Accepted Article

Cyclopentyl-methyl ether (CPME): Versatile eco-friendly solvent for applications in Biotechnology and Biorefineries.

Gonzalo de Gonzalo[a]*, Andrés R. Alcántara[b]*, Pablo Domínguez de María[c]*

[a] Departamento de Química Orgánica, Universidad de Sevilla, c/ Profesor García González 2, 41012, Sevilla, Spain, Tel: 00-34-954559997.

E-mail: gdegonzalo@us.es

[b] Department of Chemistry in Pharmaceutical Sciences. Section of Organic and Pharmaceutical Chemistry. Faculty of Pharmacy. Complutense University of Madrid. Plaza de Ramón y Cajal, s/n. E-28040 Madrid, Spain. Tel: 00-34-913941820.

E-mail: andalcan@ucm.es

[c]Sustainable Momentum, SL. Av. Ansite 3, 4-6, Las Palmas Gran Canaria E-35011, Canary Islands, Spain. Tel. 00-34-609565237.

E-mail: dominguez@sustainable-momentum.net

TABLE OF CONTENTS

1.- Introduction. Motivation for eco-friendly solvents and for CPME.

2.- CPME as (co)solvent in biotransformations.

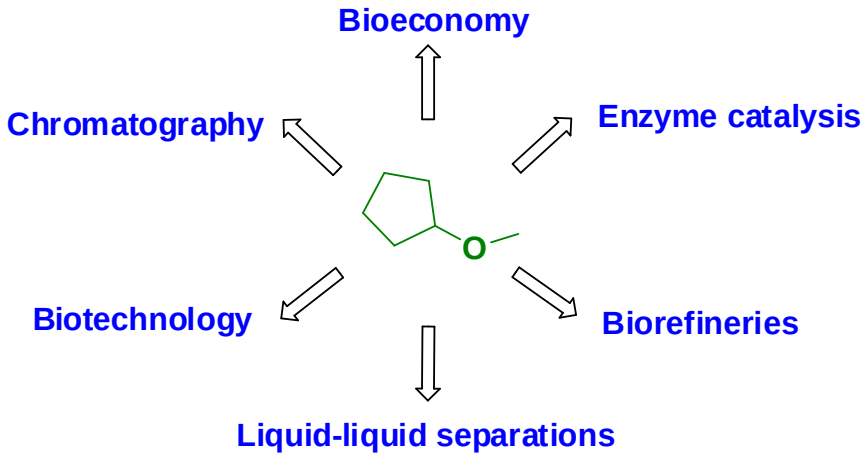
3.- CPME and its use in Biorefineries and in extractive strategies.

4.- Other emerging applications of CPME in Bioeconomy: Chromatography, solid-phase peptide synthesis.

KEYWORDS

Green Chemistry/ Bio-based solvents/ Biorefineries/ Biocatalysis/ Extractions

GRAPHICAL ABSTRACT



Abstract

The quest of sustainable solvents is currently matter of intense research and development, as solvents significantly contribute to the wastes generated by chemical industries. Cyclopentyl-methyl ether (CPME) is a promising eco-friendly solvent, because of its valuable properties such as low peroxide formation rate, stability at basic and acidic conditions, relatively high boiling point, etc. This review discusses the potential use of CPME for applications in Biotechnology (*e.g.* biotransformations, as solvent or co-solvent), Biorefineries and Bioeconomy (*e.g.* for furan synthesis, as extractive agent in Liquid-Liquid separations, etc.), as well as in other areas like chromatography or peptide synthesis. Albeit CPME is currently produced by petrochemical means with a remarkably high atom economy, its biogenic production can be envisaged from substrates like cyclopentanol or cyclopentanone, which can be derived from furfural or from (bio-based) adipic acid, respectively. The combination of the promising properties of CPME as (co)solvent with a future (economic) biogenic origin would be advantageous for setting strategies aligned with the Sustainable Chemistry principles.

1.- Introduction. Motivation for eco-friendly solvents and for CPME.

Solvents account for an important aliquot of the impact generated by chemical, pharmaceutical and manufacturing industries with respect to environmental degradation and waste generation. The quest for eco-friendly and biogenic alternatives that may replace hazardous solvents is currently matter of intense research, with remarkable successful examples such as 2-methyl-tetrahydrofuran (2-MeTHF) or deep-eutectic-solvents, to cite some of them.[1,2] In this area, over the last decade the use of cyclopentyl-methyl ether (CPME) as eco-friendly solvent has started to gain momentum as well.[3,4] Advantageous is that CPME has a manageable boiling point (106 ºC), with low solubility in water (1.1 g CPME / 100 g), what enables the set-up of biphasic media for synthetic reactions. Moreover, CPME can be effectively dried, being an effective advantage for its use in anhydrous reactions (when needed). Likewise, it exerts low toxicity, negligible peroxide formation rate, a narrow explosion range, and remains stable under strong acidic and basic conditions.[3] Overall, this confers promising features for its use as solvent and co-solvent in many chemical segments, from synthetic purposes or catalysis, to extraction or separation technologies.

The industrial synthesis of CPME has remained petrochemical until now, involving cyclopentene and methanol to afford CPME with excellent atom economy.[3.4] To close the loop with respect to eco-friendly solvents, biogenic synthetic pathways for CPME should be established. Herein, biorefineries are expected to play a significant role in the future sustainable chemical processes.[5] In particular several biomass-based routes have focused on the generation of chemical precursors that may be used for CPME production, such as cyclopentanone or cyclopentanol. Both precursors can be synthetized from furfural – which is derived from the dehydration of pentoses, mostly xylose –, thus

creating a potential biogenic pathway for CPME.[6] The synthesis of cyclopentanol is believed to follow a Piancatelli rearrangement of furfural.[7] Moreover, in classic petroleum refineries, cyclopentanone can be synthetized from the decarboxylation of adipic acid.[8] Notably, adipic acid can be derived from biomass as well, either through fermentative routes using sugars,[9] or by using lignin as feedstock (*via* muconic acid route).[10] Conclusively, biorefineries could take the lead to produce CPME as biogenic solvent in the future (Figure 1). It must be noted, though, that the biogenic origin of the raw materials is not sufficient to warrant a sustainable process. In addition to that, the synthetic procedures to deliver chemicals and solvents within a biorefinery must fulfil the Green Chemistry postulates as well, in terms of wastes, energy consumption and resource depletion (e.g. use of precious metals, etc.). In that respect, fermentative processes seem to be a good alternative, provided that straightforward downstream processing can be set. In the particular case of CPME, an excellent example is the fermentative approach to produce adipic acid, starting from lignin residues. A recent Life-Cycle-Assessment (LCA) study has shown that the biogenic route leads to a minimized impact, compared to the petrochemical classic approach.[10] Nevertheless, the subsequent reaction to convert adipic acid in cyclopentanone, and ultimately in CPME appears more challenging, in terms of sustainability (e.g. energy input, waste generation, etc.). Surely LCA technologies will be crucial to calibrate the proper synthetic options, on a case-by-case basis.

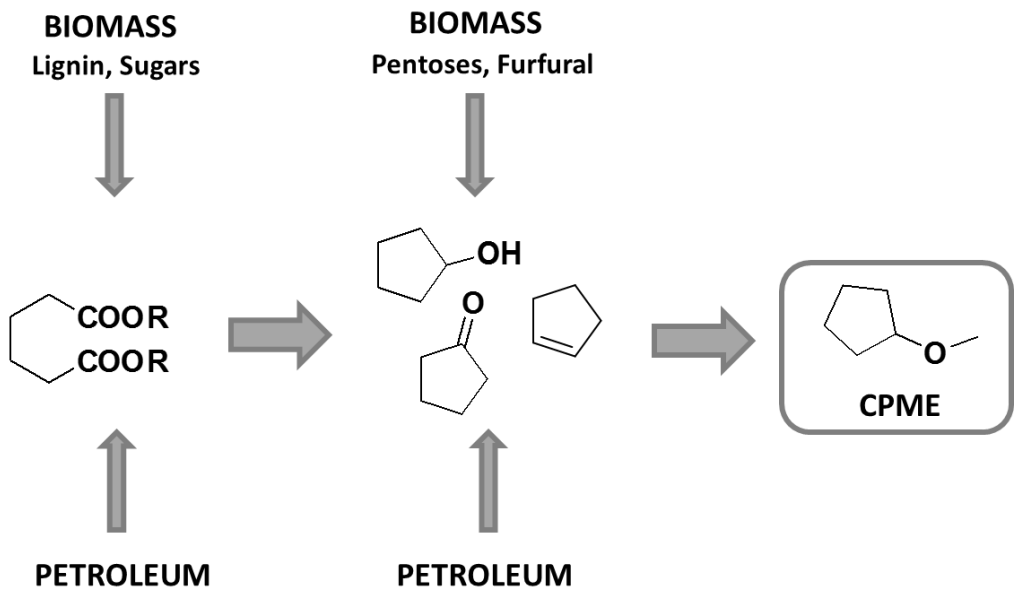


Figure 1. Synthetic options for CPME preparation, involving petroleum-based alternatives (current ones), and biogenic options ranging from adipic acid biosynthesis, or furfural production.

Given the promising characteristics as solvent that CPME shows, synthetic procedures involving organometallic, organocatalytic, radical or acid-base reactions have been reported by many researchers worldwide, and comprehensively reviewed recently by Azzena and coworkers.[4] Importantly, besides those applications, CPME offers many other potential alternatives in Bioeconomy, in fields like biotechnology, biorefineries, peptide synthesis, or chromatographic applications. In this review, these alternatives are contextualized thoroughly discussed.

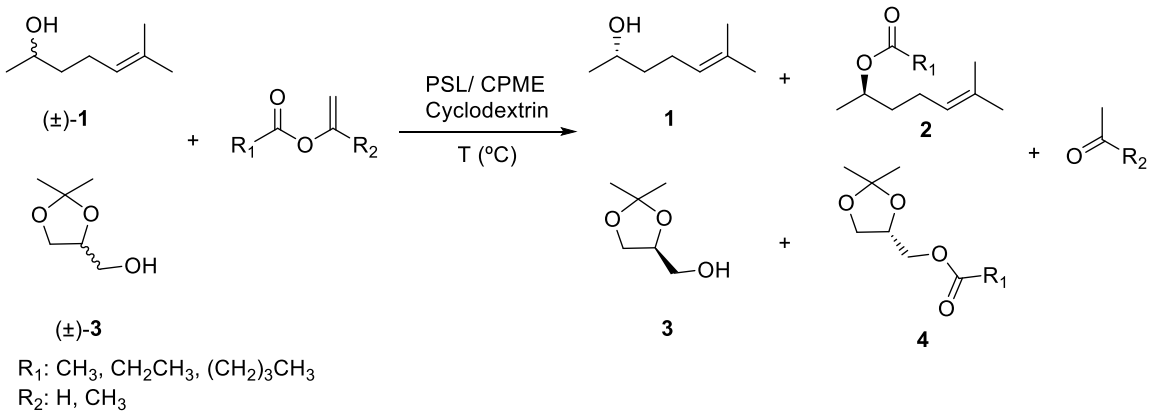
2.- CPME as (co)solvent in biotransformations.

The application of biocatalysis on a large scale may fit with the principles of Green Chemistry to establish sustainable processes with low environmental impact, while being

economically feasible as well.[11] To fully reach Green Chemistry postulates, though, not only catalysts are important to be considered; also reaction media, water consumption, wastewater generation and solvents must be holistically taken into account. In this area, the quest for green solvents derived from renewable sources in biocatalytic reactions has experienced a great development. Thus, processes using both 2-MeTHF,[2] and more recently CPME as eco-friendly solvents in enzyme-catalyzed reactions have been published. With respect to CPME, many of the reported biotransformations involve lipases, as these are very robust biocatalysts, useful for proof-of-concept applications. Moreover, these ubiquitous enzymes (belonging to hydrolases) are widely used with industrial purposes, due to their accessibility, no requirement of cofactors, high stability and accessible cost. Furthermore, they remain active in organic solvents (the so-called non-conventional media), accepting a broad substrate range, and often with high selectivity.[11,12] Likewise, lipases can catalyze different organic reactions, including hydrolysis, transesterifications, aminolysis, and they have even shown catalytic promiscuity, being even involved in some carbon-carbon bond formation processes.[11-13]

CPME has been employed as solvent in the lipase-catalyzed selective transesterification of racemic 6-methyl-5-hepten-2-ol (sulcatol, **1**) and racemic 2,2-dimethyl-1,3-dioxolane-4-methanol (solketal, **3**), with different enol ethers using *Pseudomonas cepacia* lipase (PSL). CPME and *di*isopropyl ether (DIPE) were assessed as solvents (Scheme 1).[14] For the kinetic resolution of sulcatol CPME did not result a proper solvent with none of the tested acyl donors, resulting DIPE a better choice, especially for those acyl donors with a more hydrophobic acyl chain. However, for solketal as substrate, enzymatic activities resulted higher in CPME. Subsequently, both solvents were employed in transesterifications with vinyl butyrate catalyzed by PSL co-lyophilized with different cyclodextrins, as these compounds have been successfully used

as additives to increase the activity and selectivity of lipases.[15] Again, CPME resulted a superior solvent, leading to higher enzymatic activities. The reaction temperature showed an important effect in the kinetic resolution of solketal with vinyl butyrate, using the lipase co-lyophilized with the cyclodextrin $Me_{1.78}$βCyD. Thus, the enzyme activity increased linearly with the temperature (10-60ºC), yet at the cost of decreasing the enantioselectivity. As a compromise, reactions at 30ºC displayed optimal values of activity and selectivity to develop an effective procedure for the biocatalytic preparation of optically active solketal.



Scheme 1. Kinetic resolution of racemic sulcatol (**1**) and solketal (**3**) catalyzed by PSL in CPME using cyclodextrins as additives.[14,15]

CPME has also been employed as solvent in the *Candida antarctica* lipase B (CalB) catalyzed hydrolysis of a racemic monoacylated binaphthol to obtain optically active BINOL.[16] Preliminary studies showed that the temperature was a critical parameter for performing the kinetic resolution of the starting materials in presence of *n*-butanol. No reaction was observed at 30ºC, whereas low reactivities were obtained at 60ºC. Thus, hydrolysis were carried out at 80ºC, being possible to achieve the formation of (*R*)-BINOL after 72 hours with 43% yield and 91% *ee* in a process with a good

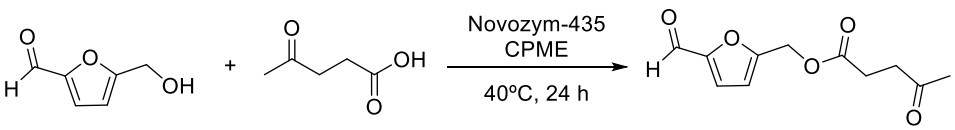
enantioselectivity. Further studies showed that toluene resulted a better solvent for this substrate and analogues, as excellent yields and higher selectivities were obtained.

The synthesis of the heart-rate reducing agent ivabradine (Procoralan®) has been recently through a chemoenzymatic protocol combining lipases and ω-transaminases.[17] The first step of this process consist in the kinetic resolution of the starting material, a racemic primary amine, employing different lipases through catalyzed acylation employing different acyl donors. The best results were achieved using the PSC-II and PS IM lipases in presence of diethyl carbonate in alkoxycarbonylation reactions, leading to the (*S*)-carbamate and the (*R*)-enantiomer of the remaining amine. Solvent optimization showed that, among others, the reactions could be developed in CPME, being obtained a 30% conversion after 24 hours with a good selectivity.

In an analogous area, the kinetic resolution of structurally different racemic alcohols such as (±)-menthol, (±)-sulcatol and (±)-α-cyclogeraniol, has been conducted through transesterifications with vinyl acetate and different lipases, comparing eco-friendly solvents, such as 2-MeTHF and CPME, with other *classical* solvents, such as toluene or *tert*-butyl methyl ether (MTBE).[18] The kinetic resolution of (±)-menthol showed the highest activity with *Candida rugosa* lipase (CRL) (yet with low selectivity), and CPME resulted a good solvent for such enzyme. Conversely, lipase AK from *Pseudomonas fluorescens* led to high selectivity, but with low activities and reaction rates, CPME leading to the highest enantioselectivity. Finally, CalB, CRL and lipase AK showed a high reaction rate in the transesterification of (±)-sulcatol. Remarkably, the use of CalB in both eco-friendly solvents led to a significant increase in the enantioselectivity when compared with toluene. Almost all lipases tested were very active in the kinetic resolution of a primary alcohol like (±)-α-cyclogeraniol. In all cases, moderate enantioselectivity was observed independently of the solvent employed with these

lipases. On the other hand, the effect of the lipase formulation for these kinetic resolutions was also studied; thus, after dissolving it at pH 8.0 and a subsequent lyophilization, lipase AK showed a higher activity in the acetylation of (±)-menthol in CPME. This effect was increased when the lyophilization was performed in the presence of additives such as MeOPEG or sucrose.[18] Therefore, the combination of eco-friendly solvents with an adequate biocatalyst design may enable powerful synergies for synthetic reactions.

With respect to other substrates, 5-hydroxymethylfurfural (HMF, **5**) is a biomass-based platform chemical that can be transformed in several high-added value compounds.[5,19] A promising HMF valorization option is esterification, as HMF esters can be employed with several purposes. The enzymatic esterification of HMF has been reported employing lipases as mild and environmentally-friendly catalysts.[20,21] In this area, the enzymatic esterification of HMF with levulinic acid (**6**) – another platform chemical[5] –, affords HMF levulinate ((5-formylfuran-2-yl)methyl 4-oxopentanoate, **7**), which can be used as fuel additive.[22] The reaction was firstly assessed with different lipases, resulting Novozym-435 (an immobilized CalB) the best candidate. Subsequent solvent optimization led to excellent conversions of >90% after 24 hours. Even shorter reaction times were achieved with 2-MeTHF (95% conversion after 12 hours). Thus, the use of eco-friendly solvents can be combined with biocatalyst to diminish the environmental impact that synthetic procedures may have.



5 **6** **7**, 92%

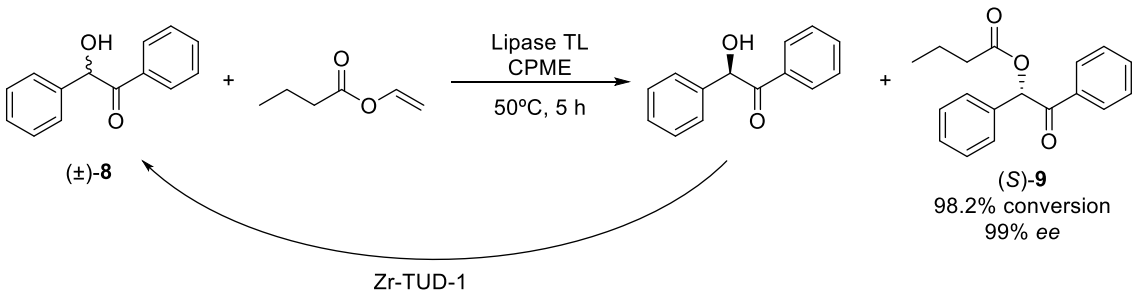
Scheme 2. Novozym-435-catalyzed esterification of HMF with levulinic acid in CPME.[22]

Other hydrolases have been studied in CPME. For example, phosphatidylserine (PS) is a phospholipid widely employed in the pharmaceutical and food industry. One of the most extended methods for its preparation requires the use of phospholipases D (PLD),[23] which catalyze the conversion of phosphatidylcholine into the desired compound in a trans-phosphatidylation procedure performed in a mixture aqueous buffer/organic solvent. Thus, ScPLD, a phospholipase D from *Streptomyces chromofuscus* overexpressed in *E. coli* (employed as lyophilized cells), has recently shown to display a high performance for the PS synthesis.[24] Some reaction co-solvents were tested, being observed an optimal transphosphatidylation conversion (87.6%) when using CPME as solvent in a biphasic system. The effect of modifying the buffer:CPME ratio from 1:1 to 1:5 showed that increasing the CPME to 1:3 led to a higher transphosphatilydation rate (96.8%; higher organic co-solvent contents did not improve the rate). The molar ratio between L-serine and phosphatidylcholine was also optimized, achieving high conversions at a molar ratio 5:1. Once optimized, the reaction was demonstrated in a 100-g scale; thus, the addition of 2.0 g of lyophilized cells of ScPLD per mol of phosphatidylcholine led to a PS concentration of 106.2 g/L (93.4% transphosphatilydation rate) after 2 hours, which corresponds to a space-time yield of 53.1 g/L h. Overall, this represents an excellent example on the potential of CPME for biocatalytic reactions, involving free enzymes as well as whole cell biocatalysts.

Notably, CPME has found applications for pickering emulsions (PEs) combined with biocatalysis as well. PEs are nanoparticle stabilized emulsions in which enzymes can be immobilized in water droplets stabilized by nanoparticles and surrounded by solvent molecules containing the substrates.[25] These systems can be applied when working with largely hydrophobic compounds. Recently, a continuous transesterification in PEs was developed using CPME as organic solvent.[26] Thus, 1-phenylethanol was

continuously subjected to transesterification with vinyl butyrate catalyzed by *Candida antarctica* lipase A (CalA)[27] with space time yields around 120 mg L^{-1} h^{-1}.[24] In this particular example, biocatalysis is combined with medium engineering and with eco-friendly solvents to provide synergies.

Likewise, CPME has been successfully employed as solvent in dynamic kinetic resolution processes (DKR) catalyzed by lipases.[28] Thus, the DKR of racemic benzoin (**7**) using lipase from *Pseudomonas stutzeri* (Lipase TL) and the chemo-catalyst Zr-TUD-1 has been recently described in different dry solvents.[29] An immobilized preparation of *Pseudomonas stutzeri* lipase (Lipase TL) in Accurel MP1001 catalyzes the selective acylation of benzoin (**8**) with vinyl butyrate yielding to the (*S*)-benzoin butyrate (*S*)-**9**, whereas the remaining (*R*)-benzoin is racemized in presence of the chemo-catalyst. Among the different solvents tested for the kinetic resolution of benzoin, dry CPME was able to dissolve 10 g/L of benzoin at room temperature and 20 g/L at 50 ºC, what is significant, given the challenging dissolution profile of benzoin. Remarkably, the activity of immobilized lipase TL in CPME resulted higher than in toluene, 2-MeTHF and 1,3-dioxolane. Moreover, the enzyme showed a high stability in CPME, as its half-time life was 1.5-fold higher that in toluene. The activity of Zr-TUD-1 in CPME was also high, racemizing (*R*)-benzoin after 10-12 hours. Once selected CPME as the proper solvent, the DKR was carried out in batch. After 5 hours at 50ºC, (*S*)-benzoin butyrate was obtained with a 98.2% conversion and 99% *ee*, a result slightly better than the one achieved in toluene. The DKR in dry CPME was also performed in a continuous way. The highest conversion (40%) was obtained after 2.5 hours, decreasing this value to 11% at 76 hours. The optical purity of (*S*)-**9** remained constant in 98% *ee* for the complete process.



Scheme 3. Dynamic kinetic resolution of racemic benzoin catalyzed by lipase TL and the chemo-catalyst Zr-TUD-1 employing CPME as solvent.[29]

Apart from hydrolases, other enzyme types, such as reductases, have shown promising results with CPME. These biocatalysts catalyze the transfer of protons and hydrides from/to the substrates by the mediation of cofactors.[11,30] CPME has been applied as (co)solvent in some biocatalytic reductions in which ketones, imines and activated carbon-carbon double bonds were selectively reduced to optically active alcohols and amines, respectively. Thus, ketoreductases (KREDs) catalyze the reversible transformation of carbonyl compounds into the corresponding alcohols, requiring the presence of nicotinamide cofactors [NAD(P)H] to perform their activity.[31]

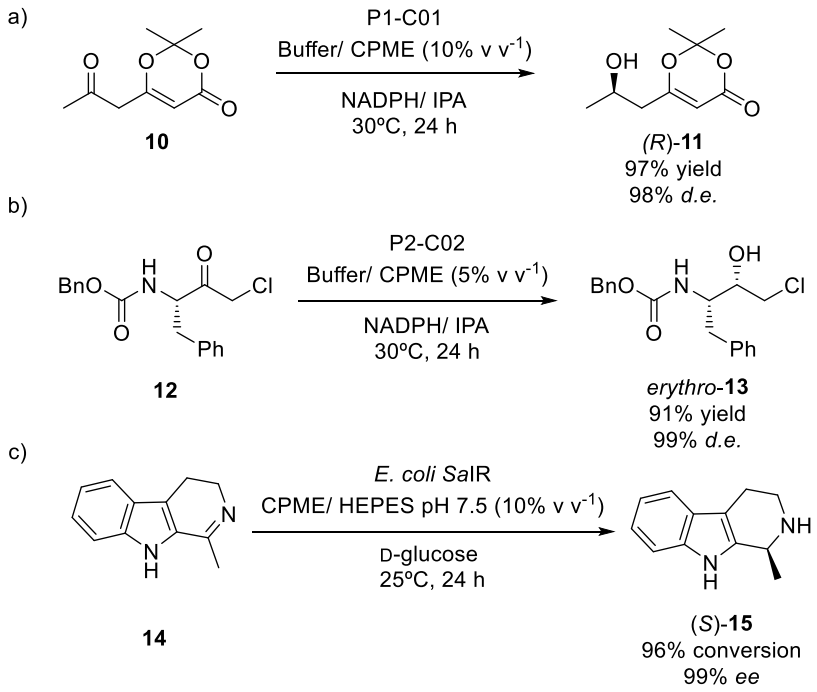
Optically active β-hydroxydioxinones are valuable building blocks in organic chemistry. One of the methods for their preparation was described in 2017, starting from the corresponding β-ketodioxinones and employing KREDs for their enantioselective reduction (Scheme 4a).[32] The use of a commercial set of engineered ketoreductases (Codex® KRED Screening Kit by Codexis) in the bioreduction of compound **10** led to the best results for the preparation of (*R*)-**11** when employing P01-H08 and P01-C01 in buffer containing NADPH and *i*-propanol for the nicotinamide cofactor regeneration. With these two enzymes, the final product was obtained with high yield (>90%) and 98% diastereomeric excess (*d.e.*) after 24 hours. When the bioreductions were developed in

presence of ethereal solvents as diisopropyl ether, 2MeTHF or CPME at 10% v/v concentration, the reaction yields were increased. For instance, (*R*)-**11** was recovered with 97% yield and 98% *d.e.* using CPME. These optimized conditions were extended to the bioreduction of other β-ketodioxinones, obtaining the final products with excellent yields (>90%) and selectivities (>90% dr) for almost all the substrate. A larger scale reduction of **10** was developed using a KRED P01-C01 loading of 0.5 wt% and a substrate concentration of 100 g/L. After 72 hours, 20 g of (*R*)-**11** were isolated with 99% yield and >98% *d.e.* after a simple extraction.

The synthesis of the HIV protease inhibitor Nelfanivir has been recently reported employing a chemo-enzymatic methodology. Herein, one of the key-steps is the bioreduction of a α-chloroketone (**12**) into the corresponding optically active chlorohydrin (*erythro*-**13**) employing the Codex® KRED Screening Kit, and using an excess of *i*-propanol for cofactor regeneration (Scheme 4b).[33] From all the enzymes tested, two of them, P1-A04 and P2-C02, were active, but the conversions were very low due to the poor solubility of the ketone in the reaction medium (aqueous buffer pH 7.0 containing *i*-propanol). To overcome this, organic co-solvents were studied at 5% v/v concentration. The use of CPME in the reaction catalyzed by P2-C02 led to excellent results, being possible to obtain the *erythro* clorohydrin with 99% *d.e.* and full conversion. The scale-up of the process at these conditions led to 91% yield of the optically pure compound after silica gel purification.

In the same area of oxidoreductases, imine reductases (IRED) are a novel type of oxidoreductases that catalyze the selective reduction of imines to yield optically active secondary or tertiary amines using NADPH as cofactor.[34] These enzymes have gained huge interest over the last years, as chiral amines are very valuable building blocks. The IRED from *Streptomyces aurantiacus* (*Sa*IR) expressed in *E. coli* cells was employed in

the selective reduction of harmane (**14**) and 1-methyl-3,4-dihydroisoquinoline to the corresponding amines in micro-aqueous medium, that is, in reaction medium containing very low water contents (Scheme 4c).[35] Imine reductions led to the highest conversions using HEPES buffer at pH 7.5 as part of this aqueous medium, being both CPME and methyl isobutyl ketone (MIBK) suitable solvents for the IRED. Herein, the reductions were conducted in the organic solvent with and a low content of HEPES buffer (5-15% v/v). CPME presents an advantage over MIBK, as the *E. coli* cells expressing the IRED remained well distributed at buffer contents up to 15%, whereas some cell clumping was observed in MIBK. Thus, this example shows that CPME can be employed as solvent or co-solvent of biocatalytic processes not only for purified enzymes, but also with whole cell systems. The bioreduction of harmane in CPME and HEPES buffer at pH 7.5 was very slow at low buffer proportions (5-7.5% v/v), significantly increasing at buffer contents of 10-15% v/v (still a non-conventional media of CPME in which buffer is used as co-solvent). At buffer amounts of 10% v/v the chiral (*S*)-amines were obtained with excellent optical purities (99% *ee*) and moderate-to-high conversions (96% for harmane and 48% for isoquinoline) after 24 hours at 25ºC using D-glucose as co-substrate for the NADPH regeneration.



Scheme 4. Bioreduction of β-ketodioxinones (a)[32] and Nelfanivir intermediate (b),[33] catalyzed by ketoreductases in presence CPME. (c) Use of imine reductases for the reduction of harmane in buffer containing CPME.[35]

Ene reductases have been widely employed as biocatalysts for the enantioselective reduction of activated C=C bonds.[36] Most of these biocatalysts are flavin-dependent proteins, belonging to the Old Yellow Enzyme family. While the addition of the hydride to the alkene comes from the flavin, it must be regenerated by using nicotinamide cofactors that have to be recycle, in general, using the system glucose dehydrogenase and glucose. In 2015, a library of ene reductases was tested in the selective bioreduction of different activated alkenes, analysing, among others, the effect of different organic solvents (20% v/v).[37] Some activity was observed when employing CPME for the ene reductases Gox-ER from *Gluconobacter oxydans* and NCR from *Zymomonas mobilis*, but the conversions were lower respecting the optimized conditions.

As emphasized in this section, the implementation of eco-friendly solvents in biocatalysis is becoming an important trend. Thus, researchers have also started to include

these solvents in screening programs, either to identify new enzymes, or to improve a certain process development step. This is becoming particularly evident for 2-MeTHF,[2] and CPME is following the same consideration as well. As an example, the groups of Ward and Hailes have recently screened a household drain metagenome to identify 29 novel transaminases with improved solvent tolerance, including CPME in the analysis.[38] As a future perspective, the combination of molecular biology tools with genetic design will pave the way for the future generation of robust biocatalysts, able to conduct industrial reactions in sustainable solvents such as CPME.

3.- CPME and its use in Biorefineries and in extractive strategies.

As stated above, biorefineries are considered to become the future processing (bio)chemical plants where chemicals and biofuels will be produced while valorizing wastes, lignocellulosic residues, and other forms of biomass.[5] Hence, biorefineries may produce bio-based solvents as well, either to be delivered to chemical plants, or to be used in-house for biorefining procedures. In this area, 2-MeTHF has found applications for biorefinery-like strategies, such as lignocellulosic pretreatment, furan production, or other subsequent valorization steps, typically setting up biphasic media for product *in situ* extraction.[39] In general, solvents are a necessary element for many steps in biorefineries.[40]

In this area, CPME has started to be assessed as well as an eco-friendly solvent with uses in biorefineries. One area of research is the production of furans, namely furfural from pentoses (mostly xylose),[41] and HMF from hexoses (mostly glucose).[17] Herein, many solvents have been screened for establishing biphasic systems.[42] CPME has been successfully used by several groups for furfural and HMF synthesis, using

different feedstock´s and with different catalysts. The formed furans are *in situ* extracted in the organic phase (Figure 2). In particular, CPME has been modelled for ternary mixtures of CPME-furfural-water in a broad range of temperatures, showing conditions for outstanding extractions of > 98% of furfural.[43]

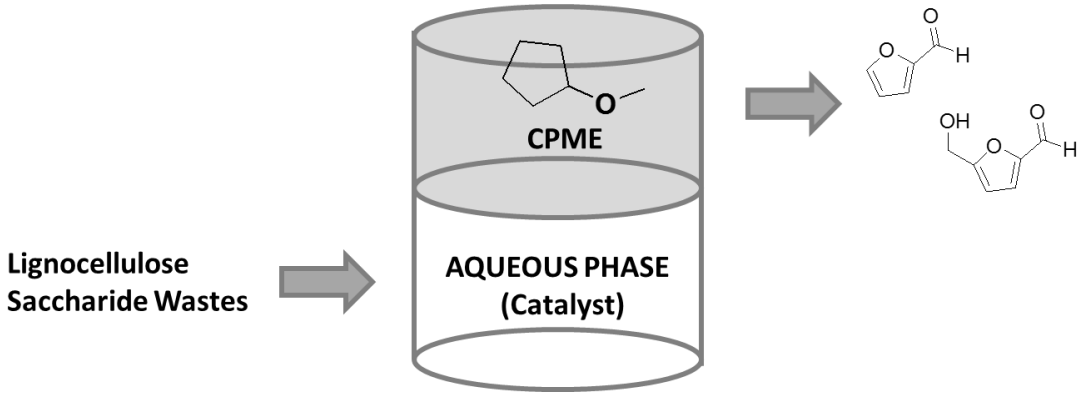


Figure 2. Conceptual approach for the synthesis of furan from lignocellulosic residues.

The López-Granados group reported the use of CPME as solvent for the dehydration of xylose with sulphuric acid as catalyst in a biphasic media.[44] To enhance the conversion in furfural, NaCl was added to the aqueous phase. Once the reaction was set with pure xylose, the concept was also successfully reported with real biomass from *Cynara cardunculus* (cardoon), with loadings of up to 4 wt% of lignocellulose.[44] Subsequently, the same group investigated the use of silica xerogel-poly(styrene sulphonic acid) nanocomposites as acid catalysts for the reaction, studying the best conditions in terms of temperature and polymer concentration. The catalyst could be reused several times.[45] In this area, the screening of (reusable) heterogeneous catalysts has become an important research line, to save costs associated to the process. Thus, nafion (a sulfonated tetrafluoroethylene based fluoropolymer-copolymer) was also

successfully studied as catalyst, again with NaCl addition and using xylose and different xylans.[46] In this case, the biphasic media was complemented with a microwave-assisted source of energy, at temperatures of 170-190 ºC. The optimal ratio water–CPME was 1:3 (v/v), affording furfural yields of up to 80 % at xylose loadings of 150 g/L. Nafion could be successfully reused up to four cycles.[46] Other groups have reported the use of a sulfonated swelling mesoporous polydivinylbenzene (PDVB-SO_3H) as catalyst, using *Camellia oleifera* shells as feedstock. Apart from CPME, also γ-valerolactone (another eco-friendly solvent) was assessed, together with DMSO and some ionic liquids.[47] Besides furfural, the synthesis of HMF has also been assessed in biphasic media containing CPME. Thus, SO_4^{2-}/SnO_2^- MMT solid catalysts were used to afford HMF (and also furfural) from corncob hydrolysates, using different biphasic systems, among them CPME.[48] Likewise, sulphuric acid has been used to synthetize both HMF and levoglucosenome {(1*S*,5*R*-6,8-Dioxabicyclo[3.2.1]oct-2-en-4-one)}.[49] The synergy between Lewis acids and Brønsted acids has also been successfully described for the synthesis of HMF, starting from corncob acid hydrolysis residues (as waste), and using $AlCl_3$ and HCl as catalytic system. Remarkably, HMF could be formed directly from glucose, instead of fructose. The aqueous phase could be reused, containing the catalysts for the performance.[50] Other analogous systems, e.g. 2-MeTHF-water media using $FeCl_3$ for furfural synthesis, have led to similar strategies and results.[39d]

Apart from the synthesis of furans, CPME has also been used as a solvent for the subsequent valorization of furfural and HMF, using different precious metal-free catalysts at different conditions. Thus, furfuryl alcohol was synthetized from furfural using a Copper-based heterogeneous catalyst (Cu/TiO_2) with microwave-assisted irradiation, leading to 100 % conversion of furfural in 3 hours, with an outstanding 99 % selectivity in furfuryl alcohol, at 125 ºC, with 10 bar of H_2. The catalyst could be successfully reused

for three times.[51] Likewise, a copper-zinc alloy nano-powder was used for the conversion of HMF into clear mixtures of 2,5-dimethylfuran and 2,5-dimethyltetrahydrofuran (fuel precursors) with yields of 97 % at 200-220 ºC with 20-30 bar of H_2.[52] Overall, these results show the potential of CPME as a solvent of choice for many applications in biorefineries.

Within biorefineries, another important line is the use of CPME for Liquid-Liquid Extractions (LLE). Herein, extractants are commonly dissolved in water-immiscible solvents to simultaneously control the concentration and reduce viscosity, while diluents should display high solubility for the extractant and conversely low solubility for water.[53] Ethereal solvents, due to its moderate polarity, represent a good choice for amphiphilic extractants. Nevertheless, archetypical ethers (Et_2O, THF or 1,4-dioxane), because of their low boiling points and relatively high water-solubility, are often not the desired options. Thus, CPME is an excellent alternative, being more hydrophobic (log P=1.59) and less soluble in water than the previously-mentioned ethers.[54]

CPME is particularly attractive for the extraction of different lipids from biomass,[55] which constitutes a promising option for the sustainable production of liquid biofuels. Breil *et al.*[56] compared the behaviour of CPME with other solvents in extracting lipids from oleaginous yeast. An experimental study (based on the oil extraction from *Yarrowia lipolytica* IFP29, using gas chromatography and high-performance thin-layer chromatography, HPTLC), was compared with two theoretical approaches: the first one took into consideration Hansen solubility parameters (HSPs),[57] which evaluates the interactions between solvents and the different components [free fatty acids (FFAs), monoglycerides (MAGs), diglycerides (DAGs), triglycerides (TGAs) and phospholipids (PLs)] present in the oil sample; and a second one, more precise, by using Conductor-like Screening Model for Realistic Solvation (COSMO-RS).[58] Aside from CPME, other

tested solvents were 2-MeTHF, *i*-propanol (IPA), ethanol (EtOH), ethyl acetate (EtOAc), ethyl lactate, dimethyl carbonate (DMC), *p*-cymene, *d*-limonene, α-pinene and hexane. The theoretical results using Hansen parameters (hydrogen bonding capability, van der Waals forces and dipolar interactions) showed that CPME was one of the best options for replacing hexane not only in the extraction of TAGs but also for DAGs and FFAs, whereas its performance for extracting PLs was less successful. On the other hand, COSMO-RS also reinforced the excellent efficiency of CPME, while experimental data indicated that solvents had no influence on the extraction yields, but rather on the distribution of lipid classes, confirming the theoretical data. Interestingly, these authors also studied other parameters (such as boiling point, LogP, toxicity category and energy required for solvent evaporation), crucial for the choice of an extraction solvent in industry, concluding that CPME, as well as 2-MeTHF and EtOAc, are the best bio-solvents options for replacing hexane.

Similarly, Probst *et al.*[59] evaluated CPME for extracting oil or TAGs from wet cells of the oleaginous yeast *Lipomyces starkeyi*, to assess the possibility of replacing hexane or choloroform in the conventional biphasic "Bligh and Dyer" (BD) method (chloroform:methanol:water). A monophasic system of CPME or a biphasic system of CPME:water (1:0.7 v/v) performed poorly (low TAG extraction efficiency and TAG selectivity) compared to other monophasic systems of hexane and chloroform and the biphasic BD method. Hereafter, biphasic systems of CPME:water:alcohol (methanol/ethanol/1-propanol) were tested, choosing methanol as the best option. Finally, these authors concluded that the highest TAG extraction efficiency (9.9 mg/mL) and TAG selectivity (64.6%) was obtained using a CPME:methanol:water starting ratio of 1:1.7:0.6 and a final ratio of 1:1:0.8. These results were analogous to those obtained with the classical BD method (TAG extraction efficiency, 10.2 mg/mL; TAG selectivity of

66.0%). Furthermore, the FFAs profile remained constant, confirming that the solvent choice was not specific for any certain fatty acid. Thus, CPME is an excellent alternative for replacing choloroform in such extractive strategies. In a similar study,[60] it was reported the assessment of different bio-solvents in the extraction of components from salmon fish oil (especially rich in long-chain Omega-3 polyunsaturated fatty acids, LCn-3PUFAs). Again, theoretical data from HSPs pointed towards the effectiveness of CPME in solvating all lipidic components of this oil, being more specific than the rest of solvents evaluated for each lipid class. Moreover, COSMO-RS data showed that CPME, EtOAc, and 2-MeTHF could solvate TAGs, DAGs, FFAs in the same extent, as well as ergosterol compounds. Experimental data (Soxhlet extraction, quantification by GC-FID) indicated that the amounts of fatty acids extracted by using each solvent were similar.

Microalgal biomass, due to its high lipid accumulation and growth rate, is a valuable renewable energy feedstock for biodiesel production. Mahmood *et al.*[61] described lipid extraction on two microalgal strains, *Chlorella vulgaris* and *Nannochloropsis sp*., *via* the Soxhlet method using various eco-friendly solvents (CPME, 2-MeTHF, EtOAc and ethyl lactate) stablishing a comparison with benchmark VOC solvent (hexane). These authors concluded that all the solvents tested displayed a higher extraction capacity when compared to hexane, with 2-MeTHF and ethyl lactate, respectively, increasing two-fold and three-fold the lipid extraction yield. Furthermore, all solvents were able to decrease the fraction of PUFAs extracted from the microalgal biomass, hence increasing the quality of the biodiesel for practical applications. Particularly, the use of CPME (as well as 2-MeTHF) allowed the extraction of a similar fraction of saponifiable lipids for *Chlorella vulgaris* compared to hexane. Very recently, it has been reported that CPME is not only a more sustainable alternative (compared to hexane) in LLE´s for biodiesel purification through acid-catalyzed *in situ*

transesterification of microalga *Chlorella pyrenoidosa* dry biomass,[62] but also useful as solvent in column chromatography for biodiesel purification (purity level higher than 96.5%). Although the biodiesel purified with eco-friendly solvents showed higher densities and viscosities than those of the obtained with hexane, it is compatible with the European and North American quality standards. Thus, authors conclude that the higher price of CPME and other bio-solvents compared to petroleum-derived solvents is the only factor limiting their large-scale use. Further optimization of the concepts, together with the implementation of biogenic routes for producing CPME, may create novel sustainable approaches for many areas.

With respect to extractions, Yada-Varón *et al.*[63] applied the previously-mentioned dual approach (theoretical using both HSPs and COSMO-RS, and experimental quantified by HPLC and UV-spectroscopy) to evaluate the capability of five eco-friendly solvents (CPME, 2-MeTHF, IPA, EtOAc and DMC) as hexane-substitutes for the extraction of carotenoids from carrots. Based on the HSPs analysis, non-polar or slightly polar solvents were the most suitable solvents for extraction of carotenoids, while COSMO-RS analysis showed a higher probability of solubility for all the carotenoids from carrot in CPME, 2-MeTHF and ethyl acetate compared with hexane. The experimental results, using a conventional solid-liquid extraction by maceration, confirmed that the best alternative solvents were CPME, 2-MeTHF and ethyl acetate, consistent with the predictive results from COSMO-RS. More specifically, the highest carotenoid content (78.4 mg per 100 g of dry vegetable matter, 66% β-carotene, 34% α-carotene) was extracted using CPME. In some other cases, CPME is described as a good choice for LLE of interesting compounds from bio-oil/water mixtures. It must be noted, however, that its high price is still a serious drawback, as described in a patent from KiOR,

Inc. (USA) reporting the extraction from a biomass obtained by thermo-catalytic pyrolysis of southern yellow pine wood chips.[64]

Apart from LLE of lipidic components of oils, CPME has also proven its utility in the extraction of membrane proteins such as FhuA (ferric hydroxamate uptake protein component A),[65] one of the largest β-barrel membrane proteins in *Escherichia coli*. These proteins are valuable hosts for hybrid catalysts in which reactions are controlled through space,[66] although its production and extraction in gram scale is challenging due to their hydrophobicity. Most of the reported FhuA extraction protocols involve the use of several detergents, which interact with the FhuA protein and solubilize it in mixed lipid-protein-detergent micelles. This generally leads to low yields and not very high purities.[67] In some other cases, mixtures of chloroform/methanol have been employed for this particular extraction,[68] although the use of this biphasic system requires a very precise selection of solvents ratio to ensure the extraction of only the target protein from other non-membrane proteins. To improve FhuA extraction, Tenne *et al.*[65] reported the use of 2-MeTHF or CPME in a 4-steps purification procedure, involving: (a) cell disruption; (b) extraction of impurities with *n*-octyl-poly-oxyethylene (oPOE); (c) 2-MeTHF or CPME membrane dissolution leading to FhuA precipitation, and (d) renaturation employing urea and polyethylene-polyethyleneglycol (PE-PEG). The eco-friendly solvents acting on stage (c) promote the dissolution of lipidic membranes, therefore causing the efficient aggregation and precipitation of embedded membrane proteins, which need to be re-solubilized and re-folded in the final step. Thus, the final enzymatic solution contains up to 95% of protein (70 mg/L fermenter broth), fully functional as confirmed by CD data and a translocation functionality assay.

Another research area in which CPME has been successfully used is the extraction of base metal ions. Oshima *et al.*[69] reported the extraction of transition metal ions by

using di-2-(ethyl-2-hexyl)phosphoric acid (D2EHPA, one of the most frequently used acidic extractants) diluted in CPME. An extraction order in CPME (quantified by atomic absorption) of Fe(III) > Zn(II) > Cu(II) ≈ Mn(II) ≈Ca(II) >Co(II) ≈ Mg(II) ≈Ni(II), was comparable to the previously reported study using kerosene.[70] Selectively, Cu(II) extraction was studied, confirming the formation of a 2:1 complex between dimeric D2EHPA and Cu(II) in CPME, measuring also rate constants. Besides, the implication of the ethereal oxygen of CPME in the octahedral coordination of Ni(II) was postulated. These same group also stated the efficiency of CPME for the extraction of Au(III) from hydrochloric acid media in a selective manner from other precious metal ions and base metal ions,[71] compared to the traditionally used long-chair alkanols. In fact, although *n*-hexanol, cyclohexanol or *n*-dodecanol are effective for extracting Au(III), his relatively higher solubility in water precludes its appropriate use. In this sense, CPME is very effective, quantitatively extracting Au(III) from 5.0 M HCl, thus allowing also the extraction of the $AuCl_4^-$ anion, in acidic solutions containing other metals, such as Pd(II), Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Pb(II), In(III), La(III), Rh(III) or Pt(IV). Application in mining technologies might be of interest as well for future research involving eco-friendly solvents.

Finally, CPME was evaluated in the LLE from aqueous samples of degradation products and precursors of some chemical weapons (mustards and V-agents), by using a derivatization and extraction technique named dispersive derivatization liquid–liquid extraction (DDLLE), which speeds up the analysis process by removing the requirement for drying of the sample.[72] In this technique, the derivatization process takes place at the interface between the analyte-containing aqueous phase and the organic phase where the derivatization agent, 1-(heptafluorobutyryl)imidazole in this particular case, is dispersed,

so that the total surface area is increased. In this case, CPME did not perform better than other classical solvents such as dichloromethane.

4.- Other emerging applications of CPME in Bioeconomy: Chromatography, solid-phase peptide synthesis.

There is a growing interest in the development and application of the Green Chemistry principles in analytical chemistry, to implement analytical methods that may prevent or minimize the generation of hazardous wastes, employ renewable reagents and solvents, and demand lower energy input.[73] In this context, the substitution of classic petroleum-derived organic solvents, widely used in great quantities as mobile phases, by greener alternatives is increasingly been postulated. Unfortunately, the use of CPME as alternative solvent in chromatography is still hampered by its high price (around 180 €/L) and his commercial unavailability in HPLC grade. Anyhow, many theoretical studies are published about CPME performance in binary, ternary or even quaternary mixtures with other solvents,[74] so its behaviour is well-known. Furthermore, in a recent paper, Tobiszewski *et al*.[75] have reported the result of the application of several chemometric tools for describing the physicochemical parameters of multiple solvents (CPME included) and predicting those missing variables (e.g. bioconcentration factors, water-octanol and octanol-air partitioning constants) not easily available. For such purpose, Estimation Programs Interface (EPI) Suite software was successfully applied to predict missing values for solvents commonly considered as "green", so that the theoretical data for such kind of solvents properties may be accessible for theoretical considerations.

Hence, CPME has been applied as an alternative to chloroform in lipid classes separation of non-polar cholesteryl ester from highly polar phospholipids by high-

performance liquid chromatography on bare silica stationary phase and evaporative light-scattering detection.[76] In this normal phase liquid chromatography, Prache *et al.* describe how the more apolar component of the mobile phase (*n*-heptane) can be advantageously replaced by hexamethyldisiloxane (HMDS), while polar chloroform might be replaced by 2-MeTHF, isopentyl acetate or CPME. Binary mixtures of HMDS and alternative solvents provided an altered elution order of lipid classes, as sterols are eluted before fatty acids with *n*-heptane-chloroform gradient, while they appear after fatty acids with gradients using alternative solvents. Furthermore, also the adequate performance of ternary mixtures of solvent (HDMS/CPME/ethanol:water) is described. In another example, CPME has been used as solvent for ultra-high performance supercritical fluid chromatography (UHPSFC) under generic gradient conditions.[77] Accordingly, 11 probe analytes (either acid, basic or neutral common drugs) were tested (UV detector) using this technique, to determine the possibility of injecting large sample volumes (up to 10 µL) in UHPSFC for maximizing UHPSFC sensitivity, without compromising peak integrity. Several aprotic solvents such as MTBE, DCM, acetonitrile (MeCN) or CPME were well adapted for the injection of high volume in UHPSFC. More specifically, MeCN and CPME displayed the additional advantages of a lower volatility, which could be important in the case of quantitative analysis.

Furthermore, CPME has been also used as a polar modifier for the supercritical fluid chromatographic separation of enantiomers on immobilized chiral stationary phases.[78] Thus, resolution of a group of nine commercially available racemates was tested using CO_2-based eluents containing the polar modifier (CPME and others), for determining the effect on increasing/decreasing retention times and on stability/solubility of analytes. More specifically, these authors found that the use of an equimolecular mixture of CPME with methanol afforded increased retention and resolution.

Finally, Amarouche *et al.*[79] described the application of ternary biphasic system composed of CPME/DMF/water (49:40:11, v/v) for the purification of a lipophilic, protected octapeptide, key intermediate in the preparation of bivalirudin (Angiomax®), a reversible direct thrombin inhibitor. For the purification of this octamer (H-Glu(OBzl)-Glu(OBzl)-Ile-Pro-Glu(OBzl)-Glu(OBzl)-Tyr(Bzl)-Leu-OBz, in which all ionic groups except the *N*-terminal end of the peptide are protected by a benzyl group), the technique of displacement mode is used; this technique, used in centrifugal partition chromatography (CPC)[80] and counter-current chromatography (CCC),[81] implies dissolving an eluter – also named displacer –, in the mobile phase and a retainer or an ion-exchanger in the stationary phase. When an acid or a base is added to the stationary phase as a retainer, this technique is known as pH-zone refining mode.[82] By using this procedure in CPC, the purification of the octapeptide was achieved with a purity of about 99.04% and a recovery of 94%, in the descending pH-zone refining mode with triethylamine (28 mM) as retainer and methanesulfonic acid (18 mM) as eluter. Thus, CPME could efficiently substitute less environment-friendly MTBE in the ternary biphasic system.

Another area of use of CPME is peptide synthesis. Peptides are essential compounds in the pharmaceutical industry, either for the preparation of final drugs or peptidomimetic or non-peptide pharmaceuticals.[83] As they are highly specific in the interaction with their *in vivo* targets, at the same time displaying scarce negative side effects, their role in the future of drug discovery is foreseen to be even higher.[84] In fact, as pointed out in a recent paper from Lawreson *et al.*,[85] presently there are more than 60 peptide-based drugs approved for use by the US Food & Drug Administration, around 140 peptide drugs currently in clinical trials and over 500 in preclinical trials. Going into

economic terms, overall peptidic drugs market in 2015 was estimated around US$ 14.1 billion, and was predicted to grow up to around US$ 25.4 billion by 2018.[83b]

Unfortunately, traditional peptide synthesis cannot be regarded as sustainable, since many auxiliary reagents are required as protecting or activating groups, and generally toxic solvents such as DMF or DMF/DCM mixtures are used. This scenario becomes more challenging when considering solid-phase peptide synthesis (SPSS), which requires hefty amounts of solvents for multiple washing of resins between procedures to remove excess reagents and by-products.[85,86] Thus, reprotoxic polar aprotic solvents, dimethylformamide (DMF), dimethylacetamide (DMA) and *N*-methyl pyrrolidone (NMP) are commonly used, although all of them are included amongst the Substances of Very High Concern (SVHC) under Registration, Evaluation, Authorization and restriction of Chemicals (REACH), indicating that their use will be restricted in near future.[86] Needless to say, DCM and Et_2O used for synthetic work-ups are also problematic.

Therefore, the replacement of the above-mentioned solvents by more sustainable alternatives is one of the areas in which Green Chemistry is being applied for peptide synthesis. A pioneering paper by Albericio *et al*.[87] reported the use of MeCN as a replacement for DMF in solid-phase peptide synthesis (SPPS). Albeit acetonitrile cannot be properly considered an eco-friendly solvent, it appears less problematic when compared to DMF, due to its lower viscosity and boiling point. Since that work, CPME has commonly been included into the group of solvents tested as potential environmentally-friendly alternatives. In 2013 it was reported a very detailed study in which they tested the performance of CPME and some other organic solvents (TBME, DCM, CH_2Cl_2, DMF, EtOAc, IPA and 2-MeTHF),[88] in the synthesis of four model amides: i) 4-methyl-*N*-phenylbenzamide (**16**, aryl acid coupled to a aryl amine, reaction

#1); ii) *N*-(4-methoxybenzyl)-4-methylbenzamide (**17**, aryl acid-alkyl amine, reaction #2); iii) *N*-2-diphenylacetamide (**18**, alkyl acid-aryl amine, reaction #3), and iv) *N*-(4-methoxybenzyl)-2-phenylacetamide (**19**, alkyl acid-alkyl amine, reaction #4), as shown in Figure 3.

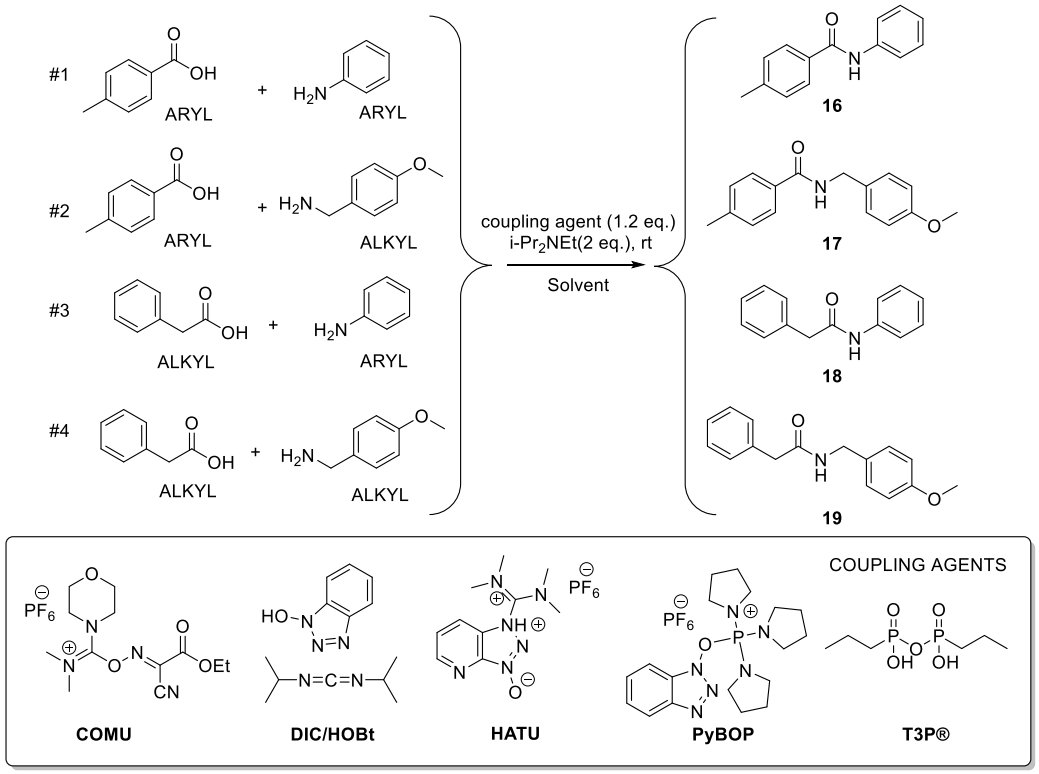


Figure 3. Assessment of organic solvent in the synthesis of model amides (**16**-**19**) using different coupling agents.[88]

The coupling agents tested were: (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (**COMU**), *N*,*N*′-diisopropylcarbodiimide/hydroxybenzotriazole (**DIC/HOBt**), *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methyl-methanaminium hexafluorophosphate *N*-oxide (**HATU**), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (**PyBOP**) and *n*-

propylphosphonic anhydride (**T3P®**). These authors reported that CPME was a good choice for the more reactive alkyl–alkyl coupling (reaction 4), as well as for reaction #1 using **HATU**, **PyBOP**, and **T3P®**, and reaction #2 using **T3P®**, leading to complete conversion for reaction times between 1-4 hours.

On the other hand, Jad *et al.*[89] reported the comparative behaviour of CPME and 2-MeTHF in peptide synthesis (Scheme 5), analyzing their ability to dissolve both amino acid derivatives and coupling reagents, as well as the swelling capacity of different resins (polystyrene, PS, and polyethylene glycol, PEG) in solid-phase synthesis. Moreover, coupling efficiency and the possibility of un-desired racemization were also evaluated. The observed behaviour was as follows:

i. Amino acids solubility was checked using Fmoc-Gly-OH as a representative control, subsequently expanding the analysis to other compounds such as Fmoc-Asn(Trt)-OH, Fmoc-Phe-OH, Fmoc-Val-OH, Fmoc-Tyr(*t*Bu)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Trp(Boc)-OH. While all of them were soluble in 2-MeTHF in concentrations up to 0.5 M, CPME was useful only for Fmoc-Trp(Boc)-OH (up to 1.4 M) and Fmoc-Val-OH (0.2 M). For the other Fmoc-protected amino acids, solubility was below 0.03 M.

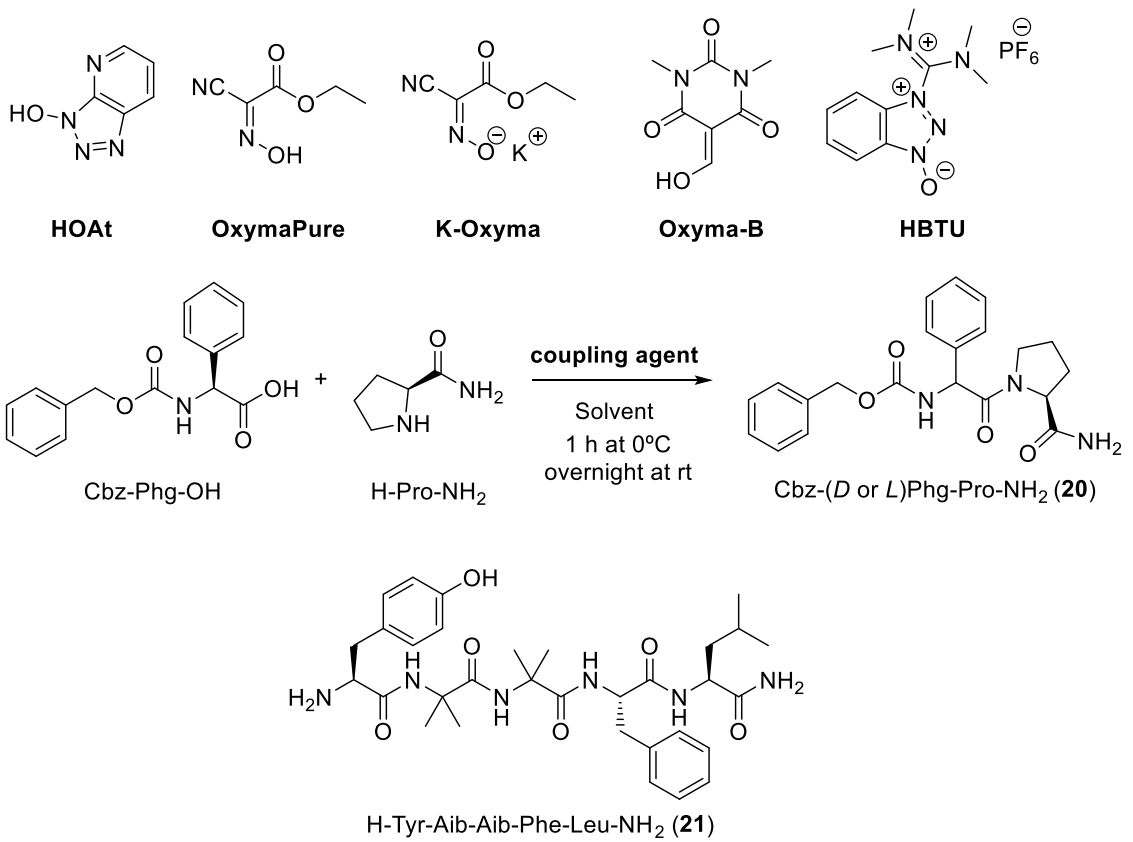
ii. Regarding the coupling agents, apart from HOBt, HATU and COMU (shown in Figure 3), some other were tested: 3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-ol (1-Hydroxy-7-azabenzotriazole, **HOAt**), ethyl (Z)-2-cyano-2-(hydroxyimino)acetate (**OxymaPure**), its potassium salt (**K-Oxyma**), 5-(hydroxymethylene)-1,3-dimethylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**Oxyma-B**) and 3-[Bis(dimethylamino)methyliumyl]-3*H*-benzotriazol-1-oxide hexafluorophosphate (Hexafluorophosphate Benzotriazole Tetramethyl Uronium,

HBTU). The higher solubility in CPME was found for **OxymaPure** (1.1 M), while for the other linking reagents solubility was much smaller.

iii. The swelling of PS resin in both CPME and 2-MeTHF was better than in MeCN, similar to DMF but lower than in DCM and THF. Furthermore, they swelled the PEG resin with lesser efficiencies than all of the other solvents but with enough capacity to warrant further investigation

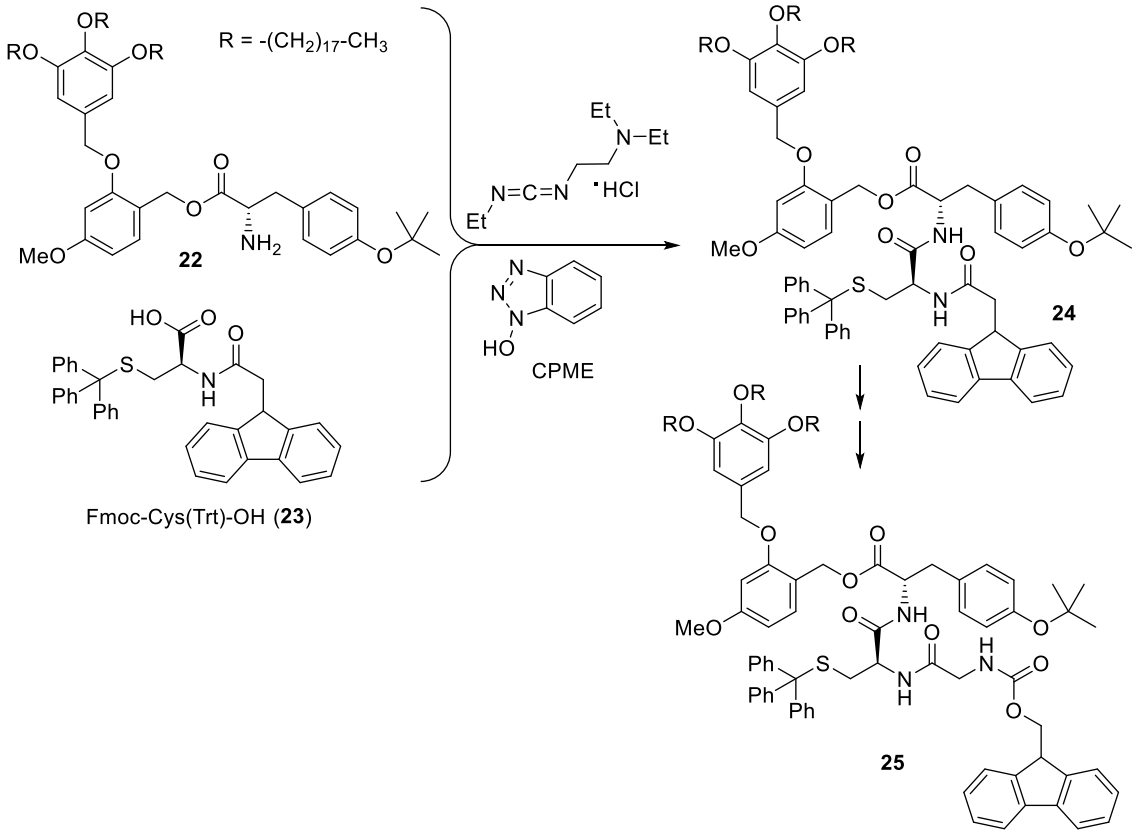
iv. The solution-phase synthesis of dipeptide Z-Phg-Pro-NH_2 (**20**, shown in Scheme 5) was selected for evaluating an eventual racemization, due to the high sensitivity of the α-phenyl moiety of phenyl-glycine (Phg). CPME yielded decent results with DIC/**HOBt** and DIC/**OxymaPure** (conversion higher than 90%, and reduced racemization), although 2-MeTHF performed generally better with most coupling agents.

v. The efficiency of these solvents for solid-phase synthesis of a complex peptide (Aib-enkephalin pentapeptide: H-Tyr-Aib-Aib-Phe-Leu-NH_2, **21**) was evaluated using standard Fmoc strategy. Thus, Fmoc-RinkAmide-AM-PS or H-Rink-Amide-AM-ChemMatrix resins were used as solid supports, and different coupling agents were also assessed. CPME led to less pure products compared to 2-MeTHF, DMF or THF, but for DIC/**HOBt**, **HOAt** and **OxymaPure**, CPME rendered the pentapeptide in higher purity versus classical and toxic DMF.



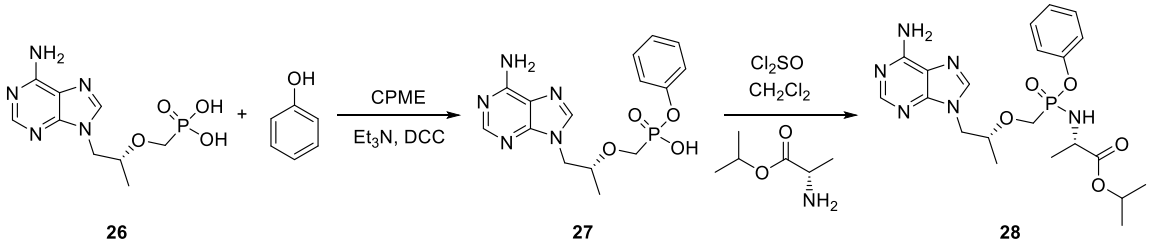
Scheme 5. Use of CPME as solvent in the peptide synthesis developed by Jad *et al.*[89]

In analogous area, the patent from Ajinomoto Co. Inc. described the use of CPME in the synthesis of several dipeptides by reacting Fmoc-protected amino acids with an α-amino ester **22** previously synthesized by carbodiimide coupling with a conveniently substituted benzylic alcohol (derived from gallic acid) and Fmoc-Tyr(Trt)-OH.[90] This is exemplified in Figure 5 for Fmoc-Tyr(Trt)-OH **23**; in all cases, CPME is used combined with EDC/HOBt for activating the carboxylic moiety of **23**, leading to excellent yields (**24**, > 90%). After basic deprotection of the Fmoc, an ulterior coupling with another FMoc-protected amino acid (FMoc-Gly-OH in Scheme 6, and also FMoc-Ala-OH or FMoc-Pro-OH) would lead to a *N*-protected tripeptide, such as **25**, also with excellent yields.



Scheme 6. Synthesis of dipeptide **24** and tripeptide **25** employing CPME as solvent.[90]

Another example on the use of CPME was reported in an Indian patent from Laurus Labs Private Ltd.,[91] as shown in Scheme 7. CPME is used in the first step of the synthesis of tenofovir alafenamide (**28**, Vemlidy™, Gilead), a nucleotide reverse transcriptase inhibitor and a prodrug of tenofovir. This antiviral compound does not contain a carboxylic amide (-CO-NH-) but a phosphinic amide, and was prepared in two-steps starting from the correspondent phosphonic acid **26**, *via* cardodiimide coupling with phenol in CPME to produce the intermediate phosphonate **27** and subsequent formation of **28**.



Scheme 7. Synthesis of tenofovir alafenamide (**28**) in CPME as solvent.[91]

Finally, CPME has been also used in peptide synthesis, not as the reaction medium for the coupling reaction, but rather in purification steps. For instance, it was described the effectiveness of CPME (as Et_2O or MTBE surrogate) in the precipitation of different peptides at the end of a peptidic synthesis.[92] In fact, in solid-phase peptide synthesis, using Fmoc and Boc strategies, the final step requires an acidic treatment of the peptidyl resin, for the removal of the protecting groups and the subsequent release the peptide from the resin.[93] This step, usually termed "global deprotection", entails the use of scavengers, in order to trap any reactive carbocation derived from the protecting groups. In the classic workup, it implies the addition of cold Et_2O to precipitate the peptide out while maintaining non-volatile scavengers and any other non-polar by-products in solution. MTBE can be also used to this same purpose, but some undesired *tert*-butylation of the peptidic chain can be produced because of the acidic decomposition of this ether. Then, these authors checked the usefulness of CPME for the precipitation of five peptides possessing different number of amino acidic residues (from 5 to 28), and concluded that, except for a short pentapeptide (Leu-enkephalin), which was not precipitated by CPME (rather maintaining the pentamer in solution), in the rest of the cases the precipitation obtained with CPME was similar to that one induced by Et_2O, in terms of recovery percentage and purity. Furthermore, LC-MS analysis confirmed the absence of any alkylation by-product, confirming that CPME is stable in trifluoroacetic acid for 8 hours at room temperature.

4.- Summary and Outlook

The need of using eco-friendly solvents in many chemical segments is nowadays necessary to reach sustainability targets. In this respect, CPME appears to be an excellent candidate, due to its properties and potential applications, as described in this review and

elsewhere.[3,4] Albeit CPME is currently synthetized *via* petrochemical routes (with high atom economy), some bio-based alternatives have become available, paving the way for a future biogenic source of the solvent. Herein, not only raw materials, but synthetic procedures must be sustainable, leading to minimized waste production and energy consumption. Combining the outstanding options of CPME with a biomass-derived origin (provided that a competitive price can be reached) would be certainly relevant for Sustainable Chemistry. In this review, applications of CPME have covered its use in biotransformations, in biorefineries, in extractive alternatives (*e.g*. Liquid-Liquid extractions), peptide synthesis, etc. Other applications of CPME have been recently reviewed as well.[4] Overall, there is a very broad diversity of topics and areas in which CPME may successfully replace other hazardous solvents.

Acknowledgments

Gonzalo de Gonzalo thanks MINECO (Ramón y Cajal Program) for personal funding.

References.

[1] a) C. J. Clarke, W. C. Tu, O. Levers, A. Bröhl, J. P. Hallett, *Chem. Rev*., **2018**, *118*, 747-800; b) F. G. Calvo-Flores, M. J. Monteagudo-Arrebola, J. A. Dobado, J. Isac-García, *Top Curr. Chem*., **2018**, 36:18; c) N. Guajardo, C. Müller, R. Schrebler, C. Carlesi, P. Domínguez de María, *ChemCatChem* **2016**, *8*, 1020-1027; d) C. Jiménez-González, P. Poechlauer, Q. B. Broxterman, B.-S. Yang, D. am Ende, J. Baird, C. Bertsch, R.E. Hannah, P. Dell´Orco, H. Noorman, S. Yee, R. Reintjens, A. Wells, V. Massonneau, J. Manley, *Org. Proc. Res. Dev*. **2011**, *15*, 900-911.

[2] a) A. R. Alcántara, P. Domínguez de María, *Curr. Green Chem.* **2018**, *5*, 85-102; b) V. Pace, P. Hoyos, L. Castoldi, P. Domínguez de María, A. R. Alcántara, *ChemSusChem* **2012**, *5*, 1369-1379.

[3] a) K. Watanabe, *Molecules* **2013**, *18*, 3183-3194; b) V. Antonucci, J. Coleman, J.B. Ferry, N. Johnson, M. Mathe, J.P. Scott, J. Xu, *Org. Process Res. Dev*. **2011**, *15*, 939-941; c) K. Watanabe, N. Yamagiwa, Y. Torisawa, *Org. Process Res. Dev*. **2007**, *11*, 251-258.

[4] U. Azzena, M. Carraro, L. Pisano, S. Monticelli, R. Bartolotta, V. Pace, *ChemSusChem* **2018**, *12*, 40-70.

[5] P. Domínguez de María (Ed.:). *Industrial Biorenewables – a practical viewpoint*, John Wiley & Sons, Hoboken, **2016**.

[6] Selected articles on the synthesis of cyclopentanone and cyclopentanol from furfural: a) N. Pino, G. Hincapié, D. López, *Energy & Fuels* **2018**, *32*, 561-573; b) M. Dahmen, W. Marquardt, *Energy & Fuels* **2017**, *31*, 4096-4121; c) G. S. Zhang, M. M. Zhu, Q. Zhang, Y. M. Liu, H. Y. He, Y. Cao, *Green Chem.* **2016**, *18*, 2155-2164; d) J. Guo, G. Xu, Z. Han, Y. Zhang, Y. Fu, Q. Guo, *ACS Sust. Chem. Eng*. **2014**, *2*, 2259-2266; e) M. Hronec, K. Fulajtarová, M. Micusik, *Appl. Catal. A: Gen*. **2013**, *468*, 426-431; f) M. Hronec, K. Fulajtarová, *Catal. Commun*. **2012**, *24*, 100-104; g) M. Hronec, K. Fulajtarová, T. Liptaj, *Appl. Catal. A: Gen*. **2012**, *437-438*, 104-111; h) M. Minai, US4970345, **1990**

[7] a) Recent review on Piancatelli rearrangement applications: C. Piutti, F. Quartieri, *Molecules* **2013**, *18*, 12290-12312; Original work on the Piancatelli rearrangement: b) G. Piancantelli, A. Scettri, *Tetrahedron Lett*. **1976**, *17*, 3555-3558; c) G. Piancantelli, A. Scettri, *Tetrahedron* **1977**, *33*, 69-72.

[8] a) P. Sudarsanam, L. Katta, G. Thrimurthulu, B.M. Reddy, *J. Ind. Eng. Chem*. **2013**, 19, 1517-1524; b) J. Vojtko, *Pet. Coal* **2005**, *47*, 1-4; c) M. Renz, A. Corma, *Eur. J. Org. Chem*. **2004**, *9*, 2036-2039; d) M. Decker, H. Wache, W. Franzischka, US4895985. **1990**.

[9] a) K. Raj, S. Partow, K. Correia, A.N. Khusnutdinova, A.F. Yanukin, R. Mahadevan, *Metab. Eng. Commun*. **2018**, *6*, 28-32; b) T. Beardslee, S. Picataggio, *Lipid Tech*. **2012**, *24*, 223-225.

[10] A. Corona, M. J. Biddy, D. R. Vardon, M. Birkved, M. Z. Hauschild, G. T. Beckham, *Green Chem*. **2018**, *20*, 3857-3866.

[11] a) R. A. Sheldon, J. M. Woodley, *Chem. Rev*. **2018**, *118*, 801-838; b) G. de Gonzalo, P. Domínguez de María (Eds.:), *Biocatalysis: an Industrial Perspective*, Royal Society of Chemistry, Oxford, **2017**; b) K. Faber, W.-D. Fessner, N. J. Turner (Eds.:), *Science of Synthesis: Biocatalysis in Organic Synthesis*, Thieme Verlag, Stuttgart, **2015**; c) K. Drauz, H. Gröger, O. May (Eds.:), *Enzyme Catalysis in Organic Synthesis*, 3rd Ed., Wiley-VCH, Weinheim, **2012**

[12] a) E. Busto, V. Gotor-Fernández, V. Gotor, *Chem. Rev*. **2011**, *111*, 3998-4035; b) A. Ghanem, *Tetrahedron* **2007**, *63*, 1721-1754.

[13] M. López-Iglesias, V. Gotor-Fernández. *Chem. Rec*. **2015**, *4*, 743-759.

[14] Y. Mine, L. Zhang, K. Fukunaga, Y. Sugimura, *Biotechnol. Lett*. **2005**, *27*, 383-387.

[15] See for instance: a) L. Bungaruang, A. Gutmann, B. Nidetzky, *Adv. Synth. Catal*. **2016**, *358*, 486-493; b) Y. Mine, K. Fukunaga, M. Yoshimoto, K. Nakao, Y. Sugimura, *J. Biosci. Bioeng*. **2003**, *95*, 441-447.

[16] N. Aoyagi, N. Ogawa, T. Izumi,. *Tetrahedron: Lett*. **2006**, *47*, 4797-4801.

[17] S. Pedragosa-Moreau, A. Le Flohic, V. Thienpondt, F. Lefoulon, A.-M. Petit, N. Ríos-Lombardía, F. Morís, J. González-Sabín, *Adv. Synth. Catal*. **2017**, *359*, 485-493.

[18] A. Belafriekh, F. Secundo, S. Serra, Z. Djeghaba, *Tetrahedron: Asymmetry*, **2017**, *28*, 473-478.

[19] R.-J. van Putten, J. C. van der Waal, E. de Jong, C. Rasrendra, H. J. Heeres, J. G. de Vries, *Chem. Rev*. **2013**, *113*, 1499-1597.

[20] M. Krystof, M. Pérez-Sánchez, P. Domínguez de María, *ChemSusChem* **2013**, *6*, 630-634.

[21] P. Domínguez de María, N. Guajardo, *ChemSusChem* **2017**, *10*, 4123-4134.

[22] Y.-Z. Qin, M.-H. Zong, W.-Y. Lou, N. Li, *ACS Sustain. Chem. Eng*. **2016**, *4*, 4050-4054.

[23] J. Damnjanovic, Y. Iwasaki, *J Biosci. Bioeng*. **2013**, *116*, 271-280.

[24] W. Qin, C. Wu, W. Song, X. Chen, J. Liu, Q. Luo, L. Liu, *Process Biochem*. **2018**, *66*, 146-149.

[25] M. Pera-Titus, L. Leclercq, J.-M. Clacens, F. DeCampo, V. Nardello-Rataj, *Angew. Chem*. **2015**, *127*, 2028-2044; *Angew. Chem. Int. Ed*. **2015**, *54*, 2006-2021.

[26] A. Heyse, C. Plikat, M. Grün, S. Delaval, M. Ansorge-Schumacher, *Process Biochem*. **2018**, *72*, 86-95.

[27] For a review on the biocatalytic applications of CAL-A see: P. Domínguez de María, C. Carboni-Oerlemans, B. Tuin, G. Bargeman, A. van der Meer, R. van Gemert, *J. Mol. Cat. B: Enzym*. **2005**, *37*, 36-46.

[28] For selected reviews on DKRs, see: a) P. Hoyos, V. Pace, A. R. Alcántara, *Adv. Synth. Catal*. **2012**, *354*, 2585-2611; b) J. H. Lee, K. Han, M.-J. Kim, J. Park, *Eur. J. Org. Chem*. **2010**, 999-1015.

[29] A. Petrenz, P. Domínguez de María, A. Ramanathan, U. Hanefeld, M. B. Ansorge-Schumacher, S. Kara, *J. Mol. Catal. B: Enzym*. **2015**, *114*, 42-49.

[30] D. Gamenara, P. Saénz-Méndez, G. Seoane, P. Domínguez de María, *Redox Biocatalysis: Fundamentals and Applications*, John Wiley & Sons, Hoboken-US. **2012**.

[31] a) Y.-G. Zheng, H.-H. Yin, D.-F. Yu, X. Chen, X.-L. Tang, X.-J. Zhang, Y.-P. Zue, Y.-J. Wang, Z.-Q. Liu, *Appl. Microbiol. Biotechnol*. **2017**, *101*, 987-1001; b) R. Kratzer, J. M. Woodley, B. Nidetzky, *Biotechnol. Adv*. **2015**, *33*, 1641-1652; c) G. W. Huisman, J. Liang, A. Krebber, *Curr. Opin. Chem. Biol*. **2010**, *14*, 122-129.

[32] R. C. Betori, E. R. Miller, K. A. Scheidt, *Adv. Synth. Catal*. **2017**, *359*, 1131-1137.

[33] L. Castoldi, L. Ielo, P. Hoyos, M. J. Hérnaiz, L. De Luca, A. R. Alcántara, W. Holzer, V. Pace, *Tetrahedron* **2018**, *74*, 2211-2217.

[34] On imine reductases: a) J. Mangas-Sanchez, S. P. France, S. L. Montgomery, G. A. Aleku, H. Man, M. Sharma, J. I. Ramsden, G. Grogan, N. J. Turner, *Curr. Opin. Chem. Biol*. **2017**, *37*, 19-25; b) G. Grogan, N. J. Turner, *Chem. Eur. J*. **2016**, *22*, 1900-1907; c) J.H. Schrittwieser, S. Velikogne, W. Kroutil, *Adv. Synth. Catal*. **2015**, *357*, 1655-1685; d) D. Gamenara, P. Domínguez de María, *Org. Biomol. Chem*. **2014**, *12*, 2989-2992.

[35] Z. Maugeri, D. Rother, *Adv. Synth. Catal*. **2016**, *358*, 2745-2750.

[36] For some reviews on ene reductases: a) H. S. Toogood, N. S. Scrutton, *ACS Catal*. **2018**, *8*, 3532-3549; b) C. K. Winkler, K. Faber, M. Hall, *Curr. Opin. Chem. Biol*. **2018**, *43*, 97-105; c) G. Brown, T. S. Moody, M. Smyth, S. J. C. Taylor in *Biocatalysis: an*

Industrial Perspective (Eds. G. de Gonzalo, P. Domínguez de María) Royal Society of Chemistry, Cambridge, **2017**; pp. 229–256

[37] T. Reβ; W. Hummel, S. P. Hanlon, H. Iding, H. Gröger, *ChemCatChem* **2015**, *7*, 1302-1311.

[38] L. Leipold, D. Dobrijevic, J.W.E. Jeffries, M. Bawn, T.S. Moody, J.M. Ward, H.C. Hailes, *Green Chem*. **2019**, *21*, 75-86.

[39] a) D. Weidener, H. Klose, W. Leitner, U. Schurr, B. Usadel, P. Domínguez de María, P.M. Grande, *ChemSusChem* **2018**, *11*, 2051-2056; b) P.M. Grande, J. Viell, N. Theyssen, W. Marquardt, P. Domínguez de María, W. Leitner, *Green Chem*. **2015**, *17*, 3533-3539; c) P.M. Grande, C. Bergs, P. Domínguez de María, *ChemSusChem* **2012**, *5*, 1203-1206; d) T. vom Stein, P.M. Grande, W. Leitner, P. Domínguez de María, *ChemSusChem* **2011**, *18*, 1592-1594; e) T. vom Stein, P.M. Grande, H. Kayser, F. Sibilla, W. Leitner, P. Domínguez de María, *Green Chem*. **2011**, *13*, 1772-1777.

[40] L. Shuai, J. Luterbacher, *ChemSusChem* **2016**, *9*, 133-155.

[41] Selected reviews on furfural: a) F. Delbecq, Y. Wang, A. Muralidhara, K. El Quardi, G. Marlair, C. Len, *Front. Chem*. **2018**, *6*, 146; b) J.P. Lange, E. van der Heide, J. van Buijtenen, R. Price, *ChemSusChem* **2012**, *5*, 150-166.

[42] X. Hu, R.J.M. Westerhof, D. Dong, L. Wu, C.Z. Li, *ACS Sust. Chem. Eng*. **2014**, *2*, 2562-2575.

[43] M. Männistö, J.P. Pokki, L. Fournis, V. Alopacus, *J. Chem. Thermodynamics* **2017**, *110*, 127-136.

[44] M.J. Campos Molina, R. Mariscal, M. Ojeda, M. López Granados, *Bioresour. Technol*. **2012**, *126*, 321-327.

[45] M.J. Campos Molina, R. Mariscal, M. López Granados, *J. Sol-Gel Sci. Technol*. **2015**, *75*, 164-179.

[46] a) S. Le Guenic, D. Gergela, C. Ceballos, F. Delbecq, C. Len, *Molecules* **2016**, *21*, 1102.; b) S. Le Guenic, F. Delbecq, C. Ceballos, C. Len, *J. Mol. Catal. A: Chem*. **2015**, *410*, 1-7.

[47] L. Zhang, Y. He, Y. Zhu, Y. Liu, X. Wang, *Bioresour. Technol*. **2018**, *249*, 536-541.

[48] Q. Qing, Q. Guo, L. Zhou, Y. Wan, Y. Xu, H. Ji, X. Gao, Y. Zhang, *Bioresour. Technol*. **2017**, *226*, 247-254.

[49] J. He, M. Liu, K. Huang, T.W. Walker, C.T. Maravelias, J.A. Dumesic, G. Huber, *Green Chem*. **2017**, *19*, 3542-3653.

[50] C. Wang, L. Zhang, T. Zhou, J. Chen, F. Xu, *Sci. Rep*. **2017**, *7*, 40908.

[51] P. N. Romano, J.M.A.R. de Almeida, Y. Carvalho, P. Priecel, E. Fallabella Sousa-Aguilar, J.A. López-Sánchez, *ChemSusChem* **2016**, *9*, 3387-3392.

[52] G. Bottari, A.J. Kumalaputri, K.K. Krawczyk, B.L. Feringa, H.J. Heeres, K. Barta, *ChemSusChem* **2015**, *8*, 1323-1327.

[53] a) J.-P. Duroudier, in *Liquid-Liquid and Solid-Liquid Extractors* (Ed.; J.-P. Duroudier), Elsevier, Oxford, **2016**, pp 1-37; b) J. Zhang, B. Hu, in *Separation and Purification Technologies in Biorefineries* (Eds.: S. Ramaswamy, H. Huang, B. V. Ramarao), John Wiley & Sons, Hoboken, **2013** pp 61-78.

[54] K. Watanabe, *Molecules* **2013**, 18, 3183-3194.

[55] L. Soh, M. J. Eckelman, *ACS Sustain. Chem. Eng*. **2016**, *4*, 5821-5837.

[56] C. Breil, A. Meullemiestre, M. Vian, F. Chemat, *Molecules* **2016**, 21, 196/1-196/14.

[57] C. M. Hansen, *Hansen Solubility Parameters: a User's Handbook*, CRC Press, Boca Raton, **2007**.

[58] F. Eckert, A. Klamt, *Aiche J*. **2002**, *48*, 369-385.

[59] K. V. Probst, M. D. Wales, M. E. Rezac, P. V. Vadlani, *Biotechnol. Prog*. **2017**, *33*, 1096-1103.

[60] M. M. Cascant, C. Breil, S. G. M. de la Guardia, A. S. Fabiano-Tixier, F. Chemat, *Anal. Bioanal. Chem*. **2017**, *409*, 3527-3539.

[61] W. M. A. W. Mahmood, C. Theodoropoulos, M. González-Miquel, *Green Chem*. **2017**, *19*, 5723-5733.

[62] S. S. de Jesus, G. F. Ferreira, M. R. W. Maciel, R. Maciel, *Fuel* **2019**, *235*, 1123-1130.

[63] E. Yara-Varon, A. S. Fabiano-Tixier, M. Balcells, R. Canela-Garayoa, A. Bily, F. Chemat, *RSC Adv*. **2016**, *6*, 27750-27759.

[64] a) B. Moore, V. Sánchez, E. Smith, US20130306557A1, **2013**; b) V. Sánchez, B. Moore, E. Smith, WO2014182499A1, **2014.**

[65] S. J. Tenne, J. Kinzel, M. Arlt, F. Sibilla, M. Bocola, U. Schwaneberg, *J. Chromatogr. B* **2013**, *937*, 13-17.

[66] a) J. W. Fairman, N. Noinaj, S. K. Buchanan, *Curr. Opin. Struct. Biol*. **2011**, *21*, 523-531; b) W. Tian, M. S. Lin, K. Tang, J. Liang, H. Naveed, *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, 1511-1516.

[67] M. Nallani, S. Benito, O. Onaca, A. Graff, M. Lindemann, M. Winterhalter, W. Meier, U. Schwaneberg, *J. Biotechnol*. **2006**, *123*, 50-59.

[68] T. L. Winstone, K. A. Duncalf, R. J. Turner, *Protein Expr. Purif.* **2002**, *26*, 111-121.

[69] T. Oshima, I. Fujiwara, Y. Baba, *Solvent Extr. Res. Dev. Jpn*. **2015**, *22*, 119-125.

[70]C. Y. Cheng, *Hydrometallurgy* **2000**, *56*, 369-386.

[71] T. Oshima, N. Ohkubo, I. Fujiwara, T. Horiuchi, T. Koyama, K. Ohe, Y. Baba, *Solvent Extr. Res. Dev. Jpn*. **2017**, *24*, 89-96.

[72] M. Palit, G. Mallard, *J. Chromatogr. A* **2011**, *1218*, 5393-5400.

[73] a) A. I. Olives, V. González-Ruiz, M. A. Martin, *ACS Sustain. Chem. Eng*. **2017**, *5*, 5618-5634; b) S. Ekins, A. M. Clark, A. J. Williams, *ACS Sustain. Chem. Eng*. **2013**, *1*, 8-13; c) A. B. Eldin, O. A. Ismaiel, W. E. Hassan, A. A. Shalaby, *J. Anal. Chem*. **2016**, *71*, 861-871; d) A. Galuszka, Z. Migaszewski, J. Namiesnik, *TrAC, Trends Anal. Chem*. **2013**, *50*, 78-84; e) M. Koel, *Green Chem.* **2016**, *18*, 923-931.

[74] a) H. X. Zhang, *J. Chem. Eng. Data* **2015**, *60*, 1371-1376; b) W. J. Jeong, H. K. Cho, J. S. Lim, *Korean J. Chem. Eng*. **2016**, *33*, 2961-2967; c) M. D. Wales, C. R. Huang, L. B. Joos, K. V. Probst, P. V. Vadlani, J. L. Anthony, M. E. Rezac, *J. Chem. Eng. Data* **2016**, *61*, 1479-1484; d) M. Mannisto, J. P Pokki, L. Fournis, V. Alopaeus, *J. Chem. Thermodyn*. **2017**, *110*, 127-136; e) A. Randova, O. Vopicka, L. Bartovska, K. Friess, *Chem. Pap*. **2018**, *72*, 947-954; f) H. X. Zhang, *J. Chem. Eng. Data* **2018**, *63*, 3338-3344; g) M. Mannisto, J. P. Pokki, V. Alopaeus, *J. Chem. Thermodyn*. **2018**, *119*, 61-75.

[75] M. Tobiszewski, M. Nedyalkova, S. Madurga, F. Pena-Pereira, J. Namiesnik, V. Simeonov, *Ecotox. Environ. Safe*. **2018**, *147*, 292-298.

[76] N. Prache, S. Abreu, P. Sassiat, D. Thiebaut, P. Chaminade, *J. Chromatogr. A* **2016**, *1464*, 55-63.

[77] V. Desfontaine, A. Tarafder, J. Hill, J. Fairchild, A. G. G. Perrenoud, J. L. Veuthey, D. A. Guillarme, *J. Chromatogr. A* **2017**, *1511*, 122-131.

[78] J. O. DaSilva, B. Coes, L. Frey, I. Mergelsberg, R. McClain, L Nogle, C. J. Welch, *J. Chromatogr. A* **2014**, *1328*, 98-103.

[79] N. Amarouche, L. Boudesocque, N. Borie, M. Giraud, L. Forni, A. Butte, F. Edwards, J. H. Renault, *J. Sep. Sci*. **2014**, *37*, 1222-1228.

[80] G. Audo, Centrifugal Partition Chromatography: The Key to Green Preparative *Chromatography. Am. Lab*. **2018**, 50, 28-29.

[81] X. Y. Huang, S. Ignatova, P. Hewitson, D. L. Di, *TRaC, Trends Anal. Chem*. **2016**, *77*, 214-225.

[82] Y. Ito, *J. Chromatogr. A* **2013**, *1271*, 71-85.

[83] a) N. Qvit, S. J. S. Rubin, T. J. Urban, D. Mochly-Rosen, E. R. Gross, *Drug Discov. Today* **2017**, *22*, 454-462; b) K. Fosgerau, T. Hoffmann, *Drug Discov. Today* **2015**, *20* (1), 122-128; c) M. Sánchez-Navarro, M. Teixido, E. Giralt, *Acc. Chem. Res*. **2017**, *50*, 1847-1854.

[84] a) A. Henninot, J. C. Collins, J. M. Nuss, *J. Med. Chem*. **2018**, *61*, 1382-1414; b) M. Erak, K. Bellmann-Sickert, S. Els-Heindl, A. G. Beck-Sickinger, *Bioorg. Med. Chem*. **2018**, *26*, 2759-2765; c) J. L. Lau, M. K. Dunn, *Bioorg. Med. Chem.* **2018**, *26*, 2700-2707.

[85] S. B. Lawrenson, R. Arav, M. North, *Green Chem*. **2017**, *19*, 1685-1691.

[86] S. B. Lawrenson, *Pure Appl. Chem*. **2018**, *90*, 157-165.

[87] G. A. Acosta, M. del Fresno, M. Paradis-Bas, M. Rigau-DeLlobet, S. Cote, M. Royo, F. J. Albericio, *J. Pept. Sci*. **2009**, *15*, 629-633.

[88] D. S. MacMillan, J. Murray, H. F. Sneddon, C. Jamieson, A. J. B. Watson, *Green Chem.* **2013**, *15*, 596-600.

[89] Y. E. Jad, G. A. Acosta, S. N. Khattab, B. G. de La Torre, T. Govender, H. G. Kruger, A. El-Faham, F. Albericio, *Amino Acids* **2016**, *48*, 419-426.

[90] D. Takahashi, WO2012165545A1, **2012**.

[91] Thatipally, S.; Dammalapati, V. L. N. R.; Kallam, V. S. R. R.; Gorantla, S. R. A.; Chava, S. WO2015040640A2, 2015.

[92] O. Al Musaimi, Y. E. Jad, A. Kumar, J. M. Collins, A. Basso, B. G. de la Torre, F. Albericio, *Curr. Opin. Green Sustain. Chem*. **2018**, *11*, 99-103.

[93] D. M. M. Jaradat, *Amino Acids* **2018**, *50*, 39-68.