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Synthesis of β-Aminosulfonopeptides Activated Through Selective N-Nitration of a Taurine Amide Unit

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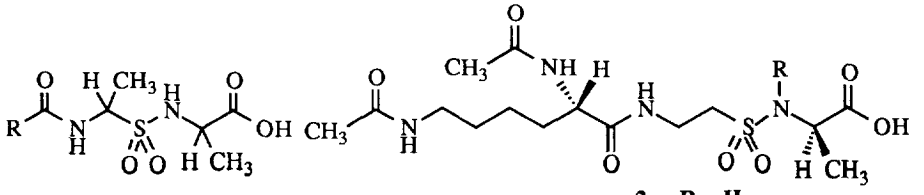
Abstract: β-Sulfonopeptides bearing a taurine in place of a penultimate amino acid unit were designed and synthesized as inhibitors of D-alanyl-D-alanine transpeptidases; N-nitration of the sulfonamide bond in the presence of multiple carboxamide groups was selectively accomplished through use of NO_2BF_4.

In connection with our efforts to synthesize novel and potent inhibitors of penicillin-sensitive-enzymes, especially D-alanyl-D-alanine transpeptidases,[1] we earlier synthesized an α-aminosulfonopeptide, **1**; unfortunately, it proved to be exceedingly unstable in aqueous media.[2] D-Alanyl-D-alanine transpeptidases catalyze transfer reactions (or hydrolysis) of the C-terminal alanine from the natural substrate UDP-N-acetylmuramyl-L-alanyl-D-glutamyl-L-lysyl-D-alanyl-D-alanine, a precursor in bacterial cell wall biosynthesis.[1] Based on recent reports that the synthetic substrate Ac_2-L-Lys-D-Ala-D-Ala showed excellent activity as a substrate for the R61, R39, and albus G enzymes, whereas Ac-D-Ala-D-Ala was a poor substrate,[3] it appeared that substrate activity may be partly relatable to the blocking group of the N-terminal alanine unit. Along other lines, a few peptide analogs[4] containing a taurine unit have been introduced based on the principle of transition-state analogy.

We now report the synthesis of a β-sulfonopeptide, **2**, in which the penultimate amino acid unit of D-ala-D-ala was replaced with a taurine residue and which was designed as an inhibitor of the cross-linking enzymes involved in bacterial cell-wall construction. The introduction[5] of an N-nitro group into the sulfonamide moiety of Ac_2-L-lysyltauryl-D-alanine (**2a**) activates the amide bond to reaction with the nucleophilic OH group of a critical serine residue in the active site of the transpeptidases. The expected stability of the enzyme-sulfonate



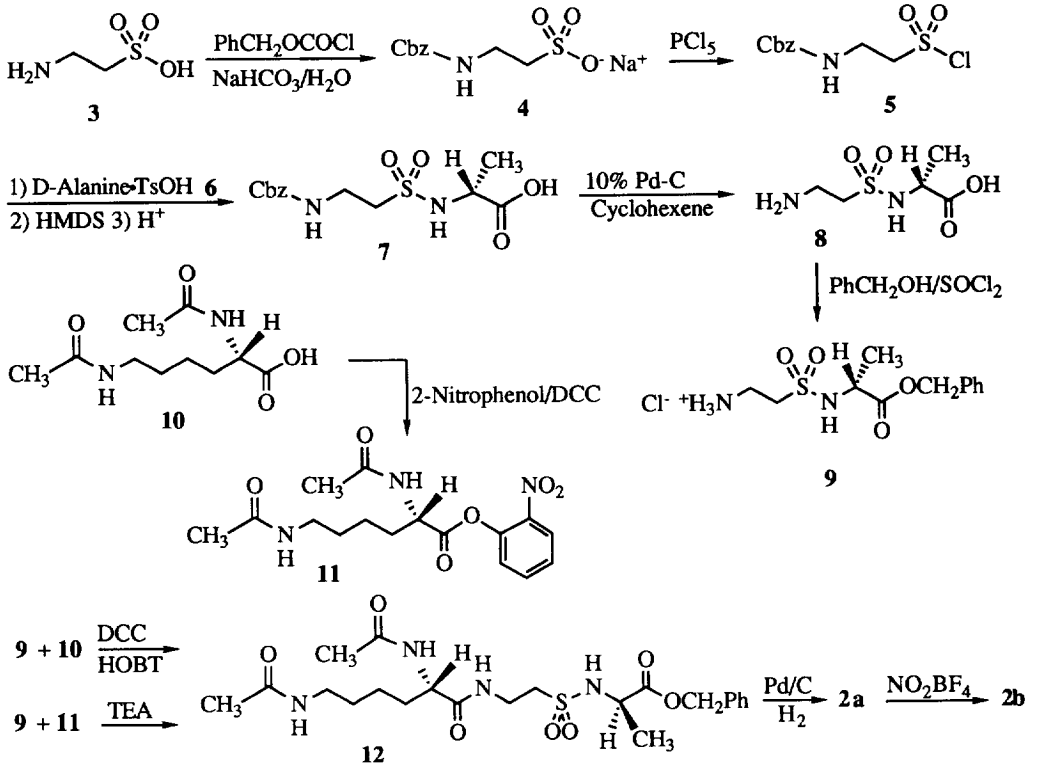
1

2 a: R = H
b: R = NO_2

ester formed could be expected to lead to irreversible inhibition of the target enzymes.[6] Further, the sulfonamide group[4] might serve as a transition-state analog[7] of the tetrahedral intermediate formed in the normal hydrolysis or transpeptidation of the D-Ala-D-Ala linkage. We report here the first synthesis of taurine-containing β-sulfonopeptides in which the penultimate residue is activated with respect to reaction with nucleophiles by an N-nitro group.

Our synthetic approach to prepare target molecule **2** through use of a simple and direct coupling of the alanine and taurine moieties (*vide infra*) is straightforward (Scheme 1) compared to the methods of Liskamp, *et. al.*,[4] in which a halogen oxidation of cysteamine to form the corresponding sulfonylchloride was used followed by a coupling reaction with the amino acid moiety. Taurylalanine **8** was readily obtained by a direct coupling of D-alanine with Cbz-tauryl chloride **5** prepared from the reaction[9] of taurine and benzyl chloroformate followed by treatment with PCl_5. During the course of our studies on α-sulfonopeptides (**1**) a convenient coupling of α-ethoxycarbonylethanesulfonyl chloride and p-toluenesulfonic acid salts of amino acids through use of hexamethyldisilazane (HMDS) without organic bases was developed.[2] In a similar manner, N,O-bis-trimethylsilylalanine, prepared *in situ* from the p-toluenesulfonic acid salt of D-alanine (**6**) and HMDS at room temperature, was treated with sulfonyl chloride **5**; aqueous work-up afforded almost pure solid product **7** (mp 127-128°C) in 25-45% yields (impurities in **5** can seriously decrease this yield). Product **5** was used directly in the next step without further purification. Removal of the benzyloxycarbonyl group from **7** was effected cleanly by catalytic transfer hydrogenation[10] (cyclohexene ,10% Pd/C) to yield taurylalanine **8** (mp 205°C dec) in quantitative yield (25% overall yield from **3**). Protection[11] of the free carboxyl group of **8** was achieved through treatment with benzyl alcohol in the presence of thionyl chloride to give 70% of benzyl ester **9**. N^{α}, N^{ε}-Diacetyl-L-lysine (**10**) was prepared and used directly in the next step without further purification by the neutralization of L-lysine hydrochloride (**2**) with silver acetate, followed by treatment with acetic anhydride by the method of Greenstein, *et al.*[12] The coupling of benzyl ester **9** with diacetyllysine (**10**) in the presence of 1-hydroxybenzotriazole (HOBT) using the DCC method[13] produced the desired diacetyl-L-lysyltauryl-D-alanine benzyl ester (**12**) after silica gel column chromatography (57%). An alternate route to product **12** using **9** and active ester **11**, prepared from acetyllysine **10** and o-nitrophenol in the presence of DCC, yielded **12** (60%), which was identical to the product obtained by the DCC method. Subsequent hydrogenolysis of **12** (H_2 and 10% Pd/C) gave the highly hygroscopic diacetyl-L-lysyltauryl-D-alanine (**2a**; mp 60-65°C; δ 3.91, alanyl CH) in a quantitative yield.[16]

Our preliminary study of the N-nitration of sulfonopeptides under classic nitration conditions (HNO_3/Ac_2O) resulted in non-selective N-nitration in low yields; the products were difficult to isolate due to rapid decomposition, possibly catalyzed by nitric acid during work-up. A direct nitration of **2a** with dinitrogen pentoxide (N_2O_5), which has been extensively used for the nitration of a variety of substrates such as amines, amides, and ureas[14] was then investigated. The treatment of **2a** with 2 equiv of N_2O_5 in CD_3CN at temperatures ranging from -20°C to 25°C resulted in the formation of a mixture of N-nitrated products; N-nitration took place approximately equally at both the acetamido and sulfonamido positions. The use of pyridine or lutidine as base along with N_2O_5 led to more complicated product mixtures; again, no selectivity was observed. We next attempted the reaction of **2a** with NO_2SbF_6 in $CDCN_3$ at 0°C. Surprisingly, the treatment of **2a** with 1 equivalent of NO_2SbF_6 in CD_3CN yielded a clear solution without any detectable N-nitrated



Scheme 1

product; the addition of 2 more equivalents of NO_2SbF_6 gave a mixture of **2b** (ca 50%) and unchanged **2a** (ca 50%); the use of 6 more equivalents of NO_2SbF_6 led cleanly to compound **2b**. Thus, the selective N-nitration of the more acidic sulfonamide group of **2a** was accomplished in the absence of bases (pyridine or 2,6-lutidine) (the presence of base is reported to generally give a mixture of N-nitroso and N-nitro compounds through the migration of nitroso and nitro groups to N after initial O-nitration).[5a] However, the product obtained in the NO_2SbF_6 nitration, after aqueous work-up, contained a considerable amount of inorganic salts; a large amount of fluorine was detected by ^{19}F NMR analysis. Attempted purification by an aqueous work-up failed to remove the impurity. Finally, treatment of **2a** with 8 equivalents of the more water-soluble nitronium tetrafluoroborate (NO_2BF_4) at 0°C for 1 h, followed by aqueous work-up, gave rise to the desired **2b** (mp 91°C dec; δ 5.34, alanyl CH) in the form of a complex with solvent (**2b**: ethyl acetate: ether = 1 : 1 : 0.5); recrystallization from acetonitrile and chloroform yielded white crystals in the form of a complex with ether (**2b**: ether = 1: 0.5). Subjecting this complex to a vacuum of 0.02 Torr for 72 h at 25°C did not result in the loss of the solvent.[16] Decomposition of compound **2b** in a pD 7.8, 0.5 mM phosphate buffer (D_2O), with a half-life of approximately 16 h at 25°C, produced N-nitroalanine[15] and diacetyllysyltaurine (100%). The details of enzyme assay of

compound **2** and its derivatives will be reported elsewhere.

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16. **2a**: mp 60-65 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.07 (br s, 2H), 7.98 (br d, 1H), 7.80 (br d, 1H), 4.10 (m, 1H, lysyl CH), 3.91 (m, 1H, alaninyl CH), 3.27 (m, 2H), 3.12 (m, 2H), 2.97 (m, 2H), 1.83 (s, 3H), 1.77 (s, 3H), 1.65-1.19 (br m, 6H), 1.30 (d, 3H); IR (KBr) 3500-2500, 1736, 1654, 1545, 1305, 1134 cm^{-1}. Anal. Calcd for $C_{15}H_{28}N_4O_7S$: C, 44.11; H, 6.91; N, 13.72. Found: C, 44.47; H, 7.07; N, 14.29.

    2b: mp 91 °C dec ; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.20 (br m, 1H, NH), 8.00 (br d, 1H, NH), 7.81 (br t, 1H, NH), 5.34 (q, J = 6.96 Hz, 1H, $CHCH_3$), 4.12 (m, 1H, lysyl CH), 4.00 (t, J = 6.96 Hz, 2H, tauryl CH_2SO_2), 3.56 (m, 2H, tauryl CH_2), 2.97 (q, J = 5.7 Hz, lysyl CH_2NH), 1.84 (s, 3H), 1.78 (s,3H), 1.55 (d, J = 6.96 Hz, 3H, $CHCH_3$), 1.65-1.20 (br m, 6H); IR (KBr) 3500-2400, 1732, 1652, 1581, 1557, 1379, 1165 cm^{-1}. Anal. Calcd for $C_{15}H_{27}N_5O_9S \cdot 1/2(C_2H_5)_2O$: C, 41.63; H, 6.58; N, 14.28. Found: C, 42.02; H, 6.83; N, 14.31.

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