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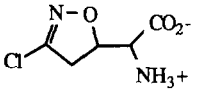
COMMUNICATION

Synthesis, Chemistry, and Absolute Configuration of Novel Transglutaminase Inhibitors Containing a 3-Halo-4,5-dihydroisoxazole[1]

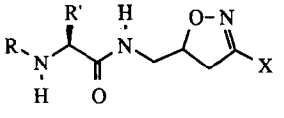
The preparation of potent transglutaminase inhibitors containing a 3-halo-4,5-dihydroisoxazole and the determination of their absolute configuration are described. Interestingly, reaction of halodihydroisoxazoles with thiolate is dependent on the nature of the halogen atom, with the bromide primarily undergoing ring cleavage and the chloride undergoing displacement with the ring intact. This result may have implications as regards mechanisms of transglutaminase inhibition by 3-halo-4,5-dihydroisoxazoles.

Transglutaminases (TG)[2] (EC 2.3.2.13) are a class of enzymes that have been implicated in a variety of conditions including acne (*1*), psoriasis (*2*), cataracts (*3*), and immunologic disorders (*4*), yet no examples of potent and specific inhibitors of these enzymes have been reported. TG catalyzes the covalent coupling of the γ-carboxamide group of peptide bound glutamine residues with an ε-amino group of peptide bound lysine residues. The critical intermediate in the catalytic sequence is a thioester acyl-enzyme formed between a glutaminyl-peptide acyl donor and the active site cysteine residue (Fig. 1) (*5*).

The natural product Acivicin, **1** (*6*), described as a glutamine antagonist, has been shown to inactivate anthranilate synthetase by modifying the active site cysteine-83 (*7*). We reasoned that the 3-chloro-4,5-dihydroisoxazole moiety of **1** might have broader utility as a latent reactive group in inhibitors targeted for other enzyme types containing a cysteine active site residue. Indeed, our efforts have resulted in the identification of peptidyl halodihydroisoxazoles **2** as novel and potent TG inhibitors.



Acivicin **1**



2

The synthesis of **2** is accomplished by utilizing either of two general methods (Schemes 1 and 2). As in Scheme 1, diastereomers are obtained which can be separated by HPLC, whereas in Scheme 2 single isomers are obtained by perform-

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[2] Abbreviations used: TG, transglutaminases; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; DMAP, dimethylaminopropylamine; Boc, *tert*-butoxycarbonyl; Cbz, benzyloxycarbonyl; BET, bovine epidermal TG; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; DMF, dimethylformamide.

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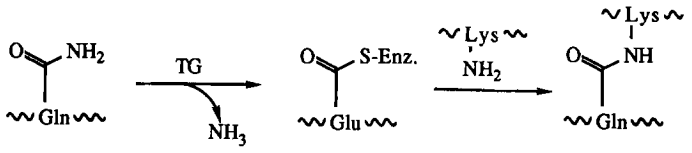
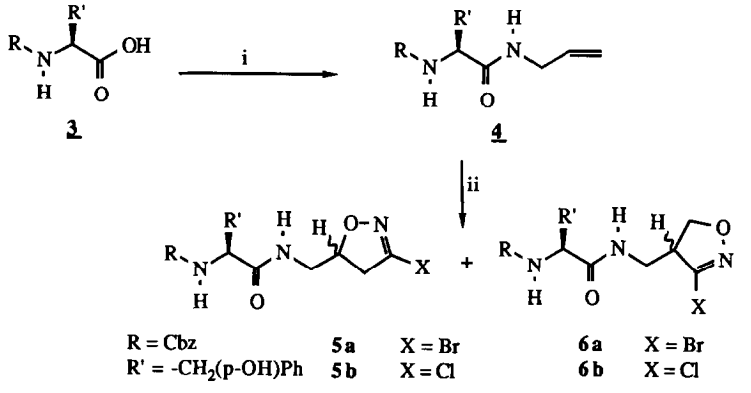


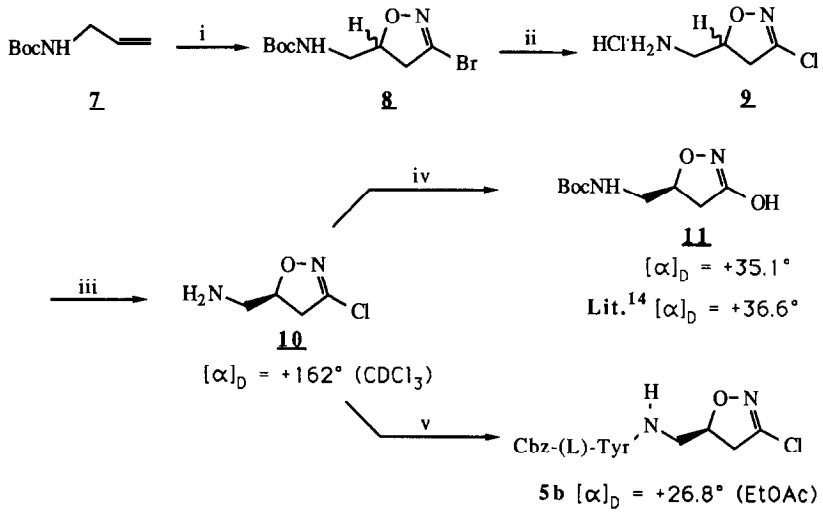
FIG. 1. Acyl transfer catalyzed by transglutaminases.

ing the resolution of intermediate **10.** For example, *N*-Cbz-L-tyrosine is condensed with allyl amine using EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride)/DMAP to produce **4** in 67% yield. The allyl amine, **4,** then undergoes a high yield (2 + 3) cycloaddition with bromonitrile oxide (*8*) to give a 1 : 1 mixture of diastereomers **5a** in 74% isolated yield. The following analogs of **5a** were prepared in a similar fashion: *N*-Cbz-L-phenylalanyl (95%), *N*-Ac-L-naphthylalanyl (95%), *N*-Cbz-glycyl (67%), *N*-Cbz-L-isoleucyl (64%), *N*-9-fluorenylmethoxycarbonyl-L-phenylalanyl (63%), and *N*-Boc-O-benzyl-L-threonyl (96%). The regioisomers **6a** are also obtained in 2–3% yield. Cycloaddition with chloronitrile oxide (*9*) gives **5b** in slightly lower yields and with a greater proportion (5–7%) of the minor regioisomers **6b.** The diastereoisomers **5a** (or **5b**) can be separated by HPLC, utilizing a Whatman M20 partisil 10 semi-preparative column with 40% EtOAc/hexane as eluant. Interestingly, the minor regioisomers **6a** or **6b** and the less polar diastereomers corresponding to structure **5a** or **5b** are relatively inactive in comparison with their more polar cognates (**5a** or **5b**). At 1 μM, **5b** (C_5-(*S*)) exhibits rapid, time-dependent inactivation ($t_{1/2}$ = 10 min) of bovine epidermal TG (BET) at 37°C, pH 8.1, 10 mM $CaCl_2$ in the presence of 1 mM monodansylcadaverine and 0.5 mM dithiothreitol. In contrast, much slower time-dependent inactivation of BET was observed with **5b** (C_5-(*R*)) ($t_{1/2}$ = 48 min at 10 μM).

A more practical synthesis of optically pure **5b** (or **5a**) which allows assignment of absolute configuration at C-5 of the dihydroisoxazoyl moiety, is outlined in Scheme 2. The preparation of **8** from the *N*-Boc allyl amine, **7,** proceeds in 86% isolated yield (*10*). Deprotection and halogen exchange is accomplished with



SCHEME 1. (i) $H_2NCH_2CH{=}CH_2$/EDCI/DMAP; (ii) $Br_2CNOH/NaHCO_3$/EtOAc/H_2O or Cl_2CNOH/$AgNO_3/CH_2Cl_2$.



SCHEME 2. (i) $Br_2CNOH/NaHCO_3/EtOAc/H_2O$; (ii) HCl/THF/RT; (iii) resolution via (*S*)-mandelic acid and then Na_2CO_3; (iv) $(Boc)_2O$ and then NaOH/DMSO; (v) Cbz-L-Tyr/EDCI/DMAP.

$HCl_{(g)}$ in dry THF conveniently providing the expected 3-chloro analog **9** quantitatively (*9, 11*). The halogen exchange process, **8** to **9,** is preferred over direct addition of chloronitrile oxide to **7** since it is a safer procedure with higher yields and greater regiospecificity. After neutralization of racemic **9,** the resulting amine is resolved with (*S*)-(*d*)-mandelic acid by fractional crystallization in $EtOH/H_2O$.[3] The optically active amine **10** is obtained following neutralization with Na_2CO_3. The assignment of configuration of **10** was established by chemical correlation to *N*-Boc-dihydromuscimol, **11,** resolved earlier and correlated to the (*S*)-(+) enantiomer of 3-hydroxy-4-aminobutyric acid by Krogsgaard-Larsen *et al.* (*12*). Hence, **10** was acylated with $(Boc)_2O$ in CH_2Cl_2 and the halide displaced with 2.0 N NaOH/DMSO/RT/48 h to give **11.** Use of methanol as solvent in the hydroxide treatment generates the 3-methoxy analog, **8c** (*10*). The sign and magnitude of the optical rotation exhibited by **11** indicate that the precursor amine, **10,** is optically pure and possesses the (*S*) stereochemistry at C-5. Amine **10** was subsequently coupled with *N*-Cbz-L-tyrosine using EDCI/DMAP[4] to give **5b**[5] in 71% yield.

[3] The first crystallization typically gives a ~95 : 5 ratio of diastereomeric mandelic acid salts as determined by HPLC at the stage of **5** in about 40% yield. The compound **10** (*S*)-(*d*)-mandelic acid salt has $[\alpha]_D = +130.6°$ (H_2O).

[4] In some preparations, the phenol group is also acylated with activated Cbz–Tyr resulting in 11% of the O-(Cbz-tyrosinoyl) analog of **5b.** This side product is hydrolyzed to **5b** and Cbz–Tyr–OMe with $MeOH/NEt_3$ at room temperature.

[5] Compound **5b** (C_5-(*S*)) has: mp 83–85°C; ir (KBr): ν_{max} 3325 (br s), 1682 (s), 1660 (s), 1530 (br), 1258 (s), 890 cm^{-1} (s); ^1H NMR (300 MHz, DMSO-d_6): δ 2.6–2.95 (m, 2H, $ArCH_2$), 2.97–3.29 (m, 2H, C_4–H), 3.32–3.47 (m, 2H, $HNCH_2$), 4.12–4.22 (m, 1H, α-CH), 4.73–4.82 (m, 1H, C_5–H), 4.95 (s, 2H, $PhCH_2$), 6.64 (AA′, 2H, Line spacing = 8.4 Hz, Ar C_3–H and C_5–H), 7.04 (BB′, 2H, Line spacing = 8.4 Hz, Ar C_2–H and C_6–H), 7.22–7.38 (m, 5H, Ph), 7.40 (d, 1H, OC(O)NH), 8.28 (t, 1H, –C(O)NH (exch.)), 9.15 (s, 1H, OH (exch.)); MS (EI): *m/z* 431 (M^+), 325, 280, 177, 147, 107, 91. *Anal.* Calcd for $C_{21}H_{22}N_3O_5Cl$: C, 58.40; H, 5.13; N, 9.73; Cl, 8.21. Found: C, 58.65; H, 5.12; N, 9.44.

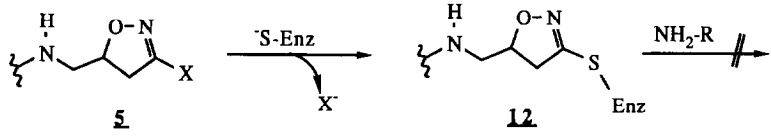
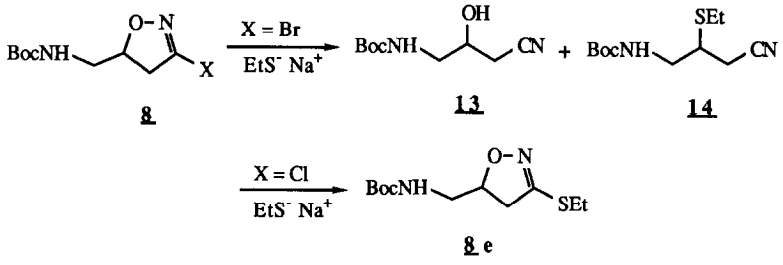


FIG. 2. Proposed mechanism of TG inhibition by peptidyl-halodihydroisoxazoles.



SCHEME 3. **8a**, X = Br; **8b**, X = Cl; **8c**, X = OMe; **8d**, X = imidazol-1-yl.

Spectroscopic data, HPLC properties of this product, and the kinetics of enzyme inactivation correspond to those of the more polar isomer **5b**.

Halodihydroisoxazoles **2** were conceived as TG inhibitors on the assumption that the active site cysteine thiol of the enzyme would displace halide and give a stable thioimine enzyme adduct, **12**, unable to participate in the next reaction with an ε-amino group of a lysine residue (Fig. 2). However, the nonenzymatic reaction of thiolate with the halodihydroisoxazole **8** is not so straightforward and is dependent on the nature of the halogen. For example, we have found that displacement of chloride from **8b** with EtS^-Na^+ (3.0 eq) in DMSO provided **8e** in 97% yield (Scheme 3). Bromide displacement also occurred from **8a** with MeO^-Na^+ in MeOH to give **8c** (75%) and with sodium imidazolide in DMF to furnish the novel 3-(imidazol-1-yl) analog, **8d** (38%).[6] However, treatment of **8a** with EtS^-Na^+ in DMSO or DMF resulted primarily in ring fragmentation with formation of the β-hydroxyl nitrile **13** (38%) plus **14** and **8e** as minor components (~10%) (*13*).[7] This observation is all the more intriguing, since both the bromide and chloride analogs **5a, 5b** and **2a, 2b** (Table 1) are effective irreversible inhibitors of TG and may have important implications as regards mechanisms of TG inactivation, which are currently under investigation in our laboratories.

Second-order rate constants derived from the pseudo-first-order kinetics are listed in Table 1 for a variety of 3-substituted 4,5-dihydroisoxazoles. From the

[6] **8d:** ir (KBr): ν_{max} 3378, 2921, 1678, 1630 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$): δ 1.4 (s, 9H, Boc), 3.1–3.7 (m, 4H, $2CH_2$), 4.8–5.2 (m, 2H, CH, NH), 7.15, 7.38, 7.81 (3 br s, 3H, Im. C–H). **8e:** ir (KBr): ν_{max} 3325, 2985, 1709, 1690 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$): δ 1.4 (t, 3H, CH_3CH_2), 1.45 (s, 9H, Boc), 2.5–3.5 (m, 6H, $3CH_2$), 4.5–5.0 (m, 2H, CH, NH).

[7] Dihydroisoxazoles with a variety of substituents at C-3 are known to fragment under specific conditions.

TABLE 1

Inactivation of TG by 3-Substituted 4,5-Dihydroisoxazoles **2** (R = Cbz; R' = $PhCH_2$–)

Cpd	X	Epidermal TG $K/[I]$ (M^{-1} min^{-1})
2a	Br	53,800
2b	Cl	13,400
2c	OMe	<100
2e	SEt	<100
2f	CH_3	Inactive

limited data, it appears that the potency of the inhibitors is related to the leaving group X. This notion is reinforced by the observation that the isostere **2f**[8] is inactive.

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[8] Compound **2f** was prepared as in Scheme 2 by adding methyl nitrile oxide to *7* (*14*).

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