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Department of Chemistry, Centre for Analysis and Synthesis

Design, synthesis, and evaluation of 1-phenylethanol and C2 threitol derivatives as non-pyranose galactose-mimicking inhibitors of Galectin-3

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Popular summary (English)

Galectins are proteins involved in several biological functions such as cell division, cell migration and cell death amongst many others. Their presence is also substantial in diseases including acute inflammation, scar formation, allergies, and cancer. The galectin family consists of 15 members where the majority of them are found in humans. Especially, galectin-3 is an interesting protein to study since it has been shown to be mainly active in cell division of cancer cells.

LacNAc is a sugar-containing fragment which has been shown to bind the best and inhibit Galectin-3. By considering and retaining the essential bindings of LacNAc leading to excellent interactions with the amino acids in the binding site, synthetic molecules can be developed. In this project, small and relatively hydrophobic molecules have been synthesized serving as potential inhibitors of Galectin-3. It is predicted that they would be able to enter the central nervous system and suppress the protein's function in cell division of cancer cells.

Populärvetenskaplig sammanfattning (Svenska)

Galektiner är proteiner som är involverade i många biologiska funktioner så som celldelning, cellvandring och celldöd bland många fler. Deras närvaro är betydande i sjukdomar som akut inflammation, ärrbildning, allergier och cancer. Galektinfamiljen består av 15 medlemmar där majoriteten av dem finns i människor. Speciellt galektin-3 är ett intressant protein att studera eftersom det har visats vara mestadels aktiv i celldelning av cancerceller.

LacNAc är ett socker-innehållande fragment som har visat sig binda bäst och hämmar Galektin-3. Genom att ta hänsyn till och bevara de viktiga bindningarna av LacNAc som leder till utmärkta interaktioner med aminosyrorna i bindningsfickan kan man utveckla syntetiska molekyler. I detta projekt har små och relativt hydrofobiska molekyler syntetiserats för att möjligen inhibera galektin-3 förutsatt att de ska kunna passera det centrala nervsystemet och dämpa proteinets funktion i celldelning av cancerceller.

Abstract

Galectins are cytosolic protein family involved in several biological functions such as cell differentiation, cell migration and apoptosis amongst many others. Their presence is also substantial in diseases including acute inflammation, fibrosis, allergies, and cancer. This protein family consists of 15 members where the majority of them are found in humans and they are divided into three subgroups: prototype, chimera and tandem-repeat. Being the only member of the chimera group, Galectin-3 is an interesting protein to study since it has also been shown to for example promote tumor immune evasion.

LacNAc is a fragment, consisting of galactose and glucose, which has been shown to bind to Galectin-3 effectively. By considering and retaining the essential bindings of LacNAc leading to interactions with the amino acid residues in the binding site, synthetic molecules can be developed. By synthesizing small and relatively nonpolar potential inhibitors of Galectin-3, it is predicted that they would be able to pass through the blood-brain barrier and suppress the protein's function in cell differentiation of cancer cells positioned in the central nervous system.

In this project, the derivatives of 1-phenylethanol and their respective enantiomers with S and R configuration, were studied with styrene compounds as starting materials. The reason behind it is to investigate whether a specific enantiomer will interact better with the active binding site of Galectin-3. Analyses of the compounds' configuration were performed and it was concluded that the OH-group could have adapt its conformation to fit into the binding site. Thus, no significant difference was seen in the binding affinity of S and R-enantiomers. Meanwhile, it was also investigated if C2 threitol derivatives would enhance the affinity of other earlier studied threitol derivatives by interacting with Tryptophan 181. Starting from a D-galactose, a threitol compound can be obtained through a series of reactions where modifications can be further made on C2. In this case, it was concluded that converting the hydroxyl group of C2 to a ketone could have caused possible repulsion when interaction with Trp 181.

List of abbreviations

Chemicals

ACN	acetonitrile
Bu_2SnO	dibutyltin oxide
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
EtOAc	ethyl acetate
HPLC	high performance liquid chromatography
IPA	isopropyl alcohol
LacNAc	N-acetyllactosamine
p-TsCl	p-toluene sulfonyl chloride
TLC	thin-layer chromatography

Amino acids

Arg	Arginine
Asn	Asparagine
Glu	Glutamine
His	Histidine
Ile	Isoleucine
Ser	Serine
Trp	Tryptophan

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1. Introduction

1.1 General knowledge about galectins

A common denominator among diseases such as allergies, acute and chronic inflammation, cancer, fibrosis, and autoimmune diseases is the participation of galectins. These proteins partake in signaling pathways, cell differentiation, cell migration and apoptosis. Galectins are a cytosolic protein family found in different species, cell types and tissues expressed in various patterns. They are soluble, non-membrane bound proteins which interacts with galactoside-containing ligands. Galectin-1, -2, -3, -4, -7, -8, -9, -10, -12, -14 are found in vertebrates and mammals, whilst galectin-5 and -6 are found only in rodents and galectin-11, -14 and -15 in ruminants. The mammalian galectins have different structures and are divided into three groups: prototype, chimera and tandem-repeat as seen in Figure 1. In the prototype group are galectins composed of having only one carbohydrate recognition domain (CRD), while tandem-repeat have two CRDs. Lastly, chimera, involving only galectin-3, can exist as a monomeric or a pentameric form in which the latter is assisted and formed by the protein's non-lectin domain. Continuing the focus on galectin-3, it has been shown to contribute to nuclear splicing of pre-mRNAs in gene expression, regulating transcription, and organizing microtubules along with downregulation of T-cell receptors and cell apoptosis regulation.[1][2][3]

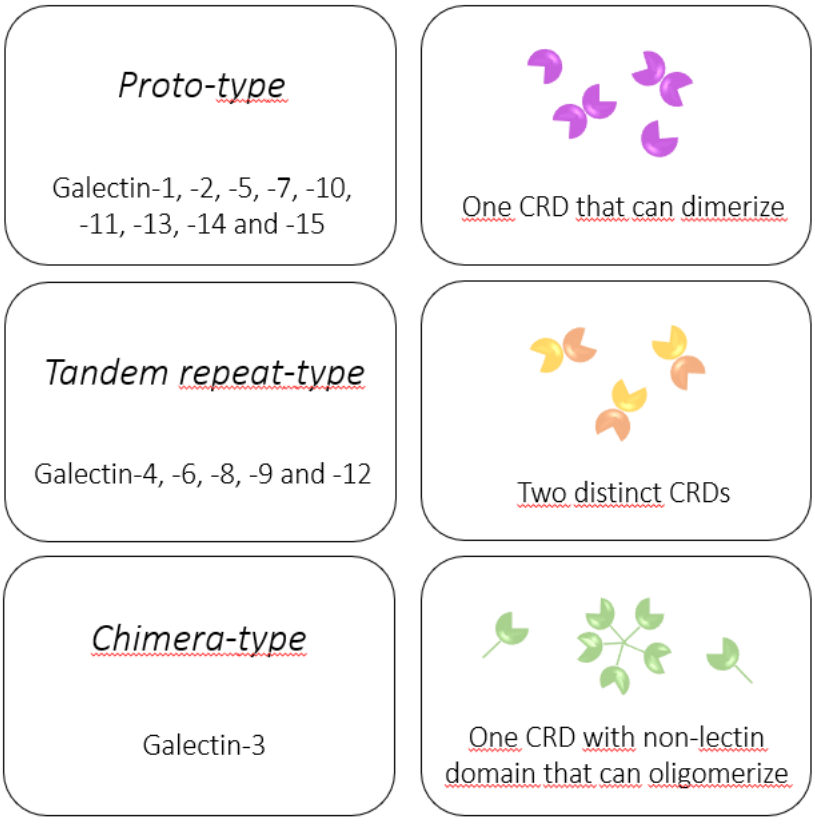


Figure 1. An illustration of the galectins' protype groups. Source: Adapted from [2].

1.2 Galectin-3 protein structure and binding site

To determine the protein structure of galectin-3 in complex with the disaccharide N-acetyllactosamine (gal-β-(1-4)-glcNAc), an X-ray structure of the complex has been solved as illustrated in Figure 2.[4] It was found that galectin-3's CRD is made up of 5-stranded (F1-F5) and 6-stranded (S1-S6a/6b) β-sheets lying on top of each other resulting in a β-sandwich. Additionally, the β-strand F1 and S1 form a surface towards the solvent while S4-S6 β-strands formed a cleft serving as the binding site. LacNAc turned out to bind to galectin-3's binding site with 72% of its surface area. The most essential hydroxyl group being the one bound to C4 resulting into acceptance of hydrogen bond from residues of His-158 and Arg-162. The binding is also enhanced by the hydroxyl group of C6 where hydrogen bonds are also interacting with Glu-184 and Asn-174. Meanwhile, C3, C4, C5 and C6 are forming a planar conformation with van der Waals interaction with the aromatic side chain of Trp-181.[4]

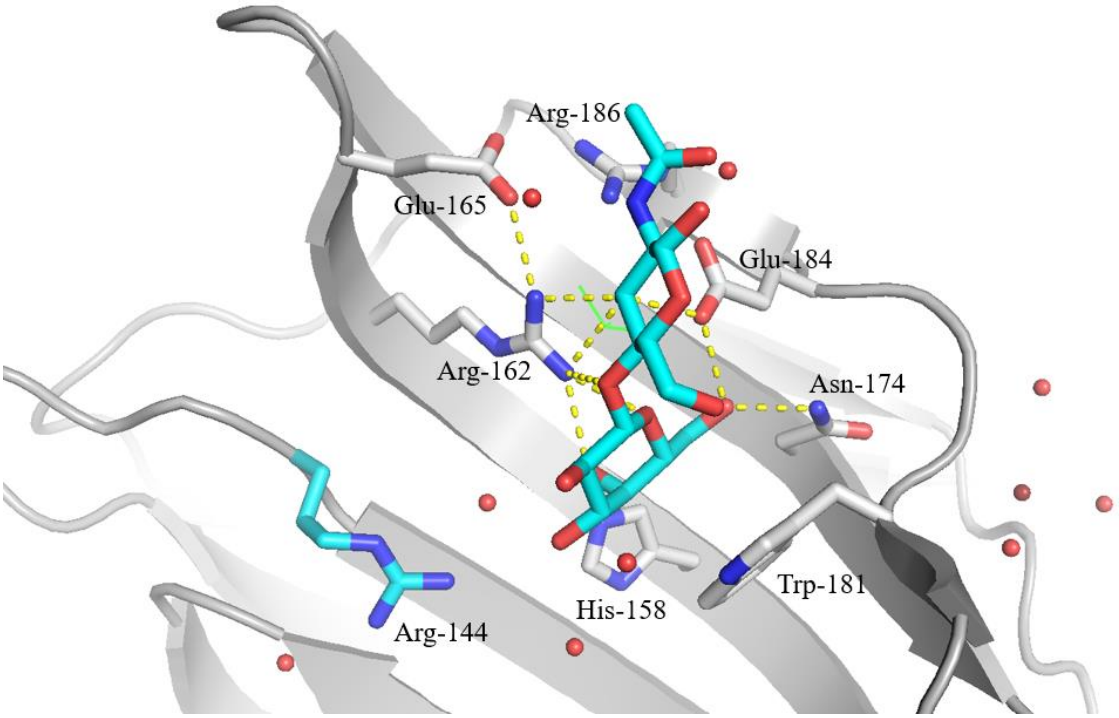


Figure 2. A graphic demonstration of how LacNAc binds to the binding site of galectin-3. The figure was generated from PDB ID 1KJL with PyMol Schrödinger LLC.

1.3 Non-natural inhibitor derivatives

Since Lac and LacNAc has been shown to be good natural disaccharide fragment ligands of galectin-3, modifications have been done to see if synthetic inhibitors will bind better to the protein. LacNAc with attached triazole derivatives have been investigated to utilize a cation-π-interactions with Arg-144 to bind better to galectin-3. A library of inhibitors has been synthesized in earlier studies as showed in Chart 1. First of all, LacNAc with a methoxy group in C1 instead of a hydroxyl group named as compound **1** in Figure 2 exhibited an affinity of K_D = 59 μM. By removing the glucose part in LacNAc and retaining the C1 methoxy group in the galactose, stated as compound **2**, expectedly decreased the affinity enormously with almost 75-fold. Nevertheless, substitution of the C1-methoxy to a C1-thiomethyl group and introduction of triazole to C3 where a phenyl group is bound to the C4 of the triazole,

compound **3**, increased the protein-ligand binding with an affinity approaching compound **1**.[5] Evidently, addition of a trifluorophenyl group bound to C4 of the triazole complemented the complex interaction by utilizing the binding with Arg-144, Ile-145 and Ser-237 through fluorine-amide interactions. Also, by substituting the thiomethyl group to a thiotolyl leading to compound **4**, the affinity increased with almost 30-fold.[6] With further investigation, it has been shown that some hydroxyl groups in the galactose ring were not actively binding to galectin-3. Cleavage of the ring generated the corresponding threitol derivative, compound 5, which exhibited a decrease in affinity as expected.[7] Lastly, it was also investigated how compound **6** with only an ethanol scaffold would bind to galectin-3 having less of the essential hydroxyl groups and it bound conceivably weaker thus decreasing the affinity greatly.[8]

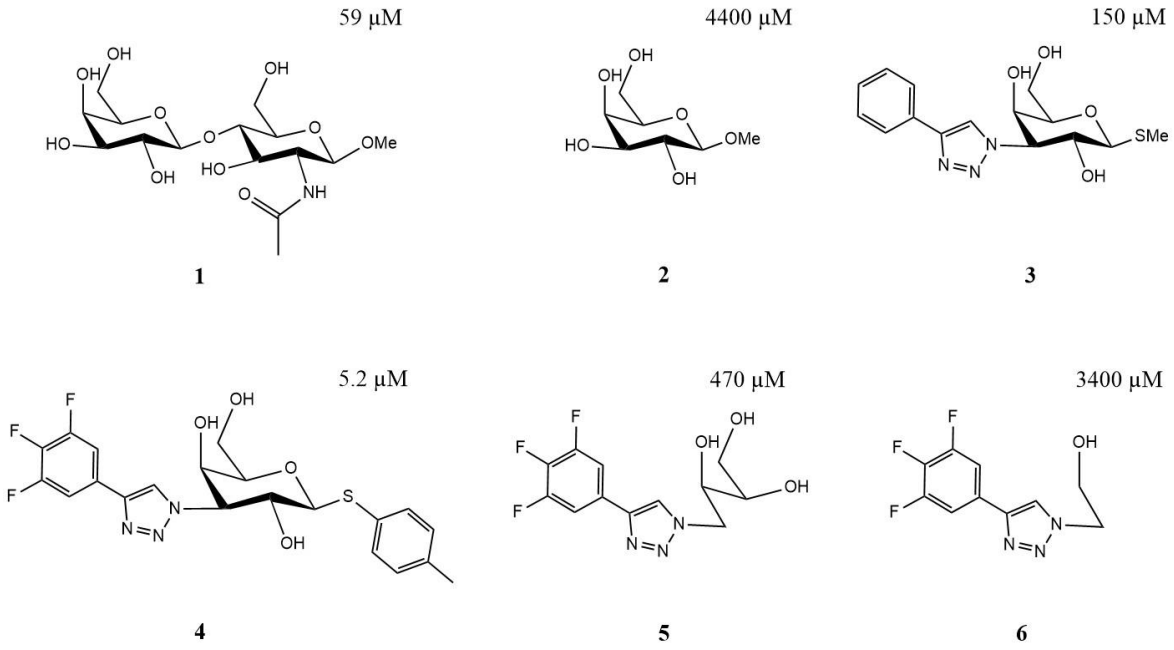


Chart 1. Synthetic inhibitors of galectin-3 showing their development.

1.4 Binding affinity: Fluorescence polarization

A measurement of how strong the interaction is between a protein and a ligand is expressed by the equilibrium dissociation constant, K_D. If the protein-ligand complex has a strong affinity then the K_D value is low and when the binding is weaker, K_D becomes higher. Affinity determination of ligand-protein binding can be done with several different methods , of which fluorescence polarization (FP) is a reliable, quick and material-economic method.[9] FP depends on a probe molecule's emitted fluorescence degree of polarization (DOP) relative to that of the excitation light in its measurements aided by a fluorescent tag. The probe molecule bound by a larger protein rotates and tumbles slower, which causes fluorescence emission with higher DOP than that of an unbound probe molecule. Once the complex is exposed to a potential protein inhibitor, the fluorescent probe may be outcompeted by the inhibitor to bind to the protein leading to a decrease in DOP. The measurements obtained from FP are values expressed in units of polarization that are then converted to anisotropy

values. The reason for this conversion is that anisotropy is better correlated to the amount of bound probe. The anisotropy values at the known varied inhibitor concentrations, along with fixed probe and protein concentrations are used to calculate K_D values. The calculations of K_D values for galectin-3 in this work are done under the assumption that each protein have only one ligand site.[10]

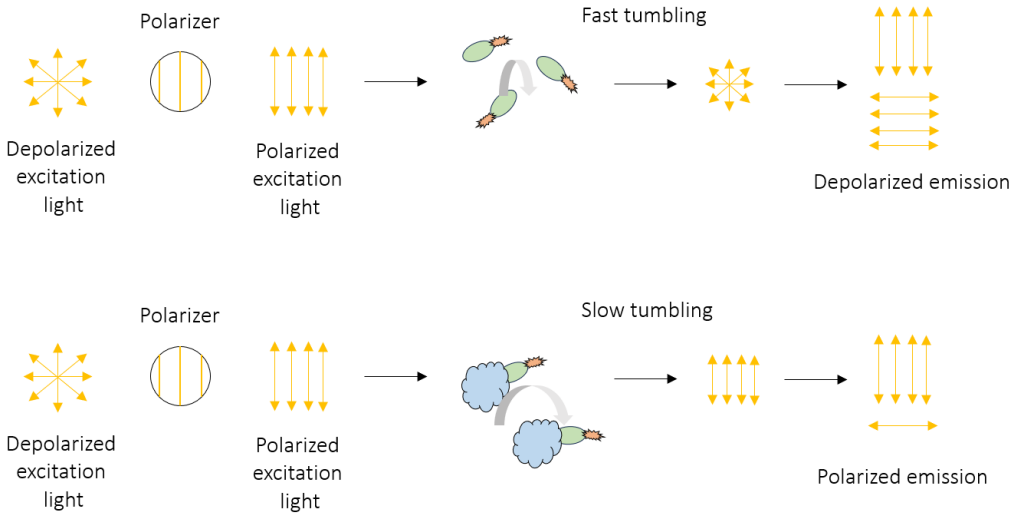


Figure 3. A graphic description of how the depolarization of light emission is affected by the rotational speed of ligand. Source: Adapted from [11].

1.5 Chiral column chromatography

Chirality in molecules can affect binding affinity, pharmacodynamics, pharmacokinetics, and toxicity of drugs. Enzyme systems can process stereoisomers differently and can cause unpredicted effects. Thus, ensuring the right isomer when developing drugs is very important.[12] When synthesizing possible drug molecules, chiral column chromatography is one of the methods used to separate enantiomers. Its principle is the same as an HPLC with a stationary phase and a mobile phase. The stationary phase in chiral HPLC consists of silica particles functionalized with a chiral structure. Different enantiomers will bind with different strength to the chiral stationary phase and thus elute at different retention times. A possible disadvantage of this method is that a variety of columns may need to be evaluated and it may be difficult to find the right one for the desired compound since it depends on the stereochemical nature of both the column stationary phase and the compound analyzed.[13] Once the analysis has been run and isomers has been separately distinguished, information including peak areas, percentage of peak areas and retention time is generated which correlates to the concentration of the compound. With the obtained information, the purity of the enantiomers is measured by enantiomeric excess (ee) which is calculated as follows,

$$ee = \frac{[(S)-(R)]}{[(S)+(R)]} \times 100\% \qquad \text{(Eq.1)}$$

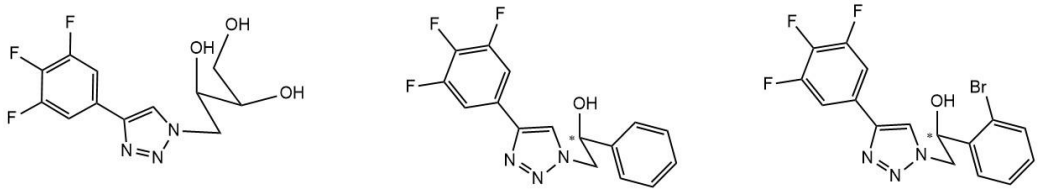
where (S) is the area% of the S enantiomer and (R) is the area% of the R-enantiomer.[13]

1.6 Project aim

As mentioned, and illustrated above, many derivatives of LacNAc can be synthesized to create a better binding to galectin-3 leading to inhibition and potential drug development. Galactose has been the backbone of these derivatives but to further get an interesting take on this development, molecules mimicking galactose is an alternative approach. The ultimate goal is to discover a small and relatively non-polar molecule which can pass through the blood-brain barrier and thus, reaching to the central nervous system to target cancer cells.

The essential binding of the galactose's 4-OH would want to be kept as it is mimicked by 1-OH of 1-phenylethanol. Already studied were the S- and R-enantiomers of the 1-phenylethanol named as compound **7** in Chart 2, which displayed affinities against galectin-3 of 740 µM and 1736 µM respectively.[14] Meanwhile, a racemic mixture of 2-bromo-1-phenylethanol, compound **8**, showed a K_D value of 160 µM.[15] By separately synthesizing the enantiomers of compound **8**, is it possible to distinguish any difference in binding affinity whether it has an S- or R-configuration?

Meanwhile, it has been known that compound **5** in Chart 2 showed an affinity of 470 µM. Thus, further modifications can be done to discover a molecule that accomplishes a stronger binding with galectin-3. By retaining the interactions of 4-OH and 6-OH of the galactose, which is mimicked by the 1-OH and 3-OH of the threitol **5**, can modifications on C2 be possible to increase the affinity through additional interactions with Trp 181?



5 **7** **8**

Chart 2. Earlier-studied synthetic inhibitors of 1-phenylethanol **7**-**8** and C2 threitol **5** derivatives.

2. Results and discussion

2.1 1-Phenylethanol derivatives

Initiation of the project was done by synthesizing the 2-bromophenyl-1-ethanol, as illustrated in Figure 4, to obtain the enantiomeric products through usage of commercially available AD-mixes.

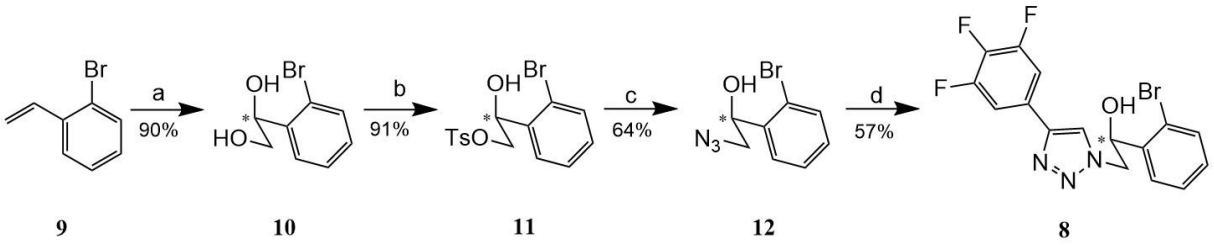


Figure 4. Synthesis steps for enantiomeric products of 1-phenylethanol derivative with 2-bromophenyl. (a) AD-mix-α for S-enantiomer or AD-mix-β for R-enantiomer, t-BuOH, H_2O, 2-bromostyrene, 0°C, 20h and quenched with sat. Na_2SO_3, rt, 40 min. (b) Bu_2SnO, *p*-TsCl, Et_3N, THF, rt, 18h. (c) NaN_3, anhydrous DMF, under N_2, 80°C, 24h. (d) 5-ethynyl-1,2,3-trifluorobenzene, CuI, Et_3N, dry DMF, under N_2, rt, 18h.

Asymmetric dihydroxylation. The synthesis is initiated with 2-bromostyrene (**9**) and designated AD-mixes reacting together with t-BuOH and H_2O as it was cooled down to 0°C with an ice bath. Analyzing compound **9** together with Figure 5, by appointing that R_L is the 2-bromo-phenyl group, R_M=R_S=H and applying the priority group rules on compound **10**, it is determined that using AD-mix-α will obtain the S-enantiomer and AD-mix-β will obtain the corresponding R-enantiomer. Both has been used to synthesize compound **8**. With the Sharpless asymmetric dihydroxylation, diol is obtained as compound **9** where a primary and secondary alcohols are present. Since using AD-mixes forms osmium tetroxide (OsO_4), a poisonous compound, an aqueous solution of sodium sulfite was used to quench and reduce OsO_4 to a less hazardous form.

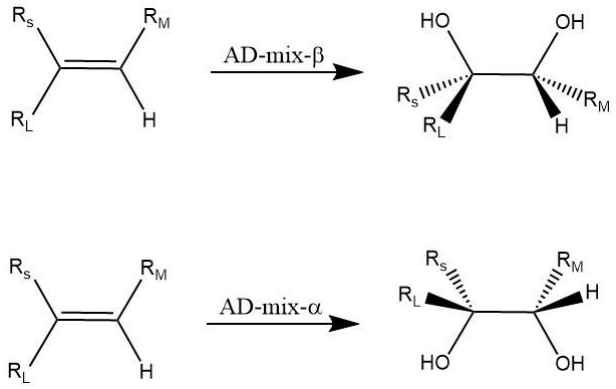


Figure 5. An illustration of how AD-mix-α and AD-mix-β produces enantiomeric products where R_L is the largest substituent, R_M is the medium-sized substituent and R_S is the smallest substituent. Source: Adapted from [16].

Tosylation. It is known that hydroxide is a poor leaving group based on the fact that it's a strong base and by introducing a better leaving group, efficient substitutions can be done. Thus, for step b in Figure 4, the tosyl group is introduced onto the primary alcohol with a sulfonyl nucleophilic substitution obtaining compound **11**. A catalytic amount of Bu_2SnO is added to create an intermediate with the *p*-TsCl attaining better selectivity for tosylation on the primary alcohol.

Azide introduction. With a better leaving group OTs, which is a weak base due to its ability of delocalizing charges between oxygens, an S_N2 substitution can be done through a nucleophilic attack. Utilizing the nucleophilicity obtained by NaN_3, the tosyl group is efficiently substituted with N_3 giving compound **12**. The reaction was heated up to 80°C to speed up the reaction and since azide ions are very nucleophilic, no other reagents were present in the reaction. The reaction is done under nitrogen and with DMF, having a boiling point of 153°C, which avoids the usage of reflux. Due to DMF's high boiling point, an evaporation difficulty occurred, but with the help of co-evaporation with toluene, the evaporation went better. Further on, partitioning with EtOAc:H_2O was enough to eliminate any excess of NaN_3 with the aqueous layer. The product azide **12** showed only small amounts of impurities and excess solvent peaks in ^{1}H-NMR as seen in Appendix A.

Triazole formation. An efficient reaction involving organic azides is copper-catalyzed azide-alkyne cycloaddition (CuAAC), also known as one of the classic Click reactions. This reaction between the azide **12** and 5-ethynyl-1,2,3-trifluorobenzene gave the 1,4-regioselective triazole compound **8**. DMF was again used as a solvent, but ACN worked just as well, having the advantage of simplifying solvent evaporation. An additional precautionary action regarding this reaction is to flush the flask with nitrogen. Forgetting to do so showed to oxidize the copper from Cu(I) to Cu(II) and the mixture turned from yellow/orange to green. Luckily, the reaction was already done before this oxidation occurred and products were still formed as shown in the TLC.

Once compound **8** was synthesized in both S- and R-enantiomeric forms, their purity and enantiomeric excess (ee) were investigated with chiral column chromatography. Both products were weighed carefully to the same mass and dissolved in 99% cyclohexane and 1% IPA. From there, equal amounts of each enantiomer were combined in a vial to create a racemic mixture. Shown in Figure 6 is believed, according to the AD-mix mechanism theory, to be the S-enantiomer with a retention time of t=11.0 and 98.6% ee as calculated from the data given in Table 1. The corresponding R-enantiomer is shown in Figure 7 with 98.9% ee and t=12.8. It is observed that in both cases, the undesired enantiomer respectively is found in very small amounts. To confirm if we have S- and R-enantiomers of the same compound, the racemic mixture was analyzed as well. As seen in Figure 8 with Rac-**8**, two peaks are observed where the retention times are only decimals different compared to the pure enantiomers. Therefore, given from the chromatograms obtained along with identical NMR and mass analysis, we have strong evidence that the two compounds analyzed are enantiomers of the same product. It also implies that the AD-mixes are excellent reagents to attain pure enantiomers of 1-phenylethanol derivative.

Table 1. Summarized data from chromatogram integration of compound **11** and calculated ee from Eq.1.

Compound	Enantiomer type	Retention time (min.)	Area%	Calculated ee
(S)-**8**	S	11.0	99.3	98.6%
	R	12.9	0.695	
(R)-**8**	S	11.1	0.552	98.9%
	R	12.8	99.4	
Rac-**8**	S	11.0	50.0	
	R	12.8	50.0	

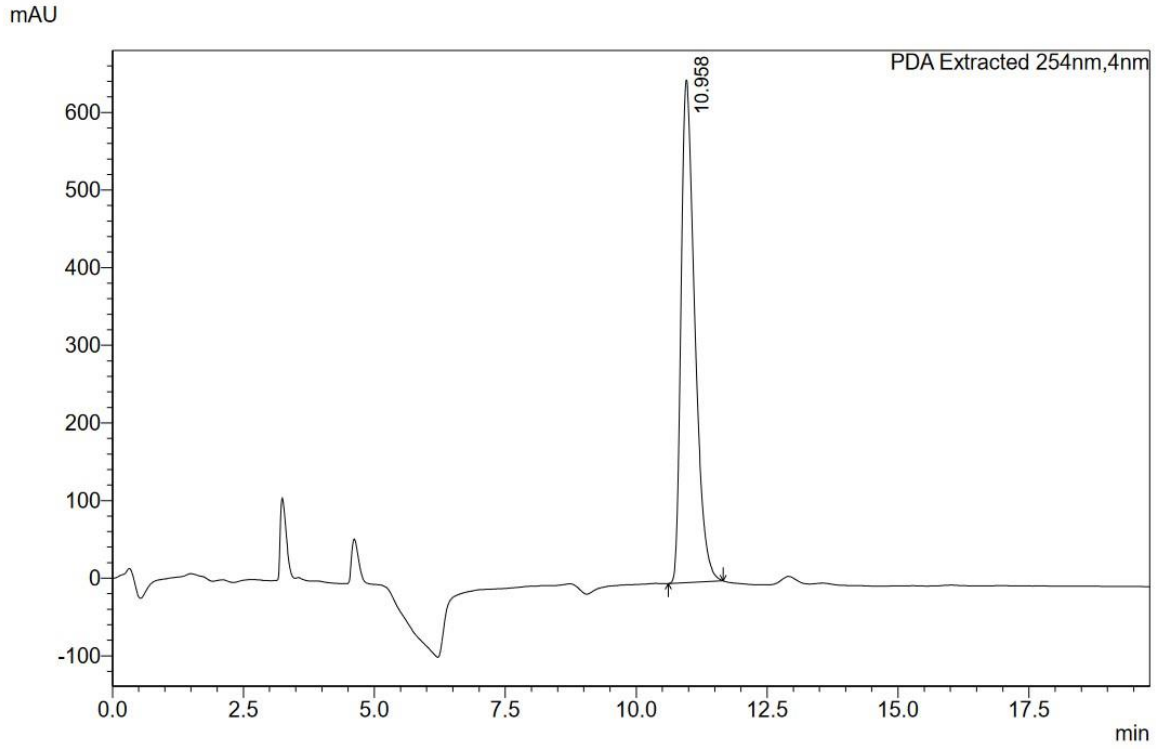


Figure 6. Chiral HPLC chromatogram of (S)-**8** where the intensity (mAU) is plotted against retention time (min.) at wavelength λ= 254 nm.

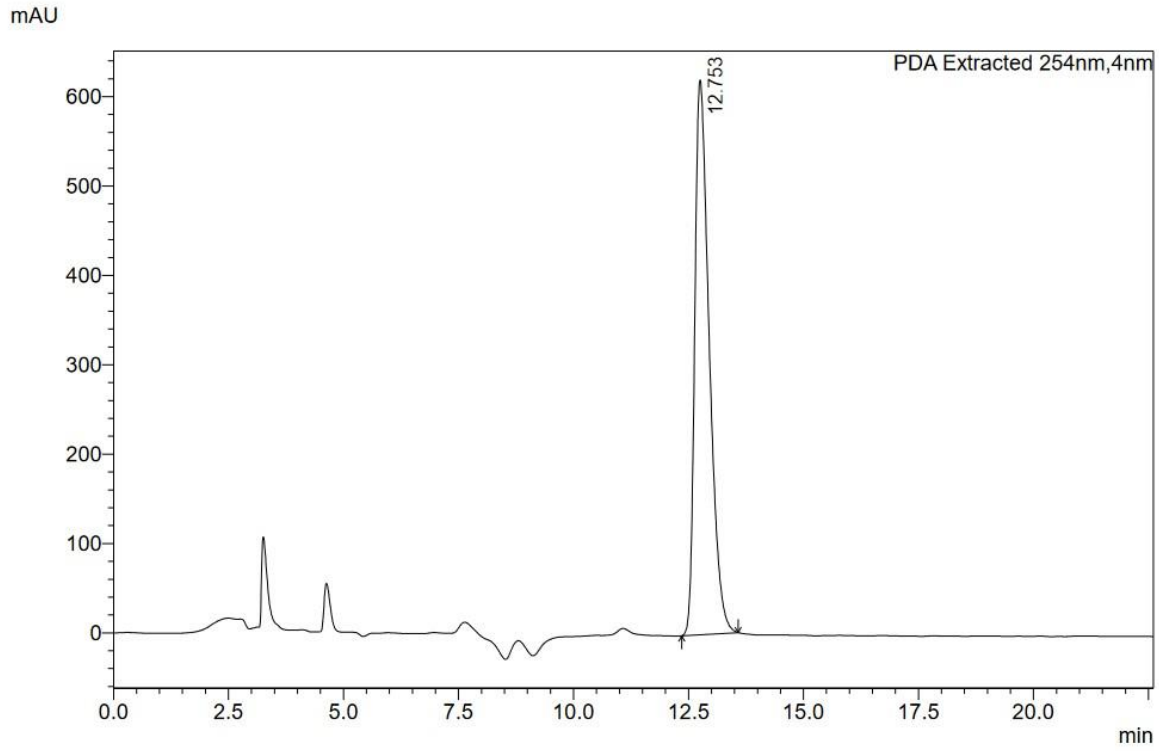


Figure 7. Chiral HPLC chromatogram of (R)-**8** where the intensity (mAU) is plotted against retention time (min.).

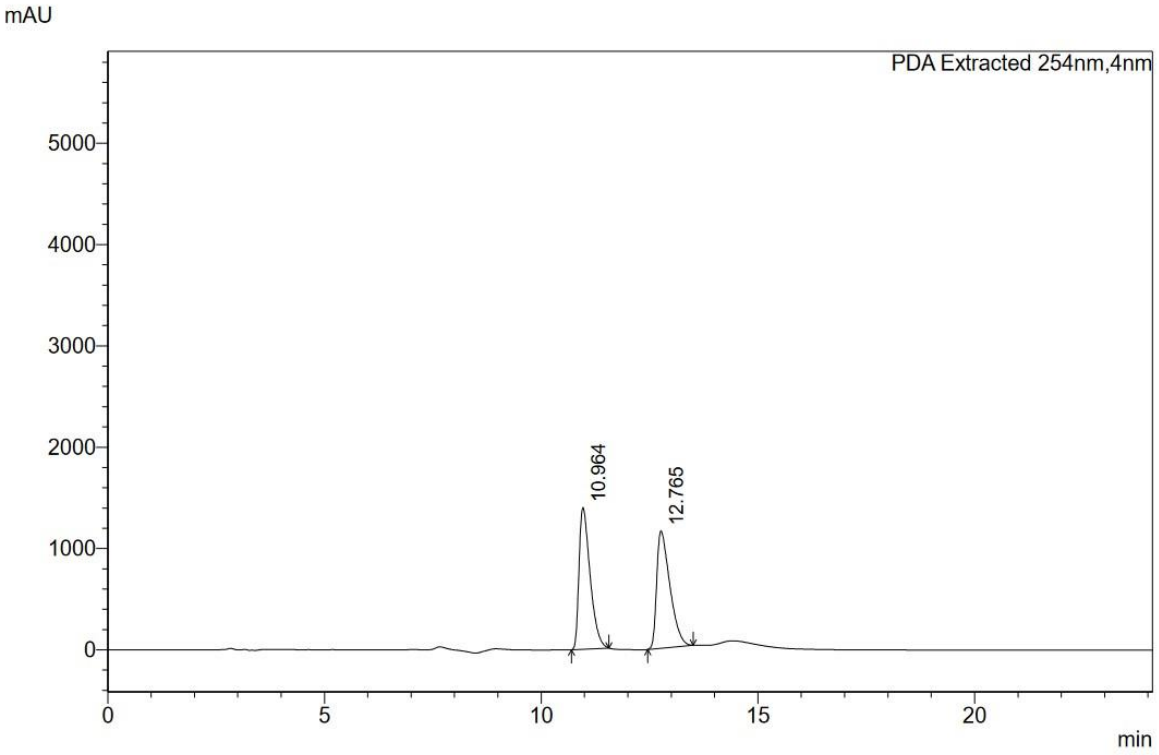
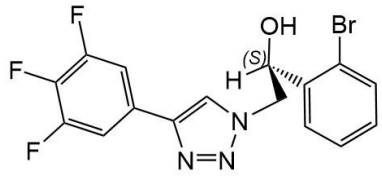
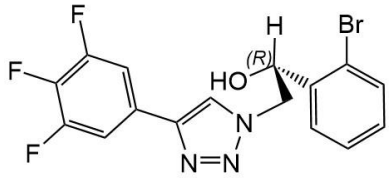
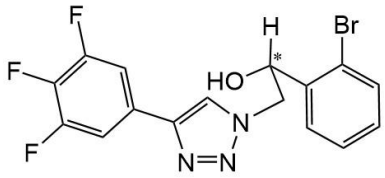


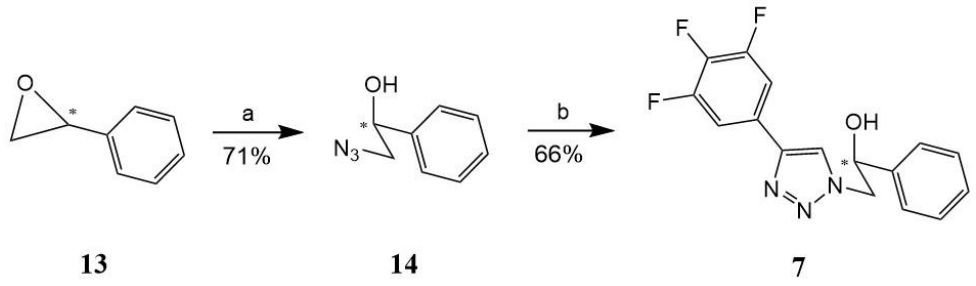
Figure 8. Chiral HPLC chromatogram of equal racemic mixture from (S)-**8** and (R)-**8** where the intensity (mAU) is plotted against retention time (min.).

Investigation of the protein-ligand binding with the enantiomeric final compounds of **8** were performed by fluorescence polarization measurements. The compounds were diluted in a series of concentrations with PBS-buffer and DMSO where the latter is used to assist the solubility issue of ligands. Although, too high concentration of DMSO could cause denaturing of the protein. In the experiments performed, it was observed repeatedly that even going to as low as 5% DMSO as final concentrations in the wells resulted in difficulties regarding ligand solubility. Thus, the measurements gave the similar K_D values as the probe which implies that no binding of the ligand was happening. In earlier experiments preceding this work it was shown that Rac-**8** bound with K_D=160 µM and hence, affinities in the same range were expected. A reason behind the worse affinities could be that the compounds were not properly pipetted and thus affecting the final well concentrations. A different way of preparing the concentration series was tried to solve the solubility issue. The new dilution method was to dilute the entire series in neat DMSO prior to diluting with PBS-buffer specifically resulting in 5% DMSO in all experiment wells. The solubility issue persisted in the dilutions with higher concentrations, but it was observed in the lower concentrations that the ligands solubilized as desired and inhibition results were obtained as showed in Table 2.

Table 2. Fluorescence polarization measurement results of 2-bromo-1-phenylethanol compounds showing affinity against galectin-3 expressed in K_D. A racemic reference from earlier studies, labelled as "Ref", is included for comparison.

Compound	K_D [µM]
(S)-**8** 	1700 ± 74
(R)-**8** 	1600 ± 280
Rac-**8**  Ref	1200 ± 210

As seen in Table 2, the values for (S)-**8** and (R)-**8** showed higher K_D values than it showed for the reference, Rac-**8**. Already mentioned was that the racemic mixture had a K_D value of 160 µM as it was tested at the time it was newly synthesized more than two years ago. It has been stored in the freezer since then and was thawed again to be tested together with the enantiomerically pure bromo derivates and it showed an almost 10-fold increase in K_D value. Also, it may seem that (R)-**8** binds better than the (S)-**8** due to its lower K_D value but looking at the standard error of the mean, they might as well have the same binding affinity. Probably, since **8** has several rotatable bonds, it can easily adopt to a conformation fitting the binding site of galectin-3, whether it be the S- or R-enantiomer. This concept has been studied earlier but with different molecules.[17] Due to the results obtained, an investigation was initiated and a sample of the old batch Rac-**8** was taken to run an LC/MS analysis which showed an extra peak compared to the analysis originally performed. It clearly indicates that a contamination has occurred that could have happened at any point and time during testing and synthesis. The ^{1}H-NMR spectrum for reference, Rac-**8**, was checked and very small amounts of impurities were found. The reason for the affinity values deviating could be based on the handling of FP analysis which covers human errors and material quality. Another reason for the deviation is the inaccuracy of the weighing scale which could have shown a lower mass than the actual one resulting in more compound molecules that could bind and thus generating a low K_D value or vice versa.

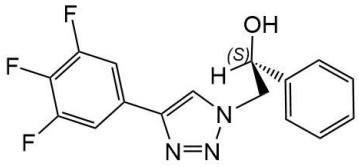
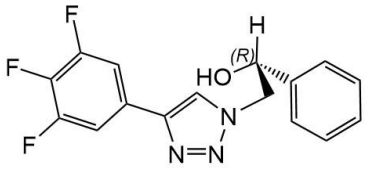
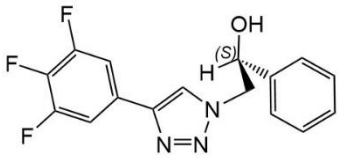


13 **14** **7**

Figure 9. Synthesis of 1-phenyl-2-(4-(3,4,5-trifluorophenyl)-1H-1,2,3-triazol-1-yl)ethanol. (a) (S)-styrene oxide or (R)-styrene oxide, MeOH, H_2O, NH_4Cl, NaN_3, rt, 16h. (b) 5-ethynyl-1,2,3-trifluorobenzene, CuI, Et_3N, ACN, rt, 24h.

Moving forward, it was decided to synthesize the enantiomers of compound **7** hoping for more clarification and promising results. Seen in Figure 9, the synthesis is started with commercially available enantiomers of styrene oxide, compound **13**. Under basic conditions, the strong nucleophilic azide ion attacks the least substituted part of the epoxide through S_N2 substitution and thus opening the ring to obtain compound **14**. From there, a CuAAC with the alkyne is once again performed to generate enantiomeric products of compound **7**. Observing the results in Table 3, the reference of (S)-**7** also showed higher affinity compared to when it was tested before displaying K_D=740 µM. Again, it could be because of the reasons mentioned above. Following the trend of the bromo derivatives, (R)-**7** also seem to bind better than (S)-**7** but then again, the difference is within the margin of error and thus not significant.

Table 3. Fluorescence polarization measurement results of 1-phenylethanol compounds showing affinity against galectin-3 expressed in K_D. Racemic **7** from earlier studies, labelled as "Ref", is included for comparison.

Compound	K_D [µM]
(S)-**7** 	2300 ± 1600
(R)-**7** 	1400 ± 990
(S)-**7**  Ref	2400 ± 460

Interestingly for both cases, the respective molecules' affinity lies around each other's K_D values and it is observed that addition of the bromo in the phenyl group possibly slightly increases the protein-ligand affinity. It is also important to point out that in this discussion, we only assume the compounds' enantiomeric configurations based on what is theoretically formed depending on the AD-mix used. If a thorough investigation is desired, to study which enantiomer has been proven to be obtained, then X-ray crystallography of the protein-ligand complex could be done.

2.2 C2 threitol derivatives

Molecular dynamic simulations of the threitol derivative **5** suggested that modifications at C2 or O2 could be tolerated while retaining the essential protein-ligand interactions mentioned earlier. By introducing an exo-methylene at C2, it seemed that the π orbitals of the double bond may engage in additional interactions with the aromatic ring of Trp 181.

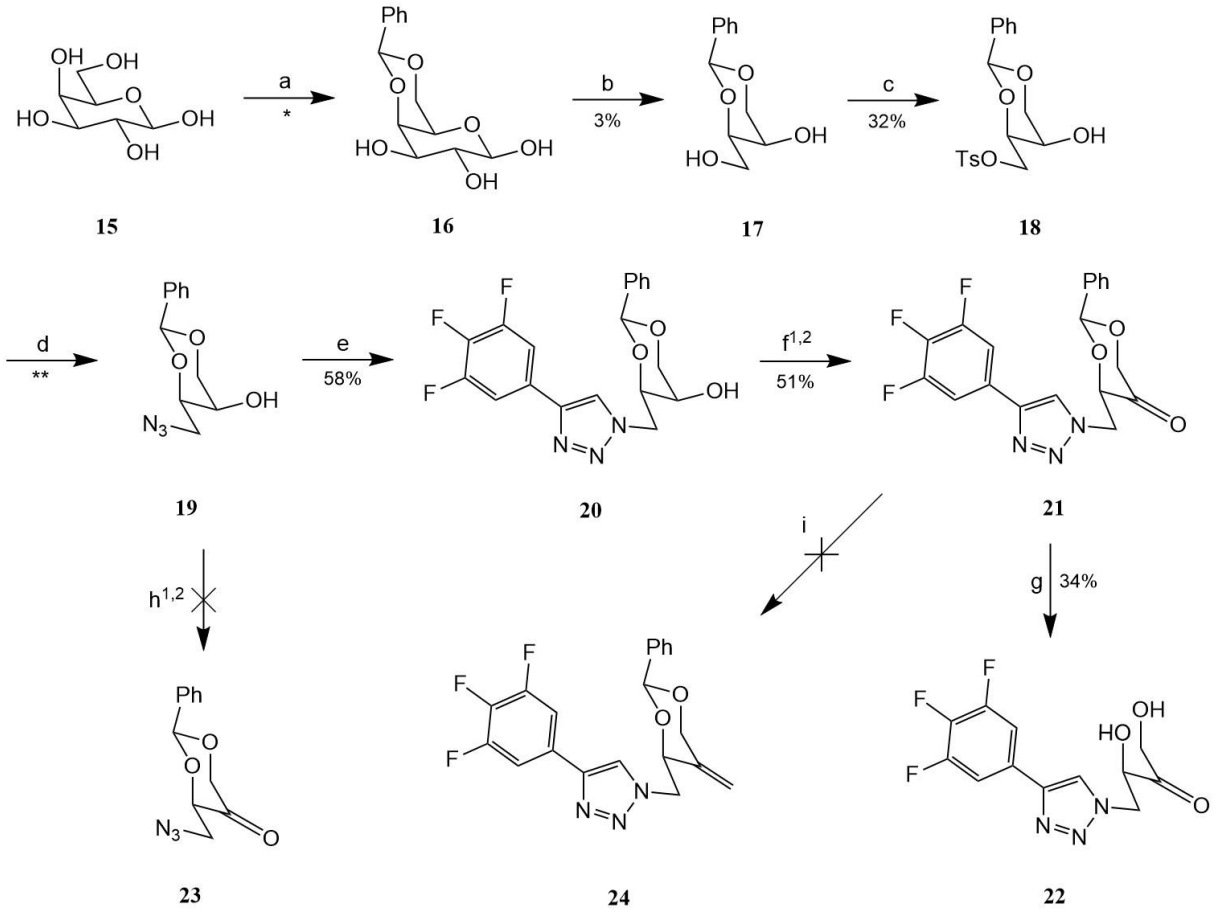


Figure 10. Synthesis steps for C2 threitol oxo- and exo-methylene derivatives. Steps with X on its arrow were unsuccessful reactions. *the yield was calculated over two steps **this step gave quantitative yield. (a) D-galactose, D-camphor-sulfonic acid, benzaldehyde dimethyl acetal, anhydrous DMF, 60°C, 250 mbar, 1.5h (b) $NaIO_4$, $NaHCO_3$, H_2O, dry THF, rt, 1h then $NaBH_4$, 0°C, 1h (c) Bu_2SnO, *p*-TsCl, Et_3N, dry DCM, under N_2, rt, 18h. (d) NaN_3, anhydrous DMF, under N_2, 80°C, 18h. (e) 5-ethynyl-1,2,3-trifluorobenzene, CuI, Et_3N, ACN, under N_2, rt, 20h. (f) Dess-Martine periodinane, dry DCM, rt, 6h then quenched with sat. $Na_2S_2O_3$, 16h. (g) $NaHSO_4$, MeOH, H_2O, 60°C, 3.5h (h^1,f^1) (COCl)2, dry DMSO, under argon, -78°C (acetone/dry ice), 5 min. then alcohol compound, 15 min. then Et_3N, 5 min. then warm up to rt. (h^2,f^2) Dess-Martine periodinane, dry DCM, rt, 6h then quenched with sat. $Na_2S_2O_3$, 16h. (i) Dimethyltitanocene, THF, 60°C, 18h.

Cleavage of the galactose ring. Pertaining to Figure 10, the synthesis is initiated with a benzylidene protection of D-galactose, to protect 4-OH and 6-OH which were essential for binding as mentioned, through acetalization to obtain **16**. Thereafter, an oxidative cleavage of the vicinal diols gave compound **17**. The bicarbonate subsequently hydrolyses the intermediate formate to an alcohol and reduction of the intermediate aldehyde to alcohol

occurs with the addition of $NaBH_4$ in the reaction. Tosylation on the primary alcohol is once again conducted to give **18**, followed by a further azidation obtaining compound **19**. CuAAC gave the corresponding triazole **20**.

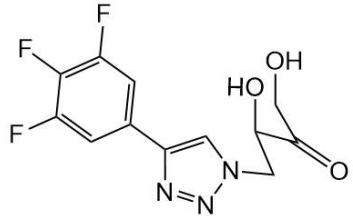
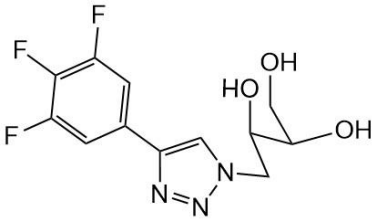
From there on, the synthesis path was unknown to the literature and the approaches towards **21**-**24** needed to be developed. Alcohol oxidation to a ketone could be envisioned before or after triazole formation, i.e. on **19** or **20** (step h or f). Oxidation of the azide **19** would allow for the formation of different triazoles, why this was initially tried with Swern and Dess-Martin conditions. These two oxidations were preferred to start with to avoid toxic reagents like chromium.

Oxidation of the azide compound. Starting with the first experiment of Swern oxidation, named as step h^1, the reactive and water-sensitive oxalyl chloride was used together with the driest DMSO available in the lab. An acetone/dry ice bath was prepared to lower the temperature to -78°C due to avoidance of possible side reactions as DMSO, acting as a nucleophile, attacks the electrophilic oxalyl chloride to form dimethylchlorosulfonium ion that would then attack the alcohol. The hydrogen directly attached to the carbon with the OH is then taken by the base, Et_3N, to form a ketone and dimethyl sulfide, which has a distinct smell and implies product conversion. Even though a small conversion has been seen in the TLC with vague spots, it also indicated that there was still a lot of the alcohol, compound **19**, left unreacted. Speculations were made that the DMSO used was not as dry as desired and, it was dried with 4Å molecular sieves to ensure a drier reagent. The experiment was performed again and the same results were obtained which could have been due to the oxalyl chloride being an old bottle resulting into possible contamination or decomposition through the years. Parallel to Swern, Dess-Martin oxidation, pertained to as step h^2, was also performed on the azide where periodinane is an excellent oxidizing agent due to the iodine having a high oxidation state. Compound **19**, acting as a nucleophile again, attacks the iodine to release an acetate anion. This anion also then acts as a base to do an E2 elimination to take the hydrogen bound to the OH-carbon and producing the ketone. However, it was also troublesome to get a sufficient conversion of the ketone due to vague product spot and issues with reappearing and disappearing spots which were possibly caused by the periodinane derivatives reacting with the silica in TLC.

Oxidation after triazole formation. Since products upon attempted oxidation of **19** could not be observed on TLC due to vague spots, it was decided to do the CuAAC on the azide first to enhance the UV indication aided by the additional phenyl group. Swern and Dess-Martin oxidation, referred to as steps f^1 and f^2 respectively, were tested alongside each other. With Swern, it still indicated much presence of the alcohol which is why excessive reagents amount were added to the reaction to push it towards generating products but it was not successful, instead byproducts were generated and no clear product was seen due to further vague spots after work-up. Meanwhile, Dess-Martin finally showed a promising reaction result where the potential product was clearly shown in TLC. The product spot was isolated with flash column chromatography and ^{1}H-NMR was taken to identify the product. Anticipated product peaks were recognized but the spectrum also indicated some impurities, ^{13}C-NMR was also recorded to confirm the presence of the ketone **21**. A ketone-carbon would show a peak at around 200 ppm and the product **21** showed a peak at 202 ppm, thus confirming the presence of a ketone functionality in **21**.

Olefination. As **21** was confirmed, the next step was to achieve the alkene, **24** from the ketone **21** through a Petasis olefination. Aided by heating, the Petasis reagent undergoes an α-elimination which generates the reactive intermediate $Cp_2Ti{=}CH_2$ and methane. The intermediate then reacts with the ketone to form a four membered intermediate which then releases the alkene and the oxygen binds to titanocene. TLC showed a possible product but some unreacted ketone **21** was also seen. Only a limited amount of the product **24**, together with some impurities, was obtained after doing a flash column chromatography. Thus, not enough **24** was obtained to test the alkene for galectin-3 affinity. It was then decided to finish project with a benzylidene acetal hydrolysis of compound **21**, which is the reversed reaction of step a, to give the ketone **22**.

Table 4. Fluorescence polarization measurement results of C2 threitol derivatives showing affinity against galectin-3 expressed in K_D where references from earlier studies are also tested and labelled as "Ref" for comparison.

Compound	K_D [μM]
22 	> 2000
5  Ref	320 ± 37

The K_D value of the reference threitol derivative **5** is compared with the ketone **22** in Table 4. The ketone **22** binds to galectin-3 significantly weaker, possibly due to the carbonyl oxygen repelling the electron-rich indole moiety of Trp 181 as seen in Figure 11. The alkene **24** would have been interesting to evaluate to investigate if the alkene π orbitals would engage in a more favorable interaction with the aromatic rings of Trp 181.

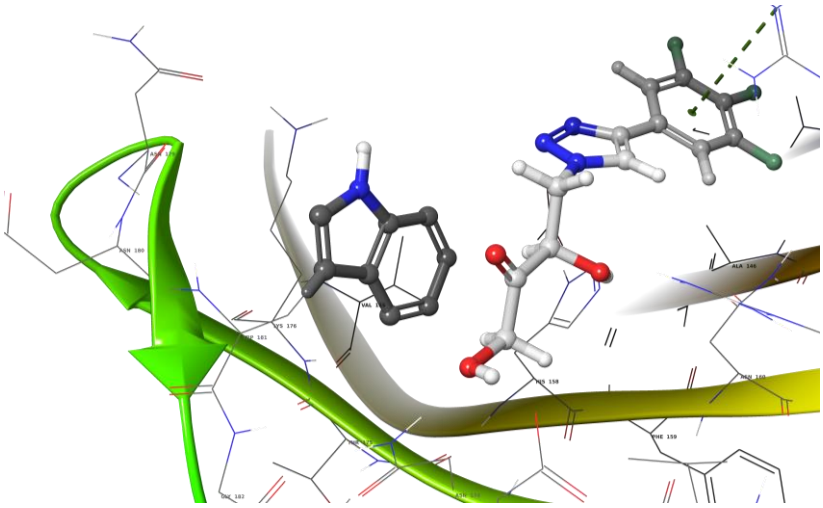


Figure 11. A snapshot from a molecular dynamic simulation illustrating the carbonyl oxygen of **22** near the Trp 181 side chain.

3. Conclusion

In regard to the 1-phenylethanol project, it may indicate that the R-enantiomers bound better than the S-enantiomers. However, the difference is not significant since the standard error of mean covered both affinity values. Also, it has been studied earlier that stereoisomeric hydroxyl groups can adapt its conformation to the binding site of galectin-3.[17] Thus, there is no assurance which enantiomer binds better than the other from this experiment. Furthermore, addition of the meta-bromo in the phenyl group had no significant affinity-enhancing effect compared to the 1-phenylethanol. It is also important to point out that the R- and S-configurations are only based on the theory about what AD-mixes generate for enantiomeric products. Determination of the absolute stereochemistry of a compound, X-ray crystallography on the protein-ligand complex should be performed.

For the C2 threitol project, no significant binding was observed for the ketone, compound **22**, which is possibly due to its repulsive interaction with Trp 181. The alkene, compound **24**, would have been perfect to test the hypothesis that the π orbitals of a double bond would interact with the aromatic ring of Trp 181.

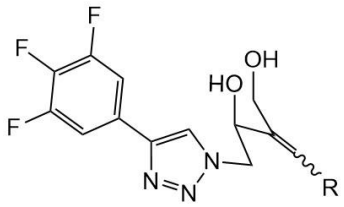
4. Future advancements

4.1 1-Phenylethanol derivative

- Substitution of bromo in compound **8** to other groups or atoms for affinity enhancement
- Position substitution of the halogen from ortho to meta or para to investigate affinity
- Addition of more halogens in the phenyl ring to hopefully increase the affinity and discover new interaction between the protein and the ligand
- To further investigate the chirality of the molecules, the optical rotation of the S- and R-enantiomers can be studied

4.2 C2 threitol derivatives

- Synthesize the alkene, compound **7**, through Wittig olefination
- If a product with E-configuration is desired, then a Horner-Wodsworth-Emmons (HWE) may be done from the ketone directly
- Introduction of alcohol to the alkene could be done through hydroboration
- Different R-groups can be attached onto the alkene or the homologated alcohol to test which group binds galectin-3 better and further develop the molecule from there on



5. References

[1] L. Johannes, R. Jacob, and H. Leffler, "Galectins at a glance," *J Cell Sci*, vol. 131, no. 9, May 2018, doi: 10.1242/jcs.208884.

[2] F.-T. Liu and G. A. Rabinovich, "Galectins: regulators of acute and chronic inflammation," *Ann N Y Acad Sci*, vol. 1183, no. 1, pp. 158–182, Jan. 2010, doi: 10.1111/j.1749-6632.2009.05131.x.

[3] M. Wang *et al.*, "Galectin-14 Promotes Trophoblast Migration and Invasion by Upregulating the Expression of MMP-9 and N-Cadherin," *Front Cell Dev Biol*, vol. 9, Mar. 2021, doi: 10.3389/fcell.2021.645658.

[4] J. Seetharaman, A. Kanigsberg, R. Slaaby, H. Leffler, S. H. Barondes, and J. M. Rini, "X-ray Crystal Structure of the Human Galectin-3 Carbohydrate Recognition Domain at 2.1-Å Resolution," *Journal of Biological Chemistry*, vol. 273, no. 21, pp. 13047–13052, May 1998, doi: 10.1074/jbc.273.21.13047.

[5] B. A. Salameh, I. Cumpstey, A. Sundin, H. Leffler, and U. J. Nilsson, "1H-1,2,3-Triazol-1-yl thiodigalactoside derivatives as high affinity galectin-3 inhibitors," *Bioorg Med Chem*, vol. 18, no. 14, pp. 5367–5378, Jul. 2010, doi: 10.1016/j.bmc.2010.05.040.

[6] K. Peterson *et al.*, "Systematic Tuning of Fluoro-galectin-3 Interactions Provides Thiodigalactoside Derivatives with Single-Digit nM Affinity and High Selectivity," *J Med Chem*, vol. 61, no. 3, pp. 1164–1175, Feb. 2018, doi: 10.1021/acs.jmedchem.7b01626.

[7] M. L. Verteramo, "Design, Synthesis & Thermodynamic Studies of Galectin Ligands," Lund University, Lund, 2019.

[8] A. Sahakjan, "Design and Synthesis of New Glycerol and Ethanol Scaffold derivatives as Galectin-1 and -3 Ligands," Lund University, 2019.

[9] A. M. Rossi and C. W. Taylor, "Analysis of protein-ligand interactions by fluorescence polarization," *Nat Protoc*, vol. 6, no. 3, pp. 365–387, Mar. 2011, doi: 10.1038/nprot.2011.305.

[10] P. Sörme, B. Kahl-Knutsson, M. Huflejt, U. J. Nilsson, and H. Leffler, "Fluorescence polarization as an analytical tool to evaluate galectin–ligand interactions," *Anal Biochem*, vol. 334, no. 1, pp. 36–47, Nov. 2004, doi: 10.1016/j.ab.2004.06.042.

[11] BMG Labtech, "Fluorescence polarization," 2023. https://www.bmglabtech.com/en/fluorescence-polarization/#7-page-nav, (2023) (accessed Jun. 01, 2023).

[12] M. M. Coelho, C. Fernandes, F. Remião, and M. E. Tiritan, "Enantioselectivity in Drug Pharmacokinetics and Toxicity: Pharmacological Relevance and Analytical Methods," *Molecules*, vol. 26, no. 11, p. 3113, May 2021, doi: 10.3390/molecules26113113.

[13] K. Robards, P. R. Haddad, and P. E. Jackson, "High-performance Liquid Chromatography—Separations," in *Principles and Practice of Modern*

Chromatographic Methods, Elsevier, 2004, pp. 305–380. doi: 10.1016/B978-0-08-057178-2.50009-1.

[14] Erik Mogard, “Synthesising 2-(4-(3,4,5-trifluorophenyl)-1H-1,2,3- triazol-1-yl)ethan-1-ol derivatives to inhibit galectin-1 and galectin-3,” Lund, 2020.

[15] M. Nkamino, “Design and synthesis of 1-phenyl ethanol derivatives as non-sugar galectin inhibitors,” Lund University, Lund, 2021.

[16] ScienceDirect, “Sharpless Asymmetric Dihydroxylation.” https://www.sciencedirect.com/topics/chemistry/sharpless-asymmetric-dihydroxylation (accessed Jun. 02, 2023).

[17] M. L. Verteramo *et al.*, “Interplay between Conformational Entropy and Solvation Entropy in Protein–Ligand Binding,” *J Am Chem Soc*, vol. 141, no. 5, pp. 2012–2026, Feb. 2019, doi: 10.1021/jacs.8b11099.

6. Experimental section

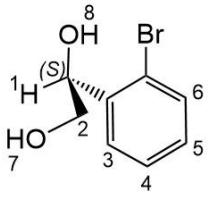
6.1 General equipment

Sigma Aldrich, Alfa Aesar or Apollo Scientific were the main suppliers of all the chemicals that has been used without further purification unless stated otherwise. Column chromatography was performed with silica gel (technical grade, 60Å. 230-400 mesh, 40-63 µM, Supelco. Meanwhile, all analytical TLC carried out was aided by silica gel 60 F 254 (Supelco) and visualization with UV-light (254 nm) and/or with cerium molybdate stain made up of ammonium molybdate, ceric ammonium molybdate and concentration sulfuric acid. Analyzed NMR spectra were recorded on a 400 MHz Bruker AVANCE II spectrometer where chemical shifts are given in ppm. Assignment of designated peaks were performed by using mainly ^{1}H, ^{1}H-COSY and ^{13}C, and while in some cases, HMQC and HMBC were used as additional support. LC/MS generating LRMS results was performed using an Agilent 1260 system on a C-18 (1.8µM, 2.1x50 mm) column with an H_2O: ACN gradient containing 0.1% formic acid. A Hewlett-Packard 1100 series DAD was used for UV-VIS detection and the mass spectra were recorded using an Agilent 6120 single quadrupole. Preparative HPLC was performed using an Agilent 1260 Infinity system with a H_2O:ACN gradient containing 0.1% formic acid on a C-18 (5 µm, 19x250 mm) column with the product collection determined by UV absorption at 254 nm. Chiral HPLC was performed using Shimadzu Prominence LC-2030C with gradient n-hexane: IPA using a CHIRALPAK IB SiO_2 column (5 µm, 4.6x250 mm).

6.2 Synthetic methods

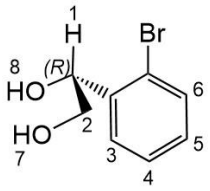
(S)-1-(2-Bromophenyl)ethane-1,2-diol ((S)-**10**): To a mixture of t-BuOH (14 mL) and H_2O (14 mL), AD-mix-α (3.69 g, 4.74 mmol, 1.8 eq.) was added then let to cool down to 0°C with an ice bath. Once cooled, addition of compound **9** (482 mg, 2.63 mmol) was done and the reaction mixture was let to react for 20h at 0°C. The reaction was then quenched with a sat. aq. solution of Na_2SO_3 (2.7 mL) and was let to stir for 40 minutes at room temperature. Thereafter, extraction was performed where the aqueous layer was washed with EtOAc (3x15 mL). After that, the combined organic layers were dried with $MgSO_4$, filtered and the solvent was removed in vacuo. Purification of the product was performed with flash column chromatography (stationary phase: SiO_2, mobile phase: 1:1 hep:EtOAc, wet packing, wet loading). The afforded product, (S)-**10** (512 mg, 2.36 mmol, 90%), turned out to be a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (dd, *J* = 7.8, 1.8 Hz, 1H, H-6), 7.53 (dd, *J* = 8.0, 1.2 Hz, 1H, H-3), 7.35 (td, *J* = 7.6, 1.2 Hz, 1H, H-5), 7.16 (td, *J* = 7.7, 1.8 Hz, 1H, H-4), 5.19 (dt, *J* = 7.8, 2.9 Hz, 1H, H-1), 3.91 (m, *J* = 10.1, 5.0 Hz, 1H, H-2a), 3.63 – 3.50 (m, 1H, H-2b), 2.75 (d, *J* = 3.3 Hz, 1H, H-8), 2.14 (t, 1H, H-7).



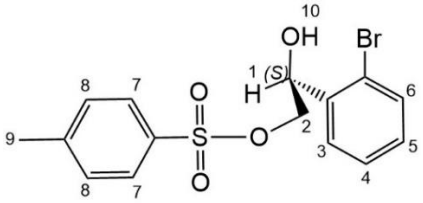
(R)-1-(2-Bromophenyl)ethane-1,2-diol ((R)-**10**): To a mixture of t-BuOH (14 mL) and H_2O (14 mL), AD-mix-β (3.83 g, 4.91 mmol, 1.8 eq.) as added then let to cool down to 0°C with an ice bath. Once cooled, addition of compound **9** (500 mg, 2.73 mmol) was done and the reaction mixture was let to react for 20h at 0°C. The reaction was then quenched with a sat. aq. solution of Na_2SO_3 (2.7 mL) and was let to stir for 40 minutes at room temperature. Thereafter, extraction was performed where the aqueous layer was washed with EtOAc (3x15 mL). After that, the combined organic layers were dried with $MgSO_4$, filtered and the solvent was removed in vacuo. Purification of the product was performed with flash column chromatography (stationary phase: SiO_2, mobile phase: 1:1 hep:EtOAc, wet packing, wet loading). The afforded product, (R)-10 (420 mg, 1.94 mmol, 71%), turned out to be a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (dd, *J* = 7.8, 1.7 Hz, 1H, H-6), 7.51 (dd, *J* = 8.0, 1.2 Hz, 1H, H-3), 7.33 (td, *J* = 7.5, 1.2 Hz, 1H, H-5), 7.14 (td, *J* = 7.7, 1.8 Hz, 1H, H-4), 5.18 (dt, *J* = 7.9, 3.0 Hz, 1H), 3.88 (m, *J* = 11.4, 6.4, 3.1 Hz, 1H, H-2a), 3.54 (m, *J* = 11.5, 7.8, 3.5 Hz, 1H, H-2b), 3.37 (d, *J* = 3.5 Hz, 1H, H-8), 2.81 (t, *J* = 6.0 Hz, 1H, H-7).



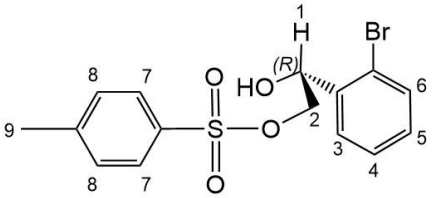
(S)-1-(2-Bromophenyl)-2-*O*-tosyl-ethane-1,2-diol ((S)-**11**): The (S)-**10** (251 mg, 1.16 mmol) was dissolved in THF (4.7 mL). Then Et_3N (237 mg, 2.34 mmol, 2.02 eq.), Bu_2SnO (14.3 mg, 0.0574 mmol, 0.0496 eq.) and *p*-TsCl (332 mg, 1.74 mmol, 1.5 eq.) were added to the reaction mixture to let stir for 18h at ambient temperature. An extraction was performed by addition of DCM (7 mL) and H_2O (7 mL) and the aqueous phase was washed with DCM (3x7 mL). Thereafter, the combined organic layers were dried with $MgSO_4$, filtered and the solvent was removed in vacuo. Purification of the product was performed with flash column chromatography (stationary phase: SiO_2, mobile phase: 4:1 hep:EtOAc, wet packing, wet loading). The product (S)-**11** (386 mg, 1.05 mmol, 91%) was obtained as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 – 7.76 (m, 2H, H-7), 7.60 – 7.54 (m, 1H, H-6), 7.48 (dd, *J* = 8.0, 1.2 Hz, 1H, H-3), 7.36 – 7.31 (m, 3H, H-5, H-8), 7.16 (td, *J* = 7.7, 1.8 Hz, 1H, H-4), 5.32 (d, *J* = 2.6 Hz, 1H, H-10), 5.31 – 5.28 (m, 1H, H-1), 4.28 (dd, *J* = 10.6, 2.6 Hz, 1H, H-2a), 3.97 (dd, *J* = 10.6, 8.3 Hz, 1H, H-2b), 2.45 (s, 3H, H-9).



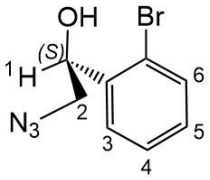
(R)-1-(2-Bromophenyl)-2-*O*-tosyl-ethane-1,2-diol ((R)-**11**): The (R)-**10** (250 mg, 1.15 mmol) was dissolved in THF (4.6 mL). Then Et_3N (233 mg, 2.30 mmol, 2.02 eq.), Bu_2SnO (14.2 mg, 0.0571 mmol, 0.0496 eq.) and *p*-TsCl (329 mg, 1.73 mmol, 1.5 eq.) were added to the reaction mixture to let stir for 18h at ambient temperature. An extraction was performed by addition of DCM (7 mL) and H_2O (7 mL) and the aqueous phase was washed with DCM (3x7 mL). Thereafter, the combined organic layers were dried with $MgSO_4$, filtered and the solvent was removed in vacuo. Purification of the product was performed with flash column chromatography (stationary phase: SiO_2, mobile phase: 4:1 hep:EtOAc, wet packing, wet loading). The product (R)-**11** (336 mg, 0.905 mmol, 79%) was obtained as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.75 (m, 2H, H-7), 7.57 (dd, *J* = 7.6, 1.9 Hz, 1H, H-6), 7.48 (dd, *J* = 8.0, 1.2 Hz, 1H, H-3), 7.38 – 7.31 (m, 3H, H-5, H-8), 7.16 (td, *J* = 7.7, 1.8 Hz, 1H, H-4), 5.31 (dd, *J* = 8.3, 2.6 Hz, 1H, H-1), 4.28 (dd, *J* = 10.6, 2.6 Hz, 1H, H-2a), 3.97 (dd, *J* = 10.6, 8.3 Hz, 1H, H-2b), 2.45 (s, 3H, H-9).



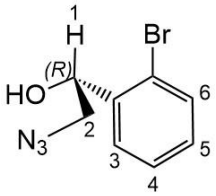
(S)-2-Azido-1-(2-bromophenyl)ethan-1-ol ((S)-**12**): Compound (S)-**11** (370 mg, 0.997 mmol) and NaN_3 (194 mg, 2.99 mmol, 3 eq.) were put together in a solution of anhydrous DMF (20 mL) under N_2. The reaction mixture was left to stir for 42h at 80°C. After completion, DMF was evaporated in vacuo aided by toluene. Purification of the product was performed with flash column chromatography (stationary phase: SiO_2, mobile phase: 2:1 hep:EtOAc, wet packing, wet loading). The product (R)-**12** (154 mg, 0.636 mmol, 64%) was afforded as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 7.8, 1.8 Hz, 1H, H-6), 7.53 (dd, *J* = 8.0, 1.2 Hz, 1H, H-3), 7.37 (td, *J* = 7.6, 1.2 Hz, 1H, H-5), 7.18 (td, *J* = 7.7, 1.8 Hz, 1H, H-4), 5.26 (dd, *J* = 8.2, 2.9 Hz, 1H, H-1), 3.58 (dd, *J* = 12.7, 2.9 Hz, 1H, H-2a), 3.35 (dd, *J* = 12.7, 8.2 Hz, 1H, H-2b).



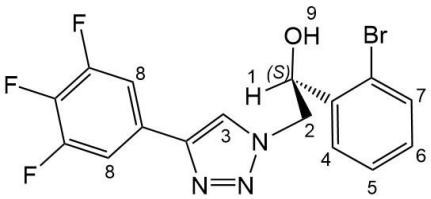
(R)-2-Azido-1-(2-bromophenyl)ethan-1-ol ((R)-**12**): Compound (R)-**11** (326 mg, 0.878 mmol) and NaN_3 (171 mg, 2.63 mmol, 3 eq.) were put together in a solution of anhydrous DMF (18 mL) under N_2. The reaction mixture was left to stir for 24h at 80°C. After completion, DMF was evaporated in vacuo aided by toluene. Purification of the product was performed with flash column chromatography (stationary phase: SiO_2, mobile phase: 2:1 hep:EtOAc, wet packing, wet loading). The product (R)-**12** (161 mg, 0.636 mmol, 76%) was afforded as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 7.8, 1.7 Hz, 1H, H-6), 7.53 (dd, *J* = 8.0, 1.2 Hz, 1H, H-3), 7.40 – 7.34 (td, 1H, H-5), 7.18 (td, *J* = 7.7, 1.8 Hz, 1H, H-4), 5.26 (dd, *J* = 8.2, 2.9 Hz, 1H, H-1), 3.58 (dd, *J* = 12.7, 2.9 Hz, 1H, H-2a), 3.35 (dd, *J* = 12.7, 8.2 Hz, 1H, H-2b).



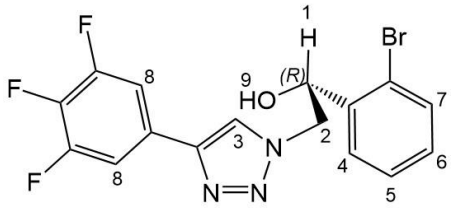
(S)-1-(2-Bromophenyl)-2-[4-(3,4,5-trifluorophenyl)-1*H*-1,2,3-triazol-1-yl]ethan-1-ol ((S)-**8**): Compound (S)-**12** (66.0 mg, 0.273 mmol) and CuI (519 mg, 2.73 mmol, 10 eq.) was solubilized in dry DMF (3 mL) under N_2. Addition of 5-ethynyl-1,2,3-trifluorobenzene (42.6 mg, 0.273 mmol, 1 eq.) and Et_3N (71.4 mg, 0.706 mmol, 2.59 eq.) were done and the reaction mixture was left to stir for 18h. After indication of completion through TLC, DMF was removed in vacuo repeatedly with toluene until all solvents were gone. Purification of the product was performed with flash column chromatography (stationary phase: SiO_2, mobile phase: 2:1 hep:EtOAc, wet packing, dry loading) which afford the product (S)-**8** (68.7 mg, 0.173 mmol, 57%) as white solid. HRMS calculated for [$C_{16}H_{11}BrF_3N_3O$] ($M+H^+$): 398.0116 m/z. Found: 398.0124 m/z.

^{1}H NMR (400 MHz, Acetone) δ 8.50 (s, 1H, H-3), 7.76 – 7.66 (m, 2H, H-8), 7.63 (dd, 1H, H-7), 7.60 (dd, *J* = 8.0, 1.2 Hz, 1H, H-4), 7.40 (td, *J* = 7.8, 1.3, 0.5 Hz, 1H, H-6), 7.25 (td, *J* = 7.7, 1.8 Hz, 1H, H-5), 5.51 – 5.49 (m, 1H, H-9), 5.46 (dd, *J* = 8.2, 3.0 Hz, 1H, H-1), 4.76 (dd, *J* = 14.0, 3.0 Hz, 1H, H-2a), 4.51 (dd, *J* = 14.0, 8.2 Hz, 1H, H-2b).



(R)-1-(2-Bromophenyl)-2-[4-(3,4,5-trifluorophenyl)-1*H*-1,2,3-triazol-1-yl]ethan-1-ol ((R)-**8**): Compound (R)-**12** (70.0 mg, 0.289 mmol) and CuI (551 mg, 2.89 mmol, 10 eq.) were solubilized in dry DMF (3 mL) under N_2. Addition of 5-ethynyl-1,2,3-trifluorobenzene (45.1 mg, 0.289 mmol, 1 eq.) and Et_3N (75.8 mg, 0.749 mmol, 2.59 eq.) were done and the reaction mixture was left to stir for 18h. After indication of completion through TLC, DMF was removed in vacuo repeatedly with toluene until all solvents were gone. Purification of the product was performed with flash column chromatography (stationary phase: SiO_2, mobile phase: 2:1 hep:EtOAc, wet packing, dry loading) which afford the product (R)-**8** (65.6 mg, 0.165 mmol, 57%) as white solid. HRMS calculated for [$C_{16}H_{11}BrF_3N_3O$] (M+H): 398.0116 m/z. Found: 398.0118 m/z.

^{1}H NMR (400 MHz, Acetone) δ 8.51 (s, 1H, H-3), 7.72 (dd, *J* = 9.2, 6.7 Hz, 2H, H-8), 7.64 (dd, *J* = 7.8, 1.8 Hz, 1H, H-7), 7.61 (dd, *J* = 8.0, 1.2 Hz, 1H, H-4), 7.40 (td, *J* = 7.5, 1.2 Hz, 1H, H-6), 7.26 (td, *J* = 7.7, 1.8 Hz, 1H, H-5), 5.54 (dd, *J* = 4.5, 0.9 Hz, 1H, H-9), 5.47 (dt, *J* = 8.0, 3.9 Hz, 1H, H-1), 4.76 (dd, 1H, H-2a), 4.52 (dd, *J* = 14.0, 8.1 Hz, 1H, H-2b).

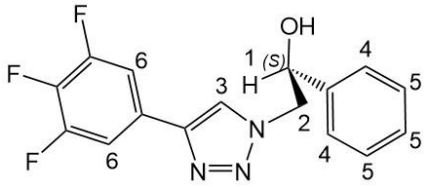


(S)-2-Azido-1-phenylethan-1-ol ((S)-**14**): Deionized water (0.4 mL) and methanol (3.2 mL) were mixed in a flask where NH_4Cl (106 mg, 1.98 mmol, 2 eq.) was added to let dissolve. Once dissolved, (S)-**13** (119 mg, 0.990 mmol) was added followed by NaN_3 (515 mg, 7.92 mmol, 8 eq) and the reaction mixture was left to stir at ambient temperature for 16h. Extraction was performed by addition of EtOAc (5 mL) and water (5 mL). The organic phase was washed with brine (3x6 mL) and dried with $MgSO_4$ and filtered. The solvent was then removed in vacuo and product purification was performed with flash column chromatography (stationary phase: SiO_2, mobile phase: 4:1 hep:EtOAc, wet packing, wet loading). To distinguish the product, the TLC plate was stained. The product (S)-**14** (115 mg, 0.705 mmol, 71%) was afforded as a yellow oil and was used directly in the next step.

(R)-2-Azido-1-phenylethan-1-ol ((R)-**14**)**:** Deionized water (0.4 mL) and methanol (3.2 mL) were mixed in a flask where NH_4Cl (107 mg, 2.00 mmol, 2 eq.) was added to let dissolve. Once dissolved, (R)-**13** (120 mg, 0.999 mmol) was added followed by NaN_3 (520 mg, 8.00 mmol, 8 eq) and the reaction mixture was left to stir at ambient temperature for 16h. Extraction was performed by addition of EtOAc (5 mL) and water (5 mL). The organic phase was washed with brine (3x6 mL) and dried with $MgSO_4$ and filtered. The solvent was then removed in vacuo and product purification was performed with flash column chromatography (stationary phase: SiO_2, mobile phase: 4:1 hep:EtOAc, wet packing, wet loading). To distinguish the product, TLC plate was stained. The product (R)-**14** (83.0 mg, 0.509 mmol, 51%) was afforded as a yellow oil and was used directly in the next step.

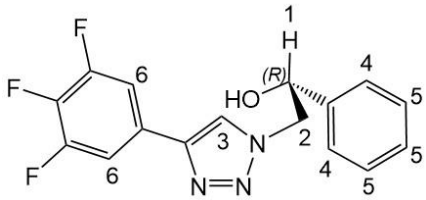
(S)-2-[4-(3,4,5-Trifluorophenyl)-1*H*-1,2,3-triazol-1-yl]-1-phenylethan-1-ol ((S)-**7**): In a flask, (S)-**14** (119 mg, 0.729 mmol) was solubilized with ACN (2.1 mL) together with 5-ethynyl-1,2,3-trifluorobenzene (120 mg, 0.766 mmol, 1.05 eq.). Thereafter, CuI (27.8 mg, 0.146 mmol, 0.2 eq.) and Et_3N (148 mg, 1.46 mmol, 2 eq.) were and the reaction mixture was left to stir at room temperature for 24h. Extraction was performed by addition of EtOAc (4 mL) and water (4 mL) where the organic phase was washed with brine (3x4 mL) and dried with $MgSO_4$ and filtered. Then, the solvent was removed in vacuo. Product purification was done with flash column chromatography (stationary phase: SiO_2, mobile phase: 2:1 hep:EtOAc, wet packing, wet loading). The product (S)-**7** (153 mg, 0.479 mmol, 66%) was obtained as a white solid. HRMS calculated for [$C_{16}H_{12}F_3N_3O$] (M+H): 320.1011 m/z. Found: 320.1017 m/z.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1H, H-3), 7.48 – 7.35 (m, 5H, H-5, H-6), 7.29 – 7.24 (m, 3H, H-4), 5.68 (dd, *J* = 8.1, 3.8 Hz, 1H, H-1), 4.63 (dd, *J* = 12.4, 8.1 Hz, 1H, H-2a), 4.24 (dd, *J* = 12.4, 3.8 Hz, 1H, H-2b).



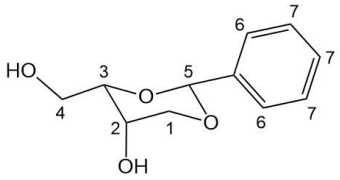
(R)-2-[4-(3,4,5-trifluorophenyl)-1*H*-1,2,3-triazol-1-yl]-1-phenylethan-1-ol ((R)-**7**): In a flask, (R)-**14** (83.0 mg, 0.509 mmol) was solubilized with ACN (1.50 mL) together with 5-ethynyl-1,2,3-trifluorobenzene (83.4 mg, 0.534 mmol, 1.05 eq.). Thereafter, CuI (19.4 mg, 0.102 mmol, 0.2 eq.) and Et_3N (103 mg, 1.02 mmol, 2 eq) were added and the reaction mixture was left to stir at room temperature for 24h. Extraction was performed by addition of EtOAc (4 mL) and water (4 mL) where the organic phase was washed with brine (3x4 mL) and dried with $MgSO_4$ and filtered. Then, the solvent was removed in vacuo. Product purification was done with flash column chromatography (stationary phase: SiO_2, mobile phase: 2:1 hep:EtOAc, wet packing, wet loading). The product (R)-**7** (53.0 mg, 0.166 mmol, 33%) was obtained as a white solid. HRMS calculated for [$C_{16}H_{12}F_3N_3O$] (M+H): 320.1011 m/z. Found: 320.1011 m/z.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1H, H-3), 7.48 – 7.37 (m, 5H, H-5, H-6), 7.32 – 7.20 (m, 2H, H-4), 5.68 (dd, *J* = 8.1, 3.8 Hz, 1H, H-1), 4.63 (dd, *J* = 12.4, 8.1 Hz, 1H, H-2a), 4.24 (dd, *J* = 12.4, 3.8 Hz, 1H, H-2b).



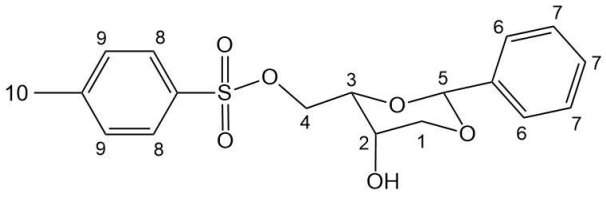
1,3-*O*-Benzylidene-D-threitol (**17**): To a solution of D-galactose (20 g, 111 mmol) in anhydrous DMF (200 mL), D-camphor-sulfonic acid (2.58 g, 11.1 mmol, 0.1 eq.) was added followed by benzaldehyde dimethyl acetal (20 mL, 133 mmol, 1.2 eq.). The reaction mixture was put in the rotary evaporator to warm up to 60°C and reduced pressure of 250 mbar for 1.5h. The white solution turned into a clear solution which indicated reaction completion. Thereafter, DMF was removed in vacuo aided by toluene and no purification step was done since it was decided to use the crude product for the next step. In the flask with crude compound **16** (10 g), $NaIO_4$ (16.3 g, 76.4 mmol, 2.05 eq.), $NaHCO_3$ (8.39 g, 99.9 mmol, 2.68 eq.), H_2O (267 mL) and dry THF (100 mL) were added to let stir for 1h at room temperature. The mixture was then cooled down to 0°C with an ice bath. Then, $NaBH_4$ (2.89 g, 76.4 mol, 2.05 eq.) in small amounts was added and was let to stir for another 1h. The built-up precipitation was filtered off and the filtrate was extracted with EtOAc (2x100 mL). The extract was washed with sat. aq. $Na_2S_2O_3$ (100 mL) followed by sat. aq. solution of $NaHCO_3$ (100 mL). The solvents were concentrated in vacuo and purification through flash column chromatography (stationary phase: SiO_2, mobile phase: 100% EtOAc, wet packing, dry loading) to obtain a yellow solid of **17** (0.628 g, 2.99 mmol, 2.7% over two steps). An easier identification of the product was aided by staining the TLC plate.

^{1}H NMR (400 MHz, MeOD) δ 7.57 – 7.48 (m, 2H, H-6), 7.39 – 7.25 (m, 3H, H-7), 5.59 (s, 1H, H-5), 4.11 (m, *J* = 1.4 Hz, 2H, H-1), 3.99 (ddd, *J* = 6.8, 5.4, 1.6 Hz, 1H, H-3), 3.79 – 3.66 (m, 2H, H-4), 3.60 (q, *J* = 1.7 Hz, 1H, H-2).



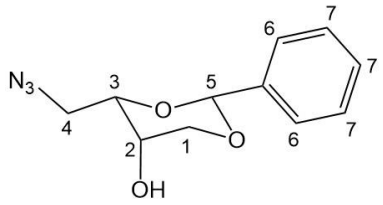
1,3-*O*-Benzylidene-4-*O*-tosyl-D-threitol (**18**): To a solution of **17** (628 mg, 2.99 mmol) in dry DCM (15 mL), Bu_2SnO (149 mg, 0.597 mmol, 0.2 eq.) was added together with Et_3N (308 mg, 3.05 mmol, 1.02 eq) and the mixture was put under N_2. Additionally, *p*-TsCl (854 mg, 4.48 mmol, 1.5 eq.) was dissolved in dry DCM to be pipetted into the flask and was let stirring at room temperature for 18h. Reaction completion was indicated by TLC and the crude was concentrated in vacuo. Purification through flash column chromatography (stationary phase: SiO_2, mobile phase: 1:1 hep:EtOAc, wet packing, wet loading) was performed and the product was stained with ceric ammonium molybdate solution. The reaction gave **18** (353 mg, 0.969 mmol, 32%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 – 7.77 (m, 2H, H-8), 7.45 – 7.39 (m, 2H, H-6), 7.39 – 7.34 (m, 3H, H-7), 7.34 – 7.29 (m, 2H, H-9), 5.55 (s, 1H, H-5), 4.27 – 4.16 (m, 4H, H-1a, H-3, H-4), 4.08 (dd, *J* = 12.1, 1.4 Hz, 1H, H-1b), 3.63 (d, *J* = 1.4 Hz, 1H, H-2), 2.44 (s, 3H, H-10).



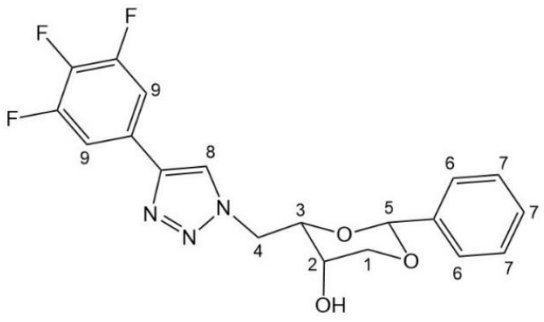
4-Azido-1,3-*O*-benzylidene-4-deoxy-D-threitol (**19**): Compound **18** (345 mg, 0.948 mmol) was mixed together with NaN_3 (185 mg, 2.84 mmol, 3 eq.) in a flask to be put under N_2 with anhydrous DMF (20 mL). The reaction was left to stir for 18h at 80°C. TLC with eluent system 2:1 hep:EtOAc indicated product conversion as it was stained with ceric ammonium molybdate solution and DMF was then evaporated together with toluene. As it was observed that there were azide salts in the reaction flask, extraction was performed by addition of H_2O (20 mL) and EtOAc (20 mL) where the organic phase was washed with H_2O (2x20 mL) as a purification step. It was then dried with Na_2SO_4 and filtered to remove excess water and the solution was concentrated. H-NMR implied that the obtained product does not need to be purified further. The product **19** (quantitative yield) was a grey solid which indicates impurities and was used directly to the next step.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 – 7.47 (m, 2H, H-6), 7.44 – 7.34 (m, 3H, H-7), 5.64 (s, 1H, H-5), 4.26 (dd, *J* = 12.0, 1.9 Hz, 1H, H-4a), 4.15 – 4.06 (m, 2H, H-1a, H-3), 3.67 (dd, *J* = 12.9, 7.8 Hz, 1H, H-4b), 3.59 (q, *J* = 1.6 Hz, 1H, H-2), 3.39 (dd, *J* = 12.9, 5.1 Hz, 1H, H-1b).



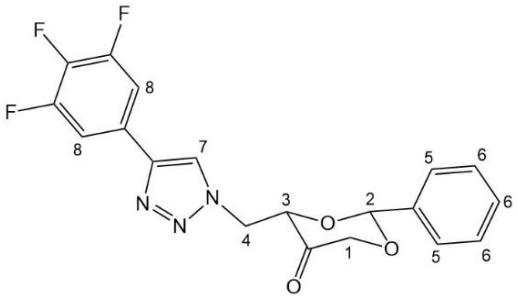
1,3-*O*-Benzylidene-4-deoxy-4-[4-(3,4,5-trifluorophenyl-1*H*-1,2,3,-triazol-1-yl]-D-threitol (**20**): In the flask under N_2 with **19** (749 mg, 3.18 mmol), 5-ethynyl-1,2,3-trifluorobenzene (522 mg, 3.34 mmol, 1.05 eq.) was added and both were solubilized with ACN (9.3 mL). Thereafter, CuI (121 mg, 0.637 mmol, 0.2 eq) and Et_3N (644 mg, 6.37 mmol, 2 eq.) were added and the reaction was let to react at room temperature for 20h. As conversion was indicated by TLC, extraction was performed by addition of EtOAc (20 mL) and water (20 mL) where the organic phase was washed with H_2O (1x20 mL) then brine (1x20 mL) and dried with $MgSO_4$ then filtered. Then, the solvent was removed in vacuo. For further purification step, flash column chromatography (stationary phase: SiO_2, mobile phase: 2:1 hep:EtOAc, wet packing, wet loading) was done. The purified product gave **20** (724 mg, 1.85 mmol, 58%) as a white fluffy solid. LRMS (ESI + mode) calculated for [$C_{19}H_{16}F_3N_3O_3$] ($M+H^+$): 392.11 m/z. Found: 392.1 m/z.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H, H-8), 7.47 – 7.37 (m, 7H, H-6, H-7, H-9), 5.57 (s, 1H, H-5), 4.74 (dd, *J* = 14.2, 5.2 Hz, 1H, H-4a), 4.65 (dd, *J* = 14.2, 7.9 Hz, 1H, H-4b), 4.44 (ddd, *J* = 8.0, 5.2, 1.3 Hz, 1H, H-3), 4.28 (dd, *J* = 12.2, 1.9 Hz, 1H, H-1a), 4.11 (dd, *J* = 12.2, 1.3 Hz, 1H, 1b), 3.58 (d, *J* = 1.8 Hz, 1H, H-2).



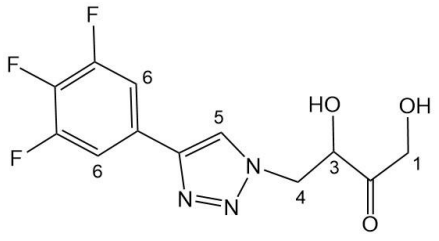
1,3-*O*-Benzylidene-4-[4-(3,4,5-trifluorophenyl)-1*H*-1,2,3-triazol-1-yl]-D-butan-1-one (**21**): To a solution of **20** (500 mg, 1.28 mmol) in DCM (10 mL), Dess-Martin periodinane (5.42 g, 1.28 mmol, 10 eq.) was added and the solution was stirred for 6h at room temperature. In accordance with the TLC result, the reaction was quenched with sat. aq. $Na_2S_2O_3$ (10 mL) to neutralize the iodine and it was left to stir 16h. The mixture was then further diluted with DCM (2x6 mL) to be able to perform an extraction with H_2O (2x6 mL). The combined organic layers were then dried with Na_2SO_4 and filtered to remove excess water and the solvents were evaporated in vacuo. For purification purposes, flash column chromatography (stationary phase: SiO_2, mobile phase: 1:1 hep:EtOAc, wet packing, wet loading) was done to obtain **21** (0.253 g, 0.000650 mol, 51%) as a white solid. LRMS (ESI + mode) calculated for $[C_{19}H_{16}F_3N_3O_3]$ $(M+H_2O+H^+)$: 408.11 m/z. Found: 408.1 m/z.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H, H-7), 7.47 – 7.38 (m, 7H, H-5, H-6, H-8), 6.01 (s, 1H, H-2), 5.09 (dd, *J* = 14.5, 3.6 Hz, 1H, H-4a), 4.96 (dd, *J* = 6.9, 3.6 Hz, 1H, H-4b), 4.75 (dd, *J* = 14.5, 6.9 Hz, 1H, H-3), 4.50 (s, 2H, H-1).

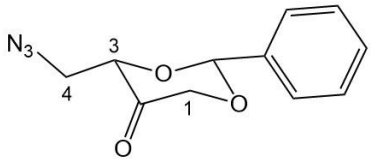


4-[4-(3,4,5-triflurophenyl)-1*H*-1,2,3-triazol-1-yl]-1,3-dihydroxy-D-butan-1-one (**22**): Methanol (1 mL) was added to a flask of **21** (10 mg, 0.0257 mmol) where NaHSO4 (9.25 mg, 0.0771 mmol, 3 eq.) was also added. The reaction was stirred for 3.5h at room temperature. Then, H2O (0.4 mL) was added and the reaction went on for another 15 min. TLC was performed with 1:2 hep:EtOAc which indicated product formation. The solvents were evaporated and crude was dissolved in milliQ water and ACN for HPLC preparation. A white fluffy solid was obtained after running HPLC and freeze drying which afforded **22** (2.60 mg, 0.00863 mmol, 34%). HRMS calculated for $[C_{12}H_{10}F_3N_3O_3]$ $(M+H^+)$: 302.0753 m/z. Found: 302.0745 m/z.

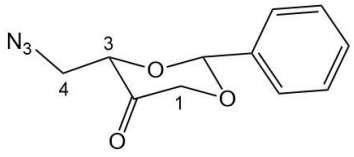
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H, H-5), 7.42 (dd, *J* = 8.3, 6.4 Hz, 2H, H-6), 4.91 (dd, *J* = 13.7, 4.5 Hz, 1H, H-4a), 4.85 – 4.81 (m, 1H, H-3), 4.78 (dd, *J* = 13.7, 3.3 Hz, 1H, H-4b), 4.68 (d, *J* = 19.7 Hz, 1H, H-1), 4.59 (d, *J* = 19.5 Hz, 1H, H-1).



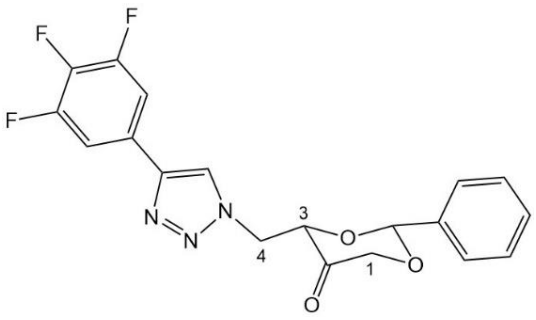
4-Azido-1,3-*O*-benzylidene-4-deoxy-D-butan-1-one (**23-h[1]**): An acetone/dry ice bath was prepared to decrease the temperature to -78°C. The reaction flask with only DCM (3 mL) under argon was put in the cold bath, then $(COCl)_2$ (0.017 mL, 0.196 mmol, 2.2 eq) and dry DMSO (33.4 mg, 0.428 mmol, 4.8 eq.) were added in the flask to react for 5 min. Then, compound **19** (21.0 mg, 0.0893 mmol) dissolved in DCM (2 mL)was added and it was let to react for another 15 min. Thereafter, Et_3N (606 mg, 5.99 mmol, 67 eq.) was added to be stirred for addition in 5 min in the cold bath and then it was let to warm up to room temperature. Possible products were detected with TLC. Extraction was done with DCM (2x6 mL) and water (2x6 mL) where the combined organic layers were washed with brine and then dried with $MgSO_4$ and filtered. The solvent was evaporated and TLC showed very vague spots leading to undistinguishable product. No yield was obtained.



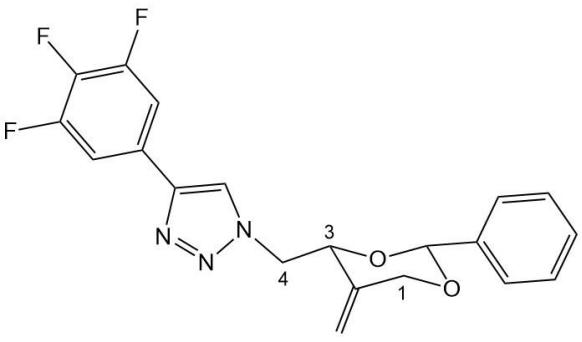
4-Azido-1,3-*O*-benzylidene-4-deoxy-D-butan-1-one (**23-h[2]**): To a solution of compound **19** (20.0 mg, 0.0850 mmol) in DCM (0.3 mL), Dess-Martin periodinane (114 mg, 0.268 mmol, 3.15 eq.) was added and the reaction mixture was stirred for 6h at room temperature. Thereafter, sat. aq. $Na_2S_2O_3$ was added to neutralize the iodine and it was let to stir 16h. Then, Et_2O (5 mL) was added to dilute the crude and H_2O (2x5 mL) was used to wash the organic layer. The combined organic layers were then dried with Na_2SO_4 and the precipitation was filtered off. Issues regarding vague possible product spot and another spot appearing and disappearing in TLC lead to the decision of aborting this experiment. Thus, no yield was obtained.



1,3-*O*-Benzylidene-4-deoxy-4-[4-(3,4,5-trifluorophenyl)-1*H*-1,2,3-triazol-1-yl]-D-butan-1-one (**21-f**[1]): An acetone/dry ice bath was prepared to decrease the temperature to -78°C. The reaction flask with only DCM (3 mL) under argon was put in the cold bath, then $(COCl)_2$ (0.017 mL, 0.196 mmol, 2.2 eq) and dry DMSO (33.4 mg, 0.428 mmol, 4.8 eq.) was added in the flask to react for 5 min. Then, compound **19** (20.0 mg, 0.0511 mmol) dissolved in DCM (2 mL) was added and it was let to react for another 15 min. Thereafter, Et_3N (606 mg, 5.99 mmol, 67 eq.) was added to be stirred for addition in 5 min in the cold bath and then it was let to warm up to room temperature. TLC indicated a lot of **19** which is why excessive amounts of the reagents were added, specifically $(COCl)_2$ (+5.5 eq), dry DMSO (+4.8 eq) and Et_3N (+33 eq). TLC indicated no existence of **19** therefore, extraction with DCM (2x6 mL)and water (2x6 mL) was done. TLC was checked again after work-up but possible products displayed very vague spots. Conclusively, no yield was obtained.

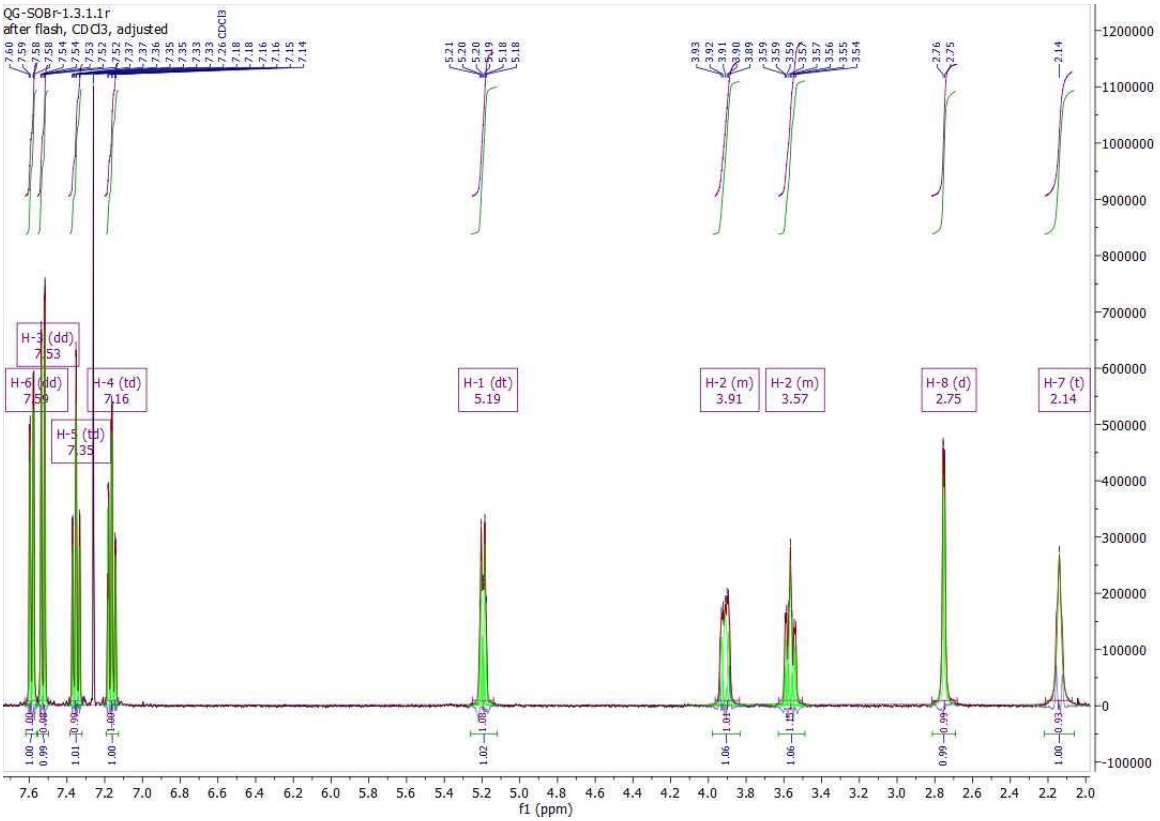


1,3-*O*-Benzylidene-2,4-dideoxy-4-[4-(3,4,5-trifluorophenyl)-1*H*-1,2,3-triazol-1-yl]-2-methylene-D-threitol (**24**): Dimethyltitanocene (24.1 mg, 0.116 mmol, 3 eq.) and compound **21** (15.0 mg, 0.0385 mol) were dissolved in THF (2 mL) and the reaction mixture was let to warm up to 60°C for 18h. Once full product conversion was indicated by TLC, petroleum ether was added to filtrate off the built-up precipitation. Then the solvent was evaporated and purification was done through flash column chromatography with (stationary phase: SiO_2, mobile phase: 1:1 hep:EtOAc, wet packing, wet loading) to isolate the product. Unfortunately, not enough product was afforded and ^{1}H-NMR implied impurities. Thus, no yield was obtained.

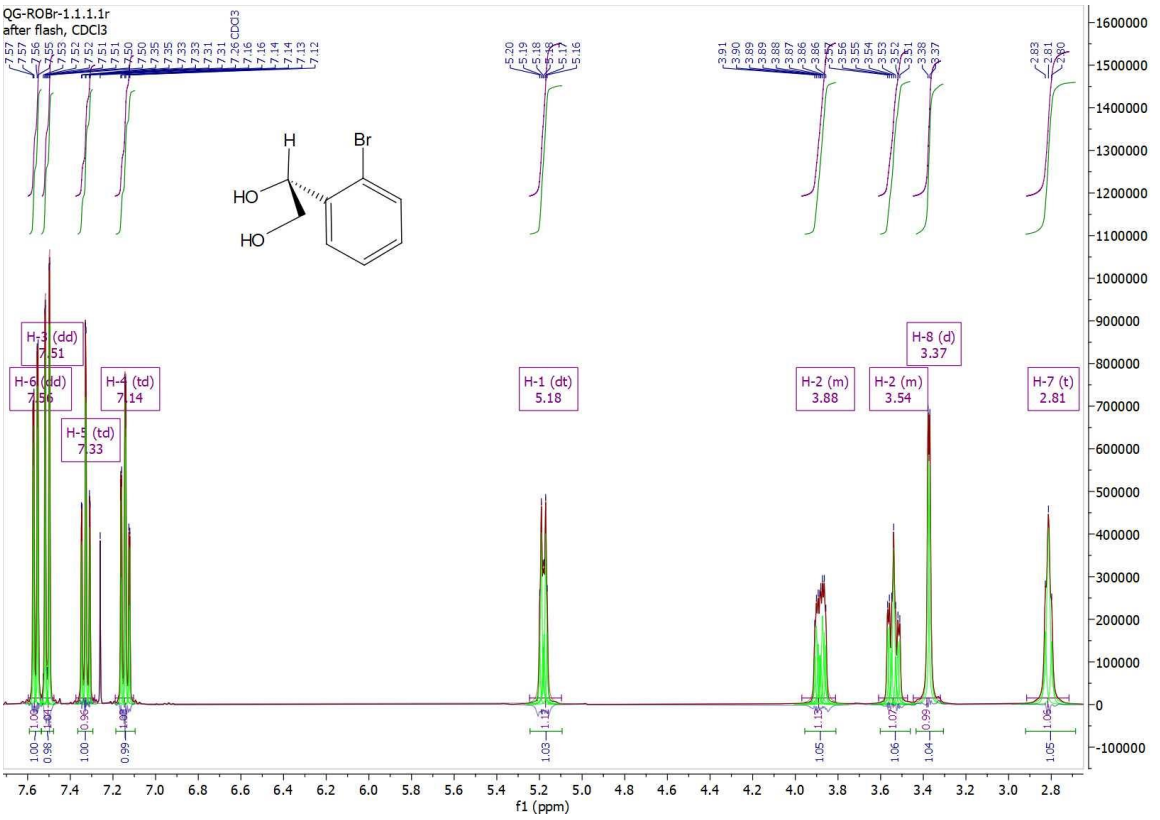


Appendix A: ^{1}H-NMR spectra

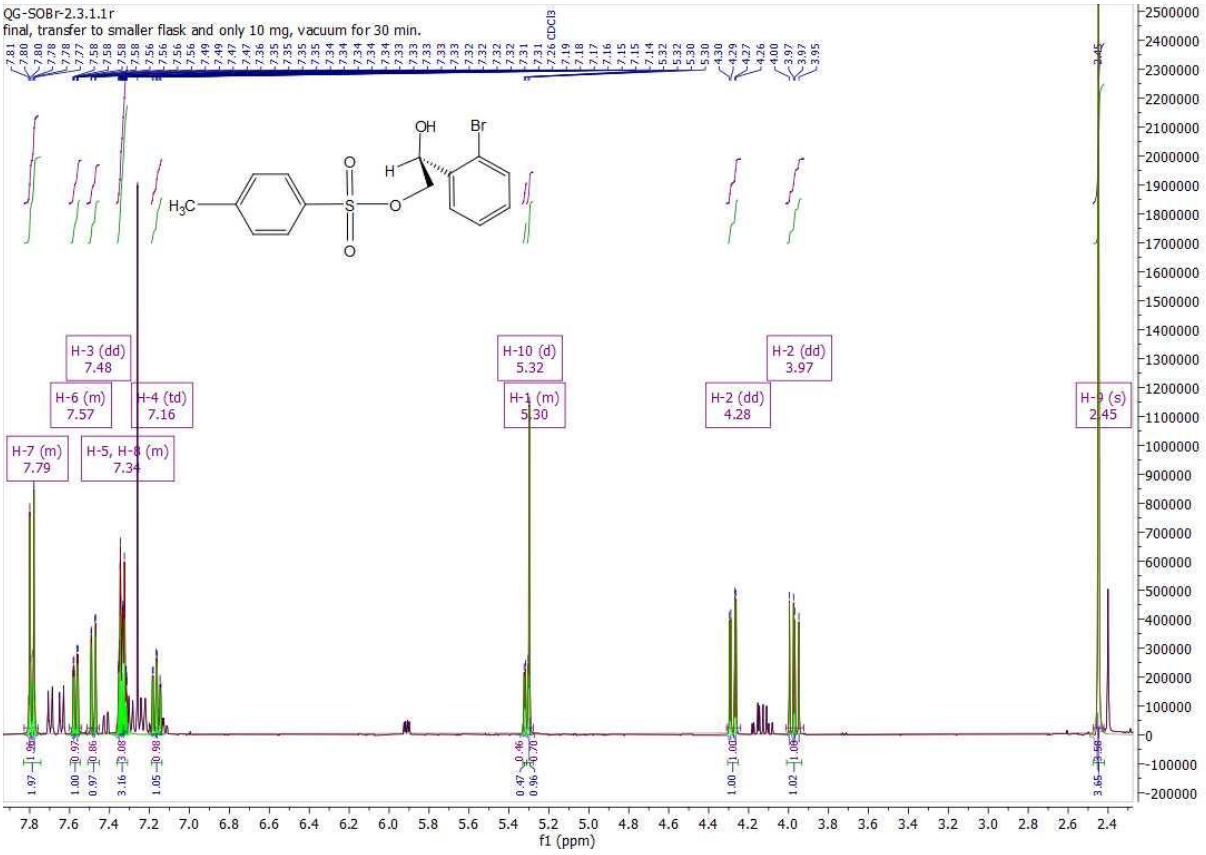
Compound (S)-**10**: QG-SOBr-1



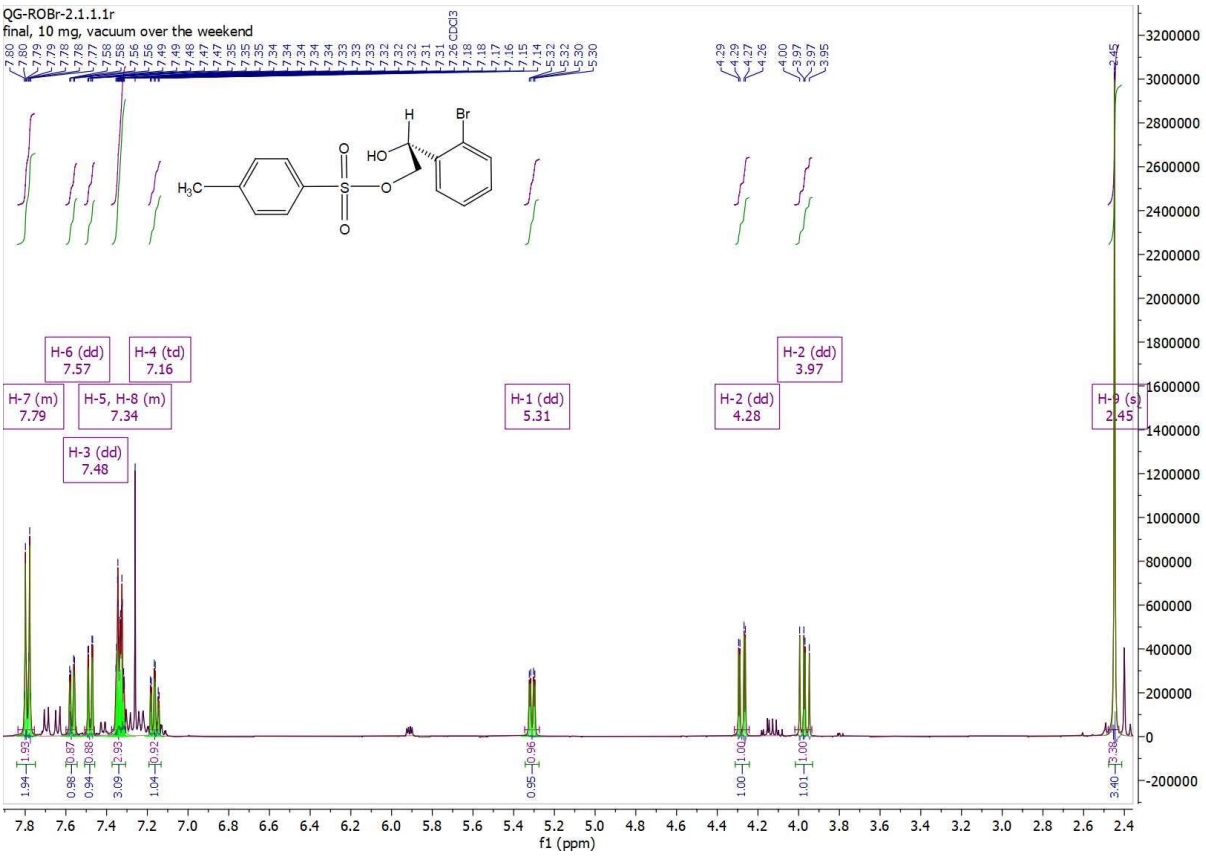
Compound (R)-**10**: QG-ROBr-1



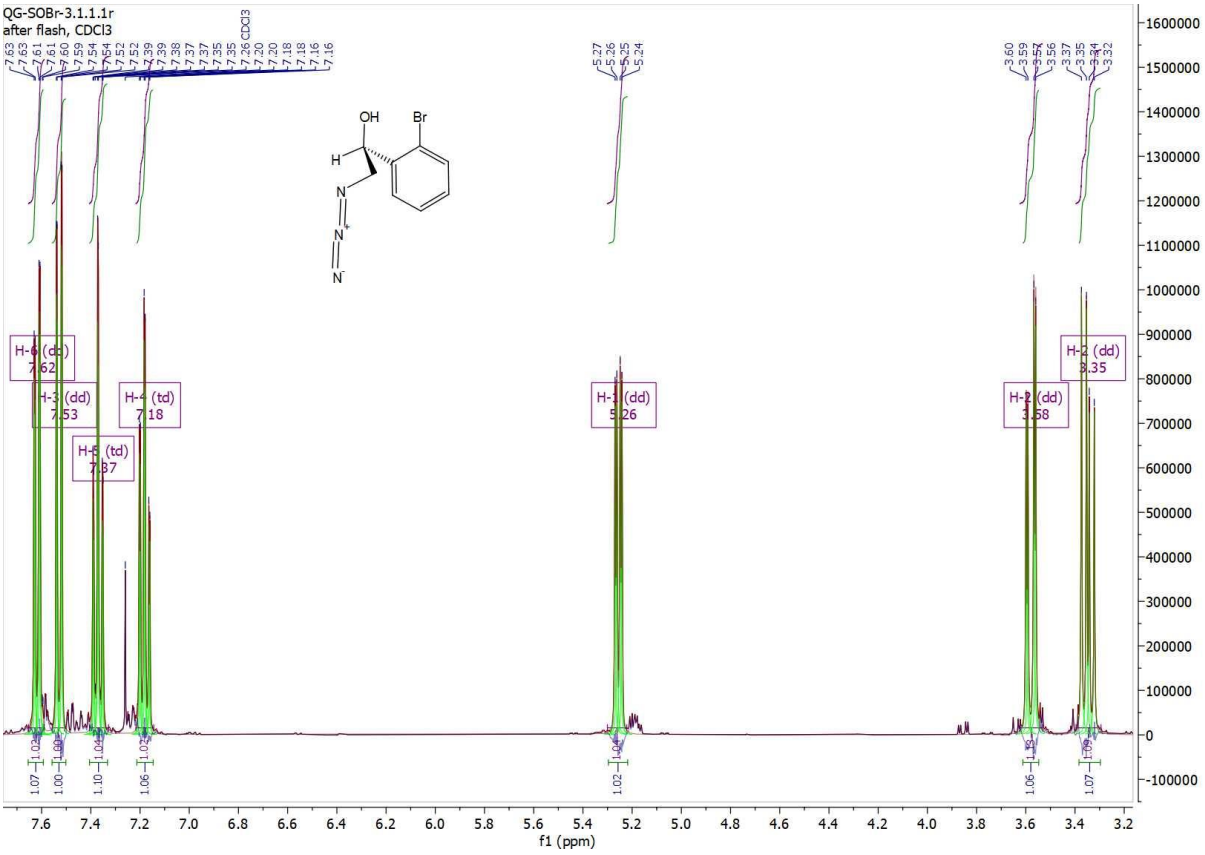
Compound (S)-**11**: QG-SOBr-2



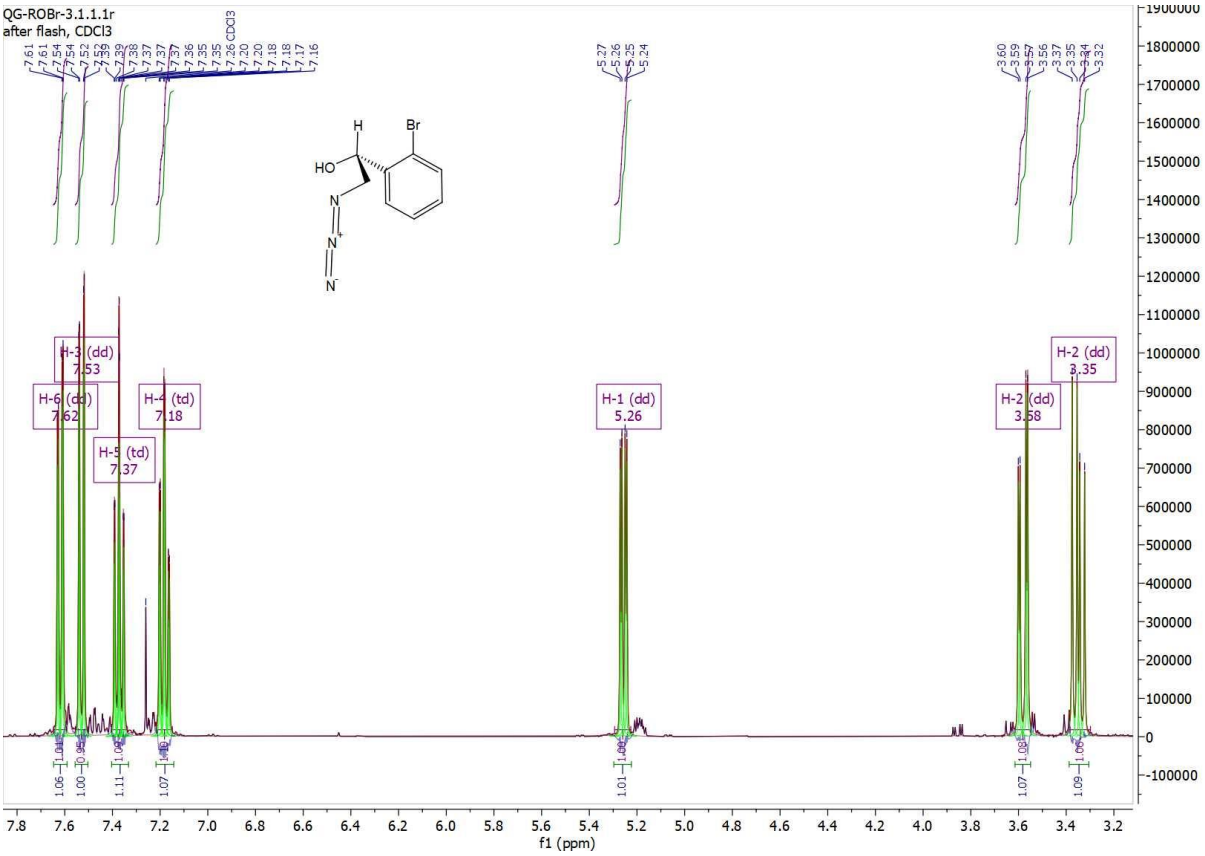
Compound (R)-**11**: QG-ROBr-2



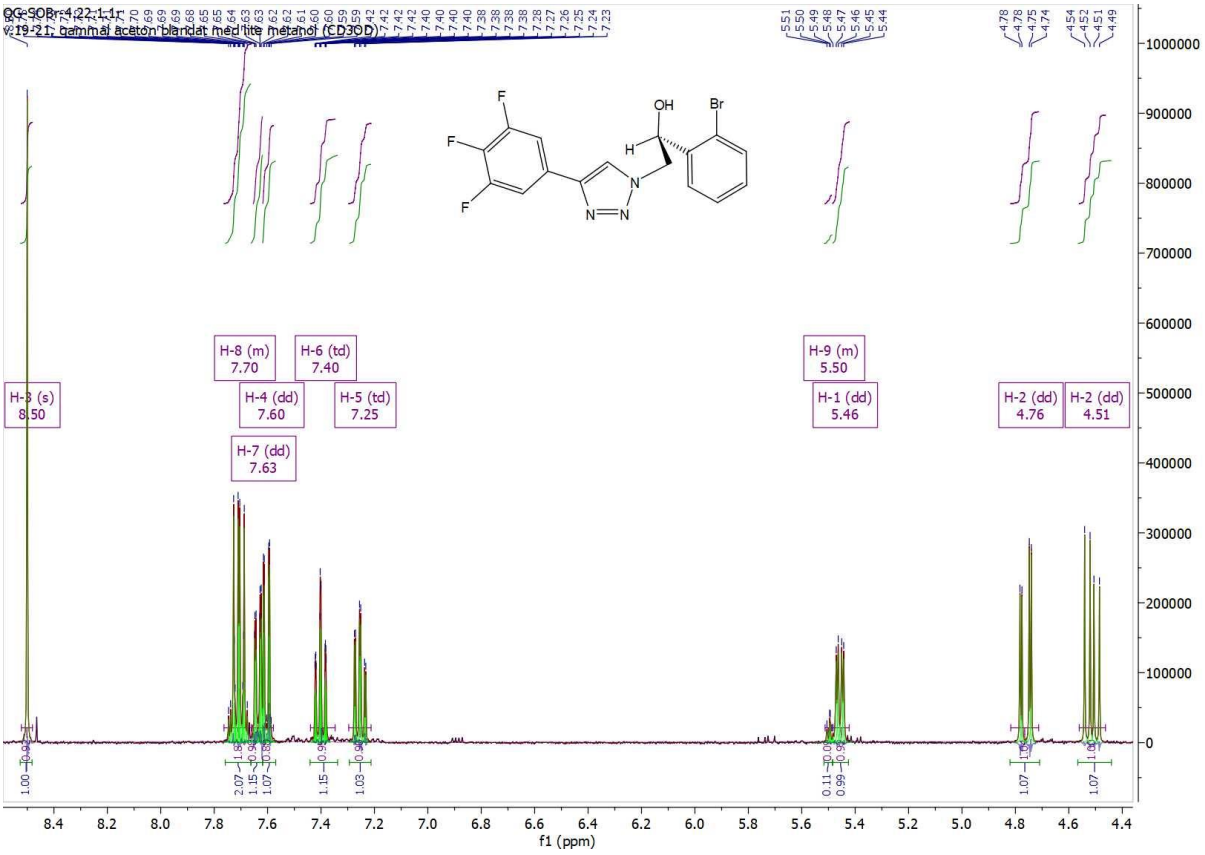
Compound (S)-**12**: QG-SOBr-3



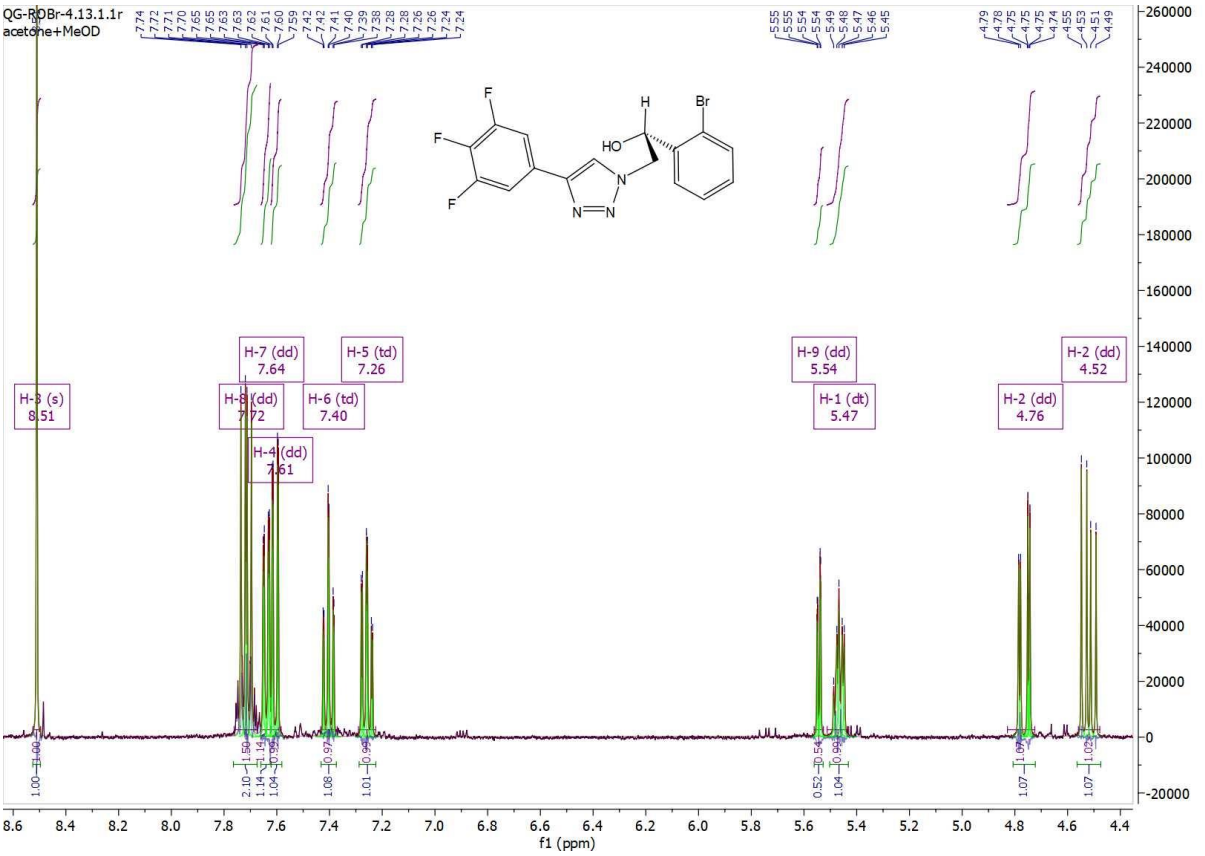
Compound (R)-**12**: QG-ROBr-3



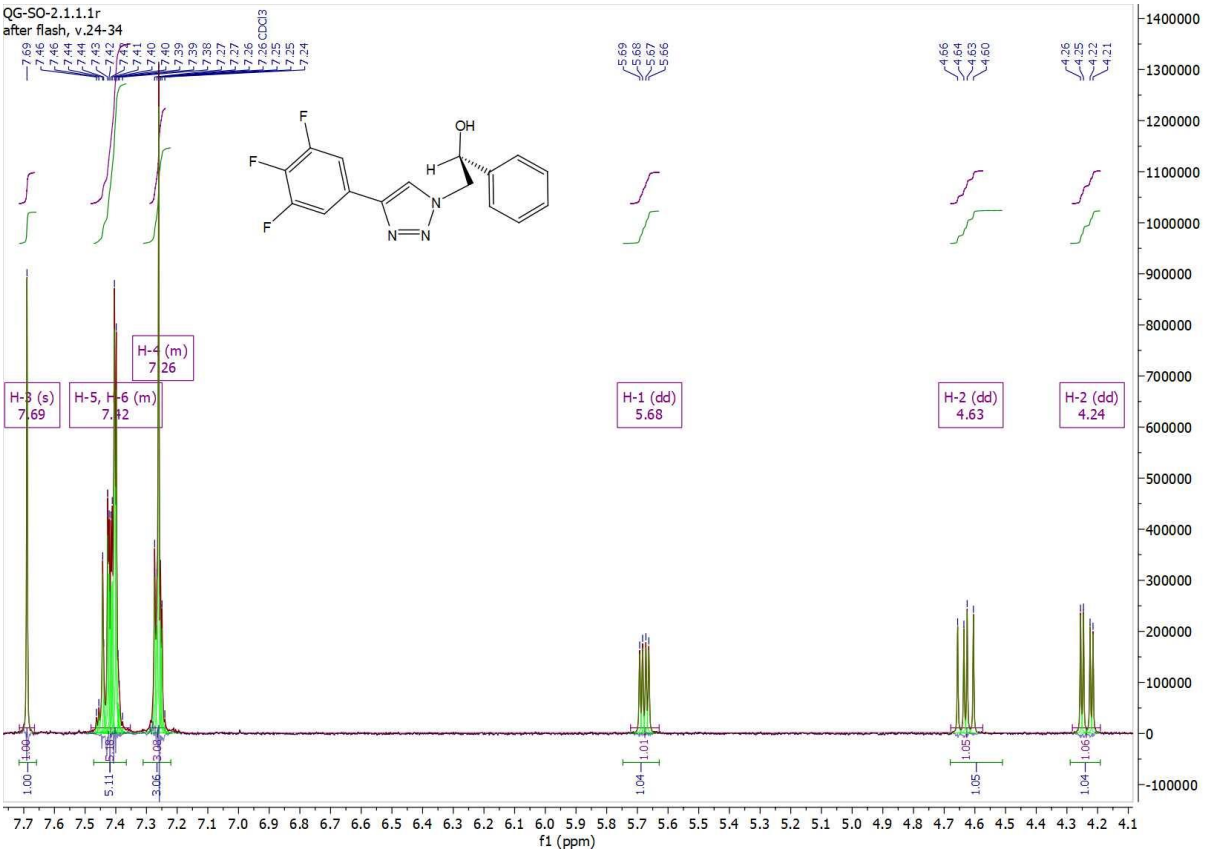
Compound (S)-**8**: QG-SOBr-4



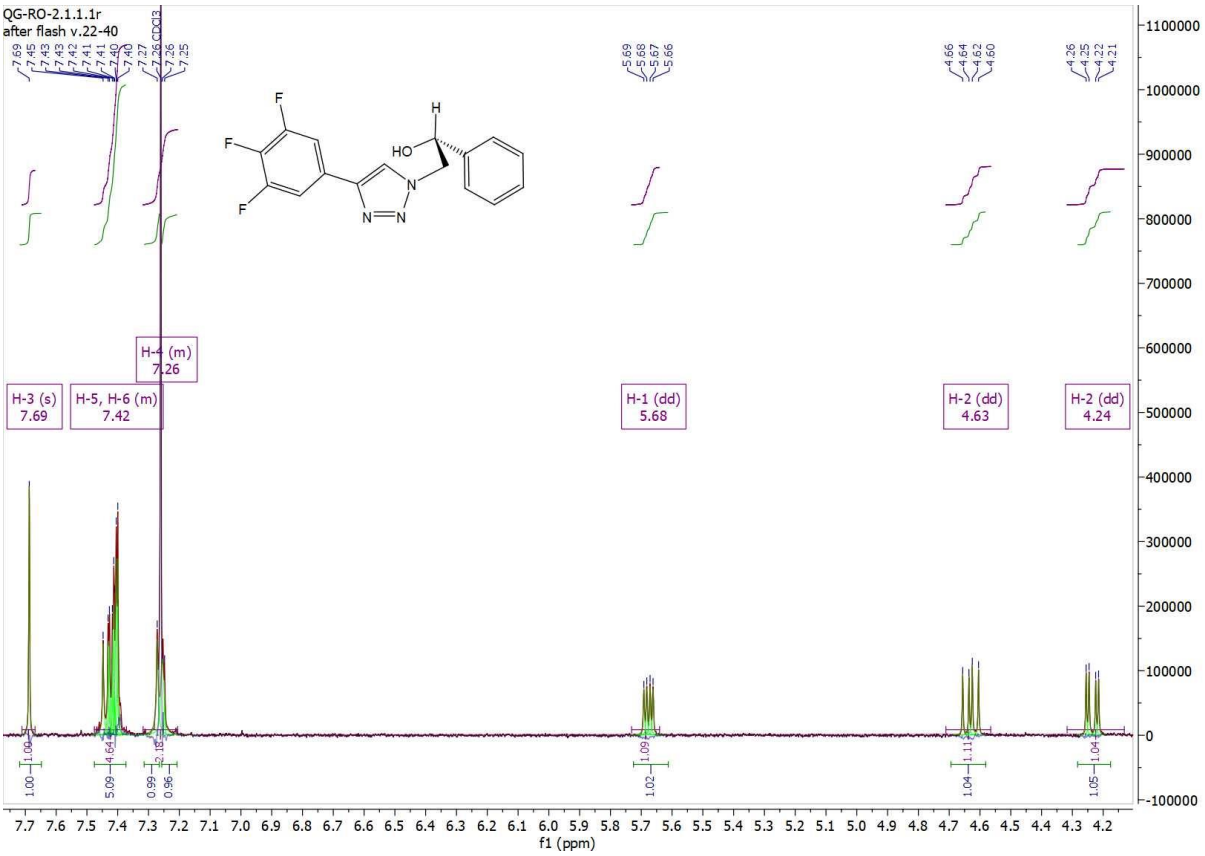
Compound (R)-**8**: QG-ROBr-4



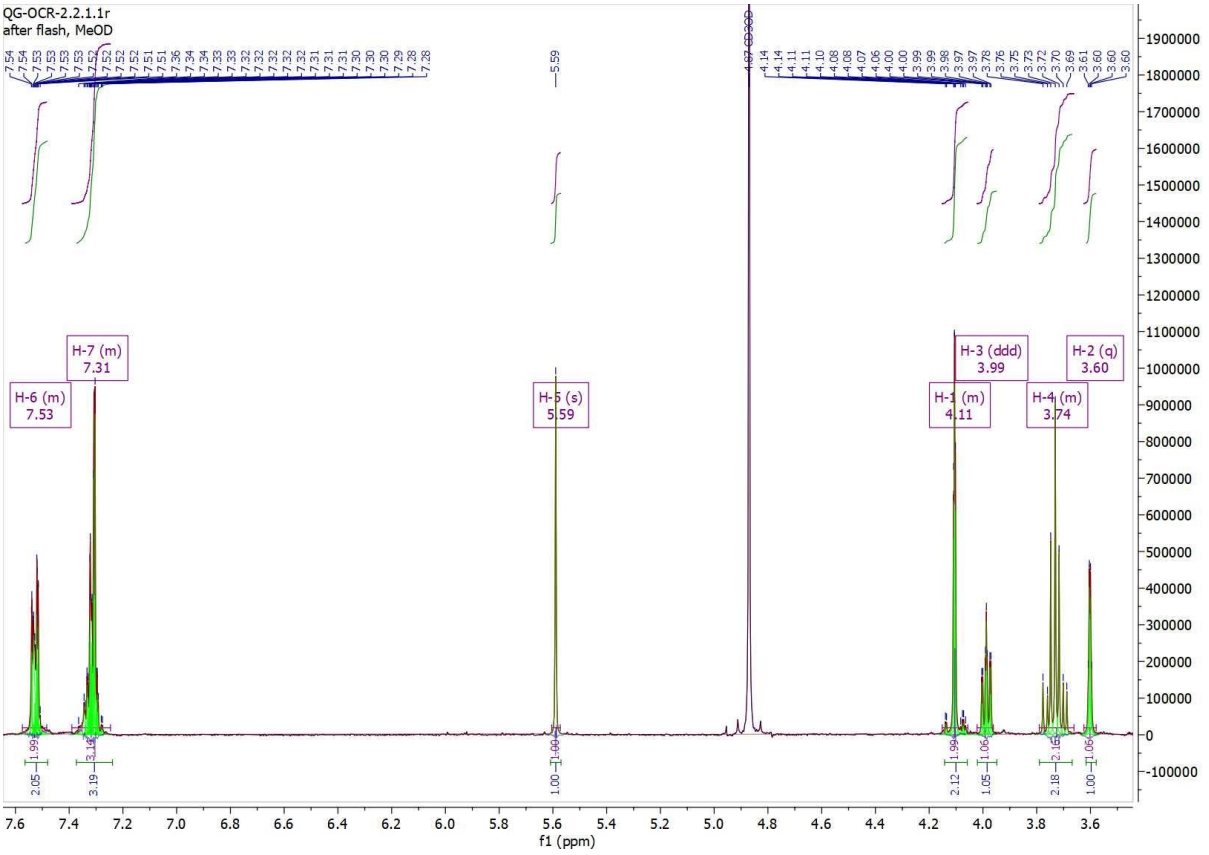
Compound (S)-**7**: QG-SO-2



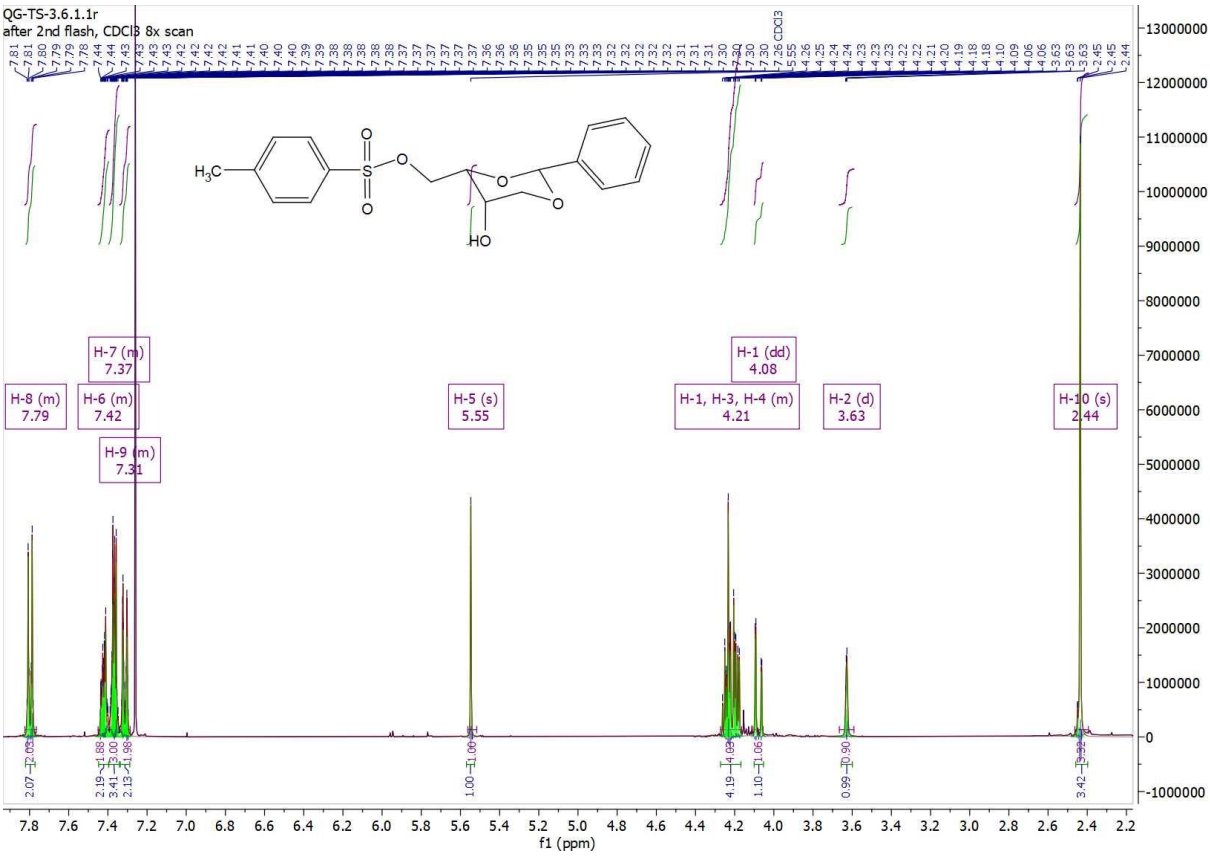
Compound (R)-**7**: QG-RO-2



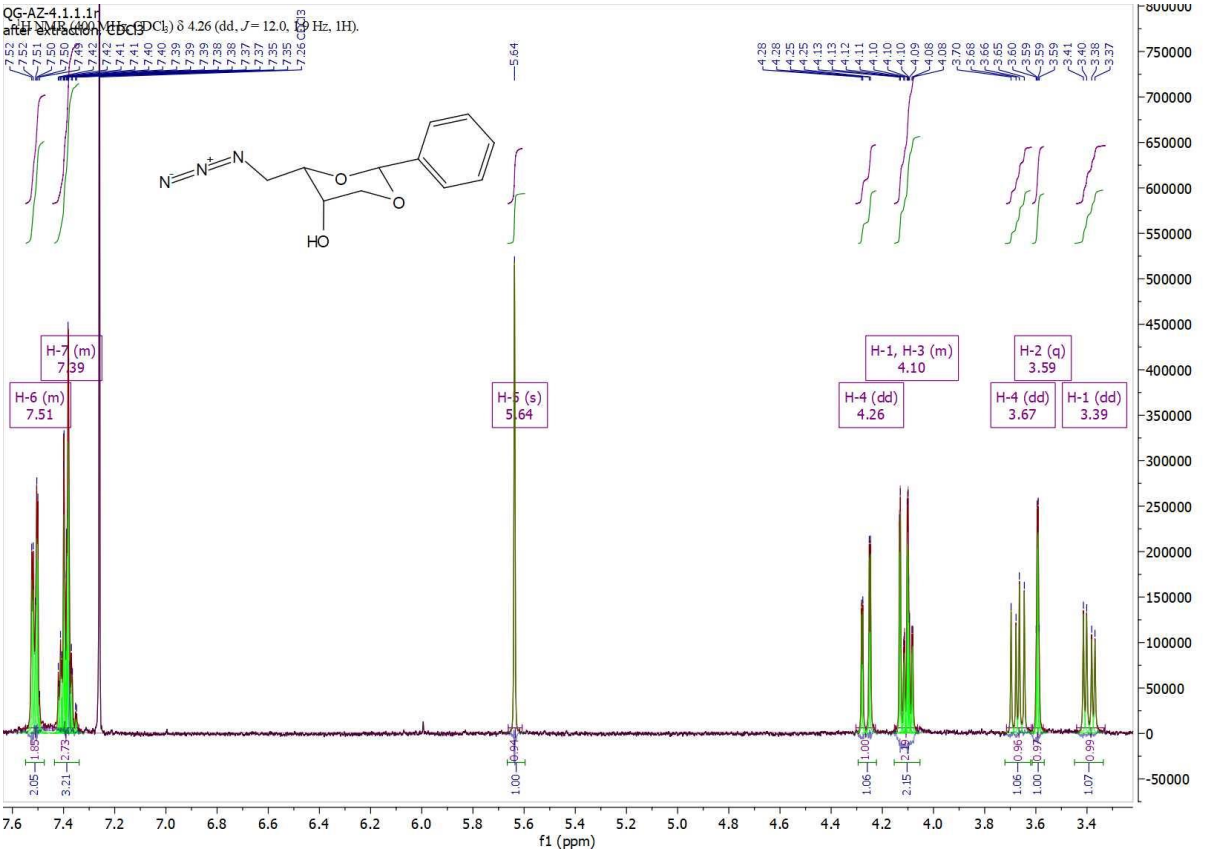
Compound **17**: QG-OCR-2



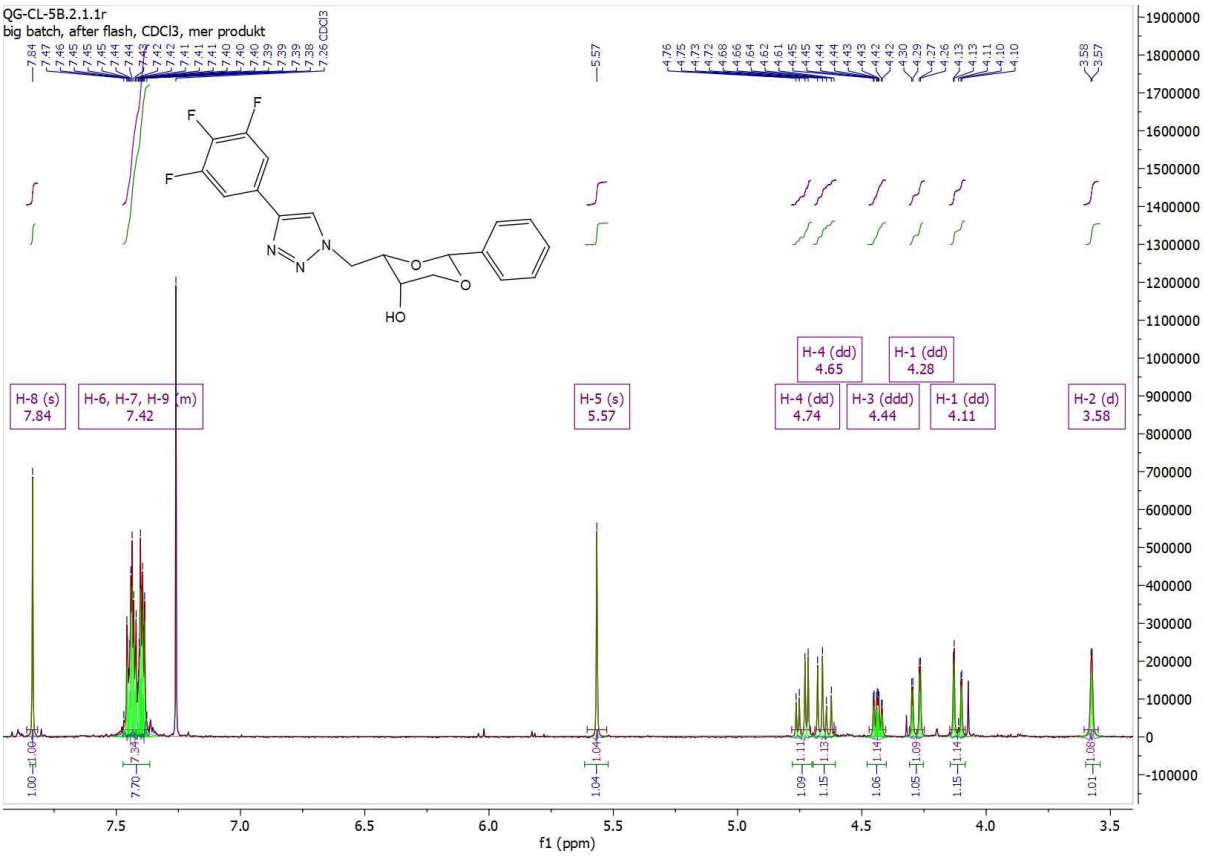
Compound **18**: QG-TS-3



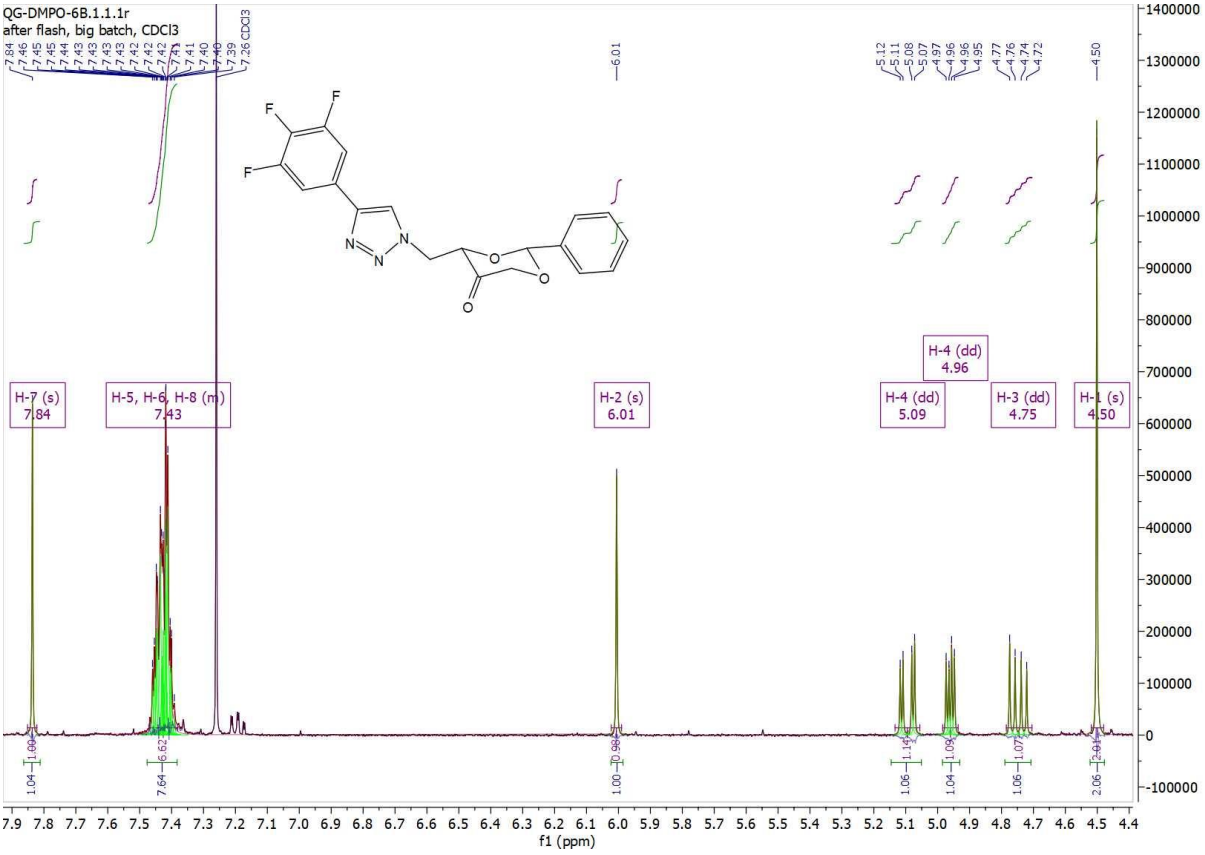
Compound **19**: QG-AZ-4



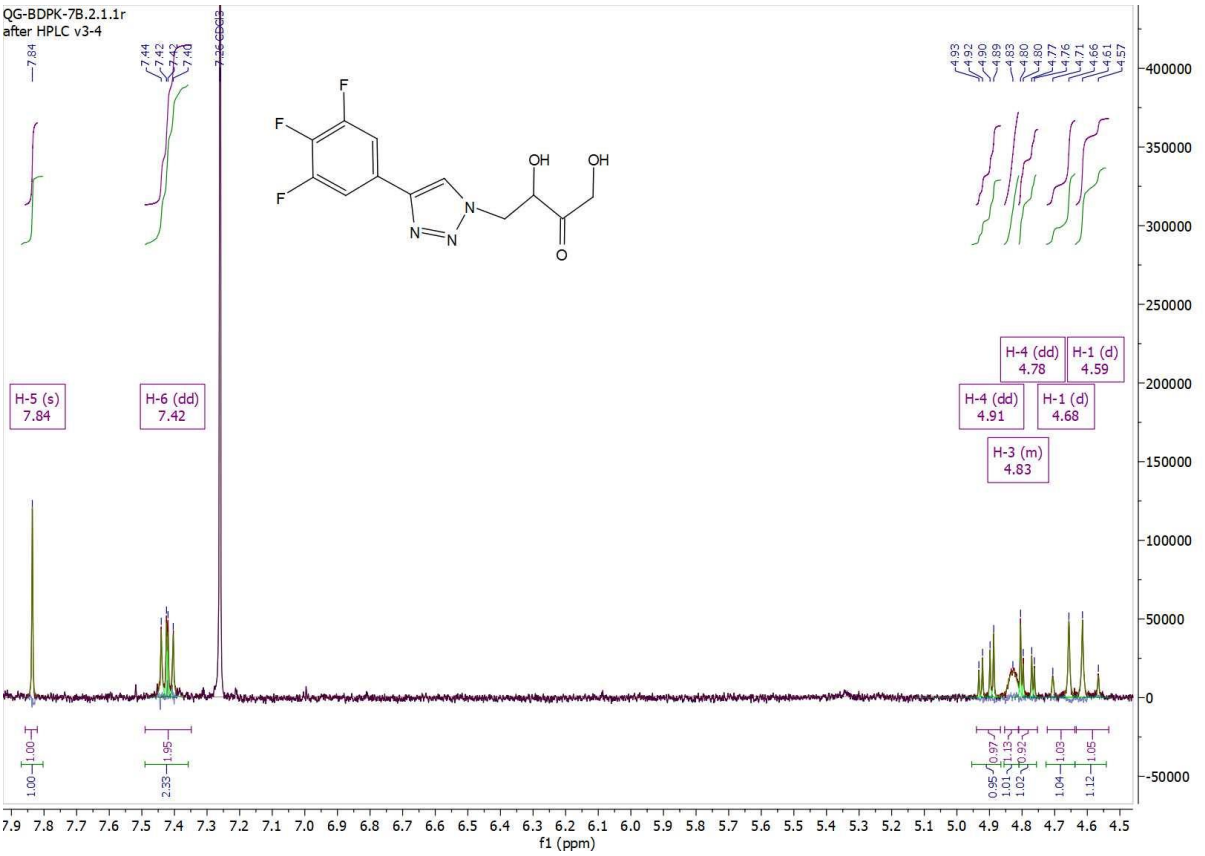
Compound **20**: QG-CL-5



Compound **21**: QG-DMPO-6



Compound **22**: QG-BDPK-7



Appendix B: ^{13}C-NMR spectrum

Compound **21**: QG-DMPO-6