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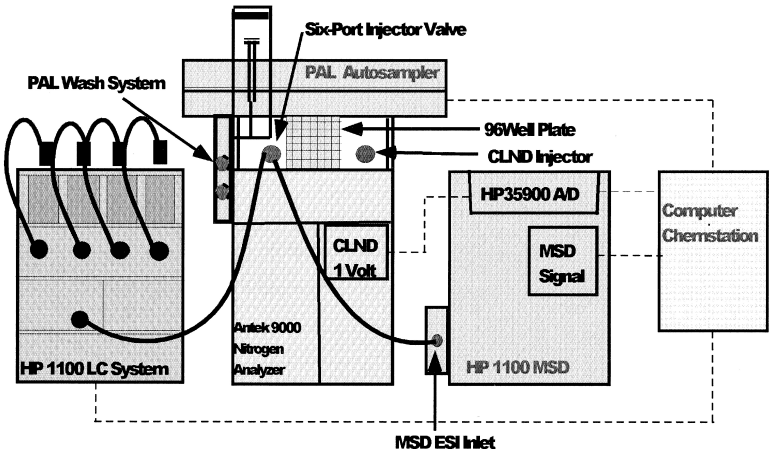

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Articles

A Novel Approach to High-Throughput Quality Control of Parallel Synthesis Libraries

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Combinatorial chemistry is a powerful tool to enhance drug discovery efforts in the pharmaceutical industry. One type of combinatorial chemistry, parallel synthesis, is now widely used to prepare numerous compounds of structural diversity. A novel high-throughput method for quality control of parallel synthesis libraries has been developed. The method uses flow injection MS, for proof of structure and estimation of purity, and a novel direct injection CLND technique for quantitation of amount. Following the synthesis of a small molecule library, compounds analyzed using this technique were characterized by mass spectrometry, and an accurate concentration of the compound was assessed by CLND. Characterization of one compound is completed in 60 s, allowing for up to 1000 compounds to be analyzed in a single day. The data is summarized using pass/fail criteria using internally developed software.

Combinatorial chemistry[1] is a powerful tool to enhance drug discovery efforts in the pharmaceutical industry. Its most striking feature is the capacity to produce a large number of compounds in a short period of time, as well as the ease of automation. One type of combinatorial chemistry, parallel synthesis, is now widely used to prepare numerous compounds of structural diversity. One of the major challenges in solid-phase synthesis is the development of standard analytical techniques not only to monitor reaction progress but also to identify the products, purities, and their concentrations. Analytical methods useful in combinatorial synthesis have been covered in reviews of solid-phase methodologies[2] and in more specific publications concerned with analytical aspects of combinatorial chemistry.[3] A widespread procedure for characterization of parallel synthesis libraries is LC/UV/MS. This allows for qualitative analysis, i.e., estimation of the purity of a sample and confirmation of structure. However, in a drug discovery setting it is ideal to also have quantitative information on synthesized compounds. Fitch[4] and Taylor[5] have reported the use of HPLC with a chemiluminescent nitrogen detector (CLND) as a means of estimating compound concentration. The technique is limited since chromatographic problems can be encountered due to the restrictions on mobile-phase solvents available for LC/CLND.

Here, we report our evaluation of the use of simultaneous flow injection mass spectrometry (FIA-MS) system in combination with direct injection CLND as a general tool for high-throughput quality control of combinatorially synthesized compounds. Following the synthesis of a small molecule library, compounds analyzed using this technique were characterized by mass spectrometry, and an accurate concentration of the compound was assessed by CLND. Characterization of one compound is completed in 60 s, allowing for up to 1000 compounds to be analyzed in a single day. The data are summarized using pass/fail criteria and highlighted with information of percent purity, percent BPC, and concentration using internally developed software.

Experimental Section

Chemicals. The set of compounds used to calibrate the CLND consisted of drug reference standards with a purity of >99%, nitrobenzene, glycine, and various chemicals purchased from Aldrich Chemical Co. (St. Louis, MO). All other compounds were synthesized at Affymax (Santa Clara, CA) and provided as solutions in methanol. HPLC grade methanol, water, and other solvents were purchased from Burdick and Jackson (Muskegon, MI).

Instrumentation. An HP1100 LC/MSD system (Agilent Technologies, Palo Alto, CA) was used in conjunction with the CLND model 9000 nitrogen analyzer with the vertical pyrolysis tube fitted with Merlin microseal (Antek Instruments, Houston, TX) and CTC-PAL autosampler (Leap Technologies, Raleigh, NC). Data from the MS and CLND

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were collected using the chemstation software (Rev. A.06.01) and a model HP35900 analog-to-digital converter (Agilent Technologies, Palo Alto, CA).

Preparation of Test Parallel Synthesis Library. Argo-Gel-Rink-NH-Fmoc resin (15 g, loading = 0.34 mmol/g) was added to a 250 mL peptide vessel. The resin was washed with dichloromethane (DCM, 3 × 100 mL) and *N*,*N*-dimethylformamide (DMF, 2 × 100 mL). The Fmoc group was then removed by treatment with a 30% piperidine solution in DMF (100 mL) for 1 min, washed with DMF (2 × 100 mL), and treated again with a 30% piperidine solution in DMF (100 mL) for 10 min. The resin was then washed with DMF (3 × 100 mL), DCM (3 × 100 mL), and DMF (3 × 100 mL). DMF (100 mL), Fmoc-L-Lys-(Boc)-OH (12.3 g, 5 equiv), [*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate] (HATU, 9.6 g, 5 equiv), and diisopropylethylamine (DIEA, 4.5 mL, 5 equiv) were then added to the resin. The reaction mixture was gently shaken for 3 h and then washed with DMF (3 × 100 mL), DCM (3 × 100 mL), and DMF (3 × 100 mL). A Kaiser test was performed on the resin. The resin was capped by treating with a 1:1:5 mixture of acetic anhydride:pyridine:DMF for 20 min and then washed with DMF (3 × 100 mL), DCM (3 × 100 mL), and DMF (3 × 100 mL). The Fmoc group was removed as described above. The resin was washed with DMF (3 × 100 mL), DCM (3 × 100 mL), and DMF (3 × 100 mL) and then suspended in DMF/DCM and distributed into 96 tared 8 mL disposable filtration tubes, washed with MeOH, and stored under vacuum. Each reaction was done on 17.7 μmol of resin loaded lysine.

The contents of tubes 1−32 were washed with acetonitrile and then treated with acetonitrile (4 mL), 32 different aldehydes (20 equiv), mercaptosuccinic acid (30 equiv), and 4Å molecular sieve (100 mg). The reactions were heated at 70 °C for 16 h, the sieves were removed, and the resin was washed exhaustively with DMF and MeOH, followed by DCM (3×) and MeOH (3×).

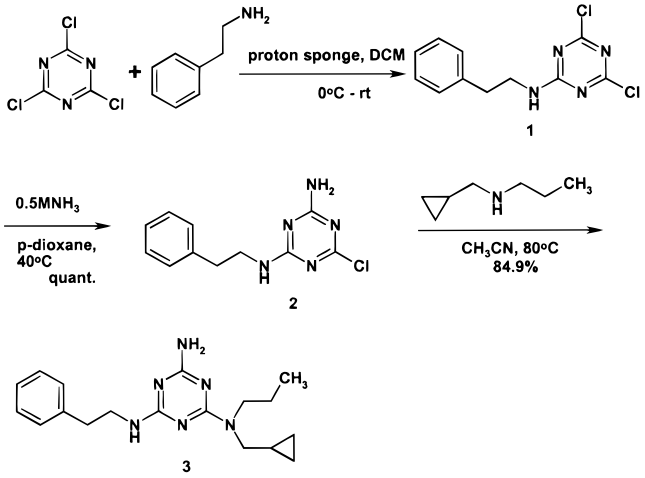
The contents of tubes 33−52 were washed with DMF and treated with 0.5 M pyridine in DMF (4 mL) and with 20 different isocyanates (20 equiv). The reactions were heated at 40 °C for 16 h. The resins were washed with DMF (3×), DCM (3×), and MeOH (3×).

The contents of tubes 53−64 were washed with DMF and treated with 0.5 M pyridine in DMF (4 mL) and with 12 different sulfonyl chlorides (20 equiv). The reactions were heated at 40 °C for 16 h. The resins were washed with DMF (3×), DCM (3×), and MeOH (3×).

The contents of tubes 65−96 were washed with DMF and treated with DMF (4 mL), HATU (20 equiv), DIEA (20 equiv), and 32 different carboxylic acids (20 equiv). The reactions were shaken at room temperature for 16 h. The resins were washed with DMF (3×), DCM (3×), and MeOH (3×).

The contents from tubes 1−96 were subjected to identical conditions and dried under high vacuum for 3 days, weights were recorded, and product was cleaved from the resin by treatment with 95% TFA/DCM for 1 h and collected into a tared vial. The solvent was removed using a speed-vac, and final crude weights were recorded. A total of 1 μL of the 2 mg/mL solution was injected for LC and LC/MS analysis.

Scheme 1. Triazine Synthesis (AF16199)



Preparation of Triazine Test Compounds As Described in Reaction Scheme 1. Preparation of 1,3-dichloro-5-(2-phenyl-ethylamino)-triazine (1): To a stirred solution of cyanuric chloride (1.844 g, 10 mol) in DCM (50 mL) at 0 °C (in ice bath) was added a solution of phenethylamine (crude, 1.21 g, 10 mmol) in DCM (20 mL) in a dropwise fashion. After addition is complete, the ice bath was removed and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with 50 mL of DCM and washed sequentially with saturated NaCl aqueous solution and 10% citric acid aqueous solution. The organic layer was dried over anhydrous Na_2SO_4, and solvents were removed in a vacuum. The crude product was then eluted through a pad of silica gel (ca. 5 cm high) with DCM. After removal of the solvent, the colorless solid of **1** (2.88 g, 96.6%) was obtained.

Preparation of 1-amino, 3-chloro-5-(2-phenyl-ethyl-amino)-triazine (2): The compound **1** (1.25 g, 5 mmol) was treated with 0.5 N NH_3 in *p*-dioxane (50 mL) at 40 °C for 14 h. The reaction mixture was concentrated in a vacuum. The residue was triturated with DI water. The resulting solid of **2** (1.181 g, 94% yield) was filtered, washed with water, dried under high vacuum, and used in the next step without purification.

Preparation of 1-amino, 3-(*N*-proprylcyclopropane-methylamino)-5-(2-phenyl-ethylamino)-triazine (3): The EtOAc (20 mL) and ^{i}PrOH (20 mL) solution of **2** (874 mg, 3.5 mmol) and *N*-proprylcyclopropanemethylamine (1.243 g, 11 mmol) was heated at 80 °C for 3 h. Solvents were removed in a vacuum. The residue was extracted with EtOAc (100 mL) and washed with 5% citric acid aqueous solution and saturated NaCl solution. The EtOAc layer was dried with Na_2SO_4, and solvent was removed in a vacuum. Recrystallization of the crude product in ether/hexane gave pure **3** (1.052 g, 91.7%), which was characterized with HPLC, LC/MS, and proton NMR.

Results and Discussion

Because chromatographic run times for acceptable resolution are usually 10 min or greater, normally only a statistical

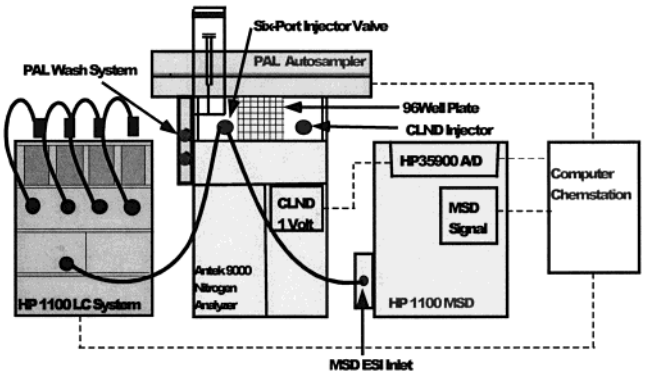


Figure 1. Schematic of FIA/CLND/MS system.

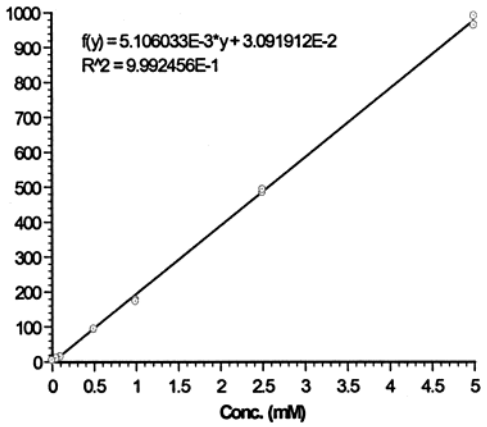


Figure 2. CLND linearity with nitrobenzene.

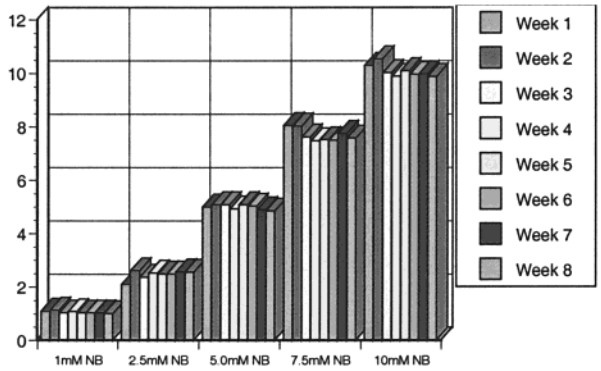


Figure 3. CLND reproducibility with nitrobenzene.

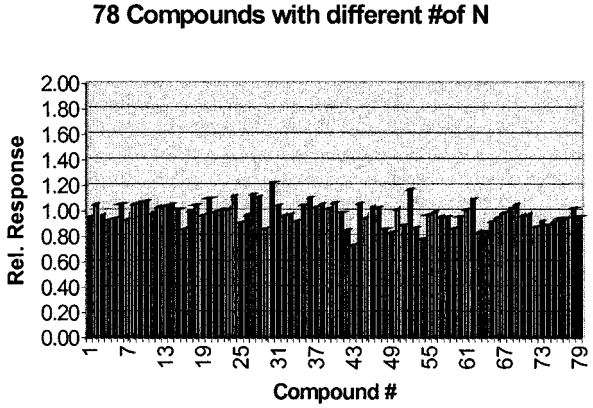


Figure 4. Relative response factors of 78 commercial compounds.

subset of a library is assay. Dulery et al.[6] reported using high-resolution liquid chromatography, mass spectrometry, and nuclear magnetic resonance techniques to assay 25% of a combinatorial library. In our ongoing effort to develop more rapid techniques, which will allow 100% testing, we have evaluated the use of a simultaneous flow injection mass spectrometry (FIA-MS) system in combination with direct injection CLND as a general tool for high-throughput quality control of combinatorially synthesized compounds. In drug discovery this is often useful for measuring accurate potencies (IC_{50} or EC_{50}) when most compounds synthesized by parallel syntheses are >80% pure or the synthesis is considered failed.

Instrument Setup. The complete system was assembled as shown in Figure 1. The mobile phase from the HPLC was plumbed into the six-port switching valve on the PAL autosampler. The outlet of the six-port valve was connected to the LC/MSD equipped with electrospray ion source. Typically, a 5 μL sample from a 96-well plate was injected on the six-port valve followed by a 5 μL direct injection into the CLND injector. The other important feature of this approach was to acquire the entire 96-well plate data in a single data file using a special custom cable connected from PAL to the LC/MSD and the use of Chemstation FIA series protocol. The external standard nitrobenzene calibration was applied to the Chemstation acquisition method. The run time for the method was 1 min/sample and 96 min/plate.

CLND Settings. Inlet oxygen = 25 mL/min, inlet argon = 140 mL/min, ozone = 30 mLs/min, pyro oxygen = 400 mLs/min. Thermo electric coolant = 5 °C, oxidation furnace = 1050 °C, high voltage = 750 V, reaction cell back pressure = 1. Injection volume: 5 μL.

HPLC and MS Settings. Mobile phase: methanol:water + 0.2% formic acid (80:20) isocratic. Flow rate: 0.4 mL/min. Ionization and polarity: electrospray, positive mode. Scan range: 100−1000 Da. Nebulizer and capillary: normal settings. Injection volume: 5 μL (10 μL loop).

CLND Linearity and Reproducibility. Because this application provides absolute nitrogen concentration for individual samples, it was essential to follow strict calibration and sample preparation protocols. The CLND was calibrated using a nitrobenzene standard. A standard stock solution was prepared at 10 mM concentration in methanol, and serial dilutions in methanol were made. The CLND calibration curve is shown in Figure 2. The curve is linear from 0.05 to 5.0 mM concentration with $R^2 = 0.9992$. The reproducibility of the detector was tested using nitrobenzene standards injected on different days of the week for eight weeks. Figure 3 illustrates that the detector shows very little drift over a period of 2 months. For this application, the ultimate CLND sensitivity was not critical. We can operate in this 0.05−5.0 mM range because parallel synthesis normally provides adequate amounts and samples.

Proof of Concept. Previous reports have hypothesized that all nitrogen compounds give equal response. To further support this hypothesis, we tested 78 diverse classes of compounds with different numbers of nitrogen. The compounds were chosen from a wide collection of commercially available building blocks and included amino acids, amines, indoles, isocyanates, hydroxylamines, amides, and carboxylic

Table 1. List of 78 Compounds Used in the Proof of Concept Study

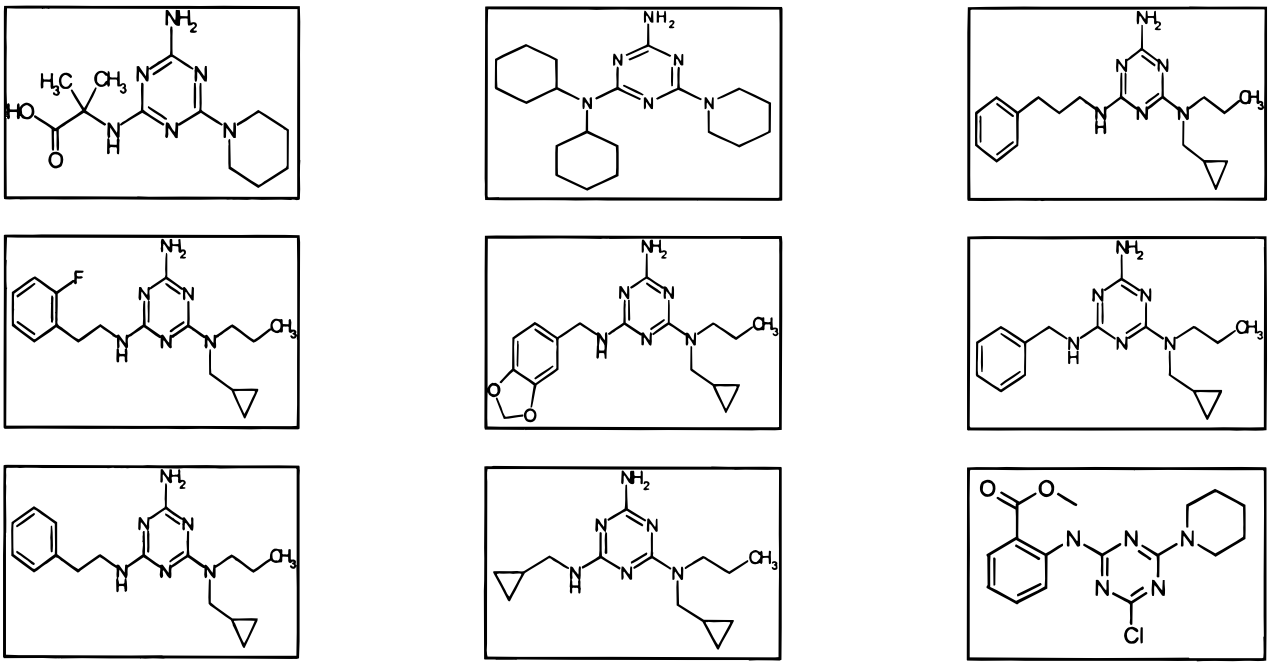
	name		name
1	FmocSerine(tBu)	**40**	2-(1-cyclohexyl)-ethylamine
2	FmocTyrosine(tBu)	**41**	2-(1-cyclohexyl)-methylamine
3	FmocProline	**42**	3-(dibutylamino)-propylamine
4	FmocLysine(Boc)	**43**	1-(3-aminopropyl)-2-pyrollidinone
5	FmocPhenylalanine	**44**	octylamine
6	FmocAlanine	**45**	heptylamine
7	FmocHistamine(Boc)	**46**	2-thiophenemethylamine
8	FmocAsparagine(Trt)	**47**	4-phenylbutylamine
9	FmocAspartic acid(OtBu)	**48**	4-fluorophenethylamine
10	FmocThreonine(tBu)	**49**	2-methoxyphenethylamine
11	FmocGlycine	**50**	*N*-phenethylenediamine
12	FmocValine	**51**	3-dimethylaminopropylamine
13	heptylamine	**52**	4-aminomorpholine
14	isoamylamine	**53**	1-(2-aminoethyl)piperazine
15	aminomethylcyclopentane	**54**	1-indoleacetic acid
16	1-naphthalenemethylamine	**55**	indole-6-carboxylic acid
17	4-trifluoromethylbenzylamine	**56**	4-methoxyindole
18	3-phenyl-1-propylamine	**57**	5-hydroxyindole
19	2-fluorophenethylamine	**58**	ethyl 5-hydroxyindole-2-carboxylate
20	2-fluorobenzylamine	**59**	ethyl 6-isocyanatohexanoate
21	3-fluorobenzylamine	**60**	*n*-amyl isocyanate
22	4-fluorobenzylamine	**61**	ethyl isocyanatoacetate
23	furfurylamine	**62**	ethyl 3-isocyanatopropionate
24	2-methoxyethylamine	**63**	*tert*-butyl isocyanate
25	3,4-dimethoxyphenethylamine	**64**	*O*-(trimethylsilyl)hydroxylamine
26	4-methoxyphenethylamine	**65**	methoxylamine hydrochloride
27	*N*,*N*-dimethylenediamine	**66**	hydroxylamine-*O*-sulfonic acid
28	1-(2-aminoethyl)pyrrolidine	**67**	*O*-benzylhydroxylamine hydrochloride
29	*N*-(3-aminopropyl)imidazole	**68**	*O*-(2,3,4,5,6-trifluorobenzyl hydroxylamine
30	2-(2-aminoethyl)pyridine	**69**	*O*-ethylhydroxylamine hydrochloride
31	4-(2-aminoethyl)morpholine	**70**	*O*-allylhydroxylamine hydrochloride
32	butylamine	**71**	*O*-tritylhydroxylamine
33	4-(*tert*-butyl)-aniline	**72**	5-(chloro)-2-mercaptoaniline hydrochloride
34	2-methoxybenzylamine	**73**	L-penicillamine
35	3,4-dimethoxybenzylamine	**74**	2-aminothiophenol
36	3-methoxybenzylamine	**75**	2,5-diamino-1,4-benzenedithiol dihydrochloride
37	cyclohexylamine	**76**	2-amino-4-(trifluoromethyl)benzenethiol hydrochloride
38	aniline	**77**	2-amino-5-nitrobenzophenone
39	diaminopropane	**78**	2-amino-5-chlorobenzophenone

acids. The standards were prepared at 1 mM concentration in methanol prior to direct injection for CLND analysis. The relative response factors were derived from the measured concentration divided by the number of nitrogens in the compound and were calibrated against nitrobenzene. CLND is indeed a nearly universal detector for compounds containing different numbers of nitrogen. The response factor values were comparable to that of nitrobenzene. Figure 4 shows the relative response factors of different compounds analyzed by CLND. The average relative response factor for the 78 compounds is 0.95. The raw data and compound identifications are included as Table 1. The CLND vendor, Antek, has found[7] that drugs that can decompose thermally to give molecular nitrogen will not reliably yield CLND response as it is well established that nitrogen gas will not give a CLND signal. These molecules will necessarily contain N−N bonds, such as azides or tetrazoles. A search of the MDDR and Glaxo Wellcome databases show that 13% of drug-like N-containing molecules contain the N−N bond and may give spurious results.

Test Compound Synthesis (Triazines). To further strengthen the validity of the proof of concept, a set of test compounds were prepared using the triazine chemistry.[8] Synthesis Scheme 1 was followed with the representative compound structures as shown in Chart 1. In this test synthesis, the compounds obtained were of high purity and did not require further purification. All compounds were dried overnight under vacuum and weighed accurately to prepare individual 1 mM solutions in methanol. The subsequent dilutions were analyzed by the same protocol as described earlier. The measured CLND concentrations of 96 test compounds were compared against their expected concentration as shown in Figure 5. The CLND gave an average value of 95% when compared against their theoretical 1 mM solutions.

Case Study. A library of 96 different substituted lysines was prepared (Scheme 2). The 96 R-groups are identified in Table 2. *N*-Boc-*N*′-Fmoc-lysine was coupled to ArgoGel-Rink resin which was deprotected with piperidine to give the common intermediate shown. This was then coupled with aldehydes and 2-mercaptosuccinic acid to give thiazolidinones (**1**−**32**), isocyanates to give ureas (**33**−**52**), sulfonyl chlorides to give sulfonamides (**53**−**64**), or acid chlorides to give amides (**65**−**96**). Products were cleaved with acid and dried to constant weight but were not purified. The weights were used to calculate traditional yields for comparison to yield measurement by the CLND method. Each well was characterized by LC/UV/MS as well as by CLND/

Chart 1. Representative Triazine Structures from the Test Set



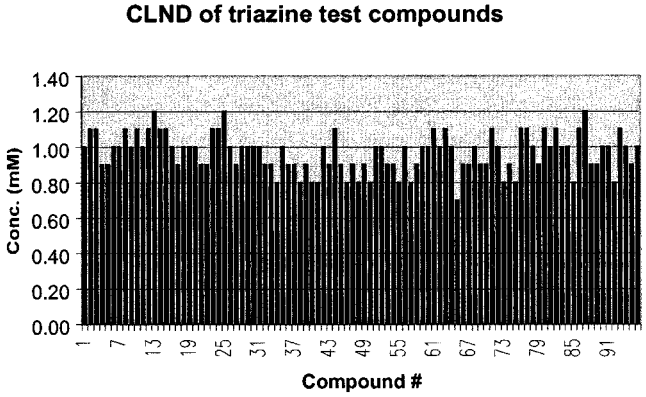
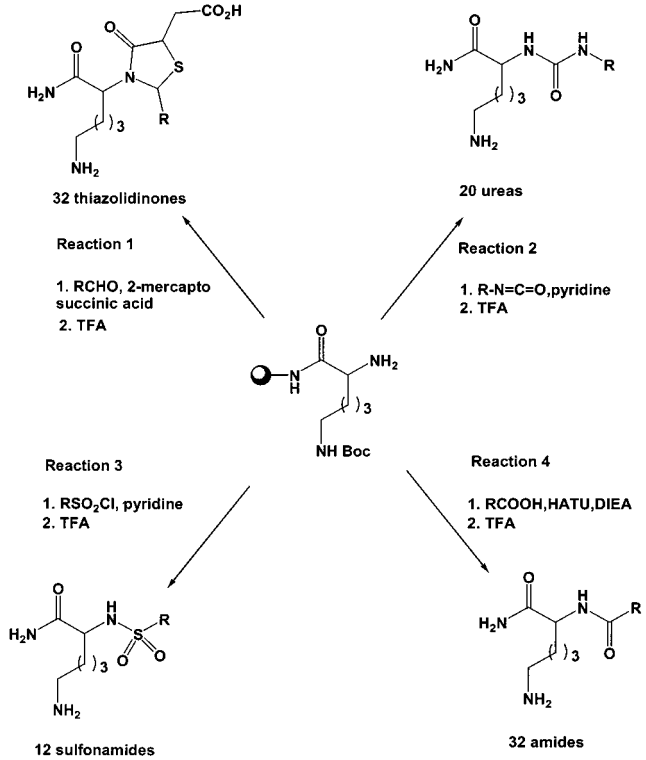


Figure 5. Measured CLND concentration of a 1 mM test triazine set.

MS. Six of the wells did not yield the expected product for various reasons. These wells are ignored for this study. All other compounds gave the predicted mass spectrum with the indicated UV percent purity. Analysis by CLND was performed after dissolution of each sample to a nominal concentration of 10 mM in methanol.

The 96 samples were analyzed using the same CLND/MS protocol described earlier. The qualitative mass spectral data and quantitative CLND data were automatically converted into text files using our customized Chemstation macros. These data were further evaluated using the Capture[9] software program. The Capture-generated summary of this data is shown in Figure 6. CLND and MS data of well E06 (M + H = 336.1) are displayed in Figure 7. This software has enabled us to view the mass spectrum, CLND derived concentration, and structure of the compound with a single click of a button. Well F08 contains a phosphate moiety and required a separate negative ion MS confirmation. The molecular weight of sample H02 was less than our initial

Scheme 2. Synthesis of 96 Test Compounds[a]



[a] The R groups are identified in Table 2.

scan range and was confirmed manually. Wells A05, G03,and H07 contain mixtures and did not meet the criteria for acceptance.

Table 3 shows the gravimetric percent yield (not corrected for purity) for each reaction, the LC/UV purity of each product, and the CLND measured percent yield. This last value is calculated by measuring the CLND nitrogen concentration in each well and dividing by the number of nitrogens in that structure formula and correcting for dilution.

Table 2. Structures of Case Study Compounds[a]

sample	reaction	R	sample	reaction	R
A01	1	H	D11	2	propyl
A02	1	pentyl	D12	2	3,5-dimethylphenyl
A03	1	1-pyrenyl	E01	2	methyl
A04	1	3-pentyl	E02	2	thien-2-yl
A05	1	2,3,6,7-tetrahydro-8-hydroxy-1*H*,5*H*-benzo[*ij*]quinolizin-9-yl	E03	2	phenyl
			E04	2	1-methoxycarbonyl-2-*tert*-butoxyethyl
A06	1	3-methylphenyl	E05	3	naphth-2-yl
A07	1	1-phenyl-4-methylpent-1-enyl	E06	3	naphth-1-yl
A08	1	1-phenyl-3-methylbut-1-enyl	E07	3	3-nitrophenyl
A09	1	2-fluorophenyl	E08	3	3,4-dichlorophenyl
A10	1	pent-1-enyl	E09	3	2-nitrobenzyl
A11	1	2-methylpropyl	E10	3	methyl
A12	1	5-nitrothien-3-yl	E11	3	thien-2-yl
B01	1	2-benzyloxyphenyl	E12	3	styryl
B02	1	1-phenylprop-2-en-2-yl	F01	3	4-bromophenyl
B03	1	2-hydroxy-4-methoxyphenyl	F02	3	2,5-dimethoxyphenyl
B04	1	(5-chloroindol-3-yl	F03	3	4-(1,1-dimethylethyl)phenyl
B05	1	4-ethoxyphenyl	F04	3	2-(1-naphthyl)ethyl
B06	1	4-(1-methyl-5-trifluoromethylpyrazol-3-yl)thien-2-yl)	F05	4	6-methylpyrid-2-yl
			F06	4	cyclopentyl
B07	1	tetrazolo[1,5-*a*]naphth-3-yl)	F07	4	3-cyanophenyl
B08	1	1-chloro-3,4-dihydro-6-methoxy-naphth-2-yl	F08	4	2-phosphoethyl
			F09	4	5-methoxy-2-nitrophenyl
B09	1	5-bromo-2-methoxyphenyl	F10	4	2-acetylphenyl
B10	1	4-ethylthien-2-yl	F11	4	2-methylpyrid-3-yl
B11	1	3-bromophenyl	F12	4	2-fur-3-yleth-1-enyl
B12	1	4-allyloxyphenyl	G01	4	4-methoxyphenyl
C01	1	4-methoxyphenyl	G02	4	3-methoxyphenyl
C02	1	3-quinolinyl	G03	4	cyclohex-1-enyl
C03	1	1-methylpyrrol-2-yl	G04	4	3-methylbutyl
C04	1	1,5-dimethyl-2-phenylpyrazol-3-on-4-yl	G05	4	2-benzyloxybenzyl
			G06	4	2,5-dimethylphenoxymethyl
C05	1	4-phenylphenyl	G07	4	2-methoxyphenoxymethyl
C06	1	3-cyano-4-*N*,*N*-dimethylamino-2-fluorophenyl	G08	4	3-bromopropyl
			G09	4	2,3-dimethylphenoxymethyl
C07	1	2,3-dihydro-2-oxo-1*H*-imidazol-4-yl	G10	4	naphth-2-yl
C08	1	pentafluorophenyl	G11	4	2-oxo-2H-1-benzopyran-3-yl
C09	2	1-methylethyl	G12	4	fur-3-yl
C10	2	4-acetylphenyl	H01	4	2-ethoxyphenyl
C11	2	2-phenylphenyl	H02	4	ethynyl
C12	2	4-(1-methylethyl)phenyl	H03	4	4-(1-methylethyl)phenoxymethyl
D01	2	4-butylphenyl	H04	4	2,5-dimethoxybenzyl
D02	2	3,4-difluorophenyl	H05	4	2-methoxypyrid-3-yl
D03	2	pentyl	H06	4	1-methylcyclopropyl
D04	2	4-methylphenyl	H07	4	4-methoxyphenyl
D05	2	allyl	H08	4	4-ethylphenyl
D06	2	3-methylthiophenyl	H09	4	adamant-1-yl
D07	2	hexyl	H10	4	4-(1-methylethyl)phenoxymethyl
D08	2	4-butoxyphenyl	H11	4	pyrid-4-yl
D09	2	heptyl	H12	4	naphth-1-yloxymethyl
D10	2	1-ethoxycarbonyl-2-phenylethyl			

It is easily seen that the two yield measurements are not comparable. The average gravimetric yield is 116%. The average CLND calculated yield is 36%. Typical LC/UV purities were between 70 and 100%, but we know from the CLND that "real"purities and yields are much lower because of variable amounts of water or TFA in the dried products. The biggest discrepancy between gravimetric and CLND derived yield was compound C04, where CLND gave an erroneously high value, due to the mixture of byproducts and impurities seen by LC and MS. The presence of the MS data is invaluable for realistic characterization of these types of impure compounds.

We utilized the Capture program to help organize our quality control data. This program brings together the chemical structures of each library member with its analytical data. Figure 6 shows the red/green analysis for this library. Green indicates that a well has FIA-MS with the correct molecular ion that is at least 30% of the mass spectrum, base peak. Also shown (in small print) are the CLND nitrogen concentrations, corrected for the number of nitrogens. These values can be used to adjust assay results so they take account of actual compound concentration.

Conclusion

A novel high-throughput method for quality control of parallel synthesis libraries has been developed. The method uses a flow injection MS for proof of structure and estimation of purity, and it uses a novel direct injection CLND technique for quantitation of amount. Additional support for the hypothesis that all nitrogen-containing compounds give

Table 3. Library Data Shows MW, Gravimetric Yield, CLND Yield, and HPLC Purity

sample ID	MW	%grav. yield	%CLND yield	%HPLC (purity)	sample ID	MW	%grav. yield	%CLND yield	%HPLC (purity)	sample ID	MW	%grav. yield	%CLND yield	%HPLC (purity)
A01	289.1	259	41	95	C09	230.2	181	35	90	F05	264.2	90	22	95
A02	359.2	119	29	90	C10	306.2	181	36	82	F06	241.2	107	39	95
A03	489.2	84	21	95	C11	340.2	126	52	83	F07	274.1	79	28	95
A04	359.2	90	19	70	C12	306.2	164	43	63	F08	279.1	39	8	0
A05	476.2	125	33	0	D01	320.2	118	47	95	F09	324.1	73	25	95
A06	379.2	124	31	95	D02	300.1	61	53	65	F10	291.2	52	10	0
A07	447.2	90	25	70	D03	258.2	131	46	61	F11	264.2	106	30	95
A08	433.2	83	18	70	D04	278.2	192	40	58	F12	265.1	81	18	84
A09	383.1	120	29	90	D05	228.2	98	55	65	G01	279.2	131	40	66
A10	357.2	120	22	90	D06	310.1	80	45	95	G02	279.2	146	58	95
A11	345.2	144	40	70	D07	272.2	52	47	54	G03	253.2	162	51	60
A12	416.1	133	34	95	D08	336.2	47	46	95	G04	243.2	159	50	80
B01	471.2	60	10	95	D09	286.2	35	42	70	G05	369.2	111	47	95
B02	405.2	125	29	70	D10	364.2	46	53	95	G06	307.2	147	49	95
B03	411.1	109	32	40	D11	230.2	100	56	46	G07	309.2	111	50	95
B04	438.1	164	35	0	D12	292.2	59	49	83	G08	293.1	88	31	15
B05	409.2	139	38	95	E01	202.1	107	59	95	G09	307.2	126	44	95
B06	519.1	95	20	95	E02	298.1	78	13	46	G10	299.2	122	42	95
B07	457.2	118	17	50	E03	308.1	87	36	59	G11	317.1	156	58	72
B08	479.1	169	40	40	E04	290.2	67	49	95	G12	239.1	117	40	95
B09	473.1	71	7	85	E05	335.1	1	29	95	H01	293.2	139	55	60
B10	399.1	137	31	95	E06	335.1	125	28	95	H02	197.1	143	47	90
B11	443.1	148	47	95	E07	330.1	147	18	90	H03	321.2	107	43	95
B12	421.2	165	42	95	E08	353	131	18	60	H04	323.2	107	42	95
C01	395.2	149	42	95	E09	344.1	122	8	51	H05	280.2	115	38	95
C02	416.2	150	32	90	E10	223.1	193	22	89	H06	269.2	128	39	70
C03	368.2	144	10	0	E11	291.1	135	38	70	H07	279.2	116	43	60
C04	475.2	276	115	15	E12	311.1	133	19	75	H08	277.2	140	45	75
C05	441.2	135	44	90	F01	363	112	24	79	H09	307.2	119	37	75
C06	451.2	141	32	80	F02	345.1	121	29	66	H10	321.2	0	0	0
C07	373.1	158	35	95	F03	341.2	120	38	62	H11	250.1	103	30	100
C08	455.1	94	21	60	F04	363.2	115	21	78	H12	329.2	131	48	100

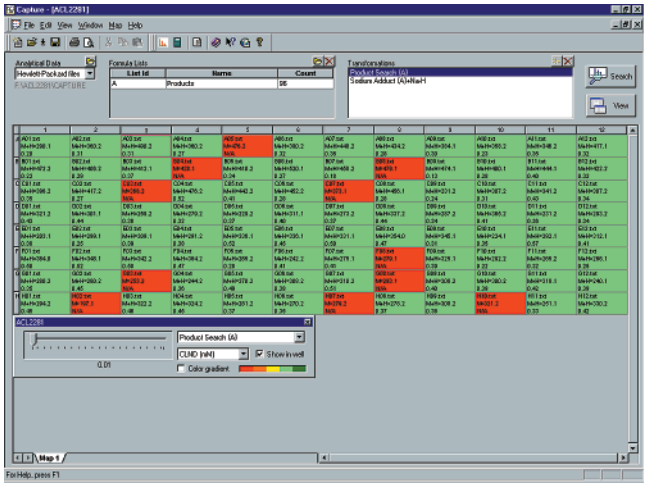


Figure 6. Library data viewed in Capture.

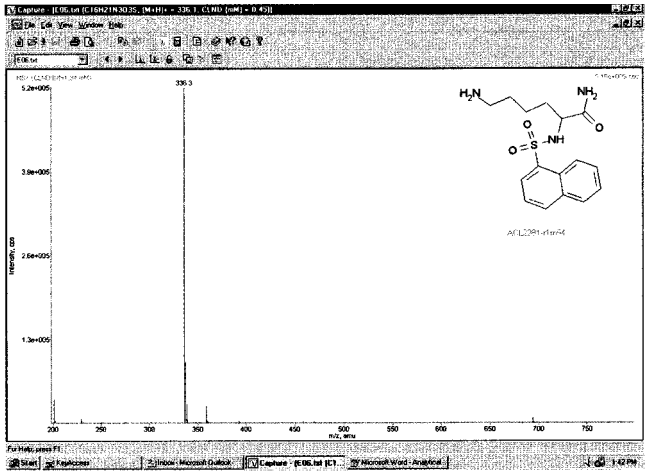


Figure 7. Data from well E06 displayed.

equimolar response to CLND is provided by a set of 174 simple organic molecules and triazines. These compounds showed an average response relative to nitrogen of 95%. The usefulness of this approach is illustrated with a simple parallel synthesis of 96 lysine derivatives. Quality control of this library using the FIA/CLND/MS technique was much faster than the traditional LC/MS method and gave essentially equivalent information.

Direct injection CLND produced a linear respnse from 0.05 to 5.0 mM nitrogen that was equivalent for a set of chemically and structurally diverse compounds. Over the entire linear range, the absolute response exhibited an average error of <10% among the compounds. The results clearly demonstrate that direct injection CLND in conjunction with FIA is a technique of choice for rapid and accurate quantitation down to low picomole levels, using a single external standard. The identity and concentration of compounds of interest were assessed from a simultaneous flow injection mass spectrometry (FIA-MS) system in combination with direct injection CLND from a 96-well plate. When operated in this mode, it takes about 96 min to run one plate. This approach is simple and can easily provide a high-throughput quality control tool for parallel synthesis. We expect this method to be useful for the majority of compounds. When large amounts of impurities and byproducts

are present and when the compound of interest is present at very low concentration, it may be necessary to use LC/CLND and LC/MS.

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