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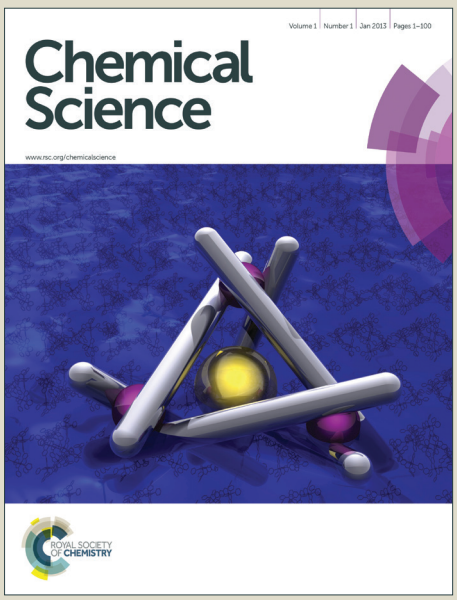

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EDGE ARTICLE

Synthesis and Chemoselective Ligations of MIDA Acylboronates with *O*-Me Hydroxylamines

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Hidetoshi Noda and Jeffrey W. Bode*

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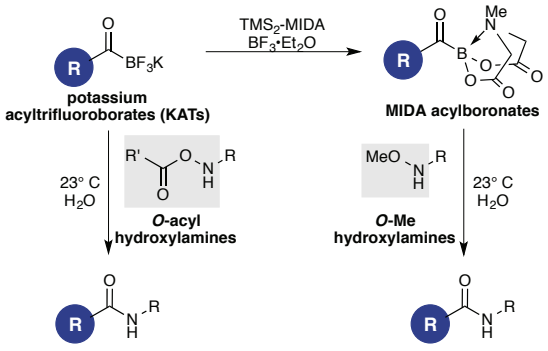
N-Methyliminodiacetyl (MIDA) acylboronates undergo chemoselective amide-bond forming ligations in water with *O*-Me hydroxylamines, including unprotected peptide substrates. These bench-stable boronates were easily prepared from potassium acyltrifluoroborates (KATs) in one step. The reactivity of MIDA acylboronates with *O*-alkylhydroxylamines – which are unreactive with KATs – was attributed to the nature of the neutral MIDA boronates versus the ionic KATs, leading to differences in the stability of likely intermediates and propensity for elimination.

Introduction

The development of new chemoselective reactions[1] is a continuing mission of synthetic organic chemistry and is critical to the synthesis of large molecules such as proteins[2] and for applications in chemical biology.[3] In our own work, we have identified the α-ketoacid-hydroxylamine (KAHA) ligation[4] and applied this to several chemical syntheses of proteins.[5] The KAHA ligation works well for the purpose of the coupling of peptide fragments, but its reaction rate[6] requires relatively high concentration of reactants (5 mM) and moderate temperatures (40–60 ˚C), which are not ideal for working with proteins or other high molecular weight molecules.

As part of ongoing efforts towards faster amide-bond forming reactions,[7] we recently reported that potassium acyltrifluoroborates[8] (KATs) undergo amide-formation with *O*-acylhydroxylamines.[9] The KAT ligation tolerates all common unprotected functional groups and takes only minutes at 23 ˚C, even at 1 mM and with a 1:1 ratio of reactants. For further improvement of this ligation, we were particularly interested in identifying a new ligation partner that reacts with *O*-alkylhydroxylamines. These hydroxylamines are smaller and more chemically stable than *O*-acyl variants, making them excellent candidates for incorporation into expressed biological materials. They are also widely synthesized and used for labelling applications with aldehyde-containing peptides and proteins.[10] Unfortunately neither α-ketoacids nor KATs form amides with *O*-alkylhydroxylamines at mild temperatures. We therefore imagined improving the reactivity of the KAT ligation by replacing the fluorine atoms with a suitable ligand on boron.



Scheme 1. Overview of the KAT ligation and this work

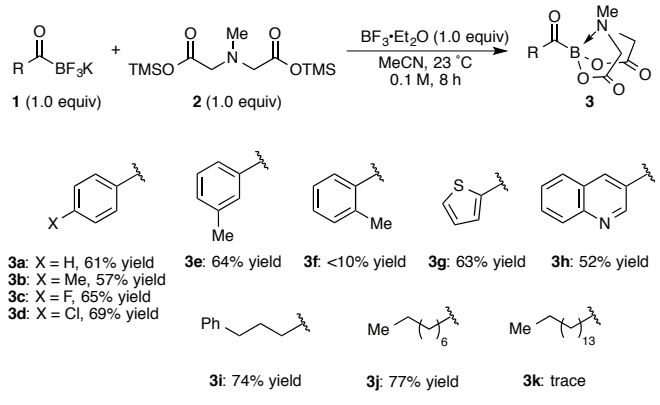
The recent report by Yudin[11] that *N*-methyliminodiacetyl (MIDA) acylboronates are bench-stable solids provided an ideal opportunity to examine their reactivity in ligation reactions with hydroxylamines. Both potassium trifluoroborates[12] and MIDA boronates[13] are commonly used as boronic acid surrogates in Suzuki-Miyaura cross couplings, but they have different physical properties and reactivities.[14] We therefore expected their acyl derivatives to behave differently in amide-forming ligations. This hypothesis proved correct, and we now demonstrate that MIDA acylboronates are considerably more reactive than KATs and undergo ligation with *O*-Me hydroxylamines in water at room temperature (Scheme 1). We also report that the MIDA acylboronates can be easily prepared in one step from KATs.

Results and discussion

Synthesis of MIDA Acylboronates

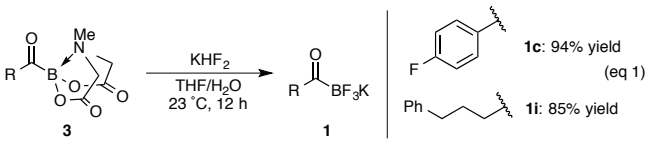
The synthesis of MIDA acylboronates reported by Yudin employs a somewhat lengthy route from vinyl MIDA boronates, which often requires several steps to prepare. For improved access, we sought to convert KATs, which we can easily prepare on gram scale from aldehydes[15] or organolithium reagents,[16] to MIDA acylboronates. Pioneering work by Vedejs[17] demonstrated that $PhBF_2$ was smoothly formed from $PhBF_3K$ by treatment with TMSCl in dry acetonitrile,[18] a procedure we have previously used to manipulate organotrifluoroborates.[19] Combined with a recent example of the formation of MIDA boronates from organodichloroboranes,[20] we believed that treatment of KATs with a suitable fluorophile followed by MIDA bis(trimethylsilyl) ester (**2**, TMS_2-MIDA) could offer a simple synthesis of MIDA acylboronates. This proved to be the case;[21] treatment of KAT **1** with BF_3•Et_2O in the presence of TMS_2-MIDA **2** in dry acetonitrile resulted in the formation of MIDA acylboronate **3** in good yield after isolation by column chromatography (Scheme 2).

Aromatic KATs with both electron withdrawing and donating groups were successfully converted to the corresponding MIDA acylboronates in moderate to good yield. *meta*-Substitution had no effect on complexation, furnishing the product in good yield. *ortho*-Substituted substrates, however, were problematic; the products were formed in good yield as determined by NMR experiments on the unpurified reaction mixtures, but significant decomposition occurred during purification and less than 10% of the desired product was isolated. Heteroaromatic KATs were suitable substrates for this transformation, and the products were obtained in moderate to good yield. Aliphatic KATs gave the product in better yield. One limitation of this transformation was substrates with poor solubility in acetonitrile. For example, only trace amounts of MIDA palmitoylboronate **3k** were observed in the NMR spectrum of the unpurified reactions.



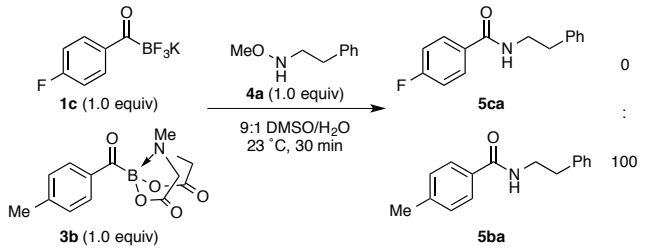
Scheme 2. Scope and limitation of MIDA acylboronate synthesis

MIDA acylboronates can be converted back to KATs simply by treatment with aqueous KHF_2 (eq 1). Both aromatic and aliphatic substrates formed the corresponding KATs in excellent yield. This reaction promises the possibility to utilize MIDA acylboronate as a protecting group for KATs.



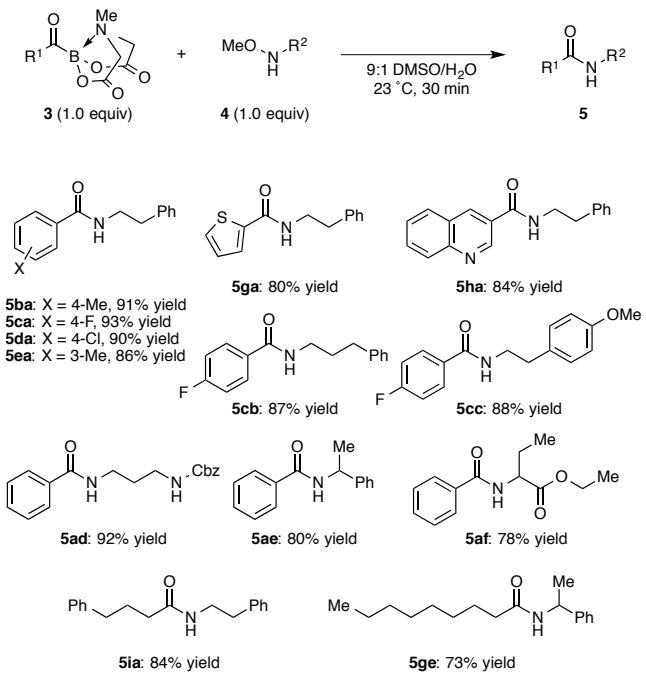
Amide-Formation with MIDA Acylboronates and *O*-Alkylhydroxylamines

We were pleased to find that MIDA acylboronates formed amides with *O*-Me hydroxylamines in water. The KATs, in contrast, did not give any product in a competition experiment (Scheme 3). This promising result led us to examine the reaction more closely. A solvent study revealed that water is essential for the rapid rate of the reaction. Among organic co-solvents screened, DMSO was preferred due to better solubility of substrates; other solvents such as THF, acetonitrile and *N*-methyl-2-pyrrolidone (NMP) were also suitable with a wide range of water ratio (Table S2). The organic solvents are not necessary for the ligation but rather for solubilizing the substrates. A screening of additives also showed that MIDA acylboronates were stable to Brønsted acids, amine bases and nucleophiles in DMSO (Table S3).



Scheme 3. A competition experiment between KAT **1c** and MIDA boronate **3b** with *O*-Me hydroxylamine **4a**

The substrate scope of this reaction was examined under the optimized condition with equimolar reactants (Scheme 4). Aromatic MIDA acylboronates smoothly provided the amides in excellent yield regardless of the nature of the substituents. Heteroaryl substrates participated in amide-formations to give the products in good yield. Substituted phenethylamine derived hydroxylamine also underwent amide-formation without problem. α-Branched hydroxylamines furnished the products in good yield. The result of an amino acid derived substrate is especially important to us, as it is encouraging for future application to peptides and proteins. Aliphatic MIDA acylboronates showed the similar reactivity to aromatic variants and gave the amides with both linear and branched hydroxylamines in excellent yield.



Scheme 4. Substrate scope of the amide-formation

Substituents on the hydroxylamine oxygen were not limited to methyl. The sterically more demanding benzyl group was tolerated and the amide was obtained in excellent yield under the identical conditions. As with the chemoselective amide-forming reactions previously developed in our group, MIDA acylboronates also underwent amide-formation with *O*-Bz hydroxylamine **6b** in water. Competition experiments showed that *O*-Bz hydroxylamine reacted much faster than the *O*-Me derivatives (Scheme S8).[22] The *O*-unsubstituted hydroxylamine **6c** formed the amide along with nitrone **6d**.[23] This amide formation also has limitations. Sterically congested *N*-tert-butylhydroxylamine **6e** did not form any product. A tertiary hydroxylamine **6f** did not react with MIDA acylboronate.

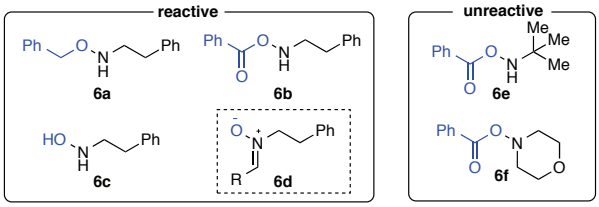
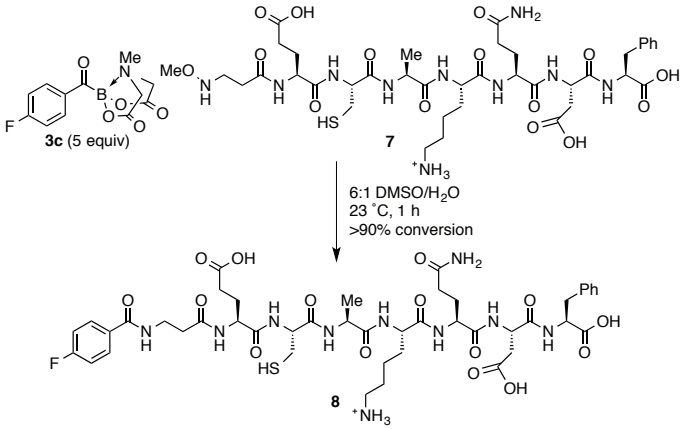


Fig 1. Further scope and limitation of hydroxylamines

In order to evaluate the chemoselectivity of this ligation, we incorporated a *β*-alanine-derived hydroxylamine into a peptide having a variety of unprotected functional groups including primary amines (Lys), thiols (Cys) and carboxylic acid (Glu/Asp). This ligation was chemoselective and the only observed peptide products were the starting material **7** and the ligated peptide **8** (Scheme 5). Unfortunately MIDA acylboronates appear to be somewhat unstable in the slightly acidic reaction media and this ligation requires 5 equiv of the acylboronate **3c** for full conversion. Attempts to reduce the decomposition of the MIDA acylboronate by modulating the reaction pH were not successful. Efforts to improve the stability of the acylboronates by ligand design are ongoing.



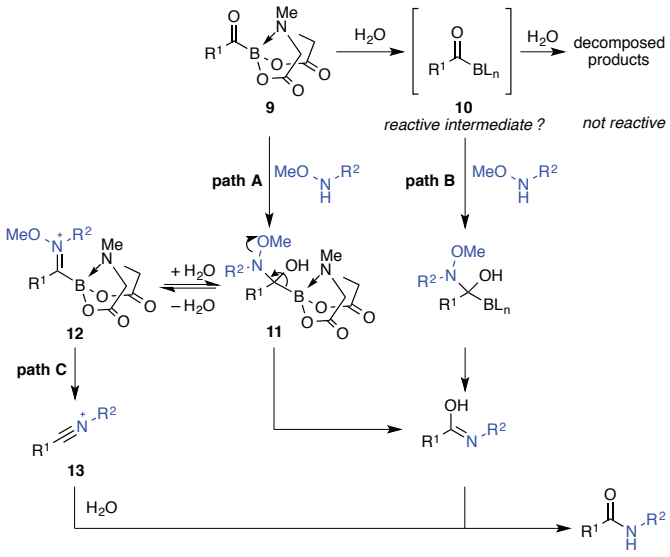
Scheme 5. A chemoselective amide-forming ligation of MIDA acylboronate **3c** and peptide hydroxylamine **7** in water

Mechanism of the Amide-Formation

The apparent instability of the acyl MIDA compounds implied that a hydrolysed intermediate might be responsible for the higher reactivity. Initially, we speculated that the acyl MIDA was converted to an intermediate **10,** which could either undergo ligation (path B) or decomposition (Scheme 6). Alternatively, the MIDA acylboronates may react directly with the hydroxylamine to form a hemiaminal, followed by a concerted elimination to give the amide (path A). The hemiaminal **11** can also lead to nitrilium **13** via iminium intermediate **12** (path C).

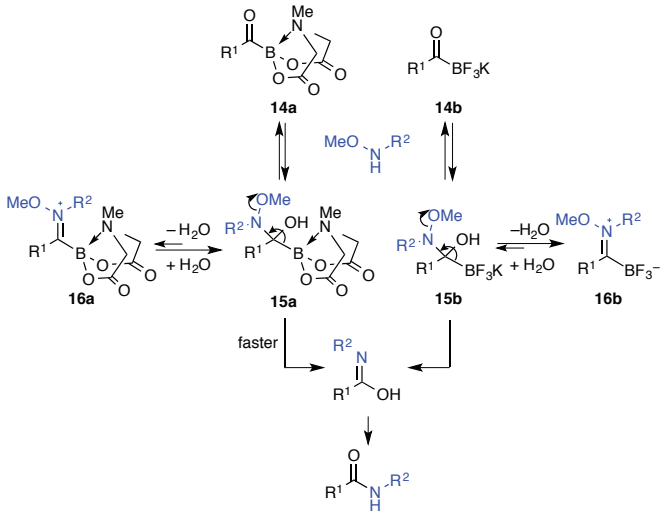
At present, we speculate that path A is most likely. Reactions conducted in MeOH as solvent gave only the amide products; the *O*-Me imidates expected from a nitrilium intermediate (path C) were not observed. MIDA acylboronates gradually decomposed in water to products that do not react with hydroxylamines. Likewise, no amide formations were observed when acyldifluoroboranes were prepared from KATs *in situ* and added to an aqueous solution of hydroxylamines (Table S5). Together, these results disfavour a mechanism involving the formation of an acylboronic acid or other hydrolysed intermediate. Although we cannot completely rule out that a reactive intermediate is involved in this amide-formation, our observations to date favour pathways involving an intact MIDA boronate.

Further support for path A is found in the dramatic effect of water on the ligations. The ligations with MIDA acylboronates occur in anhydrous DMSO, albeit at a far slower rate. This suggests that hydrolysis of the MIDA moiety is not essential and implicates hemiaminal **11** as a key intermediate. Ligations in protic solvents, such as MeOH or hexafluoroisopropanol (HFIP), did not cause decomposition of the MIDA but proceeded at a slower rate than reactions in water (see the Supporting Information for details).



Scheme 6. Possible mechanistic pathways for the amide-formation

Our current understanding of the higher reactivity of the acyl MIDAs with *O*-alkylhydroxylamines is depicted in Scheme 7. MIDA acylboronates and KATs react with *O*-Me hydroxylamines to form hemiaminals **15a** and **15b**, which are in equilibrium with their corresponding iminium intermediates **16a** and **16b**. In the MIDA case, iminium **16a** is cationic and the equilibrium favours hemiaminal **15a**. In the case of KATs, formation of the neutral species **16b** – which we can observe by ^{1}H NMR[24] – results in a much lower concentration of **15b**. The rate of the elimination could also vary based on the structure of the boron group, MIDA vs BF_3K. This step may be related to the well-studied eliminations of β-hydroxy[25,26] boranes. In these 1,2-eliminations, an oxygen atom usually coordinates with the empty p-orbital on the boron atom to facilitate eliminations. The spontaneous eliminations of intact MIDA boronates drawn in Schemes 6 and 7 are not well established by literature precedents,[27] and it is also possible that the elimination occurs after unmasking the p-orbital by the hydrolysis of MIDA moiety.[28] On the other hand, potassium β-alkoxytrifluoroborates were reported to be stabilized significantly by a potassium cation bridging one fluorine and the β-oxygen, making them less susceptible to elimination.[29] We conclude that the higher reactivity of MIDA acylboronates derives from both the higher concentration of the hemiaminal **15a** and the faster elimination step.



Scheme 7. Mechanistic differences between MIDA acylboronates and KATs in the amide-formation

Conclusions

The reactivity of the KAT ligation can be enhanced by a MIDA ligand on boron. The MIDA acylboronates underwent chemoselective amide-forming ligations with *O*-methylhydroxylamines, including unprotected peptides in water. These bench-stable boronates were prepared from the corresponding KATs in one step. The slightly unstable nature of MIDA acylboronates under aqueous conditions hampers the coupling with an equimolar ratio of reactants at lower concentrations, but their high reactivity with *O*-alkylhydroxylamines will find further applications. The MIDA boronates, which are neutral compounds while the KATs are salts, are likely to be more reactive due to differential stability of the postulated intermediates in the amide-forming ligation. Our results also imply that the acylboron-hydroxylamine ligation can be tuned by modulating the boron group to improve reactivity and stability. Further development of new ligands for the boronates should also lead to more stable derivatives that ligate with less reactive hydroxylamines.

Acknowledgements

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Notes and references

Laboratorium für Organische Chemie, Departemnt of Chemistry and Applied Bioscience, ETH Zürich, 8093 Zürich, Switzerland. *E-mail:* bode@org.chem.ethz.ch; *Web:* http://www.bode.ethz.ch

† Electronic Supplementary Information (ESI) available: Experimental procedures and spectroscopic data for all new compounds. See DOI: 10.1039/b000000x/

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