JOURNAL OF combinatorial CHEMISTRY

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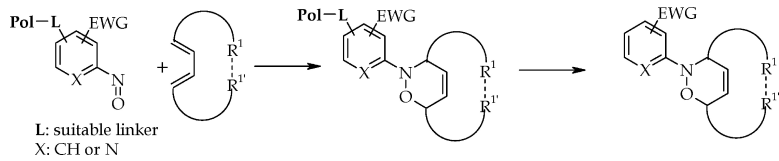
J. Comb. Chem., **2008**, 10 (1), 104-111• DOI: 10.1021/cc7001414 • Publication Date (Web): 07 December 2007



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Solid-Supported Nitroso Hetero Diels–Alder Reactions. 2. Arylnitroso Dienophiles: Scope and Limitations

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Received August 27, 2007

Immobilized arylnitroso dienophiles were prepared and used in hetero Diels–Alder (HDA) reactions with a variety of dienes. Polymer-supported arylnitroso species were prepared on several linkers cleavable by different cleavage reagents and used for (i) optimization of reaction conditions for HDA reactions, (ii) evaluation of the reaction outcome with various dienes, (iii) comparison of relative reactivities of dienes, and (iv) assessment of the stability of HDA adducts toward cleavage conditions typically used in solid-phase preparation (TFA). The outcome of the HDA reactions has been evaluated for a set of 19 dienes, and the relative reactivities of dienes that yielded the expected HDA adducts were compared. Cleaved products were submitted to biological assays, and the results are reported.

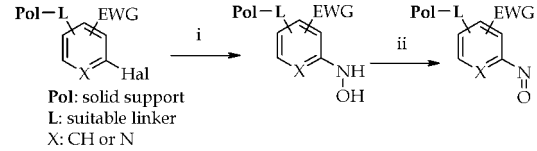
Introduction

The arylnitroso hetero-Diels–Alder (HDA) reaction was described by Wichterle[1] six decades ago, and since then, it has been demonstrated to be a useful chemical transformation for the creation of unique structural and functional diversity.[2–5] Products of HDA reactions, *N*-aryldihydro[1,2]oxazines, are amenable to further transformations in a manner analogous to acylnitroso HDA adducts. In the previous paper,[6] we described the results of HDA reactions of dienes with polymer-supported acylnitroso dienophiles. Herein, we focus on preparation of resin-bound arylnitroso species and their use in polymer-supported HDA reactions.

Results and Discussion

Arylnitroso HDA Reactions. The HDA reaction between arylnitroso dienophiles and dienes has not been previously studied on solid-phase supports. Thus, at first we synthesized polymer-supported model compounds designed to evaluate the scope and limitations of this transformation. As shown in Scheme 1, we prepared the resin-bound arylnitroso species by aromatic nucleophilic substitution of halides with hydroxylamine in electron-deficient aromatic and heteroaromatic halides, followed by oxidation of the resulting *N*-aryl hydroxylamines to the corresponding nitroso compounds. This route parallels the steps used for generation of acylnitroso species by oxidation of polymer-supported hydroxamates. However, in this case most of the nitroso species are stable enough to allow separation of the oxidation step from the subsequent HDA reaction. Electron-deficient aryl and heteroaryl halides were attached to the solid support (**Pol**) *via* a suitable linker (**L**). Traditional Merrifield copoly(styrene−1% divinylbenzene) resin was used as the solid support.

Scheme 1. Preparation of Polymer-Supported Arylnitroso Dienophiles[a]



[a] Reagents and conditions: (i) $NH_2OH \cdot HCl$, base; (ii) nBu_4NIO_4 or Dess−Martin periodinane.

Feasibility Study. To immobilize the starting synthons, we took advantage of the commercial availability of electron-deficient fluoro(hetero)aromatic carboxylic acids **1a**−**d** and attached them *via* an acid-cleavable amide bond to the Rink amide resin.[7] This linker, developed for the synthesis of peptide amides, requires the use of acidic cleavage cocktails including those containing trifluoroacetic acid (TFA), and it is not appropriate for use with dienes affording HDA adducts that are not stable toward acid-mediated cleavage. However, this linker was used to test the reaction conditions, particularly to optimize the aromatic nucleophilic substitution, oxidation, and HDA reaction. These solid-phase reagent forming conditions were then used in the second generation polymer-support substrate based on a silicon-based linker that enabled cleavage of target compounds by a mild TBAF procedure and testing of a wide range of dienes.

Briefly, the Rink resin **2** was quantitatively acylated with four electron deficient fluoro(hetero)aromatic carboxylic acids **1a**−**d** (Figure 1), the completeness of the acylation process was determined by use of the bromophenol blue test.[8] Although nucleophilic aromatic substitution reactions on solid phase are extensively used,[9] there is no precedent for the use of hydroxylamine as the nucleophile. Few reports described conversion of (hetero)aromatic halides to the

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10.1021/cc7001414 CCC: $40.75
Published on Web 12/07/2007

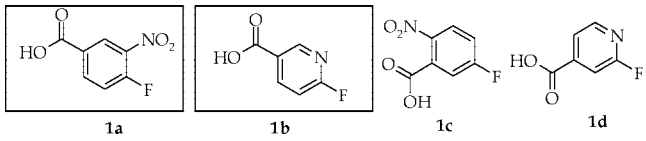


Figure 1. Electron-deficient fluoro (hetero)aromates.

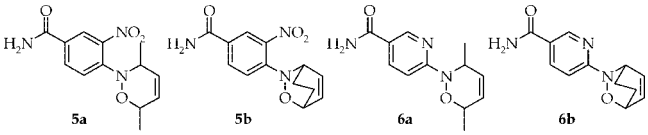


Figure 2. 2,4-Hexadiene and 1,3-cyclohexadiene arylnitroso adducts.

Table 1. Nucleophilic Aromatic Substitution of **1a**−**d** with Hydroxylamine[a]

substrate	temp	heating	time	base	solvent	conversion
1a	RT		1 h	pyridine	pyridine	23%
1a	RT		ON	pyridine	pyridine	90%
1a	RT		1 h	DIEA	DMSO	>99%
1b	100 °C	μw	5 min	pyridine	pyridine	~5%
1b	100 °C	μw	5 min	DIEA	DMSO	16%
1b	70 °C	conv	ON	DIEA	DMSO	>99%
1c	100 °C	μw	5 min	pyridine	pyridine	<1%
1d	80 °C	μw	5 min	pyridine	pyridine	<1%
1d	100 °C	μw	5 min	DIEA	DMSO	<1%
1d	100 °C	conv	ON	DIEA	DMSO	~2%

[a] μw refers to irradiation in a microwave cavity; conv stands for conventional heating, and ON refers to overnight (typically 16 h).

corresponding hydroxylamines in solution using hydroxylamine in THF,[10] ethanol,[13] or DMSO,[10] hydroxylamine hydrochloride in DMSO in the presence of $NaHCO_3$,[11] in chloroform and TEA,[12] or in pyridine[14] at elevated temperatures. For syntheses on solid phase, we carried out reactions of the resin-bound (hetero)aromatic fluorides with hydroxylamine hydrochloride in pyridine and in DMSO in the presence of tertiary base (DIEA). The results are summarized in Table 1.

The most reactive among the selected substrates **1a**−**d**, the polymer-supported 4-fluoro-3-nitrobenzoic acid **2a**, was quantitatively converted to the corresponding hydroxylamine derivative **3a** during overnight reaction at ambient temperature. The less reactive 2-fluoro-5-pyridinecarboxylic acid, **1**, required elevated temperature for quantitative nucleophilic aromatic substitution to take place. 5-Fluoro-2-nitrobenzoic acid **1c** and 2-fluoro-4-pyridinecarboxylic acid **1d** reacted sluggishly and were not used in subsequent experiments. Two electron-deficient (hetero)arylnitroso substrates **3a** and **3b** allowed us to assess the effect of a nitro group in the vicinity of the reaction center on the outcome of HDA reaction and relative reactivities of various dienes.

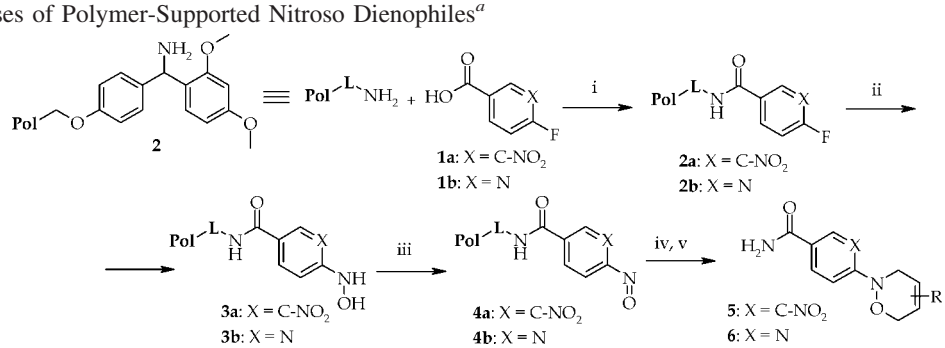
Oxidations of the resin-bound hydroxylamine derivatives **3a** and **b** were carried out by treatment with a tetrabutylammonium periodate solution in DCM or a Dess−Martin periodinane solution in DCM at ambient temperature. Clean oxidation on a cleaved sample was observed in both cases, and the nitroso species was stable overnight on resin, as judged by a sample cleaved from the resin and analyzed by LCMS. We did not study prolonged stability of the polymer-bound nitroso compounds as we typically carried out the oxidation immediately prior to the HDA reaction with dienes and stored the more stable hydroxylamine precursor for extended periods of time.

Having the polymer-supported arylnitroso dienophiles **4a** and **b** in hand, we studied the related HDA reactions. In a typical experiment, we used a 3- to 6-fold excess of diene in DCM at ambient temperature. For examples in which the dienes were poorly soluble, THF or MeOH was used as a cosolvent. THF was used as the only solvent for experiments carried out at elevated temperature in a microwave reactor. Reaction with 2,4-hexadiene and 1,3-cyclohexadiene provided clean conversion (purities of crude target compounds were >90% from HPLC analyses with no indication of remaining starting material) to the target compounds **5** and **6** (ambient temperature, overnight reaction), as judged by analyses of samples cleaved from the resin by TFA. The products were isolated, purified by reverse-phase HPLC, and characterized by MS and ^{1}H and ^{13}C NMR spectroscopy.

Reactivity of Dienes in Polymer-Supported Arylnitroso HDA Reactions. To evaluate the reactivity of dienes, including those that gave HDA adducts not stable toward acidic cleavage reagents such as TFA, we designed and synthesized polymer-supported arylnitroso dienophiles on a TBAF cleavable silyl linker, analogously to the synthesis of polymer-supported acylnitroso species described in the previous report.[6] At the same time, we focused on studies of polymer-supported dienophiles amenable to combinatorial syntheses with diversification of the N-substituent on the oxazine rings formed by the HDA reactions.

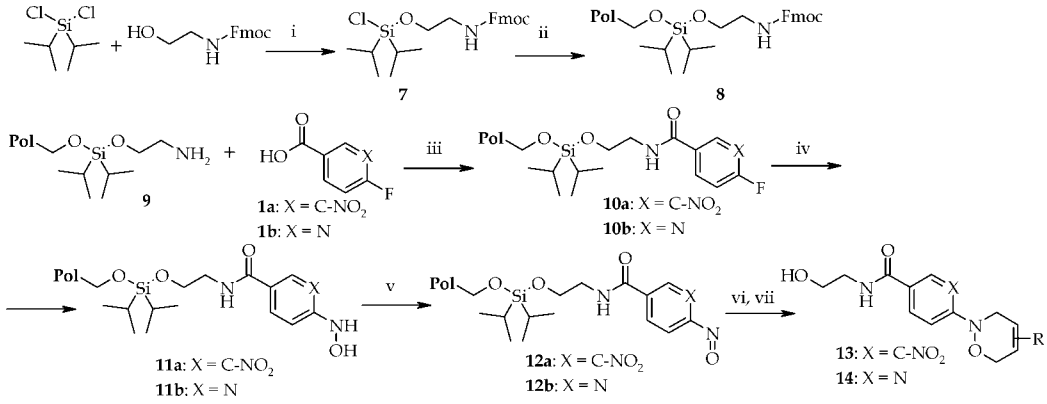
The sequence of steps leading to arylnitroso dienophiles on the TBAF cleavable linker is shown in Scheme 3. The target compounds **13** and **14** include up to four points of diversification using amino alcohols, electrophiles, electron-deficient (hetero)aryl halides, and dienes. The first building

Scheme 2. Syntheses of Polymer-Supported Nitroso Dienophiles[a]



[a] **L** stands for the Rink amide linker. Reagents and conditions: (i) DIC, HOBt, NMP, RT, 16 h; (ii) $NH_2OH \cdot HCl$, pyridine, RT, 2 h; (iii) 0.1 M nBu$_4$NIO$_4$ or Dess−Martin periodinane, DCM, RT, 1 h, (iv) diene, DCM or THF, RT, overnight, (v) TFA, DCM, RT, 30 min.

Scheme 3. Synthesis of HDA Adducts via Polymer-Supported Nitroso Dienophiles[a]



[a] Reagents and conditions: (i) imidazole, NMP, RT, 1 h; (ii) hydroxymethyl resin, solution from the previous reaction step, RT, 1 h; (iii) DIC, HOBt, NMP, RT, 16 h; (iv) $NH_2OH \cdot HCl$, pyridine, RT, 2 h; (v) 0.1 M nBu_4NIO_4 or Dess−Martin periodinane, DCM, RT, 1 h, (vi) diene, DCM or THF, RT, overnight, (vii) 0.1 M TBAF, 30 min.

Table 2. Nucleophilic Substitution of Fluoride in 2-Fluoro-5-pyridinecarboxate **10b**[a]

entry	temp	heating	time	base	solvent	conversion	cleaved
1	RT		ON	pyridine	pyridine	<1%	3%
2	75 °C	conv	ON	pyridine	pyridine	74%	54%
3	75 °C	conv	ON	DIEA	DMSO	85%	69%
4	50 °C	conv	ON	DIEA	DMSO	80%	43%
5	100 °C	μw	5 min	DIEA	DMSO	15%	ND

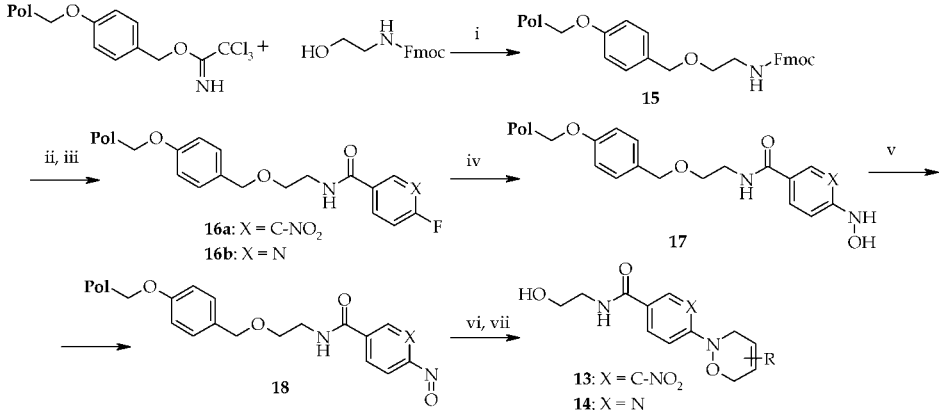
[a] μw refers to irradiation in a microwave cavity; conv stands for conventional heating.

block, the amino alcohol, was immobilized by attachment to a diisopropylsilyl linker,[15] successfully used for the solid-phase syntheses of polyketides.[16] Two different synthetic routes were evaluated. Dichlorodiisopropylsilane was exposed to Fmoc ethanolamine, followed by reaction of **7**, with hydroxymethyl resin. In a reversed manner, the hydroxymethyl resin was reacted with a 5-fold excess of dichlorodiisopropylsilane, followed by reaction with Fmoc-ethanolamine. Both routes provided the expected polymer-supported amino alcohol **8**. The former route typically afforded higher resin loading, and it was used in the subsequent experiments. The loading was determined by quantification of the UV response from a sample cleaved by TFA (TBAF cleaves the Fmoc). Using hydroxymethyl resin with the declared substitution of 0.98 mmol/g, we obtained resin-bound amino alcohol loadings of 0.5 mmol/g (75%).

The protecting Fmoc group on the resin-bound amino alcohol **8** was removed by piperidine in DMF, and the free amino group of **9** was separately and quantitatively acylated by two fluoro(hetero)aryl carboxylic acids **1a** and **b**. The nucleophilic aromatic substitution was carried out using reaction conditions developed for the previous substrates **2**. Nucleophilic aromatic substitution with substrate **10a** derived from 4-fluoro-3-nitrobenzoic acid proceeded quantitatively without any premature cleavage of the product from the resin. However, under the conditions used to displace the fluoride in the less reactive 2-fluoro-5-pyridinecarboxylic acid derivative **10b** (elevated temperature, overnight), we observed partial cleavage of the product from the resin.

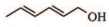
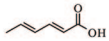
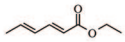
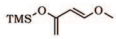
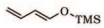
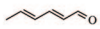
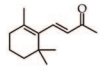
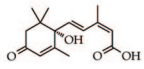
Quantification experiments (Table 2) indicated a range of about 40−70% cleavage from the resin, depending on the reaction conditions. Shortening the reaction time or lowering the temperature did not provide satisfactory conversion of pyridyl fluoride to the corresponding hydroxylamine derivative. Compromised conditions used in further experiments decreased the effective resin loading to 0.2 mmol/g.

Scheme 4. Alternative Acid Cleavable Linker[a]



[a] Reagents and conditions: (i) $BF_3 \cdot Et_2O$, THF, RT, 30 min; (ii) piperidine, DMF, 15 min, (iii) 2-fluoro-5-pyridinecarboxylic acid, DIC, HOBt, RT, NMP, 16 h; (iv) $NH_2OH \cdot HCl$, pyridine, RT, 2 h; (v) 0.1 M nBu_4NIO_4 or Dess−Martin periodinane, DCM, RT, 1 h, (vi) diene, DCM or THF, RT, overnight, (vii) 10% TFA, DCM, 30 min.

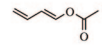
Table 3. Formation of HDA Adducts **13** and **14** Compared to Acylnitroso Cycloadditions (HDA Adducts P15) from Dienes[a]

Diene	Structure	HDA Adduct P15	HDA Adduct 13	HDA Adduct 14
Da				
Db		84:16	95:5	85:15
Dc		82:18	>99:<1	>99:<1
Dd			20%	
De				
Df		>99:1	>99:1	>99:1
Dg				
Dh		72:28	75:25	90:10
Di		9:1	>99:1	>99:1
Dj		>99:1	>99:1	>99:1
Dk				20%
Dl				10%
Dm		>99:1		>99:1
Dn		>99:1		
Do				
Dp				
Dr				
Ds			10-20%	50%
Dt				5%

[a] HPLC purity of HDA adducts and relative ratios of regioisomers from unsymmetrical dienes. Note that HDA adducts **P15** refer to acylnitroso HDA adducts described in the previous paper. The HDA product from acylnitroso dienophile and diene **Dh** cleaved by TBAF was not detected because of the instability of the adduct towards TBAF: HDA adducts with purity >90%, green; no product detected, red; the rest of HAD adducts, not color-coded.

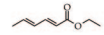
We used hydroxylamine resin **11b** in the HDA reaction with dienes because premature cleavage during the nucleophilic substitution lowered the loading of the resin but did not compromise the purity. However, the unexpected cleavage of the hydroxylamine intermediate from the resin prompted us to explore alternative solutions. First, we tried

Table 4. Formation of HDA Adducts **13** and **14** with Dienes[a]

Diene	Structure	HDA Adduct 13		HDA Adduct 14	
		Rt	μw	Rt	μw
Da		>90%	NT	>90%	>90%
Dd		20%	20%	80%	80%
Dh		>90%	>90%	>90%	NT
Dm		<1%	40%	>90%	NT
Dn		<1%	40%	<1%	75%

[a] RT refers to ambient temperature; μw refers to irradiation in a microwave cavity, THF, 100 °C, 5 min; NT, not tested. Color coding is the same as that described in Table 3.

Table 5. Relative Reactivities of Dienes toward Resin-Bound Nitroso Reagents[a]

Diene	Structure	Stability		HDA Adduct 13			HDA Adduct 14		
		TFA	TBAF						
Dc		P	Y	430			270		
Da		Y	Y	100			100		
Db		N	Y	56			160		
De		Y	Y	<1	100		8.3	100	
Dh		Y	Y		31		61	670	
Dj		Y	N		4.8	97			43
Di		Y	Y		4.9	100			100
Ds		N	Y			59			14
Dm		P	Y			<1			140

[a] TFA stability: Y, stable; N, not stable; P, partially stable.

to replace the diisopropyl silicon linker with the more stable diphenyl linker. Following an analogous sequence of reaction steps described for the incorporation of the diisopropylsilyl linker, we reacted dichlorodiphenyl silane with Fmoc-ethanolamine and, subsequently, with the hydroxymethyl resin. The loading was disappointingly low, only 0.1 mmol/g, probably because of the bulkiness of the phenyl ring when compared with the isopropyl groups.

In an alternative linker, we replaced the silicon linker with an electron-rich benzyl ether derived from the Wang linker.[17] The target compounds prepared on this benzyl ether linker were identical to compounds made on the silicon linker. Cleavage from this linker was carried out by TFA for the preparation of acid-stable HDA adducts and by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to prepare acid-labile target products.

The synthesis was straightforward: trichloroacetimidate resin, from a commercial source or prepared following the

Table 6. Analytical Data of HDA Products[a]

		HDA adduct **13**		HDA adduct **14**	
product	diene	MS ESI$^+$	RT	MS ESI$^+$	RT
13a/14a	1,3-cyclohexadiene (CHD)	320.1	4.8	276.2	3.8
13b/14b	α-terpinene	376.2	7.2	332.1	5.8
13c/14c	1,3,5,5-tetramethyl-CHD	376.1	7.4	332.1	6.4
13d/14d	cycloheptatriene	332.1		288.1	4.0
13e/14e	2,4-hexadiene	322.1	5.9	278.2	4.9
13g/14g	1,4-diphenyl-1,3-butadiene	446.2	8.0	402.2	7.6
13h/14h	2,4-hexadien-1-ol	338.1	4.4	294.1	3.8
13i/14i	sorbic acid	352.2	2.7	308.1	2.1
13j/14j	ethyl sorbate	380.1	5.9	336.1	5.1
13m/14m	1-acetoxy-1,3-butadiene			308.1	3.9
13n/14n	1-methoxy-CHD	350.1	4.6	NT	
13s/14s	*β*-ionone	432.2	6.7	388.2	5.7

[a] Each compound exhibited a signal corresponding to $[M + H]^+$ in electrospray ionization (ESI^+) mass spectrometry analyses. RT refers to retention time on analytical HPLC using a 3 × 50 mm Pro C18 YMC reverse-phase column and a 10 min gradient from 5 to 80% of acetonitrile in 10 mM aqueous ammonium acetate at a flow rate of 0.7 mL/min.

Table 7. HETCOR NMR Cross-Peaks Used for Structure Determination of the Structures of Isomers **13** and **14**

product	^{13}C NMR C6-O	^{1}H NMR H-C6	C6-R^1	^{13}C NMR C3-N	^{1}H NMR H-C3	C3-R^2
13h1	79.05	4.39	$-CH_2OH$	52.01	4.39	$-CH_3$
13h2	73.08	4.37	$-CH_3$	57.61	4.37	$-CH_2OH$
13i	79.20	4.50	−COOH	51.57	4.20	$-CH_3$
13j	75.94	5.31	−COOEt	50.67	4.86	$-CH_3$
14c	77.09	*a*	$-CH_3$	65.73	4.69	−H
14h	70.55	4.55	$-CH_3$	54.81	4.99	$-CH_2OH$
14j	75.94	5.31	−COOEt	50.68	4.86	$-CH_3$
14m	90.19		$-OCOCH_3$	46.20		−H

[a] No cross-peak in the HETCOR NMR spectra.

reported protocol,[18] was reacted with Fmoc-ethanolamine.[19] The loading of resin **15**, determined by quantification of the UV response in a TFA cleaved sample, was 0.38 mmol/g, which is comparable with reported results.[19] The polymer-supported ethanolamine **15** was subjected to the reaction sequence analogous to the silicon linker-based dienophile, yielding the hydroxylamine substrate **17** without further loss of resin loading.

Oxidations of polymer-supported *N*-arylhydroxylamines **11** and **17** were carried out using nBu$_4$NIO$_4$ or Dess−Martin periodinane. The subsequent HDA reactions of nitroso dienophiles **12** and **18** with dienes, followed by cleavage from the resin, provided target HDA adducts **13** and **14**, identical to both linkers.

Reactions of Solid-Supported Nitroso Reagents with a Set of Dienes. The resin-bound arylnitroso dienophiles were used in HDA reactions with a set of dienes to evaluate the effect of structural and functional features of individual dienes on (i) the outcome of the reaction, (ii) relative reactivity with the resin-bound dienophiles, and (iii) stability of their HDA adducts. In addition, two resin-bound arylnitroso dienophiles allowed assessment of the effect of a nitro group in close proximity to the reaction center. For comparison, formation of HDA adducts with acylnitroso species are also included. Syntheses of acylnitroso dienophiles and their HDA reactions with dienes were described in the preceding paper.[6] The set of dienes included both acyclic and cyclic versions with peripheral electron-donating or -withdrawing groups (Table 3). The reactions were carried out in DCM at ambient temperature overnight. Selected dienes were also tested at elevated temperature in THF using microwave irradiation (Table 4). The outcome of the reactions was evaluated by LCMS analyses of samples cleaved by TBAF and TFA for comparison. The detection of HDA adducts by LCMS was very sensitive because of the efficient ionization of HDA adducts by the MS electrospray source; thus even a trace amount of product was reliably detected.

The results are summarized in Table 3. With respect to the formation of the product, the dienes can be divided into three groups. Dienes that provided clean conversion to the expected HDA adducts (purity >90% as judged by integration of the LC traces), dienes that yielded complex mixtures containing the expected products, and dienes that did not afford significant amounts of the products (less than 5% as judged by analysis of LCMS traces). The results in Table 3 are color-coded for simple visual interpretation. The HDA adducts in green boxes were typically synthesized on preparative scale (typically 100–300 mg of resin), purified by semipreparative HPLC, and characterized by MS and ^{1}H and ^{13}C NMR. Analytical data are included in the Supporting Information.

Initially, the reactions were carried out at ambient temperature overnight. Simple dienes, both cyclic and acyclic yielded the expected HDA adducts as did acyclic dienes with electron-donating (hydroxymethyl) and -withdrawing (carboxylate) substituents. However, dienes with alkoxy and silyloxy substituents at the terminal olefin carbon failed to yield the expected products. When compared to arylnitroso dienophiles, the HDA reactions with acylnitroso species appeared to be substantially less forgiving to the structure of the dienes because only a subset of tested dienes provided the corresponding HDA products.

We also carried out the HDA reaction at elevated temperature in a microwave cavity. First, we reacted dienes **Da** and **Dh** that provided good results at ambient temperature to prove that the reaction conditions are compatible with the HDA reaction. Then, we applied those conditions to dienes **Dm** and **Dn** that did not yield the target HDA adducts at ambient temperature. While the reaction at elevated temperature did not influence the yield of the HDA adduct with triene **Dd**, the more vigorous conditions promoted reactions with the other two dienes and induced formation of the expected HDA adducts. The HDA adduct with 1-methoxycyclohexadiene (**Dn**) was found to not be stable.[20]

The next set of experiments assessed the relative reactivity of dienes that afforded the expected HDA adducts. The resin-bound nitroso species were exposed to a 6-fold excess of an equimolar mixture of two dienes, the products were cleaved from the resin, and their ratio determined by integration of the analytical HPLC traces (UV diode array 200–400 nm). The results are summarized in Table 5.

Interestingly, we found more than 4 orders of magnitude difference in the reactivities of the dienes. Accordingly, we used three different dienes as the reference compounds, namely 1,3-cyclohexadiene (**Da**), 2,4-hexadiene (**De**), and sorbic acid (**Di**) (green boxes in Table 4) to fully cover the entire scale of relative reactivities. The reactivities of cyclic

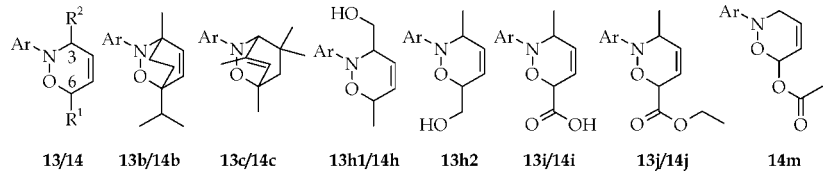


Figure 3. Structure of isolated regioisomers.

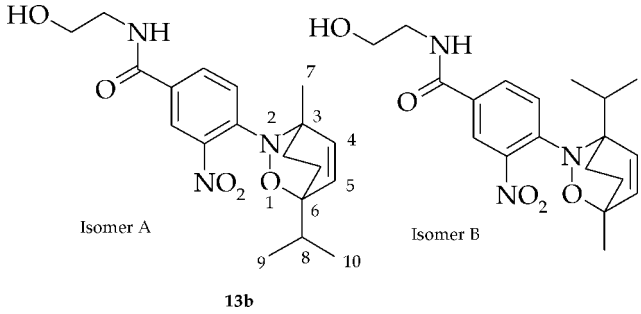


Figure 4. Structure of regioisomers of the α-terpinene HDA adducts.

six-membered dienes, 1,3-cyclohexadiene (**Da**), α-terpinene (**Db**), and 1,3,5,5-tetramethylcyclohexadiene (**Dc**) were in the same range. The addition of alkyl substituents increased the electron density and, as expected, dienes **Da** and **Dc** were more reactive toward the nitroso pyridyl dienophile than was 1,3-cyclohexadiene (**Da**). α-Terpinene (**Db**) was less reactive than 1,3-cyclohexadiene (**Da**) with the 2-nitrophenyl dienophile because of the steric influence of the neighboring nitro group.

However, even the most reactive acyclic dienes were 2 orders of magnitude less reactive than their cyclic analogs. In reactions with the pyridyl nitroso reagent, the electron-donating effect of the hydroxyl group in 2,4-hexadien-1-ol (**Dh**) contributed to its higher reactivity when compared with the unsubstituted 2,4-hexadiene (**De**). The presence of electron-withdrawing groups exhibited an expected opposite effect (dienes **Di** and **Dj**). On the other hand, any substituent present on the 2,4-hexadiene (**De**) scaffold reduced the relative reactivity of the corresponding acyclic dienes with the 2-nitrophenyl-based nitroso reagent, again reflecting the steric effect of the ortho nitro group.

The influence of a combination of an electron-withdrawing effect and steric hindrance was especially pronounced in the reactions of 1-acetoxy-1,3-butadiene (**Dm**). While its reactivity with the pyridyl nitroso reagent was comparable to that of sorbic acid (**Di**), no product was observed upon reaction with the 2-nitrophenyl nitroso species.

In summary, both electron-deficient pyridyl and nitroaryl, and thus reactive, nitroso species provided expected HDA adducts with numerous dienes. However, the presence of the nitro group in close proximity to the reaction center was shown to have a detrimental effect on reactivity with sterically more demanding dienes. While the more electron rich α-terpinene (**Db**) and 1,3,5,5-tetramethylcyclohexadiene (**Dc**) were expectedly more reactive than 1,3-cyclohexadiene (**Da**) with the pyridyl-based nitroso reagent, the relative reactivities with the *o*-nitroaryl reagent were reversed, documenting its adverse steric effect. The same effect was observed in reactions with 2,4-hexadiene (**De**) and 2,4-hexadien-1-ol (**Dh**). The increased electron density of the diene component of 2,4-hexadien-1-ol (**Dh**) relative to 2,4-hexadiene (**De**) induced enhanced reactivity with the pyridyl-based dienophile, while the *o*-nitroaryl reagent showed the reversed order of reactivity.

In addition to their use for determination of relative reactivities of their respective diene precursors, HDA adducts synthesized on the silicon linker allowed their release either by TBAF or TFA and thus allowed comparison of stabilities of the HDA adducts. Target compounds prepared from electron-rich dienes, α-terpinene (**Db**) and 1,3,5,5-tetramethylcyclohexadiene (**Dc**) were not stable toward the TFA cleavage reagent and decomposed. Structural features leading to instability toward TFA and preparative exploitation of acid-mediated reactivity for post-HDA transformations will be addressed in a dedicated report.

Structures of Regioisomers Obtained from Reactions with Unsymmetrical Dienes. As expected, unsymmetrical dienes formed two regioisomers at various relative ratios upon reaction with the resin bound nitroso reagents. With the exception of HDA adduct **13f**, predominantly one isomer was identified and isolated. In each case, the structure of the isomer prepared from reactions of unsymmetrical dienes that contained at least one proton on either the C3 or C6 carbon of the HDA adduct was determined using data from COSY and HETCOR NMR spectra. The cross-peaks in the HETCOR spectra identified protons attached to the C3, C6, or both carbons. COSY spectra were used to identify the positions of the R^1, R^2, or both groups by cross-peaks between the H-C3, H-C6, or both protons and protons on the R^1, R^2, or both groups. All isolated isomers contained the bulkier group at the C6 carbon. This observation is consistent with previous reports and theoretical predictions.[3] Dominant formation of the isomer with the bulkier substituent on the C6 carbon was observed in reaction of nitrosobenzene with methyl sorbate.[21] This isomer was also observed as the only product of an HDA reaction with 1-methoxy-1-trimethylsiloxy-1,3,5-hexatriene.[22] Dienes with electron-donating and electron-withdrawing substituents produced the same regioisomer with *p*-chloronitrosobenzene.[23]

However, the structure of the major regioisomer of HDA adducts with 2,4-hexadien-1-ol (**Dh**) was dependent on the nitroso dienophile. The HDA reaction with nitroaryl-derived nitroso dienophile **12a** yielded a mixture of regioisomers in a ratio of 75:25 in favor of the isomer having the hydroxymethyl group attached to the C3 carbon. Previous reports indicated that 2,4-hexadien-1-ol (**Dh**) provided regioisomers in a 57:28 ratio upon reaction with nitrosobenzene[24] and 61:39 with *p*-chloronitrosobenzene,[23] favoring the same regioisomer (hydroxymethyl group on the C3 carbon). Using pyridyl-derived nitroso dienophile **12b**, we observed formation of regioisomers in a ratio of 10:90 in favor of the opposite isomer having the hydroxymethyl group attached

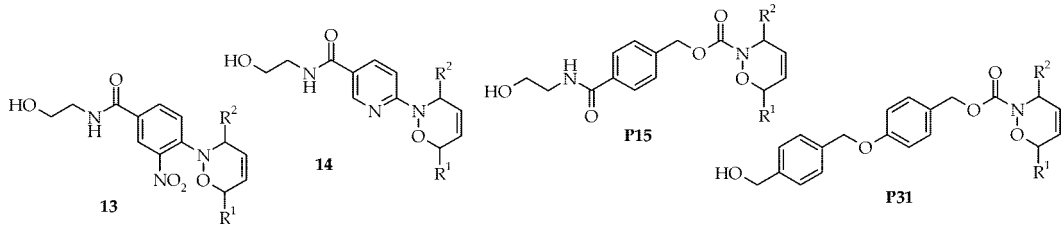


Figure 5. Acyl and arylnitroso HDA adducts.

to the C6 carbon. Both isomers, **13h1** and **13h2**, derived from the 2-nitroaryl dienophile were isolated, while only the major isomer, **14h**, was isolated from the pyridyl-based HDA adduct.

The HDA adducts **13b** and **14b** derived from α-terpinene did not contain any proton at either the C3 or C6 carbons. Thus, the structure of isomer **13b** was established from 2D NMR spectra. Proton resonance signals were assigned to the individual hydrogen atoms based on their chemical shift (δ values), multiplicities, and coupling constants (J values). The signals of all carbons with directly attached protons were assigned using HETCOR spectra. Finally, HMBC spectra were used to assign quaternary carbons and to check the correctness of the connectivities established by the interpretation of the other spectra.

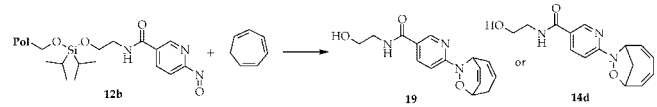
In the HMBC spectrum, the resonance signals of the C7 methyl protons (δ 1.1) exhibited cross-peaks with the C3 quaternary carbon (linked to nitrogen) signal at δ 59.51. The resonance signal of the C9 and C10 methyl protons (δ 1.0) revealed a cross-peak with the C6 quaternary carbon signal at δ 78.83. In addition, the C8 proton (δ 1.92) showed a cross-peak with the C6 quaternary carbon signal at δ 78.83; confirming the structure of the product as isomer A.

Synthesized acyl and arylnitroso HDA adducts represent a series of structurally related compounds for which isomer structural assignments were made based on ^{13}C NMR resonances of the C6 carbon. The resonance signals of carbons C6, each with a methyl substituent, were observed at δ 74.2 (**13e**) and δ 73.1 (**13h2**), whereas the resonance signals of carbons C6 with bulkier substituents were at δ 76.6 (**13j**) and δ 79.2 (**13i**). A similar trend was observed in the pyridyl-based series **14** and in both series derived from acylnitroso dienophiles. Using this set of data, we assigned the structure of acylnitroso adducts **P15** and **P31** (reported in the previous paper).[6]

The only triene included in our HDA reactions was cycloheptatriene (**Dd**). It was anticipated to be able to undergo either a 1,4- or 1,6-cycloaddition (or a competitive combination) to provide isomers **19** or **14d**. The HDA reaction yielded a single product that was isolated and purified. Analysis of COSY NMR spectra revealed the presence of conjugated dienes, confirming the structure of the product as 1,6-adduct **14d**. Our structure is in agreement with previously reported results.[25] Interestingly, the related HDA reaction with acylnitroso dienophile afforded the norcaradiene-derived cycloadduct.[6]

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

Scheme 5. Two Potential Isomers of the Cycloheptatriene HDA Adduct[a]



[a] Reagents and conditions: DCM, RT, 16 h.

Table 8. ^{13}C Resonances of C6 Carbon in Acyl and Arylnitroso HDA Adducts[a]

entry	diene	13	14	P31	P15
1	1,3-cyclohexadiene (CHD)	69.7	69.7	70.0	70.1
2	α-terpinene	78.7	80.2	81.5	81.6
3	1,3,5,5-tetramethyl-CHD	77.0	77.1	77.4	77.6
4	2,4-hexadiene	74.2	73.1	73.2	73.4
5	4-methyl-penta-1,3-diene	NT	NT	NT	79.1
6	2,4-hexadien-1-ol	79.1	NT	78.4	78.6
7	2,4-hexadien-1-ol	73.1	70.6	NT	NT
8	sorbic acid	79.2	ND	NT	78.8
9	ethyl sorbate	76.6	75.9	NP	75.6

[a] Acylnitroso HDA adducts **P15** and **P31** were described in the previous report. NT, corresponding compound was not isolated and tested; ND, corresponding compound was not detected; NP, not prepared (compound was decomposed by TBAF).

Antimicrobial activity was assayed by agar diffusion tests using a panel of Gram-positive and Gram-negative bacteria, yeasts, and fungi. Compound **13g** exhibited weak activity against mycobacteria and Gram-positive bacteria; compound **5b** exhibited activity only against mycobacteria. HDA adducts **13j** and **13t** were active against yeast cells.

In parallel, both compounds **13j** and **13t** have moderate antiproliferative (L-929 and K-562 cells) and cytotoxic (HeLa cells) activities. Compounds **13c** and **13e** were cytotoxic (HeLa cells) only.

Antiinflammatory activity, comparable to the standard of ibuprofen, was exhibited by HDA adduct **13b** in the 3α-hydroxysteroid dehydrogenase (3 α-HSD) assay. Several compounds (**13c**, **14a**, **14c**, **14d**, **14j**) revealed weak antiinflamatory activity below the standard. No conclusive SAR could be drawn from the weakly active compounds.

Conclusion

We described conditions for solid-phase supported HDA reaction with immobilized acyl and arylnitroso dienophiles. Dienophiles were attached to different linkers allowing cleavage by traditional TFA-based reagents and also by DDQ and TBAF for acid-sensitive HDA adducts. Linear and cyclic dienes containing electron-withdrawing and -donating groups provided expected HDA adducts. Large differences in the relative reactivities of dienes were found, with favorable spatial arrangement being a critical factor for fast reactivity. The structures of regioisomers were determined by two-dimensional NMR techniques. Except the 2,4-hexadien-1-

ol HDA adducts with arylnitrosodienophiles, isomers with bulkier groups attached to the C6 carbon of the dihydro[1,2] oxazine ring were formed predominantly. Assays indicated that products derived from even simple dienes possess hints of activity that might be useful as leads in the development of biologically active compounds.

Acknowledgment. The work was supported by the Department of Chemistry and Biochemistry, University of Notre Dame, and the NIH (GM075855). We thank Uta Wohlfeld, Ursula Stöckel, and Birgit Weber for their skillful technical assistance.

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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CC7001414