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# Apparatus and Method for Cleavage of Compounds from Solid Support by Gaseous Reagents

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Polymer-supported library compounds have been cleaved from acid sensitive linkers using gaseous reagents hydrogen fluoride and hydrogen chloride. Compounds attached to Wang, Rink, AMEBA, and MBHA linkers were cleaved. The reaction gas container for gaseous cleavage was made of a polypropylene tube enclosed by two side-plates. Fast and reliable filling by a reagent gas was achieved by introducing the gas into the evacuated reaction container rather than gradually replacing the gas under atmospheric pressure.

The most striking feature of combinatorial synthesis of library compounds on solid phase is the vast number compounds that are prepared per unit of time. As a consequence, new techniques that make concurrent handling of large numbers of reaction vessels manageable are needed. In this article we wish to describe an apparatus and method for gaseous cleavage of library compounds from solid-phase support.

At the end of solid-phase organic synthesis, products are cleaved from the insoluble support. Depending on the type of linker, various reagents were employed to enable the release of synthesized compounds. In most cases a liquid cleavage cocktail has been used. An alternative method of cleaving compounds from resin beads is gaseous cleavage. The use of ammonia gas has been applied to cleave the ester bond used for immobilizing of peptides to pins[1] or Merrifield resin.[2] Ammonia gas under pressure has been described for the deprotection and cleavage steps during the large-scale synthesis of oligonucleotide.[3] We have already reported the use of gaseous HCl and TFA vapors for cleavage of acids, alcohols, and amines attached to the trityl linker.[4] We also described cleavage of compounds from the *p*-methylbenzhydrylamine linker using gaseous HF at room temperature.[5,6]

## Results and Discussion

To be able to cleave compounds from a large number of reaction vessels at one time, we built a simple cleavage station. The apparatus consisted of a reagent gas container where the resin-bound compounds were exposed to the reagent gas, and a series of four trap bottles for absorbing the reagent gas after the cleavage was finished. The original reaction gas container that we used for the gaseous cleavage of compounds from resin beads was a simple polypropylene container.[6] Since it was not possible to evacuate this container, the air was gradually displaced by an inert gas (nitrogen) and reagent gas then slowly displaced nitrogen until the reaction gas container was saturated. A great deal of reagent gas was vented along with the nitrogen during this slow introduction period before the container was saturated. Monitoring the stage of saturation of the container by the reagent gas was problematic. Experiments indicated that a lengthy introduction period was necessary to ensure proper reagent gas exposure and complete cleavage. This slow saturation of the reaction gas container took about 15 min. The second shortcoming of the original design was difficulty in achieving airtight sealing of the gas container.

The new reaction gas container was built from a polypropylene tube (enclosed by two polypropylene side-plates) that was evacuated prior to the introduction of reagent gas. Introducing the gas quickly and saturating the evacuated reaction gas container cut this time substantially. The new apparatus was also significantly less prone to leaks. When HF gas contacts moisture in the air, it turns white, and any leak is visible. No evidence of gas leakage was observed. We have been changing the polypropylene seals frequently; however, the seals are inexpensive and easy to make.

We expected removal of the reagent gas following cleavage to be much quicker by pumping the gas out of the chamber (evacuating the chamber) rather than displacing the reagent gas in the chamber with inert gas. Reagent gas could be pumped out completely from the reaction gas container, and the chamber could then be filled with nitrogen and evacuated two more times to clear out residual reagent gas before removing cleaved compounds. This procedure could be done in 5−10 min, but there are at least two problems associated with this fast method of removing reagent gas.

When the reagent gas was removed by gradual displacement with nitrogen, the venting gases bubbled through the water in the traps. The reagent gas dissolved in the water, and other nonsoluble gases escaped into the next trap bottle. When flushing with nitrogen, a certain amount of insoluble nitrogen is always present in the gas being vented from the apparatus. When HF gas is pumped from the reaction gas container, all the venting gas is soluble and is dissolved in the trap solutions. Insoluble gas in the trap bottles is displaced by soluble reagent gas which dissolves. Pressure in the trap bottles begins to drop. The water solutions in the trap bottles begin to move backward through the apparatus. If left unchecked, the first normally empty trap bottle will fill with

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**Table 1.** Gaseous Cleavage of Model Compounds from Wang Linker[a]

| | | | HCl | | HF | |
|---|---|---|---|---|---|---|
| entry | amino acid | subst. | yield | % | yield | % |
| 1 | Val | 0.64 | 0.20 | 31 | 0.46 | 72 |
| 2 | Gly | 0.46 | 0.24 | 52 | 0.37 | 80 |
| 3 | Pro | 0.75 | 0.43 | 57 | 0.50 | 80 |
| 4 | Ala | 0.71 | 0.22 | 31 | 0.52 | 73 |
| 5 | Phe | 0.51 | 0.14 | 27 | 0.41 | 80 |
| 6 | Ile | 0.62 | 0.17 | 27 | 0.43 | 76 |
| 7 | Leu | 0.58 | 0.23 | 40 | 0.42 | 72 |
| 8 | Nle | 0.65 | 0.20 | 31 | 0.44 | 75 |
| 10 | Ala-crown | 33[b] | 3.6[b] | 11 | 29[b] | 88 |

[a] Wang resin esterified with Fmoc protected amino acids. Substitution and yield are in mmol/g; HCl and HF exposures were for 2 h. [b] Rink linker derivatized crown. Substitution is expressed in $\mu$mol per crown.

solution. A sufficient volume of nitrogen must be introduced during removal of reagent gas to prevent this backflow from occurring.

The second problem was connected with the high flow of reagent gas into trap bottles. The pump delivered a high volume of reagent gas that did not have sufficient time to dissolve when passing through the traps. The reagent gas reached the last trap immediately. Bromophenol blue acid−base indicator, present in the solution, changed color when reagent gas was pumped out of the reaction chamber. This color change only occurred in the last trap bottle when the reagent gas was pumped out, but not when nitrogen was used for flushing the reagent gas container. This color change indicates that reagent gases are reaching the last trap bottle and may still be present in gases leaving the apparatus entirely.

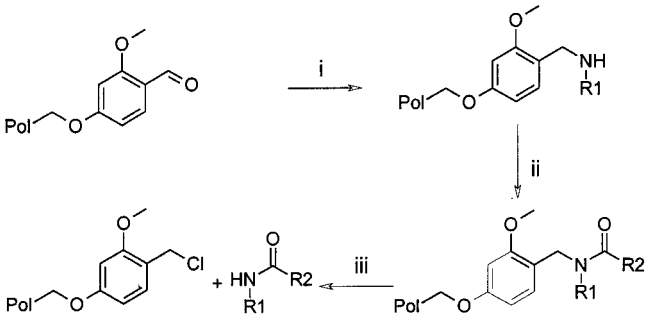
The risk of backflow and the possibility of releasing reagent gas made quick removal of reagent gas less desirable. Therefore, on a routine basis we flushed out the reaction container with a flow of nitrogen. If a regulator valve was installed that reduced the gas flow rate, it might be possible to safely pump the reagent gas from the reaction gas container, but this might not resolve the backflow problem.

To establish the cleavage efficiency from individual linkers, we exposed model compounds attached to Wang, Rink, AMEBA, and MBHA linkers to gaseous reagents. All selected linkers are routinely used in solid-phase synthesis. Standard cleavage conditions include trifluoroacetic acid in the case of Wang, Rink, and AMEBA linkers; compounds attached to the MBHA linker are cleaved by liquid HF or trifluoromethanesulfonic acid.

The Wang resin esterified with Fmoc protected amino acids was exposed to gaseous HCl or gaseous HF. The results are summarized in Table 1. With the exception of Gly and Pro, 2 h exposure to HCl gas cleaved less than 50% of Fmoc protected amino acids. HF exposure for the same time resulted in 72−88% cleavage yield. A shorter reaction time of 1 h lowered the yield by 80% when compared to the 2 h exposure. Extending exposure to 4 h did not increase the yield. Nonquantitative yield may be caused by incomplete extraction, we have not observed significant decomposition of the Fmoc amino acids by HF gas.

Rink linker derivatized SynPhase crowns were acylated with Fmoc-Ala and the resin-bound product cleaved by HCl and HF. Whereas HCl cleaved only 11% of Fmoc-Ala, the HF exposure provided 88% yield.

**Scheme 1**[a]




[a] Reagents and conditions: (i) 0.5 M amine in 5% AcOH in DMF, rt, overnight, $NaBH(AcO)_3$, rt, 5 h; (ii) acid chloride, DIEA in DCM, rt, overnight; (iii) HF or HCl gas, rt, 2 h.

**Table 2.** Cleavage of Acyl Amino Acid Esters from AMEBA Linker[a]

| entry | amine | HF yield | HCl yield |
|---|---|---|---|
| 1 | Ala-OMe | 113 | nd |
| 2 | Glu(OMe)-OMe | 121 | nd |
| 3 | Phe-OMe | 118 | nd |
| 4 | Gly-OMe | 124 | nd |
| 5 | Ile-OMe | 105 | 97 |
| 6 | Lys-OMe | nd | 99 |
| 7 | Leu-OMe | 133 | 92 |
| 8 | Met-OMe | nd | 101 |
| 9 | Val-OMe | 125 | 96 |
| 10 | Tyr-OMe | 131 | 94 |
| 11 | Ala-OtBu | 128 | 102 |

[a] HF yields over 100% are due to a common contaminant as mentioned in the text; nd = not determined.

Cleavage from the AMEBA linker was evaluated the following way. The 4-(4-formyl-3-methoxyphenoxy) butyryl resin was reductively aminated with a set of amino acid esters. The polymer-supported secondary amine was acylated with 4-($\alpha$,$\alpha$,$\alpha$-trifluoromethyl)benzoyl chloride (Scheme 1). The resin-bound product was exposed to HCl or HF for 2 h. The results summarized in Table 2 demonstrated complete cleavage of products in HCl. Yield calculations are based upon the recovered mass vs expected mass. The analytical HPLC traces revealed high purity (95−99%) of products obtained by HCl cleavage; all products cleaved by HF contained a common contaminant detectable both at 215 and 254 nm. The contaminant prevented accurate calculations of the yield. It is also worthwhile to mention that gaseous HCl completely cleaved both the Boc group (entry 6) and the tBu ester (entry 11). The Met derivative was partially decomposed in HF.

The MBHA resin was acylated by Fmoc-amino acids, the Fmoc group was removed, and the amine was acylated with 4-($\alpha$,$\alpha$,$\alpha$-trifluoromethyl)benzoyl chloride. Acylated amino acids were cleaved from the MBHA resin quantitatively by HF in 2 h; negligible cleavage by HCl was detected.

Cleavage of model compounds was designed to assess the scope and limitation of the method. We used the gaseous reagents for cleavage of numerous heterocyclic compounds, including 2-aminobenzimidazoles,[7] quinoxalines, benzodiazepines, and triazines (unpublished results).

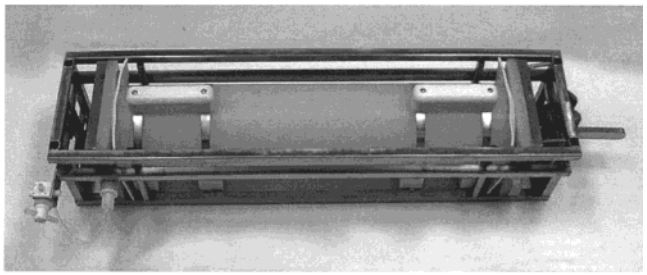


**Figure 1.** Apparatus for gaseous exposure.

We also observed that proper exposure to HF gas was accompanied by a color change that occurred to some degree in the resin, but primarily in the material of the polypropylene plate used to carry the resin. The initial white/gray color of the plates changed to a dark brown after exposure to HF. Once the plate was placed in the neutralization container with solid NaOH, the plate quickly changed its color again to a deep purple. After complete neutralization overnight, the plate was gray, slightly darker than its initial color. When plates have not received this level of exposure to HF, the color was only a light brown immediately after HF exposure and faded to pink rather than deep purple when neutralization began. Plates that exhibited these unexpected color changes were typically resubjected to another round of HF gas. Although the compounds in these plates might have been sufficiently cleaved, we considered it safer to resubject the plates to cleavage conditions until they exhibit the expected color change.

It is worth mentioning that when the HF flow was stopped immediately after the first bubbles appeared in the trap bottles, the plates appeared not to have been properly exposed to HF, even though they were exposed to HF gas for 2 h. Therefore we continued introduction of reagent gas for a period of 3 min after the pressure inside container was equilibrated with the atmospheric pressure. This additional period was sufficient to produce the expected color change in the plates.

## Materials and Methods

The synthesis of model compounds was performed on Wang resin[8] esterified with Fmoc protected amino acids, 4-(4-formyl-3-methoxyphenoxy)butyryl (AMEBA) resin[9] (all Novabiochem, Laufelfingen, Switzerland), MBHA resin (Advanced ChemTech, Louisville, KY), or Rink[10] derivatized SynPhase crowns (Chiron Technologies, Clayton Victoria, Australia).

Polypropylene 96-well plates (March Biomedical products, Rochester, NY) or polypropylene syringes equipped with a frit at the bottom (Torviq, Tucson, AZ) were used as reaction vessel for gaseous cleavage.

**Apparatus for Gaseous Cleavage.** The reaction gas container for gaseous cleavage was constructed from a polypropylene tube (diameter 143 mm, wall thickness 12 mm, length 724 mm) and enclosed by two polypropylene side-plates (thickness 19 mm), using a cross-linked polyethylene foam liner (VWR, Phoenix, AZ) as a seal (Figure 1). One side-plate was stationary, the second one was mobile, and the airtight seal was achieved by firmly pressing the mobile side-plate against the tube. Two side-plates also accommodated the inlet and outlet into and from the reaction gas container. The entire reaction gas container was placed in a stainless steel cage. The polypropylene tube was removed from the cage to load and unload reaction vessels containing resin-bound compounds (Figure 2). The tube accommodated 13 96-well 1.2 mL plates: eight side by side on top and five end to end on the bottom. A rigid polypropylene sheet with holes to allow the free circulation of reagent gas separates the two rows of plates.

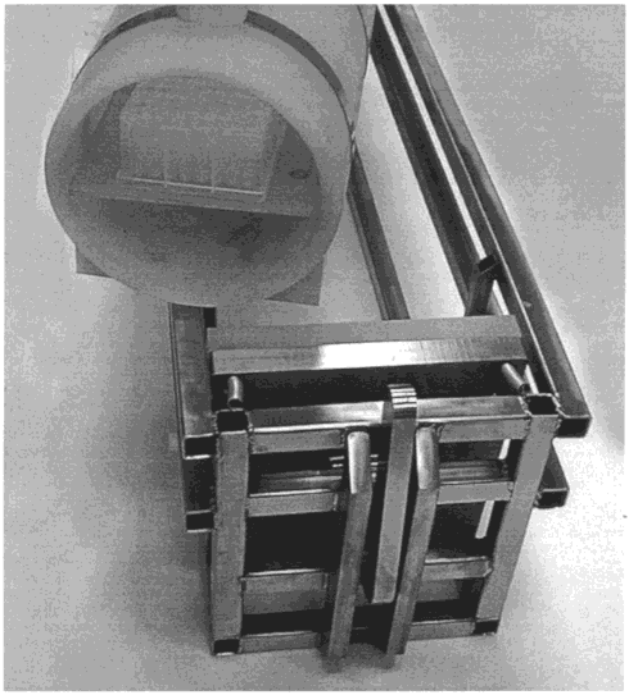


**Figure 2.** Reaction container with a plate.

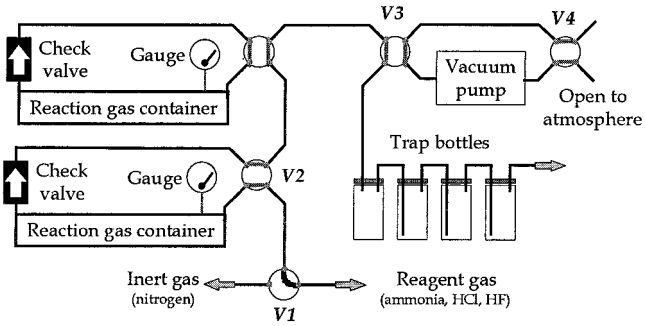



**Figure 3.** Apparatus with vacuum compatible reaction gas container.

The connections of the reaction gas container to the source of gas and trap bottles are shown in Figure 3 (arrangement with two reaction gas containers is shown). Vetted parts of valves (Partek, Tucson, AZ) were made of Teflon, and $^1/_4$ in. Teflon tubing was used for connections. Two ports of the three-port valve V1 connected the source of an inert gas (nitrogen) and a reagent gas (HF or HCl). The third port was linked to the four-port valve V2. The outlet and inlet of the reaction gas container were connected to the neighboring ports of the four-port valve V2. A check valve (Partek, Tucson, AZ) was attached to the outlet of the reaction gas container. The last port was connected to the four-port valve V3. The V3 valve connected the vacuum pump (KNF,

Trenton, NJ). The suction port of the pump was connected to the V3 valve via the four-port valve V4. The remaining ports of the V4 valve were connected to the atmosphere. The last port of the V3 valve went to the first trap bottle. There were four 1 L polypropylene trap bottles, the first one empty, the next two filled with 0.5 L of water, the last filled with 0.5 L of 10 mM NaOH and an acid−base indicator.

A vacuum gauge was attached to the apparatus for testing. The apparatus was evacuated, and the gauge indicated that it was holding sufficient vacuum, although the pressure did rise very slowly. The vacuum gauge was removed before introducing reagent gas.

**Gas Cleavage Procedure.** The resin-bound products were placed into either polypropylene 96-well plates or plastic syringes (with a frit, without a plunger) and sealed inside the reaction gas container. The reaction gas container was first evacuated with a pump. Since no vacuum gauge was used during the evacuation, we monitored the course of evacuation by disappearance of bubbles in the trap bottles. The disappearance of bubbles was also an indication of an airtight seal of the apparatus (continuous bubbling indicates a leak). Once the reaction gas container was evacuated, the valve V3 was turned to connect the reaction gas container directly to the trap bottles. The valve V4 was then opened to the atmosphere, and the pump was turned off. The check valve prevented the air from coming into the container. The reagent gas was introduced into the evacuated reaction gas container via the valve V1. It usually took less than 1 min before the bubbles started to appear in the trap bottles. This was a sign that the pressure inside the reaction gas container had equilibrated with atmospheric pressure.

Reagent gas was being absorbed into the resin at this point. If the flow of reagent gas were stopped immediately, the pressure in the container would begin to drop and cleavage may be incomplete. The flow of reagent gas was slowed but remained at a rate such that bubbles continued to appear in the traps. This introduction of gas was maintained for 3 min to fully saturate the resin. The valve V2 was closed, and the compounds were exposed to reagent gas for a time necessary to cleave the resin-bound compounds (in most cases approximately 2 h). During the HCl cleavage, additional reagent gas was introduced into the container after 1 h of exposure. Following exposure, reagent gas was flushed with nitrogen or pumped out. In either case the container was filled with nitrogen before removing cleaved compounds.

The volume of nitrogen necessary to sufficiently displace the reaction gas container is dependent upon the size of the reaction gas container. Assuming complete diffusion of nitrogen into the reagent gas (gases introduced will not diffuse immediately throughout the chamber, so these calculations may overestimate the concentration of residual reagent gas), each container volume of nitrogen injected into the reaction gas container leaves behind residual gas according to eq 1. Gas remains in the reaction gas container according to eq 2 where $n$ is the number of container volumes of nitrogen injected. The reaction gas container volume of 11.6 L will have less than 0.6% residual reagent gas (eq 3) after flushing with nitrogen for 30 min at 2 L/min.

$$\lim_{x\to\infty}\left(\frac{x-1}{x}\right)^x = \frac{1}{e} \tag{1}$$

$$\text{residual gas concentration} = \left(\frac{1}{e}\right)^n \tag{2}$$

$$\left(\frac{1}{e}\right)^n = 0.00567 \tag{3}$$

$$(30\ \text{min})(2\ \text{L/min}) = 60\ \text{L}, \quad n = 60\ \text{L}/11.6\ \text{L} = 5.17$$

Since the reaction vessels and compounds exposed to HF still contained residual adsorbed HF, they were placed in a sealed container with solid NaOH. Alternatively, the plates were left in the reaction gas container together with a dish with NaOH and evacuated. For convenience, we neutralized the HF fumes by reacting with the NaOH overnight; however, this time can be shortened.

**Model Cleavage Experiments.** Model resin-bound compounds were prepared in 50 mg batches of resin on manually operated Domino Blocks[11] using standard protocols for reductive alkylation and acylation. Dry resin-bound compounds in plastic syringes equipped with a frit at the bottom (without a plunger) were placed into the reaction gas container and exposed to the reagent gas for a given time. After the reagent gas was washed with nitrogen, the reaction vessels were left over sodium hydroxide overnight. The compounds were extracted with MeOH and MeOH/DCM mixture, the combined extracts were evaporated, and the yield was determined gravimetrically. Alternatively, the compounds were extracted five times with DMF, the extracts were pooled, and the concentration was determined spectrophotometrically.

**Safety Concerns.** HF is hazardous, and proper handling and disposal are required. Venting of reagent gas and disposal of concentrated acid waste are the primary safety concerns with HF gas cleavage. It is a good practice to have calcium gluconate gel (Calgonate Corp., RI) on hand to treat skin exposure to HF in the event of an accident. All concentrated acid waste must be neutralized prior to disposal. Procedures have been developed for disposal of the concentrated acid waste generated during cleavage.

Solutions of more than 40% hydrogen fluoride begin fuming. Overly concentrated acid cannot be neutralized without exposing the solution and allowing HF to escape. The traps must be changed frequently to prevent the solutions from becoming more than 40% hydrogen fluoride. The concentration was measured crudely by the increase in the volume of the first trap solution as it absorbs reagent gas.

Great care was necessary when neutralizing the concentrated acid waste because of the heat generated and the danger of a concentrated HF spill. Liquid waste in 500 mL quantities in a 1 L beaker was placed in an ice bath and mixed continuously with a stirring bar. The solid NaOH/NaF waste that was generated when neutralizing cleaved reaction vessels with resin was used to neutralize the acid waste. The NaOH/NaF waste was added to the acid waste slowly until the solution was sufficiently neutralized for disposal. Bromophenol blue was used as the indicator. The

solution changed color from yellow to blue when the pH reached about 4. This waste was then placed in the acid waste stream.

Waste neutralized by NaOH contains a large volume of precipitated sodium fluoride due to its low solubility (4 g/100 mL). Potassium hydroxide produces the more soluble potassium fluoride (92 g/100 mL).

## Conclusion

An apparatus for gaseous cleavage of library compounds from solid support has been designed and manufactured. The reaction gas container of the new apparatus is less prone to gas leaks and it can be evacuated, ensuring fast and dependable filling with a reagent gas. The apparatus is used routinely for cleavage from solid support with monthly throughput of 30 000 compounds.

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