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# Synthesis and Investigation with Creatine Kinase of *trans*-2-Imino-1,3-diazabicyclo[3.3.0]octane-8-carboxylic Acid, a Bicyclic Analog of Creatine

ROBERT F. DIETRICH AND GEORGE L. KENYON[1]

*Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143*

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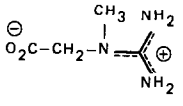
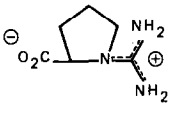
A synthesis of racemic *trans*-2-imino-1,3-diazabicyclo[3.3.0]octane-8-carboxylic acid in six steps from the known compound 2-benzylcarbamyl-5-carbethoxypyrrolidine is described. The compound, which is a bicylic analog of creatine, was shown to be neither a substrate nor an inhibitor of creatine kinase. 

## INTRODUCTION

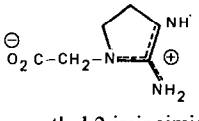
In their investigations of the mechanism of action of creatine kinase, Rowley *et al.* (*1*) and McLaughlin *et al.* (*2*) synthesized a series of substrate analogs of creatine (**1**) and examined the interactions of these compounds with the enzyme. Included in this series were two monocyclic, conformationally restricted creatine analogs (*3, 4*) that showed activity as substrates in the creatine kinase reaction, namely compounds **2** and **3**.



**1** Creatine



**2** *R-N*-Amidinoproline



**3** 1-Carboxymethyl-2-iminoimidazolidine

Compound **2** was shown to have a $V_{max}$ value approximately 1% of that of creatine in the creatine kinase reaction, while compound **3** had a $V_{max}$ value 90% of that of creatine (*2*). A logical extension of these findings is to fuse the main structural features of compounds **2** and **3** into a bicyclic compound, 2-imino-1,3-diazabicyclo[3.3.0]octane-8-carboxylic acid (**4**), and to examine its interaction with creatine kinase. Compound **4** exists in two diastereoisomeric forms, the *trans* isomer (**4a**) and the *cis* isomer (**4b**). In this paper we describe the synthesis and characterization of the *trans*-isomer (**4a**) as its racemate.

[1] To whom correspondence should be addressed.



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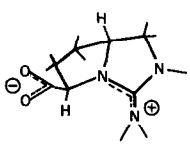
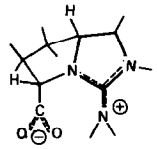


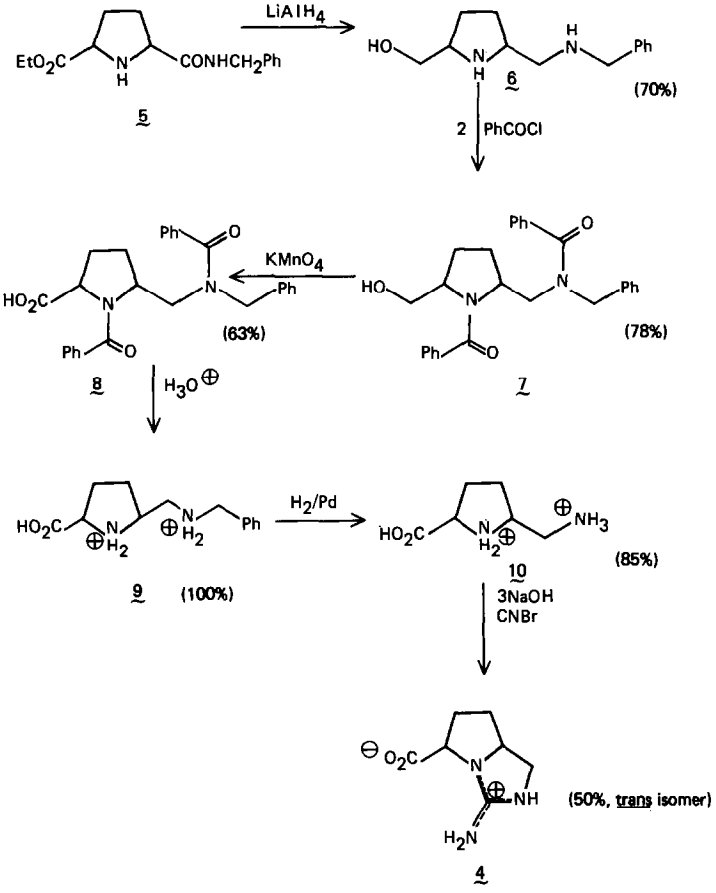
**4a**



**4b**

## RESULTS AND DISCUSSION

The successful route used in the synthesis of bicyclic creatine analog **4a** is outlined in Scheme I.




In the execution of the synthetic sequence described in Scheme I, which uses compound **5a** prepared by the method of Cignarella *et al.* (*6*) as starting material, there exists the possibility of both a *cis* isomer and a *trans* isomer for compounds **5** through **10**. While in theory it would have been possible to separate the *cis* and *trans* isomers of each product, this was considered neither practical nor necessary. A sample of compound **7** was separated into its *cis*- and *trans*-diastereoisomers by column chromatography on silica gel using 50/50 ethyl acetate/hexane as eluant. The two isomers, whose mass spectra were nearly identical, were isolated

in a 2.7:1 ratio although the *cis/trans* stereochemistry was not assigned. The conversion of compound **7** to compound **8,** however, was carried out on the mixture of *cis* and *trans* isomers.

It was also discovered that the *cis* and *trans* isomers of compound **9** could be easily separated. The *cis* isomer of the dihydrochloride salt of **9** slowly cyclized to what is apparently the corresponding lactam in aqueous solution, a process that could be monitored by proton NMR spectroscopy. The phenyl peak of the original product mixture decreased in intensity with the concomitant formation of a new peak in the aromatic region. After several days at room temperature, the reaction had stopped, leaving an approximately 50/50 mixture. The putative lactam could then be separated from the *trans*-diamino acid dihydrochloride by chromatography on anion-exchange resin ($^-$OH form). When eluted with water, the lactam was washed from the column, while the diamino acid remained on the column. The bound diamino acid could then be removed as the dihydrochloride salt by elution with 1.0 N HCl. The lactam could then be converted back to the *cis*-diamino acid by heating at reflux in 6 N HCl for several hours, consistent with the expected chemistry for the hydrolysis of an amide bond.

The *cis*- and *trans*-diamino acids were next separately converted to *cis*- and *trans*-**10**, and each of these products was treated with cyanogen bromide in an attempt to prepare the two diastereomeric forms of compound **4.** The *trans*-diamino acid **10** gave a product which, when isolated by preparative thin-layer chromatography, gave both proton and carbon-13 NMR spectra and an elemental analysis consistent with the structure of compound **4.** Although we are unable to make an absolute assignment based on the spectral data, it evidently is the *trans* isomer of **4,** compound **4a**, since it was derived from the precursor that did not undergo the apparent lactam formation in aqueous solution. When the *cis*-diamino acid was treated with cyanogen bromide, a complex product mixture formed. No compound consistent with *cis* compound **4b** was isolated. Since these results showed no worthwhile benefit in the effort involved in the separation of *cis* and *trans* compound **9**, subsequent syntheses of **4a** were carried out on the *cis, trans* mixtures.

It should be noted that the isomers of **10** may be considered conformationally restricted analogs of the amino acid lysine, compounds of great potential interest in their own right since they may interact with lysine-requiring enzymes. We suggest the trivial name "cyclolysine" for **10**.

*Enzymatic studies.* When creatine analog **4a** was investigated for substrate activity in the creatine kinase reaction using the polyethylenimine-cellulose thin-layer chromatographic assay developed by Rowley and Kenyon (*5*), no evidence of product formation (phosphocreatine analog) could be detected. Since this assay has been shown to be sensitive enough to detect product formation in analogs that react approximately 10,000 times more slowly than creatine, **4a** is at best an extremely poor substrate for creatine kinase.

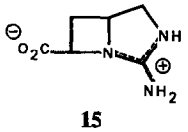
In order to examine the possibility that **4a** might bind to the creatine binding site of creatine kinase, but not act as a substrate for the enzyme, **4a** was also examined as a competitive inhibitor of creatine binding. The initial rate of the enzyme-catalyzed reaction of 15 mM creatine and 4 mM ATP was shown to be identical in the presence and absence of **4a**. These results indicate that **4a** not only does not

act as a substrate for creatine kinase, but also does not bind to the enzyme to any appreciable extent.

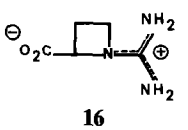
Previous studies (*1, 4*) have shown creatine kinase to have very tight steric requirements in the region of the active site for the binding of creatine. While some steric bulk is tolerated, as is shown for analogs **2** and **3**, the fact that the *S* enantiomer of **2** is not a substrate (*2*) shows the rigid conformational and steric requirements for creatine binding.

The bicyclic structure of **4a** apparently either forces the groups necessary for substrate binding into a conformation that does not allow for sufficient binding interactions, or, possibly, one of the five-membered rings is twisted into a position where steric bulk is not tolerated by the enzyme. Yet another possibility, which in fact is a more intriguing one, is that **4a** is *too rigid*, and that some conformational flexibility is needed in the creatine analog in the course of the enzyme-catalyzed phosphorylation reaction.

One way to begin to distinguish among these possibilities to explain the lack of reactivity of **4a** in the enzymatic reaction is to prepare the corresponding bicyclic analog that contains a four-membered ring in place of one of the five-membered rings, i.e., compound **15**:



**15**



**16**

This bicyclic analog is even more rigid than **4**, but contains less bulk. Compound **15** is an especially attractive analog since the four-membered ring-containing, monocyclic creatine analog *R-N*-amidinoazetidine-2-carboxylic acid (**16**) has recently been shown to have a $V_{max}$ value approximately 30% that of creatine itself in the creatine kinase reaction (*4*). A synthesis of **15**, based on the successful preparation of **4a**, has been initiated.

## EXPERIMENTAL PROCEDURES

*General.* Proton NMR spectra were taken either at 60 MHz on a Varian A60A spectrometer or on a Varian XL-100 spectrometer operating in the pulsed mode at 100.1 MHz as indicated. The carbon-13 spectra were also obtained on a Varian XL-100 spectrometer utilizing a Nicolet Multi Observe Nuclei Accessory (MONA) operating at 25.158 MHz. Chemical shifts values for both proton NMR and $^{13}C$ NMR spectra are relative to tetramethylsilane. Chemical ionization mass spectra were obtained on an Associaated Electronic Industries MS-902 spectrometer and electron-impact mass spectra on an Associated Electronic Industries MS-12 spectrometer. Melting points were uncorrected, and microanalyses were obtained from the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley, California.

Inhibition studies were carried out on a Radiometer TT2 pH-Stat, and the initial

rate in the creatine kinase catalyzed reaction between 4 mM ATP and 15 mM creatine compared in the presence and absence of 30 mM **4a.**

2-(*N-Benzylaminomethyl*)-5-*hydroxymethylpyrrolidine* (**6**). 2-Benzylcarbamyl-5-carbethoxypyrrolidine (**5**) (26.0 g, 94.2 mmol), as a mixture of the diastereoisomers (*6*), was dissolved in 300 ml anhydrous ether and added dropwise to an ice-bath-cooled, stirred suspension of $LiAlH_4$ (20 g, 526 mmol) in 300 ml ether, under an $N_2$ atmosphere. After the addition was complete, the reaction mixture was heated at reflux for 32 hr. The reaction mixture was then cooled to 0°C, and the excess hydride was destroyed by careful sequential addition of 20 ml $H_2O$, 20 ml 15% NaOH, and 60 ml $H_2O$. Stirring was continued for an additional hour, and the insoluble salts were removed by filtration followed by thorough washing with ether. The filtrate was then dried over anhydrous $K_2CO_3$ and concentrated at reduced pressure to leave a yellow oil. The oil was distilled at high vacuum (140–145°C/0.015 Torr) to give 14.6 g (70% yield) of the desired diamino alcohol (**6**) as a diastereoisomeric mixture.

*Anal.* Calcd. for $C_{13}H_{20}N_2O$: C, 70.87; H, 9.15; N, 12.71. Found: C, 70.75; H, 9.08; N, 12.75.

The 100-MHz proton NMR spectrum ($CDCl_3$) showed peaks at δ 1.0–2.0 (4H, complex m), 2.4–2.8 (2H, m), 3.04 (3H, s, $D_2O$-exchangeable), 3.1–3.7 (4H, complex m), 3.80 (2H, s), and 7.36 (5H, s).

*N,N'-Dibenzoyl-2*(*N-benzylaminomethyl*)-5-*hydroxymethylpyrrolidine* (**7**). 2-(*N*-Benzylaminomethyl)-5-hydroxymethylpyrrolidine (**6**) (11.8 g, 53.6 mmol), as a mixture of its diastereoisomers, was dissolved in 200 ml benzene with 26 g anhydrous $Na_2CO_3$, and the stirred suspension was cooled to 8°C in an ice bath. Benzoyl chloride (14.8 g, 12.2 ml, 105 mmol) in 50 ml benzene was added dropwise to the stirred mixture, at such a rate that the temperature did not rise above 10°C. After addition was complete, the reaction mixture was stirred and heated at reflux for an additional 2 hr. After cooling to room temperature, the insoluble salts were removed by filtration, and the filtrate was extracted first with 100 ml 2% NaOH, followed by extraction with 100 ml 5% HCl. The organic fraction was then dried over anhydrous $K_2CO_3$ and concentrated at reduced pressure to a solid white foam. Thin-layer chromatography on silica gel plates showed two spots, $R_f \sim 0$ and $R_f = 0.1$, when developed with ethyl acetate/hexane (50/50, v/v) and visualized with iodine vapor. A 1.1-g sample of the crude product was applied to a 52 × 1.8-cm silica gel column (Bio-Rad, Bio-Sil A, 100–200 mesh) and eluted with 50/50 ethyl acetate/hexane. The fractions (50 ml) were monitored by thin-layer chromatography until the compound at $R_f = 0.1$ had been completely eluted. The column was then washed with 300 ml ethyl acetate to remove the compound of lower $R_f$ value. While the proton NMR spectra of the two compounds were only similar, their electron-impact mass spectra were nearly identical. The crude mixture of the two diastereoisomers was converted from the solid foam to a white, powdery solid by trituration in a small amount of hot ether, followed by dropwise addition of absolute ethanol. The total yield of the two isomers of compound **7** was 18 g (78% yield), mp 120–130°C.

*Anal.* Calcd. for $C_{27}H_{28}N_2O_3$: C, 75.68; H, 6.59; N, 6.54. Found: C, 75.36; H, 6.53; N, 6.39.

The electron-impact mass spectrum showed the molecular ion at *m/e* 428 (1.0% of base peak) and the following peaks greater than 5% of the base peak: *m/e* 323, 217, 204, 106, 105 (base), 91, 82, and 77.

*N,N'-Dibenzoyl-2-(N-benzylaminomethyl)pyrrolidine-5-carboxylic acid* (**8**). An aqueous solution of $KMnO_4$ (5.3 g, 34 mmol in 100 ml $H_2O$) was added dropwise to a heated (40–50°C), stirred solution of *N,N'*-dibenzoyl-2-(*N*-benzylaminomethyl)-5-hydroxymethylpyrrolidine (**7**) (8.0 g, 19 mmol) in 75 ml glacial acetic acid. After addition was complete (~2 hr), heating and stirring were continued for an additional 4 hr. At this point an additional 1.5 g $KMnO_4$ in 50 ml water was added, and heating and stirring was continued for an additional 2 hr. After treating the reaction mixture with about 10 ml of a saturated aqueous solution of $NaHSO_3$ to destroy the excess permanganate and convert the insoluble $MnO_2$ to a water-soluble salt, the crude product was concentrated to a slightly brown, viscous oil. The residue was then taken up in $CHCl_3$ and extracted with 1% aqueous $NaHSO_3$. The colorless chloroform solution was concentrated to a white solid foam under vacuum and thoroughly triturated with 300 ml 0.5 N NaOH. The insoluble material was removed by filtration and the filtrate was then carefully acidified to pH 1 with 2.4 N HCl. The crude precipitate, 6 g, was thoroughly dried and recrystallized from ethyl acetate/hexane to give 5.2 g (63% yield) of the desired product, mp 148–153°C, as a mixture of the diastereoisomers.

*Anal.* Calcd. for $C_{27}H_{26}N_2O_4$: C, 73.28; H, 5.92; N, 6.33. Found: C, 72.91; H, 6.10; N, 6.42.

The 100-MHz proton NMR spectrum ($CDCl_3$) showed peaks at δ 1.4–2.6 (4H, complex m), 2.8–3.5 (2H, m). 4.0–5.2 (4H, complex m), 7.0–8.0 (15H, aromatic multiplets), and 8.6 (1H, broad s).

The electron-impact mass spectrum showed the molecular ion at *m/e* 442 (1.6% of the base peak) and the following peaks greater than 5% of the base peak: *m/e* 224, 218, 106, 105 (base), 91, and 77.

*2-(N-Benzylaminomethyl)pyrrolidine-5-carboxylic acid* (**9**). *N,N*-Dibenzoyl-2-(*N*-benzylaminomethyl)pyrrolidine-5-carboxylic acid (**8**) (6.6 g, 15 mmol) was heated at reflux for 18 hr in 100 ml 6.0 N HCl. After cooling to room temperature, the reaction mixture was extracted three times with 100-ml portions of ether to remove benzoic acid. Removal of the aqueous acid *in vacuo* left 4.6 g (quantitative yield) of *cis*- and *trans*-**9** dihydrochloride salt as a slightly yellow glass.

The chemical ionization mass spectrum (isobutane as reagent gas) gave an M + 1 peak, accurately mass measured at *m/e* 235.142 (calculated for $C_{13}H_{19}N_2O_2$; 235.144). Other significant peaks in spectrum were *m/e* 217, 145, 115, and 91.

The 100-MHz proton NMR spectrum ($D_2O$) showed peaks at δ 1.9–2.9 (4H, m), 3.0–4.25 (4H, m), 4.38 (2H, s), and 7.56 (5H, s).

*2-Aminomethylpyrrolidine-5-carboxylic acid* (*cyclolysine*, **10**). 2-(*N*-Benzylaminomethyl)pyrrolidine-5-carboxylic acid dihydrochloride (**9**) (2.79 g, 9.05 mmol) was dissolved in 200 ml 90% aqueous ethanol and hydrogenated at 50 psi in the presence of 1.5 g 10% palladium on charcoal on a Parr apparatus. After 18 hr, the catalyst was removed by filtration and the solvent was evaporated *in vacuo*. The crude product was shown by proton NMR spectroscopy to be a mixture of *cis*- and *trans*-**10**-along with ~10% of the lactam formed from the cyclization of the

starting material, compound **9.** This lactam side product was removed by anion-exchange chromatography ($^-$OH form); when the mixture was applied to an Ag-1-X8 Bio-Rad column, the lactam was eluted from the column with water. The diamino acid was then removed from the column by elution with 1.0 N HCl to give an 85% yield of the *cis* and *trans* mixture of **10** dihydrochloride as a slightly yellow glass.

The chemical ionization mass spectrum (isobutane as reagent gas) gave an M + 1 peak, accurately mass measured at *m/e* 145.0987 (calculated for $C_6H_{12}N_2O_2$; 145.0977). Other significant peaks in the chemical ionization mass spectrum included *m/e* 127 and 115.

The 100-MHz proton NMR spectrum ($D_2O$) showed peaks at δ 1.6–2.8 (4H, complex m) and 3.3–4.8 (4H, overlapping m).

*trans-2-Imino-1,3-diazabicyclo*[3.3.0]*octane-8-carboxylic acid* (**4a**). 2-Aminomethylpyrrolidine-5-carboxylic acid dihydrochloride (**10**) (230 mg, 1.1 mmol) was dissolved in 3 ml 1.1 N NaOH. To this stirred solution cyanogen bromide (128 mg, 1.2 mmol) in 10 ml methanol was added slowly over a period of 4 hr. The reaction mixture was stirred an additional 2 hr, and the solvents were removed *in vacuo*. Analytical thin-layer chromatography on Merck silica gel plates eluted with $CHCl_3$/MeOH/aqueous $NH_3$ (40/40/10, v/v/v) showed two spots, one pinkish-violet ($R_f$ = 0.12) and the other blue ($R_f$ = 0.28), when visualized with sodium nitroprusside–potassium ferricyanide solution (*7*). The product mixture was dissolved in approximately 1 ml methanol, the insoluble salts were removed by filtration, and 0.5 ml of the methanol solution was applied to a 2000-μl-thick Merck preparative silica gel plate that had been prerun with methanol. The two bands were visualized by blocking the majority of the plate and spraying with the sodium nitroprusside–potassium ferricyanide solution. They were then separately scraped from the plate and extracted with methanol. The compound at $R_f$ = 0.28 was isolated in ~50% yield by dissolving the methanol-extracted compound in a small amount of water, followed by careful precipitation with acetone to give **4a** as a partial hydrate, mp 260–265°C (dec.).

*Anal.* Calcd. for $C_7H_{11}N_3O_2 \cdot 2/3H_2O$: C, 46.40, H, 6.86; N, 23.19. Found: C, 46.47; H, 6.66; N, 23.02.

The 60-MHz proton NMR spectrum ($D_2O$) showed peaks at δ 1.3–2.8 (4H, complex m) and 3.0–4.8 (4H, overlapping m).

The 25.2-MHz carbon-13 NMR spectrum (*8*) of **4a** showed peaks at δ 178.4 ($CO_2^-$), 160.2 (guanidinium carbon), 64.7 (-CH-, $J$ = 145 Hz), 61.9 (-CH-, $J$ = 151 Hz), 49.3 (-$CH_2$-, $J$ = 144 Hz), 36.3 (-$CH_2$-, $J$ = 135 Hz), and 29.1 (-$CH_2$-, $J$ = 135 Hz).

The carbon-13 NMR spectrum of the hydrochloride salt of **4a** showed peaks at δ 175.0 ($CO_2H$), 159.9 (guanidinium carbon), 64.4 (-CH-, $J$ = 145 Hz), 59.2 (-CH-, $J$ = 151 Hz), 49.2 (-$CH_2$-, $J$ = 147 Hz), 36.0 (-$CH_2$-, $J$ = 135 Hz), and 28.9 (-$CH_2$-, $J$ = 135 Hz).

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