

N-Acyl Benzisothiazolinones (N-acyl BITs) Enable Efficient Amide Bond Formation in Aqueous and Organic Media

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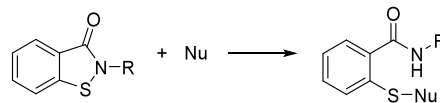
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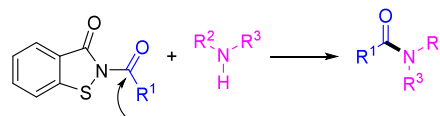
Supporting Information

ABSTRACT: N-Acyl benzisothiazolinones (N-acyl BITs) are introduced as a class of bench-stable acylating reagents that undergo efficient acyl transfer to amines under mild conditions. Unlike the well-known N–S bond cleavage pathways of benzisothiazolinone derivatives, N-acyl BITs operate through direct acyl–nitrogen bond activation, enabling rapid amide formation without additives in a wide range of organic solvents as well as mixed organic/water solvent systems. These reagents display broad compatibility with primary, secondary, and aromatic amines and exhibit enhanced resistance to hydrolysis compared with commonly used N-hydroxysuccinimide esters. A BIT–biotin derivative further demonstrates the applicability of this platform to amine modification in mixed solvent environments, achieving efficient N-terminal biotinylation of peptides under the employed reaction conditions. Together, these results establish N-acyl BITs as practical and robust reagents for amide bond formation under water-compatible conditions.

Conventional BIT Chemistry: N–S Bond Cleavage



This Work: Acyl Transfer from N-Acyl BITs (No N–S Bond Cleavage)



- High Aqueous Stability
- Additive-Free Amide Formation
- Distinct Acyl Transfer Reactivity
- Broad Acyl & Amine Scope

Chemoselective amine acylation in aqueous media represents a fundamental transformation in organic and chemical biology fields, finding wide application in the synthesis of pharmaceuticals, peptides, and bioconjugates. Developing acylation reagents that can efficiently operate under such mild, water-compatible conditions remains a long-standing challenge due to the intrinsic competition between acyl transfer to nucleophiles and hydrolysis in aqueous media.^{1–3}

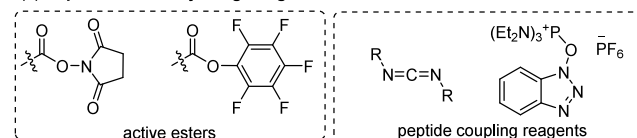
Traditionally, a variety of strategies have been employed for amine acylation.⁴ Activated esters such as N-hydroxysuccinimide (NHS),⁵ p-nitrophenol (PNP),⁶ and pentafluorophenol (PFP) esters⁷ are widely used due to their convenient reactivity profiles. Other representative classes include N-acyl imidazoles,⁸ acyl fluorides,⁹ and peptide coupling reagents such as carbodiimides¹⁰ or phosphonium salts (Scheme 1a).¹¹

Despite their utility, many of these methods suffer from limited hydrolytic stability under aqueous or bioconjugation conditions, the necessity of preactivation of carboxylic acids, or reliance on additional additives or catalysts. Moreover, maintaining chemoselectivity among multiple nucleophilic sites often presents a significant challenge. As a result, expanding the available toolbox of acylation reagents remains an important task for both synthetic and biological chemistry.

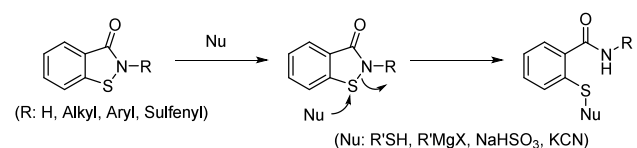
Benzisothiazolinone (BIT) derivatives are known for their versatile reactivity and have been utilized in various transformations, particularly upon reaction with thiols, which induces N–S bond cleavage to form disulfide linkages (Scheme 1b).^{12–15} Motivated by this inherent thiol reactivity, we initially considered the BIT scaffold as a platform for

Scheme 1. Previously Developed Acylating Reagents and N-acyl BIT

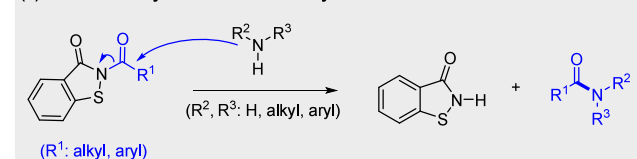
(a) Representative Acylating Reagents



(b) N–S Bond Cleavage Pathway of BITs with Nucleophiles



(c) This work: Acyl Transfer from N-Acyl BITs to Amines



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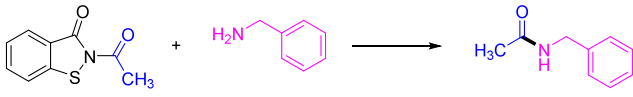


developing highly reactive thiol-selective conjugation reagents for cysteine-containing biomolecules. Because efficient thiol conjugation under aqueous or biologically relevant conditions generally requires reagents with sufficient electrophilicity, acyl substitution at the nitrogen was examined as a means to modulate the reactivity of the BIT scaffold. Under the examined conditions, *N*-acyl BITs were found to undergo efficient acyl transfer to amines including in aqueous media. This reactivity is consistent with activation of the acyl-nitrogen bond and proceeds without involvement of the conventional N–S bond cleavage pathway (Scheme 1c).

Structurally related *N*-acyl saccharins were previously reported in 2016 as acyl transfer reagents in palladium-catalyzed Sonogashira and Heck reactions.^{16,17} Earlier studies also described reactions of *N*-benzoylsaccharin with amines, which typically proceed through the formation of ring-opened adducts and require basic additives, elevated temperatures, or highly polar solvents to afford the corresponding amides. In these systems, amide formation is strongly dependent on substrate basicity and reaction conditions and is not generally observed under mild or aqueous conditions.¹⁸ In contrast, the BIT scaffold offers a distinct electronic environment at the acyl–nitrogen bond, along with practical advantages such as bench stability, crystallinity, and facile synthesis. Yet, despite these appealing features, *N*-acyl BITs themselves have remained largely unexplored as direct acylating reagents. These observations prompted us to investigate *N*-acyl BITs as a previously unrecognized class of acyl-transfer reagents with potentially distinct reactivity from those of established activated esters.

N-Acyl BIT derivatives were readily prepared from BIT and the corresponding carboxylic acids by EDC·HCl-mediated coupling in CH₂Cl₂, followed by simple aqueous trituration (see the Supporting Information). Initial evaluation of *N*-acetyl BIT (1a) with benzylamine (2a) revealed that the reaction proceeded cleanly under additive-free conditions (1.05 equiv of 1a, rt, 1 min, CH₂Cl₂), providing >99% conversion without any observable side reactions. Given the essentially quantitative outcome under these very mild conditions, we then conducted a solvent-screening study to assess both reactivity and stability of *N*-acetyl BIT across different media (Table 1). Quantitative conversion was observed within 1 min in nonpolar (toluene), chlorinated (chloroform, CH₂Cl₂), polar aprotic (DMF, DMSO), and polar protic solvents (methanol). Given its consistent performance and practical solubility characteristics,

Table 1. Solvent Screening of the Reaction between *N*-acetyl BIT (1a) and Benzylamine (2a)



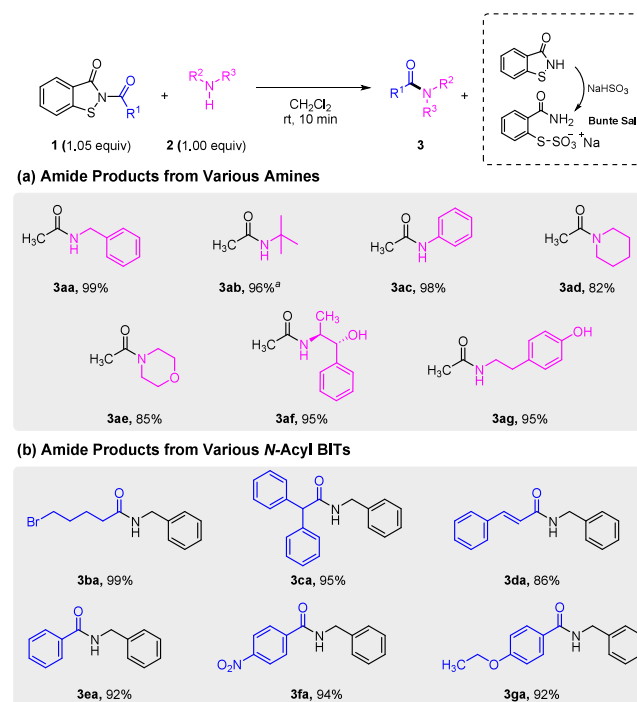
Solvent	Temperature	Reaction Time (min)	Conversion (%) ^a
CH ₂ Cl ₂	rt	~1	>99
CHCl ₃	rt	~1	>99
Toluene	rt	~1	>99
DMF	rt	~1	>99
DMSO	rt	~1	>99
MeOH	rt	~1	>99

^aConversions were determined by ¹H NMR analysis.

CH₂Cl₂ was selected as the standard reaction medium for subsequent substrate-scope studies.

The generality of the amine nucleophile scope was evaluated using *N*-acetyl BIT (1a) as a representative reagent, which displayed broad compatibility with primary, secondary, and aromatic amines (Scheme 2a). A representative primary amine,

Scheme 2. Substrate Scope Study



^aFor *t*-butylamine (2b), reaction was conducted with 1a (1.05 mmol) and 2b (1.00 mmol) in CHCl₃ (1.00 mL) at 50 °C for 8 h.

benzylamine, delivered the corresponding amide (3aa) in 99% isolated yield. The sterically demanding *tert*-butylamine also underwent efficient acylation with 1a using stoichiometric amounts of the reagent under heating (50 °C, 8 h) to give the *N*-acyl amide (3ab) in 96% isolated yield. The reaction also proceeded efficiently with less nucleophilic aromatic amines such as aniline. Aniline underwent rapid acylation to afford the amide (3ac) in 98% yield. Secondary amines such as piperidine and morpholine also provided the corresponding acyl amides (3ad, 3ae) in good yields (82 and 85%, respectively) under the standard conditions. To probe chemoselectivity, substrates bearing competing nucleophilic sites were examined. (+)-Nor-ephedrine underwent exclusive *N*-acylation to give product (3af) in 95% yield without detectable *O*-acylation. Tyramine similarly afforded the acyl amide product (3ag) with complete selectivity over the phenolic hydroxyl group, demonstrating the strong preference of *N*-acyl BITs for amines over alcohol or phenol functionalities.

We next evaluated the acyl scope using benzylamine (2a) as a representative nucleophile (Scheme 2b). *N*-(5-Bromopent-1-en-1-yl) BIT afforded the amide (3ba) in 99% yield. Even the sterically demanding *N*-(diphenylacetyl) derivative underwent efficient acyl transfer, providing the product (3ca) in 95% yield. An α,β -unsaturated acyl group was also compatible: *N*-(*trans*-cinnamoyl) BIT furnished the corresponding amide (3da) in 86% yield. Furthermore, aromatic acyl groups—including benzoyl and *para*-substituted derivatives bearing

either electron-withdrawing ($-\text{NO}_2$) or electron-donating ($-\text{OEt}$) substituents—were effectively transferred, delivering the amides (**3ea–3ga**) in 92–94% yields. Collectively, these results show that *N*-acyl BITs efficiently transfer both aliphatic and aromatic acyl groups with diverse electronic properties.

To further assess the applicability of *N*-acyl BITs to stereochemically sensitive substrates, we investigated whether *N*-acyl BIT formation and the subsequent acyl-transfer reaction proceed without erosion of stereochemical integrity. The *N*-acetyl-*L*-phenylalanine-BIT conjugate (*N*-Ac-*L*-Phe-BIT) was prepared via EDC/HOBt-mediated coupling and then reacted with *racemic*-, (*R*)-, and (*S*)-1-phenylethylamine in CDCl_3 at rt. Gratifyingly, ^1H NMR analyses of these three independent reactions showed that both the EDC/HOBt-mediated coupling to form *N*-Ac-*L*-Phe-BIT and the subsequent acyl transfer to 1-phenylethylamine proceeded with high stereochemical fidelity under the employed conditions (98:2 and 1:99 dr, respectively, see Supporting Information section 2-3).

An additional advantage of *N*-acyl BITs lies in their ease of purification. After the reaction, both unreacted *N*-acyl BIT and the byproduct BIT can be removed by treatment with saturated aqueous sodium bisulfite, which converts the BIT scaffold into water-soluble Bunte salts.¹⁵ These can then be easily separated from the desired amide products by simple aqueous extraction.

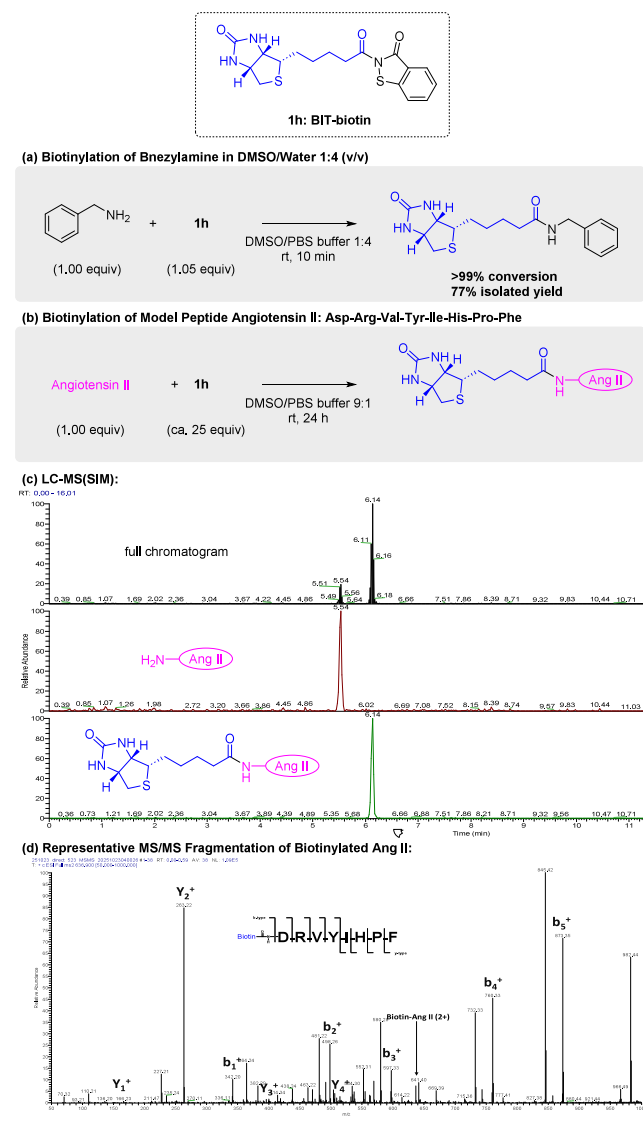
Because hydrolytic stability critically influences the practical utility of acylating reagents in aqueous or bioconjugation settings, we next evaluated the hydrolytic stability of *N*-acyl BITs. To directly compare their hydrolytic stability to the most commonly used NHS esters, two independent water-soluble analogs were prepared by introducing a pyridinium group at the ω -position of the corresponding esters derived from pentanoic acid. The resulting compounds were dissolved in D_2O -based PBS buffer (pD = 7.8), and their hydrolysis was monitored over time at room temperature by ^1H NMR spectroscopy (see Supporting Information section 2-4). After 48 h, the *N*-acyl BIT derivative retained 33% of its initial concentration, whereas the NHS ester had fully hydrolyzed to the corresponding carboxylic acid and *N*-hydroxysuccinimide. From the kinetic analysis, the half-life of *N*-acyl BIT was determined to be 31 h, compared to 7.6 h for the NHS ester—demonstrating that *N*-acyl BIT exhibits approximately 4-fold greater hydrolytic stability under aqueous conditions.

Given that increased stability is often accompanied by reduced intrinsic reactivity, we next examined whether such a stability-reactivity trade-off was observed for *N*-acyl BITs. The initial amine-acylation rates of *N*-acetyl BIT (**1a**) and a representative NHS ester were therefore compared under conditions that suppress competing hydrolysis. Both reagents were dissolved in CDCl_3 or $\text{DMSO}-d_6$, and 0.25 equiv of benzylamine was added to measure initial rates under pseudo-first-order conditions. Under these hydrolysis-free conditions, *N*-acetyl BIT exhibited moderately reduced rates relative to the NHS ester (0.68-fold in CDCl_3 and 0.32-fold in $\text{DMSO}-d_6$). These results are consistent with the expected stability-reactivity trade-off and indicate that *N*-acyl BITs maintain practically useful intrinsic reactivity while exhibiting enhanced resistance to hydrolysis in aqueous media.

To demonstrate that *N*-acyl BIT reagents can be directly applied in representative bioconjugation reactions, we prepared a BIT–biotin conjugate (**1h**) and examined its reactivity in mixed organic/aqueous solvent systems. BIT–biotin was first evaluated using benzylamine in PBS buffer (pH = 7.4) with

DMSO as a cosolvent, and the reaction progress was monitored by ^1H NMR spectroscopy (Scheme 3a). The

Scheme 3. Biotinylation of Amine and Peptide Using BIT–biotin in DMSO/Water Mixture



fragmentation patterns consistent with the expected biotin-peptide conjugate (Scheme 3d).

Notably, a single product peak with an increased retention time was observed, consistent with selective N-terminal biotinylation and no detectable modification at other residues under these conditions. These results demonstrate that N-acyl BIT derivatives enable efficient N-terminal biotinylation of peptides under the employed conditions.

In summary, we have developed a class of N-acyl BIT reagents that enable rapid and efficient amine acylation under mild conditions. These reagents can be readily prepared, are stable in water, and react swiftly with a wide range of amines, including sterically hindered amines, anilines, and peptide substrates. In particular, BIT-biotin serves as a practical biotinylating agent, providing high conversion in peptide labeling under simple mixing conditions. We anticipate that the modularity and operational simplicity of this platform will facilitate broad applications in chemical biology and bioconjugation. The balance of hydrolytic stability and intrinsic acylating reactivity establishes N-acyl BIT as a useful platform for amide-bond formation across diverse reaction environments.

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information. FAIR data, including the primary NMR FID files for compounds: **1a–1h**, **3aa–3ag**, **3ba–3ga**.

■ Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.orglett.5c05019>.

Experimental procedures and characterization data (PDF)

FAIR data, including the primary NMR FID files, for compounds **1a–1h**, **3aa–3ag**, **3ba–3ga** (ZIP)

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Author Contributions

Synthetic experiments were performed by J.M. The mass spectrometry experiments and curation were carried out by S.Y.L. and H.B.O. Conceptualization, data interpretation, and

manuscript preparation were conducted by J.M. and B.M. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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(19) Conjugation efficiencies were evaluated at various DMSO/H₂O ratios and BIT-biotin stoichiometries. The reaction still proceeded at higher water content and with lower BIT-biotin loading, affording a moderate conjugation efficiency (65% in 1:1 DMSO:H₂O with 5.0 equiv BIT-biotin; see [Supporting Information section 2-6](#), page 35). A higher reagent loading (25 equiv) was therefore employed to ensure consistent and reproducible conversion within the examined reaction time.