Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 6209–6213

Diastereoselective addition of alkynylalanes to carbohydrate-derived nitrones

Christelle Pillard,[a] Valérie Desvergnes[b,*,†] and Sandrine Py[a,*]

[a]*Département de Chimie Moléculaire (SERCO) UMR-5250, ICMG FR-2607, CNRS—Université Joseph Fourier, BP 53, 38041 Grenoble Cedex 09, France*
[b]*Institut de Chimie Organique et Analytique, UMR 6005, Université d'Orléans—CNRS, Rue de Chartres, BP 6759, F-45067 Orléans Cedex 2, France*

Received 9 May 2007; revised 8 June 2007; accepted 18 June 2007
Available online 22 June 2007

Abstract—Propargylic *N*-hydroxypyrrolidines were prepared by diastereoselective addition of pre-formed alkynylalanes to various highly functionalized carbohydrate-derived endocyclic nitrones. Excellent diastereoisomeric excesses were obtained using dimethyl-2-phenylethynylalane. Addition of other alkynylalane derivatives to such type of nitrones is also reported.

Recently, we got involved in a synthetic program aiming to the preparation of iminosugar-type glycomimetics from cyclic nitrones. In these approaches, nitrones were used either as dipole[1] or as nucleophile precursors[2,3] under controlled reductive conditions (SmI_2-mediated formation of α-amino nucleophilic species[4]). As a complementary route to access iminosugars, carbohydrate-derived nitrones[5] were also used as electrophiles, in reactions with unsaturated organometallic species (see Scheme 1).

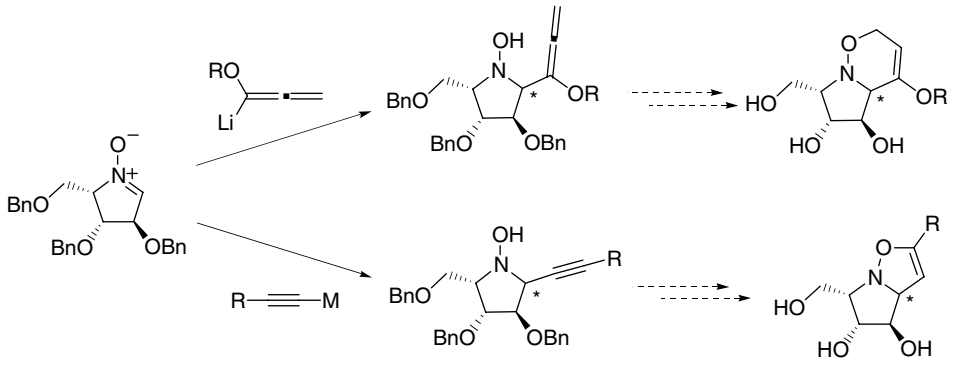
Nitrones are known to exhibit better reactivity than imines in nucleophilic addition reactions.[6,7] They proved useful for the preparation of *N*-hydroxypropargylic amines,[8] by addition of lithium,[9] magnesium,[10] or zinc[11] acetylides. On the other hand, aluminum acetylides have proved to be useful reagents in nucleophilic additions to iminiums[12] (generated in situ from oxazolidines) and acyl chlorides[13] in the pioneering work of Micouin and co-workers. However, no example of addition of such reagents to nitrones had been reported until lately.[14] In this Letter, we present our results in this field and in particular our investigations in the stereochemical aspects of the addition of various alkynylalanes to enantiopure, carbohydrate-derived nitrones.

In this study, dimethylalkynylalanes were pre-formed by heating trimethylaluminum (2 M solution in toluene) and alkyne, in the presence of a catalytic amount of triethylamine (10%), as previously described (Scheme 2).[13] The resulting mixtures were cooled to 0 °C, then the nitrones were added.

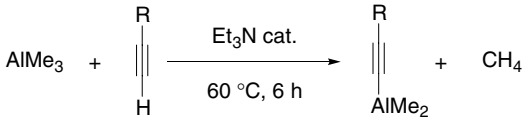
Encouragingly, when 4 equiv of the phenylacetylene-derived dimethylalane was reacted with nitrone **1**[15] (obtained from D-arabinose) in toluene, the desired propargylic *N*-hydroxylamine **2** was obtained in 96% yield, with a good diastereoselectivity (Table 1, entry 1). The amount of alkynylalane was next decreased: similar results were obtained using 4 or 2 equiv (entries 1 and 2). However, when only 1 equiv of dimethylalkynylalane was reacted with nitrone **1** the reaction was not completed after 24 h and the diastereoisomeric ratio decreased to 75:25 (entry 3). The lower diastereoselectivity observed in this case was not due to equilibration during the time of the reaction, as no change in diastereomeric ratio was observed when quenching the reaction after 3 h, 18 h or 24 h. The use of THF as a solvent (entry 4), as well as pre-complexation of the nitrone **1** with diethylaluminum chloride[16] (entry 5) also led to a significant decrease of diastereoisomeric excesses.

Keywords: Carbohydrate nitrones; Alkynylation; Alanes; Diastereoselective nucleophilic addition; *N*-Hydroxypyrrolidine.
* Corresponding authors. Tel.: +33 540 00 62 87 (V.D.); tel.: +33 476 51 48 03 (S.P.); e-mail addresses: v.desvergnes@ism.u-bordeaux1.fr; sandrine.py@ujf-grenoble.fr
† Present address: Université Bordeaux I, Institut des Sciences Moléculaires, UMR 5255-CNRS, 33405 Talence Cedex, France.

0040-4039/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2007.06.083



Scheme 1.



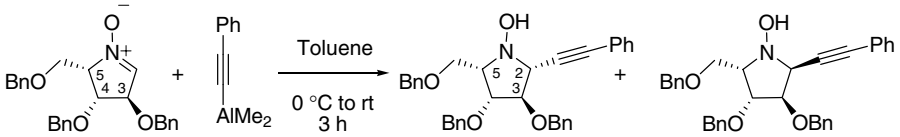
Scheme 2.

The configuration at the new stereocenter was assigned unambiguously from the connectivities determined by NOESY experiments: a cis relationship was observed between H-2 and H-5 in the major diastereoisomer in agreement with a 2,3-trans configuration.

At this stage, it is noteworthy to mention that using the 'one pot' process recently described by the group of Micouin,[14] alkynylation of nitrone **1** with phenylacetylene was sluggish. The products of methylation **3a**,**b** were major and only 20% yield of the expected propargylic *N*-hydroxylamine **2a** was obtained (Scheme 3).[17] This observation suggests that in the case of polybenzylated five-membered ring cyclic nitrones such as **1**, which may be less basic than the nitrones used in Micouin's work, the 'substrate-catalyzed' metallation process is less efficient.

The best conditions found in our preliminary study (Table 1, entry 2) were then used for the addition of various alkynylalanes to nitrone **1** (Table 2).[18] The obtained propargylic *N*-hydroxypyrrolidines were generally isolated in good yields, except when ethynylcyclohexene was used as the alkyne (entry 2). Surprisingly however,

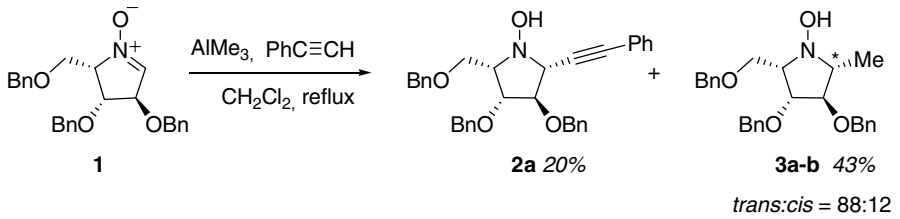
Table 1.



1 **2a** **2b**

Entry	Equiv of alane	Additive	Solvent	Yield (%)	dr[a] (**2a**:**2b**)
1	4	—	Toluene	96	93:7
2	**2**	—	**Toluene**	**96**	**92:8**
3[b]	1	—	Toluene	96	75:25
4	4	—	THF	>98[c]	68:32
5[d]	2	Et_2AlCl	Toluene	>98[c]	65:35

[a] Diastereoisomeric ratio determined after integration of signals on 2D-HSQC NMR maps of crude reaction mixtures.
[b] Reaction time: 24 h.
[c] Conversion from NMR analysis of crude mixtures. No traces of starting material were observed.
[d] Nitrone **1** was precomplexed with 1 equiv of Et_2AlCl prior to the addition to alkynylalane.

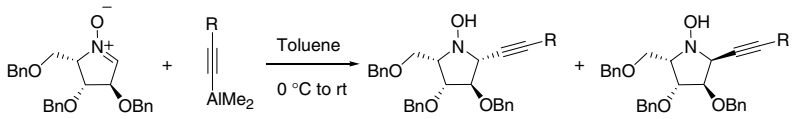


1 **2a** *20%* **3a-b** *43%*

trans:cis = 88:12

Scheme 3.

Table 2.[a]



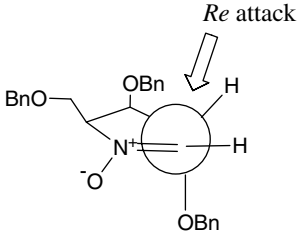
1 4-8a 4-8b

Entry	R	Product	Reaction time (h)	Yield (%)	dr[b] (**a**:**b**)
1	CH_2Ph	**4**	3	76	63:37
2		—	24	—	—
3	$(CH_2)_3Cl$	**5**	3	76	59:41
4		**6**	3.5	88	40:60
5	CH_2NMe_2	**7**	17	42[c]	34:66
6	$(CH_2)_4CH_3$	**8**	3	93	37:63
7[d]	$(CH_2)_4CH_3$	**8**	3	98	65:35

[a] Reactions were performed using 2 equiv of alkynylalane, unless otherwise stated.
[b] Diastereoisomeric ratio determined after integration of signals on 2D-HSQC NMR maps of crude reaction mixtures.
[c] Diastereomeric products were not separated.
[d] 4 equiv of alkynylalane were used.

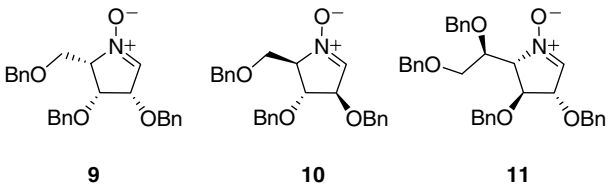
when R groups were different from phenyl, diastereoselectivities were not as good, and diastereoisomeric ratios were even inverted with *N*-containing alkynes (entries 4 and 5). Particularly intriguing were the results obtained when dimethylheptynylalane was added to nitrone **1**, a significant influence of its amount in reaction mixtures being observed (entries 6 and 7).

Understanding the stereoselectivities observed in this work is not straightforward.[19] However, when no heteroelement is present in the alkyne substrate, the major product (2,3-trans isomer) results from addition on the *Re* face of nitrone **1**. Such an attack can be rationalized by the classical Felkin–Anh model (Fig. 1).



Felkin-Anh model

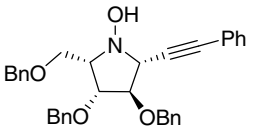
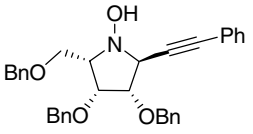
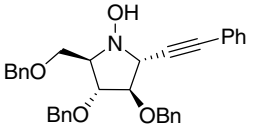
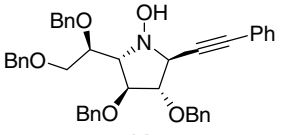
Figure 1. Predictive model for nucleophilic addition onto nitrone **1**.



9 **10** **11**

Figure 2.

Table 3. Addition of dimethyl-2-phenylethynylalane onto nitrones **1**, **9**–**11**[a]

Entry	Nitrone	Products[b]	Yield (%)	dr[c] trans:cis
1	**1**	 **2**	96	92:8
2	**9**	 **12**	88	>98:2[d]
3	**10**	 **13**	96	96:4
4	**11**	 **14**	92	>98:2[d]

[a] Conditions (see typical procedure in Ref. 18): 1 equiv of nitrone in toluene and 2 equiv of alkynylalane were stirred from 0 °C to room temperature for 3 h.
[b] All new products were fully characterized by the usual analytical and spectroscopic methods, and their relative configuration at the new stereocenter was assigned from NOESY experiments.
[c] Diastereoisomeric ratio determined after integration of signals on 2D-HSQC NMR maps of crude reaction mixtures.
[d] The cis isomer could not be detected by NMR.

In order to estimate the relative importance of the configuration of each stereocenter in various nitrones and to extend the synthetic value of this approach, carbohydrate-derived nitrones **9**,[20] **10**[2,21] and **11**[1] were also prepared and used in this reaction (Fig. 2).

Thus, dimethyl-2-phenylethynylalane was added to nitrones **9**–**11** to yield the propargylic *N*-hydroxypyrrolidines **12**–**14**, respectively, in high yields and with good diastereoisomeric excesses (Table 3). In the case of propargylic *N*-hydroxypyrrolidines **12** and **14**, a single isomer was detected by NMR (^{1}H spectra and HSQC 2D maps). In nitrone **9**, all the substituents being on the same side of the cycle, excellent facial selectivity occurs with attack on the opposite *Si* face (entry 2). In the hexofuranose-derived nitrone **11**, the C-5 substituent is bulkier than in nitrone **10**, leading to an even better facial selectivity (entry 4). In all cases, the 2,3-trans diastereoisomer was obtained as the main product, confirming that the *configuration at C-2 plays a major role in steering the approach of the alane nucleophile*, while configuration at C-4 (compare entry 3 to entry 1) and at C-3 (compare entry 4 to entry 2) seem to have little influence on the stereochemical outcome of alane additions.

In conclusion, alkynylanes turn out to be efficient, non basic organometallic reagents for nucleophilic addition onto nitrones. We report herein the diastereoselective addition of alkynylalanes on carbohydrate-derived nitrones, leading to highly functionalized intermediates for iminosugar syntheses.

Acknowledgments

This work was funded by the 'Agence Nationale pour la Recherche' (Grant No. ANR-05-JCJC-0130-01). Christelle Pillard thanks the A.N.R. for a postdoctoral fellowship. The authors are also grateful to Professor O. R. Martin (ICOA, Université d'Orléans), Professor P. Dumy (DCM, Université de Grenoble) and to the CNRS for additional support.

References and notes

1. Liautard, V.; Christina, A. E.; Desvergnes, V.; Martin, O. R. *J. Org. Chem.* **2006**, *71*, 7337–7345.
2. Desvergnes, S.; Py, S.; Vallée, Y. *J. Org. Chem.* **2005**, *70*, 1459–1462.
3. Desvergnes, S.; Desvergnes, V.; Martin, O. R.; Itoh, K.; Liu, H-w; Py, S. *Bioorg. Med. Chem.*, in press, doi:10.1016/j.bmc.2007.06.059.
4. Masson, G.; Py, S.; Vallée, Y. *Angew. Chem., Int. Ed.* **2002**, *41*, 1772–1775; Masson, G.; Cividino, P.; Py, S.; Vallée, Y. *Angew. Chem., Int. Ed.* **2003**, *42*, 2265–2268; Riber, D.; Skrydstrup, T. *Org. Lett.* **2003**, *5*, 229–231.
5. For a recent review on enantiopure cyclic nitrones, including carbohydrate-derived nitrones, see: Revuelta, J.; Cicchi, S.; Goti, A.; Brandi, A. *Synthesis* **2007**, 485–504.
6. For reviews on nucleophilic additions to various C=N bond-containing substrates, see: Bloch, R. *Chem. Rev.* **1998**, *98*, 1407–1438; Enders, D.; Reinholdt, U. *Tetrahedron: Asymmetry* **1997**, *8*, 1895–1946.
7. For additions of unsaturated organometallics species on nitrones, see: Merino, P. *C.R. Chimie* **2005**, *8*, 775–788; Lombardo, M.; Trombini, C. *Synthesis* **2000**, 759–774; see also: Helms, M.; Schade, W.; Pulz, R.; Watanabe, T.; Al-Harrasi, A.; Fisera, L.; Hlobilova, I.; Zahn, G.; Reissig, H.-U. *Eur. J. Org. Chem.* **2005**, 1003–1019, and references cited therein.
8. For a review on the preparation of propargylamines through direct addition of alkynes to C=N bonds, see: Zani, L.; Bolm, C. *Chem. Commun.* **2006**, 4263–4275.
9. Merino, P.; Anoro, S.; Castillo, E.; Merchan, F.; Tejero, T. *Tetrahedron: Asymmetry* **1996**, *7*, 1887–1890; Merino, P.; Franco, S.; Merchan, F. L.; Tejero, T. *Tetrahedron: Asymmetry* **1997**, *8*, 3489–3496; Denis, J.-N.; Tcherchian, S.; Tomassini, A.; Vallée, Y. *Tetrahedron Lett.* **1997**, *38*, 5503–5506; Merino, P.; Castillo, E.; Franco, S.; Merchan, F. L.; Tejero, T. *Tetrahedron: Asymmetry* **1998**, *9*, 1759–1769; Merino, P.; Franco, S.; Merchan, F. L.; Tejero, T. *J. Org. Chem.* **1998**, *63*, 5627–5630; Merino, P.; Franco, S.; Gascon, J. M.; Merchan, F. L.; Tejero, T. *Tetrahedron: Asymmetry* **1999**, *10*, 1867–1871; Ohtake, H.; Imada, Y.; Murahashi, S.-I. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 2737–2754; Dagoneau, C.; Denis, J.-N.; Vallée, Y. *Synlett* **1999**, 602–604; Dagoneau, C.; Tomassini, A.; Denis, J.-N.; Vallée, Y. *Synthesis* **2001**, *1*, 150–154; Goti, A.; Cicchi, S.; Mannucci, V.; Cardona, F.; Guarna, F.; Merino, P.; Tejero, T. *Org. Lett.* **2003**, *5*, 4235–4238; Patel, S. K.; Murat, K.; Py, S.; Vallée, Y. *Org. Lett.* **2003**, *5*, 4081–4084.
10. Tronchet, J.-M. J.; Mihaly, E. *Helv. Chim. Acta* **1972**, *55*, 1266–1271; Marco, J. A.; Carda, M.; Murga, J.; Portoles, R.; Falomir, E.; Lex, J. *Tetrahedron Lett.* **1998**, *39*, 3237–3240; Murga, J.; Portolés, R.; Falomir, E.; Carda, M.; Marco, J. A. *Tetrahedron: Asymmetry* **2005**, *16*, 1807–1816; Bonanni, M.; Marradi, M.; Cicchi, S.; Faggi, C.; Goti, A. *Org. Lett.* **2005**, *7*, 319–322.
11. Frantz, D. E.; Fässler, R.; Carreira, E. M. *J. Am. Chem. Soc.* **1999**, *121*, 11245–11246; Fässler, R.; Frantz, D. E.; Oetiker, J.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2002**, *41*, 3054–3056; Pinet, S.; Pandya, S. U.; Chavant, P.-Y.; Ayling, A.; Vallée, Y. *Org. Lett.* **2002**, *4*, 1463–1466; Patel, S. K.; Py, S.; Pandya, S. U.; Chavant, P.-Y.; Vallée, Y. *Tetrahedron: Asymmetry* **2003**, *14*, 525–528; Cantagrel, F.; Pinet, S.; Gimbert, Y.; Chavant, P.-Y. *Eur. J. Org. Chem.* **2005**, 2694–2701; Xu, Q.; Rozners, E. *Org. Lett.* **2005**, *7*, 2821–2824; Topic, D.; Aschwanden, P.; Fässler, R.; Carreira, E. M. *Org. Lett.* **2005**, *7*, 5329–5330.
12. Blanchet, J.; Bonin, M.; Chiaroni, A.; Micouin, L.; Riche, C.; Husson, H.-P. *Tetrahedron Lett.* **1999**, *40*, 2935–2938; Blanchet, J.; Bonin, M.; Micouin, L.; Husson, H.-P. *J. Org. Chem.* **2000**, *65*, 6423–6426; Blanchet, J.; Bonin, M.; Micouin, L.; Husson, H.-P. *Tetrahedron Lett.* **2001**, *42*, 3171–3173; Feuvrie, C.; Blanchet, J.; Bonin, M.; Micouin, L. *Org. Lett.* **2004**, *6*, 2333–2336.
13. Wang, B.; Bonin, M.; Micouin, L. *J. Org. Chem.* **2005**, *70*, 6126–6128.
14. Bunlaksananusorn, T.; Lecourt, T.; Micouin, L. *Tetrahedron Lett.* **2007**, *48*, 1457–1459.
15. Cicchi, S.; Marradi, M.; Vogel, P.; Goti, A. *J. Org. Chem.* **2006**, *71*, 1614–1619.
16. For reports on the effect of precomplexation of nitrones with Et_2AlCl on the diastereoselectivity of nucleophilic additions see: Dondoni, A.; Junquera, F.; Merchan, F. L.; Merino, P.; Schermann, M.-C.; Tejero, T. *J. Org. Chem.* **1997**, *62*, 5484–5496, and references cited therein; Merino, P.; Tejero, T.; Revuelta, J.; Romero, P.; Cicchi, S.; Mannucci, V.; Brandi, A.; Goti, A. *Tetrahedron: Asym-*

metry **2003**, *14*, 367–379; Merino, P.; Jimenez, P.; Tejero, T. *J. Org. Chem.* **2006**, *71*, 4685–4688.

17. Major diastereomer **3a**: (2*S*,3*R*,4*R*,5*R*)-3,4-bis-benzyloxy-2-benzyloxymethyl-5-methyl-pyrrolidin-1-ol: $[\alpha]_D^{20}$ +38 (*c* 0.78, MeOH). ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm) 7.34–7.23 (m, 15H), 5.63 (br s, 1H), 4.70–4.38 (m, 6H), 3.96 (dd, 1H, $J = 1.9$, 6.3 Hz), 3.92–3.76 (m, 2H), 3.49 (dd, 1H, $J = 1.9$, 6.6 Hz), 3.34–3.27 (m, 1H), 2.84 (ps. qt, 1H, $J = 6.4$ Hz), 1.30 (d, 3H, $J = 6.3$ Hz). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ (ppm) 138.2, 138.0, 128.5–127.7, 87.4, 80.2, 73.5, 72.3, 71.9, 69.6, 68.2, 68.0, 17.3. (+) MS (ESI) *m*/*z* 434 $[M+H]^+$, 456 $[M+Na]^+$. IR (NaCl, cm^{-1}) 3370, 3068, 3031, 2920, 2868, 1497, 1454, 1365, 1216, 1110, 1032, 1028, 751. Anal. Calcd for $C_{27}H_{31}NO_4$: C, 74.81; H, 7.21; N, 3.24. Found: C, 74.65; H, 7.63; N, 3.31.
18. *Representative procedure:* a solution of nitrone **1** (0.200 g, 0.48 mmol) in dry toluene (2 mL) was added to the alane solution (0.96 mmol, prepared in situ as in Ref. 13 at 0 °C. The reaction mixture was stirred for 3 h from 0 °C to room temperature and then quenched with an aqueous solution of 2 M Rochelle's salt (gas evolution). After 10 min of vigorous stirring, ethyl acetate (10 mL) was added. The organic phase was separated and the aqueous phase was extracted with ethyl acetate (3×10 mL). The combined organic layers were dried over $MgSO_4$. The solvent was evaporated and the crude product was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate 90/10, 85/15 then 80/20) to give the 2 diastereoisomers; (2*S*,3*R*,4*R*,5*R*)-3,4-Bis-benzyloxy-2-benzyloxymethyl-5-phenylethynyl-pyrrolidin-1-ol **2a**: beige solid; mp 121 °C. $[\alpha]_D^{20}$ +10 (*c* 1.10, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm) 7.47–7.43 (m, 2H), 7.31–7.20 (m, 18H), 5.95 (br s, 1H), 4.71–4.44 (m, 6H), 4.08 (dd, 1H, $J = 1.9$, 6.6 Hz), 4.01 (dd, 1H, $J = 1.9$, 6.6 Hz), 3.92 (dd, 1H, $J = 7.9$, 9.1 Hz), 3.84–3.78 (m, 2H), 3.42–3.34 (m, 1H). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ (ppm) 138.2, 138.0, 137.6, 131.9, 128.5–127.0, 122.9, 87.6, 86.3, 84.2, 80.3, 73.5, 72.1, 72.0, 69.1, 67.7, 65.5. (+) MS (ESI) *m*/*z* 520 $[M+H]^+$, 542 $[M+Na]^+$. IR (NaCl, cm^{-1}) 3568, 3057, 2977, 2918, 2853, 2307, 1587, 1492, 1453, 1264. (2*S*,3*R*,4*R*,5*S*)-3,4-Bis-benzyloxy-2-benzyl-oxymethyl-5-phenylethynyl-pyrrolidin-1-ol **2b**: yellow oil. $[\alpha]_D^{20}$ −63 (*c* 1.30, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm) 7.43–7.23 (m, 20H), 5.58 (br s, 1H), 4.71–4.47 (m, 7H), 4.24 (dd, 1H, $J = 4.4$, 8.0 Hz), 4.12 (dd, 1H, $J = 4.4$, 6.0 Hz), 3.90 (dd, 1H, $J = 6.9$, 9.4 Hz), 3.81–3.68 (m, 2H). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ (ppm) 138.4, 138.2, 137.7, 132.1, 128.5–127.6, 122.8, 89.3, 82.8, 82.3, 81.4, 73.6, 72.7, 72.4, 68.1, 67.0, 61.0. (+) MS (ESI) *m*/*z* 520 $[M+H]^+$, 542 $[M+Na]^+$. IR (NaCl, cm^{-1}) 3380, 3062, 3030, 2865, 1953, 1598. HRMS (ESI): Calcd: $m/z = 542.23073$ $[M+Na]^+$. Found $m/z = 542.2321$ (2 ppm) $[M+Na]^+$; Calcd: $m/z = 558.20467$ $[M+K]^+$. Found $m/z = 558.2066$ (4 ppm) $[M+K]^+$.
19. A non-chelated TS is favored in our case because of the cyclic nature of the substrates. Aggregated mixed-aluminum species could be involved in these reactions, but attempts to characterize them by NMR were unsuccessful.
20. Nitrone **9** was prepared through an adaptation of a previously described method (see Ref. 2). (2*S*,3*R*,4*S*)-3,4-Bis-benzyloxy-2-benzyloxymethyl-3,4-dihydro-*H*-pyrrole 1-oxide **9**: beige solid; mp 91–92 °C. $[\alpha]_D^{20}$ +51 (*c* 1.08, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.32–7.26 (m, 15H), 6.86 (dd, 1H, $J = 1.2$, 1.9 Hz), 4.74–4.57 (m, 7H), 4.40 (br t, 1H, $J = 5.6$, 5.8 Hz), 4.18–4.07 (m, 3H). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ (ppm) 138.1, 137.7, 137.6, 133.1, 128.8–127.9, 77.9, 74.8, 74.4, 73.7, 73.4, 72.8, 66.9. (+) MS (ESI) *m*/*z* 418 $[M+H]^+$, 440 $[M+Na]^+$. IR (NaCl, cm^{-1}) 3028, 2920, 2867, 1571, 1453, 1206, 1111, 725.
21. Cardona, F.; Faggi, E.; Liguori, F.; Cacciarini, M.; Goti, A. *Tetrahedron Lett.* **2003**, *44*, 2315–2318; Carmona, A. T.; Whigtman, R. H.; Robina, I.; Vogel, P. *Helv. Chim. Acta* **2003**, *86*, 3066–3073.