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The chemistry of N-pentenyl glycosides: Synthetic, theoretical, and mechanistic ramifications

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Abstract

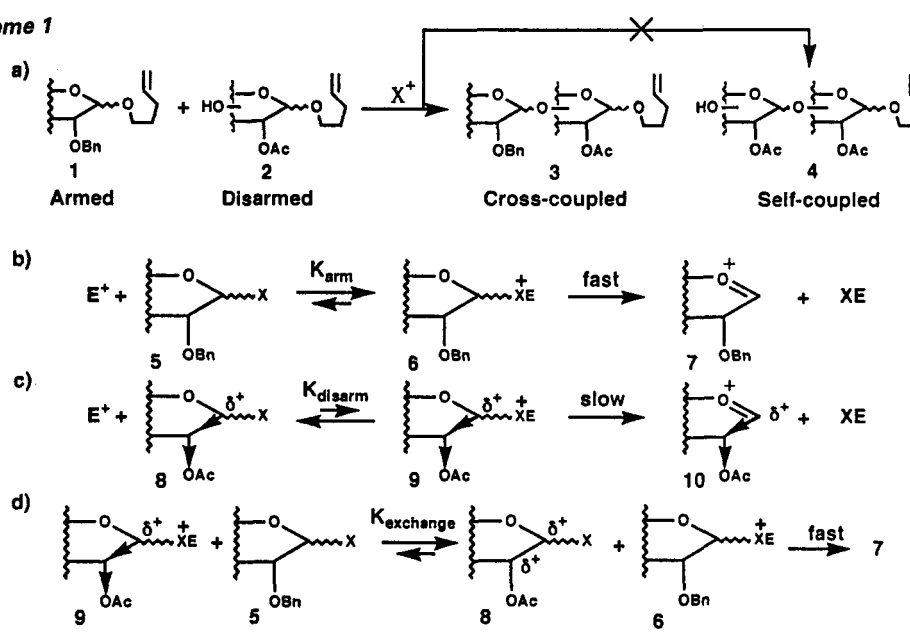
A feature of N-pentenyl glycosides (NPGs) is the ability to dibrominate the pentenyl double bond and, subsequently, regenerate it by reductive elimination. This allows a given NPG to serve (a) immediately as a glycosyl donor, or (b) after dibromination, as a glycosyl acceptor and, after subsequent reductive elimination as a glycosyl donor. These properties have been exploited to develop methodology for rapid assembly of homoglycans.

Based upon results from the oxidative hydrolysis of conformationally restrained NPGs, ab initio calculations to determine transition states of protonated axial and equatorial 2-methoxytetrahydropyran have been undertaken. The relevance of our results to the theory of stereoelectronic control (antiperiplanar lone pair hypothesis) and to the action of lysozyme is discussed.

SYNTHETIC STUDIES

The armed/disarmed strategy for saccharide coupling was first demonstrated with n-pentenyl glycosides (NPGs)[1]. Exploratory studies with these novel substrates had revealed substantial differences in reactivity depending on the nature of the C2 substituent, ethers being much more reactive than esters[2]. On this basis we showed that coupling of two pentenyl glycosides, **1** and **2**, could be regulated to give the cross-coupled product **3** in preference to the self-coupled product **4** (Scheme 1a). Shortly thereafter investigations in other laboratories[3] extended the procedure to other glycosyl donors, thereby indicating that the armed/disarmed strategy could have general applicability. We suggested the general rationalization shown in Scheme 1b and c[4].

Scheme 1



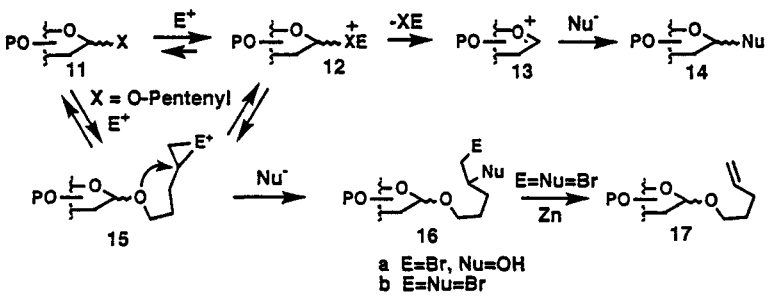
Because of the electron withdrawing group at C2 of **8** the activated intermediate, **9**, is seen to be destabilized by contiguous full and partial positive charges thereby favoring the reverse reaction, **9**--->**8**. Thus in a medium where **5** and **8** are made to compete for the electrophile, E^+, the situation depicted in Scheme 1d would prevail. The unstable intermediate **9** would transfer the electrophile to the armed partner **5** leading to the more stable species **6** which would then react rapidly to give the oxocarbenium ion **7** and thence the cross-coupled product **3**.

The situation in Scheme 1d represents a classic example of Le Chatelier's principle and would apply to the reaction of any glycosyl donor having an appropriate pairing of the anomeric substituent **X** and electrophile E^+.

Subsequent investigations gave rise to two seminal observations which are exploited in the work presented in this lecture. Firstly, the reactivity of disarmed NPGs was found to be highly dependent on the nature and source of the electrophile.[5] Thus substrates that reacted sluggishly, if at all, with iodonium dicollidine perchlorate were found to react "instantaneously" with iodonium ion derived from N-iodosuccinimide and triflates (ROTf; R = H, Ag, or Et_3Si). This observation was soon extended to the reactions of thioglycosides[3a,6,7] and can undoubtedly be extended to other glycosyl donors.

However the second observation, which follows, is restricted to NPGs.[4] It depends on the fact that whereas the activation step **11**--->**12** (Scheme 2) is a single event for other glycosyl donors, for NPGs there are two pre-equilibria, **11**--->**15** and **15**--->**12**. It is the second of these that controls activation of the glycosyl oxygen.[4]

Scheme 2

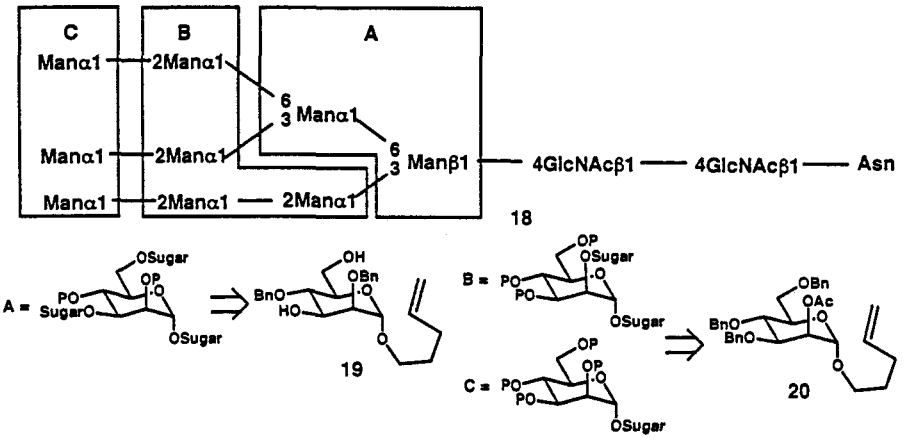


In this connection it is appropriate to ask why the reactions of an NPG in the presence of water leads to an aldose, **14**, (Nu = OH) rather than a halohydrin, **16a**. (Indeed a bromohydrin had been the desired product in the reaction sequence from which the chemistry of NPGs emanated).[8] We rationalized that the rate of the intramolecular reaction, **15**--->**12**, was so fast that the bimolecular reaction with water, to give a halohydrin could not compete. An increase in the concentration of water would enhance the rate of the bimolecular reaction without affecting the unimolecular process. Indeed added water led to formation of the bromohydrin **16a**. Similarly the vicinal dibromide **16b** could be obtained quantitatively by incorporating an excess of bromide ion in the reaction medium. This result was salutary, since treatment with zinc under conditions of reductive elimination readily regenerated the double bond in **17**.[4]

This circumstance provided us with the option of using a given NPG as a glycosyl donor or glycosyl acceptor, immediately or subsequently, depending on whether the double bond was "free" or "blocked" as the dibromide. We explored the potential of these options in the context of the high mannose glycoprotein **18**,[9] which attracts intensified interest because of its presence on the conserved V3 loop of the viral coat of HIV1.[10] Retrosynthetic analysis of the mannan component leads to three mannose subunits, **A**, **B**, and **C** which are attached to three, two, and one mannose residues respectively. Upon further retrosynthetic speculation, the NPG **20** can be seen to correspond to either **B** or **C** depending on whether the C2-ester serves as a temporary protecting group, as required for **B**, or permanent, as required for **C**.

*The retroanalysis therefore suggests that the nonamannose component of **18** can be assembled from only two differentially protected mannopyranose NPGs **19** and **20**.*

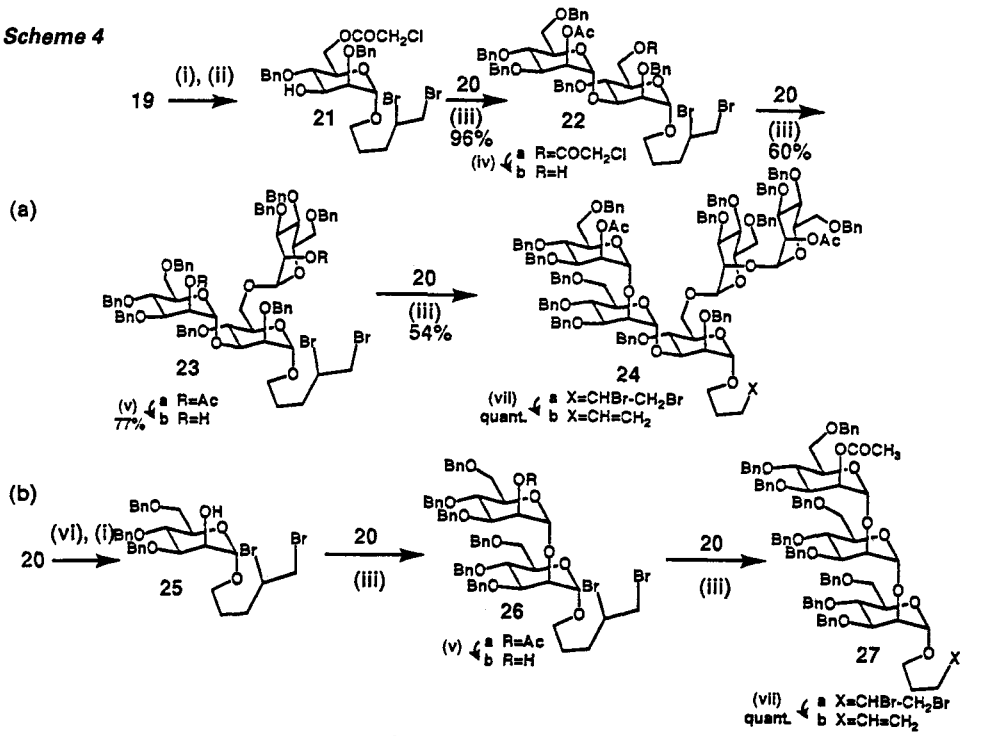
Scheme 3



These compounds were readily synthesized by adapting Ogawa's procedures for the corresponding methyl glycosides.[11]

The glycosyl donor **20** is disarmed while the alcohol bearing partner **19** is armed. This pairing is the reverse of that shown in Scheme 1a where it is the disarmed partner that bears the free hydroxyl. On the basis of Scheme 1, self-coupling of **19** would be the expected outcome. However the analysis in Scheme 2 suggests a method for reversal of the standard armed/disarmed roles.

Scheme 4



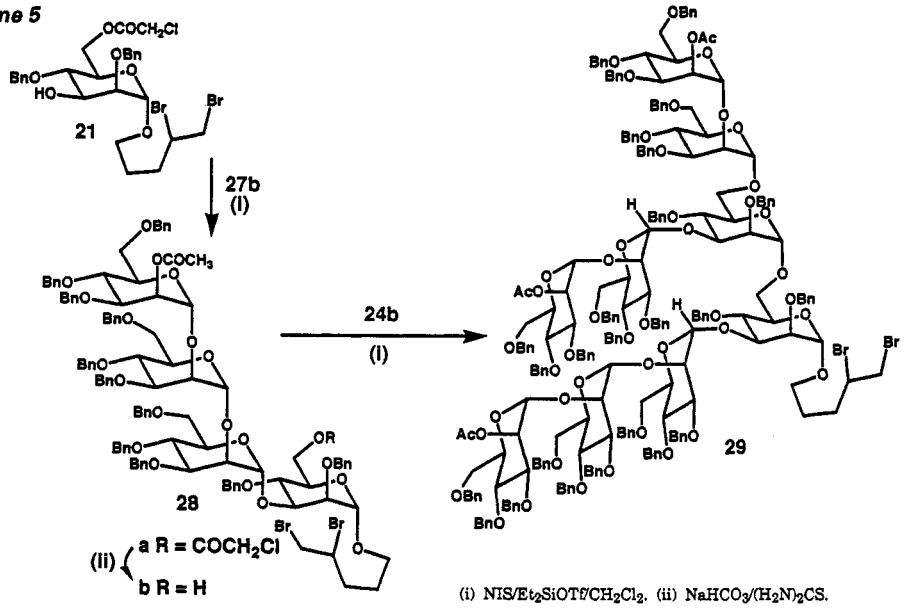
(i) Et_4NBr/Br_2.(ii) $ClCH_2COCl$. (iii) $NIS/Et_3SiOTf/CH_2Cl_2$. (iv) $NaHCO_3/(H_2N)_2CS$. (v) $NH_3/MeOH$. (vi) $K_2CO_3/MeOH$. (vii) $Zn/Bu_4NI/EtOH/EtOAc$.

Accordingly, compound **19** was treated with Br_2/Et_4NBr, and the resulting vicinal dibromide was selectively acylated to give **21** (Scheme 4a). Coupling of the latter with the disarmed glycosyl donor **20** under the standard conditions (see below) was "instantaneous" and gave **22a** in nearly quantitative yield. Sequential episodes of hydroxyl deprotection and coupling led to the pentasaccharide **24a**.

A similar strategy was applied to obtain the lowest antenna (Scheme 4b). Thus deacetylation of **20** followed by dibromination gave compound **25** which could then be

coupled with its progenitor **20**. The resulting disaccharide, **26a**, was then processed, following the pattern described above, to obtain trisaccharide **27a**.

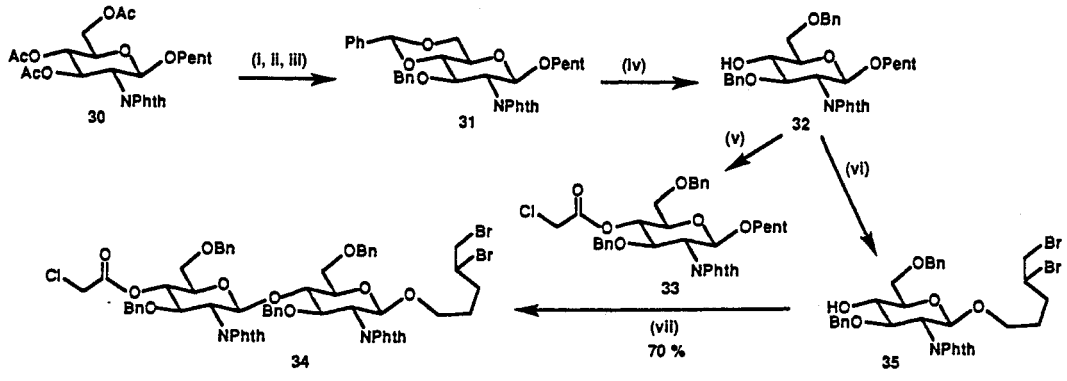
Scheme 5



Further reaction of the oligomannans **24a** and **27a** now required that their double bonds be freed. The most satisfactory method of effecting this transformation involved sonication with zinc in the presence of tetra-n-butyl ammonium iodide whereby **24b** and **27b** were obtained. Coupling of trimannan **27b** to previously described **21** proceeded smoothly to give the tetramannan **28a** (Scheme 5). Deprotection followed by coupling to the pentasaccharide **24b** then afforded the nonasaccharide **29**.

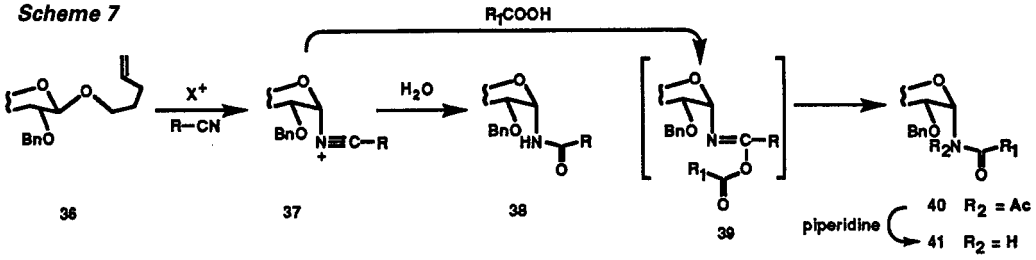
The exploratory studies depicted in Schemes 4 and 5 show that only two *primary* precursors are required for synthesis of the oligomannan, the pentenyl mannosides **19** and **20**. By application of the same basic strategy (see Scheme 6), the chitobiose moiety of the high mannose glycoprotein **18** (Scheme 3) required a single precursor, **32**, obtainable from the β-glucosaminide **30** by standard steps. Thus **32** was processed to give both glycosyl donor and acceptor, **33** and **35** respectively, and these were coupled to give the disaccharide **34**.

Scheme 6



(i) acetone, H_2O, HCl, 97%; (ii) $PhCH(OCH_3)_2$, TsOH, CH_3CN, 84%; (iii) NaH, BnBr, DMF, 83%; (iv) $NaCNBH_3$, HCl, THF, 96%; (v) $(ClAcO)_2O$, pyridine, CH_2Cl_2, 87%; (vi) Et_4NBr, Br_2, CH_2Cl_2, 35%; (vii) 1.3 eq **31**, NIS, Et_3 SiOTf, CH_2Cl_2, 70%.

Scheme 7

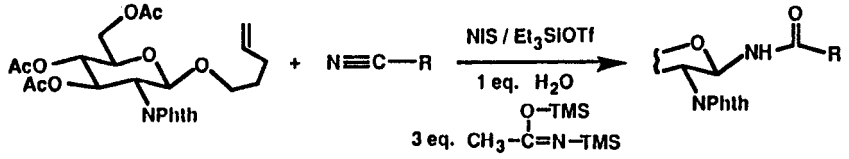


With regard to the β-N-linked aspartoyl residue of **18**, new chemistry had to be developed. Recent reports from our laboratory have described the process shown in Scheme 7 in which the glycosyl oxocarbenium ion from **36** is trapped by acetonitrile to give an acetonitrilium ion **37**.[12] This intermediate can be further trapped by water to obtain the amide **38**. However of greater seminal value was the fact that **37** could also be trapped by a carboxylic acid, R_1COOH, to give an imidic anhydride, **39**, which rearranged in *in situ* to give the N,N-diacyl derivative **40**.[13] Thus in the presence of a protected aspartic acid, an N-acetyl-N-aspartoyl product, corresponding to **40** was obtained. A procedure for selective N-deacetylation was developed whereby the asparagine **41** (R_1CO=aspartoyl) could be obtained in good yield.[14]

As indicated in Scheme 7, only the α product was obtained by this process and this proved valuable for the synthesis of an unusual glycopeptide.[14] (Our methodology has recently been adapted for thioglycosides by Sasaki and coworkers).[15] However a route to prepare the β-linked asparagine indicated in Scheme 3 was clearly desirable.

Use of neighboring group participation to control anomeric stereoselectivity was an obvious ruse. However an N-acetyl residue led to complex mixtures, and we therefore focussed attention on a phthalimido derivative. In view of the hydration reaction, **37**--->**38**, in Scheme 7, it was desirable to see whether this simple process could be effected for obtaining the desired β-oriented product. Indeed there was some success as is evident from Table 1, entries a, b and c. However complete failure was experienced with the crucial substrate in entry d.

Table 1 Hydration of Nitrilium Intermediates



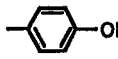
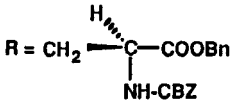
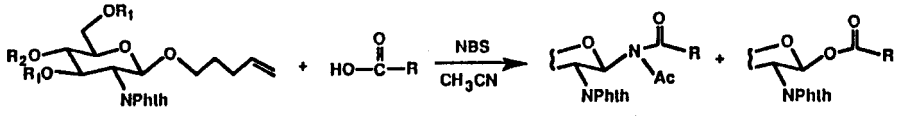
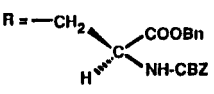
	nitrile	amide		nitrile	amide
a	R = CH_3	(48 %)	c	R = 	(22 %)
b	R = CH_2CH_3	(32 %)	d	R = 	none

Table 2 Trapping Acetonitrilium Ions with Carboxylic Acids

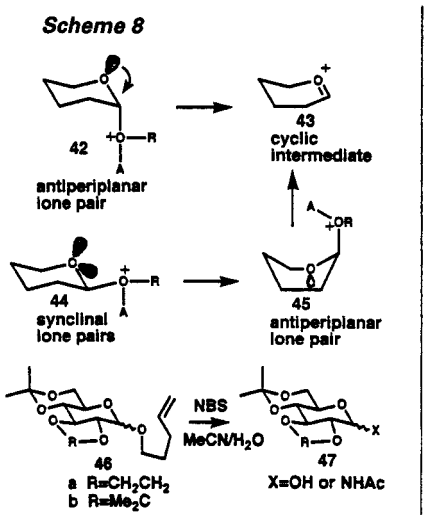


a	$R_1 = R_2 =$ Ac	R = 	(9 %)	(72 %)
b	ditto	R = 	(40 %)	(15 %)
c	R_1 = Bn R_2 = ClAc	ditto	(33%)	(9%)

This failure prompted us to test whether the two stage route **37**--->**39**--->**40** could be developed for synthesis of the β-anomer. The standard exploratory test using orthochlorobenzoic acid was unsuccessful as is apparent from Table 2 entry a, the major product being the glycosyl ester. However we were gratified to find that with the protected aspartic acid the product distribution was reversed, and the N,N-diacyl derivative could indeed be obtained as the major product. The results in entries b and c were encouraging and are currently being pursued further.

MECHANISTIC AND THEORETICAL STUDIES

The advantages offered by NPGs are not only in the realm of synthesis. Glycoside cleavage is of central importance to many synthetic and biological processes and as a result has always attracted mechanistic scrutiny.[16] A seminal postulate arising from the theory of stereoelectronic control[17] (or the antiperiplanar lone pair hypothesis[18]-ALPH) is illustrated in Scheme 8. Thus protonated α-glycosides, **42**, are presumed to cleave directly to give **43**, while β-glycosides must first proceed to a boat conformation, **44**--->**45**, wherein the leaving group is presented with an antiperiplanar lone pair.[17]



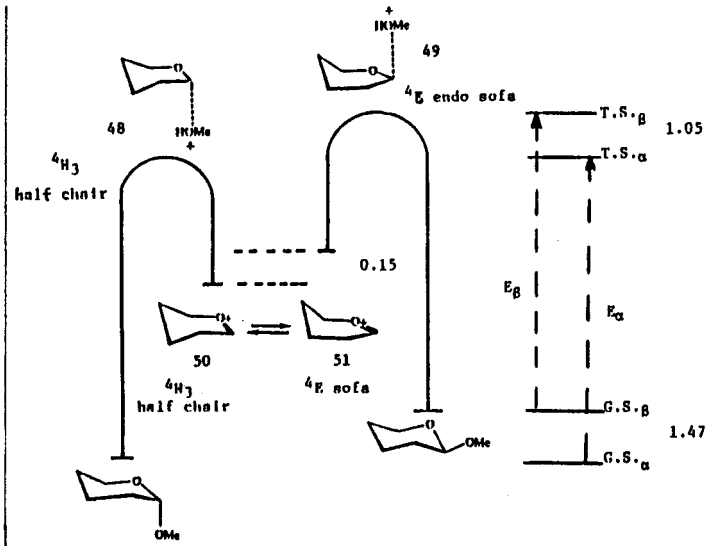
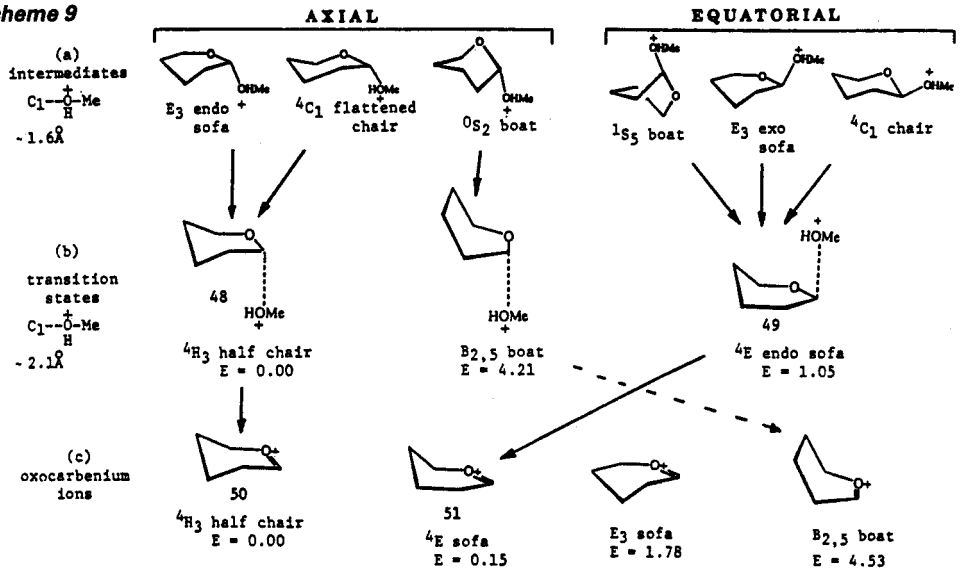


Figure 1. Energetics of Cleavage of 2-Methoxytetrahydropyran Anomers

The advent of NPGs provided an unique opportunity to examine the specific question of boat-like intermediates. The isopropylidenated glucosides, **46**, are immobile and their boat conformations are prohibitively high-energy species.[12] Attempts to use acids to study their anomeric reactivities would be futile since the isopropylidene rings would be cleaved before the glycosidic bonds. However the fact that NPGs can be activated with N-bromosuccinimide allowed us to examine the cleavage reactions under neutral conditions. In the event it was found that the β/α ratio for oxidative hydrolysis was 2:1 for **46a**, and ~ 1:1 for **46b**.[12] The 2:1 β/α ratio for cleavage of conformationally restrained **46a** is of the same order of magnitude as that observed for conformationally **mobile** glycosides.[19] Since boat conformations are impossible for the former (ie. **46**), there is no compelling reason to invoke their intermediacy for the latter.

A logical extension of these studies led us to probe,at the 6-31G* level, the intricate conformational changes which occur during glycoside hydrolysis using axial and equatorial 2-methoxytetrahydropyran as representatives of α- and β-glycosides respectively. Details of our methodology have been published[21] and some salient data are summarized in Scheme 9. The favored intermediates, based on structural and/or energetic grounds, are shown in (a). As indicated, their glycosidic bond lengths are ~ 1.6Å. Assuming a bond length of 2.1Å in the transition state, Scheme 9b shows some possible candidates along with their associated energies. Our investigations showed that the progress from intermediates to transition states is as indicated by the arrows.

Scheme 9



Thus the preferred transition states are $^4H_3$ half-chair, **48**, for the axial pyranoside, and ^4E endo sofa, **49**, for the equatorial counterpart.[21]

The energy distribution for various oxocarbenium ions is shown in (c). The stereochemical correspondence of the two low-energy ions, **50** and **51**, with the favored transition states in (b) was gratifying since it is consistent with a late transition state, a fact which has been established by kinetic investigations of glycoside hydrolysis.[22]

How valid are the transition state structures **48** and **49**? As a test we undertook[21] some calculations based on the energy data in Figure 1. We have found the difference in ground state energies for axial and equatorial 2-methoxytetrahydropyran to be 1.47 kcal, in good agreement with the values obtained by Wiberg and Murcko.[23] On the assumption that entropies and solvation energies are the same for the α- and β-anomers, and that the pre-exponential factors are equal, the Arrhenius equation can be applied as shown below, to compare rate constants for axial and equatorial

$$E_\beta + 1.47 = E_\alpha + 1.05 \qquad \therefore \qquad E_\alpha - E_\beta = 1.47 - 1.05 = 0.42$$

$$k_\beta/k_\alpha = \exp(-(E_\beta - E_\alpha))/RT = \exp(E_\alpha - E_\beta)/RT$$

cleavage. For a temperature of 75°C the β/α rate ratio is 1.8 which is very much in the range reported for several systems by Isbell and Frush.[19] At 23°C the predicted ratio is 1.9 and this agrees with the value of 2.0 found for the anomers of **46a**.[12]

The above-mentioned postulate[17] concerning the boat intermediate **45** (Scheme 8) takes on special significance in view of its relevance to the continuing inquiry into the action of lysozyme. A suggestion by Post and Karplus[24] that the transition state in lysozyme is chair-like and that protonation occurs on the ring oxygen added further complications. However a detailed analysis at 1.5Å resolution of a trisaccharide bound to subsites B, C, and D in the active-site cleft of hen egg-white lysozyme has recently been published by Strynadka and James[25] which appeared while our manuscript was in press. They show that the D-residue is distorted into a sofa corresponding to that which is depicted in Scheme 9 as E_3 exo. From their results and our own, the conclusion therefore appears to be that the enzyme preorganizes the substrate into a preferred conformation which lies on the pathway towards the transition state.

Our studies in this area as well as for the refinement of NPG methodology are continuing and will be reported in due course.

Acknowledgement

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