Organic LETTERS

Letter

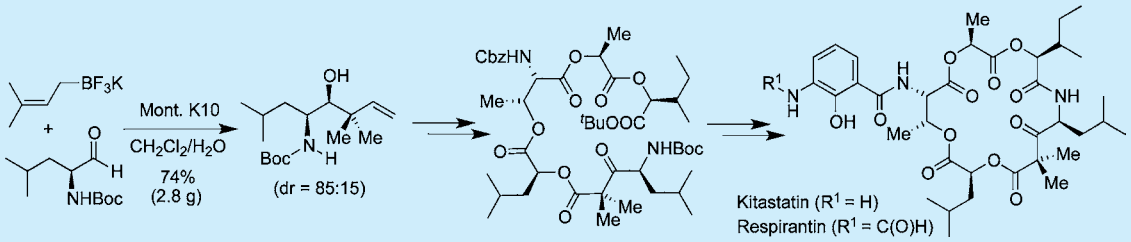
pubs.acs.org/OrgLett

An Organotrifluoroborate-Based Convergent Total Synthesis of the Potent Cancer Cell Growth Inhibitory Depsipeptides Kitastatin and Respirantin

Ramsay E. Beveridge and Robert A. Batey*

Davenport Research Laboratories, Department of Chemistry, University of Toronto, 80 St. George Street, Toronto ON, Canada, M5S 3H6

Supporting Information



ABSTRACT: The total syntheses of the highly cytotoxic neo-antimycin macrocyclic depsipeptide natural products kitastatin and respirantin have been accomplished in a convergent manner using MNBA promoted esterifications and an efficient C- and N-terminus bis-deprotection/HATU promoted macrolactamization. The first examples of using a prenyltrifluoroborate reagent in additions to carbonyl groups are disclosed including a diastereoselective multigram scale montmorillonite K10 catalyzed prenylation of *N*-Boc-L-leucinal to install the structurally unique gem-dimethyl-*β*-keto-ester fragment.

Macrocyclic depsipeptides constitute an important class of synthetic targets due to their structural complexity and diverse range of biological activities.[1] Notable members of this class are the well-known antimycin family of depsipeptide natural products (AAs, Figure 1), which have attracted considerable

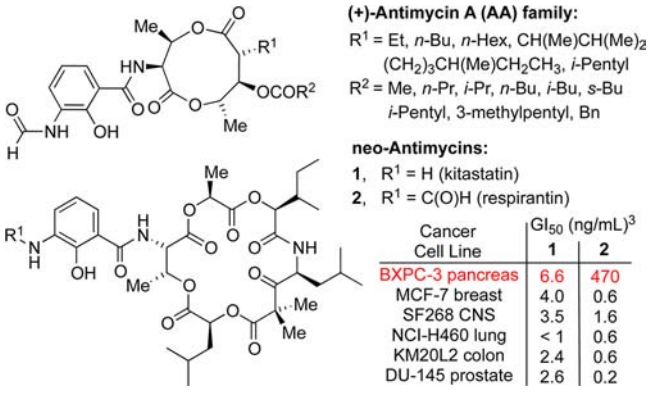


Figure 1. Antimycin A natural product family and the cytotoxic neo-antimycin cyclic depsipeptides kitastatin **1** and respirantin **2**.

synthetic attention due to their antifungal and antitumoral effects.[2] In addition to these, Pettit and co-workers reported in 2007 the isolation of two related neo-antimycin[3] depsipeptide macrocycles (kitastatin **1** and respirantin **2**, Figure 1) with potent nanogram cancer cell growth inhibitory activity.[4] Structurally, kitastatin and respirantin are related to the antimycin family through a shared threonine connected 3-amino-salicylic acid group, but are differentiated by their larger macrocycle component which contains an unusually high number of ester linkages and a rare gem-dimethyl-*β*-keto-ester fragment. Kitastatin **1** is particularly interesting since it appears to have preferential cytotoxic activity against pancreatic tumor cells and is one of only two members[5] of the antimycin/neo-antimycin class to contain a des-formamide amino-salicylic acid fragment. The potent antitumor activity of **1** and **2** combined with the potential selectivity of kitastatin for growth inhibition of high-mortality pancreatic tumor cells makes these compounds attractive potential cancer treatment candidates. Unfortunately, further preclinical testing on compounds **1** and **2**, including elucidation of their tumor-killing mechanism, is limited by the small quantities that can be obtained via extraction from the natural bacterial source (e.g., 380 L of fermentation broth was required to obtain 2.6 mg of kitastatin).[4]

This supply issue as well as the synthetic challenge presented by this 18-membered ester rich macrocycle have prompted us to target these compounds for synthesis. A previous linear total synthesis of respirantin **2**[6] by Pettit and co-workers involving a low yielding (28%) late-stage *β*-keto-ester dialkylation route to the gem-dimethyl-*β*-keto-ester group inspired us to examine alternative modes to construct this fragment as well as the key macrocycle. In this report, we outline a total synthesis of **1** and **2** utilizing a convergent macrolactamization to secure the core macrocycle and demonstrate a prenyltrifluoroborate aldehyde

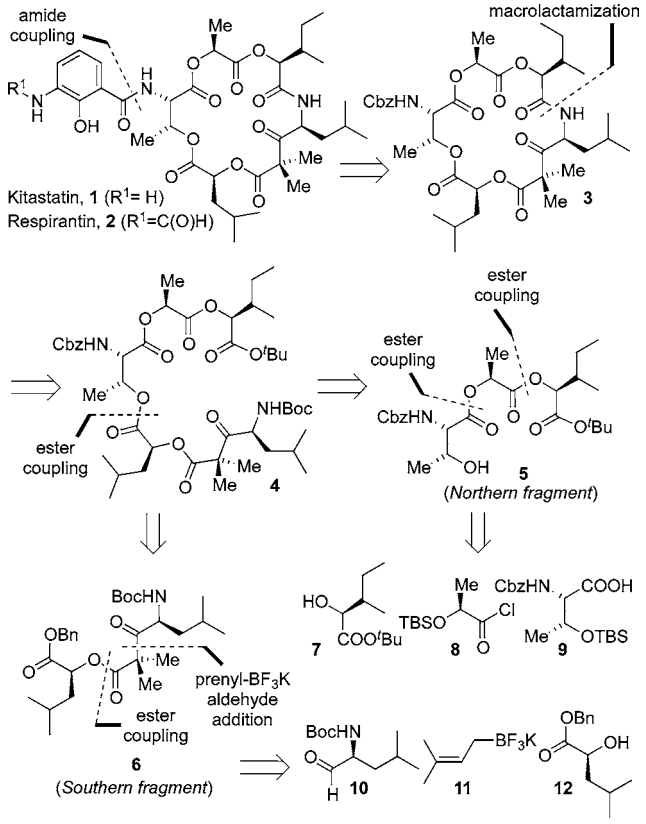
Received: February 17, 2014
Published: April 14, 2014

   dx.doi.org/10.1021/ol500484f | *Org. Lett.* 2014, 16, 2322–2325

addition route to the gem-dimethyl-β-keto-ester. In addition to being the first documented total synthesis of kitastatin[7] and the first carbonyl prenylation using a prenyltrifluoroborate reagent, our work represents a nonlinear and scaleable approach to these medicinally interesting depsipeptides.

Our overall synthetic strategy to these natural products was designed around a ring disconnection through the only amide bond in the depsipeptide structure (Scheme 1). To streamline

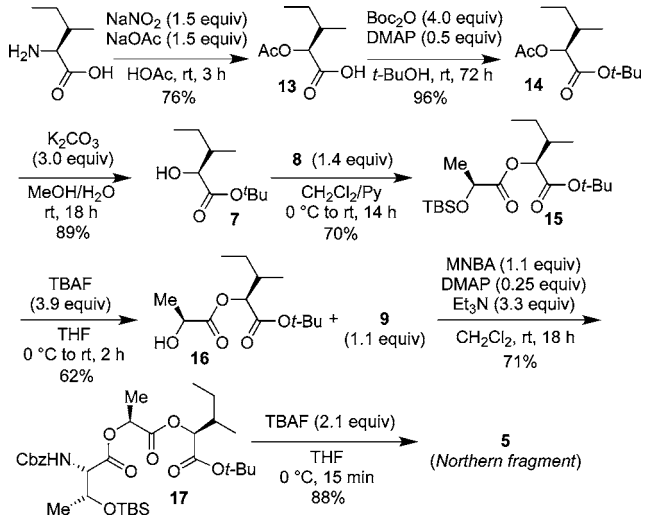
Scheme 1. Synthetic Strategy to Compounds 1 and 2



late-stage deprotections, the linear cyclization precursor **4** was designed to allow for possible simultaneous removal of both the C- and N-termini protecting groups using simple acidic conditions prior to macrolactamization. A convergent approach to **4** was envisaged through disconnection of the central ester linkage to give northern and southern fragments **5** and **6** as synthetic targets. The resulting key challenges in addressing the synthesis of **5** and **6** was the development of a general protecting group strategy and the synthesis of the gem-dimethyl-β-keto-ester functionality in **6**. For example, an early attempt to prepare **6** in the ketone oxidation state present in the natural products via an enolate addition to an acyl imidazole of L-leucine resulted in significant decarboxylation during ester deprotection.[8] To overcome this decarboxylation issue, an alternative strategy was envisaged, in which the ketone of **6** would be protected in a reduced alcohol oxidation state and generated after ester bond formation. One such approach could utilize a prenylation of aldehyde **10**, followed by an oxidative cleavage of the resultant terminal alkene to a gem-dimethyl-β-hydroxy carboxylic acid, which should ultimately provide access to the gem-dimethyl-β-keto-ester functionality of **6**. Conditions for the prenylation of **10** would need to be mild enough to avoid potential epimerization of the sensititve chiral α-aminoaldehyde[9] and occur with acceptable levels of diastereoselectivity. Recent successful applications of potassium allyl and crotyltrifluoroborate additions to carbonyl groups[10] suggested that the related prenyltrifluoroborate reagent **11** might achieve such an addition to **10**. More generally, we were interested in examining whether reagent **11**, which has not previously been explored as a carbonyl addition reagent,[11] would possess the same attractive handling and reactivity characteristics of the related potassium allyl and crotyl trifluoroborate reagents to generate γ-prenylated alcohols via addition to aldehydes.

Synthesis of northern fragment **5** began with a large-scale acetate ion nucleophile diazotization reaction of L-isoleucine to generate α-acetoxy-acid compound **13** (Scheme 2). Protection of

Scheme 2. Synthesis of the Northern Fragment 5

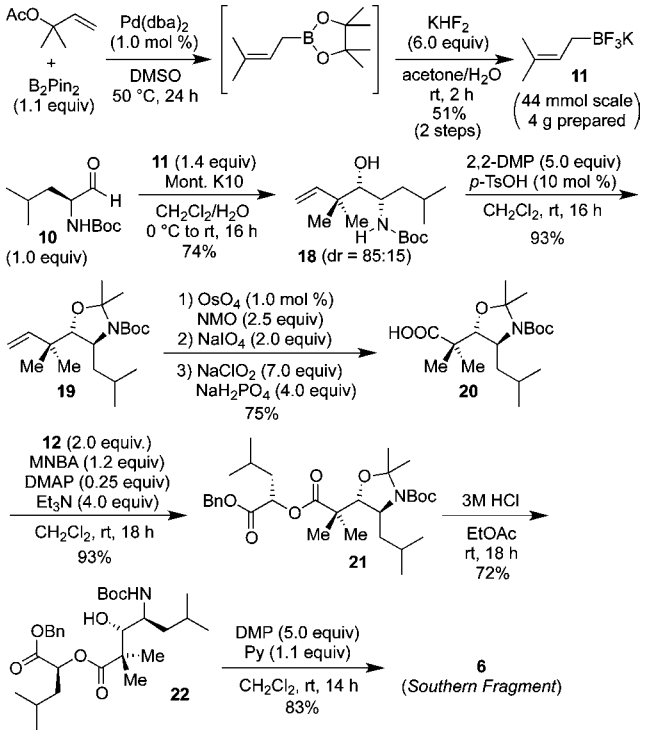


13 as the *tert*-butyl ester using Boc-anhydride and DMAP,[12] followed by acetate deprotection, furnished α-hydroxy ester **7**. Reaction of **7** with acid chloride **8** provided ester **15**, which after deprotection with TBAF was coupled with protected threonine **9** under MNBA[13] conditions to provide O-TBS protected western fragment **17**. TBAF induced desilylation provided the desired northern fragment **5** in 18% overall yield in 7 steps from L-isoleucine.

To examine the planned aldehyde prenyl-addition strategy, synthesis of the southern fragment **6** was initiated by preparing mulitple grams of prenyltrifluoroborate reagent **11** (Scheme 3). Miyaura's palladium catalyzed B_2pin_2 borylation[14] of 1,1-dimethylallyl acetate followed by KHF_2 treatment of the crude prenylpinacolboronate secured 4 g of reagent **11** as a stable, storable white solid. Reaction of **11** with *N*-Boc-L-leucinal[15] **10** was then evaluated under conditions previously reported for potassium allyl- and crotyltrifluoroborate additions to carbonyl groups. Use of $BF_3{\cdot}OEt_2$[10a−e] proved too acidic, and product formation was not observed due to possible *N*-Boc carbamate deprotection. In contrast, phase-transfer catalyzed conditions[10f,g] facilitated prenylation of this α-chiral aldehyde in a 5:1 ratio of diastereomers (crude ^{1}H NMR) and resulted in a 59% isolated yield of the major isomer **18**. Optimal conditions were established on multigram scale using a convenient montmorillonite K10 catalyzed protocol[10h] to provide the major *syn*[16] amino-alcohol isomer **18** in 74% isolated yield. The observed 85:15 reaction diastereoselectivity (established by ^{1}H NMR of the crude reaction mixture) is consistent with prenyl addition occurring anti to the isobutyl group in a closed transition state

dx.doi.org/10.1021/ol500484f | Org. Lett. 2014, 16, 2322−2325

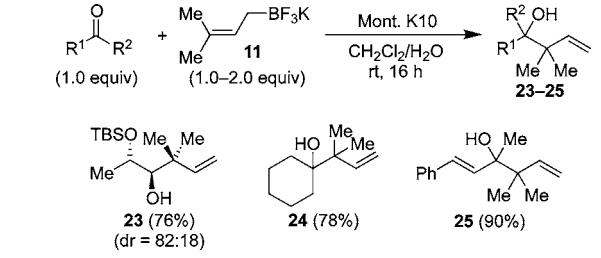
Scheme 3. Synthesis of the Southern Fragment 6



with the aldehyde in a Felkin–Ahn conformation. These results represent the first example of an aldehyde prenylation using a prenyltrifluoroborate reagent, which, to the best of our knowledge, is also the only example of a prenylboron reagent addition to a protected α-aminoaldehyde.[17] Notably, this aldehyde prenylation strategy successfully circumvented the decarboxylation issue via acetonide protection of amino-alcohol **18** followed by oxidative olefin cleavage to give stable carboxylic acid **20**. MNBA promoted esterification of **20** with **12**, followed by acetonide deprotection and oxidation of the resistant neopentylic alcohol of **22** with an excess of Dess-Martin periodinane, provided the target southern fragment ketone **6** (8 steps, 29% yield from *N*-Boc-L-leucinal).

In addition, preliminary results with other carbonyl compounds show that prenyltrifluoroborate **11** may prove useful as a general reagent for carbonyl prenylation (Scheme 4). In the case

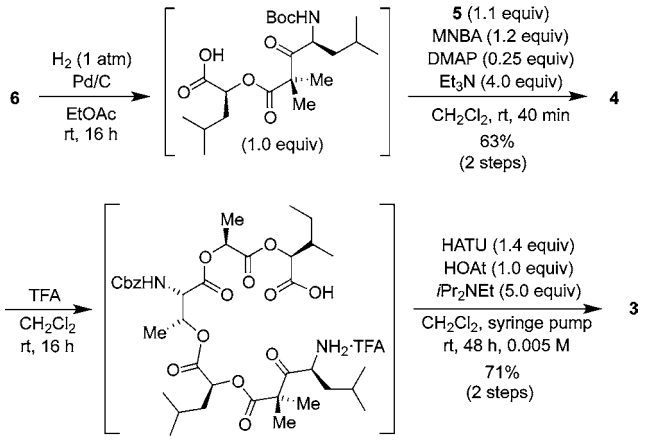
Scheme 4. Montmorillomite K10 Catalyzed Carbonyl γ-Prenylation with Prenyltrifluoroborate 11



of compound **23**, the *anti*-isomer is presumed to be the major diastereomer by analogy with previous allyltrifluoroborate additions to this aldehyde.[10b] Overall, reagent **11** has desirable preparation, stability, storage, and handling properties compared to alternative alkyl prenylboron derivatives[18] and readily reacts with aldehydes and ketones under mild conditions versus the elevated temperature and long reaction times (3–8 days)[19] typically required for prenylpinacolboronate reagent addition.

With the southern fragment now in hand, **6** was deprotected by hydrogenolysis and coupled with northern fragment **5** under MNBA conditions to provide linear cyclization precursor **4** in 63% yield for two steps (Scheme 5). Deprotection of both the

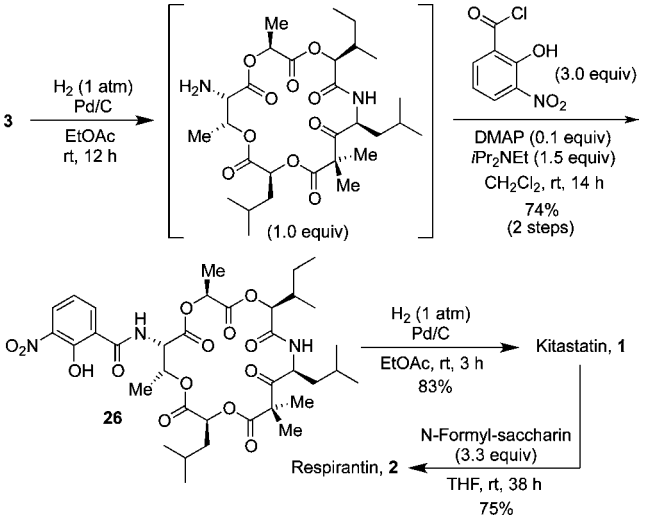
Scheme 5. Coupling of the Northern and Southern Fragments 5 and 6 and Macrolactamization to the Cyclodepsipeptide Core



Boc-carbamate and *t*-Bu-ester of compound **4** was accomplished using TFA and the resulting crude deprotected TFA salt used directly in the macrocyclization. Under optimized conditions, the key macrolactamization was performed by dual syringe pump addition of crude deprotected TFA salt and Hünig's base to a stirring room temperature solution of HATU and HOAt with an overall final concentration of 0.005 M. The core macrocyclic depsipeptide **3** was thus obtained in 71% yield over two steps utilizing a convenient C- and N-terminus bis-deprotection strategy.

With the macrocycle secured, our attention turned to attaching the amino-salicylic acid component to complete the synthesis of **1** and **2** in a step-minimized manner (Scheme 6). Deprotection of **3** by hydrogenolysis and reaction of the resulting amino-macrocycle intermediate with the acid chloride of commercially

Scheme 6. Completion of the Synthesis of 1 and 2



dx.doi.org/10.1021/ol500484f | Org. Lett. 2014, 16, 2322–2325

available 3-nitro-salicylic acid gave nitro-aryl compound **26** in 74% yield over two steps. Conveniently, **26** serves as a single intermediate for completing the synthesis of both natural product targets enabling an alternative approach to install amino-salicylic acid components characteristic of this natural product class. Hydrogenation of the nitro-group of **23** afforded **1** in 83% yield, thus completing the first total synthesis of the cytotoxic natural product kitastatin **1** in 7.9% overall yield in 12 linear steps and only 9 chromatographic purifications from *N*-Boc-L-leucinal. Finally, reaction of **1** under neutral conditions with the recently disclosed *N*-formylsaccharin reagent[20] converted the aniline of kitastatin into the target formamide of **2** to complete the total synthesis of respirantin in 5.9% yield over 13 linear steps and 10 chromatographic purifications.

In conclusion, we have completed the total syntheses of the potent depsipeptide neo-antimycin cytotoxic agents kitastatin **1** and respirantin **2** in a convergent and scaleable manner. Importantly, the application of a prenylation strategy solved the issues associated with decarboxylation of gem-dimethyl-β-keto-acids and validated the use of prenyltrifluoroborate aldehyde additions to α-chiral gem-dimethyl-β-keto-ester units and related prenyl-alcohol derivatives. Work is currently underway to use this synthetic process to generate analogues of both the amino-salicylic acid and macrocycle components and to evaluate the clinical potential of this class of compounds.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental details and characterization data for all compounds including ^{1}H and ^{13}C NMR, and comparison of spectral data of **1** and **2** with reported data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: rbatey@chem.utoronto.ca.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank the University of Toronto and the Natural Science and Engineering Research Council of Canada (NSERC) for financial support. R.E.B. is grateful to the University of Toronto for an Edwin Walter Warren Scholarship and to the Ontario Ministry of Education for an Ontario Graduate Scholarship. We thank Dr. Matthew Forbes (Toronto) for MS analysis and Dr. Alan Lough (Toronto) for X-ray structure determination of **18**. Frontier Scientific Inc. is gratefully acknowledged for their generous donation of B_2Pin_2.

■ REFERENCES

(1) For a review of the total synthesis of selected antimicrobial and antitumor cyclic depsipeptides, see: Li, W.; Schlecker, A.; Ma, D. *Chem. Commun.* **2010**, *46*, 5403−5420.

(2) Antimycin A synthesis reviews: (a) Kiyota, H. *Top. Heterocycl Chem.* **2006**, *6*, 181−214. (b) Yang, Y.-Q.; Wu, Y. *Org. Prep. Proced. Int.* **2007**, *39*, 135−152. (c) A recent asymmetric total synthesis of 14 antimycin A family members: Inai, M.; Nishi, T.; Tanaka, A.; Kaku, H.; Horikawa, M.; Tsunoda, T. *Eur. J. Org. Chem.* **2011**, 2719−2729.

(3) In addition to kitastatin and respirantin, there are six other known neo-antimycins: (a) JBIR-06: Ueda, J.-Y.; Nagai, A.; Izumikawa, M.; Chijiwa, S.; Takagi, M.; Shin-ya, K. *J. Antibiot.* **2008**, *61*, 241−244. (b) JBIR-04, JBIR-05: Izumikawa, M.; Ueda, J.-Y.; Chijiwa, S.; Takagi, M.; Shin-ya, K. *J. Antibiot.* **2007**, *60*, 640−644. (c) Prunustatin A and SW-163A: Umeda, Y.; Furihata, K.; Sakuda, S.; Nagasawa, H.; Ishigami, K.; Watanabe, H.; Izumikawa, M.; Takagi, M.; Doi, T.; Nakao, Y.; Shin-ya, K. *Org. Lett.* **2007**, *9*, 4239−4242 and references therein. (d) Neo-antimycin: Cagliote, L.; Misiti, D.; Selva, A.; Arcamone, F.; Cassinelli, G. *Tetrahedron* **1969**, *25*, 2193−2221.

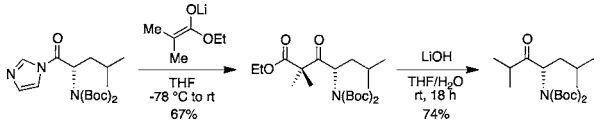
(4) Pettit, G. R.; Tan, R.; Pettit, R. K.; Smith, T. H.; Feng, S.; Doubek, D. L.; Richert, L.; Hamblin, J.; Weber, C.; Chapuis, J.-C. *J. Nat. Prod.* **2007**, *70*, 1069−1072.

(5) The neo-antimycin natural product JBIR-05 also contains a des-formamide 3-amino-salicylic acid unit. See ref 3b.

(6) Pettit, G. R.; Smith, T. H.; Feng, S.; Knight, J. C.; Tan, R.; Pettit, R. K.; Hinrichs, P. A. *J. Nat. Prod.* **2007**, *70*, 1073−1083.

(7) The synthesis of kitastatin **1** is claimed in a patent; however, no experimental details or yields were included regarding its preparation. Pettit, G. R.; Smith, T. H.; Feng, S. U.S. Pat. Appl. 065991, 2008.

(8) Decarboxylation observed during a preliminary attempt to access the southern fragment in the ketone oxidation state:



(9) Configurational stability study: Rittle, K. E.; Homnich, C. F.; Ponticello, G. S.; Evans, B. E. *J. Org. Chem.* **1982**, *47*, 3016−3018.

(10) $BF_3{\cdot}OEt_2$ conditions: (a) Batey, R. A.; Thadani, A. N.; Smil, D. V. *Tetrahedron Lett.* **1999**, *40*, 4289−4292. (b) Batey, R. A.; Thadani, A. N.; Smil, D. V. *Synthesis* **2000**, 990−998. (c) Li, S.-W.; Batey, R. A. *Chem. Commun.* **2004**, 1382−1383. (d) Nowrouzi, F.; Batey, R. A. *Angew. Chem., Int. Ed.* **2013**, *52*, 892−895. (e) Ramadhar, T. R.; Bansagi, J.; Batey, R. A. *J. Org. Chem.* **2013**, *78*, 10362−10368. Phase-transfer catalyzed conditions: (f) Thadani, A. N.; Batey, R. A. *Org. Lett.* **2002**, *4*, 3827−3830. (g) Thadani, A. N.; Batey, R. A. *Tetrahedron Lett.* **2003**, *44*, 8051−8055. Montmorillonite K10 catalyzed conditions: (h) Nowrouzi, F.; Thadani, A. N.; Batey, R. A. *Org. Lett.* **2009**, *11*, 2631−2634. Indium catalyzed conditions: (i) Nowrouzi, F.; Janetzko, J.; Batey, R. A. *Org. Lett.* **2010**, *12*, 5490−5493.

(11) The only related example of potassium prenyltrifluoroborate carbonyl-like reactivity is an interesting Rh-catalyzed enantioselective reaction with cyclic sulfonyl-imines; see: Luo, Y.; Hepburn, H. B.; Chotsaeng, N.; Lam, H. W. *Angew. Chem., Int. Ed.* **2012**, *51*, 8309−8313.

(12) Takeda, K.; Akiyama, A.; Nakamura, H.; Takizawa, S.-I.; Mizuno, Y.; Takayanagi, H.; Harigaya, Y. *Synthesis* **1994**, 1063−1066.

(13) In the course of this work we have examined numerous coupling conditions for the various ester formations and have found MNBA ("Shiina's Reagent") to be generally optimal: (a) Shiina, I.; Miyao, R. *Heterocycles* **2008**, *76*, 1313−1328. (b) Shiina, I.; Kubota, M.; Oshiumi, H.; Hashizume, M. *J. Org. Chem.* **2004**, *69*, 1822−1830.

(14) Ishiyama, T.; Ahiko, T.-A.; Miyaura, N. *Tetrahedron Lett.* **1996**, *37*, 6889−6892.

(15) Used within 3 h of preparation according to: Rich, D. H.; Sun, E. T.; Boparai, A. S. *J. Org. Chem.* **1978**, *43*, 3624−3626.

(16) The major isomer **18** was determined to be *syn* by X-ray crystallography; see Supporting Information.

(17) Prenylation of α-aminoaldehydes: (a) Rubsam, F.; Seck, S.; Giannis, A. *Tetrahedron* **1997**, *53*, 2823−2834. (b) Hanessian, S.; Park, H.; Yang, R.-Y. *Synlett* **1997**, 353−354.

(18) (a) Triprenylborane: Bubnov, Y. N.; Zhun, I. V.; Klimkina, E. V.; Ignatenko, A. V.; Starikova, Z. A. *Eur. J. Org. Chem.* **2000**, 3323−3327. (b) Prenyl-9-BBN: Kramer, G. W.; Brown, H. C. *J. Organomet. Chem.* **1977**, *132*, 9−27.

(19) (a) Breitfelder, S.; Schlapbach, A.; Hoffmann, R. W. *Synthesis* **1998**, 468−478. (b) Hoffmann, R. W.; Schlapbach, A. *Tetrahedron* **1992**, *48*, 1959−1968.

(20) Cochet, T.; Bellosta, V.; Greiner, A.; Roche, D.; Cossy, J. *Synlett* **2011**, 1920−1922.