Pergamon

Tetrahedron Letters, Vol 35, No. 44, pp. 8243-8246, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01757-3

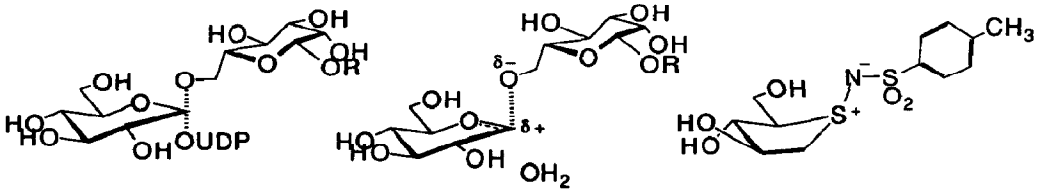
Synthesis of Iminothiasugar as a Potential Transition-State Analog Inhibitor of Glycosyltransfer Reactions

Hideya Yuasa,[§] Tetsuya Kajimoto,*,[§] and Chi-Huey Wong*,[§,¶]

§ Laboratory for Glycotechnology, Frontier Research Program, The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako, 351-01, Japan, and ¶ Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California 92037, USA

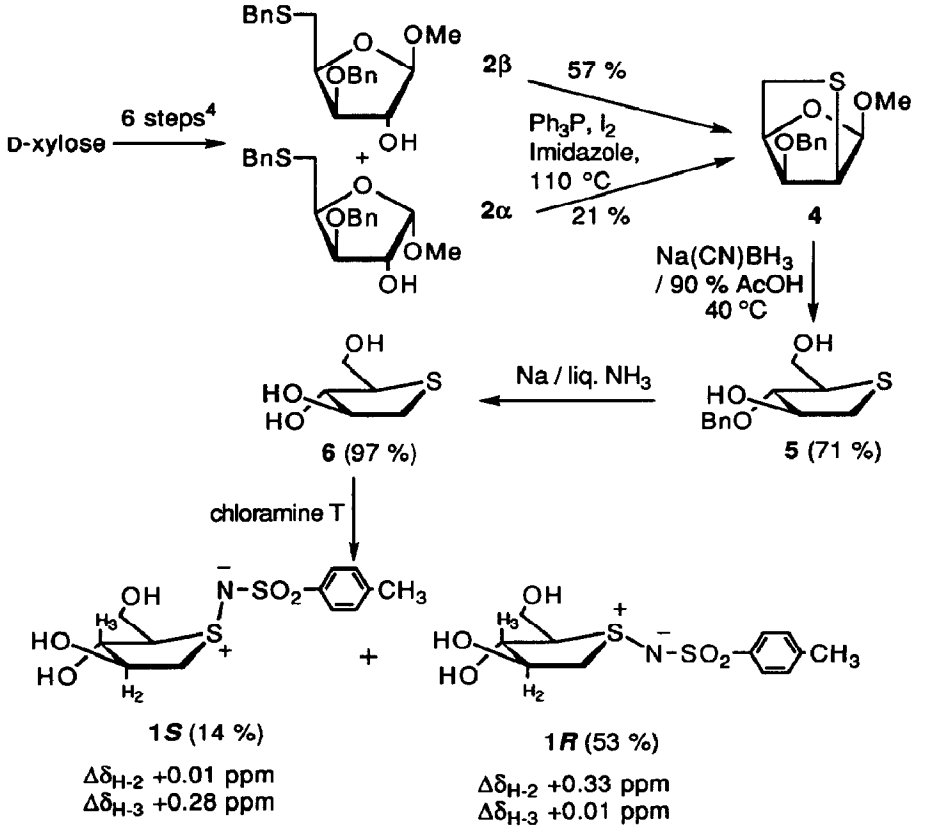
Abstract: Iminothiasugar **1**, a potential transition-state analog inhibitor of glycosidases, was synthesized in 10 steps from D-xylose.

Two important aspects that should be overcome or improved in the glycotechnology area are the facile syntheses of oligosaccharides and the control of the biosyntheses of them.[1] If a very strong and specific inhibitor of a certain glycosyltransferase is available, we may be able to block the biosynthesis of a certain oligosaccahride, and thereby control carbohydrate-mediated cell to cell recognition phenomena. One viable approach is to design a molecule mimicking the transition-state (TS) of glycosyltransfer reactions as such a specific inhibitor. Considering the TS of a glycosyltransfer reaction, we designed **1** as a mimic of the TS for glycosidases. Model consideration of compound **1** gives us the following features: i) the five-membered ring portion would mimic the half chair conformation of the TS, as many five-membered azasugars are potent inhibitors of glycosidases;[2] ii) the long C-S bond length (≈ 1.8 A) and the acute C-S-C angle (≈ 95°) would place the sulfur atom near the anomeric carbon of the TS; iii) the charge separation of the S-N bond would mimic the TS of the glycosidic bond ; and iv) the aromatic ring would mimic the hydrophobic face of the aglycon sugar in the TS. Iminothiasugars may also be used as haptens to elicit antibodies to catalyze aglycosyltransfer reaction.[3] To assess if these favorable features are valid, we synthesized **1** and tested its inhibitory effect on some glycosidases.

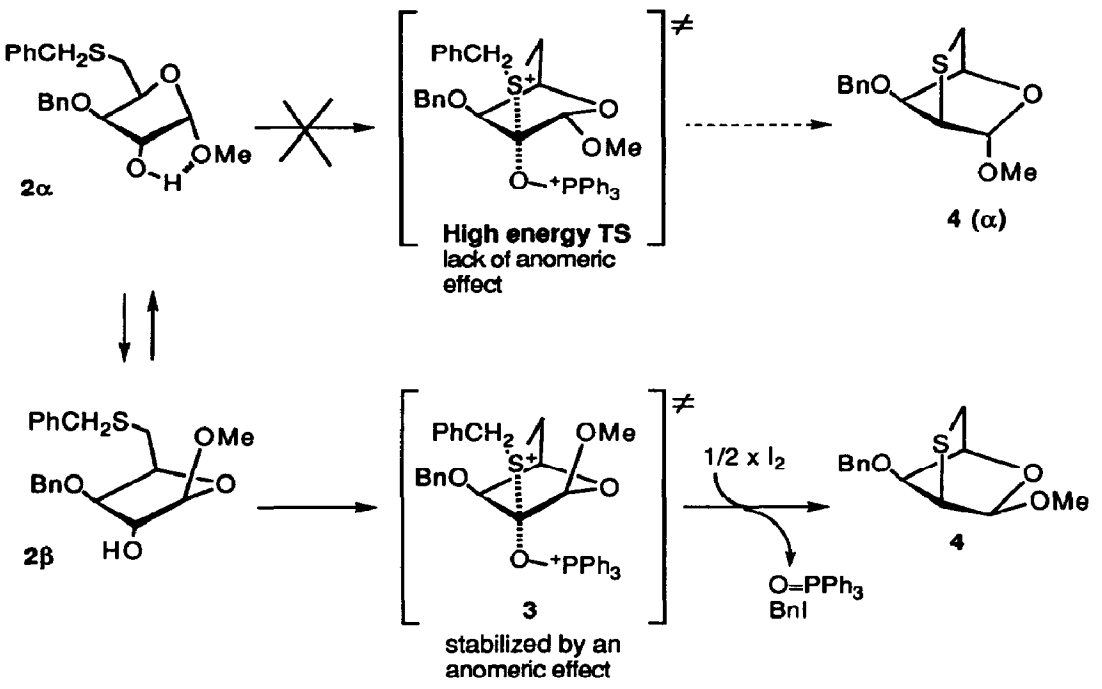


TS for glycosyltransferases TS for glycosidases 1

The synthesis (Scheme 1) was achieved in 10 steps starting from D-xylose which was first transformed in 6 steps into methyl 3,5-di-*O*-benzyl-5-thio-α and β-D-xylofuranoside (2α and 2β) as reported.[4] Both 2α and 2β were then subjected to an intramolecular cyclization[5] between 5-S and C-2 under the condition of iodination, using iodine, triphenylphosphine, and imidazole, to give exclusively compound **4**, with β configuration at the anomeric carbon, in 57 % from 2β and 21 % from 2α. This result suggests an equibrium between 2α and 2β in the reaction condition and the cyclization proceeds only from 2β possibly because of the anomeric effect on the reaction TS **3** (Scheme 2).[6] The anomerization of 2α is perhaps through a conformational change and a neighboring group assistance (general acid catalysis), followed by a glycosidic cleavage and reformation. The simultaneous hydrolysis and reduction, *in situ*, of **4** with $Na(CN)BH_3$ in aqueous acetic acid afforded **5**, which was then deprotected by Birch reduction, to give compound **6**. The ^1H and ^{13}C NMR spectra unambiguously indicated the structure of **6**.[7] Imination of **6** using chloramine T gave **1*S*** and **1*R*** in 14 % and 53 % yield, respectively.[8] The stereochemistry of **1** was determined by comparing the chemical shift of the ^1H NMR spectra with that of **6**. Significantly large downfield shifts, probably due to the anisotropy or the proximity effect from the S-N bond, were observed for H-2 in the *R* isomer and H-3 in the *S* isomer.[9]



Scheme 1



Scheme 2

The inhibitory effect of **1*R*** and **1*S*** toward β-glucosidase from almonds, *N*-acetyl-β-glucosaminidase from bovine kidney, and α-glucosidase from brewers yeast was then examined. Preliminary results showed that only **1*S*** would reduce the β-glucosidase activity. The other enzymes tested showed no inhibition by **1*R*** or **1*S*** at 1 mM. Kinetic measurements using Dixon plot on β-glucosidase and **1*S*** indicated K_i to be 1.7 ± 0.2 mM ($\Delta G \approx$ -3.8 kcal/mol) at 25 °C and 2.5 ± 0.3 mM ($\Delta G \approx$ -3.7 kcal/mol) at 37 °C (*p*-nitrophenyl-β-D-glucopyranoside as a substrate, pH 5.5, K_m = 4.0 mM at both temperatures). These results indicate that **1*S*** binds to β-glucosidase more strongly than the substrate but it is a weak inhibitor. Perhaps the lack of the 2-OH group in **1** and the aglycon moiety are attributed to the weak binding.[10] The fact that increase in temperature reduces the binding ability of **1*S*** suggests no entropical benefits from binding of **1** to the enzyme and thus that the hydrophobic face of the aromatic ring has little effect. This observation is consistent with the fact that the attachment of an aromatic ring to a strong inhibitor, as an aglycon mimic, usually has little effect on their inhibitory activity.[11]

In conclusion, though the inhibitory effect of **1*S*** is not as strong as expected, the results presented here represent an interesting entry to deoxythiosugars and iminothiasugars, and the concept of using trivalent sulfur atom as a mimic of anomeric center in the TS of glycosyltransfer reaction seems worth the effort for further development. Incorporation of iminothiasugar as a part of "bisubstrate inhibitor"[12] for glycosyltransferase is underway.

Acknowledgement

Support from the ministry of Education, Science, and Culture, Japan (Grant No. 06740500) is gratefully acknowledged. We thank Dr. Yasuo Shida, Tokyo College of Pharmacy, for measuring the mass spectra.

References

1. Kanie, O.; Hindsgaul, O. *Curr. Opin. Struct. Biol.* **1992**, *2*, 674–681. Halcomb, R. L.; Wong, C.-H. *ibid.* **1993**, *3*, 694–700. Look, G. C.; Fotsch, C. H.; Wong, C.-H. *Acc. Chem. Res.* **1993**, *26*, 182–190.
2. Takaoka, Y.; Kajimoto, T.; Wong, C.-H. *J. Org. Chem.* **1993**, *58*, 4809–4812; references cited therein.
3. The catalytic antibodies having a glycosidase activity have been explored: Reymond, J.-L.; Janda, K. D.; Lerner, R. A. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1711–1713. Yu, J.; Hsieh, L. C.; Kochersperger, L.; Yonkovich, S.; Stephans, J. C.; Gallop, M. A.; Schultz, P. G. *ibid.* **1994**, *33*, 339–341.
4. Nestadt, B.; van Es, T. *Tetrahedron* **1968**, *24*, 1973–1980.
5. Classon, B.; Garegg, P. J.; Sammuelsson, B.; Liu, Z. *J. Carbohydr. Chem.* **1987**, *6*, 593–597.
6. The conformation of **2**α and **2**β is supported by *J*-values in the ^{1}H NMR: **2**α (^{0}E), $J_{1,2}$ 5, $J_{2,3}$ 3, $J_{3,4}$ 6 Hz; **2**β ($^E_0$), $J_{1,2}$ 0, $J_{2,3}$ 3, $J_{3,4}$ 6 Hz.
7. **6**: $[\alpha]_D^{27}$ +40.2° (c 1.28, MeOH); ^{1}H NMR (CD_3OD), δ* 4.11 (1H, q, *J* 6 Hz, H-2), 3.84 (1H, t, *J* 6 Hz, H-3), 3.81 (1H, dd, $J_{4,5a}$ 5.3, $J_{5a,5b}$ 11.2 Hz, H-5a), 3.59 (1H, dd, $J_{4,5b}$ 6.6 Hz, H-5b), 3.22 (1H, q, *J* 6 Hz, H-4), 2.99 (1H, dd, $J_{1a,2}$ 5.6, $J_{1a,1b}$ 10.9 Hz, H-1a), 2.70 (1H, dd, $J_{1b,2}$ 6.3 Hz, H-1b); ^{13}C NMR (CD_3OD), δ** 81.4 (C-3), 80.0 (C-2), 66.3 (C-5), 54.5 (C-4), 35.5 (C-1). (* CH_3OH 3.3 ppm as standard, ** CD_3OD 49.8 ppm as standard); FAB MS m/z 151 (M+H).
8. **1*S***: R_f, 0.23 (CH_3Cl-MeOH 3:1); $[\alpha]_D^{24}$ +115.5° (c 0.73, H_2O); ^{1}H NMR (D_2O), δ* 7.58, 7.26 (each 2H, each d, *J* 7.9 Hz, aromatic), 4.12 (2H, m, H-2, H-3), 3.84 (3H, m, H-1a, H-5a, H-5b), 3.36 (1H, q, *J* 6.9 Hz, H-4), 2.62 (1H, dd, $J_{1b,2}$ 5.3, $J_{1a,1b}$ 14.2 Hz, H-1b), 2.26 (3H, s, CH_3Ar); ^{13}C NMR (D_2O), δ** 143.2, 137.6, 129.0, 125.0, 124.9 (aromatic), 75.4, 73.8 (C-2, C-3), 61.5 (C-4), 54.6 (C-5), 50.7 (C-1), 19.7 (CH_3Ar); FAB MS m/z 320 (M+H).

   1*R*: R_f 0.19 (CH_3Cl-MeOH 3:1); $[\alpha]_D^{24}$ –4.8° (c 0.90, H_2O); ^{1}H NMR (D_2O), δ* 7.53, 7.20 (each d, *J* 7.3 Hz, aromatic), 4.44 (1H, q, *J* 5.6 Hz, H-2), 3.85 (1H, t, *J* 5.6 HZ, H-3), 3.68 (1H, dd, $J_{4,5a}$ 4.6, $J_{5a,5b}$ 12.5 Hz, H-5a), 3.52 (1H, dd, $J_{4,5b}$ 8.2 Hz, H-5b), 3.36 (1H, dd, $J_{1a,2}$ 6.6, $J_{1a,1b}$ 13.5 Hz, H-1a), 3.18 (1H, m, H-4), 3.15 (1H, dd, $J_{1b,2}$ 5.0 Hz, H-1b), 2.22 (3H, s, CH_3Ar); ^{13}C NMR (D_2O), δ** 143.3, 137.2, 129.0, 125.3, 125.0 (aromatic), 74.8 (C-3), 74.2 (C-2), 74.0 (C-4), 57.2 (C-5), 50.7 (C-1), 19.7 (CH_3Ar), (* CH_3CN 1.9 ppm as standard, ** CH_3CN 118.2 ppm as standard); FAB MS m/z 320 (M+H).
9. Similar proximity effect of a sulfoxide bond on the ^{1}H NMR of 5-thio-D-glucopyranose *S*-oxide has been observed: Yuasa, H.; Takenaka, A.; Hashimoto, H. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 3473–3479.
10. Kajimoto, T.; Liu, K. K.-C.; Pederson, R. L.; Zhong, Z.; Ichikawa, Y.; Porco, J. A., Jr.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 6187–6196.
11. Blériot, Y.; Genre-Grandpierre, A.; Tellier, C. *Tetrahedron Lett.* **1994**, *35*, 1867–1870.
12. Palcic, M. M.; Heerze, L. D.; Srivastava, O. M.; Hindsgaul, O. *J. Biol. Chem.* **1989**, *264*, 17174–17181.

(*Received in Japan* 29 *June* 1994; *accepted* 19 *August* 1994)