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Incorporation of Carbohydrates and Peptides into Large Triazine-Based Screening Libraries Using Automated Parallel Synthesis

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Abstract: A procedure for the sequential, selective derivatization of cyanuric chloride that allows for the incorporation of carbohydrates and peptides has been elucidated. As a result, large combinatorial arrays of individual derivatives, over 40,000 in all, have been produced in 50μmole quantities using automated parallel solution phase synthesis. The use of this technology in a search for protease inhibitors, glycopeptide surrogates and other bioactive compound classes will also be discussed.

INTRODUCTION

The use of parallel synthesis for the production of large arrays of individual compounds is gaining wide acceptance because of its dual applicability to both lead discovery and accelerated lead optimization. The underlying precepts of parallel synthesis are listed below.[1]

- Geometrical expansion of both the number and diversity of compounds in a product or intermediate set is readily achieved by matrix combination of a row of functionally identical but structurally diverse building blocks with a column of diverse building blocks bearing complementary functionality, thereby introducing multiple parallel processing into the synthesis of single compounds.
- Precisely defined criteria must be applied to the selection of building blocks. These criteria address such issues as the yield and purity expectations of the reaction involved, the number and type of unit operations associated with the chemistry, the nature and quantity of co-products, spent reagents, catalysts, etc., left in the product, and the pharmacophoric nature and availability of building blocks .
- When framed in terms of a repeating set of unit operations, synthetic organic chemistry can be adapted to laboratory robotics - with enormous gain in function.
- Analysis, without which synthesis is meaningless, can be readily integrated into robotic synthesis as an on-line QC function with automated data acquisition and storage.
- The 96-well microtiter plate which serves as the foundation of high throughput data management can serve equally well as the basis for chemical data management. When arrayed in numbered 96-well plates, organic compounds acquire a new nomenclature derived from plate numbers and row-column intercepts - descriptors which are ideally suited to electronic storage and retrieval of structural databases.

Using these principles, large compound arrays from a wide range of reaction types and compound classes are created in which each array member shares some structural homology with its neighbors - a fact that leads to instant SAR insights when biologically active compounds are found in the array. The system becomes still more powerful when the array design incorporates structural motifs known to interact with specific types of disease-related biomolecules. The rest of this paper describes the automated parallel synthesis of over 40,000 compounds to date that include arrays containing carbohydrates and peptides, molecules known to be involved in protein/protein interactions. Additional arrays have been synthesized that incorporate aminimides and α-ketoamides, molecules known to be protease inhibitors.

We have developed a simple and efficient route for the production of a large array of individual compounds with the potential of interfering with specific, biologically-relevant functions. Our approach was to sequentially and selectively displace chlorine atoms from cyanuric chloride with molecules capable of targeting and, then, interfering with various protein dependent biological functions. Cyanuric chloride is a commercially

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available reagent that is inexpensive (1 kg, $25). Although previous work exists in the derivatization of cyanuric chloride[2-6], none of these studies have incorporated molecules such as carbohydrates, peptides, aminimides and α -ketoamides or have done so in a large array format that produces a single compound per reaction in high purity.

RESULTS AND DISCUSSION

Optimization of the variables of time, temperature, solvent and base for the trisubstitution of cyanuric chloride with amines is shown in Figure 1. By simply controlling the temperature, sequential, selective derivatization could be accomplished using N,N-diisopropylethylamine (DIPEA) as a base and acetonitrile as a solvent. Anilines were found to be a good class of amines to use in the first displacement due to the very reactive nature of the cyanuric chloride. Even anilines, if they were electron rich (ie.*p*-anisidine), produced small amounts of disubstituted product if the temperature of the reaction was allowed to rise above -20°C. The second substitution proceeds at room temperature and in the vast majority of cases the product is stable enough to sit for long periods due to the relative unreactivity of the third position. The third position requires heating to 80°C for a period of 5 hours. Due to the volatility of acetonitrile, the reactions are capped or dioxane is added to serve as solvent. Under these conditions, only secondary amines lead to complete displacement of the chloride to yield products of the type **II**. Primary amines give incomplete reaction.

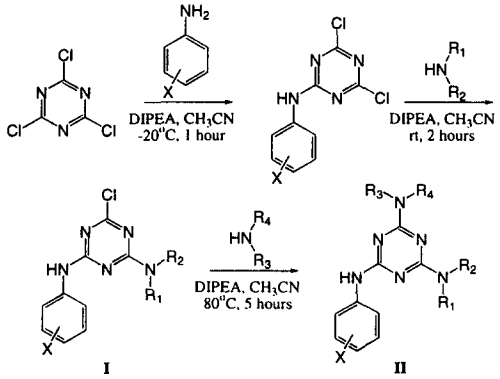


Figure 1

The viability of these conditions using automated parallel synthesis was proven in the construction of an array where 40 anilines (list 1) were crossed with 160 amines (list 2) to produce a disubstituted product array (Figure 1). The resulting array (**I**) of 6400 compounds had an average purity greater than 85% as determined by mass spectral analysis and reverse phase HPLC (UV detection). The entire process, from starting the array to completion of purity assessment, took just over three weeks.

The ability to produce a trisubstituted array was demonstrated by the construction of an array where 20 anilines (list 3) were crossed with 16 primary amines (list 4), followed by 20 secondary amines (list 5) used as the third constituent. This yielded an array (**II**) of 12,800 compounds.

Having shown that di and trisubstituted arrays could be constructed using the procedure as depicted in Figure 1, the incorporation of more biologically relevant molecules was investigated. The ability to incorporate carbohydrates in an automated format was first accomplished with an array using 1-deoxy-1-(methylamino)-D-galactitol in the third substitution on the triazine (Figure 2). The array was similar to the previous array having 40 anilines (list 1) crossed with 160 amines (list 2). The reduced aminosugar was then added as the third component to yield an array (**III**) of 6400 compounds. The production of this library also demonstrated that solvents other than acetonitrile could be tolerated. The solubility of the sugar was minimal in acetonitrile, so the sugar was added in a small amount of water. The modified procedure successfully produced the desired product without any detectable hydrolytic side products.

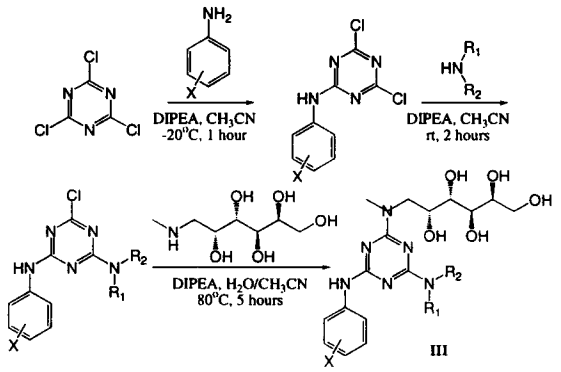


Figure 2

The commercially available nitrophenylpyranosides provided a way to incorporate sugars in the first step of the substitution scheme. Nitrophenylpyranosides are easily reduced to aminophenylpyranosides using palladium on carbon and hydrogen. The aminophenylpyranosides are functionally equivalent to an aniline for the purpose of the substitution reaction and, as such, could be used in the first step of the procedure. The insolubility of aminophenylpyranosides in acetonitrile required the discovery of another aprotic solvent that could be successfully used in this procedure. The high reactivity of cyanuric chloride precludes the use of water. It was found that if the carbohydrate was dissolved in minimal DMSO, the procedure worked effectively under the previously determined conditions for temperature, time and base (Figure 3). Eight commercially available nitrophenyl pyranosides were found. An array (**IV**) using 8 of these pyranosides (list 6) crossed with 160 amines (list 2) has been synthesized.

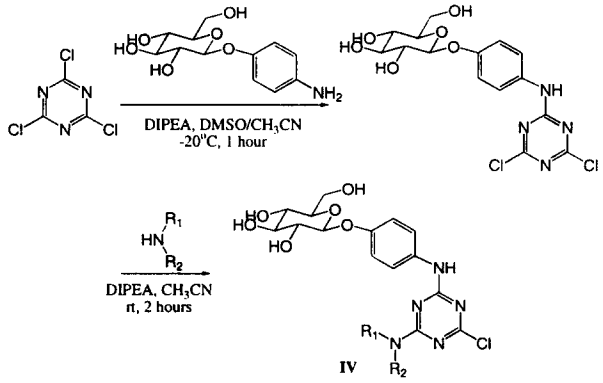


Figure 3

Having accomplished the incorporation of carbohydrates into triazine arrays, the addition of peptides was investigated. The original procedure only had to be modified slightly. For this array (**V**), the first substitution was performed with an aniline (list 1) as discussed previously. The peptide could be dissolved in minimal water and added as the second constituent (Figure 4). Dipeptides containing an amide at the carboxyl terminus were the reagents of choice (list 7). Because the dipeptides all contain primary amines at the amino terminus, this necessitated adding them as the second constituent to obtain an acceptable conversion. Peptides

have been successfully incorporated into compounds containing anilines as the first constituent and either alkylamines or aminosugars as the third component.

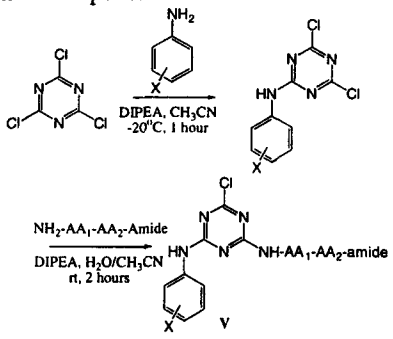


Figure 4

Sugars and peptides were incorporated into triazine derivatives because they are known recognition elements in peptide/protein and peptide/cell wall interactions. By including these molecules in the arrays, the potential for producing compounds that interfere in these biological interactions is enhanced. Another approach to producing inhibitors of specific interactions is to include molecules that are already proven to be inhibitors and modulating their activity through introduction of other substituents onto the triazine. At ArQule, we have expertise in the production of two classes of protease inhibitors: aminimides and α-ketoamides. Aminimides and α -ketoamides are both peptide mimetics. We have produced aminimides that have been shown by collaborators to have activity against both HIV-protease and elastase[7-8]. They were designed to be transition state analog inhibitors. In order to be incorporated into a triazine array (**VI**), a series of aminimides containing anilines was synthesized. A cross of 7 aminimides (list 8) with 160 amines (list 2) as the second constituent was accomplished. Minimal DMSO served as the solvent for the aminimides as their solubility in acetonitrile was low (Figure 5). The aminimides have also successfully been crossed with dipeptides. It was envisioned that by incorporating a peptide whose sequence mimics that of a known protease substrate in the scissile bond region, the aminimde could be more effectively delivered to the catalytic domain of a protease. Such compounds may be more active than just the aminimides would be alone.

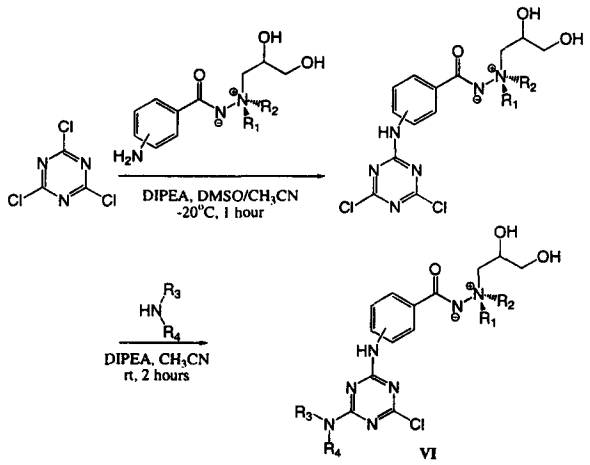


Figure 5

α -Ketoamides have been shown to be effective inhibitors of several proteases[9-11]. The mode of inhibition by α -ketoamides in the case of serine proteases is the production of a stable enzyme-substrate complex. The serine hydroxyl adds to the active carbonyl of the α -ketoamide and the complex, although reversible, is thermodynamically stable. Other researchers at ArQule have developed a facile and efficient synthesis of mono-α -ketoamides from various types of diamines. The procedure for array production proceeds much the same as it does for the aminimides. The α -ketoamides have anilines incorporated into their structure. They are dissolved into minimal DMSO and used to perform the first substitution (Figure 6). Both amines and dipeptides have been effectively brought in as second substituents. An automated array (**VII**) crossing 60 α -ketoamides (list 9) with 160 amines (list 2) to yield 9600 derivatives has been produced. An array crossing 20 α -ketoamides (list 10) with 32 dipeptides (list 7) produced an array (**VIII**) of putative protease inhibitors with enhanced recognition factors.

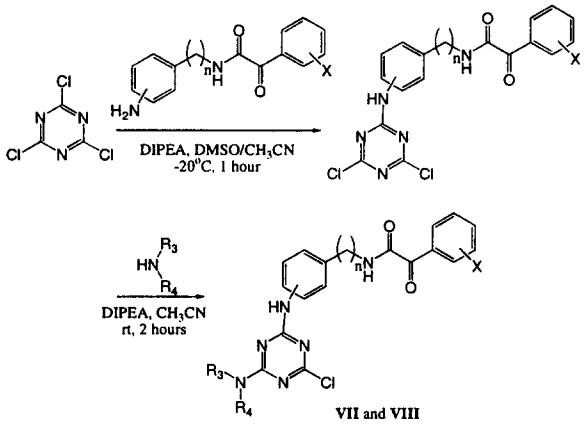
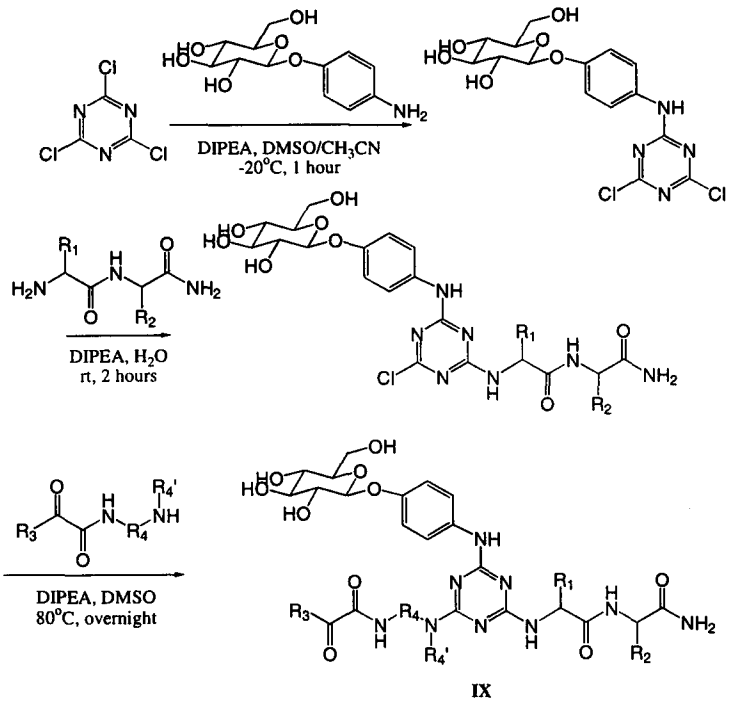


Figure 6

In the final array to be discussed, we wanted to see if it was possible to incorporate both sugars and peptides into the same array. This could be useful for a number of reasons. The presence of both a sugar and a peptide in a molecule could serve as a more efficacious recognition factor in the delivery of the protease inhibitors discussed. In addition, a molecule containing both a sugar and a peptide would be a potential glycopeptide mimic. Glycopeptides are found on the surface of many membrane and serum proteins. By synthesizing small molecules containing glycopeptide mimics one could inhibit various protein/protein interactions.[12] In this array (**IX**, Figure 7), the sugars (list 6) in DMSO performed the first substitution. The dipeptides (list 11) in water were brought in second and the α-ketoamides (list 12) in DMSO were used in the third substitution.

Two possible modes of action are envisioned for the arrays incorporating either aminimides or α -ketoamides with peptides. As previously discussed, the peptide can be used as a recognition element to deliver the inhibitory component to the appropriate locus of action. The aminimides and α -ketoamides can then inhibit through their proven modes of action. Another possibility arises with the presence of the triazine core. Once in the active site, the protein could add to the third position. We have shown that the third position can be substituted, although stringent conditions are necessary. In the close confines of the active site, an appropriately situated nucleophile could displace the chloride.

Once the arrays were prepared, they were checked by both MS and HPLC. The data for the arrays **I-IX** is presented in Table 1. The HPLC data is summarized as an average percent purity based on ELSD. The number for the MS data shows what percent of the samples had the correct molecular ion present. Some representative members of various arrays are shown in Figure 8.



IX

Figure 7

Array	# of Compounds Analyzed	Average % Purity (ELSD)	% Molecular Ion Present (MS)
I	**1600**	**85.2**	**98.5**
II	**3200**	**84.8**	**98.3**
III	**1600**	**84.6**	**95.6**
IV	**320**	**85.9**	**97.2**
V	**400**	**84.6**	**97.3**
VI	**262**	**86.7**	**100**
VII	**2400**	**85.3**	**97.7**
VIII	**160**	**84.9**	**97.2**
IX	**320**	**72.1**	**94.2**

Table 1. Summary of the Analyses for Arrays **I-IX**

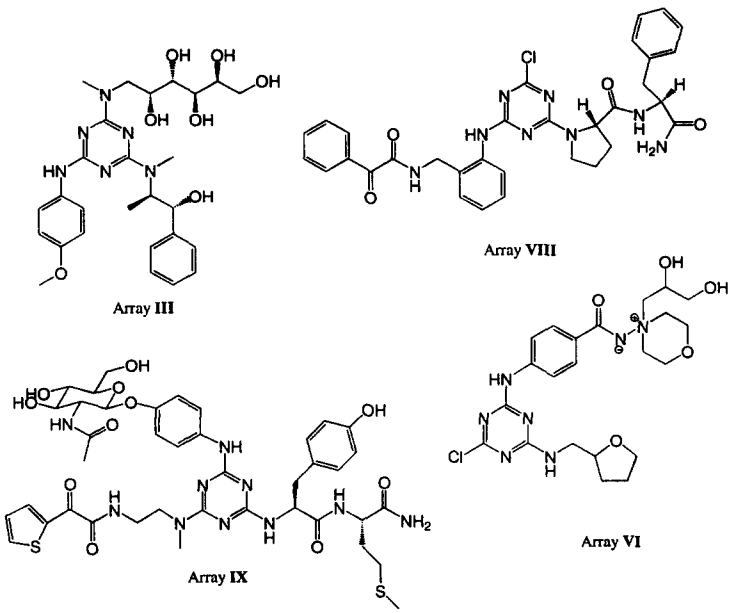


Figure 8

High throughput assays were used to screen these libraries for inhibition of the proteases Factor Xa and plasmin. Not only were compounds found that inhibit the proteases but there appears to be some indication of selectivity between the proteases dependent on the array componenets. Array **I** which crossed anilines and amines and array **VII** which crossed α -ketoamides and amines both contained compounds that demonstrated greater than 50% inhibition of plasmin at a concentration of 10μM (table 2). When these libraries were screened against Factor Xa almost no active compounds were found. Conversely, when array **VI** was screened 25 hits showed greater than 50% inhibition and 2 greater than 75% inhibition of Factor Xa while no actives were seen in the plasmin assay. The most potent inhibitor of Factor Xa from array **VI** was shown to have a 700 nM Ki. The activities of these libraries against these proteases are notable, especially for a first pass. A thorough study of the SAR resulting from the screening of these two libraries will be used in the construction of second generation libraries that will seek to produce even more potent inhibitors.

Array	Compounds Tested	Factor Xa Inhibition	Plasmin Inhibition
I	1280	0 hits > 25%	27 hits > 25% 4 hits > 50%
VI	1200	129 hits >25% 25 hits > 50% 2 hits > 75%	0 hits > 25%
VII	640	3 hits > 25%	168 hits > 25% 32 hits > 50%

Table 2. Summary of Biological Activity of Selected Arrays

In conclusion, a procedure has been developed for the sequential, selective derivatization of cyanuric chloride with functionally diverse sets of amines using automated parallel solution phase synthesis. This procedure produces large combinatorial arrays in a 96-well format with a single compound per well in high

purity. The 96-well format allows the arrays to proceed directly to a high throughput screening effort. This 96-well format also serves as the basis of the chemical data management system which allows for the rapid retrieval of structural information and formulation of SAR. The procedure for derivatization of cyanuric chloride successfully incorporated carbohydrates, peptides, aminimides and α -ketoamides into various arrays. The arrays containing these molecules are potentially therapeutically relevant as was shown by the activity in the various assays for protease inhibitors. The ease with which the initial procedure was adapted to incorporate these different molecules demonstrates its versatility and potential for the incorporation of other therapeutically relevant moieties that contain amines.

Reagent Lists

List 1

3-(1-Hydroxyethyl)aniline
3-Fluoro-*p*-anisidine
3-Methoxy-5-(trifluoromethyl)aniline
2-Amino-5-methylbenzylalcohol
5-Methoxy-2-methyl-aniline
5-Aminoindan
4-Morpholinoaniline
2-Benzylaniline
4-Methoxy-2-methyl-aniline
3-Chloroaniline
Aniline
p-Toluidine
1-Aminonaphthalene
5-Phenyl-*o*-anisidine
o-Phenetidine
2-Phenoxyaniline
2-Aminofluorene
4-Phenoxyaniline
4-Aminophenethyl alcohol
2,4-Dimethylaniline
p-Anisidine
m-Phenetidine
3-Fluoro-4-methylaniline
3-Amino-4-methylbenzylalcohol
3,5-Dimethoxyaniline
3,4-Dimethylaniline
2-Methoxy-5-methylaniline
2,5-Dimethoxyaniline
2-Aminonaphthalene
4-Cyclohexylaniline
3-Benzyloxyaniline
3,4-(Methylenedioxy)-aniline
3-Phenoxyaniline
2,4-Dimethoxyaniline
4-(Trifluoromethyl)aniline
4-Butoxyaniline
m-Anisidine
m-Toluidine
o-Anisidine
p-Phentidine

List 2

2-(2-Aminoethoxy)ethanol
Phenethylamine
2,3-Dimethylcyclohexylamine
2-Methylcyclohexylamine
3-Ethoxypropylamine
2-Methoxybenzylamine
4-Fluorobenzylamine
3-(Dibutylamino)-propylamine
4-Methoxybenzylamine
Ethanolamine
4-Methoxyphenethylamine
3-Methoxyphenethylamine
1,2,3,6-Tetrahydropyridine
1-(3-Aminopropyl)-2-pipecoline
4-Methylbenzylamine
3,4-Dimethoxyphenethylamine
3,4,5-Trimethoxybenzylamine
β-Methylphenethylamine
2-(1-Cyclohexenyl)ethylamine
3-Fluorophenethylamine
2,3-Dimethoxybenzylamine
2-Fluorophenethylamine
3-Phenyl-1-propylamine
3-Methylbenzylamine
2-(Trifluoromethyl)benzylamine
4-(Trifluoromethyl)benzylamine
1-(1-Naphthyl)ethylamine
3-Dimethylaminopropylamine
2-Amino-1-methoxypropane
2-(Ethylamino)ethanol
3-Amino-1-propanol
4-Fluorophenethylamine
1-Benzylpiperazine
(+-)-3-Amino-1,2-propanediol
3-Fluorobenzylamine
3,5-Dimethoxybenzylamine
5-Amino-1-pentanol
3,3-Diphenylpropylamine
(+-)-*exo*-2-Aminonorbornane
(S)-(+)-Isoleucinol
(+-)-2-Amino-3-methyl-1-butanol
3-Diethylaminopropylamine

1-Naphthalenemethylamine
2,4-Difluorobenzylamine
2-Methylbenzylamine
4-Amino-1-butanol
(S)-(+)-Leucinol
Cyclobutylamine
2-Amino-5-diethylaminopentane
DL-2-Amino-1-propanol
1-Aminoindan
Tetrahydrofurfurylamine
(+-)-α-Methylbenzylamine
1,2,3,4-Tetrahydro-1-naphthylamine
2-Methoxyethylamine
Cyclopentylamine
DL-1-Amino-2-propanol
1-Ethylpropylamine
2,2-Diphenylethylamine
Piperonylamine
1-Methyl-3-phenylproplylamine
4-Benzypiperidine
2-Aminobutanol
3-Methoxypropylamine
Benzylamine
Veratrylamine
Cyclohexylamine
N-Benzylmethylamine
N-Butylbenzylamine
Cyclohexanemethylamine
3-(Trifluoromethyl)benzylamine
1,2,3,4-Tetrahydroisoquinoline
Allylamine
N-Methylisoproplyamine
1,2-Diphenylethylamine
2-Amino-2-methyl-1-propanol
N-Methylproplamine
3,3-Dimethylpiperidine
4-Amino-1-benzylpiperidine
1,3,3-Trimethyl-6-azabicyclo[3.2.1]octane
3-Piperidinemethanol
2-Ethoxybenzylamine
N-Ethylbutylamine
N-Methylphenethylamine
Decahydroquinoline
Dipropylamine
(1R,2S)-(-)-Ephedrine
1-(2-Aminoethyl)piperidine
1-(α,α,α-Trifluoro-m-tolyl)piperazine
1-Phenylpiperazine
N-Propylcyclopropanemethylamine
1-(4-Fluorophenyl)-piperazine
N-Isopropylbenzylamine
3-Pyrrolidinol
3-Isopropoxypropylamine
N-Ethylcyclohexylamine
1-(2-Methoxyphenyl)-piperazine
4-Methylpiperidine
Cyclopropylamine
4-Hydroxypiperidine
(1R,2R)-(-)-Psuedoephedrine
Diethylamine
2-Amino-1-phenylethanol
(1S,2R)-(+)-Norephedrine
2-Methylaminomethyl-1,3-dioxolane
1-(2-Aminoethyl)-pyrrolidine
Thiomorpholine
(+-)-2-Piperidinemethanol
Bis(2-methoxyethyl)amine
2-Ethylpiperidine
1-(2-Hydroxyethyl)piperazine
1-Piperonylpiperazine
N-Ethylbenzylamine
α-(Methylaminomethyl)benzyl alcohol
Diethanolamine
3-Methylpiperidine
3,5-Dimethylpiperidine
1-Methylpiperazine
Piperidine
Diisopropanolamine
1,2,3,4-Tetrahydroquinoline
(Aminomethyl)cyclopropane
Dibenzylamine
Diallylamine
Morpholine
N-Benzylethanolamine
2,6-Dimethylmorpholine
Hexamethyleneimine
1-Methyl-4-(methylamino)piperidine
N-Benzyl-2-phenethylamine
4-Methylpiperidine
2,5-Difluorobenzylamine
1,4-Benzodioxan-6-amine
Serinol
N-Ethyl-2-methylallylamine
N-Methylallylamine
2-Fluorobenzylamine
N,N, N'-Trimethyl-1,3-propanediamine
N,N-Dimethylethylenediamine
N,N-Diethylethylenediamine
N,N-Diethyl-1,4-phenylenediamine
N,N,N'-Triethylenediamine
N'-Benzyl-*N,N*-dimethylethylenediamine
1-(4-Chlorophenyl)-2-methypiperazine
1-(2-Pyridyl)piperazine
Ethyl 1-piperazine carboxylate
1(-4-Methylphenyl)2-methylpiperazine
1-(2-Methoxyphenyl)piperazine
1-(2,6-Dimethylphenyl)piperazine
4-Benzyloxyaniline HCl
1-(2-Cyanophenyl)piperazine
1-(3,4,5-Trimethoxyphenyl)piperazine HCl
1-(2-Nitrophenyl)piperazine
1-(4-Methoxyphenyl)-2-methylpiperazine

1-(3-Trifluoromethyl-4-chlorophenyl)-piperazine
1-(4-Fluorophenyl)piperazine
1-(4-Ethoxphenyl)piperazine
1-(4-Chlorophenyl)piperazine
1-(2,4-Dimethoxyphenyl)piperazine
2-Methyl-1-(3-methylphenyl)piperazine

List 3

Aniline
3-Chloroaniline
3-(1-Hydroxyethyl)aniline
2-Amino-5-methylbenzylalcohol
5-Aminoindan
4-Morpholinoaniline
3,5-Dimethoxyaniline
2-Aminonaphthalene
3-Benzyloxyaniline
3,4-(Methylenedioxy)aniline
4-(Trifluoromethyl)aniline
p-Toluidine
p-Anisidine
2-Phenoxyaniline
3-Fluoro-4-methylaniline
2-Aminofluorene
4-Phenoxyaniline
4-Aminophenethyl alcohol
m-Anisidine
p-Phentidine

List 4

Tyramine
4-Methoxyphenethylamine
1,2,3,4-Tetrahydroisoquinoline
Benzyl amine
Allylamine
3-Hydroxypiperidine HCl
Cyclohexylamine
3-Dimethylaminopropylamine
Dipropylamine
Serinol
(+-)-3-Amino-1,2-propanediol
Cyclopropylamine
2-(2-Aminoethoxy)ethanol
Decahydroquinoline
1,2,3,4-Tetrahydro-1-naphthylamine
4-Methoxybenzylamine
4-(Trifluoromethyl)benzylamine
Tetrahydrofurfurylamine
1-(2-Aminoethyl)-pyrrolidine
(+-)-*exo*-2-Aminonorbornane
1-(3-Aminopropyl)-2-pipecoline
1-(2-Aminoethyl)piperidine
1-Aminoindan
4-Amino-1-benzyl-piperidine
1,2,3,4-Tetrahydroquinoline
4-Fluorobenzylamine
3-Fluorophenethylamine
3,3-Diphenylpropylamine
(S)-(+)-Leucinol
(1R,2R)-(-)-Psuedoephedrine
(1S,2R)-(+)-Norephedrine
1,4-Benzodioxan-6-amine

List 5

1-Phenylpiperazine
N-Benzylmethylamine
1-(2-Hydroxyethyl)piperazine
Piperidine
Morpholine
Diethanolamine
Bis(2-methoxyethyl)amine
Diallylamine
N-Methylphenethylamine
1-(4-Fluorophenyl)-piperazine
Piperonylamine
(+-)-2-Piperidinemethanol
4-Hydroxypiperidine
1-Piperonylpiperazine
3-Pyrrolidinol
1-Methyl-4-(methylamino)piperidine
1-(4-Methoxyphenyl)-2-Methylpiperazine
1-(2-Cyanophenyl)piperazine
N,N,N'-Triethylenediamine
α-(Methylaminomethyl)benzylalcohol

List 6

p-Nitrophenyl-β-D-glucopyranoside
p-Nitrophenyl-β-D-galactopyranoside
p-Nitrophenyl-α-D-glucopyranoside
p-Nitrophenyl-α-D-galactopyranoside
p-Nitrophenyl-2-deoxy-2-acetamido-β-D-glucopyranoside
p-Nitrophenyl-α-L-arabinopyranoside
o-Nitrophenyl-β-D-glucopyranoside
o-Nitrophenyl-β-D-galactopyranoside

List 7

Amine hyp val amide HCl
Amine ser pro amide HCl
Amine arg leu amide HCl
Amine glu leu amide HCl
Amine hyp leu amide HCl
Amine phe leu amide HCl
Amine pro leu amide HCl
Amine sar leu amide HCl
Amine tyr leu amide HCl
Amine glu phe amide HCl
Amine leu phe amide HCl

Amine pro phe amide HCl
Amine sar phe amide HCl
Amine ser phe amide HCl
Amine thr phe amide HCl
Amine val phe amide HCl
Amine ala sar amide HCl
Amine glu sar amide HCl
Amine gly sar amide HCl
Amine ile sar amide HCl
Amine leu sar amide HCl
Amine met sar amide HCl
Amine phe sar amide HCl
Amine trp sar amide HCl
Amine val sar amide HCl
Amine asp thr amide HCl
Amine glu thr amide HCl
Amine ile thr amide HCl
Amine leu thr amide HCl
Amine met thr amide HCl
Amine pro thr amide HCl
Amine sar thr amide HCl

List 8

(4-Aminophenyl)-*N*-[(2,3-dihydroxypropyl)dimethylamino]formamide
(4-Aminophenyl)-*N*-[4-(2,3-dihydroxypropyl)morpholin-4-yl]formamide
(3-Aminophenyl)-*N*-[(2,3-dihydroxypropyl)dimethylamino]formamide
(3-Aminophenyl)-*N*-[(2,3-dihydroxypropyl)piperidyl]formamide
(2-Aminophenyl)-*N*-[(2,3-dihydroxypropyl)dimethylamino]formamide
(2-Aminophenyl)-*N*-[(2,3-dihydroxypropyl)piperidyl]formamide
(2-Aminophenyl)-*N*-[4-(2,3-dihydroxypropyl)morpholin-4-yl]formamide

List 9

N-[2-(4-Aminophenyl)methyl]benzoylformamide
N-[2-(4-Aminophenyl)ethyl]benzoylformamide
N-[2-(Aminophenyl)ethyl]benzoylformamide
N-[2-(2-Aminophenyl)methyl]benzoylformamide
N-[2-(2-Amino-6-fluorophenyl)methyl]benzoylformamide
N-[2-(4-Aminophenyl)methyl]-3-methylbenzoylformamide
N-[2-(4-Aminophenyl)ethyl]-3-methylbenzoylformamide
N-[2-(Aminophenyl)ethyl-3-methylbenzoylformamide]
N-[2-(2-Aminophenyl)methyl]-3-methylbenzoylformamide
N-[2-(2-Amino-6-fluorophenyl)methyl]-3-methylbenzoylformamide
N-[2-(4-Aminophenyl)methyl]-4-*tert*-butylbenzoylformamide
N-[2-(4-Aminophenyl)ethyl]-4-*tert*-butylbenzoylformamide
N-[2-(Aminophenyl)ethyl]-4-*tert*-butylbenzoylformamide
N-[2-(2-Aminophenyl)methyl]-4-*tert*-butylbenzoylformamide
N-[2-(2-Amino-6-fluorophenyl)methyl]-4-*tert*-butylbenzoylformamide
N-[2-(4-Aminophenyl)methyl]cyclopropanoylformamide
N-[2-(4-Aminophenyl)ethyl]cyclopropanoylformamide
N-[2-(Aminophenyl)ethyl]cyclopropanoylformamide
N-[2-(2-Aminophenyl)methyl]cyclopropanoylformamide
N-[2-(2-Amino-6-fluorophenyl)methyl]cyclopropanoylformamide
N-[2-(2-Aminophenyl)methyl]-3-methyl-2-oxo-butanamide
N-[2-(2-Aminophenyl)methyl]-2-oxo-4-phenylbutanamide
N-[2-(2-Aminophenyl)methyl]-4-bromobenzoylformamide
N-[2-(2-Aminophenyl)methyl]-4-phenylbenzoylformamide
N-[2-(2-Aminophenyl)methyl]-3-fluorobenzoylformamide
N-[2-(2-Aminophenyl)methyl]-4-methoxybenzoylformamide
N-[2-(4-Aminophenyl)methyl]-3-methyl-2-oxo-butanamide
N-[2-(4-Aminophenyl)methyl]-2-oxo-4-phenylbutanamide
N-[2-(4-Aminophenyl)methyl]-4-bromobenzoylformamide
N-[2-(4-Aminophenyl)methyl]-4-phenylbenzoylformamide
N-[2-(4-Aminophenyl)methyl]-3-fluorobenzoylformamide
N-[2-(4-Aminophenyl)methyl]-4-methoxybenzoylformamide
N-[2-(4-Aminophenyl)ethyl]-3-methyl-2-oxo-butanamide
N-[2-(4-Aminophenyl)ethyl]-2-oxo-4-phenylbutanamide
N-[2-(4-Aminophenyl)ethyl]-4-bromobenzoylformamide
N-[2-(4-Aminophenyl)ethyl]-4-phenylbenzoylformamide
N-[2-(4-Aminophenyl)ethyl]-3-fluorobenzoylformamide
N-[2-(4-Aminophenyl)ethyl]-4-methoxybenzoylformamide
N-[2-(Aminophenyl)ethyl]-3-methyl-2-oxo-butanamide
N-[2-(Aminophenyl)ethyl]-2-oxo-4-phenylbutanamide
N-[2-(Aminophenyl)ethyl]-4-bromobenzoylformamide
N-[2-(Aminophenyl)ethyl]-4-phenylbenzoylformamide
N-[2-(Aminophenyl)ethyl]-3-fluorobenzoylformamide
N-[2-(Aminophenyl)ethyl]-4-methuxybenzoylformamide
N-[2-(2-Aminophenyl)methyl]-4-nitrobenzoylformamide
N-[2-(4-Aminophenyl)methyl]-4-nitrobenzoylformamide
N-[2-(4-Aminophenyl)ethyl]-4-nitrobenzoylformamide
N-[2-(Aminophenyl)ethyl]-4-nitrobenzoylformamide
N-[2-(2-Aminophenyl)methyl]-4,4-dimethyl-2-oxopentanamide
N-[2-(4-Aminophenyl)methyl]-4,4-dimethyl-2-oxopentanamide
N-[2-(4-Aminophenyl)ethyl]-4,4-dimethyl-2-oxopentanamide
N-[2-(Aminophenyl)ethyl]-4,4-dimethyl-2-oxopentanamide
N-[2-(2-Aminophenyl)methyl]-3-methoxybenzoylformamide
N-[2-(2-Aminophenyl)methyl]-4-methylbenzoylformamide
N-[2-(4-Aminophenyl)ethyl]-3-methoxybenzoylformamide
N-[2-(4-Aminophenyl)ethyl]-4-methylbenzoylformamide
N-[2-(4-Aminophenyl)methyl]-3-methoxybenzoylformamide
N-[2-(4-Aminophenyl)methyl]-4-methylbenzoylformamide
N-[2-(Aminophenyl)ethyl]-3-methoxybenzoylformamide
N-[2-(Aminophenyl)ethyl]-4-methylbenzoylformamide

List 10

N-[2-(4-Aminophenyl)ethyl]pyruvamide
N-[2-(4-Aminophenyl)ethyl]-2-oxo-4-phenylbutanamide
N-[2-(4-Aminophenyl)ethyl]benzoylformamide
N-[2-(4-Aminophenyl)ethyl]-3-methyl-2-oxo-butanamide
N-[2-(4-Aminophenyl)ethyl]-3-methoxybenzoylformamide

N-[2-(4-Aminophenyl)ethyl]-3,4-(isopropylidenyl)benzoylformamide
N-[2-(Aminophenyl)ethyl]benzoylformamide
N-[2-(Aminophenyl)ethyl]-3-methyl-2-oxo-butanamide
N-[2-(Aminophenyl)ethyl]-3-methoxybenzoylformamide
N-[2-(Aminophenyl)ethyl]-3,4-(isopropylidenyl)benzoylformamide
N-[2-(4-Aminophenyl)ethyl]-4-methoxybenzoylformamide
N-[2-(4-Aminophenyl)ethyl]-2-(2-benzofuryl)-2-oxoacetamide
N-[2-(Aminophenyl)ethyl]-2-(2-thienyl)-2-oxoacetamide
N-[2-(Aminophenyl)ethyl]cyclopropanoylformamide
N-[2-(Aminophenyl)ethyl]-3-methoxybenzoylformamide
N-[2-(Aminophenyl)ethyl]-3-fluorobenzoylformamide
N-[2-(2-Amino-6-fluorophenyl)methyl]-2-(2-thienyl)-2-oxoacetamide
N-[2-(2-Amino-6-fluorophenyl)methyl]cyclopropanoylformamide
N-[2-(2-Amino-6-fluorophenyl)methyl]-3-methoxybenzoylformamide
N-[2-(2-Amino-6-fluorophenyl)methyl]-3,4-(isopropylidenyl)benzoylformamide

List 11

Amine leu val amide HCl
Amine leu gly amide HCl
Amine ile met amide HCl
Amine ser ile amide HCl
Amine thr ile amide HCl
Amine ile val amide HCl
Amine phe val amid HCl
Amine tyr met amide HCl
Amine ser met amide HCl
Amine leu ala amide HCl
Amine pro gly amide HCl
Amine ala pro amide HCl
Amine tyr pro amide HCl
Amine ala phe amide HCl
Amine ser phe amide HCl
Amine phe thr amide HCl

List 12

N-[2-(4-Aminophenyl)ethyl]cyclopropanoylformamide
N-[2-(1-Piperazino)ethyl]cyclopropanoylformamide
N-[2-(1-Piperazino)ethyl]-4-methoxybenzoylformamide
N-[2-(Aminomethyl)ethyl]-2-(2-thienyl)-2-oxoacetamide
N-[2-(Aminomethyl)ethyl]cyclopropanoylformamide]
N-[2-(Aminomethyl)ethyl]-4-methoxybenzoylformamide
N-[2-(Aminomethyl)ethyl]-2-(2-benzofuryl)-2-oxoacetamide
N-[2-(1-Piperazino)ethyl]-2-(2-thienyl)-2-oxoacetamide
N-[2-(1-Pipeazino)ethyl]-2-(2-benzofuryl)-2-oxoacetamide
N-[2-(4-Aminophenyl)ethyl]-2-(2-thienyl)-2-oxoacetamide

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EXPERIMENTAL

General Methods. NMR spectra were recorded on a Varian Gemini 300MHz NMR. Deuterated solvents were obtained from Aldrich and tetramethylsilane was used as an internal standard. In order to obtain NMR spectra, the products were purified by a flash silica gel column. Mass spectra were obtained on a Micromass Platform 2 using a Gilson 215 autosampler and an HP 1050 LC. HPLCs were run using UV detection at 254 nm (on a Shimadzu 10AS) and/or ELSD (on a Sedex). The products were injected onto a YMC reverse phase (C18, 3 micron, 3 x 100 mm) column. The retention times reported in this experimental were obtained using the following conditions. Five microliters of a one milligram per millliliter solution of the reaction product in acetonitrile was injected onto the column. The flow rate was 0.75 mL per minute. The linear gradient elution profile for the acetonitrile / water eluent was from 20 to 100% acetonitrile over ten minutes. The elution solvents contained 0.1% TFA. The libraries were produced using the following equipment. Reagents were weighed out and diluted to a concentration of 0.5M with the aid of a Zymark radial arm. Reagents were dispensed to the reaction blocks using a Tecan Genesis RSP 150. QC plates were prepared using a Gilson 215. Products were moved from reaction blocks to shipping plates using a Quadra.

General Procedure for the Production of Array I (Figure 1). A 0.5M stock solution of all reagents were prepared. Into each well of a reaction block was measured 100µl of a stock solution of cyanuric chloride dissolved in acetonitrile. The reaction blocks were covered with a self-adhesive aluminum rectangle and then cooled to -20°C for 1 hr. While the reaction blocks were cold, 100µl of a solution of the appropriate aniline and one equivalent of diisopropylethylamine (DIPEA) dissolved in acetonitrile were dispensed. The reaction blocks were re-covered with a self-adhesive aluminum rectangle, vortexed and then cooled to -20°C for 1 hr. The

reaction blocks were placed at room temperature and 100μl of a solution of the appropriate amine and one equivalent of DIPEA dissolved in acetonitrile were dispensed. The reaction blocks were re-covered with a self-adhesive aluminum rectangle, vortexed and then left at room temperature for 2 hrs. The volatiles were removed in vacuo. Spectral data for compound **Ia** where X = 4-morpholino and R_1, R_2 = [-$(CH_2)_5$-] (Figure 1). MS (ES+) m/z 375.2 (M+H). HPLC, ELSD (C18) r.t = 5.81 min. ^{1}H NMR ($CDCl_3$) δ 1.6 (m, 6H), 3.1 (t, 4H), 3.8 (m, 4H), 3.9 (t, 4H), 6.9 (d, 2H), 7.4 (d, 2H).

General Procedure for the Production of Array **II** (Figure 1). The first two steps are identical to that given for Array **I**. After the second step, 100μl of a solution of the appropriate secondary amine and one equivalent of DIPEA dissolved in acetonitrile were dispensed. The reaction vials were capped, vortexed and heated at 80°C overnight. The volatiles were removed in vacuo. Spectral data for compound **IIa** where X = 2-methyl, R_1 = H, R_2 = benzyl, R_3, R_4 = 1-(4-fluorophenyl)-piperazine (Figure 1). MS (ES+) m/z 470.2 (M+H). HPLC, ELSD (C18) r.t = 5.33 min. ^{1}H NMR ($CDCl_3$) δ 2.3 (s, 3H), 3.1 (t, 4H), 3.9 (m, 4H), 4.6 (d, 2H), 6.9-7.4 (m, 13H).

General Procedure for the Production of Array **III** (Figure 2). The first two steps are identical to that given for Array **I**. After the second step, 100μl of a solution of 1-deoxy-1-methylamino-D-galactitol amine dissolved in water were dispensed, followed by 100ml of a DIPEA solution. The reaction vials were capped, vortexed and heated at 80°C overnight. The volatiles were removed in vacuo. Spectral data for compound **IIIa** where X = 4-methoxy, R_1 = H, R_2 = 4-methoxyphenethyl (Figure 2). MS (ES+) m/z 545.0 (M+H). HPLC, ELSD (C18) r.t = 3.22 min. ^{1}H NMR (DMSO-d_6) δ 2.8 (t, 2H), 3.2 (m, 2H), 3.6 (t, 2H), 3.7 (s, 3H), 3.9-4.6 (m, 16H), 6.9 (d, 2H), 7.1 (d, 2H), 7.4 (d, 2H), 7.5 (d, 2H).

General Procedure for the Production of Array **IV** (Figure 3). After the cyanuric chloride was dispensed and cooled to -20°C, 100μl of a solution of the aminophenyl pyranoside dissolved in DMSO was added followed by 100ml of a DIPEA solution. The reaction blocks were re-covered with a self-adhesive aluminum rectangle, vortexed and then cooled to -20°C for 1 hr. The reaction blocks were placed at room temperature and 100μl of a solution of the appropriate amine and one equivalent of DIPEA dissolved in acetonitrile were dispensed. The reaction blocks were re-covered with a self-adhesive aluminum rectangle, vortexed and then left at room temperature for 2 hrs. The volatiles were removed in vacuo. Spectral data for compound **IVa** where R_1= isopropyl, R_2= benzyl and the sugar was 4-aminophenyl-β-D-galactopyranoside (Figure 3). MS (ES+) m/z 490.4 (M+H). HPLC, ELSD (C18) r.t = 2.94 min. ^{1}H NMR (DMSO-d_6) δ 1.0 (d, 3H), 1.1 (d, 3H), 3.1 (m, 1H), 3.4-3.9 (m, 7H), 4.2 (s, 2H), 4.6-5.3 (m, 5H), 6.9 (d, 2H), 7.1-7.3 (m, 5H), 7.4 (d, 2H).

General Procedure for the Production of Array **V** (Figure 4). The procedure was identical to that of array **I** except in the second step 100μl of a solution of a dipeptide dissolved in water was added and this was followed by 100μl of a DIPEA solution. Spectral data for compound **Va** where X= 2-methoxy-5-methyl, AA_1= proline and AA_2= methionine (Figure 4). MS (ES+) m/z 495.3 (M+H). HPLC, ELSD (C18) r.t = 2.80 min. ^{1}H NMR (DMSO-d_6) δ 2.0–2.8 (m, 14H), 3.6 (m, 2H), 3.9 (s, 3H), 4.1 (t, 1H), 4.4 (t, 1H), 6.6 (d, 1H), 6.7 (d, 1H), 6.9 (s, 1H).

General Procedure for the Production of Array **VI** (Figure 5). The procedure was identical to that of array **IV** except in the first step 100μl of a solution of an aminimide dissolved in DMSO was added and this was followed by 100μl of a DIPEA solution. Spectral data for compound **VIa** where the aminimide has the amino group at the 2-position of the phenyl ring, R_1 = ethyl, R_2 = butyl (Figure 5). MS (APCI+) m/z 506.1 (M+H). HPLC, ELSD (C18) r.t = 4.88 min. ^{1}H NMR (DMSO-d_6) δ 1.0 (t, 3H), 1.2 (t, 3H), 1.5 (m, 2H), 1.6-2.1 (m, 8H), 3.3-3.7 (m, 8H), 3.8 (m, 1H), 3.9 (m, 2H), 4.2 (m, 2H) 6.8 (t, 1H), 7.2 (t, 1H), 7.3 (t, 1H), 7.9 (t, 1H).

General Procedure for the Production of Array **VII** (Figure 6). The procedure was identical to that of array **IV** except in the first step 100µl of a solution of an α -ketoamide dissolved in DMSO was added and this was followed by 100µl of a DIPEA solution. Spectral data for compound **VIIa** where the α-ketoamide has the amino group at the 4-position of the phenyl ring, n=2, X=H, R_1 = H, and R_2 = 2-(trifluoromethyl)-benzyl (Figure 6). MS (APCI+) m/z 554.7 (M+H). HPLC, ELSD (C18) r.t = 6.34 min. ^{1}H NMR ($CDCl_3$) δ 2.9 (t, 2H), 3.1 (t, 2H), 4.4 (d, 2H), 7.1-7.8 (m, 13H).

General Procedure for the Production of Array **VIII** (Figure 6). The procedure was identical to that of array **I** except in the first step 100µl of a solution of a α -ketoamide dissolved in DMSO was added and this was followed by 100µl of a DIPEA solution. In the second step, 100µl of a solution of a dipeptide dissolved in water was added and this was followed by 100µl of a DIPEA solution. Spectral data for compound **VIIIa** where the α-ketoamide has the amino group at the 4-position of the phenyl ring, n=2, X=4-methoxy, R_1 = H, and R_2 = gly-gly-amide (Figure 6). MS (ES+) m/z 545.0 (M+H). HPLC, ELSD (C18) r.t = 3.88 min. ^{1}H NMR (DMSO-d_6) δ 2.9 (t, 2H), 3.1 (t, 2H), 3.8 (s, 3H), 3.9 (s, 3H), 4.0 (s, 3H), 4.4 (d, 2H), 7.0 (d, 2H), 7.4 (d, 2H).

General Procedure for the Production of Array **IX** (Figure 7). The procedure was identical to that of array **I** except in the first step 100µl of a solution of the aminophenyl pyranoside dissolved in DMSO was added followed by 100µl of a DIPEA solution. In the second step, 100µl of a solution of a dipeptide dissolved in water was added and this was followed by 100µl of a DIPEA solution. After the second step, 100µl of a solution of an α-ketoamide dissoved in DMSO and one equivalent of DIPEA dissolved in acetonitrile were dispensed. The reaction vials were capped, vortexed and heated at 80°C overnight. The volatiles were removed in vacuo. Spectral data for compound **IXa** where R_1 = threonine, R_2 = isoleucine-amide, R_3 = cyclopropyl and R_4 and R_4·= 1-(2-aminoethyl)piperazine (Figure 7). MS (ES+) m/z 815.0 (M+H). HPLC, ELSD (C18) r.t = 3.78 min. Compound had a complex ^{1}H NMR spectrum in which each of the three components were evident.

Generic synthesis of aminimde reagent for array **VI**. The appropriate nitrobenzoyl chloride (50 mmol) was dissolve in toluene (200 ml, 0.25M). Freshly distilled glycidol (50 mmol) was added to the solution followed by triethylamine (100 mmol). The solution was stirred vigorously overnight (15 hrs) at room temperature. The triethylamine hydrochloride was filtered off and washed once with toluene. Toluene was removed by rotary evaporation and the solid residue was dried under high vacuum. The solid (the nitoglycidic ester, 50 mmol) was dissolved in isopropyl alcohol (100 ml, 0.5M). The appropriate N,N-dialkylhydrazine (50 mmol) was added to the solution. The mixture was heated at 60°C for 24 hours. The solvent was removed and the residue recrystallized twice from isopropyl alcohol. This yielded the nitrophenyl aminimide. The nitro group was reduced to the amine by catalytic hydrogenation. The nitrophenyl aminimide was dissolved in methanol to give a 0.25M solution. The solution was placed in a 3-neck round bottomed flask. The flask was flushed with nitrogen. Lindlar's catalyst (10% by weight of the nitrophenyl aminimide) was added to the solution. A balloon filled with H_2 was placed onto one of the necks to initiate the reaction. After 8 hours, The reaction mixture was filtered through celite. Concentration of the solution yielded an oil which crystallizes under vacuum. These products were sufficiently pure to be used directly in the array production.

Enzyme assays. Human Factor Xa was purchased from American Diagnostica (Greenwich, CT). Human plasmin was purchased from DiaPharma (Franklin, OH). Synthetic substrates were obtained from DiaPharma: N-α-Z-D-Arg-Gly-Arg-pNA (S-2765; factor Xa); H-D-Val-Leu-Lys-pNA (S-2251; plasmin). ArQule compounds were dissolved in DMSO, and tested at a final concentration of 10µM (final solvent: 2% DMSO in aqueous pH buffer). The enzyme was incubated for 20 minutes at room temperature with its substrate in 50mM Tris-Cl, 150mM NaCl, 0.1%(w/v) PEG-8000, pH 7.5 buffer. Proteolysis was stopped by the addition of 0.5% acetic acid, and extent of hydrolysis was measured by the release of p-nitroaniline (A_{405nm}).

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