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Total Synthesis of *ent*-Pregnanolone Sulfate and Its Biological Investigation at the NMDA Receptor

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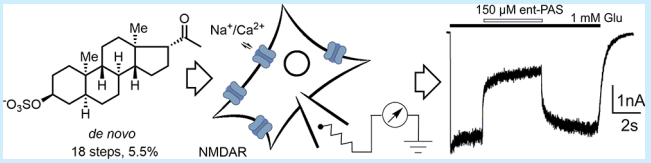
Supporting Information

ABSTRACT: A unique asymmetric total synthesis of the unnatural enantiomer of pregnanolone, as well as a study of its biological activity at the NMDA receptor, is reported. The asymmetry is introduced by a highly atom-economic organocatalytic Robinson annulation. A new method for the construction of the cyclopentane D-ring consisting of Cu^{I}-catalyzed conjugate addition and oxygenation followed by thermal cyclization employing the persistent radical effect was developed. *ent*-Pregnanolone sulfate is surprisingly only 2.6-fold less active than the natural neurosteroid.



Steroids are stereochemically the most conservative class of signaling molecules and mediators in *Eukaryota*, serving as hormones over long distances between tissues. In contrast, neurosteroids are locally biosynthesized in nervous tissue and directly influence the activity of a broad variety of ion channels.[1−3] Among these, glutamate receptors are the most prominent in the brain, being present at ca. 80−90% of synapses.[4] An especially important subclass is the *N*-methyl-D-aspartate receptors (NMDAR) modulating calcium transport into neuronal cells. Pathological processes associated with overactivation of these receptors contribute to a plethora of neurodegenerative diseases and conditions.[4,5] Neurosteroids, such as pregnanolone sulfate, modulate the activity of NMDAR, and therefore, understanding the steroid−NMDAR interactions is crucial for the rational design of neuroprotective compounds, which could significantly slow the progress of neurodegenerative conditions. However, little is known about the mode and place of their interaction. On the basis of recently published X-ray structures of the NMDAR,[6,7] single-point mutations, as well as molecular modeling, it was suggested that inhibitory steroids bind to the ion channel vestibule located just above the cytoplasmic membrane. However, interactions via the membrane cannot be excluded because of the difficult characterization of binding at transmembrane proteins (Figure 1).[8]

We hypothesized that *ent*-pregnanolone sulfate (*ent*-**1**) would be an excellent tool to shed light on the action and binding mode of inhibitory steroids on the NMDAR since it may show distinct effects on direct binding but negligible differences by mere membrane perturbation.[9−14] The only previous total synthesis of *ent*-**1** was accomplished by Covey et al. by a 10-step modification of *ent*-testosterone in 10% yield.[15] The latter was in turn synthesized according to an approach developed by Rychnovsky

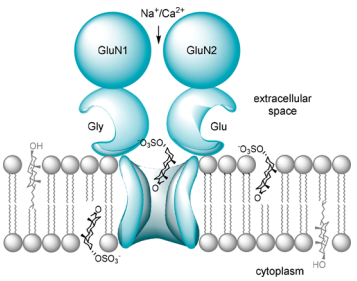


Figure 1. Schematic depiction of the NMDA receptor in the cytoplasmic membrane. A hypothetical binding site of inhibitory neurosteroids is located in an extracellular vestibule. The membrane serves as a reservoir.

in 16% yield over 10 steps using a CD → BCD → ABCD ring formation strategy.[16]

We opted for a new approach to *ent*-pregnanolone sulfate (*ent*-**1**) in which the AB ring system would be installed first, followed by annulation of rings C and D (Scheme 1). This provides the additional advantage to introduce various side chains, in this case that of *ent*-progesterone (*ent*-**2**). The desired side chain of *ent*-**1** is identical to that of *ent*-progesterone (*ent*-**2**). Disconnection of the C-18 methyl group leads to **3**, in which the D-ring and the oxygen function in position C-20 are ideally positioned to develop a new tandem process consisting of copper-catalyzed conjugate addition to tricyclic enone **5** and a radical 5-*exo* cyclization with oxygenative termination, linked by single-electron transfer (SET) oxidation to **4**.[17] Enone **5** can be traced to alternative tricycle **6** and

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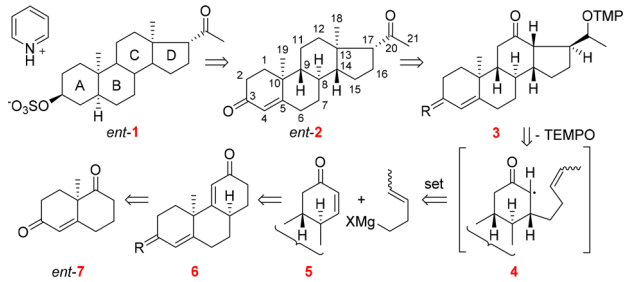
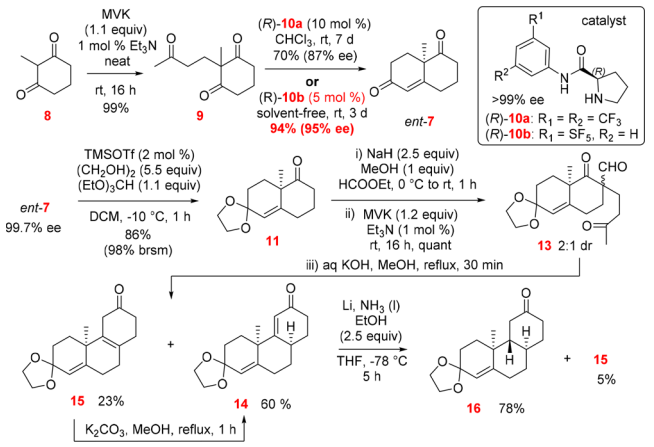
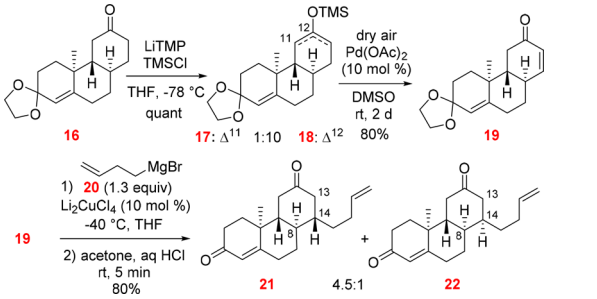
Scheme 1. Retrosynthetic Analysis and Numbering of *ent*-1



Scheme 2. Robinson Annulation Approach to Tricycle 16



Scheme 3. Preparation of Enone 19 and Initial Conjugate Addition



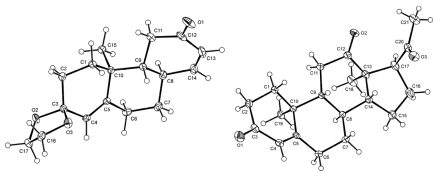
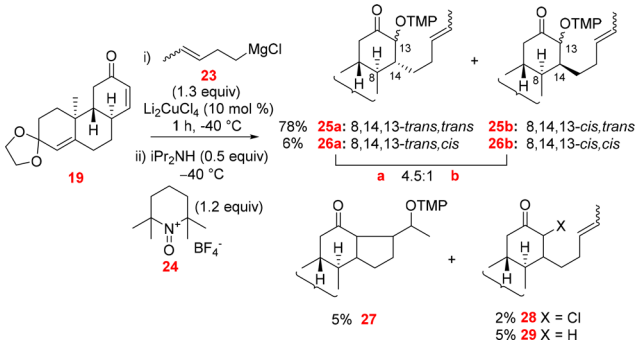


Figure 2. X-ray crystal structure of **19** and the product of oxidative deprotection of **32**.

Scheme 4. Tandem Conjugate Addition/Oxygenation Reaction of Enone 19



subsequently to Wieland–Miescher ketone *ent*-7 by enantio- and diastereoselective Robinson annulations.

The synthesis commenced with Et_3N-catalyzed Michael addition of **8** to methyl vinyl ketone in quantitative yield (Scheme 2).[18] In contrast to the Hajos–Parrish ketone, proline is a relatively inefficient catalyst for the preparation of octaline **7**,[19−22] but proline anilides are known to give better yield and enantioselectivity.[23,24] However, the asymmetric Robinson annulation of **9** using the most efficient anilide **10a**[25] reported so far gave suboptimal results. In contrast, the new SF_5-substituted anilide **10b** proved to be superior with respect to enantioselectivity and reaction times and is thus to our knowledge the best available catalyst for preparation of the Wieland-Miescher ketone **7** and potentially other aldol-type products.

The selective protection of conjugated enone **7** succeeded under nonequilibrating conditions without migration of the double bond, provided that the temperature was kept at −10 °C or lower. These conditions are superior to Noyori's ketalization employing bis(trimethylsilyl) ethylene glycol, which requires a week at −78 °C to go to completion.[26] The subsequent substrate-controlled Robinson annulation was performed in three steps via enol **12** and aldehyde **13** affording a thermodynamic 3:1 mixture of separable double bond isomers **14** and **15**. The undesired minor isomer **15** was recycled under basic conditions giving **14** in a combined 80% yield. Reduction of **14** by lithium in liquid ammonia furnished tricycle **16** with the desired *trans*–B-C ring junction. Some deconjugation of the $\Delta^{9(11)}$ double bond was observed, but the byproduct was removed in the next step.

Ketone **16** was selectively converted to the kinetic trimethylsilyl enolate **18** with lithium tetramethylpiperidide as a base, and the crude 1:10 mixture of regioisomers **17**/**18** was subjected to the Larock modification of the Saegusa oxidation (Scheme 3).[27] The configuration of enone **19** was proved by X-ray crystallography (Figure 2). The conjugate addition step was tested with 3-butenyl Grignard reagent **20**. Dilithium tetrachlorocuprate, formed in situ from $CuCl_2$ and LiCl, was found to be the optimal precatalyst giving good yields and reasonable diastereoselectivity.[28,29] For analytical purposes, hydrolysis of the ketal unit to diones **21** and **22** was performed.

All attempts to perform the initially envisaged tandem copper-catalyzed conjugate addition/radical cyclization (cf. Scheme 1) met with limited success. These results indicated that the cyclization to the steroid skeleton **27** could not compete with oxygenation to **25** and **26** (Scheme 4) and therefore we decided to develop the tandem conjugate addition/oxygenation reaction further and promote the projected radical cyclization subsequently under thermal conditions (vide infra). Under optimized conditions,[28] tricyclic oxygenated products **25** and **26** were obtained in combined 84% yield with reasonable 4.5:1 selectivity for 8,14-*trans*/*cis*-**25a**/**b** and excellent 13:1 13,14-*trans*,*cis*-**25**/**26** diastereoselectivity by performing the conjugate addition with Grignard reagent **23** and direct enolate oxygenation using *N*-oxoiminium salt **24**.[17] Byproducts **27**, **28**, and **29** were formed only to a very small extent.

Taking advantage of the known thermal lability of alkoxyamines, **25**/**26** were subjected to a radical cyclization based on the persistent radical effect. Such reactions, in which reversible generation and reaction of a transient radical are regulated by the

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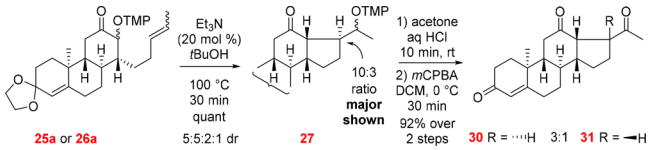
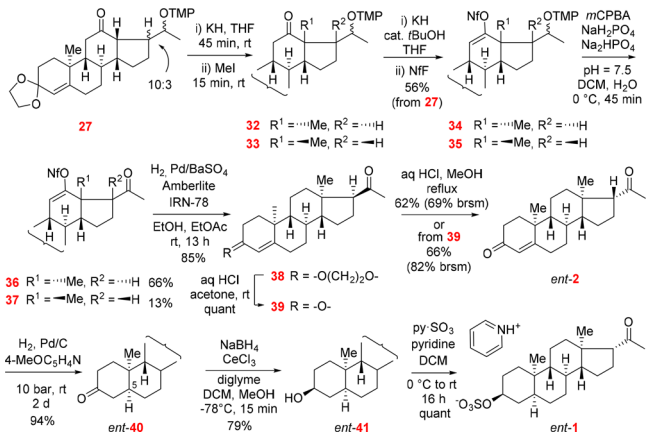
Scheme 5. Radical Cyclization of Keto Alkoxyamines 25a or 26a Forming the D Ring



Scheme 6. Conversion of 27 to *ent*-Pregnanolone Sulfate



presence of the persistent radical are an attractive alternative to standard cyclizations.[30−32] Their power has been rarely demonstrated in total syntheses.[33,34] Heating a mixture of all alkoxyamines **25** and **26** to 100 °C triggered a smooth cyclization of **25a** and **26a** to steroid derivative **27** in quantitative yield as a 5:5:2:1 diastereomeric mixture (Scheme 5). The ratio of epimers at C-17 was found to be 3:1 after oxidative deprotection of the alkoxyamine unit to triketones **30** and **31**. Remarkably, the undesired C-14 epimer **25b** remained unchanged, thus facilitating the otherwise difficult separation of both *trans*-alkoxyamines **25a** and **25b**. The formation of diastereomers of **27** during the cyclization is inconsequential, since they converge into the target steroid *ent*-**1** (vide infra).

The steroid skeleton was completed by alkylation of the thermodynamic enolate generated from **27** (Scheme 6). A screening of bases singled out KH as the only viable option for the synthesis of **32**. The resulting diastereomeric mixture of **32** and **33** was hard to separate and was therefore used directly in the next step. The correct stereochemistry at C-13 after methylation was confirmed by X-ray crystallography (Figure 2).

Removal of the C-12 keto group in **32** was unexpectedly difficult, possibly because of steric hindrance caused by the alkoxyamine unit. After much experimentation, conversion to enol nonaflate by deprotonation with KH in the presence of a catalytic amount of *t*BuOH and reaction with nonaflyl fluoride (NfF), followed by hydrogenolysis proved to be optimal.[35,36] In contrast, LDA, LiHMDS, and KHMDS were less effective, converting only one of the diastereomers. The four diastereomers **34**/**35** were isolated in 56% yield over two steps from **27**. Direct hydrogenation of **34**/**35** was unsuccessful, therefore the alkoxyamine was first oxidatively deprotected by buffered *m*-CPBA to furnish two separable diastereomers **36** and **37** and a small amount of unreacted **34** as a single diastereomer. Nonaflate **36** was selectively hydrogenated to ketal **38** in good yield. The solid-supported Amberlite base was crucial for good conversion, as the reaction seemed to be inhibited by coformed nonaflate salts in its absence.[28] Mild acidic deprotection of the labile ketal gave **39** in

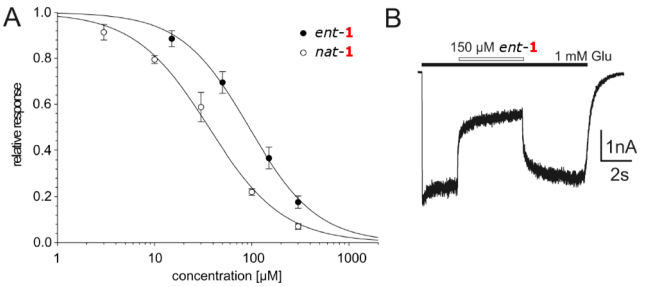


Figure 3. (A) Concentration−response curve for the effect of *nat*-**1** (open circles) and *ent*-**1** (filled circles) at GluN1/GluN2B receptors (n = 5 cells, mean ± SD). (B) Example of trace obtained by simultaneous application of *ent*-**1** (150 μM) with 1 mM glutamate (duration of application is indicated by open and filled bars, respectively).

quantitative yield. The side chain of **39** was epimerized to provide *ent*-progesterone (*ent*-**2**),[37] which was thus en passant obtained.[38−40]

Its hydrogenation stereoselectively afforded diketone *ent*-**40**.[41] The sterically more accessible ketone function at C-3 was subsequently stereoselectively reduced by $NaBH_4/CeCl_3$ in very good yield. Treatment of alcohol *ent*-**41** with the pyridine−sulfur trioxide complex led to quantitative conversion to the target pregnanolone sulfate (*ent*-**1**), totaling 18 steps and 5.5% overall yield for the whole synthesis. Compounds *ent*-**1**, *ent*-**2**, *ent*-**40**, and *ent*-**41** may serve to access other *ent*-neurosteroids.

The biological activity of *ent*-**1** was determined by whole cell voltage clamp on recombinant HEK293 cells expressing GluN1/GluN2B receptors (Figure 3). Currents induced by 1 mM glutamate were recorded at a holding potential of −60 mV, applying different concentrations of steroid sulfate *nat*-**1** or *ent*-**1**.[28] The known neurosteroid *nat*-**1** showed an IC_{50} = 36.4 ± 3.9 μM (Hill coefficient h = 1.1 ± 0.1) in accordance with previous measurements. In contrast, the unnatural enantiomer *ent*-**1** showed lower but significant inhibitory activity with IC_{50} = 94.4 ± 15.7 μM (h = 1.2 ± 0.1).

This difference in affinity toward NMDAR suggests a direct involvement of inhibitory neurosteroids with the protein, in contrast to an expected negligible difference for mere membrane effect. On the other hand, the 2.6-times lower inhibitory activity of unnatural *ent*-**1** rules out a specific binding pocket at the protein. It seems, therefore, more likely that inhibitory neurosteroids act inside the channel as blockers, held in the pore mostly by electrostatic interactions.[8] A similar phenomenon was very recently observed for *nat*- or *ent*-batrachotoxin inside a voltage-gated Na^+-channel.[42] A dominant interaction of neurosteroids with the protein−membrane interface seems to be unlikely but cannot be completely ruled out.[8] The lack of enantiodiscrimination described here, together with previous structure−activity studies of neuroactive steroids[43] suggest that simpler analogs with absolute *ent* stereochemistry may serve well as channel blockers since they will be less prone to metabolic degradation and will not interfere with endocrine signaling mediated by native steroids.[10]

In conclusion, a conceptually new synthetic approach to *ent*-pregnanolone sulfate (*ent*-**1**) was developed. The use of novel catalyst **10b** in the asymmetric Robinson annulation allowed for an exceptionally effective synthesis of the widely used Wieland−Miescher ketone (*ent*-**7**). The tandem conjugate addition/oxygenation sequence with a TEMPO surrogate and the subsequent thermal radical cyclization were effective for annulation of the D-ring. A study of the generality of this reaction sequence is underway. A remarkably selective hydrogenation of

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an enol nonaflate serves as an excellent way to deoxygenate sterically hindered ketones. The results of electrophysiological testing clearly demonstrated that the purported steroid binding pocket at the NMDAR is stereochemically relatively nonspecific but ruled out neurosteroid action mediated solely by the change in membrane physicochemical properties. Further synthetic effort is directed toward truncated steroid congeners to study minimum binding requirements for neuroactive steroid analogues.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b03838.

Experimental details, characterization data of all compounds, and electrophysiological experiments (PDF)

Accession Codes

CCDC 1048883 and 1048885−1048888 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Giatti, S.; Romano, S.; Pesaresi, M.; Cermenati, G.; Mitro, N.; Caruso, D.; Tetel, M. J.; Garcia-Segura, L. M.; Melcangi, R. C. *Steroids* **2015**, *103*, 23.

(2) Korinek, M.; Kapras, V.; Vyklicky, V.; Adamusova, E.; Borovska, J.; Vales, K.; Stuchlik, A.; Horak, M.; Chodounska, H.; Vyklicky, L. *Steroids* **2011**, *76*, 1409.

(3) Baulieu, E. E.; Robel, P.; Schumacher, M. Neurosteroids and Brain Function. In *International Review of Neurobiology*; Biggio, G., Purdy, R. H., Eds.; Academic Press: San Diego, CA, 2001; Vol. *46*, pp 1−32.

(4) Traynelis, S. F.; Wollmuth, L. P.; McBain, C. J.; Menniti, F. S.; Vance, K. M.; Ogden, K. K.; Hansen, K. B.; Yuan, H.; Myers, S. J.; Dingledine, R. *Pharmacol. Rev.* **2010**, *62*, 405.

(5) Vyklicky, V.; Korinek, M.; Smejkalova, T.; Balik, A.; Krausova, B.; Kaniakova, M.; Lichnerova, K.; Cerny, J.; Krusek, J.; Dittert, I.; Horak, M.; Vyklicky, L. *Physiol. Res.* **2014**, *63* (Suppl 1), S191.

(6) Karakas, E.; Furukawa, H. *Science* **2014**, *344*, 992.

(7) Lee, C.-H.; Lü, W.; Michel, J. C.; Goehring, A.; Du, J.; Song, X.; Gouaux, E. *Nature* **2014**, *511*, 191.

(8) Vyklicky, V.; Krausova, B.; Cerny, J.; Balik, A.; Zapotocky, M.; Novotny, M.; Lichnerova, K.; Smejkalova, T.; Kaniakova, M.; Korinek, M.; Petrovic, M.; Kacer, P.; Horak, M.; Chodounska, H.; Vyklicky, L. *Sci. Rep.* **2015**.

(9) Covey, D. F. *Steroids* **2009**, *74*, 577.

(10) Biellmann, J.-F. *Chem. Rev.* **2003**, *103*, 2019.

(11) Alakoskela, J.-M.; Covey, D. F.; Kinnunen, P. K. J. *Biochim. Biophys. Acta, Biomembr.* **2007**, *1768*, 131.

(12) Katona, B. W.; Krishnan, K.; Cai, Z. Y.; Manion, B. D.; Benz, A.; Taylor, A.; Evers, A. S.; Zorumski, C. F.; Mennerick, S.; Covey, D. F. *Eur. J. Med. Chem.* **2008**, *43*, 107.

(13) Covey, D. F.; Nathan, D.; Kalkbrenner, M.; Nilsson, K. R.; Hu, Y.; Zorumski, C. F.; Evers, A. S. *J. Pharmacol. Exp. Ther.* **2000**, *293*, 1009.

(14) Li, W.; Jin, X.; Covey, D. F.; Steinbach, J. H. *J. Pharmacol. Exp. Ther.* **2007**, *323*, 236.

(15) Nilsson, K. R.; Zorumski, C. F.; Covey, D. F. *J. Med. Chem.* **1998**, *41*, 2604.

(16) Rychnovsky, S. D.; Mickus, D. E. *J. Org. Chem.* **1992**, *57*, 2732.

(17) Kafka, F.; Holan, M.; Hidasová, D.; Pohl, R.; Císařová, I.; Klepetářová, B.; Jahn, U. *Angew. Chem., Int. Ed.* **2014**, *53*, 9944.

(18) Bradshaw, B.; Etxebarría-Jardi, G.; Bonjoch, J.; Viózquez, S. F.; Guillena, G.; Nájera, C. *Adv. Synth. Catal.* **2009**, *351*, 2482.

(19) Bui, T.; Barbas, C. F., III *Tetrahedron Lett.* **2000**, *41*, 6951.

(20) Cañellas, S.; Ayats, C.; Henseler, A. H.; Pericàs, M. A. *ACS Catal.* **2017**, *7*, 1383.

(21) Gallier, F.; Martel, A.; Dujardin, G. *Angew. Chem., Int. Ed.* **2017**, *56*, 12424.

(22) Guo, H.-C.; Ma, J.-A. *Angew. Chem., Int. Ed.* **2006**, *45*, 354.

(23) Bradshaw, B.; Bonjoch, J. *Synlett* **2012**, *23*, 337.

(24) Zlotin, S. G.; Kucherenko, A. S.; Beletskaya, I. P. *Russ. Chem. Rev.* **2009**, *78*, 737.

(25) de Arriba, Á. L. F.; Simón, L.; Raposo, C.; Alcázar, V.; Morán, J. R. *Tetrahedron* **2009**, *65*, 4841.

(26) Tsunoda, T.; Suzuki, M.; Noyori, R. *Tetrahedron Lett.* **1980**, *21*, 1357.

(27) Larock, R. C.; Hightower, T. R.; Kraus, G. A.; Hahn, P.; Zheng, D. *Tetrahedron Lett.* **1995**, *36*, 2423.

(28) See the Supporting Information.

(29) Reetz, M. T.; Kindler, A. *J. Organomet. Chem.* **1995**, *502*, C5.

(30) Fischer, H. *Chem. Rev.* **2001**, *101*, 3581.

(31) Studer, A. *Chem. - Eur. J.* **2001**, *7*, 1159.

(32) Focsaneanu, K.-S.; Scaiano, J. C. *Helv. Chim. Acta* **2006**, *89*, 2473.

(33) Xu, J.; Caro-Diaz, E. J. E.; Lacoske, M. H.; Hung, C.-I.; Jamora, C.; Theodorakis, E. A. *Chem. Sci.* **2012**, *3*, 3378.

(34) Amatov, T.; Pohl, R.; Císařová, I.; Jahn, U. *Angew. Chem., Int. Ed.* **2015**, *54*, 12153.

(35) Cacchi, S.; Morera, E.; Ortar, G. *Tetrahedron Lett.* **1984**, *25*, 4821.

(36) Cacchi, S.; Morera, E.; Ortar, G. *Org. Synth.* **2011**, *88*, 260.

(37) Butenandt, A.; Schmidt-Thomé, J.; Paul, H. *Ber. Dtsch. Chem. Ges. B* **1939**, *72*, 1112.

(38) Johnson, W. S.; Gravestock, M. B.; McCarry, B. E. *J. Am. Chem. Soc.* **1971**, *93*, 4332.

(39) Auchus, R. J.; Kumar, A. S.; Boswell, C. A.; Gupta, M. K.; Bruce, K.; Rath, N. P.; Covey, D. F. *Arch. Biochem. Biophys.* **2003**, *409*, 134.

(40) Stork, G.; McMurry, J. E. *J. Am. Chem. Soc.* **1967**, *89*, 5464.

(41) Tsuji, N.; Suzuki, J.; Shiota, M.; Takahashi, I.; Nishimura, S. *J. Org. Chem.* **1980**, *45*, 2729.

(42) Logan, M. M.; Toma, T.; Thomas-Tran, R.; Du Bois, J. *Science* **2016**, *354*, 865.

(43) Slavikova, B.; Chodounska, H.; Nekardova, M.; Vyklicky, V.; Ladislav, M.; Hubalkova, P.; Krausova, B.; Vyklicky, L.; Kudova, E. *J. Med. Chem.* **2016**, *59*, 4724.

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