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Application of an almost traceless linker in the synthesis of 2-alkylthiobenzimidazole combinatorial libraries

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Summary

Resin-bound triphenylphosphine was coupled to 4-fluoro-3-nitrobenzyl bromide, and 2-alkylthiobenzimidazoles were synthesized on resin in 4 steps using standard chemistries. Cleavage of the compounds from the resin was achieved with 10% NaOH in MeOH to leave a methyl group at the attachment point. A total of 47 amines and 40 electrophiles were evaluated, defining the scope of the reactions, culminating in the synthesis of an 80-member test library of high purity as determined by HPLC.

Abbreviations: DCM, dichloromethane; ESI MS, electron spray ionization mass spectrometry; NBS, N-bromosuccinamide; NMP, N-methylpyrrolidinone; DMF, dimethylformamide; TCD, thiocarbonyldiimidazole; TFA, trifluoroacetic acid; rt, room temperature.

Introduction

Combinatorial chemistry and parallel synthesis techniques, both in solution and solid phase, have become commonplace in drug discovery efforts. One challenge in solid phase chemistry is controlling the nature of the functional group left on the product after its cleavage from the solid support. Amide, acid and hydroxyl moieties are common functional groups that remain on the product after cleavage. However, it is often desirable to leave a non-polar memory of attachment to the resin, particularly for SAR studies. Toward this goal, a practical application of a previously reported phosphonium linker [1], which leaves a methyl group on the scaffold after cleavage from the resin, is herein described.

We chose the synthesis of 2-alkylthiobenzimidazoles to evaluate the phosphonium linker (Figure 1). This scaffold provided a straightforward yet rigorous test of the linker system using standard solid-phase chemistries [2]. Concurrent with our work, a literature report was published following the same efficient synthetic route [3]. Not surprisingly, these previous syntheses all used an amide linkage to attach the benzimidazole ring to the resin.

A number of test libraries were synthesized to evaluate the scope and limitations of the two sites of diversity, i.e. the primary amine and the electrophile components. It was found that the amine site could tolerate, in general, electron-rich benzylic, unhindered, alkenyl, alkynyl, ether- and amide-containing amines (see Table 1). Anilines, hydrazines, electron-deficient benzylic amines, and pyrazoles were either low yielding or unsuccessfully coupled. In certain cases, the amine sidechain may itself be alkylated in the presence of an electrophile later in the synthesis. Products for all evaluation libraries were analyzed by positive and negative mode ESI MS, HPLC at 3 wavelengths (220, 254 and 280 nm; the benzimidazole chromophore absorbs most strongly at about 295 nm), and LCMS for products with multiple HPLC peaks and/or without a strong MH+ ion in ESI MS. For samples with more than 4 peaks by HPLC, no further characterization was performed and the sidechain was not subsequently used. A portion of the compounds in the amine testing

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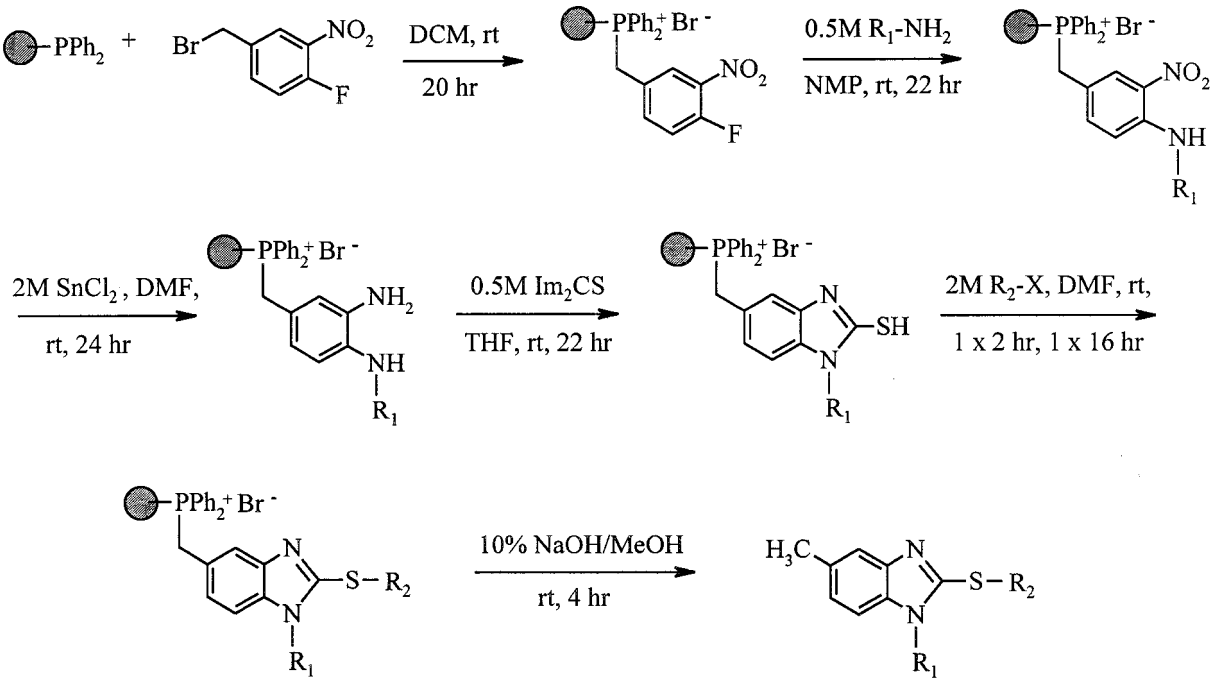

Figure 1. Synthesis of 2-alkylthiobenzimidazoles with an almost traceless linker.

library (15%) was analyzed by ^{1}H NMR for purity, and dichloroethane was added to one sample as an internal standard to determine an absolute yield of 50% after cleavage

The electrophile diversity position was similarly explored (Table 2). Unactivated secondary bromides, secondary chlorides, electron-deficient halides, hindered epoxides, triflates, tosylates and mesylates do not react satisfactorily. The major product obtained in these cases is unsubstituted thiol. In contrast, α-bromoketones, most benzyl bromides and chlorides, monosubstituted epoxides and simple halides work well. A portion of the compounds in the electrophile testing library (17%) was analyzed by ^{1}H NMR for purity, and in one example, addition of an internal standard (dichloroethane) established an absolute overall yield of 50%.

Cleavage of the benzimidazole products from the resin to leave the 'almost traceless' methyl group was achieved with a 10% NaOH in MeOH cleavage cocktail. The cocktail was added to DCM-swollen resin, the slurry was shaken at room temperature for 4 h, and the cleaved product was obtained by filtration. The solution was neutralized (HCl) and addition of chloroform precipitated the NaCl/NaBr salts, which were subsequently filtered off. The bromide ion present from the initial scaffold loading was useful for monitoring the loading level of the resin (via elemental analysis), and was conveniently removed during cleavage of the product from the resin. The presence of minor amounts (5–10 mg) of NaCl and NaBr salts is tolerable for screening purposes, and is often preferred over TFA salts in certain assays. If the presence of salts is problematic, an aqueous extraction process can easily remove them. For example, using cyclohexylamine and benzyl bromide as the diversity elements, the final 2-benzylthiobenzimidazole product showed a single peak by HPLC (280 nm), clean MS and NMR spectra (^{1}H and ^{13}C), and was present in a 60% overall yield after aqueous extraction.

Synthesis of an 80-member library, using a subset of the validated amines and electrophiles listed in the tables above, proceeded smoothly and all products were analyzed by HPLC and MS (ESI). Additionally, 34% of the library was analyzed by LCMS and 21% of the library by TLC. In this library, 70% of the compounds were obtained in greater than 80% purity by HLPC (280 nm), and 85% of the compounds were present as the major component in the well.

In summary, the utility of a phosphonium linker that leaves only a methyl group attached to the product has been demonstrated. This linker can be used for the synthesis of small libraries, and investigation of other routes for cleavage is underway.

Table 1. Primary amine reactivities

Primary amine	HPLC purity (280 nm)
Furfurylamine	100%
Cyclohexylamine	100%
4-(Aminomethyl)pyridine	100%
3-Methoxypropylamine	100%
Propargylamine	95%
4-(Aminomethyl)benzene sulfonamide	94%
N-(3′-Aminopropyl)-2-pyrrolidinone	92%
4-Methoxybenzylamine	90%
2-(1-Cyclohexyl)ethylamine	90%
N-Acetylethylenediamine	90%
Piperonylamine	89%
4-Chlorobenzylamine	82%
2-(Aminomethyl)pyridine	80%
Allylamine	79%
Isopropylamine	77%
3,3-Diphenyl-propylamine	77%
4-Methylsulfonylbenzylamine	73%
Tetrahydrofurfurylamine	70%
1-(2-Aminoethyl)piperidine	60%
2-(Aminomethyl)benzimidazole	55%
p-Anisidine	50%
4-(2-Aminoethyl)morpholine	<10%
t-Amylamine	<10%
Phenylhydrazine	<10%
Benzhydrylamine	<10%
N-(3-Aminopropyl)imidazole	<10%
α-Aminoacetophenone	<10%
Benzamidine	<10%
5-Amino-3-methyl-1-phenylpyrazole	<10%
2-Amino-4′-bromoacetophenone	<10%
3-Amino-5-tert-butylisoxazole	<10%
2-Ethylphenylhydrazine	<10%
3-Amino-1-propanol vinyl ether	<10%
p-Toluidine	<10%
4-Nitroaniline	<10%
3,4-Dihydroxybenzylamine	<10%
3-Amino-1-phenyl-2-pyrazolin-5-one	<10%
2-Aminopyrimidine	<10%
Benzhydrazide	<10%
4-Nitrobenzylamine	<10%
4-Dimethylaminobenzylamine	<10%
Acetamidine	<10%
o-Toluic hydrazide	<10%
5-Amino-1,3-dimethylpyrazole	<10%
1-(2-Aminoethyl)-2-methyl-5-nitroimidazole	<10%
2-Amino-4′-methoxyacetophenone	<10%
3-Amino-1H-isoindole	<10%

Table 2. Electrophile reactivities

Electrophile	HPLC purity (280 nm)
Iodomethane	98%
Ethyl bromoacetate	97%[a]
4-Chlorobenzyl bromide	97%
Benzyl bromide	97%
4-Bromobenzyl bromide	97%
4-Fluorobenzyl bromide	97%
2-(Chloromethyl)quinoline	96%
4-Methoxybenzyl chloride	94%
α-Bromopropriophenone	94%
1-Bromopinacolone	94%
2-(Chloromethyl)benzimidazole	91%
3-Bromopropylamine	88%
2-(Bromomethyl)-5-nitrofuran	87%
4-Methylsulphonylbenzylbromide	87%
Phenyl glycidyl ether	84%
Propylene oxide	77%
2-Bromo-4′-methoxyacetophenone	77%
Allyl glycidyl ether	70%
3-Nitrobenzylbromide	62%
Propyleneimine	53%
4-Nitrobenzyl bromide	51%
3-(2-Bromoethyl)indole	49%
Propargyl bromide	47%
Allyl iodide	34%
Bromoacetonitrile	21%
Ethyl p-toluenesulfonate	<10%
2-Bromobutane	<10%
2-Bromoethyl ethyl ether	<10%
N,N-Diethyl-chloroacetamide	<10%
Styrene oxide	<10%
4-Nitrobenzylbromide	<10%
4-Bromopiperidine	<10%
Ethyl methanesulfonate	<10%
Ethyl triflate	<10%
3-Chloro-2-butanone	<10%
1-Aziridineethanol	<10%
Cyclohexene oxide	<10%
3-Bromocyclohexene	<10%
4-Acetylphenyl triflate	<10%
3-Nitrophenacyl bromide	<10%

[a] The ethyl group cleaved during the cleavage step.

Experimental

This chemistry was developed using phosphorous resin purchased from Fluka (triphenylphosphine polymer bound, cat # 93094, 100–200 mesh). The batch used for the procedures described below was tested for phosphorus content by elemental analysis (Desert Analytics; Tucson, AZ), showing a loading of 1.37 mequiv/gm. Reactions were performed in both polypropylene syringes containing a teflon frit and 96-well filter plates. Resin in individual syringes was mixed by tumbling on a Barnstead/Thermolyne Labquake rotisserie, and resin in plates was shaken on a Labline titer plate shaker. Filter plates were washed and drained with a Univac vacuum apparatus from Polyfiltronics. Clamps for the filter plates, to prevent leaking from the bottom during reaction incubations, were custom fabricated, although Polyfiltronics' Combiclamps were also used successfully. Following cleavage and neutralization, the final products were collected in 2-mL 96-well deep well plates and dried (Savant SpeedVac).

Synthesis of 4-fluoro-3-nitrobenzyl bromide

4-Fluoro-3-nitrotoluene was dissolved in carbon tetrachloride (0.32 to 0.46 M), 1.1 equiv of NBS was added, then 0.2 equiv of benzoyl peroxide. The suspension was heated in an oil bath and refluxed (approx. 77 °C) for 16–27 h. By TLC (9:1 pet ether/EtOAc), the starting material does not completely disappear, and further addition of NBS and benzoyl peroxide did not force it to completion. To limit formation of the di-brominated side product, the reaction was stopped after about 16 h. The suspension was cooled to room temperature and filtered, the solid was washed with carbon tetrachloride, and the solute stripped to a yellow oil which solidified on standing. ^{1}H NMR of this crude product ($CDCl_3$) shows ratios of approx. 60% desired mono-bromo product, 20% starting reagent, and 20% of the undesired di-bromo side product, as determined by integration of the aryl peaks. The mixture was flash chromatographed (silica gel) using the same 9:1 solvent system as used for TLC. Crude material, of only approx. 60% purity, has been loaded onto the resin and shown to give lower loading efficiencies than pure scaffold, although the cleavage products from all are indistinguishable by ^{1}H NMR. Rf's of products (9:1 pet ether/EtOAc, visualization by UV): 0.40 = SM, 0.33 = di-Br and 0.29 = mono-Br. ^{1}H NMR of the desired mono-Br scaffold ($CDCl_3$): 4.49 ppm (s, 2H), 7.30 (t, 1H), 7.67 (m, 1H), 8.11 (d, 1H). ^{1}H of the di-Br side product ($CDCl_3$): 6.66 ppm (m, 1H), 7.54 (m, 1H), 7.91 (m, 1H), 8.29 (1H). ^{1}H of the starting material ($CDCl_3$): 2.42 ppm (s, 3H), 7.18 (t, 1H), 7.42 (m, 1H), 7.87 (d, 1H). ^{13}C NMR was not helpful, as the fluorine-induced splitting pattern was uninterpretable. MS of these materials was inconclusive, as the compounds do not ionize under ESI conditions. HPLC was not performed. Alternatively, this compound can be synthesized from 4-fluoro-3-nitrobenzylic acid via nitration, reduction, and bromination, as described in the literature [4]. For our larger library syntheses, we obtained material from Organic Consultants, Inc., Eugene, OR.

Scaffold loading

A 0.65 M solution of the scaffold (3 equiv) in DCM was added to pre-swollen resin, and the slurry was shaken for 20 h at ambient temperature. The resin was washed (5 × DCM) and a sample of the resin was dried with nitrogen purge and submitted for elemental analysis (Br content) to determine the loading level. Loading is typically 84–97% of theory. Cleavage of the scaffold from the resin at this stage provided 4-fluoro-3-nitrotoluene in 84% isolated yield after extractive workup.

Nucleophilic aromatic substitution of the aryl fluoride with primary amines

A 0.5 M solution of the amine (3.6 equiv) in NMP was added to preswollen resin, and the slurry was shaken for 20 h at ambient temperature. The resin was washed with NMP, DMF, and DCM. Cleavage of the scaffold from the resin at this stage, after substitution with cyclohexylamine, provided 4-aminocyclohexyl-3-nitrotoluene in 83% isolated yield. ^{1}H NMR and MS (ESI) were consistent with the expected product.

Reduction of the aryl nitro group with tin chloride

A 2.0 M solution of tin (II) chloride dihydrate (at least 5 equiv) in DMF was added to preswollen resin, and the slurry was shaken for 24 h at ambient temperature. The resin was washed with DMF, 50% aqueous DMF, MeOH, and DCM. ^{1}H NMR and MS (ESI) were consistent with the expected product.

Cyclization with thiocarbonyldiimidazole (TCD)

A 0.5 M solution of TCD (10 equiv) in DMF was added to preswollen resin, and the slurry was shaken for 22 h at ambient temperature. Dichloromethane, toluene, and THF have each been used as the reaction solvent in polypropylene syringes for 22 h with

excellent results, but these solvents will warp a filter plate after 6–12 h at room temperature. The resin was washed with DMF and DCM. Cleavage of a portion of resin from the scaffold at this stage, substituted with cyclohexylamine, provided 1-cyclohexyl-5-methylbenzimidazole-2-thiol in 70% yield. This sample gave ^{1}H NMR, ^{13}C NMR, and MS (ESI) data which were consistent with the expected product.

Alkylation of the thiol

A 2.0 M solution of the electrophile (at least 12 equiv) in DMF was added to preswollen resin, and the slurry was shaken for 2 h at ambient temperature. The reaction mixture was drained, the resin rinsed with 2 × DMF, then a second portion of the alkylating agent was added and the slurry was shaken for another 2–15 h. The resin was washed with DMF and DCM. Cleavage of the scaffold at this stage, substituted with cyclohexylamine and alkylated with benzyl bromide, provided a 60% isolated yield of 1-cyclohexyl-2-benzylthio-5-methylbenzimidazole after extractive workup. ^{1}H NMR, ^{13}C NMR, and MS (ESI) were consistent with the expected product.

Cleavage of the 2-alkylthiobenzimidazole product from the resin

Cleavage reactions were done using a freshly prepared cleavage cocktail of 10% NaOH in MeOH, using 1.0 mL of cocktail for reactions with ≥100 mg of resin/syringe and 0.7 mL of cocktail for reactions with less than 100 mg resin, such as in filter plates. The solution was added to the resin and the resin was shaken/tumbled for 4 h at room temperature. The resin was washed with MeOH and DCM, the washes were combined, diluted with DCM and water, neutralized with HCl, and either extracted or the plate was dried, samples resuspended in $CHCl_3$ and filtered. Analysis of the cyclohexylamine/iodomethane-substituted product showed ^{1}H NMR, ^{13}C NMR, and MS (ESI) consistent with expected product. Using an internal standard (dichloroethane) in an ^{1}H NMR experiment, integration of the product peak vs integration of the DCE indicates a yield of approx. 50% (+/– 10%) of theoretical, with the remaining mass attributed to be inorganic salts as no organic impurities show up by NMR or HPLC.

Diversity evaluation

Each site of diversity was evaluated in 2 small libraries prior to the final test library synthesis. For these libraries, sidechains with various functionality and steric components were chosen to explore their compatibility with the chemistry for the synthesis. Certain sidechains were used in more than one library to double-check reactivity and/or to monitor consistency. In total, 47 different amines and 39 different electrophiles were tested. The results are summarized in Tables 1 and 2 in the text.

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