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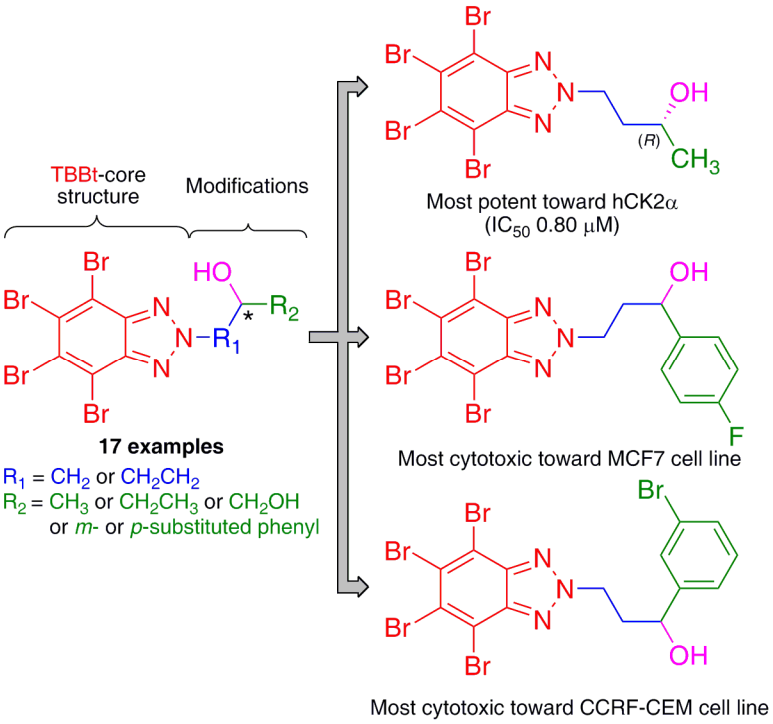

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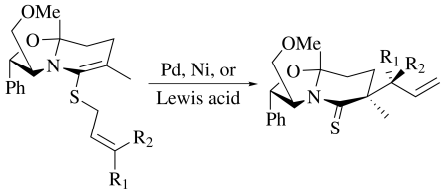

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Synthesis of novel chiral TBBt derivatives with hydroxyl moiety. Studies on inhibition of human protein kinase CK2α and cytotoxicity properties.

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ABSTRACT

The efficient method for the synthesis of novel 4,5,6,7-tetrabromo-1*H*-benzotriazole (TBBt) derivatives bearing a single stereogenic center has been developed. New compounds with a variety of substituents at the *meta*- and *para*-position of the phenyl ring are reported. All of the presented compounds were obtained using classical synthetic methods, such as bromination of benzotriazole, and its subsequent alkylation by monotosylated arylpropane-1,3-diols, which in turn have been synthesized through reduction of the corresponding prochiral β-keto esters, and the selective monotosylation of the primary hydroxyl group. The influence of the new and previously reported *N*-hydroxyalkyl TBBt derivatives on the activity of human protein kinase CK2α catalytic subunit was examined. The most active were derivatives with *N*-hydroxyalkyl substituents (IC_{50} in 0.80–7.35 μM range). A binding mode of (*R*)-1-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)butan-3-ol **7b** to hCK2α has been proposed based on *in silico* docking studies. Additionally, MTT-based cytotoxicity tests demonstrated high activities of novel 1-aryl-3-TBBt-propan-1-ol and 3-TBBt-propan-1,2-diol derivatives against human peripheral blood T lymphoblast (CCRF-CEM), and moderate anti-tumor activities against human breast adenocarcinoma (MCF7) cell lines.

1. Introduction

Human protein kinase CK2 (formerly known as casein kinase II) catalyzes the reversible phosphorylation of proteins, which is the most common post-translational modification in signal transduction. CK2 plays a key role in the regulation of numerous signaling pathways and diverse cellular functions such as: cell cycle progression, development, signal transduction, apoptosis, metabolism, differentiation (proliferation), cell morphology and migration as well as gene expression and secretion of cellular proteins [1-6]. Besides all the above mentioned fundamental processes of normal eukaryotic cell life, in which CK2 protein kinase participates, it has been experimentally confirmed that when CK2 becomes over-expressed or up-regulated as a result of i.e. mutations [7-9], it can display an uncontrolled activity leading to tumor growth promotion. As CK2 abnormal level was functionally associated with different cancer types [10-14], including prostate, lung, kidney, mammary gland, head and neck, it became obvious that this enzyme constitutes an attractive druggable target for cancer therapy. Therefore, the development of highly specific, cell-permeable, and ATP-competitive CK2 inhibitors appears as a promising strategy for treatment of several tumors.

In the last decade a great number of inhibition studies have led to the development of various classes of ATP-site directed inhibitors of CK2 [15-18]. Among them, condensed polyphenolic compounds [19,20], polyhalogenated tetrabromobenzimidazole / triazole derivatives [21,22], indolo- [23] and pyrrolo- [24] quinazolines were suggested as the most promising drug candidates. It has been reported that among the above mentioned inhibitors, the most efficient and selective are 4,5,6,7-tetrabromo-1*H*-benzotriazole TBBt (K_i = 0.4 μM) [25,26] and its derivative 2-dimethylamino-4,5,6,7-tetrabromo-1*H*-benzimidazole DMAT (K_i = 0.04 μM) [27]. However, the cytotoxicity tests showed TBBt as almost inactive anti-cancer compound [28]. Attempts to modify the TBBt scaffold in order to increase its selectivity and/or inhibitory activity toward CK2 were not successful up to now. In turn, the recent report [29] showed highly potent inhibitor ARC-1502 based on 4,5,6,7-tetrabromo-1*H*-benzimidazole (TBBi) conjugated with oligo-aspartate-containing peptide, which possess sub-nanomolar affinity (K_i = 0.5 nM) towards both CK2α, and the holoenzyme. However, to our best knowledge the inhibition of tumor cell lines growth was not reported for this compound.

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The aim of this study was to obtain novel TBBt derivatives, efficient inhibitors of CK2 with better cellular permeability than the parent TBBt-structure. Since the binding of TBBt at CK2 active site is based on hydrophobic interactions between the enzyme ATP-binding pocket and bromine atoms as well as hydrogen bond between benzotriazole ring and conserved water molecule deep inside the pocket [28], therefore to improve the activity of TBBt derivatives it seemed reasonable to introduce polar functional group on appropriately long rotatable hydrocarbon chain. Bearing in mind that this modification may allow for the interaction of the inhibitor molecule with polar residues located around hydrophobic pocket (e.g. Asp^{175} and Lys^{68}) [30,31], we have followed our previous research concept as its rational continuation [32-34]. Using the reported TBBt inhibitor core structure, as the lead compound, this study was set up to verify the above mentioned hypothesis, and thus evaluate whether incorporation of hydroxyl moiety with different substituents into a side chain of the newly designed and synthesized compounds, could increase the affinity for the enzyme and hence lower inhibitory concentration. Those modifications of parent TBBt-structure was also motivated by desire of increasing solubility and cellular permeability of potential inhibitors.

2. Results and discussion

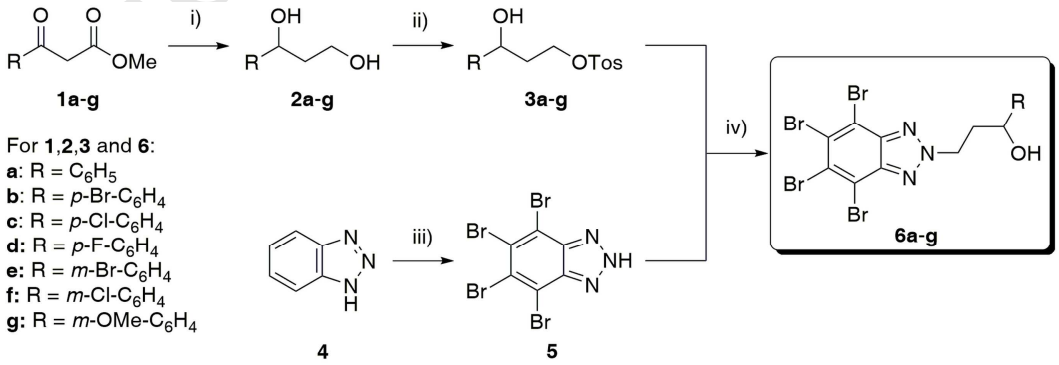
Herein, we disclose our study on designing and synthesis of new benzo-fused nitrogen heterocycles corresponding to TBBt, using simple synthetic methodologies. The planned four-step reaction sequence (**Scheme 1**) consists of the synthesis of appropriate 1,3-diols **2a-g** from corresponding β-keto esters **1a-g**, which after selective tosylation yield monotosylated derivatives **3a-g** further used as alkylation agents for 4,5,6,7-tetrabromo-1*H*-benzotriazole **5**, synthesized from commercially available benzotriazole **4**.

2.1. Synthesis of 1-aryl-3-TBBt-propan-1-ol derivatives 6a-g

The simultaneous reduction of carbonyl and carboxyl group of β-keto esters **1a-g** is well-known, and several protocols of such convenient reaction were reported [35-38]. Surprisingly, using very common reducing agent ($LiAlH_4$) in several variants of the reaction resulted in the obtaining of desired 1,3-diols **2a-g** with very poor yields (ca. 25%). The reason of this was a multiple side-products formation, what also significantly hampered the product isolation procedure. To develop a more efficient method we replaced $LiAlH_4$ by $NaBH_4$ as a less powerful reducing agent. Therefore, the reaction condition was changed to milder according to De Castro et. al. [39] and Kim et. al. [40] suggestions, respectively. In our hands, neither of these methods proved efficient, with respect to very low reaction rates. Looking for more favorable conditions we examined methanolic solution of sodium borohydride ($NaBH_4$-MeOH system) as the source of *in situ* generated $NaHB(OCH_3)_3$ complex, which is capable to reduce carbonyl group in both: ketones and esters [41]. Sodium borohydride was used in considerable 8-fold molar excess at reflux temperature. Thereby, with the optimal conditions in hand, **2a-g** were isolated in high 78-96% yields (see the Experimental Section).

In the next step, 1-aryl-propano-1,3-diols **2a-g** were submitted to the selective tosylation of their primary hydroxyl groups using 1 equiv of *p*-toluenesulfonyl chloride (TosCl) in the presence of 1.1 equiv of freshly distilled triethylamine and catalytic amount of 4-(dimethylamino)pyridine (DMAP) dissolved in dry CH_2Cl_2 according to the method reported in the literature [42-44]. The adopted procedure allowed the reaction to proceed smoothly, affording the expected products in moderate to high yields (33-82%) (see the Experimental Section).

The required 4,5,6,7-tetrabromo-1*H*-benzotriazole **5** was primarily prepared by exhaustive bromination of benzotriazole **4** with Br_2 in concentrated HNO_3, according to the original [45] and generic [46] literature procedures. However, the first attempted bromination reaction failed giving mixture of di-, tri- and tetra-bromobenzotriazoles even after elongation of the reaction time up to 5 days. The negative result of this process was likely due to larger scale used by us in comparison with the parent generic procedure [46]. Therefore, in order to solve the up-scaling problems, a modified synthetic strategy was proposed. This includes additional UV irradiation of the reacting mixture. It was performed to verify if the synthesis of tetrabromo benzo-fused nitrogen heterocycles of this type was possible not only by means of an *in situ* generated electrophilic bromonium cation (Br_2^+) formed in the presence of strong oxidizing agent (HNO_3) in course of the reaction, but if it was partially dependent on free-radical halogenation mechanism ($Br^{\cdot}$) as well. The employed change in the reaction condition resulted in desired sole product of >99% purity and in 59% yield accomplished after 48 h.

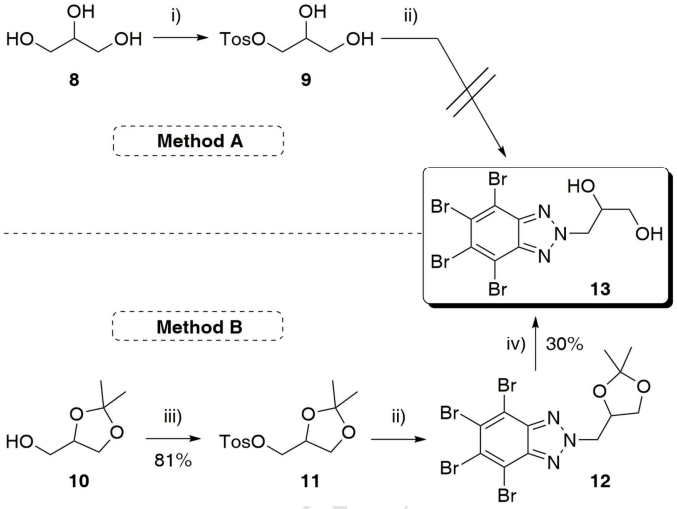


Scheme 1. Synthesis of 1-aryl-3-TBBt-propan-1-ol derivatives. Reagents and conditions: (i) $NaBH_4$ (8 equiv), MeOH, 12 h at reflux; (ii) dry Et_3N (1.1 equiv), DMAP (10 mg), TosCl (1 equiv), CH_2Cl_2 anh., 12 h at -15 °C, then 1 h at rt; (iii) Br_2 (5.99 equiv), 69% HNO_3 and 100% HNO_3, hv, 48 h at 60 °C; (iv) TBBt **5** (1 equiv), K_2CO_3 (3 equiv), acetone/acetonitrile (anh. mixture, 1:1, v/v), 48 h at reflux.

This was confirmed by nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry analyses. It is worth to note that this methodology in contrast to the original one [45], ensures good reproducibility of the reaction yield. Bearing in mind that regioselective *N*-alkylation of TBBt **5** is often problematic, we decided to investigate how the most crucial parameters affect the overall outcome of this reaction. These included both: TBBt-deprotonation approach and the selection of the appropriate reaction medium. Adopting the general procedure described by us previously [33], several bases were investigated (NaH, KOH, K_2CO_3). It turned out that the sequential deprotonation in the presence of 3-fold molar excess of anhydrous K_2CO_3 proved to be sufficient toward strong acidic proton of TBBt, thus allowing the anion formation, and subsequent reaction with monotosylated derivative **3a** as the model compound. In comparison to other tested bases, anhydrous K_2CO_3 proved to be the best, also concerning problems of the product isolation. From among several solvents applied (acetonitrile, acetone, DMF), the mixture composed of acetone and acetonitrile (1:1, v/v) turned out to be the medium of choice, since it was the only one in which the reaction ran with a reasonable rate. Under the optimized reaction conditions, at least the alkylation at *N*-1 atom has been significantly suppressed, thus improving the yield of desired *N*-2 substituted compound up to 42%. The other very important observation made, that should be briefly noted herein, refers to the manner of reaction progress monitoring. In the course of the experiments, it turned out that visualization of the TLC plates with the standard UV light (λ = 254 nm) did not allow to follow the alkylation reaction rate, since the spots representing monotosylated substrates **3a**-**g** and the corresponding major products **6a**-**g** had the same R_f values in various eluent systems applied. Initially, we had problems to recognize this phenomenon as it is not obvious to foresee that two such different structures in terms of molecular weight and polarity could behave identically on the silica matrix. Trying to overcome the above drawback, we found that the expected product could be distinguished from the corresponding monotosyl-derivative in UV light of λ = 312 nm. The isolated yields of the obtained products **6a-g** were in the range of 33-42% (see the Experimental Section).

2.2. Synthesis of 3-TBBt-propane-1,2-diol 13

With the aim to explore if an addition of one more hydroxyl group into side chain of 3-TBBt-propan-1-ol structure could influence kinase CK2 inhibitory activity, we also designed novel 3-TBBt-propane-1,2-diol **13**. To obtain desired compound **13**, we followed at first the synthetic approach shown in **Scheme 2** (Method A), which included selective tosylation of glycerol **8** and its subsequent reaction with TBBt **5**. Since this strategy suffered from tedious purification procedure, we have planned an alternative synthetic route (**Scheme 2**, Method B). Our main objective was to eliminate the difficulties in separation of TBBt **5** and 3-TBBt-propane-1,2-diol **13** present in the crude reaction mixture. Therefore, we decided to start from ready-to-use commercially available 1,2-*O*-isopropylidene glycerol (IPG) **10**, also called solketal, which was smoothly converted to tosyl derivative **11** with very high yield (81%) according to the published procedure [47]. The tosylated solketal **11** was subsequently reacted with TBBt **5** yielding TBBt-ketal **12**. Finally, acidic hydrolysis of ketal **12** using 0.5 *N* HCl according to Oh et al. [48], allowed to afford 3-TBBt-propane-1,2-diol **13** in fairly good 30% overall yield after two-steps. In accordance with our predictions the isolation and purification of compound **13** obtained through alternative pathway (Method B) went more easily when compared to Method A.



Scheme 2. Synthesis of 3-TBBt-propane-1,2-diol. Reagents and conditions: (i) dry Et_3N (1.1 equiv), DMAP (10 mg), TosCl (1 equiv), CH_2Cl_2 anh., 12 h at -15 °C, then 1 h at rt; (ii) TBBt **5** (1 equiv), K_2CO_3 (3 equiv), acetone/acetonitrile (anh. mixture, 1:1, v/v), 48 h at reflux; (iii) dry Et_3N (1.5 equiv), DMAP (0.1 equiv), TosCl (1.2 equiv), CH_2Cl_2 anh., 5 h at 0-5 °C; (iv) HCl aq., 6 h at reflux.

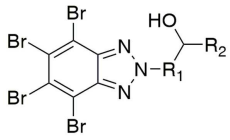
2.3. Inhibitory activity against human CK2*α in vitro*

The *in vitro* inhibitory activities against human protein kinase CK2 catalytic subunit (hCK2α) of the obtained 1-aryl-3-TBBt-propan-1-ol derivatives **6a**-**g**, and previously synthesized compounds **7a**-**c** of a similar structure [49] as well as novel 3-TBBt-propane-1,2-diol **13** are presented and compared with TBBt **5** in **Table 1**. Inhibition of hCK2α by above mentioned TBBt derivatives was determined using P81 filter isotopic assay [50] (see the Experimental Section and the Supporting Information for details). Herein studied TBBt derivatives **6a**-**g**, **7a**-**c**, **13** contain a stereogenic center, which has not been reported for this class of inhibitors heretofore. We decided to determine biochemical activity of all the compounds **6a**-**7c** and **13** as racemic mixtures at first, since activity of racemate should not drastically differs from the activity of a pure enantiomer.

However, since the aliphatic derivatives **7a**-**c** separation had been performed before [49] and isolated enantiopure (>99% ee) compounds were available, we determined the activity of those compounds as well. Basing on these results, we tried to investigate structure-activity relationship of TBBt derivatives in terms of their stereochemistry. The structure optimization also assumed the introduction of electron-withdrawing and electron-donating groups to the *para*- and *meta*-position of the phenyl ring attached directly to the chiral carbon, which contains hydroxyl group. This allows us to evaluate not only the effect of the phenyl ring itself, but also the influence of delocalized electron density by the type of substitution.

Biochemical assays revealed that aliphatic **7a**-**c**, **13** and aromatic **6a**-**g** derivatives of 3-TBBt-propan-1-ol show very different affinity towards hCK2α subunit. The aliphatic chiral derivatives **7a**-**c**, **13** showed slightly lower inhibitory activity than the lead structure **5**, IC_{50} values within the range 0.8-7.2 μM. On the other hand, derivatives with aromatic, bulky substituent **6a**-**g** have shown hardly any inhibitory activity (**Table 1**). In turn, 3-TBBt-propane-1,2-diol **13** was almost equipotent with (*S*)-**7b** and **7c** (IC_{50} in 2.17–2.50 μM range), what suggest that in spite of our expectations this compound turned out to be moderate inhibitor.

Table 1. hCK2α inhibitory activity of 4,5,6,7-tetrabromo-1*H*-benzotriazole derivatives with hydroxyl-aryl (**6a-g**) and -alkyl (**7a-c**, **13**) substituents.



Compound	R_1	R_2	IC_{50} (μM) CK2α	CK2α activity[a] (%)		log*P*[d]
				20 μM	200 μM	
5	-	-	0.32	-	-	3.74
6a	CH_2CH_2	C_6H_5	N.D.[b]	93.00	31.09	5.52
6b	CH_2CH_2	*p*-Br-C_6H_4	N.D.[b]	97.42	33.45	6.22
6c	CH_2CH_2	*p*-Cl-C_6H_4	N.D.[b]	95.60	45.38	6.13
6d	CH_2CH_2	*p*-F-C_6H_4	N.D.[b]	91.42	35.35	5.58
6e	CH_2CH_2	*m*-Br-C_6H_4	N.D.[b]	96.61	43.36	6.22
6f	CH_2CH_2	*m*-Cl-C_6H_4	N.D.[b]	74.90	23.77	6.13
6g	CH_2CH_2	*m*-OMe-C_6H_4	N.D.[b]	95.12	87.51	5.41
7a	CH_2	CH_3	5.40	-	-	1.25
(*S*)-**7a**[c]	CH_2	CH_3	5.60	-	-	-
(*R*)-**7a**[c]	CH_2	CH_3	7.35	-	-	-
7b	CH_2CH_2	CH_3	4.16	-	-	1.35
(*S*)-**7b**[c]	CH_2CH_2	CH_3	2.50	-	-	-
(*R*)-**7b**[c]	CH_2CH_2	CH_3	0.80	-	-	-
7c	CH_2	CH_2CH_3	2.50	-	-	1.73
(*S*)-**7c**[c]	CH_2	CH_2CH_3	3.60	-	-	-
(*R*)-**7c**[c]	CH_2	CH_2CH_3	N.D.[b]	-	-	-
13	CH_2	CH_2OH	2.17	-	-	0.39

[a] hCK2α kinase assays were performed in the absence or presence of a 20 or 200 μM solution of tested compound. Results are expressed as a percentage of the control activity without inhibitor.
[b] Not determined.
[c] Obtained in absolutely enantiopure form (>99% ee) (see Ref. [49]).
[d] Logarithm of the partition coefficient of a given inhibitor between *n*-octanol and water according to ChemBioDraw Ultra 13.0 software indications.

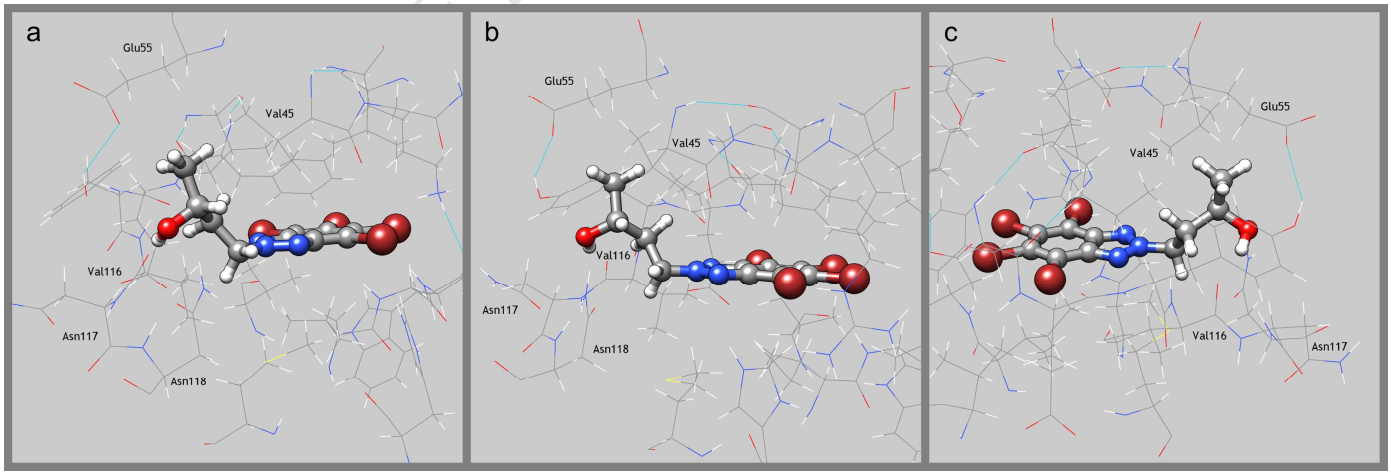


Fig. 1. The binding mode of compound (*R*)-**7b** in complex with the human CK2α active site (PDB code: 1J91). The inhibitor molecule shown as ball-and-stick model with carbon atoms in grey, hydrogen in white, oxygen in red, nitrogen in blue and bromine in brown from three different perspectives. Enzyme model is presented as a wire model. View **a** corresponds to that presented in **Fig. 2**. High-resolution graphics were generated with UCSF Chimera.

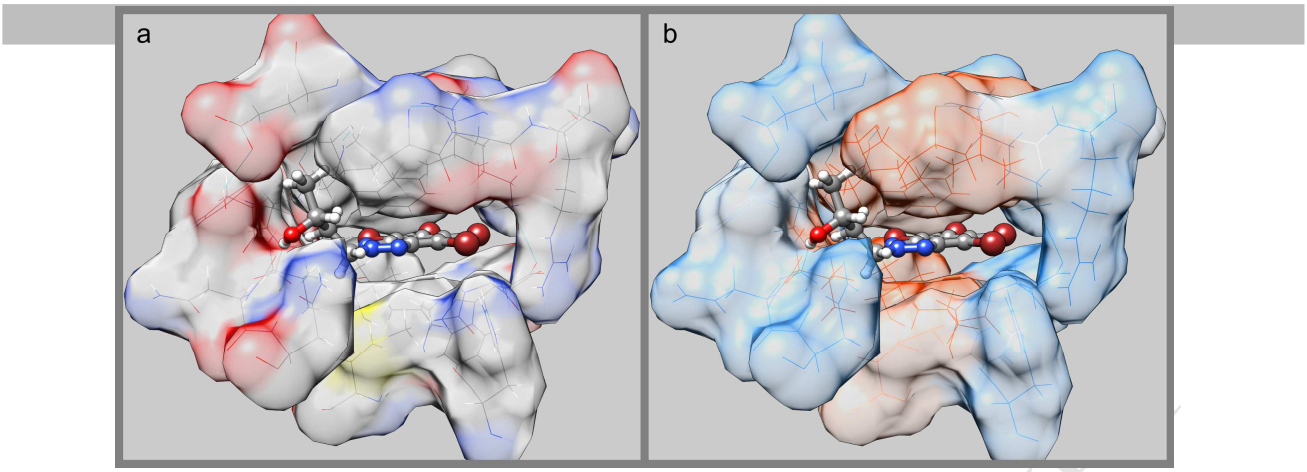


Fig. 2. The binding mode of compound (*R*)-**7b** in complex with the human CK2α active site (PDB code: 1J91). The enzyme residues are shown with the molecular surface colored a) according to the residue atom type (see **Fig. 1**), b) according to the residue hydrophobicity. Hydrophilic residues are depicted in blue, while hydrophobic are orange-red. Hydrophobic ATP-binding pocket is surrounded by hydrophilic residues. Molecular surfaces were generated with UCSF Chimera.

The lowest IC_{50} value (0.80 μM) was obtained for enantiopure (*R*)-**7b** compound, which was twice as active as the racemic mixture **7b**, and almost 4-fold more potent than the opposite (*S*)-enantiomer (*S*)-**7b**. The difference in affinities of (*S*)- and (*R*)-enantiomers of compound **7** may be explained with assistance of molecular docking. We have docked all of the synthesized compounds with Autodock 4.2 package and received ligand-receptor structures, which are useful in the explanation of this phenomenon. For the most potent inhibitor (*R*)-**7b** binding model for its complex with hCK2α is presented in **Fig. 1** and **2**. While in both cases polar interactions of the hydroxyl group with Val^{116} and Asn^{118} were observed, in (*S*)-molecule, in order to avoid clash of the terminal methyl group, hydroxyl group is located farther from Val^{116} backbone and carbonyl group of Asn^{118} residue, thus may form weaker hydrogen bonds.

All the aromatic-substituted derivatives **6a-g** have shown considerably lower inhibitory activity, with an IC_{50} value in the range 100-300 μM. This could be explained by an unfavorable interaction between the phenyl ring and the amino acid residues surrounding the ATP-binding site, which suggest that bulky substituent of hydrophobic nature is less willingly present in a distance of about 5 Å from the pocket. An additional conclusion is that those compounds with *para*-substituted phenyl ring **6b-d** were relatively more active comparing to *meta*-derivatives **6e-g**.

2.4. *In vitro* anticancer screening

As CK2 inhibitory activity does not always correlate with cytotoxicity properties of the studied compounds, the influence on the cell viability of newly **6a-g**, **13** and previously synthesized compounds **7a-b** was evaluated in MCF7 (human breast adenocarcinoma), and CCRF-CEM (acute lymphoblastic leukemia) cell lines (**Table 2** and **3**). Such an evaluation was motivated by the fact that the apoptosis of tumor cells can be induce not necessarily by down-regulation of a particular CK2 enzyme, but also with disparate molecular mechanism of action, which discovery is always of considerable interest.

Table 2. Effects of TBBt derivatives (**6a-g**, **7a-b**, **13**) on viability of MCF7 adherent cells (human breast adenocarcinoma) after 24 h and 48 h treatment (by MTT assay).

Compound	Cell viability % ± SD[a]							
	After 24 h incubation				After 48 h incubation			
	12.5μM	25 μM	50 μM	100 μM	12.5μM	25 μM	50 μM	100 μM
5	111 ± 16	108 ± 14	120 ± 2	109 ± 3	N.D.[b]	N.D.[b]	N.D.[b]	N.D.[b]
6a	115 ± 2	99 ± 3	28 ± 8	13 ± 0	113 ± 3	105 ± 4	23 ± 8	0 ± 0
6b	82 ± 1	59 ± 1	10 ± 0	0 ± 0	107 ± 1	105 ± 5	15 ± 4	0 ± 0
6c	90 ± 5	96 ± 8	19 ± 2	0 ± 0	100 ± 4	92 ± 2	9 ± 5	0 ± 0
6d	116 ± 7	101 ± 9	16 ± 1	0 ± 0	109 ± 6	97 ± 6	7 ± 1	0 ± 0
6e	81 ± 7	52 ± 9	14 ± 2	1 ± 0	102 ± 8	106 ± 2	11 ± 2	0 ± 0
6f	97 ± 7	96 ± 3	28 ± 4	0 ± 0	107 ± 1	101 ± 7	12 ± 2	0.5±1
6g	121 ± 5	71 ± 4	22 ± 7	16 ± 2	97 ± 6	96 ± 2	20 ± 2	6 ± 0
7a	118 ± 5	96 ± 6	60 ± 2	15 ± 2	99 ± 4	83 ± 3	16 ± 2	6 ± 2
(*S*)-**7a**	119 ± 5	67 ± 1	58 ± 5	25 ± 4	101 ± 3	64 ± 5	10 ± 2	8 ± 2
7b	99 ± 5	102 ± 6	90 ± 6	57 ± 1	79 ± 1	73 ± 1	76 ± 2	0 ± 0
(*S*)-**7b**	112 ± 6	102 ± 4	105 ± 6	31 ± 2	100 ± 1	89 ± 6	87 ± 0	6 ± 1
(*R*)-**7b**	109 ± 5	105 ± 3	102 ± 4	18 ± 2	86 ± 3	77 ± 6	64 ± 1	0 ± 1
13	145 ± 4	156 ± 5	98 ± 2	53 ± 5	116 ± 5	111 ± 3	109 ± 1	8 ± 3

[a] Standard deviation; the results are expressed in percentage of cell viability relative to control (cells without inhibitor in 0.5% DMSO) and are divided into two groups for each tumor cell line depending on the treatment time (24 h and 48 h). Experiments were performed in triplicate and the % of viable cells are mean values of three independent experiments.

[b] Not determined.

Table 3. Effects of TBBt derivatives (**6a-g**, **7a-b**, **13**) on viability of CCRF-CEM suspension cells (human acute lymphoblastic leukemia) after 24 h and 48 h treatment (by MTT assay).

Compound	Cell viability % ± SD[a]							
	After 24 h incubation				After 48 h incubation			
	12.5µM	25 µM	50 µM	100 µM	12.5µM	25 µM	50 µM	100 µM
5	115 ± 12	105 ± 24	118 ± 27	61 ± 18	N.D.[b]	N.D.[b]	N.D.[b]	N.D.[b]
6a	122 ± 2	123 ± 4	66 ± 2	21 ± 2	89 ± 2	84 ± 6	0 ± 0	0 ± 0
6b	101 ± 6	109 ± 1	58 ± 0	0 ± 0	89 ± 4	69 ± 4	0 ± 0	0 ± 0
6c	105 ± 2	106 ± 3	56 ± 2	0 ± 0	93 ± 4	69 ± 4	0 ± 0	0 ± 0
6d	110 ± 4	104 ± 4	46 ± 6	0 ± 0	101 ± 1	88 ± 8	0 ± 0	0 ± 0
6e	102 ± 5	123 ± 8	54 ± 6	0 ± 0	118 ± 8	54 ± 3	0 ± 0	0 ± 0
6f	110 ± 4	118 ± 2	71 ± 8	0 ± 0	70 ± 9	68 ± 8	0 ± 0	0 ± 0
6g	110 ± 4	106 ± 8	58 ± 4	48 ± 4	96 ± 6	50 ± 2	2 ± 1	2 ± 0
7a	125 ± 4	102 ± 7	108 ± 4	9 ± 6	83 ± 8	78 ± 9	53 ± 5	0 ± 0
(*S*)-**7a**	104 ± 7	104 ± 12	72 ± 7	9 ± 3	105 ± 2	92 ± 3	58 ± 5	0 ± 0
7b	106 ± 5	113 ± 8	89 ± 7	3 ± 1	94 ± 4	86 ± 4	45 ± 4	0 ± 0
(*S*)-**7b**	126 ± 8	112 ± 6	86 ± 4	36 ± 2	92 ± 9	89 ± 8	42 ± 3	10 ± 3
(*R*)-**7b**	88 ± 8	81 ± 7	69 ± 4	36 ± 2	94 ± 7	71 ± 9	23 ± 4	24 ± 3
13	78 ± 3	89 ± 3	47 ± 6	8 ± 1	60 ± 4	56 ± 5	27 ± 1	1 ± 0

[a and b] see **Table 2**.

We found that most of the tested compounds showed cytotoxicity toward both MCF7 and CCRF-CEM cells at micromolar ranges. Among them, compounds **6b-f** exerted a strong effect on MCF7 cells viability (**Table 2**). Only 10-20% of the cells survived in the presence of **6b-e** at 50 µM concentration after 24 h treatment. In turn, treatment of MCF7 for 48 h resulted in the most cytotoxic effect for *para*-fluoro substituted derivative **6d**. The cytotoxicity data obtained for CCRF-CEM (**Table 3**) revealed the cytotoxic activity of all compounds bearing a phenyl ring with the exception of **6a** and **6g**, when CCRF-CEM cells were treated with 100 µM solution of the compounds. The most active compounds **6c-e** exhibited significant effect on CCRF-CEM cells viability (46-56%) at a concentration of 50 µM after 24 h of incubation. After 48 h of treatment, tumor cell viability reached 50% of the control in the presence of 25 µM concentration of **6g** derivative.

The logarithm of a partition coefficient (log*P*) is an important parameter, which has some influence on pharmacological properties of the biologically active compounds. We have employed the ChemBioDraw Ultra 13.0 software to define the values of log*P* for each of TBBt derivatives **6a-g**, **7a-b**, **13** and the results are summarized in **Table 1**. The lipophilicity of phenyl-containing compounds **6a-g** (log*P* 5.41-6.22) were higher compared with TBBt **5** (log*P* 3.74), and its alkyl derivatives **7a-b** and **13** (log*P* 0.39-1.73), what may result in enhanced ability of those compounds to penetrate into the cell and may explain higher antitumor activity. Importantly, new derivatives exhibited significantly higher cytotoxicity activities than almost inactive parent TBBt **5**. We found, that the inhibitory activities towards hCK2α were inconsistent with MTT cytotoxicity assay results, since compounds containing phenyl ring **6a-g** in spite of being weak inhibitors of hCK2α, turned out to display anti-tumor activity at an acceptable level, and *vice versa*, TBBt alkyl-containing derivatives **7a-b**, **13** with promising hCK2α inhibitory activity showed a moderate cytotoxic properties. In general, compounds with alkyl substituents **7a-b**, **13** were substantially less active than those with additional aryl moiety **6a-g** in both cell lines what may suggest that aromatic substituent in this position is important for the TBBt derivatives to exhibit cytotoxicity.

The analysis of the obtained data revealed that the nature of the aryl substituent has little influence on cytotoxic activity of particular compound. The compounds with bromine **6b**, **6e** and fluorine **6d** substituent located at the appropriate *meta*- or *para*-position of the phenyl ring showed slightly increased cytotoxicity comparing to unsubstituted aryl derivative **6a**, and the *meta*-methoxy substituted **6g**, which decreased viability of both cell lines only to 13-48% range at 100 µM after 24 h treatment. Undoubtedly, study of structural optimization of TBBt derivatives should be continued.

3. Conclusion

Although 4,5,6,7-tetrabromo-1*H*-benzotriazole (TBBt, **5**) is considered as a promising precursor for the synthesis of CK2 inhibitors, it has no obvious effect on studied cancer cells (Jurkat, HL-60) treated in typical culture conditions (medium supplemented with 10% serum) [28]. Therefore, it was of interest to modify its structure in order to obtain new compounds active against hCK2α and/or inhibiting tumor cells growth. This was achieved by straightforward, four-step synthesis leading through simple organic reaction methodology and with the use of cheap chemical materials. The products were obtained *via* the following reaction sequence: (i) $NaBH_4$-reduction of the appropriate β-keto esters **1a-g**, (ii) selective monotosylation of a primary hydroxyl group of **2a-g** to obtain **3a-g**, (iii) bromination of benzotriazole to obtain 4,5,6,7-tetrabromo-1*H*-benzotriazole **5**, (iv) subsequent alkylation of **5** with the appropriate derivative **3a-g**. Human CK2α inhibitory activity of the newly **6a-g**, **13** and previously synthesized compounds **7a-c** was estimated at micromolar concentrations. After the *in vitro* screening we found that aliphatic TBBt derivatives **7a-c** and **13** suppressed the activity of hCK2α more efficiently than the new compounds with phenyl ring **6a-g**.

Experimental data obtained for (*R*)-**7b** revealed that this compound is the most active TBBt derivative studied in this work, with similar affinity for the hCK2α as TBBt **5**. This ability is connected with an optimal orientation of the hydroxyl group of that inhibitor in the ATP binding pocket, which is consistent with the predictions made by the computational docking method and suggests that such algorithms can serve as an aid in the designing of TBBt-based inhibitors. Despite the low hCK2α inhibitory

activity of compounds **6a-g**, **13** and moderate activity of previously received alkyl derivatives **7a-b**, cytotoxic effect of all compounds was tested on two human tumor cell lines and led to the discovery of their antitumor activity. *In vitro* biological evaluation showed that most of the compounds exhibit moderate cytotoxicity against human breast cancer cells (MCF7), in particular compounds **6b-f** posses antitumor activity against the leukemic cells (CCRF-CEM). This higher cytotoxicity than for alkyl derivatives **7a-b**, **13** is likely due to their cell penetration ability and may be related to some other mechanism of action rather than the result of CK2α kinase inhibition.

A new strategy for the herein presented synthesis allows the preparation of novel TBBt derivatives **6a-g** and **13** with significantly increased solubility in common organic solvents, what can be considered as advantage as compared with low solubility of TBBt **5**.

4. Experimental

4.1. General details

All commercially available reagents (Aldrich, Fluka and POCH) for chemistry purposes were used without further purification. Solvents (methylene chloride, acetonitrile, acetone) were dried by simply allowing them to stand over activated (predried at 300 °C for 24 h) 3 Å molecular sieves [20% mass/volume (m/v) loading of the desiccant] at least for 48 h before use [51]. Dimethyl Sulphoxide (DMSO), Molecular Biology grade used as a solvent for all stocks of the chemical agents was obtained from Roth, [gamma-P32] ATP (3000 Ci/mmol) was obtained from Hartmann Analytic GmbH; P81 (2.3 cm) circles were from Whatman. hCK2 substrate peptide (RRRDDDSDDD) was purchased from Biaffin GmbH & Co KG. All other reagents used in this study were of analytical grade. Melting points were obtained with MPA100 Optimelt SRS apparatus. Thin-layer chromatography was carried on TLC aluminium plates with silica gel Kieselgel 60 F_{254} (Merck) (0.2 mm thickness film) using UV light as a visualizing agent. Preparative separations were carried out by: (i) column chromatography using Silica gel with grain size 40-63 μm or 15-40 μm, respectively or by (ii) PLC Silica gel 60 F_{254} (20 × 20 cm with 2 mm thickness layer) glass plates from Merck. ^{1}H and ^{13}C NMR spectra were measured with a Varian Mercury 400BB spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C nuclei, chemical shifts (δ) are given in parts per million (ppm) on the delta scale. The solvent peak was used as reference value; signal multiplicity assignment: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; the type of signals: br., broad; coupling constant (*J*) are given in hertz (Hz). All of the fid documents of ^{1}H and ^{13}C NMR spectra were analyzed by ACD/NMR Processor Academic Edition 12.0 (freeware software). Mass spectrometry was recorded on Micro-mass ESI Q-TOF spectrometer at IBB PAN Warsaw. IR spectra of neat samples were recorded on a Perkin Elmer System 2000 FTIR Spectrometer equipped with a Pike Technologies GladiATR attenuated total reflectance (ATR) accessory with a monolithic diamond crystal stage and a pressure clamp; FTIR spectra were recorded in transmittance mode in the 300-4000 cm^{-1} range, in ambient air at room temperature, with 2 cm^{-1} resolution and accumulation of 32 scans.

*4.1. 1. General procedure for the synthesis 1,3-diols (**2a-g**) from β-keto esters (**1a-g**):*

$NaBH_4$ (8 equiv) was added portion-wise to the solution of an appropriate β-keto ester **1a-g** (1.5 g) in MeOH (15 mL) at room temperature. After 20 min the heterogeneous white reaction mixture was heated to reflux until all starting material was consumed (approx. 12 h, TLC). The cooled mixture was concentrated under reduced pressure and partitioned between distilled water (35 mL) and EtOAc (40 mL). The layers were separated, and the aqueous phase was back-extracted with EtOAc (3 x 40 mL). The combined extracts were dried (Na_2SO_4), concentrated under reduced pressure, and purified by chromatography on silica gel using mixture of $PhCH_3$/AcOEt (1:1, v/v) as an eluent to give the corresponding 1,3-diol **2a-g**.

*4.1.1.1. 1-Phenylpropane-1,3-diol (**2a**)*:

Colorless oil; 82% yield; R_f [$PhCH_3$/AcOEt (1:1, v/v)] 0.29; ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ*: 1.84-2.02 (m, 2H, CH_2), 3.26 (br. s., 2H, O*H*), 3.74-3.84 (m, 2H, CH_2OH), 4.90 (dd, *J*=8.8, 3.8 Hz, 1H, C*H*), 7.24-7.38 (m, 5H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*: 40.3 (*C*H_2), 61.1 (*C*H_2OH), 73.9 (*C*H), 125.6 (2C, *o*-Ph), 127.5 (*p*-Ph), 128.4 (2C, *m*-Ph), 144.2 (Ph).

*4.1.1.2. 1-(4-Bromophenyl)propane-1,3-diol (**2b**):*

Colorless oil; 96% yield; R_f [$PhCH_3$/AcOEt (1:1, v/v)] 0.24; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 1.81-2.00 (m, 2H, CH_2), 2.94 (br. s., 2H, O*H*), 3.77-3.88 (m, 2H, CH_2OH), 4.90 (dd, *J*=8.4, 3.8 Hz, 1H, C*H*), 7.18-7.25 (m, 2H, *o*-Ph), 7.43-7.51 (m, 2H, *m*-Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 40.3 (*C*H_2), 61.3 (*C*H_2OH), 73.6 (*C*H), 121.2 (*p*-Ph), 127.4 (2C, *o*-Ph), 131.5 (2C, *m*-Ph), 143.2 (Ph).

*4.1.1.3. 1-(4-Chlorophenyl)propane-1,3-diol (**2c**):*

Yellowish oil; 81% yield; R_f [$PhCH_3$/AcOEt (1:1, v/v)] 0.21; ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ*: 1.82-2.02 (m, 2H, CH_2), 2.77 (br. s., 2H, O*H*), 3.78-3.90 (m, 2H, CH_2OH), 4.94 (dd, *J*=8.6, 3.6 Hz, 1H, C*H*), 7.27-7.35 (m, 4H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*: 40.4 (*C*H_2), 61.4 (CH_2OH), 73.6 (*C*H), 126.9 (2C, *o*-Ph), 128.5 (2C, *m*-Ph), 133.0 (*p*-Ph), 142.6 (Ph).

*4.1.1.4. 1-(4-Fluorophenyl)propane-1,3-diol (**2d**):*

Colorless oil; 78% yield; R_f [$PhCH_3$/AcOEt (1:1, v/v)] 0.16; ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ*: 1.81-2.01 (m, 2H, CH_2), 3.20 (s, 2H, O*H*), 3.74-3.86 (m, 2H, CH_2OH), 4.90 (dd, *J*=8.8, 3.6 Hz, 1H, C*H*), 6.97-7.07 (m, 2H, *m*-Ph), 7.27-7.34 (m, 2H, *p*-Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*: 40.4 (*C*H_2), 61.2 (*C*H_2OH), 73.5 (*C*H), 115.2 (d, *J*=21.0 Hz, 2C, *m*-Ph), 127.2 (d, *J*=7.6 Hz, 2C, *o*-Ph), 140.0 (d, *J*=2.9 Hz, 1C, Ph), 162.1 (d, *J*=245.3 Hz, 1C, *p*-Ph).

*4.1.1.5. 1-(3-Bromophenyl)propane-1,3-diol (**2e**):*

Yellowish oil; 85% yield; R_f [$PhCH_3$/AcOEt (1:1, v/v)] 0.24; ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ*: 1.86-2.01 (m, 2H, CH_2), 2.65 (br. s., 2H, O*H*), 3.79-3.90 (m, 2H, CH_2OH), 4.92 (dd, *J*=8.1, 4.1 Hz, 1H, C*H*), 7.17-7.30 (m, 2H, *m*-Ph and one of *o*-Ph), 7.33-7.43 (m, 1H, *p*-Ph), 7.50-7.54 (m, 1H, *o*-Ph-situated closer to Br); ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*: 40.3 (*C*H_2), 61.4 (*C*H_2OH), 73.6 (C*H*), 122.5 (*m*-Ph-Br), 124.2 (*o*-Ph), 128.7 (*o*-Ph-situated closer to Br), 130.0 (*m*-Ph), 130.4 (*p*-Ph), 146.5 (Ph).

*4.1.1.6. 1-(3-Chlorophenyl)propane-1,3-diol (**2f**):*

Yellowish oil; 86% yield; R_f [$PhCH_3$/AcOEt (1:1, v/v)] 0.20; ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ*: 1.85-2.01 (m, 2H, CH_2), 2.99 (br. s., 2H, O*H*), 3.76-3.90 (m, 2H, CH_2OH), 4.92 (dd, *J*=8.1, 4.3 Hz, 1H, C*H*), 7.18-7.30 (m, 3H, Ph), 7.33-7.38 (m, 1H, *o*-Ph-situated much further from Cl); ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*: 40.3 (*C*H_2), 61.3 (*C*H_2OH), 73.5 (*C*H), 123.7 (*o*-Ph), 125.8 (*o*-Ph-situated closer to Cl), 127.5 (*p*-Ph), 129.7 (*m*-Ph), 134.3 (*m*-Ph-Cl), 146.2 (Ph).

*4.1.1.7. 1-(3-Methoxyphenyl)propane-1,3-diol (**2g**):*

Yellowish oil; 95% yield; R_f [$PhCH_3$/AcOEt (1:1, v/v)] 0.22; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.84-2.06 (m, 2H, CH_2), 2.92 (br. s., 2H, O*H*), 3.80 (s, 3H, OCH_3), 3.81-3.87 (m, 2H, CH_2OH), 4.91 (dd, *J*=8.6, 3.8 Hz, 1H, C*H*), 6.78-6.83 (m, 1H, Ph), 6.90-6.95 (m, 2H, Ph), 7.22-7.29 (m, 1H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 40.4 (*C*H_2), 55.2 (O*C*H_3), 61.3 (*C*H_2OH), 74.1 (*C*H), 111.1 (*o*-Ph-situated closer to OCH_3), 112.9 (*p*-Ph), 117.8 (*o*-Ph), 129.4 (*m*-Ph), 145.9 (Ph), 159.6 (*m*-Ph-OCH_3).

*4.1.2. General procedure for the synthesis **3a-g** by the monotosylation of the 1,3-diols (**2a-g**):*

To a flame-dried flask, containing the 1,3-diol **2a-g** (1 g) dissolved in dry CH_2Cl_2 (15 mL), freshly distilled triethylamine (1.1 equiv) and 4-(dimethylamino)pyridine (10 mg) were added at -15 °C under an nitrogen atmosphere. After 15 min, *p*-toluenesulfonyl chloride (1 equiv) suspended in anhydrous CH_2Cl_2 (6 mL) was added dropwise *via* syringe with stirring over a period of 1 h under fast stream of nitrogen. Upon completion of the addition, the reaction mixture was maintained at -15 °C for 12 h and then allowed to warm to room temperature and stirred for a further 1 h. Next, the content of the flask was cooled to 0 °C, portion of CH_2Cl_2 (30 mL) was added, and the solution was quenched successively by the careful addition of cold 1% HCl (2 x 15 mL), washed with saturated sodium bicarbonate (2 x 15 mL), brine (2 x 15 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to yield the crude product. The purification by column chromatography on silica gel with mixture of ethyl acetate/cyclohexane (4:1 or 1:1, v/v – depending on the substance purified) as an eluent afforded the desired monotosylated alcohol **3a-g**.

*4.1.2.1. 3-Hydroxy-3-phenylpropyl 4-methylbenzenesulfonate (**3a**):*

Yellowish oil; 41% yield; R_f [AcOEt/Cyclohexene (4:1, v/v)] 0.67; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.97-2.06 (m, 2H, CH_2), 2.10 (br. s., 1H, O*H*), 2.45 (s, 3H, CH_3), 4.00-4.10 (m, 1H, CH_2OSO$_2$), 4.22-4.33 (m, 1H, CH_2OSO$_2$), 4.76-4.83 (m, 1H, C*H*), 7.24-7.38 (m, 7H, Ph), 7.76-7.82 (m, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.7 (*C*H_3), 38.0 (*C*H_2), 67.6 (*C*H_2OSO$_2$), 70.15 (*C*H), 125.5 (2C, *o*-Ph), 127.8 (*p*-Ph), 127.8 (2C, *o*-PhTos), 128.5 (2C, *m*-PhTos), 129.8 (2C, *m*-Ph), 132.8 (*p*-PhTos), 143.4 (Ph), 144.7 (PhTos).

*4.1.2.2. 3-(4-Bromophenyl)-3-hydroxypropyl 4-methylbenzenesulfonate (**3b**):*

Colorless oil; 61% yield; R_f [AcOEt/Cyclohexene (1:1, v/v)] 0.76; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.98 (td, *J*=6.55, 5.42 Hz, 2H, CH_2), 2.23 (br. s., 1H, O*H*), 2.46 (s, 3H, CH_3), 3.96-4.07 (m, 1H, CH_2OSO$_2$), 4.27 (dt, *J*=10.0, 6.6 Hz, 1H, CH_2OSO$_2$), 4.79 (t, *J*=6.6 Hz, 1H, C*H*), 7.11-7.17 (m, 2H, Ph), 7.32-7.38 (m, 2H, Ph), 7.39-7.45 (m, 2H, Ph), 7.74-7.80 (m, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.7 (*C*H_3), 38.0 (*C*H_2), 67.3 (*C*H_2OSO$_2$), 69.5 (*C*H), 121.5 (*p*-Ph), 127.3 (2C, *o*-Ph), 127.9 (2C, *o*-PhTos), 129.9 (2C, *m*-PhTos), 131.6 (2C, *m*-Ph), 132.7 (*p*-PhTos), 142.4 (Ph), 144.9 (PhTos).

*4.1.2.3. 3-(4-Chlorophenyl)-3-hydroxypropyl 4-methylbenzenesulfonate (**3c**):*

Yellowish oil; 33% yield; R_f [AcOEt/Cyclohexene (4:1, v/v)] 0.73; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.94-2.03 (m, 2H, CH_2), 2.33 (br. s., 1H, O*H*), 2.45 (s, 3H, CH_3), 3.98-4.05 (m, 1H, CH_2OSO$_2$), 4.22-4.30 (m, 1H, CH_2OSO$_2$), 4.79 (t, *J*=6.7 Hz, 1H, *C*H), 7.17-7.22 (m, 2H, Ph), 7.23-7.29 (m, 2H, Ph), 7.31-7.38 (m, 2H, Ph), 7.73-7.79 (m, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.6 (*C*H_3), 38.0 (*C*H_2), 67.3 (*C*H_2OSO$_2$), 69.4 (*C*H), 127.0 (2C, *o*-Ph), 127.9 (2C, *m*-Ph), 128.6 (2C, *o*-PhTos), 129.9 (2C, *m*-PhTos), 132.7 (*p*-PhTos), 133.4 (*p*-Ph), 141.9 (Ph), 144.9 (PhTos).

*4.1.2.4. 3-(4-Fluorophenyl)-3-hydroxypropyl 4-methylbenzenesulfonate (**3d**):*

Colorless oil; 66% yield; R_f [AcOEt/Cyclohexene (1:1, v/v)] 0.71; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.94-2.06 (m, 2H, CH_2), 2.22 (br. s., 1H, O*H*), 2.45 (s, 3H, CH_3), 3.98-4.06 (m, 1H, CH_2OSO$_2$), 4.22-4.31 (m, 1H, CH_2OSO$_2$), 4.79 (dd, *J*=7.9, 5.7 Hz, 1H, C*H*), 6.95-7.03 (m, 2H, *m*-Ph), 7.20-7.27 (m, 2H, *o*-PhTos), 7.33-7.36 (m, 2H, *m*-PhTos), 7.74-7.80 (m, 2H, *o*-Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.6 (*C*H_3), 38.1 (*C*H_2), 67.4 (*C*H_2OSO$_2$), 69.5 (*C*H), 115.3 (d, *J*=21.2 Hz, 2C, *m*-Ph), 127.3 (d, *J*=7.6 Hz, 2C, *o*-Ph), 127.8 (2C, *o*-PhTos), 129.8 (2C, *m*-PhTos), 132.7 (*p*-PhTos), 139.2 (d, *J*=3.8 Hz, 1C, Ph), 144.9 (PhTos), 162.2 (d, *J*=246.3 Hz, 1C, *p*-Ph).

*4.1.2.5. 3-(3-Bromophenyl)-3-hydroxypropyl 4-methylbenzenesulfonate (**3e**):*

Yellowish oil; 82% yield; R_f [AcOEt/Cyclohexene (4:1, v/v)] 0.67; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.97-2.01 (m, 2H, CH_2), 2.06 (br. s., 1H, O*H*), 2.46 (s, 3H, CH_3), 4.06 (dt, *J*=10.2, 5.2 Hz, 1H, CH_2OSO$_2$), 4.30 (ddd, *J*=10.1, 8.1, 5.3 Hz, 1H, CH_2OSO$_2$), 4.79 (dd, *J*=8.4, 5.0 Hz, 1H, C*H*), 7.14-7.22 (m, 2H, Ph), 7.31-7.47 (m, 5H, Ph), 7.76-7.83 (m, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.7 (*C*H_3), 38.1 (*C*H_2), 67.2 (*C*H_2OSO$_2$), 69.4 (*C*H), 122.7 (*m*-Ph-Br), 124.2 (*o*-Ph), 127.9 (2C, *o*-PhTos), 128.7 (*o*-Ph-situated closer to Br), 129.9 (2C, *m*-PhTos), 130.1 (*m*-Ph), 130.8 (*p*-Ph), 132.7 (*p*-PhTos), 144.9 (Ph), 145.8 (PhTos).

*4.1.2.6. 3-(3-Chlorophenyl)-3-hydroxypropyl 4-methylbenzenesulfonate (**3f**):*

Yellowish oil; 40% yield; R_f [AcOEt/Cyclohexene (4:1, v/v)] 0.71; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.98 (dtd, *J*=8.0, 5.2, 5.2, 3.1 Hz, 2H, CH_2), 2.27 (br. s., 1H, O*H*), 2.45 (s, 3H, CH_3), 3.99-4.08 (m, 1H, CH_2OSO$_2$), 4.29 (ddd, *J*=10.1, 7.8, 5.4 Hz, 1H, CH_2OSO$_2$), 4.78 (dd, *J*=8.0, 5.3 Hz, 1H, C*H*), 7.11-7.17 (m, 1H, Ph), 7.20-7.28 (m, 3H, Ph), 7.33-7.37 (m, 2H, Ph), 7.75-7.81 (m, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.7 (*C*H_3), 38.0 (*C*H_2), 67.3 (*C*H_2OSO$_2$), 69.4 (*C*H), 123.8 (*o*-Ph), 125.8 (*o*-Ph-situated closer to Cl), 127.8 (3C, *p*-Ph and *o*-PhTos), 129.8 (*m*-Ph), 129.9 (2C, *m*-PhTos), 132.7 (*p*-PhTos), 134.4 (*m*-Ph-Cl), 144.9 (Ph), 145.6 (PhTos).

*4.1.2.7. 3-(3-Methoxyphenyl)-3-hydroxypropyl 4-methylbenzenesulfonate (**3g**):*

Yellowish oil; 39% yield; R_f [AcOEt/Cyclohexene (4:1, v/v)] 0.64; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.96-2.04 (m, 2H, CH_2), 2.05 (br. s., 1H, O*H*), 2.45 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 4.01-4.11 (m, 1H, CH_2OSO$_2$), 4.27 (dt, *J*=9.9, 6.7 Hz, 1H, CH_2OSO$_2$), 4.78 (t, *J*=6.7 Hz, 1H, C*H*), 6.76-6.88 (m, 3H, Ph), 7.17-7.27 (m, 1H, Ph), 7.29-7.39 (m, 2 H, Ph), 7.79-7.81 (m, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.6 (*C*H_3), 38.0 (*C*H_2), 55.2 (O*C*H_3), 67.6 (*C*H_2OSO$_2$), 70.1 (*C*H), 111.0 (*o*-Ph-situated closer to OCH_3), 113.3 (*p*-Ph), 117.8 (*o*-Ph), 127.9 (2C, *m*-PhTos), 129.6 (*m*-Ph), 129.9 (2C, *o*-PhTos), 132.8 (*p*-PhTos), 144.8 (Ph), 145.2 (PhTos), 159.7 (O*C*H_3).

*4.1.3. Synthesis of 4,5,6,7-tetrabromo-1H-benzotriazole (**5**):*

1*H*-Benzotriazole **4** (6 g, 50.4 mmol) was dissolved in a mixture of 69% HNO_3 (150 mL) and fuming 100% HNO_3 (10 mL). Next, the solution was heated to a temperature of 80 °C and Br_2 (48.3 g, 302 mmol, 15 mL) was added dropwise within 1 h.

The reaction mixture was stirred vigorously using mechanical stirrer at 60 °C for 48 h and irradiated by exposure to UV light. Subsequently, content of the flask was cooled to room temperature, the excess of unreacted bromine was removed in the gentle flow of nitrogen and trapped into a 20 % solution of sodium pyrosulfite (150 mL). The suspension was poured into mixture of ice-cold H_2O (300 mL) and saturated $Na_2S_2O_5$ (20 mL). The resulting yellowish precipitate was filtered off, and washed with H_2O (100 mL) and EtOH (100 mL), respectively. The obtained crude product was refluxed several times in the mixture of MeOH (50 mL) and *i*-PrOH (25 mL), and hot saturated solution was filtered off yielding white crystals **5** (13 g, 29.9 mmol, 59%). The purity of the compound **5** was confirmed by high-resolution mass spectrometry (HRMS). The ^{1}H-, ^{13}C-NMR and m.p. were consistent with the literature data [46]. White solid; mp 264-266 °C; yield: 40%; R_f [$CHCl_3$/MeOH (99:1, v/v)] 0.16; HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 435.6941, $[M+H]^+_{found}$ = 435.6629.

*4.1.4. General procedure for the synthesis of **6a-g**:*

To the solution of TBBt **5** (1 equiv) in the mixture of absolute acetone (15 mL) and dry acetonitrile (10 mL), anhydrous K_2CO_3 (3 equiv) was added. The content of the flask was flushed with nitrogen for several times, and stirred for 3 h at reflux. Next, the reaction mixture was cooled to room temperature and the solution of appropriate 3-hydroxy-3-arylpropyl-4-methylbenzenesulfonate **3a-g** (400 mg) in acetonitrile (5 mL) was added dropwise. After stirring at reflux for 48 h, the crude reaction mixture was cooled to room temperature, and both the white precipitate as well as the red colored solution were transferred into separator and washed with saturated solution of potassium carbonate (100 mL). Water phase was extracted with $CHCl_3$ (2 x 150 mL) and AcOEt (3 x 100 mL), and the collected organic layers were partially condensed on rotator evaporator, and dried (Na_2SO_4). The purification procedure was performed on preparative layer plates (PLC) coated with unmodified silica gel matrices, using mixture of $CHCl_3$/Et_2O (7:3 or 6:4, v/v) or $CHCl_3$/acetone (95:5, v/v), depending on substance purified. The appropriate fraction was removed, placed in the round-bottomed flask, suspended in the mixture of $CHCl_3$/acetone (150 mL, 2:1, v/v), and vigorously stirred for 1 h at room temperature. Next, the silica gel was filtered, and washed with $CHCl_3$/acetone (2 x 50 mL, 1:1, v/v). After partial solvent removal *in vacuo*, solution of the filtrate (approx. 1.5 mL) was kept in the refrigerator until white precipitate occurred. Next, the liquid phase was decanted off by Pasteur pipette tipped with cotton wool, and the remaining solid was washed with ice-cold acetone (1 mL), and then ethanol (1 mL) respectively to obtain desired product **6a-g** as a white solid, which was further high-vacuum-dried in the desiccator over phosphorus pentoxide (P_2O_5) overnight.

*4.1.4.1. 1-Phenyl-3-(4,5,6,7-tetrabromo-2H-benzotriazol-2-yl)propan-1-ol (**6a**):*

White solid; 40% yield; mp 135-136 °C; R_f [$CHCl_3$/Et_2O (7:3, v/v)] 0.67; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.31 (br. s., 1H, O*H*), 2.48-2.62 (m, 2H, CH_2), 4.82 (dd, *J*=7.5, 5.6 Hz, 1H, C*H*), 4.85-5.02 (m, 2H, CH_2N), 7.23-7.40 (m, 5H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 38.6 (*C*H_2), 54.4 (*C*H_2N), 71.4 (*C*H), 113.7 (2C, TBBt-internal), 125.6 (2C, TBBt-external), 126.3 (2C, *o*-Ph), 128.0 (2C, *m*-Ph), 128.6 (*p*-Ph), 143.0 (Ph), 143.1 (TBBt=N); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 569.7673, $[M+H]^+_{found}$ = 569.8090; (ESI$^-$, m/z): $[M+H]^-_{calcd}$ = 567.7516, $[M+H]^-_{found}$ = 567.7919; FTIR ν_{max}(neat): 3549, 3417, 1533, 1490, 1430, 1272, 1178, 1162, 1086, 1057, 975, 765, 748, 698, 606, 516.

*4.1.4.2. 1-(4-Bromophenyl)-3-(4,5,6,7-tetrabromo-2H-benzotriazol-2-yl)propan-1-ol (**6b**):*

White solid; 33% yield; mp 125-126 °C; R_f [$CHCl_3$/acetone (95:5, v/v)] 0.55; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.97 (br. s., 1H, O*H*), 2.44-2.61 (m, 2H, CH_2), 4.78 (dd, *J*=7.8, 5.1 Hz, 1H, C*H*), 4.83-5.02 (m, 2H, CH_2N), 7.14-7.25 (m, 2H, *o*-Ph), 7.38-7.47 (m, 2H, *m*-Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 38.4 (*C*H_2), 54.2 (*C*H_2N), 70.8 (*C*H), 113.7 (TBBt-internal), 121.7 (*p*-Ph), 126.5 (2C, TBBt-external), 127.4 (2C, *o*-Ph), 131.6 (2C, *m*-Ph), 141.9 (Ph), 143.1 (TBBt=N); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 649.6758, $[M+H]^+_{found}$ = 649.7219; (ESI$^-$, m/z): $[M+H]^-_{calcd}$ = 647.6601, $[M+H]^-_{found}$ = 647.7287; FTIR ν_{max}(neat): 3417, 1588, 1533, 1486, 1426, 1309, 1295, 1274, 1176, 1159, 1073, 1011, 979, 926, 876, 823, 772, 750, 718, 609, 544, 509.

*4.1.4.3. 1-(4-Chlorophenyl)-3-(4,5,6,7-tetrabromo-2H-benzotriazol-2-yl)propan-1-ol (**6c**):*

White solid; 41% yield; mp 112-114 °C; R_f [$CHCl_3$/Et_2O (6:4, v/v)] 0.51; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.42-2.58 (m, 2H, CH_2), 2.71 (br. s., 1H, O*H*), 4.79 (dd, *J*=8.1, 5.0 Hz, 1H, C*H*), 4.91 (dt, *J*=19.1, 6.9 Hz, 2H, CH_2N), 7.23-7.27 (m, 4H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 38.5 (*C*H_2), 54.1 (*C*H_2N), 70.6 (*C*H), 113.6 (2C, TBBt-internal), 126.4 (2C, TBBt-external), 127.0 (2C, *m*-Ph), 128.6 (2C, *o*-Ph), 133.5 (*p*-Ph), 141.4 (Ph), 142.9 (Tbbt=N); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 603.7283, $[M+H]^+_{found}$ = 603.7826; (ESI$^-$, m/z): $[M+H]^-_{calcd}$ = 601.7127, $[M+H]^-_{found}$ = 601.7653; FTIR ν_{max}(neat): 3387, 1739, 1701, 1490, 1426, 1366, 1270, 1230, 1218, 1174, 1089, 1074, 1012, 978, 832, 770, 750, 607, 530.

*4.1.4.4. 1-(4-Fluorophenyl)-3-(4,5,6,7-tetrabromo-2H-benzotriazol-2-yl)propan-1-ol (**6d**):*

White solid; 33% yield; mp 73-75 °C; R_f [$CHCl_3$/acetone (95:5, v/v)] 0.44; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.10 (br. s., 1H, O*H*), 2.47-2.57 (m, 2H, CH_2), 4.80 (dd, *J*=7.4, 5.7 Hz, 1H, C*H*), 4.84-5.02 (m, 2H, CH_2N), 6.98-7.06 (m, 2H, *m*-Ph), 7.29-7.37 (m, 2H, *o*-Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 38.7 (*C*H_2), 54.3 (*C*H_2N), 70.7 (*C*H), 113.7 (2C, TBBt-internal), 115.5 (d, *J*=21.9 Hz, 2C, *m*-Ph), 126.4 (2C, TBBt-external), 127.3 (d, *J*=7.6 Hz, 2C, *o*-Ph), 138.8 (d, *J*=2.9 Hz, 1C, Ph), 143.1 (TBBt=N), 162.3 (d, *J*=246.4 Hz, 1C, *p*-Ph); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 587.7579, $[M+H]^+_{found}$ = 587.8105; (ESI$^-$, m/z): $[M+H]^-_{calcd}$ = 585.7422, $[M+H]^-_{found}$ = 585.7770; FTIR ν_{max}(neat): 3401, 1604, 1531, 1507, 1426, 1395, 1344, 1298, 1271, 1217, 1174, 1157, 1100, 1073, 1013, 977, 929, 883, 835, 771, 746, 608, 567, 544.

*4.1.4.5. 1-(3-Bromophenyl)-3-(4,5,6,7-tetrabromo-2H-benzotriazol-2-yl)propan-1-ol (**6e**):*

White solid; 36% yield; mp 88-91 °C; R_f [$CHCl_3$/acetone (95:5, v/v)] 0.55; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.22 (br. s., 1H, O*H*), 2.44-2.63 (m, 2H, CH_2), 4.79 (dd, *J*=8.2, 4.6 Hz, 1H, C*H*), 4.84-5.02 (m, 2H, CH_2N), 7.16-7.22 (m, 1H, Ph), 7.23-7.29 (m, 1H, Ph), 7.35-7.40 (m, 1H, Ph), 7.48-7.51 (m, 1H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 38.5 (*C*H_2), 54.1 (*C*H_2N), 70.6 (*C*H), 113.6 (2C, TBBt-internal), 122.7 (*m*-Ph-Br), 124.2 (2C, TBBt-external), 126.4 (*o*-Ph), 128.7 (*m*-Ph), 130.2 (*o*-Ph-situated closer to Br), 130.9 (*p*-Ph), 143.0 (TBBt=N), 145.3 (Ph); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 647.6778, $[M+H]^+_{found}$ = 647.7335; (ESI$^-$, m/z): $[M+H]^-_{calcd}$ = 645.6622, $[M+H]^-_{found}$ = not determined; FTIR ν_{max}(neat): 3397, 1740, 1591, 1573, 1534, 1478, 1426, 1342, 1305, 1275, 1241, 1146, 1172, 1100, 1070, 1035, 1014, 1004, 977, 878, 800, 788, 753, 693, 667, 608, 585, 511, 443.

*4.1.4.6. 1-(3-Chlorophenyl)-3-(4,5,6,7-tetrabromo-2H-benzotriazol-2-yl)propan-1-ol (**6f**):*

White solid; 42% yield; mp 114-115 °C; R_f [$CHCl_3$/acetone (95:5, v/v)]; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.68 (br. s., 1H, O*H*), 2.44-2.64 (m, 2H, CH_2), 4.80 (dd, *J*=8.3, 4.5 Hz, 1H, C*H*), 4.83-5.05 (m, 2H, CH_2N), 7.16-7.29 (m, 3H, Ph), 7.34 (t, *J*=1.8 Hz, 1H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 38.5 (*C*H_2), 54.2 (*C*H_2N), 70.7 (*C*H), 113.7 (2C,TBBt-internal), 123.7 (2C, TBBt-external), 125.8 (*o*-Ph), 126.4 (*o*-Ph-situated closer to Cl), 128.0 (*m*-Ph), 129.9 (*p*-Ph), 134.5 (*m*-Ph-Cl), 143.1 (Ph), 145.1 (TBBt=N); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 603.7283, $[M+H]^+_{found}$ = 603.7560; FTIR ν_{max}(neat): 3429, 1596, 1573, 1535, 1476, 1445, 1428, 1370, 1335, 1277, 1201, 1173, 1146, 1099, 1072, 1051, 1041, 1010, 977, 881, 868, 798, 791, 777, 767, 718, 700, 688, 609, 500, 455.

*4.1.4.7. 1-(3-Methoxyphenyl)-3-(4,5,6,7-tetrabromo-2H-benzotriazol-2-yl)propan-1-ol (**6g**):*

White solid; 33% yield; mp 79-80 °C; R_f [$CHCl_3$/acetone (95:5, v/v)] 0.42; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.00 (br. s., 1H, O*H*), 2.50-2.61 (m, 2H, CH_2), 3.80 (s, 3H, OCH_3), 4.79 (dd, *J*=7.4, 5.5 Hz, 1H, C*H*), 4.93 (td, *J*=13.7, 6.4 Hz, 2H, CH_2N), 6.77-6.80 (m, 1H, Ph), 6.89-6.94 (m, 2H, Ph), 7.21-7.26 (m, 1H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 38.5 (*C*H_2), 54.4 (*C*H_2N), 55.2 (O*C*H_3), 71.3 (*C*H), 111.1 (*p*-Ph), 113.3 (*o*-Ph-situated closer to OCH_3), 113.7 (*o*-Ph), 117.8 (TBBt-internal), 126.3 (TBBt-external), 129.7 (*m*-Ph), 143.1 (TBBt=N), 144.7 (Ph), 159.8 (*m*-Ph-OCH_3); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 599.7779, $[M+H]^+_{found}$ = 599.8348; (ESI$^-$, m/z): $[M+H]^-_{calcd}$ = 597.7622, $[M+H]^-_{found}$ = not determined; FTIR ν_{max}(neat): 3529, 3447, 2932, 2831, 1735, 1604, 1579, 1533, 1482, 1429, 1377, 1306, 1284, 1273, 1256, 1235, 1176, 1166, 1146, 1095, 1062, 1041, 995, 977, 933, 913, 886, 834, 785, 770, 739, 700, 605, 566, 525, 475.

*4.1.5. Synthesis of (2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate (**11**):*

Tosyl chloride (2.07 g, 10.89 mmol) was added in small portions (during 1 h) to a solution of 2,2-dimethyl-1,3-dioxolane-4-methanol **10** (1.20 g, 9.08 mmol), triethylamine (1.38 g, 13.62 mmol, 1.90 mL), and DMAP (112 mg, 0.9 mmol) in dry CH_2Cl_2 (20 mL), with magnetic stirring at 0-5 °C. After 5 h, Et_2O (15 mL) was added, and the solution was washed with 10% water solution of HCl (2 x 15 mL), saturated aqueous $NaHCO_3$ (20 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by column chromatography over silica gel using mixture of hexane/ethyl acetate (5:1, v/v) as an eluent, to give the product **11** as white solid (2.1 g, 7.33 mmol, 81%) with >99% purity according to GC. White solid; 81% yield; mp 50-52 °C [Lit. [52]: mp 49-50 °C after recrystallization from petroleum ether/diethyl ether (10:1, v/v)]; R_f [hexane/ethyl acetate (5:1, v/v)] 0.36; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.31 (d, *J*=11.5 Hz, 6H, 2xCH_3), 2.43 (s, 3H, *p*-CH_3), 3.74 (dd, *J*=8.8, 5.2 Hz, 1H, partially CH_2), 3.92-4.06 (m, 3H, C*H* and partially CH_2 and partially CH_2OSO$_2$), 4.21-4.31 (m, 1H, partially CH_2OSO$_2$), 7.34 (d, *J*=8.1 Hz, 2H, Ph), 7.78 (d, *J*=8.1 Hz, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.6 (*p*-*C*H_3), 25.0 (*C*H_3), 26.5 (*C*H_3), 66.0 (CH*C*H_2O), 69.4 (*C*H_2OSO$_2$), 72.8 (*C*H), 109.9 (*C*), 127.9 (2C, *m*-Tos), 129.8 (2C, *o*-Tos), 132.5 (*p*-Tos), 145.0 (PhOSO$_2$); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 287.0953, $[M+H]^+_{found}$ = 287.0884; GC [100-260 (10 °C/min)]: t_R = 14.82 min.

*4.1.6. Synthesis of 4,5,6,7-tetrabromo-2-[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]-2H-benzotriazole (**12**):*

This compound was prepared by using the procedure as described in synthesis detail for compounds **6a-g**. Briefly, equimolar amounts of both reagents: **11** (400 mg, 1.4 mmol) and **5** (610 mg, 1.4 mmol) in the presence of 3-fold molar excess of K_2CO_3 (582 mg, 4.21 mmol) were stirred in refluxing mixture of absolute acetone (15 mL) and dry acetonitrile (10 mL) for 48 h. Crude product **12** was partially purified by simple filtration on short silica pad by eluting with mixture of $CHCl_3$/acetone (7:3, v/v) just to remove unreacted **5**. As the high resolution mass spectrometry analysis confirmed presence of desired product **12**, and absence of compound **5**, it was allowed to use in the next step without further purification. HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 549.7622, $[M+H]^+_{found}$ = 549.7753.

*4.1.7. Synthesis of 3-(4,5,6,7-tetrabromo-2H-benzotriazol-2-yl)propane-1,2-diol (**13**):*

To the compound **12** (165 mg, 0.3 mmol) dissolved in MeOH (3 mL), HCl (0.5 *N*, 0.3 mL) was added dropwise, and the resulting mixture was heated to reflux. After 4 h acetone and methanol were slowly distilled off. Additional portion of MeOH (1 mL) and HCl (0.5 *N*, 0.2 mL) was added, and the mixture was kept at room temperature until ketal hydrolysis was completed (approx. 2 h, TLC). The mixture was diluted with careful addition of saturated $NaHCO_3$ until effervescence ceased, and the water phase was extracted with EtOAc (3 x 10 mL). The organic extracts were combined, washed with brine (5 mL), dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified by preparative chromatography (PLC) using mixture of $CHCl_3$/acetone (9:1, v/v) as an eluent, affording target compound **13** as hygroscopic white solid (95 mg, 0.19 mmol, 62%). White semi-solid; 30% yield (after two steps: from **11** to **13**); R_f [$CHCl_3$/acetone (9:1, v/v)] 0.78; ^{1}H-NMR (MeOD, 400 MHz) δ: 3.59-3.74 (m, 2H, CH_2OH), 4.16-4.42 (m, 1H, C*H*OH) 4.76 (s, 1H, CH_2N), 4.93-5.21 (m, 1H, CH_2N); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 53.2 (*C*H_2N), 63.5 (*C*H_2OH), 71.0 (*C*HOH), 115.7 (TBBt-internal), 125.4 (TBBt-external), 142.6 (TBBt=N); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 509.7309, $[M+H]^+_{found}$ = 509.7447.

4.1.8. Biological Assays

4.1.8.1. Cloning, expression and purification of human CK2α:

The coding region of human CK2α was amplified by polymerase chain reaction using the following primers: 5'-CGCGGATCCGTCGGGACCCGTGCCAA (upstream primer) and 5'-CCCAAGCTTCTGCTGAGCGCCAGCGGCA (downstream primer) and I.M.A.G.E. clone as a template. The product was cloned into the vector pETDuet-1 (MCS1) using the restriction sites BamHI and HindIII and the bacterial strain DH5α. The sequence of the obtained clone was confirmed. Expression of the resulting N-terminal histidine-tagged hCK2α was done in the bacterial strain BL21(DE3)pLysS growing in superbroth medium after induction with 0.5 mM IPTG for 20 h at 20 °C. The cell pellet was resuspended in extraction buffer [composed of: 20 mM NaH_2PO_4 (pH 8.0), 500 mM NaCl, 10 mM imidazole, O-complete inhibitor cocktail (Roche), lysozyme (1 mg/mL)] and sonicated. The supernatant of the pellet from 200 mL of bacterial culture was loaded onto Ni-NTA agarose (Qiagene) column (2 mL bed volume). hCK2α was eluted with 300 mM imidazole. Fractions containing His-tagged hCK2α were dialyzed against 20 mM Tris-HCl (pH 8.5), 500 mM NaCl, 1 mM DTT, 0-20% glycerol and stored at -20 °C. The protein concentration in final solution was 12.68 mg/mL (determined by Bradford method and bovine serum albumin as the standard) [53].

4.1.8.2. Computational docking methods

Computer molecular dynamics simulations (docking studies) were carried out using AutoDock 4.2 program (http://autodock.scripps.edu/) [54]. Ligand molecules were prepared with ChemAxon MarvinSketch 5.12.3 (http://www.chemaxon.com/marvin/) and saved as .PDB files. The crystallographic structure of CK2α (PDB code: 1J91) was obtained from RCSB Protein Data Bank (PDB, http://www.rcsb.org/pdb/). Non-protein molecules were removed, polar hydrogen were added and Gasteiger charges were calculated with AutoDock Tools 1.5.6 package (ADT, http://mgltools.scripps.edu/) [55]. Docking was performed in 70 x 70 x 70 units grid box centered on the enzyme hydrophobic pocket with the grid spacing of 0.325 Å. For each ligand molecule 100 independent runs were performed, using Lamarckian genetic algorithm, with at most 10^6 energy evaluations and maximum number of generations of 27 000. Remaining parameters were set as default. The docking results were clustered into groups with RMSd lower than 2.0 Å. The docked ligand conformations were analyzed using ADT and Chimera 1.8 package (http://www.cgl.ucsf.edu/chimera/) [56].

4.1.8.3. Assays of CK2α subunit activity and inhibition studies:

The activity of hCK2α was tested using P81 filter isotopic assay [50]. The reaction mixture contained 20 mM Tris-HCl, pH 7.5, 20 mM $MgCl_2$, 20 µM DTT, 50 µM CK2 substrate peptide (RRRDDDSDDD), 10 µM ATP (200-300 CPM/pmol) and 200 ng hCK2α. The reaction was initiated with enzyme in a total volume of 50 µl, incubated at 30 °C, and performed for 20 min. 10 µl of a reaction mixture was spotted onto P81 paper circle. The filter papers were washed 3 x with 0.6 % phosphoric acid and once with 95% ethanol before counting in a scintillation counter (Canberra-Packard). IC_{50} values for studied compounds were determined at 4% DMSO with minimum 7 concentrations of each tested inhibitor at the range of 0.064-1000 µM and calculated by fitting the data to sigmoidal dose-response (variable slope) Y = Bottom + (Top-Bottom) / (1+10^((LogIC50-X)*HillSlope) equation in GraphPad Prism Software (La Jolla, CA, USA).

4.1.8.4. Cell culture and treatment:

MCF7 adherent cells (human breast cancer cell line) were cultured in DMEM advanced medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Sigma-Aldrich), 2 mM L-glutamine, antibiotics (100 U/ml penicillin, 100 µg/mL streptomycin) and 10 µg/mL of human recombinant insulin. CCRF-CEM suspension cells (human peripheral blood T lymphoblast cell line) were cultured in RPMI medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum and antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin). Cells were grown in 25 cm^2 cell culture flasks (Sarstedt), in a humidified atmosphere of CO_2/air (5/95%) at 37 °C.

4.1.8.5. MTT-based cytotoxicity assay:

Before the treatment MCF7 cells were trypsinized in 0.25% trypsin-EDTA solution (Sigma-Aldrich) and seeded into 96-well microplates at a density of 1.5-3 x 10^4 cells/well. Cells were treated with specific compounds dissolved in DMSO or DMSO (0.5%) at the corresponding concentrations 18 h after plating (at 70% of confluency). CCRF-CEM were seeded at 2-3 x 10^4 cells/well and treated with compounds. MTT stock solution (Sigma-Aldrich) was added to each well to a final concentration of 0.5 mg/mL. After 4 h of incubation at 37 °C water-insoluble dark blue formazan crystals were dissolved in DMSO (200 µL) (37 °C/10 min incubation), and Sorensen's glycine buffer (0.1 M glycine, 0.1 M NaCl, pH 10.5) was added (25 µL per well). The absorbance were measured at 570 nm using Synergy H4 BioTek microplate reader. All measurements were carried out in triplicate and the results are expressed in percentage of cell viability relative to control (cells without inhibitor in 0.5% DMSO). At such conditions, IC_{50} for standard anti-cancer inhibitor - doxorubicin was 1.4 µM for both MCF7 and CCRF-CEM lines.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at (…)

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- A series of 4,5,6,7-tetrabromo-1*H*-benzotriazole derivatives were synthesized.
- The most potent inhibitor of CK2α was the (*R*)-enantiomer of **7b** (IC_{50} 0.80 μM).
- The obtained results were rationalized by means of *in silico* docking experiments.
- Cytotoxicity was assessed in parallel on the human CCRF-CEM and MCF7 cell lines.

Supporting Information

for

Synthesis of novel chiral TBBt derivatives with hydroxyl moiety. Studies on inhibition of human protein kinase CK2α and cytotoxicity properties.

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General details

All commercially available reagents (Aldrich, Fluka and POCH) for chemistry purposes were used without further purification. Solvents (methylene chloride, acetonitrile, acetone) were dried by simply allowing them to stand over activated 3Å molecular sieves [20% mass/volume (m/v) loading of the desiccant] at least for 48 h before use [1]. Dimethyl Sulphoxide (DMSO), Molecular Biology grade used as a solvent for all stocks of the chemical agents was obtained from Roth, [gamma-P32] ATP (3000 Ci/mmol) was obtained from Hartmann Analytic GmbH; P81 (2.3 cm) circles were from Whatman. CK2 substrate peptide (RRRDDDSDDD) was purchased from Biaffin GmbH & Co KG. All other reagents used in this study were of analytical grade. Melting points were obtained with an MPA100 Optimelt SRS apparatus. Thin-layer chromatography was carried on TLC aluminium plates with silica gel Kieselgel 60 F_{254} (Merck) (0.2 mm thickness film) using UV light as a visualizing agent. Preparative separations were carried out by: (i) column chromatography using silica gel with grain size 40-63 μm or 15-40 μm, respectively or by (ii) PLC PSC-Fertigplatten Kieselgel 60 F_{254} (20 × 20 cm with 2 mm thickness layer) glass plates from Merck. ^{1}H and ^{13}C NMR spectra were measured with a Varian Mercury 400BB spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C nuclei, chemical shifts (δ) are given in parts per million (ppm) on the delta scale. The solvent peak was used as reference value; signal multiplicity assignment: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; the type of signals: br., broad; coupling constant (*J*) are given in hertz (Hz); All samples were performed in fully deuterated chloroform ($CDCl_3$) or methanol (CD_3OD), respectively. All of the ^{1}H and ^{13}C NMR spectra were created by ACD/NMR Processor Academic Edition 12.0. Mass spectrometry was recorded on Micro-mass ESI Q-TOF spectrometer at IBB PAN. IR spectra of neat samples were recorded on a Perkin Elmer System 2000 FTIR Spectrometer equipped with a Pike Technologies GladiATR attenuated total reflectance (ATR) accessory with a monolithic diamond crystal stage and a pressure clamp; FTIR spectra were recorded in transmittance mode in the 300-4000 cm^{-1} range, in ambient air at room temperature, with 2 cm^{-1} resolution and accumulation of 32 scans. Computer molecular dynamics simulations (docking studies) were carried out using AutoDock 4.2 program (http://autodock.scripps.edu/) [2]. Ligand molecules were prepared with ChemAxon MarvinSketch 5.12.3 (http://www.chemaxon.com/marvin/) and saved as .PDB files. The crystallographic structures of CK2α (codes: 1daw, 1j91) were obtained from RCSB Protein Data Bank (PDB, http://www.rcsb.org/pdb/). Non-protein molecules were removed, polar hydrogen were added and Gasteiger charges were calculated with AutoDock Tools 1.5.6 package (ADT,

http://mgltools.scripps.edu/) [3]. Docking was performed in 70 x 70 x 70 units grid box centered on the enzyme hydrophobic pocket with the grid spacing of 0.325 Å. For each ligand molecule 100 independent runs were performed, using Lamarckian genetic algorithm, with at most 10^6 energy evaluations and maximum number of generations of 27 000. Remaining parameters were set as default. The docking results were clustered into groups with RMSd lower than 2.0 Å. The docked ligand conformations were analyzed using ADT and Chimera 1.8 package (http://www.cgl.ucsf.edu/chimera/) [4].

Compound characterization data and representative synthetic procedures:

General procedure of preparing 1,3-diols (2a-g) from β-keto ester (1a-g):

$NaBH_4$ (8 equiv) was added portion-wise to a solution of β-keto ester **1a-g** (1.5 g) in MeOH (15 mL) at room temperature. After 20 min the heterogeneous white reaction mixture was heated to reflux until all starting material was consumed (approx. 12 h, TLC). The cooled mixture was concentrated under reduced pressure and partitioned between distilled water (35 mL) and EtOAc (40 mL). The layers were separated, and the aqueous phase was back-extracted with EtOAc (3 x 40 mL). The combined extracts were dried (Na_2SO_4), concentrated under reduced pressure, and purified by chromatography on silica gel using mixture of $PhCH_3$/AcOEt (1:1, v/v) as an eluent to give the corresponding 1,3-diol **2a-g**.

1-Phenylpropane-1,3-diol (2a):

Colorless oil; yield: 82%; R_f [$PhCH_3$/AcOEt (1:1, v/v)] 0.29; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.84-2.02 (m, 2H, CH_2), 3.26 (br. s., 2H, O*H*), 3.74-3.84 (m, 2H, CH_2OH), 4.90 (dd, *J*=8.81, 3.84 Hz, 1H, C*H*), 7.24-7.38 (m, 5H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 40.32 (*C*H_2), 61.12 (*C*H_2OH), 73.94 (*C*H), 125.60 (2C, *o*-Ph), 127.46 (*p*-Ph), 128.41 (2C, *m*-Ph), 144.21 (Ph).

1-(4-Bromophenyl)propane-1,3-diol (2b):

Colorless oil; yield: 96%; R_f [$PhCH_3$/AcOEt (1:1, v/v)] 0.24; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.81-2.00 (m, 2H, CH_2), 2.94 (br. s., 2H, O*H*), 3.77-3.88 (m, 2H, CH_2OH), 4.90 (dd, *J*=8.36, 3.84 Hz, 1H, C*H*), 7.18-7.25 (m, 2H, *o*-Ph), 7.43-7.51 (m, 2H, *m*-Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 40.25 (*C*H_2), 61.25 (*C*H_2OH), 73.56 (*C*H), 121.20 (*p*-Ph), 127.35 (2C, *o*-Ph), 131.51 (2C, *m*-Ph), 143.24 (Ph).

1-(4-Chlorophenyl)propane-1,3-diol (2c):

Yellowish oil; yield: 81%; R_f [$PhCH_3$/AcOEt (1:1, v/v)] 0.21; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.82-2.02 (m, 2H, CH_2), 2.77 (br. s., 2H, O*H*), 3.78-3.90 (m, 2H, CH_2OH), 4.94 (dd, *J*=8.58, 3.61 Hz, 1H, C*H*), 7.27-7.35 (m, 4H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 40.41 (*C*H_2), 61.39 (CH_2OH), 73.64 (*C*H), 126.92 (2C, *o*-Ph), 128.50 (2C, *m*-Ph), 133.04 (*p*-Ph), 142.64 (Ph).

1-(4-Fluorophenyl)propane-1,3-diol (2d):

Colorless oil; yield: 78%; R_f [$PhCH_3$/AcOEt (1:1, v/v)] 0.16; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.81-2.01 (m, 2H, CH_2), 3.20 (s, 2H, O*H*), 3.74-3.86 (m, 2H, CH_2OH), 4.90 (dd, *J*=8.81, 3.57

Hz, 1H, C*H*), 6.97-7.07 (m, 2H, *m*-Ph), 7.27-7.34 (m, 2H, *p*-Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 40.38 (*C*H_2), 61.20 (*C*H_2OH), 73.47 (*C*H), 115,22 (d, *J*=20.95 Hz, 2C, *m*-Ph), 127.23 (d, *J*=7.60 Hz, 2C, *o*-Ph), 139.97 (d, *J*=2.85 Hz, 1C, Ph), 162.06 (d, *J*=245.33 Hz, 1C, *p*-Ph).

1-(3-Bromophenyl)propane-1,3-diol (2e):

Yellowish oil; yield: 85%; R_f [$PhCH_3$/AcOEt (1:1, v/v)] 0.24; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.86-2.01 (m, 2H, C*H*$_2$), 2.65 (br. s., 2H, O*H*), 3.79-3.90 (m, 2H, C*H*$_2$OH), 4.92 (dd, *J*=8.13, 4.07 Hz, 1H, C*H*), 7.17-7.30 (m, 2H, *m*-Ph and one of *o*-Ph), 7.33-7.43 (m, 1H, *p*-Ph), 7.50-7.54 (m, 1H, *o*-Ph-situated closer to Br); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 40.34 (*C*H_2), 61.37 (C*H*$_2$OH), 73.61 (C*H*), 122.54 (*m*-Ph-Br), 124.15 (*o*-Ph), 128.69 (*o*-Ph-situated closer to Br), 129.97 (*m*-Ph), 130.44 (*p*-Ph), 146.51 (Ph).

1-(3-Chlorophenyl)propane-1,3-diol (2f):

Yellowish oil; yield: 86%; R_f [$PhCH_3$/AcOEt (1:1, v/v)] 0.20; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.85-2.01 (m, 2H, C*H*$_2$), 2.99 (br. s., 2H, O*H*), 3.76-3.90 (m, 2H, C*H*$_2$OH), 4.92 (dd, *J*=8.13, 4.29 Hz, 1H, C*H*), 7.18-7.30 (m, 3H, Ph), 7.33-7.38 (m, 1H, *o*-Ph-situated much further from Cl); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 40.29 (*C*H_2), 61.26 (*C*H_2OH), 73.54 (*C*H), 123.67 (*o*-Ph), 125.75 (*o*-Ph-situated closer to Cl), 127.48 (*p*-Ph), 129.66 (*m*-Ph), 134.25 (*m*-Ph-Cl), 146.22 (Ph).

1-(3-Methoxyphenyl)propane-1,3-diol (2g):

Yellowish oil; yield: 95%; R_f [$PhCH_3$/AcOEt (1:1, v/v)] 0.22; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.84-2.06 (m, 2H, C*H*$_2$), 2.92 (br. s., 2H, O*H*), 3.80 (s, 3H, OC*H*$_3$), 3.81-3.87 (m, 2H, C*H*$_2$OH), 4.91 (dd, *J*=8.58, 3.84 Hz, 1H, C*H*), 6.78-6.83 (m, 1H, Ph), 6.90-6.95 (m, 2H, Ph), 7.22-7.29 (m, 1H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 40.38 (*C*H_2), 55.22 (O*C*H_3), 61.33 (C*H*$_2$OH), 74.07 (C*H*), 111.05 (*o*-Ph-situated closer to OCH_3), 112.85 (*p*-Ph), 117.82 (*o*-Ph), 129.39 (*m*-Ph), 145.88 (Ph), 159.55 (*m*-Ph-OCH_3).

General procedure of preparing (3a-g) by the monotosylation of the 1,3-diols (2a-g):

To a flame-dried flask, containing the 1,3-diol **2a-g** (1 g) dissolved in dry CH_2Cl_2 (15 mL), freshly distilled triethylamine (1.1 equiv) and 4-(dimethylamino)pyridine (10 mg) were added at -15 °C under an nitrogen atmosphere. After 15 min, *p*-toluenesulfonyl chloride (1 equiv) suspended in anhydrous CH_2Cl_2 (6 mL) was added dropwise *via* syringe with stirring over a period of 1 h under fast stream of nitrogen. Upon completion of the addition, the reaction

mixture was maintained at -15 °C for 12 h and then allowed to warm to room temperature and stirred for a further 1 h. Next, the content of the flask was cooled to 0 °C, portion of CH_2Cl_2 (30 mL) was added, and the solution was quenched successively by the careful addition of cold 1% HCl (2 x 15 mL), washed with saturated sodium bicarbonate (2 x 15 mL), brine (2 x 15 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to yield the crude product. The purification by column chromatography on silica gel with mixture of ethyl acetate/cyclohexane (4:1 or 1:1, v/v – depending on the substance purified) as an eluent afforded the desired monotosylated alcohol **3a-g**.

3-Hydroxy-3-phenylpropyl 4-methylbenzenesulfonate (3a):

Yellowish oil; yield: 41%; R_f [AcOEt/Cyclohexene (4:1, v/v)] 0.67; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.97-2.06 (m, 2H, CH_2), 2.10 (br. s., 1H, O*H*), 2.45 (s, 3H, CH_3), 4.00-4.10 (m, 1H, CH_2OSO$_2$), 4.22-4.33 (m, 1H, CH_2OSO$_2$), 4.76-4.83 (m, 1H, C*H*), 7.24-7.38 (m, 7H, Ph), 7.76-7.82 (m, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.66 (*C*H$_3$), 38.03 (*C*H$_2$), 67.57 (*C*H$_2$OSO$_2$), 70.15 (*C*H), 125.54 (2C, *o*-Ph), 127.75 (*p*-Ph), 127.82 (2C, *o*-PhTos), 128.49 (2C, *m*-PhTos), 129.79 (2C, *m*-Ph), 132.79 (*p*-PhTos), 143.36 (Ph), 144.74 (PhTos).

3-(4-Bromophenyl)-3-hydroxypropyl 4-methylbenzenesulfonate (3b):

Colorless oil; yield: 61%; R_f [AcOEt/Cyclohexene (1:1, v/v)] 0.76; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.98 (td, *J*=6.55, 5.42 Hz, 2H, CH_2), 2.23 (br. s., 1H, O*H*), 2.46 (s, 3H, CH_3), 3.96-4.07 (m, 1H, CH_2OSO$_2$), 4.27 (dt, *J*=9.99, 6.63 Hz, 1H, CH_2OSO$_2$), 4.79 (t, *J*=6.66 Hz, 1H, C*H*), 7.11-7.17 (m, 2H, Ph), 7.32-7.38 (m, 2H, Ph), 7.39-7.45 (m, 2H, Ph), 7.74-7.80 (m, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.65 (*C*H$_3$), 37.98 (*C*H$_2$), 67.27 (*C*H$_2$OSO$_2$), 69.49 (*C*H), 121.52 (*p*-Ph), 127.34 (2C, *o*-Ph), 127.85 (2C, *o*-PhTos), 129.87 (2C, *m*-PhTos), 131.61 (2C, *m*-Ph), 132.68 (*p*-PhTos), 142.43 (Ph), 144.93 (PhTos).

3-(4-Chlorophenyl)-3-hydroxypropyl 4-methylbenzenesulfonate (3c):

Yellowish oil; yield: 33%; R_f [AcOEt/Cyclohexene (4:1, v/v)] 0.73; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.94-2.03 (m, 2H, CH_2), 2.33 (br. s., 1H, O*H*), 2.45 (s, 3H, CH_3), 3.98-4.05 (m, 1H, CH_2OSO$_2$), 4.22-4.30 (m, 1H, CH_2OSO$_2$), 4.79 (t, *J*=6.66 Hz, 1H, *C*H), 7.17-7.22 (m, 2H, Ph), 7.23-7.29 (m, 2H, Ph), 7.31-7.38 (m, 2H, Ph), 7.73-7.79 (m, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.64 (*C*H$_3$), 38.00 (*C*H$_2$), 67.33 (*C*H$_2$OSO$_2$), 69.43 (*C*H), 127.00 (2C, *o*-Ph), 127.85 (2C, *m*-Ph), 128.63 (2C, *o*-PhTos), 129.87 (2C, *m*-PhTos), 132.65 (*p*-PhTos), 133.36 (*p*-Ph), 141.94 (Ph), 144.93 (PhTos).

3-(4-Fluorophenyl)-3-hydroxypropyl 4-methylbenzenesulfonate (3d):

Colorless oil; yield: 66%; R_f [AcOEt/Cyclohexene (1:1, v/v)] 0.71; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.94-2.06 (m, 2H, CH_2), 2.22 (br. s., 1H, O*H*), 2.45 (s, 3H, CH_3), 3.98-4.06 (m, 1H, CH_2OSO$_2$), 4.22-4.31 (m, 1H, CH_2OSO$_2$), 4.79 (dd, *J*=7.86, 5.71 Hz, 1H, C*H*), 6.95-7.03 (m, 2H, *m*-Ph), 7.20-7.27 (m, 2H, *o*-PhTos), 7.33-7.36 (m, 2H, *m*-PhTos), 7.74-7.80 (m, 2H, *o*-Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.60 (*C*H$_3$), 38.08 (*C*H$_2$), 67.41 (*C*H$_2$OSO$_2$), 69.49 (*C*H), 115.34 (d, *J*=21.23 Hz, 2C, *m*-Ph), 127.28 (d, *J*=7.60 Hz, 2C, *o*-Ph), 127.84 (2C, *o*-PhTos), 129.84 (2C, *m*-PhTos), 132.74 (*p*-PhTos), 139.19 (d, *J*=3.80 Hz, 1C, Ph), 144.89 (PhTos), 162.17 (d, *J*=246.26 Hz, 1C, *p*-Ph).

3-(3-Bromophenyl)-3-hydroxypropyl 4-methylbenzenesulfonate (3e):

Yellowish oil; yield: 82%; R_f [AcOEt/Cyclohexene (4:1, v/v)] 0.67; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.97-2.01 (m, 2H, CH_2), 2.06 (br. s., 1H, O*H*), 2.46 (s, 3H, CH_3), 4.06 (dt, *J*=10.22, 5.17 Hz, 1H, CH_2OSO$_2$), 4.30 (ddd, *J*=10.11, 8.07, 5.31 Hz, 1H, CH_2OSO$_2$), 4.79 (dd, *J*=8.36, 4.97 Hz, 1H, C*H*), 7.14-7.22 (m, 2H, Ph), 7.31-7.47 (m, 5H, Ph), 7.76-7.83 (m, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.68 (*C*H$_3$), 38.06 (*C*H$_2$), 67.24 (*C*H$_2$OSO$_2$), 69.43 (*C*H), 122.68 (*m*-Ph-Br), 124.24 (*o*-Ph), 127.87 (2C, *o*-PhTos), 128.69 (*o*-Ph-situated closer to Br), 129.90 (2C, *m*-PhTos), 130.14 (*m*-Ph), 130.82 (*p*-Ph), 132.73 (*p*-PhTos), 144.94 (Ph), 145.83 (PhTos).

3-(3-Chlorophenyl)-3-hydroxypropyl 4-methylbenzenesulfonate (3f):

Yellowish oil; yield: 40%; R_f [AcOEt/Cyclohexene (4:1, v/v)] 0.71; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.98 (dtd, *J*=7.99, 5.21, 5.21, 3.05 Hz, 2H, CH_2), 2.27 (br. s., 1H, O*H*), 2.45 (s, 3H, CH_3), 3.99-4.08 (m, 1H, CH_2OSO$_2$), 4.29 (ddd, *J*=10.05, 7.79, 5.42 Hz, 1H, CH_2OSO$_2$), 4.78 (dd, *J*=8.02, 5.31 Hz, 1H, C*H*), 7.11-7.17 (m, 1H, Ph), 7.20-7.28 (m, 3H, Ph), 7.33-7.37 (m, 2H, Ph), 7.75-7.81 (m, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.65 (*C*H$_3$), 38.00 (*C*H$_2$), 67.27 (*C*H$_2$OSO$_2$), 69.44 (*C*H), 123.75 (*o*-Ph), 125.75 (*o*-Ph-situated closer to Cl), 127.84 (3C, *p*-Ph and *o*-PhTos), 129.82 (*m*-Ph), 129.90 (2C, *m*-PhTos), 132.66 (*p*-PhTos), 134.39 (*m*-Ph-Cl), 144.94 (Ph), 145.58 (PhTos).

3-(3-Methoxyphenyl)-3-hydroxypropyl 4-methylbenzenesulfonate (3g):

Yellowish oil; yield: 39%; R_f [AcOEt/Cyclohexene (4:1, v/v)] 0.64; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.96-2.04 (m, 2H, CH_2), 2.05 (br. s., 1H, O*H*), 2.45 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 4.01-4.11 (m, 1H, CH_2OSO$_2$), 4.27 (dt, *J*=9.94, 6.66 Hz, 1H, CH_2OSO$_2$), 4.78 (t, *J*=6.66 Hz, 1H, C*H*), 6.76-6.88 (m, 3H, Ph), 7.17-7.27 (m, 1H, Ph), 7.29-7.39 (m, 2 H, Ph),

7.79-7.81 (m, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.64 (CH_3), 37.97 (CH_2), 55.20 (OCH_3), 67.55 (CH_2OSO_2), 70.06 (*C*H), 110.99 (*o*-Ph-situated closer to OCH_3), 113.30 (*p*-Ph), 117.81 (*o*-Ph), 127.87 (2C, *m*-PhTos), 129.61 (*m*-Ph), 129.85 (2C, *o*-PhTos), 132.78 (*p*-PhTos), 144.82 (Ph), 145.15 (PhTos), 159.73 (OCH_3).

Preparation of 4,5,6,7-tetrabromo-1*H*-benzotriazole (5):

1*H*-Benzotriazole **4** (6 g, 50.4 mmol) was dissolved in a mixture of 69% HNO_3 (150 mL) and fuming 100% HNO_3 (10 mL). Next, the solution was heated to a temperature of 80 °C and Br_2 (48.3 g, 302 mmol, 15 mL) was added drop by drop within 1 h. The reaction mixture was stirred vigorously by using mechanical stirrer at 60 °C for 48 h and irradiated by exposure to UV light. Subsequently, content of the flask was cooled to room temperature, the excess of unreacted bromine was removed in the gentle flow of nitrogen and trapped into a 20 % solution of sodium pyrosulfite (150 mL). After this, the suspension was poured into mixture of ice-cold distilled H_2O (300 mL) and saturated $Na_2S_2O_5$ (20 mL). The resulting yellowish precipitate was filtered off, and washed with H_2O (100 mL) and EtOH (100 mL), respectively. The obtained crude product was refluxed several times in the mixture of MeOH (50 mL) and *i*-PrOH (25 mL), and hot saturated solution was filtered off yielding white crystals **5** (13 g, 29.9 mmol, 59%). The purity of the desired compound **5** was confirmed by high-resolution mass spectrometry (HRMS). The rest of the analyses made (*vide* ^{1}H-, ^{13}C-NMR, m.p.) were consistent with the literature data [5]. White solid; m.p.: 264-266 °C; yield: 40%; R_f [$CHCl_3$/MeOH (99:1, v/v)] 0.16; HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 435.6941, $[M+H]^+_{found}$ = 435.6629.

General procedure for the introduction of the Tbbt moiety [synthesis of (6a-g)]:

To a solution of TBBt **5** (1 equiv) in the mixture of absolute acetone (15 mL) and dry acetonitrile (10 mL) anhydrous K_2CO_3 (3 equiv) was added. The content of the flask was flushed with nitrogen for several times, and stirred for 3 h at reflux. Next, the reaction mixture was cooled to room temperature and the solution of appropriate 3-hydroxy-3-arylpropyl-4-methylbenzenesulfonate **3a-g** (400 mg) in acetonitrile (5 mL) was added drop by drop by using syringe over 1 h. After stirring the mixture at reflux for 48 h, the crude reaction was cooled to room temperature, and both the white precipitate as well as the red colored solution were transferred into separator and washed with saturated solution of potassium carbonate (100 mL). Water phase was extracted with $CHCl_3$ (2 x 150 mL) and AcOEt (3 x 100 mL), and the collected organic layers were partially condensed on rotator evaporator, and dried over dry

Na_2SO_4. The purification procedure was performed on preparative layer plates (PLC) coated with unmodified silica matrices, using mixture of $CHCl_3/Et_2O$ (7:3 or 6:4, v/v) or $CHCl_3$/acetone (95:5, v/v), depending on substance purified. The appropriate fraction was removed from the glass plate with SiO_2, placed in the round-bottomed flask, suspended in the mixture of $CHCl_3$/acetone (150 mL, 2:1, v/v), and stirred for 1 h at room temperature. Next, the silica gel was filtered on a sintered glass funnel, and washed with $CHCl_3$/acetone (2 x 50 mL, 1:1, v/v). After partial solvent removal *in vacuo*, solution of the filtrate (approx. 1.5 mL) was kept in the refrigerator until white precipitate occurred. Next, the liquid phase was decanted off by Pasteur pipette tipped with cotton wool, and the remaining solid was washed with portions of ice-cold acetone (1 mL), and then ethanol (1 mL) respectively to obtained desired product **6a-g** as a white solid, which was further high-vacuum-dried in the desiccator over phosphorus pentoxide (P_2O_5) overnight.

1-Phenyl-3-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)propan-1-ol (6a):

White solid; m.p.: 135-136 °C; yield: 40%; R_f [$CHCl_3/Et_2O$ (7:3, v/v)] 0.67; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.31 (br. s., 1H, O*H*), 2.48-2.62 (m, 2H, CH_2), 4.82 (dd, *J*=7.45, 5.65 Hz, 1H, C*H*), 4.85-5.02 (m, 2H, CH_2N), 7.23-7.40 (m, 5H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 38.61 (*C*H_2), 54.42 (*C*H_2N), 71.40 (*C*H), 113.69 (2C, TBBt-internal), 125.61 (2C, TBBt-external), 126.32 (2C, *o*-Ph), 127.98 (2C, *m*-Ph), 128.62 (*p*-Ph), 143.01 (Ph), 143.08 (TBBt=N); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 569.7673, $[M+H]^+_{found}$ = 569.8090; (ESI$^-$, m/z): $[M+H]^-_{calcd}$ = 567.7516, $[M+H]^-_{found}$ = 567.7919; FTIR ν_{max}(neat): 3549.27, 3416.85, 1533.15, 1489.52, 1429.48, 1271.50, 1177.95, 1162.39, 1086.05, 1056.91, 974.64, 765.01, 747.77, 697.57, 606.17, 515.80.

1-(4-Bromophenyl)-3-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)propan-1-ol (6b):

White solid; m.p.: 125-126 °C; yield: 33%; R_f [$CHCl_3$/acetone (95:5, v/v)] 0.55; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.97 (br. s., 1H, O*H*), 2.44-2.61 (m, 2H, CH_2), 4.78 (dd, *J*=7.79, 5.08 Hz, 1H, C*H*), 4.83-5.02 (m, 2H, CH_2N), 7.14-7.25 (m, 2H, *o*-Ph), 7.38-7.47 (m, 2H, *m*-Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 38.40 (*C*H_2), 54.15 (*C*H_2N), 70.79 (*C*H), 113.68 (TBBt-internal), 121.70 (*p*-Ph), 126.52 (2C, TBBt-external), 127.35 (2C, *o*-Ph), 131.60 (2C, *m*-Ph), 141.87 (Ph), 143.08 (TBBt=N); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 649.6758, $[M+H]^+_{found}$ = 649.7219; (ESI$^-$, m/z): $[M+H]^-_{calcd}$ = 647.6601, $[M+H]^-_{found}$ = 647.7287; FTIR ν_{max}(neat): 3417.18, 1588.37, 1533.29, 1485.73, 1425.84, 1308.92, 1295.42, 1274.19, 1175.75, 1159.37, 1073.38, 1011.10, 979.00, 926.17, 876.54, 822.85, 771.60, 750.05, 718.53, 608.83, 543.95, 508.55.

1-(4-Chlorophenyl)-3-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)propan-1-ol (6c):

White solid; m.p.: 112-114 °C; yield: 41%; R_f [$CHCl_3$/Et_2O (6:4, v/v)] 0.51; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.42-2.58 (m, 2H, CH_2), 2.71 (br. s., 1H, O*H*), 4.79 (dd, *J*=8.13, 4.97 Hz, 1H, C*H*), 4.91 (dt, *J*=19.14, 6.92 Hz, 2H, CH_2N), 7.23-7.27 (m, 4H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 38.46 (*C*H_2), 54.14 (*C*H_2N), 70.57 (*C*H), 113.57 (2C, TBBt-internal), 126.42 (2C, TBBt-external), 126.96 (2C, *m*-Ph), 128.60 (2C, *o*-Ph), 133.51 (*p*-Ph), 141.39 (Ph), 142.94 (Tbbt=N); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 603.7283, $[M+H]^+_{found}$ = 603.7826; (ESI$^-$, m/z): $[M+H]^-_{calcd}$ = 601.7127, $[M+H]^-_{found}$ = 601.7653; FTIR ν_{max}(neat): 3387.41, 1739.34, 1700.79, 1489.54, 1425.63, 1365.48, 1270.44, 1229.73, 1217.47, 1173.57, 1089.40, 1074.43, 1012.15, 977.71, 832.05, 770.11, 749.58, 607.20, 529.61.

1-(4-Fluorophenyl)-3-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)propan-1-ol (6d):

White solid; m.p.: 73-75 °C; yield: 33%; R_f [$CHCl_3$/acetone (95:5, v/v)] 0.44; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.10 (br. s., 1H, O*H*), 2.47-2.57 (m, 2H, CH_2), 4.80 (dd, *J*=7.38, 5.71 Hz, 1H, C*H*), 4.84-5.02 (m, 2H, CH_2N), 6.98-7.06 (m, 2H, *m*-Ph), 7.29-7.37 (m, 2H, *o*-Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 38.70 (*C*H_2), 54.33 (*C*H_2N), 70.72 (*C*H), 113.67 (2C, TBBt-internal), 115.47 (d, *J*=21.86 Hz, 2C, *m*-Ph), 126.43 (2C, TBBt-external), 127.33 (d, *J*=7.60 Hz, 2C, *o*-Ph), 138.82 (d, *J*=2.85 Hz, 1C, Ph), 143.09 (TBBt=N), 162.29 (d, *J*=246.43 Hz, 1C, *p*-Ph); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 587.7579, $[M+H]^+_{found}$ = 587.8105; (ESI$^-$, m/z): $[M+H]^-_{calcd}$ = 585.7422, $[M+H]^-_{found}$ = 585.7770; FTIR ν_{max}(neat): 3400.54, 1603.65, 1530.65, 1507.41, 1425.71, 1394.99, 1344.03, 1297.48, 1270.62, 1216.71, 1174.22, 1156.57, 1100.42, 1073.21, 1013.16, 976.66, 928.84, 882.96, 835.06, 770.74, 745.98, 607.49, 566.47, 544.29.

1-(3-Bromophenyl)-3-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)propan-1-ol (6e):

White solid; m.p.: 88-91 °C; yield: 36%; R_f [$CHCl_3$/acetone (95:5, v/v)] 0.55; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.22 (br. s., 1H, O*H*), 2.44-2.63 (m, 2H, CH_2), 4.79 (dd, *J*=8.24, 4.63 Hz, 1H, C*H*), 4.84-5.02 (m, 2H, CH_2N), 7.16-7.22 (m, 1H, Ph), 7.23-7.29 (m, 1H, Ph), 7.35-7.40 (m, 1H, Ph), 7.48-7.51 (m, 1H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 38.48 (*C*H_2), 54.12 (*C*H_2N), 70.56 (*C*H), 113.64 (2C, TBBt-internal), 122.68 (*m*-Ph-Br), 124.20 (2C, TBBt-external), 126.44 (*o*-Ph), 128.70 (*m*-Ph), 130.15 (*o*-Ph-situated closer to Br), 130.90 (*p*-Ph), 143.01 (TBBt=N), 145.33 (Ph); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 647.6778, $[M+H]^+_{found}$ = 647.7335; (ESI$^-$, m/z): $[M+H]^-_{calcd}$ = 645.6622, $[M+H]^-_{found}$ = not determined; FTIR ν_{max}(neat): 3396.57, 1740.41, 1591.39, 1573.26, 1533.54, 1477.79, 1426.25, 1341.93, 1304.67, 1274.68, 1240.74, 1145.62, 1172.03, 1100.35, 1069.74, 1035.20, 1014.10, 1004.29, 977.15, 877.76, 800.31, 788.28, 753.38, 692.91, 666.99, 608.00, 585.16, 511.23, 442.87.

1-(3-Chlorophenyl)-3-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)propan-1-ol (6f):

White solid; m.p.: 114-115 °C; yield: 42%; R_f [$CHCl_3$/acetone (95:5, v/v)]; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.68 (br. s., 1H, O*H*), 2.44-2.64 (m, 2H, CH_2), 4.80 (dd, *J*=8.33, 4.52 Hz, 1H, C*H*), 4.83-5.05 (m, 2H, CH_2N), 7.16-7.29 (m, 3H, Ph), 7.34 (t, *J*=1.79 Hz, 1H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 38.49 (*C*H_2), 54.15 (*C*H_2N), 70.68 (*C*H), 113.67 (2C,TBBt-internal), 123.73 (2C, TBBt-external), 125.81 (*o*-Ph), 126.44 (*o*-Ph-situated closer to Cl), 127.99 (*m*-Ph), 129.87 (*p*-Ph), 134.53 (*m*-Ph-Cl), 143.06 (Ph), 145.09 (TBBt=N); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 603.7283, $[M+H]^+_{found}$ = 603.7560; FTIR ν_{max}(neat): 3429.45, 1596.23, 1573.06, 1534.71, 1476.43, 1444.67, 1428.30, 1370.38, 1334.89, 1276.90, 1201.11, 1172.60, 1146.23, 1099.14, 1072.06, 1051.12, 1040.93, 1009.52, 976.79, 880.73, 867.62, 798.02, 791.15, 776.73, 766.58, 718.30, 700.06, 687.80, 608.63, 500.13, 455.35.

1-(3-Methoxyphenyl)-3-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)propan-1-ol (6g):

White solid; m.p.: 79-80 °C; yield: 33%; R_f [$CHCl_3$/acetone (95:5, v/v)] 0.42; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.00 (br. s., 1H, O*H*), 2.50-2.61 (m, 2H, CH_2), 3.80 (s, 3H, OCH_3), 4.79 (dd, *J*=7.38, 5.47 Hz, 1H, C*H*), 4.93 (td, *J*=13.69, 6.43 Hz, 2H, CH_2N), 6.77-6.80 (m, 1H, Ph), 6.89-6.94 (m, 2H, Ph), 7.21-7.26 (m, 1H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 38.51 (*C*H_2), 54.37 (*C*H_2N), 55.24 (O*C*H_3), 71.34 (*C*H), 111.14 (*p*-Ph), 113.30 (*o*-Ph-situated closer to OCH_3), 113.70 (*o*-Ph), 117.81 (TBBt-internal), 126.31 (TBBt-external), 129.69 (*m*-Ph), 143.08 (TBBt=N), 144.66 (Ph), 159.77 (*m*-Ph-OCH_3); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 599.7779, $[M+H]^+_{found}$ = 599.8348; (ESI$^-$, m/z): $[M+H]^-_{calcd}$ = 597.7622, $[M+H]^-_{found}$ = not determined; FTIR ν_{max}(neat): 3528.67, 3446.53, 2932.07, 2830.51, 1735.04, 1604.03, 1579.07, 1533.16, 1482.10, 1429.36, 1376.82, 1305.88, 1284.04, 1272.96, 1256.28, 1235.27, 1176.17, 1166.31, 1145.49, 1095.20, 1062.25, 1040.59, 995.10, 977.14, 933.18, 913.19, 886.37, 833.89, 785.01, 770.28, 739.31, 700.25, 604.77, 566.11, 525.32, 474.47.

Preparation of (2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate (11):

Procedure was adopted from literature [6]. Tosyl chloride (2.07 g, 10.89 mmol) was added in small portions (during 1 h) to a solution of 2,2-dimethyl-1,3-dioxolane-4-methanol **10** (1.20 g, 9.08 mmol), triethylamine (1.38 g, 13.62 mmol, 1.90 mL), and DMAP (112 mg, 0.9 mmol) in dry dichloromethane (20 mL), with magnetic stirring at 0-5 °C. After 5 h, Et_2O (15 mL) was added, and the solution was washed with 10% water solution of HCl (2 x 15 mL), saturated aqueous $NaHCO_3$ (20 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The crude product was chromatographed over silica gel using mixture of hexane/ethyl acetate (5:1, v/v) as an eluent, obtaining the product **11** as an white solid (2.1 g, 7.33 mmol, 81%)

with >99% purity according to GC. White solid; m.p.: 50-52 °C [Lit [7]: m.p.: 49-50 °C after recrystallization from petroleum ether/diethyl ether (10:1, v/v)]; yield: 81%; R_f [hexane/ethyl acetate (5:1, v/v)] 0.36; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.31 (d, J=11.52 Hz, 6H, 2xCH_3), 2.43 (s, 3H, *p*-CH_3), 3.74 (dd, J=8.81, 5.19 Hz, 1H, partially CH_2), 3.92-4.06 (m, 3H, CH and partially CH_2 and partially CH_2OSO_2), 4.21-4.31 (m, 1H, partially CH_2OSO_2), 7.34 (d, J=8.13 Hz, 2H, Ph), 7.78 (d, J=8.13 Hz, 2H, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.55 (*p*-CH_3), 25.03 (CH_3), 26.52 (CH_3), 66.03 ($CHCH_2O$), 69.40 (CH_2OSO_2), 72.78 (CH), 109.91 (C), 127.85 (2C, *m*-Tos), 129.80 (2C, *o*-Tos), 132.47 (*p*-Tos), 144.95 ($PhOSO_2$); HRMS (ESI^+, m/z): $[M+H]^+_{calcd}$ = 287.0953, $[M+H]^+_{found}$ = 287.0884; GC [100-260 (10 °C/min)]: t_R = 14.82 min.

Preparation of 4,5,6,7-tetrabromo-2-[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]-2*H*-benzotriazole (12):

This compound was prepared by using the procedure as described in synthesis detail for compounds **6a-g**. Briefly, equimolar amounts of both reagents: **11** (400 mg, 1.4 mmol), and **5** (610 mg, 1.4 mmol) in the presence of 3-fold molar excess of K_2CO_3 (582 mg, 4.21 mmol) were reacted]. Crude product **12** was partially purified by simple filtration on short silica pad by eluting with mixture of $CHCl_3$/acetone (7:3, v/v) just to remove unreacted **5**. As the high resolution mass spectrometry analysis confirmed presence of desired product **12**, and absence of compound **5** it was allowed to use in the next step without further purification. HRMS (ESI^+, m/z): $[M+H]^+_{calcd}$ = 549.7622, $[M+H]^+_{found}$ = 549.7753.

Preparation of 3-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)propane-1,2-diol (13):

Procedure was adopted from literature [8]. Compound **12** (165 mg, 0.3 mmol) was dissolved in MeOH (3 mL). Then, 0.5 *N* HCl (0.3 mL) was added dropwise, and the resulting mixture was heated to reflux. After 4 h acetone and methanol were slowly distilled off. Additional portion of MeOH (1 mL) and 0.5 *N* HCl (0.2 mL) was added, and the mixture was kept at room temperature until ketal hydrolysis was completed (approx. 2 h, TLC). The mixture was diluted with careful addition of saturated $NaHCO_3$ until effervescence ceased, and the water phase was extracted with EtOAc (3 x 10 mL). The organic extracts were combined, washed with brine (5 mL), dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified by preparative chromatography (PLC) using mixture of $CHCl_3$/acetone (9:1, v/v) as an eluent, affording target compound **13** as hygroscopic white solid (95 mg, 0.19 mmol, 62%). White semi-solid; yield: 30% (after two steps: from **11** to **13**); R_f [$CHCl_3$/acetone (9:1, v/v)] 0.78; ^{1}H-NMR (MeOD, 400 MHz) δ: 3.59-3.74 (m, 2H, CH_2OH),

4.16-4.42 (m, 1H, C*H*OH) 4.76 (s, 1H, C*H*$_2$N), 4.93-5.21 (m, 1H, C*H*$_2$N); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 53.23 (*C*H$_2$N), 63.53 (*C*H$_2$OH), 71.02 (*C*HOH), 115.72 (TBBt-internal), 125.40 (TBBt-external), 142.60 (TBBt=N); HRMS (ESI$^+$, m/z): $[M+H]^+_{calcd}$ = 509.7309, $[M+H]^+_{found}$ = 509.7447.

Biological Assays

Cloning, expression and purification of hCK2α:

The coding region of human CK2α was amplified by polymerase chain reaction using the following primers: 5'-CGCGGATCCGTCGGGACCCGTGCCAA (upstream primer) and 5'-CCCAAGCTTCTGCTGAGCGCCAGCGGCA (downstream primer) and I.M.A.G.E. clone as a template. The product was cloned into the vector pETDuet-1 (MCS1) using the restriction sites BamHI and HindIII and the bacterial strain DH5α. The sequence of the obtained clone was confirmed. Expression of the resulting N-terminal histidine-tagged hCK2α was done in the bacterial strain BL21(DE3)pLysS growing in superbroth medium after induction with 0.5 mM IPTG for 20 h at 20 °C. The cell pellet was resuspended in extraction buffer [composed of: 20 mM NaH_2PO_4 (pH 8.0), 500 mM NaCl, 10 mM imidazole, O-complete inhibitor cocktail (Roche), lysozyme (1 mg/mL)] and sonicated. The supernatant of the pellet from 200 mL of bacterial culture was loaded onto Ni-NTA agarose (Qiagene) column (2 mL bed volume). hCK2α was eluted with 300 mM imidazole. Fractions containing His-tagged hCK2α were dialyzed against 20 mM Tris-HCl (pH 8.5), 500 mM NaCl, 1 mM DTT, 0-20% glycerol and stored at -20 °C. The protein concentration in final solution was 12.68 mg/mL (determined by Bradford method and bovine serum albumin as a standard) [9].

Assays of CK2 alpha subunit activity and inhibition studies:

The activity of hCK2α was tested using P81 filter isotopic assay [10]. The reaction mixture contained 20 mM Tris-HCl, pH 7.5, 20 mM $MgCl_2$, 50 µM DTT, 20 µM hCK2 substrate peptide (RRRDDDSDDD), 10 µM ATP (200-300 CPM/pmol) and 200 ng hCK2α. The reaction was initiated with enzyme in a total volume of 50 µl, incubated at 30 °C, and performed for 20 min. 10 µl of a reaction mixture was spotted onto P81 paper circle. The filter papers were washed 3 x with 0.6 % phosphoric acid and once with 95% ethanol before counting in a scintillation counter (Canberra-Packard). IC_{50} values for studied compounds were determined at 4% DMSO with minimum 7 concentrations of each tested inhibitor at the range of 0.064-1000 µM and calculated by fitting the data to sigmoidal dose-response

(variable slope) Y = Bottom + (Top-Bottom) / (1+10^((LogIC50-X)*HillSlope) equation in GraphPad Prism.

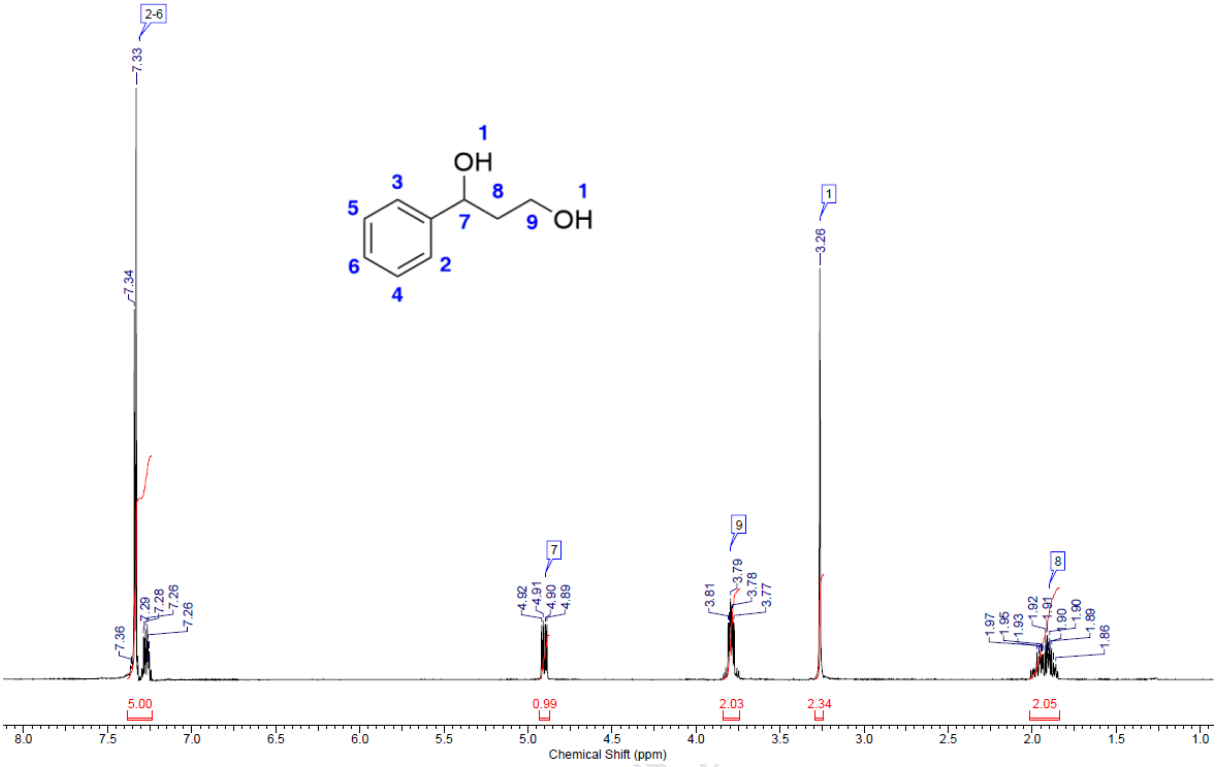
Cell culture and treatment:

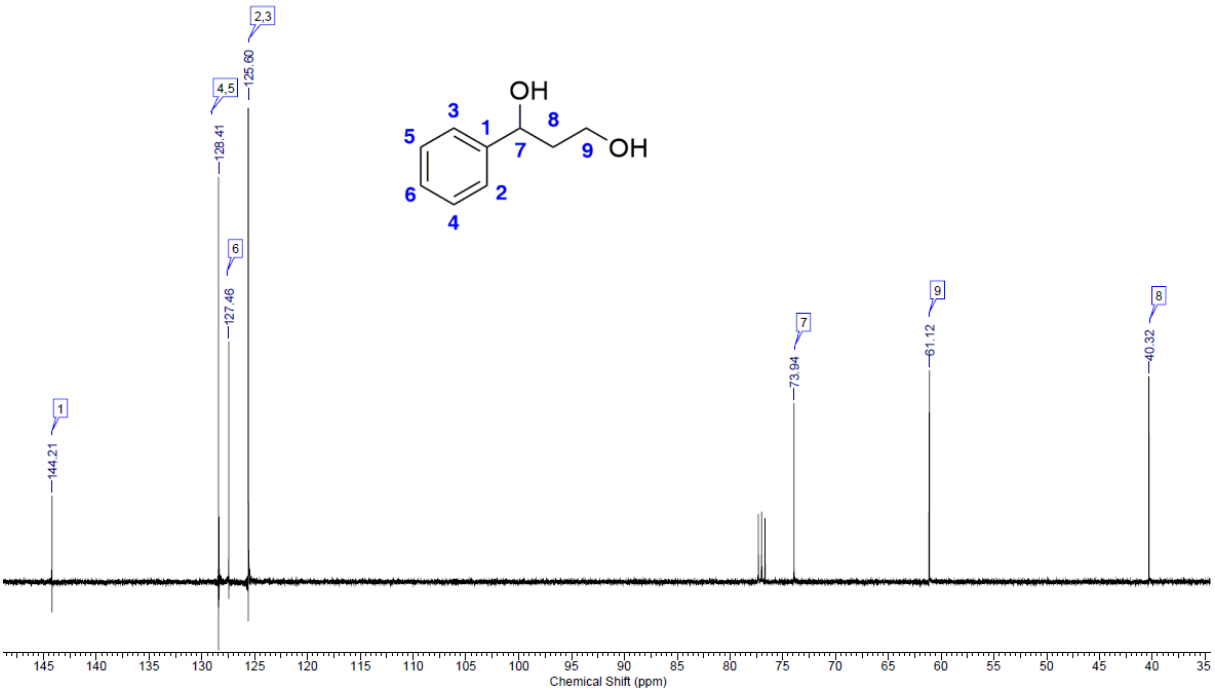
MCF7 adherent cells (human breast cancer cell line) were cultured in DMEM advanced medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Sigma-Aldrich), 2 mM L-glutamine, antibiotics (100 U/ml penicillin, 100 µg/mL streptomycin) and 10 µg/mL of human recombinant insulin. CCRF-CEM suspension cells (human peripheral blood T lymphoblast cell line) were cultured in RPMI medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum and antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin). Cells were grown in 25 cm^2 cell culture flasks (Sarstedt), in a humidified atmosphere of CO_2/air (5/95%) at 37 °C.

MTT-based cytotoxicity assay:

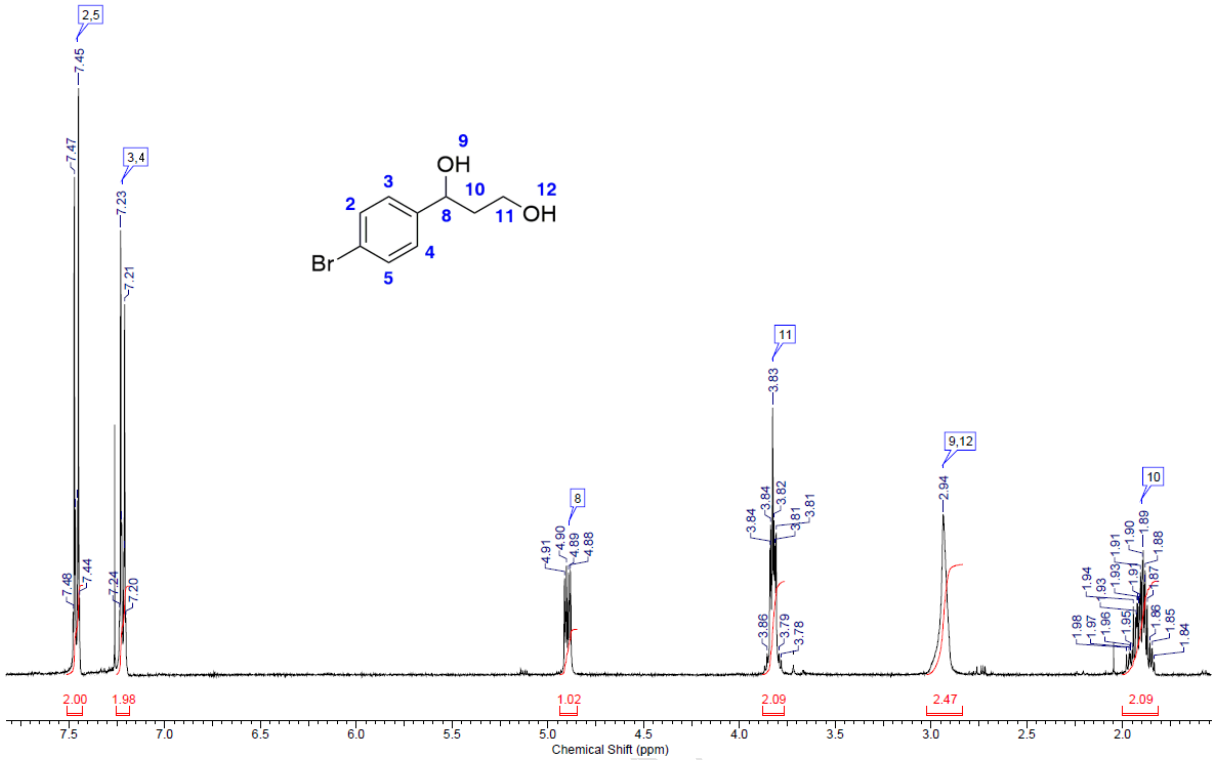
Before the treatment MCF7 cells were trypsinized in 0.25% trypsin-EDTA solution (Sigma-Aldrich) and seeded into 96-well microplates at a density of 1.5-3 x 10^4 cells/well. Cells were treated with specific compounds dissolved in DMSO or DMSO (0.5%) at the corresponding concentrations 18 h after plating (at 70% of confluency). CCRF-CEM were seeded at 2-3 x 10^4 cells/well and treated with compounds. MTT stock solution (Sigma-Aldrich) was added to each well to a final concentration of 0.5 mg/mL. After 4 h of incubation at 37 °C water-insoluble dark blue formazan crystals were dissolved in DMSO (200 µL) (37 °C/10 min incubation), and Sorensen's glycine buffer (0.1 M glycine, 0.1 M NaCl, pH 10.5) was added (25 µL per well). Optical densities were measured at 570 nm using Synergy H4 BioTek microplate reader. All measurements were carried out in triplicate and the results are expressed in percentage of cell viability relative to control (cells without inhibitor in 0.5% DMSO). At such conditions, IC_{50} for standard anti-cancer inhibitor - doxorubicin was 1.4 µM for both MCF7 and CCRF-CEM lines.

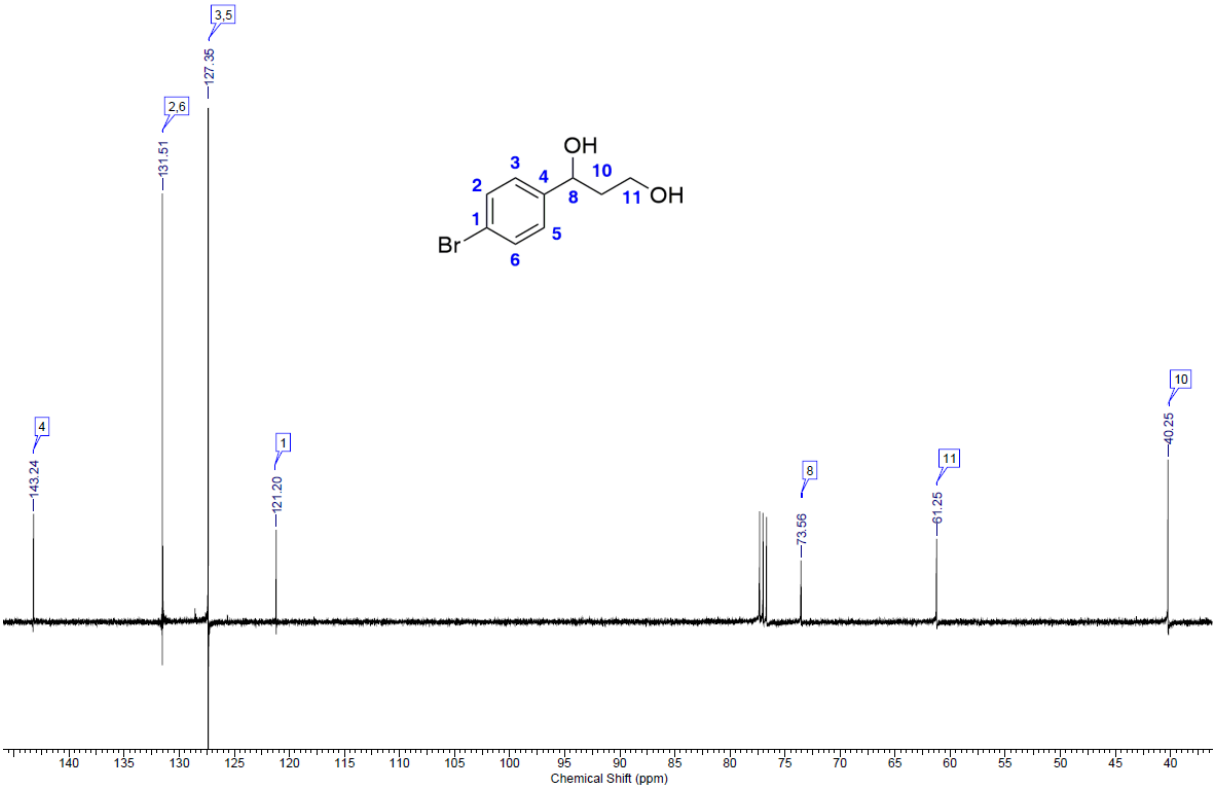
1-Phenylpropane-1,3-diol (2a):



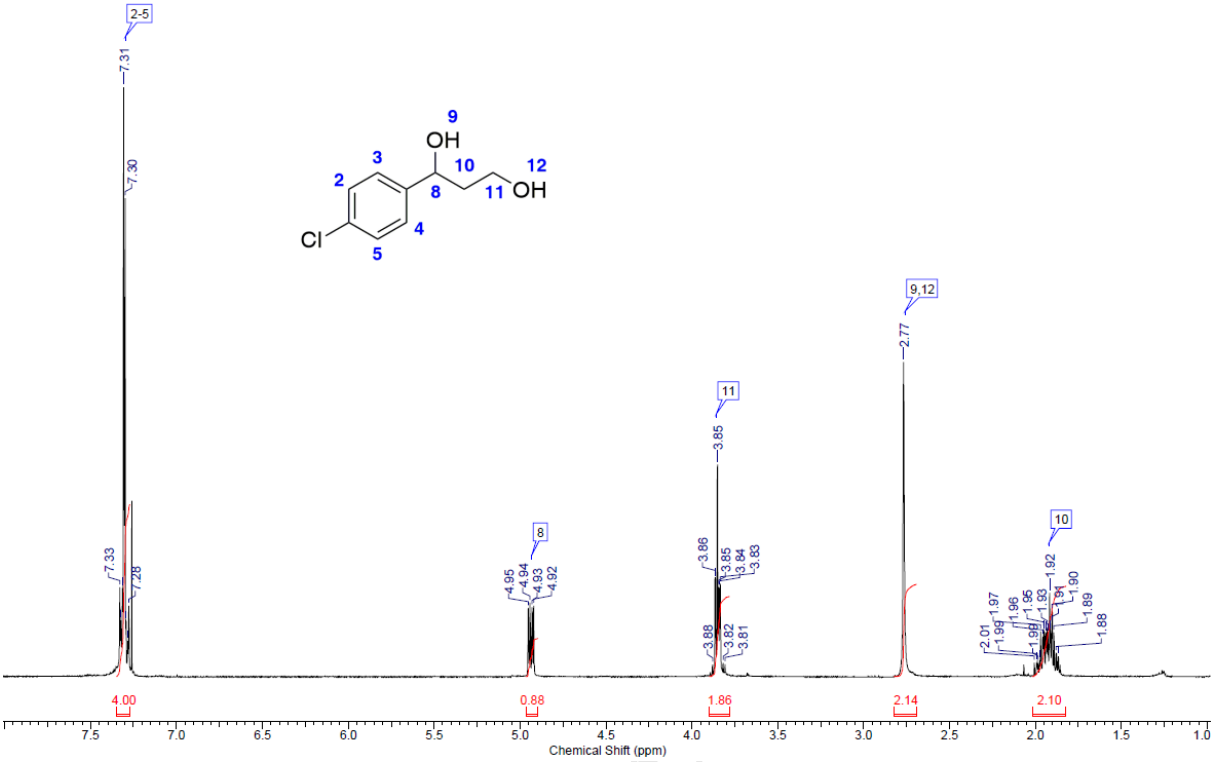


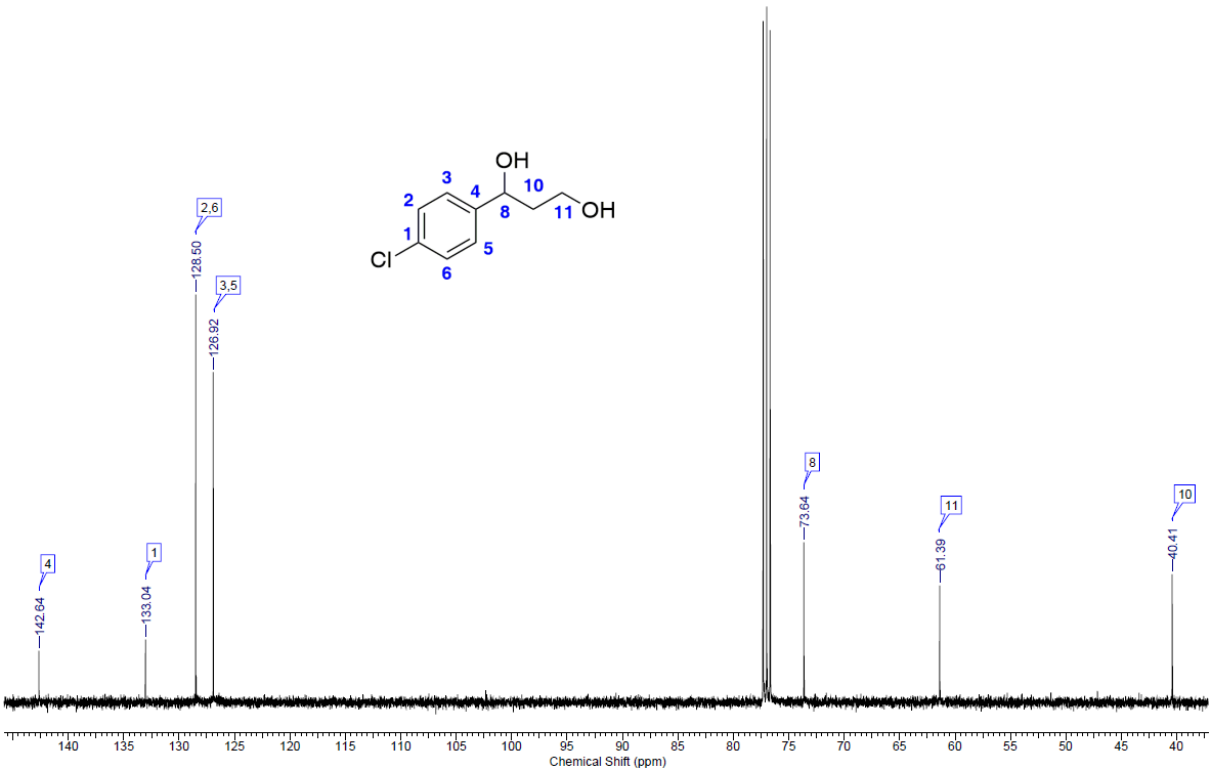
1-(4-Bromophenyl)propane-1,3-diol (2b):





1-(4-Chlorophenyl)propane-1,3-diol (2c):





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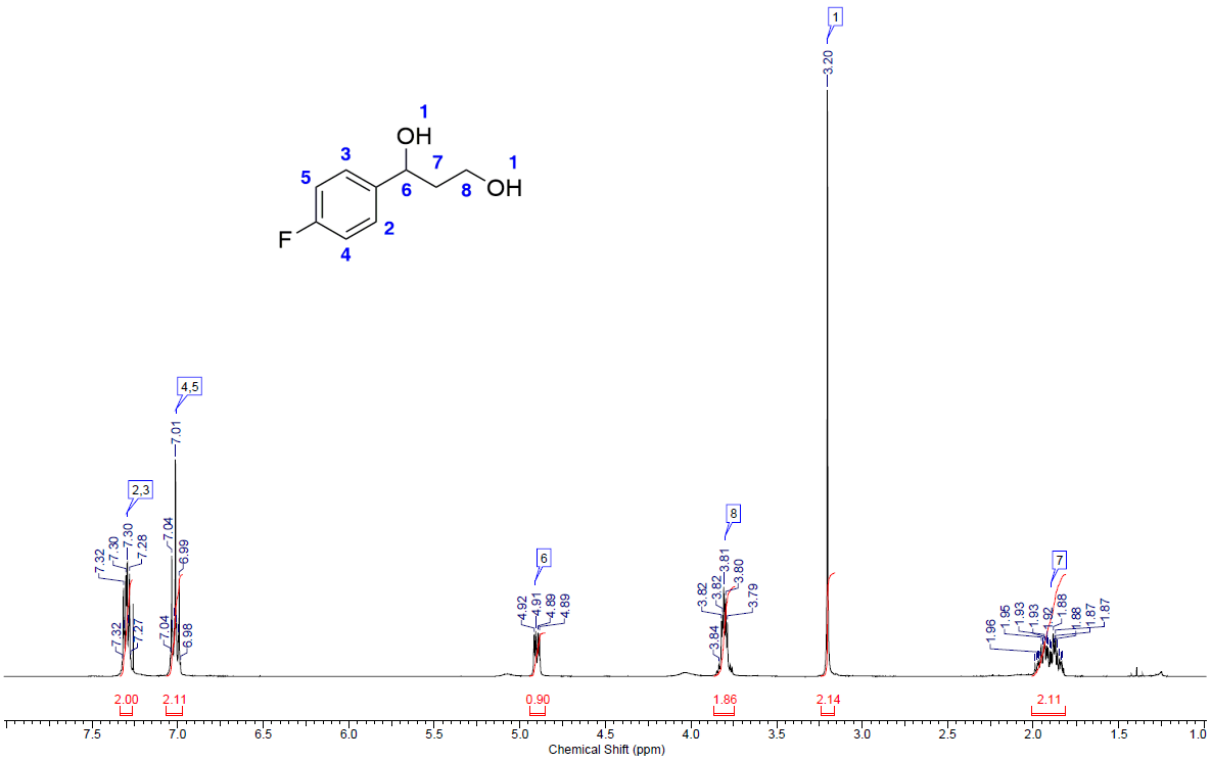

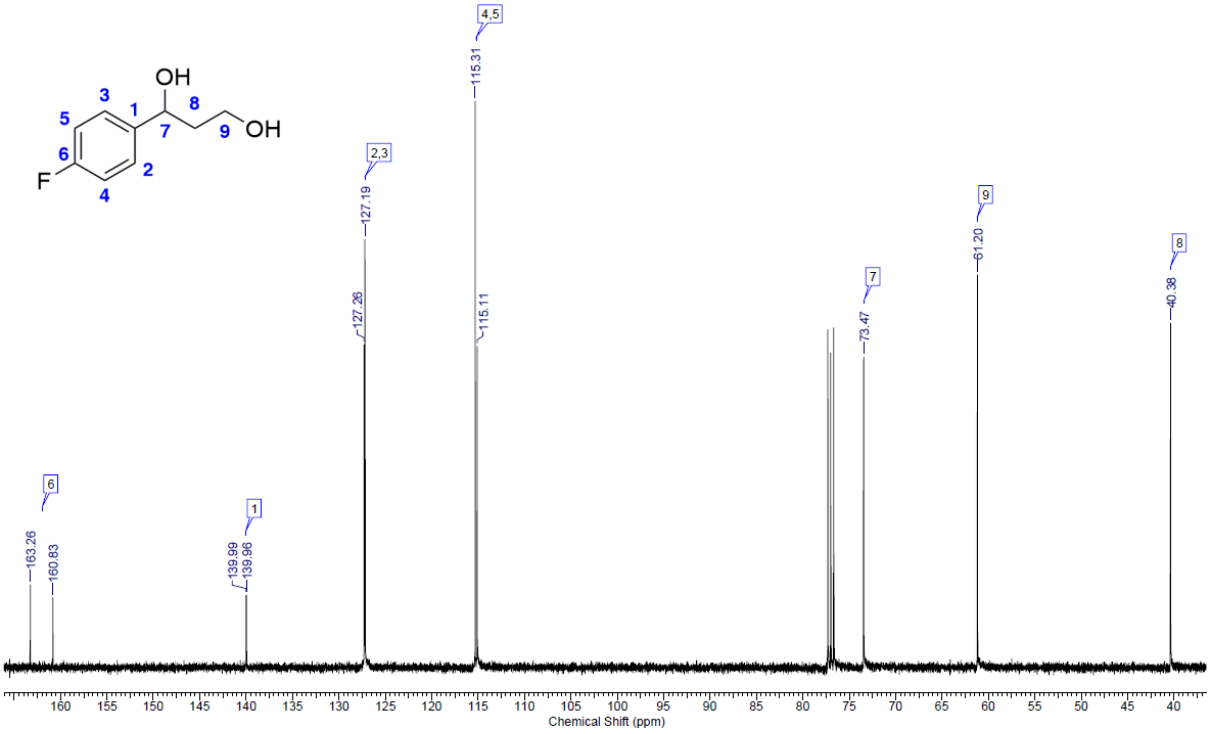

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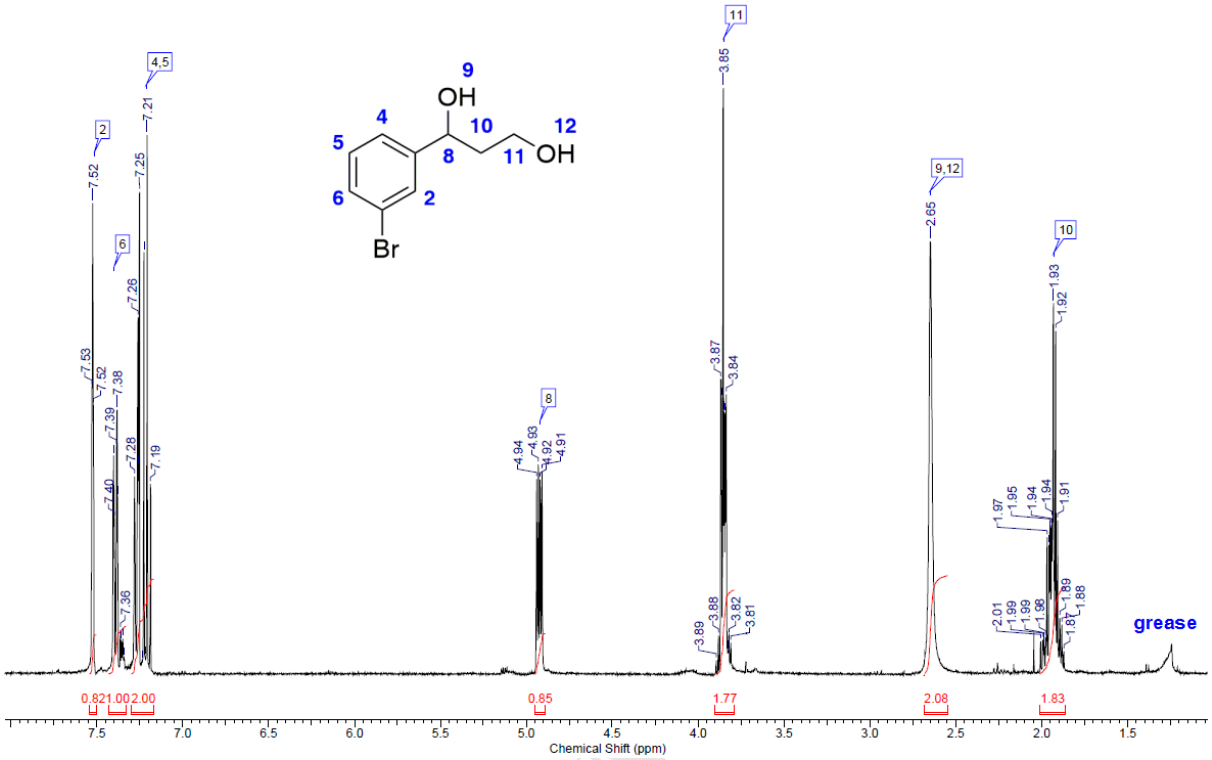

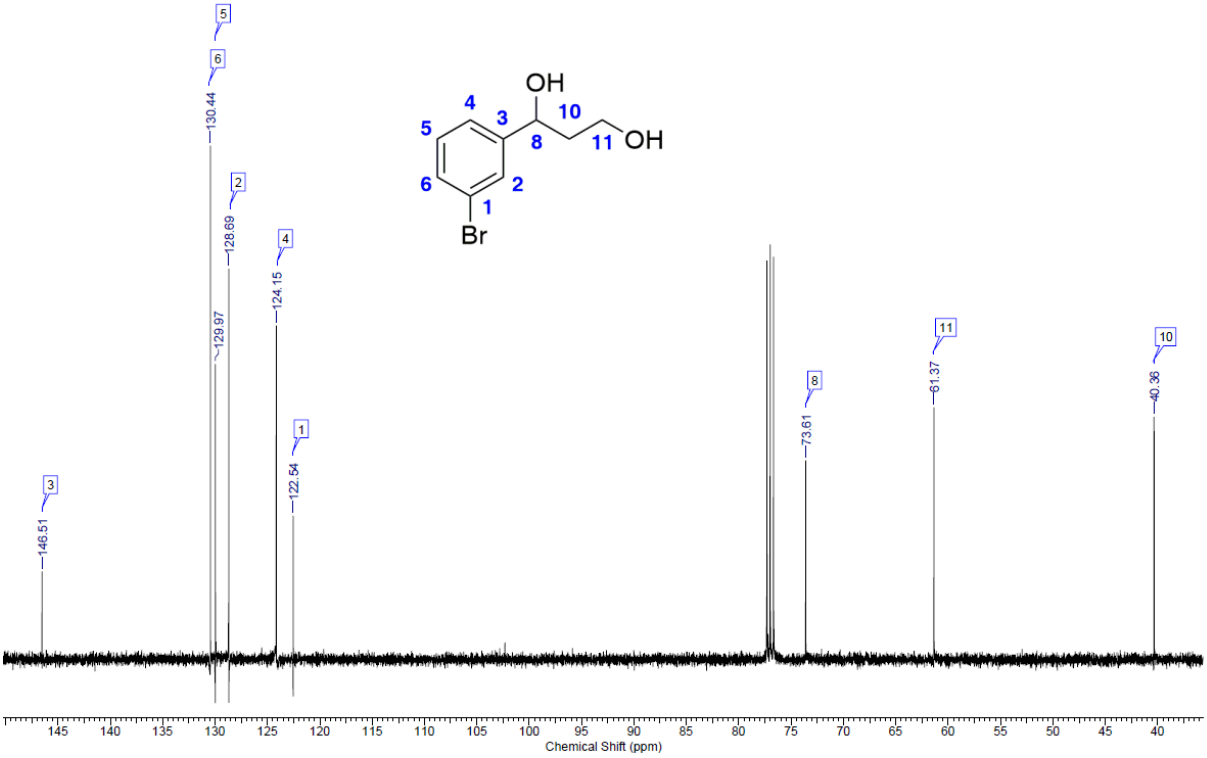

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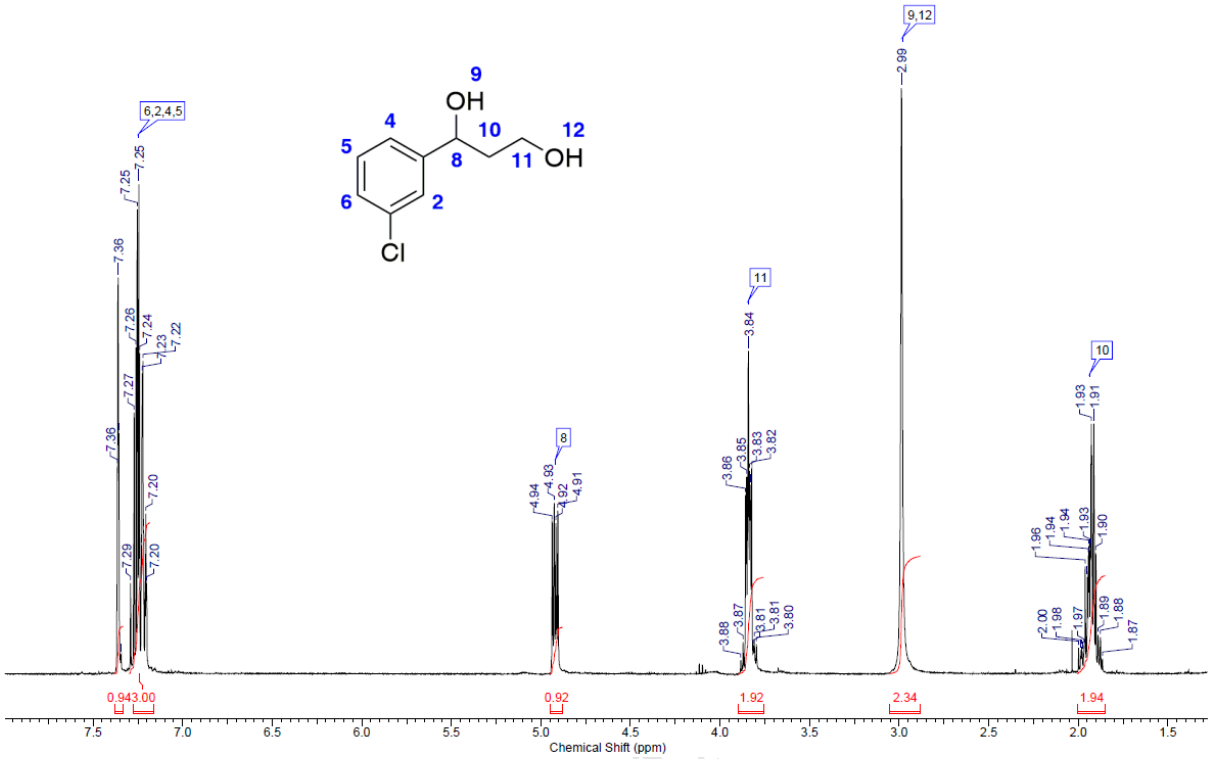
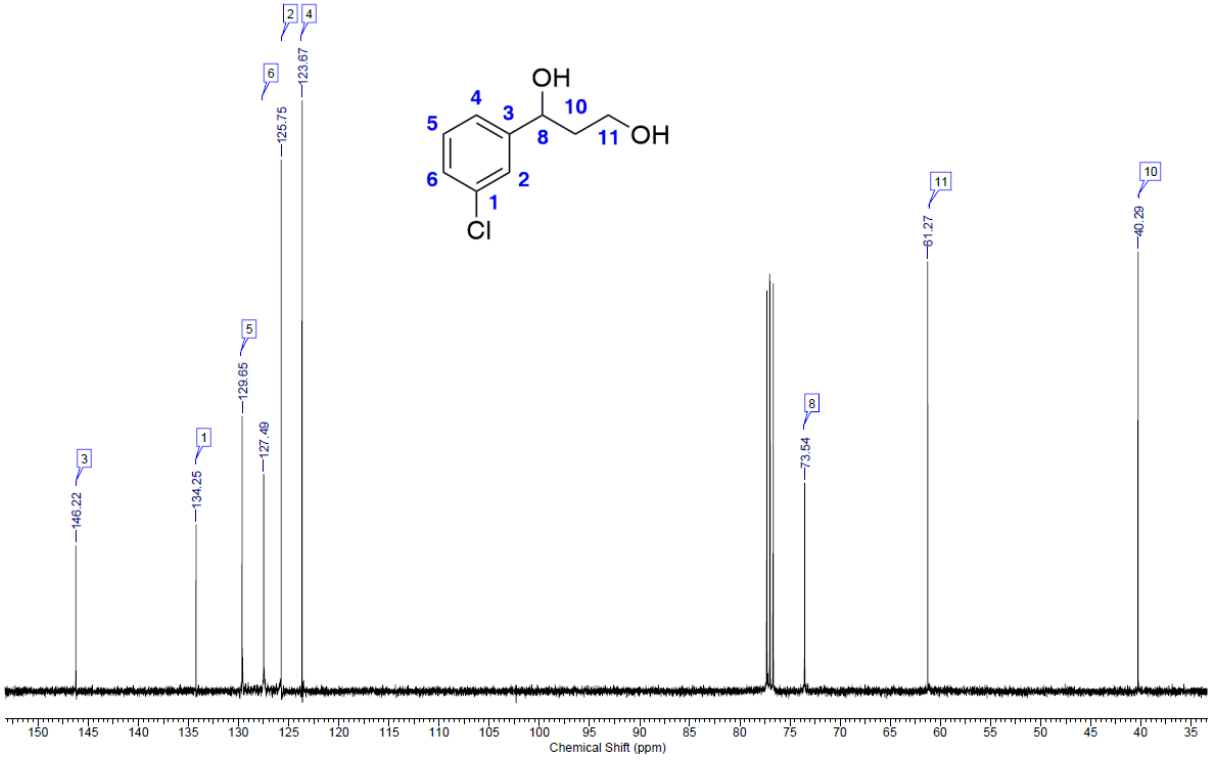
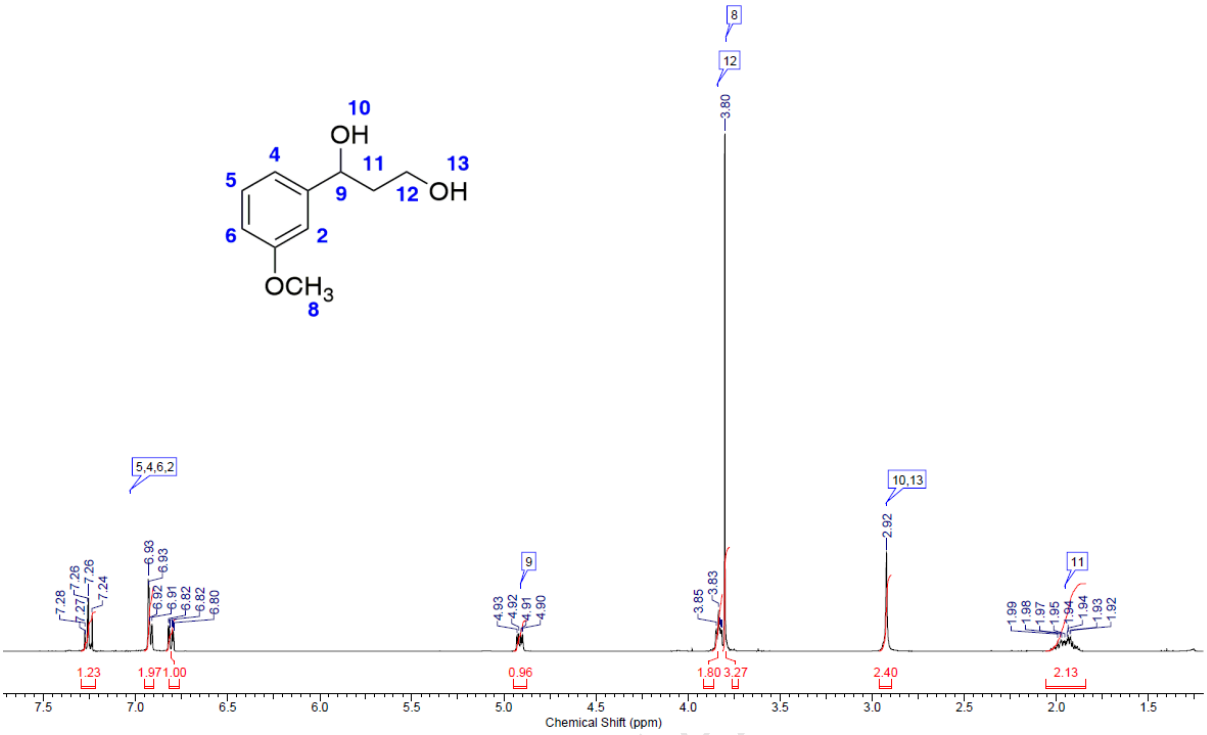
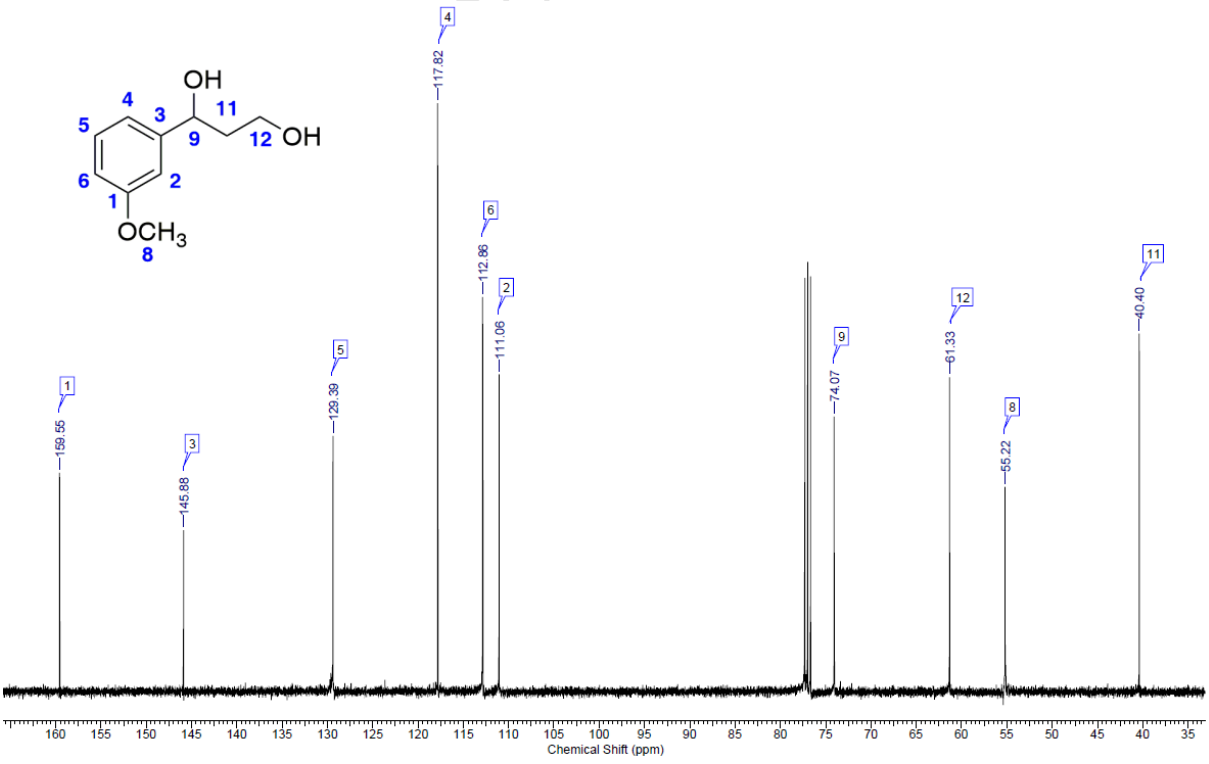


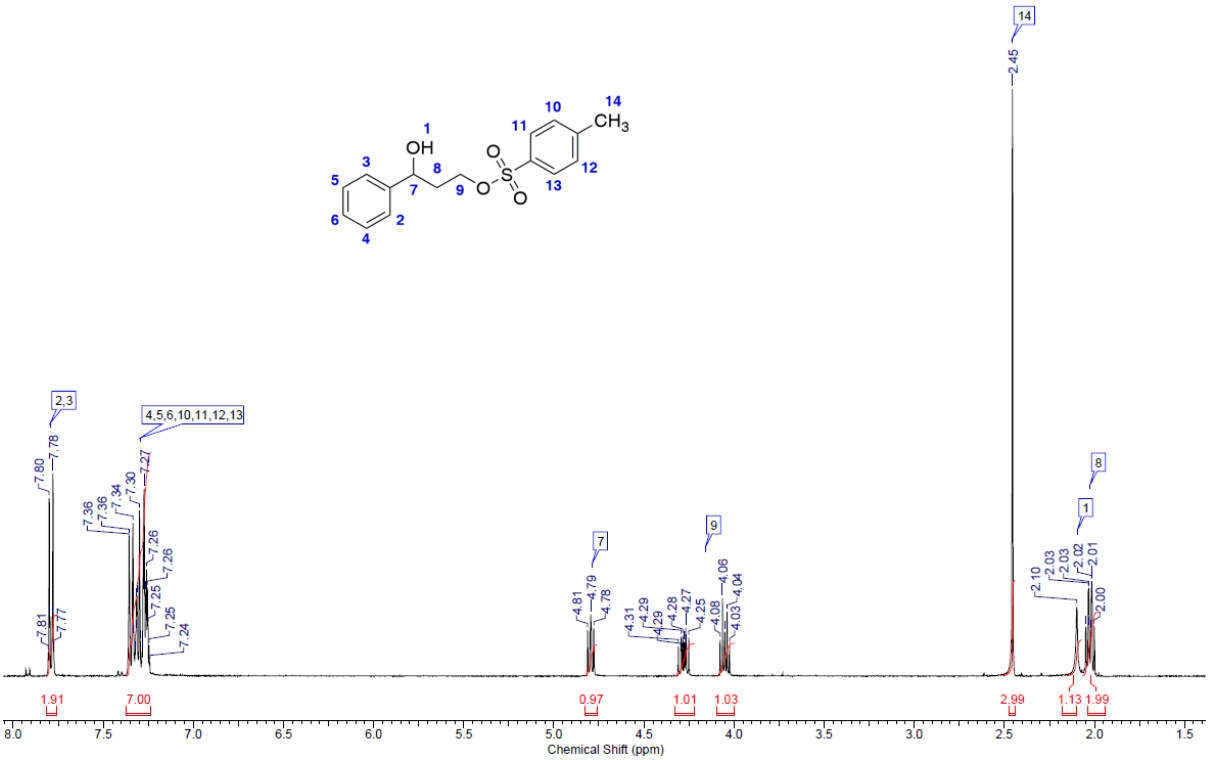


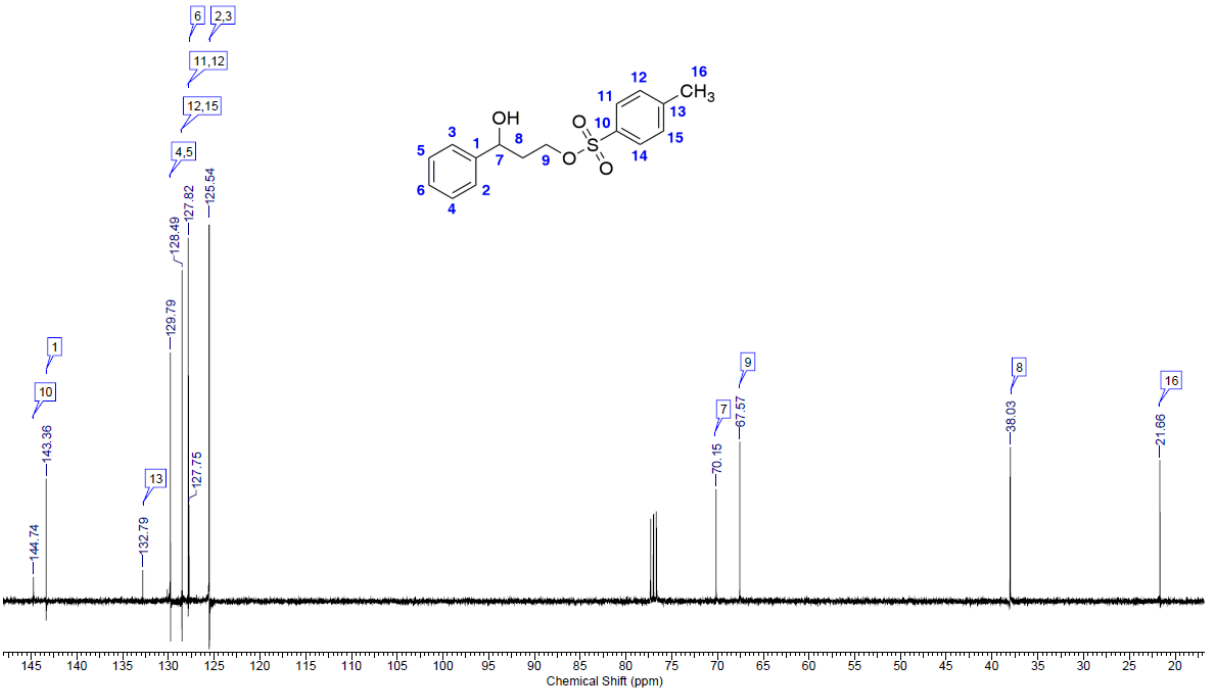
1-(3-Methoxyphenyl)propane-1,3-diol (2g):





3-Hydroxy-3-phenylpropyl 4-methylbenzenesulfonate (3a):





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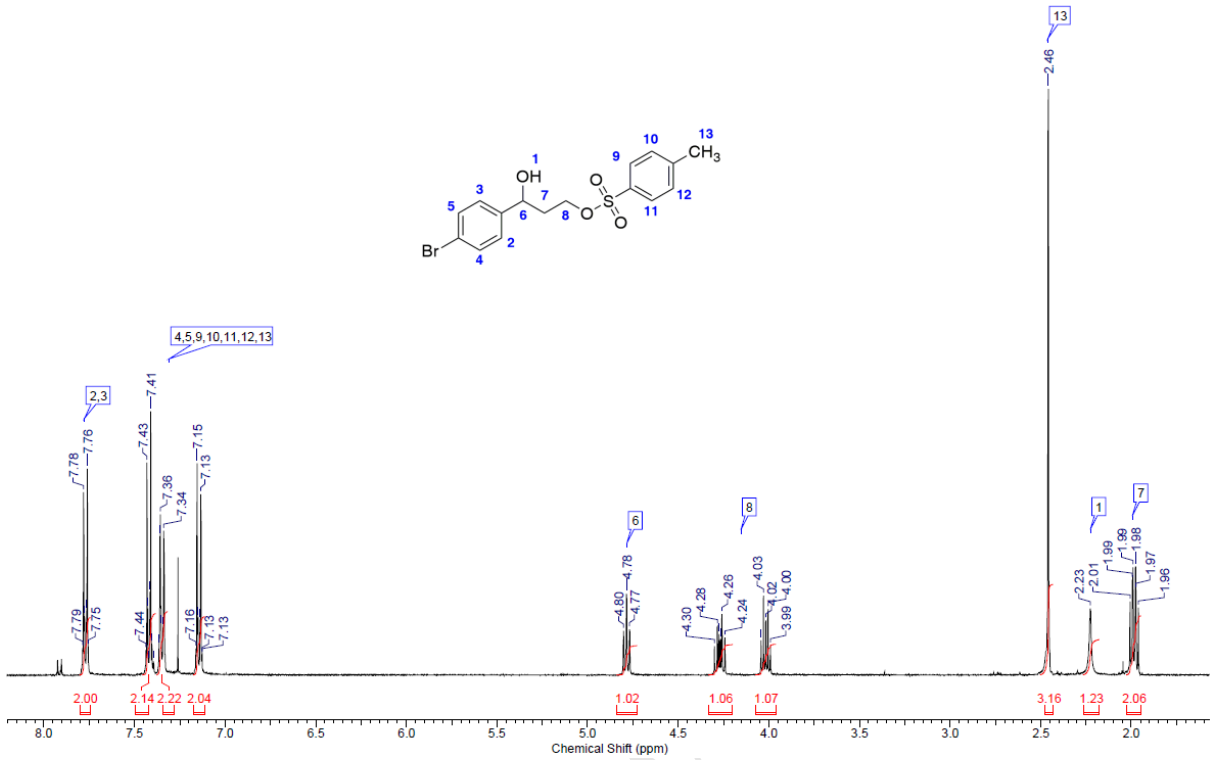

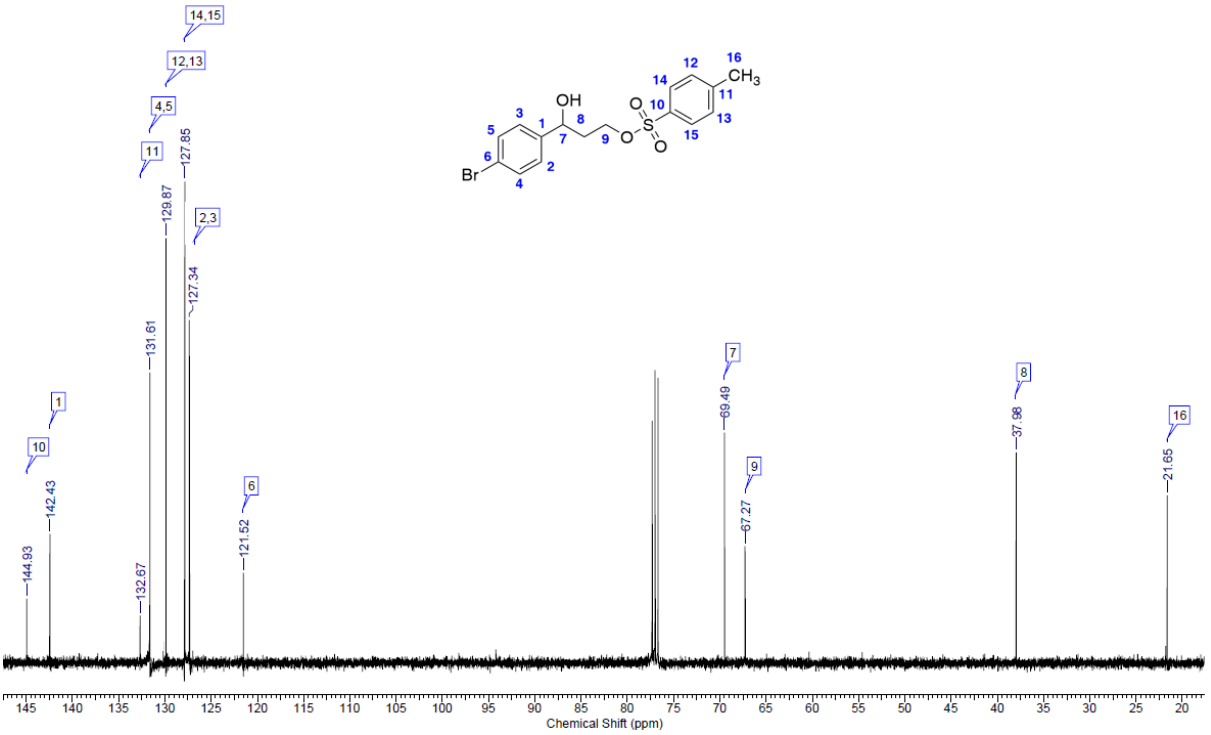

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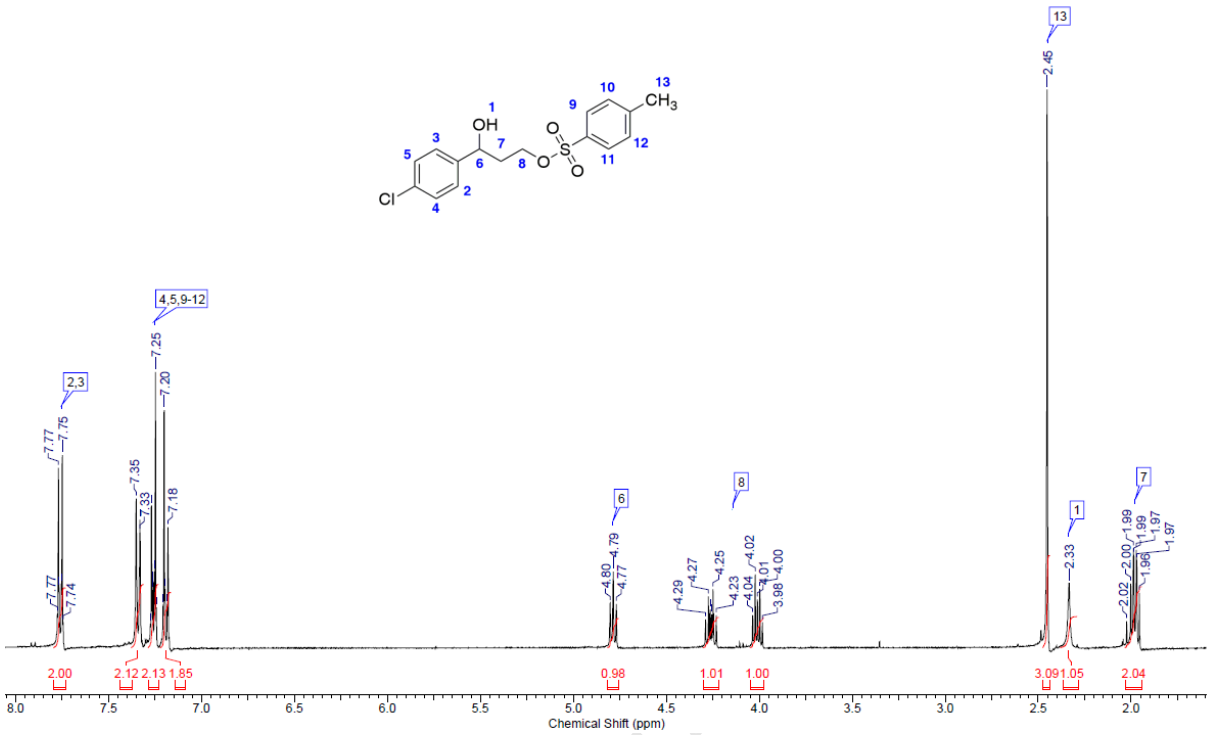

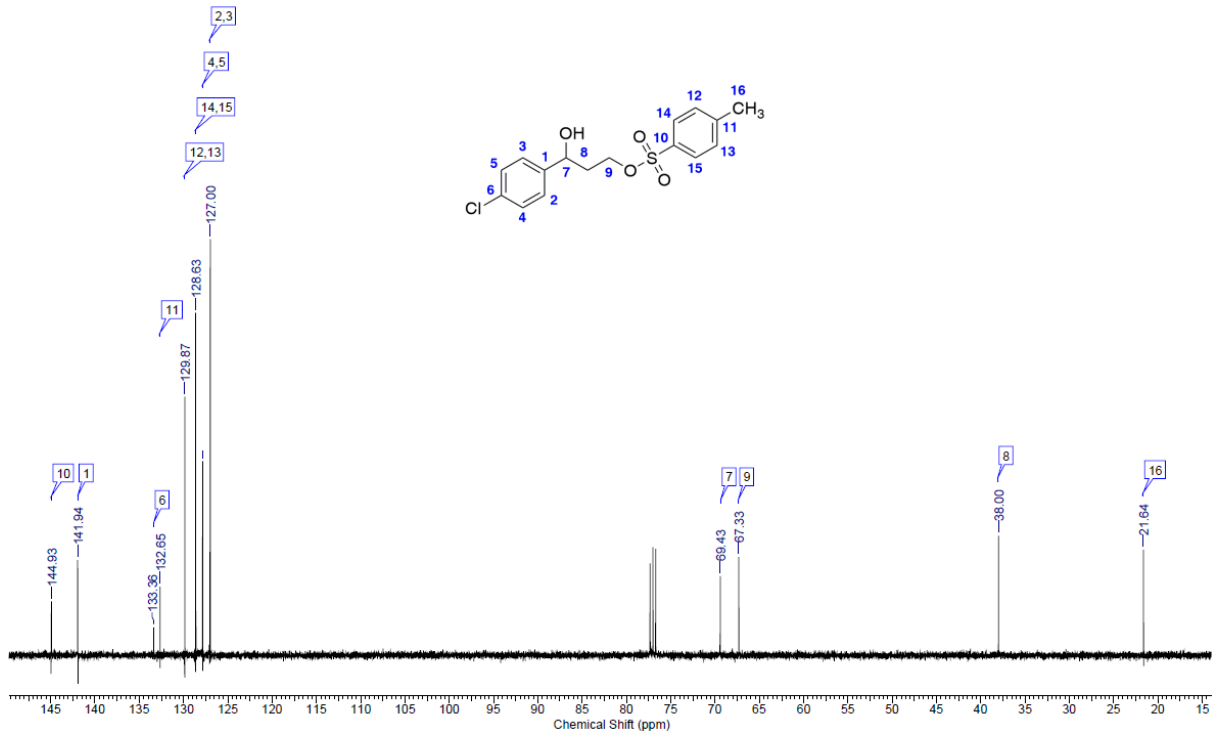

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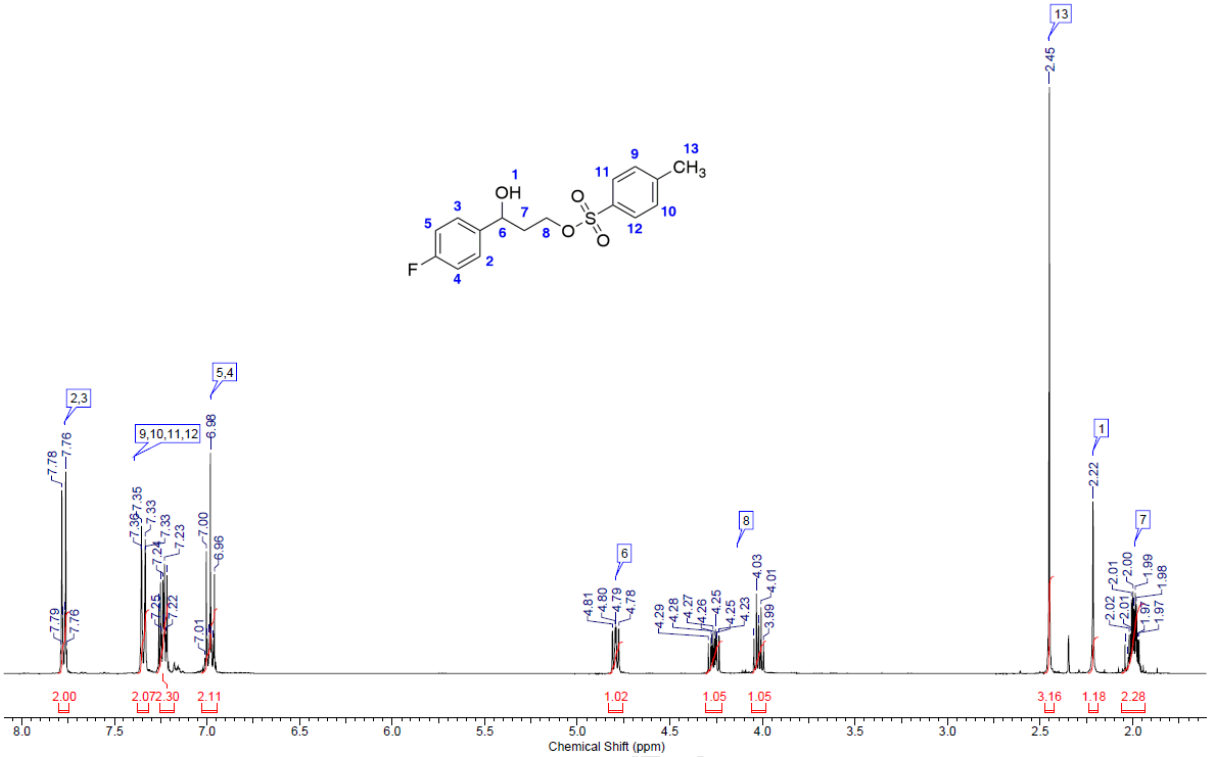

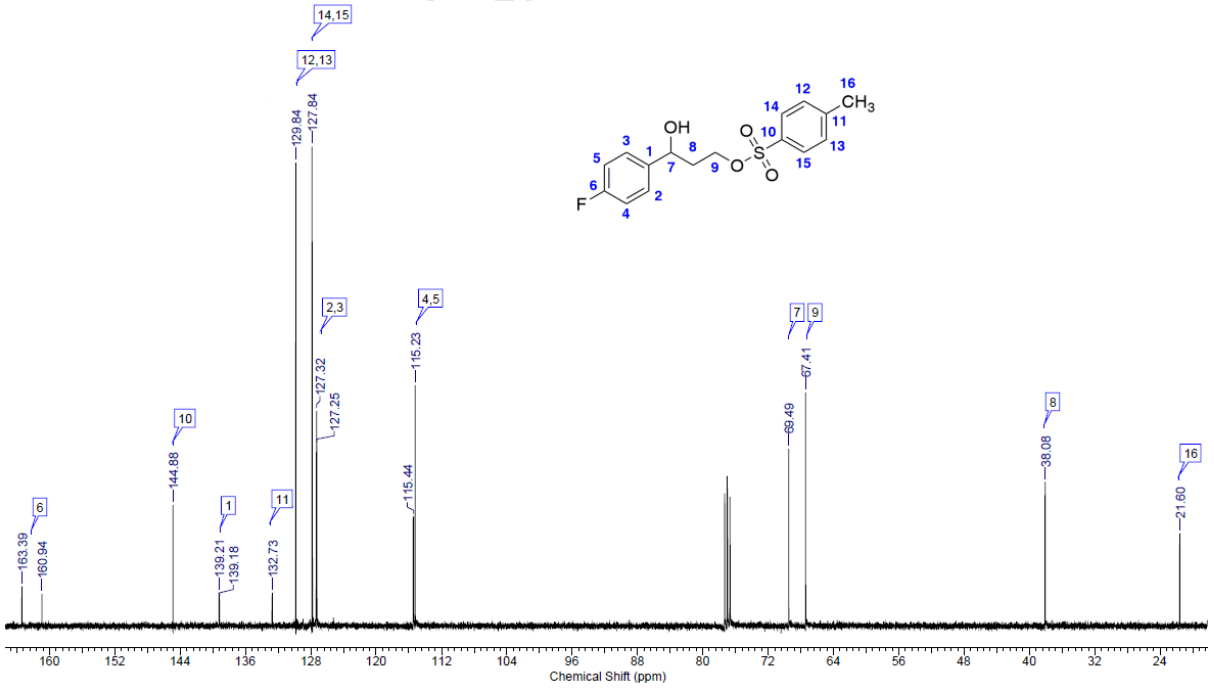

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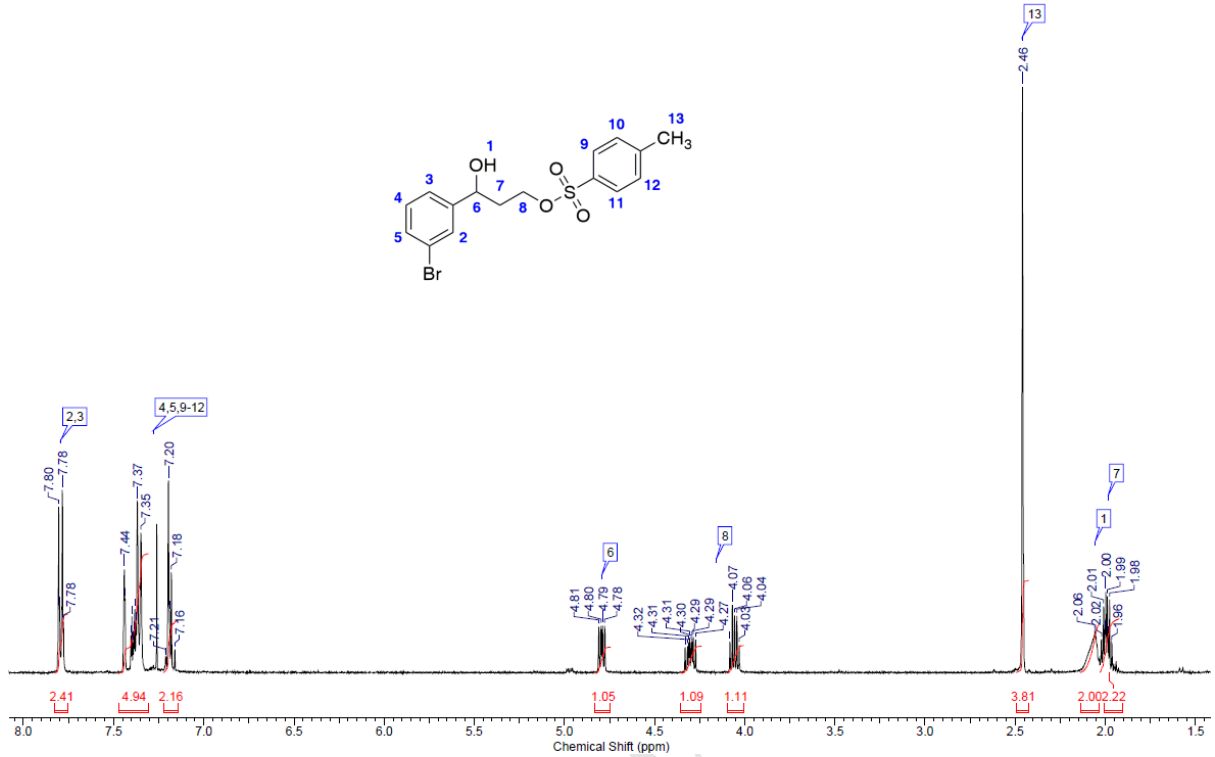

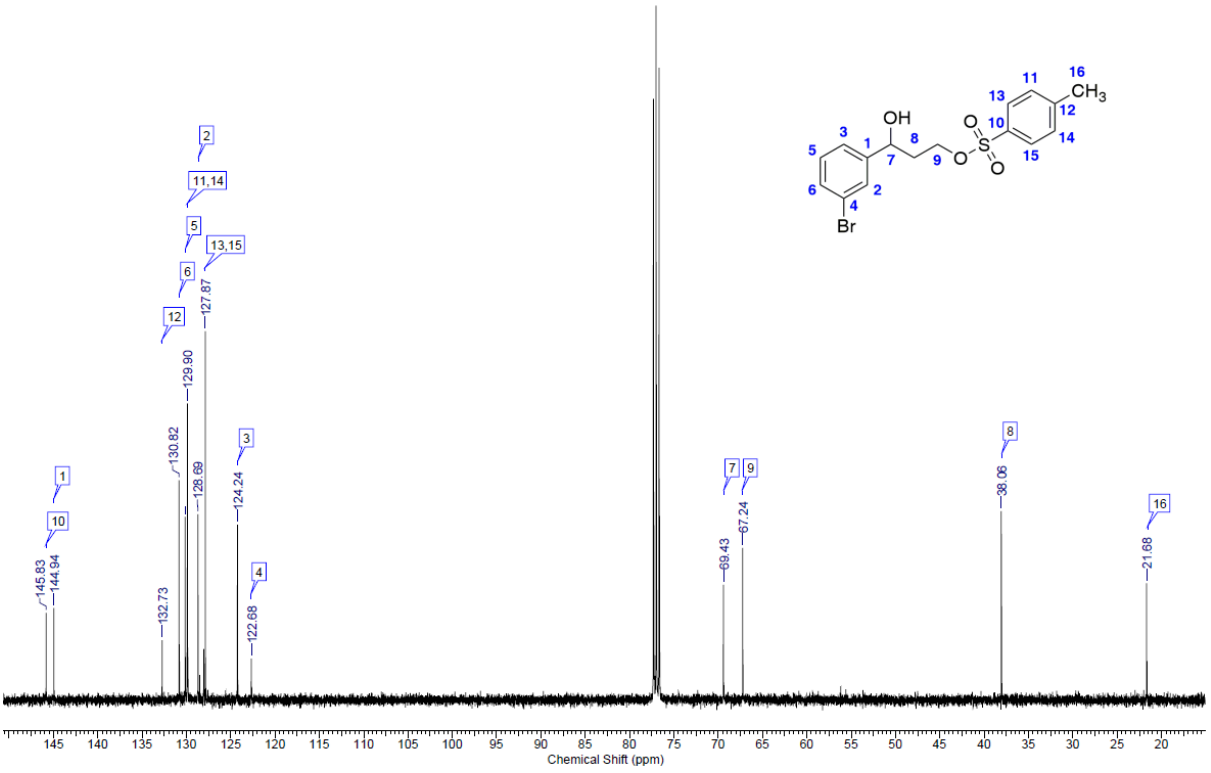

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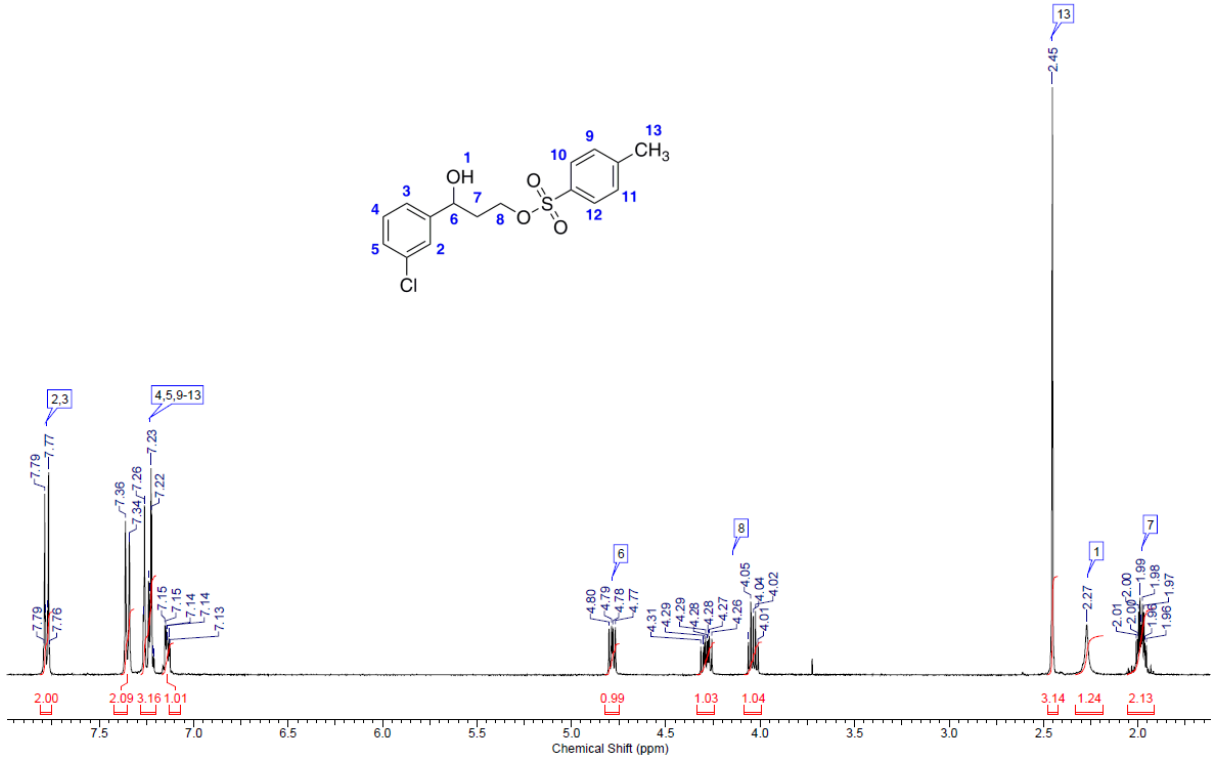

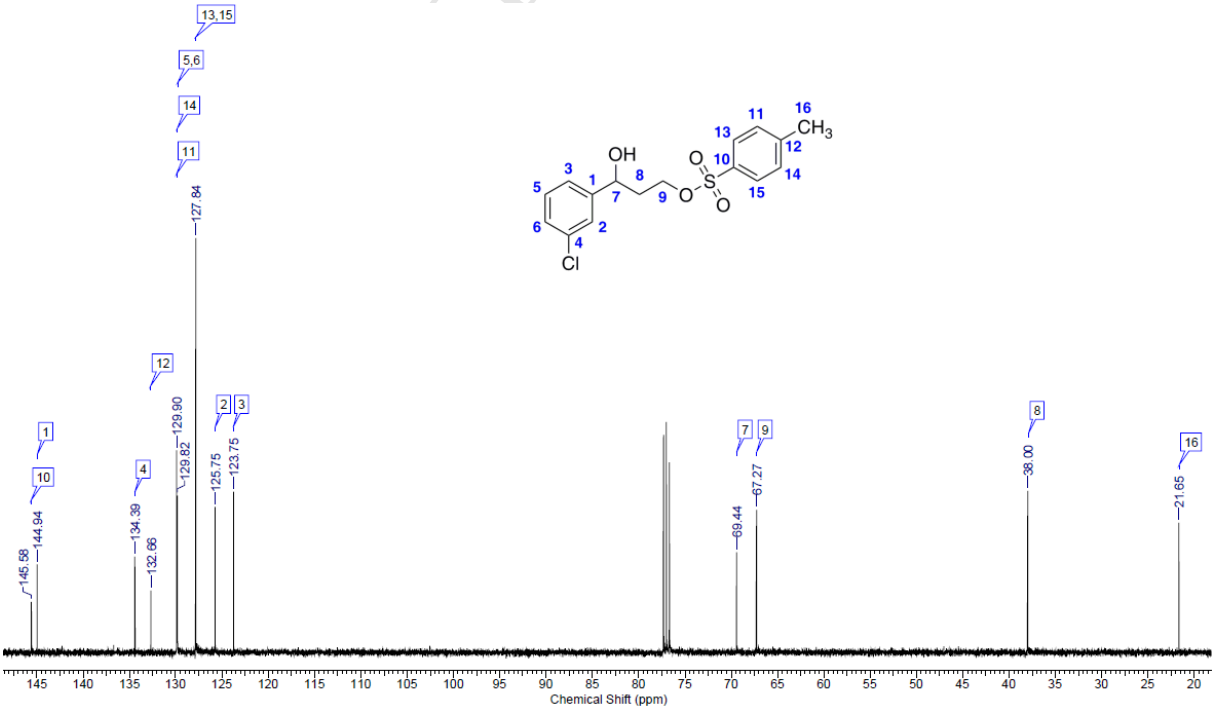

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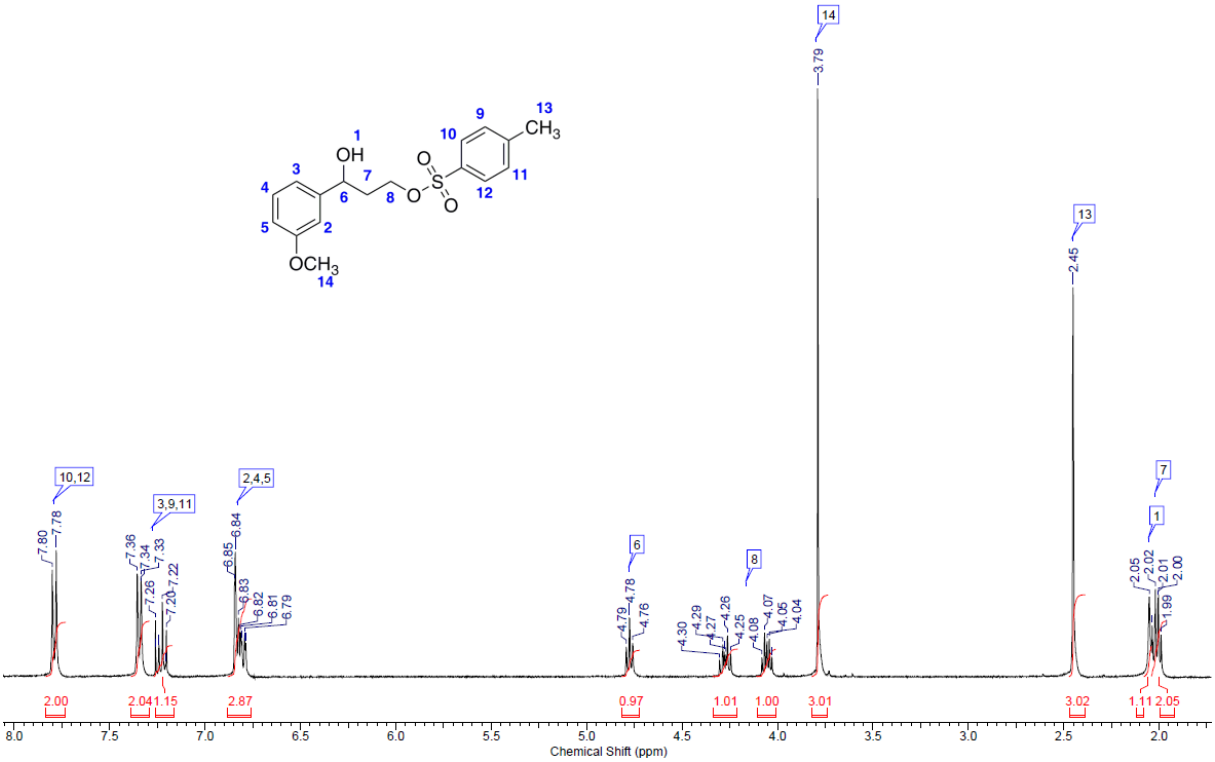
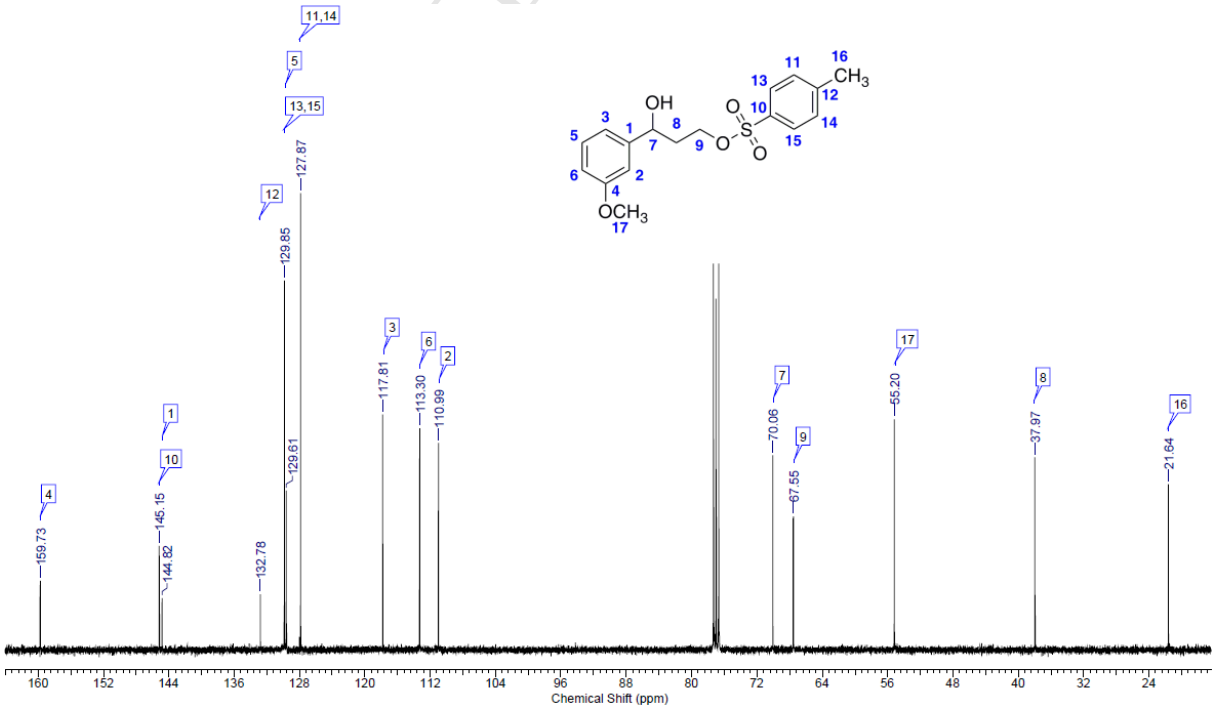
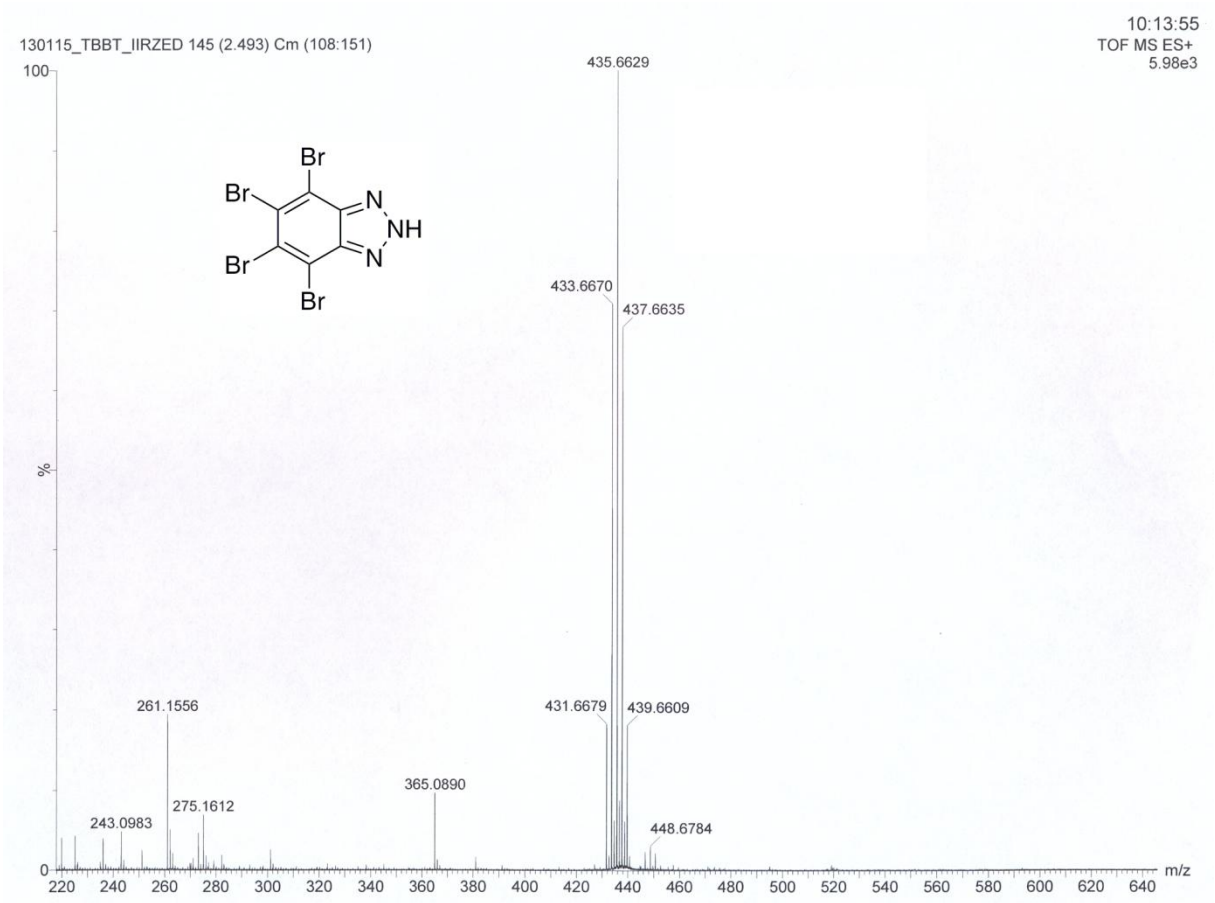
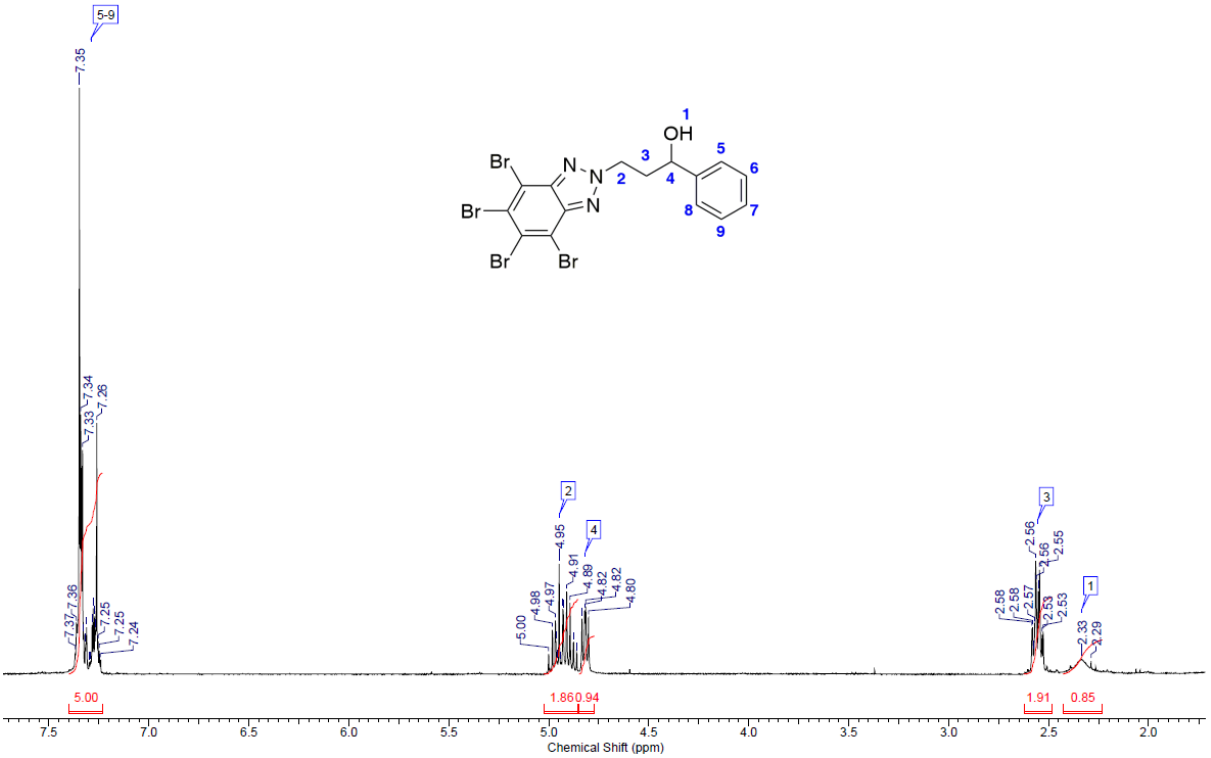


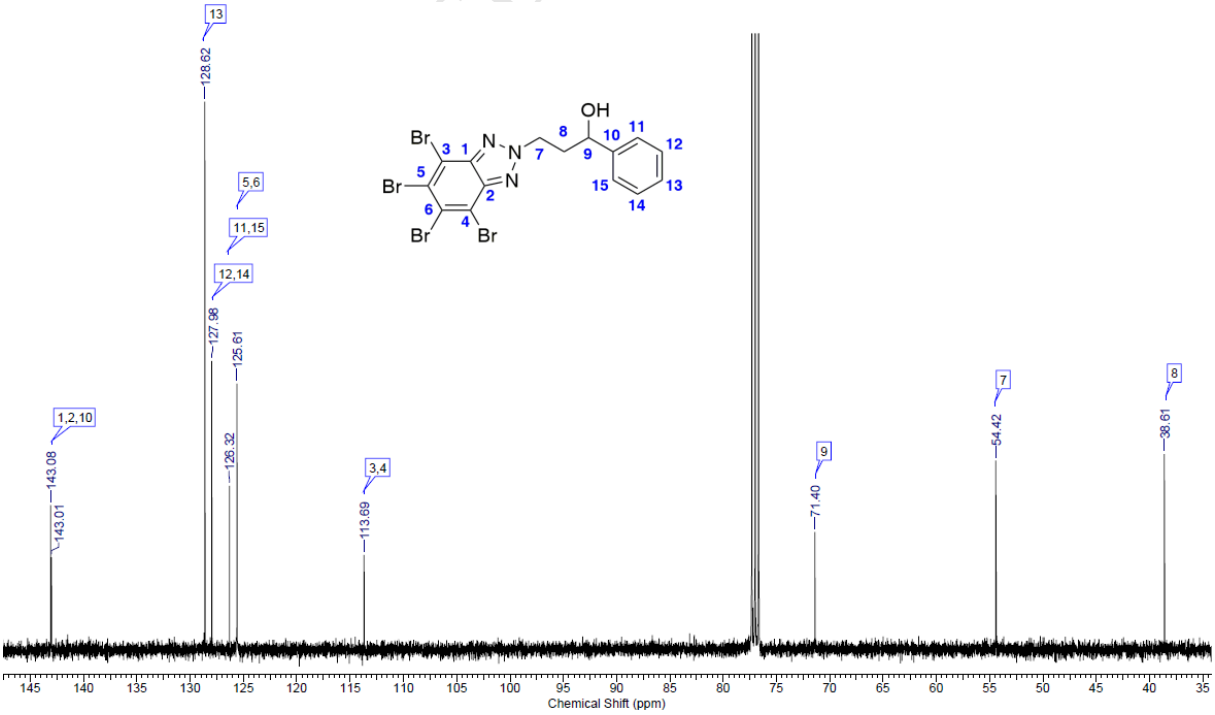


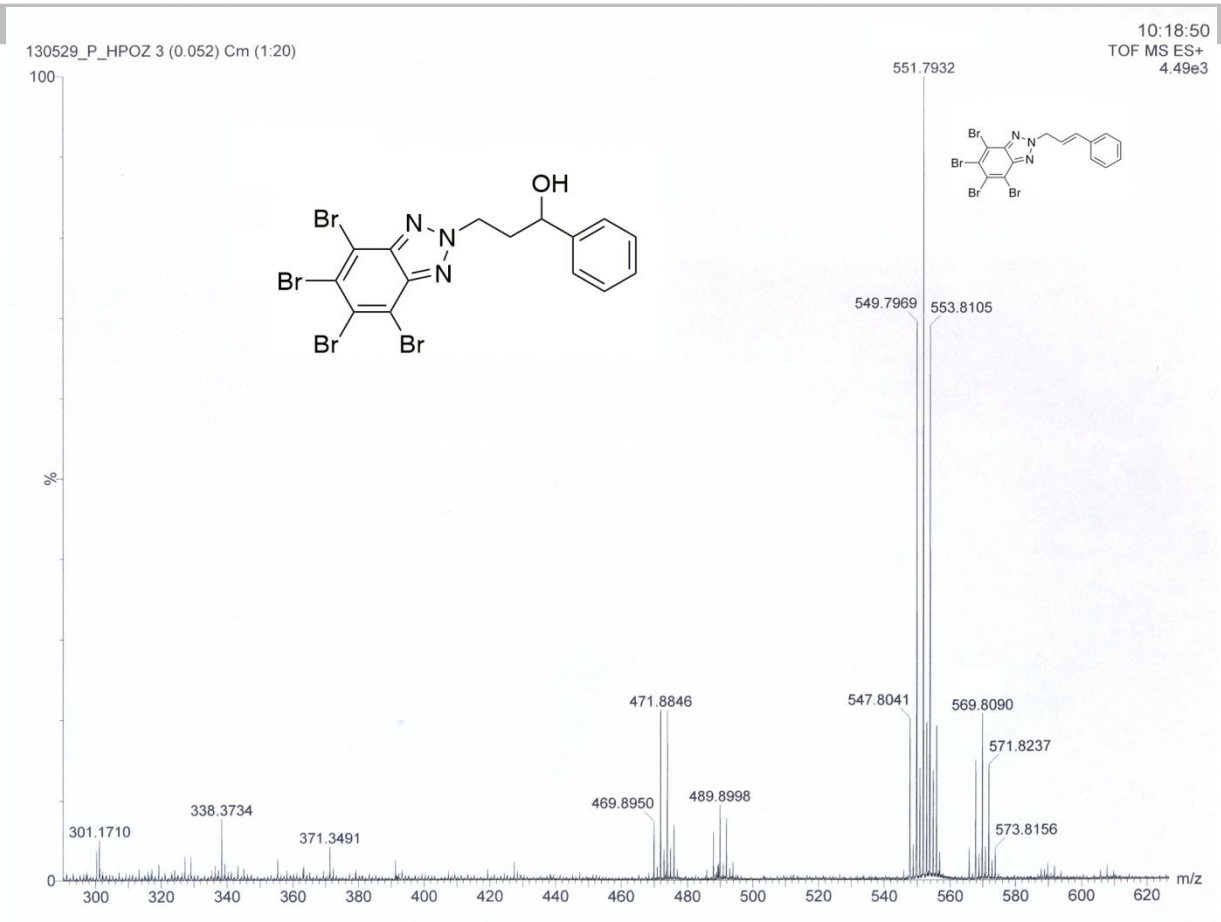
4,5,6,7-Tetrabromo-1*H*-benzotriazole (5):



1-Phenyl-3-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)propan-1-ol (6a):



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%
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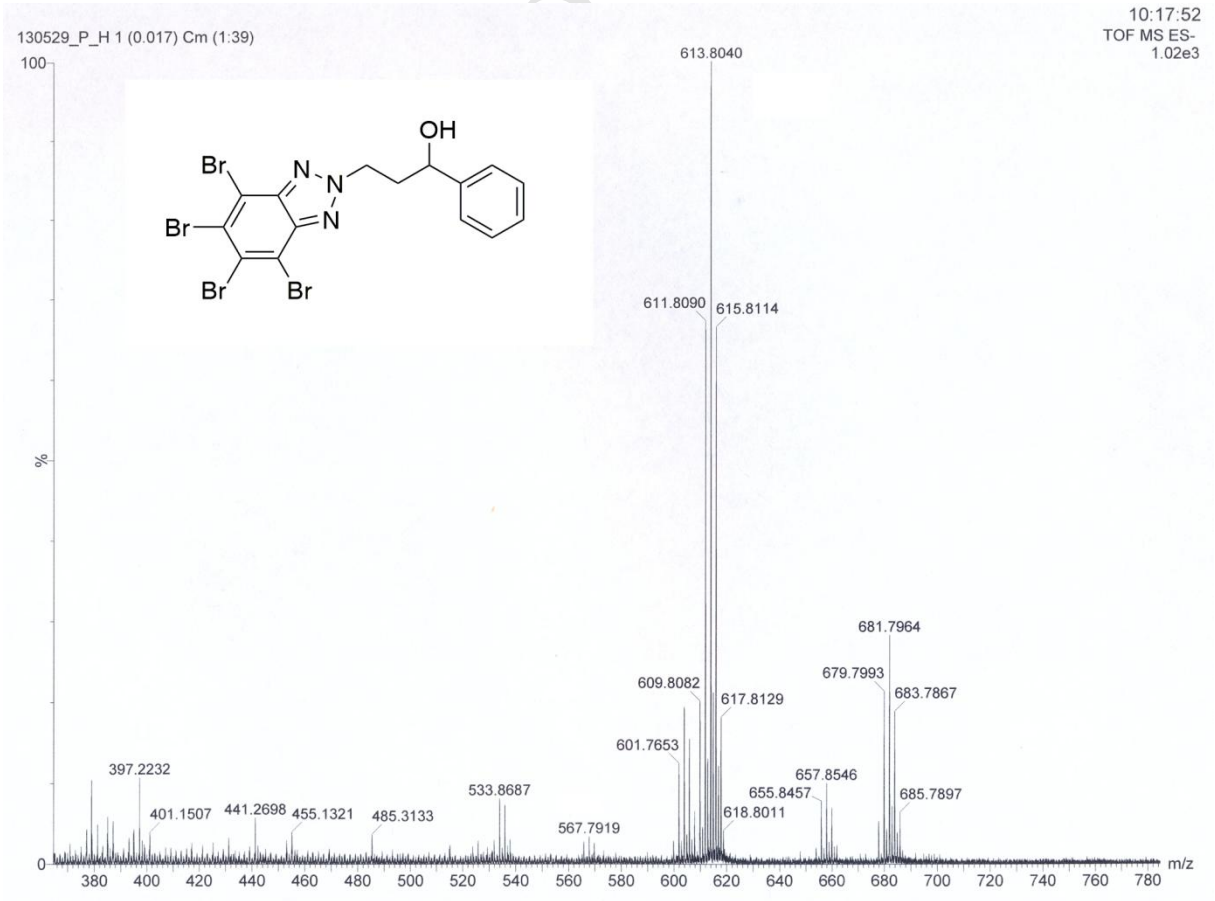
m/z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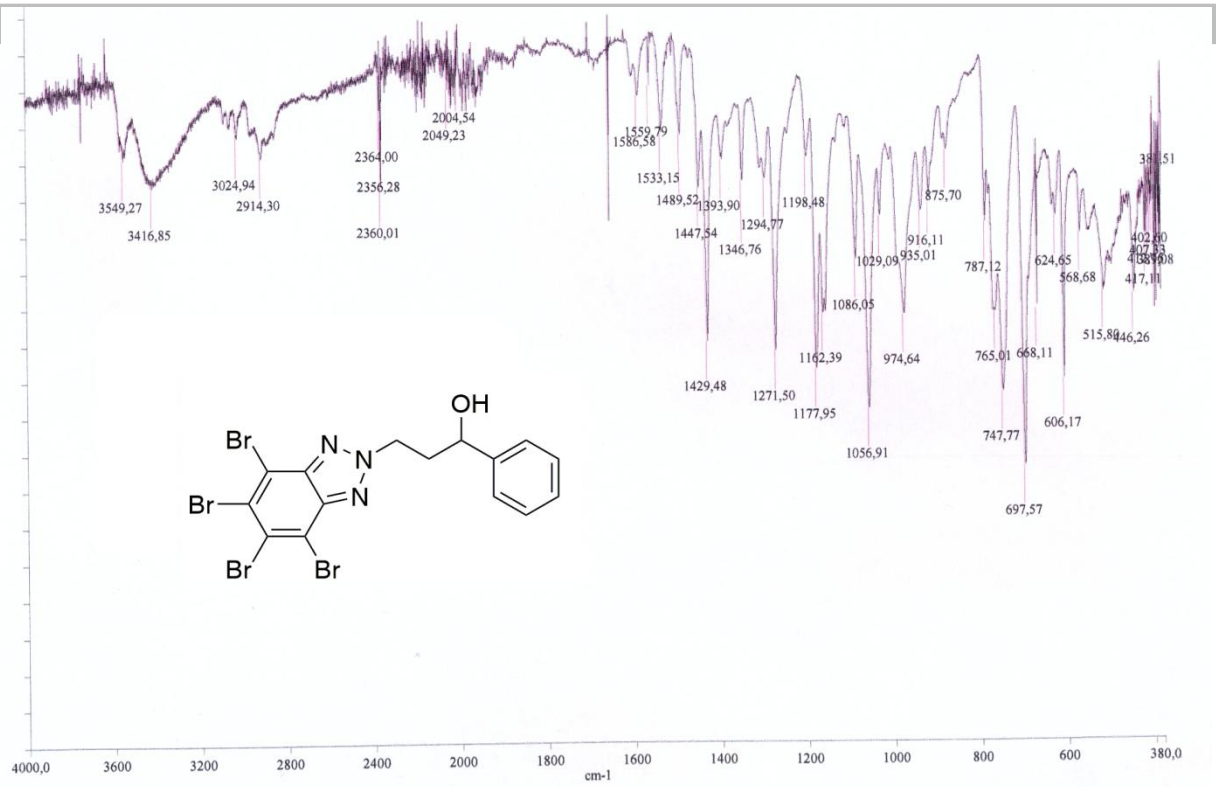
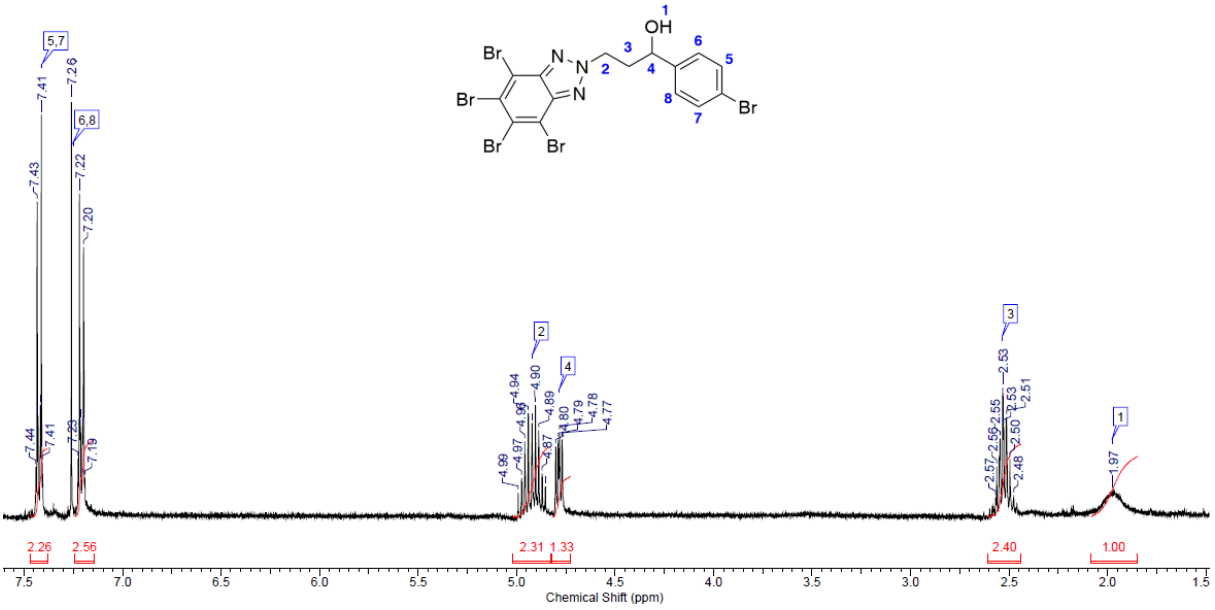
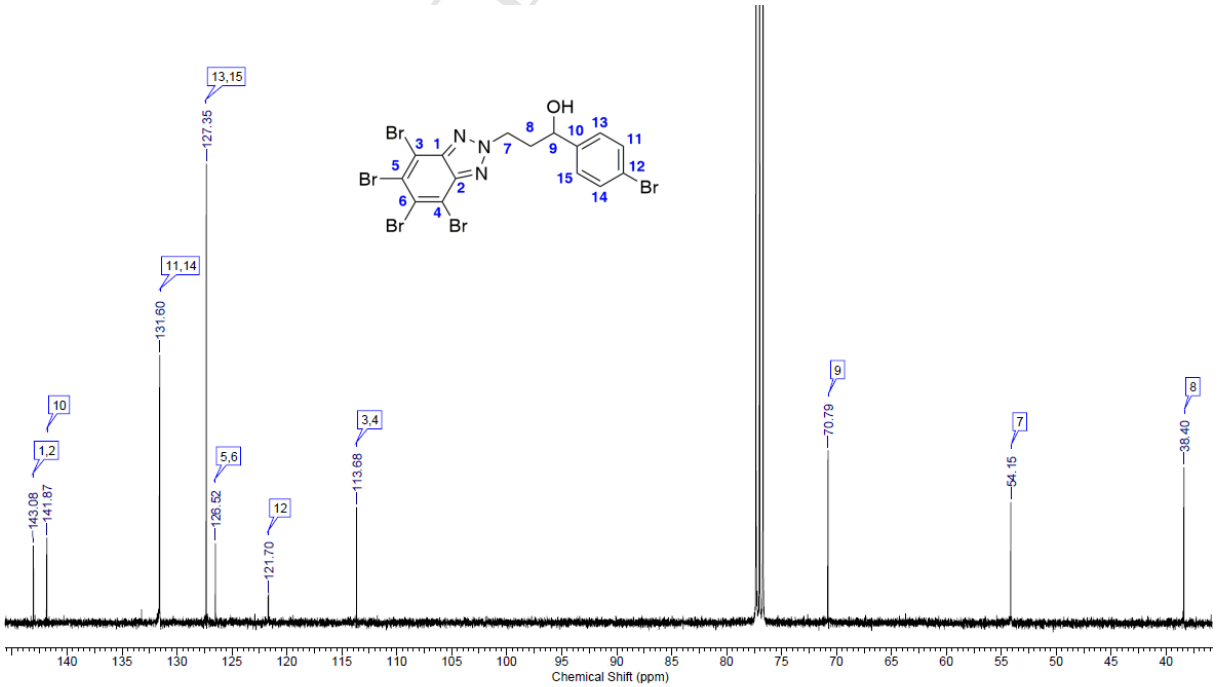
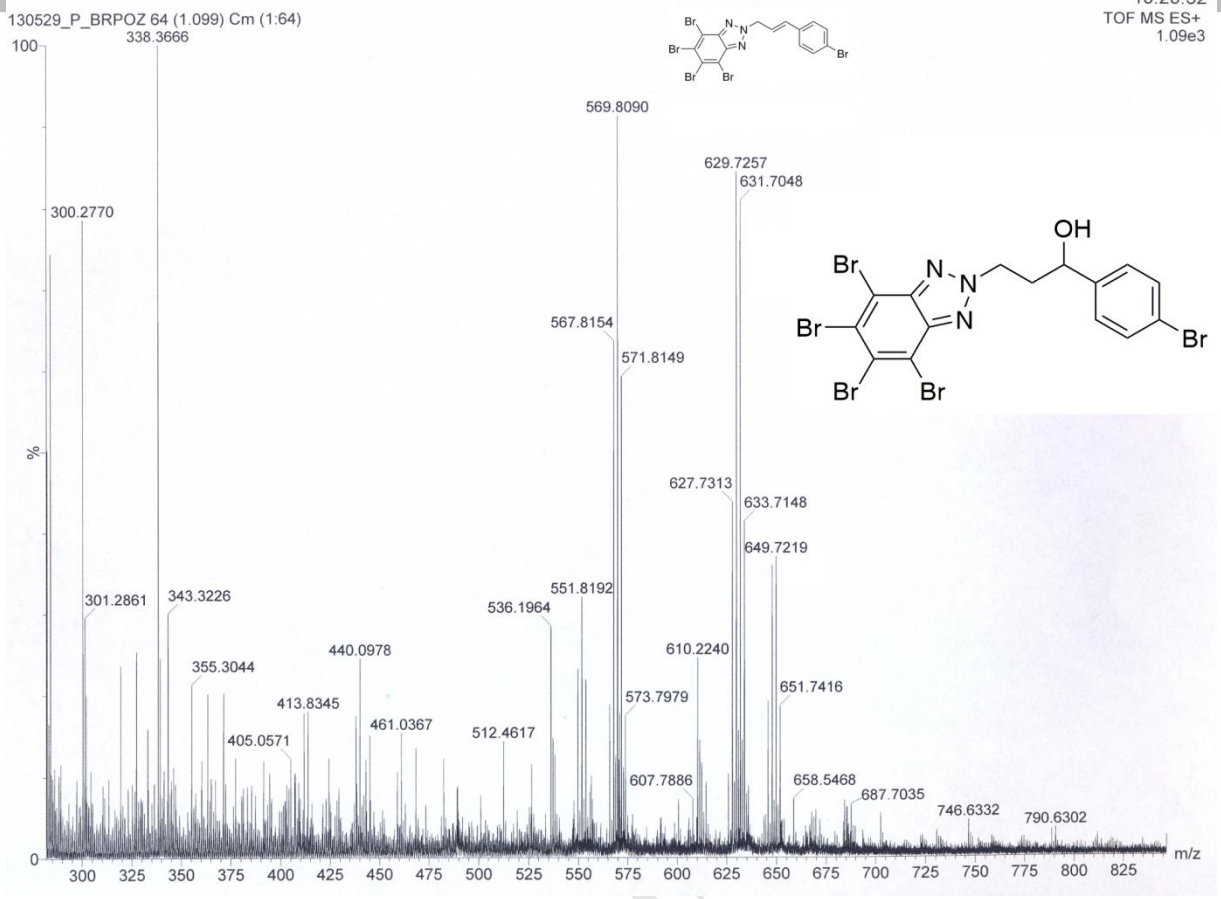
Br
380 400 420 440 460 480 500 520 540 560 580 600 620 640 660 680 700 720 740 760 780
m/z

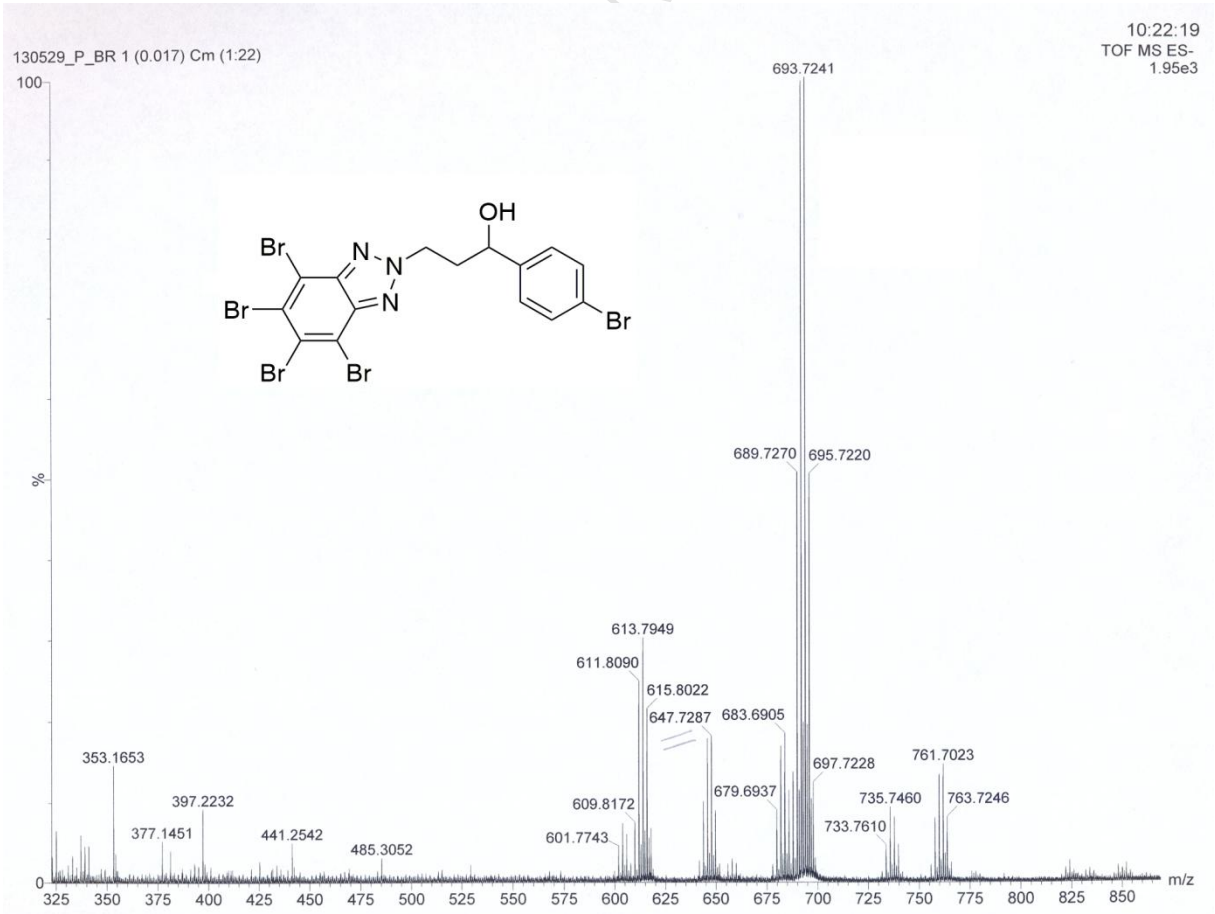


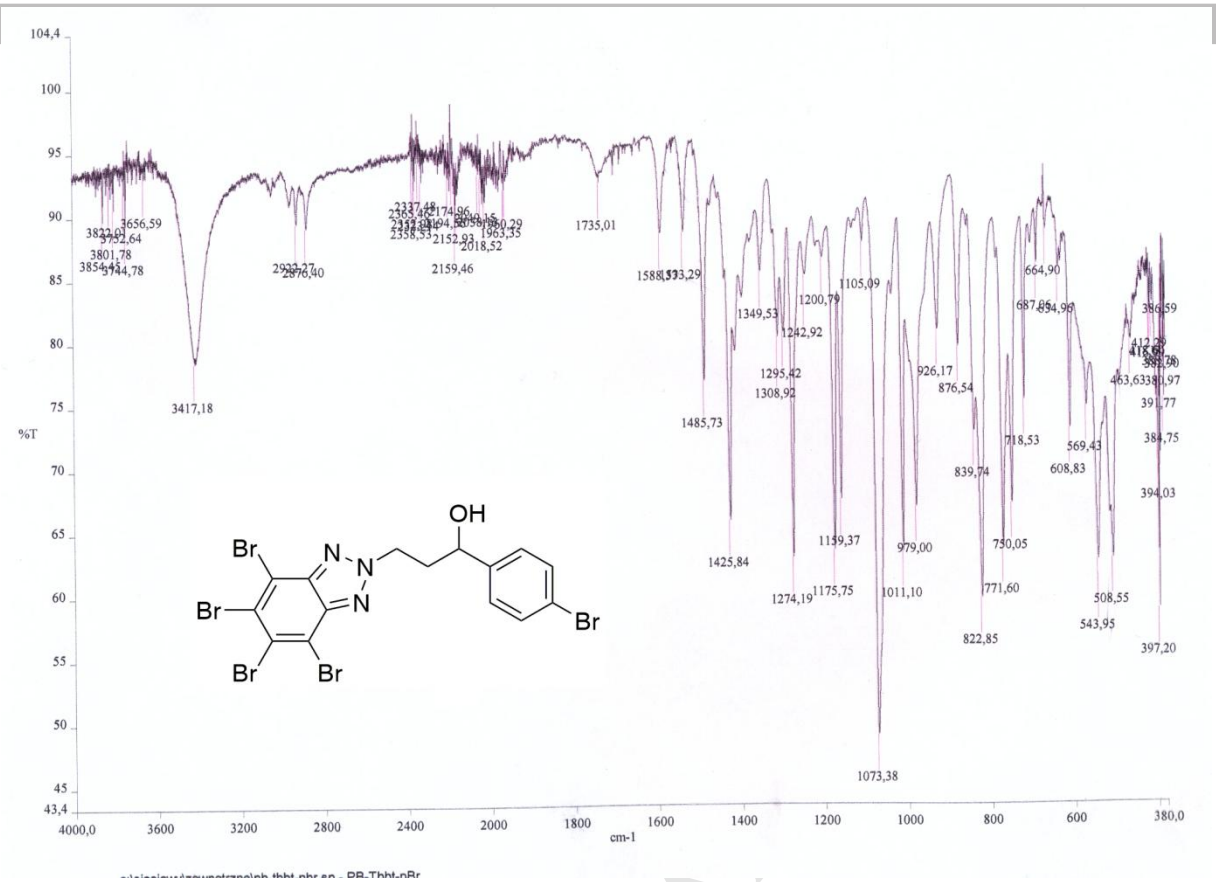
1-(4-Bromophenyl)-3-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)propan-1-ol (6b):



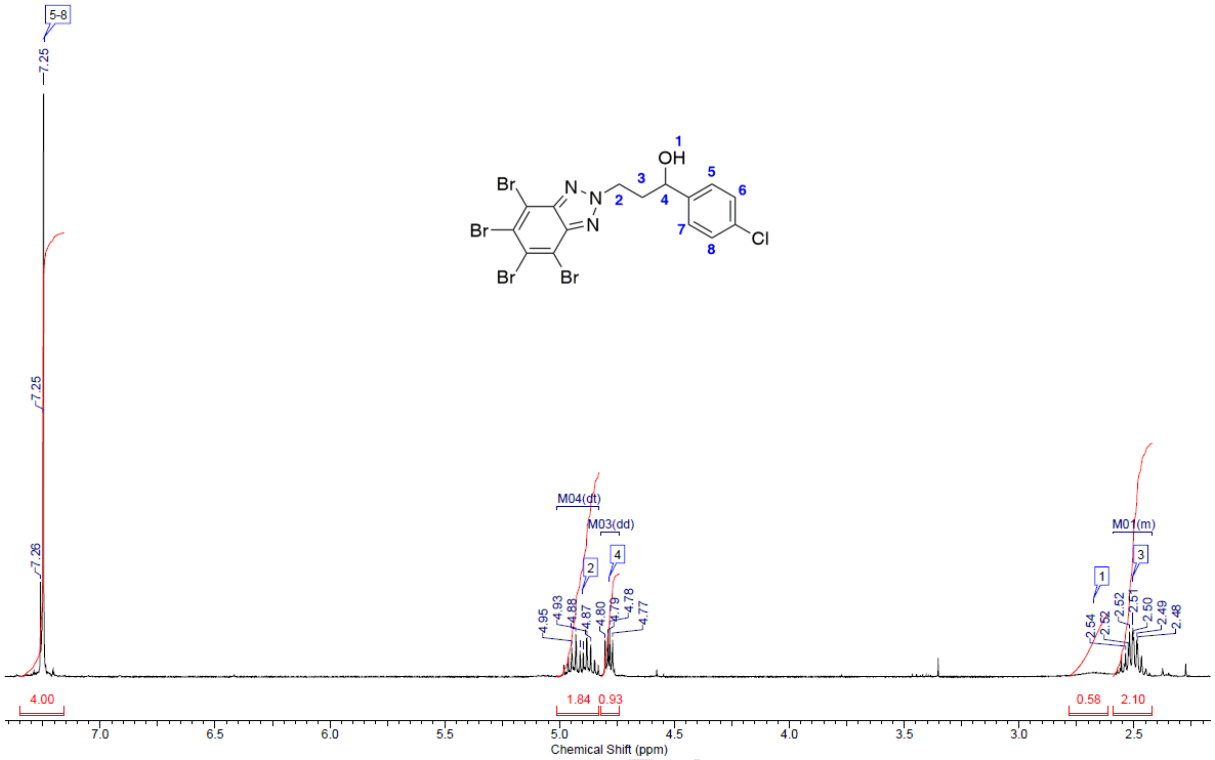


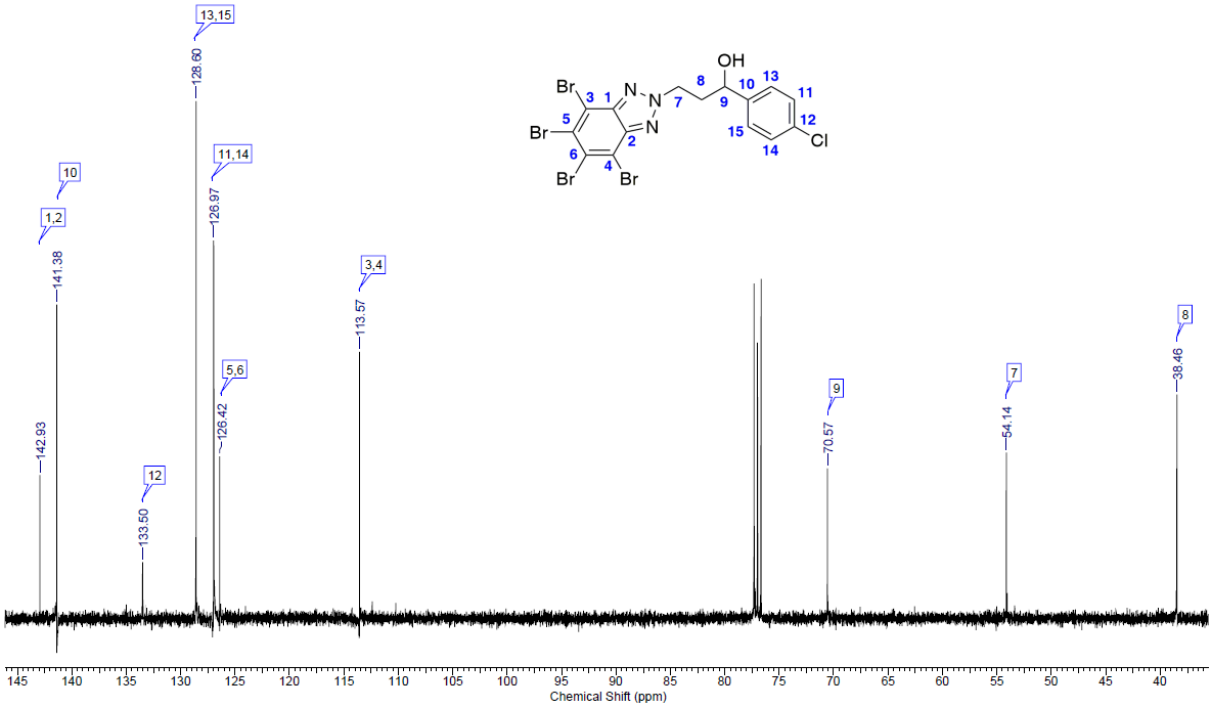

130529_P_BRPOZ 64 (1.099) Cm (1:64)
10:20:52
TOF MS ES+
1.09e3
100
%
0
338.3666
569.8090
629.7257
631.7048
300.2770
567.8154
571.8149
627.7313
633.7148
649.7219
301.2861
343.3226
551.8192
536.1964
610.2240
440.0978
355.3044
651.7416
573.7979
413.8345
461.0367
512.4617
405.0571
607.7886
658.5468
687.7035
746.6332
790.6302
300 325 350 375 400 425 450 475 500 525 550 575 600 625 650 675 700 725 750 775 800 825
m/z
OH
Br
N
N
N
Br
Br
Br
Br
Br

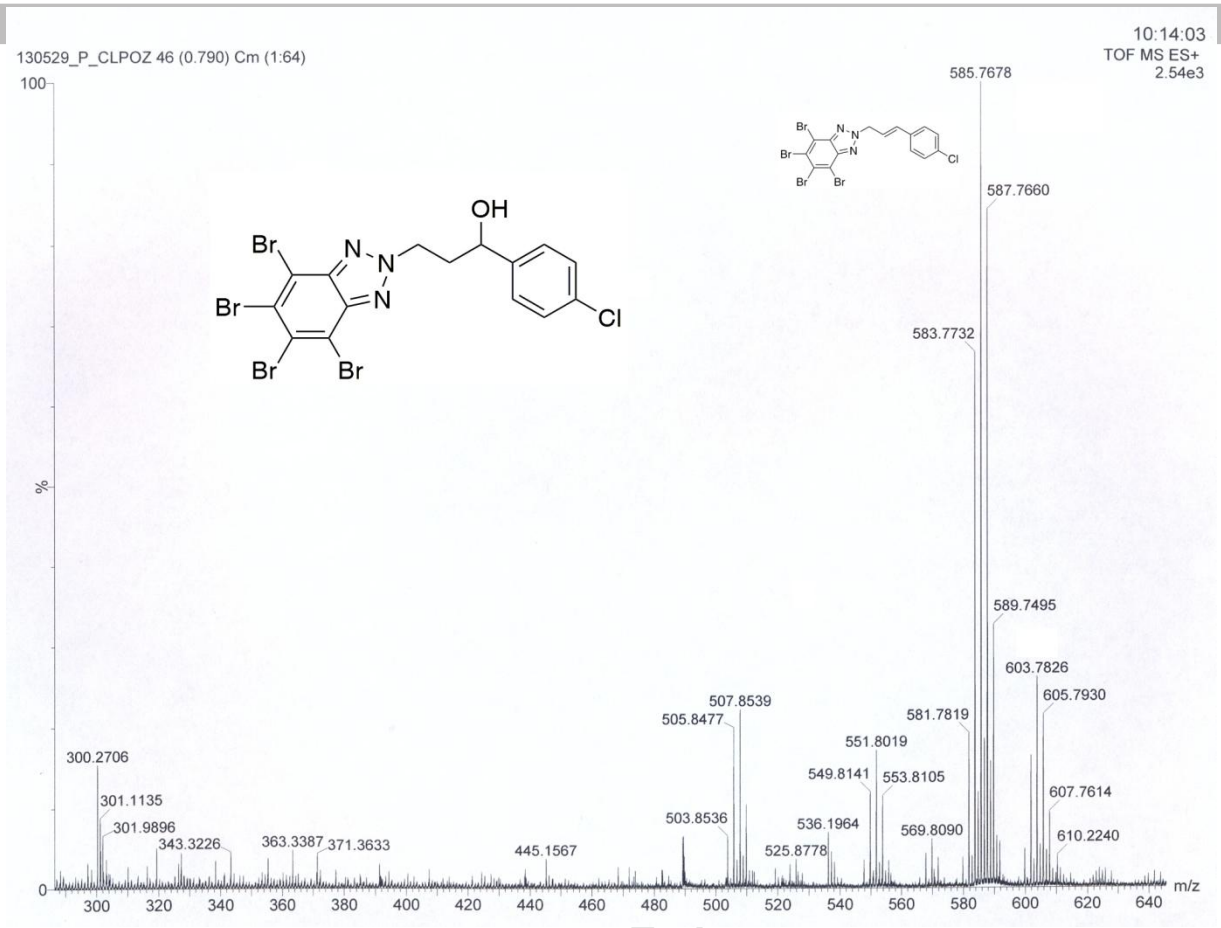

130529_P_BR 1 (0.017) Cm (1:22)
10:22:19
TOF MS ES-
1.95e3
100
%
0
693.7241
689.7270
695.7220
613.7949
611.8090
615.8022
647.7287
683.6905
353.1653
697.7228
761.7023
397.2232
679.6937
609.8172
735.7460
763.7246
377.1451
441.2542
601.7743
733.7610
485.3052
325 350 375 400 425 450 475 500 525 550 575 600 625 650 675 700 725 750 775 800 825 850
m/z
OH
Br
N
N
N
Br
Br
Br
Br
Br

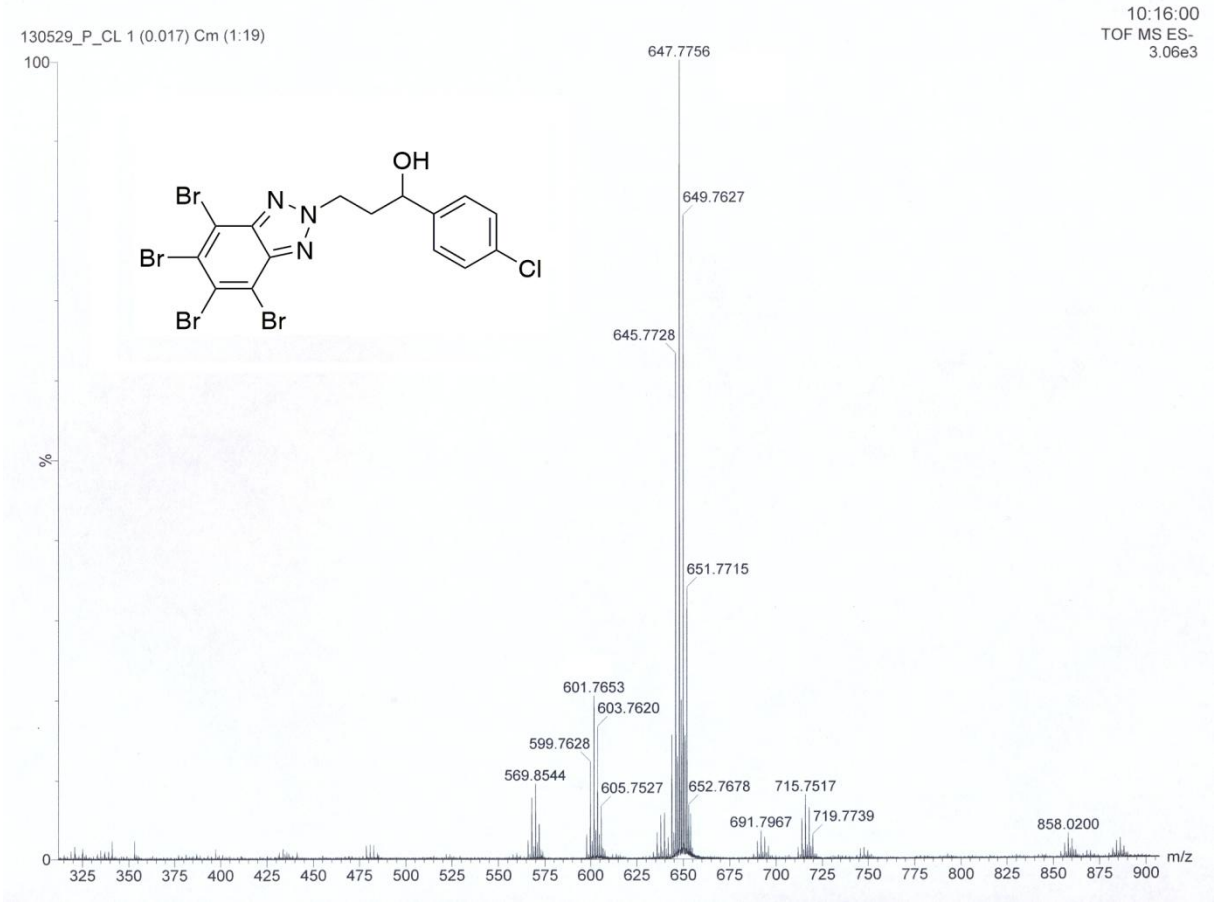

104,4
100
95
90
85
80
75
70
65
60
55
50
45
43,4
%T
4000,0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
380,0
cm-1
3854,45
3822,03
3801,78
3744,78
3656,59
3752,64
3417,18
2923,27
2876,40
2337,48
2159,46
2152,93
2018,52
1963,35
1735,01
1588,53
1485,73
1425,84
1349,53
1308,92
1295,42
1274,19
1242,92
1200,79
1175,75
1159,37
1105,09
1073,38
1011,10
979,00
926,17
876,54
839,74
822,85
771,60
750,05
718,53
687,85
664,90
634,96
608,83
569,43
543,95
508,55
463,63
397,20
394,03
391,77
384,75
OH
Br
Br
Br
Br
Br
N
N
N

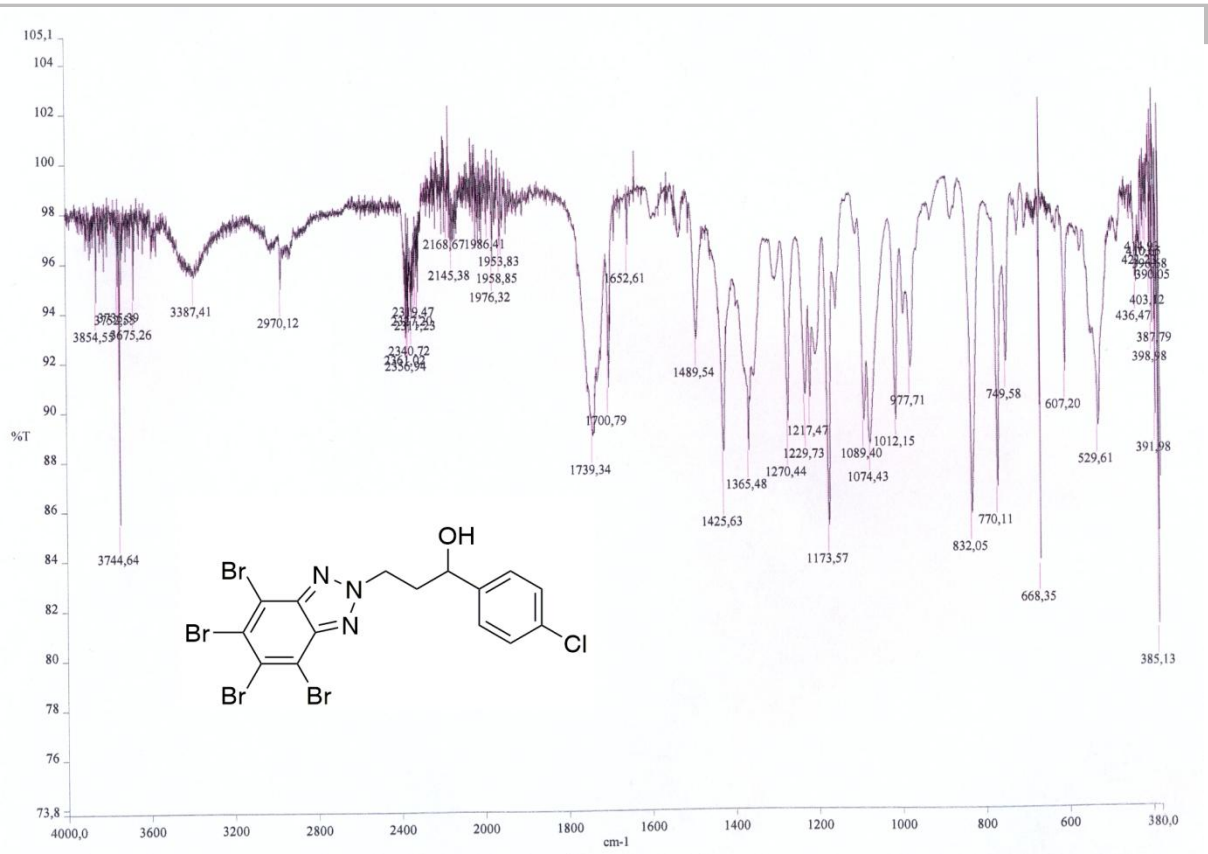
1-(4-Chlorophenyl)-3-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)propan-1-ol (6c):



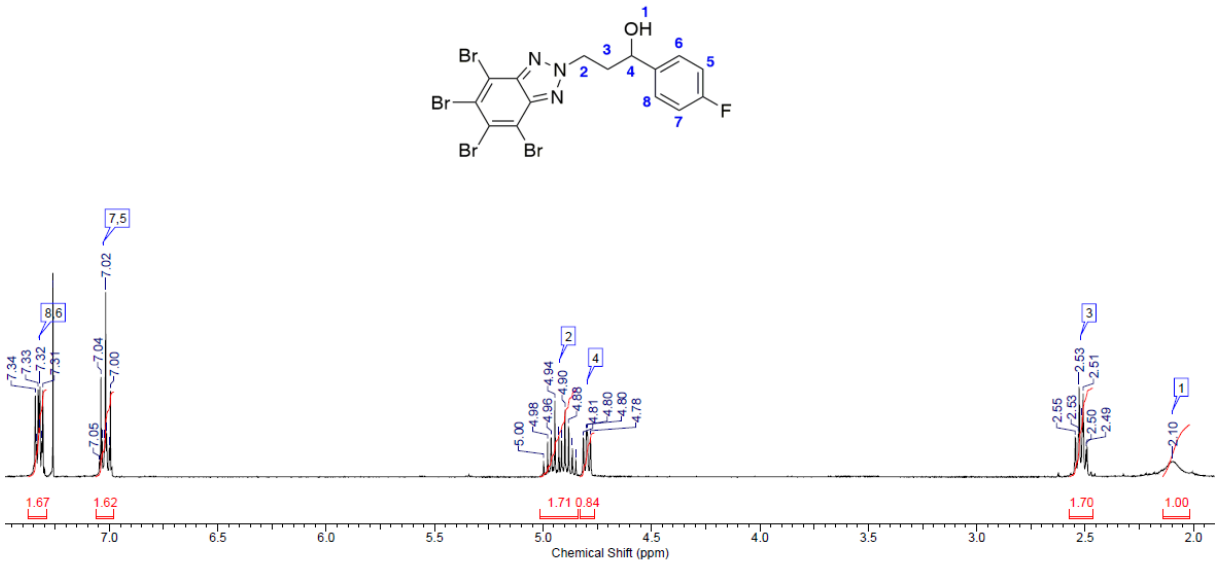


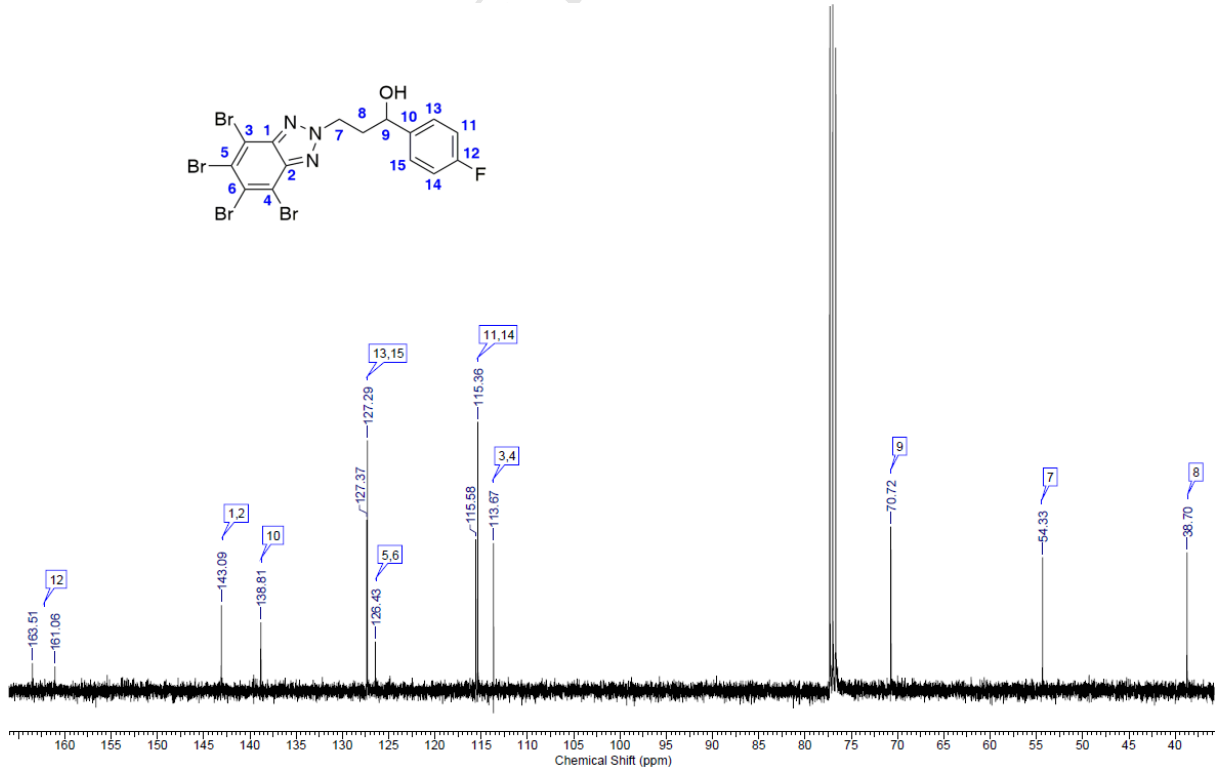

10:14:03
130529_P_CLPOZ 46 (0.790) Cm (1:64)
TOF MS ES+
2.54e3
100
%
0
585.7678
587.7660
583.7732
589.7495
603.7826
605.7930
581.7819
507.8539
505.8477
551.8019
549.8141
553.8105
607.7614
610.2240
569.8090
536.1964
503.8536
525.8778
300.2706
301.1135
301.9896
343.3226
363.3387
371.3633
445.1567
m/z
300 320 340 360 380 400 420 440 460 480 500 520 540 560 580 600 620 640
OH
Br
Br
Br
Br
N
N
N
Cl

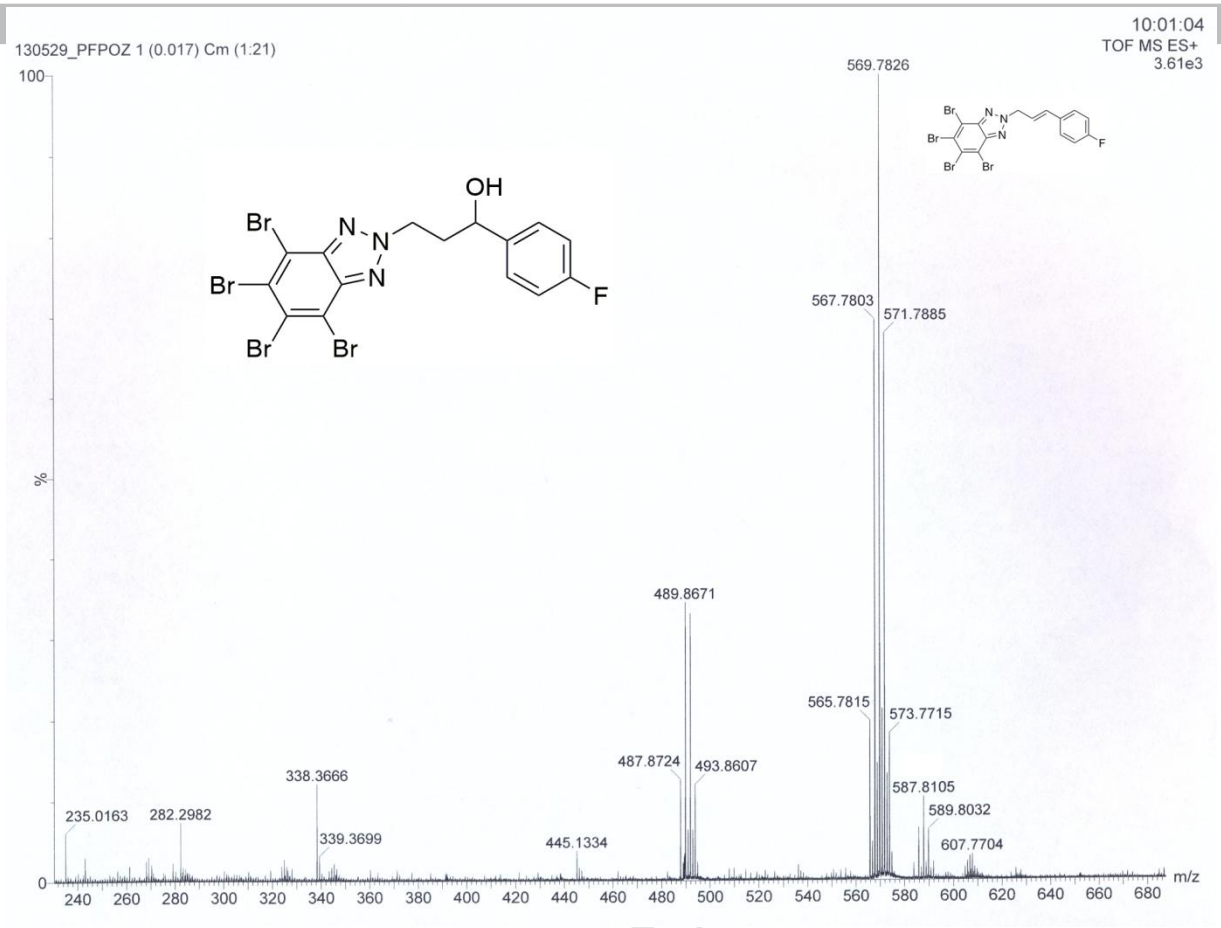

10:16:00
130529_P_CL 1 (0.017) Cm (1:19)
TOF MS ES-
3.06e3
100
%
0
647.7756
649.7627
645.7728
651.7715
601.7653
603.7620
599.7628
569.8544
605.7527
652.7678
715.7517
719.7739
691.7967
858.0200
m/z
325 350 375 400 425 450 475 500 525 550 575 600 625 650 675 700 725 750 775 800 825 850 875 900
OH
Br
Br
Br
Br
N
N
N
Cl

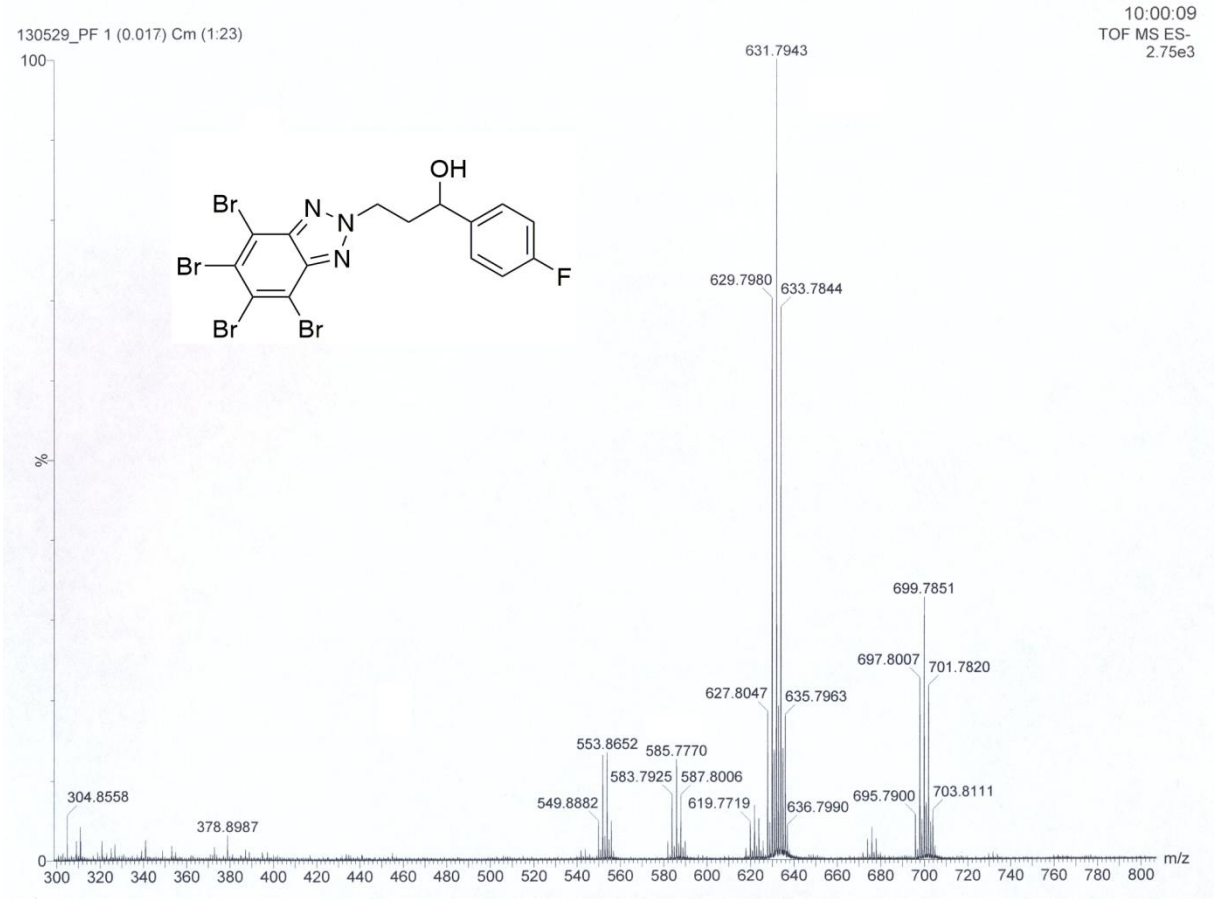

%T
cm-1
3744,64
3387,41
2970,12
2168,67
2145,38
1986,41
1953,83
1958,85
1976,32
1652,61
1700,79
1739,34
1489,54
1425,63
1365,48
1270,44
1217,47
1229,73
1173,57
1089,40
1074,43
1012,15
977,71
832,05
770,11
749,58
668,35
607,20
529,61
403,12
436,47
387,79
398,58
391,98
385,13
OH
Br
Br
Br
Br
N
N
N
Cl

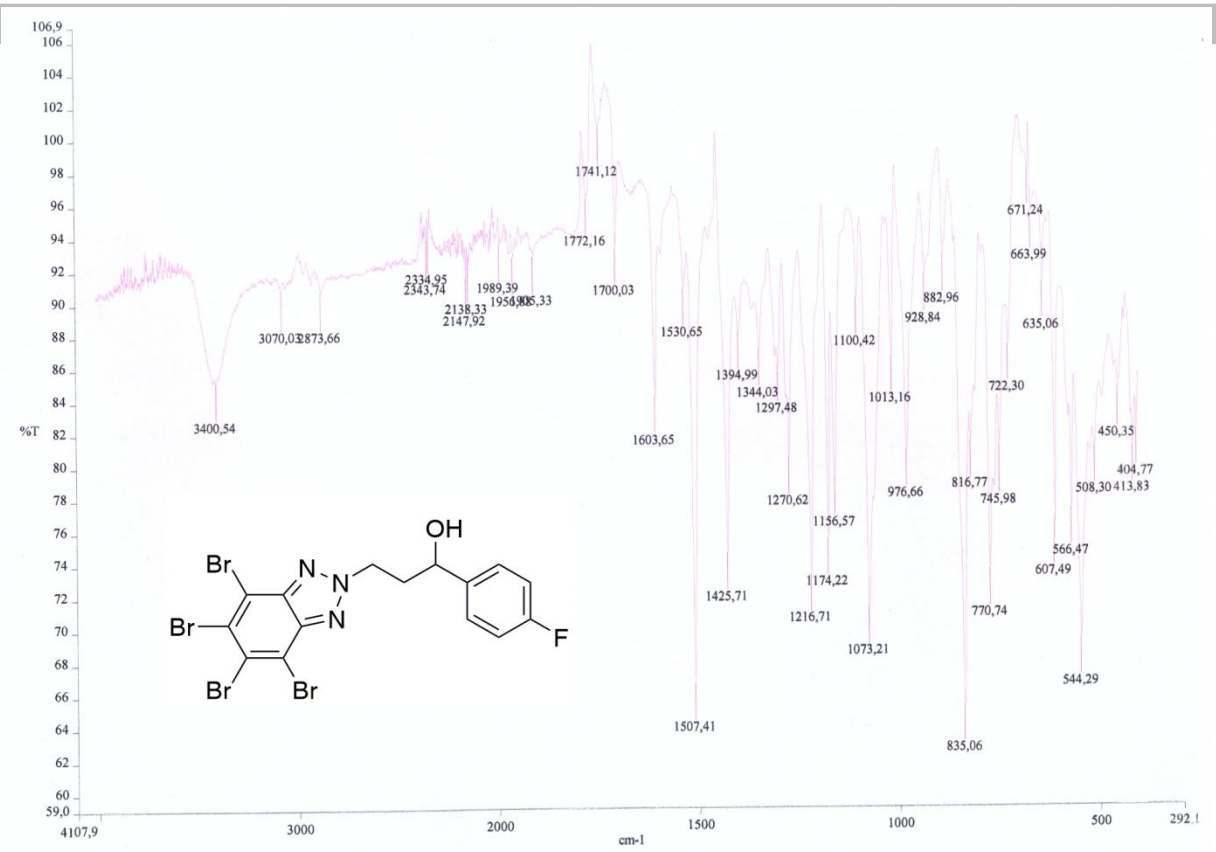
1-(4-Fluorophenyl)-3-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)propan-1-ol (6d):



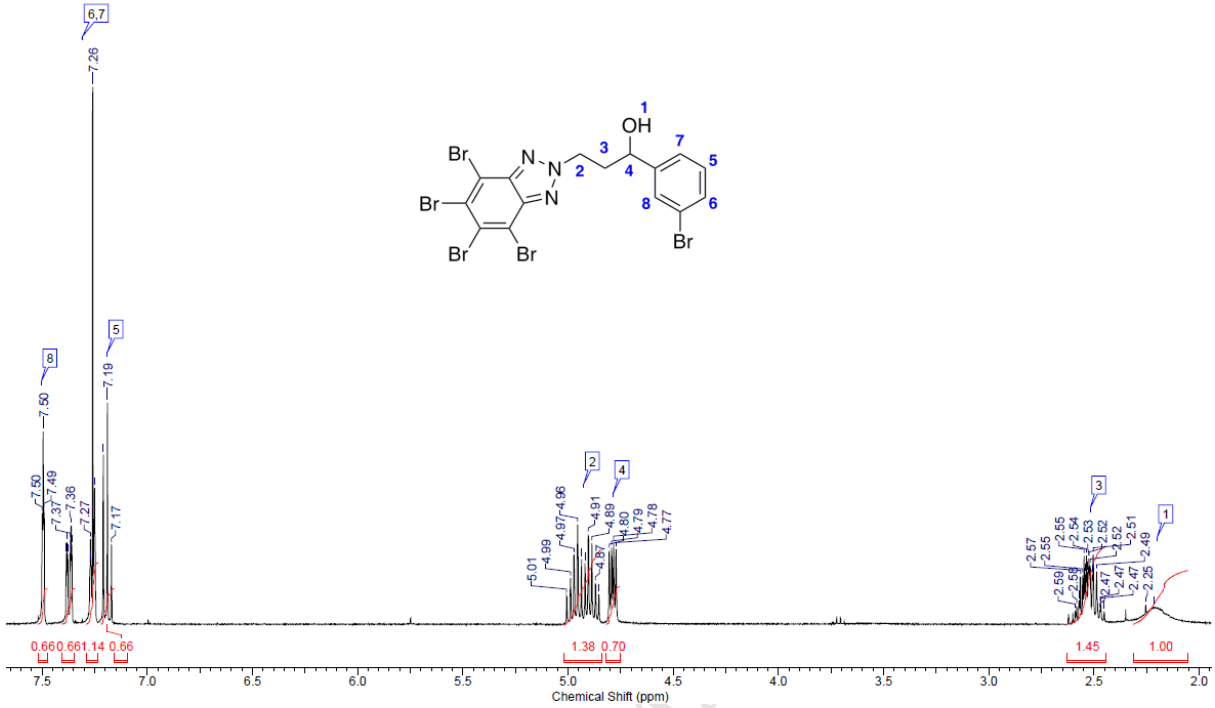


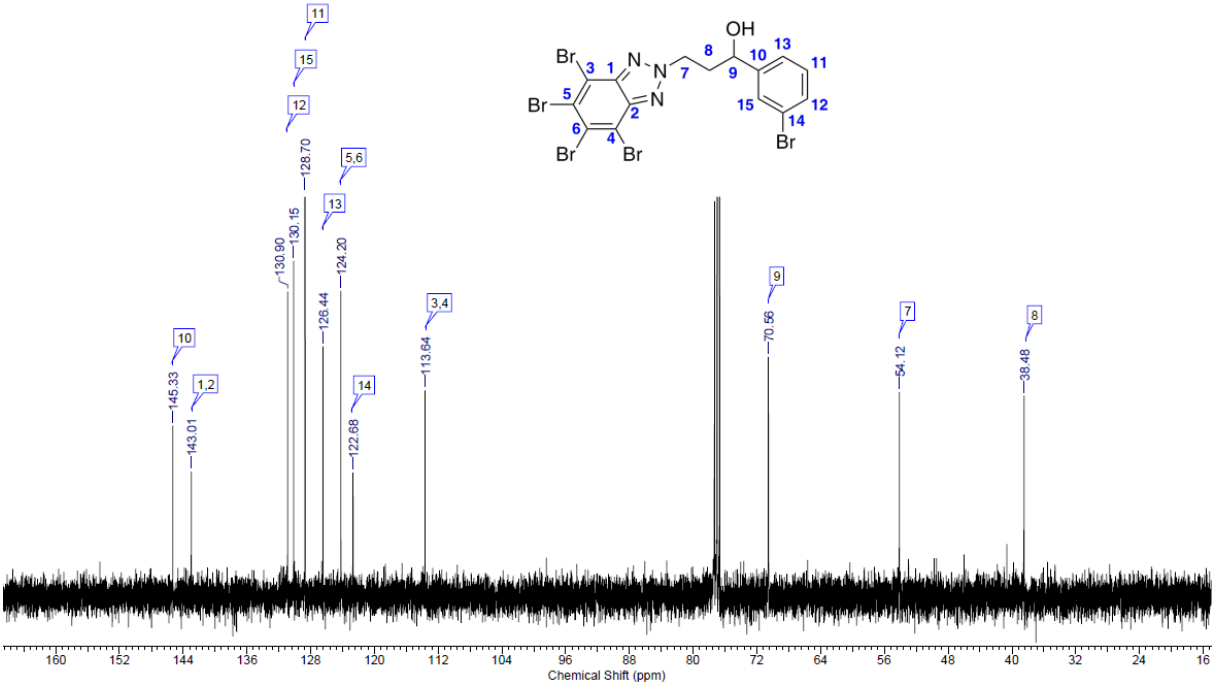

10:01:04
TOF MS ES+
3.61e3
130529_PFPOZ 1 (0.017) Cm (1:21)
100
%
0
569.7826
567.7803
571.7885
489.8671
565.7815
573.7715
487.8724
493.8607
587.8105
589.8032
338.3666
235.0163
282.2982
339.3699
445.1334
607.7704
240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540 560 580 600 620 640 660 680
m/z
OH
Br
Br
Br
Br
N
N
N
F

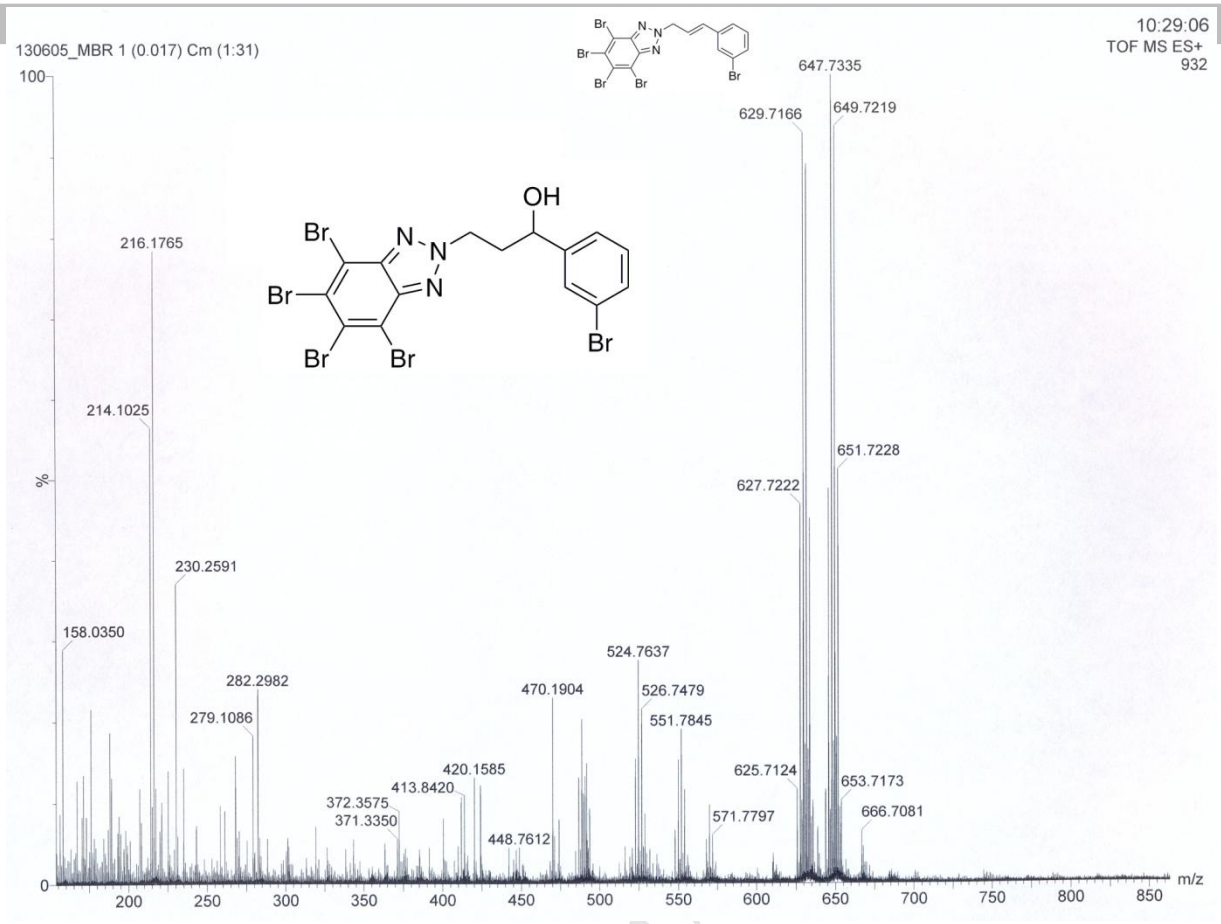

10:00:09
TOF MS ES-
2.75e3
130529_PF 1 (0.017) Cm (1:23)
100
%
0
631.7943
629.7980
633.7844
699.7851
697.8007
701.7820
627.8047
635.7963
553.8652
585.7770
583.7925
587.8006
304.8558
549.8882
619.7719
636.7990
695.7900
703.8111
378.8987
300 320 340 360 380 400 420 440 460 480 500 520 540 560 580 600 620 640 660 680 700 720 740 760 780 800
m/z
OH
Br
Br
Br
Br
N
N
N
F

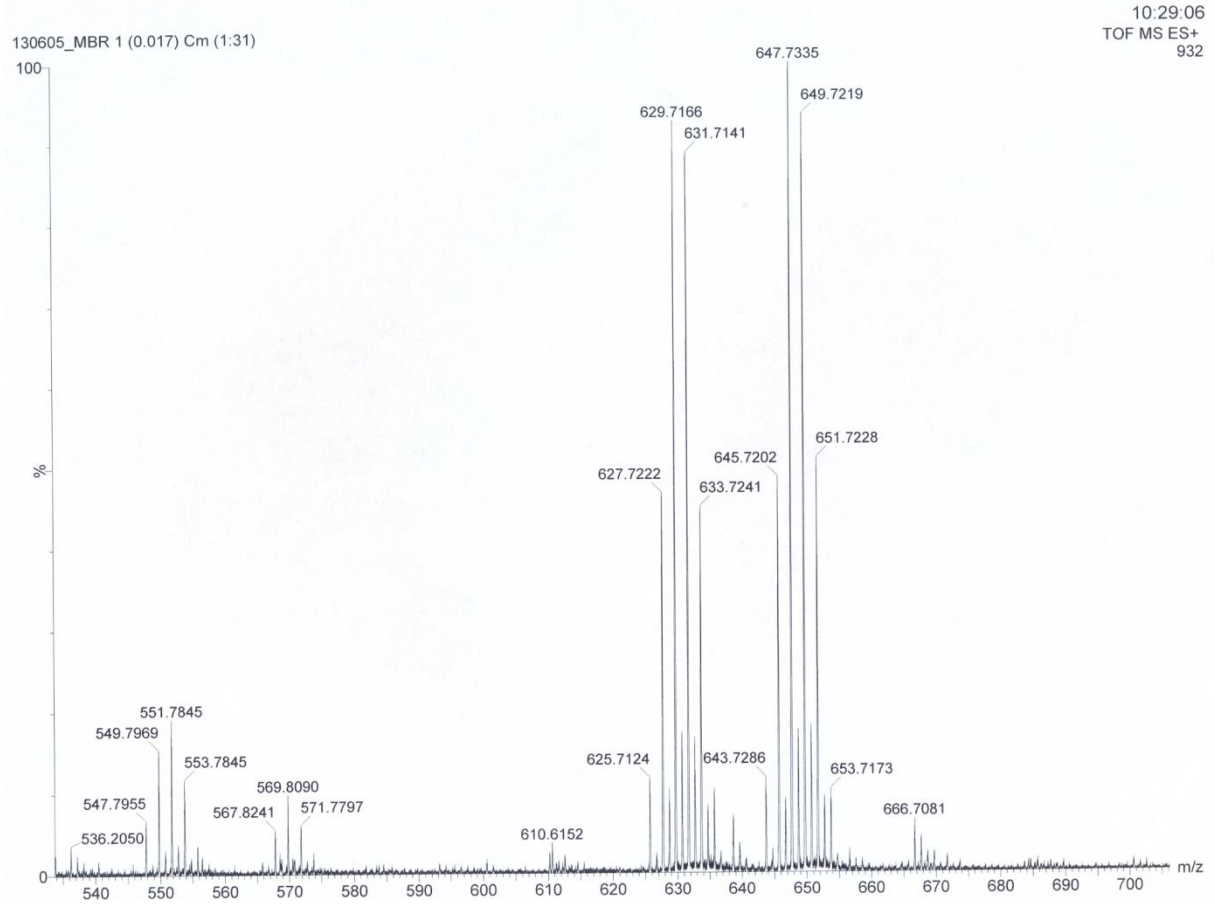

%T
cm-1
4107,9
3000
2000
1500
1000
500
292,1
3400,54
3070,03
2873,66
2334,95
2343,74
2138,33
2147,92
1989,39
1741,12
1772,16
1700,03
1603,65
1530,65
1507,41
1425,71
1394,99
1344,03
1297,48
1270,62
1216,71
1156,57
1174,22
1100,42
1073,21
1013,16
976,66
928,84
882,96
835,06
816,77
770,74
745,98
722,30
671,24
663,99
635,06
607,49
566,47
544,29
508,30
450,35
413,83
404,77
OH
Br
Br
Br
Br
N
N
N
F

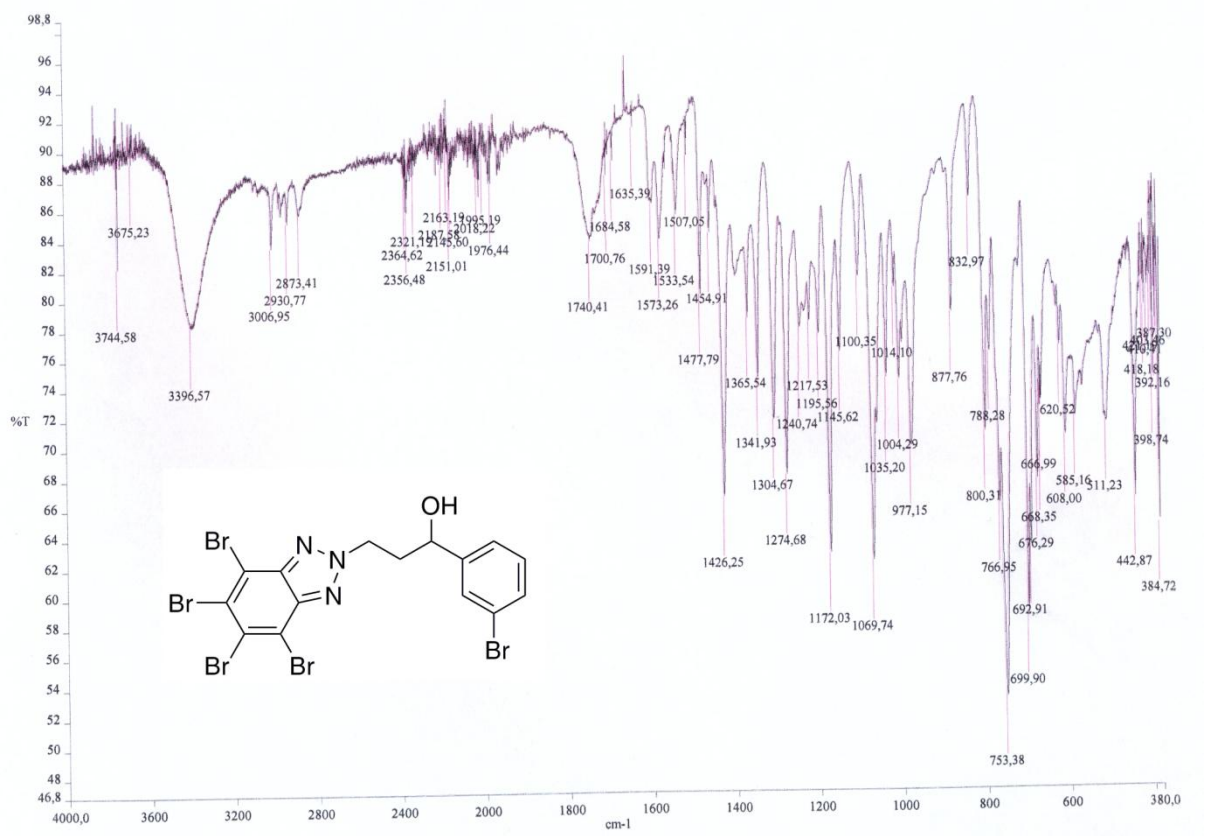
1-(3-Bromophenyl)-3-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)propan-1-ol (6e):



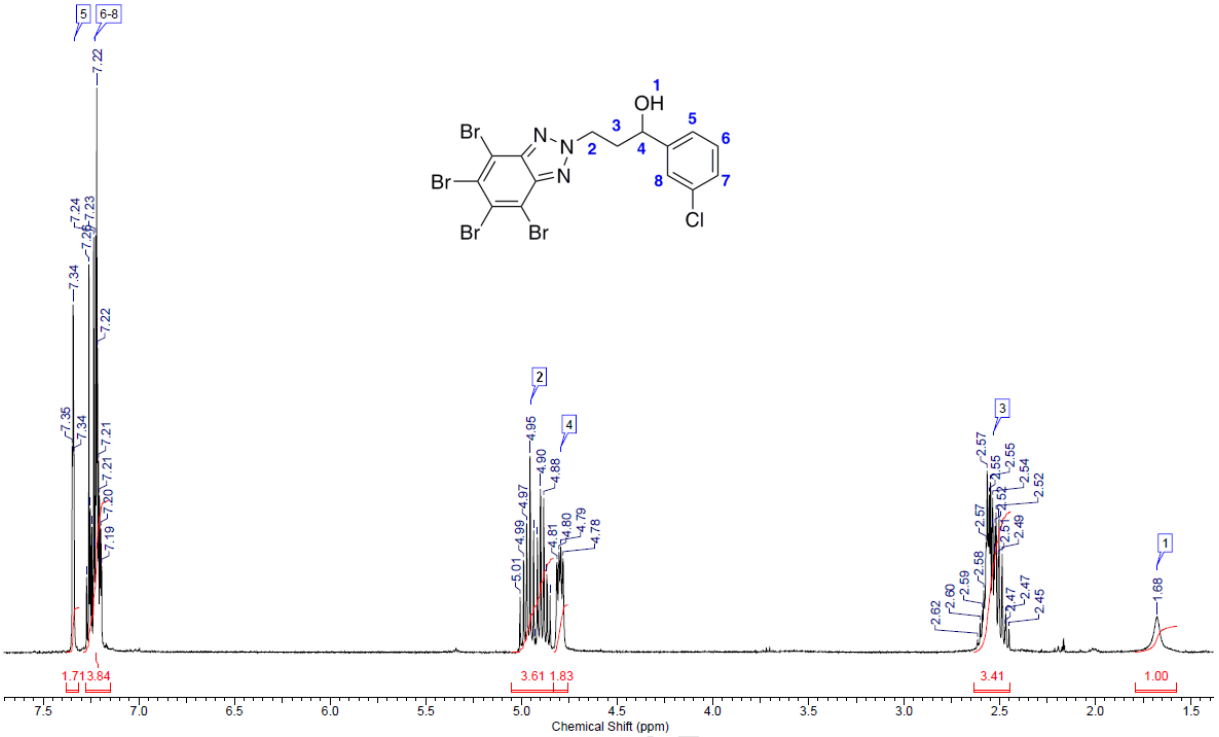


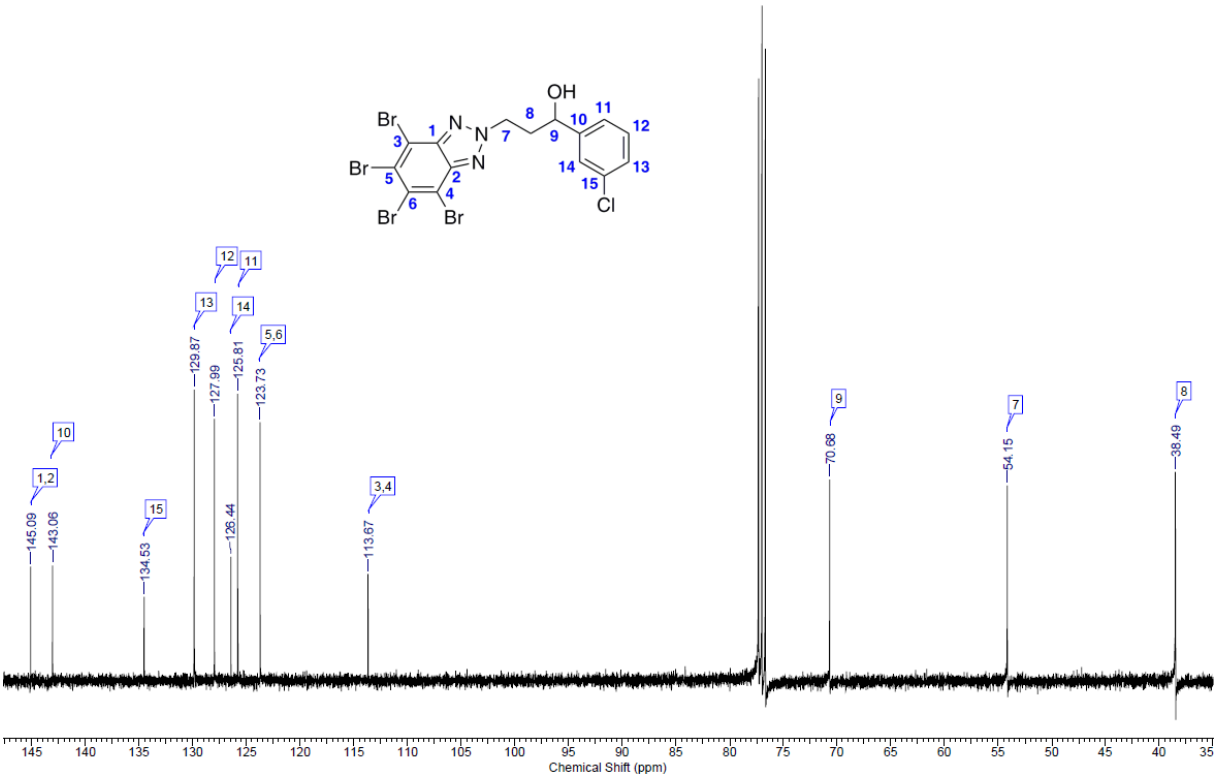

130605_MBR 1 (0.017) Cm (1:31)
10:29:06
TOF MS ES+
932
100
%
0
m/z
647.7335
649.7219
629.7166
216.1765
214.1025
651.7228
627.7222
230.2591
158.0350
524.7637
282.2982
470.1904
526.7479
279.1086
551.7845
420.1585
625.7124
413.8420
653.7173
372.3575
571.7797
666.7081
371.3350
448.7612
200
250
300
350
400
450
500
550
600
650
700
750
800
850
OH
Br
N
Br
Br
Br
Br

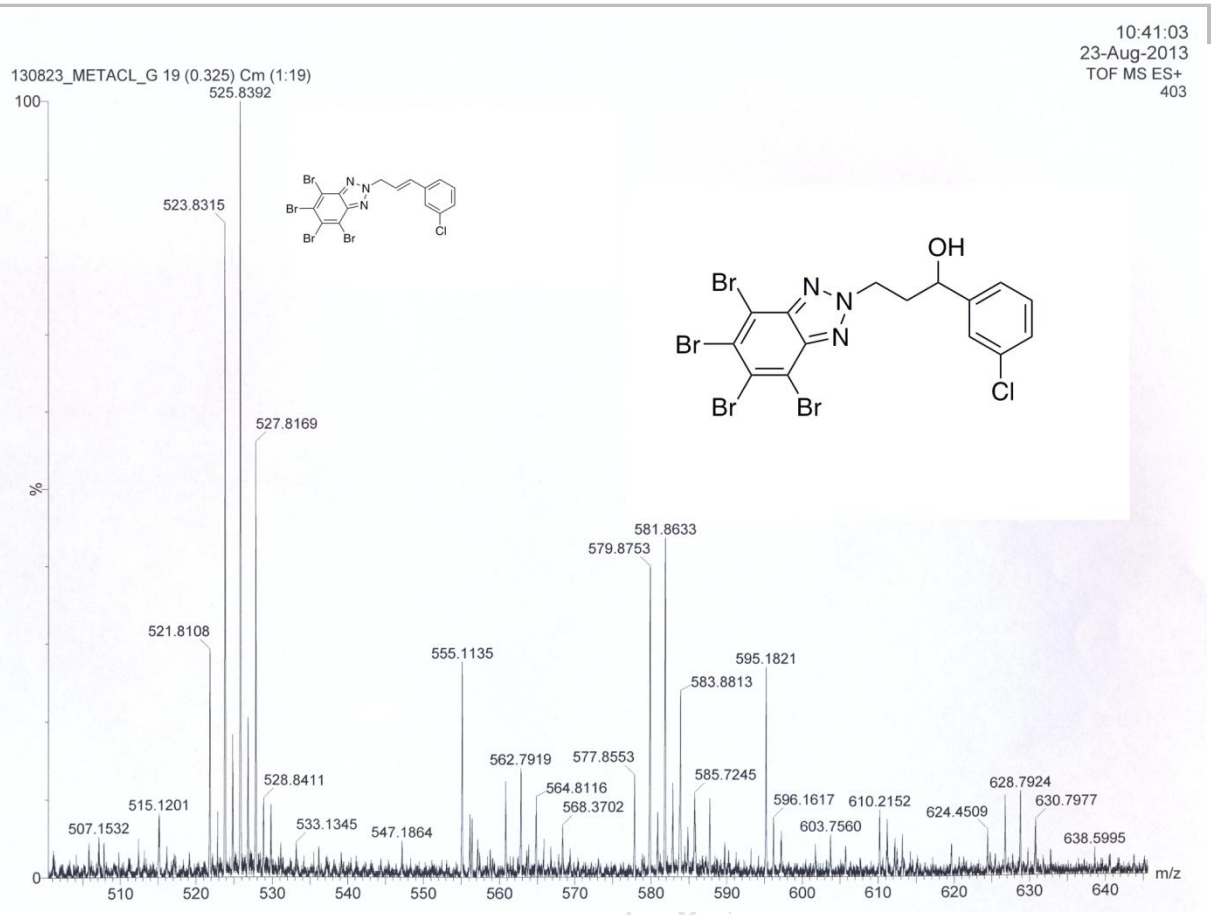

130605_MBR 1 (0.017) Cm (1:31)
10:29:06
TOF MS ES+
932
100
%
0
m/z
647.7335
649.7219
629.7166
631.7141
651.7228
645.7202
627.7222
633.7241
551.7845
549.7969
553.7845
625.7124
643.7286
653.7173
569.8090
547.7955
571.7797
567.8241
666.7081
536.2050
610.6152
540
550
560
570
580
590
600
610
620
630
640
650
660
670
680
690
700

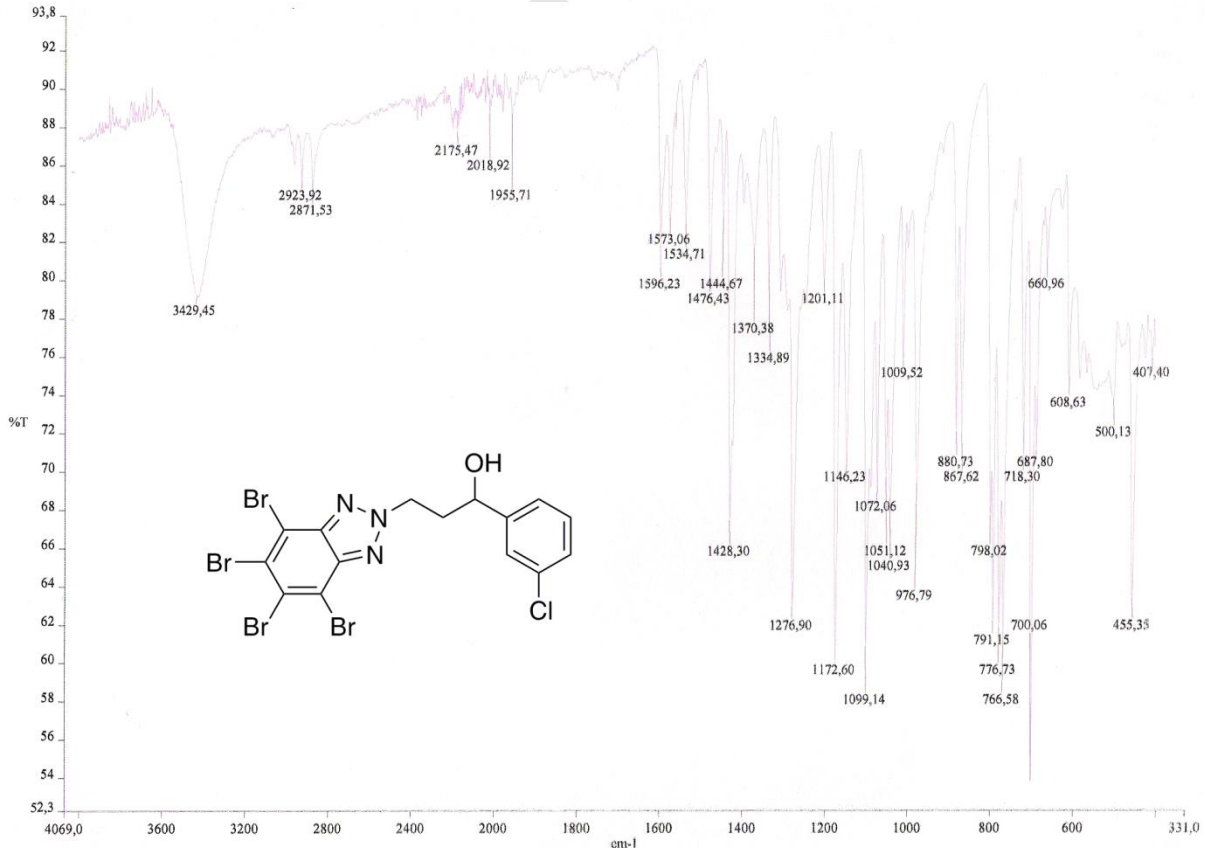


1-(3-Chlorophenyl)-3-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)propan-1-ol (6f):

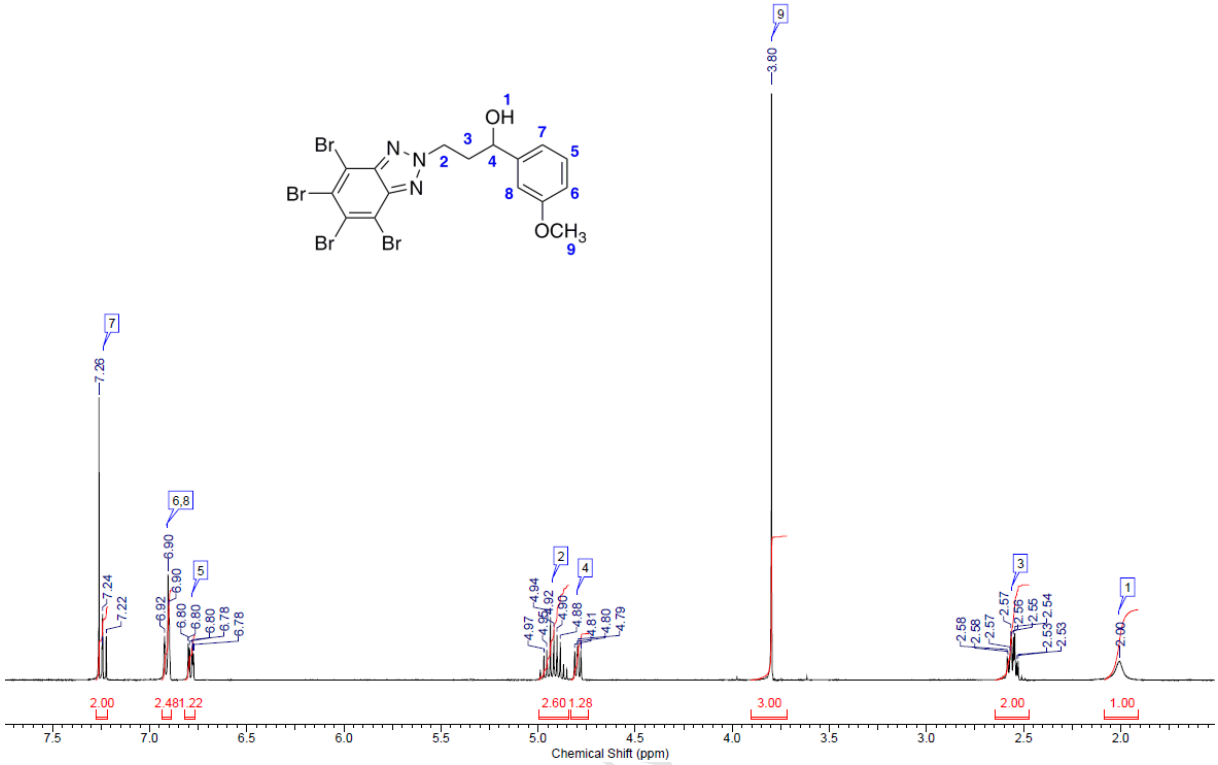


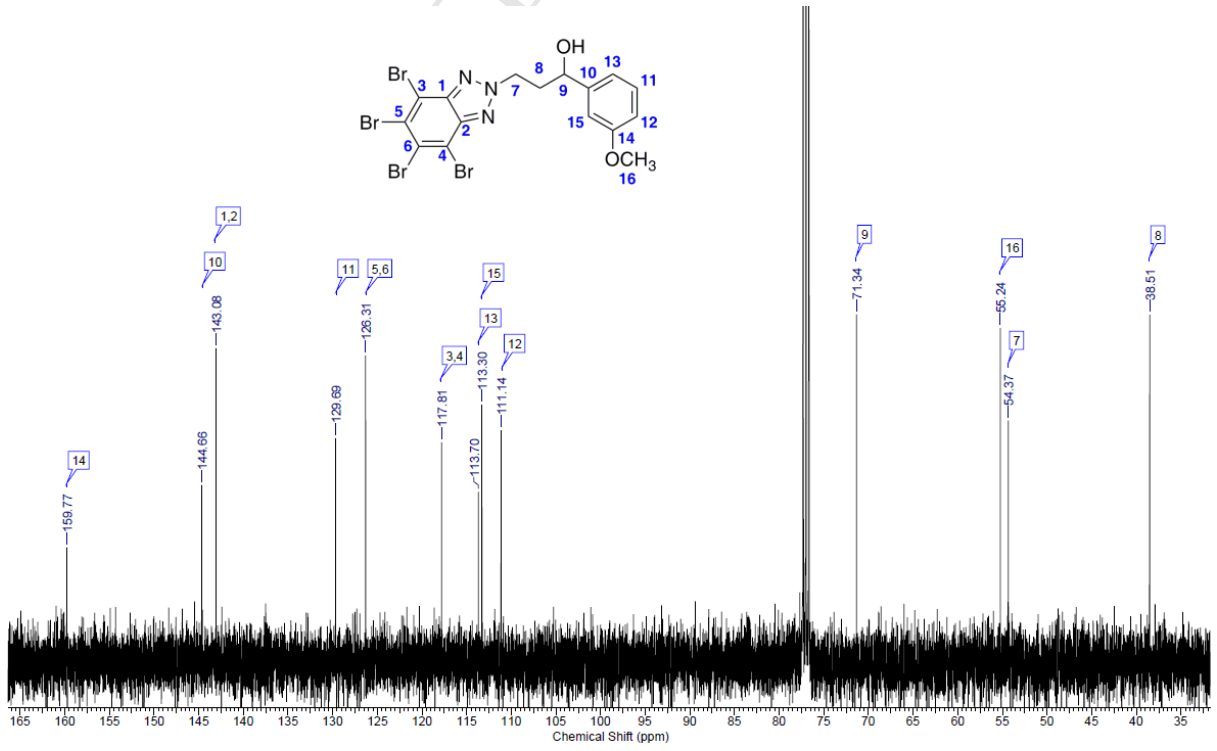


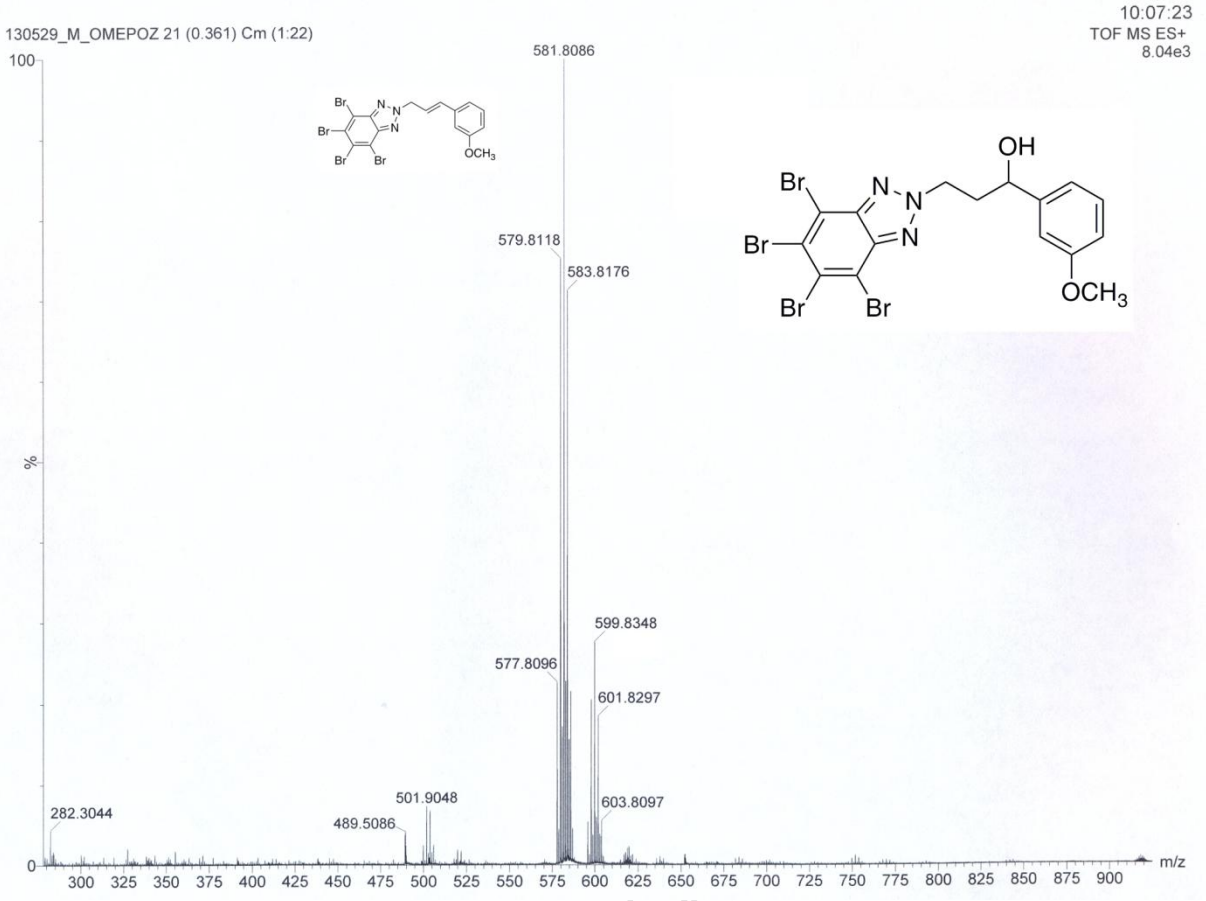

10:41:03
23-Aug-2013
TOF MS ES+
403
130823_METACL_G 19 (0.325) Cm (1:19)
100
%
0
525.8392
523.8315
527.8169
521.8108
555.1135
581.8633
579.8753
595.1821
583.8813
562.7919
577.8553
585.7245
528.8411
564.8116
568.3702
515.1201
507.1532
533.1345
547.1864
596.1617
610.2152
624.4509
628.7924
630.7977
603.7560
638.5995
510
520
530
540
550
560
570
580
590
600
610
620
630
640
m/z
Br
Br
Br
Br
N
N
N
OH
Cl

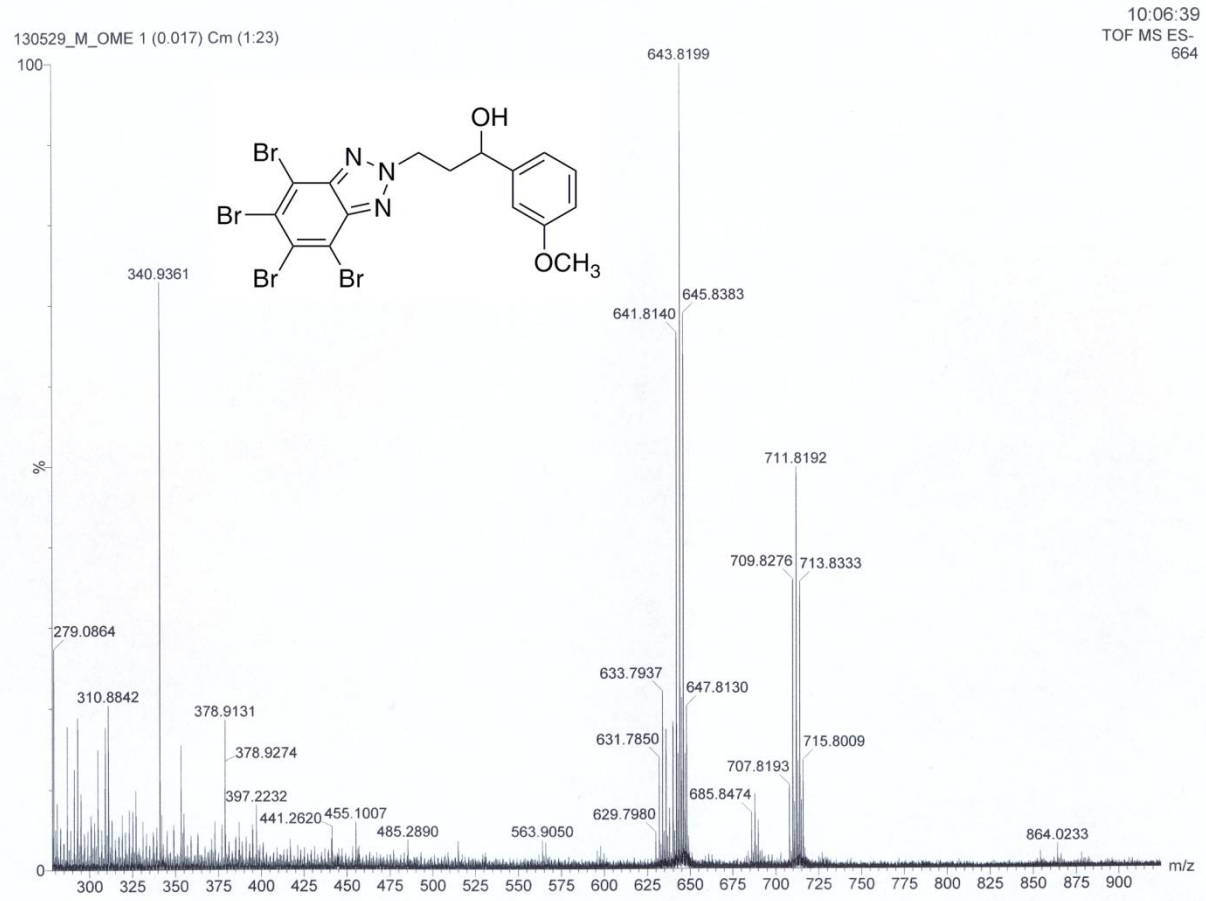

%T
93,8
92
90
88
86
84
82
80
78
76
74
72
70
68
66
64
62
60
58
56
54
52,3
4069,0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
331,0
cm-1
3429,45
2923,92
2871,53
2175,47
2018,92
1955,71
1573,06
1534,71
1596,23
1444,67
1476,43
1201,11
660,96
1370,38
1334,89
1009,52
407,40
608,63
500,13
880,73
867,62
687,80
718,30
1146,23
1072,06
1428,30
1051,12
1040,93
798,02
976,79
1276,90
700,06
455,35
791,15
1172,60
776,73
1099,14
766,58
Br
Br
Br
Br
N
N
N
OH
Cl

1-(3-Methoxyphenyl)-3-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)propan-1-ol (6g):






130529_M_OMEPOZ 21 (0.361) Cm (1:22)
10:07:23
TOF MS ES+
8.04e3
100
%
0
581.8086
579.8118
583.8176
599.8348
577.8096
601.8297
603.8097
501.9048
489.5086
282.3044
m/z
300 325 350 375 400 425 450 475 500 525 550 575 600 625 650 675 700 725 750 775 800 825 850 875 900
OH
Br
Br
Br
Br
N
N
N
OCH3


130529_M_OME 1 (0.017) Cm (1:23)
10:06:39
TOF MS ES-
664
100
%
0
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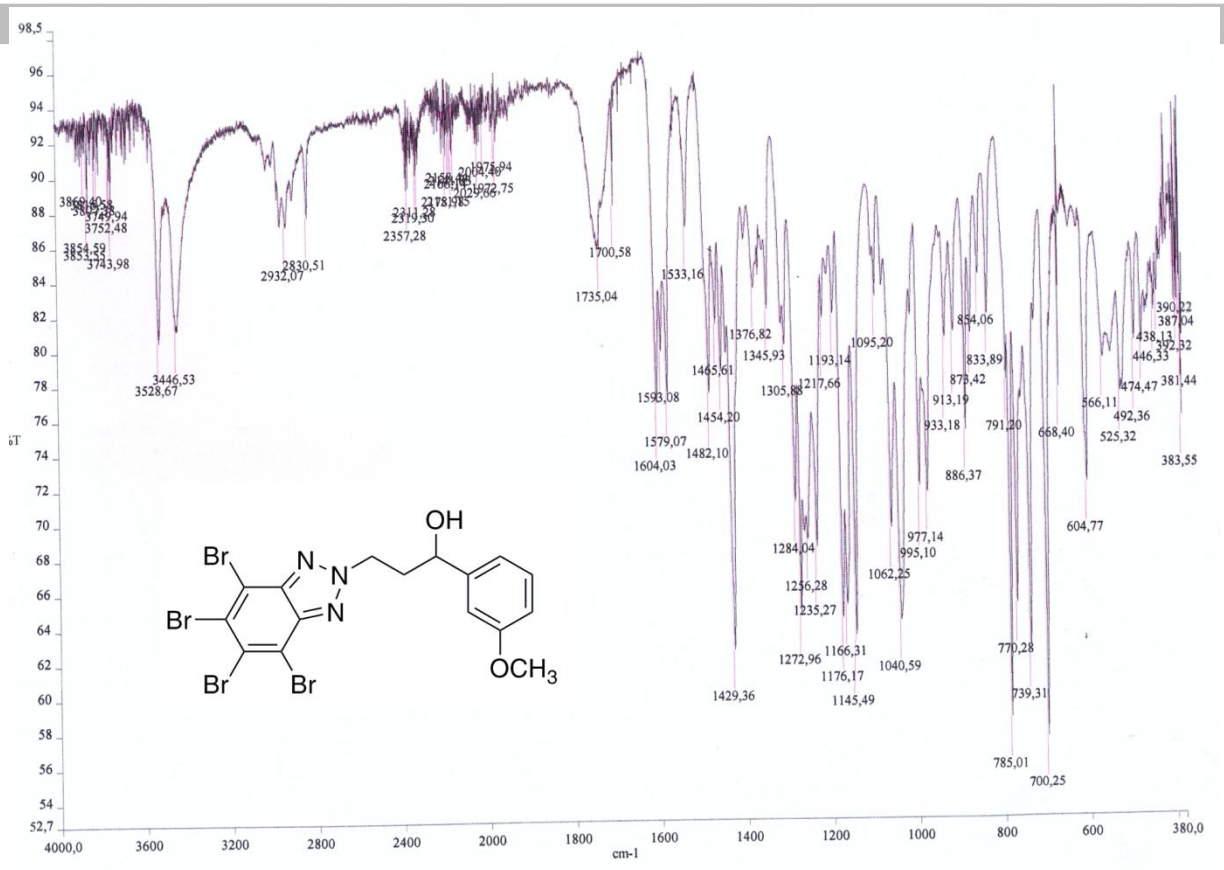
340.9361
645.8383
641.8140
711.8192
709.8276
713.8333
279.0864
633.7937
647.8130
310.8842
378.9131
631.7850
715.8009
378.9274
707.8193
397.2232
685.8474
441.2620
455.1007
629.7980
485.2890
563.9050
864.0233
m/z
300 325 350 375 400 425 450 475 500 525 550 575 600 625 650 675 700 725 750 775 800 825 850 875 900
OH
Br
Br
Br
Br
N
N
N
OCH3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cm-1
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3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
380,0
3752,48
3743,98
3446,53
3528,67
2932,07
2830,51
2357,28
1735,04
1700,58
1604,03
1579,07
1533,16
1482,10
1454,20
1465,61
1429,36
1376,82
1345,93
1305,88
1284,04
1256,28
1235,27
1217,66
1193,14
1166,31
1176,17
1145,49
1095,20
1062,25
1040,59
977,14
995,10
933,18
913,19
886,37
878,42
854,06
833,89
791,20
770,28
739,31
700,25
668,40
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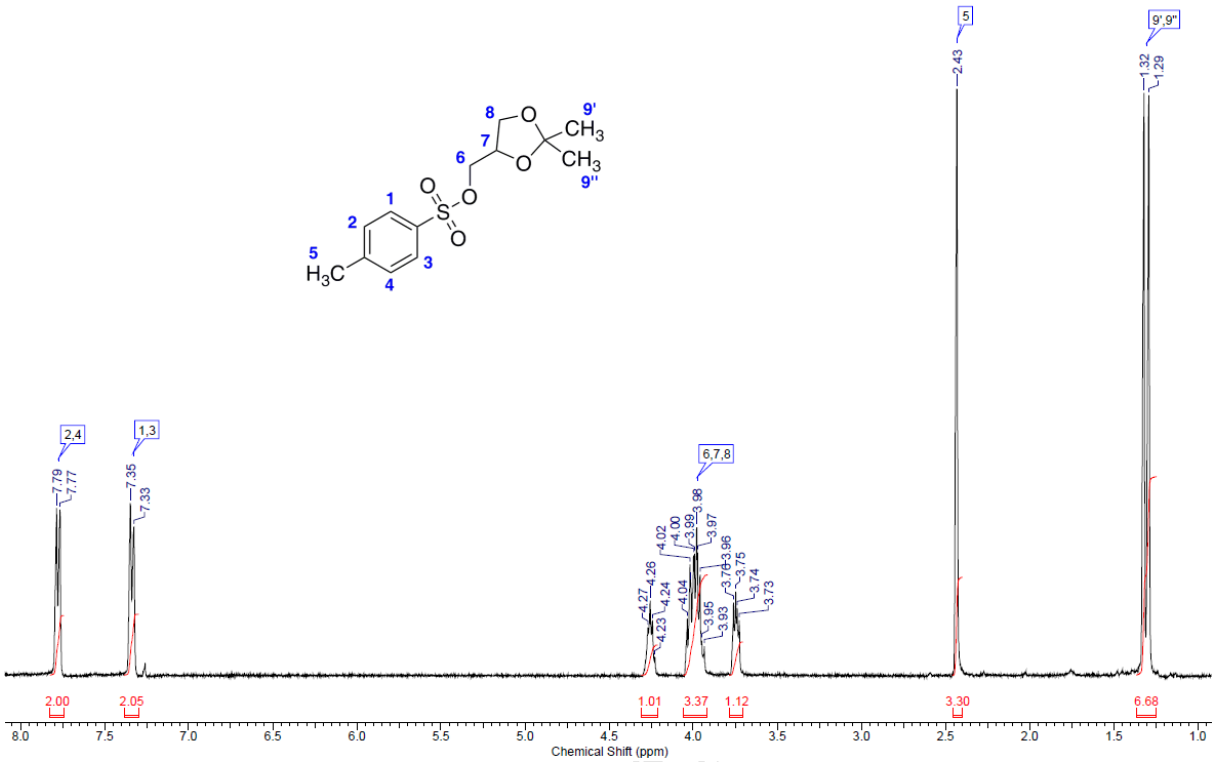
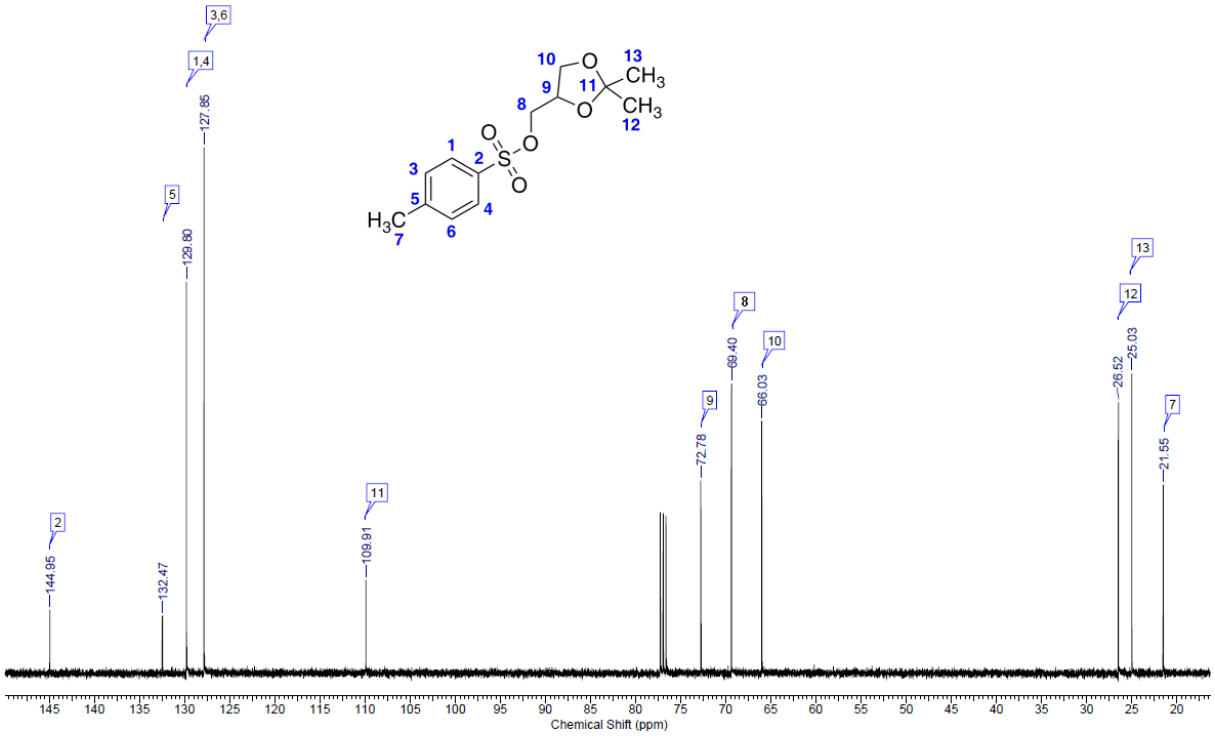
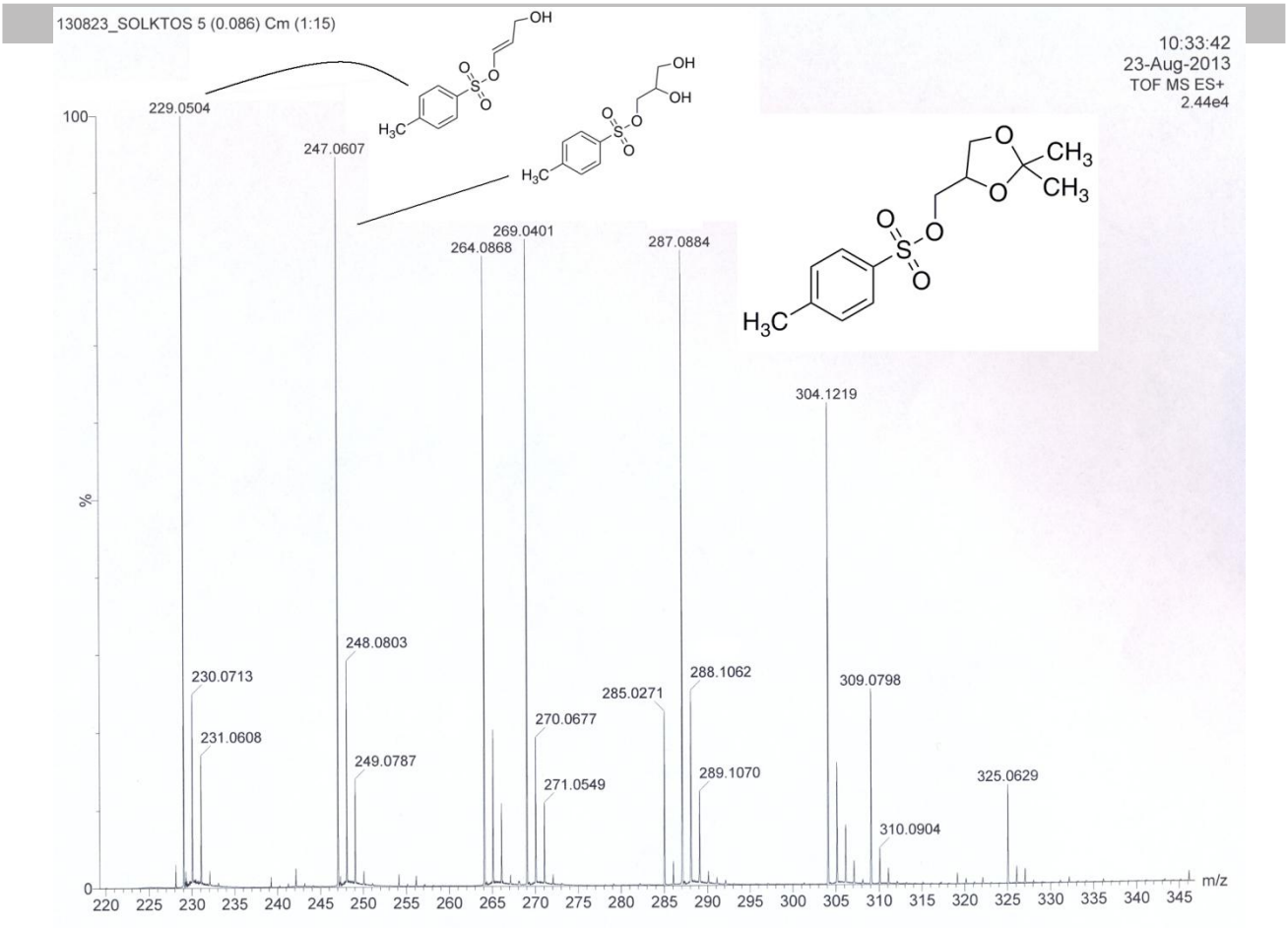
566,11
525,32
492,36
474,47
446,33
438,13
390,22
387,04
383,55
381,44
OH
Br
Br
Br
Br
N
N
N
OCH3

(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate (11):






130823_SOLKTOS 5 (0.086) Cm (1:15)
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23-Aug-2013
TOF MS ES+
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%
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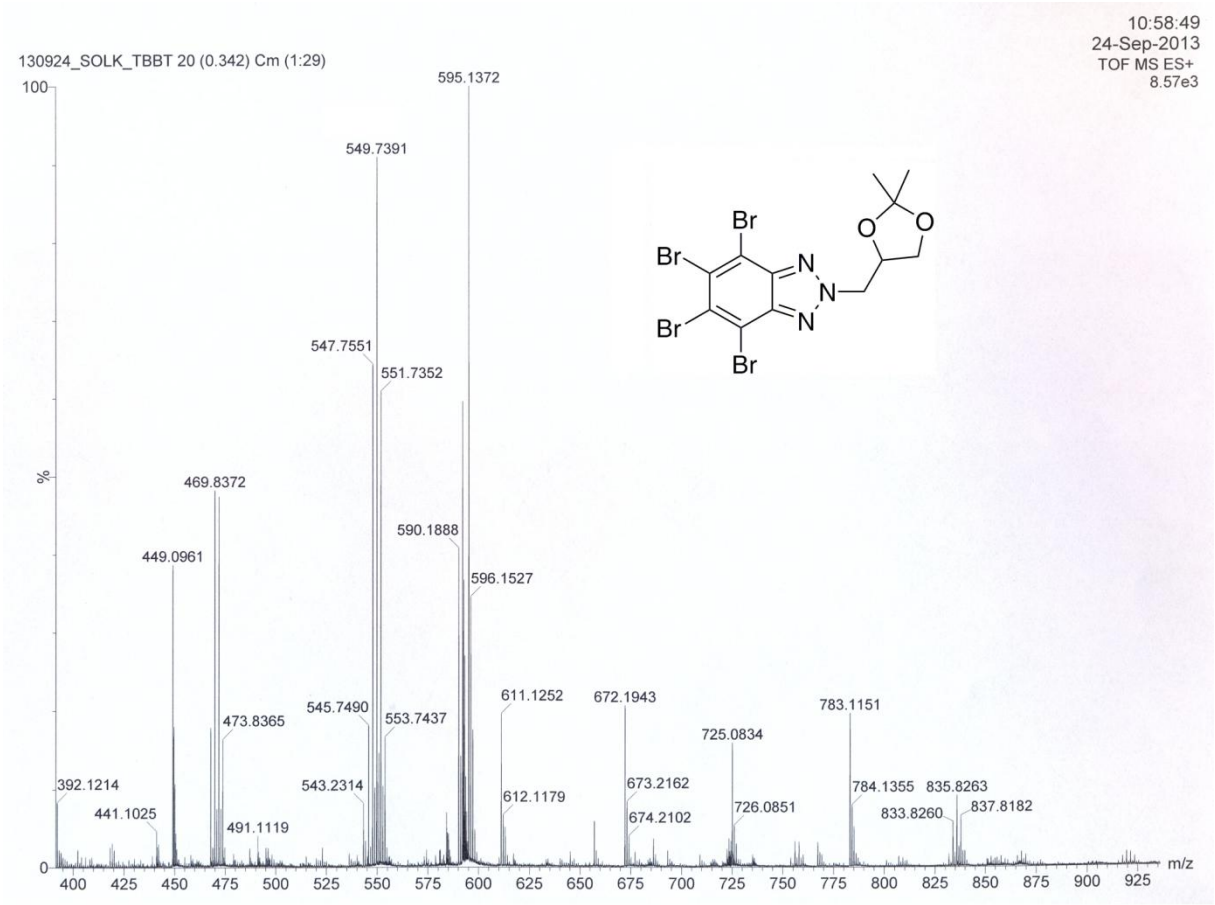
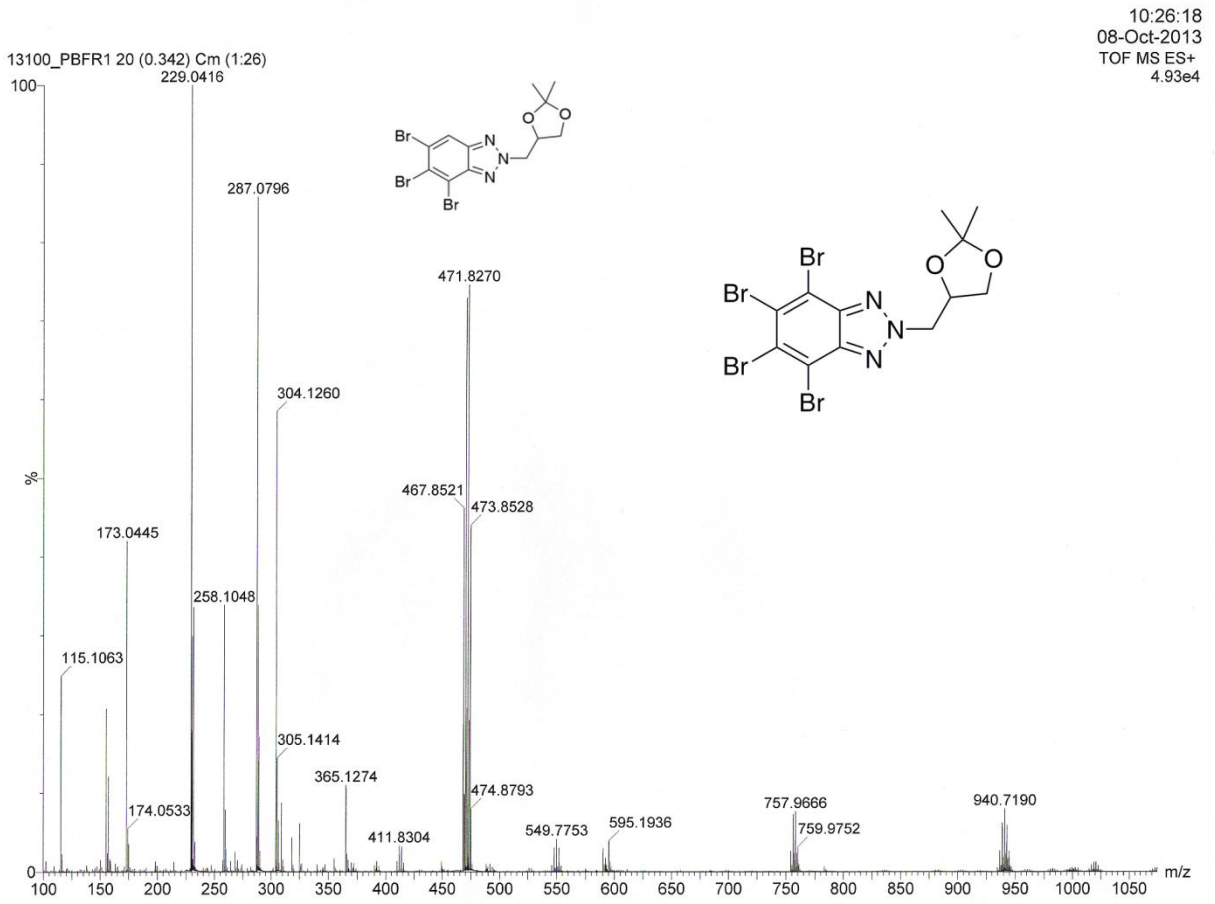
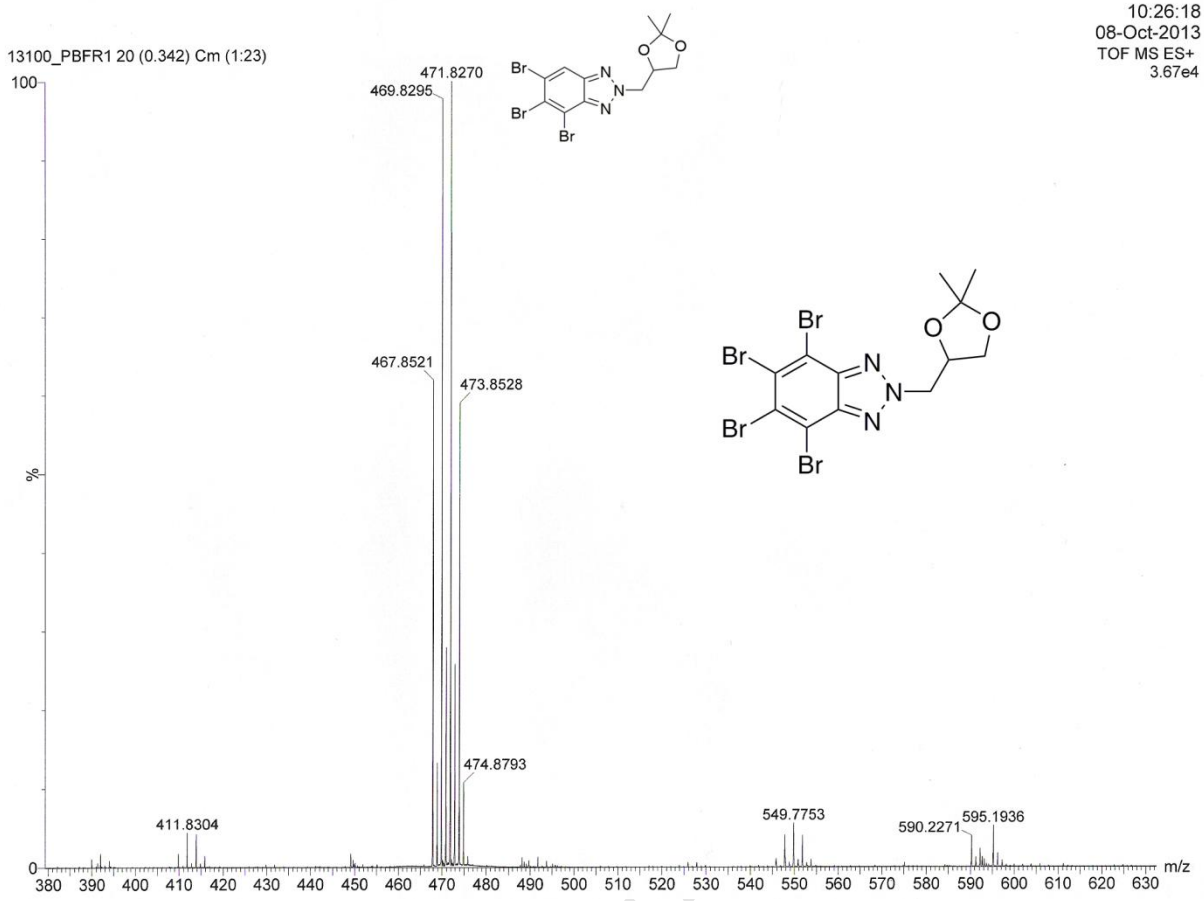
247.0607
264.0868
269.0401
287.0884
304.1219
248.0803
230.0713
231.0608
249.0787
285.0271
288.1062
270.0677
271.0549
289.1070
309.0798
310.0904
325.0629
m/z
220 225 230 235 240 245 250 255 260 265 270 275 280 285 290 295 300 305 310 315 320 325 330 335 340 345

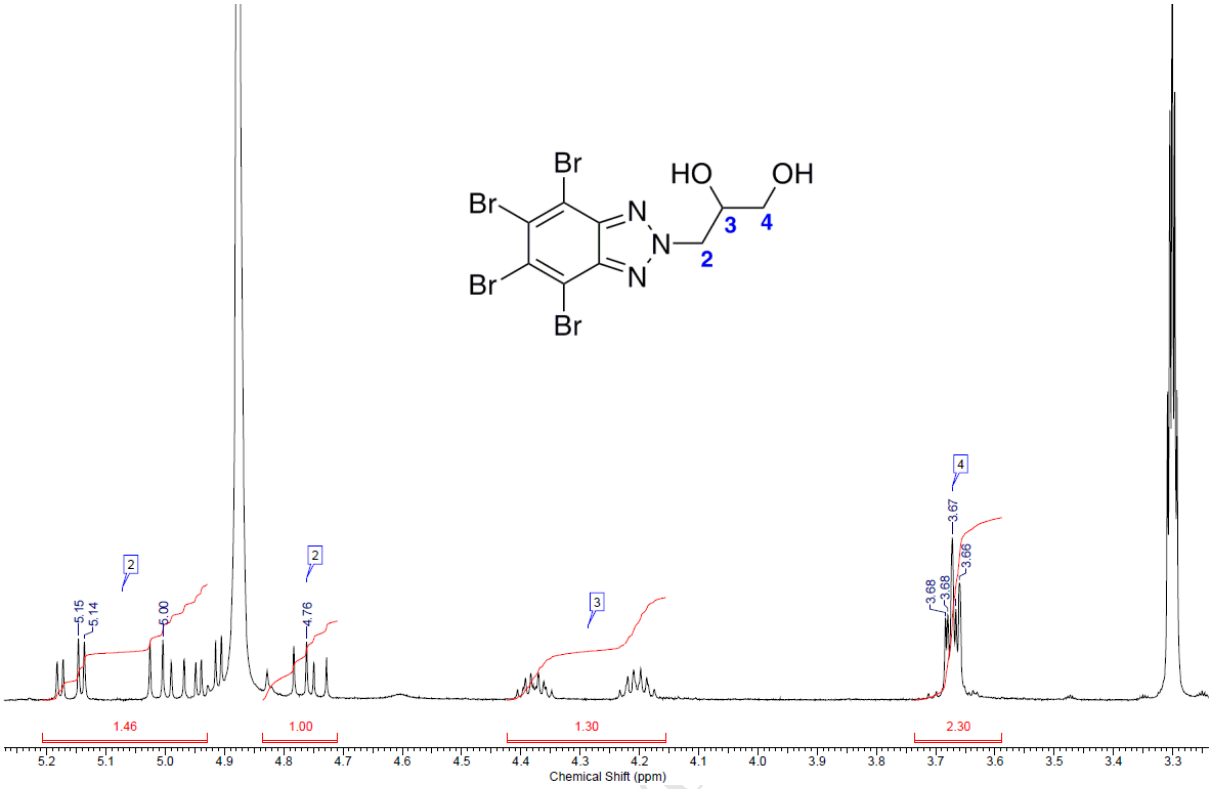
4,5,6,7-Tetrabromo-2-[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]-2*H*-benzotriazole (12):

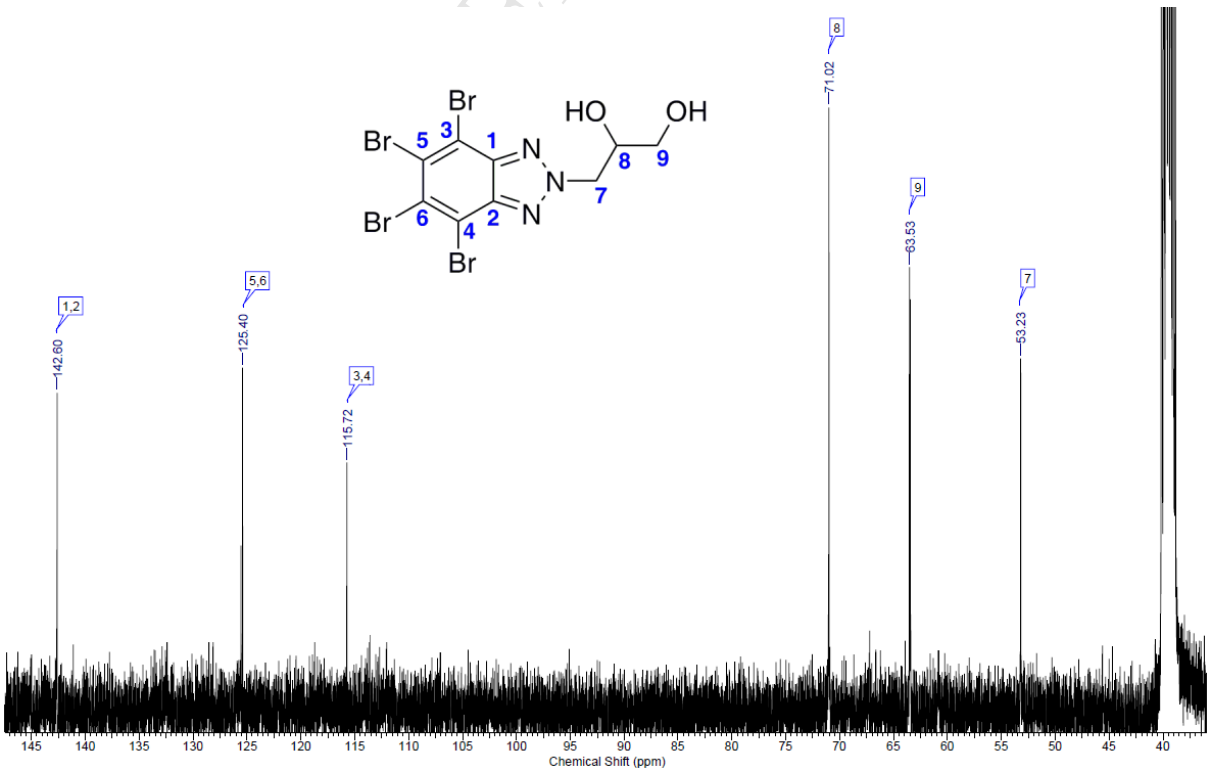


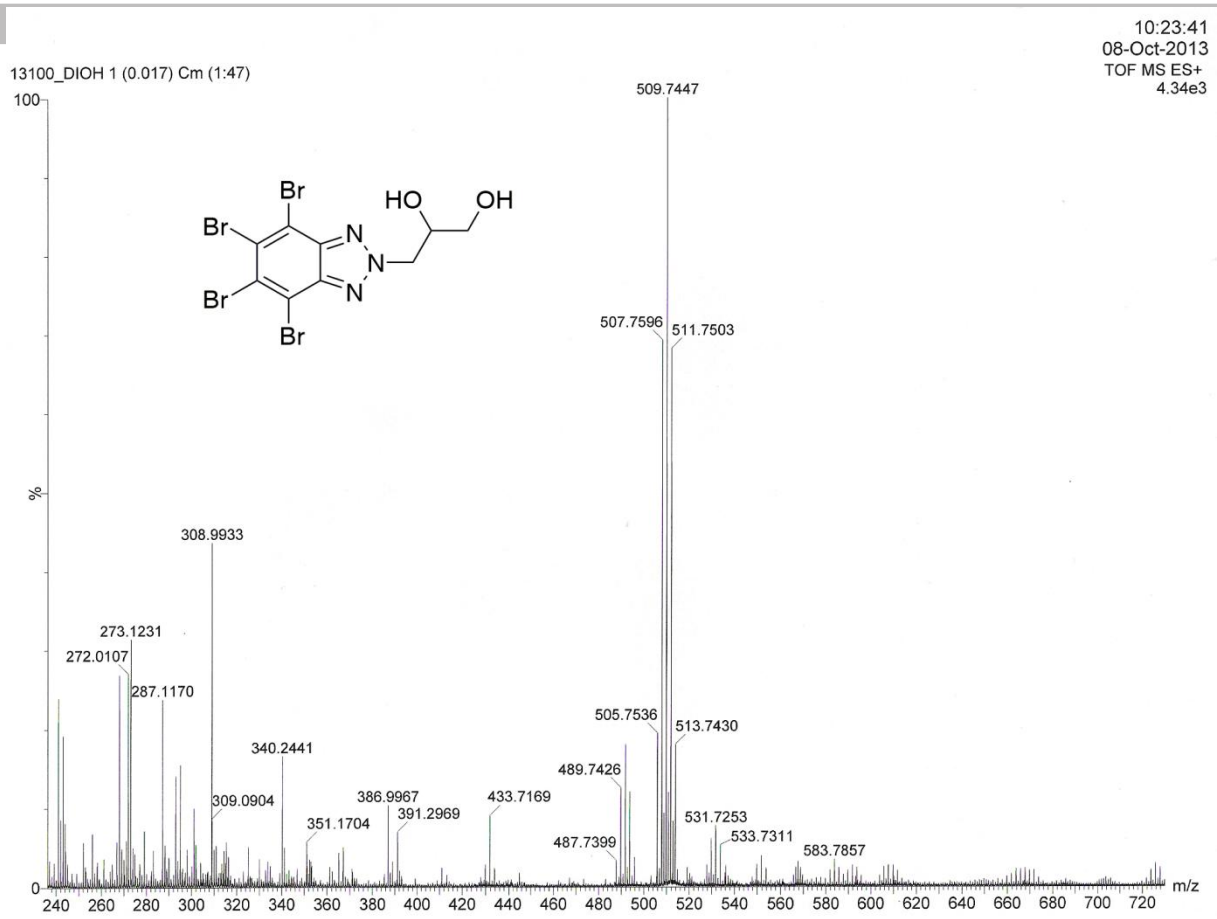




3-(4,5,6,7-Tetrabromo-2*H*-benzotriazol-2-yl)propane-1,2-diol (13):







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