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HYDROXYOXAZOLIDINES AS α-AMINOACETALDEHYDE EQUIVALENTS: NOVEL INHIBITORS OF CALPAIN

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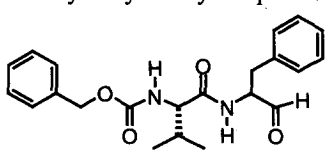
Abstract: The synthesis of [1-[(5-hydroxy-4-(phenylmethyl)-3-oxazolidinyl)carbonyl]-2-ethylpropyl]carbamic acid phenylmethyl ester (**2**; MDL 104,903), a potent inhibitor of calpain, is described. Synthesis of related compounds, which offer insights into the mechanism of action for **2**, are also described, as is an O-acetyl prodrug derivative of **2**.

In brain ischemia, elevated intracellular calcium levels initiate a cascade of events that includes the activation of calpain, a normally quiescent cysteine proteinase. Inhibition of calpain provides significant neuroprotection in global and focal ischemia by attenuating the proteolysis of structural and regulatory proteins.[1] The dipeptide aldehyde **1** (MDL 28,170) is a potent inhibitor of calpain (K_i value of 10 nM)[2] and is active in several animal models of stroke.[3]

We recently described the preparation and reactions of optically active 4-hydroxy-1,3-dioxolanes, which are 2-hydroxy aldehyde equivalents.[4] This report describes the leverage of this methodology to the synthesis of 2-amino aldehyde equivalents, which as dipeptide aldehyde equivalents are mechanism-based inhibitors of calpain.



1, MDL 28,170

Our initial target was oxazolidine **2**, which we envisioned could inhibit calpain in one of the ways shown in Figure 1. Compound **2** could act as a prodrug and be converted either hydrolytically or in an enzyme-mediated process, to either MDL 28,170 (**1**) or its N-hydroxymethyl derivative **3**. Alternatively, oxazolidine **2** might function as a transition state analog to mimic the product of enzyme addition to **1** (e.g., structure **5**). A third possibility that we envisioned was the enzyme-mediated dehydration of **2** to the oxonium ion **7**. Although oxonium ion **7** is presumably a high energy species, it may be stabilized by the enzyme active site. In addition, charged species have previously been suggested as intermediates which may be formed in enzyme active sites and a recent article predicts the intermediacy of a stabilized carbene in the mechanism of substrate processing for orotidine monophosphate decarboxylase.[5]

Also shown in Figure 1 are mimics for the suggested modes of inhibition. A simple mimic for N-hydroxymethyl compound **3** would be N-methyl compound **4**. An effective mimic for the transition state analog hypothesis would be the carbon analog of **2** (e.g., pyrrolidine **6**). And, we felt a suitable mimic for oxonium ion **7** would be a compound such as **8** or its equivalent, which placed an electrophilic sp2 center at the same relative site as in oxonium ion **7**. Thus, we set out to prepare mimics **4**, **6**, and **8** and additional compounds related to **2** to investigate the mechanism of calpain inhibition displayed by **2**. We also proposed to study potential prodrugs of **2**. This report details these synthesis studies.

The synthesis of the proposed inhibitory oxazolidine **2** is shown in Scheme 1. Treatment of Cbz-Val-Phe-OH (**9**) with paraformaldehyde in benzene with catalytic *p*-toluenesulfonic acid gave oxazolidinone **10** in 42% yield.[6–10] Reduction of **10** at low temperature with diisobutylaluminum hydride in toluene gave hydroxyoxazolidine **2** (MDL 104,903) in 33% yield.[11] As shown, the stereochemistry was predominantly as expected from our previous reductions of related 4-substituted-5-oxo-1,3-dioxolanes.[4] Acetylation of **2** with acetyl chloride gave acetoxyoxazolidine **11** in 73% yield.[12] Characteristic of the *trans* stereochemistry of **11** was the acetoxy methine proton that appeared as a doublet, J = 8.6 Hz, at δ 6.15. The same proton in the lesser amount of the *cis* isomer that was present appeared as a doublet, J = 5.2 Hz, at δ 5.47.

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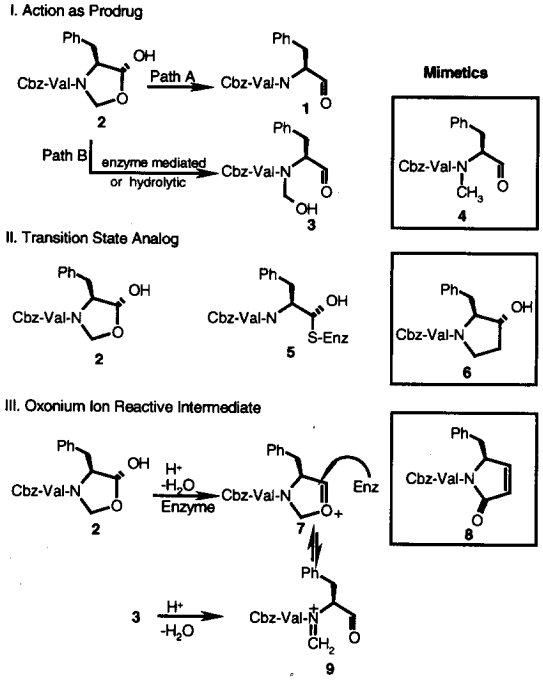
Preparation of the phenylalanyl N-methyl derivative of **1** (compound **4**), the mimic of hydroxymethyl compound **3**, is shown in Scheme 2. Cbz-Val-OH (**12**) was coupled with N-methylphenylalaninol (**13**) using carbonyldiimidazole to give a mixture of the desired N-methylamide **14** and an ester arising from coupling of **12** with the primary alcohol group of **13**. These compounds were separated by silica gel flash chromatography and **14** was subjected to Swern oxidation conditions to provide aldehyde **4** in 78% yield.

Figure 1. Potential Inactivation Mechanisms for **2**

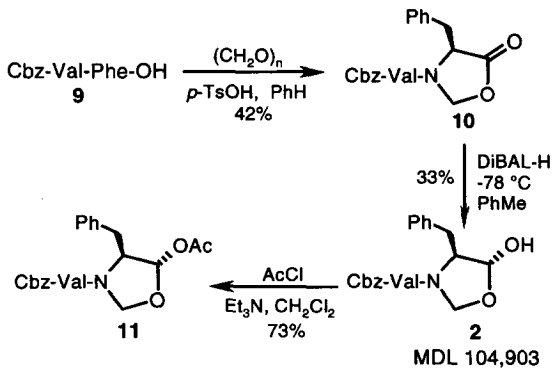


The carbon analog of hydroxyoxazolidine **2**, compound **6**, was prepared as shown in Scheme 3. Treatment of Boc-Phe-OH (**15**) with isopropenyl chloroformate and Meldrum's acid in the presence of dimethylaminopyridine (DMAP), followed by brief heating in methanol, gave the known pyrrolidinedione in quantitative yield (mp 121–122 °C; lit.[13] mp 120–121 °C). Treatment of this intermediate with sodium borohydride in methylene chloride and acetic acid afforded clean reduction of the ketone carbonyl from the less hindered face to give hydroxypyrrolidone **16** in 39% yield. Reduction of the amide carbonyl group was effected with borane-dimethyl sulfide complex in tetrahydrofuran to give hydroxypyrrolidine **17** in 39% yield. The *cis* carbon analog of **2** was prepared in a two-step sequence from **17**. Removal of the *tert*-butyloxycarbonyl group with trifluoroacetic acid followed by coupling of the resulting pyrrolidinol with Cbz-Val-OH using EDCI and HOBt gave the *cis* 2,3-disubstituted pyrrolidinol **18a** in 81% overall yield. Mitsonobu inversion of the hydroxyl group in **17** with triphenylphospine and diisopropyl azodicarboxylate in acetic acid gave *trans*-acetoxy compound **19** in 45% yield. Removal of the *tert*-butyloxycarbonyl group of **19** followed by coupling with Cbz-Val-OH as described for **17** gave the *trans*-acetoxy compound **20a** in 96% yield, which was clearly different from the isomeric *cis*-acetoxy compound **18b** which was made by acetylation of **18a** for comparison. Hydrolysis of acetate **20a** with lithium hydroxide then gave the carbon analog of MDL 104,903 (**6**) in 72% yield.

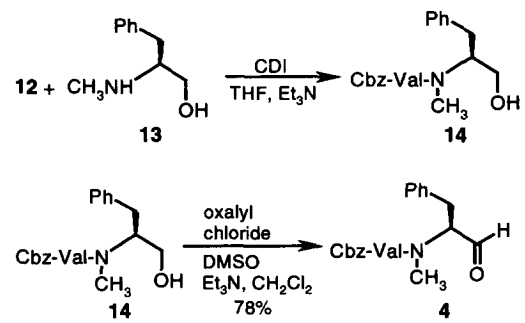
We next attempted to prepare the mimic of the proposed oxonium ion intermediate **7** (e.g., pyrrolidinone **8**). Cbz-Val-Phe-OH (**9**) was activated with isopropenyl chloroformate and treated with Meldrum's acid in the presence of DMAP. Cyclization with decarboxylation afforded the keto pyrrolidinone **21**. Reduction of **21** with sodium borohydride in methylene chloride and acetic acid (10:1) gave hydroxypyrrolidinone **22** in 41% overall yield from **9**. Again, the *cis* stereochemistry of **22** was clearly established by ^{1}H NMR. Using hydroxypyrrolidinone as a model compound for **22**, we investigated methods for the dehydration of **16** to pyrrolidone **24**. We were able to operate on the hydroxy group of **16**. Thus, Mitsonobu inversion of **16** by activation with diisopropyl azodicarboxylate gave the *trans*-hydroxypyrrolidinone **23** in 78% yield. However, when we attempted to dehydrate **16**, we were unsuccessful with the conditions we chose. Treatment of **16** with trifluoromethanesulfonic anhydride, an effective dehydrating agent under mild conditions,[14] led only to recovered starting material. The synthesis of a methylene homolog of **2** (e.g., morpholine **29**), is shown in Scheme 5. Compound **29** was of interest because it should be inherently more stable than **2** to hydrolytic conditions (e.g., opening of the lactol would not provide an intermediate that could lose formaldehyde in an irreversible process). Treatment of Cbz-Val-OH (**12**) with benzyl N-(2-hydroxyethyl)phenylalaninate (**25**)[15] using a standard coupling protocol gave ester **26** in 60% yield.

The key step in the synthesis of **29** was the intramolecular Weinreb amidation[16] of aminoester **26** which provided N-hydroxyethylamide **27** in 47% yield. Cyclization of **27** with potassium carbonate gave 74% of morpholine **28**, which was treated with DIBAL-H to give 86% of hydroxymorpholine **29**.

Scheme 1

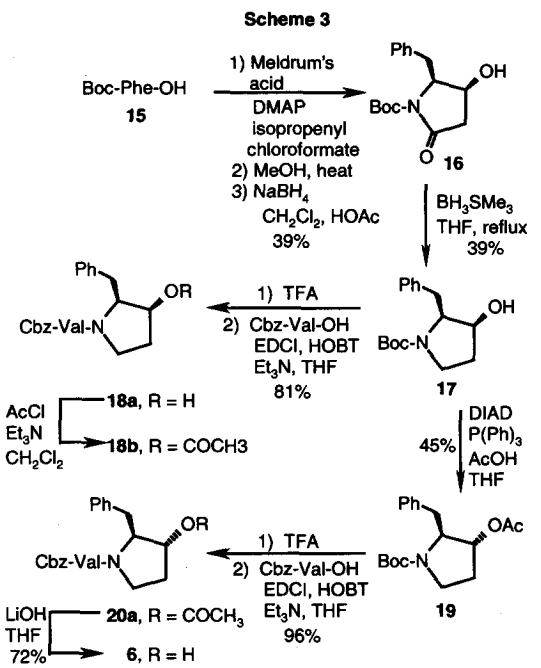


Scheme 2

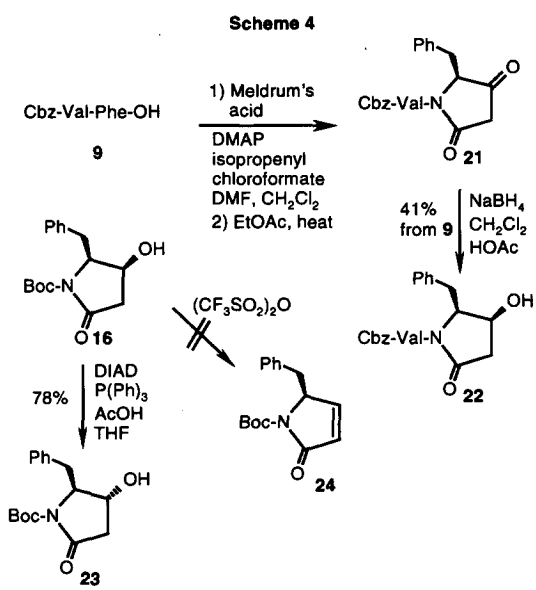


A homology model for calpain was developed on the basis of structural data, which has been reported for cathepsin B.[17] In Figure 2 is shown our homology model with compound **2** docked in the active site. Note that this model of **2** with calpain represents a *cis*-amide P_1-P_2 positioning of the ligand as a dipeptide mimetic.

Scheme 3



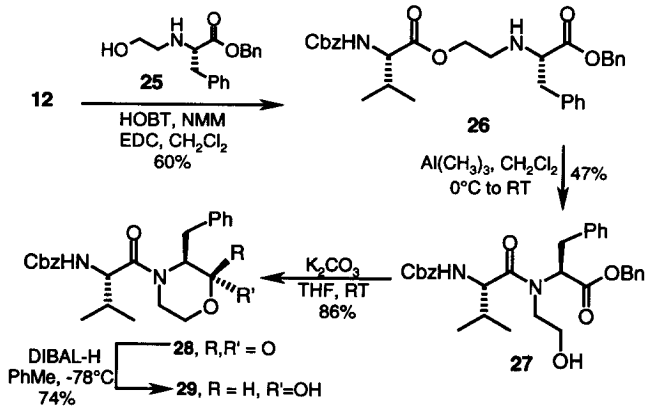
Scheme 4



We did try docking **2** in other rotomer conformations and concluded that the most favorable model for this interaction was as shown in Figure 2, in terms of ligand energetics and overall ligand–enzyme fit. Also shown in Figure 2, overlaid with inhibitor **2**, is the six-membered homolog **29**.

The cysteine sulfur is assumed to displace the alcohol group of **2** and **29**. The conformation adopted for **2** is assumed to be similar to Cbz-Arg-Ser(OBz)chloromethylketone in Cathepsin B. With **29**, the six-membered

Scheme 5



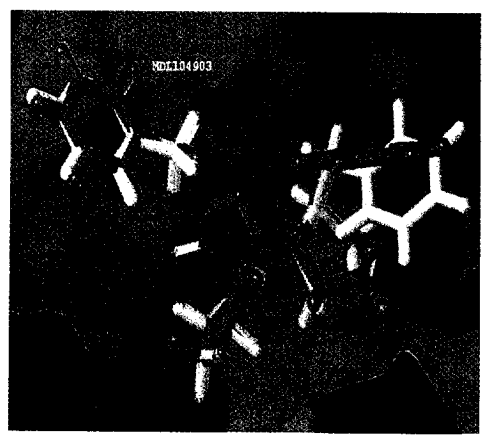


Figure 2. Placement of MDL 104,903 (**2**) (blue) and 6-Membered Ring Homolog **29** (yellow) in a Homology Model of Calpain

ring is in a 'boat' conformation, and there is severe bumping (in red) with the histidine in the structurally conserved region (HAIRILG) in the active site.

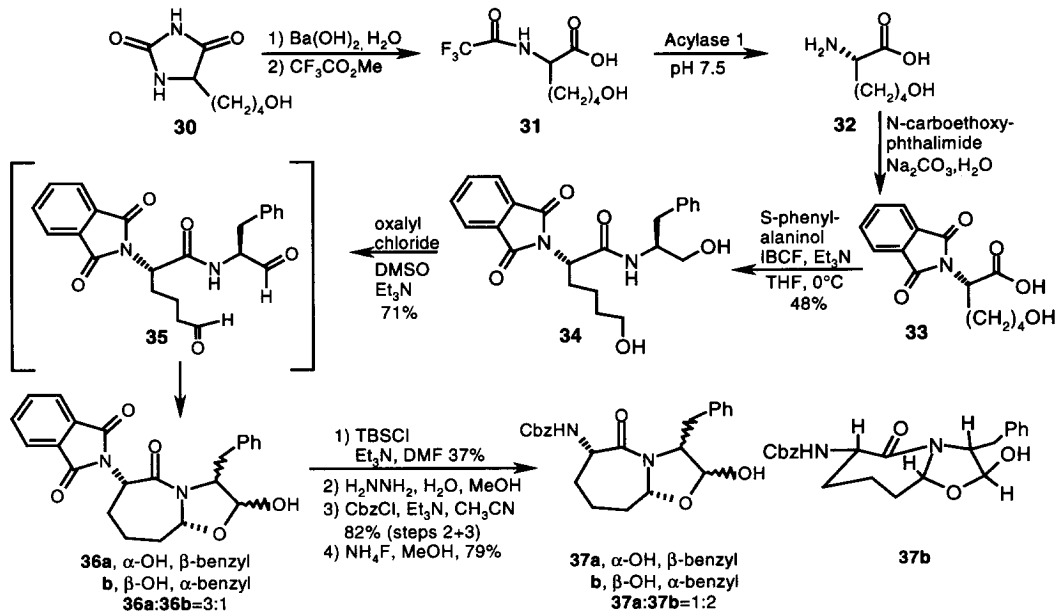
Since the P_1-P_2 *cis*-amide rotomer of **1** appeared to provide the best fit with calpain, we pursued the preparation of a doubly constrained dipeptide mimic in which was imposed a P_1-P_2 constraint to lock in the *cis*-amide architecture. Fused azepine **37** was chosen; docking of **37** with our homology model of calpain appeared to provide a reasonable interaction. Hydantoin **30**[18] was hydrolyzed with barium hydroxide to provide racemic ε-hydroxynorleucine, which was acylated with methyl trifluoroacetate to provide the N-Trifluoroacetyl derivative **31** as shown in Scheme 6. Treatment of **31** with Acylase I at pH 7.5 provided S-ε-hydroxynorleucine **32**. Protection of the N-terminus of **32** was accomplished by transimidation with N-carboethoxy-phthalimide. Elaboration to the dipeptide backbone was accomplished by coupling of S-phenylalaninol to **33**, using the mixed anhydride method, to give **34** in 48% yield. This bis-diol **34** was then oxidized using the Swern procedure[19] to provide azepinooxazolidine **36**[20] in 71% yield.

Presumably this conversion involves the intermediacy of bis-aldehyde **35**, which undergoes intramolecular condensation to provide azepine **36**. We have recently described a related bis-Swern oxidation of a diol to produce a δ-keto aldehyde.[21]

Exchange of the phthalimide protecting group for carbobenzyloxy was accomplished with a four-step procedure. The hydroxyl group was protected by treatment with *tert*-butyldimethylsilyl chloride (37% yield; **36** was recovered in 47% yield). Removal of the phthalimido group with hydrazine followed by acylation with carbobenzyloxy chloride gave the penultimate product in 82% overall yield. Finally, deprotection of the hydroxyl group with ammonium fluoride gave a 79% yield of **37** as a mixture of diastereomers as shown. NMR studies revealed that the two diastereomers analagous to **36a** and **b** were present; however, additional epimerization at the oxazolidine carbon bearing the benzyl group had occurred during the conversion of **36** to **37**. We propose that epimerization of the lactol occurs to enhance the proportion of the α-benzyl diastereomer which may occupy a thermodynamically more stable pseudo-equatorial conformation in **37b** (Scheme 6).

The K_i values for MDL 104,903 (**2**) and related compounds as inhibitors of calpain are shown in Table 1. Hydroxyoxazolidine **2** was a potent inhibitor of calpain, whose K_i value was 33 nM. The K_i value for **2** versus bovine cathepsin B was 120 nM. Two of the three compounds proposed as reaction mechanism mimetics were evaluated, and provide information on the validity of the potential mechanisms. N-Methylamide **4**, the mimetic for N-hydroxymethyl compound **3**, was significantly less potent (K_i value of 400 nM) than **2**, suggesting that **2** was not a prodrug for **4**. Moreover, none of MDL 28,170 (**1**) could be detected by HPLC in the in vitro enzyme

Scheme 6



assay buffer used for **2** versus calpain, which further suggested that **2** was not a prodrug for **1**. Hydroxypyrrolidine **6**, the transition state analog mimetic, was inactive, which ruled out this mechanistic possibility and underscored the importance of the oxygen atom in the oxazolidine ring. We were unable to prepare oxonium ion mimetic **8**. Compound **22**, a potential bioprecursor of **8**, was prepared and was inactive. However, since **16** could not be chemically dehydrated, it is perhaps unlikely that **22** would be enzymatically dehydrated. Thus, by elimination, and the finding that the oxygen atom of the oxazolidine ring was an absolute requirement for activity, we propose that oxonium ion **7** is the reactive intermediate responsible for the inhibition of calpain by **2**.

Table 1. K_i Values for **2** and Related Compounds versus Calpain

Cpd No	K_i (nM)[a]
1	10
2	33
4	400
6	NA[c]
11	2,000[b]
18a	NA[c]
18b	NT[d]
20a	NT[d]
22	NA[c]
29	56,000
36a,b	3,200
37a,b	1,500

[a] K_i values were determined as in reference 22. [b]This compound was converted to **2** in the presence of human plasma. The formation of **1** was also observed in this experiment. [c]Not active. [d]Not tested.

Additional useful information is contained in Table 1. O-Acetyl compound **11** had intrinsic micromolar affinity and was converted to **2** in human plasma. The six-membered ring analog of **2** (e.g., compound **29**) was three orders of magnitude less active than **2**. The explanation for this difference may be evident from Figure **2**, which shows the poor steric fit for **29** with respect to **2** in the catalytic site of the homology model for calpain. Both of the azepines (e.g., **36** and **37**) displayed low micromolar affinity. Perhaps this decreased affinity with respect to **2** was also due to steric compression.

In summary, we have described the synthesis of the novel, hydroxyoxazoline calpain inhibitor **2** (MDL 104,903) and related compounds which addressed potential mechanisms of action and proposed fit into the homology modeled active site of calpain. We propose that **2** inhibits calpain through the intermediacy of the oxonium ion reactive intermediate **7**.

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11. For **2**: $[\alpha]_D^{20}$ -46.13° (*c* 0.98, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) consistent with structure; IR (KBr) 3406 (br), 3032, 2964, 1710, 1640, 1529, 1454 cm^{-1}; MS (chemical ionization) *m/e* 411 ($M + H^+$), 383, 339, 275, 91. Anal. calcd for $C_{23}H_{28}N_2O_5$: C, 66.98; H, 6.83; N, 6.79. Found: C, 66.88; H, 7.07; N, 6.81.
12. For **11**: $[\alpha]_D^{20}$ -36.13° (*c* 0.64, CHCl3); ^1H NMR (300 Mhz, $CDCl_3$) δ 0.7, 0.89 and 1.00 [3d, 6H, *J* = 6.6 Hz, $CH(CH_3)_2$, rotamers cause doubling here and elsewhere], 1.98 and 1.87 (2 s, 3H, acetyl), 2.00 [m, 1H, $CH(CH_3)_2$], 2.73, 2.93 and 3.14 (dd, d, dd, 2H, *J* = 13.6, 9.6 and 7.1 Hz, and 13.8, 3.9 Hz, $CH_2C_6H_5$), 3.89 and 4.01 [2 t, 1H, *J* = 8.4 and 8.8 Hz, $CHCH_2(CH_3)_2$], 4.43 and 4.77 (dd and t, 1H, *J* = 3.9, 9.5 and 7.4 Hz, $CHCH_2C_6H_5$), 5.00-5.30 (set of m, 4H, $OCH_2C_6H_5$ and NCH_2O), 5.34 and 5.53 (2 d, 1H, *J* = 9.0 and 10.0 Hz, NH), 5.47 and 6.15 (2 d, 1H, ratio 1:2.1, *J* = 5.2 and 8.6 Hz, $CHOCOCH_3$), 7.2-7.5 (set of m, 10H, phenyls); IR (neat) 3298 (br), 3032, 2965, 1750, 1715, 1651, 1233 cm^{-1}; MS (chemical ionization) *m/e* 455 ($M + H^+$), 395, 351, 252, 234, 162, 91. Anal. calcd for $C_{25}H_{30}N_2O_6$: C, 66.07; H, 6.65; N, 6.16. Found: C, 65.76; H, 6.60; N, 6.16.
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