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Synthesis, hydrolyses and dermal delivery of *N*-alkyl-*N*-alkyloxycarbonylaminomethyl (NANAOCAM) derivatives of phenol, imide and thiol containing drugs

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Abstract—Synthesis, characterization and hydrolysis in aqueous buffers of novel *N*-alkyl-*N*-alkyloxycarbonylaminomethyl (NANAOCAM) derivatives of substituted phenols, theophylline (Th) and 6-mercaptopurine (6MP) were carried out. The mechanism of hydrolysis was further investigated by synthesis, characterization and hydrolysis of *N*-aryl-*N*-alkyloxycarbonylaminomethyl (NArNAOCAM) derivatives of phenols. The hydrolysis follows pseudounimolecular first order kinetics and operates by way of an S_N1-type mechanism. Topical delivery of selected derivatives of acetaminophen (APAP), Th and 6MP was examined in in vitro diffusion cell experiments from IPM across hairless mice skins. The prodrug of APAP and 6MP increased permeation across the skin by about 2- and 4-fold, respectively, compared to the parent drug. NANAOCAM promoieties can act as novel prodrug derivatives of phenol, imide and thiol containing drugs for enhancing topical absorption.

Drugs containing polar functional groups like phenols, thiols or imides pose problems of membrane permeability and biphasic solubility which limit their dermal delivery. The prodrug approach, which involves masking these polar functional groups as simple alkyl esters or acyloxymethyl (ACOM, $R'COOCH_2$–) and alkyloxycarbonyloxymethyl (AOCOM, $R'OCOOCH_2$–) esters which then hydrolyse to the native drug either enzymatically or chemically, has proven useful in numerous cases.[1] However, these derivatives may prove to be too unstable or may not have the desired biphasic properties to permeate the skin effectively. Replacing the oxygen atom in OCH_2 with nitrogen (N–R, R = alkyl) in $R'OCOOCH_2$– to give a NANAOCAM ($R'OCONRCH_2$–, R = alkyl) promoiety could give medicinal chemists an additional handle and flexibility to improve solubility (better balance between solubilities in lipid and water) and stability (enzymatic vs chemical) of prodrugs of polar molecules. Recent studies have shown that a drug or a prodrug needs to have adequate lipid as well as water solubility to permeate the skin effectively.[1b,c] Introduction of heteroatoms like N into a promoiety has been shown to reduce crystal lattice energy and increase biphasic solubility of drug molecules. Only one example of the use of a NANAOCAM promoiety has been reported: 6MP.[2] The use of a close analogue of NANAOCAM, $R'CONRCH_2$–, has been reported for carboxylic acids.[3] Here we extend the use of the NANAOCAM promoiety to phenols and imide containing compounds like Th, and investigate the mechanism of chemical hydrolysis. In addition, we will present data from dermal penetration studies of some NANAOCAM prodrugs of APAP (phenol containing drug), Th (imide containing drug) and 6MP (thiol containing drug).

The NANAOCAM derivatives of phenols, Th, 6MP and dimethylaminobenzoic acid were synthesized by alkylating the parent compound with NANAOCAM chloride in the presence of triethylamine and CH_2Cl_2[4] (Table 1, compounds **1**–**10**). The corresponding alkylating agent was synthesized as reported by Siver and Sloan[2] by reacting a molar equivalent of formaldehyde with methyl amine to generate 1,3,5-trimethylhexahydrotriazene. Reaction of the 1,3,5-trimethylhexahydrotriazene with the appropriate chloroformate gave the corresponding NANAOCAM chlorides (Scheme 1).

For the synthesis of NArNAOCAM derivatives of phenols,[4] we used a protocol developed by Moreira et al.[3c] for the synthesis of alkylcarbonylaminomethyl chloride.

Keywords: NANAOCAM; NArNAOCAM; APAP; Th; 6MP; Prodrugs; Dermal delivery.
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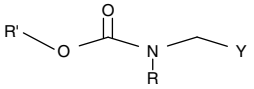
Table 1. Hydrolysis of NANAOCAM derivatives of substituted phenols, Th, 6MP and dimethylaminobenzoic acid, and NArNAOCAM derivatives of *p*-nitrophenol at pH 8.8 and 46 °C, and the influence of pK_a of leaving groups and substituents on rates of hydrolysis

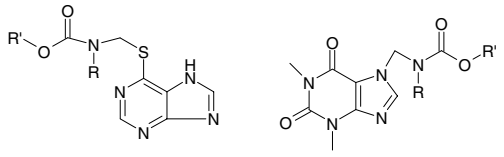


Y = Phenol, Th, 6MP, dimethylaminobenzoic acid

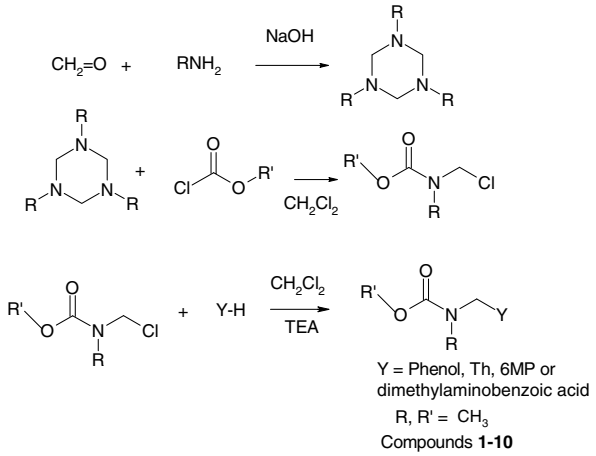
R'= CH_3; R = Alkyl or Aryl.

Compound	R	Y	log k (s^{-1})	$t_{1/2}$ (min)	pK_a[8]	σ^-
1	CH_3	*p*-MeOCHN–C_6H_4O–	−5.4937	3600	9.5	0.19
2	CH_3	*p*-NC–C_6H_4O–	−4.11	149	7.95	0.99
3	CH_3	*p*-OHC–C_6H_4O–	−3.81	75	7.66	0.94
4	CH_3	*o*-OHC–C_6H_4O–	−3.026	12	6.79	
5	CH_3	*p*-MeOC–C_6H_4O–	−4.21	189	8.05	0.82
6	CH_3	*p*-MeOOC–C_6H_4O–	−4.39	290	8.47	0.74
7	CH_3	*p*-O_2N–C_6H_4O–	−3.28	22	7.14	1.25
8	CH_3	6-MP	−3.22	19	7.5	
9	CH_3	Th	−4.56	420	8.6	
10	CH_3	*p*-Me_2N–C_6H_4COO–	−1.2953	0.22	5.03	
11	*p*-MeO–C_6H_4–	*p*-O_2N–C_6H_4O–	−4.54	400		
12	*p*-EtOOC–C_6H_4–	*p*-O_2N–C_6H_4O–	—	>24 h		
13	C_6H_5	*p*-O_2N–C_6H_4O–	−4.65	519		

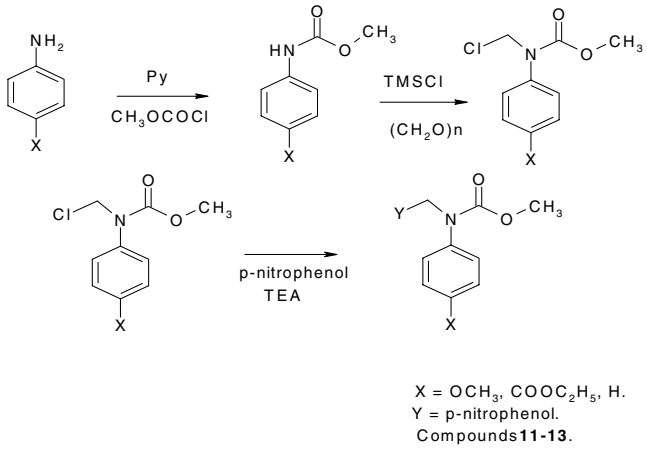
Representative prodrug structures:



6MP Th



Scheme 1.



Scheme 2.

We have expanded the synthesis to NANAOCAM chloride and NArNAOCAM chloride[5] in this case. A substituted aryl amine was reacted with pyridine and methylchloroformate to generate a *N*-arylcarbamic acid methyl ester. The *N*-arylcarbamic acid methyl ester was then reacted with excess trimethylsilylchloride and paraformaldehyde to generate the desired alkylating agent, NArNAOCAM chloride (Scheme 2). NArNAOCAM chloride was then used to alkylate a phenol (Table 1, compounds **11**–**13**). All compounds synthesized were fully characterized by UV,[6] NMR[6] and elemental analysis.

To be therapeutically useful, a prodrug must hydrolyse to the active drug molecule at an appropriate rate at the target site. Kinetic hydrolysis studies on NANAOCAM prodrugs were thus carried out. Compound **8** reverted to 6MP with a half-life of 91 min in pH 7.1 buffer at 32 °C and an S_N1 mechanism of hydrolysis was proposed initially by Siver and Sloan[2] but not firmly

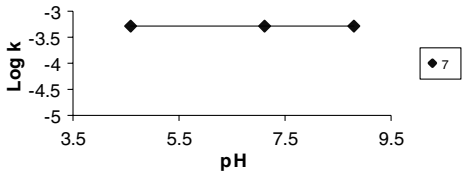
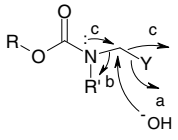


Figure 1. pH rate profile of **7**.

established. Hydrolysis of NANAOCAM derivatives of phenols at 32 °C was much slower so rates of hydrolysis were determined at 46 °C and in pH 8.8 buffer for derivatives **1**–**10** (Table 1).[7] Thus, rates of hydrolysis were determined where the leaving group Y was phenol, Th, 6MP or dimethylaminobenzoic acid in order to determine the effect of the pK_a of YH on rate.

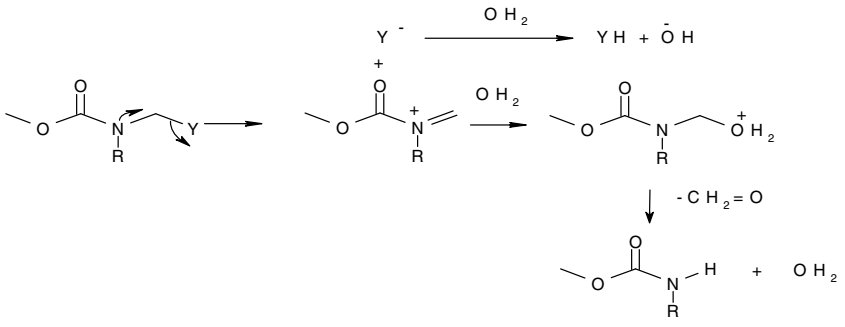
When log k (rate constant) was plotted against the pK_a of the leaving group (Y^-), a negative correlation was found (slope = −0.93, $r^2 = 0.98$; plot not shown). Rate of hydrolysis of NANAOCAM-Y was dependent on the nucleofugacity of the parent compound. The effect of electron-withdrawing groups in the para position of the phenol on rates of hydrolysis was also quantified using the Hammett plots between σ^- substituents[9] versus log k. A plot of log k versus sigma (σ^-) for **1**–**7** was linear and indicated that rates of hydrolysis were dependent on the electron-withdrawing effect imparted by the substituent at the para position (slope = $\rho = 0.96$; plot not shown). Rates of hydrolysis were also independent of the pH of the buffer. When rates were studied in various buffers (pH 4.6, 7.1 and 8.8) at 46 °C for **7** and log k was plotted against buffer pH, a line of zero slope was obtained (Fig. 1).

Several mechanisms for base catalysed hydrolysis of NANAOCAM-Y can be proposed.



Path 'a' shows an S_N2-type of pathway where hydroxide ion acts as a nucleophile and displaces Y to give a hydroxymethyl-*N*-alkylcarbamic acid alkyl ester. Similarly the hydroxide anion can attack the methylene carbon and lead to the expulsion of the *N*-alkylcarbamic acid alkyl ester as in path 'b.' This particular mechanism however can be ruled out because of the lower pK_a of YH (~5.2–9.5) compared to the *N*-alkylcarbamic acid alkyl ester (~14) making Y^- a better leaving group than the *N*-alkylcarbamic acid alkyl ester anion.

A S_N1 pathway is another possibility (path 'c,' Scheme 3). The lone pair of electrons on nitrogen can donate its electrons to stabilize an incipient carbocation with Y^- leaving. This carbocation can subsequently react with water to give hydroxymethyl-*N*-alkylcarbamic acid alkyl ester which then falls apart to give *N*-alkylcarbamic acid alkyl ester. However, none of the approaches provides an insight into the mechanism of hydrolysis since both S_N1 and S_N2 mediated hydrolyses are dependent on the leaving group ability of the ionized parent molecule, Y^-. However, the fact that hydrolyses were pH independent strongly favours an S_N1 mechanism. To more clearly define the mechanism of hydrolysis, we next synthesized prodrugs with an aryl group on the nitrogen instead of a simple alkyl group. If the mechanism is S_N2, the rate of hydrolysis should be accelerated by an electron-withdrawing substituent on the N-aryl group where a positive charge on CH_2 in N–CH_2–O is increased making the CH_2 a better target for nucleophilic attack. While if the mechanism were S_N1, the rate of hydrolysis should be decelerated by an electron-withdrawing substituent on the N-aryl group where a positive charge on CH_2 in N–CH_2–O is destabilized. Thus, NArNAOCAM (R′OCONRCH_2–, R = aryl, R′ = CH_3) promoieties were used to alkylate *p*-nitrophenol (Table 1, **11**–**13**). The half-lives of hydrolyses clearly illustrate that when an N-alkyl group (Table 1, $t_{1/2}$ for **7** = 22 min) was replaced by N-aryl (Table 1, $t_{1/2}$ for **11** and **13** = 400–520 min, while $t_{1/2}$ for **12** > 24 h), the rates of hydrolyses were slower. An electron-donating substituent on the N-aryl ring like –OCH_3 stabilizes the S_N1 transition state more than an electron-withdrawing group like –$COOC_2H_5$ or –H and the half-lives are in the order of **12** > **13** > **11**. Thus, hydrolysis of NAr-NAOCAM derivative of phenols follows an S_N1-type of mechanism and by inference so do NANAOCAM derivatives of phenols, Th, 6MP and carboxylic acids like dimethylaminobenzoic acid. Evidence in the literature supports the conclusion that the mechanism of hydrolysis is S_N1. *N*-Alkylamidomethyl esters of carboxylic acids also hydrolyse by an S_N1 mechanism.[3]



Scheme 3.

Table 2. Water solubility (S_{AQ}), solubility in IPM (S_{IPM}), log partition coefficients between IPM and pH 4.0 buffer and flux from IPM through hairless mice skins (J_{MIPM}) of NANAOCAM prodrugs and parent drugs

Compound	S_{AQ} (mM)	S_{IPM} (mM)	$\log K_{IPM:4.0}$	J_{MIPM} (μmole cm^2 h^{-1})	% of parent drug obtained after diffusion
APAP	100	1.91	−1.72	0.51	—
1	45.71	14.13	−0.45	1.11	23
Th	46	0.34	−0.51	0.48	—
9	9.33	2.69	−0.04	0.16	63
6MP	1.12	0.02	−1.75	0.0038	—
8[13]	1.45	1.35	0.03	0.015	100

NANAOCAM-carboxylic acid conjugates are chemically too unstable to serve as useful derivatives (compared to phenols which are chemically stable) because the leaving group has a pK_a of ~3–5 (compared to 6.8–9.5), e.g., **10**, $t_{1/2} \sim 6$ min at pH 7.4 and 37 °C. The NArNAOCAM promoiety enhances the chemical stability of phenolic derivatives compared to NANAOCAM promoiety, and by analogy they should increase the stability of carboxylic acid conjugates too. Thus, the implication of mechanism of hydrolysis of NANAOCAM-Y conjugates in prodrug design is the design of a more stable NArNAOCAM analogue of carboxylic acids which can be conveniently formulated but would hydrolyze independent of enzymes and be clinically useful. We are currently investigating the application of NArNAOCAM prodrug technology to carboxylic acids like naproxen.

Although NANAOCAM-phenols hydrolyse by an S_N1-type of pathway, they are chemically too stable to revert to the parent drug at a sufficient rate to be effective prodrugs based on chemical hydrolysis. To evaluate if these derivatives hydrolyse on their passage through the skin and increase permeation, selected prodrugs **1**, **8** and **9** were analyzed in in vitro skin permeation studies (Table 2). The physicochemical characterization of these selected NANAOCAM derivatives[10] and the parent drugs was also carried out (Table 2). In vitro skin permeation studies through hairless mouse skin were carried out using suspensions in IPM.[10] The steady-state flux was calculated from the slope of cumulative amounts of drug permeated versus time. The in vitro diffusion cell experiments from IPM through hairless mouse skin show reversion of NANAOCAM prodrugs to the parent drug. The permeation of **1** and **8** was enhanced compared to the parent drug, while the permeation of **9** was lower than that of Th.[11]

The increase in permeation of prodrugs **1** and **8** can be attributed to the increase in biphasic solubility of the prodrugs compared to the parent,[1b,c,12] while lower permeation of **9** was because of the large decrease in water solubility which could not be compensated by the increase in lipid solubility of the prodrug compared to Th. Thus, NANAOCAM derivatives of phenols, imides and thiols represent a novel class of prodrugs which are sufficiently chemically stable to allow formulation but sufficiently enzymatically labile to revert to the parent drug at a useful rate. Shorter chain NANAOCAM, prodrugs of phenols, have both higher lipid and water solubilities compared to ACOM and AOCOM phenolic conjugates.[11] Thus, replacing the oxygen atom in O–CH_2 of ROCOOCH_2 with a substituted nitrogen (N–R; R = alkyl) makes it possible to increase solubility in a membrane and increases permeability across a biological barrier such as skin.

In conclusion, NANAOCAM and NArNAOCAM promoieties can act as soft alkyl derivatives of polar drugs. The mechanism of chemical hydrolysis is believed to be S_N1 with phenol, Th or 6MP acting as the nucleofuge. Proof-of-mechanism being S_N1 comes from the increased stability of NArNAOCAM derivatives compared to that of NANAOCAM prodrugs. These NArNAOCAM derivatives can potentially be useful in designing stable carboxylic acid prodrugs whose pharmaceutical formulation should be easier than that of their NANAOCAM counterparts. The delivery of phenol and thiol containing drugs like APAP and 6MP can be enhanced by utilizing the NANAOCAM promoiety. The flux of APAP was increased by 2- and 4-fold for 6MP from IPM across hairless mouse skin. Synthesis of homologous series of NANAOCAM prodrugs of APAP, Th and 6MP in order to optimize flux across the skin is currently under progress. A more water-soluble NANAOCAM derivative should further enhance permeation of APAP and 6MP and also increase the permeation of Th across the skin.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.03.061.

References and notes

1. (a) Bundgard, H. Design and Application of Prodrugs. In *A Textbook of Drug Design and Development*; Krogsgaard-Larsen, P., Bundgard, H., Eds.; Harwood: Reading, UK, 1991; pp 113–191; (b) Sloan, K. B.; Wasdo, S. *Med. Res. Rev.* **2003**, *23*, 763; (c) Rautio, J.; Nevalainen, T.; Taipale, H.; Vepsalainen, J.; Gynther, J.; Pederson, T.; Jarvinen, T. J. *J. Med. Chem.* **2000**, *43*, 1489.
2. Siver, K. G.; Sloan, K. B. *J. Pharm. Sci.* **1990**, *79*, 66.
3. (a) Moreira, R.; Calheiros, T.; Cabrita, J.; Mendes, E.; Pimentel, M.; Iley, J. *Pharm. Res.* **1996**, *13*, 70; (b) Iley, J.; Moreira, R.; Calheiros, T.; Mendes, E. *Pharm. Res.* **1997**, *14*, 1634; (c) Moreira, R.; Mendes, E.; Calheiros, T.; Bacelo, M. J.; Iley, J. *Tetrahedron Lett.* **1994**, *35*, 7107.
4. General procedure for alkylation reactions: the alkylation of phenols with NANAOCAM-Cl and NArNAOCAM-Cl was carried out by refluxing equimolar equivalents of

phenol, TEA in CH_2Cl_2 for 1 h followed by addition of the alkylating agent to the reaction mixture. The contents were stirred overnight and worked up by washing with water. The CH_2Cl_2 solution was dried over Na_2SO_4 and then concentrated to an oil which was purified by crystallization or column chromatography.

5. Majumdar, S.; Sloan, K. B. *Synth. Commun.* submitted for publication.
6. ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si) of **1**: δ 7.6 (s, 1H), δ 7.39 (d, 2H), δ 6.96–6.87 (2d, 2H), δ 5.28–5.21 (2s, 2H), δ 3.72–3.7 (2s, 3H), δ 3.0–2.97 (2s, 3H), δ 2.0 (s, 3H). UV of **1**: λ_{max} (pH 8.8 buffer)/nm 243.4 (ε/L mol^{-1} cm^{-1} 0.95×10^4). UV: λ_{max} (pH 7.1 buffer)/nm 240 (ε/L mol^{-1} cm^{-1} 1.01×10^4, 0.09×10^4). ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si) of **12**: δ 8.2 (d, 2H), δ 7.27–7.42 (m, 5H), δ 7.05 (d, 2H), δ 5.67 (s, 2H), δ 3.75 (s, 3H). UV of **12**: λ_{max} (pH 8.8 buffer)/nm 305 nm (ε/L mol^{-1} cm^{-1} 1.19×10^4).
7. The rates of hydrolysis in aqueous buffers were determined by UV spectroscopy. An aliquot (100 μL) of a stock solution of compound dissolved in acetonitrile was added to 2.9 mL of buffer in a cuvette such that the final concentration was about 10^{-5} M. Half-lives were calculated from the plot of $\log(A_\infty - A_t)$ or $\log(-(A_\infty - A_t))$ versus time.
8. Jencks, W. P.; Regenstein, J. *In Handbook of Biochemistry and Molecular Biology: Physical and Chemical Data*, 3rd ed.; Fasman,G. D., Ed.; CRC: Cleveland,1976, Vol. I, pp 305–351.
9. Exner, O. *Correlation Analysis of Chemical Data*; Plenum Press: New York, 1988.
10. Experimental protocol for determination of solubilities, partition coefficients and flux through hairless mouse skin is reported in: Wasdo, S.; Sloan, K. B. *Pharm. Res.* **2004**, *21*, 940, and references cited in this paper.
11. Majumdar, S.; Sloan, K. B. 231st *American Chemical Society National Meeting and Exposition Abstracts*, Atlanta, GA, March 26–20, 2006.
12. Roberts, W. J.; Sloan, K. B. *J. Pharm. Sci.* **1999**, *88*, 515.
13. Physicochemical analysis of this derivative was reanalyzed and new values are reported in this paper.