DESIGN OF CONFORMATIONALLY LOCKED INOSITOLS AND HEXOSES THROUGH CARBOCYCLIC ANNULATION

GOVERDHan MEHTA*, SENAIIAR S RAMESH AND SAIKAT SEN

Department of Organic Chemistry, Indian Institute of Science, Bangalore – 560012 (India)
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By conceptualizing the tactic of carbocyclic ring annulation, a new family of bicyclic, conformationally locked, bipolarofacial inositolis has been designed. These trans-fused annulated inositolis, based on decalin and hydrazide frameworks exist in unnatural axial-rich conformations. For example, bicyclic myo-inositol exist in (5a/1c) conformation compared to the (5e/1a) conformation present in the parent myo-inositol. The carbocyclic ring annulation stratagem has been extended to carbohydrates, thereby resulting in the synthesis of novel annulated glucose and gulose, which are locked in unnatural axial rich conformation. The synthetic sequence towards these novel inositol and hexose entities has emanated from simple and readily available aromatic precursors like indane, tetralin and naphthalene. These annulated inositol and hexoses show a columnar arrangement of molecules in the solid state, the O-H...O hydrogen bonds defining a channel-like architecture with a hydrophobic (annulus) exterior and a hydrophilic interior (inositol/ sugar moiety).

Key Words: Annulation; Cyclitols; Axial-rich; Carbohydrates; Conformational analysis; Dihydroxylaton; Myo-inositol

Introduction

Carbohydrates and inositolis are important and ubiquitous biomolecules. Carbohydrates as part of glyconjugates like the glycoproteins, glycolipids, etc are key elements in a repertoire of life sustaining processes in the biological system like cell-cell recognition, cell growth, cell development, cell-cell adhesion, inflammation, and metastasis. Carbohydrate recognition events have been implicated in the progression of a number of diseases, as the cell surface carbohydrates are known to mediate pathogen binding.1,2 Despite this realization of the fundamental and central role of the carbohydrates in a repertoire of biological events, full exploration of the carbohydrate based therapeutics has been lacking until recently. This can be largely attributed to the sheer structural complexity associated with oligosaccharides and lack of proper analytical tools to study minuscule quantities of carbohydrates regulating biological events among other things. However, the recent advances in the synthesis of carbohydrates and availability of new structural tools and analytical techniques has stimulated development of new carbohydrate based therapeutics and vaccines based on carbohydrate-carbohydrate and carbohydrate-protein interactions. Furthermore, the discovery of diverse biological roles for oligosaccharides and glycoconjugates is fueling interest in the development of chemical entities (carbohydrate mimics) that can mimic the carbohydrates involved in various signaling and recognition events, but with improved physicochemical properties with regard to the stability, specificity, affinity and synthetic availability.3,4

Closely related to carbohydrates are the inositolis 1 (perhydroxylated cyclohexanes) and their phosphate derivatives which regulate a wide range of biological processes. Among the nine known stereoisomeric inositolis, myo-inositol 2 is the most abundant and its trisphosphate, [Ins(1,4,5)P3] 3 acts as a second messenger in the intracellular signal transduction pathway that regulates the release of calcium ions from intracellular stores, which in turn initiates a large number of cellular responses.5 In addition, inositolis are also involved in insulin stimulation, intracellular trafficking of vesicles and covalent anchoring of proteins to membranes. The recognition of the fundamental cellular role played by myo-inositol phosphates has led to extensive biochemical investigations to unravel the details of the complex pathway involving them. However,
therapeutic potential of the inositol signaling pathway remains largely unexplored. In this context, the synthetic analogues of inositols with novel structural attributes may have interesting biological role to play. Such unnatural entities could help explore the structural requirements for binding to and activating the receptor. Besides, such synthetic entities can also find applications as therapeutic agents. In this regard, conformationally constrained analogues of inositols may offer interesting prospects as they can be used to gauge the spatial requirements for effective protein-substrate interactions and provide insights into structure-activity relationships.

Conformationally Restricted Inositols
The quest for the new synthetic analogues of inositols has been extensive and attempts towards generation of inositol analogues have largely centered on ring modification, side arm and phosphate variations. Structure-activity investigation have showed that all the high-affinity agonists of Ins\((1,4,5)\)P\(_3\) receptor must contain groups equivalent to the vicinal 4R,5R-trans-dequitiatorial bisphosphate, Fig. 1.\(^{2,3a}\) On this premise, a variety of inositols phosphate analogues were designed and synthesized retaining the phosphates at 4- and 5- position, but none could match the affinity of \textit{Ins}(1,4,5)P\(_3\) itself. For example, the \textit{Ins}(2,4,5)P\(_3\), 4 was recognized by IP\(_3\)R\(_s\), but with some 25-fold lower affinity than \textit{Ins}(1,4,5)P\(_3\).\(^{3e}\)

Besides the inositol variants derived from phosphate variations, there have been persistent efforts to generate structurally modified, particularly conformationally restricted analogues.\(^{3a,b}\) With the aim of decreasing the conformational mobility of the phosphate groups, Potter et al., synthesized two bicyclic inositol variants 5a and 5b Scheme 1.\(^5\) Synthesis of these bicyclic inositol phosphates commenced from the appropriately protected myo-inositol derivative 6 and the cis-diol moiety in it was protected using 3-chloro-2-chloromethyl-1-propene to deliver 7 and subsequent synthetic maneuvers furnished the bicyclic trisphosphates 5a, b Scheme 1. Disappointingly, none of these bicyclic analogues showed any encouraging affinities towards \textit{Ins}(1,4,5)P\(_3\) receptors.

![Scheme 1](image-url)

van Boom et al. synthesized two glucose based, spiro-fused bicyclic analogues, “spirohostins” 8a, b\(^6\) as conformationally restricted analogues of adenophostins A 9\(^7\) (a naturally occurring fungal metabolite, which exhibited high affinity for the inositol receptors) but these structural entities showed affinities about 20 fold lower than \textit{Ins}(1,4,5)P\(_3\).
Potter et al. synthesized conformationally restricted cyclic phosphate analog of Ins(1,4,5)P₃, 10, constraining the one phosphate group, thereby arresting the inositol moiety in a chair conformation. Synthesis of 10 commenced from the inositol orthoformate derivative 11 and a sequence of protection/deprotection protocols led to the diol 12. Further, sequential phosphorylation led to cyclic phosphate ester 10, Scheme 2. However, the analog 10 was found to be weak agonist of Ins(1,4,5)P₃, diacetoxydiene 14 which in turn was readily obtained from cyclooctatetraene (COT). The endo-peroxide 15 was reduced and the resulting diol was protected. Stereoselective dihydroxylation generated the oxygenation pattern in 13.

In search for a suitable agonist for Ins(1,4,5,6)P₄ and antagonists for Ins(3,4,5,6)P₄, which regulate the trans-epithelial chloride secretion and transcription events, Schultz et al. have synthesized a bicyclic oxepin 16 and a tricyclic analogue 17 recently, Balcı et al. reported a new structural variant of the inositols, namely bis-homoinositol 13, Scheme 3. The six-four ring fused bicyclic inositol was synthesized via a singlet oxygen mediated oxyfunctionalisation protocol on of the InsP₄. The synthetic sequence, depicted in Scheme 4, emanated from suitably protected myo-inositol precursor and involved ring closing metathesis on the bis-allylated inositol derivative 18 as the key step. The tricyclic analogue 17 was
synthesized, from the double bond migrated compound 19, which on oxidative cleavage and intramolecular acetalization furnished tricyclic framework.

**Carbocyclic Ring Annulated Inositols**

To access a brand new family of conformationally locked inositols, we have conceived novel variants like 20, wherein a cycloalkane (5- and 6-membered) ring was appended, in trans fashion, to the inositol moiety. A notable feature of such entities is that the trans-ring fusion locks the inositol moiety in an unnatural axial-rich conformation, while retaining the natural configuration of the parent inositol. In addition, the hydrophobic ring annulus present in 20 could also serve as a handle to fine tune the hydrophobic-hydrophilic balance in the polar inositols. The efficacy of this approach has been demonstrated through the synthesis of annulated myo-inositols 21, 22 and chiro-inositols 23, 24. Parent myo-inositol 2 is known to exist in a stable conformation (1a/5e) with one axial and five equatorially disposed hydroxyl groups. However, the annulated myo-inositol 22 has been shown to exist in an axial-rich conformation, with five hydroxyl groups in axial orientation (5a/1e). Similarly, the annulated chiro-inositol 24 has a four axial and two equatorial (4a/2e) hydroxyl disposition, quite in contrast to the parent chiro-inositol having two axial-four equatorial hydroxyl disposition (2a/4e) in its stable conformation. Our synthesis of the newly conceived ring annulated inositols commenced from readily available aromatics like indane 25 and tetralin 26, through the intermediacy of annulated trans-cyclohexadiene diol (trans-CHD) derivatives like 27 and 28 as depicted in Scheme 5.

The trans-cyclohexadiene diol (trans-CHD) derivatives 27 and 28 are versatile, readily available building blocks for a wide variety of synthetic maneuvers and herein we detail their elaboration to a range of inositols. Monoepoxides 29, 30 derived from trans-CHD derivatives 27, 28, on acid catalyzed cleavage led to a deep-seated rearrangement ("acetate dance", Scheme 6) to give cis-1,4-diol derivatives 31, 32 and trans-1,2-diols 33, 34, respectively. The trans-diols 31, 32 were further elaborated to the chiro-inositol 23, 24, respectively, through dihydroxylation and functional group alterations. In a similar manner, trans-diols 33, 34 led to the annulated myo-inositols 21, 22, respectively, Scheme 5.
Following a modified approach, involving sequential oxyfunctionalization maneuvers, synthesis of annulated chiro-inositol 35 and neo-inositol 36, with two additional hydroxyl functionalities on the cyclohexane annulus, starting from naphthalene has been realized, Scheme 7.\textsuperscript{12}

The new inositos 35, 36 due to trans fusion of the cyclohexane annulus, were also locked in unnatural axial-rich conformations, while retaining the natural configuration of the parent inositol. It
is expected that the two additional hydroxyl groups in the annulated ring as in 35, 36 can act as promoters of additional binding interactions along with the inositol core. The availability of novel bicyclic inositol derivatives with chemo-differentiated hydroxyl groups and novel spatial orientations augurs well for implementing phosphate variations and subsequent biological evaluation. Efforts along these lines could be quite rewarding.

**Annulated Carbohydrates**

In recent years, carbohydrates have been extensively used as ‘chirons’ and as chiral auxiliaries (‘chirality transfer agents’) in a variety of enantioselective syntheses. However, given the central role of carbohydrates in a variety of cellular processes, structurally modified carbohydrates, particularly hexoses, can serve as interesting scaffolds for biochemical applications. Considerable efforts have been devoted to access a range of functionality and ring modified hexoses. In this context, carbocyclic ring annulation offers an interesting variant to design conformationally restricted and functionally modified hexoses with fine tuning of the hydrophilic-hydrophobic balance.

Several strategies based on inter- and intramolecular Diels-Alder reactions, Robinson annulation, radical cyclization, aldol condensation and other methods have been devised to gain access to mono- and bis-annulated hexoses. A few notable strategies adopted by different research groups for this purpose are discussed here.

The research group of Fraser-Reid has reported annulated pyranosides, with ring annulation varying from cyclopropane to the cyclohexane ring and each of these annulated entities has been utilized for the synthesis of various natural products. The basic strategy in this case was to fuse a carbocyclic ring to the hexopyranoside to give the annulated sugar and then cleave the sugar ring to leave behind the carbocyclic, with the transfer of chirality from the sugar moiety to the carbocyclic portion. Along these lines, the cyclopropanated pyranoside 37, obtained from epoxide 38, was exploited towards enantioselective synthesis chrysanthemum dicarboxylic acid 39, Scheme 8. The cyclobutane annulated hexoses 40a,b were obtained by photochemical [2+2] cycloaddition of ethylene to the enone 41, Scheme 9. The conformationally immobile cyclohexano-annulated pyranoside 42 were obtained through the Diels-Alder reaction of the butadiene and carbohydrate derived enone 41, Scheme 10. Cyclopentane annulated sibling 43 was obtained from the cyclohexenyl furanose 44 by oxidative cleavage of the cyclohexenyl ring in 42 followed by Dieckmann cyclisation, Scheme 10.
47 obtained through diastereoselective free-radical cyclisation of suitable chiral haloalkenes 48 or dithiocarbonate 49, derived from diacetone glucose 50, Scheme 11. The same research group, in their efforts towards iridoid class of natural products, have generated a range of the cyclopentane annulated pyranosides like 51, Scheme 12. The annulation in these systems was achieved via a Pauson-Khand reaction of conveniently functionalized 1,6-enynes like 52 on carbohydrate template derived from the D-glucal derivatives.

Sinou et al. have reported the synthesis of the bis-annulated pyranoside 53, 54 via a palladium acetate mediated 5-exo-trig cascade cyclization protocol on a bis-O-allylated carbohydrate template 55, 56 respectively (Scheme 13). The O,O-bis-allylated 55 and N, O-bis-allylated pyranoside 56 were readily obtained from the O-allylated glycal 57.

An intramolecular Horner-Wadsworth-Emmons olefination of appropriate vicinal β-ketophosphonate of pyranoside 58, 59 was adopted by Ermolenko et al. for the construction of cyclopentane 60 and cyclohexane annulated pyranoside 61. The phosphonates 58, 59 were obtained β-epoxide 62, Scheme 14.
Jenkins et al. have generated a range of annulated carbohydrates starting from conveniently protected carbohydrates using different strategies for generating the annulus. The annulated appendage was introduced using (a) Robinson annulation protocol on the pyranoside 63 derived ketone derivative 64, (b) intramolecular aldol based cyclopentaannulation of a glucose-derived diketone 65 to give enone 66, (c) intramolecular [2+2] photoannulation catalysed by copper (I) triflate of dienes 67 on a carbohydrate scaffold to access tricyclic pyranoside 68, and more recently (d) ring-closing metathesis (RCM) approach to the stereoselective preparation of enantiomerically pure annulated carbohydrates (69-70). The RCM protocol to generate the bicyclic sugar entities 70 has been extended by Ghosh et al for the construction of cyclic ether annulation on to furanosugar to give 71, Scheme 16. The synthesis of 71 started from O-allylated precursor 72, obtained from diacetone glucose 50.

Cyclopropanated and spirocyclopropane-annulated sugar derivative, as well as their nucleosides, are of potential interest with regard to their physiological activity. The high strain energy of the cyclopropane moiety was expected to induce enhanced reactivity in such compounds and their
metabolic intermediates. In this context, Nagarajan et al. have synthesized a range of 1,2-cyclopropanated-sugar derivative 73 from tribenzylated glycal 74 adopting the Simmons-Smith protocol or the dichlorocarbene addition-dehalogenation for the introduction of the cyclopropane ring, Scheme 17. de Meijere and co-workers have synthesized spirocyclopropane-annulated hexopyranoside derivatives 75, using high-pressure-induced inverse-electron-demand hetero-Diels-Alder reaction to realize the pyran moiety in 76, Scheme 18. These novel constructs were evaluated against various glycosidases, but did not elicit any glycosidase inhibitory activity.

Recently, Sinay et al. have applied the strategy of conformational locking to carbohydrates to ascertain the active conformation of the L-iduronic acid, a typical monosaccharide component of heparin, responsible for the antithrombotic activity of the latter. They synthesized three pentasaccharides in which the single L-iduronic residue was conformationally locked, either in 1C, 4C, or 5C form and they have shown the skew-boat conformation of L-iduronic acid governs the antithrombotic activity of heparin, Fig. 2.

Nielsen and co-workers have introduced novel 3',4'-trans-linked bicyclic nucleosides 77, 78 with sugar moiety locked in an S-type conformation, wherein the annulation was achieved by cyclic ether formation or ring-closing metathesis methodology, Scheme 19. The synthesis emanated from the allylated bis-acetone glucose derivative 79, which was elaborated to the alcohol 80 and diene 81, which served as suitable precursors for generation of 77 and 78, respectively.

**Carbocyclic Ring Annulated Hexoses (“New sugars”)**

Having tasted the success in carbocyclic annulation tactic to generate axial-rich and conformationally restricted inositol, it was of immediate interest to extend the same strategy to hexose sugars. Thus, cyclohexane ring annulation, in trans fashion, to the carbohydrate moiety, could lead to a range of annulated carbohydrates 82 a-d ("new sugars"), wherein the sugar moiety is locked in an unnatural, axial-rich conformation, while retaining its natural configuration. Consequently, a trans-cyclohexane ring annulation to β-glucose 83, having an all
undertake a synthesis of annulated β-glucose 84 and this endeavor also commenced from the annulated trans-cyclohexadiene diol (trans-CHD) derivative 28, which had proved its efficacy towards the synthesis of annulated inositols (vide supra). As depicted in Scheme 19, the allylic alcohol 85 derived from the trans-CHD derivative 28 was subjected to ozonolytic cleavage, with concomitant intramolecular acetolization to generate the pyran moiety bearing tricyclic acetel 86, which after a sequence of protection-deprotection maneuvers was elaborated the annulated β-glucopyranose 84. In another structural variation, following essentially a similar synthetic sequence, the homoallylic alcohol 87, also derived from 28, was elaborated to the annulated α- glucofuranose 88, through ozonolytic cleavage of the double bond and intramolecular acetolization serving as the key step to realize the glucofuran moiety as in 89, Scheme 20.

Additionally, the carbocyclic ring annulation strategy effectively used to generate conformationally constrained sugar variants was also employed to generate carbohydrate diversity. This was exemplified through synthesis of the two annulated galopyranosides 90 and 91 differing only in the site of the ring annulation on the sugar moiety. A notable feature was that the synthesis of both 90 and 91 emanated from the same trans-CHD 28, making it a truly versatile building block, Schemes 21 and 22. The synthesis of annulated galactose derivative 91 employed a modified ozonolysis protocol and led to chemo-differentiated termini which on further intramolecular acetolization generated the pyran moiety of 92, Scheme 22.
feature observed in the packing of cyclohexaannulated inositols and sugars in the crystalline phase is the formation of fascinating channel like architecture, formed by a hydrophobic exterior and an extensively O-H...O hydrogen bonded hydrophilic interior (Fig. 3). Interestingly enough such a phase separation disappears completely in case of cyclopentaannulated inositols.

An obvious explanation to this phenomenon lies in the fine modulation of the hydrophilic character of the cyclitols by a subtle alteration in the size of the hydrocarbon annulus. It was possible to quantify approximately this contribution from the hydrophobic appendage by a close examination of effective change in the polar surface area (PSA) of the cyclitols as brought about by a change in the size of the hydrocarbon ring (Table I). A map of the molecular lipophilic potential (MLP) surface showed too the trend in the modulation of the hydrophilic surface brought about by hydrophobic appendage (Fig. 4).

Besides demonstrating the capability of a hydrocarbon annulus to fine tune the hydrophilic

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**Fig. 3** Packing diagrams of (a) cyclohexaannulated chiro-inositol, (b) cyclohexaannulated myo-inositol and (c) cyclohexaannulated methyl-a-gulopyranoside showing the channel like architecture, and (d) cyclopentaannulated chiro-inositol.
Table I

<table>
<thead>
<tr>
<th>Molecular surface (Å²)</th>
<th>PSA (Å²)</th>
<th>Ratio (polar : non-polar surface)</th>
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<tbody>
<tr>
<td>331.3</td>
<td>254.5</td>
<td>3.31</td>
</tr>
<tr>
<td>360.0</td>
<td>197.1</td>
<td>1.20</td>
</tr>
<tr>
<td>373.0</td>
<td>194.2</td>
<td>1.09</td>
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*All molecular surface calculations were performed using the software VEGA online (http://galaxy.farma.unimi.it/vegawe/desktop.htm) using the atomic co-ordinates of the respective molecules as determined by X-ray crystallography as the input.

Fig. 4. Relative molecular lipophilic surface of (a) natural chiro-inositol, (b) cyclopentaannulated chiro-inositol and (c) cyclohexaannulated chiro-inositol. The standard MLP coloring scheme has been followed. Molecular surfaces were mapped using Chemscape Chime® (version 2.6 SP3), MDL Information System Inc. (http://www.mdlchime.com)

character of cyclitols, the solid state architecture of cyclohexaannulated inositol and sugars also bears evidence to their bipolarofacial character, a feature that distinguishes them from their non-annulated congeners. In fact it is the manifestation of this bipolarofacial behavior and the greater populace of axially locked hydroxyl groups that can be observed in the channel like solid state architecture of annulated inositols and sugars.

**Conclusion**

The tool of cycloalkane ring annulation to lock inositols and hexose sugars in unnatural, axial rich conformations has been conceptualized to create new polycyclitols. trans-Cyclohexadienediols (trans-CHDs), derived from simple aromatic precursors like tetralin, naphthalene and indane have served as starting materials for the synthesis of annulated inositols and carbohydrates through stereocontrolled oxyfunctionalization protocols. Synthesis of regioisomeric variants of annulated gulopyranoses highlights the efficacy of ring annulation strategy for generating carbohydrate diversity. The annulated carbohydrate and inositol entities prepared by us exhibit bipolarofacial architecture in the solid state, further testifying to the fine tuning of the hydrophilic-hydrophobic balance in them. It is interesting to speculate on the nature of the oligosaccharides derived from the axially rich, annulated hexoses which are bound to unleash some interesting physicochemical properties.

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