Organic LETTERS

Letter

pubs.acs.org/OrgLett

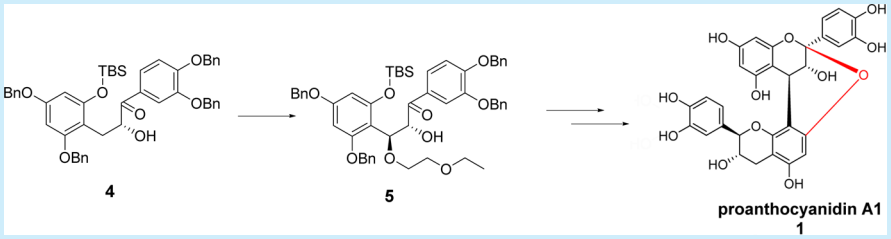
Total Synthesis of Proanthocyanidin A1, A2, and Their Stereoisomers

Pradeep K. Sharma,*[,†,§] Leo J. Romanczyk, Jr.,[‡] Leelakrishna Kondaveti,[†] Bollu Reddy,[†] Jeeva Arumugasamy,[†] Richard Lombardy,[†] Yanni Gou,[†] and Hagen Schroeter[‡]

[†]Chemical Process Research & Development, Johnson Matthey Pharmaceutical Materials & Pharma Services, Devens, Massachusetts 01434, United States

[‡]MARS Incorporated, 6885 Elm Street, McLean, Virginia 22101, United States

Supporting Information



ABSTRACT: The first novel stereoselective synthesis of naturally occurring A1 (**1**) and A2 proanthocyanidins (**2**) has been achieved. The key synthetic steps involved (a) the formation of a coupled product (**13** or **14**) between an open chain C-ring C-4 hydroxyethoxy analogue of either (+)-catechin or (−)-epicatechin with 5,7,3′,3′-tetra-*O*-benzyl-(+)-catechin/-(−)-epicatechin in the presence of bentonite clay K-10, (b) removal of benzyl protecting groups under mild catalytic hydrogenation conditions to form the desired A-type compound *in situ* as a mixture of diastereomers via ketal/oxonium ion/carbonium ion formation, and (c) separation of the diasteromers via silica gel column chromatography. The structures of A1 and A2 proanthocyanidins were unequivocally established by analytical comparison to the natural products. Following this methodology, an additional six diastereomers of proanthocyanidins A1 and A2 have been synthesized. A plausible mechanism for the formation of the A-type linkage in proanthocyanidins has been proposed.

Proanthocyanidins are naturally occurring polyphenolic compounds that have diverse biological activities.[1] As shown in Figure 1, the most commonly known A-type

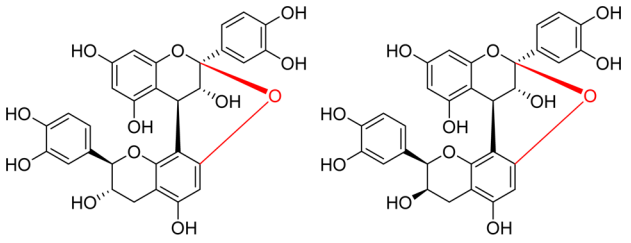


Figure 1. Structures of proanthocyanidins A1 and A2.

proanthocyanidins are where the two units, catechin and/or epicatechin, are connected through the linkages occurring between both C-2 and C-4 of the upper unit and the oxygen at C-7 and positions 6 or 8, respectively, of the lower unit.

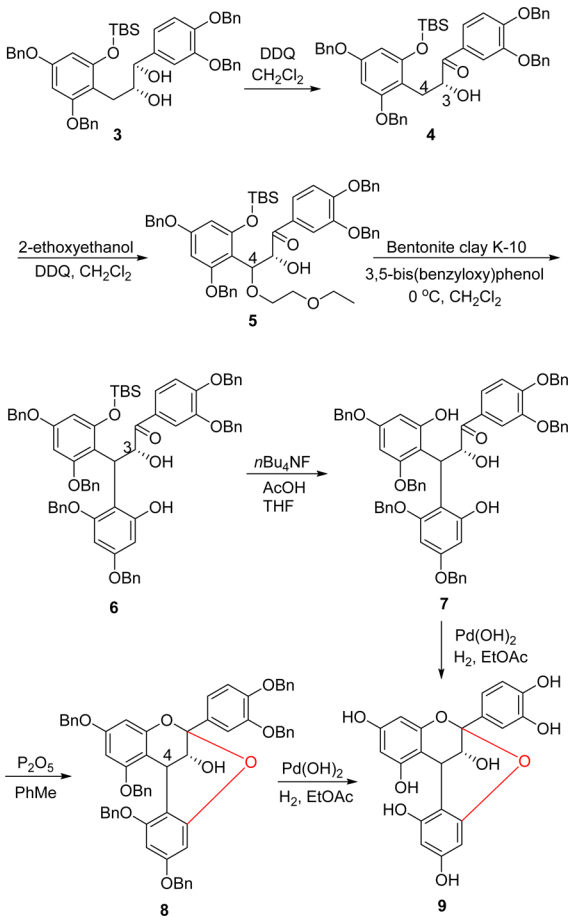
The first identified A-type proanthocyanidin was A2, which was isolated from the shells of the fruits of *Aesculus hippocastanum*.[2] Since the isolation of this compound in 1966, many more A-type proanthocyanidins have been found in plants, including dimers, trimers, tetramers, pentamers, and others.[3−5] In addition to the naturally occurring compounds **1** and **2**, others are expected as the result of stereoisomer combinations of (+)-catechin and (−)-epicatechin. Numerous attempts have been made to synthesize these compounds. This includes the transformation of natural B-type procyanidins to A-types in an alkaline/H_2O_2 system and in neutral conditions with DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals,[6] flavylium perchloride with (+)-catechin in methanol,[7] and by microwave chemistry.[8] Despite these claims, reactions either produce low yields or analogues possessing pyrogallol- type B-rings (prorobinetinidins and prodelphinidins), which are classes of compounds structurally dissimilar to the procyanidins.[9] The conversion of procyanidin B-2 to A-2 by the enzyme laccase has also been reported.[10] This result suggests that, in plants, the transformation of B-type procyanidins to A-types might involve an enzyme catalyzed oxidation reaction rather than a radical driven process as suggested by Kondo et al.[6] It has also been reported that A-types can be converted from the oxidation of their accompanying B-type analogues.[11] There is an additional report[12] claiming the synthesis of A-types, but the resultant compounds were structurally dissimilar to procyanidin based A-types. Thus, the total synthesis of substantive amounts of A-type proanthocyanidins has yet to be achieved. Additionally, the formation and stability of A-type linkages (linkage between C-2

Received: March 4, 2015

DOI: 10.1021/acs.orglett.5b00646
Org. Lett. XXXX, XXX, XXX−XXX

and C-7 via an oxygen atom) has not been thoroughly investigated. Herein, we report a unique and facile method for the synthesis of A-type proanthocyanidins. To understand the challenge that resides in the formation and stability of the A-type linkage, our initial efforts focused on the synthesis of model compound **9** (Scheme 1), which lacked the 2-(3,4-dihydroxyphenyl)-3-hydroxy-dihydropyran fragment from the lower unit of **1** and **2**. We envisaged that the construction of model compound **9** would provide a better understanding about the impact of the hydroxyl groups and A-linkage under the potential reaction conditions for the ultimate target compounds **1** and **2**.

Scheme 1. Synthesis of 9



As depicted in Scheme 1, the selective oxidation of **3**[13] with DDQ in CH_2Cl_2 at rt produced **4**, which was reacted with 2-ethoxyethanol in the presence of DDQ in CH_2Cl_2 at rt to yield **5** as a diastereomeric mixture (α/β, 7/3).[14] The diasteromeric mixture was separated by silica gel column chromatography, and structures were established by ^1H, ^{13}C NMR, and C−H correlation. However, to fulfill the objective, the mixture of diastereomers of compound **5** was used directly without separation. The reaction of **5** with 3,5-bis(benzyloxy)phenol in the presence of Bentonite K-10 in CH_2Cl_2 at 0 °C yielded **6** in 70% yield. The stereochemistry at C-4 linkage of **6** was not determined, but expected to be a mixture of diastereomers.[13] Treatment of **6** with nBu$_4$NF/AcOH in THF generated **7**,[14] which was envisioned to be a key intermediate for the synthesis of the A-type linkage. The reaction of **7** with P_2O_5 in refluxing toluene resulted in the formation of the desired A-type compound **8** in 50% yield,[15] and the structure was established by ^1H, ^{13}C NMR, and C−H correlation. No attempt was made to separate the mixture of diastereomers for **8**. However, the diastereomeric ratio (~7/3) was determined by NMR and HPLC. Debenzylation of **8** under catalytic hydrogenation conditions provided **9**. A proposed mechanism for the transformation of **7** to **9** is presented in Figure 2.

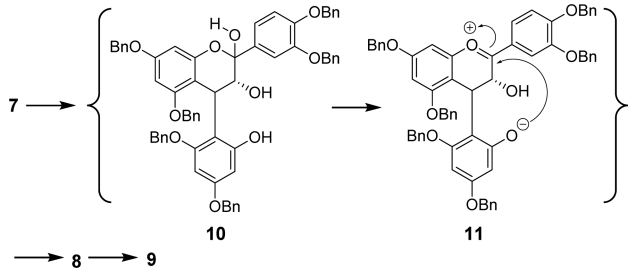


Figure 2. Proposed mechanism for the formation of **9**.

Upon treatment of **7** with P_2O_5 in toluene, the unstable intermediate hemiketal **10** would form. The hemiketal **10** would further lose a mole of water to form the oxonium ion **11**, which would react with phenoxide *in situ* to form the stable ketal **8**. We envisaged that the removal of the benzyl groups of **7** might generate the intermediate(s) that would trigger a cascade to produce the A-type linkage. Hence, **7** was subjected to catalytic hydrogenation conditions ($Pd(OH)_2$/C, EtOAc) using a H_2 balloon at rt for 0.5 h producing **9** in quantitative yield as a mixture of diastereomers. This implied that use of P_2O_5 was not required for the conversion of **7** to **9**, which completely eliminated the preparation of intermediate **8**. These results contrast those of Jacques et al.[16] who reported that the A-type linkage could be cleaved when the molecule was subjected to catalytic hydrogenation conditions at 40−50 psi at room temperature in the presence of 10% Pd/C. The formation of **9** in the absence of P_2O_5 would still involve a potentially similar mechanism. Thus, the reaction sequence for the formation of **9** indicated that the A-type linkage was stable under neutral, mildly acidic, and basic conditions along with the neutral hydrogenation at low H_2 pressure.

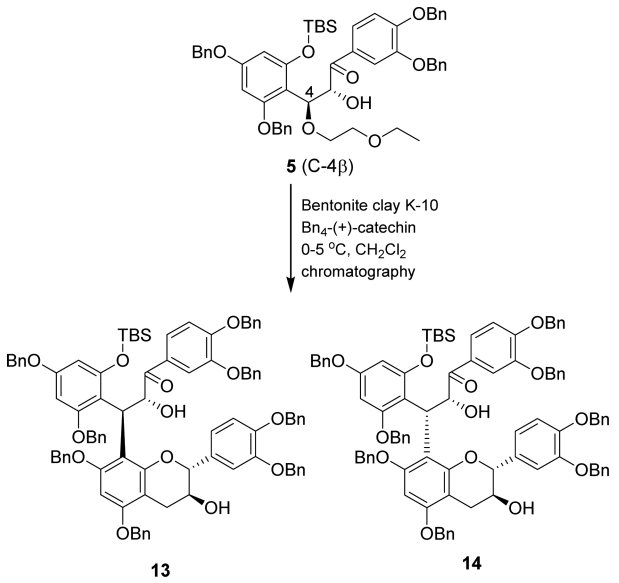
Having established the conditions for the formation of the imperative A-type linkage, the next goal was to use this method for the synthesis of **1** and **2**. The reaction of C-4 β-diastereomer **5** with 5,7,3′,4′-tetra-*O*-benzyl-(+)-catechin [Bn$_4$-(+)-C] **12** in the presence of Bentonite clay K-10 in CH_2Cl_2 at 0 °C provided **13** and **14** after chromatography in 32% and 24% yields, respectively. Thus, the formation of a mixture of **13** and **14** under these conditions was not selective. The stereochemistry of **13** and **14** at C-4 was established from NMR and literature precedence (Scheme 2).[17,18]

Treatment of **13** with nBu$_4$NF in AcOH and THF resulted in the removal of the TBS group, producing **15** in 86% yield after chromatography. Once **15** was available, the stage was set for catalytic hydrogenation where the removal of the benzyl groups and subsequent cyclization would occur for the formation of **1**. Thus, **15** was subjected to catalytic hydrogenation at room temperature using a H_2 balloon for 1 h. The crude product was further purified by silica gel chromatography using EtOAc/CH_2Cl_2/CH_3OH/AcOH (150/150/2/0.3, v/v/v/v) to produce **1** as an off-white solid (Scheme 3).

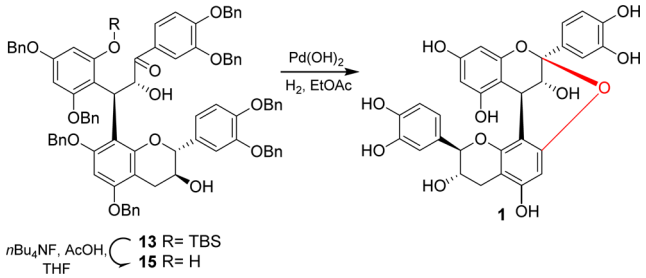
The structure of **1** was confirmed by detailed NMR analysis and comparison to values reported in the literature.[18]

DOI: 10.1021/acs.orglett.5b00646
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 2. Synthesis of 13 and 14

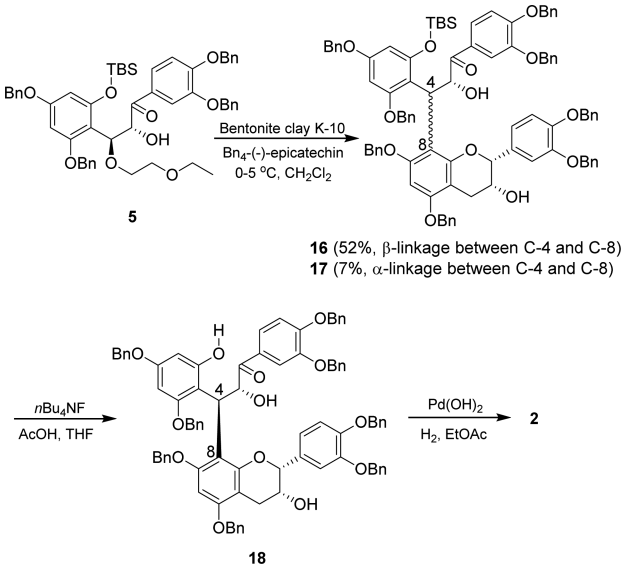


Scheme 3. Synthesis of 1



The synthesis of **2** was achieved as depicted in Scheme 4. The coupling reaction between C-4 *β*-diastereomer **5** with 5, 7, 3′, 4′-tetra-*O*-benzyl-(−)-epicatechin [Bn_4-(−)-EC][20] in the presence of bentonite clay K-10 in CH_2Cl_2 produced a mixture of **16** and **17** in 73−80% yield. Compounds **16** and **17** were isolated in 52% and 7% yield, respectively, after chromatography.

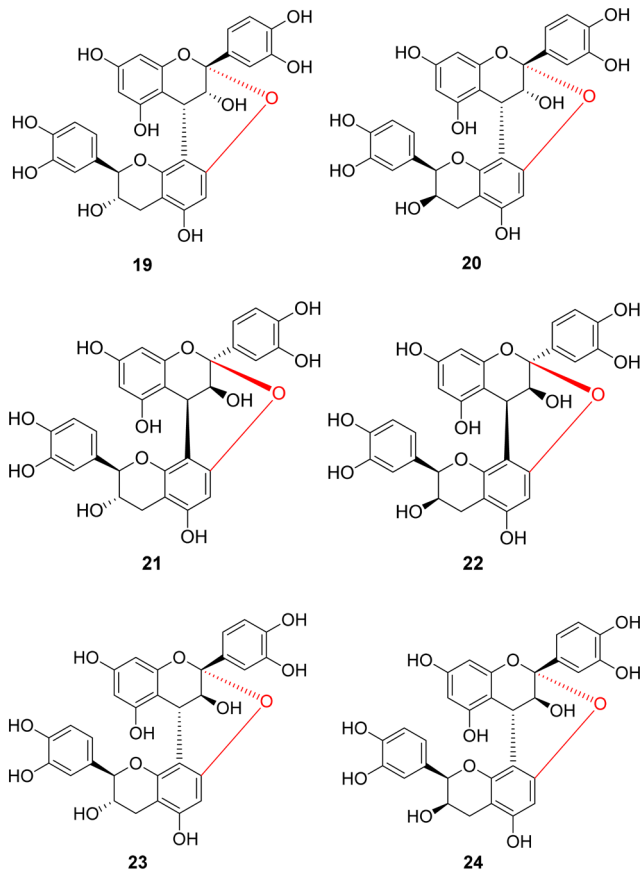
Scheme 4. Synthesis of 2



The reaction of **16** with a mixture of nBu$_4$NF/AcOH in CH_2Cl_2 at 0−5 °C afforded **18** in 85% yield. It was also observed that replacement of nBu$_4$NF/AcOH with HF/pyridine complex in THF also produced **18** in 82% yield. However, attempts to deprotect the TBS group with 1 N hydrochloric acid in THF at rt were unsuccessful due to slow reaction and the formation of additional byproducts, which were difficult to separate. Catalytic hydrogenation of **18** with $Pd(OH)_2$/C in EtOAc at rt produced the desired proanthocyanidin **2** in 93% yield after chromatography. Again, the structure of **2** was confirmed by comparison with an authentic sample[19] and NMR.[20]

After accomplishing the synthesis of the target compounds **1** and **2**, the methodology was further extended to the synthesis of other potential stereoisomers of proanthocyanidins, namely **19**−**24** (Figure 3).[18]

In conclusion, the first total syntheses of naturally occurring A-type proanthocyanidins **1** and **2** have been accomplished. The key step involved the formation of the interflavan linkage between the two flavan-3-ols at C-4 of the upper unit and C-8



Note that the nomenclature of compounds **19** to **24** are as follows.

19: *ent*-Catechin-(2α→O-7, 4α→8)-catechin
20: *ent*-Catechin-(2α→O-7, 4α→8)-epicatechin
21: *ent*-Catechin-(2β→O-7, 4β→8)-catechin
22: *ent*-Catechin-(2β→O-7, 4β→8)-epicatechin
23: *ent*-Epicatechin-(2α→O-7, 4α→8)-catechin
24: *ent*-Epicatechin-(2α→O-7, 4α→8)-catechin

Figure 3. Structures of proanthocyanidins **19** to **24**.

DOI: 10.1021/acs.orglett.5b00646
Org. Lett. XXXX, XXX, XXX−XXX

of the lower unit. The use of benzyl groups as a preferred protecting group provided access for easy removal under neutral catalytic conditions and created the foundation for the formation of the A-type linkage. The reaction conditions that were used in the synthesis suggested that A-type linkage was stable under mildly acidic and neutral conditions as well as at rt under normal handling conditions. The methodology developed was further extended to the stereoselective synthesis of additional isomers of proanthocyanidins, **19**−**24**, which to the best of our knowledge have not yet been either isolated or synthesized. This concise and flexible synthetic methodology presented here offers opportunities to create analogues with A-type linkage and focused libraries to evaluate their biological activity against various therapeutic targets.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures, spectra, and characterization data for all new compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b00646.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: sharmap76@yahoo.com; pradeep.sharma@ariad.com.

Present Address

[§]ARIAD Pharmaceuticals, Inc., 26 Landsdowne Street, Cambridge, MA 02139.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank Drs. Heather Taft and Jurjus Jurayj of Johnson Matthey Pharma Services for their constant feedback and encouragement and the AD group at JMPS for analyzing the samples in a timely manner.

■ REFERENCES

(1) Selected publications: (a) He, F.; Pan, Q.-H.; Shi, Y.; Duan, C.-Q. *Molecules* **2008**, *13*, 3007. (b) Bruyne, T. D.; Pieters, L.; Deelstra, H.; Vlietinck, A. *Biochem. Syst. Ecol.* **1999**, *27*, 445. (c) Haggerman, A. E.; Buttler, C. G. *J. Biol. Chem.* **1981**, *206*, 4494. (d) Kennedy, J. A.; Powell, K. J. *Aust. J. Chem.* **1985**, *38*, 879. (e) Weber, H. A.; Hodges, J. R.; Guthrie, B. M.; O'Brien, D.; Robwaugh, D.; Clark, A. P.; Harris, R. K.; Algaier, L. J. W.; Smith, C. S. *J. Agric. Food Chem.* **2007**, *55*, 148. (f) Scalbert, A. *Photochemistry* **1991**, *30*, 3875. (g) Iijma, K.; Yoshizumi, M.; Ouchi, Y. *Mech. Ageing Dev.* **2002**, *123*, 1033. (h) Buzzini, P.; Turchetti, B.; Ieri, F.; Goretti, M.; Branda, E.; Mulinacci, N.; Romani, A. *Top. Heterocycl. Chem.* **2007**, *10*, 239. (i) Slade, D.; Ferreira, D.; Marais, J. P. *J. Phytochemistry* **2005**, *66*, 2177. (j) Marles, M. A. S.; Ray, H.; Gruber, M. Y. *Phytochemistry* **2003**, *64*, 367. (k) Xie, D.-Y.; Dixon, R. A. *Phytochemistry* **2005**, *66*, 2127.

(2) Mayer, W.; Goll, L.; von Arndt, E. M.; Mannschreck, A. *Tetrahedron Lett.* **1966**, *2*, 429.

(3) Porter, L. J. *Tannins. Methods in Plant Biochemistry* **1989**, *1*, 389.

(4) Porter, L. J. In *Flavans and Proanthocyanidins*; Harbone, J. B., Ed.; The Flavanoids: Advances in Research since 1986; Chapman & Hall: London, 1993; p 23.

(5) Ferreira, D.; Slade, D. *Nat. Prod. Rep.* **2002**, *19*, 517.

(6) (a) Kondo, K.; Kurihara, M.; Fukuhara, K.; Tanaka, T.; Suzuki, T.; Miyata, N. *Chem. Pharm. Bull.* **1987**, *35*, 4717. (b) Kondo, K.; Kurihara, M.; Fukuhara, K.; Tanaka, T.; Suzuki, T.; Miyata, N.; Toyoda, M. *Tetrahedron Lett.* **2000**, *41*, 485.

(7) Pomilio, A. *Liebigs Ann. Chem.* **1977**, 597.

(8) Selenski, C.; Pettus, T. R. R. R. *Tetrahedron* **2006**, 5298.

(9) http://pharmacy.olemiss.edu/biomolecularsciences/team/dr-daneel-ferreira/.

(10) Osman, A. M.; Wong, K. K. Y. *Tetrahedron Lett.* **2007**, *48*, 1163.

(11) Marais, J. P. J.; Ferreira, D. *5th Tannins Conference at the 232nd ACS National Meeting*, San Francisco, CA, September 2006, CELL 104.

(12) Kraus, G. A.; Yuan, Y.; Kempema, A. *Molecules* **2009**, *14*, 807.

(13) (a) Sharma, P. K.; He, M.; Jurayj, J.; Gao, D.-M.; Lombardy, R.; Romanczyk, L. J., Jr.; Schroeter, H. *Molecules* **2010**, *15*, 5595. (b) Mull, E. S.; van Zandt, M.; Golebiowski, A.; Beckett, R. P.; Sharma, P. K.; Schroeter, H. *Tetrahedron Lett.* **2012**, *53*, 1501.

(14) Sharma, P. K.; Kolchinski, A.; Shea, H. A.; Nair, J. J.; Gou, Y.; Romanczyk, L. J., Jr.; Schmitz, H. H. *Org. Process Res. Dev.* **2007**, *11*, 422 and references cited therein.

(15) (a) Sharma, P. K.; He, M.; Romanczyk, L. J., Jr.; Schroeter, H. *J. Labelled Compd. Radiopharm.* **2010**, *53*, 605. (b) von Klaus, W.; Hans, T. *Liebigs Ann. Chem.* **1971**, *743*, 203.

(16) Jacques, D.; Haslam, E.; Bedford, D.; Greatbanks, P. J. *J. Chem. Soc., Perkin Trans. 1* **1974**, 2663.

(17) von Klaus, W.; Theobald, H. *Liebigs Ann. Chem.* **1971**, *743*, 203.

(18) Supporting Information.

(19) An authentic sample of **2** was provided by Masterfoods, a Division of MARS, Incorporated, for comparison.

(20) Tückmantel, W.; Kozikowski, A. P.; Romanczyk, L. J., Jr. *J. Am. Chem. Soc.* **1999**, *121*, 12073.

DOI: 10.1021/acs.orglett.5b00646
Org. Lett. XXXX, XXX, XXX−XXX