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# ***N*-Alkyl-1,5-Dideoxy-1,5-Imino-L-Fucitols as Fucosidase Inhibitors: Synthesis, Molecular Modelling and Activity Against Cancer Cell Lines**

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## **Abstract**

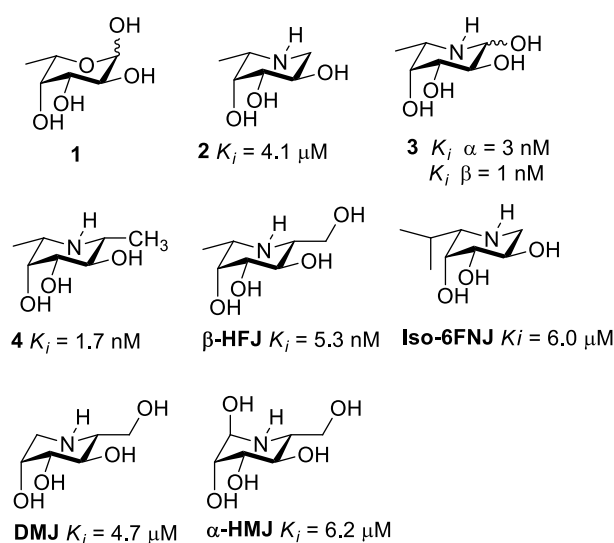
1,5-Dideoxy-1,5-imino-L-fucitol (1-deoxyfuconojirimycin, DFJ) is an iminosugar that inhibits fucosidases. Herein, *N*-alkyl DFJs have been synthesised and tested against the  $\alpha$ -fucosidases of *T. maritima* (bacterial origin) and *B. taurus* (bovine origin). The *N*-alkyl derivatives were inactive against the bacterial fucosidase, while inhibiting the bovine enzyme. Docking of inhibitors to homology models, generated for the bovine and human fucosidases, was carried out. *N*-Decyl-DFJ was toxic to cancer cell lines and was more potent than the other *N*-alkyl DFJs studied.

**Keywords:** 1-deoxyfuconojirimycin, *N*-alkylation, iminosugar, fucosidase, homology modelling, anti-cancer activity.

## **1. Introduction**

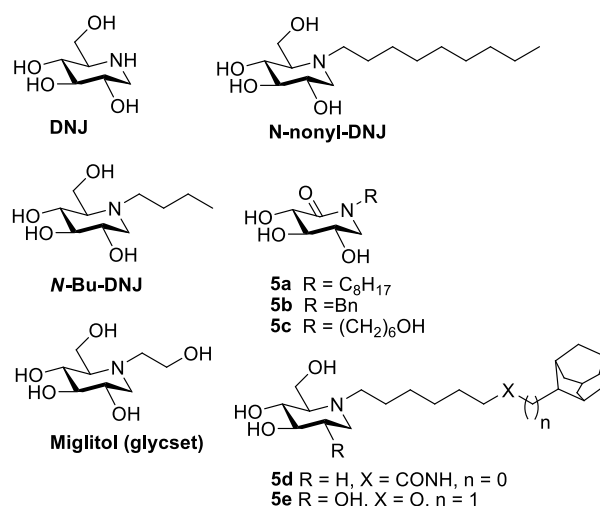
Iminosugars and their derivatives are inhibitors of enzymes of medicinal interest. These include glycosidases, glycosyltransferases [1, 2], glycogen phosphorylases [3], nucleoside-processing enzymes [4], a sugar nucleotide mutase [5, 6], metalloproteinases [7] and others [8]. Iminosugars inhibit glycosidases, due to their ability to bind at the active sites of these enzymes [9-11]. The range of enzymes inhibited by iminosugars indicates they have promise as new medicines for diseases such as diabetes, viral infections or lysosomal storage disorders [12]  $\alpha$ -Fucosidase is involved in the removal of non-reducing terminal L-fucose residues that are connected to oligosaccharides via  $\alpha$ -1,2;  $\alpha$ -1,3;  $\alpha$ -1,4 or  $\alpha$ -1,6-linkages. L-Fucose (**1**) is found on glycans that participate in cell-cell interactions and cell migration. These events are connected to physiological and pathological processes such as fertilization, embryogenesis, lymphocyte trafficking, immune responses, and cancer metastasis [13-

15]. A variety of physiological and pathological events are associated with fucose containing glycoconjugates. For instance, an aberrant distribution of  $\alpha$ -fucosidase has been reported as being relevant to inflammation [16], cancer [17], and cystic fibrosis [18]. These enzymes have been recognized as diagnostic markers for the early detection of colorectal [19] and hepatocellular cancers, and this is due to the presence of  $\alpha$ -fucosidase in patient serums.  $\alpha$ -Fucosidase inhibitors may be used to study their functions and could form the basis of developing therapeutic agents [20]. Fuconojirimycins **3**, **4** and the 1-deoxy analogue **2** (DFJ) are key inhibitors of  $\alpha$ -L-fucosidases. Other compounds assessed for their fucosidase inhibitory properties are also shown in **Fig. 1** with their reported  $K_i$  values.



**Fig. 1.** Various fucosidase inhibitors: L-fuconojirimycin **3** & **4**) [21]; natural or semi-synthetic inhibitors contains 1-deoxyfuconojirimycin (**DFJ**),  $\beta$ -hydroxymethyl 1-deoxyfuconojirimycin ( **$\beta$ -HFJ**), 1-deoxymannojirimycin (**DMJ**), mannojirimycin ( **$\alpha$ -HMJ**) [22, 23].

In recent years, *N*-alkylated iminosuars have shown improved *in vivo* and *in vitro* activities, mainly due to their improved lipophilicity, which may facilitate their crossing of the plasma membrane into cells [24-26]. In some cases, the appended alkyl groups are involved in hydrophobic interactions in the hydrophobic pocket of the target proteins. *N*-Butyl-1-deoxynojirimycin (zavesca<sup>®</sup>) has been approved for Gaucher disease and *N*-hydroxyethyl-DNJ (glyset<sup>®</sup>) is used for type-II diabetes associated complications [27]. Also, *N*-alkyl DNJs [28, 29] such as zavesca<sup>®</sup>, *N*-nonyl-DNJ and **5a-c** [30] (shown in **Fig. 2**) act as highly potent pharmacological chaperones for the potential treatment of Gaucher [31] and Pompe [32] diseases by ‘rescuing’ mutant enzymes. Kelly et al [33] and Overkleeft et al [34] have shown that additional attachment of a large lipophilic substituent (such as the adamantyl group) (see **5d-e** in **Fig. 2**) increases the interaction with the glucocerebrosidase involved in lysosomal glycosphingolipid processing.



**Fig. 2.** Bioactive *N*-alkylated iminosugars

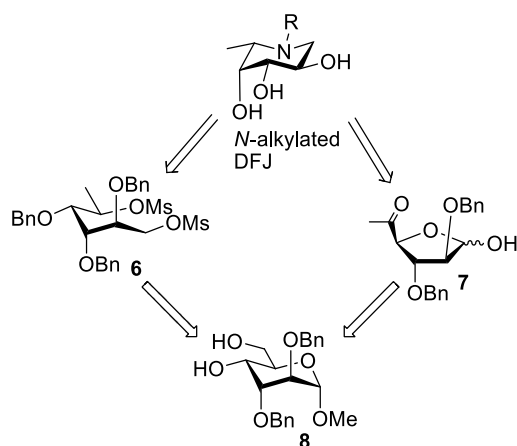
A number of *N*-alkyl DFJ derivatives have been synthesised previously. This includes an *N*-(aminopropyl) derivative prepared by Hung's group which had an IC<sub>50</sub> of 70 nM as an inhibitor of the fucosidase from *T. maritima* [35]. Some *N*-alkyl DFJ derivatives have been evaluated against fucosyl transferases [36]. *N*-Methylated-DFJ showed weak anti-HIV activity and no cytotoxicity in a study where various glycosidase inhibitors were screened [37]. Conformationally constrained *N*-alkyl DFJ derivatives were inhibitors of bovine epididymis  $\alpha$ -L-fucosidase [38]. An analogue of castanospermine with the L-fuco configuration and its fucosidase activity was described by Paulsen and co-workers [39].

Herein, the synthesis of new *N*-alkylated-1-deoxyfuconojirimycins and their testing against  $\alpha$ -fucosidases and cancer cell lines has been carried out. Molecular modelling has been used to generate hypotheses about their modes of binding. *N*-Decyl-1-deoxyfuconojirimycin (*N*-decyl-DFJ) was found to be toxic against various cancer cell lines.

## 2. Results and Discussion

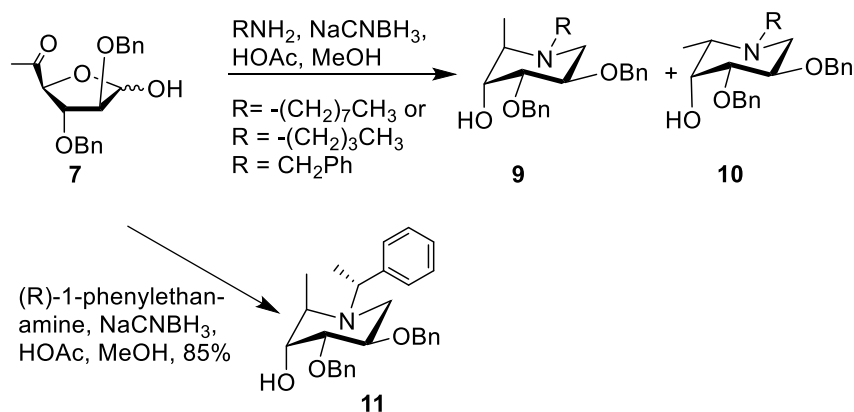
### 2.1 Chemistry

The synthetic work investigated herein is summarized in **Scheme 1**. The attempted preparation of the *N*-alkylated DFJ derivatives was investigated initially from the altrose derivative **7** prepared from **8** as previously described [40, 41]. However, synthesis from the Fleet bis-mesylate **6** [42] was ultimately the more successful route.



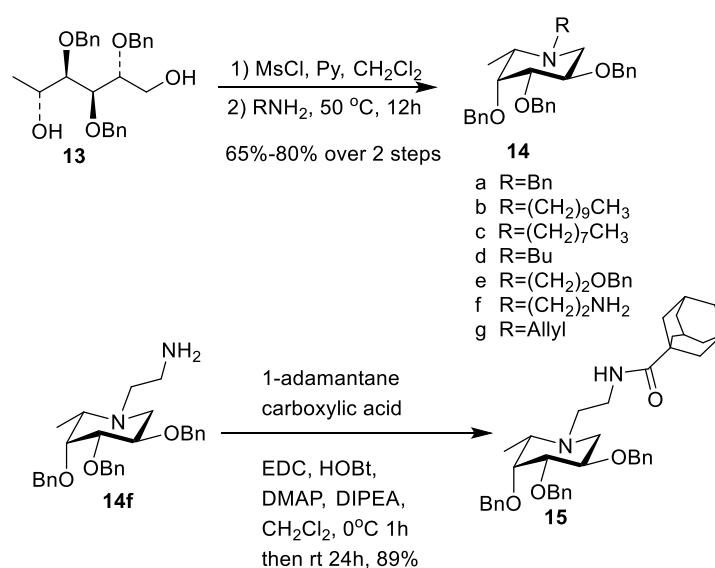
**Scheme 1.** Summary of the synthesis.

Compound **7**, which adopted a furanose structure was a mixture of anomers ( $\alpha:\beta = 2:1$ ). A double reductive amination reaction from **7** was initially investigated (**Scheme 2**) with a view to generating the required benzylated DFJ derivative [43, 44]. *N*-Butyl amine was hence reacted with **7** in the presence of  $\text{NaBH}_3\text{CN}$  and acetic acid in methanol at room temperature. The NMR analysis of the product mixture indicated formation of an inseparable mixture of 1,6-dideoxyaltronojirimycin (DAJ) derivative **9** and DFJ derivative **10** (ratio ~1:1, 79%). Instead,  $\text{NaBH}(\text{OAc})_3$  at various temperatures (-60 to -10 °C) was tried but a mixture of stereoisomers was still obtained [44]. The same approach using *N*-octylamine had a similar outcome. When benzyl amine was used, the epimers **9** ( $R = \text{Bn}$ ) and **10** ( $R = \text{Bn}$ ) could be separated and they were isolated in approximate equal amounts (36% for **9** and 40% for **10**). It was possible to improve the stereoselectivity of the reductive amination reaction by using a chiral amine [45, 46]. When (*R*)-1-phenylethanamine was used, a single isomer **11** was obtained in good yield (85%). However, NMR experiments showed that **11** had an altrose configuration with its ring adopting the  ${}^1\text{C}_4$  conformation; this was supported by coupling constants observed in the  ${}^1\text{H}$ -NMR spectrum ( $J_{1a,2} = 8.6 \text{ Hz}$ ;  $J_{1e,2} = 4.5 \text{ Hz}$ ). The reaction of the enantiomeric amine (*S*)-1-phenylethanamine was not stereoselective (**Scheme 2**).



**Scheme 2.** Double reductive amination study from **7**.

The bis-mesylate **6** (Scheme 3) which Fleet [42] and his group used in a stereoselective synthesis of 1-deoxyfuconojirimycin was next prepared. The diol **13**<sup>28</sup> was converted to **6** and without chromatographic purification the freshly generated bis-mesylate was gently heated in the presence of various primary amines, giving N-alkyl DFJs. A number of N-substituted DFJs **14a-g** were thus obtained in 65-80 % yield over 2 steps from the diol **13**. Adamantyl containing compounds have shown broad activities, including antiviral activity and for this reason were incorporated. Terminating the N-alkyl chains with a hydrophobic adamantyl group can lead to hydrophobic interactions with a target [47]. Thus, the N-ethylamine derivative **14f** was coupled with 1-adamantanecarboxylic acid in the presence of EDC and HOBt to give the adamantyl derivative **15** in 89% yield.



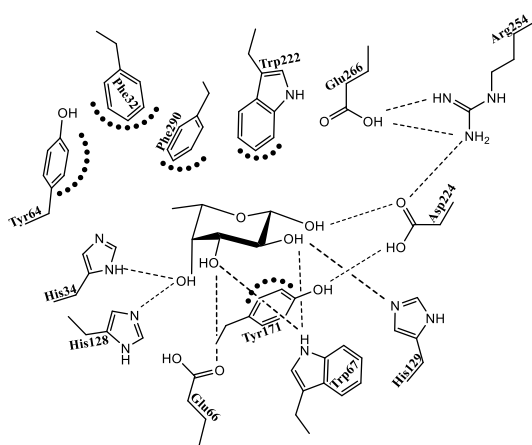
**Scheme 3.** The synthesis of **14** and **15**.

The benzyl protecting groups were removed from **14a-14e** and **15** by catalytic hydrogenation (Table 1, Scheme 4). The deprotection was first validated by the reaction of **14e** with Pd-C in the presence of hydrogen in THF-H<sub>2</sub>O-HOAc (4:2:1) and this gave **16** in 78% yield after ion-exchange chromatography [48]. When **14a** was reacted in the same way then compound **22** was isolated. This is explained by the formation of DFJ **2** after removal of all the benzyl groups from **14a**. The DFJ **2** then reacted with traces of tetrahydrofuran-2-ol (or 4-hydroxybutanal) which could be formed from dihydrofuran (DHF) present in THF. The reaction of **2** with 4-hydroxybutanal would give the iminium ion **21** which would be further reduced with hydrogen to give **22** [49]. When the solvent was changed to methanol instead of THF then only **2** was obtained from **14a**. The reaction of **14b-e** and **15** gave **16-20** (Table 1). The hydrogenation reaction of **9** (R=Bn) and **11** gave 1,6-dideoxyltronojirimycin **5** (R=H) while reaction of **10** gave 1-deoxyfuconojirimycin **2**.



## 2.2 Inhibition of fucosidases and structure activity relationship

The N-alkyl DFJs were tested as inhibitors of the fucosidase from *T. maritima* (bacterial origin) and *B. taurus* (bovine origin), and the IC<sub>50</sub> values obtained are summarized in **Table 2**. The *T. maritima* enzyme shares 38% identity with its human counterpart and is speculated to have a role in modification of hemicelluloses [48]. The bovine derived fucosidase has been widely used in N-glycan, blood group oligosaccharide and glycolipid analysis. Only DFJ (**2**) showed moderate inhibition (IC<sub>50</sub>, 8 μM) of the fucosidase from *T. maritima*. The active site of the fucosidase from *T. maritima* did not tolerate the presence of a butyl or hydroxybutyl group on the piperidine. Examination of the binding pose of L-fucose with the α-fucosidase of *T. maritima* in a co-crystal structure [53] indicated that the L-fucose is tightly enveloped by residues in the enzyme. These residues (see in **Fig. 3**) could prevent binding of N-substituted iminosugars assuming the fucopyranose mimetic **2** binds in a similar manner to L-fucopyranose.



**Fig. 3.** The interactions between α-fucosidase from *T. maritima* and α-L-fucopyranose. H-bonds are shown as dashed lines, and van der Waals contacts are shown as bold dotted lines.

The *N*-butyl derivative **18** did not inhibit, at concentrations up to 100 μM, the fucosidase *B. taurus* from bovine kidney (Table 2), whereas the butanol derivative **22** had an IC<sub>50</sub> of 30 μM. We determined an IC<sub>50</sub> of 0.3 μM for **2** which agreed with a reported IC<sub>50</sub> for **2** (0.4 μM) for the fucosidase from *B. taurus* [54]. However, lengthening the alkyl chain led to improved activity for as seen for *N*-decyl derivative **16** (IC<sub>50</sub> = 2.5 μM). By comparing the aliphatic side chains of **16-18**, it can be concluded that having a longer alkyl group gives rise to improved inhibitory activity. Compound **20** which had an adamantyl group on the *N*-side chain was less potent with an IC<sub>50</sub> of 90 μM. The *N*-ethanol derivative **19** showed IC<sub>50</sub> value of 27 μM, similar to that of the *N*-butanol derivative. The altronojirimycin derivative **5** (Table 1) did not show any inhibition towards the tested α-fucosidases.

In addition to the IC<sub>50</sub> determination, some physiochemical parameters (LogP, LipE and logD) were calculated. In terms of oral administration, LogP values should be less than 5 according to Lipinski's rules of five, which most of the *N*-alkylated derivatives show [55]. LogP values between 2 and 3 are often considered optimal to achieve a

compromise between permeability and first pass clearance, which is quite comprehensively shown by compound **16**. LipE is another parameter which links the potency (IC<sub>50</sub>) and lipophilicity (logD) in an attempt to estimate drug-likeness of a particular structure. A satisfactory LipE value would indicate selectivity for the target of interest versus a generic hydrophobic environment [56]. A LipE measurement of 6 represents one-million-fold selectivity for the target versus a generic hydrophobic environment while a LipE of zero indicates no selectivity. Compounds **2** and **19** had values higher than 6.

**Table 2.** IC<sub>50</sub> values (μM) and other parameters

Compound	IC <sub>50</sub> (fucosidase from <i>B. taurus</i> )	IC <sub>50</sub> (fucosidase from <i>T. maritima</i> )	LogP <sup>d</sup>	LogD	For fucosidase from <i>B. taurus</i>	
					pIC <sub>50</sub>	LipE <sup>e</sup>
<b>2 (DFJ)</b>	0.3 (0.4)[54]	8	-1.71	-2.23	6.52	8.75
<b>17</b>	25	<i>N. D.</i> <sup>b</sup>	1.723	1.02	4.60	3.57
<b>16</b>	2.5	<i>N. D.</i>	2.60	2.04	5.60	3.55
<b>18</b>	>100	>100	-0.04	-1.02	<4	<i>N. A.</i>
<b>19</b>	27	<i>N. D.</i>	-2.13	-1.83	4.56	6.40
<b>22</b>	30	>100	-0.07	1.05	4.52	3.47
<b>20</b>	90	<i>N. D.</i>	-1.25	-1.81	4.04	5.86
<b>5</b>	>100	>100	-1.71	-2.23	<4	<i>N. A.</i>

<sup>b</sup> *N. D.* = not determined; <sup>d</sup> based on Labute *et al* method calculated by CCG MOE2018.01; <sup>e</sup> LipE = pIC<sub>50</sub> - log D [56];

### 2.3 Molecular modelling

Molecular modelling was used in order to hypothesise how the fuconojirimycin derivatives may interact with the fucosidases and to provide a basis for the development of more potent inhibitors. The 3D-coordinates for the fucosidase from *T. maritima* (PDB code: 2ZXD) were retrieved from Royal Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB-PDB) [57]. The fucosidase of bovine origin was modelled using the α-L-fucosidase of *T. maritima* as a template. The sequence for the bovine fucosidase *B. taurus* was obtained from NCBI (National Center for Biotechnology Information). A BlastP search was then conducted which showed a number of possible templates for constructing the homology model (results from the BlastP search are provided in the supplementary information). Five templates were found with >35% of residues matching, and the length of each template was ≥ 85% of that of the *B. taurus* input sequence and therefore homology models were built for each of these. These homology models were then evaluated based on their qualitative structural uniformity. Model-4 (Table 3) developed based on the template of a fucosyl hydrolase from a strain of *T. maritima* was selected based on the data presented in Table 3 for further molecular modelling studies. Three residues were found to be outliers in this model, and they were later were energy minimised. The plots obtained before and

after minimisation are provided in the supplementary information. Comparing the pairwise sequence alignment of fucosidase of *B. taurus* with that from *T. maritima* showed the similarity of their structures (**Figure 4**).

**Table 3** Evaluation of the constructed homology models prepared from five available PDBs with identity  $\geq 35\%$  in their amino acid sequence. Qualitative structural measurements are provided in the form of Ramachandran plot, ERRAT plot, Verify-3D, ProA, Z-Score and superpose of homology model with their templates

Models	Ramachandran Plot <sup>a</sup>				ERRA T Plot	Verif y 3D	Superpose RMSD (Å)	ProSa Z-Score		
	% residue in favoured region	% residue in additional allowed region	% residue in generously allowed region	% residue in disallowed region				Templ ate	Homolog y model	Templ ate
1	85.1	7.3	0.6	0.9	84.57	80.65	1HL9_B (0.739)	-7.26	-9.87	0.26
2	84.5	13	1.5	0.9	86.74	89.25	2ZWY_A (1.174)	-7.47	-10.29	0.27
3	88.2	9.3	1.5	0.9	82.20	80.11	1HL9_A (1.050)	-6.96	-9.33	0.25
4	85.1	12.1	1.9	0.9	86.04	87.37	2WSP_A (0.926)	-7.69	-10.27	0.25
5	69.7	25.7	3.1	1.5	79.73	86.24	1ODU_A (1.89)	-6.75	-9.81	0.31

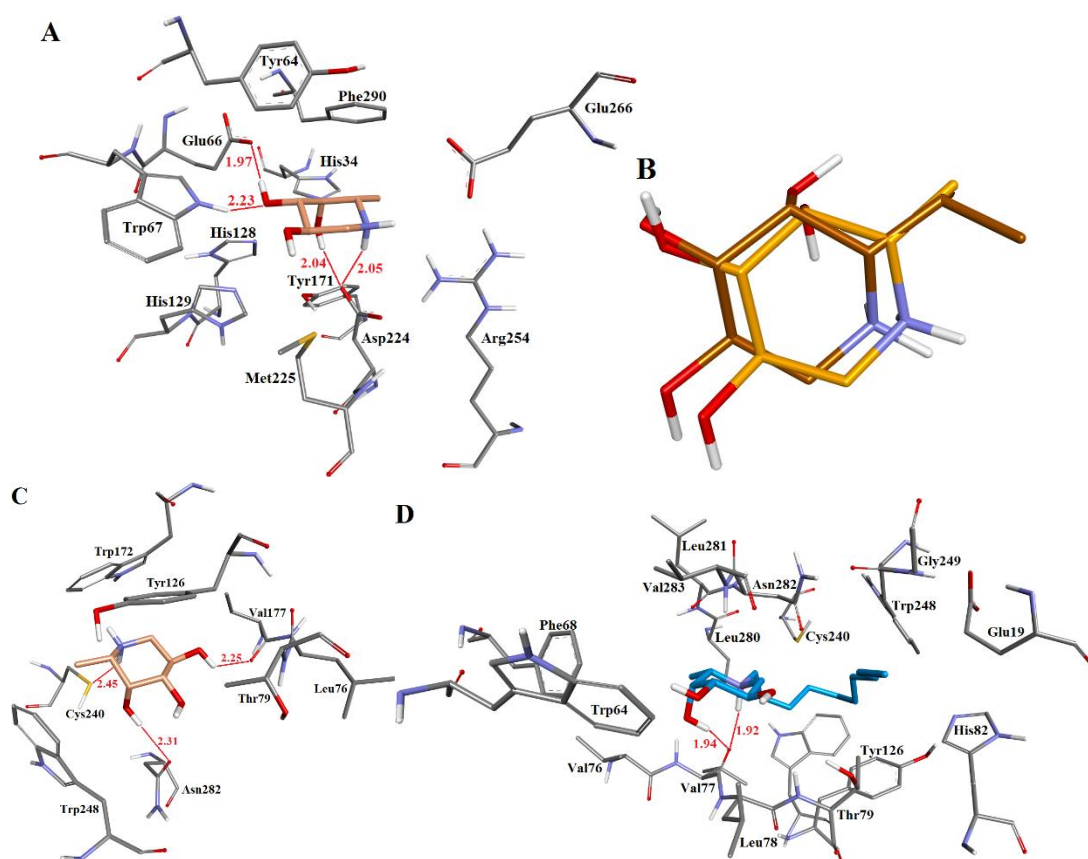
<sup>a</sup> residues in Ramachandran plot are calculated as  $\sim 0.3 = 1$



**Fig. 4.** The sequence alignment of model 4 with its template 2wsp\_A showing evolutionary conserved residues.

The docking of **2** with the crystal structure for the fucosidase from *T. maritima* was carried out and it suggested a H-bond acceptor/donor network, where hydroxyl groups of the iminosugar interacted with the side chain of Glu66 (1.97 Å), Trp67 (2.33 Å) and Asp224 (2.04 Å), as shown in **Fig. 5A**. The protonated nitrogen of the iminosugar had a H-bond donor interaction with the carboxyl group in the side chain of Asp224 (2.05

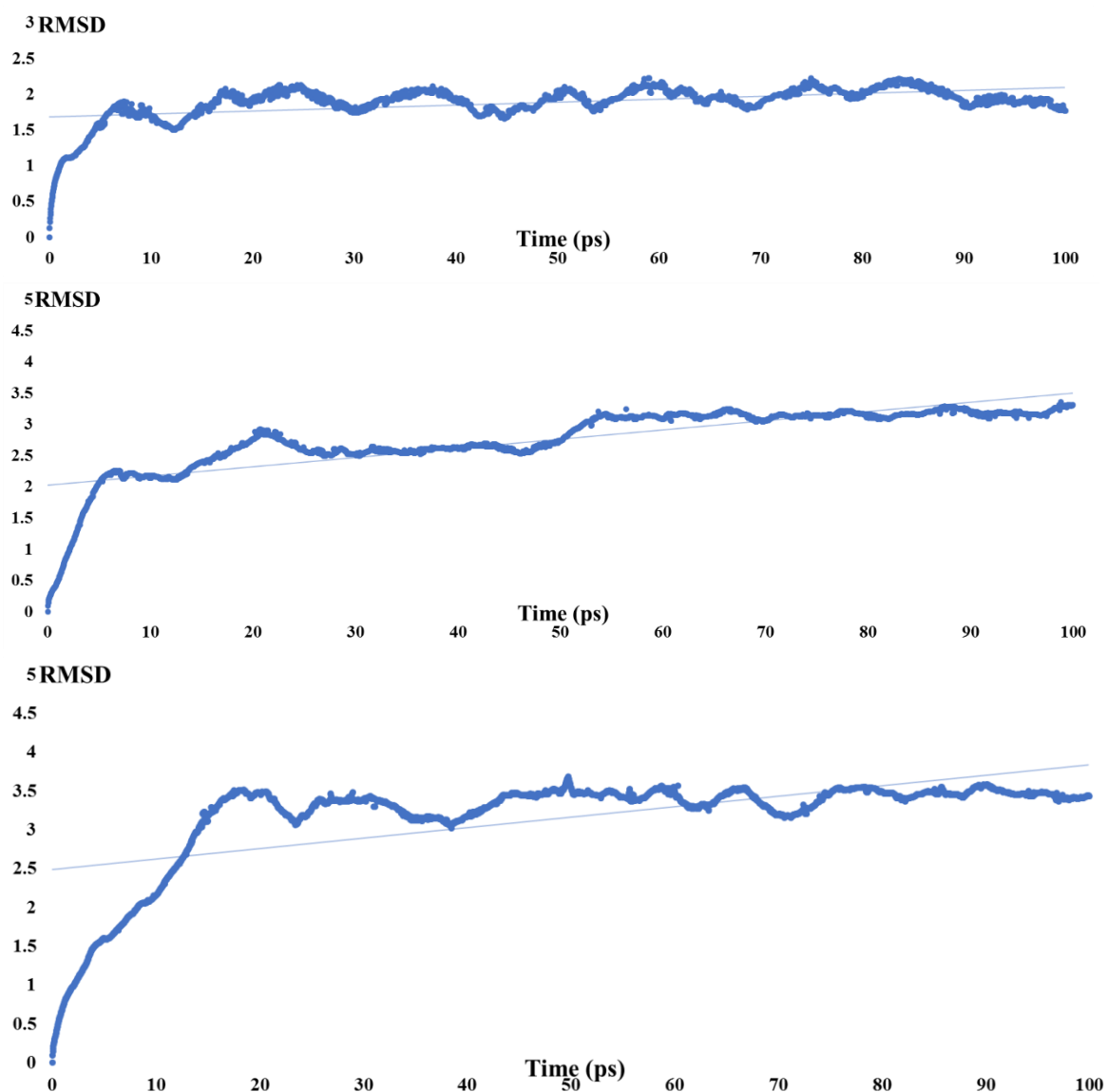
Å). The docking showed how closely the calculated binding mode of **2** resembles the already reported binding of iso-6FNJ in the crystal structure bound to the fucosidase from *T. maritima* (see **Fig. 5B**). Furthermore, the docking of **2** with the homology model of the *B. taurus* fucosidase indicated similar binding interactions to those seen for *T. maritima*. The hydroxyl groups of **2** had H-bond acceptor/donor interactions with Val77 (2.25 Å) and Asn282 (2.31 Å), while the protonated nitrogen was predicted to have H-bond donor interactions with Cys240 (2.45 Å), see in **Fig. 5C**. Also, a similar trend was noted after docking of **16** as its C-4 hydroxyl group was involved in a H-bond donor interaction with Cys240 (1.94 Å), while the protonated nitrogen had a H-bond donor interaction with Cys240 (1.92 Å). The nonane chain of **16** was placed by docking into a hydrophobic cavity constituted by aromatic residues (His82, Tyr126, Trp172) on one side while residues 281-283 and Trp248 were found on the other side of the domain. These are shown in **Fig. 5D**.



**Fig. 5.** (A) The docking pose of **2** (orange) with the fucosidase of *T. maritima*; (B) superpose of binding pose of **2** (orange) with co-crystallised ligand (brown) of 2WSP\_A (model 4, Table 3); (C) docking pose of **2** with fucosidase of *B. taurus*; (D) docking pose of **16** (blue) with fucosidase of *B. taurus*.

In order, to gain confidence regarding the reliability of the docked poses for **2** and **16**, they were selected for 100 ps molecular dynamics simulations (MD). The complex of **2** with the fucosidase of *T. maritima* showed stability after 10 picoseconds (ps) of the simulation (RMSD was consistently  $\sim 1.9$  Å, **Fig. 6A**). The same protocol was applied

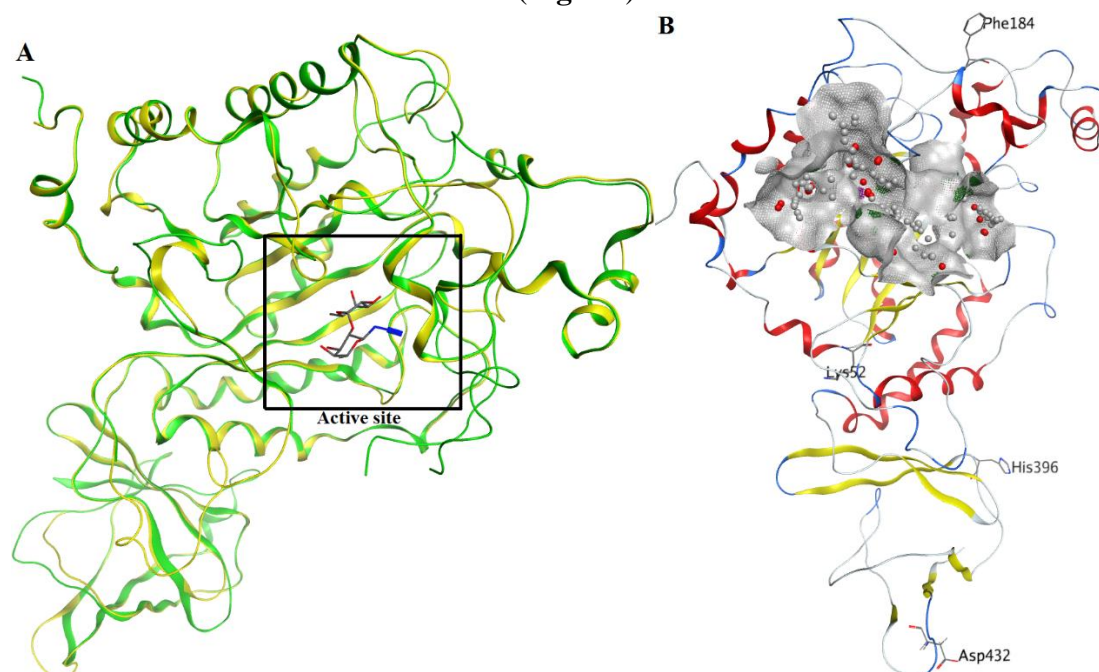
on the docked complex of **2** with the fucosidase of *B. taurus*; the ligand-protein complex showed fluctuation in first 10 ps and then the RMSD maintained a stable value  $\sim 2.8$  Å (**Fig. 6B**). The complex of **16** to fucosidase of *B. taurus* complex showed stability after 20 ps with an RMSD value of  $\sim 3.2$  Å (**Fig. 6C**). The MD simulation demonstrated the docked ligand-protein complexes were stable and generated confidence that they form the basis of a reasonable hypothesis as to the nature of inhibitor binding [58].



**Figure 6.** MD run over 100ps, (A) compound **2** (with fucosidase of *T. maritima*) with RMSD 1.88 Å; (A) Compound **2** with *B. taurus* fucosidase has RMSD 2.76 Å (C) Compound **16** with *B. taurus* fucosidase had an RMSD of 3.20 Å.

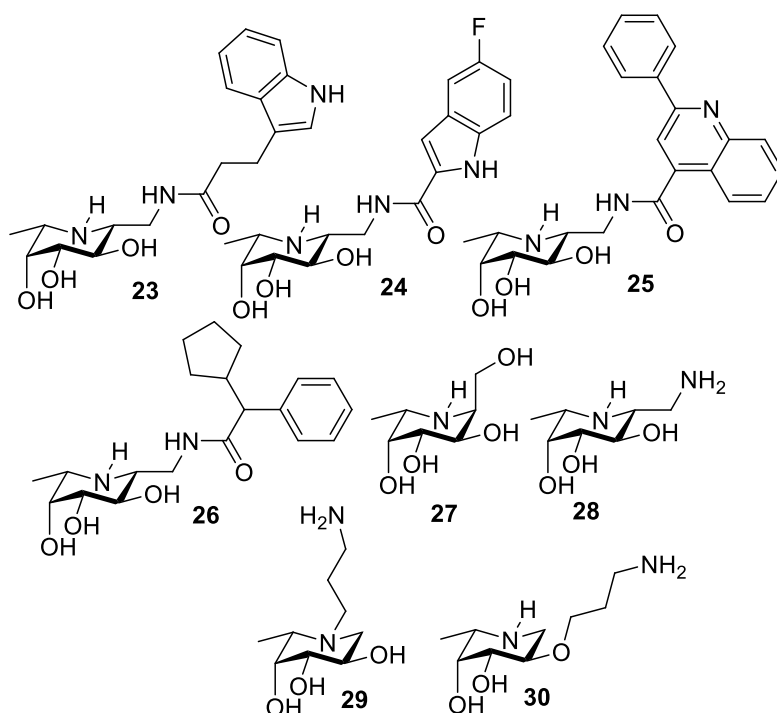
To further evaluate molecules against human fucosidase, a reverse docking strategy was performed [59-62]. The consistency and suitability of docking placement methods (in MOE 2018.01) with respect to the activities of compounds were evaluated. Hence the 3D structure of human alpha fucosidase was next modelled using the same template which was used for fucosidase of *B. taurus* origin (2WSP\_A). The quality of the human

homology model generated (**Fig. 7A**) was evaluated using Verify 3D (88.81%) and Errat (88.86), Ramachandran plot (residues in most favoured regions (86.9%), residues in additional allowed regions (10.5%), residues in generously allowed regions (1.4%) residues in disallowed regions (1.1%)) and ProSA Z-score (plots are available in *supporting information*). The residues Lys52, Phe184, His396 and Asp432 were found as outliers and are not in the active site (**Fig. 7B**)



**Figure 7.** (A) The RMSD value between the template (2WSP, yellow) and the human fucosidase homology model (green), which are superposed, was 0.71. The black rectangle outlines the active site. (B) The binding site is shown in grey. The red spheres are atoms with potential to interact with hydrophobic groups, whereas the grey spheres correspond to atoms that have potential as H-bonding donors/acceptors.

The  $IC_{50}/K_D$  values (Table 4) for inhibitors (Fig. 8) of the human fucosidase (HuF) from the work of Ho *et al* [63] were used to generate scatter plots (see **Fig. 9**). Trend lines were generated and  $R^2$  values were determined; these showed an association between the docking placement method with respect to the inhibitory properties. The docking scores are shown in **Table 4**.



1  
2 **Figure 8.** The compounds of Ho *et al* [35] used in the docking study.

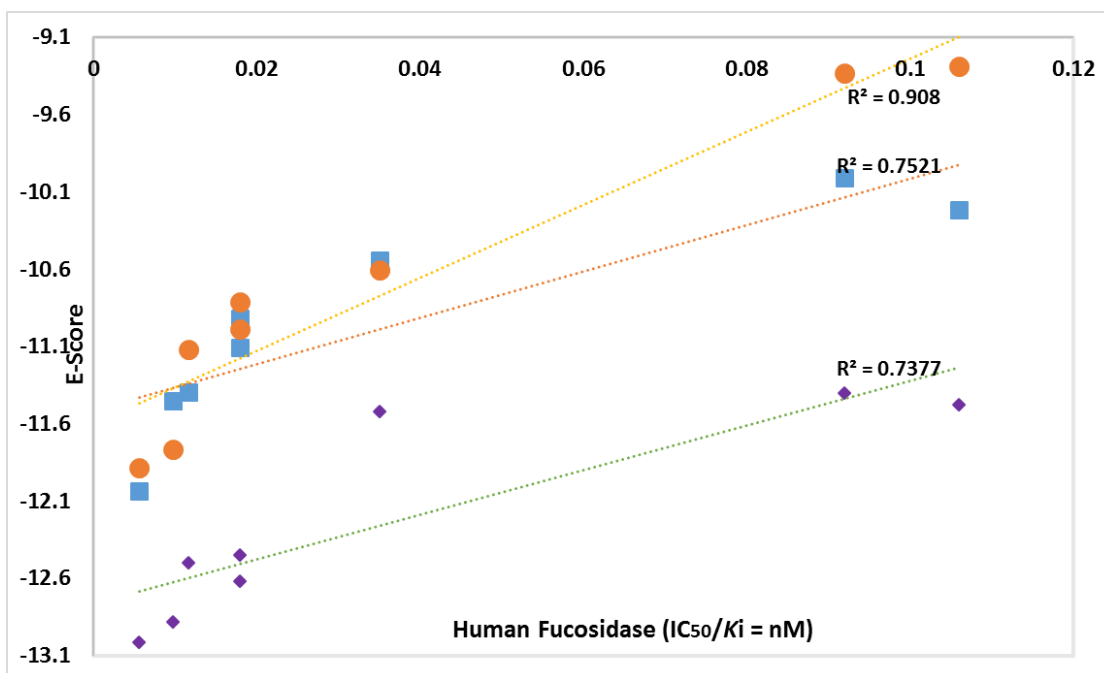
3  
4 **Table 4.** Comparison of scores attained from docking placement methods against the  
5 IC<sub>50</sub>/K<sub>D</sub> values for the compounds against human fucosidase (HuF) and *T. maritima*  
6 fucosidase <sup>TM</sup>.

Compd	K <sub>i</sub> /IC <sub>50</sub> for HuF (μM)	K <sub>i</sub> /IC <sub>50</sub> for TM (μM)	Alpha Triangle		Triangle Matcher		Alpha PMI	
			Method	Method	Method	Method	Method	Method
			HuF <sup>a</sup>	TM <sup>b</sup>	HuF <sup>a</sup>	TM <sup>b</sup>	HuF <sup>a</sup>	TM <sup>b</sup>
<b>23</b>	0.0056 <sup>c</sup>	0.000105 <sup>c</sup>	-12.037	-12.114	-11.887	-12.199	-13.011	-12.357
<b>24</b>	0.0097 <sup>c</sup>	0.000259 <sup>c</sup>	-11.451	-11.78	-11.764	-12.002	-12.882	-12.41
<b>25</b>	0.0117 <sup>c</sup>	0.00119 <sup>c</sup>	-11.393	-11.491	-11.119	-11.833	-12.50	-12.042
<b>26</b>	0.018 <sup>c</sup>	0.00101 <sup>c</sup>	-11.108	-11.542	-10.810	-11.809	-12.619	-11.945
<b>27</b>	0.018 <sup>d</sup>	0.052 <sup>d</sup>	-10.920	-11.304	-10.991	-10.892	-12.451	-11.807
<b>28</b>	0.035 <sup>d</sup>	0.064 <sup>d</sup>	-10.543	-10.928	-10.605	-10.712	-11.521	-11.065
<b>29</b>	0.092 <sup>d</sup>	0.070 <sup>d</sup>	-10.008	-10.637	-9.332	-10.288	-11.402	-10.42
<b>30</b>	0.106 <sup>d</sup>	0.267 <sup>d</sup>	-10.219	-10.419	-9.290	-9.074	-11.477	-10.317

7 <sup>a</sup> Human fucosidase homology model; <sup>b</sup> fucosidase of *T. maritima* <sup>c</sup> K<sub>i</sub> values in μM; <sup>d</sup> IC<sub>50</sub> values in μM.

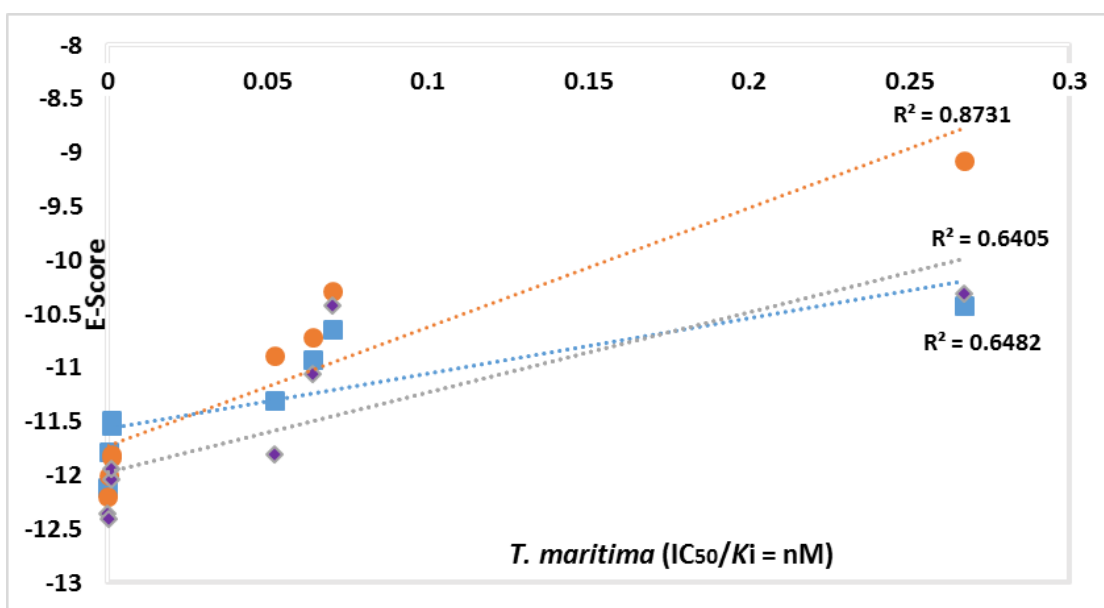
8  
9 For this study, three different docking placement methods, available in MOE 2018.01,  
10 were investigated. Of the docking methods, the triangle matcher placement method  
11 performed better than the alpha triangle and alpha PMI placement methods. With this  
12 method there were coefficients of determination of 0.908 and 0.8731, which were  
13 higher than those of the other two methods (see **Fig. 9** and **10**).

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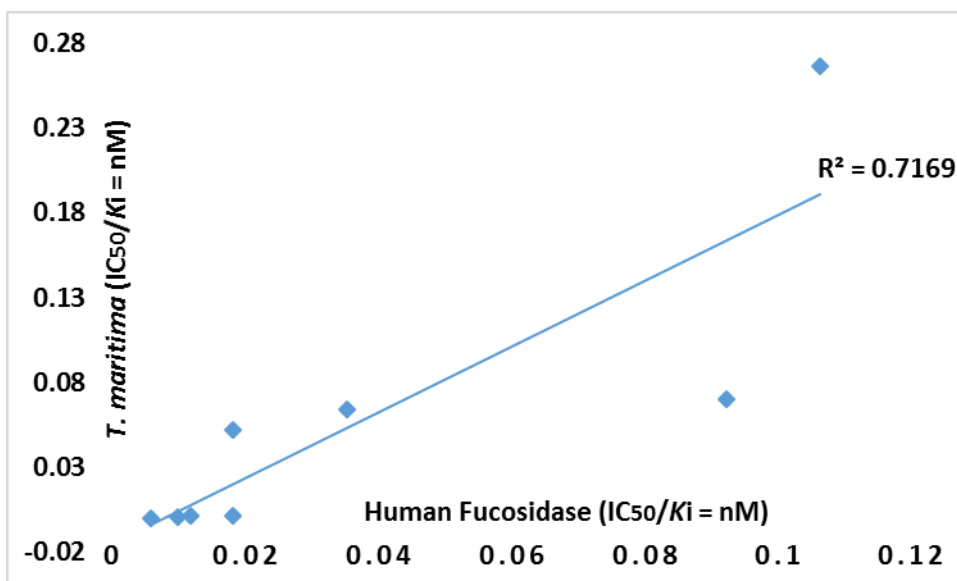
**Figure 9.** Plots of docking E-score (y-axis) and  $K_i/IC_{50}$  values (nM) for human fucosidase (x-axis) for three docking placement methods (See Table 4 for data used).



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**Figure 10.** Plots of E-score (y-axis) and  $K_i/IC_{50}$  values (x-axis) for inhibitors of the *T. maritima* fucosidase for three docking placement methods (See Table 4 for data used)

The available enzyme inhibitory data from Ho *et al* [35] for the two different enzymes were used to generate the plot in **Fig. 11**. These gave a trendline with an  $R^2$  value of 0.7169. The  $R^2$  values observed are sufficiently high to imply that the enzyme inhibitory properties for compounds are associated with binding to the specified active site cavity in the fucosidases [64].



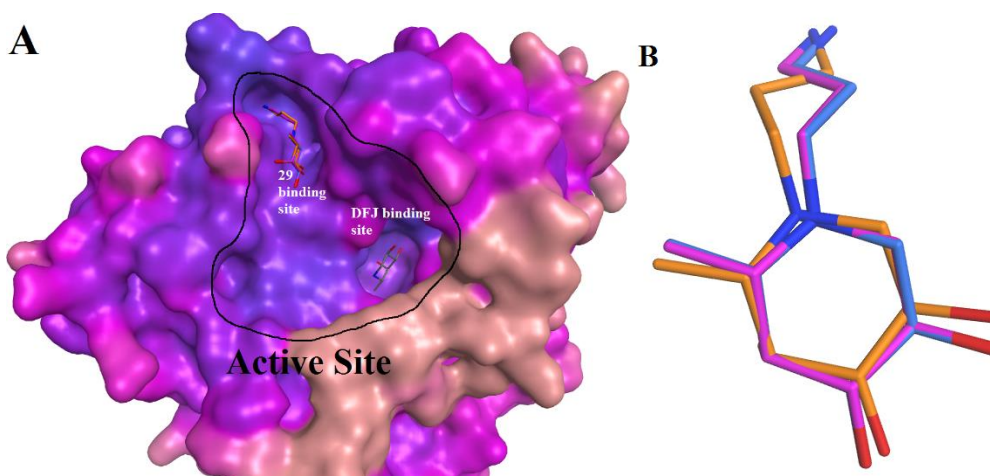
30

31 **Figure 11.** Plots of IC<sub>50</sub>/K<sub>D</sub> values for inhibitors of human fucosidase (x-axis) versus  
 32 those of *T. maritima* fucosidase (y-axis, see Table 4 for the data used).

33

34 The three docking methods all predicted that the *N*-propyl amine derivative **29** could  
 35 have a different binding location within the active site (E-Score = -10.288) compared  
 36 to other DFJ derivatives (**Fig. 12**).

37



38

39 **Figure 12.** (A) The black contour line outlines the active binding site in fucosidase of  
 40 *T. maritima*. Here **29** was predicted to differ in binding position with respect to DFJ **2**;  
 41 (B) The docked conformations of **29**, corresponding to the three docking methods, are  
 42 shown overlaid in blue (triangle matcher), magenta (alpha triangle), orange (alpha  
 43 PMI).

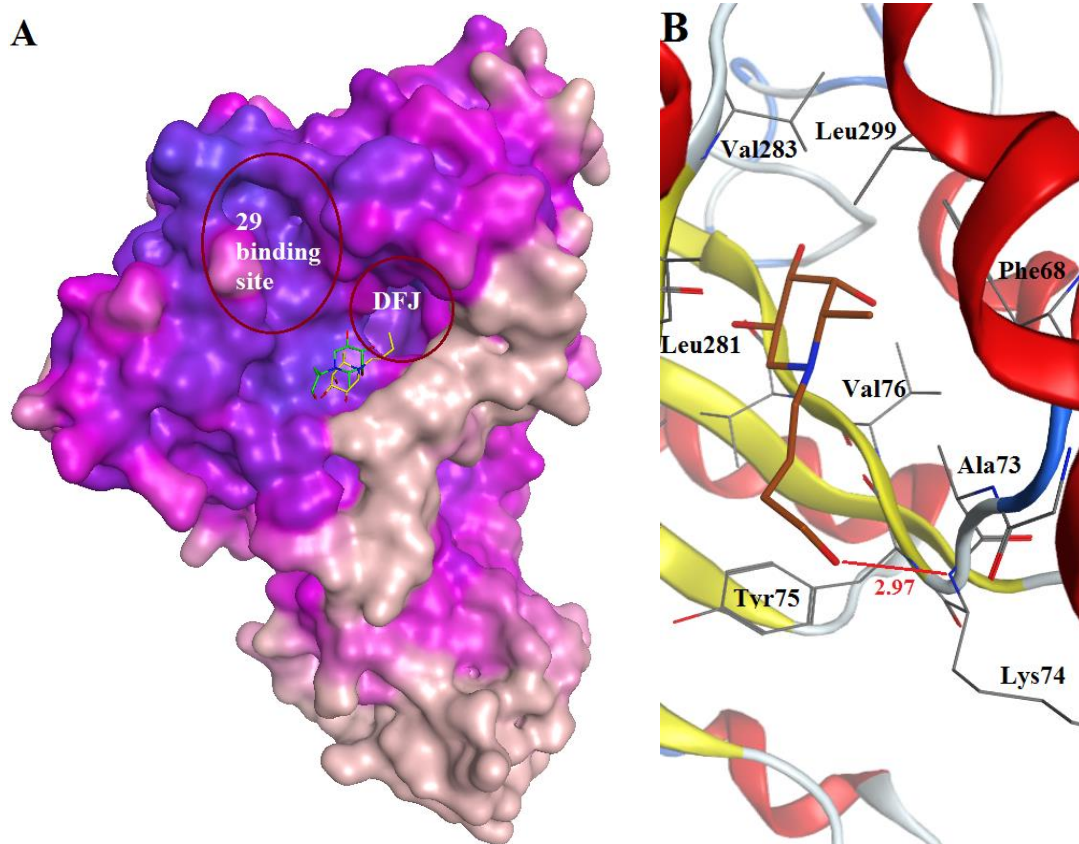
44

45 In the docking of **18** (E-score = -5.613) and **22** (E-Score = -6.175) high E-scores were  
 46 found, which are consistent with the lack of inhibition of the enzymes observed  
 47 experimentally.

48

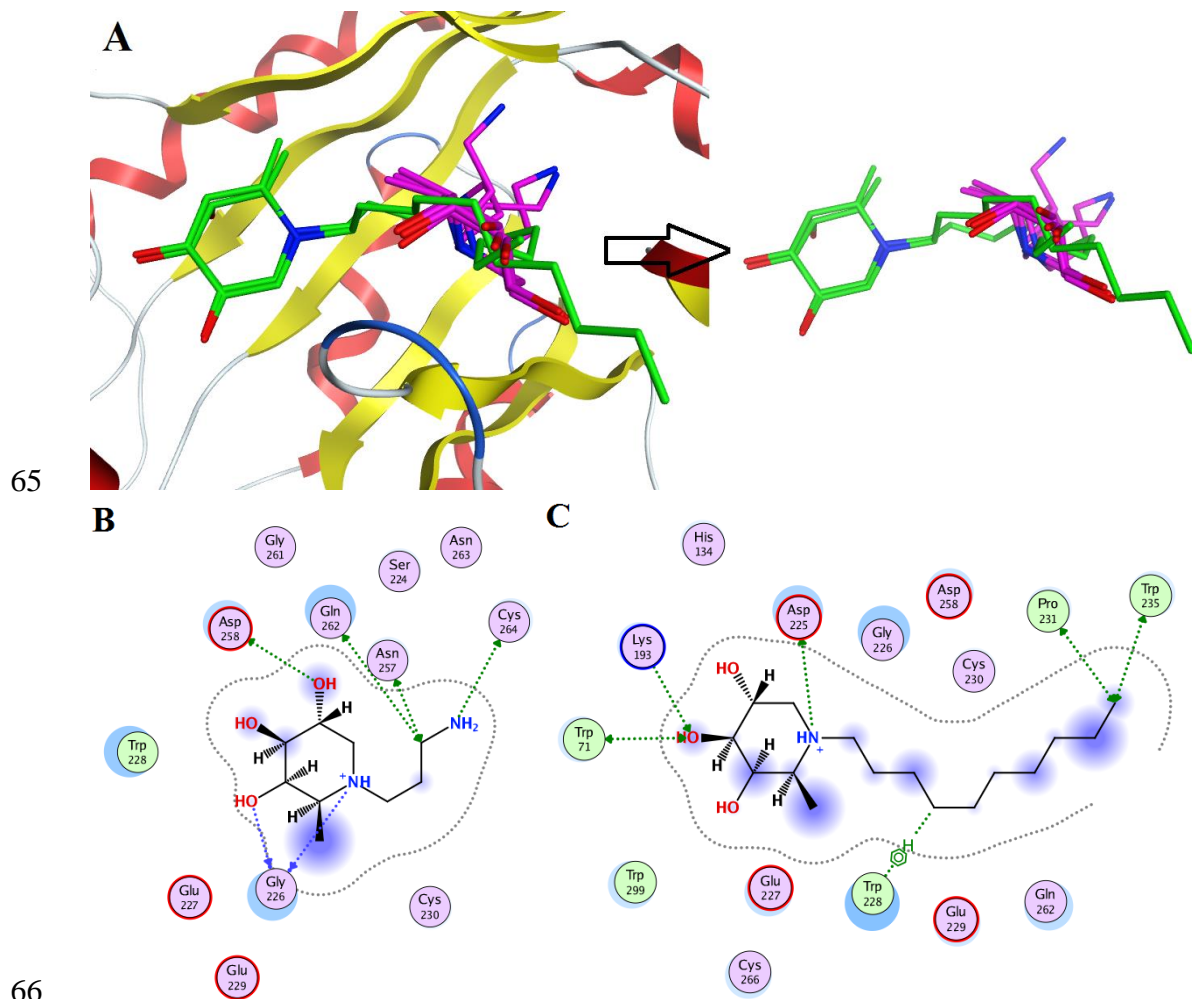
49 On the other hand, the *N*-butyl alcohol derivative **22** did show inhibitory activity  
 (IC<sub>50</sub> = 30 μM) towards the fucosidase of *B. taurus* and docking indicated this could be

50 reasonably supported by the OH group of the butyl chain being involved in a hydrogen  
51 bonding interaction with the peptide backbone of Lys74 (2.97 Å) of this fucosidase.  
52



53  
54 **Figure 13.** (A) The binding of **18** (yellow colour) and **22** (cyan colour) in fucosidase  
55 of *T. maritima*. This showed **18** and **22** outside the binding regions predicted to be  
56 occupied by **29** and **DFJ**. (B) The docking pose of **22** with respect to the fucosidase of  
57 *B. taurus* shown with predicted H-bonding interaction (green line) of Lys74.

58  
59 The docking was also performed on human fucosidase (HuF) where binding of both the  
60 *N*-propylamine derivative **29** (E-score = -9.322) and *N*-decyl derivative **16** (E-score =  
61 -8.724) is predicted to occur (**Fig. 14A**). The difference between the binding score of  
62 **16** (E-score = -8.724) to HuF and to the fucosidase of *T. maritima* (E-score = -6.901)  
63 indicates it may be of interest to evaluate **16** for its inhibition of human fucosidase in  
64 due course.



### Cytotoxicity against BT-474, MCF-7 and DU-145 cell lines using the MTT Assay

The BT-474 (breast cancer), MCF-7 (breast cancer) and DU-145 (prostate cancer) cell lines, available at the National University of Ireland Galway (NUI Galway), were used to independently determine whether compounds **5**, **16**, **18**, **20** and **22** were cytotoxic using the MTT assay. Compound **16** was found to be the most toxic against all cell lines exhibiting a greater potency towards prostate cancer cell line (DU-145). Toxicity of pleurotin, a positive control, is also given in Table 5 [64, 65]

**Table 5** Cytotoxicity evaluation using the MTT colorimetric assay. IC<sub>50</sub> values were obtained after the incubation of cells with the test compounds in DMSO for 72 h.

Compound	IC <sub>50</sub> BT-474 (μM)	IC <sub>50</sub> MCF-7 (μM)	IC <sub>50</sub> DU-145 (μM)
Pleurotin (control)	1.94 ± 0.49*	0.28 ± 0.03*	0.43 ± 0.06*
<b>5</b>	>100	>100	>100
<b>16</b>	57.39 ± 3.38	30 ± 0.04	13.54 ± 0.75
<b>18</b>	>100	77 ± 1.53	60.99 ± 3.15
<b>20</b>	>100	>100	>100
<b>22</b>	>100	>100	>100

### Inhibition of proliferation of human head and neck carcinoma cell lines

Compound **16** was further tested for its inhibition of proliferation of human head and neck carcinoma cells (HNO41, HNO97, HNO210), as well that of patient-derived glioblastoma-initiating cells (NCH644 IDH1-wt GBM) using the CellTiter-Glo assay. Compound **16** inhibited the proliferation of all these cell lines with IC<sub>50</sub> values ranging from 12 μM to 17.6 μM (Table 6). The inhibition by pleurotin, a positive control, is also reported for these various cell types.

**Table 6.** Inhibition of proliferation of cancer cell lines by **16**

Cell line	IC <sub>50</sub> of <b>16</b> [μM]	IC <sub>50</sub> of puromycin [μM]
NCH644	17.6	0.27
HNO210	17.4	0.16
HNO97	12.0	0.24
HNO41	13.8	0.24

### Conclusions

A series of *N*-alkylated fuconojirimycin analogues have been prepared and evaluated as inhibitors of fucosidases of bacterial and bovine origin. The best inhibitory activity for the new compounds, was observed for *N*-decyl-DFJ against the bovine fucosidase (IC<sub>50</sub> = 2.5 μM). The pairwise sequence alignment for the two enzymes showed their close resemblance, sharing evolutionary conserved domains. Homology models of the fucosidases from human and bovine origin were thus both constructed and used in docking and molecular dynamics. *N*-Decyl-DFJ was shown to inhibit growth of various

cell lines, including IDH wild type glioblastoma cells [65] and could form the basis for development of more potent inhibitors of tumour cell growth.

## 1. Experimental section

### 1.1 General methods for preparative chemistry

Optical rotations were determined with a Perkin-Elmer 343 model polarimeter at the sodium D line at 20 °C. Deuterated chloroform (CDCl<sub>3</sub>), CD<sub>3</sub>OD or D<sub>2</sub>O were used as NMR solvents, unless otherwise stated. NMR spectra were recorded (30 °C) with 400 or 500 MHz spectrometers. Chemical shifts are reported relative to internal Me<sub>4</sub>Si in CDCl<sub>3</sub> (δ 0.0) or HOD for D<sub>2</sub>O (δ 4.79) or CD<sub>2</sub>HOD (δ 3.31) for <sup>1</sup>H and Me<sub>4</sub>Si in CDCl<sub>3</sub> (δ 0.0) or CDCl<sub>3</sub> (δ 77.0) or CD<sub>3</sub>OD (δ 49.05) for <sup>13</sup>C. <sup>1</sup>H-NMR signals were assigned with the aid of COSY. <sup>13</sup>C-NMR signals were assigned with the aid of DEPT, gHSQCAD and/or gHMBCAD. Coupling constants are reported in hertz. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and br, broad. Low and high-resolution mass spectra were in positive and/or negative mode as indicated in each case. All anhydrous reactions were performed in flame-dried or oven-dried glassware under a positive pressure of dry argon or nitrogen. Air or moisture-sensitive reagents and anhydrous solvents were transferred with oven-dried syringes or cannulae. All flash chromatography was performed with E. Merck silica gel 60 (230-400 mesh). All solution phase reactions were monitored using analytical thin layer chromatography (TLC) with 0.2mm pre-coated silica gel aluminum plates 60 F254. Components were visualized by illumination with a short-wavelength (254 nm) ultraviolet light and/or staining (ceric ammonium molybdate, potassium permanganate, or phosphomolybdate stain solution). Flash chromatography was carried out with silica gel 60 (0.040-0.630 mm) and using a stepwise solvent polarity gradient correlated with TLC mobility. CH<sub>2</sub>Cl<sub>2</sub>, MeOH, toluene, CH<sub>3</sub>CN and THF reaction solvents were used as obtained from a Pure Solv™ Solvent Purification System. Anhydrous DMF, pyridine were used as purchased. Chromatography solvents were used as obtained from suppliers. All other reagents were obtained from Sigma-Aldrich unless otherwise stated.

### 1.2. Experimental procedures

**1.2.1 *N*-Benzyl-2,3-di-*O*-benzyl-1,5,6-trideoxy-1,5-imino-D-altritol 9 and *N*-benzyl-2,3-di-*O*-benzyl-1,5-dideoxy-1,5-imino-L-fucitol 10.** Compound 7 (39 mg, 0.11 mmol) was dissolved in MeOH (2 mL). Benzylamine (20 μL, 0.17 mmol) in MeOH (1 mL) was mixed with acetic acid (13 μL, 0.22 mmol) to adjust the pH value to 4-5, and this solution was added to the reaction mixture over a period of 0.5 h by cooling over an ice-water bath. A portion of NaBH<sub>3</sub>CN (22 mg, 0.33 mmol) was then added, and the mixture was stirred for 18 h at room temp. The mixture was quenched with 0.5 mL 1N HCl aqueous solution. After removal of the solvent, the residue was dissolved in 10% Na<sub>2</sub>CO<sub>3</sub> (10 mL), and extracted with EtOAc (3 x 7 mL). The EtOAc

layers were combined, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and the solvent was removed. Chromatography of the residue on silica gel gave **9** (16 mg, 36 %) and compound **10** (19 mg, 40%); **9** was eluted with cyclohexane-EtOAc (7:1), while **10** was eluted with cyclohexane-EtOAc (5:1).

**Analytical data for 9:**  $[\alpha]_D^{20}$  -14.6 (*c* 1.6, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.25-7.33(m, 15H, H<sub>Ar</sub>), 4.69 (d, *J* = 11.7 Hz, 1H, PhCHH), 4.64 (d, *J* = 11.7 Hz, 1H, PhCHH), 4.57 (d, *J* = 11.7 Hz, 1H, PhCHH), 4.53 (d, *J* = 11.7 Hz, 1H, PhCHH), 3.78 (d, *J* = 13.0 Hz, 1H, PhCHH), 3.76 (m, 1H, H<sub>2</sub>), 3.72 (dd, *J* = 4.6, 3.4 Hz, 1H, H<sub>4</sub>), 3.62 (dd, *J* = 7.4, 3.4 Hz, 1H, H<sub>3</sub>), 3.50 (d, *J* = 13.0 Hz, 1H, PhCHH), 2.91 (m, 1H, H<sub>5</sub>), 2.67 (dd, *J* = 12.0, 4.5 Hz, 1H, H<sub>1-eq</sub>), 2.55 (dd, *J* = 12.0, 8.1 Hz, 1H, H<sub>1-ax</sub>), 1.69 (s, 1H, OH), 1.09 (d, *J* = 6.70 Hz, 1H, CH<sub>3</sub>), <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 138.82, 138.55, 138.36 (each C), 128.68, 128.38, 128.34, 128.31, 128.15, 127.78, 127.69, 127.67, 127.53, 127.08, 126.95 (each CH), 78.84 (C<sub>3</sub>), 74.95 (C<sub>2</sub>), 72.27 (PhCH<sub>2</sub>), 71.36 (C<sub>4</sub>), 57.83 (PhCH<sub>2</sub>), 57.80 (C<sub>5</sub>), 48.77 (C<sub>1</sub>), 10.42 (CH<sub>3</sub>, C<sub>6</sub>); HRMS: Calcd for C<sub>27</sub>H<sub>32</sub>NO<sub>3</sub>, [M+H]<sup>+</sup>, 418.2387; Found, 418.2386.

**Analytical data for 10:**  $[\alpha]_D^{20}$  16.4 (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.41 – 7.20 (m, 15H, H<sub>Ar</sub>), 4.74 (dd, *J* = 14.0, 12.0 Hz, 2H, PhCH<sub>2</sub>), 4.67 (d, *J* = 11.6 Hz, 1H, PhCHH), 4.53 (d, *J* = 11.6 Hz, 1H, PhCHH), 3.95 (d, *J* = 13.6 Hz, 1H, PhCHH), 3.82 (dd, *J* = 3.1, 1.4 Hz, 1H, H<sub>4</sub>), 3.81 – 3.75 (m, 1H, H<sub>2</sub>), 3.32 (dd, *J* = 9.1, 3.2 Hz, 1H, H<sub>3</sub>), 3.29 (d, *J* = 13.6 Hz, 1H, PhCHH), 2.99 (dd, *J* = 11.5, 5.1 Hz, 1H, H<sub>1-eq</sub>), 2.48 – 2.37 (m, 1H, H<sub>5</sub>), 1.94 – 1.84 (m, 1H, H<sub>1-ax</sub>), 1.34 (d, *J* = 6.5 Hz, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 138.81, 138.64, 138.34 (each C), 129.06, 128.51, 128.46, 128.41, 127.90, 127.78, 127.76, 127.63, 127.22 (each CH), 83.45 (C<sub>3</sub>), 75.88 (C<sub>2</sub>), 73.13 (PhCH<sub>2</sub>), 72.18 (PhCH<sub>2</sub>), 72.05 (C<sub>4</sub>), 58.88 (C<sub>5</sub>), 56.81 (PhCH<sub>2</sub>), 54.84 (C<sub>1</sub>), 16.94 (CH<sub>3</sub>, C<sub>6</sub>). HRMS: Calcd for C<sub>27</sub>H<sub>32</sub>NO<sub>3</sub>, [M+H]<sup>+</sup>, 418.2387; Found, 418.2382.

### 1.2.2 *N*-((*R*)-1-Phenylethyl)-2,3-di-*O*-benzyl- 1,5,6-trideoxy-1,5-imino-*D*-altritol (**11**)

Compound **7** (29 mg, 0.085 mmol) was dissolved in methanol. (*R*)-Phenylethylamine (22 μL, 0.17 mmol) in MeOH (1 mL) was treated with acetic acid to adjust the pH value to 4-5, and this solution was added to the reaction mixture at room temp. A portion of NaBH<sub>3</sub>CN (16 mg, 0.25 mmol) was then added, and the mixture was stirred at 60 °C for 2 hr then left at room temp overnight. The mixture was quenched with 0.5 mL 1N HCl aqueous solution. After removal of the solvent, the residue was dissolved in 10% Na<sub>2</sub>CO<sub>3</sub> (8 mL), and extracted with EtOAc (3 x 6 mL). The EtOAc layers were combined, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and the solvent was removed. Chromatography of the residue on silica gel (cyclohexane-EtOAc, 8:1) gave **11** (31 mg, 85%) as a white solid;  $[\alpha]_D^{20}$  -1.8 (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.39 – 7.13 (m, 15H, H<sub>Ar</sub>), 4.70 (d, *J* = 11.9 Hz, 2H, PhCH<sub>2</sub>), 4.45 (d, *J* = 11.7 Hz, 1H, PhCHH), 4.36 (d, *J* = 11.7 Hz, 1H, PhCHH), 3.82 – 3.72 (m, 2H, 1x NPhCHH & H<sub>4</sub>), 3.65 (td, *J* = 8.3, 4.7 Hz, 1H, H<sub>2</sub>), 3.60 (dd, *J* = 7.9, 3.1 Hz, 1H, H<sub>3</sub>), 3.38 – 3.29 (m, 1H, H<sub>5</sub>), 2.75 (d, *J* = 6.6 Hz, 1H, OH), 2.63 (dd, *J* = 12.1, 4.5 Hz, 1H, H<sub>1-eq</sub>), 2.29 (dd, *J* = 12.1, 8.6 Hz, 1H, H<sub>1-ax</sub>), 1.28 (d, *J* = 6.7 Hz, 3H, CH<sub>3</sub>), 1.05 (d, *J* = 6.8 Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 145.61 (C), 138.68 (C), 138.64 (C), 128.54 (CH), 128.52 (CH), 128.38

(CH), 127.96 (CH), 127.79 (CH), 127.73 (CH), 127.58 (CH), 127.26 (CH), 127.06 (CH), 79.43 (C<sub>3</sub>), 75.59 (C<sub>2</sub>), 72.41 (PhCH<sub>2</sub>), 72.34 (PhCH<sub>2</sub>), 71.57 (C<sub>4</sub>), 59.12 (PhCH(CH<sub>3</sub>)N), 54.89 (C<sub>5</sub>), 45.84 (C<sub>1</sub>), 19.19 (PhCH(CH<sub>3</sub>)N), 9.78 (CH<sub>3</sub>, C<sub>6</sub>). IR (film, CHCl<sub>3</sub>):  $\nu_{\max}$  3025, 2920, 2358, 1450, 1369, 1260, 1205, 1092, 799, 738, 698; ES-HRMS: Found 432.2523, C<sub>28</sub>H<sub>34</sub>NO<sub>3</sub> requires 432.2539 [M+H]<sup>+</sup>.

### 1.2.3 *N*-Benzyl-2,3,4-tri-*O*-benzyl-1,5-dideoxy-1,5-imino-L-fucitol (**14a**)

The diol **13** (95 mg, 0.46 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (8 mL), and triethylamine (0.14 mL, 1.01 mmol) was added and the solution was cooled to 0 °C, methanesulfonyl chloride (75  $\mu$ L, 0.96 mmol) was added dropwise and stirred for 4 h. The solvent was removed under reduced pressure and the residue was used for next step without further purification. The bis-mesylate was dissolved in THF (1 mL) and benzylamine (0.2 mL, 1.84 mmol) was added at room temp, and the mixture was heated at 40 °C for 8 h, after which TLC indicated complete consumption of the bis-mesylate. The solution was cooled to room temp, and the solvent removed under reduced pressure. The residue was dissolved in EtOAc (30 mL), and washed with 2 M NaOH (2 x 20 mL). The organic phase was separated, further washed with brine and dried with Na<sub>2</sub>SO<sub>4</sub>. Removal of the solvent under reduced pressure and flash column chromatography of the residue on silica gel (cyclohexane-EtOAc, 10:1) gave **14a** (80 mg, 77%) as a colourless oil; R<sub>f</sub> 0.65 (cyclohexane-EtOAc, 4:3);  $[\alpha]_{\text{D}}^{20}$  -9.4 (*c* 0.85, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  7.45 – 7.17 (m, 20H, H<sub>Ar</sub>), 4.91 (d, *J* = 11.8 Hz, 1H, PhCHH), 4.82 – 4.71 (m, 2H, PhCH<sub>2</sub>), 4.66 (d, *J* = 11.8 Hz, 1H, PhCHH), 4.62 (d, *J* = 11.7 Hz, 1H, PhCHH), 4.55 (d, *J* = 11.7 Hz, 1H, PhCHH), 3.93 (d, *J* = 3.6 Hz, 1H, H<sub>2</sub>), 3.85 – 3.69 (m, 2H, PhCHH & H<sub>4</sub>), 3.59 (d, *J* = 14.0 Hz, 1H, PhCHH), 3.47 (d, *J* = 6.5 Hz, 1H, H<sub>3</sub>), 3.00 (dd, *J* = 11.9, 4.0 Hz, 1H, H<sub>1-eq</sub>), 2.62 (m, 1H, H<sub>5</sub>), 2.22 – 2.06 (m, 1H, H<sub>1-ax</sub>), 1.21 (d, *J* = 6.5 Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  139.21, 138.99, 138.23 (each C), 129.38, 128.43, 128.40, 128.31, 128.28, 128.26, 127.75, 127.55, 127.54, 127.51, 127.03 (each CH), 83.37 (C<sub>3</sub>), 79.10 (C<sub>4</sub>), 75.83 (C<sub>2</sub>), 74.29 (PhCH<sub>2</sub>), 73.11 (PhCH<sub>2</sub>), 72.51 (PhCH<sub>2</sub>), 57.36 (C<sub>5</sub>), 57.04 (PhCH<sub>2</sub>), 52.58 (C<sub>1</sub>), 14.98 (CH<sub>3</sub>, C<sub>6</sub>); ES-HRMS: Found 508.2844, C<sub>34</sub>H<sub>38</sub>NO<sub>3</sub> [M+H]<sup>+</sup> requires 508.2852.

### 1.2.4 *N*-Decyl-2,3,4-tri-*O*-benzyl-1,5-dideoxy-1,5-imino-L-fucitol (**14b**)

Reaction of the bis-mesylate (58 mg, 0.098 mmol) as described in the preparation of **14a** with *n*-decylamine gave **14b** (45 mg, 83%) as a white solid after column chromatography (cyclohexane-EtOAc, 7:1);  $[\alpha]_{\text{D}}^{20}$  12.0 (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  7.43–7.21 (m, 15H, H<sub>Ar</sub>), 4.91 (d, *J* = 12.0, 1H, 1 x PhCHH), 4.73 (m, 5H, 5 x PhCHH), 4.04 – 3.92 (m, 1H, H<sub>2</sub>), 3.64 (m, 1H, H<sub>4</sub>), 3.41 (d, *J* = 7.0, 1H, H<sub>3</sub>), 3.08 (dd, *J* = 10.0, 4.5, 1H, H<sub>1-eq</sub>), 2.63 – 2.45 (m, 2H, CH<sub>2</sub>), 2.41 (m, 1H, H<sub>5</sub>), 2.23 (t, *J* = 10.0, 1H, H<sub>1-ax</sub>), 1.47–1.08 (m, 16H, 8 x CH<sub>2</sub>), 1.00 (d, *J* = 6.5, 3H, NCHCH<sub>3</sub>), 0.88 (t, *J* = 6.5, 3H, CH<sub>2</sub>CH<sub>3</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  139.06 (C), 138.91 (C), 128.74, 128.30, 128.28, 128.05, 127.74, 127.46, 127.44, 127.38 (each CH), 84.31 (C<sub>3</sub>), 78.54 (C<sub>4</sub>), 76.32 (C<sub>2</sub>), 74.38 (PhCH<sub>2</sub>), 73.03 (PhCH<sub>2</sub>), 72.75 (PhCH<sub>2</sub>), 57.12 (C<sub>5</sub>), 54.15 (NCH<sub>2</sub>Ph), 53.19 (C<sub>1</sub>), 31.89, 30.32, 29.62, 29.59, 29.55, 29.31, 27.66, 22.66 (each CH<sub>2</sub>), 15.49 (CH<sub>3</sub>, C<sub>6</sub>), 14.09 (CH<sub>3</sub>). IR (film, CHCl<sub>3</sub>):  $\nu_{\max}$  3661, 2924, 2346,

1732, 1455, 1365, 1096, 734  $\text{cm}^{-1}$ ; HRMS: Calcd for  $\text{C}_{37}\text{H}_{52}\text{NO}_3$ ,  $[\text{M}+\text{H}]^+$ , 558.3947; Found, 558.3975.

#### 1.2.4 *N*-Octyl-2,3,4-tri-*O*-benzyl-1,5-dideoxy-1,5-imino-*L*-fucitol (**14c**)

Reaction of the bis-mesylate (44 mg, 0.074 mmol) as described in the preparation of **14a** with *n*-octylamine gave **14c** (33 mg, 80%) as a white solid after chromatography (cyclohexane-EtOAc, 8:1);  $[\alpha]^{20}_{\text{D}}$  11.2 (*c* 1.5,  $\text{CHCl}_3$ );  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.42–7.21 (m, 15H,  $\text{H}_{\text{Ar}}$ ), 4.91 (d,  $J = 12.0$ , 1H, 1 x  $\text{PhCHH}$ ), 4.83–4.62 (m, 5H, 5 x  $\text{PhCHH}$ ), 3.99 (td,  $J = 9.0$ , 4.5, 1H,  $\text{H}_2$ ), 3.64 (s, 1H,  $\text{H}_4$ ), 3.41 (dd,  $J = 9.0$ , 2.5, 1H,  $\text{H}_3$ ), 3.08 (dd,  $J = 11.5$ , 4.5, 1H,  $\text{H}_{1\text{-eq}}$ ), 2.53 (m, 2H,  $\text{CH}_2$ ), 2.41 (m, 1H,  $\text{H}_5$ ), 2.23 (t,  $J = 10.5$ , 1H,  $\text{H}_{1\text{-ax}}$ ), 1.48–1.35 (m, 1H, 1 x  $\text{CHHCH}_2$ ), 1.35 – 1.07 (m, 11H, 11 x  $\text{CHHCH}_2$ ), 1.00 (d,  $J = 6.5$ , 3H,  $\text{CHCH}_3$ ), 0.87 (t,  $J = 7.0$ , 3H,  $\text{CH}_2\text{CH}_3$ );  $^{13}\text{C NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  139.07, 138.92 (each C), 128.74, 128.31, 128.29, 128.06, 127.75, 127.47, 127.46, 127.45, 127.39 (each CH), 84.26 ( $\text{C}_3$ ), 78.56 ( $\text{C}_4$ ), 76.32 ( $\text{C}_2$ ), 74.39 ( $\text{PhCH}_2$ ), 73.03 ( $\text{PhCH}_2$ ), 72.75 ( $\text{PhCH}_2$ ), 57.13 ( $\text{C}_5$ ), 54.14 ( $\text{C}_1$ ), 53.19 ( $\text{NCH}_2\text{Ph}$ ), 31.82, 29.56, 29.28, 27.67, 23.19, 22.65 (each  $\text{CH}_2$ ), 15.70 ( $\text{CH}_3\text{-C}_6$ ), 14.09 ( $\text{CH}_3$ ); IR (film,  $\text{CHCl}_3$ ):  $\nu_{\text{max}}$  3389, 3034, 2925, 2347, 1955, 1603, 1455, 1367, 1269, 1096  $\text{cm}^{-1}$ . HRMS: Calcd for  $\text{C}_{35}\text{H}_{48}\text{NO}_3$ ,  $[\text{M}+\text{H}]^+$ , 530.3634; Found, 530.3654.

#### 1.2.5 *N*-Butyl -2,3,4-tri-*O*-benzyl- 1,5 -dideoxy-1,5-imino-*L*-fucitol (**14d**)

Compound **14d** was prepared from bis-mesylate (81 mg, 0.136 mmol) as described in the preparation of **14a**, yielding **14d** (50 mg, 78%) as white solids after column chromatography (cyclohexane-EtOAc 8:1);  $[\alpha]^{20}_{\text{D}}$  16.4 (*c* 1.0,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.42 – 7.22 (m, 15H,  $\text{H}_{\text{Ar}}$ ), 4.92 (d,  $J = 11.8$  Hz, 1H, 1 x  $\text{PhCHH}$ ), 4.80 (d,  $J = 12.0$  Hz, 1H, 1 x  $\text{PhCHH}$ ), 4.71 (m, 4H, 4 x  $\text{PhCHH}$ ), 3.99 (td,  $J = 9.0$ , 4.5 Hz, 1H,  $\text{H}_2$ ), 3.65 (t,  $J = 2.3$  Hz, 1H,  $\text{H}_4$ ), 3.41 (dd,  $J = 8.8$ , 2.7 Hz, 1H,  $\text{H}_3$ ), 3.08 (dd,  $J = 11.0$ , 4.5 Hz, 1H,  $\text{H}_{1\text{-eq}}$ ), 2.58 (ddd,  $J = 13.6$ , 11.0, 5.0 Hz, 1H,  $\text{NCHHCH}_2$ ), 2.50 (ddd,  $J = 13.6$ , 11.0, 5.0 Hz, 1H,  $\text{NCHHCH}_2$ ), 2.41 (m, 1H,  $\text{H}_5$ ), 2.23 (dd,  $J = 11.0$ , 9.8 Hz, 1H,  $\text{H}_{1\text{-ax}}$ ), 1.48 – 1.16 (m, 4H, 2 x  $\text{CH}_2$ ), 1.01 (d,  $J = 6.5$  Hz, 3H,  $\text{CHCH}_3$ ), 0.89 (t,  $J = 7.3$  Hz, 3H,  $\text{CH}_2\text{CH}_3$ );  $^{13}\text{C NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  139.05 (C), 138.90 (C), 128.73, 128.29, 128.28, 128.05, 127.74, 127.46, 127.43, 127.38 (each CH), 84.48 ( $\text{C}_3$ ), 78.56 ( $\text{C}_4$ ), 76.31 ( $\text{C}_2$ ), 74.39 ( $\text{PhCH}_2$ ), 73.02 ( $\text{PhCH}_2$ ), 72.74 ( $\text{PhCH}_2$ ), 57.13 ( $\text{C}_5$ ), 54.27 ( $\text{C}_1$ ), 52.86 ( $\text{NCH}_2$ ), 25.33 ( $\text{CH}_2$ ), 20.81 ( $\text{CH}_2$ ), 15.36 ( $\text{CH}_3\text{-C}_6$ ), 14.04 ( $\text{CH}_2\text{CH}_3$ ); HRMS: Calcd for  $\text{C}_{31}\text{H}_{40}\text{NO}_3$ ,  $[\text{M}+\text{H}]^+$ , 474.3008; Found, 474.3024.

#### 1.2.6 *N*-(2-(Benzyloxy)ethyl)-2,3,4-tri-*O*-benzyl-1,5-dideoxy-1,5-imino-*L*-fucitol (**14e**)

Compound **14e** was prepared from bis-mesylate (85 mg, 0.144 mmol) as described in the preparation of **14a**, yielding **14e** (60 mg, 75%) as white solids after column chromatography (cyclohexane-EtOAc 4:1);  $[\alpha]^{20}_{\text{D}}$  2.9 (*c* 1.25,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.41 – 7.22 (m, 20H,  $\text{H}_{\text{Ar}}$ ), 4.91 (d,  $J = 12.0$ , 1H, 1 x  $\text{PhCHH}$ ), 4.80 (d,  $J = 12.0$ , 1H, 1 x  $\text{PhCHH}$ ), 4.77 – 4.66 (m, 3H, 3 x  $\text{PhCHH}$ ), 4.64 (d,  $J = 12.0$ , 1H, 1 x  $\text{PhCHH}$ ), 4.47 (s, 2H, 2 x  $\text{PhCHH}$ ), 3.97 (td,  $J = 9.0$ , 4.5, 1H,  $\text{H}_2$ ), 3.65 (m, 1H,  $\text{H}_4$ ), 3.61 – 3.48 (m, 2H,  $\text{NCH}_2\text{CH}_2$ ), 3.43 (dd,  $J = 9.0$ , 2.5, 1H,  $\text{H}_3$ ), 3.16 (dd,  $J = 11.0$ , 4.5,

1H, H<sub>1-eq</sub>), 2.93 – 2.78 (m, 2H, CH<sub>2</sub>OBn), 2.54 (m, 1H, H<sub>5</sub>), 2.42 (t, J = 11.0, 1H, H<sub>1-ax</sub>), 1.05 (d, J = 6.5, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 139.04 (C), 138.86 (C), 138.38 (C), 128.65, 128.33, 128.30, 128.27, 128.07, 127.73, 127.54, 127.50, 127.45, 127.38 (each CH), 84.48 (C<sub>3</sub>), 78.56 (C<sub>4</sub>), 76.00 (C<sub>2</sub>), 74.39 (PhCH<sub>2</sub>), 73.18 (PhCH<sub>2</sub>), 73.04 (PhCH<sub>2</sub>), 72.67 (PhCH<sub>2</sub>), 67.21(NCH<sub>2</sub>), 57.72 (C<sub>5</sub>), 54.38 (C<sub>1</sub>), 51.94 (CH<sub>2</sub>), 15.74 (CH<sub>3</sub>, C<sub>6</sub>). ES-HRMS: Found 552.3114, C<sub>36</sub>H<sub>42</sub>NO<sub>4</sub> [M+H]<sup>+</sup> requires 552.3122.

### 1.2.7 N-(2-Aminoethyl)-2,3,4-tri-O-benzyl-1,5-dideoxy-1,5-imino-L-fucitol (14f)

Compound **14f** was prepared from bis-mesylate (130 mg, 0.22 mmol) as described in the preparation of **14a**, yielding **14f** (82 mg, 81%) as white foam after column chromatography (cyclohexane/EA 2:1); [α]<sup>20</sup><sub>D</sub> -0.8 (c 2.3, CHCl<sub>3</sub>); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 7.38 – 7.25 (m, 15H, H<sub>Ar</sub>), 4.89 (d, J = 11.4, 1H, 1 x PhCHH), 4.62 – 4.81 (m, 5H, 5 x PhCHH), 3.96 (m, 1H, H<sub>2</sub>), 3.70 (m, 1H, H<sub>4</sub>), 3.48 (dd, J = 7.2, 1.5, 1H, H<sub>3</sub>), 3.08 (dd, J = 10.5, 3.5, 1H, H<sub>1-eq</sub>), 2.80 – 2.66 (m, 3H, NCHHCH<sub>2</sub> & CH<sub>2</sub>NH<sub>2</sub>), 2.55 (m, 1H, H<sub>5</sub>), 2.42 (t, J = 6.0, 1H, NCHHCH<sub>2</sub>), 2.21 (t, J = 10.5, 1H, H<sub>1-ax</sub>), 1.09 (d, J = 6.5, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 139.03 (C), 138.92 (C), 138.81(C), 128.34, 128.32, 128.26, 128.18, 127.73, 127.53, 127.50, 127.46, 127.42 (each CH), 83.16 (C<sub>3</sub>), 78.87 (C<sub>4</sub>), 75.86 (C<sub>2</sub>), 74.20 (PhCH<sub>2</sub>), 73.04 (PhCH<sub>2</sub>), 72.67 (PhCH<sub>2</sub>), 57.96 (C<sub>5</sub>), 55.51(CH<sub>2</sub>), 53.12 (C<sub>1</sub>), 38.13 (CH<sub>2</sub>), 14.66 (CH<sub>3</sub>). IR (film, CHCl<sub>3</sub>): ν<sub>max</sub> 3360, 2915, 1364, 1095 cm<sup>-1</sup>. ES-HRMS: Found 461.2802, C<sub>29</sub>H<sub>37</sub>N<sub>2</sub>O<sub>3</sub> [M+H]<sup>+</sup> requires 461.2804.

### 1.2.8 N-Allyl- 2,3,4-tri-O-benzyl-1,5-dideoxy-1,5-imino-L-fucitol (14g)

Compound **14g** was prepared from bis-mesylate (90 mg, 0.15 mmol) as described in the preparation of **14a**, yielding **14g** (59 mg, 85%) as colorless oil after column chromatography (cyclohexane/EA 6:1); R<sub>f</sub> 0.52 (cyclohexane-EtOAc, 2:1); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.56 – 7.09 (m, 15H, H<sub>Ar</sub>), 5.97 – 5.77 (m, 1H, NCH<sub>2</sub>CH=CH<sub>2</sub>), 5.19 – 5.08 (m, 2H, NCH<sub>2</sub>CH=CH<sub>2</sub>), 4.92 (d, J = 11.8 Hz, 1H, 1 x PhCHH), 4.85 – 4.61 (m, 5H, 5 x PhCHH), 4.10 – 3.94 (m, 1H, H<sub>2</sub>), 3.66 (s, 1H, H<sub>4</sub>), 3.42 (dd, J = 7.6, 1.0 Hz, 1H, H<sub>3</sub>), 3.26 (dd, J = 14.3, 6.0 Hz, 1H, NCHHCH=CH<sub>2</sub>), 3.16 – 3.02 (m, 2H, H<sub>1-eq</sub> & NCHHCH=CH<sub>2</sub>), 2.41 (m, 1H, H<sub>5</sub>), 2.19 (t, J = 10.4 Hz, 1H, H<sub>1-ax</sub>), 1.03 (d, J = 6.4 Hz, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 139.17, 139.00, 138.98 (each C), 133.64 (CH<sub>2</sub>CH=CH<sub>2</sub>), 128.92, 128.46, 128.43, 128.23, 127.89, 127.65, 127.62, 127.60, 127.56 (each CH), 118.38 (CH<sub>2</sub>CH=CH<sub>2</sub>), 84.24 (C<sub>3</sub>), 78.64 (C<sub>4</sub>), 76.26 (C<sub>2</sub>), 74.56 (PhCH<sub>2</sub>), 73.24(PhCH<sub>2</sub>), 72.87(PhCH<sub>2</sub>), 57.50 (C<sub>5</sub>), 56.47(NCH<sub>2</sub>CH), 54.39 (C<sub>1</sub>), 15.44 (CH<sub>3</sub>, C<sub>6</sub>). ES-HRMS: Found 458.2702, C<sub>30</sub>H<sub>36</sub>NO<sub>3</sub> [M+H]<sup>+</sup> requires 458.2695.

### 1.2.9 N-((Adamantylcarbonylamino)-ethyl)-2,3,4-tri-O-benzyl-1,5-dideoxy-1,5-imino-L-fucitol (15)

The 1-adamantanecarboxylic acid (5.6 mg, 0.031mmol) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub>, cooled to 0 °C and EDC (9.2 mg, 0.048 mmol), HOBT (6.3 mg, 0.048 mmol), and DMAP (5.7 mg, 0.048 mmol) were added. After stirring for 1hr, the amine **14f** (11mg, 0.024 mmol) was added. The reaction was warmed to room temp and stirred for 24 h.

The mixture was then quenched with NH<sub>4</sub>Cl (sat.) and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic fractions were washed with satd. NaHCO<sub>3</sub>, brine and dried with Na<sub>2</sub>SO<sub>4</sub>. The crude product was purified by flash chromatography on silica gel eluting with cyclohexane-EtOAc(1:1) to yield **15** (13 mg, 88%); R<sub>f</sub> 0.18 (cyclohexane-EA, 1:1); [α]<sup>20</sup><sub>D</sub> -4.4 (*c* 1.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.41 – 7.21 (m, 15H, H<sub>Ar</sub>), 4.85 (d, *J* = 11.5 Hz, 1H, 1 x PhCHH), 6.32 (s, 1H, NH), 4.80 – 4.71 (m, 2H, 2 x PhCHH), 4.65 (m, 3H, 3 x PhCHH), 3.86 (s, 1H, H<sub>2</sub>), 3.72 (s, 1H, H<sub>4</sub>), 3.54 (d, *J* = 5.7 Hz, 1H, H<sub>3</sub>), 3.26 (d, *J* = 4.9 Hz, 2H, NCH<sub>2</sub>CH<sub>2</sub>), 3.06 (dd, *J* = 11.8, 3.4 Hz, 1H, H<sub>1-eq</sub>), 2.71 (m, 2H, 1 x NCHHCH<sub>2</sub> & H<sub>5</sub>), 2.52 – 2.39 (m, 1H, NCHHCH<sub>2</sub>), 2.24 (m, 1H, H<sub>1-ax</sub>), 1.93 (s, 3H, 3 x CH), 1.77 (d, *J* = 2.0 Hz, 6H, 3 x CH<sub>2</sub>), 1.67 (d, *J* = 12.0 Hz, 3H, 3 x CHH), 1.60 (d, *J* = 12.0 Hz, 3H, 3 x CHH), 1.12 (d, *J* = 6.5 Hz, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 178.12 (C=O), 139.13, 139.11, 138.81 (each C), 128.50, 128.46, 128.32, 127.98, 127.72, 127.58 (each CH), 84.18 (C<sub>3</sub>), 78.69 (C<sub>4</sub>), 75.88 (C<sub>2</sub>), 73.94 (PhCH<sub>2</sub>), 73.22 (PhCH<sub>2</sub>), 72.59 (PhCH<sub>2</sub>), 57.70 (C<sub>5</sub>), 51.05 (NCH<sub>2</sub>CH<sub>2</sub>), 40.70 (C), 39.48, 39.38, 36.70, 36.62, 35.97, 28.29 (each CH<sub>2</sub>), 28.25 (CH), 15.40 (CH<sub>3</sub>). HRMS: Calcd for C<sub>40</sub>H<sub>50</sub>N<sub>2</sub>O<sub>4</sub>, [M+H]<sup>+</sup>, 623.3849; Found, 623.3839.

#### 1.2.10 1,5-Dideoxy-1,5-imino-L-fucitol (**2**)

A mixture of **14a** (23mg, 0.045 mmol) in MeOH (3mL) was added 0.2 mL 6N HCl and 10% Pd-C (5 mg). The mixture was stirred for 24 h under H<sub>2</sub> atmosphere. The catalyst was then removed by filtration through Celite, and the filtrate was concentrated. The residue was dissolved in 1.0 M methanolic solution of HCl (1.0 mL) and the solvent was evaporated. The residue, dissolved in H<sub>2</sub>O, was introduced to a Dowex-50W-X8-100 (H<sup>+</sup>) packed ion-exchange column (pre-washed with MeOH and H<sub>2</sub>O), washed with water (100 mL) and MeOH (50 mL) and then the product was eluted with 1.0 M aqueous solution of NH<sub>4</sub>OH. The combined pure fractions were evaporated *in vacuo* to give **2** (6.5 mg, 91%) as a white solid after lyophilization. The NMR data of the free amine **2** were in excellent agreement to those reported in literature;<sup>28,35</sup> [α]<sup>20</sup><sub>D</sub> -42.4 (*c* 0.48, MeOH); <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ 3.70 – 3.51 (m, 2H, H<sub>4</sub> & H<sub>2</sub>), 3.30 (d, *J* = 9.4 Hz, 1H, H<sub>3</sub>), 2.97 (d, *J* = 8.4 Hz, 1H, H<sub>1-eq</sub>), 2.78 (m, 1H, H<sub>5</sub>), 2.30 (t, *J* = 11.4 Hz, 1H, H<sub>1-ax</sub>), 0.94 (d, *J* = 6.6 Hz, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O) δ 75.65 (C<sub>3</sub>), 72.99 (C<sub>4</sub>), 68.06 (C<sub>2</sub>), 54.45 (C<sub>5</sub>), 49.19 (C<sub>1</sub>), 16.70 (C<sub>6</sub>). HRMS: Calcd for C<sub>6</sub>H<sub>14</sub>NO<sub>3</sub>, [M+H]<sup>+</sup>, 148.0974; Found, 148.0971.

#### 1.2.11 N-(4-Hydroxybutyl)- 1,5-dideoxy-1,5-imino-L-fucitol (**22**)

A mixture of **14a** (23.0 mg, 0.045 mmol) and 10% Pd-C (10.0 mg) in HOAc (0.25 mL), H<sub>2</sub>O (0.5 mL), and THF (1.0 mL) was stirred for 24 h under H<sub>2</sub> atmosphere. The catalyst was then removed by filtration through Celite, and the filtrate was concentrated. The residue was dissolved in 1.0 M methanolic solution of HCl (1.0 mL) and the solvent was evaporated. The residue, dissolved in H<sub>2</sub>O, was introduced to a Dowex-50W-X8-100 (H<sup>+</sup>) packed ion-exchange column (pre-washed with MeOH and H<sub>2</sub>O), washed with water (100 mL) and MeOH (50 mL) and then the product was eluted with 1.0 M aqueous solution of NH<sub>4</sub>OH. The combined pure fractions were evaporated *in vacuo* to give **22** (8.5 mg, 85%) as a white powder after lyophilization. <sup>1</sup>H NMR (500 MHz,

CD<sub>3</sub>OD)  $\delta$  3.88 (td,  $J = 10.0, 4.9$  Hz, 1H, H<sub>2</sub>), 3.73 (d,  $J = 1.5$  Hz, 1H, H<sub>4</sub>), 3.70 – 3.57 (m, 2H, CH<sub>2</sub>OH), 3.33 (dd,  $J = 9.3, 3.3$  Hz, 1H, H<sub>3</sub>), 3.14 (dd,  $J = 11.3, 4.9$  Hz, 1H, H<sub>1-eq</sub>), 2.91 – 2.78 (m, 1H, 1x NCHHCH<sub>2</sub>), 2.70 – 2.53 (m, 2H, 1x NCHHCH<sub>2</sub> & H<sub>5</sub>), 2.25 (t,  $J = 10.7$  Hz, 1H, H<sub>1-ax</sub>), 1.71 – 1.62 (m, 2H, 1x NCH<sub>2</sub>CH<sub>2</sub>), 1.62 – 1.54 (m, 2H, CH<sub>2</sub>), 1.30 (d,  $J = 6.6$  Hz, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD)  $\delta$  77.06 (C<sub>3</sub>), 74.59 (C<sub>4</sub>), 68.73 (C<sub>2</sub>), 62.65 (CH<sub>2</sub>OH), 60.20 (C<sub>5</sub>), 57.33 (C<sub>1</sub>), 53.79 (NCH<sub>2</sub>), 31.53 (CH<sub>2</sub>), 21.81 (CH<sub>2</sub>), 16.05 (CH<sub>3</sub>, C<sub>6</sub>). HRMS: Calcd for C<sub>10</sub>H<sub>22</sub>NO<sub>4</sub>, [M+H]<sup>+</sup>, 220.1549; Found, 220.1544.

#### 1.2.12 *N*-Decyl-1,5-Dideoxy-1,5-imino-L-fucitol (16)

Compound **16** was prepared from **14b** as described in the preparation of **2**, yielding **16** (90%) as a solid after lyophilization; <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD)  $\delta$  3.80 (td,  $J = 9.4, 4.9$  Hz, 1H, H<sub>2</sub>), 3.65 (dd,  $J = 3.3, 1.4$  Hz, 1H, H<sub>4</sub>), 3.25 (dd,  $J = 9.4, 3.3$  Hz, 1H, H<sub>3</sub>), 3.01 (dd,  $J = 11.2, 4.9$  Hz, 1H, H<sub>1-eq</sub>), 2.77 – 2.63 (m, 1H, NCHH), 2.57 – 2.39 (m, 2H, H<sub>5</sub> & NCHH), 2.14 (t,  $J = 10.8$  Hz, 1H, H<sub>1-ax</sub>), 1.49 (m, 2H, CH<sub>2</sub>), 1.39 – 1.24 (m, 14H, 7 x CH<sub>2</sub>), 1.21 (d,  $J = 6.6$  Hz, CH<sub>3</sub>, H<sub>6</sub>), 0.91 (t,  $J = 7.0$  Hz, 3H, CHCH<sub>3</sub>). <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD)  $\delta$  77.31(C<sub>3</sub>), 74.84 (C<sub>4</sub>), 69.02 (C<sub>2</sub>), 59.77 (C<sub>5</sub>), 57.74 (C<sub>1</sub>), 53.96 (NCH<sub>2</sub>CH<sub>2</sub>), 33.03, 30.70, 30.66, 30.60, 30.42, 28.58, 24.65, 23.70 (each CH<sub>2</sub>), 16.32(C<sub>6</sub>), 14.41(CH<sub>3</sub>). HRMS: Calcd for C<sub>16</sub>H<sub>34</sub>NO<sub>3</sub>, [M+H]<sup>+</sup>, 288.2539; Found, 288.2539.

#### 1.2.13 *N*-Octyl-1,5-dideoxy-1,5-imino-L-fucitol (17)

Compound **17** was prepared from **14c** as described in the preparation of **2**, yielding **17** (85%) as a solid after lyophilization; <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD)  $\delta$  3.83 (td,  $J = 9.9, 4.9$  Hz, 1H, H<sub>2</sub>), 3.69 (d,  $J = 3.0$  Hz, 1H, H<sub>4</sub>), 3.29 (dd,  $J = 9.3, 3.0$  Hz, 1H, H<sub>3</sub>), 3.07 (dd,  $J = 11.0, 4.8$  Hz, 1H, H<sub>1-eq</sub>), 2.85 – 2.71 (m, 1H, NCHH), 2.70 – 2.52 (m, 2H, H<sub>5</sub> & NCHH), 2.26 (t,  $J = 10.5$  Hz, 1H, H<sub>1-ax</sub>), 1.53 (m, 2H), 1.32 (m, 10H), 1.24 (d,  $J = 6.6$  Hz, 3H, CH<sub>3</sub>), 0.91 (t,  $J = 6.9$  Hz, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD)  $\delta$  76.92 (C<sub>3</sub>), 74.42 (C<sub>4</sub>), 68.62 (C<sub>2</sub>), 60.11 (C<sub>5</sub>), 57.21 (C<sub>1</sub>), 53.97 (NCH<sub>2</sub>), 32.95, 30.51, 30.35, 28.45, 24.46, 23.68 (each CH<sub>2</sub>), 15.95 (C<sub>6</sub>), 14.39 (CH<sub>3</sub>). HRMS: Calcd for C<sub>14</sub>H<sub>30</sub>NO<sub>3</sub>, [M+H]<sup>+</sup>, 260.2226; Found, 260.2232.

#### 1.2.14 *N*-Butyl-1,5-dideoxy-1,5-imino-L-fucitol (18)

Compound **18** was prepared from **14d** as described in the preparation of **2**, yielding **18** (88 %) as a solid after lyophilization; <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$  4.13- 4.18(m, 1H, H<sub>2</sub>), 4.11(s, 1H, H<sub>4</sub>), 3.72 (d,  $J = 9.5$ , 1H, H<sub>3</sub>), 3.52-3.60 (m, 2H, H<sub>1-eq</sub> & H<sub>5</sub>), 3.30 (dt,  $J = 12.0, 5.5$  Hz, 1H, NCHH), 3.20 (dt,  $J = 12.0, 5.5$ Hz, 1H, NCHH) 3.03 (t,  $J = 12.0$  Hz, 1H, H<sub>1-ax</sub>) 1.73-1.81 (m, 2H, CH<sub>2</sub>), 1.48 (d,  $J = 7.0$ , 3H, CH<sub>3</sub>), 1.42-1.49 (m, 2H, CH<sub>2</sub>), 1.02 (t,  $J = 7.5$ , 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O)  $\delta$  75.48 (C<sub>3</sub>), 73.71 (C<sub>4</sub>), 67.40 (C<sub>2</sub>), 63.15 (C<sub>5</sub>), 55.90 (C<sub>1</sub>), 55.14 (NCH<sub>2</sub>), 26.09 (CH<sub>2</sub>), 21.86 (CH<sub>2</sub>), 15.99 (CH<sub>3</sub>, C<sub>6</sub>), 15.33 (CH<sub>3</sub>). HRMS: Calcd for C<sub>10</sub>H<sub>21</sub>NO<sub>3</sub>, [M+H]<sup>+</sup>, 204.1600; Found, 204.1604.

### 1.2.15 *N*-(2-Hydroxyethyl)-1,5-dideoxy-1,5-imino-L-fucitol (**19**)

Compound **19** was prepared from **14e** as described in the preparation of **2**, yielding **19** (78 %) as a solid after lyophilization; <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ 3.85 (m, 4H, H<sub>2</sub> & H<sub>4</sub> & CH<sub>2</sub>OH), 3.51 (d, *J* = 9.7 Hz, 1H, H<sub>3</sub>), 3.28 – 3.15 (m, 1H, H<sub>1-eq</sub>), 3.07 – 2.93 (m, 1H, NCHHCH<sub>2</sub>), 2.82 – 2.63 (m, 2H, H<sub>5</sub> & NCHHCH<sub>2</sub>), 2.36 (t, *J* = 10.7 Hz, 1H, H<sub>1-ax</sub>), 1.13 (d, *J* = 6.4 Hz, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O) δ 74.91(C<sub>3</sub>), 73.13 (C<sub>4</sub>), 66.94 (C<sub>2</sub>), 58.49 (C<sub>5</sub>), 57.51 (CH<sub>2</sub>OH), 56.07 (C<sub>1</sub>), 53.16 (NCH<sub>2</sub>), 15.24 (C<sub>6</sub>). HRMS: Calcd for C<sub>8</sub>H<sub>8</sub>NO<sub>4</sub>, [M+H]<sup>+</sup>, 192.1236; Found, 192.1228.

### 1.2.16 *N*-Adamantylcarbonylaminoethyl-1,5-Dideoxy-1,5-imino-L-fucitol (**20**)

Compound **20** was prepared from **15** as described in the preparation of **2**, yielding **20** (83 %) as solids after lyophilization; <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) δ 3.98 (ddd, *J* = 14.2, 9.5, 4.4 Hz, 1H, H<sub>2</sub>), 3.85 (m, 1H, H<sub>4</sub>), 3.49 (dd, *J* = 10.5, 6.0 Hz, 2H, NCH<sub>2</sub>), 3.45 (dd, *J* = 9.1, 2.4 Hz, 1H, H<sub>3</sub>), 3.39 (dd, *J* = 11.9, 4.9 Hz, 1H, H<sub>1-eq</sub>), 3.29 – 3.22 (m, 1H, H<sub>5</sub>), 3.22 – 3.17 (m, 1H, CHHNH), 3.08 – 3.00 (m, 1H, CHHNH), 2.76 (t, *J* = 11.2 Hz, 1H, H<sub>1-ax</sub>), 2.04 (s, 3H, 3 x CHCH<sub>2</sub>), 1.88 (d, *J* = 2.4 Hz, 6H, 3 x CHCH<sub>2</sub>), 1.78 (m, 6H, 3x CHCH<sub>2</sub>), 1.39 (d, *J* = 6.6 Hz, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD) δ 180.88 (C=O), 74.11 (C<sub>3</sub>), 71.60 (C<sub>4</sub>), 65.74(C<sub>2</sub>), 60.60 (C<sub>5</sub>), 55.23(C<sub>1</sub>), 52.55 (CH<sub>2</sub>), 40.42(C), 38.85, 38.70, 36.18, 36.11, 34.68 (each CH<sub>2</sub>), 28.17(CH), 13.43 (C<sub>6</sub>, CH<sub>3</sub>); HRMS: Calcd for C<sub>19</sub>H<sub>33</sub>N<sub>2</sub>O<sub>4</sub>, [M+H]<sup>+</sup>, 353.2440; Found, 353.2451.

### 1.2.17 1,5,6-Trideoxy-1,5-imino-D-altritol (**5**)

Compound **5** was prepared from **9** as described in the preparation of **2**, yielding **5** (93%) or prepared from **11** in 83% yield, as solids after lyophilization. [ $\alpha$ ]<sup>20</sup><sub>D</sub> 0.9 (c 0.55, MeOH) [lit. [ $\alpha$ ]<sup>20</sup><sub>D</sub> 2 (c 1.1, MeOH)]; <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ 3.93 – 3.78 (m, 2H, H<sub>2</sub> & H<sub>3</sub>), 3.55 (d, *J* = 8.9 Hz, 1H, H<sub>4</sub>), 2.93 (m, 2H, H<sub>1-eq</sub> & H<sub>5</sub>), 2.76 (dd, *J* = 14.0, 2.4 Hz, 1H, H<sub>1-ax</sub>), 1.35 (d, *J* = 6.6, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O) δ 70.82 (C<sub>3</sub>), 69.96 (C<sub>4</sub>), 68.67 (C<sub>2</sub>), 50.49 (C<sub>5</sub>), 44.23 (C<sub>1</sub>), 15.86 (C<sub>6</sub>). HRMS: Calcd for C<sub>6</sub>H<sub>14</sub>NO<sub>3</sub>, [M+H]<sup>+</sup>, 148.0974; Found, 148.0967.

## 1.3 Fucosidase Inhibition Assays

The substrate used for the enzyme assays was 4-nitrophenyl  $\alpha$ -L-fucopyranoside. Substrate solutions were made from a stock solution (10 mM) in 10 mL batches, as required, by dissolving the appropriate mass of substrate in the correct buffer solution for the enzyme. These were kept at 4 °C when not in use. Assays were carried out in triplicate, using water as a blank in place of the inhibitor. The concentrations of the enzyme were adjusted so that the reading for the final absorbance was in the range of 1.0-2.0 units over a reaction time of 20 min. Linearity over the time course of the reaction was checked using a series of incubation times. The following were combined in the well of a flat-bottomed 96-well (300  $\mu$ L) microtitre plate: 10  $\mu$ L of enzyme solution; 10  $\mu$ L inhibitor solution; 80  $\mu$ L substrate solution. The reaction mix was incubated at 25 °C for 20 min and was quenched by the addition of 100  $\mu$ L of 2 M Na<sub>2</sub>CO<sub>3</sub>. Absorbance at 405 nm was measured immediately using a microtitre plate reader. Percentage inhibition was plotted against the log of the inhibitor concentration

and a trendline was fitted using Microsoft Excel. The IC<sub>50</sub> value for each compound calculated from the value of the log of the inhibitor concentration at 50% inhibition of enzyme activity.

#### 1.4 Molecular modelling

The PDB for fucosidase of *T. maritima* was available from the RCSB-PDB, while for the fucosidase of *B. taurus*, a homology model was constructed. The sequence for the *B. taurus* fucosidase (Accession: AAI12589.1 GI: 86827599) was obtained from NCBI (National Center for Biotechnology Information). Next a BlastP search along with the BLOSSUM62 substitution matrix, generated putative template PDBs with the minimum identity of 35% and 87% sequence coverage, provided in **Table S1**, in supplementary information. Later, homology modelling on templates having more than 35 % identity and at least 85% of coverage of the query sequence was conducted. The software programme MOE 2018.01 implementing the Amber 99 forcefield was used [66]. After generated homology models were assessed. Initially, the generated homology model and the template were superimposed giving an RMSD difference, which was less than 1 Å [67]. See **Fig. S1** in the *supplementary information*. The Z-score for the templates and homology models, was assessed using ProSA, a protein structure analysis tool, respectively ([68]. See **Fig. S2** in *supplementary information*. Next, the Ramachandran plot was generated. In the Ramachandran plot, the core or the allowed regions are those satisfying the  $\psi/\phi$  angle pairs for residues in a protein. See **Fig. S4** in *supplementary information* [69, 70]. For those amino acids which did not comply with the requirements of the Ramachandran plot, further energy minimization was done. ERRAT plots, which are for the determination of errors in model building were performed. See **Fig. S3** in the *supplementary information*) [71]. Verify 3D was used to determine the compatibility of the constructed homology model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar etc); a score of >80% was considered acceptable (Figure 4) [72]. The active site for docking was identified with the active site finder tool of MOE and later, the docked pose of the largest ensembled cluster within a RMSD of 2 Å, was selected [73]. The triangle matcher placement method and GBVI/WSA dG scoring was used for the docking. Molecular dynamics (MD) was performed on the selected docked poses of compounds, **2** and **16** [58]. This analysis was carried out also using MOE 2018. 01 software [66]. Partial charges were calculated and energy minimizations were done. MD was carried for a duration of 100 ps. The adopted docking procedure was validated using the co-crystallized structure of iso-6FNJ binding to fucosidase was obtained from the PDB (2ZXD). After docking iso-6FNJ to the fucosidase, an RMSD value of 0.391 Å between the co-crystallized structure and the docked structure indicated that the adopted docking procedure was reliable. [74].

#### 1.5 Cell Culture and Cytotoxicity Evaluation

##### 1.5.1 Materials and Cell Lines

The MCF-7 breast cancer and DU-145 prostate cancer cell lines were obtained from Dr. Stephen Rea, National University of Ireland, Galway. The BT-474 breast cancer cell line was obtained from Dr. Helen Dodson, National University of Ireland, Galway. MCF-7 were cultured in Dulbecco's modified Eagle's medium (DMEM) containing high glucose (4.5 g/mL) and supplemented with 1% penicillin-streptomycin and 10% heat-inactivated fetal bovine serum (FBS). DU-145 were cultured in RPMI-1640 medium and supplemented with 1% 2 mM L-glutamine, 1% penicillin-streptomycin and 10% non heat-inactivated fetal bovine serum (FBS). BT-474 were cultured in Dulbecco's modified Eagle's medium (DMEM)/Nutrient mixture F-12 Ham and supplemented with 1% penicillin-streptomycin and 10% non heat-inactivated fetal bovine serum (FBS). All cells grew as adherent cultures. Cell culture reagents were obtained from Sigma-Aldrich. Disposable sterile plasticware was obtained from Sarstedt (Numbrecht, Germany).

### **1.5.2 Cytotoxicity measurements using the MTT assay**

The MTT colorimetric assay was used to determine cell viability. Cells were added to 96-well plates at a cell density of 1000 cells per well (MCF-7, 200  $\mu$ L per well), 2000 cells per well (DU-145, 200  $\mu$ L per well) and 3000 cells per well (BT-474, 200  $\mu$ L per well) and allowed to adhere over 24 hours. Compound solutions in DMSO were added after 24 hours (1% v/v final concentration in well). The control cells were exposed to the same concentration of the vehicle control alone (DMSO). All cells were incubated at 37 °C and 5% CO<sub>2</sub> (humidified atmosphere) for 72 hours. MTT (20  $\mu$ L, 5 mg/mL solution) was added after 72 hours and the cells were incubated for a further 3 hours. The supernatant was then removed using a multi-transfer pipette and DMSO (100  $\mu$ L) added to dissolve the MTT formazan crystals. The absorbance was determined using a plate reader at 550 nm with a reference at 690 nm. Cell viability is expressed as a percentage of the vehicle-only treated control (DMSO). Dose-response curves were analyzed by non-linear regression analysis and IC<sub>50</sub> values were determined using GraphPad Prism software, v 8.0 (GraphPad Inc., San Diego, CA, USA). The *in vitro* activity of the drugs towards all cell lines is expressed as IC<sub>50</sub> (i.e. concentration required for the reduction of the mean cell viability to 50%).

### **1.6 Tumor cell proliferation assay using HNO41, HNO97, HNO210 & NCH644 cell lines**

To further assess the influence of **16** on tumor cell proliferation well-characterized human head and neck carcinoma cell lines (HNO41, HNO97, HNO210; [75-77] as well as glioblastoma-initiating cells (NCH644, [63]) were incubated with increasing concentrations (0.015 $\mu$ M - 45 $\mu$ M) of **16** for 72h. Cells were grown either in media containing 10% fetal calf serum (HNO41, HNO97, HNO210) or under serum free conditions as described before [72-74]. Each concentration was measured in four technical replicates with the CellTiter-Glo® 3D Cell Viability Assay. After adding the CellTiter-Glo substrate the plate was shaken for 5 min and afterwards incubated for 30 min to get a stable luminescence signal. This was measured as relative light units by a Tecan Infinite F200 Pro. IC<sub>50</sub> was determined by a sigmoidal fit in version 5 of the GraphPad Prism software.

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