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Enzymatic fluorination using fluoride ion generated from degradation of fluorinated materials

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ABSTRACT

The recycle of fluoride ion generated from the degradation of 1-methyl-3-butylimidazolium tetrafluoroborate, diethyl methyl methoxyethylammonium tetrafluoroborate in the presence of Tris–HCl buffer solution and/or the biodegradation of fluorobenzene and benzotrifluoride, was described. The generated fluoride ion was reused to produce 5′-fluoro-5′-deoxyadenosine (5′-FDA), fluoroacetate and/or 4-fluorothreonine.

1. Introduction

The challenge in chemistry to develop the 3R (reuse, reduce and recycle) based on the idea of green chemistry is one of the most important issues in the scientific society [1,2]. In the fluorine science, the recycle of fluorine resource is important factor for the purpose of the sustainable development. While fluorinated materials are most stable compounds in the environment, nature seems to be reluctant to develop biological defluorination concerning *p*-fluoroaniline with horseradish peroxidase [3], fluoroacetate with monofluoroacetate dehalogenase (*Ipaeudomonas indoloxidans*, *Pseudomonas cepacia*, *Moraxella* sp., *Burkholderia* sp.) [4,5] and biodegradability of trifluoroacetic acid [6]. Further, fluoraromatic compounds are biodegraded under aerobic conditions, although anaerobic degradation has also been reported [7]. Recently, it is reported that a silylium-carborane catalyst could break C–F bonds in perfluoroalkyl groups [8]. Further, we have also reported the biological transformation of 2,2-difluoroethanol to difluoroacetic acid [9] and defluorination of fluorinated materials by bacteria. Until now, various kinds of organic synthetic [10] and/or biosynthetic methods [11] in fluorine chemistry have been reported, however it remained the recycle of fluoride ion generated from the degradation or biodegradation of fluorinated materials, producing fluorinated materials by the enzymatic fluorination.

In this paper, we would like to report some processes for the retransformation of a fluoride ion derived from the degradation of fluorinated materials by fluorinase or *Streptomyces cattleya* NBRC14057, producing 5′-fluoro-5′-deoxyadenosine (5′-FDA) or 4-fluorothreonine and fluoroacetate.

2. Results and discussion

In attempting to develop the recycling of a fluorine atom, at first, we have examined the generation of fluoride ion derived from the degradation of several types of fluorinated materials in the presence of Tris–HCl buffer (pH 8.0) and/or water. Until now, the degradation of 1-allyl-3-ethylimidazolium tetrafluoroborate in the presence of water, was reported [12]. Indeed, after screening of the fluorinated materials in Tris–HCl buffer (pH 8.0) and/or water, we have found that several kinds of BF_4 ionic liquids were degradated smoothly. To make clear the degradation of BF_4 ionic liquids, we have examined the time course for the degradation of several types of BF_4 ionic liquids in water and/or Tris–HCl buffer solution at 30 °C. From the results shown in Table 1, we have found that ionic liquid ([bmim][BF_4]) smoothly degradated in water in time dependent manner. After degradating for 1, 3, 5 and 72 h, about 0.04, 0.13, 0.27 and 6.69% of 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF_4]) was degradated in water. Further, Table 1 shows time course of the degradation of several types of BF_4 ionic liquids such as ([bmim][BF_4] and *N*,*N*-diethyl-*N*-methyl-*N*-(methoxyethyl)ammonium tetrafluoroborate [DEME][BF_4]) by water, however, some kinds of ionic liquids such as 1-butyl-3-

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Table 1
Time course of degradation (%) of ionic liquid.

Time (h)	[bmim][BF_4]	[DEME][BF_4]	[bmim][PF_6]	[bmim][TFSA]
1	0.04	0.05	0.00	0.00
2	0.07	0.10	0.00	0.00
3	0.13	0.15	0.00	0.00
4	0.19	0.21	0.00	0.00
5	0.27	0.27	0.00	0.00
6		1.75		
72	6.69	3.40		
96	7.90	4.16		
168	16.4	8.03		
366	17.6	11.8		
648	22.8	17.3	0.06	0.00

Table 2
Conversion yield.

Entry	Ionic liquid or Tris–HCl buffer	Conv. yield (%)
1	[bmim][BF_4]	11.9
2	[DEME][BF_4]	5.1
3	Tris–HCl buffer[a]	5.7

[a] The mixture of fluorinase (200 μl, 1 mg/ml), KF (2 μmol), SAM (50 nmol) in Tris–HCl buffer solution (1 ml) was stirred for 5 h at 37 °C.

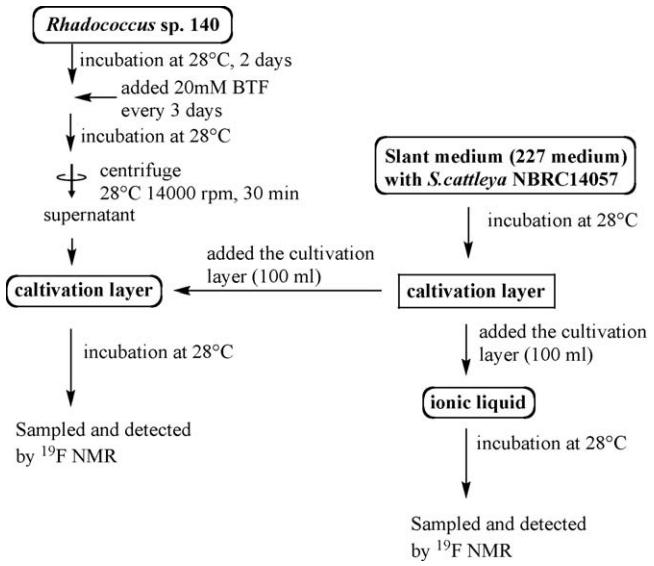
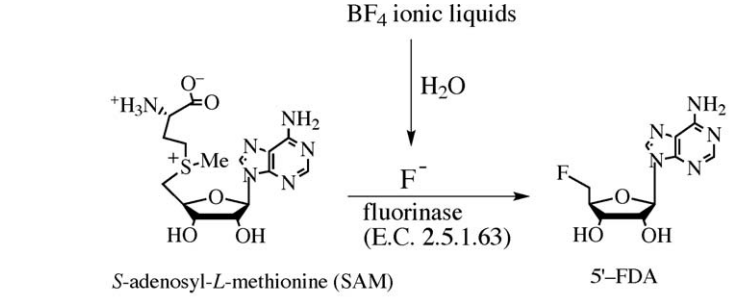


Fig. 1. The recycle of fluorine resource generated from BTF and/or ionic liquid with *Streptomyces cattleya* NBRC14057.

methyl-imidazolium hexafluorophosphate ([bmim][PF6]) and 1-butyl-3-methylimidazolium bis(trifluoromethanesulfonyl)amide ([bmim][TFSA]) did not degradate in water as shown in Table 1 Further, $NaBF_4$ do not release a fluoride ion in water at room temperature. Until now, it is not clear the reason for the degradation of BF_4 ionic liquids [13].

In the next step, we have examined the circulatory of the fluorine resource to introduce the generated fluoride ion into *S*-adenosyl-*L*-methionine (SAM) to produce 5′-FDA with fluorinase. We examined the assembly of the fluorine resource circulatory system exploiting fluorinase using the above system which was constructed from the *flA* protein, SAM, Tris–HCl buffer solution (pH 8.0) and BF_4 ionic liquid. After incubating for 18 h at room temperature, 5′-FDA was extracted with diethyl ether, and analyzed by HPLC. From the results shown in Table 2, we have found that fluorination of SAM with fluorine enzyme (fluorinase) was smoothly proceeded by using fluoride ion derived from the degradation of BF_4 ionic liquids. In the above reaction system, there is no fluorine resource except the degradation of [bmim][BF_4]. When we used [bmim][PF_6] and/or [bmim][TFSA] in the above reaction system, the fluorination did not proceed. In the meanwhile, we have revealed that the general fluorination reaction with fluorinase (200 μl, 1 mg/ml)–Tris–HCl buffer solution (pH 8.0, 1 ml) in the presence of KF (2 μmol, 1 mM in H_2O) afforded 5′-FDA in 5.7% conversion yield based on SAM (50 nmol) used. In the case of [bmim][BF_4] as a fluorine resource, we have found that the conversion yield was increased up to 2 times more than that of KF system (Scheme 1).



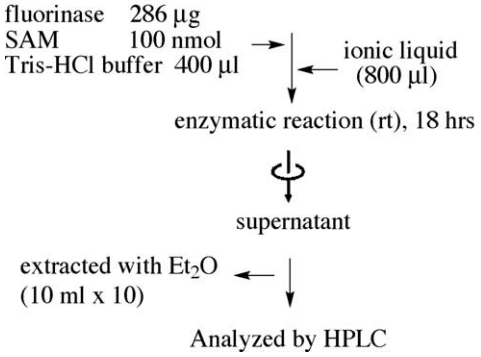
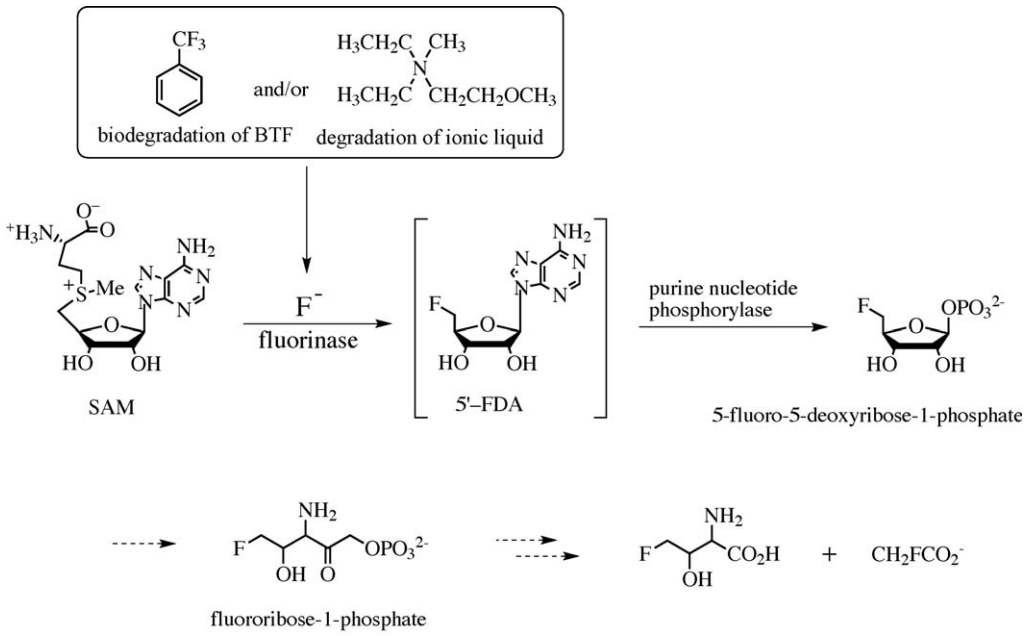


Table 2. Conversion yield

Entry	Ionic liquid or Tris-HCl buffer	Conv. Yield (%)
1	[bmim][BF_4]	11.9
2	[DEME][BF_4]	5.1
3	Tris-HCl buffer[a]	5.7

[a] The mixture of fluorinase (200 μl, 1 mg/ml), KF (2 μmol), SAM (50 nmol) in Tris-HCl buffer solution (1 ml) was stirred for 5 hrs at 37°C.

Scheme 1. Recycle of fluorine resource exploiting fluorinase.



Scheme 2. Synthetic routes to 4-fluorothreonine and fluoroacetate using fluoride ion generated from BTF and ionic liquid [DEME][BF_4] with *Streptomyces cattleya* NBRC14057.

Further, as we have reported that it was possible to degradate fluorobenzene (FB) and/or benzotrifluoride (BTF) by microorganism [14], we have examined the reuse of the fluorine atom released from the degradation of FB and/or BTF by microorganism to make clear the recycle of the fluorine resource. In the biodegradation, fluoride ion (FB: 141 nmol/ml; BTF: 126 nmol/1 ml) was detected by fluoride ion selective electrode. In the recycle of fluorine resource, the fluorination in the reaction system derived from *flA* protein, SAM, Tris–HCl buffer and the above cultivation layer was carried out for 6 h at 37 °C. The fluorination using the cultivation layer obtained the biodegradation of fluorobenzene and/or benzotrifluoride proceeded smoothly to produce 5′-FDA (1.14 nmol from fluorobenzene cultivation layer and 2.07 nmol from benzotrifluoride cultivation layer).

Furthermore, we have examined the recycle of fluorine resource with *S. cattleya* NBRC14057 instead of fluorinase enzyme. The cultivation layer based on the biodegradation of BTF was added into the cultivation layer of *S. cattleya* NBRC14057, and then the mixture was cultivated for 3 weeks at 28 °C (250 rpm) (Fig. 1). The fluorinated products (fluoroacetate and 4-fluorothreonine) were detected by ^{19}F NMR spectrum [15–17]. In this ^{19}F NMR spectrum, we have determined the productivity of fluoroacetate (537 nmol) and 4-fluorothreonine (253 nmol) by the use of the difluoroacetic acid as an internal standard. In the fluorination reaction by *S. cattleya* NBRC14057, it is reported that the produced 5′-FDA is transformed smoothly to 5-fluoro-5-deoxyribose-1-phosphate and then fluororibose-1-phosphate with several kinds of enzymes shown in Scheme 2, and as the final products fluoroacetate and 4-fluorothreonine are detected [11,17]. In our above fluorination system, the transformation of fluoride ion from cultivation layer to fluorinated materials such as fluoroacetate and 4-fluorothreonine was proceeded smoothly via 5′-FDA, 5-fluoro-5-deoxyribose-1-phosphate and then fluororibose-1-phosphate. Furthermore, an ionic liquid [DEME][BF_4] was added into the cultivation layer of *S. cattleya* NBRC14057, and then the mixture was cultivated for 2 months at 28 °C (250 rpm). We have found that it is possible to use an ionic liquid (DEME)[BF_4] for the recycle of fluorine resource, producing fluoroacetate (163 nmol) and 4-fluorothreonine (115 nmol) (Fig. 1 and Scheme 2).

3. Conclusion

We have established the enzymatic fluorination of SAM with a fluoride ion generated from the degradation of BF_4 ionic liquids and biodegradation of fluorobenzene and benzotrifluoride, giving 5′-FDA. Furthermore, we have also mentioned the enzymatic syntheses of fluoroacetate and 4-fluorothreonine using a fluoride ion generated from the degradation of fluorinated materials. This is the first example of the recycle of fluorine resource.

4. Experimental

4.1. General

All commercially available reagents were used without further purification. The ^{19}F NMR (470 MHz) spectra were recorded in ppm downfield from internal standard C_6F_6 in $CDCl_3$ using a VXR 500 instrument.

4.2. Materials and methods

4.2.1. Bacterial strains and plasmids

E. coli JM109 and BL21(DE3) pLysS were used for cloning and enzyme engineering, respectively. The fluorinase gene (*flA*) was amplified from the *S. cattleya* NBRC14057 genome using primers reported previously [7]. Only a single, silent mutation in *flA* was detected by CEQ8000 DNA analysis (Beackman Coulter). The gene was cloned in pET28b+ (Novagen) and consequently fused to the His-tag-containing peptide in the *N*-terminal of the enzyme, termed pETflA. It was sub-cloned into pALTER1 (Promega) using DraIII and SphI restriction enzymes, termed pALTERflA. K_m and k_{cat} of fluorinase were reported in the literature [18].

4.3. General procedure

(a) The mixture of the *flA* protein (200 μl, 1 mg/ml), 50 nmol of SAM, Tris–HCl buffer (1 ml), KF (2 μmol) was incubated. After incubating for 6 h at 37 °C, 5′-FDA was extracted with diethyl ether (10 ml x 10). After removing the solvent, the mixture

solution of buffer solution (40 μl, KH_2PO_4, pH 7.0) and MeOH (10 μl) was added into the above residue. The product peak and yields (5.7%) from SAM to 5′-FDA were determined by HPLC analyses (column; shim-pack VP-ODS 150L x 4.6; SHIMADZU) according to a previous report [16].

(b) The mixture of the *flA* protein (286 ng, 1 mg/ml), 100 nmol of SAM, Tris–HCl buffer (400 μl) and ionic liquid (800 μl) was incubated. After incubating for 18 h at room temperature, 5′-FDA was extracted with diethyl ether (10 ml × 10), and then worked-up similarly.

4.4. Degradation of benzotrifluoride (BTF) and/or fluorobenzene

Into the test tube production medium (4 ml) derived from starch (0.5%), sucrose (0.5%), N.Z.Amine (0.25%), peptone (0.25%), yeast extract (0.2%), extract ehlich (0.1%), KH_2PO_4 (0.1%), $MgSO_4{\cdot}7H_2O$ (0.05%) and buffer solution consisted of Na_2HPO_4 and KH_2PO_4, 200 μl of culture was inoculated. Into the above test tube, benzotrifluoride (or fluorobenzene; 4–16 mM) was added, and then the degradation of benzotrifluoride (or fluorobenzene) was carried out at 28 °C for 2 weeks aerobically. Fluoride ion (fluorobenzene: 198 nmol/1.4 ml; benzotrifluoride: 126 nmol/1 ml) was detected by ISE combination fluoride (ION pH/mV/ORP). In the next step, the cultivation layer containing fluoride ion was used for the recycle of fluorine resource to introduce the fluorine atom onto SAM to produce 5′-FDA.

4.5. Fluorination using the cultivation layer of the degradation of benzotrifluoride (BTF) and/or fluorobenzene

(a) Into the mixture of the *flA* protein (200 μl, 0.2 mg/ml), 85 nmol of SAM and Tris–HCl buffer (pH 8.0, 1 ml), the above cultivation layer (1 ml; FB: concentration of fluoride ion 141 nmol/ml) was added. After incubating for 6 h at 37 °C, 5′-FDA was extracted with diethyl ether (10 ml × 10), and 5′-FDA (1.14 nmol: consumed fluoride ion; 0.58%) was produced by the analysis with HPLC (column; shim-pack VP-ODS 150L x 4.6; SHIMADZU).

(b) In the above reaction, the cultivation layer (1 ml; BTF: concentration of fluoride ion 126 nmol/ml) and SAM (50 nmol) were used, and work-up similarly, giving 5′-FDA (2.07 nmol: consumed fluoride ion; 1.64%).

4.6. General procedure with S. cattleya NBRC14057

(a) *S. cattleya* NBRC14057 was cultivated in the 227 medium (0.4% dried yeast extract, 1.0% malt extract 0.4% glucose) and buffer solution consisted of Na_2HPO_4 and NaH_2PO_4 for 2 weeks at 28 °C. Into the above medium, the cultivation layer (BTF: fluoride ion concentration 1.25 mmol) was added, and then the mixture was cultivated for 3 weeks at 28 °C (250 rpm). The fluorinated products (monofluoroacetate and 4-fluorothreonine) were detected by ^{19}F NMR spectrum, and their productions were determined by the ^{19}F NMR integration using CHF_2CO_2H as an internal standard. ^{19}F NMR. Fluoroacetate: −54.0 (t, J = 48.8 Hz) ppm. 4-fluorothreonine: −68.6 (td, J = 47.3, 24.4 Hz) ppm from C_6F_6.

(b) *S. cattleya* NBRC14057 was cultivated in the 227 medium (0.4% dried yeast extract, 1.0% malt extract 0.4% glucose) and buffer solution consisted of Na_2HPO_4 and NaH_2PO_4 for 2 weeks at 28 °C. Into the above medium, an ionic liquid (*N*,*N*-diethyl-*N*-methyl-*N*-(methoxyethyl)-ammonium tetrafluoroborate: [DEME][BF_4]) was added to keep the substrate concentration (20 mmol) in medium (4 ml), and then the mixture was cultivated for 2 months at 28 °C (250 rpm). The production of fluorinated materials (fluoroacetate (163 nmol) and 4-fluorothreonine (115 nmol)) was determined by the ^{19}F NMR integration using CHF_2CO_2H as an internal standard.

References

[1] P. Tundo, P.T. Anastas, Green Chemistry: Challenging Perspectives, Oxford Science, Oxford, 1999.
[2] R. Natarajan, R. Azerad, B. Badet, E. Copin, J. Fluorine Chem. 126 (2005) 424–442.
[3] G.M.K. Hughes, B.C. Saunders, Chem. Ind. (1954) 1265.
[4] (a) N. Horiuchi, J. Agric. Chem. Soc. Jpn. 35 (1961) 870–873;
(b) P. Goldman, G.W.A. Milne, J. Biol. Chem. 241 (1966) 5557–5559;
(c) H. Kawasaki, K. Tsuda, I. Matsushita, K. Tonomura, J. Gen. Microbiol. 138 (1992) 1317–1323;
(d) T. Kurihara, T. Yamauchi, S. Ichiyama, H. Takahata, N. Esaki, J. Mol. Catal. B: Enzym. 23 (2003) 347–355.
[5] A.H. Neilson, A.-S. Allard, A.H. Neilson (Eds.), Degradation and Transformation of Organic Fluorine Compounds, Organofluorines, vol. 3, Springer-Verlag, Berlin, 2002, pp. 137–202.
[6] B.R. Kim, M.T. Suidan, J.T. Wallington, X. Du, Environ. Eng. Sci. 176 (2000) 337–342.
[7] (a) B. Song, N.J. Palleroni, M.M. Haggblom, Appl. Environ. Microbiol. 66 (2000) 3446–3453;
(b) C. Vargas, B. Song, M. Camps, M.M. Haggblom, Appl. Microbiol. Biotechnol. 53 (2000) 342–347;
(c) M.F. Carvalho, R.F. Jorge, C.C. Pacheco, P.De Marco, P.M.L. Castro, Environ. Microbiol. 7 (2005) 294–298.
[8] C. Douvris, O.V. Ozerov, Science 321 (2008) 1188–1190.
[9] Y. Morii, F. Hasumi, T. Kitazume, J. Fluorine Chem. 125 (2004) 731–733.
[10] (a) M. Hudlicky, E. Paviath, Chemistry of Organic Fluorine Compounds II: A Critical Review, ACS, Washington, DC, 1995;
(b) I. Ojima, J.R. McCarthyl, J.T. Welch (Eds.), Biomedical Frontiers of Fluorine Chemistry, ACS Symposium Series 639, ACS, Washington, DC, 1996;
(c) R.D. Chambers (Ed.), Organofluorine Chemistry: Technique and Synthons, 193 Topics in Current Chemistry, Springer-Verlag, Berlin, 1997;
(d) V. Soloshonok (Ed.), Enantiocontrolled Synthesis of Fluoro-Organic Compounds, John Wiley & Sons, NY, 1999;
(e) P.V. Ramachandran (Ed.), Asymmetric Fluororganic Chemistry, ACS Symposium Series 746, ACS, Washington, DC, 1999;
(f) T. Kitazume, T. Yamazaki, Experimental Method in Organic Fluorine Chemistry, Kodansha & Gordon and Breach Science, Tokyo, 1998.
[11] (a) C. Deng, F. Huang, H. Deng, C. Schaffrath, J.B. Spencer, D. O'Hagan, J.H. Naismith, Nature 427 (2004) 561–565;
(b) S.L. Cobb, H. Deng, J.T.G. Hamilton, R.P.D. McGlinchey, D. O'Hagan, Chem. Commun. (2004) 592–593;
(c) S.L. Cobb, H. Deng, J.T.G. Hamilton, R.P.D. McGlinchey, D. O'Hagan, C. Schaffrath, Bioorg. Chem. 33 (2005) 393–401;
(d) H. Deng, S.L. Cobb, A.R. McEwan, R.P. McGlinchey, J.H. Naismith, D. O'Hagan, D.A. Robinson, J.B. Spencer, Angew. Chem. Int. Ed. 45 (2006) 759–762;
(e) C.D. Cadicamo, J. Courtieu, H. Deng, A. Meddour, D. O'Hagan, ChemBioChem 5 (2004) 685–690.
[12] T. Inoue, The Chemical Times 209 (2008) 19–21.
[13] (a) H. Ohno (Ed.), Electrochemical Aspects of Ionic Liquids, John Wiley & Sons, Inc Publication, 2005;
(b) R.D. Rogers, K.R. Seddon (Eds.), Ionic Liquids as Green Solvents: Progress and Prospects, ACS Symposium Series, vol. 856, ACS, Washington, DC, 2003;
(c) M.A. Abraham, L. Moens (Eds.), Clean Solvents, ACS, Washington, DC, 2002.
[14] N. Iwai, R. Sakai, S. Tsuchida, M. Kitazume, T. Kitazume, J. Fluorine Chem. 130 (2009) 434–437.
[15] (a) M. Sanada, T. Miyano, S. Iwadare, J.M. Williamson, B.H. Arison, J.L. Smith, A.W. Douglas, J.M. Liesch, E.J. Inamine, Antibiotics 39 (1986) 259–265;
(b) T. Tamura, M. Wada, E. Esaki, K. Soda, J. Bacteriol. 177 (1995) 2265–2269.
[16] (a) S.J. Moss, C.D. Murphy, J.T.G. Hamilton, W.C.O. McRoberts, D. O'Hagan, C. Schaffrath, D.B. Harpeer, Chem. Commun. (2000) 2281–2282;
(b) C.D. Murphy, D. O'Hagan, C. Schaffrath, Angew. Chem. Int. Ed. 40 (2001) 4479–4481.
[17] (a) D. O'Hagan, C. Schaffrath, S. Cobb, J.T.G. Hamilton, C.D. Murphy, Nature 416 (2002) 279;
(b) D. O'Hagan, R.J.M. Goss, A. Meddour, J. Courtieu, J. Am. Chem. Soc. 125 (2003) 379–387;
(c) H. Deng, S.L. Cobb, A.D. Gee, A. Lockhart, M. Laurent, R.P. McGlinchey, D. O'Hagan, M. Onega, Chem. Commun. (2006) 652–654;
(d) S.L. Cobb, H. Deng, A.R. McEwan, J.H. Naismith, D. O'Hagan, D.A. Robinson, Org. Biomol. Chem. 4 (2006) 1458–1460.
[18] X. Zhu, D.A. Robinson, A.R. McEwan, D. O'Hagan, J.H. Naismith, J. Am. Chem. Soc. 129 (2007) 14597–14604.