Journal of Fluorine Chemistry 177 (2015) 80–89

Contents lists available at ScienceDirect

Journal of Fluorine Chemistry

journal homepage: www.elsevier.com/locate/fluor

Aerobic biotransformation studies of two trifluoromethoxy-substituted aliphatic alcohols and a novel fluorinated C3-based building block

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ARTICLE INFO

Article history:
Received 28 January 2015
Received in revised form 11 June 2015
Accepted 16 June 2015
Available online 22 June 2015

Keywords:
PFAS
Biotransformation
Mass spectrometry
HPLC–MS

ABSTRACT

Fluorinated substances play a significant role for many industrial and consumer products, but many of these chemicals are attributed with an adverse ecological profile and persistence in the environment. Herein, three potentially more environmentally benign substitutes were assessed for aerobic biotransformation, namely 3-(trifluoromethoxy)-propan-1-ol (TFMPrOH), 6-(trifluoromethoxy)-hexan-1-ol (TFMHxOH) and 1-(2,2,3,3,4,4,4-heptafluorobutoxy)-propan-2-ol (HFBPrOH). Analytical techniques involved different HPLC–ESI-MS/MS techniques as well as determination of fluoride in order to assess the extent of mineralization.

The two trifluoromethoxy-substituted alcohols showed very different results concerning mineralization. Whereas TFMPrOH only yielded approximately 15% fluoride, TFMHxOH reached nearly quantitative release of fluoride after 37 days. The latter one yielded 6-trifluoromethoxy hexanoic acid (TFMHxA) as well as trifluoromethyl carbonate (TFMC) as transient transformation products, both of which were entirely degraded. TFMC was also detected during biotransformation of TFMPrOH, however, the major transformation product was 3-trifluoromethoxy-propanoic acid (TFMPrA), which did not show any further degradation within a 47 days period.

The third compound under investigation, HFBPrOH, expectedly yielded perfluorobutanoic acid as a stable biotransformation product. Several acidic biotransformation intermediates as well as heptafluorobutan-1-ol were identified and a biotransformation pathway was postulated. TFMHxOH might therefore be incorporated into fluorinated substances, for instance by ester linkages assuming that biotransformation of such substances would yield the alcohol.

1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) have been used for decades since they can provide properties not achievable by non-fluorinated chemistry [1]. However, their detrimental effects regarding toxicity, ecotoxicity and bioaccumulation have become a cause for concern and have led to a restraint of several such substances carrying C_8 fluorinated alkyl chains [2]. These concerns are reinforced by the fact that perfluoroalkyl acids cannot be entirely degraded by microorganisms due to the stability of the carbon–fluorine bond and thus are persistent in the environment [3–6].

Substitutes for the legacy chemicals containing fluorinated C_8 chemistry are mainly shorter chained substances, mainly those incorporating fluorinated C_6 or C_4 chains, but yet, such substances cannot be entirely degraded due to the stable perfluoroalkyl chains [7–9]. In search of appropriate biodegradable substitutes, a series of substances with different fluorine-containing end groups was studied, including 4-trifluoromethylphenoxy-, N,N-bis(trifluoromethyl)amino- and trifluoromethoxy (TFM)-substituted n-alkane-1-sulfonates [10–12]. Within this framework, it was shown that trifluoromethoxy-decane-1-sulfonates are mineralized to approximately 90% based on fluoride release, whereas the other substances showed inferior degradation extents. The biotransformation route for TFM-alkane-1-sulfonates was initiated by desulfonation and formation of ω-TFM-alkyl carboxylates, which were then shortened by β-oxidation. Eventually, trifluoromethanol (TFMeOH) was assumed to be formed which first decays to difluorophosgene and further to fluoride and carbon dioxide [13]. This proved that

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http://dx.doi.org/10.1016/j.jfluchem.2015.06.015
0022-1139/

TFM-based compounds can be degraded if the group is attached to a biodegradable linker, for instance alkane-1-sulfonates.

In the present study, TFM-based 1-alkanols are investigated in terms of their biodegradation behavior. Such alcohols might be possible building blocks in TFM-based polyesters, similar to fluorotelomer alcohols in legacy oligomeric or polymeric PFASs [1]. These TFM-alcohols might therefore occur as biotransformation intermediates due to enzymatic or even chemical hydrolysis. Since substances carrying only one trifluoromethyl group cannot provide the same unique properties as those with higher fluorine content, this study was carried out as proof of principle for biodegradability of substances carrying the TFM group.

Moreover, a novel fluorinated building block with a C_3 perfluoroalkyl chain length was studied, namely 1-(2,2,3,3,4,4,4-heptafluorobutoxy)-propan-2-ol (HFBPrOH). In this case, the focus was set to examine whether perfluorobutanoic acid (PFBA) was formed, since ecotoxicological data for this compound exist suggesting lower concern compared with their longer-chained analogs [14,15]. Similarly to TFMPrOH and TFMHxOH, HFBPrOH contains an alcohol group and thus can be used to synthesize oligomers or polymers where these structures are implemented in the side chains.

2. Methods and materials

2.1. Chemicals

All solvents and mobile phase modifiers used for HPLC, MS and biodegradation study applications were at least of HPLC grade. Ultrapure water was prepared using a Millipore Simplicity 185 with a Slimpak 2 Cartridge (Millipore, Milford, USA). If not stated differently, this water was used for preparation of solutions and dilution.

3-(Trifluoromethoxy)-propan-1-ol (TFMPrOH, 98% purity), 6-(trifluoromethoxy)-hexan-1-ol (TFMHxOH, unknown purity) and HFBPrOH (87% purity, 5% methyl isomer impurity), 2,2,3,3,4,4,4-heptafluorobutanol (HFBOH) present in unknown fraction) were synthesized and supplied by Merck (Darmstadt, Germany).

2.2. Fluoride determination

Fluoride was determined by anion exchange chromatography on a Metrohm modular system consisting of a 709 IC Pump, a 752 Pump Unit, a 733 Separation Center, a 732 IC Detector and a 762 IC Interface (all from Metrohm, Herisau, Switzerland). Separation was carried out on a Metrosep A Supp 5 100 × 4.0 mm (Metrohm, Herisau, Switzerland) and a Varian C_{18} 3 μm precolumn (Varian, Frankfurt, Germany). The eluent was aqueous Na_2CO_3 (1.6 mM) and $NaHCO_3$ (0.5 mM) at a flow rate of 0.8 mL/min.

A fluoride stock solution was prepared from analytical grade sodium fluoride (Sigma, Seelze, Germany) at a concentration of approximately 1 mg mL^{-1}. Standards were prepared at fluoride concentrations of 0.1 mg L^{-1}, 0.25 mg L^{-1}, 0.5 mg L^{-1}, 0.75 mg L^{-1}, 1 mg L^{-1} and 2.5 mg L^{-1}.

In order to determine the amount of organically bound fluorine, one sample of TFMPrOH biotransformation assay was additionally measured with and without UV decomposition by use of an ion-selective electrode from Metrohm (Zofingen, Switzerland) as explained previously [11].

2.3. HPLC–ESI-MS analysis

Substance-specific analysis and structure elucidation was carried out by HPLC–ESI-MS/MS analysis. The chromatographic setup consisted of two Series 200 Micro Pumps, a Series 200 vacuum degasser, and a Series 200 autosampler (Perkin Elmer, Norwalk, CT, USA). The chromatograph was coupled to a hybrid triple quadrupole – linear ion trap tandem mass spectrometer (3200 Q Trap, Applied Biosystems, Foster City, CA, USA) and a Turbo Ionspray interface in ESI ("Turbo Spray") mode. Nitrogen used for MS was generated by a Membrane nitrogen generator NGM-22-LC/MS (CMC, Eschborn, Germany) coupled to a SF 4 FF oil-free orbiting scroll compressor (Atlas Corpo, Stockholm, Sweden). Operation and data acquisition of the HPLC–MS system was carried out via Analyst Software, versions 1.4 and 1.5, respectively. IonSpray Voltage was +5.5 kV in positive mode and −4.5 kV in negative mode, respectively, Curtain Gas was set to 25 psi, Nebulizer Gas was 55 psi, Turbo Gas was 65 psi and CAD Gas was set to 'medium' equalling 5 on an arbitrary scale from 1 to 12 when fragmentation was intended.

For target compound analysis, optimization of the MS parameters was performed via syringe pump injection of a solution of the analyte at an approximate concentration of 0.1–20 μg mL^{-1} depending on response factors. Optimization was either carried out automatically by the Analyst software.

For biodegradation experiments of TFMHxOH and HFBPrOH, standards of the parent compounds were always prepared from 10% (or lower) of the theoretical concentration up to approximately 125–150% of the initial concentration. At least five calibration points were used. Detailed HPLC–ESI-MS settings and parameters are given in the Supplementary material.

2.4. Biotransformation experiments

Biotransformation experiments were carried out under aerobic conditions with effluent water from a municipal wastewater treatment plant (Beuerbach, Hesse, Germany) in amber glass bottles. Effluent water was chosen as inoculum to facilitate detection of unknown transformation intermediates by MS, as the more commonly used activated sludge may cause severe ion suppression in ESI-MS. The substances under investigation were spiked at a level of approximately 10 mg L^{-1}. If possible, aqueous stock solutions were used, but if water solubility was too low for spiking purposes, methanolic stock solutions were prepared.

For all assays, sterile controls were prepared in the same way as the active assay by addition of 10 g L^{-1} sodium azide. Blanks were prepared like the active assay without addition of the active test substance, but including methanol, if used for preparation of the stock solution. The bottles were placed on an automatic shaker which was set at 100 rpm during the entire biotransformation assay. Experiments were carried out at room temperature (between 20 °C and 25 °C).

After addition of all substances needed for the assay, the bottles were gently mixed for 30–45 min, after which the initial sample was drawn. For TFM-alcohols, the bottles were then closed with a glass plug and parafilm for four days to avoid volatilization of the poorly water-soluble alcohols. Afterwards, the bottles were aerated with an aquarium pump for 30 min daily. HFBPrOH bottles were aerated daily from the beginning.

Sampling was carried out daily in the beginning of the experiment and the sampling interval was then constantly increased to weekly after three weeks. Samples were frozen at −30 °C until analysis and ultrasonicated for 10 min after thawing before samples were prepared.

Further details on biotransformation assay and preparation of samples for analytical methods are given in the Supplementary material.

3. Results and discussion

3.1. 3-(Trifluoromethoxy)-1-propanol

Even though mass spectrometric signals were observed for TFMPrOH, the study of biotransformation suffered from the

incapability of measuring the initial compound by HPLC–ESI-MS. The reason for this could be suppression of the very weakly basic aliphatic hydroxyl group due to early elution from the HPLC and ion suppression effects. Also GC-EI/MS was tested as a commonly applied method for volatile analytes, but no signal of the initial compound was observed either.

Thus, focus was set on transformation products, mainly on acidic ones, since aliphatic alcohols are commonly transformed to acids under aerobic conditions. Since TFM-substituted compounds had been previously measured in our laboratory [10], negative ion CID fragmentation of compounds bearing this functional group was known. Fragmentation usually yielded the trifluoromethanolate anion at *m/z* 85. In such cases, precursor ion scans can give valuable information about unknown compounds. Here, a compound with an *m/z* ratio of 157 was detected (see Supplementary material). The molecular mass of 158 Da (*m/z* 157 in negative ESI polarity) suggests an ω-oxidized transformation product, namely 3-trifluoromethoxypropionic acid (TFMPrA). Furthermore, a second peak was detected at *m/z* 129 (data not shown), which suggests the presence of trifluoromethyl carbonate (TFMC). Their structure was verified by 'enhanced product ion scans' using the linear ion trap to scan the product ions (see Supplementary material). While TFMC showed neutral loss of CO and CO_2 during collision-induced dissociation, respectively, TFMPrOH merely gives rise to the trifluoromethanolate anion by loss of acrylic acid. It should be pointed out that no confirmation of the detected metabolites was made by comparison with authentic standards, thus chemical identities of transformation product remains tentative.

The temporal evolution of the two transformation products detected was investigated in single ion monitoring mode and is presented in Fig. 1.

As described in the experimental section, the assay was kept closed for the first four days to prevent volatilization of TFMPrOH or volatile transformation products. Therefore, no data points are available for this time period. Both TFMC and TFMPrA showed a rapid increase in peak area. TFMC reached a maximum after seven days and was then completely transformed showing no signal anymore after 14 days, whereas TFMPrA reached a plateau just at this time point. Even after 34 days, no decline was observed suggesting stability of substance.

This is striking since so far, it was assumed that longer ω-TFM substituted alkanoic acids were degraded by β-oxidation until TFMeOH is generated [10]. The stability of TFMPrA was also verified by different fluoride measurements, as summarized in Table 1. Besides free inorganic fluoride, fluoride was also measured after UV decomposition of organic compounds, and after filtration and UV decomposition. This is a very valuable method to affirm results obtained from organic analysis, such as LC–ESI-MS, and to determine the percentage contribution of biotransformation pathways in case of multiple pathways [11,12].

Herein, fluoride measurement after UV decomposition yielded 4.54 mg L^{-1}, which is ca. 17% higher than the theoretical value, as calculated based on the initially spiked test compound. This value can be substantiated by evaporation of the biodegradation medium, which was kept at room temperature and aerated regularly contributing to evaporation of water. Assumed that only biotransformation of TFMC can lead to inorganic fluoride and that this pathway yields 100% fluoride, it accounts for 0.75/4.54 = 16.5%.

The tentative biotransformation pathway is presented in Fig. 2. The first pathway is likely achieved by ω-oxidation of the hydroxyl group to the carboxylate group yielding TFMPrOH, probably via the short-lived aldehyde.

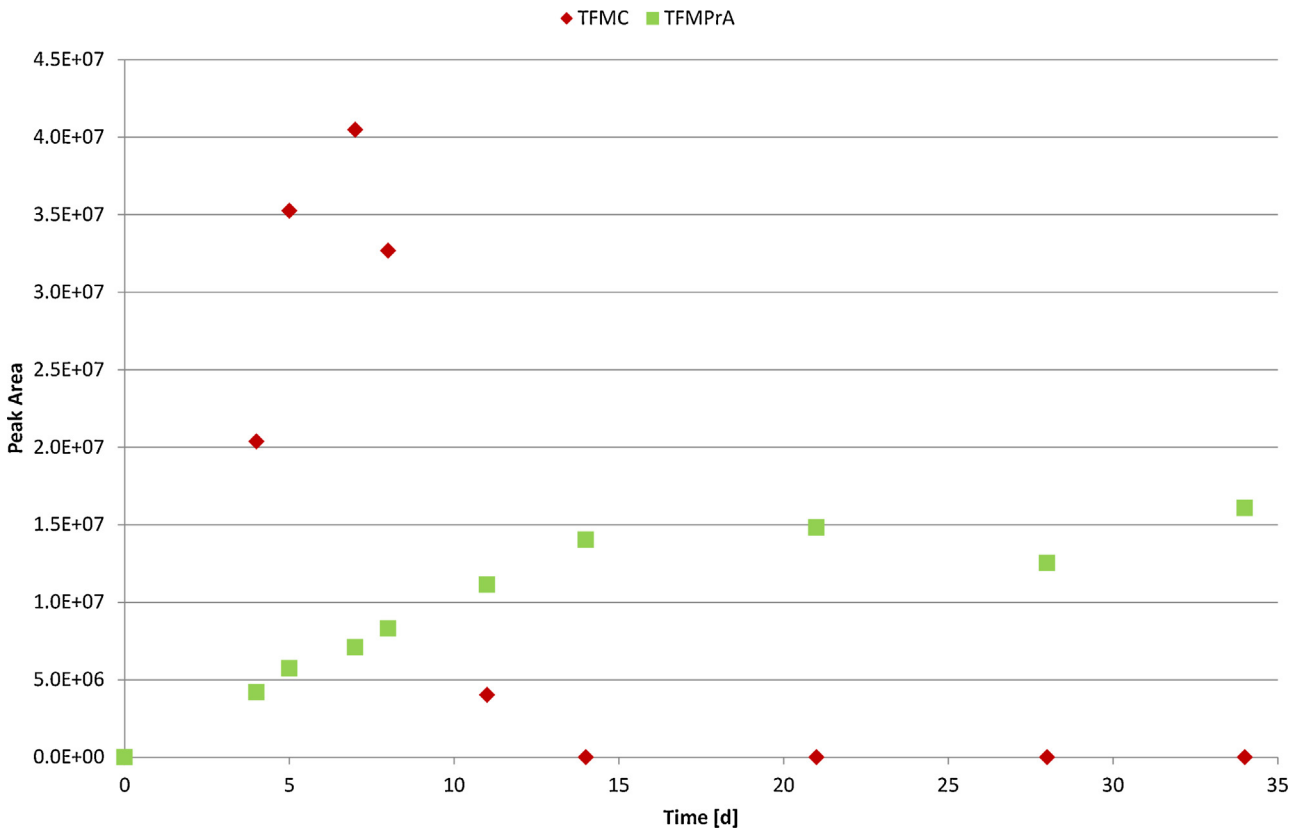

Fig. 1. Temporal evolution of TFMPrOH metabolites TFMPrA and TFMC expressed as peak area by HPLC–ESI-MS versus time.

Table 1
Concentrations of inorganic fluoride and organically bound fluorine in TFMPrOH biodegradation samples after 77 days.

	β (F^-) [mg L^{-1}]	Fluoride [% theoretical]
Inorganic	0.75	19.3
After filtration and UV decomposition	4.50	116
After UV decomposition of the whole sample	4.54	117

It must be highlighted that the pathway leading to TFMC and inorganic fluoride is highly speculative, since no intermediates were detected. These intermediates were screened for in single ion monitoring (SIM), but none of them were detected, probably due to the transient nature of these intermediates. However, it can be stated that no link between TFMPrA and the second pathway can be established, since the HPLC–MS signal of TFMPrA remains constant. Thus, a second pathway must exist. The detection of TFMC must have been preceded by an insertion of an oxygen atom into the alkyl chain. A possible but unproven explanation is given as follows.

A likely reaction would be the in-chain oxidation of the alkyl chain in proximity to the TFM group yielding the hemiacetal **I**, which can be oxidized to the ester **II**. A Baeyer–Villiger-oxidation-like reaction, that is an insertion of an oxygen atom leading to the 3-hydroxypropyl trifluoromethyl carbonate **III**. Ester hydrolysis leads to TFMC, which subsequently decays to TFMeOH and thus finally yields fluoride and carbon dioxide.

As the ω-oxidation pathway apparently proceeds much more rapidly, the 3-(trifluoromethoxy)propoxy group does not seem to be a good candidate for environmentally friendly substitutes of PFASs assuming that in potential fluorosurfactants, the 3-(trifluoromethoxy)propoxy group is cleaved off as TFMPrOH. Whereas at least 15% of the organically bound fluorine can be released as inorganic fluoride, the long-term fate and effects of the remaining major transformation product TFMPrA are questionable and cannot be assessed without further laborious studies.

3.2. 6-(Trifluoromethoxy)-1-hexanol

TFMHxOH biotransformation was initially investigated by the temporal assessment of the test compound itself. In this case it was possible by measuring the protonated molecule $[M+H]^+$ in positive ESI mode.

The assay suffered from loss of TFMHxOH also in the sterilized experiment (see Fig. 3a). This can be reasoned by a mixture of volatilization and adsorption to glassware or particles present in the inoculum, which is typical for biodegradation studies of fluorinated substances [16]. Yet, the reduction in concentration of TFMHxOH proceeds more rapidly in the active assay indicating biotransformation. Complete primary transformation was achieved after 11 days.

Similarly to the biodegradation assay of TFMPrOH, two acidic transformation products were detected for TFMHxOH as well, in this case 6-(trifluoromethoxy)hexanoic acid (TFMHxA) and again TFMC.

The identity of TFMHxA was verified by performing product ion scans (see Supplementary material). Besides the common and indicative trifluoromethanolate anion at *m/z* 85, a second product ion at *m/z* 113 was observed, which represents an ω-unsaturated alkenoate anion. This ion results from cleavage of neutral TFMeOH from the precursor ion. This ion species had also been detected in measurements of Peschka et al. when examining 10-(trifluoromethoxy)decane-1-sulfonate [10].

Both compounds were generated rapidly with TFMC showing a maximum after only four days and no signal after 11 days, as shown in Fig. 3b. TFMHxA showed its maximum concentration after five days and – in stark contrast to TFMPrA – was completely degraded afterwards showing complete degradation also after 11 days. The main difference between the degradation between TFMPrOH and TFMHxOH, however, is a very high degree of

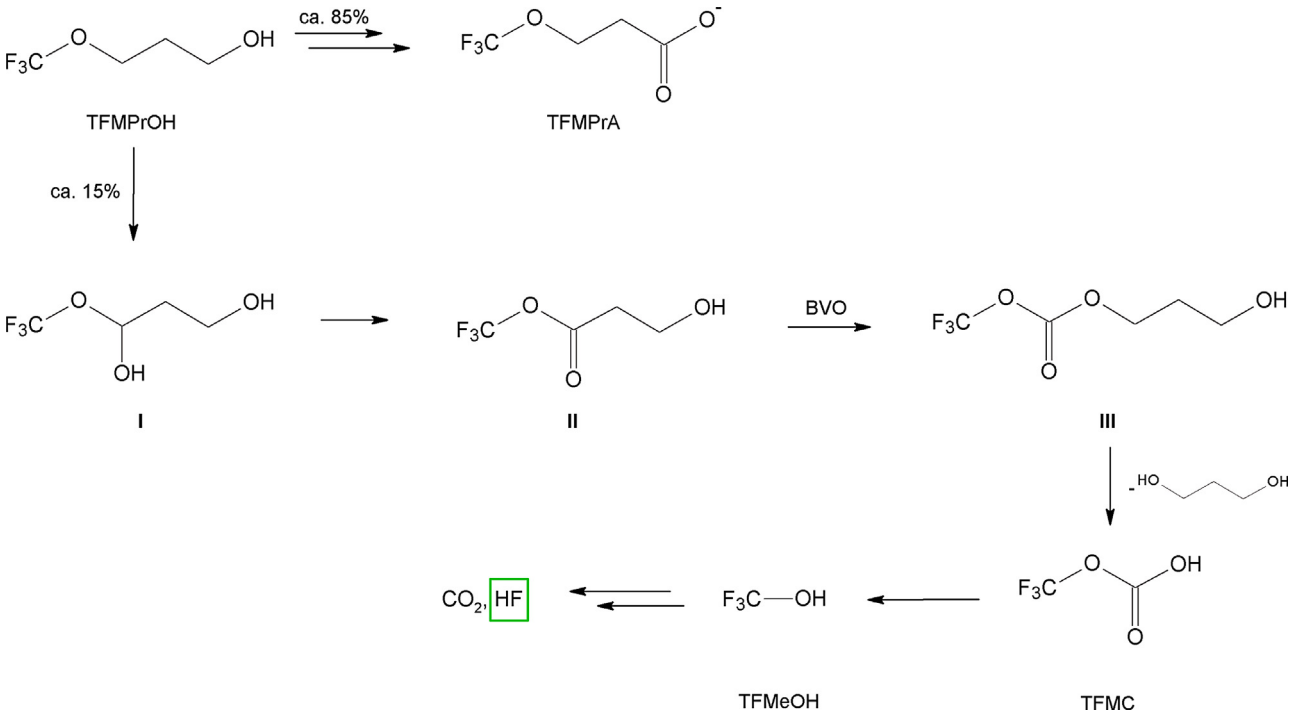

Fig. 2. Proposed biotransformation pathways of TFMPrOH; BVO, Baeyer–Villiger oxidation. The dotted rectangles indicate the transformation products identified by HPLC–ESI-MS/MS.

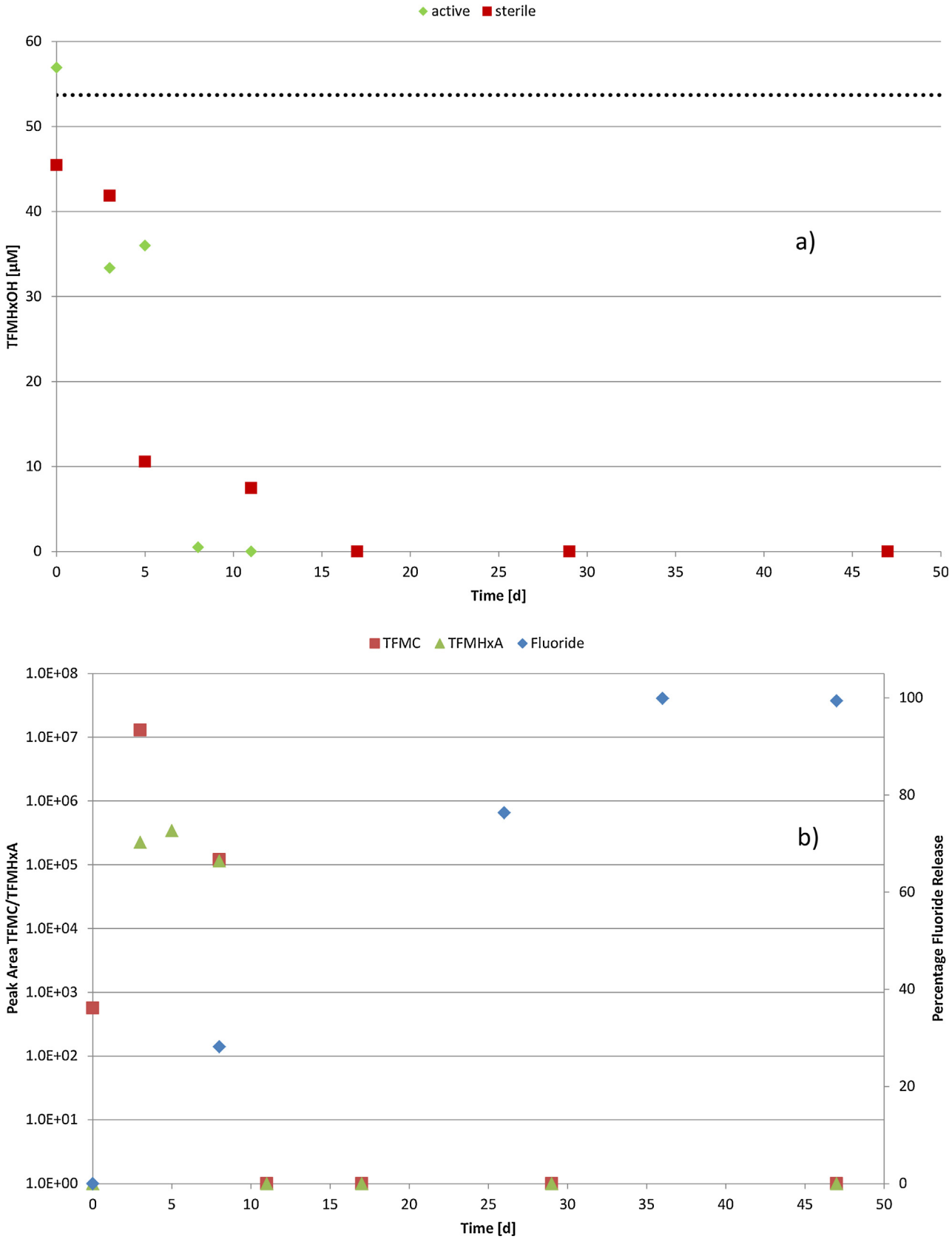

Fig. 3. (a) Temporal evolution of TFMHxOH in active and sterile biodegradation assays. The black dotted line indicates the theoretical initial TFMHxOH concentration and (b) temporal evolution of TFMC and TFMHxA during biotransformation (please note the logarithmic scale) and percentage fluoride release.

defluorination, which accounted for nearly 100% of the theoretical value for TFMHxOH.

This difference in mineralization yield between TFMPrOH and TFMHxOH cannot be entirely explained, because no further transformation products were detected in the course of this experiment. Thus, the degradation pathways presented here are speculative (see Fig. 4). It is assumed that TFMHxOH is first oxidized in a similar manner as TFMPrOH, leading to TFMHxA. Unlike TFMPrA, TFMHxA is further metabolized, as suggested in Fig. 4. In contrast to TFMPrA, beta oxidation was considered one way to accomplish chain shortening of TFMHxA. In the latter one, there is a longer spacer between the fluorinated functional group and the carboxylic acid group thus facilitating attack of enzymes.

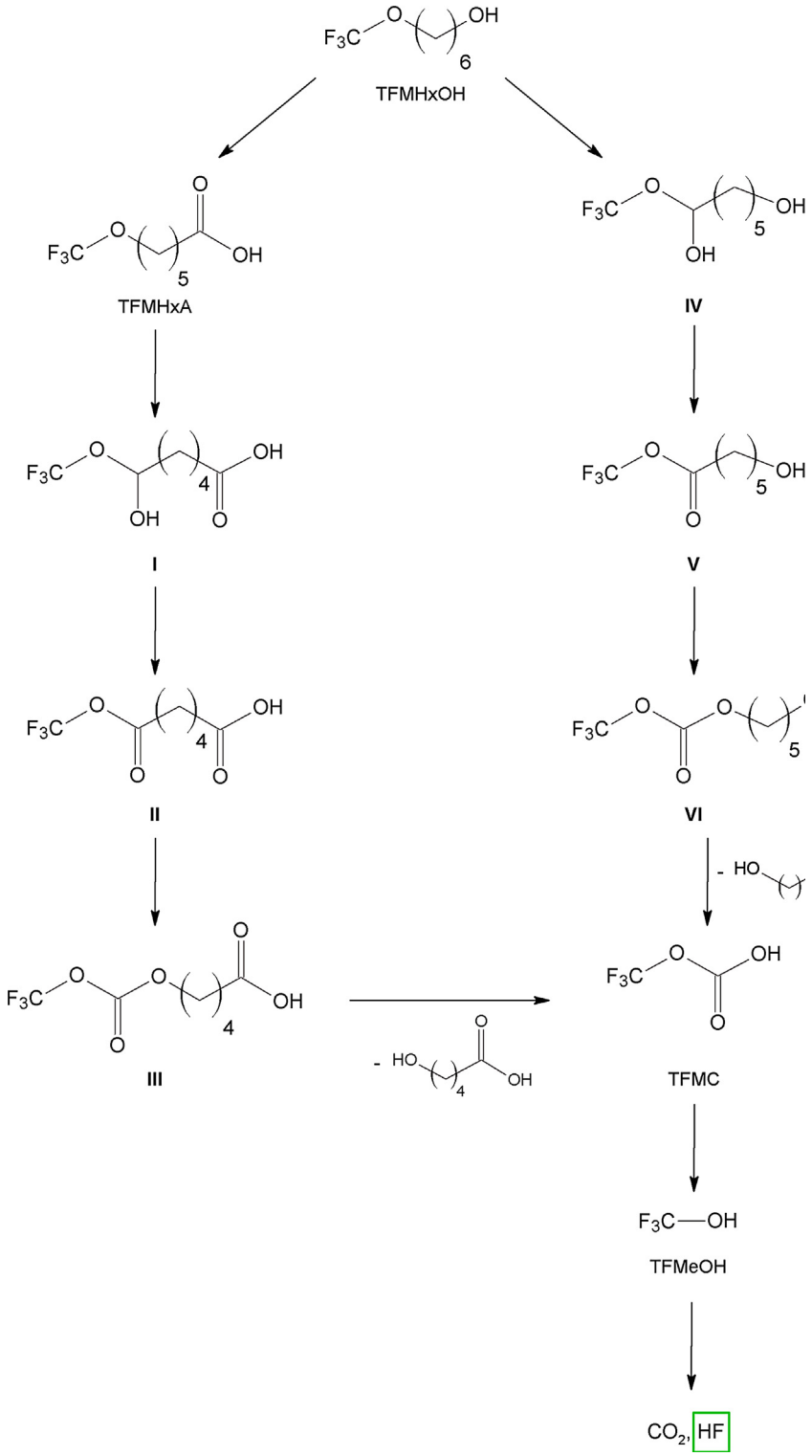

Fig. 4. Proposed degradation pathways of TFMHxOH; please note that possible transitions from the pathway A to pathway B are possible by oxidation, but not depicted here. For further information, see text. The dotted rectangles indicate the transformation products identified by HPLC–ESI-MS/MS.

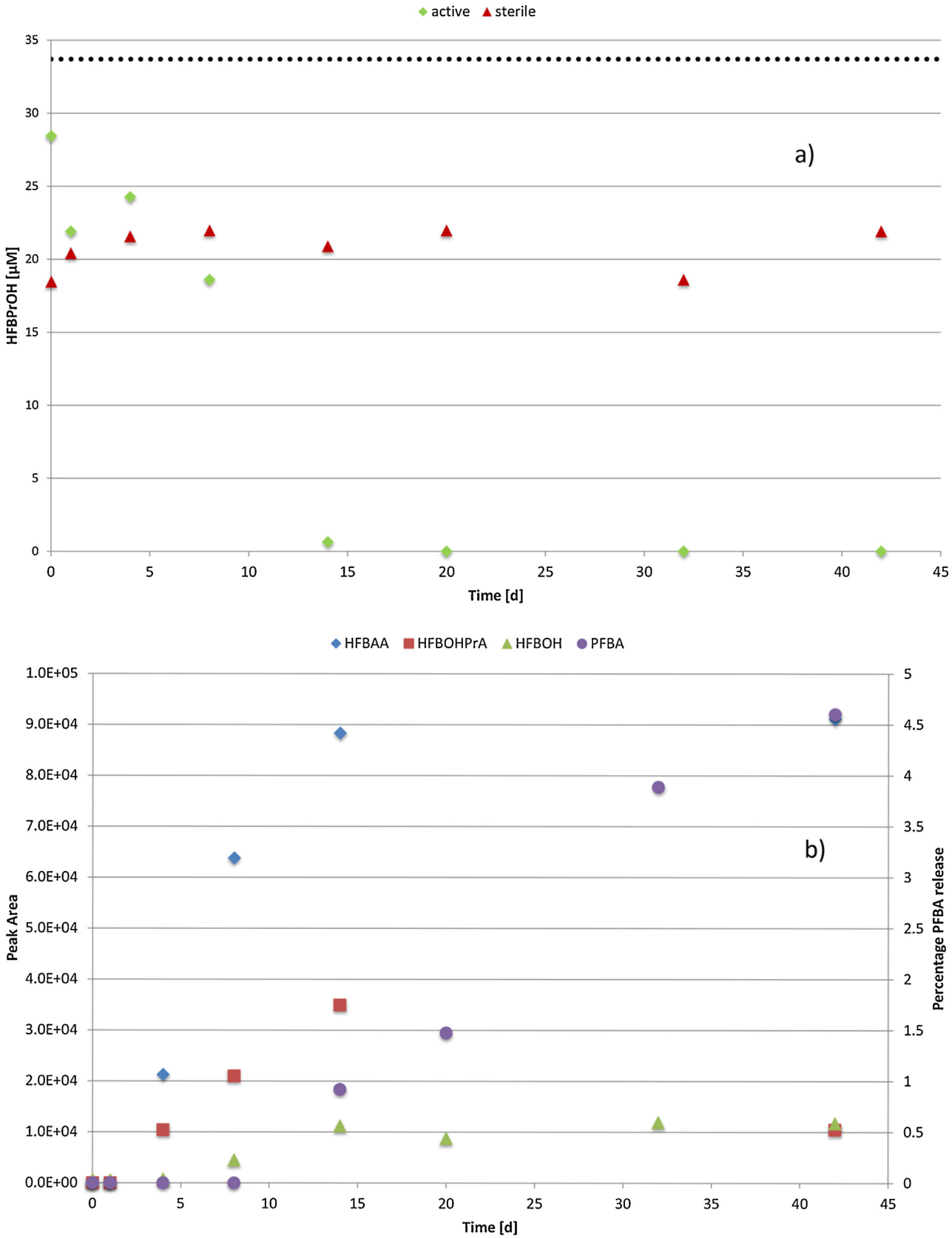

Fig. 5. (a) Temporal evolution of HFBPrOH in the active and sterilized biodegradation assay. The dotted line indicates the theoretical initial concentration and (b) temporal evolution of biotransformation products of HFBPrOH expressed as their peak area in HPLC–MS.

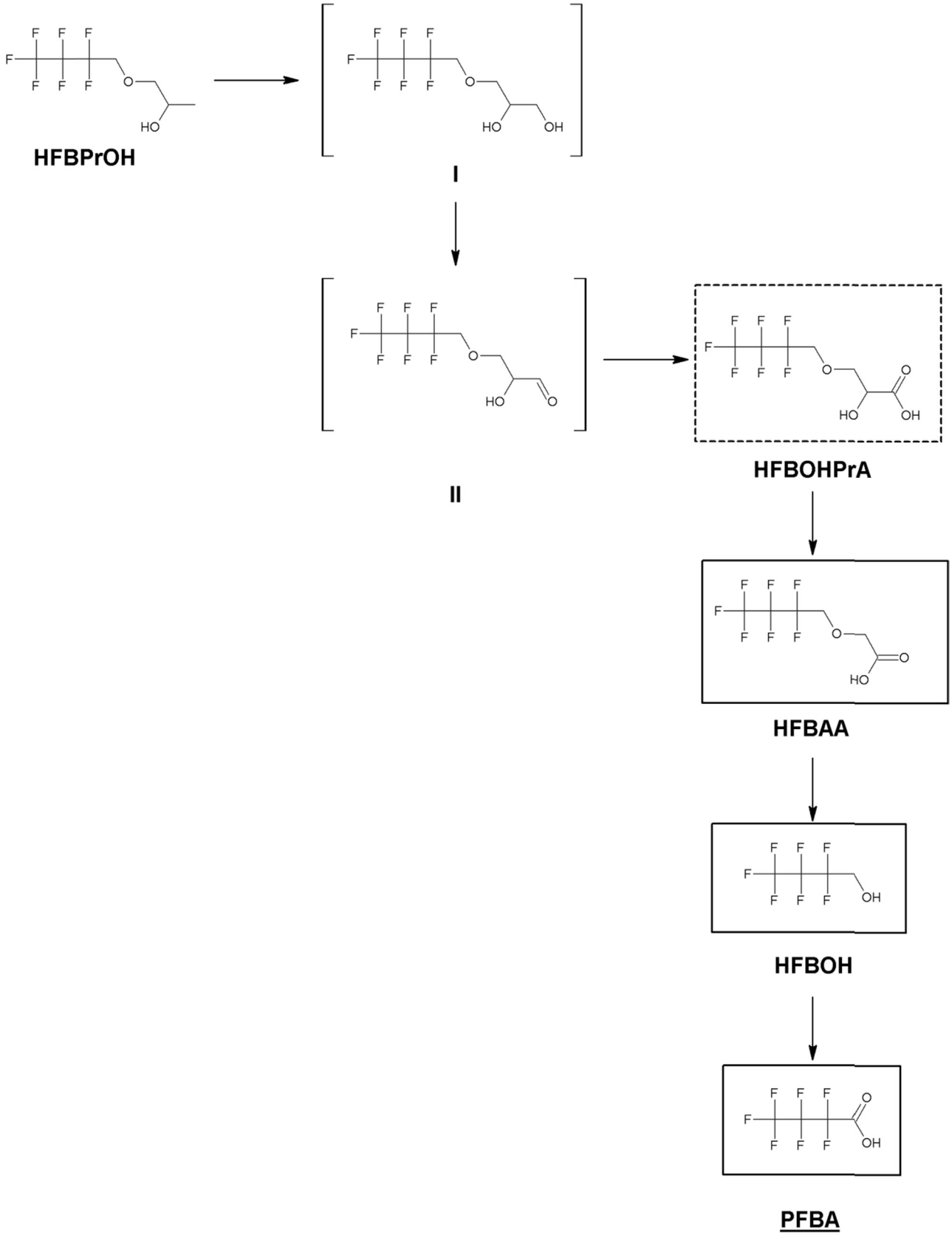

Fig. 6. Proposed degradation pathway of HFBPrOH with PFBA being the dead-end transformation product. The dotted rectangle indicates detection of the transformation product without confirmation by MS/MS, the rectangle indicates detected and confirmed transformation products. T **I** and **II** were not detected.

Since both TFMHxA and TFMC are degraded and no further organic transformation product was detected, it is hypothesized that both transformation products yield inorganic fluoride. Since the curves for TFMHxA and TFMC are not temporally shifted, no link between the two transformation products can be made, and yet, TFMHxA is assumed to be transformed to TFMC in a similar manner as TFMHxOH is metabolized itself. That is, TFMHxA could be oxidized to an alcohol in proximity to the ether group yielding the hemiacetal **I**. In turn, this can be oxidized to the ester **II**, which is oxidized to the Baeyer–Villiger-like carbonate **III**. Ester hydrolysis yields TFMC, which is eventually mineralized.

Simultaneously, oxidation of TFMHxOH analogous to TFMPrOH proceeds. Even more complex, transformation products **IV** to **VI** might be oxidized to the respective molecule on the left side. For instance, **IV** might be oxidized to **I** by oxidation of the terminal hydroxyl group to the carboxylic acid. Which of these reactions take place cannot be scrutinized here because some of these reactions are supposed to proceed very rapidly and thus the intermediates remain undetected.

Alternatively, TFMHxA might be shortened via β-oxidation, as was initially presumed during the investigation of 10-(trifluoromethoxy)decane-1-sulfonate by Peschka et al. [10] and also in other studies carried out with ω-substituted alkane-1-sulfonates, which were initially transformed to the respective carboxylates [11,12]. This would involve the two transient transformation products **VII** and **VIII** as shown in pathway C, whereas subsequent β-oxidation of **VIII** would contribute to the presence of TFMeOH. However, a β-oxidation pathway would not form TFMC, indicating that at least one other transformation pathway must be involved. Also, **VII** and **VIII** were not detected by MS, even though they are supposed to be analytes that can be detected in negative ESI mode in a straightforward way being at least semi-strong acids.

The complete defluorination renders the 6-(trifluoromethoxy)hex-1-oxy group a promising building block for environmentally friendly substitutes of PFASs. If implemented in larger organic molecules via an ester bridge, TFMHxOH is a likely to be formed by chemical or enzymatic ester hydrolysis. The transformation rate is probably highly depending on the structure of the whole molecule, especially when it comes to enzymatic cleavage, where steric aspects may predominate.

3.3. 1-(2,2,3,3,4,4,4-Heptafluorobutoxy)-propan-2-ol

HFBPrOH was found to be measurable as the acetate adduct $[M+acetate]^-$, similar to FTOHs [17]. In order to increase reproducibility of the quantitative analysis, 6:2-FTOH was used as an internal standard.

As illustrated in Fig. 5a, the initially spiked concentration of HFBPrOH could not be verified by HPLC–MS measurement, which is probably due to pronounced adsorption of the compound. In the sterilized assay, the concentration remains stable around 20 μM for 45 days, whereas in the active assay, a drop in concentration can be observed after 8 days. Primary biodegradation then proceeds rapidly so that almost no HFBPrOH can be detected after 14 days.

Different transformation products were sought for by HPLC–MS/MS in MRM mode or SIM mode with subsequent verification by MS/MS measurements. Non-acidic potential transformation products, i.e. those carrying only ether and hydroxyl groups, were monitored as $[M+acetate]^- \rightarrow$ acetate. The structures of these transformation products are shown in the Supplementary material.

Several transformation products were observed, which were neither detected in the sterilized nor in the control assay. Unfortunately, for non-acidic compounds, no further verification of the structure could be made, since acetate adducts fragment by dissociation to an acetate ion and the neutral molecule it was attached to, which disallows further measurement by MS. The structure of HFBOHPrA could not be verified. In SIM mode, a compound with an m/z of 287 was detected and its temporal evolution recorded as shown in. However, no significant product ions were detected in enhanced product ion mode as a result of the low intensity. Thus, its structure was only postulated but not confirmed. For HFBAA however, verification by MS/MS experiments was carried out. The corresponding product ion spectrum and explanation of product ions is shown in the Supplementary material.

The temporal evolution of the transformation products is illustrated in Fig. 5a. The first substances were detected after four days, except for PFBA, which was only measured above the LOD after 14 days, which suggests that PFBA is generated via an indirect pathway, i.e. via non-detected transient transformation products.

It is suggested that PFBA is generated by a complex pathway as shown in Fig. 6. Starting with a hydroxylation in α-position (**I**) and subsequent oxidation of this terminal hydroxyl group to the aldehyde (**II**), the lactic acid derivative 3-(2,2,3,3,4,4,4-heptafluorobutoxy)-2-hydroxypropanoic acid (HFBOHPrA) could be formed. This in turn is transformed to (2,2,3,3,4,4,4-heptafluoroboxy)acetic acid (HFBAA) by α-oxidation. β-Oxidation then leads to 2,2,3,3,4,4,4-heptafluorobutan-1-ol (HFBOH), which is easily converted to PFBA, probably via the corresponding aldehyde. As expected, PFBA seems to be the dead-end transformation product of HFBPrOH, since no fluoride was detected. The transformation products **I** and **II** were not detected, but highly reductive compounds such as aldehydes or vicinal diols are rarely detected in degradation studies.

4. Conclusion

Three different candidates for building blocks in novel fluorinated surfactants were investigated with respect to their degradability.

The two structurally related TFMPrOH and TFMHxOH showed analogous transformation products, but a drastic difference in mineralization yield, expressed as the molar percentage release of fluoride. This is due to the stability of TFMPrA, the carboxylic acid associated to TFMPrOH, which is generated in the biotransformation assay, but not further degraded. In contrast to this, the carboxylic acid derivative of TFMHxOH, TFMHxA, is generated and completely transformed to other transformation products. Thus, TFMHxOH biotransformation yielded nearly 100% of the theoretical fluoride, but TFMPrOH was only defluorinated to an extent of 15%. It is assumed that the remaining 85% are accumulated in form of TFMPrA, whose environmental behavior is unknown. Thus, from an environmental point of view, TFMHxOH would be a suitable substitute for longer-chained PFASs. However, it is questionable whether the compound can compete with long-chained fluorinated compounds in terms of performance.

The reason for the dissimilar stability of TFMHxA and TFMPrA cannot be entirely explained with the knowledge obtained. One possible reason is a different accessibility of the methylene group in vicinity to the TFM group in the two compounds. In TFMPrA, the methylene group might be shielded by the polar carboxylic acid function, so that it cannot be hydroxylated enzymatically, which in turn could be possible in TFMHxA, where the carboxylic acid group is separated by more methylene groups. However, these suggestions remain speculative unless one or several transient transformation products are detected in other studies.

Interestingly, the implementation of ether bridges as in TFMHxOH and TFMPrOH does not imply biotransformation. Such ether bridges are also implemented into new substitutes for traditional PFASs, such as perfluoroether carboxylic acids and

perfluoroether sulfonic acids, which were also found not to be biodegraded [8].

From an environmental point of view, HFBPrOH exhibits both advantageous and disadvantageous properties. Whereas it does not lead to precarious and hazardous transformation products like PFOS or PFOA, it is also not mineralized, which would represent the ideal case. Instead, the already known substance PFBA is generated via biotransformation. PFBA is not a novel PFAS and has been detected in environmental samples for several years [18–21]. PFBA has been shown to exhibit less environmentally adverse effects, such as less pronounced bioaccumulation and toxicity [22]. Ecotoxicity of PFBA has been investigated and it also gives rise to dramatically reduced effects when compared with longer chained PFCAs [14,15]. Whereas most properties and environmental effects of short-chained PFASs seem to be more benign as compared with their long-chained counterparts, there is at least one drawback of the short perfluoroalkyl chain: their mobility. It was shown that PFBA is not retained during soil passage [23], which in turn indicates that it may easily reach groundwater and eventually drinking water, where it has already been detected [24,25], sometimes even up to the $\mu g\,L^{-1}$ range as PFBA is as persistent in the environment as its longer-chained homologs [6,9]. Thus, it can be stated that this C_3 perfluorinated building block represents a compromise between satisfactory performance and acceptable environmental and toxicological profile.

Acknowledgement

The authors would like to thank Karl-Heinz Bauer for fluoride measurements.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfluchem.2015.06.015.

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