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Irreversible Conjugation of Aldehydes in Water To Form Stable 1,2,4-Oxadiazinan-5-ones

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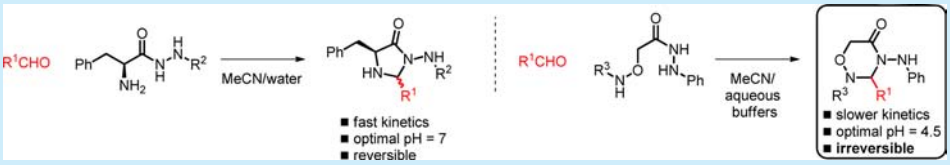
Supporting Information

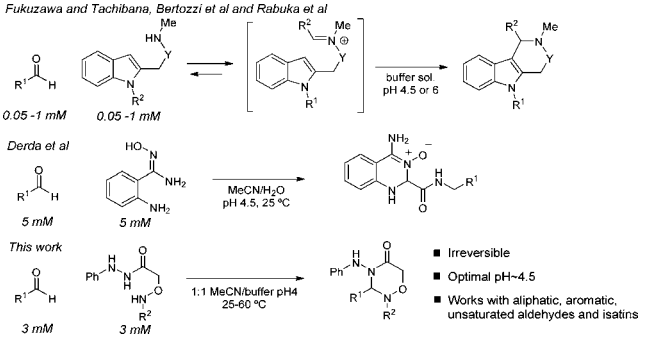


ABSTRACT: A new, irreversible aldehyde conjugation reaction in aqueous media was developed. α-Aminooxy acetohydrazides undergo irreversible condensation reactions with aliphatic, aromatic, or unsaturated aldehydes and isatins in a mixture of acetonitrile and acetate buffer at pH 4 to yield 1,2,4-oxadiazinan-5-one heterocycles in excellent isolated yields (40−99%). This class of heterocycles proved to be hydrolytically stable throughout a wide range of temperatures and pH (4.5−7).

Aldehydes have unique reactivity ideally suited for bioorthogonal chemistry,[1] and interest in their use has inspired improved, chemoselective methods for introducing aldehydes into biomolecules, particularly proteins. N-terminal glyoxaldehydes can be introduced by a mild oxidation of N-terminal Ser/Thr residues[2] or by biomimetic PLP-mediated transamination.[3] This strategy has allowed the selective modification of antibodies[4] and filamentous phage.[5] Internal formylglycine residues can be created by enzymatic oxidation of cysteine side chains embedded in a specific pentapeptide sequence.[6] More recently, Bode et al. stablished a novel oxyproline reagent for the total synthesis of relevant proteins using the KA−HA ligation[7] that upon interaction with a ketoacid forms a new amide bond, creating an aspartyl aldehyde residue.[8]

One of the most popular conjugation methods using aldehydes is the formation of oximes from aminooxy groups, which is a rapid and selective process when performed at pH 3−5.[9] Unfortunately, the oxime products are prone to hydrolysis over time.[10] To avoid the instability of oxime and the related hydrazones, the Fukuzawa and Tachibana groups reported in 2008 the use of tryptamine to seek an irreversible Pictet−Spengler reaction with a modified wild-type myoglobin having a N-terminal aldehyde.[11] Bertozzi et al. reported the kinetic improvement of this method through substrate optimization (Scheme 1).[12] By using an aminooxy-functionalized indole, conjugation into isobutyraldehyde occurred in less than 1 h at pH 4.5 (k = 10 M^{-1} s^{-1}, 1 mM, 22 °C). The oxacarboline formed was shown to be stable for at least 2 days in pH 4.5 and 5 buffers (Scheme 1). The kinetics of the ligation using the hydrazine derivative at the more neutral pH 6 were found to be superior to those of the aminooxy analogue.[13] Derda et al. reported the rapid reaction of 2-aminobenzamidoxime derivatives with aldehydes in water (Scheme 1). The reaction proceeds 4 times faster than Pictet−Spengler under the optimal pH conditions (pH 4.5). The product is fluorescent and displays high long-term stability.[14]

Scheme 1. Recent Examples of Aldehyde Condensation Reactions in Aqueous Media



Inspired by these concepts and recent developments from Bane[15] and Gillingham,[16] we now describe several classes of hydrazine reagents that react rapidly with aldehydes to form stable conjugates. One class—α-aminooxy acetohydrazides—form stable 1,2,4-oxadiazinan-5-ones in aqueous media and under mild conditions. These adducts are resistant toward hydrolysis and aldehyde exchange and provide a new approach to aldehyde conjugation.

At the outset of our work, we hypothesized that α-amino acid amides could trap aldehydes in water as electron-deficient aminals which might present good stability against hydrolysis. This idea is supported by work from us and others on the synthesis of stable geminal bisamide aminals of ketones.[17]

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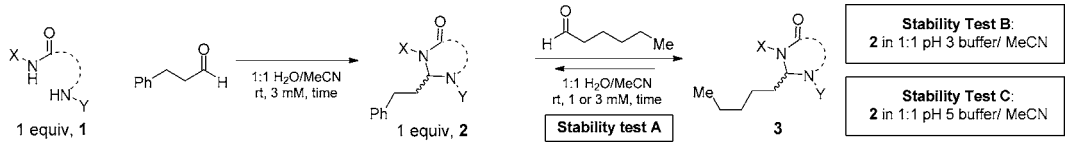
Table 1. Evaluation of Amino Amide Derivatives for Aldehyde Conjugation and Adduct Stability (N.R. = No Reaction; N.D. = Not Determined)



entry	amino amide	adduct	conv (%)[a]	% isolated yields of **2**[b]	test A % **3a-j** formed	test B % hydrolysis **2**	test C % hydrolysis **2**
1	(1a)	n/a	N.R (24 h)	-	-	-	-
2	(1b)	n/a	N.R (24 h)	-	-	-	-
3	(1c)	(2c)	95 (0.5 h) 98 (1 h)[c]	77 (1 h)	25 (2 h)[d]	55 (2 h)	N.D.
4	(1d)	(2d)	80 (0.5 h)[e]	N.D.	15 (2.5 h)[d]	N.D.	N.D.
5	(1e)	(2e)	75 (0.5 h)[e]	N.D.	25 (2.5 h)[d]	N.D.	N.D.
6	(1f)	(2f)	70 (0.5 h)[e]	N.D.	45 (2 h)[d]	N.D.	N.D.
7	(1g)	(2g)	75 (0.5 h)	97 (1 h)	3 (2 h)[d] 10 (12 h)[f]	21 (12 h)	17 (12 h)
8	(1h)	(2h)	80 (0.5 h)	92 (2.5 h)	8 (13 h)[f]	17 (12 h)	9 (12 h)
9	(1i)	(2i)	67 (0.5 h)	74 (4 h)	8 (12 h)[f]	N.D.	N.D.
10	(1j)	(2j)	97 (0.5 h)	82 (13 h)	26 (12 h)[f]	72 (12 h)	15 (12 h)
11	(4)	(5)	5 (0.5 h)[g] 75 (28 h)[g]	92 (20 h)[g,h]	4 (81 h)[f,g,i]	1 (72 h)[g]	1 (24 h)[j]

[a]HPLC conversion after 30 min, [reagents] = 3 mM (for further details, see SI section 7, Table S1). [b][Reagents] = 49 mM. [c]1:1 CH_3CN/LB media as solvent. [d][Reagents] = 3 mM. [e]Identified in crude ^{1}H NMR. [f][Reagents] = 1 mM. [g]1:1 acetate buffer, pH 4, 50 mM/CH_3CN as solvent. [h][Reagents] = 60 mM. [i]Formation of adduct 9. [j]1:1 acetate buffer, pH 7, 50 mM/CH_3CN as solvent.

We began our study with amides **1a** and **1b**, which unfortunately did not undergo any reaction with hydrocinnamaldehyde under our preferred conditions: 3 mM in CH_3CN/H_2O at 23 °C (Table 1, entries 1 and 2). Phenylalanine *N*-tosyl hydrazide **1c** gave exclusively imidazolidin-4-one **2c** as a mixture of diastereoisomers,[18] with over 95% conversion in 30 min (entry 3). This encouraging result was confirmed to be highly chemoselective, as imidazolidin-4-one **2c** was obtained selectively with similar conversion and reaction rate in the presence of protein digests (LB media). Examples of imidazolidin-4-one synthesis in aqueous media are rare and require excess amounts of aldehyde and heating at 60 °C.[19] Our initial studies showed that the conversion extent of hydrazide **1c** into adduct **2c** is controlled by the pH of the reaction (see Supporting Information SI section 7). We continued the substrate screening by altering the substitution at the α-nitrogen and hydrazide moieties to evaluate their effect on reactivity and product stability. The ranking of reactivity was constructed by checking substrate conversion by HPLC after 30 min. The stability of the product in regard to their hydrolysis was studied using two sets of assays. The first assay determined the extent of formation of products **3c–j** as a result from the cross-exchange reaction between adducts **2c–j** and hexanal (test A). A second stability assay evaluated the extent of hydrolysis of freshly

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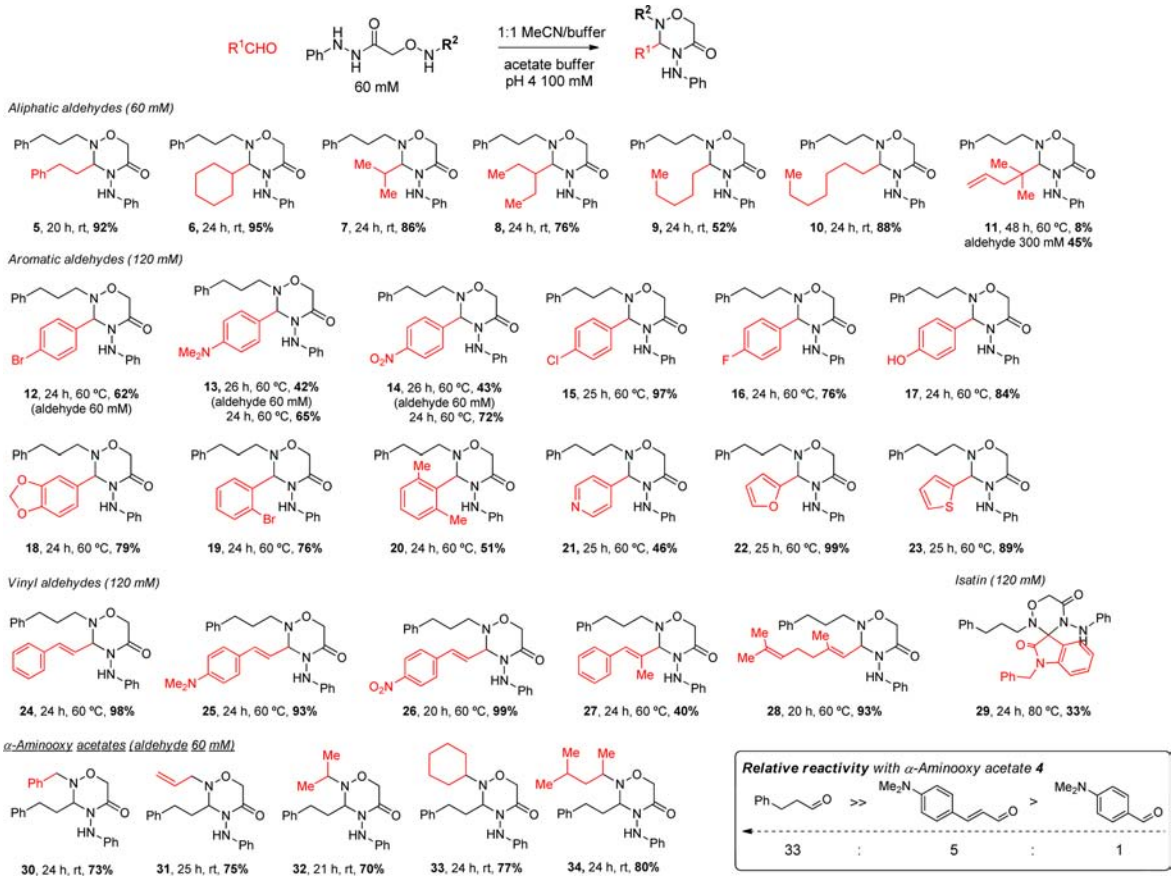
Scheme 2. Substrate Scope for 1,2,4-Oxadiazinan-5-one Formation



prepared samples of adducts **2c**–**j** in the presence of acidic buffers (tests B and C). We found that monosubstituted hydrazides **1d**–**f** displayed generally decreased reactivity when compared with that of hydrazide **1c** (Table 1, entries 4–6) but also afforded exclusively the respective undesired *N*-acyl hydrazones **2d**–**f**.[9a] Phenyl hydrazide derivative **1g** led to decreased reactivity, which could be slightly improved by using more nucleophilic anisyl hydrazide **1h** (Table 1, entries 7 and 8).

Replacement of the aromatic hydrazide moiety by a *tert*-butyl hydrazide (substrate **1i**) further reduced the overall reactivity, most likely due to increased steric hindrance. *O*-Benzyl hydroxamic acid **1j** displayed high reactivity, forming imidazolidin-4-one **2j** in 97% yield after 30 min (Table 1, entry 10). From all substrates, only **1c** and **1j** led to a complete reaction after 30 min (see SI section 7, Table S1).

In terms of product stability, imidazolidin-4-one **2c** readily exchanged with hexanal, leading to 25% of imidazolidin-4-one **3c** in just 2 h. The *N*-acyl hydrazones obtained from hydrazides **1d**–**f** (which had previously displayed similar reactivity between themselves) displayed quite distinct levels of stability, suggesting some sort of influence from the α-amine substituent. Interestingly, adduct **2g** that was obtained from the less reactive phenyl hydrazide **1g** displayed an increased stability toward hexanal exchange, allowing only 3% conversion into adduct **3g** after 2 h. This increase in stability was also observed for adducts **2h** and **2i**, which had undergone about 10% exchange with hexanal after 12 h. From this screening, we identified hydrazide **1g** and **1h** as the most promising substrates, whose adduct stability was also evaluated against acidic buffers. The extent of hydrolysis observed for adducts **2g** and **2h** after 12 h ranged from 9 to 21%, being higher in the presence of more acidic buffers. Disappointingly, *O*-benzyl hydroxamic acid-based adduct **2j**, which featured a quite promising reactivity, showed a poorer overall stability when compared with adducts **2g** and **2h**. The data collected highlight the importance that the nucleophile strength has on an effective aminal formation from aldehydes in aqueous media. Unfortunately, imidazolidin-4-ones **2c**–**j** are not hydrolytically stable and are, in fact, hydrolyzed faster in more acidic buffer solutions (for mechanistic rationale, see SI section 10). These observations led us to study a different class of reagents—as exemplified by aminooxy acetate hydrazide **4**—which would not only form more thermodynamically stable six-membered adducts but also are expected to display higher stability in lower pH values. Compared to hydrazides **1c**–**j**, aminooxy acetate hydrazide **4** is considerably less reactive, affording only 5% of 1,2,4-oxadiazinan-5-one **5** after 30 min in acetonitrile/acetate buffer at pH 4 (Table 1, entry 11). However, the yield reached 75% after 28 h, giving 1,2,4-oxadiazinan-5-one **5** as the single product. This compound was found to be highly resistant toward hydrolysis/exchange reactions at room temperature up to 2 days (only at 40 °C and in the presence of acidic buffer, about 10% hydrolysis was observed after 24 h). We also found that 1,2,4-oxadiazinan-5-one is stable against cysteine in aqueous DMSO (see SI section 9). Even though 1,2,4-oxadiazinan-5-one **5** is formed slower, it displays improved stability compared with oxime[10] and oxacarboline[20] ligation products (see SI section 8, Tables S2 and S3).

3-Substituted 1,2,4-oxadiazinan-5-one heterocycles have been long overlooked, and their synthesis in an intermolecular fashion was never reported in aqueous media.[21] In 1989, Urogdi et al.

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synthesized for the first time this class of compounds in an intermolecular fashion through azeotrope distillation.[22] We proceeded afterward to evaluate the method scope by studying several classes of carbonyl compounds in water and at room temperature (Scheme 2). Hydrazide **4** reacted with hydrocinnamaldehyde (1 equiv, 60 mM) within 20 h at room temperature to yield 1,2,4-oxadiazinan-5-one **5** in 92% isolated yield. Under the same conditions, this condensation reaction proceeded very efficiently with other branched and nonbranched aliphatic aldehydes, affording the respective 1,2,4-oxadiazinan-5-ones in good to excellent yields (adducts **6**−**10**, 52−95% yield). Tertiary aliphatic aldehydes were found to be less reactive, but upon prolonged heating at 60 °C with an excess of aldehyde, we isolated 1,2,4-oxadiazinan-5-one **11** in 45% yield. We also screened benzaldehyde derivatives having halogens, free amines and hydroxyls, acetals, NO_2 and alkyl groups as substituents in the *para* and/or *ortho* positions, and heteroaromatic aldehydes. With the exception of 4-pyridinecarboxaldehyde, all aromatic aldehydes were converted in the respective 1,2,4-oxadiazinan-5-ones **12**−**20** and **22**−**23** in good to excellent yields (62−99%) upon heating at 60 °C. α,β-Unsaturated aromatic and aliphatic aldehydes also provided the respective products in near quantitative yields after 20−24 h at 60 °C (1,2,4-oxadiazinan-5-ones **24**−**26** and **28**). It is worth noting that, despite being very hindered, 2,6-dimethylbenzaldehyde, 2-bromobenzaldehyde, and 2-methylcinnamaldehyde still yielded the respective 1,2,4-oxadiazinan-5-ones **19**, **20**, and **27** in 51, 76, and 40% yield. In the same way, benzylic, allyl, or secondary aliphatic substitution in the hydroxylamine moiety were also tolerated, as the respective 1,2,4-oxadiazinan-5-ones **30**−**34** were obtained in high yields (70−80%). Between cyclohexanone, isophore, acetophenone, and *N*-benzyl isatin, we found that only the latter cyclized at 80 °C with hydrazide **4** to give spirocyclic 1,2,4-oxadiazinan-5-one **29** in 33% yield. To further comprehend the relative reactivity between all three classes of aldehydes tested herein, we performed a set of competitive experiments to show that aliphatic and α,β-unsaturated aldehydes are, respectively, 33 and 5 times more reactive than aromatic aldehydes (see SI section 6).

This study highlights that the α-effect present in the hydrazide moiety has a vital role in controlling the kinetics of imidazolidin-4-one[19c] formation from α-amino hydrazides and their stability in aqueous media. Although the imidazolidin-4-ones were found not to be hydrolytically stable, the knowledge gathered from studying their properties in aqueous solutions fueled the design of α-aminooxy acetyl phenylhydrazides, which undergo irreversible condensation reactions with aldehydes in slightly acidic aqueous media. This class of hydrazides forms 3-substituted 1,2,4-oxadiazinan-5-one heterocycles by conjugation with aliphatic, α,β-unsaturated, or aromatic aldehydes and isatins. Most importantly, this class of heterocycles is stable in aqueous media throughout a relevant range of pH (4.5−7) and temperatures and will find application for chemoselective aldehyde functionalization in aqueous media.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01889.

Experimental procedures, spectral data, and copies of all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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