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Synthesis of β-aminoethanesulfonyl fluorides or 2-substituted taurine sulfonyl fluorides as potential protease inhibitors

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ABSTRACT

Substituted taurine sulfonyl fluorides derived from taurine or protected amino acids are conveniently synthesized from β-aminoethanesulfonyl chlorides using KF/18-crown-6 or from β-aminoethanesulfonates using DAST.

Probably one of most well-known and commonly used protease inhibitors is PMSF (phenyl methyl sulfonyl fluoride). This compound or the less known AEBSF (4-(2 aminoethyl)-benzenesulfonyl fluoride hydrochloride, Fig. 1) is often present in protease inhibitor 'cocktails'[1] or is added together with, for example, leupeptin, during isolation or handling procedures of proteins to prevent their premature proteolytic degradation.[2] PMSF is capable of inhibition of a broad spectrum of serine proteases, for example, chymotrypsin, trypsin and thrombin. It is cheap and relatively easy to handle. Its moderate reactivity prevents rapid (chemical) degradation by hydrolysis or fast reaction with a variety of nucleophiles. In this respect, the sulfonyl fluoride, which is the reactive moiety, is suitable for incorporation into potential protease inhibitors. Despite this, and to our knowledge, the synthesis of other functionalized sulfonyl fluorides has not been described in the literature together with applications as protease inhibitors or in other areas. This may be due to a relative lack of synthetic procedures for the mild preparation of aliphatic sulfonyl fluorides.

Recently, our successful efforts towards an efficient synthesis of amino acid-derived sulfonyl chlorides[3] provided us with a possible attractive entry for obtaining functionalized, amino acid-based sulfonyl fluorides for possible future use as selective protease inhibitors and for proteomics applications. Our results are reported herein.

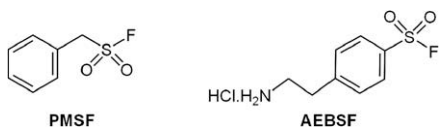
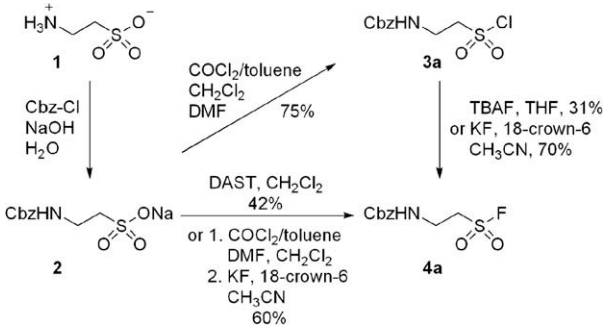


Figure 1. The structures of PMSF and AEBSF.

It was decided to start with the most simple sulfonyl fluoride derived from taurine. To this end, the amino group of taurine (**1**) was Cbz-protected using Cbz-chloride and NaOH (Scheme 1) leading to sodium sulfonate **2**, which was treated with a solution of phosgene in toluene to afford sulfonyl chloride **3a**. In an initial attempt to convert the sulfonyl chloride into a sulfonyl fluoride, TBAF in THF was used,[4] which afforded sulfonyl fluoride **4a** in only 31% yield. Although the TBAF solution was dried over molecular sieves before use, a significant amount of the sulfonyl chloride was hydrolyzed. Also, it was difficult to monitor the reaction on TLC since both the sulfonyl chloride and sulfonyl fluoride ran and stained similarly. Furthermore, repetition of this reaction showed that the yield was not reproducible (0–73%), probably because of the varying quantities of residual water in the TBAF solution. Therefore, it was decided to try a procedure using dry potassium fluoride and 18-crown-6 in acetonitrile,[5] which afforded sulfonyl fluoride **4a** in a good yield of 70%. The progress of the reaction was monitored from the shift of the SO_2CH_2 signals in the ^1H NMR spectrum. After developing this successful procedure, we were interested in whether it could also be applied to the crude



Scheme 1. Synthesis of taurine-derived sulfonyl fluoride **4a**.

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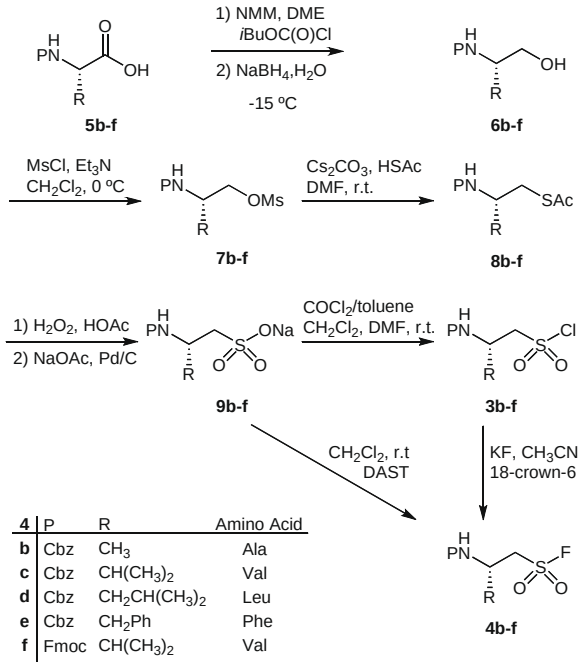
sulfonyl chloride in a one-pot reaction. Thus, sodium sulfonate **2** was converted to sulfonyl chloride **3a** and subsequently treated with potassium fluoride and 18-crown-6 after evaporation of excess phosgene and solvents. Sulfonyl fluoride **4a** was obtained in 60% yield, which was higher than that of the procedure with purification of the intermediate sulfonyl chloride **3a** (53%). The higher yield combined with omitting one purification step made this the procedure of choice. Although having found a good two-step, one-pot procedure for the preparation of the amino acid-based sulfonyl fluoride, we were left wondering whether diethylamino sulfur trifluoride (DAST)[6] could also be used to transform sodium sulfonate **2** directly into sulfonyl fluoride **4a**. This was indeed possible and **4a** was obtained, albeit in a lower yield (42%). Therefore, it was decided to use the former two-step, one-pot procedure with KF via the crude sulfonyl chloride.



4	P	R	Amino Acid
b	Cbz	CH_3	Ala
c	Cbz	$CH(CH_3)_2$	Val
d	Cbz	$CH_2CH(CH_3)_2$	Leu
e	Cbz	CH_2Ph	Phe
f	Fmoc	$CH(CH_3)_2$	Val

Scheme 2. Synthesis of amino acid-based sulfonyl fluorides **4b–f** starting from *N*-protected amino acids.

Table 1
Yields (%) for the synthesis of sulfonyl fluorides **4b–f** from Cbz- or Fmoc-protected amino acids

Amino acid	7	8	3[a]	4	4[a,d]
Cbz-Ala-OH (**b**)	82	91	61	65[c]	65
Cbz-Val-OH (**c**)	50	65	51	nd	40
Cbz-Leu-OH (**d**)	—	58[a]	44	45[c]	nd
Cbz-Phe-OH (**e**)	80[e]	86[e]	87[e]	67[c]	62
Fmoc-Val-OH (**f**)	90	86	71	30[b]	nd

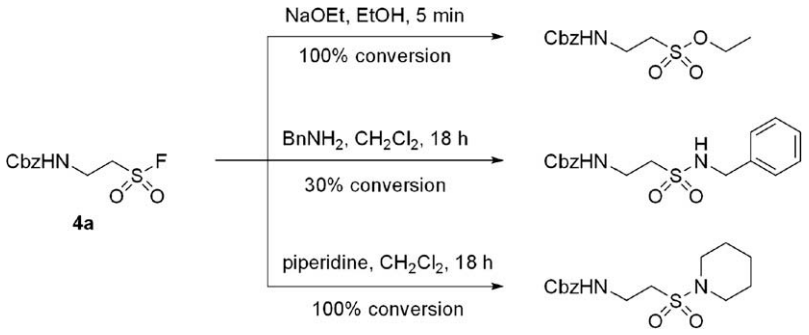
[a] Yield over two steps.
[b] Yield obtained using the TBAF procedure.
[c] Yield obtained using the KF/18-crown-6 ether procedure.
[d] Yield obtained using the DAST procedure.
[e] Ref. 4.

After preparation of taurine-derived sulfonyl fluoride **4a**, the next step was the preparation of sulfonyl fluorides derived from a variety of side-chain-containing amino acids. Thus, Cbz-protected amino acids **5b–e** (Ala, Val, Leu and Phe) were first reduced to the corresponding alcohols **6b–e** (Scheme 2) using sodium borohydride treatment of the in situ prepared mixed anhydride.[7]

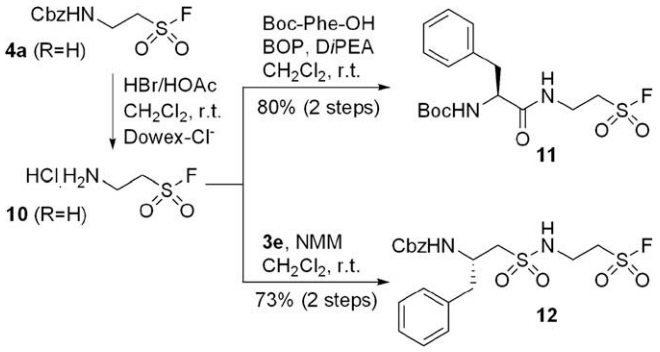
Mesylates **7b–f** were obtained in reasonable to high yields (Table 1) by reaction of alcohols **6b–f** with methanesulfonyl chloride in the presence of base (Et_3N). Next, thioacetates **8b–f** were prepared in good to high yields (65–91%) by reaction with in situ prepared cesium thioacetate. Oxidation of the thioacetates using aqueous hydrogen peroxide and subsequent addition of sodium acetate afforded sodium sulfonates **9b–f**. These sodium sulfonates were treated with a phosgene solution, and after evaporation, the crude sulfonyl chlorides **3b–f** were reacted directly with potassium fluoride in the presence of 18-crown-6 in acetonitrile. Unfortunately, the yields (Table 1) were much lower compared to taurine-derived sulfonyl fluoride **4a** (Scheme 1). Due to the more complex ^{1}H NMR spectra combined with some impurities, it was tedious to monitor these reactions. Therefore, it was decided to perform the substitution reaction with potassium fluoride and 18-crown-6 after purification of the sulfonyl chlorides. The reactions were now monitored more easily and sulfonyl fluorides **4b, 4d and 4e** were isolated in acceptable to good yields (45–67%). Using the TBAF procedure, a sulfonyl fluoride containing an Fmoc-protecting group was prepared (**4f**); this is useful since most of the earlier prepared amino acid-based sulfonyl chlorides were Fmoc-protected.[3] However, the yield was only moderate (30%), probably due to removal of the Fmoc group by the basic fluoride ion.

For comparison with taurine-derived sulfonyl fluoride **4a**, sodium sulfonates **9b–e** were reacted with DAST in dichloromethane. Surprisingly, the yields of sulfonyl fluorides **4b–e** (40–65%, two steps from the thioacetates (**8b–e**)) obtained (Table 1) were similar or even higher than the yield of **4a** (42%). In addition, the yields with DAST were higher than the yields obtained via the KF/crown ether procedure (20–58%, three steps from the thioacetates (**8b–e**)), thereby providing a good alternative procedure. Furthermore, by using DAST, the acidic phosgene reaction is avoided, and therefore acid labile protecting groups for functionalized amino acids can be used.

For future biological evaluation of the prepared amino acid-based sulfonyl fluorides **4a–f**, it was decided to check the reactivity towards different nucleophiles. To this end, sulfonyl fluoride **4a** was subjected to reactions with an alkoxide, a primary amine and a secondary amine (Scheme 3).



Scheme 3. Reactions of sulfonyl fluoride **4a** with different nucleophiles.



Scheme 4. Modification of the N-terminus of sulfonyl fluoride **4a**.

When sulfonyl fluoride **4a** was treated with sodium ethoxide in ethanol, full conversion was observed after stirring for only 5 min at room temperature. Treatment with benzylamine in dichloromethane gave only 30% conversion after 18 h of stirring at room temperature. A higher conversion was expected after reaction with the more nucleophilic piperidine, and after 18 h at room temperature, all the sulfonyl fluoride had been consumed. These test-reactions with sulfonyl fluoride **4a** clearly showed the potential of the amino acid-based sulfonyl fluorides **4a–f** as possible serine and cysteine protease inhibitors. Since they react slowly with primary and secondary amines compared to alkoxides they will probably also react slowly with nitrogen group-containing residues present in enzymes, for example, lysine, histidine and tryptophan.

It is expected that selective protease inhibitors can be prepared by further functionalization of the sulfonyl fluorides at the N-terminus. Therefore, the Cbz-group was cleaved from taurine sulfonyl fluoride **4a** using HBr in acetic acid (Scheme 4).

After treatment with HBr, an ion exchange resin was used to obtain the non-hygroscopic hydrochloride salt **10**. Taurine-derived hydrochloride salt **10** was coupled to Boc-Phe-OH using BOP and D*i*PEA as a base and sulfonyl fluoride **11** was obtained in high yield (80%, two steps). Hydrochloride salt **10** was also reacted with phenylalanine-derived sulfonyl chloride **3e** using NMM as the base to give sulfonyl fluoride **12** in high yield (73%, two steps). The ability of the sulfonyl fluoride to resist these harsh deprotection conditions points to its relative stability as a 'reactive' group.

In conclusion, we have developed a successful synthesis of substituted β-aminoethanesulfonyl fluorides, which can be prepared, starting in principle, from any Cbz- or Fmoc-protected amino acid. Different procedures were used for fluorination of which DAST and KF/18-crown-6 were the best. By using DAST for introduction of the fluorine atom, strong acidic conditions were avoided, which allows the use of acid labile protecting groups present in functionalized amino acids. Under present investigation are the protease inhibitory properties of these new functionalized sulfonyl fluorides.

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