latter.

N-Substituted Dihydro[1,2]oxazine: Wang Linker. An alternative route for the syntheses of HDA adducts, **15**, was developed using a Wang linker that allows release of products using DDQ (Scheme 5),[46,47] in addition to the customary TFA-based reagent. Thus, the Wang imidate resin, **16**,[52] was reacted with Fmoc ethanolamine according to the optimized procedure developed for amino alcohols.[53] The loading of resin **17**, determined by quantification of the UV response in a TFA cleaved sample, was 0.38 mmol/g, comparable with reported results.[53] The polymer-supported ethanolamine, **17**, was subjected to the reaction sequence analogous to that used with the silicon linker-based dienophile **13**. HDA reactions yielded resin bound adducts **18** that, upon cleavage with TFA or DDQ, provided HDA adducts **15** that were identical to products obtained from the silicon linker, **9**.

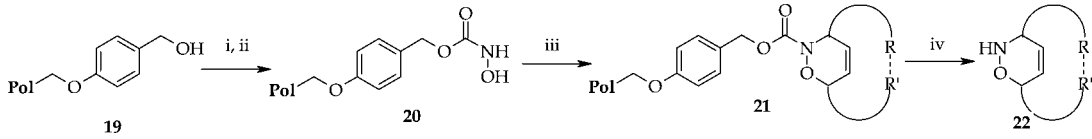
This route was used for the synthesis of TFA-stable HDA adducts by releasing the target adducts **15** with TFA. The small amount of TFA ester that formed during cleavage[47] was hydrolyzed by addition of water. TFA-sensitive HDA

Scheme 5. Synthesis of the HDA Adducts on a DDQ Cleavable Linker[a]



[a] Reagents and conditions: (i) $BF_3 \cdot Et_2O$, THF, RT, 30 min; (ii) 10% TFA, RT, 30 min; (iii) DDQ, DCM, water, RT, 1 h.

Scheme 6. Solid-Supported HDA Reaction on Wang Resin[a]



[a] Reagents and conditions: (i) CDI, pyridine, DCM, 1 h, RT; (ii) $NH_2OH \cdot HCl$, pyridine, RT, 2 h; (iii) 0.1 M nBu$_4$NIO$_4$, diene, DCM, RT, 1 h; (iv) 50% TFA, DCM, 30 min.

Table 2. HDA Adducts Prepared from Wang Resin-Derived Dienophiles[a]

product	diene	$[M + H]^+$	isomers	yield
22a	1,3-cyclohexadiene	112.1		37%
22b	α-terpinene	168.2	55:45	36%
22d	9,10-dimethylanthracene (DMA)	238.2		12%
22e	2,4-hexadiene	114.1		40%
22h	trans,trans-2,4-hexadien-1-ol	130.1	72:28	72%
22i	sorbic acid	143.1	91:9	42%

[a] Electrospray ionization (ESI) mass spectrometry provided the expected $[M + H]^+$ signal for each product. The yields and relative ratios of regioisomers were estimated from the corresponding ^{1}H NMR spectra.

adducts were cleanly cleaved in a high yield by DDQ.[46,47] In addition, as will be described in a subsequent paper, we used this linker for the synthesis of post-HDA reaction modification of TFA-labile HDA adducts.

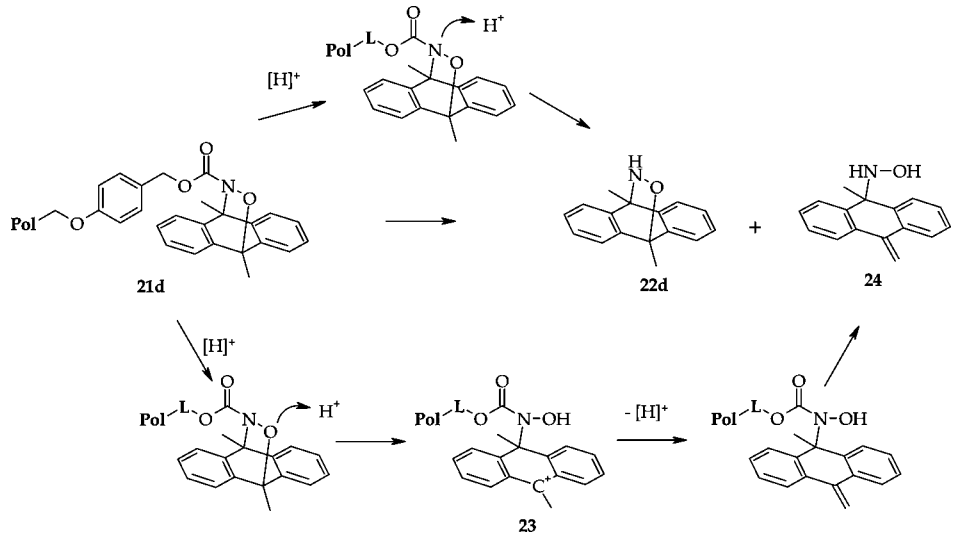
N-H Dihydro[1,2]oxazine: Wang Linker. In this section, we focus on route B of Scheme 1 that, as indicated earlier, was designed for preparation of HDA adducts that do not contain a substituent at the dihydro[1,2]oxazine nitrogen. These adducts would correspond to the equivalent of having performed a cycloaddition reaction with "N═O", itself. The most straightforward reaction sequence to provide the requisite resin-bound hydroxamate was modification of the Wang resin, **19**. The polymer-supported Wang linker was treated with a solution of CDI in DCM, followed by addition of hydroxylamine hydrochloride in pyridine. The resulting hydroxamate, **20**, was oxidized by tetrabutylammonium periodate in DCM in the presence of a diene and the resin-bound product, **21**, was treated with TFA to release the HDA adduct, **22** (Scheme 6). A small set of dienes was evaluated in this HDA reaction and the results are summarized in Table 2.

We observed formation of the HDA adducts with simple dienes, including those that were cyclic (**22a**, **22b**) and acyclic (**22e**) with electron-donating (**22h**) and -withdrawing (**22i**) groups. No products were detected with 1-methoxy-1,3-cyclohexadiene and β-ionone. Unsymmetrical dienes formed both regioisomers and their relative ratio was estimated from the ^{1}H NMR spectra of crude products.

We also evaluated the HDA reaction with DMA to carry out the retro-HDA reaction and trapping with other added dienes. LCMS analysis of a TFA-cleaved sample revealed the presence of two major components with the relative ratio dependent on the concentration of TFA. NMR analyses of HPLC-purified samples confirmed that one of the products was the expected HDA adduct, **22d**. The NMR spectrum of the second component was consistent with a product, **24**, formed from carbon−oxygen bond cleavage. The ^{1}H NMR spectrum contained a proton signal from only one methyl group and a signal of two protons from the methylene group. The formation of this side-product, **24**, was caused by acid-induced cleavage of the carbon−oxygen bond, while the HDA adduct was still attached to the resin (Scheme 7). We observed that milder cleavage conditions (10% TFA for 30 min) caused predominant formation of the side-product (57% when compared to 24% of the expected adduct), while a higher concentration of TFA (50%) reduced the formation of the side-product to 20%. Prolonged exposure to the cleavage cocktail did not increase the relative amount of the side-product. An independent confirmation of this cleavage pattern was provided by studies of the next model substrate designed not to cleave the carbamate linkage (cf., the previous section). In general, the acid instability of the HDA adducts was found to depend on the ability to stabilize the carbocation (shown for the DMA adduct in structure **23**) formed by scission of the carbon−oxygen bond. We observed this type of carbon−oxygen bond cleavage on several occasions, and its potential for the preparative use by trapping the carbocation by a nucleophile will be addressed in a separate paper.

When compared with the lability of DMA adduct **21d** on a Wang linker, **5f** is considerably less stable. The release of the benzyloxycarbonyl group from the dihydro[1,2]oxazine seemed to stabilize the DMA adduct toward TFA.

Scheme 7. Side-Product Formation during TFA Cleavage of the HDA Adduct with DMA[a]



[a] **L** stands for the Wang linker. Reagents and conditions: 10–50% TFA, DCM, 30 min.

The use of a Wang linker-immobilized acylnitroso precursor provided the expected HDA products with simple dienes; however, individual steps of the reaction sequence were difficult to monitor, and optimization of reaction conditions was not trivial. In addition, the reaction conditions excluded the use of dienes that were incompatible with acidic media (TFA) used for cleavage of target compounds from the resin. Absence of the product in a cleaved sample could have been caused either by failure of the HDA reaction or decomposition of the product. Although this linker is synthetically very useful for the preparation of TFA-resistant HDA products without any N-substitution on the dihydro[1,2]oxazine ring, to evaluate the scope and limitations of the solid-supported HDA reaction with various dienes, we designed and synthesized a dual linker that circumvented the above-mentioned problems. We have already successfully used dual linkers in unrelated studies.[54–56]

***N*-H Dihydro[1,2]oxazine: Dual Si/Wang linker.** By analogy to the syntheses of N-substituted acid-sensitive HDA adducts, we introduced the TBAF cleavable silicon-based linker into the dual Si/Wang construct (Scheme 8, structure **30**). The first linker allowed mild cleavage of a product (and its subsequent analysis) after each solid-phase transformation. Use of the mild cleavage reagent TBAF allowed release of all solid-supported components from a silicon-based linker. The HDA adduct was released from a silicon linker, still attached to the Wang linker. This step enabled evaluation of dienes for their applicability in HDA reactions. Treatment with TFA cleaved the HDA adduct from the Wang linker and enabled assessment of the stability of the products to TFA.

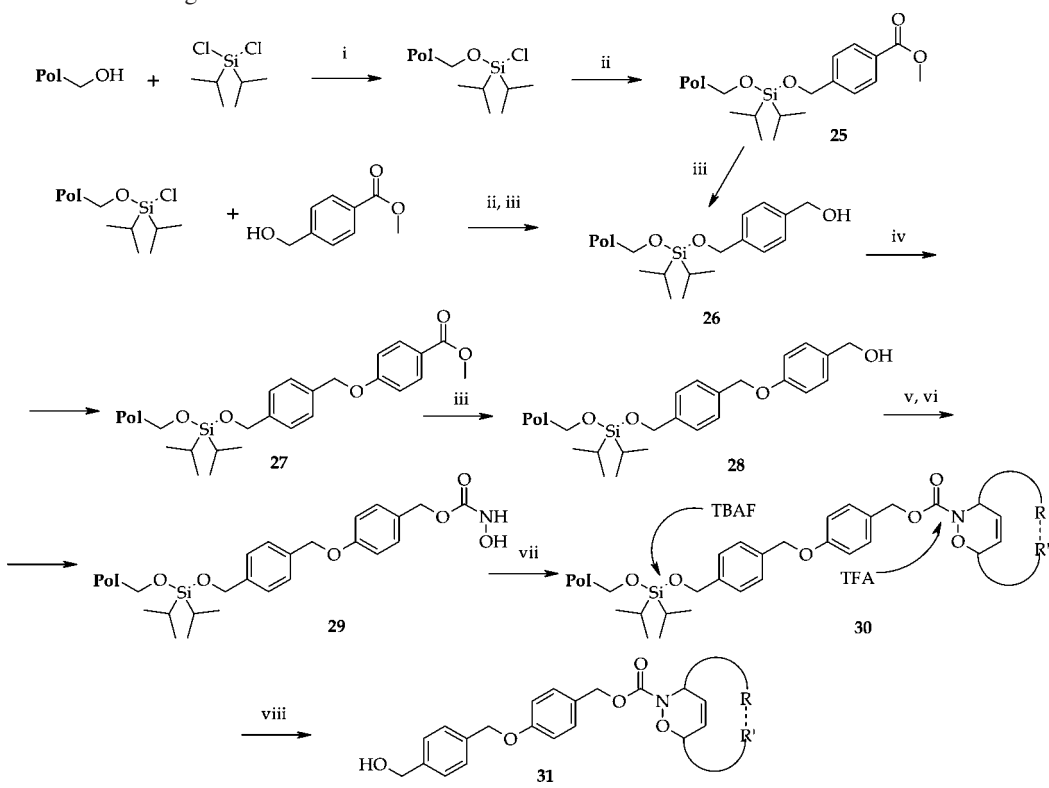
The diisopropylsilyl linker[50] can be prepared from commercially available synthons and allows immobilization of a variety of alcohols and phenols. The first building block, 4-hydroxymethylbenzoic acid, was immobilized on the diisopropyl silyl linker using the reported procedure.[50] The polymer-supported ester, **25**, was reduced by DIBAL,and the new polymer-supported alcohol, **26**, was reacted under Mitsunobu conditions with methyl 4-hydroxybenzoate. The resulting ester, **27**, was reduced to alcohol **28** by treatment with DIBAL, and further transformations to hydroxamate **29** followed the route already described for the Wang-based hydroxamate **20**. Hydroxamate **29** was then oxidized in the presence of dienes. Different reaction conditions were tested. Low temperature (−18 or 0 °C) did not substantially increase product yield or purity when compared to oxidation at ambient temperature. Use of Dess−Martin periodinane rather than tetrabutylammonium periodate did not produce considerable differences. The reaction was carried out in DCM to facilitate proper swelling of the polystyrene-based resin.

Treatment of polymer-supported HDA adduct, **30**, on the dual Si/Wang linker with TBAF, released the transformed diene as a *N*-(4-(4-hydroxymethylbenzyloxy)-benzyloxycarbonyl) derivative, **31**. Alternatively, TFA-induced cleavage liberated the HDA adduct without substitution on the nitrogen and addressed the stability of HDA reaction products toward acidic cleavage conditions. Because the dual Si/Wang linker contains two sites cleavable by TFA, analyses of cleaved products also allowed evaluation of the cleavage rate of HDA adducts from the Wang linker. The presence of compounds **31** in the TFA-released samples would indicate incomplete cleavage of the 4-alkoxybenzyloxycarbonyl group from the dihydro[1,2]oxazine rings. A sample obtained by treatment with 50% TFA for 30 min was analyzed by LCMS and did not reveal the presence of compound **31**, thus providing direct evidence that the cleavage from the Wang linker was complete. This information was used for syntheses of HDA adducts, **22**, on a Wang linker. We have previously used the dual linker concept for assessing the cleavage rate of unrelated target compounds.[54]

Using this linker, we evaluated the outcome of the HDA reactions with a set of 20 dienes. Tested dienes included cyclic and acyclic versions, as well as dienes containing electron-withdrawing or -donating groups to address both steric and electronic effects. The results will be discussed in the last section of this report, together with results of syntheses of N-substituted HDA adducts.

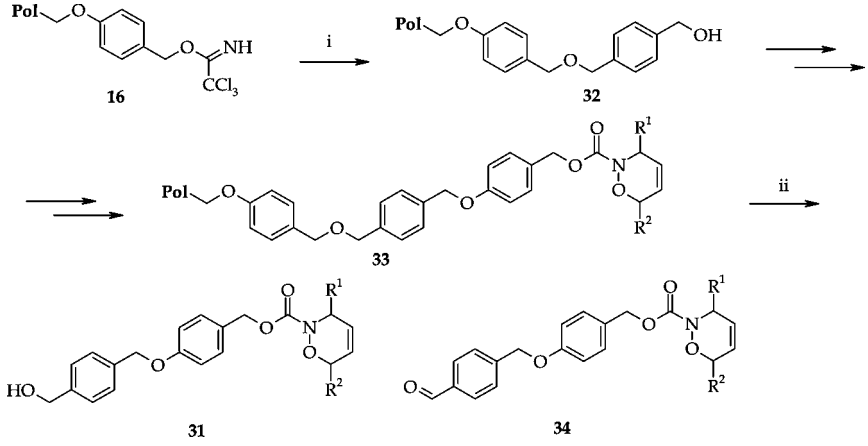
***N*-H Dihydro[1,2]oxazine: Dual Wang/Wang Linker.** For the syntheses of N-derivatized HDA adducts we used two linkers that are cleavable by the nonacidic reagents,

Scheme 8. Synthesis of Si/Wang Dual Linker for Evaluation of Dienes in HDA Reactions[a]



[a] Reagents and conditions: (i) TEA, DMAP, DCM, RT, 1 h; (ii) TEA, DMAP, DMF/DCM, RT, 1 h; (iii) DIBAL, THF, 1 h; (iv) 4-hydroxybenzoic acid methyl ester, DIAD, PPh_3 THF, 5 h; (v) CDI, pyridine, DCM, RT, 1 h; (v) $NH_2OH \cdot HCl$, pyridine, RT, 16 h; (vii) 0.1 M $n Bu_4NIO_4$, diene, DCM, RT, 1 h; (viii) 0.1 M TBAF in THF, 30 min.

Scheme 9. Synthesis of Wang/Wang Dual Linker for Evaluation of Dienes in HDA Reactions[a]



[a] Reagents and conditions: (i) 1,4-benzenedimethanol, $BF_3 \cdot Et_2O$, THF, RT, 30 min; (ii) DDQ, DCM, water, RT, 1 h.

TBAF and DDQ. Application of the same concept toward a dual linker for the synthesis of *N*-H HDA adducts led to the design and studies of a Wang/Wang dual linker. Although this dual linker contains two benzyl ethers, the 4-alkylbenzylether is not cleaved by DDQ.[46]

The dual Wang/Wang linker was prepared from Wang imidate resin **16**.[52] The resin was reacted with 1,4-benzenedimethanol and the Wang-linker immobilized alcohol **32** was subjected to reaction steps identical to those used for the synthesis of the Si/Wang inker-supported hydroxamate, **29**. After oxidation in the presence of dienes, the polymer-supported HDA adducts, **33**, were subjected to DDQ cleavage. Analyses of crude reaction products revealed, in addition to the expected products **31**, another minor component in each case. MS and NMR analyses of the compounds isolated by semipreparative HPLC indicated that the benzyl alcohols were oxidized to the corresponding aldehydes, **34** (Scheme 9).[57,58]

Reactivity of Dienes in Polymer-Supported Acylnitroso HDA Reactions. Because of the rich structural and functional diversity of dienes, including numerous complex natural products, we considered it important to assess the performance of individual dienes with representative features (acyclic, cyclic, substituted with electron-donating and -withdrawing groups, as well as dienes containing bulky groups) in polymer-supported HDA reactions. The diene evaluation included reactivity in HDA reactions with polymer-supported acylnitroso dienophiles, the effect of structure on

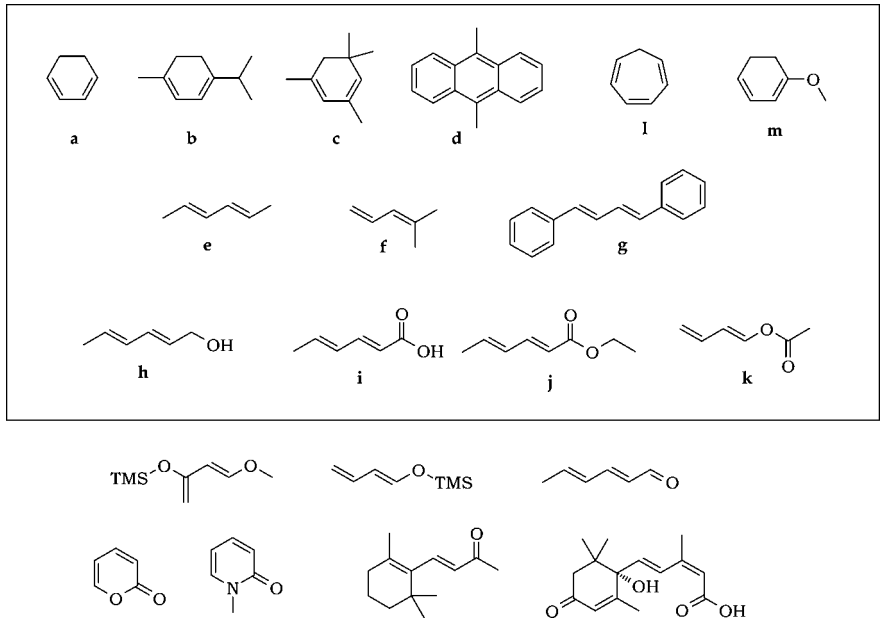


Figure 1. Dienes tested in the acylnitroso HDA reaction (dienes that provided HDA adducts are shown in the box).

Table 3. Analytical Data of HDA Products[a]

product	diene	TFA stability	$[M + H]^+$	isomers	yield
15a	1,3-cyclohexadiene (CHD)	P	335.5		82%
15b	α-terpinene	N	389.5	82:18	87%
15c	1,3,5,5-tetramethyl-CHD	N	389.5	81:19	84%
15d	9,10-dimethylanthracene (DMA)	N	459.1		91%
15e	2,4-hexadiene	Y	333.5		77%
15f	4-methyl-penta-1,3-diene	P	335.3	>99:1	48%
15g	1,4-diphenyl-1,3-butadiene	Y	459.3		63%
15h	trans,trans-2,4-hexadien-1-ol	Y	393.5	80:20	87%
15i	sorbic acid	Y	365.4	94:6	83%
15j	ethyl sorbate	Y	393.5	>99:1	70%
31a	1,3-cyclohexadiene (CHD)	Y	338.3		65%
31b	α-terpinene	N	394.3	84:16	58%
31c	1,3,5,5-tetramethyl-CHD	N	394.5	82:18	ND
31d	9,10-dimethylanthracene (DMA)	N	464.3		ND
31e	2,4-hexadiene	Y	340.1		ND
31h	*trans*,*trans*-2,4-hexadien-1-ol	Y	356.2	72:28	48%

[a] TFA stability (50% TFA in DCM, 30 min): Y, stable; N, not stable; P, partially stable.

Table 4. Retro HDA Reaction of DMA Adduct[a]

adduct	diene	$[M + H]^+$	isomers	yield
15a	cyclohexadiene (CHD)	332.5		95%
15c	1,3,5,5-tetramethyl-CHD	389.6	79:21	92%
15e	2,4-hexadiene	335.5		85%

[a] Electrospray ionization (ESI) mass spectrometry provided the expected $[M + H]^+$ signals. Yields of the HDA reactions were calculated with respect to loading of the DMA adduct.

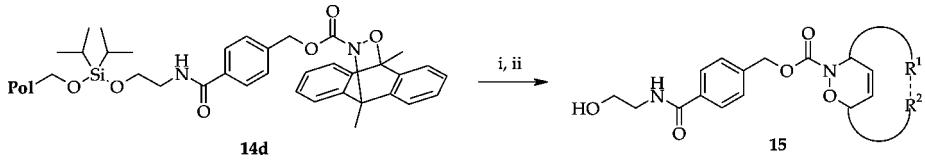
the relative reactivity of dienes yielding the expected products, stability of HDA adducts, and their potential for further chemical transformations.

Using polymer-supported dienophile precursors **13** and **29**, which are cleavable under mild conditions with TBAF, we evaluated the outcome of the HDA reactions with a set of 20 dienes (Figure 1). Six carbocyclic dienes provided clean HDA adducts (Table 3). The HDA reaction of cycloheptatriene with **13** afforded product **15l**, corresponding to a cycloadduct with norcaradiene rather than cycloheptatriene, an analogy to DA reactions with other dienophiles.[59–63] The 1-methoxycyclohexadiene adduct **15m** was not stable, confirming a previous report.[64] Acyclic dienes containing either electron-donating or -withdrawing groups afforded expected products. Interestingly, the HDA adduct with ethyl sorbate was not stable to the TBAF cleavage conditions, but the expected product was isolated after TFA-mediated cleavage from polymer-supported HDA adduct **14j**. Trimethylsilyloxy-derivatized acyclic dienes, pyran-2-one and 1-methyl-1*H*-pyridin-2-one did not afford the expected HDA adduct. We also evaluated the reactivity of two more complex dienes, *β*-ionone and abscisic acid, as representative examples of natural products. Neither provided the target HDA adduct under the standard reaction conditions. The purity of crude HDA adducts from thirteen dienes that provided expected products was >90% as judged by the LC profiles of crude preparations. The data obtained with acylnitroso dienophiles are compared with the results of arylnitroso HDA reaction described in the accompanying paper. The accompanying paper also addresses regioisomerism of HDA adducts prepared from unsymmetrical dienes.

Adducts **15** revealed expected $[M + H]^+$ peaks in their MS spectra. Products **31** exhibited a MS signal corresponding to a fragment $[M - CO_2 + H]^+$ in electrospray ionization (ESI^+) mass spectrometry analyses. Yields were estimated from NMR spectra of crude products. NMR spectra are included in the Supporting Information (ND, not determined).

Treatment of polymer-supported HDA adducts with TFA revealed structural features contributing to instability toward acidic cleavage conditions. The presence of electron-donating groups on dienes contributed to the stability of carbocation intermediates generated during cleavage and, thus, to instability of HDA adducts. However, the fate of HDA adducts also largely depended on the N-substituent. While *N*-H dihydro[1,2]oxazine derivatives were found to be substantially more stable, the presence of a carbamate group made the HDA adducts more labile toward TFA. We took

Scheme 10. Comparison of Direct and Retro-HDA Reactions[a]



[a] Reagents and conditions: (i) diene, THF, 100 °C in a microwave cavity, 5 min; (ii) TBAF, THF, 30 min.

advantage of the instability of HDA adducts for preparative useful post-HDA modifications and will report our findings.

Retro-HDA Reaction. With different linkers for tethering the acid-sensitive DMA HDA adducts, we evaluated the conditions for the polymer-supported retro-HDA reactions using a few dienes with the silicon linker-supported HDA adduct **14d**. The retro-HDA reaction was carried out at elevated temperature in a microwave field. The liberated DMA was washed, and the TBAF-mediated cleavage provided clean products in high yields (Table 4). The retro-HDA products were analyzed by LCMS and ^{1}H NMR spectra, and they were found to be identical to adducts prepared directly from acylnitroso dienophiles (Scheme 10). The retro-HDA reaction was also performed using conventional heating (70 °C, 2 h).

All synthesized compounds were subjected to a panel of biological assays that included tests for antimicrobial, antiinflammatory, antiproliferative, cytotoxic, and cytostatic activities. Details of tests are described in the Supporting Information.

Antimicrobial activity was tested against a panel of Gram-positive and Gram-negative bacteria, yeasts, and fungi by agar diffusion tests. Compounds **15c1**, **15d**, and **15g** exhibited weak activity against Gram-positive bacteria. Compounds **15b1**, **15d**, **15g**, **31b**, **31c**, **31e**, and **31h** inhibited growth of mycobacteria. HDA adducts containing a 4-(4-hydroxymethyl-benzyloxy)-benzyl moiety and a very hydrophobic and bulky substituted oxazine ring inhibited growth of mycobacteria but not Gram-positive bacteria. We did not observe any activity against Gram-negative bacteria. Very weak inhibitory activity was exhibited by compounds **15f**, **31b**, and **31e** against yeast cells.

The HDA adduct **31b** showed antiinflammatory activity in the 3α-hydroxysteroid dehydrogenase (3 α-HSD) assay, comparable to the standard ibuprofen.

Compounds **15j**, **15k**, and **15e** exhibited moderate to strong antiproliferative activity against K-562 cells. Moderate to strong antiproliferative and cytotostatic activities were demonstrated by compounds **6a**, **6e**, **15a**, **15c2**, and **31b**. HDA adducts **31b**, **31c**, and **31d** were found to have antiproliferative (L-929 and K-562 cells) and cytotoxic (HeLa cells) activity. A common feature of these HDA adducts is the presence of a 4-(4-hydroxymethyl-benzyloxy)-benzyl moiety and a very hydrophobic and bulky substituted oxazine ring.

Compound **31b** was broadly active in antimicrobial, antiinflammatory, antiproliferative, and cytotoxicity assays.

Conclusion

In summary, we described HDA reactions of dienes with polymer-supported acylnitroso dienophiles. Linkers that allowed syntheses of acid-sensitive HDA adducts, both N-unsubstituted and N-derivatized, were developed. A set of 20 dienes was used to assess their reactivity in HDA reactions on solid-phase and stability of HDA adducts toward conditions typically used for solid-phase synthesis. Assays indicated that products derived from even simple dienes possess interesting biological activity. Relative reactivity of dienes, determination of structures of regioisomers, and comparison of acylnitroso and arylnitroso HDA reactions on solid phase is a subject of the accompanying paper.[44]

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Supporting Information Available. Details of experimental procedures and spectroscopic data for synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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