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AN EFFICIENT METHOD FOR THE SYNTHESIS OF GUANIDINO PRODRUGS

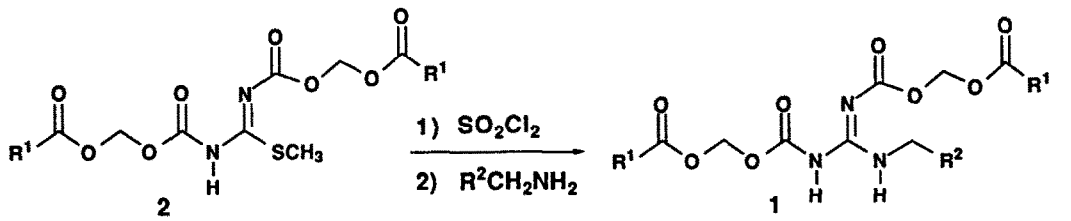
Mark G. Saulnier*, David B. Frennesson, Milind S. Deshpande, Steven B. Hansel, and Dolatrai M. Vyas

Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, CT 06492.

ABSTRACT: *A new class of guanidino prodrug is efficiently synthesized from an amine and an appropriate N,N'-bis(acyloxymethoxycarbonyl)-S-methylisothiourea. The N,N'-bis(acyloxymethoxycarbonyl)-S-methylisothiourea is readily prepared from S-methylisothiourea and the corresponding acyloxymethyl carbonochloridate in good yield. The N,N'-bis(acyloxymethyl carbamate) derivatives of the highly basic guanidino group serve as lipophilic, uncharged, esterase-activatable prodrugs of simple guanidine containing molecules.*

Several biologically active substances contain the highly basic, hydrophilic guanidino moiety (pKa = 13.5). For example, the spider venom toxin, *argiopine*[1], and the antitumor, antibiotic *spergualin*[2], represent two such agents bearing a positively charged guanidino group. In the case of *argiopine*, as well as for many serine protease inhibitors[3], the guanidino substituent occurs in an arginine residue. The presence of the guanidino function may give rise to certain problems in the formulation and overall bioavailability of potential drug candidates. Such barriers to effective drug delivery might be overcome by the synthesis of the corresponding prodrugs[4] (bioreversible derivatives) of the guanidine containing drug.

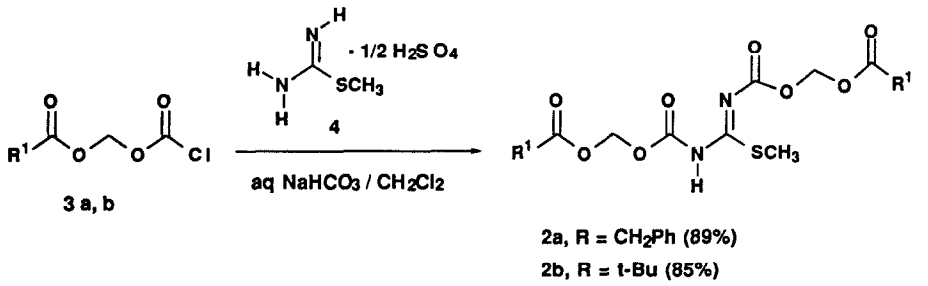
The studies of Gogate[5] show that N-(acyloxymethyl) carbamates serve as useful prodrugs for secondary, but not for primary amines. The extension of this methodology to the more basic guanidino nitrogen, as indicated by the N,N'-bis(acyloxymethyl carbamate) derivatives **1**, suggests novel guanidino prodrug targets. We now describe the synthesis of these esterase-activatable guanidino prodrugs **1** from the corresponding N,N'-bis(acyloxymethoxycarbonyl)-S-methylisothioureas **2** and a primary amine. To our knowledge, this chemistry represents the first example of a prodrug for the guanidino group.



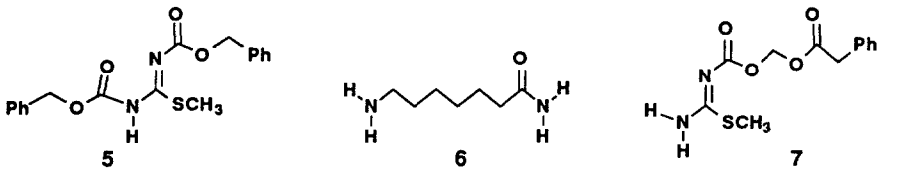
2 **1**

Reagents **2** are readily fashioned from known[6] acyloxymethyl carbonochloridates **3** and commercially available S-methylisothiourea sulfate **4** as shown in **Scheme I**. Thus, a dichloromethane solution of the chloroformates **3a-b**, and a saturated aqueous sodium bicarbonate solution of **4** (0.5 equiv), is stirred at room temperature for 22 h. Following purification by flash chromatography[7], the N,N'-bis(acyloxymethoxycarbonyl)-S-methylisothioureas **2a** (89%) and **2b** (85%) are isolated. Small amounts of the corresponding, more polar mono-acylated adducts are also obtained.

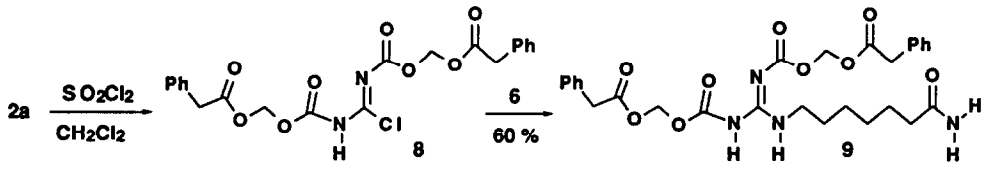
SCHEME I



The desired transformation of reagents **2** into the corresponding guanidino prodrug targets **1**, derives precedent[8] from the reaction of the known[9] N,N'-bis(benzyloxycarbonyl)-S-methylisothiourea **5** with primary amines. Thus, the displacement of the thiomethyl moiety in **5** by 7-aminoheptanamide **6** proceeds to give the desired protected guanidino amide.[8] However, the analogous chemoselectivity of **2a** does not obtain following its reaction with amine **6**. Instead, attack of **6** at the ester carbonyl gives the mono-acylated adduct **7** as the major product.

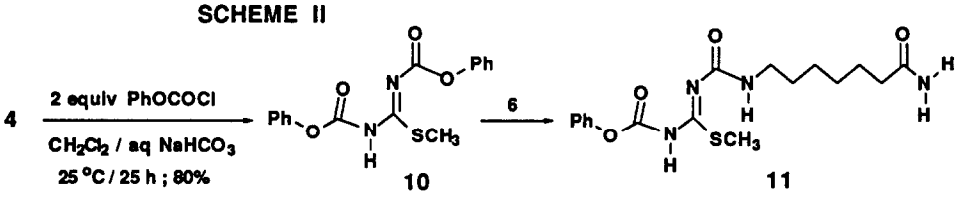


This chemoselectivity problem is circumvented by the reaction of **2a** with sulfuryl chloride (3 equiv, CH_2Cl_2, 25 °C, 3 h) to provide reactive intermediate **8**. Following evaporation of the solvent and drying at 0.05 torr, **8** is redissolved in CH_2Cl_2, and then readily condenses with amino amide **6** at room temperature, and with complete chemoselectivity in the desired sense, to give target **9** in 60% overall yield.[10]

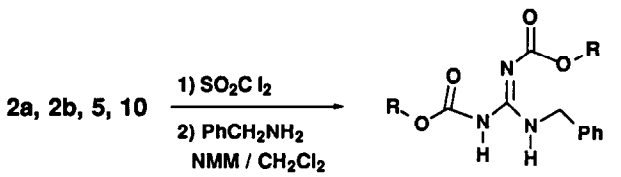


To further illustrate the scope of this chemistry, N, N'-bis(phenyloxycarbonyl)-S-methylisothiourea (**10**) is synthesized (80%)[10] as shown in **Scheme II**. The reaction of **10** with amine **6** (THF, N-methylmorpholine (NMM), 30 °C, 3 h) proceeds to give adduct **11** as the only isolable product. This result shows that the undesired selectivity of phenoxide displacement, over that of the methylthio moiety of **10**, is preferred.

SCHEME II



A general, chemoselective synthesis of N^2,N^3- bis(acyloxycarbonyl) guanidines is now available from the corresponding N,N'-bis(acyloxycarbonyl)-S-methylisothioureas (**2a**, **2b**, **5**, **10**) and primary amines, using this SO_2Cl_2 activation protocol. To illustrate, the benzyl amine-derived N^2,N^3-bis(acyloxycarbonyl) guanidines **12a-d** are synthesized in good yield as shown below.[10] For the synthesis of **12c** from **5**, the SO_2Cl_2 procedure provides a more rapid and efficient (77 %) protocol, as the direct[8] reaction of **5** with benzyl amine does not proceed to completion after 48 h reflux in 1,2-dichloroethane.[11]



	R	Yield
12a	CH_2OCOCH_2Ph	46 %
12b	$CH_2OCOC(CH_3)_3$	53 %
12c	CH_2Ph	77 %
12d	Ph	77 %

The N^2,N^3-bis(acyloxycarbonyl) guanidine **9** is rapidly and quantitatively converted into the known[12] free guanidino amide **13** by pig liver esterases in vitro (T1/2 < 5 min at 37 °C, pH = 7.4). In similar fashion, both **12a** and **12b** yield the known[13] free guanidine **14**, with the more hindered ester derivative **12b** showing the expected slower cleavage over its unhindered counterpart **12a** .

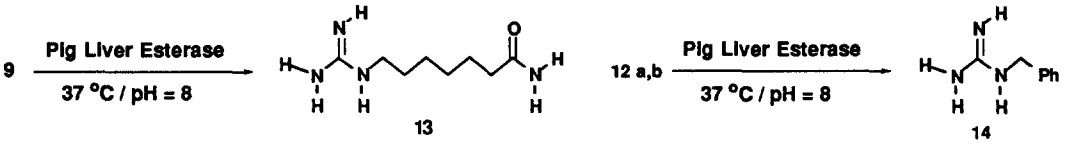
Table 1

In vitro data for guanidino prodrugs

Compound	*In vitro* Plasma $t_{1/2}$ (min)	Caco -2 P_C (nm/sec)[a]
14	n.d.	22.4 ± 0.4
12a	0.13	85.5 ± 10
12b	1.12	36.9 ± 12.8
12c	12.5	5.1 ± 0.4
12d	0.46	64.5 ± 7.2

n.d. = no detectable degradation over 2 hours.

a = (n = 2). ^{14}C-Mannitol, a paracellular marker, has a P_C = 5.2 nm/sec. ^{14}C-testosterone, completely absorbed in humans, has a P_C = 98.5 nm/sec



The stabilities of these four potential prodrugs (**12a** - **12d**) were evaluated *in vitro* in rat plasma.[14] All compounds were analyzed by HPLC.[15] "Drug" (**14**) was stable in plasma over 2 hours (Table 1). Intermediate products, presumably the monosubstituted guanidino forms, were detected in plasma during the incubation of each prodrug (**12a** - **12d**). However, **14** was generated only during incubations of **12a** and **12b**. "Drug"**14** was not detected in any plasma samples over a one hour incubation with **12c** or **12d**. All N^2,N^3-bis(acyloxycarbonyl) guanidines degraded more quickly in rat plasma than in water at 37°C (data not shown). In separate experiments, these prodrugs were incubated in porcine liver esterase suspensions (0.5 u/ml, Tris-base pH = 8.0), resulting in the formation of **14** from **12a** and **12b**, but not from **12c** and **12d**. These results are consistent with the plasma data. The bis(phenylcarbamate) **12d** yielded a product other than **14** in both plasma and esterase experiments, suggesting incomplete conversion. Furthermore, **12c** was stable in the presence of esterase, indicating that its plasma degradation is not due to the action of this enzyme. Thus, **12a** and **12b** function as esterase-labile prodrugs for the corresponding free "drug" **14**. In addition, **12c** and **12d**, which were synthesized as model compounds, as expected do not function as viable prodrugs in this regard.

Caco-2 monolayer experiments[16] were performed to determine if the absorptive potential of these guanidino-containing compounds was improved over that of "drug" **14**. Table 1 shows the calculated permeability coefficients (P_C), which reflect all molecular species detected on the receiver side that could potentially give rise to **14** (i.e. intact prodrug, monosubstituted form of the prodrug, or generated **14**). When **14**, **12a**, or **12b** were dosed onto monolayers the major species detected on the receiver side was **14**. Intermediates were the major species found on the receiver side when **12c** and **12d** were dosed. Surprisingly, there is an inverse relationship between the P_C values in Caco-2 monolayers and compound stability *in vitro* in rat plasma, suggesting that an increased stability towards enzymatic cleavage does not necessarily translate into an enhancement of intestinal absorption. The low P_C coefficient for **12c** is unexplainable, although this observation is unimportant inasmuch as **12c** is not a prodrug.

Most importantly, **12a** and **12b** significantly improve the permeability coefficients in Caco-2 cell monolayers. These results would predict increased oral absorption *in vivo*. Thus the N^2,N^3-bis(acyloxycarbonyl) guanidines **12a** and **12b**, can serve as functional prodrugs for the positively charged, free guanidines.

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10. All new compounds in this communication gave satisfactory analytical and spectroscopic data in full accord with their assigned structures. Yields are reported following purification by flash chromatography[7] over silica gel.
11. Representative spectroscopic data for compounds **2b** and **12b** follows. **2b**: ^{1}H NMR ($CDCl_3$) δ 5.80 (s, 4 H), 2.42 (s,3H), 1.20 (s,18 H). Anal. Calcd for $C_{16}H_{26}N_2O_8S$: C, 47.28; H, 6.45; N, 6.89.Found: C, 47.48; H, 6.43; N, 6.50. MS (DCI) m/z 407 (M^+ + H). **12b**: ^{1}H NMR ($CDCl_3$) δ 7.40-7.28 (m, 5 H), 5.79 (d, 4 H), 4.65 (d, 2 H), 1.22 (s, 18 H). ^{13}C NMR ($CDCl_3$) δ 177.1, 176.6, 162.2, 156.0,

152.4, 136.4, 128.8, 127.9, 127.7, 81.2, 80.6, 45.3, 38.7, 26.9, 26.8. Anal. Calcd for $C_{22}H_{31}N_3O_8$: C, 56.76; H, 6.71; N, 9.03. Found: C, 56.37; H, 6.56; N, 9.00. MS (DCI) m/z 466 ($M^+ + H$).

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14. All compounds were examined at 37°C in rat plasma, water, or esterase suspension. All prodrugs were dissolved in methanol prior to dosing plasma. At appropriate times, 0.1 ml samples were removed and added to 0.2 ml methanol to stop the reaction. The suspension was centrifuged (16,000 g x 3 min) and the supernatant was analyzed as described. All plasma kinetics were determined to be linear at an initial concentration of 0.04 or 0.1 mM.

15. All prodrugs were analyzed by isocratic HPLC conditions using a Zorbax Rx-C8 column with 10 mM Na phosphate (pH = 3.5) and 5 mM octane sulfonic acid as buffer (30 - 45 %), mixed with 55 - 70 % acetonitrile (total flow = 1 ml/min). All prodrugs were detected by UV at 240 nm except **12a** which used 210 nm. "Drug" **14** was detected with greater sensitivity by a post-column derivatization method, using separately pumped 0.75 N NaOH and 0.3% ninhydrin, with subsequent flourescence detection (EX = 375 nm, EM = 470 nm).

16. Caco-2 cells were grown to confluency on polycarbonate microporous membranes (24.5 mm, 3.0 μM pore size). Monolayer integrity was monitored by both transepithelial electrical resistance and ^{14}C-mannitol flux. The donor and receiver chambers consisted of Hank's balanced salt solution plus 10 mM HEPES at pH = 7.4. All prodrugs were dissolved in methanol prior to dosing monolayers, although the percent organic in the dosing solution never exceeded 1%. Dosing solutions consisted of 400 μM model compound, except for **12a** where the solubility did not allow a concentration above 32.5 μM. The P_C was calculated using the following equation

$$P_c = \frac{V_R}{A \cdot C_0} \cdot \frac{dC_R}{dt}$$

where V_R is the receiver volume, A is the membrane surface area, C_0 is the dosing solution concentration, and dC_R/dt is the rate of concentration change on the receiver side. For further details and review, see, Hilgers, A. R.; Conradi, R. A.; Burton, P. S. *Pharm. Res.* **1990**, *7*, 902.

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