Enantioseparation of novel chiral sulfoxides on chlorinated polysaccharide stationary phases in supercritical fluid chromatography

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Abstract

Asymmetric sulfoxides is a particular case of chirality that may be found in natural as well as synthetic products. Twenty-four original molecules containing a sulphur atom as a centre of chirality were analysed in supercritical fluid chromatography on seven polysaccharide-based chiral stationary phases (CSP) with carbon dioxide – methanol mobile phases. While all the tested CSP provided enantioseparation for a large part of the racemates, chlorinated cellulosic phases proved to be both highly retentive and highly enantioselective towards these species.

Favourable structural features were determined by careful comparison of the enantioseparation of the probe molecules. Molecular modelling studies indicate that U-shaped (folded) conformations were most favourable to achieve high enantioresolution on these CSP, while linear (extended) conformations were not so clearly discriminated.

For a subset of these species adopting different conformations, a broad range of mobile phase compositions, ranging from 20 to 100% methanol in carbon dioxide, were investigated. While retention decreased continuously in this range, enantioseparation varied in a non-monotonous fashion. Abrupt changes in the tendency curves of retention and selectivity were observed when methanol proportion reaches about 60%, suggesting that a change in the conformation of the analytes and/or chiral selector is occurring at this point.

Keywords:

Conformation; enantiomer separation; molecular modelling; polysaccharide-based chiral stationary phase; sulfoxides; supercritical fluid chromatography.

1. Introduction

Asymmetrically substituted carbon atoms are most frequently encountered in chiral species, but other atoms may also play a role of stereogenic centre, like the sulfur atom in sulfoxides. In sulfoxides, the sulfur atom is the apex of a trigonal pyramid, and is linked to one oxygen atom and two other substituents. For the sulfoxide to be a chiral center, the susbtituents must be different. The energy required to invert the stereocentre is high enough to ensure that the interconversion is very slow [1], thus racemization usually does not occur at room temperature. Chiral sulfoxides are encountered in natural products (like alliin, a precursor of garlic flavor) or synthetic drugs (omeprazole or its pure enantiomer esomeprazole, albendazole, modafinil, sulindac). They may also be found as metabolites of sulfur-containing pesticides (methiocarb, fenamifos, etc.). Naturally, as observed for most chiral molecules, the two enantiomers may have different bioactivities [2], hence the interest in achieving their resolution.

Enantioselective separations of sulfoxides are scarce in the literature.

Capillary electrophoresis with cyclodextrins as chiral selectors was employed to separate pesticide metabolites [3].

HPLC enantioseparations are naturally more frequent. Pirkle *et al.* [4,5] described the enantioseparation of twelve sulfoxide enantiomers on an original fluoro-alcoholic chiral stationary phase (CSP). Berthod *et al.* achieved the enantioseparation of thirty-one chiral sulfoxides with five macrocyclic glycopeptide stationary phases, in the normal-phase, reversed-phase and polar organic modes [6]. Cass and co-workers [7,8] explored multimodal elution (normal phase, reversed-phase and polar organic modes) to resolve chiral sulfoxides with polysaccharide-based CSP. Zongde *et al.* separated albendazole sulfoxide enantiomers with an amylose tris-(3,5-dimethylphenylcarbamate) CSP operated in the normal-phase mode [9], with the aim of transposing the method to preparative scale. Materazzo *et al.* separated albendazole sulfoxides and fenbendazole sulfoxides and their precursors and metabolites with immobilized amylose stationary phases in reversed-phase HPLC mode [10]. Dixit *et al.* resolved four chiral sulfoxide drugs with a chlorinated cellulose CSP in the normal-phase and polar organic modes [11]. Chankvetadze and co-workers separated chiral sulfoxides in polar organic, polar aqueous-organic

and normal-phase HPLC [12,13] with several polysaccharide stationary phases. In particular, some chlorinated polysaccharide CSP provided high enantiorecognition, with impressive enantioseparation factors reaching 112 as highest value in one instance [14].

Supercritical fluid chromatography (SFC) was employed to separate albendazole enantiomers [15] and benzimidazole enantiomers including omeprazole [16,17] with amylose tris-(3,5-dimethylphenylcarbamate) CSP and cellulose tris-(3,5-dimethylphenylcarbamate). Mobile phase composition and temperature effects were both explored. Generally improved resolutions (sometimes with values over 10) with lower analysis times were obtained with SFC, as compared to normal-phase HPLC conditions.

SFC also had the additional benefit of limited solvent consumption, as liters of toxic hexane are replaced by non-toxic carbon dioxide, retaining only the alcohol co-solvent.

Polysaccharide CSP have been the most popular chiral selectors in chromatography for over twenty years [18]. In particular, several halogenated phases originally developed by Chankvetadze and co-workers [19–21] have been made commercially available in the recent years. Fundamental studies conducted in SFC have demonstrated that chlorinated polysaccharide CSP interact with the analytes in a manner that is significantly different from non-chlorinated polysaccharide CSP having otherwise comparable structure [22]. The differences were attributed mainly to the effect of chlorine atoms present on the aromatic ligand on the hydrogen-bonding capabilities of the carbamate function attaching the aromatic ring to the polysaccharide. Halogen bonds should also be considered as possible contributors to the specific retention and separation behavior of chlorinated CSP. Halogen bonds may occur between a halogen atom acting as an electrophile and a nucleophile (like oxygen, sulfur or nitrogen atom, or the π electrons of an aromatic ring).

While chlorinated polysaccharide CSP have proven to be promising for the separation of chiral sulfoxide species in the past, we were interested in examining the SFC retention and separation in more detail In the present paper, a group of twenty-four synthetic chiral sulfoxides are analyzed with seven polysaccharide CSP, four of them being chlorinated. The high resolution observed on chlorinated CSP prompted more detailed investigation of the enantioseparation on these CSP, with the assistance of molecular modelling. 4

2. Material and methods

2.1. Stationary phases

The columns selected for this study were all polysaccharide stationary phases provided by Phenomenex (Le Pecq, France). They were: Lux Amylose-1 (150 x 4.6 mm, 5 µm), Lux Amylose-2 (250 x 4.6 mm, 3 µm), Lux Cellulose-1 (250 x 4.6 mm, 5 µm), Lux Cellulose-2 (250 x 4.6 mm, 5 µm), Lux Cellulose-3 (250 x 4.6 mm, 5 µm), Lux Cellulose-4 (250 x 4.6 mm, 5 µm) and Lux i-Cellulose-5 (250 x 4.6 mm, 5 µm). Because different column dimensions and particle size were available, only retention and separation factors will be considered when comparing the results from different columns. The structures of the chiral stationary phases are presented in Figure 1.

2.2. Chemicals

The solvent used was HPLC-grade methanol (MeOH) provided by VWR (Fontenay-sous-Bois, France). Carbon dioxide was provided by Messer (Puteaux, France).

The structure of analytes can be found in Figure 2. Solutions of all probe analytes were prepared in MeOH at 1 mg/mL. The analytes were only available as racemates thus the elution order was never assessed.

2.3. Apparatus and conditions

A Waters Acquity UltraPerformance Convergence Chromatography^{TM} (UPC^2) system was used. Operating conditions were as follows: CO_2-methanol in varying proportions as indicated in the text, 25°C and 150 MP bara outlet pressure, with a flow rate of 3 mL/min, except for the experiments were methanol percentage was varied from 20 to 100%, where the flow rate was 1 mL/min to avoid reaching the upper pressure limit of the pumping system.

Injection volume was 1 µL for all compounds. Retention factors (*k*) were calculated based on the retention time t_R, determined using the peak maximum and on the hold-up time t_0 measured on the first negative peak due to the unretained sample solvent.

2.4. Molecular modelling

First, the 3D sulfoxide structures were prepared from 2D coordinates. The 3D conformations were generated using the Structure Preparation function and hydrogens atoms were added using Protonate3D function in MOE2014.09 (Chemical Computing Group Inc., Montreal, Canada). Second, the structures were submitted to conformational search using LowModeMD [23] with default parameters. The MMFF94x force field with Born solvation was used and the lowest energy conformer was kept in this study. Structures were then observed and copied from Discovery Studio 4.0 Visualizer (Accelrys, San Diego, California, USA).

3. Results and discussion

3.1. Examination of the set of racemates

First, let us examine the structures of the racemates analyzed. The structures are presented in Figure 2, organized to enlighten the similarities and structural variations between them. There is one common feature to all of them: a sulfoxide function directly attached to a phenyl ring (that will be called “A ring” in the following). On the other side of the sulfoxide, there can be a simple methyl group (**oMAm,M**), a propyl group (**oMAM,P**), or a benzyl group (all others). The latter benzyl ring (that will be called “B ring” in the following) may be substituted with a bromide atom in the *meta* (**odMAm,mB**, **oMAm,mB**, **oAm,mB**, **oBzAm,mB**) or in the *para* position (**odMAm,pB**, **oMAm,pB**); with a trifluoromethyl group (**oMAm,3F**) or with a nitro group
(**oMAm,N**) in the *para* position, or will remain unsubstituted (all others).

A carboxyl group is also attached on the A ring, which can be placed in *para* (**pMAm**, **pAc**, **pEs**), *meta* (**mMAm**, **mAm**, **mAC**, **mEs**) or *ortho* position (all others). The carboxyl group is most often a primary amide function that may be unsubstituted (**oAm,mB**, **oAm**, **mAm**), N,N-dimethyl (**odMAm,mB**, **odMAm,pB**, **odMAm**) or N-substituted (all other amides). In the latter case, the N-substituent is most often a methyl group but may also be a phenyl or benzyl group (**oPAm**, **oBzAm**, **oBzAm,mB**, **oNAm**), which will be called “C-ring” in the following.

Finally, the carboxyl function is not always an amide but can be an acid group (**oAc**, **mAc**, **pAc**) or a methyl ester (**oEs**, **mEs**, **pEs**).

All in all, the twenty-four racemates provide an interesting diversity of structural features to investigate enantioseparation mechanisms.

3.2. Comparison of retention characteristics

First of all, it was observed that retention was significantly higher on some stationary phases than on others. More precisely, the most retentive phases towards these analytes were the three chlorinated cellulosic stationary phases: Cellulose-2, Cellulose-4 and i-Cellulose-5. Typically, 10% methanol as a co-solvent were sufficient to elute most analytes with a reasonable analysis time from Amylose-1, Amylose-2, Cellulose-1 and Cellulose-3, while it was necessary to increase the co-solvent percentage to 30% to obtain comparable retention on the other chlorinated cellulose CSP. In two cases (**oAm** and **oAm,mB** on Cellulose-2), even higher elution strength was required (50% methanol) to achieve measurable retention (still over one hour for the second enantiomer). It can be observed that the chlorinated amylose stationary phase did not exhibit particularly strong retention, only chlorinated cellulose phases. The retention range observed on each column with these settings (10 or 30% methanol) can be observed on Figure 3.

Stronger retention on the chlorinated cellulose phases may be explained by stronger hydrogen bonding between the carbamate function of the CSP ligands and the amide group of the chiral sulfoxides, and/or through halogen bonding between the chlorine atoms in the CSP ligands and the S, O or N atoms and aromatic rings of the analytes. Strong hydrogen bonding is associated to the larger portion of free carbamate groups in chlorinated polysaccharide CSP, as compared to their non-chlorinated counterparts where more carbamate groups are involved in intra-molecular hydrogen bonding [12,19–21,24].

3.3. Preliminary comparison of separation characteristics

In enantioselective chromatography, retention does not usually correlate with enantioresolution, because strong interactions may occur between analytes and CSP, while these interactions do not necessarily contribute to the enantioselectivity. Indeed, when observing scatter plots of enantioseparation (α values) vs. retention (*k* values) on Amylose-1, Amylose-2, Cellulose-1 and Cellulose-3, the points were widespread in the whole space, showing no trend. However, when observing the same plots on Cellulose-2, Cellulose-4 and i-Cellulose-5, some tendency could be observed, meaning that large retention values were related to large separation factors. Sample figures are presented in Figure 4 with the examples of Cellulose-1

and Cellulose-2. With the chlorinated phase, it appears that small enantioseparation values are essentially clustered in a small area, while large enantioseparation values increase proportionally to retention. In addition, it is worth noting that, although the figures represent the retention of the second eluted enantiomer only, similar trends (or absence of trends) were observed when the retention of the first enantiomer was plotted. This observation indicates that both enantiomers interact more strongly with the stationary phase, thus the improved enantioseparation is not solely associated to stronger enantioselective interactions (that would cause increased retention of one enantiomer) but probably also to stronger non-enantioselective interactions. This is in accordance with previous observations that chlorinated phases display strong hydrogen bonding with electron donors, while this type of interaction was not necessarily favorable to enantiorecognition, especially to strongly retained molecules [22].

Thus in the present case, the three stationary phases that yielded the highest retention values also provided the highest success rate to resolve the sulfoxide enantiomers. Indeed, even with the high elution strength employed (30% methanol in CO_2) for the chlorinated cellulose phases, average enantioselectivity was 4.9, 2.7 and 3.0 respectively on Cellulose-2, Cellulose-4 and i-Cellulose-5, while it ranged between 1.1 and 1.3 on the other four columns. We may also note that the proportion of racemates for which some enantioselectivity was observed ($\alpha > 1.0$) was above 80% for the chlorinated cellulose phases, and in the 70-75% range on the four other phases.

Because the chlorinated cellulose phases were so clearly superior for the separation of chiral sulfoxides, the following discussion will focus solely on these three stationary phases.

3.4. Relation between structural features and enantioseparation

Because the set of racemates employed here contains several types of structural variations, as pointed out in section 3.1 above, the effects of structure on enantioseparation can be assessed. Firstly, it was observed that the effect of a benzyl group directly attached to the sulfoxide chiral centre (B ring) was very significant to enantioseparation. Indeed, the two racemates that possessed a simple methyl or propyl chain in this place (**oMAM,M**, **oMAm,P**) were among the most difficult to separate: while

enantioseparation occurred on all three CSP for both racemates, the separation factors were among the smallest values observed. The variation of enantioseparation when changing this group can be seen in Figure 5a. We may conclude from this observation that some interactions between a benzyl group and the chiral stationary phase should contribute favorably to chiral recognition. This hypothesis is supported by the further observation that any substituent on the B ring tends to decrease enantioseparation. In Figure 5b, the separation factors measured for the racemates substituted with p-trifluoromethyl (**oMAm,3F**), p-nitro (**oMAm,N**), p-bromo
(**oMAm,pB**) or m-bromo (**oMAm,mB**) groups are compared to those observed when no substituent is present on the benzyl ring. It should be clear from this figure that any substituent, possibly limiting a close π-π interaction between the B ring and stationary phase aromatic groups, is negatively impacting the enantioseparation. There is a single exception as the meta-bromine (**oMAm,mB**) is slightly favored on i-Cellulose-5 as compared to the absence of substituent (**oMAm**). Large substituents (trifluoromethyl and nitro) are obviously more impacting than smaller substituents (bromine atom). Moreover, the bromine halogen in *para* position is less favorable than bromine in *meta* position.

Considering the amide group substitution, it was observed on the contrary that a simple methyl substitution was far more favorable than aromatic rings in the C position (Figure 5c). A phenyl group (**oPAm**) is the least favorable substituent in this position, while a p-methoxybenzyl group (**oBzAm**) is slightly better. We may then infer that an aromatic ring in the C position is essentially limiting the steric fit in the chiral cavities rather than providing additional, favorable interactions with the chiral selector.

The substitution on the A ring in *ortho*, *meta* or *para* positions, has a very significant effect on amide and methyl-amide racemates (Figure 5d). The *ortho* position (**oMAm**) is largely favored, yielding much higher enantioseparation factors than the *meta* position (**mMAm**), which is a little more favorable than the *para* position (**pMAm**). With esters, the same ordering is observed, although the difference is not so significant between the *ortho* compound (**oEs**) and the other two (**mEs** and **pEs**). However, with acids, the contrary is observed. The para-acid (**pAc**) is actually the only acid racemate being resolved on all three chlorinated cellulose stationary phases while the *ortho*-acid (**oAc**) is never resolved and the *meta*-acid (**mAc**) only once.

Finally, modifications on the amide function (-NH_2, -$NHCH_3$, -$N(CH_3)_2$, acid or ester) yield a diversity of behaviors, depending on the substitution of the A ring (*ortho*, *meta* or *para*). However, a constant observation is that the carboxylic acid function (**oAc**, **mAc** or **pAc**) is the least favorable to enantioseparation.

To understand why some racemates were so much better resolved than others, we proceeded to compute three-dimensional conformations of each racemate. Indeed, observing molecules in two dimensions can be deceiving as they seem most similar, while in 3D space they may appear to have different lowest energy conformations. Interestingly, the lowest energy conformers of the racemates obtained from conformational search are essentially divided into two groups: folded (bent or U-shaped) conformations and extended (linear or Z-shaped) conformations. The smaller species having no B-ring (**oMAm,M** and **oMAm,P**) do not really fall in any of these categories. Sample figures are depicted in Figure 6 where one of the best separated racemate (**oMAm**) appears to be in a U-shaped conformation, while a racemate with much smaller enantioselectivity values (**oMAm,N**) appears to be in a Z-shaped conformation. Considering all racemates in this set, it appears that the average enantioseparation factors among the U-shaped racemates are much larger than among the Z-shaped racemates. Average values were: 9.2 vs. 1.8 on Cellulose-2; 4.2 vs. 1.9 on Cellulose-4 and 5.2 vs. 1.5 on i-Cellulose-5. On the other four stationary phases, no difference was observed between the two groups. A possible explanation for such significant difference on the chlorinated cellulose phases is that the folded (U-shaped) conformations may better fit the chiral selector cavities, especially when two aromatic rings (A ring and B ring) can interact favorably with two aromatic rings or chlorine atoms (via halogen bond) from the chiral stationary phase. On the other hand, the extended (Z-shaped) conformations may have difficulties penetrating the chiral cavities entirely with at least one of the aromatic rings (A or B) remaining outside, thereby reducing compound-stationary phase interactions and thus the possibilities for enantioselective interactions. This observation is in accordance with previous works indicating that, to strongly retained analytes, sphericity of the molecule is a favorable feature to enantiorecognition on chlorinated cellulose phases [22], while linear conformations are less efficiently discriminated. This is also an interesting example of conformationally-driven chiral recognition [25].

3.5. Variation of enantioseparation over a broad range of mobile phase compositions

Previous works indicated that normal-phase liquid and supercritical fluid conditions provide significantly different elution patterns and enantiorecognition [26]. However, to the best of our knowledge, polar organic HPLC mode was never compared to SFC mode. Analyzing the same set of chiral sulfoxide racemates in liquid polar organic mode (results not shown) indicated that significant differences should exist between the liquid conditions and the SFC conditions explored above. To shed some light on these differences, we selected a subset of six racemates as being representative of the different conformations observed (U-shaped, Z-shaped or other) and analyzed them with increasing mobile phase elution strength, varying methanol proportion from 20 to 100%. Selected results are presented in Figure 7 to illustrate the observations on the three classes of conformations. Sample chromatograms are presented in Figure 8.

As expected, increasing methanol percentage caused decreased retention in all cases, due to increasing elution strength of the mobile phase [27]. In SFC, similarly to liquid-phase adsorption processes, logarithmic values of retention factors can usually be related to the logarithmic values of mobile phase composition in a close-to-linear fashion, due to non-linear variation of elution strength in carbon dioxide - co-solvent mixtures [27,28]. In the present case, the most linear curves were observed for U-shaped species (as observed in Figure 7c). For other conformations, linearity was good between 20 and 60% methanol, then some curvature can be observed when methanol is further increased up to 100% (Figure 7a and 7b).

Decreasing retention values may be associated to varied trends of enantioseparation, although it is most often seen to decrease when co-solvent percentage increases. In the present case, the trends of enantioseparation vs. mobile phase composition were significantly different between the different analyte conformations. As observed in Figure 7f, logarithmic values of enantioseparation factors decreased regularly, even nearly linearly for the U-shaped species (represented by **oMAm**). For other conformations, a significant change of trend was observed when methanol percentage reached 60%. In some cases, as represented with the Z-shaped conformation (**oMAm,N**) in Figure 7e, the trend was even reversed as enantioseparation increased between 20 and 60% methanol, then decreased between 60 and 100% methanol.

Besides, the variation of apparent dead volume also showed a change of trend at 60% methanol. Firstly, the dead time increased from 20 to 60% methanol, indicating the apparent dead volume increased. This suggests that the volume of stationary phase is decreasing in this range. Two possible reasons can explain a change in stationary phase volume: either through adsorption of mobile phase components, or through changes in the polysaccharide conformation. Secondly, the dead time stabilized and remained constant from 60 to 100%, suggesting that the amount of mobile phase components adsorbed in the stationary phase did not vary anymore at this point, and/or that the conformation of stationary phase did not change over this range.

Based on these observations, it is reasonable to assume that (i) U-shaped structures access different regions of the stationary phase from Z-shaped structures and (ii) some change in the stationary phase conformation is occurring around 60% methanol, explaining a change in retention and separation trends of Z-shaped structures. Similar discrepancies between HPLC elution modes (reversed-phase, normal-phase or polar organic) were also noted in previous works [8,12].

The high diffusivities afforded by carbon dioxide-based mobile phases is usually advantageous to column efficiency, as compared to more viscous liquids. In the present case, column efficiency showed different variation trends between analytes, probably because diffusion in the stationary phase was also varying with methanol proportion, not only mobile phase diffusivity. However, resolution always decreased when increasing methanol percentage. As efficiency and separation factors exhibited a diversity of patterns, decreasing resolution must essentially result from the strongly decreasing retention. A practical conclusion issuing from these experiments is that carbon dioxide offers the possibility to enhance retention for those analytes that would be too weakly retained in the polar organic mode, thereby increasing resolution, even when peak width is not significantly improved by lower mobile phase viscosity.

4. Conclusions

Chlorinated cellulose stationary phases have proven to be both highly retentive and highly enantioselective towards chiral sulfoxide species. The molecules that could adopt a folded U-shaped conformation were most efficiently discriminated compared

to extended conformations, indicating that a conformationally-driven enantiorecognition process was taking place. In other words, not only the type of interactions occurring between analyte and stationary phase, but also the steric fit of the analyte in the chiral grooves were shown to be significant features of the enantiorecognition process. The discrimination between bent and linear conformations was not observed in other polysaccharide chiral stationary phases. Changing the mobile phase composition from SFC to polar organic HPLC mode showed that the chiral selector must adopt a different conformation in the two operating modes, thus that separations observed in one mode are not necessarily transposable to the other mode. In all instances, SFC offered superior resolution.

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Figure Caption

Figure 1. Structures and names of the seven chiral stationary phases employed in this study.

Figure 2. Structures of the 24 sulfoxide racemates analyzed in this study with full and abbreviated names. (o,m,p) is related to the ortho, meta or para substitution of the A ring. (Am, Ac, Es) refer to the amide, acid or ester function between A and C groups. (A, dM, P, Bz) relate to methyl, dimethyl, phenyl or benzyl substitution of the amide function. (mB, pB, P, 3F, N) refer to the meta or para substituent on B ring.

Figure 3. Comparison of retention ranges (*k* values for both enantiomers) on the seven polysaccharide stationary phases in Figure 1 for the 24 racemates in Figure 2. CO_2-methanol 90:10 or 70:30 as indicated, 25°C, 15 MP0 baar, 3 mL/min. The red cross indicates average retention, the red line indicates median retention.

Figure 4. Examination of the relation between enantioseparation and retention of the second eluted enantiomer on a non-chlorinated cellulose phase (Lux Cellulose-1; CO_2-methanol 90:10) and a chlorinated cellulose phase (Lux Cellulose-2; CO_2-methanol 70:30); 25°C, 15 MPa, 3 mL/min..

Figure 5. Influence of structural features on enantioseparation with the chlorinated cellulose stationary phases. CO_2-methanol 70:30, 25°C, 150 barMPa, 3 mL/min.

Figure 6. Molecular modelling highlighting the differences in three-dimensional conformations between the investigated racemates: a small molecule with no B-ring (**oMAm,M**); a typical Z-shaped (extended) conformation (**oMAm,N**) and a typical U-shaped (folded) conformation (**oMAm**) Bottom view shows a different point of view evidencing the bent and linear conformations.

Figure 7. Variation of retention factors (a,b,c) and enantioseparation factors (d,e,f) when moving from SFC to liquid polar organic mode for the three racemates with different conformations presented in Figure 6: (a,d) **oMAm,M** ; (b,e) **oMAm,N** ; (c,f)

oMAm. Lux i-Cellulose-5, CO_2 with methanol varying 20 to 100%, 25°C, 15 from MPa, 3 mL/min.

Figure 8. Chromatograms obtained for three selected racemates with different conformations presented in Figure 6: (a) oMAm,M ; (b) oMAm,N ; (c) oMAm. Lux i-Cellulose-5, CO_2 with methanol varying from 20 to 90% as indicated, 25°C, 15 MPa, 3 mL/min.

Figure 1

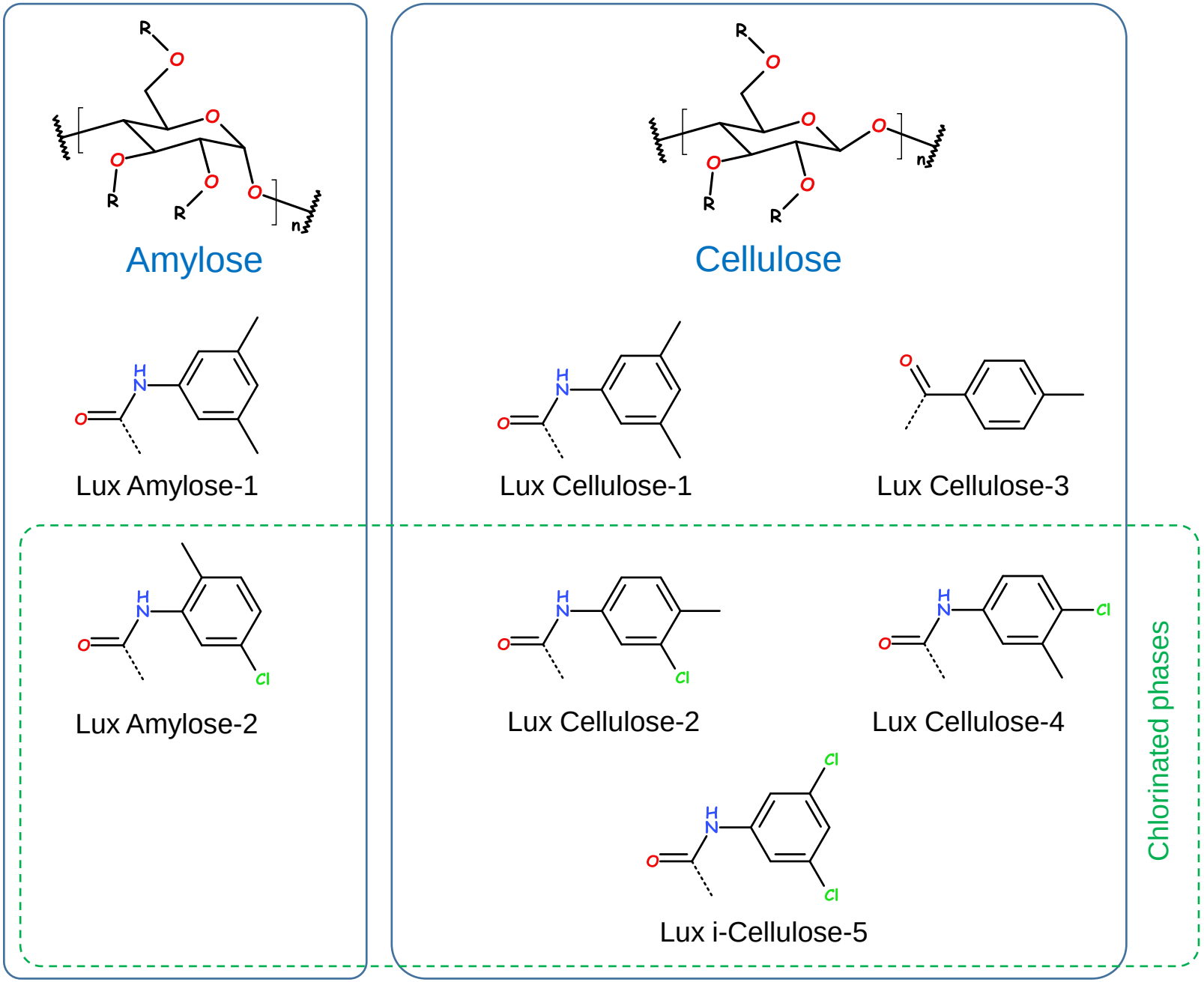

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Figure 2

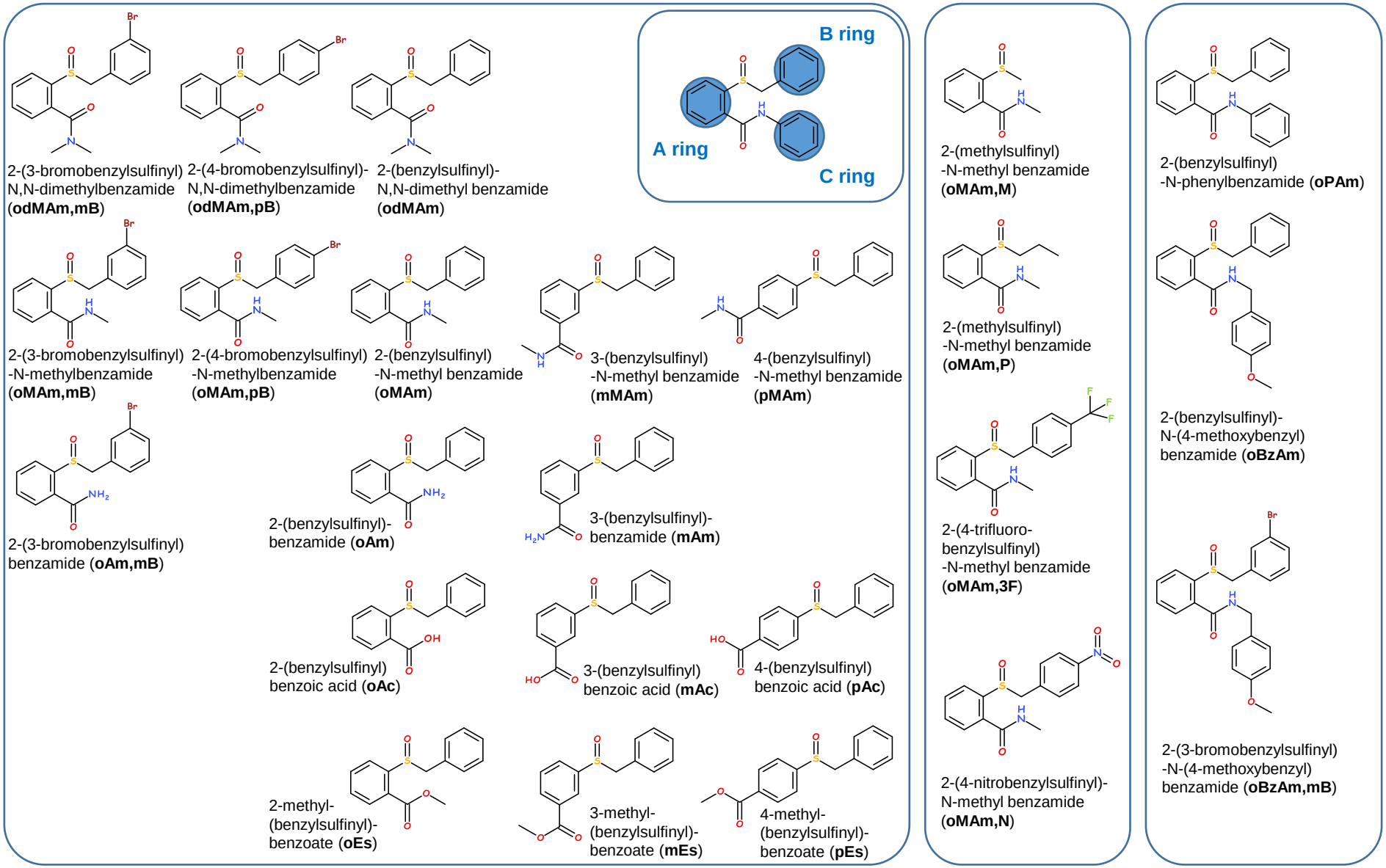

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Figure 3

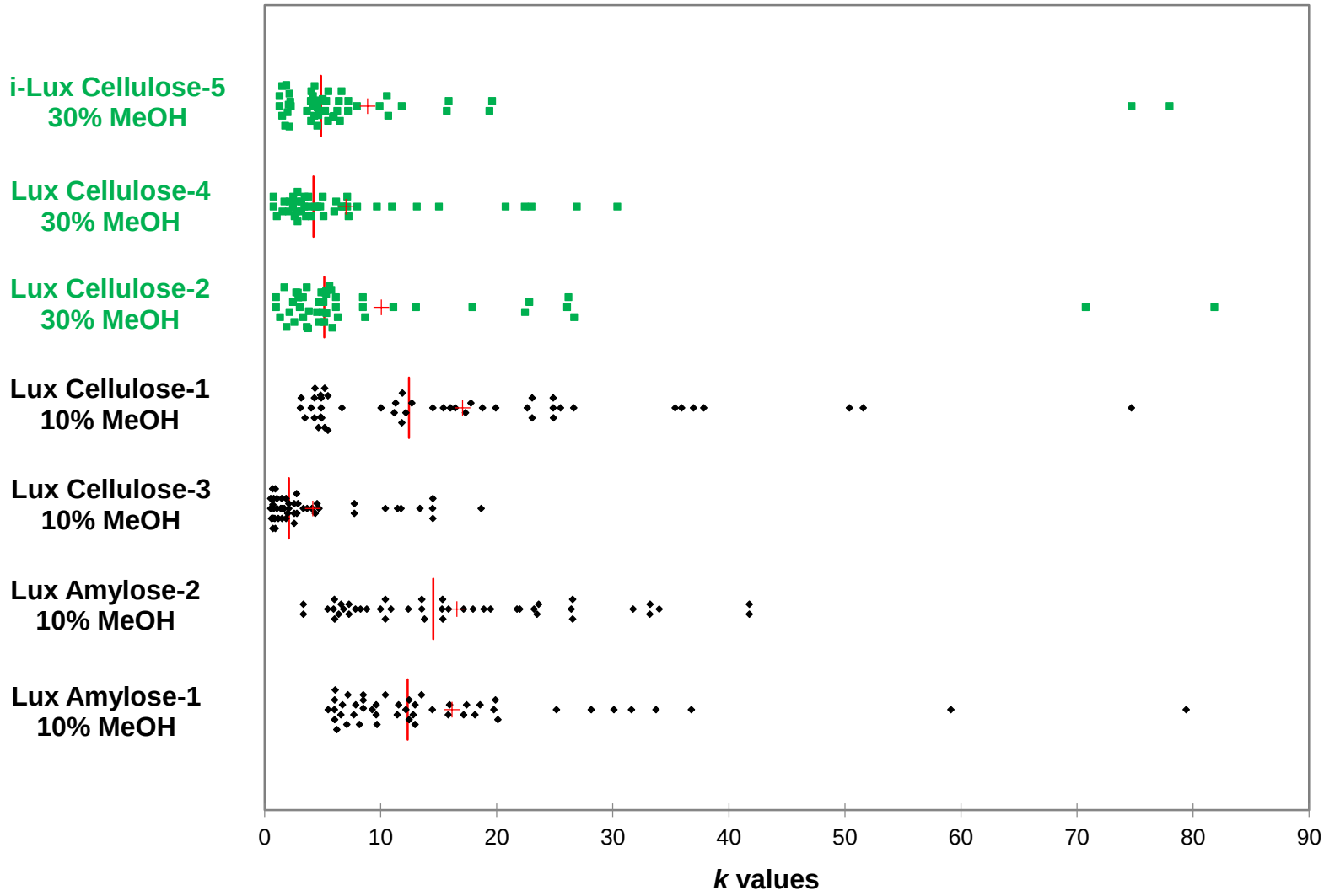

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Figure 4

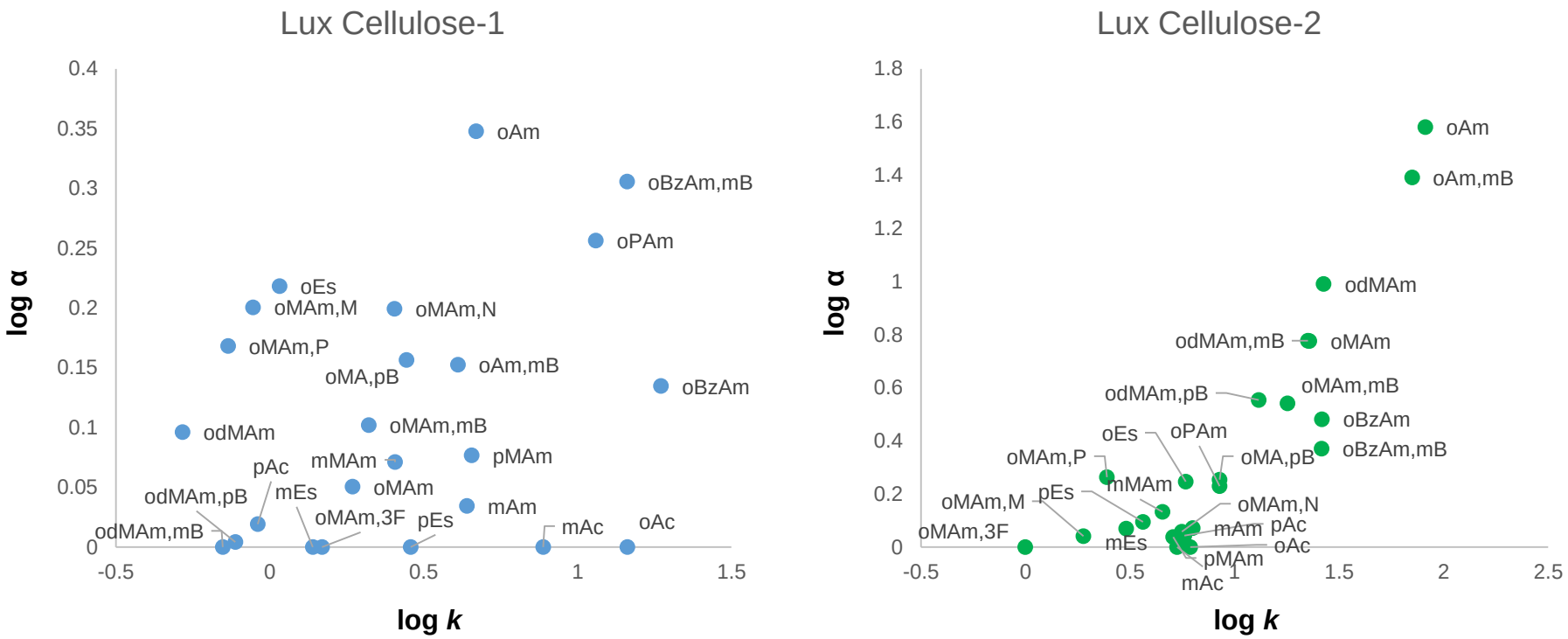

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Figure 5

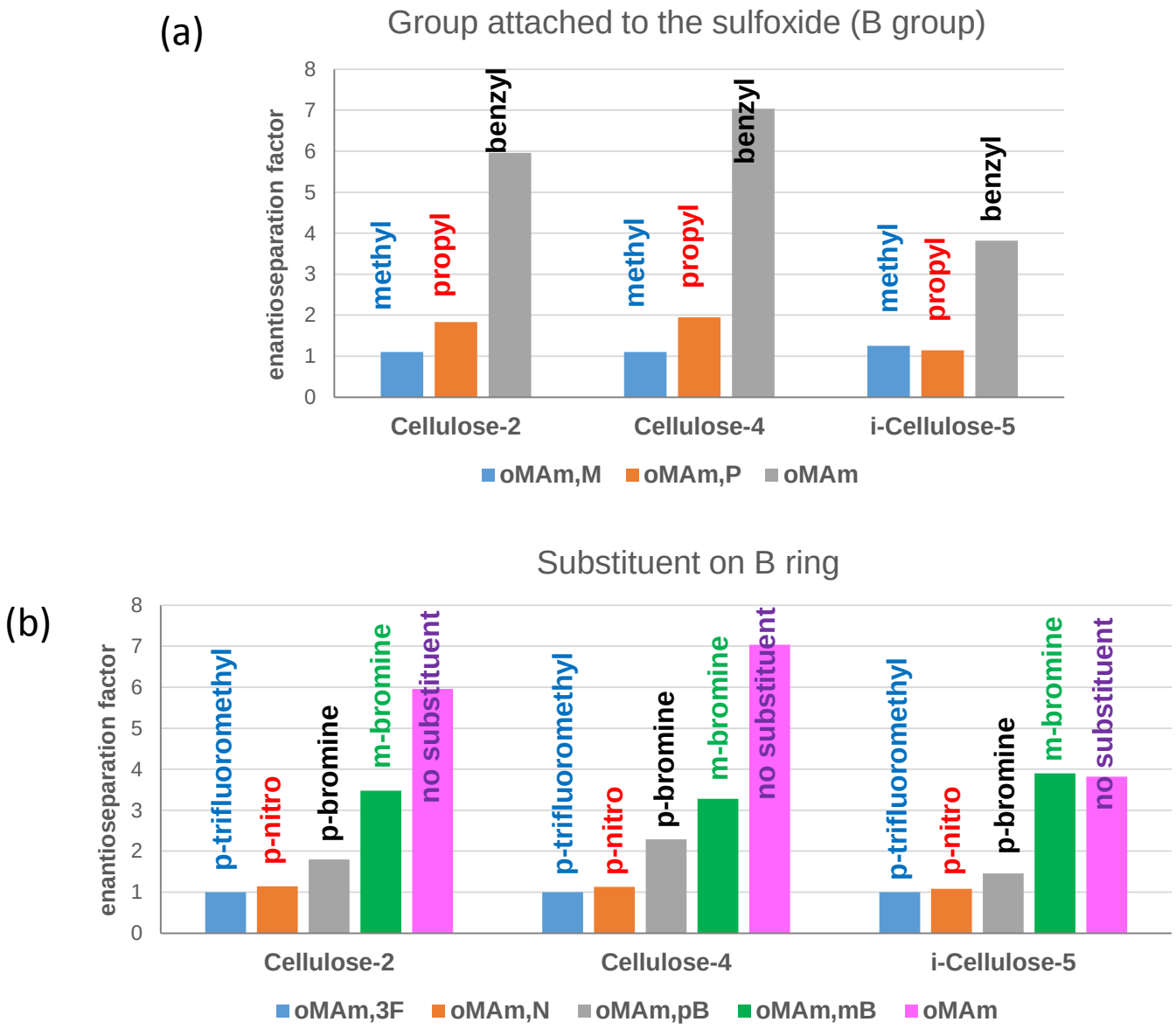
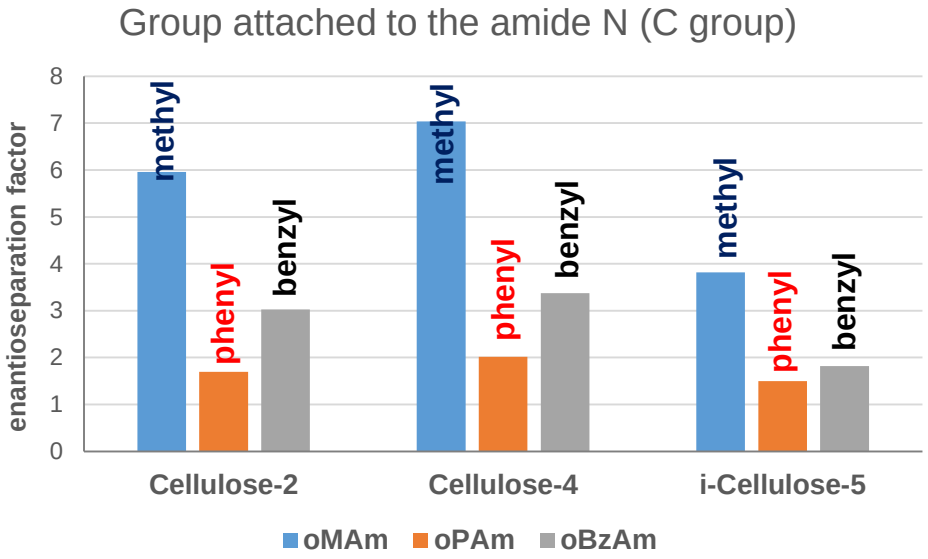


Figure 5. Influence of structural features on enantioseparation with the chlorinated cellulose stationary phases. CO_2-methanol 70:30, 25°C, 150 bar, 3 mL/min.

(c)



(d)

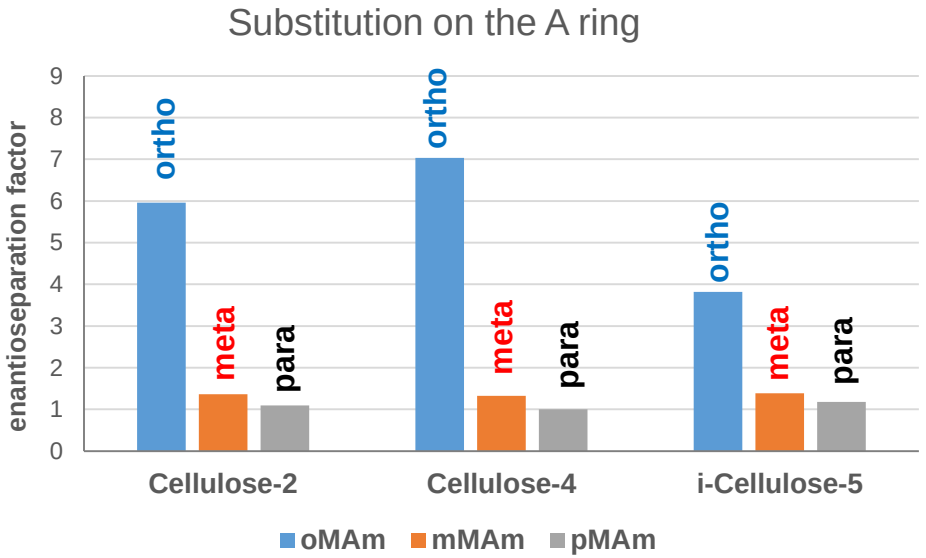
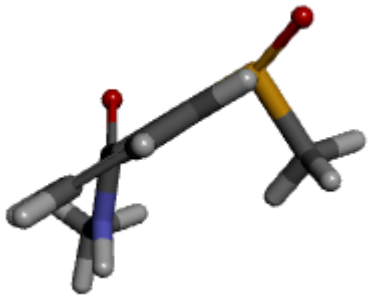
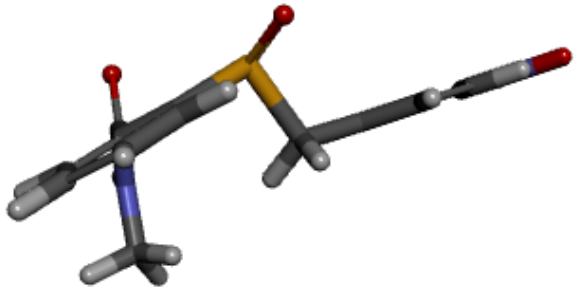


Figure 5 (continued). Influence of structural features on enantioseparation with the chlorinated cellulose stationary phases. CO_2-methanol 70:30, 25°C, 150 bar, 3 mL/min.

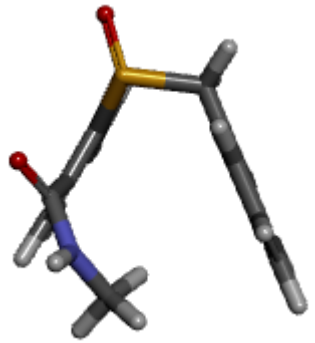
Figure 6



2-(methylsulfinyl)-N-methyl benzamide (**oMAm,M**)



2-(4-nitrobenzylsulfinyl)-N-methyl benzamide (**oMAm,N**)



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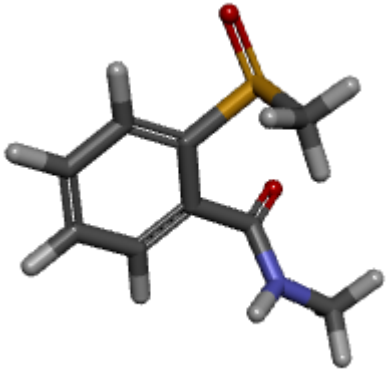

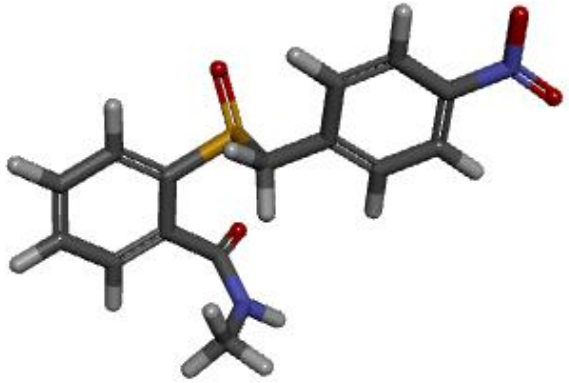

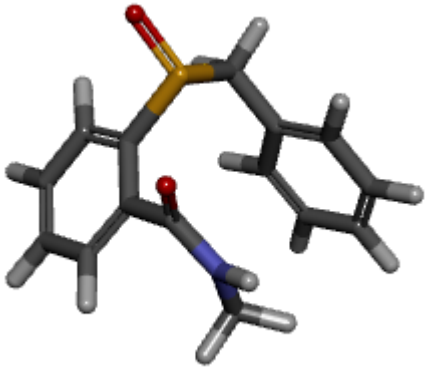

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Figure 7

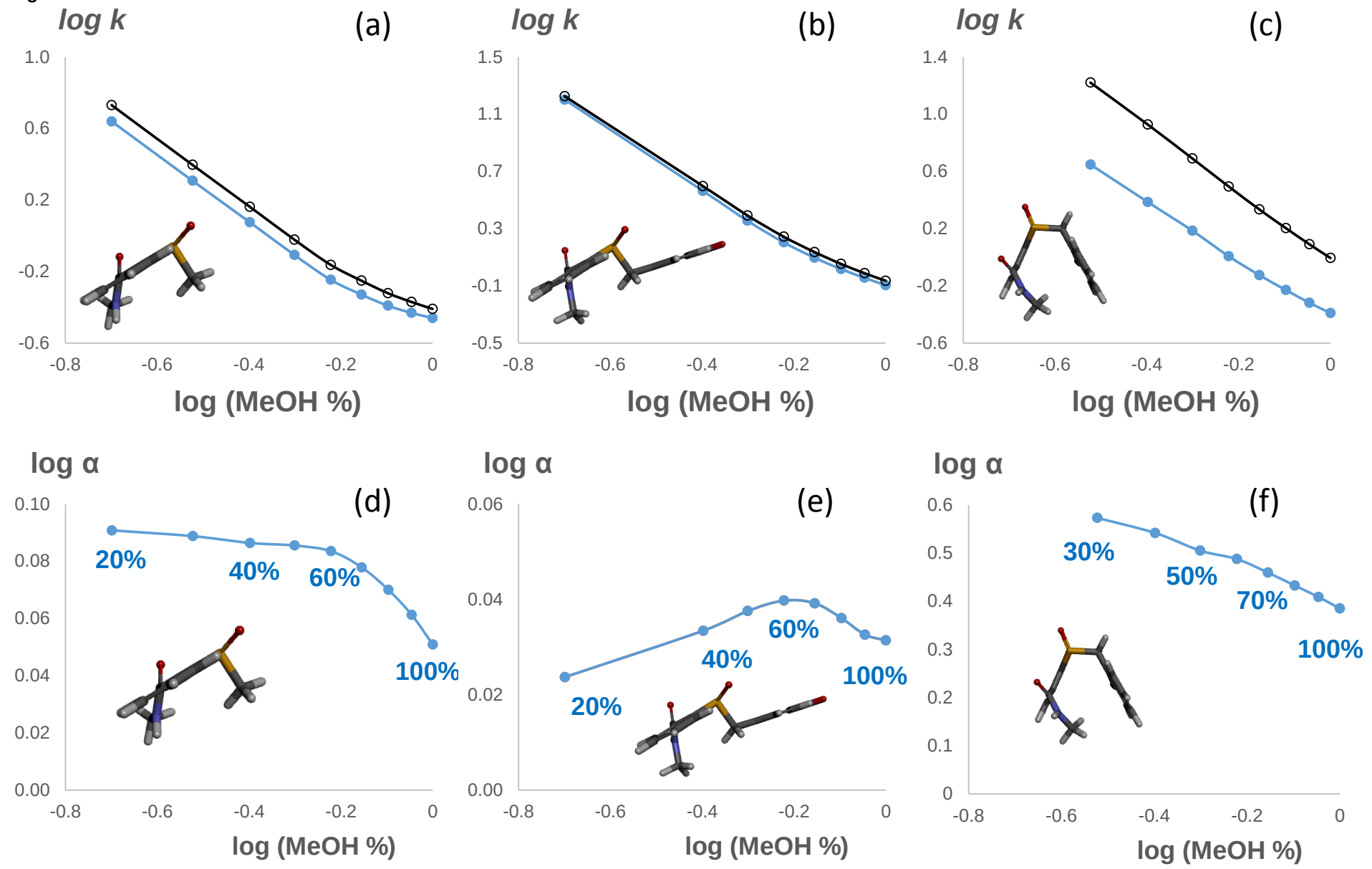

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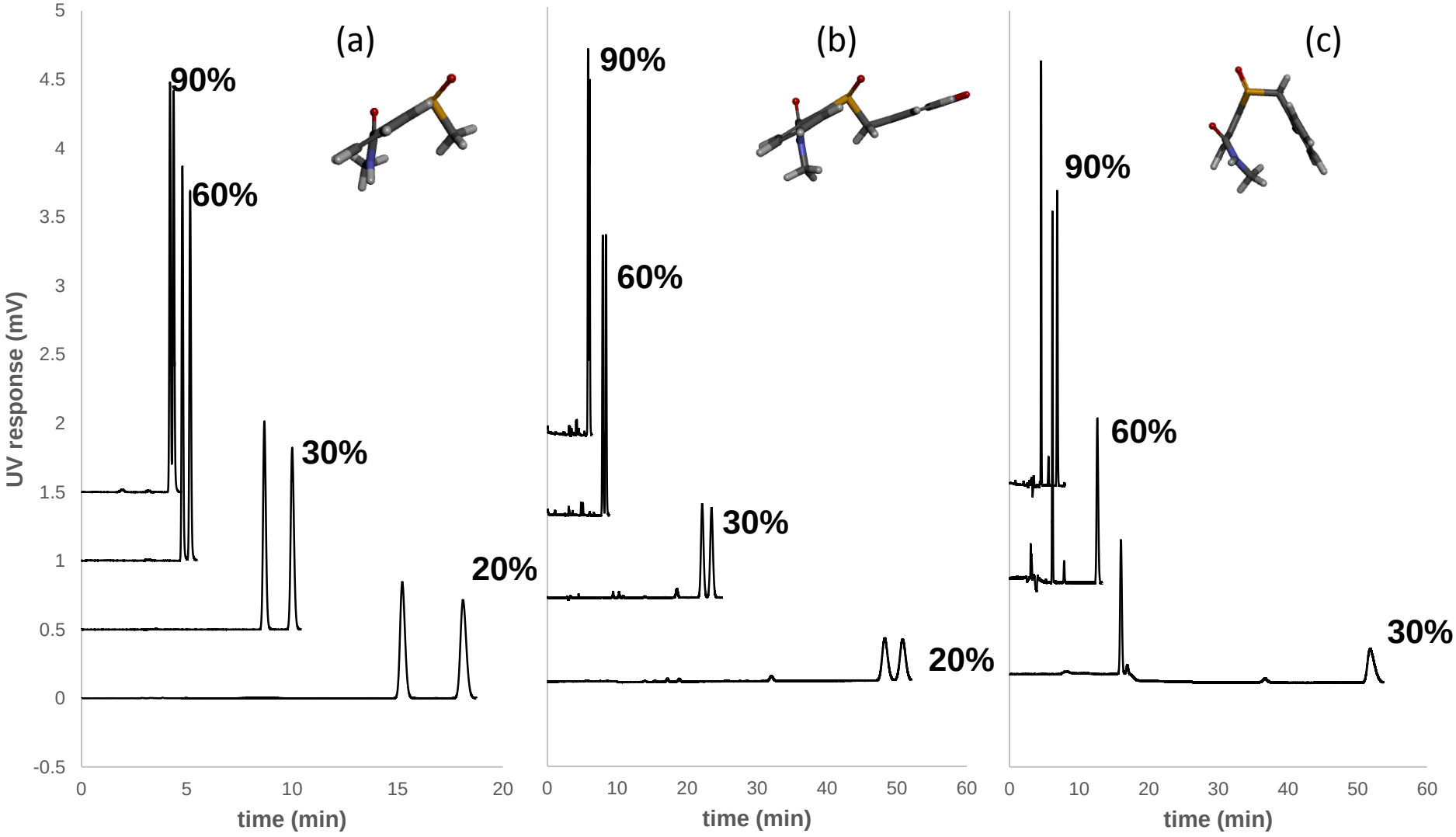
Figure 8



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