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Chemistry

# Liquid-phase combinatorial synthesis

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**ABSTRACT** **A concept termed liquid-phase combinatorial synthesis (LPCS) is described. The central feature of this methodology is that it combines the advantages that classic organic synthesis in solution offers with those that solid-phase synthesis can provide, through the application of a linear homogeneous polymer. To validate this concept two libraries were prepared, one of peptide and the second of nonpeptide origin. The peptide-based library was synthesized by a recursive deconvolution strategy [Erb, E., Janda, K. D. & Brenner, S. (1994) *Proc. Natl. Acad. Sci. USA* 91, 11422–11426] and several ligands were found within this library to bind a monoclonal antibody elicited against β-endorphin. The nonpeptide molecules synthesized were arylsulfonamides, a class of compounds of known clinical bactericidal efficacy. The results indicate that the reaction scope of LPCS should be general, and its value to multiple, high-throughput screening assays could be of particular merit, since multimilligram quantities of each library member can readily be attained.**

The typically long and arduous process of new drug discovery and development has prompted a reorientation in thinking, along with an infusion of new technologies into the drug discovery process. A product of this renaissance has been the introduction of automated high-throughput screening. However, the overall impact that a robotic benchmark assay can impart is in one sense directly related to the number of new chemical entities that can be created. Consequently, combinatorial chemistry, a technology which allows the parallel synthesis of diverse molecular structures, has been anointed as the pharmaceutical chemical methodology of the future (1). It is within this technology that traditional methods of serial chemistry are surpassed by combinatorial techniques, having the potential to create molecular diversity exponentially (2).

A central tenet ingrained into all combinatorial chemistry is the availability of general reaction strategies and protocols which can lead to high-yield reaction products. As such, polymer-supported synthesis has emerged as one of the most important tools in research efforts focused on the construction of combinatorial libraries (3–6). Yet the "solid-phase" method in a combinatorial format, as successful as it has been, still has certain drawbacks. The most notable liability is the heterogeneous reaction conditions, which can exhibit several of the following problems: nonlinear kinetic behavior, unequal distribution and/or access to the chemical reaction, solvation problems, the use of insoluble reagents or catalysts, and pure synthetic problems associated with solid-phase synthesis. Because of the limitations that solid-phase synthesis presents, we have sought alternative methodologies for the generation of combinatorial libraries.

In essence what we have done is apply "liquid-phase" synthesis (7) to the combinatorial process. By adopting such a tack, the difficulties found in solid-phase combinatorial synthesis can be avoided, while its positive aspects are preserved. We have termed this strategy liquid-phase combinatorial synthesis (LPCS).

The cornerstone of LPCS is a soluble, linear homopolymer [polyethylene glycol monomethyl ether (MeO-PEG)] which also serves as a terminal protecting group for the library of compounds synthesized. This monofunctional polymer was selected as the homopolymer protecting group of choice, because of its successful application in peptide, oligonucleotide, and oligosaccharide synthesis (7–9). Two properties that are inherent in this homopolymer's structural makeup provide the necessary elements for it to be attractive in a combinatorial format. First, due to its helical structure MeO-PEG has a strong propensity to crystallize (10); thus as long as the polymer remains unaltered during the construction of the library, purification by cystallization can be accomplished at each stage of the combinatorial process. Second, MeO-PEG has remarkable solubilizing effects in a variety of aqueous and organic solvents (11). This solublizing feature, found in the liquid-phase process, can be used to advantage if the homopolymer is treated as a reagent and used in large excess. Another virtue of MeO-PEG's favorable solubility properties is that all manipulations in the LPCS method, including split synthesis, may be carried out under homogeneous conditions. Further, because LPCS is a solution-phase process, a recursive deconvolution strategy can be used to create and screen the library of interest (12). Lastly, yields from the individual combinatorial reaction steps can be monitored by either $^{13}C$ or $^{1}H$ NMR spectroscopy.

To demonstrate the overall potential of LPCS, a two-pronged approach was undertaken. First, we validated LPCS by integrating the combinatorial recursive deconvolution strategy into the process. By this tactic we have synthesized a library of peptides and identified several ligands from within this library that bind an acceptor molecule. This key molecule is a monoclonal antibody against β-endorphin which binds the peptide sequence Tyr-Gly-Gly-Phe-Leu (YGGFL) with great affinity. Second, we have extended the types of chemistry and also the classes of compounds that can be generated within LPCS to a small set of bioavailable organic compounds (sulfonamides) which have known bactericidal efficacy.

## MATERIALS AND METHODS

**Construction of the Pentapeptide Library.** The pentapeptide library was constructed manually on a MeO-PEG ($M_r$ 5000) polymer support with *tert*-butyloxycarbonyl (Boc)-protected amino acids by split synthesis (13) and Bayer and Mutter's protocol (7) with the following modifications. Boc-L-Leu, Boc-Gly, Boc-L-Phe, and Boc-Tyr(2-BrCbz) (where 2-BrCbz is 2-bromobenzyloxycarbonyl) were amino acid components for the library construction. The first amino acid residue was anchored to MeO-PEG by the *N,N'*-dicyclohexylcarbodiimide/*N,N*-dimethylaminopyridine coupling method (14). The coupling efficiency was determined to be >99%, based on the absorbance of a phenyl carbamate derivative ($\varepsilon_{236}$ = 17,500 $M^{-1}\cdot cm^{-1}$) which was quantitatively formed by the reaction between the unreacted hydroxyl groups of MeO-PEG and phenyl

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Abbreviations: Boc, *tert*-butyloxycarbonyl; BSA, bovine serum albumin; LPCS, liquid-phase combinatorial synthesis.

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isocyanate in the presence of a catalytic amount of dibutyltinlaurate. The next amino acids were added sequentially with the aid of 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate and diisopropylethylamine (15). Each coupling reaction was run in a mixed solvent of methylene chloride and dimethylformamide until the ninhydrin test of Kaiser *et al.* (16) was negative; acetic anhydride was used to cap any uncoupled amino groups. After each coupling step, a portion of the polymer was saved and labeled for future use according to the recursive deconvolution method of combinatorial chemical libraries (12). The final removal of *N*-Boc and *O*-(2-BrCbz) groups by iodotrimethylsilane (17) completed the construction of the pentapeptide library.

**Preparation of the [Leu$^5$]Enkephalin–Bovine Serum Albumin (BSA) Conjugate (BSA-1).** [Leu$^5$]Enkephalin was coupled to BSA, making BSA-**1** (Fig. 1). Note that the coupling of **1** to BSA requires the reformulation of BSA to a sulfhydrylated protein by Traut's reagent (2-iminothiolane).

**Partial Library Competition ELISA for Anti-β-Endorphin Monoclonal Antibody.** Each well of a Costar 96-well plate was initially coated with 25 μl of BSA-**1** (5–20 mg/ml) in 60 mM sodium bicarbonate/30 mM sodium carbonate, pH 9.3, overnight. The wells were washed with deionized water and blocked with 100 μl of blotto (nonfat dry milk/antifoam A solution with 1% thimerosal) to prevent nonspecific adsorption. After incubation for 30 min at 37°C in a moist chamber, the blotto was shaken out, and 25 μl of blotto and 25 μl of the partial library pool (competing antigen) were added to the first well and serially diluted across the plate; the same process was then continued in the first well of the second row. Well 12 was used as the positive control (this serial dilution step was used for all competing partial library pools). The anti-β-endorphin antibody was added to each well (25 μl) and the plate was incubated at 37°C for 2 hr. The plate was washed 20 times with deionized water, and 25 μl of a 1:1000 dilution of goat anti-mouse IgG glucose oxidase conjugate (Cappel) was added to each well and the plate was incubated at 37°C for 1 hr. The plates were washed 20 times with deionized water, and bound antibody was detected by the addition of 50 μl of developing agent [0.6 μl of 20% glucose, 40 μl of 91 mM 2,2′-azinobis(3-ethylbenzthiazolinesulfonate), and 40 μl of 25 μM horseradish peroxidase in 5 ml of phosphate buffer, pH 6.0] to each well. Thirty minutes later the plates were read at 405 nm.

**Construction of a Sulfonamide Library.** The arylsulfonamide library was constructed on the MeO-PEG support by parallel synthesis. MeO-PEG was incubated with 4-(chlorosulfonyl)phenyl isocyanate in the presence of a catalytic amount of dibutyltinlaurate to give the *N*-[4-(chlorosulfonyl)-phenyl]carbamate of MeO-PEG, compound **6** (see Fig. 3). The compound **6** preparation was divided into six portions for reaction with six different amines in the presence of pyridine to generate sulfonamides **7.** The basic hydrolysis of these MeO-PEG sulfonamides completed the construction of an arylsulfonamide library consisting of six members (see Fig. 3).

O-*(MeO-PEG)* N-*[4-(chlorosulfonyl)phenyl]carbamate.* 4-(Chlorosulfonyl)phenyl isocyanate (0.653 g, 3 mmol) was added to MeO-PEG (5 g, 1 mmol) in methylene chloride (50 ml) and two drops of dibutyltinlaurate were added (18). After 5 hr of stirring at room temperature, diethyl ether was slowly added to the vigorously stirred reaction mixture. The precipitate was collected on a glass filter and thoroughly washed with diethyl ether. The precipitate was dried under vacuum to yield the desired product quantitatively.

O-*(MeO-PEG)* N-*[(4-alkylaminosulfonyl)phenyl]carbamate.* The *N*-[4-(alkylaminosulfonyl)phenyl]carbamate of MeO-PEG was prepared by continuously bubbling ammonia gas through *O*-(MeO-PEG) *N*-[4-(chlorosulfonyl)phenyl]carbamate (0.5 g, 95.8 μmol) in methylene chloride (5 ml) containing pyridine (20 eq) for 24 hr at room temperature (method A), by stirring *O*-(MeO-PEG) *N*-[4-(chlorosulfonyl)phenyl]carbamate (0.5 g, 95.8 μmol) with an excess of amine (15 eq) in methylene chloride (5 mL) containing pyridine (20 eq) for 24 hr at room temperature (method B) (19), or by heating the reaction mixture in pyridine solvent at 65°C for an hour (method C) (20). The MeO-PEG polymer was precipitated from the homogeneous solution by the addition of diethyl ether, washed with ethanol, and dried under vacuum to give the desired product quantitatively.

*Sulfonamide.* *O*-(MeO-PEG) *N*-[4-(alkylaminosulfonyl)phenyl]carbamate (0.45 g) was dissolved in 0.5 M NaOH (10 ml) and heated at 90°C for 30 min (19, 20). The reaction mixture was cooled to 4°C and neutralized to pH 6–8 with concentrated HCl. The reaction mixture was extracted with ethyl acetate three times, and the combined ethyl acetate layer was washed with brine and dried over $MgSO_4$. The removal of solvent gave an analytically pure product (as judged from the NMR spectrum). The reaction yield was typically 95–97%.

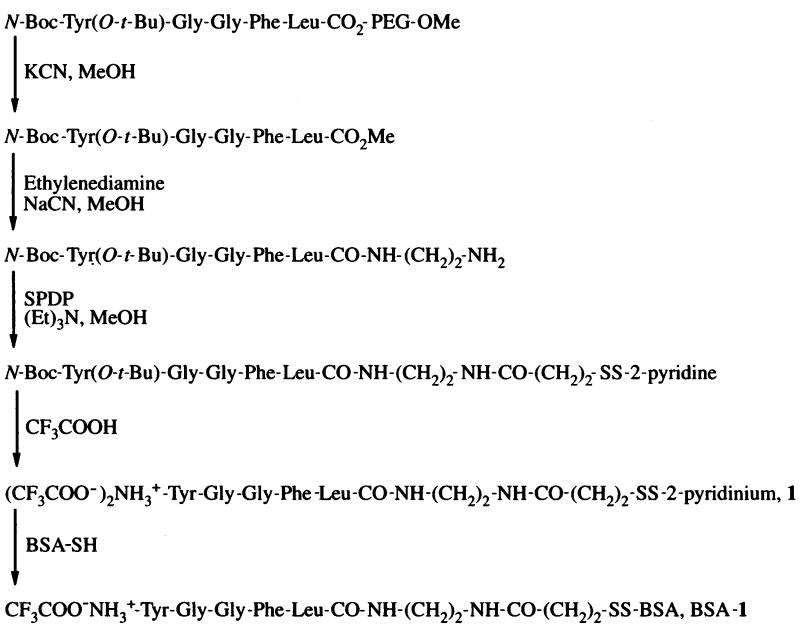


FIG. 1. Preparation of [Leu$^5$]enkephalin–BSA conjugate. SPDP, *N*-succinimidyl 3-(2-pyridyldithio)propionate; BSA-SH, BSA after introduction of sulfhydryl by reaction with Traut's reagent (2-iminothiolane).

## RESULTS AND DISCUSSION

**Synthesis of the MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Naa$_{[4]}$-Naa$_{[5]}$ Peptide Library.** Validation of the LPCS method was achieved by synthesis of a peptide library using the recursive deconvolution methodology. The essence of recursive deconvolution is to build and hold a set of partially synthesized combinatorial libraries (12). The first LPCS library contained four components (Tyr, Gly, Phe, and Leu) and five partial sublibraries, to give a total library size of 1024. Because there were four components, four channels of synthesis were used, each involving the addition of a single component at any time.

Initiating the process required the splitting of MeO-PEG into four equal pools, in which Tyr, Gly, Phe, or Leu was coupled to the homopolymer. After the coupling reactions, MeO-PEG-Naa (where Naa is Tyr, Gly, Phe, or Leu) was precipitated by the addition of diethyl ether. This allowed the removal of excess coupling reagents by filtration of the MeO-PEG-Naa. The more polar contaminants were removed by simple recrystallization of the MeO-PEG-coupled product. The importance of this step is that crystallization avoids the possibility of inclusions, which may occur with gelatinous precipitates. Additionally, the excess of protected amino acids can be removed quantitatively. Portions of each of these sublibraries were set aside and catalogued as partial libraries p(1). The remaining MeO-PEG-Naa was combined, solubilized, and separated into four portions; Tyr, Gly, Phe, or Leu was attached as before; and polymer sublibraries were precipitated and crystallized. Again, an aliquot of each sublibrary was set aside as a partial library p(2), which consisted of four pools made up of MeO-PEG-Naa$_{[1]}$-Tyr, MeO-PEG-Naa$_{[1]}$-Gly, MeO-PEG-Naa$_{[1]}$-Phe, or MeO-PEG-Naa$_{[1]}$-Leu (subscript numbers in brackets indicate positions, not multiples; note that Naa$_{[1]}$ is the C-terminal residue). The remainder was again pooled and split, and the entire process was repeated for the assembly of sublibraries p(3) and p(4) and a final sublibrary, p(5) (MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Naa$_{[4]}$-Tyr, MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Naa$_{[4]}$-Gly, MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Naa$_{[4]}$-Phe, or MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Naa$_{[4]}$-Leu).

**Recursive Deconvolution of the MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Naa$_{[4]}$-Naa$_{[5]}$ Peptide Library: Screening for Anti-β-Endorphin Ligands.** A competition ELISA method was devised which, when integrated into the recursive deconvolution strategy, allowed us to define the optimum ligands that inhibited the binding of [Leu$^5$]enkephalin (Tyr-Gly-Gly-Phe-Leu-OH) to anti-β-endorphin monoclonal antibody 3E7 (21). This antibody binds to its natural epitope with high affinity ($K_d$ = 7.1 nM) (22).

To set up the competition ELISA, attachment of the true ligand onto a protein of sufficient hydrophobicity had to be accomplished. We chemically synthesized the C-terminal pyridinium disulfide derivative **1** (Fig. 1). This activated pentapeptide was swiftly and cleanly coupled to bovine serum albumin (BSA) which had been modified with Traut's reagent. This BSA-**1** conjugate thus provided a way to display the pentapeptide ligand on an ELISA plate. This strategy also allowed the coupling process to be monitored, since thiopyridine absorbs at 343 nm. This BSA-Tyr-Gly-Gly-Phe-Leu unit affixed to an ELISA plate allowed quantitation of Tyr-Gly-Gly-Phe-Leu or its analogs in solution by competition for binding of anti-β-endorphin to the immobilized Tyr-Gly-Gly-Phe-Leu. The amount of bound anti-β-endorphin could then be quantified by ELISA.

The diverse solubilizing power of MeO-PEG provided a direct way to screen the saved and catalogued MeO-PEG sublibraries in a homogeneous competition ELISA assay for binding to the β-endorphin antibody (Table 1). However, it should be noted that the library could be "deprotected" and the MeO-PEG removed to provide just the library of ligands. These sublibrary mixtures could also be searched in an analogous manner for prospective binding ligands, and the binding affinities detected were quite similar (Table 1).

The deconvolution sequence could be monitored by examining the $IC_{50}$ values determined for each p($n$) sublibrary in Table 1. Thus, starting with the four pools of the pentapeptide sublibrary p(5), where only the N-terminal amino acid is defined, the MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Naa$_{[4]}$-Tyr pool gave the only detectable binding, $IC_{50}$ = 51 μM. Based on the recursive strategy, Tyr was coupled to the four saved and catalogued p(4) sublibraries, giving MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Gly-Tyr, MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Phe-Tyr, MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Leu-Tyr, and MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Tyr-Tyr. Assay of these four new pools provided an enrichment step and, more importantly, deconvoluted the next residue, glycine (MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Gly-Tyr, $IC_{50}$ = 7.7 μM). These results allowed for a logical procession to the next saved sublibrary, p(3), wherein both tyrosine and glycine were coupled to the four p(3) pooled sequences. Solving for the third amino acid did not give a unique result but MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Gly-Gly-Tyr, the sequence corresponding to that of the native epitope, was the strongest binder, with $IC_{50}$ = 1.1 μM. The p(2) sublibrary was solved in a similar manner (see below), but now two pools—one containing the predicted sequence, MeO-PEG-Naa$_{[1]}$-Phe-Gly-Gly-Tyr ($IC_{50}$ = 0.18 μM), and one containing the sequence MeO-PEG-Naa$_{[1]}$-Leu-Gly-Gly-Tyr ($IC_{50}$ = 4.0 μM)—were uncovered. At this point, deduction of alternative active members could have been accomplished by tracing in succession both sequences, Tyr-Gly-Gly-Phe and Tyr-Gly-Gly-Leu. However, because this same pentapeptide library had already been examined by a solid-phase recursive deconvolution strategy (12), we decided to follow only the most active component (Tyr-Gly-Gly-Phe) through the iterative process. The final p(1) sublibrary provided us with the native epitope and several other potent binders (Table 1).

Table 1. Recursive deconvolution of peptide library containing the antigenic determinant Tyr-Gly-Gly-Phe-Leu recognized by monoclonal antibody 3E7

| Library mixture | $IC_{50}$, μM |
|---|---|
| p(5) | |
| Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Naa$_{[4]}$-Tyr | 46 |
| MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Naa$_{[4]}$-Tyr | 51 |
| MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Naa$_{[4]}$-Leu | >1000 |
| MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Naa$_{[4]}$-Gly | >1000 |
| MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Naa$_{[4]}$-Phe | >1000 |
| p(4) | |
| MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Gly-Tyr | 7.3 |
| MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Leu-Tyr | >250 |
| MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Phe-Tyr | >250 |
| MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Naa$_{[3]}$-Tyr-Tyr | >250 |
| p(3) | |
| MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Gly-Gly-Tyr | 1.1 |
| MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Leu-Gly-Tyr | 32 |
| MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Phe-Gly-Tyr | 54 |
| MeO-PEG-Naa$_{[1]}$-Naa$_{[2]}$-Tyr-Gly-Tyr | 43 |
| p(2) | |
| MeO-PEG-Naa$_{[1]}$-Phe-Gly-Gly-Tyr | 0.18 |
| MeO-PEG-Naa$_{[1]}$-Leu-Gly-Gly-Tyr | 4.0 |
| MeO-PEG-Naa$_{[1]}$-Gly-Gly-Gly-Tyr | 19 |
| MeO-PEG-Naa$_{[1]}$-Tyr-Gly-Gly-Tyr | 32 |
| p(1) | |
| MeO-PEG-Leu-Phe-Gly-Gly-Tyr | 0.034 |
| MeO-PEG-Phe-Phe-Gly-Gly-Tyr | 0.049 |
| MeO-PEG-Tyr-Phe-Gly-Gly-Tyr | 0.091 |
| MeO-PEG-Gly-Phe-Gly-Gly-Tyr | 0.21 |

Note that Naa$_{[1]}$ is the C-terminal residue.

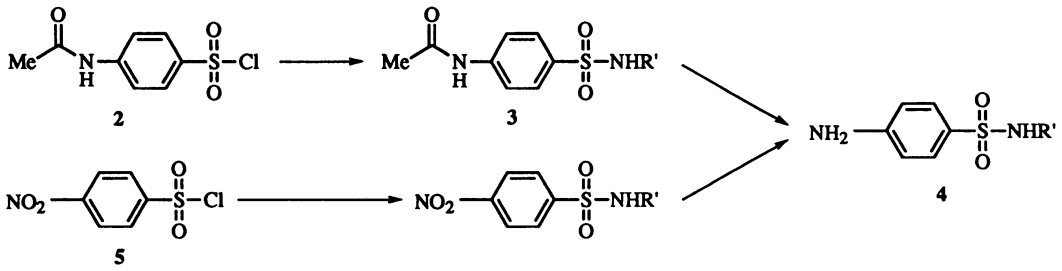

FIG. 2. Two classic arylsulfonamide preparation methods.

**Liquid-Phase Synthesis and Characterization of Nonpeptide, Nonoligomeric Molecules: Sulfonamides.** The LPCS process should allow for the synthesis of any class of molecular entity as long as the chemistry employed does not interact with or adversely affect the polymer's properties. As a starting point for the examination of the MeO-PEG support under conditions other than peptide linking and deprotection reactions, we investigated the polymer's potential in the context of synthesizing a class of compounds known as sulfonamides. Sulfonamides, because of their low cost and undeniable efficacy in susceptible infections, have for years spurred the preparation of numerous analogs (23). However, because of bacterial resistance, a relatively narrow antibacterial spectrum, and unacceptable side effects in some patients, the antibacterial sulfonamides no longer enjoy the clinical vogue they once had. Interestingly, because of these extensive clinical studies several pleasant surprises came out of this work—namely, a number of the arylsulfonamides which showed poor antibacterial potency now provided leads to new classes of drugs (24). These include new classes of endothelin antagonists (25) and antitumor agents (26) and/or possess antiarrhythmic activity (27). The arylsulfonamide nucleus thus appears to be a significant pharmacophore on which to build a combinatorial library.

Before a library of any magnitude can be secured, a general synthetic scheme with reliable protocols for a variety of chemistries must be investigated. Past syntheses of arylsulfonamides that have led to drugs have been achieved by one of two fairly straightforward routes (Fig. 2) (27). In the first route, chlorosulfonation of acetanilide gives the corresponding sulfonyl chloride **2**, and reaction with the appropriate amine gives the intermediate **3**. Hydrolysis in either acid or base leads to the sulfanilamide **4**. In an alternative approach, the amide formation is performed on *p*-nitrobenzenesulfonyl chloride, **5**. Reduction by either chemical or catalytic methods directly affords the desired product. We envisioned an arylsulfonyl chloride like **2** (Fig. 2) to be the key intermediate in our MeO-PEG synthesis, and while both routes provide such an intermediate, neither presents a convenient handle for the attachment of the arylsulfonyl chloride appendage.

A new route (Fig. 3) was devised which provides the flexibility for added diversity and embraces in a simple manner the desired arylsulfonyl chloride. By starting with 4-(chlorosulfonyl)phenyl isocyanate the MeO-PEG support is functionalized and the desired sulfonyl chloride intermediate **6** is obtained in a single step. Most impressive is that there is no competing nucleophilic process at the chlorosulfonic acid moiety during this coupling reaction. Equally important is that this linkage allows the reaction to be followed by $^1$H NMR (Table 2) and is compatible with a variety of sulfonyl chloride nucleophilic addition reactions; yet at the end of the synthesis the carbamate which links the arylsulfonamide to the MeO-PEG is readily cleaved (with NaOH) and the product can be isolated from the homogeneous support. Employing the reaction scheme shown in Fig. 3 we have synthesized the structurally diverse arylsulfonamides **8** in multimilligram quantities (Fig. 3 and Table 2). While the key intermediate is sulfonyl chloride **6**, the overall success of the arylsulfonamides synthesized, as shown in Table 2, is highly dependent on the $pK_a$ of the nucleophile. Therefore very poor nucleophiles such as **7e** and **7f** require longer reaction times and more stringent temperatures (Table 2).

**Concluding Remarks.** Peptide libraries secured on solid supports were the first chemically synthesized combinatorial libraries (28–31). As important as this work was, the need for greater chemical diversity was quickly recognized and an explosion of nonoligomeric heterocyclic libraries has begun to dominate the combinatorial scene (32–35). An outgrowth of these classes of libraries is the fervent pace to try and adapt solid-phase synthesis to multistep organic reaction sequences. We have proposed and implemented a technology termed LPCS to simplify and thus further accelerate this process. This methodology combines the advantages that classic organic synthesis offers in solution with those that solid-phase synthesis can provide.

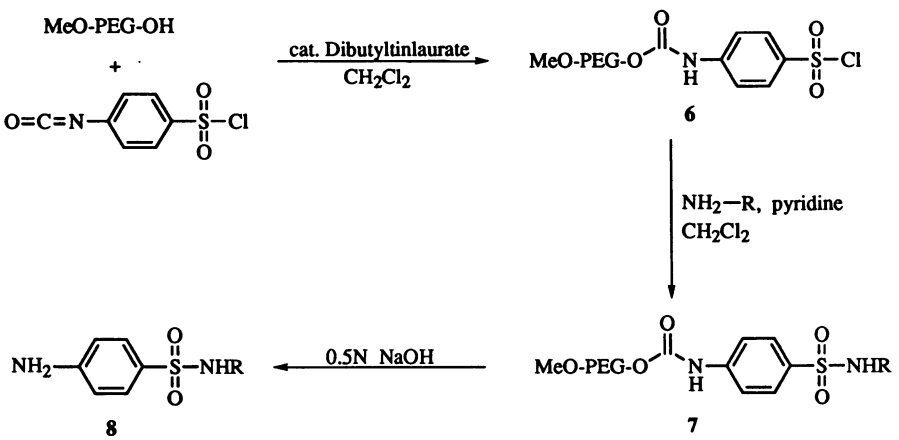

FIG. 3. Construction of an arylsulfonamide library. R = hydrogen, benzyl, isobutyl, phenyl, 2-pyridyl, or 2-(4,6-dimethyl)pyrimidyl.

Table 2. Arylsulfonamide derivatives 7

| Derivative | R | $pK_a$ | Method |
|---|---|---|---|
| **7a** | Hydrogen | 9.2 | A |
| **7b** | Isobutyl | 10.75 | B |
| **7c** | Benzyl | 9.3 | B |
| **7d** | 2-Pyridyl | 6.82 | B |
| **7e** | 2-(4,6-Dimethyl)pyridyl | 4.8 | C |
| **7f** | Phenyl | 4.63 | C |

All compounds were characterized by $^1H$ NMR. In the NMR spectrum, the integration of R protons versus carbamate protons ($-CH_2O-$) at 4.35 ppm was used to determine the extent of the displacement reaction of sulfonyl chloride with amine. For further details, see *Materials and Methods* and specifically methods A, B, and C.

The results reported in this article indicate that the reaction scope of LPCS should be general. Its value to multiple, high-throughput screening assays could be of particular merit, since multimilligram quantities of each library member can be attained. The principles and methods outlined for LPCS should be applicable to the synthesis of complex chemical structure libraries as well as to other processes that fall under the heading of chemical diversity.

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