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Letter

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Hydroxymethyl-Branched Polyhydroxylated Indolizidines: Novel Selective α-Glucosidase Inhibitors

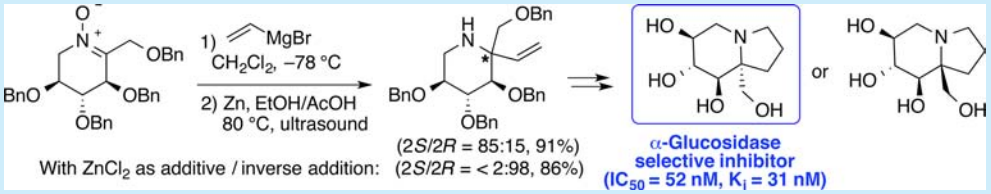
Julien Boisson,[†,‡] Amélia Thomasset,[†,‡] Emilie Racine,[†,‡] Pascale Cividino,[†,‡] Thomas Banchelin Sainte-Luce,[†,‡] Jean-François Poisson,[†,‡] Jean-Bernard Behr,[§] and Sandrine Py*[,†,‡]

[†]Univ. Grenoble Alpes, DCM, F-38000 Grenoble, France

[‡]CNRS, DCM, F-38000 Grenoble, France

[§]Université de Reims Champagne-Ardenne, Institut de Chimie Moléculaire de Reims (ICMR), CNRS UMR 7312, UFR Sciences Exactes et Naturelles, BP 1039, 51687 Reims Cedex 2, France

Supporting Information



ABSTRACT: α,α-Disubstituted piperidines and conformationally constrained polyhydroxylated indolizidines bearing a hydroxymethyl substituent in position 8a were synthesized from a readily available L-sorbose-derived ketonitrone. Diastereoselective vinylation under two sets of complementary conditions allowed access to both configurations of the newly formed quaternary stereocenter. Subsequent *N*-allylation and ring-closing metathesis afforded 8a-branched indolizidines in high yield. The newly prepared iminosugars demonstrated highly potent inhibition of α-glucosidases. Most interestingly, compound **9b** exhibits very high selectivity toward this class of enzymes, with an unusual mode of binding.

Glycosidase inhibition is a valuable therapeutic strategy for the treatment of metabolic disorders, cancer, and viral infections.[1] Intense research on glycosidase inhibition by iminosugars[2] is ongoing in both academic laboratories and pharmaceutical companies.[3] However, the main limitation of glycosidase inhibition as a therapeutic approach is the ubiquity of this class of enzymes in living organisms, and therefore the inherent difficulty of selective inhibition without affecting normal cell functions. It is thus a timely challenge to design glycosidase inhibitors that are not only potent but also highly selective toward specific enzymes. In this letter, we describe the synthesis of novel polyhydroxylated indolizidines bearing a C-8a quaternary center, among which a potent and highly selective inhibitor of α-glucosidase has been identified.

Deoxynojirimycin (DNJ, Figure 1) was the first iminosugar to be recognized as a potent inhibitor of glucosidases from various animal, plant, or microbial sources.[4] Castanospermine, a natural polyhydroxylated indolizidine isolated from *Castanospermum australe*, share the same inhibition profile and might be considered as a conformationally restricted analogue of DNJ.[5] In the search for more potent and selective glycosidase inhibitors, the design of constrained analogues of chemical leads has been a classical approach. To reach this goal, a first method consists in introducing a second cycle on the parent structure to reduce its conformational mobility and adjust the substituent's orientation.[6] Another method aims at building quaternary centers to afford C-branched iminosugars featuring an additional substituent, which may improve the affinity for

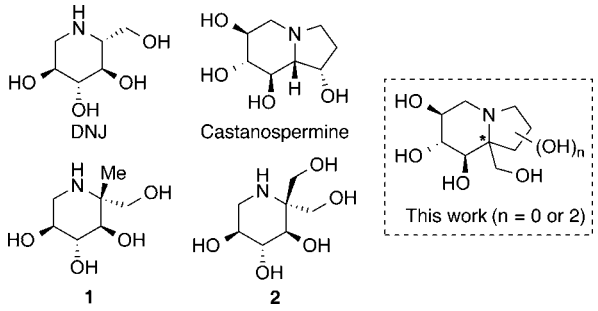


Figure 1. Analogues of DNJ as α-glucosidase inhibitors.

the active site of specific glycosidases.[7] As examples, α,α-disubstituted piperidine **1** is a slightly better inhibitor of human α-glucosidase (IC_{50} = 1 μM) than DNJ (IC_{50} = 1.44 μM),[8] and α,α-disubstituted piperidine **2** (IC_{50} = 32 nM) inhibits rice α-glucosidase more selectively than DNJ.[9] In a combination of both approaches, we envisioned the synthesis of novel quaternary castanospermine analogues bearing a hydroxymethyl substituent in position 8a. The latter may mimic the hydroxymethyl in position 6 of a glucosyl residue and could improve affinity and/or selectivity for glucosidases. The synthesis of compounds containing a quaternary stereocenter remains a challenging endeavor, and only two reports on the

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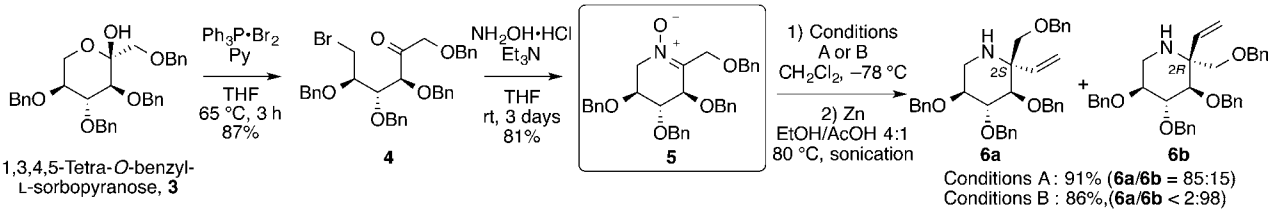
Scheme 1. Preparation of Nitrone 5 and Stereodivergent Vinylation



Conditions A: Addition of vinylMgBr (1.2 equiv) to nitrone **5**;
Conditions B: Addition of nitrone **5** to a (1:1) solution of $ZnCl_2$ and vinylMgBr (1.2 equiv), then vinylMgBr (2.4 equiv).

synthesis of 8a-substituted polyhydroxylated indolizidines are described.[10]

In continuation of our interest in the synthesis of glycosidase inhibitors,[11] we have previously reported the synthesis of a six-membered ring ketonitrone from D-fructose, which was used for the synthesis of deoxymannojirimycin.[12] To synthesize the targeted castanospermine analogues, we thought to start from nitrone **5**, of the D-*gluco* configuration, which could be prepared from readily available L-sorbose. The previous route to synthesize such ketonitrones was significantly improved by direct activation of the primary alcohol to a bromide:[13] 1,3,4,5-tetra-*O*-benzyl-L-sorbopyranose (**3**)[14] was treated with PPh_3·Br_2 and pyridine, in refluxing THF, to yield **4** in 87% yield. The latter was next converted into nitrone **5** in a single step, by treatment with an excess of hydroxylamine hydrochloride and triethylamine, in THF. Nitrone **5** was thus obtained in only 2 steps from **3**, with a 70% overall yield (Scheme 1).

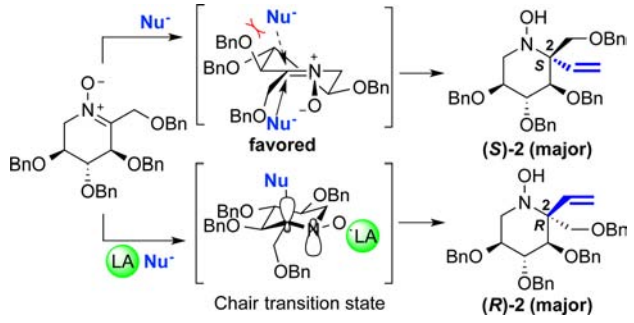
The first approach to prepare indolizidines from ketonitrones using SmI_2-mediated *umpolung* being unsuccessful,[15] a strategy involving ring closing metathesis to build up the five-membered ring fused to the piperidine moiety was chosen, requiring diastereoselective addition of a vinyl group to nitrone **5**. The addition of organometallics to five-membered polyalkoxylated aldonitrones has been amply described to occur with good stereoselectivities.[16] In contrast, the addition of organometallics to six-membered aldonitrones proceeds with lower selectivities[17] and such reactions have never been reported on ketonitrones.

After a screening of solvents (see Supporting Information (SI)), we found that vinylmagnesium bromide added on nitrone **5** in high yields, with the best selectivity in dichloromethane at −78 °C (dr = 85:15). Reduction of the crude mixture of the resulting unseparable hydroxylamines with zinc[18] and acetic acid afforded piperidines **6a** and **6b** (Scheme 1), which were separated by chromatography (overall yield: 91%). The configuration of their quarternary center was assigned unambiguously by NMR (see SI).

With the aim to invert the selectivity and favor isomer **6b**, the addition of vinylmagnesium bromide to nitrone **5** was performed in the presence of various additives. Both Et_2AlCl[19] and $MgBr_2$·OEt_2[20] are known to tune, and in some cases to invert, the diastereoselectivity of addition of organometallics to α-alkoxy-substituted nitrones. However, these additives gave unsatisfactory results in this case (**6a**/**6b** ≈ 75:25; see SI). In contrast, complexation of nitrone **5** with TMSOTf, $Zn(OTf)_2$, or $ZnCl_2$ prior to addition of vinylmagnesium bromide resulted in inversion of diastereoselectivity (**6a**/**6b** ≈ 20:80; see SI). Most satisfyingly, the desired (2*R*)-vinyl-*N*-hydroxypiperidine was formed as a single diastereoisomer when nitrone **5** (solution in dichloromethane) was added to an equimolar mixture of $ZnCl_2$ and vinylmagnesium bromide previously cooled to −78 °C. After reduction with zinc, only piperidine **6b** was isolated in 86% yield (Scheme 1).[21]

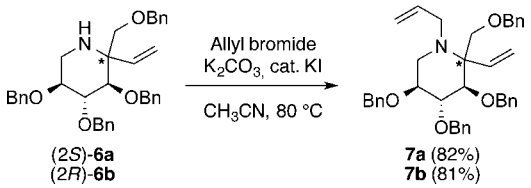
The observed diastereoselectivity (*trans*-addition with vinylmagnesium bromide only and *cis*-addition in the presence of $ZnCl_2$ as a Lewis acid) is in accordance with the models proposed by Davis et al.[8] and Cheng et al.[17b] for nucleophilic additions to endocyclic C═N bonds (Scheme 2). In the absence of an additive, the Grignard addition is sterically controlled, with a favored attack opposite to the benzyloxy group at C3, yielding a *N*-hydroxypiperidine of (*S*)-configuration at C2. In the presence of a Lewis acid, we hypothesize the dominance of stereoelectronic control, favoring axial attack of the nucleophile in a chairlike transition state forming with the development of an antiperiplanar nonbonding doublet at the nitrogen atom and pyramidalization of the electrophilic carbon atom. This can explain the prevalent formation of the *N*-hydroxypiperidine of (*R*)-configuration at C2 when a Lewis acid efficiently coordinates nitrone **5**.

Scheme 2. Stereoselectivity of Vinylation



Next, vinylpiperidines **6a** and **6b** were converted to dienes **7a** (82%) and **7b** (81%) respectively (Scheme 3). Ring closing metathesis proceeded smoothly in the presence of 5 mol % of Grubbs II catalyst, in refluxing dichloromethane, yielding **8a** (85%) from **7a** and **8b** (93%) from **7b** (Scheme 4). Hydrogenation of the double bond and benzyl deprotection were effected in one step (5 bar of H_2, Pearlman's catalyst,

Scheme 3. *N*-Allylation of Piperidines 6a and 6b



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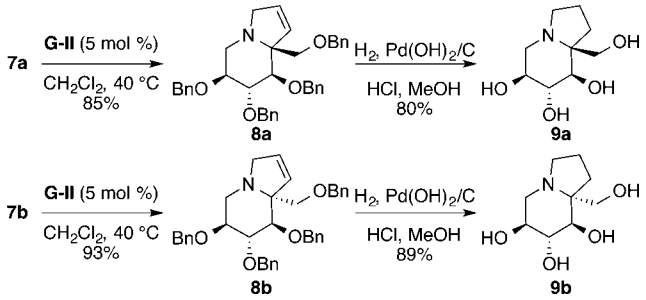
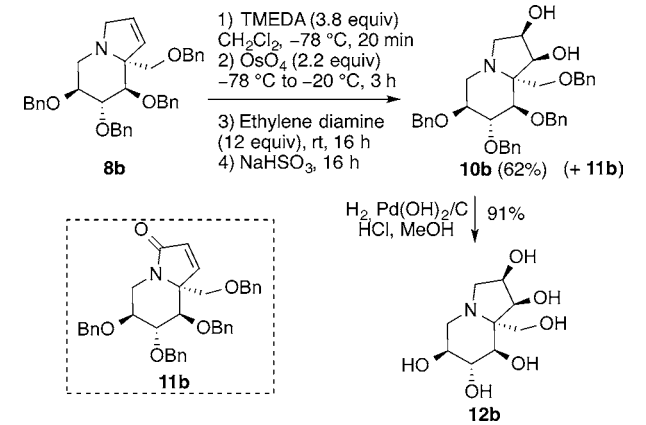
Scheme 4. Elaboration of Tetrahydroxylated Indolizidines



HCl) to afford the 8a-branched tetrahydroxy indolizidines **9a** (80%) and **9b** (89%).

Dihydroxylation of the double bond in **8b** was next studied. Osmylation of **8b** under classical conditions (cat. OsO_4, NMO)[22] afforded only 20% of the *cis*-diol **10b** as a single isomer (see SI for configuration assignment). One of the isolated byproducts was the lactam **11b** (14%), resulting from allylic oxidation.[23] To circumvent this side reaction, the osmylation was next performed in the presence of acids. Introduction in the reaction media of $Ti(OiPr)_4$ (1.4 equiv) or HCl (1.1 equiv) resulted in only a slight yield improvement (27% and 38% respectively). To avoid isolation of the rather unstable alkene **8b**, one-pot metathesis/dihydroxylation was also attempted,[24,25] with no success. At last, inspired by the recent work of Jarosz, we decided to treat **8b** with a stoichiometric amount of osmium tetraoxide in the presence of TMEDA to form a stable complex, which was next converted into the expected diol upon treatment with ethylene diamine.[26] Under these conditions, the diol **10b** was isolated in an improved yield of 62% (Scheme 5). Finally, **10b** was converted

Scheme 5. Dihydroxylation of 8b

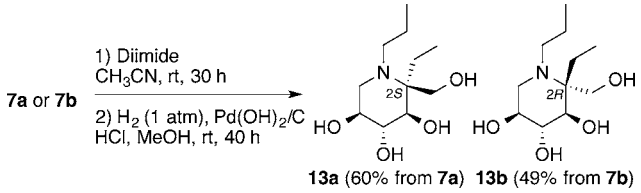


to **12b** (91%) by hydrogenolysis. Application of these dihydroxylation conditions to the isomer **8a** only led to complex mixtures of products, from which the expected diol could not be isolated.

With the aim to evaluate the importance of the constrained bicyclic scaffold on the activity and selectivity of our products toward glycosidases, *N*-allylated piperidines **7a** and **7b** were also converted into tetrahydroxy α,α-disubstituted piperidines **13a** (60%) and **13b** (49%), following a two-step sequence involving diimide reduction[27] and debenzylation (Scheme 6).[28]

The inhibitory activity of compounds **9a**, **9b**, and **12b** and their monocyclic analogues **13a** and **13b** was evaluated against

Scheme 6. Synthesis of Tetrahydroxylated *N*-Propyl α,α-Disubstituted Piperidines



a panel of commercially available glycosidases under a standard protocol,[29] and percent inhibition was evaluated at 1 mM concentration of inhibitor (Table 1). In case of complete inactivation (>96%) at this concentration, IC_{50} values were determined further.

Table 1. Inhibitory Activity against Glycosidases[a,b]

enzyme	9a	9b	12b	13a	13b
α-glucosidase *S. cerevisiae*	80%	82%	71%	96%	95%
α-glucosidase rice	99%[c]	100%[d]	96%[e]	95%	99%[f]
β-glucosidase almond	89%	NI	20%	NI	47%
β-galactosidase *A. orizae*	10%	NI	19%	6%	7%
α-mannosidase Jack beans	NI	NI	10%	NI	6%
β-mannosidase *H. pomatia*	NI	NI	10%	4%	NI
α-rhamnosidase *A. niger*	53%	24%	90%	NI	NI

[a]Expressed as % inhibition at 1 mM concentration of drug. [b]NI means no inhibition. [c]IC_{50} = 2.2 μM. [d]IC_{50} = 0.052 μM, K_i = 31 nM, K_i' = 67 nM. [e]IC_{50} = 1.5 μM. [f]IC_{50} = 2.3 μM.

All the synthesized *C*-branched iminosugars were found to exhibit inhibitory potency against glycosidases, and in some cases the inhibition revealed to be selective toward the two α-glucosidases tested (Table 1). In particular, indolizidine **9b**, the structure that fits best with that of DNJ or castanospermine, was the most active (IC_{50} = 52 nM, rice α-glucosidase). Interestingly, as revealed by Lineweaver−Burk plots (see SI), *a mixed inhibition pattern* was observed for **9b** (K_i = 31 nM, K_i' = 67 nM), in sharp contrast with the more standard competitive behavior of DNJ or castanospermine. Moreover, and strinkingly, compound **9b** is a highly selective inhibitor of α-glucosidases, in contrast to DNJ and castanospermine that are also inhibitors of β-glucosidases. The discovery of a novel, noncompetitive mode of inhibition of α-glucosidases could be of great interest and should open new opportunities in the development of drugs with more specific action. Further studies are currently underway to unravel the specificity of the mode of interaction of compound **9b** with α-glucosidases and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Characterization data, full experimental procedures, and copies of ^1H and ^{13}C NMR spectra of all new compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01505.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: sandrine.py@ujf-grenoble.fr.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Zechel, D. L.; Withers, S. G. *Acc. Chem. Res.* **2000**, *33*, 11−18. (b) Lopez, O. M.-M.; Martos, S.; Gonzalez-Benjumea, A. *Carbohydr. Chem.* **2012**, *38*, 215−262. (c) Gloster, T. M. *Biochem. Soc. Trans.* **2012**, *40*, 913−928.

(2) (a) Compain, P.; Martin, O. R. *Iminosugars: From synthesis to therapeutic applications*; Wiley: Chichester, U.K., 2007. (b) Horne, G.; Wilson, F. X.; Tinsley, J.; Williams, D. H.; Storer, R. *Drug Discovery Today* **2011**, *16*, 107−118. (c) Nash, R. J.; Kato, A.; Yu, C.-Y.; Fleet, G. W. J. *Future Med. Chem.* **2011**, *3*, 1513−1521.

(3) Bras, N. F.; Cerqueira, N. M. F. S. A.; Ramos, M. J.; Fernandes, P. A. *Expert Opin. Ther. Pat.* **2014**, *24*, 857−874.

(4) Asano, N.; Oseki, K.; Kizu, H.; Matsui, K. *J. Med. Chem.* **1994**, *37*, 3701−3706.

(5) (a) Hohenschutz, D. L.; Bell, E. A.; Jewess, P. J.; Leworthy, D. P.; Pryce, R. J.; Arnold, E.; Clardy, J. *Phytochemistry* **1981**, *20*, 811−814. (b) Molyneux, R. J.; Roitman, J. N.; Dunnheim, G.; Szumilo, T.; Elbein, A. D. *Arch. Biochem. Biophys.* **1986**, *251*, 450−457. (c) Pandey, G.; Dumbre, S. G.; Pal, S.; Khan, M. I.; Shabab, M. *Tetrahedron* **2007**, *63*, 4756−4761. (d) Sánchez-Fernández, E. M.; Rísquez-Cuadro, R.; Chasseraud, M.; Ahidouch, A.; Mellet, C. O.; Ouadid-Ahidouch, H.; Fernández, J. M. G. *Chem. Commun.* **2010**, *46*, 5328−5330.

(6) (a) Borges de Melo, E.; da Silveira Gomes, A.; Carvalho, I. *Tetrahedron* **2006**, *62*, 10277−10302. (b) Behr, J.-B.; Gainvors-Claisse, A.; Belarbi, A. *Nat. Prod. Res.* **2006**, *20*, 1308−1314. (c) Arora, I.; Kashyap, V. K.; Singh, A. K.; Dasgupta, A.; Kumar, B.; Shaw, A. K. *Org. Biomol. Chem.* **2014**, *12*, 6855−6868. For a recent review on bicyclic iminosugars, see: Lahiri, R.; Ansari, A. A.; Vankar, Y. D. *Chem. Soc. Rev.* **2013**, *42*, 5102−5118.

(7) Furneaux, R. H.; Gainsford, G. J.; Mason, J. M.; Tyler, P. C.; Hartley, O.; Winchester, B. G. *Tetrahedron* **1997**, *53*, 245−268.

(8) Maughan, M. A. T.; Davies, I. G.; Claridge, T. D. W.; Courtney, S.; Hay, P.; Davis, B. G. *Angew. Chem., Int. Ed.* **2003**, *42*, 3788−3792.

(9) Pawar, N. J.; Parihar, V. S.; Chavan, S. T.; Joshi, R.; Joshi, P. V.; Sabharwal, S. G.; Puranik, V. G.; Dhavale, D. D. *J. Org. Chem.* **2012**, *77*, 7873−8782.

(10) (a) Langlois, N.; Le Nguyen, B. K.; Retailleau, P.; Tarnus, C.; Salomon, E. *Tetrahedron: Asymmetry* **2006**, *17*, 53−60. (b) Duran-Lara, E. F.; Shankaraiah, N.; Geraldo, D.; Santos, L. S. *J. Braz. Chem. Soc.* **2009**, *20*, 813−819.

(11) (a) Behr, J.-B.; Chevrier, C.; Defoin, A.; Tarnus, C.; Streith, J. *Tetrahedron* **2003**, *59*, 543−553. (b) Desvergnes, S.; Py, S.; Vallée, Y. *J. Org. Chem.* **2005**, *70*, 1459−1462. (c) Masson, G.; Philouze, C.; Py, S. *Org. Biomol. Chem.* **2005**, *3*, 2067−2069. (d) Ceccon, J.; Greene, A. E.; Poisson, J.-F. *Org. Lett.* **2006**, *8*, 4739−4742. (e) Laroche, C.; Behr, J.-B.; Szymoniak, J.; Bertus, P.; Schutz, C.; Vogel, P.; Plantier-Royon, R. *Bioorg. Med. Chem.* **2006**, *14*, 4047−4054. (f) Ceccon, J.; Danoun, G.; Greene, A. E.; Poisson, J.-F. *Org. Biomol. Chem.* **2009**, *7*, 2029−2031. (g) Danoun, G.; Ceccon, J.; Greene, A. E.; Poisson, J.-F. *Eur. J. Org. Chem.* **2009**, *2009*, 4221−4224.

(12) Racine, E.; Bello, C.; Gerber-Lemaire, S.; Vogel, P.; Py, S. *J. Org. Chem.* **2009**, *74*, 1766−1769.

(13) Chery, F.; Cronin, L.; O'Brien, J. L.; Murphy, P. V. *Tetrahedron* **2004**, *60*, 6597−6608.

(14) Helleur, R.; Rao, V. S.; Perlin, A. S. *Carbohydr. Res.* **1981**, *89*, 83−90. See Supporting Information for modification of the original experimental procedures described in this paper.

(15) Racine, E.; Py, S. *Org. Biomol. Chem.* **2009**, *7*, 3385−3387.

(16) For general reviews, see: (a) Merino, P.; Tejero, T. *Synlett* **2011**, *2011*, 1965−1977. (b) Merino, P. *C. R. Chim.* **2005**, *8*, 775−788. (c) Lombardo, M.; Trombini, C. *Curr. Org. Chem.* **2002**, *6*, 695−713. (d) Lombardo, M.; Trombini, C. *Synthesis* **2000**, *2000*, 759−774. (e) Merino, P.; Franco, S.; Merchan, F. L.; Tejero, T. *Synlett* **2000**, 442−454. For vinylation of nitrones, see: (f) Cardona, F.; Moreno, G.; Guarna, F.; Vogel, P.; Schuetz, C.; Merino, P.; Goti, A. *J. Org. Chem.* **2005**, *70*, 6552−6555. (g) Delso, I.; Tejero, T.; Goti, A.; Merino, P. *Tetrahedron* **2010**, *66*, 1220−1227. (h) Kaliappan, K. P.; Das, P.; Chavan, S. T.; Sabharwal, S. G. *J. Org. Chem.* **2009**, *74*, 6266−6274.

(17) (a) Archibald, G.; Lin, C.-P.; Boyd, P.; Barker, D.; Caprio, V. *J. Org. Chem.* **2012**, *77*, 7968−7980. (b) Chan, T.-H.; Chang, Y.-F.; Hsu, J.-J.; Cheng, W.-C. *Eur. J. Org. Chem.* **2010**, *2010*, 5555−5559. (c) Zhao, H.; Wang, W.-B.; Nakagawa, S.; Jia, Y.-M.; Hu, X.-G.; Fleet, G. W. J.; Wilson, F. X.; Nash, R. J.; Kato, A.; Yu, C.-Y. *Chin. Chem. Lett.* **2013**, *24*, 1059−1063.

(18) Cicchi, S.; Bonanni, M.; Cardona, F.; Revuelta, J.; Goti, A. *Org. Lett.* **2003**, *5*, 1773−1776.

(19) (a) Shibata, T.; Uemae, K.; Yamamoto, Y. *Tetrahedron: Asymmetry* **2000**, *11*, 2339−2346. (b) Lombardo, M.; Fabbroni, S.; Trombini, C. *J. Org. Chem.* **2001**, *66*, 1264−1268. (c) Delso, I.; Marca, E.; Mannucci, V.; Tejero, T.; Goti, A.; Merino, P. *Chem. - Eur. J.* **2010**, *16*, 9910−9919. See also ref 17c.

(20) (a) Giovannini, R.; Marcantoni, E.; Petrini, M. *J. Org. Chem.* **1995**, *60*, 5706−5707.

(21) Notably, when $ZnCl_2$ or Et_2AlCl was used as an additive, excess vinylmagnesium bromide was necessary for complete conversion of nitrone **5**. While transmetalation could be considered between the Grignard reagent and these additives, divinylzinc failed to react with nitrone **5** at −78 °C and led to poor diastereoselectivities at higher temperatures.

(22) Wang, N.; Zhang, L.-H.; Ye, X.-S. *Org. Biomol. Chem.* **2010**, *8*, 2639−2649.

(23) (a) Sletten, E. M.; Liotta, L. J. *J. Org. Chem.* **2006**, *71*, 1335−1343. (b) Hottin, A.; Wright, D. W.; Steenackers, A.; Delannoy, P.; Dubar, F.; Biot, C.; Davies, G. J.; Behr, J.-B. *Chem. - Eur. J.* **2013**, *19*, 9526−9533.

(24) (a) Beligny, S.; Eibauer, S.; Maechling, S.; Blechert, S. *Angew. Chem., Int. Ed.* **2006**, *45*, 1900−1903. (b) For a recent application in iminosugar synthesis, see: Malik, M.; Ceborska, M.; Witkowski, G.; Jarosz, S. *Tetrahedron: Asymmetry* **2015**, *26*, 29−34.

(25) Scholte, A. A.; An, M. H.; Snapper, M. L. *Org. Lett.* **2006**, *8*, 4759−4762.

(26) Malik, M.; Witkowski, G.; Ceborska, M.; Jarosz, S. *Org. Lett.* **2013**, *15*, 6214−6217.

(27) Marsh, B. J.; Carbery, D. R. *J. Org. Chem.* **2009**, *74*, 3186−3188.

(28) Pd- or Raney-Ni-catalyzed hydrogenation/hydrogenolysis of **7a** yielded significant amounts of *N*-deallylation products.

(29) Gossan, D.; Alabdul Magid, A.; Kouassi-Yao, P.; Behr, J.-B.; Ahibo, A.; Djakoure, L.; Harakat, D.; Voutquenne-Nazabadioko, L. *Phytochemistry* **2015**, *109*, 76−83.

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