Anionic- and Lipophilic-Mediated Surface Binding Inhibitors of Human Leukocyte Elastase

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We report the synthesis of a series of diphenylmethane-based oligomers containing anionic and lipophilic functionalities that are potent inhibitors of human leukocyte elastase (HLE). The enzyme inhibition is regulated by the size of the oligomer, as well as, the number of charges. Lipophilicity is an important element in determining potency and specificity against other basic enzymes. Compounds whose scaffolds contain three phenoxyacetic acid groups and three alkyl ethers are competitive and specific inhibitors of HLE with $K_i = 20$ nM. The mechanism of action of this class of compounds is believed to involve multidendate interactions with the surface of HLE near the active site which prevents substrate access to the catalytic site.

Introduction

Human leukocyte elastase (HLE, EC 3.4.21.37) is a highly basic, 30 kDa, serine protease contained within azurophilic granules of polymorphonuclear leukocytes.[1] The earliest recognized role of HLE was in the degradation of phagocytized proteins.[2,3] More recently, it has been suggested that HLE may be externalized in small quantities and bound to surface "receptors" on leukocytes during migration from the surface of the endothelium into extravascular connective tissues.[4,5] Upon acute activation, it may also be exocytized along with other granular contents. An imbalance between free HLE and its natural inhibitors, α_1-proteinase and α_2-macroglobulin in plasma[6] and secretory leukocyte protease inhibitor on mucosal surfaces,[7] has been suggested as a causative factor in emphysema,[8] chronic bronchitis,[9] acute respiratory distress syndrome,[9] cystic fibrosis,[10] and arthritis.[11]

Several types of chemical strategies have emerged to identify agents which inhibit HLE.[12] Active site directed inhibitors have been constructed with reactive functionalities appended onto substrate peptides as a method to control the proteolytic process. Examples include the transition state analog inhibitors such as peptidyl trifluoromethyl ketones[13] (e.g. **1**, ICI 200,355,[14] and analogs[15]), peptidyl ketobenzoxazoles,[16] peptidyl aldehydes,[17] peptidyl boronic acid esters,[18] peptidyl α-keto amides,[19] and peptidyl α,α-difluoro-β-keto amides and ketones.[20] HLE inactivation through active site acylation has been accomplished by β-lactams,[21] isoxazolines,[22] saccharins,[23] and benzisothiazolones.[24] Also reported as inhibitors of HLE are the chemical classes of phenylbutyrates,[25] oligonucleotides,[26] polyanionic chelators,[27] heparin derivatives,[28] peptidyl carbamates,[29] sulfonamidobenzoylglycines,[30] isocoumarins,[31] and biphenyldisulfonic acid copolymer.[32]

We have previously reported that certain linear aromatic anionic polymers (e.g. **2**, RG 13577, M_w = 1000−5000) can modulate the activity of biological systems via charge−charge interactions of the polymer with basic residues in proteins and produce inhibitory effects,[33] including the inhibition of HLE (IC_{50} = 40−130 ng/mL).[34] It was our belief that these polymers share a common bioactive motif. That is, a certain fragment length, composed of repeating monomer units containing anionic charges, is responsible for the observed biological activity. As a consequence, the remainder of the polymer backbone does not contribute to the biological efficacy. With this premise in mind and coupled with our ability to assemble discrete anionic oligomers contained in a polymeric mixture,[35] we undertook the task of establishing whether small, well-defined anionic oligomeric compounds could also provide potent charge-mediated inhibition of HLE.[36] From the diversity of our polymer scaffolds[33a] we decided that the manifold for carrying the anionic charges should be constructed of phenyl rings linked in a *para* relationship by methylene groups, as illustrated by diphenylmethane-based oligomer **3a**,**b**.

In addition to its pathophysiological significance,[8−11] HLE was chosen as a target for this study due to its unique molecular surface topology. The high basicity of HLE is due to 18 arginine residues on its surface which are balanced by only 6 acidic residues. Approximately 40% of the total amino acid side chains are hydrophobic and accessible to bulk water molecules.[37] Four Arg residues, Arg 36, Arg 147, Arg 177, and Arg 217, are located adjacent to the active site, span a total of approximately 40 Å, and are separated by hydrophobic amino acids (e.g. Leu 35, Phe 41, Val 62, Val 99, Leu 143, Ile 151, Phe 215, Val 216). Thus, HLE represents an attractive chemical landscape from which to explore the hypothesis that potent and specific enzyme inhibition is attainable with a synthetic molecule bound, not in, but rather, adjacent to the active site by a combination of anionic and lipophilic interactions.[38,39] For a compound to be an effective and specific inhibitor of HLE, it should be designed to chemically

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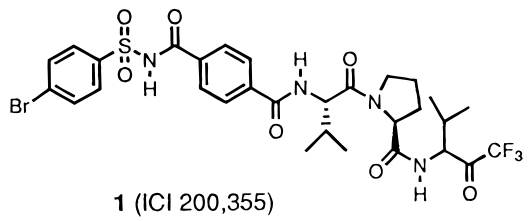
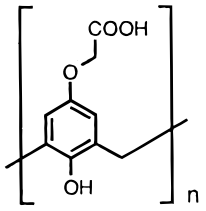
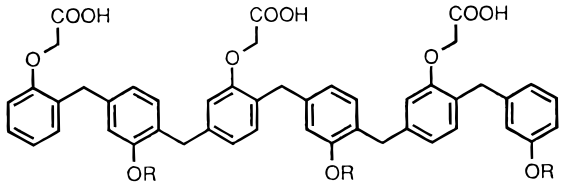


1 (ICI 200,355)



2 (RG 13577)



3a: R = CH_2COOH
3b: R = alkyl

match, as closely as possible, the electronic features near the active site. That is, the target molecule should possess appropriately positioned carboxylic acids to bind with the arginine's guanidinium groups and also hydrophobic groups to interact at the hydrophobic domains. Other important features would include a flexible backbone that could conformationally adapt to the surface topology and be resistant to proteolytic degradation. It would be anticipated that compounds of this description could tightly adhere to the surface of HLE via multiple interactions, sterically prevent access of the peptide substrate to the catalytic site, and, thereby, halt the proteolytic process. Tetramer **4**, pentamer **5**, and hexamers **6** and **7a**−**f** were designed to fulfill these criteria. Thus, the construction and *in vitro* inhibition of these compounds is the subject of this report.

Chemistry

The retrosynthetic strategy applied to assemble hexamer **6** is outlined in Scheme 1. Palladium-mediated coupling of dimer benzylic zinc **8** with tetramer aryl iodide **9** would provide the hexamer scaffold. Tetramer **9** would be assembled from the coupling of dimers **8** and **10**. Monomers **11** and **12** would serve as precursors for both dimers **8** and **10**.

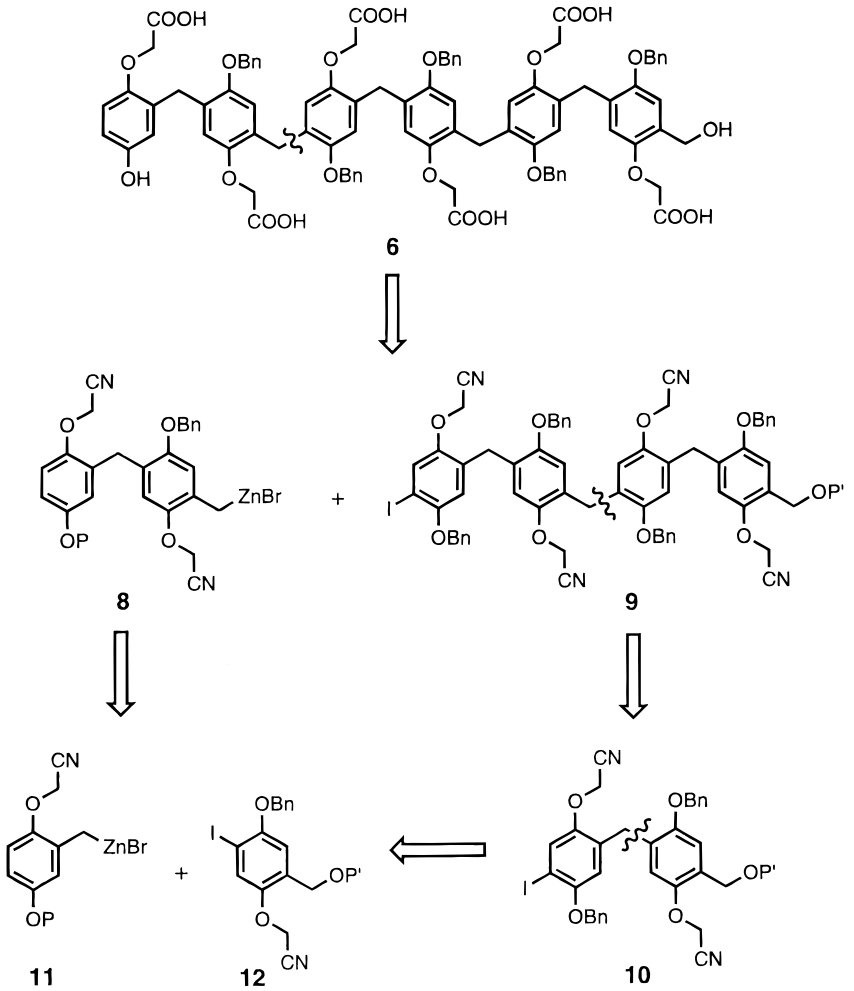
The synthesis of tetramer **4** is described in Scheme 2. Treatment of 2,5-dihydroxybenzaldehyde (**13**) with benzoic anhydride followed by monoester hydrolysis by K_2CO_3 in methanol produced **14**. Alkylation of **14** with bromoacetonitrile furnished phenoxyacetonitrile **15** in 73% overall yield. Sodium borohydride reduction of aldehyde **15**, protection of the alcohol as the *tert*-butyldiphenylsilyl (TBDPS) ether and benzoate ester hydrolysis using NaOMe at low temperature gave sequentially **16**, **17,** and **18** in 92% overall yield from **15**. Iodination[40] (I_2, morpholine) of phenol **18** provided **19** as the sole regioisomer in 84% yield, and **19** was converted to benzyl phenyl ether **20** using NaH and benzyl bromide. Alcohol **16** was transformed to benzyl bromide **21** in 65% isolated yield with NBS and triphenylphosphine. Treatment of **21** with freshly activated Zn powder[41] followed by aryl iodide **20** and catalytic $[(Ph)_3P]_4Pd$ provided dimer **22** in 88% yield. Processing of **22** to dimers **25** and **27** was accomplished with similar reaction conditions as those described above. That is, **22** was hydrolyzed (NaOMe), iodinated (I_2, morpholine), and alkylated (NaH, benzyl bromide) to give sequentially **23**, **24**, and **25** in 80% overall yield. Also, **22** was treated with tetra-*n*-butylammonium fluoride (TBAF), and the resulting alcohol, **26**, was converted (NBS, Ph_3P) to benzyl bromide **27** in 81% overall yield. The conversion of the dimer aryl iodide, **25**, and the benzylic zinc species[41] derived from dimer **27** into tetramer **28** was accomplished by Pd(0)-mediated coupling in 89% yield. Saponification of the four nitriles into carboxylic acids, removal of the benzoate ester, and silyl ether hydrolysis of **28** by the action of KOH in hot methanol and THF provided **4** in 45% yield.

The preparation of pentamer **5** is outlined in Scheme 3. Tetramer **28** was transformed into an aryl iodide **31** in 76% overall yield by exposure to NaOMe at low temperature to provide phenol **29**, I_2 and morpholine[40] to give **30** and subsequently NaH and benzyl bromide. Coupling of the benzylic zinc intermediate derived from **21** with **31**, mediated by Pd(0), furnished pentamer **32** in 79% yield. Hot KOH in methanol saponified the five nitriles to carboxylic acids, benzoate ester to phenol, and silyl ether to hydroxmethyl and gave **5** in 53% yield.

The construction of hexamer **6** is shown in Scheme 4. The hexamer scaffold, **33**, was obtained in 55% yield from the coupling of the benzylic zinc derivative of **27**[41] and tetramer **31** mediated by Pd(0). Conversion of **33** to target molecule **6** was accomplished by the action of KOH in hot methanol and THF. The hydrolysis of the six nitriles to carboxylic acids, the benzoate ester to phenol, and the silyl ether to hydroxy methyl occurred in 88% yield.

The construction of hexamer **7f**, whose scaffold is composed of three phenoxyacetic acid units alternating with three cyclohexylmethyl aryl ethers, is described in Scheme 5. Conversion of phenol **14** to aryl iodide **37** was readily accomplished in four steps and in 22% overall yield by treatment of **14** with NaH and MEM chloride (**34**), reduction of the aldehyde with $NaBH_4$, protection of the benzylic alcohol as a TBDPS silyl ether, benzoate ester hydrolysis (NaOMe) (**35**), iodination (*N*-iodosuccinamide/morpholine[40]) (**36**), and alkylation of the phenol with methyl iodide (**37**). Dimer **38** was obtained in 73% yield from the Pd(0)-mediated coupling of the benzylic zinc species from **21**[41] and aryl iodide **37**. The TBDPS protecting group of **38** was removed (TBAF) to give alcohol **39**, which was transformed (NBS/Ph_3P) to benzyl bromide **40** in 48% yield. In addition, **38** was converted to aryl iodide **43** by sequential treatment with NaOMe (**41**, 86%), I_2 and morpholine (**42**, 85%), and NaH and MeI (99%). Tetramer **44** was produced from aryl iodide **43** and the benzylic zinc intermediate from **40** with the use of Pd(0) in 28% yield. The conversion of **44** to aryl iodide **47** was realized using

Scheme 1. Retrosynthetic Strategy for the Synthesis of **6**



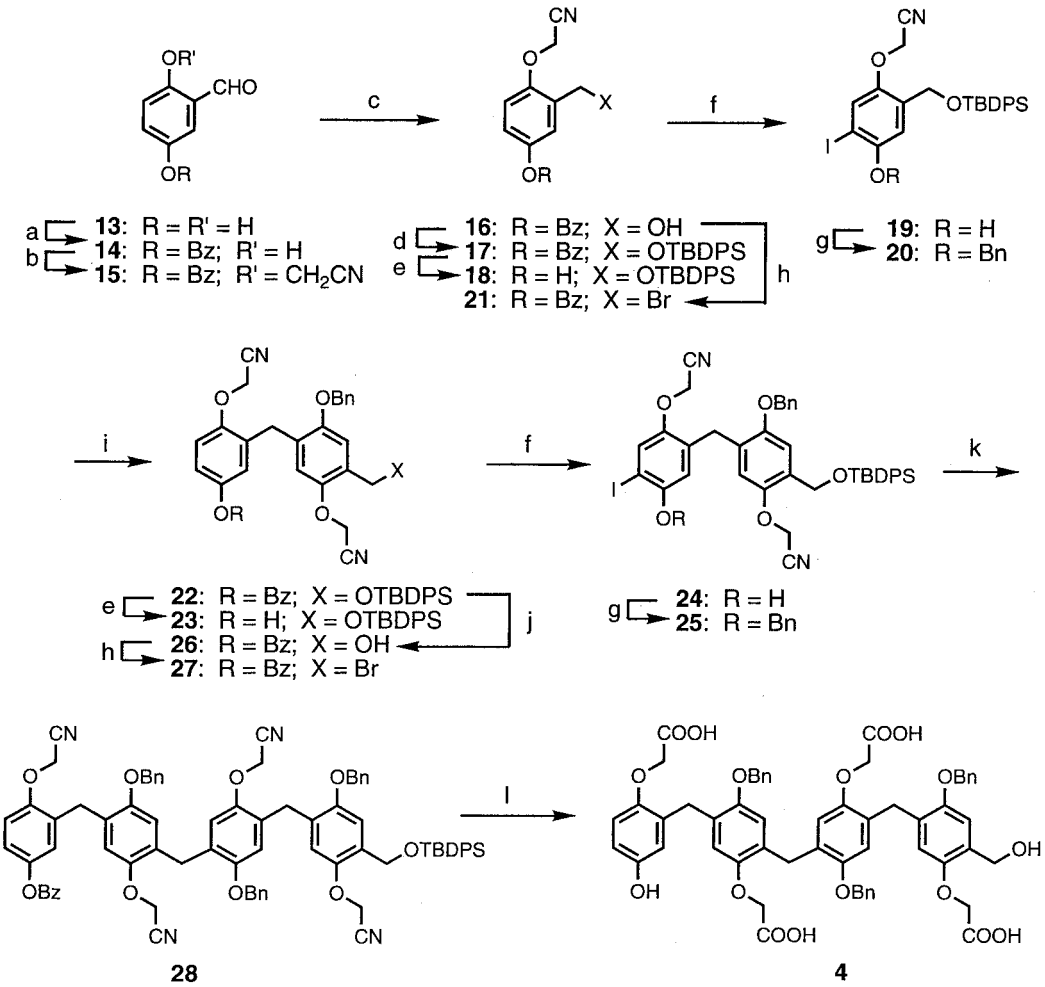
reaction conditions similar to those described for the preparation of **43**. That is, benzoate hydrolysis (NaOMe) gave **45**, iodination (I_2/morpholine) provided **46**, and alkylation of the phenol (NaH/MeI) furnished **47** in 85% overall yield. Coupling of the benzylic zinc derivative of **40** and **47**, using Pd(0) as catalyst, provided hexamer **48** in 68% yield. Conversion of the TBDPS ether in **48** to a methyl group was accomplished in 43% overall yield by exposure of **48** to TBAF to give alcohol **49**, bromination with NBS and triphenylphosphine to furnish **50**, and tri-*n*-butyltin hydride/AIBN reduction to provide **51**. Benzoate hydrolysis (NaOMe) of **51** produced phenol **52**, which was methylated (NaH, MeI) and gave **53** in 73% yield for the two steps. Removal of the three MEM protecting groups from the phenols in **53** was accomplished in 95% yield with PPTS and furnished **54**. Alkylation of the phenolic hydroxylic groups in **54** with cyclohexylmethyl bromide (K_2CO_3, DMF) gave **55** in 44% yield. The three nitriles in **55** were hydrolyzed with KOH and produced triacid **7f** in 56% yield.

Results and Discussion

Our first program goal was to establish the mechanism by which these compounds inhibit HLE. Figure 1 shows the inhibition of **6** vs the substrate pGlu-Pro-Val-pNA (S-2484) at pH 7.0 with 120 mM NaCl in HEPES buffer. Kinetic analysis indicates that **6** is a competitive inhibitor (K_i = 190 nM) since it increases the K_m values without affecting V_m.

The second objective was to discern the effect of the oligomer scaffold size on the *in vitro* inhibition of HLE. Examples of other anionic-mediated inhibitors that are size sensitive include sulfated saccharides as antiproliferative agents[42] and the platelet aggregation inhibitor aurintricarboxylic acid polymer.[43] These two classes of compounds have demonstrated that a minimum molecular size is required for biological activity, and once this has been achieved, further increases in chain size do not significantly improve efficacy. Table 1 summarizes the relative abilities of compounds **1** and **4**−**6** to competitively inhibit HLE *in vitro* vs the substrate Suc-Ala-Ala-Pro-Phe-pNA in HEPES buffer containing 200 mM NaCl at pH 7.5. Tetramer **4** was substantially less potent than pentamer **5** and hexamer **6** (K_i = 8300, 130, and 120 nM, respectively). No improvement in biological efficacy was observed with octamers or hexadecamers from a similar class of compounds (data not shown). In addition, no inhibitory potency was observed with the phenoxyacetonitrile precursors of these compounds (i.e. **32**). Thus, a minimum scaffold size consisting of five phenoxyacetic acid units is needed to effectively span the relevant surface of HLE and bind with the guanidinium groups to retard the proteolytic activity. It is worthwhile to note that calix[8]arene **56**,[44] despite an appropriate number of phenoxyacetic acids, displayed modest inhibitory activity against HLE with K_i = 1300 nM (Table 1). The cyclic array of charges of **56** is capable of only spanning approximately the same

Scheme 2. Synthesis of Tetramer **4**[a]



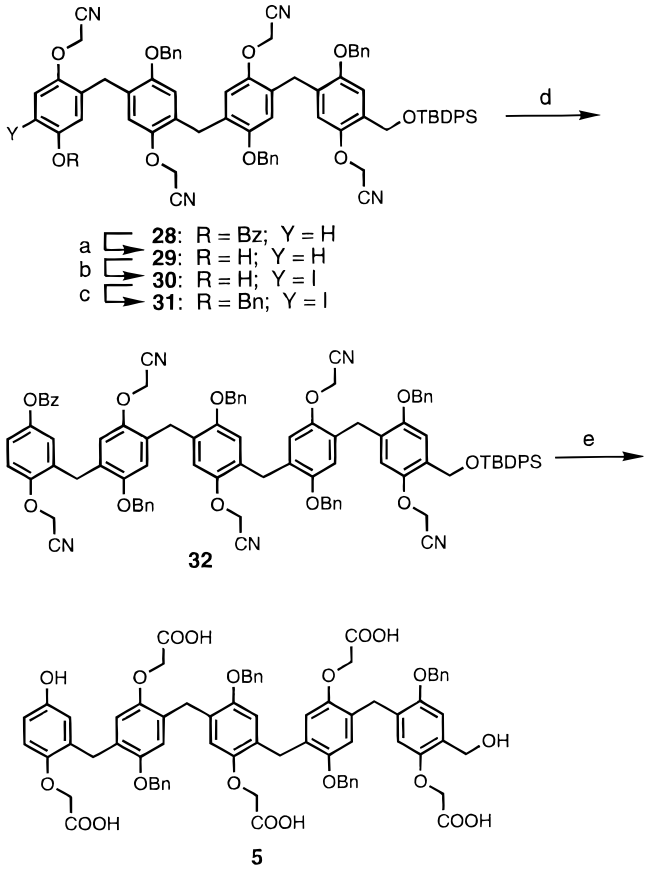
[a] Abbreviations: Bn = CH_2Ph, Bz = C(O)Ph, TBDPS = Si(*t*-Bu)Ph_2. Reagents: (a) (1) $(PhCO)_2O/Et_3N/CH_2Cl_2$, (2) K_2CO_3/MeOH/25 °C; (b) $BrCH_2CN/K_2CO_3/CH_3CN$/80 °C; (c) $NaBH_4$/THF/diglyme/−70 °C; (d) *t*-Bu$(Ph)_2SiCl$/imidazole/DMAP/CH_2Cl_2; (e) NaOMe/MeOH/−50 to −30 °C; (f) I_2/morpholine/CH_2Cl_2; (g) NaH/$PhCH_2Br$/DMPU/THF; (h) NBS/$(Ph)_3P$/THF; (i) **21**/Zn/THF/0−5 °C then **20**/$[(Ph)_3P]_4Pd$/THF/60 °C; (j) $(n\text{-Bu})_4NF/MeCO_2H$/THF/25 °C; (k) **27**/Zn/THF/0−5 °C then **25**/$[(Ph)_3P]_4Pd$/THF/69 °C; (l) KOH/MeOH/THF/reflux.

distance as a tetramer, such as **4**. Also, **56** may not be properly aligned or flexible enough to productively bind to the enzyme surface. Taken together, these results are consistent, although not conclusive, with our proposed mechanism of HLE inhibition by electostatic and hydrophobic interactions with the enzyme surface.

The third goal of the program was the identification of target molecules which would carry fewer charged groups and include other hydrophobic moieties. The rationale for pursuing this strategy was the belief that compounds devoid of noninteracting carboxylic acids and with an array of anionic charge and lipophilicity that better matched the cationic charge and lipophilic surface character of HLE might prove to be superior inhibitory agents. Hydrophobic amino acids adjacent to the Arg residues near the active site are exposed to bulk solvent, and therefore, lipophilic groups attached to the oligomer scaffold would be expected to interact at these sites and improve efficacy. As an initial venture that was guided by the requirements of the surface topology of HLE, analogs were prepared wherein alternating acetic acids on the backbone of **6** were replaced with ethers of varying lipophilicity (i.e. **3b**). In addition, for ease of synthesis, methyl ethers were substituted for the benzyl ethers of **6**. As a result, the scaffolds consisted of three phenoxyacetic acid units linked with three phenols (**7a**) or three phenolic ethers (**7b**−**f**). The competitive *in vitro* inhibitory activity of **7a**−**f** against HLE is shown in Table 2. Compounds **7a** and **7b** containing alternating phenoxyacetic acids with phenol and phenolic MEM ethers, respectively, were weakly inhibitory (K_i = 1000−1300 nM). *In vitro* potency was improved for those compounds endowed with additional lipophilic character. For example, the benzyl, **7c**, and phenylpropyl, **7d**, derivatives showed a 5−10-fold enhancement in activity with K_i values of 100−200 nM. Compounds **7e** and **7f**, containing *n*-hexyl and cyclohexylmethyl phenyl ethers, respectively, were the most potent with K_i values of 20 nM. The alkyl ethers analogs were somewhat more potent than the alkyl aryl ethers[45] as seen in **7e** and **7f** vs **7c** and **7d**. Thus, increases in inhibitory activity against HLE by **7e** and **7f** were secured, in addition to the methyl to benzyl ether exchange, by deleting three nonproductively binding acetic acid groups from **6** and replacing them with aliphatic ethers. A 65-fold improvement in *in vitro* potency was observed between **7a** and **7f** and supports the importance of the hydrophobic interactions for these compounds. Molecules with this motif apparently provide a better match with the electronic properties on the surface of HLE, and this improved binding is reflected in enhanced biological activity.[46,47]

In addition to the evaluation of **4**−**6** and **7a**−**f** against HLE, the compounds were evaluated *in vitro* for their

Scheme 3. Synthesis of Pentamer **5**[a]



[a] Abbreviations: Bn = CH_2Ph, Bz = C(O)Ph, TBDPS = Si(*t*-Bu)Ph_2. Reagents: (a) NaOMe/MeOH/−50 to −22 °C; (b) I_2/morpholine/CH_2Cl_2; (c) NaH/$PhCH_2Br$/DMPU/THF; (d) **21**/Zn/THF/0−5 °C then **31**/$[(Ph)_3P]_4Pd$/THF/60 °C; (e) KOH/MeOH/THF/reflux.

inhibition against other basic enzymes. Our goal was to determine if the geometric array of anionic charges on the oligomers was specific only for the positive charges on HLE. Cathepsin G, a serine protease also found in human neutrophils, is more basic than HLE. This enzyme has been shown to be inhibited by heparin fragments[28a] and a biphenyldisulfonic acid copolymer.[32] Thrombin, a serine protease, contains a heparin binding domain on its surface and can interact with heparin.[48] The selectivity of **4**−**6** and **7a,c**−**f** for HLE versus cathepsin G and thrombin was between 4 and >750 as shown in Table 3. This data suggested that the molecular size or electronic or topological properties of these oligomers do not provide the satisfactory matches that are required for productive binding to these other basic enzymes. Compound **7b**, containing three MEM ethers, was a more potent inhibitor of thrombin (K_i = 240 nM) than HLE (K_i = 1 μM).

To further explore the issue of biological specificity, hexamer **6** was evaluated for its ability to inhibit HLE *in vitro* in the presence of physiological concentrations of various purified human plasma proteins. Albumin, γ-globulin, fibrinogen, and fibronectin were individually incubated with **6**. The rate of HLE hydrolysis of the substrate MeO-Suc-Ala-Pro-Val-pNA by **6** was not changed when the plasma protein was present in reaction medium. These results suggest that **6** has a higher binding affinity for HLE than albumin, γ-globulin, fibrinogen, and fibronectin.

Conclusions

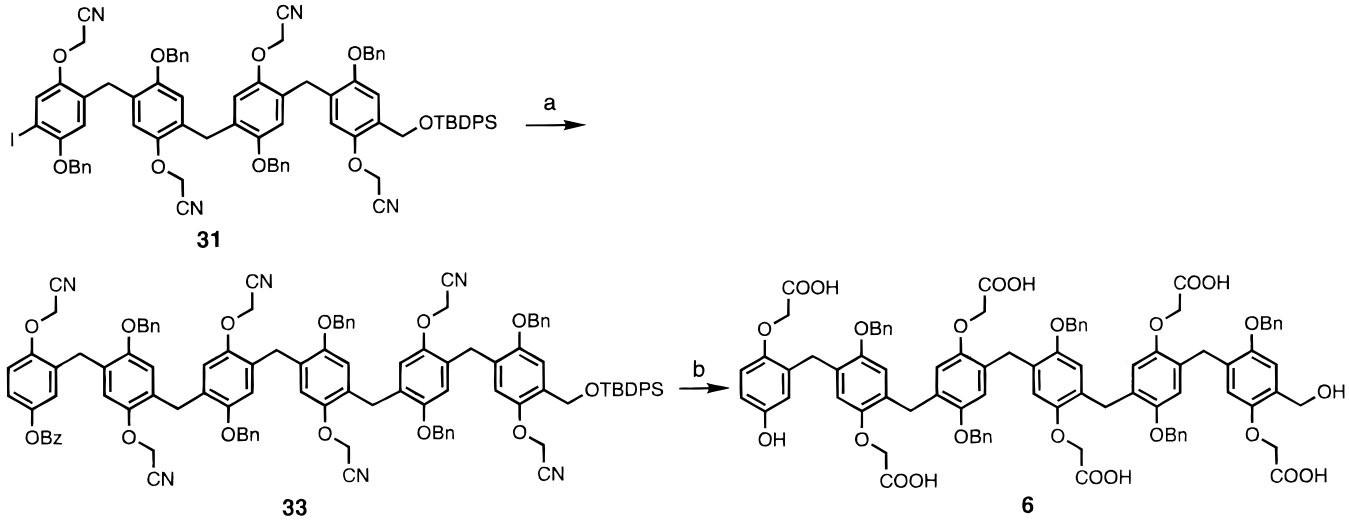
We have shown that competitive inhibition of HLE can be effectively accomplished by the ability of an anionic and hydrophobic-based oligomer to bind to the enzyme via charge−charge interactions with the basic amino acid residues and in conjuction with hydrophobic interactions on the surface. We believe the inhibition is occurring as a result of multiple interactions of the oligomer with the basic residues that are adjacent to the active site and with surrounding hydrophobic residues.[49] Consequently, the substrate is denied access to the enzyme. This type of HLE inhibition requires a minimum size of the oligomer scaffold as seen by the activity of **4** vs **5**. Furthermore, an increase in the number of charges does not provide additional inhibitory potency as observed with **5** vs **6** and **56**. Non-enzyme-interacting anionic charges can be deleted from the oligomer manifold and potency retained. Hydrophobic interactions with the enzyme surface were realized with aliphatic ethers appended to the oligomer and produced improvements in HLE inhibition as shown with **7a** vs **7f**. Moreover, specificity versus other proteins and basic enzymes was obtained through the construction of oligomers that are endowed with functional groups and a charge distribution pattern that are complimentary to the surface of HLE (compare **7b** with **7f**). Thus, the surface topology and electronic properties of HLE have been shown to be an exploitable physiological feature from which potent and specific inhibitors can be designed.

Further application of this principle can be applied to any protein (enzyme or receptor) whose solvent accessable domains are composed of charged and hydrophobic amino acid residues. These regions, if bound to a synthetic inhibitor, should be capable of producing a biologically modulating affect by sterically preventing protein−ligand or protein−protein interactions. It is also our belief that every protein's surface is a unique composition of charged, hydrophobic, and hydrophilic domains. Biological specificity for a single protein can be achieved with a compound with electronically complimentary functionality attached to a scaffold capable of adapting to the topology of the protein surface. Therefore, as an aid to future drug discovery efforts compounds with protein-surface-matched electronics and topology can be considered for synthesis.

Experimental Procedures

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorected. NMR spectra were measured on a Varian EM-390 or Bruker AC-F 300 NMR spectrometer using tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ) from the tetramethylsilane resonance in the indicated solvent. Mass spectra were run on a Varian MATT 112. TLC analytical separations were conducted with E. Merck silica gel F-254 plates of 0.25 mm thickness and were visualized with UV or I_2. Flash chromatographies were run according to the procedure of Still *et al.* (EM Science Kieselgel 60, 70−230 mesh) or on a Waters Prep LC/System 500 using Prep Pak-500 silica cartridges in the indicated solvent. The commercially available starting materials and anhydrous solvents were used as obtained. All buffer salts and substrates were obtained from Sigma and were of the highest purity avaliable. Human α-thrombin was obtained from Enzyme Research Laboratories (South Bend, Indiana). HLE was purchased from Elastin Products (St. Louis, Mo).

Scheme 4. Synthesis of Hexamer **6**[a]



[a] Abbreviations: Bn = CH_2Ph, Bz = C(O)Ph, TBDPS = Si(*t*-Bu)Ph_2. Reagents: (a) **27**/Zn/THF/0−5 °C then **31**/$[(Ph)_3P]_4Pd$/THF/69 °C; (b) KOH/MeOH/THF/reflux.

2-(Hydroxymethyl)-4-(benzyloxy)-5-[4-[4-[4-[2-[[(hydroxycarbonyl)methyl]oxy]-5-hyroxybenzyl]-3-(benzyloxy)-5-[[(hydroxycarbonyl)methyl]oxy]benzyl]-3-(benzyloxy)-5-[[(hydroxycarbonyl)methyl]oxy]benzyl]-3-(benzyloxy)-5-[[(hydroxycarbonyl)methyl]oxy]benzyl]-phenoxyacetic Acid (5). To a room temperature solution of **32** (0.100 g, 0.065 mmol) in 1 mL of 2:1 EtOH/H_2O was added potassium hydroxide (0.087 g, 1.56 mmol), and the solution was stirred and warmed to 55−60 °C. Dioxane (∼200 μL) was added to obtain complete solubility. The mixture was stirred overnight and cooled to room temperature and the pH adjusted to 1 with 1 N HCl. The resulting cloudy mixture was diluted with ethyl acetate and extracted with half-saturated brine (5×) followed once by brine. After sitting overnight the aqueous layer was filtered to obtain 45 mg (53%) of product. ^{1}H NMR (DMSO-d_6): δ 3.81 (m, 8H), 4.52 (m, 18H), 4.97 (s, 2H), 6.32 (br s, 1H), 6.46 (dd, J = 12, 3 Hz, 1H), 6.62 (m, 2H), 6.70 (d, J = 6 Hz, 2H), 6.83 (s, 1H), 6.88 (s, 1H), 6.92 (s, 1H), 7.05 (br s, 3H), 7.15−7.32 (m, 14H), 7.40 (t, J = 9 Hz, 4H), 7.68 (br s, 1H). IR (KBr, cm^{-1}): 3060 m, 2911 m, 1726 s, 1503 s, 1405 m, 1283 w, 1218 s, 1063 m, 730 w, 696 w. MS (FAB pNBA): m/z 1279 (M + H$^+$). Combustion analysis: C, H.

2-(Hydroxymethyl)-4-(benzyloxy)-5-[4-[4-[4-[4-[2-[[(hydroxycarbonyl)methyl]oxy]-5-hydroxybenzyl]-3-(benzyloxy)-5-[[(hydroxycarbonyl)methyl]oxy]benzyl]-3-(benzyloxy)-5-[[(hydroxycarbonyl)methyl]oxy]benzyl]-3-(benzyloxy)-5-[[(hydroxycarbonyl)methyl]oxy]benzyl]-3-(benzyloxy)-5-[[(hydroxycarbonyl)methyl]oxy]benzyl]-phenoxyacetic Acid (6). A solution of **33** (0.703 g) in 15 mL of 3 M KOH, 4 mL of methanol, and 4 mL of THF was heated to reflux for 72 h, cooled to room temperature, acidified to pH 1 with aqueous HCl, and diluted with THF. The organic layer was washed with brine and dried ($MgSO_4$), and the volatiles were removed *in vacuo*. The residue was precipitated from a hot solution of THF by the addition of ethyl acetate and subsequent cooling and provided 0.539 g (88%) of product. ^{1}H NMR (DMSO-d_6): δ 3.76 (s, 4H), 3.81 (s, 2H), 3.90 (s, 2H), 4.45 (s, 2H), 4.50 (s, 8H), 4.52 (s, 8H), 4.97 (s, 2H), 6.33 (d, J = 2.6 Hz, 1 H), 6.45 (dd, J = 8, 2.6 Hz, 1H), 6.60 (s, 1H), 6.62 (s, 2H), 6.70 (s, 1H), 6.71 (s, 1H), 6.83 (s, 1H), 6.86 (s, 1H), 6.93 (s, 1H), 7.0−7.5 (m, 21H). MS (FAB pNBA): m/z 1184 (M + H). Combustion analysis: C, H.

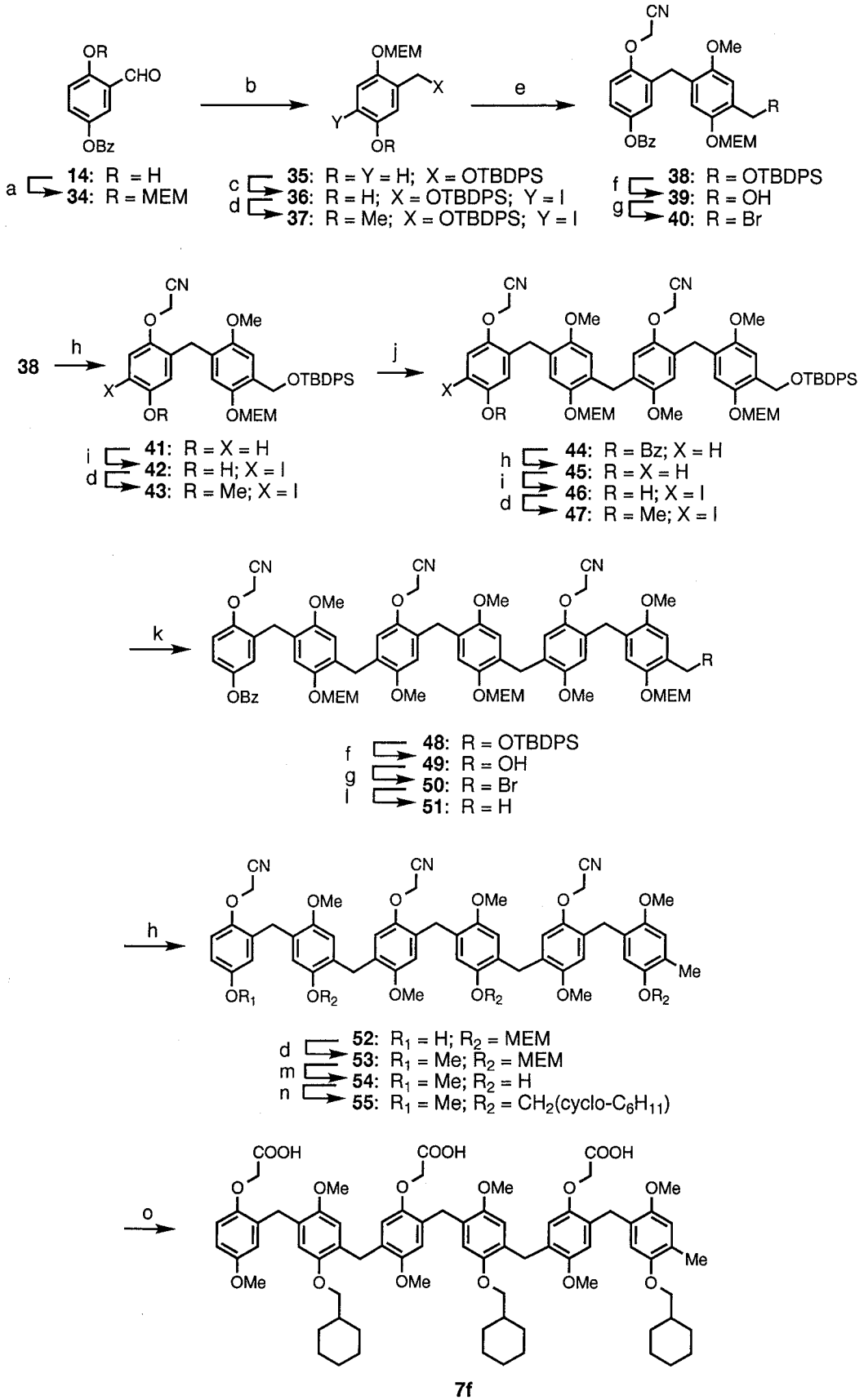
2-[4-[4-[4-[4-(2-Methoxy-4-methyl-5-hydroxybenzyl)-2-methoxy-5-[[(hydroxycarbonyl)methyl]oxy]benzyl]-2-methoxy-5-hydroxybenzyl]-2-methoxy-5-[[(hydroxycarbonyl)methyl]oxy]benzyl]-2-methoxy-5-hydroxybenzyl]-4-methoxyphenoxyacetic Acid (7a). Prepared from **7b** as described for the synthesis of **54** followed by NaOH hydrolyses of the propyl esters (dioxane, ethanol, 1 N NaOH, 25 °C, 18 h). Purification of the material was accomplished by flash chromatography using a solution of 4% formic acid and 33% THF in $CHCl_3$ as the eluent. Concentration *in vacuo* of the product-rich fractions and trituration of the residue with 50% ether in $CHCl_3$ provided the solid product in 13% yield. ^{1}H NMR (DMSO-d_6): δ 6.85−6.35 (m, 13 H), 4.60 (s, 2 H), 4.45 (s, 4 H), 3.85−3.5 (m, 28 H with singlets, 3 H, at 3.71, 3.67, 3.66, 3.63, and 3.61), 2.08 (s, 3 H). MS (FAB-pNBA): m/z 1004 (M$^+$). Combustion analysis: C, H.

2-[4-[4-[4-[4-[2-Methoxy-4-methyl-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-2-methoxy-5-[[(hydroxycarbonyl)methyl]oxy]benzyl]-2-methoxy-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-2-methoxy-5-[[(hydroxycarbonyl)methyl]oxy]benzyl]-2-methoxy-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-4-methoxyphenoxyacetic Acid (7b). Prepared from **53**, as described for the synthesis of **7f**, in 91% yield, mp 65−70 °C. ^{1}H NMR ($CDCl_3$): δ 6.95−6.65 (m, 11 H), 6.4 (m, 2 H), 5.10 (s, 2 H), 5.01 (s, 2 H), 4.95 (s, 2 H), 4.52 (s, 2 H), 4.39 (s, 4 H), 3.95−3.7 (m, 36 H) containing singlets at 3.94, 3.89, 3.83, 3.80, 3.77, 3.76, 3.74), 3.65 (m, 2 H), 3.53 (m, 2 H), 3.41 (m, 2 H), 3.35 (s, 3 H), 3.30 (s, 3 H), 2.19 (s, 3 H). MS (FAB-pNBA): m/z 1257 (M + H), 1280 (M + Na). Combustion analysis: C, H.

2-[4-[4-[4-[2-Methoxy-4-(hydroxymethyl)-5-(benzyloxy)benzyl]-2-methoxy-5-[[(hydroxycarbonyl)methyl]-oxy]benzyl]-2-methoxy-5-(benzyloxy)benzyl]-2-methoxy-5-(benzyloxy)benzyl]-4-hydroxyphenoxyacetic Acid (7c). Prepared in 35% yield by hydrolysis of the nitriles (KOH, ethanol, dioxane, 40−50 °C, 14 h) and purification by flash chromatography using a solution of 15% THF and 2% formic acid in $CHCl_3$ as the eluent. Concentration *in vacuo* of the product-rich fractions and trituration of the residue with ethyl acetate and CH_2Cl_2 gave the solid product, mp 185−9 °C. ^{1}H NMR ($CDCl_3$): δ 7.35−7.2 (m, 15 H), 7.05−6.45 (m, 13 H), 4.87 (s, 2 H), 4.81 (s, 2 H), 4.77 (s, 2 H), 4.66 (s, 2 H), 4.35 (s, 2 H), 4.1−3.65 (m, 29 H containing singlets at 3.98, 3.92, 3.88, 3.79, 3.75, and 3.71). MS (FAB-pNBA): m/z 1265 (M + H). Combustion analysis: C, H.

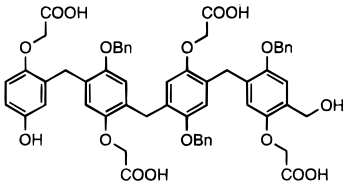
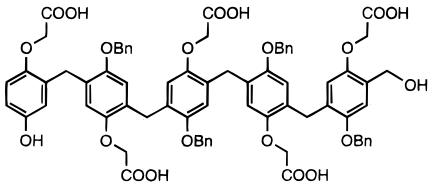
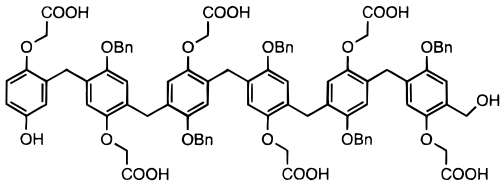
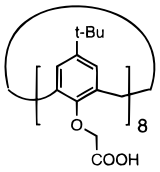
2-[4-[4-[4-[4-[2-Methoxy-4-methyl-5-[(3-phenylpropyl)-oxy]benzyl]-2-methoxy-5-[[(hydroxycarbonyl)methyl]oxy]-benzyl]-2-methoxy-5-[(3-phenylpropyl)oxy]benzyl]-2-methoxy-5-[[(hydroxycarbonyl)methyl]oxy]benzyl]-2-methoxy-5-[(3-phenylpropyl)oxy]benzyl]-4-methoxyphenoxyacetic Acid (7d). Prepared from **54** by alkylation (1-bromo-3-phenylpropane, K_2CO_3, DMF, 85 °C, 18 h), hydrolysis (KOH, dioxane, ethanol, 65 °C, 5 h), and purification (flash chromatography using a solution of 11% THF and 2% formic acid in $CHCl_3$ as the eluent) in 50% overall yield. ^{1}H NMR (DMSO-d_6) δ 7.3−7.05 (m, 15 H), 6.8−6.5 (m, 13 H), 4.59 (s, 2 H), 4.44 (s, 4 H), 3.85−3.50 (m, 34 H), 2.65 (m, 6 H), 2.14

Scheme 5. Synthesis of Hexamer **7f** [a]



[a] Abbreviations: Bz = C(O)Ph; MEM = $CH_2O(CH_2)_2OMe$; TBDPS = Si(*t*-Bu)Ph_2. Reagents: (a) NaH/MEM-Cl/THF/0−25 °C; (b) (1) $NaBH_4$/THF/−60 °C, (2) *t*-Bu$(Ph)_2$SiCl/imidazole/CH_2Cl_2, (3) NaOMe/MeOH/THF/−25 °C; (c) NIS/morpholine/CH_2Cl_2; (d) NaH/MeI/DMPU/THF/0−25 °C; (e) **21**/Zn/THF/0−5 °C then **37**/$[(Ph)_3P]Pd$/THF/65 °C; (f) $(n\text{-Bu})_4NF/CH_3COOH$/THF/25 °C; (g) NBS/$(Ph)_3P$/THF; (h) NaOMe/MeOH/THF/−55 °C; (i) I_2/morpholine/CH_2Cl_2/0−25 °C; (j) **40**/Zn/THF/0−5 °C then **43**/$[(Ph)_3P]Pd$/THF/70 °C; (k) **40**/Zn/THF/0−5 °C then **47**/$[(Ph)_3P]Pd$/THF/70−80 °C; (l) $(n\text{-Bu})_3SnH$/AIBN/toluene/reflux; (m) PPTS/$(Me)_2CO/(Me)_2CHOH$/65 °C/3 days; (n) $(c\text{-}C_6H_{11})CH_2Br/K_2CO_3$/DMF/85 °C; (o) KOH/EtOH/dioxane/65 °C/18 h.

Table 1. *In Vitro* Inhibition of HLE by **1**, **4**−**6**, and **56**

Structure	Compound	Formula[a]	HLE Inhib.[b] Ki (nM)
ICI 200,355	**1**		4[c]
	**4**	$C_{57}H_{52}O_{17}\cdot 2H_2O$	8300
	**5**	$C_{73}H_{66}O_{21}\cdot H_2O$	130
	**6**	$C_{89}H_{80}O_{25}\cdot 2H_2O$	120
	**56**		1300

[a] All compounds exhibited satisfactory spectral data consistent with their structures. Analytical results are within ± 0.4% of the theoretical value. [b] Inhibition of HLE at pH 7.5 in HEPES buffer containing 200 mM NaCl with the substrate Suc-Ala-Ala-Pro-pNA. See the Experimental Section for details. [c] Twenty minute incubation of HLE and test compound.

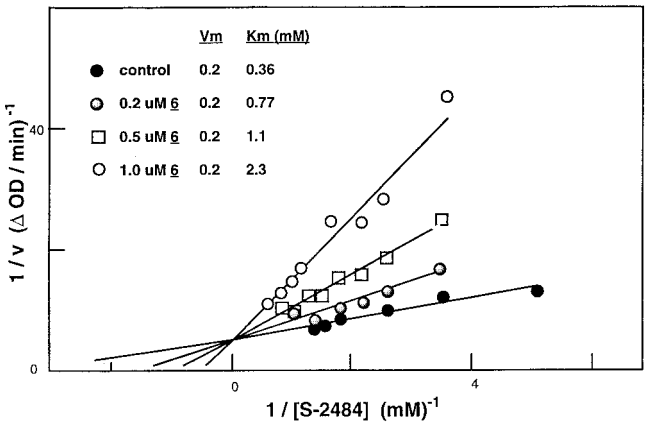
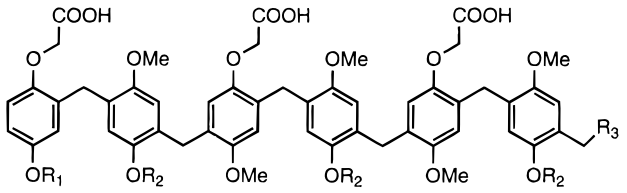


Figure 1. HLE kinetics: compound **6** vs S-2484. Legend: Inhibition kinetic analysis of HLE activity with compound **6**. Each reaction was performed in 600 μL of HEPES buffer containing 15 nM HLE, compound **6** at the indicated concentrations, and the peptide substrate S-2484 at varying concentrations. The absorbance at 405 nm over a period of 5 min was recorded. Shown are the double-reciprocal plots and the determined apparent V_m and K_m values at each inhibitor concentration.

(s, 3 H), 1.9 (m, 6 H). MS (FAB-pNBA): *m*/*z* 1347 (M + H). Combustion analysis: C, H.

2-[4-[4-[4-[2-Methoxy-4-methyl-5-(hexyloxy)benzyl]-2-methoxy-5-[[(hydroxycarbonyl)methyl]oxy]benzyl]-2-methoxy-5-(hexyloxy)benzyl]-2-methoxy-5-(hexyloxy)-benzyl]-4-methoxyphenoxyacetic Acid (7e). Prepared from **54** by alkylation (iodohexane, K_2CO_3, DMF, 85 °C, 18 h, 54%) followed by hydrolysis (KOH, ethanol, dioxane, 65 °C, 5

Table 2. *In Vitro* Inhibition of HLE by Hexamers **7a**−**f**



7a - f

compd	R_1	R_2	R_3	formula[a]	HLE inhib[b] K_i (nM)
7a	Me	H	H	$C_{54}H_{56}O_{18}\cdot 4HO$	1300
7b	Me	$CH_2O(CH_2)_2OMe$	H	$C_{66}H_{80}O_{24}\cdot H_2O$[c]	1000
7c	H	CH_2Ph	OH	$C_{74}H_{72}O_{19}\cdot 2H_2O$[d]	200
7d	Me	$(CH_2)_3Ph$	H	$C_{81}H_{86}O_{18}\cdot H_2O$	100
7e	Me	$(CH_2)_5Me$	H	$C_{72}H_{92}O_{18}\cdot 3H_2O$	17
7f	Me	CH_2-(cyclo)C_6H_{11}	H	$C_{75}H_{92}O_{18}\cdot H_2O$	20

[a] All compounds exhibited satisfactory spectral data consistent with their structures. Analytical results are within ±0.4% of the theoretical value. [b] Inhibition of HLE at pH 7.5 in HEPES buffer containing 200 mM NaCl with the substrate Suc-Ala-Ala-Pro-Phe-pNA. See the Experimental Section for details. [c] Anal. C: calcd, 62.16; found, 62.6. [d] Anal. C: calcd, 69.15; found, 68.59.

h) and purification (flash chromatography using a solution of 9% THF and 1% formic acid in $CHCl_3$ as the eluent) in 57% yield. ^{1}H NMR (DMSO-d_6): δ 6.85−6.5 (m, 13 H), 4.61 (s, 2 H), 4.47 (s, 4 H), 3.90−3.25 (m, 34 H, with singlets at 3.80, 3.73, 3.64, 3.62, 3.34), 2.10 (s, 3 H), 1.60 (m, 6 H), 1.24 (m, 18 H), 0.83 (m, 9 H). MS (FAB-pNBA): *m*/*z* 1245 (M + H). Combustion analysis: C, H.

2-[4-[4-[4-[4-[2-Methoxy-4-methyl-5-[(cyclohexyl-methyl)oxy]benzyl]-2-methoxy-5-[[(hydroxycarbonyl)-methyl]oxy]benzyl]-2-methoxy-5-[(cyclohexylmethyl)-

Table 3. Specificity of **4**, **5**, **6**, and **7a**−**f**

compd	K_i (nM) HLE	cathepsin G[a]	thrombin[b]
4	8300	>15000	>15000
5	130	>15000	750
6	120	>15000	>15000
7a	1300	>15000	>15000
7b	1000	>15000	240
7c	200	1500	>15000
7d	100	>15000	1700
7e	17	>15000	1400
7f	20	>15000	>15000

[a] Inhibition of cathepsin G at pH 7.5 in HEPES buffer containing 200 mM NaCl with the substrate Suc-Ala-Ala-Pro-Phe-pNA. See the Experimental Section for details. [b] Inhibition of thrombin at pH 7.5 in HEPES buffer containing 200 mM NaCl with the substrate Bz-Phe-Val-Arg-pNA. See the Experimental Section for details.

oxy]benzyl]-2-methoxy-5-[[(hydroxycarbonyl)methyl]oxy]-benzyl]-2-methoxy-5-[(cyclohexylmethyl)oxy]benzyl]-4-methoxyphenoxyacetic Acid (7f). A mixture of **55** (27 mg) and KOH (70 μL of a 12.5 N solution) in ethanol (500 μL) and dioxane (200 uL) was heated at 65 °C for 5 h, the volume was reduced by 50% by removal of the volatiles *in vacuo*, and the mixture was diluted with H_2O, acidified with HCl, and extracted with ethyl acetate. The combined organic extracts were washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by flash chromatography using as the eluent a solution of 1% formic acid and 2% THF in $CHCl_3$. Concentration *in vacuo* of the product-rich fractions gave 16 mg (56%) of tan solid. MS (FAB-pNBA): *m*/*z* 1281 (M + H). Combustion analysis: C, H.

5-(Benzoyloxy)-2-hydroxybenzaldehyde (14). A solution of 2,5-dihydroxybenzaldehyde (**13**, 84.3 g, 0.610 mol), benzoic anhydride (296 g, 1.28 mol), and triethylamine (137.3 g., 1.34 mol) in 800 mL of CH_2Cl_2 was stirred at room temperature overnight and the volume reduced by ~50% by concentration *in vacuo*. The residue was diluted with ether and ethyl acetate, washed with H_2O and brine, and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was dissolved in 700 mL of anhydrous methanol, and 85 g of anhydrous K_2CO_3 was added. The mixture was stirred 2 h with occassional heating, diluted with H_2O, and extracted with ether. The combined organic layers were washed with 2 N HCl, saturated Na_2HPO_4, and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided 145 g (98%) of the tan solid which was used without further purification. MS: *m*/*z* 242 (M^+).

5-(Benzoyloxy)-2-[(cyanomethyl)oxy]benzaldehyde (15). A mixutre of **14** (37.4 g, 0.154 mol), bromoacetonitrile (22.9 g., 0.185 mol), and K_2CO_3 (25.9 g, 0.185 mol) in 300 mL of acetonitrile was heated at 80 °C for 10 h under a N_2 atmosphere, cooled to 0−5 °C, neutralized with 1 N HCl, and diluted with ether. The organic layer was washed with H_2O and brine and dried ($MgSO_4$), and the volatiles were removed *in vacuo*. Recrystallization of the residue with hexanes and ethyl acetate and purification of the mother liquor on HPLC using 20% ethyl acetate in hexanes as the eluent provided 32.0 g (74%) of the solid, mp 100−1 °C. MS: *m*/*z* 281 (M^+). ^{1}H NMR ($CDCl_3$): δ 4.92 (s, 2H), 7.13 (d, J = 8.9 Hz), 7.46−7.52 (m, 3H), 7.63 (t, J = 7.6 Hz, 2H), 7.72 (d, J = 3 Hz, 1H), 8.15 (d, J = 7.1 Hz, 2H) 10.39 (s, 1H).

4-(Benzoyloxy)-2-(hydroxymethyl)phenoxyacetonitrile (16). To a −70 °C solution of **15** (32.0 g, 0.114 mol) in 300 mL of anhydrous THF was added $NaBH_4$ (114 mL of 0.5 M in diglyme, 56.9 mmol). The mixture was stirred 5 h and quenched with 60 mL of 2 N HCl and ther. The organic layer was washed with H_2O and brine and dried ($MgSO_4$), and the voltiles were removed *in vacuo*. Recrystallization of the residue with hexanes−ethyl acetate and purification of the mother liquor on HPLC using 43% ethyl acetate in hexanes as the eluent provided 30 g (93%) of the white solid. MS: *m*/*z* 283 (M^+).

2-[[[(1,1-Dimethylethyl)diphenylsilyl]oxy]methyl]-5-hydroxyphenoxyacetonitrile (18). To a solution of **16** (29.1 g, 0.103 mol) in 300 mL of CH_2CI_2 were added imidazole (8.31 g, 0.125 mol), 4-(dimethylamino)pyridine (DMAP) (1.26 g, 10.3 mmol), and then *tert*-butylchlorodiphenylsilane (29.5 mL). The mixture was stirred 1 h, diluted with ether, washed with H_2O and brine, and dried ($MgSO_4$), and the volatiles were removed *in vacuo* to provide **17**. This material was dissolved in 400 mL of THF, cooled to −50 °C, and sodium methoxide (24.7 mL of a 25% methanol solution) was added. The mixture was stirred 2 h while warming to −30 °C and quenched with 2 N HCl (53 mL) and ether. The organic layer was washed with water and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo*, recrystallization of the residue with hexanes and ethyl acetate, and purification of the mother liquor by flash chromatography using 30% ethyl acetate in hexanes as the eluent provided 42.9 g (99%) of solid product.

2-[[[(1,1-Dimethylethyl)diphenylsilyl]oxy]methyl]-4-hydroxy-5-iodophenoxyacetonitrile (19). To a solution of **18** (42.9 g, 0.103 mol) and morpholine (22.4 mL, 0.258 mol) in 300 mL of CH_2Cl_2 at 0−5 °C was added I_2 (24.7 g, 97.3 mmol). The mixture was warmed to room temperature, stirred for 1.5 h, and quenched with 1 N HCl (155 mL) and ether. The organic layer was washed with H_2O, saturated sodium bisulfite, and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by flash chromatography using 35% ether in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions furnished 46.8 g (84%) of the oily product.

4-(Benzyloxy)-2-[[[(1,1-dimethylethyl)diphenylsilyl]-oxy]methyl]-5-iodophenoxyacetonitrile (20). To a suspension of NaH (60%, 3.62 g, 90.5 mmol) in 75 mL of anhydrous THF at −20 °C was added a solution of **19** (46.8 g, 86.2 mmol), benzyl bromide (11.8 mL, 99.2 mmol), and DMPU (63 mL) in 185 mL of THF. The mixure was warmed to room temperature, stirred for 2.5 h, and quenched with 1 N HCl (20 mL) and ether. The organic layer was washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by flash chromatography using 10% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions gave 52.8 g (97%) of the oily product.

4-(Benzoyloxy)-2-(bromomethyl)phenoxyacetonitrile (21). To a solution of **16** (27.9 g, 98.5 mmol) and triphenylphosphine (36.5 g, 0.138 mol) in 300 mL of anhydrous THF was added portionwise *N*-bromosuccinimide (NBS) (21.1 g, 0.118 mol). After 1 h additional triphenylphosphine (4.3 g) and NBS (2.1 g) were added. The mixture was stirred for 30 min, and the volatiles were removed *in vacuo*. The residue was dissolved in $CHCl_3$, applied to a plug of silica gel, and eluted with 25% ethyl acetate in hexanes. Purification of the filtrate by HPLC using 20% ethyl acetate in hexanes as the eluent provided 22.2 g (65%) of the pale yellow solid, mp 87−8 °C. MS: *m*/*z* 347, 345 (M^+).

4-(Benzyloxy)-5-[2-[(cyanomethyl)oxy]-5-(benzolyl-oxy)benzyl]-2-[[[(1,1-dimethylethyl)diphenylsilyl]oxy]-methyl]phenoxyacetonitrile (22). A suspension of Zn powder (325 mesh, 12.6 g, 0.193 mol) and 0.8 mL of freshly distilled 1,2-dibromoethane in 12 mL of anhydrous THF was warmed to 80 °C for 1.5 min and cooled to 0−5 °C. To the suspension was added **21** (17.4 g, 50.3 mmol) in 70 mL of anhydrous THF over 2.75 h. After the addition was complete the mixture was added via cannula to a mixture of **20** (30.0 g, 47.3 mmol) and tetrakis(triphenylphosphine)palladium (3.0 g). The zinc was washed with 30 mL of THF, and the washings were added to the mixture. The resulting solution was heated at 60 °C for 4.25 h, cooled to room temperature, and diluted with ether and 5% aqueous NH_3. The organic layer was washed with 5% aqueous NH_3 and brine and dried ($MgSO_4$), and the volatiles were removed *in vacuo*. Purification of the residue by flash chromatography using 40% ether, 10% CH_2Cl_2 in hexanes as the eluent provided 32.02 g (88%) of the product, mp 100−1 °C. ^{1}H NMR ($CDCl_3$): δ 1.07 (s, 9H), 4.0 (s, 2H), 4.56 (s, 2H), 4.69 (s, 2H), 4.75 (s, 2H), 5.01 (s, 2H), 6.77 (s, 1H), 6.94 (d, J = 9 Hz, 1H), 7.01 (d, J = 2.8 Hz, 1H), 7.07 (dd, J = 9, 2.8 Hz, 1H), 7.1−7.41 (m, 12H), 7.50 (t, J =

7.5 Hz, 2H), 7.60−7.67 (m, 5H), 8.15 (dd, J = 8.1, 1.4 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 19.98, 27.5, 31.1, 54.8, 55.2, 61.2, 71.1, 112.3, 113.3, 115.9, 121.1, 125.5, 128.1, 128.3, 128.4, 128.5, 129.19, 129.23, 130.4, 130.8, 131.9, 134.0, 134.3, 136.2, 137.7, 146.6, 147.5, 153.0, 153.1, 166.0. IR (KBr, cm^{-1}): 3070 w, 2922 m, 2851 m, 1722 s, 1509 m, 1495 s, 1380 m, 1281 s, 1200 s, 1077 s, 706 s. MS (FAB-pNBA): m/z 771 ($M - H^+$). Combustion analysis: C, H.

4-(Benzyloxy)-5-[2-[(cyanomethyl)oxy]-5-hydroxybenzyl]-2-[[[(1,1-dimethylethyl)diphenylsilyl]oxy]methyl]-phenoxyacetonitrile (23). To a −50 °C solution of **22** (16.21 g) in 80 mL of THF was added sodium methoxide (4.5 mL of a 25% solution in methanol). The mixture was stirred 50 min while warming to −30 °C and quenched with ether and 1 N HCl (21 mL). The organic layer was washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by flash chromatography using 60% ether in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions yielded 13.8 g (98%) of a viscous oil. ^{1}H NMR ($CDCl_3$): δ 1.09 (s, 9H), 3.92 (s, 2H), 4.50 (s, 2H), 4.54 (s, 2H), 4.75 (s, 2H), 5.00 (s, 2H), 5.13 (s, 1H) 6.58 (d, J = 2.9 Hz, 1H), 6.62 (dd, J = 8.6, 2.9 Hz, 1H), 6.70 (s, 1H), 6.76 (d, J = 8.6 Hz, 1H), 7.24 (s, 1H), 7.28−7.42 (m, 11H), 7.66 (dd, J = 7.6, 1.4 Hz, 4H). ^{13}C NMR ($CDCl_3$): δ 20.0, 27.5 30.6, 55.2, 55.5, 61.2, 71.1, 112.4, 114.4, 115.0, 116.2, 116.4, 119.0, 128.2, 128.4, 128.5, 128.6, 129.2, 130.4, 130.5, 132.0, 134.0, 136.2, 137.7, 147.4, 149.3, 152.0, 153.0.

4-(Benzyloxy)-5-[2-[(cyanomethyl)oxy]-4-iodo-5-hydroxybenzyl]-2-[[(1,1-dimethylethyl)diphenylsiloxy]-methyl]phenoxyacetonitrile (24). To a solution of **23** (4.10 g, 6.14 mmol) in 20 mL of CH_2Cl_2 at 0−5 °C were added morpholine (1.3 mL, 14.9 mmol) and then I_2 (1.6g, 6.30 mmol). The mixture was warmed to room temperature, stirred 2.5 h, and diluted with ether and aqueous HCl (9.5 mL of a 1 N solution). The organic layer was washed with H_2O and brine and dried ($MgSO_4$) and the volatiles were removed *in vacuo*. Purification of the residue by flash chromatography using 40% ethyl acetate in hexanes as the eluent provided 4.0 g (82%) of product.

4-(Benzyloxy)-5-[2-[(cyanomethyl)oxy]-4-iodo-5-(benzyloxy)benzyl]-2-[[[(1,1-dimethylethyl)diphenylsilyl]oxy]-methyl]phenoxyacetonitrile (25). To a suspension of NaH (60%, 0.20 g, 5.0 mmol) in 5 mL of anhydrous THF at −20 °C was added a solution of **24** (4.0 g, 5.04 mmol), DMPU (3.8 mL), and benzyl bromide (0.77 mL) in 12 mL of THF. The mixture was warmed to room temperature, stirred for 2.75 h, and quenched with ether and aqueous HCl (5 mL of a 0.1 N solution). The organic layer was washed with water and dried ($MgSO_4$). Removal of the volatles *in vacuo* provided a residue which was purified by flash chromatography using 40% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions furnished 4.4 g (99%) of the product.

4-(Benzoyloxy)-5-[2-[(cyanomethyl)oxy]-5-(benzoyloxy)-benzyl]-2-(hydroxymethyl)phenoxyacetonitrile (26). A solution of **22** (40.9 g, 53.0 mmol), acetic acid (30 mL of a 2.0 M solution in THF), and tetra-*n*-butylammonium fluoride (TBAF) (60 mL of a 1.0 M solution in THF) in 120 mL of THF was stirred at room temperatue 2.5 h and diluted with 20% CH_2Cl_2 in ether and H_2O. The organic layer was washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by flash chromatography using 20% hexanes in ether followed by 20% CH_2Cl_2 in ether as the eluents. Concentration *in vacuo* of the product-rich fractions gave 26.0 g (92%) of the product, mp 115−6 °C.

4-(Benzyloxy)-5-[2-[(cyanomethyl)oxy]-5-(benzoyloxy)-benzyl]-2-(bromomethyl)phenoxyacetonitrile (27). To a solution of **26** (2.81 g, 5.26 mmol) in 35 mL of anhydrous THF were added triphenylphosphine (1.77 g, 6.76 mmol) and NBS (1.11 g, 6.23 mmol). The mixture was stirred 25 min, and the volatiles were removed *in vacuo*. Purification of the residue by flash chromatography using CH_2Cl_2 as the eluent provided 2.76 g (88%) of the product.

2-[[[(1,1-Dimethylethyl)diphenylsilyl]oxy]methyl]-4-(benzyloxy)-5-[4-[4-[2-[(cyanomethyl)oxy]-5-(benzoyloxy)benzyl]-5-(benzyloxy)-2-[(cyanomethyl)oxy]benzyl]-5-(benzyloxy)-2-(cyanomethyloxy)benzyl]phenoxyacetonitrile (28). A mixture of Zn dust (3.2 g, 48.9 mmol) and 1,2-dibromoethane (200 μL) in 3 mL of anhydrous THF was heated to reflux for 2 min and cooled to 0−5 °C, and a solution of **27** (4.5 g, 7.54 mmol) in 20 mL of THF and 4 mL of DMF was added dropwise over the course of 1.1 h. The mixture was stirred 15 min, and the supernatant was added to a mixture of **25** (4.4 g, 4.98 mmol) and $[(Ph)_3P]_4Pd]$ (0.36 gm). The mixture was heated to 69 °C for 2.75 h, cooled to room temperature, and diluted with ether and 5% aqueous ammonia. The organic layer was dried ($MgSO_4$), and the volatiles were removed *in vacuo*. Purification of the residue by flash chromatography using 30% ethyl acetate, 30% CH_2Cl_2 in hexanes as the eluent and concentration *in vacuo* of the product-rich fractions furnished 5.62 g (89%) of product.

2-[[[(1,1-Dimethylethyl)diphenylsilyl]oxy]methyl]-4-(benzyloxy)-5-[4-[4-[2-[(cyanomethyl)oxy]-5-hydroxybenzyl]-5-(benzyloxy)-2-[(cyanomethyl)oxy]benzyl]-5-(benzyloxy)-2-[(cyanomethyl)oxy]benzyl]-5-(benzyloxy)-2-[(cyanomethyl)oxy]benzyl]phenoxyacetonitrile (29). A solution of **28** (4.9 g, 3.86 mmol) and sodium methoxide (840 μL of a 25% methanol solution) in 15 mL of THF was stirred at −50 to −22 °C during the course of 2 h and quenched with aqueous HCl (3.9 mL of a 1 N solution) and 20% CH_2Cl_2 in ether. The organic layer was washed with H_2O and brine and dried ($MgSO_4$), and the volatiles were removed *in vacuo*. Purification of the residue by flash chromatography using 30% ethyl acetate, 30% CH_2Cl_2 in hexanes as the eluent and concentration *in vacuo* of the product-rich fractions gave 4.3 g (96%) of the product.

2-[[[(1,1-Dimethylethyl)diphenylsilyl]oxy]methyl]-4-(benzyloxy)-5-[4-[4-[2-[(cyanomethyl)oxy]-4-iodo-5-hydroxybenzyl]-5-(benzyloxy)-2-[(cyanomethyl)oxy]benzyl]-5-(benzyloxy)-2-[(cyanomethyl)oxy]benzyl]phenoxyacetonitrile (30). To a 0−5 °C solution of **29** (3.7 g, 3.18 mmol) in 15 mL of CH_2Cl_2 were added morpholine (688 μL, 7.87 mmol) and then I_2 (0.802 g, 3.16 mmol). The mixture was warmed to room temperature, stirred 2.5 h, and diluted with 20% CH_2Cl_2 in ether and water. The organic layer was washed with H_2O and brine and dried ($MgSO_4$), and the volatiles were removed *in vacuo*. Purification of the residue by flash chromatography using 20% ethyl acetate and 30% CH_2Cl_2 in hexanes as the eluent and concentration *in vacuo* of the product-rich fractions furnished 3.41g (83%) of product.

2-[[[(1,1-Dimethylethyl)diphenylsilyl]oxy]methyl]-4-(benzyloxy)-5-[4-[4-[2-[(cyanomethyl)oxy]-4-iodo-5-(benzyloxy)benzyl]-5-(benzyloxy)-2-[(cyanomethyl)oxy]benzyl]-5-(benzyloxy)-2-[(cyanomethyl)oxy]benzyl]-phenoxyacetonitrile (31). To a suspension of NaH (60%, 109 mg, 2.73 mmol) in 2 mL of anhydrous THF at −20 °C was added a solution of **30** (3.4 g, 2.62 mmol) and benzyl bromide (0.4 mL) in 2 mL of DMPU and 6 mL of THF. The mixture was warmed to room temperature, stirred for 2 h, and diluted with ether and aqueous HCl (5 mL of a 0.1 M solution). The organic layer was washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by flash chromatography using 20% ethyl acetate and 20% CH_2Cl_2 in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions furnished 3.46 g (95%) of product.

2-[[[(1,1-Dimethylethyl)diphenylsilyl]oxy]methyl]-4-(benzyloxy)-5-[4-[4-[4-[2-[(cyanomethyl)oxy]-5-(benzoyloxy)benzyl]-3-(benzyloxy)-5-[(cyanomethyl)oxy]benzyl]-3-(benzyloxy)-5-[(cyanomethyl)oxy]benzyl]-3-(benzyloxy)-5-[(cyanomethyl)oxy]benzyl]phenoxyacetonitrile (32). A suspension of Zn powder (325 mesh, 3.03 g, 0.046 mol) and 0.2 mL of freshly distilled 1,2-dibromoethane in 7 mL of anhydrous THF was warmed to 80 °C for 1.5 min and cooled to 0−5 °C. To the suspension was added **21** (3.46 g, 10 mmol) in 10 mL of anhydrous THF over 1 h. After the addition was complete, 4.16 mL of the supernatant was added via syringe to a mixture of **31** (1.0 g, 0.8 mmol) and tetrakis-(triphenylphosphine)palladium (37 mg). The resulting solution was heated at 60 °C for 3 h, cooled to room temperature, and diluted with ether and 5% aqueous NH_3. The organic layer was washed with 5% aqueous NH_3 and brine and dried

($MgSO_4$), and the volatiles were removed *in vacuo*. Purification of the residue by flash column using 35−50% ethyl acetate in hexanes as the eluent provided 870 mg (79%) of the product. ^{1}H NMR ($CDCl_3$): δ 1.08 (s, 9H), 3.92 (m, 8H), 4.48 (s, 2H), 4.54 (s, 2H), 4.61 (m, 8H), 4.68 (s, 2H), 4.73 (s, 2H), 4.98 (s, 2H), 6.72 (m, 6H), 6.90 (d, J = 9 Hz, 1H), 6.97 (d, J = 3 Hz, 1H), 7.06 (dd, J = 9, 3 Hz, 1H), 7.15−7.55 (m, 30H), 7.65 (d, J = 9 Hz, 6H), 8.12 (d, J = 9 Hz, 2H). MS (FAB-pNBA): m/z 1526 (M + H).

2-[[[(1,1-Dimethylethyl)diphenylsilyl]oxy]methyl]-4-(benzyloxy)-5-[4-[4-[4-[4-[2-[(cyanomethyl)oxy]-5-(benzoyloxy)benzyl]-3-(benzyloxy)-5-[(cyanomethyl)oxy]benzyl]-3-(benzyloxy)-5-[(cyanomethyl)oxy]benzyl]-3-(benzyloxy)-5-[(cyanomethyl)oxy]benzyl]-3-(benzyloxy)-5-[(cyanomethyl)oxy]benzyl]phenoxyacetonitrile (33). A mixture of Zn dust (3.2 g, 48.9 mmol) and 1,2-dibromoethane (200 μL) in 2 mL of anhydrous THF was heated at reflux for 2 min and cooled to 0−5 °C.

A solution of **27** (0.85 g, 1.42 mmol) in 4 mL of THF was added dropwise over 30 min. The mixture was stirred for 15 min, and the supernatant was added to a mixture of **31** (1.38 g, 0.996 mmol) and $[(Ph)_3P]_4Pd$ (0.11 mg). The mixture was heated at 60 °C for 1.25 h, cooled to room temperature, and diluted with THF, ether, and 5% aqueous NH_3. The organic layer was washed with brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was dissolved in hot ethyl acetate, cooled, filtered, and provided 0.983 g (55%) of product.

2-[[(2-Methoxyethoxy)methyl]oxy]-5-(benzoyloxy)benzaldehyde (34). To a suspension of NaH (60%, 13.0 g, 0.321 mol) in 20 mL of anhydrous THF under an Ar atmosphere at 0−5 °C was added a solution of **14** (67.67 g, 0.279 mol), (2-methoxyethoxy)methyl chloride (90%, 46.4 g, 0.335 mol), and DMPU (30 mL) in 400 mL of THF. The mixture was slowly warmed to room temperature, stirred for 90 min, and quenched with aqueous HCl (330 mL of a 1 N solution) and ether. The organic layer was washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by HPLC using 25% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions provided 75.5 g (82%) of the pale yellow solid. MS: m/z 300 (M^+).

3-[[[(1,1-Dimethylethyl)diphenylsilyl]oxy]methyl]-4-[[(2-methoxyethoxy)methyl]oxy]phenol (35). To a −60 °C solution of **34** (75.52 g, 0.229 mol) in 250 mL of anhydrous THF was added dropwise $NaBH_4$ (230 mL of 0.5 M in diglyme, 0.115 mol). The mixture was stirred under a N_2 atmosphere for 90 min, and additional $NaBH_4$ (23 mL) was added. After 45 min of stirring, the reaction was quenched by the addition of aqueous HCl (250 mL of a 1 N solution). The mixture was diluted with H_2O and extracted with ether. The combined organic extracts were washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was dissolved in 300 mL of CH_2Cl_2 and added to an aged (15 min) mixture of imidazole (32 g, 0.458 mol) and *tert*-butyldiphenylsilyl chloride (62.95 g, 0.229 mol) in 500 mL of CH_2Cl_2. The mixture was stirred for 90 min, and approximately 50% of the volatiles removed *in vacuo*. The residue was dissolved in ether, washed with 0.5% aqueous HCl, water, and brine, and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was dissolved in 500 mL of THF and cooled to −25 °C. Sodium methoxide (53 mL of a 25% methanol solution, 0.229 mol) was added, and the mixture was stirred for 30 min and quenched with aqueous HCl (250 mL of a 1 N solution) and ether. The organic layer was washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by HPLC using 20% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions gave 68.6 g (64%) of the oily product. MS: m/z 465 (M − H).

3-[[[(1,1-Dimethylethyl)diphenylsilyl]oxy]methyl]-4-[[(2-methoxyethoxy)methyl]oxy]-6-iodophenol (36). To a solution of **35** (68.6 g, 0.147 mol) and morpholine (25.6 g, 0.294 mol) in 600 mL of anhydrous CH_2Cl_2 at 0−5 °C was added portionwise *N*-iodosuccinamide (NIS) (35 g, 0.147 mol). The mixture was warmed to room temperature over 2 h, and additional morpholine (7 mL) and NIS (8.75 g) were added. After 1 h the mixture was diluted with ether, washed with 1 N HCl, H_2O, aqueous $Na_2S_2O_5$, H_2O, and brine, and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by HPLC using 11% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions gave 70.8 g (81%) of the product. MS (FAB-pNBA): m/z 593 (M − H).

3-[[[(1,1-Dimethylethyl)diphenylsilyl]oxy]methyl]-4-[[(2-methoxyethoxy)methyl]oxy]-6-iodoanisole (37). To a suspension of NaH (60%, 4.75 g, 0.119 mol) in 20 mL of anhydrous THF at 0−5 °C was added a solution of phenol **36** (70.3 g, 0.119 mol), iodomethane (33.8 g, 0.238 mol), and DMPU (20 mL) in 230 mL of THF. The mixture was warmed to room temperature, stirred for 90 min, quenched with aqueous HCl (125 mL of a 1 N solution), and extracted with ether. The organic extracts were washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by HPLC using 5% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions gave 37.0 g (51%) of the gummy product. MS: FAB 608 (M^+). ^{1}H NMR ($CDCl_3$): δ 7.65 (m, 4 H), 7.35 (m, 7 H), 7.22 (d, 1 H), 5.05 (s, 2 H), 4.75 (s, 2 H), 3.82 (s, 3 H), 3.65 (m, 2 H), 3.5 (m, 2 H), 3.35 (s, 3 H), 1.1 (s, 9 H).

2-[(2-Methoxy-4-[[[(1,1-dimethylethyl)diphenylsilyl]-oxy]methyl]-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-4-(benzoyloxy)phenoxyacetonitrile (38). A mixture of zinc (70.0 g, 1.07 mol) and 1,2-dibromoethane (5.02 g, 0.027 mol) in 30 mL of anhydrous THF was heated at 75 °C for 5 min and cooled to 0−5 °C, and a solution of **21** (37.0 g, 0.107 mol) in 60 mL of THF was added dropwise. After the addition was complete (4.5 h), the supernatant was added to a solution of **37** (65.0 g, 0.107 mol) and $(Ph_3P)_4$ Pd (6.18 g, 5.35 mmol) in 60 mL of degassed THF. The mixture was heated at 65 °C for 5 h, cooled to room temperature, and diluted with ether and 5% NH_4OH solution. The organic layer was washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by HPLC using 25% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions furnished 58.4 g (73%) of the orange gum. ^{1}H NMR ($CDCl_3$): δ 8.15 (d, 2 H), 7.8−7.3 (m, 12 H), 7.24 (d, 2 H), 7.1−6.9 (m, 4 H), 5.05 (s, 2 H), 4.82 (s, 2 H), 4.80 (s, 2 H), 3.95 (s, 2 H), 3.8 (s, 3 H), 3.65 (m, 2 H), 3.45 (m, 2 H), 3.30 (s, 3 H), 1.1 (s, 9 H). MS (FAB-pNBA): m/z 745 (M^+).

2-[(2-Methoxy-4-(hydroxymethyl)-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-4-(benzoyloxy)phenoxyacetonitrile (39). A solution of **38** (34.4 g, 46.2 mmol), tetrabutylammonium fluoride (TBAF) (69 mL of a 1.0 M THF solution, 69.3 mmol), and acetic acid (4.45 g, 74.0 mmol) in 100 mL of anhydrous THF was stirred for 3 h, and additional acetic acid (900 μL) and TBAF (14 mL) were added. After 1 h the mixture was diluted with ether and H_2O. The organic layer was washed with brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by HPLC using 50% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions gave 19.3 g (82%) of the orange gum. MS: FAB 507 (M^+). ^{1}H NMR ($CDCl_3$): δ 5.18 (s, 2 H), 4.78 (s, 2 H), 4.65 (s, 2 H), 3.95 (s, 2 H), 3.75 (m, 5 H), 3.5 (m, 2 H), 3.3 (s, 3 H), 1.6 (bs, 1 H).

2-[2-Methoxy-4-(bromomethyl)-5-[[(2-methoxyethoxy)-methyl]oxy]benzyl]-4-(benzoyloxy)phenoxyacetonitrile (40). To a mixture of **39** (19.3 g, 38 mmol) and triphenylphosphine (16.1 g, 60.8 mmol) in 140 mL of THF was added portionwise NBS (9.50 g, 53.2 mmol). The mixture was stirred for 90 min, and the volatiles were removed *in vacuo*. Purification of the CH_2Cl_2 soluble residue by flash chromatography using 33% ethyl acetate in hexanes as the eluent provided 12.8 g (59%) of the tan solid. ^{1}H NMR ($CDCl_3$): δ 5.25 (s, 2 H), 4.75 (s, 2 H), 4.5 (s, 2 H), 3.9 (s, 2 H), 3.82 (m, 2 H), 3.75 (s, 3 H), 3.5 (m, 2 H), 3.32 (s, 3 H). MS (FAB-pNBA): m/z 571, 569 (M^+).

2-[2-Methoxy-4-[[[(1,1-dimethylethyl)diphenylsilyl]oxy]-methyl]-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-4-hydroxyphenoxyacetonitrile (41). A −55 °C solution of **38** (24.08 g, 32.3 mmol) and sodium methoxide (7.4 mL of a 25%

methanol solution, 32.3 mmol) in 120 mL of anhydrous THF was stirred under Ar for 30 min, and additional sodium methoxide (0.75 mL) was added. After 20 min the mixture was quenched with aqueous HCl (40 mL of 1 N solution) and ether. The organic layer was washed with brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* and purification of the residue by HPLC using 33% ethyl acetate in hexanes as the eluent provided 17.74 g (86%) of the tan solid. MS (FAB-pNBA): *m/z* 641 (M^+).

2-[2-Methoxy-4-[[[(1,1-dimethylethyl)diphenylsilyl]oxy]-methyl]-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-4-hydroxy-5-iodophenoxyacetonitrile (42). To a cold (0−5 °C) solution of **41** (17.74 g, 27.6 mmol) and morpholine (5.30 g, 60.8 mmol) in 60 mL of anhydrous CH_2Cl_2 was added a solution of I_2 (7.02 g, 27.6 mmol) in 210 mL of CH_2Cl_2. The mixture was stirred 2 h, warmed to room temperature, stirred for 2 h, and diluted with ether and aqueous HCl (80 mL of 1 N solution). The organic layer was washed with H_2O, saturated $Na_2S_2O_4$, and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* and purification of the residue by HPLC using 25% ethyl acetate in hexanes as the eluent provided 17.93 g (85%) of the tan solid. MS (FAB-pNBA): *m/z* 767 (M^+).

2-[2-Methoxy-4-[[[(1,1-dimethylethyl)diphenylsilyl]oxy]-methyl]-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-4-methoxy-5-iodophenoxyacetonitrile (43). To a suspension of NaH (60%, 0.935 g, 23.4 mmol) in 10 mL of anhydrous THF at 0−5 °C was added **42** (17.93 g, 23.4 mmol), DMPU (10 mL), and iodomethane (6.63 g, 46.7 mmol) in 100 mL of THF. The mixture was warmed to room temperature, stirred for 1 h, and quenched with ether and aqueous HCl (30 mL of a 1 N solution). The organic layer was washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* and purification of the residue by HPLC using 20% ethyl acetate in hexanes as the eluent provided 18.1 g (99%) of the brown gum. ^{1}H NMR ($CDCl_3$): δ 7.7 (dd, 4 H), 7.4 (m, 6 H), 7.2 (s, 1 H), 6.82 (s, 1 H), 6.7 (s, 1 H), 5.05 (s, 2 H), 4.78 (s, 2 H), 4.65 (s, 2 H), 3.85 (s, 2 H), 3.80 (s, 3 H), 3.75 (s, 3 H), 3.6 (m, 2 H), 3.4 (m, 2 H), 3.3 (s, 3 H), 1.1 (s, 9 H). MS (FAB-pNBA): *m/z* 781 (M^+).

2-[4-[4-[2-Methoxy-4-[[[(1,1-dimethylethyl)diphenylsilyl]oxy]methyl]-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-3-[(cyanomethyl)oxy]-6-methoxybenzyl]-2-methoxy-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-4-(benzoyloxy)phenoxyacetonitrile (44). A mixture of Zn dust (9.2 g, 0.14 mol) and 1,2-dibromoethane in 5 mL of anhydrous THF was heated at reflux for 3 min and cooled to 0−5 °C, and a solution of **40** (8.00 g, 14.0 mmol) in 25 mL of anhydrous THF was added dropwise over 3 h. The supernatant was added to a degassed solution of **43** (11.0 g, 14.0 mmol) and $(Ph_3P)_4$ Pd (0.81 g, 0.70 mmol) in 25 mL of THF. The mixture was heated at 70 °C overnight, cooled to room temperature, diluted with 5% NH_4OH, and extracted with ether. The combined organic extracts were washed with brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* and purification of the residue by HPLC using 50% ethyl acetate in hexanes as the eluent provided 4.54 g (28%) of the white solid product. ^{1}H NMR ($CDCl_3$): δ 8.15 (d, 2 H), 7.70 (dd, 4 H), 7.6−6.6 (m, 18 H), 5.15 (s, 2H), 5.0 (s, 2 H), 4.78 (s, 4 H), 4.55 (s, 2 H), 3.95 (s, 2 H), 3.90 (s, 2 H), 3.88 (s, 2 H), 3.80 (s, 3 H), 3.75 (m, 2 H), 3.70 (s, 3 H), 3.65 (s, 3 H), 3.60 (m, 2 H), 3.45 (m, 2 H), 3.40 (m, 2 H), 3.35 (s, 3 H), 3.28 (s, 3 H), 1.1 (s, 9 H). MS: FAB 1145 (M + H). TLC (4:3 hexanes:ethyl acetate): R_f 0.24. Also recovered was 4.4 g (40%) of **43**.

2-[4-[4-[2-Methoxy-4-[[[(1,1-dimethylethyl)diphenylsilyl]oxy]methyl]-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-3-[(cyanomethyl)oxy]-6-methoxybenzyl]-2-methoxy-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-4-methoxy-5-iodophenoxyacetonitrile (47). A solution of **44** (4.50 g, 3.93 mmol) and sodium methoxide (0.92 mL of a 25% sodium methoxide in methanol solution) in 30 mL of anhydrous THF at −50 °C was stirred for 60 min, quenched with aqueous HCl (5 mL of 1 N solution) and H_2O, and extracted with ether. The combined organic extracts were washed with brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo*, purification of the residue by flash chromatography with 50% ethyl acetate in hexanes as the eluent, and concentration *in vacuo* of the product-rich fractions gave a quantitative yield of yellow gum **45**. To this material and morpholine (0.767 g, 8.80 mmol) in 15 mL of anhydrous CH_2Cl_2 at 0−5 °C was added dropwise a solution of I_2 (1.02 g, 4.0 mmol) in 45 mL of CH_2Cl_2. The mixture was warmed to room temperature, stirred for 3 h, diluted with ether, washed with 1 N HCl, H_2O, saturated $Na_2S_2O_5$, and brine, and dried ($MgSO_4$). Removal of the volatiles *in vacuo*, purification of the residue by flash chromatography using 50% ethyl acetate in hexanes as the eluent, and concentration of the product-rich fractions gave 3.92 g (86%) of the tan solid **46**. This material, iodomethane (0.952 g, 6.70 mmol), and DMPU (2 mL) in 24 mL of anhydrous THF was added to a suspension of NaH (60%, 0.135 g, 3.35 mmol) in 1 mL of THF at 0−5 °C. The mixture was warmed to room temperature, stirred for 2 h, acidified with aqueous HCl (5 mL of a 1 N solution), and diluted with ether and H_2O. The organic layer was washed with brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* and purification of the residue by flash chromatography using 50% ethyl acetate in hexanes as the eluent and concentration *in vacuo* of the product-rich fractions furnished 3.93 g (99%) of a yellow gum. ^{1}H NMR ($CDCl_3$): δ 7.7 (d, 4 H), 7.7−7.15 (m, 8 H), 6.89 (s, 1 H), 6.84 (s, 1 H), 6.72 (s, 1 H), 6.71 (s, 1 H), 6.70 (s, 1 H), 6.6 (s, 1 H), 5.1 (s, 2 H), 5.00 (s, 2 H), 4.79 (s, 2 H), 4.69 (s, 2 H), 4.59 (s, 2 H), 3.89 (s, 4 H), 3.86 (s, 2 H), 3.80 (s, 3 H), 3.77 (s, 3 H), 3.74 (m, 2 H), 3.73 (s, 3 H), 3.70 (s, 3 H), 3.61 (m, 2 H), 3.44 (m, 2 H), 3.39 (m, 2 H), 3.34 (s, 3 H), 3.28 (s, 3 H), 1.1 (s, 9 H). MS (FAB-pNBA): *m/z* 1185 (M + H).

2-[4-[4-[4-[4-[2-Methoxy-4-[[[(1,1-dimethylethyl)diphenylsilyl]oxy]methyl]-5-[[(2-methoxyethoxy)methyl]oxy]-benzyl]-2-methoxy-5-[(cyanomethyl)oxy]benzyl]-2-methoxy-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-2-methoxy-5-[(cyanomethyl)oxy]benzyl]-2-methoxy-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-4-(benzoyloxy)phenoxyacetonitrile (48). A mixture of Zn dust (1.43 g, 21.8 mmol) and 1,2-dibromoethane (0.103 g, 0.545 mmol) in 2 mL of anhydrous THF was heated at reflux for 3 min and cooled to 0−5 °C. A solution of **40** (1.25 g, 2.18 mmol) in 5 mL of THF was added dropwise over 90 min. After 15 min of stirring the supernatant was added to a mixture of **47** (1.80 g, 1.53 mmol) and $(Ph_3P)_4$ Pd (0.13 g) in 2 mL of THF. The mixture was heated at 70−80 °C overnight, cooled to room temperature, and diluted with ether and 5% NH_4OH. The organic layer was washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by flash chromatography using 50% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions gave 1.60 g (68%) of the tan solid. MS (FAB pNBA): *m/z* 1543 (M^+).

2-[4-[4-[4-[4-[2-Methoxy-4-(hydroxymethyl)-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-2-methoxy-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-2-methoxy-5-[(cyanomethyl)oxy]benzyl]-2-methoxy-5-[[(2-methoxyethoxy)methyl]oxy]benzy]-4-(benzoyloxy)phenoxyacetonitrile (49). In a manner similar to the preparation of **26**, **48** (3.35 g, 2.17 mmol) was treated with TBAF (3.26 mmol) and acetic acid (3.47 mmol) in 10 mL of THF for 2 h at room temperature. Workup and purification by flash chromatography using 25% hexanes in ethyl acetate as the eluent gave 2.00 g (71%) of white solid. ^{1}H NMR ($CDCl_3$): δ 8.15 (dd, 2 H), 7.61 (t, 1 H), 7.49 (t, 2 H), 7.1−6.95 (m, 3 H), 6.71 (bs, 3 H), 6.7−6.6 (m, 7 H), 5.16) (s, 2 H), 5.15 (s, 2 H), 5.11 (s, 2 H), 4.79 (s, 2 H), 4.65 (s, 2 H), 4.57 (s, 2 H), 4.55 (s, 2 H), 3.92 (s, 2 H), 3.89 (b s, 6 H), 3.85 (s, 2 H), 3.80 (s, 3 H), 3.75 (s, 6 H), 3.74 (s, 3 H), 3.72 (s,3 H), 3.5 (m, 6 H), 3.32 (s, 3 H), 3.31 (s, 3 H), 3.30 (s, 3 H). MS (FAB-pNBA): *m/z* 1305 (M^+).

2-[4-[4-[4-[4-[2-Methoxy-4-(bromomethyl)-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-2-methoxy-5-[(cyanomethyl)oxy]benzyl]-2-methoxy-5-[[(2-methoxyethoxy)-methyl]oxy]benzyl]-2-methoxy-5-[(cyanomethyl)oxy]benzyl]-2-methoxy-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-4-(benzoyloxy)phenoxyacetonitrile (50). In a manner similar to the preparation of **27**, **49** (1.95 g, 1.49 mmol) was treated with NBS (2.1 mmol) and triphenylphosphine (2.4 mmol) in 10 mL of THF, purified by flash chromatography

using 44% hexanes in ethyl acetate as the eluent, and gave 1.5 g (73%) of product. MS (FAB-pNBA): *m/z* 1369 (M + H).

2-4-[4-[4-[4-(2-Methoxy-4-methyl-5-[[(2-methoxyethoxy)-methyl]oxy]benzyl]-2-methoxy-5-[(cyanomethyl)oxy]benzyl]-2-methoxy-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-2-methoxy-5-[(cyanomethyl)oxy]benzyl]-2-methoxy-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-4-(benzoyloxy)phenoxyacetonitrile (51). A mixture of **50** (0.90 g, 0.66 mmol), tri-*n*-butyltin hydride (0.24 g, 0.792 mmol), and AIBN (12 mg) in 5 mL of anhydrous toluene was heated at reflux for 90 min, and the volatiles were removed *in vacuo*. Purification of the residue by flash chromatography using 44% hexanes in ethyl acetate as the eluent and concentration *in vacuo* of the product-rich fractions provided 0.7 g (82%) of white solid. ^{1}H NMR ($CDCl_3$): δ 5.15 (s, 2 H), 5.13 (s, 2 H), 5.11 (s, 2 H), 4.79 (s, 2 H), 4.57 (s, 2 H), 4.54 (s, 2 H), 3.76 (s, 3 H), 3.72 (s, 3 H), 3.71 (s, 3 H), 3.69 (s, 3 H), 3.68 (s, 3 H), 3.34 (s, 3 H), 3.32 (s, 3 H), 3.30 (s, 3 H), 2.21 (s, 3 H). MS (FAB-pNBA): *m/z* (M + H).

2-[4-[4-[4-[4-[2-Methoxy-4-methyl-5-[[(2-methoxyethoxy)-methyl]oxy]benzyl]-2-methoxy-5-[(cyanomethyl)oxy]benzyl]-2-methoxy-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-2-methoxy-5-[(cyanomethyl)oxy]benzyl]-2-methoxy-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-4-hydroxyphenoxyacetonitrile (52). In a manner similar to the preparation **29**, **51** (0.91 g, 0.71 mmol) was treated with sodium methoxide (0.71 mmol) in methanol and THF at −50 °C. Workup and purification by flash chromatography using 40% hexanes in ethyl acetate as eluent gave 0.66 g (80%) of the white solid. MS (FAB-pNBA): *m/z* 1186 (M + H).

2-[4-[4-[4-[4-[2-Methoxy-4-methyl-5-[[(2-methoxyethoxy)-methyl]oxy]benzyl]-2-methoxy-5-[(cyanomethyl)oxy]benzyl]-2-methoxy-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-2-methoxy-5-[(cyanomethyl)oxy]benzyl]-2-methoxy-5-[[(2-methoxyethoxy)methyl]oxy]benzyl]-4-methoxyphenoxyacetonitrile (53). To NaH (60%, 23 mg, 0.557 mmol) was added a solution of **52** (0.66 g, 0.557 mmol), iodomethane (0.158 g, 1.11 mmol), and DMPU (300 μL) in 5 mL of anhydrous THF. The mixture was stirred for 1 h, acidified with aqueous HCl (1 mL of a 1 N solution), and diluted with ether and H_2O. The organic layer was washed with brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by flash chromatography using 40% hexanes in ethyl acetate as the eluent. Concentration *in vacuo* of the product-rich fractions gave 0.61 g (91%) of the gummy product. ^{1}H NMR ($CDCl_3$): δ 6.95−6.65 (m, 13 H), 5.13 (s, 4 H), 5.12 (s, 2 H), 4.69 (s, 2 H), 4.58 (s, 2 H), 4.57 (s, 2 H), 3.88 (s, 6 H), 3.85 (s, 4 H), 3.77 (s, 3 H), 3.74 (s, 3 H), 3.73 (s, 3 H), 3.72 (s, 3 H), 3.71 (s, 3 H), 3.70 (s, 3 H), 3.5 (m, 6 H), 3.34 (s, 3 H), 3.33 (s, 3 H), 3.31 (s, 3 H), 2.22 (s, 3 H). MS (FAB-pNBA): *m/z* 1200 (M + H).

2-[4-[4-[4-[4-(2-Methoxy-4-methyl-5-hydroxybenzyl)-2-methoxy-5-[(cyanomethyl)oxy]benzyl]-2-methoxy-5-hydroxybenzyl]-2-methoxy-5-[(cyanomethyl)oxy]benzyl]-2-methoxy-5-hydroxbenzyl]-4-methoxy-phenoxyacetonitrile (54). A mixture of **53** (0.405 g, 0.338 mmol) and PPTS (0.312 g, 1.21 mmol) in acetone (2 mL) and 2-propanol (6 mL) was heated at 65 °C for 3 days, cooled to room temperature, diluted with ethyl acetate, washed with H_2O and brine, and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by flash chromatography using 50% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions gave 0.30 g (95%) of white solid. MS (FAB-pNBA): *m/z* 936 (M + H).

2-[4-[4-[4-[4-[2-Methoxy-4-methyl-5-[(cyclohexylmethyl)oxy]benzyl]-2-methoxy-5-[(cyanomethyl)oxy]benzyl]-2-methoxy-5-[(cyclohexylmethyl)oxy]benzyl]-2-methoxy-5-[(cyanomethyl)oxy]benzyl]-2-methoxy-5-[(cyclohexylmethyl)oxy]benzyl]-4-methoxyphenoxyacetonitrile (55). A mixture of **54** (47 mg, 0.0502 mmol), cyclohexylmethyl bromide (135 μL, 0.905 mmol), and K_2CO_3 (255 mg, 1.81 mmol) in anhydrous DMF (0.5 mL) was heated at 85 °C overnight, cooled to room temperature, diluted with ether, washed thoroughly with H_2O and brine, and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue, which was purified by flash chromatography using 25% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fraction gave 27 mg (44%) of a tan solid. ^{1}H NMR ($CDCl_3$): δ 6.9 (m, 1 H), 6.75−6.55 (m, 12 H), 4.68 (s, 2 H), 4.56 (s, 4 H), 3.88 (m, 6 H), 3.8−3.6 (m, 28 H), 2.20 (s, 3 H), 1.9−1.0 (m, 33 H). MS (FAB-pNBA): *m/z* 1223 (M^+).

***K*$_i$ Determinations.** All assays are based on the ability of the test compound to inhibit the enzyme-catalyzed hydrolysis of a peptide *p*-nitroanalide substrate. In a typical K_i determination substrate is prepared in DMSO and diluted into an assay buffer consisting of 50 mM HEPES and 200 mM NaCl at pH 7.5. The final concentration for each of the substrates is listed below. All substrate concentrations are at least 10-fold lower than the experimentally determined value for K_m. Test compounds are prepared as a 0.16 mg/mL solution in DMSO. Dilutions are prepared in DMSO, yielding eight final concentrations encompassing a 200-fold concentration range. Enzyme solutions are prepared at the concentrations listed below in assay buffer.

Inhibition kinetic studies of HLE activity with the test compound at a fixed concentration has been performed in the presence of varying concentrations of the peptide substrate. The velocity of the reaction was calculated as the increase of the absorbance at 405 nm/min. Double-reciprocal plots of the data were used to determine the apparent V_m and K_m values and therefore to analyze the mode of inhibition of the HLE activity by the compound. Figure 1 shows the result with **6**.

In a typical K_i determination, into each well of a 96-well plate were pipetted 280 μL of substrate solution and 10 μL of inhibitor solution, and the plate was allowed to thermally equilibrate at 37 °C in a Molecular Devices plate reader for >10 min. Reactions were initiated by the addition of a 10 μL aliquot of enzyme, and the absorbance increase at 405 nm is recorded for 15 min. Data corresponding to more than 90% of inhibition of substrate hydrolysis was used in the calculations. Using the protocol of I. H. Segel the ratio of the velocity (rate of the change in absorbance as a function of time) for a sample containing inhibitor is divided by the velocity of a sample containing no inhibitor and is plotted as a function of inhibitor concentration. The data is fit to the equation for a competitive inhibitor

$$v_i/v_c = K_i/(K_i + I) \quad \text{when} \quad [S] \ll K_m$$

and from the fit, values for K_i are determined (SD = ±0.90).

Elastase. Elastase activity was assesed as the ability to hydrolyze the substrate Suc-Ala-Ala-Pro-Phe-pNA. Substrate solutions were prepared at a concentration of 10 μM (10 μM < K_m = 200 μM). The final DMSO concentration was 0.3%. Purified human leukocyte elastase was diluted into assay buffer. Final reagent concentrations were [elastase] = 3 nM, [Suc-Ala-Ala-Pro-Phe-pNA] = 10 μM.

Thrombin. Thrombin activity was assesed as the ability to hydrolyze the substrate Bz-Phe-Val-Arg-pNA. Substrate solutions were prepared at a concentration of 60 μM (60 μM ≪ K_m = 1.2 mM). The final DMSO concentration was 0.3%. Purified human α-thrombin was diluted into assay buffer to a concentration of 450 nM. Final reagent concentrations were [thrombin] = 30 nM, [Bz-Phe-Val-Arg-pNa] = 45 μM.

Cathepsin G. Cathepsin G activity was assesed as the ability to hydrolyze the substrate Suc-Ala-Ala-Pro-Phe-pNA. Substrate solutions were prepared at a concentration of 22 μM (22 μM ≪ K_m = 240 μM) in assay buffer. The final DMSO concentration was 0.3%. Purified Cathepsin G was diluted into assay buffer to a concentration of 225 nM. Final reagent concentrations were [cathepsin G] = 15 nM, [Suc-Ala-Ala-Pro-Phe-pNA] = 22 μM.

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