Synthesis of Symmetric and Dissymetric Bisperfluoroalkanesulfonylimides and Evaluation of Their Inhibition on Bovine Carbonic Anhydrase

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ABSTRACT: *This study describes a synthesis of symmetric and dissymmetric bis[(perfluoroalkane)sulfonyl]imides by the reaction of the sodium salt of perfluoroalkanesulfonamide $R_F SO_2 NH^- Na^+$ ($R_F = C_4F_9$, C_6F_{13}, C_8F_{17}) with hexamethyldisilazane and perfluoroalkanesulfonylfluoride $R_F SO_2 F$ ($R_F = C_4F_9$, C_6F_{13}, C_8F_{17}). They are obtained, in two steps, in moderate overall yield. Moreover, this paper provides a study of their inhibition on bovine carbonic anhydrase.* © 2008 Wiley Periodicals, Inc. Heteroatom Chem 19:542–548, 2008; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.20452

INTRODUCTION

It has been known for a few years that the substitution of a hydrogen atom by a fluorine atom in organic compounds can modify their physical, chemical, and biological properties. Consequently, the synthesis of organofluorine compounds has become an important area of chemistry both in academia and industry [1–3].

Perfluoroalkanesulfonimides are of increasing interest for their high Brönsted acidity, electrochemical properties, and high thermal and chemical stability.

Salts of anions of bis[(perfluoroalkane)sulfonyl]-imides have been found to serve as solutes for polymer electrolytes leading to dramatically improved performance in lithium batteries and fuel cells [4–6]. They also present a significant interest as intermediates of electrophilic fluorinating agents [7,8], in ionic liquids [9], as Lewis acid catalysts do for Diels Alder [10] and Friedel–Crafts acylation [11]. However, until now they have never been studied for their biological properties, in particular for their activity on bovine carbonic anhydrase (bCA).

Our research team has developed new methodologies of syntheses and aim at reaching to various families of fluorinated molecules, in particular those comprising the following sulfur functions: sulfonic acids and sulfinic acids derivatives [12–14]. We were interested with the perfluoroalkanesulfonimides because it is known that sulfonamides [15,16], in particular trifluoromethanesulfonamide was an inhibitor of bCA [17]. Because of the analogy of structure of perfluoroalkanesulfonylimides with perfluoroalkanesulfonamides, we thought that these compounds could inhibit the bCA enzyme.

The perfluoroalkanesulfonimides could be prepared by different methods. They involve a multistep

reaction using sodium perfluoroalkanesulfonamides with hexamethyldisilizane and perfluoroalkanesulfonylfluorides [18–20], or a one-pot synthesis by the reaction of perfluoroalkanesulfonylfluoride with liquid NH_3 in the presence of triethylamine as a base under $-40°C$ [21]. Also the reaction could be made from perfluoroalkanesulfonamides with perfluoroalkanesulfonylhalides in the presence of nonnucleophilic base such as lithium carbonate [22], or a one-pot reaction of perfluoroalkanesulfonylhalides with acetamide derivatives in the presence of K_2CO_3 [23], or a protonation of N-sulfonyl trichlorophosphazenes with sulfonic acids [24].

In the present paper, we describe the synthesis of symmetric and dissymmetric bis[(perfluoroalkane)sulfonyl]imides using a sodium salt of perfluoroalkanesulfonamides, hexamethyldisilazane (HMDS), and perfluoroalkanesulfonylfluoride. Then, we have evaluated their inhibition in vitro on bCA.

RESULTS AND DISCUSSION

Chemistry

In our experiments, the method was based on that used by Desmarteau et al. to prepare perfluoroalkanesulfonylimides [18,19]. The procedure to synthesize bis[(perfluoroalkane)sulfonyl]imides is shown in Scheme 1.

The sodium perfluoroalkanesulfonamides (**1**) were synthesized starting from perfluoroalkanesulfonamides and sodium methylate; their synthesis was previously described [25].

The N-silyl form (**2**) was prepared by the reaction of sodium perfluoroalkanesulfonamide (**1**) with an excess of HMDS in acetonitrile at $110°C$ for 24 h. In our experiment, it was difficult to analyze this intermediate (**2**) by NMR spectroscopy or mass spectroscopy because of its great moisture sensitivity. We considered that compound **2** was formed in
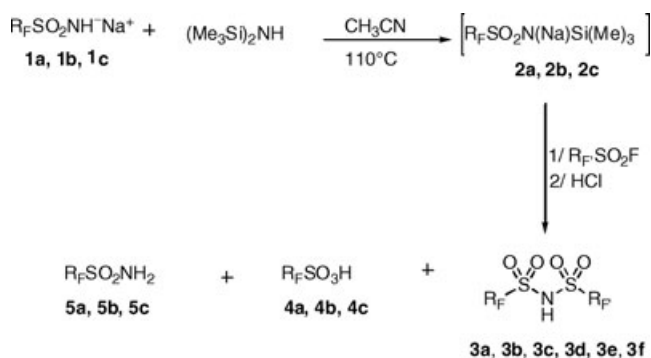
the reaction media. Finally, the N-silyl form (**2**) react with an excess of perfluoroalkanesulfonylfluoride, in THF at $70°C$ from 12 to 24 h, and lead to bis[(perfluoroalkane)sulfonyl]imides (**3**) after acidification by aqueous solution of HCl in a range varying from 46% to 62% yield based on starting amount of **1**.

In the last reaction described in Scheme 1, three different products were obtained. In the first part, there was the formation of the expected product: the perfluoroalkanesulfonylimide (**3**), in the second part the formation of the perfluoroalkanesulfonic acid (**4**), and in the third part perfluoroalkanesulfonamides (**5**). These compounds were identified and characterized by ^{19}F NMR spectroscopy and FAB spectroscopy.

We determined the conversion of each chemical species by ^{19}F NMR spectroscopy (Table 1).

We can note that the formation of the perfluoroalkanesulfonamides (**5**) was probably due to the hydrolysis of the N-silyl form (**2**) at the time of the acid treatment, which can be interpreted by the fact that the N-silyl form intermediate did not completely react.

The formation of the perfluoroalkanesulfonic acid (**4**) was probably related to the hydrolysis of the perfluoroalkanesulfonylfluoride (in excess in the reaction), which did not react.

The perfluoroalkanesulfonylimides (**3**) were purified by silica gel chromatography and were characterized by ^{19}F NMR spectroscopy and HRMS.

Carbonic Anhydrase Inhibition

Carbonic anhydrases (CAs) are zinc metalloenzymes, which catalyze the reversible hydration of carbon dioxide into bicarbonate and therefore are vital to many biological and physical functions. The inhibition of these enzymes may be clinically exploited in the treatment or prevention of a variety of disorders. In consequence, CA inhibitors (CAIs) possess a variety of applications in therapy. CA inhibition with sulfanilamide discovered by Mann and Keilin was the beginning of a great scientific adventure that led to important drugs such as the antihypertensives of benzothiadiazine and high-ceiling-type diuretics, the sulfonamides with CA inhibitory properties are mainly used as antiglaucoma agents, some antithyroid drugs, hypoglycemic sulfonamides, and ultimately some novel types of anticancer agents [15,26,27].

In this paper, we prepared bis[(perfluoroalkane)sulfonyl]imides and they were evaluated for inhibition on bCA. Not only does CA catalyze the reversible hydration of CO_2 and dehydration of HCO_3^-

SCHEME 1

TABLE 1 Determination of the Conversion of Each Species by ^{19}F NMR Spectroscopy

Reactants		Products					
1	$R_F SO_2 F$	**3**	Yields[a]	**4**	Yields[a]	**5**	Yields[a]
1a	$C_4F_9SO_2F$	**a**	64	**A**	23	**a**	13
1a	$C_8F_{17}SO_2F$	**b**	61	**C**	25	**a**	14
1b	$C_6F_{13}SO_2F$	**c**	69	**B**	25	**b**	6
1b	$C_4F_9SO_2F$	**d**	78	**A**	13	**b**	9
1c	$C_8F_{17}SO_2F$	**e**	58	**C**	19	**c**	23
1c	$C_6F_{13}SO_2F$	**f**	66	**B**	24	**c**	10

[a]The conversions were determined by ^{19}F NMR spectroscopy (d_6-acetone) after treatment of the crude product (the residue was dissolved in Et_2O and washed with 1 N HCl and water). They were also determined by the relative integration of the functional CF_2 signal of compounds **3, 4, 5** by the formula $(f_i/N_i)/(\Sigma f_i/N_i) \times 100$, where f_i represent the height integration of the functional CF_2 signal and N_i the number of corresponding atom.

but also the hydrolysis of many esters. We assayed the enzyme activity both with respect to hydration of CO_2 and to hydrolysis of an ester, namely *p*-nitrophenylacetate (NPA). Although the esterase activity of these enzymes was very weak, by comparison with the CA activity, it could be determined with greater ease and accuracy [28,29]. We determined esterase activity spectrophotometrically with NPA as substrate in routine assays (Fig. 1). Hydrolysis Kinetics of NPA by bCA was calculated from the tangents at time zero minute in the presence and in the absence of perfluoroalkanesulfonylimides.

It is interesting to note that we used a constant concentration of NPA (7.5×10^{-4} M) and a constant concentration of bCA (2.9×10^{-6} M) for this study.

We followed the rate of the hydrolysis of the NPA by measurements of the absorbance at 348 nm, and we deduced the rate of inhibition of the different compounds previously prepared on bCA. The results are shown in Table 2.
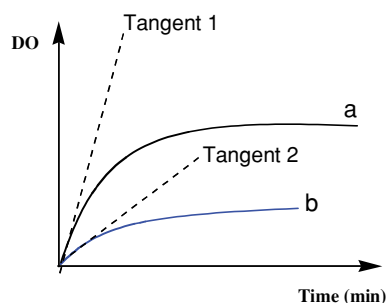

FIGURE 1 Determination of the rate of the hydrolysis of NPA by measurements of the absorbance at 348 nm. Note that curve a represents the hydrolysis of NPA (7.5×10^{-4} M) by bCA without any compound and curve b represents the hydrolysis of NPA in presence of perfluoroalkanesulfonylimides.

We thought that the sulfonimide function would complex the zinc ion of the active site like the sulfonamide function does it. Moreover, we had imagined that the F-alkylated chain of the perfluoroalkanesulfonylimides, which are known to be very hydrophobic could push back the water, which is formed at the time of the enzymatic catalysis. Unfortunately, we did not obtain the expected results.

At a concentration of 1.35×10^{-4} M, we found for the symmetric perfluoroalkanesulfonylimides (**3a, 3c, 3e**) that the compound with the C_4F_9 chain inhibits less than the compound with the C_6F_{13} and C_8F_{17} chains. These two last compounds inhibit the bCA in an equivalent way. For the dissymmetric perfluoroalkanesulfonylimides (**3b, 3d, 3f**), the inhibition properties are similar.

At a concentration of 1.35×10^{-6} M, we see that the inhibition properties disappear for all perfluoroalkanesulfonimides, whereas for acetazolamide and trifluoromethanesulfonamide, there is a good inhibition of the hydrolysis of NPA. Because of the weak inhibition properties of the perfluoroalkanesulfonylimides, we do not determinate the inhibition constants (K_i).

We may suppose on the one hand that the two F-alkylated chains were rigid and could prevent the access to the active site of the enzyme, and on the other hand that the sulfonylimide function could not complex enough the Zn metal present in the catalytic site.

CONCLUSION

In summary, we prepared a symmetric and dissymmetric perfluoroalkanesulfonimides starting from sodium salts of perfluoroalkanesulfonamides, HMDS, and perfluoroalkanesulfonylfluorides and

TABLE 2 Determination of the percent Inhibition of the Hydrolysis of the NPA on bCA by Different Compounds

Compound Concentrations Tested	Inhibition of Hydrolysis (%) Compounds							
	3a	**3b**	**3c**	**3d**	**3e**	**3f**	$CF_3SO_2NH_2$	Acetazolamide
1.35×10^{-4} M	65	97	85	87	89	87	87	85
1.35×10^{-6} M	0	0	0	0	0	0	85	85

NPA = *p*-nitrophenylacetate.

we assayed their inhibition on bCA. In spite of their current interest, perfluoroalkanesulfonylimides have not been studied for their biological properties. In consequence, we found that these compounds inhibit this enzyme at a concentration of 1.35×10^{-4} M and do not inhibit it at concentration of 1.35×10^{-6} M. Further studies of Docking could help us to understand the favorable positioning of the perfluoroalkanesulfonylimides in the enzymatic site.

EXPERIMENTAL

Chemistry

Moisture-sensitive reactions were carried out under dry nitrogen. The trifluoromethanesulfonimide and the acetazolamide were purchased from Aldrich (Saint Quentin Fallavier, France). HMDS was purchased from Aldrich and distilled once before use. Solvents were distilled from the appropriate drying agents immediately prior to use. Reactions were monitored on TLC on silica gel 60 F_{254} and visualized under iodine. For column chromatography, silica gel (Kieselgel 60) was employed.

^{1}H and ^{19}F, spectra were recorded respectively at 300.13 MHz, 282.37 MHz with a Brucker Avance 300 spectrometer, therefore chemical shifts are given in parts per million relative to Me_4Si, CCl_3F, respectively, as internal standards. Coupling constants are given in Hz. Mass spectra and HRMS were recorded on a Jeol SX 102 spectrometer.

Melting points were recorded at atmospheric pressure unless otherwise stated on a Stuart scientific SMP3 apparatus and remained without any correction.

Synthesis of Sodium Perfluoroalkanesulfonamides (1a, 1b, 1c)

The synthesis of sodium perfluorobutanesulfonamide (**1a**) and sodium perfluorooctanesulfonamide (**1c**) were described previously [25].

*Synthesis of Sodium 1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-n-hexanesulfonamide (**1b**).* A

mixture of 10 g of perfluorohexanesulfonamide (25 mmol) and 1.28 g of sodium methylate (23.68 mmol) dissolved, respectively, in 40 mL of anhydrous diethyl ether, and 5 mL of methanol was refluxed for 3 h and then filtered. The filtrate was evaporated in vacuo, and the residue was washed three times with 30 mL of diethyl ether to remove the excess of perfluorohexanesulfonamide and then dried under vacuum. An amount of 9.7 g of **2b** was obtained (92%).

^{19}F NMR (282.37 MHz, d_6-acetone): δ −125.93 (m, 2F, $CF_3CF_2(CF_2)_4$), −122.46 (m, 2F, $CF_3CF_2CF_2(CF_2)_3$), −121.56 (m, 2F, $CF_3(CF_2)_2CF_2$ $(CF_2)_2$), −120.06 (m, 2F, $CF_3(CF_2)_3CF_2CF_2$), −113.55 (m, 2F, $CF_3(CF_2)_4CF_2$), −80.83 (m, 3F, $CF_3(CF_2)_5$); MS (FAB$^-$): 398; HRMS Calcd for $C_6HO_2NF_{13}S$: 397.9482; found 397.9492.

General Procedure for the Synthesis of N-Trimethylsilane Sodium Salts of Perfluoroalkanesulfonamides $R_FSO_2NNaSi(CH_3)_3$ (2a, 2b, 2c)

Freshly distilled $HN[Si(Me)_3]_2$ (25 eq.) was added to a stirred solution of dry powdered $R_FSO_2NH^-$ Na^+ (1 eq.) dissolved in dry CH_3CN. The reaction mixture was refluxed at 110°C for 24 h. The solvent and excess of HMDS were then removed by vacuum distillation. Owing to the moisture sensitivity of the compound, the remaining solid was used directly without any further characterization and the yield was not determined.

*Synthesis of N-Timethylsilane Sodium Salts of Perfluorobutanesulfonamide (**2a**).* A total of 3 g (9.3 mmol) of **1a** was dissolved in CH_3CN (15 mL), and HMDS (49 mL) was added. In all, 3.6 g of **2a** obtained.

*Synthesis of N-Trimethylsilane Sodium Salts of Perfluorohexanesulfonamide (**2b**).* A measure of 9.7 g (23 mmol) of **1b** was dissolved in CH_3CN (37 mL), and HMDS (120 mL) was added. In all, 11 g of **2b** was obtained.

*Synthesis of N-Trimethylsilane Sodium Salts of Perfluorooctanesulfonamide (**2c**).* A total of 12 g (23 mmol) of **1c** were dissolved in CH_3CN (38 mL) and HMDS (122 mL) was added. In all, 13.4 g of **2c** were obtained.

General Procedure for the Synthesis of Perfluoroalkanesulfonylimides (*3a, 3b, 3c*)

R_FSO_2F (1.15 eq) was added to a stirred solution of dry powdered $R_FSO_2N^-NaSi(CH_3)_3$ (1 eq.) dissolved in dry THF. The reaction mixture was refluxed from 12 to 24 ho at 70°C. After cooling, all volatile parts of the mixture were removed in vacuo. The residue was dissolved in Et_2O and washed once with an aqueous solution of HCl (1 N) and twice with water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Silica gel chromatography (AcOEt/petroleum ether = 6/4 (v/v)) yielded perfluoroalkanesulfonylimides **3**.

*Synthesis of Bis((1,1,2,2,3,3,4,4,4-Nonafluorobutane)sulfonyl)imide (**3a**).* **2a** (1.21 g, 3.1 mmol) and $C_4F_9SO_2F$ (1.07 g, 3.56 mmol) dissolved in CH_3CN (10 mL) were refluxed for 12 h. The crude product was purified by silica gel chromatography (AcOEt/petroleum ether = 6/4) to give **3a** (1 g) in a 55% overall yield based on the starting amount of **1a**.

mp: 120.7°C; ^{19}F NMR (282.37 MHz, d_6-acetone): δ −125.7 (m, 4F, $(CF_3\mathbf{CF_2}(CF_2)_2SO_2)_2NH$), −120.7 (m, 4F, $(CF_3CF_2\mathbf{CF_2}CF_2SO_2)_2NH$), −113.0 (m, 4F, $(CF_3(CF_2)_2\mathbf{CF_2}SO_2)_2NH$), −80.8 (m, 6F, $(\mathbf{CF_3}(CF_2)_3SO_2)_2NH$); MS (FAB⁻): 580; HRMS Calcd for $C_8NO_4F_{18}S_2$: 579.8981; found 579.8968.

*Synthesis of S-(1,1,2,2,3,3,4,4,4-Nonafluorobutane), S'-(1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluorooctane)sulfonylimide (**3b**).* **2a** (1.18 g, 3 mmol) and $C_8F_{17}SO_2F$ (1.73 g, 3.45 mmol) dissolved in CH_3CN (10 mL) were refluxed for 12 h. The crude product was purified by silica gel chromatography (AcOEt/petroleum ether = 6/4) to give **3b** (1.22 g) in a 51% overall yield based on the starting amount of **1a**.

mp: 130.3°C; ^{19}F NMR (282.37 MHz, d_6-acetone): δ −125.7 (m, 4F, $CF_3\mathbf{CF_2}(CF_2)_2SO_2NHSO_2(CF_2)_6\mathbf{CF_2}CF_3$), −122.3 (m, 2F, $SO_2(CF_2)_5\mathbf{CF_2}CF_2CF_3$), −121.4 (m, 6F, $SO_2(CF_2)_3(\mathbf{CF_2})_3CF_2CF_3$), −120.7 (m, 2F, $CF_3CF_2\mathbf{CF_2}CF_2SO_2$), −119.6 (m, 2F, $SO_2CF_2\mathbf{CF_2}(CF_2)_5CF_3$), −113.0 (m, 2F, $CF_3(CF_2)_2\mathbf{CF_2}SO_2$), −112.9 (m, 2F, $SO_2\mathbf{CF_2}CF_2(CF_2)_5CF_3$), −80.8 (m, 6F, $\mathbf{CF_3}(CF_2)_3SO_2NHSO_2(CF_2)_7\mathbf{CF_3}$); MS (FAB⁻): 780; HRMS calcd for $C_{12}NO_4F_{26}S_2$: 779.8854; found 779.8844.

*Synthesis of Bis((1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane)sulfonyl)imide (**3c**).* **2b** (3.42 g, 6.94 mmol) and $C_6F_{13}SO_2F$ (3.21 g, 7.98 mmol) dissolved in CH_3CN (20 mL) were refluxed for 24 h. The crude product was purified by silica gel chromatography (AcOEt/petroleum ether = 6/4) to give **3c** (2.98 g) in a 54% overall yield based on the starting amount of **1b**.

mp: 132.5°C; ^{19}F NMR (282.37 MHz, d_6-acetone): δ −125.8 (m, 4F, $(CF_3\mathbf{CF_2}(CF_2)_4SO_2)_2NH$), −122.4 (m, 4F, $(CF_3CF_2\mathbf{CF_2}(CF_2)_3SO_2)_2NH$), −121.4 (m, 4F, $(CF_3(CF_2)_2\mathbf{CF_2}(CF_2)_2SO_2)_2NH$), −119.7 (m, 4F, $(CF_3(CF_2)_3\mathbf{CF_2}CF_2SO_2)_2NH$), −112.8 (m, 4F, $(CF_3(CF_2)_4\mathbf{CF_2}SO_2)_2NH$), −80.7 (m, 6F, $(\mathbf{CF_3}(CF_2)_5SO_2)_2NH$); MS (FAB⁻): 780; HRMS calcd for $C_{12}NO_4F_{26}S_2$ 779.8854; found 779.8840.

*Synthesis of S-(1,1,2,2,3,3,4,4,5,5,6,6,6 Tridecafluorohexane), S'-(1,1,2,2,3,3,4,4,4-Nonafluorobutane)sulfonylimide (**3d**).* **2b** (5.68 g, 11.5 mmol) and $C_4F_9SO_2F$ (4 g, 13.2 mmol) dissolved in CH_3CN (20 mL) were refluxed for 24 h. The crude product was purified by silica gel chromatography (AcOEt/petroleum ether = 6/4) to give **3d** (4.93 g) in a 61% overall yield based on the starting amount of **1b**.

mp: 127.2°C; ^{19}F NMR (282.37 MHz, d_6-acetone): δ −125.8 (m, 4F, $CF_3\mathbf{CF_2}(CF_2)_4SO_2NHSO_2CF_2$), −122.5 (m, 2F, $CF_3CF_2\mathbf{CF_2}(CF_2)_3SO_2$), −121.4 (m, 2F, $CF_3(CF_2)_2\mathbf{CF_2}(CF_2)_2SO_2$), −120.7 (m, 2F, $SO_2CF_2\mathbf{CF_2}CF_2CF_3$), −113.0 (m, 2F, $CF_3(CF_2)_4\mathbf{CF_2}SO_2$), −112.9 (m, 2F, $SO_2\mathbf{CF_2}(CF_2)_2CF_3$), −80.7 (m, 6F, $\mathbf{CF_3}(CF_2)_5SO_2NHSO_2(CF_2)_3\mathbf{CF_3}$); MS (FAB⁻): 680; HRMS calcd for $C_{10}NO_4F_{22}S_2$: 679.8917; found 679.8922.

*Synthesis of Bis((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluorooctane)sulfonyl)imide (**3e**).* **2c** (6.63 g, 11.1 mmol) and $C_8F_{17}SO_2F$ (6.45 g, 12.8 mmol) dissolved in CH_3CN (20 mL) were refluxed for 24 h. The crude product was purified by silica gel chromatography (AcOEt/petroleum ether = 6/4) to give **3e** (5.2 g) in a 46% overall yield based on the starting amount of **1c**.

mp: 143.3°C; ^{19}F NMR (282.37 MHz, d_6-acetone): δ −125.8 (m, 4F, $(CF_3\mathbf{CF_2}(CF_2)_6SO_2)_2NH$), −122.3 (m, 4F, $(CF_3CF_2\mathbf{CF_2}(CF_2)_5SO_2)_2NH$), −121.4 (m, 12F, $(CF_3(CF_2)_2(\mathbf{CF_2})_3(CF_2)_2SO_2)_2NH$), −119.6 (m, 4F, $(CF_3(CF_2)_5\mathbf{CF_2}CF_2SO_2)_2NH$), −112.8 (m, 4F, $(CF_3(CF_2)_6\mathbf{CF_2}SO_2)_2NH$), −80.7 (m, 6F, $(\mathbf{CF_3}(CF_2)_7SO_2)_2NH$), MS (FAB⁻): 980; HRMS Calcd for $C_{16}NO_4F_{34}S_2$: 979.8726; found 979.8703.

Synthesis of S-(1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluorooctane), S'-(1,1,2,2,3,3,4,4,5,5,6,6,6-

*Tridecafluorohexane)sulfonylimide (**3f**).* **2c** (4.51 g, 7.6 mmol) and $C_6F_{13}SO_2F$ (3.51 g, 8.7 mmol) dissolved in CH_3CN (20 mL) were refluxed for 24 h. The crude product was purified by silica gel chromatography (AcOEt/petroleum ether = 6/4) to give **3f** (3.55 g) in a 52% overall yield based on the starting amount of **1c**.

mp: 137.5°C; ^{19}F NMR (282.37 MHz, d_6-acetone): δ −125.8 (m, 4F, $CF_3\mathbf{CF_2}(CF_2)_6SO_2NHSO_2(CF_2)_4$ $\mathbf{CF_2}CF_3$), −122.3 (m, 4F, $CF_3CF_2\mathbf{CF_2}(CF_2)_5SO_2NH$ $SO_2(CF_2)_3\mathbf{CF_2}CF_2CF_3$), −121.4 (m, 8F, $CF_3CF_2CF_2$ $(\mathbf{CF_2})_3(CF_2)_2SO_2NHSO_2(CF_2)_2\mathbf{CF_2}(CF_2)_2CF_3$), −119.7 (m, 4F, $CF_3(CF_2)_5\mathbf{CF_2}CF_2SO_2NHSO_2CF_2\mathbf{CF_2}(CF_2)_3$ CF_3), −112.8 (m, 4F, $CF_3(CF_2)_6\mathbf{CF_2}SO_2NHSO_2\mathbf{CF_2}$ $(CF_2)_4CF_3$), −80.7 (m, 6F, $\mathbf{CF_3}(CF_2)_7SO_2NHSO_2$ $(CF_2)_5\mathbf{CF_3}$); MS (FAB⁻): 880; HRMS Calcd for $C_{14}NO_4F_{30}S_2$: 879.8790; found 879.8774.

Byproducts. Perfluoroalkanesulfonic Acids

4a ($C_4F_9SO_3H$) ^{19}F NMR (282.37 MHz, d_6-acetone): δ −126.8 (m, 2F, $CF_3\mathbf{CF_2}(CF_2)_2$); −122.2 (m, 2F, $CF_3CF_2\mathbf{CF_2}CF_2$); −115.5 (m, 2F, $CF_3(CF_2)_2\mathbf{CF_2}$); −81.9 (m, 3F, $\mathbf{CF_3}(CF_2)_2$); MS (FAB⁻) 299.

4b ($C_6F_{13}SO_3H$) ^{19}F NMR (282.37 MHz, d_6-acetone): δ −125.8 (m, 2F, $CF_3\mathbf{CF_2}(CF_2)_4SO_3H$); −122.4 (m, 2F, $CF_3CF_2\mathbf{CF_2}(CF_2)_3SO_3H$); −121.4 (m, 2F, $CF_3(CF_2)_2\mathbf{CF_2}(CF_2)_2SO_3H$); −120.2 (m, 2F, CF_3 $(CF_2)_3\mathbf{CF_2}CF_2SO_3H$); −114.3 (m, 2F, $CF_3(CF_2)_4$ $\mathbf{CF_2}SO_3H$); −80.8 (m, 3F, $\mathbf{CF_3}(CF_2)_5SO_3H$); MS (FAB⁻) 399.

4c ($C_8F_{17}SO_3H$) ^{19}F NMR (282.37 MHz, d_6-acetone): δ −125.8 (m, 2F, $CF_3\mathbf{CF_2}(CF_2)_6SO_3H$); −122.3 (m, 2F, $CF_3CF_2\mathbf{CF_2}(CF_2)_5SO_3H$); −121.3 (m, 6F, $CF_3(CF_2)_2(\mathbf{CF_2})_3(CF_2)_2SO_3H$); −120.2 (m, 2F, $CF_3(CF_2)_5\mathbf{CF_2}CF_2SO_3H$); −114.3 (m, 2F, CF_3 $(CF_2)_6\mathbf{CF_2}SO_3H$); −80.7 (m, 3F, $\mathbf{CF_3}(CF_2)_7SO_3H$); MS (FAB⁻) 499.

Perfluoroalkanesulfonamides

5a ($C_4F_9SO_2NH_2$). mp: 67.5°C, ^1H NMR (300.13 MHz, d_6-acetone): δ 8.2 (m, 2H, SO_2NH_2); ^{19}F NMR (282.37 MHz, d_6-acetone): δ −125.8 (m, 2F, $CF_3\mathbf{CF_2}(CF_2)_2$), −121.0 (m, 2F, $CF_3CF_2\mathbf{CF_2}CF_2$), −113.7 (m, 2F, $CF_3(CF_2)_2\mathbf{CF_2}$), −80.8 (m, 3F, $\mathbf{CF_3}(CF_2)_3$); MS (FAB⁻) 298.

5b ($C_6F_{13}SO_2NH_2$). mp: 119.8°C; ^1H NMR (300.13 MHz, d_6-acetone): δ 4.90 (m, 2H, $SO_2N\mathbf{H_2}$); ^{19}F NMR (282.37 MHz, d_6-acetone): δ −125.8 (m, 2F, $(CF_3\mathbf{CF_2}(CF_2)_4)$); −122.3 (m, 2F, $(CF_3CF_2$ $\mathbf{CF_2}(CF_2)_3)$); −121.4 (m, 2F, $(CF_3(CF_2)_2\mathbf{CF_2}(CF_2)_2)$); −119.8 (m, 2F, $(CF_3(CF_2)_3\mathbf{CF_2}CF_2)$); −113.8 (m, 2F, $(CF_3(CF_2)_4\mathbf{CF_2})$); −80.8 (m, 3F, $(\mathbf{CF_3}(CF_2)_5)$); MS (FAB⁻): 398.

5c ($C_8F_{17}SO_2NH_2$). mp: 154.6°C. ^1H NMR (300.13 MHz, d_6-acetone): δ 8.2 (m, 2H, SO_2NH_2); ^{19}F NMR (282.37 MHz, d_6-acetone): δ −125.8 (m, 2F, $CF_3\mathbf{CF_2}(CF_2)_6$), −122.3 (m, 2F, $CF_3CF_2\mathbf{CF_2}$ $(CF_2)_5$), −121.4 (m, 6F, $CF_3(CF_2)_2(\mathbf{CF_2})_3(CF_2)_2$), −119.9 (m, 2F, $CF_3(CF_2)_5\mathbf{CF_2}CF_2$), −113.5 (m, 2F, $CF_3(CF_2)_6\mathbf{CF_2}$), −80.7 (m, 3F, $\mathbf{CF_3}(CF_2)_7$); MS (FAB⁻): 498.

BIOLOGICAL

bCA was purchased as a lyophilized powder from Sigma Chemical Co. (Saint Quentin Fallavier, France) All the reagents that were used were of analytical grade. Acetazolamide was purchased from Aldrich. Native enzyme concentrations were determined from the absorbance at 280 nm, using a molar absorbance of 5.7×10^4 M^{-1} cm^{-1} for bCA. All enzyme preparations were stored in 0.05 M $TrisSO_4^{2+}$ 1 mM mercaptoethanol (pH 8.7) at 4°C. Enzyme concentration was 2.9×10^{-6} M for the bCA.

Initial rates of 4-nitrophenyl acetate hydrolysis were estimated by a modification of the method of Verpoorte et al. [28]. The increase in absorbance was followed at 348 nm for approximately 20 min. Steady-state measurements were made at 25°C in a Kontron Uvikon 860 spectrophotometer. The solution of substrate was prepared in acetone/H_2O media (1/50, v/v); the substrate concentration used was 7.5×10^{-4} M for the bCA. A molar absorption coefficient ε of 16.3×10^3 M^{-1} cm^{-1} was used for 4-nitrophenolate formed by hydrolysis, in the conditions of the experiments (pH 8). Nonenzymatic hydrolysis rates were always subtracted from the observed rates. Triplicate experiments were conducted for each substrate concentration and for each inhibitor concentration. Inhibitor solutions were prepared in distilled-deionized water with 10% (v/v) DMSO (which is not inhibitory at these concentrations), and dilutions were done thereafter with distilled-deionized water; the inhibitor concentrations varied between 1.35×10^{-4} M and 1.35×10^{-6} M. Inhibitor and enzyme solutions were preincubated together for 5 min at room temperature prior to assay. Acetazolamide was used as a specific inhibitor in our studies, its inhibitory effectiveness was determined by using 2.9×10^{-6} M bCA and varying acetazolamide concentrations from 1.35×10^{-4} M to 1.35×10^{-6} M.

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