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Synthesis of Bifunctional Potassium Acyltrifluoroborates

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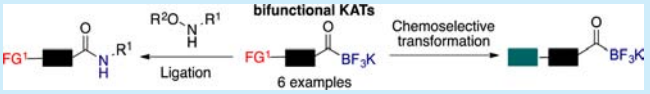
Supporting Information

ABSTRACT: New bifunctional potassium acyltrifluoroborate (KAT) substrates have been synthesized in gram scale using optimized reaction conditions. Chemoselective transformation of functional groups in the presence of an acyltrifluoroborate has been demonstrated, and orthogonal reactions of bifunctional KAT reagents are reported. This allows for the incorporation of KAT moieties into peptides and dyes.



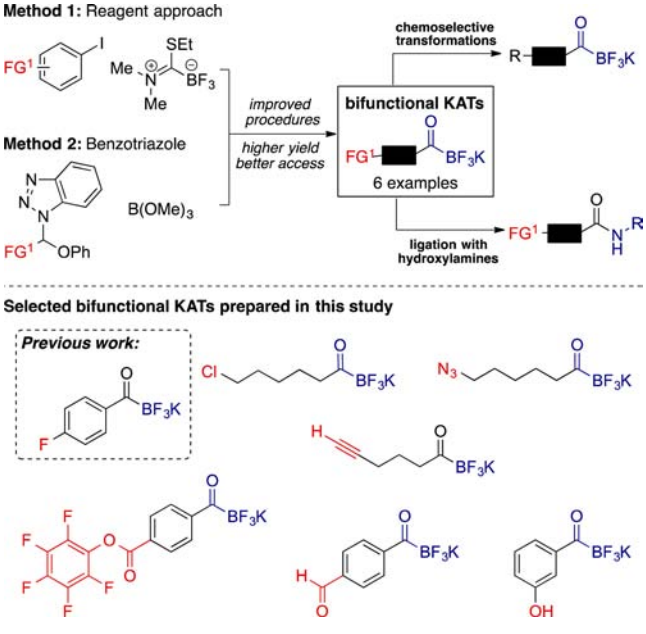
Chemoselective ligation reactions have transformed the practice of organic synthesis by enabling covalent bond construction without the need for reagents or protection of common functional groups.[1] Easy to use chemistry such as azide−alkyne click reactions and thio-Michael additions are commonly employed not only for organic chemistry but also in biology and materials.[2] The popularity of these methods, particularly azide/alkyne reactions, lies in the facile introduction of the requisite functional groups.

Recently, we reported ligations from potassium acyltrifluoroborates (KATs), a unique class of functional groups that undergo rapid, chemoselective amide-forming reactions with hydroxylamines under aqueous conditions without the need for activating agents or protecting groups.[3] With second-order rate constants of up to 20 M^{-1} s^{-1} under acidic conditions and complete compatibility with azides, strained alkynes, thiols, and acrylates, this reaction complements contemporary conjugation methods.[4−6] The KAT functional groups are extremely stable and can be introduced into simple organic molecules by several methods. However, their use for bioconjugation is currently hampered by the lack of multifunctional reagents for incorporating KATs into more complex molecules.[7]

In an effort to expand the synthetic repertoire of bifunctional KAT reagents to meet the demand for ease of incorporation into organic molecules, we envisioned bifunctional reagents that bear the unique KAT functionality as well as an easily modifiable functional group that can undergo chemoselective reaction in the presence of the KAT. We now report the preparation and use of six key reagents for this purpose (Scheme 1), suitable for reactions including copper-catalyzed azide−alkyne cycloaddition (CuAAC), acylation, Wittig reaction, alkylation, and nucleophilic substitution reactions.

To date, we have employed only one bifunctional KAT for elaborating other molecules. Potassium 4-fluorobenzoyltrifluoroborate is an excellent substrate for nucleophilic aromatic substitution (S_NAr) reactions due to the electron-withdrawing nature of the KAT moiety. Using this reagent, our group successfully prepared KATs bearing PEG-chains as well as markers including dyes and biotin.[4] These studies demonstrated that KATs can tolerate different reaction conditions and reagents, including strongly basic and strongly acidic conditions, and encouraged us to prepare a number of other bifunctional reagents.

Scheme 1. Synthetic Access to Bifunctional KATs



As depicted in Scheme 1, two general methods for KAT synthesis were exploited for the synthesis of such bifunctional reagents. In the first approach, KATs were accessed through lithium−halogen exchange and the subsequent quenching of the metalated species with reagent **2**[7] (Scheme 2). This reagent allows for the synthesis of a broad range of aromatic KATs including phenyl, pyridine, quinoline, and thiophene KAT substrates in one step and has been adapted by Sigma-Aldrich for commercial purposes (CAS No.: 1622923-36-7). The second approach, via benzotriazole chemistry, allows access to both aromatic and aliphatic KATs by utilizing the *N,O*-acetal as an acyl anion equivalent.[8]

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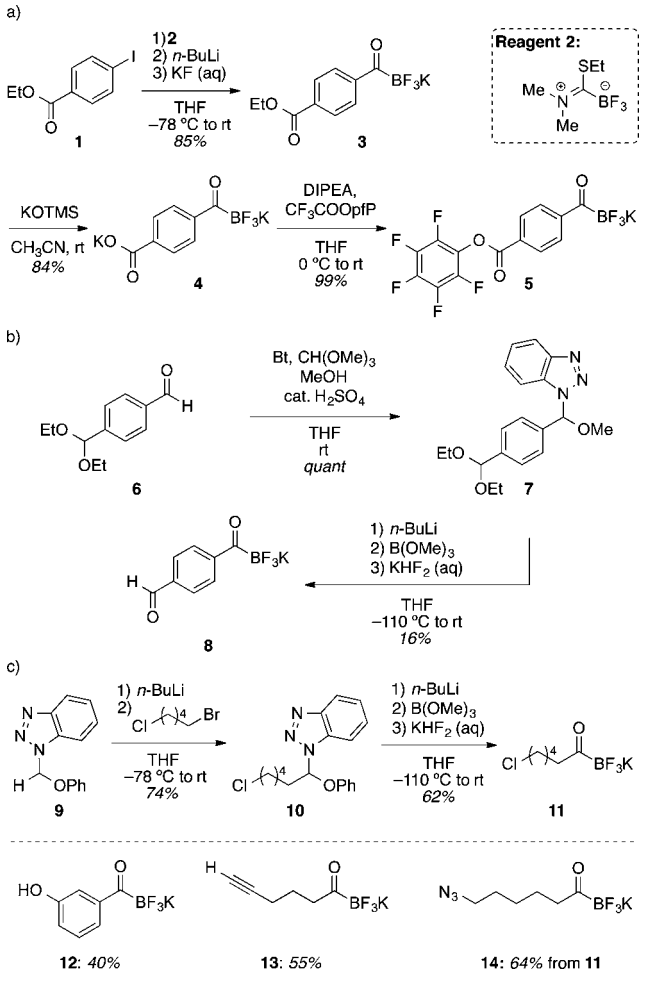
Scheme 2. Preparation of Bifunctional KATs

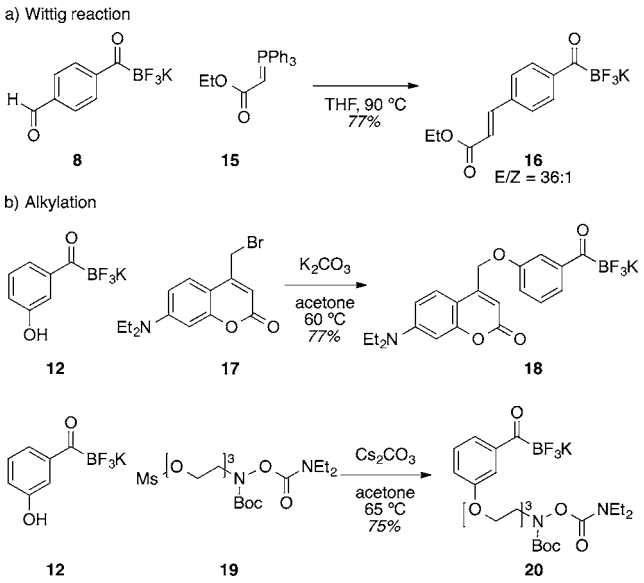


The reagent-based approach was used to prepare a KAT bearing an activated ester via the route shown in Scheme 2a. Hydrolysis of **3** gave the carboxylate salt, which was easily converted to the corresponding perfluorophenyl ester in excellent yield. For preparation of the structurally related KAT aldehyde **8**, we found that the best, although still low yielding, approach was via the benzotriazole chemistry, as shown in Scheme 2b. In the original procedure,[9] the deprotonation step was carried out at −78 °C, but we found that these conditions led to decomposition of the lithiated intermediate. Decreasing the temperature to −110 °C proved more effective, but required a prolonged lithiation step to ensure complete consumption of *n*-butyllithium to avoid the formation of the side product *n*-$BuBF_3K$, upon quenching with aqueous KHF_2.

The synthesis of bifunctional, aliphatic KATs begins with readily available starting material 1-(phenoxymethyl)-1*H*-benzotriazole **9**, which is deprotonated with *n*-buthyllithium, resulting in an anion which is allowed to react with a variety of alkyl halides.[10] The resulting KAT precursors (e.g., **10**) were deprotonated a second time, trapped with $B(OMe)_3$, and converted to the potassium acyltrifluoroborates. While the synthesis of the precursors were straightforward, the subsequent reactions to form the KATs often led to poor or no yield of the desired product when following the original protocol. For instance, KAT **11** was isolated in 30% yield while KAT **13** was only observed in trace amounts. Decreasing the temperature to −110 °C had a favorable effect on the overall yield of the reaction. Using a modified procedure with a prolonged lithiation time, **11** was isolated in 62% yield and with high purity. Subsequent substitution of the chloride with an azide provided a handle for further functionalization via a CuAAC reaction. KAT **13** was synthesized in a one-pot fashion including an *in situ* TMS deprotection (see Supporting Information). The overall isolated yield for the alkyne KAT formation was 55%.

In order to demonstrate the orthogonality of the newly installed functional group in the presence of the acyltrifluoroborate, chemoselective reactions were performed for each of the substrates (Scheme 3). In some cases, the product mixtures could not be purified by column chromatography and relied instead on a trituration-based purification. As such, full consumption of the KAT-containing starting material is vital, and these were kept as limiting reagents throughout. Refluxing KAT **8** and reagent **15** for 16 h gave exclusively the Wittig product **16**, which was isolated by trituration in 77% yield with an *E*/*Z*- isomer ratio of 36:1.

Scheme 3. Chemoselective Transformation of Reagents Bearing Acyltrifluoroborate Moieties



O-Alkylation of the phenol KAT **12** with coumarin **17**[11] was performed in acetone in the presence of a slight excess of Cs_2CO_3 to give **18** in good yield. Substitution of **19** with **12** gave the protected hydroxylamine-KAT **20** in good yield, demonstrating that that substitution works equally well on aliphatic mesylates.

Encouraged by these findings, we attempted to modify larger molecules using the bifunctional KATs and test their utility in sequential coupling reactions (Scheme 4a). Alkyne **13** could be joined with fluorescein azide **21**,[12] followed by *in situ* KAT ligation to give amide **23**.

The same approach was repeated with azide **14** and alkyne-rhodamine **24**[13] to form compound **25**. These ligations were performed *in situ*, as purification and isolation of the KAT-CuAAC products proved challenging. Interestingly, the 1,3-dipolar cycloaddition using copper(II) sulfate and sodium ascorbate as the Cu (I) source failed to give the desired product, yet the reaction proceeded to completion in the presence of an equimolar amount of copper(I) iodide and an

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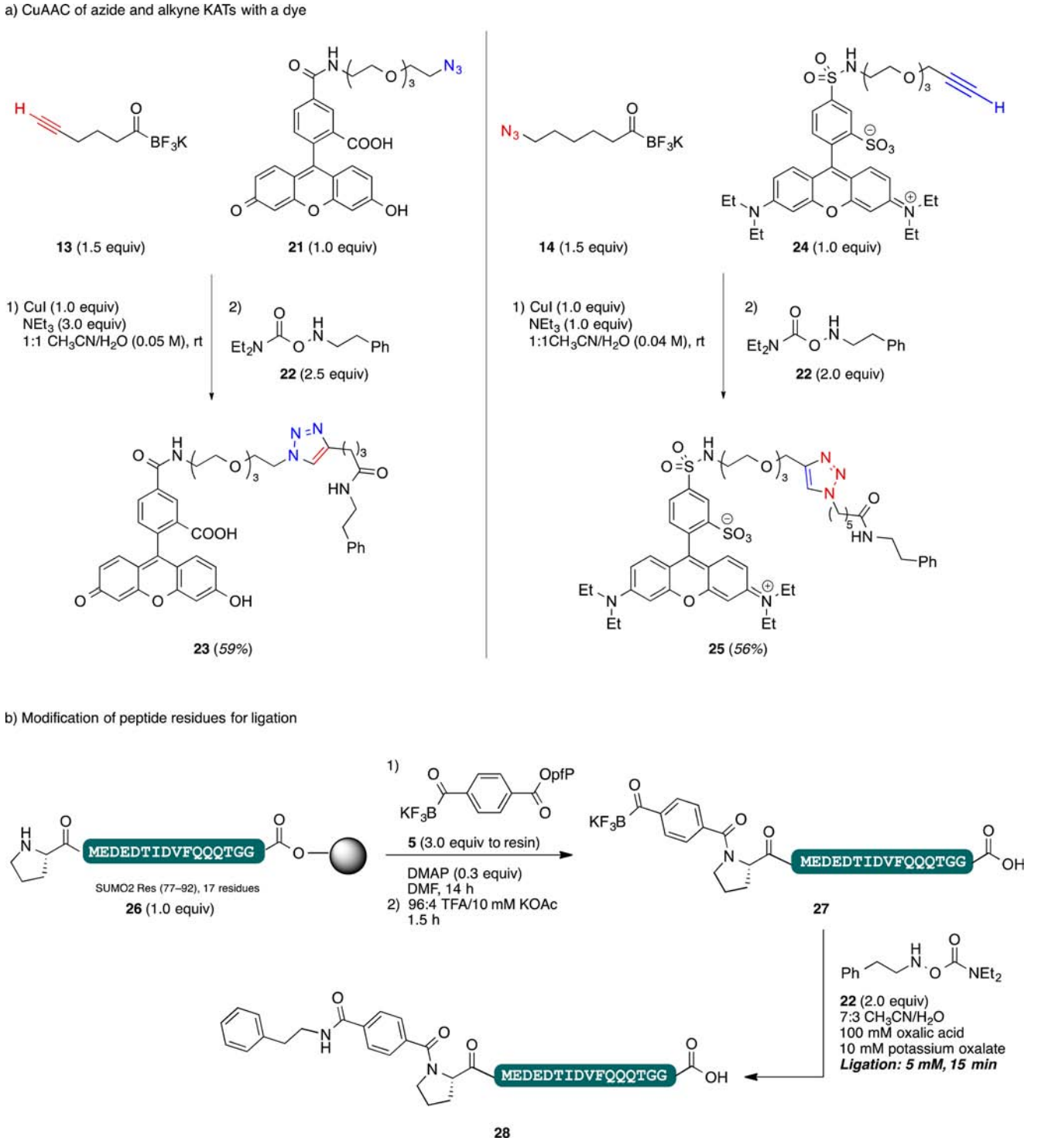
Scheme 4. Orthogonal Reaction of Potassium Acyltrifluoroborates



amine base. Adapting the protocol described above allowed cycloaddition and KAT ligation with *O*-carbamoyl hydroxylamine **22**[4] to form the corresponding amide adduct in a one-pot fashion. Purification by preparative HPLC afforded compound **23** in 59% and **25** in 56% isolated yield.

To demonstrate that this strategy is also applicable to the modification of peptides we coupled KAT **5** to a resin-bound 17-residue segment of SUMO2-**26** in the presence of DMAP in DMF (Scheme 4b). Cleavage from the resin using TFA with 10 mM potassium acetate and subsequent ligation with a hydroxylamine led to the isolation of the desired modified peptide after purification by preparative HPLC. To avoid reduction of the KAT group by hydride-based scavengers, we found that cleavage with TFA alone or with electron-rich aromatic scavengers is preferred. The addition of a potassium salt, in this case KOAc, was also beneficial for the stability of the product. This was particularly true during preparative HPLC, where we found it essential to use a buffer system containing potassium for isolation of KATs. This straightforward protocol

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allows for incorporation of KATs into peptides and subsequent labeling or conjugation.

In summary, we have developed convenient syntheses of bifunctional KAT reagents and demonstrated that the KAT groups are stable to a wide range of transformations. We also improved the benzotriazole route to aliphatic KATs, which complements our established reagent-based route for the synthesis of neutral and electron-deficient aromatic KATs. Six new bifunctional KAT reagents were developed by these routes, allowing for the incorporation of the KAT moiety into more complex molecules, including peptides. These reagents can be prepared on a multigram scale in a few synthetic steps from readily available starting materials. As showcased by their use in the incorporation of KATs into peptides and dyes, these new bifunctional molecules will allow for expansion of the current library of potassium acyltrifluoroborates, a class of molecules with great potential for bioconjugation and convergent synthesis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02652.

Experimental procedures and characterization of bifunctional KAT reagents (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) For selected examples, see: (a) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. *Science* **1994**, *266*, 776−779. (b) Dawson, P. E.; Kent, S. B. *Annu. Rev. Biochem.* **2000**, *69*, 923−960. Hackenberger, C. P. R.; Schwarzer, D. *Angew. Chem., Int. Ed.* **2008**, *47*, 10030−10074. Prescher, J. A.; Bertozzi, C. R. *Nat. Chem. Biol.* **2005**, *1*, 13−21. (c) Bode, J. W.; Fox, R. M.; Baucom, K. D. *Angew. Chem.* **2006**, *118*, 1270−1274; *Angew. Chem., Int. Ed.* **2006**, *45*, 1248−1252.

(2) For selected examples on CuAAC, see: (a) Meldal, M.; Tornoe, C. W. *Chem. Rev.* **2008**, *108*, 2952−3015. (b) Golas, P. L.; Matyjaszewski, K. *Chem. Soc. Rev.* **2010**, *39*, 1338−1354. (c) Dörner, S.; Westermann, B. *Chem. Commun.* **2005**, 2852−2854. (d) Ornelas, C.; Ruiz, J.; Cloutet, E.; Alves, S.; Astruc, D. *Angew. Chem., Int. Ed.* **2007**, *46*, 872−877. (e) Uttamapinant, C.; Tangpeerachaikul, A.; Grecian, S.; Clarke, S.; Singh, U.; Slade, P.; Gee, K. R.; Ting, A. Y. *Angew. Chem., Int. Ed.* **2012**, *51*, 5852−5856. For selected examples on thio-Michael addition, see: (f) Kuroki, Y.; Lett, R. *Tetrahedron Lett.* **1984**, *25*, 197−200. (g) Chan, J. W.; Yu, B.; Hoyle, C. E.; Lowe, A. B. *Chem. Commun.* **2008**, *40*, 4959−4961. (h) Zhang, Q.; Li, G. Z.; Becer, C. R.; Haddleton, D. M. *Chem. Commun.* **2012**, *48*, 8063−8065. (i) Koehler, K. C.; Anseth, K. S.; Bowman, C. N. *Biomacromolecules* **2013**, *14*, 538−547. (j) Gobbo, P.; Biesinger, M. C.; Workentin, M. S. *Chem. Commun.* **2013**, *49*, 2831−2833.

(3) Dumas, A. M.; Molander, G. A.; Bode, J. W. *Angew. Chem., Int. Ed.* **2012**, *51*, 5683−5686.

(4) Noda, H.; Erős, G.; Bode, J. W. *J. Am. Chem. Soc.* **2014**, *136*, 5611−5614.

(5) Saito, F.; Noda, H.; Bode, J. W. *ACS Chem. Biol.* **2015**, *10*, 1026−1033.

(6) Mazunin, D.; Bode, J. W. Manuscript submitted.

(7) Erős, G.; Kushida, Y.; Bode, J. W. *Angew. Chem., Int. Ed.* **2014**, *53*, 7604−7607.

(8) Katritzky, A. R.; Lang, H.; Wang, Z.; Zhang, Z.; Song, H. *J. Org. Chem.* **1995**, *60*, 7619−7624.

(9) Dumas, A. M.; Bode, J. W. *Org. Lett.* **2012**, *14*, 2138−2141.

(10) Katritzky, A. R.; Lang, H.; Wang, Z.; Lie, Z. *J. Org. Chem.* **1996**, *61*, 7551−7557.

(11) Seven, I.; Weinrich, T.; Gränz, M.; Grünewald, C.; Brüß, S.; Krstic, I.; Prisner, T. F.; Heckel, A.; Göbel, M. W. *Eur. J. Org. Chem.* **2014**, *2014*, 4037−4043.

(12) Serdjukow, S.; Kink, F.; Steigenberger, B.; Tomas-Gamasa, M.; Carell, T. *Chem. Commun.* **2014**, *50*, 1861−1863.

(13) Goswami, L. N.; Khan, A. A.; Jalisatgi, S. S.; Hawthorne, M. F. *Chem. Commun.* **2014**, *50*, 5793−5795.

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