Pergamon

Tetrahedron 54 (1998) 11755–11766

TETRAHEDRON

QUANTITATIVELY MONITORING OF SOLID-PHASE ORGANIC SYNTHESIS BY COMBUSTION ELEMENTAL ANALYSIS

Bing Yan,[1*] Charles F. Jewell, Jr.,[2] Stewart W. Myers[3]

[1]Departments of Core Technologies, [2]Metabolic and Cardiovascular Diseases and [3]Transplantation
Novartis Pharmaceuticals Corporation, 556 Morris Avenue, Summit, NJ 07901

Received 10 June 1998; accepted 23 July 1998

Abstract A systematic investigation on the role of combustion elemental analysis in the quantitative monitoring of solid-phase organic synthesis was done. Quantitative elemental analysis results obtained for eight resin-bound organic compounds are highly consistent with those obtained from well-established methods such as Fmoc method. A six-step peptoid synthesis and a dansylhydrazone synthesis were quantitatively monitored using elemental analysis. All results were corroborated by well-established photometric methods. Based on these data, the elemental analysis method is, in general, reproducible and accurate in the quantitative analysis of resin-bound organic compounds. Considering the severe shortage of on-resin quantitation methods at present, it adds a valuable tool for monitoring solid-phase organic synthesis steps that cannot be quantitated by current methods.

* Corresponding author's E-mail: bing.yan@pharma.novartis.com

0040-4020/98/$ - see front matter
PII: S0040-4020(98)00721-2

INTRODUCTION

Solid-phase synthesis is the primary tool for combinatorial chemistry.[1] A major advantage of solid-phase organic synthesis (SPOS) [2] is that it circumvents the tedious intermediate isolation and purification procedures. Ideally, a solid-phase reaction can be driven to completion by an excess of reagent without causing problems in the purification. In reality, most organic reactions do not go to completion and unlike solution synthesis, the resin-bound synthetic intermediate in SPOS can not be isolated and purified. The unreacted portion will accumulate until the end of the synthetic sequence and cause purity problems for the final product. Therefore, the quantitative analysis of solid-bound compounds at intermediate stages is very advantageous to guide the success of the chemistry validation and the final library synthesis.

Dye-coupling methods have been applied to quantitation of aldehyde[3] and amines[4]. However, when there are no such functional groups in the molecule, the on-resin quantitation is very difficult. Although the elemental analysis method has been occasionally used for resin samples[5], it is not generally

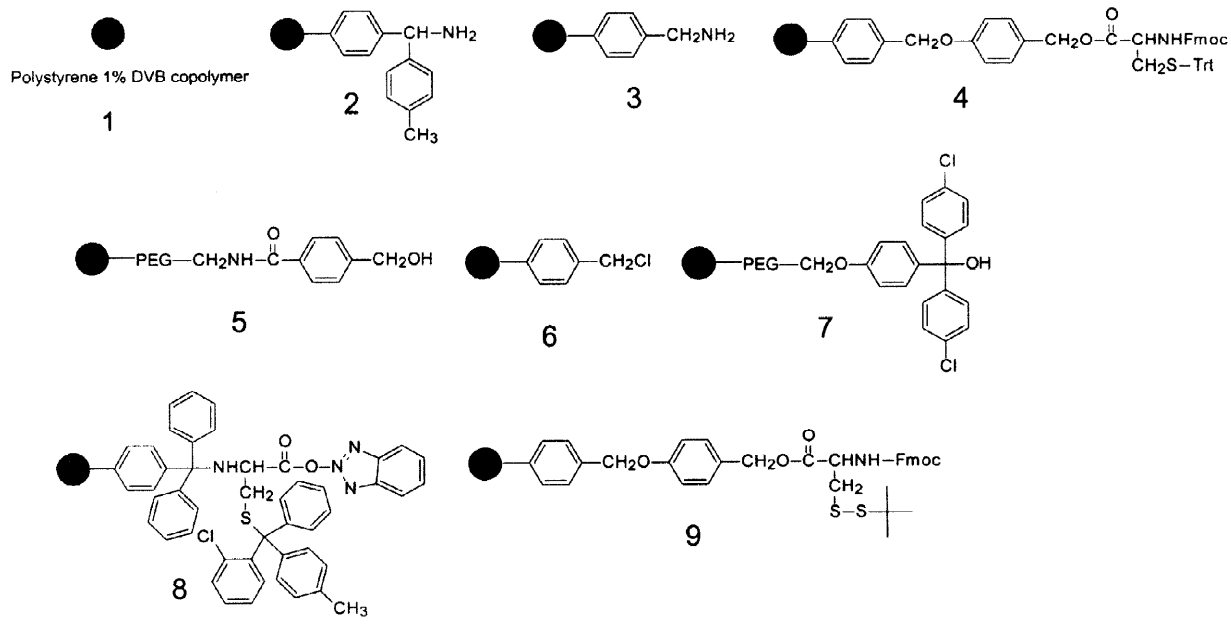


Figure 1. Chemical structures of resin-bound compounds characterized by elemental analysis.

applied as a routine quantitation tool in SPOS. In this study, we investigated the role of the elemental analysis in characterization of resin-bound organic compounds and in the quantitatively monitoring of SPOS in the reaction optimization stage of combinatorial chemistry.

RESULTS AND DISCUSSION

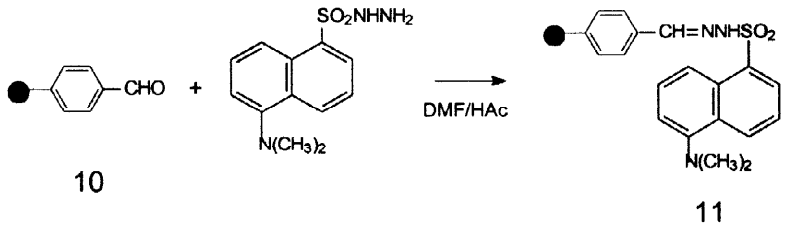
In a carbon-hydrogen-nitrogen (CHN) analysis, resin compounds were combusted and oxidized completely to form CO_2, H_2O and N_2 which are then quantitatively determined simultaneously on the basis of their different thermoconductivities relative to the carrier gas helium. All samples for CHN analysis were in reference to the standard acetanilide. 1% Divinylbenzene (DVB) - polystyrene copolymer is the basic matrix for many resins used in SPOS. The theoretical C/H ratio of the plain 1% DVB-polystyrene

Table 1. Elemental analysis of resin-bound compounds: reproducibility and accuracy

Compd.	Weight (mg per measurement)	Element	Measurements	Value (mg/g resin)	Expected[a] (mg/g resin)
1	2	C/H	4	920±2/77±1	923/77
2	2	N	4	6.7±0.2	6.44
3	2	N	4	14.4±0.3	14.7
4	2	N	4	9.9±0.2	9.1
5	2	N	4	3.8±0.2	3.9
6a[b]	15-20	Cl	2	36.5±0.01	36.1
6b	15-20	Cl	2	25.7±0.01	26.6
7	15-20	Cl	2	13.6±0.1	13.3
8	15-20	Cl	2	22.9±0.2	22.1
8	20-35	S	2	22.5±0.3	20.2
9	20-35	S	2	34.8±0.4	32.0

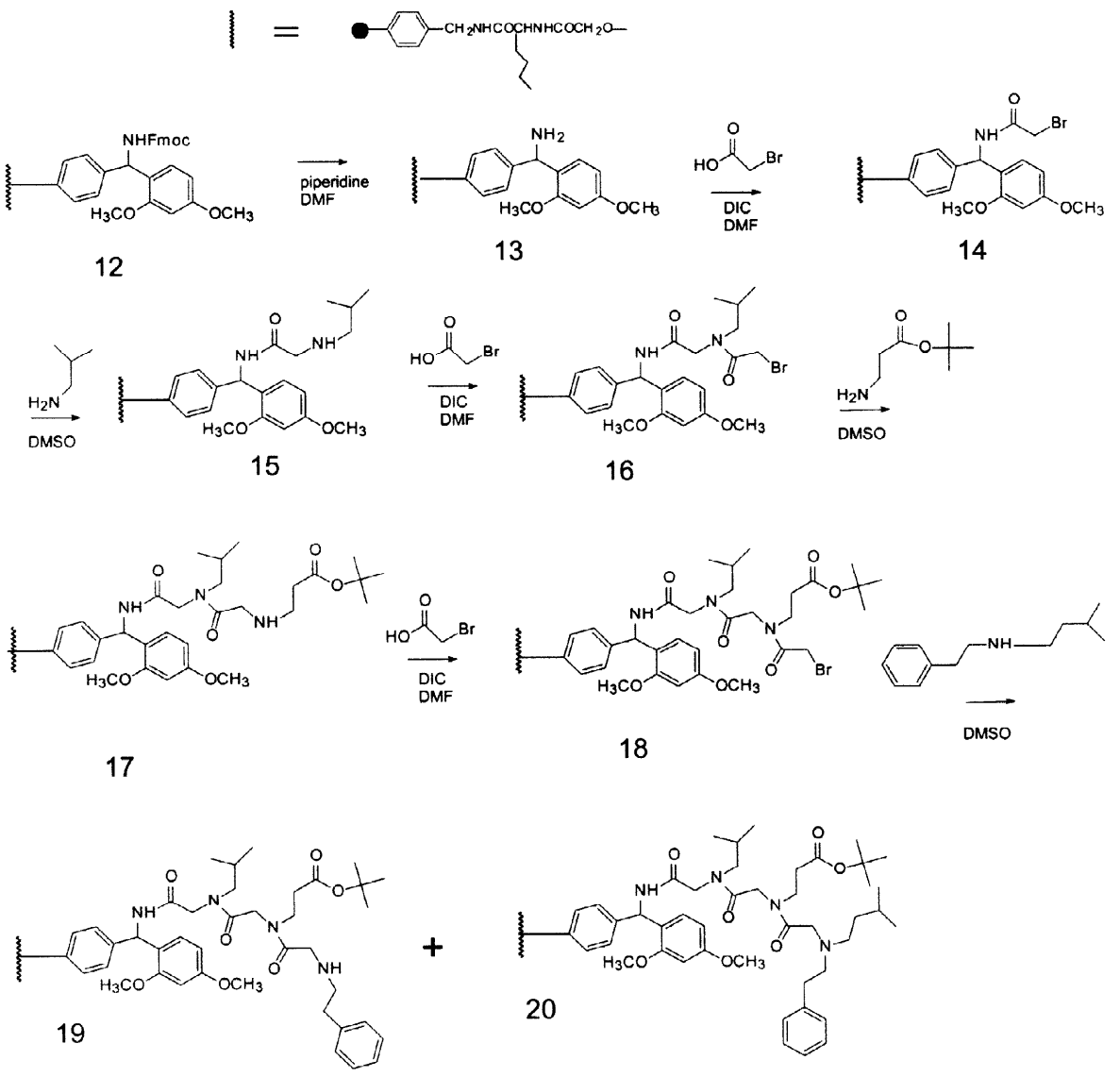
Note: a. Photometric determination of Fmoc. See experimental section for details. b. 6a and 6b are from two different batches.

Scheme 1



10 11

Scheme 2



resin based on the ratio of starting materials used for polymerization is 92.3/7.7. Elemental analysis of this resin in quadruple gave a ratio of 92.0±0.2/7.7±.01 showing excellent reproducibility and accuracy. To further investigate the reproducibility of the method, a multiple analysis of compounds **2-5** was carried out for their nitrogen contents (Table 1). The reproducibility of the data is very good as shown by the very small variations. It is important to point out that only 2 mg of resin is required for a CHN analysis and each analysis takes less than 20 min in an automated fashion. In routine analysis, a single analysis or a duplicate measurements should be sufficient.

In addition to nitrogen quantitation, a series of resin samples containing sulfur and chlorine were also analyzed (Table 1). All the expected values including those for nitrogen quantitation mentioned above came from well-established photometric analysis such as analysis of the cleavaged fluorenylmethoxy-carbonyl (Fmoc) and N'-hydroxylbenzotriazole (HOBt) groups except that the value for plain polystyrene is based on the ratio of starting materials for polymerization. Standard deviations from the expected value for most samples are below 5%, a level acceptable for quantitative analysis in SPOS.

The good reproducibility and accuracy of the elemental analysis method prompt us to apply this technique in the quantitatively monitoring of SPOS. The synthesis of a dansylhydrazone from a resin-bound aldehyde was investigated (Scheme 1). The nitrogen content was increased from <0.2 to 22.7 mg/g after the reaction (Table 2) showing a nearly quantitative conversion which is also confirmed by single bead IR and a dye-coupling quantitation method.[3]

The potential of the method for quantitatively monitoring of solid-phase organic synthesis was further substantiated by the successful monitoring of a series of peptoid synthesis reactions (Scheme 2). Peptoids or oligo-N-substituted glycines are a class of compounds with unique folding properties[6] and potential applications in DNA delivery in gene therapy[7]. Following the published procedure[8] with only minor modifications, the starting material Rink amide AM resin (**12**, 0.44 mmol/g) was first activated and also quantitated by the cleavage of the Fmoc chromophore[9]. The loading of the resulting amine resin is increased to 0.48 mmol/g due to the altered resin/functional group ratio after the leaving of Fmoc. In all SPOS, it is necessary to re-calculate the loading after each synthetic step due to the changed resin/functional group ratio. The increased weight from the new group was added to the base weight and the original loading in term of mmol/g was re-calculated after each reaction step. As more fragments of similar elemental composition are added, the relative change in the values get smaller and therefore the relative errors get larger. For this reason, we did not use nitrogen quantitation as a indicator to monitor this synthesis. The six-step synthetic reaction was quantitatively monitored by both the bromine quantitative analysis and nitro phenylisothiocyanate-O-trityl (NPIT) quantitation.[4d] The disappearance of the primary

and the secondary amines was quantitatively correlated with the formation of the brominated compounds as determined by both the NPIT test and the bromine quantitation (Table 2).

In the last step, the bromine test suggests a quantitative conversion of **18** to **20**. However, NPIT test suggests the presence of 30% of amines in the product resins. In order to investigate the inconsistency, end products were cleaved from resin and analyzed by MS and HPLC. MS confirmed the presence of both **19** and **20** and HPLC indicated the ratio of **19/20** is 1/1.82. The cause for the formation of the side product

Table 2. Elemental analysis of resin-bound compounds: reaction monitoring

Compd.	Weight (mg per measurement)	Element	Value (vs. theor) (mg/g resin)	yield Br analysis	yield NPIT
10	2	N	<0.2	--	--
11	25-35	N	22.7 (24.3)	93.4% [o]	--
12	25-35	Br	--	--	--
13	25-35	Br	0 (0.0)	--	109% [o]
14	25-35	Br	37.1 (36.7)	101% [o]	negative [s]
15	25-35	Br	0.12 (0.0)	97% [s]	100.5% [o]
16	25-35	Br	32.3 (34.9)	92.5% [o]	negative [s]
17	25-35	Br	0.08 (0.0)	complete [s]	78% [o]
18	25-35	Br	24.3 (32.3)	75.2% [o]	negative [s]
19+20	25-35	Br	0.02 (0.0)	complete [s]	30% [o]

Note: o - overall yield. s - One step yield.

19 is the 6% impurity (phenylethyamine) in the reagent for the last step synthesis. This result indicates that the purity of reagents is crucial when the impurity is more reactive. In the final step, the combination of quantitative measurements helped predict the result which was shown by HPLC and MS, after cleavage from the solid support. This type of on-resin analysis can help facilitate the reaction optimization steps required in developing on-resin synthetic processes.

SUMMARY

In this investigation, we have systematically evaluated the value of the combustion elemental analysis method in the characterization of resin-bound organic molecules and in the monitoring of SPOS. Our results show that the elemental analysis method is a reproducible and an accurate quantitation method for polystyrene- and TentaGel-bound organic compounds. It is extremely valuable in the monitoring of SPOS and the results correlated well with photometric methods. We emphasize the importance of sample washing and drying to elemental analysis results since the resin-trapped reagent or solvent molecules will cause the variable results. In practical considerations, although the combustion elemental analysis method is a destructive method and is not always sample efficient, it adds a quantitation tool for characterization of solid-bound samples and monitoring SPOS steps that currently can not be quantitated on resin supports.

EXPERIMENTAL

Materials. All resins in Fig.1 and starting materials in Scheme 1 and 2 were purchased from NovaBiochem (San Diego, CA). The loading of **4** and **9** was based on the released Fmoc groups and that of **8** on the release of HOBt group under peperidine/DMF treatment. The loadings of **2**, **3**, **5**, **6**, **7** and **10** were also based on the determination of Fmoc chromophore released from their Fmoc derivatives (coupling with Fmoc-Cl, Fmoc-hydrazide or Fmoc-amino acids). In peptoid synthesis, Rink AM Resin (which is FMOC protected, loading of 0.44 mcq/g) was purchased from Peptides International, Louisville, Kentucky, USA, catalog number RHM-1073-PI. All solvents and reagents were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin, USA, unless otherwise noted.

General synthesis methods. The reaction in Scheme 1 was carried out and monitored as described in reference 3. For reactions in Scheme 2, all solid phase reactions are carried out in various glass solid phase reactors, which can be clamped onto a Wrist Action Shaker, Model 75, from Burrell Scientific, Pittsburgh, Pennsylvania, USA. Solvents and reagents are added through an opening in the top of the reactor. Reaction vessels are drained through a valved opening at the bottom of the vessel (below the frit) with the aid of a small nitrogen pressure applied at an opening on top of the glass vessel. Glycine *tert*-butyl ester was liberated from the hydrochloride salt by suspending in THF, and bubbling ammonia through for a few minutes, then filtering off the resulting ammonium hydrochloride with the aid of a celite plug, then evaporating the filtrate on a rotary evaporator. N-phenethyl-N-isoamyl amine was prepared via the Borch procedure[10] of reductive amination of phenethylamine and isovaleraldehyde. The distilled product contained 6% phenethylamine as determined via GC and NMR. The solid phase "peptoid" synthesis was accomplished by a slightly modified version of the Chiron procedure.[8]

Combustion elemental analysis. Elemental analysis was performed by our contract laboratory Robertson Microlit Laboratories, Inc., Madison, NJ. CHN was determined simultaneously with a Perkin Elmer CHN analyzer Model 2400. For sulfur, chlorine and bromine analysis, the resin sample was first combusted and oxidized completely. The content of sulfur was determined by titration with barium acetate with dimethylsulfonazo III as an indicator; and the chlorine by titrating with mercuric acetate with diphenyl carbazone as an indicator. The reference standards were S-Benzylthiuronium chloride for both measurements. The content of bromine in the resin was determined by titrating the oxidized iodine with thiosulfate using the *p*-Nitrobenzyl bromide as a standard.

Qualitative NPIT test:[4d] To a small sample of washed resin in a small plastic filter tube was added 0.25 mL of 0.03M NPIT solution in DMF. This was allowed to stand for 5 minutes then the resin was filtered and washed 3 times with 0.5 mL DMF and 5 times with 0.5 mL CH_2Cl_2. Then 0.5 mL of 2% trifluoroacetic acid in acetonitrile was added to the resin. If no color was detected, the result was negative, meaning no reactive amines present. If the color was orange, the result was positive for the presence of reactive amines.

Quantitative NPIT test: 1 mg of washed and vacuum dried resin was put into a 1 mL plastic filter tube. About 1 mL of DMF was added to swell the resin, and then drained after 5 min. Approximately 0.25 mL of 0.03M NPIT in DMF was added. The sample was let stand for 15 minutes. The resin was drained and washed with DMF (3 X 0.5mL) and CH_2Cl_2 (5 X 0.5 mL). The DMT group was cleaved with 5 X 0.25 mL

of 2% trifluoroacetic acid/CH_2Cl_2, and then 3 X 0.25 mL of 0.2% tirfluoroacetic acid/acetonitrile. These filtrates were collected and combined into a 25 mL volumetric flask. This was diluted to 25 mL with 0.2% trifluoroacetic acid/acetonitrile. By comparison to a standard concentration curve, the concentration of dimethoxytrityl cation was extrapolated by measuring the absorption at 498 nm. The average of 2 or 3 determinations is reported.

General Procedure for the synthesis of resin-bound compound 11. The procedures for the synthesis of **11** was the same as in reference 3.

Microanalytical Data and NPIT results for Rink AM Resin 12:

Found: C, 84.86; H, 7.41; N, 1.93; Cl, <0.10; Br, <0.20; I, <0.30.

Qualitative NPIT Test: negative.

FMOC deprotection of resin 12 to give amine resin 13: Rink AM Resin (5g, .44 mmol) and 45 mL of DMF was put in a solid phase reaction vessel, and agitated via wrist action shaking for 30 min to swell the resin. The solvent was drained, and then 45 mL of 20% piperidine in DMF was added and agitated 5 minutes, then the vessel was drained, and the piperidine treatment was repeated for 30 minutes. The reaction was drained, and the resin washed with DMF (3 X 45 mL, 5 min. each), CH_2Cl_2 (3 X 45 mL, 5 min. each), and MeOH (3 X 45 mL, 5 min. each). The resin was dried on the vacuum pump for 1.5 hours to give 4.03 g of resin **13**. A 40 mg sample was collected to obtain microanalytical data and NPIT test results.

Found: C, 85.90; H, 7.65; N, 2.09; Cl, <0.10; Br, <0.20; I, <0.30.

Qualitative NPIT Test: positive.

Quantitative NPIT Test: 21.2 micromolar;109 % of the value based on the loading of the starting material.

General procedure for bromoacetylation of amine containing resin: The resin was swelled with DMF and agitated for at least 15 minutes, the solvent was drained, then a 0.5 M solution of bromoacetic acid in DMF was added and a 3.2 M solution of diisopropylcarbodiimide in DMF was added (10 eq. of bromoacetic acid and 13 eq. of DIC based on estimated loading of the resin). The reaction was agitated for 30 minutes, drained and repeated one more time for 30 minutes. The reaction was drained and the resin was washed with DMF (3 X 45 mL, 5 min. each), CH_2Cl_2 (3 X 45 mL, 5 min. each), and MeOH (3 X 45 mL, 5 min. each). The resin was dried on the vacuum pump for at least an hour. A 40 mg sample was collected to obtain microanalytical data and NPIT test results.

General procedure for amination of the bromoacetyl containing resin: The resin was swelled with DMF and agitated for at least 15 minutes, the solvent was drained, then a solution of the appropriate amine (1.8 M in DMSO) was added to the resin (10 mL per gram of resin) and the mixture was agitated for 16 hours. The reaction was drained and the resin was washed with DMF (3 X 45 mL, 5 min. each), CH_2Cl_2 (3 X 45 mL, 5 min. each), and MeOH (3 X 45 mL, 5 min. each). The resin was dried on the vacuum pump for at least an hour. A 40 mg sample was collected to obtain microanalytical data and NPIT test results.

Bromoacetylation of amine resin 13 to give resin 14: Amine resin **13** (4.03 g) was bromoacetylated using the general procedure to give 4.22g of resin **14**.
Found: C, 81.61; H, 7.24; N, 2.02; Br, 3.71 (Br indicates 101% overall yield).
Qualitative NPIT Test: negative.

Amination of resin 14 with isobutylamine to give amine resin 15: Resin **14** (4.22 g)was aminated using the general procedure to give 4.12 g of amine resin **15**.
Found: C, 84.51; H, 7.80; N, 2.68; Br, 0.12.
Qualitative NPIT Test: positive.
Quantitative NPIT Test: 18.5 micromolar;100.5 % of theoretical.

Bromoacetylation of amine resin 15 to give resin 16: Amine resin **15** (4.12 g) was bromoacetylated using the general procedure to give 4.32g of resin **16**.
Found: C, 80.27; H, 7.35; N, 2.68; Br, 3.23 (Br indicates 92.5% overall yield).
Qualitative NPIT Test: negative.

Amination of resin 16 with glycine tert-butyl ester to give amine resin 17: Resin **16** (4.32 g)was aminated using the general procedure to give 4.12 g of amine resin **17**.
Found: C, 82.47; H, 7.64; N, 2.87; Br, 0.08.
Qualitative NPIT Test: positive.
Quantitative NPIT Test: 13.29 micromolar;78% overall yield.

Bromoacetylation of amine resin 17 to give resin 18: Amine resin **17** (0.5 g) was bromoacetylated using the general procedure to give resin **18**.
Found: C, 77.15; H, 7.21; N, 2.90; Br, 2.43 (Br indicates 75.2 % overall yield).

Qualitative NPIT Test: negative.

Amination of resin 18 with N-phenethyl-N-isoamylamine to give amine resin 19/20: Resin **18** was aminated using the general procedure to give amine resin **19/20**.
Found: C, 82.12; H, 7.80; N, 3.13; Br, 0.02.
Qualitative NPIT Test: positive.
Quantitative NPIT Test: 5.5 micromolar; indicates ~30% titratable amine relative to amount of expected product. However the expected tertiary amine should not give a positive result with NPIT. HPLC of the cleaved products revealed ~ 35% yield of undesired secondary amine product (see below).

Cleavage of Product from Resin 19/20: To resin **19/20** (130 mg, ~ 0.05 mmol) was added 2.5 mL of 95% trifluoroacetic acid/water. This was stirred for 15 minutes, filtered and collected the filtrate. This resin was washed two times more with 2.5 mL 95% trifluoroacetic acid/water and one time with 1 mL of 100% water. All filtrates were combined and concentrated under high vacuum for 16 hours to give 23.2 mg of foam. HPLC (reverse phase C18 column, with gradient from 20% acetonitrile/80% water containing 0.1% trifluoroacetic acid to 50% acetonitrile over 20 min) showed two products. The lower Rf = 4.38 corresponded to an MH+ of 421 via positive mode electrospray MS and the higher Rf = 6.46 corresponded to an MH+ of 491. The ratio of these peaks was 1.82 to 1 in favor of the desired higher molecular weight compound. The lower Rf compound was the product resulting from the reaction of phenethylamine with the bromoacetyl containing resin **18**. Therefore, the primary amine contaminant present at the level of 6%, accounted for ~35% of the product, showing increased rate of reactivity of the primary amine over that of the secondary amine in the "peptoid" chemistry.

ACKNOWLEDGMENTS

We thank Dr. Wenbao Li for the synthesis of **11** and Robertson Microlit Laboratories, Inc. for elemental analysis service.

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