shown that the hydrogenolysis of chiral benzylamines over Pd catalysts tends to proceed with inversion of configuration,[9] the stereoselectivity is not necessarily high and sometimes racemization[10] and even retention of configuration[11] are observed, and the rationalization for those results is still controversial.[12] The present results provide the first clear evidence for the stereochemical course (complete inversion) of the hydrogenolysis of strained chiral benzyl-*amide* bonds over Pd catalysts.[13]

The significance of the present findings is not only the elucidation of the stereochemistry of the reaction but also its application to the synthesis of deuterium- or tritium-labeled homochiral peptides[14] since regiospecific and stereoselective labeling of C_3 positions of α-amino acid residues is extremely difficult based on conventional organic transformations.[15] The C_3-labeled homochiral peptides will play an important role (i) for the study of metabolism since C_3-labeling does not disappear through racemization (C_2-labeling will be lost by racemization), (ii) for the conformational analysis of physiologically active peptides in their binding sites by NMR spectroscopy, and (iii) for the mechanistic study of oxygenases which may produce phenylserine derivatives since such oxidation by enzymes will proceed stereoselectively distinguishing two diastereotopic benzyl protons. Although we demonstrate the usefulness of our stereoselective as well as regio- and stereospecific labeling method only with deuterium, its extention to tritium labeling is straightforward. In fact, we were successful in the stereoselective synthesis of C_3-tritiated dipeptides following the above-mentioned procedure with the use of T_2 instead of D_2 and THF instead of methanol-d_1.[16] The results will be reported elsewhere.

Further studies along this line are actively under way.

Acknowledgment. This work was supported by a grant from the Center for Biotechnology, SUNY at Stony Brook, which is sponsored by the New York State Science and Technology Foundation. The generous support from Ajinomoto Co., Inc., is also gratefully acknowledged.

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(10) (a) Dahn, H.; Garbarino, J. A. *Bull. Soc. Vaudoise Sci. Nat.* **1968**, *70*, 27-30. (b) Dahn, H.; Garbarino, J. A.; Murchu, C. O. *Helv. Chim. Acta* **1970**, *53*, 1370-1378.

(11) Mitsui, S.; Sato, E. *Nippon Kagaku Zasshi* **1965**, *86*, 416-418.

(12) The stereochemistry of the hydrogenolysis of chiral tertiary benzyl-oxygen and benzyl-nitrogen bonds was studied in 1960-1970s by several groups[9-11,17] and it was shown that benzyl-oxygen bonds were cleaved with inversion of configuration over Pd or Pt catalysts and with retention of configuration over Raney-Ni whereas the stereochemistry of benzyl-nitrogen bond cleavage was complicated and inconsistent.

(13) It is well-known that the hydrogenolysis of benzyl-nitrogen bonds is not as easy as that of benzyl-oxygen bonds and is frequently accompanied by hydrogenation of aromatic rings even in the cases of simple benzylamines.[17a] Moreover, it is also known that the hydrogenolysis of benzyl-*amide* bonds cannot be achieved under normal conditions: Addition of strong acid and high pressures are necessary to promote the reaction, which usually suffers from severe side reactions.[18] Consequently, it is apparent that the strain energy of β-lactams is indispensable for the facile and clean reductive cleavage in the present systems: See also ref 3c.

(14) At present the applicability of this method is restricted to the labeling of aromatic amino acid residues such as phenylalanine, tyrosine, tryptophan, histidine, and dopa. Nevertheless, its usefulness is obvious since there are so many physiologically important peptides which include aromatic amino acid residues.

(15) E.g.: (a) Marche, P.; Girma, J. P.; Morgat, J. L.; Fromageot, P.; Ghelis, C.; Dubrasquet, M.; Bonfils, S. *Eur. J. Biochem.* **1975**, *50*, 375-382. (b) Marche, P.; Morgat, J. L.; Fromageot, P.; Kerdelhue, B.; Jutisz, M. *FEBS Lett.* **1972**, *26*, 83-86. (c) Brundish, D. E.; Wade, R. In *Peptides—Structure, Biology and Function, Proceedings of the American Peptide Symposium*; Gross, E., Meienhofer, J., Eds.; Pierce Chem. Co.: Rockford, IL, 1979; pp 483-486. (d) Brundish, D. E.; Combe, M. G.; Wade, R. *J. Labelled Compd. Radiopharm.* **1983**, *20*, 869-886.

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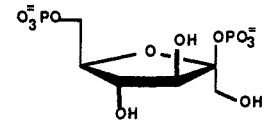
New Approaches to Enzyme Regulators. Synthesis and Enzymological Activity of Carbocyclic Analogues of D-Fructofuranose and D-Fructofuranose 6-Phosphate

Craig S. Wilcox* and John J. Gaudino

Department of Chemistry
University of Texas at Austin
Austin, Texas 78712
Received January 31, 1986

The remarkable enzyme regulatory effects of fructose 2,6-diphosphate (Fru-2,6-P_2) have been revealed only recently.[1,2] This bioregulator (**1**) is a potent positive effector for phosphofructokinase (EC 2.7.1.11) and inhibits 1,6-diphosphofructo-1-phosphatase (EC 3.1.3.11).[3] These enzymes are of major importance in controlling metabolic flux in the glycolytic pathway and the net effect of Fru-2,6-P_2 is to increase the rate of glycolysis and decrease the rate of gluconeogenesis. Analogues of fructose or fructose phosphates are therefore interesting potential agents for controlling diseases caused by errors in regulation of glycolysis.[4] This paper describes the first total syntheses of **2a** and **2b**, carbocyclic analogues of D-fructofuranose and 6-phospho-D-fructose, respectively.[5] Preliminary enzymological data for this new analogue of 6-phospho-D-fructose is also described.



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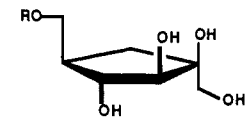

2a R = -H
2b R = -$PO_3^=$

Logical retrosynthetic considerations based on key ideas presented in our earlier work[6] suggested that the penultimate

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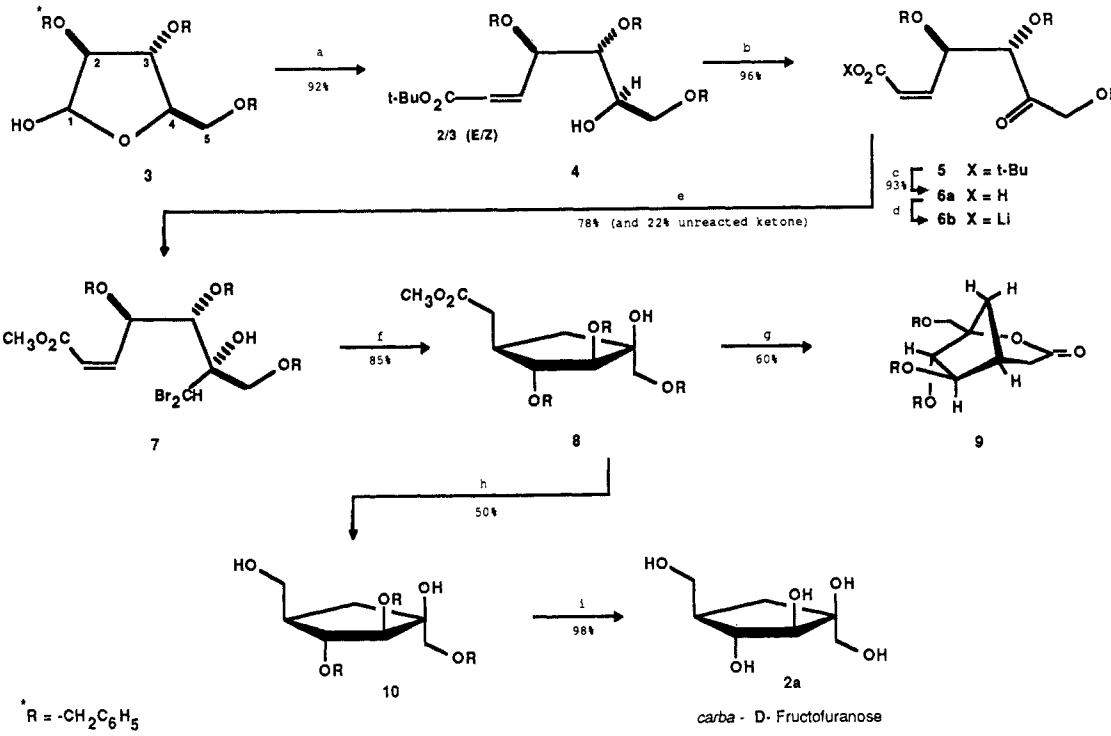
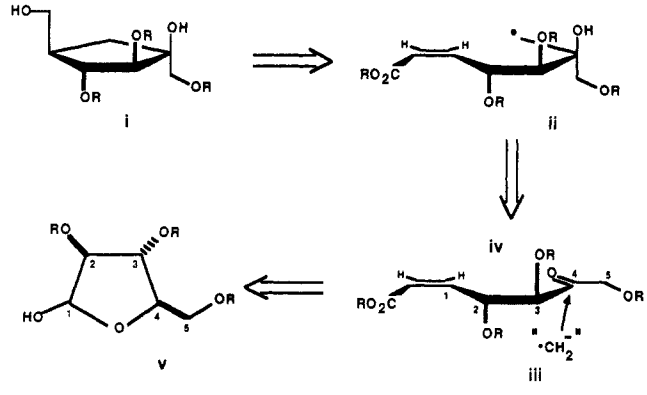
Scheme I



[a] (a) *t*-$BuCO_2CH{=}PPh_3$, CH_2Cl_2, 25 °C; (b) $Me_2SO/(COCl)_2$, Et_3N; (c) CF_3CO_2H/CH_2Cl_2, 0 °C; (d) 1.0 equiv of LDA; (e) CH_2Br_2, then LDA/−78 °C, CH_2N_2; (f) 5 equiv of Bu_3SnH, C_6H_6, 25 °C; (g) DBU, C_6H_6, 80 °C; (h) PhMgBr, then HOAc, then $O_3/(NaBH_4)$; (i) $Pd(OH)_2/C$, H_2, ethanol.

target, i, a selectively protected derivative of carba-D-fructofuranose (**2a**), might be obtained via cyclization of the radical ii. The radical ii would arise from addition of a one-carbon synthon, iii, to the ketose derivative iv. The use of this unitive synthon iii in conjunction with the radical cyclization strategy would allow an inexpensive D-arabinose derivative v to be efficiently transformed into the ultimate targets.



In practice, commercially available 2,3,5-tri-*O*-benzyl-D-arabinose (**3**) afforded, in two steps, the olefinic ketose **5** and its *E* isomer in 88% overall yield. These isomers (3:2, *Z*:*E*)[7] were simply separated by chromatography (SiO_2, EtOAc/Skelly B). Exploratory investigations wherein ketone **5** was treated with Grignard reagents, Wittig-type reagents, and alkyllithium reagents provided an expected result: anionic intermediates in these additions undergo intramolecular 1,4-addition and afford *C*-glycoside products or cause isomerization of the olefinic system. Further experimentation revealed that deprotection of the ester with trifluoroacetic acid provided acid **6a** and the carboxylate anion of this acid (**6b**) afforded good yields of ketone nucleophilic addition products without the complications observed with the ester.

The stage was thus prepared for the addition of the essential unitive synthon and closure of the cyclopentanoid ring. A search for synthetic reagents equivalent to iii resulted in the discovery that (dibromomethyl)lithium[8] reacts with keto carboxylate **6b** (formed in situ from **6a** and lithium diisopropylamide) to afford a single product stereoisomer, ester **7**, in 93% overall yield from keto ester **5** (based on recovery of 22% unreacted ketone). Subsequent cyclization of the unsaturated geminal dibromide **7** provided the desired hydroxy ester **8** in high yield. Preliminary data indicate that a cyclopentanoid bromohydrin intermediate is formed in this reaction but the rate of formation and the rate of debromination of this intermediate are closely competitive.[9]

Notably, this new protocol (featuring a unitive one-carbon synthon) results in a completely stereospecific conversion of the acyclic linear keto olefin into the desired cyclopentanoid ring in two efficient and consecutive carbon–carbon bond-forming steps. The relative stereochemistry about the two new stereogenic nuclei is confirmed by the fact that hydroxy ester **8** readily affords lactone **9**. Results of nuclear Overhauser experiments confirm the assigned structure for lactone **9** and do not support the alternative possibility. The stereochemical outcome of the nucleophilic addition is in accord with expectations based on stereoelectronic considerations. The stereochemical outcome of the radical cyclization is consistent with our earlier hypothesis on stereocontrol in such reactions.[6]

Barbier–Wieland degradation of ester **8** afforded the key intermediate **10**, a selectively protected derivative of carba-D-fructofuranose. At this stage the appropriateness of having begun with 2,3,5-tri-*O*-benzyl-D-arabinose may be appreciated: the three benzyl groups of the commercially available starting material are positioned on the D-fructose analogue exactly as required for formation of the desired phosphate derivatives. Hydrogenolytic

(6) Wilcox, C. S.; Thomasco, L. M. *J. Org. Chem.* **1985**, *50*, 546–547.

(7) This ratio may be compared with the 9:1 (*Z*:*E*) ratio obtained under similar conditions for 5-*O*-[*tert*-butyldimethylsilyl]-2,3-*O*-(1-methylethylidene)-D-ribose.[5] Obviously, these anomalously high *Z*:*E* ratios are not due *solely* to the presence of an α-alkoxy substituent.

(8) Taguchi, H.; Yamamoto, H.; Nozaki, H. *J. Am. Chem. Soc.* **1974**, *96*, 3010.

(9) Related processes which may afford practical syntheses of halohydrins are under investigation.

deprotection of **10** affords **2a**, carba-D-fructofuranose, in excellent yield (Scheme I).[10] Phosphorylation of **10** with dibenzyl-chlorophosphate and complete deprotection provided **2b**, carba-D-fructose 6-phosphate.

The important result from biological testing of **2b** is that these carba analogues of fructose are indeed substrates for the relevant enzymes of the glycolytic pathway.[11] Analogue **2b** is an excellent substrate for phosphofructokinase and 6-phosphofructo-2-kinase, with K_m from 5–20 times larger than that of the natural substrate, Fru-6-P. The analogue inhibits fructose-2,6-bisphosphatase with K_i about 500 times higher than that of Fru-6-P. Finally, preliminary results reveal that the diphosphate generated from **2b** by the action of 2-kinase is a potent positive effector for phosphofructokinase.

This work demonstrates a practical synthetic approach to important new analogues of fructofuranoid enzyme regulators. The synthesis illustrates an interesting heuristic principle that can be used to plan future syntheses: the acyclic, unbranched, chiral, and elaborately functionalized carbon chains that are readily available from carbohydrates can be neatly converted to polyhydroxylated and branched carbocycles by the use of appropriate unitive synthons. Further applications of this concept are under investigation.

Acknowledgment. The collaboration of Professor Kosaku Uyeda (University of Texas Health Science Center, Dallas) is gratefully acknowledged. This work is financially supported by a grant from the National Institute of Arthritis, Diabetes, Digestive, and Kidney Diseases.

(10) ^{1}H NMR (360 MHz, D_2O) δ 3.82 (1 H, dd, J = 8.7, 8.8 Hz) 3.75 (dd, 1 H, J = 5, 11 Hz), 3.67 (1 H, d, J = 8.7 Hz), 3.58 (dd, J = 7.3, 11 Hz), 3.51 (s, 2 H, 2.16 (dd, 1 H, J = 10, 14.6 Hz) 1.89 (m, 1 H) 1.45 (dd, J = 7.6, 14.6 Hz). ^{13}C NMR (90 Mz, D_2O) δ 81.32, 80.32, 79.24, 68.80, 66.43, 44.02, 36.21.

(11) Personal communication from Professor Kosaku Uyeda (University of Texas Health Science Center, Dalls), manuscript in preparation.

Mechanism for the Opsin Shift of Retinal's Absorption in Bacteriorhodopsin[1]

Johan Lugtenburg,* Maria Muradin-Szweykowska, Carola Heeremans, and Johannes A. Pardoen

The Department of Chemistry, State University of Leiden 2300 RA Leiden, The Netherlands

Gerard S. Harbison[†] and Judith Herzfeld[‡]

The Department of Physiology and Biophysics Harvard Medical School, Boston, Massachusetts 02115

Robert G. Griffin

The Francis Bitter National Magnet Laboratory Massachusetts Institute of Technology Cambridge, Massachusetts 02139

Steven O. Smith[§] and Richard A. Mathies

The Department of Chemistry, University of California Berkeley, California 94720

Received June 28, 1985

Dihydro derivatives of retinal were first used by Nakanishi et al.[2] in their pioneering development of a point charge model to explain how bacteriorhodopsin (BR) shifts the absorption maximum of its retinal protonated Schiff base (PSB) chromophore to the red. They measured the protein-induced shift of the absorption maximum (the "opsin shift") for selectively saturated chromophores. The opsin shift was reported to drop from 4870 cm^{-1} in the native chromophore, to 2500 cm^{-1} in the 5,6-dihydro derivative, and to only 1000 cm^{-1} in the 7,8-dihydro derivative. These data indicated that a negative bacterio opsin charge located near C_5=C_6 of the β-ionone ring is responsible for the opsin shift. A variety of BR analogue experiments have been performed subsequently to test the point charge model.[3] In our own experiments[4] it was useful to reexamine the 5,6- and 7,8-dihydroretinal data. While our 5,6-dihydro spectra are in agreement with the earlier work, our measured opsin shift for the 7,8-dihydroretinal derivative (3500 cm^{-1}) is larger than the originally published value. A large opsin shift for the 7,8-dihydro derivative would not be expected to result from a single negative point charge perturbation located near C_5=C_6. Thus it is evident that the current picture for the mechanism of the opsin shift in BR must be revised. ^{13}C NMR experiments[5] have supported the initial proposal that there is a negative protein charge near C_5=C_6 and have suggested that

Table I. Absorption Maxima and Bacterio Opsin Shifts for Retinal Analogues

		5,6-dihydro		7,8-dihydro	
chromophore	native	ref 2	this work	ref 2	this work
aldehyde, nm[a]	381	370	368	338	340
protonated Schiff base, nm[b]	440, 465[d]	425	428	395	385
pigment, nm[c]	568	476	475	400	445
opsin shift, cm^{-1}	5100, 3900[d]	2500	2300	1000	3500

[a] Synthesis described in ref 8. [b] Chloride salt of the *n*-butylamine Schiff base in methanol. [c] The sample consisted of membrane sheets suspended in H_2O at 1.5 °C.[9] [d] These values are for a planar 6-s-trans PSB as the reference state for the opsin shift rather than the twisted 6-s-cis conformer found in solution.[10]

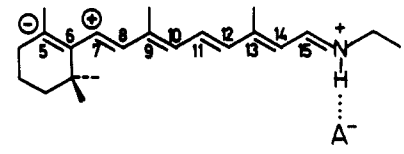


Figure 1. Model for the bacterio opsin binding site where the 6-s-trans chromophore interacts with a pair of opsin charges near C_5···C_7 The opsin shift data presented here support the idea that the retinal chromophore is perturbed by a negative charge near C_5 and a positive charge near C_7. However, it is also evident that the tendency for the negative charge near C_5 to red-shift the absorption is largely canceled out by the presence of the positive charge near C_7. This shows that much of the opsin shift is caused by protein–chromophore interactions near the Schiff base.

(1) Supported by the NIH (GM-23316, GM-23289, EY-02051, and RR-00995), the NSF (CHE-8116042 and DMR-8211416), the Netherlands Foundation for Chemical Research (SON), and the Netherlands Organization for the Advancement of Pure Research (ZWO).

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[†] Present address: Max Planck Institut for Polymerforschung, D-6500 Mainz, Federal Republic of Germany.

[‡] Present address: Chemistry Department, Brandeis University, Waltham, MA 02254.

[§] Present address: Francis Bitter National Magnet Lab, Massachusetts Institute of Technology, Cambridge, MA 02139.

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