

Synthesis of alkyne- and azide-functionalised aziridine and epoxide carbocycles for development of selective glucuronidase mechanism-based inhibitors

Helena L. Ehren,^a Seino A. K. Jongkees,^{a,b} Tom Wennekes^a

^a Department of Chemical Biology and Drug Discovery, Utrecht Institute for Pharmaceutical Sciences and Bijvoet Center for Biomolecular Research, Utrecht University, Universiteitsweg 99, 3584 CG, Utrecht, the Netherlands

^b Department of Chemistry and Pharmaceutical Sciences
Amsterdam Institute of Molecular and Life Sciences
Vrije Universiteit Amsterdam
De Boelelaan 1108, 1081 HZ Amsterdam, the Netherlands

Abstract

Heparanase (HPSE) is an endo-acting β -glucuronidase and the only known enzyme responsible for the regulation of extracellular heparan sulfate (HS) structures, a glycosaminoglycan (GAG) occurring in conjugation with a protein class called heparan sulfate proteoglycans (HSPGs) in the extracellular matrix (ECM).¹ The enzyme is found to be significantly upregulated in aggressive cancer types aiding cell proliferation by increased degradation of HS.² Inhibition of HPSE reduces cancer growth making it an interesting druggable target for cancer treatment and diagnostics. Only few of the known efficient HPSE inhibitors have progressed through clinical studies and none of them has been approved yet.¹ We here present the synthesis of three cyclophellitol scaffolds, based on known mechanism-based inhibitors of HPSE. These novel scaffolds are amiable to facile further elaboration via copper-catalysed azide-alkyne cycloadditions to aid in exploring the structure-activity relationship for selective inhibitors of HPSE.

Introduction

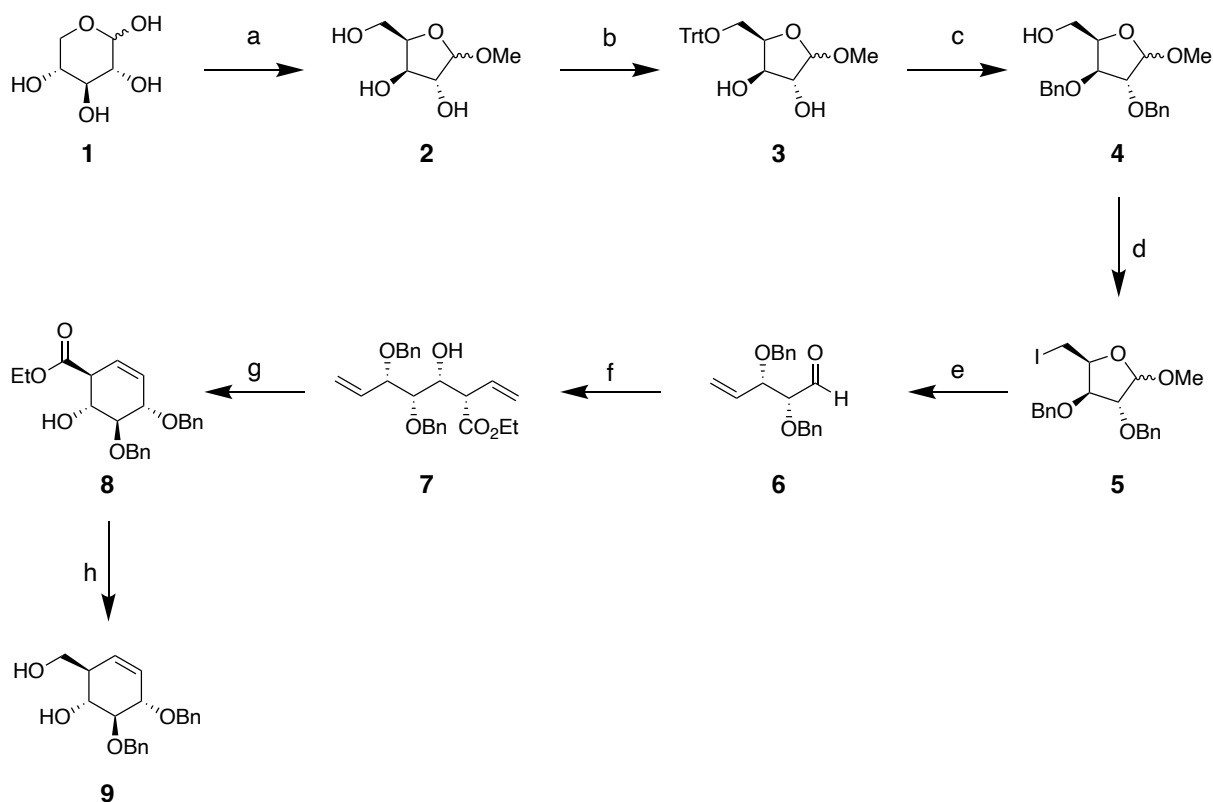
HSPGs are a class of membrane bound, transmembrane or extracellular glycoproteins involved in a plethora of physiological functions including cell migration, proliferation, and adhesion, as well as cell-cell interactions, cell signaling pathways and the maintenance of the extra cellular matrix (ECM) integrity and functionality.^{1–4} Their diversity is displayed in the number and structural heterogeneity of their attached heparan sulfate (HS) chains. HS is a glycosaminoglycan (GAG) comprised of alternating 1,4-linked β -D-glucuronic acid (GlcUA) and α -D-N-acetylglucosamine (GlcNAc) moieties. Modifications include epimerization of glucuronic to iduronic acid and substitution of N-acetylglucosamine by N-sulfo-glucosamine. These compositional alterations lead to differences in the supramolecular structure of HS, allowing for a variety of different binding partners for the same polysaccharide. HS degradation is performed by heparanase (HPSE). This process is heavily influenced by the HS macromolecular structure and limited to certain cleavage sites depending on the sulfation pattern.⁴ HPSE is the only known enzyme responsible for the degradation of extracellular heparan sulfate chains. It has been found to be overexpressed in the presence of various aggressive cancer types such as cervical cancer,⁵ breast cancer,⁶ bladder⁷ and colon cancer.^{8,9} An overactivity of HSPG breakdown in the ECM by HPSE facilitates cell migration, and releases cytokines

and growth factors stimulating cell proliferation and angiogenesis.² A mechanism that can be exploited by cancerous cells for tumor growth and migration.⁴ Furthermore, abnormal HPSE activity is linked to various other diseases including fibrosis,^{10,11} diabetes,¹² inflammations,^{13,14} and viral infections^{15–17} making it an important therapeutic target. Inhibition of HPSE is complicated by its broad distribution over intracellular, extracellular and nuclear spaces, its structural similarity to its close homologue HPSE-2 and its complex, broad range of functions.¹ Different classes of HPSE inhibitors include polyanionic saccharide based heparan sulfate mimetics,^{18–23} oligonucleotides,^{24,25} monoclonal antibodies,^{26,27} neoproteoglycans^{28,29} as well as small molecule inhibitors.^{2,30–34} So far, only the HS-like saccharide-based inhibitors have made it into clinical studies and none of them has been approved for clinical application. Their limitations are reflected in problematic product standardization and identification as a result of structural heterogeneity and poor bioavailability originating from molecular sizes and solubility issues.¹ This calls for the development of well-defined small molecule inhibitors with drug-like properties. Current small molecule inhibitors for HPSE rely for example on benzimidazoles,^{22,23} benzoxazoles,^{30,35–39} triazolo-thiadiazole^{40,41} scaffolds. Another very promising class of irreversible mechanism-based HPSE inhibitors are glucuronic acid configured cyclophellitol derivatives.^{2,34,42} This class of inhibitors have achieved nanomolar inhibition of HPSE in complex biological samples and were able to reduce cancer aggression *in cellulo* as well as murine metastasis.² To date, the best performing inhibitor is a disaccharide consisting of *glucurono*-cyclophellitol carrying a GlcNAc-unit in the 4-position. In preclinical studies however, this compound showed non-optimal drug-like properties. Modifications of the cyclophellitol scaffold in the 4-position enables increased interactions of the inhibitor with the amino acid residues within the active site of HPSE while still assuring the conformational pathway necessary for the trapping of the ⁴H₃ transition state.³⁴ Substituting cyclophellitol derivatives at the 4-position with fragments or small peptides could lead to more favorable drug-like properties and enhanced HPSE specificity through interactions with the -2 subsite, or at the aziridine to position groups in the +1 subsite. In the present work we present the synthesis of azide- and alkyne-functionalized *glucurono*-cyclophellitol scaffolds, based on the reported HSPE mechanism-based inhibitors, with short or no spacers to allow for facile future elaboration via click chemistry and testing for improved HSPE selectivity.

Results and discussion

Synthesis of the Cyclohexene-Intermediate

Retrosynthetic analysis readily identifies cyclohexene structure **9** as the core intermediate towards the synthesis of *glucurono*-cyclophellitol and *glucurono*-cyclophellitol aziridines.^{43–45} Intermediate **9** was synthesized via a 9-step synthesis pathway starting from D-xylose **1**. *Fischer* glycosylation provided methyl-glycoside **2** quantitatively. Subsequent protection of the free primary hydroxyl group gave compound **3** in a good yield of 80%. Next, the two remaining secondary alcohols were protected as benzyl ethers and the trityl group was hydrolysed under acidic conditions. Protected glycoside **4** was obtained in 73% yield over two steps and in the following subjected to *Appel* conditions leading to the formation of iodofuranoside **5** in good yield (80%). The deviation from the standard protocol which first installs the iodide at the 6-position^{43,45} was a decision we made due to better handling at large scale. Reaction conditions were adapted from a protocol published by *Cuzzupe et al.*⁴⁶

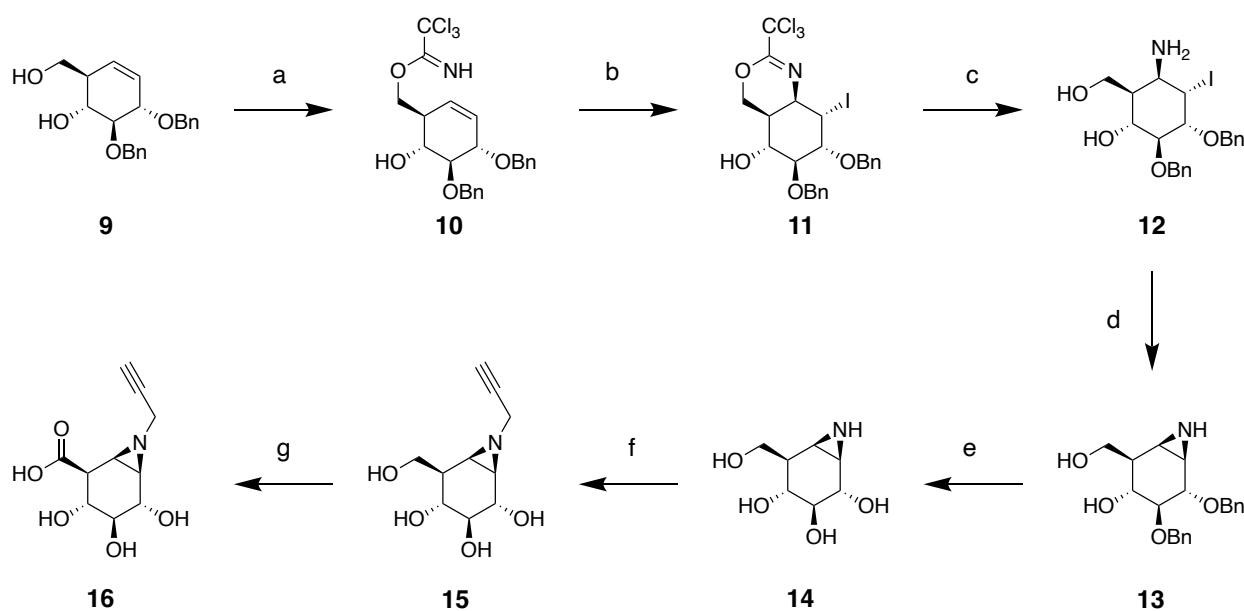


Scheme 1: Synthesis of cyclohexene **9**: (a) AcCl, MeOH, 4 °C, 5h, quant., (b) Trt-Cl, pyridine, 80%, (c) i) BnBr, NaH, ii) AcOH, 70 °C, 73%, (d) I₂, PPh₃, imidazole, 80%, (e) Zn, THF:H₂O (9/1), sonication, 94%, (f) In, ethyl-4-bromocrotonate, La(OTf)₃, H₂O, 69%, (g) Grubbs 2nd generation catalyst, 40 °C, 95%, (h) i) DiBAL-H, THF, 0 °C, 2h ii) NaBH₄, EtOAc, H₂O, o.n., 90%.

Following the installation of the iodide, a *Vasella* fragmentation gave aldehyde **6** in an excellent yield of 94%, which was then used in an indium mediated *Barbier* reaction for the formation of diene **7**. As observed before, the used reaction conditions afforded compound **7** in good yield (69%) and excellent diastereoselectivity.^{43,47} Diene **7** was then converted to cyclohexylester **8** in a ring-closing metathesis using the *Grubbs* 2nd generation catalyst (95% yield). Finally, reduction of ester **8** to the primary alcohol gave key intermediate **9** in 90% yield (26% over 9 steps).

Synthesis of *N*-propargyl-*glucurono*-cyclophellitol aziridine

For the synthesis of cyclophellitol aziridine **14** we followed a protocol published by *Overkleeft et al.* (Scheme 2): Formation of the trichloroacetimidate **10** was followed by iodocyclisation to yield compound **11**. Subsequent, hydrolysis of the cyclic imidate gave *trans*-iodo-amine **12** which was then ring-closed to the desired aziridine **13** (51% over four steps) by a nucleophilic attack (S_N2) of the amine to expel the iodide. Final deprotection of the hydroxyl functions under *Birch* conditions afforded deprotected aziridine **14** in 72% yield. At first, we envisioned to alkylate the aziridine with an azido-alkyl chain as a *click*-handle for the cycloaddition with a small alkyne-containing fragment. To ensure optimal interactions between the fragment and the active site of the enzyme we wanted the alkyl linker to be as short as possible. However, all attempts at alkylating the aziridine with 1-azido-2-iodoethan or 2-azido-ethyl-trifluoromethanesulfonate failed due to insufficient stability of the alkyl moiety (see below). Revising our initial strategy, we decided to alkynylate the aziridine and *click* it to an azide-containing fragment. Finally, alkylation of the aziridine was achieved according to a procedure described by *Åhman* and *Somfai*⁴⁸ giving the propargylated aziridine **15** in a yield of 60%. The reaction proceeds attuned to the aziridine instability under mild conditions using potassium carbonate as non-nucleophilic base in combination with 18-crown-6 to solubilize the salt. In the final step of the synthesis oxidation of the primary alcohol to the carboxylate resulted in the formation of the desired *glucurono*-cyclophellitol aziridine **16** in 50% yield (3% over 16 steps). The product proved to be sufficiently stable, a major advantage, which we attribute to the electron donating effect of the alkyne substituent.



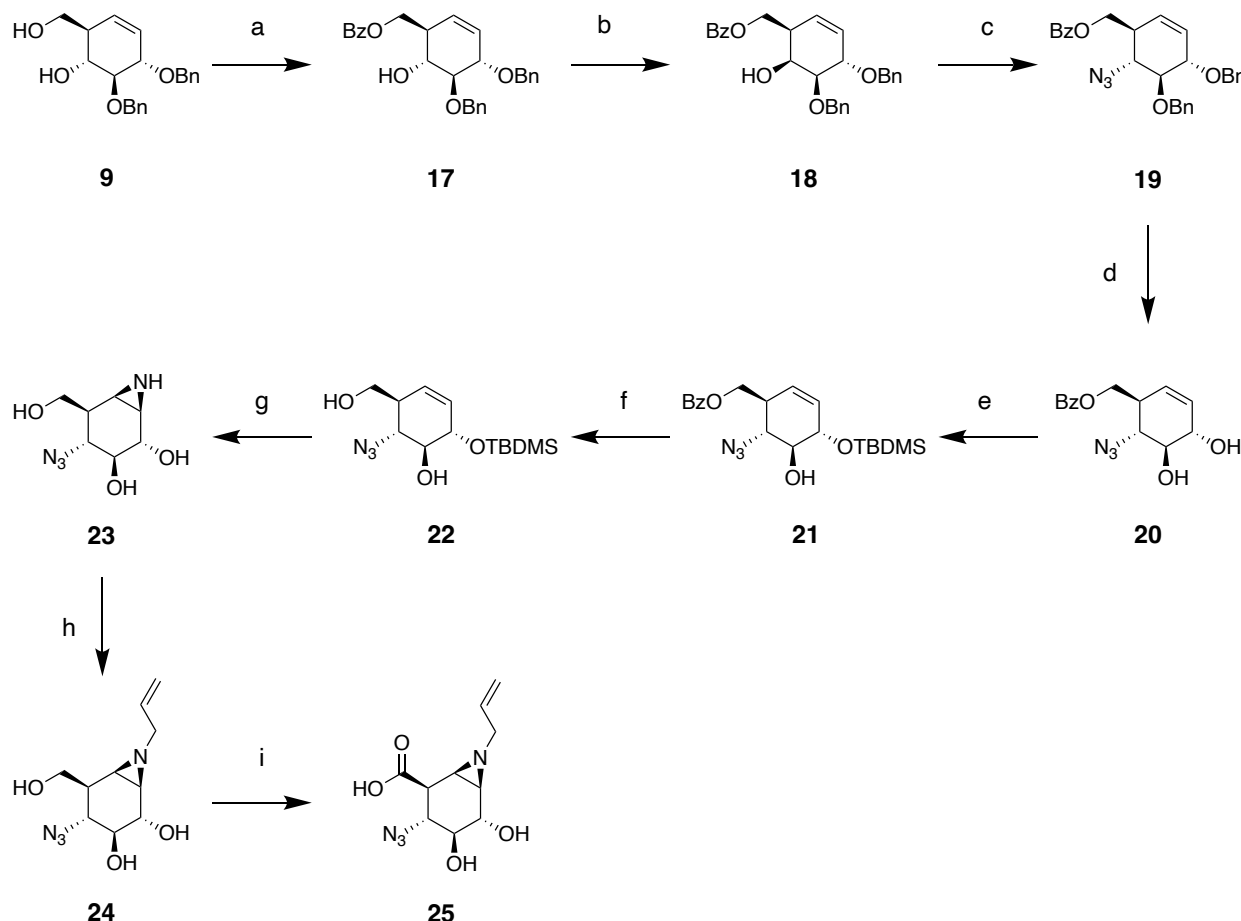
Scheme 2: Synthesis of *N*-propargyl glucurono-cyclophellitol aziridine **16**: (a) CCl_3CN , DBU, DCM, $0\text{ }^\circ\text{C} \rightarrow \text{RT}$, 2h, (b) I_2 , NaHCO_3 , H_2O , (c) 37% HCl (aq.), dioxane, $60\text{ }^\circ\text{C}$, 2h, (d) NaHCO_3 , MeOH, 4 days, RT, 51% over 4 steps, (e) Li , NH_3 (liq.), THF, $-60\text{ }^\circ\text{C}$, then Amberlite H^+ IR-120, 72%, (f) propargyl bromide, 18-crown-6, K_2CO_3 , DMF, RT, 3 days, then $50\text{ }^\circ\text{C}$, 1h, 60% (g) TEMPO, NaOCl, NaBr, H_2O , $0\text{ }^\circ\text{C}$, 50%.

Synthesis of 4-Azido-glucurono-Cyclophellitol and 4-Azido-glucurono-Cyclophellitol aziridine

In silico studies have shown that the cyclophellitol scaffold can be modified in the 4-position whilst keeping the conformational pathway the molecule has to proceed through to trap the 4H_3 transition-state intact.³⁴ Inspired by this, we decided to introduce an azide function directly onto the carbocycle. Starting from Intermediate **9** we first modified the free primary alcohol with a benzoyl protecting group (92%). Next, a hydroxyl-inversion was performed to produce the galactose configured carbocycle **18** according to literature known procedures.^{49,50} Here, the free secondary hydroxyl group was first transformed into a triflate leaving group and subsequently substituted with the nitrite via a $\text{S}_{\text{N}}2$ mechanism. The nitrite then spontaneously disintegrates under the formation of nitric oxide and the desired hydroxyl to give product **18** in 54% yield over two steps. One major limitation of this step is the migration of the benzoyl-protecting group to the 4-position during the work-up of the triflate intermediate thereby decreasing the final yield significantly. The readiness of the benzoyl-group to migrate to the 4-position also prohibits the use of higher temperatures to speed up the substitution reaction. Different protecting groups were evaluated for their performance in this step (see below) but the

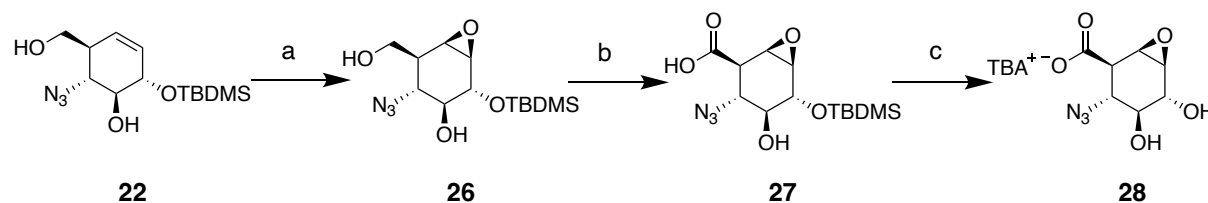
benzoyl protecting group seemed to be the best compromise considering also that it speeds up the substitution step by neighboring group participation. Next, we introduced the azide-function onto the carbocycle by transformation of the hydroxyl to a triflate followed by a S_N2-type substitution using tetrabutylammonium azide to yield the glucose-configured azido-carbocycle **19** (82%). Optimization of this step revealed the use of tetrabutylammonium azide as preferable over sodium azide because the increased solubility allows for lower reaction temperatures thereby ensuring the stability of the triflate-intermediate. Notably, we did not observe any migration of the benzoyl protecting group confirming that this process heavily relies on the conformational restraints of the cyclohexene scaffold. Selective benzyl ether deprotection was performed in the next step under mild conditions using boron trichloride^{51,52} to give monoprotected carbocycle **20** in 93% yield. We here managed to keep the benzoyl protection group in place and avoid hydrogenating conditions by which the azide would be transformed to an amine group. Selective silyl-protection of the allylic alcohol afforded compound **21** (88% yield) which was then deprotected at the primary alcohol function (compound **22**, 70%), a necessity for the stereoselective introduction of the aziridine moiety in the next step. As a minor side product, we here obtained the 3-*O*-TBDMS protected carbocycle due to migration of the silyl ether under basic conditions. Aziridination was performed according to the previously described procedure in a four-step procedure. The iodocyclisation reaction showed very slow progress under the standard conditions due to steric hinderance by the allylic silyl ether. For this reason, the reaction mixture was heated up to 50 °C and left for 3 days until TLC showed full conversion of the imidate to the iodocycle. Subsequent acidolysis of the cyclic imidate also freed up the allylic hydroxyl affording the *trans*-iodo-amine which was then closed to the aziridine under mild basic conditions. The globally deprotected azido-aziridine **23** was obtained in 56% yield over four steps. In order to oxidize the primary alcohol to the corresponding carboxylic azide, we first introduced a Boc-protection group. However, the formed carbamate was not stable under the applied oxidative reaction conditions and led to the formation of the ring-opened protected amine. We then decided to alkylate the aziridine amine with an allyl-fragment for two reasons: firstly, the electron-donating effect of the alkene linker ensures the stability of the aziridine and secondly the double bond enables chemical transformations to potentially introduce a second fragment, a fluorescent dye or affinity tag via *e.g.* metathesis, ene-reactions or other electrophilic addition reactions. Furthermore, it is possible to also deprotect the aziridine again. Applying the reaction conditions described by Åhman and

*Somfai*⁴⁸ afforded the alkylated aziridine **24** in a yield of 16% (after HPLC), which was then oxidized to the glucuronic acid configured aziridine **25** (yield: 25%). The desired product was obtained in 0.1% yield over 23 steps.



Scheme 3: Synthesis of *N*-allyl-4-azido-glucurono-cyclophellitol aziridine **25**: (a) Bz-Cl, DCM, 0 °C, 1.5 h, 92%, (b) i) Tf₂O, pyridine, DCM, -20 °C → 0 °C, 2h, ii) TBANO₂, MeCN, o.n., RT, 54%, (c) i) Tf₂O, pyridine, DCM, -20 °C → 0 °C, 2h, ii) TBAN₃, MeCN, o.n., 50 °C, 82%, (d) BCl₃, DCM, -78 °C, 2h, 91%, (e) TBDMS-OTf, 2,6-lutidine, DCM, -40 °C → -10 °C, 2h, 88%, (f) NaOMe, MeOH, 0 °C, 1 h, 70%, (g) i) CCl₃CN, DBU, DCM, 0 °C → RT, 2h, ii) I₂, NaHCO₃, H₂O, RT → 50 °C, 3 days, iii) 37% HCl (aq.), dioxane, 60 °C, 1.5 h, iv) NaHCO₃, MeOH, RT, o.n., 56% over 4 steps, (h) Allyl-Br, 18-crown-6, K₂CO₃, DMF, 50 °C, 5h, 16%, (i) TEMPO, NaOCl, NaBr, H₂O, 0 °C, 25%.

Similarly, 4-Azido-glucurono-cyclophellitol probe **28** was available via epoxidation and oxidation of intermediate **22**.

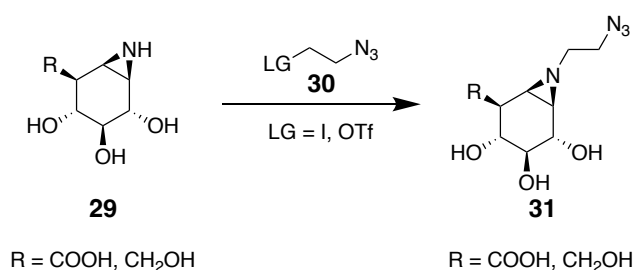


Scheme 4: Synthesis of 4-Azido-*glucurono*-cyclophellitol salt **26**: (a) *m*-CPBA, NaH₂PO₄ (aq.,1M), Na₂HPO₄ (aq.,1M), DCE, 50 °C, o.n., 75%, (b) TEMPO, BAIB, DCM:*t*-BuOH:H₂O (4/4/1), 0 °C, 74%, (c) TBAF, THF, RT 53%.

Epoxidation was achieved using *m*-CPBA in a phosphate buffered system keeping the pH close to 7. Here, the silyl protection group came in handy as it blocks one side of the carbocycle leading to the formation of only one stereoisomer of the cyclophellitol structure **26** in 75% yield.⁵³ Oxidation of the primary alcohol to the carboxylic acid **27** (yield: 74%) was performed using TEMPO in combination with the co-oxidant BAIB as previously described.⁴² Finally, deprotection of the silyl protection group using TBAF⁵³ gave the tetrabutylammonium salt **28** in 53% yield. In total the tetrabutylammonium salt of 4-azido-*glucurono*-cyclophellitol **28** was obtained in an overall yield of 1.7% over 20 steps. Next, we attempted ion-exchange chromatography using Na⁺- Amberlite and Na⁺-Dowex resins. With the Amberlite resin we were not able to exchange the TBA ion and observed ring-opening of the epoxide due to the basic conditions we employed to create the sodium resin. The Dowex resin however, proved to be suitable for the ion exchange. Unfortunately, we obtained the sodium salt in only very low yield due to the problems encountered with the Amberlite resin.

Optimization for the alkylation of the aziridine

Alkylation reactions were performed using different substrates and different linkers under varying reaction conditions. We first attempted to alkylate the aziridine with a C₂-alkyl fragment carrying an azide (Scheme 5). The linkers (**30**) were synthesized from 2-Chloro-ethanol, by first substitution of the chloride using sodium azide and subsequent formation of the triflate or *Appel* reaction to introduce the iodide:



Scheme 5: Attempts to alkylate the aziridine with a C₂-azido-linker.

The used reaction conditions are depicted in Table 1. First, we attempted alkylation of the *glucurono*-cyclophellitol aziridine (entries 1-3). We decided for this strategy because of

reported low yields for the oxidation of the primary alcohol in the presence of an alkylated aziridine. However, our attempts remained unsuccessful due to a lack of solubility of the starting material as well as poor stability of the 1-azido-2-iodoethane linker. The first two conditions lead to re-isolation of the starting material at simultaneous degradation of the linker fragment. The conditions used for entry 3 resulted in complete degradation of the starting material and the linker fragment. Addressing the solubility issues observed with the acid-carbocycle we decided to attempt alkylation of the aziridine prior to oxidation (entries 4-9). We tried different reaction temperatures, different bases and different leaving groups on the linker, but none of the used conditions lead to the formation of the desired alkylated aziridine **31**. In most cases the aziridine or the opened primary amine was reisolated while in all cases the alkyl-fragment showed multiple side reactions e.g. the formation of adducts. Also, subsequent addition of more equivalents of linker did not lead to the formation of the desired products.

Table 1: Reaction conditions used for the alkylation of aziridines **29**.

Entry	R	LG	T[°C]	Base	Solvent	t[h]
1	COOH	I	RT	K ₂ CO ₃ , 18-crown-6	DMF	48
2	COOH	I	RT	K ₂ CO ₃ , 18-crown-6	DMF	96
3	COOH	I	60°C, then RT	K ₂ CO ₃ , 18-crown-6	DMF	27
4	OH	I	70°C	K ₂ CO ₃	DMF	24
5	OH	OTf	50°C	K ₂ CO ₃	DMF	24
6	OH	OTf	RT	DIPEA	DMF	24
7	OH	OTf	-20°C→-5°C	DIPEA	DMF	27
8	OH	OTf	-20°C	2,6-Lutidine	MeCN	24
9	OH	OTf	-60 °C	DIPEA	DMF	24

Finally, we decided to reverse our strategy and alkynylate the aziridine using propargyl bromide. Using propargyl bromide together with potassium carbonate in combination with 18-crown-6 for increased solubility of the salt enabled us to perform the alkylation under mild conditions and isolate the desired product in 60% yield. The resulting alkynylated aziridine proved to be stable under oxidative conditions and was in general sufficiently stable.

Evaluation of protection groups for the double inversion step

For the introduction of an azide functionality onto the carbocycle scaffold a double inversion strategy was employed (Scheme 6). In the first step the primary alcohol of intermediate **9** was protected (**32**). Subsequently, the remaining free hydroxyl group is transformed into a leaving group by triflation. Through an S_N2-reaction the first inversion was achieved and afforded *galacto*-cyclohexene structure **33**. In the following the newly generated free hydroxyl with opposing stereoconfiguration is triflated and displaced with an azide in another S_N2-reaction, yielding the desired azido-carbocycle **34** under net retention of the glucose stereoconfiguration. Benzylether cleavage was conducted under mild conditions to form monoprotected compound **35** using BCl₃ thereby avoiding the use of hydrogenating procedures which would lead to undesired transformation of the azide to an amine.

Scheme 6: Reaction conditions: (a) for PG = Trityl: Trityl-Cl, Pyridine, DMAP, RT, o.n., for PG = Bn: i) PhCH(OMe)₂, 15% *p*-TSA, MeCN, RT, o.n., <99% ii) Et₃SiH, TfOH, DCM, molecular sieves 4Å, -78 °C, 1h, for PG = TBDPS: TBDPS-Cl, imidazole, DMF, 0 °C → RT, 2h, for PG = Bz: Bz-Cl, Pyridine, 0 °C, 1.5h, (b) i) Tf₂O, Pyridine, DCM, -20 °C → 0 °C, 1.5h, ii) TBANO₂, MeCN, RT, o.n., (c) i) Tf₂O, Pyridine, DCM, -20 °C → 0 °C, 1.5h, ii) TBAN₃, MeCN, 50 °C, o.n., (d) BCl₃, DCM, -78 °C, 1.5h.

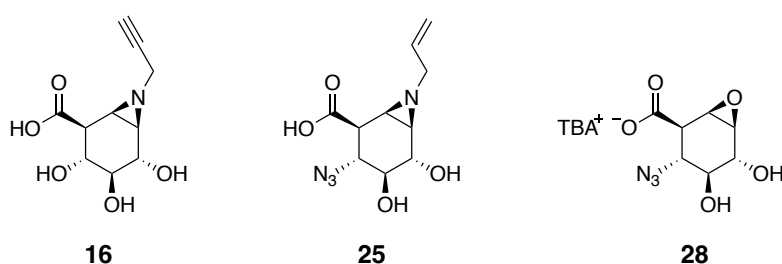
compound **TBDPS-32** in excellent yield and regioselectivity. Inversion of the hydroxyl group gave compound **TBDPS-33** in 74 – 92% yield in the following step without further complications. The substitution in the next step however did not proceed under formation of the **TBDPS-34** in desirable yields. Attributing these problems to the bulkiness of the chosen protection group, we decided to try a benzyl ether as a next step (Entries 4-6, Table 2). Trying to install the Benzyl protection group immediately from compound **9b** did not proceed with desirable regioselectivity which is why we switched to a two-step procedure, where first the benzylacetal is formed between the two remaining free hydroxyl groups and then opened to form the desired primary protected compound **Bn-32** in 53-67% yield over two steps using Et₃SiH in a combination with trifluoromethanesulfonic acid. For the hydroxyl inversion higher temperatures had to be employed in the substitution step. Here, the stability of the triflate leaving group showed to be problematic. We could isolate benzylated compound **Bn-33** in only one attempt on small scale. In all other attempts the elevated temperatures lead to significant material degradation and no formation of the desired product. Using **Bn-33** in the following azide substitution gave desired product **Bn-34** in over 99% yield. Benzyl deprotection in the next step afforded fully deprotected compound **Bn-35** in a good yield of 81%. Due to the unreliability of the hydroxyl-inversion step, we again decided to choose for a different protection group strategy. Protection of the primary hydroxyl group with benzoyl chloride led to the formation of **Bz-32** in excellent yields (entries 7-9). The following inversion of the secondary alcohol group showed to be problematic as we encountered significant migration of the benzoyl protection group to the 4-*O* position. All attempts to modify the triflate work-up procedure did not lead to improved yields (see entries 8-9, Table 2). However, the substitution reaction showed spot-to-spot conversion of the triflate to the inverted hydroxyl assisted by neighboring group participation of the benzoyl protection group and product **Bz-33** could be isolated in acceptable yields. Interestingly, we did not observe migration of the hydroxyl protection group for the *cis*-configured hydroxyls in the next step leading to good yields of 61-93% for product **Bz-34**. Here, we also modified the reaction protocol from using sodium azide in DMF to tetrabutylammonium azide in acetonitrile. This modification proofed to be a major break-through as it enabled us to keep the reaction temperatures below 60°C, leading to increased stability of the triflate and thus to much higher yields for the azide **Bz-34**. One major advantage of the use of the benzoyl group was that it is stable towards BCl₃ leading to monoprotected **Bz-35** in excellent yields (entries 7-8), enabling us to continue the synthesis route without an additional protection

step. Concluding, the benzoyl protection group seemed to be the best compromise, despite the problems we encountered due to protection group migration in the hydroxyl-inversion step. To address these problems, we tried using more stable leaving groups *e.g.* an iodide or methanesulfonate, but were not able to isolate the inverted alcohol. In hindsight, it would seem favorable to again explore the TBDPS protection group, because the problems we encountered for the azide substitution step might be overcome employing the improved reaction protocol for this step.

Table 2: Chosen protective groups and yields for intermediates **32**, **33**, **34**, **35**.

Entry	PG	Yield 32 [%]	Yield 33 [%]	Yield 34 [%]	Yield 35 [%]	Problems
1	Trityl	>99	-	-	-	unstable
2	TBDPS	85	74	36	-	low amounts
3	TBDPS	85	92	-	-	solubility of NaN ₃
4	Bn	67	60	>99	81	low amounts
5	Bn	46	-	-	-	temperature
6	Bn	53	-	-	-	temperature
7	Bz	89	54	61	99	Benzoyl-migration
8	Bz	92	42	82	90	Benzoyl-migration
9	Bz	92	20	93	60	Benzoyl-migration

Conclusions and future prospects



Scheme 7: Small molecule probes for *click*-Reactions.

In summary, three *glucurono*-cyclophellitol derivatives were synthesized as potential mechanism-based inhibitors for improved selectivity in targeting glucuronidases (scheme 7). *Glucurono*-cyclophellitol aziridine **16** was obtained in an overall yield of 3% over 16 steps. An alkyne linker attached to the aziridine function proved to be favorable over an

azido containing linker because of increased stability for both, the linker itself as well as the resulting alkylated aziridine.

4-Azido-*glucurono*-cyclophellitol aziridine **25** was obtained in 0.1% yield over 23 steps. The aziridine was functionalized with an allyl-linker that can either be deprotected or used for the modification with a fluorescent organic dyes or affinity tag. It further enables the installation of a secondary fragment via metathesis, ene-reactions or other electrophilic addition reactions to potentially engage both the +1 and -2 subsites.

4-Azido-*glucurono*-cyclophellitol **28** was obtained in an overall yield of 1.7% over 20 steps. Here, the final purification step via ion exchange column can be optimized by carefully exchanging a *Dowex* H⁺ resin with sodium ions and neutralizing it before use. For both 4-Azido-*glucurono*-cyclophellitol derivatives the synthesis route could be more efficient with better yields for the double-inversion step. Future investigations should include a protection group scheme that excludes protection group migration. Exploring the TBDPS group again, applying the improved reaction conditions for the azide substitution could lead to higher overall yields since TBDPS has shown excellent performance in the hydroxyl inversion. As a next step for all three probes, the CuAAC reaction should be optimized first on simple model compounds before expanding to library synthesis and testing. For both aziridines it could also be useful to conjugate at the 'extra' handle to fluorescent dyes or affinity tags and perform activity-based protein profiling (ABPP) using in-gel fluorescence or pull-down experiments analogous to previously reported procedures.^{2,34,42}. In conclusion, all three synthesized probes enable further (bio-)chemical investigations in different directions using *click*-chemistry to add fragments to drive selectivity or other detectable reporter groups for optimal targeting of HPSE and other retaining β -glucuronidases.

Supporting Information

Chemicals and solvents:

Chemicals were purchased from *Merck*, *Acros*, *Alfa Aesar*, *Fisher Scientific*, *Bio-Connect*, *Carbosynth* or *TCI* and were used without further purification unless stated differently in the experimental section. All moisture sensitive reactions were carried out under argon atmosphere and dry conditions. Solvents were purchased from *Biosolve* and dried over activated 3Å or 4Å molecular sieves prior to use.

Chromatography:

All chromatographical purifications were carried out using silica gel (*Avantor delivered by VWR*, 40-63 µm, or *Silicycle*, 60-200 µm, 60 Å) or C₁₈-reversed phase silica gel (*Merck*, 40-63 µm, 230-440 mesh). Thin layer chromatography was performed on silica coated aluminium plates (*Merck* TLC plates silica gel 60F₂₅₄) and spots were visualized under UV light or using acid (10% H₂SO₄ in ethanol), potassium permanganate (KMnO₄), ninhydrin or bromocresol stain.

High pressure liquid chromatography (HPLC):

HPLC runs were carried out on a *Shimadzu* semi-preparative system either using a C₁₈-column (*Dr. Maisch GmbH*, ReproSil-Pur, 120 C18-AQ, 250 mm x 10 mm, 10 µm particle size) or a Hilic column (*Waters*, XBridge, BEH Amide, 250 mm x 10 mm, 5 µm particle size). Analytes were detected by their UV absorption (214 nm, 254 nm). Buffers were used as follows: A = MeCN : NH₄CO₃ aq. (10 mM) = 95% : 5%/ B= MeCN : NH₄CO₃ aq. (10 mM) = 20% : 80%.

Nuclear Magnetic Resonance (NMR):

¹H-, und ¹³C-NMR measurements were conducted on an *Agilent* 400-MR (400 MHz) and on a *Bruker* 600 Ultrashield (600 MHz) spectrometer. Chemical shifts are given in parts per million (ppm) relative to the TMS signal. The residual proton signal of the solvent is

used as internal standard. All spectra were recorded in CDCl₃, CD₃OD, or D₂O. Multiplicities in the ¹H-spectra are given as s (singlet), d (doublet), t (triplet), and q (quartet), m (multiplet), dd (doublet of doublet), dt (doublet of triplets), td (triplet of doublets), qd (quartet of doublets), ddd (doublet of doublet of doublets), bs (broad signal). ¹³C-spectra are recorded using broadband decoupling for protons. Signals for primary, secondary, tertiary and quaternary carbons were identified by APT spectra or using a phase sensitive HSQC spectrum. For whole structure elucidations HSQC-, HMBC- and H,H-COSY-spectra were recorded. All measurements were performed at room temperature.

High resolution Mass Spectrometry (HRMS)

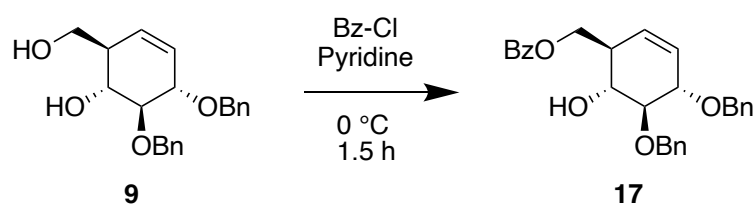
High resolution mass spectrometry was conducted on an *Agilent* 6560B DTIM-QTOF ion mobility spectrometer used in QTOF mode only. The instrument uses electron spray ionisation (ESI), a nozzle voltage of 2000 V, and a capillary voltage of 4500 V. The drying gas and the sheath gas had a temperature of 300 °C at a flow rate of 12 L min⁻¹. The nebulizer was used at a pressure of 40 psi. Masses are given in calculated and found masses.

Synthesis procedures:

Compounds **1-14** were synthesized as described in literature, the spectral data were in agreement with the published data for each compound.^{42,43,45,46}

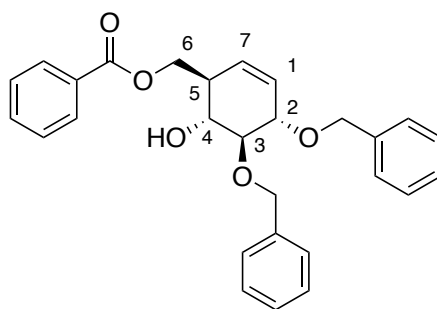
Synthesis of the Azidocarbocycles

Synthesis of ((1*R*,4*S*,5*S*,6*R*)-4,5-bis(benzyloxy)-6-hydroxycyclohex-2-en-1-yl) methyl benzoate (**17**)



Building block **9** (1.5 g, 4.41 mmol, 1.00 eq.) was dissolved in 15 ml of DCM and cooled to 0 °C. Subsequently first pyridine (80 ml) was added followed by the dropwise addition of

benzoylchloride (615 μ l, 5.30 mmol, 1.20 eq). The reaction solution was stirred for 1.5 h at 0 °C, then the reaction was quenched by the addition of methanol. The volatiles were removed under reduced pressure. The residue was dissolved in ethylacetate and washed with 1 N HCl solution. The organic layer was dried with MgSO₄ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (PE:EtOAc = 5:1). Compound **17** was obtained as a colourless oil (1.746 g, 3.93 mmol, 89%).



17

Yield: 1.746 g (3.93 mmol, 89%).

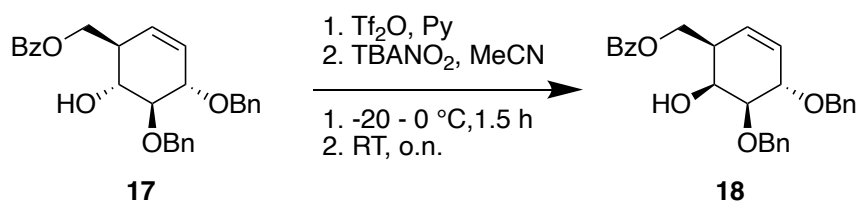
MW: (C₂₈H₂₈O₅): 444.53 g/mol.

TLC: (SiO₂, PE/EtOAc 5:1) R_f = 0.32.

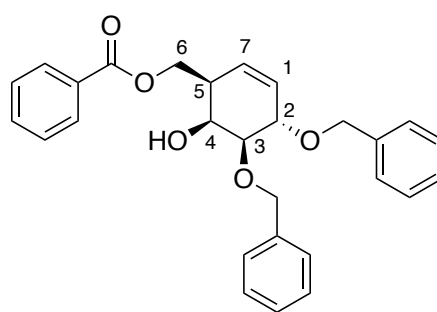
¹H-NMR: (600 MHz; CDCl₃): δ (ppm) = 8.01 (dd, J = 8.3, 1.4 Hz, 2H, H-Ar), 7.62 – 7.54 (m, 1H, H-Ar), 7.43 (t, J = 7.8 Hz, 2H, H-Ar), 7.39 – 7.29 (m, 10H, H-Ar), 5.81 (dt, J = 10.2, 2.5 Hz, 1H, H-1), 5.71 (dt, J = 10.2, 2.0 Hz, 1H, H-7), 4.91 (dd, J = 165.6, 11.2 Hz, 2H, Bn-CH₂), 4.74 – 4.65 (m, 1H, Bn-CH₂), 4.59 (dd, J = 10.9, 3.7 Hz, 1H, H-6a), 4.45 (dd, J = 10.9, 5.5 Hz, 1H, H-6b), 4.24 (ddt, J = 7.4, 3.7, 2.0 Hz, 1H, H-3), 3.75 (t, J = 9.5 Hz, 1H, H-4), 3.69 (dd, J = 9.9, 7.5 Hz, 1H, H-2), 2.80 (s, 1H, OH), 2.71 (dhept, J = 8.8, 3.0 Hz, 1H, H-5).

¹³C-NMR: (150 MHz; CDCl₃): δ (ppm) = 166.68 (1C, C=O), 138.50 (1C, Cq-Ar), 138.25 (1C, Cq-Ar), 133.16 (1C, CH-Ar), 130.22 (1C, Cq-Ar), 129.75 (2C, CH-Ar), 128.74 (2C, CH-Ar), 128.64 (2C, CH-Ar), 128.52 (2C, CH-Ar), 128.13 (2C, CH-Ar), 128.07 (1C, CH-Ar), 128.00 (2C, CH-Ar), 127.96 (1C, CH-Ar), 127.82 (1C, C-1), 127.44 (1C, C-7), 83.80 (1C, C-2), 80.38 (1C, C-3), 75.16 (1C, Bn-CH₂), 71.66 (1C, Bn-CH₂), 69.96 (1C, C-4), 64.47 (1C, C-6), 43.45 (1C, C-5).

Synthesis of ((1R,4S,5S,6S)-4,5-bis(benzyloxy)-6-hydroxycyclohex-2-en-1-yl) methyl benzoate (18)



Compound **17** (287 mg, 0.65 mmol, 1.00 eq.) was dissolved in 15 ml in dry DCM and 1.5ml of dry pyridine were added. The solution was cooled to $-20\text{ }^{\circ}\text{C}$ and trifluoromethanesulfonic anhydride (300 μl , 1.95 mmol, 3.00 eq.) was added dropwise. The reaction solution was allowed to warm up to $0\text{ }^{\circ}\text{C}$. When TLC showed full conversion of the starting material to a higher running product, the solution was diluted with EtOAc and washed with ice-cold 1 N HCl solution, aqueous NaHCO_3 solution and brine. The organic layer was dried with MgSO_4 and the solvent was removed under reduced pressure at room temperature. The crude product was coevaporated with toluene and then dissolved in 15ml of dry MeCN. Then, TBANO_2 (1.125 g, 3.90 mmol, 6.00 eq.) was added and the reaction solution was stirred overnight. After TLC showed full conversion of the starting material, the solvent was removed under reduced pressure and the crude product was purified by column chromatography (PE:EtOAc = 4:1) to give 154 mg (0.346 mmol, 54 %) of the pure product **18**.

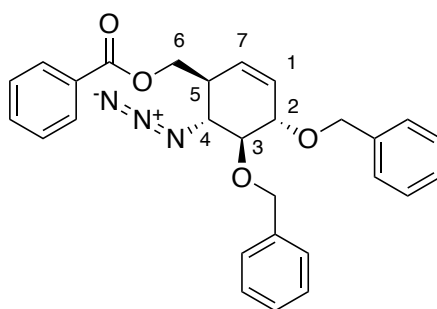


18

Yield: 154 mg (0.346 mmol, 54%).

MW: ($\text{C}_{28}\text{H}_{28}\text{O}_5$): 444.53 g/mol.

TLC: (SiO_2 , PE/EtOAc 4:1) R_f = 0.21.



19

Yield: 107 mg (0.228 mmol, 70%).

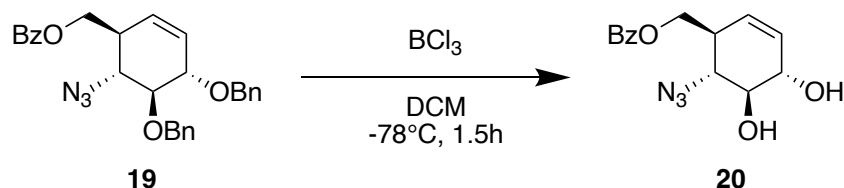
MW: (C₂₈H₂₇N₃O₄): 469.54 g/mol.

TLC: (SiO₂, PE/EtOAc 6:1) R_f = 0.38.

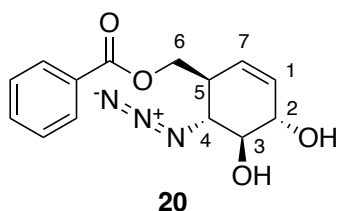
¹H-NMR: (400 MHz; CDCl₃): δ [ppm] = 8.13 – 8.02 (m, 2H, H-Ar), 7.63 – 7.57 (m, 1H, H-Ar), 7.53 – 7.44 (m, 4H, H-Ar), 7.42 – 7.38 (m, 6H, H-Ar), 7.37 – 7.32 (m, 2H, H-Ar), 5.85 (dt, *J* = 10.3, 2.4 Hz, 1H, H-1), 5.70 (dt, *J* = 10.2, 2.1 Hz, 1H, H-7), 4.99 (q, *J* = 10.7 Hz, 2H, Bn-CH₂), 4.73 (dd, *J* = 11.6, 4.6 Hz, 2H, Bn-CH₂), 4.61 (dd, *J* = 11.1, 3.6 Hz, 1H, H-6a), 4.44 (dd, *J* = 11.1, 5.0 Hz, 1H, H-6b), 4.30 (ddt, *J* = 7.5, 3.8, 2.1 Hz, 1H, H-3), 3.79 (dd, *J* = 10.5, 7.4 Hz, 1H, H-2), 3.71 (t, *J* = 10.1 Hz, 1H, H-4), 2.63 – 2.52 (m, 1H, H-5).

¹³C-NMR: (100 MHz; CDCl₃): δ [ppm] = 166.32 (1C, C=O), 138.12 (1C, Cq-Ar), 137.94 (1C, Cq-Ar), 133.22 (1C, CH-Ar), 129.87 (1C, Cq-Ar), 129.64 (2C, CH-Ar), 128.54 (2C, CH-Ar), 128.51 (2C, CH-Ar), 128.49 (2C, CH-Ar), 128.46 (1C, CH-Ar), 128.38 (2C, CH-Ar), 128.10 (1C, CH-Ar), 127.92 (1C, C-1), 127.87 (2C, CH-Ar), 127.18 (1C, C-7), 83.12 (1C, C-2), 80.21 (1C, C-3), 75.48 (1C, Bn-CH₂), 72.07 (1C, Bn-CH₂), 64.46 (1C, C-6), 62.56 (1C, C-4), 42.25 (1C, C-5).

Synthesis of ((1*S*,4*S*,5*S*,6*R*)-6-azido-4,5-dihydroxycyclohex-2-en-1-yl) methyl benzoate (20)



Starting compound **19** (93 mg, 0.198 mmol, 1.00 eq.) was dissolved in 5 ml of dry DCM. The solution was cooled to -78 °C and a 1M solution of BCl₃ in DCM (1.50 ml, 0.150 mmol, 7.5 eq.) was added slowly while keeping the temperature the same. The reaction was quenched by adding a 1:1 mixture of DCM and Methanol (1 ml) at -78 °C. Then the solvents were removed under reduced pressure and the residue was purified by flash column chromatography (DCM:MeOH = 15:1). The product **20** was obtained as a colourless syrup (52 mg, 0.180 mmol, 91%).



Yield: 52 mg (0.180 mmol, 91%).

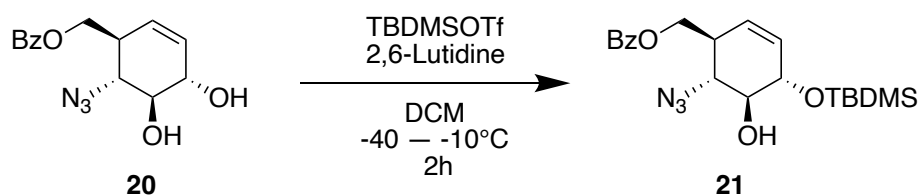
MW: (C₁₄H₁₅N₃O₄): 289.11 g/mol.

TLC: (SiO₂, DCM/MeOH 10:1) R_f = 0.50.

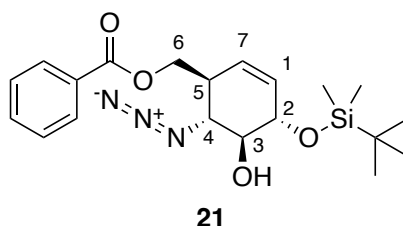
¹H-NMR: (400 MHz; CDCl₃): δ [ppm] = 8.02 – 7.98 (m, 2H, *m*-Bz-CH), 7.59 – 7.53 (m, 1H, *p*-Bz-CH), 7.43 (dd, *J* = 8.5, 7.0 Hz, 2H, *o*-Bz-CH), 5.71 (dt, *J* = 10.2, 2.3 Hz, 1H, H-1), 5.62 (dt, *J* = 10.2, 2.1 Hz, 1H, H-7), 4.53 (dd, *J* = 11.2, 3.8 Hz, 1H, H-6a), 4.37 (dd, *J* = 11.2, 5.1 Hz, 1H, H-6a), 4.30 (ddt, *J* = 7.9, 4.0, 2.1 Hz, 1H, H-3), 3.72 (dd, *J* = 10.5, 7.9 Hz, 1H, H-2), 3.54 (t, *J* = 10.2 Hz, 1H, H-4), 2.57 (dtd, *J* = 9.8, 5.2, 2.4 Hz, 1H, H-5).

¹³C-NMR: (150 MHz; CDCl₃): δ [ppm] = 166.49 (1C, C=O), 133.42 (1C, *p*-Bz CH), 130.00 (1C, C-1), 129.87 (1C, Bz-Cq), 129.75 (2C, *m*-Bz-CH), 128.65 (2C, *o*-Bz-CH), 127.13 (1C, C-7), 77.42 (1C, C-2), 72.51 (1C, C-3), 64.56 (1C, C-6), 63.23 (1C, C-4), 42.57 (1C, C-5).

Synthesis of ((1*S*,4*S*,5*S*,6*R*)-6-azido-4-((*tert*-butyldimethylsilyl)oxy)-5-hydroxycyclohex-2-en-1-yl) methyl benzoate (**21**)



Diol **20** (42 mg, 0.145 mmol, 1.00 eq.) was dissolved in 10 ml of dry DCM and the solution was cooled to -40 °C. Next, 2,6-lutidine (35 µl, 0.300 mmol, 2.00 eq.) was added followed by the addition of TBDMS triflate (39 µl, 0.170 mmol, 1.20 eq.). The reaction solution was allowed to warm up to -10 °C over the course of two hours. To quench the reaction aqueous NaHCO₃-solution was added at -10 °C. The watery phase was extracted twice with EtOAc. The combined organic layers were washed with brine and dried with MgSO₄. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography (PE:EtOAc = 6:1). The product **21** was obtained as a white solid (53 mg, 0.130 mmol, 88%).



Yield: 53 mg (0.130 mmol, 88%).

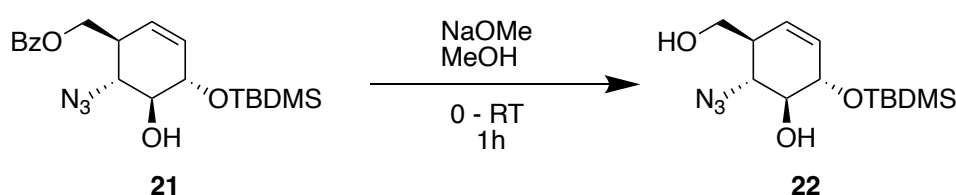
MW: (C₂₀H₂₉N₃O₄Si): 403.55 g/mol.

TLC: (SiO₂, PE/EtOAc 6:1) R_f = 0.53.

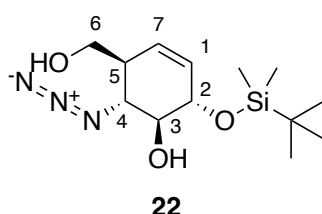
¹H-NMR: (600 MHz; CDCl₃): δ [ppm] = 8.05 – 8.00 (m, 2H, *m*-Bz-CH), 7.61 – 7.55 (m, 1H, *p*-Bz-CH), 7.48 – 7.42 (m, 2H, *o*-Bz-CH), 5.73 – 5.47 (m, 2H, H-1, H-7), 4.56 (dd, *J* = 11.1, 3.8 Hz, 1H, H-6a), 4.38 (dd, *J* = 11.1, 5.2 Hz, 1H, H-6b), 4.25 (ddt, *J* = 7.7, 3.5, 1.7 Hz, 1H, H-3), 3.77 – 3.68 (m, 1H, H-2), 3.57 (t, *J* = 10.2 Hz, 1H, H-4), 2.66 (s, *J* = 3.1 Hz, 1H, OH), 2.55 (dtt, *J* = 9.0, 3.3, 1.8 Hz, 1H, H-5), 0.92 (s, 9H, *t*-Bu-TBDMS), 0.14 (d, *J* = 6.4 Hz, 6H, Me-TBDMS).

¹³C-NMR: (150 MHz; CDCl₃): δ [ppm] = 166.48 (1C, C=O), 133.31 (1C, *p*-Bz-CH), 131.25 (1C, C-1), 129.96 (1C, Bz-Cq), 129.73 (2C, *m*-Bz-CH), 128.58 (2C, *o*-Bz-CH), 126.15 (1C, C-7), 77.54 (1C, C-2), 73.60 (1C, C-3), 64.69 (1C, C-6), 62.62 (1C, C-4), 42.31 (1C, C-5), 25.89 (3C, *t*-Bu-TBDMS), 18.20 (1C, Cq-TBDMS), -4.38 (1C, Me-TBDMS), -4.80 (1C, Me-TBDMS).

Synthesis of (1*S*,2*S*,5*S*,6*R*)-6-azido-2-((*tert*-butyldimethylsilyl)oxy)-5-(hydroxymethyl)-cyclohex-3-en-1-ol (**22**)



Compound **21** (48 mg, 0.119 mmol, 1.00 eq.) was dissolved in 25 ml of MeOH and the solution was cooled to 0 °C. Next, a solution of NaOMe in methanol (5.4 M) was added dropwise (179 µl, 0.179 mmol, 1.50 eq.). The solution was stirred for one hour at 0°C until TLC showed full conversion of the starting material to a lower running product. The pH of the reaction solution was carefully adjusted to neutral using a 1 M HCl solution. The volatiles were removed on the rotary evaporator and the crude product was purified by silica flash column chromatography (PE:EtOAc = 4:1). The pure product **22** was obtained as a white solid (24 mg, 0.080 mmol, 67%).



Yield: 24 mg (0.080 mmol, 67%).

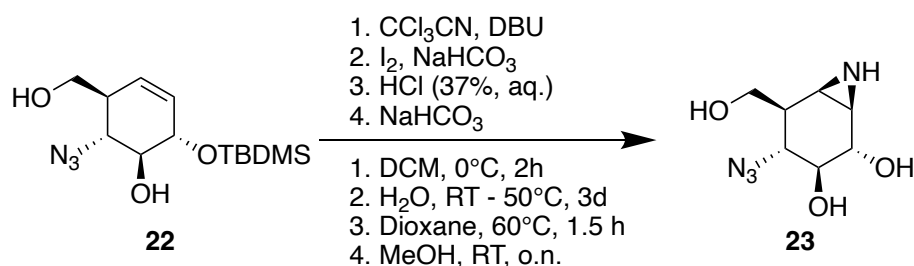
MW: (C₁₃H₂₅N₃O₃Si): 299.45 g/mol.

TLC: (SiO₂, PE/EtOAc 4:1) R_f = 0.28.

¹H-NMR: (600 MHz; CDCl₃): δ [ppm] = 5.62 (ddd, *J* = 10.2, 2.8, 1.9 Hz, 1H, H-1), 5.51 (dt, *J* = 10.2, 2.3 Hz, 1H, H-7), 4.23 (ddt, *J* = 7.6, 3.7, 2.0 Hz, 1H, H-6a), 3.78 (d, *J* = 4.1 Hz, 2H, H-6b, H-3), 3.69 (dd, *J* = 10.6, 7.6 Hz, 1H, H-2), 3.60 (t, *J* = 10.2 Hz, 1H, H-4), 2.51 (s, 1H, OH), 2.28 (dp, *J* = 9.1, 3.1, 2.2 Hz, 1H, H-5), 0.92 (s, 9H, *t*-Bu-TBDMS), 0.13 (d, *J* = 4.9 Hz, 6H, Me-TBDMS).

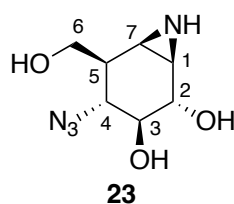
¹³C-NMR: (150 MHz; CDCl₃): δ [ppm] = 131.78 (1C, C-1), 126.76 (1C, C-7), 77.47 (1C, C-2), 73.68 (1C, C-3), 63.25 (1C, C-6), 62.05 (1C, C-4), 44.84 (1C, C-5), 25.93 (3C, *t*-Bu-TBDMS), 18.23 (1C, Cq-TBDMS), -4.26 (1C, Me-TBDMS), -4.39 (1C, Me-TBDMS).

Synthesis of (1*R*,2*S*,3*S*,4*R*,5*S*,6*R*)-4-azido-5-(hydroxymethyl)-7-azabicyclo[4.1.0]-heptane-2,3-diol (23)



Compound **22** (23 mg, 0.080 mmol, 1.00 eq.) was dried under high vacuum overnight. Subsequently, the starting material was dissolved in 2 ml of DCM and the resulting solution was cooled to 0 °C. Then, trichloroacetonitrile (10 µl, 0.100 mmol, 1.20 eq.) and DBU (1.2 µl, 0.008 mmol, 0.10 eq.) were added. The reaction was stirred at 0°C for 2h until TLC showed full conversion of the starting material to a higher running spot. Afterwards water (250 µl), iodine (63 mg, 0.250 mmol, 3.10 eq.) and NaHCO₃ (70 mg, 0.833 mmol, 10.00 eq.) were added. The reaction was stirred for 2 h at room temperature and then heated up to 50 °C for 1 h. More water (500 µl), Iodine (63 mg, 0.250 mmol, 3.10 eq.) and NaHCO₃ (70 mg, 0.833 mmol, 10.00 eq.) were added at 50 °C and the reaction was stirred at room temperature overnight. Afterwards, a 10% aqueous solution of Na₂S₂O₃ was added dropwise until the iodine was reduced completely. Then the watery phase was extracted three times with EtOAc. The combined organic phases were dried over MgSO₄ and the solvent was removed under reduced pressure. The residue was dissolved in 18 mL of dioxane and the solution was cooled to 0 °C. Then 6.20 mL of concentrated HCl were added. The reaction was heated to 60 °C for 1 h and left to stir at room temperature

overnight. Afterwards, the volatiles were removed under reduced pressure and the residue was co-evaporated three times with dioxane. Next, the residue was dissolved in 50 mL of MeOH and 3.360 g of NaHCO₃ (0.040 mol, 500 eq.) were added. The resulting suspension was stirred overnight at room temperature. Following, the suspension was diluted with DCM and filtered over a silica plug. The filtrate was collected, and the solvent was removed under reduced pressure. The crude product was purified by silica flash column chromatography (PE:EtOAc = 4:1). The pure product **23** was obtained as a white solid (9 mg, 0.045 mmol, 56%).



Yield: 9 mg (0.045 mmol, 56%).

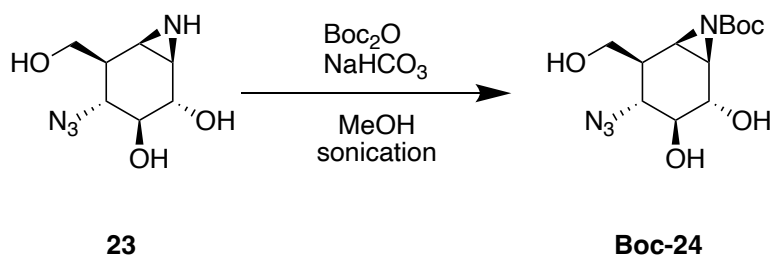
MW: (C₇H₂₂N₄O₃): 200.20 g/mol.

TLC: (SiO₂, DCM/MeOH 4:1) R_f = 0.30.

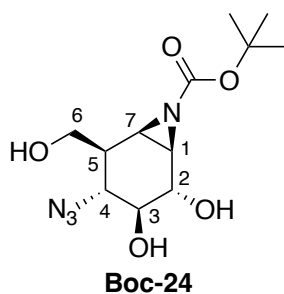
¹H-NMR: (400 MHz; MeOD): δ [ppm] = 3.90 (dd, *J* = 10.5, 3.6 Hz, 1H, H-6a), 3.64 (m, 2H, H-6b, H-2), 3.26 (dd, *J* = 10.4, 8.3 Hz, 1H, H-3), 2.89 (*pseudo-t*, *J* = 14.3 Hz, 1H, H-4), 2.56 (s, 1H, H-1), 2.26 (d, *J* = 5.7 Hz, 1H, H-7), 1.81 (m, 1H, H-5).

¹³C-NMR: (100 MHz; MeOD): δ [ppm] = 79.05 (1C, C-3), 74.19 (1C, C-2), 63.50 (1C-6), 61.88 (1C, C-4), 43.71 (1C, C-5), 36.16 (1C, C-7), 33.65 (1C, C-1).
33.66.

Synthesis of tert-butyl-(1*R*,2*S*,3*R*,4*S*,5*S*,6*R*)-3-azido-4,5-dihydroxy-2-(hydroxymethyl)-7-azabicyclo[4.1.0]heptane-7-carboxylate (Boc-24)



Compound **23** (11 mg, 0.060 mmol, 1.00 eq.) was dissolved in 9 ml of MeOH. Next, Boc₂O (20 mg, 0.090 mmol, 1.50 eq.) and NaHCO₃ (15 mg, 0.180 mmol, 3.00 eq) were added and the solution was sonicated at room temperature for 1 h. Afterwards the solution was diluted with DCM and filtered over a plug of neutralized silica. The solvents were evaporated at room temperature under reduced pressure. The clean product **Boc-24** was obtained as a white solid (12 mg, 0.040 mmol, 73%).



Yield: 12 mg (0.040 mmol, 73%).

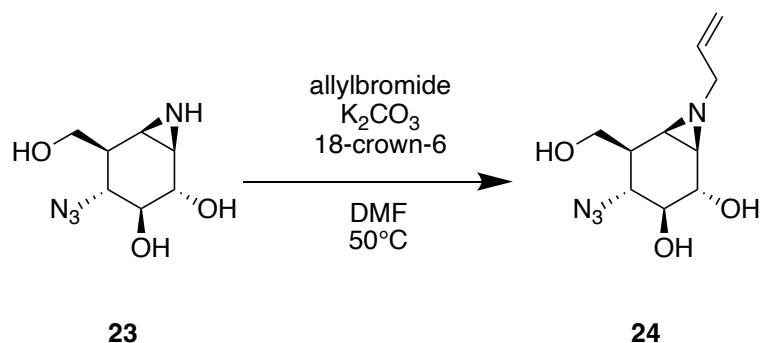
MW: (C₁₂H₂₀N₄O₅): 300.32 g/mol.

TLC: (SiO₂, DCM/MeOH 10:1) R_f = 0.73.

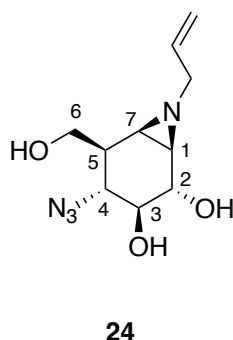
¹H-NMR: (600 MHz; MeOD): δ [ppm] = 3.93 (dd, *J* = 10.5, 3.5 Hz, 1H, H-6a), 3.74 (dd, *J* = 10.5, 8.2 Hz, 1H, H-6b), 3.70 (d, *J* = 8.0 Hz, 1H, H-2), 3.25 (dd, *J* = 10.4, 8.0 Hz, 1H, H-3), 2.99 (t, *J* = 10.6 Hz, 1H-4), 2.95 (dd, *J* = 6.0, 2.8 Hz, 1H, H-1), 2.66 (d, *J* = 6.0 Hz, 1H, H-7), 1.79 (ddt, *J* = 11.1, 8.2, 3.2 Hz, 1H, H-5), 1.46 (s, 9H, *t*-Bu).

¹³C-NMR: (150 MHz; MeOD): δ [ppm] = 163.58 (1C, C=O), 82.88 (1C, Cq-Boc), 79.02 (1C, C-3), 73.55 (1C, C-2), 63.43 (1C, C-6), 61.78 (1C, C-4), 43.62 (1C, C-5), 42.94 (1C, C-5), 41.69 (1C, C-7), 28.08 (1C, C-1).

Synthesis of (1R,2S,3S,4R,5S,6R)-7-allyl-4-azido-5-(hydroxymethyl)-7-azabicyclo-[4.1.0]heptane-2,3-diol (24)



The starting material **23** (20 mg, 0.100 mmol, 1.00 eq.) was dissolved in 4 ml of DMF. Subsequently, 18-crown-6 (7 mg, 0.027 mmol, 0.27 eq.), K₂CO₃ (45 mg, 0.326 mmol, 3.26 eq.) and allylbromide (50 µl, 0.578 mmol, 5.78 eq.) were added. The mixture was heated to 50 °C for 5 h. Afterwards, the solvent was removed at 15°C using the high vacuum pump. The product was purified by flash column chromatography using neutralized silica (DCM:MeOH = 20:1) and subsequently further purified by HPLC (linear gradient (C₁₈, A = MeCN : NH₄CO₃ aq. (10 mM) = 95% : 5%/ B= MeCN : NH₄CO₃ aq. (10 mM) = 20% : 80%, gradient = 0 → 50%). The clean product **24** was obtained as a white solid (4 mg, 0.016 mmol, 16%).



Yield: 4 mg (0.016 mmol, 16%).

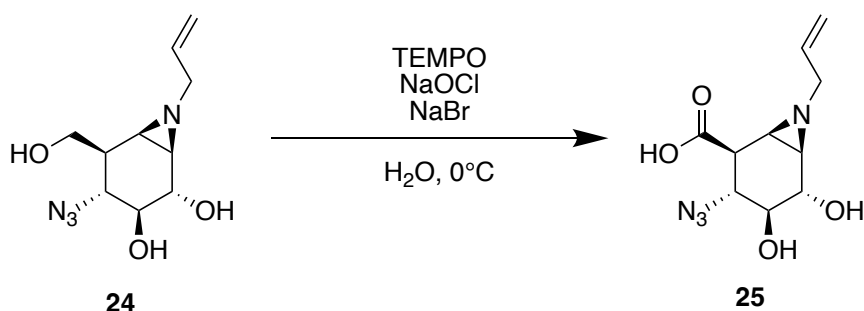
MW: (C₁₀H₁₆N₄O₃): 240.26 g/mol.

TLC: (SiO₂, DCM/MeOH 6:1) R_f = 0.46.

¹H-NMR: (600 MHz; MeOD): δ [ppm] = 5.96 (ddt, J = 16.7, 10.3, 6.0 Hz, 1H, Allyl-CH), 5.23 (dd, J = 17.1, 1.2 Hz, 1H, terminal Allyl-CH₂), 5.14 (d, J = 10.3 Hz, 1H, terminal Allyl-CH₂), 3.89 (dd, J = 10.3, 3.7 Hz, 1H, internal Allyl-CH₂), 3.62 (dd, J = 21.9, 9.3 Hz, 2H, internal Allyl-CH₂, H-2), 3.20 (dd, J = 10.4, 8.2 Hz, 1H, H-3), 3.04 (dd, J = 13.8, 5.6 Hz, 1H, H-6a), 2.90 (t, J = 10.6 Hz, 1H, H-4), 2.82 (dd, J = 13.9, 6.4 Hz, 1H, H-6b), 2.11 (dd, J = 6.3, 3.3 Hz, 1H, H-1), 1.76 (ddt, J = 10.4, 8.9, 3.5 Hz, 1H, H-5), 1.73 (d, J = 6.2 Hz, 1H, H-7).

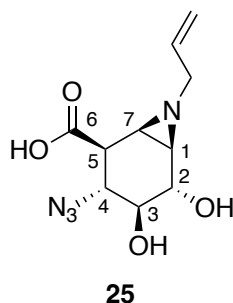
¹³C-NMR: (150 MHz; MeOD): δ [ppm] = 136.13 (1C, Allyl-CH), 117.47 (1C, terminal Allyl-CH₂), 78.95 (1C, C-3), 74.10 (1C, C-2), 63.95 (1C, C-6), 63.48 (1C, internal Allyl-CH₂), 62.66 (1C, C-4), 45.02 (1C, C-7), 43.91 (1C, C-5), 42.61 (1C, C-1).

Synthesis of (1*R*,2*S*,3*R*,4*S*,5*S*,6*R*)-7-allyl-3-azido-4,5-dihydroxy-7-azabicyclo-[4.1.0]-heptane-2-carboxylic acid (25)



Aziridine **24** (4 mg, 0.016 mmol, 1.00 eq.) was dissolved in 500 μL H₂O. The solution was cooled to 0 °C before TEMPO (1 mg, 0.006 mmol, 0.40 eq.) and NaBr (1 mg, 0.010 mmol, 0.60 eq.) were added. Subsequently, 10 μL (0.021 mmol, 1.3eq.) of a 13% sodium hypochlorite solution was added dropwise and the pH was adjusted to 10 by slowly adding a 0.5 M KOH solution. After 1 h again 10 μL (0.021 mmol, 1.3eq.) of the sodium hypochlorite solution were added while keeping the pH at 10 by the addition of 0.5 M KOH solution. The reaction was stirred at 0 °C until TLC revealed complete conversion of the starting material. Then, the reaction was quenched by the addition of 100 μL of ethanol and the pH was adjusted to 7 using a 0.5 M HCl solution. Afterwards, the volatiles were removed under reduced pressure at room temperature. The residue was purified using a benchtop C₁₈ cartridge. The product was lyophilized overnight and further purified by

HPLC (C₁₈, A = MeCN : NH₄CO₃ aq. (10 mM) = 95% : 5% / B= MeCN : NH₄CO₃ aq. (10 mM) = 20% : 80%, gradient = 0 → 50%). The product **25** was obtained as a white solid.



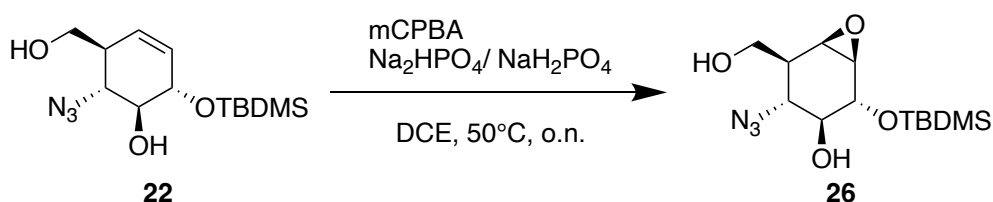
Yield: 1 mg (0.004 mmol, 25%).

MW: (C₁₀H₁₄N₄O₄): 254.25 g/mol.

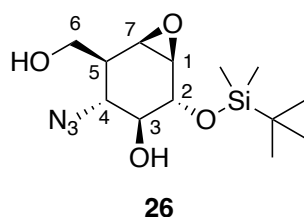
¹H-NMR: (600 MHz; D₂O): δ [ppm] = 5.87 (ddt, *J* = 16.6, 11.0, 5.8 Hz, 1H, Allyl-CH), 5.25 – 5.14 (m, 2H, terminal Allyl-CH₂), 3.75 (d, *J* = 8.7 Hz, 1H, H-3), 3.46 (t, *J* = 10.6 Hz, 1H, H-4), 3.31 (dd, *J* = 11.0, 8.9 Hz, 1H, H-2), 3.02 (dd, *J* = 14.2, 5.9 Hz, 1H, internal Allyl-CH₂), 2.85 (dd, *J* = 14.1, 5.9 Hz, 1H, internal Allyl-CH₂), 2.56 (dd, *J* = 10.6, 3.7 Hz, 1H, H-1), 2.26 (dd, *J* = 6.4, 3.8 Hz, 1H, H-5), 1.86 (d, *J* = 6.3 Hz, 1H, H-7).

¹³C-NMR: (150 MHz; MeOD): δ [ppm] = 177.56 (1C, C=O), 134.16 (1C, Allyl-CH), 117.11 (1C, terminal Allyl-CH₂), 75.75 (1C, C-2), 71.80 (1C, C-3), 61.76 (1C, C-4), 61.54, 61.37 (1C, internal Allyl-CH₂), 48.54 (1C, C7), 42.07 (1C, C5), 42.22 (1C, C1).
(from HSQC&HMBC)

Synthesis of (1*R*,2*R*,3*S*,4*R*,5*S*,6*R*)-4-azido-2-((*tert*-butyldi-methylsilyl)-oxy)-5-(hydroxy-methyl)-7-oxabicyclo[4.1.0]heptan-3-ol (26)



The diol compound **22** (50 mg, 0.167 mmol, 1.00 eq.) was dissolved in 2.4 mL of DCE. Subsequently, 1.5 mL of each 1 M Na₂HPO₄-solution and 1 M NaH₂PO₄-solution were added to the solution, followed by the addition of 80 mg (0.464 mmol, 2.78 eq.) of mCPBA. The reaction solution was heated to 50 °C and stirred at this temperature overnight. Afterwards, the solvent was removed on the rotary evaporator and the residue was co-evaporated with 1,4-dioxane to remove the residual water. The crude residue was purified by flash column chromatography using neutralized silica (4:1 *n*-Hex: EtOAc). The product **26** was obtained as colourless solid (40 mg, 0.127 mmol, 76%).



Yield: 40 mg (0.127 mmol, 76%).

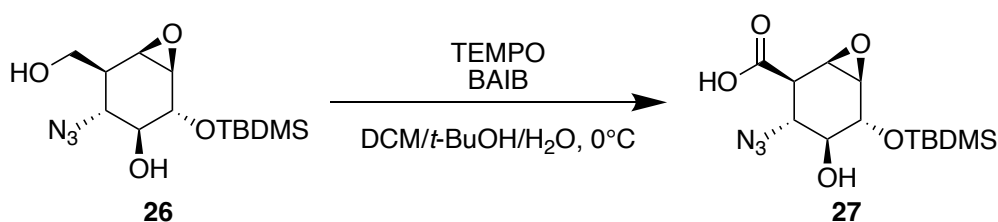
MW: (C₁₃H₂₅N₄O₃Si): 315.45 g/mol.

TLC: (SiO₂, *n*-Hex/EtOAc 4:1) R_f = 0.14.

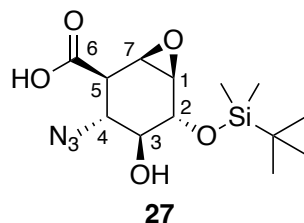
¹H-NMR: (600 MHz; MeOD): δ [ppm] = 4.03 (dd, *J* = 10.9, 3.7 Hz, 1H, H-6a), 3.90 (dd, *J* = 10.9, 6.3 Hz, 1H, H-6b), 3.79 (d, *J* = 8.0 Hz, 1H, H-3), 3.45 (dd, *J* = 10.5, 8.0 Hz, 1H, H-2), 3.36 – 3.34 (m, 1H, H-1), 3.27 (t, *J* = 10.5 Hz, 1H, H-4), 3.04 (d, *J* = 3.6 Hz, 1H, H-7), 1.96 (dddd, *J* = 10.2, 5.9, 3.6, 1.9 Hz, 1H, H-5), 0.94 (s, 9H, *t*-Bu-TBDMS), 0.18 (s, 3H, Me-TBDMS), 0.16 (s, 3H, Me-TBDMS).

¹³C-NMR: (150 MHz; MeOD): δ [ppm] = 77.34 (1C, C-2), 73.01 (1C, C-3), 62.71 (1C, C-6), 59.09 (1C, C-4), 55.69 (1C, C-7), 55.25 (1C, C-1), 42.51 (1C, C-5), 25.89 (3C, *t*-Bu-TBDMS), 18.21 (1C, Cq-TBDMS), -4.51 (2C, Me-TBDMS).

Synthesis of (1*R*,2*R*,3*R*,4*S*,5*R*,6*R*)-3-azido-5-((*tert*-butyldi-methyl-silyl)oxy)-4-hydroxy-7-oxabicyclo[4.1.0]heptane-2-carboxylic acid (**27**)



Compound **26** (40 mg, 0.127 mmol, 1.00 eq.) was dissolved in a 4/4/1 mixture of DCM, *t*-BuOH and H₂O. The solution was cooled to 0 °C before TEMPO (4 mg, 0.026 mmol, 0.20 eq.) and BAIB (102 mg, 0.318 mmol, 2.50 eq.) were added. The reaction was stirred at 0 °C until TLC revealed complete conversion of the starting material. Then, a 10% solution of NaS₂O₃ was added. Afterwards, the volatiles were removed under reduced pressure and the residue was purified using a benchtop C₁₈ cartridge. The product was lyophilized overnight and further purified by HPLC (C₁₈, A = MeCN : NH₄CO₃ aq. (10 mM) = 95% : 5%/ B = MeCN : NH₄CO₃ aq. (10 mM) = 20% : 80%, gradient = 0 → 50%). The product **27** was obtained as a white solid (31 mg, 0.094 mmol, 74%).



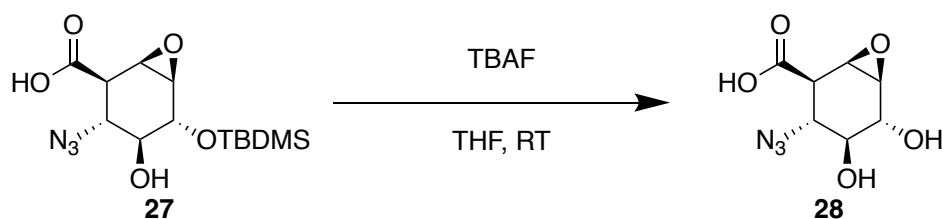
Yield: 31 mg (0.094 mmol, 74%).

MW: (C₁₃H₂₃N₃O₅Si): 329.43 g/mol.

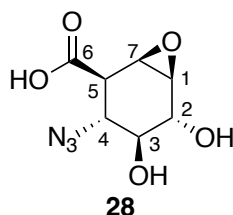
¹H-NMR: (600 MHz; MeOD): δ [ppm] = 3.80 (dd, *J* = 7.8, 0.8 Hz, 1H, H-3), 3.53 (t, *J* = 10.6 Hz, 1H, H-1), 3.47 – 3.42 (m, 1H, H-4), 3.20 (dd, *J* = 10.8, 7.8 Hz, 1H, H-2), 2.98 (d, *J* = 3.6 Hz, 1H, H-7), 2.55 (dd, *J* = 10.4, 1.9 Hz, 1H, H-5), 0.94 (s, 9H, *t*-Bu-TBDMS), 0.18 (s, 3H, Me-TBDMS), 0.17 (s, 3H, Me-TBDMS).

¹³C-NMR: (150 MHz; MeOD): δ [ppm] = 176.83 (1C, C=O), 77.66 (1C, C-2), 74.32 (1C, C-3), 62.56 (1C, C-4), 57.78 (1C, C-7), 55.54 (1C, C-1), 47.95 (1C, C-5), 26.33 (3C, *t*-Bu-TBDMS), 19.00 (1C, Cq-TBDMS), -4.28 (1C, Me-TBDMS), -4.80 (1C, Me-TBDMS).

Synthesis of (1*R*,2*R*,3*R*,4*S*,5*R*,6*S*)-3-azido-4,5-dihydroxy-7-oxabicyclo [4.1.0]heptane-2-carboxylic acid (**28**)



The protected carbocycle **27** (27 mg, 0.082 mmol, 1.00 eq.) was dissolved in 3 mL of THF. Then a 1 M solution of TBAF in THF (0.5 ml, 0.500 mmol, 6.25 eq.) was added dropwise. Due to insufficient solubility of the starting material in THF additionally 2 mL of acetonitrile were added. The reaction was stirred for 3 h at room temperature. Subsequently, the solvents were removed under reduced pressure and the residue was redissolved in water. Freshly prepared Amberlite Na⁺ resin was added to the solution and the mixture was incubated for 15 min. The resin was removed by filtration and the water was removed by co-evaporation with dioxane under reduced pressure. The crude mixture was purified using a benchtop C₁₈ cartridge. The product was lyophilized overnight and further purified by HPLC (HILIC, A = MeCN : NH₄CO₃ aq. (10 mM) = 95% : 5% / B = MeCN : NH₄CO₃ aq. (10 mM) = 20% : 80%, gradient = 100 → 0%). The resulting TBA-salt was again submitted to ion exchange column using freshly prepared Dowex Na⁺ resin. The product was lyophilized overnight and further purified by HPLC (C₁₈, A = MeCN : NH₄CO₃ aq. (10 mM) = 95% : 5% / B = MeCN : NH₄CO₃ aq. (10 mM) = 20% : 80%, gradient = 0 → 50%). Due to remaining impurities the resulting product was again submitted to HPLC using a HILIC column (HILIC, A = MeCN : NH₄CO₃ aq. (10 mM) = 95% : 5% / B = MeCN : NH₄CO₃ aq. (10 mM) = 20% : 80%, gradient = 100 → 0%). Afterwards due to remaining impurities the resulting powder was washed with ethanol (300 µL) and toluene (300 µL). The pure sodium salt of the product **28** was isolated in under 1 mg yield.



Yield: TBA-salt: 20 mg (0.044 mmol, 53%).
Na-salt: <1 mg (0.004 mmol, 5%).

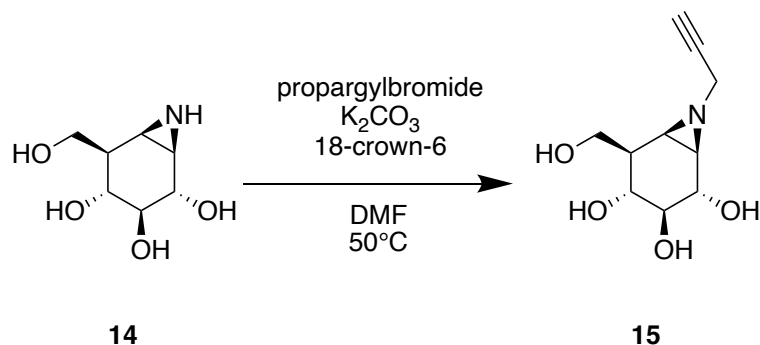
MW: TBA-salt ($C_{23}H_{44}N_4O_5$): 456.63 g/mol
Na-salt ($NaC_7H_8N_3O_5$): 237.15 g/mol.

1H -NMR: TBA-salt: (600 MHz; D_2O): δ [ppm] = 3.86 (dd, J = 8.5, 0.8 Hz, 1H, H-3), 3.59 – 3.57 (m, 1H, H-1), 3.54 (td, J = 10.6, 0.9 Hz, 1H, H-2), 3.41 (ddd, J = 10.8, 8.5, 1.0 Hz, 1H, H-4), 3.25 (dd, J = 3.8, 0.9 Hz, 1H, H-7), 3.23 – 3.17 (m, 8H, TBA- CH_2), 2.76 (ddd, J = 10.5, 1.9, 1.1 Hz, 1H, H-5), 1.71 – 1.61 (m, 8H, TBA- CH_2), 1.37 (h, J = 7.3 Hz, 8H, TBA- CH_2), 0.96 (td, J = 7.4, 0.9 Hz, 12H, Me-TBA).
Na⁺-salt: (600 MHz; D_2O): δ [ppm] = 3.86 (d, J = 8.6 Hz, 1H, H-3), 3.56 (dd, J = 3.9, 1.8 Hz, 1H, H-1), 3.53 (td, J = 10.6, 1.1 Hz, 1H, H-4), 3.44 – 3.36 (m, 1H, H-2), 3.24 (dd, J = 3.7, 1.1 Hz, 1H, H-7), 2.72 (dt, J = 10.5, 1.5 Hz, 1H, H-5).

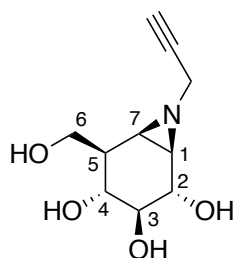
^{13}C -NMR: TBA-salt: (600 MHz; D_2O): δ [ppm] = 177.30 (1C, C=O), 74.85 (1C, C-2), 70.65 (1C, C-3), 60.50 (1C, C-4), 58.10 (4C, TBA- CH_2), 55.80 (1C, C-1), 55.65 (1C, C-7), 48.78 (1C, C-5), 23.11 (4C, TBA- CH_2), 19.13 (4C, TBA- CH_2), 12.79 (4C, Me-TBA).
Na⁺-salt: (600 MHz; D_2O): δ [ppm] = 177.30 (1C, C=O), 74.88 (1C, C-2), 70.71 (1C, C-3), 60.66 (1C, C-4), 55.80 (1C, C-1), 55.75 (1C, C-7), 49.14 (1C, C-5).

Synthesis of the glucuronic acid configured carbocycle

Synthesis of (1*R*,2*S*,3*S*,4*R*,5*R*,6*R*)-5-(hydroxymethyl)-7-(prop-2-yn-1-yl)-7-azabicyclo-[4.1.0]heptane-2,3,4-triol (15)



The starting material **14** (12 mg, 0.068 mmol, 1.00 eq.) was dissolved in 6 ml of DMF. Subsequently, 18-crown-6 (2 mg, 0.007 mmol, 0.1 eq.), K₂CO₃ (11 mg, 0.080 mmol, 1.2 eq.) and freshly distilled propargylbromide (17 mg, 0.110 mmol, 1.5 eq.) were added. The mixture was stirred at room temperature for 3 days and then heated to 70 °C for 1 h. Afterwards, the mixture was filtered over a celite plug before the solvent was removed at 15°C using the high vacuum pump. The product was purified by flash column chromatography using neutralized silica (DCM:MeOH = 6:1). The clean product **15** was obtained as a white solid (9 mg, 0.042 mmol, 60%).



15

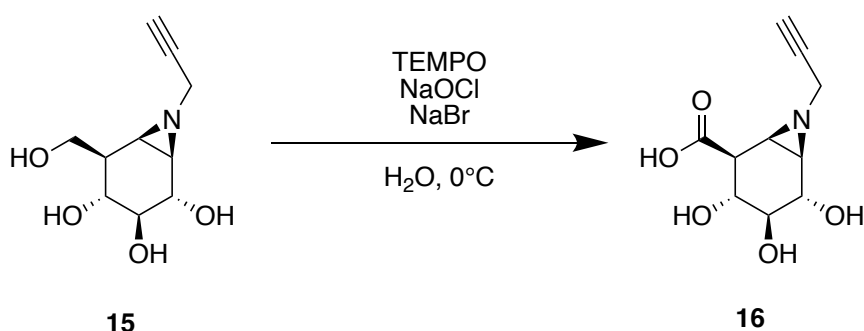
Yield: 9 mg (0.042 mmol, 60%).

MW: (C₁₀H₁₅NO₄): 213.23 g/mol.

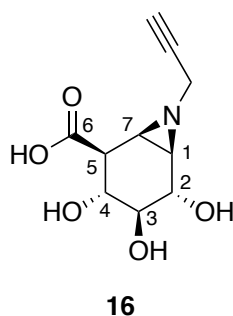
¹H-NMR: (600 MHz; MeOD): δ [ppm] = 3.98 (dd, *J* = 10.3, 4.5 Hz, 1H, H-6a), 3.68 (dd, *J* = 10.4, 8.7 Hz, 1H, H-6b), 3.61 (d, *J* = 8.1 Hz, 1H, H-3), 3.26 (ddd, *J* = 28.5, 16.5, 2.7 Hz, 2H, Propargyl-CH₂), 3.11 (dd, *J* = 10.0, 8.1 Hz, 1H, H-2), 3.05 (t, *J* = 9.8 Hz, 1H, H-4), 2.66 (t, *J* = 2.5 Hz, 1H, Propargyl-CH), 2.24 (dd, *J* = 6.4, 3.4 Hz, 1H, H-1), 1.93 (d, *J* = 6.4 Hz, 1H, H-7), 1.89 (dddd, *J* = 9.6, 8.3, 4.5, 3.4 Hz, 1H, H-5).

¹³C-NMR: (150 MHz; MeOD): δ [ppm] = 79.80 (1C, Propargyl-Cq), 79.04 (1C, C-3), 74.25 (1C, Propargyl-CH), 73.81 (1C, C-2), 70.04 (1C, C-4), 63.75 (1C, C-6), 47.65 (1C, Propargyl-CH₂), 45.41 (1C, C-5), 44.03 (1C, C-7), 41.95 (1C, C-1).

Synthesis of (1*R*,2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-7-(prop-2-yn-1-yl)-7-azabicyclo[4.1.0]heptane-2-carboxylic acid (**16**)



Aziridine **15** (9 mg, 0.040 mmol, 1.00 eq.) was dissolved in 200 μL H₂O. The solution was cooled to 0 °C before TEMPO (1 mg, 0.006 mmol, 0.15 eq.) and NaBr (2 mg, 0.020 mmol, 0.50 eq.) were added. Subsequently, 46 μL (0.098 mmol, 2.4 eq.) of a 13% sodium hypochlorite solution was added dropwise and the pH was adjusted to 10 by slowly adding a 0.5 M KOH solution. The reaction was stirred at 0 °C until TLC revealed no further conversion. Additional 115 μL of the sodium hypochlorite solution were added and the reaction was stirred until TLC revealed complete conversion of the starting material. Then, the reaction was quenched by the addition of 100 μL of ethanol and the pH was adjusted to 7 using a 1 N HCl solution. Afterwards, the volatiles were removed under reduced pressure at room temperature. The residue was redissolved in 200 μL of Milli-Q and purified using a benchtop C₁₈ cartridge. The product was lyophilized overnight and further purified by HPLC (C₁₈, A=NH₄CO₃(25 mM)/ B=MeCN 0 \rightarrow 50%). The product **16** was obtained as a white solid.



Yield: 5 mg (0.020 mmol, 50%).

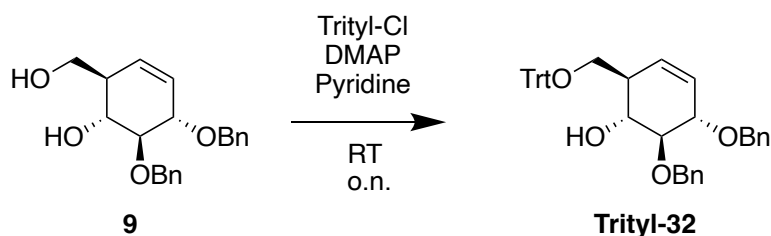
MW: (C₁₀H₁₃NO₅): 227.22 g/mol.

¹H-NMR: (600 MHz; D₂O): δ [ppm] = 3.81 (d, *J* = 8.7 Hz, 1H, H-3), 3.66 (t, *J* = 10.2 Hz, 1H, H-4), 3.41 (dd, *J* = 16.7, 2.5 Hz, 1H, Propargyl-CH₂), 3.37 (dd, *J* = 10.5, 8.7 Hz, 1H, H-2), 3.22 (dd, *J* = 16.7, 2.5 Hz, 1H, Propargyl-CH₂), 2.77 – 2.74 (m, 1H, Propargyl-CH), 2.70 (dd, *J* = 9.9, 3.8 Hz, 1H, H-5), 2.51 (dd, *J* = 6.5, 3.8 Hz, 1H, H-1), 2.10 (d, *J* = 6.4 Hz, 1H, H-7).

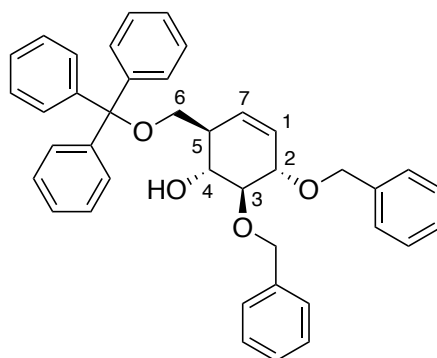
¹³C-NMR: (150 MHz; D₂O): δ [ppm] = 178.71 (C=O), 79.09 (Propargy-Cq), 76.28 (1C, C-2), 73.73 (1C, C-3), 71.92 (Propargyl-CH), 68.56 (1C, C-4), 49.87 (1C, C-5), 46.20 (Propargyl-CH₂), 41.98 (1C, C-1), 38.76 (1C, C-7).

Synthesis of Intermediates with different protecting groups

Synthesis of (1*R*,2*R*,5*S*,6*S*)-5,6-bis(benzyloxy)-2-((trityloxy)methyl)cyclohex-3-en-1-ol (Trityl-32)



The starting material **9** (101 mg, 0.297 mmol, 1.00 eq) was dissolved in 5ml (*c* = 0.06 mol/l) pyridine. Subsequently, 0.446 mmol (124 mg, 1.20 eq.) of triphenylmethylchloride and 4 mg of DMAP (0.03 mmol, 10 mol%) were added. The reaction solution was stirred at room temperature overnight. Then, the solvent was evaporated under reduced pressure and the residue was redissolved in EtOAc. The organic phase was washed with a saturated, aqueous NaHCO₃ solution, brine and then dried over MgSO₄. The solvent was removed under reduced pressure and the crude material was purified by flash column chromatography (PE: EtOAc 7:1) to give 173 mg (0.297 mmol, >99%) of **Trityl-32** as a yellow oil.



Trityl-32

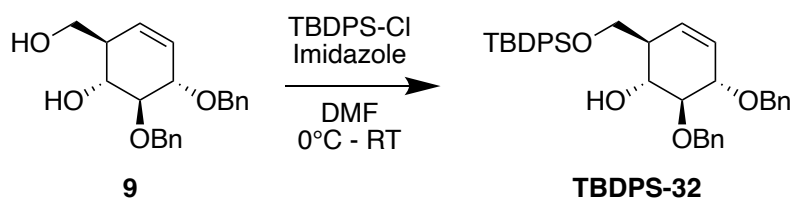
Yield: 173 mg (0.30 mmol, >99%).

MW: (C₄₀H₃₈O₄): 582.74 g/mol.

¹H-NMR: (400 MHz; CDCl₃): δ [ppm] = 7.53 (d, *J* = 7.8 Hz, 2H, H-Ar), 7.47 – 7.22 (m, 23H, H-Ar), 5.82 (dt, *J* = 10.3, 2.4 Hz, 1H, H-1), 5.67 (s, 1H, H-7), 5.08 (d, *J* = 11.3 Hz, 1H, Bn-CH₂), 4.87 (d, *J* = 11.3 Hz, 1H, Bn-CH₂), 4.76 (q, *J* = 11.6 Hz, 2H, Bn-CH₂), 4.32 – 4.28 (m, 1H, H-3), 3.90 – 3.67 (m, 2H, H-6a, H-6b), 3.41 – 3.31 (m, 1H, H-4), 3.01 (d, *J* = 15.6 Hz, 1H, H-2), 2.62 (d, *J* = 8.7 Hz, 1H, H-5).

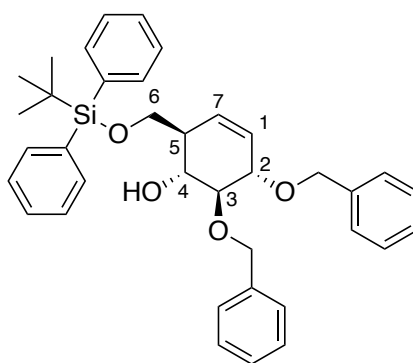
¹³C-NMR: (100 MHz; CDCl₃): δ [ppm] =

Synthesis of (1*R*,2*R*,5*S*,6*S*)-5,6-bis(benzyloxy)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)cyclohex-3-en-1-ol (TBDPS-32)



Compound **9** (500 mg, 3 mmol, 1.00 eq.) was dissolved in 18.50 ml of DMF (*c* = 0.16 mol/l). Then, 360 mg (6 mmol, 2.00 eq.) of imidazole were added and the resulting solution was cooled to 0 °C. Subsequently, 896 µl (3.50 mmol, 1.20 eq.) of TBDPS-Cl were added dropwise. After addition the ice bath was removed, and the reaction solution was

stirred at room temperature for 2 h. The reaction was quenched by the addition of demineralized water at 0°C. The aqueous phase was extracted three times with EtOAc. The combined organic phases were dried over Na₂SO₄ and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:EtOAc 5:1) to give the product **TBDPS-32** as a white crystalline solid (1,439 g, 2.56 mmol, 85%).



TBDPS-32

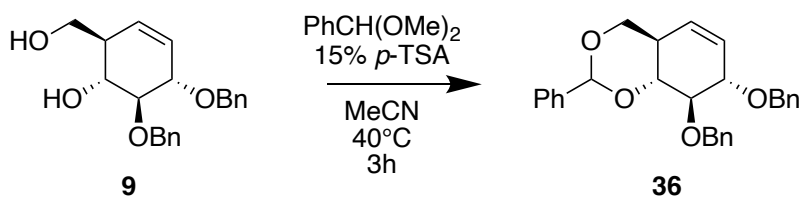
Yield: 1.439 g (2.56 mmol, 85%).

MW: (C₃₇H₄₂O₄Si): 578.82 g/mol.

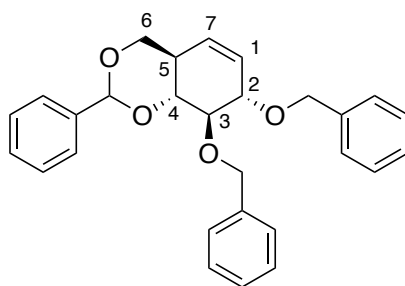
¹H-NMR: (400 MHz; CDCl₃): δ [ppm] = 7.72 (ddd, *J* = 7.9, 1.8, 0.6 Hz, 4H, H-Ar), 7.45 – 7.29 (m, 16H, H-Ar), 5.76 (dt, *J* = 10.2, 2.4 Hz, 1H, H-1), 5.62 (dt, *J* = 10.2, 2.0 Hz, 1H, H-7), 4.91 (dd, *J* = 83.3, 11.3 Hz, 2H, Bn-CH₂), 4.70 (q, *J* = 11.5 Hz, 2H, Bn-CH₂), 4.22 (ddt, *J* = 7.5, 3.6, 2.0 Hz, 1H, H-3), 3.87 – 3.75 (m, 3H, H-6a, H-6b, H-4), 3.67 (dd, *J* = 10.0, 7.6 Hz, 1H, H-2), 2.88 (d, *J* = 1.7 Hz, 1H, H-5), 2.53 – 2.43 (m, 1H, OH), 1.05 (s, 9H).

¹³C-NMR: (100 MHz; CDCl₃): δ [ppm] = 138.65 (1C, Cq-Ar), 138.34 (1C, Cq-Ar), 135.60 (1C, CH-Ar), 135.56 (1C, CH-Ar), 135.16 (1C, Cq-Ar), 134.78 (2C), 133.38 (1C, Cq-Ar), 129.68 (1C, CH-Ar), 129.62 (2C, CH-Ar), 128.64 (1C, CH-Ar), 128.48 (1C, CH-Ar), 128.42 (1C, CH-Ar), 128.01 (1C, CH-Ar), 127.82 (1C, CH-Ar), 127.75 (1C, CH-Ar), 127.70 (2C, CH-Ar), 127.67 (2C, CH-Ar), 127.66 (1C, C1), 126.63 (1C, C7), 83.95 (1C, C2), 80.33 (1C, C3), 75.14 (1C, Bn-CH₂), 71.53 (1C, Bn-CH₂), 70.65 (1C, C-4), 64.44 (1C, C6), 45.84 (1C, C5), 26.54 (3C, *t*-BuCH₃), 19.16 (1C, *t*-BuCq).

Synthesis of (4aR,7S,8R,8aR)-7,8-bis(benzyloxy)-2-phenyl-4a,7,8,8a-tetrahydro-4H-benzo[d][1,3]dioxine (36)



The starting material **9** (12 mg, 0.04 mmol, 1.00 eq.) was dissolved in 1 ml ($c = 0.04$ mol/l) dry MeCN. Subsequently, 9 μl (0.06 mmol, 1.50 eq.) of benzaldehyde dimethylacetal and 1 mg (0.01 mmol, 0.15 eq.) of *para*-toluenesulfonic acid were added. The reaction was stirred at 40°C for 3 h until complete conversion. Then, 25 μl of Et_3N were added and the solvent removed under reduced pressure. The crude product was purified via flash column chromatography (PE:EtOAc 6:1). The product **36** was obtained as colorless oil in a yield of 99% (17 mg, 0.04 mmol).



36

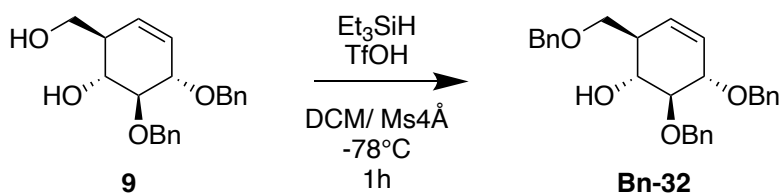
Yield: 17 mg (0.04 mmol, 99%).

MW: ($\text{C}_{28}\text{H}_{28}\text{O}_4$): 428.53 g/mol.

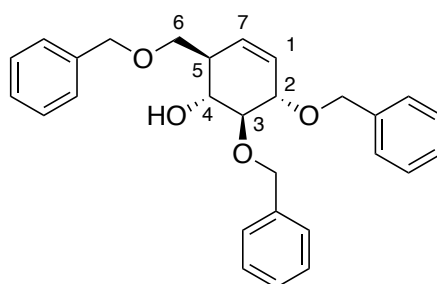
$^1\text{H-NMR}$: (400 MHz; CDCl_3): δ [ppm] = 7.57 – 7.49 (m, 2H, H-Ar), 7.44 – 7.23 (m, 13H, H-Ar), 5.75 (dt, $J = 9.9, 2.9$ Hz, 1H, H-1), 5.63 (s, 1H, H-Ph-CH), 5.39 (dt, $J = 9.9, 1.8$ Hz, 1H, H-7), 4.91 (dd, $J = 89.2, 11.3$ Hz, 2H, Bn- CH_2), 4.75 – 4.64 (m, 2H, Bn- CH_2), 4.29 (ddd, $J = 10.0, 3.7, 1.3$ Hz, 2H, H-6a, H-4), 4.00 (dd, $J = 10.3, 7.0$ Hz, 1H, H-3), 3.81 (t, $J = 9.9$ Hz, 1H, H-4), 3.64 (t, $J = 11.2$ Hz, 1H, H-6b), 2.76 – 2.64 (m, 1H, H-5).

¹³C-NMR: (100 MHz;CDCl₃): δ [ppm] = 138.86 (1C, Cq-Ar), 138.55 (1C, Cq-Ar), 138.30 (1C, Cq-Ar), 129.07 (1C, C-1), 128.91 (1C, CH-Ar), 128.52 (2C, CH-Ar), 128.45 (2C, CH-Ar), 128.34 (2C, CH-Ar), 128.26 (2C, CH-Ar), 127.97 (2C, CH-Ar), 127.79 (1C, CH-Ar), 127.71 (1C, CH-Ar), 126.11(2C, CH-Ar), 125.21 (1C, C-7), 101.65 (1C, Bn-CH), 82.30 (1C, C-4), 82.08 (1C, C-3), 80.80 (1C-C-4), 74.83 (Bn-CH₂), 72.41 (Bn-CH₂), 70.15 (1C, C-6), 38.72 (1C, C-5).

Synthesis of (1*R*,2*R*,5*S*,6*S*)-5,6-bis(benzyloxy)-2-((benzyloxy)methyl)cyclohex-3-en-1-ol (Bn-32)



Compound **9** (34 mg, 0.08 mmol, 1.00 eq.) was dissolved in 20 ml dry DCM ($c = 0.004$ mol/l). The resulting solution was cooled to -78°C and Et₃SiH (38 μl, 0.24 mmol, 3.00 eq.) and trifluoromethanesulfonic acid (24 μl, 0.27 mmol, 3.40 eq.) were added. The reaction solution was stirred for one hour at -78°C and then quenched by the addition of 25 μl of Et₃N and 0.20 ml of MeOH. Subsequently, the solution was diluted with DCM and the organic phase was washed with a saturated, aqueous solution of NaHCO₃, brine and water. After drying of the organic layer over MgSO₄, the solvent was removed under reduced pressure and the crude product was purified via flash column chromatography (PE: EtOAc 4:1). The product **Bn-32** was obtained as colorless oil in a yield of 23 mg (0.05 mmol, 67%).



Yield: 23 mg (0.05 mmol, 67%).

MW: (C₂₈H₃₀O₄): 430.54 g/mol.

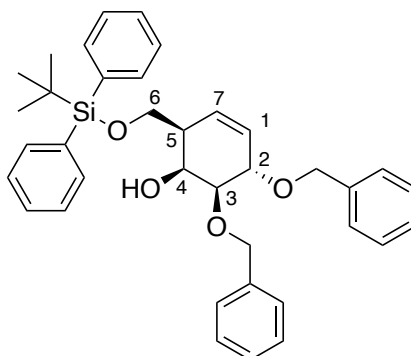
¹H-NMR: (600 MHz; CDCl₃): δ [ppm] = 7.52 – 7.18 (m, 15H, H-Ar), 5.75 (dt, *J* = 10.2, 2.3 Hz, 1H, H-1), 5.64 (dt, *J* = 10.2, 2.0 Hz, 1H, H-7), 4.91 (dd, *J* = 125.9, 11.3 Hz, 2H, Bn-CH₂), 4.68 (dd, *J* = 30.8, 11.3 Hz, 2H, Bn-CH₂), 4.55 (d, *J* = 2.8 Hz, 2H, Bn-CH₂), 4.21 (ddt, *J* = 7.5, 3.7, 2.0 Hz, 1H, H-3), 3.76 – 3.65 (m, 2H, H-2, H-4), 3.64 – 3.52 (m, 2H, H-6a, H-6b), 2.93 (s, 1H, OH), 2.55 (ddh, *J* = 8.4, 5.4, 2.7 Hz, 1H, H-5).

¹³C-NMR: (150 MHz; CDCl₃): δ [ppm] = 138.68 (1C, Cq-Ar), 138.30 (1C, Cq-Ar), 138.15 (1C, Cq-Ar), 128.52 (2C, CH-Ar), 128.47 (2C, CH-Ar), 128.42 (2C, CH-Ar), 128.23 (1C, C-7), 127.98 (2C, CH-Ar), 127.90 (2C, CH-Ar), 127.78 (1C, CH-Ar), 127.74 (1C, CH-Ar), 127.67 (1C, CH-Ar), 127.62 (2C, CH-Ar), 126.71 (1C, C-1), 83.88 (1C, C-2), 80.19 (1C, C-3), 74.94 (1C, C-4), 73.37 (1C, Bn-CH₂), 71.58 (1C, Bn-CH₂), 71.12 (1C, Bn-CH₂), 71.05 (1C, C-6), 44.02 (1C, C-5).

General procedure for the hydroxyl inversion

The starting material (1.00 eq.) was dissolved in (*c* = 0.04 mol/l) in dry DCM and dry pyridine (10:1, *c* = 0.04 mol/l). The solution was cooled to -20 °C and trifluoromethanesulfonic anhydride (3.00 eq.) was added dropwise. The reaction solution was allowed to warm up to 0 °C. Upon full conversion of the starting material to a higher running product, the solution was diluted with EtOAc and washed with ice-cold 1 N HCl solution, aqueous NaHCO₃ solution and brine. The organic layers were dried over MgSO₄, and the solvent was removed under reduced pressure at room temperature. The crude product was co-evaporated with toluene and then dissolved in dry MeCN (*c* = 0.04 mol/l). Then, TBANO₂ (6.00 eq.) was added, and the reaction solution was stirred at room temperature overnight. After TLC showed full conversion of the starting material, the solvent was removed under reduced pressure and the crude product was purified by column chromatography (PE:EtOAc).

(1*S*,2*R*,5*S*,6*S*)-5,6-bis(benzyloxy)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)cyclohex-3-en-1-ol (TBDPS-33)



TBDPS-33

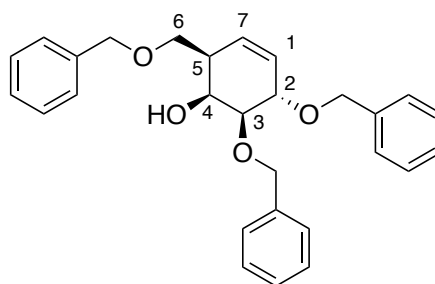
Yield: 1.335 g (2.34 mmol, 92%).

MW: (C₃₇H₄₂O₄Si): 578.82 g/mol.

¹H-NMR: (600 MHz; CDCl₃): δ [ppm] = 7.67 (td, *J* = 7.9, 1.5 Hz, 4H, H-Ar), 7.46 – 7.27 (m, 16H, H-Ar), 5.79 (dt, *J* = 10.2, 2.7 Hz, 1H, H-1), 5.48 (dq, *J* = 10.2, 1.7 Hz, 1H, H-7), 4.73 (dd, *J* = 26.5, 10.8 Hz, 4H, Bn-CH₂), 4.36 (dq, *J* = 5.2, 2.5 Hz, 2H, H-3, H-4), 3.88 (dd, *J* = 10.0, 7.9 Hz, 1H, H-6a), 3.78 (dd, *J* = 10.0, 6.1 Hz, 1H, H-6b), 3.67 (dd, *J* = 7.7, 2.2 Hz, 1H, H-2), 2.53 (dh, *J* = 5.8, 2.9 Hz, 1H, H-5), 1.06 (s, 9H, *t*-Bu).

¹³C-NMR: (150 MHz; CDCl₃): δ [ppm] = 138.76 (1C, Cq-Ar), 138.47 (1C, Cq-Ar), 135.64 (2C, CH-Ar), 135.58 (2C, CH-Ar), 133.41 (1C, Cq-Ar), 133.30 (1C, Cq-Ar), 129.79 (1C, CH-Ar), 128.46 (1C, CH-Ar), 128.38 (1C, CH-Ar), 127.84 (2C, CH-Ar), 127.83 (2C, CH-Ar), 127.76 (2C, CH-Ar), 127.75 (2C, CH-Ar), 127.74 (2C, CH-Ar), 127.57 (1C, CH-Ar), 127.40 (1C, C-1), 126.30 (1C, C-7), 82.37 (1C, C-2), 77.36 (1C, C-3), 72.34 (1C, Bn-CH₂), 72.10 (1C, Bn-CH₂), 68.51 (1C, C-4), 64.29 (1C, C-6), 43.03 (1C, C-5), 26.88 (3C, *t*-BuCH₃), 19.27 (1C, *t*-BuCq).

(1*S*,2*R*,5*R*,6*R*)-5,6-bis(benzyloxy)-2-((benzyloxy)methyl)cyclohex-3-en-1-ol (Bn-33)



Bn-33

Yield: 13 mg (0.03 mmol, 60%).

MW: (C₂₈H₃₀O₄): 430.54 g/mol.

¹H-NMR: (600 MHz; CDCl₃): δ [ppm] = 7.45 – 7.27 (m, 15H, H-Ar), 5.80 (dt, *J* = 10.2, 2.7 Hz, 1H, H-1), 5.54 – 5.49 (m, 1H, H-7), 4.79 – 4.67 (m, 4H, Bn-CH₂), 4.55 (d, *J* = 2.7 Hz, 2H, Bn-CH₂), 4.35 (ddq, *J* = 10.3, 5.1, 2.0 Hz, 2H, H-3, H-4), 3.76 – 3.65 (m, 2H, H-2, H-6a), 3.57 (dd, *J* = 9.1, 6.1 Hz, 1H, H-6b), 2.64 (ddp, *J* = 8.8, 5.9, 2.9 Hz, 1H, H-5).

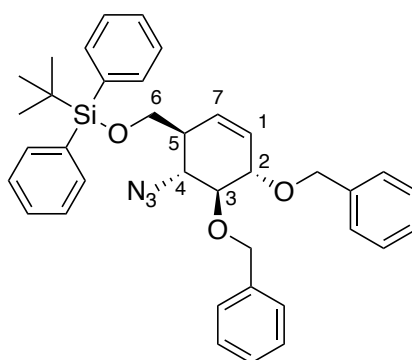
¹³C-NMR: (150 MHz; CDCl₃): δ [ppm] = 138.81 (1C, Cq-Ar), 138.46 (1C, Cq-Ar), 138.18 (1C, Cq-Ar), 128.60 (2C, CH-Ar), 128.57 (2C, CH-Ar), 128.50 (2C, CH-Ar), 127.98 (2C, CH-Ar), 127.96 (2C, CH-Ar), 127.90 (3C, CH-Ar), 127.88 (1C, CH-Ar), 127.71 (1C, CH-Ar), 127.45 (1C, C-1), 126.39 (1C, C-7), 82.37 (1C, C-2), 77.26 (1C, C-3), 73.56 (1C, Bn-CH₂), 72.49 (1C, Bn-CH₂), 72.17 (1C, Bn-CH₂), 70.55 (1C, C-6), 68.53 (1C, C-4), 41.11 (1C, C-5).

General procedure for the azide substitution

The starting material (1.00 eq.) was dissolved in DCM (*c* = 0.04 mol/l) and the solution was cooled to -20 °C. Subsequently, pyridine was added (1:10 vs. DCM), followed by the dropwise addition of trifluoromethanesulfonic anhydride (6.00 Äq.). The solution was allowed to warm up to 0 °C over the course of two hours. When TLC showed full conversion of the starting material to a higher running product, the solution was diluted with EtOAc and washed with ice-cold 1 N HCl solution, aqueous NaHCO₃ solution and

brine. The organic layer was dried with MgSO_4 and the solvent was removed under reduced pressure at room temperature. The crude triflate was dried under high vacuum for one hour. Afterwards the triflate was dissolved in dry MeCN or DMF ($c = 0.04 \text{ mol/l}$). Next, TBAN_3 (12.00 eq.) or NaN_3 (12.00 eq.) was added, and the solution was stirred at 50°C or 70°C overnight. Then, the solvent was removed under reduced pressure and the crude product was purified by column chromatography (PE:EtOAc).

(((1*S*,4*S*,5*S*,6*R*)-6-azido-4,5-bis(benzyloxy)cyclohex-2-en-1-yl)methoxy) (tert-butyl)di-phenylsilane (TBDPS-34)



TBDPS-34

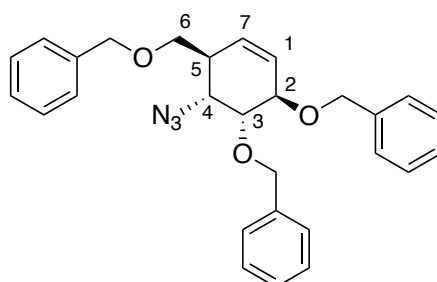
Yield: 6 mg (0.01 mmol, 36%).

MW: ($\text{C}_{37}\text{H}_{47}\text{N}_3\text{O}_3\text{Si}$): 603.84 g/mol.

$^1\text{H-NMR}$: (600 MHz; CDCl_3): δ [ppm] = 7.67 – 7.62 (m, 4H, H-Ar), 7.47 – 7.29 (m, 16H), 5.80 – 5.75 (m, 1H, H-1), 5.62 (dt, $J = 10.2, 2.1 \text{ Hz}$, 1H, H-7), 4.97 – 4.87 (m, 2H, Bn- CH_2), 4.77 – 4.67 (m, 2H, Bn- CH_2), 4.27 (ddt, $J = 7.4, 3.9, 2.0 \text{ Hz}$, 1H, H-3), 3.83 – 3.66 (m, 4H, H-6a, H-6b, H-4, H-2), 2.24 – 2.19 (m, 1H, H-5), 1.05 (s, 9H, *t*-Bu).

$^{13}\text{C-NMR}$: (150 MHz; CDCl_3): δ [ppm] = 138.41 (1C, Cq-Ar), 138.19 (1C, Cq-Ar), 135.77 (2C, CH-Ar), 135.73 (1C, CH-Ar), 135.66 (2C, CH-Ar), 133.43 (1C, Cq-Ar), 133.42 (1C, Cq-Ar), 129.92 (2C, CH-Ar), 129.30 (1C, C-1), 128.62 (2C, CH-Ar), 128.57 (2C, CH-Ar), 128.55 (2C, CH-Ar), 127.98 (2C, CH-Ar), 127.91 (2C, CH-Ar), 127.89 (1C, CH-Ar), 127.86 (2C, CH-Ar), 127.83 (1C, CH-Ar), 127.25 (1C, C-7), 83.48 (1C, C-2), 80.68 (1C, C-3), 75.58 (1C, Bn- CH_2), 72.18 (1C, Bn- CH_2), 63.61 (1C, C-4), 62.19 (1C, C-6), 44.96 (1C, C-5), 29.85 (3C, *t*-Bu- CH_3), 19.50 (1C, *t*-Bu-Cq).

(((1*R*,2*R*,5*S*,6*R*)-6-azido-5-((benzyloxy)methyl)cyclohex-3-ene-1,2-diyl)bis(oxy))bis(methylene))dibenzene (Bn-34)



Bn-34

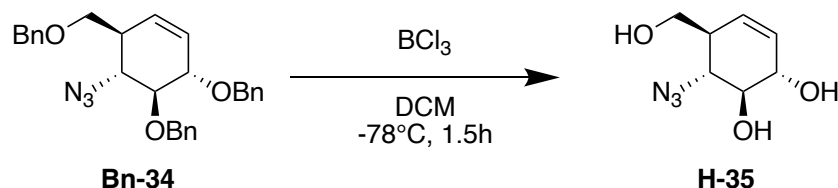
Yield: 16 mg (0.03 mmol, >99%).

MW: (C₂₈H₂₉N₃O₃): 455.55 g/mol.

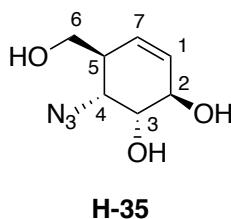
¹H-NMR: (600 MHz; CDCl₃): δ [ppm] = 7.46 – 7.28 (m, 15H, H-Ar), 5.75 (dt, *J* = 10.2, 2.4 Hz, 1H, H-1), 5.65 (dt, *J* = 10.3, 2.1 Hz, 1H, H-7), 5.01 – 4.84 (m, 2H, Bn-CH₂), 4.67 (dd, *J* = 11.5, 5.1 Hz, 2H, Bn-CH₂), 4.54 (dd, *J* = 44.2, 12.4 Hz, 2H, Bn-CH₂), 4.24 (dtd, *J* = 5.2, 3.5, 1.9 Hz, 1H, H-3), 3.70 – 3.61 (m, 2H, H-4, H-2), 3.56 (qd, *J* = 9.2, 4.1 Hz, 2H, H-6a, H-6b), 2.30 (tdd, *J* = 7.9, 4.1, 2.2 Hz, 1H, H-5).

¹³C-NMR: (150 MHz; CDCl₃): δ [ppm] = 138.33 (1C, Cq-Ar), 138.25 (1C, Cq-Ar), 138.16 (1C, Cq-Ar), 128.82 (1C, C-7), 128.60 (1C, CH-Ar), 128.59 (1C, CH-Ar), 128.54 (1C, CH-Ar), 128.43 (1C, CH-Ar), 128.03 (1C, CH-Ar), 127.92 (1C, CH-Ar), 127.91 (1C, CH-Ar), 127.89 (1C, C-1), 127.76 (1C, CH-Ar), 127.23 (1C, CH-Ar), 83.40 (1C, C-2), 80.43 (1C, C-3), 75.46 (1C, Bn-CH₂), 73.39 (1C, Bn-CH₂), 72.25 (1C, Bn-CH₂), 69.82 (1C, C-6), 62.56 (1C, C-4), 43.18 (1C, C-5).

Synthesis of (1*R*,2*R*,5*S*,6*R*)-6-azido-5-(hydroxymethyl)cyclohex-3-ene-1,2-diol (H-35)



The starting material **Bn-34** (16 mg, 0.04 mmol, 1.00 eq.) was dissolved in 5 ml ($c = 0.01$ mol/l) of anhydrous DCM. The solution was cooled to -78°C and 0.3 ml (0.30 mmol, 7.50 eq.) of a 1M solution of BCl_3 in DCM were added dropwise. The reaction was stirred at -78°C for 1.5 h and subsequently quenched with a mixture of DCM and MeOH (1:1). Then, the solvent was removed under reduced pressure and the crude product subjected to flash column chromatography (DCM:MeOH 10:1). The pure product **H-35** was obtained as a colorless liquid (6 mg, 0.03 mmol, 81%).



Yield: 6 mg (0.03 mmol, 81%).

MW: ($\text{C}_{28}\text{H}_{29}\text{N}_3\text{O}_3$): 185.18 g/mol.

$^1\text{H-NMR}$: (600 MHz; MeOD): δ [ppm] = 5.69 – 5.55 (m, 2H, H-1, H-7), 4.08 (ddd, $J = 7.8, 3.2, 1.8$ Hz, 1H, H-3), 3.73 (dd, $J = 10.9, 3.3$ Hz, 1H, H-2), 3.61 (dd, $J = 10.9, 5.3$ Hz, 1H, H-4), 3.55 – 3.38 (m, 2H, H-6a, H-6b), 2.23 – 2.09 (m, 1H, H-5).

$^{13}\text{C-NMR}$: (150 MHz; MeOD): δ [ppm] 131.43 (1C, C-1), 128.76 (1C, C-7), 78.59 (1C, C-3), 73.48 (1C, C-2), 64.22 (1C, C-4), 63.13 (1C, C-6), 46.16 (1C, C-5).

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