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Carbonic Anhydrase Inhibitors: Allylsulfonamide, Styrene Sulfonamide, *N*-allyl Sulfonamides and Some of Their Si, Ge, and B Derivatives

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Unsubstituted aromatic, heterocyclic and perfluoroalkylic sulfonamides possessing the general formula RSO_2NH_2 act as powerful inhibitors of the zinc enzyme carbonic anhydrase (CA). Unsaturated primary/substituted sulfonamides have never been investigated for their interaction with the enzyme. Here it is shown that such compounds, and more precisely allyl-sulfonamide and *trans*-styrene sulfonamide possessing the above general formula (with $R=CH_2=CH-CH_2-$ and $C_6H_5-CH=CH-$, respectively) behave as nanomolar inhibitors of the physiologically relevant isozymes CA I and CA II. Some other derivatives of these two leads (incorporating Si(IV), Ge(IV) and B(III) moieties among others) were also synthesized and investigated for their interaction with CA, but showed decreased affinity for both isozymes. The structure-activity relationship for this class of CA inhibitors is discussed. Furthermore, it was observed that allylsulfonyl chloride is a strong CA inactivator, probably by reacting with amino acid residues critical for the catalytic cycle.

Keywords: Carbonic anhydrase; Sulfonamide; Unsaturated sulfonamide; *N*-substituted-sulfonamide; Si(IV), Ge(IV), B(III) derivative

INTRODUCTION

Carbonic anhydrase (CA) inhibition by sulfanilamide discovered by Mann and Keilin in 1940[1] was the beginning of a great scientific adventure that led to important drugs such as the antihypertensives of the benzothiadiazine and high-ceiling diuretics type,[2] the sulfonamides with CA inhibitory properties mainly used as antiglaucoma agents,[2,3] to some anti-thyroid drugs,[2] to the hypoglycemic sulfonamides[4] and ultimately to some novel types of anticancer and antiviral agents.[5] Two main classes of sulfonamides have been investigated as CA inhibitors (CAIs): the aromatic and heterocyclic unsubstituted sulfonamides possessing the general formula $R-SO_2NH_2$.[2,3] Ultimately, some perfluoroalkylsulfonamides of the type $C_nF_{2n+1}-SO_2NH_2$ were also shown to possess good

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inhibitory properties.[2] Krebs[6] reported in 1948 that substitution of the sulfonamide moiety in compounds of the type $ArSO_2NHR$, drastically reduced the CA inhibitory properties as compared to the corresponding derivatives possessing primary sulfonamide groups, $ArSO_2NH_2$. As a consequence, other zinc-binding functions other than SO_2NH_2 have rarely been taken into consideration in the design of CAIs, although many other zinc enzymes are inhibited by a multitude of derivatives possessing an entire range of zinc enzymes are inhibited by a multitude of derivatives possessing an entire range of zinc binding functions, such as thiols, phosphonates, carboxylates, hydroxamates, etc.[7,8] Only recently several detailed studies regarding the possible modifications of the sulfonamido moiety, compatible with the retention of strong binding to the enzyme, have been reported.[9,10]

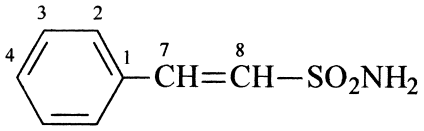
Within the context of research on new types of inhibitors of human carbonic anhydrase,[10–12] we previously studied *N*-metallated sulfonamide derivatives of *p*-toluene sulfonamide.[10,12,13] However, electron rich unsaturated sulfonamides with a double bond in the α or β position to the sulfonamide group, could act as good inhibitors of these zinc enzymes provided the new molecules were able to reach the center of the enzyme cavity; such compounds have not been previously investigated for their interaction with the enzyme.[2] Therefore we prepared unsaturated primary sulfonamides *N*-allyl secondary sulfonamides $RSO_2NH-CH_2CH=CH_2$, some derivatives of the allylsulfonamide $CH_2=CH-CH_2SO_2NRR'$ (R = H; R′ = H, $CH_2=CHCH_2$, $C_6H_5CH_2$, $C_6H_5CH=CH$) and the *trans*-styrene sulonamide $C_6H_5CH=CH-SO_2NH_2$. As derivatives of germanium or boron[10–12] were previously shown to act as good inhibitors of human carbonic anhydrase, often by complexation of the Ge or B or of one of the nitrogen atoms at the entrance of the enzyme of the enzyme cavity, we also prepared C-substituted derivatives of the above mentioned unsaturated sulfonamides, by addition across the double bonds contained in their molecules. Such compounds would be more stable toward hydrolysis than the previously *N*-metallated sulfonamides tested[12,13] and therefore more useful as CA inhibitors. We present here our results within this new series of unsaturated sulfonamides and some of their silicon, germanium and boron derivatives.

MATERIALS AND METHODS

Chemistry

All reactions were carried out under nitrogen and with dry solvents. NMR spectra were recorded on Brücker AC 80 (^1H) and AC 200 (^{13}C) spectrometers; IR spectra on a Perkin Elmer 16000 FTIR spectrometer; mass spectra on a HP 5889 in the electron impact mode (70 eV) or on a Rybermag R10–10 spectrometer operating in the electron impact mode or by chemical desorption (Dci/CH_4 or NH_3) or by electrospray. Elemental analysis were performed by the "Service Central de Microanalyse" of the "Ecole Nationale Supérieure de Chimie de Toulouse", for recrystallized or distilled compounds. The compounds of a sticky nature were characterized by Mass spectrometry and by other spectroscopic means. Melting points were measured on a Leitz microscope.

Trans-*styrene Sulfonamide (1)*



(i) *Using sodium amide*: to sodium amide (1.43 g; 0.04 mol) in suspension in 10 ml ether, was added *trans*-styrene sulfonylchloride (8.10 g; 0.04 mol). After 12 h under stirring at room temperature, the ether was evaporated, replaced by hot ethanol and the sodium chloride formed was

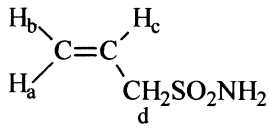
filtered. Evaporation of the ethanol led to a brown residue which was then recrystallized in hot THF, yielding 0.73 g of a white power of **1**. Yield: 10%.

Note that sodium amide has to be free of hydrolysis products to avoid the formation of $C_6H_5CH{=}CHSO_3H$ (^{1}H NMR (DMSO): 6.90 (d, 1H, H_7, $^3J_{HH} = 15$ Hz), 7.09 (d, 1H, H_8, $^3J_{HH} = 15$ Hz), 7.25–7.57 (m, 5H, C_6H_5), 14.58 (s, 1H, SO_3H). ^{13}C NMR (DMSO): 134.43 (C_1), 128.60 (C_2), 127.20 (C_3), 128.24 (C_4), 131.93 (C_7), 132.67 (C_8). MS (EI): $M^{+\cdot} = 184$ (33%), $M^{+\cdot} - SO_2 = 120$ (7%), $M^{+\cdot} - SO_3H = 103$ (27%), $M^{+\cdot} - SO_3H - H = 102$ (100%)) and $C_6H_5CH{=}CHSO_3Na$ (^{1}H NMR (DMSO): 7.19 (d, 1H, H_7, $^3J_{HH} = 11$ Hz), 7.34 (d, 1H, H_8, $^3J_{HH} = 11$ Hz), 7.31–7.70 (m, 5H, C_6H_5). ^{13}C NMR (DMSO): 134.92 (C_1), 128.65 (C_2), 127.02 (C_3), 128.41 (C_4), 130.72 (C_7), 133.88 (C_8)).

(ii) *Using ammonia solution*: to *trans*-styrene sulfonylchloride (1.00 g; 4.94 mmol), was added a large excess of ammonia solution. Addition of ether to the mixture led to the precipitation of a white powder, which was filtered and dried under vacuum and identified as **1** (0.33 g). Yield: 33%.

(iii) Using NH_3 gas: to *trans*-styrene sulfonylchloride (1.24 g; 6.11 mmol) in 19 ml of dry ether at −50°C, was added NH_3 gas. The mixture is allowed to stand at room temperature and the ether then evaporated in vacuum. The ammonium chloride formed was separated by precipitation in chloroform. Evaporation of the chloroform in vacuum led to 0.56 g of **1**. Yield: 49%. Mp 134–135°C. ^{1}H NMR (DMSO): 7.10 (s, 2H, NH_2), 7.23 (d, 1H, H_7, $^3J_{HH} = 15$ Hz), 7.32 (d, 1H, H_8, $^3J_{HH} = 15$ Hz), 7.42–7.70 (m, 5H, C_6H_5). ^{13}C NMR (DMSO): 132.86 (C_1), 128.11 (C_2), 128.94 (C_3), 130.15 (C_4), 130.30 (C_7), 136.48 (C_8). IR (nujol): 3237 and 3328 cm^{-1} (NH_2), 1128 and 1377 cm^{-1} (SO_2), 1624 cm^{-1} (C=C). MS (EI): $M^{+\cdot} = 183$ (49%), $M^{+\cdot} - SO_2 = 119$ (28%), $M^{+\cdot} - SO_2NH_2 = 103$ (44%), $M^{+\cdot} - SO_2NH_2 - H = 102$ (100%).

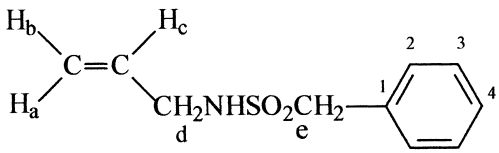
Allylsulfonamide (2)



In accord with the literature,[14] allylbromide (24.80 g; 0.21 mol) was treated with sodium sulfite (25.00 g; 0.20 mol), to yield a mixture of salts which were treated with phosphorus oxychloride. The crude allylsulfonylchloride obtained (10.65 g, 37%) was purified by distillation leading to 7.41 g of pure $CH_2{=}CHCH_2SO_2Cl$. Yield: 30%. Bp 83–85°C/20 mm Hg. ^{1}H NMR ($CDCl_3$): 4.31 (dd, 2H, H_d, $^3J_{cd} = 7$ Hz, $^3J_{bd} = 1$ Hz), 5.47–6.26 (m, 3H, H(a,b,c)). ^{13}C NMR ($CDCl_3$): 127.93 (CH_2=), 123.17 (=CH), 68.77 (CH_2SO_2Cl). IR (nujol): 1166 and 1372 cm^{-1} (SO_2), 1641 cm^{-1} (C=C). GC/MS (EI): $M^{+\cdot} - CH_2CH{=}CH_2 = 99$ (2%), $M^{+\cdot} - SO_2 = 76$ (7%), $M^{+\cdot} - SO_2Cl = 41$ (100%).

As in method (iii), used for preparation of **1**, allylsulfonylchloride (7.41 g; 0.05 mol) was treated with NH_3 gas, to yield crude oily **2**. Recrystallization in a mixture (1/1) of chloroform and pentane at −20°C led to a pure powder (2.80 g) of **2**. Yield: 44%. Mp 29–33°C. ^{1}H NMR ($CDCl_3$): 3.82 (d, 2H, H_d, $^3J_{cd} = 7$ Hz), 5.28–6.19 (m, 3H, H(a,b,c)), 5.19 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$): 124.66 (CH_2=), 125.83 (=CH), 59.30 ($CH_2SO_2NH_2$). IR (KBr): 3256 and 3344 cm^{-1} (NH_2), 1145 and 1328 cm^{-1} (SO_2), 1641 cm^{-1} (C=C). MS (EI): $M^{+\cdot} + 1 = 122$ (2%), $M^{+\cdot} - CH_2CH{=}CH_2 = 80$ (7%), $M^{+\cdot} - SO_2 + 1 = 56$ (47%), $M^{+\cdot} - SO_2NH_2 = 41$ (100%).

N-*(benzylsulfonyl)allylamine (3)*

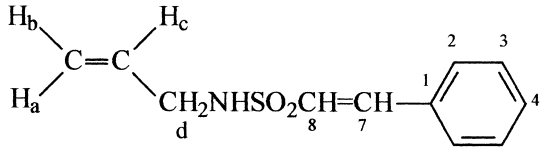


Preparation of allylaminolithium: butyllithium (7.89 mmol; 4.90 ml at 1.6 M in hexane) was

added to allylamine (0.45 g; 7.89 mmol) in 10 ml THF at −80°C and allowed to come to room temperature.

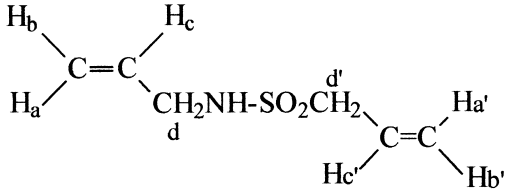
Preparation of **3**: benzylsulfonylchloride (1.50 g; 7.88 mmol) in 6 ml THF was reacted exothermically with allylaminolithium (7.89 mmol) prepared as before. After 1 h under stirring at room temperature, the solvent was evaporated under vacuum, replaced by toluene and the lithium chloride filtered off. Evaporation of the toluene under vacuum led to 1.50 g of a white powder of crude **3** which was washed twice with pentane. Yield: 90%. Mp 61–62°C. ^{1}H NMR ($CDCl_3$): 4.95–5.27 (m, 2H, H(a,b)), 5.27–5.93 (m, 1H, H_c), 3.50 (d, 2H, H_d, $^3J_{cd} = 7$ Hz), 4.17 (s, 2H, H_e), 7.31 (s, 5H, C_6H_5), 4.38 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): 129.54 (C_1), 130.79 (C_2), 128.70 (C_3), 128.56 (C_4), 59.27 (C_e), 46.02 (C_d), 117.25 (CH_2=), 134.11 (=CH). IR (nujol): 3200 (NH), 1135 and 1377 cm^{-1} (SO_2), 1645 cm^{-1} (C=C). MS (EI): $M^{+\cdot} - 1 = 210$ (7%), $M^{+\cdot} - SO_2 - 1 = 146$ (12%), $[C_6H_5CH_2]^+ = 91$ (100%).

N-(trans-*styrenesulfonyl)allylamine (4)*



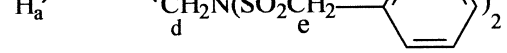
Trans-styrenesulfonylchloride (1.60 g; 7.89 mmol) in 5 ml THF reacted exothermically with allylaminolithium (7.89 mmol) prepared as before. After 1 h stirring at room temperature, THF was replaced by toluene. The lithium chloride was filtered off and the toluene was evaporated under vacuum leading to 1.58 g of the sticky brown compound identified as crude **4**. Yield: 91%. Attempts at recrystallization in different solvents (chloroform, ethanol, pentane) did not yield crystals. ^{1}H NMR ($CDCl_3$): 6.68–7.49 (m, 8H, C_6H_5), H(7,8) and NH), 5.08–5.36 (m, 2H, H(a,b)), 5.59–6.14 (m, 1H, H_c), 3.64–3.69 (m, 2H, H_d). ^{13}C NMR ($CDCl_3$): 132.65 (C_1), 128.33 (C_2), 129.13 (C_3), 128.79 (C_4), 129.22 (C_7), 141.64 (C_8), 117.72 (CH_2=), 133.57 (=CH), 45.62 (C_d). MS (EI): $M^{+\cdot} - 1 = 222$ (4%), $M^{+\cdot} - SO_2 = 159$ (23%), $M^{+\cdot} - SO_2 - 1 = 158$ (23%), $M^{+\cdot} - C_6H_5 = 146$ (67%), $[C_6H_5CH = CH]^+ = 103$ (77%).

N-*(allylsulfonyl)allylamine (5)*



Allylsulfonylchloride (2.38 g; 0.02 mol) in 5 ml THF reacted exothermically with allylaminolithium (0.02 mol) prepared as before. After one night under stirring at room temperature, THF was replaced by toluene. The lithium chloride was filtered off and the toluene was evaporated under vacuum leading to 2.84 g of a sticky brown compound identified as crude **5**. Yield: 89%. Attempts at recrystallization were unsuccessful. ^{1}H NMR ($CDCl_3$): 3.50–3.81 (m, 4H, CH_2(d,d′), 5.03–5.45 (m, 4H, H(a,a′,b,b′)), 5.47–6.10 (m, 2H, H(c,c′)). ^{13}C NMR ($CDCl_3$): 44.12 (CH_2N), 57.64 (CH_2SO_2), 116.53 and 117.71 (CH_2=), 133.44 and 134.90 (=CH). IR ($CDCl_3$): 3291 (NH), 1146 and 1327 cm^{-1} (SO_2), 1621 cm^{-1} (C=C). MS (EI): $M^{+\cdot} - SO_2 = 97$ (5%), $M^{+\cdot} - HSO_2CH_2 = CH = 71$ (100%).

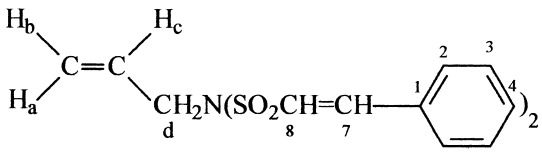
N,N′-*bis(benzylsulfonyl)allylamine (6)*



According to the procedure used for **3**, to allyldiaminolithium, formed by reaction of butyllithium (8.98 mmol; 5.60 ml at 1.6 M in hexane) on allylamine (0.26 g; 4.50 mmol) in

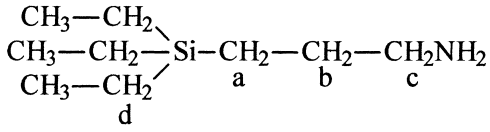
2 ml THF, was added benzylsulfonylchloride (1.71 g; 8.98 mmol) in 10 ml THF to yield, 1.38 g to **6** as a yellow powder. Yield: 84%. Mp decomposition around 60°C. ^{1}H NMR ($CDCl_3$): 4.68–5.31 (m, 7H, H(a,b,c) and CH_2e), 3.50 (d, 2H, CH_2d, $^3J_{cd} = 6$ Hz); 7.41 (s, 10H, C_6H_5). ^{13}C NMR ($CDCl_3$): 127.21 (C_1), 131.88 (C_2), 129.01 (C_3), 129.39 (C_4), 119.80 (CH_2=), 132.14 (=CH), 52.48 (CH_2N), 62.27 ($CH_2C_6H_5$). IR ($CDCl_3$): 1164 and 1372 cm^{-1} (SO_2), 1642 cm^{-1} (C=C). MS (EI): $M^{+\cdot} = 365$ (1%), $M^{+\cdot} - SO_2 = 301$ (1%), $M^{+\cdot} - C_6H_5CH_2SO_2 = 210$ (10%), $M^{+\cdot} - C_6H_5CH_2$-SO_2–$SO_2 = 146$ (17%), $M^{+\cdot} - 2(C_6H_5CH_2SO_2) = 55$ (7%).

N,N′-*bis(styrenesulfonyl)allylamine (7)*



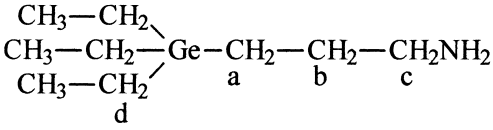
According to the same procedure used for preparation of **6**, addition of styrenesulfonylchloride (1.86 g; 9.18 mmol) in 5 ml THF to allylaminodilithium (9.12 mmol) in 2 ml THF led to a brown sticky residue of crude **7**. The starting material $C_6H_5CH{=}CHSO_2Cl$, that did not react, was precipitated as the hydrochloride using triethylamine is toluene. Pure **7** was isolated as a brown sticky compound (1.42 g). Yield: 40%. Attempts at recrystallization were unsuccessful. ^{1}H NMR ($CDCl_3$): 4.35 (d, 2H, CH_2d, $^3J_{cd} = 6$ Hz), 4.80–5.89 (m, 3H, H(a,b,c)), 6.84–7.69 (m, 14H, C_6H_5, H(7,8)). ^{13}C NMR ($CDCl_3$): 131.99 (C_1), 128.80 (C_2), 129.23 (C_3), 128.54 (C_4), 131.61 (C_7), 143.53 (C_8), 119.73 (CH_2=), 132.65 (=CH), 51.06 (C_d). IR ($CDCl_3$): 1152 and 1372 cm^{-1} (SO_2), 1614 cm^{-1} (C=C). MS (EI): $M^{+\cdot} = 389$ (1%), $M^{+\cdot} - SO_2 = 325$ (3%), $M^{+\cdot} - SO_2 + 1 = 262$ (3%).

3-triethylsilylpropanamine (8)



Triethylsilane (3.24 g; 0.03 mol) and allylamine (1.45 g; 0.03 mol) were heated under stirring in the presence of 0.1 ml of H_2PtCl_6 at 0.1 M in isopropanol. After 6 h at 100°C, the mixture was distilled to yield 3.70 g of pure **8**. Yield: 71%. Bp 270°C/760 mm Hg. ^{1}H NMR ($CDCl_3$): 0.10–0.99 (m, 19H, Et_3Si, CH_2(a,b)), 2.42 (t, 2H, CH_2C, $^3J_{bc} = 7$ Hz), 1.08 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$): 8.07 (CH_2a), 28.01 (CH_2b), 45.65 (CH_2c), 3.04 (CH_2d), 7.18 (CH_3). IR (film): 3289 and 3378 cm^{-1} (NH_2). MS (EI): $M^{+\cdot} - Et = 144$ (100%), $M^{+\cdot} - 2Et = 115$ (26%), $M^{+\cdot} - 3Et = 86$ (11%).

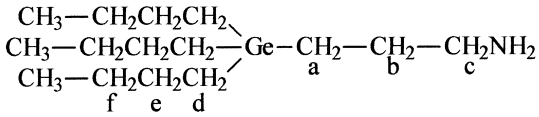
3-triethylgermylpropanamine (9)



Triethylgermane (2.47 g; 0.02 mol) and allylamine (0.95 g; 0.02 mol) were heated in the presence of 0.1 ml of H_2PtCl_6 at 0.1 M in isopropanol. After two days in a sealed tube at 80°C, the mixture was distilled to yield 0.95 g of pure **9**. Yield: 30%. Bp 130–140°C/40 mm Hg. ^{1}H NMR ($CDCl_3$): 0.20–1.15 (m, 19H, Et_3Ge, CH_2(a,b)); 2.60 (t, 2H, CH_2c, $^3J_{bc} = 7$ Hz); 1.91 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$): 8.30 (CH_2a); 29.03 (CH_2b); 45.37 (CH_2c); 3.80 (CH_2d); 8.90 (CH_3). IR (film): 3278 and 3356 cm^{-1} (NH_2). MS (EI): $M^{+\cdot} = 219$ (75%); $M^{+\cdot} - Et = 190$ (75%); $M^{+\cdot} - 2Et = 161$ (67%); $M^{+\cdot} - 3Et = 132$ (67%).

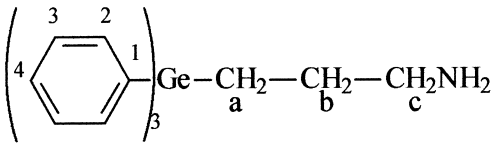
3-tributylgermylpropanamine (10)

Tributylgermane (3.56 g; 0.02 mol) and allylamine (0.83 g; 0.02 mol) were heated in the



presence of 0.1 ml of H_2PtCl_6 at 0.1 M in isopropanol. After one day in a sealed tube at 110°C, the mixture was distilled to yield 1.95 g of pure **10**. Yield: 43%. Bp 120°C/12 mm Hg. ^{1}H NMR ($CDCl_3$): 0.73–1.68 (m, 33H, Bu_3Ge, CH_2(a,b) and NH_2), 2.64 (t, 2H, CH_2c, $^3J_{bc} = 7$ Hz). ^{13}C NMR ($CDCl_3$): 9.66 (CH_2a), 29.19 (CH_2b), 45.54 (CH_2c), 12.44 (CH_2d), 26.68 (CH_2e), 27.51 (CH_2f), 13.81 (CH_3). IR (film): 3267 and 3378 cm^{-1} (NH_2). MS (EI): $M^{+\cdot} - Bu = 246$ (11%), $M^{+\cdot} - 2Bu = 189$ (9%) $M^{+\cdot} - 3Bu = 132$ (29%), $M^{+\cdot} - NH_2 - 1 = 286$ (3%).

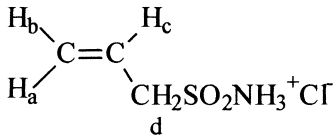
3-triphenylgermylpropanamine (11)



Triphenylgermane (0.48 g; 1.58 mmol) in 0.2 ml THF and allylamine (0.20 g; 3.52 mmol) were heated in the presence of AIBN. After 3 days in a sealed tube at 100°C, the evaporation of THF and allylamine in excess led to 0.26 g of **11** as a yellow powder. Yield: 45%. Mp 93–95°C. ^{1}H NMR ($CDCl_3$): 1.56–1.65 (m, 6H, CH_2(a,b) and NH_2), 2.71 (t, 2H, CH_2c, $^3J_{bc} = 7$ Hz), 7.31–7.53 (m, 15H, C_6H_5). ^{13}C NMR ($CDCl_3$): 11.14 (CH_2a), 29.19 (CH_2b), 45.38 (CH_2c), 137.03 (C_1), 128.25 (C_2), 135.16 (C_3), 128.95 (C_4). IR ($CDCl_3$): 3070 and 3154 cm^{-1} (NH_2). MS (EI): $M^{+\cdot} = 363$ (7%), $M^{+\cdot} - C_6H_5 = 286$ (43%).

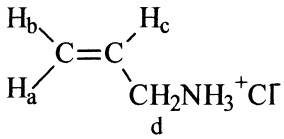
Attempts at hydrosilation or germation of **1** *and* **2**: reaction of different silanes or germanes on compounds **1** and **2** in the presence of different catalysts did not yield to any reaction (R_3M = $SiEt_3$, $GeEt_3$, $GePh_3$, $SiPh_3$, catalysts being H_2PtCl_6, $(Ph_3P)_4Pt$, AIBN or tBu_2O_2).

Allylsulfonamide Hydrochloride (12)



Allylsulfonamide (0.12 g; 0.99 mmol) and phenyldichlorogermane (0.41 g; 1.84 mmol) were heated for 2 days in a sealed tube at 80°C. Centrifugation of the mixture gave two phases, a liquid one containing an unidentified polymer and a solid identified by NMR to be allylsulfonamide hydrochloride **12**. ^{1}H NMR ($CDCl_3$): 3.83 (d, 2H, CH_2SO_2, $^3J_{cd} = 7$ Hz), 5.20–6.20 (m, 3H, H(a,b,c)), 4.80 (s, 3H, NH_3^+). ^{13}C NMR ($CDCl_3$): 59.41 (CH_2SO_2), 124.83 (CH_2=), 125.79 (=CH).

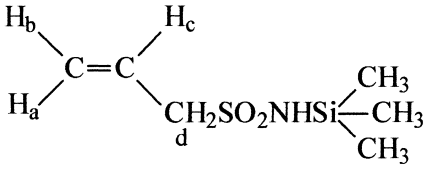
Allylamine Hydrochloride (13)



According to the procedure used for **12**, allylamine (0.09 g; 1.61 mmol) and phenyldichlorogermane (0.72 g; 3.22 mmol) were reacted exothermically to yield, besides an unidentified polymer, a solid identified as allylamine hydrochloride **13**. ^{1}H NMR ($CDCl_3$): 3.46 (d, 2H, CH_2, $^3J_{cd} = 7$ Hz), 5.10–6.20 (m, 3H, H(a,b,c)), 6.76 (s, 3H, NH_3^+). ^{13}C NMR ($CDCl_3$): 42.58 (CH_2N), 119.36 (CH_2=), 132.33 (=CH). IR (nujol): 1648 cm^{-1} (C=C) and 2800 cm^{-1} (NH_3^+). MS (electrospray, positive mode): 58 (CH_2 = $CHCH_2NH_3^+$).

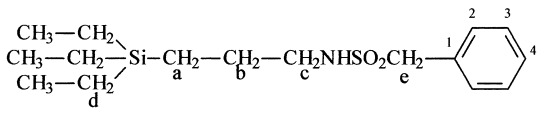
N-*trimethylsilylallylsulfonamide (14)*

To allylsulfonamide **2** (0.50 g; 4.14 mmol) in 10 ml THF at −70°C, was added butyllithium (4.14 mmol; 5.10 ml at 1.6 M in hexane). To the mixture warmed to room temperature, was added drop-wise trimethylchlorosilane (0.45 g;



4.14 mmol) in 4 ml THF. After 2 h under reflux, the THF was evaporated, replaced by toluene and the lithium chloride was filtered off. Evaporation of the toluene under vacuum led to a yellow sticky residue, which was washed twice with pentane to yield 1.01 g of yellow powder of **14**. Yield: 93%. Mp 53–55°C. ^{1}H NMR ($CDCl_3$): 0.13 (s, 9H, CH_3), 3.72 (d, 2H, CH_2d, $^3J_{cd} = 7$ Hz), 5.18–6.50 (m, 4H, H(a,b,c) and NH). ^{13}C NMR ($CDCl_3$): 121.88 (CH_2=), 128.53 (=CH), 60.50 (CH_2d), 1.73 (CH_3). IR ($CDCl_3$): 3443 (NH), 1151 and 1346 cm^{-1} (SO_2), 1641 cm^{-1} (C=C). MS (Dci/CH_4): $M + H^+ = 194$, $M + C_2H_5^+ = 222$, $M + C_3H_5^+ = 234$.

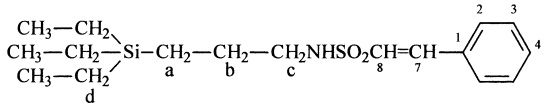
N-*(benzylsulfonyl)-3-triethylsilylpropanamine (15)*



By the same procedure, to 3-triethylsilylpropanaminolithium, formed by reaction of **8** (0.52 g; 2.98 mmol) in 10 ml THF at −75°C and butyllithium (2.98 mmol; 1.86 ml at 1.6 M in hexane), was added benzylsulfonylchloride (0.57 g; 2.99 mmol) in 5 ml THF. The sticky residue (0.88 g) obtained after evaporation of the solvent was identified as **15**. Yield: 90%. Attempts to recrystallize from different solvents were unsuccessful. ^{1}H NMR ($CDCl_3$): 0.25–1.03 (m, 19H, CH_2 (a,b,d) and CH_3), 2.90 (t, 2H, CH_2c, $^3J_{bc} = 7$ Hz), 4.21 (s, 2H, CH_2e), 7.36 (s, 6H, C_6H_5 and NH). ^{13}C NMR ($CDCl_3$): 129.56(C_1), 130.68 (C_2), 128.97 (C_3), 128.85 (C_4), 8.32 (C_a), 25.16 (C_b), 47.03 (C_c), 3.11 (C_d), 58.72 (C_e), 7.48 (CH_3). IR ($CDCl_3$): 3391 (NH), 1152 and 1327 cm^{-1} (SO_2). MS (EI), $M^{+\cdot} = 327$ (4%), $M^{+\cdot} - Et = 298$ (100%), $M^{+\cdot} - Et{-}SO_2 = 234$ (78%).

Traces of the *N*-disubstituated compound (< 5%) were characterized by mass spectroscopy.

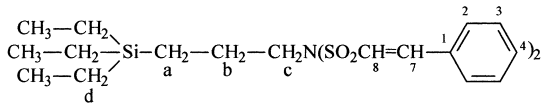
N-*(styrenesulfonyl)-3-triethylsilylpropanamine (16)*



In a similar manner to 3-triethylpropanaminolithium, formed by reaction of **8** (0.52 g; 298 mmol) in 10 ml THF at −70°C and butyllithium (2.98 mmol; 1.88 ml at 1.6 M in hexane), was added styrenesulfonylchloride (0.61 g; 3.01 mmol) in 5 ml THF. The sticky residue (0.82 g) obtained after evaporation of the solvent was identified as **16**. Yield: 90%. Attempts of recrystallization from different solvents were unsuccessful. ^{1}H NMR ($CDCl_3$): 0.41–0.96 (m, 19H, CH_2 (a,b,d) and CH_3), 3.05 (t, 2H, CH_2c, $^3J_{bc} = 7$ Hz), 6.75 (d, 1H, H_8, $^3J_{7-8(trans)} = 15$ Hz), 7.00–7.70 (m, 7H, H_7, C_6H_5 and NH). ^{13}C NMR ($CDCl_3$): 132.66 (C_1), 128.27 (C_2), 129.12 (C_3), 129.20 (C_4), 130.84(C_7), 141.53 (C_8), 8.44(C_a), 24.70 (C_b), 46.45 (C_c), 3.18 (C_d), 7.47 (CH_3). IR ($CDCl_3$): 3290 (NH), 1146 and 1327 cm^{-1} (SO_2), 1618 cm^{-1} (C=C). MS (EI):$M^{+\cdot} - Et = 310$ (100%), $M^{+\cdot} - 2Et - 1 = 280$ (9%), $M^{+\cdot} - 3Et = 253$ (1%).

Traces (< 5%) of the *N*-disubstituted compound and of $C_6H_5CH{=}CHSO_2Bu$ formed by reaction of butyllithium on starting styrenesulfonylchloride were characterized and identified using synthetic samples prepared as follows (a, b).

(a) *N*,*N*′-bis(styrenesulfonyl)-3-triethylsilylpropanamine

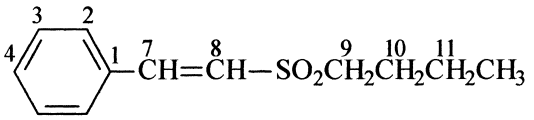


To 3-triethlsilylpropanaminodilithium, formed by reaction of **8** (0.50 g; 2.91 mmol) in 10 ml THF

at −70°C and butyllithium (5.82 mmol; 3.63 ml at 1.6 M in hexane), was added styrenesulfonylchloride (1.18 g; 5.83 mmol) in 5 ml THF. Evaporation of the solvent led to a sticky residue containing the disubstituated compound. ^{1}H NMR ($CDCl_3$): 0.10–0.98 (m, 19H, CH_2 (a,b,d and CH_3), 3.68 (t, 2H, CH_2c, $^3J_{bc} = 7$ Hz), 6.80 (d, 2H, H_8, $^3J_{7-8(trans)} = 15$ Hz), 7.00–7.55 (m, 12H, H_7 and C_6H_5). ^{13}C NMR ($CDCl_3$): 132.70 (C_1), 128.56 (C_2), 128.78 (C_3), 131.55 (C_7), 142.96 (C_8), 8.44 (C_a), 29.77 (C_b), 52.18 (C_c), 3.18(C_d), 7.47 (CH_3). MS (EI):$M^{+\cdot} - Et = 476$ (5%).

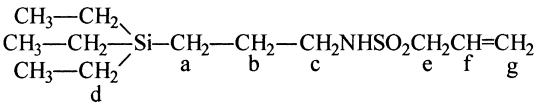
Note that under these conditions, the expected disubstitued compound was not pure and contained **16** (46%) and $C_6H_5CH{=}CHSO_2Bu$ (30%).

(b) (*trans*-styrenesulfonyl) butane



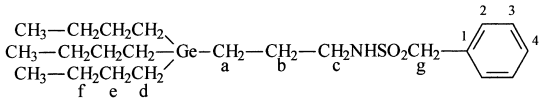
To styrenesulfonylchloride (0.29 g; 1.40 mmol) in 5 ml THF at −70°C, was added butyllithium (1.40 mmol; 0.90 ml at 1.6 M in hexane). After 2 h stirring at room temperature, the THF was evaporated under vacuum, replaced by toluene and the LiCl was filtered off. Evaporation of the solvent led to a sticky residue containing starting styrenesulfonylchloride (58%) and $C_6H_5CH{=}CHSO_2Bu$ (42%). ^{1}H NMR ($CDCl_3$): 1.36–1.74 (m, 7H, CH_2 (10,11) and CH_3), 4.17 (t, 2H, $CH_2$9, $^3J_{10-9} = 7$ Hz), 7.17–7.50 (m, 7H, H(7,8) and C_6H_5). ^{13}C NMR ($CDCl_3$): 128.54 (C_2), 129.70 (C_3), 131.52 (C_7), 144.71 (C_8), 31.00 (C_{10}); 20.20 (C_{11}), 12.75 (CH_3). MS (EI):$M^{+\cdot} - SO_2 = 160$ (30%), $M^{+\cdot} - Bu = 167$ (100%), $M^{+\cdot} - SO_2 - CH_2CH_2CH_3 = 117$ (67%).

N-*(allysulfonyl)-3-triethylsilylpropanamine (17)*



Following the procedure used for **15** the monolithium derivative of **8** (2.98 mmol) and allysulfonylchloride (0.42 g; 2.98 mmol) led to 0.75 g of a sticky residue identified as **17**. Yield: 90%. Attempts at recrystallization were unsuccessful. ^{1}H NMR ($CDCl_3$): 0.35–1.10 (m, 19H, CH_2(a,b,d) and CH_3), 3.00 (t, 2H, CH_2c, $^3J_{bc} = 7$ Hz), 3.77 (d, 2H, CH_2e, $^3J_{fe} = 7$ Hz), 5.23–6.10 (M, 4H, CH_2g, H_f and NH). ^{13}C NMR ($CDCl_3$): 8.42 (C_a), 29.76 (C_b), 46.91 (C_c), 3.20 (C_d), 57.02 (C_e), 126.14 (C_f), 123.77 (C_g), 7.47 (CH_3). MS $M^{+\cdot} - Et_2 = 248$ (14%), $M^{+\cdot} - 3Et = 190$ (25%).

N-*(benzylsulfonyl)-3-tributylgermylpropanamine (18)*

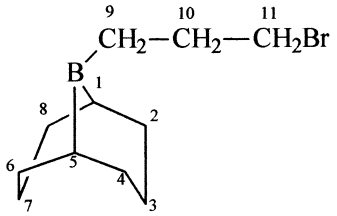


In a similar manner the monolithium derivative of **10** (0.93 mmol) and benzylsulfonylchloride (0.18 g; 0.94 mmol) led to 0.35 g of a sticky residue of crude **18**. Yield: 82%. ^{1}H NMR ($CDCl_3$): 0.50–1.50 (m, 31H, CH_2(a,b,d,e,f), CH_3), 2.94 (t, 2H, CH_2c, $^3J_{bc} = 7$ Hz), 4.23 (s, 2H, CH_2g), 7.37 (s, 6H, C_6H_5) and NH). ^{13}C NMR ($CDCl_3$): 9.77 (C_a), 29.77 (C_b), 46.83 (C_c), 12.38 (C_d), 26.64 (C_e), 27.47 (C_f), 58.68 (C_g), 13.83 (CH_3), 127.41 (C_1), 130.64 (C_2), 128.87 (C_3), 128.77 (C_4). MS (EI) $M^{+\cdot} - Bu = 400$ (43%), $M^{+\cdot} - Bu - SO_2 = 336$ (20%).

The major impurity was $Bu_3Ge(CH_2)_3N(SO_2CH_2C_6H_5)_2$ formed by dilithiation of the starting amine (10–25% according to the preparation) which could not be separated by recrystallization. ^{1}H NMR ($CDCl_3$): 0.50–1.50 (m, 31H, CH_2(a,b,d,e,f) and CH_3), 2.90 (t, 2H, CH_2c, $^3J_{bc} = 7$ Hz), 4.70 (s, 4H, CH_2g), 7.37 (s, 10H, C_6H_5). MS (EI): $M^{+\cdot} - Bu = 554$ (1%), $M^{+\cdot} - Bu - SO_2 = 490$ (1%), $M^{+\cdot} - 3Bu = 440$ (1%).

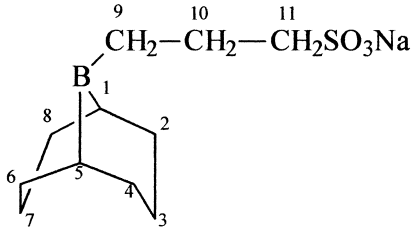
3-[9-borabicyclo[1.3.3]nonane]-1-bromopropane (19)

To 9-borabicyclo[1.3.3]nonane (0.04 mol; 68 ml at 0.5 M in THF) was added allylbromide



(4.18 g; 0.04 mol). After 5 h at room temperature, distillation yielded 2.03 g of pure **19**. Yield: 24%. Bp 160°C/20 mm Hg. ^{1}H NMR ($CDCl_3$): 0.90–2.05 (m, 18H, CH and CH_2(1–10), 3.42 (t, 2H, CH_2(11), $^3J_{10-11} = 7$ HZ). ^{13}C NMR ($CDCl_3$): 23.27 (CH_2(3,7,9)), 28.57 (CH_2(10)), 33.34(CH(1,5) and CH_2(2,4,6,8), 37.18 (CH_2(11)).IR (film): 2911 cm^{-1} (CH). MS (EI): $M^{+\cdot} = 242$ (6%), $M^{+\cdot} - (CH_2)_2 = 214$ (6%), $M^{+\cdot} - (CH_2)_3 = 200$ (12%), $M^{+\cdot} - (CH_2)_4 = 186$ (3%), $M^{+\cdot} - (CH_2)_5 = 172$ (11%), $M^{+\cdot}$ − bicyclo[3.3.0]octane + H = 133 (100%).

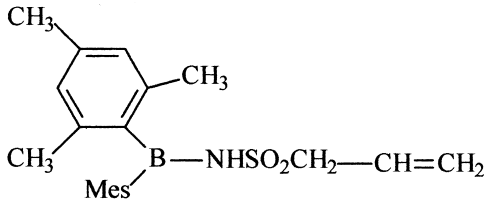
3-[9-borabicyclo[1.3.3]nonane]-1-propylsulfonic Acid, Sodium Salt (20)



To sodium sulfite (0.99 g; 7.78 mmol) in 5 ml water at 60°C was added **19** (1.89 g; 7.78 mmol) drop-wise. The reaction was exothermic. After 4 h further heating at 100°C, the water was evaporated, yielding yellow crystals of a mixture of sodium bromide and **20**. ^{1}H NMR (D_2O): 0.83–1.15 (m, 4H, CH_2(3,7)), 1.15–2.11 (m, 10H, CH_2(2,4,6,8,10)), 2.94–2.98 (m, 4H, $CH_2$9 and CH(1,5)), 3.75 (t, 2H, $CH_2$11, $^3J = 7$ Hz).

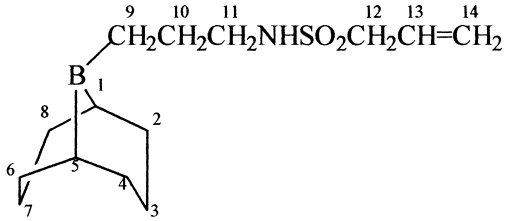
A large excess of $P(O)Cl_3$ was added to the mixture of salts. After 3 h under reflux and addition of NH_3 gas to the mixture, an NMR spectra in DMSO allowed the characterization of NH_4Cl beside a compound insoluble in organic solvents, water and DMSO, which could not be identified as the expected sulfonamide.

N-*(dimesitylboro)allylsulfonamide (21)*



As prepared in the synthesis of **14**, the monolithium derivative of **2** (3.22 mmol) was reacted with dimesitylfluoroborane (0.86 g; 3.22 mmol) in 5 ml THF leading to 1.05 g of a white powder of **21**. Yield: 88%. Mp 107–109°C. ^{1}H NMR ($CDCl_3$): 2.29 (s, 12H, oCH_3), 2.34 (s, 6H, pCH_3), 3.77 (d, 2H, CH_2SO_2, $^3J_{HH} = 7$ Hz), 5.27–6.10 (m, 4H, CH_2=CH and NH), 6.82 (s, 4H, C_6H_2). ^{13}C NMR ($CDCl_3$): 23.26 (oCH_3), 22.78 (pCH_3), 140.67 (C_2), 128.19 (C_3), 138.95 (C_4), 58.90 (CH_2SO_2), 124.83 (=CH), 124.94 (CH_2=). IR (nujol): 3210 cm^{-1} (NH), 1122 and 1397 cm^{-1} (SO_2), 1606 cm^{-1} (C=C). MS (EI): $M^{+\cdot}$ − Mes = 250 (48%), $M^{+\cdot}$ − Mes−$CH_2CHCH_2SO_2$ = 145 (100%).

Characterization Of N-*allylsulfonyl, 3-[9-borabicyclo[1.3.3]nonane]propanamine*



19 (0.61 g; 2.50 mmol) in 2 ml THF was reacted exothermically with the monolithium derivative of **2** (2.50 mmol). After 3 h at 60°C, evaporation of the solvent led to sticky yellow residue. An ^{1}H NMR spectra in $CDCl_3$ allowed the identification of the expected compound, further characterized by GC/MS: ^{1}H NMR ($CDCl_3$): 1.14–1.65 (m, 18H,

CH and CH_2 (1–10), 2.41 (t, 2H, CH_2 (11), $^3J_{10-11} = 7$ Hz), 3.70 (d, 2H, CH_2(12), $^3J_{13-12} = 7$ Hz), 5.18–6.05 (m, 4H, CH(13), CH_2(14) and NH). MS (EI): $M^{+\cdot} = 283$ (17%), $M^{+\cdot}$ − bicyclo[3.3.0]octane−H = 172 (39%). MS (Dci/CH_4): $M + H^+ = 248$, $M + C_2H_5^+ = 312$. MS (Dci/NH_3): $M + H^+ = 284$, $M + NH_4^+ = 301$. Attempts at recrystallization did not lead to a pure compound.

Reaction of Et_3SiH with Allylbromide

Triethylsilane (2.79 g; 0.02 mol) reacted exothermically with allybromide (2.86 g; 0.02 mol) in the presence of 0.1 ml H_2PtCl_6 (0.1 M in iPrOH). Spectroscopic analysis showed the formation of triethylbromosilane instead of the expected compound. ^{1}H NMR ($CDCl_3$): 0.80–1.17 (m, 15H, CH_3 and CH_2). ^{13}C NMR ($CDCl_3$): 7.09 (CH_3), 7.84 (CH_2). MS (EI): $M^{+\cdot} = 194$ (17%), $M^{+\cdot}$ − Et = 165 (100%), $M^{+\cdot}$ − 2Et = 136 (83%), $M^{+\cdot}$ − 3Et = 107 (17%).

Biochemistry

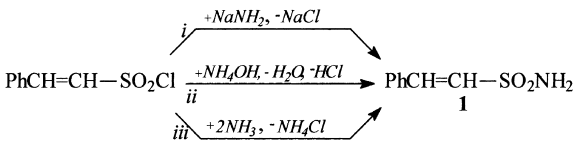
Human CAI and CAII cDNAs were expressed in *Escherichia coli* strain BL21 (DE3) from the plasmids pACA/hCAI and pACA/hCAII described by Likdskog *et al.*[17] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by this group[18] and enzymes were purified by affinity chromatography according to the method of Khalifah *et al.*[19] Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of 49 $mM^{-1}\,cm^{-1}$ for CAI and 54 $mM^{-1}\,cm^{-1}$ for CAII, respectively, based on $M_r = 28.85$ kDa for CAI, and 29.30 kDa for CAII, respectively.[20,21]

Initial rates of 4-nitrophenyl acetate hydrolysis catalyzed by different CA isozymes were monitored spectrophotometrically, at 400 nm, with a Cary 3 instrument interfaced with an IBM compatible PC.[22] Solutions of substrate were prepared in anhydrous acetonitrile, working at 25°C. A molar absorption coefficient ε of 18,400 $M^{-1}\,cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis, under the conditions of the experiments (pH 7.40), as reported in the literature.[22] Non-enzymatic hydrolysis rates were always subtracted from the observed rates. Duplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the mean of such results. Inhibitor and enzyme solutions were preincubated together for 10 min at room temperature prior to assay, in order to allow for the formation of the E–I complex. The inhibition constant K_I was determined as described by Pocker and Stone.[22]

RESULTS AND DISCUSSION

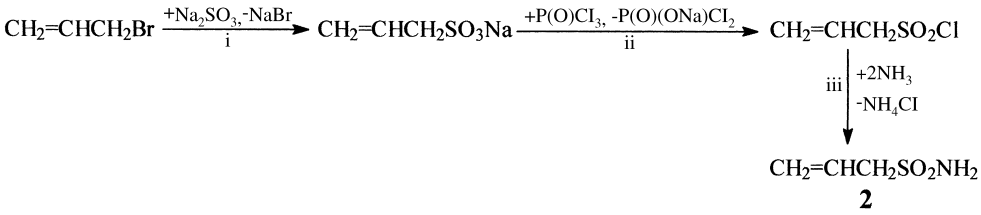
Chemistry

None of the starting unsaturated sulfonamides are commercially available. We tried a number of routes to obtain the best experimental conditions to provide a primary sulfonamide from commercially available *trans*-styrene sulfonylchloride (Scheme 1).

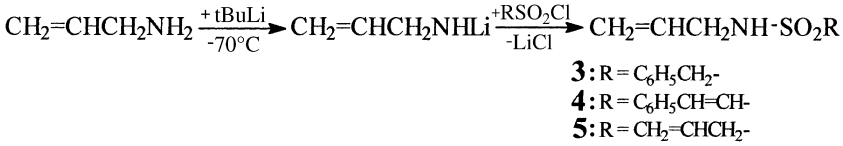


SCHEME 1

Actually **1** may be obtained by any of the three reactions of Scheme 1. However reaction (i) (Scheme 1) is relatively slow and sodium amide must be free of any traces of sodium hydroxide to prevent the formation of the corresponding inseparable sulfonic acid and its sodium salt. With aqueous ammonia (ii) (Scheme 1), **1** is formed in much lower yield than by direct ammonolysis (iii) (Scheme 1), which therefore



SCHEME 2



SCHEME 3

provides the best method of synthesis of a primary sulfonamide from the corresponding sulfonyl chloride.

Allylsulfonamide is obtained in a three steps reaction (Scheme 2) according to literature procedures[14] and allylsulfochloride and allylsulfonamide **2** were characterized by spectrochemical means.

The *N*-allylsulfonamides were prepared by reaction on *N*-lithiated allylamine with sulfonyl chlorides (Scheme 3).

There was no observable allyllic transition as seen from the absence of a CH_3 signal in the ^1H NMR. However, the dilithiated derivative of *N*-allyl amine is easily formed and led to the disulfonylamides **6**, **7** (Scheme 4).

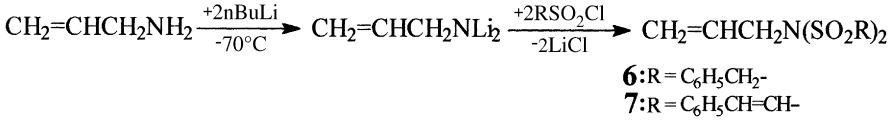
For the sterically non-hindered double bond of allylamine, addition across the bond performed in the presence of catalysts[15,16] and leads to the C-silylated corresponding amine (Eq. (1)).

$$CH_2 = CHCH_2NH_2 + R_3MH \xrightarrow[\Delta]{catalyst} R_3M(CH_2)_3NH_2 \quad (1)$$

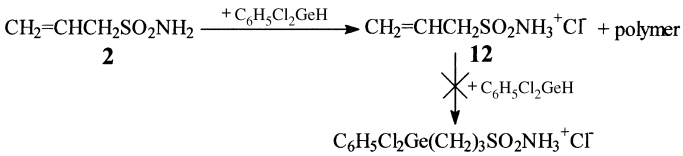
8 : $R_3M = SiEt_3$; **9** : $R_3M = GeEt_3$;
10 : $R_3M = GeBu_3$; **11** : $R_3M = GePh_3$

We verified that the same experimental conditions (temperature, catalyst, reaction time) did not induce any secondary reaction or polymerization of the allylsulfonamide. However, we were unable to repeat these reactions on either of the unsaturated sulfonamides **1**, **2** (Eq. (2)).

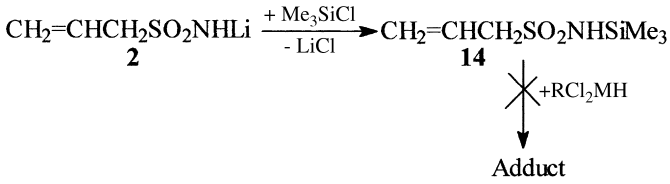
$$CH_2=CH\text{-}CH_2SO_2NH_2\ (\mathbf{2}) + R_3MH \xrightarrow[\Delta]{catalyst} \not\rightarrow \quad (2)$$



SCHEME 4



SCHEME 5

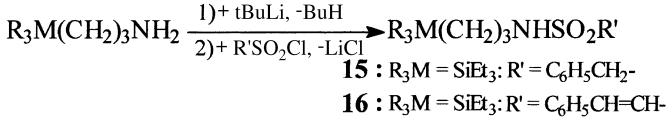


SCHEME 6

As hydrosilylation or germylation are usually easier from halohydrosilanes or germanes, we tried phenyl dichlorogermane and methyl dichlorosilane on the less sterically hindered sulfonamide **2** (Scheme 5). As expected, the first step of the reaction led to the sulfonamide hydrochloride **12**, which did not react either in the presence of H_2PtCl_6, on another equivalent of RCl_2MH (RCl_2MH=$PhCl_2GeH$, $MeCl_2SiH$). Note that starting from allylamine, we obtained the allylamine hydrochloride **13**, using the same reaction conditions.

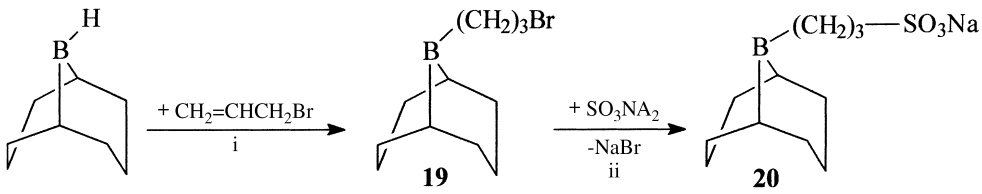
Another, *N*-protected allyl-sulfonamide such as **14** (Scheme 6), under the same conditions, did not allow either any addition across the allylic double bond.

A comparison of allylamine and allylsulfonamide **2** using molecular modeling did not explain such a change in reactivity in this reaction [the most stable conformers were obtained by minimization of the energies and dynamics from 278 to 400°C using Insight II, Discover 3, Molecular Simulation-MSI, Energy Calculation based on esff force field (9685 Scranton Road, San Diego, CA 92121–2777]. Both C=C double bonds present the same formal charges and although one face of the double bond in allylsulfonamide **2** is sterically more hindered, the other is perfectly accessible. The sulfonamide group probably acts as a poison for the transition metal catalysts, since the use of $(Ph_3P)_4Pt$ (Eq. (2)) did not improve the rate of hydrosilylation. Addition of sulfonamide **2** to the reaction mixture leading to triethylsilylpropanamine **8** (Eq. (1)) impedes the hydrosilylation reaction since the starting triethylsilane and allylamine were recovered unchanged in the presence of **2**. A more thorough investigation of the action of the sulfonamide group on the platinum catalysis, as well as other catalysts for this reaction with unsaturated



15 : $R_3M = SiEt_3$: R' = $C_6H_5CH_2$-
16 : $R_3M = SiEt_3$: R' = C_6H_5CH=CH-
17 : $R_3M = SiEt_3$: R' = CH_2=$CHCH_2$-
18 : $R_3M = GeBu_3$: R' = $C_6H_5CH_2$-

SCHEME 7



SCHEME 8

sulfamides is in progress but will be dealt with in a further work.

In order to obtain a C-metallated sulfonamide with an unsaturated radical, 3-silyl or 3-germyl propanamide lithiums of **8**, **10** were reacted with the corresponding sulfonylchlorides (Scheme 7).

Hydrosilylation of **4** does not lead to **16**. There is no selective hydrosilylation of the less sterically hindered double bond, apparently the steric hindrance on nitrogen or the SO_2 proximity here also, prevented the reaction (Eq. (3)).

$$C_6H_5CH{=}CHSO_2NHCH_2CH{=}CH_2 \quad \mathbf{4}$$

$$\xrightarrow[H_2PtCl_6,\ \Delta]{+\ Et_3SiH} C_6H_5CH{=}CHSO_2NHCH_2CH_2CH_2SiEt_3 \quad \mathbf{16} \qquad (3)$$

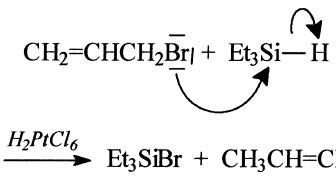
However a direct hydroboronation reaction such as in Eq. (1), cannot be used for the corresponding boron derivatives, because of the complexation of boron on the atom of nitrogen. Therefore, we tried to prepare a boron sulfonamide starting from the allylbromide, according to Scheme 8. Treatment of borosulfonate **20** with $POCl_3$, followed by amonolysis in a way similar to Scheme 2, led to ammonium chloride and to a solid insoluble in organic solvents, water and DMSO, which could not be identified as the expected sulfonamide.

Unsaturated boron sulfonamide **21** was obtained by reaction of dimesitylboron fluoride with the lithium derivative of the allylsulfonamide (Eq. (4)).

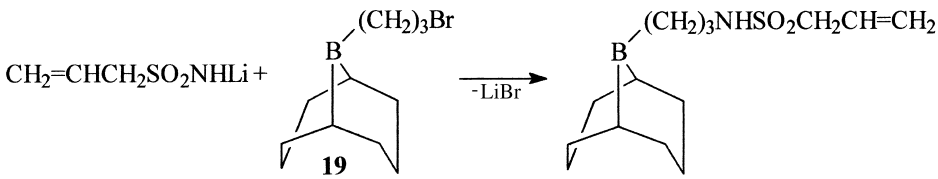
$$\begin{aligned} Mes_2BF + CH_2{=}CHCH_2SO_2NHLi &\\ &\rightarrow Mes_2BNHSO_2CH_2CH{=}CH_2 + LiF \end{aligned} \qquad (4)$$

Reaction of boron bromide **19** with the same lithium allylsulfonamide allowed the characterization of *N*-allylsulfonyl, 3-[9-borabicyclo[1.3.3]-nonane]propanamine in a mixture with secondary reaction products (Scheme 9).

Note also that the sequence of reactions in Scheme 8 cannot be used for obtaining the silicon compounds because of the halogenation of silicon. Triethylsilane reacts exothermically with allylbromide, with evolution of propene (Eq. (5)).



(5)



SCHEME 9

Biochemistry

Most of the unsaturated sulfonamides and their Si, Ge or B derivatives reported here were tested as inhibitors of two carbonic anhydrase isozymes in the quest for a better understanding of the inhibition mechanism (Table I).

As seen from the above data, three compounds behave as efficient inhibitors of both CA isoenzymes, all of them being more efficient on CAII. Two of them contain a primary sulfonamide group and unsaturation, which is either bulky (**1**) or not (**2**). If the sulfonamide group is replaced by a sulfonylchloride group (CH_2=$CHCH_2SO_2Cl$), we observe an increase in affinity for the enzyme, probably due to the reaction of nucleophilic moities (ε-NH_2 groups of Lys, imidazoles of His, etc.) with the reactive electrophile, i.e. RSO_2Cl, whereas when the sulfonamide group is replaced by an allyl moiety (**5**), there is a decrease in the activity. This confirms the fact that there are strong interactions between the sulfonamide group and the active site of the enzyme and also suggests that a sulfonylchloride group is able to produce strong inactivation due to reactions with amino-acids residues at the active site.

TABLE I Inhibition of isozymes bCAII and hCAI with inhibitors of type **1**–**21**. Sulfanilamide was included as standard as a well-known sulfonamide inhibitor

Inhibitor	bCAII K_I(nM)	hCAI K_I(μM)
Sulfanilamide	300	28
1	35	9
2	38	34
CH_2=$CHCH_2SO_2Cl$	14	39
3	398	nt*
4	405	nt
5	170	nt
6	>1000	nt
7	>1000	nt
8	>1000	nt
9	>1000	nt
10	>1000	nt
11	71	nt
14	59	nt
15	177	nt
16	222	nt
17	137	nt
20	379	nt
21	219	nt

*Not tested.

Considering compound **5**, it may be seen that when the allyl group is replaced by one more bulky substituent, such as benzyl (**3**) or styrene (**4**), the affinity for the enzyme is further decreased. The disubstitution with these two groups, as in compounds **6** and **7**, leads to completely inactive compounds.

By comparing compounds **5** and **14**, it appears that a trimethylsilyl group, even if more bulky than an allylsulfonyl group, is able to promote stronger interactions with the active site of the enzyme. The importance of the sulfonamide group for the CA inhibitory properties of these compounds is again obvious by considering compounds **8**–**11** and their substituted derivatives, **15**–**17**. The starting amines **8**–**11** are not efficient at all as CA inhibitors, but when the amino group is replaced by an allylsulfonyl (**17**), benzylsulfonyl (**15**) or styrenesulfonyl (**16**) group, forming a sulfonamide group in these substituted compounds **15**–**17**, the affinity for the enzyme is increased to the range of the sulfonamides, compounds **15**–**17** behaving as unusual inhibitors of CA. Comparing the results obtained for compound **21** and that obtained for dimesitylfluoroborane ($IC_{50} = 30\,\mu M$ for CAII), the starting material in a precedent paper,[10] the substitution of the fluorine by an allysulfonamide group greatly decreases the activity, probably because of the steric hindrance in **21** compared to dimesitylfluoroborance. In the same way, compound **20**, which is really bulky because of the 9-borabicyclo[1.3.3]nonane group, has a really low affinity for the enzyme.

The results presented here confirm the fact that two main factors are important in the design of new CA inhibitors: (i) the presence of an (unsubstituted or substituted with compact moieties) sulfonamide group, (ii) the steric hindrance around the zinc binding function, since bulky substituents considerably decrease the affinity of the inhibitors for the enzyme.[23] It seems that the presence of a double bond in the

structure of the inhibitor leads to really potent CAIs, this being the first report in the literature of strong CA inhibition by unsaturated sulfonamides. The three more active compounds in the series described here (**1**, **2**, and CH_2=$CHCH_2$-SO_2Cl), respond to these criteria and behave as efficient inhibitors of CAI and II (for **1** and **2**) and presumably as irreversible inhibitor (for the last derivative). It should be noted that unlike other enzymes (such as for example, the serine proteases),[24] CA inactivation by active-site directed irreversible inhibitors has not been investigated previously.

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