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13. ABSTRACT (Maximum 200 words) The synthesis methodology for preparing four types of novel nucleophilic scavengers for experimental treatment of HD intoxication is described. The four types include 3-alkoxyquinuclidines, 4-(dialkyl)aminopyridines, N-alkylpyridine-4-thiones, and imidazoline-2-thiones. A total of 4 compounds representing three of the classes were prepared, characterized, and submitted to WRAIR for biological evaluation. In addition, a convenient kinetic screen to rank target compounds in order of relative reactivity was established.				
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Midterm Progress Report -17 April 1991

SYSTEMIC MUSTARD GAS SCAVENGERS

Covering the Period 05 March 1990 to 04 March 1991

Ralph N. Harris III
Robert A. Sanderson

SRI Project PYU 8838

Supported by:

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick
Frederick, MD 21702-5012

Contract No. DAMD17-90-C-0034

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EXECUTIVE SUMMARY

Contract No.: DAMD17-90-C-0034
Project Title: "Systemic Mustard Gas Scavengers"
Summary Date: 03-05-90 to 03-04-91
Contract Cost: \$346,965.00
Amount Funded: \$247,925.00
Contract Expenditures: Labor \$131,810.00
M & S 7,792.00
Present Funds: \$93,500.00

Project Staffing:	Personnel	Position	%Time
	Dr. Ralph N. Harris III	Principle Investigator	27
	Mr. Robert A. Sanderson	Research Chemist	100
	Ms. Dorris L. Taylor	Research Chemist	5

Progress: During the first year of the contract, the synthesis methodology for preparing type IA quinuclidine, type IB 4-aminopyridine, type IIA N-alkylpyridine-4-thione, and type IIB imidazoline-2-thione target compounds was established. Four new experimental therapeutics representing type IA, IIA, and IB targets were submitted to WRAIR for biological evaluation as treatment agents for mustard gas intoxication. A representative example of type IIB compounds was prepared that lacks only solubility and log P determinations before the compound can be submitted to WRAIR. Finally, a simple kinetic screen using UV spectroscopy was established that may be useful for ranking compounds in priority for future biological tests.



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PROJECT SUMMARY

The objective of this project is to develop new agents for treatment of exposure to mustard gas and related chemical warfare agents. Our investigation is focused on designing and synthesizing various nucleophilic compounds that have the capability of detoxifying mustard gas both topically and systemically by direct nucleophilic attack to yield innocuous products. Our specific approach includes the following steps: prepare 3 to 5 gram quantities of selected test compounds; evaluate the compounds with respect to spectroscopic properties, solubility, and octanol-buffer partition coefficient; submit 3 to 5 grams of each compound to the Walter Reed Army Institute of Research (WRAIR) for biological evaluation; elucidate structure-activity relationships (SAR) for protective activity based on in vivo and/or in vitro test results; and design new compounds with improved protective ability.

Our project objective is to synthesize seven classes of reactive nucleophiles: 3-alkoxyquinuclidines (type IA), 4-(dialkyl)aminopyridines (type IB), N-alkylpyridine-4-thiones (type IIA), imidazoline-2-thiones (type IIB), arylphosphonothioic acids (type IIIA), arylphosphonamidodithioic acids (type IIIB), and alkylene-bis-phosphonodithioic acids (type IIIC). Synthesis methodology for preparing type I and II target compounds was established and subsequently implemented to prepare representative examples of these classes in sufficient quantity for submission to WRAIR. Work continues on establishing the synthesis methodology for preparing type III target compounds.

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INTRODUCTION

This report summarizes the technical research efforts undertaken on U.S. Army Medical Research and Development Command Contract DAMD17-90-C-0034, "Systemic Mustard Gas Scavengers." The report covers progress from March 1990 to March 1991. In addition to reviewing the background, we outline our progress on the chemical synthesis of target compounds, describe the problems we encountered in some of our originally proposed synthesis routes to compounds and how we overcame the problems, and report the results to date on our efforts to develop an in vitro kinetic screen for synthesized targets that may ultimately be used to rank compounds in priority for biological evaluation.

The powerful vesicant bis-(2-chloroethyl)sulfide (mustard gas, HD) has been known for more than a century.¹ However, no effective medical treatment yet exists for the severe pathologic effects of this chemical warfare agent. Characteristic symptoms of exposure to HD are an initial latent period of several hours followed by severe erythema, vesication, edema, pruritis, and—depending on the severity of exposure—ulceration and necrosis of the skin and respiratory tract.² The most imminent danger from the superficial damage is infection of the open lesions. If secondary complications can be prevented, the victim will slowly recover. In cases of severe exposure, HD is readily absorbed through the skin and can persist in vivo for days while causing more generalized systemic pathology. In such cases, the pathologic manifestations include a pronounced degenerative effect on the bone marrow, with subsequent leukopenia and damage to the gastrointestinal tract.² If the more generalized systemic effects occur, the possibility of secondary complications becomes correspondingly more likely and life-threatening.³ Long-term effects from HD exposure include permanent eye damage, severe respiratory impairment, and carcinogenesis.⁴ Hence there is an urgent need in medical defense for treatment and preventive agents for exposure to HD and related chemical weaponry.

BACKGROUND

Although the complete pathology of HD and related vesicants is yet to be elucidated, the biochemical basis for their toxicity seems to reside in their ability to electrophilically alkylate protein and chromosomal material in the cell.⁵ By this process, several modes of cell injury are possible, ranging from inhibition of various enzymes by alkylation of sulfhydryl or other

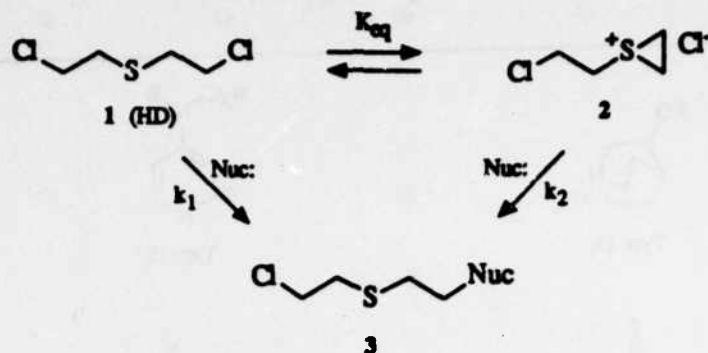
nucleophilic protein groups to alkylation of DNA bases in the cell nucleus. Because HD hydrolyzes very rapidly when solvated in water,⁶ it remains unclear how HD persists for extended periods in vivo and causes such extensive cellular damage. HD is a very lipophilic material ($\log P = 2.45$),⁷ and previous medical case studies⁸ convincingly indicate that it does indeed behave similarly to other lipophilic toxins (e.g., pesticides, polychlorinated biphenyls) by accumulating in lipid compartments in vivo. The sequestering of HD in lipid compartments where the water concentration is very low and hydrolysis kinetics are impeded could account for the apparent extended in vivo lifetime of HD and explain much of the delayed systemic pathology that is associated with this toxin.

Previous studies confirm that neutral nucleophiles such as thiols can provide limited protection for in vivo systems against mustards.⁹ It is believed that the protection afforded by such compounds is due to a direct nucleophilic reaction of nucleophile with HD which produces nontoxic products and that this reaction can occur within the cell.^{9d} The highly nucleophilic thiosulfate anion is also known to provide a degree of protection against mustards,^{9b} but only when administered immediately following exposure. It is likely that the limited effectiveness of the highly polar, hydrophilic thiosulfate ion is due to its inability to penetrate into relatively nonpolar lipid compartments where HD is known to accumulate.

In view of the foregoing, SRI is designing, synthesizing, and evaluating nucleophilic compounds that have the capability of acting both topically in a therapeutically preventive mode by deactivating HD on and under the skin and systemically to penetrate lipid compartments in vivo and deactivate lingering reservoirs of latent HD.

OBJECTIVES AND APPROACH

The mechanistic aspects of the reaction of HD and related β -chloroethyl sulfides with nucleophiles are fairly complex. However, extensive studies⁶ have determined that HD exists as a mixture of the open-chain form 1 (Scheme I) in equilibrium with the cyclic ethylenesulfonium ion 2 and that two reaction pathways for nucleophilic attack are possible—both leading to the same product 3. Ion 2 is considerably more reactive as an electrophilic species than open-chain form 1, and therefore reaction rate k_2 is much faster than reaction rate k_1 . The position of the equilibrium (K_{eq}) in Scheme I depends almost exclusively on the dielectric constant (ϵ) of the medium in which 1 is dissolved. When HD is solvated in water, which has a very high dielectric constant ($\epsilon = 80$), ion 2 is favored and HD is rapidly destroyed by hydrolysis to produce thiodiglycol. However, because HD is very hydrophobic, it apparently partitions in vivo into lipid

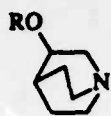


Scheme I. Mechanistic Pathways Involved in Reaction of HD with Nucleophiles

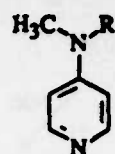
compartments such as subcutaneous fat ($\epsilon \sim 3-6$)¹⁰ in which the concentration of water is typically low. In this case, the open-chain form 1 is favored, hydrolysis kinetics are greatly slowed, and considerable systemic damage to the victim results over time.

In view of the above discussion, our objective is to develop therapeutic agents that can intervene before extensive cellular damage to the victim occurs. Our approach for accomplishing this objective is to design and prepare nucleophilic compounds that can penetrate lipid compartments in vivo and are sufficiently reactive to convert HD to a more hydrophilic derivative that will partition into the serum and be hydrolyzed or expelled through metabolic waste channels. Obviously, such compounds would also be effective topically on the skin as barrier or preventive agents. To test this approach, we are synthesizing a series of seven types of potential compounds, shown in Table 1. These compounds are designed to be very nucleophilic and, because of the appended side chains R, have the correct partitioning properties to penetrate lipophilic compartments in vivo where HD may reside. In this midterm report, we report our progress to date in synthesizing target compounds which has resulted in the delivery to WRAIR of four novel experimental therapeutics (Table 2) for HD exposure and present the results of our efforts to develop a simple, convenient kinetic screen to rank compounds according to their relative efficiency as nucleophilic scavengers for HD.

Table 1. Target Compounds



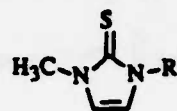
Type IA



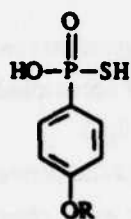
Type IB



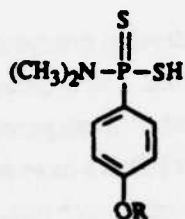
Type IIA



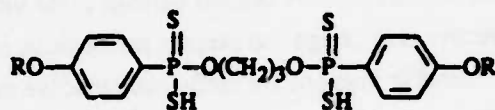
Type IIB



Type IIIA




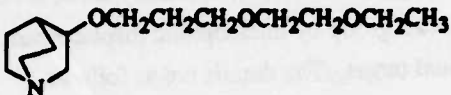
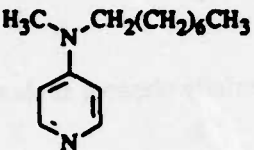
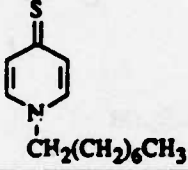
Type IIIB



Type IIIC

R = CH₂(CH₂)₅CH₃
 = CH₂CH₂CH₂OCH₂CH₂OCH₂CH₃
 = CH₂CH₂CH₂OCH₂CH₂SO₂CH₃

Table 2. Compounds Submitted to WRAIR

SRI Code No.	Compound Structure	WR Bottle No.	Quantity (g)
HS-001	 <chem>OCC(CCC)CCN12CCC3CC1C23</chem>	BM05629	3.0
HS-002	 <chem>CCOCC(CCC)CCOCCN12CCC3CC1C23</chem>	BM06582	3.0
HS-003	 <chem>CN(CCC)CC1=CC=CN=C1</chem>	BM07310	3.0
HS-004	 <chem>CCN(CCC)C1=CC=NC=S1</chem>	BM07552	5.0

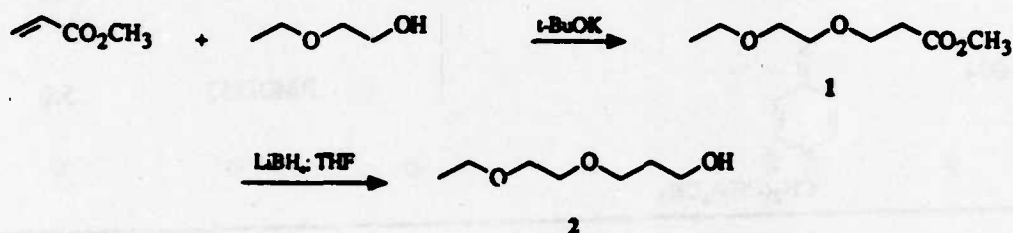
RESULTS

SYNTHESIS

The synthesis of target compounds followed the general procedure of preparing the appropriate side chain R in electrophilically activated form as a mesylate or triflate ester and then coupling it to a protected nucleophilic head group by nucleophilic displacement. The head group was then deprotected to generate the final target. The details are as follows.

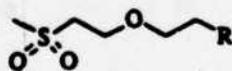
Synthesis of Side Chains

The proposed dioxanonanol side chain 2 was successfully prepared as shown in Scheme II



Scheme II

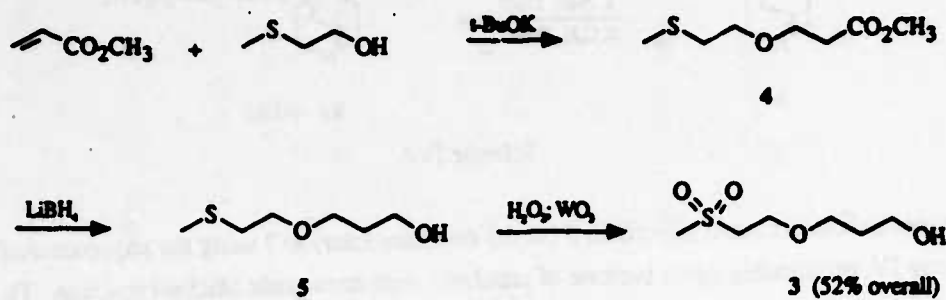
II. However, we were unsuccessful in attempting to use a similar route to prepare the methylsulfonyl-substituted side chain 3. We attribute this failure to a competing retro grade Michael reaction of ester 4 during attempted lithium borohydride reduction to give 3.



3 R = CH₂OH

4 R = CO₂CH₃

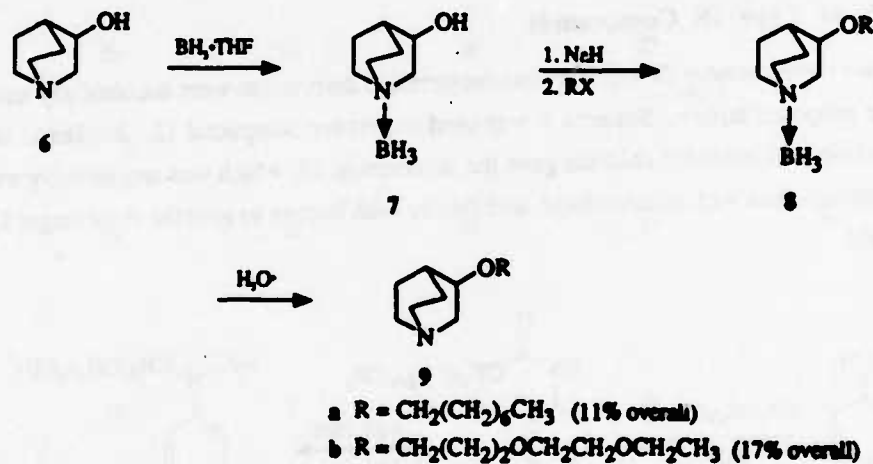
This problem ultimately forced us to consider an alternative route to 3 that was successful and is outlined in Scheme III.



Scheme III

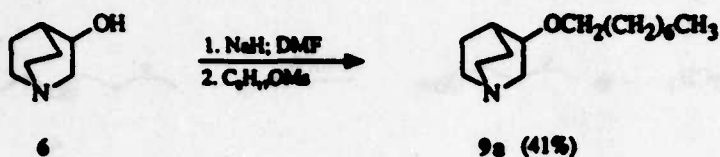
Synthesis of Type IA Compounds

Type IA compounds **9** were prepared according to Scheme IV. Thus, protection of 3-quinuclidinol (**6**) as the borane adduct **7** followed by formation of the corresponding sodium alkoxide salt with sodium hydride and reaction with the appropriate side chain gave compounds **8** in fair yield after flash chromatography. Compounds **8** were readily deprotected in methanolic



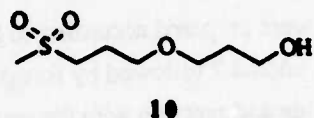
Scheme IV

acid to give the final targets **9**. Alternatively, compound **9a** was prepared directly from 3-quinuclidinol by way of the route outlined in Scheme IVA, which gave somewhat better yield.



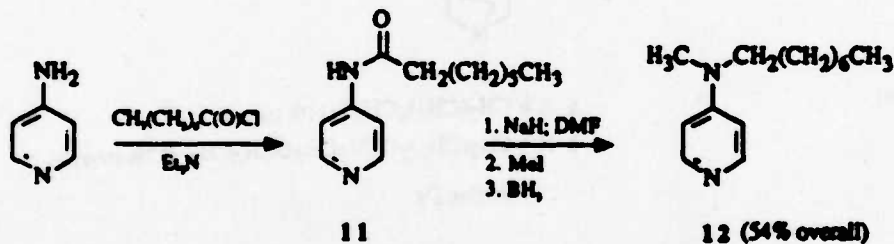
Scheme IVA

We were unable to couple side chain 3 (as the mesylate ester) to 7 using the sequence outlined in Scheme IV, presumably again because of problems with retro grade Michael reaction. To avoid this problem, we are considering the side chain 10, whose corresponding mesylate ester should behave normally under the conditions outlined in Scheme IV. Compound 10 should be readily prepared using a sequence similar to Scheme III but starting with commercially available 3-(methylthio)-1-propanol.



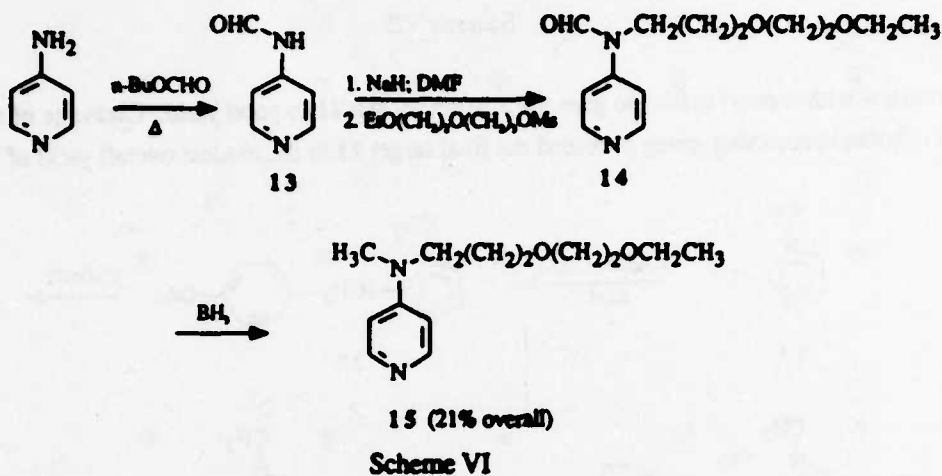
Synthesis of Type IB Compounds

Two routes to type IB 4-(dialkyl)aminopyridine derivatives were successfully used to synthesize proposed targets. Scheme V was used to prepare compound 12. Acylation of 4-aminopyridine with octanoyl chloride gave the octanamide 11, which was sequentially treated with sodium hydride, then with iodomethane, and finally with borane to give the final target 12 in good overall yield.



Scheme V

Alternatively, Scheme VI was used to prepare compound 15. Formylation of 4-aminopyridine with butyl formate was accomplished by thermal acyl transfer to give the corresponding formamide 13. Compound 13 was then carried to the final target 15 by using a sequence of reactions similar to that for the transformation of compound 11 to compound 12 (Scheme V). Although the overall yield for Scheme VI is somewhat lower than that for Scheme V, the versatility of Scheme VI makes it the route of choice for future type IB targets.

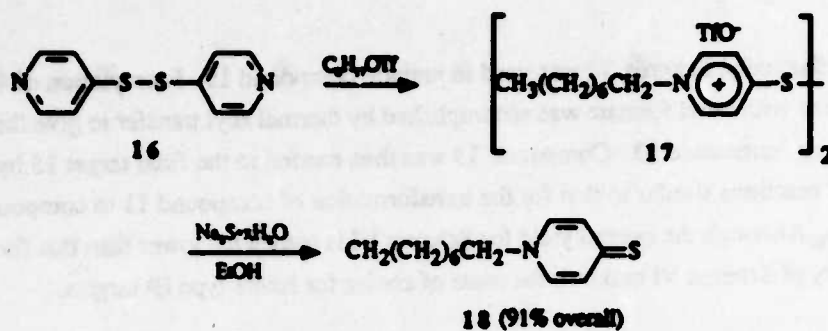


Synthesis of Type IIA Compounds

A very convenient route to type IIA N-alkylpyridine-4-thiones was established. The route begins with the commercially available disulfide 16 and proceeds as shown in Scheme VII. Bis-quaternization of 16 with n-octyl triflate proceeded in high yield to give the bis-salt 17. Reductive cleavage of the disulfide bond of 17 using the procedure of Burawoy and Turner¹¹ occurred essentially quantitatively to give the type IIA target 18 as a yellow solid. The route outlined in Scheme VII should be of general utility for the synthesis of the other type IIA targets.

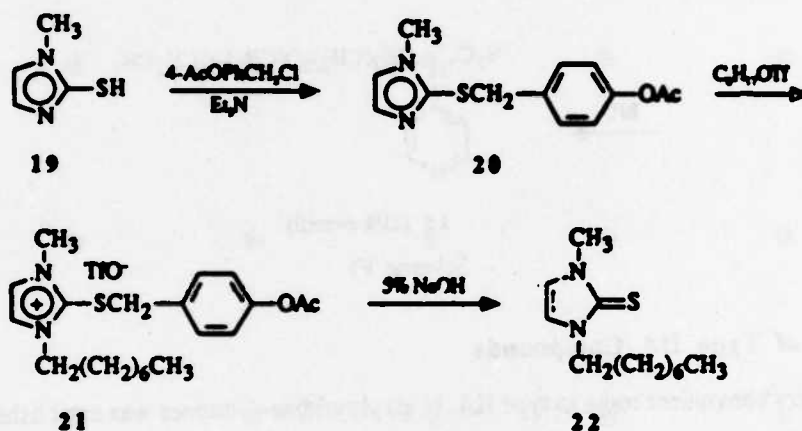
Synthesis of Type IIB Compounds

The synthesis methodology for preparing type IIB imidazoline-2-thiones was established and successfully implemented during this report period. Scheme VIII outlines the route we used to prepare compound 22. The S-protected thioimidazole 20 was prepared in modest yield and



Scheme VII

quaternized with n-octyl triflate to give the quaternary salt 21 in good yield. Cleavage of the 4-acetoxybenzyl protecting group provided the final target 22 in the modest overall yield of 14%.

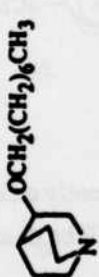
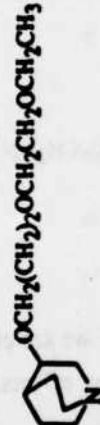
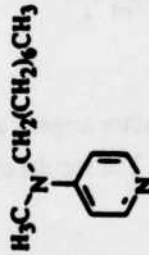
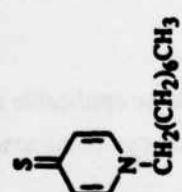


Scheme VIII

Although the yield of 22 was low, most of the steps in Scheme VIII have not been optimized and the versatility of this route makes it attractive as a means for preparing other type IIB compounds. We will refine Scheme VIII so that the remaining type IIB targets can be prepared and delivered to WRAIR.

Table 3 lists selected physical properties and other data for all compounds submitted to WRAIR during this report period.

Table 3. Selected Physical Data for Compounds Submitted to WRAIR

SRI Code No.	Cpd. No. ^a	Structure	Yield (%) ^b	mp (bp/mmHg)	log P ^c	Analysis ^d
HS-001	9a		41	100 °C/0.15	+2.08	C ₁₅ H ₂₉ NO
HS-002	9b		27	58-62 °C	+1.5	C ₁₄ H ₂₇ NO ₃ ·H ₂ O
HS-003	12		74	oil	+2.79	C ₁₄ H ₂₄ N ₂ ·1/2H ₂ O
HS-004	18		100	81-82 °C	+2.94	C ₁₃ H ₂₁ NS

^aSee text for description of synthesis routes.
^bYield from immediate precursor.

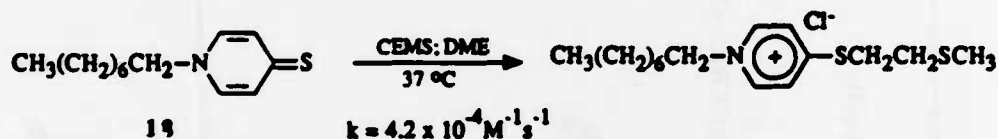
^cOctanol-buffer (pH 7.4) partition coefficient.

^dAgrees within ±0.4% of theoretical values.

KINETIC EVALUATION OF TARGET COMPOUNDS

To establish some basis for ranking target compounds as nucleophilic scavengers, we attempted to develop a convenient kinetic screen that can routinely be performed on synthesized compounds. Such a screen could be used to rank compounds in priority for biological evaluation and as a source of data to aid the design of improved compounds.

Our kinetic screen essentially consists of spectroscopically monitoring the rate of reaction of the target compound with the HD simulant 2-chloroethyl methyl sulfide (CEMS) in a solvent of appropriate dielectric constant. The first spectroscopic method we chose for monitoring kinetics was ^1H NMR. Although reaction of compound 9a with CEMS in CDCl_3 was detectable by this method, it became obvious very soon that there was far too much overlap (at 60 MHz) of the proton signals for starting materials and products for this method to be adequate for our purpose. Therefore, we turned to UV spectroscopy as a means of monitoring reactions and had somewhat more success. Thus, the change in concentration (c) of thione 18 from its initial concentration of 2.6×10^{-5} M ($\lambda_{\text{max}} = 354$ nm) in 1,2-dimethoxyethane solvent (DME, $\epsilon = 5.5$) was monitored as reaction with CEMS (c = 0.43 M) occurred under pseudo first order conditions at 37 °C. Under these conditions, $t_{1/2}$ for the disappearance of 18 was determined to be approximately 1.1 h, with a rate constant of $4.2 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$.



This method should be applicable to other targets, and we are currently refining our techniques so that all targets can be screened. The results of our efforts will be described in forthcoming reports.

CONCLUSIONS

During this report period, the synthesis methodology for preparing all side chains for target compounds was established. However, the use of the 2-[(2'-methylsulfonyl)ethoxy]ethyl side chain was precluded because of unanticipated elimination reactions that prevented the coupling of this side chain to the target head groups. The synthesis methodology for preparing type I and II target compounds was established and was used to prepare representative examples of these targets for submission to WRAIR. Finally, a simple kinetic screen to rank compounds nucleophilically as scavengers for HD was investigated and used to obtain a rate constant for reaction of a type III N-alkylpyridine-4-thione with an HD simulant.

In the coming period, we will investigate substitutes for the 2-[(2'-methylsulfonyl)ethoxy]ethyl side chain, complete the synthesis and delivery of the remaining type I and II target compounds, and continue developing conditions for the synthesis of type III compounds. In addition, we hope to refine our kinetic screen for the evaluation of all targets so that a system can be established for ranking compounds in relative order of reactivity.

EXPERIMENTAL SECTION

UV spectra were recorded on a Hewlett Packard model 8450 UV-vis spectrophotometer equipped with a Model 89100A temperature controller. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Associates EM-360 or Joel FX90 spectrometer. Chemical shifts are reported in parts per million in δ units from an internal tetramethylsilane reference. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Melting points (mp) were determined on a Hoover or Mel Temp melting point apparatus and are uncorrected. Microanalyses were performed by Desert Analytics, Tucson, Arizona.

Tetrahydrofuran (THF) was distilled from benzophenone ketyl and used immediately. All other solvents were reagent grade. All commercial starting materials were purchased from Aldrich Chemical Company.

PREPARATION OF METHYL 4,7-DIOXANONANOATE (1)

To a stirred, ice-cooled mixture of 2-ethoxyethanol (0.18 mol) and methyl acrylate (0.458 mol), a mixture of 2-ethoxyethanol (0.277 mol) and potassium *t*-butoxide (0.046 mol) was added, and the resulting mixture was stirred overnight at room temperature. The mixture was then neutralized with acetic acid and vacuum distilled to give pure compound 1 in 31% yield as a colorless oil: bp 60 °C (0.3 mmHg); $^1\text{H NMR}$ (CDCl_3) δ 4.39-3.35 (m, 11H, 4 CH_2O , OCH_3), 2.63 (t, 2H, $J = 7$ Hz, CH_2CO), 1.22 (t, 3H, $J = 7$ Hz, CH_3).

PREPARATION OF 4,7-DIOXA-1-NONANOL (2)

To a stirred, ice-cooled mixture of lithium borohydride (0.142 mol) in dry THF (80 mL), a solution of compound 1 (0.142 mol) in THF (20 mL) was added dropwise over 15-30 min. The mixture was stirred overnight at room temperature and then heated at 40 °C for 3 h. After the mixture was quenched with water (10 mL), it was taken up in dichloromethane (DCM, 100 mL), washed with saturated brine (2 x 200 mL), dried (MgSO_4), filtered, and concentrated to give a clear oil. The oil was vacuum distilled to give pure compound 2 in 44% yield as a colorless oil: bp 50 °C (0.1 mmHg); $^1\text{H NMR}$ (CDCl_3) δ 3.83-3.14 (m, 11H, 5 CH_2O , OH), 2.09-1.68 (m, 2H, CH_2), 1.24 (t, 3H, $J = 7$ Hz, CH_3).

PREPARATION OF METHYL 4-OXA-7-THIAOCTANOATE (4)

This compound was prepared in 96% yield from 2-(methylthio)ethanol and methyl acrylate using a procedure similar to that for the preparation of compound 1 and was purified by flash chromatography (DCM eluting solvent): $^1\text{H NMR}$ (CDCl_3) δ 4.47-3.38 (m, 7H, $2\text{CH}_2\text{O}$, OCH_3), 2.84-2.48 (m, 4H, CH_2S , CH_2CO), 2.12 (s, 3H, SCH_3).

PREPARATION OF 4-OXA-7-THIA-1-OCTANOL (5)

This compound was prepared in 77% yield by lithium borohydride reduction of compound 4 using a procedure similar to that described for the preparation of compound 2: $^1\text{H NMR}$ (CDCl_3) δ 3.97-3.39 (m, 7H, $3\text{CH}_2\text{O}$, OH), 2.71 (t, 2H, $J = 7$ Hz, CH_2S), 2.16 (s, 3H, SCH_3), 2.09-1.62 (m, 2H, CH_2).

PREPARATION OF 2-[2'-(METHYLSULFONYL)ETHOXY]-1-ETHANOL (3)

A tungstic acid catalyst¹² was prepared by treating 0.07 g of tungsten trioxide monohydrate in deionized water (20 mL) with 5 drops of 5% sodium hydroxide followed by sufficient acetic acid to adjust the pH of the mixture to 5.5. Alcohol 5 (8.5g, 57 mmol) was then added and the temperature of the mixture brought to 60-65 °C. While the mixture was stirred, 30% H_2O_2 (18 mL, 156 mmol) was added slowly over 2-2.5 h at the rate of ~2 mL/15 min. It was necessary to cool the mixture occasionally to maintain a reaction temperature of 60-65 °C. After the mixture was stirred an additional 20 h at room temperature, excess H_2O_2 was destroyed with 15% sodium bisulfite and the mixture was saturated with NaCl and extracted well with DCM. The extracts were dried (MgSO_4), filtered, and evaporated to give sulfone 3 in 71% yield as a colorless oil: $^1\text{H NMR}$ (CDCl_3) δ 4.02-3.15 (m, 9H, $3\text{CH}_2\text{O}$, CH_2SO_2 , OH), 3.03 (s, 3H, SO_2CH_3), 2.07-1.62 (m, 2H, CH_2).

PREPARATION OF 3-QUINUCLIDINOL-BORANE COMPLEX (7)

To a stirred, ice-cooled mixture of 3-quinuclidinol (0.197 mol) in DCM (350 mL), 1 M borane in THF (216 mL) was added dropwise over 2 h. The mixture was stirred for 20 h and concentrated to yield a yellow oil. The oil was flash-chromatographed through silica gel and eluted with DCM to give pure compound 7 as a white solid in 62% yield: mp 178 °C dec; $^1\text{H NMR}$ (CDCl_3) δ 4.31-2.50 (m, 8H, $3\text{CH}_2\text{N}$, CHOH), 2.49-1.50 (m, 5H, 2CH_2 , CH).

PREPARATION OF 3-(OCTYL-1'-OXY)QUINUCLIDINE (9a)

Compound 9a was prepared by two procedures, both of which are described as follows.

Method A. To an ice-cooled, stirred suspension of sodium hydride (12 mmol, free from oil) in THF (15 mL), borane complex 7 (12 mmol) in THF (20 mL) was added, and the mixture was warmed to room temperature and stirred for 2 h. After the mixture was again cooled in ice, *n*-octyl mesylate (10 mmol, prepared from *n*-octyl alcohol and methanesulfonyl chloride) was added and the mixture was stirred for 3 days at room temperature. The mixture was then filtered and concentrated to yield an amber oil that was treated with 5% HCl in methanol (20 mL) and stirred overnight. The mixture was concentrated, and the residue was taken up in water and washed well with ethyl ether. After the aqueous layer was basified to pH > 12 with 5% sodium hydroxide, it was extracted well with DCM. The combined extracts were dried (MgSO₄), filtered, and concentrated to give compound 9a as a pale yellow oil in 17% yield (see Table 3): ¹H NMR (CDCl₃) δ 3.48-3.20 (m, 3H, CHOCH₂), 3.16-2.55 (m, 6H, 3CH₂N), 2.15-1.19 (m, 17H, 8CH₂, CH), 0.88 (t, 3H, J = 7 Hz, CH₃).

Method B. To an ice-cooled, stirred mixture of sodium hydride (23.9 mmol) in DMF (25 mL), a solution of 3-quinuclidinol (18.4 mmol) in 50 mL of DMF was added dropwise over 30 min. The mixture was then stirred at room temperature for 3 h and *n*-octyl mesylate (19.3 mmol) was added. After the mixture was stirred for 2 days, 100 mL of water was carefully added and the mixture was extracted well with ethyl ether. The ether extracts were back-extracted with 5% HCl, and the combined HCl extracts were basified to pH > 12 with 5% sodium hydroxide and extracted well with DCM. After the combined DCM layers were washed with brine and dried over MgSO₄, they were filtered and concentrated to give compound 9a in 41% yield. This material was identical to the sample prepared by Method A.

PREPARATION OF 3-(4',7'-DIOXANONYL-1'-OXY)QUINUCLIDINE (9b)

To an ice-cooled, stirred mixture of 4,7-dioxa-1-nonanol (2, 30.7 mmol), diisopropylethylamine (32 mmol), and DCM (50 mL), a solution of methanesulfonyl chloride (32 mmol) in 25 mL of DCM was added dropwise over 30 min. The mixture was warmed to room temperature, stirred overnight, and then washed with water followed by brine. After the mixture was dried (MgSO₄), it was filtered and concentrated to give an oil that was flash-chromatographed through silica gel and eluted with DCM-methanol (95:5) to give pure 4,7-dioxa-1-nonyl mesylate as a colorless oil in 94% yield: ¹H NMR (CDCl₃) δ 4.39 (t, 2H, J = 7 Hz, CH₂OMs), 3.81-3.38

(m, 8H, 4CH₂O), 3.04 (s, 3H, CH₃SO₂), 2.23-1.81 (m, 2H, CH₂), 1.22 (t, 3H, J = 7 Hz, CH₃).

Compound 9b was prepared from borane complex 7 and 4,7-dioxa-1-nonyl mesylate following a procedure similar to that outlined as Method A above for the preparation of compound 9a, and was obtained in 17% overall yield as a crystalline hydrate (see Table 3): ¹H NMR (CDCl₃) δ 3.89-3.04 (m, 17H, 5CH₂O, 3CH₂N, CHOCH₂), 2.62-1.71 (m, 7H, 3CH₂, CH), 1.25 (t, 3H, J = 7 Hz, CH₃).

PREPARATION OF 4-[N-METHYL-N-(1'-OCTYL)]AMINOPYRIDINE (12)

To a stirred solution of 4-aminopyridine in THF (200 mL) and triethylamine (5.9 mL) cooled at -78 °C, octanoyl chloride (7.3 mL) was added dropwise over 20 min. The mixture was stirred for 30 min, warmed to 0 °C and stirred for 3 h, and then warmed to room temperature and stirred overnight. After the mixture was filtered, it was concentrated to give an oil that was taken up in DCM and washed consecutively with water and then brine. The mixture was dried over MgSO₄, filtered, and concentrated to provide pure N-(4'-pyridyl)octanamide (11) in 100% yield as a pale yellow oil: ¹H NMR (CDCl₃) δ 10.60 (br s, 1H, NH), 8.55 (d, 2H, J = 7 Hz, aryl), 7.79 (d, 2H, J = 7 Hz, aryl), 2.49 (t, 2H, J = 7 Hz, CH₂CO), 2.00-1.02 (m, 10H, 5CH₂), 0.87 (t, 3H, J = 7 Hz, CH₃).

To an ice-cooled, stirred mixture of sodium hydride (74 mmol, free from oil) in DMF (150 mL), a solution of compound 11 (71.7 mmol) in DMF (75 mL) was added dropwise over 20 min. After the mixture was stirred for 20 min, it was warmed to room temperature and stirred an additional 90 min. The mixture was again cooled in ice, and iodomethane (4.7 mL) in THF (75 mL) was added dropwise over 30 min. After careful addition of 50 mL of water to the mixture, it was concentrated by rotary evaporation to give a residue that was taken up in DCM, washed consecutively with water and brine, and dried (MgSO₄). After the mixture was filtered and concentrated, the yellow oil obtained was flash-chromatographed through silica gel and eluted with DCM-methanol (99:1) to give pure N-methyl-N-(4'-pyridyl)octanamide in 73% yield as a pale yellow oil: ¹H NMR (CDCl₃) δ 8.78 (d, 2H, J = 7 Hz, aryl), 7.30 (d, 2H, J = 7 Hz, aryl), 3.38 (s, 3H, NCH₃), 2.32 (t, 2H, J = 7 Hz, CH₂CO), 2.00-1.09 (m, 10H, 5CH₂), 0.89 (t, 3H, J = 7 Hz, CH₃).

To an ice-cooled, stirred mixture of N-methyl-N-(4'-pyridyl)octanamide (19 mmol) in THF (10 mL), 1 M borane in THF (51 mL) was added dropwise over 30 min. After the mixture was stirred for 1 h, it was warmed to room temperature and stirred an additional hour. The mixture

was again cooled in ice after which 20 mL of 37% HCl was carefully added over 30 min. The mixture was then basified to pH>12 with 5% sodium hydroxide and extracted well with DCM. The combined extracts were dried over MgSO₄, filtered, and concentrated to give an oil that was flash-chromatographed through silica gel and eluted with DCM-methanol (99:1) to provide pure compound 12 in 74% yield as a colorless oil (see Table 3): ¹H NMR (CDCl₃) δ 8.29 (dd, 2H, J = 7 Hz and 1 Hz, aryl), 6.50 (dd, 2H, J = 7 Hz and 1 Hz, aryl), 3.32 (t, 2H, J = 7 Hz, CH₂N), 2.93 (s, 3H, NCH₃), 1.90-1.10 (m, 12H, 6CH₂), 0.89 (t, 3H, J = 7 Hz, CH₃).

PREPARATION OF 4-[N-METHYL-N-(4',7'-DIOXANONYL-1')]AMINO-PYRIDINE (15)

A mixture of 4-aminopyridine (80.5 mmol) and n-butyl formate (20 mL) was stirred at 100 °C for 3 days and the mixture was concentrated under reduced pressure to give a white solid. The solid was washed well with DCM to give 4-(N-formyl)aminopyridine (13) in 51% yield and sufficient purity for subsequent use: mp 68-74 °C; ¹H NMR (CDCl₃) δ 8.76 (br s, 1H, CHO), 8.26 (d, 2H, J = 7 Hz, aryl), 6.92 (d, 2H, J = 7 Hz, aryl).

To an ice-cooled, stirred mixture of sodium hydride (41 mmol, free from oil) in DMF (100 mL), a solution of compound 13 (37 mmol) in DMF (50 mL) was added dropwise over 10 min. After the mixture was stirred for 30 min, it was warmed to room temperature and stirred an additional hour. The mixture was again cooled in ice and 4,7-dioxa-1-nonyl mesylate (39 mmol) in DMF (25 mL) was added dropwise over 30 min. After the mixture was stirred overnight, 50 mL of water was carefully added to the mixture and it was concentrated by rotary evaporation to give an oil. The oil was flash-chromatographed through silica gel and eluted with DCM-methanol (99:1) to give pure 4-[N-formyl-N-(4',7'-dioxanonyl-1')]aminopyridine (14) in 55% yield as a pale yellow oil: ¹H NMR (CDCl₃) δ 8.75 (br s, 1H, CHO), 8.54 (dd, 2H, J = 7 Hz and 1 Hz, aryl), 7.16 (dd, 2H, J = 7 Hz and 1 Hz, aryl), 3.97 (t, 2H, J = 7 Hz, CH₂N), 3.71-3.29 (m, 8H, 4CH₂O), 2.11-1.70 (m, 2H, CH₂), 1.21 (t, 3H, J = 7 Hz, CH₃).

Borane reduction of compound 14 as described above for the preparation of compound 12 gave compound 15 in 74% yield as a colorless oil (see Table 3): ¹H NMR (CDCl₃) δ 8.14 (dd, 2H, J = 7 Hz and 1 Hz, aryl), 6.50 (dd, 2H, J = 7 Hz and 1 Hz, aryl), 3.82-3.31 (m, 10H, 4CH₂O, CH₂N), 2.06-1.61 (m, 2H, CH₂), 1.21 (t, 3H, J = 7 Hz, CH₃).

PREPARATION OF N-(OCTYL-1'-)PYRIDINE-4-THIONE (18)

To an ice-cooled, stirred mixture of 4,4'-dipyridyl disulfide (Aldrithiol-4™, 3.8 mmol) in nitromethane (25 mL), n-octyl triflate (7.5 mmol, prepared from n-octyl alcohol and triflic anhydride) in 15 mL of nitromethane was added and the mixture was stirred overnight. The mixture was then concentrated under reduced pressure to give a white solid that was washed well with ethyl ether to give pure diquaternary salt 17 in 91% yield as a white solid.

Compound 17 (17.7 mmol) was dissolved in ethanol (350 mL), and sodium sulfide hydrate (25.5 g) in 150 mL of water was added. After the mixture was stirred for 5 h, it was saturated with NaCl and extracted well with DCM. The combined extracts were dried (MgSO₄), filtered, and concentrated to give an orange solid. The solid was flash-chromatographed through silica gel and eluted with DCM to give pure N-(octyl-1'-)pyridine-4-thione (18) in 93% yield as an orange solid (see Table 3): ¹H NMR (CDCl₃) δ 7.49-7.12 (m, 4H, vinylic), 3.93 (t, 2H, J = 7 Hz, CH₂N), 1.98-1.00 (m, 12H, 6CH₂), 0.89 (t, 3H, J = 7 Hz, CH₃).

PREPARATION OF 3-METHYL-1-(OCTYL-1'-)IMIDAZOLINE-2-THIONE (22)

To an ice-cooled, stirred mixture of 2-mercapto-1-methylimidazole (95.5 mmol) and triethylamine (13.3 mL) in DCM (50 mL), *p*-(chloro)methylphenyl acetate¹³ (105 mmol) in 25 mL of DCM was added. After the mixture was stirred for 30 min, it was warmed to room temperature and stirred an additional 20 h. The mixture was then washed consecutively with water and brine, dried over MgSO₄, filtered, and concentrated to give an oil. The oil was flash-chromatographed through silica gel and eluted with DCM to give pure compound 20 in 23% yield as a yellow oil: ¹H NMR (CDCl₃) δ 7.30-6.91 (m, 6H, aryl), 4.19 (s, 2H, CH₂S), 3.26 (s, 3H, NCH₃), 2.28 (s, 3H, CH₃CO).

To an ice-cooled, stirred mixture of compound 20 (22 mmol) in nitromethane (50 mL), n-octyl triflate (23 mmol) in 50 mL of nitromethane was added dropwise over 30 min. The mixture was warmed to room temperature and stirred for 18 h, and concentrated under reduced pressure to give quaternary salt 21 in sufficient purity for subsequent use: ¹H NMR (DMSO-d₆) δ 8.02 (br s, 2H, aryl), 7.42-7.02 (m, 4H, aryl), 4.45 (s, 2H, CH₂S), 3.99 (t, 2H, J = 7 Hz, CH₂N), 3.78 (s, 3H, NCH₃), 2.31 (s, 3H, CH₃CO), 1.94-1.08 (m, 12H, 6CH₂), 0.90 (t, 3H, J = 7 Hz, CH₃).

A mixture of salt 21 (22 mmol) in 200 mL of 5% sodium hydroxide was stirred for 4 h. The mixture was then extracted with DCM (3 x 200 mL). The combined extracts were washed

consecutively with 10% HCl and brine, and dried over MgSO₄. After the mixture was filtered and concentrated, a crude solid was obtained that was flash-chromatographed through silica gel and eluted with DCM to provide pure 3-methyl-1-(octyl-1'-)imidazoline-2-thione (22) in 60% yield as a yellow oil: λ_{max} (CH₃CN) 354 nm; ¹H NMR (CDCl₃) δ 6.70 (br s, 2H, aryl), 4.03 (t, 2H, J = 8 Hz, CH₂N), 3.59 (s, 3H, NCH₃), 1.95-1.02 (m, 12H, 6CH₂), 0.88 (t, 3H, J = 7 Hz, CH₃).

KINETIC EVALUATION OF 3-METHYL-1-(OCTYL-1'-)IMIDAZOLINE-2-THIONE (22)

A stock solution of compound 22 at a concentration of 2.6×10^{-5} M in DME was prepared. To this solution in a 1-cm cuvette thermostatted at 37 °C in the spectrophotometer, sufficient CEMS was added to bring the concentration of CEMS to 0.43 M. The mixture was briefly agitated to mix the reactants, and timing was started. The disappearance of compound 22 was monitored by taking UV absorbance readings at 354 nm over the next 1.7 h, after which time the concentration of compound 22 had fallen to 36% of its starting concentration. Thus;

$$T = 1/k = t_{\text{fract rem}} / \ln(1/\text{fract. rem.}) = 5650 \text{ s} / \ln(1/0.36) = 5.5 \times 10^3 \text{ s}$$

$$k_1 = 1/T = 1.8 \times 10^{-4} \text{ s}^{-1}$$

$$t_{1/2} = \ln 2 \times T = 3.8 \times 10^3 \text{ s} = 1.1 \text{ h}$$

$$k_2 = k_1 / [\text{CEMS}] = 1.8 \times 10^{-4} \text{ s}^{-1} / 0.43 \text{ M} = 4.2 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$$

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