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Accepted Article

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To be cited as: *Chem. Eur. J.* 10.1002/chem.202003367

Link to VoR: https://doi.org/10.1002/chem.202003367

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10.1002/chem.202003367

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Conceptualization and synthesis of first inosito-inositol (decahydroxydecalin, DHD): *In silico* binding to β-amyloid protein

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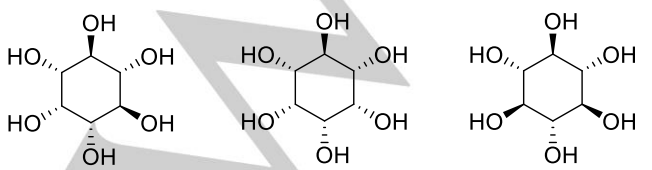
Abstract: Previously unknown entities in the form of 1,2,3,4,5,6,7,8,9,10-decahydroxydecalins (DHDs) have been conceptualized and the first member of this class, an inosito-inositol, has been synthesised from aromatic hydrocarbon naphthalene following a flexible strategy that is amenable to diversity creation. The DHD accessed here has been subjected to preliminary *in-silico* evaluation with Aβ and may hold some promise in Alzheimer's therapeutics.

Inositols represent an important class of naturally occurring bioactive, oxygenated cyclohexanoids, characterized by specific disposition of hydroxyl groups on each of their six carbon centres in variegated stereochemical patterns (Figure 1). Being formally carbasugar siblings, harboring dense oxygen functionality, inositols have been found to play a key role in numerous biological functions in plant and animal tissues. Notably they are involved in inter- and intracellular communication by mediating cell signal transductions, exhibit insulin mimetic attributes by acting as secondary messengers in the insulin intracellular pathway stimulation, phosphate storage & transfer and anchoring of certain important proteins to cell membrane, etc.[1-3] Recently, inositols have been proposed for the safe management of polycystic ovary syndrome (PCOS) in women and treatment of gestational diabetes mellitus (GDM).[3f] *myo*-inositol (**1**) and inositol phosphates (InsPs) among other siblings have been implicated and investigated for inhibiting various cancer types and for providing chemo-protection.[4] Significantly, the role of *myo*- (**1**), *epi*- (**2**) and *scyllo*- (**3**) inositols in dementia, caused by the Alzheimer's disease (AD) has created much excitement and **3** in particular has been shown to coat the surface of Aβ protofibrils and disrupt their stacking into fibrillar aggregates. In addition to the efficacy, the safety and pharmacokinetic profile of **3** led to FDA granting fast-track IND status for clinical trials.[5]



myo-inositol (**1**) *epi*-inositol (**2**) *scyllo*-inositol (**3**)

Figure 1. Inositols **1-3** with promising bioactivity potential.

In view of the wide ranging therapeutic attributes of inositols, particularly in the context of Alzheimer's disease,[6] numerous efforts have been mounted to create structural and functional diversity around their poly-oxygenated architecture to map and amplify their bioactivity potential. These forays have broadly ranged from diversified regioselective derivatization of readily available inositols,[7] synthesis and approaches to their numerous ring size variants like 4-,5-,8- and 9-membered cyclitols (**4-7**)[8] and crafting of bi- and tricyclic analogues (polycyclitols, **8-12**) that may either augment or unravel new biological activities.[9] Some of these new constructs have emanated from our group and from others and representative ones are displayed in Figure 2.

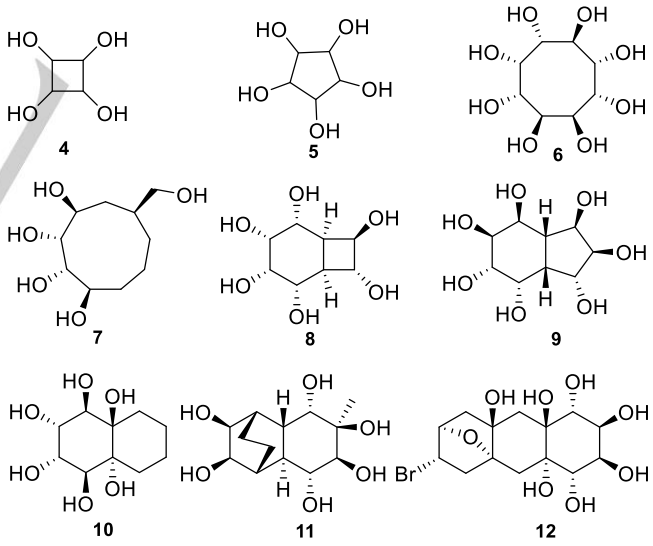
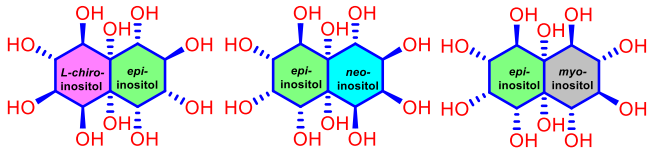


Figure 2. Assorted ring size and annulated variants of inositols.

As continuation of our long standing interest[9a-e,g] in the design of polycyclitols, we have now conceptualized a new entity in the form of 1,2,3,4,5,6,7,8,9,10-decahydroxydecalin **13** (DHD), in which each of the 10 carbon atoms of the decalin moiety carries a hydroxyl group. This ensemble can also be viewed as one in which two inositols are conjoined through a shared common ring junction, a sort of 'inosito-inositol' and embody the imprint of both the constituents and can be expected to exhibit either augmented or new bioactivity attributes. Thus, depending upon the choice of the co-joining inositol pair, a new series of DHDs can be imagined and a few prototypical constructs, **13-15** are displayed in Figure 3.



L-chiro-epi-inosito-inositol (**13**) | *epi-neo*-inosito-inositol (**14**) | *epi-myo*-inosito-inositol (**15**)

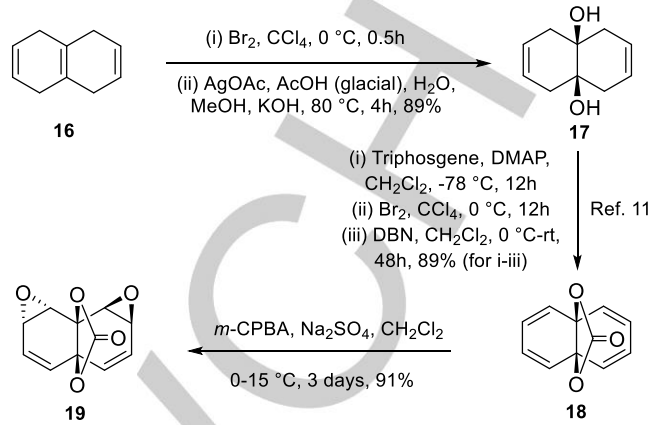
Figure 3. Newly conceptualized decahydroxydecalins (DHDs).

The attractive architecture of DHDs **13-15** held enough traction to embark on their synthesis. An added incentive for this undertaking was the observation that both hydrogen bonding and hydrophobic interactions of inositols e.g. *scyllo*-**3** are crucial to its binding to Aβ motif and their modulation by switching on additional hydrogen bonding and fine tuning of the hydrophobic surface can enhance binding efficiencies (*vide supra*) with implications in Alzheimer's disease. In our view, constructs **13-15** had the potential to measure up to such possibilities. With this backdrop of purpose, we chose DHDs **13-15** as possible targets for and intriguingly selected naphthalene, an aromatic hydrocarbon as the key starting material.

In our synthetic endeavour towards racemic DHDs **13-15**, it was imperative to select a decalin based starting material which had adequately distributed functionality for sequential, differentiated, oxyfunctionalization manoeuvres, particularly at C9 and C10 bridgehead positions. These considerations led to the identification of isotetralin **16**, a symmetrical, bicyclic skipped triene, readily and efficiently available from naphthalene via reductive dearomatization (Birch reduction), as the launch pad.[10] Regioselective bromination of the tetra-substituted bridgehead double bond in **16** followed by silver acetate mediated acetolysis and hydrolysis led to the *cis*-diol **17** in good yield. The diol **17** was internally protected as a cyclic carbonate and a bromination-dehydrobromination was executed following an adaptation of the pioneering Ginsberg protocol [11] to deliver protected tetraene **18** bearing a propellane architecture. The propellane framework of tetraene **18** was solicited in view of its known proclivity towards face-selective reactivity under stereoelectronic control. [12]

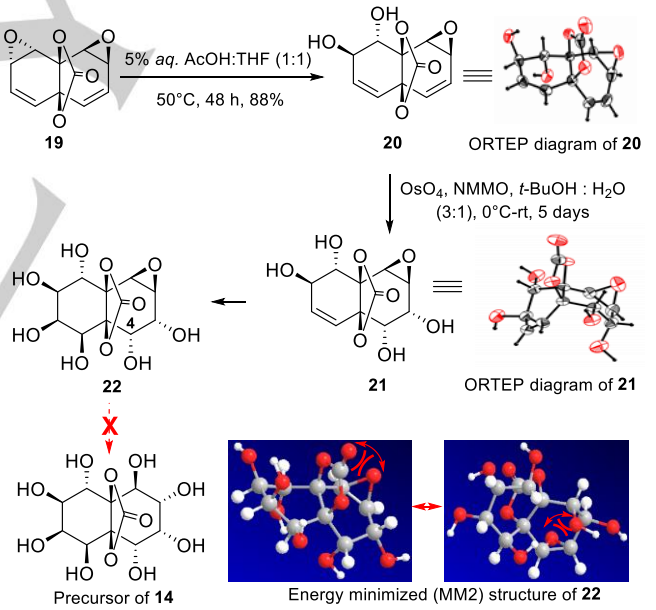
Our initial impulse was to attempt a direct shot at the target through per-dihydroxylation and/or per-epoxidation (with each epoxide serving as a surrogate for 1,2-diol) on tetraene **18** but such attempts invariably led to intractable product profile, pointing to a more calibrated step wise approach. Controlled epoxidation of tetraene **18** with *m*-CPBA led to a diepoxide **19** in high yield (91%) with epoxidation occurring from both the π-faces of the cyclohexadiene moiety, an outcome reminiscent of the cycloadditions similar to hetero-propellanes.[12] The stereostructure of diepoxide **19** was secured through X-ray crystal structure determination[13] (Scheme 1). However, diene-diepoxide **19** resisted all further attempts at epoxidation and therefore recourse was taken to an alternate tactic to amplify oxyfunctionalization of tetraene **18**.

After some exploratory experiments it emerged that the less hindered α-epoxide ring in **19**, *anti* to the carbonate bridge could be regio- and stereoselectively opened under mild acidic conditions to furnish epoxydiol **20** in decent yield and its stereostructure was established through single crystal X-ray studies. In order to amplify the oxyfunctionalization pattern in **20** enroute to the target DHD, it was subjected to exhaustive *cis*-dihydroxylation with OsO_4/NMMO under improved conditions reported by researchers at Upjohn.[14] Initially, formation of only mono-dihydroxylated epoxy tetrol **21** was encountered (structure confirmed by X-ray, Scheme 2). Prima facie this was quite intriguing in the sense that the olefinic double bond adjacent to the epoxide ring in **20** underwent preferential dihydroxylation compared to the more widely explored allylic alcohol double bond. However, when this dihydoxylation reaction was continued for a longer duration (5 days) the desired hexahydroxy epoxide **22** was obtained via the intermediacy of epoxy tetrol **21** (Scheme 2). The dihydroxylation in this case was surprisingly *anti*-Kishi rule, possibly occuring through the involvement of β-face oxygen functionality on the propellane bridge.



Scheme 1. Assembly of key decalin-diepoxide intermediate, **19**.

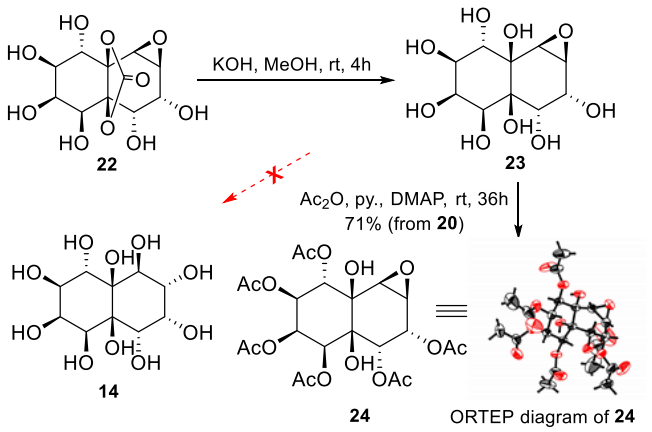
In order to advance **22** towards the target DHD, it was subjected to epoxide ring opening under various reaction regimes. However, to our disappointment, the epoxide in **22** proved to be stubborn to ring opening and deployment of AcOH, TFA combinations in water at variable temperatures, BF_3-Et_2O etc proved ineffective. Energy minimized 3-D structure of compound **22** showed that the approach of the external nucleophile towards epoxide ring is hindered from the front as well as back side and this probably is the reason for its reticence.



Scheme 2. Synthesis of epoxy-hexahydroxydecalin (HHD), **22**.

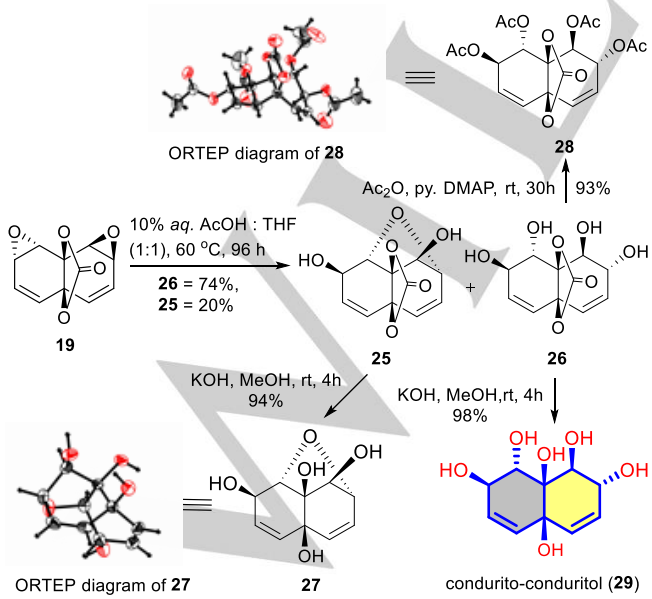
To alleviate the crowded steric environment around the epoxide ring in hexahydroxy-epoxide **22**, a carbonate deprotection maneuver was implemented in the presence of KOH/MeOH to break open the propellane architecture and reveal the decalin moiety, bearing an annulated *epi*-inositol framework **23**. The stereostructure of the resulting octahydroxy-epoxide **23** was secured through the single X-ray crystal structure determination of the derived epoxy-hexaacetate **24** (Scheme 3). With access to octahydroxy compound **23**, we were tantalizingly close to the target DHD and only the epoxide equivalence to a diol needed to be established. However, this maneuver once again proved capricious and the epoxide ring in **23** could not be opened under a variety of conditions like

*aq.*AcOH, TFA/H_2O, BF_3-Et_2O etc. that the sensitive nature of the polyhydroxylated substrate would permit (Scheme 3).



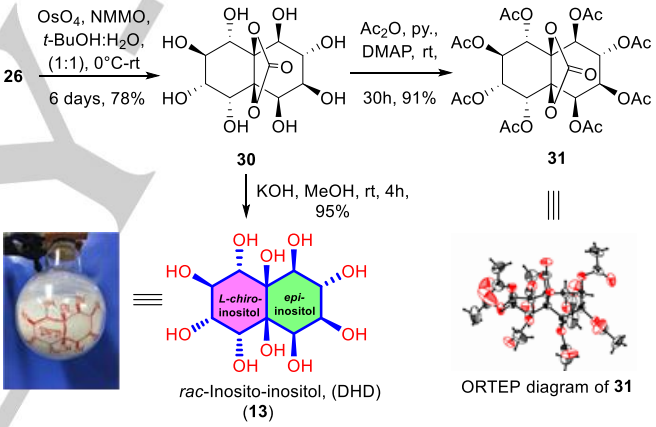
Scheme 3. Synthesis of epoxy-octahydroxydecalin (OHD), **23.**

This late stage failure with seemingly routine epoxide ring opening in **23** to deliver DHD, forced a retreat to the drawing boards and the initial launch pad, the propellane based diepoxide **19**, Scheme 1. It was observed that under modified, more acidic conditions and longer reaction time, **19** led to a (2:8) mixture of tetracyclic ether **25** (resulting from intramolecular epoxide opening) and tricyclic tetrol **26,** Scheme 4. Both the epoxide rings in **19** opened regioselectively and while two intermolecular nucleophilic openings led to tetrol **26**, tandem inter- and intramolecular epoxide ring opening led to ether **25**. Stereostructures of tetrol **26** and tetracyclic ether **25** were secured by conversion of the former to its crystalline tetra-acetate **28** and X-ray crystal structure determination and of the latter through deprotection of the carbonate moiety to crystalline tricyclic ether tetrol **27** and X-ray studies, Scheme 4. Formation of tetrol **26** was a significant, preparatory advance in the pursuit of DHDs and the decalin moiety in it was liberated through carbonate deprotection to deliver hexahydroxy decalin derivative **29** in excellent yield. Indeed, **29** can be visualised as 'condurito-conduritol', a new construct in the rich chemistry of conduritols.[15] In this context it may also be recalled that the conduritols are considered as precursors of inositols, mimicking sugars and exhibit wide ranging bioactivity [15] (Scheme 4).



Scheme 4. Synthesis of a condurito-conduritol, **29**.

With the convenient availability of propellanic tetrol-diene **26**, the stage was set for simultaneous dihydroxylation of both the double bonds of hydroxy-allyl moieties present to deliver the all-important octol **30**. Indeed prolonged exposure (6 days) of **26** to cat. OsO_4 and excess NMMO (3 eq.) resulted in the formation of propellane based octahydroxy compound **30** in decent yield and was purified by flash column chromatography on a reverse phase column. Although the stereochemistry of the newly installed dihydroxy groups in **30** could be predicted on the basis of Kishi rules[16] but given its pivotal position, an unambiguous proof of stereostructure was necessary. Thus, **30** was transformed to the corresponding crystalline octa-acetate **31** following routine acetylation protocol and a single crystal X-ray structure determination secured its formulation, (Scheme 5). Finally, unraveling of the fully hydroxylated decalin moiety embedded in hetero-propellane **30** was accomplished through base mediated deprotection of the carbonate functionality to deliver the long sought racemic DHD **13.** It has not been possible to either grow single crystals of **13** or a co-crystal with suitable hosts so far but it was obtained as an amorphous white solid, nicely depositing/coating the walls of the RB flask (see picture) and was fully characterized spectroscopically (^1H & ^{13}C NMR, HRMS etc. See Supporting Information), Scheme 5. With this first time acquisition of DHD, the stage is set for generating stereochemical diversity and developing a chiral version of the syntheses.



Scheme 5. Synthesis of first racemic decahydroxydecalin (DHD), **13**.

In-silico evaluation of inositols, 1-3 and DHD, 13

After successfully completing the synthesis of DHD **13**, it was of immediate interest to compare it with inositols (**1-3**) in respect of inhibition of polymerization and fibril formation of Aβ through *in-silico* evaluation. Initially, we mapped the molecular potential energy surface (MPES) of DHD **13** and compared the values with those of bioactive inositols **1-3** to gain some insights into the correlations between the molecular structures and extent of binding to the protein. A marked difference in terms of the specific distribution of positive and negative potentials (-6.5×10^{-2} to $+ 6.5 \times 10^{-2}$) was observed between **1-3** and **13**.[17] Whereas the positive and negative surface potential in case of the former is diffused over the entire surface, it is more pronounced and selectively distributed in the case of **13** with one side of the molecule dominated by positive potential and the other by negative potential (Figure 4). This differential in potential distribution in **13** imparts dipolar character to its surface and therefore it is expected to interact strongly and more efficiently with target protein compared to simple inositols **1-3** with lesser number of hydroxyl groups.

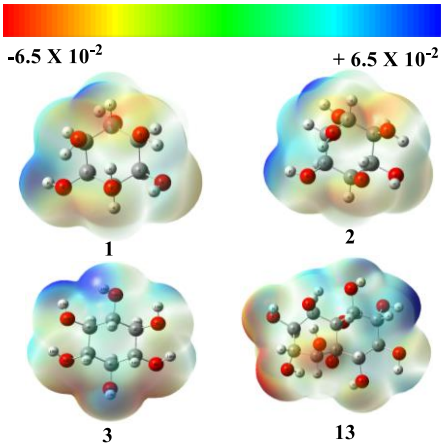


Figure 4. Comparison of molecular potential energy surfaces (MPES, calculated by B3LYP/6-31G(d) basis set) of inositols **1-3** and DHD **13**.

To understand the implication of these variable potentials, it was considered useful to assess the relative binding affinities of the inositols (**1**-**3**) and DHD **13** towards the Alzheimer's disease related monomeric and hexameric Aβ protein to provide useful information about their binding potential. The binding energy values indicate an increased binding potential for DHD **13** compared to inositols **1**-**3**. Moreover, the subtle difference in binding affinity values between monomeric and hexameric Aβ protein units further revealed that DHD **13** acts both as polymerization inhibitor as well as exhibits the potential to disrupt the protein fibrils (Figure 5).

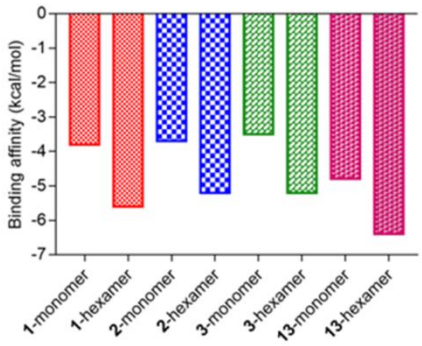


Figure 5. Comparison of binding affinity values of inositols **1-3** and DHD, **13**.

In the light of these observations, it was decided to probe into the molecular interactions of inositols **1**-**3** and DHD **13** with the concerned Alzheimer's disease related monomeric and hexameric Aβ–protein units through the tool of molecular docking using 'Autodock vina 1.1.2 program[18] and finally LigPlot$^+$ was used to plot and discern the binding interactions between the target molecules and protein units.[19] Though the nature of interactions and the amino acids involved in these interactions (Gly29, Lys28 and Ser26) are by and large same in inositols **1**-**3** and DHD **13** but the latter with more hydroxyl groups showed increased number of hydrogen bonding (Asn27) as well as hydrophobic interactions, (Figure 6). These *in-silico* results indicate better profile of DHD **13** in stabilizing the monomeric protein units thereby inhibiting polymerization and also disrupting hexameric units.

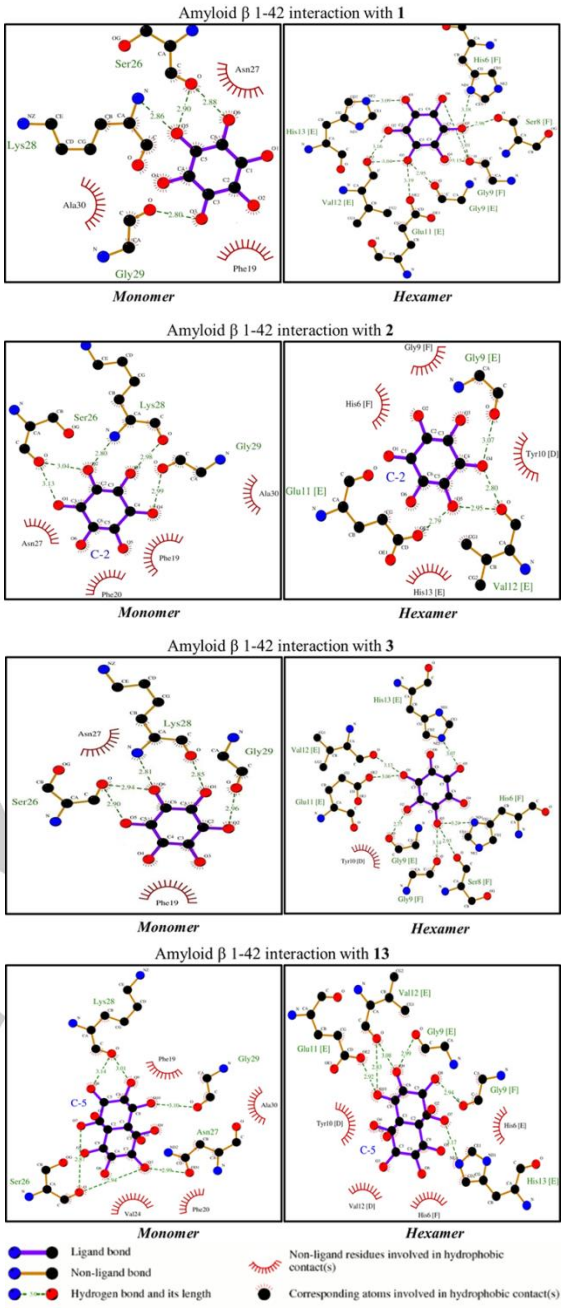


Figure 6. Lig plots of inositols **1-3** and DHD, **13** showing interactions with monomeric and hexameric Aβ-protein units.

In conclusion, a racemic synthesis of a newly conceived decahydroxydecalin (inosito-inositol) has been devised from aromatic hydrocarbon naphthalene via a series of sequentially executed oxyfunctionalization manoeuvres with attendant regio- and stereocontrol. This approach should be adaptable for diversity creation and syntheses of a host of DHDs with variegated stereochemical footprint and developing a chiral version. Initial indications from *in-silico* evaluation of this newly accessed DHD through molecular potential energy surfaces, relative binding affinities and docking studies hold promise as a disruptor of the polymerization and fibril formation of Aβ–protein implicated in Alzheimer's disease. Following on these indications, we are currently scouting for suitable bio-essays and other probes to evaluate the efficacy of DHD in Alzheimer's and diabetes.

Acknowledgments

SR wishes to thank the School of Chemistry, University of Hyderabad for hosting him. BAB acknowledges the grant support from SERB, India (CRG/2019/005179). GM thanks Dr. Reddy's Research Laboratory (DRL) for the award of Dr. Kallam Anji Reddy Chair (IIIM communication).

Keywords: inosito-inositol 1• decahydroxydecalin 2 • conduritol-conduritol 3 • Amyloid-β 4 • oxyfunctionalization 5

References

[1] a) B. V. L. Potter, *Nat. Prod. Rep.* **1990**, *7*, 1-24; b) B. V. L. Potter, D. Lampe, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1933-1972; c) Y. T. Chang, G. R. Rosania, S. K. Chung, *Exp. Opin. Ther. Patents* **2001**, ***11***, 45-59; d) R. F. Irvine, *Nature Rev. Mol. Cell Biol.* **2003**, *4*, 586-590; e) S. B. Shears, S. B. Ganapathi, N. A. Gokhale, T. M. H. Schenk, H. Wang, J. D. Weaver, A. Zaremba, Y. Zhou, *Subcell Biochem.* **2012**, *59*, 389-412.

[2] a) N. W. Brown Jr., M. Alan, A. M. Marmelstein, D. Fiedler, *Chem. Soc. Rev.* **2016**, *45*, 6311-6326; b) P. M. Rendle, F. Kassibawi, K. A. Johnston, J. B. Hart, S. A. Cameron, A. Falshaw, G. F. Painter, K. M. Loomes, *J. Med. Chem.* **2016**, *122*, 442-451. c) F. Cheng, L. Han, Y. Xiao, C. Pan, Y. Li, X. Ge, Y. Zhang, S. Yan, M. Wang, *J. Agric. Food Chem.* **2019**, *67*, 5957-5967.

[3] a) S. B. Shears, *Biochem. J.* **1989**, *260*, 313-324; b) S. Cockcroft, G. M. H. Thomas, *Biochem. J.* **1992**, *288*, 1-14; c) T. Meyer, D. Holowka, L. Stryer, *Science* **1998**, *240*, 653-656; d) J. Baillargeon, E. Diamanti-Kandarakis, R. E. Ostlund, T. Apridonidze, M. J. Iuorno, J. E. Nestler, *Diabetes Care* **2006**, *29*, 300-305; e) J. E. Nestler, V. Unfer, *Gynecol. Endocrinol.* **2015**, *31*, 501-505; f) A. Gateva, V. Unfer, Z. Kamenov, *Gynecol. Endocrinol.* **2018**, *34*, 545-550.

[4] a) K. Tanaka, S. Takenaka, K. Yoshida, *Austin J. Clin. Neurol*. **2015**, *2*, 1040; b) M. Bizzarri, S. Dinicola, A. Bevilacqua, A. Cucina, *Int. J. Endocrinol*. **2016**, *2016*, 27795708.

[5] a) J. McLaurin, M. E. Kierstead, M. E. Brown, C. A. Hawkes, M. H. Lambermon, A. L. Phinney, A. A. Darabie, J. E. Cousins, J. E. French, M. F. Lan, F. Chen, S. S. Wong, H. T. Mount, P. E. Fraser, D. Westaway, P. St George-Hyslop, *Nat. Med.* **2006**, *12*, 801-808; b) K. Ma, L. A. Thomason, J. McLaurin, *Adv. Pharmacol.* **2012**, *64*, 177-212; c) S. Sinha, Z. Du, P. Maiti, F. G. Klärner, T. Schrader, C. Wang, G. Bitan, *ACS Chem. Neurosci*. **2012**, *3*, 451-458; d) M. S. Butler, A. A. B. Robertson, M. A. Cooper, *Nat. Prod. Rep.* **2014**, *31*, 1612-1661.

[6] a) J. McLaurin, R. Golomb, A. Jurewicz, J. P. Antel, P. E. Fraser, *J. Biol. Chem.* **2000**, *275*, 18495-18502; b) S. Salloway, R. Sperling, R. Keren, A. P. Porsteinsson, C.H. van Dyck, P. N. Tariot, S. Gilman, D. Arnold, S. Abushakra, C. Hernandez, G. Crans, E. Liang, G. Quinn, M. Bairu, A. Pastrak, J. M. Cedarbaum, *Neurology* **2011**, *77*, 1253-1262.

[7] a) K. M. Sureshan, M. S. Shashidhar, T. Praveen, T. Das, *Chem. Rev.* **2003**, *103*, 4477-4503; b) Y. Sun, G. Zhang, C. A. Hawkes, J. E. Shaw, J. McLaurin, M. Nitz, *Biorg. Med. Chem*. **2008**, *16*, 7177-7184.

[8] a) W. Hartmann, R. Steinmetz, *Chem. Ber*. **1967**, *100*, 217-222; b) F. G. Cocu, Th. Posternak, *Helv. Chim. Acta* **1971**, *54*, 1676-1687; c) G. Mehta, S. Pallavi, *Chem. Commun.* **2002**, 2828-2829; d) G. Moura-Letts, L. A. Paquette, *Org. Chem.* **2008**, *73*, 7663-7670; e) G. Mehta, S. Pallavi, S. Katukojvala, *Tetrahedron Lett.* **2009**, *50*, 4519-4522.

[9] a) G. Mehta, S. S. Ramesh, *Chem. Commun.* **2000**, 2429-2430; b) G. Mehta, S. S. Ramesh, *Tetrahedron Lett*. **2001**, *42*, 1987-1990; c) G. Mehta, S. S. Ramesh, *Tetrahedron lett.* **2002**, *44*, 3105-3108; d) G. Mehta, S. S. Ramesh, M. K. Bera, *Chem. Eur. J.* **2003**, *9*, 2264-2272; e) G. Mehta, S. S. Ramesh, *Tetrahedron Lett*. **2003**, *44*, 3105-3108; f) Y. Kara, M. Balci, *Tetrahedron* **2003**, *59*, 2063-2066; g) G. Mehta, S. Sen, *Tetrahedron lett*. **2010**, *51*, 503-507.

[10] E. Vogel, W. Klug, A. Breuer, *Org. Synth.* **1974**, *54*, 11-18.

[11] P. Ashkenazi, J. Kalo, A. Ruttimann, D. Ginsburg, *Tetrahedron*, **1977**, *34*, 2161-2165.

[12] D. Ginsburg, *Acc. Chem Res.* **1974**, *7*, 286-293.

[13] G. Mehta, S. Sen, C. S. A. Kumar, *Acta Cryst.* **2013**, *E69*, o1504-o1505.

[14] V. Vanrheenen, R. C. Kelly, D. Y. Cha, *Tetrahedron Lett.* **1976**, *17*, 1973-1976.

[15] a) M. Balci, *Pure Appl. Chem.* **1997**, *69*, 97-104; b) M. S. Gultekin, M. Celik, M. Balci, *Curr. Org. Chem.* **2004**, *8*. 1159-1186; c) J. Duchek, D. R.; Adams, T. Hudlicky, *Chem. Rev.* **2011**, *111*, 4223-4258.

[16] a) J. K. Cha, W. J. Christ, Y. Kishi, *Tetrahedron lett.* **1983**, *37*, 3943-3946; b) W. J. Christ, J. K. Cha, Y. Kishi, *Tetrahedron lett.* **1983**, *37*, 3947-3950.

[17] a) A. D. Becke, *Chem. Phys.* **1993**, *98*, 1372-1377; b) P. J. Stephens, F. J. Devlin, C. F. Chabalowski, M. J. Frisch, *J. Phys. Chem. B* **1994**, *98*, 11623-11627.

[18] O. Trott, A. J. Olson, *J. Comput. Chem.* **2010**, *31*, 455-461.

[19] R. A. Laskowski, M. B. Swindells, *J. Chem. Inf. Model.* **2011**, *51*, 2778-2786.

10.1002/chem.202003367

Entry for the Table of Contents

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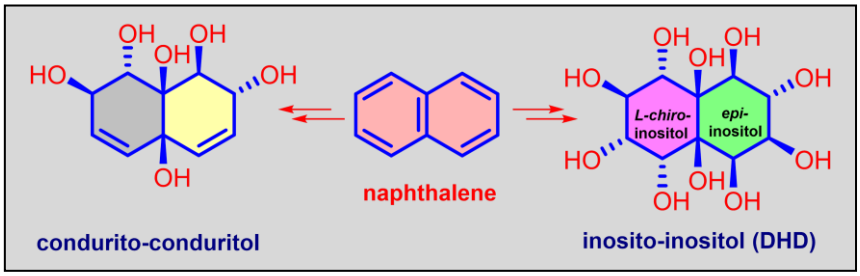

Previously unknown entities in the form of 1,2,3,4,5,6,7,8,9,10-decahydroxydecalins (DHDs) have been conceptualized and the first member of this class, an inosito-inositol, has been synthesized via a series of sequentially executed oxyfunctionalization manoeuvres with attendant regio- and stereocontrol. The DHD accessed here has been subjected to preliminary *in-silico* evaluation with Aβ and is expected to hold promise in Alzheimer's therapeutics.